# SH2B1 Regulates Insulin Sensitivity and Glucose Homeostasis by Multiple Mechanisms

**by**

**David L. Morris**

**A dissertation submitted in partial fulfillment**
**of the requirements for the degree of**
**Doctor of Philosophy**
**(Molecular and Integrative Physiology)**
**in The University of Michigan**
**2009**

**Doctoral Committee:**

**Associate Professor Liangyou Rui, Chair**
**Professor Christin Carter-Su**
**Professor Ormond A. MacDougald**
**Associate Professor Martin G. Myers, Jr.**
**Research Assistant Professor Robert C. Thompson**

## Table of Contents

## List of Figures

**Figure**

**Abstract**

Leptin and insulin are key hormonal regulators of energy balance and glucose homeostasis. SH2B1 is a PH- and SH2-domain containing adapter protein that promotes both leptin and insulin signaling in cells. Disruption of *Sh2b1* in mice causes obesity and diabetes, providing genetic evidence that SH2B1 is an essential regulator of energy balance and glucose homeostasis. Here, the contribution of SH2B1 in brain, peripheral tissues, and islets to the regulation of insulin sensitivity and glucose homeostasis was examined in vivo. Reconstitution of full length SH2B1, but not the SH2 domain alone, in neurons in *Sh2b1*-deficient mice was sufficient to prevent the development of obesity and insulin resistance. By contrast, expression of a mutant form of SH2B1 that lacked a functional SH2 domain in neurons of wild type mice promoted weight gain and impaired whole body insulin sensitivity. Thus, SH2B1 in the brain indirectly regulates glucose homeostasis by controlling energy balance and body weight. To address the role of peripheral SH2B1 in the regulation of insulin sensitivity and glucose metabolism, mice expressing SH2B1 in the brain but not in peripheral tissues (TgKO mice), were fed a high fat diet (HFD). Deletion of SH2B1 in peripheral tissues did not alter HFD-induced obesity, but significantly exacerbated HFD-induced hyperglycemia, hyperinsulinemia and glucose intolerance in TgKO mice. Insulin signaling was attenuated in muscle, liver, and white adipose tissue from HFD-fed TgKO mice. In cultured cells, SH2B1 binds to the insulin receptor, IRS-1 and IRS-2, and enhances insulin sensitivity by both promoting receptor activity and by inhibiting tyrosine dephosphorylation of IRS proteins, providina mechanism by which SH2B1 likely promotes insulin action in these tissues. SH2B1 also promotes glucose homeostasis independent of central leptin action. Leptin-deficient (*ob/ob*) mice with *Sh2b1* haploinsufficiency (*Sh2b1*$^{+/-}$*ob/ob*) developed severe hyperglycemia and glucose intolerance. Plasma insulin levels and pancreatic insulin content were reduced in *Sh2b1*$^{+/-}$*ob/ob* mice. SH2B1 is highly expressed in pancreatic islets, and these data suggest that SH2B1 in islets may regulate glucose homeostasis by

promoting insulin biosynthesis and secretion. Together, these findings indicate that SH2B1 regulates insulin sensitivity and glucose homeostasis by multiple mechanisms in vivo.

# Chapter 1

## Introduction

Overnutrition and increasingly sedentary lifestyles have led to an increased prevalence of obesity and obesity-associated metabolic diseases, including type 2 diabetes (or non-insulin dependent diabetes mellitus (NIDDM)). Obesity is primarily caused by long-term energy imbalance, which occurs when energy (food) intake exceeds metabolic expenditure. Excess energy is stored as trglyceride in adipose tissue, causing an increased in adiposity and weight gain. Increased adiposity that is associated with excessive weight gain is now considered a driving force for the development of type 2 diabetes. Increased adiposity is associated with an increase in pro-inflammatory cytokine production and a state of low grade, chronic inflammation. Systemic increases in pro-inflammatory cytokines causes impaired insulin action in peripheral tissues (insulin resistance). Insulin resistance contributes to the development of impaired glucose tolerance and hyperglycemia, and may eventually cause β-cell failure, the determinant type 2 diabetes.

Both obesity and type 2 diabetes are complex polygenic disorders, and the interaction of multiple factors contributes to the onset and severity of both diseases. Interactions between environmental risk factors (e.g. increasing sedentary lifestyle, overnutrition, and aging) and genetic susceptibility contribute to the development of obesity and diabetes. While the environmental component is difficult to quantify, it is clear that genetic susceptibility is a key determinant. Factors associated with obesity and diabetes, including dyslipidemia, hypertension, and adiposity, are highly heritable in man (220). However, single gene defects only explain a small proportion of all cases of obesity and diabetes (88, 89, 172, 189). These findings suggest that dysfunction or aberrant regulation of multiple genes, which by themselves have relatively small effect, likely interact with obesogenic and/or diabetogenic factors to promote energy imbalance

and/or impaired glucose tolerance. Therefore, identification and characterization of obesity- and diabetes-susceptibility genes promises to increase not only our basic understanding of the pathophysiology involved in these disease processes, but also increase the potential therapeutic options for treatment of these comorbidities. Mounting evidence suggests that the adapter protein SH2B1 is a candidate susceptibility gene for obesity and diabetes in man, and a novel positive regulator of both leptin and insulin action in mammals.

**Central leptin action regulates energy balance and body weight**

Body weight is normally maintained within a narrow range by a sophisticated neuroendocrine system that constantly monitors energy storage, availability, and consumption. When energy intake (food intake) exceeds metabolic demand (expenditure), excess energy is assimilated into triglyceride in adipose tissue. This leads to increased adiposity and weight gain. If this energy balance is not corrected, obesity and obesity-associated metabolic diseases ensue.

Communication between adipose tissue, which is the primary energy storage depot in mammals, and the brain is important for the long-term regulation of energy balance and body weight. While the primary function of adipose tissue is to store excess energy as neutral lipid, adipose tissue is also a key endocrine organ. In obese individuals, adipose tissue becomes the largest endocrine tissue in the body. Adipose tissue secretes many cytokines (also referred to adipokines), including leptin. Leptin- or leptin-receptor (LEPR) deficiency causes morbid obesity and metabolic syndrome in both animals and humans (42, 156, 214, 219, 250), indicating that leptin plays an essential role in the regulation of energy balance and body weight. Leptin is secreted in direct proportion to adipose mass and serum leptin levels rise as triglyceride accumulates in adipose depots (32, 86, 176, 213). Thus, leptin levels signal the relative storage and availability of energy.

The brain senses leptin and various other metabolic signals and integrates these signals into appropriate physiological responses to regulate energy homeostasis and body weight. Central administration of recombinant leptin reduces food intake and body weight in rodents (32, 85, 213). Neuron-specific deletion of LEPR in mice results in obesity (43), whereas neuron-specific restoration of functional LEPR rescues the obese

phenotype in LEPR-null (*db/db*) mice (51, 122). These data indicate that leptin acts on neurons in the brain to suppress food intake and promote energy expenditure.

**Leptin acts on multiple neuronal populations in the brain**

LEPR mRNA is highly expressed in multiple regions of the brain, including the arcuate nucleus (ARC), the ventromedial (VMH), dorsomedial hypothalamic nuclei (DMH), ventral tegmental area (VTA), hippocampus, and the brainstem (38, 62, 63, 71, 84, 97, 149, 151, 202, 203). Leptin induces expression of c-Fos, a marker of neuronal activity, and activates signal transducer and activator of transcription 3 (STAT3) in hypothalamic neurons known to control energy intake and expenditure, such as the ARC, VMH, and DMH (60, 62, 101, 162). However, restoration of LEPR expression specifically in the ARC corrects hyperphagia and obesity in LEPR-null rats, suggesting that the ARC is a key leptin target (202). Two subpopulations of neurons in the ARC (POMC-neurons and AgRP-neurons) have emerged as critical mediators for leptin action.

POMC-neurons coexpress two anorexigenic neuropeptides: proopriomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) (61, 202). POMC is proteolytically cleaved to generate α-melanocyte stimulating hormone (α-MSH) which activates melanocortin-3 and melanocortin-4 receptors (MC3R and MC4R) (35, 67, 72, 102, 191). MC3R and MC4R are G protein-coupled receptors that are highly expressed in the hypothalamus, especially in the paraventricular hypothalamus (PVN). Deletion of either MC3R or MC4R results in leptin resistance and obesity in mice (35, 102). Additionally, genetic defects in either the *POMC* or the *MC4R* genes are linked to obesity in humans (42, 124).

AgRP-neurons coexpress two orexigenic neuropeptides: agouti-related protein (AgRP) and neuropeptide Y (NPY) (83, 202). NPY potently stimulates feeding behavior and food intake (212). AgRP is a potent antagonist of both MC3R and MC4R (174). Hypothalamic AgRP-neurons are required for feeding in adult mice (82, 143). Interestingly, leptin has opposing effects on POMC- and AgRP-neurons in the hypothalamus. Leptin stimulates excitability and expression of POMC and CART in POMC-neurons; conversely, leptin inhibits both excitability and NPY/AgRP expression in AgRP-neurons (49, 61, 123, 155, 202, 203, 209, 213, 215).

The differential regulation of excitability in NPY/AgRP and POMC neurons can be explained, at least in part, by differential modulation of $K_{ATP}$ channels by leptin and other factors, including insulin and glucose (135, 175, 209, 210). Leptin-induced hyperpolarization of NPY/AgRP-neurons inhibits neuronal activity, which is supported by the observation that leptin fails to induce c-Fos expression in NPY/AgRP-neurons (60). Others have proposed that leptin directly increases the action potential frequency in POMC neurons by both regulating a nonspecific cation channel and by reducing the inhibitory tone of POMC neurons, which is imposed by GABA release from either NPY/AgRP neurons or inhibitory interneurons within the ARC (48, 49). However, POMC neurons have also been shown to be glucose- and insulin-responsive (103, 135, 175, 240), and it is likely that POMC neurons integrate multiple metabolic cues and synaptic inputs with leptin signaling to regulate energy balance.

Surprisingly, selective deletion of functional LEPR in either AgRP- or POMC-neurons results in mild obesity (9, 226). Deletion of LEPR in both AgRP- and POMC-neurons has an additive effect in terms of body weight and adiposity; however, the metabolic phenotypes in these double mutant mice are still milder than in systemic LEPR-deficient *db/db* mice (226). Interestingly, whereas *db/db* mice remain severely hyperphagic throughout life, mice lacking LEPR in both AgRP- and POMC- neurons initially exhibit periweaning hyperphagia (4-6 wks of age), but food intake gradually returns to wild type levels in adulthood (226). These observations suggest that leptin also acts on extraarcuate neurons to regulate energy balance and body weight. In the VMH, leptin activates steroidogenic factor-1 (SF1)-positive neurons and selective deletion of LEPR in this population increases susceptibility to diet-induced obesity (21, 54). Additionally, leptin suppresses motivated food-seeking behaviors by activating STAT3 in dopaminergic and GABAnergic neurons in the VTA (71, 97). These distinct leptin-responsive neurons may act redundantly, in parallel, and/or synergistically with hypothalamic neurons to fully mediate leptin action. Unraveling the complexities of these and other leptin-regulated neuronal networks will not only provide further insight into regulation of energy balance and feeding behavior by leptin, but will also likely increase our understanding of the role of leptin in other aspects of physiology, including reproduction and motivated behaviors as indicated by recent reports (133, 134, 164).

## Leptin stimulates multiple signal transduction pathways

The *LEPR* gene produces multiple leptin receptor isoforms (a, b, c, d, and e) via alternative mRNA splicing (27, 64, 69, 151). All isoforms have an extracellular leptin-binding domain, but only the longest form, LEPRb, contains a full-length intracellular domain required for cellular signaling (70). LEPRa lacks the entire cytoplasmic domain and is thought to mediate leptin transport across the blood brain barrier (94, 95). Genetic deficiency of LEPRb results in profound leptin resistance and morbid obesity in animals, indicating that LEPRb is required for leptin action (14, 36, 41, 77, 128). LEPRb belongs to the gp130 family of cytokine receptors (14, 36, 77). It constitutively binds to JAK2, a member of the Janus kinase (JAK) family of tyrosine kinases. Leptin stimulates LEPRb dimerization, resulting in JAK2 activation and autophosphorylation (10, 52, 66, 232). JAK2 also phosphorylates LEPRb and various downstream signaling molecules on tyrosines (25, 76, 113). JAK2 phosphorylates $Tyr^{985}$, $Tyr^{1077}$ and $Tyr^{1138}$ in the cytoplasmic domain of LEPRb, which then act as docking sites for downstream signaling molecules (10, 90, 136). Replacement of these three tyrosines with phenylalanines in LEPRb induces marked leptin resistance and obesity in mutant mice, indicating that phospho-$Tyr^{985}$, -$Tyr^{1077}$ and/or -$Tyr^{1138}$ mediate the activation of key downstream pathways; however, the mutant mice are less obese and less hyperglycemic than LEPRb-deficient *db/db* mice, indicating that LEPRb can mediate some of leptin's action independent of phosphorylation on these tyrosines (107).

STAT family members are SH2 domain-containing transcription factors located in the cytoplasm in quiescent cells. Cytokine-stimulated tyrosine phosphorylation of STATs induces homo- or heterodimerization, nuclear translocation and transcriptional activation (98). Leptin stimulates tyrosine phosphorylation of STAT1, 3, 5 and 6 in cultured cells (14, 77, 192, 217); however, only STAT3 and STAT5 phosphorylation has been documented in the hypothalamus of leptin-treated rodents (80, 148, 225). $Tyr^{1138}$ in LEPRb is within an YXXQ motif, a consensus binding motif for STAT3 (211). In response to leptin, STAT3 binds to phospho-$Tyr^{1138}$, allowing JAK2 to phosphorylate and activate STAT3. Mutation of $Tyr^{1138}$ abolishes the ability of leptin to activate the STAT3 but not other pathways in both cultured cells and mice (10, 13, 232). Disruption of the STAT3 binding site in LEPRb, or deletion of STAT3 in leptin-responsive neurons, causes

severe hyperphagia and morbid obesity in mice (12, 13, 50, 73, 79, 107, 178, 241). These data indicate that the LEPRb/JAK2/STAT3 pathway in the brain is required for the anti-obesity actions of leptin.

Leptin also stimulates phosphorylation of LEPRb on $Tyr^{1077}$ which recruits STAT5 and mediates STAT5 phosphorylation (80, 90, 163). Deletion of both STAT5A and STAT5B in the brain causes leptin resistance, hyperphagia and obesity, but to a lesser extent than STAT3 deletion (130). These findings suggest that activation of the LEPRb/JAK2/STAT3 pathway is also required for the anti-obesity actions of leptin.

Leptin promotes the activation of phosphoinositide-3 kinase (PI3K) in cultured cells and in the hypothalamus (18, 116, 240, 253). JAK2 phosphorylates insulin receptor substrate-2 (IRS-2) on tyrosines, which recruits the p85 regulatory subunit of PI3K. Inhibition of the PI3K pathway in the brain blocks the ability of leptin to reduce food intake and weight gain in rodents (170, 252). Likewise, deletion of IRS-2, either systemically or in hypothalamic neurons, results in hyperphagia and obesity in mice (31, 125, 140, 145). PI3K activity is negatively regulated by PTEN (Phosphatase and Tensin homolog deleted on chromosome Ten), and deletion of PTEN specifically in LEPR-expressing neurons increases leptin sensitivity and reduces adiposity (180). Several proteins have been implicated as downstream targets of the JAK2/IRS-2/PI3K pathway. These include FOXO1, phosphodiesterase 3, and ATP-sensitive potassium channels (115, 117, 154, 179, 252). Collectively, these findings indicate that the JAK2/IRS-2/PI3K pathway is required for leptin's anorexigenic actions.

SH2-containing protein tyrosine phosphatase 2 (SHP2) is a ubiquitously expressed cytoplasmic protein-tyrosine phosphatase that contains two N-terminal SH2 domains and one C-terminal phosphatase domain. SHP2 binds via its SH2 domain to phosphorylated $Tyr^{985}$ in LEPR and leptin stimulates ERK1/2 activation via phosphorylation of SHP2 in cultured cells and the hypothalamus (22, 34, 136, 184, 216, 248). Neuron-specific deletion of SHP2 results in obesity (248), and pharmacological inhibition of ERK1/2 in the hypothalamus also abrogates the ability of leptin to inhibit food intake and block weight gain (184). Thus, the SHP2/MAPK pathway is involved in mediating leptin's anorexigenic action in vivo.

Our laboratory has also reported that leptin stimulates the STAT3 and the MAPK pathways in cells that are genetically deficient of JAK2 (106). The Src tyrosine kinase family members appear to be involved in mediating JAK2-independent leptin signaling (15, 106, 157). Interestingly, overexpression of kinase-inactive JAK2 enhances leptin signaling in JAK2-deficient cells, suggesting that JAK2 functions both as a tyrosine kinase and as an adaptor to transduce leptin signals (106). The JAK2-dependent and JAK2-independent pathways appear to act synergistically to mediate leptin responses (106). However, the physiological importance of the JAK2-independent pathway has not been verified in animals.

These diverse leptin signaling pathways form a network that regulates neuronal activity in the hypothalamus to fully mediate the physiological responses to leptin. This signaling network may be differentially regulated in different hypothalamic neurons with varying degrees of cross talk and synergy between individual pathways. Additionally, individual pathways in this network may contribute differently to the regulation of various populations of LERPb-expressing neurons.

**Central leptin resistance contributes to obesity**

Obese individuals produce excessive amounts of leptin (hyperleptinemia), yet leptin apparently fails to reach or activate its hypothalamic targets (45, 87, 144). Moreover, with the exception of rare monogenic forms of leptin deficiency, leptin treatment fails to induce weight loss in nearly all cases of obesity (93). These observations have given rise to the concept that leptin resistance is a driving force in pathogenesis of obesity in man. Many factors likely contribute to leptin resistance, including impaired leptin transport into the brain and impaired activation of leptin signal transduction pathways.

Blood-borne proteins, including leptin, are separated from the brain by the blood-brain barrier (BBB), which is formed by tight junctions in the cerebral endothelium (199). Leptin is transported across the BBB by a saturable transport mechanism (11). A role for LEPRa in leptin transport has been suggested based on the observation that LEPRa is highly expressed in brain microvessels and in the choroid plexus (24, 94, 95, 110). However, circumventricular organs lack tight junctions characteristic of the BBB (199). Many of the leptin-responsive neurons in the hypothalamus lie near the highly

vascularized median eminence, which is outside the BBB. This raises the possibility that leptin may also freely diffuse from the circulation to leptin-sensitive neurons in the hypothalamus. Regardless, obese humans have a reduced cerebrospinal fluid-to-serum ratio for leptin, suggesting that reduced leptin transport into the brain contributes to obesity (33, 58, 201). Moreover, intracerebroventricular leptin injections more potently inhibit food intake than peripheral leptin administration, particularly in obese rodents (32, 85, 86, 176, 227). Collectively, these findings suggest that impaired leptin transport into the brain contributes to leptin resistance and may be a driving force in the development of obesity; however, it remains unclear whether leptin transport defects predispose to obesity or are acquired with the onset of obesity.

Leptin resistance may also be caused by diminished leptin signaling in hypothalamic neurons. Leptin-stimulated activation of the JAK2/STAT3 and the JAK2/IRS-2/PI3K pathways in hypothalamic neurons is negatively regulated by suppressor of cytokine signaling 3 (SOCS3) and protein tyrosine phosphatase 1B (PTP1B) (16, 26, 28, 37, 112, 165, 245). Leptin stimulates SOCS3 expression via the JAK2/STAT3 pathway in cultured cells as well as in the hypothalamus (26). SOCS3 provides a critical negative feedback mechanism to prevent over-activation of leptin signaling pathways in the brain. SOCS3 binds to JAK2 and inhibits JAK2 activity (26). Additionally, SOCS3 binds to phospho-$Tyr^{985}$ in LEPRb and inhibits leptin signaling (28, 66). As expected, neuron-specific deletion of SOCS3 enhances leptin sensitivity in mice (158), and SOCS3 haploinsufficiency protects mice from dietary fat-induced leptin resistance and obesity (100). Furthermore, mutation of $Tyr^{985}$ in LEPRb, the binding site for SOCS3, increases leptin sensitivity in mice (29). SOCS3 expression is significantly increased in the hypothalamus in leptin resistant animals, suggesting that increased SOCS3 expression contributes to leptin resistance (23, 65, 161, 177).

PTP1B binds to and dephosphorylates JAK2, thereby inhibiting leptin signaling (112, 165, 245). PTP1B is expressed in the ARC, VMH and DMH (245). Both systemic and neuron-specific deletion of PTP1B improves leptin sensitivity and reduces adiposity in mice (16, 37, 245). The expression of hypothalamic PTP1B is increased in leptin resistant animals, suggesting that PTP1B also contributes to leptin resistance (160, 231). Interestingly, overexpression of the JAK2 binding protein, SH2B1, counteracts PTP1B-

mediated inhibition of leptin signaling in cultured cells (185). Therefore, cellular leptin sensitivity may be determined, at least in part, by a balance between positive (e.g. SH2B1) and negative (e.g. SOCS3 and PTP1B) regulators.

## The SH2-B family of adapter proteins mediate cell signaling

The SH2-B family of adapter proteins consists of three members (SH2B1, SH2B2 (APS) and SH2B3 (Lnk)) that are encoded by different genes (147). SH2B proteins are structurally similar in that all have an N-terminal dimerization domain (DD), multiple proline-rich regions, a central pleckstrin homology (PH) domain, and a C-terminal Src homology 2 (SH2) domain. Additionally, SH2B1 also has a nuclear localization signal (NLS) between the DD and PH domains (146).

The expression of SH2B3 is restricted to hematopoietic tissues and SH2B3 negatively regulates hematopoiesis (218, 228, 243). By contrast, SH2B1 and SH2B2 are expressed in multiple tissues, including brain, skeletal muscle, liver, white and brown adipose tissue, pancreas, and spleen (56, 153, 185). Four forms of SH2B1 (α, β, γ, and δ) and two forms of SH2B2 (α and β) have been identified (137, 168). All four variants of SH2B1 have the complete DD, NLS, PH and SH2 domains but their respective N-termini differ due to alternative mRNA splicing (168). Because the key functional domains are conserved, SH2B1 variants are predicted to have similar and/or overlapping functions. SH2B2α also has conserved DD, PH and SH2 domains; however, a naturally occurring truncated form of SH2B2 (SH2B2β) has also been identified (137). SH2B2β has both DD and PH domains, but lacks the C-terminal SH2 domain (137).

SH2B1 and SH2B2 proteins have been implicated as signaling molecules for a host of growth factors and cytokines. This list includes leptin, insulin, growth hormone (GH), insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial-cell-line-derived neurotrophic factor (GDNF) (55, 120, 121, 139, 183, 190, 193-195, 229, 251). In general, SH2B1 and SH2B2 enhance cell signaling, which promotes various cellular responses ranging from mitogenesis to glucose uptake to neurite outgrowth and neuronal differentiation.

## Identification of SH2B1 as an endogenous leptin sensitizer

To examine the physiological functions of SH2B1, two groups have independently disrupted the *Sh2b1* gene in mice by homologous recombination (56, 173). SH2B1-knockout (KO) mice are obese and develop insulin resistance (56, 138, 185). SH2B1-KO are hyperleptinemic and leptin treatment fails to induce weight loss (185), indicating that SH2B1 is required for leptin action in vivo. Interestingly, neuron-specific reconstitution of SH2B1β in KO mice rescues obesity by improving leptin responses (186). Moreover, overexpression of SH2B1β in neurons prevents diet-induced leptin resistance and obesity (186). Collectively, these data indicate that SH2B1 in the brain plays an essential role in the regulation of energy balance and suggest that SH2B1 is a physiologically relevant leptin signaling molecule.

**SH2B1 promotes leptin signaling**

SH2B1 has been shown to promote leptin signaling in cells by multiple mechanisms. Leptin stimulates JAK2 autophosphorylation on $Tyr^{813}$, and SH2B1 binds via its SH2 domain to phospho-$Tyr^{813}$ to markedly enhance JAK2 activity (139). Importantly, the SH2 domain is both necessary and sufficient to promote JAK2 activity in response to leptin and to promote the activation of the leptin signaling pathways downstream of JAK2 (139). Additionally, SH2B1 also directly binds to IRS-1 and IRS-2 (55). Leptin stimulates the formation of JAK2/SH2B1/IRS protein complexes, thereby promoting JAK2-mediated phosphorylation of IRS proteins and subsequent activation of the PI3K (55, 139, 185). SH2B1 forms homodimers through its N-terminal dimerization domain (DD) (53, 169), which may also provide a platform to initiate the formation of JAK2/SH2B1/IRS protein complexes and/or to stabilize these complexes, in response to leptin. Whether these mechanisms account for SH2B1-regulation of leptin action and control of energy balance in vivo is unknown. In this Dissertation, I generated two transgenic mice models to further understand the mechanisms by which SH2B1 promotes energy balance in mice. Specifically, I tested whether the SH2 domain of SH2B1 is necessary and sufficient for regulation of energy balance and body weight.

**SH2B1 is a candidate gene for human obesity**

Jamshidi and coworkers screened obese human populations for mutations in the SH2B1 gene (105). They identified a single nucleotide polymorphism (SNP) within the human *Sh2b1* loci that was strongly associated with serum leptin levels, total fat content,

and body weight in a European population of female twins (105). Recently, two unbiased genome wide scans independently identified SNPs within the human *Sh2b1* loci that were associated with indices of obesity (188, 221). These findings implicate *Sh2b1* as a candidate gene for obesity in man, particularly when coupled to the findings that SH2B1-deficiency causes obesity in mice and overexpression of SH2B1 promotes leptin signaling.

**Additional physiological roles of SH2B1**

The obesity phenotype of SH2B1-KO mice, identification of SH2B1 as a leptin signaling molecule, and recent reports that the *Sh2b1* locus is associated with leptin resistance and obesity in humans all provide strong evidence that one of the primary physiological functions of SH2B1 is to control energy balance, adiposity, and body weight. Restoring SH2B1 expression specifically in neurons prevents energy imbalance, weight gain, and obesity in SH2B1-KO mice, indicating that central SH2B1 plays a critical role in the regulation of energy homeostasis in mice. Moreover, by controlling energy balance and adiposity, SH2B1 in the brain indirectly promotes systemic insulin sensitivity to influence lipid and glucose metabolism. However, SH2B1 is expressed in multiple tissues (skeletal muscle, white and brown adipose tissue, liver, pancreas, spleen, heart, and gonads) and may have multiple roles in mammalian physiology.

Insulin controls blood glucose homeostasis. SH2B1-KO mice develop severe insulin resistance and type 2 diabetes (56, 185). Additionally, SH2B1 is expressed in insulin-target tissues and in the pancreas (56, 185). In cells, SH2B1 promotes insulin, IGF-1 and GH signaling (56, 159). This raises the intriguing hypothesis that SH2B1 in peripheral tissues and in the pancreas contributes to the regulation of glucose metabolism by promoting both insulin action and/or insulin production.

**Hormonal regulation of glucose metabolism**

Glucose is the major energy source for mammalian cells, and the precise regulation of blood glucose levels is required to sustain life. Hypoglycemia (low blood glucose) impairs cellular metabolism and function, whereas hyperglycemia (elevated blood glucose) leads to glucose toxicity and irreversible tissue damage.

The primary glucose sensor is the endocrine pancreas, which secretes two hormones that regulate glucose homeostasis: glucagon and insulin. Glucagon is produced

and secreted by α-cells in the islets of Langerhans. Glucagon prevents prolonged hypoglycemia during fasting by promoting endogenous glucose production. The liver, which is the primary target of glucagon, generates glucose from glycogen (glycogenolysis) and from de novo synthesis (gluconeogenesis) using gluconeogenic metabolites.

Insulin is secreted by the β-cells in the islets of Langerhans in response to elevations in blood glucose, which occurs acutely after feeding. Insulin decreases blood glucose both by promoting glucose disposal and by counteracting the effects of glucagon in the liver. Insulin promotes glucose disposal in skeletal muscle and adipose tissue by stimulating glucose uptake (via glucose transporter 4 (GLUT4)). In skeletal muscle and liver, insulin promotes glycogen synthesis to facilitate glucose storage. Insulin also suppresses the transcription of key gluconeogenic enzymes in the liver, thereby counteracting the effects of glucagon. Thus, the physiological responses to insulin culminate in the rapid normalization of blood glucose levels following a meal; however, when these physiological responses are impaired or inadequate, blood glucose levels remain elevated (hyperglycemia) in the absorptive state. This impaired or inadequate response to insulin is referred to as insulin resistance. Insulin resistance in skeletal muscle and adipose tissue reduces the rate of glucose disposal, and hepatic insulin resistance results in increased hepatic glucose production.

**Peripheral insulin resistance contributes to the development of type II diabetes**

Insulin resistance precedes the development of a cluster of metabolic diseases, and is the primary defect underlying the development of type II diabetes (206, 207). More than 18 million Americans have Type II diabetes mellitus (or non-insulin dependent diabetes mellitus (NIDDM)), currently the sixth leading cause of death in the United States (75). Insulin resistance has also been implicated in the development of cardiovascular disease, obesity, and polycystic ovarian syndrome. Alarmingly, an estimated 70 to 80 million Americans may have some degree of insulin resistance (121), which increases the likelihood of developing these morbidities.

In the prediabetic state, the insulin-producing cells of the pancreas (β-cells) compensate for peripheral insulin resistance by secreting more insulin. Thus, compensatory hyperinsulinemia is also a key feature and marker of peripheral insulin

resistance. In most individuals, both hyperglycemia and hyperinsulinemia contribute to β-cell dysfunction and failure (insulin insufficiency), which is the final step in the development of type II diabetes. At that point, the intrinsic ability to regulate glucose homeostasis is lost, and administration of pharmacological levels of insulin (insulin therapy) is required to regulate blood glucose homeostasis.

Mechanistically, systemic insulin resistance primarily arises secondary to impaired cellular insulin signaling in insulin-target tissues (206, 207). Thus, understanding the cellular and molecular mechanisms by which insulin effectively regulates glucose homeostasis, how other hormones and cytokines influence cellular insulin sensitivity, and how these mechanisms are disrupted or impaired in insulin resistant individuals is not only an interesting pursuit to basic scientists, but also clinically relevant, as future treatments for multiple metabolic-related diseases may depend on these findings. The key proteins that mediate insulin signal transduction have been identified and studied in both cell culture and animal models.

**Insulin activates the IRS/PI3K/Akt pathway to control glucose homeostasis**

The insulin receptor (IR) is a member of the tyrosine-kinase receptor family and is comprised of two extracellular α-subunits and two membrane spanning β-subunits linked by disulfide bonds. Insulin binding to the α-subunits induces a conformational change in the heterotetrameric receptor, activating the intrinsic tyrosine kinase within the β subunit. The β-subunit is then autophosphorylated on multiple tyrosine residues, which serve to both enhance tyrosine kinase activity and to create docking sites for intracellular signaling proteins.

Signaling molecules that have either phosphotyrosine binding (PTB) or Src 2 homology (SH2) domains bind to phosphotyrosines within the β-subunit of the IR. Upon binding, many of these proteins become substrates for the IR and are subsequently phosphorylated on multiple tyrosine residues by the activated kinase domain. Shc, SH2B2(APS), and insulin receptor substrate (IRS) proteins are well characterized substrates for the insulin receptor. Insulin stimulated tyrosine phosphorylation of these proteins initiates the activation of multiple intracellular signaling cascades.

Shc phosphorylation triggers the activation of the Ras/Raf/MAPK pathway; this pathway is critical for insulin- and insulin-like growth factor 1 (IFG-1)-induced

mitogenesis (198). In cultured adipocytes, insulin-stimulated SH2B2(APS) phosphorylation initiates the activation of the Cbl-B/TC10 pathway, which is sufficient for the translocation of GLUT4 vesicles to the plasma membrane (39, 141); however, the physiological significance of this pathway has not been firmly established as SH2B2 knockout mice are surprisingly insulin sensitive (153). Although the Shc/Ras/Raf/MAPK and SH2B2(APS)/Cbl-B/TC10 pathways are activated by insulin, the contribution of these pathways to the regulation of blood glucose by insulin in vivo are thought to be relatively insignificant. Instead, the metabolic actions of insulin are primarily mediated through tyrosine phosphorylation of IRS proteins and subsequent activation of the PI3K/Akt pathway (44). Activation of the IRS/PI3K/Akt pathway increases glucose transport into skeletal muscle and adipose tissue. Additionally, activation of the IRS/PI3K/Akt pathway stimulates glycogen synthesis in muscle and liver, suppresses gluconeogenic enzyme transcription in the liver, and inhibits lipolysis in adipose tissue.

The IRS/PI3K/Akt pathway is activated when the regulatory subunit of PI3K (p85) binds via its SH2 domains to phosphotyrosines within IRS proteins. Binding of p85 to phosphorylated IRS proteins induces a conformational change in the holoenzyme to activate the catalytic subunit (p110). PI3K phosphorylates membrane phosphatidylinositides to produce phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 accumulation recruits the serine/threonine-protein kinase Akt to the membrane (152), where it is phosphorylated on Thr308 and Ser473 by 3-phosphoinositide-dependent protein kinases (PDK1 and PDK2) (6, 236, 242). Once activated by serine/threonine phosphorylation, Akt serves as the principal effector kinase in the PI3K-pathway, activating multiple cellular processes to promote glucose uptake and utilization and to suppress endogenous glucose production.

Akt stimulates glucose uptake in skeletal muscle and adipose by activating the translocation and fusion of GLUT4 containing vesicles to the plasma membrane; this increases the rate of facilitated glucose diffusion into these tissues. Additionally, Akt stimulates fatty acid, protein, and glycogen synthesis by regulating the activity and transcription of key enzymes involved in these processes; this further promotes cellular utilization of blood glucose. Finally, Akt suppresses endogenous glucose production by inhibiting the transcriptional activity of the forkhead transcription factor FOXO1 in the

liver (167, 182, 187). Because FOXO1 activates the transcription of rate-limiting gluconeogenic enzymes (phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6P)) in liver, Akt-mediated inhibition of FOXO1 suppresses the rate of endogenous glucose production.

Insulin-stimulated activation of the IRS/PI3K/Akt signaling pathway is vital to the regulation of glucose homeostasis in vivo. In mice models of genetic- and diet-induced obesity, insulin-stimulated activation of PI3K-mediated signaling is diminished in the liver, skeletal muscle, and adipose tissue; these defects correlate with systemic insulin resistance and hyperglycemia (92, 150, 171, 254). Genetic inactivation IRS-1 (108) or IRS-2 (126, 239), but not IRS-3 (142) or IRS-4 (68), results in peripheral insulin resistance in mice. Homozygous deletion of either isoform of the catalytic subunit of PI3K (p110α or p110β) is embryonic lethal (19, 20); however, mice heterozygous for both p110 isoforms ($p110\alpha^{+/-}$ and $p110\beta^{+/-}$) are insulin resistant (30). Finally, deletion of Akt2 also disrupts glucose homeostasis (74), whereas Akt1- and Akt3-deficient mice have impaired growth but remain insulin sensitive (40, 57). Although mice models have confirmed the essential role of PI3K-dependent signaling in the regulation of insulin sensitivity, the development of insulin resistance is a complex process and the underlying cellular mechanisms are not completely understood.

Impaired IR kinase activity and/or reduced tyrosyl phosphorylation of IRS proteins are common cellular defects associated with the development of insulin resistance. IR and IRS proteins are negatively regulated by various intracellular molecules, including PTP1B, Grb10, Grb14, SOCS1, SOCS3, JNK, PKCθ, S6K and IKKβ (1, 4, 6, 9-11, 13, 14, 25, 27-29, 33-35, 37, 44, 47). The relative contribution of these negative regulators to the progression of insulin resistance has been extensively studied (2, 17, 46, 59, 78, 96, 114, 119, 196, 200, 205, 208, 222-224, 230, 235, 244, 255). For example, PTP1B, negatively regulate insulin action by dephosphorylating critical phosphotyrosines within the IR and IRS proteins (78, 197, 204); this serves to inactivate the receptor and to disrupt IRS-p85 association. Elevated phosphatase activity may contribute to the pathogenesis of type II diabetes by inappropriately inhibiting insulin action. In vivo, transgenic expression of PTP1B in skeletal muscle impairs insulin action

and disrupts glucose homeostasis (246), whereas Ptp1b-deficient mice are hypersensitive to insulin and have improved glycemic control (245).

The proinflammatory environment that is associated with chronic obesity also drives insulin resistance. Increased adiposity leads to elevated circulating levels of free fatty acids, adipose-derived cytokines, proinflammatory cytokines, and various other metabolites. For example, levels of circulating tumor necrosis factor (TNF)-α are significantly increased in obesity. TNF-α activates c-Jun NH2-terminal kinase (JNK) in skeletal muscle, liver, and white adipose tissue (1, 99). JNK phosphorylates IRS-1 and IRS-2 on serine/threonine residues. Unlike tyrosine phosphorylation, which promotes the activation of the PI3K/Akt-dependent signaling, serine/threonine phosphorylation of IRS-1 or IRS-2 uncouples insulin signaling (1, 131). Specifically, phosphorylation of IRS-1 on serine 307 disrupts PTB domain function, inhibiting IRS-1 from binding to the IR. Serine phosphorylation also promotes ubiquitination and proteosome-mediated degradation of IRS proteins, limiting the pool of available substrate and hindering the ability of insulin to activate the PI3K/Akt pathway (233). Recent data indicate that SH2B1 may be a positive regulator of insulin signaling in vivo (4, 5, 55, 56).

**Putative role of SH2B1 in the regulation of insulin signaling**

As discussed above, genetic deletion of SH2B1 results in severe insulin resistance and type 2 diabetes in mice (8). However, SH2B1-KO mice are also severely obese due to leptin resistance (138, 185, 186), raising the possibility that insulin resistance may be secondary to obesity in SH2B1 null mice. Preliminary data generated by our laboratory indicate that peripheral SH2B1 may be involved in the regulation of glucose homeostasis in vivo.

First, the ability of exogenous insulin activate the IRS/PI3K/Akt pathway in skeletal muscle and liver is impaired in SH2B1-KO mice (56). Second, preliminary hyperglycemic-euglycemic clamp studies indicate that insulin-stimulated glucose disposal and suppression of hepatic glucose production is impaired in SH2B1-KO mice (K. Cho and L. Rui, unpublished). Finally, insulin fails to promote glucose uptake in adipocytes isolated from obese SH2B1-KO and lean TgSH2B1-KO male mice (M. Li, K. Cho., and L. Rui, unpublished). Reconstitution of SH2B1 expression ex vivo improves insulin stimulated glucose uptake in SH2B1-deficient adipocytes (K. Cho and L. Rui,

unpublished), suggesting that SH2B1 cell autonomously promotes insulin action in adipocytes. However, it is unclear whether peripheral SH2B1 directly regulates insulin sensitivity in insulin target tissues in vivo independent of body weight and increases in adiposity.

Data generated from cell culture models also suggest a role for SH2B1 in insulin signaling. Our laboratory previously reported that insulin signaling is enhanced by SH2B1 (8). SH2B1 overexpression increases IR autophosphorylation and tyrosine phosphorylation of IRS-1 and IRS-2 in cultured cells (56, 137). Similar observations were independently reported by two other groups (4, 249). SH2B1 binds via its SH2 domain to phospho-$Tyr^{1158}$ in the activation loop of IR (121, 168). $Tyr^{1158}$ phosphorylation occurs early in the activation of the insulin receptor kinase (234, 237, 247), suggesting that binding of the SH2 domain of SH2B1 to phospho-$Tyr^{1158}$ likely occurs early in the activation of the insulin signaling cascades. This interaction may promote activation of IR by stabilizing the kinase domain of IR in an active conformation. Consistent with these observations, SH2B1 complexes, which are immunoprecipitated from cell extracts, reportedly promote IR autophosphorylation by reducing the $K_m$ for ATP (249). The same report also concluded that SH2B1 dimerization was required for its stimulation of IR autophosphorylation, because treatment of cells with dimerization domain peptide mimetics inhibited IR autophosphorylation and downstream pathways (249). Alternatively, SH2B1-IR interaction may facilitate IR binding to its substrates or prevent the binding of negative regulators, such as PTP1B and Grb7/10. Additionally, SH2B1 directly binds to IRS-1 and IRS-2 in vitro (55), further suggesting that SH2B1 may be involved in the activation of the IRS/PI3K/Akt pathway.

In this dissertation, we examine the role of peripheral SH2B1 in the regulation of insulin action and glucose homeostasis. We generated a mouse model in which recombinant SH2B1 is specifically expressed in the brain of SH2B1 null mice (TgKO) using transgenic approaches (186). Since these mice have normal leptin sensitivity but lack SH2B1 in peripheral tissue, we were able to isolate the effects of SH2B1 deficiency on insulin signaling in skeletal muscle, liver, and adipose (Chapter 3). Because the mechanism(s) by which SH2B1 promotes activation of the IRS/PI3K/Akt pathway are

not entirely cleat, we further examined the regulation of insulin signaling by SH2B1 using cell culture and in vitro assays (Chapter 3).

**Putative role of SH2B1 in pancreatic β cell function**

Insulin resistance in peripheral tissues and insufficient insulin secretion (β-cell dysfunction) contribute to the development of impaired glucose tolerance and hyperglycemia, hallmarks of type 2 diabetes. Insulin is secreted by the β-cells in the islets of Langerhans in response to elevations in blood glucose. In the prediabetic state, β-cells initially compensate for insulin resistance by increasing both insulin production and secretion (91, 109, 111, 181). This compensatory mechanism, due to β-cell proliferation (or islet hyperplasia), is the reason why insulin resistant individuals have both prolonged hyperglycemia and hyperinsulinemia (elevated insulin levels) following a glucose challenge. However, the compensatory capacity of the β-cell is limited and β-cells eventually fail to secrete adequate amounts of insulin to regulate blood glucose levels, and insulin therapy (administration of exogenous insulin) is needed to normalize blood glucose levels and prevent the development of diabetic complications (cardiovascular disease, neuropathies, renal failure, and blindness).

Multiple growth factors, including insulin, insulin-like growth factor-1 (IGF-1), growth hormone (GH), and prolactin promote and support β-cell function (47, 81, 104, 118, 127, 129, 132, 166, 238, 239). Many of the intracellular signaling pathways through which these growth factors modulate β-cell function have been studied. IGF-1 and insulin activate the IRS/PI3K/Akt pathway, which inhibits FOXO1 activity to promote Pdx1 expression (118, 127, 132, 166, 238, 239). Pdx1 is a master transcriptional regulator in β-cells that regulates the expression of genes necessary for β-cell function, as well as those needed for proliferation and survival (3, 7, 8, 127). Additionally, activation of the JAK2/STAT pathways by GH and prolactin promote β-cell function and proliferation (47, 81, 104, 129).

SH2B1 is expressed in the pancreas (56, 185). SH2B1 is expressed at high levels α- and β-cells within the islet (S. Oka and L. Rui, unpublished data). Additionally, SH2B1-KO mice are sensitive to the β-cell toxin streptozotocin (STZ) (D. Ren, S. Oka, and L. Rui, unpublished data). As discussed above, SH2B1 promotes the activation of

both the IRS/PI3K/Akt and JAK2/STAT pathways in insulin-, IGF-1-, and GH-treated cells and these growth factors support β-cell survival and function (5, 25, 37, 40, 43). Therefore, SH2B1 may play a role in β-cell function, survival, and/or proliferation. However, little is known about the physiological role of SH2B1 in the α- or β-cells. In Chapter 4, we began to explore the role of SH2B1 in the pancreas in vivo, and provide evidence that SH2B1 promotes β-cell function in genetically obese mice.

**Dissertation overview**

Leptin and insulin are key hormonal regulators of energy balance and glucose homeostasis. SH2B1 is a PH- and SH2-domain containing adapter protein that promotes both leptin and insulin signaling in cells. Disruption of *Sh2b1* in mice causes obesity and diabetes, providing genetic evidence that SH2B1 is an essential regulator of energy balance and glucose homeostasis. In this dissertation, the contribution of SH2B1 in brain, peripheral tissues, and islets to the regulation of body weight, energy balance, insulin sensitivity and glucose homeostasis were examined in vivo.

The aims of this Dissertation were:

1. To determine whether the SH2 domain of SH2B1 in neurons is required and/or sufficient for the ability of SH2B1 to regulate energy balance in mice (Chapter 2).
2. To determine whether SH2B1 in peripheral tissues (skeletal muscle, liver, and white adipose tissue) directly promotes insulin action and regulates glucose homeostasis independent of central regulation of energy balance, body weight, and adiposity during diet-induced obesity (Chapter 3 and Chapter 4).
3. To define the molecular mechanisms by which SH2B1 promotes insulin signaling (Chapter 3).
4. To determine whether SH2B1 in the pancreas contributes to the regulation of glucose homeostasis by regulating β-cell function (Chapter 4).

## References


1. **Aguirre V, Uchida T, Yenush L, Davis R, and White MF.** The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem* 275: 9047-9054, 2000.

2. **Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, and White MF.** Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *J Biol Chem* 277: 1531-1537, 2002.

3. **Ahlgren U, Jonsson J, Jonsson L, Simu K, and Edlund H.** beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. *Genes Dev* 12: 1763-1768, 1998.

4. **Ahmed Z and Pillay TS.** Adapter protein with a pleckstrin homology (PH) and an Src homology 2 (SH2) domain (APS) and SH2-B enhance insulin-receptor autophosphorylation, extracellular-signal-regulated kinase and phosphoinositide 3-kinase-dependent signalling. *Biochem J* 371: 405-412, 2003.

5. **Ahmed Z and Pillay TS.** Functional effects of APS and SH2-B on insulin receptor signalling. *Biochem Soc Trans* 29: 529-534, 2001.

6. **Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, and Cohen P.** Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. *Curr Biol* 7: 261-269, 1997.

7. **Babu DA, Chakrabarti SK, Garmey JC, and Mirmira RG.** Pdx1 and BETA2/NeuroD1 participate in a transcriptional complex that mediates short-range DNA looping at the insulin gene. *J Biol Chem* 283: 8164-8172, 2008.

8. **Babu DA, Deering TG, and Mirmira RG.** A feat of metabolic proportions: Pdx1 orchestrates islet development and function in the maintenance of glucose homeostasis. *Mol Genet Metab* 92: 43-55, 2007.

9. **Balthasar N, Coppari R, McMinn J, Liu SM, Lee CE, Tang V, Kenny CD, McGovern RA, Chua SC, Jr., Elmquist JK, and Lowell BB.** Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. *Neuron* 42: 983-991, 2004.

10. **Banks AS, Davis SM, Bates SH, and Myers MG, Jr.** Activation of downstream signals by the long form of the leptin receptor. *J Biol Chem* 275: 14563-14572, 2000.

11. **Banks WA, Kastin AJ, Huang W, Jaspan JB, and Maness LM.** Leptin enters the brain by a saturable system independent of insulin. *Peptides* 17: 305-311, 1996.

12. **Bates SH, Dundon TA, Seifert M, Carlson M, Maratos-Flier E, and Myers MG, Jr.** LRb-STAT3 signaling is required for the neuroendocrine regulation of energy expenditure by leptin. *Diabetes* 53: 3067-3073, 2004.

13. **Bates SH, Stearns WH, Dundon TA, Schubert M, Tso AW, Wang Y, Banks AS, Lavery HJ, Haq AK, Maratos-Flier E, Neel BG, Schwartz MW, and Myers MG, Jr.** STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* 421: 856-859, 2003.

14. **Baumann H, Morella KK, White DW, Dembski M, Bailon PS, Kim H, Lai CF, and Tartaglia LA.** The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. *Proc Natl Acad Sci U S A* 93: 8374-8378, 1996.

15. **Beltowski J, Wojcicka G, Trzeciak J, and Marciniak A.** H2O2 and Src-dependent transactivation of the EGF receptor mediates the stimulatory effect of leptin on renal ERK and Na+, K+-ATPase. *Peptides* 27: 3234-3244, 2006.

16. **Bence KK, Delibegovic M, Xue B, Gorgun CZ, Hotamisligil GS, Neel BG, and Kahn BB.** Neuronal PTP1B regulates body weight, adiposity and leptin action. *Nat Med* 12: 917-924, 2006.

17. **Bereziat V, Kasus-Jacobi A, Perdereau D, Cariou B, Girard J, and Burnol AF.** Inhibition of insulin receptor catalytic activity by the molecular adapter Grb14. *J Biol Chem* 277: 4845-4852, 2002.

18. **Berti L, Kellerer M, Capp E, and Haring HU.** Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: evidence for a P13-kinase mediated effect. *Diabetologia* 40: 606-609, 1997.

19. **Bi L, Okabe I, Bernard DJ, and Nussbaum RL.** Early embryonic lethality in mice deficient in the p110beta catalytic subunit of PI 3-kinase. *Mamm Genome* 13: 169-172, 2002.

20. **Bi L, Okabe I, Bernard DJ, Wynshaw-Boris A, and Nussbaum RL.** Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110alpha subunit of phosphoinositide 3-kinase. *J Biol Chem* 274: 10963-10968, 1999.

21. **Bingham NC, Anderson KK, Reuter AL, Stallings NR, and Parker KL.** Selective loss of leptin receptors in the ventromedial hypothalamic nucleus results in increased adiposity and a metabolic syndrome. *Endocrinology* 149: 2138-2148, 2008.

22. **Bjorbaek C, Buchholz RM, Davis SM, Bates SH, Pierroz DD, Gu H, Neel BG, Myers MG, Jr., and Flier JS.** Divergent roles of SHP-2 in ERK activation by leptin receptors. *J Biol Chem* 276: 4747-4755, 2001.

23. **Bjorbaek C, Elmquist JK, Frantz JD, Shoelson SE, and Flier JS.** Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell* 1: 619-625, 1998.

24. **Bjorbaek C, Elmquist JK, Michl P, Ahima RS, van Bueren A, McCall AL, and Flier JS.** Expression of leptin receptor isoforms in rat brain microvessels. *Endocrinology* 139: 3485-3491, 1998.

25. **Bjorbaek C, Uotani S, da Silva B, and Flier JS.** Divergent signaling capacities of the long and short isoforms of the leptin receptor. *J Biol Chem* 272: 32686-32695, 1997.

26. **Bjorbak C, El-Haschimi K, Frantz JD, and Flier JS.** The Role of SOCS-3 in Leptin Signaling and Leptin Resistance. *J Biol Chem* 274: 30059-30065, 1999.

27. **Bjorbak C, Elmquist JK, Michl P, Ahima RS, van Bueren A, McCall AL, and Flier JS.** Expression of Leptin Receptor Isoforms in Rat Brain Microvessels. *Endocrinology* 139: 3485-3491, 1998.

28. **Bjorbak C, Lavery HJ, Bates SH, Olson RK, Davis SM, Flier JS, and Myers MG, Jr.** SOCS3 mediates feedback inhibition of the leptin receptor via Tyr985. *J Biol Chem* 275: 40649-40657, 2000.

29. **Bjornholm M, Munzberg H, Leshan RL, Villanueva EC, Bates SH, Louis GW, Jones JC, Ishida-Takahashi R, Bjorbaek C, and Myers MG, Jr.** Mice lacking inhibitory leptin receptor signals are lean with normal endocrine function. *J Clin Invest* 117: 1354-1360, 2007.

30. **Brachmann SM, Ueki K, Engelman JA, Kahn RC, and Cantley LC.** Phosphoinositide 3-kinase catalytic subunit deletion and regulatory subunit deletion have opposite effects on insulin sensitivity in mice. *Mol Cell Biol* 25: 1596-1607, 2005.

31. **Burks DJ, de Mora JF, Schubert M, Withers DJ, Myers MG, Towery HH, Altamuro SL, Flint CL, and White MF.** IRS-2 pathways integrate female reproduction and energy homeostasis. *Nature* 407: 377-382, 2000.

32. **Campfield LA, Smith FJ, Guisez Y, Devos R, and Burn P.** Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269: 546-549, 1995.

33. **Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang PL, Sinha MK, and Considine RV.** Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 348: 159-161, 1996.

34. **Carpenter LR, Farruggella TJ, Symes A, Karow ML, Yancopoulos GD, and Stahl N.** Enhancing leptin response by preventing SH2-containing phosphatase 2 interaction with Ob receptor. *Proc Natl Acad Sci U S A* 95: 6061-6066, 1998.

35. **Chen AS, Marsh DJ, Trumbauer ME, Frazier EG, Guan XM, Yu H, Rosenblum CI, Vongs A, Feng Y, Cao L, Metzger JM, Strack AM, Camacho RE, Mellin TN, Nunes CN, Min W, Fisher J, Gopal-Truter S, MacIntyre DE, Chen HY, and Van der Ploeg LH.** Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat Genet* 26: 97-102, 2000.

36. **Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, and Morgenstern JP.** Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* 84: 491-495, 1996.

37. **Cheng A, Uetani N, Simoncic PD, Chaubey VP, Lee-Loy A, McGlade CJ, Kennedy BP, and Tremblay ML.** Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. *Dev Cell* 2: 497-503, 2002.

38. **Cheung CC, Clifton DK, and Steiner RA.** Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. *Endocrinology* 138: 4489-4492, 1997.

39. **Chiang SH, Baumann CA, Kanzaki M, Thurmond DC, Watson RT, Neudauer CL, Macara IG, Pessin JE, and Saltiel AR.** Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. *Nature* 410: 944-948, 2001.

40. **Cho H, Thorvaldsen JL, Chu Q, Feng F, and Birnbaum MJ.** Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *J Biol Chem* 276: 38349-38352, 2001.

41. **Chua SC, Jr., Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, and Leibel RL.** Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. *Science* 271: 994-996, 1996.

42. **Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, and Guy-Grand B.** A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 392: 398-401, 1998.

43. **Cohen P, Zhao C, Cai X, Montez JM, Rohani SC, Feinstein P, Mombaerts P, and Friedman JM.** Selective deletion of leptin receptor in neurons leads to obesity. *J Clin Invest* 108: 1113-1121, 2001.

44. **Combettes-Souverain M and Issad T.** Molecular basis of insulin action. *Diabetes Metab* 24: 477-489, 1998.

45. **Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, and et al.** Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334: 292-295, 1996.

46. **Cooney GJ, Lyons RJ, Crew AJ, Jensen TE, Molero JC, Mitchell CJ, Biden TJ, Ormandy CJ, James DE, and Daly RJ.** Improved glucose homeostasis and enhanced insulin signalling in Grb14-deficient mice. *EMBO J* 23: 582-593, 2004.

47. **Cousin SP, Hugl SR, Myers MG, Jr., White MF, Reifel-Miller A, and Rhodes CJ.** Stimulation of pancreatic beta-cell proliferation by growth hormone is glucose-dependent: signal transduction via janus kinase 2 (JAK2)/signal transducer and activator of transcription 5 (STAT5) with no crosstalk to insulin receptor substrate-mediated mitogenic signalling. *Biochem J* 344 Pt 3: 649-658, 1999.

48. **Cowley MA, Cone RD, Enriori P, Louiselle I, Williams SM, and Evans AE.** Electrophysiological actions of peripheral hormones on melanocortin neurons. *Ann N Y Acad Sci* 994: 175-186, 2003.

49. **Cowley MA, Smart JL, Rubinstein M, Cerdan MG, Diano S, Horvath TL, Cone RD, and Low MJ.** Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* 411: 480-484, 2001.

50. **Cui Y, Huang L, Elefteriou F, Yang G, Shelton JM, Giles JE, Oz OK, Pourbahrami T, Lu CY, Richardson JA, Karsenty G, and Li C.** Essential role of STAT3 in body weight and glucose homeostasis. *Mol Cell Biol* 24: 258-269, 2004.

51. **de Luca C, Kowalski TJ, Zhang Y, Elmquist JK, Lee C, Kilimann MW, Ludwig T, Liu SM, and Chua SC.** Complete rescue of obesity, diabetes, and infertility in db/db mice by neuron-specific LEPR-B transgenes. *J Clin Invest* 115: 3484-3493, 2005.

52. **Devos R, Guisez Y, Van der Heyden J, White DW, Kalai M, Fountoulakis M, and Plaetinck G.** Ligand-independent dimerization of the extracellular domain of the leptin receptor and determination of the stoichiometry of leptin binding. *J Biol Chem* 272: 18304-18310, 1997.

53. **Dhe-Paganon S, Werner ED, Nishi M, Hansen L, Chi Y-I, and Shoelson SE.** A phenylalanine zipper mediates APS dimerization. 11: 968-974, 2004.

54. **Dhillon H, Zigman JM, Ye C, Lee CE, McGovern RA, Tang V, Kenny CD, Christiansen LM, White RD, Edelstein EA, Coppari R, Balthasar N, Cowley MA, Chua S, Jr., Elmquist JK, and Lowell BB.** Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron* 49: 191-203, 2006.

55. **Duan C, Li M, and Rui L.** SH2-B promotes insulin receptor substrate 1 (IRS1)- and IRS2-mediated activation of the phosphatidylinositol 3-kinase pathway in response to leptin. *J Biol Chem* 279: 43684-43691, 2004.

56. **Duan C, Yang H, White MF, and Rui L.** Disruption of the SH2-B gene causes age-dependent insulin resistance and glucose intolerance. *Mol Cell Biol* 24: 7435-7443, 2004.

57. **Easton RM, Cho H, Roovers K, Shineman DW, Mizrahi M, Forman MS, Lee VM, Szabolcs M, de Jong R, Oltersdorf T, Ludwig T, Efstratiadis A, and Birnbaum MJ.** Role for Akt3/protein kinase Bgamma in attainment of normal brain size. *Mol Cell Biol* 25: 1869-1878, 2005.

58. **El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, and Flier JS.** Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest* 105: 1827-1832, 2000.

59. **Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, and Kennedy BP.** Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 283: 1544-1548, 1999.

60. **Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, and Elmquist JK.** Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron* 23: 775-786, 1999.

61. **Elias CF, Lee C, Kelly J, Aschkenasi C, Ahima RS, Couceyro PR, Kuhar MJ, Saper CB, and Elmquist JK.** Leptin activates hypothalamic CART neurons projecting to the spinal cord. *Neuron* 21: 1375-1385, 1998.

62. **Elmquist JK, Ahima RS, Elias CF, Flier JS, and Saper CB.** Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. *Proc Natl Acad Sci U S A* 95: 741-746, 1998.

63. **Elmquist JK, Ahima RS, Maratos-Flier E, Flier JS, and Saper CB.** Leptin activates neurons in ventrobasal hypothalamus and brainstem. *Endocrinology* 138: 839-842, 1997.

64. **Elmquist JK, Bjorbaek C, Ahima RS, Flier JS, and Saper CB.** Distributions of leptin receptor mRNA isoforms in the rat brain. *J Comp Neurol* 395: 535-547, 1998.

65. **Enriori PJ, Evans AE, Sinnayah P, Jobst EE, Tonelli-Lemos L, Billes SK, Glavas MM, Grayson BE, Perello M, Nillni EA, Grove KL, and Cowley MA.** Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. *Cell Metab* 5: 181-194, 2007.

66. **Eyckerman S, Broekaert D, Verhee A, Vandekerckhove J, and Tavernier J.** Identification of the Y985 and Y1077 motifs as SOCS3 recruitment sites in the murine leptin receptor. *FEBS Lett* 486: 33-37, 2000.

67. **Fan W, Boston BA, Kesterson RA, Hruby VJ, and Cone RD.** Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 385: 165-168, 1997.

68. **Fantin VR, Wang Q, Lienhard GE, and Keller SR.** Mice lacking insulin receptor substrate 4 exhibit mild defects in growth, reproduction, and glucose homeostasis. *Am J Physiol Endocrinol Metab* 278: E127-133, 2000.

69. **Fei H, Okano HJ, Li C, Lee GH, Zhao C, Darnell R, and Friedman JM.** Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proc Natl Acad Sci U S A* 94: 7001-7005, 1997.

70. **Friedman JM and Halaas JL.** Leptin and the regulation of body weight in mammals. *Nature* 395: 763-770, 1998.

71. **Fulton S, Pissios P, Manchon RP, Stiles L, Frank L, Pothos EN, Maratos-Flier E, and Flier JS.** Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* 51: 811-822, 2006.

72. **Gantz I, Miwa H, Konda Y, Shimoto Y, Tashiro T, Watson SJ, DelValle J, and Yamada T.** Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. *J Biol Chem* 268: 15174-15179, 1993.

73. **Gao Q, Wolfgang MJ, Neschen S, Morino K, Horvath TL, Shulman GI, and Fu XY.** Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. *Proc Natl Acad Sci U S A* 101: 4661-4666, 2004.

74. **Garofalo RS, Orena SJ, Rafidi K, Torchia AJ, Stock JL, Hildebrandt AL, Coskran T, Black SC, Brees DJ, Wicks JR, McNeish JD, and Coleman KG.** Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta. *J Clin Invest* 112: 197-208, 2003.

75. **Gerberding JL.** Diabetes: Disabling, Deadly, and on the Rise, edited by (CDC) CfDC: Centers for Disease Control (CDC), 2004.

76. **Ghilardi N and Skoda RC.** The leptin receptor activates janus kinase 2 and signals for proliferation in a factor-dependent cell line. *Mol Endocrinol* 11: 393-399, 1997.

77. **Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, and Skoda RC.** Defective STAT signaling by the leptin receptor in diabetic mice. *Proc Natl Acad Sci U S A* 93: 6231-6235, 1996.

78. **Goldstein BJ, Bittner-Kowalczyk A, White MF, and Harbeck M.** Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B. Possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. *J Biol Chem* 275: 4283-4289, 2000.

79. **Gong L, Yao F, Hockman K, Heng HH, Morton GJ, Takeda K, Akira S, Low MJ, Rubinstein M, and MacKenzie RG.** Signal transducer and activator of transcription-3 is required in hypothalamic agouti-related protein/neuropeptide Y neurons for normal energy homeostasis. *Endocrinology* 149: 3346-3354, 2008.

80. **Gong Y, Ishida-Takahashi R, Villanueva EC, Fingar DC, Munzberg H, and Myers MG, Jr.** The long form of the leptin receptor regulates STAT5 and ribosomal protein S6 via alternate mechanisms. *J Biol Chem* 282: 31019-31027, 2007.

81. **Gorogawa S, Fujitani Y, Kaneto H, Hazama Y, Watada H, Miyamoto Y, Takeda K, Akira S, Magnuson MA, Yamasaki Y, Kajimoto Y, and Hori M.** Insulin secretory defects and impaired islet architecture in pancreatic beta-cell-specific STAT3 knockout mice. *Biochem Biophys Res Commun* 319: 1159-1170, 2004.

82. **Gropp E, Shanabrough M, Borok E, Xu AW, Janoschek R, Buch T, Plum L, Balthasar N, Hampel B, Waisman A, Barsh GS, Horvath TL, and Bruning JC.** Agouti-related peptide-expressing neurons are mandatory for feeding. *Nat Neurosci* 8: 1289-1291, 2005.

83. **Hahn TM, Breininger JF, Baskin DG, and Schwartz MW.** Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci* 1: 271-272, 1998.

84. **Hakansson ML, Hulting AL, and Meister B.** Expression of leptin receptor mRNA in the hypothalamic arcuate nucleus--relationship with NPY neurones. *Neuroreport* 7: 3087-3092, 1996.

85. **Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, and Friedman JM.** Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *PNAS* 94: 8878-8883, 1997.

86. **Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, and Friedman JM.** Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269: 543-546, 1995.

87. **Hamilton BS, Paglia D, Kwan AY, and Deitel M.** Increased obese mRNA expression in omental fat cells from massively obese humans. *Nat Med* 1: 953-956, 1995.

88. **Hegele RA.** Monogenic forms of insulin resistance: apertures that expose the common metabolic syndrome. *Trends Endocrinol Metab* 14: 371-377, 2003.

89. **Hegele RA and Pollex RL.** Genetic and physiological insights into the metabolic syndrome. *Am J Physiol Regul Integr Comp Physiol* 289: R663-669, 2005.

90. **Hekerman P, Zeidler J, Bamberg-Lemper S, Knobelspies H, Lavens D, Tavernier J, Joost HG, and Becker W.** Pleiotropy of leptin receptor signalling is defined by distinct roles of the intracellular tyrosines. *FEBS J* 272: 109-119, 2005.

91. **Herman MA and Kahn BB.** Glucose transport and sensing in the maintenance of glucose homeostasis and metabolic harmony. *J Clin Invest* 116: 1767-1775, 2006.

92. **Heydrick SJ, Jullien D, Gautier N, Tanti JF, Giorgetti S, Van Obberghen E, and Le Marchand-Brustel Y.** Defect in skeletal muscle phosphatidylinositol-3-kinase in obese insulin-resistant mice. *J Clin Invest* 91: 1358-1366, 1993.

93. **Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, and McCamish M.** Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA* 282: 1568-1575, 1999.

94. **Hileman SM, Pierroz DD, Masuzaki H, Bjorbaek C, El-Haschimi K, Banks WA, and Flier JS.** Characterizaton of short isoforms of the leptin receptor in rat cerebral microvessels and of brain uptake of leptin in mouse models of obesity. *Endocrinology* 143: 775-783, 2002.

95. **Hileman SM, Tornoe J, Flier JS, and Bjorbaek C.** Transcellular transport of leptin by the short leptin receptor isoform ObRa in Madin-Darby Canine Kidney cells. *Endocrinology* 141: 1955-1961, 2000.

96. **Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, and Hotamisligil GS.** A central role for JNK in obesity and insulin resistance. *Nature* 420: 333-336, 2002.

97. **Hommel JD, Trinko R, Sears RM, Georgescu D, Liu ZW, Gao XB, Thurmon JJ, Marinelli M, and DiLeone RJ.** Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 51: 801-810, 2006.

98. **Horvath CM.** The Jak-STAT pathway stimulated by interferon gamma. *Sci STKE* 2004: tr8, 2004.

99. **Hotamisligil GS.** Mechanisms of TNF-alpha-induced insulin resistance. *Exp Clin Endocrinol Diabetes* 107: 119-125, 1999.

100. **Howard JK, Cave BJ, Oksanen LJ, Tzameli I, Bjorbaek C, and Flier JS.** Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. *Nat Med* 10: 734-738, 2004.

101. **Hubschle T, Thom E, Watson A, Roth J, Klaus S, and Meyerhof W.** Leptin-induced nuclear translocation of STAT3 immunoreactivity in hypothalamic nuclei involved in body weight regulation. *J Neurosci* 21: 2413-2424, 2001.

102. **Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, and Lee F.** Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88: 131-141, 1997.

103. **Ibrahim N, Bosch MA, Smart JL, Qiu J, Rubinstein M, Ronnekleiv OK, Low MJ, and Kelly MJ.** Hypothalamic proopiomelanocortin neurons are glucose responsive and express K(ATP) channels. *Endocrinology* 144: 1331-1340, 2003.

104. **Jackerott M, Moldrup A, Thams P, Galsgaard ED, Knudsen J, Lee YC, and Nielsen JH.** STAT5 activity in pancreatic beta-cells influences the severity of diabetes in animal models of type 1 and 2 diabetes. *Diabetes* 55: 2705-2712, 2006.

105. **Jamshidi Y, Snieder H, Ge D, Spector TD, and O'Dell SD.** The SH2B gene is associated with serum leptin and body fat in normal female twins. *Obesity (Silver Spring)* 15: 5-9, 2007.

106. **Jiang L, Li Z, and Rui L.** Leptin Stimulates Both JAK2-dependent and JAK2-independent Signaling Pathways. *J Biol Chem* 283: 28066-28073, 2008.

107. **Jiang L, You J, Yu X, Gonzalez L, Yu Y, Wang Q, Yang G, Li W, Li C, and Liu Y.** Tyrosine-dependent and -independent actions of leptin receptor in control of energy balance and glucose homeostasis. *Proc Natl Acad Sci U S A* 105: 18619-18624, 2008.

108. **Kadowaki T, Tamemoto H, Tobe K, Terauchi Y, Ueki K, Kaburagi Y, Yamauchi T, Satoh S, Sekihara H, Aizawa S, and Yazaki Y.** Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1 and identification of insulin receptor substrate-2. *Diabet Med* 13: S103-108, 1996.

109. **Kahn SE, Hull RL, and Utzschneider KM.** Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444: 840-846, 2006.

110. **Kastin AJ, Pan W, Maness LM, Koletsky RJ, and Ernsberger P.** Decreased transport of leptin across the blood-brain barrier in rats lacking the short form of the leptin receptor**1. *Peptides* 20: 1449-1453, 1999.

111. **Kasuga M.** Insulin resistance and pancreatic beta cell failure. *J Clin Invest* 116: 1756-1760, 2006.

112. **Kaszubska W, Falls HD, Schaefer VG, Haasch D, Frost L, Hessler P, Kroeger PE, White DW, Jirousek MR, and Trevillyan JM.** Protein tyrosine phosphatase 1B negatively regulates leptin signaling in a hypothalamic cell line. *Molecular and Cellular Endocrinology* 195: 109-118, 2002.

113. **Kellerer M, Koch M, Metzinger E, Mushack J, Capp E, and Haring HU.** Leptin activates PI-3 kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. *Diabetologia* 40: 1358-1362, 1997.

114. **Kim JK, Fillmore JJ, Sunshine MJ, Albrecht B, Higashimori T, Kim DW, Liu ZX, Soos TJ, Cline GW, O'Brien WR, Littman DR, and Shulman GI.** PKC-theta knockout mice are protected from fat-induced insulin resistance. *J Clin Invest* 114: 823-827, 2004.

115. **Kim MS, Pak YK, Jang PG, Namkoong C, Choi YS, Won JC, Kim KS, Kim SW, Kim HS, Park JY, Kim YB, and Lee KU.** Role of hypothalamic Foxo1 in the regulation of food intake and energy homeostasis. *Nat Neurosci* 9: 901-906, 2006.

116. **Kim Y-B, Uotani S, Pierroz DD, Flier JS, and Kahn BB.** In Vivo Administration of Leptin Activates Signal Transduction Directly in Insulin-Sensitive

Tissues: Overlapping but Distinct Pathways from Insulin. *Endocrinology* 141: 2328-2339, 2000.

117. **Kitamura T, Feng Y, Kitamura YI, Chua SC, Jr., Xu AW, Barsh GS, Rossetti L, and Accili D.** Forkhead protein FoxO1 mediates Agrp-dependent effects of leptin on food intake. *Nat Med* 12: 534-540, 2006.

118. **Kitamura T, Nakae J, Kitamura Y, Kido Y, Biggs WH, 3rd, Wright CV, White MF, Arden KC, and Accili D.** The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. *J Clin Invest* 110: 1839-1847, 2002.

119. **Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Sharpe AH, Stricker-Krongrad A, Shulman GI, Neel BG, and Kahn BB.** Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol Cell Biol* 20: 5479-5489, 2000.

120. **Kong M, Wang CS, and Donoghue DJ.** Interaction of fibroblast growth factor receptor 3 and the adapter protein SH2-B. A role in STAT5 activation. *J Biol Chem* 277: 15962-15970, 2002.

121. **Kotani K, Wilden P, and Pillay TS.** SH2-Balpha is an insulin-receptor adapter protein and substrate that interacts with the activation loop of the insulin-receptor kinase. *Biochem J* 335 ( Pt 1): 103-109, 1998.

122. **Kowalski TJ, Liu S-M, Leibel RL, and Chua SC, Jr.** Transgenic Complementation of Leptin-Receptor Deficiency: I. Rescue of the Obesity/Diabetes Phenotype of LEPR-Null Mice Expressing a LEPR-B Transgene. *Diabetes* 50: 425-435, 2001.

123. **Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, and Hastrup S.** Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* 393: 72-76, 1998.

124. **Krude H, Biebermann H, Luck W, Horn R, Brabant G, and Gruters A.** Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nat Genet* 19: 155-157, 1998.

125. **Kubota N, Terauchi Y, Tobe K, Yano W, Suzuki R, Ueki K, Takamoto I, Satoh H, Maki T, Kubota T, Moroi M, Okada-Iwabu M, Ezaki O, Nagai R, Ueta Y, Kadowaki T, and Noda T.** Insulin receptor substrate 2 plays a crucial role in beta cells and the hypothalamus. *J Clin Invest* 114: 917-927, 2004.

126. **Kubota N, Tobe K, Terauchi Y, Eto K, Yamauchi T, Suzuki R, Tsubamoto Y, Komeda K, Nakano R, Miki H, Satoh S, Sekihara H, Sciacchitano S, Lesniak M, Aizawa S, Nagai R, Kimura S, Akanuma Y, Taylor SI, and Kadowaki T.** Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory beta-cell hyperplasia. *Diabetes* 49: 1880-1889, 2000.

127. **Kushner JA, Ye J, Schubert M, Burks DJ, Dow MA, Flint CL, Dutta S, Wright CV, Montminy MR, and White MF.** Pdx1 restores beta cell function in Irs2 knockout mice. *J Clin Invest* 109: 1193-1201, 2002.

128. **Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, and Friedman JM.** Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 379: 632-635, 1996.

129. **Lee JY, Gavrilova O, Davani B, Na R, Robinson GW, and Hennighausen L.** The transcription factors Stat5a/b are not required for islet development but modulate pancreatic beta-cell physiology upon aging. *Biochim Biophys Acta* 1773: 1455-1461, 2007.

130. **Lee JY, Muenzberg H, Gavrilova O, Reed JA, Berryman D, Villanueva EC, Louis GW, Leinninger GM, Bertuzzi S, Seeley RJ, Robinson GW, Myers MG, and Hennighausen L.** Loss of cytokine-STAT5 signaling in the CNS and pituitary gland alters energy balance and leads to obesity. *PLoS ONE* 3: e1639, 2008.

131. **Lee YH, Giraud J, Davis RJ, and White MF.** c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. *J Biol Chem* 278: 2896-2902, 2003.

132. **Leibiger IB, Leibiger B, Moede T, and Berggren PO.** Exocytosis of insulin promotes insulin gene transcription via the insulin receptor/PI-3 kinase/p70 s6 kinase and CaM kinase pathways. *Mol Cell* 1: 933-938, 1998.

133. **Leinninger GM, Jo YH, Leshan RL, Louis GW, Yang H, Barrera JG, Wilson H, Opland DM, Faouzi MA, Gong Y, Jones JC, Rhodes CJ, Chua S, Jr., Diano S, Horvath TL, Seeley RJ, Becker JB, Munzberg H, and Myers MG, Jr.** Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. *Cell Metab* 10: 89-98, 2009.

134. **Leshan RL, Louis GW, Jo YH, Rhodes CJ, Munzberg H, and Myers MG, Jr.** Direct innervation of GnRH neurons by metabolic- and sexual odorant-sensing leptin receptor neurons in the hypothalamic ventral premammillary nucleus. *J Neurosci* 29: 3138-3147, 2009.

135. **Levin BE, Dunn-Meynell AA, and Routh VH.** Brain glucose sensing and body energy homeostasis: role in obesity and diabetes. *Am J Physiol* 276: R1223-1231, 1999.

136. **Li C and Friedman JM.** Leptin receptor activation of SH2 domain containing protein tyrosine phosphatase 2 modulates Ob receptor signal transduction. *PNAS* 96: 9677-9682, 1999.

137. **Li M, Li Z, Morris DL, and Rui L.** Identification of SH2B2beta as an inhibitor for SH2B1- and SH2B2alpha-promoted Janus kinase-2 activation and insulin signaling. *Endocrinology* 148: 1615-1621, 2007.

138. **Li M, Ren D, Iseki M, Takaki S, and Rui L.** Differential role of SH2-B and APS in regulating energy and glucose homeostasis. *Endocrinology* 147: 2163-2170, 2006.

139. **Li Z, Zhou Y, Carter-Su C, Myers MG, Jr., and Rui L.** SH2B1 Enhances Leptin Signaling by Both Janus Kinase 2 Tyr813 Phosphorylation-Dependent and -Independent Mechanisms. *Mol Endocrinol* 21: 2270-2281, 2007.

140. **Lin X, Taguchi A, Park S, Kushner JA, Li F, Li Y, and White MF.** Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. *J Clin Invest* 114: 908-916, 2004.

141. **Liu J, Kimura A, Baumann CA, and Saltiel AR.** APS facilitates c-Cbl tyrosine phosphorylation and GLUT4 translocation in response to insulin in 3T3-L1 adipocytes. *Mol Cell Biol* 22: 3599-3609, 2002.

142. **Liu SC, Wang Q, Lienhard GE, and Keller SR.** Insulin receptor substrate 3 is not essential for growth or glucose homeostasis. *J Biol Chem* 274: 18093-18099, 1999.

143. **Luquet S, Perez FA, Hnasko TS, and Palmiter RD.** NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science* 310: 683-685, 2005.

144. **Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, and et al.** Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1: 1155-1161, 1995.

145. **Masaki T, Chiba S, Noguchi H, Yasuda T, Tobe K, Suzuki R, Kadowaki T, and Yoshimatsu H.** Obesity in insulin receptor substrate-2-deficient mice: disrupted control of arcuate nucleus neuropeptides. *Obes Res* 12: 878-885, 2004.

146. **Maures TJ, Chen L, and Carter-Su C.** Nucleocytoplasmic Shuttling of the Adapter Protein SH2B1{beta} is Required for Nerve Growth Factor (NGF)-Dependent Neurite Outgrowth and Enhancement of Expression of a Subset of NGF-Responsive Genes. *Mol Endocrinol* 23: 1077-1091, 2009.

147. **Maures TJ, Kurzer JH, and Carter-Su C.** SH2B1 (SH2-B) and JAK2: a multifunctional adaptor protein and kinase made for each other. *Trends Endocrinol Metab* 18: 38-45, 2007.

148. **McCowen KC, Chow JC, and Smith RJ.** Leptin signaling in the hypothalamus of normal rats in vivo. *Endocrinology* 139: 4442-4447, 1998.

149. **McMinn JE, Sindelar DK, Havel PJ, and Schwartz MW.** Leptin deficiency induced by fasting impairs the satiety response to cholecystokinin. *Endocrinology* 141: 4442-4448, 2000.

150. **Menahan LA.** Age-related changes in lipid and carbohydrate metabolism of the genetically obese mouse. *Metabolism* 32: 172-178, 1983.

151. **Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, and Trayhurn P.** Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. *FEBS Lett* 387: 113-116, 1996.

152. **Milburn CC, Deak M, Kelly SM, Price NC, Alessi DR, and Van Aalten DM.** Binding of phosphatidylinositol 3,4,5-trisphosphate to the pleckstrin homology domain of protein kinase B induces a conformational change. *Biochem J* 375: 531-538, 2003.

153. **Minami A, Iseki M, Kishi K, Wang M, Ogura M, Furukawa N, Hayashi S, Yamada M, Obata T, Takeshita Y, Nakaya Y, Bando Y, Izumi K, Moodie SA, Kajiura F, Matsumoto M, Takatsu K, Takaki S, and Ebina Y.** Increased insulin sensitivity and hypoinsulinemia in APS knockout mice. *Diabetes* 52: 2657-2665, 2003.

154. **Mirshamsi S, Laidlaw HA, Ning K, Anderson E, Burgess LA, Gray A, Sutherland C, and Ashford ML.** Leptin and insulin stimulation of signalling pathways in arcuate nucleus neurones: PI3K dependent actin reorganization and KATP channel activation. *BMC Neurosci* 5: 54, 2004.

155. **Mizuno TM, Kleopoulos SP, Bergen HT, Roberts JL, Priest CA, and Mobbs CV.** Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and [corrected] in ob/ob and db/db mice, but is stimulated by leptin. *Diabetes* 47: 294-297, 1998.

156. **Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, and O'Rahilly S.** Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 387: 903-908, 1997.

157. **Montecucco F, Bianchi G, Gnerre P, Bertolotto M, Dallegri F, and Ottonello L.** Induction of neutrophil chemotaxis by leptin: crucial role for p38 and Src kinases. *Ann N Y Acad Sci* 1069: 463-471, 2006.

158. **Mori H, Hanada R, Hanada T, Aki D, Mashima R, Nishinakamura H, Torisu T, Chien KR, Yasukawa H, and Yoshimura A.** Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nat Med* 10: 739-743, 2004.

159. **Morris DL, Cho KW, Zhou Y, and Rui L.** SH2B1 Enhances Insulin Sensitivity by Both Stimulating the Insulin Receptor and Inhibiting Tyrosine Dephosphorylation of IRS Proteins. *Diabetes* 58: 2039-2047, 2009.

160. **Morrison CD, White CL, Wang Z, Lee SY, Lawrence DS, Cefalu WT, Zhang ZY, and Gettys TW.** Increased hypothalamic protein tyrosine phosphatase 1B contributes to leptin resistance with age. *Endocrinology* 148: 433-440, 2007.

161. **Munzberg H, Flier JS, and Bjorbaek C.** Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology* 145: 4880-4889, 2004.

162. **Munzberg H, Huo L, Nillni EA, Hollenberg AN, and Bjorbaek C.** Role of signal transducer and activator of transcription 3 in regulation of hypothalamic proopiomelanocortin gene expression by leptin. *Endocrinology* 144: 2121-2131, 2003.

163. **Mutze J, Roth J, Gerstberger R, and Hubschle T.** Nuclear translocation of the transcription factor STAT5 in the rat brain after systemic leptin administration. *Neurosci Lett* 417: 286-291, 2007.

164. **Myers MG, Jr., Munzberg H, Leinninger GM, and Leshan RL.** The geometry of leptin action in the brain: more complicated than a simple ARC. *Cell Metab* 9: 117-123, 2009.

165. **Myers MP, Andersen JN, Cheng A, Tremblay ML, Horvath CM, Parisien J-P, Salmeen A, Barford D, and Tonks NK.** TYK2 and JAK2 Are Substrates of Protein-tyrosine Phosphatase 1B. *J Biol Chem* 276: 47771-47774, 2001.

166. **Nakae J, Biggs WH, Kitamura T, Cavenee WK, Wright CV, Arden KC, and Accili D.** Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. *Nat Genet* 32: 245-253, 2002.

167. **Nakae J, Park BC, and Accili D.** Insulin stimulates phosphorylation of the forkhead transcription factor FKHR on serine 253 through a Wortmannin-sensitive pathway. *J Biol Chem* 274: 15982-15985, 1999.

168. **Nelms K, O'Neill TJ, Li S, Hubbard SR, Gustafson TA, and Paul WE.** Alternative splicing, gene localization, and binding of SH2-B to the insulin receptor kinase domain. *Mamm Genome* 10: 1160-1167, 1999.

169. **Nishi M, Werner ED, Oh BC, Frantz JD, Dhe-Paganon S, Hansen L, Lee J, and Shoelson SE.** Kinase activation through dimerization by human SH2-B. *Mol Cell Biol* 25: 2607-2621, 2005.

170. **Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myers MG, Jr., and Schwartz MW.** Intracellular signalling. Key enzyme in leptin-induced anorexia. *Nature* 413: 794-795, 2001.

171. **Noguchi T and Tanaka T.** Insulin resistance in obesity and its molecular control. *Obes Res* 3 Suppl 2: 195S-198S, 1995.

172. **O'Rahilly S, Farooqi IS, Yeo GS, and Challis BG.** Minireview: human obesity-lessons from monogenic disorders. *Endocrinology* 144: 3757-3764, 2003.

173. **Ohtsuka S, Takaki S, Iseki M, Miyoshi K, Nakagata N, Kataoka Y, Yoshida N, Takatsu K, and Yoshimura A.** SH2-B is required for both male and female reproduction. *Mol Cell Biol* 22: 3066-3077, 2002.

174. **Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, and Barsh GS.** Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* 278: 135-138, 1997.

175. **Parton LE, Ye CP, Coppari R, Enriori PJ, Choi B, Zhang CY, Xu C, Vianna CR, Balthasar N, Lee CE, Elmquist JK, Cowley MA, and Lowell BB.** Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. *Nature* 449: 228-232, 2007.

176. **Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, and Collins F.** Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269: 540-543, 1995.

177. **Peralta S, Carrascosa JM, Gallardo N, Ros M, and Arribas C.** Ageing increases SOCS-3 expression in rat hypothalamus: effects of food restriction. *Biochemical and Biophysical Research Communications* 296: 425-428, 2002.

178. **Piper ML, Unger EK, Myers MG, Jr., and Xu AW.** Specific physiological roles for signal transducer and activator of transcription 3 in leptin receptor-expressing neurons. *Mol Endocrinol* 22: 751-759, 2008.

179. **Plum L, Ma X, Hampel B, Balthasar N, Coppari R, Munzberg H, Shanabrough M, Burdakov D, Rother E, Janoschek R, Alber J, Belgardt BF, Koch L, Seibler J, Schwenk F, Fekete C, Suzuki A, Mak TW, Krone W, Horvath TL, Ashcroft FM, and Bruning JC.** Enhanced PIP3 signaling in POMC neurons causes KATP channel activation and leads to diet-sensitive obesity. *J Clin Invest* 116: 1886-1901, 2006.

180. **Plum L, Rother E, Munzberg H, Wunderlich FT, Morgan DA, Hampel B, Shanabrough M, Janoschek R, Konner AC, Alber J, Suzuki A, Krone W, Horvath TL, Rahmouni K, and Bruning JC.** Enhanced Leptin-Stimulated Pi3k Activation in the CNS Promotes White Adipose Tissue Transdifferentiation. *Cell Metab* 6: 431-445, 2007.

181. **Prentki M and Nolan CJ.** Islet beta cell failure in type 2 diabetes. *J Clin Invest* 116: 1802-1812, 2006.

182. **Puigserver P, Rhee J, Donovan J, Walkey CJ, Yoon JC, Oriente F, Kitamura Y, Altomonte J, Dong H, Accili D, and Spiegelman BM.** Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. *Nature* 423: 550-555, 2003.

183. **Qian X, Riccio A, Zhang Y, and Ginty DD.** Identification and characterization of novel substrates of Trk receptors in developing neurons. *Neuron* 21: 1017-1029, 1998.

184. **Rahmouni K, Sigmund CD, Haynes WG, and Mark AL.** Hypothalamic ERK Mediates the Anorectic and Thermogenic Sympathetic Effects of Leptin. *Diabetes*, 2008.

185. **Ren D, Li M, Duan C, and Rui L.** Identification of SH2-B as a key regulator of leptin sensitivity, energy balance, and body weight in mice. *Cell Metabolism* 2: 95-104, 2005.

186. **Ren D, Zhou Y, Morris D, Li M, Li Z, and Rui L.** Neuronal SH2B1 is essential for controlling energy and glucose homeostasis. *J Clin Invest* 117: 397-406, 2007.

187. **Rena G, Guo S, Cichy SC, Unterman TG, and Cohen P.** Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. *J Biol Chem* 274: 17179-17183, 1999.

188. **Renstrom F, Payne F, Nordstrom A, Brito EC, Rolandsson O, Hallmans G, Barroso I, Nordstrom P, and Franks PW.** Replication and extension of genome-wide association study results for obesity in 4923 adults from northern Sweden. *Hum Mol Genet* 18: 1489-1496, 2009.

189. **Ridderstrale M and Groop L.** Genetic dissection of type 2 diabetes. *Mol Cell Endocrinol* 297: 10-17, 2009.

190. **Riedel H, Yousaf N, Zhao Y, Dai H, Deng Y, and Wang J.** PSM, a mediator of PDGF-BB-, IGF-I-, and insulin-stimulated mitogenesis. *Oncogene* 19: 39-50, 2000.

191. **Roselli-Rehfuss L, Mountjoy KG, Robbins LS, Mortrud MT, Low MJ, Tatro JB, Entwistle ML, Simerly RB, and Cone RD.** Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. *Proc Natl Acad Sci U S A* 90: 8856-8860, 1993.

192. **Rosenblum CI, Tota M, Cully D, Smith T, Collum R, Qureshi S, Hess JF, Phillips MS, Hey PJ, Vongs A, Fong TM, Xu L, Chen HY, Smith RG, Schindler C, and Van der Ploeg LH.** Functional STAT 1 and 3 signaling by the leptin receptor (OB-R); reduced expression of the rat fatty leptin receptor in transfected cells. *Endocrinology* 137: 5178-5181, 1996.

193. **Rui L and Carter-Su C.** Platelet-derived growth factor (PDGF) stimulates the association of SH2-Bbeta with PDGF receptor and phosphorylation of SH2-Bbeta. *J Biol Chem* 273: 21239-21245, 1998.

194. **Rui L, Herrington J, and Carter-Su C.** SH2-B is required for nerve growth factor-induced neuronal differentiation. *J Biol Chem* 274: 10590-10594, 1999.

195. **Rui L, Mathews LS, Hotta K, Gustafson TA, and Carter-Su C.** Identification of SH2-Bbeta as a substrate of the tyrosine kinase JAK2 involved in growth hormone signaling. *Mol Cell Biol* 17: 6633-6644, 1997.

196. **Rui L, Yuan M, Frantz D, Shoelson S, and White MF.** SOCS-1 and SOCS-3 Block Insulin Signaling by Ubiquitin-mediated Degradation of IRS1 and IRS2. *J Biol Chem* 277: 42394-42398, 2002.

197. **Salmeen A, Andersen JN, Myers MP, Tonks NK, and Barford D.** Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. *Mol Cell* 6: 1401-1412, 2000.

198. **Sasaoka T and Kobayashi M.** The functional significance of Shc in insulin signaling as a substrate of the insulin receptor. *Endocr J* 47: 373-381, 2000.

199. **Saunders NR, Habgood MD, and Dziegielewska KM.** Barrier mechanisms in the brain, I. Adult brain. *Clin Exp Pharmacol Physiol* 26: 11-19, 1999.

200. **Schenk S, Saberi M, and Olefsky JM.** Insulin sensitivity: modulation by nutrients and inflammation. *J Clin Invest* 118: 2992-3002, 2008.

201. **Schwartz MW, Peskind E, Raskind M, Boyko EJ, and Porte D, Jr.** Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med* 2: 589-593, 1996.

202. **Schwartz MW, Seeley RJ, Campfield LA, Burn P, and Baskin DG.** Identification of targets of leptin action in rat hypothalamus. *J Clin Invest* 98: 1101-1106, 1996.

203. **Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P, and Baskin DG.** Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. *Diabetes* 46: 2119-2123, 1997.

204. **Seely BL, Staubs PA, Reichart DR, Berhanu P, Milarski KL, Saltiel AR, Kusari J, and Olefsky JM.** Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. *Diabetes* 45: 1379-1385, 1996.

205. **Shi H, Tzameli I, Bjorbaek C, and Flier JS.** Suppressor of cytokine signaling 3 is a physiological regulator of adipocyte insulin signaling. *J Biol Chem* 279: 34733-34740, 2004.

206. **Shulman GI.** Cellular mechanisms of insulin resistance. *J Clin Invest* 106: 171-176, 2000.

207. **Shulman GI.** Cellular mechanisms of insulin resistance in humans. *Am J Cardiol* 84: 3J-10J, 1999.

208. **Smith FM, Holt LJ, Garfield AS, Charalambous M, Koumanov F, Perry M, Bazzani R, Sheardown SA, Hegarty BD, Lyons RJ, Cooney GJ, Daly RJ, and Ward A.** Mice with a disruption of the imprinted Grb10 gene exhibit altered body composition, glucose homeostasis, and insulin signaling during postnatal life. *Mol Cell Biol* 27: 5871-5886, 2007.

209. **Spanswick D, Smith MA, Groppi VE, Logan SD, and Ashford ML.** Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature* 390: 521-525, 1997.

210. **Spanswick D, Smith MA, Mirshamsi S, Routh VH, and Ashford ML.** Insulin activates ATP-sensitive K+ channels in hypothalamic neurons of lean, but not obese rats. *Nat Neurosci* 3: 757-758, 2000.

211. **Stahl N, Farruggella TJ, Boulton TG, Zhong Z, Darnell JE, Jr., and Yancopoulos GD.** Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. *Science* 267: 1349-1353, 1995.

212. **Stanley BG and Leibowitz SF.** Neuropeptide Y injected in the paraventricular hypothalamus: a powerful stimulant of feeding behavior. *Proc Natl Acad Sci U S A* 82: 3940-3943, 1985.

213. **Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, and et al.** The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 377: 530-532, 1995.

214. **Strobel A, Issad T, Camoin L, Ozata M, and Strosberg AD.** A leptin missense mutation associated with hypogonadism and morbid obesity. *Nat Genet* 18: 213-215, 1998.

215. **Takahashi KA and Cone RD.** Fasting induces a large, leptin-dependent increase in the intrinsic action potential frequency of orexigenic arcuate nucleus NPY/AgRP neurons. *Endocrinology*, 2004.

216. **Takahashi Y, Okimura Y, Mizuno I, Iida K, Takahashi T, Kaji H, Abe H, and Chihara K.** Leptin induces mitogen-activated protein kinase-dependent proliferation of C3H10T1/2 cells. *J Biol Chem* 272: 12897-12900, 1997.

217. **Takahashi Y, Okimura Y, Mizuno I, Takahashi T, Kaji H, Uchiyama T, Abe H, and Chihara K.** Leptin induces tyrosine phosphorylation of cellular proteins including STAT-1 in human renal adenocarcinoma cells, ACHN. *Biochem Biophys Res Commun* 228: 859-864, 1996.

218. **Takaki S, Watts JD, Forbush KA, Nguyen NT, Hayashi J, Alberola-Ila J, Aebersold R, and Perlmutter RM.** Characterization of Lnk. An Adapter Protein Expressed in Lymphocytes. *J Biol Chem* 272: 14562-14570, 1997.

219. **Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, and et al.** Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83: 1263-1271, 1995.

220. **Teran-Garcia M and Bouchard C.** Genetics of the metabolic syndrome. *Appl Physiol Nutr Metab* 32: 89-114, 2007.

221. **Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadottir A, Styrkarsdottir U, Gretarsdottir S, Thorlacius S, Jonsdottir I, Jonsdottir T, Olafsdottir EJ, Olafsdottir GH, Jonsson T, Jonsson F, Borch-Johnsen K, Hansen T, Andersen G, Jorgensen T, Lauritzen T, Aben KK, Verbeek AL, Roeleveld N, Kampman E, Yanek LR, Becker LC, Tryggvadottir L, Rafnar T, Becker DM, Gulcher J, Kiemeney LA, Pedersen O, Kong A, Thorsteinsdottir U, and Stefansson K.** Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet* 41: 18-24, 2009.

222. **Ueki K, Kondo T, and Kahn CR.** Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol Cell Biol* 24: 5434-5446, 2004.

223. **Ueki K, Kondo T, Tseng YH, and Kahn CR.** Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. *Proc Natl Acad Sci U S A* 101: 10422-10427, 2004.

224. **Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J, and Thomas G.** Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 431: 200-205, 2004.

225. **Vaisse C, Halaas JL, Horvath CM, Darnell JE, Jr., Stoffel M, and Friedman JM.** Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. *Nat Genet* 14: 95-97, 1996.

226. **van de Wall E, Leshan R, Xu AW, Balthasar N, Coppari R, Liu SM, Jo YH, MacKenzie RG, Allison DB, Dun NJ, Elmquist J, Lowell BB, Barsh GS, de Luca C, Myers MG, Jr., Schwartz GJ, and Chua SC, Jr.** Collective and individual functions of leptin receptor modulated neurons controlling metabolism and ingestion. *Endocrinology* 149: 1773-1785, 2008.

227. **Van Heek M, Compton DS, France CF, Tedesco RP, Fawzi AB, Graziano MP, Sybertz EJ, Strader CD, and Davis HRea.** Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *Journal of Clinical Investigation* 99: 385-390, 1997.

228. **Velazquez L, Cheng AM, Fleming HE, Furlonger C, Vesely S, Bernstein A, Paige CJ, and Pawson T.** Cytokine signaling and hematopoietic homeostasis are disrupted in Lnk-deficient mice. *J Exp Med* 195: 1599-1611, 2002.

229. **Wang J and Riedel H.** Insulin-like growth factor-I receptor and insulin receptor association with a Src homology-2 domain-containing putative adapter. *J Biol Chem* 273: 3136-3139, 1998.

230. **Wang L, Balas B, Christ-Roberts CY, Kim RY, Ramos FJ, Kikani CK, Li C, Deng C, Reyna S, Musi N, Dong LQ, DeFronzo RA, and Liu F.** Peripheral disruption of the Grb10 gene enhances insulin signaling and sensitivity in vivo. *Mol Cell Biol* 27: 6497-6505, 2007.

231. **White CL, Whittington A, Barnes MJ, Wang ZQ, Bray G, and Morrison CD.** HF diets increase hypothalamic PTP1B and induce leptin resistance through both leptin-dependent and independent mechanisms. *Am J Physiol Endocrinol Metab*, 2008.

232. **White DW, Kuropatwinski KK, Devos R, Baumann H, and Tartaglia LA.** Leptin receptor (OB-R) signaling. Cytoplasmic domain mutational analysis and evidence for receptor homo-oligomerization. *J Biol Chem* 272: 4065-4071, 1997.

233. **White MF.** IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab* 283: E413-422, 2002.

234. **White MF, Shoelson SE, Keutmann H, and Kahn CR.** A cascade of tyrosine autophosphorylation in the beta-subunit activates the phosphotransferase of the insulin receptor. *J Biol Chem* 263: 2969-2980, 1988.

235. **Wick KR, Werner ED, Langlais P, Ramos FJ, Dong LQ, Shoelson SE, and Liu F.** Grb10 inhibits insulin-stimulated insulin receptor substrate (IRS)-phosphatidylinositol 3-kinase/Akt signaling pathway by disrupting the association of IRS-1/IRS-2 with the insulin receptor. *J Biol Chem* 278: 8460-8467, 2003.

236. **Wick MJ, Dong LQ, Riojas RA, Ramos FJ, and Liu F.** Mechanism of phosphorylation of protein kinase B/Akt by a constitutively active 3-phosphoinositide-dependent protein kinase-1. *J Biol Chem* 275: 40400-40406, 2000.

237. **Wilden PA, Kahn CR, Siddle K, and White MF.** Insulin receptor kinase domain autophosphorylation regulates receptor enzymatic function. *J Biol Chem* 267: 16660-16668, 1992.

238. **Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, and White MF.** Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. *Nat Genet* 23: 32-40, 1999.

239. **Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, and White MF.** Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391: 900-904, 1998.

240. **Xu AW, Kaelin CB, Takeda K, Akira S, Schwartz MW, and Barsh GS.** PI3K integrates the action of insulin and leptin on hypothalamic neurons. *J Clin Invest* 115: 951-958, 2005.

241. **Xu AW, Ste-Marie L, Kaelin CB, and Barsh GS.** Inactivation of signal transducer and activator of transcription 3 in proopiomelanocortin (Pomc) neurons causes decreased pomc expression, mild obesity, and defects in compensatory refeeding. *Endocrinology* 148: 72-80, 2007.

242. **Yamada T, Katagiri H, Asano T, Tsuru M, Inukai K, Ono H, Kodama T, Kikuchi M, and Oka Y.** Role of PDK1 in insulin-signaling pathway for glucose metabolism in 3T3-L1 adipocytes. *Am J Physiol Endocrinol Metab* 282: E1385-1394, 2002.

243. **Yokouchi M, Suzuki R, Masuhara M, Komiya S, Inoue A, and Yoshimura A.** Cloning and characterization of APS, an adaptor molecule containing PH and SH2 domains that is tyrosine phosphorylated upon B-cell receptor stimulation. *Oncogene* 15: 7-15, 1997.

244. **Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, and Shoelson SE.** Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293: 1673-1677, 2001.

245. **Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, Kim YB, Elmquist JK, Tartaglia LA, Kahn BB, and Neel BG.** PTP1B regulates leptin signal transduction in vivo. *Dev Cell* 2: 489-495, 2002.

246. **Zabolotny JM, Haj FG, Kim YB, Kim HJ, Shulman GI, Kim JK, Neel BG, and Kahn BB.** Transgenic overexpression of protein-tyrosine phosphatase 1B in muscle causes insulin resistance, but overexpression with leukocyte antigen-related phosphatase does not additively impair insulin action. *J Biol Chem* 279: 24844-24851, 2004.

247. **Zhang B, Tavare JM, Ellis L, and Roth RA.** The regulatory role of known tyrosine autophosphorylation sites of the insulin receptor kinase domain. An assessment by replacement with neutral and negatively charged amino acids. *J Biol Chem* 266: 990-996, 1991.

248. **Zhang EE, Chapeau E, Hagihara K, and Feng G-S.** Neuronal Shp2 tyrosine phosphatase controls energy balance and metabolism. *PNAS* 101: 16064-16069, 2004.

249. **Zhang M, Deng Y, Tandon R, Bai C, and Riedel H.** Essential role of PSM/SH2-B variants in insulin receptor catalytic activation and the resulting cellular responses. *J Cell Biochem* 103: 162-181, 2008.

250. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, and Friedman JM.** Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425-432, 1994.

251. **Zhang Y, Zhu W, Wang YG, Liu XJ, Jiao L, Liu X, Zhang ZH, Lu CL, and He C.** Interaction of SH2-Bbeta with RET is involved in signaling of GDNF-induced neurite outgrowth. *J Cell Sci* 119: 1666-1676, 2006.

252. **Zhao AZ, Huan JN, Gupta S, Pal R, and Sahu A.** A phosphatidylinositol 3-kinase phosphodiesterase 3B-cyclic AMP pathway in hypothalamic action of leptin on feeding. *Nat Neurosci* 5: 727-728, 2002.

253. **Zhao AZ, Shinohara MM, Huang D, Shimizu M, Eldar-Finkelman H, Krebs EG, Beavo JA, and Bornfeldt KE.** Leptin induces insulin-like signaling that antagonizes cAMP elevation by glucagon in hepatocytes. *J Biol Chem* 275: 11348-11354, 2000.

254. **Zierath JR, Houseknecht KL, Gnudi L, and Kahn BB.** High-fat feeding impairs insulin-stimulated GLUT4 recruitment via an early insulin-signaling defect. *Diabetes* 46: 215-223, 1997.

255. **Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, Xie N, Wilcox D, Jacobson P, Frost L, Kroeger PE, Reilly RM, Koterski S, Opgenorth TJ, Ulrich RG, Crosby S, Butler M, Murray SF, McKay RA, Bhanot S, Monia BP, and Jirousek MR.** PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. *Proc Natl Acad Sci U S A* 99: 11357-11362, 2002.

## Chapter 2

## The SH2 Domain of SH2B1β in Neurons is Necessary but not Sufficient for Regulation of Body Weight and Glucose Homeostasis in Mice


### Abstract

SH2B1 enhances leptin signaling in the hypothalamus to control energy balance and body weight in mice. In cultured cells, the SH2 domain of SH2B1 alone is sufficient to promote JAK2 activation and leptin signaling. Conversely, SH2 domain defective mutants of SH2B1 and SH2B2 function as dominant negatives and impair the ability of SH2B1 to enhance leptin signaling. Here, we examined the function of the SH2 domain of SH2B1 *in vivo*. Two transgenic mice models expressing either N-terminal truncated SH2B1β (amino acids 504-670) or a dominant negative form of SH2B1β (R555E) in neurons were generated to test whether the SH2 domain of neuronal SH2B1 is necessary and sufficient for the regulation of body weight. N-terminal truncated SH2B1β (Δ504) contained an intact SH2 domain and C-terminus of SH2B1β, but lacked the dimerization domain, PH domain, and nuclear localization sequence (NLS). Expression of Δ504 in neurons was not sufficient to prevent the development of obesity and obesity-associated metabolic disease in SH2B1-knockout (KO) mice, suggesting that multiple functional domains within SH2B1 are required for neuronal SH2B1 to control energy balance and body weight in mice. Reconstitution of R555E in neurons also failed to prevent the development of obesity in SH2B1-KO mice. By contrast, overexpression of R555E in the brain of wild type mice induced obesity and caused insulin resistance. Collectively, these data indicate that a functional SH2 domain is necessary but not sufficient for regulation of energy balance and insulin sensitivity by neuronal SH2B1. Moreover, these data suggest that dominant negative mutations within *Sh2b1* could contribute to the pathogenesis of obesity.

## Introduction

Body weight is normally maintained within a narrow range by a sophisticated neuroendocrine system that controls energy (food) intake and energy expenditure. The brain senses multiple signals of nutrient storage and availability and integrates those signals into appropriate physiological and behavioral responses to maintain energy homeostasis. Obesity is caused by energy imbalance which occurs when long term energy (food) intake exceeds metabolic demand. Excess energy is assimilated into triglyceride and stored in white adipose tissue, leading to increased adiposity and weight gain. Leptin is secreted by adipocytes in direct proportion to white adipose mass, and conveys information about peripheral energy storage and availability to the hypothalamus. Leptin suppresses food intake and promotes energy expenditure (8, 25, 47, 56). Paradoxically, serum leptin levels are elevated in obese individuals (14, 26, 35), suggesting that leptin resistance may underlie the pathogenesis of obesity. Moreover, with the exception of rare monogenic forms of leptin deficiency, leptin treatment fails to induce weight loss in most cases of obesity (27). Leptin resistance may be due to either defective leptin transport across the blood brain barrier (9, 18, 55) or impaired leptin signaling in hypothalamic neurons (3-5, 18, 40).

Leptin signaling is mediated by the long form of the leptin receptor (LepRb), which is expressed in multiple neuronal subpopulations within the hypothalamus and other regions of the brain (19-21). Leptin binding to LepRb activates the receptor-associated tyrosine kinase, JAK2, which phosphorylates LepRb and downstream signaling molecules. Signal transducer and activator of transcription 3 (STAT3) is activated by JAK2 in leptin-stimulated neurons (1, 2). Disruption of the JAK2→STAT3 pathway, either by neuron-specific disruption of the STAT3 gene or genetic disruption of the STAT3 binding site in LepRb (Tyr1138→Ser1138 knock-in mutation), results in obesity (15, 24). JAK2 also activates PI3-kinase in leptin-stimulated neurons by recruiting and phosphorylating insulin receptor substrate-2 (IRS-2) (11, 16, 42, 45, 50). Systemic deletion of the IRS-2 gene or pharmacological inhibition of PI3-kinase activity results in obesity in mice models (34, 46, 57). Activation of these two pathways may be negatively or positively regulated by cellular proteins that either inhibit (e.g. SOCS3 and PTP1B) or promote (e.g. SH2B1) JAK2 activity.

Leptin-stimulated activation of the JAK2→STAT3 and the JAK2→IRS-2/PI3-kinase pathways in hypothalamic neurons is negatively regulated by SOCS3 and PTP1B (3, 5, 7, 13, 29, 38, 43, 61). Expression of both SOCS3 and PTP1B is significantly increased in the hypothalamus in leptin resistant animals (22, 40, 41, 59), whereas neuron-specific deletion of SOCS3 or PTP1B improves leptin sensitivity (3, 38). The JAK2-binding protein SH2B1 promotes leptin action in the hypothalamus (50, 51), and SH2B1 can restore leptin signaling in cells that overexpress PTP1B (50). Thus, leptin sensitivity may be controlled by a balance between positive (e.g. SH2B1) and negative regulators (e.g. SOCS3 and PTP1B).

SH2B1 is a PH and SH2 domain containing protein belonging to the SH2B family (10, 37). SH2B1 promotes leptin-stimulated activation of both the JAK2→STAT3 and the JAK2→IRS-2/PI3-kinase pathways in cells and in the hypothalamus of mice (16, 31, 33, 50). Disruption of the *Sh2b1* gene in mice results in hyperphagia, obesity, and obesity-associated metabolic disorders (32, 50, 51). Recently, our laboratory reported that neuron-specific expression of recombinant SH2B1β in SH2B1-KO mice rescues obesity and prevents the development of obesity-related dyslipidemia and insulin resistance (51). Additionally, overexpression of recombinant SH2B1β prevented diet-induced obesity in transgenic mice (51). Thus, SH2B1 in the brain controls energy balance and body weight.

SH2B1 has been shown to enhance leptin signaling by multiple mechanisms. Leptin stimulates JAK2 autophosphorylation on Tyr813, and SH2B1 binds via its SH2 domain to phospho-Tyr813 to markedly enhance JAK2 activity and promote the activation of the signaling pathways downstream of JAK2 (33, 44, 53). Importantly, the SH2 domain alone is sufficient to promote JAK2 activity in leptin-treated cells (33). Additionally, SH2B1 also directly binds to IRS-1 and IRS-2 (16), and leptin stimulates the formation of JAK2/SH2B1/IRS-1 and JAK2/SH2B1/IRS-2 protein complexes, thereby specifically promoting JAK2-mediated phosphorylation of IRS proteins and activation of PI 3-kinase (16, 33, 50). Interestingly, SH2 domain defective mutants of SH2B1 and SH2B2 impair the ability of SH2B1 to promote activation of the JAK2→IRS-2/PI3-kinase pathway in cells (31, 33). Because SH2B1 is a potent

endogenous leptin sensitizer, it is important to test these and other mechanisms *in vivo* in order to determine if SH2B1 has therapeutic potential for treatment of obesity.

Here, we generated two transgenic mouse models to test whether the SH2 domain of SH2B1 in neurons is necessary and/or sufficient for SH2B1 to regulate body weight *in vivo*. Neuron-specific expression of an N-terminal truncated form of SH2B1β that contained the entire SH2 domain and C-terminus (amino acids 504-670), but lacked other functional domains, did not correct energy imbalance in SH2B1-KO mice. These data suggest that multiple domains within neuronal SH2B1 participate in the regulation of energy balance *in vivo*. Neuron-specific expression of a dominant negative form of SH2B1β lacking a functional SH2 domain (SH2B1 R555E) also failed to prevent obesity or restore insulin sensitivity in SH2B1-KO mice. However, neuron-specific expression of dominant negative SH2B1 R555E in wild type mice (TgR555E mice) induced obesity and caused insulin resistance. These findings suggest that mutations in SH2B1 which alter SH2 domain function may contribute to leptin resistance and contribute to the development of obesity.

## Materials and Methods

**Generation of transgenic and compound mutant mice.** SH2B1 knockout (KO), TgSH2B1, and KO/TgSH2B1 mice (C57BL/6 x SJL background) have been described (17, 51). Transgenic mice that express either dominant negative (TgR555E) or truncated (TgΔ504) mutant forms of SH2B1β in neurons were generated for these studies. A 4.9 kb transgene was generated by fusing cDNA encoding a Myc epitope tagged form of rat SH2B1β (R555E) downstream of the rat NSE (neuron-specific enolase) promoter/ GH (growth hormone) enhancer sequence described previously (30, 51). In parallel, a 3.7 kb transgene was generated by fusing cDNA encoding a Myc epitope tagged, N-terminal truncation of rat SH2B1β (amino acids 504-670) downstream of the NSE promoter/ GH enhancer. Linear transgenic constructs were independently microinjected into F2 mouse oocytes (C57BL/6 x SJL) and surgically transferred to recipients by trained personnel at the University of Michigan Transgenic Animal Model Core facilities. Founder mice were genotyped by PCR. Ten TgR555E and eleven TgΔ504 founders were obtained. Two independent lines of TgR555E mice ($TgR555E^{205}$ and $TgR555E^{244}$) and two lines of TgΔ504 mice ($Tg\Delta504^{301}$ and $Tg\Delta504^{315}$), which expressed similar levels of recombinant SH2B1β R555E and SH2B1β Δ504, respectively, were selected for analysis.

Compound mutant mice were generated by crossing TgR555E or TgΔ504 mice with SH2B1-KO mice to generate KO/TgR555E or KO/TgΔ504 mice. Male mice were used for all experiments. Mice were housed on a 14-hour light/10-hour dark cycle in the Unit for Laboratory Animal Medicine (ULAM) at the University of Michigan. Animal protocols were approved by the University Committee on the Use and Care of Animals (UCUCA).

**Growth and body composition.** Body weight was recorded weekly beginning at 4 weeks of age. Mice were briefly anesthetized (2-3% isoflurane), and body composition was determined by dual-energy X-ray absorptiometry (DEXA; PIXImus2 Dexa Scanner, GE Lunar Corporation). Body fat (g) was normalized to body weight (g).

**Immunoprecipitation and immunoblotting.** Mice were fasted overnight (16-h) and anesthetized with Avertin (0.5 g tribromoethanol and 0.25 g tert-amyl alcohol in 39.5 ml of water; 0.02 ml/g body weight). Tissues were isolated, frozen in liquid nitrogen,

and stored at -80°C until analysis. Tissues were homogenized in lysis buffer (50 mM Tris [pH 7.5], 1% Nonidet P-40, 150 mM NaCl, 2 mM EGTA, 1 mM $Na_3VO_4$, 100 mM NaF, 10 mM $Na_4P_2O_7$, 1 mM phenylmethylsulfonyl fluoride, 10 g/ml aprotinin, and 10 g/ml leupeptin). Immunoprecipitation and immunoblotting were conducted as described previously (17). Proteins were visualized using the Odyssey Infrared Imaging System (Li-Cor Biosciences) or ECL (Amersham). SH2B1 antibodies have been described (17, 51).

**Measurements of food intake and energy expenditure.** Mice were individually housed and food intake was recorded daily for 5-7 days. Caloric value for the diet used was 4.6 kCal/g. Metabolic rates were measured by indirect calorimetry (Oxymax Equal Flow system; Columbus Instruments). Mice were individually housed and acclimated for 24-h in metabolic cages. After acclimation, exhaust air was sampled for a 24-h period. Samples were recorded for 1 minute at 27-minute intervals and $O_2$ and $CO_2$ content was determined. Oxygen consumption ($VO_2$) and carbon dioxide production ($CO_2$) were normalized to lean body mass.

**Serum analysis**. Blood samples were collected from the tail vein. Blood glucose concentrations were determined using a glucometer (Glucometer Elite XL; Bayer Corp., Tarrytown, NY). Plasma insulin was determined using a rat insulin ELISA kit (Crystal Chem, Inc., Chicago, IL).

**Glucose and insulin tolerance tests.** For glucose tolerance tests (GTT), mice were fasted overnight (16-h) and D-glucose (2g/kg body weight) was injected intraperitoneally. Blood glucose was measured 0, 15, 30, 60 and 120 min after glucose injection. For insulin tolerance tests (ITT), mice were fasted for 6-h and human insulin (1 U/kg) was injected intraperitoneally. Blood glucose was measured 0, 15, 30 and 60 min after injection.

**Statistical Analysis.** Data are presented as means ± SEM. Differences between groups were determined by two-tailed Student's *t* tests or ANOVA. $P< 0.05$ was considered significant.

## Results

**Generation of SH2B1-null mice expressing recombinant SH2B1β R555E and SH2B1β Δ504 in the brain.** We previously reported that neuron-specific restoration of SH2B1β was sufficient to restore leptin sensitivity, prevent obesity, and reverse obesity-associated metabolic diseases (hyperlipidemia and insulin resistance) in SH2B1-deficient (KO) mice (51). To further define the role of the SH2 domain of SH2B1 *in vivo*, two mutant forms of rat SH2B1β were cloned into the same transgenic vector used to generate TgSH2B1 mice (51). To generate TgR555E mice, cDNA encoding a Myc tagged SH2B1β mutant in which the SH2 domain is disrupted due to replacement of $Arg^{555}$ with Glu (R555E) was used (Fig. 2.1A). We have previously reported that this point mutation impairs the ability of SH2B1β to promote IRS-1 phosphorylation in leptin-treated cells (16, 17). To generate TgΔ504 mice, cDNA encoding an N-terminal truncated mutant of rat SH2B1β (amino acids 504-670) was used (Fig. 2.1A). This mutant contains an intact SH2 domain and C-terminus, but lacks the PH and dimerization (DD) domains. In cells, Δ504 is sufficient to promote leptin signaling (16, 33). Two independent lines were characterized for each SH2B1 mutant based on the criteria that the expression levels of recombinant R555E in TgR555E mice and recombinant Δ504 in TgΔ504 mice were similar to the expression of recombinant SH2B1β in TgSH2B1 mice (data not shown).

To examine the effects of the mutant forms of recombinant SH2B1 in SH2B1 deficient (KO) mice, TgR555E and TgΔ504 mice were crossed to KO mice to generate KO/TgR555E and KO/TgΔ504 compound mutant mice. We first characterized the expression of recombinant R555E and Δ504 in the brains of compound mutant mice. Two forms of endogenous SH2B1 were detected in wild type (WT) mice, but only one form of SH2B1, corresponding to recombinant SH2B1, was detected in brain extract from KO/TgSH2B1 and KO/TgR555E mice. Recombinant R555E was detected in both lines of KO/TgR555E mice (designated KO/TgR555E$^{205}$ and KO/TgR555E$^{244}$), and expression of R555E in both lines was similar to recombinant wild type SH2B1β in KO/TgSH2B1 mice (Fig. 2.1B). The SH2B1 antiserum used to immunoprecipitate endogenous and recombinant forms of SH2B1 in brain samples from WT, KO/TgSH2B1,

and KO/R555E mice did not immunoprecipitate Δ504 in extracts from KO/TgΔ504 mice, presumably because the epitope is not present in recombinant Δ504 (data not shown). However, Δ504 was immunoprecipitated successfully using a second antibody raised against Δ504. Expression of Δ504 in brain from two lines of KO/TgΔ504 mice (designated KO/TgΔ504$^{301}$ and KO/TgΔ504$^{315}$) was similar to recombinant SH2B1 in KO/TgSH2B1 mice (Fig. 2.1C). For unknown reasons, the SH2B1 antibody against Δ504 only recognized one form of endogenous SH2B1 in WT mice; nonetheless, this endogenous form co-migrated with recombinant SH2B1β from KO/TgSH2B1 mice (Fig. 2.1C), and expression of Δ504 in KO/TgΔ504 mice brain was similar to the levels of recombinant and endogenous SH2B1 in KO/TgSH2B1 and WT mice, respectfully (Fig. 2.1C).

**Neuron-specific restoration of SH2B1β, but not R555E or Δ504 mutants, rescues obesity and corrects energy imbalance in SH2B1-KO mice.** To determine whether the SH2 domain of SH2B1 is required and sufficient to normalize body weight in SH2B1-deficient mice, body weight was monitored weekly in KO/TgR555E and KO/TgΔ504 male mice fed a standard diet, and compared to WT, KO, and KO/TgSH2B1 male mice. KO mice were significantly heavier than WT mice at 9 weeks of age and continued to gain weight rapidly thereafter (Fig. 2.2A). KO mice were nearly 1.3 times heavier than WT mice at 16-weeks of age. As expected, neuron-specific restoration of wild type SH2B1β prevented weight gain in KO/TgSH2B1 mice (Fig. 2.2A). However, neuron-specific expression of SH2B1β R555E or SH2B1β Δ504 failed to prevent weight gain in KO/TgR555E or KO/TgΔ504 mice, respectfully (Fig. 2.2A). Body weight was similar in KO/TgR555E$^{205}$, KO/TgR555E$^{244}$, KO/TgΔ504$^{301}$, and KO/TgΔ504$^{315}$ male mice and comparable to KO mice (TgR555E$^{205}$: 39.5 ± 2.2 g, n = 6; KO/TgR555E$^{244}$: 41.4 ± 3.0 g, n = 8; KO/TgΔ504$^{301}$: 42.5 ± 2.6 g, n = 6; KO/TgΔ504$^{315}$: 40.9 ± 2.5, n = 10; KO: 41.9 ± 1.8 g, n = 10; 16-weeks). Moreover, both the onset and rate of weight gain in KO/TgR555E and KO/TgΔ504 mice were similar to KO mice, indicating that neuron-specific expression of R555E and Δ504 does not alter the development of obesity in KO mice. Whole body fat content in KO mice was more than 270% higher than WT mice (Fig. 2.2B). Fat content was dramatically reduced in KO/TgSH2B1 mice (Fig.

2.2B), consistent with our previous findings (51). By contrast, fat content was similar between KO, KO/TgR555E, and KO/TgΔ504 mice (Fig. 2.2B). Lean mass and bone mineral content were also similar in WT, KO, KO/TgSH2B1, KO/TgR555E and KO/TgΔ504 mice (data not shown), indicating that differences in body weight are attributed to differences in fat mass.

Increased adiposity may result from increased energy consumption, reduced energy expenditure, or a combination of both. Therefore, food intake and energy expenditure were determined in KO/TgR555E and KO/TgΔ504 mice. Average daily energy intake was more than 30% higher in KO, KO/TgR555E, and KO/TgΔ504 mice compared to WT and KO/TgSH2B1 mice at the same age (Fig. 2.2C). By contrast, energy intake was similar between WT and KO/TgSH2B1 mice (Fig. 2.2C). Previously, we reported that basal energy expenditure is increased in KO mice (50); however, energy intake still exceeds expenditure in KO mice, resulting in positive energy imbalance (50). In this study, twenty-four hour oxygen consumption and carbon dioxide production were increased to similar levels in KO, KO/TgR555E and KO/TgΔ504 mice (Fig. 2.2D-E). By contrast, both oxygen consumption and carbon dioxide production in KO/TgSH2B1 mice were reduced to WT levels (Fig. 2.2D-E). Collectively, these data indicate that neuron-specific restoration of wild type SH2B1β, but not mutant forms of SH2B1β, can correct energy imbalance in SH2B1-deficient mice. Thus, the SH2 domain is required but not sufficient for the regulation of energy balance and body weight in mice.

**Neuron-specific restoration of wild type SH2B1β, but not R555E or Δ504, improves glucose metabolism and insulin sensitivity in SH2B1-knockout mice.** We previously proposed that SH2B1 in the brain improves insulin sensitivity by controlling adiposity and body weight (51). Therefore, we examined glucose homeostasis in KO/TgR555E and KO/TgΔ504 mice to test whether expression of these mutant forms of SH2B1 in neurons altered insulin sensitivity and glucose metabolism.

Fasting glucose levels in KO, KO/TgR555E, and KO/TgΔ504 mice were 1.8-, 2.2-, and 1.7-times higher, respectfully, than WT mice at 17-19 weeks of age (Fig. 2.4A). Compared to KO mice, blood glucose levels were reduced in KO/TgSH2B1 mice, but hyperglycemia was not completely restored to WT levels (WT: 60.9 ± 4.5 mg/dl, n = 7; KO/TgSH2B1: 80.1 ± 4.4 mg/dl, n = 7; $P<0.01$; Fig. 4.3A). Hyperglycemia was detected

as early as 10 weeks of age in fasted KO, KO/TgR555E, and KO/TgΔ504 mice and blood glucose levels were comparable between these three groups at this age (data not shown), suggesting that neuron-specific expression of R555E and Δ504 do not alter the onset or severity of hyperglycemia in KO mice. Fasting insulin levels were 9 times higher in KO mice (Fig. 2.3B). Neuron-specific restoration of SH2B1β corrected hyperinsulinemia in KO/TgSH2B1 mice whereas KO/TgR555E and KO/TgΔ504 mice remained hyperinsulinemic (Fig. 2.3B).

To further examine peripheral insulin sensitivity, glucose and insulin tolerance tests were performed. At 17-18 weeks of age, KO mice were severely glucose intolerant (Fig. 2.3C). Like KO mice, glucose tolerance was also impaired in KO/TgR555E and KO/TgΔ504 mice, but improved in KO/TgSH2B1 mice (Fig. 2.3C). The area under the glucose tolerance curve was similar for KO, KO/TgR555E, and KO/TgΔ504 mice (Fig. 2.3C). During insulin tolerance tests, exogenous insulin (1U/kg body weight) reduced blood glucose levels in WT and KO/TgSH2B1 mice, but not in KO, KO/TgR555E, or KO/TgΔ504 mice (Fig. 2.3D). Blood glucose remained elevated in KO, KO/TgR555E, and KO/TgΔ504 mice, indicating that whole body insulin resistance was similar in these mice. Together, these data indicate that neuron-specific restoration of wild type SH2B1β, but not R555E or Δ504 mutants, improves glucose metabolism and insulin sensitivity in SH2B1-knockout mice. Moreover, these data support our initial conclusions that SH2B1 in the brain promotes insulin sensitivity by regulating energy balance and adiposity.

**Dominant negative SH2B1β (R555E) in the brain alters energy balance and glucose homeostasis in wild type mice.** In cells, mutation of Arg 555 to Glu (R555E) in SH2B1 largely abolishes the ability of SH2B1 to promote leptin signaling (16, 33). Additionally, a naturally occurring mutant form of SH2B2β, which lacks the SH2 domain, but has intact dimerization and PH domains, can bind to SH2B1 and antagonize the ability of SH2B1 to promote leptin signaling (31). This raises the possibility that dominant negative forms of SH2B family members may impair SH2B1 function *in vivo*.

To test this, we examined the phenotype of TgR555E mice. Expression of R555E in the brain of WT mice induced obesity (Fig. 2.4A). Body weight in TgR555E and WT

mice was comparable from 3-12 weeks of age; however, after 13 weeks of age, body weight was significantly increased in TgR555E mice and TgR555E mice continued to gain weight thereafter (Fig. 2.4A). Relative to KO mice, the onset of obesity in TgR555E mice was delayed (KO mice: 9 weeks; TgR555E: 13 weeks). Moreover, TgR555E mice were not as heavy as KO mice ($TgR555E^{205}$: 35.9 ± 2.9 g, n = 5; KO: 40.2 ± 2.0 g, n = 9; 18 weeks of age), indicating that the severity of obesity differed between mice expressing dominant negative SH2B1 and mice lacking endogenous SH2B1. Relative to wild type mice, whole body fat content was higher in TgR555E mice ($TgR555E^{205}$: 13.2 ± 3.2 %, n = 6; WT: 7.2 ± 3.2 g, n = 5), but the difference did not reach significance (p=0.22). Taken together, these data indicate that over expression of dominant negative SH2B1 in neurons of wild type induces weight gain and increases adiposity.

Food intake and energy expenditure was also determined in TgR555E mice. Food intake was similar between TgR555E and WT mice (data not shown). However, compared to WT mice, both oxygen consumption and carbon dioxide production increased in TgR555E mice (Figs. 2.4B-C). This was also observed in KO mice, suggesting that loss of neuronal, rather than peripheral, SH2B1 some how alters energy consumption such that KO and TgR555E mice actually expend more energy than WT mice.

Glucose metabolism was also examined in TgR555E mice. Compared to WT mice, fasting blood glucose levels were increased in TgR555E mice (Fig. 2.5A). Glucose tolerance and insulin tolerance tests were performed to determine if neuron-specific overexpression of R555E altered insulin sensitivity. The area under the glucose tolerance curve tended (P=0.12) to be larger for TgR555E mice than WT mice (Fig. 2.5B). However, insulin tolerance was impaired in TgR555E mice (Fig. 2.5C). Taken together, these data indicate that overexpression of dominant negative SH2B1 in neurons of wild type mice impairs systemic insulin sensitivity and alters glucose metabolism.

## Discussion

Leptin resistance is a driving force in the development of obesity and obesity-associated metabolic diseases, including hyperlipidemia and insulin resistance. Two mechanisms for leptin resistance have been proposed. First, impaired transport across the blood brain barrier appears to limit the ability of circulatory leptin to access target neurons in the hypothalamus and throughout the brain (9, 18, 55). Second, leptin signaling in target neurons is attenuated by negative regulators such as SOCS3 and PTP1B (5-7, 23, 29, 43, 61). Consistent with this, the expression of hypothalamic SOCS3 and PTP1B is increased in leptin resistant animals (4, 22, 40, 41, 48, 59). We have reported that the JAK2-binding protein SH2B1 functions as an endogenous leptin sensitizer to control body weight by enhancing JAK2 activity and promoting leptin signaling (16, 32, 33, 50, 51). In this study, we investigated the contribution of the SH2 domain of SH2B1 to the regulation of energy balance and body weight *in vivo*.

We report that restoration of an N-terminal truncated mutant of rat SH2B1β (amino acids 504-670) in neurons of SH2B1-knockout mice (KO/TgΔ504) was not sufficient to prevent the development of obesity and obesity associated metabolic disease. Presumably, the inability of Δ504 to prevent obesity in KO/TgΔ504 mice was related to the inability of Δ504 to promote leptin action *in vivo* given that body weight, fat content, and energy imbalance in KO/TgΔ504 mice is nearly identical to leptin-resistant KO mice. Additional biochemical experiments are planned to confirm that leptin signaling is indeed impaired in KO/TgΔ504 mice.

If leptin signaling is comprised in KO/TgΔ504 mice, as we predict, then the inability of the Δ504 transgene to restore energy balance would suggest that multiple functional domains within SH2B1 are required for leptin signaling in mice. SH2B1 Δ504 contains an intact SH2 domain and C-terminus, but lacks the dimerization and PH domains (Fig. 2.1A). These domains have been implicated in the regulation of cell signaling by SH2B1 and may participate in activation of the JAK2→IRS-2/PI3-kinase pathway. SH2B1 binds directly to IRS-1 and IRS-2, and a tertiary signaling complex comprised of JAK2/SH2B1/IRS-1 or JAK2/SH2B1/IRS-2 can be immunopurified from leptin-treated cells (16). SH2B1 can bind via its PH domain to both kinase-inactive

JAK2 and a mutant form of IRS-1 lacking tyrosine residues (Y18F) (16, 54). These phosphorylation-independent interactions may allow SH2B1 to scaffold latent signaling molecules in close proximity to LepRb in order to facilitate signaling upon leptin binding. Additionally, SH2B family members SH2B1 and SH2B2 form homo- and heterodimers via their respective dimerization domains (31, 49). While dimerization of SH2B1 does not seem to be required for enhancing JAK2 activity in cells (53), homo- or heterodimerization of SH2B1 may facilitate the aggregation of signaling molecules into functional complexes around activated JAK2 (16). Either of these two mechanisms might explain why neuron-specific restoration of Δ504 can not restore energy balance in KO/TgΔ504 mice.

Alternatively, the inability of Δ504 to restore energy balance may be due to altered cellular distribution of Δ504 within neurons. Altered cellular distribution of Δ504, due to deletion of a functional domain, may sequester Δ504 away from the LepRb/JAK2 signaling complex. Specifically, deletion of the PH domain may impair the ability of Δ504 to localize to the plasma membrane. Recently, SH2B1 was shown to translocate between the cytoplasmic and nuclear compartments in NGF-treated PC12 cells, a neuronal cell line (12, 36). A nuclear localization sequence (NLS) in SH2B1 between the dimerization and PH domains was identified (36). The NLS is deleted in Δ504 (Fig. 2.1A). Thus, we can not exclude the possibility that disrupting cytoplasmic-to-nuclear shuttling somehow contributes to the inability of Δ504 to restore energy balance in KO/TgΔ504 mice. Regardless, our data suggest that Δ504 lacks at least one key functional domain which is necessary for the regulation of energy balance in mice.

In cells, mutation of Arg 555 to Glu (R555E) in SH2B1 disrupts the SH2 domain and largely abolishes the ability of SH2B1 to promote leptin signaling (16, 33). As predicted, reconstitution of SH2B1 R555E in neurons of KO/TgR555E mice failed to prevent the development of obesity, indicating that a functional SH2 domain is required for regulation of energy balance and body weight by SH2B1.

Interestingly, overexpression of R555E in the brains of wild type mice induced obesity. We also found that overexpression of R555E altered energy balance such that both oxygen consumption and carbon dioxide production were increased. Oxygen consumption and carbon dioxide production are also elevated in KO mice, as

demonstrated in previous studies (50, 51). This abnormal energy expenditure phenotype is reversed after neuron-specific restoration of SH2B1β in KO mice. Collectively, these findings suggest that the mechanism that underlies abnormally elevated energy expenditure in both KO and TgR555E mice has a neuronal, rather than peripheral, origin.

Mild obesity in TgR555E mice causes mild hyperglycemia and insulin resistance. However, insulin resistance does not appear to be as severe in TgR555E mice as in KO mice at the same age (17-18 wks). The apparent difference in insulin sensitivity in TgR555E mice is likely due to the fact that endogenous SH2B1 is expressed in peripheral insulin-target tissues of these mice whereas the same tissues lack SH2B1 in KO mice. This is consistent with our recent report that peripheral SH2B1 enhances insulin signaling and contributes to the regulation of glucose homeostasis in mice (39).

Interestingly, single nucleotide polymorphisms (SNPs) within the human *Sh2b1* loci are associated with leptin resistance and obesity (28, 52, 58, 60), supporting the concept that SH2B1 plays an essential role in the regulation of energy balance and body weight in man. Although these SNPs have not been shown to be associated with altered SH2B1 function or expression, our findings suggest that mutations in the coding region of SH2B1 which alter SH2 domain function may be dominant and result in impaired leptin signaling and obesity. In support of this concept, a naturally occurring mutant form of SH2B2β, which lacks the SH2 domain, but has intact dimerization and PH domains, can bind to SH2B1 and antagonize the ability of SH2B1 to promote leptin signaling in cultured cells (31).

In summary, restoration of SH2B1, but not the SH2 domain alone, in neurons corrects obesity in SH2B1-deficient mice. Our findings suggest that multiple domains within SH2B1, possibly the DD, PH domain, or NLS, may be required for regulation of energy balance and body weight by SH2B1 *in vivo.* Additionally, we report that overexpression of dominant negative SH2B1 (R555E) can induce obesity and mild insulin resistance in mice. Collectively, these data indicate that neuron-specific expression of a functional SH2 domain of SH2B1 is not sufficient but is necessary for normal regulation of energy homeostasis and insulin sensitivity in mice.

**Acknowledgements**

This study was supported by National Institute of Health grants RO1 DK 065122, RO1 DK073601 (to Liangyou Rui) and F31NS056575 (to David L. Morris), and by American Diabetes Association award 1-09-RA-156 (to Liangyou Rui). This work utilized the cores supported by the Michigan Diabetes Research and Training Center (funded by NIH 5P60 DK20572), the University of Michigan's Cancer Center (funded by NIH 5 P30 CA46592), the University of Michigan Nathan Shock Center (funded by NIH P30AG013283), and the University of Michigan Gut Peptide Research Center (funded by NIH DK34933).

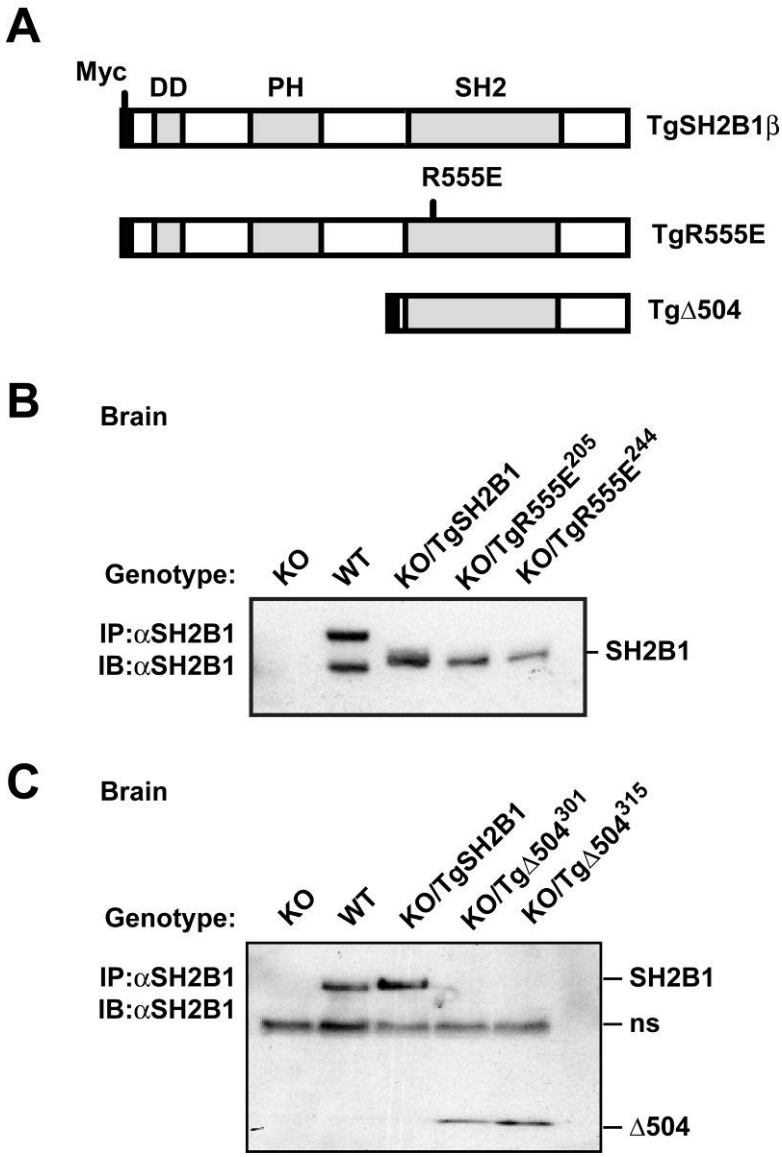


**Fig. 2.1. Generation of SH2B1-null mice expressing recombinant SH2B1β R555E and SH2B1β Δ504 in the brain.** (A) Schematic representation of TgSH2B1, TgR555E, and TgΔ504. The Myc epitope, dimerization (DD), PH, and SH2 domains are indicated. (B) Expression of SH2B1 in brain from KO, WT, KO/TgSH2B1 and two independent lines of KO/TgR555E mice. SH2B1 was immunoprecipitated (IP) with anti-SH2B1 (αSH2B1) antibody and immunoblotted (IB) with αSH2B1. (C) Expression of SH2B1 in brain from KO, WT, KO/TgSH2B1 and two independent lines of KO/TgΔ504 mice. SH2B1 was immunoprecipitated and immunoblotted with αSH2B1.

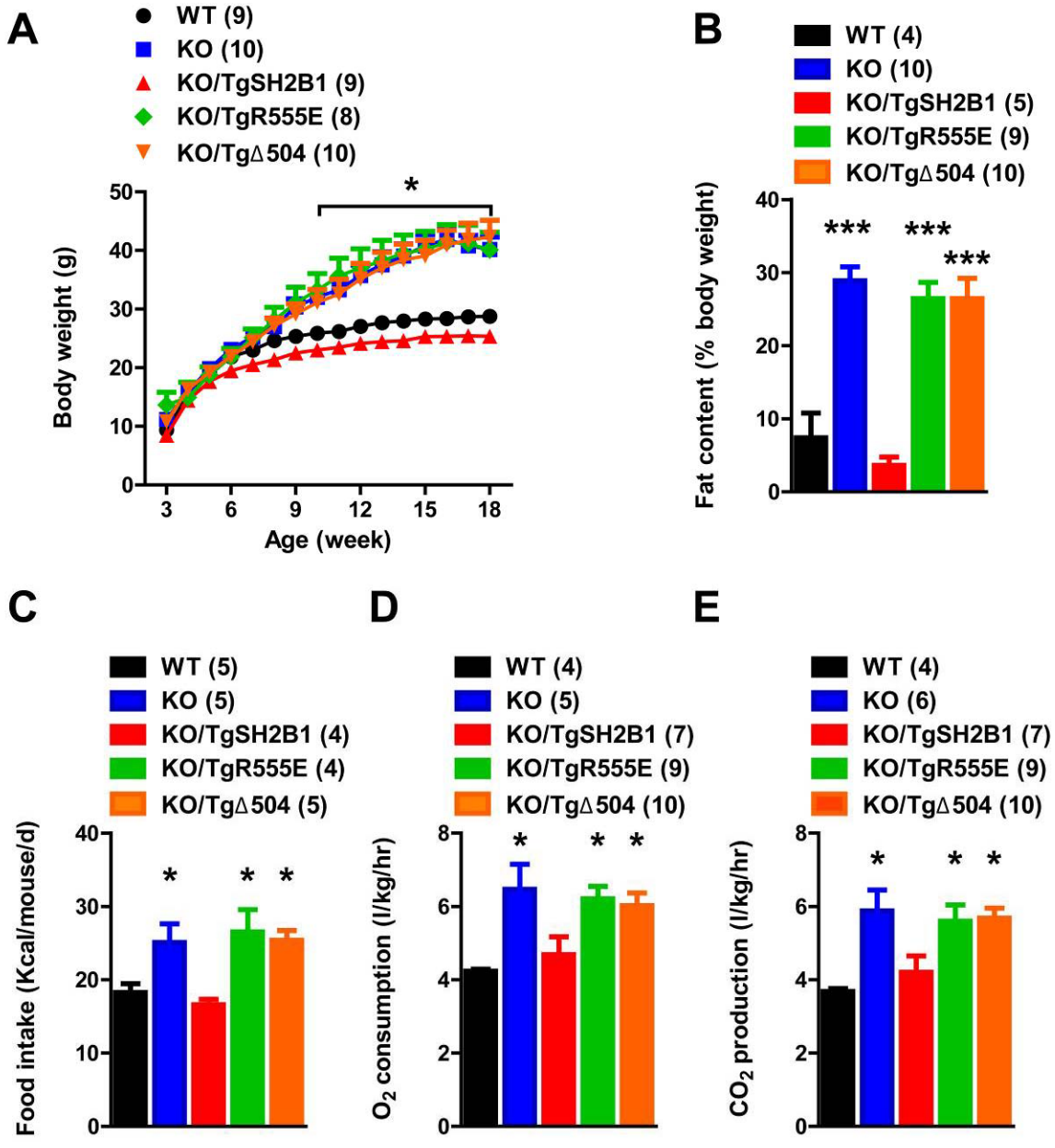


**Fig. 2.2 Restoration of wild type SH2B1β, but R555E or Δ504, in neurons prevents obesity and dyslipidemia in SH2B1-deficient mice.** (A) Growth curve. (B) Whole body fat content was normalized to body weight (15-17 wks). (C) Food intake (13-14 wks). (D-E) Energy expenditure was determined by indirect calorimetry (15-17 wks). (D) Oxygen consumption and (E) carbon dioxide production was normalized to lean body mass. The number of mice in each group is indicated in parenthesis. *P<0.05, ***P<0.001.

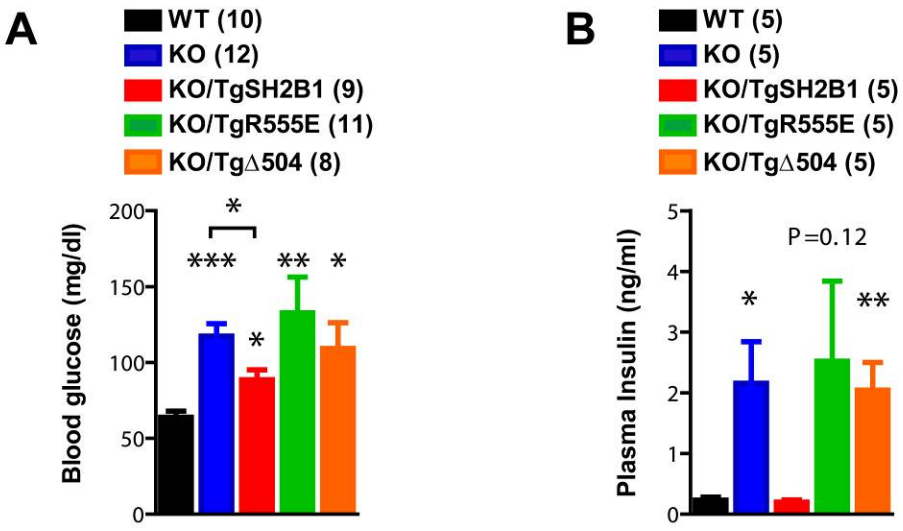


**Fig. 2.3A-B. Improved insulin sensitivity in KO/TgSH2B1, but not in KO/TgR555E or KO/TgΔ504 male mice.** (A) Fasting (16-h) blood glucose levels and (B) plasma insulin levels (17-18 wks). The number of mice in each group is indicated in parenthesis. *P < 0.05, **P<0.01, ***P<0.001.

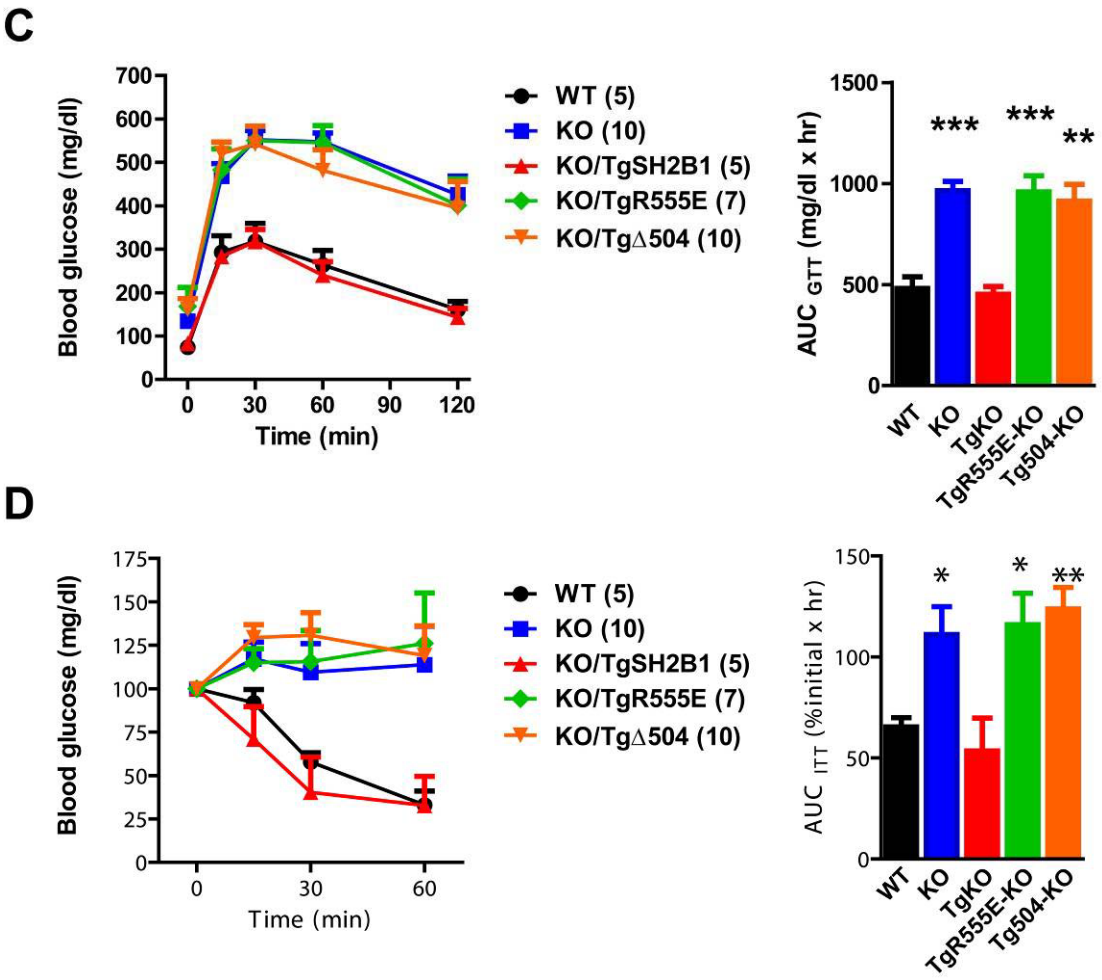


**Fig. 2.3C-D. Improved insulin sensitivity in KO/TgSH2B1, but not in KO/TgR555E or KO/TgΔ504 male mice.** (C) Glucose tolerance tests (GTT) performed on male mice (18-19 wks). Mice were fasted overnight (16-h) and D-glucose (2g/kg body weight) was administered by i.p. injection. Blood glucose levels were monitored 0, 15, 30, 60 and 120 min after injection. (D) Insulin tolerance tests (ITT) in male mice (18-19 wks). Mice were fasted for 6-h and human insulin (1U/ kg body weight) was administered by i.p. injection. Blood glucose was monitored 0, 15, 30 and 60 min after injection. Values are expressed as a percentage of initial (time 0). Area under the curve (AUC) was calculated for GTT and ITT using the trapezoidal rule. The number of mice in each group is indicated in parenthesis. *P < 0.05, **P<0.01.

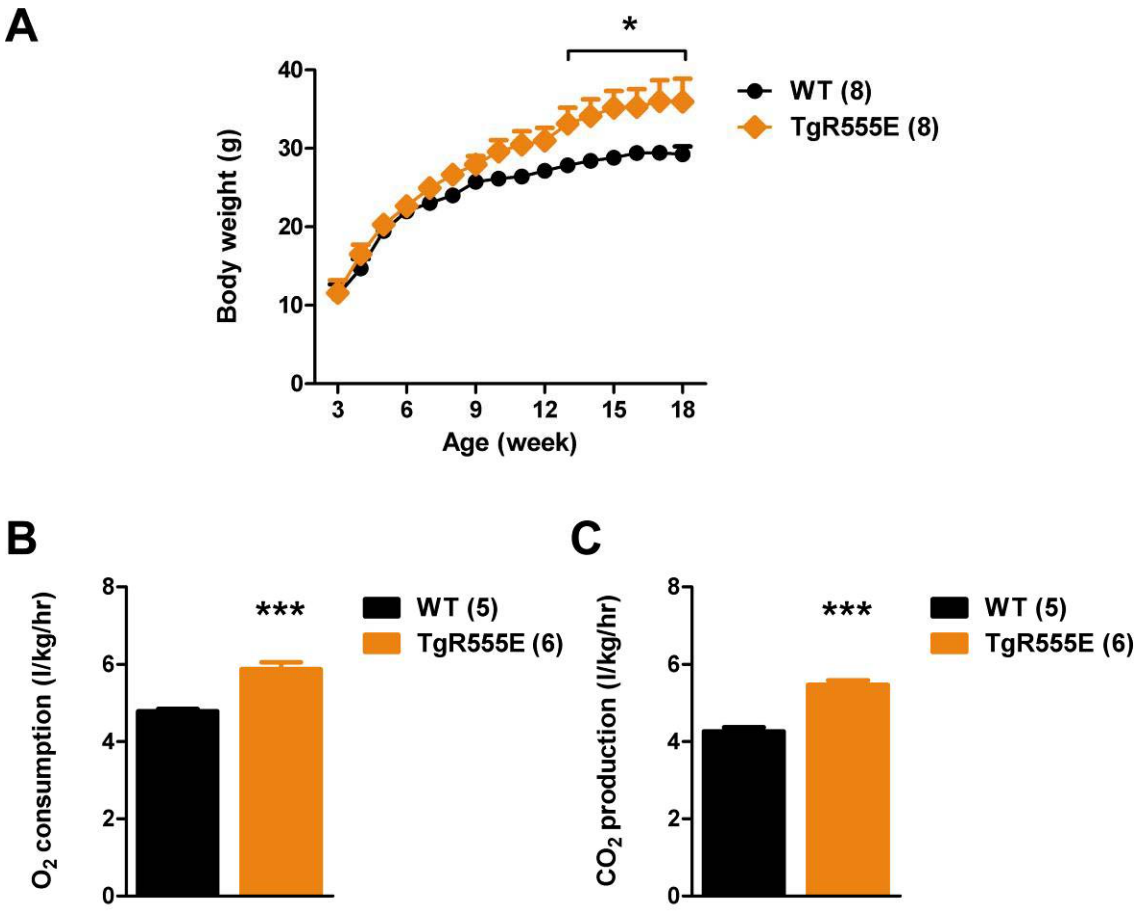


**Fig. 2.4 Expression of dominant negative SH2B1 in neurons disrupts energy homeostasis in WT mice.** (A) Growth curves. (B) Oxygen consumption in male mice (15-17 wks). VO2 was normalized to lean body mass. (C) Carbon dioxide production in male mice (15-17 weeks). VCO2 was normalized to lean body mass. ***P<0.001.

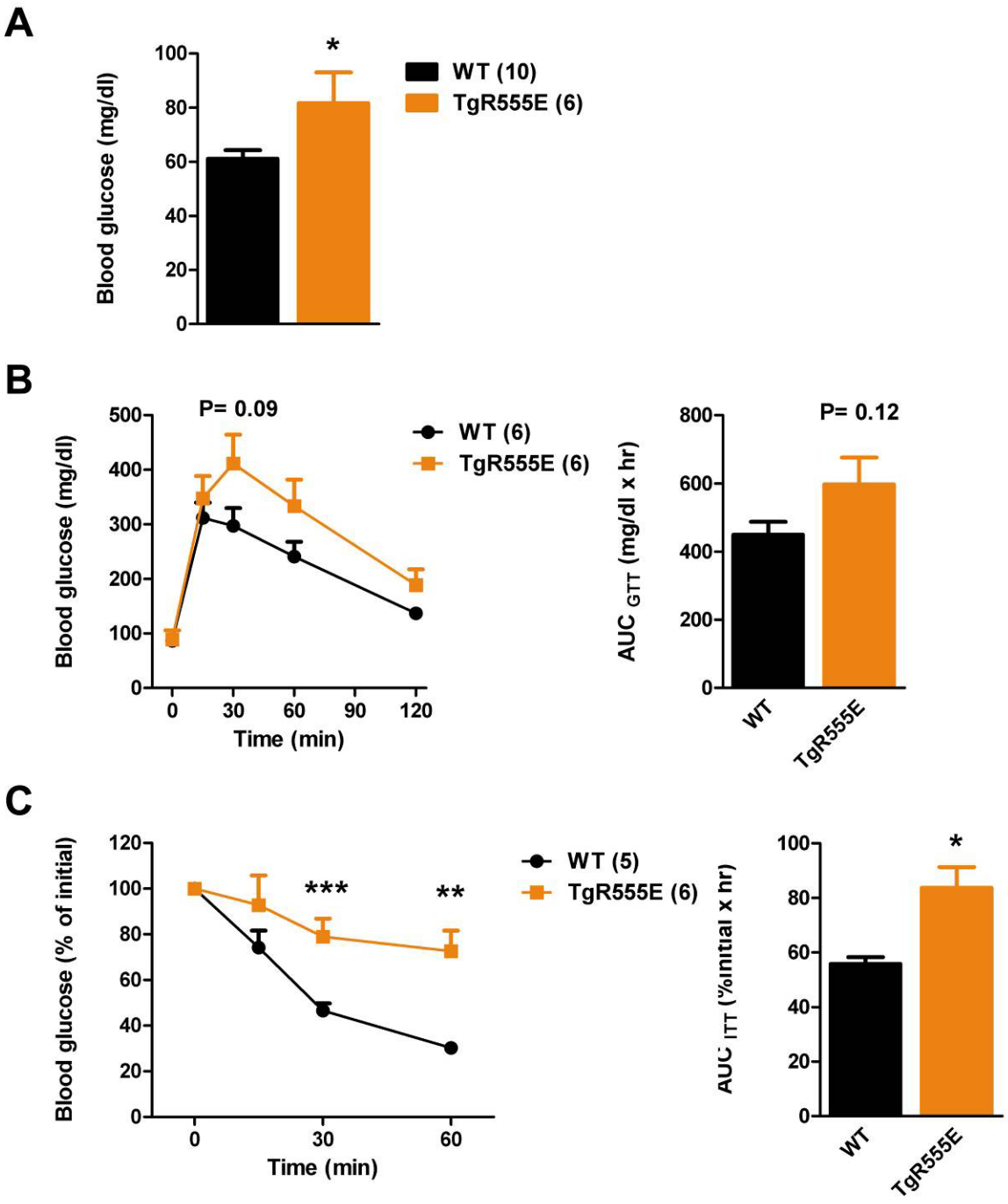


**Fig. 2.5 Expression of dominant negative SH2B1 in neurons alters glucose metabolism and insulin sensitivity.** (A) Fasting (16-h) blood glucose levels (17-18 wks). (B) Glucose tolerance tests (GTT) performed on male mice (18-19 wks). Mice were fasted overnight (16-h) and D-glucose (2g/kg body weight) was administered by i.p. injection. Blood glucose levels were monitored 0, 15, 30, 60 and 120 min after injection. (C) Insulin tolerance tests (ITT) in male mice (18-19 wks). Mice were fasted for 6-h and human insulin (1U/kg body weight) was administered by i.p. injection. Blood glucose was monitored 0, 15, 30 and 60 min after injection. Values are expressed as a percentage of initial (time 0). Area under the curve (AUC) was calculated for GTT and ITT using the trapezoidal rule. The number of mice in each group is indicated in parenthesis. *P < 0.05.

## References


1. **Bates SH, Dundon TA, Seifert M, Carlson M, Maratos-Flier E, and Myers MG, Jr.** LRb-STAT3 signaling is required for the neuroendocrine regulation of energy expenditure by leptin. *Diabetes* 53: 3067-3073, 2004.

2. **Bates SH, Stearns WH, Dundon TA, Schubert M, Tso AW, Wang Y, Banks AS, Lavery HJ, Haq AK, Maratos-Flier E, Neel BG, Schwartz MW, and Myers MG, Jr.** STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* 421: 856-859, 2003.

3. **Bence KK, Delibegovic M, Xue B, Gorgun CZ, Hotamisligil GS, Neel BG, and Kahn BB.** Neuronal PTP1B regulates body weight, adiposity and leptin action. *Nat Med* 12: 917-924, 2006.

4. **Bjorbaek C, Elmquist JK, Frantz JD, Shoelson SE, and Flier JS.** Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell* 1: 619-625, 1998.

5. **Bjorbak C, El-Haschimi K, Frantz JD, and Flier JS.** The Role of SOCS-3 in Leptin Signaling and Leptin Resistance. *J Biol Chem* 274: 30059-30065, 1999.

6. **Bjorbak C, Elmquist JK, Michl P, Ahima RS, van Bueren A, McCall AL, and Flier JS.** Expression of Leptin Receptor Isoforms in Rat Brain Microvessels. *Endocrinology* 139: 3485-3491, 1998.

7. **Bjorbak C, Lavery HJ, Bates SH, Olson RK, Davis SM, Flier JS, and Myers MG, Jr.** SOCS3 mediates feedback inhibition of the leptin receptor via Tyr985. *J Biol Chem* 275: 40649-40657, 2000.

8. **Campfield LA, Smith FJ, Guisez Y, Devos R, and Burn P.** Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269: 546-549, 1995.

9. **Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang PL, Sinha MK, and Considine RV.** Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 348: 159-161, 1996.

10. **Carter-Su C, Rui L, and Stofega MR.** SH2-B and SIRP: JAK2 binding proteins that modulate the actions of growth hormone. *Recent Prog Horm Res* 55: 293-311, 2000.

11. **Carvalheira JB, Ribeiro EB, Folli F, Velloso LA, and Saad MJ.** Interaction between leptin and insulin signaling pathways differentially affects JAK-STAT and PI 3-kinase-mediated signaling in rat liver. *Biol Chem* 384: 151-159, 2003.

12. **Chen L and Carter-Su C.** Adapter protein SH2-B beta undergoes nucleocytoplasmic shuttling: implications for nerve growth factor induction of neuronal differentiation. *Mol Cell Biol* 24: 3633-3647, 2004.

13. **Cheng A, Uetani N, Simoncic PD, Chaubey VP, Lee-Loy A, McGlade CJ, Kennedy BP, and Tremblay ML.** Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. *Dev Cell* 2: 497-503, 2002.

14. **Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, and et al.** Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334: 292-295, 1996.

15. **Cui Y, Huang L, Elefteriou F, Yang G, Shelton JM, Giles JE, Oz OK, Pourbahrami T, Lu CY, Richardson JA, Karsenty G, and Li C.** Essential role of STAT3 in body weight and glucose homeostasis. *Mol Cell Biol* 24: 258-269, 2004.

16. **Duan C, Li M, and Rui L.** SH2-B promotes insulin receptor substrate 1 (IRS1)- and IRS2-mediated activation of the phosphatidylinositol 3-kinase pathway in response to leptin. *J Biol Chem* 279: 43684-43691, 2004.

17. **Duan C, Yang H, White MF, and Rui L.** Disruption of the SH2-B gene causes age-dependent insulin resistance and glucose intolerance. *Mol Cell Biol* 24: 7435-7443, 2004.

18. **El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, and Flier JS.** Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest* 105: 1827-1832, 2000.

19. **Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, and Elmquist JK.** Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron* 23: 775-786, 1999.

20. **Elias CF, Lee C, Kelly J, Aschkenasi C, Ahima RS, Couceyro PR, Kuhar MJ, Saper CB, and Elmquist JK.** Leptin activates hypothalamic CART neurons projecting to the spinal cord. *Neuron* 21: 1375-1385, 1998.

21. **Elmquist JK, Ahima RS, Elias CF, Flier JS, and Saper CB.** Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. *Proc Natl Acad Sci U S A* 95: 741-746, 1998.

22. **Enriori PJ, Evans AE, Sinnayah P, Jobst EE, Tonelli-Lemos L, Billes SK, Glavas MM, Grayson BE, Perello M, Nillni EA, Grove KL, and Cowley MA.** Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. *Cell Metab* 5: 181-194, 2007.

23. **Eyckerman S, Broekaert D, Verhee A, Vandekerckhove J, and Tavernier J.** Identification of the Y985 and Y1077 motifs as SOCS3 recruitment sites in the murine leptin receptor. *FEBS Lett* 486: 33-37, 2000.

24. **Gao Q, Wolfgang MJ, Neschen S, Morino K, Horvath TL, Shulman GI, and Fu XY.** Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. *Proc Natl Acad Sci U S A* 101: 4661-4666, 2004.

25. **Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, and Friedman JM.** Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269: 543-546, 1995.

26. **Hamilton BS, Paglia D, Kwan AY, and Deitel M.** Increased obese mRNA expression in omental fat cells from massively obese humans. *Nat Med* 1: 953-956, 1995.

27. **Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, and McCamish M.** Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA* 282: 1568-1575, 1999.

28. **Jamshidi Y, Snieder H, Ge D, Spector TD, and O'Dell SD.** The SH2B gene is associated with serum leptin and body fat in normal female twins. *Obesity (Silver Spring)* 15: 5-9, 2007.

29. **Kaszubska W, Falls HD, Schaefer VG, Haasch D, Frost L, Hessler P, Kroeger PE, White DW, Jirousek MR, and Trevillyan JM.** Protein tyrosine phosphatase 1B negatively regulates leptin signaling in a hypothalamic cell line. *Molecular and Cellular Endocrinology* 195: 109-118, 2002.

30. **Kowalski TJ, Liu S-M, Leibel RL, and Chua SC, Jr.** Transgenic Complementation of Leptin-Receptor Deficiency: I. Rescue of the Obesity/Diabetes Phenotype of LEPR-Null Mice Expressing a LEPR-B Transgene. *Diabetes* 50: 425-435, 2001.

31. **Li M, Li Z, Morris DL, and Rui L.** Identification of SH2B2beta as an inhibitor for SH2B1- and SH2B2alpha-promoted Janus kinase-2 activation and insulin signaling. *Endocrinology* 148: 1615-1621, 2007.

32. **Li M, Ren D, Iseki M, Takaki S, and Rui L.** Differential role of SH2-B and APS in regulating energy and glucose homeostasis. *Endocrinology* 147: 2163-2170, 2006.

33. **Li Z, Zhou Y, Carter-Su C, Myers MG, Jr., and Rui L.** SH2B1 Enhances Leptin Signaling by Both Janus Kinase 2 Tyr813 Phosphorylation-Dependent and -Independent Mechanisms. *Mol Endocrinol* 21: 2270-2281, 2007.

34. **Lin X, Taguchi A, Park S, Kushner JA, Li F, Li Y, and White MF.** Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. *J Clin Invest* 114: 908-916, 2004.

35. **Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, and et al.** Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1: 1155-1161, 1995.

36. **Maures TJ, Chen L, and Carter-Su C.** Nucleocytoplasmic Shuttling of the Adapter Protein SH2B1{beta} is Required for Nerve Growth Factor (NGF)-Dependent Neurite Outgrowth and Enhancement of Expression of a Subset of NGF-Responsive Genes. *Mol Endocrinol*, 2009.

37. **Maures TJ, Kurzer JH, and Carter-Su C.** SH2B1 (SH2-B) and JAK2: a multifunctional adaptor protein and kinase made for each other. *Trends Endocrinol Metab* 18: 38-45, 2007.

38. **Mori H, Hanada R, Hanada T, Aki D, Mashima R, Nishinakamura H, Torisu T, Chien KR, Yasukawa H, and Yoshimura A.** Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nat Med* 10: 739-743, 2004.

39. **Morris DL, Cho KW, Zhou Y, and Rui L.** SH2B1 Enhances Insulin Sensitivity by Both Stimulating the Insulin Receptor and Inhibiting Tyrosine Dephosphorylation of IRS Proteins. *Diabetes* 58: 2039-2047, 2009.

40. **Morrison CD, White CL, Wang Z, Lee SY, Lawrence DS, Cefalu WT, Zhang ZY, and Gettys TW.** Increased hypothalamic protein tyrosine phosphatase 1B contributes to leptin resistance with age. *Endocrinology* 148: 433-440, 2007.

41. **Munzberg H, Flier JS, and Bjorbaek C.** Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology* 145: 4880-4889, 2004.

42. **Myers MG, Jr.** Leptin receptor signaling and the regulation of mammalian physiology. *Recent Prog Horm Res* 59: 287-304, 2004.

43. **Myers MP, Andersen JN, Cheng A, Tremblay ML, Horvath CM, Parisien J-P, Salmeen A, Barford D, and Tonks NK.** TYK2 and JAK2 Are Substrates of Protein-tyrosine Phosphatase 1B. *J Biol Chem* 276: 47771-47774, 2001.

44. **Nishi M, Werner ED, Oh BC, Frantz JD, Dhe-Paganon S, Hansen L, Lee J, and Shoelson SE.** Kinase activation through dimerization by human SH2-B. *Mol Cell Biol* 25: 2607-2621, 2005.

45. **Niswender KD, Morrison CD, Clegg DJ, Olson R, Baskin DG, Myers MG, Jr., Seeley RJ, and Schwartz MW.** Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. *Diabetes* 52: 227-231, 2003.

46. **Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myers MG, Jr., and Schwartz MW.** Intracellular signalling. Key enzyme in leptin-induced anorexia. *Nature* 413: 794-795, 2001.

47. **Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, and Collins F.** Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269: 540-543, 1995.

48. **Peralta S, Carrascosa JM, Gallardo N, Ros M, and Arribas C.** Ageing increases SOCS-3 expression in rat hypothalamus: effects of food restriction. *Biochemical and Biophysical Research Communications* 296: 425-428, 2002.

49. **Qian X and Ginty DD.** SH2-B and APS are multimeric adapters that augment TrkA signaling. *Mol Cell Biol* 21: 1613-1620, 2001.

50. **Ren D, Li M, Duan C, and Rui L.** Identification of SH2-B as a key regulator of leptin sensitivity, energy balance, and body weight in mice. *Cell Metabolism* 2: 95-104, 2005.

51. **Ren D, Zhou Y, Morris D, Li M, Li Z, and Rui L.** Neuronal SH2B1 is essential for controlling energy and glucose homeostasis. *J Clin Invest* 117: 397-406, 2007.

52. **Renstrom F, Payne F, Nordstrom A, Brito EC, Rolandsson O, Hallmans G, Barroso I, Nordstrom P, and Franks PW.** Replication and extension of genome-wide association study results for obesity in 4923 adults from northern Sweden. *Hum Mol Genet* 18: 1489-1496, 2009.

53. **Rui L and Carter-Su C.** Identification of SH2-bbeta as a potent cytoplasmic activator of the tyrosine kinase Janus kinase 2. *Proc Natl Acad Sci U S A* 96: 7172-7177, 1999.

54. **Rui L, Gunter DR, Herrington J, and Carter-Su C.** Differential binding to and regulation of JAK2 by the SH2 domain and N-terminal region of SH2-bbeta. *Mol Cell Biol* 20: 3168-3177, 2000.

55. **Schwartz MW, Peskind E, Raskind M, Boyko EJ, and Porte D, Jr.** Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med* 2: 589-593, 1996.

56. **Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, and et al.** The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 377: 530-532, 1995.

57. **Suzuki R, Tobe K, Aoyama M, Inoue A, Sakamoto K, Yamauchi T, Kamon J, Kubota N, Terauchi Y, Yoshimatsu H, Matsuhisa M, Nagasaka S, Ogata H, Tokuyama K, Nagai R, and Kadowaki T.** Both insulin signaling defects in the liver and obesity contribute to insulin resistance and cause diabetes in Irs2(-/-) mice. *J Biol Chem* 279: 25039-25049, 2004.

58. **Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadottir A, Styrkarsdottir U, Gretarsdottir S, Thorlacius S, Jonsdottir I, Jonsdottir T, Olafsdottir EJ, Olafsdottir GH, Jonsson T, Jonsson F, Borch-Johnsen K, Hansen T, Andersen G, Jorgensen T, Lauritzen T, Aben KK, Verbeek AL, Roeleveld N, Kampman E, Yanek LR, Becker LC, Tryggvadottir L, Rafnar T, Becker DM, Gulcher J, Kiemeney LA, Pedersen O, Kong A, Thorsteinsdottir U, and Stefansson K.** Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet* 41: 18-24, 2009.

59. **White CL, Whittington A, Barnes MJ, Wang ZQ, Bray G, and Morrison CD.** HF diets increase hypothalamic PTP1B and induce leptin resistance through both leptin-dependent and independent mechanisms. *Am J Physiol Endocrinol Metab*, 2008.

60. **Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, Berndt SI, Elliott AL, Jackson AU, Lamina C, Lettre G, Lim N, Lyon HN, McCarroll SA, Papadakis K, Qi L, Randall JC, Roccasecca RM, Sanna S, Scheet P, Weedon MN,**

**Wheeler E, Zhao JH, Jacobs LC, Prokopenko I, Soranzo N, Tanaka T, Timpson NJ, Almgren P, Bennett A, Bergman RN, Bingham SA, Bonnycastle LL, Brown M, Burtt NP, Chines P, Coin L, Collins FS, Connell JM, Cooper C, Smith GD, Dennison EM, Deodhar P, Elliott P, Erdos MR, Estrada K, Evans DM, Gianniny L, Gieger C, Gillson CJ, Guiducci C, Hackett R, Hadley D, Hall AS, Havulinna AS, Hebebrand J, Hofman A, Isomaa B, Jacobs KB, Johnson T, Jousilahti P, Jovanovic Z, Khaw KT, Kraft P, Kuokkanen M, Kuusisto J, Laitinen J, Lakatta EG, Luan J, Luben RN, Mangino M, McArdle WL, Meitinger T, Mulas A, Munroe PB, Narisu N, Ness AR, Northstone K, O'Rahilly S, Purmann C, Rees MG, Ridderstrale M, Ring SM, Rivadeneira F, Ruokonen A, Sandhu MS, Saramies J, Scott LJ, Scuteri A, Silander K, Sims MA, Song K, Stephens J, Stevens S, Stringham HM, Tung YC, Valle TT, Van Duijn CM, Vimaleswaran KS, Vollenweider P, et al.** Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 41: 25-34, 2009.

61. **Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, Kim YB, Elmquist JK, Tartaglia LA, Kahn BB, and Neel BG.** PTP1B regulates leptin signal transduction in vivo. *Dev Cell* 2: 489-495, 2002.

## Chapter 3

## SH2B1 Enhances Insulin Sensitivity By Both Stimulating The Insulin Receptor And Inhibiting Tyrosine Dephosphorylation Of IRS Proteins


### Abstract

SH2B1 is a SH2 domain-containing adaptor protein expressed in both the central nervous system and peripheral tissues. Neuronal SH2B1 controls body weight; however, the function of peripheral SH2B1 remains unknown. To address the role of peripheral SH2B1 in the regulation of insulin sensitivity and glucose metabolism, TgKO mice, which express SH2B1 in the brain but not in peripheral tissues, were fed a high fat diet (HFD). Deletion of peripheral SH2B1 did not alter body weight or adiposity in HFD-fed TgKO mice. However, deletion of SH2B1 in peripheral tissues exacerbated HFD-induced hyperglycemia, hyperinsulinemia and glucose intolerance in TgKO mice. Insulin signaling was dramatically impaired in muscle, liver and adipose tissue in TgKO mice. Deletion of SH2B1 impaired insulin signaling in primary hepatocytes, whereas SH2B1 overexpression stimulated tyrosine phosphorylation of insulin receptor substrates (IRS-1, IRS-2 and Shc). The SH2 domain of SH2B1 was both required and sufficient to promote insulin signaling. In vitro, recombinant SH2B1 promoted the catalytic activity of the insulin receptor, which required SH2B1 binding to Tyr1158 within the activated receptor. Additionally, insulin stimulated the binding of SH2B1 to IRS-1 or IRS-2, and this physical interaction inhibited tyrosine dephosphorylation of IRS-1 or IRS-2 and increased the ability of IRS proteins to activate the PI 3-kinase pathway. In conclusion, SH2B1 in peripheral tissues functions as an endogenous insulin sensitizer. Mechanistically, SH2B1 directly binds to the insulin receptor, IRS-1 and IRS-2, and enhances insulin sensitivity by both promoting receptor activity and by inhibiting tyrosine dephosphorylation of IRS proteins.

## Introduction

Insulin decreases blood glucose both by promoting glucose uptake into skeletal muscle and adipose tissue and by suppressing hepatic glucose production. In type 2 diabetes, the ability of insulin to reduce blood glucose is impaired (insulin resistance) due to a combination of genetic and environmental factors, resulting in hyperglycemia. Insulin resistance is not only the hallmark but also a determinant of type 2 diabetes.

Insulin binds to and activates the insulin receptor (IR). IR tyrosyl phosphorylates insulin receptor substrates (IRS-1, -2, -3 and -4). IRS proteins, particularly IRS-1 and IRS-2, initiate and coordinate multiple downstream pathways, including the PI 3-kinase/Akt pathway (38). Genetic deletion of IRS-1, IRS-2 or Akt2 causes insulin resistance in mice, indicating that the IRS protein/PI 3-kinase/Akt2 pathway is required for regulation of glucose homeostasis by insulin (3, 5, 30, 43). IR and IRS proteins are negatively regulated by various intracellular molecules, including PTP1B, Grb10, Grb14, SOCS1, SOCS3, JNK, PKCθ, S6K and IKKβ (1, 4, 6, 9-11, 13, 14, 25, 27-29, 33-35, 37, 44, 47). The relative contribution of these negative regulators to the progression of insulin resistance has been extensively studied (1, 4, 6, 9-11, 13, 14, 25, 27-29, 33-35, 37, 40, 44, 47). However, insulin signaling is likely to also be modulated by positive regulators. In this study, we demonstrate that SH2B1 is a novel endogenous insulin sensitizer.

SH2B1 is a member of the SH2B family of adapter proteins that also includes SH2B2 (APS) and SH2B3 (Lnk). SH2B1 and SH2B2 are expressed in multiple tissues, including insulin target tissues (e.g. skeletal muscle, adipose tissue, liver and the brain); by contrast, SH2B3 expression is restricted to hematopoietic tissue (32, 36). Structurally, SH2B family members have an N-terminal dimerization domain (DD), a central pleckstrin homology (PH) domain and a C-terminal Src homology 2 (SH2) domain. The DD domain mediates homodimerization or heterodimerization between different SH2B proteins (21). SH2B1 and SH2B2 bind via their SH2 domains to a variety of tyrosine phosphorylated proteins, including JAK2 and IR, in cultured cells (18). Genetic deletion of SH2B1 results in marked leptin resistance, obesity, insulin resistance and type 2 diabetes in mice, demonstrating that SH2B1 is required for the maintenance of normal body weight, insulin sensitivity and glucose metabolism (8, 17, 22, 23). Surprisingly,

SH2B2 null mice have normal body weight and slightly improved insulin sensitivity (17, 19), suggesting that SH2B1 and SH2B2 have distinct functions in vivo. However, it remains unclear whether SH2B1 cell-autonomously regulates insulin sensitivity in peripheral insulin target tissues because systemic deletion of SH2B1 causes obesity, which may cause insulin resistance in SH2B1 null mice.

We generated a mouse model in which recombinant SH2B1 is specifically expressed in the brain of SH2B1 null mice (TgKO) using transgenic approaches (23). Neuron-specific restoration of SH2B1 corrects both leptin resistance and obesity, suggesting that neuronal SH2B1 regulates energy balance and body weight by enhancing leptin sensitivity (23). Consistent with these conclusions, polymorphisms in the *SH2B1* loci are linked to leptin resistance and obesity in humans (24, 31, 42). In this work, we demonstrate that deletion of SH2B1 in peripheral tissues impairs insulin sensitivity independent of obesity in TgKO mice. Moreover, we demonstrate that SH2B1 directly promotes insulin responses by stimulating IR catalytic activity and by protecting IRS proteins from tyrosine dephosphorylation.

## Materials and Methods

**Animal Studies.** SH2B1 KO and TgKO mice have been described previously (8, 23), and were backcrossed for 6 generations onto a C57BL/6 genetic background. Mice were housed on a 12-h light/dark cycle in the Unit for Laboratory Animal Medicine at the University of Michigan, and fed either normal rodent chow (9% fat; Lab Diet) or HFD (45% fat; Research Diets) ad libitum with free access to water. Fat content was measured by dual energy x-ray absorptiometry (Norland Medical System). Blood glucose levels were determined using glucometers (Bayer Corp). Plasma insulin was measured using a rat insulin ELISA kit (Crystal Chem). Glucose tolerance tests (GTT) (2 g D-glucose/kg of body weight) and insulin tolerance tests (ITT) (1 IU/kg of body weight; Eli Lilly) were conducted as previously described (8, 22, 23). To analyze insulin signaling, mice (fasted 16-h) were anesthetized with Averin (0.5 g of tribromoethanol and 0.25 g or tert-amyl alcohol in 39.5 ml of water; 0.02 ml/g of body weight), and treated with phosphate buffered saline (PBS) or human insulin (3 U per mouse; Eli Lilly) via inferior vena cava injection. Five minutes after injection, gastrocnemius muscles, liver and epididymal fat pads were dissected, frozen in liquid nitrogen and stored at -80ºC. Tissues were homogenized in ice cold lysis buffer (50 mM Tris HCl, pH 7.5, 1.0% NP-40, 150 mM NaCl, 2 mM EGTA, 1 mM $Na_3VO_4$, 100 mM NaF, 10 mM $Na_4P_2O_7$, 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin) and extracts were immunoblotted or immunoprecipitated with indicated antibodies. Animal protocols were approved by the University Committee on Use and Care of Animals.

**Cell lines and Transfection.** COS-7 and HEK293 cells were grown in DMEM supplemented with 5% bovine serum and transfected with indicated plasmids using Lipofectamine 2000 (Invitrogen). Chinese hamster ovary ($CHO^{IR}$ and $CHO^{IR/IRS-1}$) cells were cultured in Ham's F-12 media supplemented with 8% FBS. Cells were deprived of serum for 16-h in DMEM (COS-7 and HEK293) or F-12 (CHO) containing 0.6% BSA before being treated. Primary liver cells were isolated from male mice (8 weeks) by perfusion of the liver with type II collagenase (Worthington Biochem), and plated on collagen coated plates in M199 containing 10% FBS, 100 units/ml penicillin and 100 μg/ml streptomycin. After 2-h, primary cells were rinsed in PBS and cultured for an

additional 16-h in Williams' Medium E (Sigma) supplemented with 0.6% BSA, 100 units/ml penicillin and 100 μg/ml streptomycin.

**Immunoprecipitation and Immunoblotting.** Immunoprecipitation and immunoblotting were conducted as described previously (7, 8). Proteins were visualized using the Odyssey Infrared Imaging System (Li-Cor Biosciences) or ECL (Amersham), and quantified using Odyssey 1.2 software (Li-Cor). Actin, phospho-Akt (Thr308), Akt, insulin receptor β, Myc, Shc and tubulin antibodies were from Santa Cruz. The phosphotyrosine-specific antibody was from Upstate. The AS160 antibody was from Millipore and phospho Akt substrate (PAS) antibody was from Cell Signaling. Phospho-Akt (Ser473) was from BioSource. SH2B1 and IRS-1 antibodies have been described (7, 25).

**Insulin Receptor Kinase Assay.** Cells were serum-deprived for 16-h, treated with insulin and solubilized in kinase lysis buffer (50 mM Tris HCl [pH 7.5], 0.1% Triton X-100, 150 mM NaCl, 1 mM EDTA, 1 mM $Na_3VO_4$, 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin). The insulin receptor (IR) was precipitated with wheat germ agglutinin (WGA)-conjugated agarose beads, washed three times in wash buffer (50 mM Tris HCl [pH 7.5], 0.5 M NaCl, 0.1% Triton X-100) and twice in kinase reaction buffer (20 mM Hepes [pH 7.6], 0.1% Triton X-100, 5 mM $MgCl_2$, 100 μM $Na_3VO_4$). WGA-immobilized proteins were preincubated in kinase reaction buffer supplemented with soluble glutathione-S-tranferase (GST) protein alone, GST-SH2B1 or GST-SH2 fusion proteins at 37°C. GST-IRS-1 (5-10 μg) and ATP (50 μM) were added to initiate kinase reactions at 37°C. Reactions were stopped by adding SDS-PAGE loading buffer and reaction mixtures were boiled immediately. Proteins were separated by SDS-PAGE and immunoblotted with indicated antibodies.

**Dephosphorylation Assays.** Immunopurified proteins were washed in lysis buffer and preincubated with GST-SH2B1 or GST (2 μg) in phosphatase reaction buffer (50 mM Tris-HCl [pH 8.2], 100 nM NaCl, 10 mM $MgCl_2$, 1 mM DTT) for 15 min at room temperature with constant mixing. Alkaline phosphatase (New England Biolabs) was added at the indicated concentration, and the mixtures were incubated an additional 30 min at room temperature. Reactions were stopped by adding SDS-PAGE loading

buffer and mixtures were boiled immediately. Proteins were separated by SDS-PAGE and immunoblotted with indicated antibodies.

**Statistical Analysis.** Data are presented as means ± SEM. Differences between groups were determined by two-tailed Student's *t* tests or ANOVA. $P < 0.05$ was considered significant.

## Results

**Loss of peripheral SH2B1 predisposes mice to high fat diet (HFD)-induced insulin resistance.** We previously generated SH2B1 transgenic (Tg) mice in which the expression of recombinant SH2B1 is controlled by the neuron-specific enolase promoter (23). Tg mice were crossed with SH2B1 knockout (KO) mice to generate SH2B1 transgenic/knockout compound mutant (TgKO) mice. In TgKO mice, recombinant SH2B1 is expressed in brain but not in other tissues, including liver, muscle and adipose tissue (23). Neuron-specific restoration of SH2B1 in TgKO mice fully corrected leptin resistance and obesity, and largely rescued the hyperglycemia and insulin resistance observed in SH2B1 null mice, indicating that neuronal SH2B1 indirectly regulates insulin sensitivity and glucose metabolism by controlling adiposity (23).

To determine whether loss of peripheral SH2B1 exacerbates dietary fat-induced insulin resistance, TgKO and wild type littermates (7 weeks) were fed HFD. Body weight and adiposity were similar between wild type and TgKO mice fed HFD (Figs. 3.1A-B). However, fasting (16-h) blood glucose levels were 1.3-fold higher in TgKO mice than in wild type mice fed HFD for 16 weeks (Fig. 3.1C). Fasting plasma insulin levels were 2-fold higher in TgKO mice than in wild type mice (Fig. 3.1D). To examine insulin sensitivity, glucose and insulin tolerance tests (GTT and ITT) were performed. Blood glucose levels were 23-26% higher in TgKO mice than wild type mice 15 and 30 minutes after injection of D-glucose (Fig. 3.1E). Exogenous insulin markedly reduced blood glucose in wild type but not in TgKO mice during ITT (Fig. 3.1F). These results indicate that loss of peripheral SH2B1 exacerbates HFD-induced insulin resistance, hyperglycemia, and glucose intolerance independent of obesity.

**Loss of peripheral SH2B1 impairs insulin signaling in muscle, liver and adipose tissue in HFD-fed mice.** To examine insulin signaling in skeletal muscle, liver and epididymal fat, mice (7 weeks) were fed HFD for 16 weeks and treated with insulin or PBS vehicle. Insulin markedly stimulated tyrosine phosphorylation of IRS-1 as well as IRS-1 association with p85, the regulatory subunit of the PI 3-kinase, in skeletal muscle of wild type mice (Fig. 3.2A). Both IRS-1 phosphorylation and IRS-1-p85 association were markedly reduced in TgKO muscle (Fig. 3.2A). Loss of peripheral SH2B1 also decreased insulin receptor autophosphorylation and impaired the ability of

insulin to stimulate Akt phosphorylation on $Thr^{308}$ and $Ser^{473}$ in TgKO muscle (Fig. 3.2A). Insulin-stimulated IRS-1 and Akt $Thr^{308}$ phosphorylation were reduced by 44% and 52%, respectfully, in TgKO muscle (Fig. 3.2B).

Insulin signaling was also examined in liver and white adipose tissue (WAT) from HFD-fed mice. Relative to wild type mice, basal IRS-1 phosphorylation was increased in both liver and white adipose tissue (WAT) in TgKO mice; insulin stimulated IRS-1 phosphorylation in these tissues from wild type but not TgKO mice (Figs. 3.2C and 3.2E). Akt phosphorylation ($Ser^{473}$) was also reduced in liver (Fig. 3.2D) and WAT (not shown) in TgKO mice. AS160, a Rab-GAP, is an Akt-substrate involved in GLUT4 vesicle trafficking in adipocytes (12, 26). To measure AS160 phosphorylation, WAT extracts were immunoprecipitated with anti-phospho-Ser/Thr Akt substrate antibody and immunoblotted with anti-AS160 antibody. Similar to IRS-1, basal AS160 phosphorylation was increased in adipose tissue from TgKO mice (Fig. 3.2F); however, insulin failed to further stimulate AS160 phosphorylation (Fig. 3.2F). Together, these data indicate that peripheral SH2B1 increases insulin sensitivity in mice by promoting insulin signaling, including the activation of the IRS protein/PI 3-kinase/Akt pathway, in muscle, liver and white adipose tissue.

**SH2B1 cell-autonomously promotes insulin signaling via its SH2 domain.** To determine whether endogenous SH2B1 directly enhances insulin signaling, primary hepatocyte cultures were prepared from wild type and SH2B1 KO littermates, and treated with insulin. SH2B1 was detected in wild type but not in KO hepatocytes as expected (Fig. 3.3A). Insulin stimulated tyrosine phosphorylation of IR and IRS-1, IRS-1 association with p85, and phosphorylation of Akt in wild type hepatocytes; however, IR autophosphorylation, IRS-1 phosphorylation, IRS-1 association with p85, and Akt phosphorylation were all reduced in KO hepatocytes (Fig. 3.3A). Interestingly, SH2B1 deficiency impaired IRS-1 phosphorylation to a greater extent than IR autophosphorylation. In HEK293 cells, overexpression of SH2B1 markedly increased insulin-stimulated tyrosine phosphorylation of IRS-1; in contrast, a SH2B1 mutant in which the SH2 domain is disrupted due to replacement of $Arg^{555}$ with Glu (R555E) functioned as a dominant negative to inhibit IRS-1 phosphorylation (Fig. 3.3B). To further assess the role of the SH2 domain of SH2B1, IRS-1 and IR were coexpressed with

ΔN504, an N-terminal truncated form of rat SH2B1β (amino acids 504-670) that contained the entire SH2 domain and a minimal number of adjacent amino acids. ΔN504 also promoted IRS-1 phosphorylation in insulin-treated cells (Fig. 3.3C). These data suggest that the SH2 domain of SH2B1 is not only required but also sufficient to promote IR-mediated phosphorylation of IRS-1.

To determine whether SH2B1 also promotes phosphorylation of other IR substrates, Shc was coexpressed with SH2B1 or ΔN504. SH2B1 and ΔN504 enhanced insulin stimulation of Shc phosphorylation to similar levels (Fig. 3.3D). By contrast, SH2B1 stimulated IRS-1 phosphorylation to a higher level than did ΔN504 (Fig. 3.3C), suggesting that SH2B1 promotes phosphorylation of IRS-1 and Shc by different mechanisms.

**SH2B1 stimulates IR catalytic activity through the binding of its SH2 domain to Tyr$^{1158}$ in IR.** SH2B1 directly binds via its SH2 domain to Tyr$^{1158}$ within the activation loop of IR (15, 20). To test whether this interaction modulates IR activation, CHO$^{IR}$ cells, which stably express IR, were treated with insulin, and active IR was purified using wheat germ agglutinin (WGA)-conjugated agarose beads (39). IR was then pre-incubated with purified GST-SH2B1 fusion protein, and subsequently subjected to in vitro kinase assays using GST-IRS-1 fusion protein as substrate. Tyrosine phosphorylation of GST-IRS-1 was measured by immunoblotting with anti-phospho-tyrosine antibodies. GST-SH2B1, but not GST alone, dose-dependently stimulated IR kinase activity as indicated by increased phosphorylation of GST-IRS-1 (Fig. 3.4A). In similar experiments, a GST-SH2 fusion protein prepared by fusing the SH2 domain (amino acids 524-670 of SH2B1β) to GST was preincubated with WGA-purified IR in vitro kinase assays. The SH2 domain of SH2B1 was sufficient to enhance IR catalytic activity, stimulating IRS-1 phosphorylation by ~66 % (Fig. 3.4B). Additionally, the SH2 domain of SH2B1 also promoted the catalytic activity of IR immunopurified with an anti-phospho-tyrosine antibody, increasing IRS-1 substrate phosphorylation by ~79 % (Fig. 3.4C).

To determine whether Tyr$^{1158}$ in IR is involved in SH2B1 stimulation of IR activity, Tyr$^{1158}$ was replaced with Phe (Y1158F). COS-7 cells were transiently transfected with IR or Y1158F and treated with insulin. Insulin stimulated

autophosphorylation of both IR and Y1158F, but Y1158F autophosphorylation was reduced (Fig. 3.4D). Y1158F phosphorylated IRS-1 in response to insulin (data not shown), indicating that Y1158F retains the ability to be activated and to phosphorylate its substrates. IR and Y1158F were purified using WGA-beads, preincubated with GST-SH2B1, and subjected to in vitro kinase assays. SH2B1 stimulated IR kinase activity by ~5 fold; however, SH2B1 was unable to stimulate Y1158F catalytic activity (Fig. 3.4E). Taken together, these data suggest that the physical interaction between the SH2 domain of SH2B1 and $Tyr^{1158}$ in IR is required and sufficient for stimulation of IR catalytic activity.

**SH2B1 protects IRS proteins against tyrosine dephosphorylation.** SH2B1 directly binds to IRS-1 and IRS-2 in vitro (7), and insulin stimulated coimmunoprecipitation of SH2B1 with IRS-1 in cells (Fig. 3.5A). To determine whether this physical interaction inhibits IRS-1 dephosphorylation by phosphatases, $CHO^{IR/IRS-1}$ cells, which stably express IR and IRS-1, were stimulated with insulin to promote tyrosine phosphorylation of IRS-1. Phosphorylated IRS-1 was immunopurified, preincubated with GST or GST-SH2B1, and subjected to *in vitro* dephosphorylation assays. IRS-1 bound to GST-SH2B1 but not to GST (data not shown). Alkaline phosphatase dose-dependently dephosphorylated IRS-1 on tyrosines in the GST-pretreated samples; in contrast, alkaline phosphatase was unable to dephosphorylate SH2B1-bound IRS-1 (Fig. 3.5B). Insulin also promoted the association of SH2B1 with IRS-2, and SH2B1 similarly inhibited tyrosine dephosphorylation of IRS-2 (data not shown).

To determine whether SH2B1 inhibits IRS-1 dephosphorylation in cells, IRS-1 was coexpressed with PTP1B (a protein tyrosine phosphatase) in the absence or presence of SH2B1. PTP1B dephosphorylated IRS-1, and SH2B1 dose-dependently attenuated the ability of PTP1B to dephosphorylate IRS-1 (Fig. 3.5C). To determine whether SH2B1 is able to promote IRS-1 phosphorylation without stimulating IR kinase activity, Y1158F was coexpressed with SH2B1. Although SH2B1 was unable to stimulate Y1158F kinase activity (Fig. 3.4E), SH2B1 still markedly enhanced tyrosine phosphorylation of IRS-1 in Y1158F-expressing cells (Fig. 3.5D). Thus, SH2B1 is likely to augment Y1158F-

mediated phosphorylation of IRS-1 by inhibiting IRS-1 dephosphorylation by endogenous protein phosphatase(s).

To determine whether the SH2B1-IRS interaction sterically inhibits the binding of IRS proteins to PI 3-kinase, IRS-1 and Y1158F were coexpressed with or without SH2B1 in HEK293 cells, and IRS-1-p85 association was examined by co-immunoprecipitation assays. Insulin stimulated coimmunoprecipitation of IRS-1 with p85; importantly, SH2B1 markedly enhanced insulin-stimulated p85 binding to IRS-1 (Fig. 3.5E). These data indicate that the SH2B1-IRS interaction does not interfere with IRS-PI 3-kinase interaction, but rather increases the IRS-PI 3-kinase association by inhibiting IRS dephosphorylation. Consistent with these observations, SH2B1 also enhanced insulin-stimulated, Y1158F-mediated Akt phosphorylation (Fig. 3.5F). Collectively, these data suggest that, in addition to enhancing IR catalytic activity via binding to Tyr1158, SH2B1 also promotes activation of the IRS protein/PI 3-kinase/Akt pathway by inhibiting IRS dephosphorylation.

## Discussion

Insulin resistance is the primary risk factor for various metabolic diseases, including type 2 diabetes, non-alcoholic fatty liver disease, dyslipidemia and cardiovascular disease. The molecular mechanisms underlying insulin resistance are extremely complex and not completely understood. It is commonly accepted that impairments in insulin signal transduction play a key role in the development of insulin resistance. We previously observed that insulin signaling is enhanced by SH2B1 (8). SH2B1 overexpression increases IR autophosphorylation and tyrosine phosphorylation of IRS-1 and IRS-2 in cultured cells (8, 16). Similar observations were independently reported by two other groups (2, 46). Additionally, we showed that genetic deletion of SH2B1 results in severe insulin resistance and type 2 diabetes in mice (8). However, SH2B1 null mice are also severely obese due to leptin resistance (17, 22, 23), raising the possibility that insulin resistance may be secondary to obesity in SH2B1 null mice. Therefore, it was unclear whether peripheral SH2B1 directly regulates insulin sensitivity in insulin target tissues in vivo.

We generated TgKO mice that express SH2B1 only in the brain but not in peripheral tissues (e.g. liver, muscle and adipose tissue). Body weight was similar between TgKO and wild type littermates fed either normal chow or HFD, consistent with our previous conclusion that neuronal SH2B1 controls energy balance and body weight by promoting leptin sensitivity (23). In the current study, we demonstrated that loss of peripheral SH2B1 markedly impaired insulin sensitivity independent of body weight. TgKO mice developed hyperglycemia, hyperinsulinemia and glucose intolerance to a greater extent than wild type littermates fed HFD. The ability of exogenous insulin to reduce blood glucose and to stimulate IR autophosphorylation and phosphorylation of IRS proteins and Akt in muscle, liver, and adipose tissue was significantly reduced in TgKO mice. These results suggest that peripheral SH2B1 serves as an endogenous insulin sensitizer. Insulin signaling has been shown to be attenuated by multiple intracellular signaling molecules (e.g. PTP1B, Grb10, Grb14, SOCS1, SOCS3, JNK, PKCθ, S6K and IKKβ) which contribute to the development of insulin resistance (1, 4, 6, 9-11, 13, 14, 25, 27-29, 33-35, 37, 40, 44, 47). Our data suggest that insulin sensitivity is

controlled by a balance between these negative regulators and SH2B1 in insulin target cells.

SH2B1 promotes insulin signaling by stimulating IR catalytic activity. SH2B1 binds via its SH2 domain to phospho-Tyr$^{1158}$ in the activation loop of IR (15, 20). We showed that bacteria-derived SH2B1 markedly increased the ability of purified IR to tyrosyl phosphorylate IRS-1 in vitro. In contrast, SH2B1 was unable to stimulate the catalytic activity of Y1158F, an IR mutant lacking the binding site for SH2B1. In cells, SH2B1 overexpression promotes IR autophosphorylation as well as IR phosphorylation of its substrates (e.g. IRS-1, IRS-2 and Shc). Conversely, deletion of endogenous SH2B1 impaired insulin stimulation of IR autophosphorylation and IRS-1 phosphorylation in primary hepatocyte cultures. Consistent with these observations, SH2B1 complexes, which are immunoprecipitated from cell extracts, reportedly promote IR autophosphorylation by reducing the $K_m$ for ATP (46). The same report also concluded that SH2B1 dimerization was required for its stimulation of IR autophosphorylation, because treatment of cells with dimerization domain peptide mimetics inhibited IR autophosphorylation and downstream pathways (46). However, the report did not provide evidence showing that the mimetics disrupted SH2B1 dimerization. In contrast, we observed that the SH2 domain alone was sufficient to stimulate IR catalytic activity in vitro. Moreover, ΔN504, a N-terminal truncated SH2B1 containing the intact SH2 domain but completely lacking both dimerization and PH domains, still markedly enhanced insulin-stimulated tyrosine phosphorylation of both IRS-1 and Shc. Conversely, R555E, a SH2B1 mutant with a defective SH2 domain, inhibited insulin signaling as a dominant negative mutant. These data indicate that the SH2 domain of SH2B1 is both required and sufficient to stimulate IR kinase activity. Because Tyr$^{1158}$ phosphorylation occurs early in the activation of the insulin receptor kinase (39, 41, 45), binding of the SH2 domain of SH2B1 to phospho-Tyr$^{1158}$ may stabilize IR in an active conformation. Alternatively, SH2B1-IR interaction may facilitate IR binding to its substrates.

Insulin stimulated the binding of SH2B1 to IRS-1 or IRS-2. Importantly, SH2B1 directly inhibited tyrosine dephosphorylation of IRS-1 and IRS-2 by recombinant phosphatase *in vitro* and by PTP1B in cultured cells. Although unable to stimulate

Y1158F catalytic activity, SH2B1 still enhanced Y1158F-mediated phosphorylation of IRS-1 in cultured cells, presumably by inhibiting IRS-1 dephosphorylation by endogenous tyrosine phosphatase(s). Consistent with these observations, deletion of endogenous SH2B1 impaired tyrosine phosphorylation of IRS-1 to a greater extent than IR autophosphorylation in primary hepatocyte cultures. Together, these data suggest that the SH2B1-IRS physical interaction inhibits IRS dephosphorylation by tyrosine phosphatases. Interestingly, the SH2B1-IRS interaction did not inhibit the ability of phosphorylated IRS proteins to bind to p85, the regulatory subunit of PI 3-kinase; in contrast, it enhanced insulin-stimulated IRS-p85 association and subsequent Akt phosphorylation and activation, presumably by protecting IRS proteins against dephosphorylation. These data suggest that SH2B1 does not compete with p85 for the same binding sites in IRS proteins, and that the SH2B1-IRS interaction does not sterically interfere with the IRS-p85 interaction. Therefore, the SH2B1-IRS interaction may selectively block IRS interaction with tyrosine phosphatases, thereby inhibiting IRS dephosphorylation. Alternatively, the SH2B1-IRS interaction may alter IRS conformation so that multiple tyrosine phosphorylation sites, in addition to SH2B1-bound site(s), are resistant to dephosphorylation, but still retain their ability to bind to downstream signaling molecules and activate downstream pathways including the PI 3-kinase/Akt pathway.

In conclusion, SH2B1 appears to promote insulin sensitivity in animals by multiple mechanisms (Fig. 3.6). Neuronal SH2B1 increases insulin sensitivity indirectly by reducing adiposity (23). In muscle, liver and adipose tissue, SH2B1 binds to IR and stimulates IR catalytic activity to globally activate pathways downstream of IR. SH2B1 binds to both IRS-1 and IRS-2 and protects IRS proteins from tyrosine dephosphorylation, augmenting and/or prolonging IRS protein-mediated pathways. In addition, SH2B1 forms dimers, and each SH2B1 molecule in a SH2B1 dimer may simultaneously bind to IR and IRS-1 (or IRS-2), thereby stabilizing IR/IRS-1 (or IR/IRS-2) complexes. Therefore, SH2B1 and molecules that mimic these functions of SH2B1 are potential therapeutic targets for the treatment of obesity and/or type 2 diabetes.

**Acknowledgements**

This study was supported by National Institute of Health grants RO1 DK 065122, RO1 DK073601 (to Liangyou Rui) and F31NS056575 (to David L. Morris), and by American Diabetes Association award 1-09-RA-156 (to Liangyou Rui). This work utilized the cores supported by the Michigan Diabetes Research and Training Center (funded by NIH 5P60 DK20572), the University of Michigan's Cancer Center (funded by NIH 5 P30 CA46592), the University of Michigan Nathan Shock Center (funded by NIH P30AG013283), and the University of Michigan Gut Peptide Research Center (funded by NIH DK34933). We thank Dr. Xiao Jian Sun (University of Chicago) for providing GST-IRS-1 plasmids and Dr. Liang Sheng for his assistance in primary hepatocyte cultures.

This chapter was published in *Diabetes* under the title "SH2B1 Enhances Insulin Sensitivity by Both Stimulating the Insulin Receptor and Inhibiting Tyrosine Dephosphorylation of IRS Proteins", by David L. Morris, Kae Won Cho, Yingjiang Zhou, and Liangyou Rui. Dr. Kae Won Cho and Yingjiang Zhou raised the mice used in this study and contributed data for Fig. 3.1. Dr. Yingjiang Zhou assisted in isolating primary hepatocytes for Fig. 3.3A.

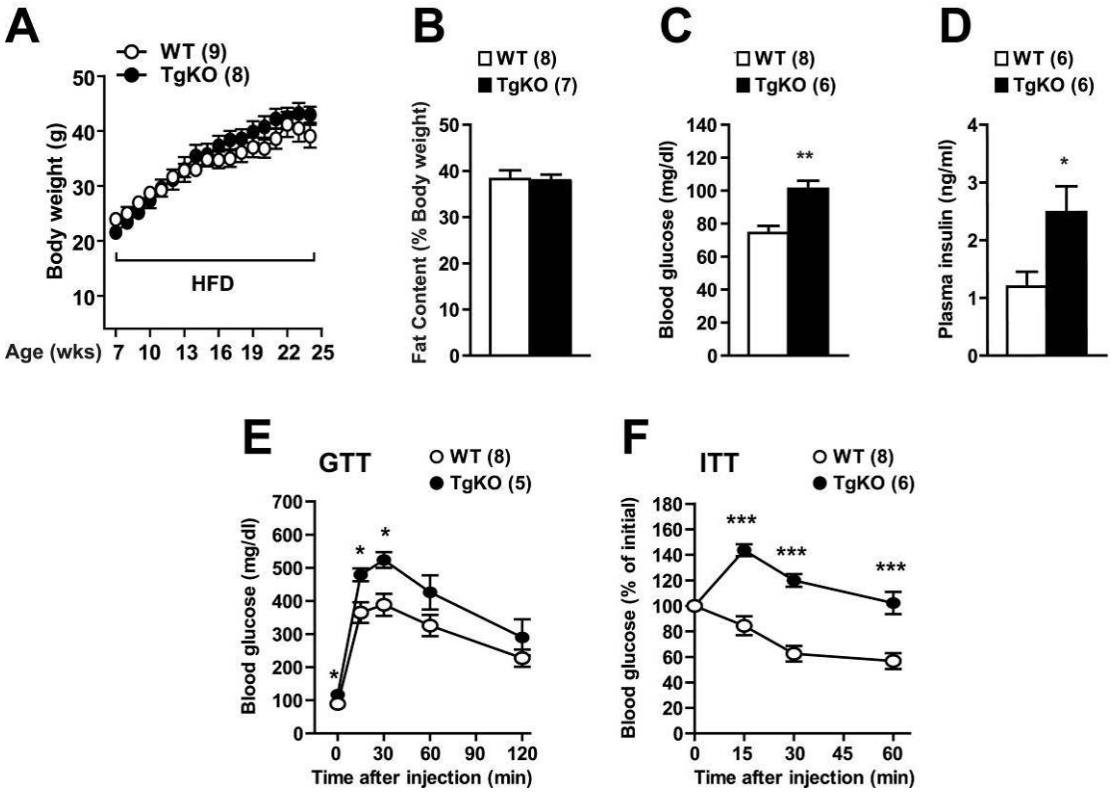


**Fig. 3.1. Peripheral SH2B1 enhances insulin sensitivity in mice.** (A-F) WT and TgKO male mice (7 wks) were fed a HFD. (A) Growth curve. (B) Body fat content after 16-wks on HFD. (C) Fasting (16-h) blood glucose levels and (D) plasma insulin levels after 16-wks on HFD. (E) Glucose tolerance tests (GTT) performed on mice fed HFD for 16-wks. (F) Insulin tolerance tests (ITT) performed on mice fed HFD for 16-wks. The number of mice is indicated in parenthesis. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

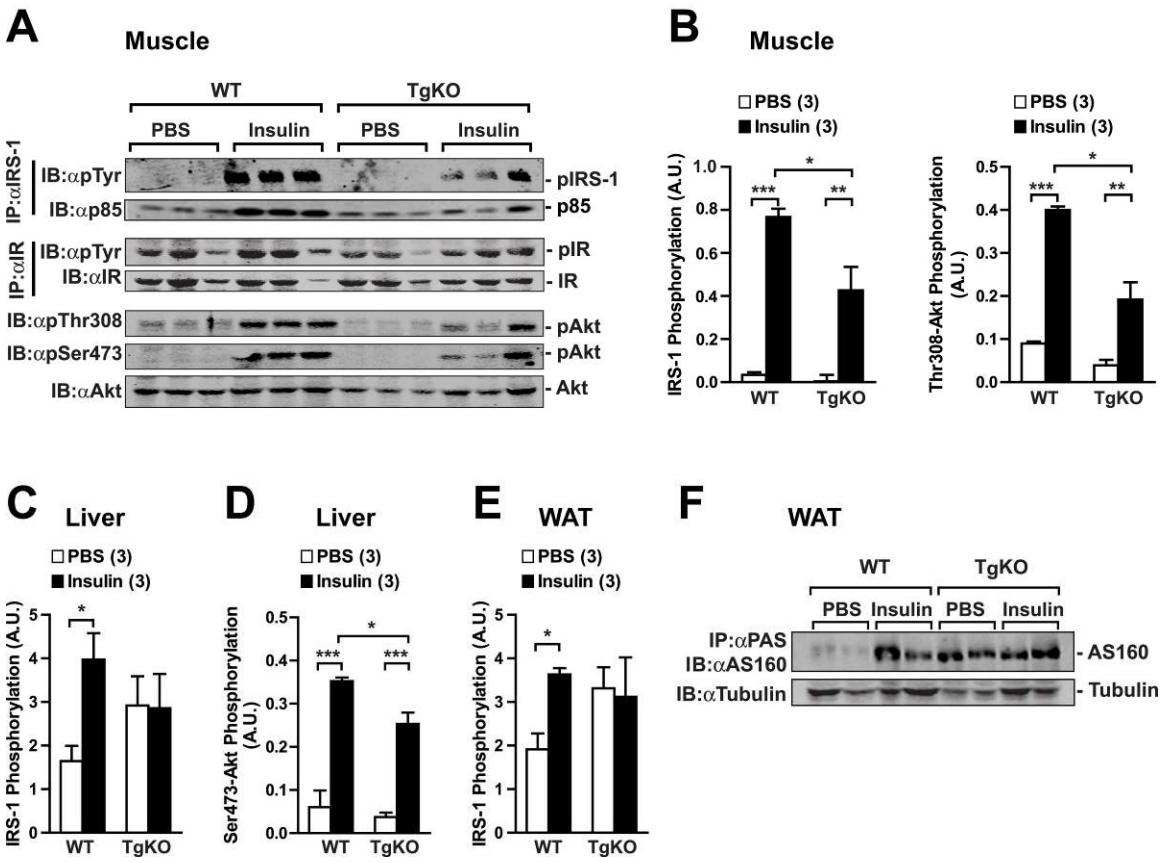


**Fig. 3.2. Deletion of peripheral SH2B1 attenuates insulin signaling in mice.** WT and TgKO males (7 wks) were fed a HFD for 16-wks. Mice were fasted for 16-h and treated with PBS or insulin (3 U per mouse). Tissue extracts were prepared 5 min after stimulation. (A) IRS-1 in muscle extracts was immunoprecipitated (IP) with anti-IRS-1 antibody (αIRS-1) and immunoblotted (IB) with anti-phosphotyrosine (αpTyr) and αp85 antibodies. IR in muscle extracts was immunoprecipitated with αIR and immunoblotted with αpTyr. Muscle extracts were immunoblotted with phospho-specific Akt antibodies against phospho-$Thr^{308}$ (αpThr308) or phospho-$Ser^{473}$ (αpSer473) and αAkt, respectively. (B) IRS-1 and Akt phosphorylation in (A) was quantified by densitometry and normalized to total IRS-1 and Akt protein levels, respectively. (C) Liver extracts were immunoprecipitated with αIRS-1 and immunoblotted with αpTyr. The same blots were reprobed with αIRS-1. IRS-1 phosphorylation was quantified and normalized to total IRS-1 protein levels. (D) Liver extracts were immunoblotted with αpSer473 and αAkt. $Ser^{473}$ phosphorylation was quantified and normalized to total Akt protein levels. (E) Epididymal fat (WAT) extracts were immunoprecipitated with αIRS-1 and immunoblotted with αpTyr and reprobed with αIRS-1. IRS-1 phosphorylation was normalized to total IRS-1 protein levels. (F) WAT extracts were immunoprecipitated with αPAS (anti-phospho-Ser/Thr Akt substrate) and immunoblotted with αAS160. Extracts were also immunoblotted with αtubulin. Three animals were examined for each condition. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

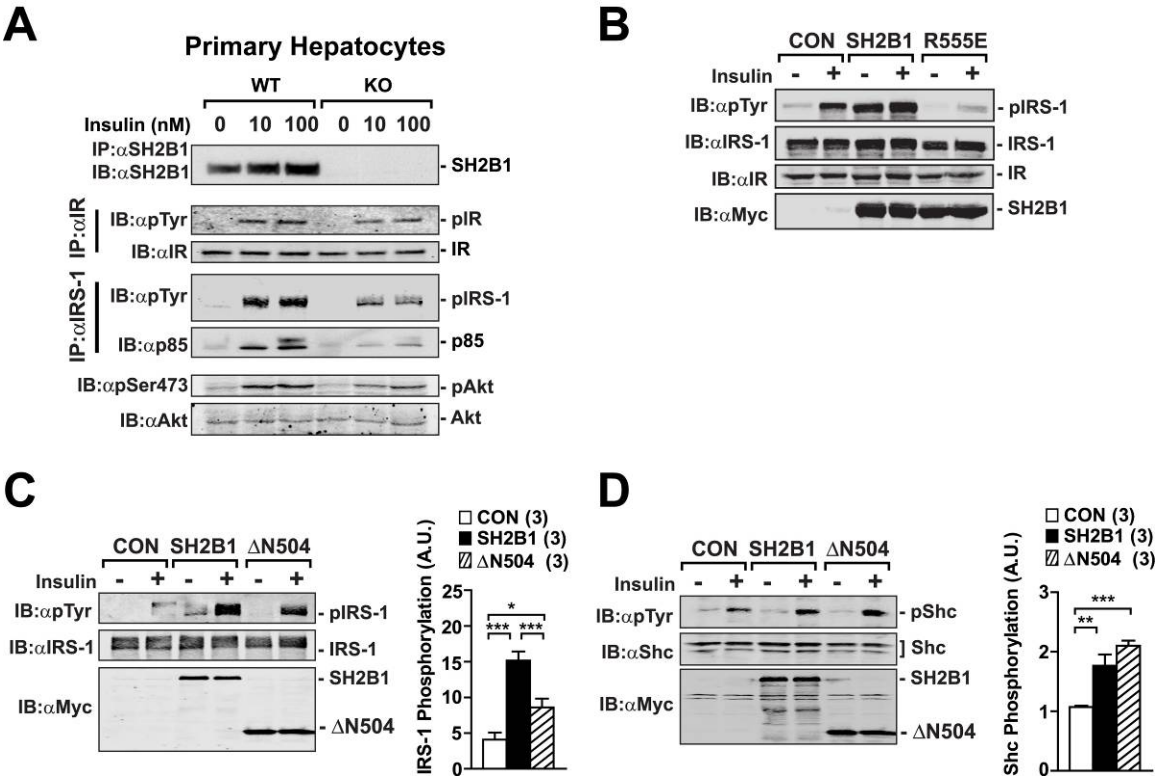


**Fig. 3.3. SH2B1 directly promotes insulin signaling in cells via its SH2 domain.** (A) Primary hepatocyte cultures were prepared from WT or KO mice (8 wks) and treated with 100 nM insulin for 10 min. Panel 1: cell extracts were immunoprecipitated with αSH2B1 and immunoblotted with αSH2B1. Panels 2-3: cell extracts were immunoprecipitated with αIR and immunoblotted with αpTyr and αIR. Panels 4-5: cell extracts were immunoprecipitated with αIRS-1 and immunoblotted with αpTyr or αp85. Panels 6-7: cells extracts were immunoblotted with αpSer473 and αAkt. (B) IRS-1 and IR were transiently coexpressed with empty vector (CON), Myc-tagged SH2B1 or R555E plasmids in HEK293 cells. Cells were treated with 100 nM insulin for 10 min and extracts were immunoblotted with indicated antibodies. (C) IRS-1 and IR were transiently coexpressed with empty vector (CON), Myc-tagged SH2B1 or ΔN504 plasmids in HEK293 cells. Cells were treated with 100 nM insulin for 10 min and extracts were immunoblotted with indicated antibodies. IRS-1 phosphorylation was normalized to total IRS-1 protein levels. (D) Shc and IR were transiently coexpressed with empty vector (CON), Myc-tagged SH2B1 or ΔN504 plasmids in HEK293 cells. Cells were treated with 100 nM insulin for 10 min and extracts were immunoblotted with indicated antibodies. Shc phosphorylation was normalized to total Shc protein levels. * P<0.05, ** P<0.01, *** P<0.001.

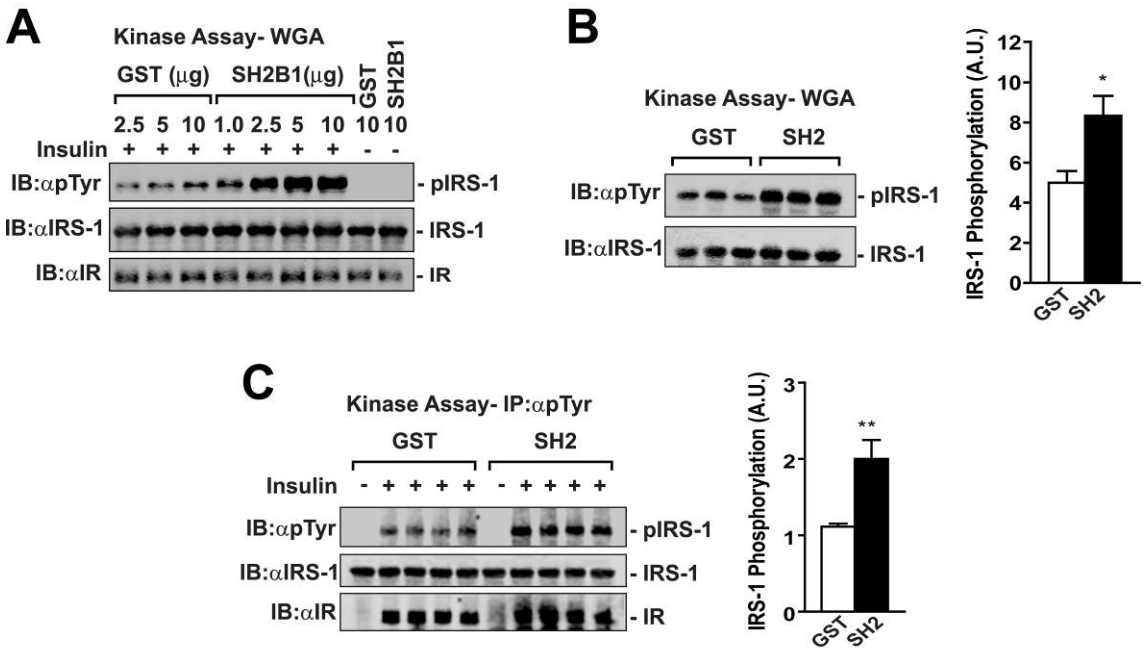


**Fig. 3.4A-C. SH2B1 directly enhances insulin receptor activity in vitro.** (A) CHO$^{IR}$ cells were treated without or with 100 nM insulin for 10 min. IR was purified with WGA-agarose beads, preincubated with indicated amounts of GST or GST-SH2B1 fusion protein, and subjected to in vitro kinase assays with GST-IRS-1 as substrate for 10 min. Reaction mixtures were immunoblotted with αpTyr, αIRS-1 or αIR. (B) WGA-purified IR was preincubated with GST or GST-SH2 fusion protein (5 μg) and subjected to in vitro kinase assays with GST-IRS-1 protein (amino acids 526-859 of rat IRS-1) as substrate for 10 min. IRS-1 phosphorylation was quantified by densitometry and normalized to total GST-IRS-1 levels. (C) CHO$^{IR}$ cells were treated without or with 100 nM insulin for 10 min. Cell extracts were immunoprecipitated with αpTyr. αpTyr-immunopurified IR was preincubated with GST or GST-SH2 fusion protein (amino acids 524-670) (5 μg), and subjected to in vitro kinase assays with GST-IRS-1 as substrate for 10 min. Reaction mixtures were immunoblotted with indicated antibodies and IRS-1 phosphorylation was quantified and normalized to total GST-IRS-1 levels. * $P<0.05$, ** $P<0.01$.

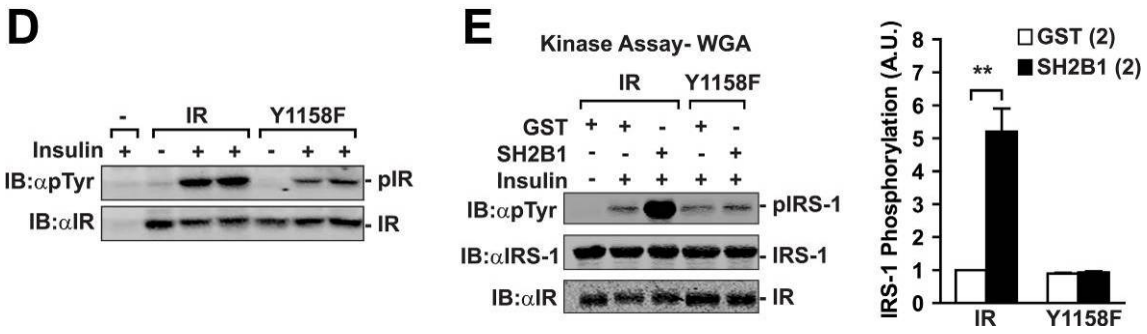


**Fig. 3.4D-E. SH2B1 directly enhances insulin receptor activity in vitro.** (D) WT or Y1158F was expressed in COS-7 cells. Cells were treated with 100 nM insulin for 10 min. Cell extracts were immunoblotted with αpTyr or αIR. (E) WT or Y1158F was expressed in COS-7 cells and treated with insulin. WT or Y1158F was purified with WGA-agarose beads, preincubated with GST or GST-SH2B1 fusion protein (5 μg), and subjected to in vitro kinase assays with GST-IRS-1 as substrate for 10 min. GST-IRS-1 phosphorylation was normalized to total GST-IRS-1 protein levels. * $P<0.05$, ** $P<0.01$.

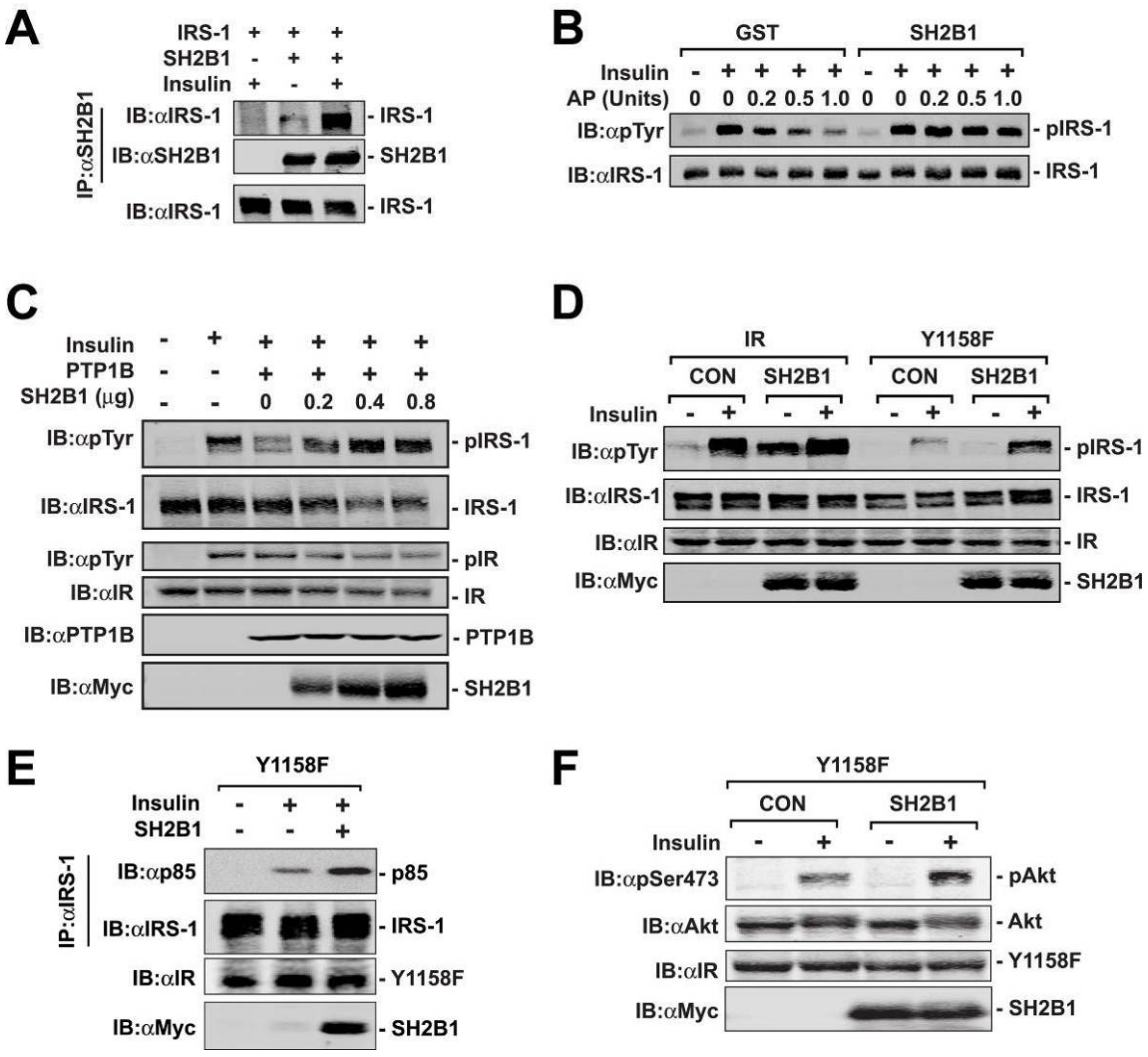


**Fig. 3.5. SH2B1 protects IRS-1 from dephosphorylation.** (A) IRS-1 and SH2B1 were transiently expressed in HEK293 cells. Cell extracts were immunoprecipitated with αSH2B1 and immunoblotted with αIRS-1 and αSH2B1. (B) $CHO^{IR/IRS-1}$ cells were stimulated with 100 nM insulin for 10 min. IRS-1 was immunoprecipitated with αIRS-1, preincubated with GST or GST-SH2B1 (2 μg), and subjected to in vitro dephosphorylation assays with indicated amounts of alkaline phosphatase for 30 min. Reaction mixtures were immunoblotted with αpTyr and αIRS-1. (C) IR and IRS-1 were transiently expressed with PTP1B (0.1 μg) and increasing amounts of Myc-tagged SH2B1 (0-0.8 μg). Cells were treated with 100 nM insulin for 10 min and extracts were immunoblotted with indicated antibodies. (D) IRS-1 was expressed with IR or Y1158F in the absence or presence of SH2B1 in HEK293 cells. Cells were treated with 100 nM insulin for 10 min and extracts were immunoblotted with indicated antibodies. (E) IRS-1 (1 μg) and Y1158F (1 μg) plasmids were transiently cotransfected with or without SH2B1 plasmids (0.8 μg) in HEK293 cells. Cells were deprived of serum overnight 48 h after transfection, and treated with 100 nM insulin for 10 min. Cell extracts were immunoprecipitated with αIRS-1 and immunoblotted with αp85 and αIRS-1. Extracts were also immunoblotted with indicated antibodies. (F) IRS-1 and Y1158F were coexpressed with or without SH2B1. Cells were treated with 100 nM insulin for 10 min and extracts were immunoblotted with indicated antibodies.

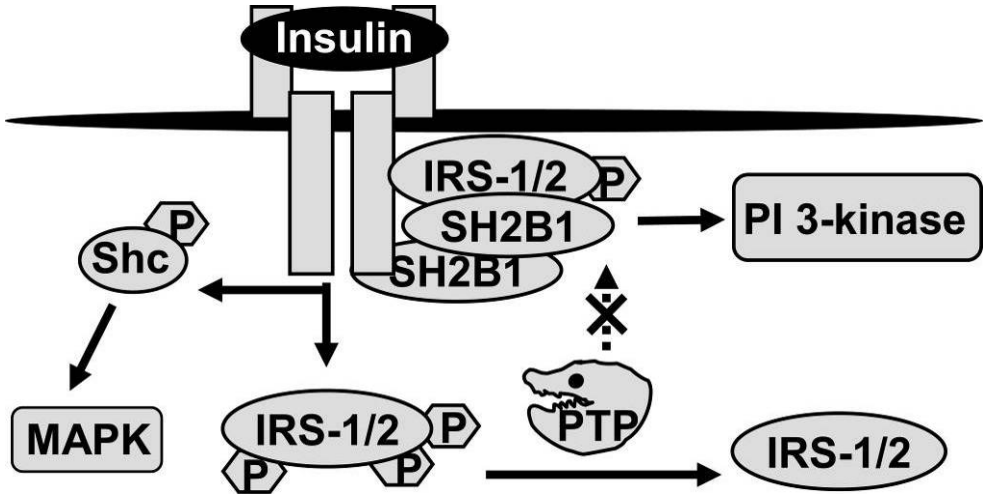


**Fig. 3.6. A model for SH2B1 regulation of insulin signaling.** In response to insulin, SH2B1 binds directly to phospho-Tyr$^{1158}$ in IR via its SH2 domain and stimulates IR kinase activity, thereby enhancing the activation of multiple signaling pathways downstream of IR (e.g. the Shc/MAPK and the IRS/PI 3-kinase pathways). SH2B1 also binds to IRS-1 or IRS-2 and inhibits their dephosphorylation on tyrosines to specifically promote the activation of IRS protein-mediated pathways. Since SH2B1 dimerizes via its DD domain, dimerized SH2B1 may further enhance insulin signaling by simultaneously binding to both IR and IRS-1 to stabilize active IR with IRS-1 or recruit IRS-1 to IR. PTP: protein tyrosine phosphatase; P: phosphate group.

## References


1. **Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, and White MF.** Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *J Biol Chem* 277: 1531-1537, 2002.

2. **Ahmed Z and Pillay TS.** Adapter protein with a pleckstrin homology (PH) and an Src homology 2 (SH2) domain (APS) and SH2-B enhance insulin-receptor autophosphorylation, extracellular-signal-regulated kinase and phosphoinositide 3-kinase-dependent signalling. *Biochem J* 371: 405-412, 2003.

3. **Araki E, Lipes MA, Patti ME, Bruning JC, Haag B, 3rd, Johnson RS, and Kahn CR.** Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* 372: 186-190, 1994.

4. **Bereziat V, Kasus-Jacobi A, Perdereau D, Cariou B, Girard J, and Burnol AF.** Inhibition of insulin receptor catalytic activity by the molecular adapter Grb14. *J Biol Chem* 277: 4845-4852, 2002.

5. **Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB, 3rd, Kaestner KH, Bartolomei MS, Shulman GI, and Birnbaum MJ.** Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science* 292: 1728-1731, 2001.

6. **Cooney GJ, Lyons RJ, Crew AJ, Jensen TE, Molero JC, Mitchell CJ, Biden TJ, Ormandy CJ, James DE, and Daly RJ.** Improved glucose homeostasis and enhanced insulin signalling in Grb14-deficient mice. *EMBO J* 23: 582-593, 2004.

7. **Duan C, Li M, and Rui L.** SH2-B promotes insulin receptor substrate 1 (IRS1)- and IRS2-mediated activation of the phosphatidylinositol 3-kinase pathway in response to leptin. *J Biol Chem* 279: 43684-43691, 2004.

8. **Duan C, Yang H, White MF, and Rui L.** Disruption of the SH2-B gene causes age-dependent insulin resistance and glucose intolerance. *Mol Cell Biol* 24: 7435-7443, 2004.

9. **Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, and Kennedy BP.** Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 283: 1544-1548, 1999.

10. **Goldstein BJ, Bittner-Kowalczyk A, White MF, and Harbeck M.** Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine

phosphatase 1B. Possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. *J Biol Chem* 275: 4283-4289, 2000.

11. **Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, and Hotamisligil GS.** A central role for JNK in obesity and insulin resistance. *Nature* 420: 333-336, 2002.

12. **Kane S, Sano H, Liu SC, Asara JM, Lane WS, Garner CC, and Lienhard GE.** A method to identify serine kinase substrates. Akt phosphorylates a novel adipocyte protein with a Rab GTPase-activating protein (GAP) domain. *J Biol Chem* 277: 22115-22118, 2002.

13. **Kim JK, Fillmore JJ, Sunshine MJ, Albrecht B, Higashimori T, Kim DW, Liu ZX, Soos TJ, Cline GW, O'Brien WR, Littman DR, and Shulman GI.** PKC-theta knockout mice are protected from fat-induced insulin resistance. *J Clin Invest* 114: 823-827, 2004.

14. **Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Sharpe AH, Stricker-Krongrad A, Shulman GI, Neel BG, and Kahn BB.** Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol Cell Biol* 20: 5479-5489, 2000.

15. **Kotani K, Wilden P, and Pillay TS.** SH2-Balpha is an insulin-receptor adapter protein and substrate that interacts with the activation loop of the insulin-receptor kinase. *Biochem J* 335 ( Pt 1): 103-109, 1998.

16. **Li M, Li Z, Morris DL, and Rui L.** Identification of SH2B2beta as an inhibitor for SH2B1- and SH2B2alpha-promoted Janus kinase-2 activation and insulin signaling. *Endocrinology* 148: 1615-1621, 2007.

17. **Li M, Ren D, Iseki M, Takaki S, and Rui L.** Differential role of SH2-B and APS in regulating energy and glucose homeostasis. *Endocrinology* 147: 2163-2170, 2006.

18. **Maures TJ, Kurzer JH, and Carter-Su C.** SH2B1 (SH2-B) and JAK2: a multifunctional adaptor protein and kinase made for each other. *Trends Endocrinol Metab* 18: 38-45, 2007.

19. **Minami A, Iseki M, Kishi K, Wang M, Ogura M, Furukawa N, Hayashi S, Yamada M, Obata T, Takeshita Y, Nakaya Y, Bando Y, Izumi K, Moodie SA, Kajiura F, Matsumoto M, Takatsu K, Takaki S, and Ebina Y.** Increased insulin sensitivity and hypoinsulinemia in APS knockout mice. *Diabetes* 52: 2657-2665, 2003.

20. **Nelms K, O'Neill TJ, Li S, Hubbard SR, Gustafson TA, and Paul WE.** Alternative splicing, gene localization, and binding of SH2-B to the insulin receptor kinase domain. *Mamm Genome* 10: 1160-1167, 1999.

21. **Nishi M, Werner ED, Oh BC, Frantz JD, Dhe-Paganon S, Hansen L, Lee J, and Shoelson SE.** Kinase activation through dimerization by human SH2-B. *Mol Cell Biol* 25: 2607-2621, 2005.

22. **Ren D, Li M, Duan C, and Rui L.** Identification of SH2-B as a key regulator of leptin sensitivity, energy balance, and body weight in mice. *Cell Metabolism* 2: 95-104, 2005.

23. **Ren D, Zhou Y, Morris D, Li M, Li Z, and Rui L.** Neuronal SH2B1 is essential for controlling energy and glucose homeostasis. *J Clin Invest* 117: 397-406, 2007.

24. **Renstrom F, Payne F, Nordstrom A, Brito EC, Rolandsson O, Hallmans G, Barroso I, Nordstrom P, and Franks PW.** Replication and extension of genome-wide association study results for obesity in 4923 adults from northern Sweden. *Hum Mol Genet* 18: 1489-1496, 2009.

25. **Rui L, Yuan M, Frantz D, Shoelson S, and White MF.** SOCS-1 and SOCS-3 Block Insulin Signaling by Ubiquitin-mediated Degradation of IRS1 and IRS2. *J Biol Chem* 277: 42394-42398, 2002.

26. **Sano H, Kane S, Sano E, Miinea CP, Asara JM, Lane WS, Garner CW, and Lienhard GE.** Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. *J Biol Chem* 278: 14599-14602, 2003.

27. **Schenk S, Saberi M, and Olefsky JM.** Insulin sensitivity: modulation by nutrients and inflammation. *J Clin Invest* 118: 2992-3002, 2008.

28. **Shi H, Tzameli I, Bjorbaek C, and Flier JS.** Suppressor of cytokine signaling 3 is a physiological regulator of adipocyte insulin signaling. *J Biol Chem* 279: 34733-34740, 2004.

29. **Smith FM, Holt LJ, Garfield AS, Charalambous M, Koumanov F, Perry M, Bazzani R, Sheardown SA, Hegarty BD, Lyons RJ, Cooney GJ, Daly RJ, and Ward A.** Mice with a disruption of the imprinted Grb10 gene exhibit altered body composition, glucose homeostasis, and insulin signaling during postnatal life. *Mol Cell Biol* 27: 5871-5886, 2007.

30. **Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y, Satoh S, and et al.** Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* 372: 182-186, 1994.

31. **Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadottir A, Styrkarsdottir U, Gretarsdottir S, Thorlacius S, Jonsdottir I, Jonsdottir T, Olafsdottir EJ, Olafsdottir GH, Jonsson T, Jonsson F, Borch-Johnsen K, Hansen T, Andersen G, Jorgensen T, Lauritzen T, Aben KK, Verbeek AL, Roeleveld N, Kampman E, Yanek LR, Becker LC, Tryggvadottir L, Rafnar T, Becker DM, Gulcher J, Kiemeney LA, Pedersen O, Kong A, Thorsteinsdottir U, and Stefansson K.** Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet* 41: 18-24, 2009.

32. **Tong W, Zhang J, and Lodish HF.** Lnk inhibits erythropoiesis and Epo-dependent JAK2 activation and downstream signaling pathways. *Blood* 105: 4604-4612, 2005.

33. **Ueki K, Kondo T, and Kahn CR.** Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol Cell Biol* 24: 5434-5446, 2004.

34. **Ueki K, Kondo T, Tseng YH, and Kahn CR.** Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. *Proc Natl Acad Sci U S A* 101: 10422-10427, 2004.

35. **Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J, and Thomas G.** Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 431: 200-205, 2004.

36. **Velazquez L, Cheng AM, Fleming HE, Furlonger C, Vesely S, Bernstein A, Paige CJ, and Pawson T.** Cytokine signaling and hematopoietic homeostasis are disrupted in Lnk-deficient mice. *J Exp Med* 195: 1599-1611, 2002.

37. **Wang L, Balas B, Christ-Roberts CY, Kim RY, Ramos FJ, Kikani CK, Li C, Deng C, Reyna S, Musi N, Dong LQ, DeFronzo RA, and Liu F.** Peripheral disruption of the Grb10 gene enhances insulin signaling and sensitivity in vivo. *Mol Cell Biol* 27: 6497-6505, 2007.

38. **White MF.** IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab* 283: E413-422, 2002.

39. **White MF, Shoelson SE, Keutmann H, and Kahn CR.** A cascade of tyrosine autophosphorylation in the beta-subunit activates the phosphotransferase of the insulin receptor. *J Biol Chem* 263: 2969-2980, 1988.

40. **Wick KR, Werner ED, Langlais P, Ramos FJ, Dong LQ, Shoelson SE, and Liu F.** Grb10 inhibits insulin-stimulated insulin receptor substrate (IRS)-phosphatidylinositol 3-kinase/Akt signaling pathway by disrupting the association of IRS-1/IRS-2 with the insulin receptor. *J Biol Chem* 278: 8460-8467, 2003.

41. **Wilden PA, Kahn CR, Siddle K, and White MF.** Insulin receptor kinase domain autophosphorylation regulates receptor enzymatic function. *J Biol Chem* 267: 16660-16668, 1992.

42. **Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, Berndt SI, Elliott AL, Jackson AU, Lamina C, Lettre G, Lim N, Lyon HN, McCarroll SA, Papadakis K, Qi L, Randall JC, Roccasecca RM, Sanna S, Scheet P, Weedon MN, Wheeler E, Zhao JH, Jacobs LC, Prokopenko I, Soranzo N, Tanaka T, Timpson NJ, Almgren P, Bennett A, Bergman RN, Bingham SA, Bonnycastle LL, Brown M, Burtt NP, Chines P, Coin L, Collins FS, Connell JM, Cooper C, Smith GD, Dennison EM, Deodhar P, Elliott P, Erdos MR, Estrada K, Evans DM, Gianniny L, Gieger C, Gillson CJ, Guiducci C, Hackett R, Hadley D, Hall AS, Havulinna AS, Hebebrand J, Hofman A, Isomaa B, Jacobs KB, Johnson T, Jousilahti P, Jovanovic Z, Khaw KT, Kraft P, Kuokkanen M, Kuusisto J, Laitinen J, Lakatta EG, Luan J, Luben RN, Mangino M, McArdle WL, Meitinger T, Mulas A, Munroe PB, Narisu N, Ness AR, Northstone K, O'Rahilly S, Purmann C, Rees MG, Ridderstrale M, Ring SM, Rivadeneira F, Ruokonen A, Sandhu MS, Saramies J, Scott LJ, Scuteri A, Silander K, Sims MA, Song K, Stephens J, Stevens S, Stringham HM, Tung YC, Valle TT, Van Duijn CM, Vimaleswaran KS, Vollenweider P, et al.** Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 41: 25-34, 2009.

43. **Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, and White MF.** Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391: 900-904, 1998.

44. **Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, and Shoelson SE.** Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293: 1673-1677, 2001.

45. **Zhang B, Tavare JM, Ellis L, and Roth RA.** The regulatory role of known tyrosine autophosphorylation sites of the insulin receptor kinase domain. An assessment by replacement with neutral and negatively charged amino acids. *J Biol Chem* 266: 990-996, 1991.

46. **Zhang M, Deng Y, Tandon R, Bai C, and Riedel H.** Essential role of PSM/SH2-B variants in insulin receptor catalytic activation and the resulting cellular responses. *J Cell Biochem* 103: 162-181, 2008.

47. **Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, Xie N, Wilcox D, Jacobson P, Frost L, Kroeger PE, Reilly RM, Koterski S, Opgenorth TJ, Ulrich RG, Crosby S, Butler M, Murray SF, McKay RA, Bhanot S, Monia BP, and Jirousek MR.** PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. *Proc Natl Acad Sci U S A* 99: 11357-11362, 2002.

# Chapter 4

## SH2B1 Supports β-cell Function Independent Of Central Leptin Action


### Abstract

Insulin resistance and β-cell dysfunction are determinants for diabetes. Systemic disruption of the *Sh2b1* gene in mice results in obesity and diabetes. Neuronal SH2B1 promotes peripheral insulin sensitivity by regulating leptin sensitivity, energy balance, and adiposity. However, SH2B1 is expressed in insulin-target tissues and in the pancreas, raising the possibility that SH2B1 in these tissues may contribute to insulin sensitivity and/or β-cell function to regulate glucose metabolism. To test these possibilities, we examined glucose metabolism, glucose tolerance and insulin sensitivity in two mouse models. *Sh2b1* haploinsufficiency exacerbated the diabetic phenotype in leptin-deficient, *ob/ob* mice. Insulin resistance was similar in *Sh2b1*$^{+/-}$*ob/ob* and *ob/ob* mice. However, *Sh2b1*$^{+/-}$*ob/ob* were severely hyperglycemic due to β-cell dysfunction. Pancreatic insulin content, but not pancreas mass or β-cell area, was dramatically reduced in *Sh2b1*$^{+/-}$*ob/ob* mice, which likely contributes glucose intolerance and decreased postprandial insulin levels in *Sh2b1*$^{+/-}$*ob/ob* mice. We generated pancreas-specific SH2B1-knockout (P-KO) mice to further examine the role of SH2B1 in the pancreas. P-KO mice were fed a high-fat diet (HFD) to induce peripheral insulin resistance. However, disruption of SH2B1 in the pancreas did not alter glucose tolerance after 10 weeks of diet-induced obesity. Collectively, these data suggest that SH2B1 has an important leptin-independent role in the regulation of glucose homeostasis. In the pancreas of severely obese mice, SH2B1 supports β-cell function, possibly by protecting β-cells from oxidative stress.

## Introduction

Impaired insulin action in peripheral tissues (insulin resistance) and insufficient insulin secretion (β-cell dysfunction) contribute to the development of impaired glucose tolerance and hyperglycemia, hallmarks of type 2 diabetes. Insulin resistance is a driving force for the development of β-cell dysfunction. In the prediabetic state, β-cells initially compensate for insulin resistance by increasing both insulin production and secretion (13, 16, 17, 33). However, the compensatory capacity of the β-cell is limited and β-cells eventually fail to secrete adequate amounts of insulin, resulting in the development of type 2 diabetes.

Multiple growth factors, including insulin, insulin-like growth factor-1 (IGF-1), growth hormone (GH), and prolactin promote β-cell function (6, 11, 15, 18, 21-23, 29, 44, 45). Many of the intracellular signaling pathways through which these growth factors modulate β-cell function have been studied. IGF-1 and insulin activate the IRS-2/PI 3-kinase/Akt2 pathway, which inhibits FoxO1 activity to promote Pdx1 expression (18, 21, 23, 29, 44, 45). Pdx1 is a master transcriptional regulator in β-cells that regulates the expression of genes necessary for β-cell function, as well as those needed for proliferation and survival (1-3, 21). Additionally, activation of the JAK2/STAT pathways by GH and prolactin promote β-cell function and proliferation (6, 11, 15, 22). The adapter protein SH2B1 is expressed in the pancreas (9, 35) and promotes the activation of both the IRS-2/PI 3-kinase/Akt2 and JAK2/STAT pathways in cells (5, 25, 37, 40, 43), raising the possibility that SH2B1 may play a role in β-cell function and/or β-cell proliferation.

SH2B1 is a PH and SH2 domain containing adapter protein that mediates cellular responses to a wide range of growth factors and cytokines, including insulin and leptin (8, 19, 20, 25, 34, 37-40, 43, 47). Disruption of the *Sh2b1* gene in mice causes both obesity and diabetes (24, 35, 36). Genetic evidence indicates that SH2B1 in the brain predominantly regulates leptin action to control energy balance (9, 24, 35, 36). SH2B1-knockout (KO) mice are severely leptin resistant (24, 35, 36). However, leptin sensitivity is restored, energy balance is corrected, and systemic insulin sensitivity is improved after reconstitution of SH2B1 expression in neurons of SH2B1-KO mice (36). Moreover, overexpression of SH2B1 in neurons prevents diet-induced obesity (36), whereas

overexpression of a dominant negative mutant of SH2B1 in neurons induces obesity (Chapter 2). Collectively, these findings indicate that SH2B1 in the brain is a leptin sensitizer that is critical for regulation of energy balance and body weight. Moreover, SH2B1 in the brain indirectly promotes systemic insulin sensitivity and glucose homeostasis by controlling body weight and adiposity.

In cells, SH2B1 promotes insulin signaling by promoting insulin receptor kinase activity and by attenuating dephosphorylation of insulin receptor substrate (IRS) proteins (28). SH2B1 is expressed in insulin-target tissues, including skeletal muscle, liver, and white adipose tissue (9, 35), and we recently reported that disruption of SH2B1 in these tissues exacerbates diet-induced hyperglycemia, hyperinsulinemia and glucose intolerance (28). These data indicate that peripheral SH2B1 directly regulates insulin signaling to promote glucose homeostasis.

SH2B1 is expressed in the pancreas (9, 35), but whether pancreatic SH2B1 has a role β-cell function is not known. The observations that SH2B1 promotes insulin, IGF-1 and GH signaling in cultured cells (9, 28) raises the possibility that SH2B1 in the pancreas may contribute to the regulation of glucose metabolism by supporting β-cell function. To test this, we examined glucose metabolism, glucose tolerance and insulin sensitivity in two mice models. First, we generated leptin-deficient (ob/ob) mice with *Sh2b1* haploinsufficiency (*Sh2b1*$^{+/-}$ *ob/ob* mice) to test whether SH2B1 promotes glucose metabolism independent of leptin action. We report that *Sh2b1* haploinsufficiency did not alter energy metabolism in leptin-deficient mice; however, *Sh2b1* haploinsufficiency exacerbated the diabetic phenotype in *ob/ob* mice. Glucose tolerance was severely impaired in *Sh2b1*$^{+/-}$ *ob/ob* mice due to β-cell dysfunction. These data support the hypothesis that SH2B1 in the pancreas contributes to the regulation of glucose homeostasis independent of the ability of SH2B1 in the brain to regulate energy balance. To further examine the contribution of SH2B1 in the pancreas to the regulation of glucose homeostasis and to test whether SH2B1 in β-cells prevents β-cell dysfunction during diet-induced obesity, we generated pancreas-specific SH2B1-knockout mice (P-KO). P-KO mice were fed a high fat diet (HFD) for 10 weeks to induce peripheral insulin resistance. Fed glucose levels were higher in HFD-fed P-KO mice than in HFD-

fed control mice. However, fasting glucose levels and glucose tolerance were similar in P-KO and control mice. Together, these data indicate that SH2B1 has an important leptin-independent role in the regulation of glucose homeostasis. In the pancreas, SH2B1 supports β-cell function in genetically obese mice, possibly by protecting β-cells from oxidative stress induced by glucotoxicity.

## Materials and Methods

**Generation of leptin-deficient (*ob/ob*) mice with reduced SH2B1 expression.** *Sh2b1*$^{+/-}$ mice have been described previously (9). *Sh2b1*$^{+/-}$ mice were backcrossed for six generations onto a C57BL/6 genetic background. *Sh2b1*$^{+/-}$ mice were crossed to *ob/+* mice (Jackson Laboratories) to generate double heterozygotes (*Sh2b1* $^{+/-}$ *ob/+*). *Sh2b1* $^{+/-}$ *ob/+* mice were crossed to generate wild-type (WT), *ob/ob*, *Sh2b1* $^{+/-}$ *ob/ob*, and *SH2B1*$^{KO}$ *ob/ob* mice. Male mice were used for experiments. Mice were housed on 14-hour light/10-hour dark cycle in the Unit for Laboratory Animal Medicine at the University of Michigan, and were fed standard rodent chow (9% fat; Lab Diet) ad libitum with free access to water. Animal protocols were approved by the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan.

**Body composition, energy expenditure, food intake and locomotor activity.** Fat content was measured by dual energy x-ray absorptiometry (Norland Medical System). Food intake, oxygen consumption ($VO_2$), carbon dioxide production ($VCO_2$), and spontaneous locomotor activity were measured using the Comprehensive Laboratory Monitoring System (CLAMS, Columbus Instruments). Mice were individually housed in metabolic chambers with free access to food and water. After 24 hours of acclimation, measurements were made continuously for 48 hours. $O_2$ and $CO_2$ in each chamber were sampled for 5 seconds at 10 minute intervals. Food intake was measured using a precision balance attached to the chamber. Locomotor activity was recorded every second in X and Z dimensions.

**Blood glucose, plasma insulin, glucose tolerance tests (GTT) and insulin tolerance tests (ITT).** Blood glucose levels were determined using glucometers (Bayer Corp). Plasma insulin was measured using a rat insulin ELISA kit (Crystal Chem). Glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were conducted as previously described (9, 35, 36).

**Immunoprecipitation and immunoblotting.** Mice were anesthesized with Averin (0.5 g of tribromoethanol and 0.25 g or tert-amyl alcohol in 39.5 ml of water; 0.02 ml/g of body weight). Tissues were isolated, rapidly frozen in liquid nitrogen, and stored at -80 ºC until analysis. Frozen tissue samples were homogenized in ice cold lysis buffer (50 mM Tris HCl, pH 7.5, 0.5% Nonidet P-40, 150 mM NaCl, 2 mM EGTA, 1 mM

$Na_3VO_4$, 100 mM NaF, 10 mM $Na_4P_2O_7$, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin) and extracts were immunoblotted or immunoprecipitated with indicated antibodies.

**Islet morphology and immunoflourescence.** Tissues were fixed in 4% paraformaldehyde overnight and protected in 30% sucrose. Frozen pancreas sections (5-8 μm) were prepared and stained with indicated antibodies. Immunoflourescence was visualized using a BX51 microscope (Olympus) and images were captured using a DP70 Digital Camera (Olympus).

**β-cell area.** To determine β-cell area, 4-5 pancreas sections >250 μm apart were obtained per mouse. Sections were stained with anti-insulin antibody to label β-cells. Sections were visualized and images were captured as described above. β cell area (in $\mu m^2$) and the area of each section was determined using ImageJ software. β cell area was calculated by dividing the area of all insulin-positive cells in the 4-5 sections by total area of the 4-5 sections examined for each mouse.

**Insulin content in pancreas.** Pancreata were isolated from fasted (16-h) mice, weighed, rapidly frozen in liquid nitrogen and stored at -80 ºC until analysis. The entire pancreas was homogenized in acid-ethanol (1.5% HCl in 70% EtOH), and extracted insulin was measured using a rat insulin RIA kit (Millipore). Insulin content was normalized to pancreas wet weight.

**Generation of *Sh2b1* $^{flox/flox}$ mice.** Unidirectional loxP sites were introduced into a 22 kb fragment of mouse genomic DNA that spanned the coding exons of the *Sh2b1* gene. One loxP site was inserted into the intron between the second and third exons and a second loxP site was inserted into the intron between the fifth and sixth exons in the *Sh2b1* gene. A Neo cassette flanked by unidirectional Flp-recombinase recognition (Frt) sites was inserted 3' of the first loxP (between exon 2 and exon 3). A thymidine kinase (TK) expression cassette were included in the targeting vector. A *Hind* III restriction site was introduced after the 3' loxP site to facilitate detection of homologous recombination by Southern blot analysis.

The linearized targeting construct was electroporated into R1 ES cells (129/Svx129/Sv-CP F1) by staff at the University of Michigan Transgenic Animal Model

Core (TAMC) facility. ES clones were first screened by PCR to detect integration of the 3' loxP site. To confirm that homologous recombination occurred between the targeting construct and the *Sh2b1* locus, genomic DNA was digested with *Hind* III and subjected to Southern blot analysis. Two ES cell clones were microinjected into C57BL/6 blastocyts to generate chimeric *Sh2b1* $^{flox\text{-}NEO/+}$ mice. Six chimeric (>90% agouti) males from each clone were breed to C57BL/6 females to identify germline founders.

The Neo cassette acts as a transcriptional block in *Sh2b1* $^{flox\text{-}NEO/+}$ mice, resulting in reduced SH2B1 expression (data not shown). Therefore, the Neo cassette was deleted from the germline by crossing *Sh2b1* $^{flox\text{-}NEO/+}$ mice with a Flp deleter strain (TgACTFLPe) that ubiquitously expresses Flp recombinase under the control of the human actin promoter (Jackson Laboratories) to produce *Sh2b1* $^{flox/+}$ mice. *Sh2b1* $^{flox/+}$ mice were intercrossed to generate mice homozygous for the conditional allele (*Sh2b1* $^{flox/flox}$).

**Generation of pancreas specific SH2B1-knockout (P-KO) mice.** Pdx1-Cre mice in which Cre recombinase is expressed under the control of the mouse Pdx1 promoter have been described (12). Pdx1-Cre mice were provided by Dr. Richard Mortensen, University of Michigan. Mice homozygous for the floxed *Sh2b1* allele (*Sh2b1* $^{flox/flox}$ ) were crossed to Pdx1-Cre transgenic mice. The resultant double heterozygous mice were crossed to *Sh2b1*$^{flox/+}$ mice to produce pancreas-specific SH2B1-knockout mice (P-KO mice: *Sh2b1* $^{flox/flox}$/Pdx1-Cre). The control group (Ctrl) consisted of both *Sh2b1* $^{flox/flox}$ and *Sh2b1* $^{+/+}$/Pdx1-Cre mice. P-KO and Ctrl male mice were fed either normal rodent chow (9% fat; Lab Diet) or a high fat diet (45% fat; Research Diets) ad libitum with free access to water.

**Statistical Analysis.** Data are presented as means ± SEM. Differences between groups were determined by two-tailed Student's *t* tests or ANOVA. $P < 0.05$ was considered significant.

## Results

**Disruption of *Sh2b1* in leptin-deficient (*ob/ob*) mice results in lethality and reduced viability.** To explore the leptin-independent regulation of glucose metabolism by SH2B1 *in vivo*, SH2B1-knockout (KO) mice were crossed onto the leptin-deficient background in an attempt to generate double mutant mice lacking both SH2B1 and leptin. Surprisingly, the number of double mutant *Sh2b1*$^{-/-}$ *ob/ob* mice produced from heterozygous matings (*Sh2b1*$^{+/-}$ *Ob/ob* x *Sh2b1*$^{+/-}$ *Ob/ob*) did not reach the expected Mendelian ratio indicating that the combined deletion of both SH2B1 and leptin in C57BL/6 mice causes either embryonic or perinatal lethality (data not shown).

Therefore, we chose to examine the effects of *Sh2b1* haploinsufficiency on glucose metabolism in leptin-deficient mice (*Sh2b1*$^{+/-}$ *ob/ob*) mice. To confirm that inactivation of one *Sh2b1* allele produced the expected 50% reduction in SH2B1 protein, we examined SH2B1 protein levels in brain and liver. Two isoforms of SH2B1 in brain and one isoform of SH2B1 in liver were detected in wild type (WT), *ob/ob* and *Sh2b1*$^{+/-}$ *ob/ob* mice (Fig. 4.1A). Compared to *ob/ob* mice, SH2B1 levels in both brain and liver were reduced in *Sh2b1*$^{+/-}$ *ob/ob* mice. Notably, SH2B1 protein levels were similar in brain and liver from wild type (WT) and *ob/ob* mice (Fig. 4.1A), indicating that leptin-deficiency does not alter SH2B1 expression in mice.

***Sh2b1* haploinsufficiency does not alter obesity or energy balance in leptin-deficient mice.** Obesity and dyslipidemia likely contribute to insulin resistance and glucose intolerance in SH2B1-KO mice (9, 24, 35, 36), making it difficult to address the contribution of SH2B1 in peripheral tissues and pancreas to the regulation of glucose metabolism. Body weight and fat content were similar in *ob/ob* and *Sh2b1*$^{+/-}$ *ob/ob* male mice (Fig. 4.1B-C). The size of individual adipocytes from epididymal fat pads was also similar in *Sh2b1*$^{+/-}$ *ob/ob* and *ob/ob* mice (data not shown). As expected, *ob/ob* mice were hyperphagic and consumed more food than age-matched WT mice; however, *Sh2b1* haploinsufficiency had no additive effect on food intake in *Sh2b1*$^{+/-}$ *ob/ob* mice (Fig. 4.1D). Moreover, *Sh2b1* haploinsufficiency did not alter energy expenditure or activity in leptin-deficient mice (Fig. 4.1E-F). Collectively, these data indicate that a reduction in SH2B1 levels is not sufficient to further alter energy imbalance in leptin-deficient mice.

***Sh2b1* haploinsufficiency exacerbates the diabetic phenotype in leptin-deficient mice.** SH2B1 is expressed in insulin-target tissues and in the pancreas (9, 35). To determine whether SH2B1 in these tissues contributes to the regulation of insulin sensitivity and glucose homeostasis in the absence of leptin signaling, glucose and insulin levels in *ob/ob* and *Sh2b1*$^{+/-}$ *ob/ob* male mice were compared. Fasting glucose levels were significantly elevated in *ob/ob* mice as early as seven weeks of age (WT: 64.8 ± 4.8 mg/dl, n = 9; *ob/ob*: 138.3 ± 6.5 mg/dl, n = 9); hyperglycemia was further exacerbated in *Sh2b1*$^{+/-}$ *ob/ob* mice (185.6 ± 16.9 mg/dl, n = 9). By 12 weeks of age, fasting and random fed glucose levels were increased by 150% and 30%, respectfully, in *Sh2b1*$^{+/-}$ *ob/ob* mice compared to *ob/ob* mice (Fig. 4.2A). Fasting insulin levels were similar in *Sh2b1*$^{+/-}$ *ob/ob* and *ob/ob* mice (Fig. 4.2B); however, insulin levels in randomly fed *Sh2b1*$^{+/-}$ *ob/ob* and *ob/ob* mice were significantly different (Fig. 4.2B). In the fed state, plasma insulin levels were 4.6-times higher in *ob/ob* mice compared to *Sh2b1*$^{+/-}$ *ob/ob* mice (*ob/ob*: 23.2 ± 6.8 ng/ml, n = 5; *Sh2b1*$^{+/-}$ *ob/ob*: 5.0 ± 1.3 ng/ml, n = 5; $p<0.01$). Comparing insulin levels in fasted versus fed mice, plasma insulin increased ~5-fold in *ob/ob* mice in response to feeding (fed: 23.2 ± 6.8 ng/ml, n = 5; fasted: 4.7 ± 6.8 ng/ml, n = 9); by contrast, insulin levels were nearly identical in fed and fasted *Sh2b1*$^{+/-}$ *ob/ob* mice (fed: 5.0 ± 1.3 ng/ml, n = 5; fasted: 4.9 ± 1.3 ng/ml, n = 9).

To examine glucose and insulin sensitivity in *Sh2b1*$^{+/-}$ *ob/ob* and *ob/ob* mice, glucose tolerance (GTT) and insulin tolerance tests (ITT) were performed. Exogenous glucose (0.6g D-glucose/kg body weight) caused a sharp rise in glucose levels in both *ob/ob* and *Sh2b1*$^{+/-}$ *ob/ob* mice; however, glucose tolerance was more significantly impaired in *Sh2b1*$^{+/-}$ *ob/ob* mice, as glucose levels remained significantly higher for a longer period after the glucose injection (Fig. 4.2C). Compared to *ob/ob* mice, the area under the GTT curve increased nearly 1.5-times in *Sh2b1*$^{+/-}$ *ob/ob* mice (*ob/ob*: 587.5 ± 51.6 mg/dl x hr, n = 7; *Sh2b1*$^{+/-}$ *ob/ob*: 875.9 ± 88.7 mg/dl x hr, n = 9; $p<0.05$). Notably, the extent to which glucose tolerance is impaired in *Sh2b1*$^{+/-}$ *ob/ob* mice is underestimated. Blood glucose levels in nearly half (3 of 7) of the *Sh2b1*$^{+/-}$ *ob/ob* mice assayed were higher than the upper detection limit (600 mg/dl) of the glucometers used both 30 and 60 minutes after injection; in those three mice, glucose levels were assigned a conservative estimate of 600 mg/dl.

To examine the extent to which insulin resistance contributed to glucose intolerance in *Sh2b1*$^{+/-}$ *ob/ob* mice, we conducted insulin tolerance tests (ITT). Both *ob/ob* and *Sh2b1*$^{+/-}$ *ob/ob* mice were severely insulin resistant compared to WT mice and required higher doses of insulin to reduce blood glucose. Insulin (4U/kg body weight) reduced glucose levels to a similar extent in *ob/ob* mice and *Sh2b1*$^{+/-}$ *ob/ob* mice, and the area under the insulin tolerance curve was similar (Fig. 4.2D). In younger mice (7-weeks of age), insulin (2U/kg body weight) reduced blood glucose levels by ~34% in *ob/ob* and by ~30% in *Sh2b1*$^{+/-}$ *ob/ob* within one hour of injection, and the area under the insulin tolerance curve was not different (*ob/ob*: 105.3 ± 3.3 mg/dl x h, n = 5; *Sh2b1*$^{+/-}$ *ob/ob*: 103.5 ± 11.6 mg/dl x h, n = 5). Taken together, these data indicate that differences in insulin resistance do not explain the severe hyperglycemia and glucose intolerance observed in *Sh2b1*$^{+/-}$ *ob/ob* mice.

***Sh2b1* haploinsufficiency reduces pancreatic insulin content in leptin-deficient mice.** Severely impaired glucose tolerance and reduced plasma insulin levels in fed, hyperglycemic *Sh2b1*$^{+/-}$ *ob/ob* mice suggest that β-cell function may be regulated by SH2B1. Therefore, we next examined the pancreas and islets from *Sh2b1*$^{+/-}$ *ob/ob* and *ob/ob* mice. β-cell area was determined by measuring the area of insulin-positive cells. Compared to WT mice, β-cell area was increased in *ob/ob* mice; however, β-cell area was similar between *Sh2b1*$^{+/-}$ *ob/ob* and *ob/ob* mice (Fig. 4.3A-B), indicating that compensatory β-cell hyperplasia is not significantly impaired in *Sh2b1*$^{+/-}$ *ob/ob* mice. Pancreas wet weight was also similar in *ob/ob* and *Sh2b1*$^{+/-}$ *ob/ob* mice (Fig. 4.3C). However, pancreatic insulin content was significantly decreased in *Sh2b1*$^{+/-}$ *ob/ob* (Fig. 4.3D). At 15 weeks of age, pancreatic insulin content was reduced by 69% in *Sh2b1*$^{+/-}$ *ob/ob* mice (*ob/ob*: 487.8± 55.5 ng insulin/mg pancreas, n = 8; *Sh2b1*$^{+/-}$ *ob/ob*: 151.1 ± 22.9 ng insulin/mg pancreas, n = 9; $P<0.001$). Collectively, these findings suggest that β-cell dysfunction contributes to both impaired glucose tolerance and decreased postprandial insulin levels in *Sh2b1*$^{+/-}$ *ob/ob* mice.

**SH2B1 is expressed in endocrine pancreas.** SH2B1 is expressed in the pancreas (9, 35), but whether SH2B1 is expressed in exocrine or endocrine pancreas has not been determined. Therefore, we examined SH2B1 expression in islets and β-cells. SH2B1 protein was detected in isolated islets from WT male mice (Fig. 4.4A), and in

MIN6 and INS-1 832/13 insulinoma cell lines (data not shown). To further examine the distribution of SH2B1 in the pancreas, pancreas sections from WT and KO mice were co-immunostained with anti-SH2B1 and anti-insulin antibodies. SH2B1 was detected in pancreas sections from WT, but not KO, mice (Fig.4.4B). SH2B1 immunoreactivity was strongest within the islet (Fig. 4.4B); however, SH2B1 immunoreactivity was largely undetected in surrounding acinar and ductal cells (Fig. 4.4B). These data indicate that SH2B1 is highly expressed in islets. Furthermore, these observations suggest that SH2B1 may cell autonomously promote insulin production and/or other aspects of β-cell biology.

**Generation of pancreas-specific SH2B1-knockout mice.** Multiple growth factors, including insulin, insulin-like growth factor-I (IGF-I), growth hormone (GH), and prolactin, support β-cell function (insulin production and secretion), proliferation and/or survival (4, 6, 10, 14, 42, 46). In cells, SH2B1 is a signaling molecule for all of these factors (5, 37, 40, 43). To examine the role of SH2B1 in the endocrine pancreas, we generated pancreas-specific SH2B1-knockout mice (P-KO) (Materials and Methods). In P-KO mice, SH2B1 protein was significantly reduced in pancreas, but present in other tissues, confirming that Cre-mediated recombination of the conditional *Sh2b1* alleles occurred specifically in pancreas (Fig. 4.5A-B). β-cell area and islet morphology was similar in P-KO and *Sh2b1* $^{flox/flox}$ mice (not shown), suggesting that SH2B1 is not required for development of the endocrine pancreas.

To determine whether SH2B1 in the pancreas is required for glucose metabolism, glucose levels in chow-fed P-KO mice (7-8 weeks) were compared to the control (Ctrl) group, which consisted of both *Sh2b1* $^{flox/flox}$ and *Sh2b1* $^{+/+}$/Pdx1-Cre male mice. Under these experimental conditions, fasting and random fed glucose levels were similar in P-KO and Ctrl mice (Fig. 4.5C). Additionally, neither glucose tolerance nor insulin sensitivity was altered by pancreas-specific deletion of SH2B1 in P-KO mice (Fig. 4.5D-E). Taken together, these data indicate that disruption of *Sh2b1* in the pancreas alone is not sufficient to alter pancreas development or glucose metabolism in young, chow-fed mice.

**Pancreas-specific deletion of SH2B1 causes hyperglycemia, but not glucose intolerance in mice fed a high fat diet.** To determine whether deletion of SH2B1 in the pancreas alters glucose homeostasis during diet-induced obesity, P-KO and Ctrl mice

were fed a high-fat diet (HFD) for 10 weeks. Body weight (Fig. 4.6A) and fat content (not shown) were similar between HFD-fed P-KO and Ctrl mice. After 10 weeks of diet-induced obesity, random fed, but not fasting, glucose levels were increased in P-KO mice (Fig. 4.6B-C). Compared to Ctrl mice, blood glucose levels were 40% higher in P-KO mice after 10 weeks on HFD (Ctrl: 202.1 ± 18.9 mg/dl, n = 24; P-KO: 289.2 ± 33.59 mg/dl, n = 13; $P<0.02$). As expected insulin resistance was similar between P-KO and Ctrl mice (Fig. 4.6D); however, glucose tolerance was not significantly impaired in HFD-fed P-KO mice relative to Ctrl mice (Fig. 4.6E). Collectively, these data indicate that disruption of SH2B1 in the pancreas only mildly affects glucose homeostasis during diet-induced obesity.

## Discussion

Insulin resistance and β-cell dysfunction are determinants of diabetes. Systemic disruption of the *Sh2b1* gene in mice results in obesity and diabetes, demonstrating that SH2B1 is required for the maintenance of both energy balance and glucose homeostasis (9, 24, 35, 36). We have demonstrated that SH2B1 in neurons promotes leptin signaling and contributes to the intrinsic regulation of energy balance and body weight (36). By regulating energy balance and adiposity, neuronal SH2B1 indirectly promotes whole body insulin sensitivity. Thus, regulation of leptin signaling in the brain may be the primary function of SH2B1 *in vivo*. However, SH2B1 is expressed in insulin-target tissues and in the pancreas (9, 35). SH2B1 promotes insulin signaling in cultured cells and deletion of peripheral SH2B1 exacerbates diet-induced insulin resistance and hyperglycemia (28). These latter observations indicate that SH2B1 directly promotes insulin action in peripheral tissues. However, the role of SH2B1 in the pancreas, and the relative contribution of pancreatic SH2B1 in the regulation of glucose metabolism has remained largely undefined.

To test the role of SH2B1 in the pancreas, we examined glucose metabolism, glucose tolerance and insulin sensitivity in two mice models. First, we generated leptin-deficient (*ob/ob*) mice with *Sh2b1* haploinsufficiency to test whether SH2B1 promotes insulin sensitivity and β-cell function independent of its role as a leptin signaling molecule. *Sh2b1* haploinsufficiency did not alter the onset or severity of obesity in *ob/ob* mice. However, the diabetic phenotype characteristic of *ob/ob* mice was dramatically exacerbated when SH2B1 was reduced. Compared to *ob/ob* mice, *Sh2b1*$^{+/-}$*ob/ob* mice were more severely hyperglycemic and glucose intolerant. However, insulin resistance as measured by insulin tolerance tests (ITT), was similar between *Sh2b1*$^{+/-}$*ob/ob* mice and *ob/ob* mice, suggesting that β cell dysfunction, rather than insulin resistance, may underlie the dramatic impairment in glucose metabolism.

Consistent with β-cell dysfunction, postprandial plasma insulin levels were significantly reduced in *Sh2b1*$^{+/-}$*ob/ob* mice relative to *ob/ob* mice. Pancreas mass and β-cell area were similar in *Sh2b1*$^{+/-}$*ob/ob* and *ob/ob* mice, but pancreatic insulin content was dramatically reduced in *Sh2b1*$^{+/-}$*ob/ob* mice. Therefore, we propose that defects in

insulin production largely explain glucose intolerance and the apparent inability of these mice to increase postprandial insulin levels. The extent to which insulin biosynthesis and glucose-stimulated insulin secretion is impaired by *Sh2b1* haploinsufficiency in β-cells of *Sh2b1*$^{+/-}$*ob/ob* mice will require further investigation. Nonetheless, these findings provide the first evidence that suggest SH2B1 can promote β-cell function in mice.

SH2B1 is expressed in the pancreas and SH2B1 expression is highest in islets, so we generated pancreas-specific SH2B1-knockout (P-KO) mice to further characterize the role of SH2B1 in β-cell function. To accomplish this, mice with conditional (floxed) *Sh2b1* alleles were crossed with mice expressing Pdx1-Cre mice. In Pdx1-Cre, the expression of Cre recombinase is controlled by the mouse Pdx1 promoter (12). Pdx1 is expressed early in pancreatic development, and Pdx1-Cre has been shown to recombine conditional alleles in progenitors that give rise to endocrine, exocrine and ductal cells in the pancreas (12). Therefore, we assume that SH2B1 is deleted in all pancreatic cells in P-KO mice. We chose the Pdx1-Cre line over RIP-Cre mice, in which Cre expression is controlled by the rat insulin II promoter. While the RIP-Cre transgene is predicted to drive recombination of the conditional *Sh2b1* alleles within β-cells, Cre-mediated recombination has also been shown to occur in regions of the hypothalamus associated with regulation of energy balance (7, 26, 30). Because recombination of the conditional *Sh2b1* alleles within the hypothalamus may induce obesity, we chose to avoid this line.

P-KO mice were fed a high-fat diet (HFD) to induce peripheral insulin resistance. After 10 weeks of diet-induced obesity, peripheral insulin resistance caused hyperglycemia in fed, but not fasted, P-KO mice. However, glucose tolerance was not affected by pancreas-specific deletion of SH2B1. This was somewhat surprising given the robust and severe diabetic phenotype in *Sh2b1*$^{+/-}$*ob/ob* mice. Whether islet area, pancreatic insulin content, or glucose-stimulated insulin secretion is altered in HFD-fed P-KO mice will require further investigation.

Nonetheless, it is noteworthy that *Sh2b1* haploinsufficiency caused such a severe β-cell phenotype in *ob/ob* mice. Although the underlying mechanism is not clear, one possibility is that SH2B1 may increase the antioxidant capacity of β-cells. β-cell dysfunction is thought to be accelerated by oxidative stressors, such as chronic

glucotoxicity and/or lipotoxicity (31, 32). Thus, β-cell dysfunction may be hastened in *Sh2b1*$^{+/-}$*ob/ob* mice due to reduced antioxidant capacity. Interestingly, SH2B1-KO mice are more sensitive to low dose streptozotocin (STZ)-induced diabetes than WT mice (S. Oka, D. Ren, and L. Rui, unpublished). Since STZ is thought to generate reactive oxygen species (ROS) in β-cells (27, 41), these data support this hypothesis. Future studies are required to elucidate the mechanisms by which SH2B1 promotes β-cell function and/or survival during metabolic stress.

In summary, SH2B1 promotes glucose metabolism independent of its role as a leptin signaling molecule in the brain. SH2B1 in the pancreas promotes β-cell function in genetically obese mice. The extent to which insulin biosynthesis and glucose-stimulated insulin secretion is impaired by disruption of SH2B1 in β-cells will require further investigation. Nonetheless, islets that lack SH2B1 may be susceptible to oxidative damage, leading to β-cell dysfunction and premature β-cell failure

**Acknowledgements**

This study was supported by National Institute of Health grants RO1 DK 065122, RO1 DK073601 (to Liangyou Rui) and F31NS056575 (to David L. Morris), and by American Diabetes Association award 1-09-RA-156 (to Liangyou Rui). This work utilized the cores supported by the Michigan Diabetes Research and Training Center (funded by NIH 5P60 DK20572), the University of Michigan's Cancer Center (funded by NIH 5 P30 CA46592), the University of Michigan Nathan Shock Center (funded by NIH P30AG013283), and the University of Michigan Gut Peptide Research Center (funded by NIH DK34933).

This chapter will be prepared for submission. Drs. Shinichi Oka and Liangyou Rui generated data for Fig. 4.4.

.

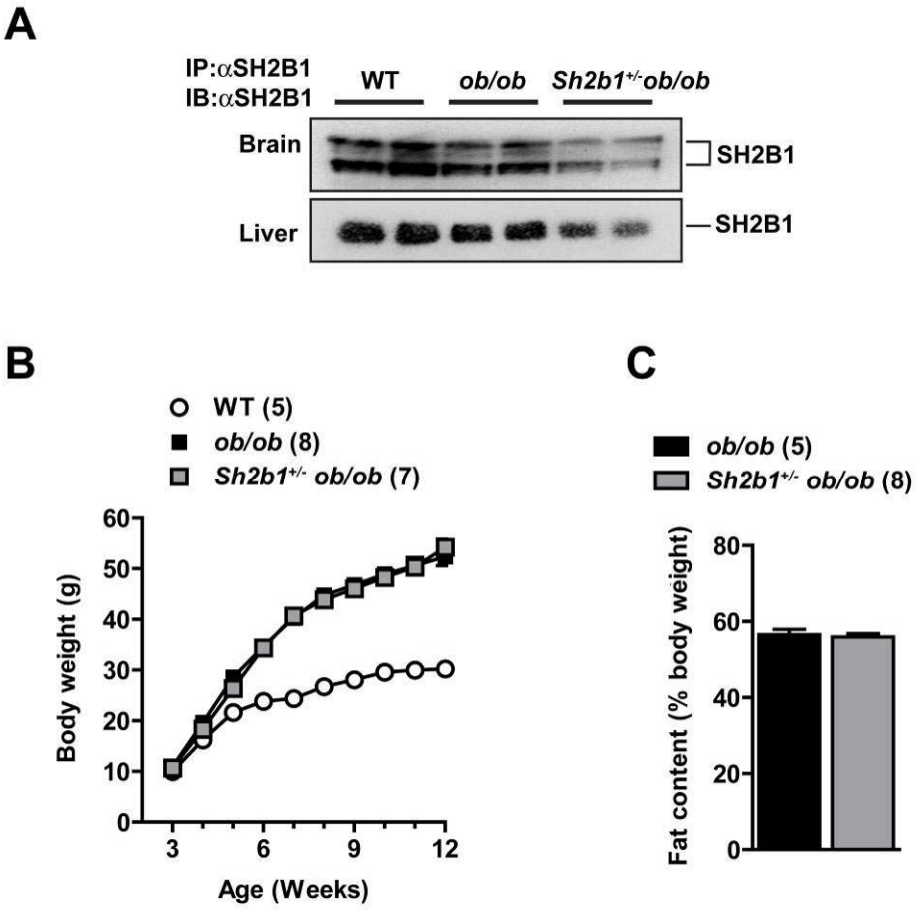


**Fig. 4.1A-C. Sh2b1 haploinsufficiency does not further alter energy balance in leptin-deficient (ob/ob) male mice.** (A) SH2B1 expression in brain and liver from WT, *ob/ob* and *Sh2b1*$^{+/-}$*ob/ob* male mice (15 wks). Each lane represents an individual mouse. (B) Growth curve. (C) Body fat content (10 wks). The number of mice per group is indicated in parenthesis.

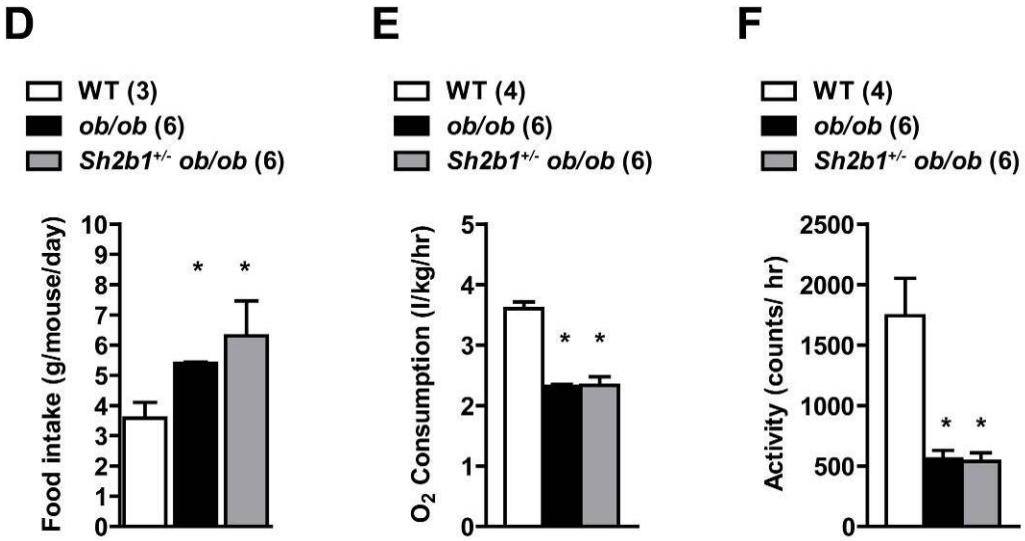


**Fig. 4.1D-F. Sh2b1 haploinsufficiency does not further alter energy balance in leptin-deficient (ob/ob) male mice.** (D) Food intake. (E) Oxygen consumption ($VO_2$). (F) Spontaneous locomotor activity. The number of mice per group is indicated in parenthesis. * P<0.05, ** P<0.01.

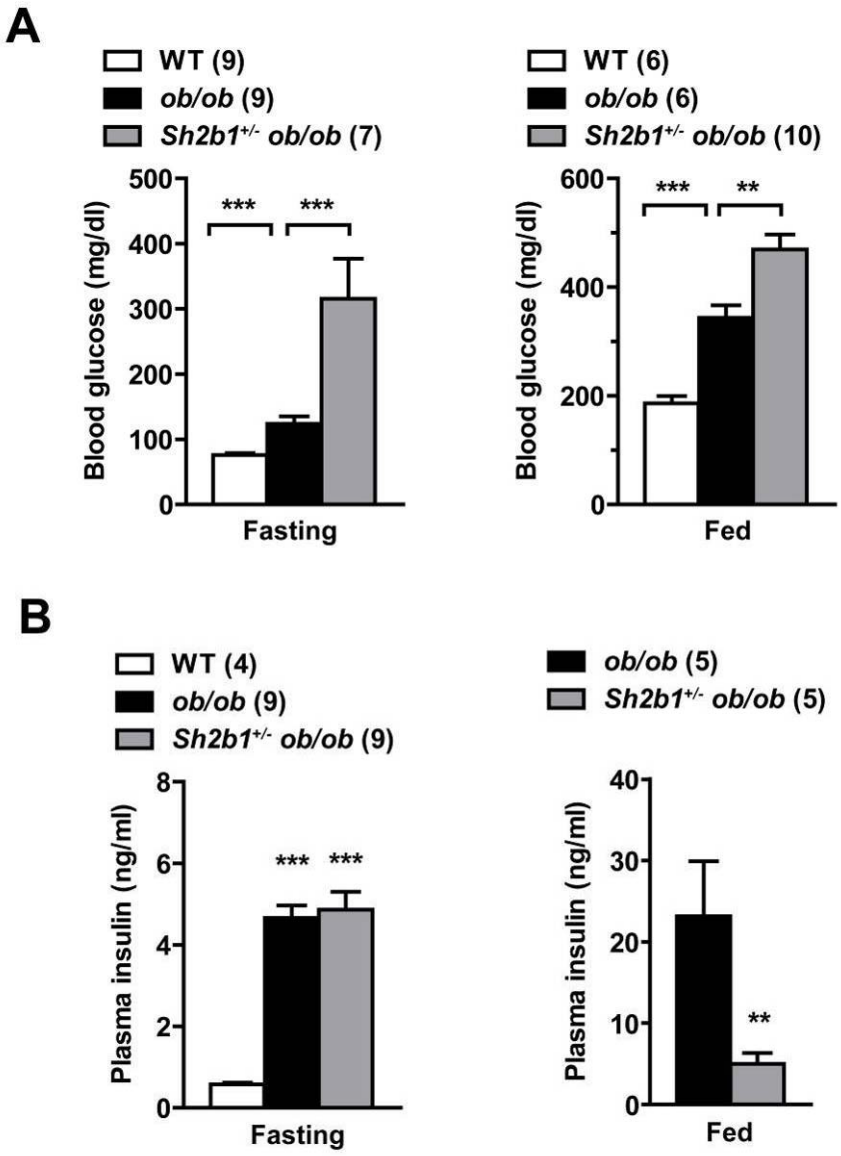


**Fig. 4.2A-B. Sh2b1 haploinsufficiency exacerbates hyperglycemia and glucose intolerance in leptin-deficient (ob/ob) male mice.** (A) Fasting (16-h) and random fed glucose levels in male mice (12 wks). (B) Fasting (16-h) and random fed plasma insulin levels (12 wks). The number of mice per group is indicated in parenthesis. ** P<0.01, *** P<0.001.

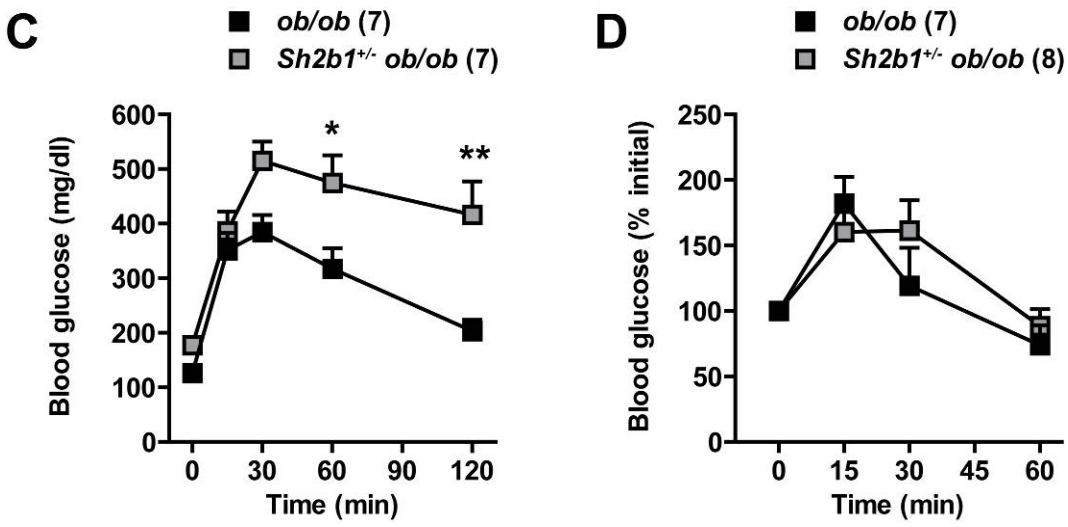


**Fig. 4.2C-D. Sh2b1 haploinsufficiency exacerbates hyperglycemia and glucose intolerance in leptin-deficient (ob/ob) male mice.** (D) Insulin tolerance tests were preformed on male mice at 12 wks (4U insulin/kg body weight). The number of mice per group is indicated in parenthesis. * $P<0.05$, ** $P<0.01$.

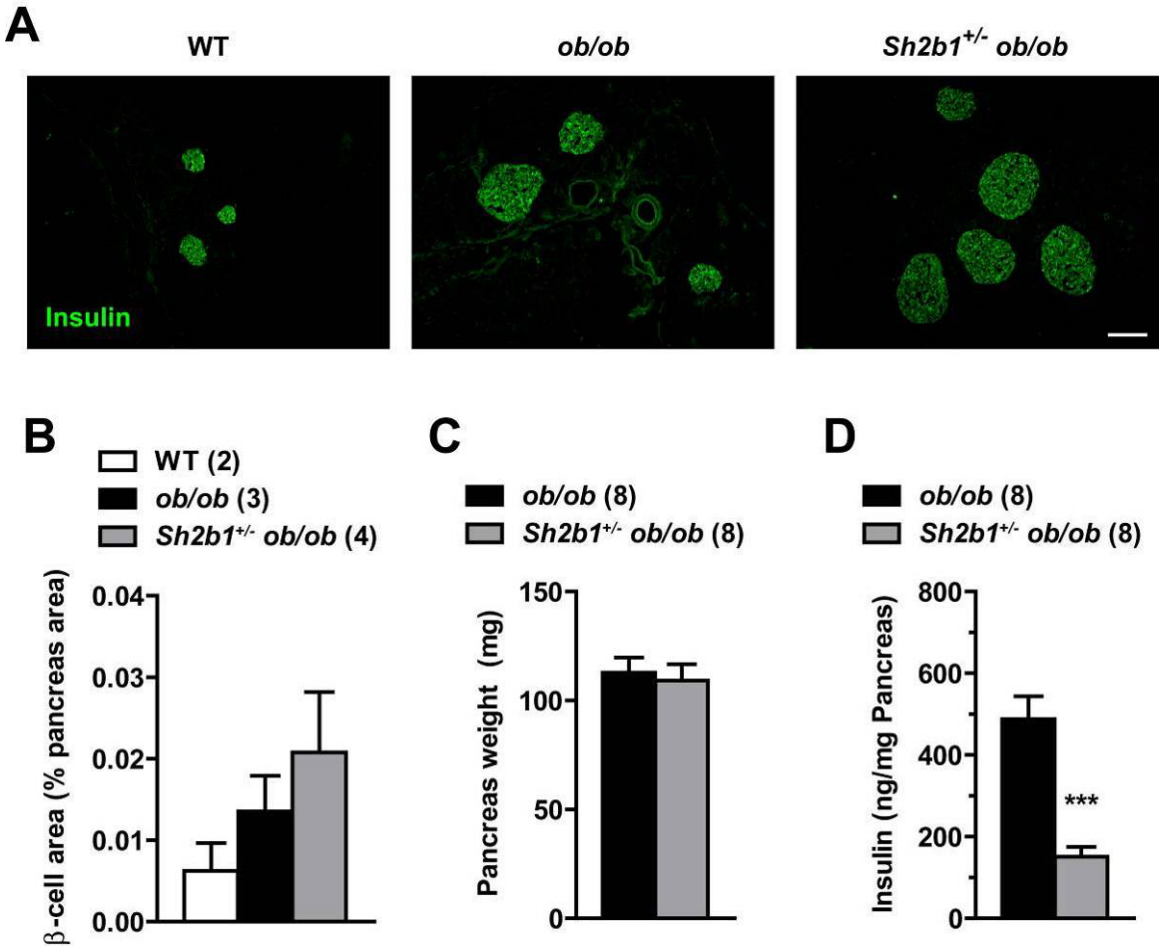


**Fig. 4.3. Insulin content, but not β-cell mass, is reduced in Sh2b1+/-ob/ob male mice.** (A) Representative images of pancreas sections stained with αinsulin antibody. Scale bar = 200μm. (B) Relative β-cell area in male mice (15 wks). The area of insulin-positive cells was normalized to total pancreas section area. 4-5 sections were measured per mouse. (C) Pancreas wet weight. (D) Insulin content in whole pancreas. The number of mice per group is indicated in parenthesis. *** P<0.001.

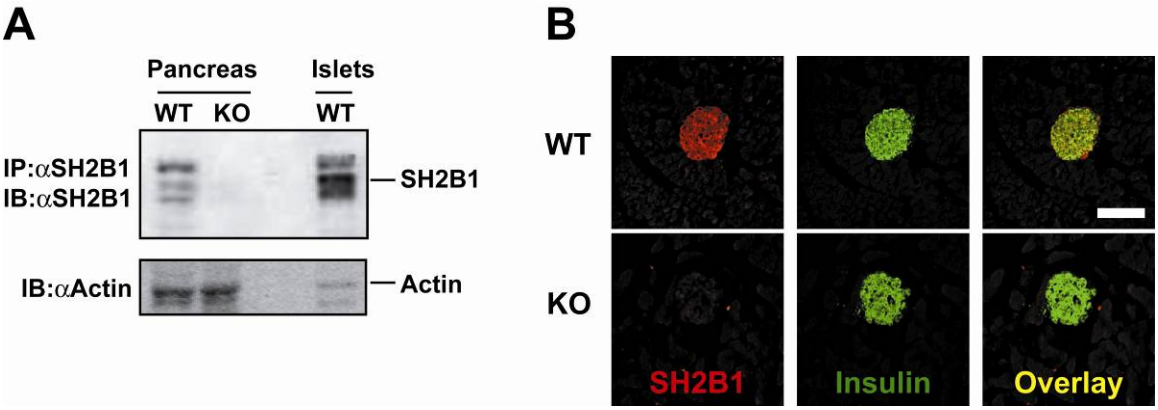


**Fig. 4.4. SH2B1 is expressed in islets.** (A) SH2B1 expression in pancreas and islet extracts from WT and SH2B1-knockout (KO) male mice. (B) SH2B1 and insulin immunoreactivity in WT and KO pancreas sections. Scale bar = 200μm.

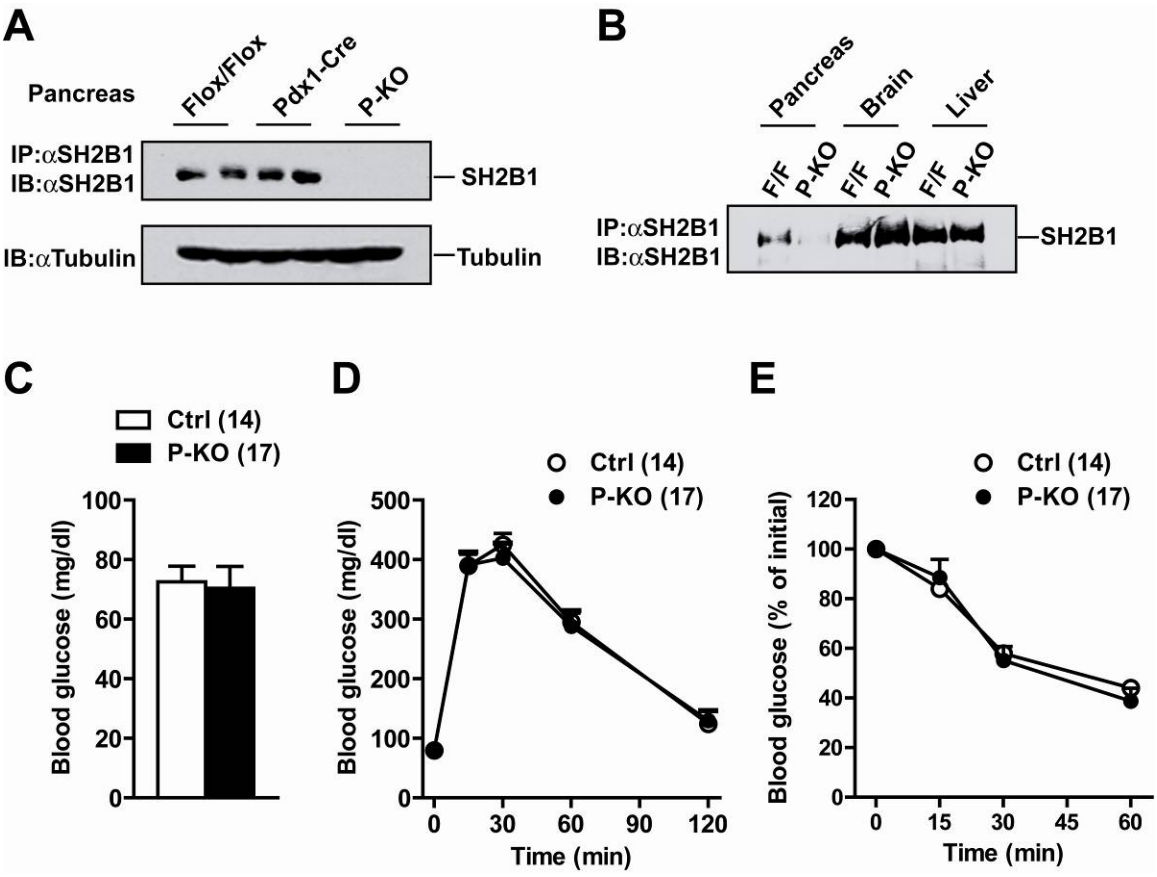


**Fig. 4.5. Generation of pancreas-specific SH2B1-knockout (P-KO) mice.** (A) SH2B1 expression in pancreas extracts from *Sh2b1* $^{flox/flox}$, *Sh2b1* $^{+/+}$/Pdx1-Cre mice, and P-KO male mice (12 wks). SH2B1 in pancreas extracts (1 mg) was immunoprecipitated with anti-SH2B1 antibody (αSH2B1) and immunoblotted with αSH2B1. In parallel, extracts were immunoblotted with αtubulin. (B) SH2B1 expression in pancreas, brain and liver from *Sh2b1* $^{flox/flox}$ and P-KO male mice. SH2B1 in pancreas (1 mg), brain (1 mg) and liver (1 mg) was immunoprecipitated with αSH2B1 and immunoblotted with αSH2B1. (C) Fasting (16-h) blood glucose levels in control (Ctrl) and P-KO male mice. (D) GTT performed on male mice (7-8 wks). Mice were fasted overnight (16-h) and D-glucose (2g/ kg body weight) was administered by i.p. injection. (E) Insulin tolerance tests (ITT) performed on male mice (7-8 wks). Mice were fasted for 6-h and human insulin (1U/kg body weight) was administered by i.p. injection. The number of mice per group is indicated in parenthesis.

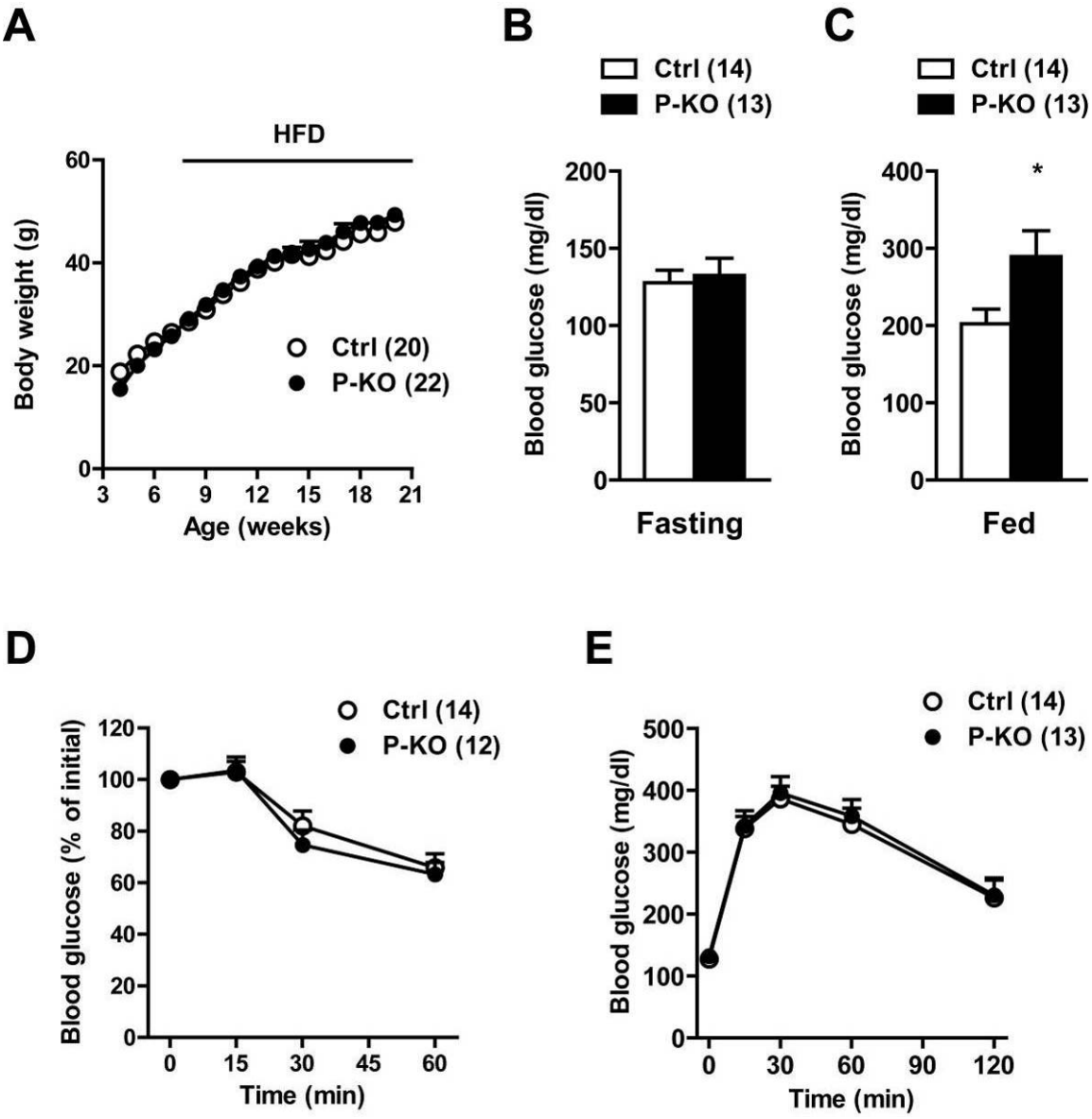


**Fig. 4.6. Disruption of Sh2b1 in the pancreas is not sufficient to alter glucose homeostasis during diet-induced obesity.** (A-E) P-KO and Ctrl male mice (7-8 wks) were fed a high fat diet (HFD) for 10 wks. (A) Growth curve. (B) Fasting (16-h) blood glucose levels after 10 wks on HFD. (C) Random fed blood glucose levels after 10 wks on HFD. (D) ITT (1U insulin/kg body weight) performed on male mice fed HFD for 10 wks. (E) GTT (1g D-glucose/kg body weight) performed on male mice fed HFD for 10 wks. The number of mice per group is indicated in parenthesis. * $P<0.05$.

## References


1. **Ahlgren U, Jonsson J, Jonsson L, Simu K, and Edlund H.** beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. *Genes Dev* 12: 1763-1768, 1998.

2. **Babu DA, Chakrabarti SK, Garmey JC, and Mirmira RG.** Pdx1 and BETA2/NeuroD1 participate in a transcriptional complex that mediates short-range DNA looping at the insulin gene. *J Biol Chem* 283: 8164-8172, 2008.

3. **Babu DA, Deering TG, and Mirmira RG.** A feat of metabolic proportions: Pdx1 orchestrates islet development and function in the maintenance of glucose homeostasis. *Mol Genet Metab* 92: 43-55, 2007.

4. **Bruning JC, Winnay J, Bonner-Weir S, Taylor SI, Accili D, and Kahn CR.** Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles. *Cell* 88: 561-572, 1997.

5. **Carter-Su C and Smit LS.** Signaling via JAK tyrosine kinases: growth hormone receptor as a model system. *Recent Prog Horm Res* 53: 61-82; discussion 82-63, 1998.

6. **Cousin SP, Hugl SR, Myers MG, Jr., White MF, Reifel-Miller A, and Rhodes CJ.** Stimulation of pancreatic beta-cell proliferation by growth hormone is glucose-dependent: signal transduction via janus kinase 2 (JAK2)/signal transducer and activator of transcription 5 (STAT5) with no crosstalk to insulin receptor substrate-mediated mitogenic signalling. *Biochem J* 344 Pt 3: 649-658, 1999.

7. **Covey SD, Wideman RD, McDonald C, Unniappan S, Huynh F, Asadi A, Speck M, Webber T, Chua SC, and Kieffer TJ.** The pancreatic beta cell is a key site for mediating the effects of leptin on glucose homeostasis. *Cell Metab* 4: 291-302, 2006.

8. **Duan C, Li M, and Rui L.** SH2-B promotes insulin receptor substrate 1 (IRS1)- and IRS2-mediated activation of the phosphatidylinositol 3-kinase pathway in response to leptin. *J Biol Chem* 279: 43684-43691, 2004.

9. **Duan C, Yang H, White MF, and Rui L.** Disruption of the SH2-B gene causes age-dependent insulin resistance and glucose intolerance. *Mol Cell Biol* 24: 7435-7443, 2004.

10. **Friedrichsen BN, Richter HE, Hansen JA, Rhodes CJ, Nielsen JH, Billestrup N, and Moldrup A.** Signal transducer and activator of transcription 5 activation is sufficient to drive transcriptional induction of cyclin D2 gene and proliferation of rat pancreatic beta-cells. *Mol Endocrinol* 17: 945-958, 2003.

11. **Gorogawa S, Fujitani Y, Kaneto H, Hazama Y, Watada H, Miyamoto Y, Takeda K, Akira S, Magnuson MA, Yamasaki Y, Kajimoto Y, and Hori M.** Insulin secretory defects and impaired islet architecture in pancreatic beta-cell-specific STAT3 knockout mice. *Biochem Biophys Res Commun* 319: 1159-1170, 2004.

12. **Gu G, Dubauskaite J, and Melton DA.** Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* 129: 2447-2457, 2002.

13. **Herman MA and Kahn BB.** Glucose transport and sensing in the maintenance of glucose homeostasis and metabolic harmony. *J Clin Invest* 116: 1767-1775, 2006.

14. **Hugl SR, White MF, and Rhodes CJ.** Insulin-like growth factor I (IGF-I)-stimulated pancreatic beta-cell growth is glucose-dependent. Synergistic activation of insulin receptor substrate-mediated signal transduction pathways by glucose and IGF-I in INS-1 cells. *J Biol Chem* 273: 17771-17779, 1998.

15. **Jackerott M, Moldrup A, Thams P, Galsgaard ED, Knudsen J, Lee YC, and Nielsen JH.** STAT5 activity in pancreatic beta-cells influences the severity of diabetes in animal models of type 1 and 2 diabetes. *Diabetes* 55: 2705-2712, 2006.

16. **Kahn SE, Hull RL, and Utzschneider KM.** Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444: 840-846, 2006.

17. **Kasuga M.** Insulin resistance and pancreatic beta cell failure. *J Clin Invest* 116: 1756-1760, 2006.

18. **Kitamura T, Nakae J, Kitamura Y, Kido Y, Biggs WH, 3rd, Wright CV, White MF, Arden KC, and Accili D.** The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. *J Clin Invest* 110: 1839-1847, 2002.

19. **Kong M, Wang CS, and Donoghue DJ.** Interaction of fibroblast growth factor receptor 3 and the adapter protein SH2-B. A role in STAT5 activation. *J Biol Chem* 277: 15962-15970, 2002.

20. **Kotani K, Wilden P, and Pillay TS.** SH2-Balpha is an insulin-receptor adapter protein and substrate that interacts with the activation loop of the insulin-receptor kinase. *Biochem J* 335 ( Pt 1): 103-109, 1998.

21. **Kushner JA, Ye J, Schubert M, Burks DJ, Dow MA, Flint CL, Dutta S, Wright CV, Montminy MR, and White MF.** Pdx1 restores beta cell function in Irs2 knockout mice. *J Clin Invest* 109: 1193-1201, 2002.

22. **Lee JY, Gavrilova O, Davani B, Na R, Robinson GW, and Hennighausen L.** The transcription factors Stat5a/b are not required for islet development but modulate pancreatic beta-cell physiology upon aging. *Biochim Biophys Acta* 1773: 1455-1461, 2007.

23. **Leibiger IB, Leibiger B, Moede T, and Berggren PO.** Exocytosis of insulin promotes insulin gene transcription via the insulin receptor/PI-3 kinase/p70 s6 kinase and CaM kinase pathways. *Mol Cell* 1: 933-938, 1998.

24. **Li M, Ren D, Iseki M, Takaki S, and Rui L.** Differential role of SH2-B and APS in regulating energy and glucose homeostasis. *Endocrinology* 147: 2163-2170, 2006.

25. **Li Z, Zhou Y, Carter-Su C, Myers MG, Jr., and Rui L.** SH2B1 Enhances Leptin Signaling by Both Janus Kinase 2 Tyr813 Phosphorylation-Dependent and -Independent Mechanisms. *Mol Endocrinol* 21: 2270-2281, 2007.

26. **Lin X, Taguchi A, Park S, Kushner JA, Li F, Li Y, and White MF.** Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. *J Clin Invest* 114: 908-916, 2004.

27. **Lukic ML, Stosic-Grujicic S, and Shahin A.** Effector mechanisms in low-dose streptozotocin-induced diabetes. *Dev Immunol* 6: 119-128, 1998.

28. **Morris DL, Cho KW, Zhou Y, and Rui L.** SH2B1 Enhances Insulin Sensitivity by Both Stimulating the Insulin Receptor and Inhibiting Tyrosine Dephosphorylation of IRS Proteins. *Diabetes* 58: 2039-2047, 2009.

29. **Nakae J, Biggs WH, Kitamura T, Cavenee WK, Wright CV, Arden KC, and Accili D.** Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. *Nat Genet* 32: 245-253, 2002.

30. **Niswender KD and Magnuson MA.** Obesity and the beta cell: lessons from leptin. *J Clin Invest* 117: 2753-2756, 2007.

31. **Poitout V.** Glucolipotoxicity of the pancreatic beta-cell: myth or reality? *Biochem Soc Trans* 36: 901-904, 2008.

32. **Poitout V and Robertson RP.** Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocr Rev* 29: 351-366, 2008.

33. **Prentki M and Nolan CJ.** Islet beta cell failure in type 2 diabetes. *J Clin Invest* 116: 1802-1812, 2006.

34. **Qian X, Riccio A, Zhang Y, and Ginty DD.** Identification and characterization of novel substrates of Trk receptors in developing neurons. *Neuron* 21: 1017-1029, 1998.

35. **Ren D, Li M, Duan C, and Rui L.** Identification of SH2-B as a key regulator of leptin sensitivity, energy balance, and body weight in mice. *Cell Metabolism* 2: 95-104, 2005.

36. **Ren D, Zhou Y, Morris D, Li M, Li Z, and Rui L.** Neuronal SH2B1 is essential for controlling energy and glucose homeostasis. *J Clin Invest* 117: 397-406, 2007.

37. **Riedel H, Yousaf N, Zhao Y, Dai H, Deng Y, and Wang J.** PSM, a mediator of PDGF-BB-, IGF-I-, and insulin-stimulated mitogenesis. *Oncogene* 19: 39-50, 2000.

38. **Rui L and Carter-Su C.** Platelet-derived growth factor (PDGF) stimulates the association of SH2-Bbeta with PDGF receptor and phosphorylation of SH2-Bbeta. *J Biol Chem* 273: 21239-21245, 1998.

39. **Rui L, Herrington J, and Carter-Su C.** SH2-B is required for nerve growth factor-induced neuronal differentiation. *J Biol Chem* 274: 10590-10594, 1999.

40. **Rui L, Mathews LS, Hotta K, Gustafson TA, and Carter-Su C.** Identification of SH2-Bbeta as a substrate of the tyrosine kinase JAK2 involved in growth hormone signaling. *Mol Cell Biol* 17: 6633-6644, 1997.

41. **Tsuji A and Sakurai H.** Generation of nitric oxide from streptozotocin (STZ) in the presence of copper(II) plus ascorbate: implication for the development of STZ-induced diabetes. *Biochem Biophys Res Commun* 245: 11-16, 1998.

42. **Vasavada RC, Garcia-Ocana A, Zawalich WS, Sorenson RL, Dann P, Syed M, Ogren L, Talamantes F, and Stewart AF.** Targeted expression of placental lactogen in the beta cells of transgenic mice results in beta cell proliferation, islet mass augmentation, and hypoglycemia. *J Biol Chem* 275: 15399-15406, 2000.

43. **Wang J and Riedel H.** Insulin-like growth factor-I receptor and insulin receptor association with a Src homology-2 domain-containing putative adapter. *J Biol Chem* 273: 3136-3139, 1998.

44. **Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, and White MF.** Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. *Nat Genet* 23: 32-40, 1999.

45. **Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, and White MF.** Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391: 900-904, 1998.

46. **Xu GG and Rothenberg PL.** Insulin receptor signaling in the beta-cell influences insulin gene expression and insulin content: evidence for autocrine beta-cell regulation. *Diabetes* 47: 1243-1252, 1998.

47. **Zhang Y, Zhu W, Wang YG, Liu XJ, Jiao L, Liu X, Zhang ZH, Lu CL, and He C.** Interaction of SH2-Bbeta with RET is involved in signaling of GDNF-induced neurite outgrowth. *J Cell Sci* 119: 1666-1676, 2006.

# Chapter 5

## Conclusions and Future Directions

### Conclusions

SH2B1 is a multifunctional adapter protein expressed in multiple tissues. Until recently, the physiological functions of SH2B1 were largely unknown. Using genetically modified mice, our laboratory has made significant strides in elucidating the essential physiological roles of SH2B1. In this Dissertation, I presented animal and biochemical data that indicates SH2B1 regulates insulin sensitivity and glucose homeostasis in mice by multiple mechanisms.

#### Central SH2B1 indirectly controls insulin sensitivity by regulating energy balance

Our laboratory previously demonstrated that neuron-specific restoration of SH2B1 corrects energy imbalance and prevents obesity in SH2B1-knockout (KO) mice (35). Restoring central SH2B1 in SH2B1-KO mice also improved systemic insulin sensitivity, presumably by controlling adiposity (35). In cultured cells, the SH2 domain of SH2B1 alone is sufficient to promote JAK2 activation and leptin signaling. Conversely, a dominant negative mutant form of SH2B1 (R555E) that contains a point mutation within the SH2 domain blocks both leptin and insulin signaling in cells. In chapter 2, I generated and characterized transgenic mice expressing either truncated SH2B1 (Δ504) or mutant SH2B1 (R555E) in neurons to test whether the SH2 domain of neuronal SH2B1 is sufficient and necessary for the regulation of body weight in mice. Surprisingly, expression of SH2B1 (Δ504) in neurons was not sufficient to prevent the development of obesity and obesity associated metabolic disease in SH2B1-KO mice. These findings suggest that multiple functional domains within SH2B1 are required for neuronal SH2B1 to control energy balance and body weight in mice.

As expected, SH2B1 (R555E) failed to prevent the development of obesity in SH2B1-KO mice, indicating that the SH2 domain of neuronal SH2B1 is required. However, overexpression of SH2B1 (R555E) in neurons of wild type mice induced obesity and caused insulin resistance. Interestingly, the onset of obesity in TgR555E mice was delayed relative to SH2B1-KO mice, and TgR555E mice were also not as heavy as age-matched SH2B1-KO mice. This finding suggests that mutations in the coding region of SH2B1 which alter SH2 domain function may be dominant and result in obesity. In support of this concept, a naturally occurring mutant form of SH2B2β, which lacks the SH2 domain, but has intact dimerization and PH domains, can bind to SH2B1 and antagonize the ability of SH2B1 to promote leptin signaling in cultured cells (21).

**Peripheral SH2B1 directly controls insulin sensitivity by regulating insulin signaling**

In chapter 3, I demonstrated that SH2B1 in peripheral tissues contributes directly to the regulation of glucose metabolism by promoting insulin signaling in skeletal muscle, liver, and white adipose tissue. We generated TgKO mice that express SH2B1 only in the brain but not in peripheral tissues (e.g. skeletal muscle, liver, and adipose tissue). Body weight was similar between TgKO and wild type littermates fed either normal chow or high fat diet (HFD), consistent with our previous conclusion that neuronal SH2B1 controls energy balance and body weight by promoting leptin sensitivity (35). However, loss of peripheral SH2B1 markedly impaired insulin sensitivity independent of body weight. TgKO mice developed hyperglycemia, hyperinsulinemia and glucose intolerance to a greater extent than wild type mice fed HFD. The ability of exogenous insulin to reduce blood glucose and to stimulate insulin receptor autophosphorylation and activate the IRS/PI3K/Akt pathway in muscle, liver, and white adipose tissue was significantly impaired in TgKO mice. It is interesting to note that this insulin resistance phenotype was observed only after TgKO mice are fed HFD. This suggests that peripheral SH2B1 is particularly important for insulin action during bouts of overnutrition.

**SH2B1 promotes insulin signaling by multiple mechanisms**

In chapter 3, I examined the molecular mechanisms by which SH2B1 regulates insulin signaling and demonstrated that SH2B1 cell autonomously promotes insulin signaling by multiple mechanisms. First, SH2B1 enhances insulin receptor (IR) activity

by binding via its SH2 domain to phospho-Tyr$^{1158}$ in the activation loop of IR. The SH2 domain was sufficient to enhance IR activity in vitro, as well as promote the activation of signaling pathways downstream of IR. Interestingly, SH2B1 still enhanced insulin signaling in cells with a mutant IR lacking the SH2B1 binding site (Y1158F). These findings led to the subsequent observation that SH2B1 binds to phosphorylated IRS proteins, and somehow protects them from dephosphorylation by both recombinant and cellular phosphatases.

Based on these findings, we proposed a new model for how SH2B1 promotes insulin action. First, SH2B1 binds to IR and stimulates IR catalytic activity to globally activate downstream signaling pathways. Second, SH2B1 binds to both IRS-1 and IRS-2 and protects IRS proteins from tyrosine dephosphorylation, augmenting and/or prolonging IRS protein-mediated pathways. Third, by forming dimers via their respective DD domains, each SH2B1 molecule in a SH2B1 dimer could simultaneously bind to IR and IRS-1 (or IRS-2), thereby stabilizing IR/IRS-1 (or IR/IRS-2) complexes.

**Pancreatic SH2B1 contributes to β cell function in leptin-deficient mice**

Multiple growth factors promote β-cell function and some of these growth factors (insulin, IGF-1, prolactin, and GH) utilize SH2B1 as a signaling mediator. SH2B1 is expressed at high levels β-cells within the islet, as well as in immortalized β-cell lines. In chapter 4, we began to explore the role of SH2B1 in the pancreas in vivo.

I demonstrated that Sh2b1 haploinsufficiency dramatically exacerbates the severity of diabetes, but not obesity, in leptin-deficient (*ob/ob*) mice. Although insulin resistance was similar in *Sh2b1*$^{+/-}$*ob/ob* and *ob/ob* mice, *Sh2b1*$^{+/-}$*ob/ob* were severely hyperglycemic due to due to β-cell dysfunction. Pancreatic insulin content, but not pancreas mass or β-cell area, was dramatically reduced in *Sh2b1*$^{+/-}$*ob/ob* mice. The defects in insulin production appear to explain glucose intolerance and the inability of these mice to increase postprandial insulin levels. These data support the overall idea that SH2B1 promotes glucose metabolism independent of its role as a leptin signaling molecule in the brain. More importantly, these findings suggest that SH2B1 in the β-cell is particularly necessary for glucose homeostasis during cases of extreme obesity.

Although the underlying mechanism for β-cell failure in genetically obese mice with reduced levels of SH2B1 is not clear, one intriguing possibility is that SH2B1 may

protect β-cells from gluco- or lipotoxicity, which is thought to contribute to β-cell dysfunction during obesity (2, 3, 18). However, pancreas-specific deletion of SH2B1 alone did not alter glucose homeostasis in mice during diet-induced obesity. Therefore, future studies are required to elucidate the mechanisms by which SH2B1 promotes β-cell function and/or survival, particularly during metabolic stress.

## Future Directions

The studies presented in this dissertation have proved new insight into the role of SH2B1 in vivo, particularly with regards to the regulation of systemic insulin sensitivity and glucose homeostasis. Additionally, cell culture studies and biochemical assays provided a new model for the regulation of insulin signaling by SH2B1. However, there is still a lot about the role SH2B1 in mammalian physiology that is unknown.

### Regulation of SH2B1 function in health and disease

Our findings indicate that SH2B1 functions as both a leptin sensitizer and insulin sensitizer in mice. SH2B1 promotes leptin and insulin action in cells by multiple mechanisms, including promoting JAK2 and IR kinase activity and preventing dephosphorylation of IRS proteins (10, 22, 30). Interestingly, overexpression of SH2B1 can counteract the negative effects of PTP1B on both leptin and insulin signaling in cultured cells (30, 34). Thus, both leptin- and insulin sensitivity may be determined by a cellular balance between negative regulators (e.g. PTP1B) and positive regulators (SH2B1). In support of this, PTP1B expression is increased in both leptin- and insulin resistant mice (31, 45, 48). Although SH2B1 is expressed in multiple tissues, relatively little is known about whether SH2B1 expression is regulated. Therefore, it is important to determine whether and how SH2B1 expression and/or function are regulated in vivo.

SH2B1 protein levels increase in 3T3-L1 preadipocytes during differentiation into adipocytes (35, 47), indicating that SH2B1 protein levels can be regulated, at least in adipocytes. Others have reported that SH2B1 mRNA abundance increased in white adipose tissue from leptin receptor-deficient (*db/db*) and from mice fed a high fat diet (47); by contrast, preliminary data from our laboratory indicate that SH2B1 protein is decreased in white adipose tissue from leptin-deficient (*ob/ob*) mice (K. Cho and L. Rui, unpublished data). Interpretation of these data is complicated by the observations that SH2B1 is expressed in both macrophages and T-cells (8, 20), two populations of cells

which infiltrate adipose tissue and accumulate during obesity (23-25, 32). Thus, it is unclear whether data from either study truly reflects changes in SH2B1 expression in adipocytes in vivo. Moreover, it is unclear whether SH2B1 expression is altered in hypothalamic neurons, skeletal muscle, liver, or β-cells in pathophysiological conditions associated with weight gain, insulin resistance, or diabetes.

SH2B1 migrates as a broad band on SDS-PAGE gels whereas in vitro dephosphorylation of immunopurified SH2B1 with alkaline phosphatase causes SH2B1 to migrate as a tight band, suggesting that SH2B1 is a phospho-protein which may be regulated by post-translational modifications. SH2B1 is phosphorylated on tyrosine residues by JAK2, TrkB, and RET (9, 33, 40, 43). By contrast, SH2B1 does not appear to be a good substrate for IR (D. Morris and L. Rui, unpublished data). The importance of tyrosine phosphorylation of SH2B1 is largely unknown, but one hypothesis is specific phosphotyrosines within SH2B1 may provide additional binding sites for other signaling molecules.

SH2B1 also contains multiple serine and threonine residues, which appear to be phosphorylated both in the absence and presence of stimulation (37, 39, 40). One serine, Ser 96, has been identified as being phosphorylated by downstream of mitogen-activated protein kinase kinase (MEK) (39); however, other specific serine phosphorylation sites in SH2B1, and their function, have not been identified. Serine/threonine phosphorylation of SH2B1 may inhibit SH2B1 function and/or alter the affinity of SH2B1 for its binding partners, analogous to inhibitory serine/threonine phosphorylation of IRS-1 (1, 19). Specifically, phosphorylation of IRS-1 on serine 307 disrupts PTB domain function, inhibiting IRS-1 from binding to the IR. Serine phosphorylation also promotes ubiquitination and proteosome-mediated degradation of IRS proteins, limiting the pool of available substrate and hindering the ability of insulin to activate the PI3K/Akt pathway (46). Therefore, additional studies are required to identify tyrosine, serine, and threonine residues which are phosphorylated and regulate SH2B1 function. Determining if phosphorylation on these sites correlates with either leptin/insulin sensitivity or resistance is also critical to understanding the regulation of SH2B1 in health and disease.

**SH2B1 in the central nervous system**

Our data indicate that SH2B1 in neurons plays a critical role in the regulation of energy homeostasis and leptin sensitivity. The leptin receptor is expressed in multiple regions of the brain, including the arcuate nucleus (ARC), the ventromedial (VMH), dorsomedial hypothalamic nuclei (DMH), ventral tegmental area (VTA), hippocampus, and the brainstem (7, 12-16, 28, 29, 41, 42). SH2B1 is expressed in the hypothalamus of mice, and preliminary immunostaining experiments have demonstrated that SH2-B protein is expressed in multiple neurons, including clusters of neurons in the ARC and VMH (D. Morris and L. Rui, unpublished data). However, the identity of SH2B1-positive neurons has not been determined. Future studies are needed to determine whether SH2B1 is expressed in neuronal populations that are relevant to leptin action.

Typically, obesity in leptin-signaling impaired mice is a result of both increased food intake and decreased energy expenditure. Surprisingly, SH2B1-KO mice are both hyperphagic and have increased energy expenditure; however, subtracting energy expenditure from total energy intake indicated that SH2B1-KO mice remain in a net positive energy balance, resulting in obesity (34). Additionally, overexpression of dominant negative SH2B1 (R555E) causes increased energy expenditure in mice, indicating that this abnormal elevation in energy expenditure is neuronal, rather than peripheral, in origin. One explanation for this aberrant increase in energy expenditure is that SH2B1 may differentially regulate leptin sensitivity in hypothalamic neurons controlling energy intake versus those controlling energy expenditure. SH2B1 may be required for leptin sensitivity in AgRP/NPY neurons which control energy intake; thus, SH2B1 deficiency induces severe leptin resistance in these neurons, resulting in hyperphagia and hyperleptinemia. In contrast, SH2B1 may play a minor role in the regulation of neurons that control energy expenditure; therefore, hyperleptinemia in SH2B1-KO mice may chronically activate neurons that have normal or only slightly impaired leptin sensitivity, resulting in increased energy expenditure. Consistent with this idea, systemic deletion of SH2B1 increases AgRP and NPY expression, whereas POMC expression remained normal (34).

Alternatively, increased energy expenditure in SH2B1-KO and TgR555E mice may be leptin-independent. SH2B1 is a signaling molecule for a number of neurotrophins, including brain-derived neurotrophic factor (BDNF) (6, 27, 33, 43).

Interestingly, BDNF haploinsufficiency or conditional deletion of BDNF in neurons results in leptin resistance, hyperphagia, and weight gain, as well as inter-male aggressiveness and increased locomoter activity (17, 26, 36). All of these phenotypes, including increased aggressiveness (L. Jiang and L. Rui, unpublished data), have been observed in SH2B1-KO mice, suggesting that the BDNF-regulated processes are also impaired in the absence of SH2B1. Interestingly, preliminary data indicate that conditional deletion of SH2B1 in neurons expressing the leptin receptor causes leptin resistance, hyperphagia, and obesity, but not aggressiveness in male mice (L. Jiang and L. Rui, unpublished data). This suggests that SH2B1 may independently promote both leptin and BDNF regulated processes in neurons.

Nerve growth factor (NGF) is critical for neuronal differentiation and survival and for synaptic plasticity (4). SH2B1 has been shown to promote both neurite outgrowth and neuronal differentiation in response to NGF (5, 11, 33, 38, 44). Whether NGF signaling is impaired in neurons lacking SH2B1 has not been explored. Thus, it is possible that impaired NGF signaling in SH2B1-KO mice may contribute to the obesity phenotype by altering the development of the neural circuitry that regulates energy homeostasis.

In summary, these possibilities suggest that neuronal SH2B1 not only regulates leptin action but possibly other processes in the central nervous system to control body weight. Regulation of BDNF and NGF signaling by central SH2B1 needs to be explored to fully appreciate the role of SH2B1 in neurons.

**SH2B1 in individual tissues**

To address the role of SH2B1 in individual tissues, we have generated conditional SH2B1-KO mice using the Cre/loxP system (chapter 4). Unidirectional loxP sites were introduced into the Sh2b1 locus in mice by homologous recombination. These mice will be used to delete SH2B1 in specific neurons, as well as the liver, skeletal muscle, white adipose tissue, and β-cells. To date, we have generated mice lacking SH2B1 in leptin-receptor expressing neurons, liver, and pancreas. Selective deletion of SH2B1 in leptin-receptor expressing neurons in mice (SH2B1-LRb-KO mice) results in obesity, further supporting our previous conclusions that SH2B1 is required for leptin sensitivity and regulation of body weight (L. Jiang and L. Rui, unpublished data). By contrast, deletion

of SH2B1 specifically in the liver (Y. Zhou and L. Rui, unpublished data) or in the pancreas (Chapter 4) resulted in very mild phenotypes, indicating that the collective actions of SH2B1 in multiple tissues may contribute to systemic insulin sensitivity and regulation of glucose homeostasis. Additional studies are required to examine the contribution of SH2B1 in other tissues to the regulation of energy balance and glucose homeostasis.

## References


1. **Aguirre V, Uchida T, Yenush L, Davis R, and White MF.** The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem* 275: 9047-9054, 2000.

2. **Bernard-Kargar C and Ktorza A.** Endocrine pancreas plasticity under physiological and pathological conditions. *Diabetes* 50 Suppl 1: S30-35, 2001.

3. **Chang-Chen KJ, Mullur R, and Bernal-Mizrachi E.** Beta-cell failure as a complication of diabetes. *Rev Endocr Metab Disord* 9: 329-343, 2008.

4. **Chao MV.** Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci* 4: 299-309, 2003.

5. **Chen L and Carter-Su C.** Adapter protein SH2-B beta undergoes nucleocytoplasmic shuttling: implications for nerve growth factor induction of neuronal differentiation. *Mol Cell Biol* 24: 3633-3647, 2004.

6. **Chen L, Maures TJ, Jin H, Huo JS, Rabbani SA, Schwartz J, and Carter-Su C.** SH2B1beta (SH2-Bbeta) enhances expression of a subset of nerve growth factor-regulated genes important for neuronal differentiation including genes encoding urokinase plasminogen activator receptor and matrix metalloproteinase 3/10. *Mol Endocrinol* 22: 454-476, 2008.

7. **Cheung CC, Clifton DK, and Steiner RA.** Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. *Endocrinology* 138: 4489-4492, 1997.

8. **Chu P, Pardo J, Zhao H, Li CC, Pali E, Shen MM, Qu K, Yu SX, Huang BC, Yu P, Masuda ES, Molineaux SM, Kolbinger F, Aversa G, de Vries J, Payan DG, and Liao XC.** Systematic identification of regulatory proteins critical for T-cell activation. *J Biol* 2: 21, 2003.

9. **Donatello S, Fiorino A, Degl'innocenti D, Alberti L, Miranda C, Gorla L, Bongarzone I, Rizzetti MG, Pierotti MA, and Borrello MG.** SH2B1beta adaptor is a key enhancer of RET tyrosine kinase signaling. *Oncogene* 26: 6546-6559, 2007.

10. **Duan C, Li M, and Rui L.** SH2-B promotes insulin receptor substrate 1 (IRS1)- and IRS2-mediated activation of the phosphatidylinositol 3-kinase pathway in response to leptin. *J Biol Chem* 279: 43684-43691, 2004.

11. **Eggert A, Ikegaki N, Liu X, Chou TT, Lee VM, Trojanowski JQ, and Brodeur GM.** Molecular dissection of TrkA signal transduction pathways mediating differentiation in human neuroblastoma cells. *Oncogene* 19: 2043-2051, 2000.

12. **Elmquist JK, Ahima RS, Elias CF, Flier JS, and Saper CB.** Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. *Proc Natl Acad Sci U S A* 95: 741-746, 1998.

13. **Elmquist JK, Ahima RS, Maratos-Flier E, Flier JS, and Saper CB.** Leptin activates neurons in ventrobasal hypothalamus and brainstem. *Endocrinology* 138: 839-842, 1997.

14. **Fulton S, Pissios P, Manchon RP, Stiles L, Frank L, Pothos EN, Maratos-Flier E, and Flier JS.** Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* 51: 811-822, 2006.

15. **Hakansson ML, Hulting AL, and Meister B.** Expression of leptin receptor mRNA in the hypothalamic arcuate nucleus--relationship with NPY neurones. *Neuroreport* 7: 3087-3092, 1996.

16. **Hommel JD, Trinko R, Sears RM, Georgescu D, Liu ZW, Gao XB, Thurmon JJ, Marinelli M, and DiLeone RJ.** Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 51: 801-810, 2006.

17. **Kernie SG, Liebl DJ, and Parada LF.** BDNF regulates eating behavior and locomotor activity in mice. *EMBO J* 19: 1290-1300, 2000.

18. **Leahy JL.** Natural history of beta-cell dysfunction in NIDDM. *Diabetes Care* 13: 992-1010, 1990.

19. **Lee YH, Giraud J, Davis RJ, and White MF.** c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. *J Biol Chem* 278: 2896-2902, 2003.

20. **Li L, Kong L, Fang X, Jiang C, Wang Y, Zhong Z, Sun Q, Gu G, Zheng D, Meng R, and Kang J.** SH2-B beta expression in alveolar macrophages in BAL fluid of asthmatic guinea pigs and its role in NGF-TrkA-mediated asthma. *Respirology* 14: 60-68, 2009.

21. **Li M, Li Z, Morris DL, and Rui L.** Identification of SH2B2beta as an inhibitor for SH2B1- and SH2B2alpha-promoted Janus kinase-2 activation and insulin signaling. *Endocrinology* 148: 1615-1621, 2007.

22. **Li Z, Zhou Y, Carter-Su C, Myers MG, Jr., and Rui L.** SH2B1 Enhances Leptin Signaling by Both Janus Kinase 2 Tyr813 Phosphorylation-Dependent and -Independent Mechanisms. *Mol Endocrinol* 21: 2270-2281, 2007.

23. **Lumeng CN, Bodzin JL, and Saltiel AR.** Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* 117: 175-184, 2007.

24. **Lumeng CN, DelProposto JB, Westcott DJ, and Saltiel AR.** Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. *Diabetes* 57: 3239-3246, 2008.

25. **Lumeng CN, Deyoung SM, Bodzin JL, and Saltiel AR.** Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. *Diabetes* 56: 16-23, 2007.

26. **Lyons WE, Mamounas LA, Ricaurte GA, Coppola V, Reid SW, Bora SH, Wihler C, Koliatsos VE, and Tessarollo L.** Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc Natl Acad Sci U S A* 96: 15239-15244, 1999.

27. **Maures TJ, Chen L, and Carter-Su C.** Nucleocytoplasmic Shuttling of the Adapter Protein SH2B1{beta} is Required for Nerve Growth Factor (NGF)-Dependent Neurite Outgrowth and Enhancement of Expression of a Subset of NGF-Responsive Genes. *Mol Endocrinol*, 2009.

28. **McMinn JE, Sindelar DK, Havel PJ, and Schwartz MW.** Leptin deficiency induced by fasting impairs the satiety response to cholecystokinin. *Endocrinology* 141: 4442-4448, 2000.

29. **Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, and Trayhurn P.** Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. *FEBS Lett* 387: 113-116, 1996.

30. **Morris DL, Cho KW, Zhou Y, and Rui L.** SH2B1 Enhances Insulin Sensitivity by Both Stimulating the Insulin Receptor and Inhibiting Tyrosine Dephosphorylation of IRS Proteins. *Diabetes* 58: 2039-2047, 2009.

31. **Morrison CD, White CL, Wang Z, Lee SY, Lawrence DS, Cefalu WT, Zhang ZY, and Gettys TW.** Increased hypothalamic protein tyrosine phosphatase 1B contributes to leptin resistance with age. *Endocrinology* 148: 433-440, 2007.

32. **Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K, Kadowaki T, and Nagai R.** CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med* 15: 914-920, 2009.

33. **Qian X, Riccio A, Zhang Y, and Ginty DD.** Identification and characterization of novel substrates of Trk receptors in developing neurons. *Neuron* 21: 1017-1029, 1998.

34. **Ren D, Li M, Duan C, and Rui L.** Identification of SH2-B as a key regulator of leptin sensitivity, energy balance, and body weight in mice. *Cell Metabolism* 2: 95-104, 2005.

35. **Ren D, Zhou Y, Morris D, Li M, Li Z, and Rui L.** Neuronal SH2B1 is essential for controlling energy and glucose homeostasis. *J Clin Invest* 117: 397-406, 2007.

36. **Rios M, Fan G, Fekete C, Kelly J, Bates B, Kuehn R, Lechan RM, and Jaenisch R.** Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol Endocrinol* 15: 1748-1757, 2001.

37. **Rui L and Carter-Su C.** Platelet-derived growth factor (PDGF) stimulates the association of SH2-Bbeta with PDGF receptor and phosphorylation of SH2-Bbeta. *J Biol Chem* 273: 21239-21245, 1998.

38. **Rui L, Herrington J, and Carter-Su C.** SH2-B is required for nerve growth factor-induced neuronal differentiation. *J Biol Chem* 274: 10590-10594, 1999.

39. **Rui L, Herrington J, and Carter-Su C.** SH2-B, a membrane-associated adapter, is phosphorylated on multiple serines/threonines in response to nerve growth factor by kinases within the MEK/ERK cascade. *J Biol Chem* 274: 26485-26492, 1999.

40. **Rui L, Mathews LS, Hotta K, Gustafson TA, and Carter-Su C.** Identification of SH2-Bbeta as a substrate of the tyrosine kinase JAK2 involved in growth hormone signaling. *Mol Cell Biol* 17: 6633-6644, 1997.

41. **Schwartz MW, Seeley RJ, Campfield LA, Burn P, and Baskin DG.** Identification of targets of leptin action in rat hypothalamus. *J Clin Invest* 98: 1101-1106, 1996.

42. **Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P, and Baskin DG.** Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. *Diabetes* 46: 2119-2123, 1997.

43. **Suzuki K, Mizutani M, Hitomi Y, Kizaki T, Ohno H, Ishida H, Haga S, and Koizumi S.** Association of SH2-B to phosphorylated tyrosine residues in the activation loop of TrkB. *Res Commun Mol Pathol Pharmacol* 111: 27-39, 2002.

44. **Wang X, Chen L, Maures TJ, Herrington J, and Carter-Su C.** SH2-B is a positive regulator of nerve growth factor-mediated activation of the Akt/Forkhead pathway in PC12 cells. *J Biol Chem* 279: 133-141, 2004.

45. **White CL, Whittington A, Barnes MJ, Wang ZQ, Bray G, and Morrison CD.** HF diets increase hypothalamic PTP1B and induce leptin resistance through both leptin-dependent and independent mechanisms. *Am J Physiol Endocrinol Metab*, 2008.

46. **White MF.** IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab* 283: E413-422, 2002.

47. **Yoshiga D, Sato N, Torisu T, Mori H, Yoshida R, Nakamura S, Takaesu G, Kobayashi T, and Yoshimura A.** Adaptor protein SH2-B linking receptor-tyrosine kinase and Akt promotes adipocyte differentiation by regulating peroxisome proliferator-activated receptor gamma messenger ribonucleic acid levels. *Mol Endocrinol* 21: 1120-1131, 2007.

48. **Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG, and Kahn BB.** Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. *J Biol Chem* 283: 14230-14241, 2008.