Supporting Online Material
Materials and methods

Animals, Treatments and Surgical Procedures. *Adrα2A*$^{-/-}$, *Osteocalcin*$^{-/-}$, *Esp*$^{-/-}$, *Atf4*$^{-/-}$, *Lep*$^{flox/flox}$, *Sf1-Cre* and *a1(I)Collagen-Cre* were previously described (*1-7*). Osteoblasts-specific *Adrβ2*-deficient (*Adrβ2*$_{osb-/-}$) mice were generated through homologous recombination in ES cells and the use of *α1(I)collagen-Cre* mice. B6.V-*Lep*^{ob}/J, B6.Cg-*m*$^{+/+}$*Lepr*^{db}/J, Tg(Pomc1-cre)16Lowl/J and FVB-Tg(AZIP/F)1Vsn/J mice were purchased from the Jackson Laboratory. Isoproterenol (3 mg kg^{-1}) was injected intraperitoneally (IP) once daily for 2 weeks. For intracerebroventricular infusion, a 28-gauge cannula (Brain infusion kit II, Alza) was implanted into the third ventricle as previously described infusing human leptin (Sigma) at 4 ng hr^{-1} for 7 days (*8*). The cannula was connected to an osmotic pump (Alza) placed in the dorsal subcutaneous space of the animal. Genotyping was performed by PCR analysis of genomic DNA. All procedures involving animals were approved by the IACUC and conform to the relevant regulatory standards.

Metabolic studies. For glucose tolerance tests (GTT) glucose (0.625 or 2 g kg^{-1}) was injected IP after an overnight fast, and blood glucose was monitored using blood glucose strips and the Accu-Check glucometer (Roche) at indicated times. For insulin tolerance test (ITT) mice were injected IP with insulin (0.5 U kg^{-1}), and blood glucose levels were measured at indicated times. ITT data are presented as percentage of initial blood glucose concentration. Hyperinsulinemic-euglycemic clamps and hyperglycemic clamps were performed at the Penn State Diabetes & Obesity Mouse Phenotyping Center. Briefly, *ob/ob*, *ob/ob;Esp*$^{-/-}$ and WT littermates mice (n = 4 for each group) were fasted overnight, and a 2-hr hyperinsulinemic (2.5 mU kg^{-1} min^{-1})-euglycemic clamp was performed following intravenous administration of [3-^{3}H] glucose and 2-deoxy-D-[1-^{14}C] glucose as previously described (*9*). *Adrβ2*$_{osb}$*-/-* and control mice (n = 4 for each group) were fasted overnight, and a 2-hr hyperglycemic clamp was conducted with a variable infusion of 20% glucose to maintain hyperglycemia at ~300 mg dl^{-1}. Blood samples were taken at 10 to 20 min intervals for measurement of plasma insulin levels (*10*).

Islet perifusion. Islet perifusion was performed as described previously (*11*). In brief, islets were isolated from WT mice (CD1) using standard collagenase digestion followed by purification through a Ficoll gradient. One hundred islets were hand picked under a light microscope and placed into a perifusion chamber (Millipore). A computer-controlled fast-performance HPLC system (625 LC System, Waters Corporation) allowed programmable rates of flow and concentration of the appropriate solutions held in a 37°C water bath. Islets were perifused with Krebs bicarbonate buffer (2.2 mM Ca^{2+}, 0.25% bovine serum albumin, 10 mM HEPES, and 95% O_2/5% CO_2 equilibration [pH 7.35]) to reach baseline hormone secretion values before the addition of the appropriate secretagogues. Samples were collected at regular intervals with a fraction collector

(Waters Corporation). Insulin content was determined using a radioimmunoassay (University of Pennsylvania Diabetes Center).

In vitro glucose stimulated insulin secretion. For assay of insulin release (*12*), 5 islets were manually selected, incubated in Krebs-Ringer solution, and stimulated at 37 °C with various concentrations of glucose in the presence or absence of osteocalcin for 1 hour. The islets were then collected by centrifugation, and the supernatant was assayed for insulin content by ELISA (Crystal Chem Kit).

Cell and molecular studies. Primary osteoblasts were prepared from calvaria of 5-day-old pups as previously described (*8*) and were cultured in αMEM/10% FBS in the presence of 100 μg ml^{-1} ascorbic acid and 5 mM β-glycerophosphate for 5 days. Real-time PCR was performed on DNaseI-treated total RNA converted to cDNA using Taq SYBR Green Supermix with ROX on an MX3000 instrument; *β-actin* amplification was used as an internal reference for each sample. ChIP assays and immunoblotting were performed as previously described (*4*) using rabbit anti-NeuroD (Cell signaling), rabbit anti-phospho-CREB (Ser133) (Cell signaling) and rabbit anti-CREB (SantaCruz) antibodies. COS cells and ROS17/2.8 were transfected as described (*4*) using 0.5 μg of various Luciferase reporter vectors, 0.05 μg of RSV-β-gal reporter vectors and 0.5 μg of each expression plasmids unless otherwise indicated. Luciferase and β-galactosidase assays were performed using standard procedures.

Biochemistry. Serum levels of insulin (Crystal Chem Kit) and c-peptide (Yanaihara Institute kit) were measured using commercial kits. For quantification of uncarboxylated levels of osteocalcin sera were incubated with hydroxyapatite slurry for 1 hour. The quantity of osteocalcin present in the unbound fraction and in the fractions eluted at 0.02 M and 1 M sodium phosphate (pH 6.8) were measured by IRMA (Immunotopics Kit).

Histology. Tissue was fixed in 10% neutral formalin, embedded in paraffin and sectioned at 5 μm; sections were stained with hematoxylin and eosin. Immunohistochemistry was performed using rabbit anti-insulin (SantaCruz) and mouse anti-Ki67 (Vector) antibodies. β-cell area represents the surface positive for insulin immunostaining divided by the total pancreatic surface. β-cell mass was calculated as β-cell area multiplied by pancreatic weight as described previously (*3*).

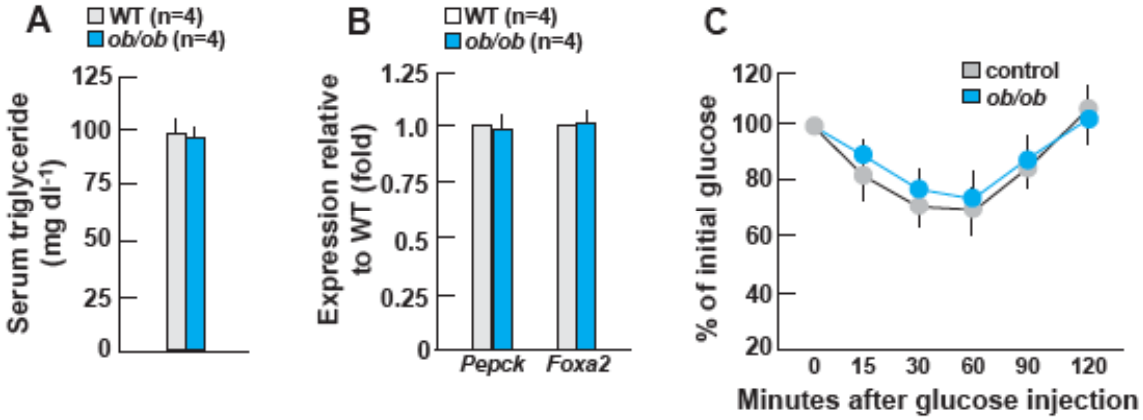
Statistical analyses. Results are given as means ± standard error of the mean (SEM). Statistical analyses were performed using unpaired, two-tailed Student's *t* or ANOVA tests.

Supporting references

1. J. D. Altman et al., *Mol. Pharmacol.* **56**, 154 (1999).
2. P. Ducy et al., *Nature* **382**, 448 (1996).
3. N. K. Lee et al., *Cell* **130**, 456 (2007).
4. X. Yang et al., *Cell* **117**, 387 (2004).
5. E. van de Wall et al., *Endocrinology* **149**, 1773 (2008).
6. H. Dhillon et al., *Neuron* **49**, 191 (2006).
7. R. Dacquin, M. Starbuck, T. Schinke, G. Karsenty, *Dev. Dyn.* **224**, 245 (2002).
8. P. Ducy et al., *Cell* **100**, 197 (2000).
9. H. J. Kim et al., *Diabetes* **53**, 1060 (2004).
10. Y. R. Cho et al., *Am. J. Physiol. Endocrinol. Metab.* **293**, E327 (2007).
11. N. Gao et al., *Cell Metab.* **6**, 267 (2007).
12. T. Kitamura et al., *Mol. Cell. Biol.* **21**, 5624 (2001).

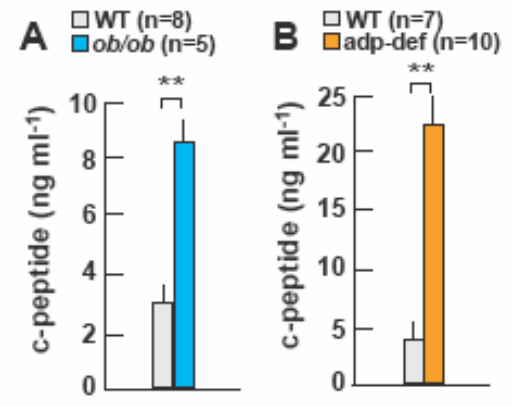
Supporting figures

Fig. S1



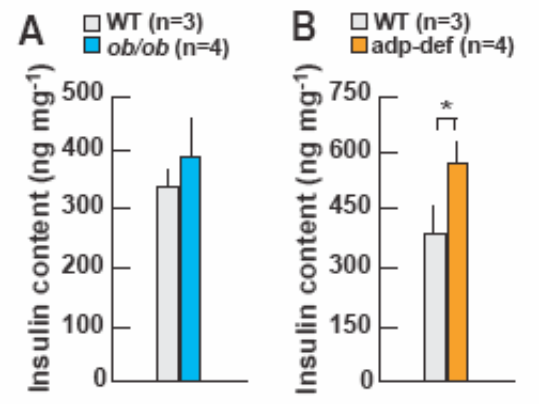
Absence of metabolic disturbance in 2 week-old *ob/ob* mice. (A) Serum triglyceride in 2 week-old *ob/ob* mice. (**B**) Expression of markers of insulin sensitivity in liver and (**C**) ITT in 2 week-old *ob/ob* mice.

Fig. S2



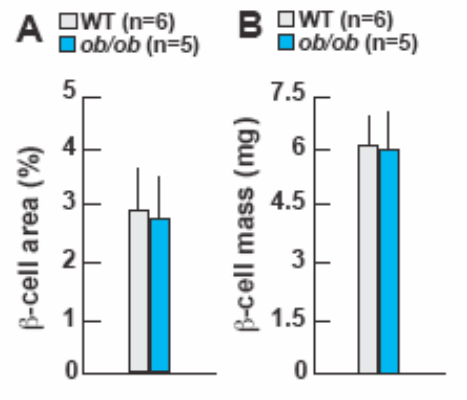
Serum c-peptide in 2 week-old *ob/ob* and adipocyte-deficient mice.

Fig. S3



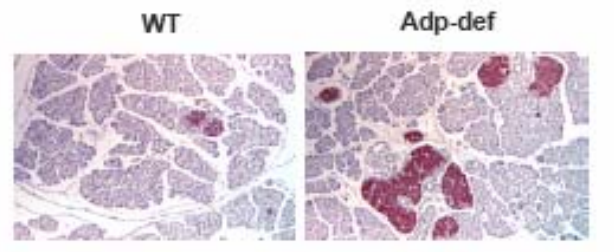
Insulin content in pancreas of 2 week-old *ob/ob* and adipocyte-deficient mice.

Fig. S4



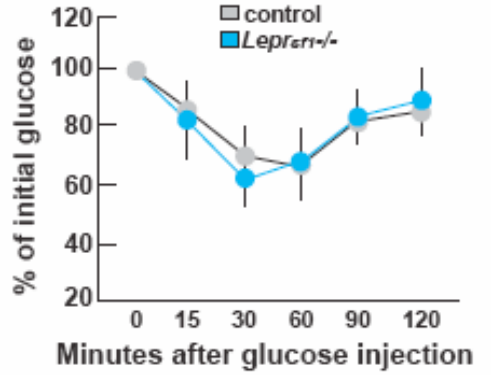
β-cell area and β-cell mass in 2 week-old *ob/ob* mice.

Fig. S5



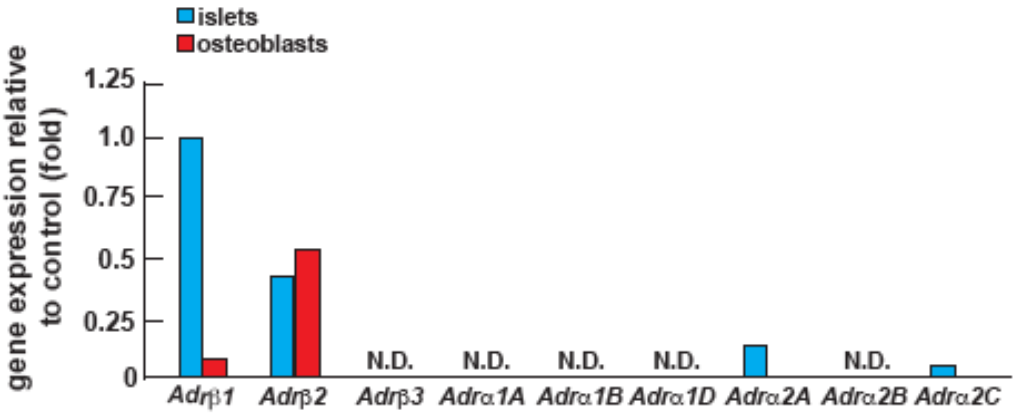
Insulin immunostaining in pancreas of 2 week-old adipocyte-deficient mice.

Fig. S6



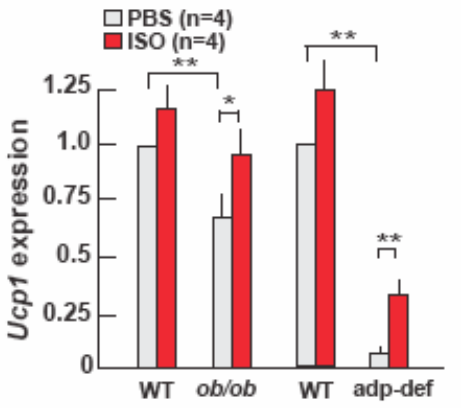
ITT in 1 month-old *Lepr*$_{sf1}$*-/-* mice.

Fig. S7



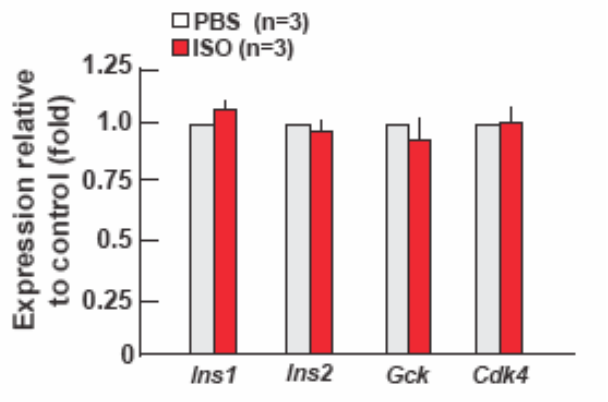
Adrenergic receptor expression in islets and osteoblasts.

Fig. S8



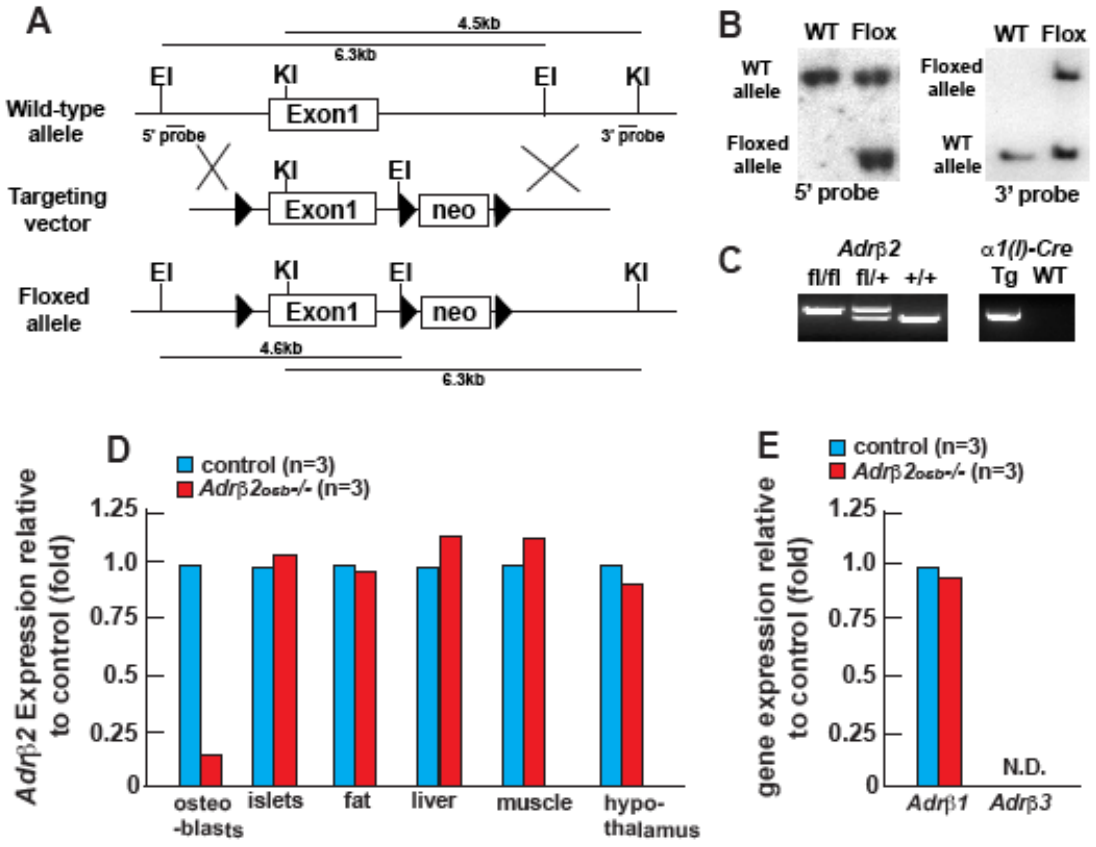
Ucp1 expression in brawn fat of *ob/ob* and adipocyte-deficient mice after daily isoproterenol injection for 2 weeks.

Fig. S9



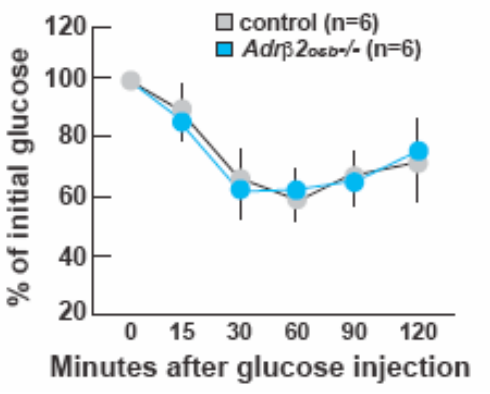
Gene expression by isoproterenol in islets.

Fig. S10



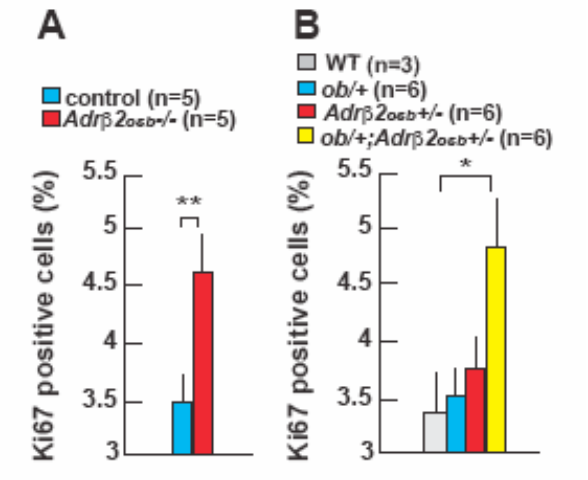
Generation of *Adrβ2*$_{osb}$-/- mice. (A) Targeting construct for conditional inactivation of *Adrβ2*. White box, exon; black triangles, LoxP sites; EI, EcoRI; KI, KpnI. **(B)** Southern blot analysis of *Adrβ2-flox* clone. The 5' probe detected a 6.3kb WT and 4.6kb targeted band while 3' probe detected 4.5kb WT and 6.3kb targeted band. **(C)** PCR genotyping of *Adrβ2*$_{osb}$-/- mice. WT and floxed allele yield 250bp and 290bp products, respectively. *α1(I)collagen-Cre* transgenic mice harbor a transgene-specific band. **(D)** *Adrβ2* expression in several tissues of *Adrβ2*$_{osb}$-/- mice. **(E)** *Adrβ1* and *Adrβ3* expression in osteoblasts of *Adrβ2*$_{osb}$-/- mice. N.D.; not detected.

Fig. S11



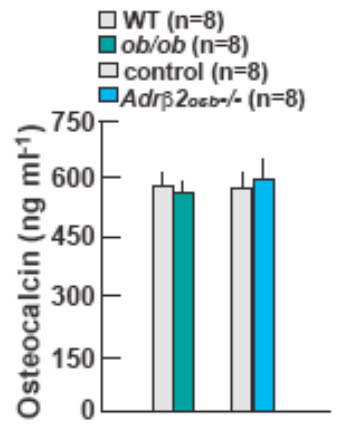
ITT in 2 month-old *Adrβ2*$_{osb}$-/- mice.

Fig. S12



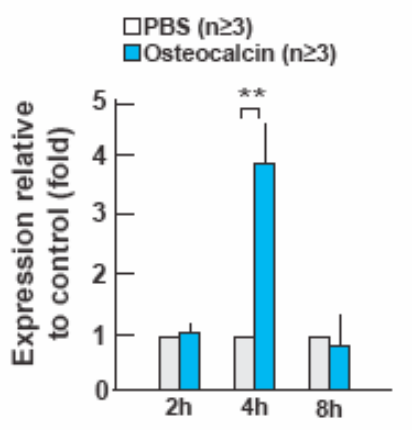
Insulin/Ki67-immunoreactive cells in islets of 2 week-old (A) *Adrβ2*$_{osb}$-/- and (B) *ob/+;Adrβ2*$_{osb}$+/- mice.

Fig. S13



Total serum osteocalcin in 2 week-old *ob/ob* and *Adrβ2*$_{osb}$-/- mice.

Fig. S14



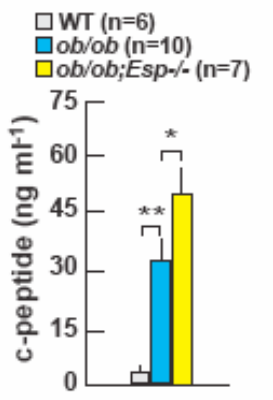
Time course of *Insulin* expression by uncarboxylated osteocalcin in islets.

Fig. S15

	Geno-type	No. of pups	% of total
ob/+ x *ob/+*	*ob/ob*	34/156	21.8
ob/+;Esp-/- x *ob/+;Esp-/-*	*ob/ob; Esp-/-*	17/143	11.9

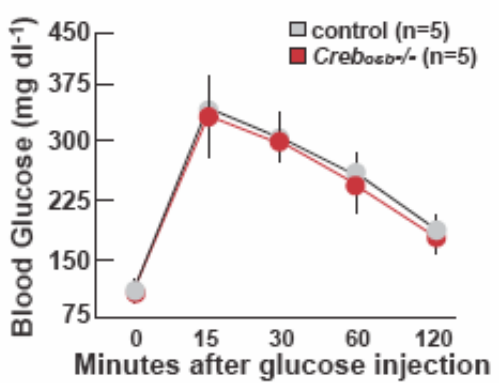
Lethality of *ob/ob;Esp*$^{-/-}$ pups.

Fig. S16



Serum c-peptide in *ob/ob;Esp*$^{-/-}$ mice.

Fig. S17



GTT in 3 month-old *Creb*$_{osb}$*-/-* mice.

Table S1. Mutant mice used in this study and their phenotypes. n.d.: not determined. Background of mice; *ob/ob*, *Atf4-/-*, *Creb*$_{osb-/-}$, *Adrβ2*$_{osb-/-}$, *ob/+;Adrβ2*$_{osb}$*+/-* and *Adrβ2*$_{osb}$*+/-;Atf4+/-*: C57BL/6 and *A-ZIP/F-1*: FVB.

Name	Blood glucose	Insulin in serum	*Insulin* genes	Fat mass	β-cell proliferation	Osteocalcin (total)	Osteocalcin (HA-low affinity)
ob/ob	↓	↑	↑	↔	↑	↔	↑
A-ZIP/F-1	↓	↑	↑	↓	↑	n.d.	n.d.
Adrβ2osb-/-	↓	↑	↑	↔	↑	↔	↑
ob/+;Adrβ2osb+/-	↓	↑	↑	↔	↑	n.d.	n.d.
Adrβ2osb+/-;Atf4+/-	↓	↑	n.d.	↔	↑	n.d.	n.d.
Crebosb-/-	↔	↔	n.d.	↔	↔	n.d.	n.d.
Atf4-/-	↓	↑	n.d.	↓	↑	↓	↑