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Neural deletion of *Sh2b1* results in brain growth retardation and reactive aggression

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ABSTRACT: Psychiatric disorders are associated with aberrant brain development and/or aggressive behavior and are influenced by genetic factors; however, genes that affect brain aggression circuits remain elusive. Here, we show that neuronal Src-homology-2 (SH2)B adaptor protein-1 (*Sh2b1*) is indispensable for both brain growth and protection against aggression. Global and brain-specific deletion of *Sh2b1* decreased brain weight and increased aggressive behavior. Global and brain-specific *Sh2b1* knockout (KO) mice exhibited fatal, intermale aggression. In a resident–intruder paradigm, latency to attack was markedly reduced, whereas the number and the duration of attacks was significantly increased in global and brain-specific *Sh2b1* KO mice compared with wild-type littermates. Consistently, core aggression circuits were activated to a higher level in global and brain-specific *Sh2b1* KO males, based on c-fos immunoreactivity in the amygdala and periaqueductal gray. Brain-specific restoration of Sh2b1 normalized brain size and reversed pathologic aggression and aberrant activation of core aggression circuits in *Sh2b1* KO males. *SH2B1* mutations in humans were linked to aberrant brain development and behavior. At the molecular level, Sh2b1 enhanced neurotrophin-stimulated neuronal differentiation and protected against oxidative stress–induced neuronal death. Our data suggest that neuronal Sh2b1 promotes brain development and the integrity of core aggression circuits, likely through enhancing neurotrophin signaling.—Jiang, L., Su, H., Keogh, J. M., Chen, Z., Henning, E., Wilkinson, P., Goodyer, I., Farooqi, I. S., Rui, L. Neural deletion of *Sh2b1* results in brain growth retardation and reactive aggression. FASEB J. 32, 1830–1840 (2018). www.fasebj.org

KEY WORDS: aggression circuits · brain development · BDNF

Aggression is an evolutionarily conserved behavior that serves to protect animals and their offspring, and it is employed to obtain or defend food and territory (1, 2). In humans, a spectrum of psychiatric disorders is associated with escalated aggression and antisocial behaviors (3). Neuroanatomic studies in primates and other mammalian species have led to the identification of a highly conserved neural network involving the medial preoptic area, lateral septum, anterior hypothalamus, ventromedial hypothalamus, periaqueductal gray (PAG), medial amygdala, and bed nucleus of the stria terminalis that is activated during aggressive behavior (4–8). Reactive aggression (also called impulsive, defensive, or affective aggression) is orchestrated by core amygdala-hypothalamus-PAG aggression circuits in response to basic threats and/or frustration (9). In humans, aggression and closely related maladaptive behaviors, such as impulsivity, have been shown to be highly heritable traits (10, 11). However, only a few genes have been directly associated with these phenotypes to date (12, 13).

Src-homology-2 (SH2)B adaptor protein-1 (SH2B1) is an intracellular scaffolding protein that mediates signaling through a number of receptor tyrosine kinases and cytokine receptors (14). Among these, the leptin receptor and the brain-derived neurotrophic factor (BDNF) receptor tropomyosin-related kinase B (TrkB) are known to have a role in energy homeostasis (15–17). We previously

ABBREVIATIONS: ASEBA, Achenbach System of Empirically Based Assessment; BDNF, brain-derived neurotrophic factor; blbp, brain lipid binding protein; FBS, fetal bovine serum; KO, knockout; MTT, 3-(4,5-dimethylthiazol-2-*yl*)-2,5-diphenyltetrazolium bromide; NGF, nerve growth factor; PAG, periaqueductal grey; *Sh2b1*, Src-homology-2 (SH2)B adaptor protein 1; Tg, transgene; TrkB, tropomyosin-related kinase B; WT, wild type

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reported that deletion of *Sh2b1* results in obesity and hyperinsulinemia in mice (15), phenotypes that are reversed by brain-specific restoration of Sh2b1β (18). In humans, we and others have shown that deletions on chromosome band 16p11.2, encompassing *SH2B1* (19), and rare heterozygous loss-of-function mutations in the *SH2B1* gene are associated with severe obesity and disproportionate insulin resistance (20, 21). In those studies, we also observed that some mutation carriers experience behavioral problems (20). However, given the few individuals available for study and the potential influence of other social, cultural, and biologic factors on human behavior, we cannot draw conclusions from those studies. We, therefore, went on to examine the role of *SH2B1* in a context in which genetic background and environment can be more precisely manipulated, by performing targeted disruption of *Sh2b1* in mice. We found that neuronal Sh2b1 promotes brain growth and protects against pathologic aggression.

MATERIALS AND METHODS

Mouse lines

The generation of whole-body *Sh2b1* knockout (KO), *Sh2b1* transgenic, and transgene (Tg)KO mice has been described previously (18). *Sh2b1*$^{f/f}$, *nestin-cre*, and brain lipid binding protein (blbp)–cre transgenic mice have been described and characterized previously (22–24). In this study, brain-specific *Sh2b1* KO mice (C57BL/6 background) were generated by crossing *Sh2b1*$^{f/f}$ with *nestin-cre* or *blbp-cre* mice. Animal experiments were conducted following the protocols approved by the University Committee on the Use and Care of Animals.

Resident–intruder aggression tests

Intruder male mice at 8–12 wk old and group housed (4/cage) were used once per test. Resident mice were individually housed for 2 wk before experiments. Two hours after the start of the dark cycle, a novel, intruder male mouse was introduced into the home cage of a resident male, and mouse activity was video-recorded using an infrared video camera. Social encounters and fighting behaviors were manually scored to calculate latencies for first attack, attack number, and the total attack durations in 15–30-min test periods. Each resident mouse was tested 3 times, and the results from the third trials are presented.

Immunostaining

Mice were anesthetized with Avertin (0.5 g tribromoethanol and 0.25 g *tert*-amyl alcohol in 39.5 ml of water; 0.02 ml/g of body weight; Sigma-Aldrich, St. Louis, MO, USA) and fixed with ice-cold 4% paraformaldehyde *via* transcardial perfusion. Brains were dissected and incubated sequentially in 4% paraformaldehyde overnight at 4°C (postfix) and in 30% sucrose overnight (cryoprotection). Frozen brain sections were prepared using a Leica cryostat (Leica Biosystems, Wetzlar, Germany). Floating brain slices were immunostained with antibody to c-fos (sc-52, 1:500 dilution; Santa Cruz Biotechnology, Dallas, TX, USA) and visualized with a BX51 microscope equipped with a DP72 digital camera (Olympus, Tokyo, Japan). Brain sections were immunostained with antibody against NeuN (12943, 1:500 dilution; Cell Signaling Technology, Danvers, MA, USA). c-fos$^+$ cells were counted manually and blindly, and NeuN$^+$ cells were counted using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

PC12 cell lines and neuronal differentiation

PC12 cells were grown on collagen-coated dishes in DMEM supplemented with 25 mM glucose, 100 U/ml penicillin, 100 μg/ml streptomycin, 10% heat-inactivated horse serum, and 5% fetal bovine serum (FBS). PC12 cells were transduced with lentiviral vectors expressing rat TrkB to generate stable PC12^{TrkB} lines. PC12^{TrkB} cells were infected with lentiviral vectors expressing human SH2B1β or SH2B1β variants to establish individual stable lines. To induce neuronal differentiation, cells were cultured for 3–6 d in DMEM supplemented with 25 mM glucose, 2% horse serum, 1% FBS, 1–5 ng/ml BDNF, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were imaged using an Noran OZ laser scanning confocal microscope (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a ×60 objective lens (Nikon, Tokyo, Japan). Cells with processes longer than 2 times the cell body were defined as neurons (20).

Immunoprecipitation and immunoblotting

Brains were rapidly dissected from anesthetized mice, and brain samples were homogenized in a 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 benzamidine, 10 μg/ml aprotinin, 10 μg/ml leupeptin; 1 mM PMSF]. Brain extracts were immunoprecipitated and immunoblotted with anti-Sh2b1 antibody. PC12 cells with 90% confluence were deprived of FBS overnight, stimulated with BDNF at 20 ng/ml for 10 min, and lysed in a lysis buffer. Cell extracts were immunoblotted with the indicated antibodies.

Viability and TUNEL assays

PC12 cells were treated with H_2O_2 or vehicle for 1 d and then incubated with colorimetric 3-(4,5-dimethylthiazol-2-*yl*)-2,5-diphenyltetrazolium bromide (MTT; 75 μg/ml) for 4 h. After extensive PBS washes, cells were solubilized in DMSO. Absorbance (570 nm) of cell extracts was measured using an AD 340 plate reader (Beckman Coulter, Brea, CA, USA). For TUNEL assays, PC12 cells were treated with H_2O_2 for 1 d, fixed in 4% paraformaldehyde, and subjected to TUNEL assays using an *In Situ* Cell Death Detection Kit (11684817910; Roche Diagnostics, Indianapolis, IN, USA) following manufacturer's instruction. TUNEL$^+$ cells were imaged using BX51 microscope, and they were counted and normalized to total DAPI$^+$ cell number.

Human studies

SH2B1 mutation carriers were recruited from the Genetics of Obesity Study, as described previously (20). Protocols were approved by the Cambridge Local Research Ethics Committee and undertaken after informed, written consent. We used age-appropriate questionnaires for both the Achenbach System of Empirically Based Assessment (ASEBA) and the Autism-Spectrum Quotient. A researcher was present at all times to answer any questions and to make sure all questionnaires were completed. Adults completed their own questionnaires, and mothers completed questionnaires relating to their children. In all cases, mothers completed the norm-referenced Child Behavior

Checklist (CBCL/16-18-2001), which rates a child's behavior and emotional problems during the previous 6 mo using a 3-point response format to establish the frequency of problem behavior. The adults completed the Adult Self Report (ASR/18-59-2003). Both the CBCL and the ASR are rated on a 3-point Likert scale (0, not true; 1, somewhat or sometimes true; or 2, very true or often true), and items were scored using Assessment Data Manager software (*http://www.aseba.org*). Raw scores for each scale were converted to norm-referenced *T* scores.

Statistical analysis

Data are presented as means ± SEM throughout and were analyzed by 2-tailed Student's *t* tests. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Neural deletion of *Sh2b1* results in pathologic intermale aggression

To determine the distribution of Sh2b1 in the brain, tissue extracts were prepared from different brain regions and immunoprecipitated and immunoblotted with anti-Sh2b1 antibody. Sh2b1 was widely expressed in the brains of wild-type (WT) but not global *Sh2b1* KO mice (**Fig. 1*A***).

To examine the role of brain Sh2b1, we generated brain-specific *Sh2b1* KO mice by crossing $Sh2b1^{f/f}$ mice with *nestin-cre* drivers (23). In $Sh2b1^{f/f}$ mice, coding exons 1–4 of *Sh2b1* are flanked by loxp sites (22). As expected, *Sh2b1* was specifically disrupted in the brain but not other tissues of $Sh2b1^{f/f}$;*nestin-cre* mice (Fig. 1*B*). $Sh2b1^{f/f}$;*nestin-cre* males displayed adult-onset, pathologic aggression in their home cages. $Sh2b1^{f/f}$;*nestin-cre* intermale aggression was fatal, with only 1 of 4 mice surviving per cage after 7–15 wk of age. We performed a quantitative analysis of aggressive behavior using the resident–intruder paradigm. In $Sh2b1^{f/f}$;*nestin-cre* male mice, latency to attack was markedly reduced, whereas both the frequency and the duration of attack were substantially increased compared with $Sh2b1^{f/f}$ male mice (**Fig. 2*A***). We replicated these findings in an independent line of brain-specific KO mice generated by crossing $Sh2b1^{f/f}$ mice with brain blbp-cre drivers (24). *Sh2b1* was specifically disrupted in the brain of $Sh2b1^{f/f}$;*blbp-cre* mice (Fig. 1*C*). Adult $Sh2b1^{f/f}$;*blbp-cre* males also fatally attacked their male littermates in their home cages and had to be singly housed after 6–7 wk of age. Latency to attack was significantly shorter, attack frequency was much greater, and the duration of attack was considerably longer in $Sh2b1^{f/f}$;*blbp-cre* males than it was in S$h2b1^{f/f}$ male littermates (Fig. 2*B*). Similar to brain-specific KO male mice, adult global *Sh2b1* KO males fatally attacked their male littermates in their home cages. They had shorter latency to attack, higher attack rates, and longer attack duration (Fig. 2*C*). As sociability can influence how much time animals spend engaged in close inspection with intruder mice, we measured the number of social contacts in 15 min after introduction of the intruder. Although the number of encounters was slightly increased in KO and

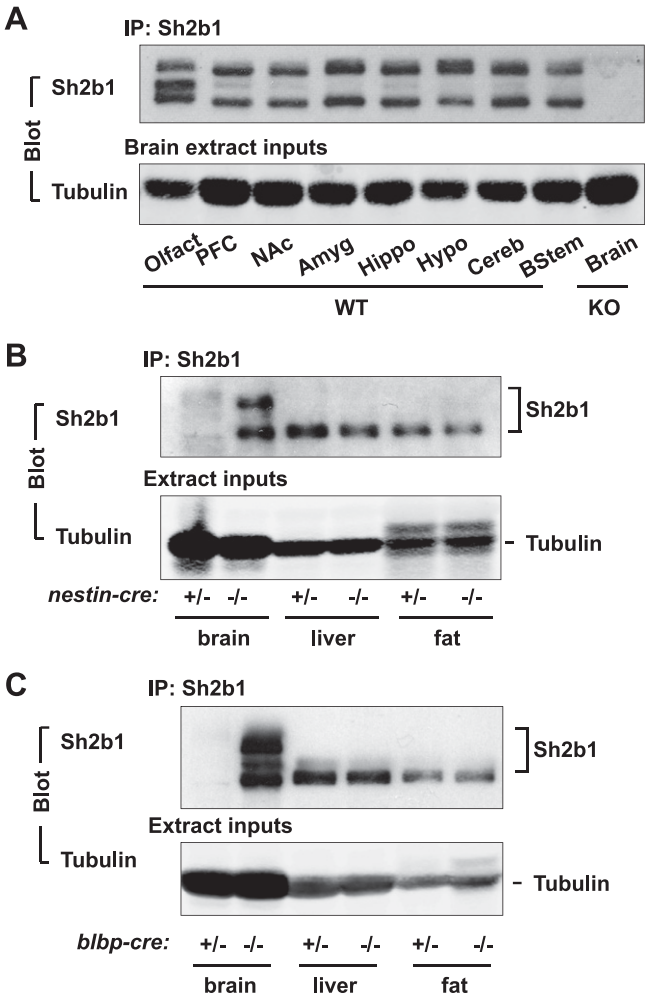

Figure 1. Generation of brain-specific *Sh2b1* KO mice. *A*) Brain extracts were prepared from the indicated brain regions, immunoprecipitated with anti-Sh2b1 antibody, and immunoblotted with anti-Sh2b1 antibody. The extracts were immunoblotted with anti-tubulin antibody. Amyg, amygdala; BStem, brainstem; Cerebe, cerebellum; Hippo, hippocampus; NAc, nucleus accumbens; Olfact, olfactory bulb; PFC, prefrontal cortex. *B*, *C*) Tissue extracts were prepared from $Sh2b1^{f/f}$;*nestin-cre* (*B*) or $Sh2b1^{f/f}$;*blbp-cre* (*C*) male mice, immunoprecipitated with anti-Sh2b1 antibody, and immunoblotted with anti-Sh2b1 antibody. Tissue extracts were also immunoblotted with anti-tubulin antibody.

$Sh2b1^{f/f}$;*nestin-cre* males, those differences were not statistically significant compared with WT animals (KO: 19 ± 3, $n = 9$, WT: 14 ± 1, $n = 9$; $P = 0.1997$; $Sh2b1^{f/f}$;*nestin-cre*: 17 ± 2, $n = 8$; S$h2b1^{f/f}$: 14 ± 1, $n = 14$; $P = 0.183$).

To test whether brain-specific restoration of *Sh2b1* was able to correct aggression in global *Sh2b1* KO mice, mice expressing a rat *SH2B1β* Tg under the control of a neuron-specific enolase promoter were crossed with global *Sh2b1* KO mice to generate TgKO mice (18). *Sh2b1* KO mice develop obesity, and brain-specific restoration of Sh2b1 fully reverses the obesity phenotypes of TgKO mice (18). Importantly, brain-specific restoration of Sh2b1 completely corrected fatal aggression in TgKO males (Fig. 2*C*). Together, these data indicate that brain-specific Sh2b1 deficiency is sufficient to cause fatal, intermale aggression.

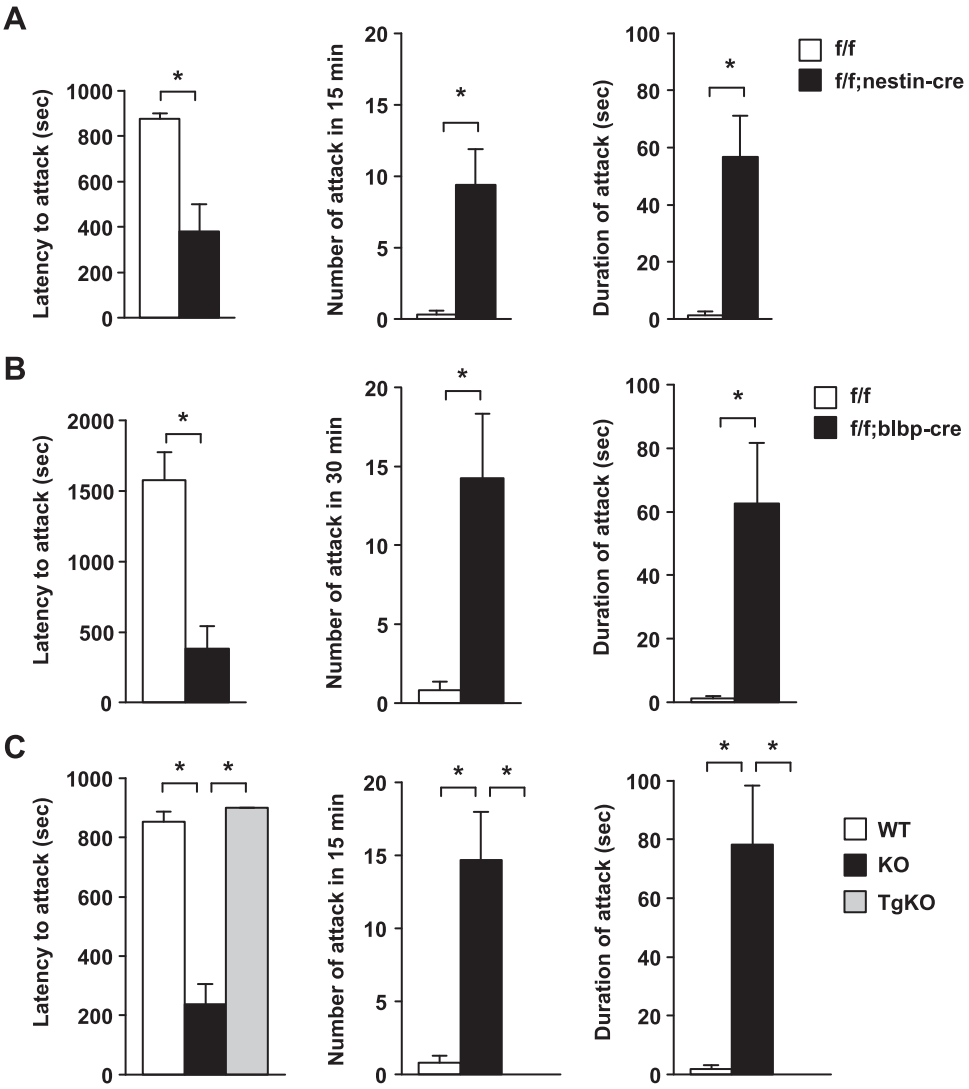

Figure 2. Brain Sh2b1 deficiency results in fatal intermale aggression. Resident–intruder experiments were performed on male mice at 18 (*A*), 8 (*B*), and 9–12 (*C*) wk of age, and attack latency, frequency, and duration were calculated. *A*) *Sh2b1*$^{f/f}$: n = 14, *Sh2b1*$^{f/f}$*;nestin-cre*: n = 8. *B*) *Sh2b1*$^{f/f}$: n = 6, *Sh2b1*$^{f/f}$*;blbp-cre*: n = 4. *C*) WT: n = 9, KO: n = 9, TgKO: n = 8. Data are presented as means ± SEM and were analyzed by 2-tailed Student's t tests. *$P < 0.05$.

Sh2b1-mediated signaling suppresses aggression-circuit activity

We next tested whether disruption of *Sh2b1* alters neuronal activity (assessed by c-fos immunoreactivity) in core aggression circuits. Brain sections were prepared from resident male mice exposed to male intruders for 15 min and immunostained with anti–c-fos antibody. The number of c-fos$^+$ neurons in the piriform cortex, amygdala, and PAG was significantly higher in KO than it was in WT mice and in *Sh2b1*$^{f/f}$*;blbp-cre* mice than it was in *Sh2b1*$^{f/f}$ littermates (**Fig. 3*A, B***). The piriform cortex is responsible for olfactory sensing (25, 26), and the amygdala-hypothalamus-PAG circuit mediates aggression in the face of basic and social threats (5). Notably, basal immunoreactivity to c-fos in the piriform cortex, amygdala, and PAG was similar between WT and KO adult male mice in the absence of exposure to intruders (Fig. 3*C*). These data raise the possibility that brain Sh2b1 deficiency may enhance the sensitivity of core aggression circuits to basic threats. To test that idea, WT and KO adult male mice were exposed to intruder bedding soils, which contained intruder pheromones. Exposure to male intruder pheromones increased c-fos immunoreactivity in the piriform cortex to a greater extent in KO than it did in WT adult male mice (Fig. 3*D*). Taken together, these results suggest that brain Sh2b1 deficiency promotes pathologic aggression at least in part by enhancing the ability of core aggression circuits to sense and/or integrate basic and/or social threats.

Neural deletion of *Sh2b1* impairs brain development

We found that brain sizes were smaller in *Sh2b1*$^{f/f}$*;nestin-cre* (*vs.* *Sh2b1*$^{f/f}$) and KO (*vs.* WT) mice (**Fig. 4*A***). Fresh brain weight was significantly less in both *Sh2b1*$^{f/f}$*;nestin-cre* and *Sh2b1*$^{f/f}$*;blbp-cre* mice, relative to their *Sh2b1*$^{f/f}$ littermates (Fig. 4*B, C*). Similarly, KO males or females had lower brain weight than WT male or female littermates had (Fig. 4*D, E*). Brain-specific restoration of *Sh2b1* increased brain weight to normal levels in both male and female TgKO mice (Fig. 4*D, E*). Brain growth retardation was observed in young KO mice within 3 wk of age and was fully normalized by brain-specific

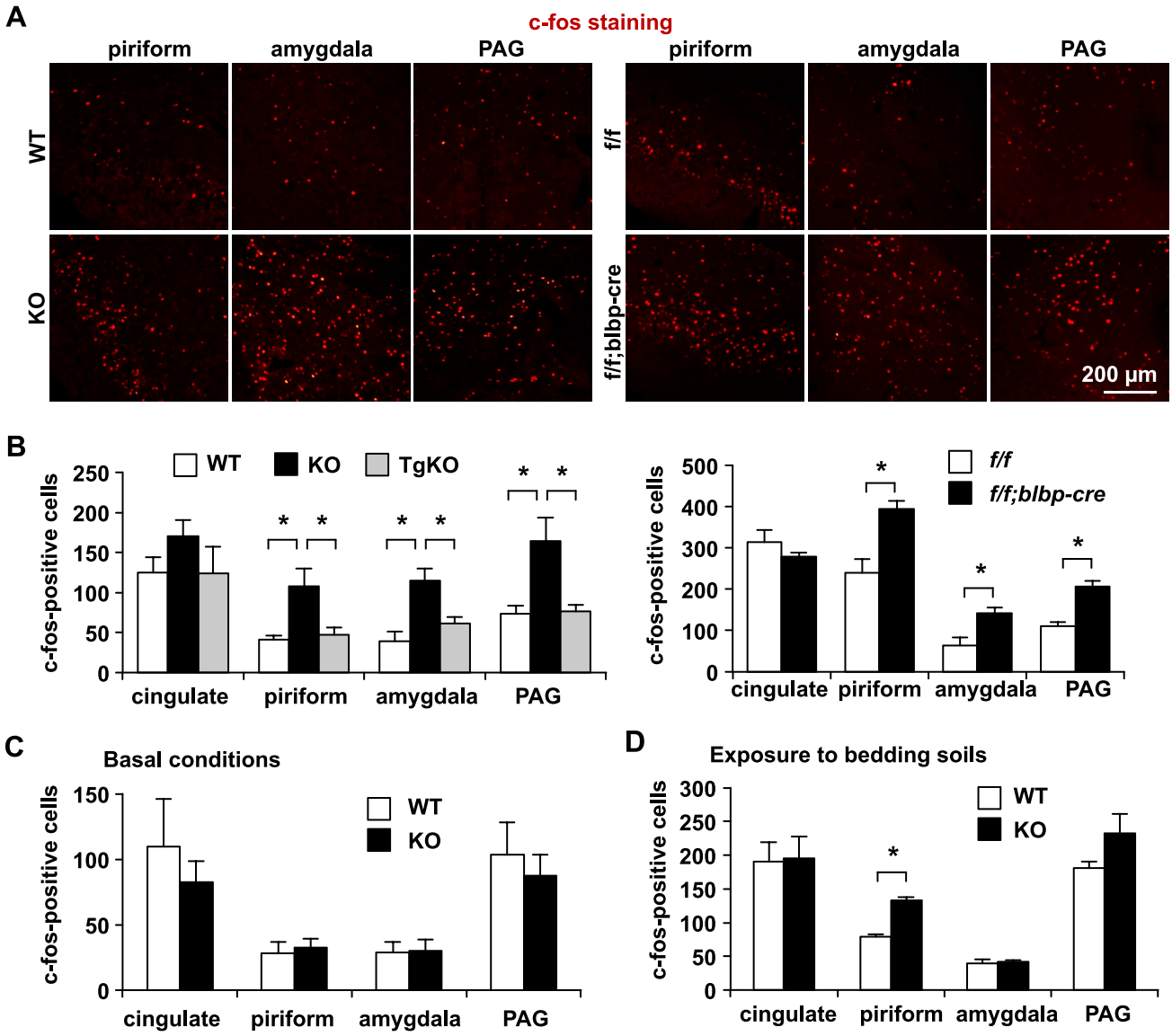

Figure 3. Sh2b1 regulates the activity of aggression circuits. *A*, *B*) Brain sections were prepared from resident mice 15 min after encountering intruders and immunostained with antibody against c-fos. *A*) Representative images. *B*) c-fos$^+$ cells were counted in the cingulate cortex, piriform cortex, amygdala, and PAG. WT: $n = 3$, KO: $n = 6$, TgKO: $n = 4$, *Sh2b1*$^{f/f}$: $n = 5$, *Sh2b1*$^{f/f}$*;blbp-cre*: $n = 5$. *C*) Basal c-fos$^+$ cells in different brain regions. WT: $n = 5$, KO: $n = 4$. *D*) Male mice were exposed to intruder bedding soils for 15 min. Brain sections were stained with antibody to c-fos. WT: $n = 3$; KO: $n = 3$. Data are presented as means ± SEM and were analyzed by 2-tailed Student's *t* tests. $^*P < 0.05$.

restoration of Sh2b1 in young TgKO mice (Fig. 4*F*). These data indicate that brain Sh2b1 is indispensable for brain development and growth.

To further investigate the critical role of Sh2b1 in brain development, we counted neuronal numbers in key brain regions. Brain sections were immunostained with antibody against NeuN, a neuronal marker. In line with reduced brain size, the total number of neurons in piriform and amygdala sections was significantly less in *Sh2b1* KO mice than it was in WT littermates (**Fig. 5*A*, *B***). Because the number of aggression-related, c-fos$^+$ neurons was higher in those regions in KO mice (Fig. 4), we hypothesize that a subpopulation of the lost neurons might provide inhibitory inputs to core aggression circuits. Therefore, their absence may induce disinhibition of core aggression circuits, contributing to pathologic aggression in *Sh2b1*-null mice.

SH2B1 enhances BDNF/TrkB-stimulated neuronal differentiation and survival

Notably, the aggression phenotype of brain-specific *Sh2b1* KO mice is reminiscent of that seen in heterozygous *Bdnf*-null mice and mice with targeted disruption of *Bdnf* in specific brain regions (27–29). We, therefore, hypothesized that the effect of Sh2b1 on behavior may be mediated, in part, by modulating BDNF signaling. We stably introduced the BDNF receptor TrkB into PC12 cells (PC12^{TrkB}) with rat Sh2b1β or SH2 domain-defective

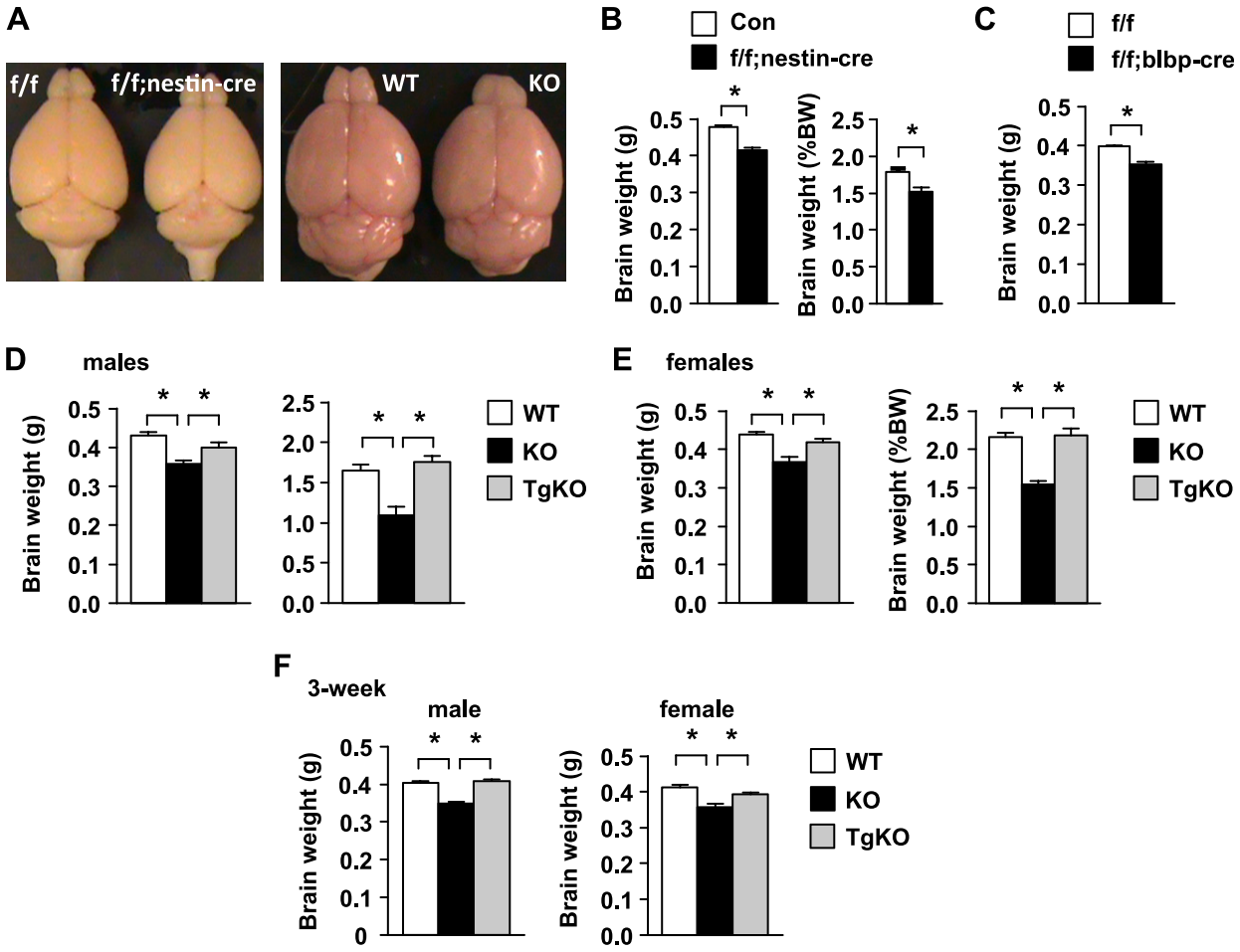

Figure 4. Neural SH2B1 is required for brain development and growth. *A*) Representative brain images. WT and KO male mice: 10 wk; *Sh2b1*$^{f/f}$ and *Sh2b1*$^{f/f}$*;nestin-cre* male mice: 20 wk. *B–E*) Brain weight and brain weight to body weight ratios in mice at 20 (*B*), 8 (*C*), 9–12 (*D*), and 9–10 (*E*) wk of age. *B*) Control (Con): $n = 9$, *Sh2b1*$^{f/f}$*;nestin-cre*: $n = 8$. *C*) *Sh2b1*$^{f/f}$: $n = 4$, *Sh2b1*$^{f/f}$*;blbp-cre*: $n = 4$. *D*) WT: $n = 12$, KO: $n = 8$, TgKO: $n = 4$. *E*) WT: $n = 11$, KO: $n = 6$, TgKO: $n = 7$. *F*) Brain weight at 3 wk of age. Males: WT: $n = 15$, KO: $n = 8$, TgKO: $n = 3$; females: WT: $n = 14$, KO: $n = 6$, TgKO: $n = 5$. Data are presented as means ± SEM and were analyzed by 2-tailed Student's *t* tests. $^*P < 0.05$.

Sh2b1β (R555E). BDNF rapidly and robustly stimulated shifts in Sh2b1 mobility (**Fig. 6*A***). Initially, tyrosine phosphorylation of Sh2b1 was undetectable, but after pretreatment with the phosphatase inhibitor Na_3VO_4, BDNF-stimulated tyrosine phosphorylation of Sh2b1 was detected (Fig. 6*A*). These data suggest that BDNF-stimulated tyrosine phosphorylation of Sh2b1 is rapidly dephosphorylated by protein phosphatases. Sh2b1 was coimmunoprecipitated with TrkB (Fig. 6*A*), confirming previous findings that Sh2b1 binds to TrkB (30). BDNF stimulated PC12 cell neuronal differentiation, which was markedly enhanced by Sh2b1β and blocked by Sh2b1β (R555E) (Fig. 6*B, C*). These results suggest that Sh2b1 acts downstream of TrkB to promote neuronal differentiation.

Next, we stably introduced human WT and mutant forms of SH2B1β into PC12^{TrkB} cells (Fig. 6*D*). These mutations have been associated with behavioral abnormalities in humans (20). Human WT SH2B1β similarly enhanced BDNF-stimulated neuronal differentiation, and the *SH2B1β* mutants had reduced ability to promote BDNF-induced neuronal differentiation and neurite growth of PC12^{TrkB} cells (Fig. 6*E*). We examined the effect of human SH2B1β on neuronal survival and death by MTT and TUNEL assays. WT SH2B1β completely blocked the detrimental effect of 0.2-mM H_2O_2 and significantly attenuated the toxic effect of 0.4-mM H_2O_2 on PC12^{TrkB} cell viability (Fig. 6*F*). In contrast, *SH2B1β* mutants had either no or very modest effect on PC12^{TrkB} cell viability (Fig. 6*F*). Additionally, WT SH2B1β, but not *SH2B1β* mutants, blocked 0.3-mM H_2O_2-induced apoptosis (Fig. 6*G*). Those observations raise the possibility that brain SH2B1 promotes brain development and growth and suppresses aggression, in part, by enhancing the ability of the BDNF/TrkB system to promote neuronal differentiation and/or survival.

Behavioral studies in humans with *SH2B1* mutations

We performed limited behavioral studies in 3 boys and 5 adults (4 women; 1 man) with heterozygous loss-of-function mutations in *SH2B1*, using the age-appropriate ASEBA questionnaires (**Table 1**). We found that 3 of 4 men

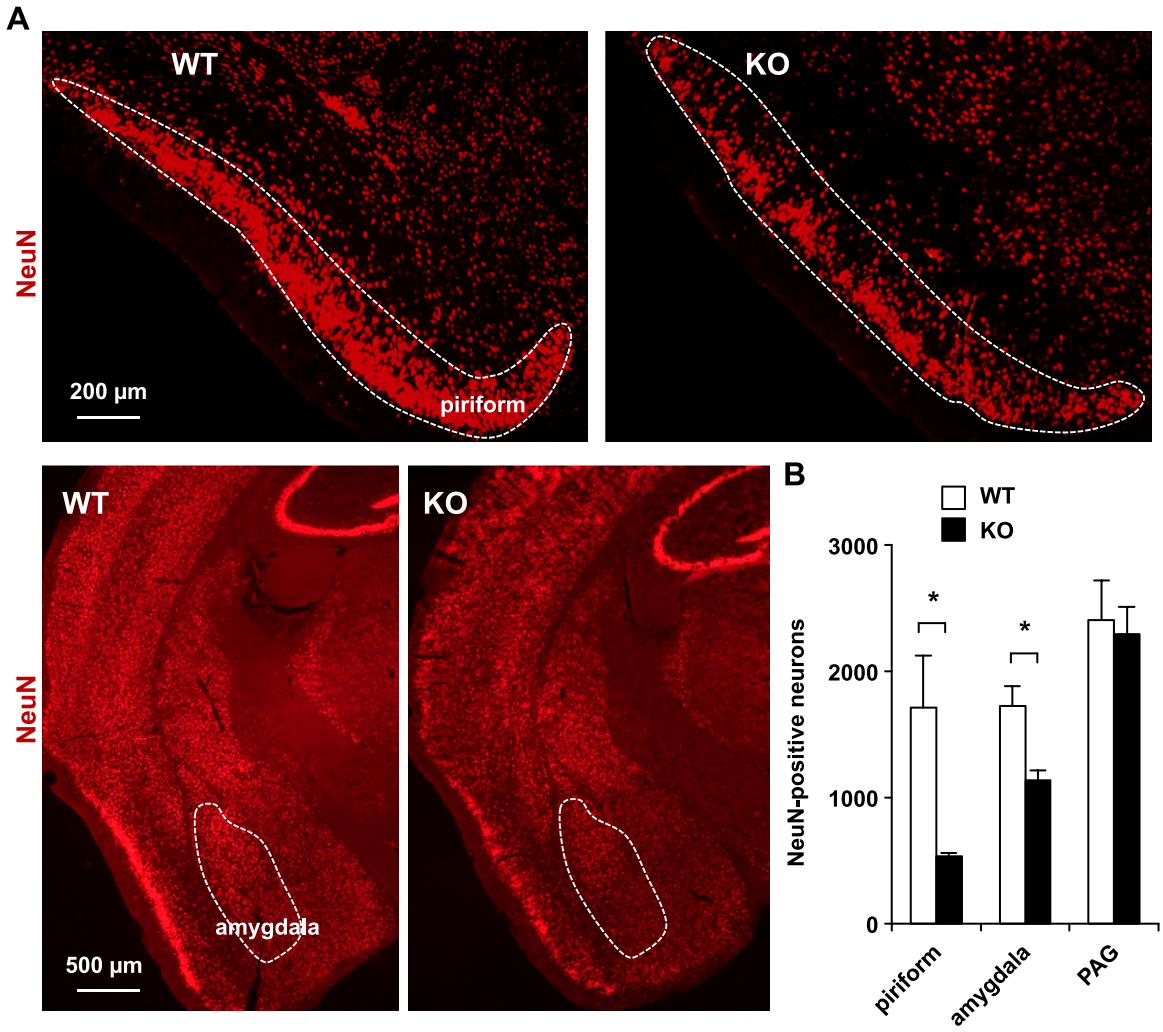

Figure 5. Sh2b1 deficiency reduces brain neuronal number. *A*, *B*) Brain sections were prepared from WT and KO male littermates at 10 wk of age and stained with anti-NeuN antibody. *A*) Representative images. The piriform and amygdala are marked. *B*) NeuN$^+$ neurons were counted in the piriform, amygdala, and PAG sections. WT: $n = 3$, KO: $n = 3$. Data are presented as means ± SEM and were analyzed by 2-tailed Student's t tests. $^*P < 0.05$.

had clinically significant/borderline significant levels of aggressive behavior compared with age- and gender-specific reference data (**Fig. 7**). Although we were unable to obtain structural brain-imaging data on mutation carriers, postmortem findings from a 15-yr-old boy carrying a *SH2B1* mutation, who died suddenly after a septicemic illness, showed markedly reduced brain weight (1244 g; mean brain weight for age, 1434 g) and extensive loss of Purkinje cells in the cerebellum. We acknowledge that the few individuals available for study and the multiple factors that influence complex human behaviors such as aggression represent a challenge for human studies. Nevertheless, our data suggest that neuronal SH2B1 similarly regulates human brain development and behavior.

DISCUSSION

We demonstrate that global *Sh2b1* KO male mice and 2 independent lines of mice with brain-specific deletion of *Sh2b1* develop pathologic aggression, fatally attacking other male mice. Brain-specific restoration of Sh2b1 completely reversed intermale aggression in TgKO mice. These findings define the role of brain Sh2b1 signaling in reactive aggression. This is distinct from motivation-driven, premeditated (instrumental) aggression (*e.g.*, predatory aggression), which was not tested in this study. Using a marker of neural activity (c-fos expression), we found that activation of neurons in the amygdala and PAG was significantly greater in both global and brain-specific KO male mice compared with WT animals exposed to intruders. These experiments compared activation of brain regions after a 15-min resident–intruder test; during which, very few of the controls, but almost all of the KO mice, attacked. Thus, although the changes we observed may suggest that those neural circuits underlie the enhanced propensity to attack, they may, in part, reflect the neural circuitry that is activated by engaging in an actual attack. Based on our genetic models as well as physiologic and behavior tests, we conclude that Sh2b1 deficiency in the brain is sufficient to elicit pathologic aggression through activating core aggression circuits.

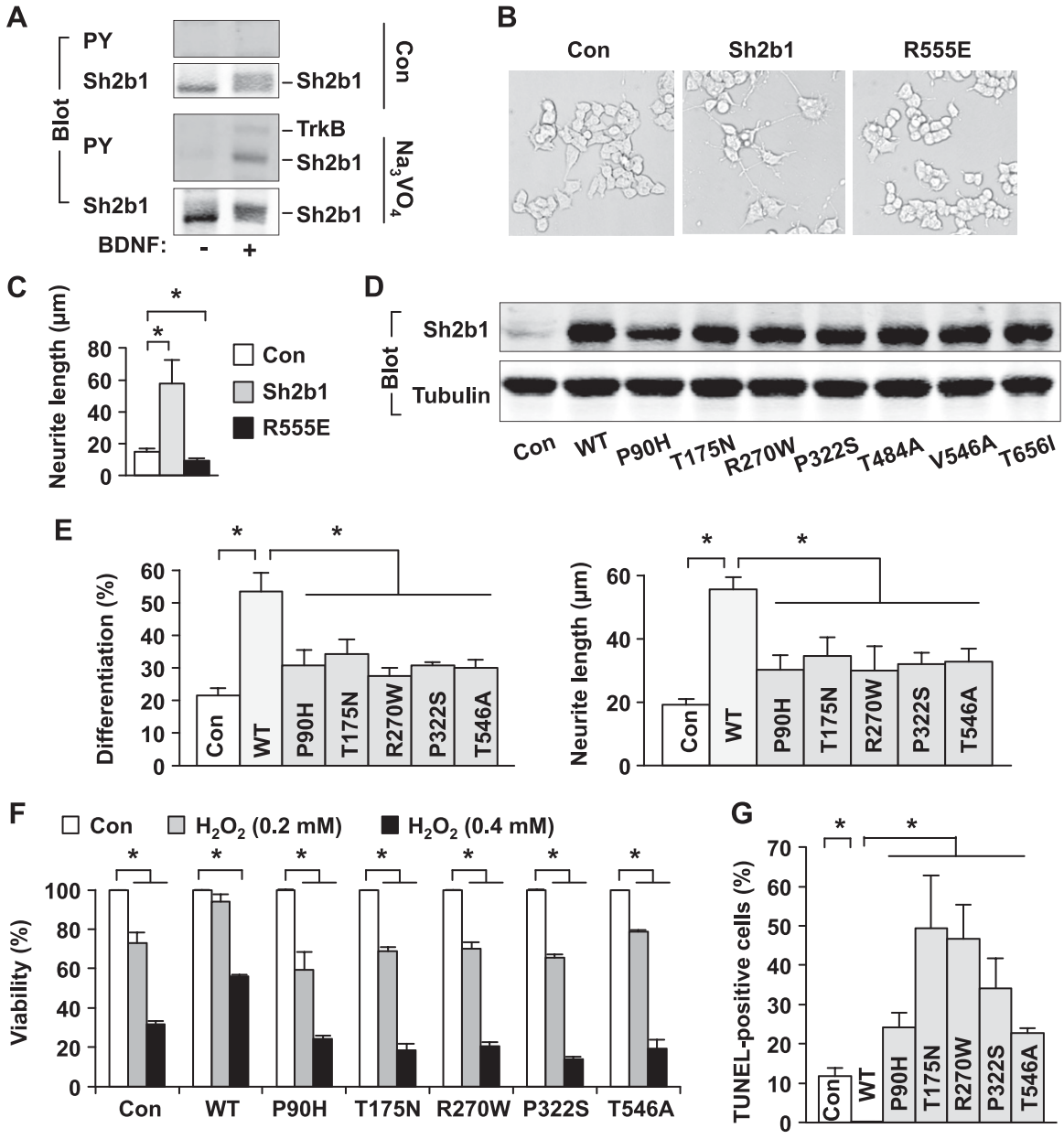

Figure 6. SH2B1 enhances BDNF-induced neuronal differentiation and protects against oxidative stress-induced neuronal death. *A*) TrkB and Sh2b1β were stably introduced into PC12 cells. Cells were stimulated with BDNF (20 ng/ml) for 10 min in the absence (upper 2 panels) or presence (lower 2 panels) of phosphatase inhibitor Na_3VO_4. Cell extracts were immunoprecipitated with anti-Sh2b1 antibody and immunoblotted with the indicated antibodies. PY, antibody to phospho-Tyrosine. *B, C*) $PC12^{TrkB}$ cells, which stably express Sh2b1β or Sh2b1β(R555E), were treated with BDNF (1 ng/ml) for 3 d. Cells were visualized with a phase-contrast microscope, and neurite length was measured. Control (con): $n = 3$, Sh2b1β: $n = 3$, Sh2b1β(R555E): $n = 3$. *D*) $PC12^{TrkB}$ cells were stably infected with empty lentiviral vectors (con) or lentiviral vectors containing individual human SH2B1 variants. Cell extracts were immunoblotted with antibodies to SH2B1 or tubulin. *E*) The indicated $PC12^{TrkB}$ cells were treated with 5 ng/ml BDNF for 1 d. Neurite length and differentiation efficiency were measured; neuron number was normalized to total cell number. Each individual line: $n = 3$. *F*) The indicated $PC12^{TrkB}$ cells were treated with H_2O_2 for 1 d, and cell viability was measured by MTT assays. *G*) The indicated $PC12^{TrkB}$ cells were treated with 0.3 mM H_2O_2 for 1 d and subsequently subjected to TUNEL assays. Data are presented as means ± SEM and were analyzed by 2-tailed Student's *t* tests. $^*P < 0.05$.

We also uncovered a critical role for neuronal Sh2b1 in brain development. Brains were smaller in *Sh2b1* KO mice, and neuron-specific reconstitution of Sh2b1 fully rescued abnormal brain growth in TgKO mice. Consistently, total neuronal number was less in the piriform and amygdala of KO mice relative to WT littermates. Those observations suggest that neuronal Sh2b1 directly promotes brain growth. In line with that conclusion, brain-specific deletion of *Sh2b1* significantly decreased brain weight in 2 independent lines of brain-specific KO mice. Notably, impaired brain development has been associated with human psychiatric disorders and pathologic aggression. Thus, brain Sh2b1-deficiency–induced impairment in brain development likely contributes, at least in part, to

TABLE 1. *Characteristics of carriers of heterozygous mutations in* SH2B1

Characteristic	Adults					Children		
Age (yr)	49	20	49	22	53	6.7	9.1	15
Gender	F	F	F	F	M	M	M	M
BMI (kg/m^2)	35.7	40.1	27.9	47.3	35	26.9	31.1	46.1
BMI SD						4	3.7	4
SH2B1 mutation	P90H	E299G	E299G	R227C	R227C	R270W	R227C	P90H

BMI, body–mass index [weight/height (kg/m^2)]; BMI SD, BMI SD score is shown for children to allow for the effects of age and gender on BMI; F, female; M, male.

escalated aggressive behavior in *Sh2b1*-null mice. Remarkably, Sh2b1 deficiency decreased total neuronal numbers and increased the aggression-activated (c-fos$^+$) neuronal subpopulation in core aggression circuits. We speculate that the neurons, which are lost in global or brain-specific *Sh2b1* KO mice, may provide inhibitory inputs to core aggression circuits to restrain inappropriate escalation of aggression. Absence of these neurons may disinhibit core aggression circuits, leading to pathologic aggression in *Sh2b1*-null male mice. This hypothesis needs to be tested in additional experiments.

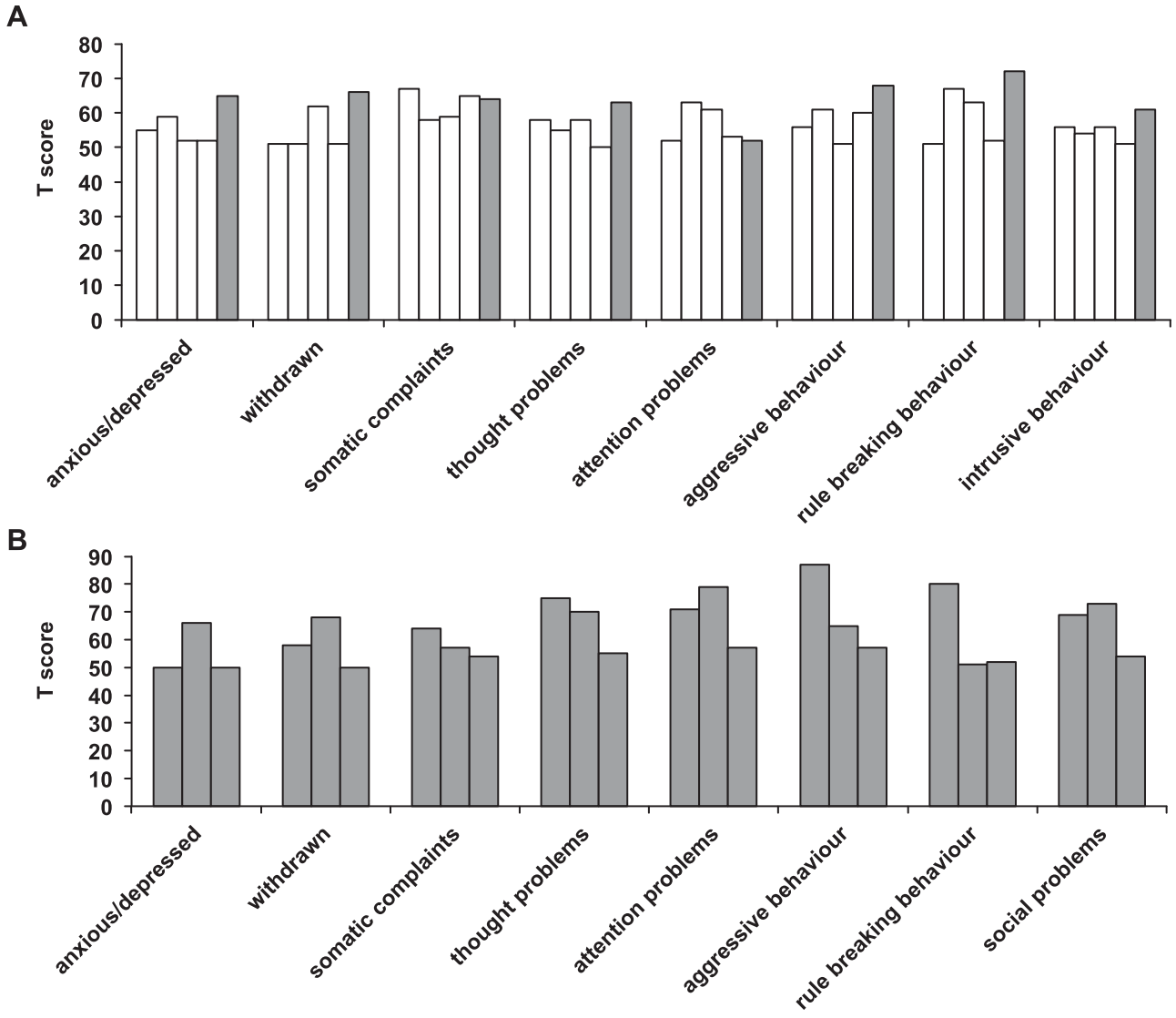

Figure 7. Behavioral scores in individuals with *SH2B1* mutations. *A*, *B*) A range of behaviors were assessed using age-appropriate ASEBA questionnaires (see Materials and Methods) during face-to-face interviews with adults (*A*) and children (*B*) with heterozygous loss-of-function *SH2B1* mutations reported previously (10, 11). Age-adjusted *T* scores were compared with reference data for males (gray) and females (white). *T* scores >60 were deemed to be borderline clinically significant, and scores ≥69 were deemed in the clinical range.

We also observed that *SH2B1* missense mutations were associated with behavioral abnormalities and impaired brain development in humans. However, the few individuals available for the study limited our ability to conduct additional analyses. In view of the data in rodents, further investigation of the contribution of human SH2B1 signaling to brain development and behavior is warranted.

Sh2b1 is involved in mediating signaling by many receptor tyrosine kinases and cytokine receptors (14). As such, establishing the precise molecular mechanisms by which neural deletion of *Sh2b1* leads to impaired brain development and aggressive behavior in mice is challenging. Although impaired leptin signaling and insulin signaling may contribute to the metabolic effects of *Sh2b1* deletion in mice, disruption of those pathways is unlikely to explain aggressive behavior because this phenotype is not seen in animals with impaired leptin/insulin signaling. Brain development is tightly regulated by neurotrophins (31). BDNF, neurotrophin 4, and nerve growth factor (NGF) promote neural stem-cell survival and differentiation, axonal growth, synaptogenesis and maturation, and refinement of synaptic connectivity (32). We previously reported that Sh2b1 binds *via* its SH2 domain to NGF receptor TrkA and promotes NGF-stimulated neuronal differentiation (33). However, NGF is mainly involved in the development and maintenance of peripheral neurons. In this study, we showed that Sh2b1 binds to a closely related BDNF receptor TrkB, which mediates the ability of BDNF to stimulate neuronal differentiation and survival in the brain. SH2B1, but not loss-of-function human *SH2B1* mutants, enhanced BDNF action and protected against oxidative stress-induced neuronal injury and death. Those data suggest that neural SH2B1 promotes brain development and growth, at least in part, by enhancing the actions of BDNF and related neurotrophic factors. We acknowledge that PC12 cell-derived neurons, which were used in this study, are not ideal for modeling neurons in the brain. Notably, in line with our findings, SH2B1 has been reported to promote BDNF-stimulated axonal growth in primary cortical neurons (34). Importantly, mice with systemic (heterozygous) or forebrain-specific deletion of *BDNF* similarly develop escalated intermale aggression (27, 35). Therefore, impaired BDNF signaling may be one of the factors that could contribute to the phenotypes observed in global and brain-specific *Sh2b1* KO mice. However, further studies, such as deletion of *Sh2b1* in TrkB-expressing neurons (including postnatal deletion), are warranted in the future to directly test that hypothesis. FJ

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AUTHOR CONTRIBUTIONS

L. Jiang, H. Su, I. S. Farooqi, and L. Rui conceived and designed the research; L. Jiang, H. Su, J. M. Keogh, Z. Chen, E. Henning, P. Wilkinson, and I. Goodyer performed the experiments; L. Jiang, H. Su, I. S. Farooqi, and L. Rui analyzed the data; L. Jiang, H. Su, I. S. Farooqi, and L. Rui wrote the manuscript; and all authors have critically reviewed the paper.

REFERENCES

1. Nelson, R. J., and Trainor, B. C. (2007) Neural mechanisms of aggression. *Nat. Rev. Neurosci.* **8**, 536–546
2. Barr, C. S., and Driscoll, C. (2014) Neurogenetics of aggressive behavior: studies in primates. *Curr. Top. Behav. Neurosci.* **17**, 45–71
3. Dorfman, H. M., Meyer-Lindenberg, A., and Buckholtz, J. W. (2014) Neurobiological mechanisms for impulsive-aggression: the role of MAOA. *Curr. Top. Behav. Neurosci.* **17**, 297–313
4. Hong, W., Kim, D. W., and Anderson, D. J. (2014) Antagonistic control of social versus repetitive self-grooming behaviors by separable amygdala neuronal subsets. *Cell* **158**, 1348–1361
5. Anderson, D. J. (2012) Optogenetics, sex, and violence in the brain: implications for psychiatry. *Biol. Psychiatry* **71**, 1081–1089
6. Takahashi, A., and Miczek, K. A. (2014) Neurogenetics of aggressive behavior: studies in rodents. *Curr. Top. Behav. Neurosci.* **17**, 3–44
7. Gregg, T. R., and Siegel, A. (2001) Brain structures and neurotransmitters regulating aggression in cats: implications for human aggression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **25**, 91–140
8. Lin, D., Boyle, M. P., Dollar, P., Lee, H., Lein, E. S., Perona, P., and Anderson, D. J. (2011) Functional identification of an aggression locus in the mouse hypothalamus. *Nature* **470**, 221–226
9. Blair, R. J. (2007) Aggression, psychopathy and free will from a cognitive neuroscience perspective. *Behav. Sci. Law* **25**, 321–331
10. Niv, S., Tuvblad, C., Raine, A., and Baker, L. A. (2013) Aggression and rule-breaking: heritability and stability of antisocial behavior problems in childhood and adolescence. *J. Crim. Justice* **41**
11. Davidson, R. J., Putnam, K. M., and Larson, C. L. (2000) Dysfunction in the neural circuitry of emotion regulation—a possible prelude to violence. *Science* **289**, 591–594
12. Brunner, H. G., Nelen, M., Breakefield, X. O., Ropers, H. H., and van Oost, B. A. (1993) Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science* **262**, 578–580
13. Bevilacqua, L., Doly, S., Kaprio, J., Yuan, Q., Tikkanen, R., Paunio, T., Zhou, Z., Wedenoja, J., Maroteaux, L., Diaz, S., Belmer, A., Hodgkinson, C. A., Dell'osso, L., Suvisaari, J., Coccaro, E., Rose, R. J., Peltonen, L., Virkkunen, M., and Goldman, D. (2010) A population-specific HTR2B stop codon predisposes to severe impulsivity. *Nature* **468**, 1061–1066
14. Rui, L. (2014) SH2B1 regulation of energy balance, body weight, and glucose metabolism. *World J. Diabetes* **5**, 511–526
15. Ren, D., Li, M., Duan, C., and Rui, L. (2005) Identification of SH2-B as a key regulator of leptin sensitivity, energy balance, and body weight in mice. *Cell Metab.* **2**, 95–104
16. Rios, M. (2014) Neurotrophins and the regulation of energy balance and body weight. *Handb. Exp. Pharmacol.* **220**, 283–307
17. Xu, B., Goulding, E. H., Zang, K., Cepoi, D., Cone, R. D., Jones, K. R., Tecott, L. H., and Reichardt, L. F. (2003) Brain-derived neurotrophic

factor regulates energy balance downstream of melanocortin-4 receptor. *Nat. Neurosci.* **6**, 736–742
18. Ren, D., Zhou, Y., Morris, D., Li, M., Li, Z., and Rui, L. (2007) Neuronal SH2B1 is essential for controlling energy and glucose homeostasis. *J. Clin. Invest.* **117**, 397–406
19. Bochukova, E. G., Huang, N., Keogh, J., Henning, E., Purmann, C., Blaszczyk, K., Saeed, S., Hamilton-Shield, J., Clayton-Smith, J., O'Rahilly, S., Hurles, M. E., and Farooqi, I. S. (2010) Large, rare chromosomal deletions associated with severe early-onset obesity. *Nature* **463**, 666–670
20. Doche, M. E., Bochukova, E. G., Su, H. W., Pearce, L. R., Keogh, J. M., Henning, E., Cline, J. M., Saeed, S., Dale, A., Cheetham, T., Barroso, I., Argetsinger, L. S., O'Rahilly, S., Rui, L., Carter-Su, C., and Farooqi, I. S. (2012) Human *SH2B1* mutations are associated with maladaptive behaviors and obesity. *J. Clin. Invest.* **122**, 4732–4736; erratum: **123**, 526
21. Pearce, L. R., Joe, R., Doche, M. E., Su, H. W., Keogh, J. M., Henning, E., Argetsinger, L. S., Bochukova, E. G., Cline, J. M., Garg, S., Saeed, S., Shoelson, S., O'Rahilly, S., Barroso, I., Rui, L., Farooqi, I. S., and Carter-Su, C. (2014) Functional characterization of obesity-associated variants involving the α and β isoforms of human SH2B1. *Endocrinology* **155**, 3219–3226
22. Chen, Z., Morris, D. L., Jiang, L., Liu, Y., and Rui, L. (2014) SH2B1 in β-cells regulates glucose metabolism by promoting β-cell survival and islet expansion. *Diabetes* **63**, 585–595
23. Sclafani, A. M., Skidmore, J. M., Ramaprakash, H., Trumpp, A., Gage, P. J., and Martin, D. M. (2006) Nestin-Cre mediated deletion of Pitx2 in the mouse. *Genesis* **44**, 336–344
24. Hegedus, B., Dasgupta, B., Shin, J. E., Emnett, R. J., Hart-Mahon, E. K., Elghazi, L., Bernal-Mizrachi, E., and Gutmann, D. H. (2007) Neurofibromatosis-1 regulates neuronal and glial cell differentiation from neuroglial progenitors in vivo by both cAMP- and Ras-dependent mechanisms. *Cell Stem Cell* **1**, 443–457
25. Stowers, L., Holy, T. E., Meister, M., Dulac, C., and Koentges, G. (2002) Loss of sex discrimination and male-male aggression in mice deficient for TRP2. *Science* **295**, 1493–1500
26. Stowers, L., Cameron, P., and Keller, J. A. (2013) Ominous odors: olfactory control of instinctive fear and aggression in mice. *Curr. Opin. Neurobiol.* **23**, 339–345
27. Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S. H., Wihler, C., Koliatsos, V. E., and Tessarollo, L. (1999) Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc. Natl. Acad. Sci. USA* **96**, 15239–15244
28. Ito, W., Chehab, M., Thakur, S., Li, J., and Morozov, A. (2011) BDNF-restricted knockout mice as an animal model for aggression. *Genes Brain Behav.* **10**, 365–374
29. Chan, J. P., Unger, T. J., Byrnes, J., and Rios, M. (2006) Examination of behavioral deficits triggered by targeting Bdnf in fetal or postnatal brains of mice. *Neuroscience* **142**, 49–58
30. Qian, X., Riccio, A., Zhang, Y., and Ginty, D. D. (1998) Identification and characterization of novel substrates of Trk receptors in developing neurons. *Neuron* **21**, 1017–1029
31. Vilar, M., and Mira, H. (2016) Regulation of neurogenesis by neurotrophins during adulthood: expected and unexpected roles. *Front. Neurosci.* **10**, 26
32. Park, H., and Poo, M. M. (2013) Neurotrophin regulation of neural circuit development and function. *Nat. Rev. Neurosci.* **14**, 7–23
33. Rui, L., Herrington, J., and Carter-Su, C. (1999) SH2-B is required for nerve growth factor-induced neuronal differentiation. *J. Biol. Chem.* **274**, 10590–10594
34. Shih, C. H., Chen, C. J., and Chen, L. (2013) New function of the adaptor protein SH2B1 in brain-derived neurotrophic factor-induced neurite outgrowth. *PLoS One* **8**, e79619
35. Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., Kuehn, R., Lechan, R. M., and Jaenisch, R. (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol. Endocrinol.* **15**, 1748–1757

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