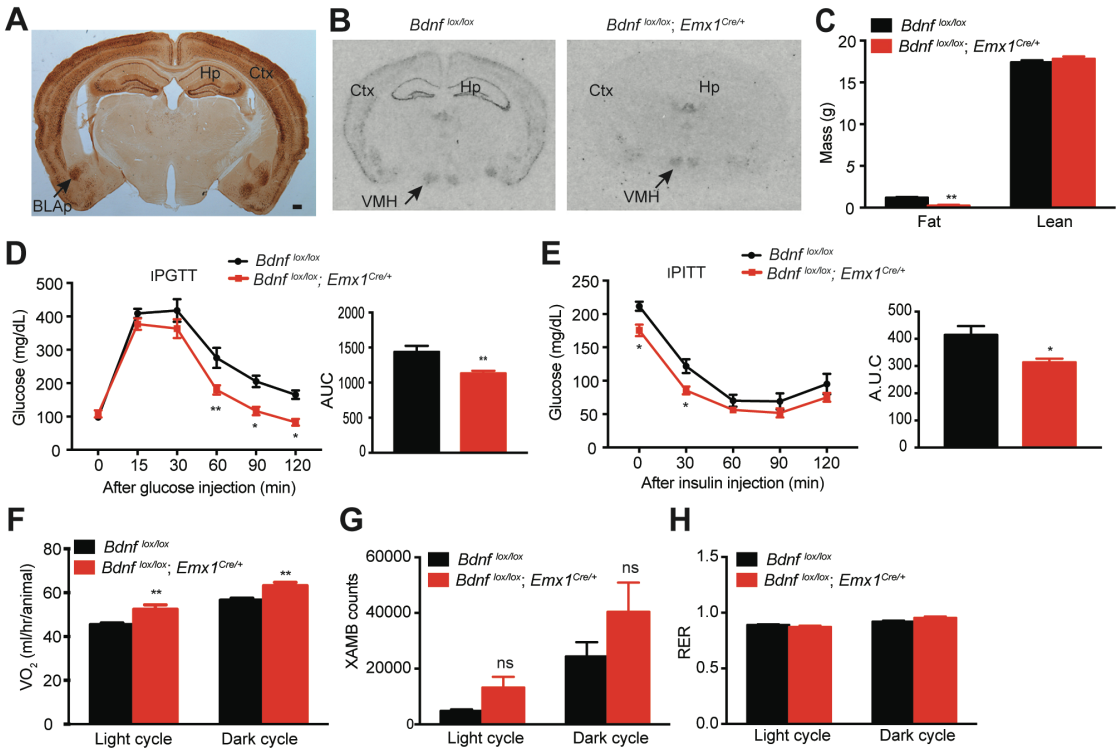

Figure S1, related to Figure 1. *Bdnf* deletion in Emx1-expressing neurons increased energy expenditure and improved glucose management

(A) Representative immunohistochemistry image showing expression of β-galactosidase in the brain of a *Bdnf*$^{klox/+}$;*Emx1*$^{Cre/+}$ mouse. Ctx, cortex; Hp, hippocampus; BLAp, posterior part of basolateral amygdala. Scale bar, 200 μm.

(B) Representative images of *in situ* hybridization revealing *Bdnf* expression in the brain. Ctx, cortex; Hp, hippocampus; VMH, ventromedial hypothalamus.

(C) Body composition of 14-week-old male mice fed chow diet. n=8-9 mice per group; **p<0.01 by Student's t test.

(D) Intraperitoneal glucose tolerance test (IPGTT) in chow-fed male mice at 12-13 weeks of age. n=8-9 mice per group; Two-way ANOVA followed by Bonferroni's test: genotype, $F_{(1,48)}$=27.07, p<0.0001; *p<0.05 and **p<0.01 when comparisons were made at individual time points. Student's t test was used to analyze area under curve (AUC) data: **p<0.01.

(E) Intraperitoneal insulin tolerance test (IPITT) in chow-fed male mice at 12-13 weeks of age. n=8-9 mice per group; Two-way ANOVA followed by Bonferroni's test: genotype, $F_{(1,60)}$=18.74, p<0.0001; *p<0.05 when comparisons were made at individual time points. Student's t test was used to analyze area under curve (AUC) data: *p<0.05.

(F) Oxygen consumption of 10-week-old male mice fed chow diet. n=8-9 mice per group; **p<0.01 by Student's t test.

(G) Ambulatory activity at the x axis (XAMB) of 10-week-old male mice fed chow diet. n=8-9 mice per group; ns, no significance by Student's *t* test.

(H) Respiratory exchange ratio (RER) of 10-week-old male mice fed chow diet. n=8-9 mice per group.

All data were presented as mean ± SEM.

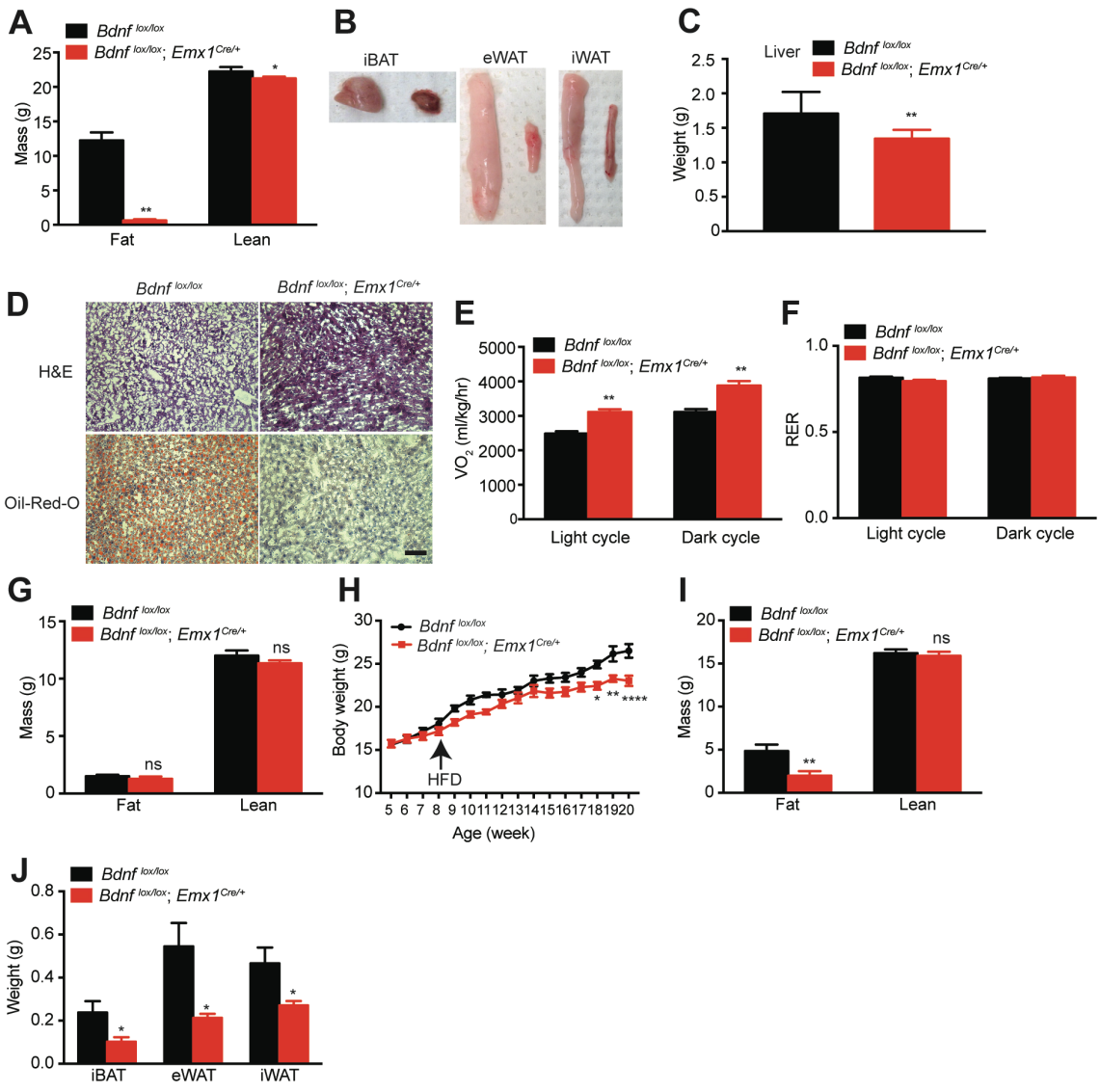

Figure S2, related to Figure 2. Elevated energy expenditure and leanness in HFD-fed *Bdnf$^{lox/lox}$;Emx1$^{Cre/+}$* mice

(A) Body composition of male mice fed HFD for 12 weeks. n=6-9 mice per group; **p<0.01 by Student's t test.

(B) Representative images of individual fat tissues from male control (left images) and mutant (right images) mice after 12 weeks of HFD feeding.

(C) Liver weight of male control and mutant mice after 12 weeks of HFD feeding. n=6-9 mice per group; **p<0.01 by Student's t test.

(D) Representative images of liver histology revealing cytoarchitecture through H&E staining (upper panel) and lipid accumulation through Oil Red O staining (lower panel). Scale bar, 20 μm.

(E) O_2 consumption of mice during the first week of HFD feeding. n=6-9 mice per group; **p<0.01 by Student's t test.

(F) RER of male mice during the first week of HFD feeding. n=6-9 mice per group.

(G) Body composition of 8-week-old female mice fed chow diet. n=6-7 mice per group. ns, no significance by Student's t test.
(H) Body weight curve. Female mice were maintained on chow diet until 8 weeks old and then fed HFD for 12 weeks. n=6-7 mice per group. Two-way ANOVA followed by Bonferroni's test: genotype, $F_{(1,160)}$=64.6, p<0.0001; *p<0.05, **p<0.01 and ****p<0.0001 when comparisons were made at individual time points.
(I) Body composition of female mice after 12 weeks of HFD feeding. n=6-7 mice per group; **p<0.01 by Student's t test.
(J) Weight of individual fat tissues dissected from female mice after 12 weeks of HFD feeding. n=6-7 mice per group; ** p<0.05 by Student's t test.
All data were presented as mean ± SEM.

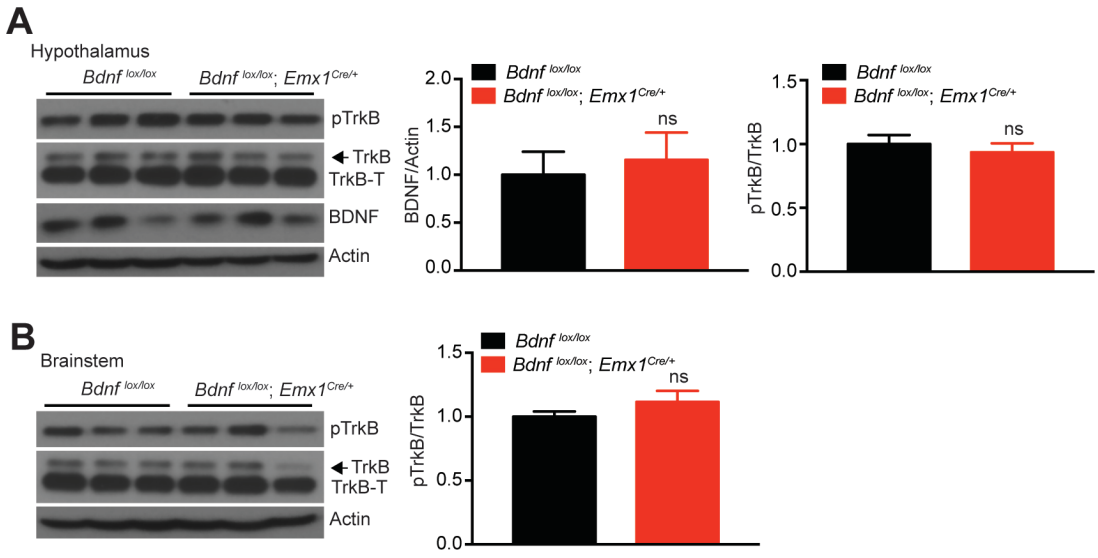

Figure S3, related to Figure 3. BDNF-TrkB signaling is unaltered in the hypothalamus and brainstem of *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice

(A) Western blotting analysis and quantification of BDNF and phosphorylated TrkB (pTrkB) in the hypothalamus of 12-week-old male mice on chow diet. TrkB, full-length TrkB; TrkB-T, truncated TrkB that lacks the kinase domain. ns, no significance, by Student's t test.

(B) Western blotting analysis and quantification of phosphorylated TrkB in the brainstem of 12-week-old male mice on chow diet. ns, no significance, by Student's t test.

All data were presented as mean ± SEM.

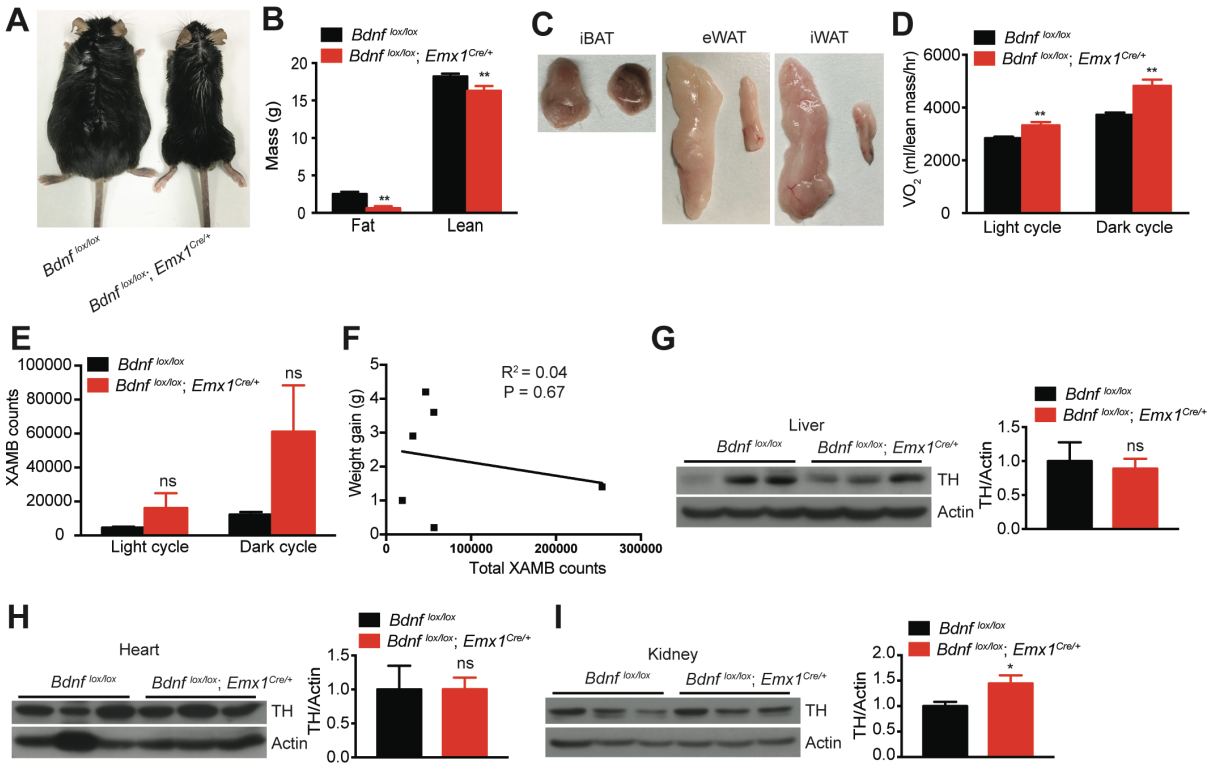

Figure S4, related to Figure 4. Male *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice showed resistance to DIO in the thermoneutral zone.

(A) Representative image of mice after 12 weeks of HFD feeding at the thermoneutral zone.

(B) Body composition of mice after 12 weeks of HFD feeding at thermoneutrality. n=6-8 mice per group; **p<0.01 by Student's t test.

(C) Representative images of individual fat tissues (Left images, control; Right images, mutant) after 12 weeks of HFD feeding at thermoneutrality.

(D) O_2 consumption of mice at thermoneutrality during the first week of HFD feeding. n=6-8 mice per group; **p<0.01 by Student's t test.

(E) Ambulatory activity as revealed by beam break counts at X axis (XAMB counts). n=6-8 mice per group; not significant (ns) by Student's t test.

(F) No correlation between ambulatory activity and weight gain on HFD in *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice.

(G) Western blotting analysis and quantification of TH in the liver of 12-week-old male mice on chow diet. ns, no significance, by Student's t test.

(H) Western blotting analysis and quantification of TH in the heart of 12-week-old male mice on chow diet. ns, no significance by Student's t test.

(I) Western blotting analysis and quantification of TH in the kidney of 12-week-old male mice on chow diet. *p<0.05 by Student's t test.

All data were presented as mean ± SEM.

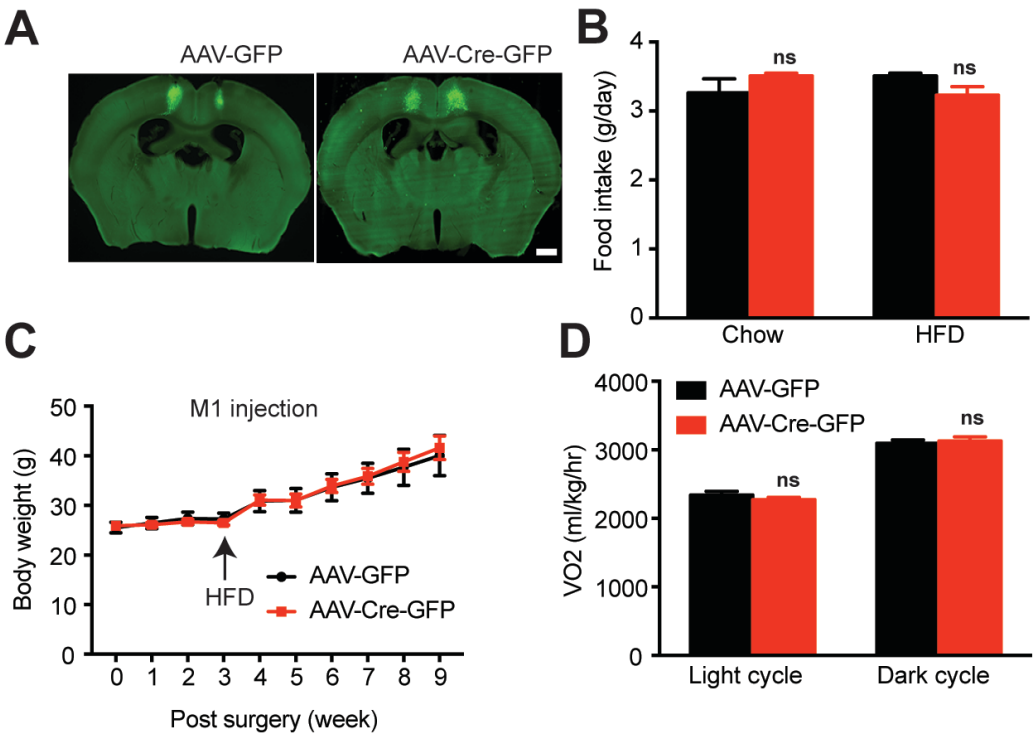

Figure S5, related to Figure 5. Deletion of the *Bdnf* gene in the M1 motor cortex did not increase energy expenditure

(A) Representative images showing injection of AAV-GFP or AAV-Cre-GFP into M1 motor cortex. AAV was injected into 8-week-old male *Bdnf*$^{lox/lox}$ mice. Scale bar, 200 μm.

(B) Daily food intake of M1-injected mice on chow diet or HFD. n=6-9 mice per group; ns, no significance by Student's t test.

(C) Body weight of male *Bdnf*$^{lox/lox}$ mice with AAV injection into the M1 motor cortex. Three weeks after AAV injection, animals were fed HFD for 6 weeks. n=6-9 mice per group.

(D) O_2 consumption of male *Bdnf*$^{lox/lox}$ mice with AAV injection into the M1 motor cortex. The measurement was performed 2 weeks after AAV injection. n=6-9 mice per group; ns, no significance by Student's t test.

All data were presented as mean ± SEM.

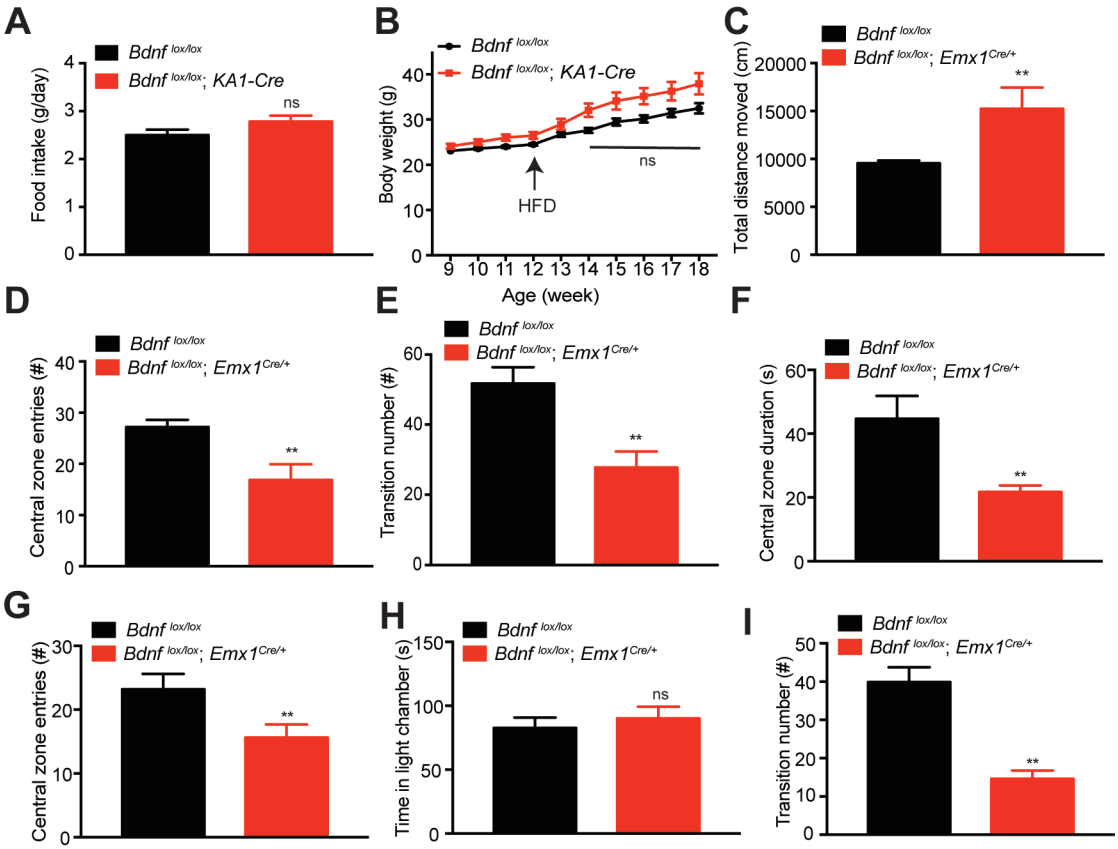

Figure S6, related to Figure 6. Anxiety, but not aggression, was associated with resistance to DIO in *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice.

(A) Daily HFD intake of male *Bdnf*$^{lox/lox}$ and *Bdnf*$^{lox/lox}$;KA1-Cre mice. n=6-7 mice per group; ns, no significance by Student's t test.

(B) Body weight of male *Bdnf*$^{lox/lox}$ and *Bdnf*$^{lox/lox}$;KA1-Cre mice. The mice were switched to the HFD from chow diet at 12 weeks of age. n=6-7 mice per group; Two-way ANOVA followed by Bonferroni's test: genotype, $F_{(1,110)}$=31.69, p<0.0001; ns, no significance.

(C) Total distance moved during 30 minutes of open-field tests in male *Bdnf*$^{lox/lox}$ and *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice. n=8-13 mice per group. ** p<0.01 by Student's t test.

(D) Central zone entries during the first 5 minutes of open field tests in male *Bdnf*$^{lox/lox}$ and *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice. n=8-13 mice per group; **p<0.01 by Student's t test.

(E) Transition number between light and dark chambers during light-dark box tests in male *Bdnf*$^{lox/lox}$ and *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice. n=8-13 mice per group; **p<0.01 by Student's t test.

(F and G) Time spent in central zone and central zone entries of female *Bdnf*$^{lox/lox}$ and *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice during the first 5 minutes of open field tests. n=10-11 mice per group; **p<0.01 by Student's t test

(H and I) Time spent in light chamber and transition number between light and dark chambers during light-dark box tests in female *Bdnf*$^{lox/lox}$ and *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice. n=10-11 mice per group; ns, no significance; **p<0.01 by Student's t test.

All data were presented as mean ± SEM.

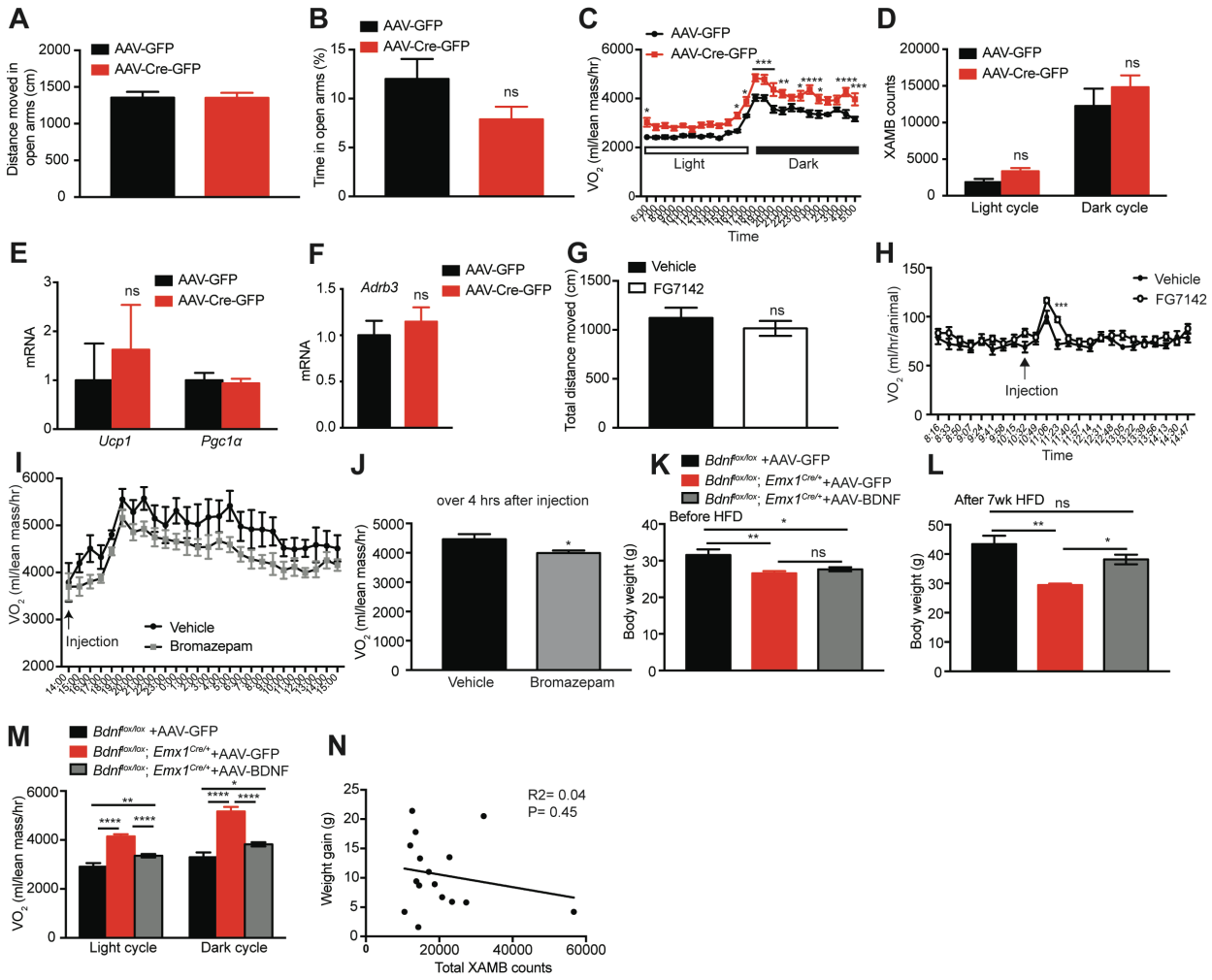

Figure S7, related to Figure 7. Effects of *Bdnf* deletion or overexpression in the amygdala on anxiety and energy expenditure

(A) Total distance moved in open arms in elevated plus maze tests. n=12-13 male *Bdnf*$^{lox/lox}$ mice per group.

(B) Time spent in open arms (%) in elevated plus maze tests. n=12-13 male *Bdnf*$^{lox/lox}$ mice per group. ns, no significance by Student's *t* test.

(C) Oxygen consumption (VO_2) of AAV-injected male *Bdnf*$^{lox/lox}$ mice during HFD feeding. n=7-8 mice per group; Two-way ANOVA followed by Bonferroni's test: genotype, $F_{(1,312)}$=252.7, p<0.0001; *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 when comparisons were made at individual time points.

(D) Ambulatory activity of AAV-injected male *Bdnf*$^{lox/lox}$ mice during HFD feeding. n=12-13 mice per group; ns, no significance by Student's t test.

(E) Expression of *Ucp1* and *Pgc1*α in iWAT of AAV-injected male *Bdnf*$^{lox/lox}$ mice after 8 weeks of HFD feeding. n=12-13 mice per group; ns, no significance by Student's *t* test.

(F) Relative levels of *Adrb3* mRNA in iWAT of AAV-injected male *Bdnf*$^{lox/lox}$ mice after 8 weeks of HFD feeding. n=12-13 mice per group; ns, no significance by Student's *t* test.

(G) Total distance moved in light chamber during light-dark box tests after FG7142 treatment (5 mg/kg). n=10 male WT mice per group; ns, no significance by Student's t test.

(H) O_2 consumption of male C57BL6 WT mice treated with vehicle or FG7142 (5 mg/kg). n=8 mice per group. Two-way ANOVA followed by Bonferroni's test: treatment, $F_{(1,336)}$=25.69, p<0.0001; ***p<0.001 when comparisons were made at individual time points.

(I) O_2 consumption of male *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice treated with vehicle or bromazepam (50 μg/kg). n=7 mice per group. Two-way ANOVA followed by Bonferroni's test: treatment, $F_{(1,286)}$=59.5. p<0.0001.

(J) O_2 consumption of male *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice over a 4-h period after treatment with vehicle or bromazepam. n=7 mice per group. *p<0.05 by Student's *t* test.

(K) Body weight of AAV-injected male *Bdnf*$^{lox/lox}$ and *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice before HFD. n=7-16 mice per group. ns, no significance; **p<0.05, **p<0.01 by one-way ANOVA.

(L) Body weight of AAV-injected male *Bdnf*$^{lox/lox}$ and *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice after 7 weeks of HFD feeding. n=7-16 mice per group. *p<0.05, **p<0.01 by one-way ANOVA.

(M) O_2 consumption of AAV-injected male *Bdnf*$^{lox/lox}$ and *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice during HFD feeding. n=7-16 mice per group. **p<0.01 by one-way ANOVA.

(N) No correlation between ambulatory activities and weight gain on HFD in AAV-BDNF-injected male *Bdnf*$^{lox/lox}$;*Emx1*$^{Cre/+}$ mice.

All data were presented as mean ± SEM.

Table S1. Sequences of qPCR primers, Related to STAR Methods

Gene	Forward	Reverse
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Pgc1α	CGGAAATCATATCCAACCAG	TGAGAACCGCTAGCAAGTTTG
Prdm16	GACATTCCAATCCCACCAGA	CACCTCTGTATCCGTCAGCA
Cidea	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
Adiponectin	AAGGACAAGGCCGTTCTCT	TATGGGTAGTTGCAGTCAGTTGG
Adrb3	TGAAACAGCAGACAGGGACA	GGCGTCCTGTCTTGACACTC
F4/80	CCCCAGTGTCCTTACAGAGTG	GTGCCCAGAGTGGATGTCT
36B4	ATCCCTGACGCACCGCCGTGA	TGCATCTGCTTGGAGCCCACGTT
Tnfα	CATCTTCTCAAAACTCGAGTGACAA	TGGGAGTAGATAAGGTACAGCCC
Corticotropin-releasing factor (Crf)	CGCAGCCCTTGAATTTCTTG	GCGGGACTTCTGTTGAGATT