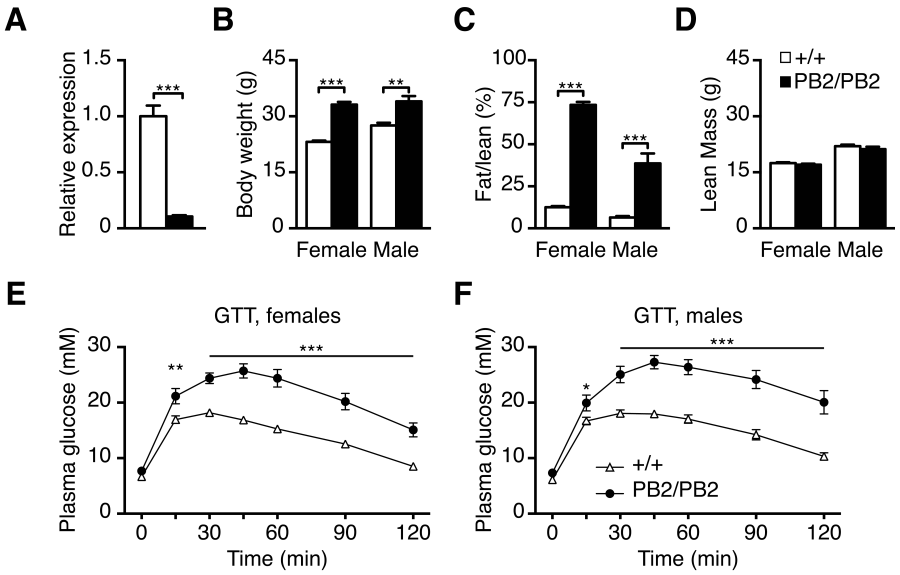
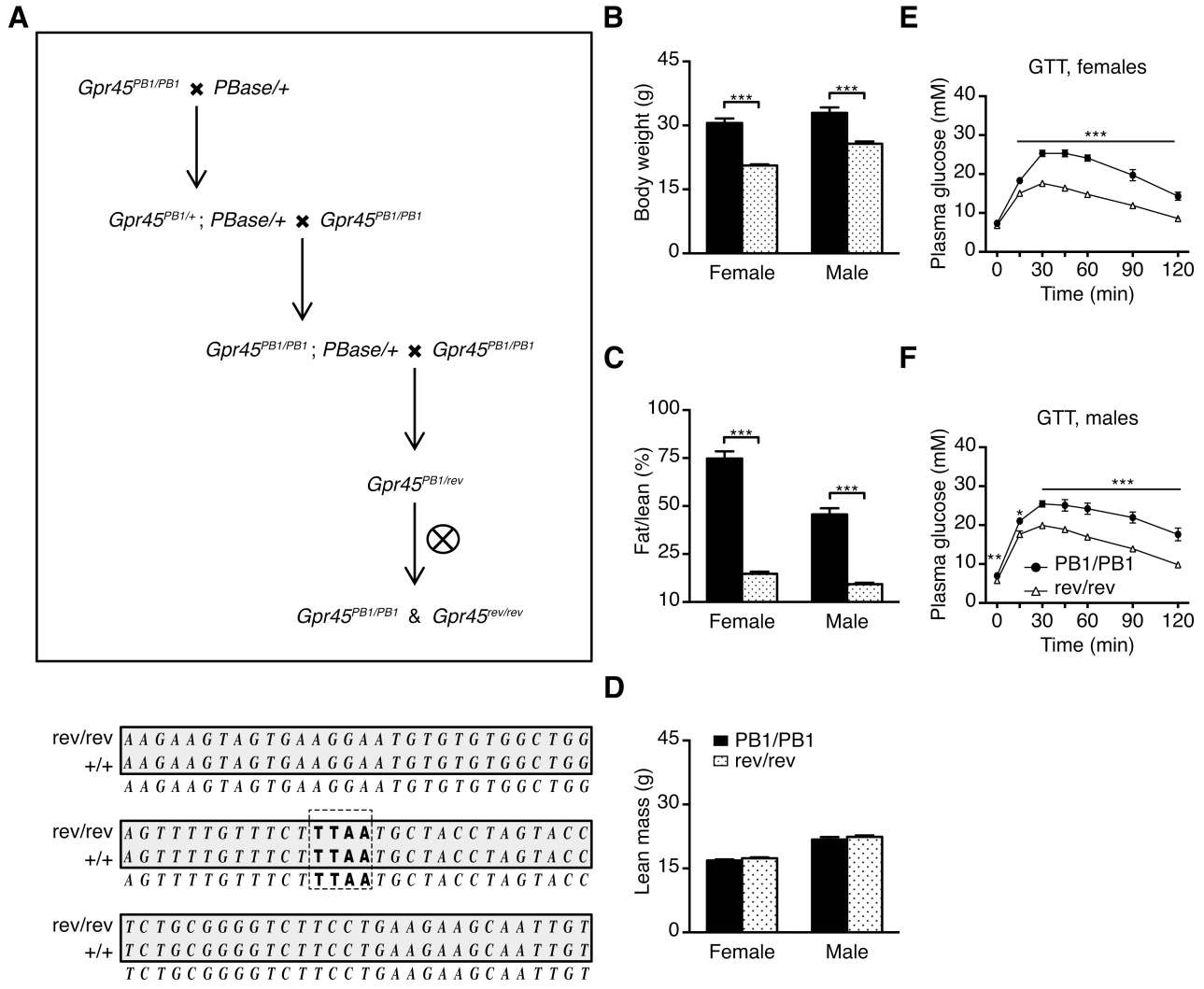
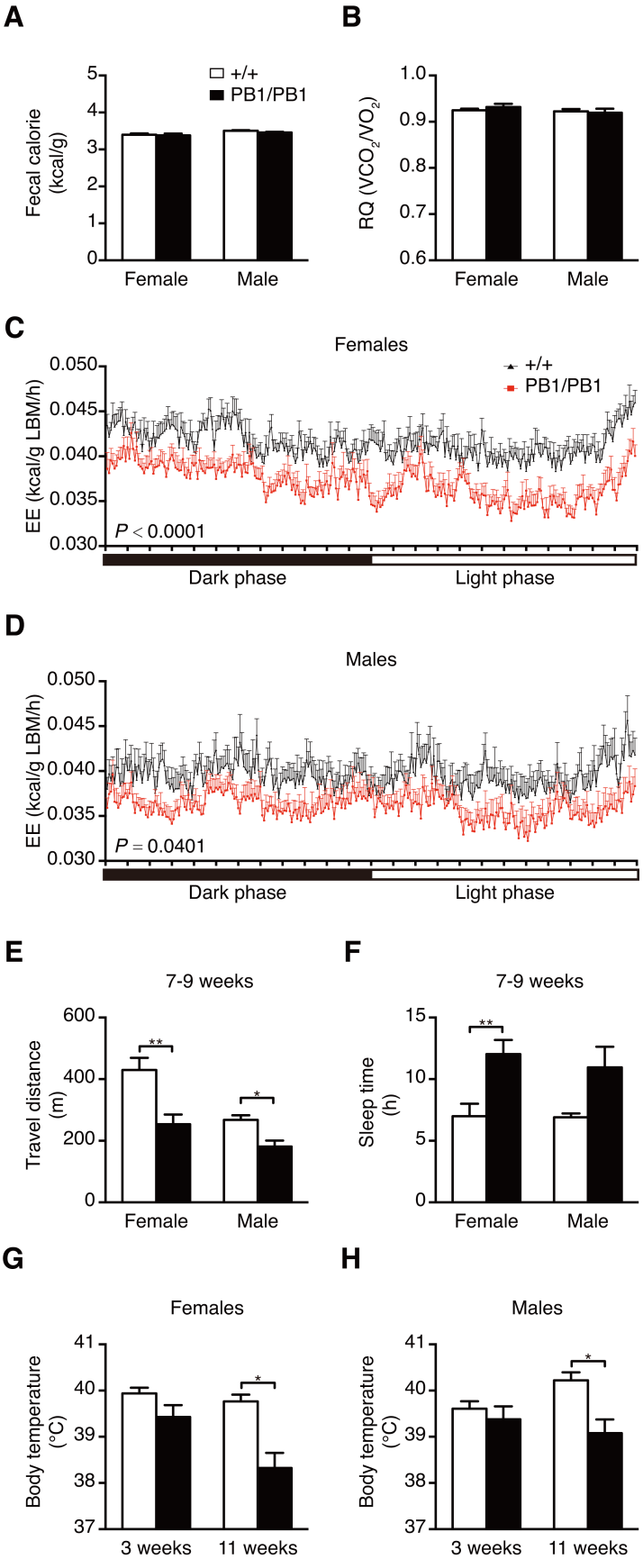


Supplemental Figure 1. Obesity and glucose intolerance in *Gpr45*$^{PB2/PB2}$ mice. (**A**) Real-time RT-PCR analysis showed reduced *Gpr45* expression in brain of P5 mutants. Number of mice: +/+, *n* = 4; PB2/PB2, *n* = 6. Expression of *Gapdh* serves as the internal control to calculate relative expression levels. (**B** and **C**) Average body weight (**B**) and fat/lean ratio (**C**) are increased in 12-week-old female (+/+, *n* = 7; PB2/PB2, *n* = 7) and male (+/+, *n* = 6; PB2/PB2, *n* = 7) mutants. (**D**) Average lean mass are normal in 12-week-old female (+/+, *n* = 7; PB2/PB2, *n* = 7) and male (+/+, *n* = 6; PB2/PB2, *n* = 7) mutants. (**E** and **F**) Glucose tolerance tests (GTTs) in 12-week-old female (**E**, +/+, *n* = 13; PB2/PB2, *n* = 10) and male (**F**, +/+, *n* = 10; PB2/PB2, *n* = 9) mice. All data are shown as the mean ± SEM. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 by Student's *t* test.

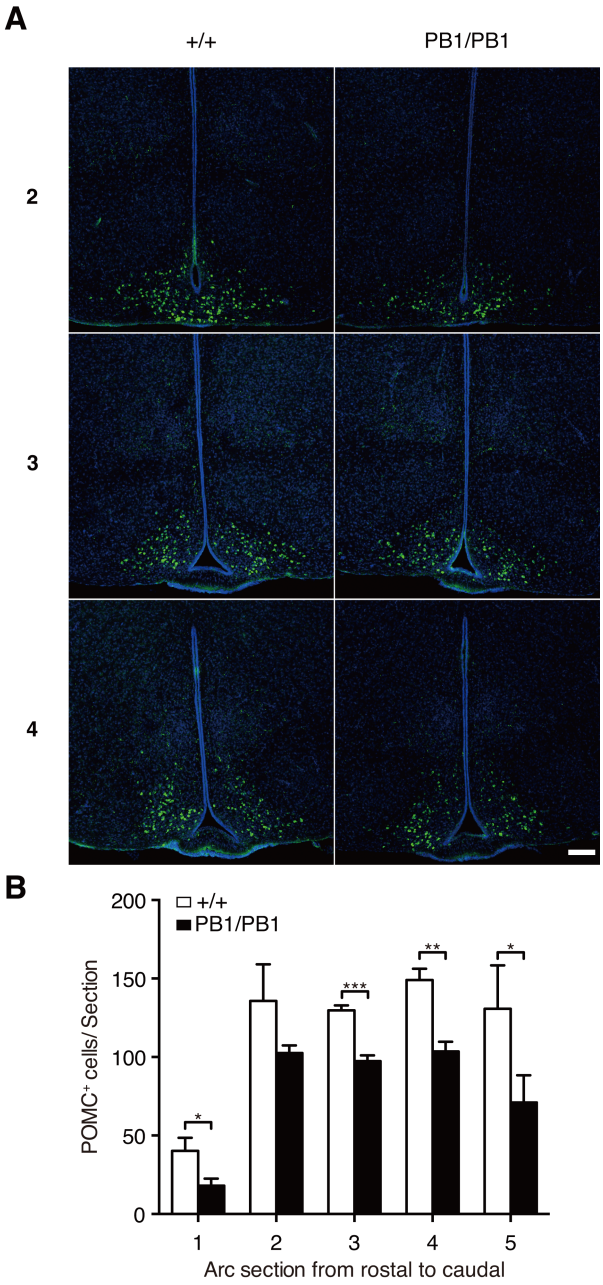


Supplemental Figure 2. Genetic revertant of *Gpr45* results in reduced obesity. (**A**) Generating a *Gpr45* revertant allele (*Gpr45*^{rev}) by excision of the *PB1* insertion. (**B**, **C** and **D**) 12-week-old female (rev/rev, *n* = 11; PB1/PB1, *n* = 10) and male (rev/rev, *n* = 13; PB1/PB1, *n* = 8) *Gpr45*$^{rev/rev}$ mice showed significantly reduced body weight (**B**) and fat/lean ratio (**C**), as well as normal lean mass (**D**). (**E** and **F**) 12-week-old female (**E**) and male (**F**) *Gpr45*$^{rev/rev}$ mice showed better tolerance upon glucose administration. All data are shown as the mean ± SEM. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 by Student's *t* test.



Supplemental Figure 3.

Physiology and behavior analysis of *Gpr45*$^{PB1/PB1}$ mice. (**A**) Similar fecal energy between wild-type (female, *n* = 4; male, *n* = 3) and mutant (female, *n* = 7; male, *n* = 3) mice at the age of 3 weeks. (**B**) Similar respiratory quotient (RQ) between wild-type (female, *n* = 22; male, *n* = 22) and mutant (female, *n* = 22; male, *n* = 22) mice at the ages among P21 to P33. (**C** and **D**) Real-time energy expenditure in 24 hours, normalized by lean mass, were decreased in female (**C**) and male (**D**) mutants (female, *n* = 18; male, *n* = 12) at P21 to P33. Energy expenditure (EE) data were analyzed by two-way ANOVA. (**E** and **F**) Compared with wild-type adult mice (9-week-old females, *n* = 8; 7-week-old males, *n* = 4), age- and sex- matched mutants (female, *n* = 8; male, *n* = 4) had shorter travel distance (**E**) but longer sleep time (**F**) in a period of 24 hours. (**G** and **H**) Similar body temperatures were observed in 3-week-old mutant (**G**, female, *n* = 7; **H**, male, *n* = 10) and wild-type (female, *n* = 8; male, *n* = 12) mice. However, mutants (female, *n* = 4; male, *n* = 5) exhibited significantly lower body temperature than the wild-type (female, *n* = 3; male, *n* = 5) mice at the age of 11 weeks. All data are shown as the mean ± SEM. **P* < 0.05 and ***P* < 0.01 by Student's *t* test.



Supplemental Figure 4. Decreased expression of POMC in *Gpr45*$^{PB1/PB1}$ mice. (**A**) Representative images of POMC immunostaining in coronal sections of brain of P14 mice. Green, POMC; blue, DAPI; Scale bar: 0.2 mm. (**B**) Quantification of POMC neurons from the most rostral ("1") to the most caudal ("5") sections (*n* = 3 animals for each genotype). Slices 1, 2, 3, 4, and 5 are approximately positioned to Bregma -1.34 mm, -1.58 mm, -1.82 mm, -2.06 mm, and -2.30 mm as described (1). Data are shown as the mean ± SEM. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 by Student's *t* test.

1. Franklin KBJ, and Paxinos G. *The mouse brain in stereotaxic coordinates.* San Diego: Academic Press; 1997;xxii p.