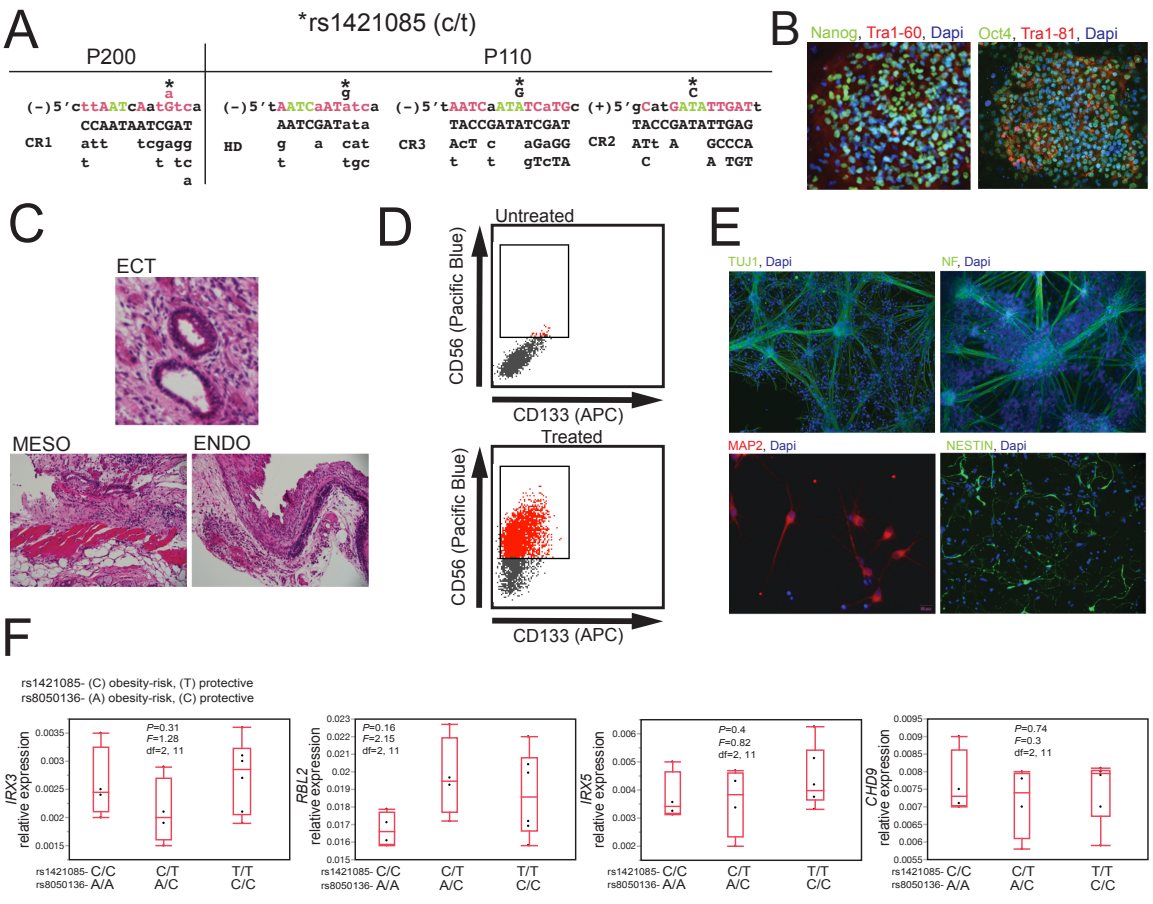
Supplemental Figures

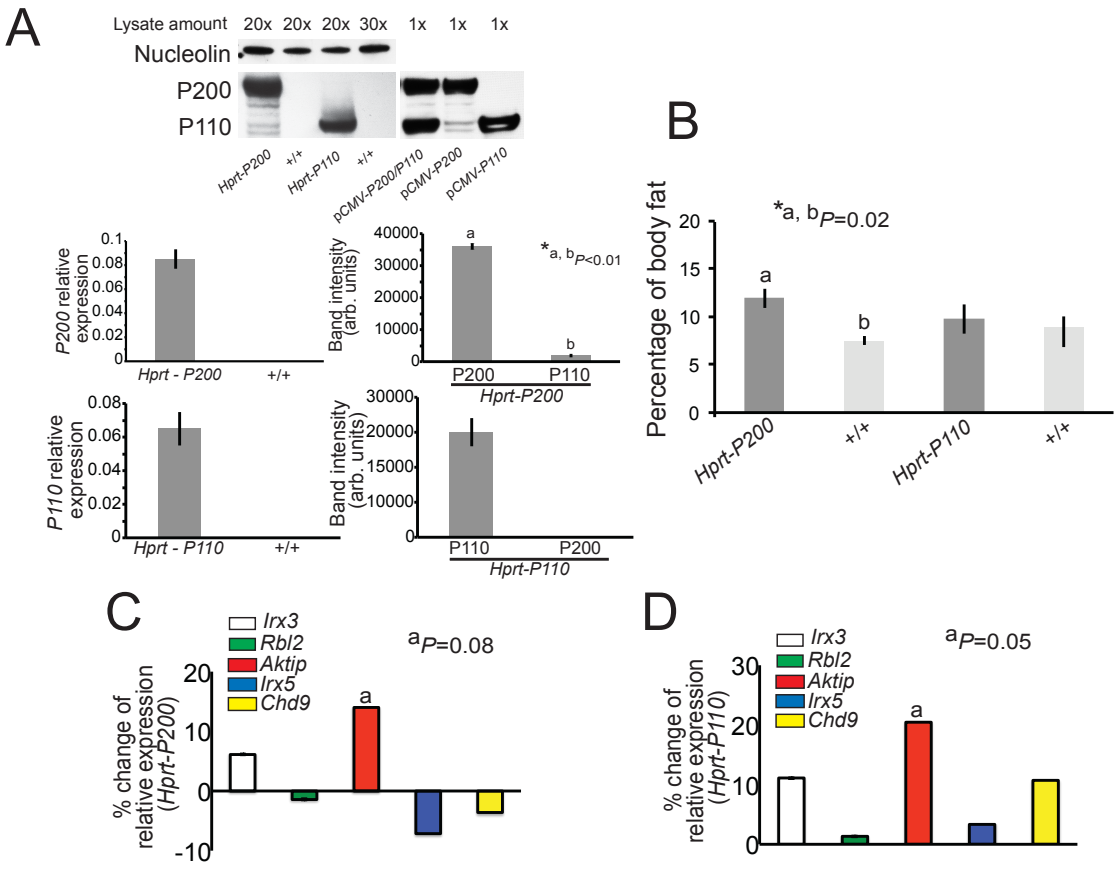
Supplementary Figure 1



CUX1 binding at rs1421085 and lack of allelic effect on vicinal genes in neurons. (**A**) Modeling the binding of CUX1 isoforms P200 and P110 at the predicted binding site including rs1421085. CR2 and CR3 domains recognize a degenerate sequence suggesting flexibility in DNA interaction, whereas CR1 or HD determines binding specificity (26). DNA consensus binding site for each DNA-binding domain (except HD domain) consists of an obligatory ATA sequence (in green). P200 is predicted to bind weakly the rs1421085 site at the

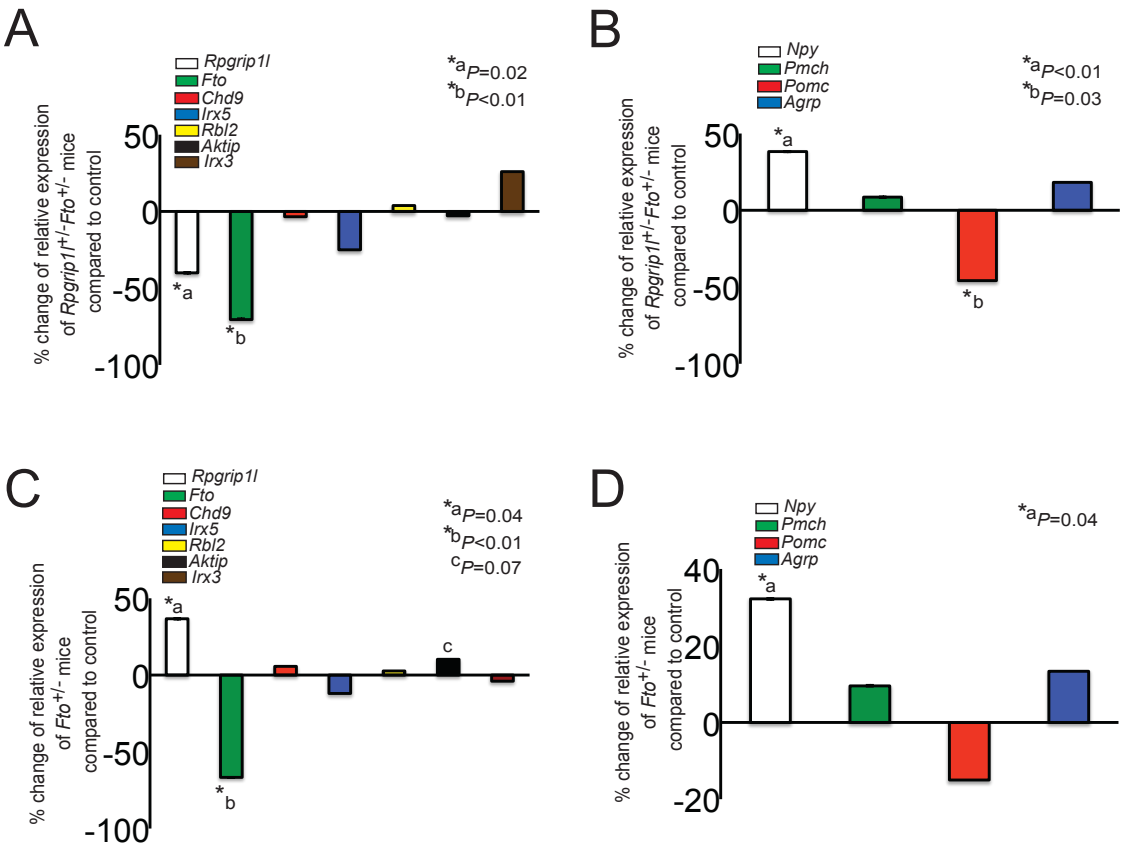
ATC core sequence. On the other hand, P110 is predicted to have higher affinity for the rs1421085 site due to the conservation of the ATC core sequence. P200 is also predicted to preferentially bind the obesity-risk C allele as it fits the CR1 consensus, whereas P110 is predicted to preferentially bind the rs1421085 T protective allele as it fits the consensus for the CR2 and CR3 domains. (**B**) Primary fibroblasts were reprogrammed to iPSC that express the canonical pluripotency markers, alkaline phosphatase, Tra 1-60, octamer-binding transcription factor 4 (Oct4), Nanog, and Tra 1-81 (*n* = 2). (**C**) Injection of iPSC into immunocompromised NSG mice resulted in the formation of teratomas that display structures of neural rosettes indicating ectoderm, muscle cells indicating mesoderm, and intestinal crypts indicating endoderm (*n* = 2). (**D**) Differentiated neurons sorted for neuronal marker CD56 and controlled for the stem cell surface marker CD133. (*n* = 14) (**E**) iPSC-derived neurons express neuronal markers, including Microtubule-associated protein 2 (MAP2), Neurofilament (NF), Nestin and Neuron-specific class III beta-tubulin (TUJ1) (*n* = 2). (**F**) *IRX3*, *IRX5*, *RBL2* and *CHD9* expression remained unchanged in neurons heterozygous (*n* = 4) or homozygous for the obesity-risk (C/A) (*n* = 4) or protective (T/C) (*n* = 6) alleles at rs1421085 and rs8050136. All error bars represent SEM. Statistical significance was determined by a two-tailed paired Student's t test or ANOVA. *P* values lower than 0.05 were considered significant (*).

Supplementary Figure 2



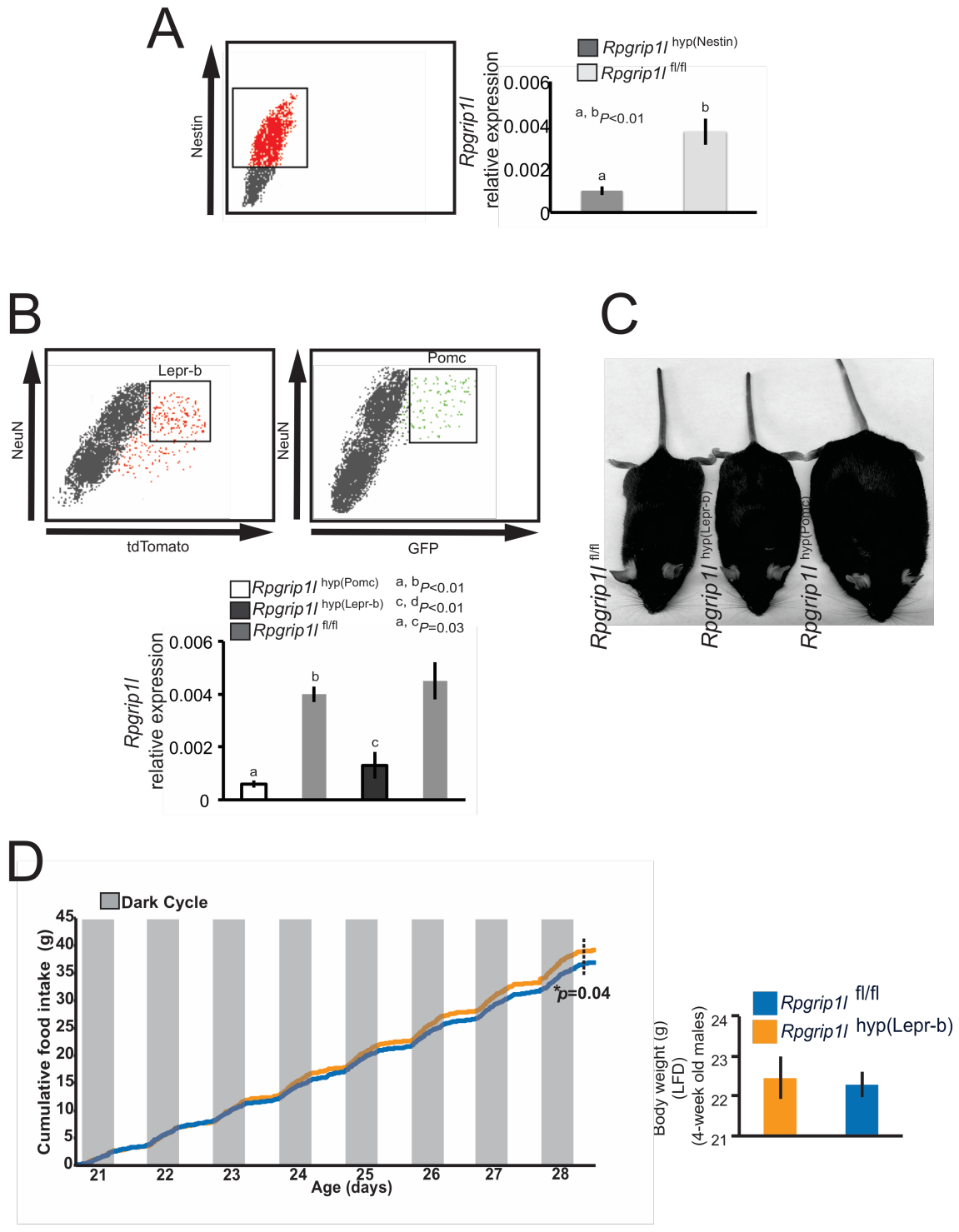
Phenotypic and expression analysis of *Hprt-P200* and *Hprt-P110* mice. (**A**) P200 protein or mRNA levels from total hypothalamic RNA or protein nuclear extracts of *Hprt-P200* and *Hprt-P110* mice or N2a cells overexpressing P200 or P110 cDNA (n = 5). Error bars represent SEM. (**B**) Body fat of *Hprt-P200* (n = 7), *Hprt-P110* (n = 9) and control (n = 8 and n = 10 respectively) mice adjusted for body weight. Error bars represent SEM. (**C**) Percent change of hypothalamic expression analysis of genes vicinal to the *FTO* locus in *Hprt-P200* (n = 5) or (**D**) *Hprt-P110* (n = 5) mice compared to +/+ (n = 5 respectively) control mice. Statistical significance was determined by a two-tailed paired Student's t test. P alpha values lower than 0.05 were considered significant (*).

Supplementary Figure 3



Transcriptional analysis of *Fto*$^{+/-}$ and *Rpgrip1l*$^{+/-}$/*Fto*$^{+/-}$ mice. Percent change of transcript levels of *Rpgrip1l*, *Fto* and vicinal genes in (**A**) *Rpgrip1l*$^{+/-}$/*Fto*$^{+/-}$ and (**C**) *Fto*$^{+/-}$ mice compared with +/+ mice (*n* = 5). Percent change of expression levels of orexigenic (*Npy, Pmch, AgRP*) and anorexigenic (*Pomc*) in (**B**) *Rpgrip1l*$^{+/-}$/*Fto*$^{+/-}$ and (**D**) *Fto*$^{+/-}$ mice compared with +/+ mice (*n* = 5). Statistical significance was determined by a two-tailed paired Student's t test. *P* values lower than 0.05 were considered significant (*).

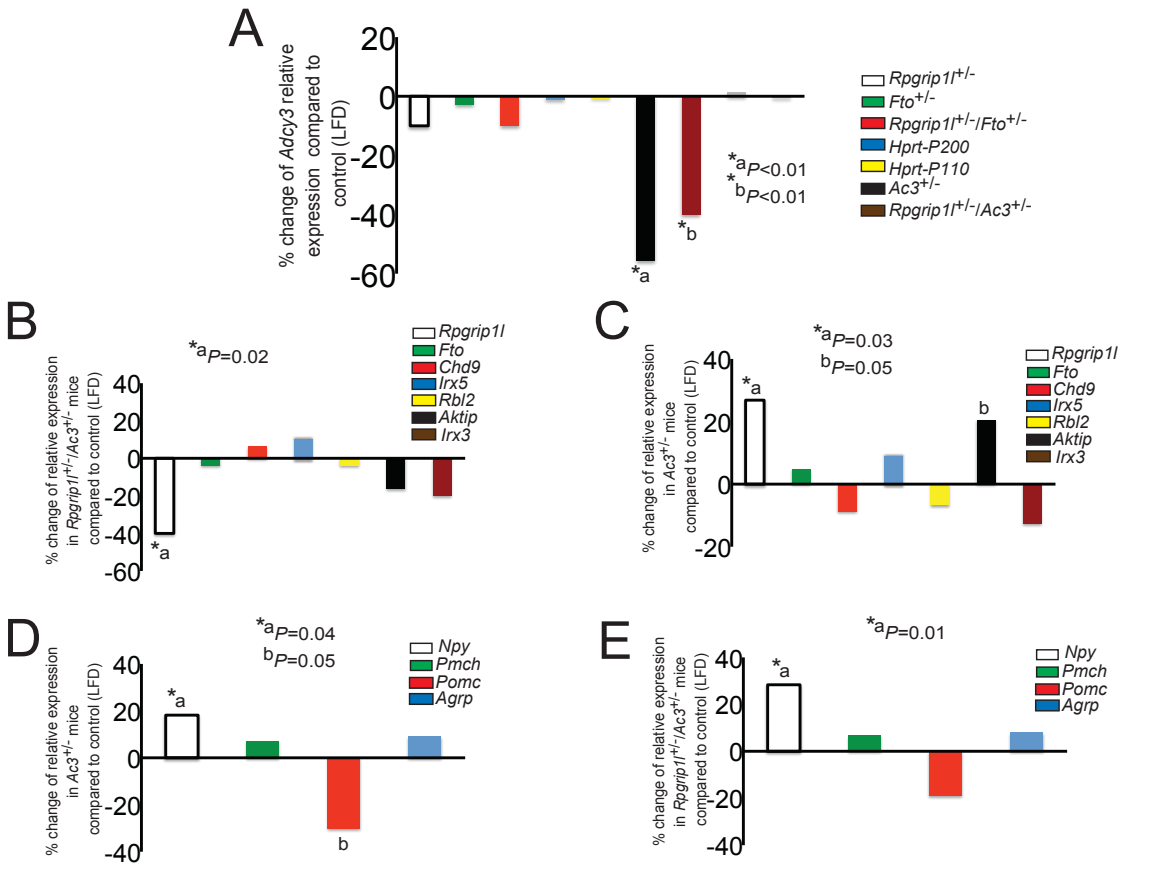
Supplementary Figure 4



***Rpgrip1l* expression and phenotypic analysis of *Rpgrip1l*$^{hyp(Pomc)}$, *Rpgrip1l*$^{hyp(Lepr-b)}$ and *Rpgrip1l*$^{hyp(Nestin)}$ mice**. Detection of *Rpgrip1l* expression

by RT-PCR in FACS-sorted (**A**) *Nestin*-expressing (*n* = 3), and (**B**) *Pomc*-expressing (*n* = 3, each data point consisted of admixed hypothalamic tissue from 3 animals) and *Lepr-b*-expressing (*n* = 3, each data point consisted of admixed hypothalamic tissue from 6 animals) hypothalamic neurons segregating for 2 floxed *Rpgrip1l* alleles. (**C**) Picture of 22-week old +/+, *Rpgrip1l*$^{hyp(Pomc)}$, and Rpgrip1l hypomorphic mice in Lepr-b expressing neurons (*Rpgrip1l*$^{hyp(Lepr\text{-}b)}$). (**D**) Body weight and time course of cumulative food intake and body weight of 4-week old *Rpgrip1l*$^{hyp(Lepr\text{-}b)}$ (*n* = 8) and control (*n* = 8) mice fed LFD. All error bars represent SEM. Statistical significance was determined by a two-tailed paired Student's t test. *P* values lower than 0.05 were considered significant (*).

Supplementary Figure 5



Hypothalamic expression analysis of *Ac3*$^{+/-}$ and *Rpgrip1l*$^{+/-}$/*Ac3*$^{+/-}$ mice. (**A**) Percent change of hypothalamic *Ac3* (*Adcy3* gene) expression in *Rpgrip1l*$^{+/-}$, *Rpgrip1l*$^{+/-}$/*Ac3*$^{+/-}$, *Ac3*$^{+/-}$, *Hprt-P110*, *Hprt-P200*, *Fto*$^{+/-}$, and *Rpgrip1l*$^{+/-}$/*Fto*$^{+/-}$ mice compared to +/+ control mice (n = 5). Percent change of hypothalamic expression of *Rpgrip1l*, *Fto* and vicinal genes in (**B**) *Rpgrip1l*$^{+/-}$/*Ac3*$^{+/-}$ and (**C**) *Ac3*$^{+/-}$ mice compared to +/+ mice (n = 5). Percent change of hypothalamic expression of *Npy*, *Pmch*, *Pomc* and *AgRP* in (**D**) *Ac3*$^{+/-}$ and (**E**) *Rpgrip1l*$^{+/-}$/*Ac3*$^{+/-}$ mice compared to +/+ mice (n = 5). Statistical significance was determined by a two-tailed paired Student's t test. P values lower than 0.05 were considered significant (*).