

Supporting Information

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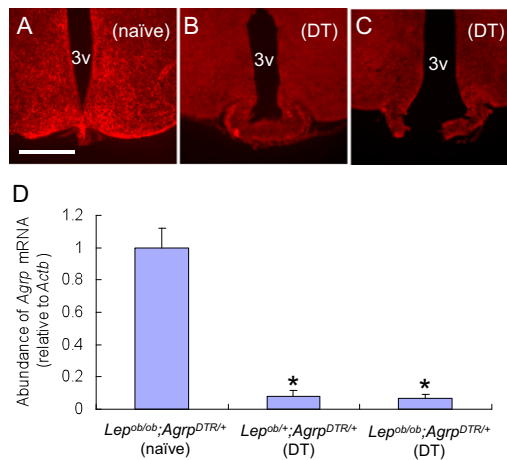


Fig. S1. Agouti-related protein (AgRP)-expressing neurons in adult leptin-deficient mice carrying the $Agrp^{DTR}$ allele are ablated by diphtheria toxin (DT) treatment. (A–C) Representative pictures of anti-AgRP immunostaining in the arcuate nucleus (ARC) of naive $Lep^{ob/ob}; Agrp^{DTR/+}$ mice without DT treatment. (Scale bar: 400 μ m for A–C.) (B) Representative pictures of anti-AgRP immunostaining in the ARC of $Lep^{ob/+}; Agrp^{DTR/+}$ mice injected with DT (50 ng/g body weight, two i.m. injections given 2 d apart). (C) Representative pictures of anti-AgRP immunostaining in the ARC of $Lep^{ob/ob}; Agrp^{DTR/+}$ mice treated with DT. (D) Quantification of the $Agrp$ transcript levels in the ARC of mice as described in A–C. The relative abundance of $Agrp$ mRNA was measured and normalized to that of the $Actb$ gene (encoding β -actin) by quantitative RT-PCR. $n = 4$ –6 per group; $*P < 0.01$, ANOVA.

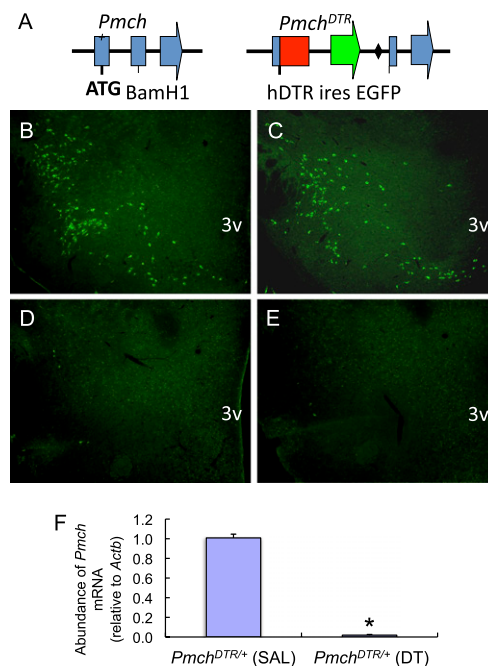


Fig. S2. Ablation of melanin-concentrating hormone (MCH)-expressing neurons after DT injection. (A) The $Pmch^{DTR}$ allele (Right) expresses the human DT receptor (hDTR) from the $Pmch$ promoter, followed by an internal ribosome entry site (ires) and EGFP. These transgenes replace parts of the first and second exons of the WT $Pmch$ allele (Left) between the initiation codon, ATG, and a BamH1 site. (B–E) Injection of DT (top of panels), but not vehicle (saline; bottom of panels), into adult mice carrying the $Pmch^{DTR}$ allele abolishes anti-MCH immunostaining in the lateral hypothalamus. The mice in B and D are leptin-replete ($Lep^{+/+}$), whereas those in C and E are leptin-deficient ($Lep^{ob/ob}$). 3v, third ventricle. (F) Quantification of $Pmch$ transcript levels between $Pmch^{DTR/+}$ mice receiving either DT or vehicle [saline (SAL)]. Error bars represent mean \pm SEM ($n = 3$ per group). $*P < 0.0001$, unpaired t test.

