Supplementary Information

AgRP neurons regulate bone mass

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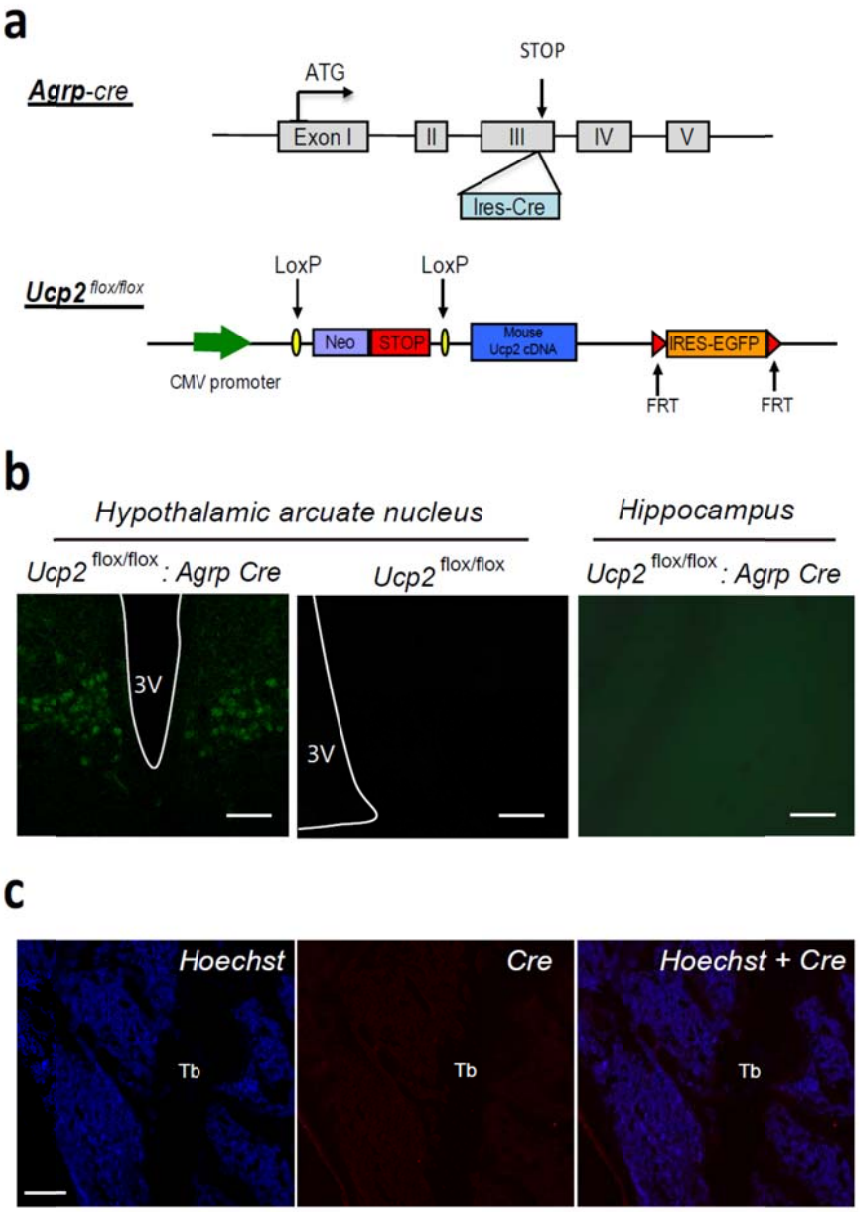
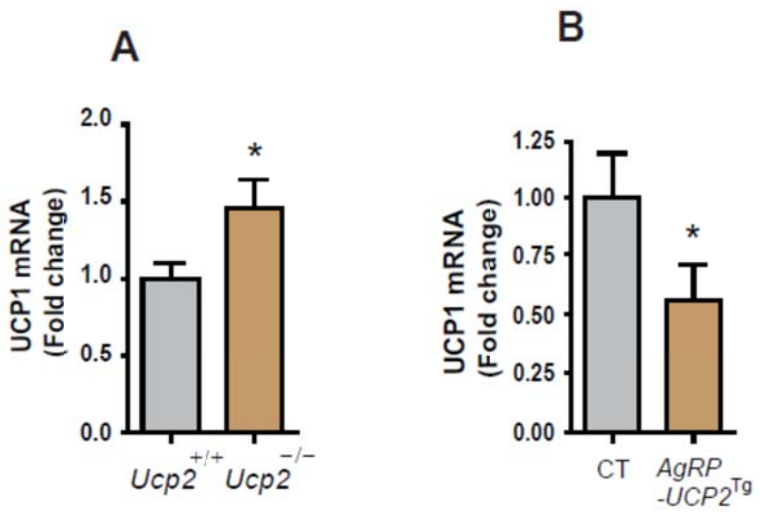
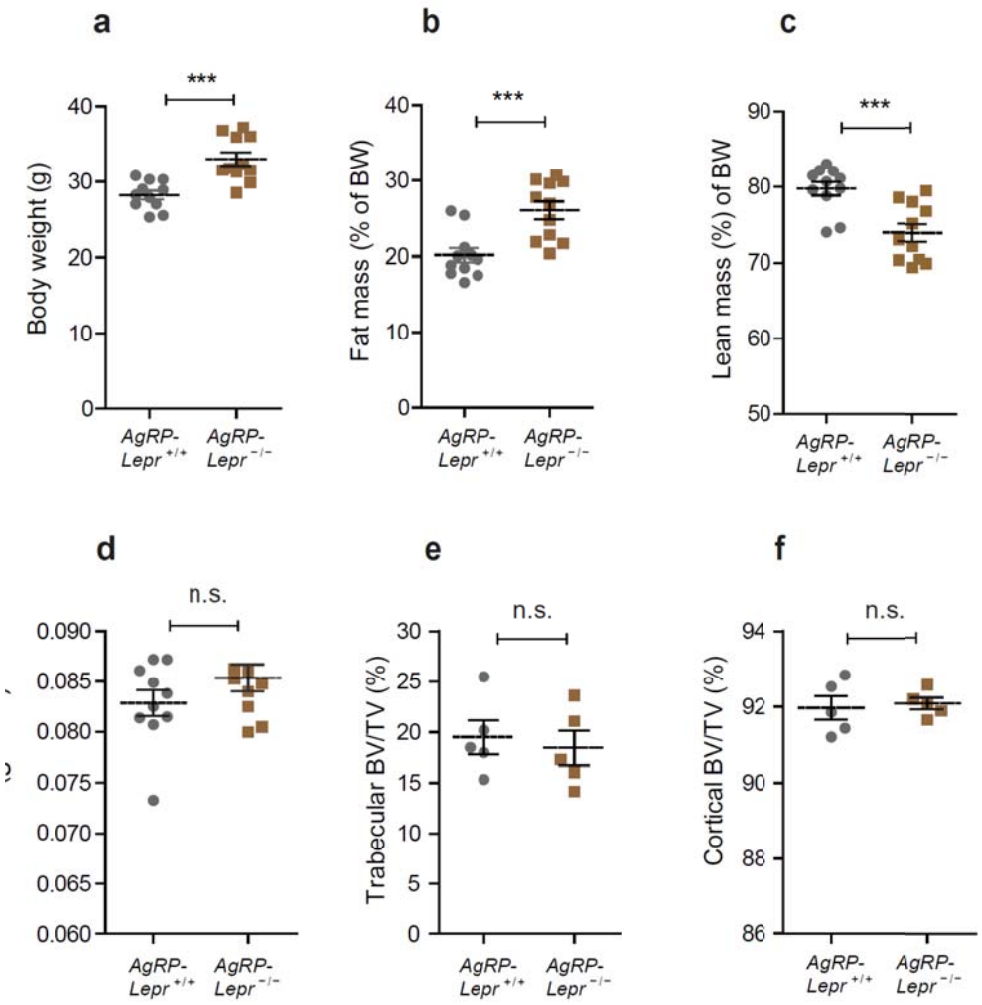


Supplementary Figure 1 (Related to Figure 1). **Generation of mice overexpressing *Ucp2* in AgRP neurons.** (**a**) Schematic diagram of the *Agrp cre* allele and the *Ucp*2 $^{flox/flox}$ transgene. **(b)** *Agrp-cre* mice were mated with *Ucp2* $^{flox/flox}$ mice. Transgenic mice express EGFP protein in the hypothalamic arcuate nucleus following cre-mediated recombination but not in hippocampus. **(c)** Immunolocalization of Cre protein in the femur of three-month-old *Agrp-cre* mice. Sections were incubated with antibody against *Cre* and counterstained with Hoechst (Blue). Immunoreactive signal of Cre (Red) was not observed in the femur of *Agrp Cre* mice. Tb, Trabecular bone. Scale bar = 100 μm.



Supplementary Figure 2 (Related to Figures 1 and 4). ***Ucp1*** **mRNA expression in brown adipose tissue (BAT) of** ***Ucp2*****$^{-/-}$ mice and** ***Agrp-Ucp2*****^{Tg} mice.** *Ucp2*$^{-/-}$ mice reveals an elevation of *Ucp1* mRNA levels in BAT (n=5 for *Ucp2*$^{+/+}$, n=6 for *Ucp2*$^{-/-}$). *Agrp-Ucp2*^{Tg} mice reveals a reduced *Ucp1* mRNA levels in BAT (n=6 for CT, n=9 for *Agrp-Ucp2*^{Tg}, p<0.05). * p<0.05. Data are presented as means ± s.e.m. P values for unpaired comparisons were analyzed by Student's *t* test.



Supplementary Figure 3 (Related to Figures 1, 2 and 3). **Impaired leptin signaling in AgRP neurons resulted in increased body weight and fat mass but did not have altered bone phenotypes.** Two-month-old male $Agrp^{Lepr-/-}$ mice exhibited increased (**a**) body weight (n=11 for $Agrp^{Lepr+/+}$, n=11 for $Agrp^{Lepr-/-}$, p<0.001) and (**b**) fat mass (n=11 for $Agrp^{Lepr+/+}$, n=11 for $Agrp^{Lepr-/-}$, p<0.001) but a decreased (**c**) lean mass (n=11 for $Agrp^{Lepr+/+}$, n=11 for $Agrp^{Lepr-/-}$, p<0.001). (**d**) DXA analysis demonstrated that BMD was not altered in $Agrp^{Lepr-/-}$ mice (n=10 for $Agrp^{Lepr+/+}$, n=10 for $Agrp^{Lepr-/-}$, p=0.0974). (**e, f**) Micro-CT analysis showed no differences in trabecular and cortical BV/TV between $Agrp^{Lepr\ +/+}$ and $Agrp^{Lepr\ -/-}$ mice (**e**: n=5 for $Agrp^{Lepr+/+}$, n=5 for $Agrp^{Lepr-/-}$, p=0.3372; **f**: n=5 for $Agrp^{Lepr+/+}$, n=5 for $Agrp^{Lepr-/-}$, p=0.3787). *** p<0.001. Data are presented as means ± s.e.m. P values for unpaired comparisons were analyzed by Student's t test.

Supplementary Method

Immunohistochemical analysis of Cre expression.

Femora were dissected from three-month-old *Agrp Cre* mice. After cleaning, they were fixed overnight in 0.1 M phosphate buffer (PB) containing 4% paraformaldehyde. Decalcification was later performed by immersing bone specimens in 15% wt/vol ethylenediaminetetraacetic acid (EDTA; Sigma) at 25°C for 3 wk. Decalcifying solution was replaced every 3 days. After being embedded in paraffin, bone specimens were cut longitudinally into 7 μm sections, which were later incubated at 37°C for 30 min in antigen retrieval solution (0.01 mg/ml proteinase K, 50 mM Tris HCl, pH 8.0, and 5 mM EDTA). To inhibit background endogenous peroxidase activity, sections were incubated for 1h with 10% H_2O_2. Nonspecific binding was blocked for 2 h by 4% bovine serum albumin, 10% normal goat serum, and 0.7% Tween-20 in PB buffer. Thereafter, sections were incubated at 4°C overnight with 1:1000 mouse primary antibody against *Cre* (MAB3120, Millipore, Billerica, MA). After being washed with PB buffer, the sections were incubated with avidin-biotinylated HRP-complex (ABC, Vector Laboratories) for 2 h and then reacted with Tyramide Signal Amplification system (NEN Life Science, Boston, MA). After processing, the sections were counterstained with Hoechst and photographed using fluorescence microscopy.