Central control of bone remodelling by Neuromedin U: a mediator of the leptin-dependent regulation of bone formation


Supplementary Figure 1

Supplementary Figure 1: High bone mass in Nmu−/− mice due to increased bone formation.
(a) BMD of each of 20 equal longitudinal divisions of the femora of 3 month-old male mice. (b,c) Histological analysis of the vertebrae of 3 month-old female mice (b) and 6 month-old male mice (c). Bone volume per tissue volume (BV/TV). (d) Histomorphometric analysis of the tibiae of 3 month-old male mice. Mineral apposition rate (MAR), bone formation rate per bone surface (BFR/BS), osteoblast surface per bone surface (Ob.S/BS), trabecular thickness (Tb. Th) and osteoclast surface per bone surface (Oc.S/BS). **: p<0.01, *: p<0.05.
Supplementary Figure 2: Decrease in bone mass by NMU icv infusion.
(a) Effect of NMU icv infusion on fat pad weight. Male mice at 3 months. (b) Effect of NMU icv infusion on body weight, fat pad weight (top) and bone mass by histology (bottom). Female mice at 3 months. Note that male Nmu^{-/-} mice have higher bone mass compared to female Nmu^{-/-} mice. Scale bar, 1 mm, *: p<0.05.
Supplementary Figure 3: Leptin does not rescue high bone mass in *Nmu*<sup>−/−</sup> mice.

(a) Effect of NMU or leptin icv infusion on fat pad weight in mice (3 month-old male). (b,c) Effect of leptin icv infusion in *Nmu*<sup>−/−</sup> mice (3 month-old male). (b) Fat pad weight. (c) Bone mass (BV/TV) of the tibiae by histology. (d) Histomorphometric analysis of leptin-infused WT male mice at 3 months. Scale bar, 1 mm, **: p<0.01, *: p<0.05.
Supplementary Figure 4: Sympathetic activation does not rescue high bone mass in Nmu$^{-/-}$ mice. (a, b) Effect of sympathetic activation by isoproterenol (ISO) injection in 3 month-old Nmu$^{-/-}$ mice. (a) Bone mass of tibiae (BV/TV) in male mice by histology. (b) Bone mass of vertebrae (BV/TV) in female mice by histology. Note that male Nmu$^{-/-}$ mice have higher bone mass compared to female Nmu$^{-/-}$ mice. Scale bar, 1 mm, **: $p<0.01$. 
Supplementary Figure 5: Expression of Adrb2 in Nmu^{-/-} osteoblasts treated with NMU.
Supplementary Figure 6: Altered Per2 expression in Nmu−/− mice. Per2 expression was decreased in Nmu−/− bone. *: p<0.05.
Supplementary Figure 7: Altered expression of Cartpt in Nmu−/− hypothalamus.

(a,b) In situ hybridization analysis of Cartpt by 35S-labeled probe (a) and digoxigenin-labeled probe (b) at the atlas-level of 38 (left), 43 (middle) and 46 (right). (c) Quantitative analysis of the Cartpt expression. Expression at PVN, Arc1, Arc2, Arc3, DMH1, DMH2 and VMH was analyzed at the atlas level of 38, 41, 43, 46, 43, 46 and 43, respectively. Note the increase in Cartpt expression in PVN, caudal part of Arc and DMH in Nmu−/− mice. Values represent arbitrary photostimulated luminescence (PSL) units. PVN, paraventricular nucleus; Arc, arcuate nucleus; DMH, dorsomedial nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus. Scale bar, 500 µm, **: p<0.01, *: p<0.05.