Supplemental Data

Neonatal Ghrelin Programs Development of Hypothalamic Feeding Circuits

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Supplemental Figure 1

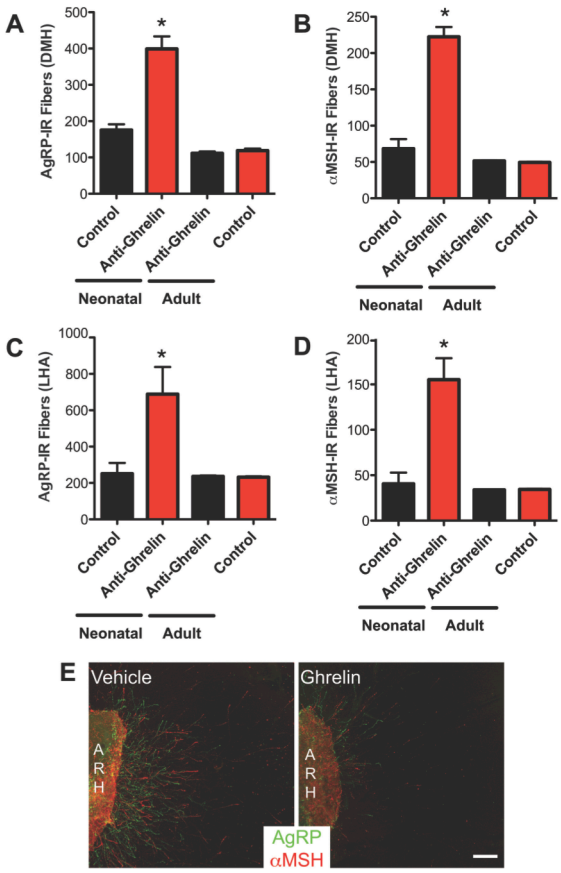

Figure S1. Neonatal ghrelin influences the normal developmental pattern of arcuate projections. Quantification of AgRP- (A, C) and α-MSH-IR (B, D) fibers at 100-120 days of age in the DMH (A-B), LHA (C-D) of mice injected with control or anti-ghrelin during neonatal (n = 6 for control; n = 7 for anti-ghrelin) or adult (n = 3 per group) life. (E) Photomicrographs of AgRP- (green fluorescence) and α-MSH-IR (red fluorescence) fibers from isolated organotypic cultures of neonatal ARH incubated for 48 h with vehicle or ghrelin (100 ng/ml). ARH, arcuate nucleus, DMH, dorsomedial nucleus, LHA, lateral hypothalamic area. Values are shown as the mean ± SEM. *P < 0.05 versus control. Scale bar, 100 um

Supplemental Figure 2

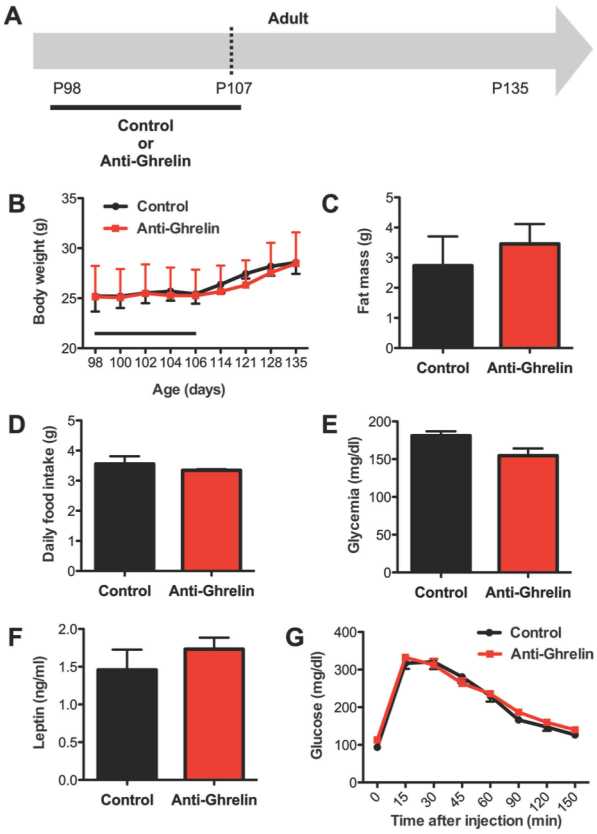

Figure S2. Adult ghrelin blockade does not cause marked metabolic alterations. (A) Schematic representation of the experimental design used to block ghrelin action during adult life. Starting at P98, mice were treated daily with intraperitoneal injections of the anti-ghrelin compound NOX-B11-2 (15 mg/kg) or an inactive control, for a total of 10 days. (B) Body weights of mice injected with control or anti-ghrelin compound (n = 5 per group); the black bar represents the injection period. (C) Fat mass of P135 animals injected with control or anti-ghrelin (n = 5 per group). (D) The daily food intake in adult mice injected with control or anti-ghrelin (n = 5 per group). (E) Blood glucose levels of P105 mice injected with control or anti-ghrelin (n = 5 per group). (F) Plasma leptin levels at 105 days of age in adult mice injected with control or anti-ghrelin (n = 5 per group). (G) Glucose tolerance test (GTT) of P108 mice injected with control or anti-ghrelin (n = 5 per group). Values are shown as the mean ± SEM.

Supplemental Figure 3

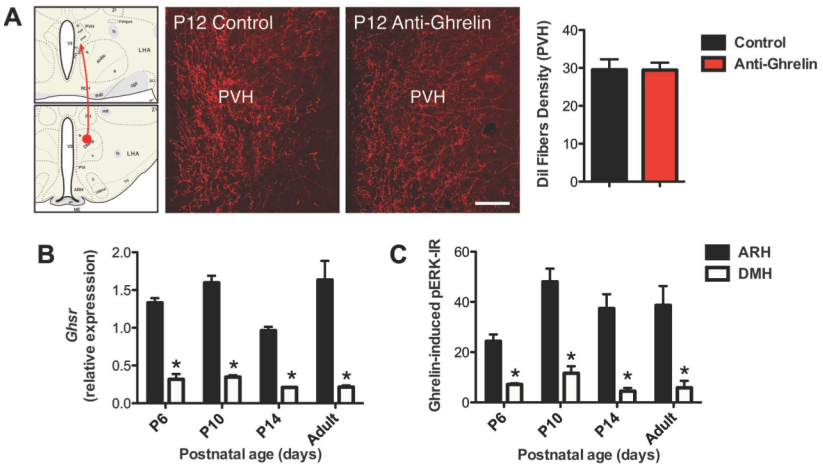

Figure S3. Normal development of DMH neural projections in mice neonatally treated with anti-ghrelin. (A) DiI crystals were implanted into the dorsomedial nucleus (DMH) of P12 mice treated with control or anti-ghrelin (n = 4 for control; n = 6 for anti-ghrelin) and neural projections to the paraventricular nucleus (PVH) were examined. The quantification revealed no difference in the density of DMH DiI-labeled fibers innervating the PVH between control-injected and anti-ghrelin injected neonates. (B) Relative expression of *Ghsr* mRNA in the arcuate nucleus (ARH) and DMH of P6, P10, P14, and adult (P90) mice (n = 6 for P10; n = 5 for P6 and P14; n = 4 for adult). (C) Quantitative comparisons of pERK-immunoreactive cells in the ARH and DMH 45 min after intraperitoneal administration of ghrelin (2 mg/kg) in P6, P10, P14, and adult (P90) mice (n = 5 for P10 and P14; n = 4 for P6 and adult). *P < 0.05 versus ARH. Scale bar, 150 um. Schematic illustrations are based on Brain Maps: Structure of the Rat Brain (Swanson, 1998).