

**Knockout of IRE1 $\alpha$  in POMC neurons decreases fat mass via increasing energy  
expenditure**

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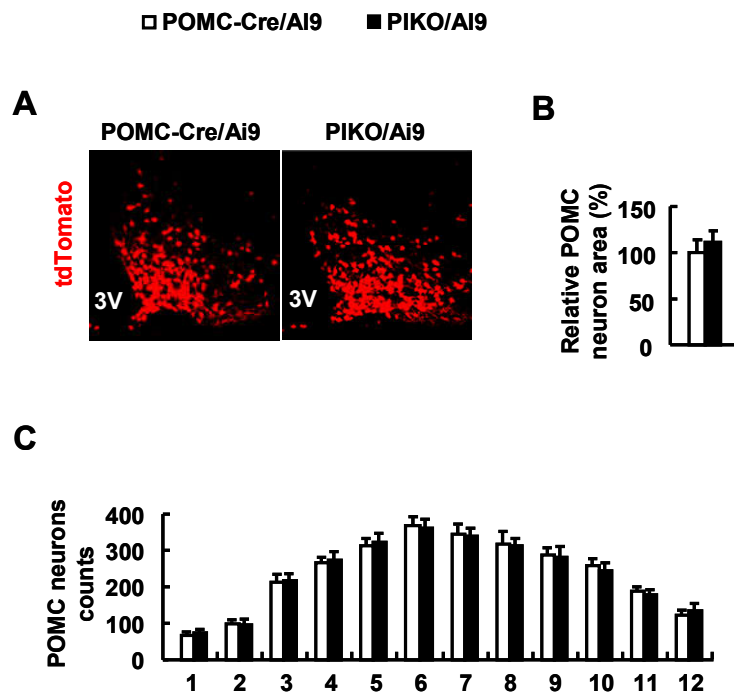
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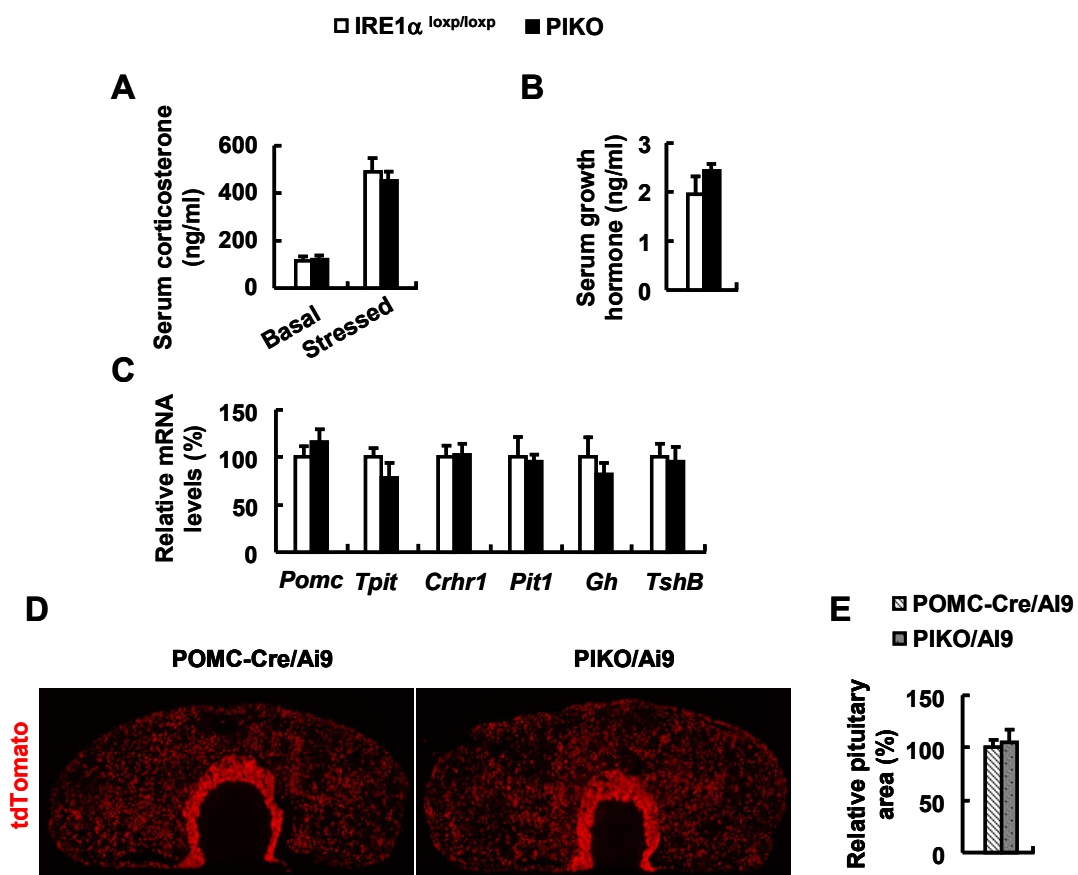
**Running title:** POMC IRE1 $\alpha$  regulates obesity

**Supplementary Figure 1. The distribution and counts of POMC neurons are not affected by IRE1 $\alpha$  deletion in POMC neurons.** (A) Representative immunofluorescence images of the arcuate nucleus (ARC), POMC neurons (red) (20 $\times$ ) 3V: third ventricle; (B) POMC neurons area; (C) POMC neurons distribution and number throughout ARC. All studies were conducted in 8-weeks old male POMC-Cre/Ai9 and PIKO/Ai9 mice maintained on a standard chow diet. Values are means  $\pm$  SEM (n = 4/group).

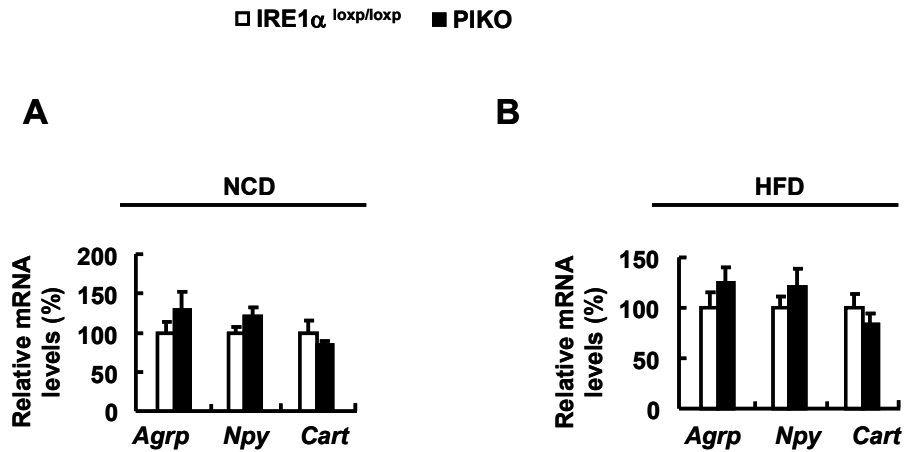


**Supplementary Figure 2. The function of the pituitary-adrenal axis is not affected by IRE1 $\alpha$  deletion in POMC neurons.** (A) Serum corticosterone levels under basal and stressed conditions; (B) Serum growth hormone levels; (C) Expression of representative pituitary genes in the pituitary; (D) Representative images of the pituitary, POMC expressing cells (red) (4 $\times$ ); (E) pituitary area. Studies

were conducted in 8-weeks old male control and PIKO mice maintained on a standard chow diet in A-C or in 8-weeks old male POMC-Cre/Ai9 mice and PIKO/Ai9 mice maintained on a standard chow diet in D and E. Values are means  $\pm$  SEM (n =6-7/group in A-C and n= 4/group in D-E).



**Supplementary figure 3. The expression of feeding neuropeptides *Agrp*, *Npy* and *Cart* are not altered in the hypothalamus of PIKO mice.** (A and B) hypothalamic *Agrp*, *Npy* and *Cart* mRNA levels. All studies were conducted in male control and PIKO mice fed on a standard chow diet (NCD) or high-fat diet (HFD) for 16 weeks, starting from 6-weeks old. Values are means  $\pm$  SEM (n = 8-10/group).



**Supplementary figure 4. The ER stress is ameliorated in the hypothalamus of PIKO mice under both a standard chow diet and high-fat diet (HFD).** (A) p-eIF2 $\alpha$ , BiP and ATF4 proteins in the hypothalamus (*left*, western blot; *right*, quantitative measurement of p-eIF2 $\alpha$ , BiP and ATF4 relative to total protein or actin); (B) Representative images showing immunofluorescence staining of ATF4 (green) in the arcuate nucleus (ARC) of mice (20 $\times$ ), 3V: third ventricle; (C) Quantification analysis of ATF4 in ARC. All studies were conducted in male control and PIKO mice fed on a standard chow diet (NCD) or HFD for 16 weeks, starting from 6-weeks old. Values are means  $\pm$  SEM (n = 8-10/group in A, n = 4/group in B-C), \*P < 0.05 for the effects of PIKO mice versus control mice in A and C, #P < 0.05 for the effects of HFD versus standard chow diet in the same genotype mice in A and C.

