Supplemental data

Supplemental Figure Legends

Supplemental Figure 1. Analysis of deletion of AMPKα2 in POMC and AgRP neurons in control and *POMCα2KO* and *AgRPα2KO* mice. (**A**) Immunofluorescence analysis for AMPKα2 (green) and POMC (red) expression in the hypothalami of control and *POMCα2KO* mice. Colocalisation of typical nuclear staining of AMPKα2 is seen in POMC neurons in control section (indicated by white arrows) and no colocalization is seen in *POMCα2KO* section. (**B**) Immunofluorescence analysis for AMPKα2 (red) and AgRPCreYFP (green) expression in the hypothalami of control and *AgRPα2KOYFP* mice. Colocalisation of typical nuclear staining of AMPKα2 is seen in AgRP neurons in control section (indicated by white arrows) and no colocalization is seen in *AgRPα2KOYFP* section. Confocal images of representative ARC fields are shown and are typical of results from 3 mice of each genotype. Scale bars: 10 μm. (**C**) Quantification of deletion of AMPKα2 in *POMCα2KO* and *AgRPα2KO* neurons. AMPKα2 staining was assessed in at least 50 POMC or AgRP neurons from 3 mice of each genotype. Results are expressed as percentage of cells in which AMPKα2 expression was absent and are means ± SEM.

Supplemental Figure 2. Unaltered neuropeptide release in hypothalamic explants from *POMCα2KO* and *AgRPα2KO* mice and normal anterior pituitary hormone gene expression in *POMCα2KO* mice. (**A**) α-MSH release in control and *POMCα2KO* hypothalamic explants (n = 15 per genotype). (**B**) AgRP and (**C**) NPY release in control and *AgRPα2KO* hypothalamic explants (n = 8 per genotype). (**D**) Pre-pro-opiomelanocortin (POMC), growth hormone (GH) and thyroid stimulating hormone beta subunit (TSHβ) mRNA expression in control and *POMCα2KO* pituitaries assessed by quantitative RT-PCR, n = 6-10. Probes for GAPDH were used to adjust for total RNA content. All values are mean ± SEM.

Supplemental Figure 3. Glucose homeostasis in *POMCα2KO* and *AgRPα2KO* mice. (**A**) Glucose tolerance in 12-week old male control and *POMCα2KO* mice on chow diet, *n* = 16. (**B**) Glucose tolerance in male control and *POMCα2KO* mice after 18 week exposure to HFD, *n* = 11-15. (**C**) Glucose tolerance in 12-week old male control and *AgRPα2KO* mice, *n* = 5-7. (**D**) Insulin tolerance in 12-week old male control and *AgRPα2KO* mice, *n* = 12-20. All values are mean ± SEM. * $P < 0.05$.

Supplemental Figure 4. AgRP neuronal anatomy is normal in *AgRPα2KO* mice. (**A**) *In situ* hybridization for *AgRP* mRNA in ARC of control and *AgRPα2KO* mice. Representative sections from 3 mice for each genotype are shown. (**B**) Immunoreactivity for AgRP in ARC of control and *AgRPα2KO* mice. Representative sections from 3 mice for each genotype are presented. Population size and distribution (**C** and **D**) for AgRP neurons within the ARC in control and *AgRPα2KO* mice (n = 2-3). AgRP somatic area (**E**) and diameter (**F**) in control and *AgRPα2KO* mice. A minimum of 100 neurons were analysed per group. 3V, 3rd ventricle. Scale bars, 50 μm. All values are mean $\pm$ SEM.

Supplemental Table 1. Body length, bone mineral content and biochemical parameters in *AMPKα2* mutant mice.

	Control	*POMCα2KO*	*α1HetPOMCα2KO*	Control	*AgRPα2KO*
Nose/anus length (cm)	9.4 ± 0.1 (8)	9.4 ± 0.1 (8)	9.6 ± 0.1 (8)	9.3 ± 0.2 (7)	9.1 ± 0.3 (8)
Bone mineral content (g/cm^2)	0.051 ± 0.001 (8)	0.054 ± 0.001 (8)	0.053 ± 0.001 (8)	ND	ND
Randomly fed blood glucose (mmol/l)	8.1 ± 0.2 (27)	8.2 ± 0.2 (25)	8.1 ± 0.4 (12)	6.8 ± 0.2 (15)	6.8 ± 0.2 (14)
Fasted blood glucose (mmol/l)	5.7 ± 0.3 (24)	5.8 ± 0.5 (23)	5.3 ± 0.4 (18)	4.7 ± 0.2 (16)	4.3 ± 0.2 (17)
Insulin (ng/ml)	0.149 ± 0.015 (7)	0.173 ± 0.052 (7)	ND	0.155 ± 0.019 (7)	0.186 ± 0.019 (7)
Adiponectin (μg/ml)	4.8 ± 0.6 (6)	4.5 ± 0.6 (7)	ND	4.0 ± 0.5 (7)	3.1 ± 0.3 (7)
Corticosterone (ng/ml)	11.1 ± 1.9 (6)	9.2 ± 1.9 (7)	ND	ND	ND
T4 (μg/dl)	6.1 ± 0.3 (6)	6.3 ± 0.7 (7)	ND	3.4 ± 0.2 (6)	3.6 ± 0.3 (7)

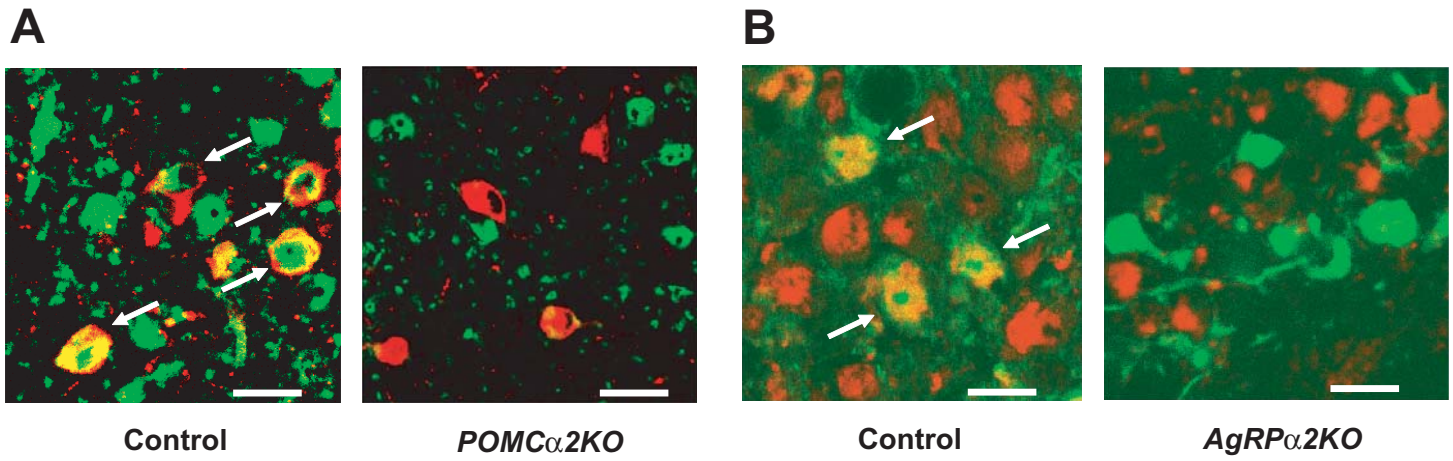
Data are expressed as mean ± SEM. The number of mice per group is shown in parenthesis. ND, not determined.

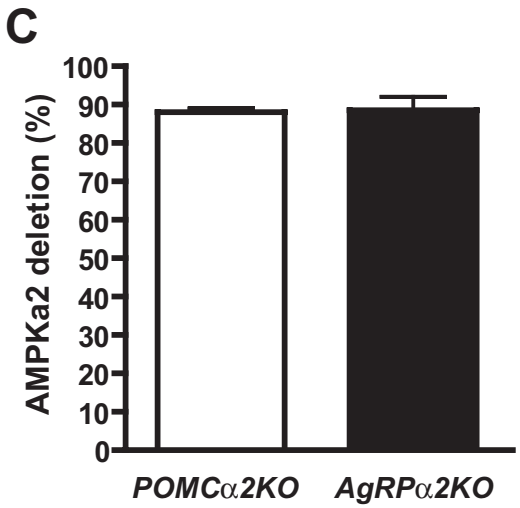
Supplemental Table 2. The biophysical properties of ARC AgRP-expressing neurons in control and *AgRPα2KO* mice.

	Control	**AgRPα2KO**
Membrane potential (mV)	-46 ± 1 (26)	-51 ± 1 (19)*
Input resistance (GΩ)	2.5 ± 0.3 (23)	2.8 ± 0.3 (17)
Spike firing frequency (Hz)	2.1 ± 0.4 (26)	2.8 ± 0.5 (19)

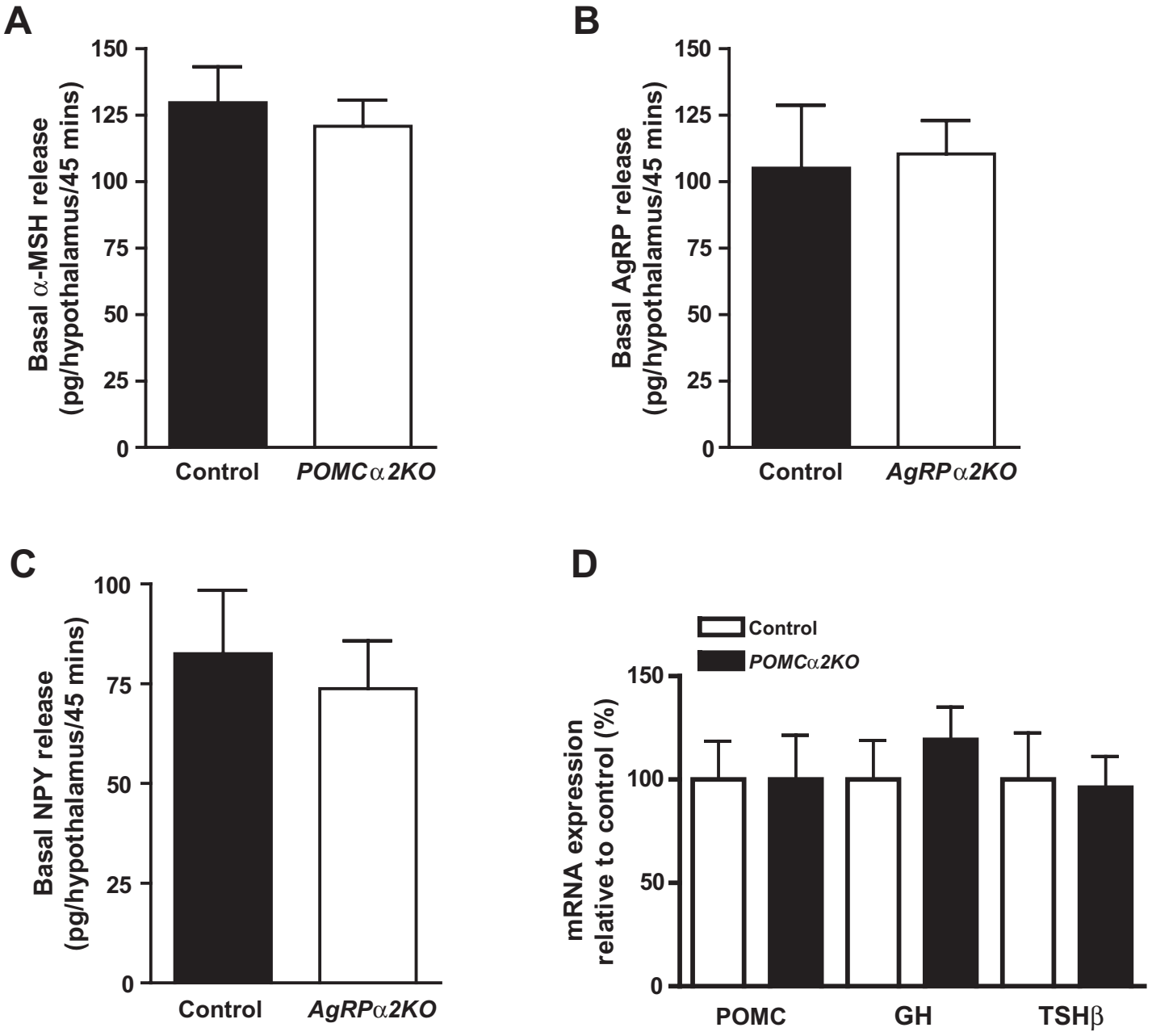
Data are expressed as mean ± SEM. The number of neurons per group is shown in parenthesis. * $P < 0.05$.

Supplemental Figure 1

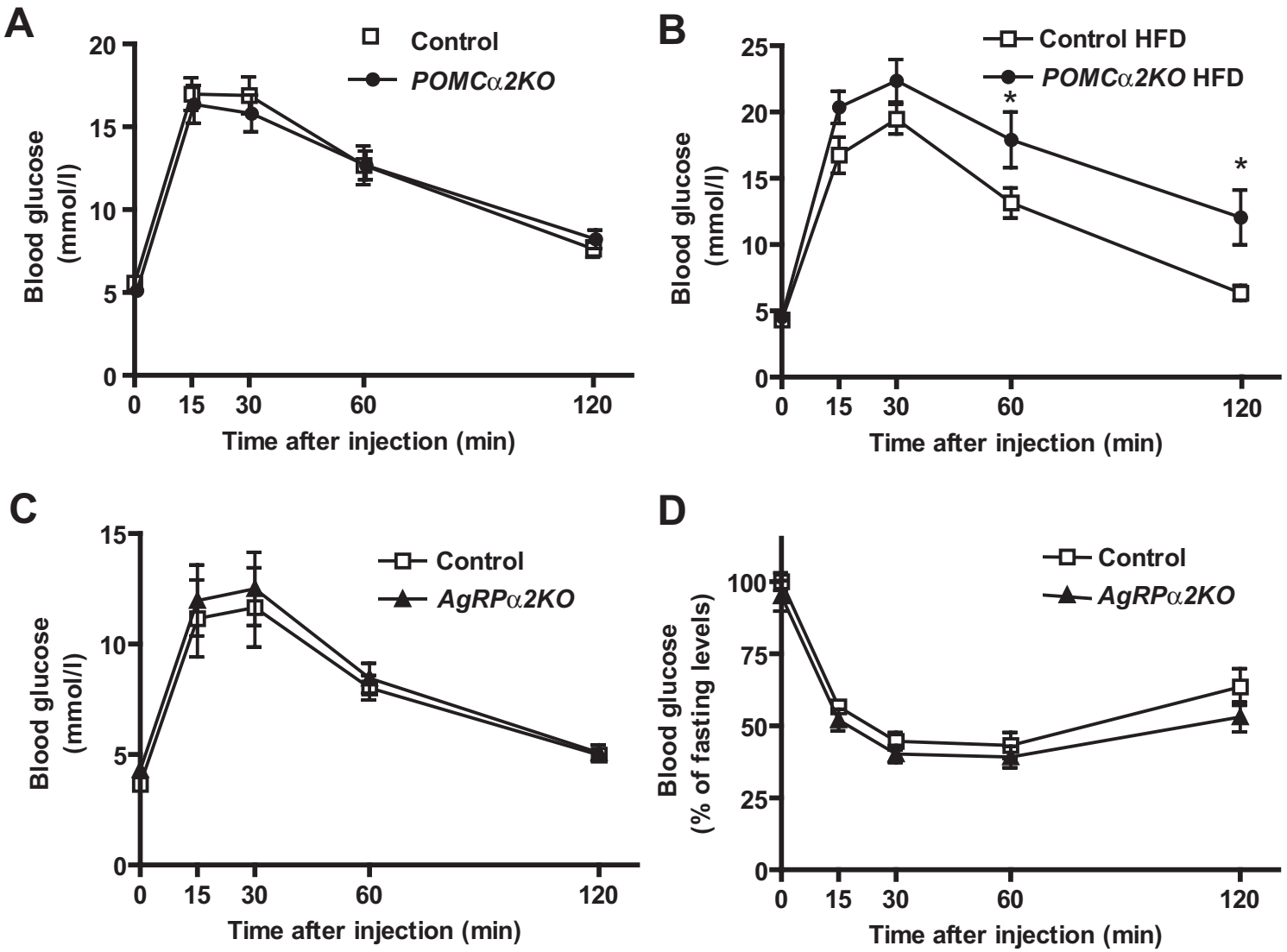




Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4