Supplemental Information

Legend for the Supplementary Figures

Figure S1: **Mitochondria changes in POMC neurons in response to food deprivation, related to Figure 2**. (A) Mitochondria density decreases in POMC neurons in response to 24h food deprivation (FD). (B) Mitochondria coverage of cell or cytosol also decreased after FD. (C) Probability distribution of mitochondria profiles cross-sectional area in POMC cells showing no effect of FD on mitochondria size. (D) Similar to C, but showing a parameter utilized to describe changes in mitochondria shape (aspect ratio – AR). No effects of FD were found. Overall, the data indicate decrease in mitochondria density in POMC neurons in response to FD, without changes in size and/or shape. N (Fed) = 852 mitochondria/15 cells/3mice. N (FD) = 616 mitochondria/15 cells/3 mice.

Figure S2: **Levels of *mfn1* and *mfn2* in Agrp neurons during different metabolic states, related to Figure 2.** We used ribose profiling to isolated RNA bound to the ribosomes selectively from Agrp neurons. (A) Data represent enrichment for *agrp, npy, pomc* and *s100b* (marker for astrocyte contamination). (B) Data from fasted, fed and high-fat diet fed mice related to fed mice. N = 3-4/group. Five animals were pooled for each N. Both male and female mice were used. * $P < 0.05$, ** $P < 0.01$.

Figure S3: **Hypothalamic transcripts and Agrp neuronal projection in mitofusin deficient mice, related to Figures 3 and 4**. (A) Amount of *agrp, npy* and *pomc* transcripts in the arcuate nucleus of the hypothalamus of littermate control and Agrp-Mfn1$^{-/-}$ mice. (B) Similar to (A), data correspond to Agrp-Mfn2$^{-/-}$ mice. Female mice were used in these studies. (C) Agrp neuronal projection was measured in the paraventricular nucleus of the hypothalamus (PVN). Quantification of fluorescent fibers in control and Agrp-Mfn1$^{-/-}$ mice. (B) Similar to A, but

comparing control and Agrp-Mfn2$^{-/-}$ mice. Both males and females were used for these studies. (E) Levels of *bip, chop, atf3, atf4, atf6 and xbp1s* in control and Agrp-Mfn1$^{-/-}$ mice. (F) Similar to (E), data correspond to Agrp-Mfn2$^{-/-}$ mice. Female mice were used in these studies. (G-H) Amount of *agrp, npy* and *pomc* transcripts in the arcuate nucleus of the hypothalamus of (G) Agrp-Mfn1$^{-/-}$ and (H) Agrp-Mfn2$^{-/-}$ mice related to littermate control mice. (I) Levels of *bip, chop, atf3, atf4, atf6 and xbp1s* in control and Agrp-Mfn1$^{-/-}$ mice. (J) Similar to (I), data correspond to Agrp-Mfn2$^{-/-}$ mice. Female mice were used in these studies. Data are expressed in relative quantities related to control mice. Bars represent mean ± SEM. *$P < 0.05$.

Figure S4: **Mitochondria fusion regulates the electrical activity of Agrp neurons in response to high-fat feeding, related to Figure 5**. (A) In normal chow conditions, both control and Agrp-Mfn2$^{-/-}$ neurons have similar frequency of action potential (AP) as recorded using slice whole-cell recording. (B) When mice were fed a HFD, Agrp-Mfn2$^{-/-}$ neurons have decreased AP frequency compared to control cells. (C) Percentage of silent Agrp neurons in control and Agrp-Mfn2$^{-/-}$ mice fed a normal chow diet. (D) In HFD, increased percentage of silent Agrp neurons in Agrp-Mfn2$^{-/-}$ mice compared to control mice ($P < 0.05$, Fisher's test). In A and B, bars represent mean ± SEM. In C and D, bars represent absolute values. All cells were recorded using perforated clamp with amphotericin B in the pipette solution.

Figure S5: **Metabolic adaptations of Agrp-Mfn1$^{-/-}$ and Agrp-Mfn2$^{-/-}$ mice fed a normal chow diet, related to Figure 7**. (A) Body weight curve of female control (grey) and Agrp-Mfn2$^{-/-}$ mice (pink). (B) Fat mass and (C) lean mass in the same animals as measured by MRI. (D) Leptin levels in two different cohorts of mice. (E) Water intake, (F) food intake, (G) energy expenditure, (H) ambulatory and vertical activities, and (I) RER in female control (grey) and Agrp-Mfn2$^{-/-}$ mice (pink). (J-L) Similar to (A-C), but data correspond to male mice. (M) Body weight curve of female control (grey) and Agrp-Mfn1$^{-/-}$ mice (blue). (N) Fat mass and (O) lean

mass in the same animals as measured by MRI. (P) Leptin levels in two different cohorts of mice. (Q) Water intake, (R) food intake, (S) energy expenditure, (T) ambulatory and vertical activities, and (U) RER in female control (grey) and Agrp-Mfn1$^{-/-}$ mice (blue). (V-X) Similar to (M-O), but data correspond to male mice. (Y) Leptin levels in male mice. In A, J, M, and V symbols represent mean ± SEM; shadow lines represent individual mouse body weight curve. Bars represent mean ± SEM. * $P < 0.05$.

Figure S6: **Glucose profile of Agrp-Mfn1$^{-/-}$ and Agrp-Mfn2$^{-/-}$ mice fed a normal chow diet, related to Figure 7**. (A) GTT and (B) ITT in female control and Agrp-Mfn1$^{-/-}$ mice. (C) Insulin level in two different cohorts of mice. (D-F) Similar to (A-C), data correspond to male mice. (G-H) GTTs in two different cohorts of female control and Agrp-Mfn2$^{-/-}$ mice. (I) ITT in the same groups of mice. (J) Insulin level in two different cohorts of mice. (K-N) Similar to (G-J), data corresponds to male Agrp-Mfn2$^{-/-}$ mice. Symbols represent mean ± SEM. Bars represent mean ± SEM. P value is stated in the graphics when a trend is observed or when statistical difference was found. Differences in GTT and ITT were tested using two-way ANOVA with time as a repeated-measure. Statistical differences in insulin levels were tested using t-test.

Figure S7: **Metabolic adaptations of Agrp-Mfn1$^{-/-}$ and Agrp-Mfn2$^{-/-}$ mice fed a high-fat diet, related to Figure 7**. (A) Food intake, (B) water intake, (C) energy expenditure, (D) activity, (E) RER, (F) GTT, (G) ITT, (H) insulin levels in female control and Agrp-Mfn1$^{-/-}$ mice. (I) GTT and (J) ITT male mice fed a HFD. (K) Leptin levels in female mice. (L) Food intake, (M) water intake, (N) energy expenditure, (O) activity, (P) RER, (Q) GTT, (R) ITT, (S) insulin levels in female control and Agrp-Mfn2$^{-/-}$ mice. (T) GTT, (U) insulin and (V) leptin levels in male mice fed a HFD. (W) Leptin levels in female mice. Symbols represent mean ± SEM. Bars represent mean ± SEM. * $P < 0.05$.

Experimental Procedures

Animals:

All transgenic mice were bred and maintained in our laboratory colony, and were in a mixed background. Wild-type control mice were also from our own colony. Tg.AgrpCre mice were in a mixed background (from Alison Xu). We also used Agrp-*Ires*-cre mice (Agrp$^{tm1(cre)Lowl}$, Jax #012899). To control for ectopic expression of cre, mice were either backcrossed to Rosa26-LSL-Lacz mice (B6.129S4-Gt(ROSA)26Sor^{tm1Sor}, Jax #003309) or genotyped for the excised allele of the target floxed gene. In both cre lines, we found a 30-60% ectopic expression of cre. Ectopic mice were excluded from this study. Mfn1 floxed mice (Mfn1^{tm2Ddd}, 029901-UCD, MMRRC) and Mfn2 floxed mice (Mfn2^{tm3Dcc}, 029902-UCD, MMRRC) were purchased from Mutant Mouse Regional Resource Center. NPY-sapphireFP mice (donated by Jeffrey Friedman), Rosa26-LSL-Lacz mice (B6.129S4-Gt(ROSA)26Sor^{tm1Sor}, Jax #003309) and Rpl22 floxed (B6.129-Rpl22tm1.1^{Psam}/J, Jax #011029) mice are available in The Jackson Laboratories.

Fixative solution:

The following solution was prepared fresh for perfusion of mice for electron microscopy analyzes: Paraformaldehyde 4%, gluteraldehyde 0.1%, picric acid 15%, in phosphate buffer (PB) 0.1 M, pH = 7.4.

Antibodies for immunostaining:

The following antibodies were used for electron microscopy studies: anti-POMC (1:4000, 48h, 4ºC - Phoenix), anti-GFP (1:5000, 48h, 4ºC - Abcam), anti-Lacz (1:6000, 48h, 4ºC - Millipore). For fluorescence microscopy: guinea pig-anti-Agrp (from Kevin Grove), 1:1500, 72 h, 4°C; goat-anti-guinea pig Alexa 568 (Invitrogen, #A11075), 1:500, 1.5 h, room temperature.

Probes:

agrp [Mm00475829_g1]

npy [Mm00445771_m1]

pomc [Mm00435874_m1]

drp1 [Mm01342903_m1]

opa1 [Mm00453879_m1]

mfn1 [Mm01289372_m1]

mfn2 [Mm01255785_m1]

atf3 (Mm_ 00476032_m1)

atf4 (Mm00515324_m1)

atf6 (Mm01295317_m1)

chop (Mm00492097_m1)

bip (Mm00517691_m1)

xbp1s (Mm034644969_m1)

Solutions used for electrophysiology recordings:

For perforated whole-cell recording, pipette solution contained (in mM): potassium gluconate 135, MgCl2 2, Hepes 10, EGTA 0.2, Mg-ATP 2, Tris-ATP 3, Na2-phosphocreatin 10 and Na2-GTP 0.3, pH 7.3 with KOH. In the perforated whole-cell recording using amphotericin B (Sigma, final concentration 1 μg/ml) was added to a modified pipette solution without Tris-ATP.