Neuron, Volume *106*

Supplemental Information

PNOC^{ARC} Neurons Promote Hyperphagia and Obesity upon High-Fat-Diet Feeding

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Figure S1, related to Figure 1

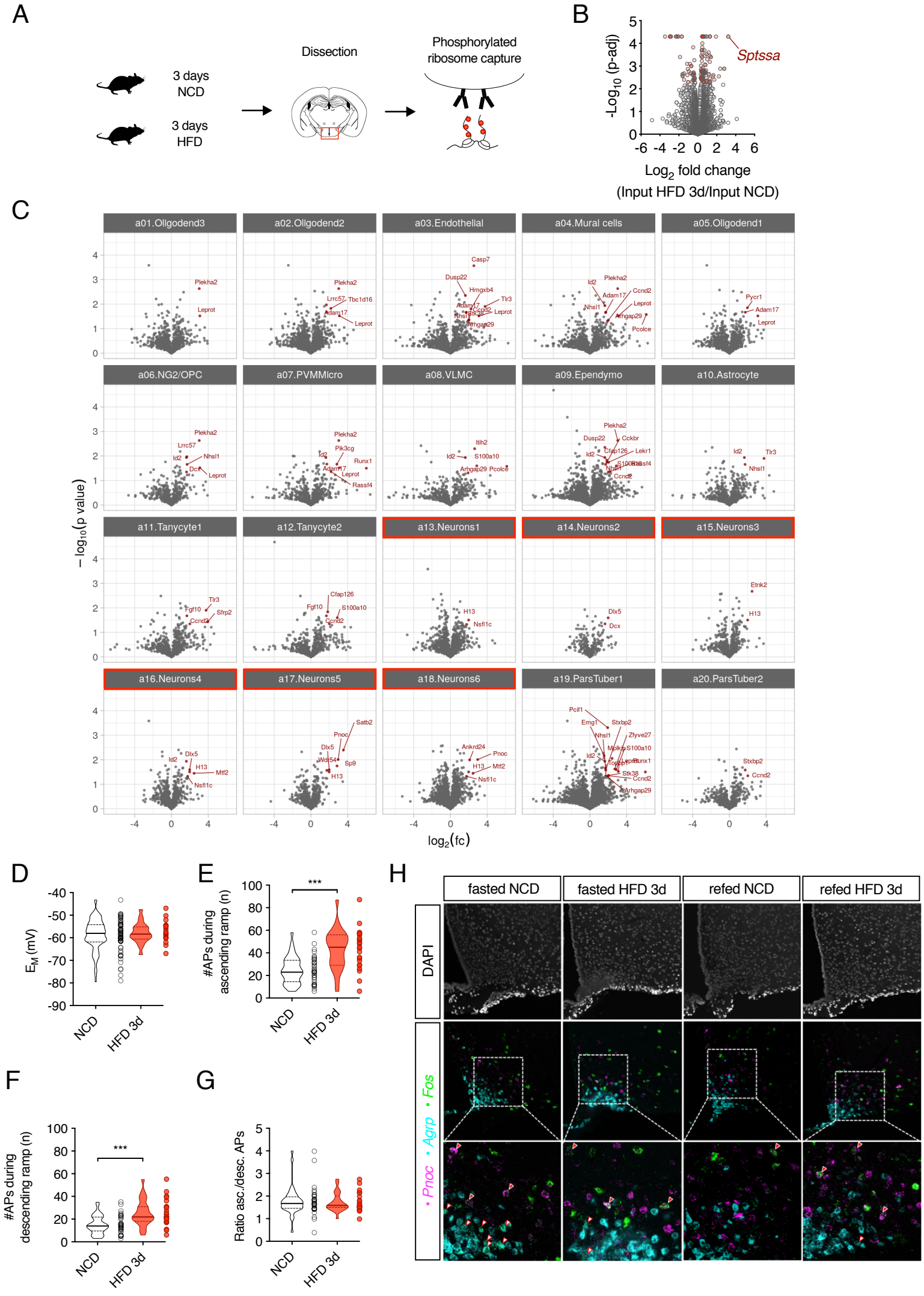


Figure S1. Related to Figure 1.

(A) Phosphoribotrap strategy for genetic identification of acute HFD-activated neurons. (B) Fold enrichment Input HFD/Input NCD and statistical significance are shown. (C) Identification of neuronal marker genes from the phosphoribotrap screen. Overlap analysis of genes from Campbell et al. (2017) and phosphoribotrap data. Top 50 HFD-regulated genes are indicated in red. (D) Membrane potential (mV) in $PNOC^{ARC}$ neurons from NCD-fed (n = 66) and HFD-fed (n = 20) mice. (E) Number of action potentials during the ascending ramp in $PNOC^{ARC}$ neurons from NCD-fed (n = 36) and HFD-fed (n = 23) mice. (F) Number of action potentials during the descending ramps in $PNOC^{ARC}$ neurons from NCD-fed (n = 36) and HFD-fed (n = 23) mice. (G) Ratio between the number of action potentials during the ascending and descending ramps in $PNOC^{ARC}$ neurons from NCD-fed (n = 36) and HFD-fed (n = 23) mice. (H) Representative confocal images showing *Pnoc* (magenta), *Agrp* (cyan) and *Fos* (green) expression in the ARC of C57BL/6N mice. White arrows indicate Fos-positive AgRP neurons. Red arrows indicated Fos-positive PNOC neurons. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as determined by Mann-Whitney test (D-G).

Supplemental Table 1. Top 50 significantly enriched transcripts upon HFD feeding. Related to Figure 1.

Ensembl ID	Gene name	FDR-adjusted p-value	Fold change (HFD/NCD, log2)	GO Term: neuropeptide signaling pathway
ENSMUSG00000038742	*Angptl6*	0.0499	93.67	no
ENSMUSG00000029718	*Pcolce*	0.0264	6.14	no
ENSMUSG00000022952	*Runx1*	0.0315	6.04	no
ENSMUSG00000020329	*Polrmt*	0.0336	4.06	no
ENSMUSG00000114649	*3110006O06Rik*	0.0357	4.06	no
ENSMUSG00000027996	*Sfrp2*	0.0367	3.97	no
ENSMUSG00000039131	*Gipc2*	0.0375	3.92	no
ENSMUSG00000031639	*Tlr3*	0.0125	3.77	no
ENSMUSG00000038331	*Satb2*	0.0040	3.54	no
ENSMUSG00000035900	*Gramd4*	0.0262	3.31	no
ENSMUSG00000097675	*1700101I11Rik*	0.0436	3.28	no
ENSMUSG00000086213	*A330040F15Rik*	0.0379	3.23	no
ENSMUSG00000047363	*Cstad*	0.0268	3.14	no
ENSMUSG00000101693	*Gm19461*	0.0061	3.11	no
ENSMUSG00000035212	*Leprot*	0.0303	3.10	no
ENSMUSG00000031557	*Plekha2*	0.0023	3.03	no
ENSMUSG00000045731	***Pnoc***	**0.0098**	**2.96**	**yes**
ENSMUSG00000079038	*D130040H23Rik*	0.0087	2.87	no
ENSMUSG00000041959	*S100a10*	0.0253	2.87	no
ENSMUSG00000016496	*Cd274*	0.0032	2.86	no
ENSMUSG00000068859	*Sp9*	0.0179	2.84	no
ENSMUSG00000094374	*Gm5435*	0.0005	2.83	no
ENSMUSG00000020573	*Pik3cg*	0.0217	2.83	no
ENSMUSG00000089957	*A830011K09Rik*	0.0233	2.82	no
ENSMUSG00000007279	*Scube2*	0.0457	2.80	no
ENSMUSG00000070572	*Trmt112-ps2*	0.0218	2.73	no
ENSMUSG00000018820	*Zfyve27*	0.0239	2.71	no
ENSMUSG00000022671	*Mzt2*	0.0112	2.71	no
ENSMUSG00000022160	*Mettl3*	0.0439	2.64	no
ENSMUSG00000037254	*Itih2*	0.0050	2.64	no
ENSMUSG00000027411	*Vps16*	0.0364	2.59	no
ENSMUSG00000025076	*Casp7*	0.0003	2.56	no
ENSMUSG00000051346	*Spryd4*	0.0269	2.55	no
ENSMUSG00000021680	*Crhbp*	0.0443	2.52	no
ENSMUSG00000048668	*Rhno1*	0.0196	2.51	no
ENSMUSG00000081010	*Gm13880*	0.0417	2.49	no
ENSMUSG00000081683	*Fzd10*	0.0173	2.48	no
ENSMUSG00000048905	*4930539E08Rik*	0.0384	2.47	no
ENSMUSG00000070644	*Etnk2*	0.0021	2.47	no
ENSMUSG00000029267	*Mtf2*	0.0358	2.46	no
ENSMUSG00000012429	*Mplkip*	0.0088	2.41	no

ENSMUSG00000017664	*Slc35c2*	0.0161	2.36	no
ENSMUSG00000096795	*Zfp433*	0.0378	2.35	no
ENSMUSG00000097292	*A230107N01Rik*	0.0174	2.34	no
ENSMUSG00000007646	*Rad51c*	0.0243	2.32	no
ENSMUSG00000038704	*Aspdh*	0.0282	2.31	no
ENSMUSG00000083474	*Gm15267*	0.0075	2.25	no
ENSMUSG00000034518	*Hmgxb4*	0.0158	2.24	no
ENSMUSG00000029034	*Ints11*	0.0258	2.24	no
ENSMUSG00000039208	*Metrnl*	0.0246	2.22	no

Table indicates the top 50 enriched transcripts (input normalized IP-associated mRNAs) after 3 days of HFD feeding. Gene Ontology term 'neuropeptide signaling pathway' (GO:0007218).

Figure S2, related to Figure 2

A

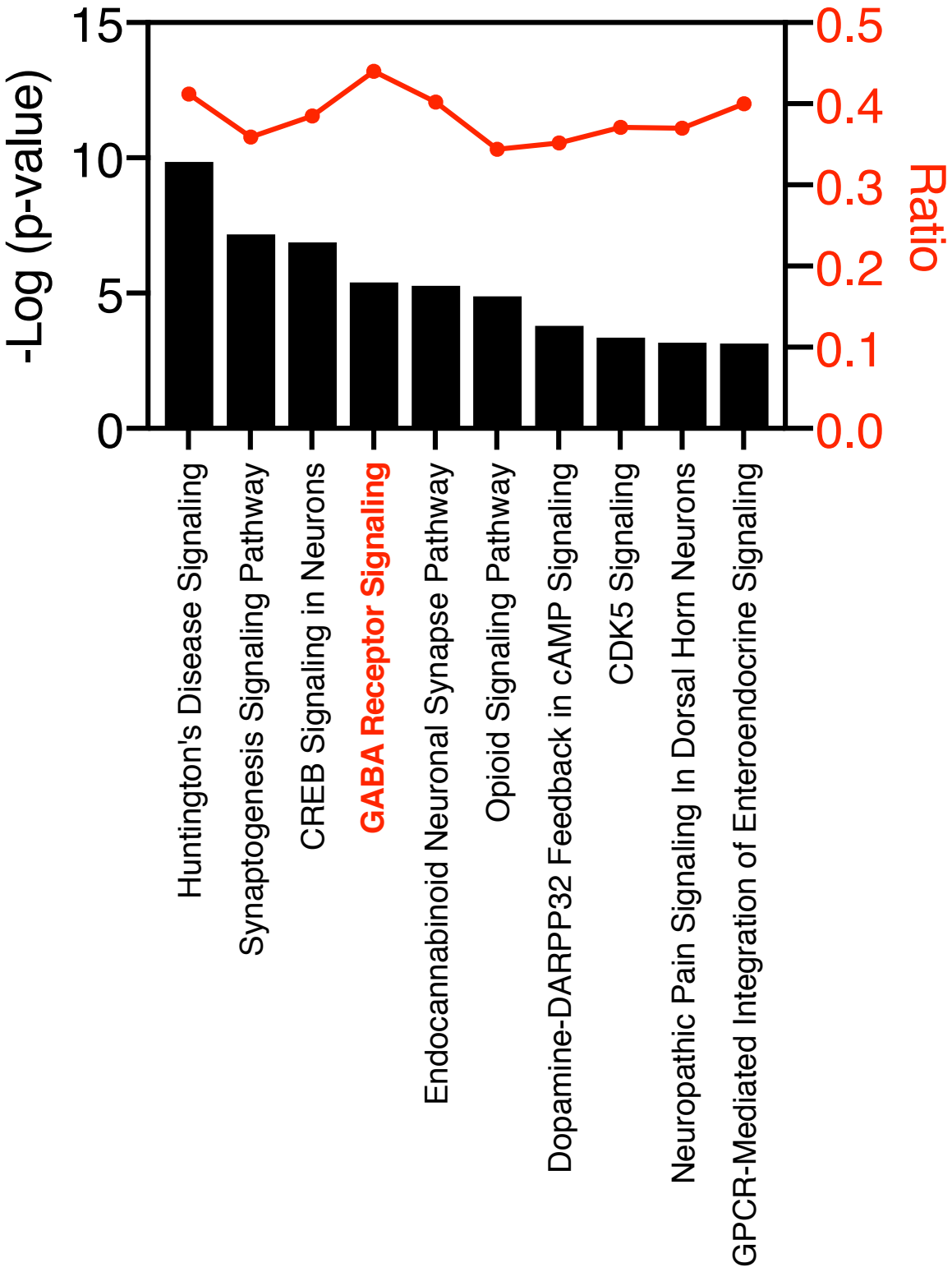


Figure S2. Related to Figure 2.

(A) Gene set enrichment analysis of Pnoc BacTRAP profile. Significantly enriched canonical pathways using the IPA canonical pathway collection ‘neurotransmitters and other nervous system signaling pathways’ are shown. The significance values for the canonical pathways are calculated by Fisher's exact test right-tailed.

Figure S3, related to Figure 3

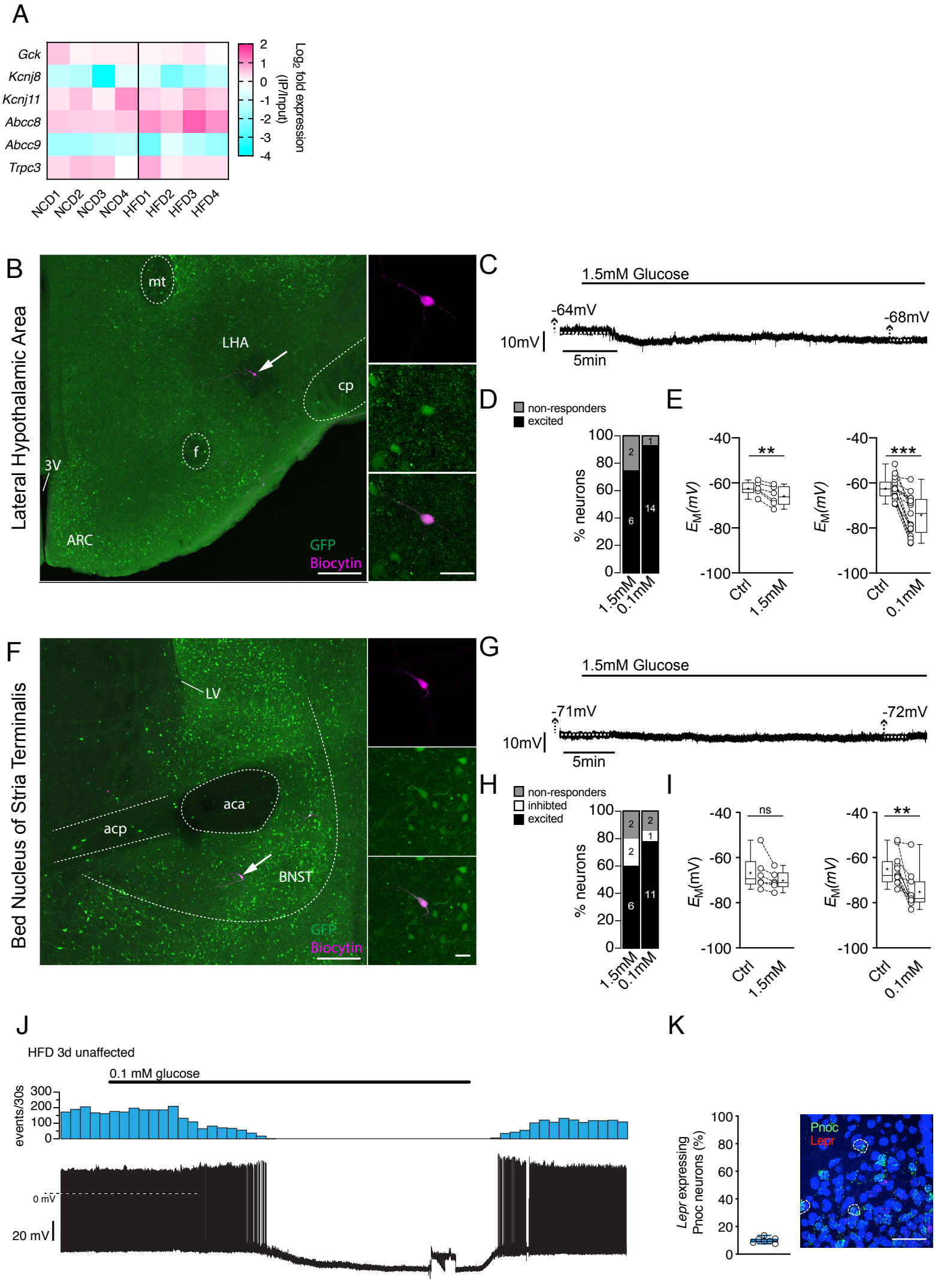


Figure S3. Related to Figure 3.

(A) Expression profiling of marker genes for glucose sensing cells and functional KATP channel-subunits in PNOC neurons using BacTRAP. (B,C) Glucose-excited LHA^{PNOC} neuron labeled with biocytin/streptavidin (B) and the corresponding current clamp recording (C). D) Percentage of glucose-excited and non-responsive LHA^{PNOC} neurons in response to changes in extracellular glucose concentration from 5 to 1.5 mM and from 5 to 0.1 mM. (E) Boxplots showing the responses of the glucose-excited neurons (5 to 1.5 mM, n=6; 5 to 0.1 mM, n=14). (F,G) Glucose-excited $BNST^{PNOC}$ neuron labeled with biocytin/streptavidin (F) and the corresponding current clamp recording (G). (H) Percentage of glucose-sensitive neurons and non-responsive $BNST^{PNOC}$ neurons in response to changes in the extracellular glucose concentration from 5 to 1.5 mM and from 5 to 0.1 mM. (I) Boxplots with means and single experiments of the glucose-excited neurons (5 to 1.5 mM, n=6; 5 to 0.1 mM, n=11). While all neurons in (I) were significantly glucose-excited, the population response was not significant. Each LHA and BNST neuron was tested individually for glucose-sensitivity as described in STAR Methods. The scale bars are 250 µm in the overviews and 20 µm in the enlargements.

3V, 3rd ventricle; aca, anterior commissure anterior; acp, anterior commissure posterior; ARC, arcuate nucleus; BNST, bed nucleus of the stria terminalis; cp, cerebral peduncle f, fornix; LHA, lateral hypothalamic area; LV, lateral ventricle; mt, mammillothalamic tract. (J) Original recording and corresponding rate histogram of a $PNOC^{ARC}$ neuron from a HFD-fed mouse that showed a strong response to a decrease in extracellular glucose from 5 to 0.1 mM. (K) Representative confocal images and quantification of *in situ* hybridization of *Pnoc* mRNA (cyan) and *Lepr* mRNA (red) with DAPI staining (blue) in the ARC of C57BL/6N mice (n = 7). Scale bar, 50 µm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as determined by paired Student's t test (E and I) or unpaired Student's t test (K).

Figure S4, related to Figure 4

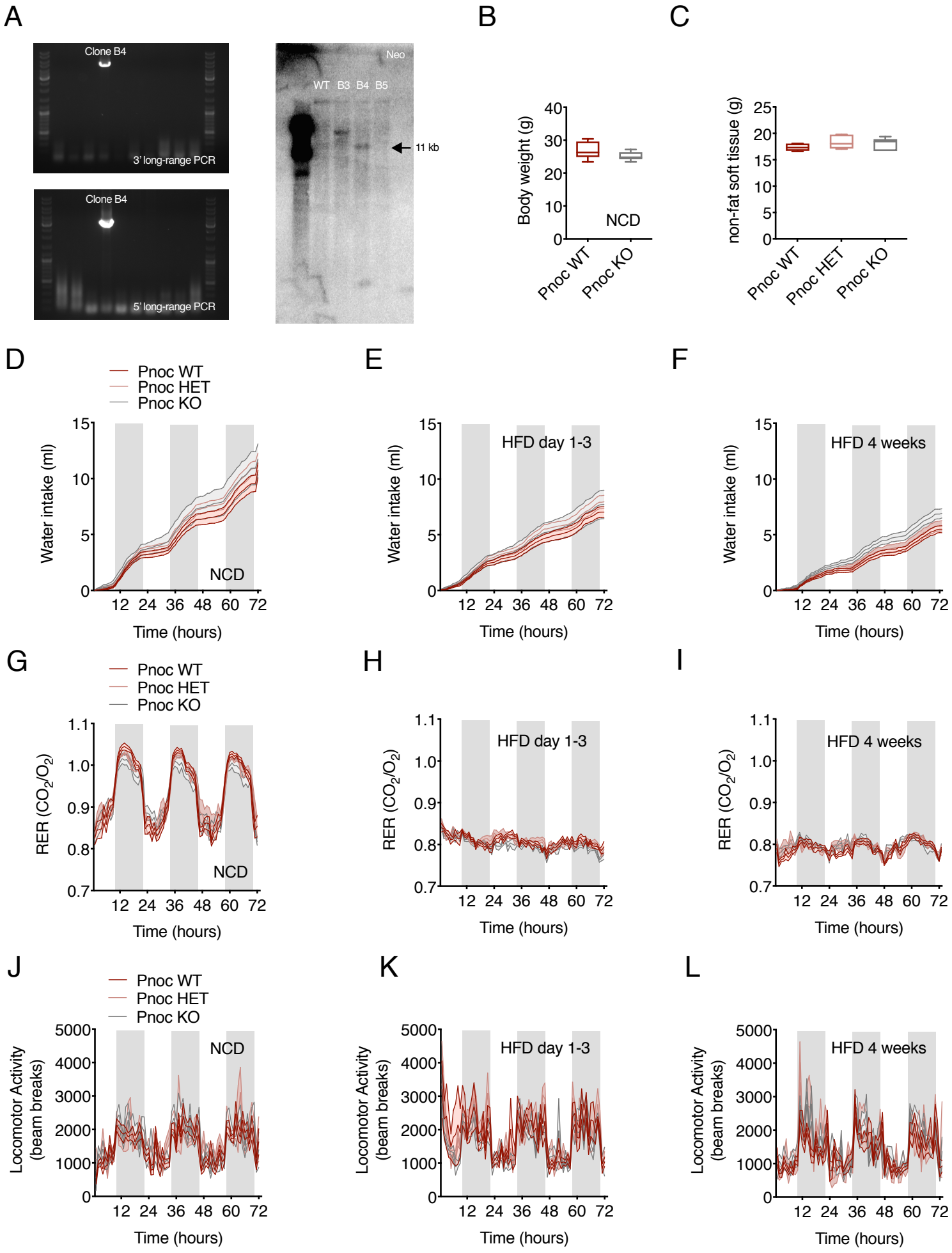
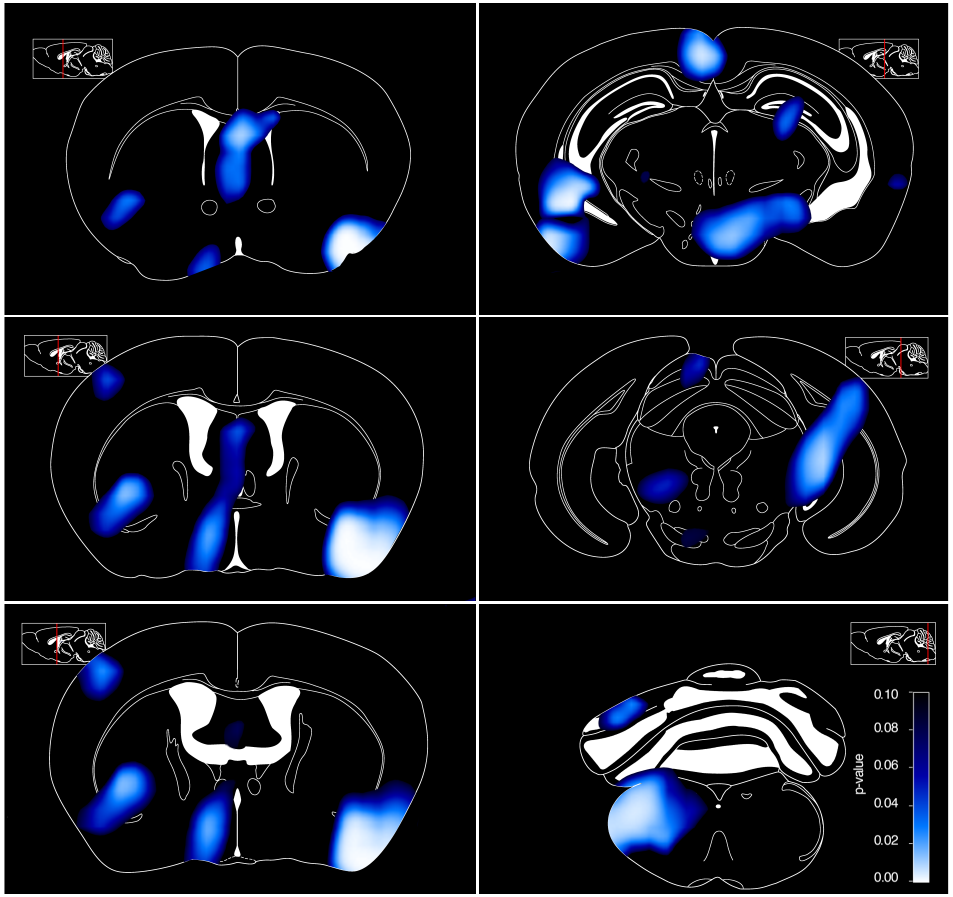


Figure S4. Related to Figure 4.

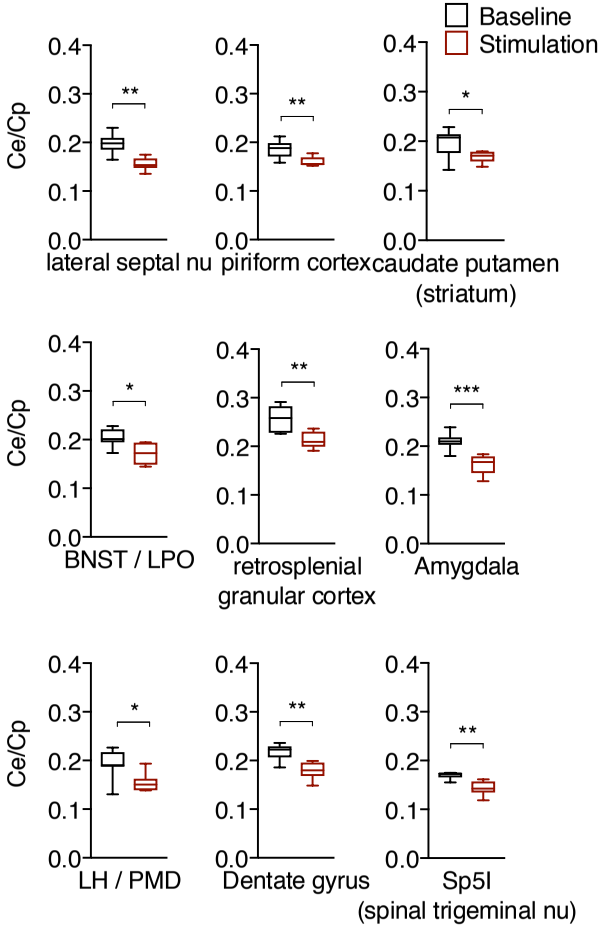
(A) Selection of positive ES cell clones using long-range PCR and Southern blot analysis of Neo cassette integration. (B) Bodyweight of Pnoc WT and Pnoc KO on NCD. (C) Lean mass of Pnoc WT, Pnoc HET and Pnoc KO mice after 4 weeks of HFD. (n = 6/5/6, one-way ANOVA followed by Tukey's post hoc test) (D) Cumulative water intake on NCD. (E) Cumulative water intake during 3 days of HFD feeding. (F) Cumulative water intake after 4 weeks on HFD. (G) Respiratory exchange ratio (RER) on NCD. (H) Respiratory exchange ratio (RER) during 3 days of HFD feeding. (I) Respiratory exchange ratio (RER) after 4 weeks on HFD.(J) Locomotor activity on NCD. (K) Locomotor activity during 3 days of HFD feeding. (L) Locomotor activity after 4 weeks on HFD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as determined by two-tailed, unpaired Student's t test (B) or one-way ANOVA followed by Tukey's post hoc test (C) or by two-way repeated measures ANOVA with Sidak's multiple comparisons test (D-L).

Figure S5, related to Figure 5

A



B



C

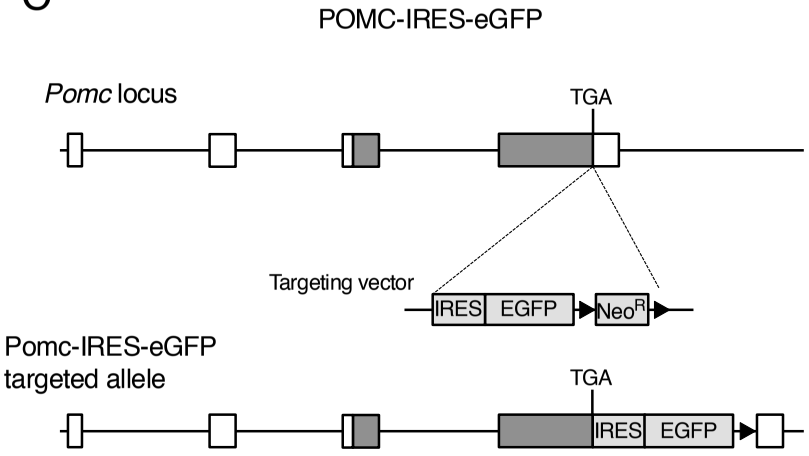


Figure S5. Related to Figure 5.

(A) Parametric maps of p values from paired t-test of differences in glucose metabolism between photostimulated $PNOC^{ARC}$ neurons and baseline (without photostimulation) determined by [18F]FDG PET in anaesthetized mice (n = 7). Blue color scale indicates regions where metabolism at baseline > stimulation (Inhibition). Sagittal reference image inserts show location of corresponding coronal plates (Franklin and Paxinos, 2013). (B) Quantification of selected VOIs from PET imaging of PNOC-Cre-ChR2 mice (n = 7). Ce/Cp is the ratio of tissue and blood glucose concentrations, a blood glucose level-insensitive measure for glucose metabolism. (C) Schematic diagram of the POMC-IRES-eGFP allele. The IRES-eGFP cassette was targeted just downstream of the stop codon in exon III of the Pomc gene.

*p < 0.05, **p < 0.01, ***p < 0.001 as determined by two-tailed, paired Student's t test.

Figure S6, related to Figure 6

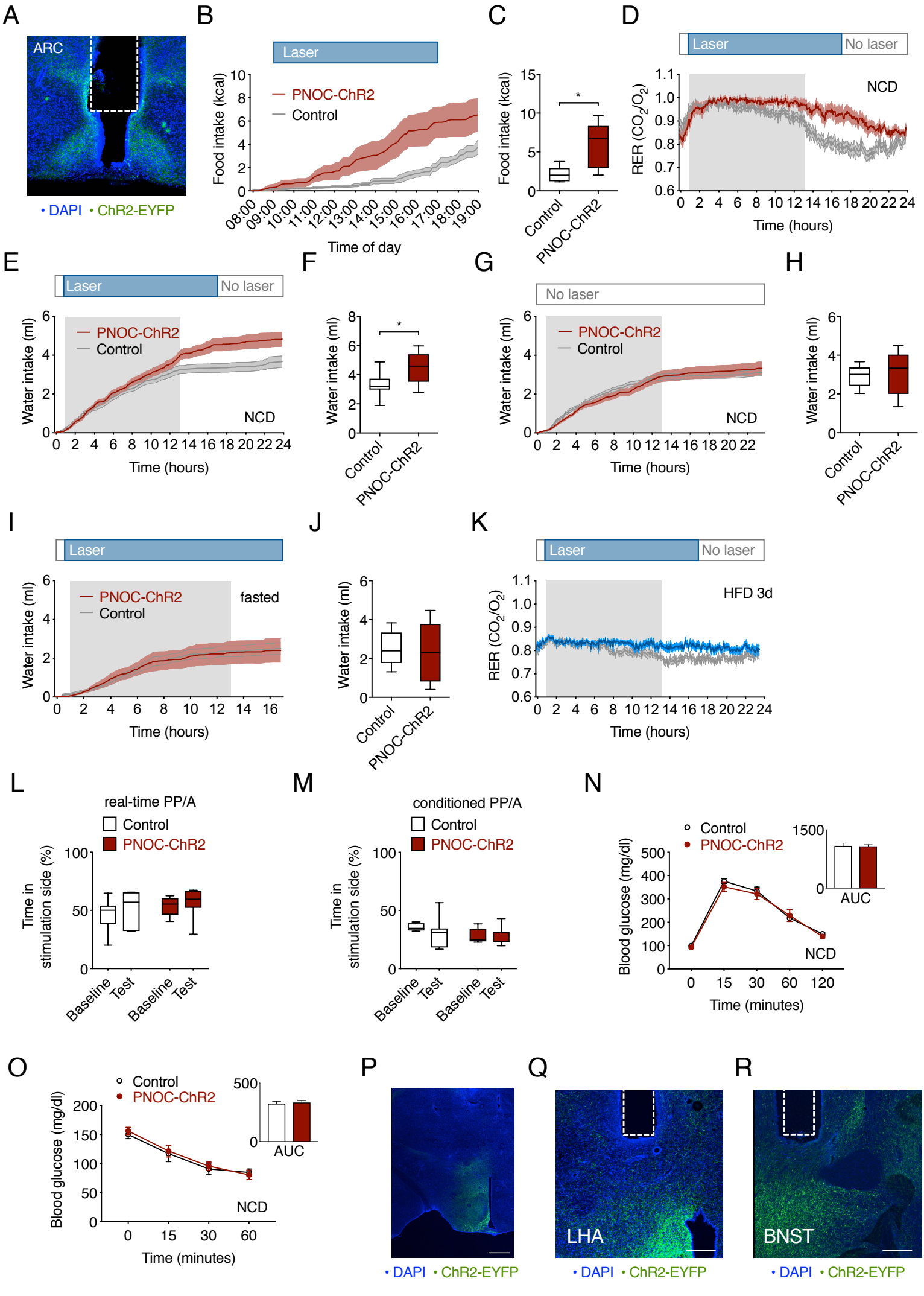
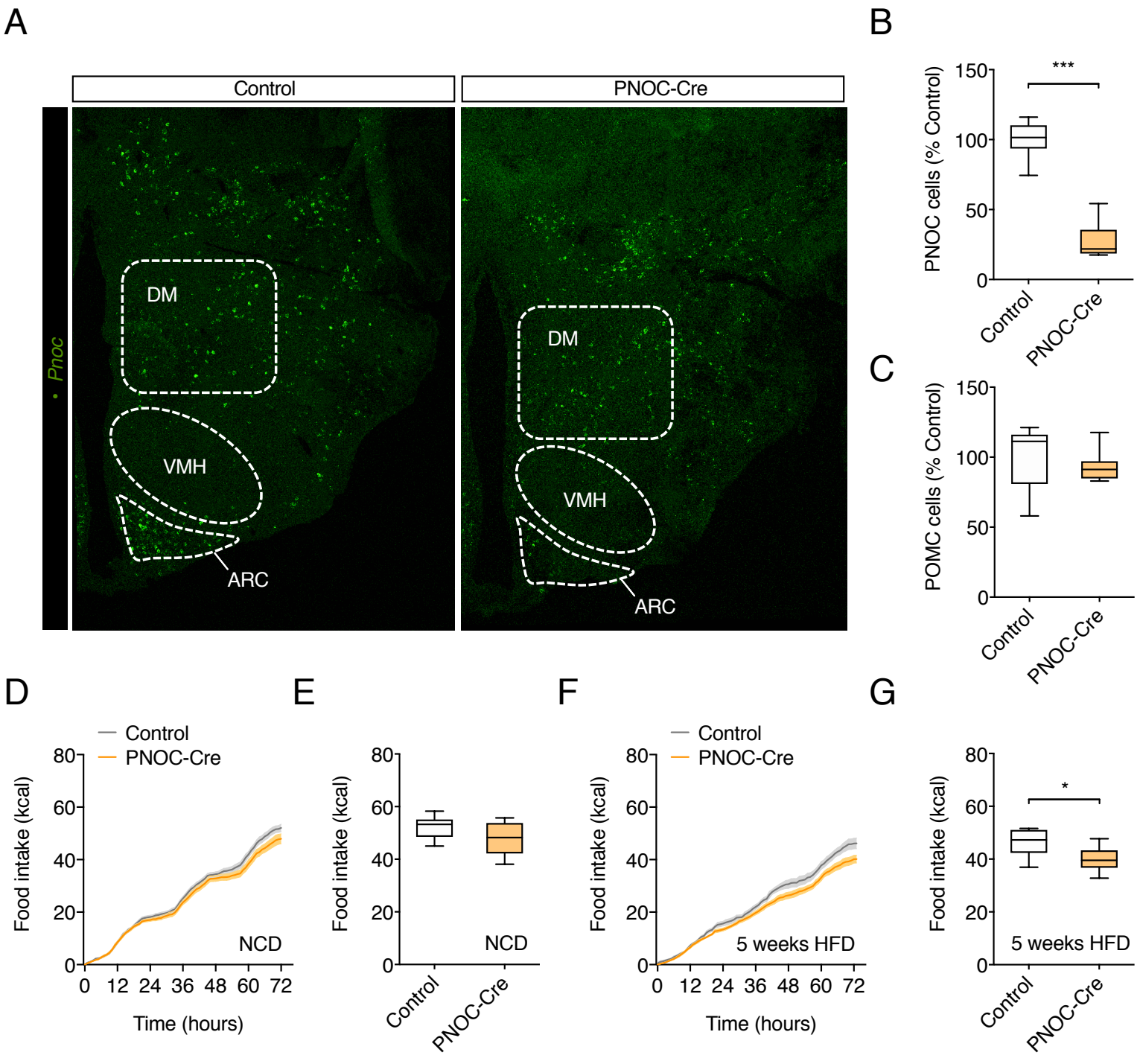


Figure S6. Related to Figure 6.

(A) Expression of ChR2 in PNOC neurons as assessed by EYFP fluorescence as well as fiber placement. (B) Cumulative food intake during light cycle photostimulation (n = 6/7). (C) Total intake after the stimulation period (8 hours). (D) Respiratory exchange ratio (RER) during photostimulation on NCD (n = 12/11). (E) Cumulative water consumption during photostimulation on NCD (n = 12/11). (F) Total water consumption during photostimulation on NCD. (G) Cumulative water consumption without stimulation on NCD (n = 12/11). (H) Total water consumption without stimulation on NCD. (I) Cumulative water consumption during photostimulation without food (n = 8/7). (J) Total water consumption during photostimulation without food. (K) Respiratory exchange ratio (RER) during photostimulation after 3 days of HFD (n = 11/11). (L) Real time place preference/aversion test during photostimulation of $PNOC^{ARC}$ neurons. (n = 7/7). (M) Conditioned place preference/aversion test during photostimulation of $PNOC^{ARC}$ neurons. (n = 7/7). (N) GTT during photostimulation. Insert shows area under the curve (AUC). (n = 6/7, two-way repeated-measures ANOVA with Sidak's multiple comparisons test; group p=0.6095, interaction p=0.5299). (O) ITT during optogenetic stimulation. Insert shows area under the curve (AUC). (n = 6/7, two-way repeated-measures ANOVA with Sidak's multiple comparisons test; group p=0.7780, interaction p=0.8274). (P) Representative image of AAV-injection site showing PNOC cell bodies in the ARC. Scale bar, 500 μm. (Q) Expression of ChR2 in $PNOC^{ARC}$ fibers in the LHA as assessed by EYFP fluorescence as well as fiber placement. Scale bar, 200 μm. (R) Expression of ChR2 in $PNOC^{ARC}$ projections in the BNST as assessed by EYFP fluorescence as well as fiber placement. Scale bar, 200 μm.

*p < 0.05, **p < 0.01, ***p < 0.001 as determined by two-tailed, unpaired Student's t test (C, F, H, J) or with two-way repeated-measures ANOVA with Sidak's multiple comparisons test (L, M, N, O).

Figure S7, related to Figure 7



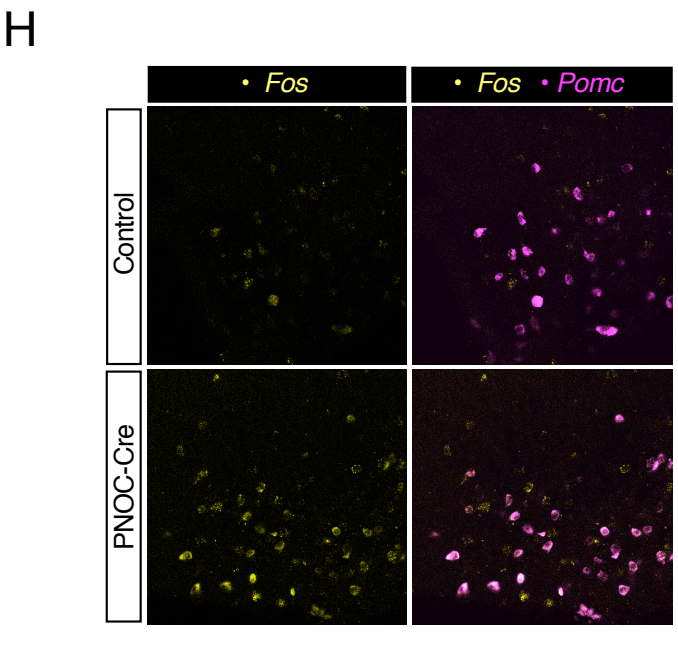


Figure S7. Related to Figure 7.

(A) Representative confocal tile-scan images of *in situ* hybridization of *Pnoc* mRNA showing the specific ablation of PNOC neurons in the ARC. (B) Quantification of $PNOC^{ARC}$ neurons in AAVCasp-injected PNOC-Cre mice and control animals (n = 10/9). (C) Quantification of POMC neurons in AAVCasp-injected PNOC-Cre mice and control animals (n = 10/9). (D) Cumulative food intake during 3 days of NCD feeding (n = 8/9). (E) Total food intake after 3 days of NCD feeding (n = 8/9). (F) Cumulative food intake after 5 weeks of HFD feeding (n = 8/9). (G) Total food intake during 3 days after 5 weeks of HFD feeding (n = 8/9). (H) Representative confocal images of *in situ* hybridization of *Fos* mRNA (yellow) and *Pomc* mRNA (magenta) expression in the ARC of in AAVCasp-injected PNOC-Cre mice and control animals. *p < 0.05, **p < 0.01, ***p < 0.001 as determined by two-tailed, unpaired Student's t test (B, C, E, G).