Supplementary Materials

Generation of *Nestin-Cre; Ptpn1$^{lox/lox}$; Socs-3$^{lox/lox}$* mice. *Socs-3*^{lox} mice were obtained from Dr Yoshimura and *Ptpn1*^{lox} mice from Drs Bence, Kahn and Neel (1; 2). Briefly, exon 2 and exons 6-8 of *Socs-3* and *Ptpn1* genes respectively were targeted with LoxP sites for Cre-mediated recombination. Nestin-Cre mice (Jackson Labs #3771) were mated with mice floxed for either *Socs-3* or *Ptpn1* or for both genes (*Ptpn1*^{lox}; *Socs-3*^{lox} mice were generated by inter-breeding both colonies). A first cohort of *Socs-3* and *Ptpn1* single and double mutants was generated by back-crossing the resulting Nestin-Cre; *Socs-3*$^{lox/+}$ with *Socs-3*^{lox} mice, Nestin-Cre; *Ptpn1*$^{lox/+}$ with *Ptpn1*^{lox} mice, and Nestin-Cre; *Ptpn1*$^{lox/+}$; *Socs-3*$^{lox/+}$ with *Ptpn1*^{lox}; *Socs-3*^{lox} mice respectively. A second cohort was generated by breeding Nestin-Cre; *Ptpn1*$^{lox/+}$; *Socs-3*$^{lox/+}$ mice with females carrying different combinations of floxed alleles in order to keep the heterogeneity in genetic background minimal. Assessed physiological parameters were similar between the two cohorts and were pooled when possible. Mice were analyzed in an open enrollment process.

Nestin-Cre and floxed control mice were either littermates of single and double mutants or derived from mating *Nestin-Cre; Ptpn1$^{lox/+}$;Socs-3$^{lox/+}$* with C57BL/6 females. Floxed mice were a mixture of mice floxed for either or both genes. Nestin-Cre transgene was always paternally transmitted. In addition to genotyping for Cre transgene and floxed alleles, mice were genotyped for possible whole-body deletion of the floxed alleles in comparison to brain samples of brain-specific KO mice (using primer set c in (1) and a/c in (2)). Up to 12% of the genotyped offspring was discarded because of whole-body deletion, suggesting early recombination events in sperm or zygote due to leaky Nestin-Cre activity.

Mice were housed in a temperature-controlled (20-22°C) barrier facility with a 12 hr light/12 hr dark cycle and provided ad libitum water and chow (Formulab #5008 composed of 17% calories from fat). Age-matched males were used in this study.

RNA extraction and real-time PCR. Total RNA was extracted from tissues using RNeasy kits (Qiagen) and cDNA synthetized using SuperScript™ II enzyme (Invitrogen). Quantification of neuropeptide, *Socs-3* and *Ptpn1* expression was performed by quantitative real-time PCR using SYBR Green (Applied Biosystems) on a Stratagene MX3000P QPCR system. Primer sequences were designed in Primer Express® (Applied Biosystems): *Ptpn1*: 5'-tctcctacctggctgtcatcga-3' and 5'-tccttccactgatcctgcactg-3', *Socs-3*: 5'-gagatttcgcttcgggacta-3' and 5'-aacttgctgtgggtgaccat-3'.

Mice for neuropeptide gene expression analysis. 7-11 month-old mice on chow diet were fasted overnight (14-17 hours) prior to intraperitoneal injection with leptin (3µg/g) or saline (PBS). Mice were sacrificed by CO2 inhalation 3 or 6 hours after injections (n=3 to 5 per time point and per genotype). The arcuate nucleus of the hypothalamus was freshly dissected out as previously described (3), flash-frozen in liquid nitrogen and stored at -80C for later use.

SUPPLEMENTARY RESULTS

In glucose tolerance tests, insulin levels of Nestin-Cre mice 30 min after glucose injection tended to be lower than those of Floxed mice, although the difference did not reach statistical significance (Nes: 0.53 ± 0.11; Floxed: 0.73 ± 0.25 ng/mL; n=5). This may reflect an underlying defect in insulin secretion in Nes-Cre mice, which could explain their lower performance in glucose tolerance test (Fig. S1E).

Figure S1. Presence of the Nestin-Cre transgene affects several physiological parameters on chow.

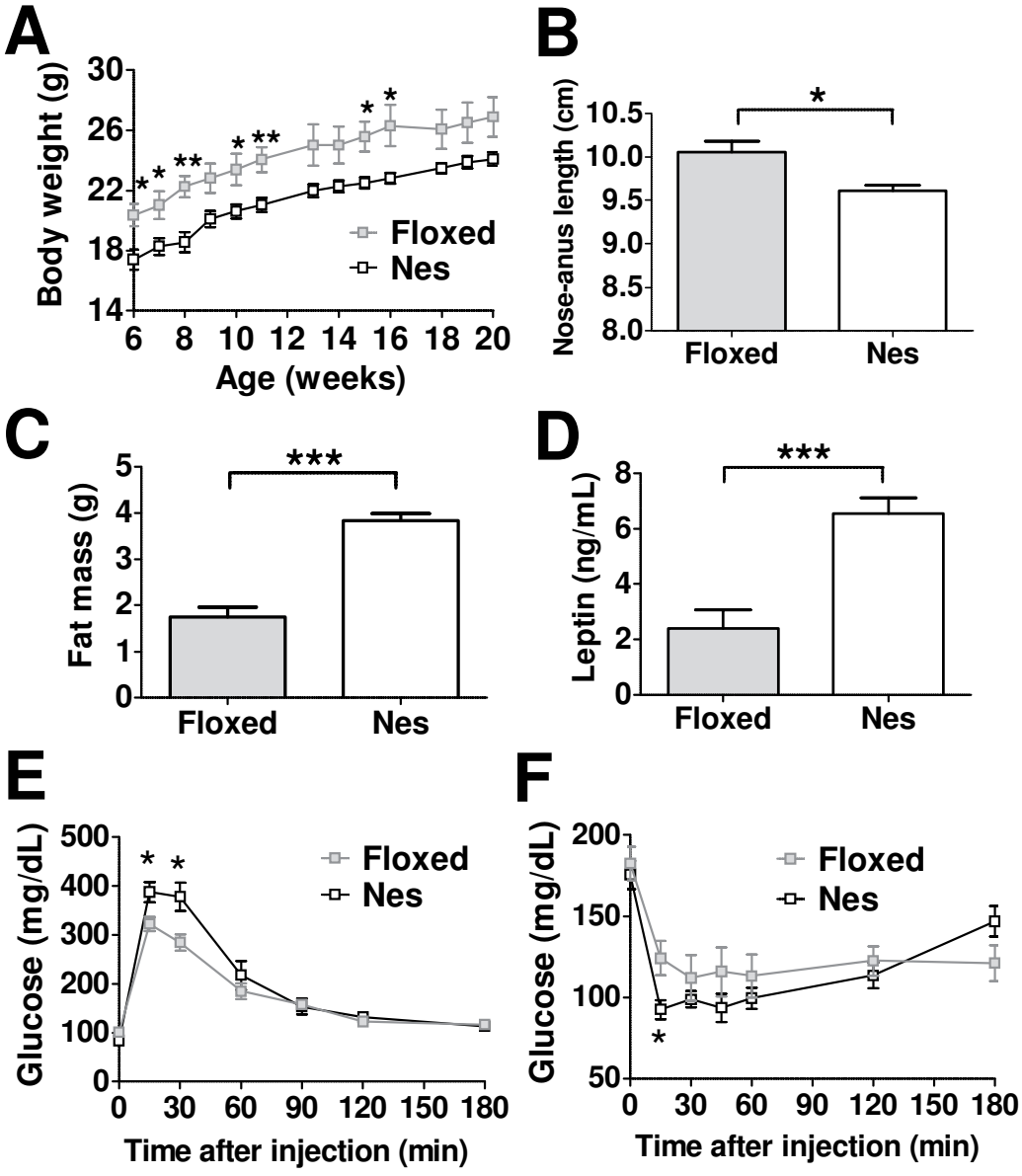
A) Body weight curve of Floxed (n=13) and Nestin-Cre mice (Nes; n=6).

B) Body length at 5 months.

C) Fat body content measured at 19 weeks by EchoMRI (n=9 Floxed and 6 Nes).

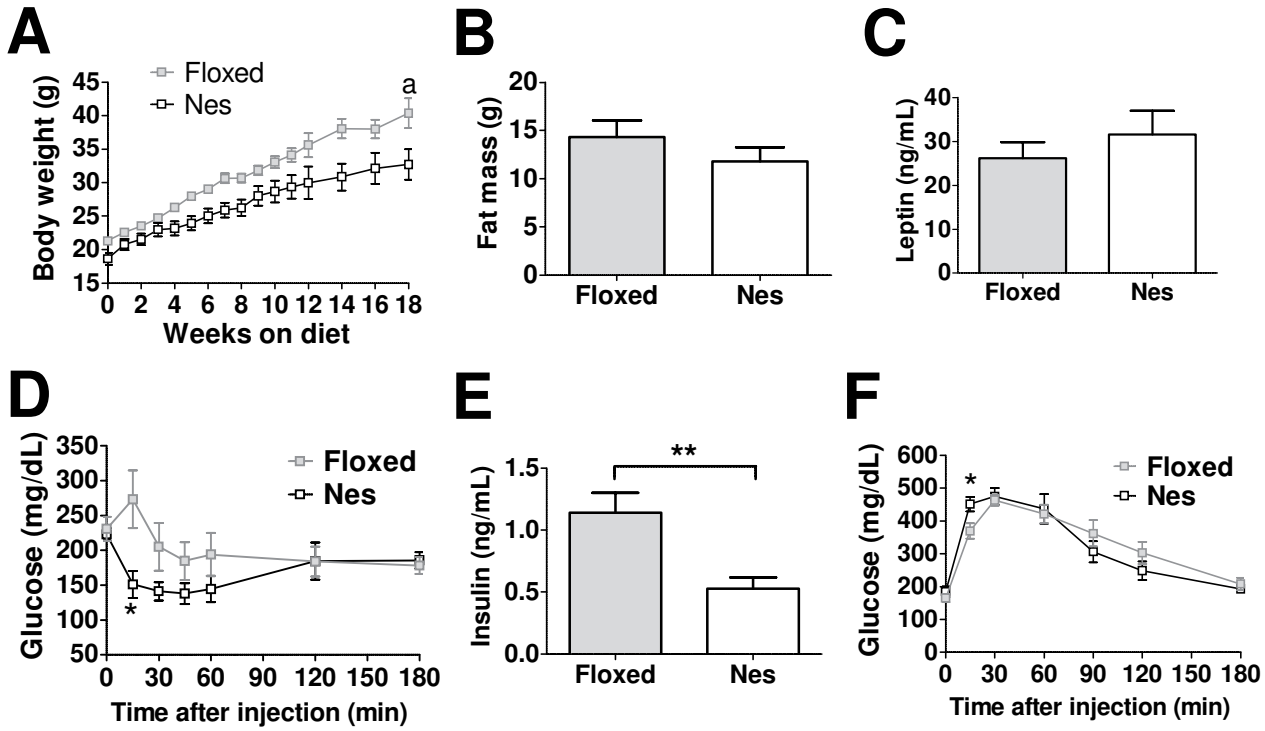
D) Circulating leptin levels in *ad libitum* fed animals (n=5 Floxed and 6 Nes).

E-F) Glucose tolerance (E) and insulin sensitivity (F) tests at 20-23 weeks (n=9 Floxed and 6 Nes). Basal glucose and insulin levels (not shown) were not different between Nes and Floxed mice.



Unpaired t test (with Welch's correction when variances were heterogeneous) was used for statistical analysis (*p<0.05, **p<0.01 and ***p<0.001).

Figure S2. Nestin-Cre mice gain less weight than Floxed mice on HFD.
A) Body weight curve on high fat diet starting at 6 weeks of age. a, $p<0.05$ by t test for every time points (except for weeks 3 and 12). Linear regression curves applied to the body weight curves have significantly different slopes (two-tailed $P<0.001$).
B) Fat mass measured by EchoMRI.
C) Circulating leptin levels after 11-13 weeks on HFD.
D) Insulin sensitivity test after 20 weeks on HFD.
E) Circulating insulin levels after 11-13 weeks on HFD.
F) Glucose tolerance test after 19 weeks on HFD.

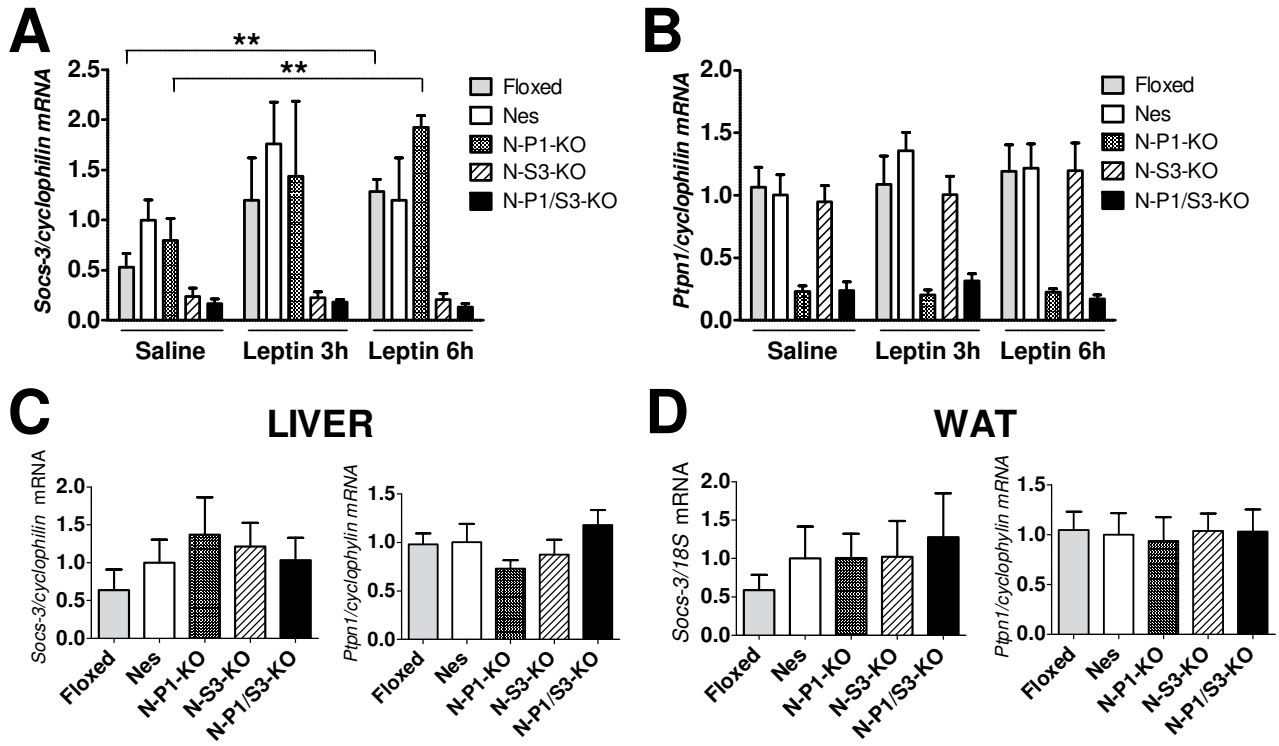


Unpaired t test was used for statistical analysis (*$p<0.05$, **$p<0.01$).

Figure S3. Confirmation of brain-specific deletions at *Socs-3* and *Ptpn1* loci in double mutant mice.

A-B) Real time PCR for *Socs-3* (A) and *Ptpn1* transcripts (B) was performed following reverse-transcription of RNA extracted from hypothalamic arcuate nuclei. Mice were either injected with PBS (saline) or recombinant leptin and sacrificed 3 or 6 hours after injections. Primers were designed in the floxed exons and hence do not enable amplification when Cre-mediated recombination events occurred. Nestin-Cre; *Ptpn1*$^{lox/lox}$; *Socs-3*$^{lox/lox}$ double mutants (N-P1/S3-KO) lack both transcripts while Nestin-Cre; *Ptpn1*$^{lox/lox}$ (N-P1-KO) and Nestin-Cre; *Socs-3*$^{lox/lox}$ (N-S3-KO) single mutants lack either *Ptpn1* or *Socs-3* transcripts respectively. Results are normalized to cyclophilin and given as fold change of saline injected Nestin-Cre (Nes) values. Saline vs. leptin injected animals were compared by unpaired t test (**p<0.01, n=3-5 per group). In line with previous reports (4-9), hypothalamic *Socs-3* mRNA levels are increased upon leptin injection in *Socs-3* expressing mice. Conversely, *Ptpn1* transcript levels were not affected by acute leptin administration in *Ptpn1*-expressing mice.

C-D) Real time PCR was performed after reverse-transcription of RNA extracted from liver (C) and white adipose tissue (WAT) (D). *Socs-3* and *Ptpn1* transcript levels (left and right histograms of each panel respectively) are normalized to cyclophilin or 18S and normalized to Nes values.



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