SUPPLEMENTARY DATA

Euglycemia Restoration by Central Leptin in Type 1 Diabetes Requires STAT3 Signaling but Not Fast-Acting Neurotransmitter Release

Yuanzhong Xu[1], Jeffrey T. Chang[1,2], Martin G. Myers Jr.[3], Yong Xu[4] and Qingchun Tong[1]

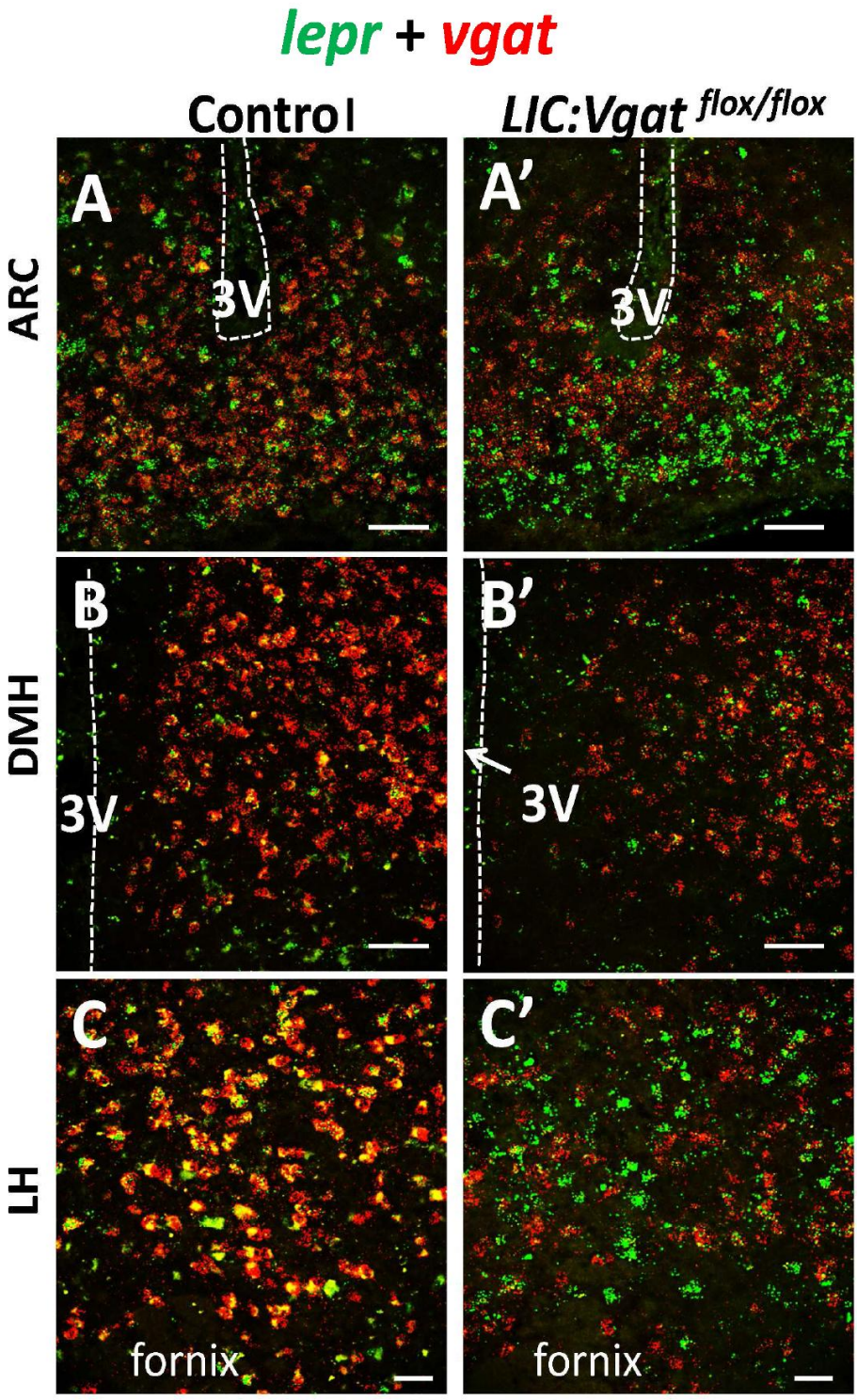
SUPPLEMENTARY DATA

Supplementary Figure S1. Deletion of Vgat by Leptin-Ires-Cre

Dual color in situ hybridization of Vgat (red) and Leptin receptor (green) was performed on hypothalamic sections from control (A-C) and Leptin-Ires-Cre (LIC):lox-Vgat mice (A'-C'). Matched sections of arcuate nucleus (Arc, A and A'), dorsomedial hypothalamus (DMH, B and B') and latheral hypothalamus (LH, C and C') were shown. Abundant colocalization between leptin receptor and Vgat was evident in panels A-C and minimal colocalization was observed in panels A'-C'. 3V: third ventricle. Scale bars = 50 μm.

RNAscope Multiple Fluorescent Assay in situ hybridization (ISH) technique designed to visualize multiple cellular RNA targets in fresh frozen tissues was used for detecting the colocalization of *Vgat* and *LepR* in the brain (Advanced Cell Diagnostics, Hayward, California). This technique is highly sensitive and allows detection of mRNA at single molecular level (Wang F., et al., *J. Mol Dign.* 14: 22-29, 2012). Probes against mouse *Vgat* and *LepR* mRNA were generated and pre-labeled with different fluorescent reagents. All ISH procedures were performed following the protocol provided by the manufacturer. Briefly, fresh frozen brain tissues were sectioned with cryostat at 15μm thickness and placed onto SuperFrost Plus slides. After fixed with pre-chilled 10% formalin at 4°C for 1 h, the sections were dehydrated with grade ethanol and stored overnight in 100% ethanol at -20°C. All sections were then air-dried for 30 min, pretreated with Pretreat 3 solution for 20 min at room temperature (RT), and followed by 2 hr incubation with pre-mixed target probes at 40°C. After 2 times washes, all slides were sequentially incubated with Amp-1FL for 30 min, Amp-2FL for 15 min, Amp-3FL for 30 min, and Amp-4FL for 15 min at 40°C. Finally, all slides were coverslipped with Prolong Gold antifade reagent. Sections were photographed with a TCS SP5 confocal microscope (Leica, Nussloch, Germany).

SUPPLEMENTARY DATA

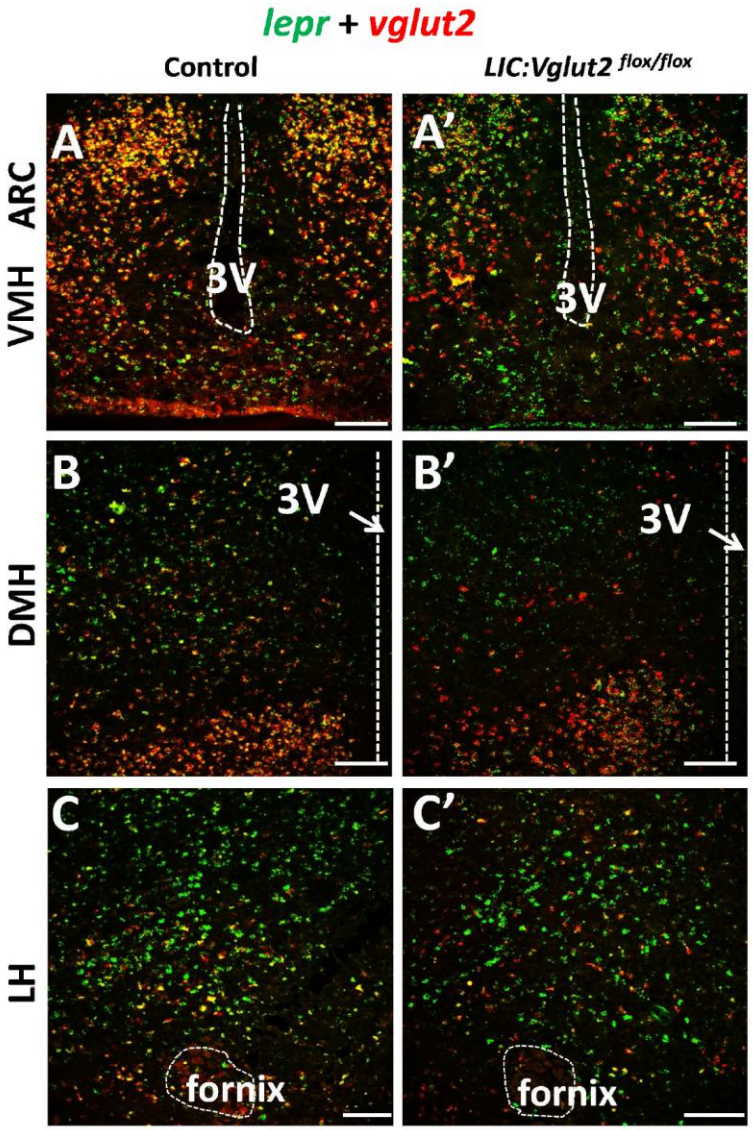


SUPPLEMENTARY DATA

Supplementary Figure S2. Deletion of Vglut2 by Leptin-Ires-Cre

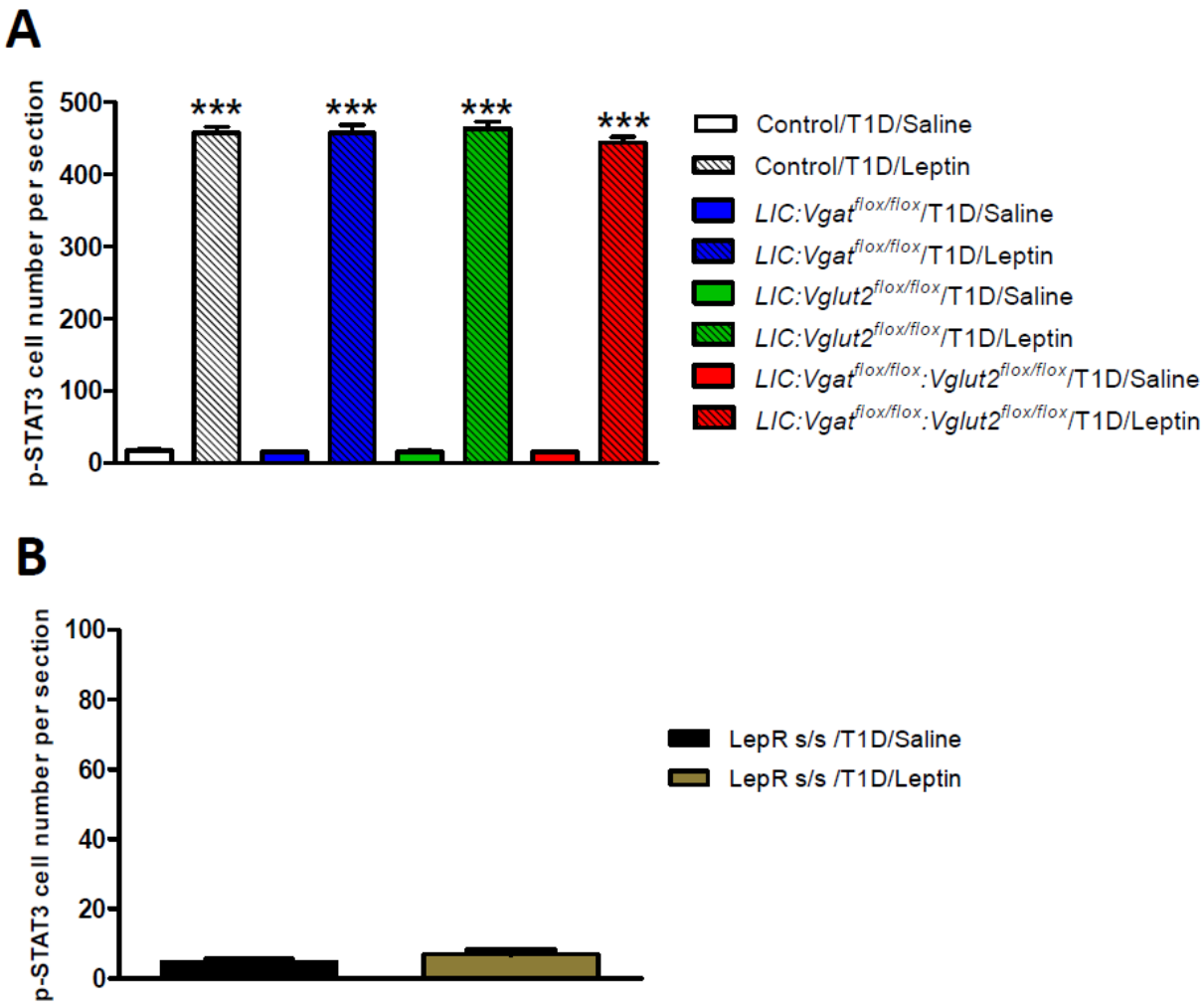
Dual color in situ hybridization for *Vglut2* (red) and Leptin receptor (green) was performed on hypothalamic sections from control (A-C) and Leptin-Ires-Cre (LIC):lox-Vglut2 mice (A'-C'). Matched sections of ventral medial thalamus (VMH) and arcuate nucleus and (Arc, A and A'), dorsomedial hypothalamus (DMH, B and B') and latheral hypothalamus (LH, C and C') were shown. Abundant colocalization between leptin receptor and Vgat was evident in VMH (A) and some colocalization was also observed in DMH and LH (panels B-C) and minimal colocalization was observed in panels A'-C'. 3V: third ventricle. Scale bars = 50 µm.

The experimental procedure was the same as described in Fig. S1. Please note that in some brain areas with densely packed stained neurons such as VMH, the appearance of merged signal (yellow) may be due to close proximity of red and green fluorescence in neighboring neurons.



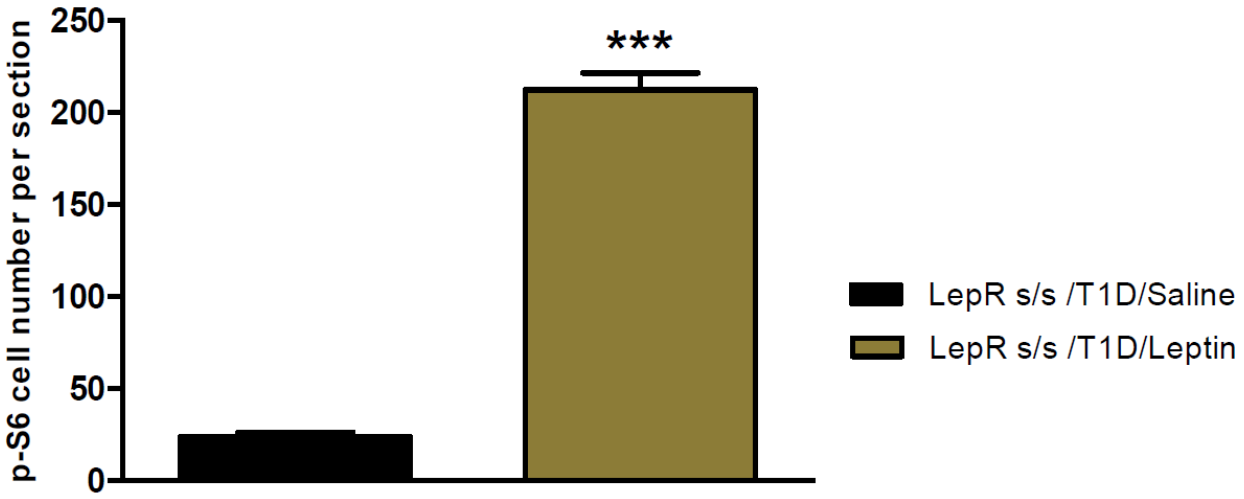
SUPPLEMENTARY DATA

Supplementary Figure S3. Statistical analysis of p-STAT3-expressing neuron number in the hypothalamus after leptin infusion. To quantify p-STAT3 positive neurons number, three sections at the corresponding rostrocaudal levels were chosen from each animal. All immunopostive cells with clear profile were counted, and the number from all animals were averaged and analyzed (*n* = 5-8) . ***p < 0.001, unpaired student's t test, saline versus leptin.



SUPPLEMENTARY DATA

Supplementary Figure S4. Quantitative analysis of p-S6 expression in the hypothalamus. A series of brain sections from all LepRs/s and control mice used in Fig. 5 was used to immunostain p-S6. Three matched sections at the corresponding rostrocaudal levels were chosen from each animal. All immunopostive cells with clear profile were counted, and the number from all animals were averaged and analyzed (*n* = 4-7). ***p < 0.001, one away ANOVA. The immunostaining protocol was described in the method section of the main text.



SUPPLEMENTARY DATA

Supplementary Figure S5. Q-PCR analysis of a few selected genes from microarray analysis. Littermate wild type male mice (mixed background) were used for this study. Procedures of generation of T1D and i.c.v. surgical perfusion of leptin were the same as described in the method section of the main text. Hypothalamic tissues were punched out from fresh brains at the 13th day after leptin or saline infusion and were used for extraction of RNAs. After cDNA synthesis in the volume of 20 µl with 1 µg RNA using reverse transcriptase kit (Applied Biosystems, Grand Island, NY), Q-PCR was performed on selected genes STAT1 (A), STAT3 (B), Cartpt (C), Ccnd3 (D) and Tyk2 (E) using a iTaq Universal SYBR green Supermix kit (Bio-Rad, Hercules, California) on a pcr machine (Bio-Rad). The primers used for STAT1: forward 5'-CTGAATATTTCCCTCCTGGG-3', reverse 5'-TCCCGTACAGATGTCCATGAT-3'; STAT3: forward 5'-CAGAAAGTGTCCTACAAGGGCG-3', reverse 5'-CGTTGTTAGACTCCTCCATGTTC-3'; Cartpt: forward 5'-CCCGAGCCCTGGACATCTA-3', reverse 5'- GCTTCGATCTGCAACATAGCG-3'; Ccnd3: forward 5'-TGGATCGCTACCTGTCCTG-3', reverse 5'-CCTGGTCCGTATAGATGCAAAG-3' and Tyk2: forward 5'-AGCCATCTTGGAAGACAGCAA-3', reverse 5'- GACTTTGTGTGCGATGTGGAT-3'. *p<0.05, One Way ANOVA tests, *n* = 5-6 each group.