Supporting Online Material

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Article Title:	Tanycytes of the Hypothalamic Median Eminence Form a Diet-Responsive Neurogenic Niche.
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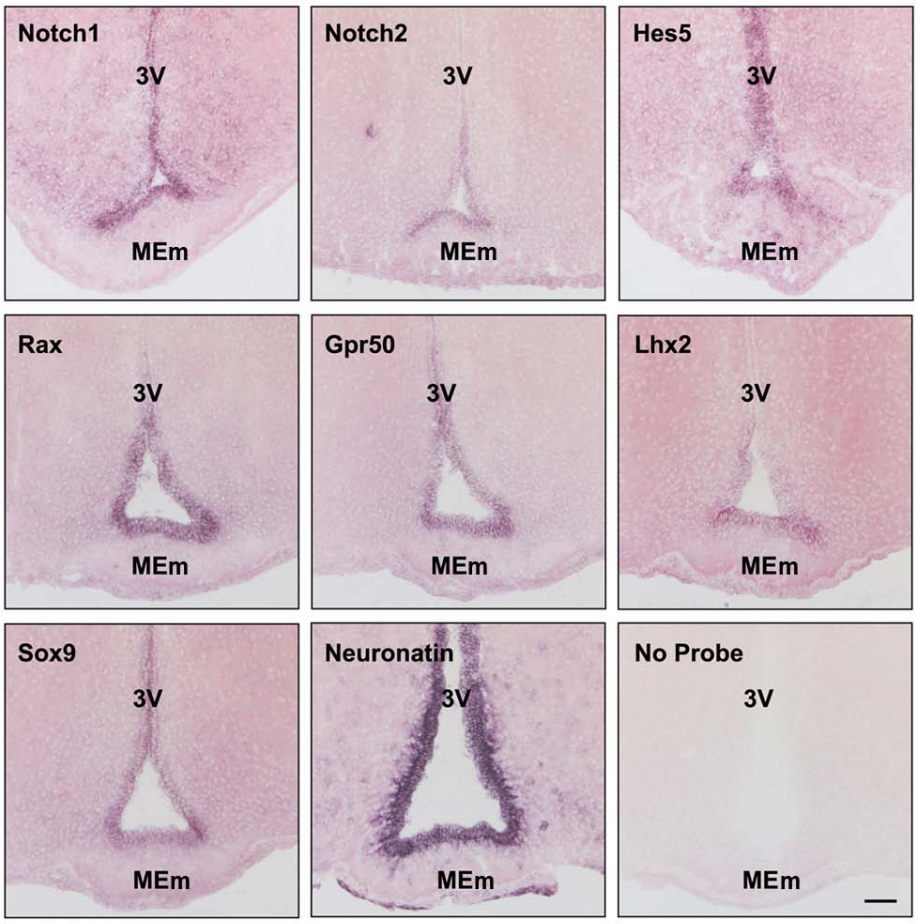
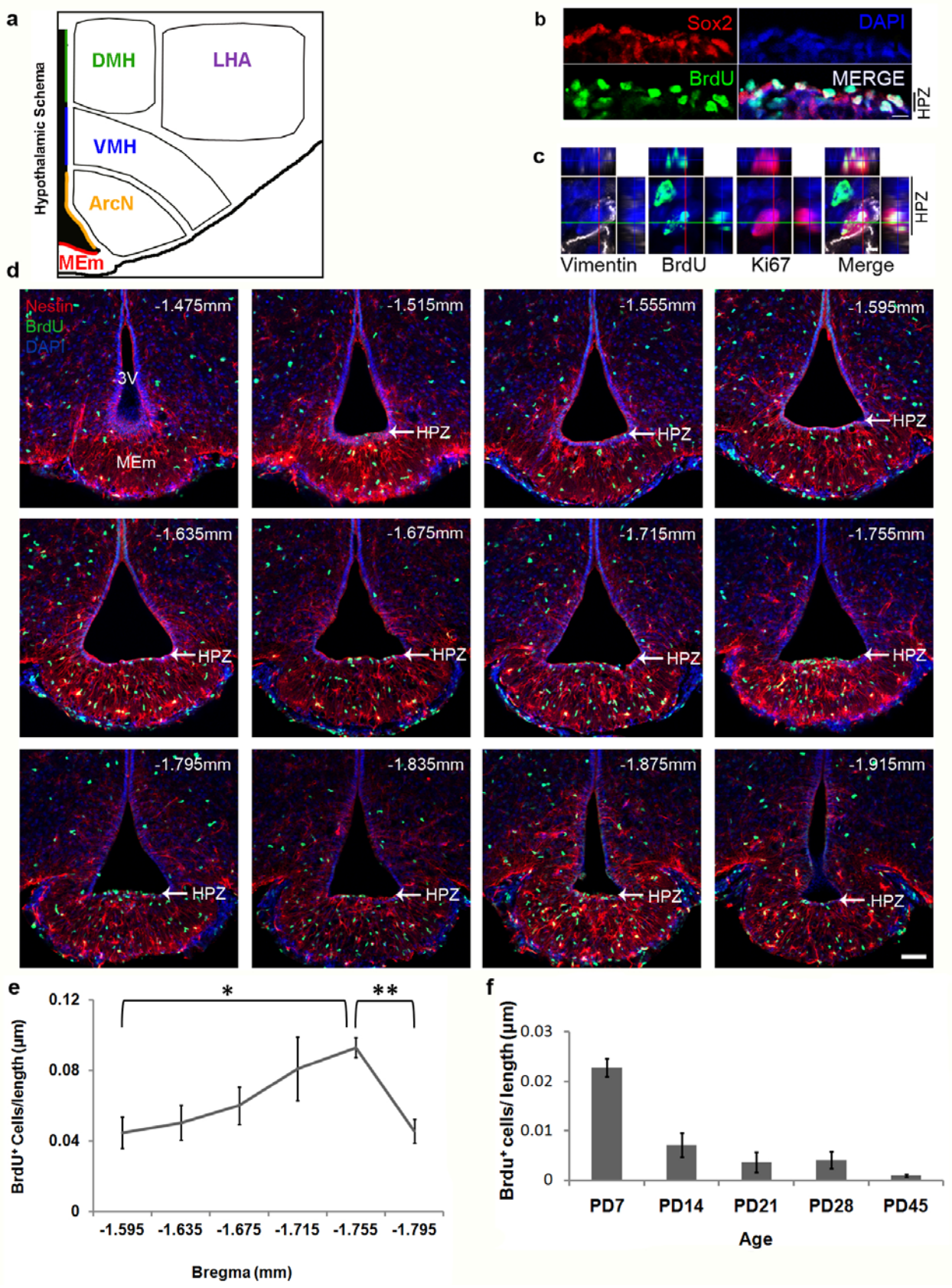
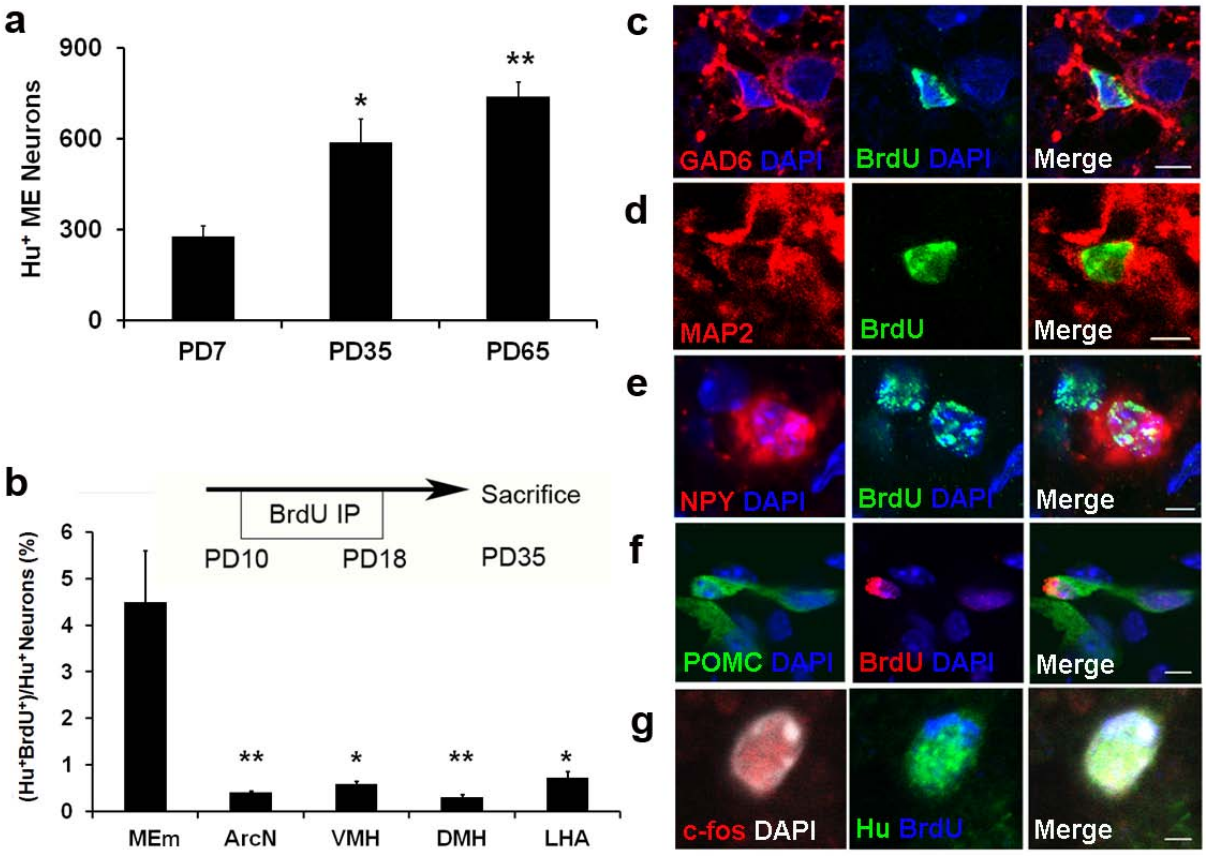
Supplementary Items included:



Supplementary Figure 1. Tanycytes express neural stem and progenitor-specific markers. Tanycytes, a radial-glia like ependymal cell lining the hypothalamic ventricular zone (HVZ) of the third ventricle (3V)[4], express stem and neural progenitor-specific markers. *In situ* hybridization of the adult hypothalamus reveals that HVZ tanycytes express numerous neural stem and progenitor specific markers, along with a number of genes selectively expressed in hypothalamic progenitor cells[6] (*Gpr50, Rax*, and *Lhx2*). MEm = median eminence. Scale bars = 50μm.

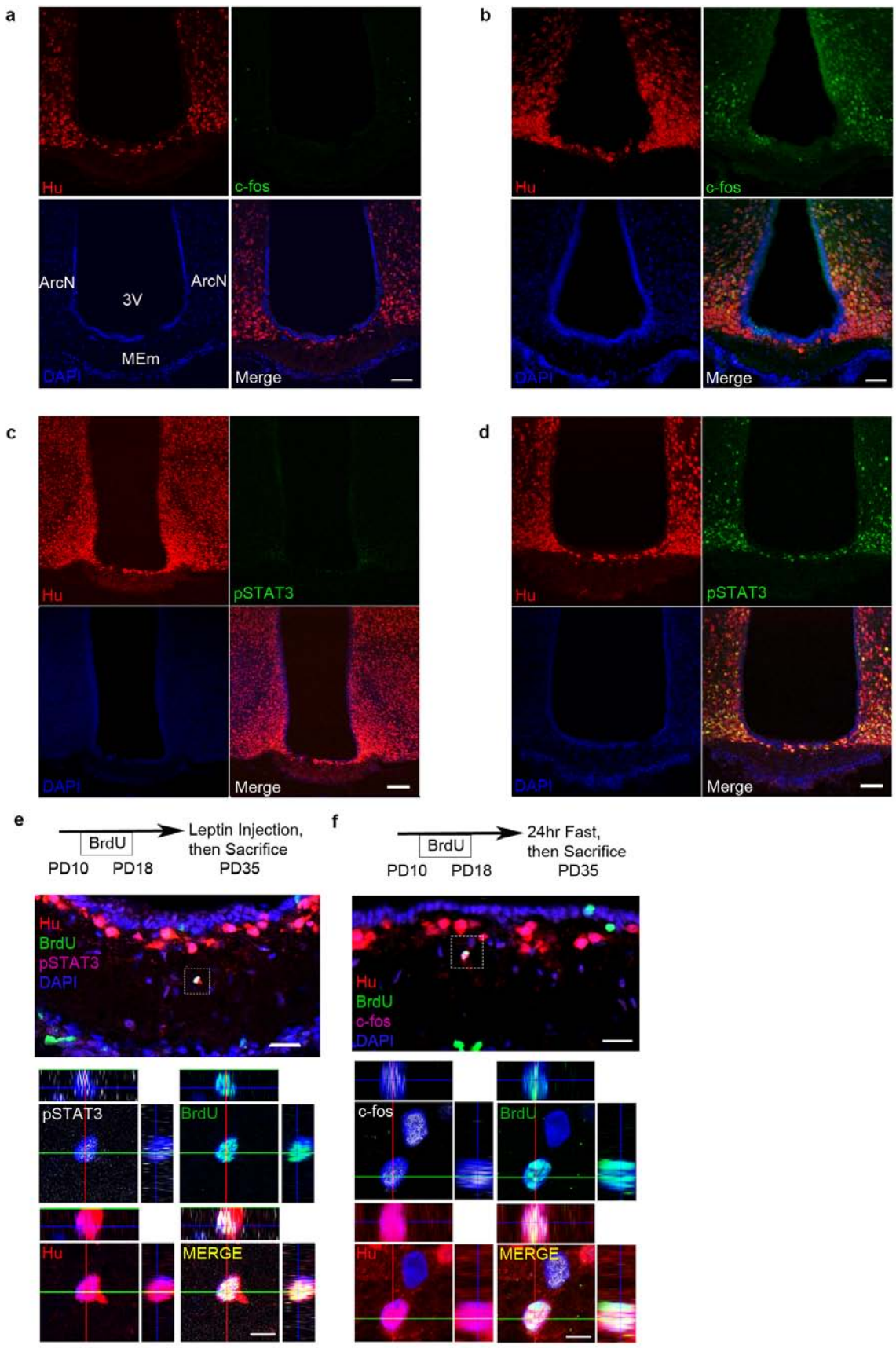

a
Hypothalamic Schema
DMH
LHA
VMH
ArcN
MEm
b
Sox2
DAPI
BrdU
MERGE
HPZ
c
HPZ
Vimentin
BrdU
Ki67
Merge
d
Nestin
BrdU
DAPI
-1.475mm
3V
MEm
-1.515mm
HPZ
-1.555mm
HPZ
-1.595mm
HPZ
-1.635mm
HPZ
-1.675mm
HPZ
-1.715mm
HPZ
-1.755mm
HPZ
-1.795mm
HPZ
-1.835mm
HPZ
-1.875mm
HPZ
-1.915mm
HPZ
e
BrdU+ Cells/length (μm)
0.12
0.08
0.04
0
*
**
-1.595
-1.635
-1.675
-1.715
-1.755
-1.795
Bregma (mm)
f
Brdu+ cells/ length (μm)
0.03
0.02
0.01
0
PD7
PD14
PD21
PD28
PD45
Age

Supplementary Figure 2. Spatial and temporal characterization of proliferating cells in the hypothalamic proliferative zone (HPZ).
(a) Coronal schema of hypothalamic nuclei: median eminence (MEm), arcuate nucleus (ArcN), ventromedial hypothalamus (VMH), and dorsalmedial hypothalamus (DMH). **(b)** Immunohistochemical staining of the HPZ with tanycyte-enriched markers revealed that HPZ $BrdU^+$ cells (green) expressed Sox2 (red). **(c)** HPZ tanycytes can re-enter the cell-cycle more than once. At PD25, $Vimentin^+BrdU^+$ (white/green) HPZ tanycytes also expressed Ki67 (red), a pan-proliferation marker, after receiving BrdU i.p. injections from PD15 to PD23. Differential levels of BrdU labeling were observed in the HPZ along the anterior-posterior axis. Serial coronal brain sections of a PD19 mouse that received BrdU ip injections from PD10–18 (Figure 1A). **(d)** Differential levels of BrdU labeling were observed in the HPZ along the anterior-posterior axis. Serial coronal brain sections of a PD19 mouse that received BrdU ip injections from PD10-18. Immunohistochemical staining for Nestin (red) and BrdU (green) revealed an enrichment of $Nestin^+BrdU^+$ cells at the third ventricle (3V) floor of the hypothalamic median eminence (MEm). This enrichment of proliferating cells was found between Bregma –1.475 and –1.915mm, with a peak at approximately –1.755mm Bregma. We named this novel proliferative domain the hypothalamic proliferative zone (HPZ) (white arrow). All sections shown were counterstained with DAPI (blue), a nuclear marker. Scale bar = 50μm (d); 5μm (b); 2μm (c). **(e)** Anterior-posterior distribution of HPZ $BrdU^+$ cells were calculated by $BrdU^+$ cells/coronal length of ependymal layer (μm). Peak enrichment is observed at –1.755mm Bregma. n=5; * = $p<0.01$; ** = $p<0.001$. **(f)** Saturation levels of BrdU were given for 12 hours by i.p. injection at PD7, 14, 21, 28, or 45 to characterization changes in proliferative capacity of the HPZ over time. Ventrobasal hypothalamus was immunostained for BrdU, and $BrdU^+$ cells along the coronal length of the ependymal layer were quantified following a 12 hour BrdU saturation period. The change in the average number of $BrdU^+$ ependymal cells normalized to the coronal length of the ependymal layer (over seven sections) is shown in graph. HPZ proliferative capacity diminishes with age with the highest level of proliferation seen at PD7.

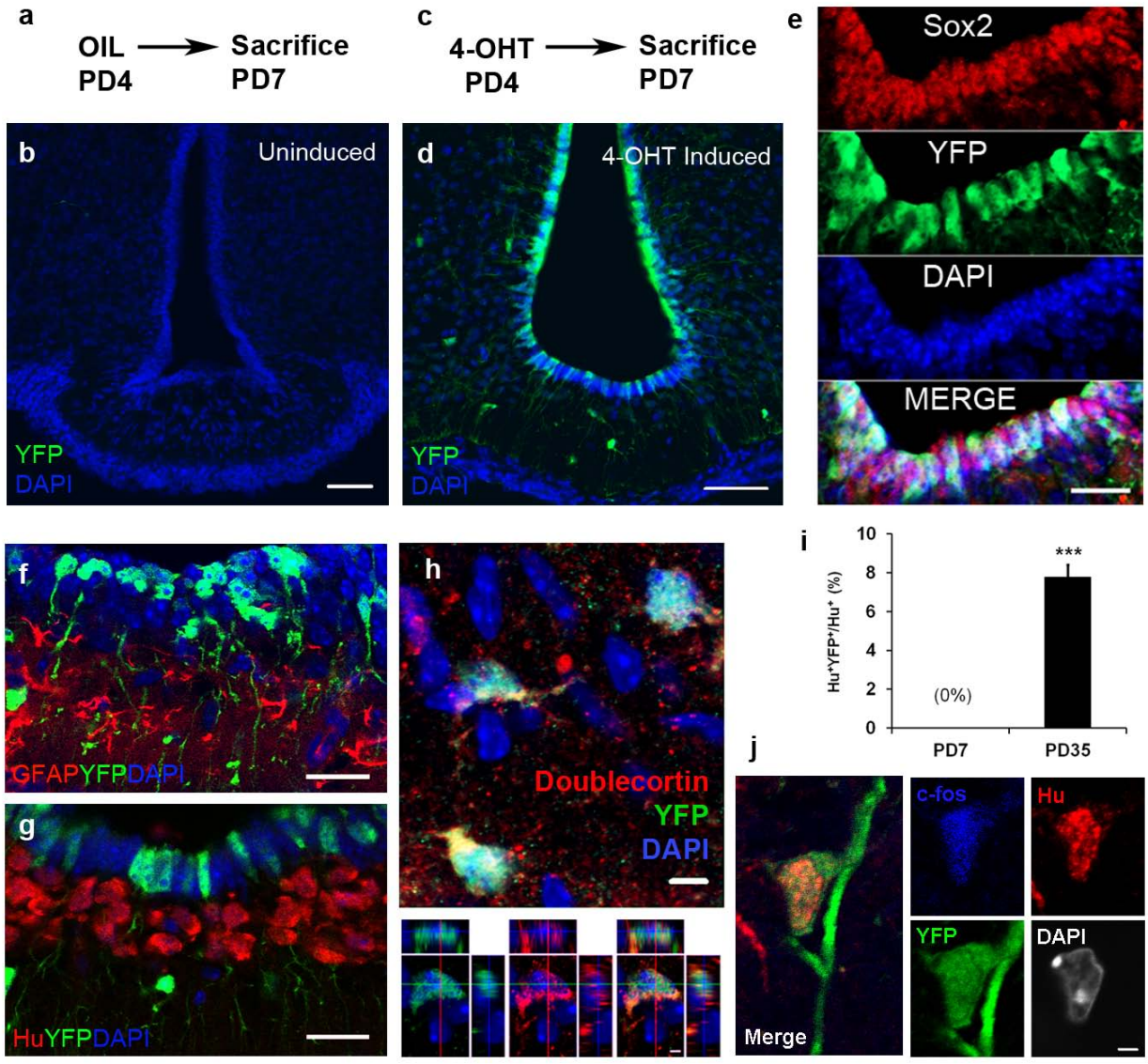


Supplementary Figure 3. Median eminence neurogenesis

Coronal ventrobasal hypothalamic sections at the level of the MEm and ArcN were analyzed by immunohistochemical staining. **(a)** Hu^+ MEm neurons were quantified at PD7 (277.9±33.2 Hu^+ neurons), PD35 (587.4±77.2 Hu^+ neurons), and PD65 (739.6±47.7 Hu^+ neurons) (n=4 for all three timepoints). Significance of neuronal counts of PD35 and PD65 were calculated relative to PD7. Student's t-test was conducted and corrected for multiple comparisons by using Dunn-Šidák correction. * = p=0.0126; ** = p<0.001. **(b-g)** Coronal hypothalamic sections of PD35 mice receiving BrdU from PD10–18. **(b)** Levels of neurogenesis $Hu^+BrdU^+DAPI^+$ neurons/Hu^+DAPI^+ neurons quantified by the following hypothalamic nuclei: MEm (4.8%±1.3), ArcN (0.4%±0.05), VMH (0.6%±0.05), DMH (0.3%±0.05), LHA (0.7%±0.1)). Significance of newborn neurons within each hypothalamic nucleus calculated relative to that observed within the MEm. * = p<0.03; ** = p<0.01. **(c)** Immunohistochemical staining of MEm sections reveals glutamic acid decarboxylase (GAD6), a GABAergic neuronal marker (red), and BrdU (green) co-labeling. **(d)** Immunohistochemical staining of MEm sections reveals MAP2, a mature neuronal marker (red), and BrdU (green) co-localization. **(e)** Co-labeling of Neuropeptide Y (NPY), a neuropeptide involved in the regulation of energy balance, detected by fluorescent *in situ* hybridization (red), and BrdU, detected by immunohistochemical staining (green). **(f)** Immunohistochemical staining of POMC:Cre;Z/EG mice reveals BrdU (red) and pro-opiomelanocortin (POMC, green) co-localization in the MEm. **(g)** Mice were fasted for 24hours. Immunohistochemical staining of MEm sections reveals c-fos (red), a transcription factor often expressed when neurons fire action potentials, and BrdU (green) co-labeling. Sections shown were counterstained with DAPI, a nuclear marker. Scale bars = 5μm (c, d, f), 2μm (e, g).


a
Hu
c-fos
ArcN
3V
ArcN
MEm
DAPI
Merge
b
Hu
c-fos
DAPI
Merge
c
Hu
pSTAT3
DAPI
Merge
d
Hu
pSTAT3
DAPI
Merge
e
BrdU
Leptin Injection,
then Sacrifice
PD10
PD18
PD35
Hu
BrdU
pSTAT3
DAPI
pSTAT3
BrdU
Hu
MERGE
f
BrdU
24hr Fast,
then Sacrifice
PD10
PD18
PD35
Hu
BrdU
c-fos
DAPI
c-fos
BrdU
Hu
MERGE

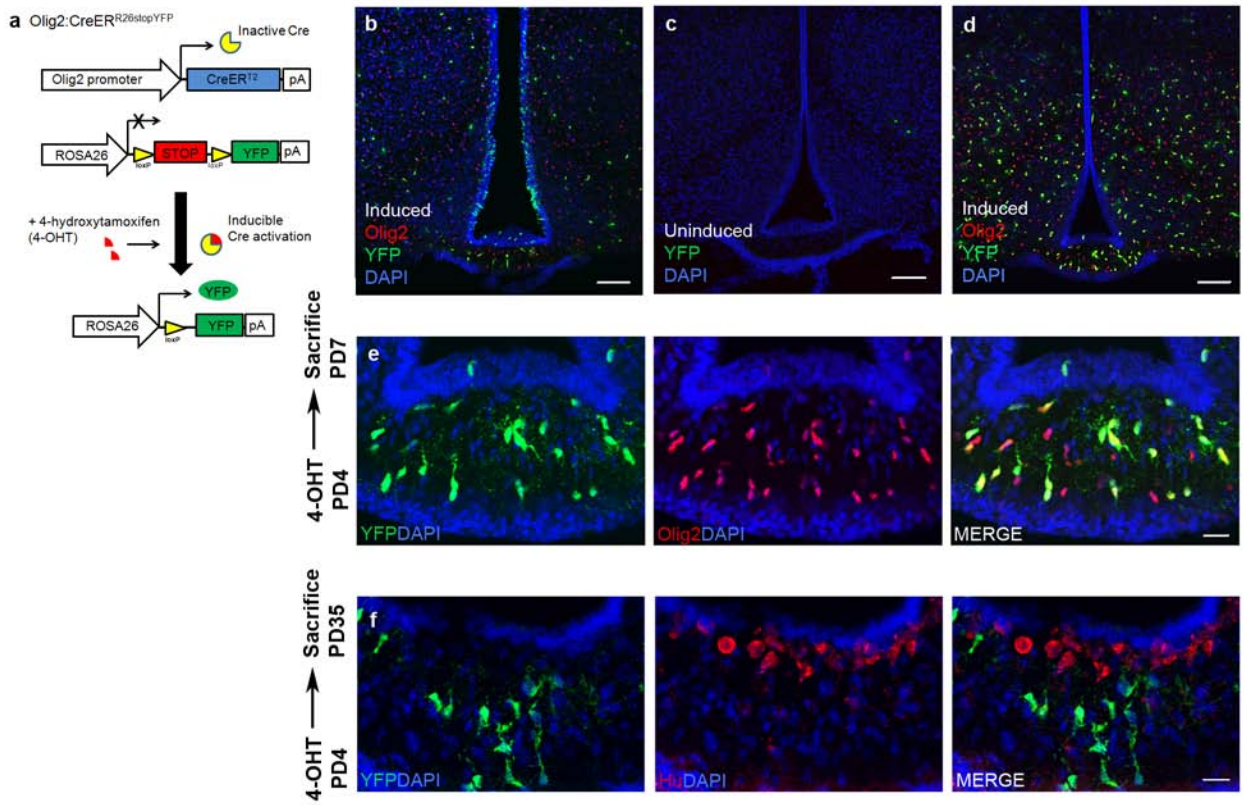
Supplementary Figure 4. pSTAT3 and c-fos expression in newborn neurons.
Immunohistochemical staining of coronal brain sections of ventrobasal hypothalamus of a PD35 mice. **(a)** PD35 mice fed normal chow *ad libitum* displayed few c-fos$^+$ cells (green) in Hu$^+$ neurons (red) of the MEm and ArcN. **(b)** PD35 mice fasted for 24 hours selectively activate c-fos expression in Hu$^+$ neurons (red) of the MEm and ArcN. **(c)** PD35 mice fasted for 24 hours (beginning at PD34) and received saline control injection (PD35) displayed little pSTAT3$^+$ expression (green). pSTAT3 expression that is present is very weakly expressed in a small subset of ventrobasal hypothalamic neurons. **(d)** PD35 mice fasted for 24 hours (beginning at PD34) and received a single leptin i.p. injection 45 minutes prior to sacrifice displayed strong and robust pSTAT3 labeling in feeding centers of the brain. Very high levels of pSTAT3 expression (green) were present in Hu$^+$ neurons (red) of the ArcN and MEm. Sections were counterstained with DAPI (blue), a nuclear marker. **(e)** Normal chow fed mice received ip injections of BrdU from PD10–18, 24hr fasted from PD34-35, and were sacrificed on PD35 following an ip injection of leptin. Immunohistochemical staining was performed on coronal sections of the hypothalamus at the level of the MEm. Leptin induces STAT3 phosphorylation within a subset of hypothalamic neurons of the arcuate nucleus and the median eminence (**Supplementary Fig. 4d**). At PD35, a subset of Hu$^+$BrdU$^+$ neurons co-expressed pSTAT3. White box indicates a higher magnification z-stack reconstruction shown below. In high fat diet (HFD)-fed mice injected with leptin at PD35, 21.2%±4.2 (72/366 total counts for n=4) of Hu$^+$BrdU$^+$ newborn neurons expressed pSTAT3 in leptin injected mice compared to 3.4%±1.7 (8/244 total counts for n=3) for 24 hour fasted control mice (data not shown). pSTAT3 expression in the fasted control mice was very weak compared to that observed in leptin injected cohorts. **(f)** Twenty–four hour food deprivation activates c-fos expression in numerous neurons of the MEm. Mice were injected with BrdU, and immunohistochemical staining revealed that Hu$^+$BrdU$^+$ neurons co-expressed c-fos. White box indicates a higher magnification z-stack reconstruction shown below. Sections were counterstained with DAPI (blue), a nuclear marker. For each figure, white boxes indicate cells for which higher-power 3D z-stack reconstruction was performed. Scale Bars = 100µm (c), 50µm (a,b,d), 20µm (e-f main), 5µm (e-f insets).



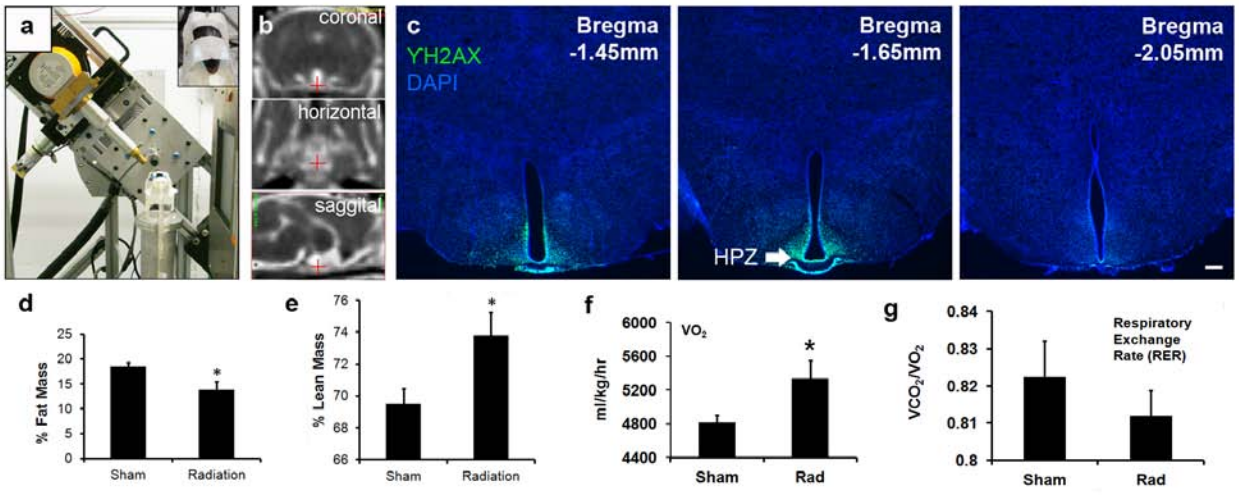
Supplementary Figure 5. Genetic fate-mapping of Tancytes

Nestin:CreER^{R26stopYFP} transgenic mice prospectively labeled tanycytes following 4-hydroxytamoxifen (4-OHT) induction. **(a)** Mice received oil vehicle at PD4, and were analyzed at PD7 for examination of ectopic recombination. **(b)** Uninduced oil-treated control animals (uninduced) animals did not display any leakiness of reporter expression in the ME. **(c)** Mice received 4-OHT at PD4, and were analyzed at PD7 for reporter expression. **(d)** 72 hrs following 4-OHT induction at PD4, Nestin:CreER^{R26stopYFP} mice show permanent labeling of tanycytes by yellow fluorescent protein (YFP). **(e)** PD7 Nestin:CreER^{R26stopYFP} mice treated with 4-OHT 72 hrs prior demonstrate specificity of tanycytic labeling via co-expression of YFP and Sox2^{+} tanycytes. **(f)** We observed no co-labeling of YFP (green) with either astrocyte marker GFAP or the **(g)** pan-neuronal marker Hu (red) at 72 hrs following PD4 4-OHT induction. **(h)** Nestin:CreER^{R26stopYFP} mice were 4-OHT induced at PD4, and examined at PD21. Doublecortin^{+} immature neurons (Dcx^{+}/YFP^{+} neurons) were present within the MEm (white and yellow arrowheads). The yellow arrowhead indicates a higher magnification z-stack reconstruction

shown below. **(i)** Nestin:CreER^{T2};R26YFP mice were 4-OHT induced at PD4, and examined at PD35. Hu^{+}/YFP^{+}/DAPI^{+} neurons were present one-month after induction (7.8%±0.6; 160/2097 total cells for n=4), but not following a 72 hour interval (0%±0; 0/1136 total cells for n=4; $p<0.001$). **(j)** Nestin:CreER^{R26stopYFP} mice were 4-OHT induced at PD4, fasted for 24 hours beginning at PD34, and examined at PD35. C-fos (blue), a transcription factor often expressed when neurons fire action potentials, is co-expressed here with neuronal marker Hu (red), prospective label YFP (green), and DAPI (white). All sections shown were counterstained with DAPI (blue), a nuclear marker. Scale bars = 50 μm (b,d); 20 μm (e-g), 10μm (h), 2μm (j).



Supplementary Figure 6. Genetic fate-mapping of Olig2$^+$ oligodendrocyte precursor cells
(a) Olig2:CreER^{R26stopYFP} transgenic mice prospectively labeled Olig2$^+$ oligodendrocyte precursor cells following 4-hydroxytamoxifen (4-OHT) induction. **(b)** Coronal brain section of ventrobasal hypothalamus of PD7 Nestin:CreER^{R26stopYFP} 72 hrs following 4-OHT induction. Tanycytes and a small subset of Olig2$^+$ cells (red) are prospectively labeled with YFP (green). **(c)** Coronal brain section of ventrobasal hypothalamus of an Olig2:CreER^{R26stopYFP} PD7 pup 72 hrs following vehicle (oil control). No YFP expression is observed in ME. **(d)** Coronal brain section of ventrobasal hypothalamus of Olig2:CreER^{R26stopYFP} P7 pup 72 hrs following 4-OHT induction. 39.4%±2.7 (336/858 total cells for n=3) of Olig2-expressing cells (red) are prospectively labeled with YFP (green). 100% of YFP$^+$ cells were Olig2$^+$ (336/336 total cells for n=3). **(e)** High power image of coronal brain section of mediotuberal hypothalamus of an Olig2:CreER^{R26stopYFP} PD7 pup 72 hrs following 4-OHT induction. Olig2-expressing cells (red) are prospectively labeled with YFP (green). **(f)** High power image of mediotuberal hypothalamus of an Olig2:CreER^{R26stopYFP} mouse at PD35, one month following 4-OHT induction. Prospectively labeled OPCs (green) do not appreciably co-label with Hu (red), a neuronal marker (n=4). Sections were counterstained with DAPI (blue), a nuclear marker. Scale Bars = 100µm (**b-d**), 20µm (**e-f**).



Supplementary Figure 7. Irradiation targeting of the ventral mediobasal hypothalamus. **(a)** Radiation (10 Gy) was delivered by the small animal radiation research platform (SARRP), a dedicated laboratory radiation device developed in-house. Mouse subject was anesthetized and placed on a robotically-controlled stage of the SARRP unit (inset). **(b)** Accuracy of this radiation targeting was assessed by iodine contrast enhanced CT-guided imaging, which allowed for visualization and targeting of third ventricle floor. **(c)** Accuracy was checked posthumously by immunostaining for γH2AX. The H2AX histone protein is phosphorylated after double-stranded DNA breaks, which occurs following irradiation and acts as a radiation dose readout. Targeting was specific to the ventrobasal hypothalamus, where the hypothalamic proliferative zone (arrow) resides. Representative samples shown along the anterior-posterior axis of the ventral mediobasal hypothalamus with Bregma to indicate position. Scale bar = 200μm. **(d-e)** Irradiated and sham treated HFD-fed mice were examined by quantitative magnetic resonance spectroscopy where % fat mass and % lean mass were measured. Irradiated mice had significantly less % fat mass and significantly more % lean mass than sham controls (n=5, * = $p<0.05$). Total mass: (Sham) 21.0±0.3g, (Irradiated) 18.86±0.4g; Lean mass: (Sham) 14.6±0.2g, (Irradiated) 13.9g±0.3g; Fat Mass: (Sham) 3.9±0.2g, (Irradiated) 2.6±0.3g (n=5, * = $p<0.05$). **(f-g)** Irradiated and sham treated adult mice were placed in a Comprehensive Lab Animal Monitoring System (CLAMS) for simultaneous measurements of food intake, physical activity, and whole-body metabolic profiling two weeks after treatment. **(f)** Following acclimation in the testing chamber, irradiated mice were observed to have significantly greater VO_2 (ml/kg/hr) compared to sham controls during the dark portion of the day (n=11,12; * = $p<0.05$). **(g)** No significant difference was observed in the respiratory exchange rate (RER) (n=11,12). ADDITIONAL CONTROLS: Irradiated mice appeared to be healthy as determined by luster of fur coat, activity, response to examiner, and hematological analysis. Whole blood was drawn (n=9) at 3 weeks post-treatment for CBC analysis, and revealed no significant difference between sham and irradiated groups. There was no statistically significant difference in food intake was observed between groups. Quantification of ArcN neurogenesis one month post-treatment did not reveal any statistically significant inhibition in neurogenesis following irradiation (n=4).

	Normal Chow ($Hu^+BrdU^+DAPI^+$)/(Hu^+DAPI^+)	High Fat Diet ($Hu^+BrdU^+DAPI^+$)/(Hu^+DAPI^+)
PD35X (P6-14 BrdU)	4.3%±0.2 101/2337 total neurons (n=4)	4.6%±0.9 101/2164 total neurons (n=3)
PD45X (P15-23 BrdU)	4.0%±0.9 105/2754 total neurons (n=4)	6.2%±0.4 173/2804 total neurons (n=4)
PD75X (P45-53 BrdU)	1.7%±0.4 38/2177 total neurons (n=4)	5.9%±1.6 114/1941 total neurons (n=4)

Supplementary Table 1. Age and diet-dependent effects on MEm neurogenesis.
Newborn Hu^+BrdU^+ MEm neurons quantified 1-month following BrdU administration under normal chow or HFD conditions (raw numbers). Diagram of experimental paradigm in **Fig. 2c**.

	Median Eminence (ME)	Arcuate Nucleus (ArcN)
Fraction of YFP^+ tanycytes ($Sox2^+YFP^+$)/$Sox2^+$ tanycytes	**0.18±.02** 1457/7949 total cells (n=3)	**0.67±0.03** 15354/23068 total cells (n=4)
Fraction of YFP^+ neurons (Hu^+YFP^+)/Hu^+ neurons	**0.08±0.01** 160/2097 total cells (n=4)	**0.007±0.0** 210/27,400 total cells (n=4)
Relative Neurogenic Index $\frac{\left[\frac{(Fraction\ of\ YFP\ ME\ neurons)}{(Fraction\ of\ labeled\ ME\ tanycytes)}\right]}{\left[\frac{(Fraction\ of\ YFP\ ArcN\ neurons)}{(Fraction\ of\ labeled\ ArcN\ tanycytes)}\right]}$	$\frac{\left[\frac{(0.08)}{(0.18)}\right]}{\left[\frac{(0.007)}{(0.67)}\right]} = 42.7$	**1**

Supplementary Table 2. ME tanycytes are more neurogenic than ArcN tanycytes.
PD35 Nestin:$CreER^{T2}$;R26YFP mice were used to prospectively label tanycytes, and examined to what degree β2 ME tanycytes were more neurogenic than other subsets of tanycytes. In order to examine this, at PD7 the number of $Sox2^+$ tanycytes labeled with YFP in the ME and ArcN following tamoxifen induction (PD4) was quantified. At PD35, the number of Hu^+ neurons labeled with YFP in the ME and ArcN was then quantified. A much smaller fraction of Hu^+/YFP^+ neurons in hypothalamic regions other than the ME was observed. When correcting for regional differences in the percentage of tanycytes labeled with the Nestin:$CreER^{T2}$;R26YFP mice, the ME showing a 43-fold greater level of relative neurogenic potential compared to the adjacent ArcN.