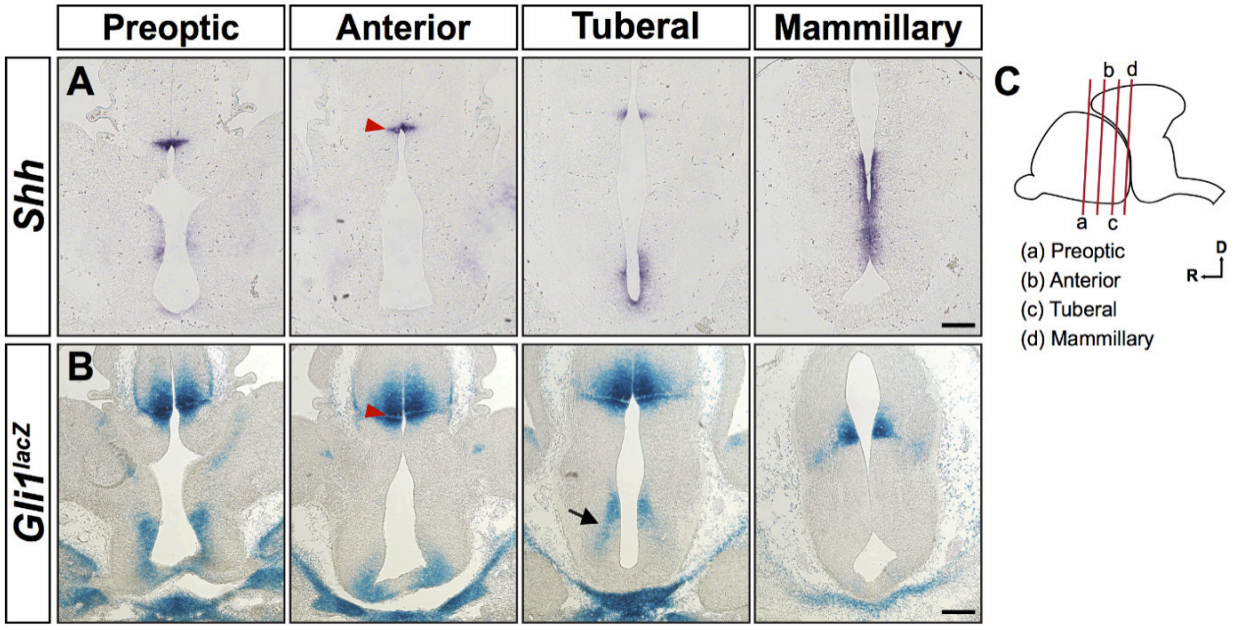

Figure S1. Persistence of *Shh* signaling during peak periods of hypothalamic neurogenesis. Coronal sections through the hypothalamus of (A) wild type and (B) *Gli1*$^{lacZ/+}$ embryos at E12.5. (A) RNA *in situ* hybridization detects *Shh* expression along the rostrocaudal axis of the hypothalamus. Weak *Shh* expression is detected in the ventral midline at preoptic and anterior hypothalamic regions. *Shh* expression is maintained in hypothalamic progenitors adjacent to the ventral midline in tuberal and mammillary regions. Shh is also observed in the zona limitans intrathalamica (red arrowhead) in the caudal diencephalon. (B) X-gal staining of *Gli1*^{lacZ} embryos along the rostrocaudal axis of the hypothalamus. X-gal staining is detected adjacent to Shh expressing domains throughout the hypothalamus. At the level of the tuberal hypothalamus, X-gal staining marks progenitors of the DMH and a population of cells that stream ventrally towards the VMH (arrows). Scale bars: 200 μm.

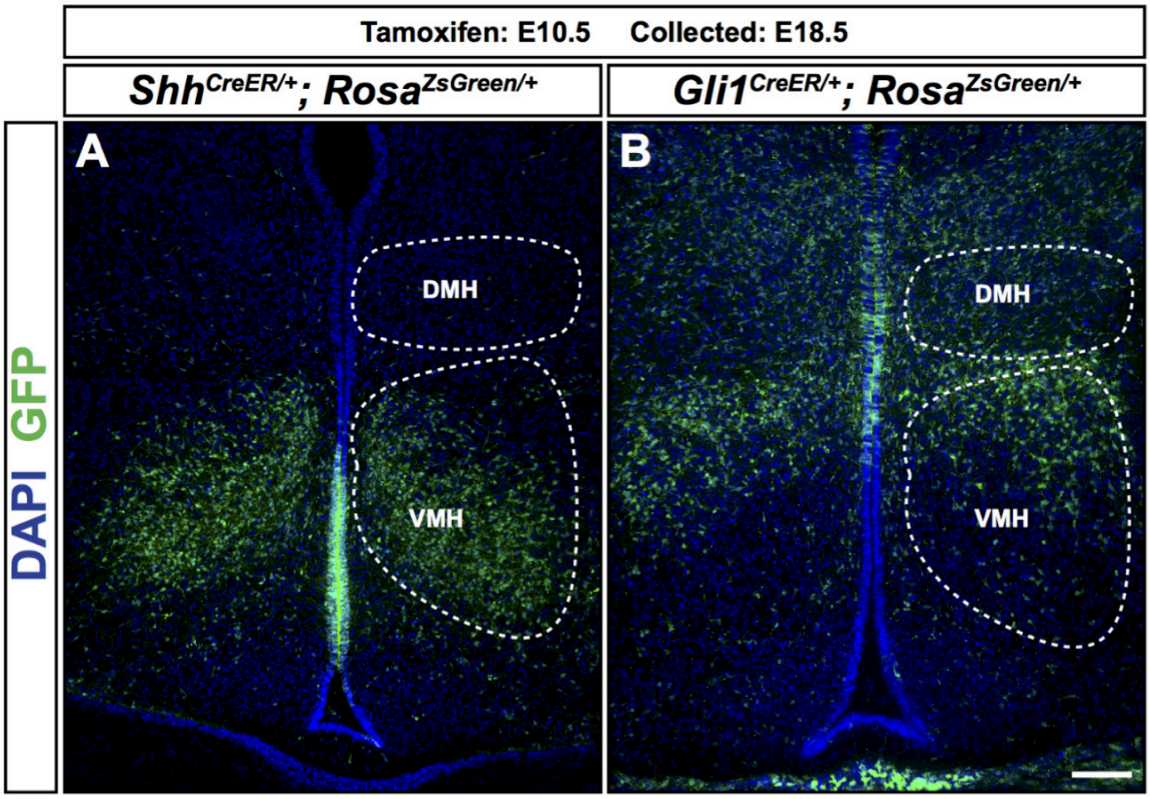

Figure S2. Shh and Gli1 lineages occupy distinct regions of the VMH. Coronal sections through the tuberal hypothalamus of (A) *Shh*$^{CreER/+}$; *Rosa*$^{ZsGreen/+}$ and (B) *Gli1*$^{CreER/+}$; *Rosa*$^{ZsGreen/+}$ embryos at E18.5 that received tamoxifen at E10.5. GFP (ZsGreen) fluorescence is shown on sections counterstained with DAPI. (A) Cells that expressed *Shh* at E10.5 contribute to the ventral and central regions of the VMH at E18.5. (B) Cells that expressed *Gli1* at E10.5 contribute to the DMH and dorsal region of the VMH at E18.5. Scale bar: 100 μm. White dashed lines outline tuberal hypothalamic nuclei.

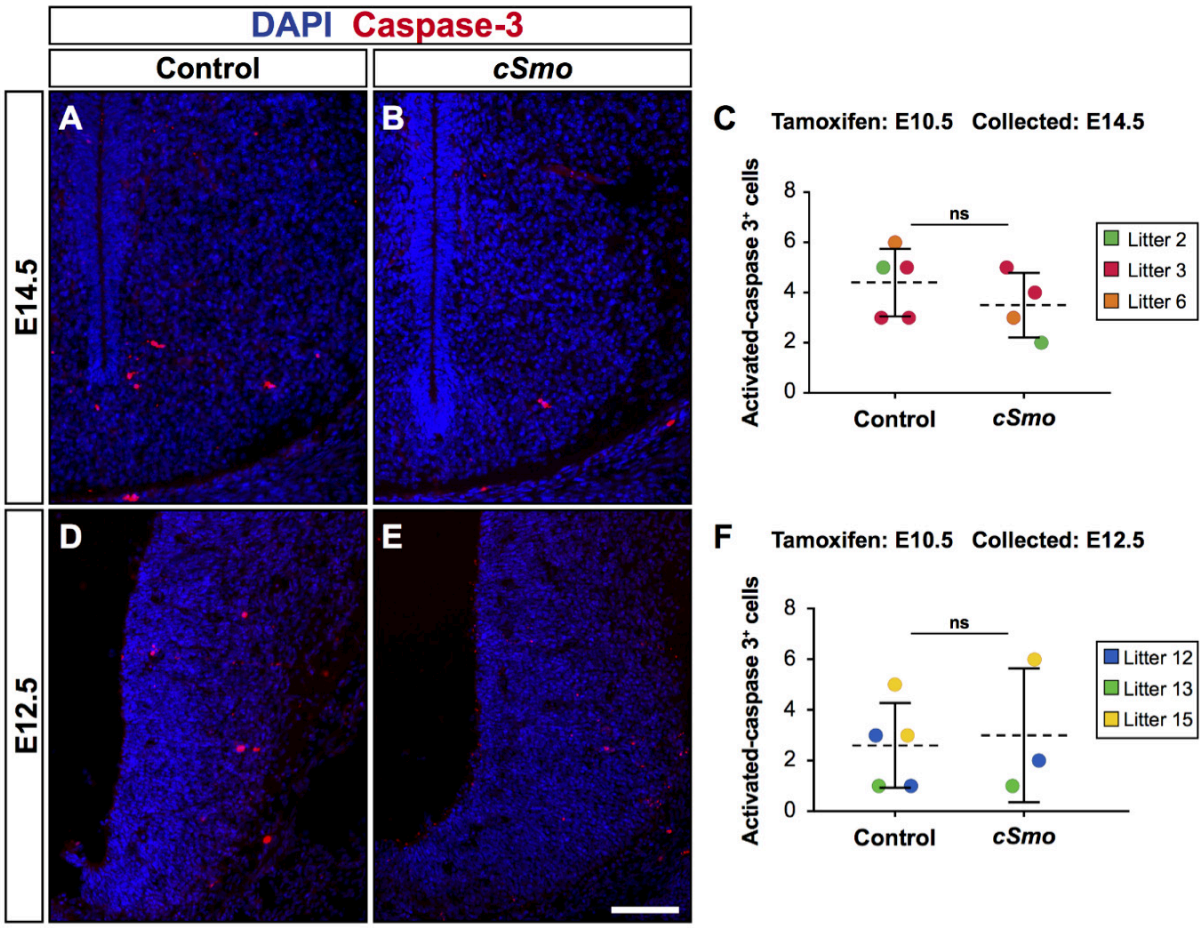

Figure S3. No difference in cell death between control and *cSmo* embryos. Coronal sections of control and *cSmo* embryos that received tamoxifen at E10.5 immunostained for activated caspase-3 and counterstained with DAPI. (A-C) No difference in apoptosis between control and *cSmo* embryos is observed at E14.5 (control n=5, 4.4 ± 1.3; *cSmo* n=4, 3.5 ± 1.3; p>0.05). (D-F) No difference in apoptosis between control and *cSmo* embryos is observed at E12.5 (control n=5, 2.6 ± 1.7; *cSmo* n=3, 3.0 ± 2.6; p>0.05). Each data point represents the number of cells expressing a given marker on a single section at equivalent levels of the tuberal hypothalamus from a single embryo color-coded by litter (n=number of embryos analyzed). Scale bars:100 µm. Statistical analysis was performed using a two-tailed unpaired *t*-test

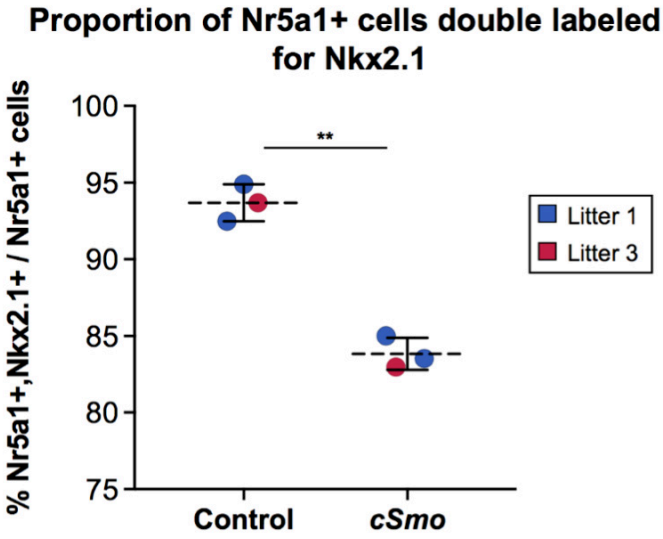

Figure S4. Fewer Nr5a1 expressing neurons co-label with Nkx2.1 in *cSmo* embryos. Quantification of Nr5a1 positive cells on a hemi-section double labeled for Nkx2.1 displayed as the proportion of Nr5a1, Nkx2.1 double positive cells over the total number of Nr5a1 positive cells (control n=3, 93.7% ± 0.7; *cSmo* n=3, 83.8% ± 0.6; **p=0.0004). Horizontal dotted line represents the mean and error bars indicate S.D. Statistical analysis was performed using a two-tailed unpaired *t*-test on arcsin-transformed data.

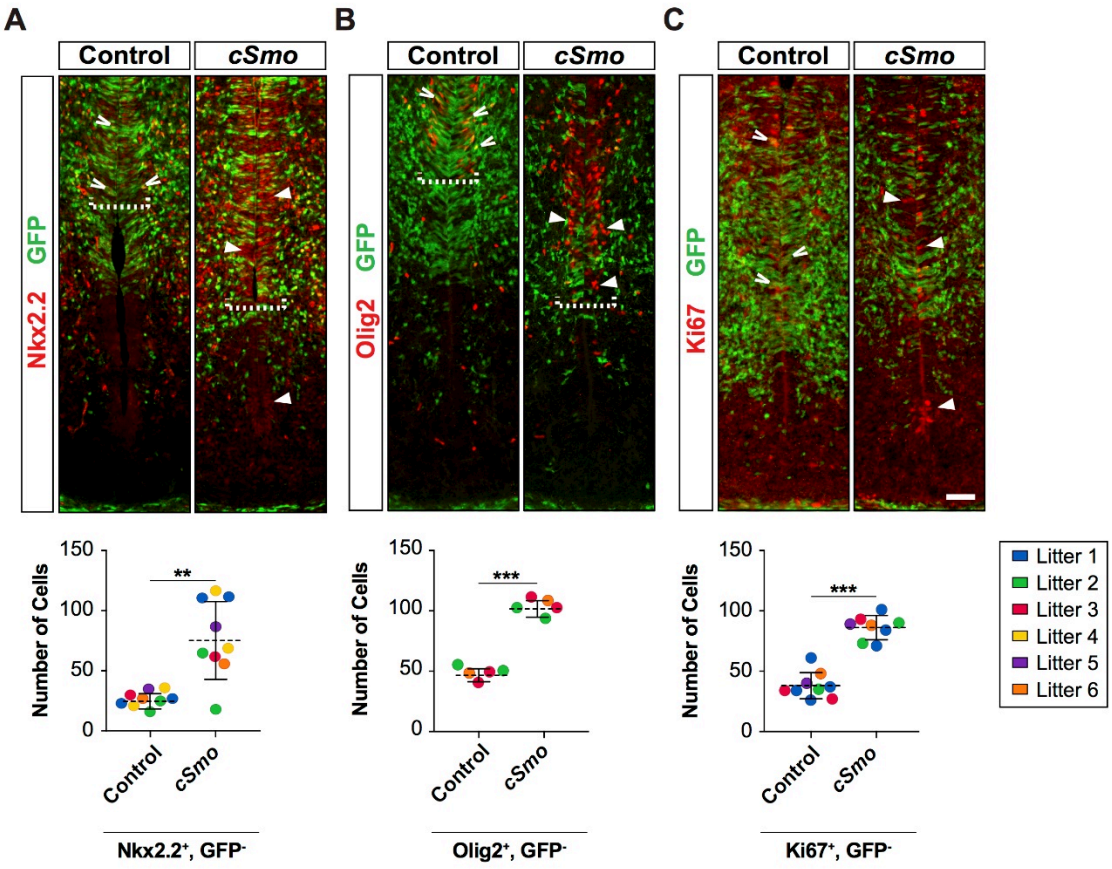

Figure S5. *cSmo* embryos show ectopic activation of Shh responsive genes in non-recombined (wild type) cells. Coronal sections through the tuberal hypothalamus of control and *cSmo* embryos at E14.5 (tamoxifen administered at E10.5). Shh responsive progenitors in control embryos frequently co-label with GFP and (A) Nkx2.2, (B) Olig2, or (C) Ki67 (open arrowhead). Most progenitors in *cSmo* embryos showing ectopic expression of Nkx2.2, Olig2, or Ki67 do not co-label with GFP (closed arrowheads), suggesting that they derive from non-recombined (wild type) cells. The ventral boundary of Nkx2.2 and Olig2 expression (white dotted bracket) is shifted ventrally in *cSmo* embryos. The same images are shown in Figure 6 without the GFP overlay. Scale bar: 50 μm.