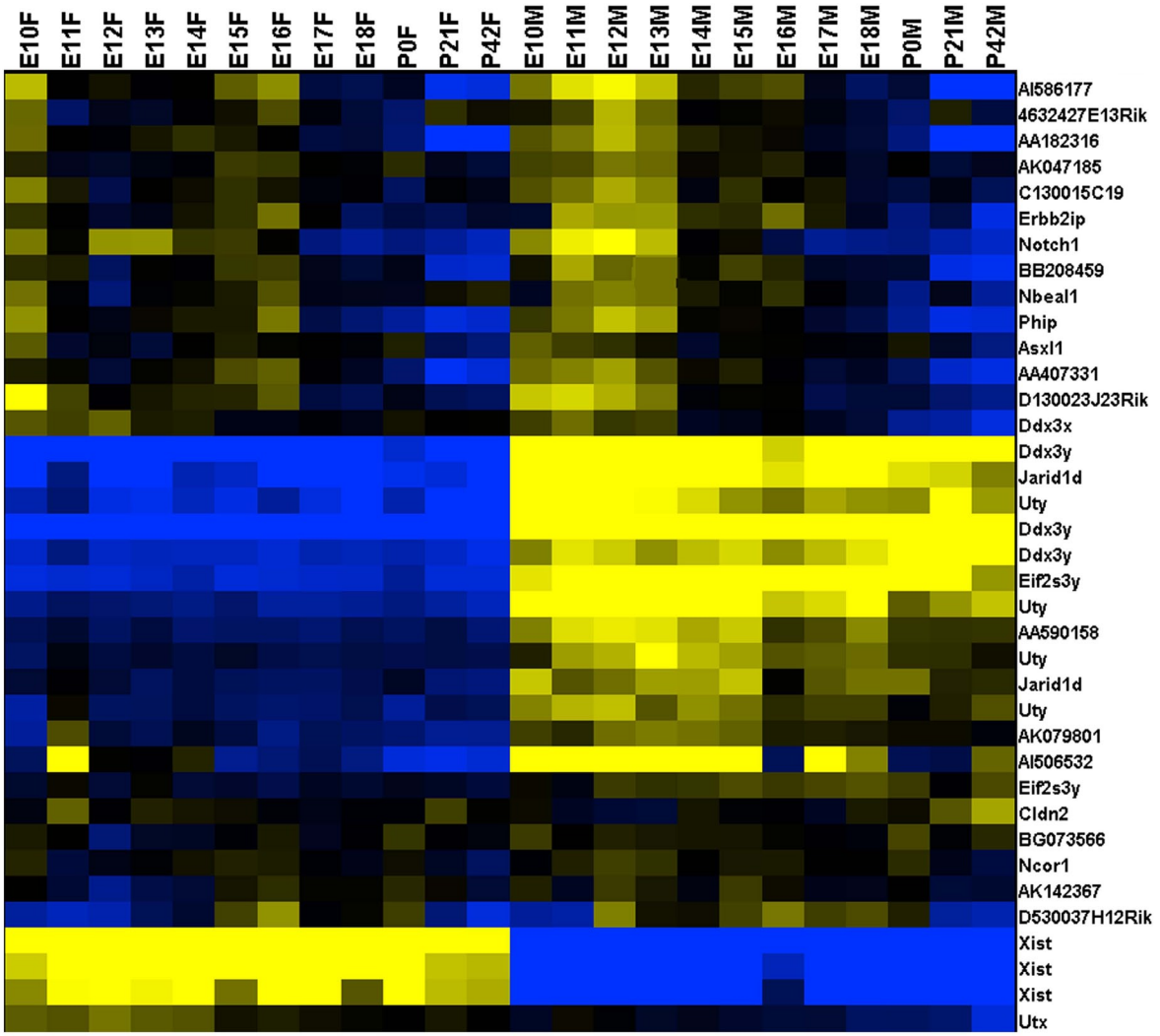
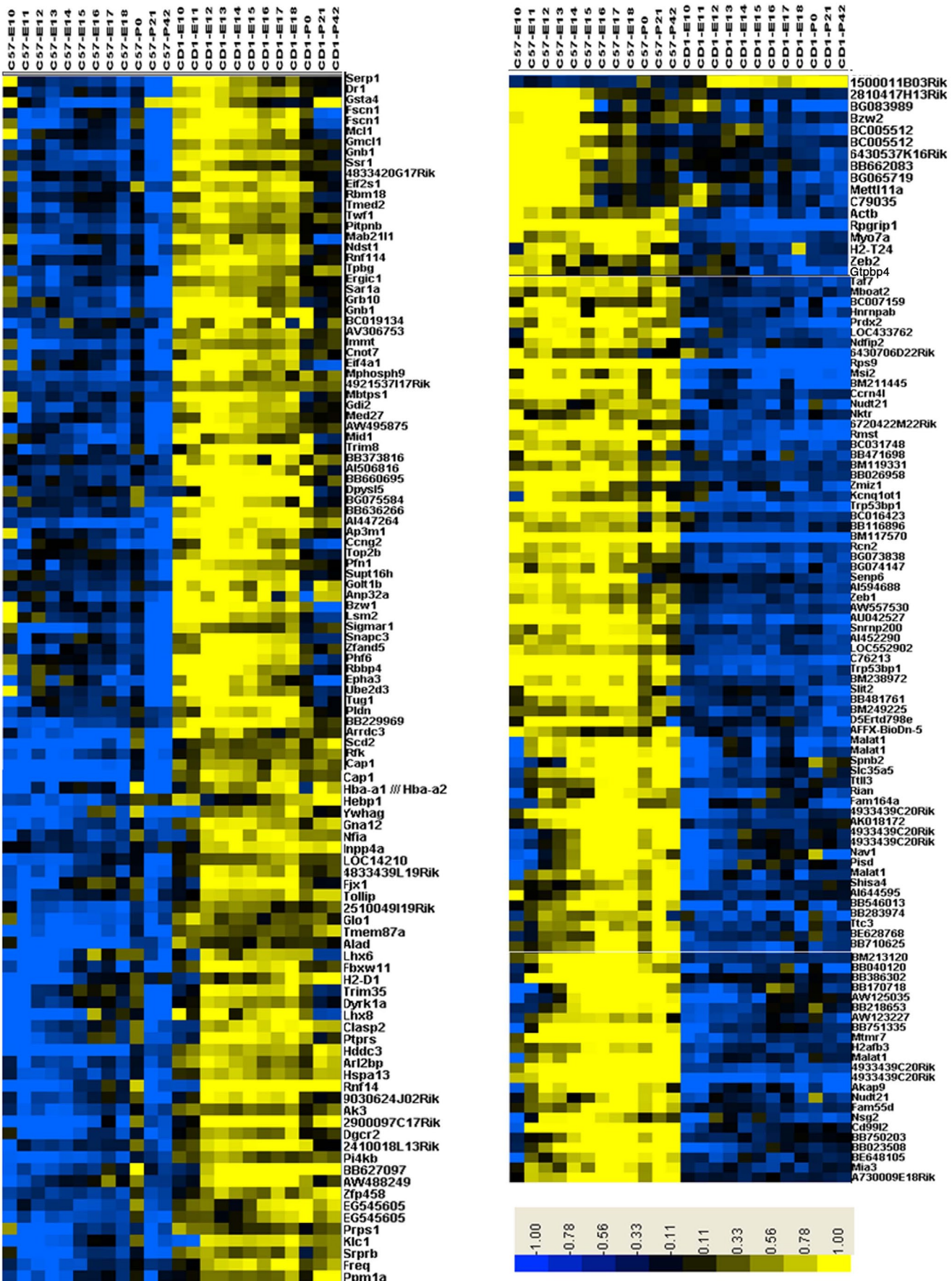


Supplementary Figure 1: SOM-based cluster analysis of the 37 probesets that showed significantly different expression between male and female mice is shown. Signal intensity from mice of C57BL/6 and CD-1 strain backgrounds are averaged for this analysis. For this analysis, three consecutive timepoints (e.g. E10.5, E11.5, E12.5) are considered for each sex when calculating statistical significance. The indicated signal intensity shows the difference between the median $\log_2$ scaled signal intensity at the indicated timepoint for either male or female samples and the median intensity observed for that probeset in all samples regardless of genotype across all timepoints tested. Chromosomal localization of differentially expressed transcripts is shown. A=autosomal exonic transcript. A*=autosomal intronic transcript.

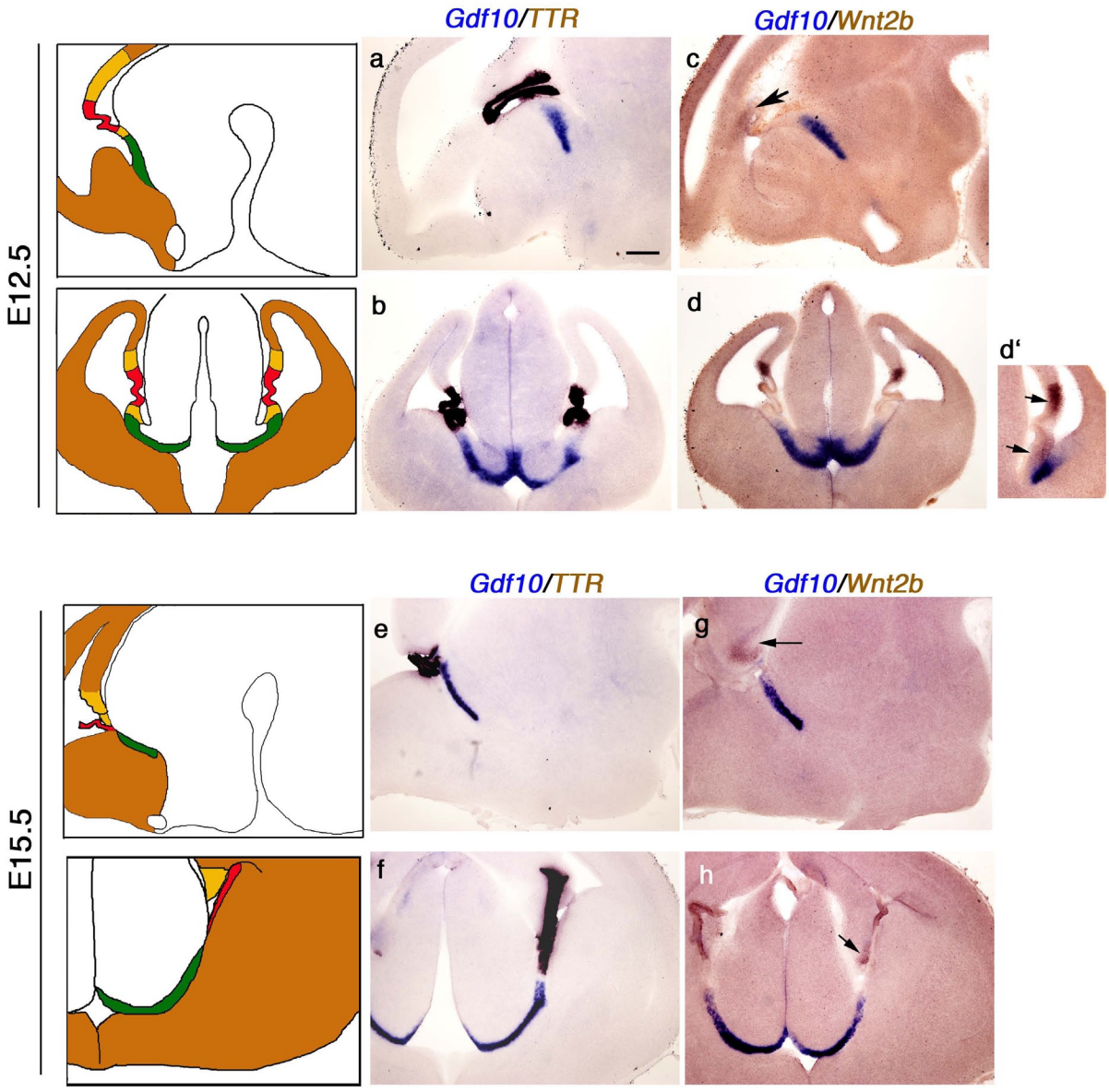


Shimogori et al., supplemental FIG 2

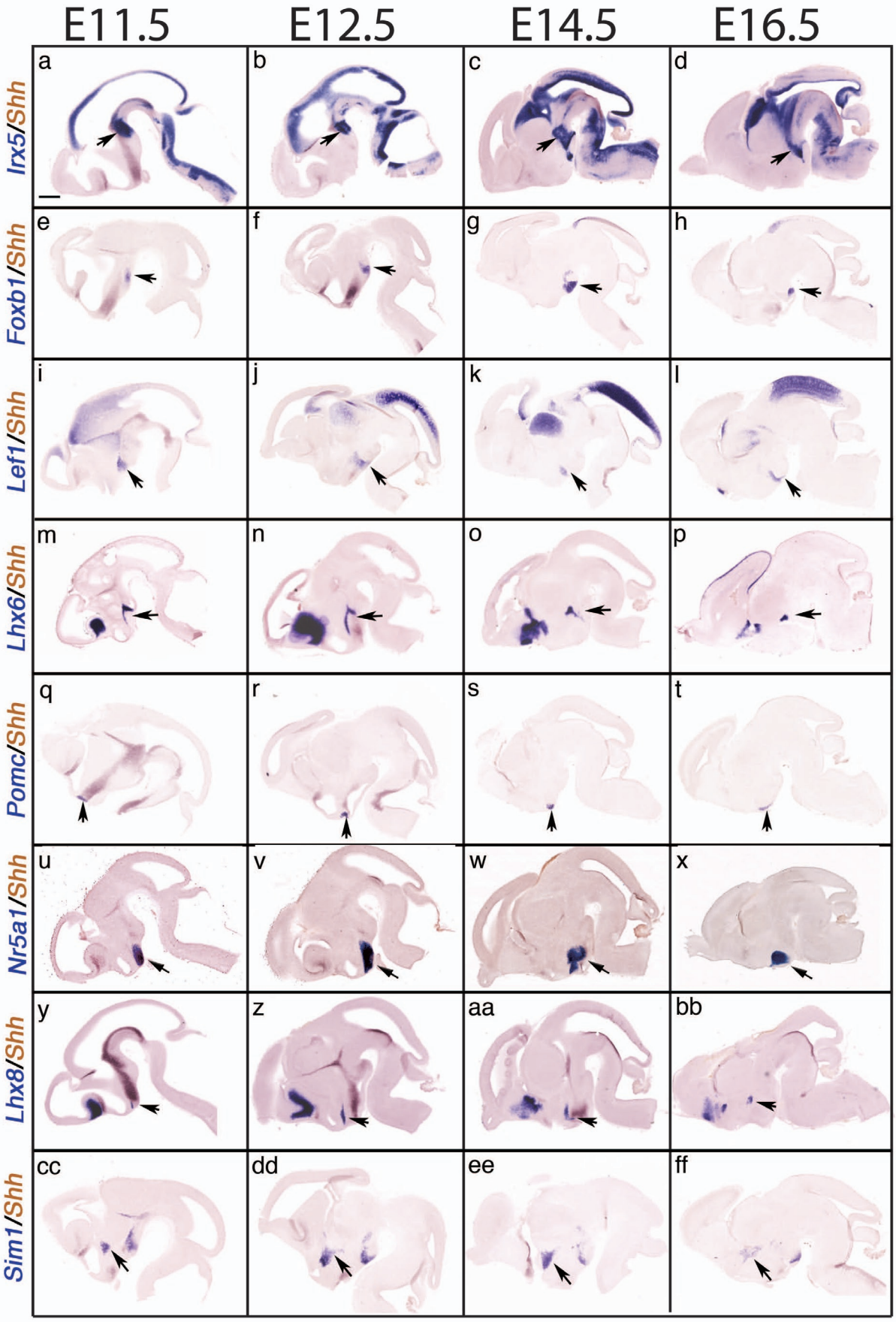
Nature Neuroscience: doi:10.1038/nn.2545

Supplementary Figure 2: SOM-based cluster analysis of the 222 probesets that showed significantly different expression between mice of C57BL/6 and CD-1 strain backgrounds is shown. Signal intensities from male and female samples are averaged for this analysis. For this analysis, three consecutive time-points are considered for each strain when calculating statistical significance. The indicated signal intensity shows the difference between the median $\log_2$ scaled signal intensity at the indicated time-point for C57BL/6 or CD-1 samples and the median intensity observed for that probeset in all samples regardless of genotype across all time-points tested.

Nature Neuroscience: doi:10.1038/nn.2545



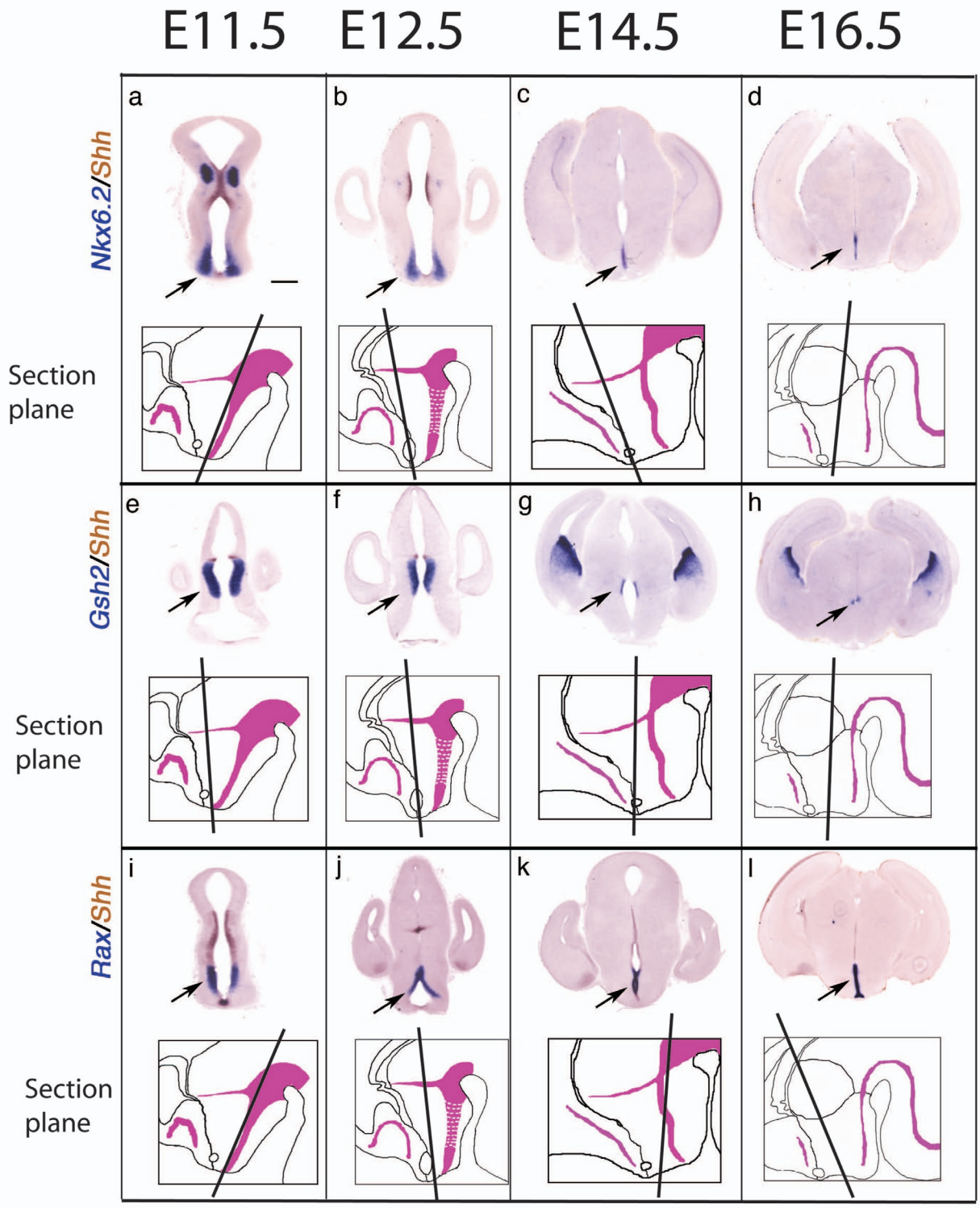
Supplementary Figure 3: *Gdf10* expression does not overlap with the cortical hem markers TTR and Wnt2b. (**a,b**) Sagittal and coronal views of two-color ISH for *Gdf10* (blue) and *TTR* (brown) at E12.5. (**c,d**). Sagittal and coronal views of two-color ISH for *Gdf10* (blue) and *Wnt2b* (brown) at E12.5. Zones of *Wnt2b* expression are indicated with black arrows. **(d')** Higher power view of coronal section of of two-color ISH for *Gdf10* (blue) and *Wnt2b* (brown) at E12.5. Zones of *Wnt2b* expression are indicated with black arrows. **(e,f)** Sagittal and coronal views of two-color ISH for *Gdf10* (blue) and *TTR* (brown) at E15.5. **(g,h)** Sagittal and coronal views of two-color ISH for *Gdf10* (blue) and *Wnt2b* (brown) at E15.5. Zones of *Wnt2b* expression are indicated with black arrows. Scale bar = 0.2 mm.



Shimogori et al., supplemental FIG 4

Nature Neuroscience: doi:10.1038/nn.2545

Supplementary Figure 4: Expression of region-specific markers that are expressed in postmitotic cells is maintained over the course of hypothalamic neurogenesis. Arrows indicate hypothalamic or prethalamic expression of the gene in question. *Shh* expression (brown) is used to confirm location of domains of gene expression at early stages of neurogenesis. **(a-d)** Specific expression of *Irx5* is observed in the SMM region from E11.5 to E16.5. **(e-h)** Specific expression of *Foxb1* is observed in the MM region. **(i-l)** Specific expression of *Lef1* is observed in PMM hypothalamus. **(m-p)**. *Lhx6* is persistently and selectively expressed in posterior DI and TT. **(q-t)** *Pomc* is stably and selectively expressed in arcuate nucleus. **(u-x)** *Nr5a1* is expressed in VMH. **(y-bb)** *Lhx8* is expressed in anterior DI from E11.5 to E14.5 and in dorsomedial hypothalamic nucleus (DMH) from E16.5. **(cc-ff)** *Sim1* is expressed in paraventricular hypothalamic neuroepithelium from E11.5 to E16.5. Scale bar in a=0.45 mm (**a,e,i,m,q,u,y,cc**); 0.5 mm (**b,f,j,n,r,v,z,dd**); 0.55 mm (**c,g,k,o,s,w,aa,ee**); and 0.6 mm (**d,h,l,p,t,x,bb,ff**).

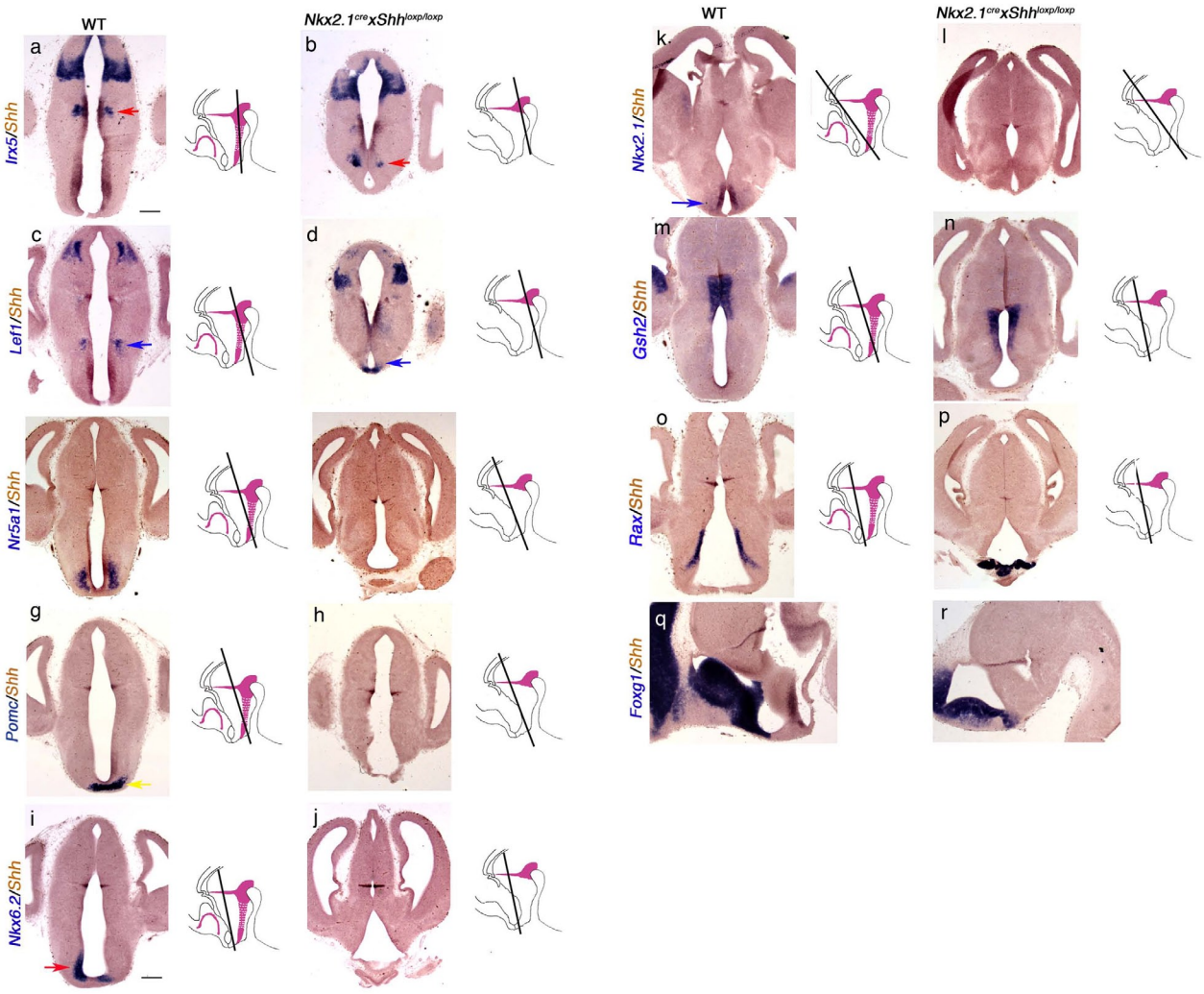


Shimogori et al., supplemental FIG 5

Nature Neuroscience: doi:10.1038/nn.2545

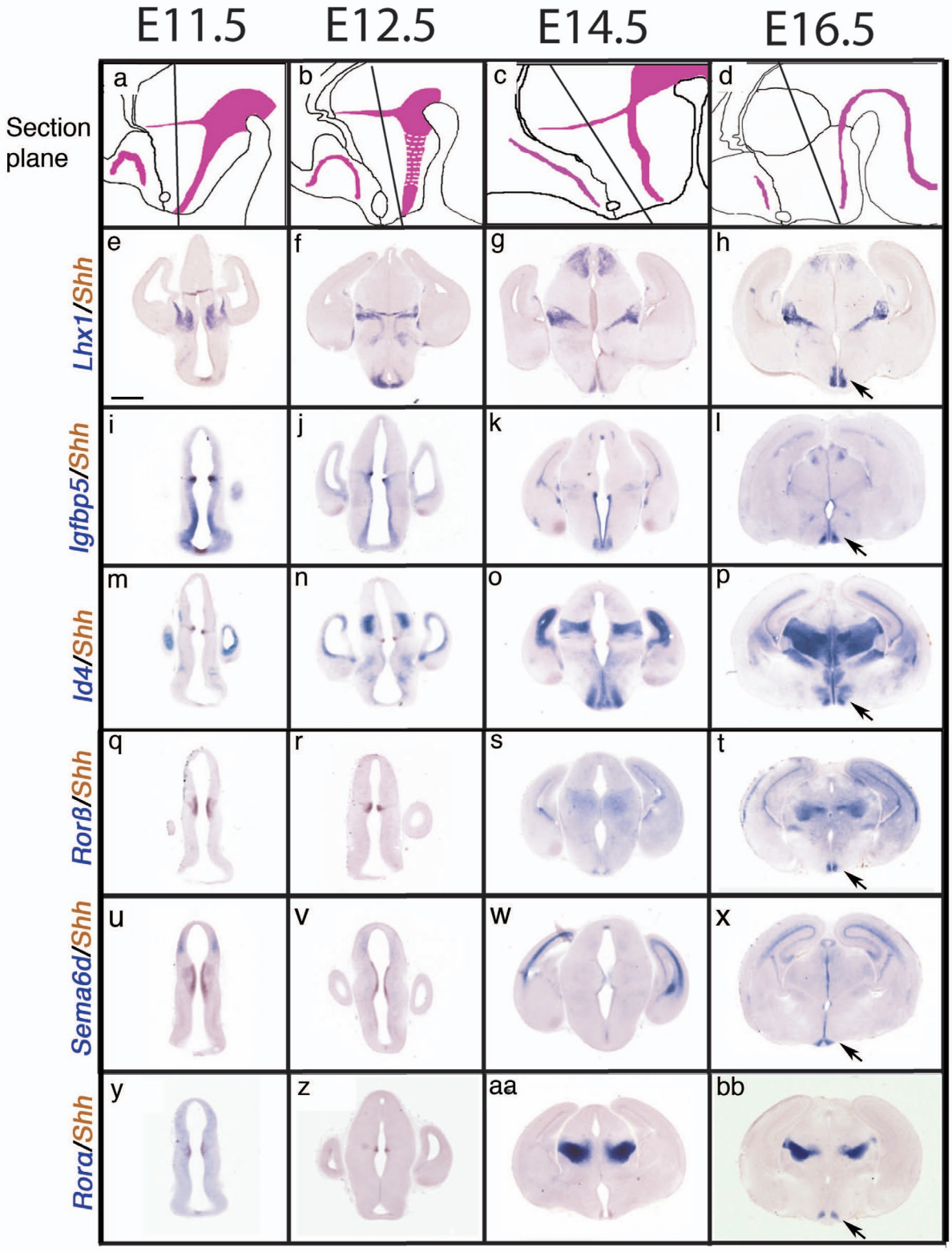
Supplementary Figure 5: Expression of region-specific markers that are expressed in mitotic progenitor cells is maintained over the course of hypothalamic neurogenesis. For each section at each developmental stage, a schematic drawing indicating both the plane of section (black) and the region of *Shh* expression (pink) is included. Arrows indicate hypothalamic or prethalamic expression of the gene in question. (**a-d**) Specific expression of *Nkx6.2* is observed in anterioventral hypothalamus from E11.5 to E16.5. (**e-h**) Specific expression of *Gsh2* is observed in prethalamic neuroepithelium. (**i-l**). Specific expression of *Rax* is observed in hypothalamic neuroepithelium. Scale bar in a= 0.35 mm (**a,e,i**); 0.4 mm (**b,f,j**); 0.45 mm (**c,g,k**); 0.5 mm (**d,h.l**).

Nature Neuroscience: doi:10.1038/nn.2545



Shimogori et al., Supplemental FIG 6

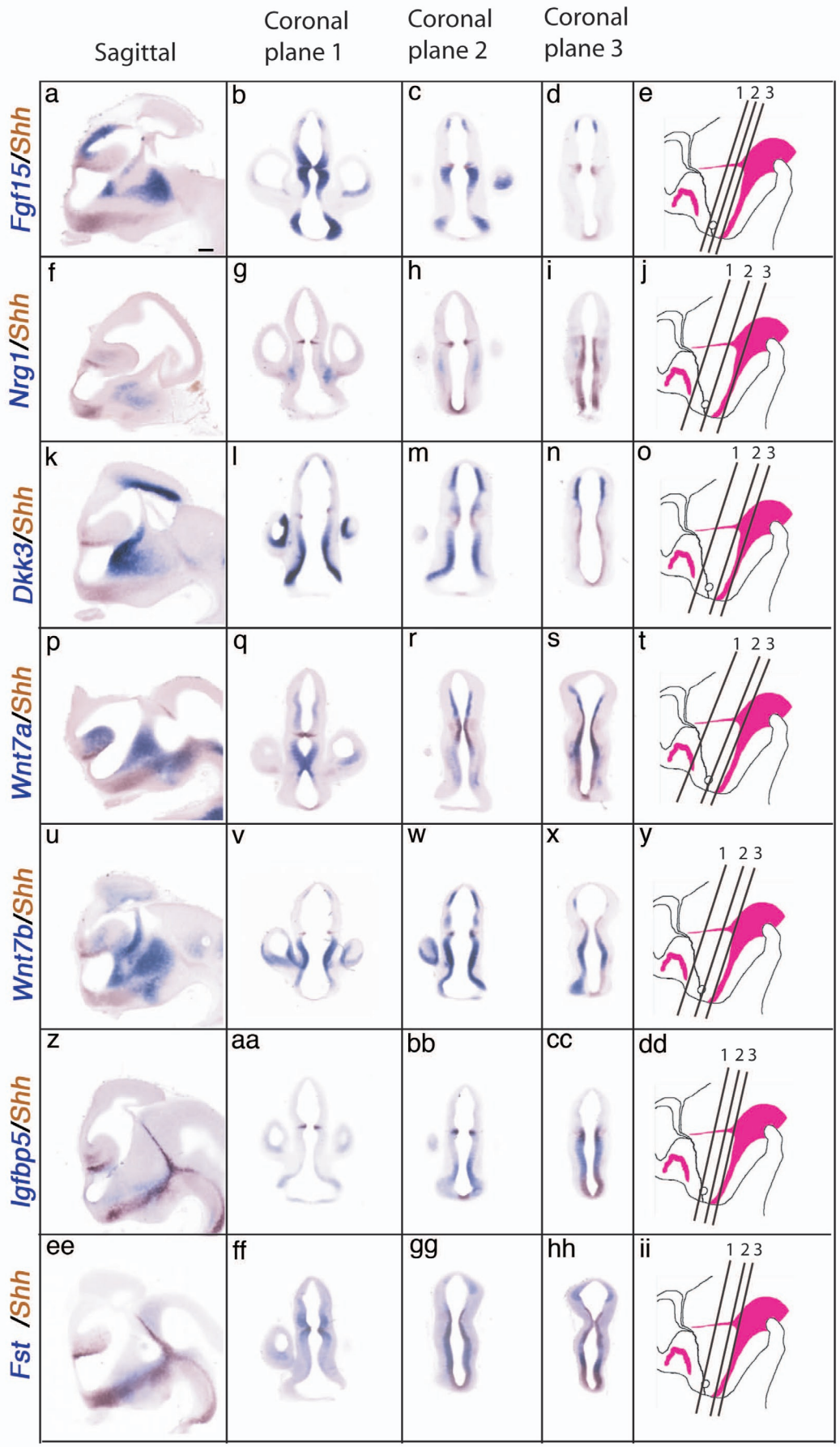
Supplementary Figure 6: Analysis of additional region-specific marker genes in *Nkx2.1-Cre x Shh* $^{lox/lox}$ mice at E12.5. Coronal sections are shown from a-p, and sagittal sections from q-v. For coronal sections of both wild type and mutant brains, a schematic drawing indicating both the plane of section (black) and the region of *Shh* expression (pink) is included. (**a,b**). *Irx5* expression in SMM hypothalamus (red arrow) is preserved in *Nkx2.1-Cre x Shh* $^{lox/lox}$ animals. (**c,d**) PMM expression of *Lef1* is preserved in *Nkx2.1-Cre x Shh* $^{lox/lox}$ mice but restricted to the ventral midline (blue arrow). (**e,f**) The Expression of the VMH-specific marker *Nr5a1* (green arrow) is absent from mutant mice. (**g,h**) Arcuate-specific *Pomc* expression (yellow arrow) is also absent from mutant animals. (**i,j**) Anterioventral hypothalamus marker *Nkx6.2* (red arrow) is absent from mutant animals. (**k,l**) *Nkx2.1* expression (blue arrow) is not detected in the basal hypothalamus of mutant animals. (**m,n**) *Gsh2* expression is preserved in prethalamic neuroepithelium of mutant animals. (**o,p**) *Rax* expression is preserved in ventral hypothalamic neuroepithelium of mutant animals. (**q,r**) *Foxg1* expression is not expanded into diencephalon of mutant animals. Scale bar in a= 0.25 mm.



Shimogori et al., supplemental FIG 7

Nature Neuroscience: doi:10.1038/nn.2545

Supplementary Figure 7: Identification of genes showing stage-specific onset of expression in developing suprachiasmatic nucleus (SCN). *Shh* (brown) is included as a second color for each ISH sample to allow reliable assignment of section plane. Expression of each test transcript (blue) in SCN is indicated by a black arrow. (**a-d**) A schematic drawing indicating both the plane of section (black) and the region of *Shh* expression (pink) is shown for each developmental stage shown. (**e-h**) *Lhx1* expression is readily detected in developing SCN by E12.5. (**i-l**) *Igfbp5* expression is detected in postmitotic cells of developing SCN at E14.5. (**m-p**) *Id4* expression is also detected in postmitotic cells of developing SCN at E14.5, as is *Rorβ* (**q-t**). (**u-x**) *Sema6d* expression is detected in the medial portion of SCN at E16.5. (**y-bb**) *Rorα* is broadly and selectively expressed in SCN beginning at E16.5. Scale bar in e= 0.45 mm (**a,e,i,m,q,u,y**); 0.5 mm (**b,f,j,n,r,v,z**); 0.55 mm (**c,g,k,o,s,w,aa**); and 0.6 mm (**d,h,l,p,t,x,bb**).

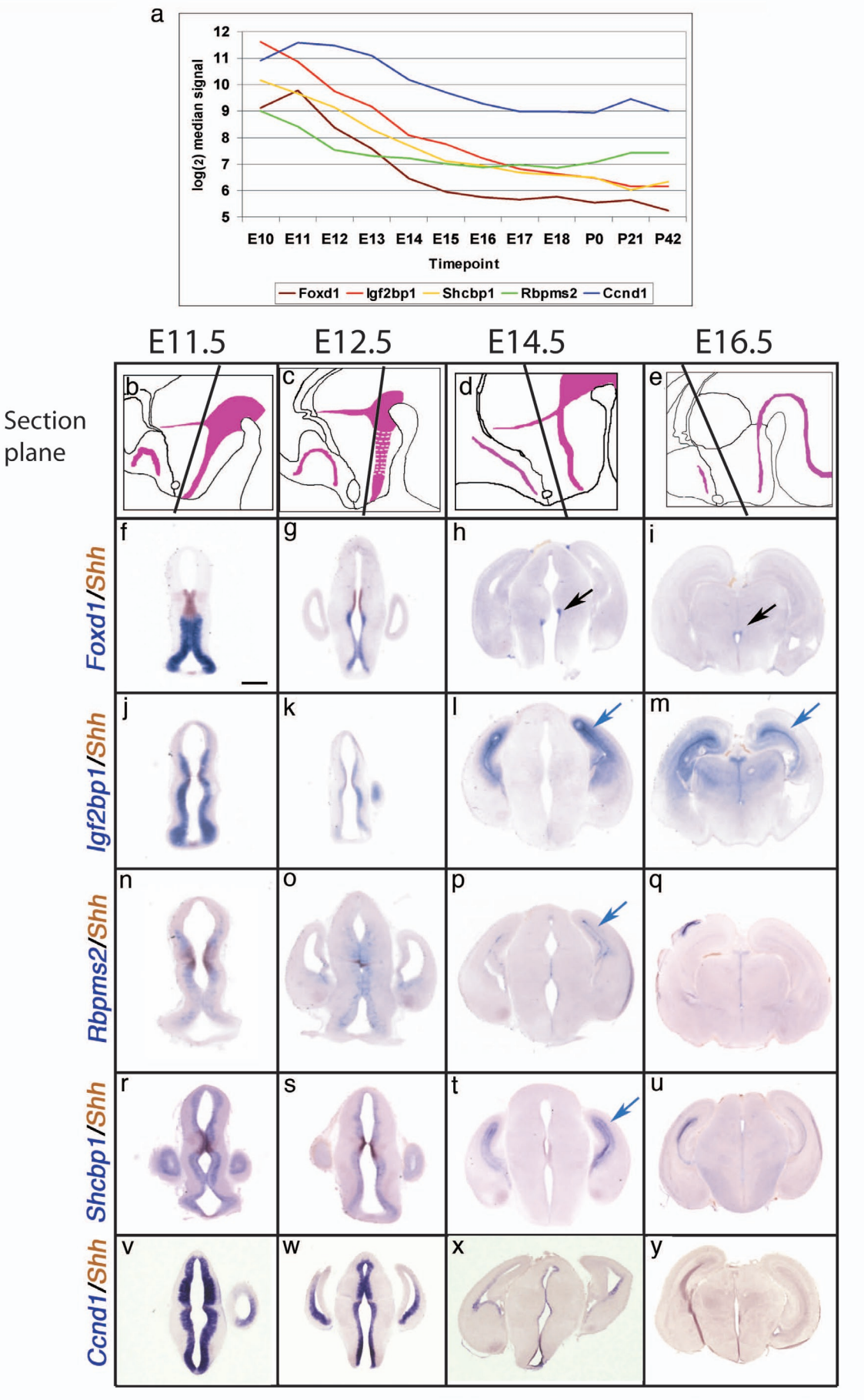


Shimogori et al., supplemental FIG 8

Nature Neuroscience: doi:10.1038/nn.2545

Supplementary Figure 8: Growth and differentiation factors are expressed in discrete domains along the dorsoventral axis of prethalamus and hypothalamus. Sagittal and coronal planes of section are shown, with the position of each coronal plane relative to the domain of *Shh* expression indicated in a schematic drawing. (**a-e**). *Fgf15* expression is found in a domain dorsal and anterior to the basal domain of Shh expression. (**f-j**). *Nrg1* expression delineates a domain in dorsomedial prethalamus and dorsal hypothalamus, with expression extending to the basal zone of *Shh* expression (arrow). (**k-o**) *Dkk3* expression is found in a zone similar of Fgf15 but extending more ventrally and stopping just short of the basal *Shh* zone. (**p-t**) Wnt7b expression encompasses the entire prethalamus and the *Sim1*-positive zone of anteriodorsal hypothalamus, with expression overlapping the *Shh*-positive basal plate and extending ventral along the TT (black arrow). (**u-y**) *Wnt7b* is expressed in a domain that is very similar to that of Wnt7a. (**z-dd**) *Igfbp5* is expressed in a region immediately dorsal to the zone of basal *Shh* expression. (**ee-ii**) *Fst1* expression overlaps with and extends slightly dorsal to the basal zone of Shh expression. Scale bar in a = 0.2mm.

Nature Neuroscience: doi:10.1038/nn.2545

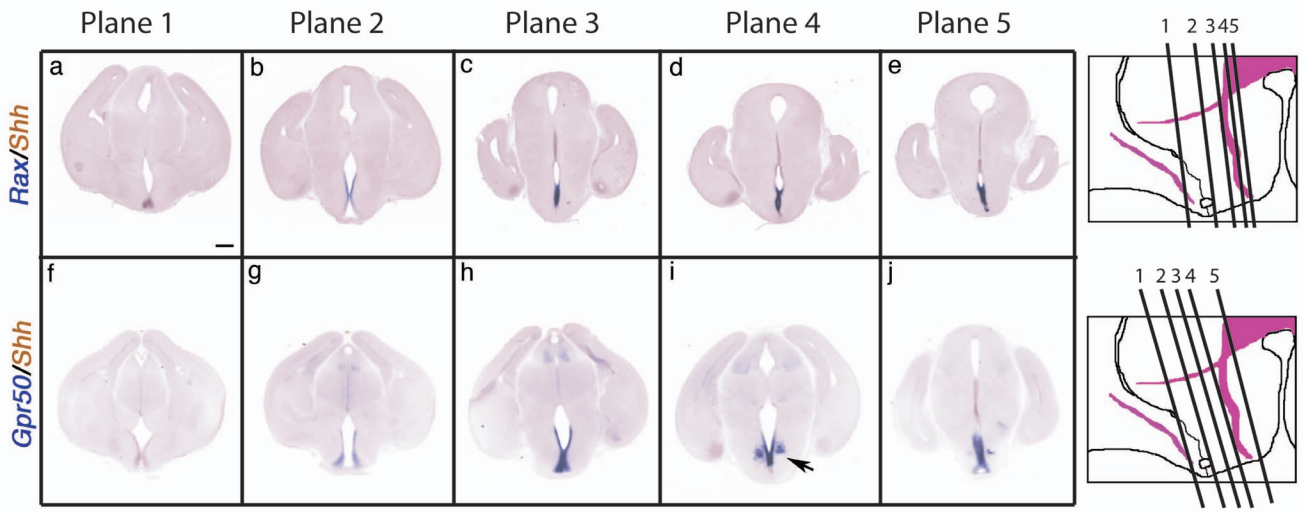


Shimogori et al., supplemental FIG 9

Nature Neuroscience: doi:10.1038/nn.2545

Supplementary Figure 9: Identification of transcripts selectively expressed in early-stage hypothalamic progenitor cells. (**a**) Median signal intensity for a subset of hypothalamic-expressed genes is plotted on $\log_2$ scale. A substantially more rapid decrease in the expression levels of these transcripts over time is observed than is observed for the panprogenitor marker *cyclin D1* (*Ccnd1*). (**b-e**) A schematic drawing indicating both the plane of section (black) and the region of *Shh* expression (pink) is shown for each developmental stage shown. (**f-i**). *Foxd1* is strongly expressed in anterior diencephalic progenitors at E11.5. Expression in notably weaker at E12.5, and is confined to a limited zone in prethalamus at E14.5 and E16.5 (black arrow). (**j-m**) Early stage-specific expression of *Igf2bp1* is observed in progenitors throughout the forebrain. Expression of *Igf2bp1* is detected in prethalamus and hypothalamus at E11.5 and E12.5, but is not detected at later stages. Expression is detected, however, in progenitor cells of the cerebral cortex at E14.5 (blue arrow), where neurogenesis occurs over a longer interval than in the diencephalon. (**n-q**). *Rbpms2* expression shows a similar temporal profile to *Igf2bp1* in anterior diencephalon, but shows stronger expression in a scattered subset of progenitor cells. Blue arrow indicates expression in progenitors of the cerebral cortex after diencephalic expression is no longer detectable. (**r-u**) *Shcbp1* shows a similar temporal expression pattern, but shows weaker expression in M-phase progenitors along the ventricular surface. Blue arrow indicates cortical expression. (**v-y**) *Ccnd1* expression serves as a general marker for mitotic progenitors in the prethalamus and hypothalamus.
Scale bar in f = 0.35 mm (**f,j,n,r,v**); 0.4 mm (**g,k,o,s,w**); 0.45 mm (**h,l,p,t,x**); 0.5 mm (**i,m,q,u,y**).

Nature Neuroscience: doi:10.1038/nn.2545



Supplementary Figure 10: *Rax* and *Gpr50* are broadly and selectively expressed in hypothalamic progenitors at E14.5. (**a-e**) A series of coronal sections indicate that *Rax* is selectively expressed in hypothalamic progenitors at E14.5. (**f-j**) *Gpr50* shows a similar pattern of selective expression in hypothalamic progenitors at E14.5. Expression of *Gpr50* is also detected in a subset of postmitotic cells of the ID at this stage (black arrow), and is detected in neurons of the dorsomedial hypothalamus (DMH) in the postnatal hypothalamus.
Scale bar in a= 0.2mm.

All two-color ISH data using candidate genes and Shh, along with all Supplementary Tables in Excel format can be accessed at: blackshaw.bs.jhmi.edu and at and in the Mouse Gene Expression Database at Jackson Labs (http://www.informatics.jax.org/expression.shtml).

Supplementary Tables:

Supplementary Table 1: Normalized $\log_{(2)}$ signal intensity is shown for all probesets and all microarray samples examined in this study. Microarray samples are identified using the following nomenclature: Strain (C57BLl/6 or CD-1)_Developmental stage (gestational age rounded down to the nearest full day)_Sex (male or female).

Supplementary Table 2: Median $\log_{(2)}$ signal intensity is shown for all 25,471 probesets that showed significantly different expression ($p<0.01$ via one-way ANOVA analysis) at one or more timepoints when compared to median intensity for that probeset across all timepoints analyzed. Probe signal intensities represent the normalized median values of all four biological replicates sampled for that timepoint.

Supplementary Table 3: Summary of all first-pass single color ISH data. The gene symbol corresponding to the transcript tested is shown, along with the accession numbers for all 1,166 ISH probes examined. An assessment of the quality of the probe signal-to-noise level (graded from poor to excellent). Whether or not the transcript in question shows prominent and/or selective expression in hypothalamus is indicated, along with whether or not a previously published report describing the hypothalamic expression pattern of the transcript in question exists. In addition, if the probe in question was tested using two-color ISH in conjunction with Shh, this fact is indicated, whether or not the two-color ISH was successful. The predominant cell type that expresses that transcript is indicated using a numerical code as follows:
0=no signal/ubiquitous.
1= mature neurons
2=mature glia
3=mitotic progenitors
4=postmitotic immature neuronal precursor cells
5=mitotic progenitors and precursors cells
6=nonneuronal cells

The overall cellular expression pattern of the transcript in question is also indicated as follows:
0=no expression/contiguous expression
1=nuclear expression (where a particular region shows clearly stronger expression than surrounding areas).
2=scattered subset of cells
3=nuclear and scattered subset of cells

Finally, it is indicated whether expression is detected over the course of hypothalamic neurogenesis in any of the following regions of the hypothalamus: anterodorsal (AD), anteroventral (AV), tubero (or medio)dorsal (TD), tuberoventral (TV), posterodorsal (PD), posteroventral (PV) or lateral (LH) hypothalamus.

Supplementary Table 4: Summary of all two-color ISH data. The expression pattern of all probes that demonstrated a particularly selective and/or dynamic expression in developing

hypothalamus in the single color ISH experiments detailed in Table ST3 were examined together, with Shh used as a second color. Only probes that showed clear signals are listed in here. The gene symbol of the transcript examined, along with the accession number of the probe, and whether a coronal or sagittal series of sections were analyzed. Expression levels for each of the probes are scored on a 0-5 point scale in a range of structures throughout the forebrain at E11.5, E12.5, E14.5 and E16.5. "ND" indicates section was absent or unscored. If gene expression shows a "nuclear" or "scattered" expression pattern within that particular structure, as defined above for Table ST3, this is indicated by either an "n", "s" or "ns" after the expression intensity. Abbreviations used for E11-16 are as follows, along with the criteria used to identify specific anatomical regions:

Ctx/VZ	Cerebral cortex/mitotic progenitors (ventricular zone)
Ctx/MZ	Cerebral cortex/postmitotic neurons (intermediate zone and cortical plate)
GE/VZ	Ganglionic eminences/mitotic progenitors (ventricular zone)
GE/MZ	Ganglionic eminences /postmitotic precursors (mantle zone)
Dorsal thal-VZ	Dorsal thalamus/mitotic progenitors (ventricular zone)
Dorsal thal -MZ	Dorsal thalamus/postmitotic precursors (mantle zone)
Prethal-VZ	Prethalamus/mitotic progenitors (ventricular zone)
Prethal -MZ	Prethalamus /postmitotic precursors (mantle zone)
POA-D-VZ	Dorsal POA (Foxg1/Sim1+ zone) (ventricular zone)
POA-D-MZ	Dorsal POA (Foxg1/Sim1+ zone) (mantle zone)
POA-D-VZ	Ventral POA (Foxg1/Six3+ zone) (ventricular zone)
POA-D-MZ	Ventral POA (Foxg1/Six3+ zone) (mantle zone)
AVH-V-VZ	Anteroventral hypothalamus/Nkx6.2+ zone (ventricular zone)
AVH-V-VZ	Anteroventral hypothalamus/Nkx6.2+ zone (mantle zone)
PvN-VZ	Anterior hypothalamic Sim1+ zone (ventricular zone)
PvN –MZ	Anterior hypothalamic Sim1+ zone (mantle zone)
ID-Ant-VZ	Anterior Intrahypothalamic Diagonal Dlx2+/Lhx1+ D-V stripe (ventricular zone)
ID-Ant-MZ	Anterior Intrahypothalamic Diagonal Dlx2+/Lhx1+ D-V stripe (mantle zone)
ID-Post-VZ	Posterior Intrahypothalamic Diagonal Dlx2+/Lhx6+ D-V stripe (ventricular zone)
ID-Post-MZ	Posterior Intrahypothalamic Diagonal Dlx2+/Lhx6+ D-V stripe (mantle zone)
Premam-VZ	Premammilary Lef1+ zone (ventricular zone)
Premam -MZ	Premammilary Lef1+ zone (mantle zone)
TT-VZ	Lhx6+ Tuberomamillary terminal (ventricular zone)
TT-MZ	Lhx6+ Tuberomamillary terminal (mantle zone)
Premam-VZ	Premamillary Lef1+ zone (ventricular zone)
Premam -MZ	Premamillary Lef1+ zone (mantle zone)
Mam-VZ	Mamillary Foxb1+ zone (ventricular zone)
Mam -MZ	Mamillary Foxb1+ zone (mantle zone)
Supramam-VZ	Supramamillary Irx5+ zone (ventricular zone)
Supramam -MZ	Supramamillary Irx5+ zone (mantle zone)

For the E16.5 timepoint, where anatomic criteria can be more readily used to	Preoptic area/mitotic progenitors (ventricular zone)

classify gene expression patterns, the following abbreviations are also used.POA/VZ	
POA/MZ	Preoptic area/postmitotic precursors (mantle zone)
AH/VZ	Anterior hypothalamus/mitotic progenitors (ventricular zone)
AH/MZ	Anterior hypothalamus/postmitotic precursors (mantle zone)
SCN//VZ	Suprachiasmatic nucleus/mitotic progenitors (ventricular zone)
SCN/MZ	Suprachiasmatic nucleus /postmitotic precursors (mantle zone)
DMH/VZ	Dorsomedial hypothalamic nucleus/mitotic progenitors (ventricular zone)
DMH/MZ	Dorsomedial hypothalamic nucleus/postmitotic precursors (mantle zone)
VMH/VZ	Ventromedial hypothalamic nucleus/mitotic progenitors (ventricular zone)
VMH/MZ	Ventromedial hypothalamic nucleus/postmitotic precursors (mantle zone)
PvN/VZ	Paraventricular hypothalamic nucleus/mitotic progenitors (ventricular zone)
PvN/MZ	Paraventricular hypothalamic nucleus/postmitotic precursors (mantle zone)
ArcN/VZ	Arcuate hypothalamic nucleus/mitotic progenitors (ventricular zone)
ArcN/MZ	Arcuate hypothalamic nucleus/postmitotic precursors (mantle zone)
PH/VZ	Posterior hypothalamus/mitotic progenitors (ventricular zone)
PH/MZ	Posterior hypothalamus/postmitotic precursors (mantle zone)

All two-color ISH data using candidate genes and Shh, along with all Supplementary Tables in Excel format can be accessed at: blackshaw.bs.jhmi.edu and at and in the Mouse Gene Expression Database at Jackson Labs **(**http://www.informatics.jax.org/expression.shtml).