Supplemental Figures and Legends

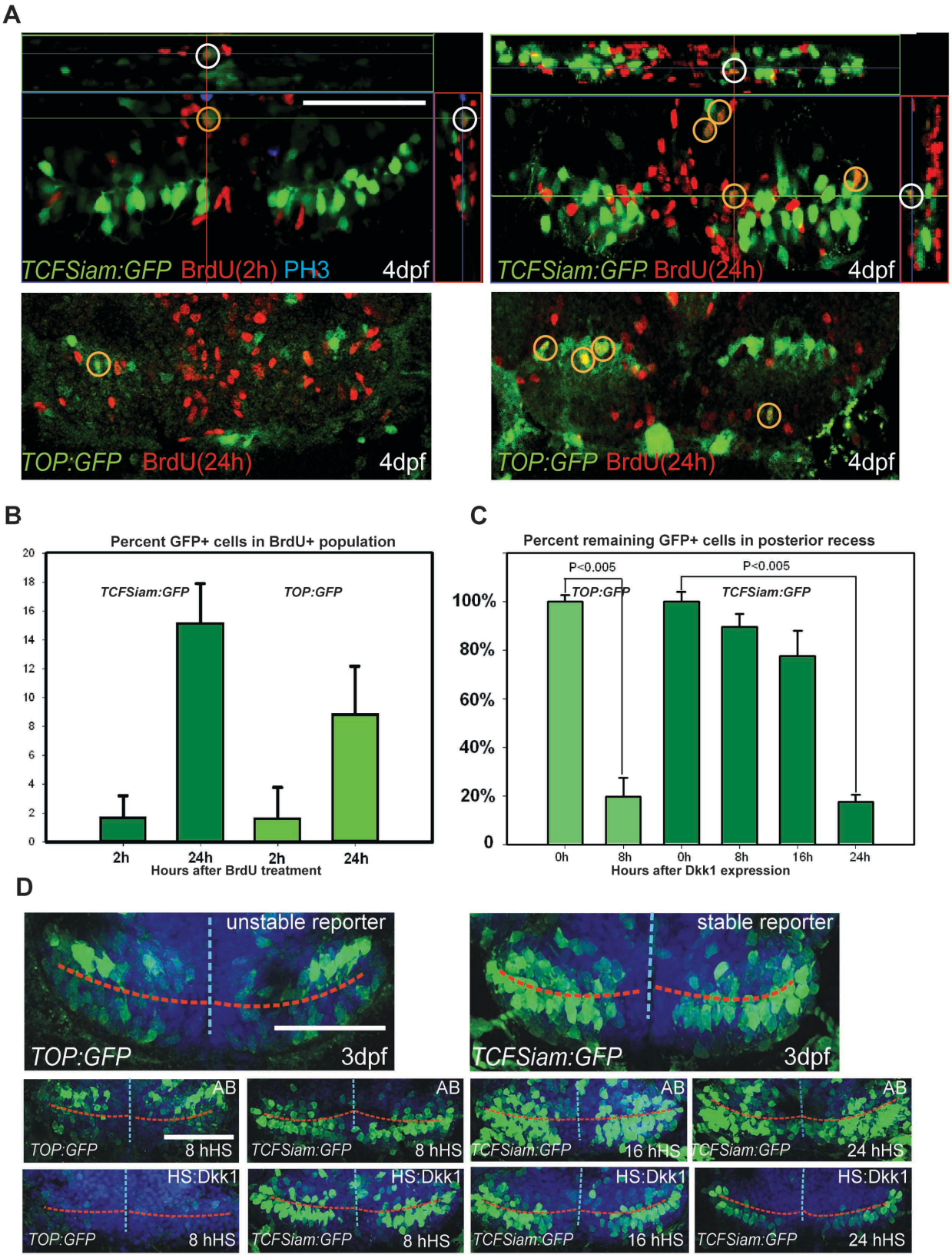

Figure S1 Analysis of Wnt reporter at 4dpf, related to Figure 1. (A) Ventral view of the posterior recess region of *TOP:GFP* and *TCFSiam:GFP* hypothalamus after short (2h) and long term (24h) BrdU labeling; observed with 50µm maximum intensity confocal Z-projections. (B) Quantification of $BrdU^+/GFP^+$ cells 2 hours or 24 hours after labeling. (C) Relative percentages of GFP^+ cells following *hs:dkk1* expression at 3dpf, compared to wild-type controls. (D) Ventral view of the posterior recess region of *TOP:GFP* and *TCFSiam:GFP* embryos, 8h, 16h, and 24h after *hs:dkk1* activation at 3dpf. Images are 50µm ventral maximum intensity confocal Z-projections. Scale bars: 80µm. Cell counts were collected from ventral maximum intensity confocal Z-projections through the posterior recess of 3 individual samples for each condition. Error=±SD.

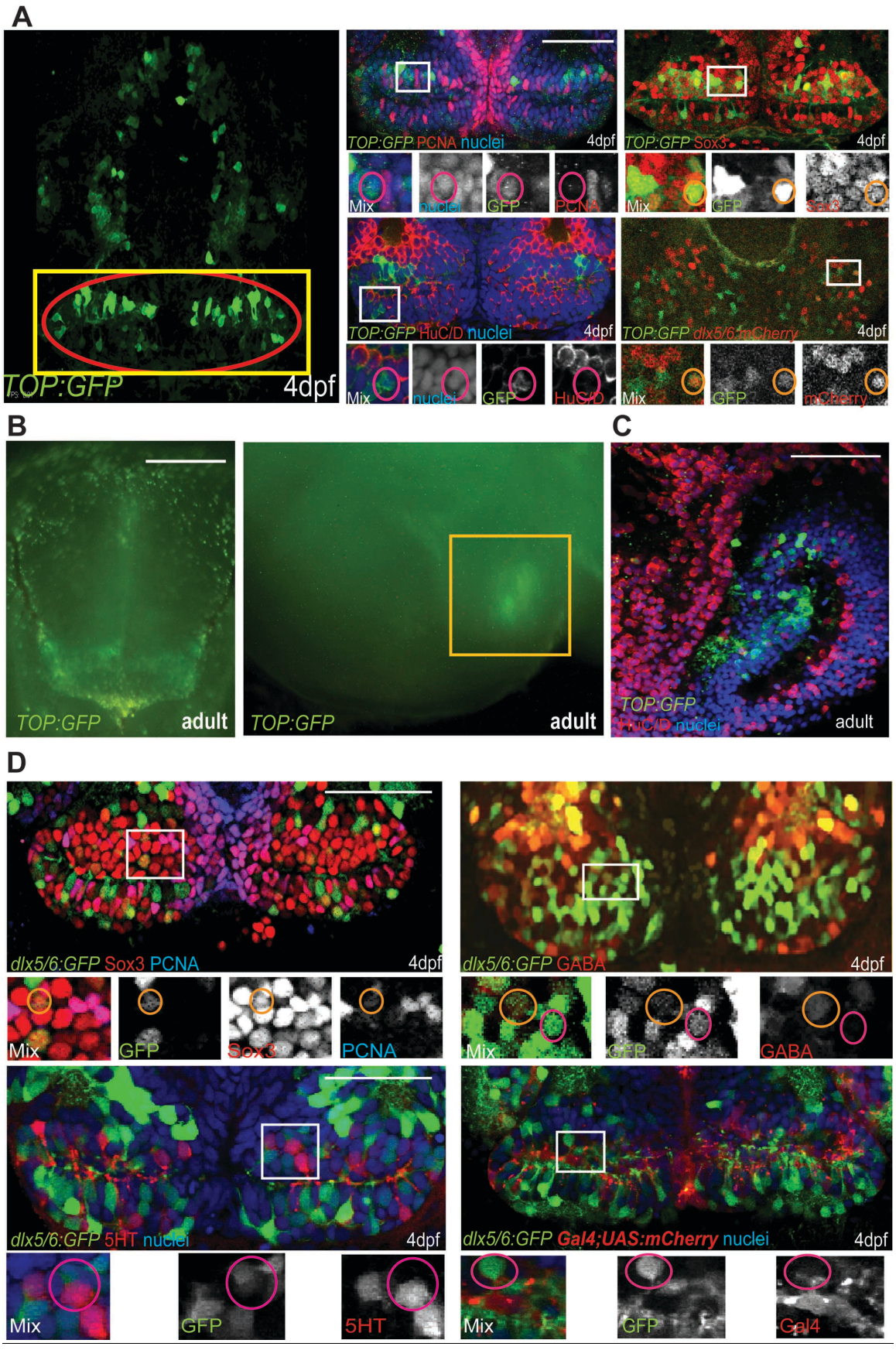
A
TOP:GFP
4dpf
TOP:GFP PCNA nuclei
4dpf
Mix
nuclei
GFP
PCNA
TOP:GFP Sox3
4dpf
Mix
GFP
Sox3
TOP:GFP HuC/D nuclei
4dpf
Mix
nuclei
GFP
HuC/D
TOP:GFP dlx5/6:mCherry
4dpf
Mix
GFP
mCherry
B
TOP:GFP
adult
TOP:GFP
adult
C
TOP:GFP
HuC/D nuclei
adult
D
dlx5/6:GFP Sox3 PCNA
4dpf
Mix
GFP
Sox3
PCNA
dlx5/6:GFP GABA
4dpf
Mix
GFP
GABA
dlx5/6:GFP 5HT nuclei
4dpf
Mix
GFP
5HT
dlx5/6:GFP Gal4;UAS:mCherry nuclei
4dpf
Mix
GFP
Gal4

Figure S2 Identity of reporter-labeled cells, related to Table 1. (A) Maximum-intensity confocal ventral projection of 4dpf *TOP:GFP* hypothalamus on left, yellow box marks the area shown on right and red oval marks the posterior recess. Co-staining of GFP with cell-type specific markers is shown on right. (B) Dissecting microscope images of *TOP:GFP* expression in the adult hypothalamus, from ventral and mid-sagittal views. Yellow box marks the area shown in (C). (C) Co-staining of GFP and HuC/D in the adult hypothalamus. (D) *dlx5/6:GFP* expression overlaps with Sox3, PCNA, and GABA, but not with 5HT or a Gal4 insertion expressed in radial glia (C). White boxes indicate enlarged regions. Small orange circles label cells with colocalization and small magenta circles label cells without colocalization. Single confocal optical sections are shown unless otherwise indicated. Scale bars: Scale bars: (A,C,D) 80μm, (B) 250μm.

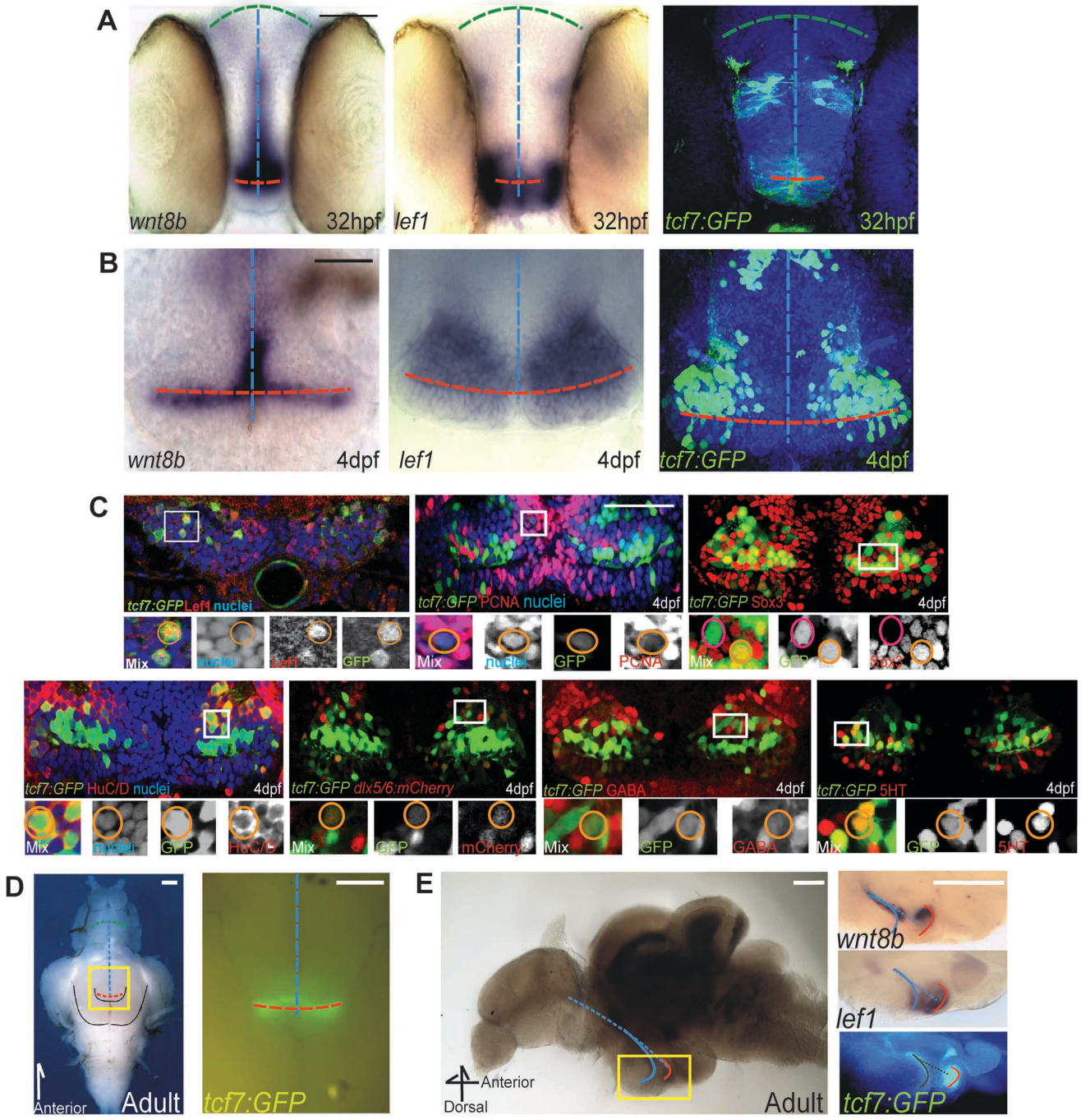
A
wnt8b
32hpf
lef1
32hpf
tcf7:GFP
32hpf
B
wnt8b
4dpf
lef1
4dpf
tcf7:GFP
4dpf
C
tcf7:GFP Lef1 nuclei
tcf7:GFP PCNA nuclei
4dpf
tcf7:GFP Sox3
4dpf
Mix
nuclei
Lef1
GFP
Mix
nuclei
GFP
PCNA
Mix
GFP
Sox3
tcf7:GFP HuC/D nuclei
4dpf
tcf7:GFP dlx5/6:mCherry
4dpf
tcf7:GFP GABA
4dpf
tcf7:GFP 5HT
4dpf
Mix
nuclei
GFP
HuC/D
Mix
GFP
mCherry
Mix
GFP
GABA
Mix
GFP
5HT
D
Anterior
Adult
tcf7:GFP
E
Anterior
Dorsal
Adult
wnt8b
lef1
tcf7:GFP

Figure S3 Expression of Wnt pathway components in zebrafish hypothalamic neural progenitors, related to Figure 2. (A,B) *wnt8b* mRNA, *lef1* mRNA, and *tcf7:GFP* expression at 32hpf (A), and 4dpf (B) in ventral whole-mount views. (C) Co-expression of *tcf7:GFP* in the 4dpf hypothalamus with cell-type specific markers. (D) Bright-field and fluorescent dissecting microscope ventral images of the adult hypothalamus expressing *tcf7:GFP*. Yellow box in the bright field image indicates the region magnified in the fluorescent image. (E) Mid-sagittal view of *wnt8b* mRNA, *lef1* mRNA, and *tcf7:GFP* expression in the adult hypothalamus. Yellow box in the left image indicates the region magnified in the right images. Blue line: 3rd ventricle; Red line: Posterior recess. A 12µm transverse cryosection is shown for Lef1 staining in (C), all other panels show single confocal optical sections from ventral views unless otherwise indicated. Scale bars: (A,B) 50µm, (C) 80µm, (D,E) 250µm.

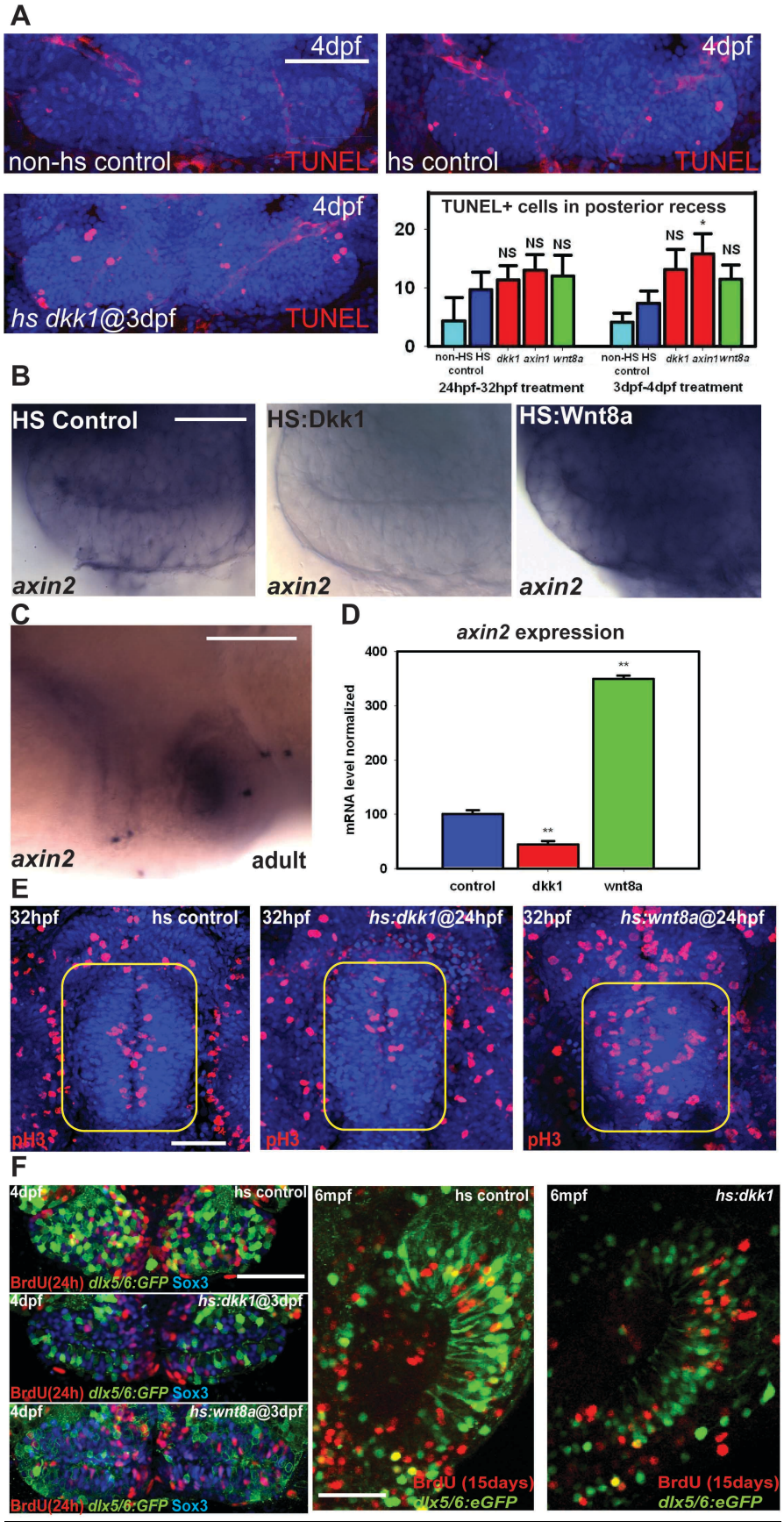
A
4dpf
non-hs control
TUNEL
4dpf
hs control
TUNEL
4dpf
hs dkk1@3dpf
TUNEL
TUNEL+ cells in posterior recess
20
10
0
NS
NS
NS
NS
NS
*
non-HS control
HS
dkk1
axin1
wnt8a
24hpf-32hpf treatment
3dpf-4dpf treatment
B
HS Control
HS:Dkk1
HS:Wnt8a
axin2
axin2
axin2
C
axin2
adult
D
axin2 expression
mRNA level normalized
400
300
200
100
0
**
**
control
dkk1
wnt8a
E
32hpf
hs control
32hpf
hs:dkk1@24hpf
32hpf
hs:wnt8a@24hpf
pH3
pH3
pH3
F
4dpf
hs control
BrdU(24h) dlx5/6:GFP Sox3
4dpf
hs:dkk1@3dpf
BrdU(24h) dlx5/6:GFP Sox3
4dpf
hs:wnt8a@3dpf
BrdU(24h) dlx5/6:GFP Sox3
6mpf
hs control
BrdU (15days)
dlx5/6:eGFP
6mpf
hs:dkk1
BrdU (15days)
dlx5/6:eGFP

Figure S4 Effects of Wnt signaling on hypothalamic progenitors, related to Figure 3. (A) Ventral confocal optical sections of TUNEL staining at 4dpf, and counts of $TUNEL^{+}$ cells at 32hpf and 4dpf. Only *hs:axin1* expression from 3-4dpf significantly increases cell death compared to heat shock alone. (B) *axin2* in situ hybridization at 4dpf following heat shock at 3dpf. (C) *axin2* in situ hybridization in a mid-sagittal section of the adult hypothalamus. Red oval marks posterior recess. (D) *axin2* mRNA expression levels in the dissected adult hypothalamus following Wnt pathway inhibition or activation for 15 days. (E) Phospho-Histone H3 staining in the 32hpf hypothalamus (yellow squares). (F) Co-localization of BrdU labeling with Sox3 and *dlx5/6:GFP* labeling in the 4dpf and adult posterior recess. Single confocal optical sections are shown in all panels. Scale bars: (A,C,F) 80μm, (B) 25μm, (E) 50μm. Cell counts were collected from ventral maximum intensity confocal Z-projections through 3 individual brains. The entire hypothalamus was counted at 32hpf, and the entire posterior recess was counted at 4dpf. *: $p<0.05$ compared to non-HS control. Error=±SD.

Supplemental Experimental Procedures

Fish strains

The following lines have been described previously: *Tg(TOP:GFP)^{w25}* (Dorsky et al., 2002), *Tg(gfap:GFP)^{mi2001}* (Bernardos and Raymond, 2006), *Tg(hsp701:tcf3-GFP)^{w26}* (Lewis et al., 2004), *Tg(hsp701:wnt8a-GFP)^{w34}* (Weidinger et al., 2005), *Tg(hsp701:dkk1-GFP)^{w32}* (Stoick-Cooper et al., 2007), *Et(T2KHG)^{nkhg21c}* (Nagayoshi et al., 2008), *Tg(1.4dlx5a/-dlx6a:GFP)^{ot1}* (Ghanem et al., 2003), and *Tg(UAS-E1b:NfsB-mCherry)^{jh17}* (Parsons et al., 2009), *Tg(UAS-E1b:Kaede)^{s1999t}* (Scott et al., 2007).

lef1^{zd11} was made by ZFN mutagenesis (described in detail below).

Tg(hsp701:GFP-axin1)^{zd13} was made by inserting a cDNA containing *GFP* fused to zebrafish *axin1* into a pCS2+ backbone vector containing the *hsp70-4* promoter. A stable transgenic line was produced by plasmid injection at the 1-cell stage followed by screening for germline transmission of heat shock-induced GFP expression.

Tg(dlx5/6:mCherry)^{zd14} was made by inserting the *1.4dlx5a/-dlx6a* enhancer/promoter (Ghanem et al., 2003) upstream of an *mCherry* cDNA in a Tol2 destination vector using multisite Gateway cloning (Kwan et al., 2007). A stable transgenic line was produced by plasmid injection with *tol2* mRNA at the 1-cell stage followed by screening for germline transmission of mCherry expression.

Tg(7xTCF-Xla.Siam:GFP)^{ia4} (*TCFSiam:GFP*) was made by inserting the 7xTCF-Siam enhancer/promoter (Maretto et al., 2003) upstream of an *eGFP* cDNA in a Tol2 destination vector using multisite Gateway cloning (Kwan et al., 2007). A stable transgenic line was produced by plasmid

injection with *tol2* mRNA at the 1-cell stage followed by screening for germline transmission of GFP.

The *Et(Gal4VP16; myl7:gfp)*^{zc1066a} enhancer-trap line was generated by plasmid injection with *tol2* mRNA at the 1-cell stage. Potential founders were crossed to *Tg(UAS-E1b:Kaede)*^{s1999t} fish for testing and identified by Kaede expression in embryos. Identified F1 transgenics were crossed to *Tg(UAS-E1b:nfsB-mCherry)*^{jh17} fish and embryos were imaged at 1, 2, and 5 dpf for identification of expression patterns.

Mice

BAT-LacZ (Maretto et al., 2003), *Hes1*^{C2} (Kopinke et al., 2011), *R26R*^{EYFP} (Srinivas et al., 2001), *R26R*^{LacZ} (Soriano, 1999), and *Ctnnb1*$^{lox(ex3)}$ (Harada et al., 1999) mice have been described previously. Floxed and germline β-catenin loss-of-function mice, Ctnnb1^{lox} and Ctnnb1$^{\Delta}$ respectively (Brault et al., 2001), were obtained from Jackson Laboratories. *Hes1*$^{C2/+}$*; R26R*$^{LacZ/+}$ mice were used for lineage tracing experiments. For ß-catenin loss-of-function experiments, *Hes1*$^{C2/+}$*; R26R*$^{EYFP/+}$*; Ctnnb1* $^{lox/\Delta}$ mice (referred to as *Ctnnb1*$^{Hes1\text{-}cKO}$) were compared to control *Hes1*$^{C2/+}$*; R26R*$^{EYFP/+}$*; Ctnnb1* $^{lox/+}$ (*Ctnnb1*$^{Hes1\text{-}het}$) animals. To assess a potential ß-catenin gain-of-function phenotype, crosses were set up to yield *Hes1*$^{C2/+}$*; R26R*$^{EYFP/+}$*; Ctnnb1* $^{lox(ex3)/+}$ (*Ctnnb1*$^{Hes1\text{-}GOF}$) and *Hes1*$^{C2/+}$*; R26R*$^{EYFP/+}$ (*Ctnnb1*$^{Hes1\text{-}ctrl}$) mice. All genotypes included the *R26R*^{EYFP} or *R26R*^{LacZ} reporter allele to follow the fate of recombined cells.

Antibodies

Primary antibodies used were: mouse anti PCNA (Sigma: P8825), rabbit anti Sox3 (Gift from M. Klymkowsky), rabbit anti GFP (Molecular Probes: A11122), mouse anti GFP (Molecular Probes:

A11120), chick anti GFP (Aves Labs: GFP-1020), mouse anti HuC/D (Molecular Probes: A21271), rabbit anti 5HT (ImmunoStar: 541016), rabbit anti GABA (Sigma: A2052), mouse anti BrdU (Sigma: B8434), rat anti BrdU (Abcam: ab6326), rabbit anti BLBP (Abcam: ab32432), mouse anti GFAP (zrf-1; ZIRC [Eugene, OR]), goat anti Sox2 (Santa Cruz: sc-17320), rabbit anti DCX (Abcam: ab18732), rabbit anti Dlx2 (Abcam: ab18188), rabbit anti GFAP (Abcam: ab7260), mouse anti NeuN (Millipore: MAB377), chick anti LacZ (Abcam: ab9361), rabbit anti pH3 (Cell Signaling: 9713), rabbit anti Lef1 (Open Biosystems), rabbit anti LEF1 (Cell Signaling: 2230). Secondary antibodies were obtained from Jackson ImmunoResearch. Hoechst33342 was used to stain cell nuclei.

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Supplemental References

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