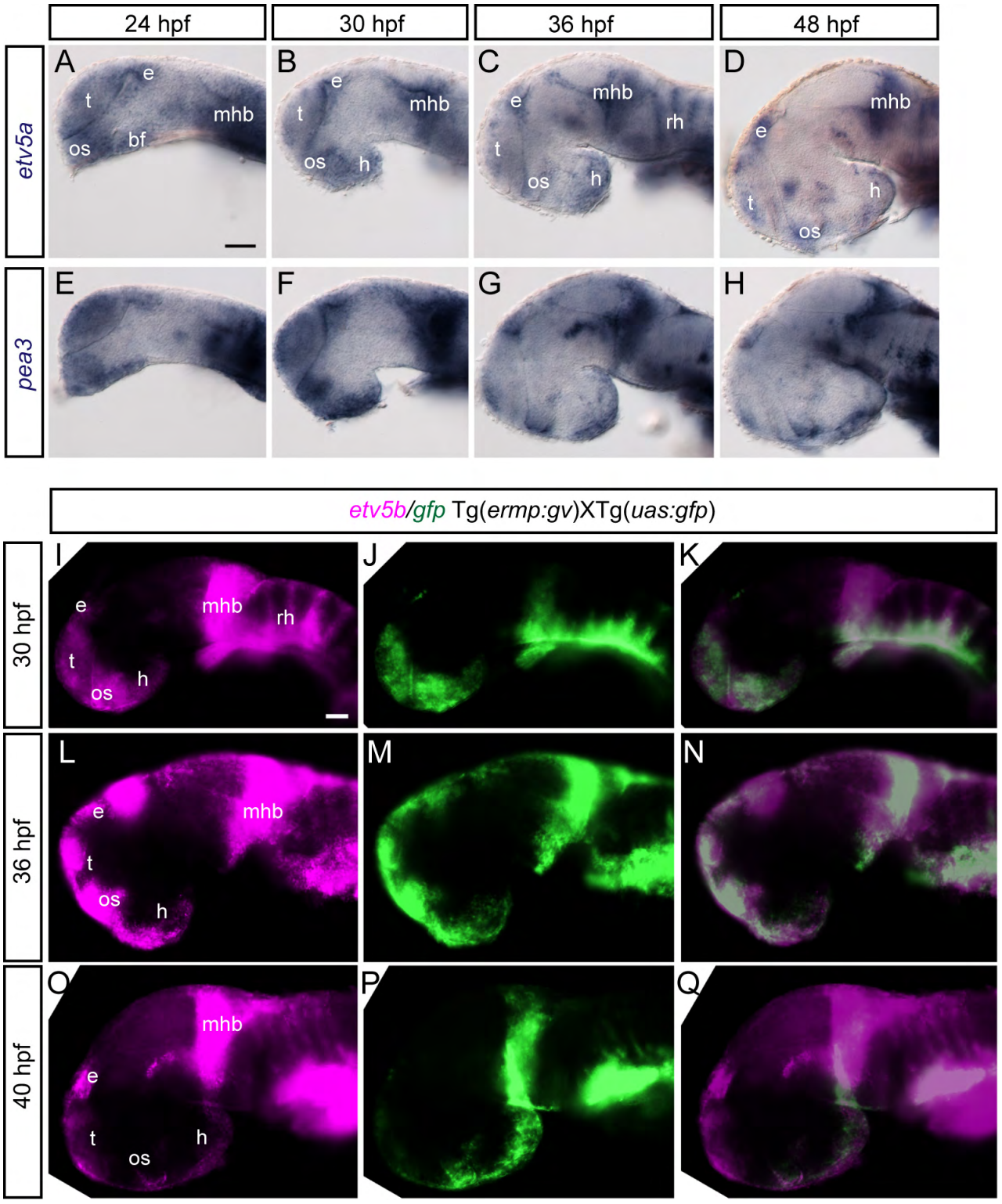

Fig. S1. *etv5a* and *pea3* are expressed by the developing hypothalamus but at lower levels/in a more restricted fashion than *etv5b*. (**A-H**) Expression of *etv5a* and *pea3* (whole-mounts). At 24 hpf, *etv5a* transcripts were present in the telencephalon (t), pineal/epithalamus (e), basal forebrain (bf), optic stalk (os), mid-hindbrain boundary (mhb) and rhombencephalon (rh). Progressively, the expression domain in the basal forebrain narrowed, and by 30 and 36 hpf it was present in the ventral/caudal hypothalamus (h). Expression persisted in this region at low levels until 48 hpf. *pea3* was expressed in a more restricted manner in the forebrain at 24 hpf, and from 30 hpf onwards its expression in the basal forebrain was limited to a stripe at the distal limits of the hypothalamus. (**I-Q**) Fluorescent microscopy images of Tg(*ermp:gv*)×Tg(*uas:gfp*) transgenic embryos (whole-mount) processed for double *gfp/etv5b* ISH. Lateral views, anterior left. Scale bars: 50 μm.

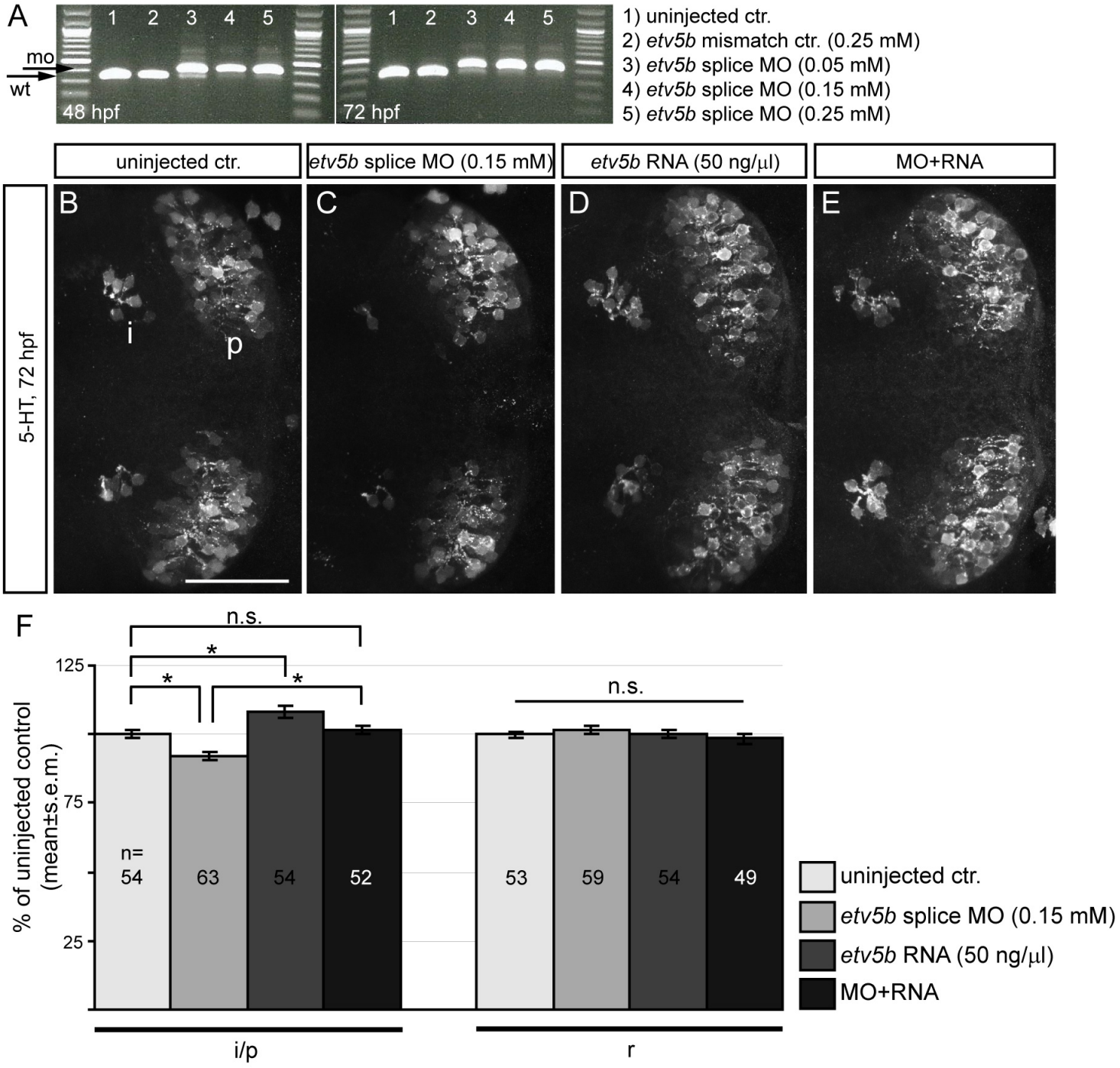

Fig. S2. *etv5b* overexpression rescues the 5-HT phenotype seen in *etv5b* splice morphants. (**A**) RT-PCR on total RNA extracted from 48 or 72 hpf pooled (n=6), uninjected or mismatch controls as well as embryos injected with increasing concentrations of *etv5b* splice MO. Injection of the splice MO results in almost complete loss of the wild-type band (wt) and the presence of a morphant band (mo) corresponding to an insertion of the entire intron 10 (79 bp), which in the amino acid sequence results in a premature stop codon. (**B-E**) Confocal maximum intensity projections showing embryos after RNA rescue of the *etv5b* splice MO phenotype. Embryos were subjected to the indicated treatments and processed for 5-HT immunohistochemistry (dissected brains). The intermediate/posterior (i./p.) clusters of the hypothalamus are shown. Ventral views, anterior left. Scale bar: 50 μm. (**F**) The number of 5-HT cells obtained in the rescue experiment in the i./p. and anterior raphe (r.) clusters at 72 hpf after the indicated treatments expressed as percentage of control. *etv5b* splice MO alone results in a significant decrease in the number of 5-HT-expressing cells as compared with uninjected control siblings (92.3±1.5%, $P=6.6\times10^{-4}$), whereas *etv5b* RNA injections led to a significant increase (108.3±2.1%, $P=2.07\times10^{-3}$). n, total number of embryos analysed for each experiment. *$P\leq0.05$; n.s., not significant.

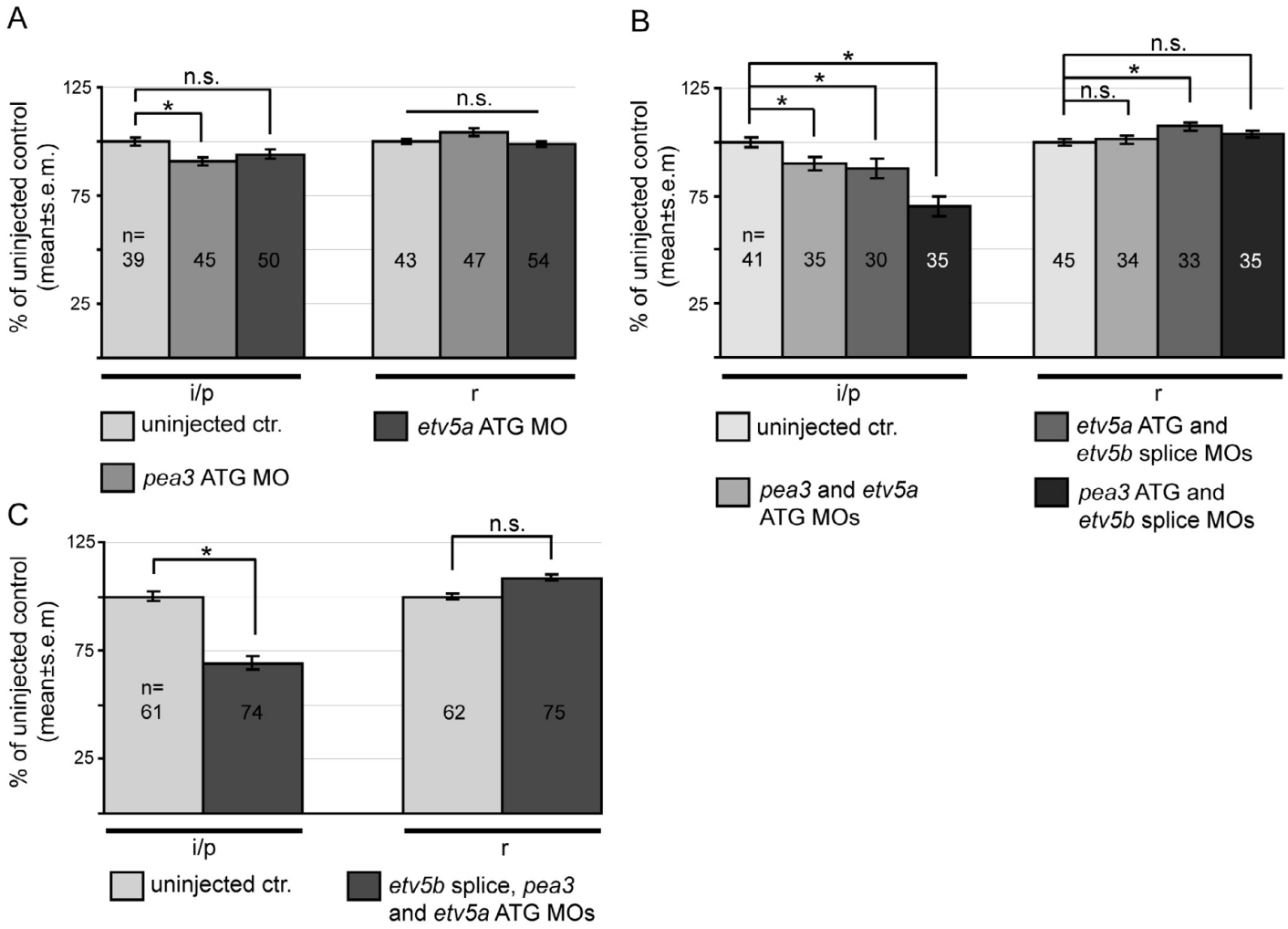

Fig. S3. *pea3*-deficient but not *etv5a*-deficient embryos exhibit a reduced number of 5-HT cells in the hypothalamus. (**A-C**) The number of 5-HT cells in the intermediate/posterior (i./p.) clusters of the hypothalamus and the anterior raphe (r.) 5-HT population at 72 hpf in controls, *pea3* (0.15 mM) or *etv5a* (0.05 mM) ATG morphants expressed as percentage of control. The *pea3* and *etv5a* ATG MOs were also co-injected with the *etv5b* splice MO (0.15 mM) to generate all possible double and triple combinations. In the triple combination, the *pea3* and *etv5b* splice MO concentrations were reduced to 0.1 mM. *n*, total number of embryos analysed for each experiment. *$P \leq 0.05$; n.s., not significant.

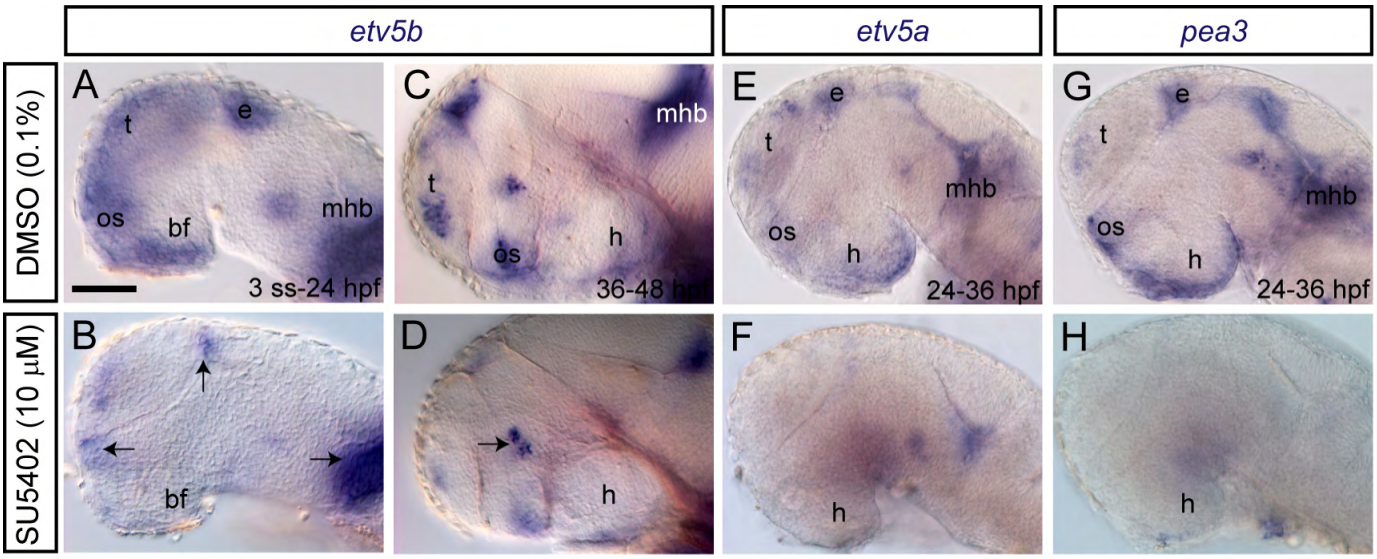

Fig. S4. Fgf loss-of-function downregulates expression of *pea3* family members. (**A-H**) Embryos were treated with SU5402 during the developmental stages indicated, fixed directly after treatment and analysed for *etv5b*, *etv5a* or *pea3* expression (whole-mounts). Following SU5402 treatment, no *etv5b* transcripts were detected in the basal forebrain (bf), including hypothalamus (h), although transcripts were still detectable in some of the domains normally expressing *etv5b*, including the telencephalon (t), mid-hindbrain boundary (mhb), optic stalk (os) and pineal/epithalamus (e) (B, arrows) and ventral thalamus (D, arrow). SU5402 treatment also abolished *etv5a* and *pea3* expression in the hypothalamus as well as in other domains where they are normally expressed. Lateral views, anterior left. Scale bar: 50 μm.

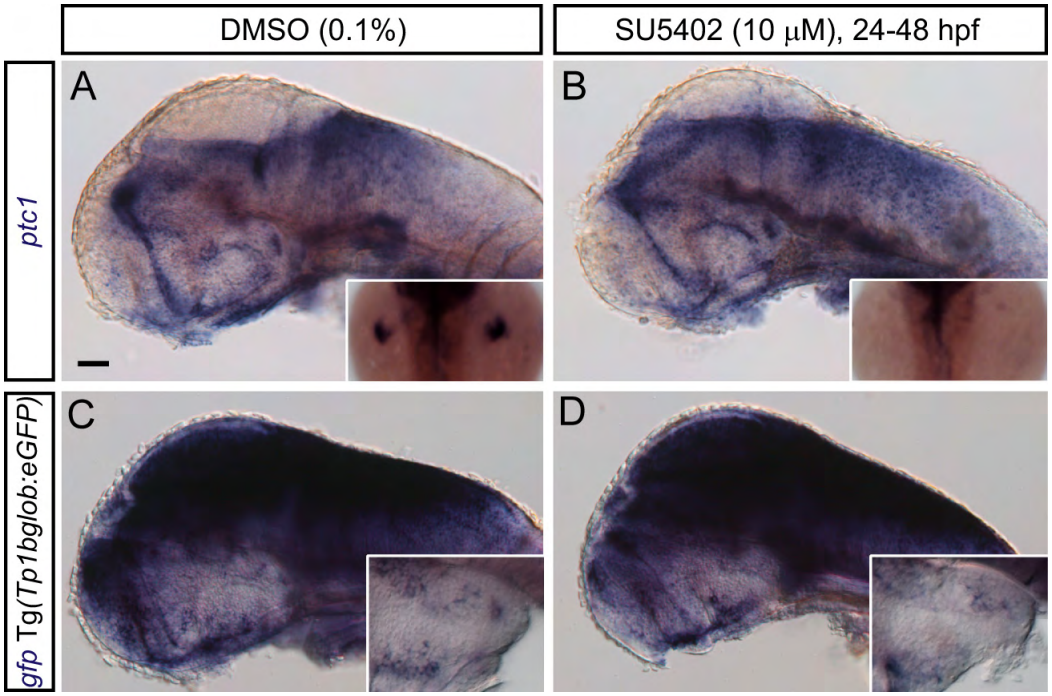

Fig. S5. Hedgehog and Notch signalling are not differentially regulated by Fgf signalling within the hypothalamus during embryonic development. Micrographs showing DMSO controls and SU5402-exposed (24-48 hpf) wild-type embryos processed for *ptc1* (**A**,**B**) or Notch reporter [Tg(*Tp1bglob:eGFP*)] embryos processed for *gfp* ISH (**C**,**D**) at 48 hpf (whole-mounts). Insets in A and B show downregulation of *ptc1* expression in pectoral fin buds (dorsal view, anterior up). Insets in C and D show *gfp* signal in hypothalamus after a shorter signal development time. No up- or downregulation of *ptc1* or *gfp* was observed in hypothalamus. Lateral views, anterior left. Scale bar: 50 μm.

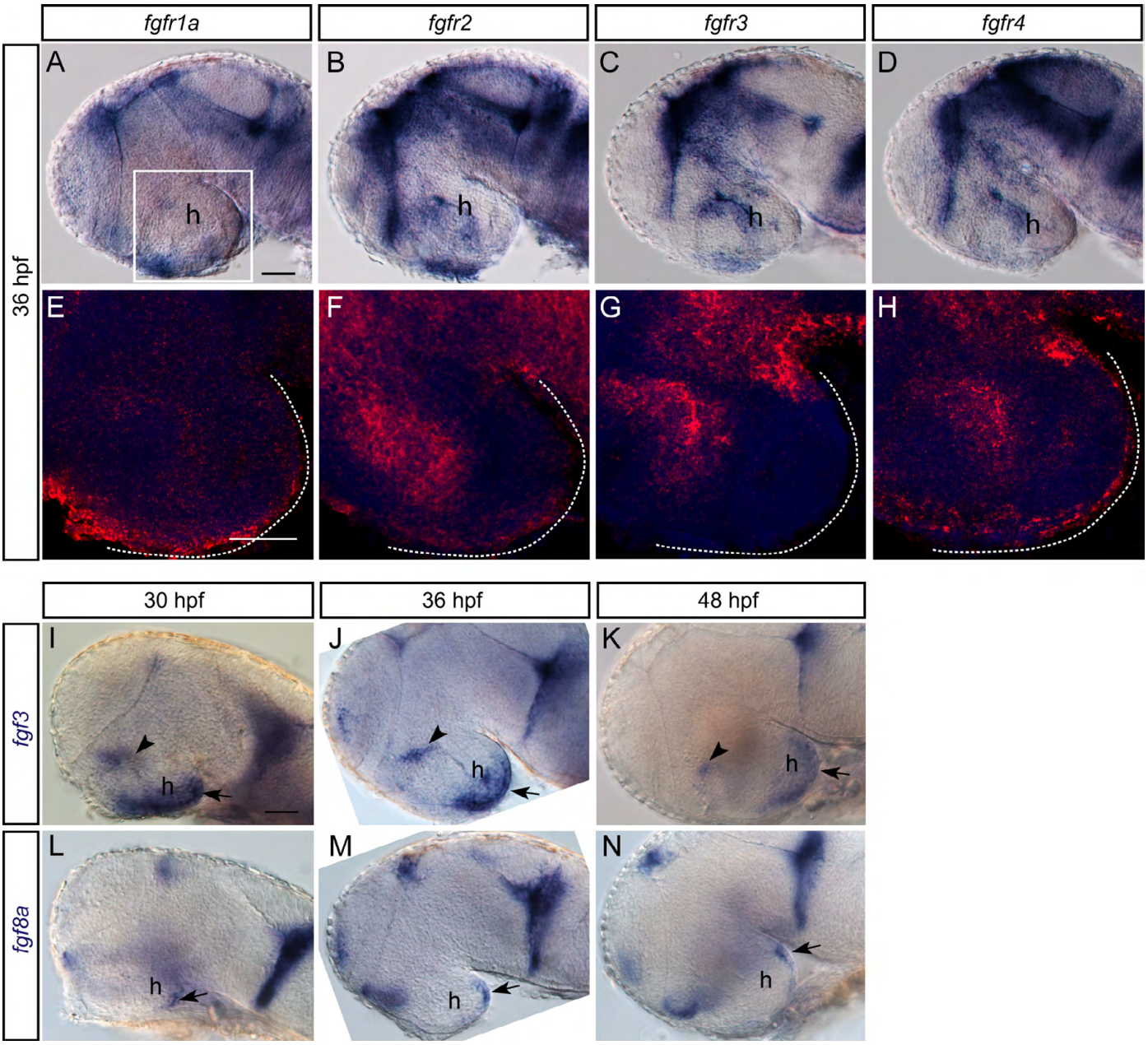

Fig. S6. Fgf receptors and ligands are expressed in the hypothalamus at a stage when 5-HT progenitors are proliferating. (**A-D**) *fgfr1a*, *2*, *3* and *4* expression in whole-mount embryos. (**E-H**) Confocal maximum intensity projections of embryos processed for fluorescent ISH (red) and counterstained with DAPI (blue) covering 30 μm around the midline corresponding to boxed area in A. Transcripts for *fgfr1a*, *2* and *4*, but not *fgfr3*, were detectable in the caudal hypothalamus (dashed line). (**I-N**) Expression of *fgf3* and *fgf8a* in whole-mount embryos. At all stages analysed, both transcripts were detectable in the hypothalamus (h). However, *fgf3* exhibited a broader expression domain covering the entire caudal/ventral hypothalamus (arrows) and a restricted part of the posterior tuberculum/hypothalamus (arrowheads) (I-K), whereas *fgf8a* was limited to an area in the most caudal/dorsal hypothalamus (arrows) (L-N). Lateral views, anterior left. Scale bars: 50 μm.

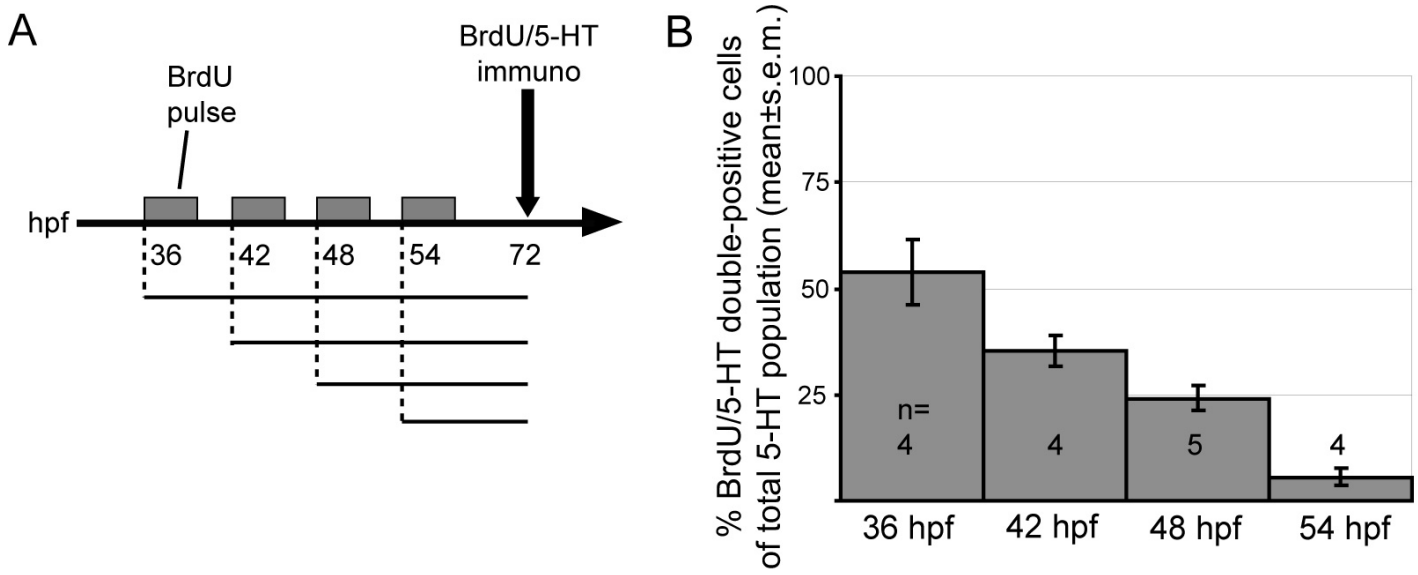

Fig. S7. Proliferating 5-HT progenitors leave the cell cycle before 54 hpf. (**A**) Scheme illustrating the procedure for temporal analysis of proliferation rate among 5-HT progenitors in the hypothalamic intermediate/posterior (i./p.) clusters by repeated BrdU treatment of embryos starting at 36, 42, 48 or 54 hpf. (**B**) The proportion of 5-HT/BrdU double-positive cells among the 5-HT-positive population in the i./p. clusters at 72 hpf after repeated BrdU pulses, expressed as percentage of control. These results suggest that the majority of the hypothalamic 5-HT progenitor population has left the cell cycle by 54 hpf. *n*, total number of embryos analysed for each experiment.

Table S1. Primary antibodies and *in situ* hybridisation probes

Antibody	Dilution	Source	Number
Rabbit anti-GFP	1:500	Acris Antibodies	TP401
Rabbit anti-5-HT	1:2500	Sigma	S5545
Rat anti-5-HT	1:100	Millipore	MAB352
Mouse anti-TH1	1:300	Millipore	MAB318
Rat anti-BrdU	1:200	Abcam	ab6326
Rabbit anti-ph-H3	1:300	Millipore	06-570
Rabbit anti-mKO2	1:100	Medical and Biological Laboratories	M168-3
Mouse anti-mAG	1:100	Medical and Biological Laboratories	PM052
Rabbit anti-cleaved caspase 3	1:250	Cell Signaling	9661
Probe	**Reference**		
avpl	Eaton et al., 2008		
etv5a	Roussigné and Blader, 2006		
etv5b	Münchberg et al., 1999		
fgf3	Kiefer et al., 1996		
fgf8a	Reifers et al., 1998		
fgf8b	Reifers et al., 2000		
fgfr1a	Rohner et al., 2009		
fgfr2	Tonou-Fujimori et al., 2002		
fgfr3	Sleptsova-Friedrich et al., 2001		
fgfr4	Sleptsova-Friedrich et al., 2001		
nkx2.1a	Rohr et al., 2001		
oxtl	Unger and Glasgow, 2003		
pea3	Münchberg et al., 1999		
ptc1	Concordet et al., 1996		
sst3	Devos et al., 2002		