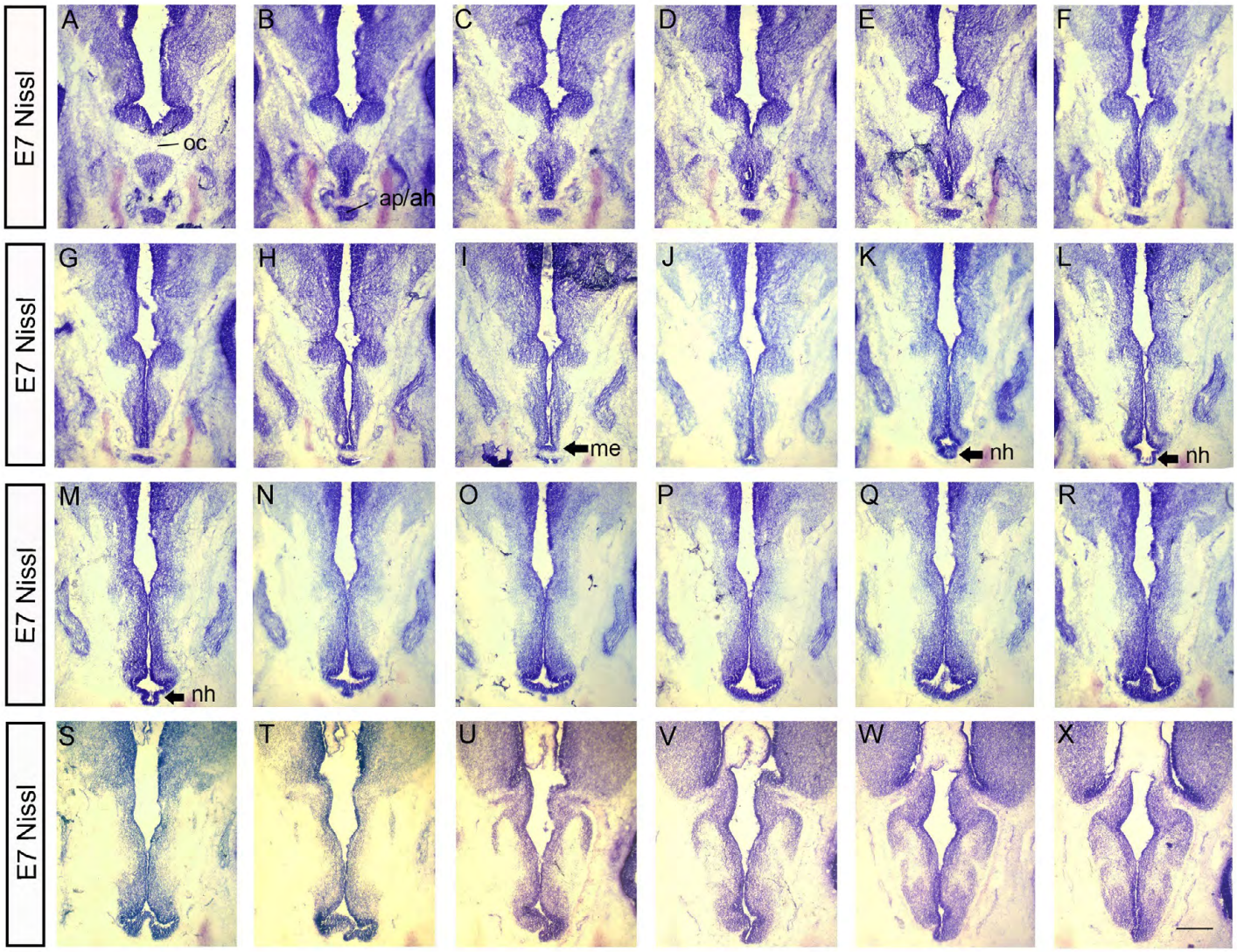

Fig. S1. Nissl staining of the E7 chick hypothalamus. (**A-X**) Nissl staining of serial transverse sections (15 µm) through the chick hypothalamus at E7 from anterior (A) to posterior (X). Anterior-most sections are from optic chiasm regions (oc, A). The anterior pituitary/adenohypophysis (ap/ah) can be identified beneath the ventral midline of the anterior hypothalamus (B-I), terminating close to the ME (me, I). The evagination of the NH can be identified (black arrows, K-M). Posterior to the NH, the recess of the third ventricle widens (N-T) then narrows (V-X). Scale bar: 200 µm.

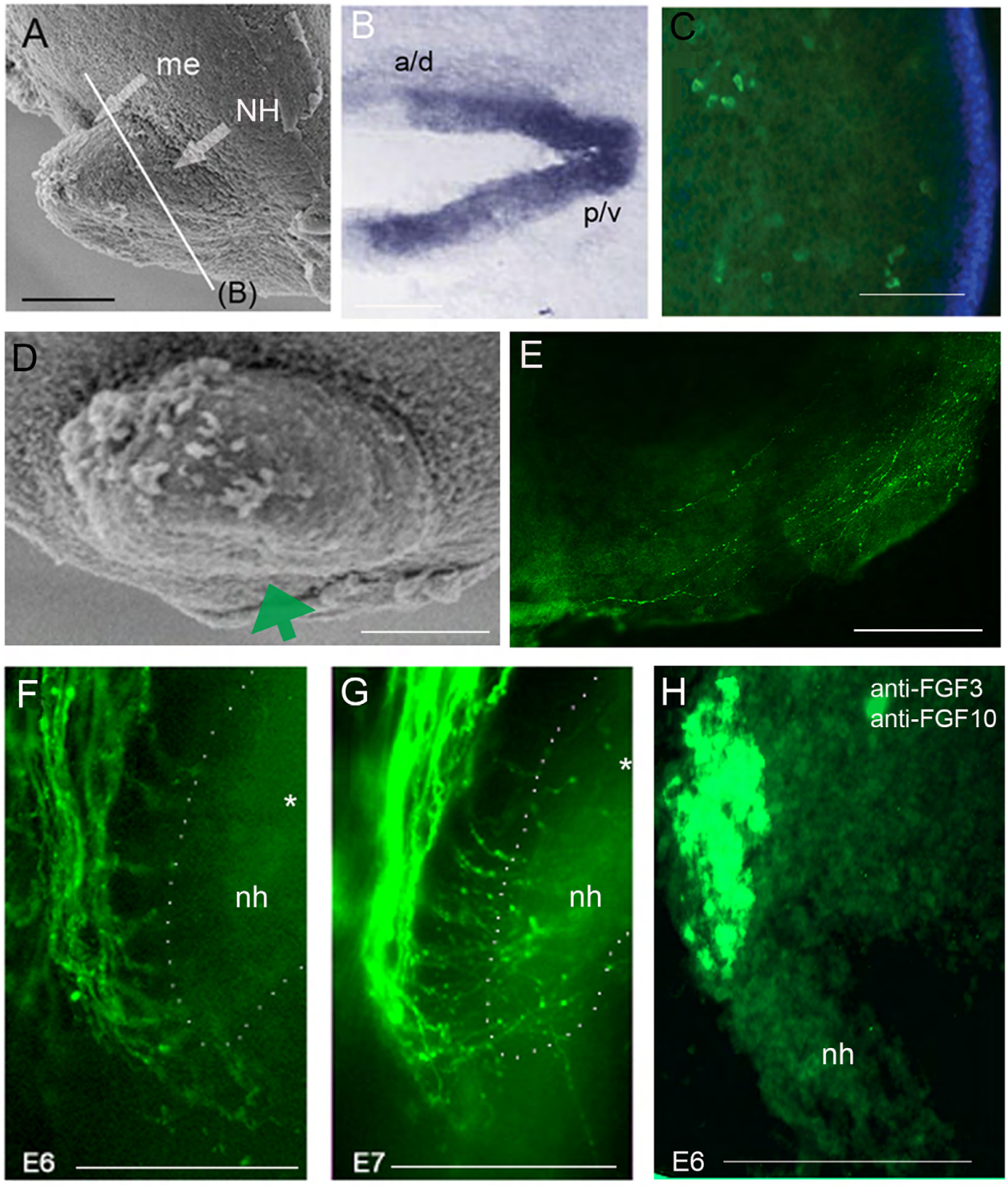

Fig. S2. Axons project to the *Fgf10*$^{+}$ NH, but do not initially project deep into it. (**A**,**D**) Scanning electron micrograph of E7 (A) or E10 (D) chick NH to indicate plane of section in B and view in E. In A, anterior is to the left; dorsal, up. In D, green arrow points to ventral side of NH. (**B**) *In situ* hybridisation analysis shows that *Fgf10* is expressed throughout the NH, with highest levels in posterior-ventral regions. (**C**) The first Vp^{+} neurons differentiate at E5.5-E6. (**E**) Ventral view of isolated hypothalamus: at E10, TUJ1^{+} axons project beneath/into the ventral-most NH. (**F**,**G**) High-powered view of an E4 GFP-transplanted [L] graft (see Fig. 2G) analysed after 2 (F) and 3 (G) days of culture. Axons grow rapidly towards the NH (E6), turning towards it, but slow/stall as they enter, and do not initially project rapidly deeper into the NH (asterisk). Dotted outline marks NH (nh). (**H**) E4 GFP-transplanted [L] graft (see Fig. 2G) cultured for 2 days in the presence of Fgf3 and Fgf10 blocking antibodies. No axons project from the graft. Scale bar: 150 µm in A,B,F-H; 70 µm in C-E.

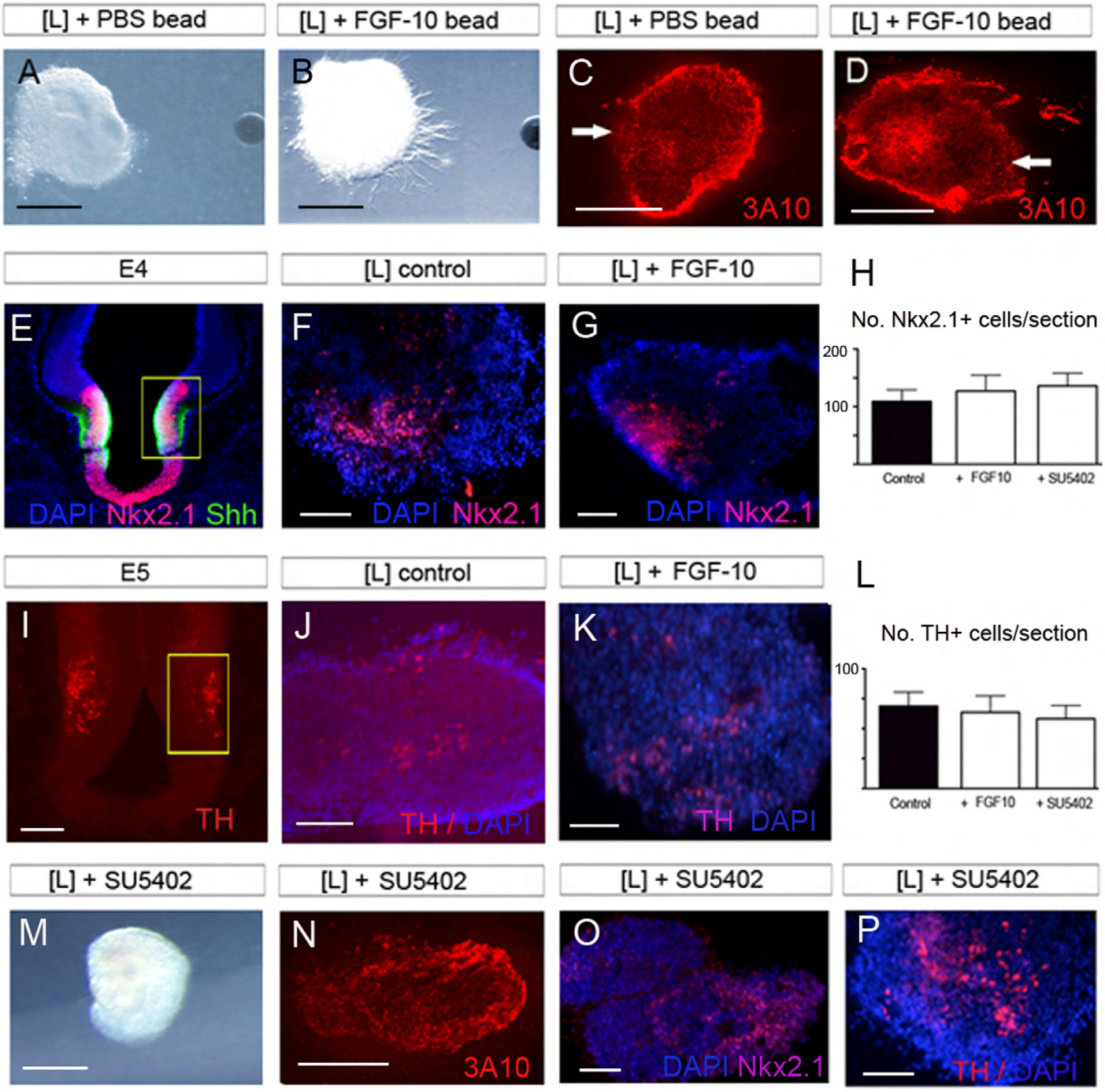

Fig. S3. FGF signalling promotes axonal re-orientation and growth without affecting cell numbers. Effects of FGFs and FGF inhibitors on chick lateral neuronal [L] explants. (**A**,**B**) Axon outgrowth is promoted by an Fgf10- but not a PBS-soaked bead. (**C**) The neurofilament marker 3A10 is detected in [L] explants cultured with a PBS-soaked bead (*n*=5); all axons remain confined within the explant. (**D**) 3A10 is similarly detected within [L] explants cultured with an Fgf10 bead but is also detected on axons that have emerged and extended towards the bead (*n*=5). Quantitative analysis reveals no apparent difference in the numbers of $3A10^{+}$ cells (137.6±12.75 with PBS beads versus 116.4±11.50 with Fgf10-beads). Arrows in C and D point to region of [L] explant with little neurofilament labelling: consistently, axons initially extend away from this region. (**E-H**,**O**) Transverse section (E) through E4 medial hypothalamus, showing expression of the transcription factor Nkx2.1 in the ventral and lateral medial hypothalamus (co-expressed with Shh in lateral regions). [L] explants cultured alone (F), with Fgf10 (G) or with SU5402 (O) show statistically similar numbers of $Nkx2.1^{+}$ progenitor cells (H). (**I-L**,**P**) Transverse section (I) through E5 medial hypothalamus, showing expression of TH in lateral regions. [L] explants cultured alone (J), with Fgf10 (K) or with SU5402 (P) show similar numbers of TH^{+} cells. (**M**,**N**) SU5402 has no effect on axon outgrowth (M) or on 3A10 expression within [L] explants (N). Error bars represent s.e.m. Scale bars: 100 µm in A-D,I,M,N; 50 µm in F,G,J,K,O,P.

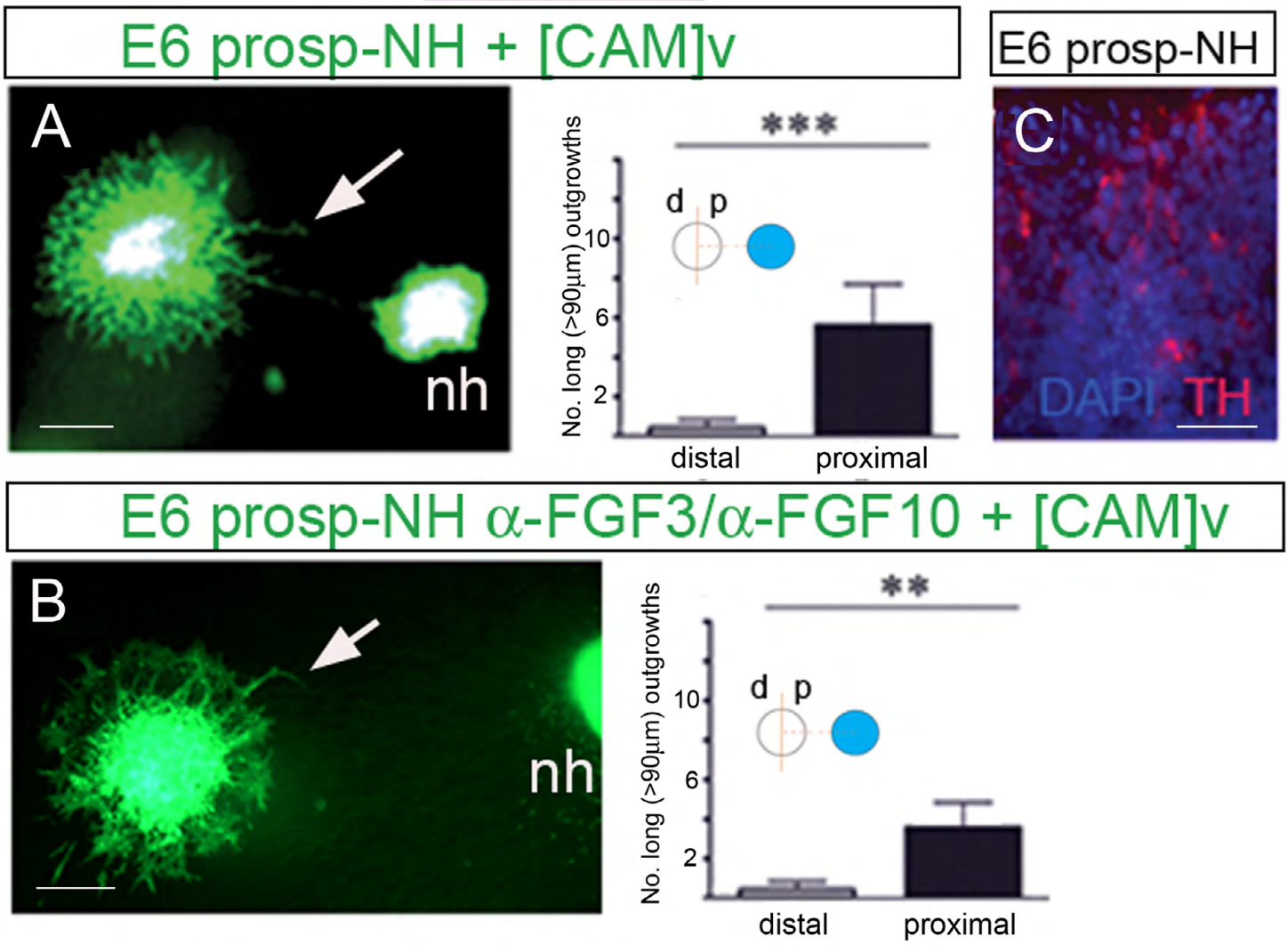

Fig. S4. E6 prosp-NH stimulates weak outgrowth in an FGF-independent manner in chick. (**A**,**B**) GFP [CAMv] explants co-cultured for 40 hours with E6 prosp-NH either untreated (A) or pre-soaked in anti-Fgf3 and anti-Fgf10 (B). A few long endothelial processes emerge from the [CAMv] explant in response to E6 prosp-NH; however, numbers are lower than upon co-culturing with E4 prosp-NH (compare Fig. 4L). Pre-treatment of E6 prosp-NH with anti-Fgf3 and anti-Fgf10 leads to a reduction of endothelial outgrowth (compare A and B), which, however, is not completely blocked, in contrast to the effect caused by anti-Fgf3 and anti-Fgf10 on E4 prosp-NH (compare B with Fig. 4W). Error bars represent s.e.m. ***P*<0.005; ****P*<0.001. d, distal; p, proximal. (**C**) Immunohistochemical analysis post-culture reveal that E6 prosp-NH explants contains some TH^+ axon termini. Scale bars: 100 µm in A,B; 50 µm in C.

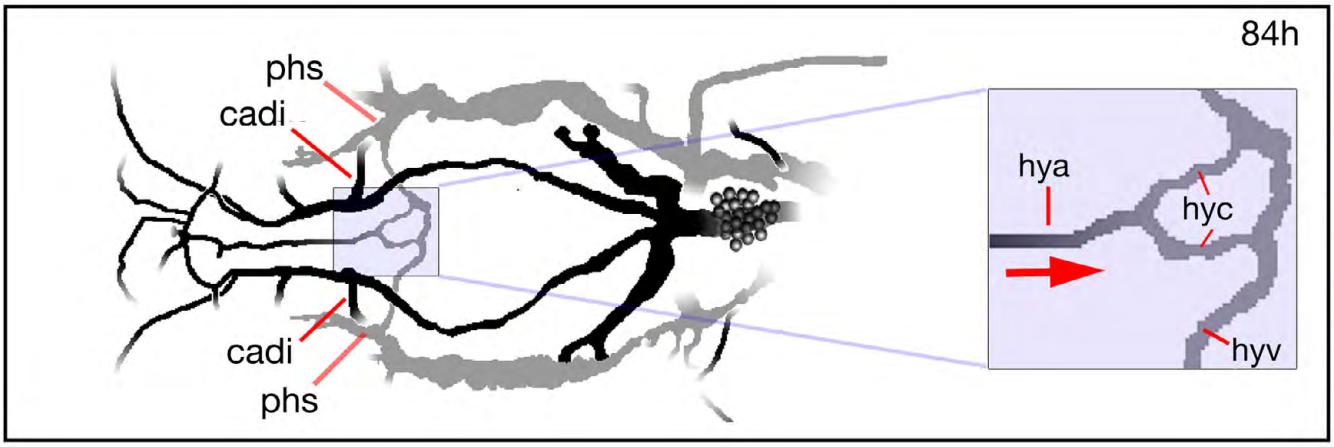

Fig. S5. Schematic summarizing microangiography analyses of zebrafish ventral brain vessels at 84 hpf. Ventral view; modified from Isogai et al. (Isogai et al., 2001). Inset shows magnified view of hypophyseal region. At 84 hpf, the basal communicating artery shown in Fig. 5O,P at 36 and 48 hpf is positioned more dorsally and not included in this scheme. cadi, caudal division of internal carotid artery; hya, hypophyseal artery; hyc; hypophyseal capillary; hyv, hypophyseal vein; phs, primary head sinus.

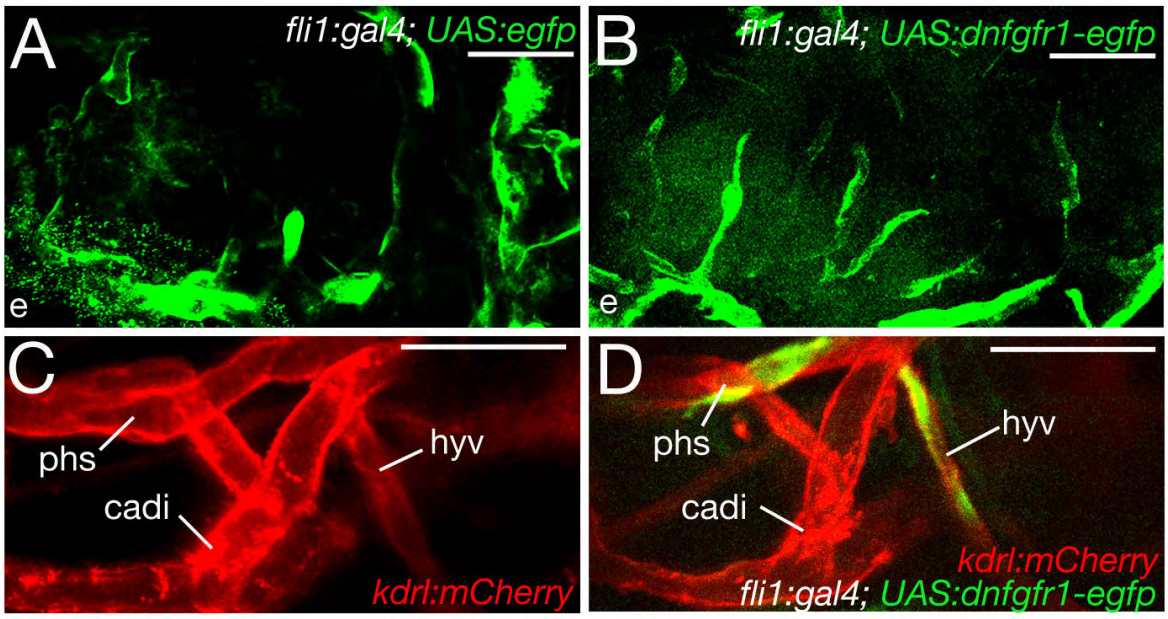

Fig. S6. Endothelial cells expressing the dominant-negative Fgfr1 receptor contribute normally to other cephalic blood vessels and do not affect morphogenesis of the hypophyseal vein. (**A-D**) Confocal *in vivo* images of different transgenic fish, as indicated; 100 hpf; ventral views, anterior to the left. Injection of the *UAS:egfp* control (A) or the *UAS:dnfgfr1-egfp* plasmid (B,D) into *tg(fli1a:gal4); tg(kdrl:mcherry)* double transgenic animals leads to mosaic expression of EGFP (A) or the truncated Fgfr1 receptor fused to EGFP in endothelial cells. For simplicity, the red channel is omitted in A and B, and the green channel omitted in C. (A,B) Endothelial cells with activation of the *UAS-egfp* transgene (A) or the *UAS:dnfgfr1-egfp* transgene (B) display comparable contributions to cephalic blood vessels. Nevertheless, their contributions to the hypophyseal artery and the two branches encompassing the pituitary differ (80% versus 40%; data not shown). (C,D) Morphogenesis of the hypophyseal vein is unaffected by endothelial cells expressing the *UAS:dnfgfr1-egfp* transgene. cadi, caudal division of internal carotid artery; e, eye; hyv, hypophyseal vein; phs, primary head sinus. Scale bars: 50 μm.

Table S1. Quantitative analyses of lateral neuronal [L] vessel explants cultured in a 3-days matrix with hypothalamic tissue, or protein-soaked beads, alone, or with FGF inhibitors

Groups (neuronal explants)	*n*	Number of axon fascicles: Proximal face (i)	Number of axon fascicles: Distal face (ii)
L alone	24	2.0 ± 0.25	1.7 ±- 0.29
L + prosp-NH	20	27.4 ± 1.2	3.0 ± 0.33
L + Fgf10 bead	15	31.6 ± 3.3	2.8 ± 0.35
L + Fgf3 bead	12	27.0 ± 3.2	2.2 ± 0.23
L + Fgf8 bead	10	2.8 ± 0.38	2.3 ± 0.34
L + PBS bead	20	2.4 ± 0.27	2.2 ± 0.47
L + Fgf10 bead + SU5402	10	3.1 ± 0.29	2.5 ± 0.35
L + Fgf3 bead + SU5402	12	2.0 ± 0.48	2.1 ± 0.46
L + prosp-NH + SU5402	15	8.3 ± 0.36	2.9 ± 0.17
L + prosp-NH + anti-Fgf3	10	18.9 ±1.2	1.8 ± 0.39
L + prosp-NH + anti-Fgf10	10	15.6 ± 3.1	2.0 ± 0.46
L + prosp-NH + anti-Fgf3/10	10	7.2 ± 2.8	3.1 ± 0.65
L + Fgf10 bead (high)	5	6.6 ± 0.9	23.0 ± 3.0

The mean number of axon fascicles (±s.e.m.) emerging from proximal and distal faces is shown. Maximal outgrowth, quantitatively similar to that evoked by prosp-NH explants, is promoted by beads soaked in 50-100 ng/µl Fgf10 and 100-200 ng/ml Fgf3. Repulsive effects occur in response to three- to fivefold higher levels of Fgf10 (300-500 ng/µl), but are not detected in response to Fgf3 (300-1000 ng/µl). *n*, number of explants analysed after 48 hours.

Table S2. Quantitative analyses of [CAMv] explants cultured in a 3-days matrix alone, with FGF proteins or with FGF inhibitors

Groups (vessel explants)	*n*	Mean number of processes *Mean length of processes* Proximal face	Mean number of processes *Mean length of processes* Distal face
[CAMv] alone	20	40.2 ± 4.5 *68.4 ± 4.2 (0%)*	38.7 ± 5.4 *69.2 ± 5.6 (0%)*
[CAMv] + E4 prosp-NH	6	45.6 ± 3.2 *148 ± 5.6 (26%)*	40.1 ± 3.3 *70.5 ± 4.3 (0%)*
[CAMv] + E4 prosp-NH (anti-Fgf3/anti-Fgf10)	6	38.2 ± 3.5; *78.4 ± 4.1 (0%)*	41.7 ± 4.4 *89.2 ± 5.2(0%)*
[CAMv] + E6 prosp-NH	6	66.7 ± 4.2 *121.5 ± 75.6 (9%)*	60.1 ± 3.9 *75.8 ± 5.3 (0%)*
[CAMv] + E6 prosp-NH (anti-Fgf3/anti-Fgf10)	6	58.2 ± 6.5; *72.4 ± 3.1 (7%)*	51.7 ± 5.4 *77.2 ± 6.2(0%)*
[CAMv] + Fgf10	15	46.2 ± 5.2 *186.2 ± 6.7 (62%)*	42.1 ± 3.5 *188.6 ± 7. 1(68%)*
[CAMv] + PBS bead	15	47.1 ± 3.5 *73.5 ± 5.6 (0%)*	45.6 ± 4.7 *72.5 ± 4.3 (0%)*
[CAMv] + Fgf10 bead	15	51.2 ± 6.3 *142.4 ± 7.8 (65%)*	39.8 ± 4.5 *68.3 ± 4.3 (0%)*
[CAMv] + Fgf3 bead	15	45.8 ± 3.2 *136.3 ± 6.3 (61%)*	37.2 ± 6.8 *43.3 ± 3.2 (0%)*
[CAMv] + Fgf10 bead (300-500 ng/μl)	15	43.9 ± 3.7 *141 ± 8.2 (59%)*	47.2 ± 3.2 *40.2 ± 4.1 (0%)*

The mean number of processes emerging from proximal and distal faces is indicated, followed by the mean process length; parentheses indicate percentage of processes with length >90 μm. Beads soaked in 50-100 ng/ml Fgf10 or in 300-500 ng/ml Fgf10 evoked a similar response. *n*, number of explants analysed after 48 hours.