SUPPLEMENTARY FIGURES, FIGURE LEGENDS AND VIDEO LEGENDS

Figure S1

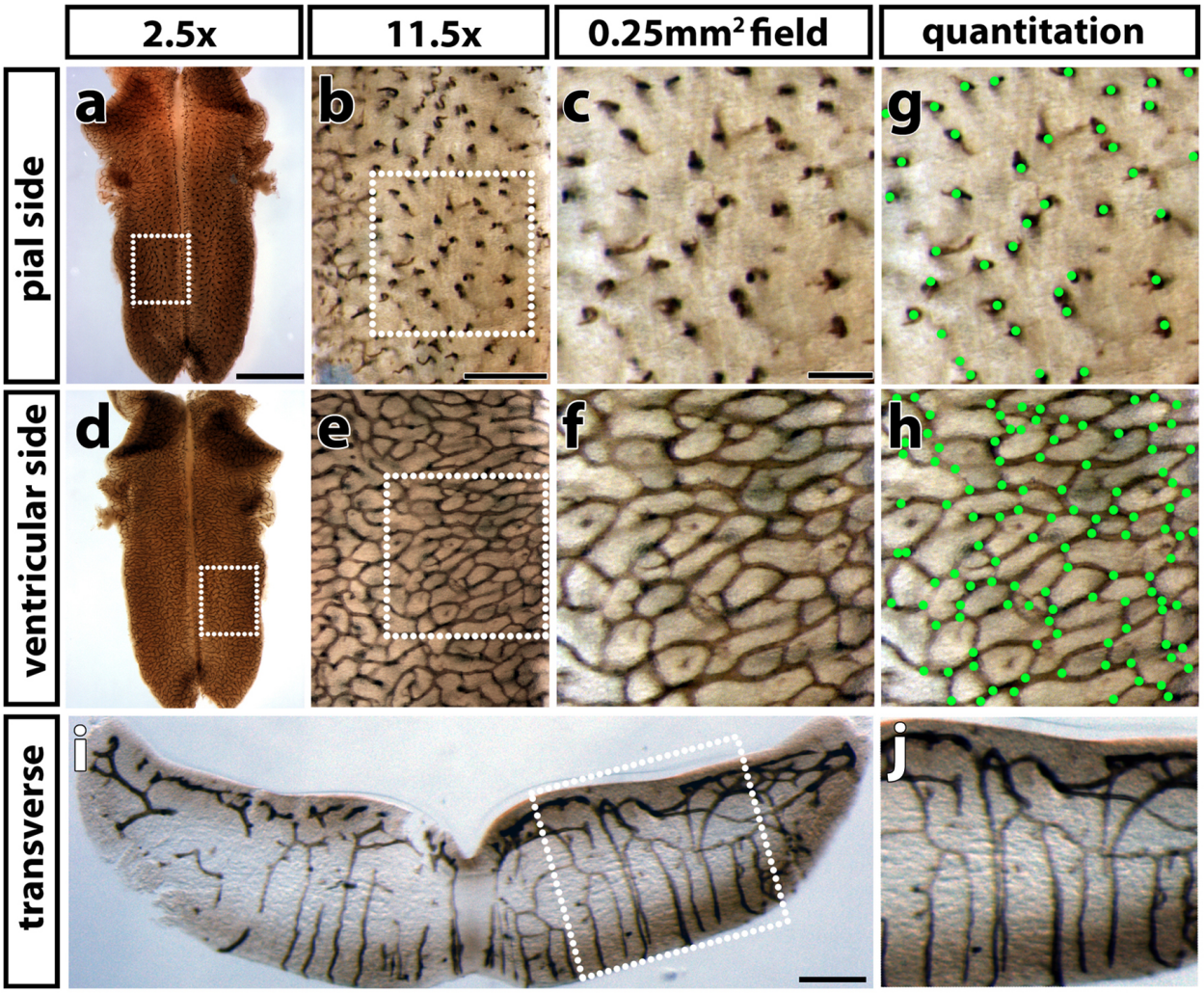

Fig. S1: Histochemical IB4 staining of an E12.5 mouse embryo hindbrain. An E12.5 hindbrain was labelled with biotinylated IB4 followed by HRP-conjugated streptavidin, flatmounted and imaged at the indicated magnifications. (a-c) Flatmounting the hindbrain with the pial side up allows visualisation of radial vessels entering the brain. (d-f) Flatmounting the hindbrain with the ventricular side up allows visualisation of the subventricular vascular plexus. The dotted boxes in (a,d) indicate the areas shown at higher magnification in (b,e), the dotted boxes in (b,e) those shown at higher magnification in (c,f), respectively; the size of each field in (c,f) is 500 µm x 500 µm, i.e. 0.25 mm^2. (g,h) Counting of radial vessels and vascular intersections in the fields shown in (c,f); green dots were used to track vessels that have been counted. (i,j) A 100 µm transverse vibratome section through the E12.5 hindbrain shown in (a); radial vessels (rv) extend from the pial side of the hindbrain and form the SVP on the ventricular side; the boxed area in (i) is shown at a higher magnification in panel (j). Scale bars: (a,d) – 1 mm; (b,e,i) – 200 µm, (c,f) - 100 µm. All animal procedures were performed in accordance with institutional and UK Home Office guidelines.

Figure S2

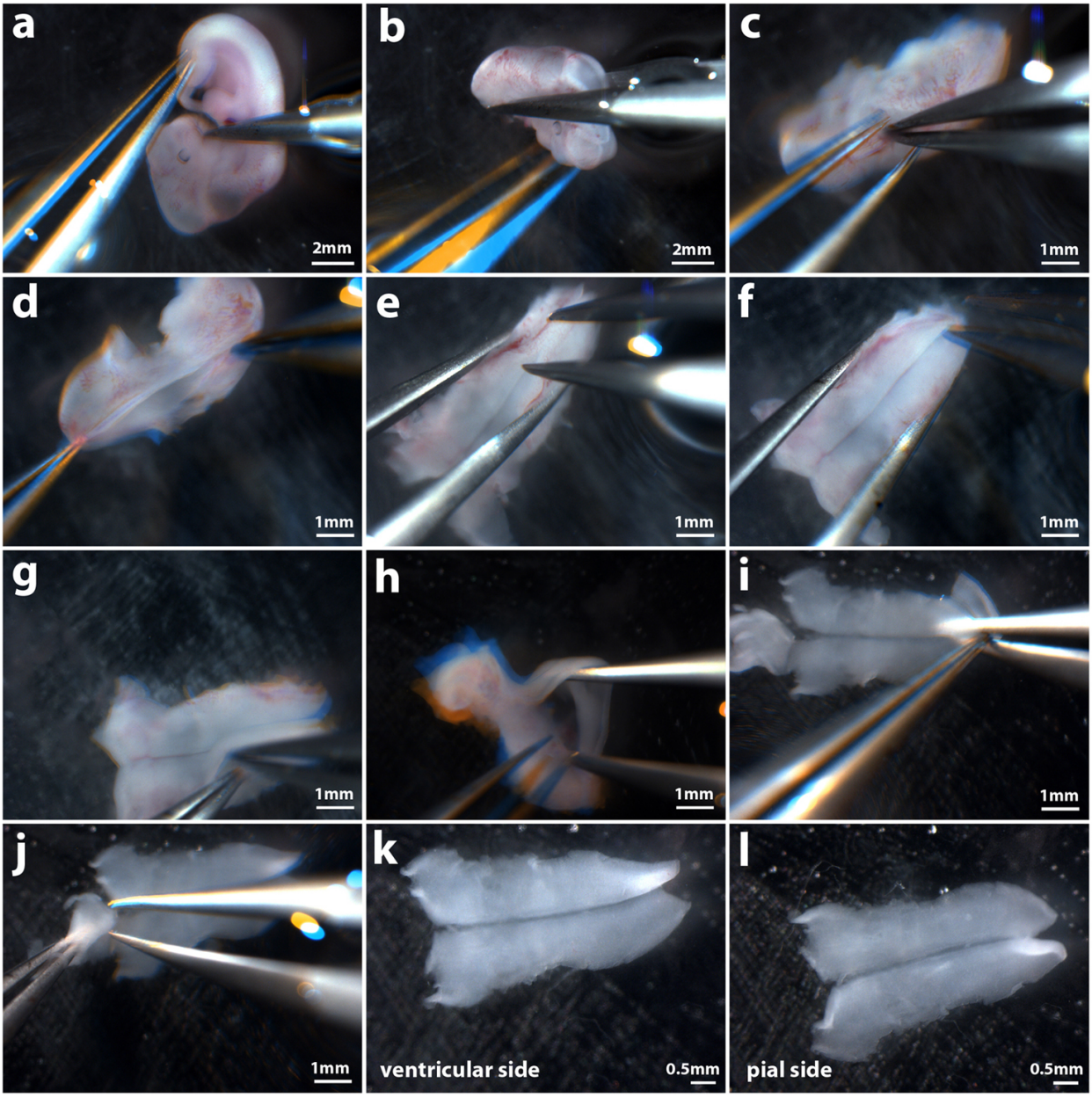

Fig. S2: Dissection of an E11.5 wild type mouse embryo hindbrain. Selected snapshots from supplemental movie 1 demonstrate key steps in hindbrains dissection. An E 11.5 embryo is decapitated roughly at shoulder height (**a**), and the anterior part of the head is removed (**b**). The hindbrain is revealed after peeling away the dorsal skin and hindbrain roof-plate membrane (**c**), allowing the hindbrain to unfurl (**d-f**). Next, the non-neural tissue underneath the hindbrain is peeled away (**g,h**). Finally, the hindbrain is trimmed to remove the spinal cord (**i**) and midbrain (**j**). The ventricular (**k**) and pial side (**l**) of the dissected hindbrain are shown. All animal procedures were performed in accordance with institutional and UK Home Office guidelines.

Supplementary video 1: Dissection of an E11.5 wild type mouse embryo hindbrain. This movie shows the procedure for dissecting an E11.5 hindbrain from a wild type mouse; the same procedure should be followed to dissect embryos at E12.5 or genetically altered mouse embryos at E11.5 or E12.5. Please turn on the sound to listen to an accompanying commentary explaining the dissection stages. All animal procedures were performed in accordance with institutional and UK Home Office guidelines.

Supplementary video 2: Dissection of an E11.5 *Tie2-Cre;Nrp1*$^{fl/-}$ mouse hindbrain. This movie shows the dissection of an E11.5 hindbrain from a *Tie2-Cre;Nrp1*$^{fl/-}$ mouse embryo that lacks NRP1 in endothelial cells. Note that vascular malformations can be observed already during the dissection procedure. All animal procedures were performed in accordance with institutional and UK Home Office guidelines.