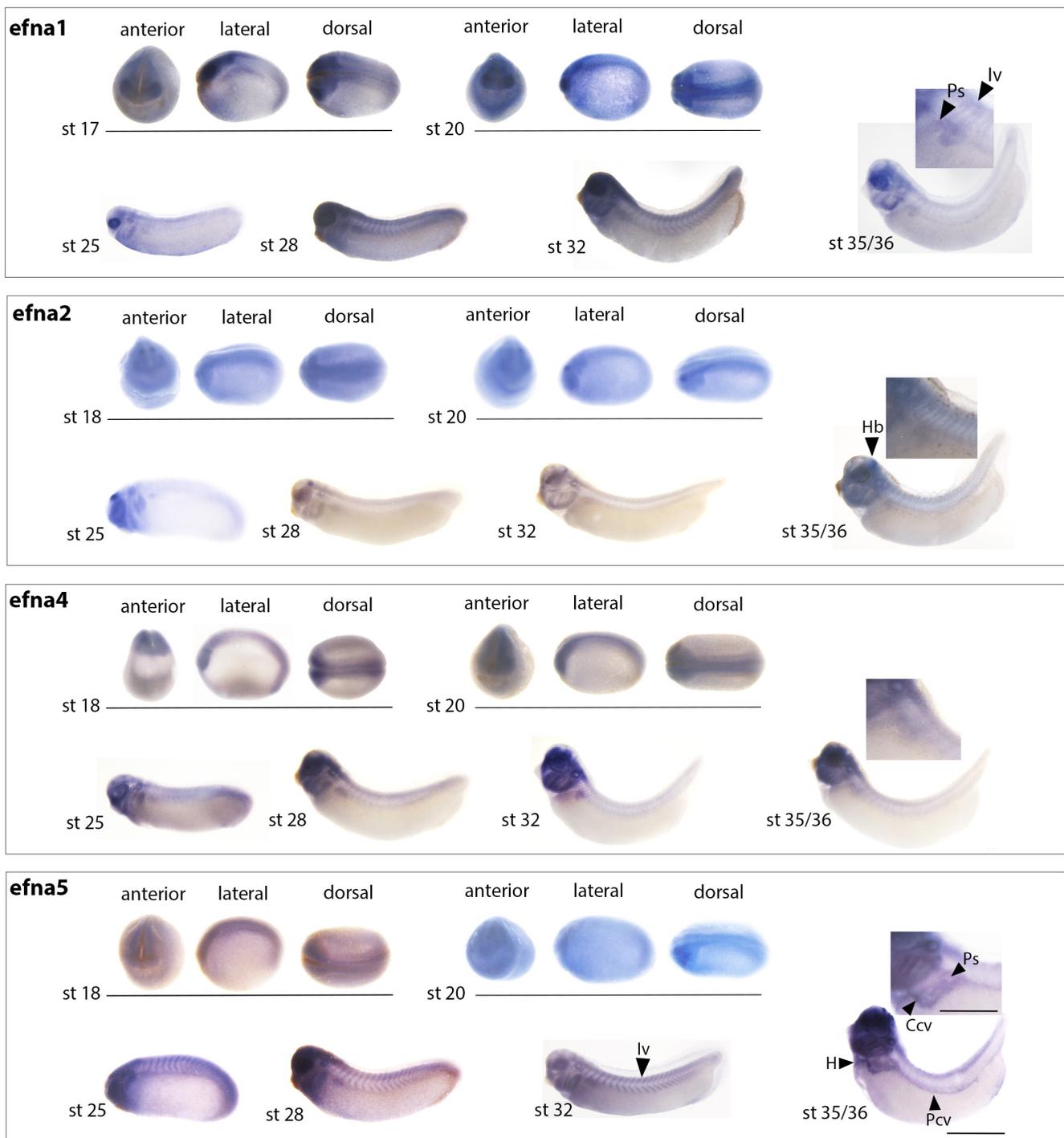


Supplementary Material

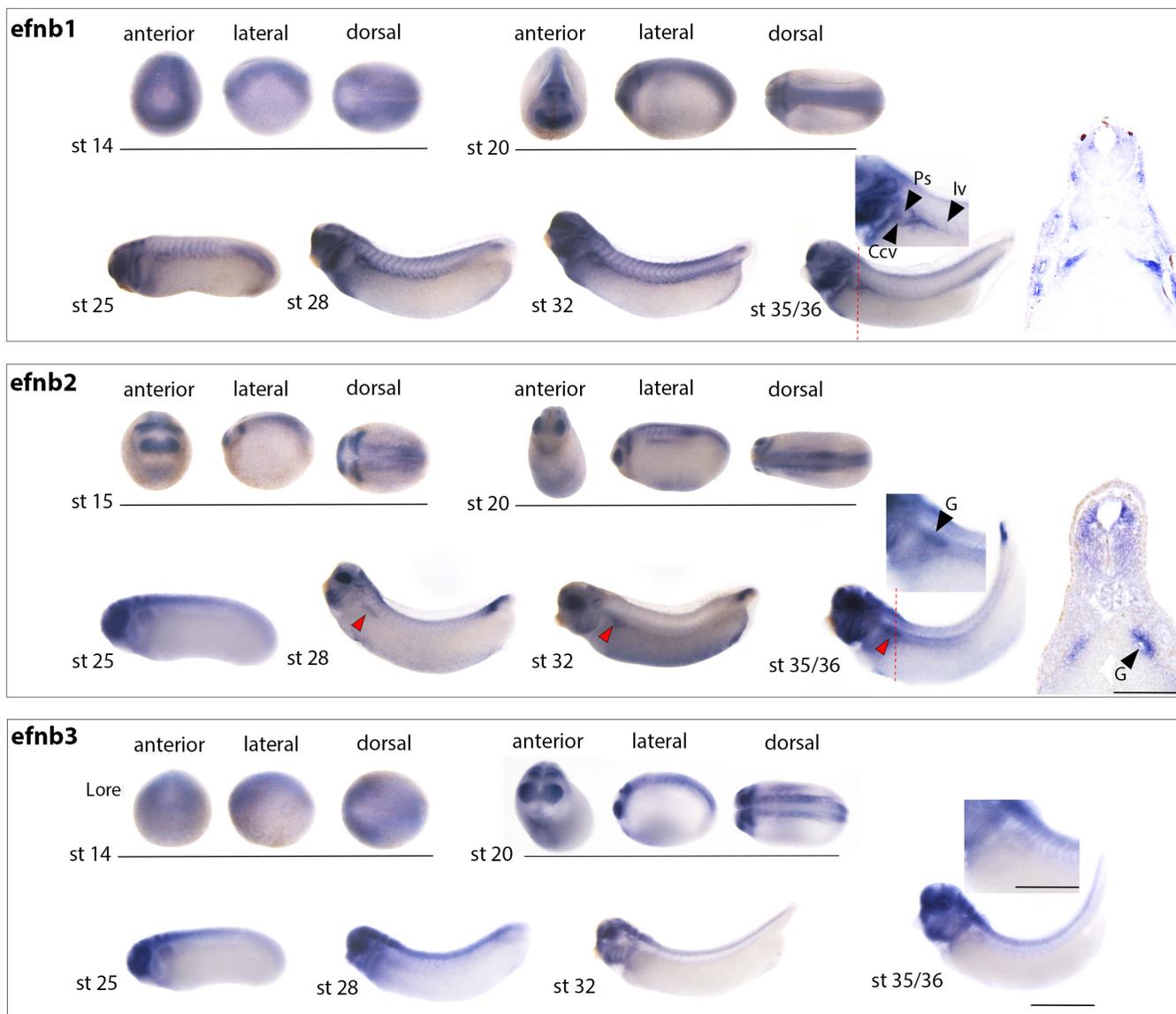
corresponding to:

Comprehensive analysis of Ephrin ligand and receptor expression reveals exclusive domains during nephrogenesis for epha4/epha7 and efna3

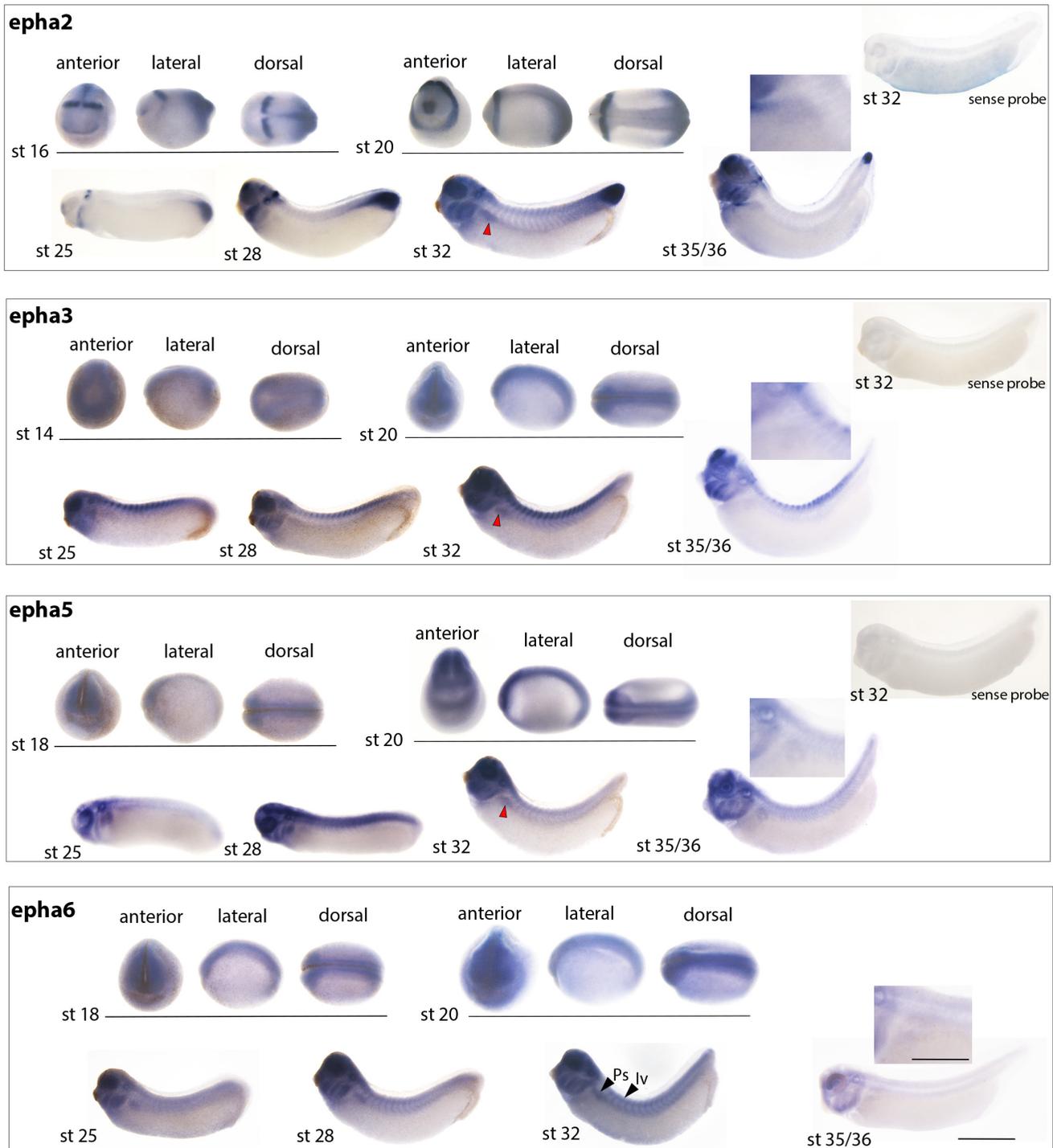
RONAN LE BOUFFANT, VALÉRIE BELLO,
ISABELLE BUISSON, MURIEL UMBHAUER, JEAN-FRANÇOIS RIOU



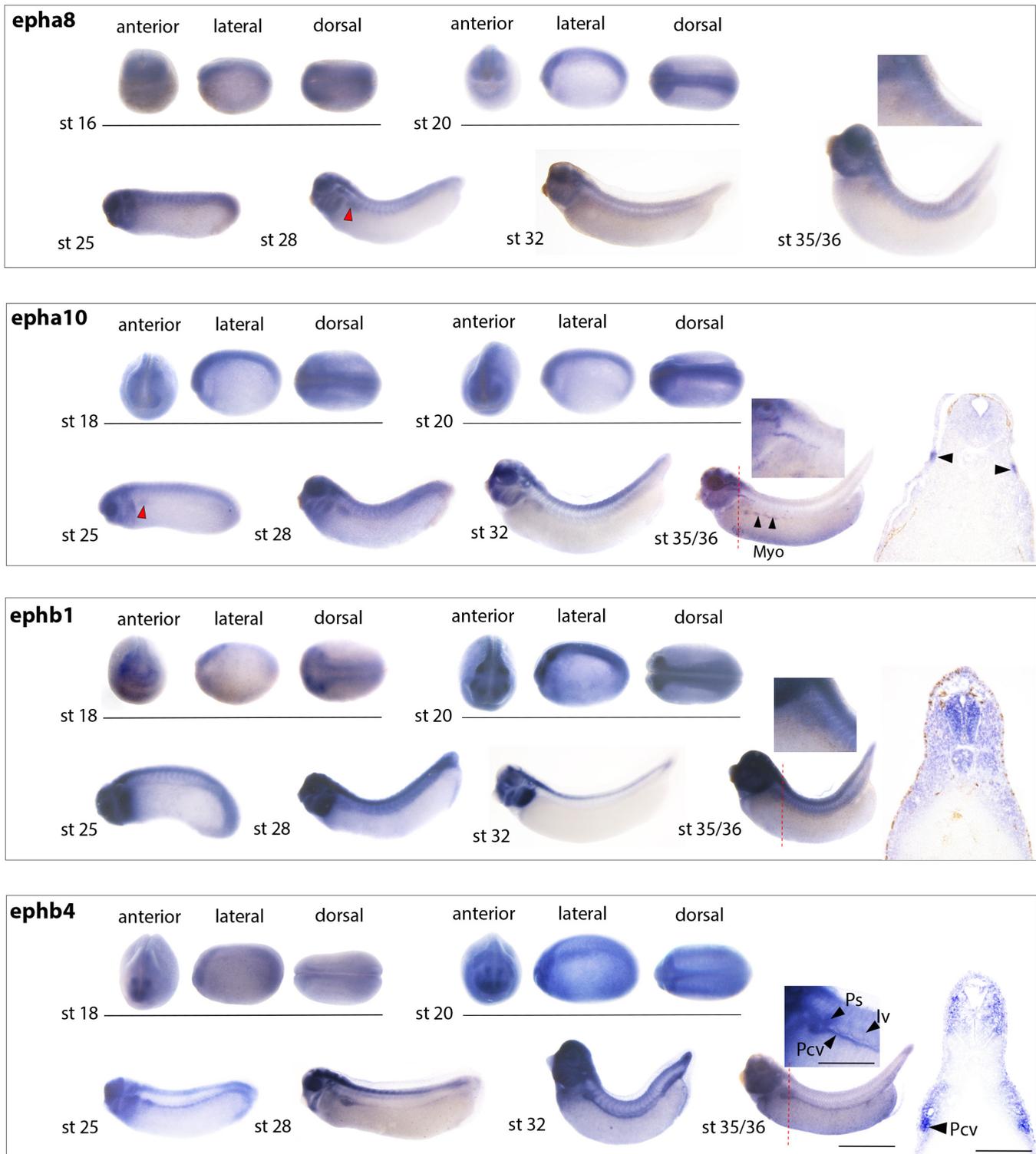
Supplemental Fig. 1. Expression of genes encoding ephrin-a ligands at stages of pronephric development from kidney field (neurula stages 17-18) to functional pronephros (early tadpole stage 35/36), except for *efna3* that is shown in detail in Fig.3. Anterior, dorsal and lateral views are shown at neurula stages (stages 17-18, 20). Only lateral views are shown for tailbud stages 25, 28 and 32. A lateral view with the enlarged pronephric region is shown at early tadpole stage 35/36. From stage 25 onward, low levels of *efna1* and *a4* expression are detected at the level of the pronephric primordium, possibly in the associated developing vasculature. At the early tadpole stage (stage 35/36), *efna1* is expressed in the vasculature associated to the pronephric region. Expression of *efna5* in the pronephric region is observed in the pronephric sinus (Ps). It is also observed in the vascular system, the intersomitic veins (lv), the posterior cardinal vein (Pcv), the common cardinal vein (Ccv) and the heart (H). Expression of *efna2* is detected in the Hindbrain (Hb) at stage 35/36. Scale bars: 1.7 mm for whole embryo, 0.5 mm for embryonic magnification.



Supplemental Fig. 2. Expression of genes encoding ephrin-b ligands at stages of pronephric development from kidney field (neurula stages 14-18) to functional pronephros (early tadpole stage 35/36). Anterior, dorsal and lateral views are shown at neurula stages (st14-18 and 20). Only lateral views are shown for tailbud stages 25, 28 and 32. A lateral view with the enlarged pronephric region is shown at early tadpole stage 35/36. Images of sectioned st 35/36 embryos are shown for *efnb1* and *efnb2* with red dotted lines indicating the corresponding levels on whole mounts (note that the sections were not made from the whole mount specimen shown). Only *efnb2* appears to be expressed in the developing pronephros. Its pronephric expression is detected from tailbud stage 28 in the dorsal region of the pronephric anlage. At tadpole stage 35/36, it appears to be expressed in the glomus (G) at the level of differentiating podocytes (arrowhead on section) (White *et al.*, 2010). *Efnb1* is expressed in the pronephric region, but rather in the pronephric sinus (Ps). It is also detected in other vascular components, the intersomitic veins (Iv) and the common cardinal vein (Ccv). *Efnb3* is not expressed in the developing pronephros. The red arrowheads indicate the pronephric anlage at stage 28 and the pronephros at stage 32 and 35/36. Scale bars: 1.7 mm for whole embryo, 0.5 mm for embryonic magnification and 280 μ m for section.



Supplemental Fig. 3. Expression of genes encoding receptors *epha2*, *a3*, *a5* and *a6* at stages of pronephric development from kidney field (neurula stages 14-18) to functional pronephros (early tadpole stage 35/36). Anterior, dorsal and lateral views are shown at neurula stages (st14-18 and 20). Only lateral views are shown for tailbud stages 25, 28 and 32. A lateral view with the enlarged pronephric region is shown at early tadpole stage 35/36. Expression of *epha2*, *a3* and *a5* is observed in the region of the developing pronephros from stage 32 onward. *Epha6* is detected as soon as the tailbud stage 25. At stage 32 it is detected in the forming intersomitic veins (Iv) and pronephric sinus (Ps). For *epha2*, *a3* and *a5*, *in situ* hybridization with the sense probes was shown at stage 32 as control. Scale bar: 1.7 mm for whole embryo, 0.5 mm for embryo magnification.



Supplemental Fig. 4. Expression of genes encoding receptors *epha8*, *a10*, *b1* and *b4* at stages of pronephric development from kidney field (neurula stages 16-18) to functional pronephros (early tadpole stage 35/36). Anterior, dorsal and lateral views are shown at neurula stages (st16-18 and 20). Only lateral views are shown for tailbud stages 25, 28 and 32. A lateral view with the enlarged pronephric region is shown at early tadpole stage 35/36. Images of sectioned st 35/36 embryos are shown for *epha10*, *ephb1* and *ephb4* with red dotted lines indicating the corresponding levels on whole mounts (note that the sections were not made from the whole mount specimen shown). *Epha8* expression is observed in the developing pronephros (red arrowhead) from tailbud stage 28, while *epha10* is transiently detected in the pronephros (red arrowhead) from tailbud stage 25 to 32. At tadpole stage 35/36 *epha10* is not anymore expressed in pronephros, but rather in migrating hypaxial myoblasts (Myo). *Ephb1* and *ephb4* are not detected in the developing pronephros. Vascular *ephb4* expression in posterior cardinal vein (PcV), pronephric sinus (Ps), and intersomitic veins (Iv) has been previously described (Helbling *et al.*, 2000). Scale bar: 1.7 mm for whole embryo, 0.5 mm for embryo magnification and 280 μ m for section.