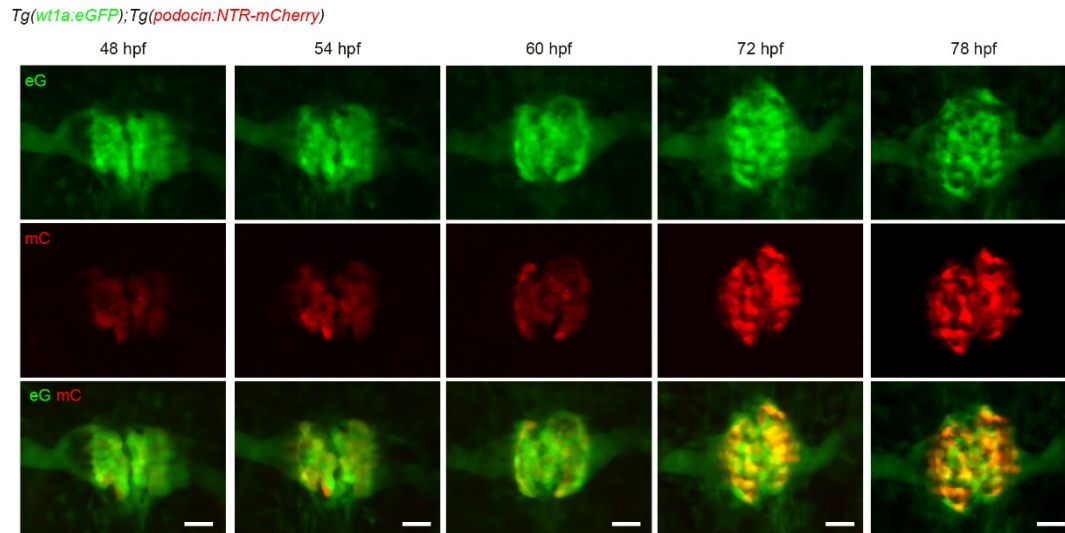


**SUPPLEMENTARY MATERIALS**

**Blood Flow Regulates Glomerular Capillary Formation in Zebrafish Pronephros**

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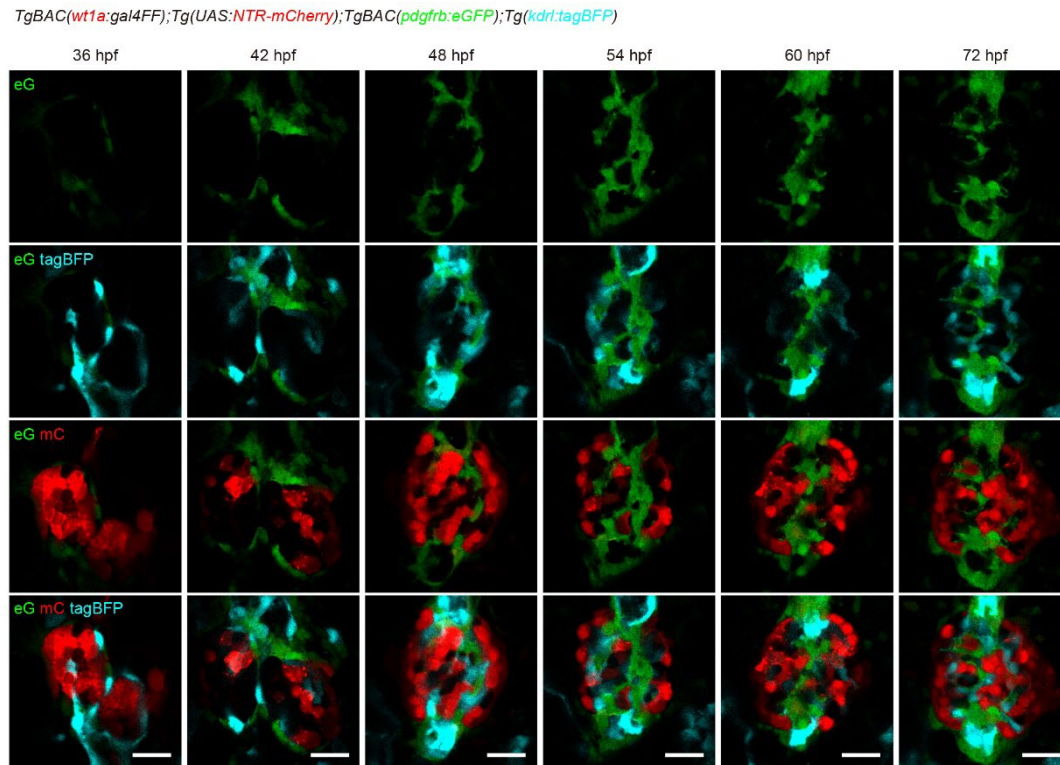
**SUPPLEMENTRAY FIGURES**



Nishimura, Ishii et al. Supplementray Figure 1

**Supplementary Figure 1. Differentiation of podocytes during pronephric glomerulus development**

Confocal z-projection images of the pronephric glomeruli in the *Tg(wt1a:eGFP);Tg(podocin:NTR-mCherry)* embryos at the stage indicated at the top of each column. Dorsal view images; anterior to the top. Upper row, *wt1a:eGFP* images (eG: green); middle row, *podocin:NTR-mCherry* images (mC: red); bottom row, the merged images of *wt1a:eGFP* and *podocin:NTR-mCherry*. Scale bars; 20  $\mu$ m.

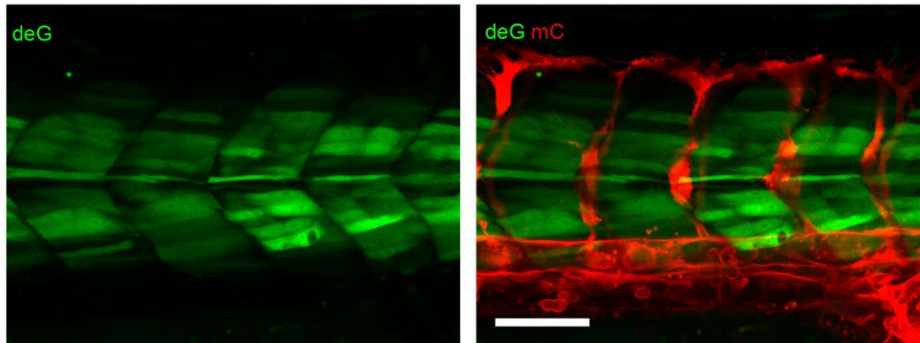


Nishimura, Ishii et al. Supplementray Figure 2

### Supplementary Figure 2. Differentiation of mesangial cells during pronephric glomerulus development

Confocal single plane images of the pronephric glomeruli in *Tg(wt1a:gal4FF);Tg(UAS:NTR-mCherry);Tg(UAS:NTR-mCherry);TgBAC(pdgfrb:eGFP);Tg(kdrl:tagBFP)* embryos and larvae at the stage indicated at the top of each column. Dorsal view images; anterior to the top. Top row, *wt1a:eGFP* images (eG: green); second row, the merged images of *wt1a:eGFP* and *kdrl:tagBFP* (tBFP, cyan); third row, the merged images of *wt1a:eGFP* and *wt1a:gal4FF;UAS:NTR-mCherry* (mC: red); bottom row, the merged images of *wt1a:eGFP*, *wt1a:gal4FF;UAS:NTR-mCherry*, and *kdrl:tagBFP*. Scale bars; 20  $\mu$ m.

*TgBAC(vegfaa:deGFP);Tg(fli1a:Myr-mCherry)*, 28 hpf



Nishimura, Ishii et al. Supplementray Figure 3

**Supplementary Figure 3. *vegfaa* promoter-driven expression of deGFP in the somites of zebrafish embryos**

Confocal z-projection images of the trunk region in the *TgBAC(vegfaa:deGFP);Tg(fli:Myr-mCherry)* embryo at 28 hpf. Left, *vegfaa:deGFP* image (deG; green); right, the merged image of *vegfaa:deGFP* and *fli1a:Myr-mCherry* (mC; red). Scale bars; 20  $\mu$ m.

## SUPPLEMENTARY MOVIE LEGENDS

**Supplementary Movie 1.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos at 28 hpf as observed in Figure 1B. Left, *fli1a:Myr-mCherry* image; right, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*. Scale bar, 20  $\mu$ m.

**Supplementary Movie 2.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos at 32 hpf as observed in Figure 1B. Left, *fli1a:Myr-mCherry* image; right, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*. Scale bar, 20  $\mu$ m.

**Supplementary Movie 3.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos at 39 hpf as observed in Figure 1B. Left, *fli1a:Myr-mCherry* image; right, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*. Scale bar, 20  $\mu$ m.

**Supplementary Movie 4.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos at 48 hpf as observed in Figure 1B. Left, *fli1a:Myr-mCherry* image; right, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*. Scale bar, 20  $\mu$ m.

**Supplementary Movie 5.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos at 96 hpf as observed in Figure 1B. Left, *fli1a:Myr-mCherry* image; right, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*. Scale bar, 20  $\mu$ m.

**Supplementary Movie 6.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos injected with Qdot705 at 35 hpf as observed in Figure 3B. Left, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*; middle, the merged image of *fli1a:Myr-mCherry* and Qdot705; right, Qdot705 image. Scale bar, 20  $\mu$ m.

**Supplementary Movie 7.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos injected with Qdot705 at 38 hpf as observed in Figure 3B. Left, the merged image of *wt1a:EGFP* and

*fli1a:Myr-mCherry*; middle, the merged image of *fli1a:Myr-mCherry* and Qdot705; right, Qdot705 image. Scale bar, 20  $\mu\text{m}$ .

**Supplementary Movie 8.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos injected with Qdot705 at 42 hpf as observed in Figure 3B. Left, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*; middle, the merged image of *fli1a:Myr-mCherry* and Qdot705; right, Qdot705 image. Scale bar, 20  $\mu\text{m}$ .

**Supplementary Movie 9.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos injected with Qdot705 at 46 hpf as observed in Figure 3B. Left, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*; middle, the merged image of *fli1a:Myr-mCherry* and Qdot705; right, Qdot705 image. Scale bar, 20  $\mu\text{m}$ .

**Supplementary Movie 10.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos injected with Qdot705 at 48 hpf as observed in Figure 3B. Left, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*; middle, the merged image of *fli1a:Myr-mCherry* and Qdot705; right, Qdot705 image. Scale bar, 20  $\mu\text{m}$ .

**Supplementary Movie 11.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in 54 hpf *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos treated with vehicle from 36 hpf as observed in Figure 4D. Left, *fli1a:Myr-mCherry* image; right, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*. Scale bar, 20  $\mu\text{m}$ .

**Supplementary Movie 12.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in 54 hpf *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos treated with BDM from 36 hpf as observed in Figure 4D. Left, *fli1a:Myr-mCherry* image; right, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*. Scale bar, 20  $\mu\text{m}$ .

**Supplementary Movie 13.** Dorsal to ventral confocal z-stack series of the pronephric glomerulus in 54 hpf *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos treated with tricine from 36 hpf as observed in Figure 4D. Left, *fli1a:Myr-mCherry* image; right, the merged image of *wt1a:EGFP* and *fli1a:Myr-mCherry*. Scale bar, 20  $\mu\text{m}$ .

**Supplementary Movie 14.** Time-lapse confocal imaging of morphogenetic processes of pronephric glomeruli in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos as observed in Figure 5A. Images of *wt1a:eGFP* (green) and *fli1a:Myr-mCherry* (red) were obtained every 15 min from 33 hpf for 15 h. Elapsed time (hr:min) is shown in the upper right corner. Scale bar, 50  $\mu$ m.

**Supplementary Movie 15.** Time-lapse confocal imaging of morphogenetic processes of pronephric glomeruli in *Tg(wt1a:EGFP);Tg(fli1a:Myr-mCherry)* embryos in the presence of BDM as observed in Figure 5B. Images of *wt1a:eGFP* (green) and *fli1a:Myr-mCherry* (red) were obtained every 15 min from 33.5 hpf for 14.5 h. Elapsed time (hr:min) is shown in the upper right corner. Scale bar, 50  $\mu$ m.