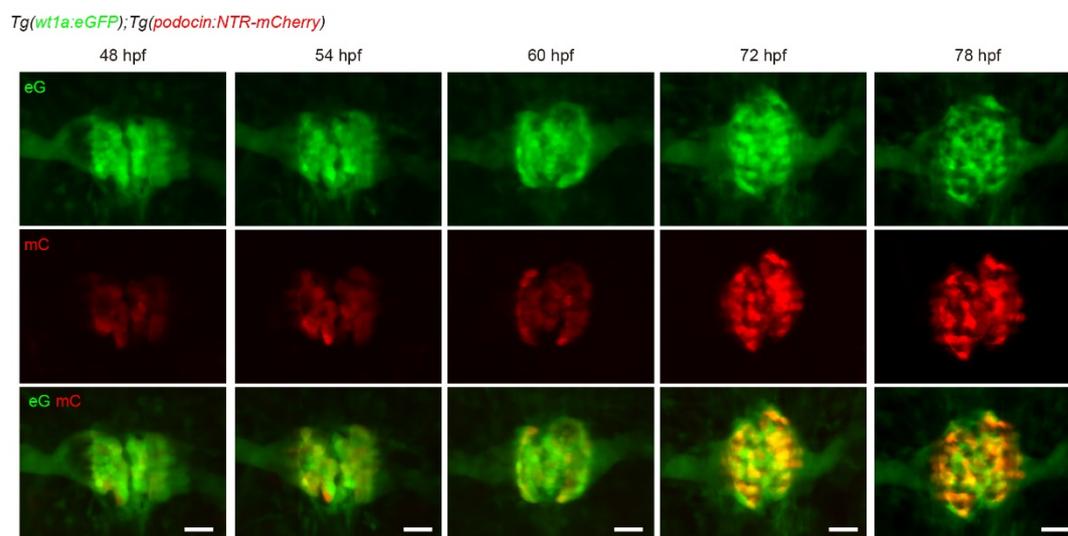


SUPPLEMENTARY MATERIALS

Blood Flow Regulates Glomerular Capillary Formation in Zebrafish Pronephros

Yusuke Nishimura, Tomohiro Ishii, Koji Ando, Shinya Yuge, Hiroyuki Nakajima,
Weibin Zhou, Naoki Mochizuki, Shigetomo Fukuhara

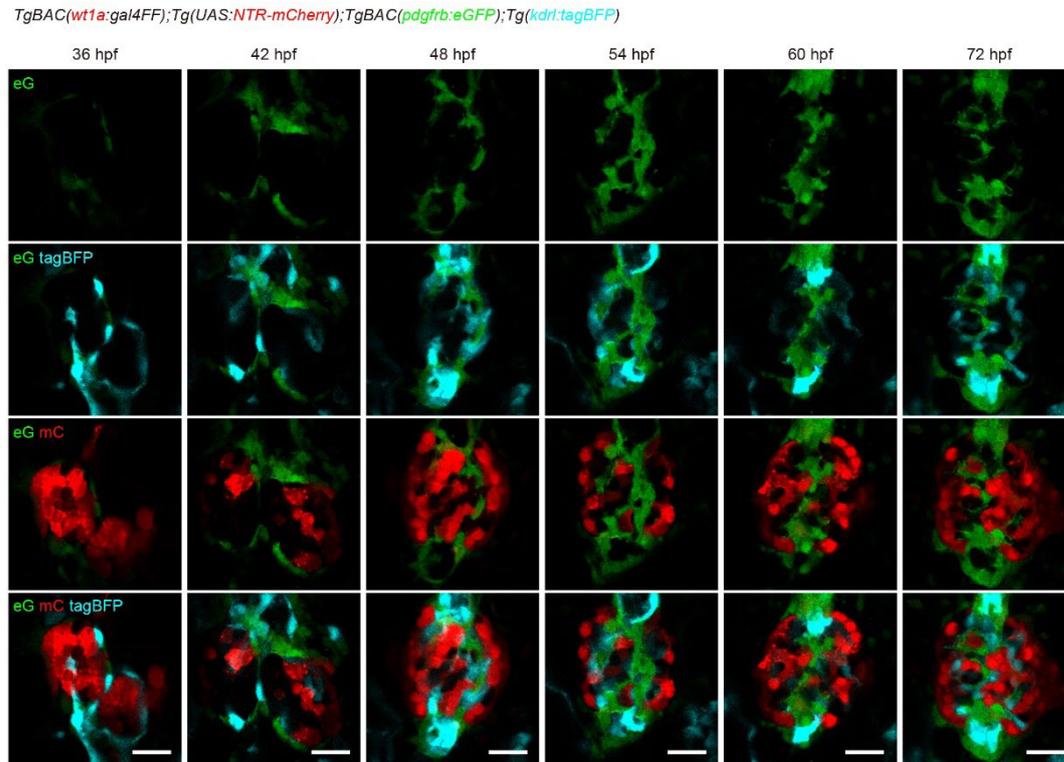
SUPPLEMENTRAY FIGURES



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 μ m.

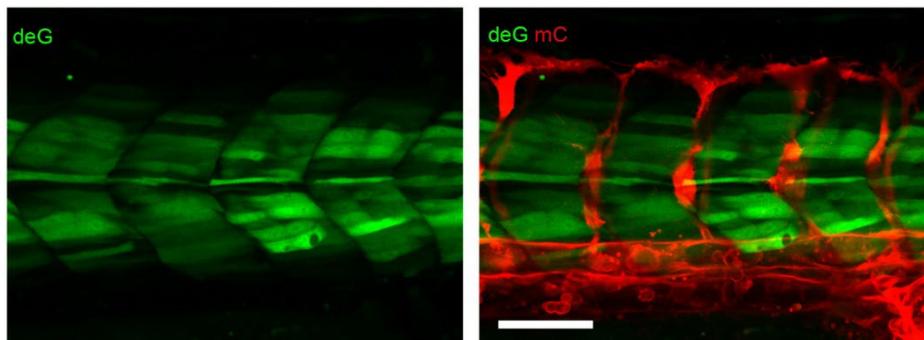


Nishimura, Ishii et al. Supplementray Figure 2

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 μ 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 μ 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 μ 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 μ 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 μ 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 μ 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 μ 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 μ 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 μm .

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 μm .

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 μm .

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 μm .

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 μm .

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 μm .

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 μm .

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 μ 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 μ m.