

Description of Additional Supplementary Files

File Name: Supplementary Data 1

Description: CRISPR sites, primers, and Ct values.

File Name: Supplementary Movie 1

Description: Brightfield live imaging (4.13 msec/frame) of a 54 hpf wild-type heart. The movie depicts the AV flow profile (anterograde, no-flow, and retrograde) during 3 cardiac cycles.

Scale bar: 50 μm .

File Name: Supplementary Movie 2

Description: Brightfield live imaging (4.13 msec/frame) of a 78 hpf wild-type heart. The movie depicts the AV flow profile (anterograde, no-flow, and retrograde) during 3 cardiac cycles.

Scale bar: 50 μm .

File Name: Supplementary Movie 3

Description: Brightfield live imaging (4.13 msec/frame) of a 102 hpf wild-type heart. The movie depicts the AV flow profile (anterograde, no-flow, and retrograde) during 3 cardiac cycles.

Scale bar: 50 μm .

File Name: Supplementary Movie 4

Description: Brightfield live imaging (4.13 msec/frame) of a 78 hpf *pkd1a* mutant heart. The movie depicts the AV flow profile (anterograde, no-flow, and retrograde) during 3 cardiac cycles. Scale bar: 50 μm .

File Name: Supplementary Movie 5

Description: Brightfield live imaging (4.13 msec/frame) of a 78 hpf *pkd111*; *pkd1a*; *pkd2* triple mutant heart. The movie depicts the AV flow profile (anterograde, no-flow, and retrograde) during 3 cardiac cycles. Scale bar: 50 μm .

File Name: Supplementary Movie 6

Description: Calcium live imaging (30 msec/frame) of a 78 hpf wild-type heart using the *fli1a:Gal4FF*; *UAS:GCamP6s* endothelial calcium sensor line during 3 cardiac cycles. Maximal projection of a 4D-assembled spinning disc movie. Scale bar: 50 μm .

File Name: Supplementary Movie 7

Description: Calcium live imaging (30 msec/frame) of a 78 hpf *pkd111*; *pkd1a*; *pkd2* triple mutant heart using the *fli1a:Gal4FF*; *UAS:GCamP6s* endothelial calcium sensor line during 3 cardiac cycles. Maximal projection of a 4D-assembled spinning disc movie. Scale bar: 50 μm .

File Name: Supplementary Movie 8

Description: Calcium live imaging (30 msec/frame) of a 78 hpf *tnnt2a* morphant heart using the *fli1a:Gal4FF*; *UAS:GCamP6s* endothelial calcium sensor line during 3 cardiac cycles. Maximal projection of a 4D-assembled spinning disc movie. Scale bar: 50 μm .