Supplemental Data: Figures, Tables and Videos
Figure S1. Validation of the TrackMate obtained velocities. The movement of the same red blood cell (RBC) in zebrafish #28 of the 2 dpf set imaged under 180 fps is shown in (A). For the validation, the velocity against time profile of the tracked RBC was obtained using TrackMate software and compared against the manual tracking calculation (B). The TrackMate software tracking algorithm provided velocity values in close correspondence with the manual tracking method.
Figure S2. Fåhræus–Lindqvist (FL) effect model for microvessels, where the blood viscosity is dependent on plasma viscosity, lumen tube hematocrit (Ht) and vessel lumen diameter; these calculations are performed using equations 13 to 17 of the main article: Biphasic viscosity against vessel diameter behavior for blood flow in microvessels with a comparatively wide range of diameters over which viscosity reduces with diameter reduction (A and B) and a trend reversal at capillary diameters (C). Examples of the FL effect model is showed on whole blood with plasma viscosity of 0.0012 Pa·s (i) and 0.00146 Pa·s (ii).
Figure S3. Zebrafish heart rate across development for two different experiment sets. Circle points indicate the mean level heartbeat, and whisker bars represent the standard deviation ranges. Set A (blue): 6, 15, 26, 34, 32 and 15 zebrafish were measured for heart rate at 1, 2, 3, 4, 5 and 6 dpf. Set B (green): 18, 30, 32, 38, 35 and 29 zebrafish were measured for heart rate at 1, 2, 3, 4, 5 and 6 dpf. Set B zebrafish were used for all analysis performed and discussed in the main article. The average of two sets data (red) is a pooling of set A and set B data.
Figure S4. Validation of the hematocrit assessment using RBC reduction in networks by morpholino (MO) treatment targeting Gata1. Varied dosage delivery resulted in three hematocrit levels: normal or typical levels for control MO samples C1 (A) and C2 (B); moderate reduction in morphants M1 (C), M2 (D) and M3 (E); and greatly reduced levels in M4 (F) and M5 (G). Red heat color indicates the Gata1:dsRed RBC markers and green indicates the kdrl:EGP lumen EC marker. Yellow arrows denote regions where the diameters and hematocrits were measured for the validation. Videos of circulating RBCs in these respective samples can be seen in SV 6 – SV12. The comparison of hematocrit evaluation by counting against the proposed average intensity correlation method is shown for the DA/CA (H), PCV/CV (I) and ISV (J).
Figure S5. Validation of lumen diameter assessment by comparing method in paper (red) against EC marker peak to peak distance assessment (green) for the DA/CA (Ai), PCV/CV (Bi) and ISV (Ci). Judging against the hematocrit in Aii, Bii and Cii, the discrepancy between the two methods grows as vessel lumen diameters decrease and hematocrits become vanishingly low (hematocrit fraction < 0.01).
Figure S6. Examples of the DA/CA diameter calculation using the dsRed signal and compared against the peak to peak distance evaluation from the kdrl:EGFP lumen EC marker signal. First the RBC core width is estimated using Eqn.(2) in the main text after applying a super-gaussian fitting (red dash) of the maximum projection signal (red bold). The RBC core width ($D_{\text{core}}$) is determined by the full-width half maximum of the super-gaussian and is shown by the purple dash lines in the graphs. Evaluation of the CFL thickness ($\delta_{\text{CFL}}$) was performed iteratively using Eqn. (5) and lumen width ($D$) was updated iteratively using Eqn. (7) until the iteration residual fell < 0.001.
Figure S7. Examples of the PCV/CV diameter calculation using the dsRed signal and compared against the peak to peak distance evaluation from the kdrl:EGFP lumen EC marker signal. First the RBC core width is estimated using Eqn.(2) in the main text after applying a supergaussian fitting (red dash) of the maximum projection signal (red bold). The RBC core width ($D_{core}$) is determined by the full-width half maximum of the supergaussian and is shown by the purple dash lines in the graphs. Evaluation of the CFL thickness ($\delta_{CFL}$) was performed iteratively using Eqn. (5) and lumen width ($D$) was updated iteratively using Eqn. (7) until the iteration residual fell < 0.001.
Figure S8. Examples of the ISV diameter calculation using the dsRed signal and compared against the peak to peak distance evaluation from the kdrl:EGFP lumen EC marker signal. First the RBC core width is estimated using Eqn.(2) in the main text after applying a super-gaussian fitting (red dash) of the maximum projection signal (red bold). The RBC core width ($D_{core}$) is determined by the full-width half maximum of the super-gaussian and is shown by the purple dash lines in the graphs. Evaluation of the CFL thickness ($\delta_{CFL}$) was performed iteratively using Eqn. (5) and lumen width ($D$) was updated iteratively using Eqn. (7) until the iteration residual fell < 0.001.
Figure S9. Validation of the direct correspondence between heartbeat rate and velocity pulsation frequency in the DA. Using zebrafish C2 of the validation experiments data set, the velocity pulsation was monitored in the anterior region of the DA in (boxed region in Ai) and a pulsation frequency of 180 bpm was obtained based on the observation of 30 pulsation cycles over 10 seconds of velocity sampling (Aii). The heartbeat in C2 was obtained by examining the heart wall displacement along the yellow arrow line indicated in Bi: Kymograph pattern along this line presented 12 wall pulsation cycles over 4 seconds, which works out to be a heart rate of 180 bpm (Bii). Since both methods indicated the same 180 bpm frequency, the assumption that velocity pulsation in the DA gives the heartbeat rate is a valid assumption.
Figure S10. Dorsal aorta/caudal artery trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 2 dpf zebrafish.
Figure S11. Dorsal aorta/caudal artery trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 3 dpf zebrafish.
Figure S12. Dorsal aorta/caudal artery trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $W_{\text{SSpeak}}$ [Pa] in 4 dpf zebrafish.
Figure S13. Dorsal aorta/caudal artery trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity Vpeak [µm/s], lumen diameter D [µm], discharge hematocrit fraction Hd [max is 1], apparent blood viscosity η [Pa·s] and systolic wall shear stress WSSpeak [Pa] in 5 dpf zebrafish.
Figure S14. Dorsal aorta/caudal artery trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity \(V\text{peak} \,[\mu m/s]\), lumen diameter \(D \,[\mu m]\), discharge hematocrit fraction \(H_d \,[\text{max is 1}]\), apparent blood viscosity \(\eta \,[Pa\cdot s]\) and systolic wall shear stress \(WSS\text{peak} \,[Pa]\) in 6 dpf zebrafish.
Figure S15. Posterior cardinal vein/caudal vein trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [$\mu$m/s], lumen diameter $D$ [$\mu$m], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 2 dpf zebrafish.
Figure S16. Posterior cardinal vein/caudal vein trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{peak}$ [$\mu$m/s], lumen diameter $D$ [$\mu$m], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{peak}$ [Pa] in 3 dpf zebrafish.
Figure S17. Posterior cardinal vein/caudal vein trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 4 dpf zebrafish.
Figure S18. Posterior cardinal vein/caudal vein trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 5 dpf zebrafish.
Figure S19. Posterior cardinal/caudal vein trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity \( V_{\text{peak}} \) [µm/s], lumen diameter \( D \) [µm], discharge hematocrit fraction \( H_d \) [max is 1], apparent blood viscosity \( \eta \) [Pa·s] and systolic wall shear stress \( WSS_{\text{peak}} \) [Pa] in 6 dpf zebrafish.
Figure S20. Arterial intersegmental vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{peak}$ ($\mu$m/s), lumen diameter $D$ ($\mu$m), discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{peak}$ [Pa] in 2 dpf zebrafish.
Figure S21. Arterial intersegmental vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [$\mu$m/s], lumen diameter $D$ [$\mu$m], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 3 dpf zebrafish.
Figure S22. Arterial intersegmental vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 4 dpf zebrafish.
Figure S23. Arterial intersegmental vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $W_{\text{SSpeak}}$ [Pa] in 5 dpf zebrafish.
Figure S24. Arterial intersegmental vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 6 dpf zebrafish.
Figure S25. Venous intersegmental vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 2 dpf zebrafish.
Figure S26. Venous intersegmental vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [$\mu$m/s], lumen diameter $D$ [$\mu$m], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 3 dpf zebrafish.
Figure S27. Venous intersegmental vessel trends along the normalized anterior-posterior axis ($x$-axis of graphs) for systolic velocity ($V_{peak}$ [µm/s]), lumen diameter ($D$ [µm]), discharge hematocrit fraction ($H_d$ [max is 1]), apparent blood viscosity ($\eta$ [Pa·s]) and systolic wall shear stress ($WSS_{peak}$ [Pa]) in 4 dpf zebrafish.
Figure S28. Venous intersegmental vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ (max is 1), apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 5 dpf zebrafish.
Figure S29. Venous intersegmental vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [$\mu$m/s], lumen diameter $D$ [$\mu$m], discharge hematocrit fraction $H_d$ (max is 1), apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 6 dpf zebrafish.
Figure S30. Dorsal longitudinal anastomotic vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{peak}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{peak}$ [Pa] in 2 dpf zebrafish.
Figure S31. Dorsal longitudinal anastomotic vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{peak}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{peak}$ [Pa] in 3 dpf zebrafish.
Figure S32. Dorsal longitudinal anastomotic vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{peak}$ [µm/s], lumen diameter $D$ [µm], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{peak}$ [Pa] in 4 dpf zebrafish.
Figure S33. Dorsal longitudinal anastomotic vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity $V_{\text{peak}}$ [$\mu$m/s], lumen diameter $D$ [$\mu$m], discharge hematocrit fraction $H_d$ [max is 1], apparent blood viscosity $\eta$ [Pa·s] and systolic wall shear stress $WSS_{\text{peak}}$ [Pa] in 5 dpf zebrafish.
Figure S34. Dorsal longitudinal anastomotic vessel trends along the normalized anterior-posterior axis (x-axis of graphs) for systolic velocity Vpeak [µm/s], lumen diameter D [µm], discharge hematocrit fraction Hd [max is 1], apparent blood viscosity η [Pa·s] and systolic wall shear stress WSSpeak [Pa] in 6 dpf zebrafish.
Figure S35. Linear regression fit to describe the anterior-posterior (AP) trend for analyzed AP-position averaged quantities in the DA/CA (see Fig. 2C of main article for definition of AP-position averaging) after data pooling from multiple zebrafish at same developmental day [dpf]. Trends for individual fish were shown in figures S10 – S34.
Supplementary table 1. Regression statistics for pooled DA/C4A data

| DA/C4A | Peak velocity table | Discharge hematocrit table | Discharge WSSpeak table | Peak WSS table |
|--------|---------------------|-----------------------------|-------------------------|-----------------|
| d.f. n | Regression model for data from g > 0.2 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 |
| 2 704 | peak = -1593.4 + 1438.7 4.1832 | 0.001314 | 0.001314 | 0.001314 |
| 3 1058 | peak = -1662.2 + 1930.9 0.15714 | 0.001314 | 0.001314 | 0.001314 |
| 4 1213 | peak = +1392.5 ± 1935.6 3.597 | 0.001314 | 0.001314 | 0.001314 |
| 5 991 | peak = -1855.7 ± 1585.4 4.0238 | 0.001314 | 0.001314 | 0.001314 |
| 6 802 | peak = -15899.1 ± 805.4 0.14829 | 0.001314 | 0.001314 | 0.001314 |
| Lumen diameter table | Regression model for data from g > 0.2 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 |
| d.f. n | Regression model for data from g > 0.2 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 |
| 2 704 | peak = -1593.4 + 1438.7 4.1832 | 0.001314 | 0.001314 | 0.001314 |
| 3 1058 | peak = -1662.2 + 1930.9 0.15714 | 0.001314 | 0.001314 | 0.001314 |
| 4 1213 | peak = +1392.5 ± 1935.6 3.597 | 0.001314 | 0.001314 | 0.001314 |
| 5 991 | peak = -1855.7 ± 1585.4 4.0238 | 0.001314 | 0.001314 | 0.001314 |
| 6 802 | peak = -15899.1 ± 805.4 0.14829 | 0.001314 | 0.001314 | 0.001314 |
| Viscosity table | Regression model for data from g > 0.2 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 |
| d.f. n | Regression model for data from g > 0.2 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 |
| 2 704 | peak = -1593.4 + 1438.7 4.1832 | 0.001314 | 0.001314 | 0.001314 |
| 3 1058 | peak = -1662.2 + 1930.9 0.15714 | 0.001314 | 0.001314 | 0.001314 |
| 4 1213 | peak = +1392.5 ± 1935.6 3.597 | 0.001314 | 0.001314 | 0.001314 |
| 5 991 | peak = -1855.7 ± 1585.4 4.0238 | 0.001314 | 0.001314 | 0.001314 |
| 6 802 | peak = -15899.1 ± 805.4 0.14829 | 0.001314 | 0.001314 | 0.001314 |
| WSSpeak table | Regression model for data from g > 0.2 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 |
| d.f. n | Regression model for data from g > 0.2 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 | D = 0.15495 ± 0.0084 |
| 2 704 | peak = -1593.4 + 1438.7 4.1832 | 0.001314 | 0.001314 | 0.001314 |
| 3 1058 | peak = -1662.2 + 1930.9 0.15714 | 0.001314 | 0.001314 | 0.001314 |
| 4 1213 | peak = +1392.5 ± 1935.6 3.597 | 0.001314 | 0.001314 | 0.001314 |
| 5 991 | peak = -1855.7 ± 1585.4 4.0238 | 0.001314 | 0.001314 | 0.001314 |
| 6 802 | peak = -15899.1 ± 805.4 0.14829 | 0.001314 | 0.001314 | 0.001314 |
### Supplementary table 2. Regression statistics for pooled CV/CV data

**Peak velocity table**

| dP| n | Regression model from data | F | S.E.R | p value |
|---|---|-----------------------------|---|-------|---------|
| 2 | 723 | \[\dot{v}_{peak} = 463.422 + 159.32 \times D \] | 0.080891 | 0.515697 | 3.75 < 0.05 |
| 3 | 527 | \[\dot{v}_{peak} = 350.883 + 1.52 \times D \] | 0.591937 | 0.752568 | 3.43 < 0.05 |
| 4 | 886 | \[\dot{v}_{peak} = 423.742 + 562.8 \times D \] | 0.011074 | 0.577945 | 3.56 < 0.05 |
| 5 | 920 | \[\dot{v}_{peak} = 430.113 + 673.74 \times D \] | 0.137825 | 0.912997 | 3.06 < 0.05 |
| 6 | 647 | \[\dot{v}_{peak} = 45.0813 + 36.82 \times D \] | 0.007078 | 0.265549 | 4.06 < 0.05 |

**Lumen diameter table**

| dP| n | Regression model from data | F | S.E.R | p value |
|---|---|-----------------------------|---|-------|---------|
| 2 | 723 | \[D = 14.092 + 2.59 \times D \] | 0.029171 | 0.203645 | 3.43 < 0.05 |
| 3 | 527 | \[D = 16.085 + 31.30 \times D \] | 0.171087 | 0.925657 | 3.56 < 0.05 |
| 4 | 886 | \[D = 10.364 + 21.84 \times D \] | 0.004025 | 0.404439 | 4.06 < 0.05 |
| 5 | 920 | \[D = 11.435 + 24.67 \times D \] | 0.168783 | 1.012952 | 3.56 < 0.05 |
| 6 | 647 | \[D = 7.5428 + 21.63 \times D \] | 0.239531 | 0.823880 | 3.56 < 0.05 |

**Lumbar blood flow table**

| dP| n | Regression model from data | F | S.E.R | p value |
|---|---|-----------------------------|---|-------|---------|
| 2 | 723 | \[\dot{H} = -0.02622 + 0.1775 \times D \] | 0.000402 | 0.801248 | 3.87 < 0.05 |
| 3 | 527 | \[\dot{H} = 0.01304 + 0.1871 \times D \] | 0.032381 | 0.797228 | 3.87 < 0.05 |
| 4 | 886 | \[\dot{H} = 0.02159 + 0.2011 \times D \] | 0.005857 | 0.266326 | 4.06 < 0.05 |
| 5 | 920 | \[\dot{H} = 0.0317 + 0.2221 \times D \] | 0.011391 | 0.824076 | 3.87 < 0.05 |
| 6 | 647 | \[\dot{H} = 0.04126 + 0.2693 \times D \] | 0.011391 | 0.824076 | 3.87 < 0.05 |

**Peak SS table**

| dP| n | Regression model from data | F | S.E.R | p value |
|---|---|-----------------------------|---|-------|---------|
| 2 | 723 | \[SSpeak = 0.1332 + 1.45 \times D \] | 0.000402 | 0.801248 | 3.87 < 0.05 |
| 3 | 527 | \[SSpeak = 0.3200 + 0.2011 \times D \] | 0.032381 | 0.797228 | 3.87 < 0.05 |
| 4 | 886 | \[SSpeak = 0.40402 + 0.8711 \times D \] | 0.005857 | 0.266326 | 4.06 < 0.05 |
| 5 | 920 | \[SSpeak = 0.50112 + 0.4761 \times D \] | 0.011391 | 0.824076 | 3.87 < 0.05 |
| 6 | 647 | \[SSpeak = 0.0112 + 0.1046 \times D \] | 0.000384 | 0.801248 | 3.87 < 0.05 |
**Regression statistics for pooled aSV data**

Supplementary table 3.

| n | Regression model for data from n = 0.02 | df | p value | F | r.s.r. | p value | gradient | df | r.s.r. | p value | %change | %change |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 208 | Spear = 326.156 + 115.51 & 0.043868 56.14 2.35012 0.004033 151.26 124.75 768.75 25.0 |
| 3 | 366 | Spear = 117.792 + 1403.1 0.179013 111.395 0.21217 0.014863 111.15 1402.75 768.75 25.0 |
| 4 | 404 | Spear = 56.215 + 864.78 0.004652 135.316 1.005 0.0048 84.78 58.21 768.75 25.0 |
| 5 | 397 | Spear = 397.36 + 680.46 0.211401 154.4484 6.525 0 80.46 197.30 600.84 238.30 |
| 6 | 179 | Spear = -50.991 + 405.61 0.093295 205.724 0.465 0.00453 405.63 155.99 379.42 258.64 |

Lumen diameter table

| n | Regression model for data from n = 0.02 | df | p value | F | r.s.r. | p value | gradient | df | r.s.r. | p value | %change | %change |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 208 | D = 2.38E + 5.643 0.10724 2.998 0.01944 0.02958 0.0611 106.56 |
| 3 | 366 | D = 1.84E + 9.046 0.00366 1.321 0.00842 0.00984 0.0709 78.74 |
| 4 | 404 | D = 3.03E + 7.846 0.59379 0.20489 0.00488 0.0558 0.00488 0.00488 |
| 5 | 397 | D = 1.34E + 0.185 0.757 0.113 0.01578 0.07065 0.3326 20.08 |
| 6 | 179 | D = 1.49E + 10.039 0.00842 0.7190 0.0103 0.149 0.1149 0.1149 |

Discharge hematocrit table

| n | Regression model for data from n = 0.02 | df | p value | F | r.s.r. | p value | gradient | df | r.s.r. | p value | %change | %change |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 208 | H = 0.37E + 0.02 0.02117 0.0125 0.00298 0.00416 106.56 |
| 3 | 366 | H = 0.027E + 0.029 0.00005 0.000 0.000 0.000 |
| 4 | 404 | H = 0.038E + 0.023 0.04371 0.04507 0.0024 0.004 0.0048 0.0048 |
| 5 | 397 | H = 0.038E + 0.023 0.04371 0.04507 0.0024 0.004 0.0048 0.0048 |
| 6 | 179 | H = 0.038E + 0.023 0.04371 0.04507 0.0024 0.004 0.0048 0.0048 |

Visibility table

| n | Regression model for data from n = 0.02 | df | p value | F | r.s.r. | p value | gradient | df | r.s.r. | p value | %change | %change |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 208 | & 7.36E + 0.125 0.1213 0.1046 0.00213 0.00213 0.0120 1.95 |
| 3 | 366 | & 1.84E + 0.125 0.01317 0.0038 0.00112 0.00112 0.00112 0.00112 |
| 4 | 404 | & 1.36E + 0.125 0.00044 0.0057 0.00044 0.00044 0.00044 0.00044 |
| 5 | 397 | & 6.31E + 0.125 0.01199 0.0069 0.0012 0.0012 0.0012 0.0012 |
| 6 | 179 | & 7.08E + 0.125 0.1213 0.1046 0.00213 0.00213 0.0120 1.95 |

WSSpeak table

| n | Regression model for data from n = 0.02 | df | p value | F | r.s.r. | p value | gradient | df | r.s.r. | p value | %change | %change |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 208 | WSSpeak = -50.991 + 405.61 0.093295 205.724 0.465 0.00453 405.63 155.99 379.42 258.64 |
| 3 | 366 | WSSpeak = 0.40E + 0.017 0.757 0.113 0.01578 0.07065 0.3326 20.08 |
| 4 | 404 | WSSpeak = 0.34E + 0.045 0.30979 0.3976 0.00488 0.00488 0.00488 0.00488 |
| 5 | 397 | WSSpeak = 0.39E + 0.045 0.30979 0.3976 0.00488 0.00488 0.00488 0.00488 |
| 6 | 179 | WSSpeak = 0.018E + 0.357 0.14705 0.4302 0.0027 0.00428 0.00428 0.00428 |

Peak PSR table

| n | Regression model for data from n = 0.02 | df | p value | F | r.s.r. | p value | gradient | df | r.s.r. | p value | %change | %change |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 208 | PSR = 7.08E + 0.03 0.05 54.16 0.00121 0.00121 0.00121 0.00121 |
| 3 | 366 | PSR = 8.51E + 1.242 0.0027 0.0027 0.0027 0.0027 |
| 4 | 404 | PSR = 1.36E + 1.25 0.00044 0.00044 0.00044 0.00044 |
| 5 | 397 | PSR = 0.96E + 1.151 0.07382 0.841778 7.95E 0.0151 0.096 0.096 0.096 |
| 6 | 179 | PSR = 8.52E + 0.03 0.05 54.16 0.00121 0.00121 0.00121 0.00121 |

Regression statistics for pooled aSV data
Supplementary table 4. Regression table for pooled vLSV data

| dfp | n | Regression model for data from | R² | S.E.R | p < 0.05 | p < 0.01 | %change | %change | %change |
|-----|---|--------------------------------|----|------|---------|---------|---------|---------|---------|
| 2   | 192 | ypeak + 167.991 + 760.46 | 0.001058 | 276.6949 | 1.075 | NO | 760.46 | 760.46 | 760.46 |
| 2   | 395 | ypeak + 41.310 e + 747.56 | 0.001058 | 276.6949 | 1.075 | NO | 747.56 | 747.56 | 747.56 |
| 2   | 424 | ypeak + 12.724 e + 574.44 | 0.170444 | 747.56 | 2.956 | YES | 424 | 424 | 424 |
| 2   | 384 | ypeak + 111.166 e + 473.51 | 0.072859 | 244.4551 | 7.186 | YES | 473.51 | 473.51 | 473.51 |
| 2   | 385 | ypeak + 51.769 e + 297.29 | 0.001038 | 117.9216 | 0.870 | YES | 297.29 | 297.29 | 297.29 |

Lumen diameter table

| dfp | n | Regression model for data from | R² | S.E.R | p < 0.05 | p < 0.01 | %change | %change | %change |
|-----|---|--------------------------------|----|------|---------|---------|---------|---------|---------|
| 2   | 192 | D = 0.0518e + 1.6808 | 0.001058 | 276.6949 | 1.075 | NO | 1.6808 | 1.6808 | 1.6808 |
| 2   | 395 | D = 0.1212e + 1.0427 | 0.001058 | 276.6949 | 1.075 | NO | 1.0427 | 1.0427 | 1.0427 |
| 2   | 424 | D = 0.1256e + 0.7714 | 0.170444 | 747.56 | 2.956 | YES | 0.7714 | 0.7714 | 0.7714 |
| 2   | 384 | D = 0.8332e + 9.7884 | 0.072859 | 244.4551 | 7.186 | YES | 9.7884 | 9.7884 | 9.7884 |
| 2   | 385 | D = 0.8494e + 10.376 | 0.072859 | 244.4551 | 7.186 | YES | 10.084 | 10.084 | 10.084 |

Discharge hemodynamic table

| dfp | n | Regression model for data from | R² | S.E.R | p < 0.05 | p < 0.01 | %change | %change | %change |
|-----|---|--------------------------------|----|------|---------|---------|---------|---------|---------|
| 2   | 192 | Hal + 0.0556e + 0.3672 | 0.001058 | 276.6949 | 1.075 | NO | 0.3672 | 0.3672 | 0.3672 |
| 2   | 395 | Hal + 0.0087e + 0.0092 | 0.001058 | 276.6949 | 1.075 | NO | 0.0092 | 0.0092 | 0.0092 |
| 2   | 424 | Hal + 0.0734e + 0.0972 | 0.170444 | 747.56 | 2.956 | YES | 0.0972 | 0.0972 | 0.0972 |
| 2   | 384 | Hal + 0.1223e + 0.1564 | 0.072859 | 244.4551 | 7.186 | YES | 0.1564 | 0.1564 | 0.1564 |
| 2   | 385 | Hal + 0.0734e + 0.1564 | 0.072859 | 244.4551 | 7.186 | YES | 0.1564 | 0.1564 | 0.1564 |

Ventricular table

| dfp | n | Regression model for data from | R² | S.E.R | p < 0.05 | p < 0.01 | %change | %change | %change |
|-----|---|--------------------------------|----|------|---------|---------|---------|---------|---------|
| 2   | 192 | n + 3.44e -1.2103 | 0.001058 | 276.6949 | 1.075 | NO | 1.2103 | 1.2103 | 1.2103 |
| 2   | 395 | n + 2.85e -1.2103 | 0.001058 | 276.6949 | 1.075 | NO | 1.2103 | 1.2103 | 1.2103 |
| 2   | 424 | n + 5.31e -1.2103 | 0.170444 | 747.56 | 2.956 | YES | 1.2103 | 1.2103 | 1.2103 |
| 2   | 384 | n + 7.20e -1.2103 | 0.072859 | 244.4551 | 7.186 | YES | 1.2103 | 1.2103 | 1.2103 |
| 2   | 385 | n + 7.49e -1.2103 | 0.072859 | 244.4551 | 7.186 | YES | 1.2103 | 1.2103 | 1.2103 |

Peak VW5 table

| dfp | n | Regression model for data from | R² | S.E.R | p < 0.05 | p < 0.01 | %change | %change | %change |
|-----|---|--------------------------------|----|------|---------|---------|---------|---------|---------|
| 2   | 192 | VWpeak + 0.0051e + 0.446 | 0.001058 | 276.6949 | 1.075 | NO | 0.446 | 0.446 | 0.446 |
| 2   | 395 | VWpeak + 0.0051e + 0.446 | 0.001058 | 276.6949 | 1.075 | NO | 0.446 | 0.446 | 0.446 |
| 2   | 424 | VWpeak + 0.0151e + 0.7077 | 0.170444 | 747.56 | 2.956 | YES | 0.7077 | 0.7077 | 0.7077 |
| 2   | 384 | VWpeak + 0.0151e + 0.7077 | 0.072859 | 244.4551 | 7.186 | YES | 0.7077 | 0.7077 | 0.7077 |
| 2   | 385 | VWpeak + 0.0151e + 0.7077 | 0.072859 | 244.4551 | 7.186 | YES | 0.7077 | 0.7077 | 0.7077 |

Peak PSR table

| dfp | n | Regression model for data from | R² | S.E.R | p < 0.05 | p < 0.01 | %change | %change | %change |
|-----|---|--------------------------------|----|------|---------|---------|---------|---------|---------|
| 2   | 192 | PSR = 7.734 e + 7.9683 | 0.001058 | 276.6949 | 1.075 | NO | 7.9683 | 7.9683 | 7.9683 |
| 2   | 395 | PSR = 7.8655 e + 8.9309 | 0.001058 | 276.6949 | 1.075 | NO | 8.9309 | 8.9309 | 8.9309 |
| 2   | 424 | PSR = 10.3679 e + 9.6205 | 0.170444 | 747.56 | 2.956 | YES | 9.6205 | 9.6205 | 9.6205 |
| 2   | 384 | PSR = 11.151 e + 24.548 | 0.072859 | 244.4551 | 7.186 | YES | 24.548 | 24.548 | 24.548 |
| 2   | 385 | PSR = 11.151 e + 24.548 | 0.072859 | 244.4551 | 7.186 | YES | 24.548 | 24.548 | 24.548 |
## Supplementary table 5. Regression statistics for pooled DLAV data

### Peak velocity table

| ddp | n | Regression model for data from E < 0.2 | R | S.E.R | p<0.05? | gradient | % change btw p<0.2 and p<1 |
|-----|---|--------------------------------------|---|-------|---------|----------|----------------------------|
| 2   | 197 | ypeak = 197.32 + 0.84x | 0.7463 | 37.239 | 0.0803 | 1.0082 | 13.30102 5.14E-03 | 9.84 0.85 |
| 3   | 408 | ypeak = 1.2E+05 + 1.8249x | 0.3819 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |
| 4   | 409 | ypeak = 0.27E+05 + 1.0082x | 0.0163 | 0.0197 | 10.42 9.705 |
| 5   | 366 | ypeak = 1.2E+05 + 1.8249x | 0.3941 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |
| 6   | 128 | ypeak = 1.2E+05 + 1.8249x | 0.3941 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |

### Discharge rate table

| ddp | n | Regression model for data from E < 0.2 | R | S.E.R | p<0.05? | gradient | % change btw p<0.2 and p<1 |
|-----|---|--------------------------------------|---|-------|---------|----------|----------------------------|
| 2   | 197 | D = 0.76E+03 + 1.2384x | 0.7463 | 37.239 | 0.0803 | 1.0082 | 13.30102 5.14E-03 | 9.84 0.85 |
| 3   | 408 | D = 1.2E+05 + 1.8249x | 0.3819 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |
| 4   | 409 | D = 0.27E+05 + 1.0082x | 0.0163 | 0.0197 | 10.42 9.705 |
| 5   | 366 | D = 0.137E+05 + 1.8249x | 0.3941 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |
| 6   | 128 | D = 0.67E+05 + 1.8249x | 0.3941 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |

### Viscosity table

| ddp | n | Regression model for data from E < 0.2 | R | S.E.R | p<0.05? | gradient | % change btw p<0.2 and p<1 |
|-----|---|--------------------------------------|---|-------|---------|----------|----------------------------|
| 2   | 197 | η = 2.0E+03 + 1.2384x | 0.7463 | 37.239 | 0.0803 | 1.0082 | 13.30102 5.14E-03 | 9.84 0.85 |
| 3   | 408 | η = 2.0E+05 + 1.8249x | 0.3819 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |
| 4   | 409 | η = 0.27E+05 + 1.0082x | 0.0163 | 0.0197 | 10.42 9.705 |
| 5   | 366 | η = 4.4E+05 + 1.8249x | 0.3941 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |
| 6   | 128 | η = 4.3E+05 + 1.8249x | 0.3941 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |

### Discharge & PSR table

| ddp | n | Regression model for data from E < 0.2 | R | S.E.R | p<0.05? | gradient | % change btw p<0.2 and p<1 |
|-----|---|--------------------------------------|---|-------|---------|----------|----------------------------|
| 2   | 197 | PSR = 0.03122 + 1.1325x | 0.7463 | 37.239 | 0.0803 | 1.0082 | 13.30102 5.14E-03 | 9.84 0.85 |
| 3   | 408 | PSR = 5.85E+03 + 25.14x | 0.3941 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |
| 4   | 409 | PSR = 0.25E+05 + 11.345x | 0.0163 | 0.0197 | 10.42 9.705 |
| 5   | 366 | PSR = 1.2E+05 + 11.345x | 0.3941 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |
| 6   | 128 | PSR = 0.28E+05 + 11.345x | 0.3941 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |

### Lumen diameter table

| ddp | n | Regression model for data from E < 0.2 | R | S.E.R | p<0.05? | gradient | % change btw p<0.2 and p<1 |
|-----|---|--------------------------------------|---|-------|---------|----------|----------------------------|
| 2   | 197 | D = 0.76E+03 + 1.2384x | 0.7463 | 37.239 | 0.0803 | 1.0082 | 13.30102 5.14E-03 | 9.84 0.85 |
| 3   | 408 | D = 1.00E+05 + 1.2384x | 0.7463 | 37.239 | 0.0803 | 1.0082 | 13.30102 5.14E-03 | 9.84 0.85 |
| 4   | 409 | D = 0.27E+05 + 1.0082x | 0.0163 | 0.0197 | 10.42 9.705 |
| 5   | 366 | D = 0.137E+05 + 1.8249x | 0.3941 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |
| 6   | 128 | D = 0.67E+05 + 1.8249x | 0.3941 | 5.984 | 0.0161 | 0.0197 | 10.42 9.705 |
Legend for supplemental videos

Video 1: Representative RBC flow in zebrafish from the high throughput experiment at 2 dpf. Shown in the video is zebrafish #28 from the 2 dpf population group with flow recorded at 180 fps for 1000 frames. The video is played back at 180 fps.

Video 2: Representative RBC flow in zebrafish from the high throughput experiment at 3 dpf. Shown in the video is zebrafish #30 from the 3 dpf population group with flow recorded at 180 fps for 1000 frames. The video is played back at 180 fps.

Video 3: Representative RBC flow in zebrafish from the high throughput experiment at 4 dpf. Shown in the video is zebrafish #37 from the 4 dpf population group with flow recorded at 120 fps for 1000 frames. The video is played back at 120 fps.

Video 4: Representative RBC flow in zebrafish from the high throughput experiment at 5 dpf. Shown in the video is zebrafish #33 from the 5 dpf population group with flow recorded at 100 fps for 1000 frames. The video is played back at 100 fps.

Video 5: Representative RBC flow in zebrafish from the high throughput experiment at 6 dpf. Shown in the video is zebrafish #29 from the 6 dpf population group with flow recorded at 100 fps for 1000 frames. The video is played back at 100 fps.

Video 6: RBC flow in zebrafish at 2 dpf imaged at 80x magnification for the validation experiments. Shown in the video is zebrafish C1 that received control morpholino treatment resulting in normal hematocrit levels. The blood flow was recorded at 100 fps for 2000 frames. The video is played back at 100 fps.

Video 7: RBC flow in zebrafish at 2 dpf imaged at 80x magnification for the validation experiments. Shown in the video is zebrafish C2 that received control morpholino treatment resulting in normal hematocrit levels. The blood flow was recorded at 100 fps for 2000 frames. The video is played back at 100 fps.

Video 8: RBC flow in zebrafish at 2 dpf imaged at 80x magnification for the validation experiments. Shown in the video is zebrafish M1 that received gata1 morpholino treatment resulting in moderately reduced hematocrit levels. The blood flow was recorded at 100 fps for 2000 frames. The video is played back at 100 fps.

Video 9: RBC flow in zebrafish at 2 dpf imaged at 80x magnification for the validation experiments. Shown in the video is zebrafish M2 that received gata1 morpholino treatment resulting in moderately reduced hematocrit levels. The blood flow was recorded at 100 fps for 2000 frames. The video is played back at 100 fps.

Video 10: RBC flow in zebrafish at 2 dpf imaged at 80x magnification for the validation experiments. Shown in the video is zebrafish M3 that received gata1 morpholino treatment resulting in moderately reduced hematocrit levels. The blood flow was recorded at 100 fps for 2000 frames. The video is played back at 100 fps.

Video 11: RBC flow in zebrafish at 2 dpf imaged at 80x magnification for the validation experiments. Shown in the video is zebrafish M4 that received gata1 morpholino treatment resulting in greatly reduced hematocrit levels. The blood flow was recorded at 100 fps for 2000 frames. The video is played back at 100 fps.

Video 12: RBC flow in zebrafish at 2 dpf imaged at 80x magnification for the validation experiments. Shown in the video is zebrafish M5 that received gata1 morpholino treatment resulting in greatly reduced hematocrit levels. The blood flow was recorded at 100 fps for 2000 frames. The video is played back at 100 fps.

SV13: Video montage showing the spatial distribution map of hemodynamic and morphological data in all zebrafish obtained from the high throughput experiment after automated spatial bin averaging