Supplemental Materials

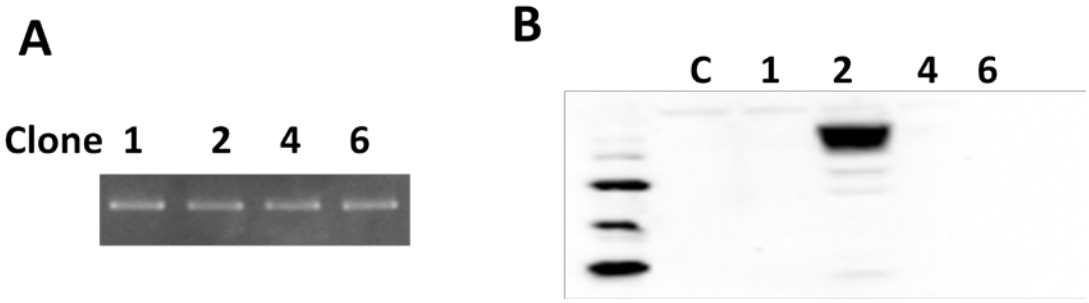
Supplemental Methods:

Cell viability assay

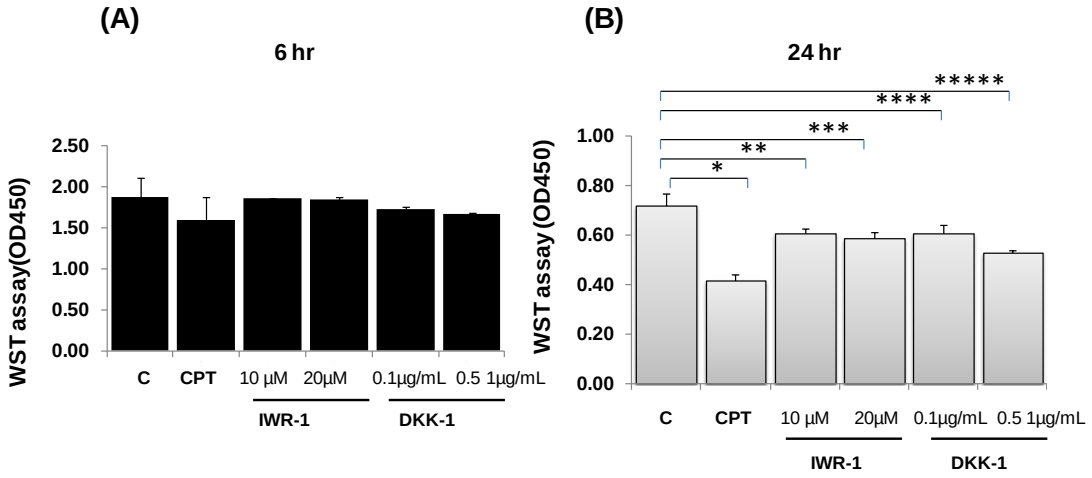
The effects of Wnt inhibitors on endothelial cell apoptosis were assessed with WST-1 cell viability assay (Cayman Chemicals). Briefly, HAEC were seeded in the 96 well plate and grown to sub-confluence. Cells were then treated with or without the Wnt inhibitors, IWR-1 (at 10µM and 20 µM) or Dkk-1 (at 0.1µg/mL and 0.5µg/mL) or a positive control of apoptosis Camptothecin (CPTs at 10 uM) for 6 hours versus 24 hours. 5uL ofWST-1 reagent was then added into cells. After 2 hour incubation, , absorbance at 450nm was measured as an indication of cell viability.

Supplemental Figures

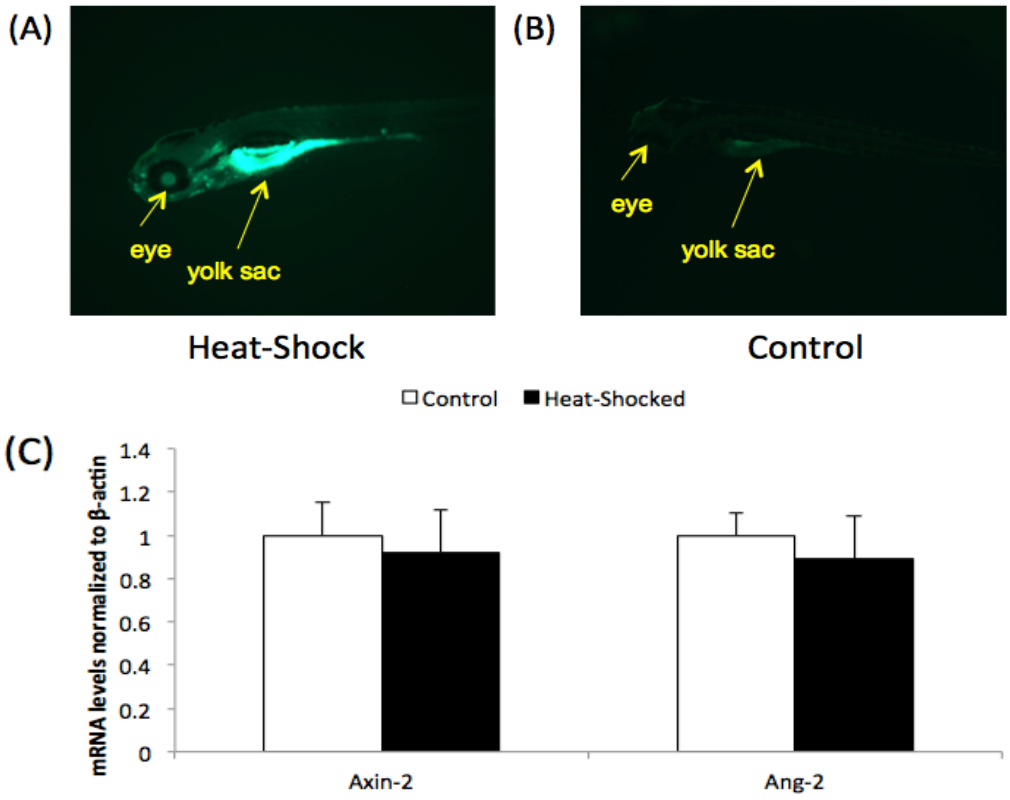
Supplemental Figure I. Verification of HA-tagged zAng2 expression. (**A**) pCS2-zAng-HA plasmids were transfected into HEK-293 cells. RNA was isolated to verify zAng2-HA mRNA expression by qRT-PCR. (**B**) zAng2-HA protein expression was verified by Western blots using anti-HA antibody.



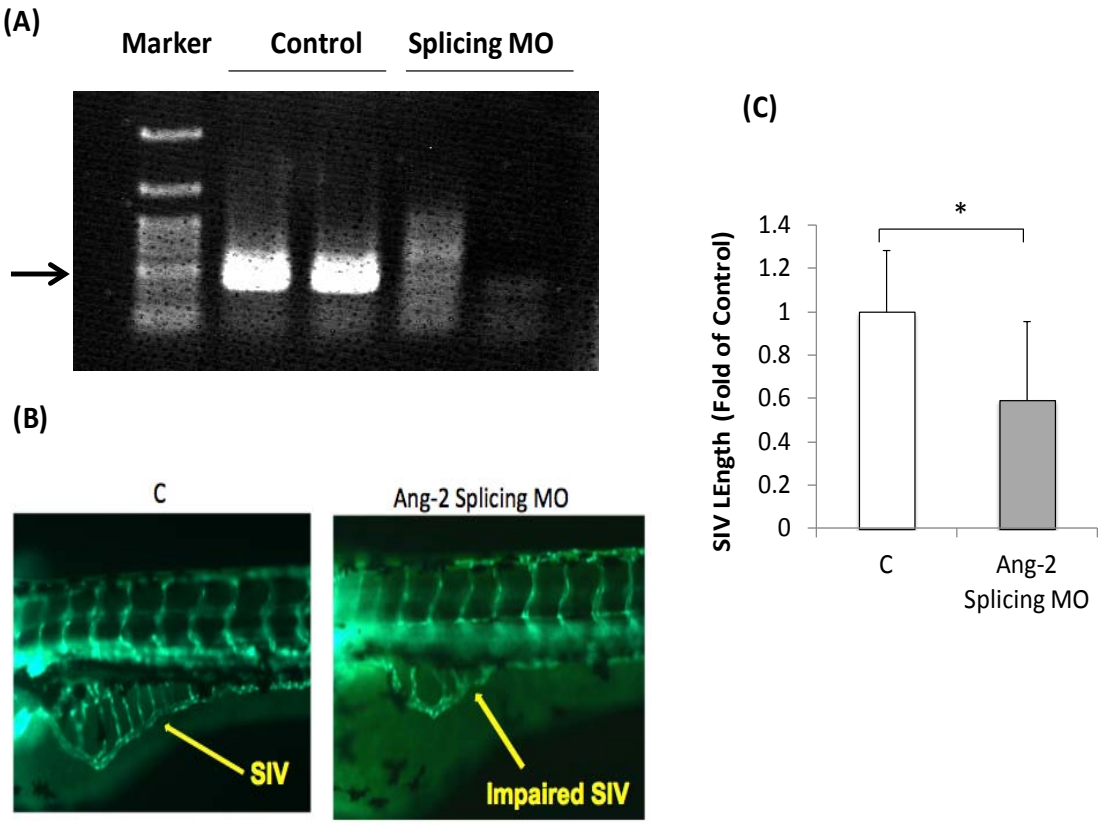
Supplemental Figure II. **Inhibition of Wnt signaling and cell viability**. (**A**) After 6 hours of incubation, IWR-1 and DKK did not significantly reduce cell viability ($p > 0.05$ vs. control, n=3). (**B**) After 24 hours of incubation, both IWR-1 (at 10µM and 20 µM) and Dkk-1 (at 0.1µg/mL and 0.5µg/mL) significantly reduced HAEC viability (*, **, ***, ****, *****$p<0.05$ vs. control, n=3). C denotes the control, CPT (Camptothecin) was used as a positive control for apoptosis.



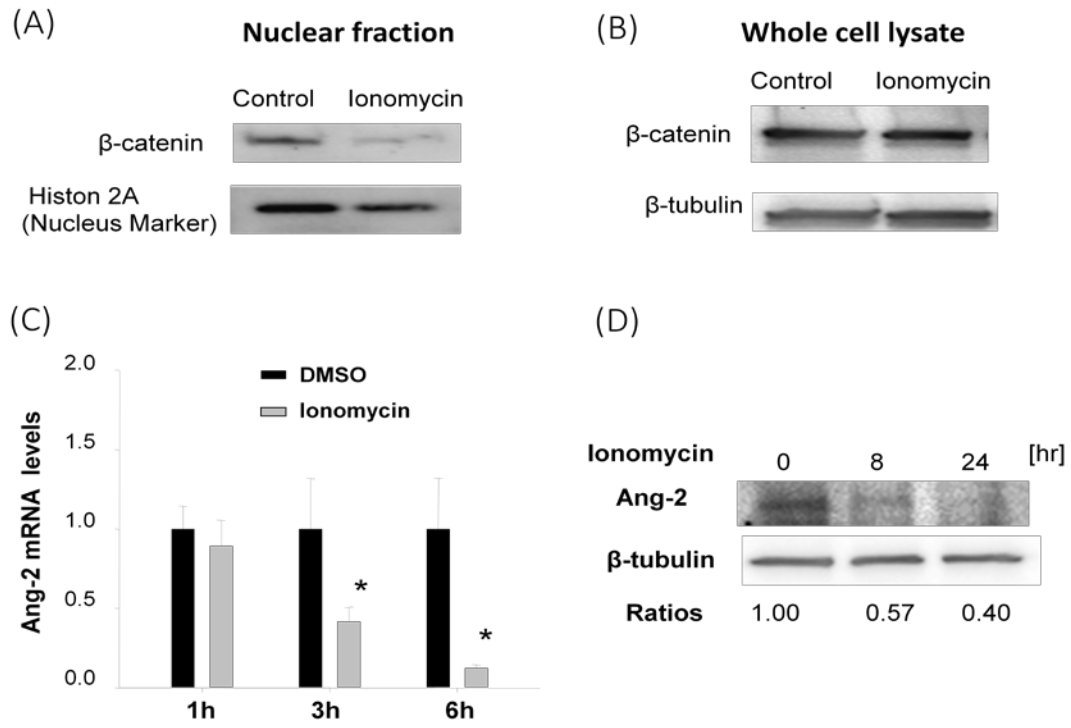
Supplemental Figure III. The effect of heat shock on non-transgenic fish on Axin2 and Ang-2 mRNA expression. (A) Heat-shock induction of the *Tg(hsp70l:Dkk1-GFP)* embryos at 48 hpf at 37°C for 1 hour resulted in an increase in DKK-1-GFP expression. **(B)** In the absence of heat-shock induction, DKK-1-GFP was not expressed. **(C)** Heat-shock of non-transgenic (wild-type) fish showed no changes in Axin-2 or Ang-2 expression as compared to control ($p > 0.05$, n=3).



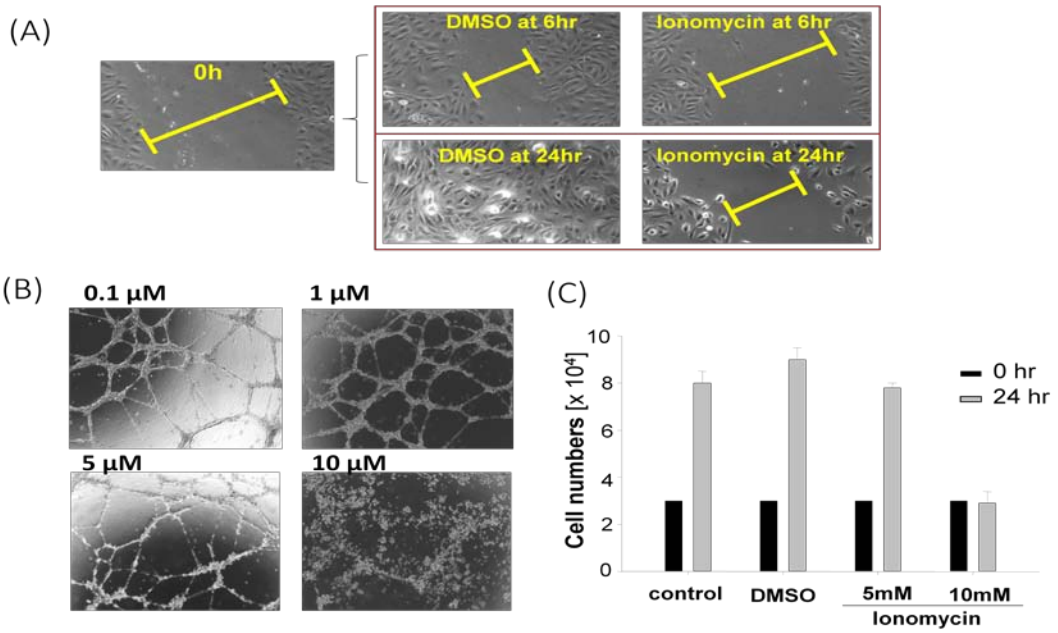
Supplemental Figure IV. zAng-2 splicing morpholino inhibited SIV formation. **(A)** RT-PCR was performed to validate the effect of Ang-2 splicing MO using primers that covered the exon 2-intron 2 boundary of Ang-2 (Lamont RE1, Vu W, Carter AD, Serluca FC, MacRae CA, Childs SJ. **Hedgehog signaling via angiopoietin1 is required for developmental vascular stability**. Mech Dev. 2010 Apr;127(3-4):159-68). The splicing morpholino injection resulted in a decrease in the wild type band intensity (arrow) in comparison with the controls. **(B)** The splicing morpholino micro- injection into the 2-egg stage resulted in an impaired SIV formation at 72 hpf (representative photos) . **(C)** Quantification of the SIV length revealed a 41% reduction in SIV length in response to Ang-2 splicing MO (* $p <$ 0.01, $n = 20$).



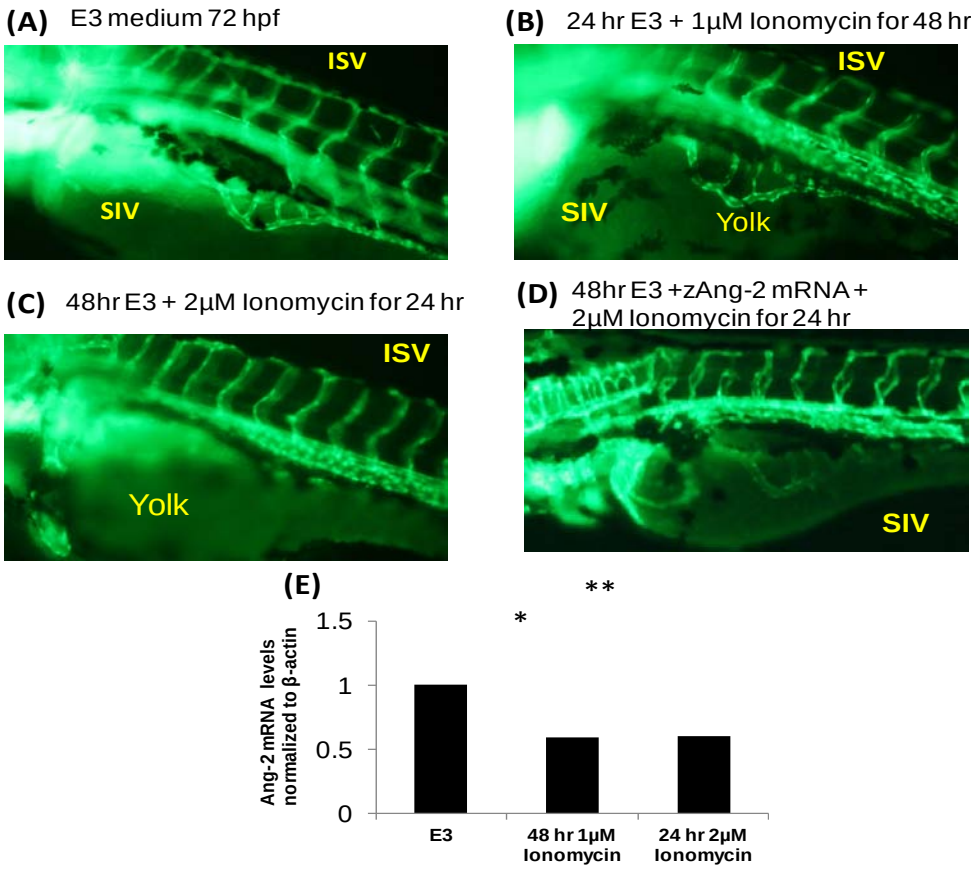
Supplemental Figure V. Ionomycin reduced Ang-2 expression by attenuating nuclear translocation of β-catenin. (A) Ionomycin treatment at 5 μmol/L attenuated nuclear fraction of β-catenin in HUVEC. (B) Ionomycin treatment did not alter the overall β-catenin quantity in the whole cell lysate. (C) RNAs were isolated HUVEC that were treated with 5μmol/L of Ionomycin for 1h, 3h, and 6h, respectively. Ionomycin attenuated Ang-2 mRNA expression in HUVEC as normalized to GAPDH (*$p < 0.05$ vs. Control cells with 0.1%DMSO treatment, n = 3). (D) Ionomycin attenuated Ang-2 protein levels. Entire cell lysates were collected by RIPA buffer and 50 μg of entire cell protein was prepared for Ang-2 protein levels. The relative expression was normalized to β-tubulin from density scan data. The blots were representative of two independent experiments with identical results.



Supplemental Figure VI. Wnt signaling pathway influenced endothelial cell migration and tube formation via Angiopoietin-2. (A) Ionomycin inhibited cell migration in HUVEC monolayer scratch assay. (B) HUVEC were cultured on the Matrigel in the presence of 0.1 – 10 μmol/L of Ionomycin for 8 hours. Ionomycin inhibited tube formation in a dose-dependent manner. (C) HUVEC were seeded in the 6-well plates and cultured for 24 hours in the presence or absence of Ionomycin at the indicated concentration, and were then trypsinized and counted with hemocytometer. HUVEC treated with the high concentration of Ionomycin ceased to proliferate.



Supplemental Figure VII. Ionomycin impaired SIV formation that was rescued by Ang-2. (**A**) Presence of SIV at 72 hpf in E3 medium. (**B**) 1μM Ionomycin starting at 24 hpf disrupted SIV formation, or (**C**) 2μM Ionomycin starting at 48 hpf inhibited SIV formation. (**D**) Micro-injection of 9 ng zAng-2 mRNA at the two-cell stage rescued SIV formation in the presence of 2μM Ionomycin. (**E**) qRT-PCR revealed that treatment at 48 hpf with 2 μM Ionomycin for 24 hr significantly down-regulated Ang-2 expression (**p* < 0.01 vs. E3; ***p* <0.01 vs. E3, n=3).



Supplemental Table:

Supplemental Table I. List of genes in the Stem Cell RT^2 Profiler^{TM} PCR Array (SuperArray®)

■Stem Cell Specific Markers:
Cell Cycle Regulators: Apc, Axin1, Ccna2, Ccnd1, Ccnd2, Ccne1, Cdc2a, Cdc42, Ep300, Fgf1, Fgf2, Fgf3, Fgf4, Myc, Notch2, Pard6a, Rb1.
Chromosome and Chromatin Modulators: Gcn5l2, Hdac1, Hdac2, Myst1, Myst2, Rb1, Tert.
Genes Regulating Symmetric/Asymmetric Cell Division: Dhh, Notch1, Notch2, Numb, Pard6a.
Self-Renewal Markers: Hspa9, Myst1, Myst2, Neurog2, Sox1, Sox2.
Cytokines and Growth Factors: Bmp1, Bmp2, Bmp3, Cxcl12, Fgf1, Fgf2, Fgf3, Fgf4, Gdf2, Gdf3, Igf1, Jag1.
Genes Regulating Cell-Cell Communication: Dhh, Dll1, Gja1, Gjb1, Jag1.
Cell Adhesion Molecules: Acan (Agc1), Apc, Bglap1, Cd4, Cd44, Cdh1, Cdh2, Ctnna1, Cxcl12, Ncam1.
Metabolic Markers: Abcg2, Aldh1a1, Aldh2, Fgfr1.
■Stem Cell Differentiation Markers:
Embryonic Cell Lineage Markers: Actc1, Ascl2, Foxa2, Pdx1 (Ipf1), Isl1, Krt15, Msx1, Myod1, T.
Hematopoietic Cell Lineage Markers: Cd19, Cd3d, Cd4, Cd8a, Cd8b1, Mme.
Mesenchymal Cell Lineage Markers: Acan (Agc1), Bglap1, Col1a1, Col2a1, Col9a1, Pparg.
Neural Cell Lineage Markers: Cd44, Ncam1, Oprs1, S100b, Tubb3.
■Signaling Pathways Important for Stem Cell Maintenance:
Notch Pathway: Dll1, Dll3, Dtx1, Dtx2, Dvl1, Ep300, Gcn5l2, Hdac1, Hdac2, Jag1, Notch1, Notch2, Numb.
Wnt Pathway: Adar, Apc, Axin1, Btrc, Ccnd1, Frat1, Fzd1, Myc, Ppard, Wnt1.

Stem-cell specific markers, stem cell differentiation markers, and genes in signaling pathways important for stem cells maintenance were analyzed using qRT-PCR array. We analyzed expression of a focused panel of genes related to stem cell biology in response to fluid shear stress. Bioinforamtics approaches were based on the above functional gene groupings. Based on the initial grouping of the genes into different categories, we have first identified gene subsets in the Wnt pathway whose gene expression levels were significantly elevated at specific time points in response to shear stress. In the Wnt pathway, genes cooperate with each other to perform certain functions. Thus, the expression levels of genes in the Wnt pathway were highly correlated.

Supplemental Movies:

Supplemental Video I: Micro-injection with the control MO displayed a normal blood circulation in dorsal aorta (DA), posterior cardinal vein (PCV), and subintestinal vein (SIV) at 72 hpf.

Supplemental Video II: Micro-injection with Ang-2 MO (0.5uM) led to absence of circulation despite cardiac contraction at 72 hpf.

Supplemental Video III: *Tg(hsp70l:DKK1-GFP)* fish were imaged for blood flow at 72hpf without heat-shock.

Supplemental Video IV: *Tg(hsp70l:DKK1-GFP)* fish were imaged for blood flow at 72hpf after one hour heat-shock at 48 hpf to induce DKK1 expression. DKK-1 did not have apparent effect on blood flow.