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Supplementary Materials for

3D curvature-instructed endothelial flow response and tissue vascularization

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/38/eabb3629/DC1)

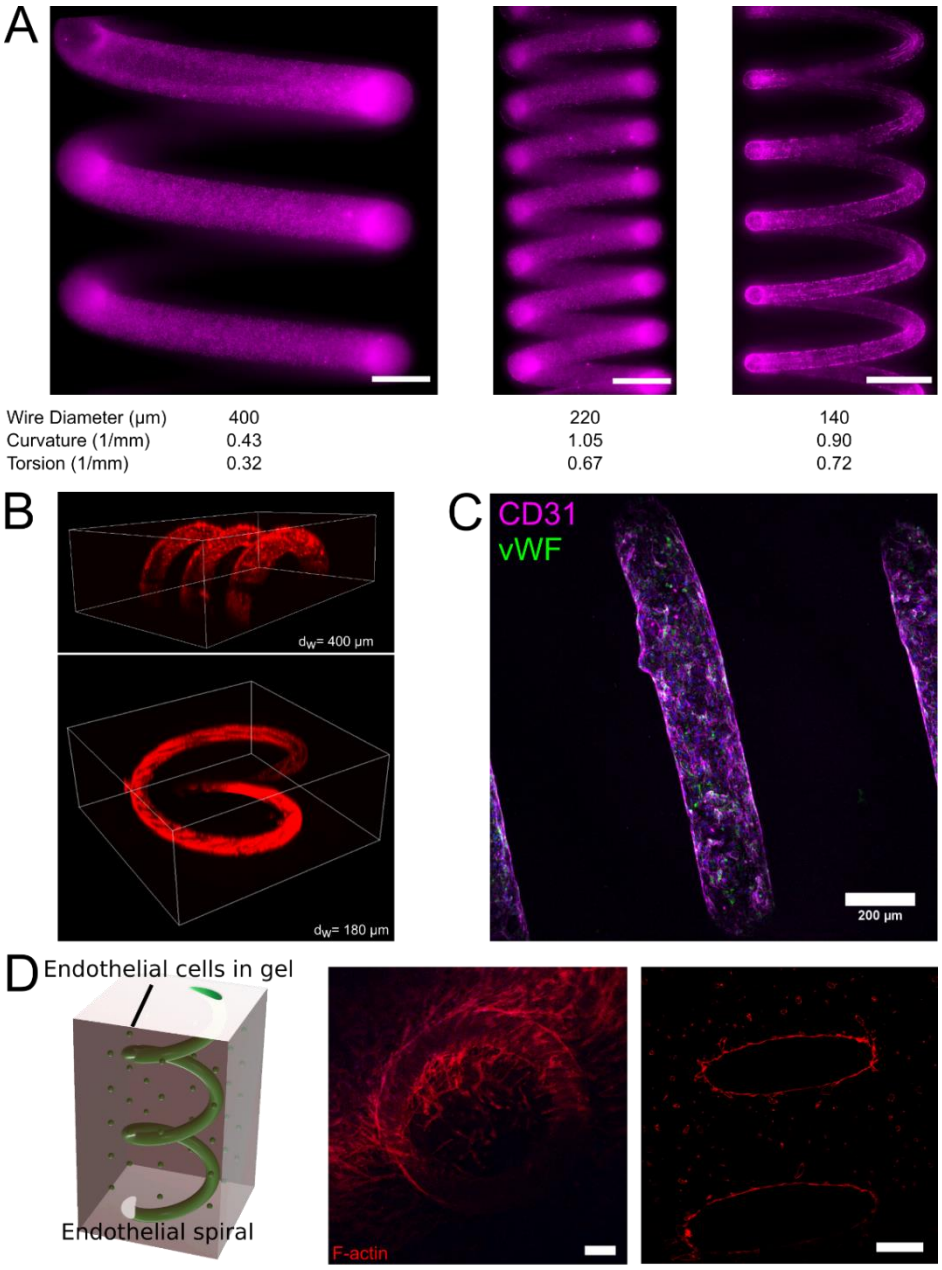

Fig. S1 Spiral vessel dimensions, cell seeding, and vasculogenesis model. (A) Representative spiral vessels formed in 7.5 mg/mL collagen hydrogel and corresponding vessel diameter, curvature, and torsion values (scale = 500 μm). (B) 3D reconstructions of confocal z-stack images of 400 μm (upper) and 180 μm (lower) spiral vessels in collagen hydrogel perfused with fluorescent beads (1 μm diameter). (C) Maximum intensity projection (MIP) of representative endothelialized 180 μm spiral vessel. Magenta, CD31; green, von Willebrand Factor; blue, nuclei. (D) Anastomoses of spiral vessel from the lumen with self-assembled

endothelial cell network in the bulk collagen matrix. Left panel: schematic; center panel: MIP of a single spiral loop in XY plane (scale = 200 μm); and optical section of the side view of spiral vessel connected with self-assembled vascular network (scale = 400 μm).

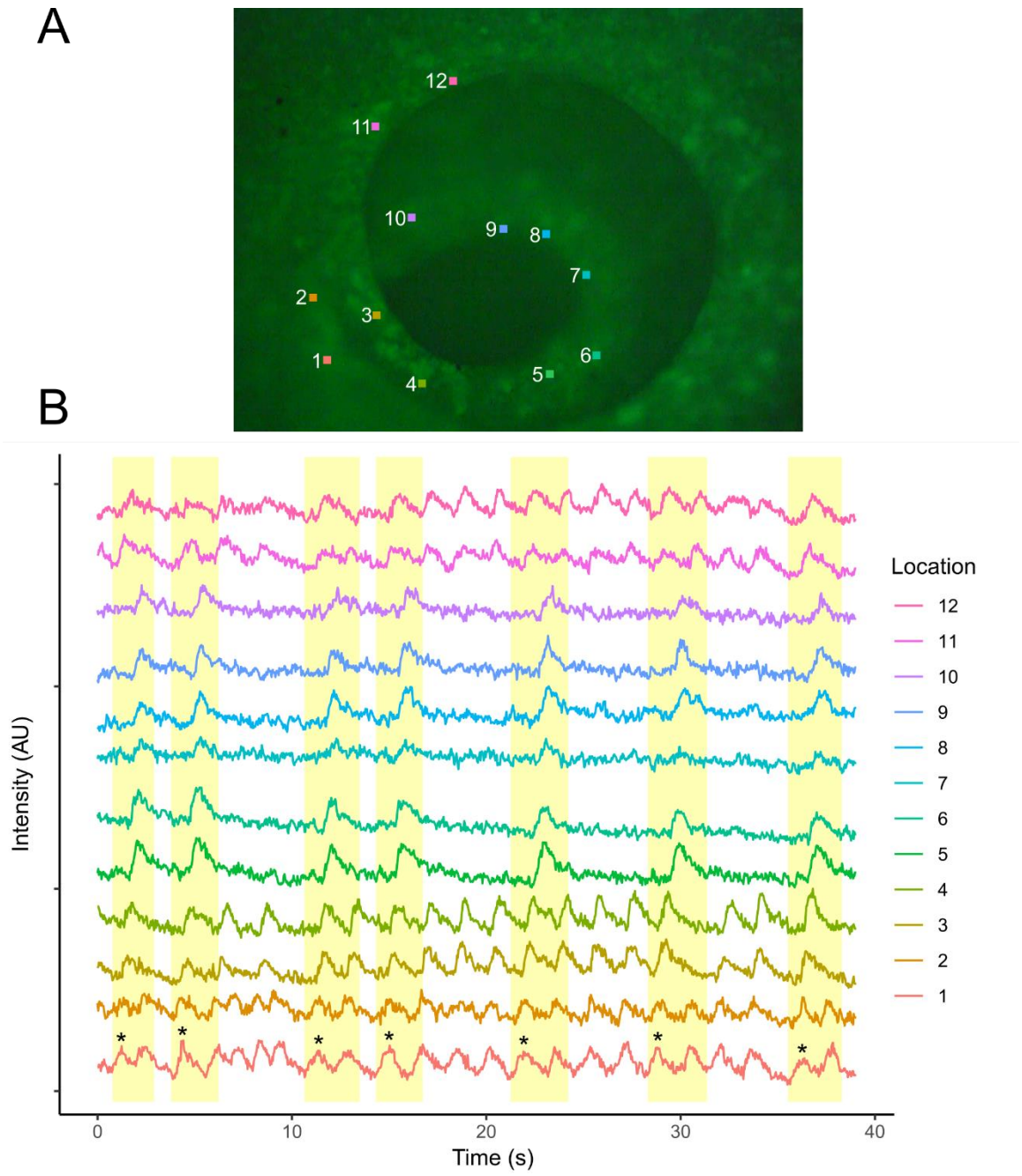

Fig. S2. Vascularized heart chamber calcium wave propagation. (A) Snapshot frame from Movie S2 with 12 regions of interest along the spiral vessel. Regions 1, 2, 11, and 12 are on the same plane at the top surface, regions 3 – 10 extend into the tissue. (B) GCaMP3 intensity for each region of interest plotted over the time. Highlighted regions indicate calcium waves that travel from the top surface to the bottom of the chamber with the initiating wave marked by a (*).

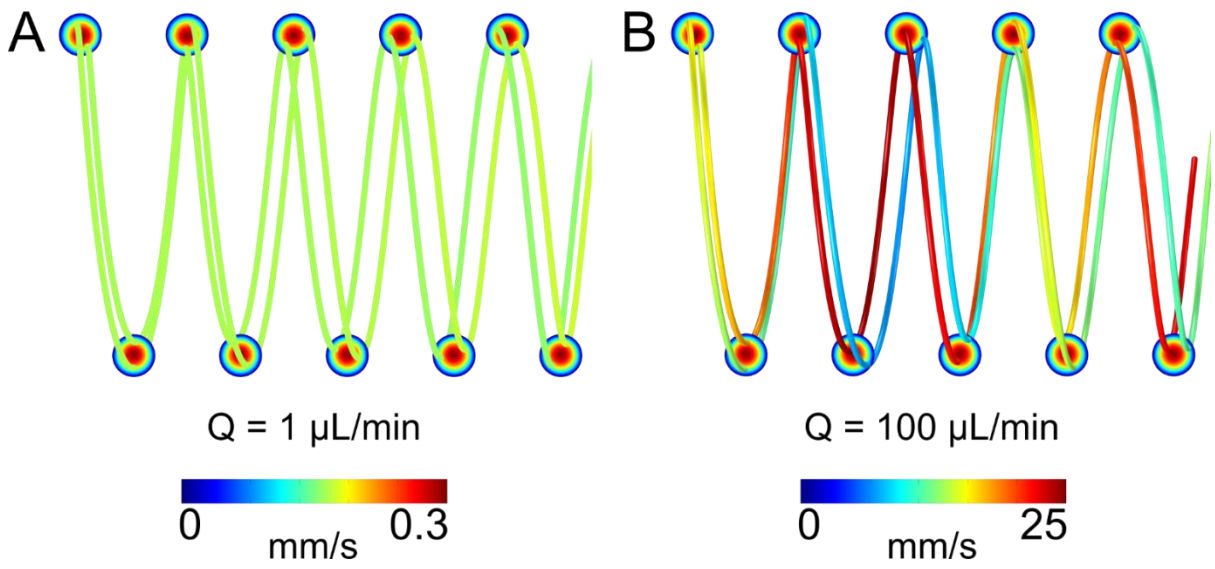

Fig. S3. Streamlines in spiral vessels at 1 and 100 µL/min. Computational fluid dynamics plots of spiral vessel streamlines (color expressed with primary velocity magnitude) at Q = 1 µL/min (A) and Q = 100 µL/min (B).

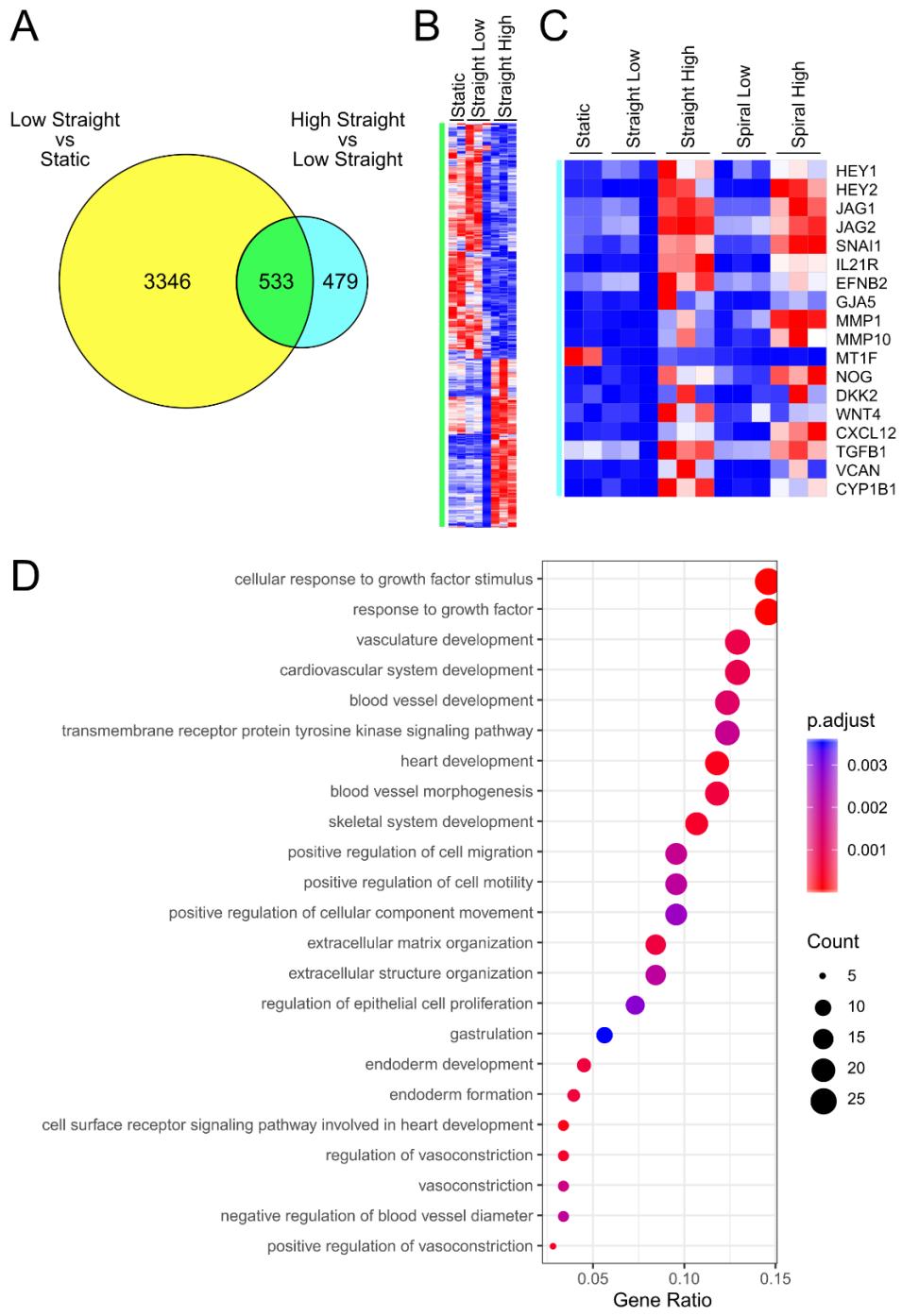

Fig. S4. Bulk RNAseq comparison of spiral and straight flow to static conditions. (A) Venn diagram of significantly regulated transcripts in ECs cultured at low flow in straight tubes compared with static culture and those in high flow compared to low flow conditions in straight tubes. (B) CPM heatmap of overlapping region (green) of (A). (C) CPM heatmap of selected genes significantly regulated by increasing flow (corresponding blue region of A).

(D) GO enriched terms associated with upregulated genes identified in straight high versus straight low, but not in low straight versus static.

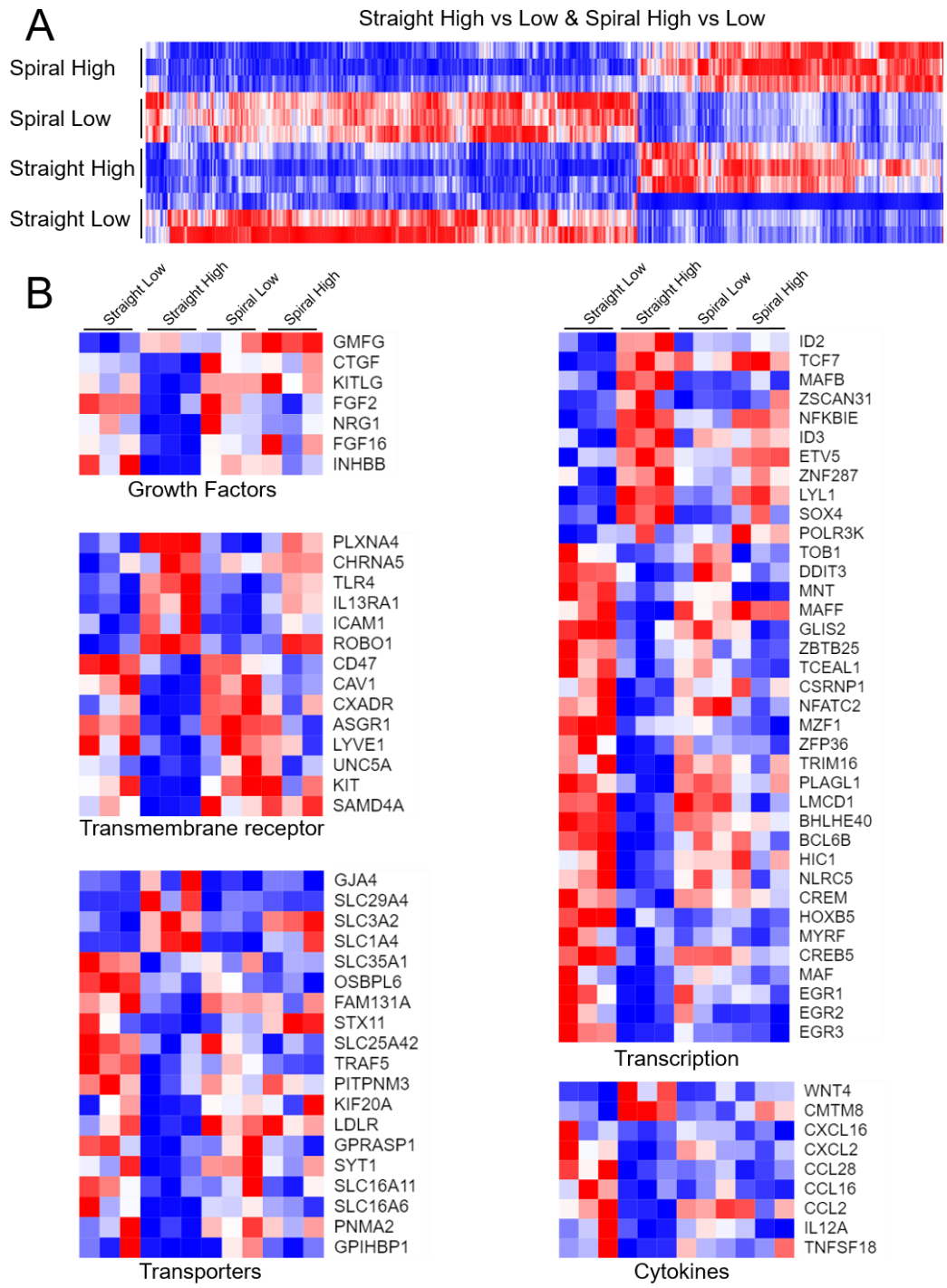

Fig. S5. Genes that are significantly regulated when flow is increased in spiral or straight geometries. (A) CPM heatmaps of all genes that are significantly changed by increasing flow in both spiral and straight geometries. (B) CPM heatmaps of selected genes associated with growth factors, transmembrane receptors, transporters, transcription factors, and cytokines

that are significantly changed by increasing flow in straight vessels, but not changed by increasing flow in spiral vessels.

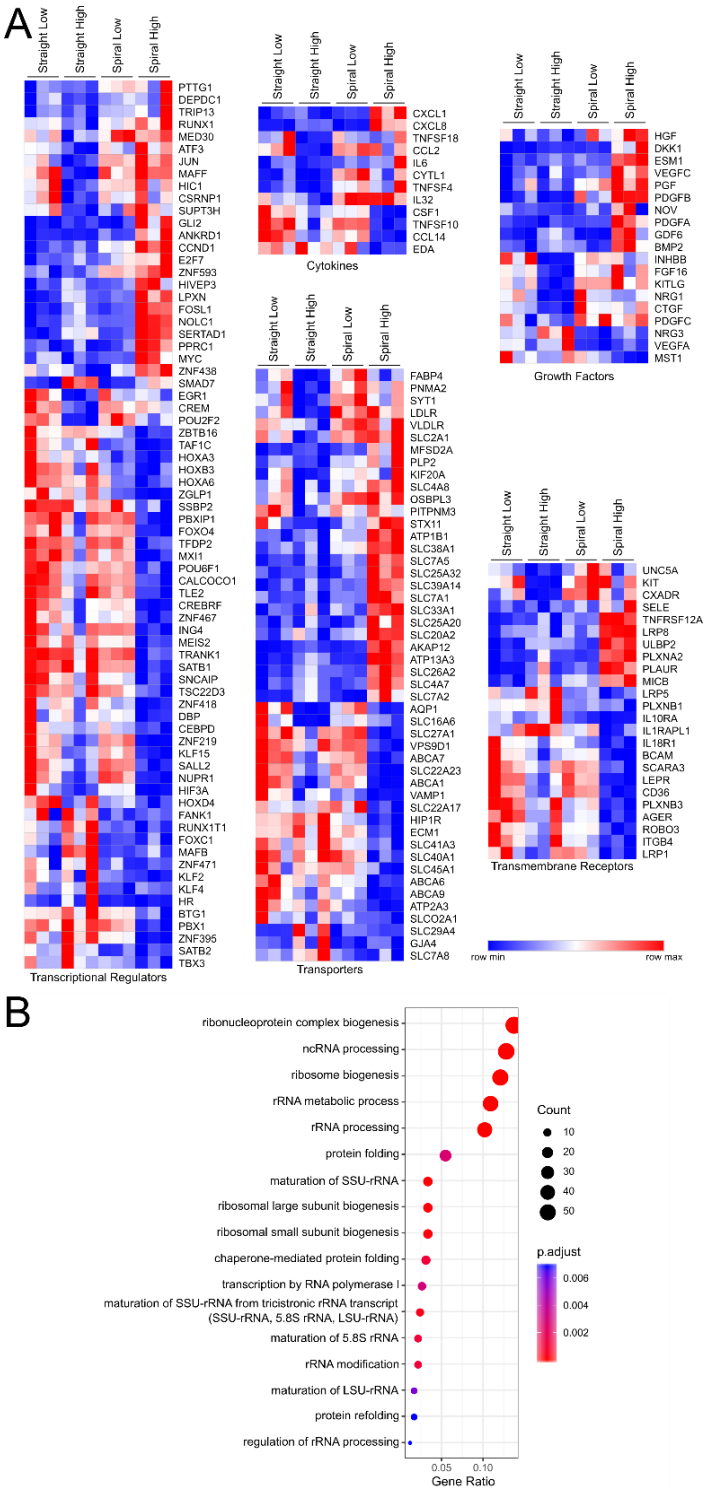

Fig. S6. Genes that are significantly changed in high spiral flow. (A) CPM heatmaps of selected genes associated with transcriptional regulators, cytokines, transporters, growth factors, and transmembrane receptors that are significantly changed by increasing flow in spiral vessels, but not in straight vessels. (B) Enriched GO terms from genes significantly upregulated in spiral high flow versus low flow, but not in straight high flow versus low flow.

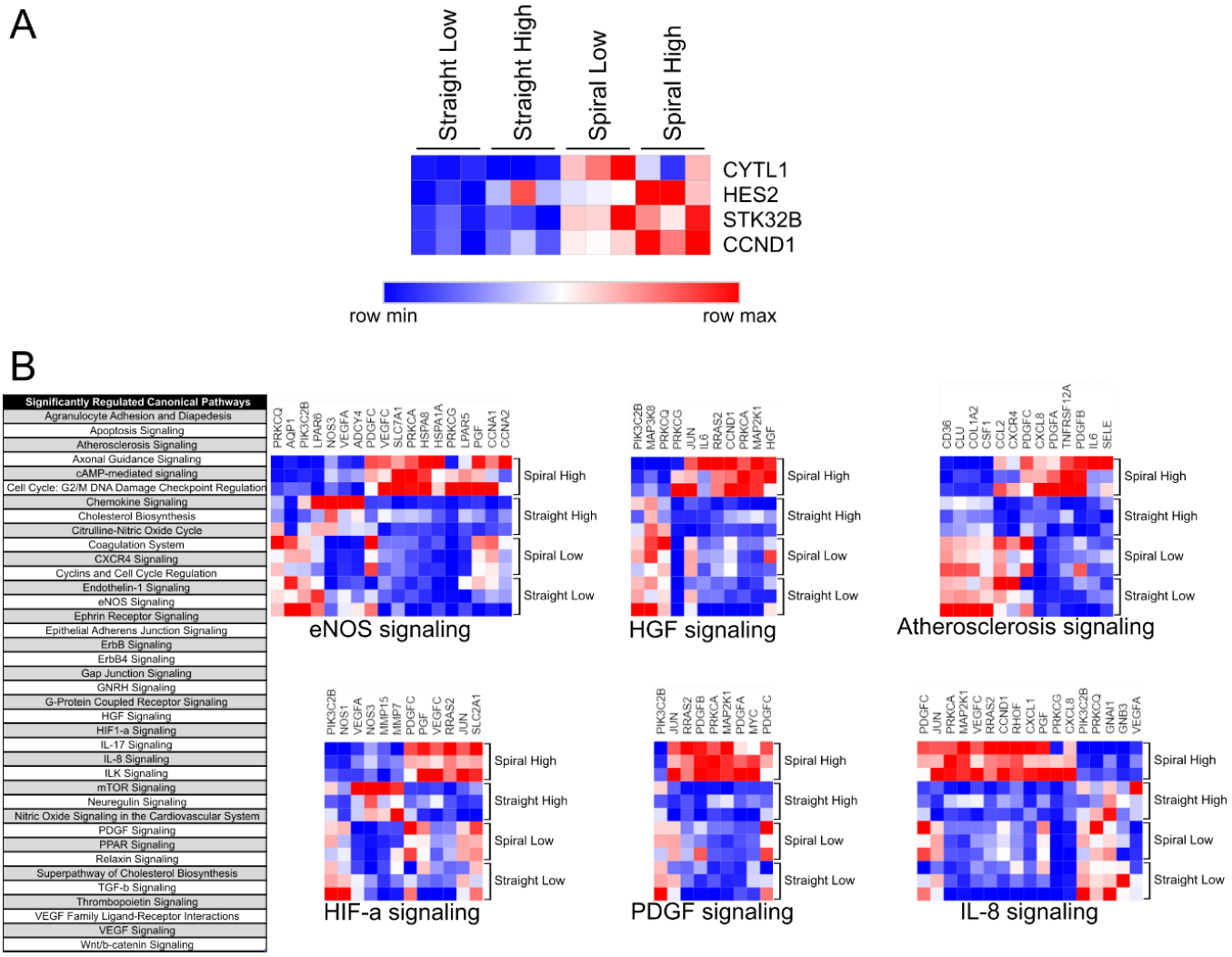

Fig. S7. Significant differences in gene expression in low flow and Ingenuity Pathway Analysis. (A) Heatmap of all significantly upregulated genes in spiral low flow versus straight low flow. (B) Heatmaps of significantly regulated canonical pathways identified by Ingenuity Pathway Analysis (IPA) and heatmaps of genes associated with selected signaling pathways.

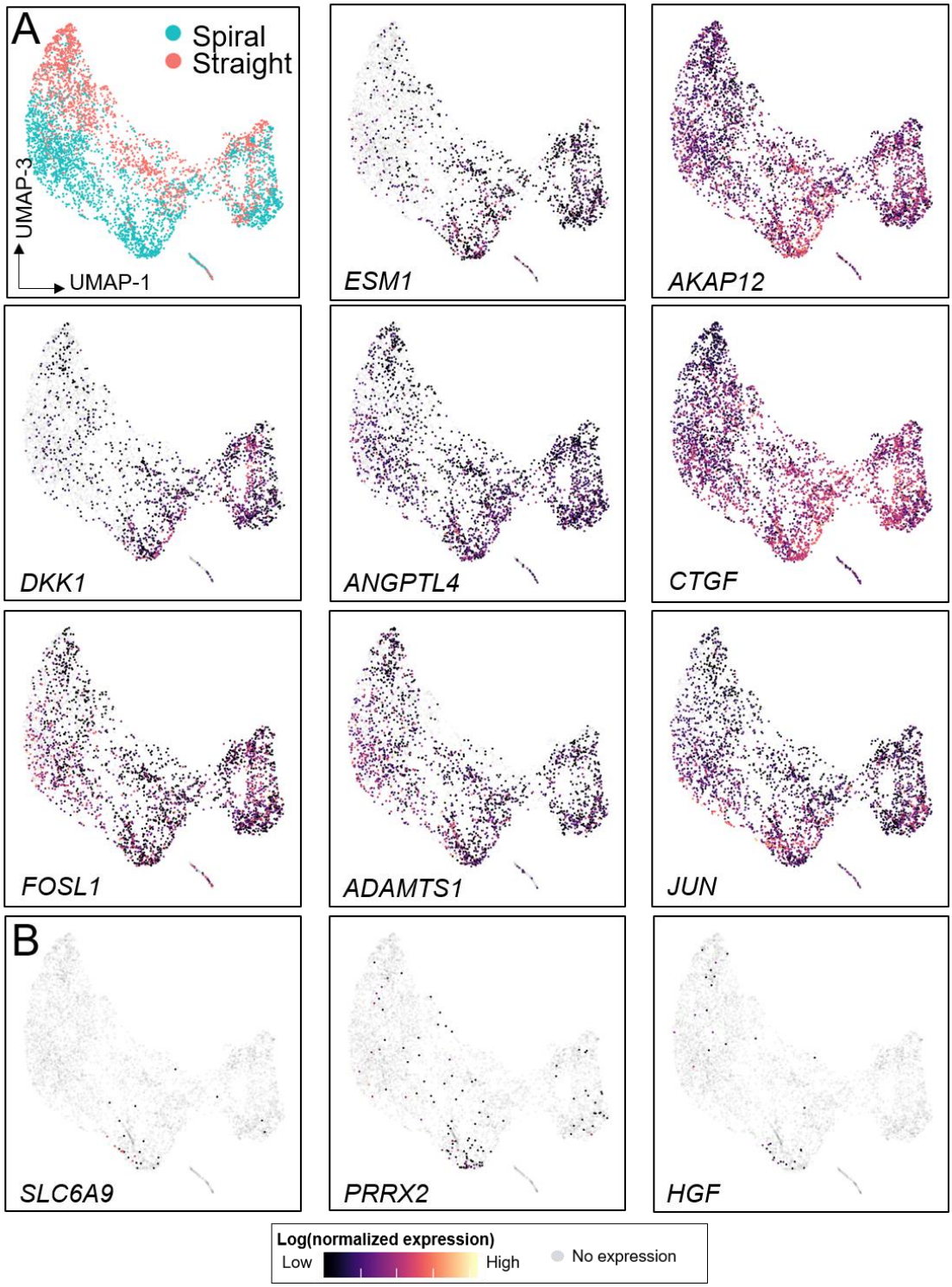

Fig. S8. UMAP distribution of significantly regulated genes in both bulk and single cell RNA sequencing. (A) Selected genes that were significantly different in spiral versus straight vessels under high flow. (B) Differentially expressed genes detected in scRNAseq, but not in bulk RNA sequencing.

Movie S1. Perfusion of 220 μm diameter spiral vessel in collagen with 1 μm fluorescent beads at 1 μL/min (upper), 10 μL/min (middle), and 100 μL/min (lower).

Movie S2. GCaMP3 signal in a vascularized cardiac chamber construct showing 3D calcium wave propagation along the spiral vessel.