SUPPLEMENTAL MATERIALS

Single-Cell RNA Sequencing Reveals Endothelial Cell Transcriptome Heterogeneity under Homeostatic Laminar Flow

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Running title: EC gene expression variance under flow

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Major Resources Table

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot #	Persistent ID / URL
rabbit anti-HMGN2	Cell Signaling	9437S	1:500 (2 µg/ml)		
Alexa647-mouse-anti-human PECAM1	Biolegend	303112	1:250 (2 µg/ml)		
rabbit anti-ANKRD1	Invitrogen	PA5-30548	1:100 (10 µg/ml)		
mouse anti-NPM1	Invitrogen	32-5200	1:250 (2 µg/ml)		
rabbit anti-DEK	Abcam	ab221545	1:500 (0.9 µg/ml)		
rabbit anti-HMGA1	Invitrogen	PA5-78007	1:500 (2 µg/ml)		
rabbit anti-ERG	Abcam	ab92513	1:100 (10 µg/ml)		
rabbit anti-LMNA	Abcam	ab26300	1:1000 (1 µg/ml)		
rabbit anti-KLF2	Invitrogen	PA5-72410	1:200 (5 µg/ml)		
rabbit anti-SUN1	Abcam	ab124770	1:500 (2 µg/ml)		
mouse anti-SUN2 (clone 3.1E)	Millipore-Sigma	MABT880	1:1000 (1 µg/ml)		
donkey-anti-mouse-Alexa555	Abcam	ab150110	1:250 (8 µg/ml)		
donkey-anti-rabbit-Alexa488	Invitrogen	A21206	1:250 (8 µg/ml)		
DAPI	Sigma	10236276001	0.04 µg/ml		

Cultured Cells

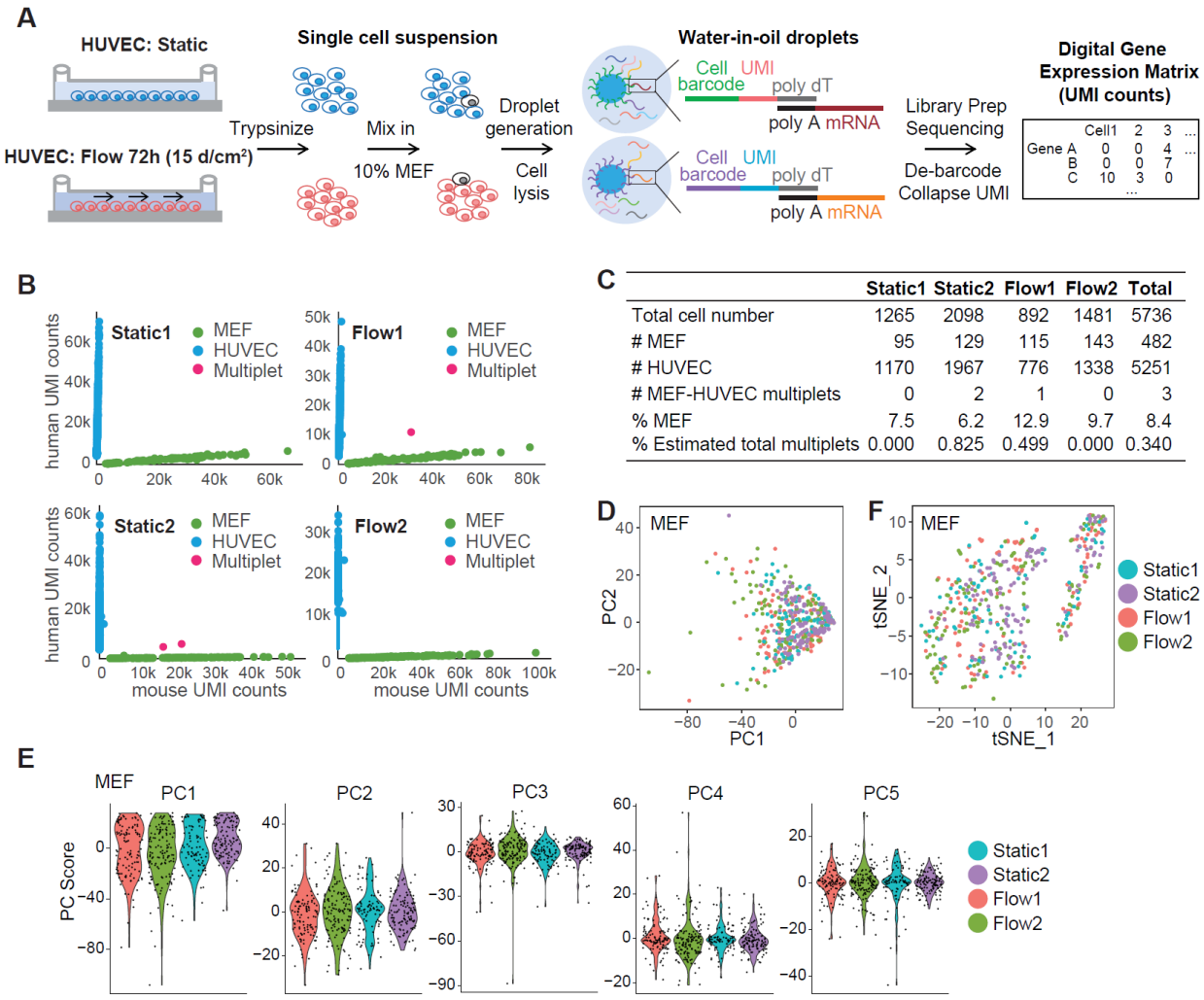
Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID
Human umbilical vein endothelial cells (HUVEC)	Lonza	unknown	C2519A
Mouse embryonic fibroblasts (MEF)	Isolated from E13.5 CD1 mice	unknown	

Data & Code Availability

Description	Source / Repository	Persistent ID
Raw (.fastq) and processed (UMI counts) single-cell RNA-seq data	GEO database	GSE151867

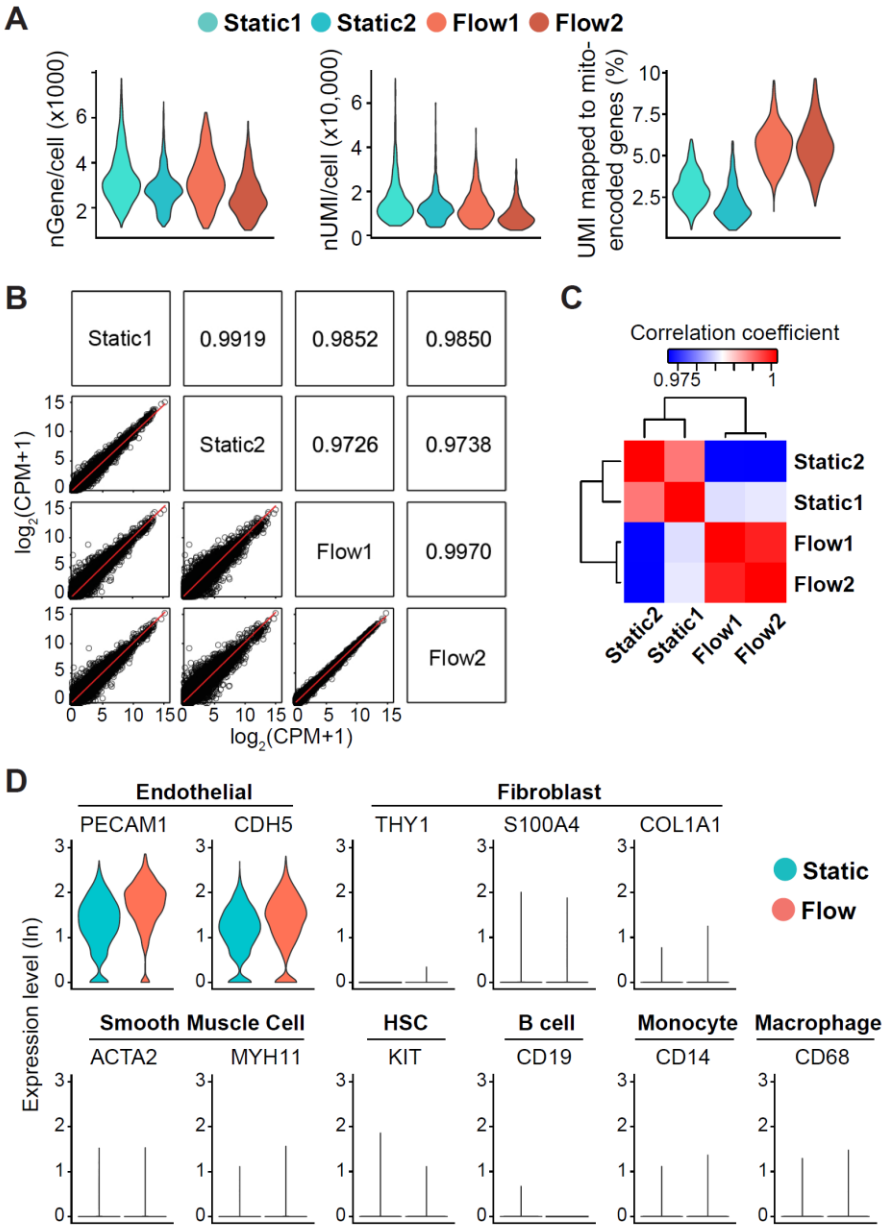
Other

Description	Source / Repository	Persistent ID
fibronectin	Roche	11080938001
µ channel slide	ibidi	80176
Endothelial Cell Growth Medium (EGM)-2 Bullet Kit	Lonza	CC-3162
Chromium™ Single Cell 3' Library and Gel Bead Kit v2, 4 rxns	10x Genomics	120267
Chromium™ Single Cell A Chip Kit, 16 rxns	10x Genomics	1000009
Chromium™ i7 Multiplex Kit, 96 rxns	10x Genomics	120262

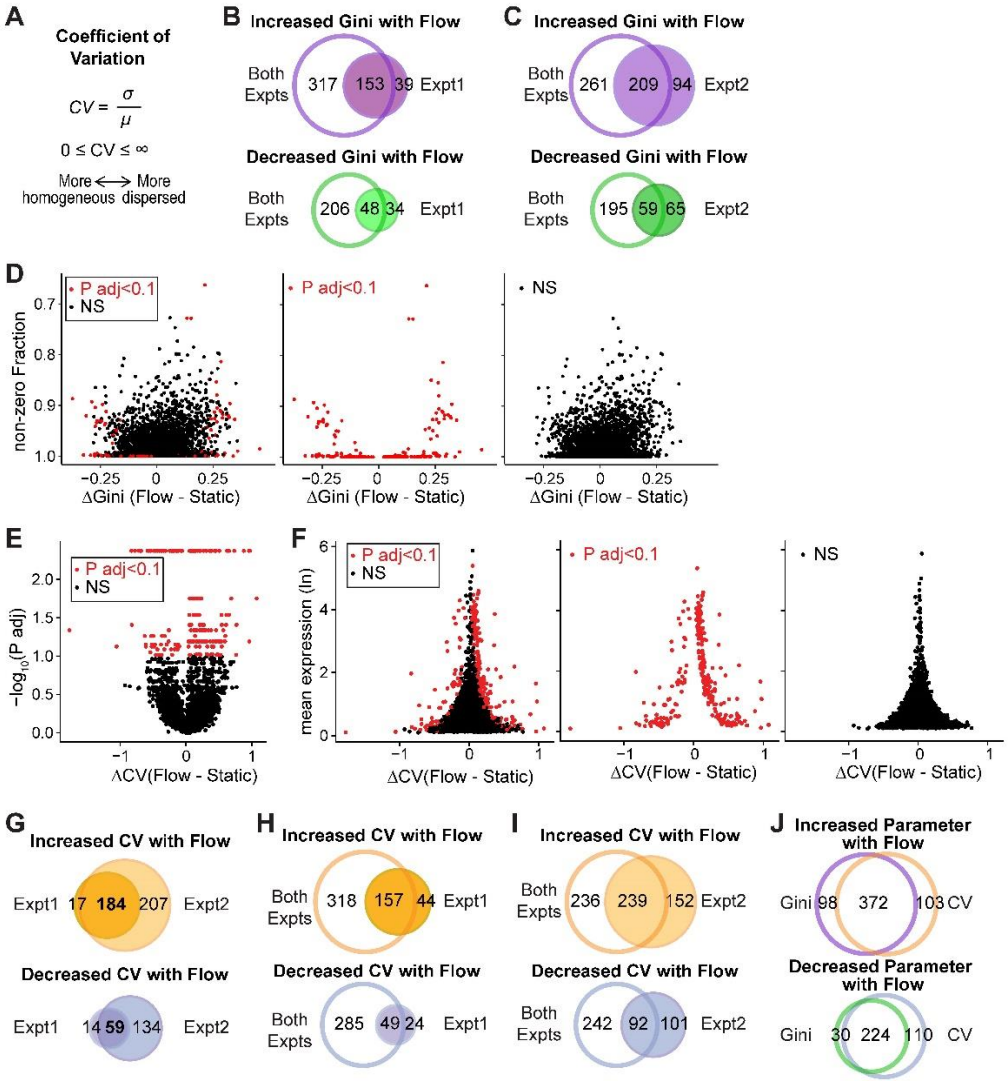


Supplemental Figure I. Single-cell RNA-seq experiment workflow and quality control.

(**A**) Experimental workflow. Beads are coated with numerous DNA oligos containing 14 bp cell barcode (one oligo sequence/bead, different sequences among different beads), a 10 bp unique molecular identifier (UMI, unique sequence for each oligo on the same bead), and a poly dT sequence to capture mRNA. (**B**) Multiplet discrimination of indicated samples by plotting UMI counts of each cell barcode that map to human (HUVEC, blue dots), mouse (MEF, green dots), or both (red dots) genomes. (**C**) Summary of cell number and multiplet rates. (**D**) PCA of MEF from indicated samples calculated with top 2000 highly variable genes. (**E**) Score for PC1-PC5 of PCA. (**F**) tSNE plot of MEF from indicated samples calculated with PC1-PC10.

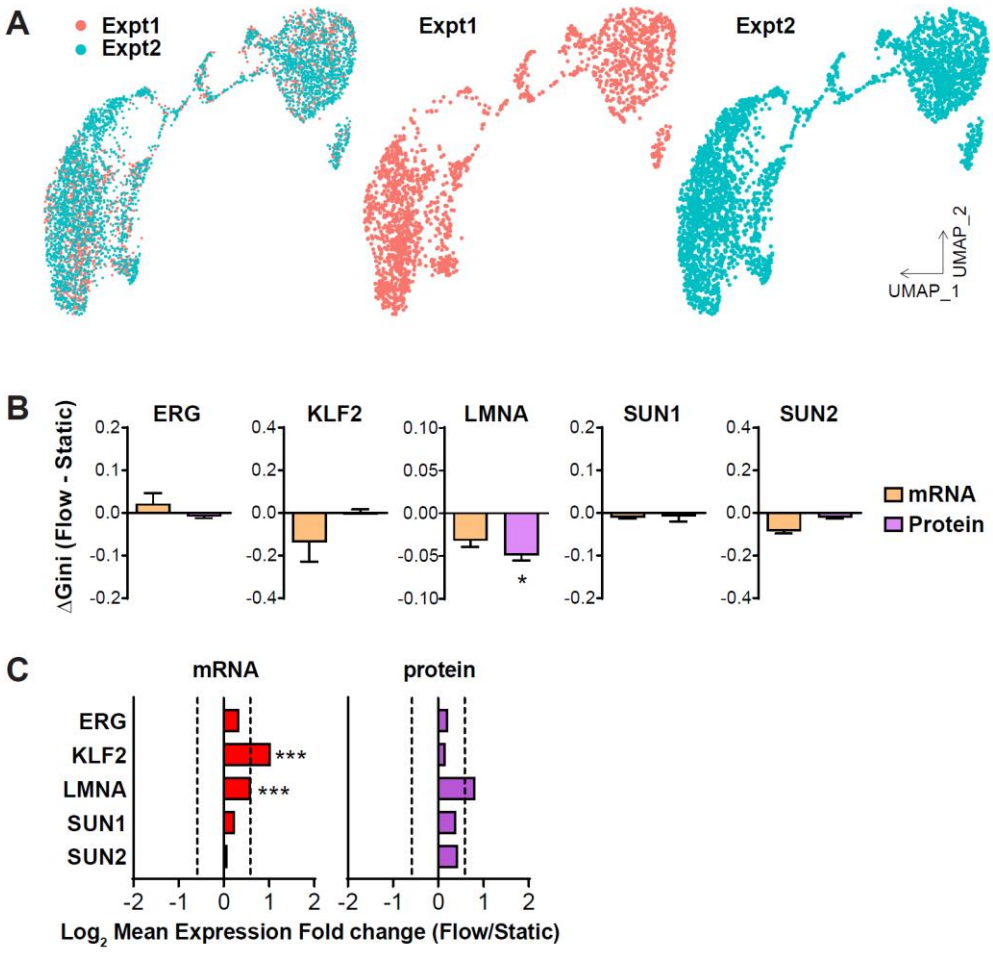


Supplemental Figure II. HUVEC single-cell RNA-seq data quality control.
(**A**) Distribution of detected genes/cell (left), UMI counts/cell (middle), and % UMI counts mapped to mitochondrial-encoded genes (right). (**B-C**) Pearson correlation analysis among pseudo-bulk samples assembled from the single-cell RNA-seq data showing a higher correlation between biological replicates than across conditions. (**B**) Dot plots with trend lines. (**C**) Heatmap color-coded by correlation coefficient. (**D**) Expression of canonical markers of EC, fibroblast, smooth muscle cells, hematopoietic stem cells, B cells, monocytes, and macrophages. Expression of several other markers were undetected in the dataset: CD45 (blood cells), CD235a (red blood cells), CD11b (macrophage), CD3 (T cells), CD15 (neutrophil).



Supplemental Figure III. Transcriptomic heterogeneity of EC under homeostatic laminar flow.

(A) CV formula. (**B-C**) Differential Gini test of HUVEC transcriptomes between static and flow performed with batch as a covariate. Resulting significant gene lists were compared to results of each independent experiment without batch correction **(Fig. 1F-I)** — experiment 1 in (**B**) and experiment 2 in (**C**). (**D**) Each gene's Gini change plotted against DESCEND-deconvolved non-zero fraction. (**E-J**) CV was calculated for each gene with DESCEND and differential test of CV was performed for each experiment. (**E-F**) Each gene's CV change plotted against p value (**E**) or mean expression (**F**). Red dots, significant genes (P adj < 0.1), black dots, non-significant genes. (**G**) Venn plots showing overlap of genes with significant CV change in the two replicate experiments. (**H-J**) Differential CV test of HUVEC between static and flow performed for each gene with batch as a covariate. Resulting significant gene lists were compared to results of each independent experiment without batch correction — experiment 1 in (**H**) and experiment 2 in (**I**). (**J**) Venn plots showing overlap of genes with significant Gini vs. CV changes after batch correction.



Supplemental Figure IV. Quality control for subpopulation analysis of single-cell RNA-seq and additional protein heterogeneity of EC under homeostatic laminar flow.

(**A**) UMAP plots of single-cell HUVEC transcriptome colored by experimental duplicates. **(B-C)** HUVEC immunostained for nuclear genes under flow vs. static. (**B**) Gini change under flow vs. static for tested nuclear genes at the RNA (single-cell RNA-seq, all non-significant by DESCEND) and protein level (immunostaining). Error bars, mean ± SEM from multiple replicates. Statistics for protein, paired t test, * $p < 0.05$. (**C**) Mean expression level fold change for tested genes at the RNA (single-cell RNA-seq) and protein level (mean fluorescence intensity from 3 independent experiments). Dotted lines, Fold change = 1.5. Statistics for mRNA, Wilcoxon rank-sum test, *** $p < 0.001$. Statistics for protein, ratio paired t test, all non-significant.