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Supplemental Information

Endothelial Reprogramming by Disturbed Flow Revealed by Single-Cell RNA and Chromatin Accessibility Study

Aitor Andueza, Sandeep Kumar, Juyoung Kim, Dong-Won Kang, Hope L. Mumme, Julian I. Perez, Nicolas Villa-Roel, and Hanjoong Jo

SUPPLEMENTARY FIGURES

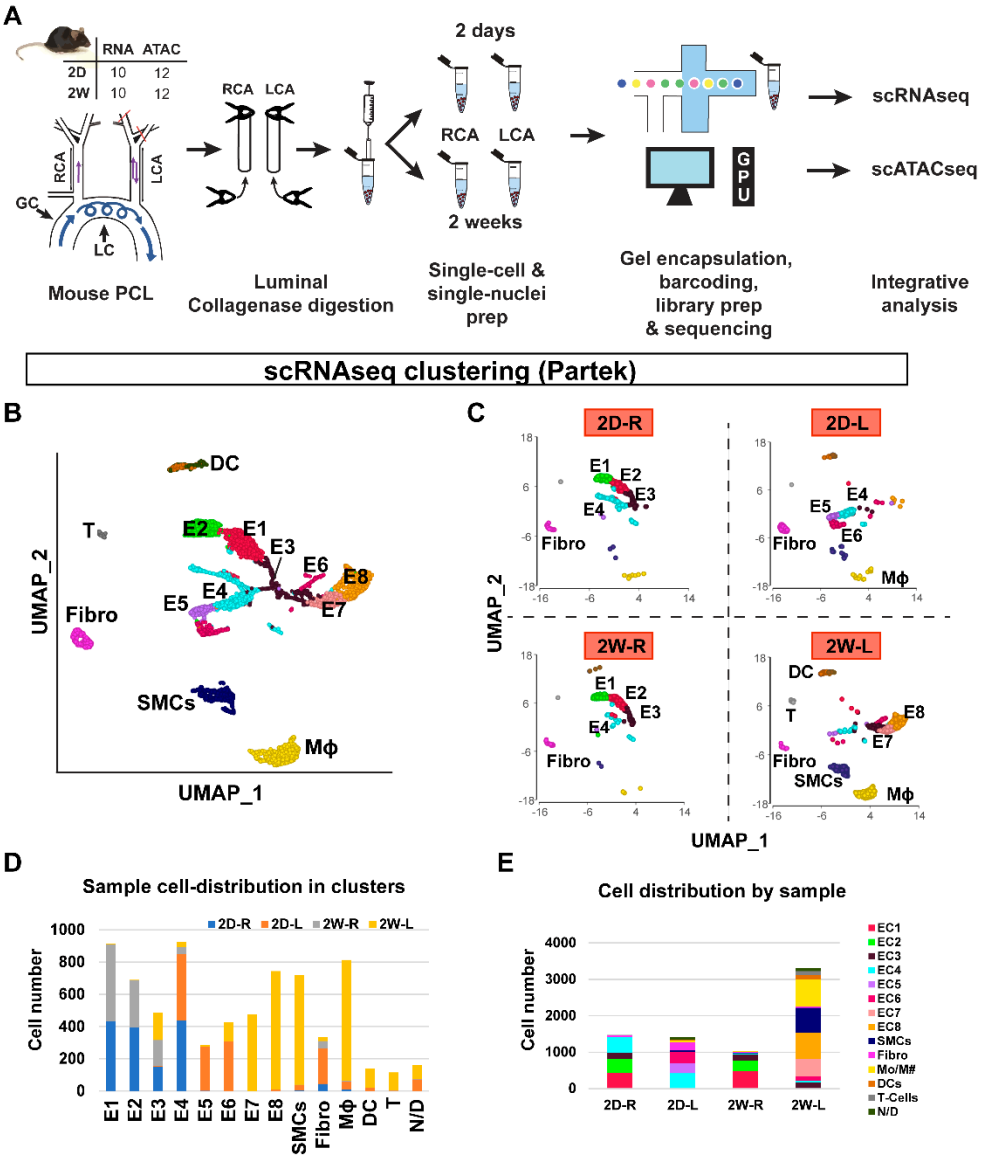


Figure S1 (related Figure 1). Overview of scRNA and scATAC studies, and scRNAseq clustering and cell identification using Partek Flow® software. (A) Partial carotid ligation (PCL) was performed on mice (n=44) to induce *d-flow* in the left carotid arteries (LCA) using the right (RCA) as a *s-flow* control. Single cells were enzymatically isolated from the lumens of RCAs and LCAs at 2 days and 2 weeks post PCL. For scRNAseq, 10 RCAs and LCAs were pooled at each timepoint and single-cells were encapsulated, barcoded, cDNA library was prepared, and sequenced. For the scATACseq study, 12 RCAs and LCAs were pooled at each time point to prepare single-nuclei, which were encapsulated, treated with transposase, barcoded and sequenced. scRNAseq and scATACseq data were analyzed with Seurat, respectively. **(B and C)** scRNAseq data representing 2-day RCA and LCA (2D-R and 2D-L) and 2-weeks RCA and LCA (2W-R and 2W-L) were plotted on a single UMAP to identify major cell populations. **(B)** shows a UMAP representing all 4 samples while **(C)** represents individual UMAP plots for each condition. Major cell populations include endothelial cells (ECs, E1-8), smooth muscle cells (SMCs), fibroblasts (Fibro), Monocytes/Macrophages (Mϕ), dendritic cells (DCs), and T-cells (T). **(D)** Graph shows the cell numbers in each cell cluster for 4 different experimental conditions whereas **(E)** represents the number of cells in each sample and cluster.

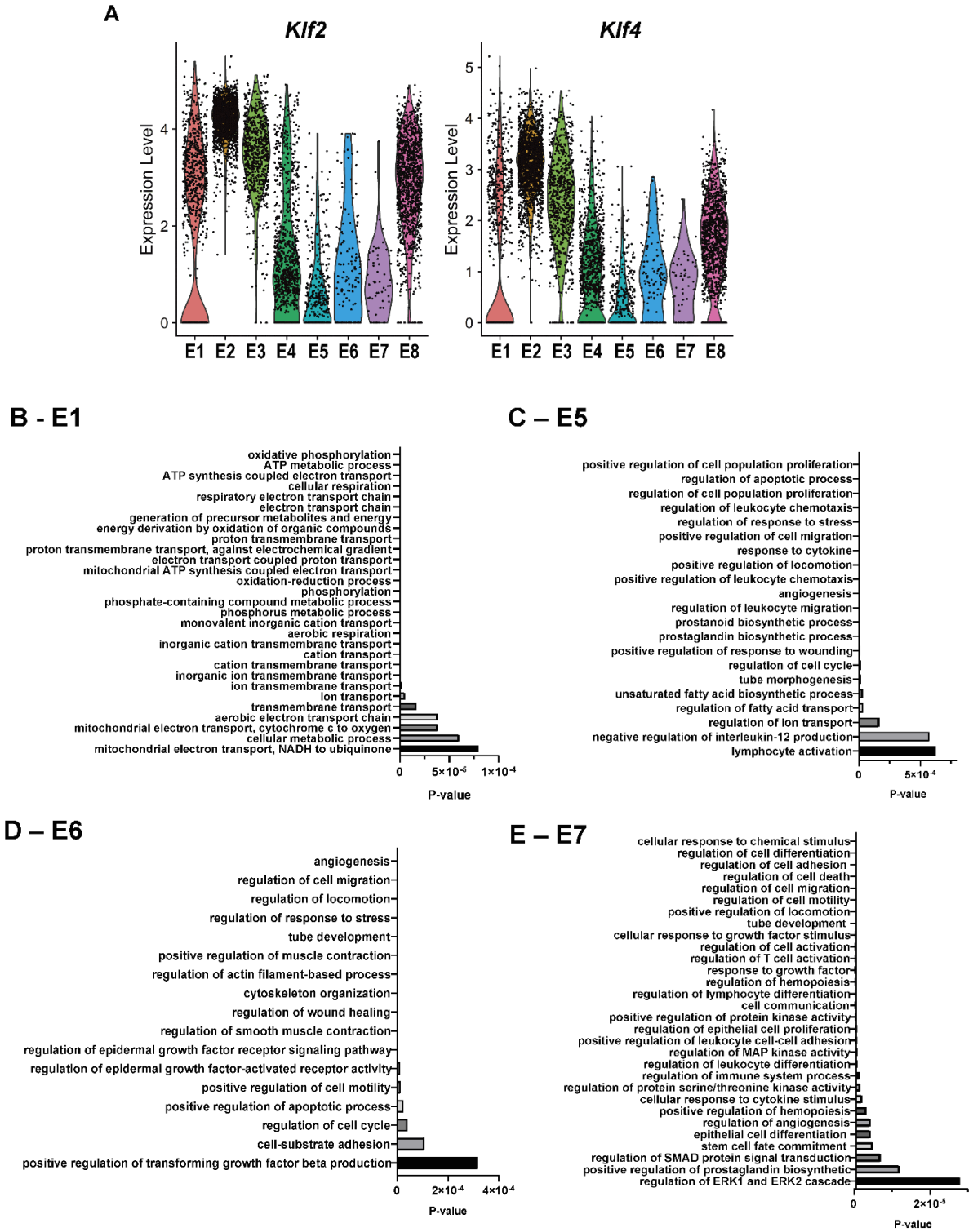


Figure S2 (related Figure 1 and 2). D-flow reduces the gene expression of *Klf2* and *Klf4*, and activates pro-atherogenic pathways in ECs. (A) The violin plots of scRNAseq data show expression of *Klf2* and *Klf4*, two well-known flow-sensitive genes, in all EC clusters, E1 to E8. **(B-E)** Gene Ontology pathway analysis was performed using the upregulated genes in E1 through E7 clusters in comparison to E2 representing the *s-flow* phenotype. Since not enough genes were differentially regulated in E3 and E4 in comparison to E2, the results are not shown. Shown are E1 vs. E2 (B), E5 vs. E2 (C), E6 vs. E2 (D), E7 vs. E2 (E), and X-axis shows the p-values.

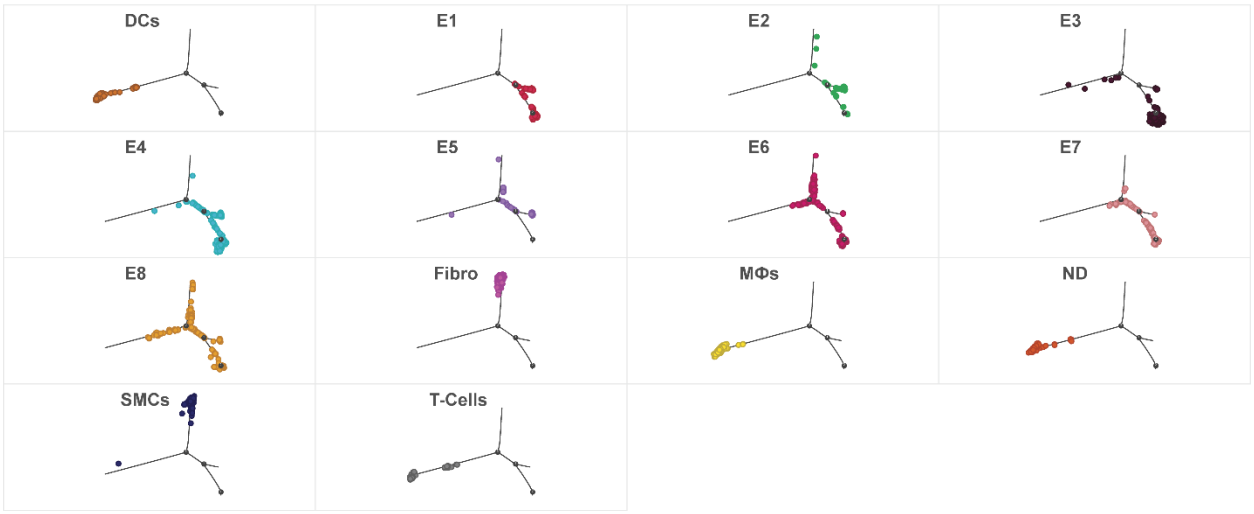


Figure S3 (related Figure 3). scRNAseq-based pseudotime trajectory analysis divided by cell clusters. Each cluster is colored and labeled with its corresponding color and name.

SUPPLEMMENTARY TABLES

Table S1 (related Figure 1). scRNAseq information.

	Sample	**Mice #**	**Study groups**	**Single cells**	**Mean reads/ cell**	**Genes/ cell**
scRNAseq	2D-R	10	2 day, RCA	1,867	55,593	3,376
	2D-L		2 day, LCA	2,119	76,050	3,284
	2W-R		2 wk, RCA	1,263	88,631	3,537
	2W-L		2 wk, LCA	4,460	34,142	2,770
	Sample	**Mice #**	**Study groups**	**Single cells**	**Mean fragments/ cell**	**Total reads**
scATACseq	2D-R	12	2 day, RCA	1,291	24,459	304,487,130
	2D-L		2 day, LCA	5,351	22,201	372,162,757
	2W-R		2 wk, RCA	5,826	19,690	336,611,489
	2W-L		2 wk, LCA	5,856	22,398	370,557,062

Table S2 (related Figure 3). Chronic d-flow induces genes related to EndMT, EndICLT, EndoHT, and APC.

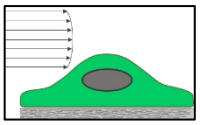
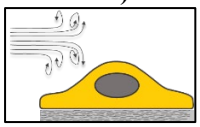
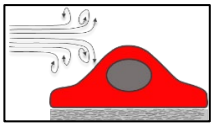
Process	Genes names	s-flow (E2 cluster) 	Acute d-flow (scRNAseq: EC5 & scATACseq EC6) 	Chronic d-flow (E8 cluster) 	References
Endothelial markers	*Pecam1*	+++	++	++	*(Kalluri et al., 2019)*
	Cdh5	+++	+++	+++	
	Icam2	+++	++	++	
	Tie1	+++	++	++	
EndMT	*Tagln*	-	+++	++	*(Lai et al., 2018; Mahmoud et al., 2017; Moonen et al., 2015)*
	Cnn1	--	--	+	
	Acta2	-	+++	+++	
	Snai1	--	-	++	
EndHT	*Sox7*	+++	+	+	*(Ottersbach, 2019)*
	Sox17	+++	+	+	
	Gata2	+++	+	+	
	Kit	--	-	++	
	Notch1	+++	+	+	
	EPRC	+	+++	++	
	Tie2	+++	+	+	
	Bmp4	+	++	+++	
ESC/EPC	*CD157*	++	-	++	*(Wakabayashi et al., 2018; Xiao et al., 2006)*
	Sca1	++	+	+	
APC	*H2-aa*	-	-	++	*(Santambrogio et al., 2019)*
	H2-Ab1	-	-	++	
	H2-Eb1	-	-	++	
	Cd74	-	-	++	
EndICLT	*C1qa*	--	--	+	
	C1qb	--	--	+	
	C5ar1	--	--	+	
	Tnf	--	--	+	
	Lyz2	--	--	++	

Table S3 (related Figure 4). List and sequences of qPCR primers for mRNA expression

Primer Name	Nucleotide Sequence
KLF2 Forward	5'-CCAAGAGTTCGCATCTGAAGGC-3'
KLF2 Reverse	5'-CCGTGTGCTTTCGGTAGTGGC-3'
KLF4 Forward	5'-ATCTTTCTCCACGTTCGCGTCTG-3'
KLF4 Reverse	5'-AAGCACTGGGGGAAGTCGCTTC-3'
C1QC Forward	5'-AGGATGGGTACGACGGACTG-3'
C1QC Reverse	5'-GTAAGCCGGGTTCTCCCTTC-3'
C5AR1 Forward	5'-TCCTTCAATTATACCACCCCTGA-3'
C5AR1 Reverse	5'-GGAAGACGACTGCAAAGATGA-3'
SNAI1 Forward	5'-GCACGGCCTAGCGAGTGGTT -3'
SNAI1 Reverse	5'-GGGCTGCTGGAAGGTAAACTCTGG-3'
TAGLN Forward	5'-CCTGGCTAGGGAAACCCACCCT-3'
TAGLN Reverse	5'-TCTGGGGAAAGCTCCTTGGAAGT-3'
18S Forward	5'-AGGAATTGACGGAAGGGCACCA-3'
18S Reverse	5'-GTGCAGCCCCGGACATCTAAG-3'