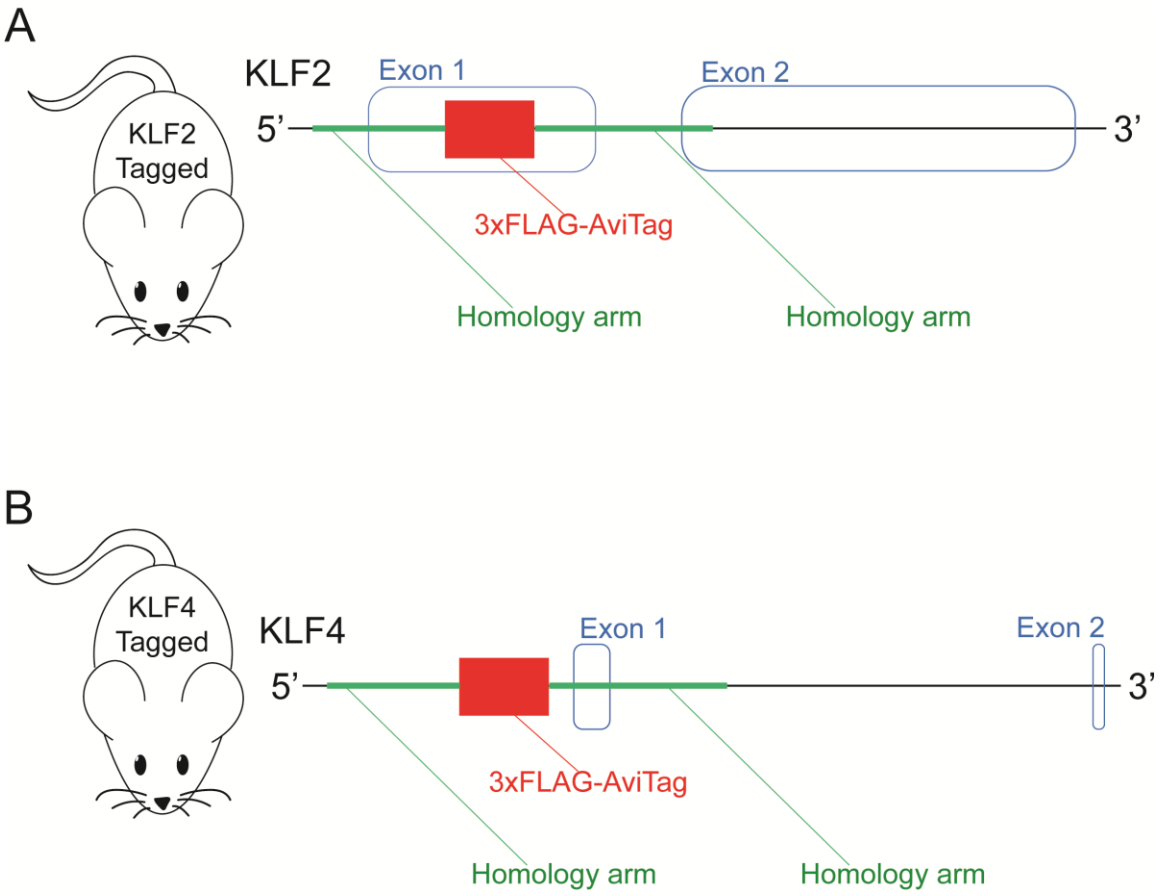
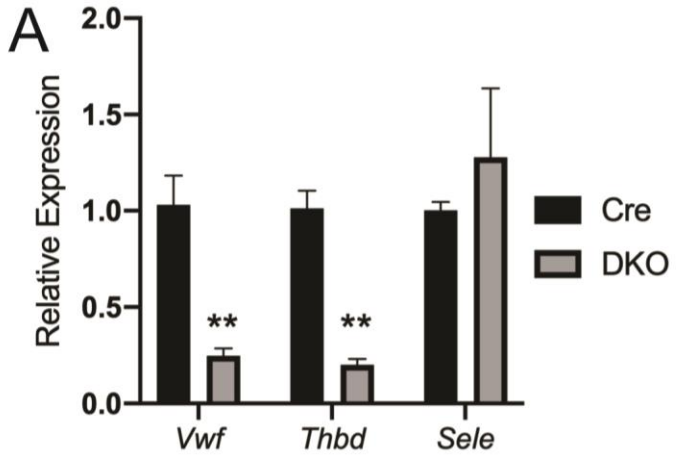
SUPPLEMENTAL MATERIAL

Figure S1. Models of KLF2 and KLF4 tagged mice.

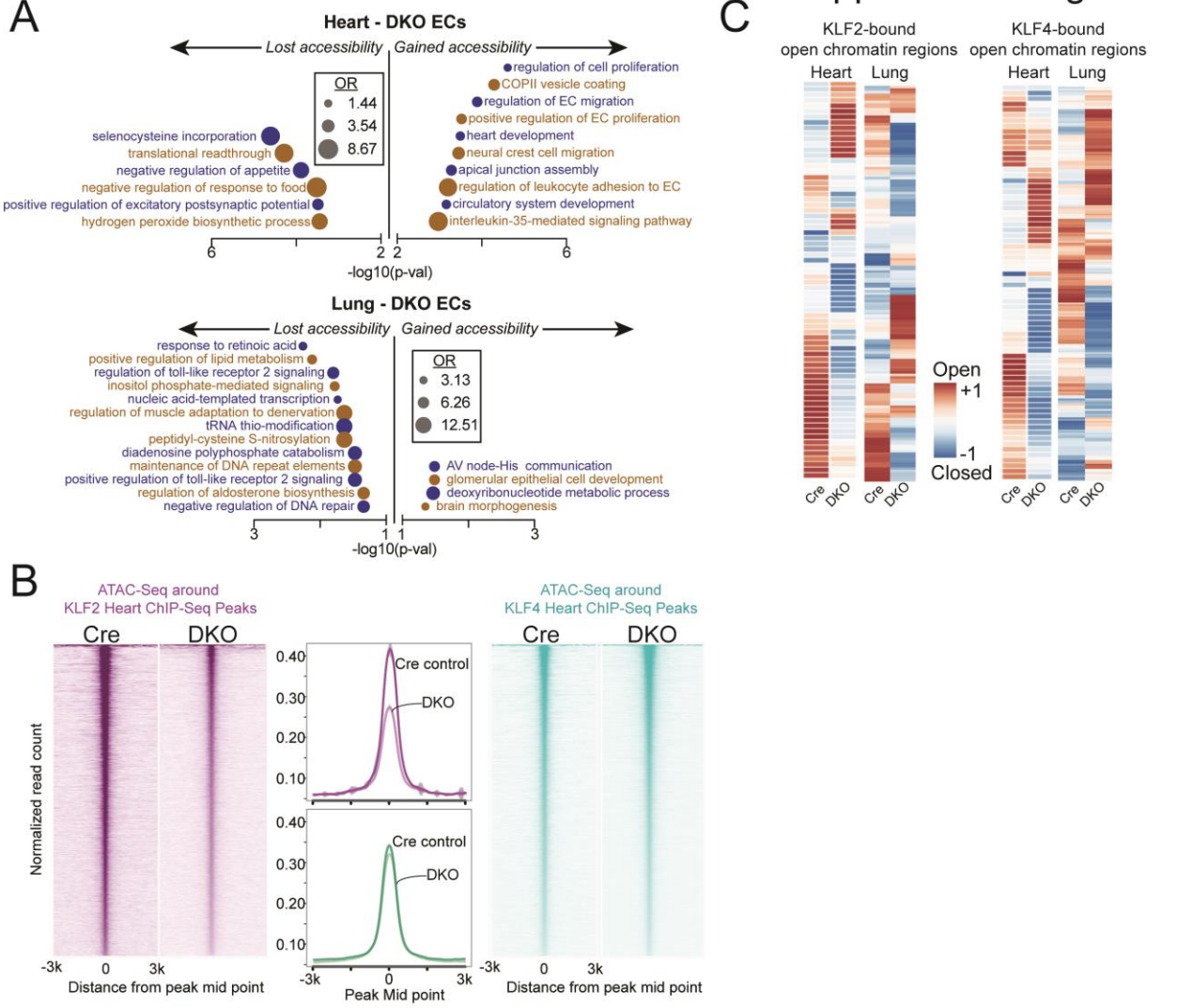


(A) Depiction of 3xFLAG-AviTag cassette insertion into the endogenous KLF2 locus. **(B)** Depiction of 3xFLAG-AviTag cassette insertion into the endogenous KLF4 locus.

Figure S2. Confirmation of changes in transcription due to loss of KLF2 and KLF4 in lung ECs.

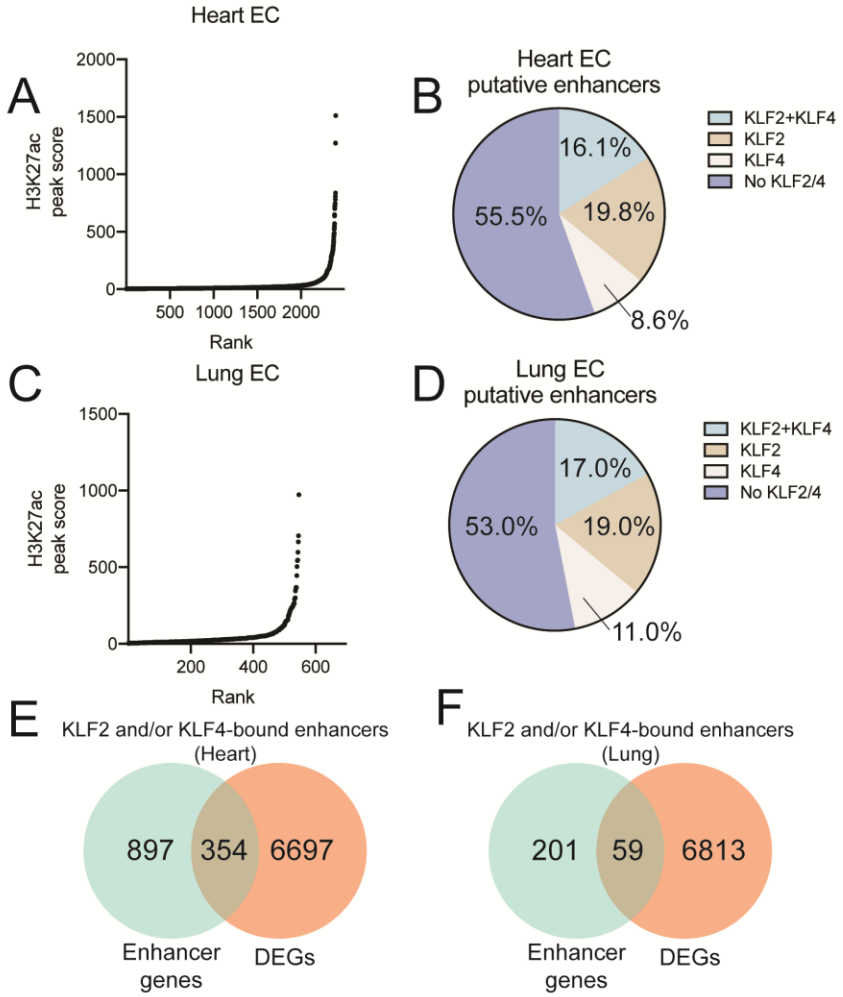


Supplemental Figure 3



Supplemental Figure 3: *Endothelial KLFs increase chromatin accessibility with factor-specific bias.* **(A)** Gene ontology biological process (GO BP) for regions with gained/lost accessibility in heart and lung DKO ECs. Node size corresponds to odds ratio (OR) of computed mean rank of geneset compared to those of several random genesets (EnrichR). **(B)** Heatmap of ATAC-seq reads from Cre and DKO plotted with respect to corresponding ChIP-seq peak midpoints in heart (KLF2 in magenta, KLF4 in teal). Composite plots quantify the occupancy (reads) in a ±3kb window from peak midpoints. **(C)** Heatmaps showing KLF2- and KLF4-bound open (red) and closed (blue) regions in Cre compared to DKO in heart and lung ECs.

Figure S4. Comparative assessment of heart and lung EC enhancers.



G **KLF2+KLF4 bound enhancers (heart)**

GO Biological Process	p-val
Leukocyte rolling	7.37e-34
Cell extravasation	1.46e-21
Cell junction maintenance	4.49e-20
Chromatin disassembly	7.33e-20
Actin-myosin filament sliding	1.81e-16

H **KLF2+KLF4 bound enhancers (lung)**

GO Biological Process	p-val
Leukocyte rolling	8.33e-38
Cell extravasation	8.06e-28
Chromatin disassembly	2.51e-21
Actin-myosin filament sliding	1.29e-18
Lamellipodium morph.	1.28e-15

(A, C) Hockey stick plot depicting called putative enhancer peaks in heart and lung ECs (from Cre mice). **(B,D)** Proportion of heart/lung enhancers bound by KLF2, KLF4, or KLF2+KLF4. **(E)** Differential expression of genes associated with enhancers with KLF2 and/or KLF4 bound in heart and **(F)** lung. These represent any enhancer with at least one of these two factors present. **(G)** Functional enrichment of gene ontology (GO) biological process terms of genes associated with KLF2+KLF4-bound enhancers in heart and **(H)** lung.