SUPPLEMENTAL MATERIAL

Single-cell RNA-seq Unveils Unique Transcriptomic Signatures of Organ-Specific Endothelial Cells

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Supplemental Tables

Supplemental Table I. Genes used to annotate endothelial cells in each organ from the *Tabula Muris* dataset

Tissue	**EC markers**
Aorta	*Cav1*, *Cdh5*, *Fabp4*, *Pecam1*
Adipose	*Cd34*, *Cdh5*, *Pecam1*
Brain	*Cldn5*, *Ocln*, *Pecam1*, *Slco1c1*
Diaphragm	*Pecam1*
Heart	*Cav1*, *Cdh5*, *Ehd3*
Kidney	*Plvap*
Liver	*Kdr, Nrp1*, *Oit3*, *Pecam1*
Lung	*Pecam1*
Mammary Gland	*Esam*, *Pecam1*
Pancreas	*Cdh5*, *Kdr*, *Pecam1*
Skeletal Muscle	*Pecam1*
Trachea	*Pecam1*

Supplemental Table II. Expression profile of non-endothelial genes in endothelial cells from *Tabula Muris*

	Marker	**% ECs not expressing marker**	**% ECs expressing marker**
Neuronal	Snap25	95.92	4.48
	Rbfox3	99.02	0.98
	Syp	97.9	2.1
	L1cam	97.06	2.94
Hepatocyte	Alb	23.08	76.92
	Ttr	60.44	39.56
	Afp	100	0
	Hnf4a	96.7	3.3
Cardiomyocyte	Actn2	95.75	4.25
	Tnnt2	79.01	20.99
	Tnni3	76.98	23.02
	Nppa	79.61	20.39
Kidney	Nphs2	100	0
	Kirrel	99.21	0.79
	Slc12a1	99.21	0.79
	Slc12a3	97.62	2.38
Lung	Igfbp2	91.92	8.08
	Scgb1a1	92.64	7.36
	Sftpc	90.76	9.24
	Trp63	99.13	0.87

Supplemental Table III. List of all differentially expressed genes in organ-specific endothelial cells

In a downloadable Excel spreadsheet titled ‘Supplemental Table III’

Supplemental Table IV. Unique genes associated with common pathways in organ-specific endothelial cells

Wnt signaling	
Brain	*Axin2, Fzd6, Jun, Lef1, Nkd1, Ppp3cc, Prkcb, Tcf7*
Heart	*Ccnd1, Ctnnbip1, Plcb4*
Liver	*Apc, Ep300, Lrp6, Ppp2r5e, Prickle1, Tcf7l1, Wnt2*
Lung	*Daam1, Nkd1, Prickle1, Prickle2*

MAPK signaling	
Adipose Tissue	*Atf4, Ddit3, Fos, Gadd45b, Hspa1b, Nr4a1*
Brain	*Dusp22, Fos, Hspa1a, Jun, Nr4a1, Ppp3cc, Prkcb, Ptprr, Sos1, Stmn1, Tgfb2*
Kidney	*Cd38, Cmpk2, Ldhb, Lpcat1, Pola2, Ptgs1, Upp1*
Lung	*Arrb1, Fgfr3, Mapt, Rps6ka1, Stk4, Stmn1*
Mammary Gland	*Atf4, Dusp5, Gadd45b, Gadd45g, Map2k3, Myc, Relb*
Pancreas	*Fgfr1, Ntf3, Pla2g1*
Trachea	*Atf4, Dusp2, Gadd45b, Map2k3, Map3k8, Nr4a1*

Cytokine-cytokine receptor interaction	
Aorta	*Csf1r, Lifr, Tgfbr2*
Brain	*Flt1, Ifnar1, Il10rb, Il6ra, Tgfb2, Tnfrsf12a, Tnfrsf19*
Liver	*Bmp2, Bmpr2, Cntfr, Csf1, Csf1r, Cxcl16, Flt4, Hgf, Il13ra1, Il1a, Il1r1, Il6st, Inhbb, Kdr, Kit, Lifr, Tgfb1, Tnfrsf1a*
Lung	*Bmpr2, Ghr, Il2rg, Kit, Tnfsf10, Vegfa*
Mammary Gland	*Csf2rb2, Csf3, Il4ra, Il6, Inhbb, Osmr, Tnfrsf10b, Tnfrsf12a, Tnfrsf1b*
Skeletal Muscle	*Csf1, Cxcl1, Cxcl10, Cxcl2, Cxcl9, Il6, Osmr, Tnfrsf10b, Tnfrsf12a*

Metabolic pathways	
Adipose Tissue	*Acox1, Gda, Papss2, Polr2a*
Lung	*Acer2, Adk, Agpat4, Aldh2, B4galt1, Guk1, Hyal2, Impdh1, Inpp4a, Kdsr, Stt3b*
Skeletal Muscle	*Adh1, Dgkh, Mgat4a, Odc1, Pfkp, Pnp, Sat1, Sphk1, Ugdh*

Supplemental Table V. Comprehensive list of predicted ligand-receptor pairs between endothelial and all parenchymal cells

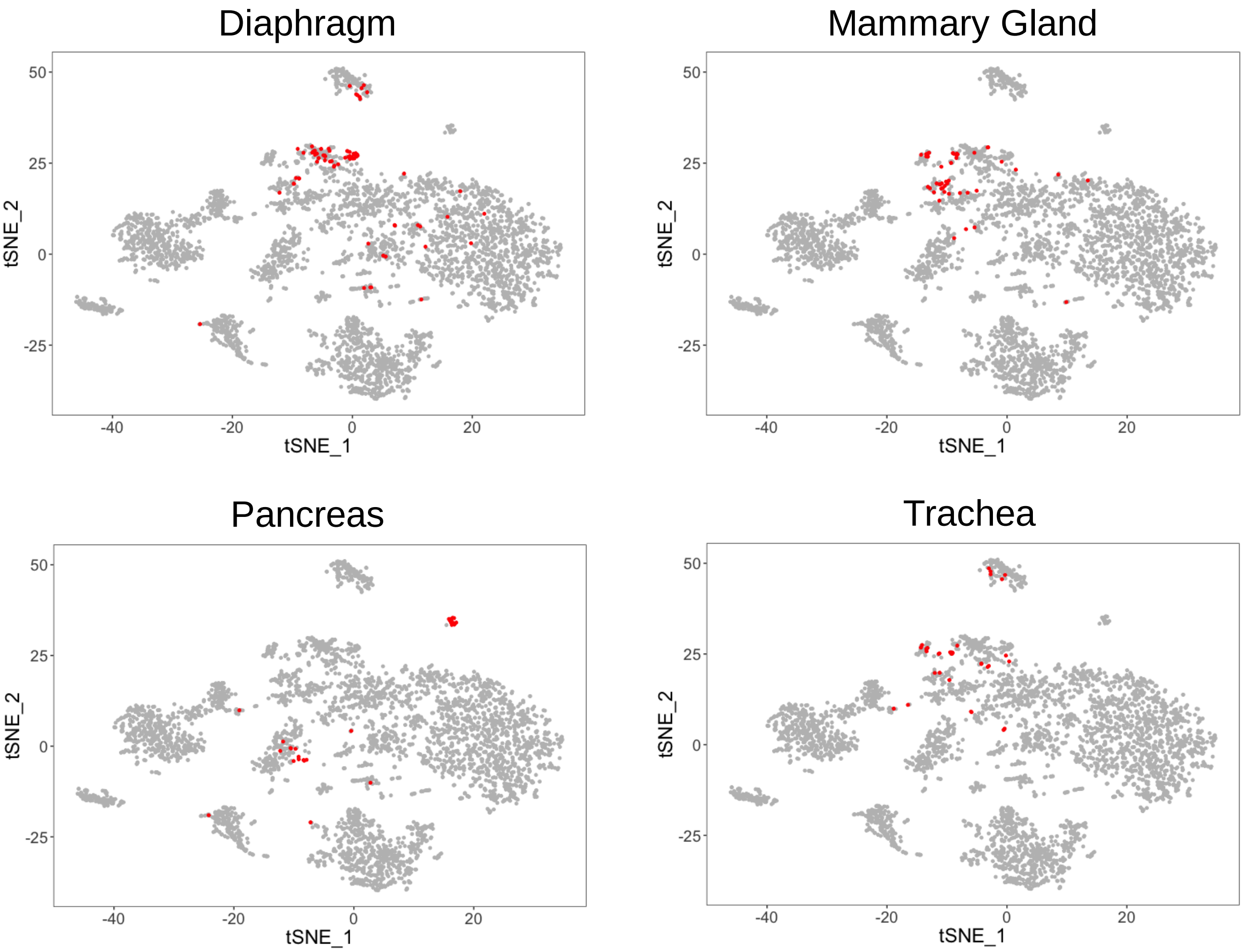
In a downloadable Excel spreadsheet titled ‘Supplemental Table V’

Supplemental Table VI. Top enriched genes that define the 13 unsupervised clusters

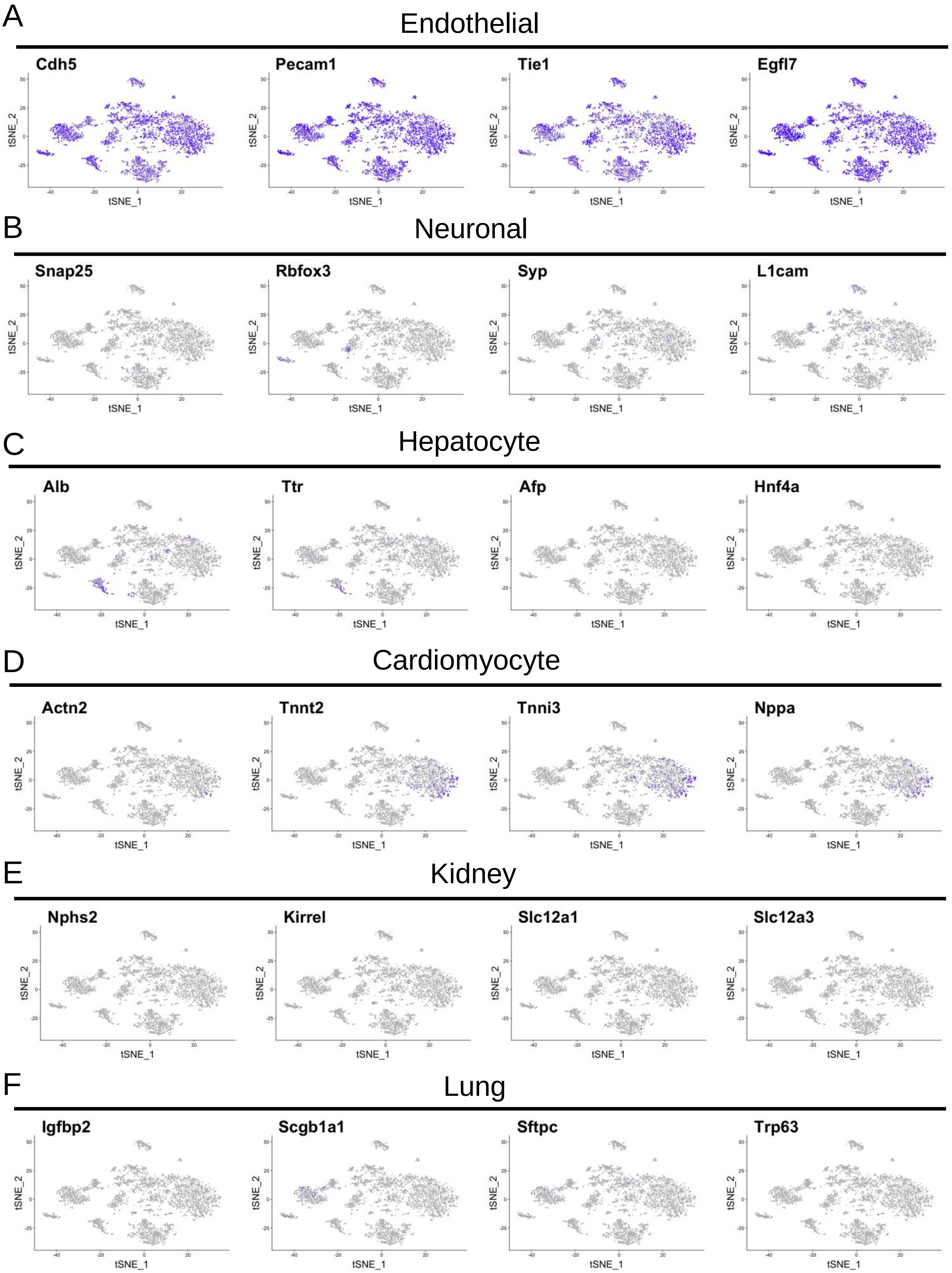
Cluster	Genes
Cluster 0	*Slc28a2, Eepd1, Car8, Kcna5, Meox2, Chek2, Lpl, Myadm, C1qtnf9, Trp53i11*
Cluster 1	*Slco1c1, Slco1a4, Slc22a8, Pglyrp1, Spock2, Mfsd2a, Stra6, Ddc, Slc38a5, Slc38a3*
Cluster 2	*Csf2rb, Angptl41, Col8a1, Scd1, Car3, Gpr160, Egr2, Tmem182, Cldn15, Flbn5*
Cluster 3	*P2ry122, Anxa4, Eci1, Rbp7, Tcf15, Aqp7, Larp4b, Szrd1, Trim37, Ccdc124*
Cluster 4	*Tmem221, Glp1r, H2-Aa, Sox11, Grtp1, Casc4, Nckap5, Adrb1, H2-Eb1, H2-Ab1*
Cluster 5	*Il6, Cxcl10, Adamts4, Rnd1, Sphk1, Cxcl1, Fosl1, Tubb6, Myc, Rab20*
Cluster 6	*Esm1, Tmem26, Dram1, Igfbp5, Fam101a, Dkk2, Col4a3, Pbx1, Ptpru, Chrm3*
Cluster 7	*Dnas1l3, Clec4g, Fcgr2b, Stab2, Oit3, Mup20, Hpx, Serpina3k, Bmp2, Mup3*
Cluster 8	*Cytl1, Sfrp1, Mgp, Kcne3, Gpr126, Plek2, Sulf1, Plac8, Ptgis, Tmem45a*
Cluster 9	*Mmrn1, Slc45a3, Apba2, Rein, Lrrn4cl, Fgl2, Gpm6a, Dtx1, Pdpn, Sh3gl3*
Cluster 10	*Myh11, Rgs4, Acta2, Nrip2, Vtn, Crispld2, Tpm2, Myl9, S1pr3, Lum*
Cluster 11	*Fibin, Pcdhac2, Rprml, Xpnpep2, Adamtsl5, D630045J12Rik, Tmeff2, Ccdc68, Gpnmb, Dpysl5*
Cluster 12	*Cela2a, Prss2, Pnlip, Cela1, Try4, Ctrb1, Cela3b, Cpa1, Cel, Clps*

Supplemental Table VII. Comprehensive list of sex-specific differentially expressed genes

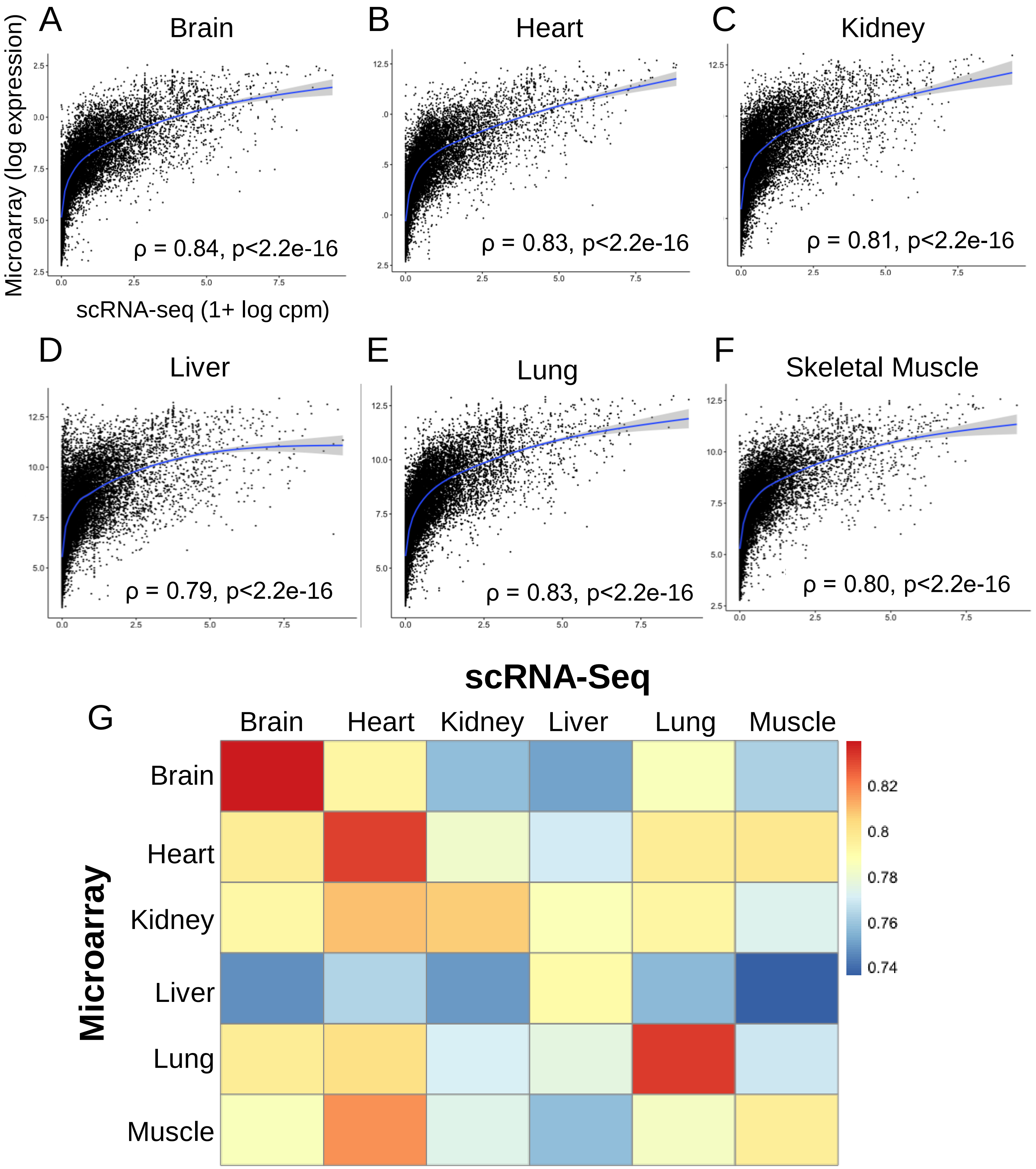
In a downloadable Excel spreadsheet titled ‘Supplemental Table VII’



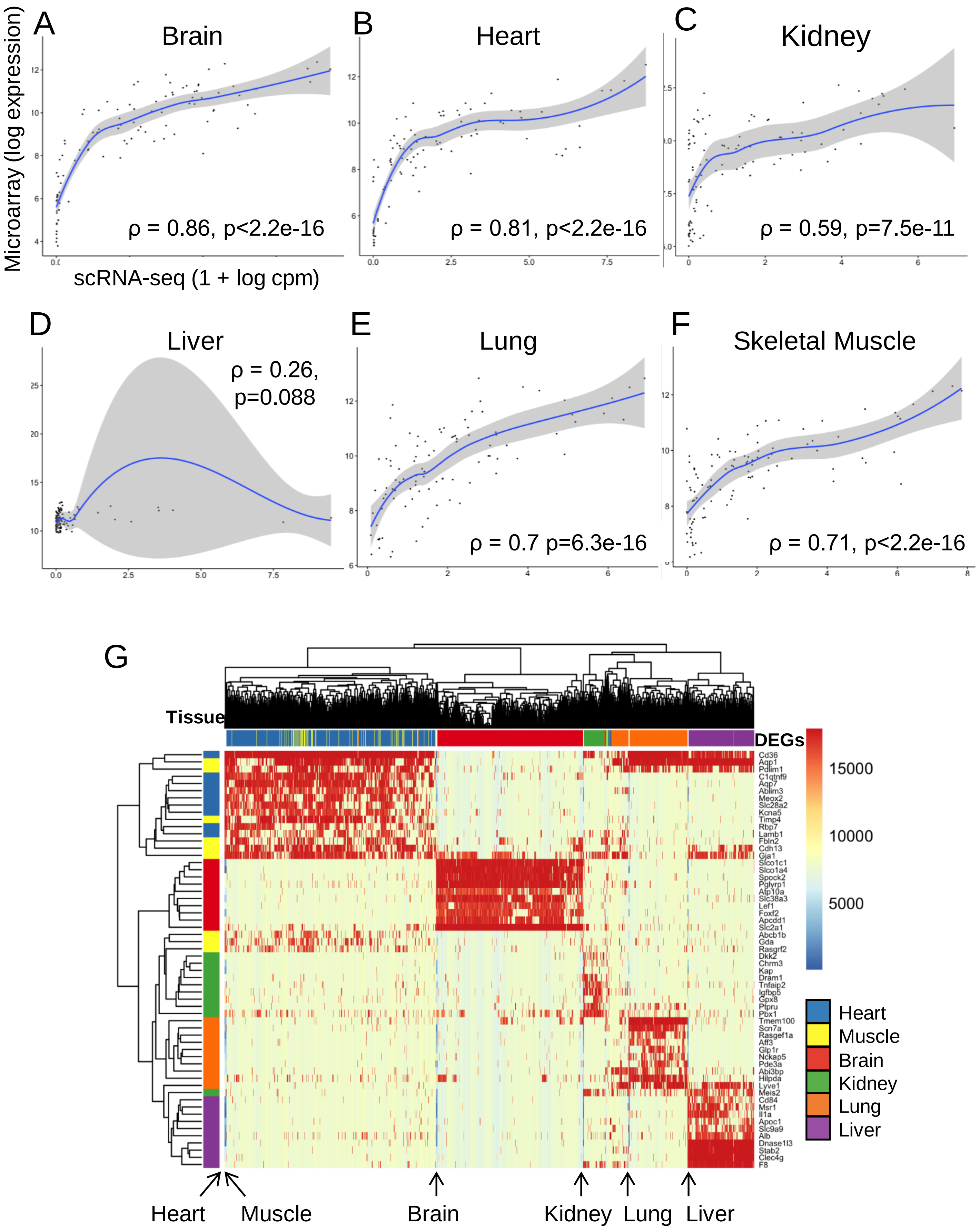
Supplemental Figure I. Tissue-specific endothelial cells. Feature plots of endothelial cells from diaphragm, mammary gland, pancreas, and trachea on 2-dimensional t-distributed stochastic neighbor embedding (t-SNE) projections.


A
Endothelial
Cdh5
Pecam1
Tie1
Egfl7
B
Neuronal
Snap25
Rbfox3
Syp
L1cam
C
Hepatocyte
Alb
Ttr
Afp
Hnf4a
D
Cardiomyocyte
Actn2
Tnnt2
Tnni3
Nppa
E
Kidney
Nphs2
Kirrel
Slc12a1
Slc12a3
F
Lung
Igfbp2
Scgb1a1
Sftpc
Trp63
tSNE_2
50
25
0
-25
tSNE_1
-40
-20
0
20

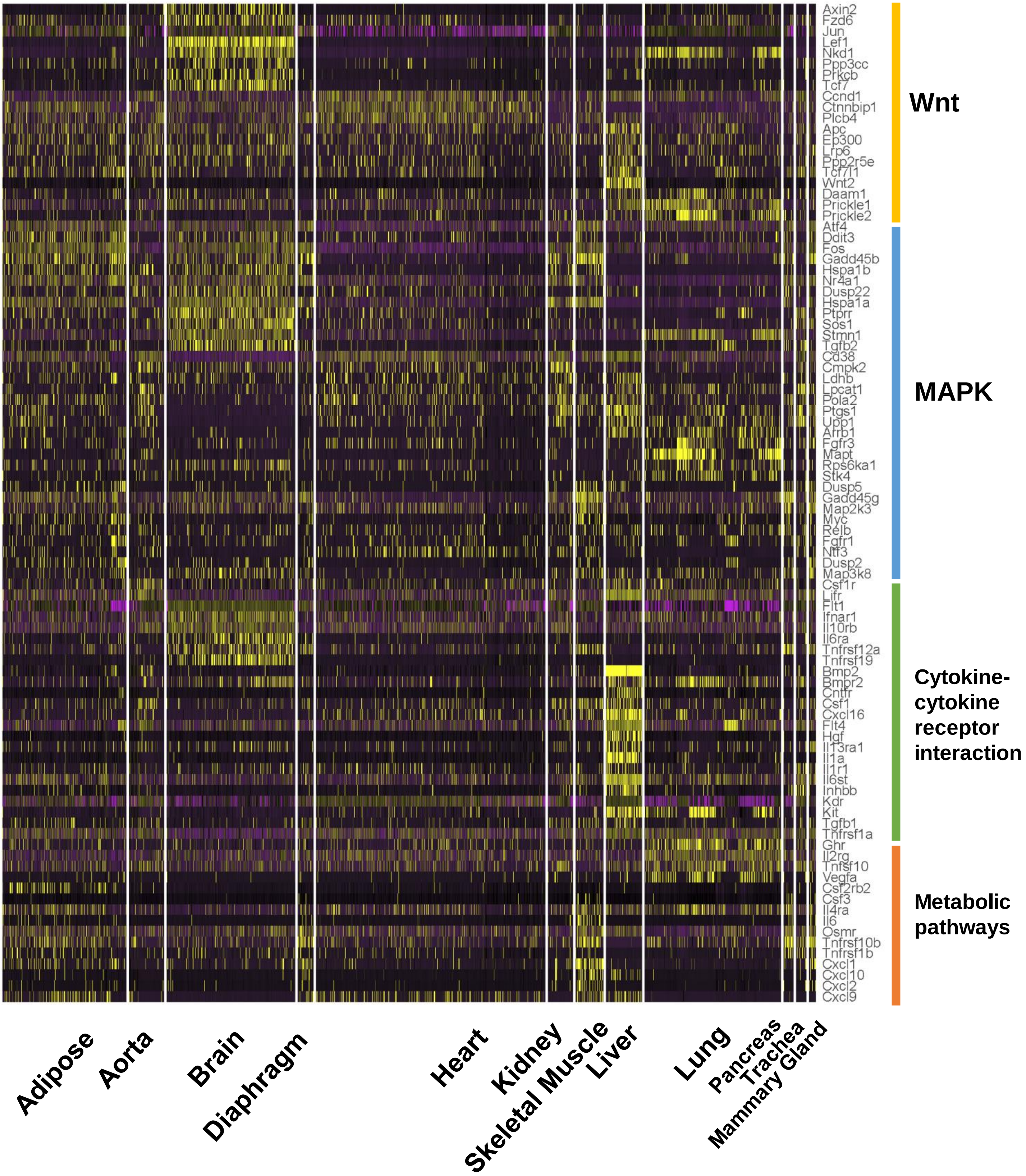
Supplemental Figure II. Expression levels of various parenchymal markers in endothelial cells. Single-cell transcriptome of all (**A**) endothelial cells extracted and analyzed from the *Tabula Muris* dataset show little to no expression of genes representative in (**B-F**) non-endothelial cell types. Blue and grey indicate cells with high and low expression of the select marker, respectively. In the kidney, Nphs2 and Kirrel are podocyte markers, Slc12a1 is a marker of epithelial cells in the ascending limb of the loop of Henle, and Slc12a3 is a marker of epithelial cells in the distal convoluted tubule. In the lung, Igfbp2 is a marker of alveolar type I cells, Scgb1a1 is a marker of club cells, Sftpc is a marker of alveolar type II cells, and Trp63 is a basal cell marker.



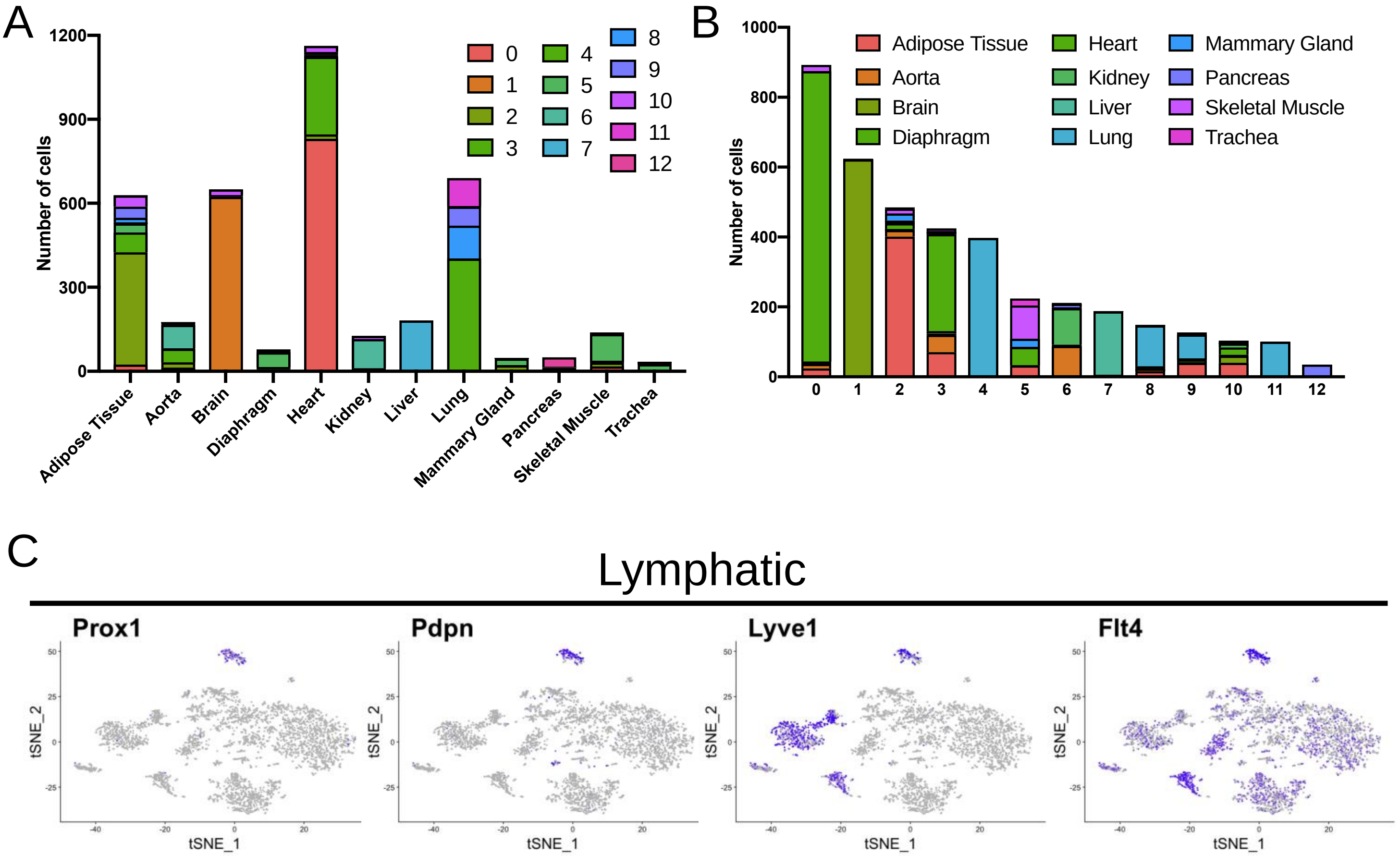
Supplemental Figure III. Correlation of tissue-specific endothelial cell gene expression between microarray- and single-cell RNA sequencing-based transcriptomic measurements. (A-F) Each panel shows gene expression determined by either scRNA-seq (x-axis) or microarray (y-axis) in endothelial cells from a specific tissue. Expression measurements for each gene were averaged across all cells (scRNA-seq) or samples (microarray). Spearman's rank correlation coefficients are shown in each panel. **(G)** Heatmap shows Spearman correlation coefficients determined from the similarity of endothelial gene expression between different tissues and transcriptomic techniques.


A
Brain
B
Heart
C
Kidney
Microarray (log expression)
scRNA-seq (1 + log cpm)
ρ = 0.86, p<2.2e-16
ρ = 0.81, p<2.2e-16
ρ = 0.59, p=7.5e-11
D
Liver
ρ = 0.26,
p=0.088
E
Lung
ρ = 0.7 p=6.3e-16
F
Skeletal Muscle
ρ = 0.71, p<2.2e-16
G
Tissue
DEGs
15000
10000
5000
Cd36
Aqp1
Pdlim1
C1qtnf9
Aqp7
Ablim3
Meox2
Slc28a2
Kcna5
Timp4
Rbp7
Lamb1
Fbln2
Cdh13
Gja1
Slco1c1
Slco1a4
Spock2
Pglyrp1
Atp10a
Slc38a3
Lef1
Foxf2
Apcdd1
Slc2a1
Abcb1b
Gda
Rasgrf2
Dkk2
Chrm3
Kap
Dram1
Tnfaip2
Igfbp5
Gpx8
Ptpru
Pbx1
Tmem100
Scn7a
Rasgef1a
Aff3
Glp1r
Nckap5
Pde3a
Abi3bp
Hilpda
Lyve1
Meis2
Cd84
Msr1
Il1a
Apoc1
Slc9a9
Alb
Dnase1l3
Stab2
Clec4g
F8
Heart
Muscle
Brain
Kidney
Lung
Liver
Heart
Muscle
Brain
Kidney
Lung
Liver

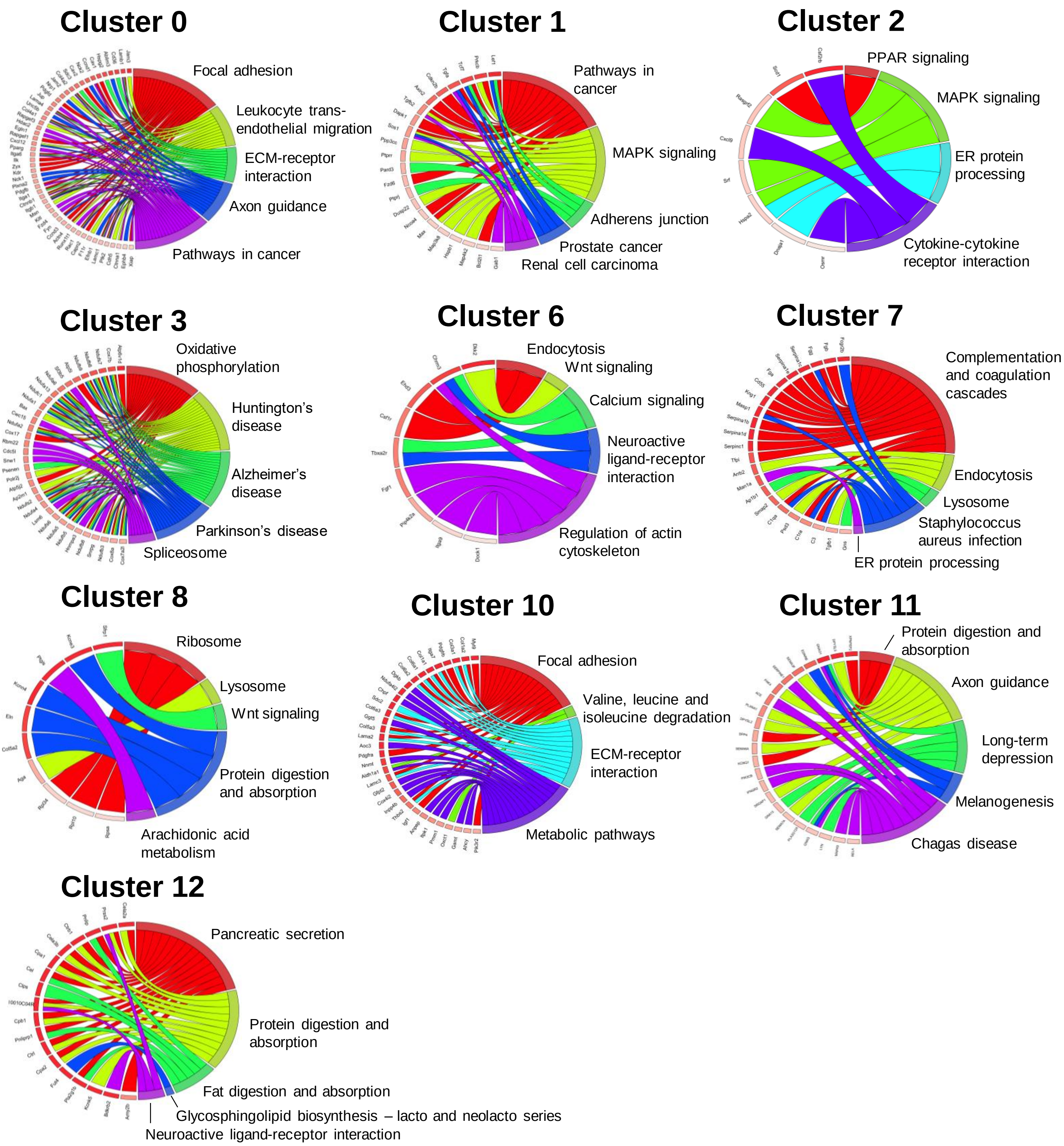
Supplemental Figure IV. Correlation of differentially expressed genes in tissue-specific endothelial cells between microarray and single-cell RNA sequencing-based transcriptomic measurements. (A-F) Each panel shows scRNA-seq (x-axis) or microarray (y-axis)-based expression measurements of the top 100 differentially expressed genes (DEGs), identified by microarray, in tissue-specific endothelial cells. Expression measurements for each DEG were averaged across all cells (scRNA-seq) or samples (microarray). Spearman's rank correlation coefficients are shown in each panel. **(G)** Heatmap shows the ranked expression of top tissue-specific DEGs in endothelial cells from various tissues. Red and blue indicate regions of high and low expression similarity, respectively. Tissue-specific DEG lists were generated by taking the intersection of tissue-specific DEGs determined by microarray and scRNA-seq. Hierarchical clustering of individual samples and DEGs is shown on the x and y axes, respectively. Arrows labeled with tissue names indicate the location of the microarray samples.



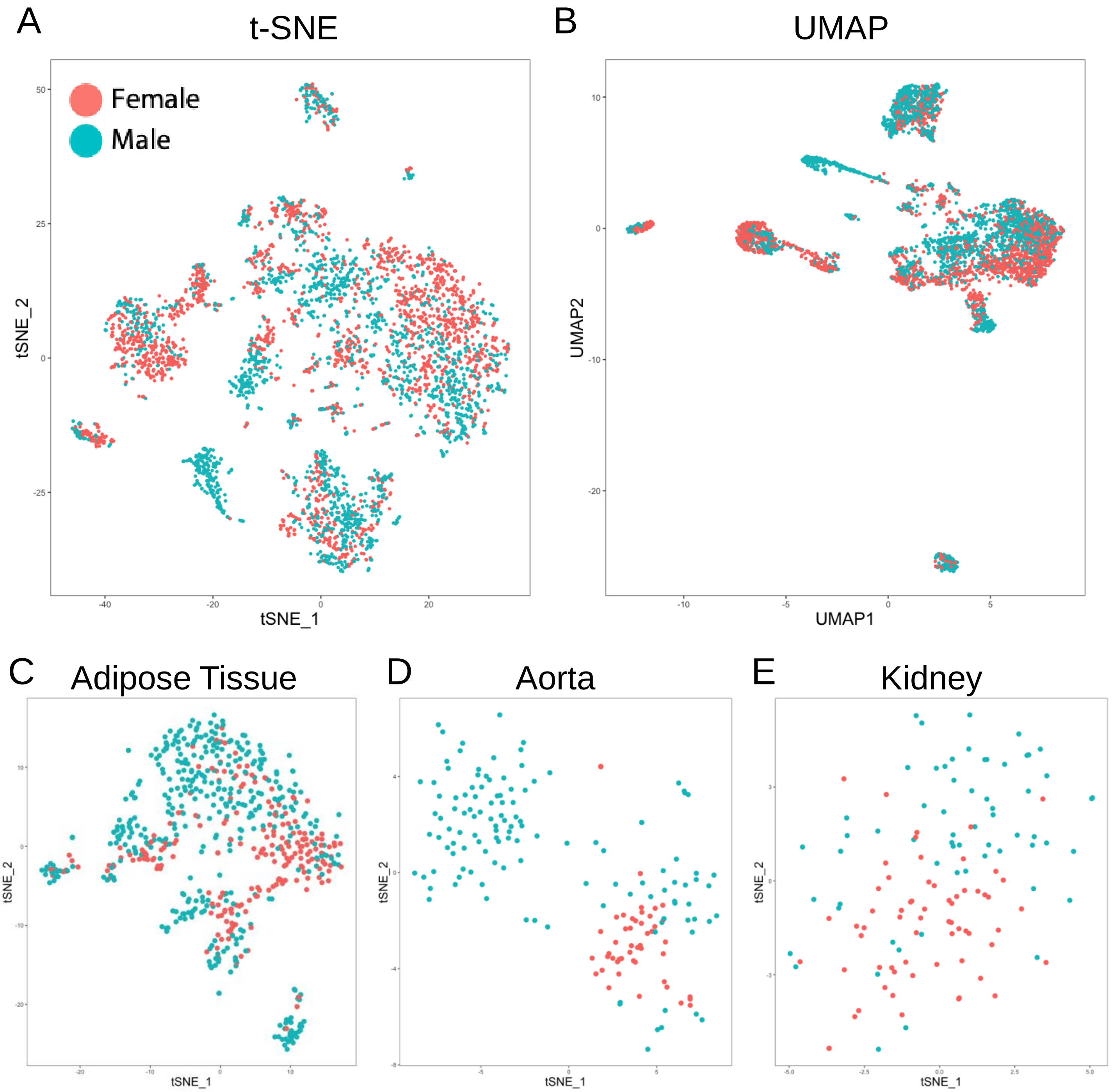
Supplemental Figure V. Heatmap of genes associated with common molecular pathways in organ-specific endothelial cells. Within a common molecular pathway, such as Wnt, MAPK, cytokine-cytokine receptor interaction, or metabolism-related pathways, unique sets of genes were found to be enriched in each organ-specific ECs. Yellow represents high gene expression and purple represents low gene expression.



Supplemental Figure VI. Unsupervised clustering and endothelial subtypes. (A) Stacked histogram shows the number of endothelial cells in each tissue that were assigned to different clusters. **(B)** Stacked histogram shows the number of endothelial cells in each cluster coming from various tissues. **(C)** Expression of putative lymphatic endothelial markers in endothelial cells. Blue and grey indicate cells with high and low expression of the select marker, respectively.



Supplemental Figure VII. Pathway enrichment analysis of endothelial clusters identified by unsupervised clustering. KEGG pathway enrichment analysis based on genes enriched in endothelial subtypes identified by unsupervised clustering. Chord plots show enriched pathways and associated genes.



F

Adipose Tissue	
Male	**Female**
Ddx3y	Wtap
Lars2	4921513D23Rik
Gcg	Arglu1
Gabpb2	Ppp1r16b
Stt3b	Slc39a1

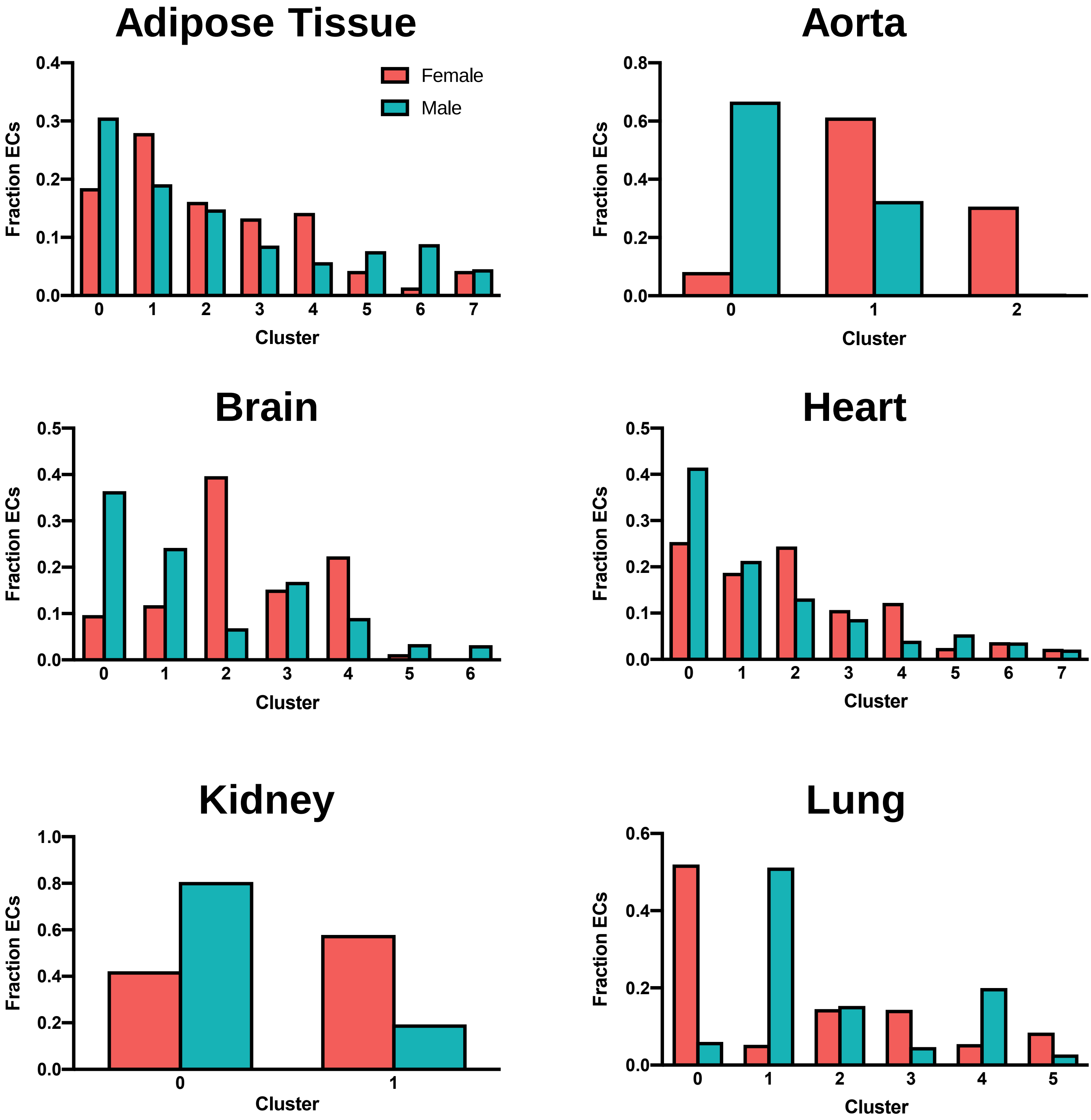
G

Aorta	
Male	**Female**
Esm1	Cxcl9
Igfbp5	Ptrf
Enpep	Sik1
Ptpru	Hspa8
Ehd3	Fabp4

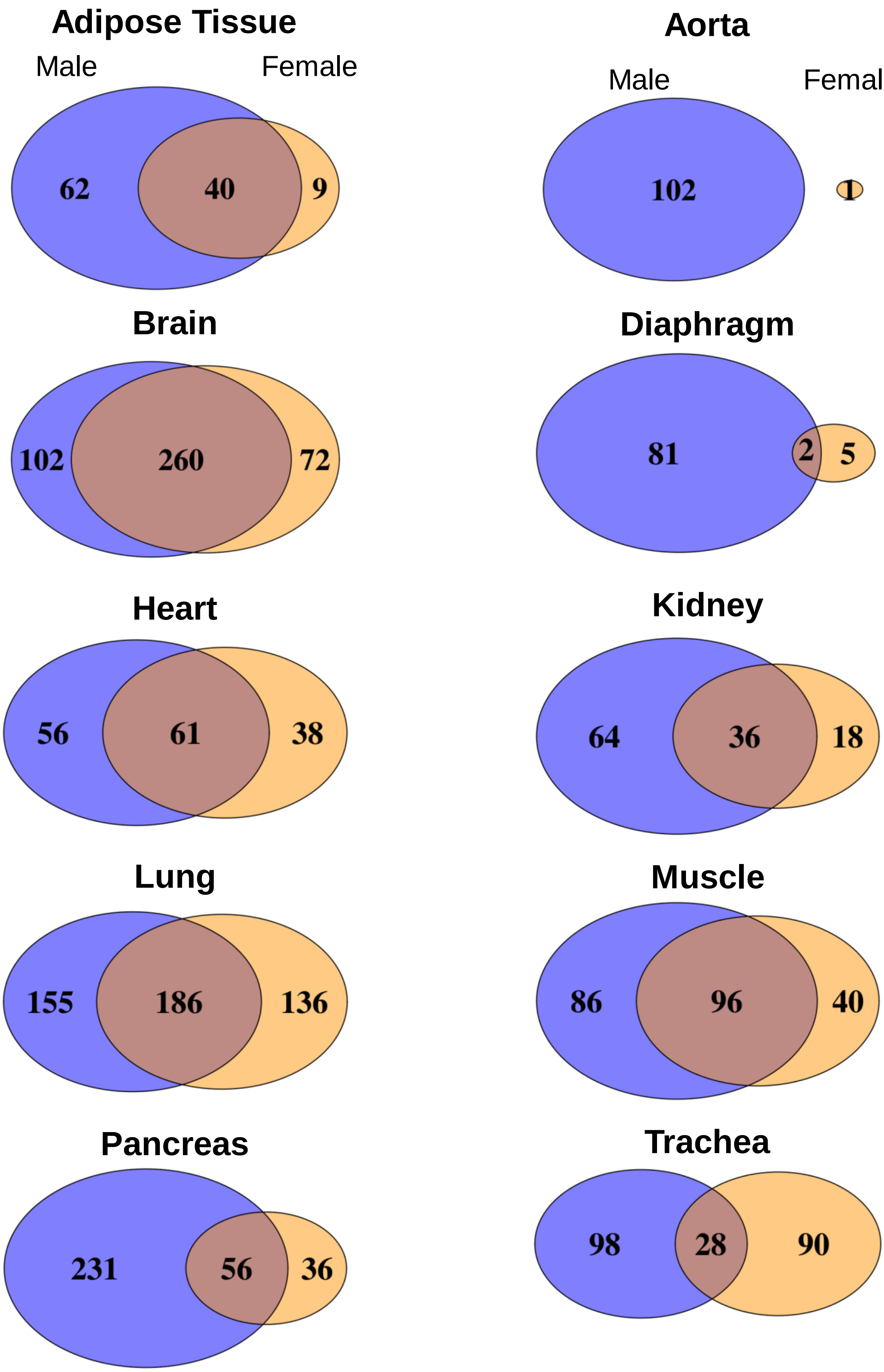
H

Kidney	
Male	**Female**
Ddx3y	Kdm6b
Lars2	Hspa1a
Per1	Sod1
Sox13	Ubb
Hist1h4d	

Supplemental Figure VIII. Sex differences in tissue-specific endothelial gene expression. 2-dimensional **(A)** t-SNE and **(B)** UMAP projections of endothelial cells from female (pink) and male (blue) mice. **(C-E)** t-SNE projections of endothelial cells from male and female mice in select organs. **(F-H)** Differentially expressed endothelial genes between male and female mice from select organs. UMAP, uniform manifold approximation and projection.



Supplemental Figure IX. Segregation of tissue-specific endothelial subclusters by sex. Fraction of tissue-specific male or female endothelial cells assigned to tissue-specific endothelial subclusters.



Supplemental Figure X. Sex differences in identification of tissue-specific endothelial markers. Venn diagrams show the overlap (pink) of differentially expressed genes identified in tissue-specific endothelial cells from male (blue) and female (orange) mice.