Supplemental Information

Single-cell transcriptome analysis of embryonic and adult endothelial cells allows to rank the hemogenic potential of post-natal endothelium

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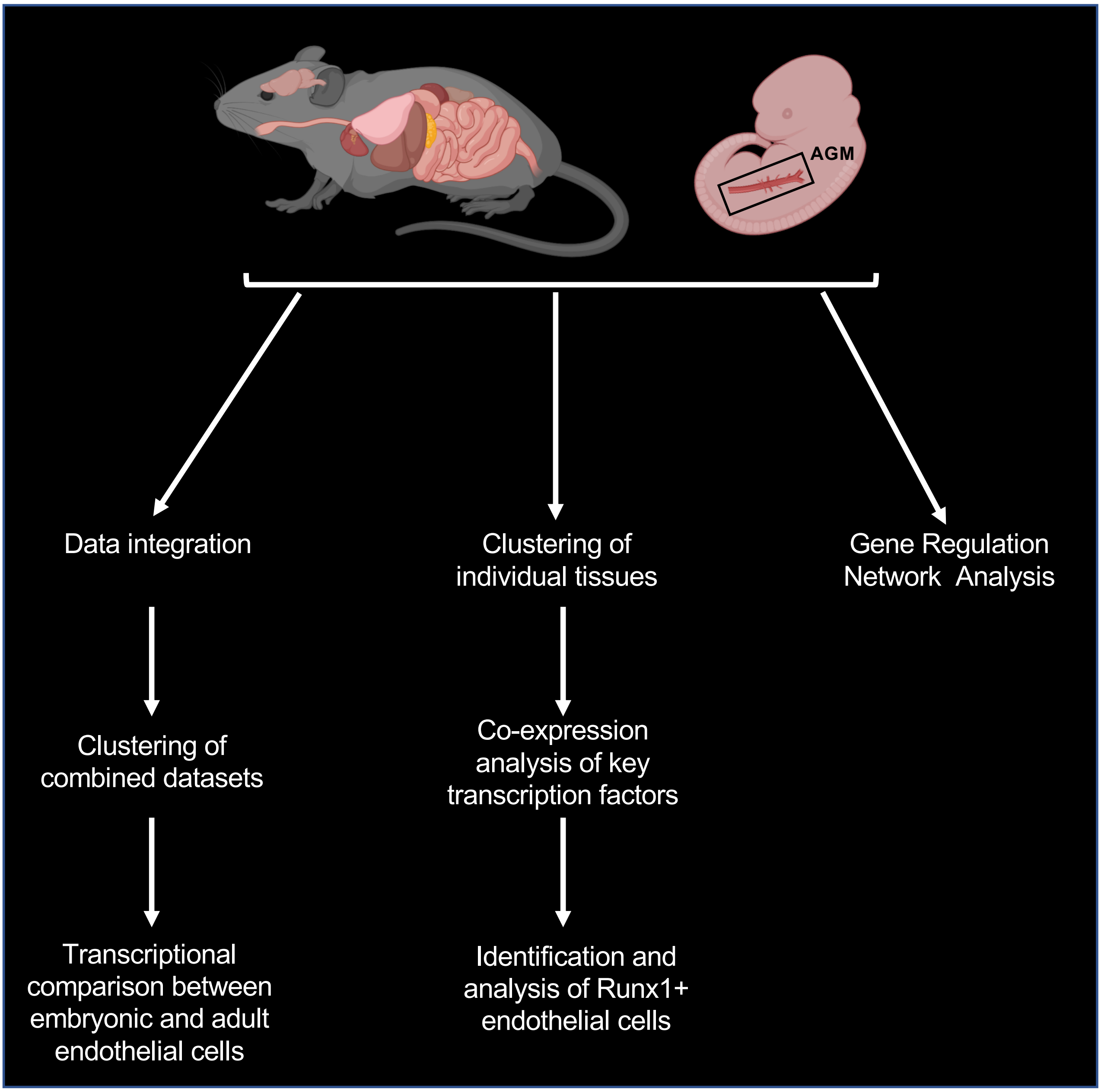
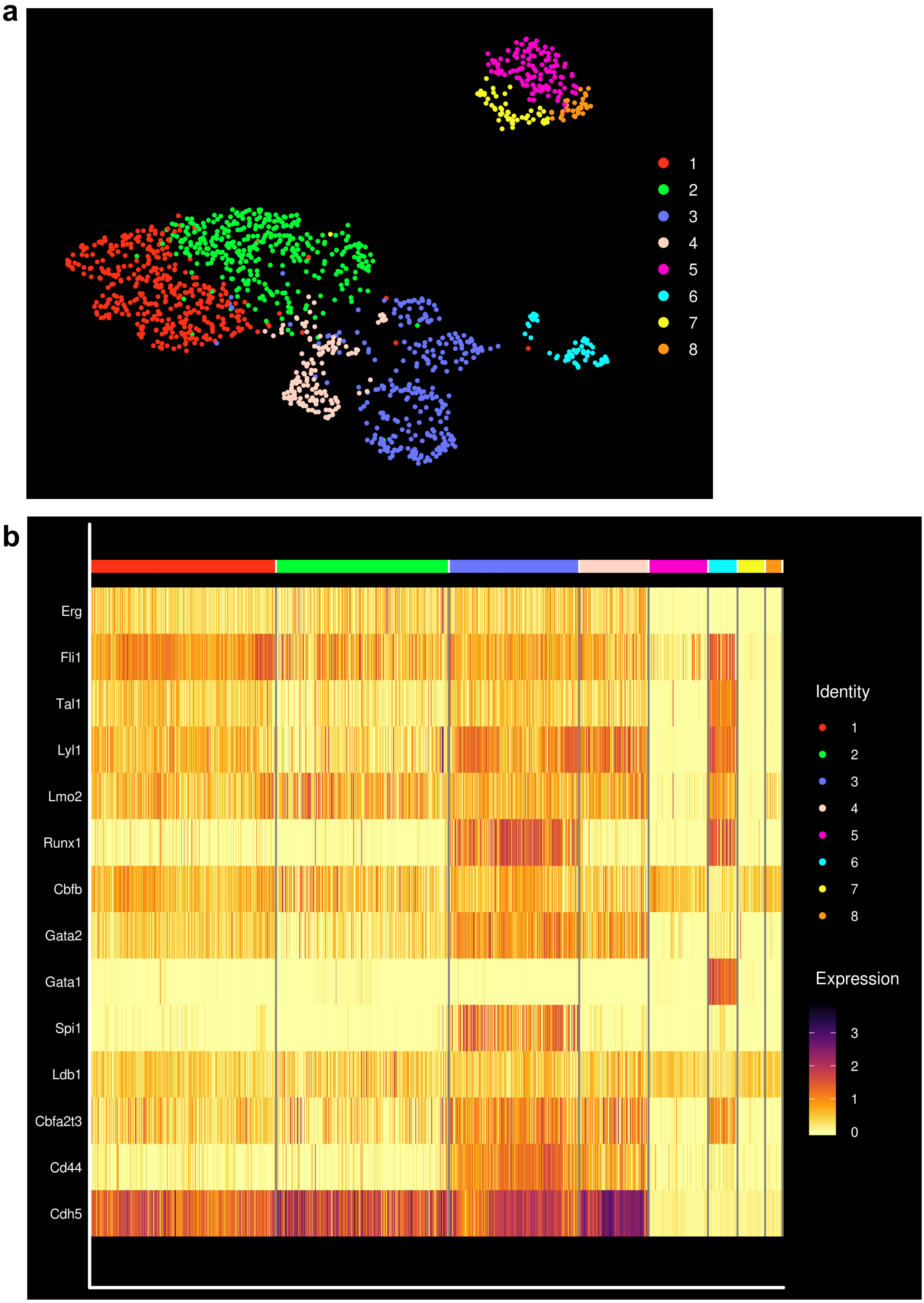
Supplementary Fig. 1



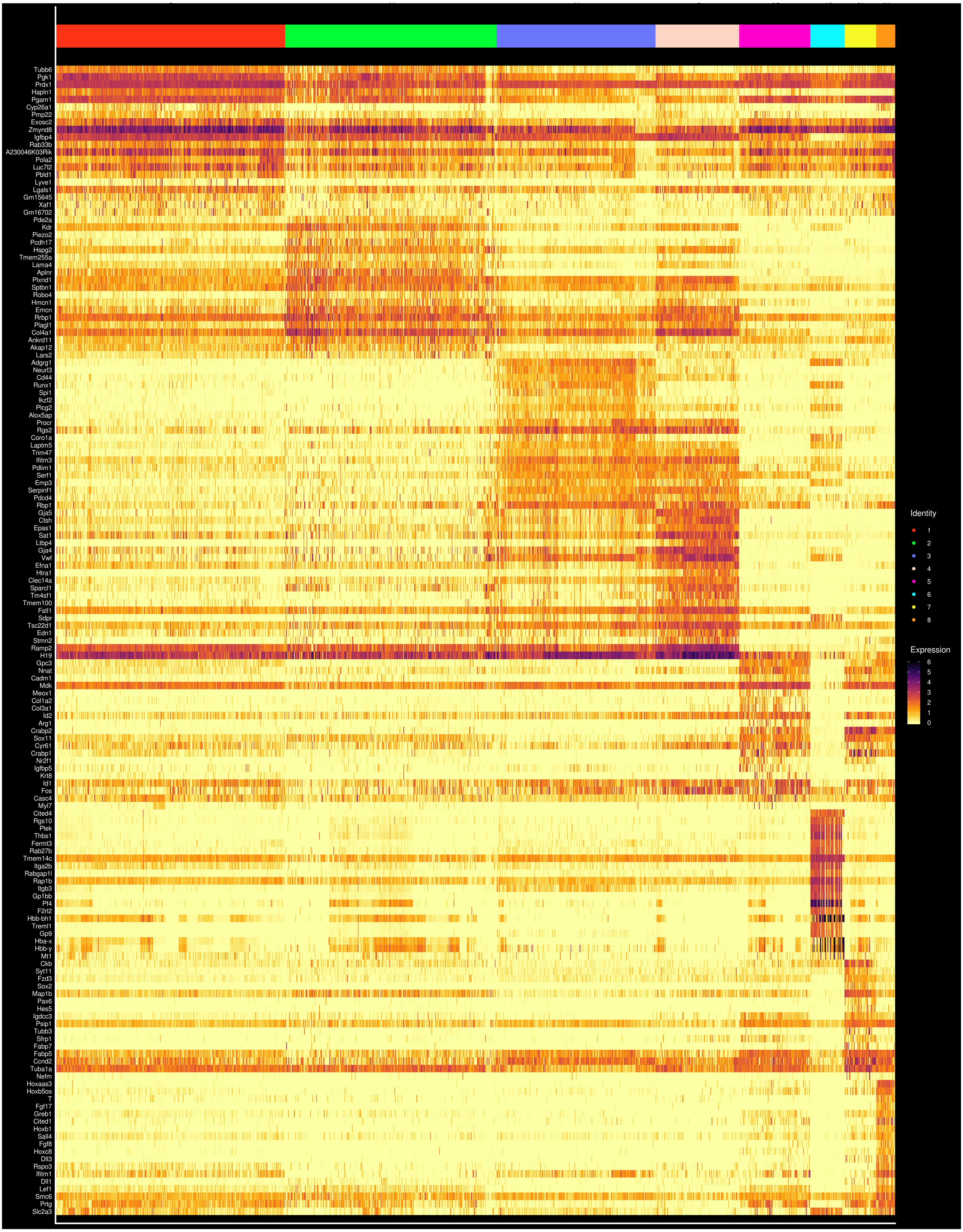
Supplementary Fig.1: Comparative analysis of murine embryonic and adult endothelial cells. Figure was made in BioRender - biorender.com.

Supplementary Fig. 2

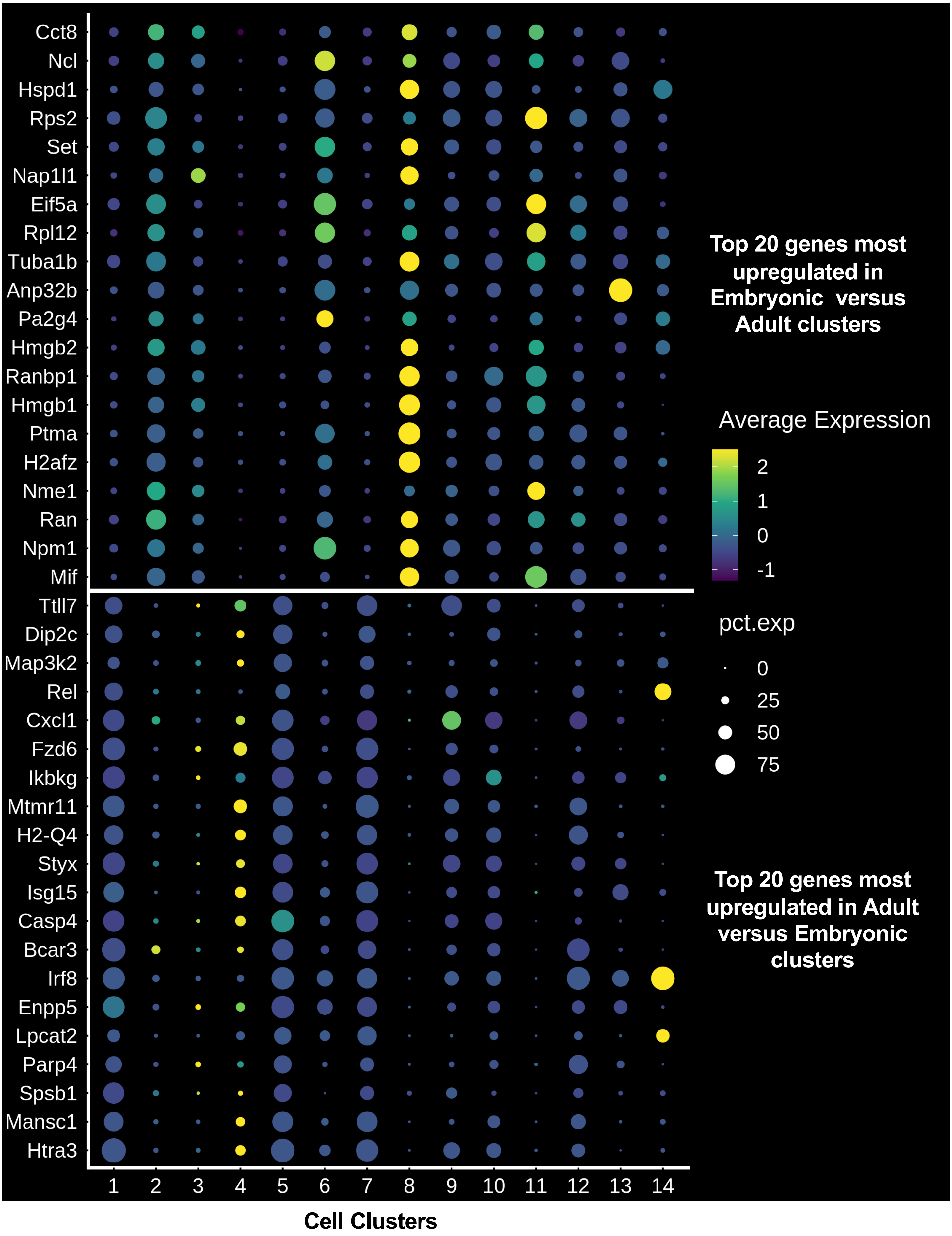


Supplementary Fig. 2: Clustering analysis of Embryo_dataset_1. a) UMAP plots showing the clustering analysis result; **b)** Gene expression heatmap for a selection of key genes involved in the EHT process.

Supplementary Fig. 3

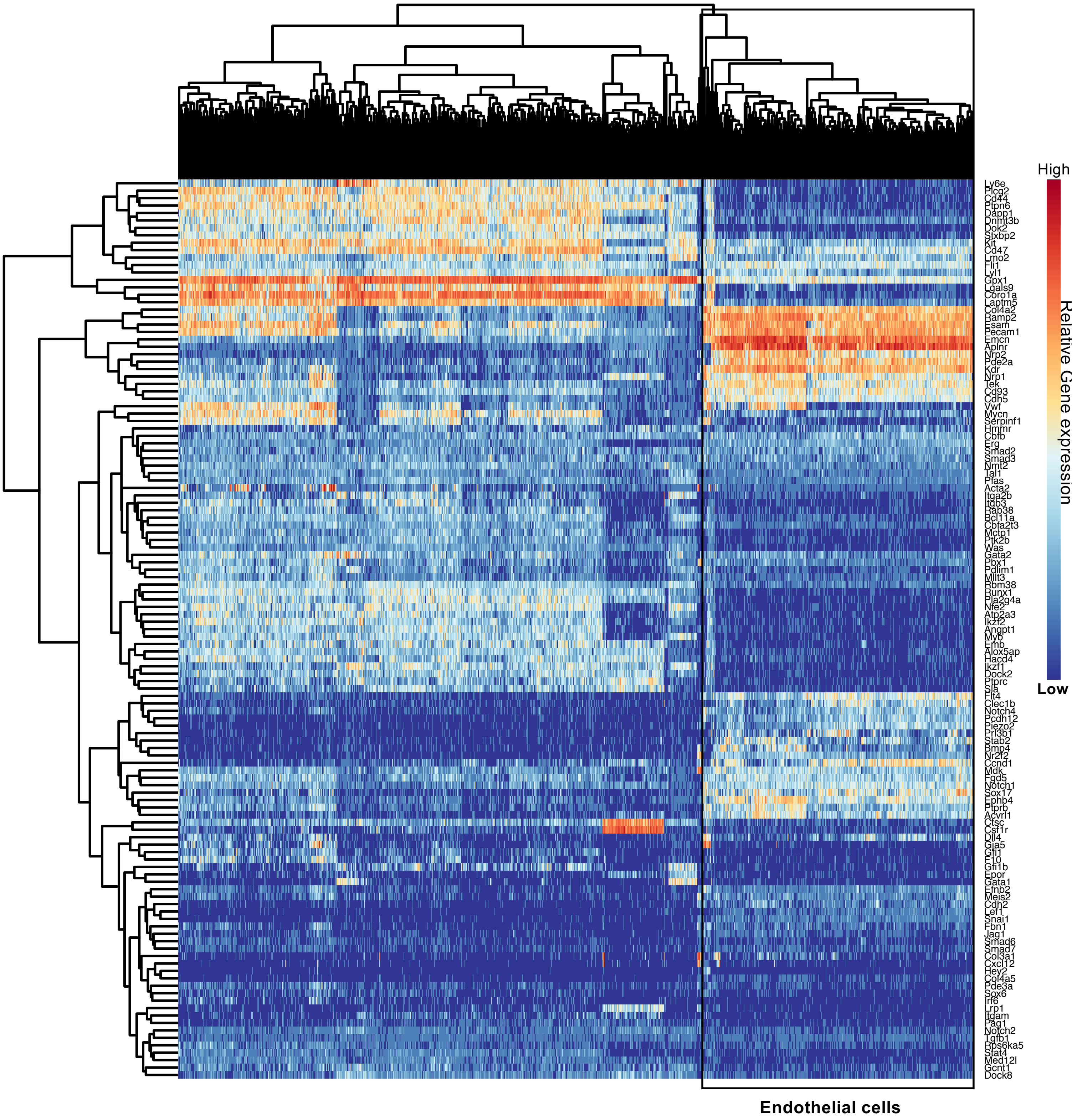


Supplementary Fig. 3: Marker genes of Embryo_dataset_1 clusters. Expression heatmap for marker genes for the eight indicated clusters.


Cct8
Ncl
Hspd1
Rps2
Set
Nap1l1
Eif5a
Rpl12
Tuba1b
Anp32b
Pa2g4
Hmgb2
Ranbp1
Hmgb1
Ptma
H2afz
Nme1
Ran
Npm1
Mif
Ttll7
Dip2c
Map3k2
Rel
Cxcl1
Fzd6
Ikbkg
Mtmr11
H2-Q4
Styx
Isg15
Casp4
Bcar3
Irf8
Enpp5
Lpcat2
Parp4
Spsb1
Mansc1
Htra3
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Cell Clusters
Top 20 genes most upregulated in Embryonic versus Adult clusters
Average Expression
2
1
0
-1
pct.exp
0
25
50
75
Top 20 genes most upregulated in Adult versus Embryonic clusters

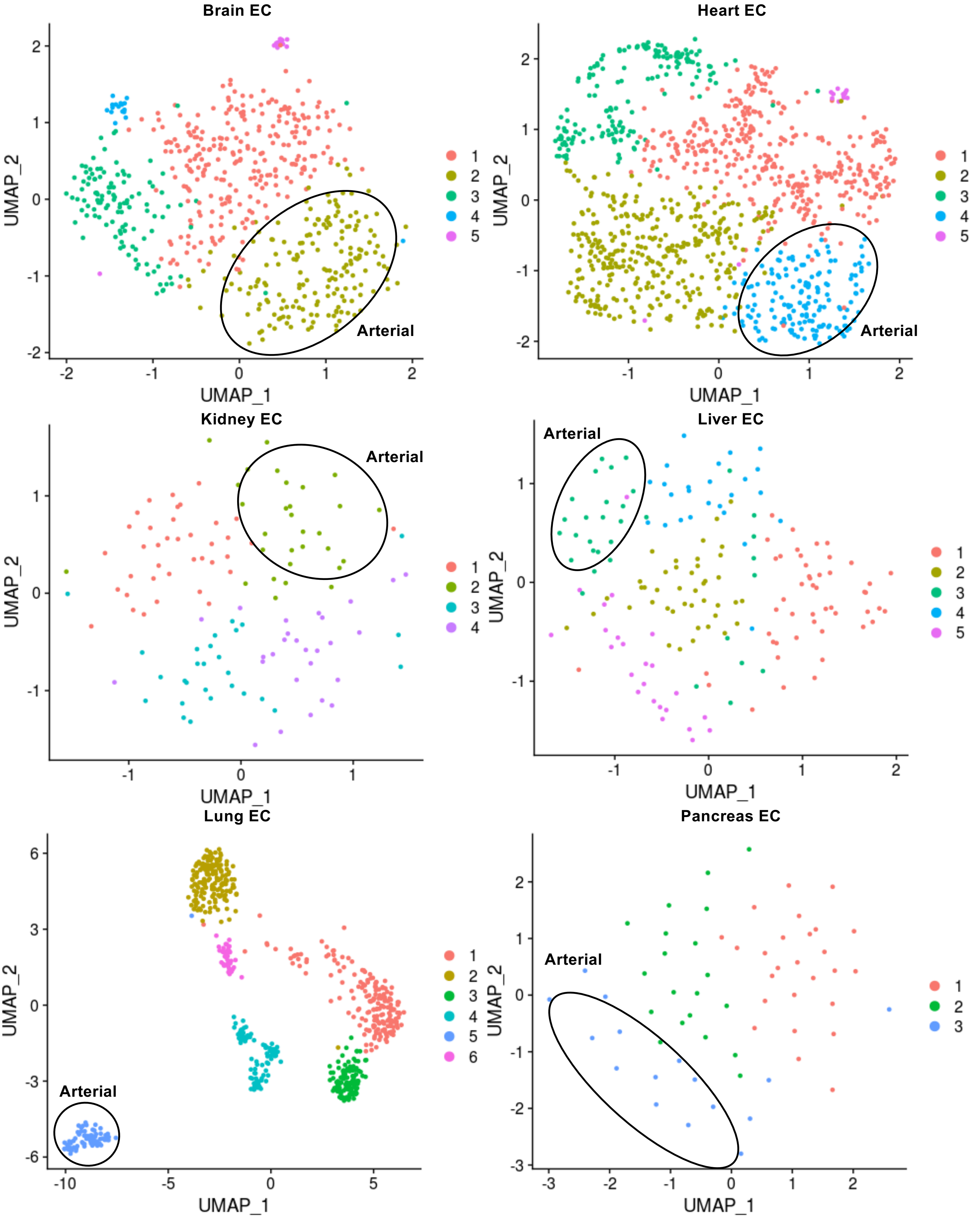
Supplementary Fig. 4: Differential gene expression analysis between the embryonic and adult derived cell clusters from Figure 2. Clusters 1, 5, 7, 10, 12, 13 were put together in the Adult group. On the other hand, Clusters 2, 3, 6, 8, 11 and 14 were affiliated to the Embryonic group. A differential expression test of Embryonic vs Adult group was performed. Dot plots indicating the expression and percentage of expression of the genes listed in the graph.

Supplementary Fig. 5



Supplementary Fig. 5: Clustering analysis of cells from Embryo_dataset_2. Heatmap plot of gene expression in the cells from the Embryo_dataset_2 using a gene list defined in Oatley et al. (2020). The endothelial cells are highlighted. Color scale bar indicates relative gene expression.

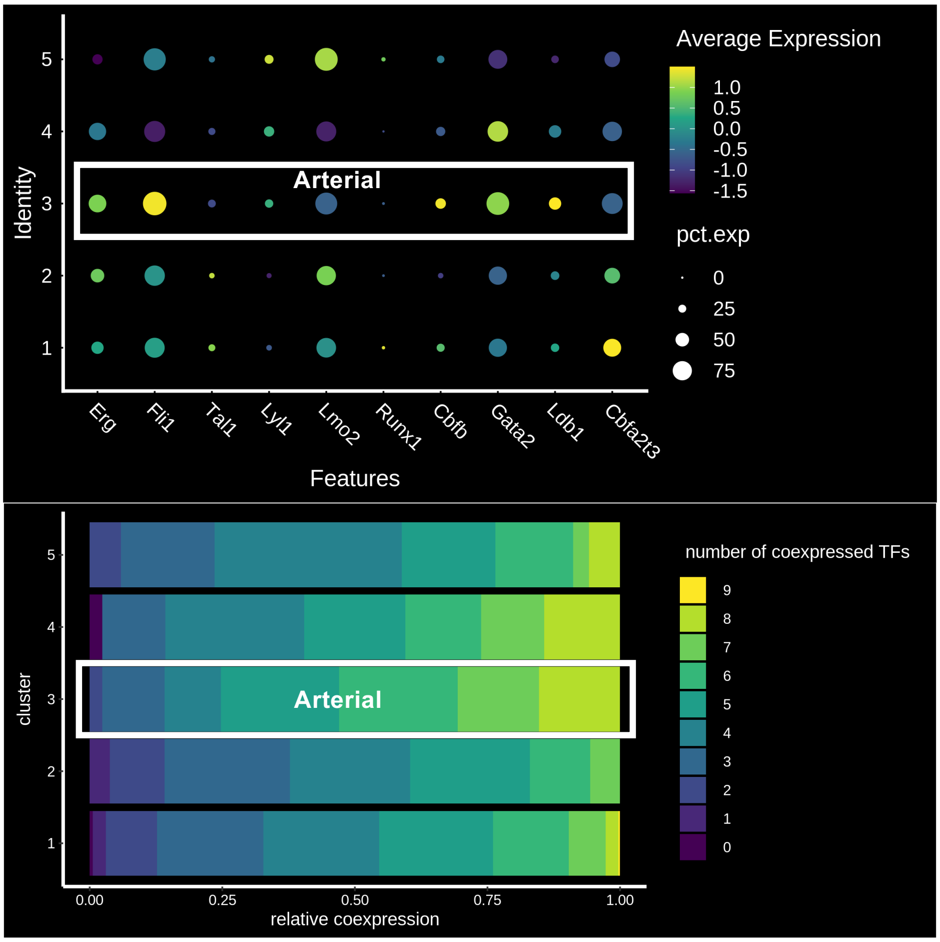
Supplementary Fig. 6



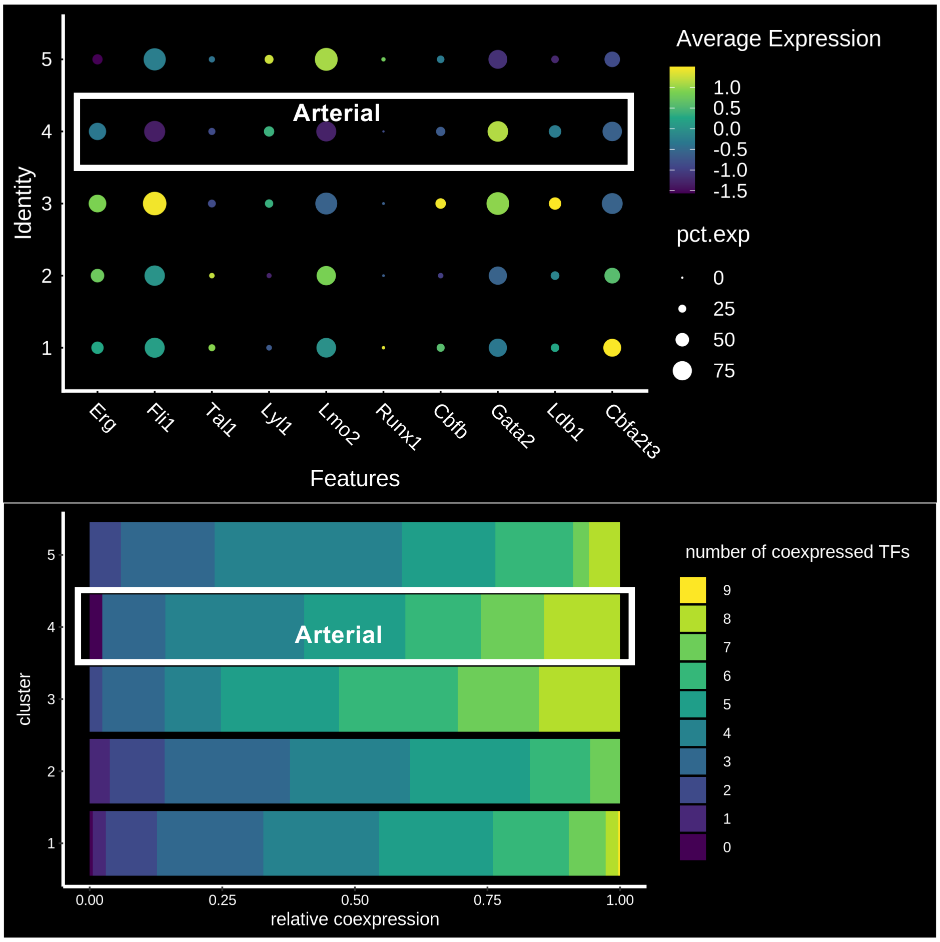
Supplementary Fig. 6: Clustering analysis of endothelial cells from Tabula Muris. Umap plots of the results of the endothelial cell clustering analysis of the six indicated tissues. Clusters expressing arterial markers are highlighted with an ellipse.

Supplementary Fig. 7

Brain EC

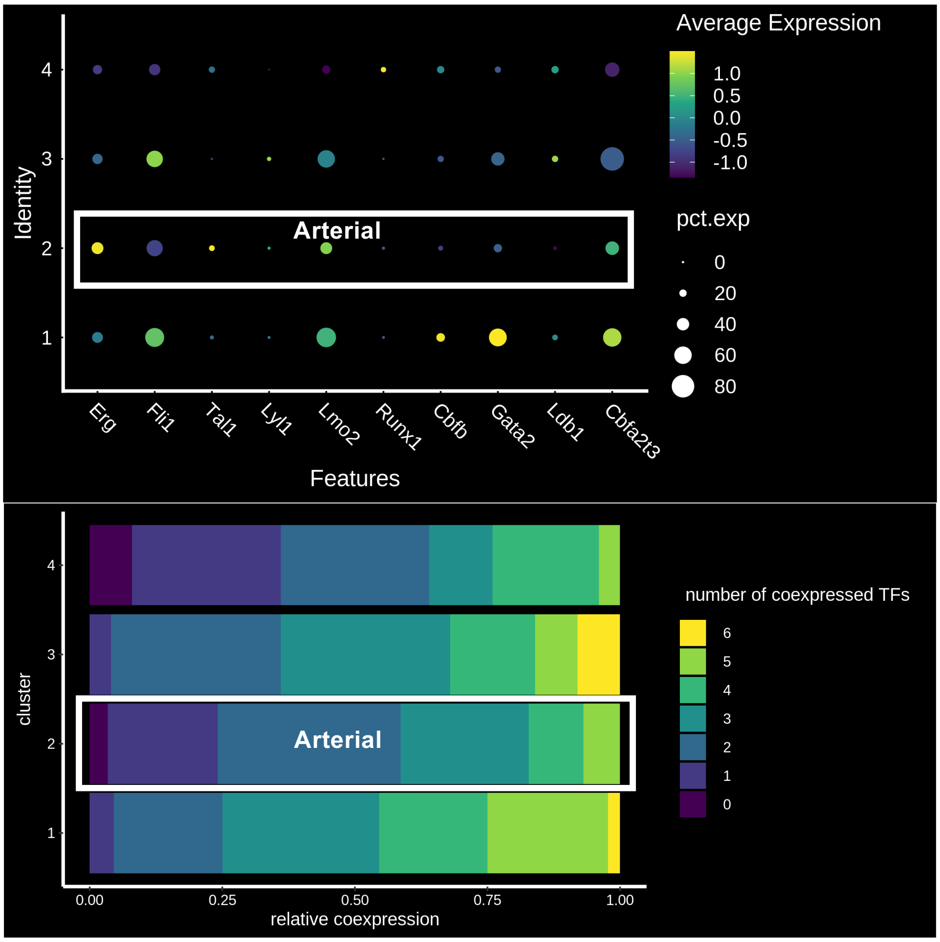


Heart EC

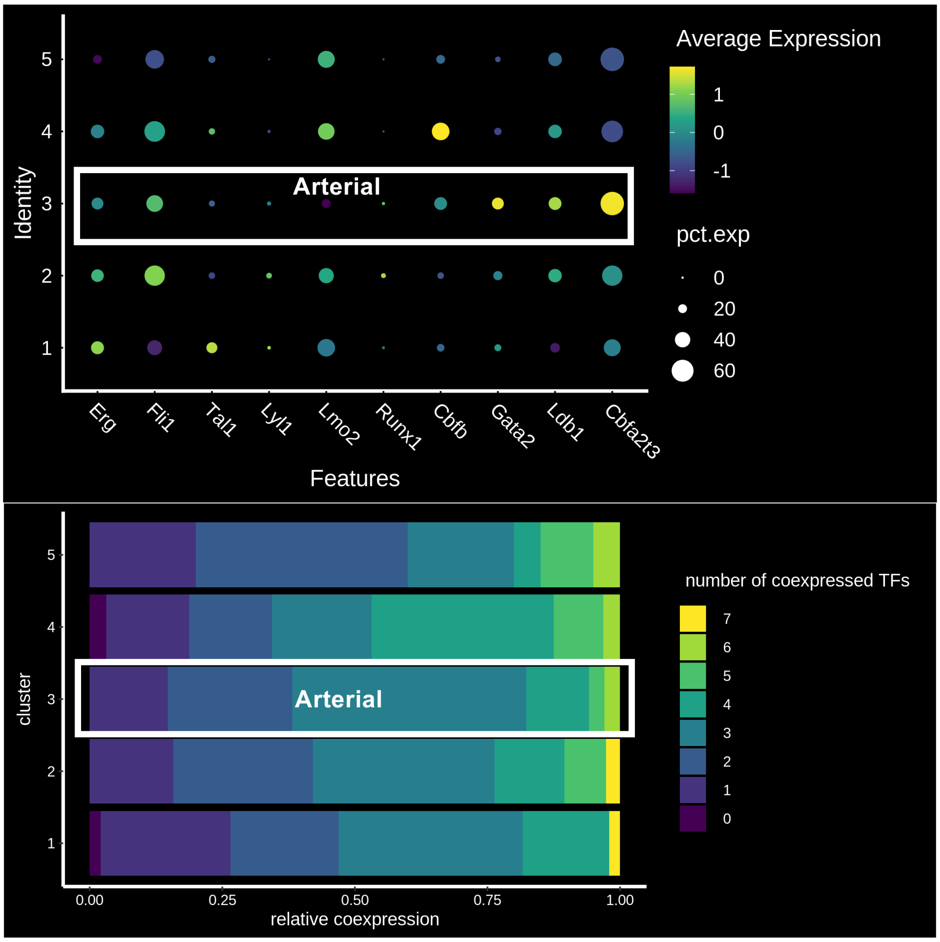


Supplementary Fig. 7

Kidney EC

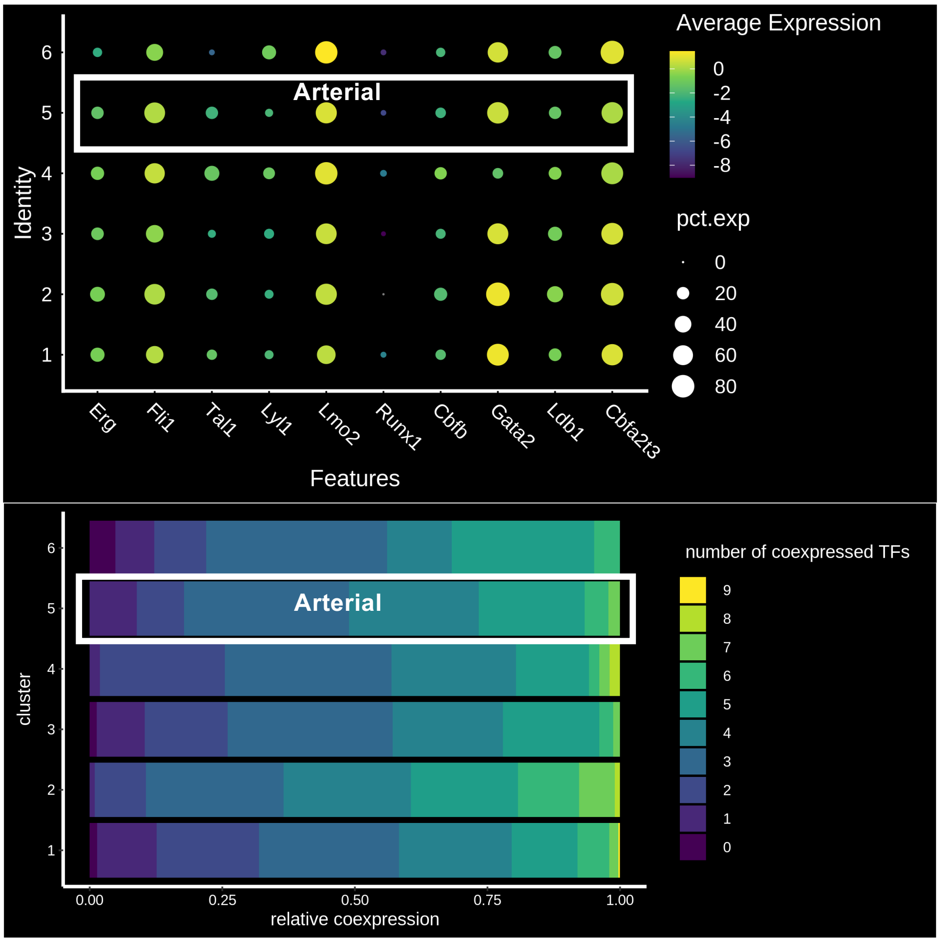


Liver EC

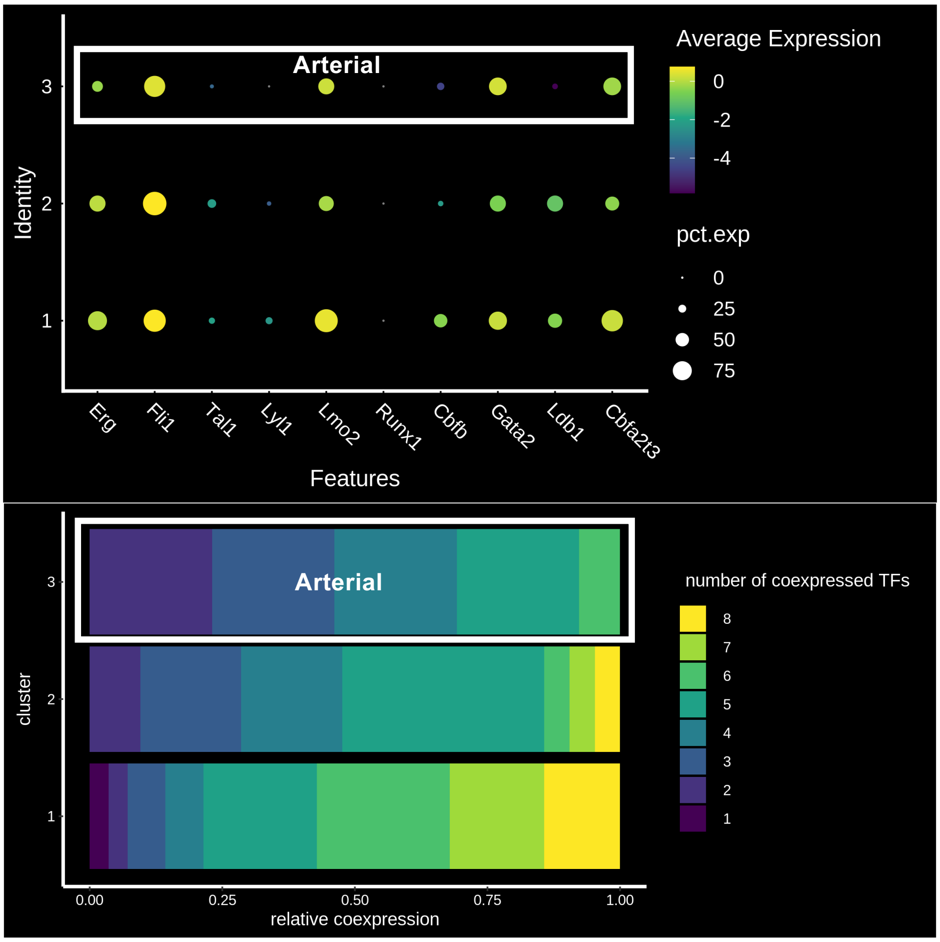


Supplementary Fig. 7

Lung EC

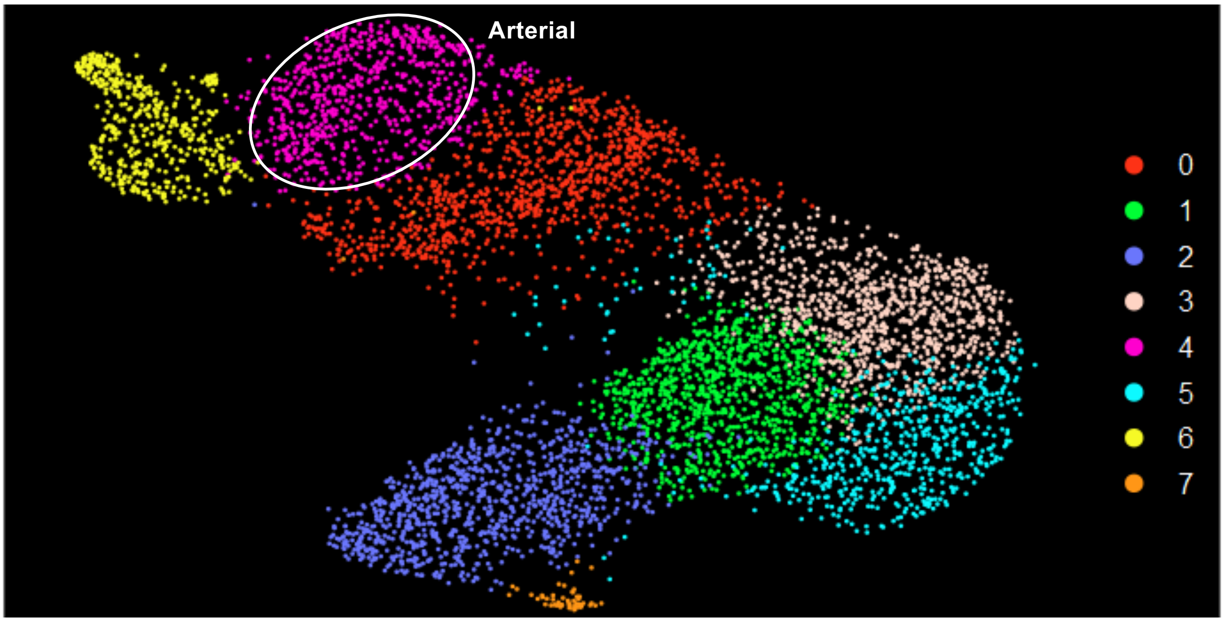


Pancreas EC

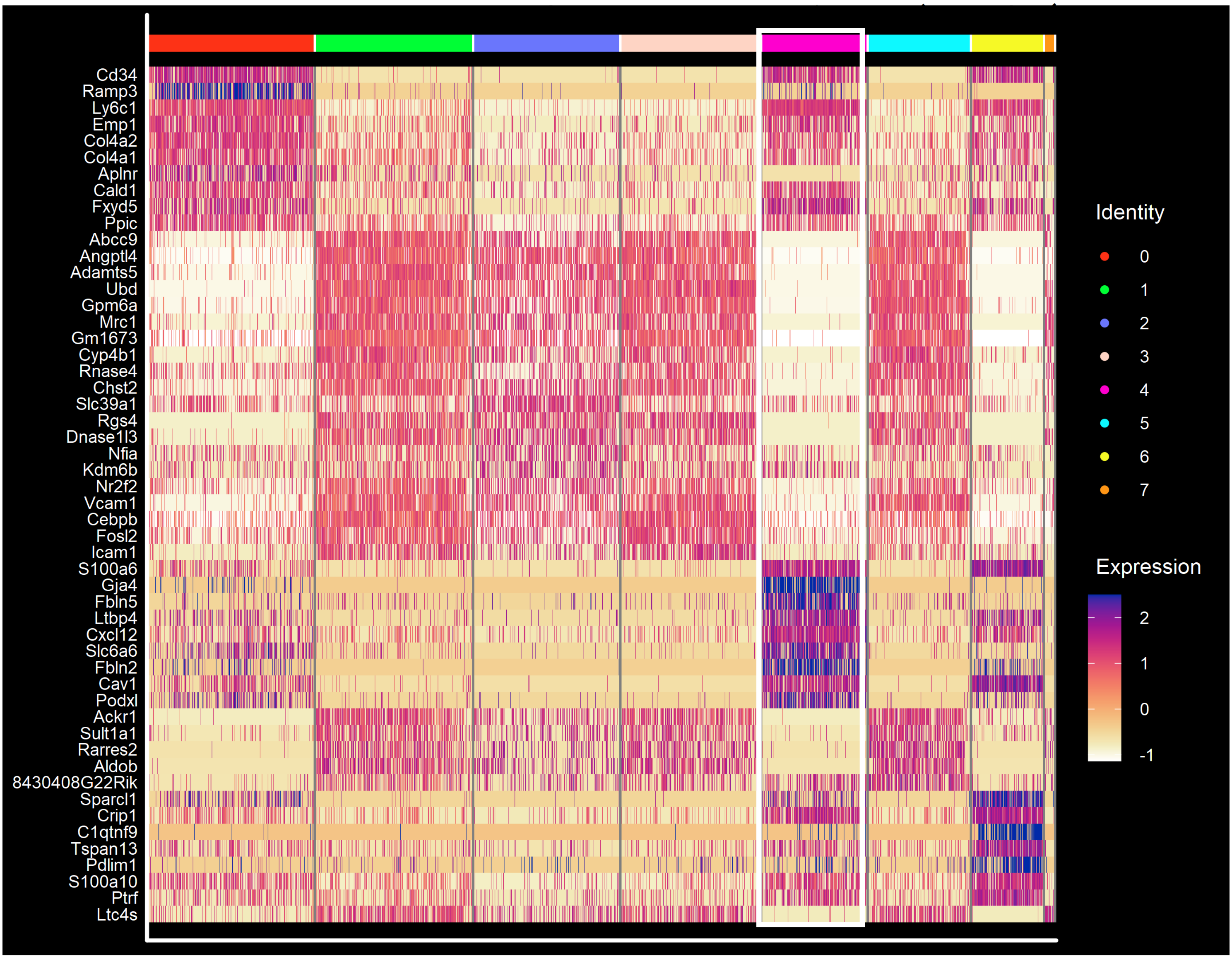


Supplementary Fig. 7: Analysis of transcription factor coding genes in endothelial cell clusters. For each tissue, the top panel shows a dot plot of the expression and percentage of expression of the indicated transcription factors in the endothelial cell clusters; the bottom panel shows the analysis of the co-expression between the transcription factors. The rectangles highlight arterial endothelial cell clusters.

a

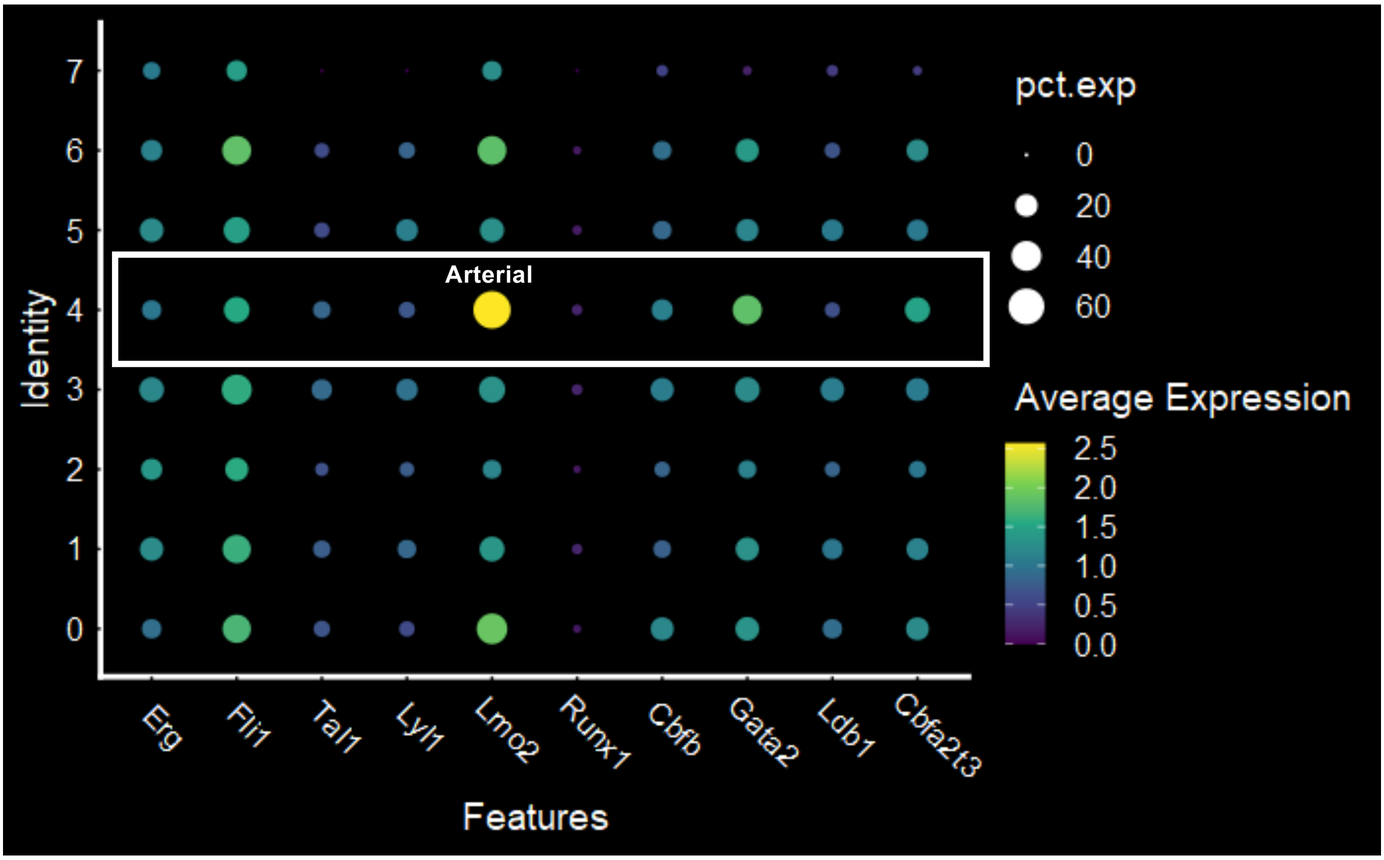

Arterial
0
1
2
3
4
5
6
7

b

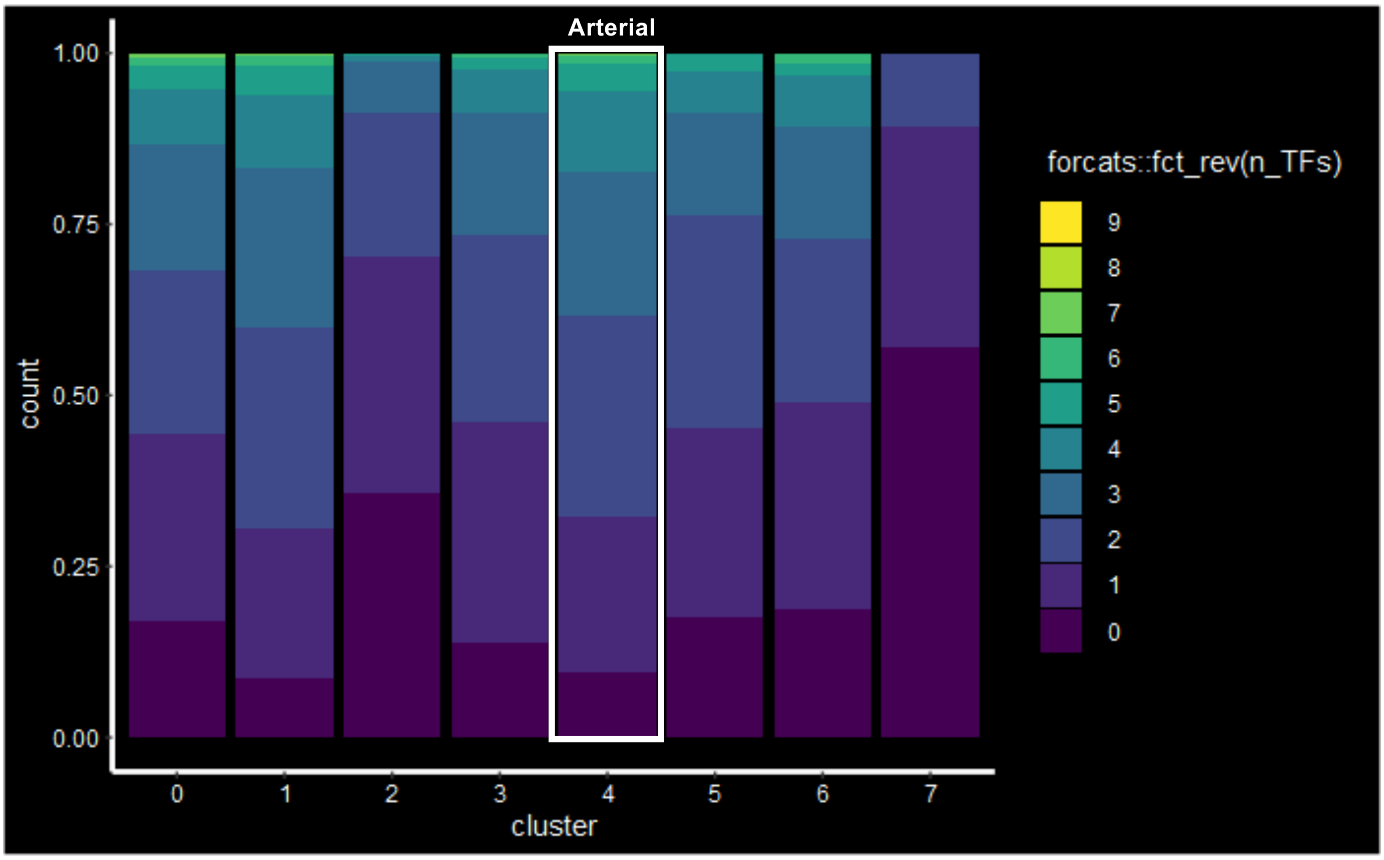

Cd34
Ramp3
Ly6c1
Emp1
Col4a2
Col4a1
Aplnr
Cald1
Fxyd5
Ppic
Abcc9
Angptl4
Adamts5
Ubd
Gpm6a
Mrc1
Gm1673
Cyp4b1
Rnase4
Chst2
Slc39a1
Rgs4
Dnase1l3
Nfia
Kdm6b
Nr2f2
Vcam1
Cebpb
Fosl2
Icam1
S100a6
Gja4
Fbln5
Ltbp4
Cxcl12
Slc6a6
Fbln2
Cav1
Podxl
Ackr1
Sult1a1
Rarres2
Aldob
8430408G22Rik
Sparcl1
Crip1
C1qtnf9
Tspan13
Pdlim1
S100a10
Ptrf
Ltc4s
Identity
0
1
2
3
4
5
6
7
Expression
2
1
0
-1

Supplementary Fig. 8 Results of the scRNA-seq clustering analysis of the bone marrow endothelial cells. a) UMAP plot highlighting the endothelial cell ; b) Heatmap of marker genes for the indicated cell clusters.

a

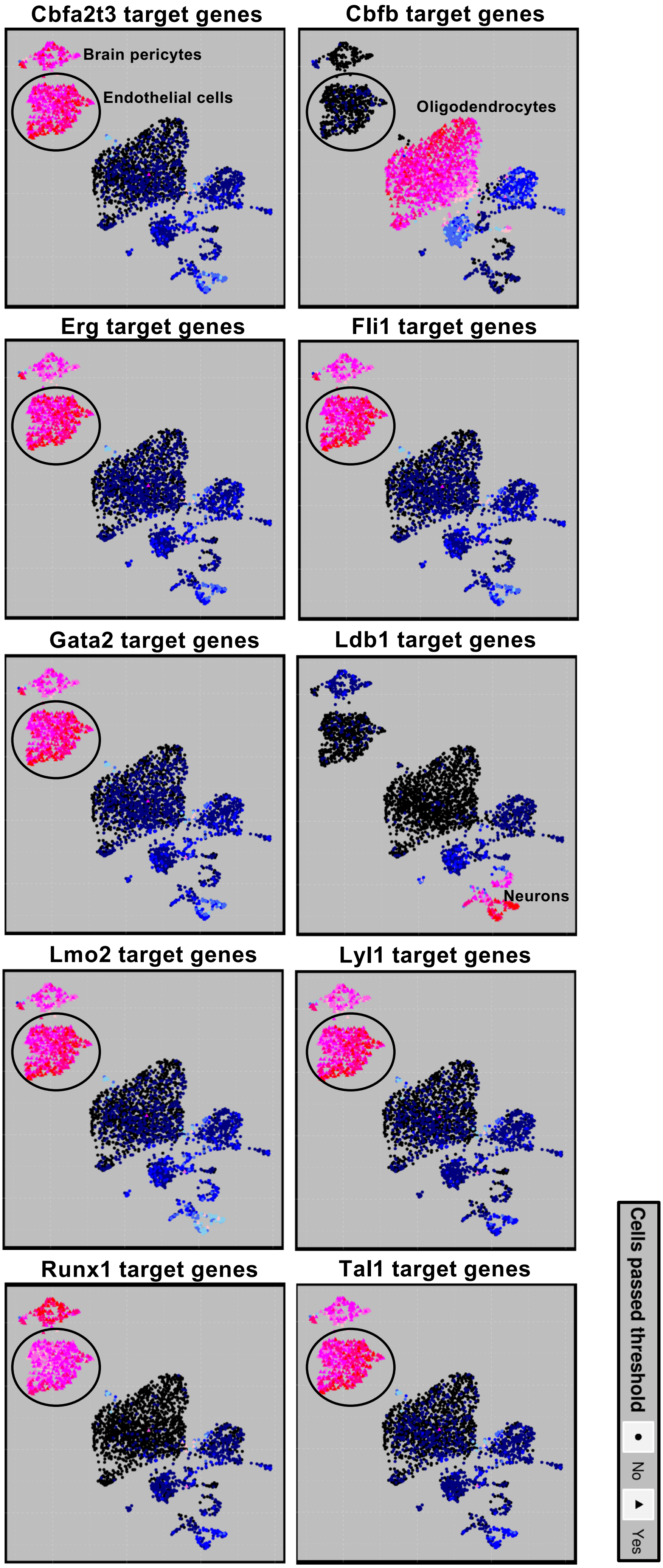

Arrterial
Arterial
Identity
7
6
5
4
3
2
1
0
Features
Erg
Fli1
Tal1
Lyl1
Lmo2
Runx1
Cbfb
Gata2
Ldb1
Cbfa2t3
pct.exp
0
20
40
60
Average Expression
2.5
2.0
1.5
1.0
0.5
0.0

b


Arterial
count
1.00
0.75
0.50
0.25
0.00
cluster
0
1
2
3
4
5
6
7
forcats::fct_rev(n_TFs)
9
8
7
6
5
4
3
2
1
0

Supplementary Fig. 9: Analysis of transcription factor gene expression in the bone marrow endothelial cell clusters. a) Dot plot showing the expression and percentage of expression of the indicated transcription factors in the endothelial cell clusters; **b)** Analysis of the co-expression between the transcription factors.

Supplementary Fig. 10


Cbfa2t3 target genes

Cbfb target genes

Erg target genes

Fli1 target genes

Gata2 target genes

Ldb1 target genes

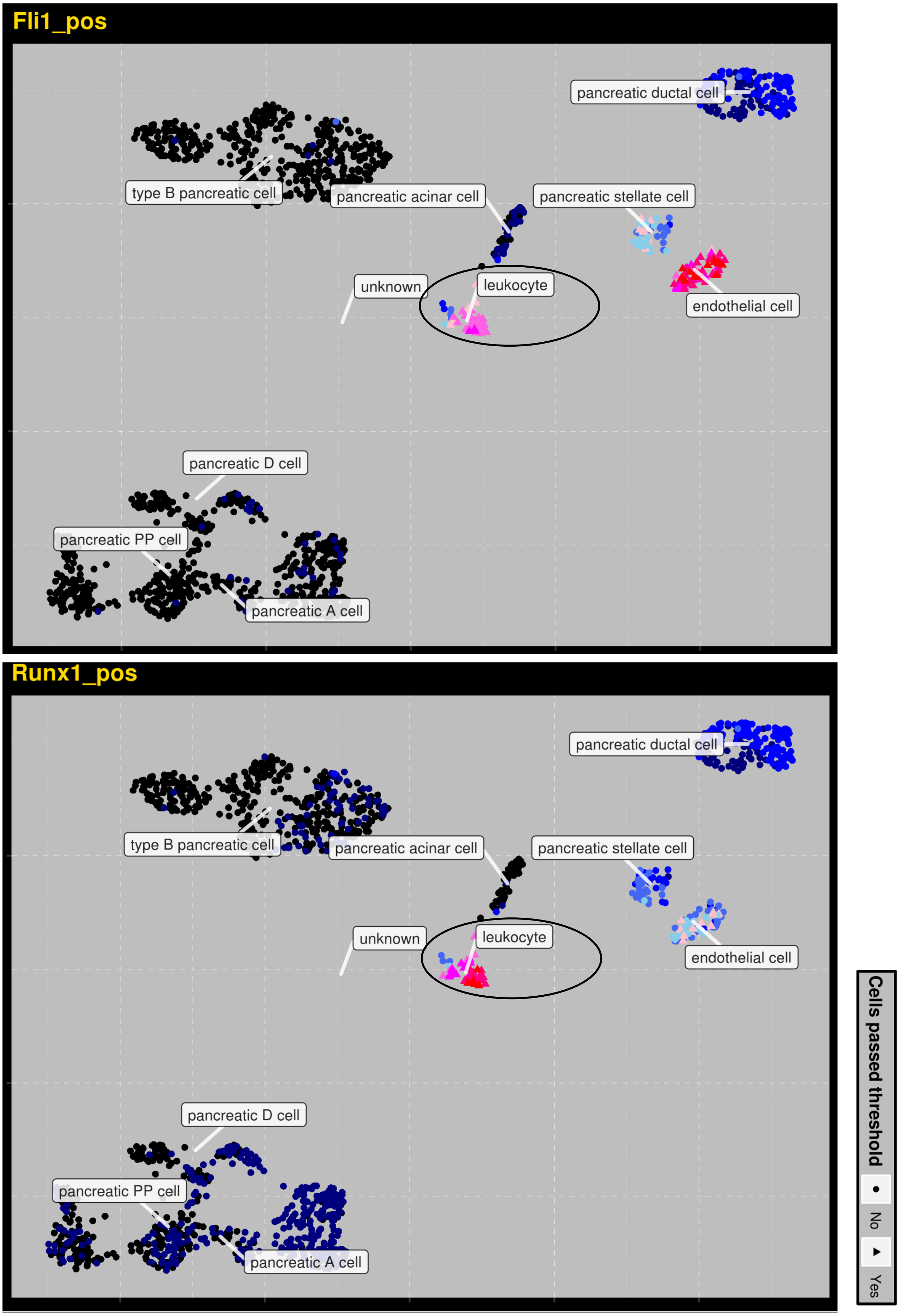
Lmo2 target genes

Lyl1 target genes

Runx1 target genes

Tal1 target genes

Supplementary Fig. 10: UMAP plot highlighting the clusters in which the target genes of a given seed TFs are expressed. Only positive relationships are highlighted. The ellipse shows the endothelial cell cluster. Colors represent AUC value – cells that passed AUC threshold colored in pink, others in blue.


Fli1_pos
pancreatic ductal cell
type B pancreatic cell
pancreatic acinar cell
pancreatic stellate cell
unknown
leukocyte
endothelial cell
pancreatic D cell
pancreatic PP cell
pancreatic A cell
Runx1_pos
pancreatic ductal cell
type B pancreatic cell
pancreatic acinar cell
pancreatic stellate cell
unknown
leukocyte
endothelial cell
pancreatic D cell
pancreatic PP cell
pancreatic A cell
Cells passed threshold
● No
▲ Yes

Supplementary Fig. 11: Overlap of genes between Fli1 and Runx1 only in leukocytes cluster. UMAP plot highlighting the clusters in which the target genes of a given seed TFs are expressed. Only positive relationships are highlighted. The ellipse shows the leukocytes cell cluster. Colors represent AUC value – cells that passed AUC threshold colored in pink, others in blue.

	c1	**c2**	**c3**	**c4**	**c5**	**c6**	**c7**	**c8**	**c9**	**c10**	**c11**	**c12**	**c13**	**c14**
Embryo_dataset1	0	22	20	30	1	8	0	97	0	0	11	0	0	100
Embryo_dataset2	3	5	5	2	5	1	5	2	20	9	3	6	4	0
Embryo_dataset3	0	71	75	44	0	87	0	1	8	0	84	3	0	0
Aorta	5	0	0	1	3	0	0	0	1	5	0	0	0	0
Brain	19	0	0	3	8	1	3	0	0	8	0	14	0	0
Diaphragm	3	0	0	0	1	0	0	0	2	1	0	3	0	0
Fat	7	1	0	4	21	1	64	0	27	23	1	31	0	0
Heart	23	1	0	4	45	1	18	0	3	15	1	15	95	0
Kidney	4	0	0	0	2	1	1	0	1	2	0	9	0	0
Limb_muscle	4	0	0	1	3	0	2	0	2	4	0	5	0	0
Liver	7	0	0	0	1	0	0	0	0	1	0	3	0	0
Lung	19	0	0	10	7	0	3	0	31	19	0	5	1	0
Mammary_gland	1	0	0	0	1	0	1	0	1	4	0	1	0	0
Pancreas	2	0	0	0	1	0	1	0	0	4	0	2	0	0
Trachea	3	0	0	1	1	0	2	0	4	5	0	3	0	0

Supplementary Table 1: Composition of the clusters from figure 2. The values represent percentage.

a

GO.ID	Term	p-value (FDR)
GO:0044703	Multi-organism reproductive process	0.0053
GO:0040007	Growth	0.0074
GO:0006457	Protein folding	0.0103
GO:0010033	Response to organic substance	0.0181
GO:0033043	Regulation of organelle organisation	0.0185

b

GO.ID	Term	p-value (FDR)
GO:0071496	Cellular response to external stimulus	0.0027
GO:0009314	Response to radiation	0.0098
GO:0043405	Regulation of MAP kinase activity	0.0102
GO:0006469	Negative regulation of protein kinase activity	0.0138
GO:0071248	Cellular response to metal ion	0.0209

Supplementary Table 2: Gene ontology analysis of adult and embryonic cell clusters from figure 2.

a) List of the top 5 GO term of genes most differentially expressed in the embryonic cell clusters.

b) List of the top 5 GO term genes most differentially expressed in the adult cell clusters.

Gene symbol	**Number of common tissues**	**Name of tissues**
Cd63	5	fat, kidney, limb_muscle, lung, trachea
Gsn	5	fat, heart, kidney, limb_muscle, trachea
Mt1	5	diaphragm, fat, kidney, limb_muscle, trachea
Myl9	5	brain, diaphragm, fat, limb_muscle, trachea
Rgs5	5	aorta, brain, diaphragm, limb_muscle, trachea
Acta2	4	diaphragm, fat, limb_muscle, trachea
Art3	4	brain, diaphragm, kidney, mammary_gland
Bgn	4	brain, kidney, limb_muscle, trachea
Cd44	4	diaphragm, kidney, limb_muscle, mammary_gland
Ndrg2	4	aorta, diaphragm, limb_muscle, trachea
Notch2	4	aorta, diaphragm, kidney, limb_muscle
Pex7	4	aorta, kidney, limb_muscle, lung
Stip1	4	aorta, fat, kidney, mammary_gland

Supplementary Table 3: Identification of marker genes in Runx1$^+$ adult endothelial populations.