

SUPPLEMENTAL MATERIAL

Single-cell analysis of salt-induced hypertensive mouse aorta reveals cellular heterogeneity and state changes

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Supplemental Table 1: EC subpopulation features (or functions) gene lists

Inflammator y response to wounding	Lymph vessel development	Migration					Lipid transport
Grn	Nr2f2	Ephb4	Cyp1b1	Vash1	Card10	Lgals8	Cd36
Ager	Foxc1	Mef2c	Plcg1	Dcn	Itgb3	Cxcl13	Mfsd2a
Alox5	Vegfc	Tgfr1	Hmox1	Clec14a	Adgra2	Adam17	ApoE
	Syk	Nus1	Tnf	Zc3h12a	Gata2	Efnb2	Slc27a4
	Lgals8	Pik3c2a	SrpX2	Vegfa	TdGF1	Try4	Fabp5
	Foxc2	Smoc2	Sox18	Sp1	Bmper	Try5	Slc27a1
	Efnb2	Stat5a	Nr4a1	Pdcd6	Rhoa	Try10	
	Ppp3cb	Rhob	Prkca	Prkx	Rras	Prss3	
	Tmem204	Rock2	Ceacam10	EfnA1	Nr2e1	Gm5771	
	Tbx1	Mmrn2	Ceacam2	Gpi1	Hrg	Gm10334	
	Heg1	Atp2b4	Ceacam1	Pten	Ptk2b	Prss1	
	Ptpn14	Fgfr1	Stc1	Csnk2b	Rhoj	Prss2	
	Flt4	Pxn	Egr3	Scarb1	Gm42906	Adtrp	
	Ccbe1	Grn	Apoa1	Hif1a	Stard13	Gadd45a	
	Pkd1	Atp5b	Loxl2	Ets1	Dpp4	Adgrb1	
	Pdpn	Tgfb1	Acvrl1	Aamp	Notch1	Fgf4	
	Lgals12	Hmgb1	P2rx4	Gata3	Gpx1	Hdac7	
	Epha2	Fgfl	Ptgs2	Zfp580	Anxa3	Emp2	
	Bmpr2	Gpld1	Robo1	Angpt1	Myh9	Gdf2	
	Prox1	Cdh13	Alox12	Edn1	Dnaja4	Meox2	
	Sox18	Angpt2	Sash1	Map2k3	Plk2	Bmp10	
	Svep1	Wnt7a	Nrp1	Pdcd10	Slit2	Rin2	
	Acvrl1	Prp	Akt1	Met	ApoH	Pdgfb	
	Vash1	Wnt5a	Mecp2	Agtr2	Tmsb4x	Rgcc	
	Vegfa	Ptn	Fgfl6	Map3k3	Gm49510	Itgb2	
	Acvr2b	Hspb1	Nrp2	Gm49337	Sh3bp1	Itgb2l	
		Fbxw7	Akt3	Paxip1	Thbs1	Ptprm	
		Tnfsf12	Amot	Tek	Agt	Id1	
		Dll4	Epha2	Lgmn	Svbp	Nf1	
		Hdac9	Dab2ip	Grem1	Jcad	Atoh8	
		Sema4a	Pdprk1	Fut1	Ptk2	Prkd1	
		S100a2	Adamts9	Cib1	Tbxa2r	Lgals12	
		Pik3ca	Flt4	Kdr	Map2k5	Srf	
		Coro1b	Fgf2	Spred1	Glul	Rab13	
		Cdh5	Pecam1	Prox1	Bcar1	Scg2	
		Sema5a	Anxa1	Fap	Ccbe1	Plekhg5	
		Jup	Bmpr2	Lpxn	Pparg	Gipc1	
		Calr	ApoE	Sparc	Itgb1	Ccn3	
		Sirt1	Cd40	Foxc2	Amotl1	Abi1	

		Fgf18	Plxnd1	Angpt4	Bcas3	Hdac5	
		Fgfbp1	Atp5a1	Itgb1bp1	Klf4	Nos3	
		Krit1	Prkd2	Plpp3	Vegfc	Ptp4a3	
		Nfe2l2	Serpinf1	Bmp4	Pik3r3	Nr2f2	
		Emc10					

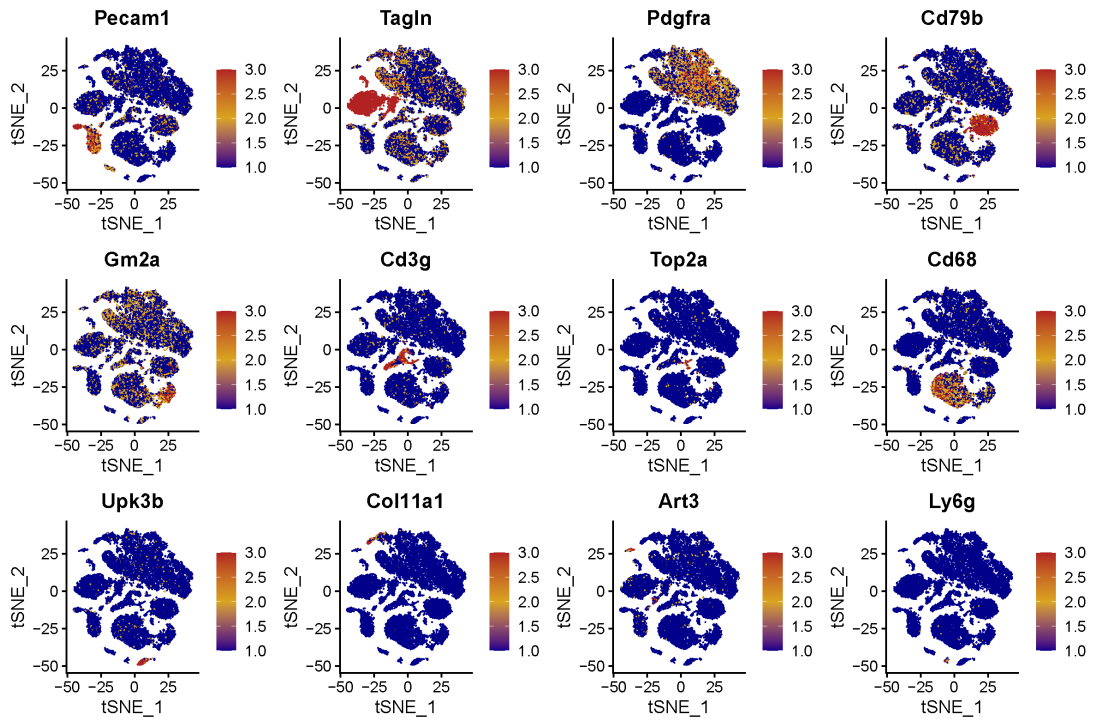
Contractile	Collagen	MHC protein complex	Proteoglycan	Differentiation	ROS-related enzymes	Proliferation
Actg2	Col1a2	B2m	Fmod	Ccm2	Nox1	Itga4
Tpm2	Col4a2	B2m	Prelp	Dll1	Nox4	Mef2c
Myo1d	Col27a1	Cd74	Srgn	Rbpj	Nox2	Stat3
Myh11	Col5a2	Cd74	Gpc3	Hey2	Nox3	Ccl2
Tpm4	Col8a1	H2-Aa	Gpc4	Tmem100	Nox5	Hmgb1
Actb	Col4a4	H2-Aa	Agtn	Hey1	Sod1	Fgfr1
Actg1	Col4a3	H2-Aa	Gpc1	Foxj2	Sod2	Pdpk1
Myo19	Col5a1	H2-Ab1	Sdc2	Nrp1	Sod3	Sirt6
Acta2	Col4a1	H2-Ab1	Aspn	Notch1	Ptgs1	Adam17
Actc1	Col3a1	H2-D1	Omd		Ptgs2	Ghsr
Tnnt3	Col14a1	H2-DMa	Ogn		Cybb	Col4a3
Myo18a	Col9a2	H2-DMb1	Lum		Xdh	Plcg1
Myo9a	Col6a1	H2-DMb2	Sdc3			Igf2
Myo1e	Col16a1	H2-Eb1	Smc3			Sp1
Myh9	Col28a1	H2-K1	Cd44			Apln
Myo1f	Col5a3	H2-L	Bgn			Fgf2
Myo5c	Col15a1	H2-M3	Sdc4			Akt3
Myo9b	Col18a1	H2-Oa	Acan			Pparg
Myo6	Col6a2	H2-Ob	Hspg2			Ghrl
Myo15b	Col25a1	H2-Q1	Vcan			Flt1
Myh10	Col6a3	H2-Q2	Podn			
Myo1c	Col8a2	H2-Q4	Cspg4			
Tpm3	Col12a1	H2-Q6	Tgfbr3			
Tpm3-rs7	Col13a1	H2-Q7	Gpc6			
Myo1b	Col1a1	H2-Q9	Dcn			
Myo5a	Col4a5	H2-Q10				
Myo10		H2-T23				
Tpm1		H2-T23				
		Mr1				

List of genes used to define EC subpopulation features (or functions) scores

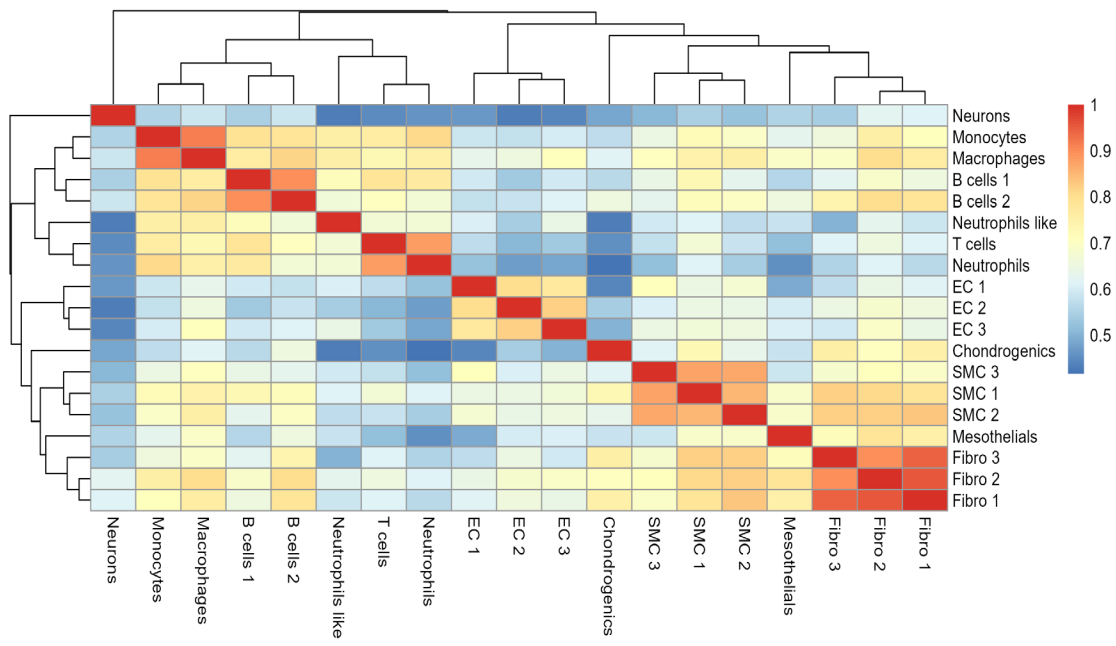
Supplemental Table 2: contractile SMC markers gene list

contractile SMC markers
Acta2
Myh11
Myh9
Myh7
Myh6
Myh13
Myh10
Myh2
Myh4
Myh14
Myh3
Myh1
Tagln
Smtn
Mylk
Vcl
Des

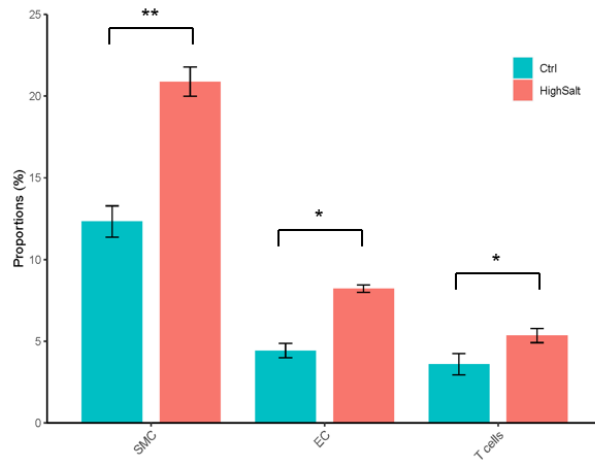
List of genes used to define contractile SMC.



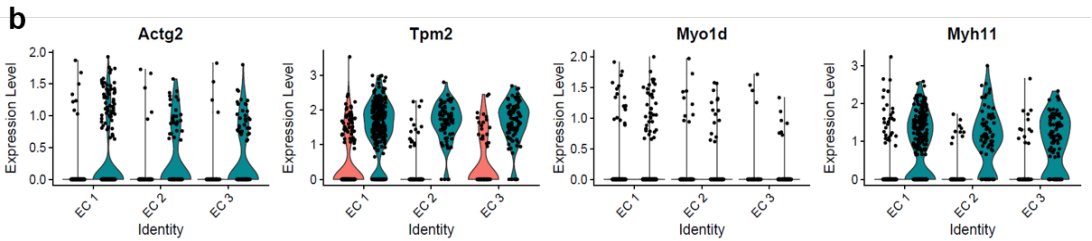
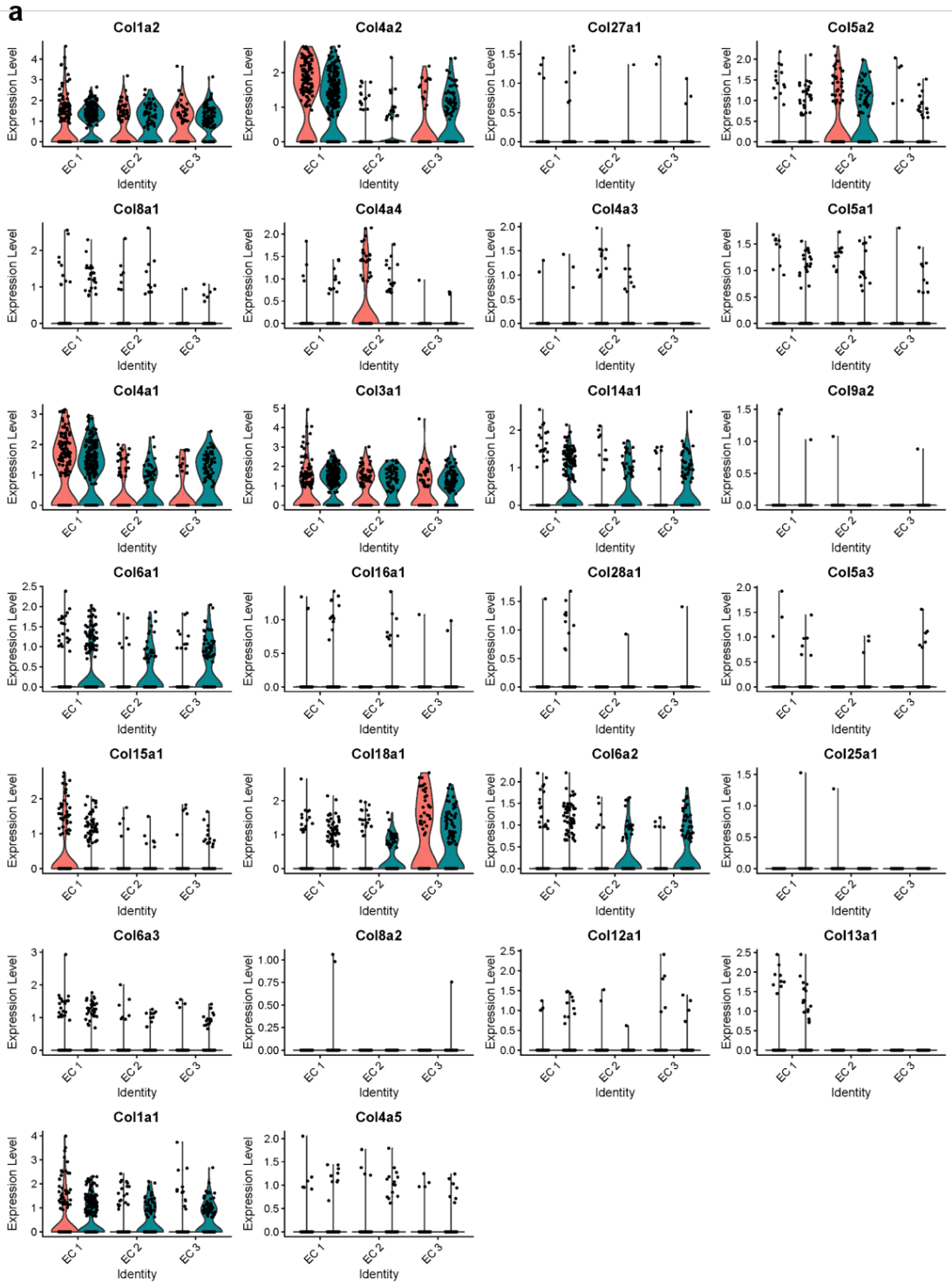
Supplemental Figure 1 Expression of genes correlated with major cell types onto a t-SNE plot

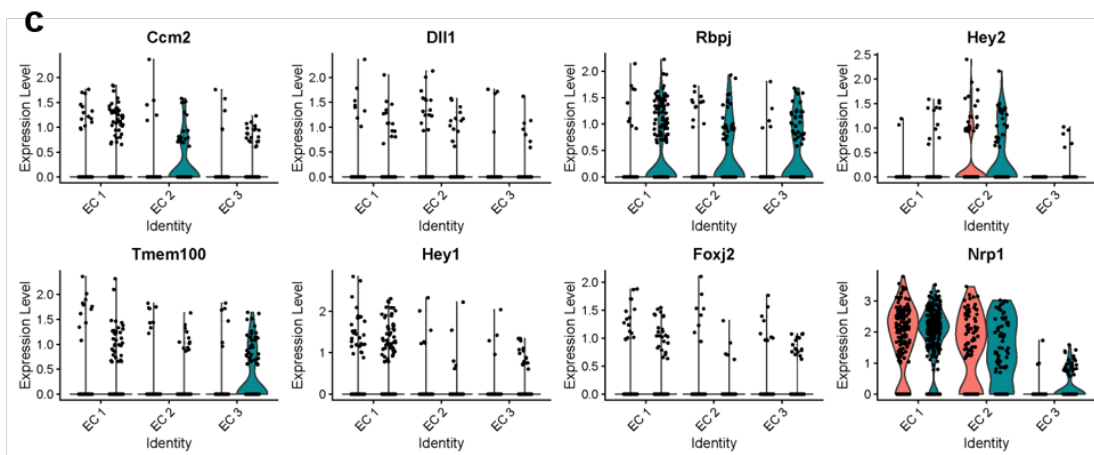
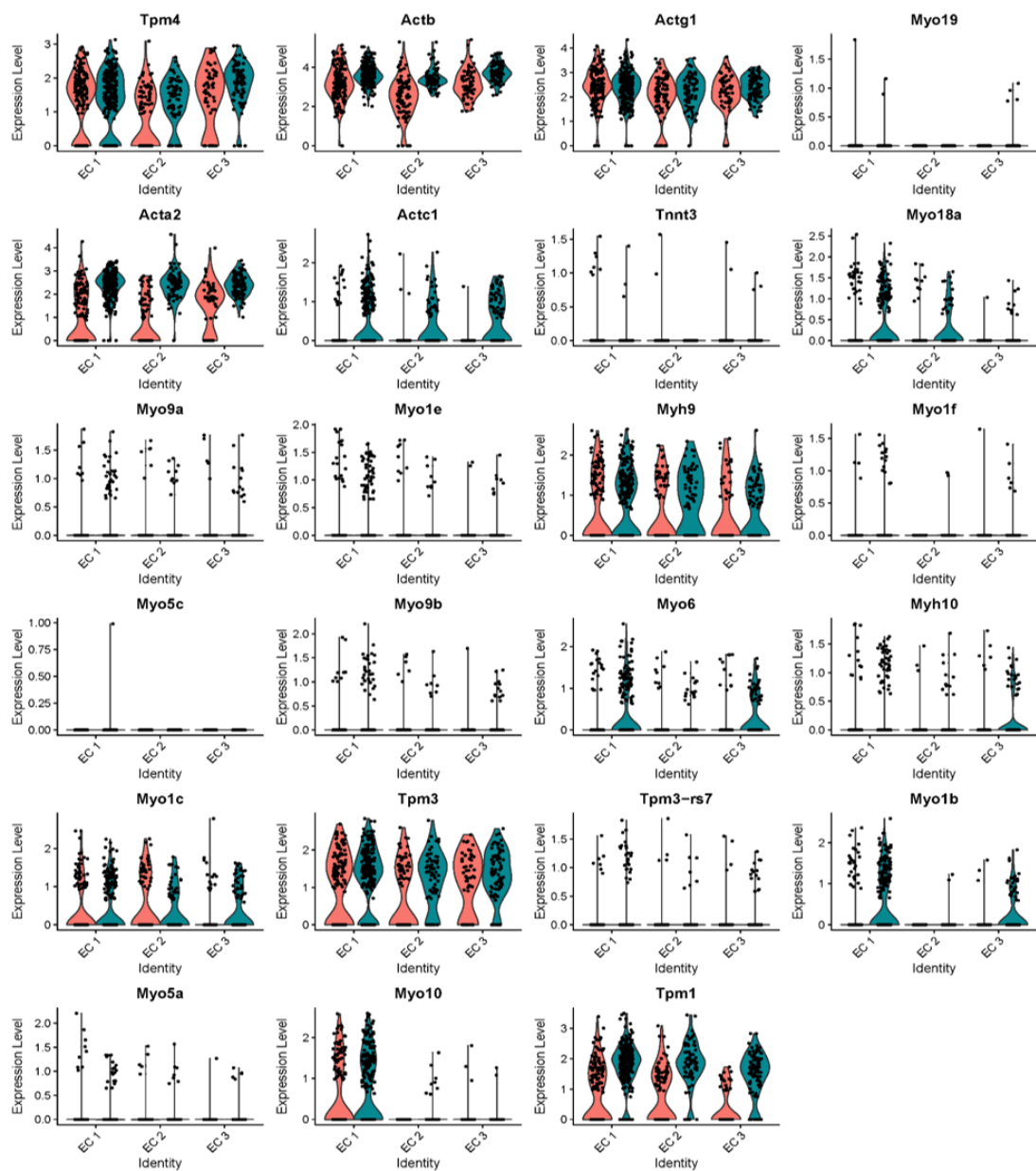


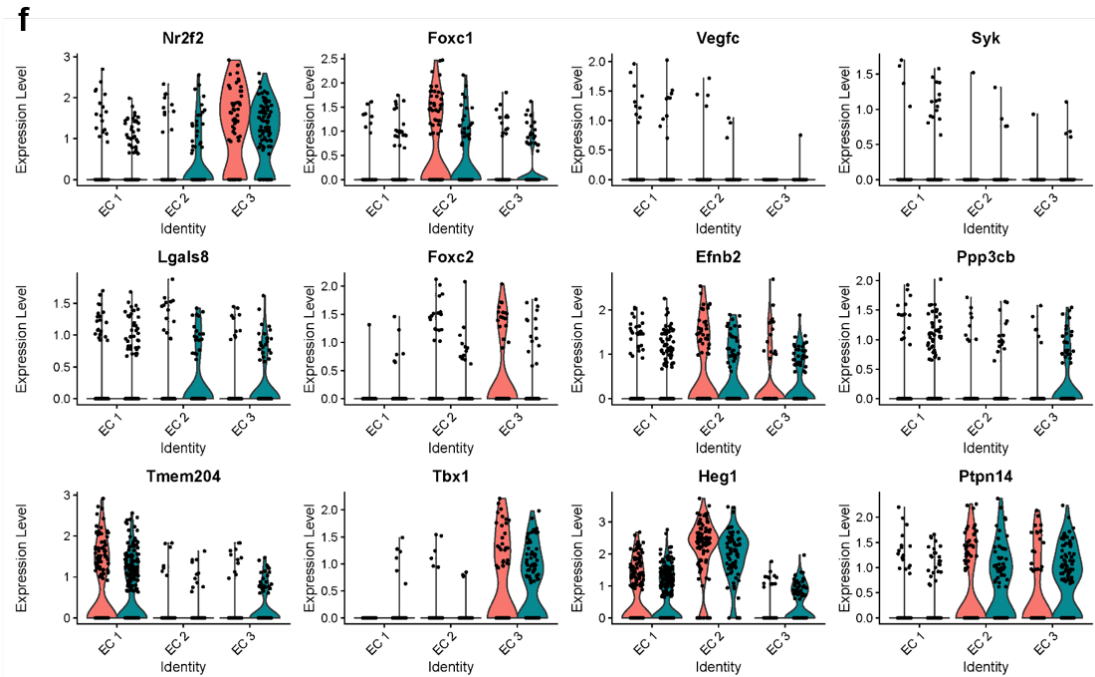
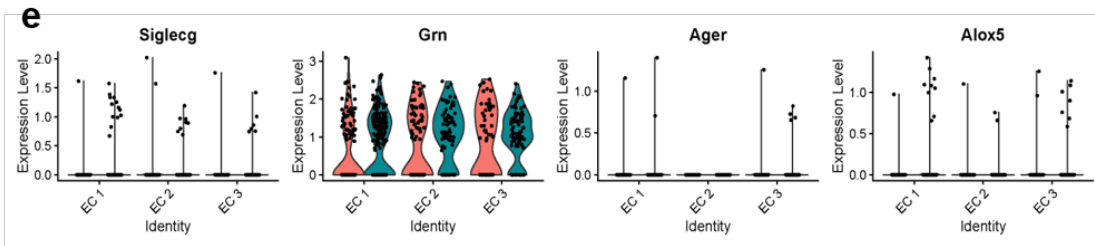
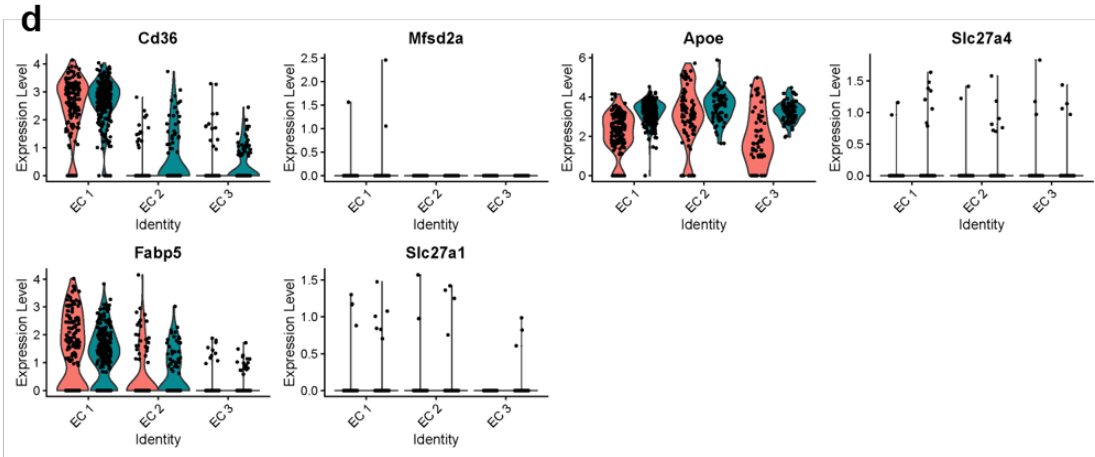
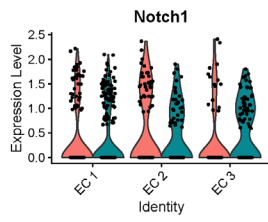
Supplemental Figure 2 Correlation of 19 cell types in the mouse aortic arch

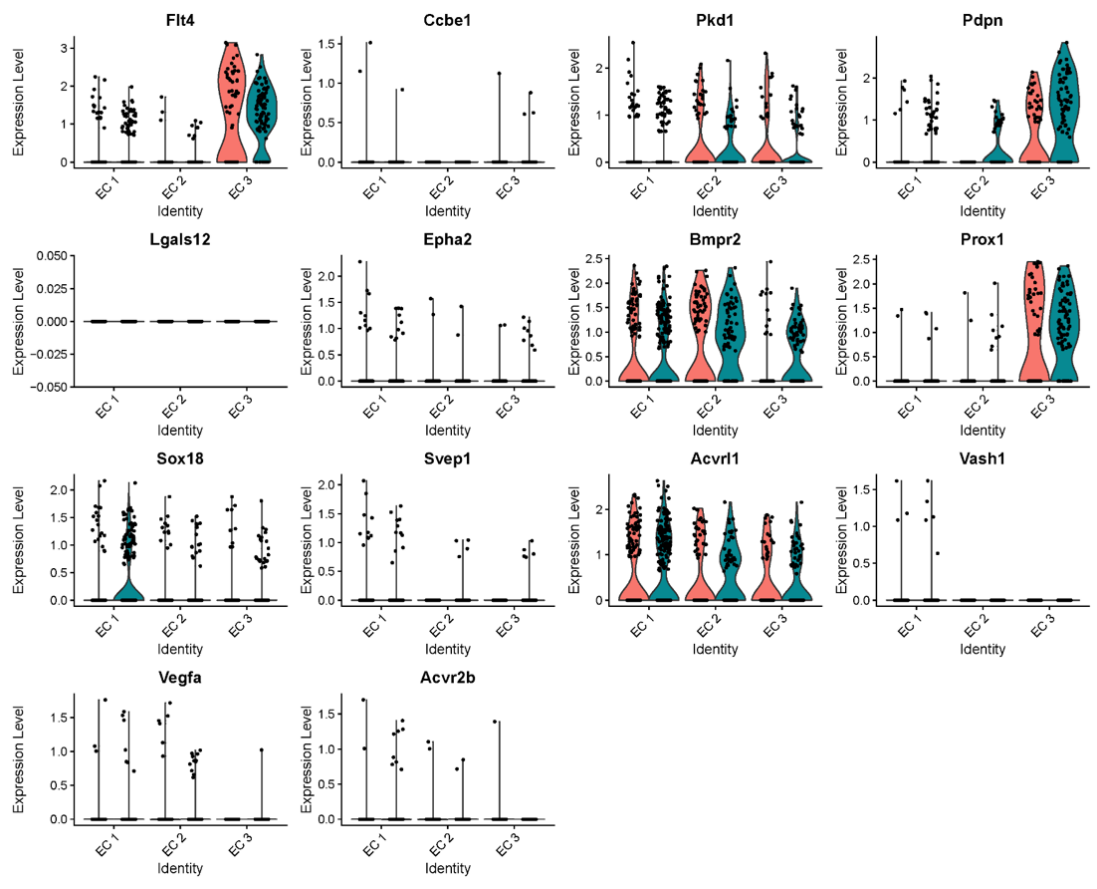


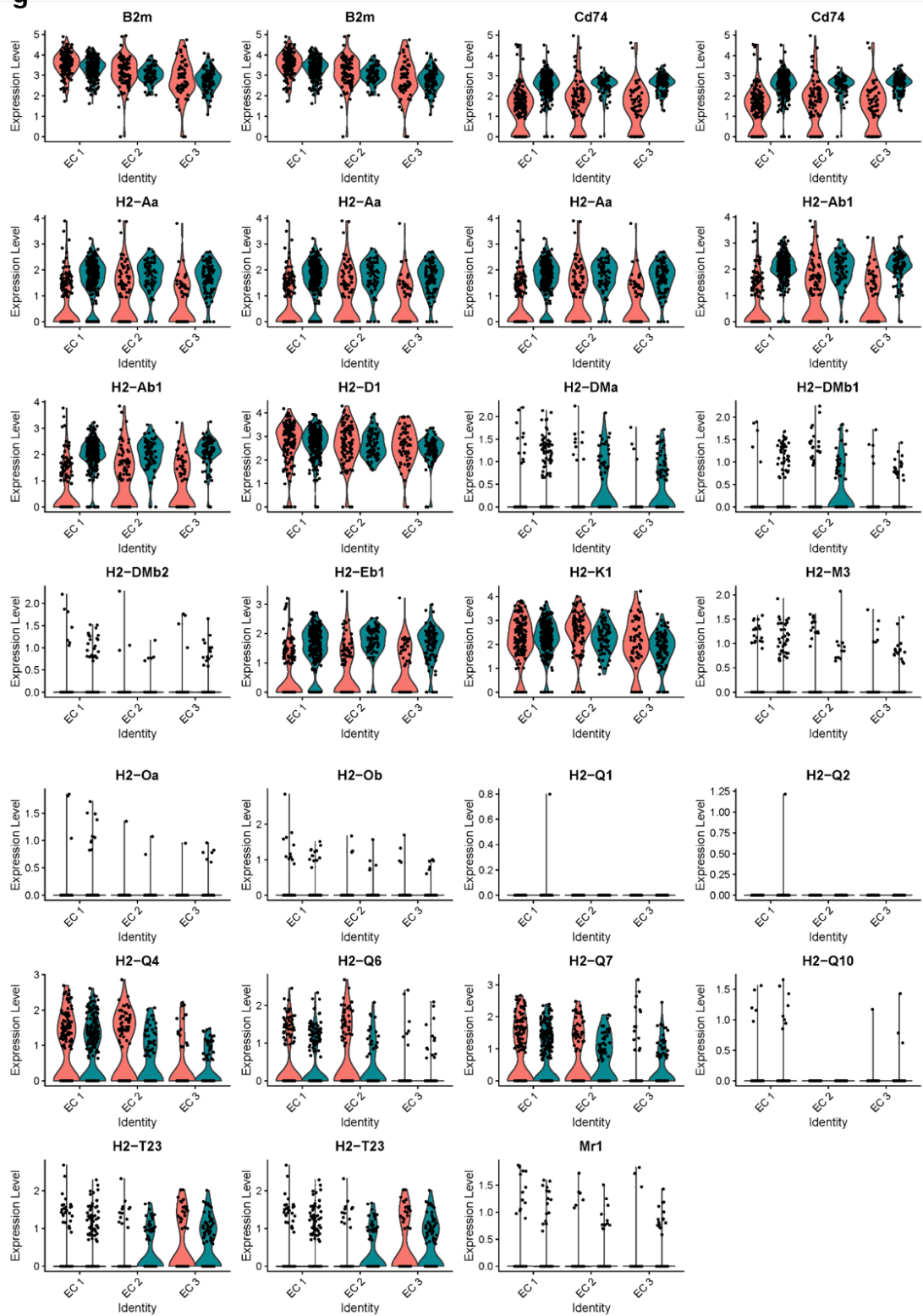
Supplemental Figure 3 The percentages of the SMC, EC and T cells population from control and high-salt diet mice. Data are represented as the mean \pm SEM. ** $P < 0.01$ and * $P < 0.05$ by Student's t test. $n=4$ control vs. $n=3$ high-salt diet mice.

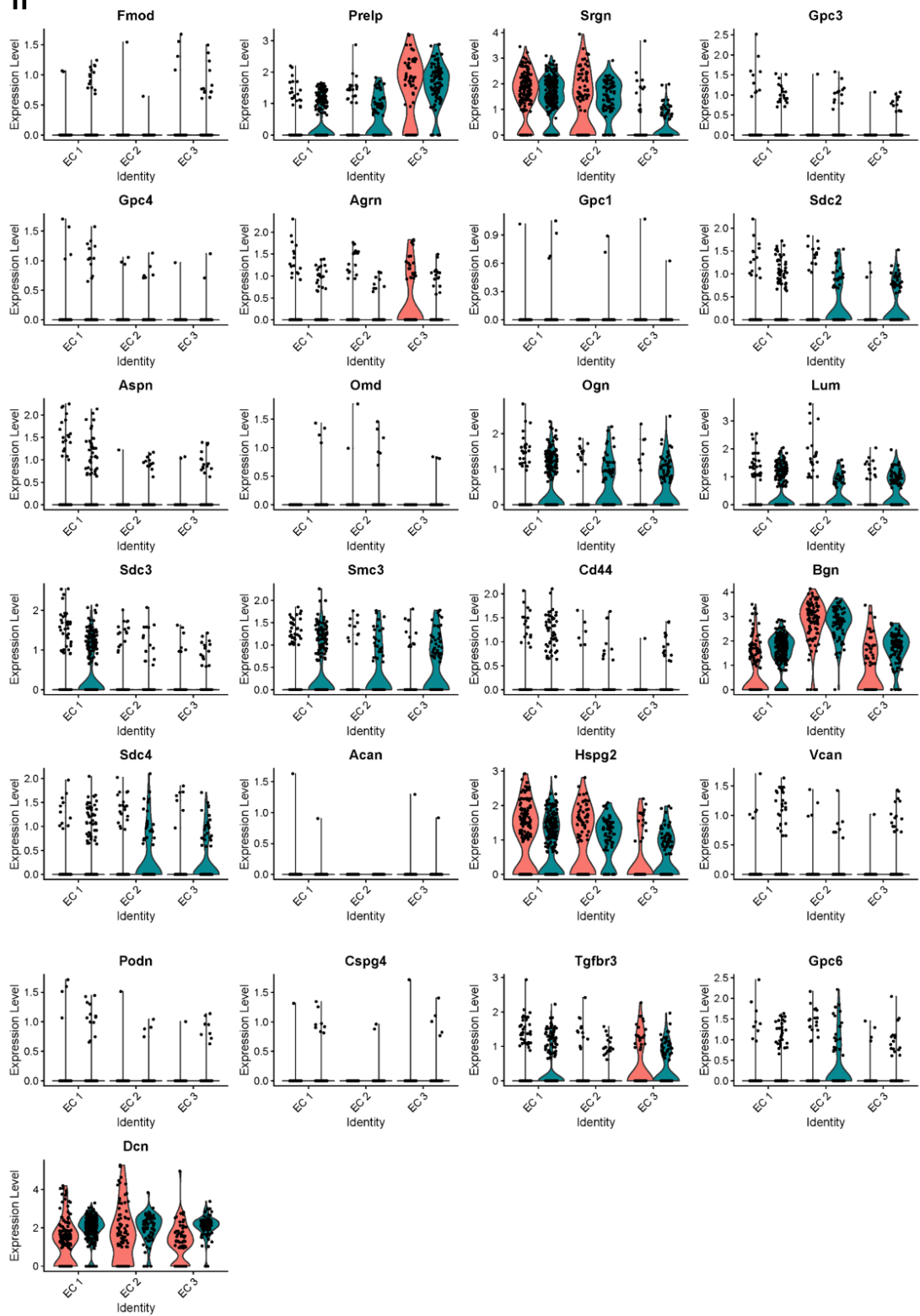


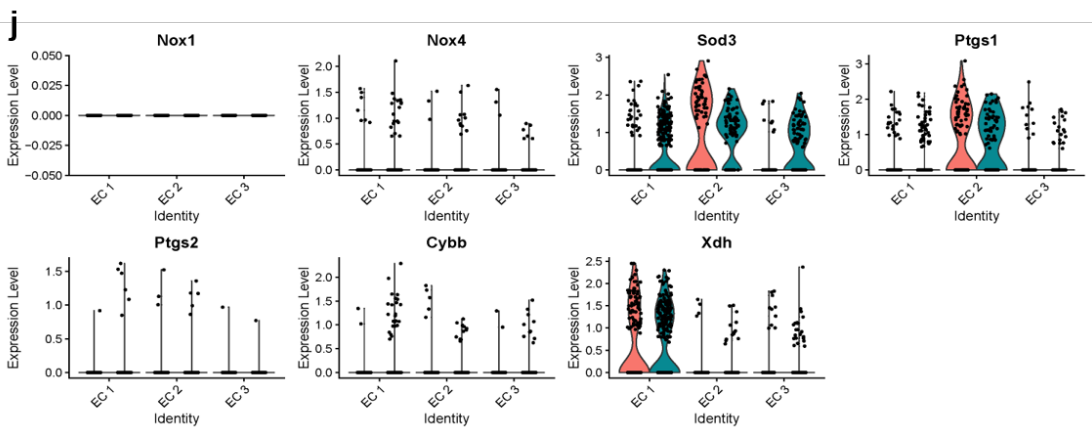
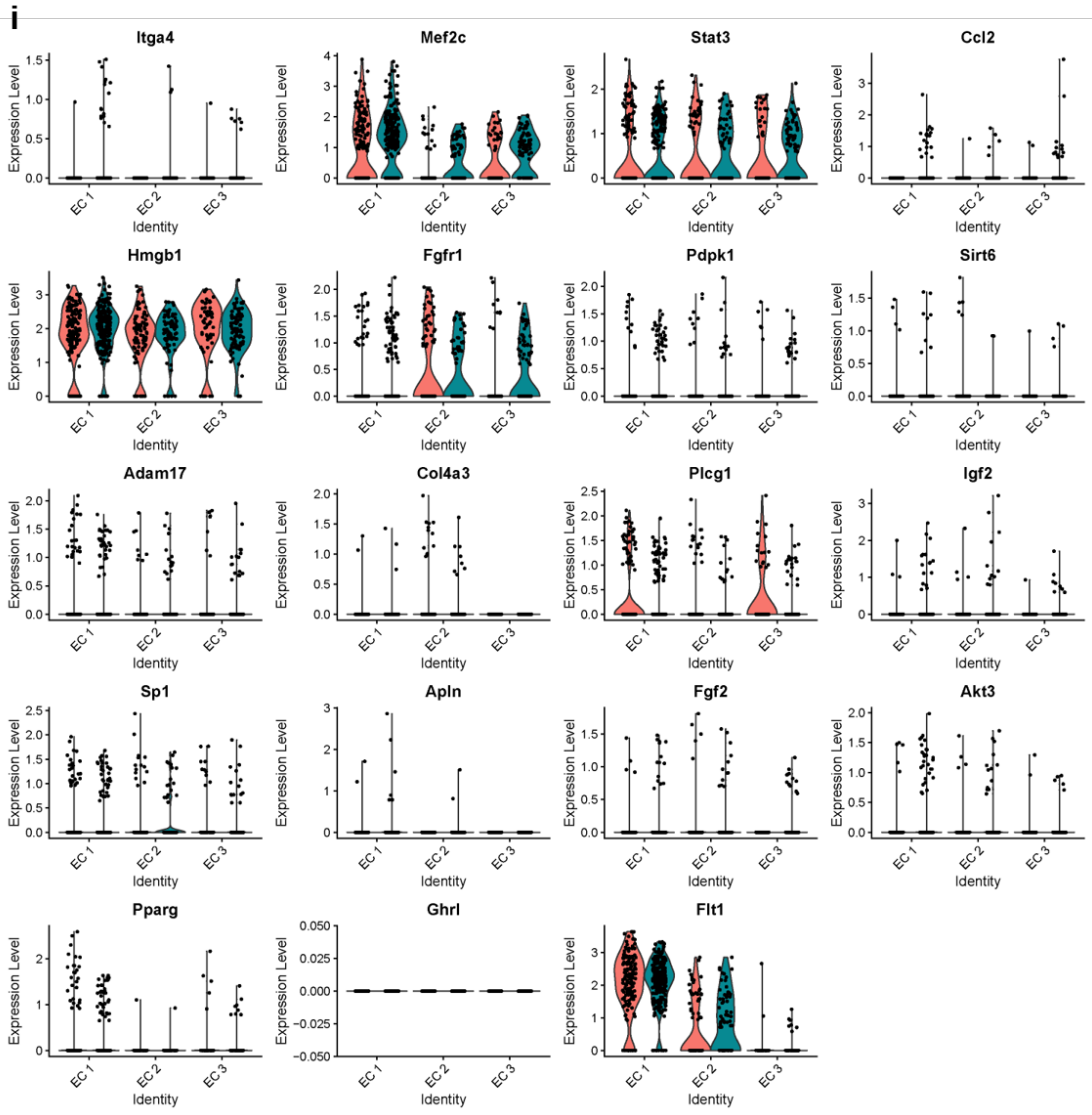


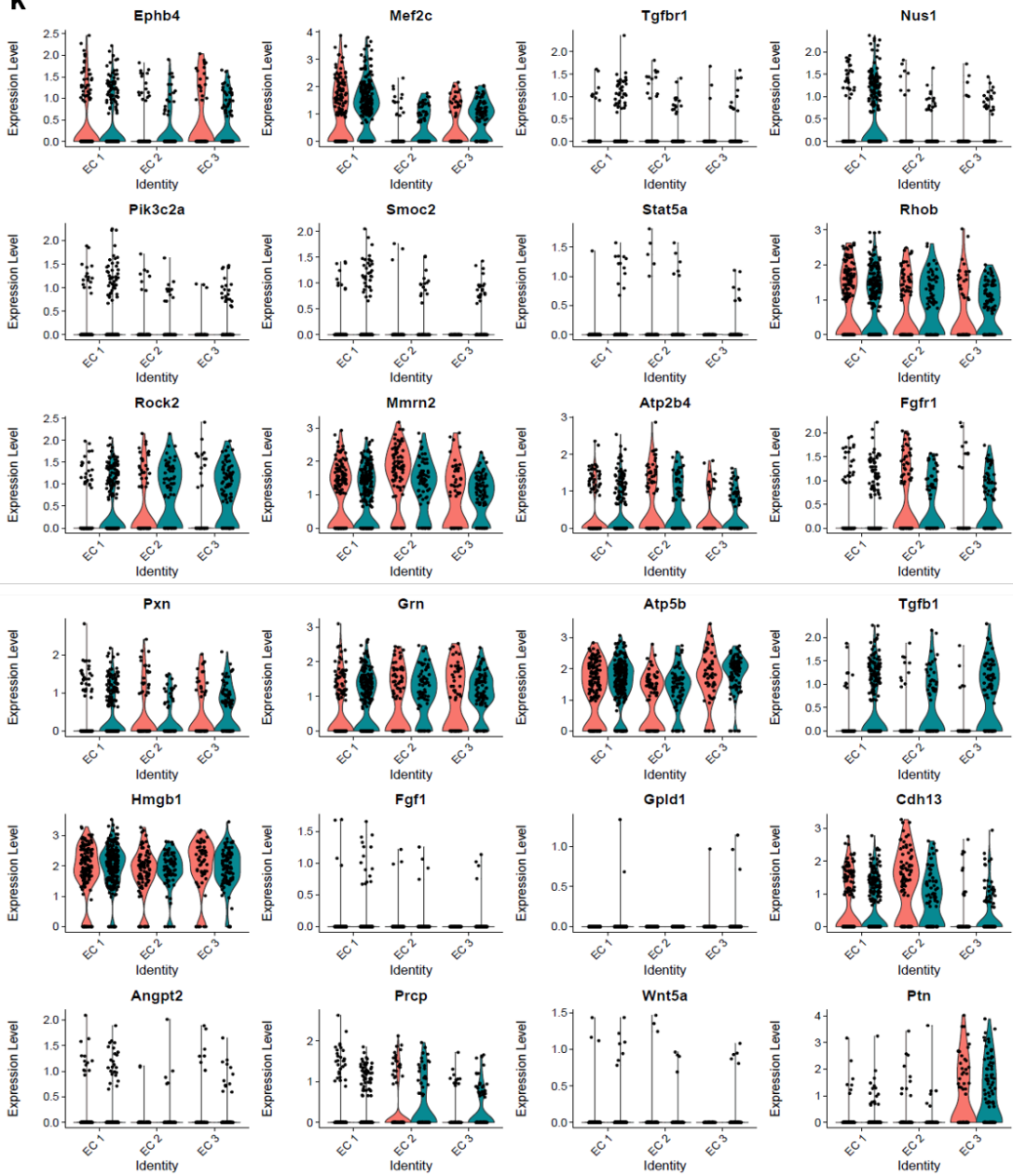


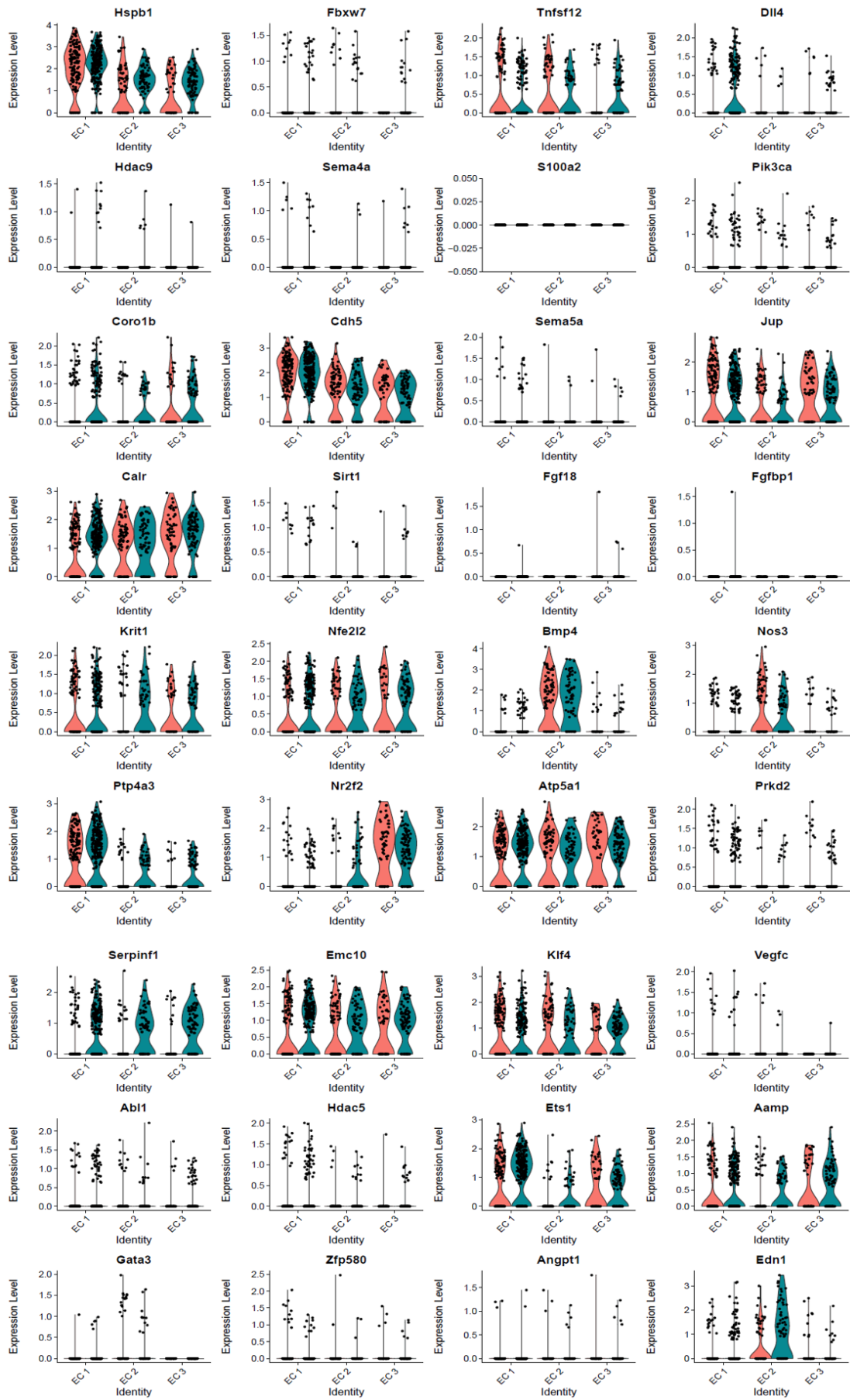


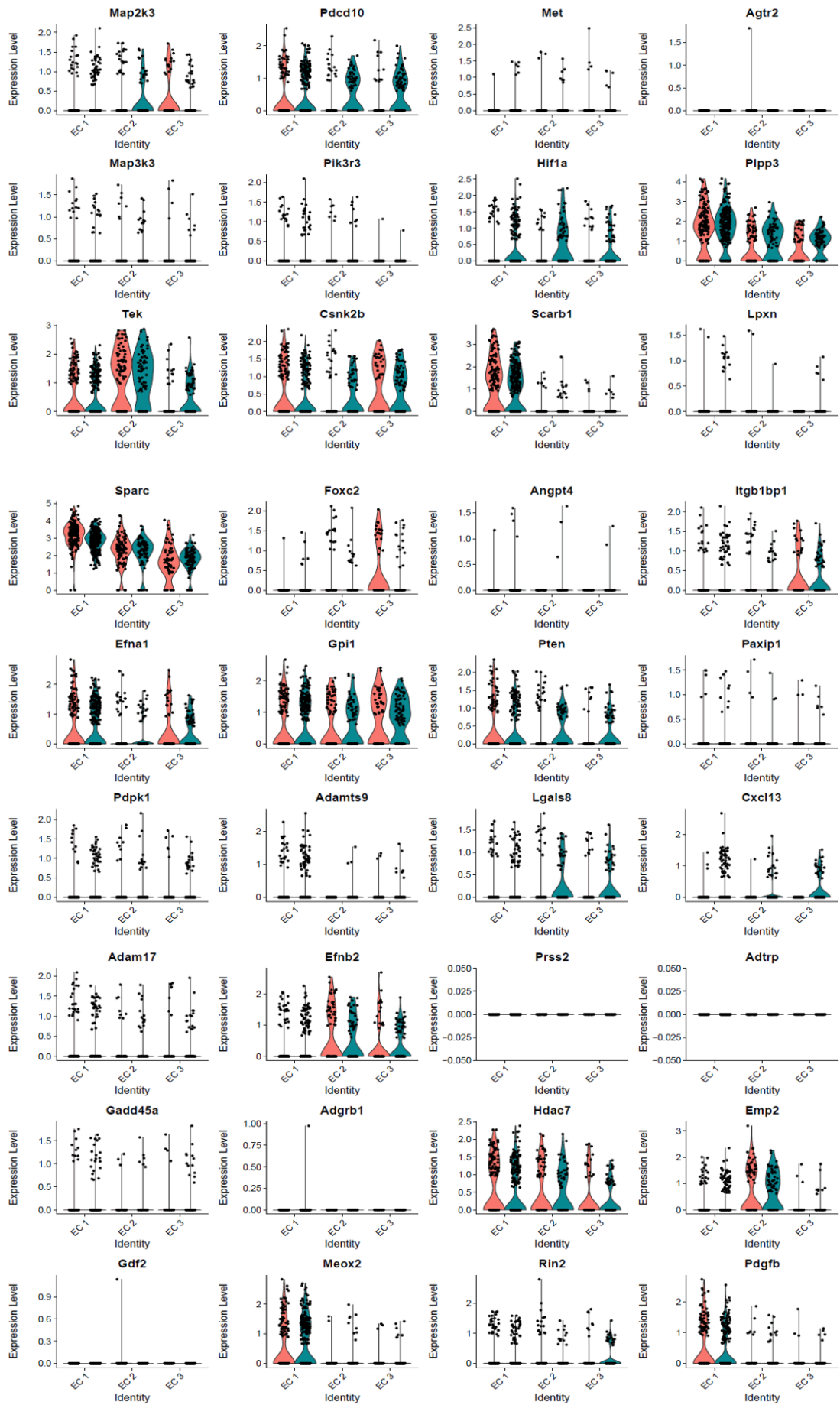
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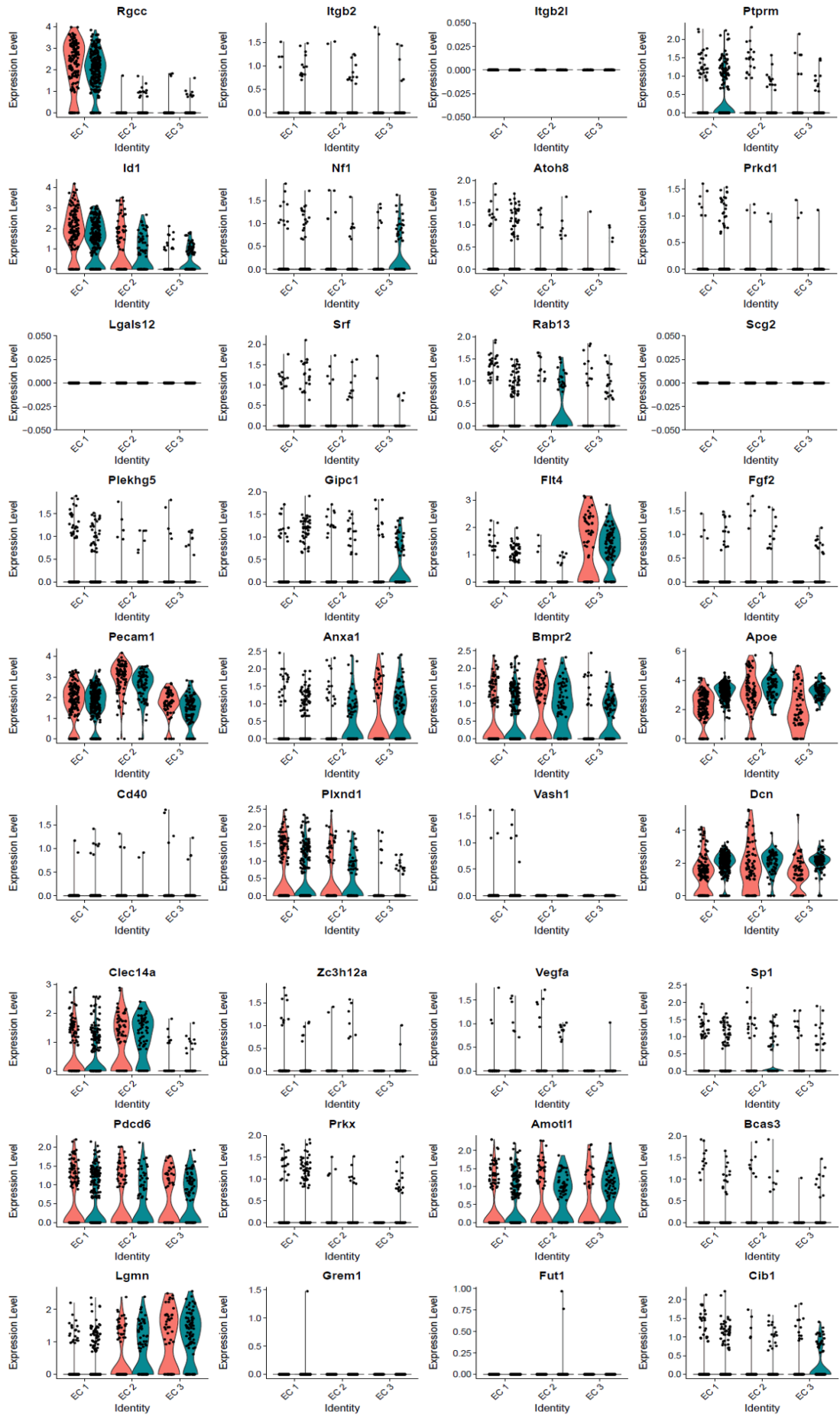
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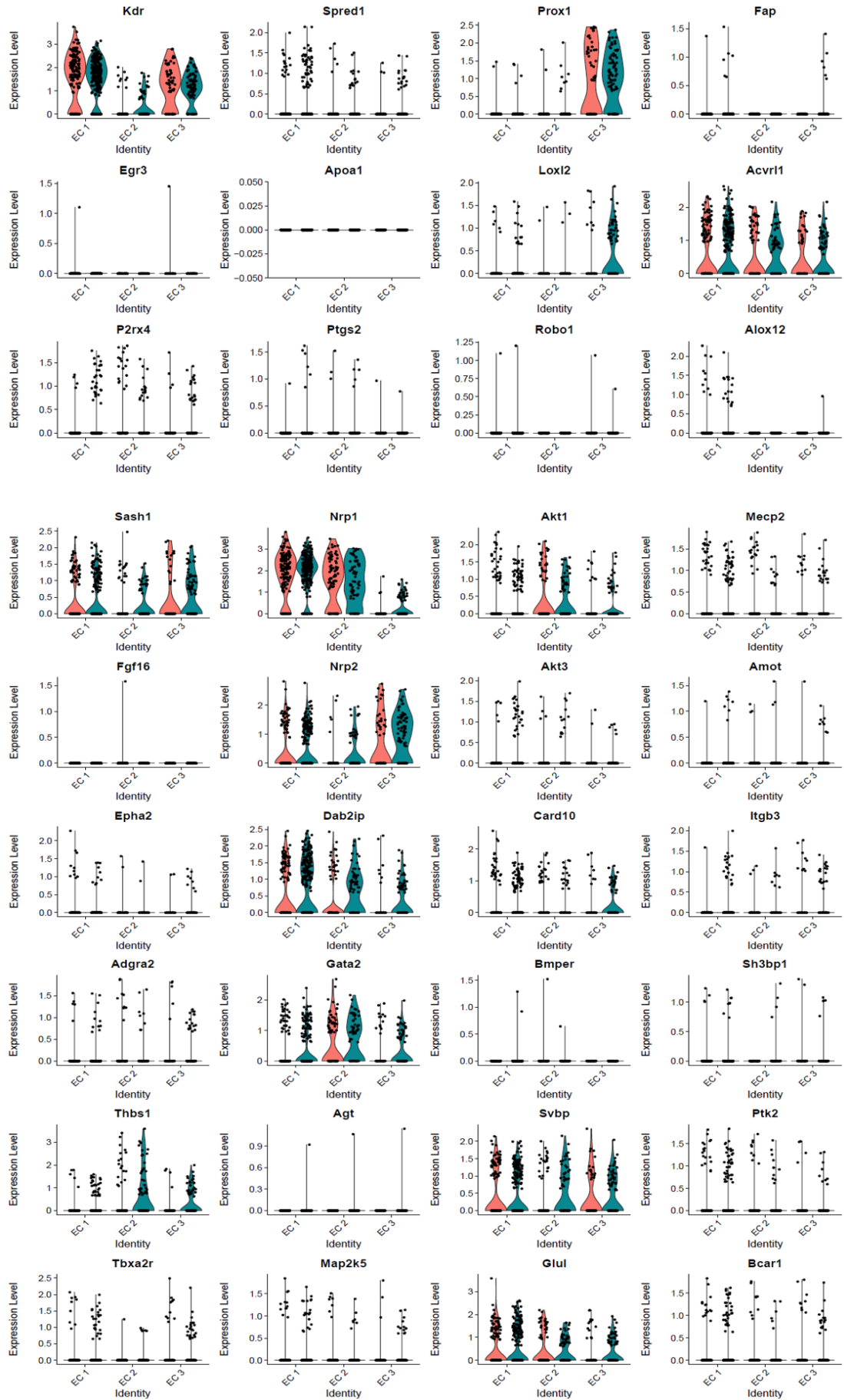


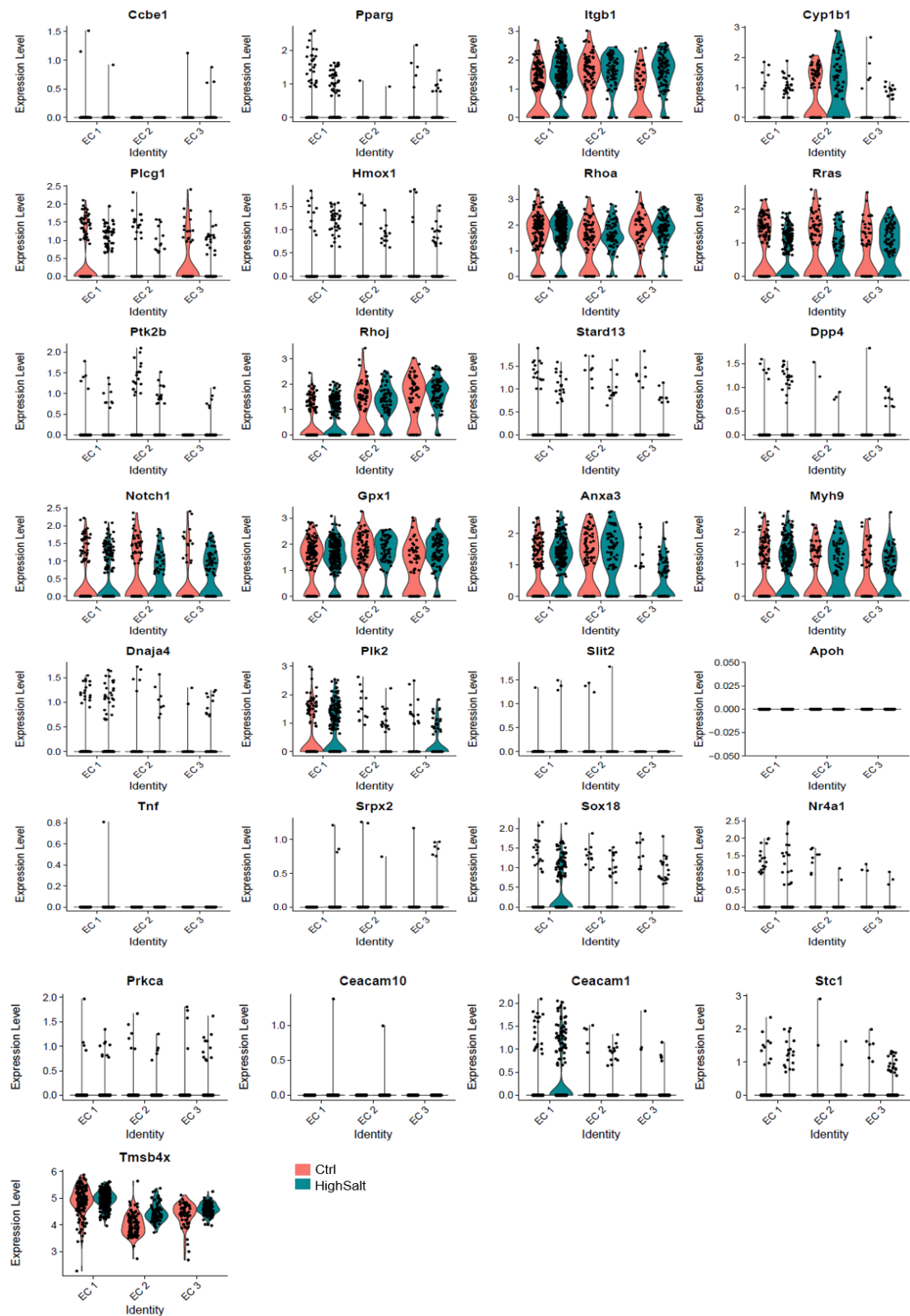
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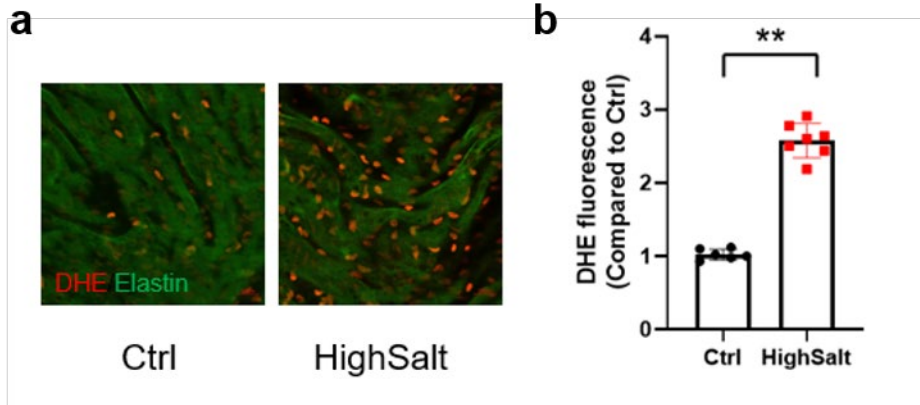




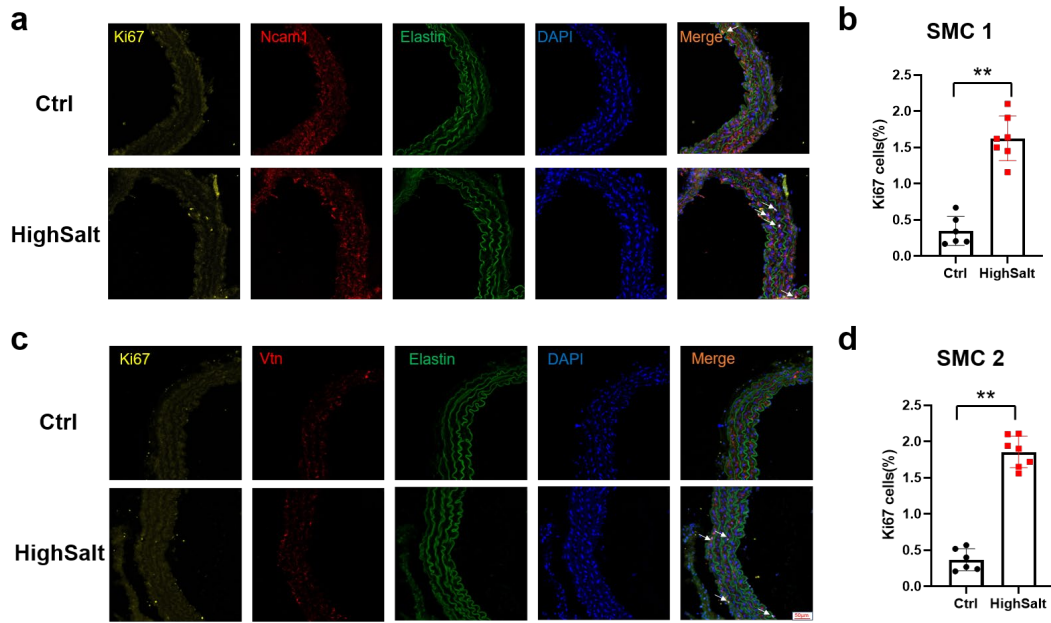




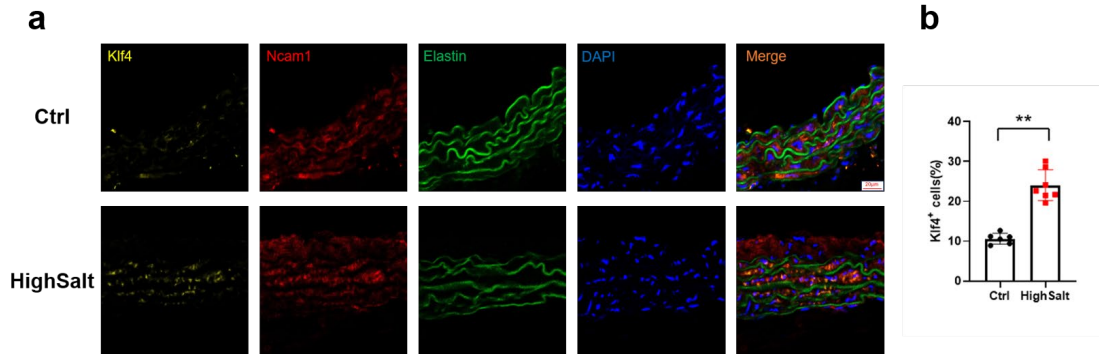
Supplemental Figure 4 Violin plot showing the expression of genes related to endothelial cell features (or functions). a: Collagen, b: Contractile, c: Differentiation, d: Lipid transport, e: Inflammatory response to wounding, f: Lymph vessel development, g: MHC protein complex, h: Proteoglycan, i: Proliferation, j: ROS-related enzymes, k: Migration.



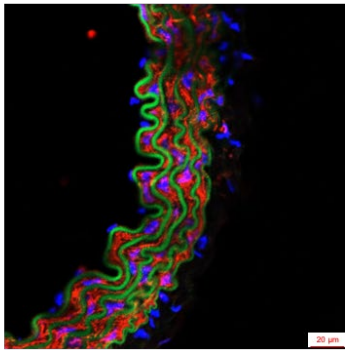
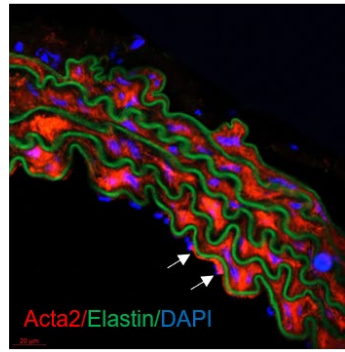
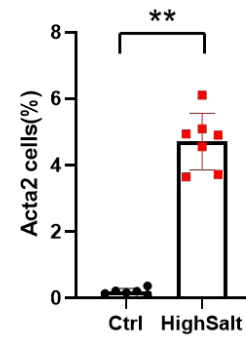
Supplemental Figure 5 High-salt diet enhanced endothelial reactive oxygen species (ROS) production. **a** (representative images) and **b** (summarized data), ROS measured by DHE in the en face endothelium of aortae from control and High-salt diet mice. Bar: 20 μ m. Data are represented as the mean \pm SEM. **P < 0.01 by Student's t test. n = 6-7.



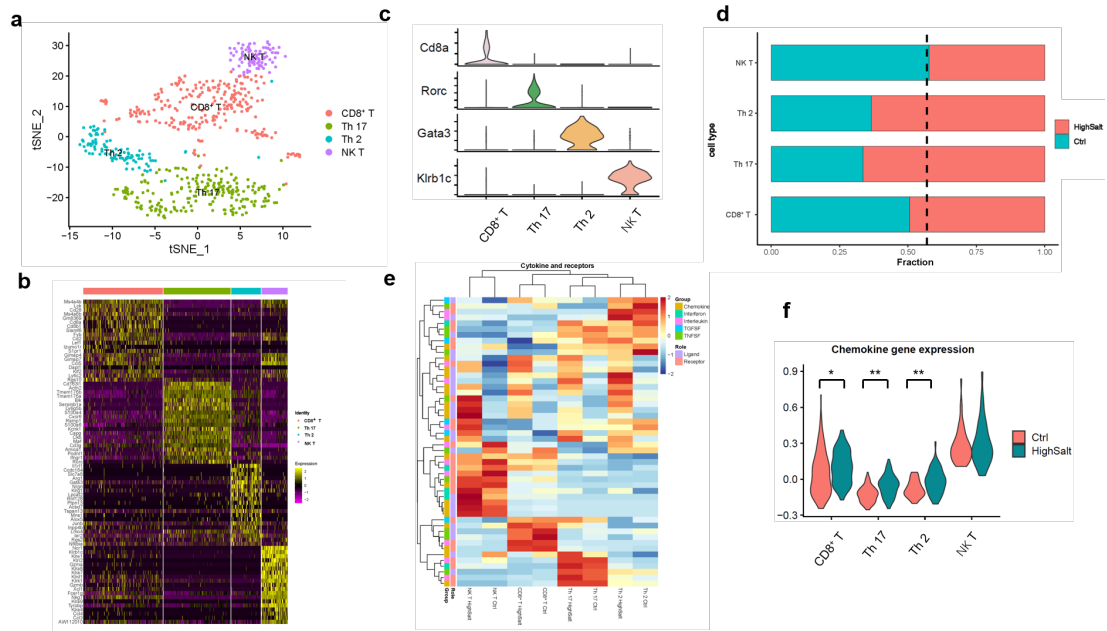
Supplemental Figure 6 Quantification of double-immunostained cells with Ncam1/Vtn (SMC1/SMC2 marker) and Ki67 markers in aortic arch section. a and c are representative of immunostaining for Ncam1/Vtn (red, SMC1/SMC2) and Ki67 (yellow). b and d are summarized data. DAPI detection of nuclei is represented in blue, and green represents elastin autofluorescence. Arrows point to proliferating SMC. Scale bar: 50 μ m. Data are represented as the mean \pm SEM. **P < 0.01 by Student's t test. n = 6-8.



Supplemental Figure 7 High-salt diet induces increased Klf4 expression in SMC
(a) Representative of immunostaining for Ncam1 (red, SMC1) and Klf4(yellow). **(b)** Summarized data. DAPI detection of nuclei is represented in blue, and green represents elastin autofluorescence; scale bar: 20 μ m. Data are represented as the mean \pm SEM. **P < 0.01 by Student's t test. n = 6-8.

a**Ctrl****HighSalt****b**

Supplemental Figure 8 Examples of ACTA2 expression in monolayers of cells lining the vasculature on histological sections of the aortic arch in control and high-salt diet mice. a (representative images) and **b** (summarized data). Arrows point to Acta2-expressing endothelial cells. Scale bar: 20 μm . Data are represented as the mean \pm SEM. ** $P < 0.01$ by Student's t test. $n = 6-8$.



Supplemental Figure 9 Characteristics of aortic T cells and their changes in Hypertension. (a) t-SNE plots of the T cells subpopulations from control and hypertensive tissue (control, n = 447 cells. hypertension, n = 587 cells. CD8⁺T, n= 390 cells; Th17, n= 364 cells; Th2, n=159 cells; NK T, n=121 cells). (b) Heatmap showing the top 10 genes [by average Log(fold change)] for each T cells subpopulation. (c) Expression levels of T cells subpopulation marker genes. (d) Bar graph of the composition of each T cells subpopulation (dashed black line, expected proportion of cells in the control group). (e) Mean expression of cytokine and receptors genes in T cluster. (f) Chemokine gene set score of T cells subpopulations differentiates control and hypertensive. *Mann–Whitney U test P<0.05, **Mann–Whitney U test P<0.01.