

Supplemental Table 1. Differentially expressed genes (DEGs) in female control adrenal glands between 9- and 6-weeks of age.

	Gene Name	Description	Log2Fold Change	padj
Up Regulated	<i>Gm13394</i>	predicted gene 13394	2.54	2.44E-05
	<i>Jchain</i>	immunoglobulin joining chain	1.59	8.23E-04
	<i>Igkv10-96</i>	immunoglobulin kappa variable 10-96	1.55	2.42E-03
	<i>Igkc</i>	immunoglobulin kappa constant	1.35	8.57E-03
	<i>Tmem196</i>	transmembrane protein 196	1.29	2.04E-02
	<i>Igkv17-121</i>	immunoglobulin kappa variable 17-121	1.49	2.04E-02
	<i>C1rb</i>	complement component 1, r subcomponent B	0.91	3.11E-02
	<i>Ighv3-6</i>	immunoglobulin heavy variable 3-6	1.41	4.28E-02
	<i>Insr</i>	insulin receptor	0.41	4.54E-02
	<i>Unc5c</i>	unc-5 netrin receptor C	0.56	4.54E-02
	<i>C1s2</i>	complement component 1, s subcomponent 2	1.28	4.54E-02
	<i>Gpr31c</i>	G protein-coupled receptor 31, D17Leh66c region	1.33	4.54E-02
	<i>Fgb</i>	fibrinogen beta chain	0.00	4.90E-02
	<i>Ociad2</i>	OCIA domain containing 2	0.37	4.90E-02
	<i>Igha</i>	immunoglobulin heavy constant alpha	1.12	4.90E-02
	<i>Gm5436</i>	predicted pseudogene 5436	1.17	4.90E-02
	<i>Siglech</i>	sialic acid binding Ig-like lectin H	1.27	4.90E-02
Down Regulated	<i>Cyb5r1</i>	cytochrome b5 reductase 1	-0.38	4.08E-03
	<i>Cisd1</i>	CDGSH iron sulfur domain 1	-0.31	9.94E-03
	<i>Padi2</i>	peptidyl arginine deiminase, type II	-1.47	1.42E-02
	<i>Gm16587</i>	predicted gene 16587	-1.73	4.54E-02
	<i>Omp</i>	olfactory marker protein	-1.31	4.54E-02
	<i>Plin5</i>	perilipin 5	-1.29	4.90E-02

Statistical analysis was performed using the Wald test, p adj <0.05.

Supplemental Table 2. scRNAseq clusters in whole adrenals isolated from 6-week versus 9-week female *Znrf3* cKO mice.

Group	Cluster	6-Week <i>Znrf3</i> cKO (%)	9-Week <i>Znrf3</i> cKO (%)
Adrenal	Capsule	0.11	0.09
	Cortex Zona Glomerulosa (zG)	15.36	9.19
	Cortex Zona Fasciculata (zF)	38.17	7.39
Myeloid	Medulla	0.02	0.09
	Macrophages	10.97	22.07
	Monocytes	1.55	2.87
	Conventional Dendritic Cells 1 (cDCs1)	2.88	10.11
	Conventional Dendritic Cells 2 (cDCs2)	0.89	1.41
	Monocyte-derived Dendritic Cells (MoDCs)	0.2	0.95
	Myeloid-derived Suppressor Cells (MDSCs)	0.24	0.66
	Suppressive Neutrophils	2.5	5.46
	Immature Neutrophils	0.01	2.26
Lymphoid	Natural Killer (NK) Cells	4.07	9.64
	T Cells	0.73	2.09
	B Cells	1.71	3.26
	Plasmacytoid Dendritic Cells (pDCs)	0.35	0.89
Other	Endothelial Cells	18.25	16.49
	Proliferating Immune	1.13	4.08
	Unknown	0.91	1.00

Supplemental Table 3. Immune cell signatures and patient outcome in TCGA-ACC

Signature Name	Gene List	Overall Survival	Progression-Free Survival	Male vs Female
Macrophage	<i>C1QA, C1QB, APOE, MERTK</i>	p=0.406	p=0.181	*p=3.94x10 ⁻²
Phag. Mac.	<i>CD68, TREM2, TYROBP</i>	p=0.296	*p=4.18x10 ⁻²	**p=2.91x10 ⁻³
Dendritic	<i>CD209, ITGAE, IRF8, TLR3</i>	p=0.653	p=0.111	p=0.823
Neutrophil	<i>S100A8, S100A9, MMP9, CSF3R</i>	p=0.971	p=0.324	*p=1.82x10 ⁻²
B Cell	<i>CD79A, CD79B, RAG1, CD22</i>	**p=4.70x10 ⁻³	**p=4.47x10 ⁻³	p=0.792
NK Cell	<i>GZMA, GZMB, CCL5, NKG7</i>	p=0.092	***p=3.58x10 ⁻⁴	*p=3.86x10 ⁻²
T Cell	<i>CD3E, CD3D, CCR7, CTLA4</i>	p=0.103	**p=1.62x10 ⁻³	p=0.477
Mo-Derived	<i>ITGAX, CD68, CCR2, ITGAM</i>	*p=3.28x10 ⁻²	**p=2.49x10 ⁻³	*p=2.17x10 ⁻²
AMRS	<i>ITGAM, CD33, CD68, MSR1</i>	*p=3.55x10 ⁻²	*p=1.00x10 ⁻²	*p=4.60x10 ⁻²
Proliferation	<i>MKi67</i>	**p=1.30x10 ⁻³	****p=2.25x10 ⁻⁵	p=0.111

Analysis of immune cell signatures as well as established prognostic marker High-*MKi67* in TCGA-ACC data. Statistical analysis was performed using log-rank Mantel-Cox test.

Abbreviations: TCGA, The Cancer Genome Atlas; ACC, adrenocortical carcinoma; Phag. Mac., phagocytic macrophages; NK, natural killer; Mo-Derived, monocyte-derived; AMRS, adrenal myeloid response score.

Supplemental Table 4. Summary of ACC patient characteristic

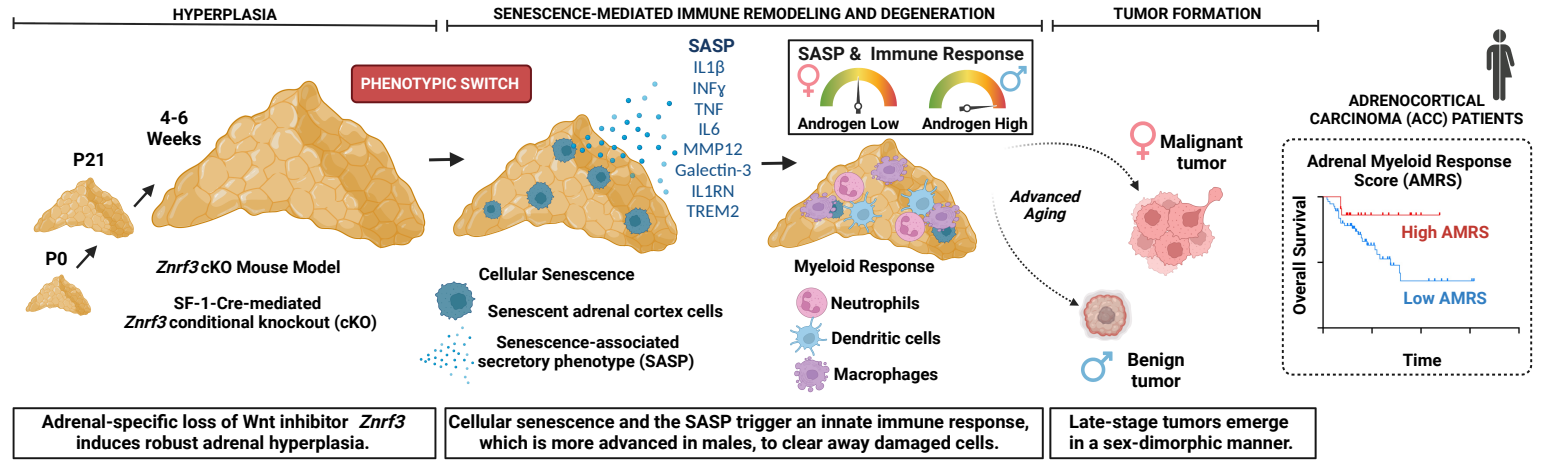
N	Age (years)	Sex (male/female)	Overall CD68-Index (%)	Overall Ki67-Index (%)
38	49.1 (21-76)	17/21	11.52 (2.51-32.46)	8.67 (0.53-29.96)

Data represent total number or average values with (range).

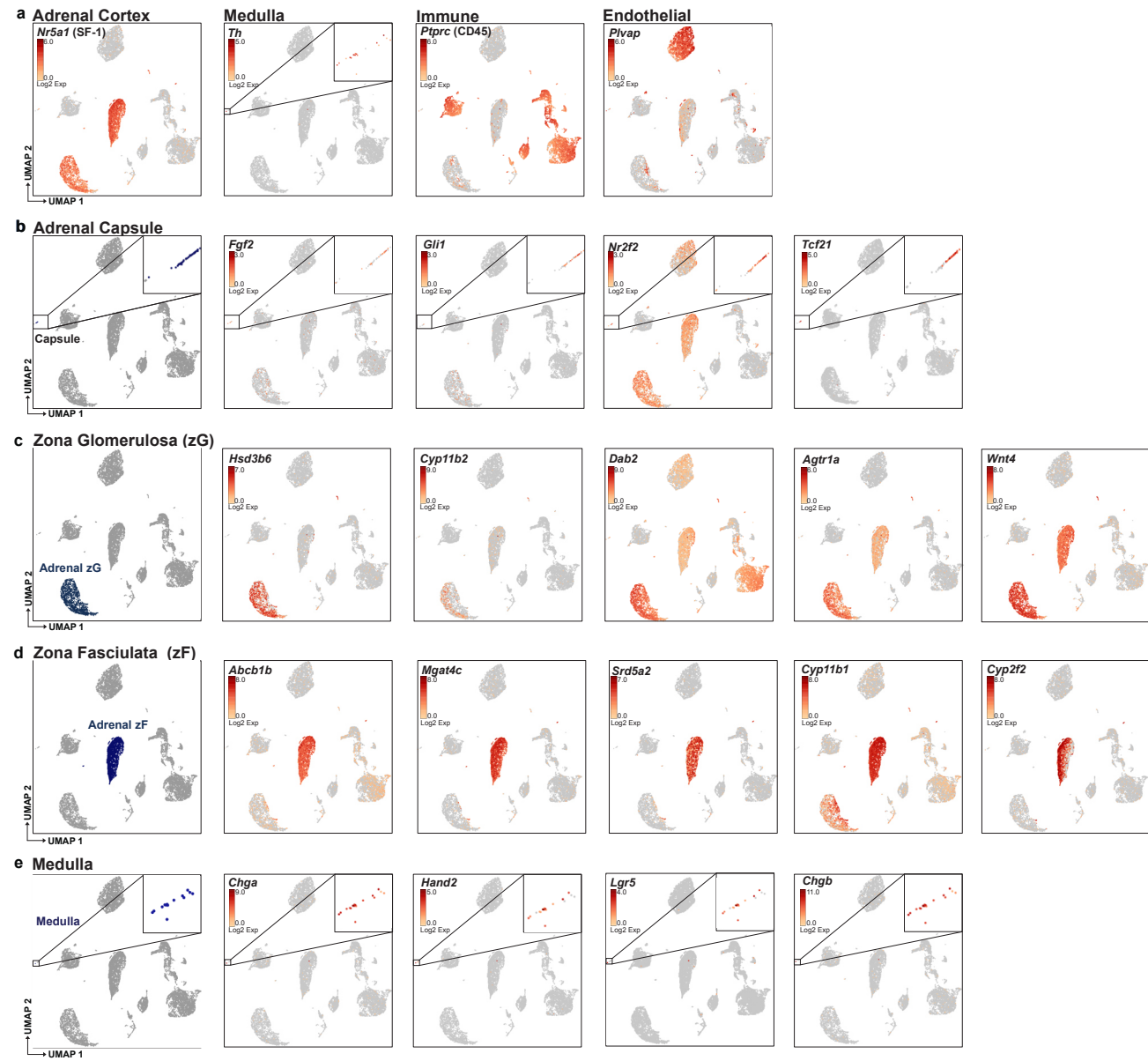
Supplemental Table 5. List of antibodies and staining conditions for immunohistochemistry in mouse adrenal tissue.

Target	Company, Catalog #	Species	Unmasking	Dilution	Blocking Buffer	Antibody Diluent
Cleaved caspase 3	Cell Signaling, 9664	Rabbit	10mM NaCitrate, pH 6.0, 0.05% tween 20	1:200	2.5% HS + 1% BSA	2.5% HS + 0.1% BSA
Ki67	Thermo Fisher, MA5-14520	Rabbit	10mM NaCitrate, pH 6.0, 0.05% tween 20	1:200	2.5% HS	2.5% HS
53BP1	Novus Biologicals, NB100-304	Rabbit	10mM NaCitrate, pH 6.0, 0.05% tween 20	1:1000	2.5% HS + 1% BSA	2.5% HS + 0.1% BSA
p21	Abcam, ab188224	Rabbit	10mM NaCitrate, pH 6.0, 0.05% tween 20	1:7500	2.5% HS + 1% BSA	2.5% HS + 0.1% BSA
CDKN2A (p16 ^{INK4a})	Abcam, ab211542	Rabbit	10mM NaCitrate, pH 6.0, 0.05% tween 20	1:1000	2.5% HS + 1% BSA	2.5% HS + 0.1% BSA
GFP	Abcam, ab5450	Goat	10mM NaCitrate, pH 6.0	1:1000	2.5% HS + 3% BSA	2.5% HS + 1% BSA
Ki67	Thermofisher, 14-5698-80	Rat	10mM NaCitrate, pH 6.0	1:2000	2.5% GS	2.5% GS
F4/80	Cell Signaling, 70076	Rabbit	10mM Tris, 1mM EDTA, 0.05% Tween, pH 9.0	1:250	2.5% HS + 3% BSA	2.5% HS + 1% BSA
CD11b	Abcam, ab133357	Rabbit	10mM NaCitrate, pH 6.0	1:10,000	2.5% HS + 3% BSA	2.5% HS + 1% BSA
CD11c	Cell Signaling, 97585	Rabbit	10mM Tris, 1mM EDTA, pH 9.0	1:300	2.5% HS + 3% BSA	2.5% HS + 1% BSA
Ly6g	Cell Signaling, E6Z1T	Rabbit	10mM Tris, 1mM EDTA, 0.05% Tween, pH 9.0	1:200	2.5% HS + 3% BSA	2.5% HS + 1% BSA
CD3e	Cell Signaling, 99940	Rabbit	10mM Tris, 1mM EDTA, 0.05% Tween, pH 9.0	1:250	2.5% HS + 3% BSA	2.5% HS + 1% BSA
CD68	Abcam, ab125212	Rabbit	10mM NaCitrate, pH 6.0	1:1000	2.5% HS	2.5% HS

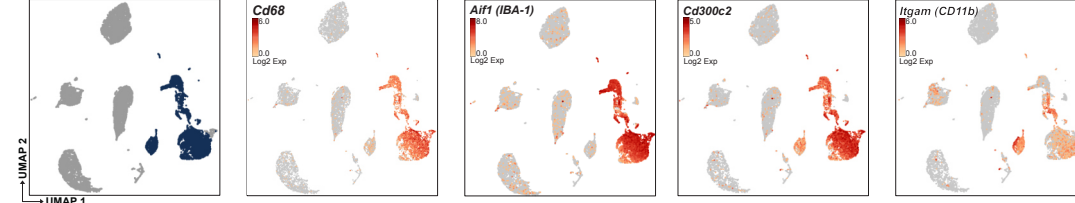
Supplemental Fig. 1: Graphical Abstract. Created with BioRender.com



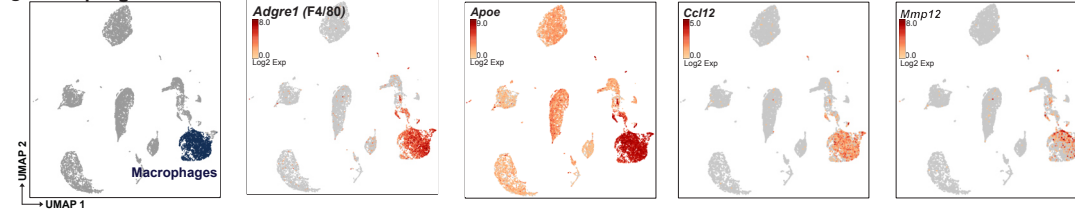
Supplemental Fig. 2: Validation of major cell types identified by scRNAseq



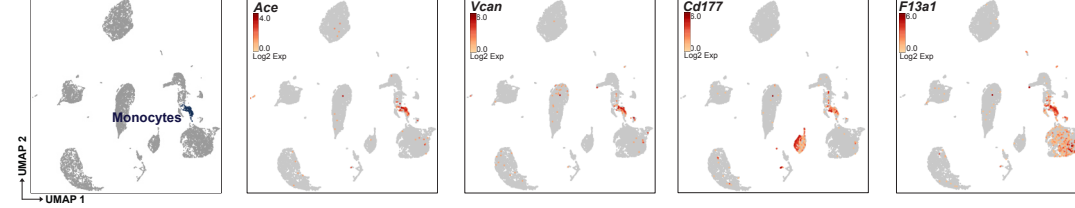
f Myeloid Cells



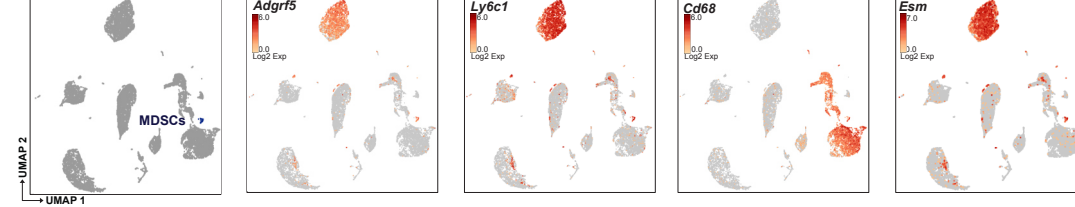
g Macrophages



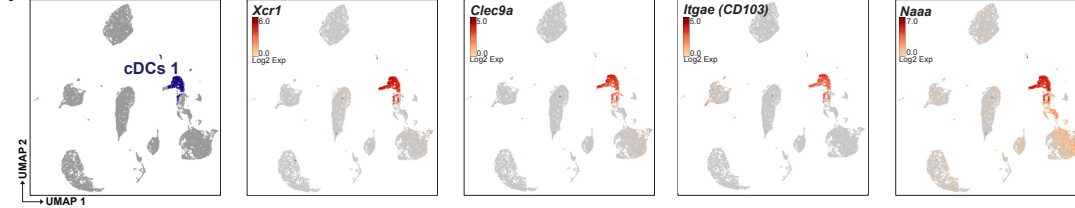
h Monocytes



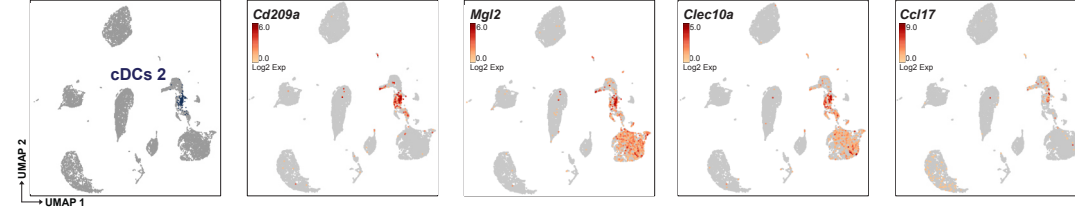
i MDSCs



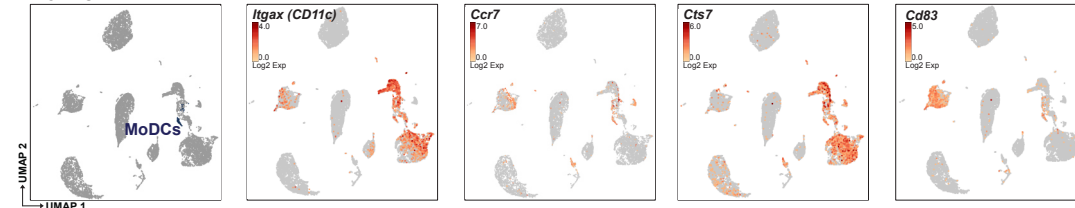
j cDCs 1



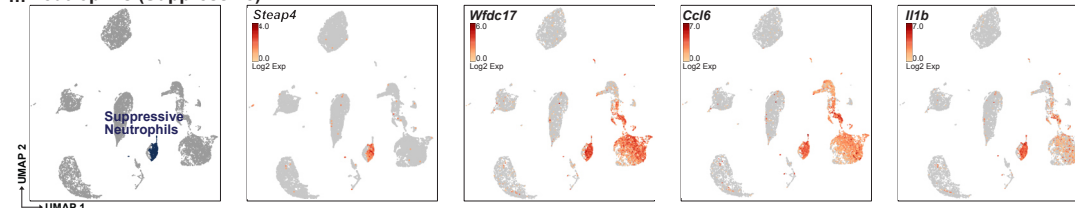
k cDCs 2



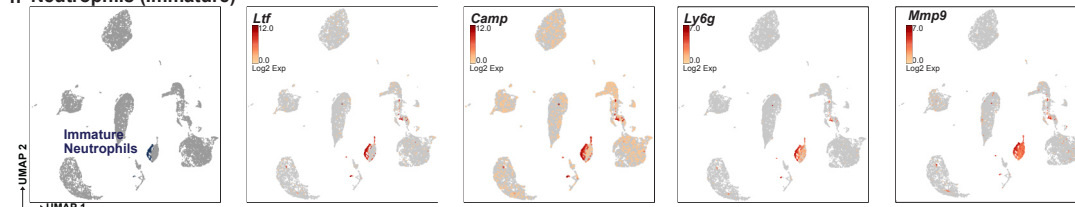
l MoDCs



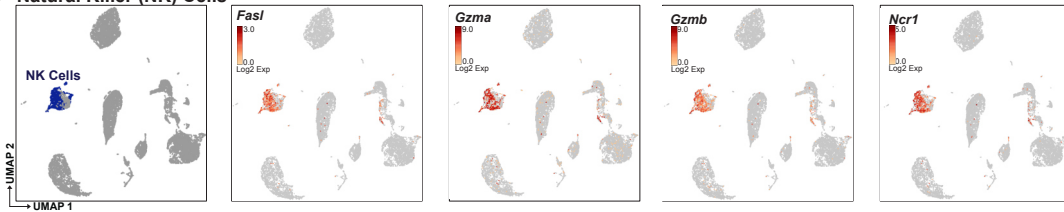
m Neutrophils (Suppressive)



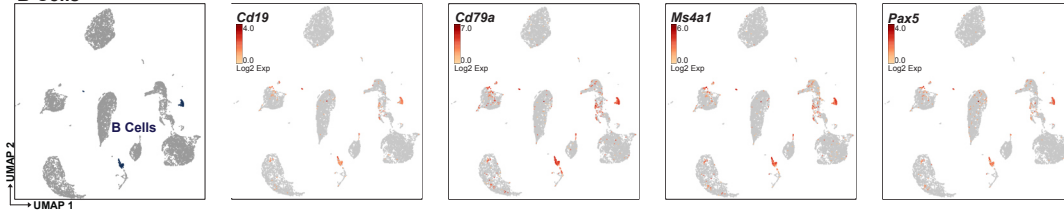
n Neutrophils (Immature)



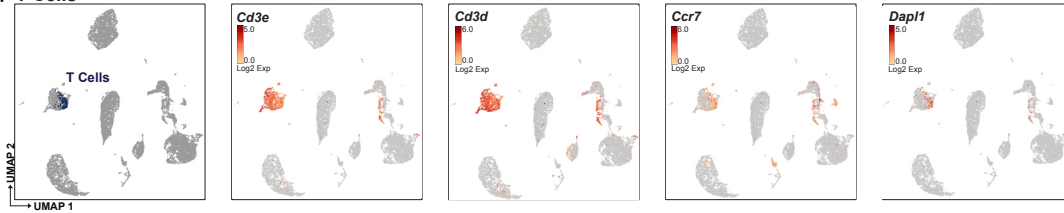
o Natural Killer (NK) Cells



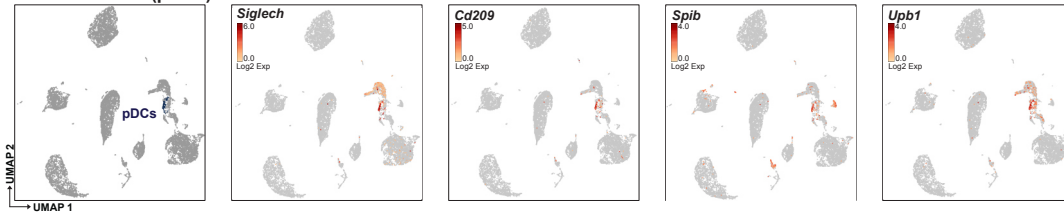
p B Cells



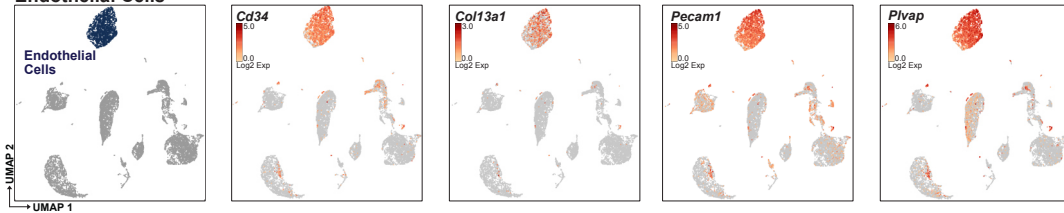
q T Cells



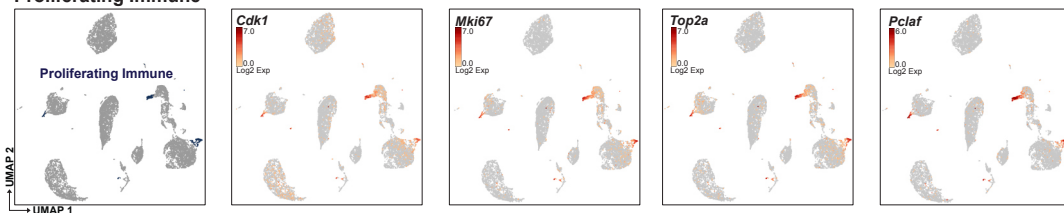
r Plasmacytoid Dendritic Cells (pDCs)



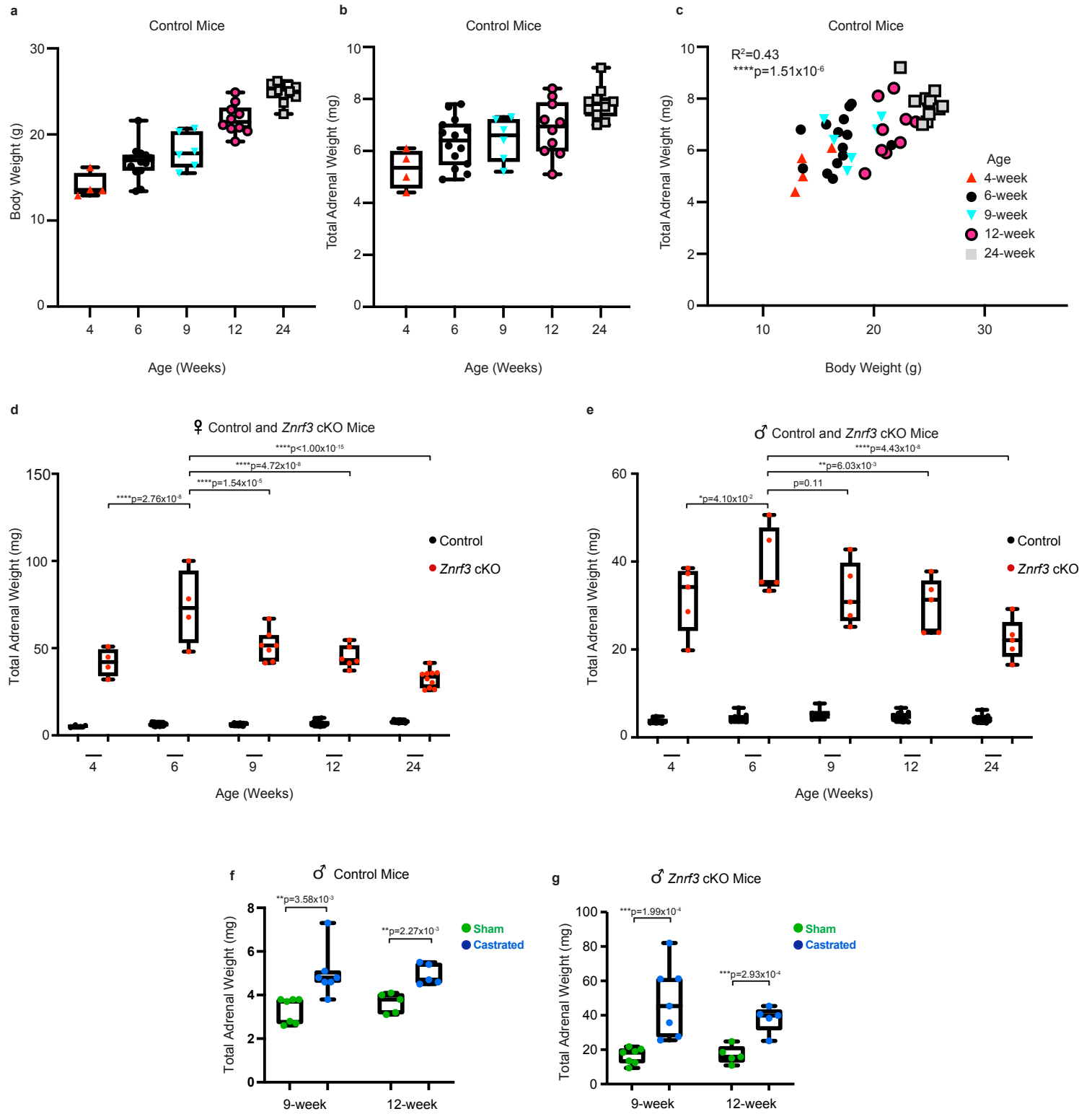
s Endothelial Cells



t Proliferating Immune

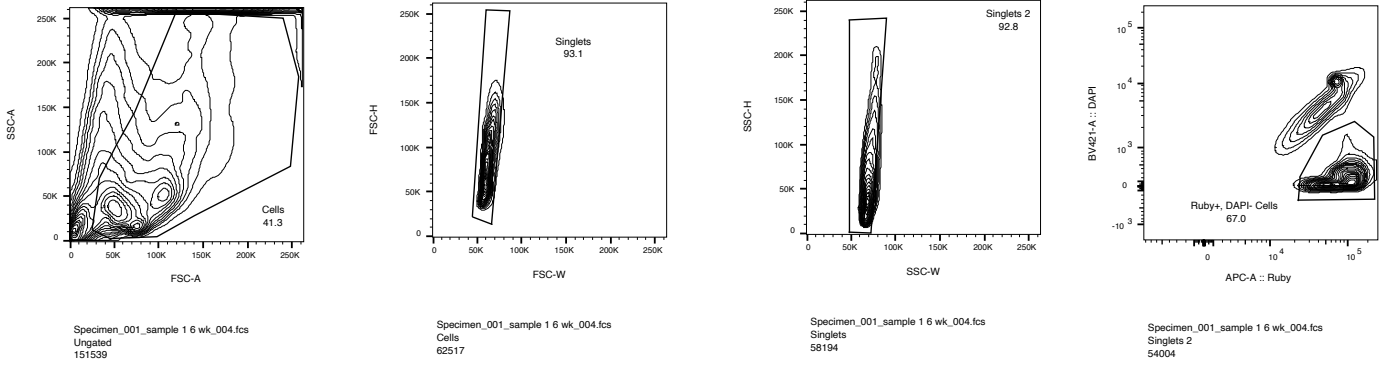


Supplemental Fig. 3: Extended adrenal weight analysis.



Supplemental Fig. 4: FACS gating strategy for isolation of dissociated adrenal cells for scRNAseq

(a) 6-week *Znrf3* cKO Female



(b) 9-week *Znrf3* cKO Female

