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Supplemental Information

Single-Cell RNA Profiling Reveals Adipocyte to Macrophage Signaling Sufficient to Enhance Thermogenesis

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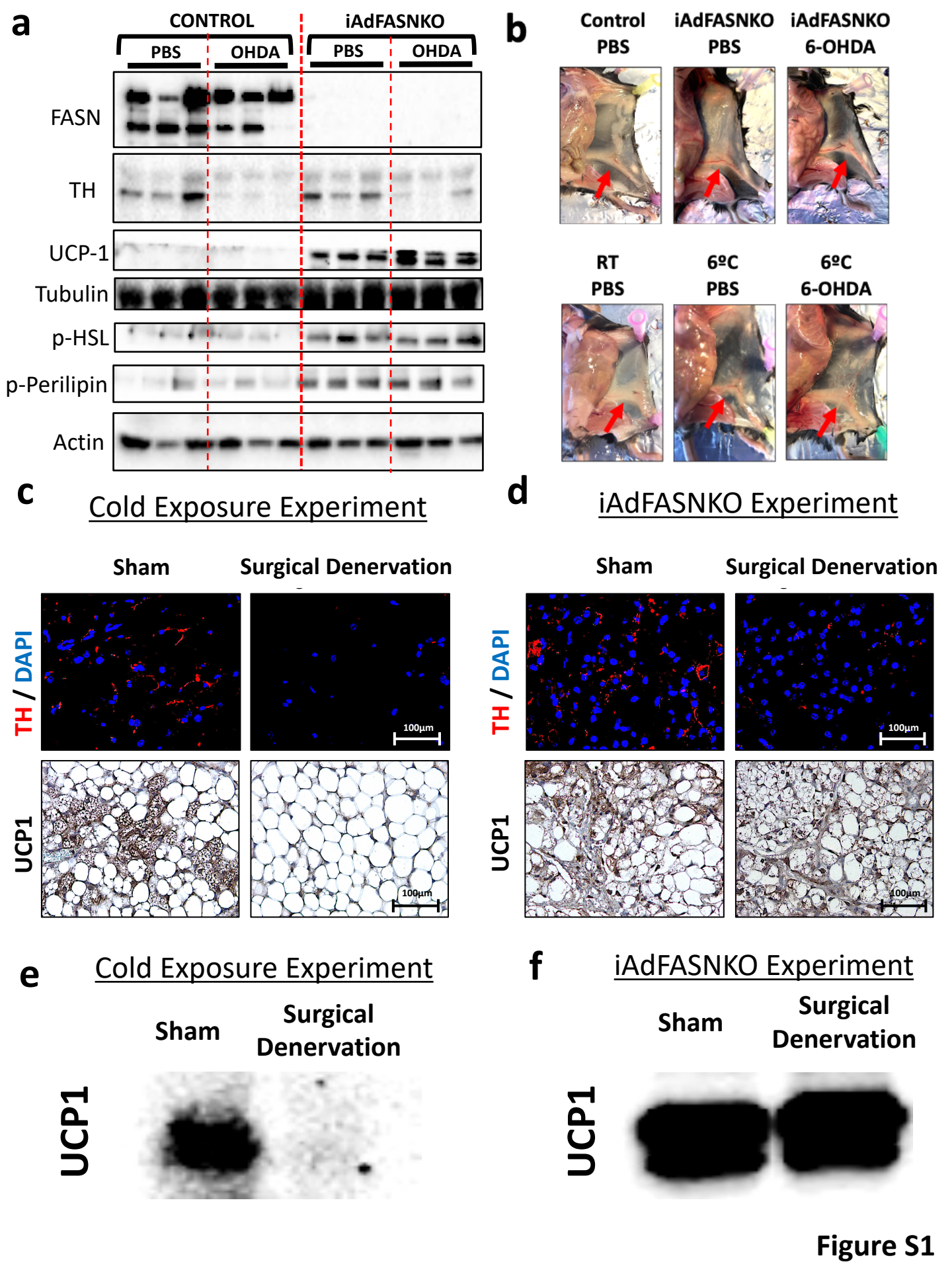
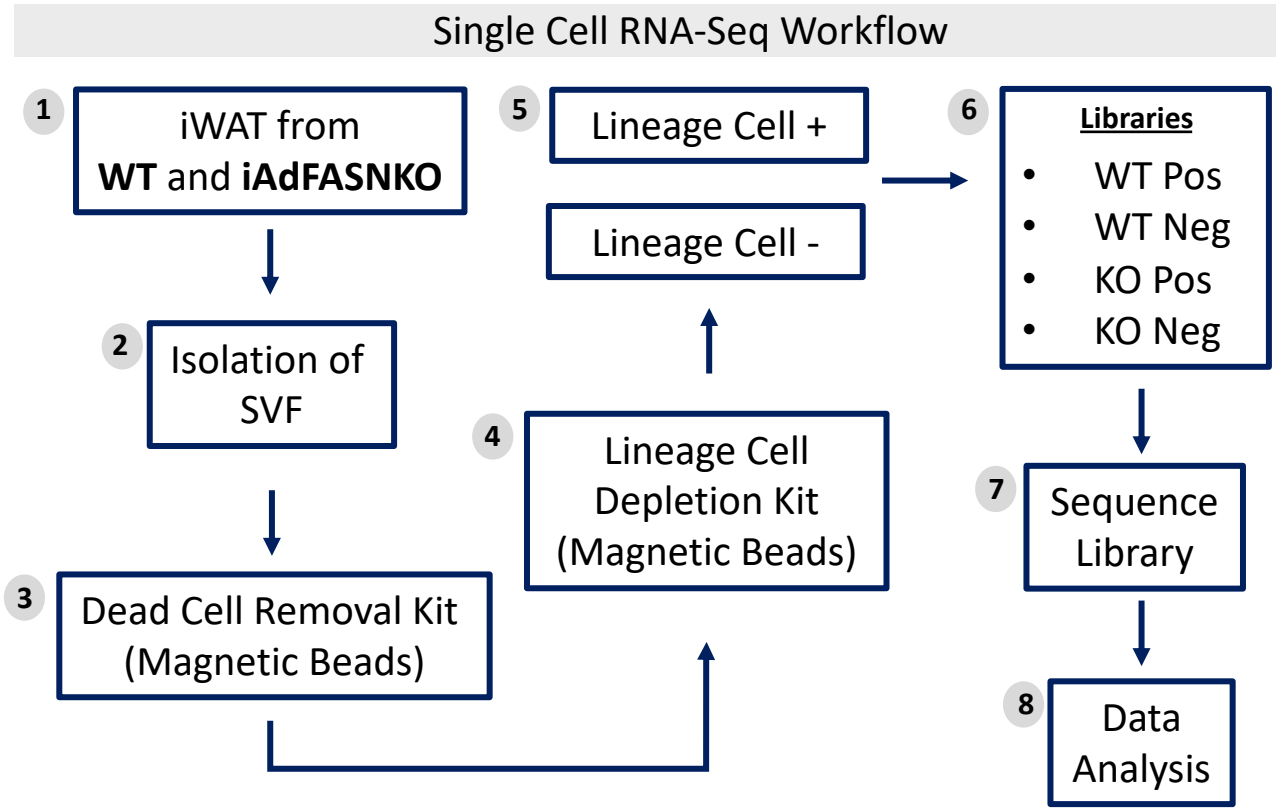

a
CONTROL
iAdFASNKO
PBS
OHDA
PBS
OHDA
FASN
TH
UCP-1
Tubulin
p-HSL
p-Perilipin
Actin
b
Control
PBS
iAdFASNKO
PBS
iAdFASNKO
6-OHDA
RT
PBS
6ºC
PBS
6ºC
6-OHDA
c
Cold Exposure Experiment
Sham
Surgical Denervation
TH / DAPI
UCP1
100µm
d
iAdFASNKO Experiment
Sham
Surgical Denervation
TH / DAPI
UCP1
100µm
e
Cold Exposure Experiment
Sham
Surgical Denervation
UCP1
f
iAdFASNKO Experiment
Sham
Surgical Denervation
UCP1

Figure S1

Figure S1: Chemical and surgical denervation fail to block upregulation of PKA signaling, TH and UCP1 expression levels in iWAT from iAdFASNKO, related to Figure 2. (a): Depicted are representative immunoblots to detect FASN, TH, UCP1, phospho-HSL, phospho-perilipin, tubulin and actin protein levels in iWAT from control and iAdFASNKO mice, treated with PBS or chemically denervated with 6OHDA. **(b):** Representative images of mice emphasizing the iWAT (see red arrows) after chemical denervation from iAdFASNKO and cold exposure group. **(c-d)** Confirmation that TH levels were reduced in the iWAT after surgical denervation. TH signals in iWAT were detected by immunofluorescence staining of tissue samples from **(c)** cold exposed mice and **(d)** iAdFASNKO mice. To evaluate the UCP1 protein level in the iWAT, immunohistochemistry and western blotting for UCP1 was performed for **(c, e)** cold exposure and **(d, f)** iAdFASNKO. N = 3-4 per group. These results are representative of five independent experiments.

a


Single Cell RNA-Seq Workflow
1
iWAT from
WT and iAdFASNKO
2
Isolation of
SVF
3
Dead Cell Removal Kit
(Magnetic Beads)
4
Lineage Cell
Depletion Kit
(Magnetic Beads)
5
Lineage Cell +
Lineage Cell -
6
Libraries
WT Pos
WT Neg
KO Pos
KO Neg
7
Sequence
Library
8
Data
Analysis

b

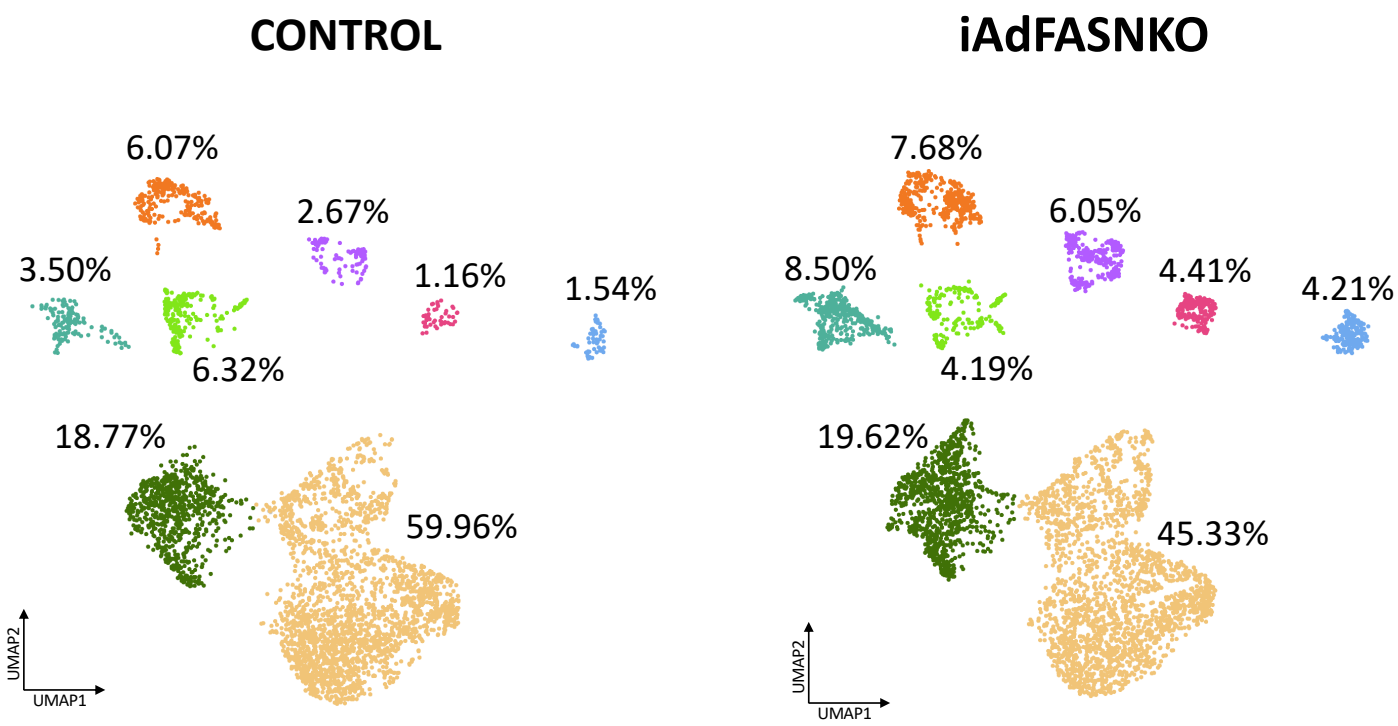

CONTROL
6.07%
2.67%
3.50%
1.16%
1.54%
6.32%
18.77%
59.96%
UMAP2
UMAP1
iAdFASNKO
7.68%
6.05%
8.50%
4.41%
4.21%
4.19%
19.62%
45.33%
UMAP2
UMAP1

Figure S2

Figure S2: Single-Cell RNA-Seq Workflow and UMAP plot from control vs. iAdFASNKO mice, related to Figure 4. **(a)** Workflow overview exhibiting all steps for the single-cell RNA-seq. See the methods section for the complete details. **(b)** UMAP plot from figure 4 split into the cells from control and iAdFASNKO. The percentage of the total number of cells is shown for each cluster.

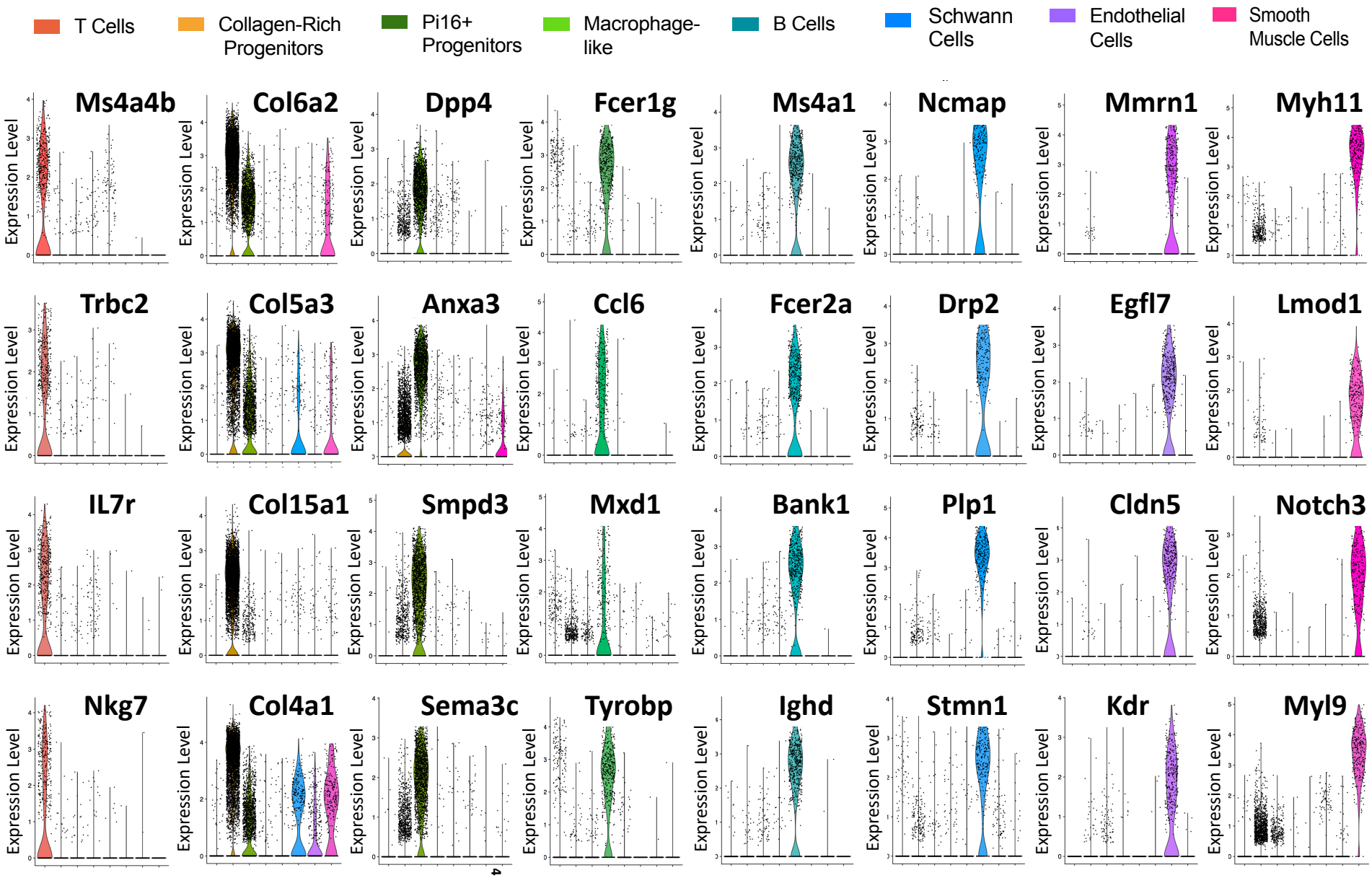

T Cells
Collagen-Rich Progenitors
Pi16+ Progenitors
Macrophage-like
B Cells
Schwann Cells
Endothelial Cells
Smooth Muscle Cells
Ms4a4b
Col6a2
Dpp4
Fcer1g
Ms4a1
Ncmap
Mmrn1
Myh11
Trbc2
Col5a3
Anxa3
Ccl6
Fcer2a
Drp2
Egfl7
Lmod1
IL7r
Col15a1
Smpd3
Mxd1
Bank1
Plp1
Cldn5
Notch3
Nkg7
Col4a1
Sema3c
Tyrobp
Ighd
Stmn1
Kdr
Myl9
Expression Level

Figure S3

Figure S3: Violin plots of the top expression levels for representative genes in each different cluster, related to Figure 4. Violin plots of $\log_e$ (normalized values) for the top representative genes from each cluster in the aggregated dataset. Cluster-enriched genes are shown in columns below the indicated cluster.

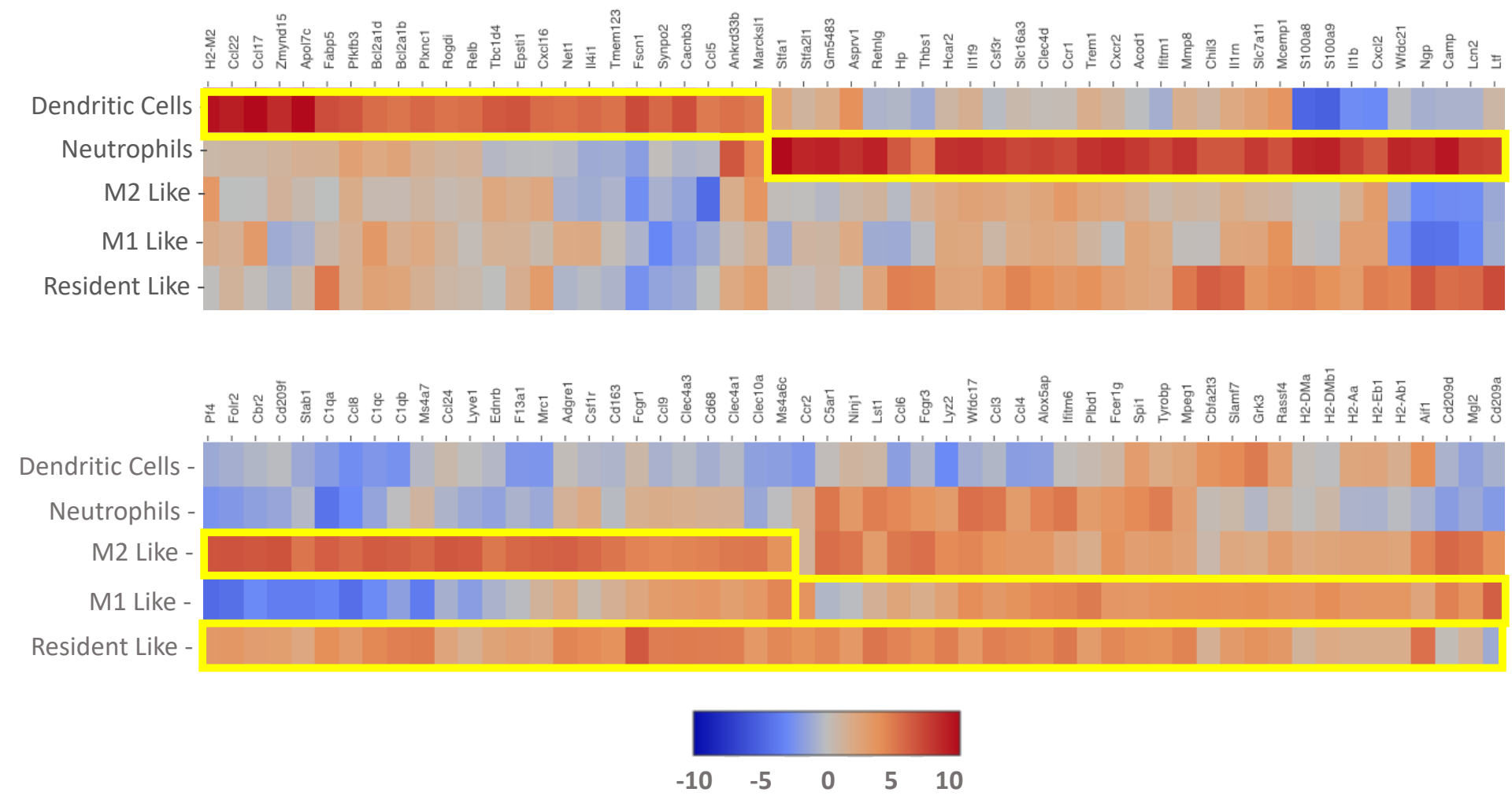

H2-M2
Ccl22
Ccl17
Zmynd15
Apol7c
Fabp5
Pfkfb3
Bcl2a1d
Bcl2a1b
Plxnc1
Rogdi
Relb
Tbc1d4
Epsti1
Cxcl16
Net1
Il4i1
Tmem123
Fscn1
Synpo2
Cacnb3
Ccl5
Ankrd33b
Marcksl1
Stfa1
Stfa2l1
Gm5483
Asprv1
Retnlg
Hp
Thbs1
Hcar2
Il1f9
Csf3r
Slc16a3
Clec4d
Ccr1
Trem1
Cxcr2
Acod1
Ifitm1
Mmp8
Chil3
Il1rn
Slc7a11
Mcemp1
S100a8
S100a9
Il1b
Cxcl2
Wfdc21
Ngp
Camp
Lcn2
Ltf
Dendritic Cells
Neutrophils
M2 Like
M1 Like
Resident Like
Pf4
Folr2
Cbr2
Cd209f
Stab1
C1qa
Ccl8
C1qc
C1qb
Ms4a7
Ccl24
Lyve1
Ednrb
F13a1
Mrc1
Adgre1
Csf1r
Cd163
Fcgr1
Ccl9
Clec4a3
Cd68
Clec4a1
Clec10a
Ms4a6c
Ccr2
C5ar1
Ninj1
Lst1
Ccl6
Fcgr3
Lyz2
Wfdc17
Ccl3
Ccl4
Alox5ap
Ifitm6
Plbd1
Fcer1g
Spi1
Tyrobp
Mpeg1
Cbfa2t3
Slamf7
Grk3
Rassf4
H2-DMa
H2-DMb1
H2-Aa
H2-Eb1
H2-Ab1
Aif1
Cd209d
Mgl2
Cd209a
Dendritic Cells
Neutrophils
M2 Like
M1 Like
Resident Like
-10
-5
0
5
10

Figure S4

Figure S4: Heatmap showing scaled expression of differentially expressed genes for each different macrophage-like sub-cluster identified, related to Figure 5. The heatmap shows the top 25 differentially expressed genes between sub-clusters obtained from graph-based log2 fold changes generated from the Loupe browser. Colors (values) are z-score of the average log fold-change between sub-clusters for each gene (so z l is based on fold change across the five sub-clusters). Cluster names were manually determined based on the top-25 upregulated genes related to differential gene expression between clusters and knowledge of canonical cell markers.

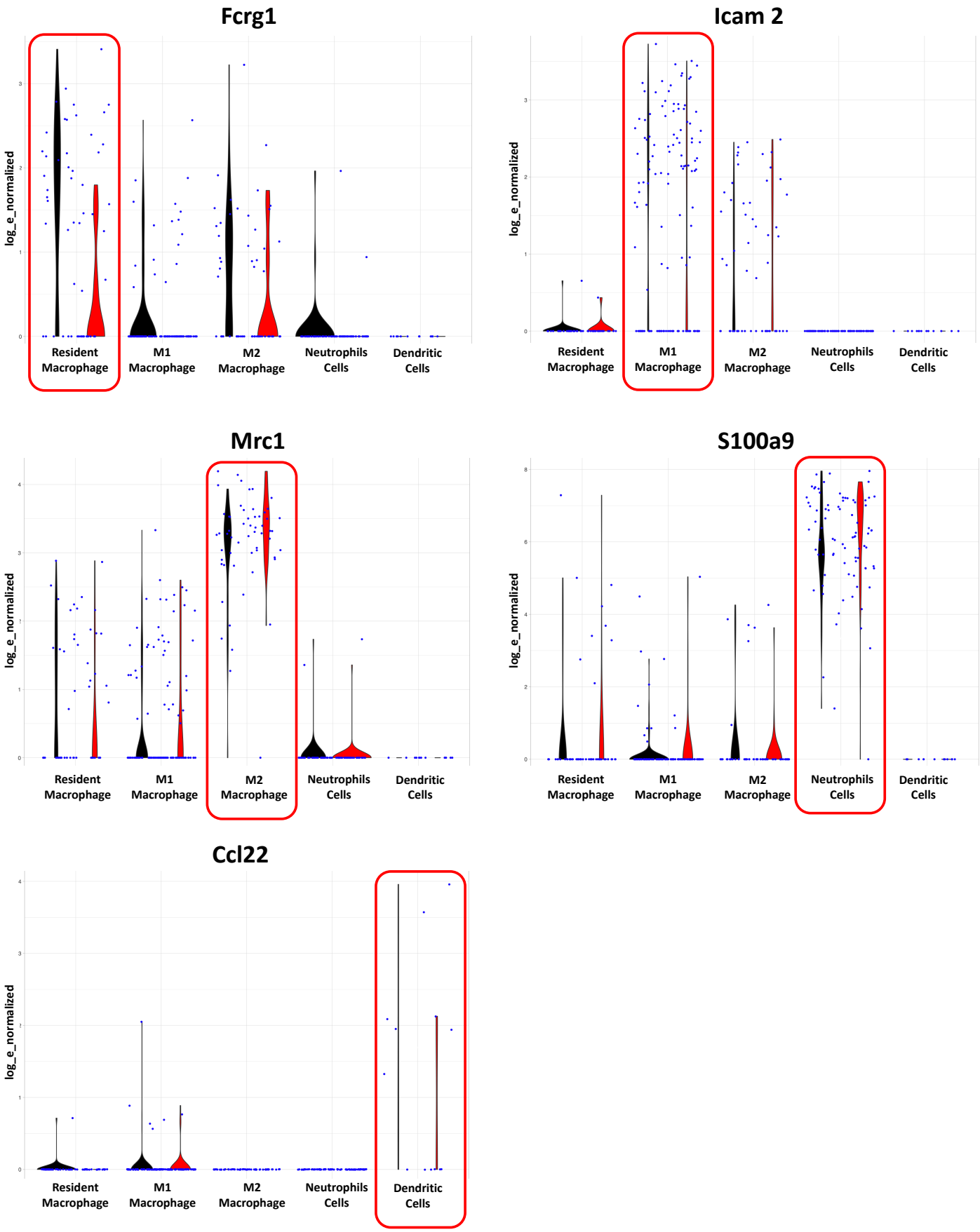

Fcrg1
log_e_normalized
Resident Macrophage
M1 Macrophage
M2 Macrophage
Neutrophils Cells
Dendritic Cells
Icam 2
log_e_normalized
Resident Macrophage
M1 Macrophage
M2 Macrophage
Neutrophils Cells
Dendritic Cells
Mrc1
log_e_normalized
Resident Macrophage
M1 Macrophage
M2 Macrophage
Neutrophils Cells
Dendritic Cells
S100a9
log_e_normalized
Resident Macrophage
M1 Macrophage
M2 Macrophage
Neutrophils Cells
Dendritic Cells
Ccl22
log_e_normalized
Resident Macrophage
M1 Macrophage
M2 Macrophage
Neutrophils Cells
Dendritic Cells

Figure S5

Figure S5: Representative marker for each different macrophage-like sub-cluster identified, related to Figure 5. Violin plots show the $\log_e$ of normalized expression values for representative genes in the different Macrophage-like sub-clusters. These representative genes were used to identify and name the following clusters: Resident Macrophage (Fcgr1), M1 Macrophage (Icam2), M2 Macrophage (Mrc1), Neutrophils Cells (S100a9) and Dendritic Cells (Ccl22).

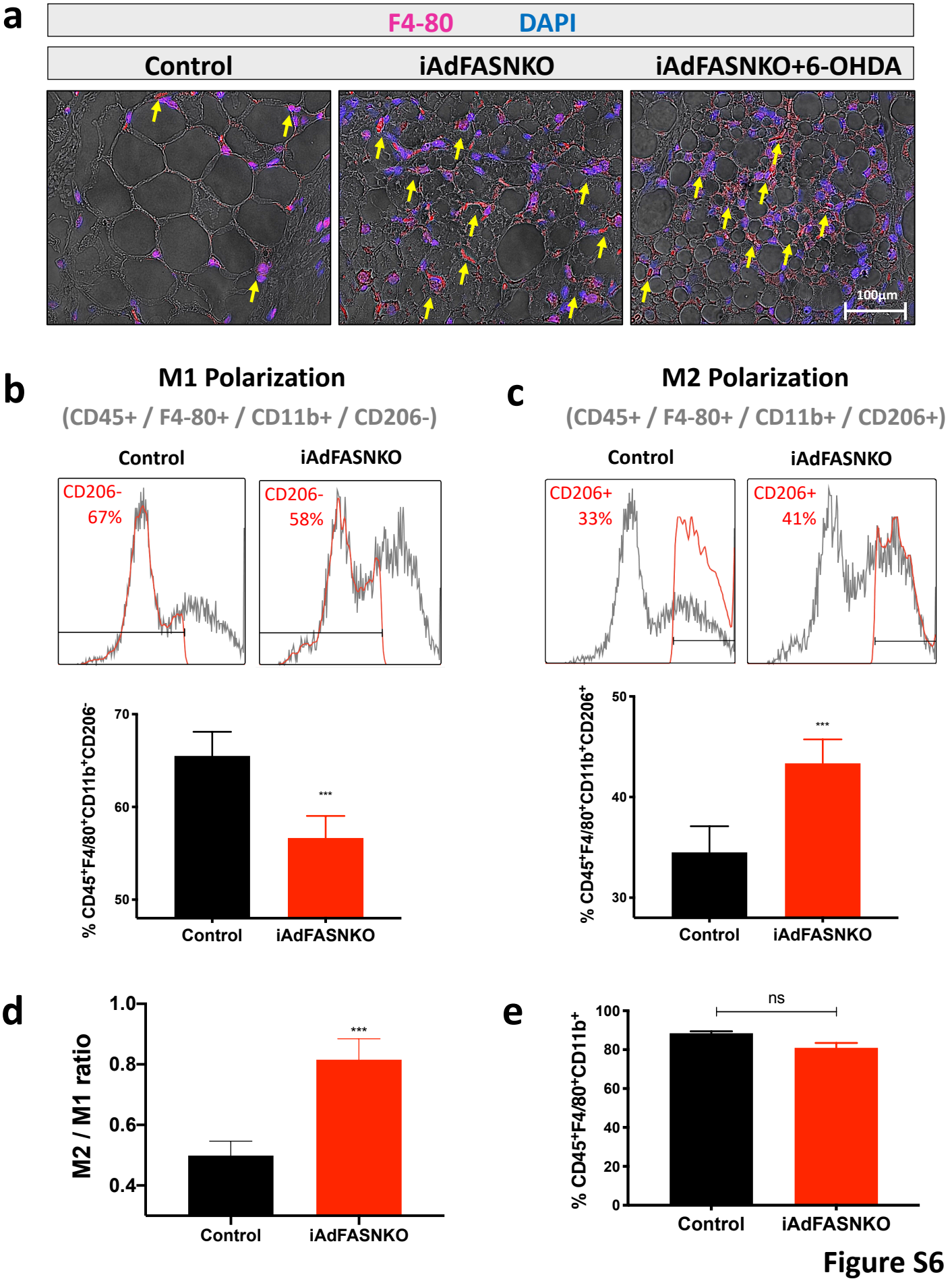
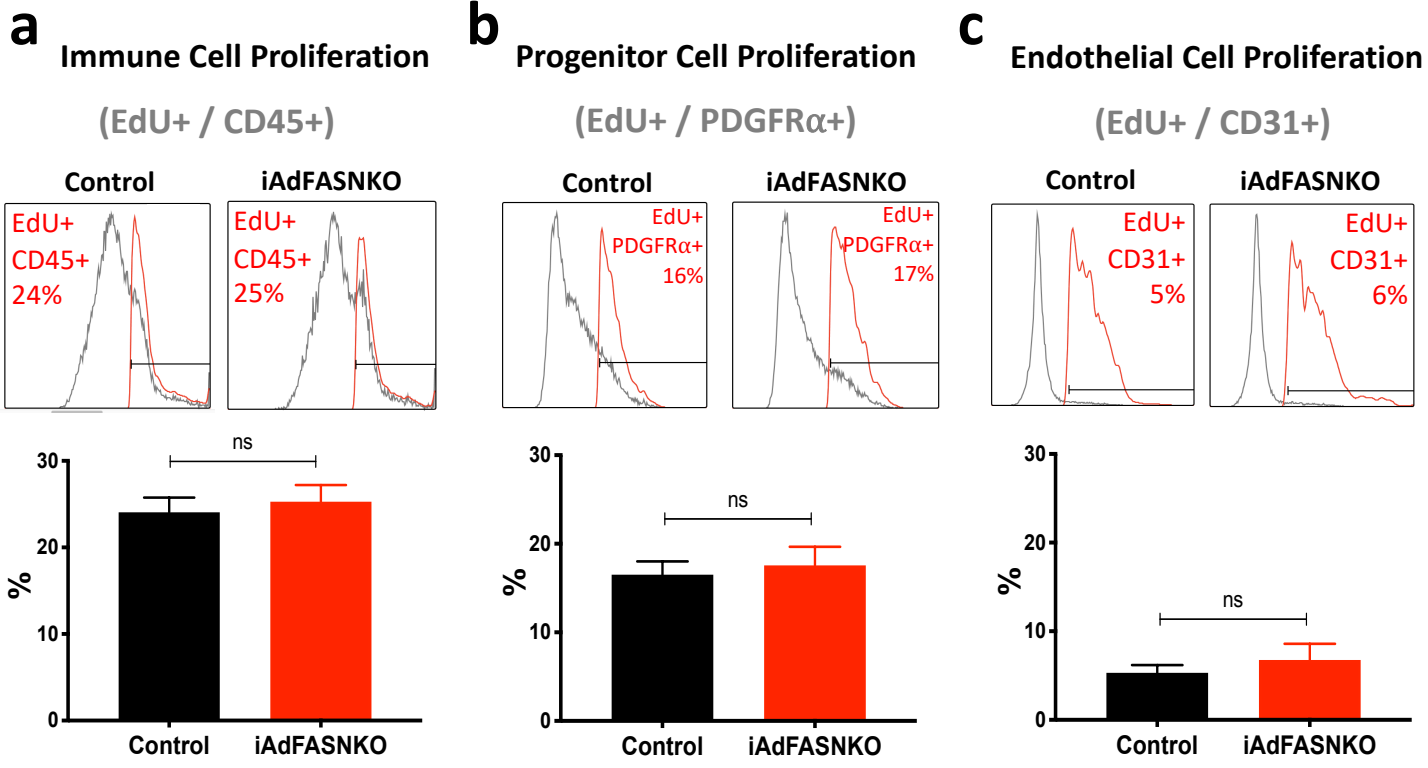
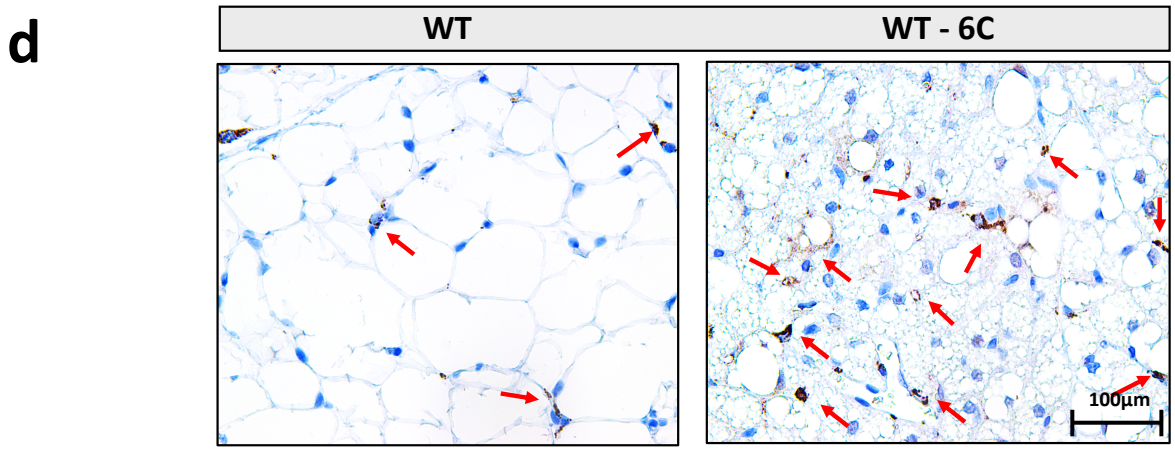

a
F4-80
DAPI
Control
iAdFASNKO
iAdFASNKO+6-OHDA
100μm
b
M1 Polarization
(CD45+ / F4-80+ / CD11b+ / CD206-)
Control
iAdFASNKO
CD206-
67%
CD206-
58%
% CD45+F4/80+CD11b+CD206-
70
60
50
***
Control
iAdFASNKO
c
M2 Polarization
(CD45+ / F4-80+ / CD11b+ / CD206+)
Control
iAdFASNKO
CD206+
33%
CD206+
41%
% CD45+F4/80+CD11b+CD206+
50
40
30
***
Control
iAdFASNKO
d
M2 / M1 ratio
1.0
0.8
0.6
0.4
***
Control
iAdFASNKO
e
% CD45+F4/80+CD11b+
100
80
60
40
20
0
ns
Control
iAdFASNKO

Figure S6

Figure S6: FACS analysis confirms the enrichment of M2-type macrophage populations in the iWAT of iAdFASNKO mice. (a): Representative images for immunofluorescent staining of paraffin-embedded tissue sections for pan-macrophage marker F4/80 (red), DAPI (blue) and bright-field (BF, grey) in the iWAT from the different experimental groups (Control, iAdFASNKO and iAdFASNKO+6-OHDA). Yellow arrows indicate cell F4/80+ cells located in the browning area (multilocular adipocytes) of iWAT from the iAdFASNKO and iAdFASNKO+6-OHDA group. **(b-c):** Stromal vascular fractions (SVF) were isolated from iWAT by collagenase digestion for each different group. Flow cytometric analysis of SVF was conducted using fluorescent-conjugated antibodies against CD45, F4/80, CD11b and CD206. Adipose tissue macrophages were defined as $CD45^{+}F4/80^{+}CD11b^{+}$. **(b)** M1 and **(c)** M2 macrophages were defined as $CD45^{+}F4/80^{+}CD11b^{+}CD206^{-}$ and $CD45^{+}F4/80^{+}CD11b^{+}CD206^{+}$, respectively. **(d)** M2/M1 adipose tissue macrophage ratio in the iWAT from control vs. iAdFASNKO. **(e)** The total number of macrophages was defined as $CD45^{+}F4/80^{+}CD11b^{+}$. Representative results of flow cytometry are shown. N = 4 per group. Graphs show the mean ± SEM. Two-way ANOVA determined statistical significance, ***$P < 0.001$.


a
Immune Cell Proliferation
(EdU+ / CD45+)
Control
iAdFASNKO
EdU+
CD45+
24%
EdU+
CD45+
25%
ns
%
30
20
10
0
Control
iAdFASNKO
b
Progenitor Cell Proliferation
(EdU+ / PDGFRα+)
Control
iAdFASNKO
EdU+
PDGFRα+
16%
EdU+
PDGFRα+
17%
ns
%
30
20
10
0
Control
iAdFASNKO
c
Endothelial Cell Proliferation
(EdU+ / CD31+)
Control
iAdFASNKO
EdU+
CD31+
5%
EdU+
CD31+
6%
ns
%
30
20
10
0
Control
iAdFASNKO


d
WT
WT - 6C
100μm

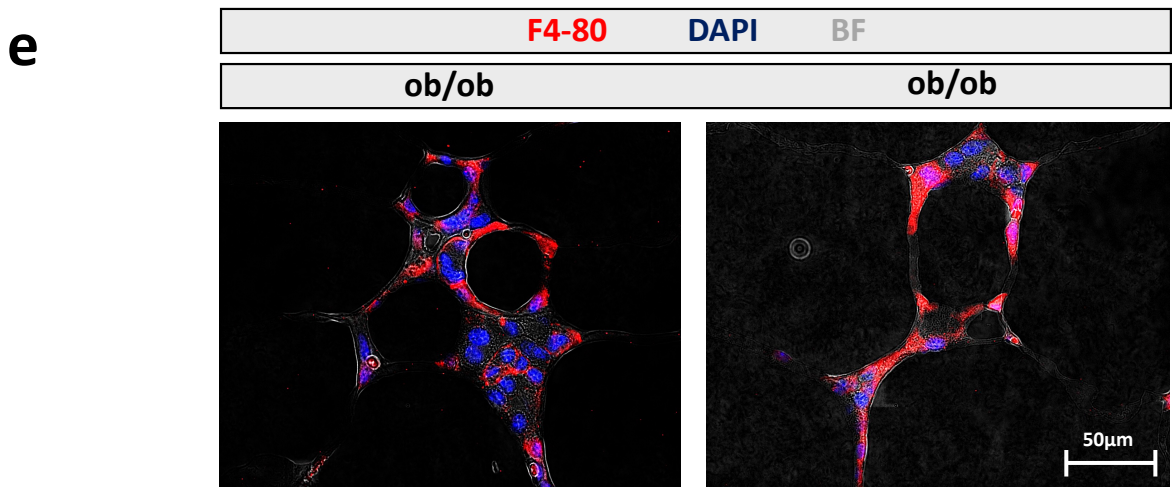

e
F4-80
DAPI
BF
ob/ob
ob/ob
50μm

Figure S7

Figure S7: Cell proliferation analyses and immune cell infiltration, related to Figure 6. (a-c) The Click-iT® EdU assay and flow cytometric analysis were conducted to measure the proliferation ratios of different types of cells present in the SVF derived from iWAT. Immune cells were defined as CD45+, progenitor cells as PDGFRα^+ and endothelial cells as CD31+. Representative results of flow cytometry are shown. N = 4 per group. Graphs show the mean ± SEM. ns = not significant. **(d)** Immunohistochemistry for detection of F4/80 in the iWAT from wild-type mice housed at 22°C or 6°C. Red arrows indicated the presence of F4/80 positive cells in between the multilocular cells (beige cells). Bars are indicated in the panel. **(e)** Validation of the anti-F4/80 antibody. Immunofluorescent analysis to detect F4/80 signals in eWAT from ob/ob mice. Positive control for enhanced macrophage infiltration in iWAT, confirming the specificity of the anti-F4/80 antibody used. Scale bars are provided in the panel.

GENE	Forward	Reverse
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Fasn	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
Cidea	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
Gsα	ACAAGCAGGTCTACCGGGCC	CTCCGTTAAACCCATTAACATGCA
Nrg4	CACGCTGCGAAGAGGTTTTTC	CGCGATGGTAAGAGTGAGGA
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
CD206	CTCTGTTCAGCTATTGGACGC	TGGCACTCCCAAACATAATTTGA
CD11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTCA
CD68	TGTCTGATCTTGCTAGGACCG	GAGAGTAACGGCCTTTTTGTGA
36B4	TCCAGGCTTTGGGCATCA	CTTTATCAGCTGCACATCACTCAGA
β*2m*	CATGGCTCGCTCGGTGAC	CAGTTCAGTATGTTCGGCTTCC
18S	CGAACGTCTGCCCTATCAACTT	CCGGAATCGAACCCTGATT

Table S1

Table S1: Primer sequences used for qRT-PCR.