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Supplemental information

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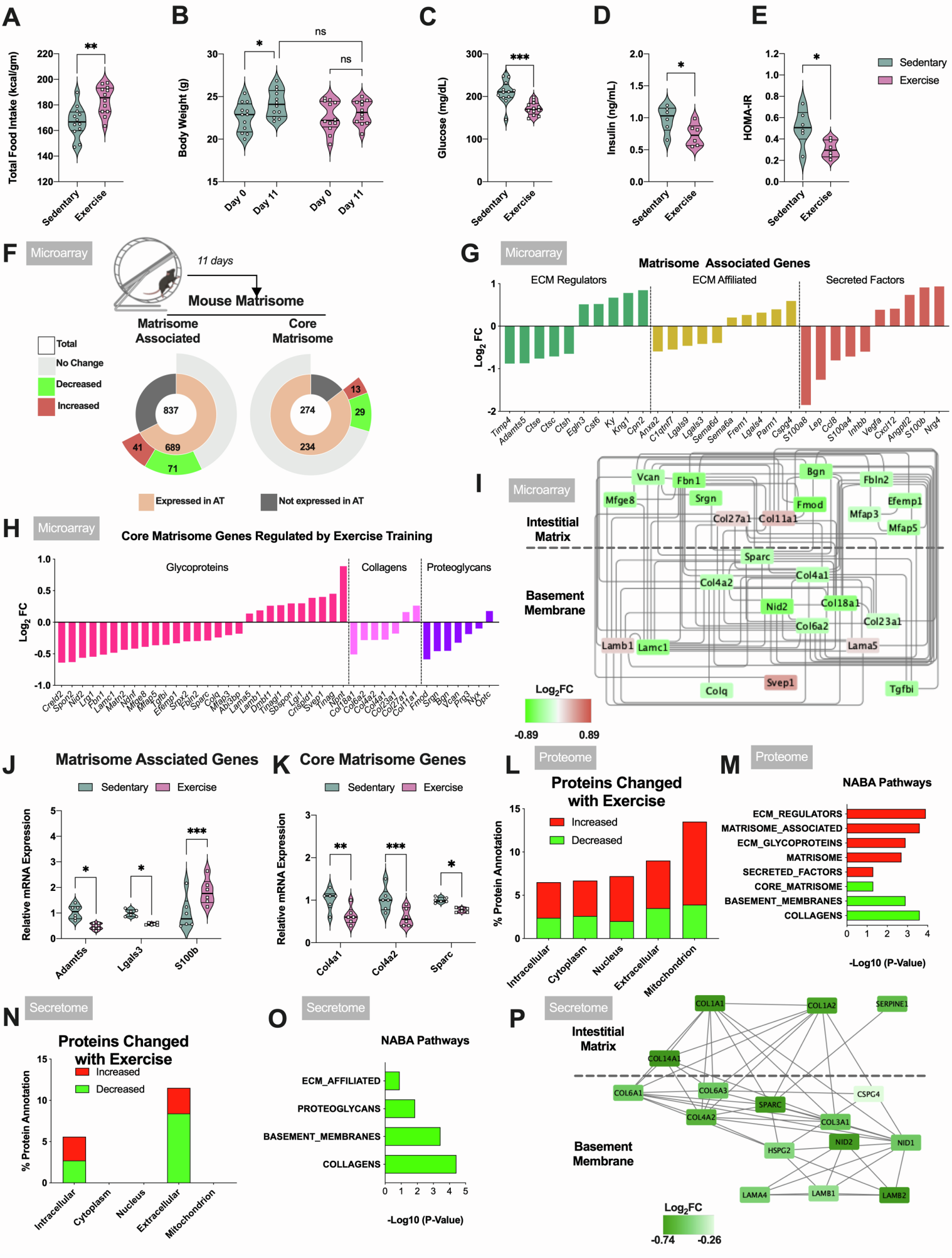
Exercise Training Remodels Inguinal White Adipose Tissue Through Adaptations in Innervation, Vascularization and the Extracellular Matrix

Pasquale Nigro[17], Maria Vamvini[1,2,7], Jiekun Yang[3], Tiziana Caputo[1,3], Li-Lun Ho[3], Nicholas P. Carbone[1], Danae Papadopoulos[3], Royce Conlin[1], Jie He[1], Michael F. Hirshman[1], Joseph D. White[4], Jacques Robidoux[4], Robert C. Hickner[4,5], Søren Nielsen[6], Bente K. Pedersen[6], Manolis Kellis[3], Roeland J. W. Middelbeek[1,2] and Laurie J. Goodyear[1,8,9*.]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Total Food Intake (kcal/gm)
Sedentary
Exercise
B
Body Weight (g)
Day 0
Day 11
ns
C
Glucose (mg/dL)
D
Insulin (ng/mL)
E
HOMA-IR
Sedentary
Exercise
F
Microarray
11 days
Mouse Matrisome
Matrisome Associated
Core Matrisome
Total
No Change
Decreased
Increased
837
689
41
71
274
234
13
29
Expressed in AT
Not expressed in AT
G
Microarray
Matrisome Associated Genes
ECM Regulators
ECM Affiliated
Secreted Factors
Log2 FC
Timp4 Adamts5 Ctse Ctsc Ctsh Eglin3 Csf6 Ky Kng1 Cpn2 Anxa2 C1qtnf7 Lgals9 Lgals3 Sema6d Sema6a Frem1 Lgals4 Parm1 Cspg4 S100a8 Lep Ccl8 S100a4 Inhbb Vegfa Cxcl12 Angptl2 S100b Nrg4
H
Microarray
Core Matrisome Genes Regulated by Exercise Training
Glycoproteins
Collagens
Proteoglycans
Log2 FC
Creld2 Spon2 Nid2 Lrg1 Fbn1 Lamc1 Matn2 Ndnf Mfge8 Mfap5 Tgfbi Efemp1 Srpx2 Fblin2 Sparc Colq Mfap3 Abi3bp Lama5 Lamb1 Dmbt1 Tinagl1 Sbspon Lgi1 Crispld1 Svep1 Tinag Npnt Col18a1 Col6a2 Col4a2 Col4a1 Col23a1 Col27a1 Col11a1 Fmod Srgn Bgn Vcan Prg3 Nyx Optc
I
Microarray
Intestitial Matrix
Basement Membrane
Vcan
Fbn1
Bgn
Fbln2
Mfge8
Srgn
Fmod
Efemp1
Col27a1
Col11a1
Mfap3
Mfap5
Sparc
Col4a1
Col4a2
Nid2
Col18a1
Col6a2
Col23a1
Lamb1
Lamc1
Lama5
Svep1
Colq
Tgfbi
Log2FC
-0.89
0.89
J
Matrisome Assciated Genes
Sedentary
Exercise
Relative mRNA Expression
Adamts5
Lgals3
S100b
K
Core Matrisome Genes
Sedentary
Exercise
Relative mRNA Expression
Col4a1
Col4a2
Sparc
L
Proteome
Proteins Changed with Exercise
Increased
Decreased
% Protein Annotation
Intracellular
Cytoplasm
Nucleus
Extracellular
Mitochondrion
M
Proteome
NABA Pathways
ECM_REGULATORS
MATRISOME_ASSOCIATED
ECM_GLYCOPROTEINS
MATRISOME
SECRETED_FACTORS
CORE_MATRISOME
BASEMENT_MEMBRANES
COLLAGENS
-Log10 (P-Value)
N
Secretome
Proteins Changed with Exercise
Increased
Decreased
% Protein Annotation
Intracellular
Cytoplasm
Nucleus
Extracellular
Mitochondrion
O
Secretome
NABA Pathways
ECM_AFFILIATED
PROTEOGLYCANS
BASEMENT_MEMBRANES
COLLAGENS
-Log10 (P-Value)
P
Secretome
Intestitial Matrix
Basement Membrane
COL1A1
COL1A2
SERPINE1
COL14A1
COL6A1
COL6A3
CSPG4
SPARC
COL4A2
COL3A1
NID2
NID1
HSPG2
LAMA4
LAMB1
LAMB2
Log2FC
-0.74
-0.26

Figure S1 Systemic phenotypic effects of 11 days of exercise training on C57BL/6 male mice, with qPCR data for ECM genes, related to Figure 1.

(A-C) Total food consumption (A), body weight change (B), and glucose (C) for all sedentary and trained mice used in the study (*n=12/group*).

(D-E) Serum insulin level (D) and insulin resistance index HOMA-IR (E) for a sub cohort of n=6/group mice.

(F) Overview of exercise training effect on mouse matrisome gene categories in iWAT using microarray dataset (GSE68161).

(G) List of top 10 matrisome associated genes changing with exercise training versus sedentary mice ($p<0.05$) divided in subcategories: ECM regulators (*green*), ECM affiliated (*yellow*) and secreted factors (*red*).

(H) List of core matrisome genes changing with training ($p<0.05$) divided in subcategories: glycoproteins (*magenta*), collagens (*pink*), and proteoglycans (*purple*).

(I) Protein-protein interaction (PPI) networks analysis for iWAT matrisome genes changed with exercise training.

(J-K) mRNA expression level for matrisome associated genes (J) and core matrisome genes (K)(*n=6/group*).

(L) Categorization of identified proteins in proteome modulated by exercise training using GO Cellular Component terms.

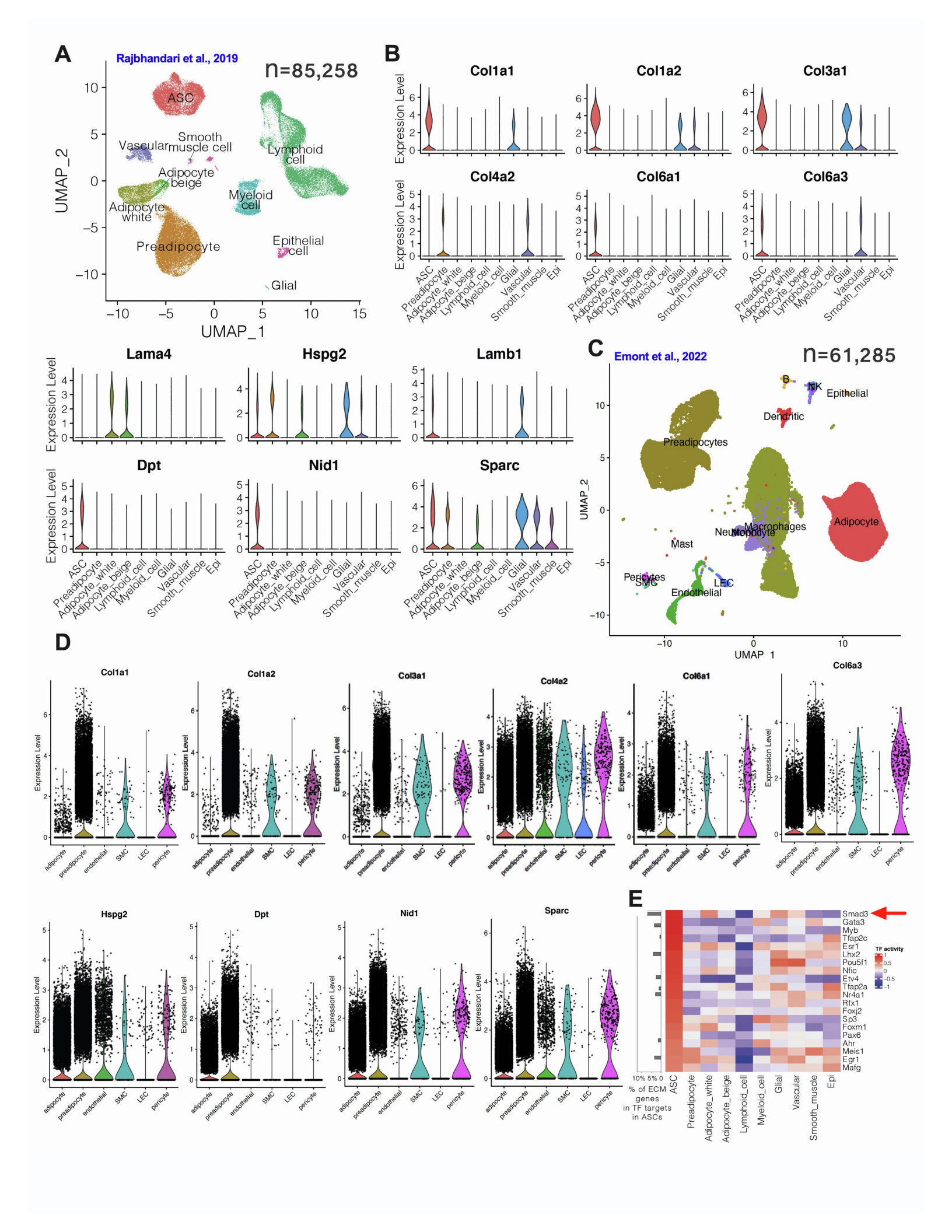
(M) Pathway analysis for the exercise-regulated proteins using the matrisome gene sets available in the Molecular Signature Database (MSigDB v5.0).

(N) Categorization of identified proteins in secretome modulated by exercise training using GO Cellular Component terms.

(O) Pathway analysis for the exercise-regulated proteins using the matrisome gene sets.

(P) Protein-protein interaction (PPI) networks analysis for iWAT conditioned media secreted proteins that are decreased with exercise training.

Data are presented as mean ± SEM and were compared using unpaired two-tailed Student's t test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.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Rajbhandari et al., 2019
n=85,258
ASC
Smooth muscle cell
Vascular
Lymphoid cell
Adipocyte beige
Myeloid cell
Adipocyte white
Preadipocyte
Epithelial cell
Glial
UMAP_1
UMAP_2
B
Col1a1
Col1a2
Col3a1
Col4a2
Col6a1
Col6a3
Expression Level
ASC
Preadipocyte
Adipocyte_white
Adipocyte_beige
Lymphoid_cell
Myeloid_cell
Glial
Vascular
Smooth_muscle
Epi
Lama4
Hspg2
Lamb1
Dpt
Nid1
Sparc
C
Emont et al., 2022
n=61,285
B
NK
Epithelial
Dendritic
Preadipocytes
Adipocyte
Macrophages
Neutrophil
Mast
Pericytes
SMC
LEC
Endothelial
UMAP_1
UMAP_2
D
Col1a1
Col1a2
Col3a1
Col4a2
Col6a1
Col6a3
Hspg2
Dpt
Nid1
Sparc
Expression Level
adipocyte
preadipocyte
endothelial
SMC
LEC
pericyte
E
Smad3
Gata3
Myb
Tfap2c
Esr1
Lhx2
Pou5f1
Nfic
Etv4
Tfap2a
Nr4a1
Rfx1
Foxj2
Sp3
Foxm1
Pax6
Ahr
Meis1
Egr1
Mafg
TF activity
% of ECM genes in TF targets in ASCs
ASC
Preadipocyte
Adipocyte_white
Adipocyte_beige
Lymphoid_cell
Myeloid_cell
Glial
Vascular
Smooth_muscle
Epi

Figure S2 Unsupervised analysis of two independent single-cell transcriptomics datasets, related to Figure 2.

(A) Unsupervised clustering of 85,258 cells from the iWAT of pooled male C57BL/6 mice. 10 distinct cell groups were represented on a UMAP plot.

(B) Individual violin plots showing the expression levels and distribution of representative genes for the core matrisome proteins decreased with exercise training detected in iWAT conditioned media (secretome analysis). The y-axis is the log-scale normalized read count.

(C) Unsupervised clustering of 61,285 cells from the iWAT of pooled male C57BL/6 mice. 13 distinct cell groups were represented on a UMAP plot.

(D) Individual violin plots showing the expression levels and distribution of representative genes for the core matrisome proteins decreased with exercise training detected in iWAT conditioned media (secretome analysis). The y-axis is the log-scale normalized read count.

(E) Inferred TF activity based on target gene expression levels across cell types identified in the single-cell data presented in panel (A). The TFs were ranked by their activity levels in ASCs. The percentage of ECM genes in the targets of the individual TFs was plotted to the left of the heatmap.

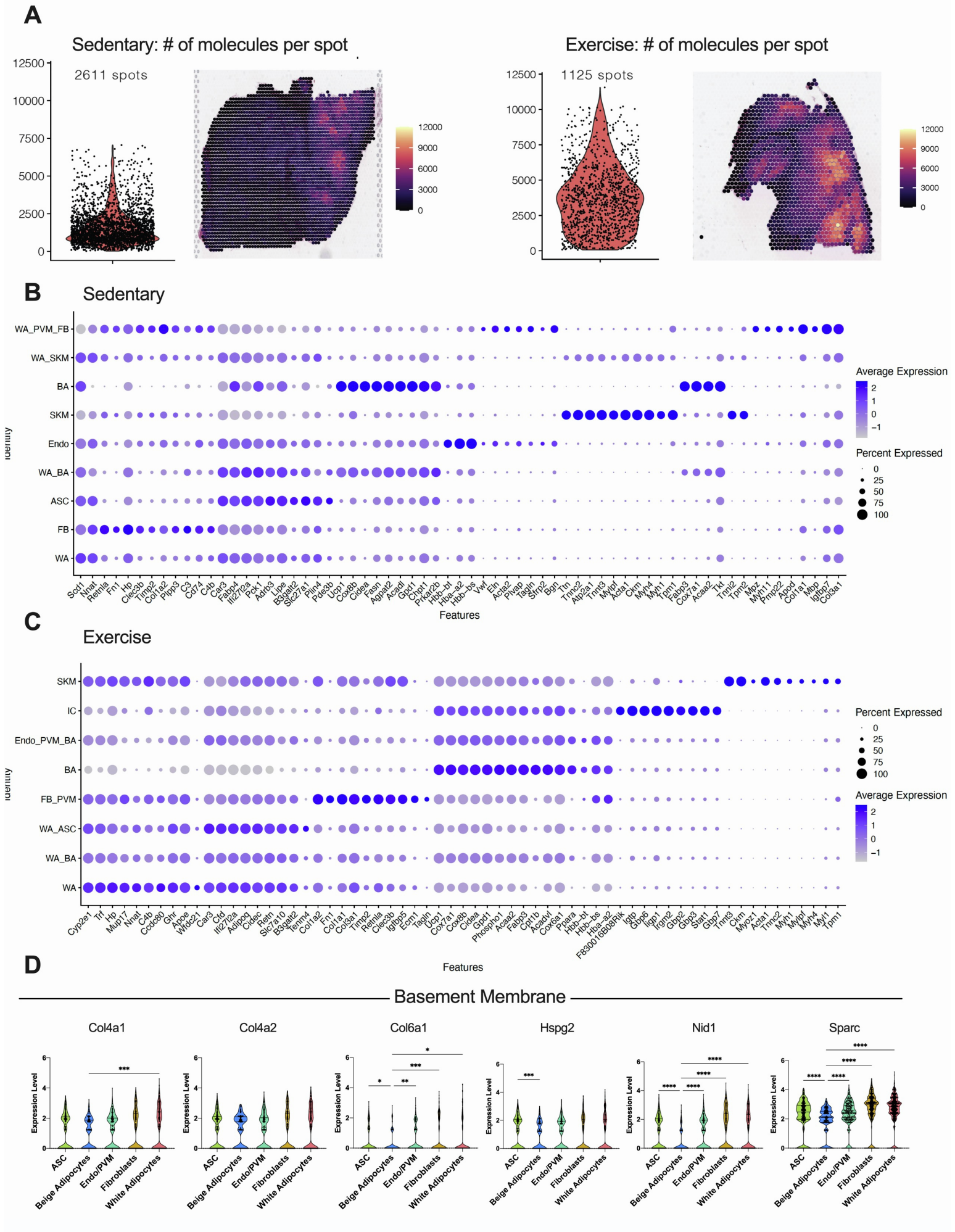

A
Sedentary: # of molecules per spot
2611 spots
Exercise: # of molecules per spot
1125 spots
B
Sedentary
C
Exercise
Identity
Features
Average Expression
Percent Expressed
D
Basement Membrane
Col4a1
Col4a2
Col6a1
Hspg2
Nid1
Sparc
Expression Level
ASC
Beige Adipocytes
Endo/PVM
Fibroblasts
White Adipocytes

Figure S3 Spatial transcriptomics analysis of iWAT from sedentary and exercise training mice, related to Figure 2.

(A) Violin plots showing the variance in molecular counts across spots and the total number of spots counted in iWAT from sedentary (*left*) and exercise training (*right*) mice. (B-C) Dotplot visualization listing cell clusters identified with spatial transcriptomics on iWAT from sedentary (B) and exercise training (C) mice. Cell clusters listed on the y-axis, showing unbiased gene expression for the top genes per cluster identified by log fold change; genes (features) are listed along the x-axis. Dot size reflects percentage of cells in a cluster expressing each gene; dot color reflects expression level (as indicated in the legend).

(D) Individual violin plots showing the expression level for basement membrane collagens species across 5 selected cell type clusters. Data are presented as mean ± SEM and were compared using One-way ANOVA. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

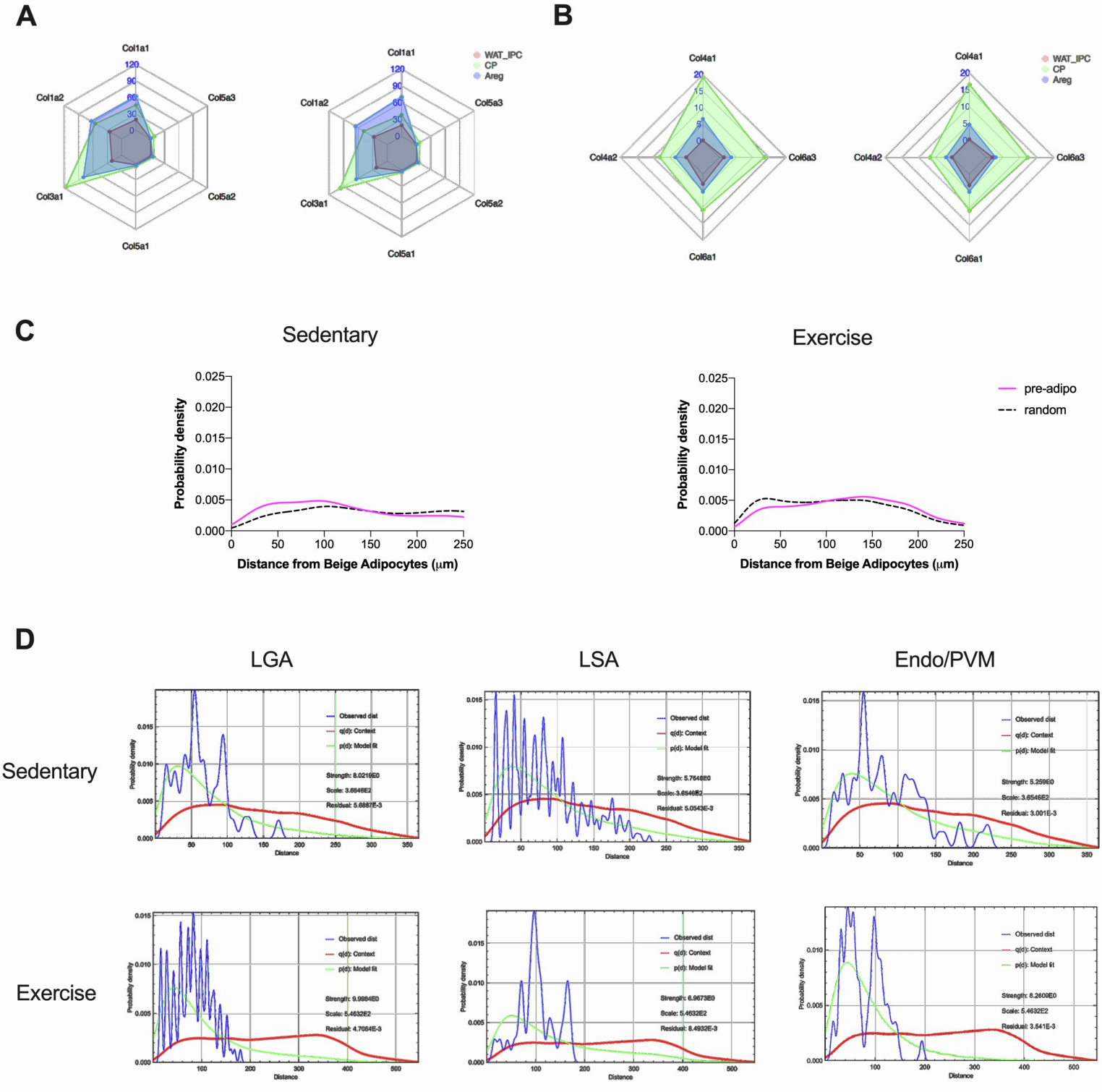

A
Col1a1
Col5a3
Col5a2
Col5a1
Col3a1
Col1a2
WAT_IPC
CP
Areg
B
Col4a1
Col6a3
Col6a1
Col4a2
C
Sedentary
Exercise
Probability density
Distance from Beige Adipocytes (μm)
pre-adipo
random
D
LGA
LSA
Endo/PVM
Sedentary
Exercise
Observed dist
q(d): Context
p(d): Model fit
Strength: 8.0219E0
Scale: 3.6546E2
Residual: 5.6887E-3
Strength: 5.7648E0
Residual: 5.0543E-3
Strength: 5.259E0
Residual: 3.001E-3
Strength: 9.9984E0
Scale: 5.4632E2
Residual: 4.7064E-3
Strength: 6.9673E0
Residual: 8.4932E-3
Strength: 8.2609E0
Residual: 3.541E-3
Distance

Figure S4 Spatial transcriptomics analysis of iWAT from sedentary and exercise training mice, related to Figure 2.

(A) Interstitial matrix components' gene expression level in the three different ASC subclusters (CP, ICP and Areg). The gene expression level is presented as a scale ranging from zero to 120, where 120 represents the highest expression level.

(B) Basement membrane components' gene expression level in the three different ASC subclusters (CP, ICP and Areg). The gene expression level is presented as a scale ranging from zero to 20, where 20. represents the highest expression level.

(C) Probability density distribution plots showing the distance relative to the beige adipocyte from preadipocytes (pre-adipo) in iWAT from sedentary (*left*) and exercise training (*right*) mice. Dashed black lines indicate the random distribution among the clusters.

(D) Interaction analysis for LGA, LSA and Endo/PVM clusters in iWAT from sedentary (*top*) and exercise training (*bottom*). The plots show the observed nearest-neighbor (NN) distance distribution (*blue*) between two arbitrary point patterns, the model fits (*green*) to the observed NN distance distribution and the random context distribution (*red*).

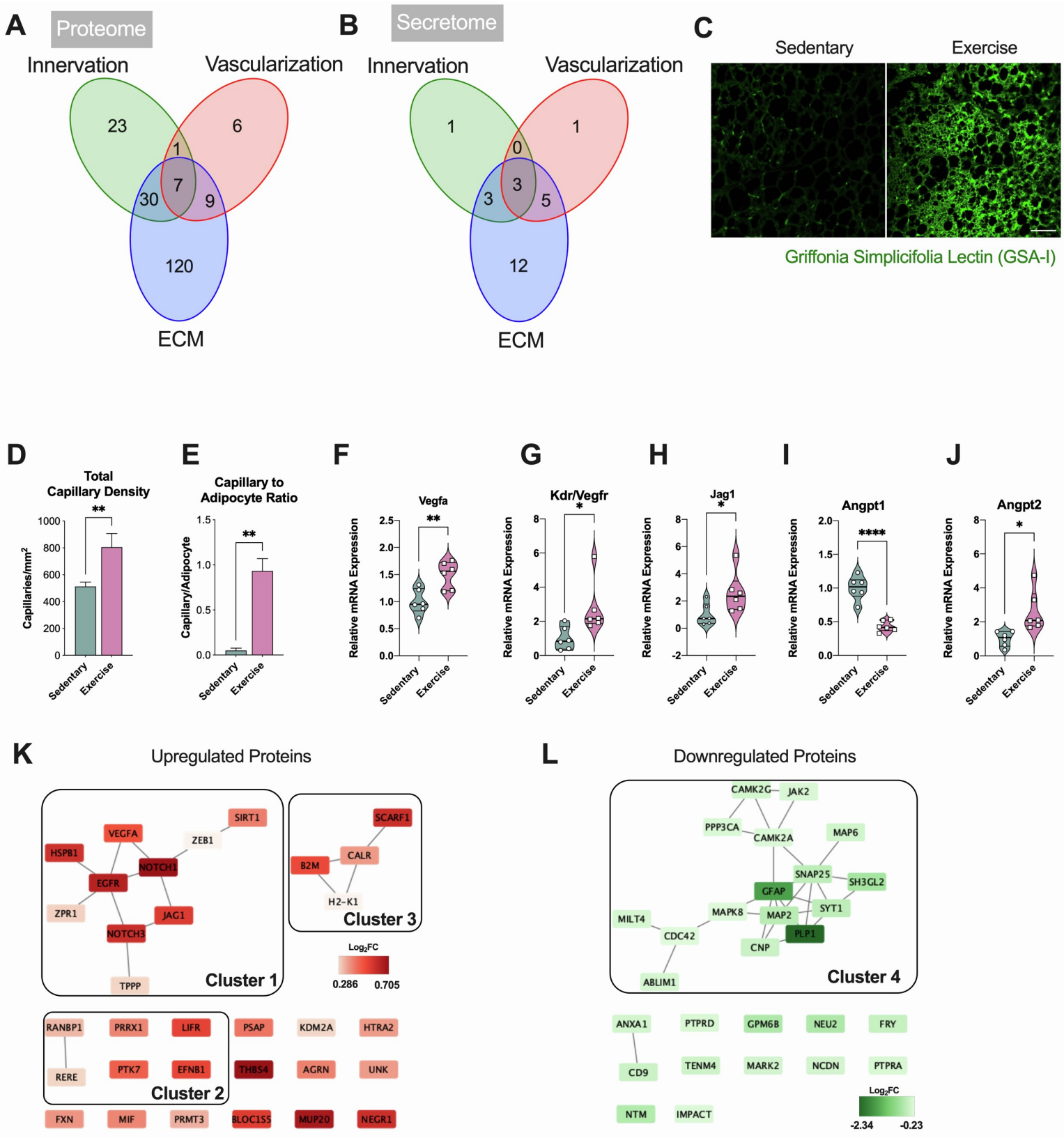

A
Proteome
Innervation
Vascularization
23
6
1
7
30
9
120
ECM
B
Secretome
Innervation
Vascularization
1
1
0
3
3
5
12
ECM
C
Sedentary
Exercise
Griffonia Simplicifolia Lectin (GSA-I)
D
Total Capillary Density
Capillaries/mm²
Sedentary
Exercise
E
Capillary to Adipocyte Ratio
Capillary/Adipocyte
F
Vegfa
Relative mRNA Expression
G
Kdr/Vegfr
H
Jag1
I
Angpt1
J
Angpt2
K
Upregulated Proteins
SIRT1
VEGFA
ZEB1
HSPB1
NOTCH1
EGFR
ZPR1
JAG1
NOTCH3
TPPP
Cluster 1
SCARF1
CALR
B2M
H2-K1
Cluster 3
Log2FC
0.286
0.705
RANBP1
PRRX1
LIFR
PSAP
KDM2A
HTRA2
RERE
PTK7
EFNB1
THBS4
AGRN
UNK
Cluster 2
FXN
MIF
PRMT3
BLOC1S5
MUP20
NEGR1
L
Downregulated Proteins
CAMK2G
JAK2
PPP3CA
CAMK2A
MAP6
SNAP25
SH3GL2
GFAP
SYT1
MAPK8
MAP2
MILT4
CDC42
PLP1
CNP
ABLIM1
Cluster 4
ANXA1
PTPRD
GPM6B
NEU2
FRY
CD9
TENM4
MARK2
NCDN
PTPRA
NTM
IMPACT
Log2FC
-2.34
-0.23

Figure S5 ECM proteins are key components for vasculature and innervation remodeling in iWAT, related to Figure 4 and 5.

(A-B) Venn diagram illustrating quantified proteins regulated by exercise training in proteome (A) and secretome (B) datasets, annotated as members of innervation, vascularization and ECM pathways.

(C) Representative images of iWAT from sedentary and trained mice stained with Griffonia Simplicifolia Lectin (GSA-I). Scale bar: 50 μm

(D-E) Quantification of capillary density (D) and capillary per adipocyte ratio (E) in iWAT from sedentary and trained mice (*n=6/group; calculated from 10 fields/mouse*).

(F-J) Relative mRNA expression of pro-angiogenic markers *Vegfa* (F), *Kdr/Vegfr* (G), *Jag1*(H), *Angpt1*(I), *Angpt2*(J) in iWAT from sedentary and trained mice (*n=6/group*).

(K-L) Protein-protein interaction (PPI) networks analysis for iWAT proteins up-regulated (K) and down-regulated (L) with exercise training detected in proteome dataset and annotated in the innervation pathway.

Data are presented as mean ± SEM and were compared using unpaired two-tailed Student's t test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.0001$.

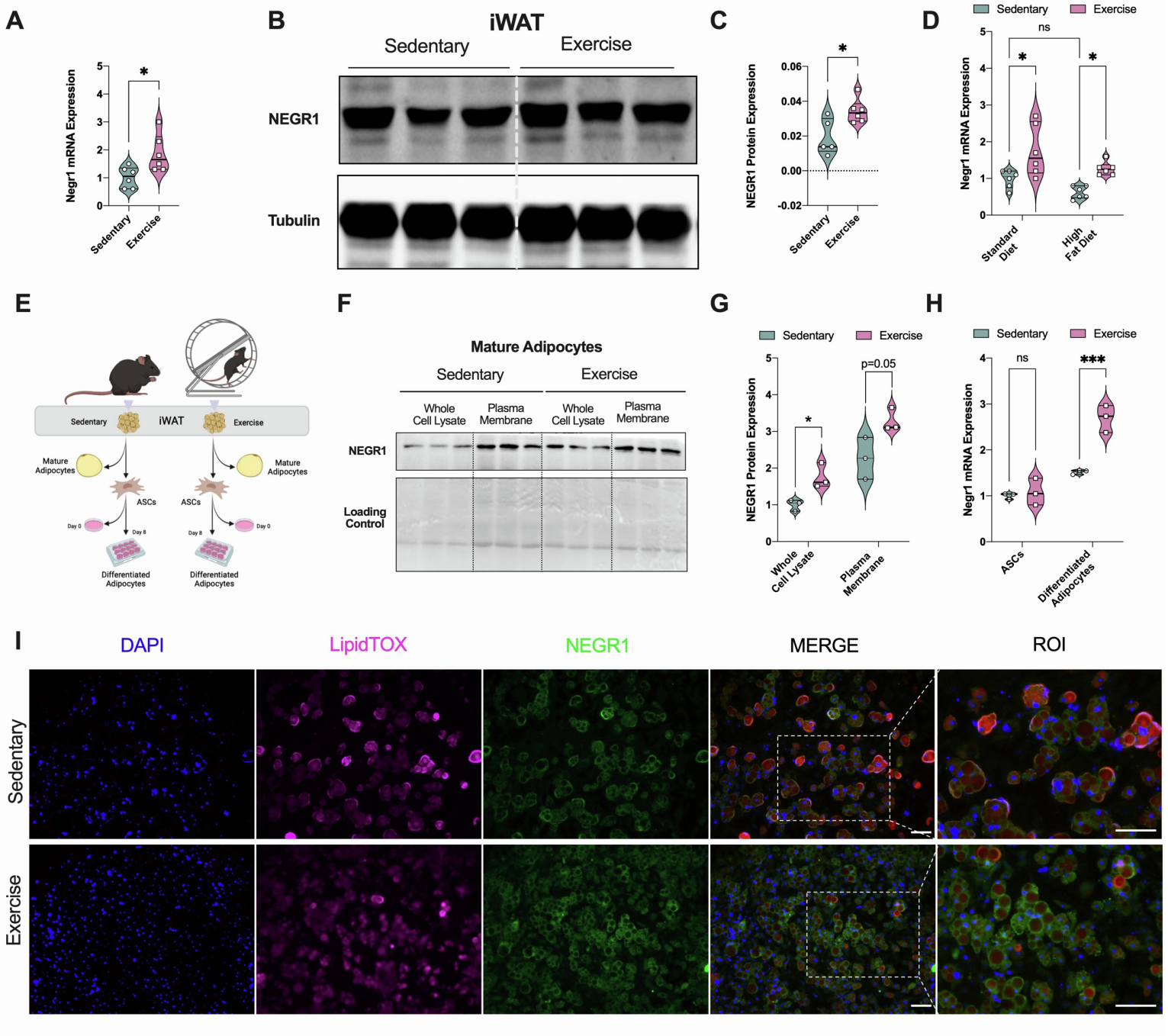

A
Negr1 mRNA Expression
Sedentary
Exercise
B
iWAT
Sedentary
Exercise
NEGR1
Tubulin
C
NEGR1 Protein Expression
Sedentary
Exercise
D
Sedentary
Exercise
ns
Negr1 mRNA Expression
Standard Diet
High Fat Diet
E
Sedentary
iWAT
Exercise
Mature Adipocytes
ASCs
Day 0
Day 8
Differentiated Adipocytes
F
Mature Adipocytes
Sedentary
Exercise
Whole Cell Lysate
Plasma Membrane
NEGR1
Loading Control
G
Sedentary
Exercise
p=0.05
NEGR1 Protein Expression
Whole Cell Lysate
Plasma Membrane
H
Sedentary
Exercise
ns
***
Negr1 mRNA Expression
ASCs
Differentiated Adipocytes
I
DAPI
LipidTOX
NEGR1
MERGE
ROI
Sedentary
Exercise

Figure S6 NEGR1 is expressed in mature adipocytes.

(A) Relative mRNA expression of *Negr1* in iWAT from sedentary and trained mice (*n=6/group*).

(B-C) Representative images of NEGR1 protein by western blot (B) in sedentary and trained mice with relative quantification (C) (*n=6/group*).

(D) Relative mRNA expression of *Negr1* in iWAT from sedentary and trained mice under standard diet (20% fat) and high fat diet (60% fat) (*n=6/group*).

(E) Study design to collect fresh mature adipocytes and adipose stem cells (ASCs) from sedentary and trained mice (*n=12/group, 4 mice were pooled to reach n=3*).

(F-G) Representative images of NEGR1 protein by western blot in fresh mature adipocytes isolated from sedentary and trained mice with relative quantification (G)(*n=3/group*).

(H) Relative mRNA expression of *Negr1*in undifferentiated ASCs and differentiated mature adipocytes isolated from sedentary and trained mice (*n=3/group*).

(I) Representative images of differentiated mature adipocytes isolated from sedentary and trained mice immunostained for NEGR1, DAPI (*nuclei*) and LipidTOX for neutral lipid stain (*n=3/group*). Scale bar: 50 µm (20x).

Data are presented as mean ± SEM and were compared unpaired two-tailed Student's t test and Two-way ANOVA followed by Tukey's multiple comparisons test, $^{*}p < 0.05$, $^{***}p < 0.001$.

A

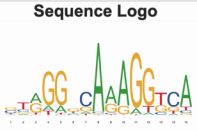
M. Musculus -Negr1

Transcription Factor Motif

Matrix ID:MA0065.2

Name: Pparg::Rxra

Sequence Logo



>FP004797 Negr1_1 :+U EU:NC; range -2000 to 100.

Jaspar Score

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GCTGCAAACCTCTCCGTTAATTGCTGAGCCTCGCAGGGAATCTTCTGCAGGGTGGTGATG
TTGCTTAGAGGCTCCTGGATAGTCAGTCTGGGTGGTCCCGGGTGATGTGAATTGCTGGCC
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GGTACCACCAACATCTATGACTCCTGCCTGCCTCATCAAGAGCTCAGTCTGGGGAGGAAG
CCGGATGCTGACCCCTGCAGAGATCCTTGCTTCAGTGCGCTCTGCCGCGGGGCCCTGCTG
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GCCTGGAAGACCTCTAGGATCTTAGGGTTGAAAATGAGTGCCAGAAAGGGGGTGGGGGGG
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TTTAAAGCCGAGGAATGCCTTGGCTTCAACCGAATGCGCAAGCCTTCCGCCCAGTAGGCT
CAAATGGGTTAAACTGCCTCTTACATAACAGGACCGCCTAGCTCCACACAATTAAAACAC
TGATTGCAGCCTCCTCCAACTCTGCATCCCAGTAAGGCCCGCCCATCCCTCCCGTTGCGC
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GACGCACAGAGAAGGGCAGGCGTTCTCCAGTACGCACAGGCGGACTCACACACGCACTAG
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GGGGAGGGGAGAGGAGAGGAGAGGAGAGGAGAGGAGAAGAGAGAGAAGAGAGAGCTA
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CAACAAAAAAAGCCAACTCACTCAGTCTCAGGCGTCATGATGGCGCCGACCCCAACTTGA
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GCCCTGACTTCCCAGCCCCGCGGCTCGCAAAGCTCTCTAGTTCGCGCTGCCAAGCCGGAC
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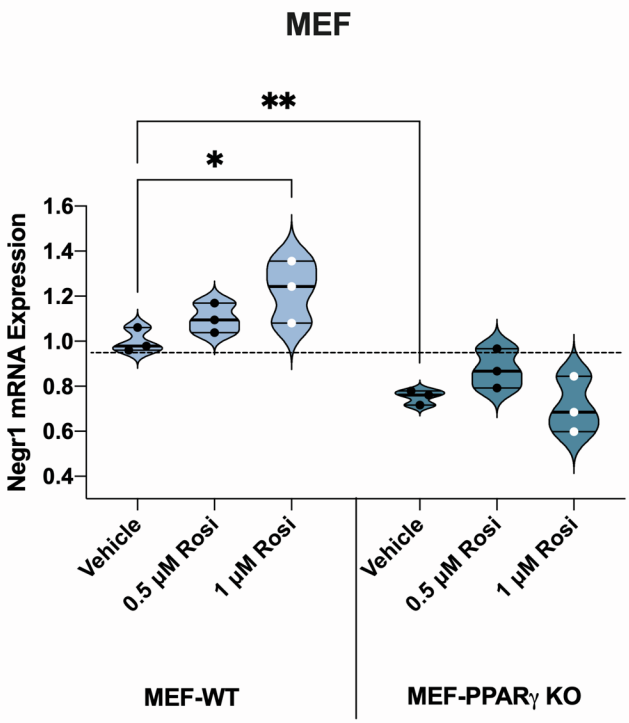
CGCCCCGCGCTGCCTGCGCCGCCTCCCGCGCTCTGCTCGCAGCCTCGCAGGACGGTGTGC
GGCCGCCAGGGATTAGCCACGCTCCGGACGCGGCCGGGCGCCCCGAAGGAGGTGGAGCGC
G

Score	Relative Score
9.371807	0.8233357058458314

Score	Relative Score
9.959607	0.8329075675829608
Score	Relative Score
10.944433	0.8489446868973355

←TSS

B



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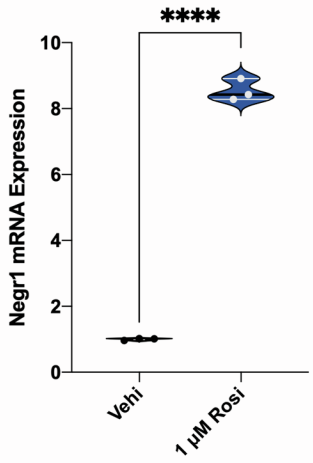

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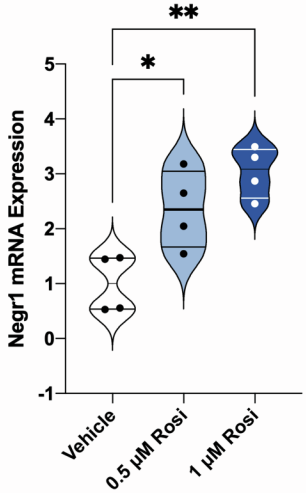


E

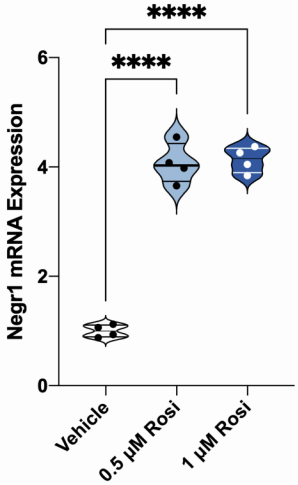


Figure S7 Pparg is the master regulator of NEGR1 expression.

(A)The sequence analysis of murine *Negr1* gene promoter region (-2000bp relative to the transcription start site, TSS). The highlighted regions in blue, green and yellow depict the three high scored consensus sequences for the Pparg::Rxra transcription factor complex.

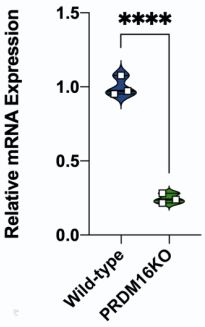
(B)Relative mRNA expression of *Negr1* in wildtype and Pparg KO MEFs treated with and without 0.5 and 1μM rosiglitazone for 24hrs (*n=3/group*).

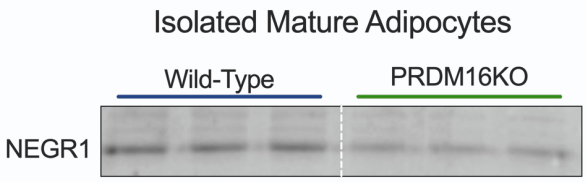
(C)Relative mRNA expression of *Negr1* in fresh mature adipocytes isolated from sedentary mice treated with and without 1μM rosiglitazone for 24hrs (*n=12/group, 4 mice were pooled to reach n=3*).

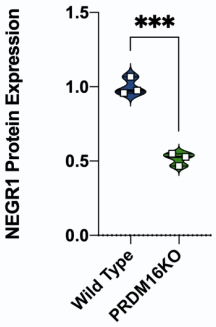
(D)Relative mRNA expression of *Negr1* in ASCs isolated from sedentary mice treated with 0.5 and 1μM rosiglitazone for 24hrs (*n=12/group, 3 mice were pooled to reach n=4*)

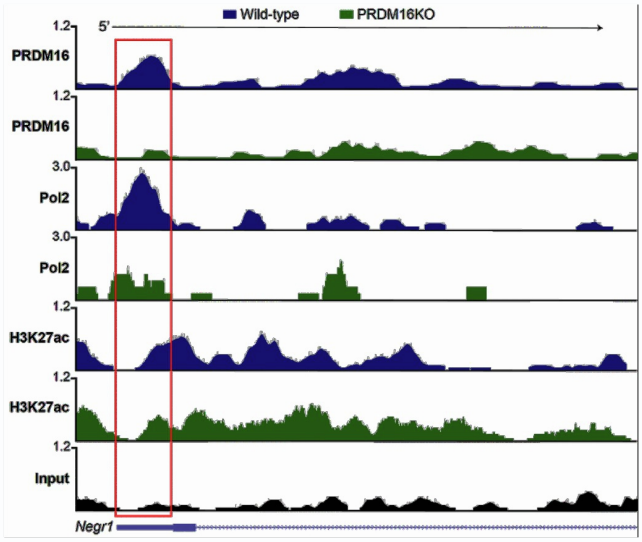
(E) Relative mRNA expression of *Negr1* in differentiated adipocytes for 7 days using 0.5 and 1μM rosiglitazone (*n=12/group, 3 mice were pooled to reach n=4*)

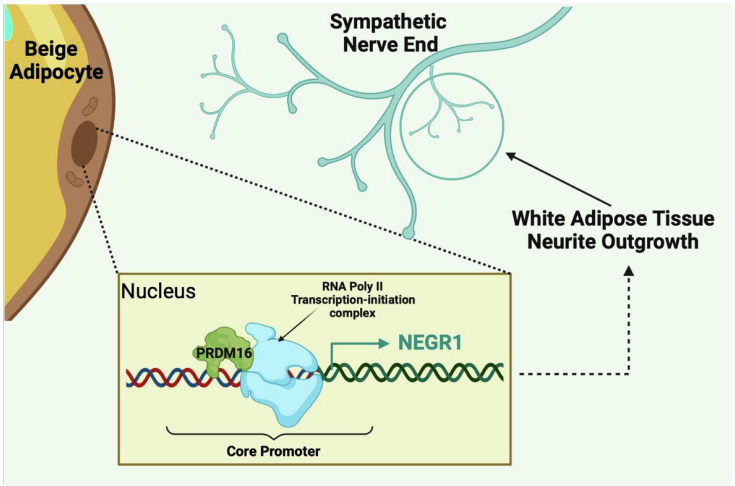
Data are presented as mean ± SEM and were compared using unpaired two-tailed Student's t test and Two-way ANOVA followed by Tukey's multiple comparisons test $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{****}p < 0.0001$.

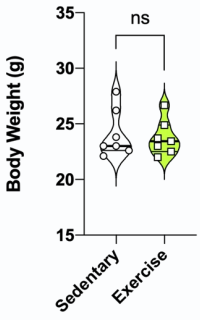

A
Relative mRNA Expression
1.5
1.0
0.5
0.0
****
Wild-type
PRDM16KO

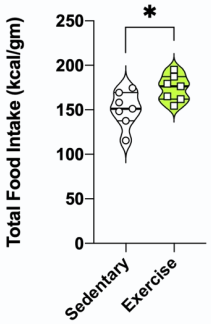

B
Isolated Mature Adipocytes
Wild-Type
PRDM16KO
NEGR1


C
NEGR1 Protein Expression
1.5
1.0
0.5
0.0
***
Wild Type
PRDM16KO


D
ChIP-Seq Data Analysis
(Harms et al. 2015; Kajimura et al. 2008)
Wild-type
PRDM16KO
5'
PRDM16
PRDM16
Pol2
Pol2
H3K27ac
H3K27ac
Input
Negr1


E
Beige Adipocyte
Sympathetic Nerve End
White Adipose Tissue Neurite Outgrowth
Nucleus
RNA Poly II Transcription-initiation complex
PRDM16
NEGR1
Core Promoter


F
Body Weight (g)
35
30
25
20
15
ns
Sedentary
Exercise


G
Total Food Intake (kcal/gm)
250
200
150
100
50
0
*
Sedentary
Exercise


H
Glucose (mg/dL)
400
300
200
100
0
**
Sedentary
Exercise

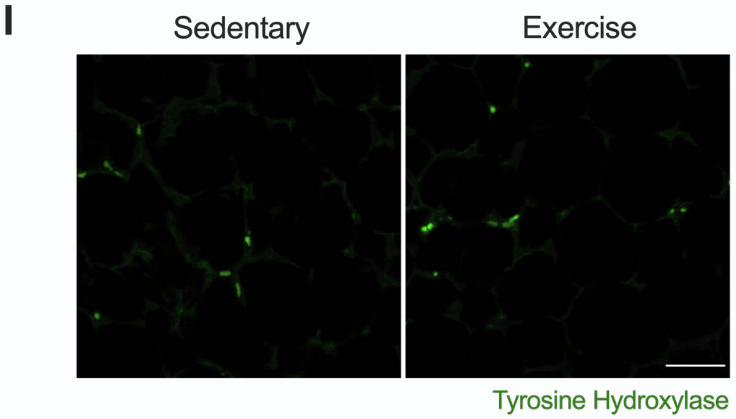

I
Sedentary
Exercise
Tyrosine Hydroxylase

Figure S8 PRDM16 transcriptional complex mediated exercise-induced iWAT remodeling, related to Figure 6.

(A) Relative mRNA expression of *Negr1* in fresh mature adipocytes isolated from wild type and PRDM16KO mice (*n=12/group, 4 mice were pooled to reach n=3*).

(B-C) Representative images of NEGR1 protein by western blot (B) in fresh mature adipocytes isolated from wild type and PRDM16KO mice with relative quantification (C) (*n=12/group, 4 mice were pooled to reach n=3*).

(D) ChIP-seq profiles in reads per million total reads (RPM) for *PRDM16*, *RNA Polymerase II* (Pol2) and *H3K27ac* in iWAT at *Negr1* for wild type (*blue*) and PRDM16 knockout (*green*) mice.

(E) Summary cartoon for the transcriptional regulation of *Negr1* in beige adipocytes and its potential role in the white adipose tissue neurite outgrowth.

(F-H) Body weight (F), total food consumption (G), and glucose (H) for sedentary and trained PRDM16KO mice (*n=6/group*)

(I) Representative immunofluorescence staining images of iWAT for the sympathetic innervation marker Tyrosine Hydroxylase (TH). Scale bar: 50 μm

Data are presented as mean ± SEM and were compared using unpaired two-tailed Student's t test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

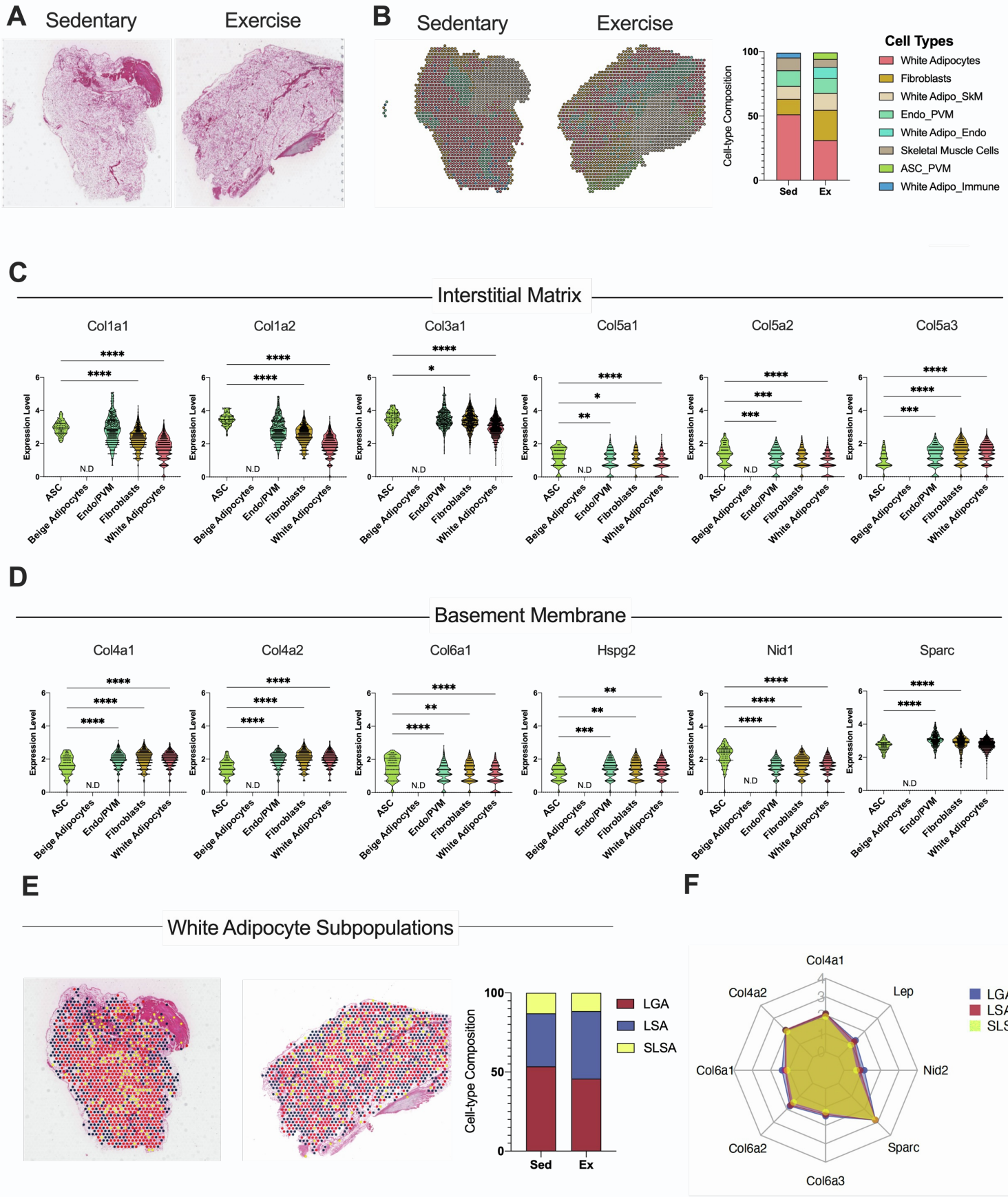

A
Sedentary
Exercise
B
Sedentary
Exercise
Cell-type Composition
100
50
0
Sed
Ex
Cell Types
White Adipocytes
Fibroblasts
White Adipo_SkM
Endo_PVM
White Adipo_Endo
Skeletal Muscle Cells
ASC_PVM
White Adipo_Immune
C
Interstitial Matrix
Col1a1
Col1a2
Col3a1
Col5a1
Col5a2
Col5a3
Expression Level
N.D
ASC
Beige Adipocytes
Endo/PVM
Fibroblasts
White Adipocytes
D
Basement Membrane
Col4a1
Col4a2
Col6a1
Hspg2
Nid1
Sparc
E
White Adipocyte Subpopulations
LGA
LSA
SLSA
F
Col4a1
Lep
Nid2
Sparc
Col6a3
Col6a2
Col6a1
Col4a2

Figure S9 Spatial transcriptomics analysis of iWAT from sedentary and exercise training PRDM16KO mice, related to Figure 6.

(A-B) Magnified view of H&E-stained sections (A) and relative spatial RNA-seq barcoded spot (B) indicating the cell clusters detected in iWAT from sedentary (*left*) and exercise training (*right*) PRDM16KO mice. Scale bar: 2 mm

(C) Individual violin plots showing the expression level for interstitial matrix collagens species across 5 selected cell type clusters.

(D) Individual violin plots showing the expression level for basement membrane collagens species across 5 selected cell type clusters.

(E) Spatial distribution maps showing the three white adipocyte subpopulations lipid-scavenging adipocytes (LSA), stressed lipid-scavenging adipocytes (SLSA) and lipogenic adipocytes (LGA) detected in iWAT from sedentary (*left*) and exercise training (*right*) PRDM16KO mice along with the relative proportion plot.

(F) Basement membrane components gene expression level in LSA and LGA white adipocyte subpopulations. The gene expression level is presented as a scale ranging from zero to four, where four represents the highest expression level.

Data are presented as mean ± SEM and were compared using One-way ANOVA. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.