Meflin defines mesenchymal stem cells and/or their early progenitors with multilineage differentiation capacity across multiple tissues

Hara *et al*.

Supporting Information

Figure S1

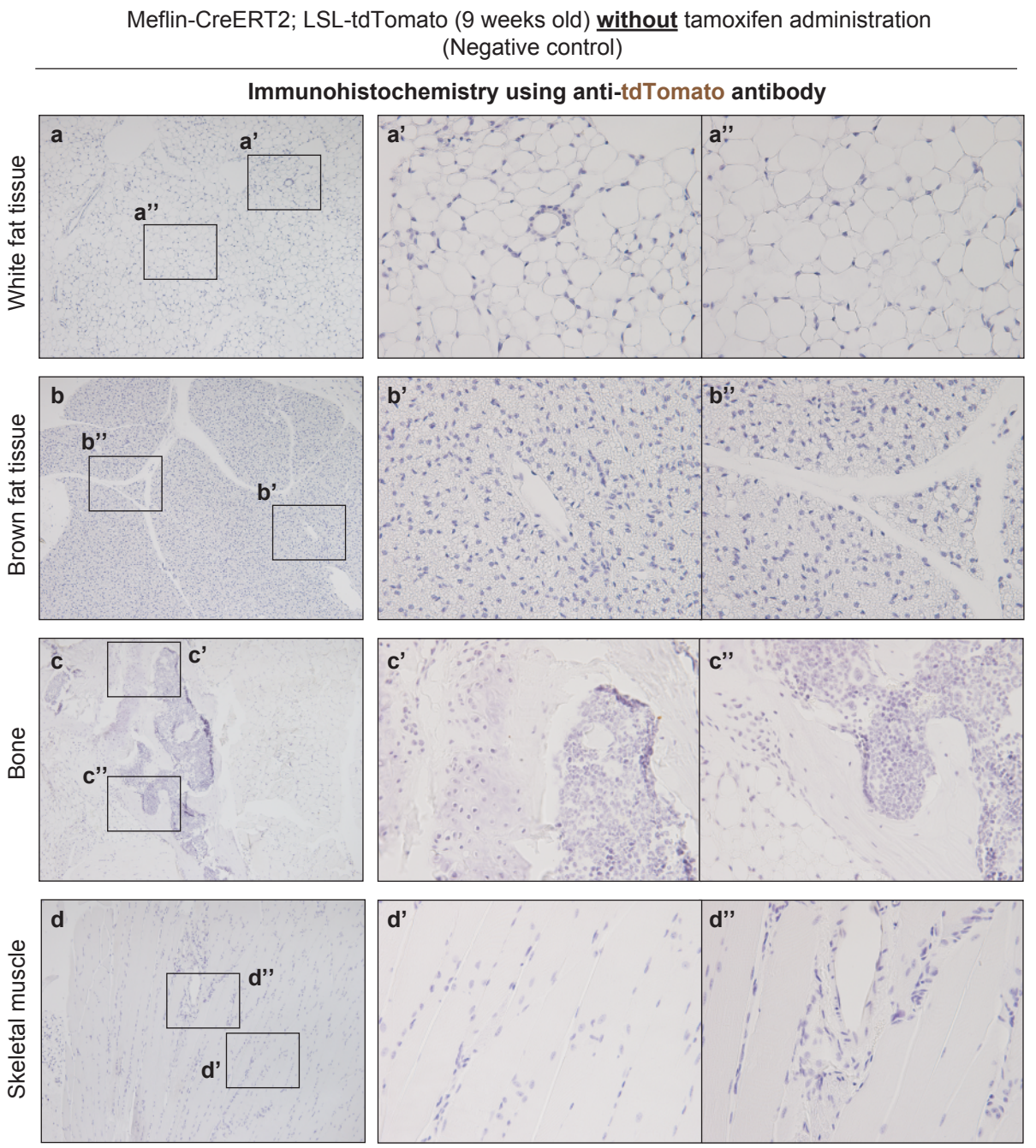
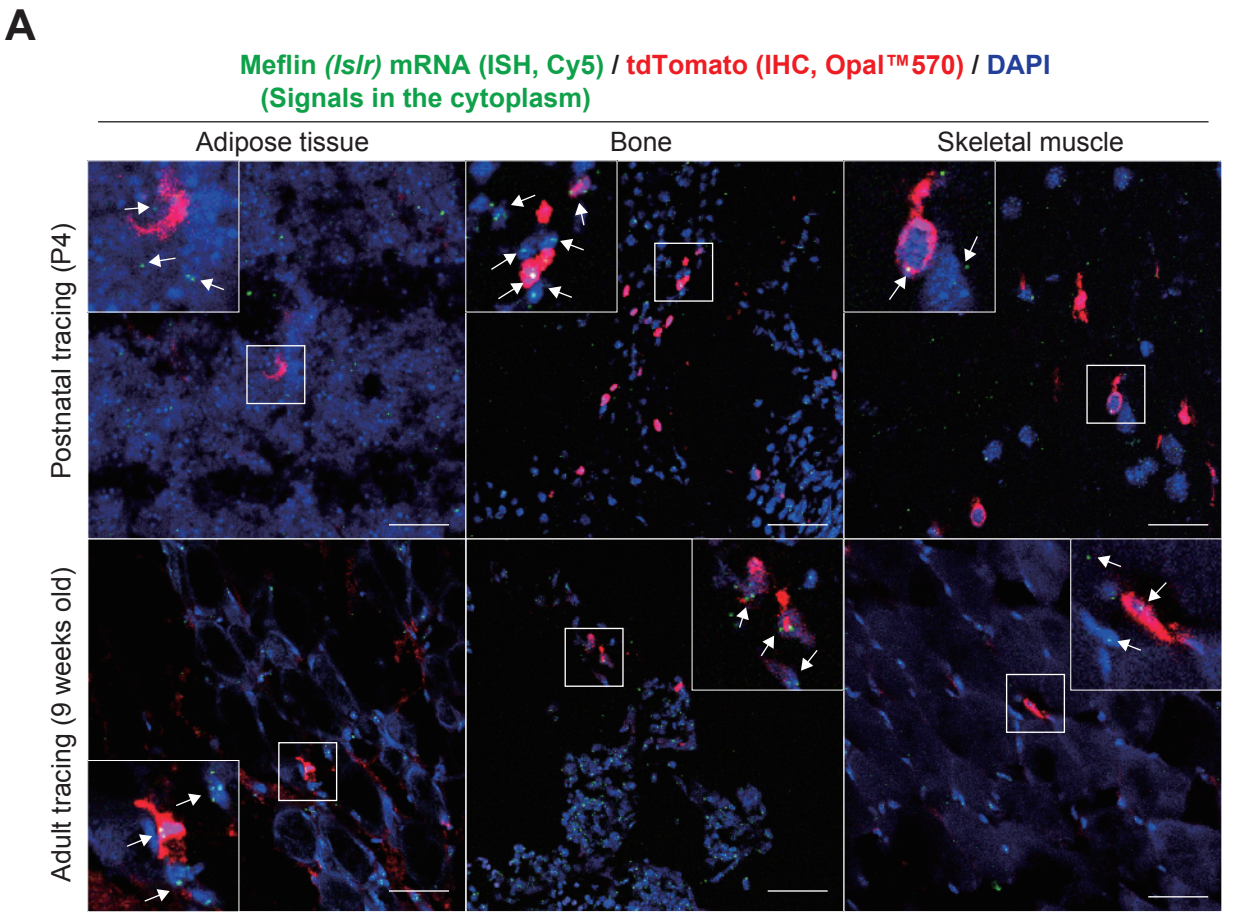

Meflin-CreERT2; LSL-tdTomato (9 weeks old) without tamoxifen administration
(Negative control)
Immunohistochemistry using anti-tdTomato antibody
White fat tissue
Brown fat tissue
Bone
Skeletal muscle
a
a'
a''
b
b''
b'
c
c'
c''
d
d''
d'

Figure S1. A negative control study for IHC using anti-tdTomato antibody

The indicated FFPE tissues harvested from Meflin-CreERT2; LSL-tdTomato mice not administered TAM (9-weeks-old) were stained for tdTomato by immunohistochemistry (IHC). Boxed regions **(a', a", b', b", c', c", d' and d")** are magnified in adjacent panels.

Figure S2


A
Meflin (Islr) mRNA (ISH, Cy5) / tdTomato (IHC, Opal™570) / DAPI
(Signals in the cytoplasm)
Adipose tissue
Bone
Skeletal muscle
Postnatal tracing (P4)
Adult tracing (9 weeks old)

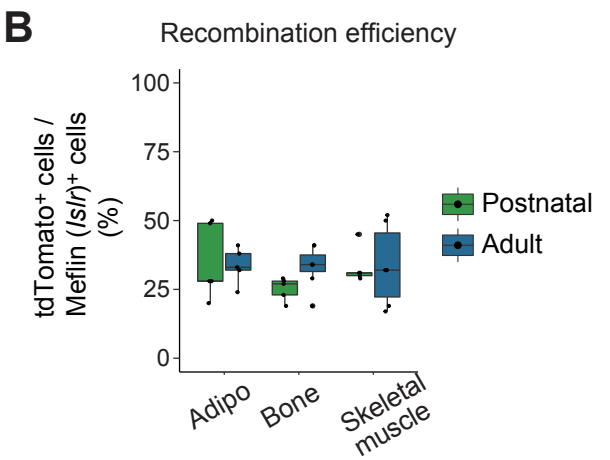

B
Recombination efficiency
tdTomato+ cells / Meflin (Islr)+ cells (%)
100
75
50
25
0
Adipo
Bone
Skeletal muscle
Postnatal
Adult

Figure S2. Evaluation of Cre-mediated recombination efficiency in Meflin$^+$ cells of mesenchymal tissues of Meflin-CreERT2; LSL-tdTomato mice

(A) FFPE tissues were stained for tdTomato by immunofluorescenct (IF) staining using Opal fluorophores (red), followed by Meflin mRNA detection by *in situ* hybridization (ISH) using the RNAscope technology (green). Arrows indicate Meflin mRNA-positive cells. Boxed areas were enlarged in insets. Nuclei were visualized by DAPI staining. Scale bars, 100 μm.

(B) The sections were prepared from the same tissue blocks that were used for the lineage trace experiments, followed by IHC for tdTomato and ISH for Meflin mRNA. The tdTomato positivity in all Meflin mRNA-positive cells was calculated in the indicated tissues, followed by quantification. The values were calculated from 5 independent images randomly taken from the stained sections.

Figure S3

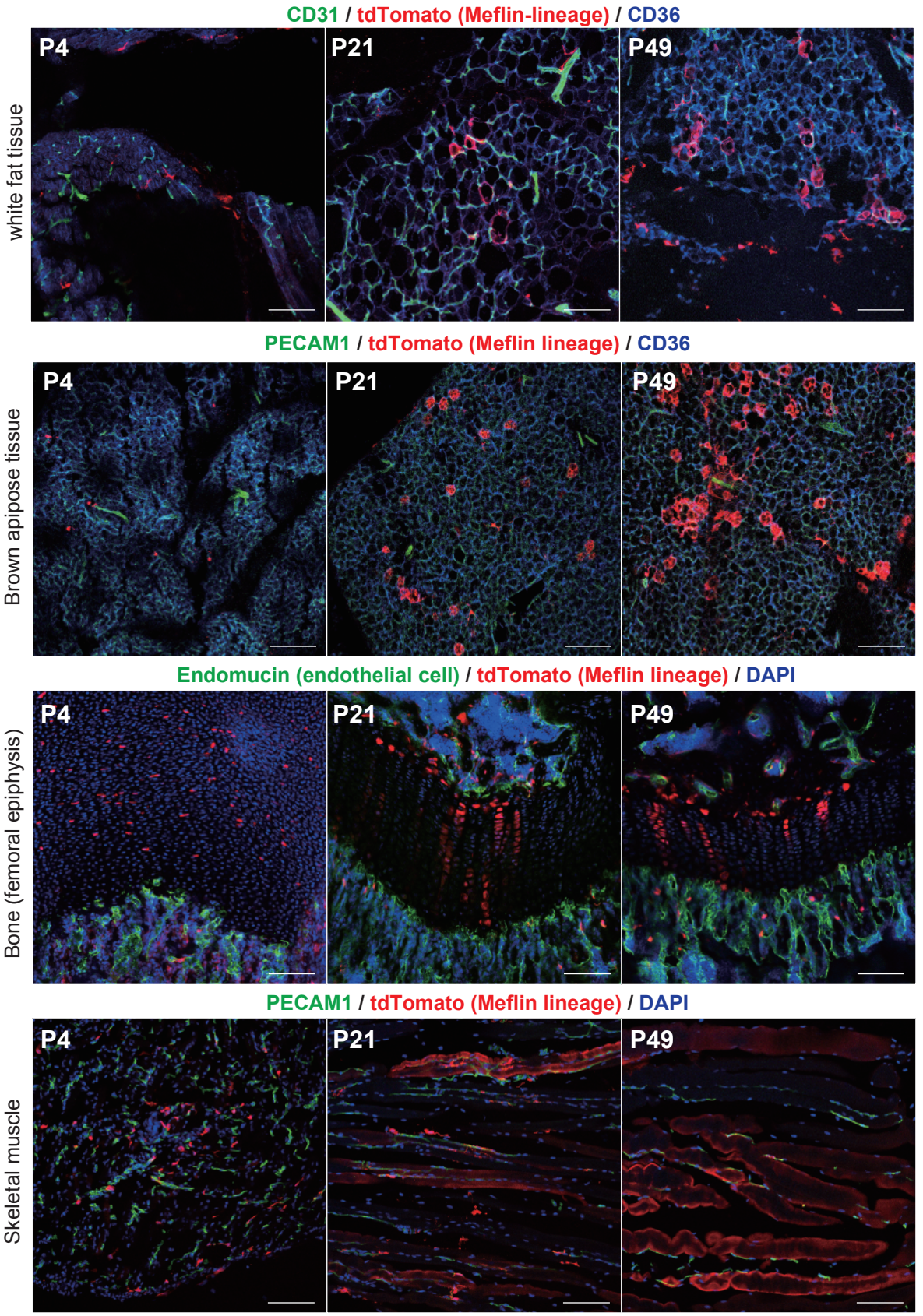

CD31 / tdTomato (Meflin-lineage) / CD36
white fat tissue
P4
P21
P49
PECAM1 / tdTomato (Meflin lineage) / CD36
Brown apipose tissue
P4
P21
P49
Endomucin (endothelial cell) / tdTomato (Meflin lineage) / DAPI
Bone (femoral epiphysis)
P4
P21
P49
PECAM1 / tdTomato (Meflin lineage) / DAPI
Skeletal muscle
P4
P21
P49

Figure S3. Meflin^{+} cells do not give rise to endothelial cells in the postnatal period

The indicated tissues harvested from Meflin-CreERT2; LSL-tdTomato mice after TAM administration were stained for the endothelial cell markers CD31 and Endomucin by IF (green). CD36 is a marker of adipocytes and endothelial cells. The data show that tdTomato^{+} cell do not express CD31 nor Endomucin throughout P4, P21 and P49. Scale bars, 200 μm.

Figure S4

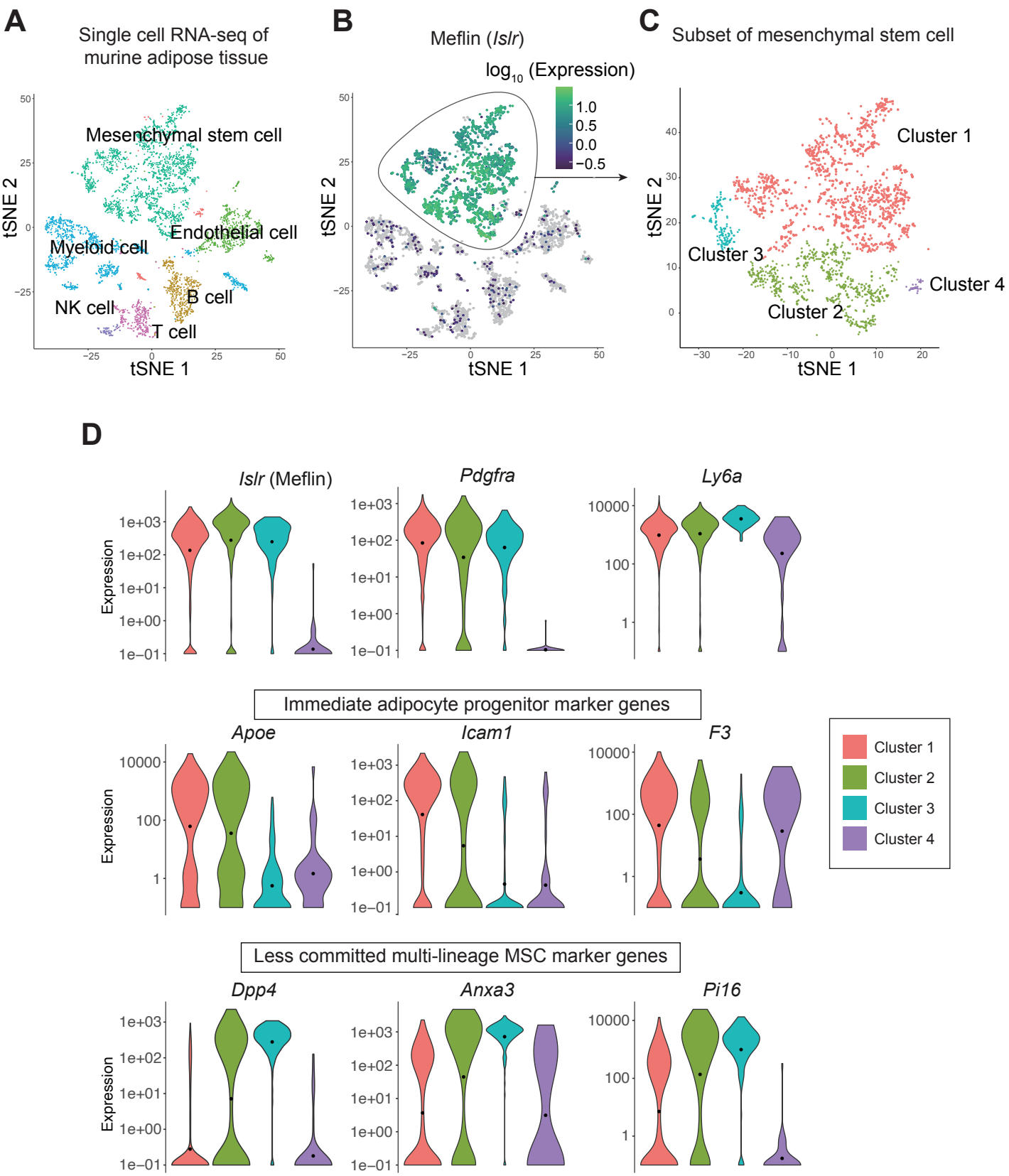

A
Single cell RNA-seq of murine adipose tissue
Mesenchymal stem cell
Endothelial cell
Myeloid cell
B cell
NK cell
T cell
tSNE 1
tSNE 2
B
Meflin (Islr)
log10 (Expression)
1.0
0.5
0.0
−0.5
C
Subset of mesenchymal stem cell
Cluster 1
Cluster 2
Cluster 3
Cluster 4
D
Islr (Meflin)
Pdgfra
Ly6a
Expression
Immediate adipocyte progenitor marker genes
Apoe
Icam1
F3
Less committed multi-lineage MSC marker genes
Dpp4
Anxa3
Pi16

Figure S4. Meflin is expressed in MSC subpopulations that are enriched for PDGFRα and Sca-1 in adipose tissue

(A-C) t-SNE plots showing distinct cell populations that were identified by single-cell RNA sequencing of all cells isolated from the mouse brown, gonadal, mesenteric and subcutaneous fat tissue (Schaum et al., GEO accession number GSM2967049). The t-SNE plot shown in **(B)** indicates Meflin (*Islr*) expression in the MSC population (the area enclosed by a line), which was further expanded in **(C)**, which identified four subsets of MSCs (Cluster 1 – 4).

(D) Violin plots depicting normalized expression of Meflin (*Islr*), PDGFRα, Sca-1 (*Ly6a*), and markers of immediate adipocyte progenitors and less committed multi-lineage MSCs as determined by Merrick *et al* (Science. 364, eaav2501). The data showed that these markers are differentially expressed in distinct subsets of MSCs in the adipose tissue. Meflin is preferentially expressed in MSC subsets 1-3 that are highly enriched for PDGFRα and Sca-1 expression.

Figure S5

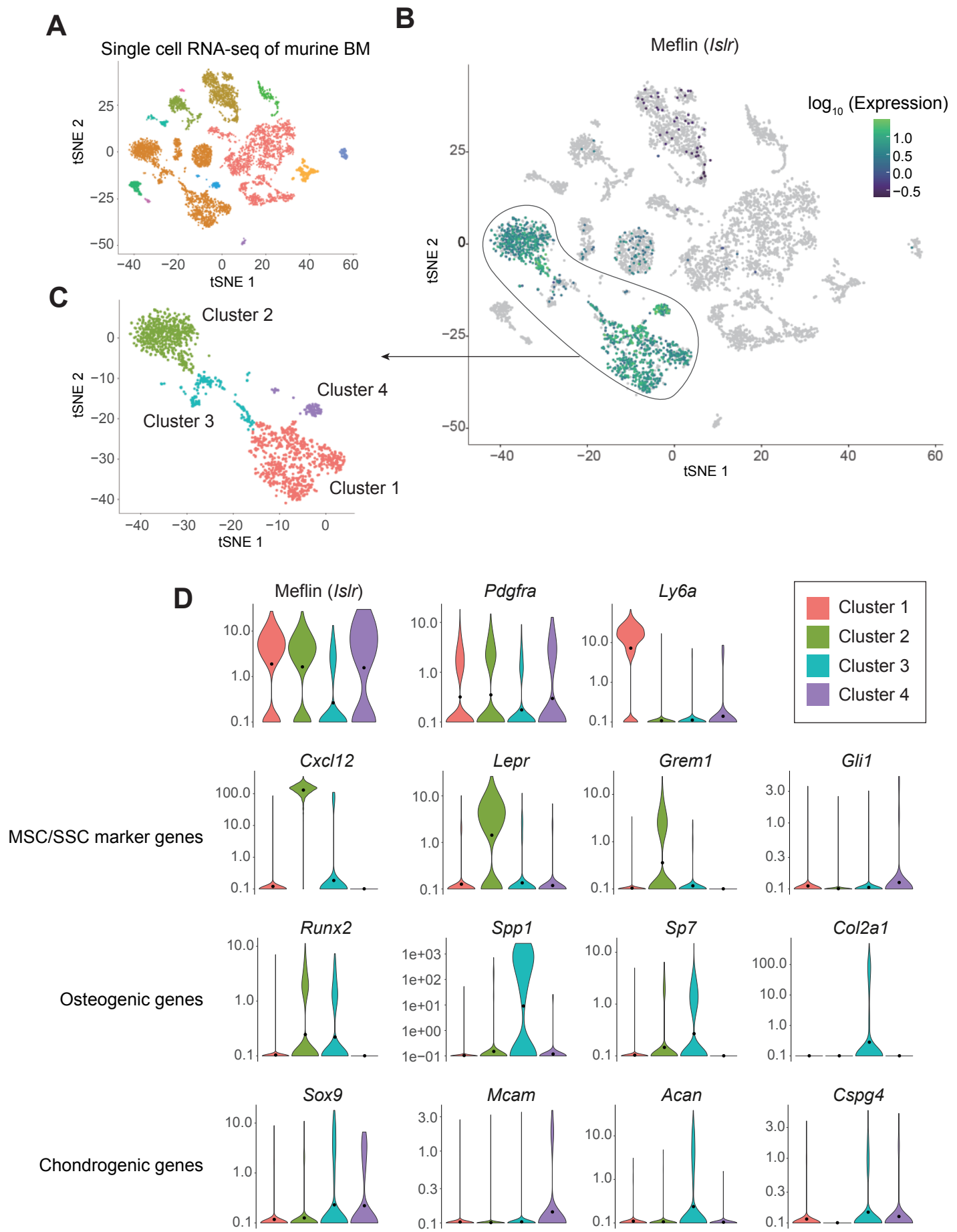

A
Single cell RNA-seq of murine BM
tSNE 2
tSNE 1
B
Meflin (Islr)
log10 (Expression)
1.0
0.5
0.0
−0.5
tSNE 2
tSNE 1
C
Cluster 2
Cluster 4
Cluster 3
Cluster 1
tSNE 2
tSNE 1
D
Meflin (Islr)
Pdgfra
Ly6a
Cluster 1
Cluster 2
Cluster 3
Cluster 4
MSC/SSC marker genes
Cxcl12
Lepr
Grem1
Gli1
Osteogenic genes
Runx2
Spp1
Sp7
Col2a1
Chondrogenic genes
Sox9
Mcam
Acan
Cspg4

Figure S5. Meflin is expressed in MSC subpopulations including one that is enriched for PDGFRα and Sca-1 in bone marrow (BM)

(A - C) t-SNE plots showing distinct cell populations that were identified by single-cell RNA sequencing of all cells isolated from the mouse BM (Baccin et al., 2020 GEO accession number GSE122465). The t-SNE plot shown in **(B)** indicates Meflin (*Islr*) expression in the MSC population (the area enclosed by a line), which was further extracted in **(C)** and further divided into 4 subsets (Clusters 1 – 4).

(D) Violin plots depicting normalized expression of Meflin (*Islr*), PDGFRα, Sca-1 (*Ly6a*), marker genes of MSCs/SSCs, and osteogenic and chondrogenic genes. The data showed that these markers are differentially expressed in distinct subsets of MSCs in the BM. Meflin is widely expressed in the MSC subsets including one (Cluster 1) that is highly enriched for PDGFRα and Sca-1 expression.

Figure S6

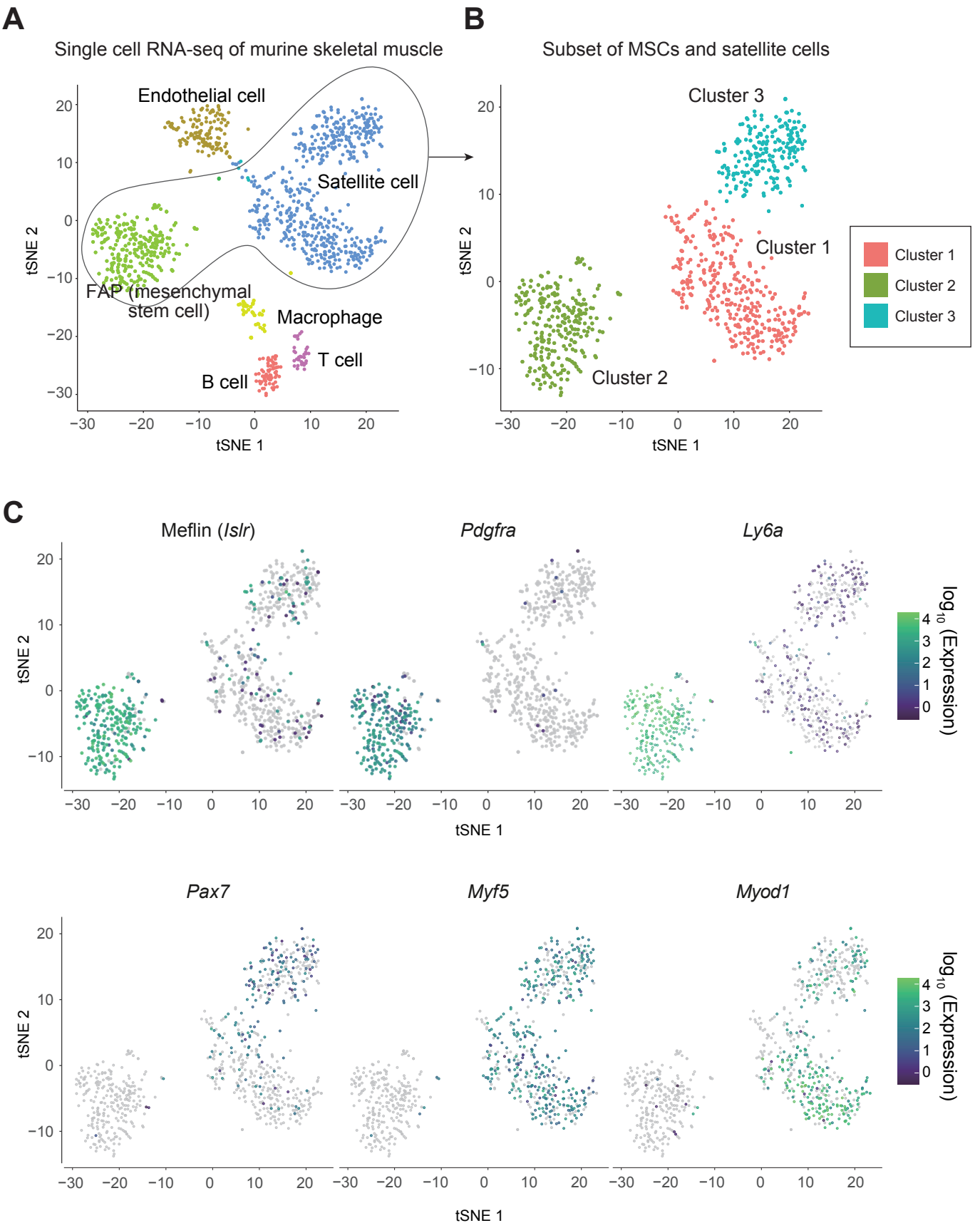

A
Single cell RNA-seq of murine skeletal muscle
Endothelial cell
Satellite cell
FAP (mesenchymal stem cell)
Macrophage
T cell
B cell
tSNE 1
tSNE 2
B
Subset of MSCs and satellite cells
Cluster 3
Cluster 1
Cluster 2
Cluster 1
Cluster 2
Cluster 3
C
Meflin (Islr)
Pdgfra
Ly6a
Pax7
Myf5
Myod1
log10 (Expression)

Figure S6. Meflin is expressed in both satellite cells and FAPs in skeletal muscle tissue

(A, B) t-SNE plots showing distinct cell populations that were identified by single-cell RNA sequencing of all cells isolated from the mouse skeletal muscle tissue (Schaum et al., GEO accession number GSM2967056). A cell population that includes satellite cells and FAPs in **(A)** (the area enclosed by a line) was expanded in **(B)**, which identified 3 stem cells subsets (Clusters 1 – 3).

(C) Violin plots depicting normalized expressions of Meflin (*Islr*), PDGFRα (a marker of FAPs), Sca-1 (*Ly6a*), and markers of satellite cells, myoblasts and myocytes. The data showed that these markers are differentially expressed in distinct subsets of stem cells in skeletal muscle tissue. Meflin is preferentially expressed in FAPs that are highly enriched for PDGFRα and Sca-1 expression but also to a lesser extent in satellite cells that are positive for Pax7.