Cell Reports, Volume *42*

Supplemental information

Dynamic interplay between IL-1 and WNT pathways in regulating dermal adipocyte lineage cells during skin development and wound regeneration

Lixiang Sun, Xiaowei Zhang, Shuai Wu, Youxi Liu, Christian F. Guerrero-Juarez, Wenjie Liu, Jinwen Huang, Qian Yao, Meimei Yin, Jiacheng Li, Raul Ramos, Yanhang Liao, Rundong Wu, Tian Xia, Xinyuan Zhang, Yichun Yang, Fengwu Li, Shujun Heng, Wenlu Zhang, Minggang Yang, Chi-Meng Tzeng, Chao Ji, Maksim V. Plikus, Richard L. Gallo, and Ling-juan Zhang

	State 1	State 2		State 3				
	perifollicular (C2,5,9)	**PAP (C7)**	**RET (C3)**	**AP /pAd (C4)**	**pAd /eAd (C8)**	**HI-pAd (C10)**	**Areg (C6)**	**HI-AP (C11)**
	CD24 hi	*Entpd1*	*Ptgfr*	*Cebpb*	*Pparg*	*Agt*	*Mgp*	*Wnt2*
	Alpl	*Lrig1*	*Cyp4b1*	*Lgr5*	*Fabp4*	*Icam1*	*Fmo2*	*Smpd3*
	Sox2	*Grem1*	*Sfrp2*	*Icam1*	*Plin1*	*Lpl*	*F3*	*Cd34*
	Lef1	*Lum hi*	*Col1a1*	*Plin2*	*Plin2*	*Plin2*	*Sfrp4*	*Col14a1*
	Inhba				*Lpl*			*Fn1 hi*
	Col23a1				*Agt*			
Trps1	high	med	low	low	low	low	low	low
Dlk1	low	low	med	med	med	high	high	high
Dpp4	low	med	low	low	low	low	low	high
Ly6a	low	low	low	med	med	med	med	high

Table S1. Key marker genes for indicated dFB cell clusters in neonatal mouse skin.

Table S2. List of Abbreviations:

Abbreviations	Definitions
AP	Adipocyte progenitor
AD	Adipocyte progenitor
APM	Arrector pili muscle
AREG	Adipogenesis regulators
dFB	Dermal fibroblast
DP	Dermal papilla
DS	Dermal sheath
dWAT	Dermal white adipose tissue
EC	Endothelial cell
ECM	Extracellular matrix
FACS	Fluorescence activated cell sorting
GT	Grannule tissue
HF	Hair follicle
HB	Hair bulb
HI-AP	Hypodermal interstitial adipocyte progenitor
IHC	Immunohistochemistry
KC	Keratinocyte
KC-IFE	Keratinocyte-interfollicular epidermis
LV	Lymphatic vessel
myoFB	Myo-fibroblast
NB	Neonatal fibroblast
pAd	Pre-adipocyte
PAP	Papillary
PHA	Phalloidin
RET	Reticular
RET-AP	Reticular-adipocyte progenitor
scRNA-seq	Single-cell RNA sequencing
TAM	Tamoxifen
TFs	Transcriptional factors
tSNE	t-distributed stochastic neighbor embedding
UMAP	Uniform manifold approximation and projection
VSMC	Vscular smooth muscle cell
w.d.	Wound day

Table S3 List of protein and gene symbols:

Proteins	Protein symbols	Gene symbols
Actin Alpha 2	ACTA2	*Acta2*
Adiponectin	ADIPOQ	*Adipoq*
RAC-alpha serine/threonine-protein kinase	AKT	*Akt*
Alkaline phosphatase, tissue-nonspecific isozyme	ALPL	*Alpl*
Patatin-like phospholipase domain-containing protein 2	ATGL	*Pnpla2*
Axin-2	AXIN2	*Axin2*
Cathelicidin antimicrobial peptide	CAMP	*Camp*
Signal transducer CD24	CD24	*Cd24*
T-cell surface glycoprotein CD3	CD3	*Cd3*
Hematopoietic progenitor cell antigen CD34	CD34	*Cd34*
Platelet glycoprotein 4	CD36	*Cd36*
Receptor-type tyrosine-protein phosphatase C	CD45	*Ptprc*
CCAAT/enhancer-binding protein beta	CEBPB	*Cebpb*
Collagen	COL	*Col*
Cellular retinoic acid-binding protein 1	CRABP1	*Crabp1*
Cyclic AMP-responsive element-binding protein 1	CREB	*Creb1*
CCN family member 2	CTGF	*Ccn2*
C-X-C motif chemokine	CXCL	*Cxcl*
Decorin	DCN	*Dcn*
Protein delta homolog 1	DLK1	*Dlk1*
Dipeptidyl peptidase 4	DPP4	*Dpp4*
Ectonucleoside triphosphate diphosphohydrolase 1	ENTPD1	*Entpd1*
Mitogen-activated protein kinase	ERK	*Mapk*
Coagulation Factor III	F3	*F3*
Dimethylaniline monooxygenase [N-oxide-forming] 2	FMO2	*Fmo2*
Fibronectin	FN1	*Fn1*
Green fluorescent protein	GFP	*Gfp*
Glycogen synthase kinase-3	GSK3	*Gsk3*
Intercellular adhesion molecule 1	ICAM1	*Icam1*
Interleukin-1	IL1	*Il1*
Interleukin-1 receptor type 1	IL1R1	*Il1r1*
Proliferation marker protein Ki-67	Ki67	*Mki67*
Keratin	KRT	*Krt*
Lymphoid enhancer-binding factor 1	LEF1	*Lef1*
Leucine-rich repeat-containing G-protein coupled receptor 5	LGR5	*Lgr5*
Lipoprotein lipase	LPL	*Lpl*

Leucine-rich repeats and immunoglobulin-like domains protein 1	LRIG1	*Lrig1*
Lumican	LUM	*Lum*
Lymphocyte antigen 6A-2/6E-1	LY6A	*Ly6a*
Lysozyme C-2	LYZ2	*Lyz2*
Matrix Gla protein	MGP	*Mgp*
Myosin regulatory light chain 2, skeletal muscle isoform	MYLPF	*Mylpf*
Myogenin	MYOG	*Myog*
Nuclear factor NF-kappa-B p105 subunit	NFKB	*Nfkb*
Protein naked cuticle homolog 2	NKD2	*Nkd2*
Proliferating cell nuclear antigen	PCNA	*Pcna*
Platelet-derived growth factor receptor alpha	PDGFRA	*Pdgfra*
Perilipin	PLIN	*Plin*
Peroxisome proliferator-activated receptor gamma	PPARG	*Pparg*
Transcription factor SOX-2	SOX2	*Sox2*
Sterol regulatory element-binding protein 1	SREBF1	*Srebf1*
Transgelin	TAGLN	*Tagln*
Transforming growth factor beta	TGFB	*Tgfb*
Transforming growth factor-beta-induced protein ig-h3	TGFBI	*Tgfbi*
Thy-1 membrane glycoprotein	THY1	*Thy1*
Wnt Family Member 2	WNT2	*Wnt2*

Table S4. List of primers used for RT-qPCR of mouse genes:

Gene	Strand	Primer sequence
Tbp	Forward	CCTTGTACCCTTCACCAATGAC
	Reverse	ACAGCCAAGATTCACGGTAGA
Camp	Forward	CAAGGAACAGGGGGTGG
	Reverse	TCCGGCTGAGGTACAAGTTT
Dlk1	Forward	TGGCTGGGACGGGAAATTC
	Reverse	CACGCAAGTTCCATTGTTGGC
Col1a1	Forward	GCTCCTCTTAGGGGCCACT
	Reverse	ATTGGGGACCCTTAGGCCAT
Thy1	Forward	CCTTACCCTAGCCAACTTCAC
	Reverse	AGGATGTGTTCTGAACCAGC
Pdgfra	Forward	ATGAGAGTGAGATCGAAGGCA
	Reverse	CGGCAAGGTATGATGGCAGAG
Ly6a	Forward	GAGGCAGCAGTTATTGTGGAT
	Reverse	CGTTGACCTTAGTACCCAGGA
Pparg1	Forward	AAGAAGCGGTGAACCACTGA
	Reverse	GGAATGCGAGTGGTCTTCCA
Adipoq	Forward	CACACCAGGCCGTGATGGCA
	Reverse	GAAGCCCCGTGGCCCTTCAG
Fabp4	Forward	GTGGGAGTGGGCTTTGCCACA
	Reverse	CACCAGGGCCCCGCCATCTA
Cd24a	Forward	TTCTGGCACTGCTCCTACC
	Reverse	GCGTTACTTGGATTTGGGGAA
Il6	Forward	ACAAAGCCAGAGTCCTTCAGAGAGA
	Reverse	AGCCACTCCTTCTGTGACTCCAG
Acta2	Forward	GGCACCACTGAACCCTAAGG
	Reverse	ACAATACCAGTTGTACGTCCAGA
Spp1	Forward	TCTCCTTGCGCCACAGAATG
	Reverse	GGCTTTCATTGGAATTGCTTGG
Dpp4	Forward	TATGCCCAGTTTAACGACACAG
	Reverse	ACAGTTGGATTCACAGCTCCT
Sox2	Forward	GCGGAGTGGAAACTTTTGTCC
	Reverse	CGGGAAGCGTGTACTTATCCTT
Alpl	Forward	CCAACTCTTTTGTGCCAGAGA
	Reverse	GGCTACATTGGTGTTGAGCTTTT
Inhba	Forward	TGTGGGTAAAGTGGGGGAGA
	Reverse	CACGCTCCACTACTGACAGG
Lef1	Forward	AACGAGTCCGAAATCATCCCA
	Reverse	GCCAGAGTAACTGGAGTAGGA
Crabp1	Forward	CAGCAGCGAGAATTTCGACGA
	Reverse	CGCACAGTAGTGGATGTCTTGA

Lrig1	Forward	TTGAGGACTTGACGAATCTGC
	Reverse	CTTGTTGTGCTGCAAAAAGAGAG
Col23a1	Forward	CCCCATCTGAGTGCATCTGTC
	Reverse	CTTGCCGTCCAGACCTAGAG
Col14a1	Forward	TTTGGCGGCTGCTTGTTTC
	Reverse	CGCTTTTGTTGCAGTGTTCTG
Lgr5	Forward	CCTACTCGAAGACTTACCCAGT
	Reverse	GCATTGGGGTGAATGATAGCA
Cebpb	Forward	CAAGAGCCGCGACAAGGCCA
	Reverse	CTCGCGACAGCTGCTCCACC
Cd36	Forward	AGATGACGTGGCAAAGAACAG
	Reverse	CCTTGGCTAGATAACGAACTCTG
Lpl	Forward	GGGAGTTTGGCTCCAGAGTTT
	Reverse	TGTGTCTTCAGGGGTCCTTAG
Lipe	Forward	AGGATCGAAGAACCGCAGTC
	Reverse	GTCTTCTGCGAGTGTCACCA
Smpd3	Forward	TCATGGACGTGGCCTATCAC
	Reverse	GCAGGCGATGTACCCAACAA
Wnt2	Forward	CTCGGTGGAATCTGGCTCTG
	Reverse	CACATTGTCACACATCACCCT
Il1r1	Forward	GTGCTACTGGGGCTCATTTGT
	Reverse	GGAGTAAGAGGACACTTGCGAAT
Il4ra	Forward	TCTGCATCCCGTTGTTTTGC
	Reverse	GCACCTGTGCATCCTGAATG
Cxcl12	Forward	TGCATCAGTGACGGTAAACCA
	Reverse	TTCTTCAGCCGTGCAACAATC
Cxcl5	Forward	TGCCCTACGGTGGAAGTCATA
	Reverse	TGCATTCCGCTTAGCTTTCTTT
Axin2	Forward	CGAGTGTGAGATCCACGGAA
	Reverse	GGACATGGAATCGTCGGTCA
F3	Forward	GCCATTTACAAACGCCCCAA
	Reverse	GCAGGGTGAGGAATGTACCA
Atgl	Forward	AACGCCACTCACATCTACGG
	Reverse	CAATCAGCAGGCAGGGTCTT
Tnc	Forward	CAGCTACCGACGGGATCTTC
	Reverse	TTCCGGTTCAGCTTCTGTGG
Il1b	Forward	GAAATGCCACCTTTTGACAGTG
	Reverse	TGGATGCTCTCATCAGGACAG
Ly6g	Forward	GACTTCCTGCAACACAACTACC
	Reverse	ACAGCATTACCAGTGATCTCAGT
Nkd2	Forward	GAGCGGAAGAAACGGACCG
	Reverse	CCTTAGGGTCTCCATTGAGCA
Col3a1	Forward	CCTGGCTCAAATGGCTCAC

	Reverse	CAGGACTGCCGTTATTCCCG
Pparg2	Forward	TCGCTGATGCACTGCCTATG
	Reverse	GAGAGGTCCACAGAGCTGATT
Col7a1	Forward	ACCACGTTTCTGACCGTGTC
	Reverse	AGCTGTGTCCACTAAATCTTGG
Plin1	Forward	CTGTGTGCAATGCCTATGAGA
	Reverse	CTGGAGGGTATTGAAGAGCCG
Cxcl1	Forward	CACCCGCTCGCTTCTCTG
	Reverse	TCTTGAGGTGAATCCCAGCC
Col4a1	Forward	TCCGGGAGAGATTGGTTTCC
	Reverse	CTGGCCTATAAGCCCTGGT

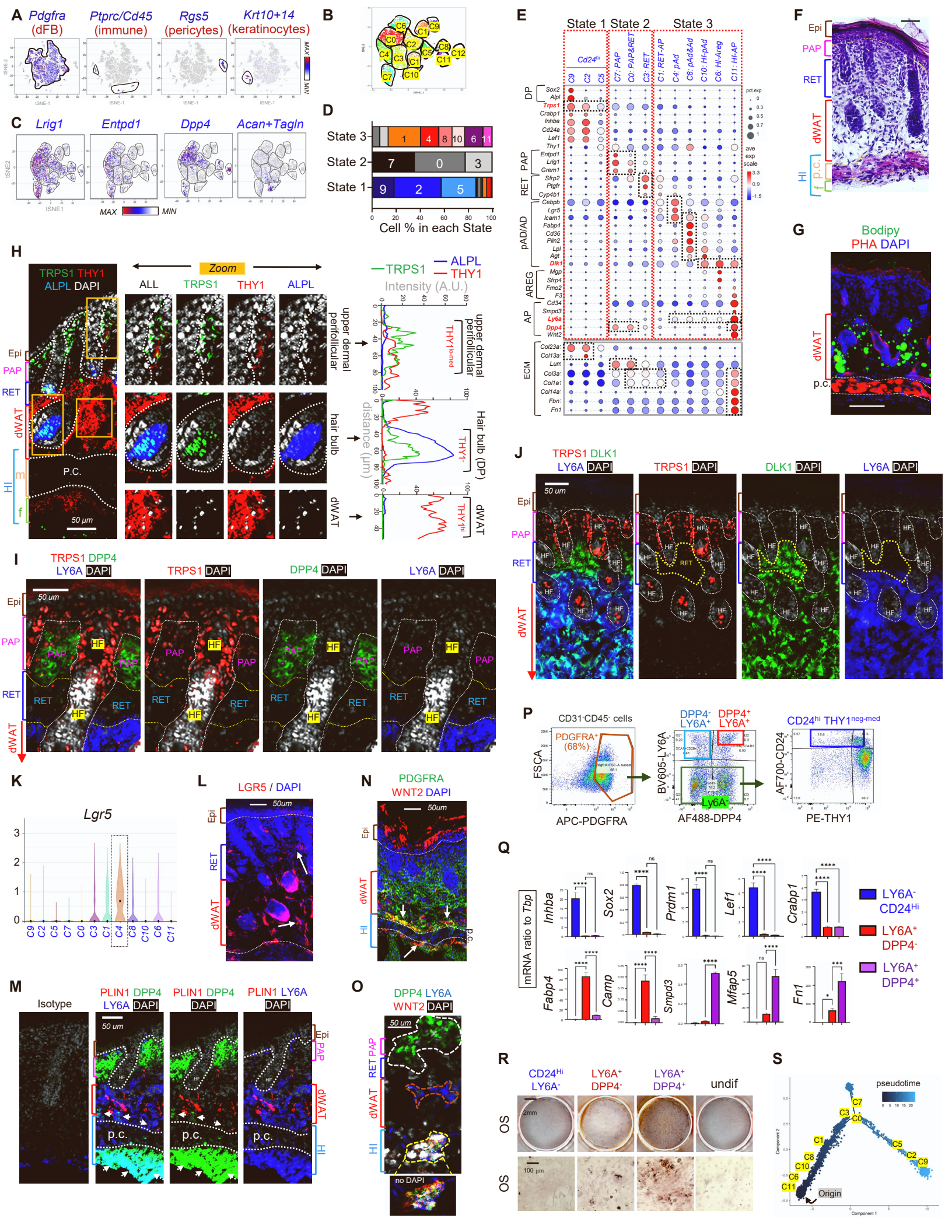
A
Pdgfra (dFB)
Ptprc/Cd45 (immune)
Rgs5 (pericytes)
Krt10+14 (keratinocytes)
MAX
MIN
B
C
Lrig1
Entpd1
Dpp4
Acan+Tagln
D
State 3
State 2
State 1
Cell % in each State
E
State 1 State 2 State 3
DP
PAP
RET
pAD/AD
AREG
AP
ECM
F
Epi
PAP
RET
dWAT
HI
p.c.
f
G
Bodipy
PHA DAPI
dWAT
p.c.
H
TRPS1 THY1
ALPL DAPI
Zoom
ALL
TRPS1
THY1
ALPL
Intensity (A.U.)
distance (μm)
upper dermal perifollicular
hair bulb
dWAT
Hair bulb (DP)
P.C.
50 μm
I
TRPS1 DPP4
LY6A DAPI
TRPS1 DAPI
DPP4 DAPI
LY6A DAPI
50 um
Epi
PAP
RET
dWAT
HF
J
TRPS1 DLK1
LY6A DAPI
TRPS1 DAPI
DLK1 DAPI
LY6A DAPI
50 um
K
Lgr5
L
LGR5 / DAPI
50um
N
PDGFRA
WNT2 DAPI
50um
M
Isotype
PLIN1 DPP4
LY6A DAPI
PLIN1 DPP4
DAPI
PLIN1 LY6A
DAPI
50 um
p.c.
O
DPP4 LY6A
WNT2 DAPI
50 um
no DAPI
P
CD31-CD45- cells
PDGFRA+ (68%)
FSCA
APC-PDGFRA
DPP4- LY6A+
DPP4+ LY6A+
BV605-LY6A
AF488-DPP4
CD24hi THY1neg-med
AF700-CD24
PE-THY1
Q
mRNA ratio to Tbp
Inhba
Sox2
Prdm1
Lef1
Crabp1
Fabp4
Camp
Smpd3
Mfap5
Fn1
LY6A- CD24Hi
LY6A+ DPP4-
LY6A+ DPP4+
R
CD24Hi LY6A-
LY6A+ DPP4-
LY6A+ DPP4+
undif
OS
2mm
100 μm
S
pseudotime
Origin

Figure S1 (related to Figure 1). Characterization of heterogeneous dermal adipocyte lineage cells in developing neonatal skin
(**A**) tSNE plots showing the expression of *Pdgfra*, *Ptprc/Cd45*, *Rgs5*, and *Krt10+Krt14* in neonatal mouse skin cell clusters.
(**B-C**) Neonatal mouse *Pdgfra*$^{+}$ dFBs were re-clustered into 13 clusters, and t-SNE plots for cell distribution by clusters (**B**) and indicated marker genes expression (**C**) were shown. Note that to focus on clusters related to adipogenesis, clusters including *Dpp4*$^{-}$ *Entpd1*$^{+}$*Lrig1*$^{+}$ PAP dFB clusters and an *Acan*$^{+}$*Tagln*$^{+}$ dermal sheath cluster were excluded from the rest of this study.
(**D**) Neonatal dFBs were grouped into 3 cell states by trajectory analysis with Monocle, and the percentage of clusters in each cell state is shown.
(**E**) Bubble plots of indicated genes in neonatal dFB clusters
(**F**) HE staining of neonatal skin sections. Scale bar, 50 μm.
(**G**) Staining of neonatal skin sections with Bodipy (green dye for lipid) and phalloidin (red dye for actin fiber) staining
(**H**) Immunostaining of neonatal skin sections with TRPS1 (green), THY1 (red) and ALPL (blue), and DAPI was counterstained in white. White dotted line marks junction between the dermis and the epidermis or hair follicles. Scale bar, 50 μm. The right panel is the quantified results showing the fluorescent intensity (arbitrary unit, AU) of TRPS1 (green), ALPL (blue), and THY1 (red) from top to bottom of the indicated zoomed images (representative of n=3/group).
(**I-J**) Immunostaining of neonatal skin sections with TRPS1 (red), LY6A (blue) and DPP4 (green) in **I** or DLK1 (green) in **J**, and DAPI in white. Dotted line marks junction between the papillary dermis , reticular dermis, and the epidermis or hair follicles. In **J**, a reticular dermal region (RET), that was TRPS1^{-}DLK1^{+}LY6A^{-}, was circled by yellow dotted lines. Scale bar, 50 μm. (**K**) Violin plot showing the expression of *Lgr5* across various *Pdgfra*$^{+}$ dFB clusters.
(**K**) Violin plot showing the expression of *Lgr5* across various *Pdgfra*$^{+}$ dFB clusters.
(**L**) Immunostaining of neonatal skin sections with LGR5 (red) and DAPI was counterstained in blue. Scale bar, 50 μm. White arrows indicate LGR5+ pAds in the reticular dermis or dWAT layer.
(**M**) Immunostaining of neonatal skin sections with PLIN1 (red), LY6A (blue) and DPP4 (green), and DAPI was counterstained in white. Arrows mark DPP4^{+}LY6A^{+} cells in the HI region above p.c. muscle. Scale bar, 50 μm.
(**N**) Immunostaining of neonatal skin sections with WNT2 (red) and PDGFRA (green), and DAPI was counterstained in blue. Arrows mark WNT2^{+}PDGFRA^{+} cells in the HI region. Scale, 50 μm.
(**O**) Immunostaining of neonatal skin sections with WNT2 (red), DPP4 (green) and LY6A (blue), and DAPI was counterstained in white. Scale bar, 50 μm.
(**P**) Flow sorting strategies to sort various dFB sub-populations, including the perifollicular dFBs (CD24^{hi}LY6A$^{–}$), pAds (DPP4$^{–}$LY6A^{+}) and HI-APs (DPP4^{+}LY6A^{+}).
(**Q**) Sorted cells were subjected to qRT-PCR analysis of indicated dFB genes (n = 3 per group).
(**R**) Sorted cells were subjected to in vitro osteocyte differentiation assay, and osteocytes were stained with Alizarin Red (AR). Zoom-out images in the lower panel.
(**S**) Cell trajectory analysis of neonatal dFB clusters (C0~C11), and pseudotime (arbitrary units) is depicted from dark to light blue (left).
All error bars indicate mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

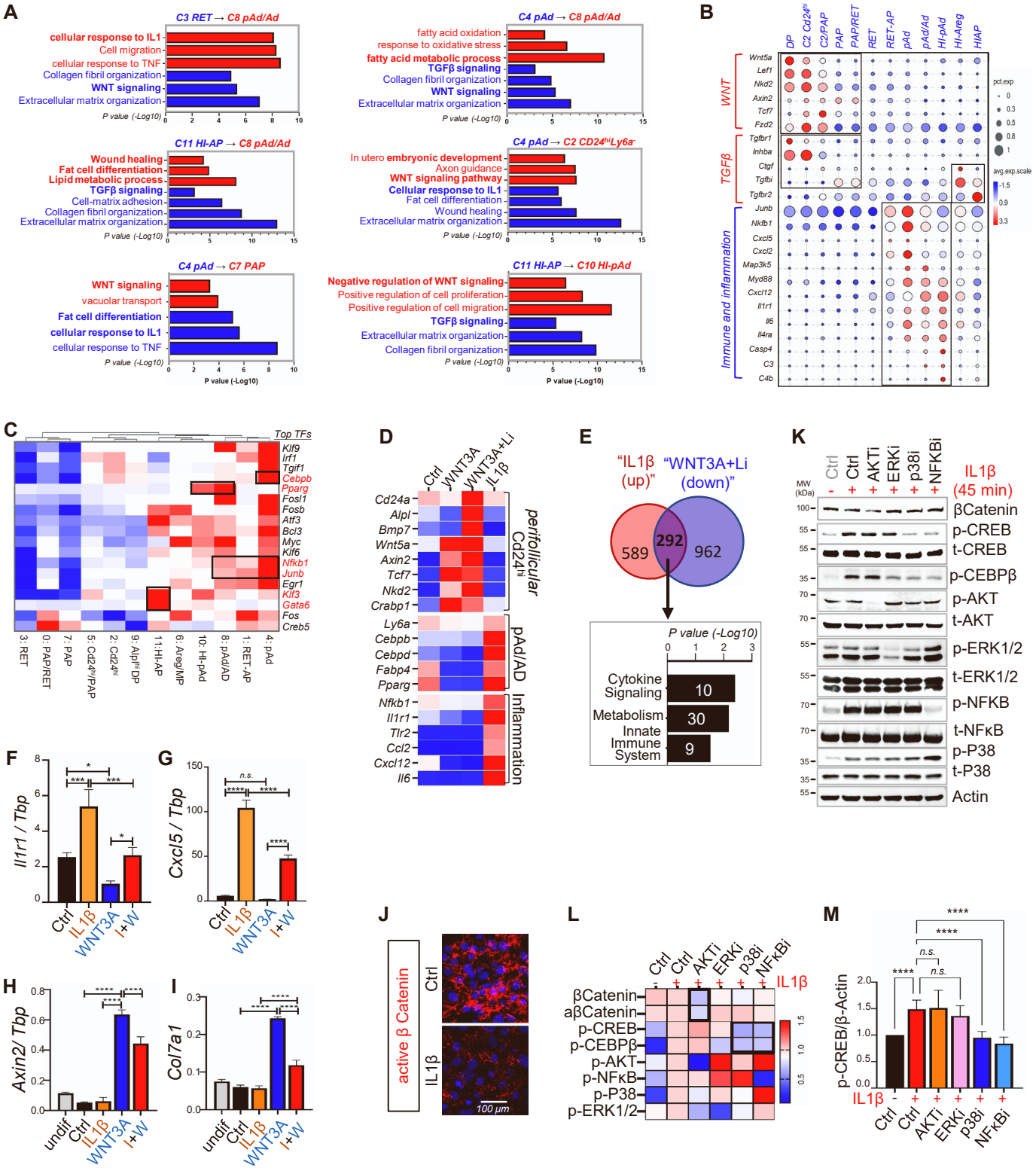
A
C3 RET → C8 pAd/Ad
cellular response to IL1
Cell migration
cellular response to TNF
Collagen fibril organization
WNT signaling
Extracellular matrix organization
C4 pAd → C8 pAd/Ad
fatty acid oxidation
response to oxidative stress
fatty acid metabolic process
TGFβ signaling
Collagen fibril organization
WNT signaling
Extracellular matrix organization
C11 HI-AP → C8 pAd/Ad
Wound healing
Fat cell differentiation
Lipid metabolic process
TGFβ signaling
Cell-matrix adhesion
Collagen fibril organization
Extracellular matrix organization
C4 pAd → C2 CD24hiLy6a-
In utero embryonic development
Axon guidance
WNT signaling pathway
Cellular response to IL1
Fat cell differentiation
Wound healing
Extracellular matrix organization
C4 pAd → C7 PAP
WNT signaling
vacuolar transport
Fat cell differentiation
cellular response to IL1
cellular response to TNF
C11 HI-AP → C10 HI-pAd
Negative regulation of WNT signaling
Positive regulation of cell proliferation
Positive regulation of cell migration
TGFβ signaling
Extracellular matrix organization
Collagen fibril organization
P value (-Log10)
B
DP
C2 Cd24hi
C2/PAP
PAP
PAP/RET
RET
RET-AP
pAd
pAd/Ad
HI-pAd
HI-Areg
HIAP
WNT
Wnt5a
Lef1
Nkd2
Axin2
Tcf7
Fzd2
TGFβ
Tgfbr1
Inhba
Ctgf
Tgfbi
Tgfbr2
Immune and inflammation
Junb
Nkfb1
Cxcl5
Cxcl2
Map3k5
Myd88
Cxcl12
Il1r1
Il6
Il4ra
Casp4
C3
C4b
pct.exp
avg.exp.scale
C
Top TFs
Klf9
Irf1
Tgif1
Cebpb
Pparg
Fosl1
Fosb
Atf3
Bcl3
Myc
Klf6
Nfkb1
Junb
Egr1
Klf3
Gata6
Fos
Creb5
3: RET
0: PAP/RET
7: PAP
5: Cd24hi/PAP
2: Cd24hi
9: Alplo DP
11:HI-AP
6: Areg/MP
10: HI-pAd
8: pAd/AD
1: RET-AP
4: pAd
D
Ctrl
WNT3A
WNT3A+Li
IL1β
perifollicular Cd24hi
Cd24a
Alpl
Bmp7
Wnt5a
Axin2
Tcf7
Nkd2
Crabp1
pAd/AD
Ly6a
Cebpb
Cebpd
Fabp4
Pparg
Inflammation
Nfkb1
Il1r1
Tlr2
Ccl2
Cxcl12
Il6
E
"IL1β (up)"
"WNT3A+Li (down)"
589
292
962
P value (-Log10)
Cytokine Signaling
Metabolism
Innate Immune System
10
30
9
K
MW (kDa)
Ctrl
Ctrl
AKTi
ERKi
p38i
NFKBi
IL1β (45 min)
βCatenin
p-CREB
t-CREB
p-CEBPβ
p-AKT
t-AKT
p-ERK1/2
t-ERK1/2
p-NFKB
t-NFκB
p-P38
t-P38
Actin
F
Il1r1 / Tbp
Ctrl
IL1β
WNT3A
I+W
G
Cxcl5 / Tbp
n.s.
H
Axin2/ Tbp
undif
I
Col7a1
J
active β Catenin
Ctrl
IL1β
100 μm
L
βCatenin
aβCatenin
p-CREB
p-CEBPβ
p-AKT
p-NFκB
p-P38
p-ERK1/2
M
p-CREB/β-Actin
n.s.

Figure S2

Figure S2 (related to Figure 2) Interplay between the WNT-β-catenin and IL1-immune signaling pathways during the conversion of dermal fibroblasts to adipocytes

(**A**) KEGG/GO pathway analysis showing the top activated (red) or suppressed (blue) pathways during cell transition between indicated neonatal dFB clusters.

(**B**) Bubble plots showing the expression of indicated genes belonging to WNT, TGFβ, or immune/inflammation pathways across various neonatal dFB clusters.

(**C**) SCENIC analysis showing the top enriched transcriptional factors in neonatal dFB clusters.

(**D**) Neonatal dFBs were treated with WNT3A, Lithium, or IL1β for 48 hrs then subjected to bulk RNA-Seq. Heatmap showing mRNA expression of indicated genes**.**

(E) Venn diagram comparing genes up-regulated by IL1β and genes downregulated by WNT3A and lithium and top enriched pathways is shown in the lower panel**.**

(**F-I**) qRT-PCR analysis of the expression of indicated genes in neonatal dFBs treated with WNT3A and/or IL1β under undifferentiated condition (**F-G**) or adipocyte differentiation condition (**H-I**) (n=4/group).

(**J**) Neonatal dFBs were treated with IL1β for 48 hours then subjected to immunostaining analysis of active β-catenin (red) and DAPI (blue) (Scale bar, 100 μm). Note that β-catenin, upon being phosphorylated by GSK3, is targeted to proteasome for degradation, and the active β-catenin antibody we used specifically recognized the non-phosphorylated form of β-catenin, which cannot be degraded.

(**K-M**) Neonatal dFBs were pretreated with specific inhibitors followed with stimulation with IL1β for 45 mins. Cell extracts were subjected to phospho-blotting analysis (**K**) using indicated antibodies and quantified results of heatmap are shown in **L** (average of n=3/group). Bar graph showing ratio of p-CREB /β-Actin is shown in **M** (n=3/group).

All error bars indicate mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

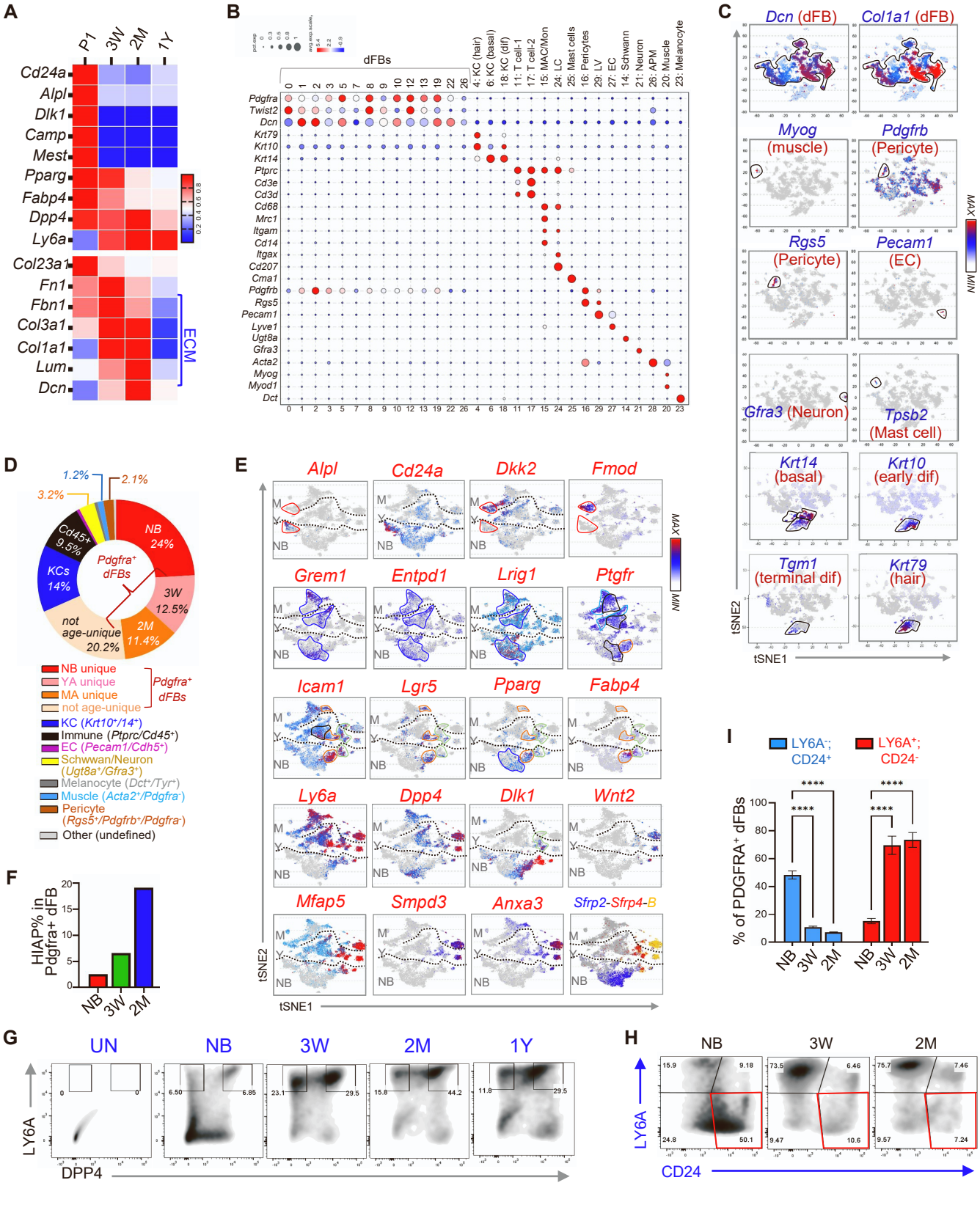
A
P1
3W
2M
1Y
Cd24a
Alpl
Dlk1
Camp
Mest
Pparg
Fabp4
Dpp4
Ly6a
Col23a1
Fn1
Fbn1
Col3a1
Col1a1
Lum
Dcn
ECM
B
dFBs
4: KC (hair)
6: KC (dif)
18: KC (dif)
11: T cell-1
17: T cell-2
15: MAC/Mon
24: LC
25: Mast cells
16: Pericytes
29: LV
27: EC
14: Schwann
21: Neuron
26: APM
20: Muscle
23: Melanocyte
Pdgfra
Twist2
Dcn
Krt79
Krt10
Krt14
Ptprc
Cd3e
Cd3d
Cd68
Mrc1
Itgam
Cd14
Itgax
Cd207
Cma1
Pdgfrb
Rgs5
Pecam1
Lyve1
Ugt8a
Gfra3
Acta2
Myog
Myod1
Dct
C
Dcn (dFB)
Col1a1 (dFB)
Myog (muscle)
Pdgfrb (Pericyte)
Rgs5 (Pericyte)
Pecam1 (EC)
Gfra3 (Neuron)
Tpsb2 (Mast cell)
Krt14 (basal)
Krt10 (early dif)
Tgm1 (terminal dif)
Krt79 (hair)
MAX
MIN
tSNE2
tSNE1
D
1.2%
2.1%
3.2%
Cd45+ 9.5%
NB 24%
KCs 14%
Pdgfra+ dFBs
3W 12.5%
2M 11.4%
not age-unique 20.2%
NB unique
YA unique
MA unique
not age-unique
Pdgfra+ dFBs
KC (Krt10+/14+)
Immune (Ptprc/Cd45+)
EC (Pecam1/Cdh5+)
Schwann/Neuron (Ugt8a+/Gfra3+)
Melanocyte (Dct+/Tyr+)
Muscle (Acta2+/Pdgfra-)
Pericyte (Rgs5+/Pdgfrb+/Pdgfra-)
Other (undefined)
E
Alpl
Cd24a
Dkk2
Fmod
Grem1
Entpd1
Lrig1
Ptgfr
Icam1
Lgr5
Pparg
Fabp4
Ly6a
Dpp4
Dlk1
Wnt2
Mfap5
Smpd3
Anxa3
Sfrp2-Sfrp4-B
M
Y
NB
MAX
MIN
tSNE2
tSNE1
F
HIAP% in Pdgfra+ dFB
NB
3W
2M
I
LY6A-; CD24+
LY6A+; CD24-
% of PDGFRA+ dFBs
****
NB
3W
2M
G
UN
NB
3W
2M
1Y
LY6A
DPP4
H
NB
3W
2M
LY6A
CD24

Figure S3

Figure S3 (related to Figure 3). Tracing dermal adipogenesis and fibrogenesis during skin maturation.

(A) Heatmap showing the mRNA expression kinetics (based on bulk RNAseq) of listed dFB/Ad marker or ECM genes in mouse skin at the indicated age.

(**B~C**) Bubble chart (**B**) and t-SNE plots(**C**) showing the expression of indicated marker genes in total skin cell clusters.

(**D**) Pie chart showing age-related changes in the percentage of various cell types, including Pdgfra+ dFBs (68%), Krt10/14+ keratinocytes (14%), Ptprc/Cd45+ immune cells (9.5%), Schwann/neurons (3.2%), pericytes/vascular smooth muscle cells (VSMC, 2.1%), Myog+ muscle cells (1.2%), melanocytes (0.7%), endothelial cells (EC, 0.6%), and arrector pili muscle (APM, 0.5%)

(**E**) t-SNE plots showing the expression of indicated marker genes in the reclustered *Pdgfra*$^{+}$ dFB clusters. Cells derived from different ages are separated with dotted lines. NB, newborn; Y, young (3 weeks of age); M, mature (2 months of age)

(**F**) Quantified percentage of HI-AP in indicated ages based on scRNA-seq (n=1/group).

(**G**) FACS plots showing the expression of DPP4 and LY6A in CD31^{-}CD45^{-}PDGFRA^{+} dFBs (representative of n=3/group).

(**H-I**) FACS plots (**H**) showing the expression of LY6A and DPP4 in CD31^{-}CD45^{-}PDGFRA^{+} dFBs, and quantified bar graphs of indicated cell population percentage are shown in **I** (n=3/group). All error bars indicate mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

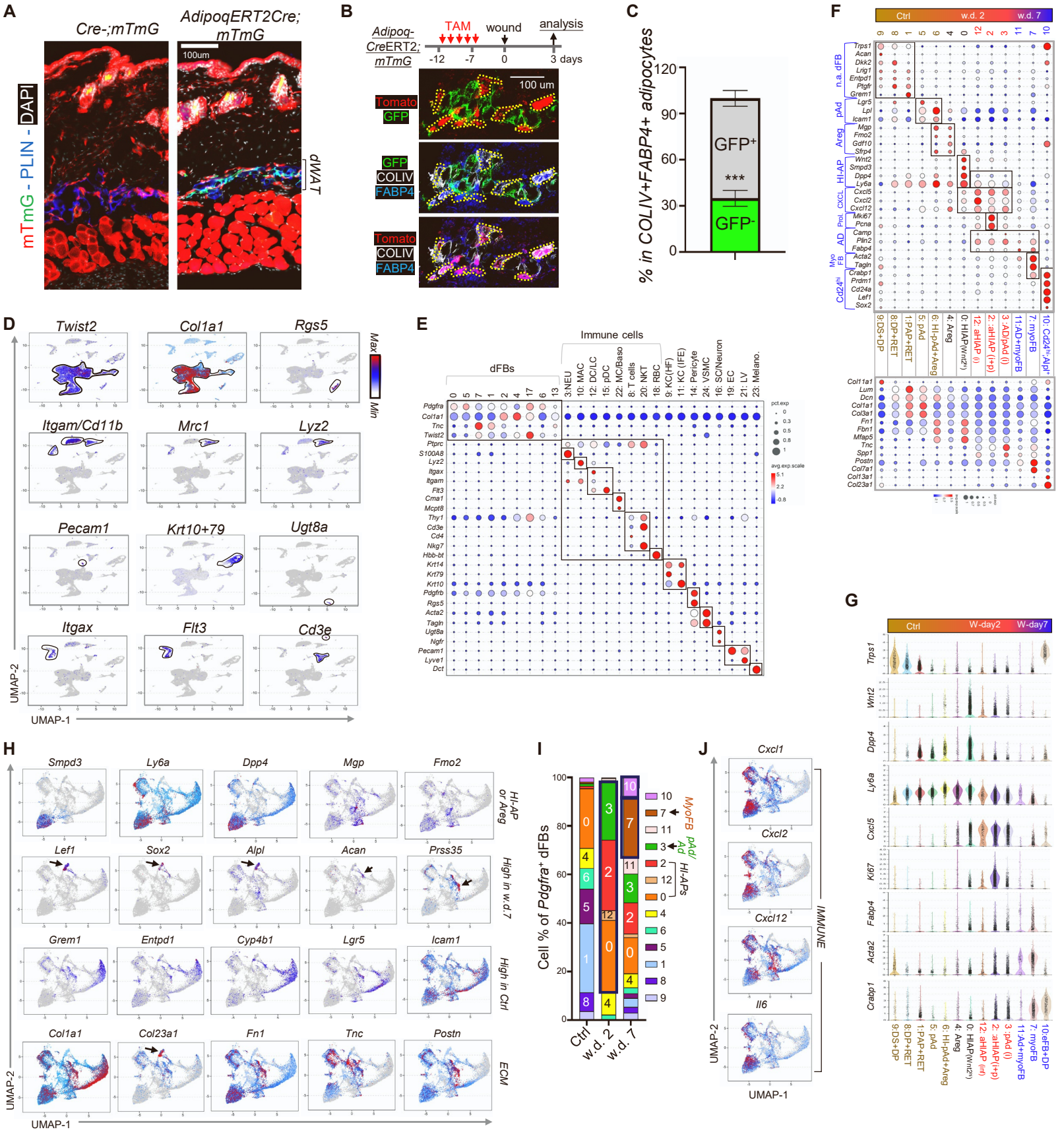
A
Cre-;mTmG
AdipoqERT2Cre; mTmG
100um
mTmG - PLIN - DAPI
dWAT
B
Adipoq-CreERT2; mTmG
TAM
wound
analysis
-12
-7
0
3 days
100 um
Tomato
GFP
GFP
COLIV
FABP4
Tomato
COLIV
FABP4
C
% in COLIV+FABP4+ adipocytes
120
90
60
30
0
GFP+
***
GFP-
F
Ctrl
w.d. 2
w.d. 7
n.a. dFB
pAd
Areg
HI-AP
CXCL
Prol.
AD
Myo FB
Cd24hi
Trps1
Acan
Dkk2
Lrig1
Entpd1
Ptgfr
Grem1
Lgr5
Lpl
Icam1
Mgp
Fmo2
Gdf10
Sfrp4
Wnt2
Smpd3
Dpp4
Ly6a
Cxcl5
Cxcl2
Cxcl12
Mki67
Pcna
Camp
Pln2
Fabp4
Tagln
Acta2
Crabp1
Prdm1
Cd24a
Lef1
Sox2
9:DS+DP
8:DP+RET
1:PAP+RET
5: pAd
6: HI-pAd+Areg
4: Areg
0: HIAP(Wnt2+)
12: aHIAP (i+p)
2: aHIAP (i+p)
3: ADj/pAd (i)
11:AD+myoFB
7: myoFB
10: Cd24hi Alpl+
Col1a1
Lum
Dcn
Col1a1
Col3a1
Fn1
Fbn1
Mfap5
Tnc
Spp1
Postn
Col7a1
Col13a1
Col23a1
D
Twist2
Col1a1
Rgs5
Max
Min
Itgam/Cd11b
Mrc1
Lyz2
Pecam1
Krt10+79
Ugt8a
Itgax
Flt3
Cd3e
UMAP-2
UMAP-1
E
Immune cells
dFBs
0
5
7
1
2
4
17
6
13
3:NEU
10: MAC
12: DC/ILC
15: pDC
22: MC/Baso
8: T cells
20: NKT
18: RBC
9: KC(HF)
11: KC (IFE)
14: Pericyte
24: VSMC
16: SC/Neuron
19: EC
21: LV
23: Melano.
Pdgfra
Col1a1
Tnc
Twist2
Ptprc
S100A8
Lyz2
Itgax
Itgam
Flt3
Cma1
Mcpt8
Thy1
Cd3e
Cd4
Nkg7
Hbb-bt
Krt14
Krt79
Krt10
Pdgfrb
Rgs5
Acta2
Tagln
Ugt8a
Ngfr
Pecam1
Lyve1
Dct
pct.exp
0
0.3
0.5
0.8
1
avg.exp.scale
5.1
2.2
-0.8
G
Ctrl
W-day2
W-day7
Trps1
Wnt2
Dpp4
Ly6a
Cxcl5
Ki67
Fabp4
Acta2
Crabp1
9:DS+DP
8:DP+RET
1:PAP+RET
5: pAd
6: HI-pAd+Areg
4: Areg
0: HIAP(Wnt2+)
12: aHIAP (inf)
2: aHIAP (i+p)
3 :pAd (i)
11:Ad+myoFB
7: myoFB
10:eF&D+DP
H
Smpd3
Ly6a
Dpp4
Mgp
Fmo2
HI-AP or Areg
Lef1
Sox2
Alpl
Acan
Prss35
High in w.d.7
Grem1
Entpd1
Cyp4b1
Lgr5
Icam1
High in Ctrl
Col1a1
Col23a1
Fn1
Tnc
Postn
ECM
UMAP-2
UMAP-1
I
Cell % of Pdgfra+ dFBs
100
80
60
40
20
0
Ctrl
w.d. 2
w.d. 7
10
7
11
3
2
12
0
4
6
5
1
8
9
MyoFB
pAd/ Ad
HI-APs
J
Cxcl1
Cxcl2
Cxcl12
Il6
IMMUNE
UMAP-2
UMAP-1

Figure S4

Figure S4 (related to Figure 4) Tracing dermal adipogenesis and fibrogenesis during skin wound healing.

(**A**) *Adipoq-Cre*ERT2;mTmG mice were administrated with tamoxifen (TAM) from day 12 ~ 7 prior to wounding, and unwounded control tissues were collected for immunostaining of COLIV (white), and GFP (green) and tomato (red) were overlaid to confirm that $PLIN1^+$ adipocytes were successfully labeled by GFP (representative of n=3/group). Scale bar, 100 μm.

(**B**) A*dipoq-Cre*ERT2*;mTmG* mice were administrated with tamoxifen (TAM) from day 12 ~ 7 prior to wounding, and wound tissues were collected at w.d. 3 for immunostaining of COLIV (white), and GFP (green) and tomato (red) were overlaid. Scale bar, 100 μm.

(**C**) Stacked bar graphs showing the quantification of the percentage of GFP^+ or GFP^- cells in $COLIV^+FABP4^+$ adipocytes as shown in **B** (n=3/group).

(**D-E**) W.d. 2, W.d. 7 and unwounded control skin samples were collected and subjected to scRNA-seq analysis. UMAP plot (**D**) or bubble chart (**E**) or showing the expression of indicated marker genes for each cluster.

(**F-J**) *Pdgfra*$^+$ dFBs were reclustered as shown in Fig. 4G. Bubble plots (**F**), violin plots (**G**), and UMAP plots (**H**) showing the expression of indicated marker genes. Stacked bar graph (**I**) showing the percentage of each dFB cluster in ctrl, w.d. 2 or w.d. 7 samples. UMAP plots showing the expression of inflammatory cytokines/chemokines are shown in **J**.

All error bars indicate mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

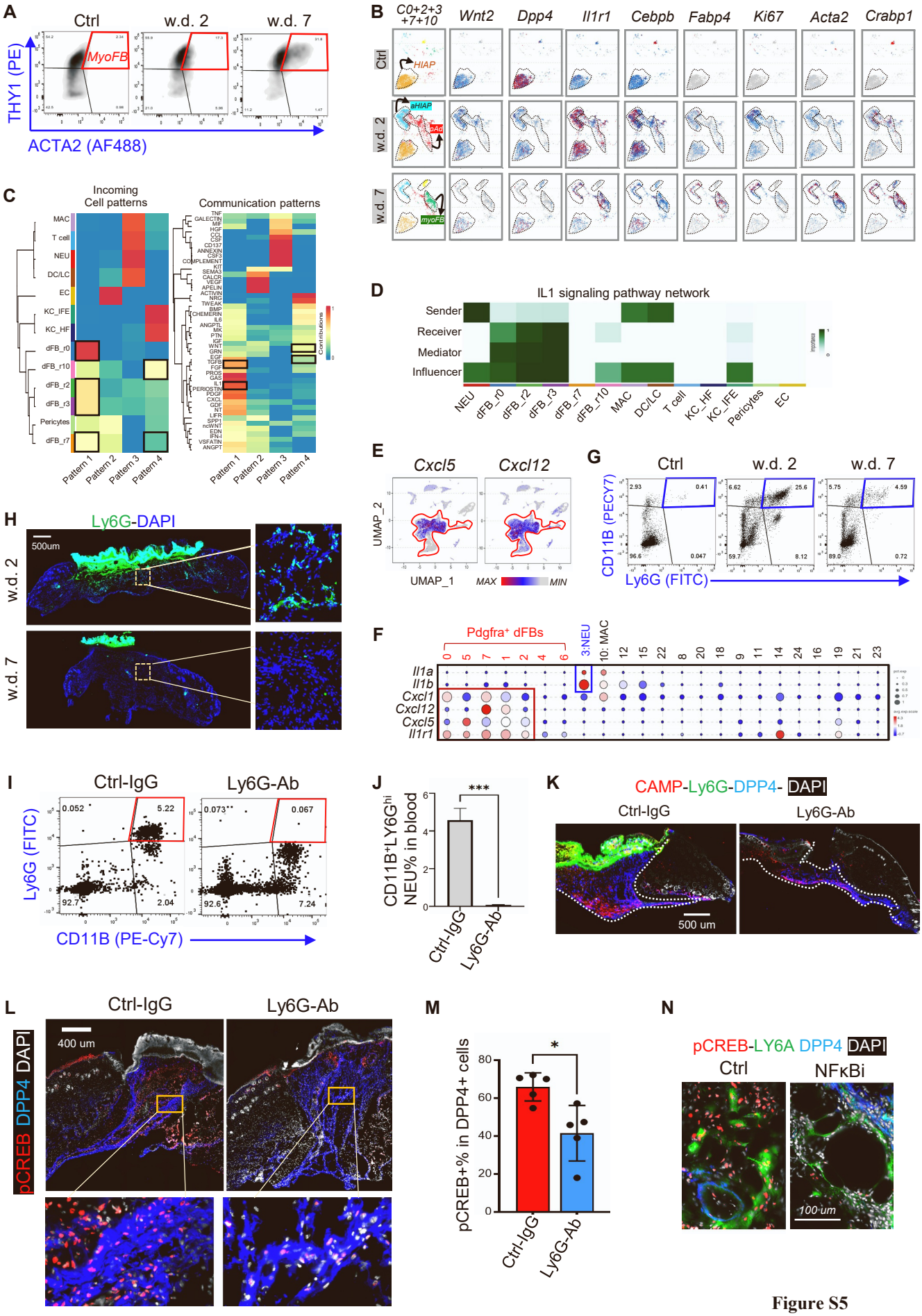
A
Ctrl
w.d. 2
w.d. 7
MyoFB
THY1 (PE)
ACTA2 (AF488)
B
C0+2+3 +7+10
Wnt2
Dpp4
Il1r1
Cebpb
Fabp4
Ki67
Acta2
Crabp1
Ctrl
w.d. 2
w.d. 7
HIAP
aHIAP
dAd
myoFB
C
Incoming Cell patterns
Communication patterns
MAC
T cell
NEU
DC/LC
EC
KC_IFE
KC_HF
dFB_r0
dFB_r10
dFB_r2
dFB_r3
Pericytes
dFB_r7
Pattern 1
Pattern 2
Pattern 3
Pattern 4
Contributions
D
IL1 signaling pathway network
Sender
Receiver
Mediator
Influencer
NEU
dFB_r0
dFB_r2
dFB_r3
dFB_r7
dFB_r10
MAC
DC/LC
T cell
KC_HF
KC_IFE
Pericytes
EC
E
Cxcl5
Cxcl12
UMAP_2
UMAP_1
MAX
MIN
G
Ctrl
w.d. 2
w.d. 7
CD11B (PECY7)
Ly6G (FITC)
2.93
0.41
96.6
0.047
6.62
25.6
59.7
8.12
5.75
4.59
89.0
0.72
H
Ly6G-DAPI
500um
w.d. 2
w.d. 7
F
Pdgfra+ dFBs
3:NEU
10: MAC
Il1a
Il1b
Cxcl1
Cxcl12
Cxcl5
Il1r1
I
Ctrl-IgG
Ly6G-Ab
Ly6G (FITC)
CD11B (PE-Cy7)
0.052
5.22
92.7
2.04
0.073**
0.067
92.6
7.24
J
CD11B+LY6Ghi NEU% in blood
***
Ctrl-IgG
Ly6G-Ab
K
CAMP-Ly6G-DPP4- DAPI
Ctrl-IgG
Ly6G-Ab
500 um
L
Ctrl-IgG
Ly6G-Ab
pCREB DPP4 DAPI
400 um
M
pCREB+% in DPP4+ cells
*
Ctrl-IgG
Ly6G-Ab
N
pCREB-LY6A DPP4 DAPI
Ctrl
NFκBi
100 um

Figure S5

Figure S5 (related to Figure 5). Wound-induced adipogenesis is regulated by neutrophils and the IL1 signaling axis.
(**A**) FACS plots showing $THY1^+SMA^+$ myoFBs in control, w.d. 2 and w.d. 7 skin samples (representative of n=3/group).
(**B**) UMAP plots showing how the expression of indicated genes were dynamically changed in C0, C2, C3, C7 and C10 dFB clusters during wound healing.
(**C**) The inferred incoming communication patterns of skin cells showing the correspondence between the inferred latent patterns and cell groups and signaling molecules.
(**D**) Heatmap showing the relative importance of each cell cluster as signal sender, receiver, mediator or influencer in IL1β signaling network.
(**E**) tSNE plots showing the expression of *Cxcl5* and *Cxcl12* in all wound cell clusters.
(**F**) Bubble plot showing the expression and/or distribution of indicated genes in all wound cell clusters.
(**G**) FACS plots analyzing the presence of neutrophils ($Ly6G^+CD11B^+$) (representative of n=3/group).
(**H**) Immunostaining of Ly6G (green) and DAPI (white) of w.d. 2 and w.d. 7 skin tissues. Scale bar, 500 μm.
(I-M) Mice were injected with isotype (ctrl) or anti-Ly6G antibodies to deplete neutrophils. FACS plots (**I**) and quantified bar graph (**J**) showing the presence of $Ly6G^+CD11B^+$ neutrophils in blood samples from mice injected with ctrl-IgG or antiLy6G antibody (n=3/group). (**K-M**) Skin wounds were collected at w.d. 3. for immunostaining of CAMP (red), Ly6G (green) and DPP4(blue) (**K**), or pCREB (red) and DPP4 (blue) (**L**). Nuclei were stained with DAPI (white). Scale bar, 400 μm. (**M**). Bar graphs showing quantified percentage of pCREB+ cells in DPP4+ cells (n=5/group).
(**N**) Mice were injected *i.p.* with DMSO (ctrl) or NF-κB inhibitor and skin wounds were collected for immunostaining of pCREB (red), LY6A (green) and DPP4 (blue). Scale bar, 100 μm.
All error bars indicate mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

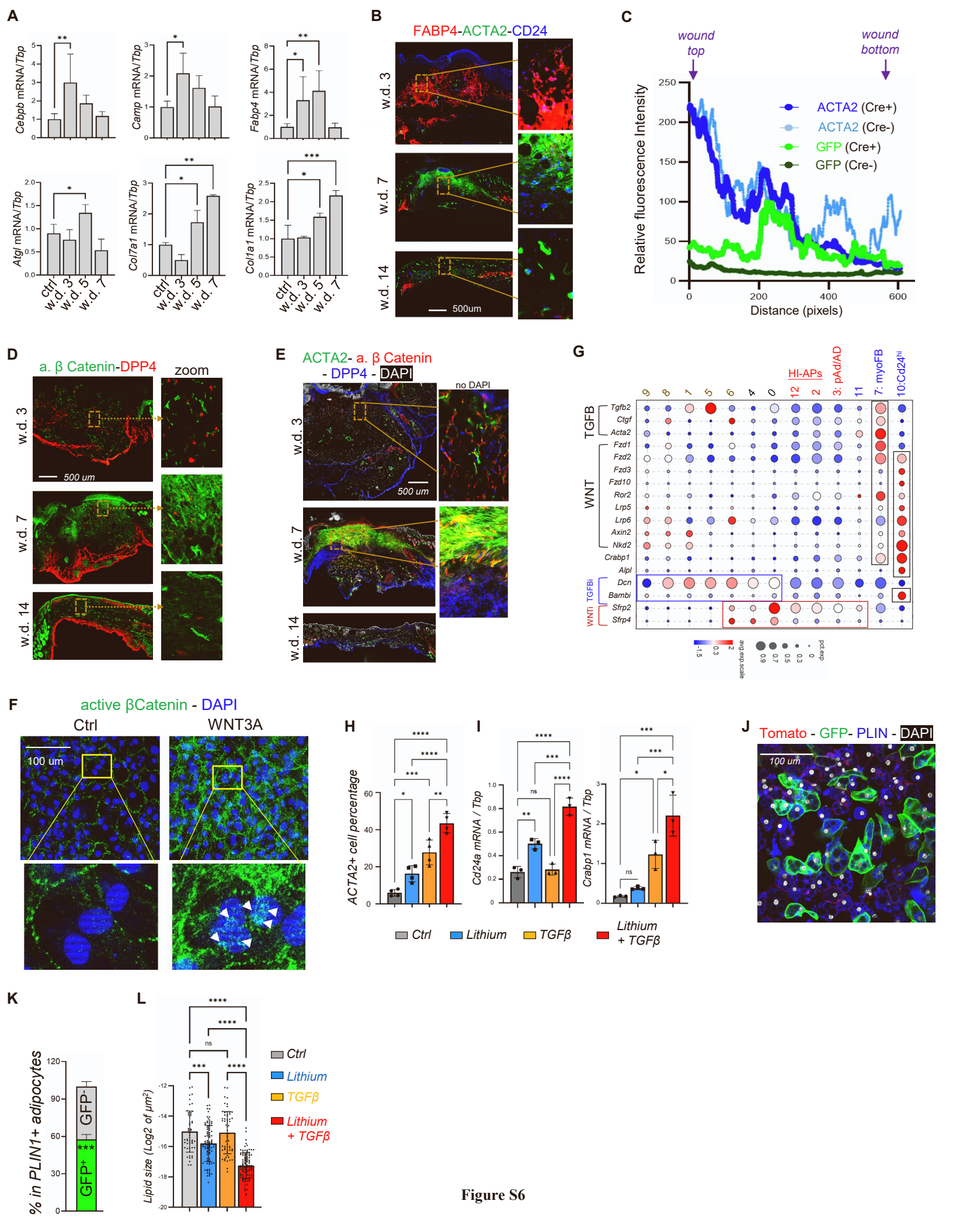
A
Cebpb mRNA/Tbp
Camp mRNA/Tbp
Fabp4 mRNA/Tbp
Atgl mRNA/Tbp
Col7a1 mRNA/Tbp
Col1a1 mRNA/Tbp
ctrl
w.d. 3
w.d. 5
w.d. 7
B
FABP4-ACTA2-CD24
w.d. 3
w.d. 7
w.d. 14
500um
C
wound top
wound bottom
Relative fluorescence Intensity
Distance (pixels)
ACTA2 (Cre+)
ACTA2 (Cre-)
GFP (Cre+)
GFP (Cre-)
D
a. β Catenin-DPP4
zoom
w.d. 3
w.d. 7
w.d. 14
500 um
E
ACTA2- a. β Catenin
- DPP4 - DAPI
no DAPI
w.d. 3
w.d. 7
w.d. 14
500 um
G
HI-APs
3: pAd/AD
7: myoFB
10:Cd24hi
TGFB
WNT
WNTi TGFBi
Tgfb2
Ctgf
Acta2
Fzd1
Fzd2
Fzd3
Fzd10
Ror2
Lrp5
Lrp6
Axin2
Nkd2
Crabp1
Alpl
Dcn
Bambi
Sfrp2
Sfrp4
avg.exp.scale
pct.exp
F
active βCatenin - DAPI
Ctrl
WNT3A
100 um
H
ACTA2+ cell percentage
I
Cd24a mRNA / Tbp
Crabp1 mRNA / Tbp
Ctrl
Lithium
TGFβ
Lithium + TGFβ
J
Tomato - GFP- PLIN - DAPI
100 um
K
% in PLIN1+ adipocytes
GFP-
GFP+
L
Lipid size (Log2 of μm²)
Ctrl
Lithium
TGFβ
Lithium + TGFβ

Figure S6

Figure S6 (related to Figure 6) Activation of WNT and TGFβ signaling pathways in adipocyte lineage cells during the wound-proliferative phase.

(**A**) Bar graphs showing the mRNA expression kinetics of listed genes during skin wound healing (n=3 per group). All error bars indicate mean ± SEM. *p <0.05, **p < 0.01, ***p < 0.001.

(**B**) Mouse wound tissues were immuno-stained with indicated antibodies. Scale bar, 500 μm.

(**C**) Quantified intensity profiles of Fig.6B image showing signals from ACTA2 or GFP in Cre- or Cre+ wounds from wound top to bottom (representative of n=3/group).

Supplemental result for Fig. S6C: Indeed, $ACTA2^{+}GFP^{-}$ myofibroblasts were also detected in the wound, indicating that myofibroblasts can also be derived from other cell types, such as papillary and reticular fibroblasts, HI-APs and preadipocytes that do not express Adipoq.

(**D-E**) Wound tissues were stained with active β-catenin, ACTA2 and/or DPP4 as indicated. Scale bar, 500 μm.

(**F**) Primary dFBs were treated with WNT3A (10ng/mL) or vehicle control for 24 hrs, and cells were collected for immunostaining analysis with active β-catenin antibody (green), and nuclei were counterstained with DAPI (blue).

However, it is difficult to distinguish β-catenin within nuclei from that within the cytosol in cells that are tightly packed within whole wound tissue. Note that clear nuclear signal of β-Catenin could be detected in cultured primary dFBs stimulated with WNT ligand, although the majority of active β-Catenin was retained in the cytosol or membrane (Fig. S6F).

(**G**) Bubble plots showing the expression of indicated genes across various dFB clusters shown in Fig. 4G-H. **Supplemental results for Fig. S6G:** Interestingly, we found that all dFB clusters, except the r7 and r10 dFBs, expressed several TGFβ and/or WNT pathway inhibitor genes (Fig. S6G), which may dampen the responsiveness of these cells to external TGFβ and/or WNT signaling.

(**H-I**) Differentiated mature adipocytes were treated with Lithium ± TGFβ2 as shown in Fig. 6E. (**H**) quantification of the percentage of ACTA2+ cells (n=4/group). (**I**) qRT-PCR analyses of the expression of indicated genes (n=3/group).

(**J-L**) Mature adipocytes were differentiated from dFBs isolated from *Adipoq-Cre*ERT2;mTmG mice in the presence of tamoxifen. (**J**) Cells were immunostained with PLIN1 (blue) to determine the efficiency of the genetic labeling of PLIN1+ adipocytes by GFP. Scale bar, 100 μm. (**K**) Stacked bar graphs showing the quantification of the percentage of GFP^{+} or GFP^{-} cells in $PLIN1^{+}$ adipocytes (n=3/group). (**L**) Bar graph showing the quantification of changes in lipid droplet size as shown n Fig. 6I (LD quantified from n=5 fields/group).

Results for J-K: Approximately 60% of the differentiated $PLIN1^{+}$ adipocytes were successfully labeled with membrane-bound GFP.

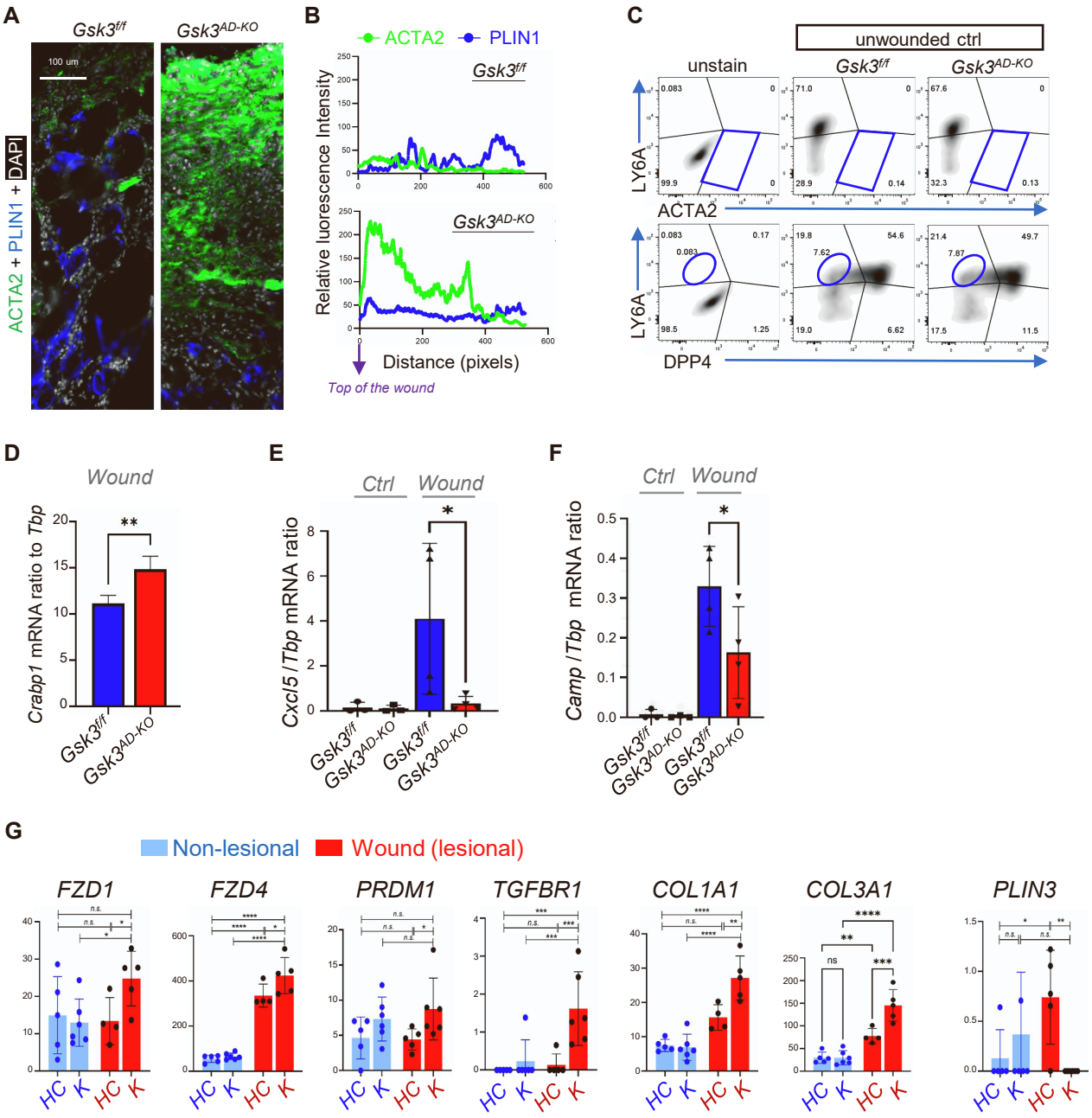

Figure S7 (related to Figure 7) WNT activation in adipocytes promotes myofibroblast formation during wound healing.

(**A-B**) Skin wound tissues (w.d. 7) were collected from wildtype control (*Gsk3*$^{flox/flox}$) or *Gsk3* adipocyte conditional knockout (*Adipoq-Cre*ERT2;*Gsk3*$^{flox/flox}$) mice. (**A**) Wound tissues were stained with ACTA2 (green) and DPP4 (blue), and DAPI in white. Scale bar, 100 μm. (**B**) Quantified intensity profiles of Fig.S6A showing signals from all three fluorescent channels from wound top to bottom (representative of n=3/group).

(**C-F**) Unstained control or unwounded control FACS plots for Fig. 7E showing the percentage of ACTA2^{+}LY6A myofibroblasts or LY6A^{+}DPP4^{-} in PDGFRA^{+} dFBs (representative of n=3/group). (**D**) qRT-PCR analyses showing the mRNA expression of *Crabp1* in the wound granular tissues (n=3/group). (**E-F**) qRT-PCR analysis of *Cxcl5* (**E**) and *Camp* (**F**) (n=3/group).

(**G**) Bar graphs showing relative mRNA expression (based on RNA-seq FPKM values) of listed genes in non-lesional and wound tissues from health control (HC) or keloid (K) individuals (n=4~5/group).

All error bars indicate mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.