Supplemental Information

Age-related loss of innate immune antimicrobial function of dermal fat is mediated by TGFβ

Ling-juan Zhang*, Stella Xiang Chen, Christian F. Guerrero-Juarez, Fengwu Li, Yun Tong, Yuqiong Liang, Marc Liggins, Xu Chen, Hao Chen, Min Li, Tissa Hata, Ye Zheng, Maksim V. Plikus, Richard L. Gallo*

*corresponding authors: lingjuan.zhang@xmu.edu or l6zhang@ucsd.edu (L.J.Z.) or rgallo@ucsd.edu (R.L.G)

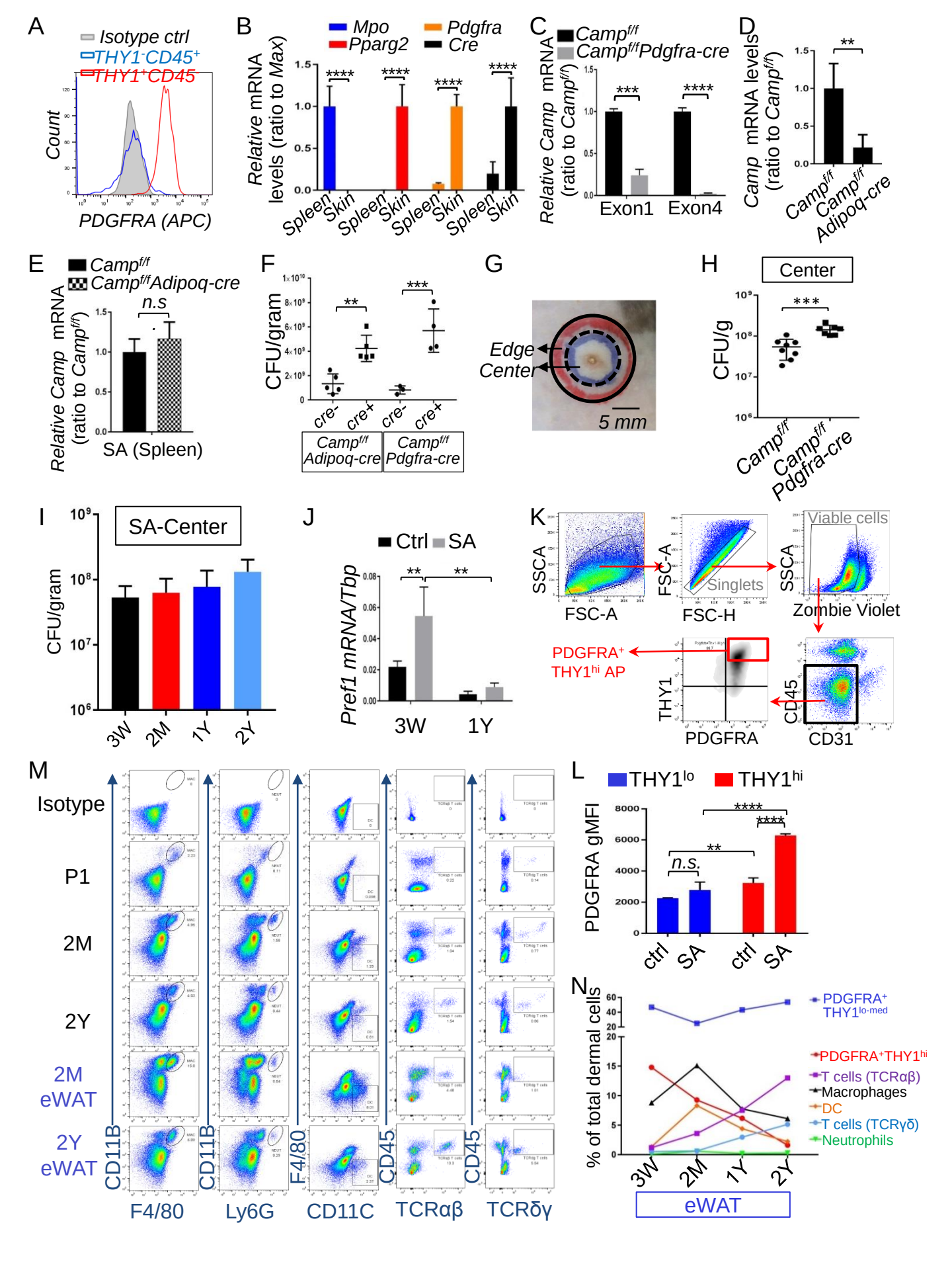

A
Isotype ctrl
THY1-CD45+
THY1+CD45-
Count
PDGFRA (APC)
B
Mpo
Pparg2
Pdgfra
Cre
Relative mRNA levels (ratio to Max)
Spleen
Skin
C
Campf/f
Campf/fPdgfra-cre
Relative Camp mRNA (ratio to Campf/f)
Exon1
Exon4
D
Camp mRNA levels (ratio to Campf/f)
Campf/f
Campf/f Adipoq-cre
E
Campf/f
Campf/fAdipoq-cre
n.s
Relative Camp mRNA (ratio to Campf/f)
SA (Spleen)
F
CFU/gram
cre-
cre+
Campf/f Adipoq-cre
Campf/f Pdgfra-cre
G
Edge
Center
5 mm
H
Center
CFU/g
Campf/f
Campf/f Pdgfra-cre
I
SA-Center
CFU/gram
3W
2M
1Y
2Y
J
Ctrl
SA
Pref1 mRNA/Tbp
3W
1Y
K
SSCA
FSC-A
FSC-H
Singlets
Viable cells
Zombie Violet
PDGFRA+ THY1hi AP
THY1
PDGFRA
CD45
CD31
L
THY1lo
THY1hi
PDGFRA gMFI
ctrl
SA
M
Isotype
P1
2M
2Y
2M eWAT
2Y eWAT
CD11B
F4/80
Ly6G
CD11C
CD45
TCRαβ
TCRδγ
N
% of total dermal cells
PDGFRA+ THY1lo-med
PDGFRA+THY1hi
T cells (TCRαβ)
Macrophages
DC
T cells (TCRγδ)
Neutrophils
eWAT

Figure S1 (related to Figure 1). Age-dependent loss of dermal PDGFRA^{+}THY1^{high} adipocyte progenitors and their antimicrobial response. (A). FACS analysis of surface expression of PDGFRA (APC) on Thy1^{-}CD45^{+} myeloid immune cells or Thy1^{+} CD45^{-} dermal non-immune cells in cells isolated from *S. aureus* infected skin. Isotype control is shown as background staining. (**B).** The spleen or skin tissues from *S. aureus* infected *Camp$^{f/f}$*;*Pdgfra-cre* mice were subjected to RTqPCR analyses for *Mpo* (neutrophil marker), *Pparg2* (adipocyte marker), *Pdgfra* (preadipocyte marker) or *Cre* (n=3~4/group). (**C**). Skin tissues collected from *Camp$^{f/f}$* (WT) or *Camp$^{f/f}$*;*Pdgfra-cre* were subjected to RTqPCR analyses for *Camp* mRNA expression using primers specific for exon1 or exon4 of *Camp* as indicated. Relative fold changes to *Camp$^{f/f}$* were shown (n=3~4/group). (**D**). *Camp$^{f/f}$* (WT) or *Camp$^{f/f}$*;*Adipoq-cre* littermate mice (7 weeks of age) were infected intradermally with *S. aureus* (SA), and *Camp* mRNA expression levels (ratio to housekeeping gene *Tbp*) at control or infected site were measured by RTqPCR analyses (n=3~5/group). (**E**). Spleen tissue from infected *Camp$^{f/f}$* (WT) or *Camp$^{f/f}$*;*Adipoq-cre* littermate mice were subjected to RTqPCR analysis for *Camp* mRNA expression (n=3~5/group). (**F**). *Camp* deletion in Pdgfra^{+} fibroblasts (*Camp$^{f/f}$*;*Pdgfra-cre*) or adipocytes (*Camp$^{f/f}$*;*Adipoq-cre*) lead to increased *S. aureus* susceptibility as measured by bacterial CFU counts from the whole infected skin (n=5/group). (**G-H**) *Camp$^{f/f}$* (WT) or *Camp$^{f/f}$*;*Pdgfra-cre* littermate mice (2 month) were infected intradermally with *S. aureus*, and skin samples were collected at day3 for analysis. (**G**). Gross picture of infected skin. Solid circle marked infected area (11mm in diameter), and dotted circle marked infection center (6 mm in diameter). Area within the solid line was collected as infection center and area between the solid and dotted line was collected as infection edge. **H**. Bacterial CFU was measured from the infection center area (n=6~7/group). **I.** 3 weeks, 2 months, 1 year or 2 years wildtype mice were infected i.d. with *S. aureus*, and bacteria CFU were measured from the infection center area as indicated (n=4~5/group). (**J**). 3 weeks or 1 year old WT mice were infected with SA and *Pref1* expression in the infected skin or controls skin were analyzed by RTqPCR (ratio to *Tbp*) (n=3~5/group). (**K**). FACS gating strategies to gate CD31^{-}CD45- PDGFRA^{+}Thy1^{hi} adipocyte progenitors from total dermal cells. (**L**). FACS quantification of the geometric MFI (gMFI) of PDGFRA surface expression in Thy1^{lo} or Thy1^{hi} CD31^{-}CD45-PDGFRA^{+} dFB in control or SA-infected skin (day1) (n=3~4/group). (**M**). Representative FACS plot for gating myeloid-derived skin resident immune cells, including CD11B+F4/80+ macrophage, CD11B+Ly6G^{hi} neutrophils, CD11C+F4/80- dendritic cells (DC), CD45+TCRγδ+ T cells and CD45+TCRαβ+ T cells in total viable single cells isolated from skin dermis (n=3~4/group). (**N**). Age-dependent changes of the percentage of THY1$^{low\text{-}med}$PDGFRA^{+} fibroblasts, THY1^{hi}PDGFRA^{+} fibroblasts, .CD11B^{+}F4/80^{+} macrophages, CD11B^{+}Ly6G+ neutrophils, CD11C^{+}F4/80^{-} dendritic cells, CD45^{+}TCRγδ+ T cells and CD45^{+}TCRαβ+ T cells in total viable single cells isolated from eWAT as indicated (n=3~4/group). All error bars indicate mean ± s.e.m. * P<0.05, ** P<0.01, *** P<0.001 (one way Anova).

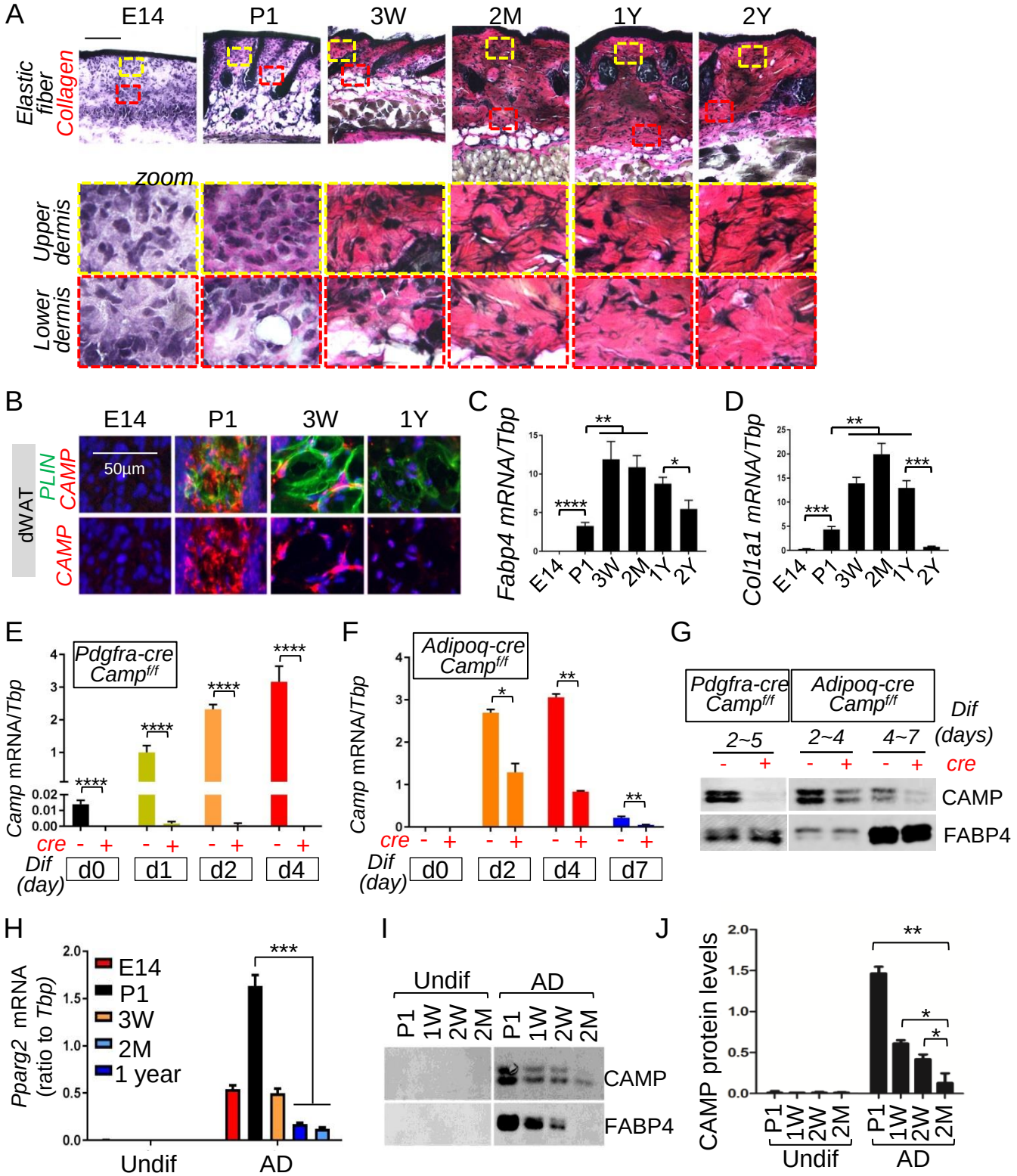

A
E14
P1
3W
2M
1Y
2Y
Elastic fiber
Collagen
zoom
Upper dermis
Lower dermis
B
E14
P1
3W
1Y
dWAT
PLIN
CAMP
50μm
CAMP
C
Fabp4 mRNA/Tbp
D
Col1a1 mRNA/Tbp
E
Pdgfra-cre Camp f/f
Camp mRNA/Tbp
cre
Dif (day)
d0
d1
d2
d4
F
Adipoq-cre Camp f/f
d7
G
Pdgfra-cre Camp f/f
Adipoq-cre Camp f/f
Dif (days)
2~5
2~4
4~7
CAMP
FABP4
H
Pparg2 mRNA (ratio to Tbp)
E14
P1
3W
2M
1 year
Undif
AD
I
Undif
AD
P1
1W
2W
2M
CAMP
FABP4
J
CAMP protein levels

Figure S2 (related to Figure 2). Loss dermal immature fat and adipogenic-antimicrobial function of primary dFB during development and aging of mouse skin. (**A**). Elastic and collagen staining of skin sections from mice at indicated age (representative of n=3~6/group). Elastic fiber is shown as black cytoplasmic staining. Nuceli are black nuclear staining. Collagen is red. Note that while dermal collagen increased during post-natal development and aging, elastic fiber also in dFB also developed postnatally during adulthood. During aging, elastic fiber became less dense in the lower dermis, whereas dFB in the upper dermis remained heavily packed with elastic fiber through adulthood and aging. (**B**). Representative cathelicidin staining in dWAT of mouse skin at indicated ages (n=3~5/age group). Scale, 50 μm. (**C-D**). RTqPCR analyses showing the kinetics of *Fabp4* (**C**) or *Col1a1* (**D**) mRNA expression (ratio to housekeeping gene *Tbp*) in mouse skin at indicated age (n=3/group). (**E-G**). Primary dermal dFB isolated from *Camp*$^{f/f}$ (wildtype *cre*- control), *Camp*$^{f/f}$;*Pdgfra-cre*+ or *Camp*$^{f/f}$;*Adipoq-cre*+ skin were differentiated to adipocyte in vitro. (**E-F**) *Camp* mRNA expression during adipocytes (AD) differentiation in time course as indicated (n=3/group). (**G**). Western blots measuring cathelicidin and FABP protein secretion during AD differentiation. (**H).** RTqPCRs measuring *Pparg2 mRNA* expression in dFBs at indicated age in undifferentiated or differentiated adipocytes (AD) (n=3/age group). (**I**): Westernblots measuring CAMP and FABP protein secretion from dFB at indicated age from differentiating adipocytes. (**J**). Quantification of CAMP protein secretion by dot blots (n=3/group). All error bars indicate mean ± s.e.m. * P<0.05, ** P<0.01, *** P<0.001 (one way Anova).

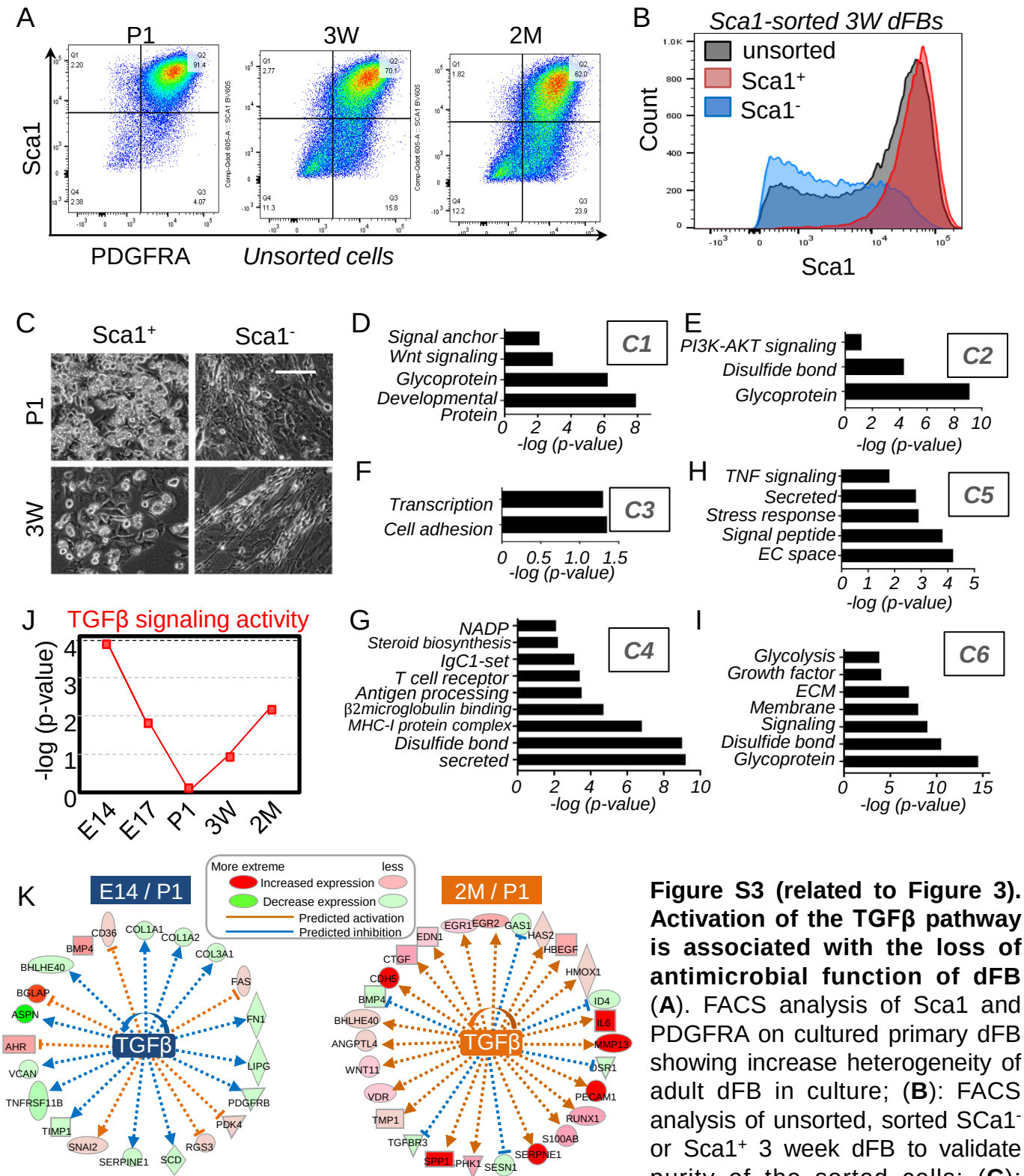


Figure S3 (related to Figure 3). Activation of the TGFβ pathway is associated with the loss of antimicrobial function of dFB (**A**). FACS analysis of Sca1 and PDGFRA on cultured primary dFB showing increase heterogeneity of adult dFB in culture; (**B**): FACS analysis of unsorted, sorted SCa1^{-} or Sca1^{+} 3 week dFB to validate purity of the sorted cells; (**C**): Purified SCa1^{+} or SCa1^{-} P1 or 3W dFB were differentiated to adipocytes. Phase contrast images of day4 differentiated cells showing that SCa1^{+} but not SCa1^{-} P1 or 3W dFB can differentiate into adipocytes. (**D-I**).Gene ontologies (GOs) of six groups of differentially expressed genes in primary dFB over developmental time course (C1~C6 as shown in Fig. 3B). (**J**). Comparison analysis by IPA showing p values of TGFβ signaling activity of dFB at indicated compared to P1 dFB. (**K**). TGFβ was predicted to be inhibited in E14 cells compared to P1 cells whereas TGFβ was predicted to be activated in 2M cells compared to P1 cells by IPA analysis.

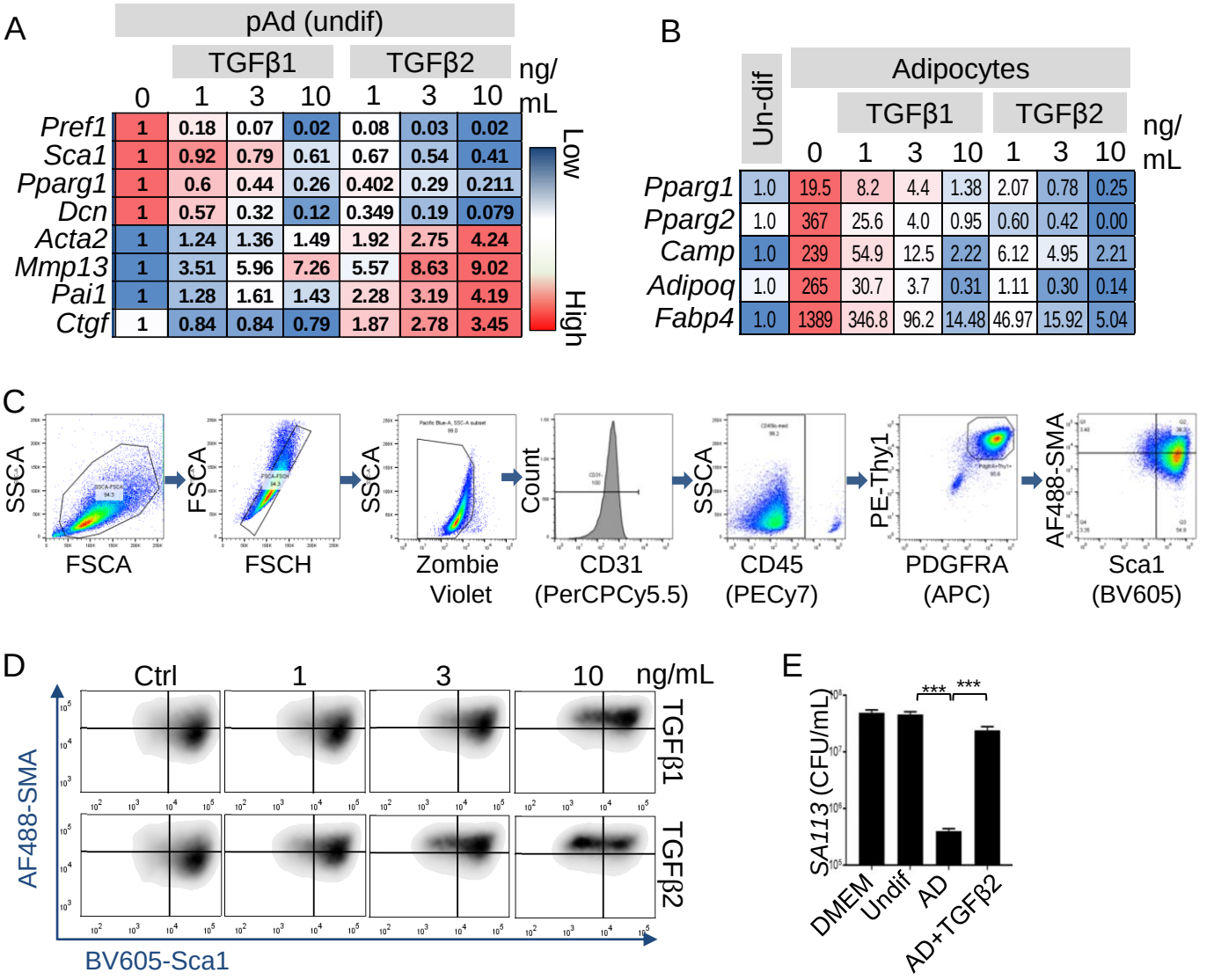


Figure S4 (related to Figure 4). TGFβ2 drives a loss of adipogenic-antimicrobial function of neonatal dFB. (**A**). Heatmap showing the dose-dependent effect of TGFβ1 or TGFβ2 in suppressing the expression of listed pro-adipogenic genes and inducing the expression of listed pro-fibrotic genes in undifferentiated neonatal dFB. (**B**). Heatmap showing the dose-dependent effect of TGFβ1 or TGFβ2 in suppressing the induction of Camp and other adipocyte genes in differentiating neonatal dFB. (**C**). FACS gating strategies to analyze SMA and Sca1 expression on PDGFRA+THY1+ dFB in culture. (**D**). FACS plots for Sca1 and SMA in neonatal dFB treated 2 days with indicated doses of TGFβ1 or TGFβ2 as indicated. (**E**). TGFβ2 abolished the antimicrobial function of adipocytes against *SA113*. CFU count of *SA113 in* conditioned medium from undifferentiated P1-dFB or differentiated adipocytes treated with or without TGFβ2 as indicated (n=3~5/group). All error bars indicate mean ± s.e.m. * P<0.05, ** P<0.01, *** P<0.001 (one way Anova).

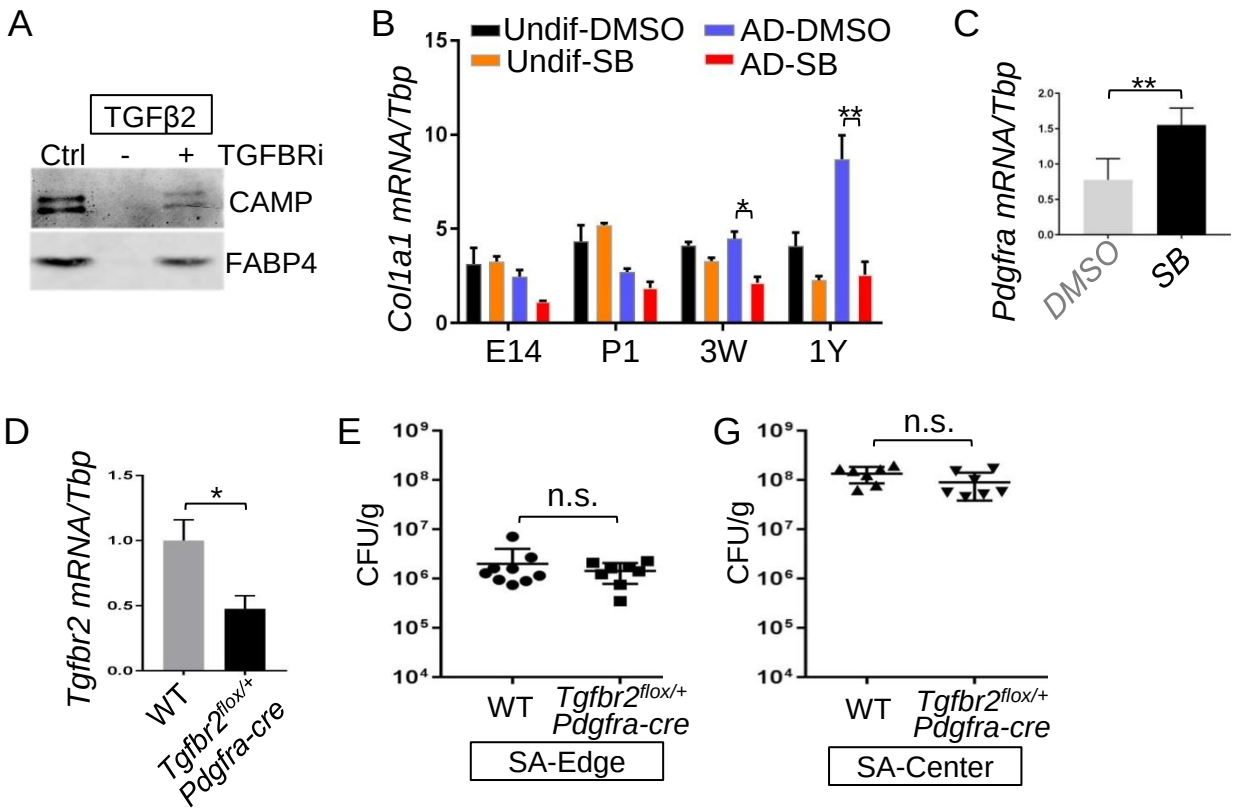
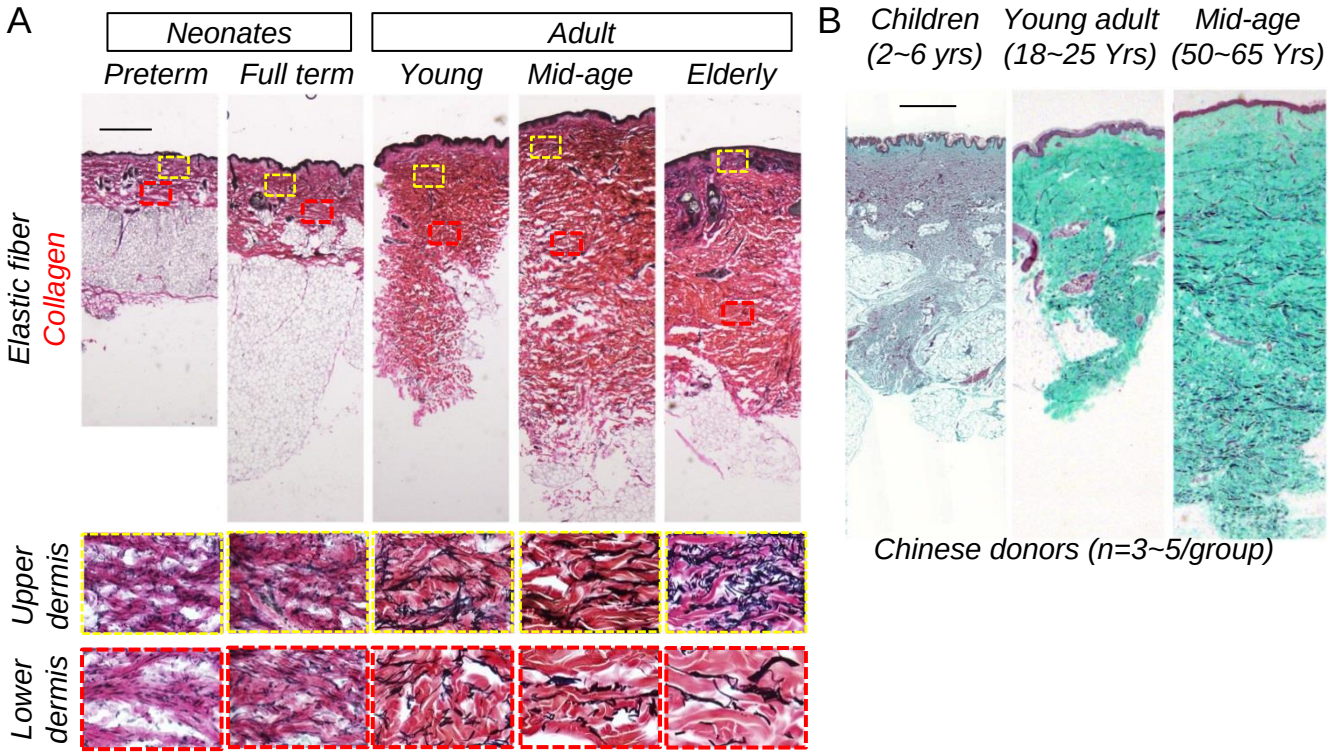


Figure S5 (related to Figure 5 and Figure 6). Inhibition of TGFBR function boosts dFB adipogenic potential and enhances resistance to skin infection. (**A**). Neonatal dFB were pretreated with TGFBR inhibitor (SB431542) then treated with TGFβ2. Western blots measuring cathelicidin (CAMP) and FABP protein secretion from differentiating adipocytes. (**B**). RTqPCR analyses of *Col1a1* expression in undifferentiated or adipocyte-differentiated dermal fibroblasts treated with SB431542 or DMSO control. All error bars indicate mean ± s.e.m. * P<0.05, ** P<0.01 (one way Anova). (**C**). 1 year old C57BL6 mice were infected intradermally with *S. aureus* then treated intradermally with DMSO or SB431542 at day 1 and 2, and skin biopsies were collected at day 3 for RTqPCR analysis of *Pdgfra*, mRNA expression in the infected skin treated with DMSO or SB indicated (n=3~5 mice/group). (**D**).RTqPCR analysis of *Tgfbr2* mRNA expression in primary dFB isolated from WT or *Tgfbr2*$^{Pd-/+}$*Pdgfra-cre* mouse skin (n=3/group). (**E-G**). WT or *Tgfbr2*$^{Pd-/+}$*Pdgfra-cre* mice were infected *i.d.* with *S. aureus*. Bacterial CFU was measured from the infection edge or center area at day 3 (n=7~8 mice/group).



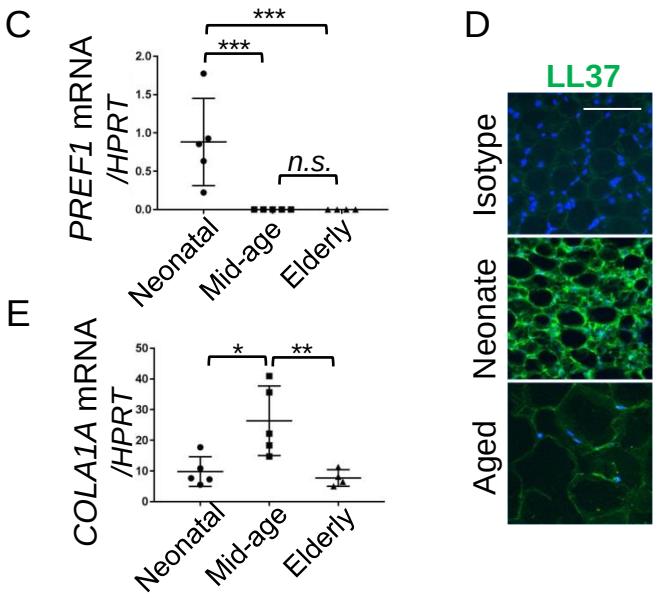


Figure S6 (related to Figure 7). Age-related changes of dermal collagen, elastic fiber and fat in human skin. (**A**). Collagen bundle and elastic fibers became thicker with age. Elastic staining of human skin sections from indicated age group (representative of n=3~6/group). Neonates (pre-term: gestation week, GW 29~32 or full term GW 38~40), young adult (18~25 years), mid-age adult (50~65 years) and elderly (>75 years). All subjects are white Caucasians (n=3~6/age group). Elastic fiber is shown as black cytoplasmic staining. Collagen was stained as red. Nuceli were stained as black nuclear staining. Scale bar, 100 μM. While the elastic fiber became thicker but less dense in lower dermis during aging, the upper dermis became heavily packed with elastic fiber with advancing age. Increase of elastic fiber in the upper dermis maybe driven by accumulated UV exposure to the area during adulthood. (**B**). Representative collagen trichrome staining images of human back skin sections from young children (2~6 years), young adult (18~25 years) and mid-age adult (50~65 years). All subjects are Chinese donors (n=3~6/age group). Scale bar, 100 μM. (**C**). RTqPCR analyses of *PREF1* mRNA expression (ratio to housekeeping *HPRT*) in human neonatal, mid-age or elderly skin (all Caucasians) as indicated. (**D**). LL37 immunostaining in DWAT from neonatal or aged human skin sections as indicated (n=3/group). (**E**). RTqPCR analyses of *COL1A1* mRNA expression (ratio to housekeeping *HPRT*) in human neonatal, mid-age or elderly skin (all Caucasians) as indicated. All error bars indicate mean ± s.e.m. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 (one way Anova).

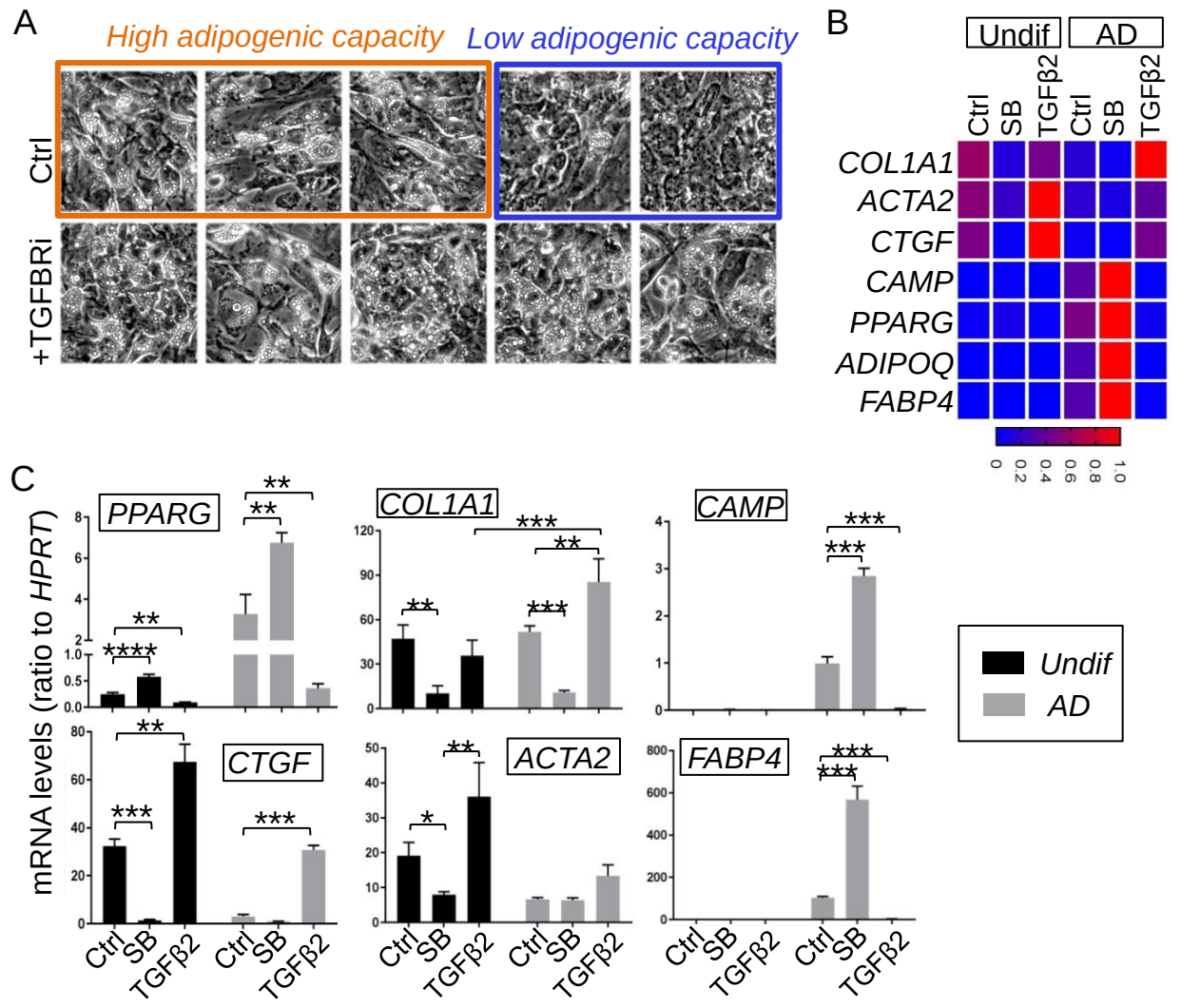


Figure S7 (related to Figure 7). TGFβ promotes loss of adipogenic and antimicrobial defense function of primary human dermal fibroblasts. (**A**). Human primary neonatal dFB isolated from 5 neonatal donors were differentiated to adipocytes with or without TGFBR inhibitor (SB). Basal adipogenic capacity (high or low) in each neonatal dFB was defined by the ability to form lipid-filled adipocytes as shown by phase contrast images. RTqPCR analyses of *PREF1* mRNA expression (ratio to housekeeping *HPRT*) in human neonatal, mid-age or elderly skin (all Caucasians) as indicated. (**B-C**). Primary human neonatal dFB (with low basal A.C.) were differentiated to adipocytes with or without TGFBR inhibitor (SB) or recombinant TGFβ2. Heatmap (**B**) or bar graphs (**C**) showing the expression of listed pro-fibrotic genes adipocyte genes in undifferentiated or adipocyte differentiated dFB treated with vehicle control, SB or TGFβ2 as indicated. All error bars indicate mean ± s.e.m. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 (one way Anova).