BMP2-induced Adam12^{+} Fibroblasts Dictate Wound-associated Skin Scarring and Fibrosis

Jun-Yi Chen[a,1], Jin-Ru Song[a,1], Ke-Ai Li[a,b,1], Xue-Yan Xu[a], Ding-Heng Zhu[a], Lian Zhang[a], Zi-Shuo Chen[a], Qing Cheng[a], Liu-Yi Yao[a], Yi-Qi Shen[a], Zhili Rong[a,c,*], Bin Yang[a,*], Cheng-Cheng Deng[a,*]

[a]Dermatology Hospital, Southern Medical University, Guangzhou 510091, China.

[b]The First School of Clinical Medicine, Southern Medical University, Guangzhou 510515, China.

[c]Cancer Research Institute, School of Basic Medical Sciences, State Key Laboratory of Organ Failure Research, National Clinical Research Center of Kidney Disease, Key Laboratory of Organ Failure Research (Ministry of Education), Southern Medical University, Guangzhou 510515, China.

[1]These authors contributed equally to this work.

*Correspondence: Cheng-Cheng Deng, Dermatology Hospital, Southern Medical University, Guangzhou 510091, China. Email: dengchch@smu.edu.cn; or Bin Yang, Dermatology Hospital, Southern Medical University, Guangzhou 510091, China. Email: yangbin1@smu.edu.cn; or Zhili Rong, Dermatology Hospital, Southern Medical University, Guangzhou 510091, China. Email: rongzhili@smu.edu.cn

This file includes: Supplementary Figure S1–6

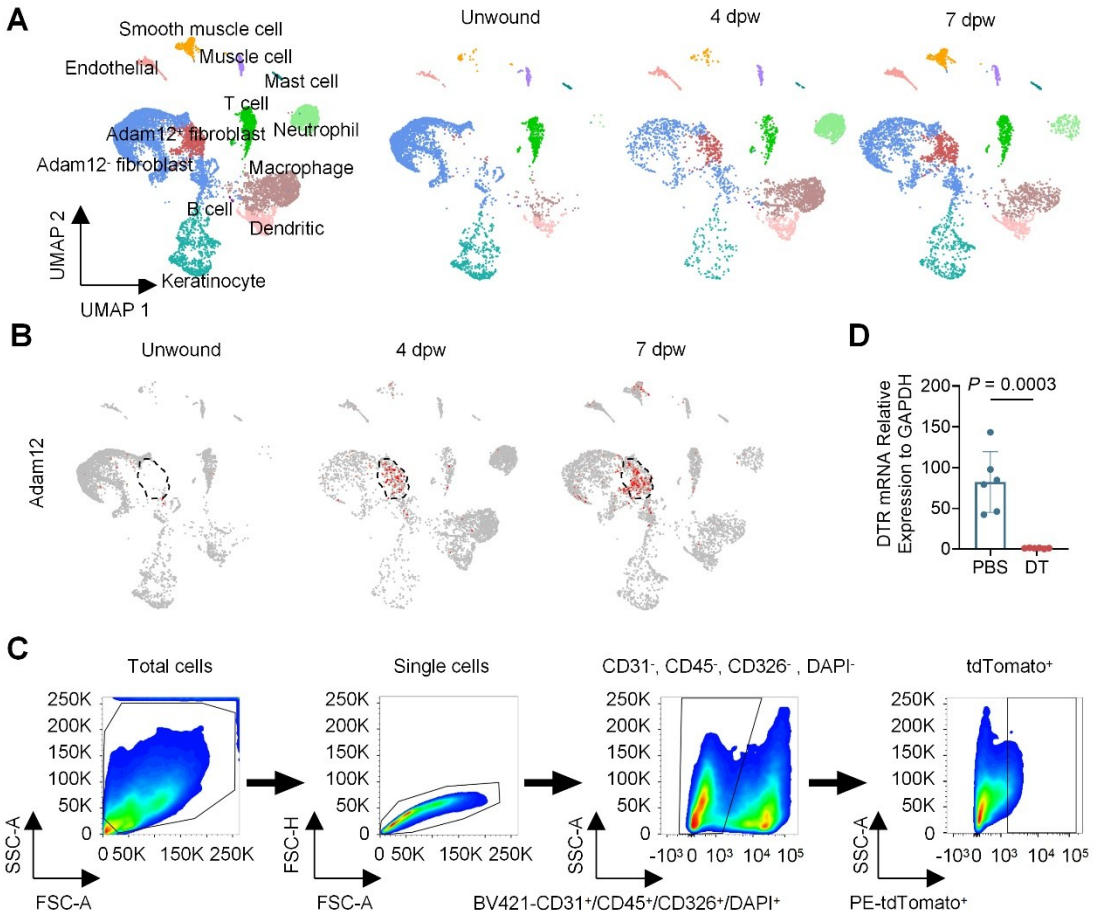

Figure S1. Adam12^{+} fibroblasts increase after skin injury. A Reanalyzing single cell RNAseq data of unwound and wound (day 4 post-wounding, 4dpw and 7dpw) mice skin from Remy Vu et al. (Cell Reports, 2022). **B** Expression of Adam12 in single cell RNAseq data of unwound and wound (4dpw and 7dpw) mice skin. **C** Flow sorting strategy of Fig.1b. A lineage gate (Lin) for hematopoietic (CD45), endothelial (CD31), and epithelial (CD326) cell markers was used as a negative gate to isolate fibroblasts (Lin-). **D** qRT-PCR analysis of DTR indicated that Adam12^{+} cells were efficiently ablated after DT administration. Error bars represent SD (n=6 wounds). Two-tailed Student's unpaired t-test was used to determine statistical significance in **D**.

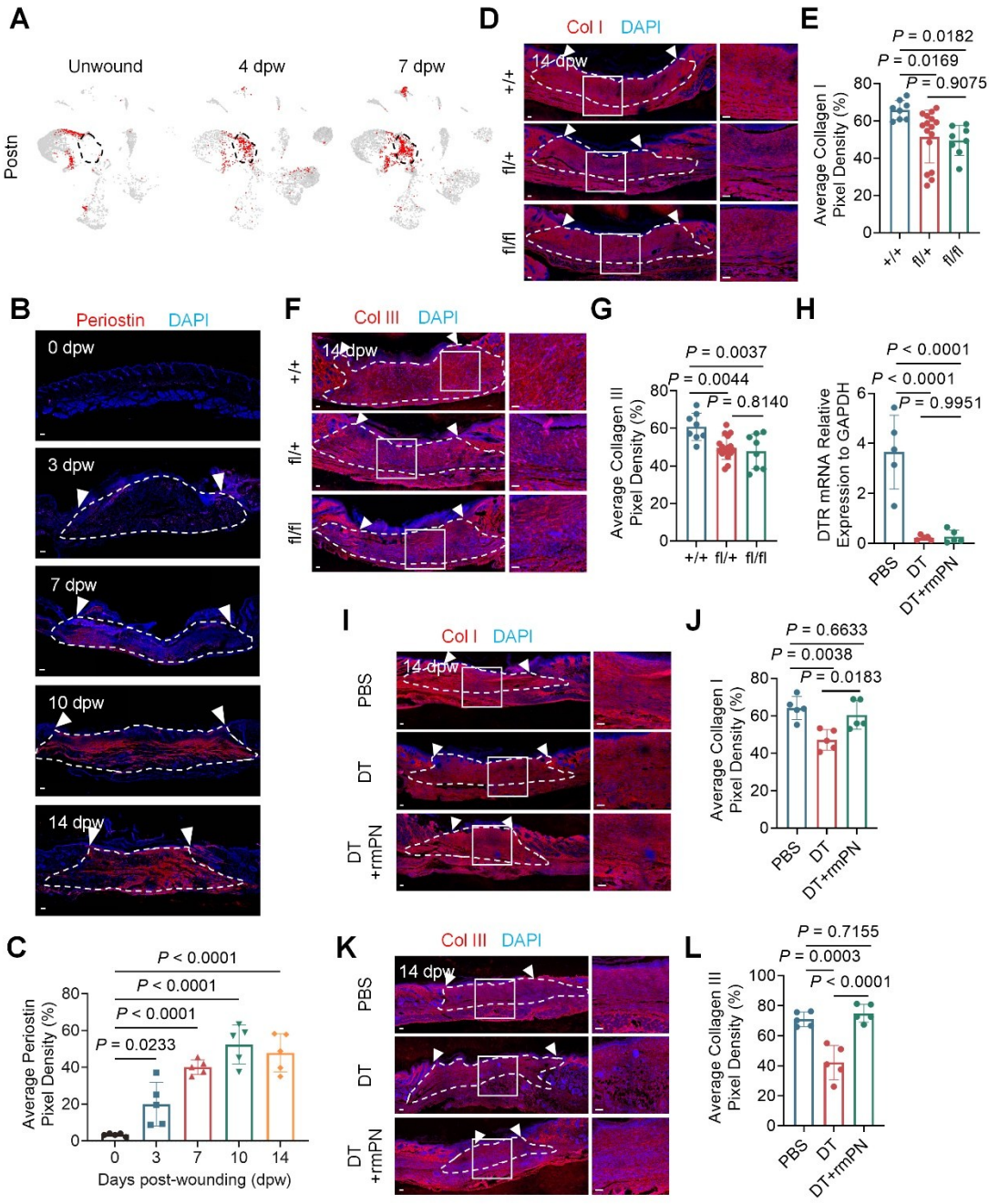

Figure S2. Periostin expression increases after skin injury and are essential for Adam12^{+} fibroblasts' functions in skin scarring and fibrosis. **A** Expression of Postn in single cell RNAseq data of unwound and wound (4dpw and 7dpw) mice skin. **B, C** Immunofluorescence staining analysis for Periostin in C57 mice on unwound and wounded back skin at 0dpw, 3dpw, 7dpw, 10dpw and 14dpw. Error bars represent SD (n=5 wounds). **D-G** Immunofluorescence staining analysis for collagen I and collagen III on wound tissues of Adam12^{CreERT2};Postn$^{+/+}$, Adam12^{CreERT2};Postn$^{fl/+}$, and Adam12^{CreERT2};Postn$^{fl/fl}$ mice at 14dpw. Error bars represent SD (n=8-16 wounds). Scale bar = 100μm. **H** qRT-PCR analysis of DTR indicated that Adam12^{+} cells were efficiently ablated after DT

administration. Error bars represent SD (n=5 wounds). **I-L** Immunofluorescence staining analysis for collagen I and collagen III on wound tissues of PBS group, DT group and DT+rmPOSTN group Adam12^{CreERT2};R26$^{LSL\text{-}tdTomato\text{-}2A\text{-}DTR}$ mice at 14dpw. Error bars represent SD (n=5 wounds). Scale bar = 100μm. One-way ANOVA test was used to determine statistical significance in **C, E, G, H, J** and **L**.

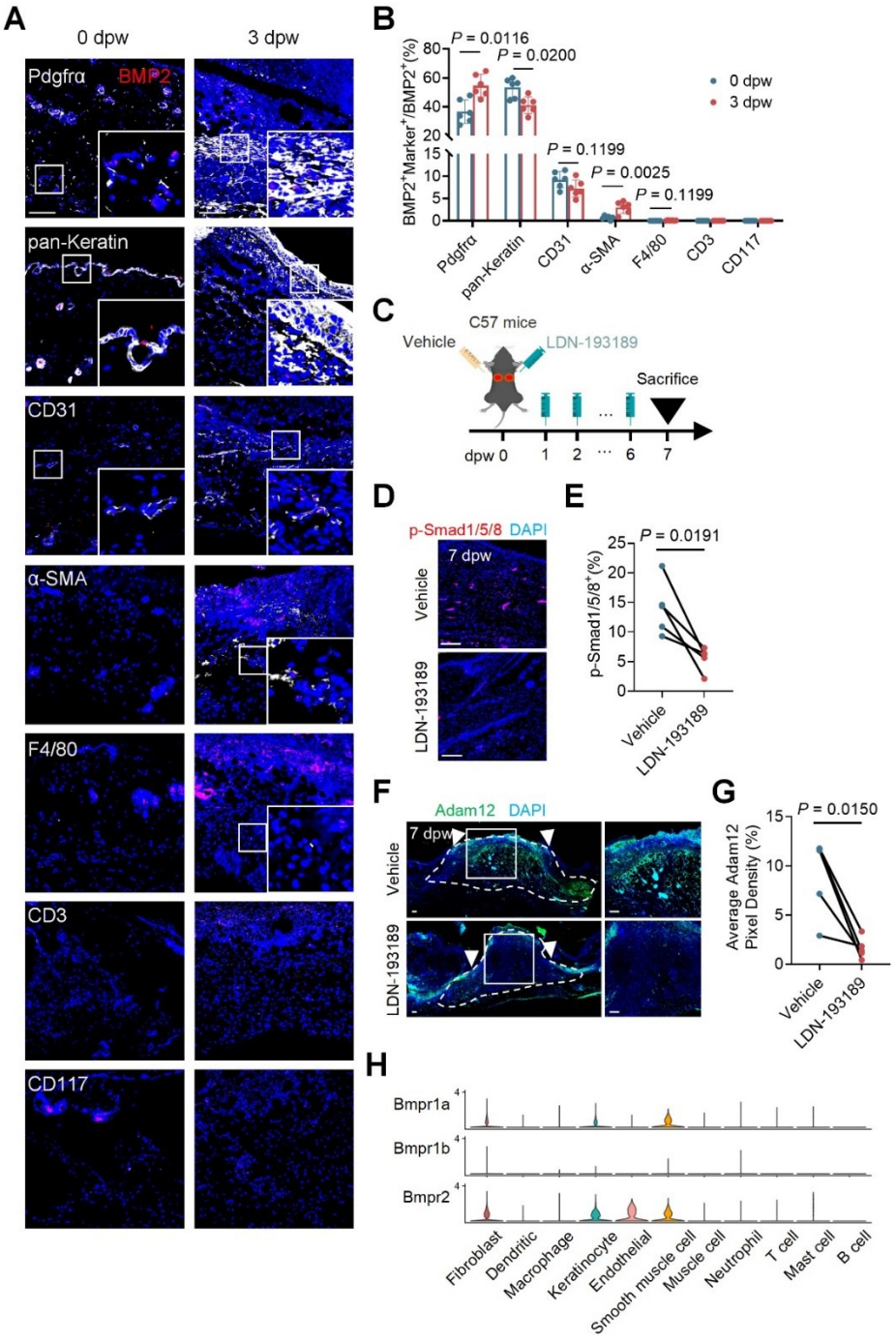

Figure S3. BMP2 signaling is necessary for Adam12^{+} fibroblasts generation. A, B RNAscope analysis of BMP2 RNA expression on unwound and wounded back skin at 3dpw. Pdgfrα, Keratin, CD31, α-SMA, F4/80, CD3 and CD117 antibodies were used to mark fibroblasts, keratinocytes, endothelial cells, pericytes, macrophages, T cells and mast cells. Error bars represent SD (n=6 wounds). **C** Schematic depicting wounding, vehicle injection and 20μg BMP2 inhibitor LDN-193189 2HCl injection of C57 mice. **D, E** Immunofluorescence staining analysis for p-Smad1/5/8 on vehicle injection and LDN-193189 2HCl injection wounds of C57 mice. Error bars represent SD

(n=5 wounds). **F, G** Immunofluorescence staining analysis for Adam12 on vehicle injection and LDN-193189 2HCl injection wounds of C57 mice. Error bars represent SD (n=5 wounds). **H** Expression of Bmpr1a, Bmpr1b and Bmpr2 in single cell RNAseq data of mice skin from Remy Vu et al. (Cell Reports, 2022). Two-tailed Student's unpaired t-test was used to determine statistical significance in **B**. Two-tailed Student's paired t-test was used to determine statistical significance in **E** and **G**.

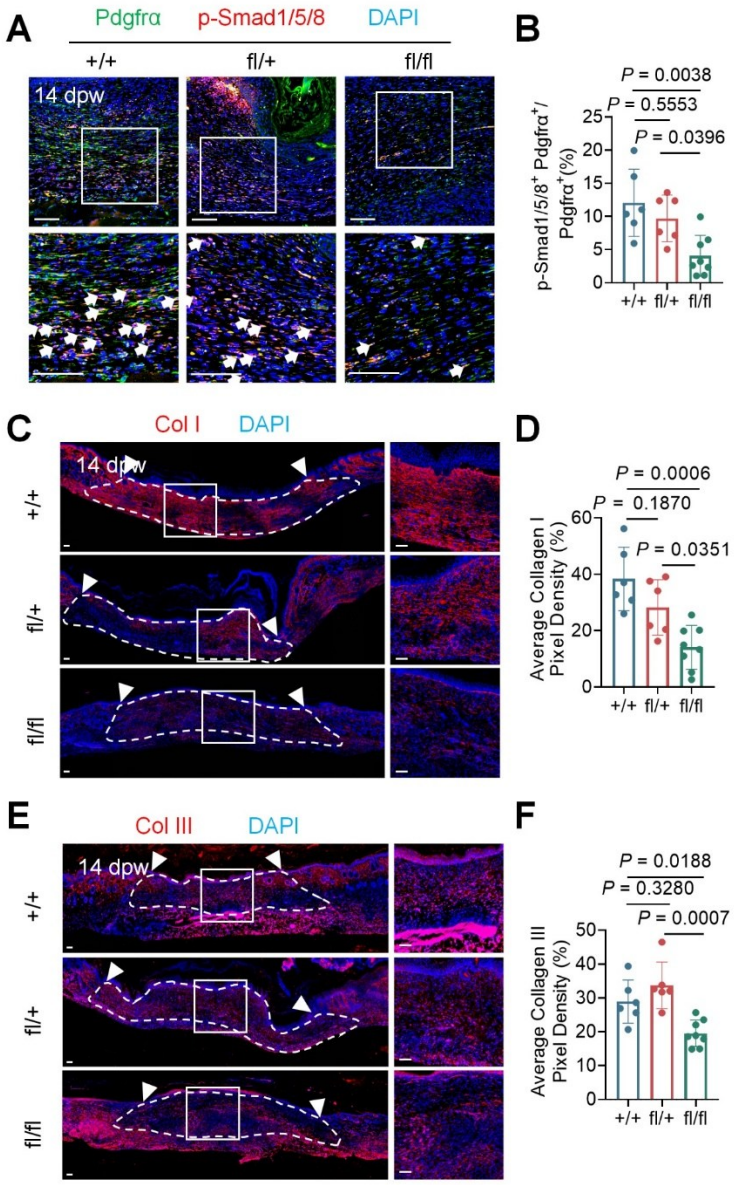

Figure S4. BMP2 signaling is required for the lineage commitment of resident dermal fibroblasts into pro-fibrogenic Adam12^{+} fibroblasts. A, B Immunofluorescence staining analysis for p-Smad1/5/8 and Pdgfrα on wound tissues of Pdgfra^{CreERT2};Bmpr2$^{+/+}$, Pdgfra^{CreERT2};Bmpr2$^{fl/+}$, and Pdgfra^{CreERT2};Bmpr2$^{fl/fl}$ mice at 14dpw. Error bars represent SD (n=6 wounds for Pdgfra^{CreERT2};Bmpr2$^{+/+}$, Pdgfra^{CreERT2};Bmpr2$^{fl/+}$ and n=8 wounds for Pdgfra^{CreERT2};Bmpr2$^{fl/fl}$). **C-F** Immunofluorescence staining analysis for collagen I and collagen III on wound tissues of Pdgfra^{CreERT2};Bmpr2$^{+/+}$, Pdgfra^{CreERT2};Bmpr2$^{fl/+}$, and Pdgfra^{CreERT2};Bmpr2$^{fl/fl}$ mice at 14dpw. Error bars represent SD (n=6 wounds for Pdgfra^{CreERT2};Bmpr2$^{+/+}$, Pdgfra^{CreERT2};Bmpr2$^{fl/+}$ and n=8 wounds for Pdgfra^{CreERT2};Bmpr2$^{fl/fl}$). One-way ANOVA test was used to determine statistical significance in **B, D** and **F**.

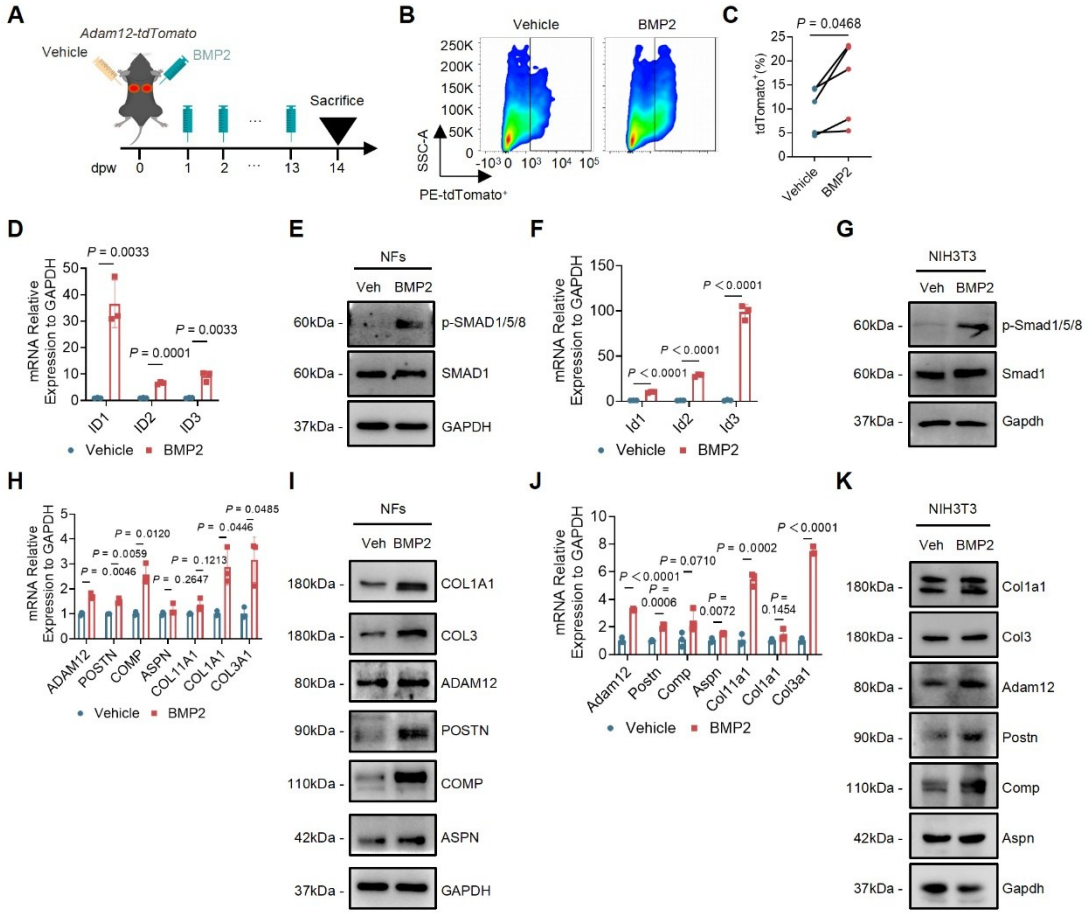

Figure S5. BMP2 upregulated the number of Adam12+ fibroblasts and induces a profibrotic response. A Schematic depicting wounding, vehicle injection and 25ng BMP2 injection of Adam12-tdTomato mice. **B, C** Flow cytometry analysis of tdTomato on vehicle injection and BMP2 injection wounds of Adam12-tdTomato mice. Error bars represent SD (n=5 wounds). **D** qPCR analysis of BMP2 target genes (ID1, ID2, ID3) in NFs following BMP2 stimulation. Error bars represent SD (n=3). **E** Western blot analysis of phosphorylated SMAD1/5/8 in NFs following BMP2 stimulation. **F** qPCR analysis of BMP2 target genes (ID1, ID2, ID3) in NIH3T3 cells following BMP2 stimulation. Error bars represent SD (n=3). **G** Western blot analysis of phosphorylated SMAD1/5/8 in NIH3T3 cells following BMP2 stimulation. **H** qPCR analysis of an ADAM12^{+} fibroblast marker and collagens (COL1A1, COL3A1) in NFs following BMP2 stimulation. Error bars represent SD (n=3). **I** Western blot analysis of an ADAM12^{+} fibroblast marker and collagen I/III in NFs following BMP2 stimulation. **J** qPCR analysis of an ADAM12^{+} fibroblast marker and collagens (Col1a1, Col3a1) in NIH3T3 cells following BMP2 stimulation. Error bars represent SD (n=3). **K** Western blot analysis of an ADAM12^{+} fibroblast marker and collagen I/III in NIH3T3 cells following BMP2

stimulation. Two-tailed Student's paired t-test was used to determine statistical significance in **C**. Two-tailed Student's unpaired t-test was used to determine statistical significance in **D, F, H** and **J**.

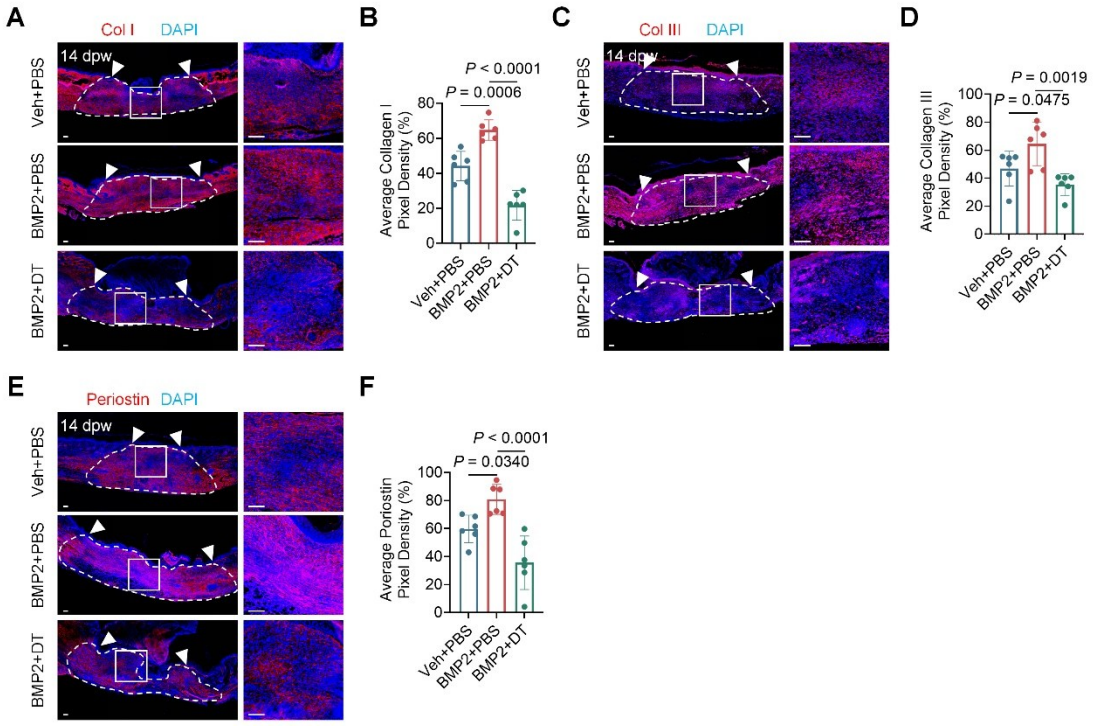

Figure S6. Enhancing BMP2 signaling increases the number of Adam12^{+} fibroblasts and aggravate skin scarring and fibrosis. A-F Immunofluorescence staining analysis for collagen I, collagen III and periostin on wound tissues of vehicle+PBS group, BMP2+PBS group and BMP2+DT group Adam12^{CreERT2};R26$^{LSL\text{-}tdTomato\text{-}2A\text{-}DTR}$ mice at 14dpw. Error bars represent SD (n=6 wounds). One-way ANOVA test was used to determine statistical significance in **B, D and F**.

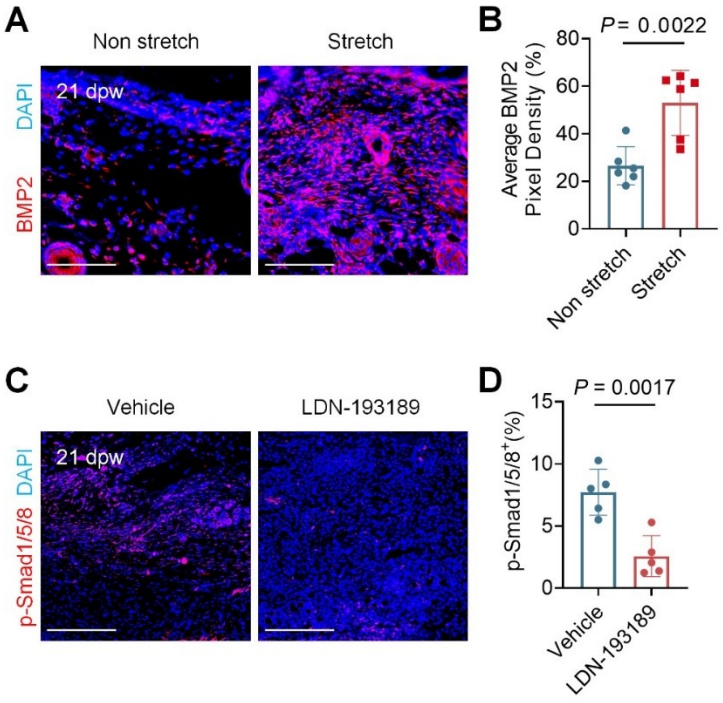

Figure S7. Immunofluorescence staining analysis for BMP2 and p-Smad1/5/8 in tension induced hypertrophic scar mice model. A, B Immunofluorescence staining analysis for BMP2 in non-stretched and stretch-induced hypertrophic scar mice model at 21dpw. Error bars represent SD (n=6 wounds). **C, D** Immunofluorescence staining analysis for p-Smad1/5/8 on vehicle injection and LDN-193189 2HCl injection wounds of tension induced hypertrophic scar mice model at 21dpw. Error bars represent SD (n=5 wounds). Two-tailed Student's unpaired t-test was used to determine statistical significance in **B** and **D**.