## SUPPLEMENTAL METHODS

***Second Harmonic Generation (SHG) microscopy.*** Paraffin-embedded 4 μm thick skin sections were visualized using a multi-photon laser scanning upright microscope (Nikon A1 MP) tuned to 850 nm for two-photon excitation to capture SHG images. Digital images were captured at 512x512 pixels and averaged over two frames to improve the signal-to-noise ratio. Three randomly selected fields per mouse were analyzed using ImageJ (NIH) to quantify the SHG signal intensity representing the concentration and organization of collagen bundles [20]. A look up table pseudocolor scale correlation (Rainbow RGB scale, ImageJ) was applied to the images to highlight collagen deposition in the dermis.

## LEGENDS TO SUPPLEMENTAL FIGURES

**Figure S1. Skin fibrosis in scleroderma is accompanied by intradermal fat loss.** Skin biopsies were obtained by punch biopsy from lesional forearm from patients with diffuse cutaneous SSc (S1, S2) or from forearm of age-matched healthy subjects (N1). **A,** H&E stain. Note thickened dermis with reduced intradermal adipose layer in SSc (S1 and S2) biopsies relative to normal control (N1). High magnification images (insets) highlight the change in adipocyte number and size. Scale bar, 20 μm. **B,** Masson's Trichrome stain. Note replacement of adipose tissue by collagen bundles. Scale bar, 100 μm. **C,** Immunostain using antibodies to perilipin (red). Note reduced adipocyte number and change in adipocyte morphology in SSc biopsies. Nuclei counterstain with DAPI (blue). Scale bar, 100 μm. Images representative of 20 SSc and 10 control skin biopsies.

**Figure S2. Time-dependent increase in dermal thickness and collagen accumulation**. C57BL6/J mice were given daily subcutaneous injections of bleomycin (BLEO) or PBS (CONTROL) in parallel for up to 14 days. Skin tissue isolated from the areas of injection was harvested at indicated time points, and imaged by second harmonic generation (SHG) microscopy. Pseudocolor-encoded images show low-black to high-red collagen deposition. Note increased collagen deposition in dermis at day 21 (1.82-fold, p=0.001). Dotted lines outline epidermis (epi) and dermis. Scale bar, 100 μm.

**Figure S3. Expression of aP2-Cre is not restricted exclusively to adipocytes. A,** aP2-Cre transgenic mice were crossed with Ai14 (tdTomato) or R26R (lacZ) reporter mice to generate progeny that express reporter protein restricted to aP2-lineage cells. Skin was harvested from the interscapular region from untreated 6-week old female. **B,** Confocal images showed no background staining from the unrecombined Ai14 allele in the absence of aP2-Cre, whereas in aP2-Cre$^{+}$; tdTomato$^{+/f}$ mice the tdT reporter was expressed in multiple cell types throughout the dermis. Nuclear counterstain using Hoechst 33342 (blue); scale bar, 50μm. **C,** aP2-Cre directed β-galactosidase expression in multiple non-adipose cells demonstrating the non-specificity of aP2-Cre in the skin. The control mouse shows no β-Galactosidase expression in the absence of aP2-Cre. Dotted lines outline epidermis (epi), dermis and intradermal adipose (adipose). Nuclear counterstain with fast red; scale bar, 100μm. *n*= 2 mice for group.

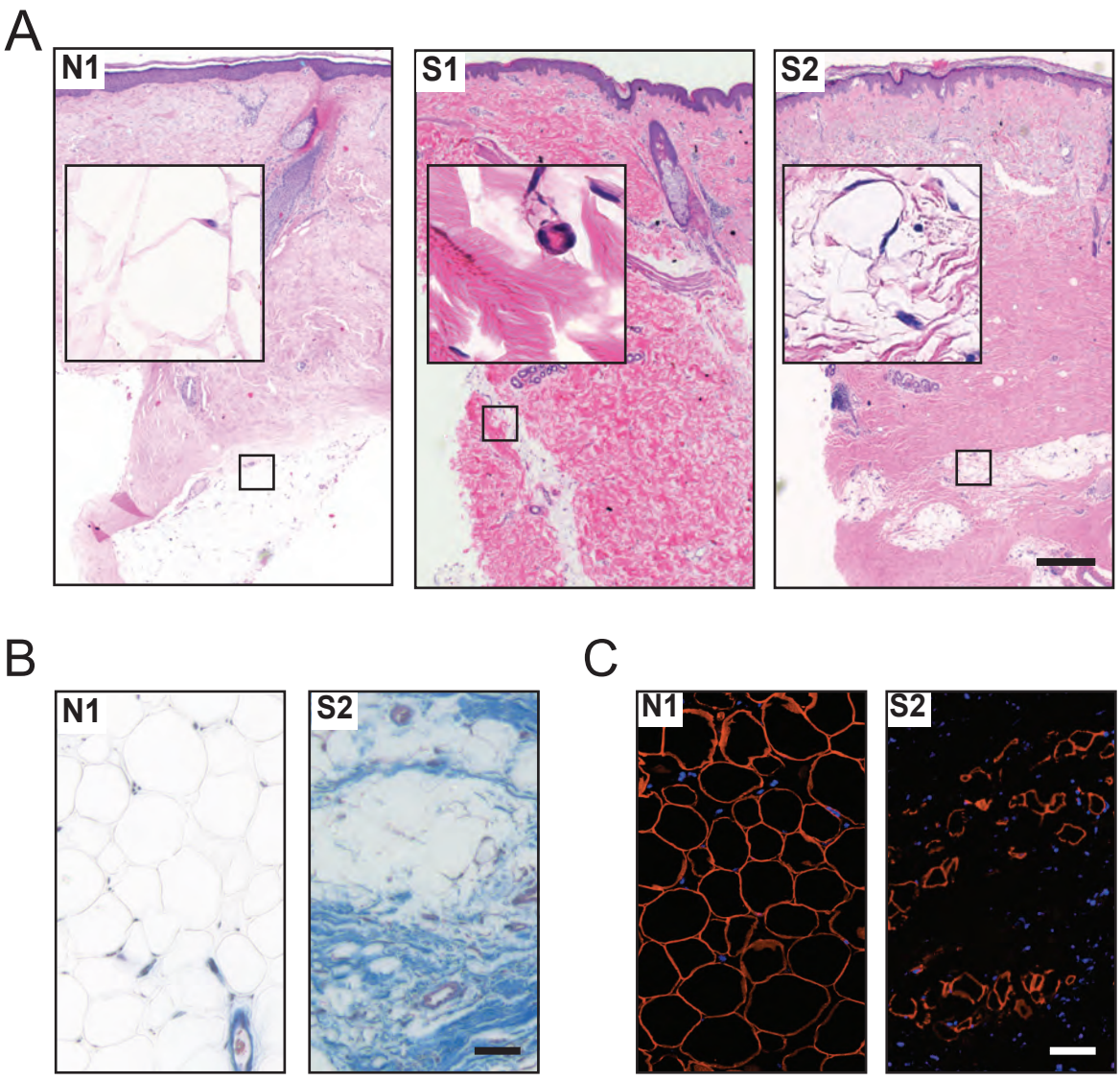
**Figure S4. tdT-positive cells are absent in the dermis in early fibrogenesis.** AdipoP-Cre$^{+}$; tdTomato$^{+/f}$ mice were given daily subcutaneous injections of bleomycin (BLEO) or PBS (CONTROL) and lesional skin was harvested at day 5. **A,** H&E stain. Note decrease in intradermal adipose while no increase in dermal thickness. Scale bar, 100 μm. **B,** Thickness of dermis. Results are shown as –fold change of the means ± SEM of five determinations/hpf from 4 mice per group, n.s. = non significant. **C,** Split image fluorescence micrographs. Note endogenous tdTomato lineage label restricted to adipocytes. Dotted lines outline epidermis (epi), dermis and intradermal adipose (adipose). Nuclear counterstain with Hoechst 33342 (blue). Scale bar, 50 μm. Representative images, *n*= 4 mice for group.

**Figure S5. Adipocyte-derived cells populating fibrotic dermis do not express inflammatory cell or adipocyte markers.** AdipoP-Cre$^{+}$; tdTomato$^{+/f}$ mice were given daily subcutaneous injections of bleomycin (BLEO) or PBS (CONTROL) for 14 days and lesional skin was harvested at day 21. Endogenous tdT label and immunofluorescence using antibodies to **A,** CD31 (green); **B,** CD45 (green); **C,** F4/80 (green) or **D**, Perilipin (green). Staining of intradermal adipose in control mice and dermis in both control and bleomycin-treated mice are shown. Note that there is no co-localization of tdT-derived cells in the dermis with endothelial, leukocyte, macrophage or adipocyte cell markers. Nuclear counterstain with Hoechst 33342 (blue). Scale bar, 25 μm.

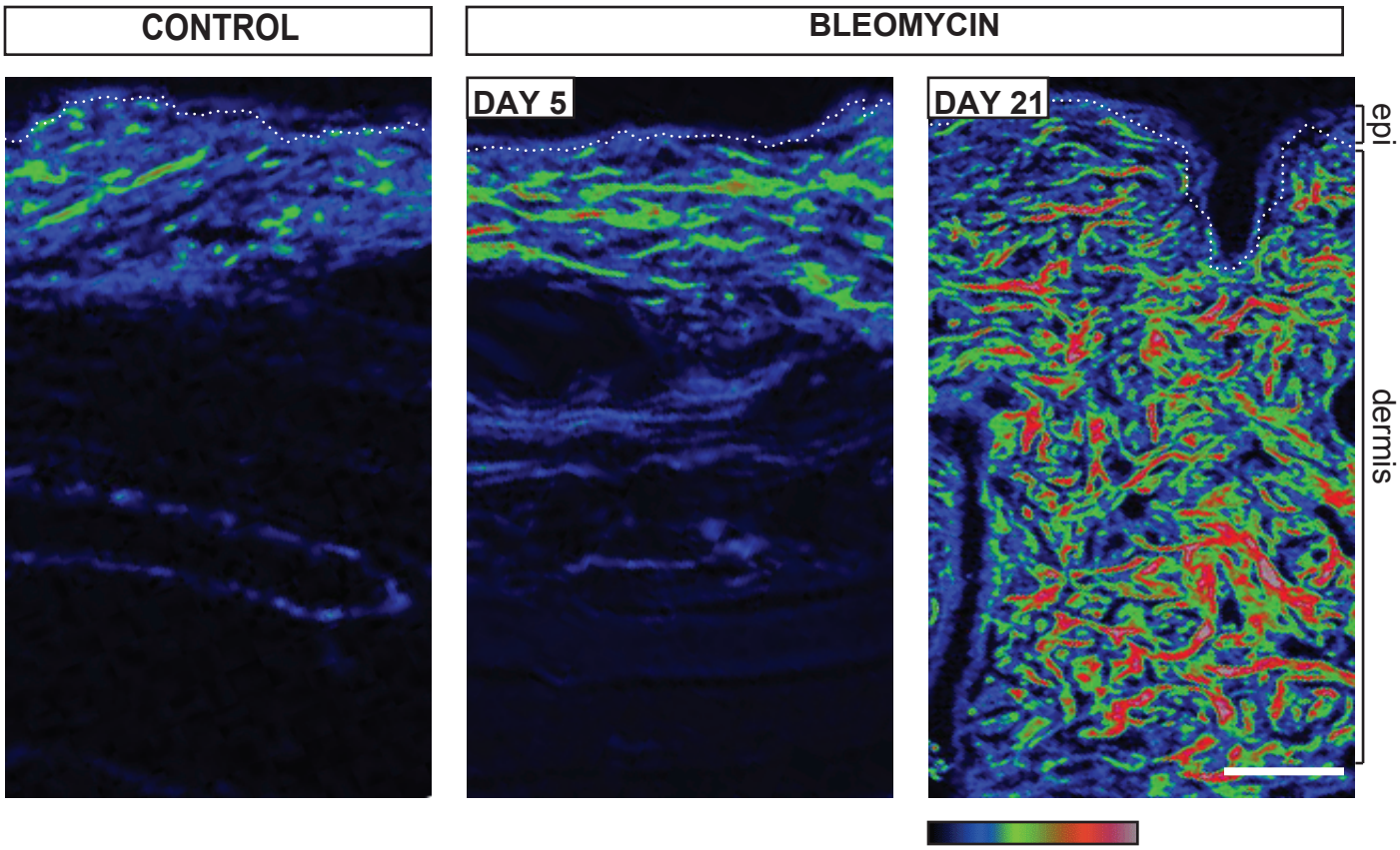
**Figure S6. Adipocyte-derived cells in the dermis are S100A4-positive.** AdipoP-Cre$^{+}$; tdTomato$^{+/f}$ mice were given daily subcutaneous injections of bleomycin (BLEO) or PBS (CONTROL) for 14 days, lesional skin was harvested at day 21 and immunostained with antibodies to S100A4. Merge image fluorescence micrographs of dermis. Note co-localization (yellow) of endogenous tdT (red) and S100A4 immunolabel (green) in BLEO. Nuclear counterstain with Hoechst 33342 (blue). *Inset* (right panel) highlights S100A4 and tdT double labeled cell. Dotted lines outline epidermis (epi) and dermis. Representative images. Scale bar, 20 μm. Quantification of tdT-positive cells that co-express S100A4 and S100A4 cells that co-express tdT. Results are means ± SEM, * = $p < 0.001$.

**Table S1. Early downregulation of adipogenic genes.** C57BL6/J mice were given daily subcutaneous injections of bleomycin (BLEO) for up to 5 days. Skin tissue isolated from the areas of injection was harvested at day 0 and day 5. RNA was isolated hybridized to Illumina MouseRef8 V2.0 Microarrays. Quality control, filtering and normalization were performed as described in the methods section. The fold change indicates the mean gene expression ratio of day 5 compared to day 0 (n=3 mice for group).

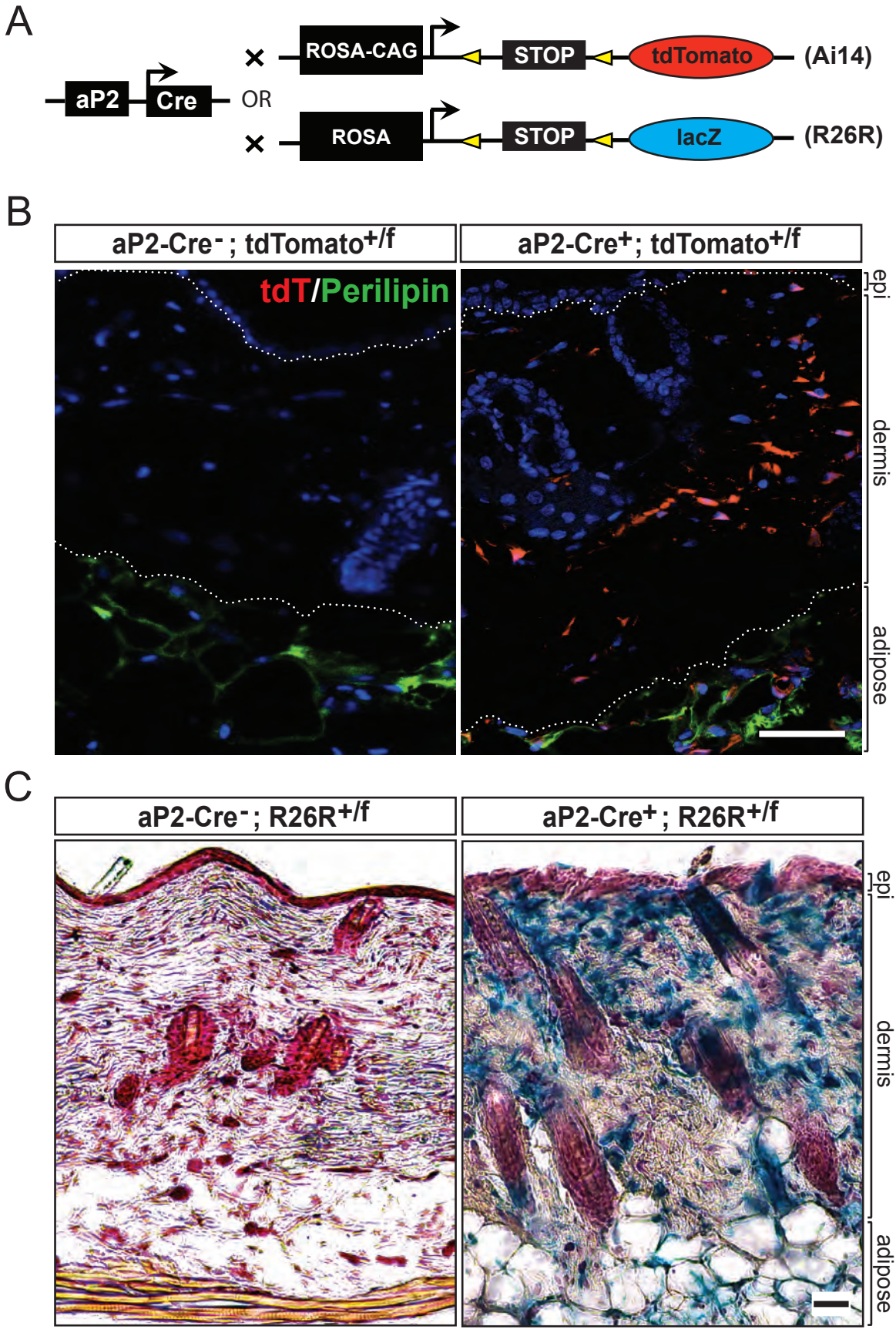
**Goncalves Marangoni et al., Figure S1**



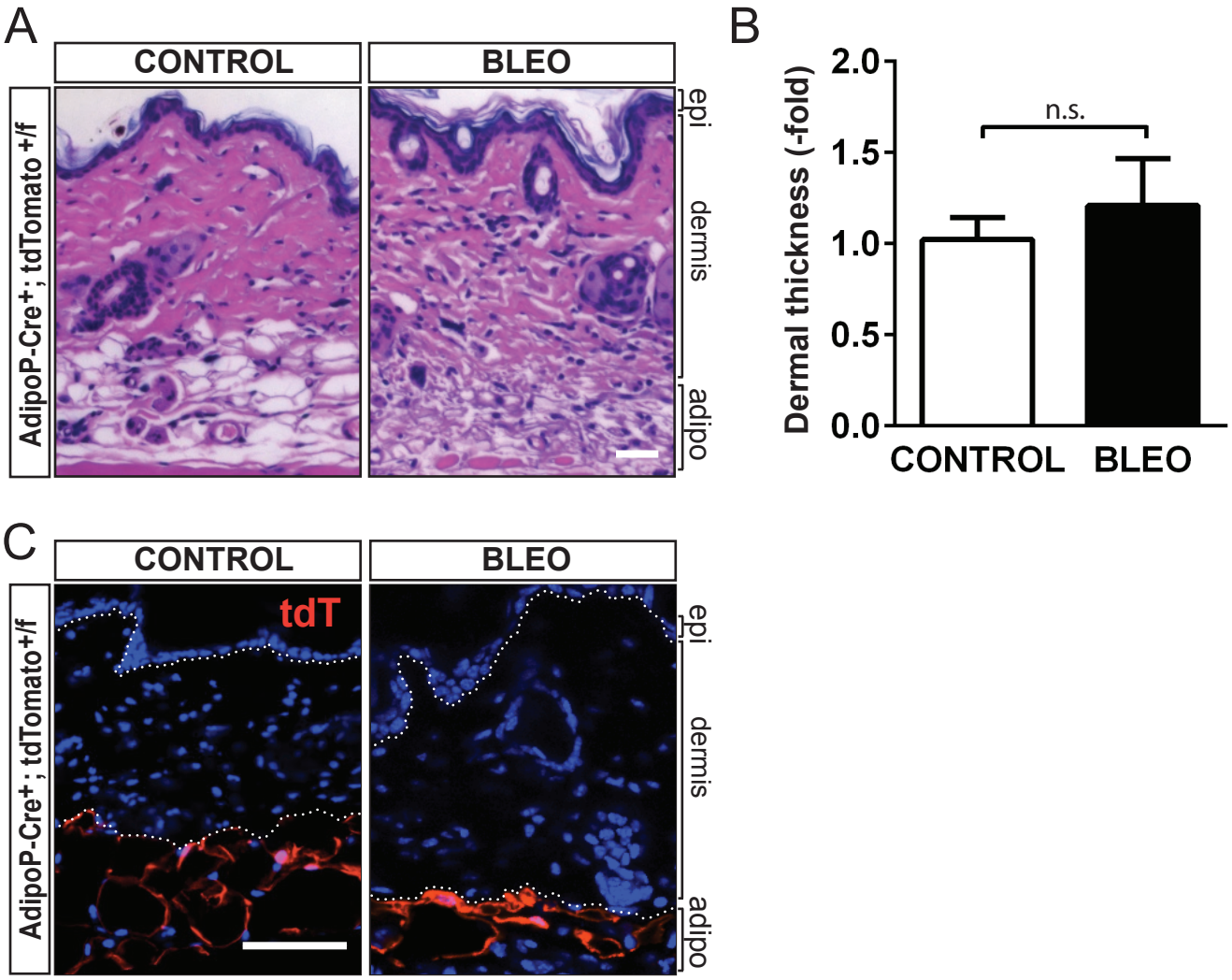
# Goncalves Marangoni et al., Figure S2



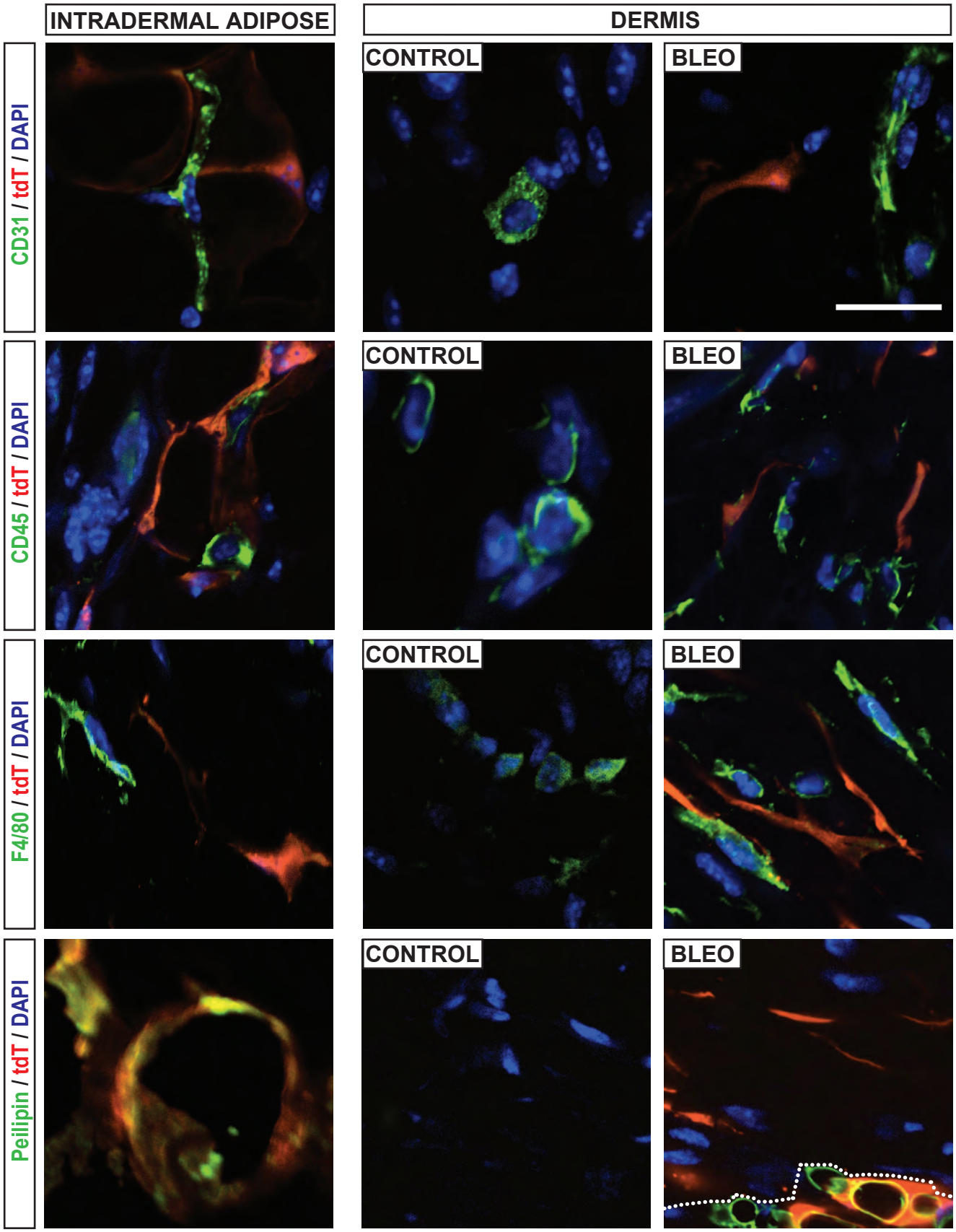
Goncalves Marangoni et al., Figure S3



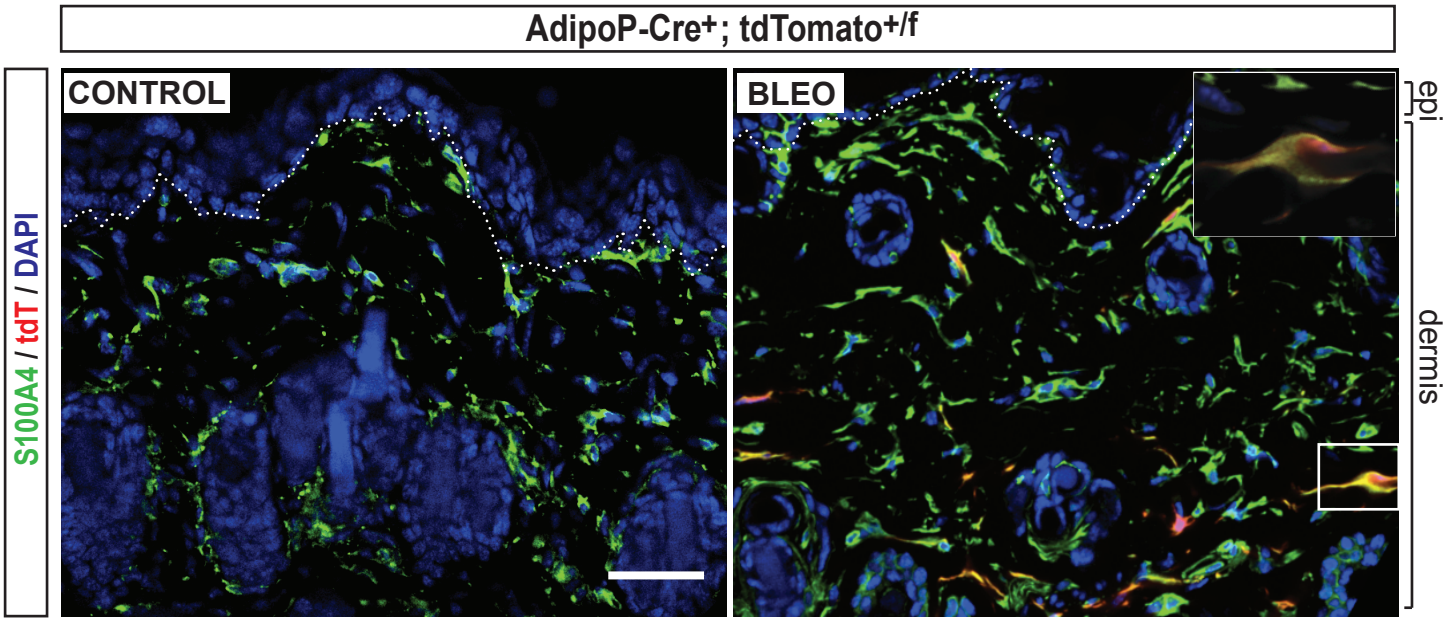
# Goncalves Marangoni et al., Figure S4

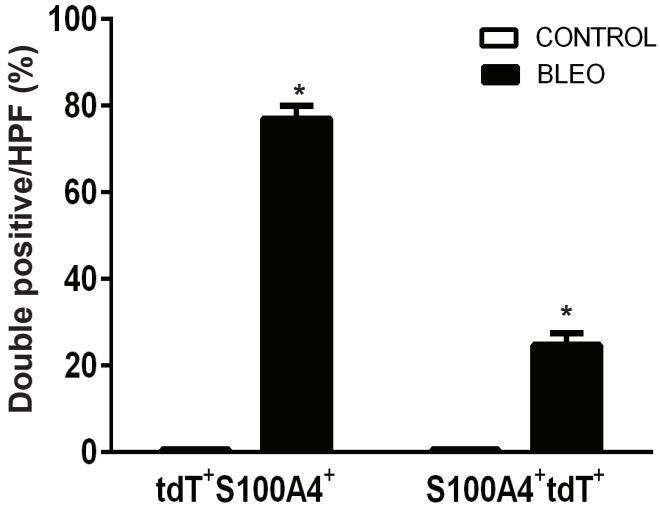


# Goncalves Marangoni et al., Figure S5



# Goncalves Marangoni et al., Figure S6



| IlluminaID | Gene Symbol | Gene name | Avg Expression Control (log2) | Average Expression day 5 BLEO (log2) | fold change | p-value |
|---|---|---|---|---|---|---|
| ILMN_2835423 | cfd | Complement factor d (adipsin) | 13.99 | 12.91 | 0.48 | 4.16E-03 |
| ILMN_1215252 | bmp4 | Bone morphogenic protein 4 | 8.81 | 7.89 | 0.53 | 1.89E-04 |
| ILMN_1248843 | gata3 | GATA binding protein 3 | 10.73 | 9.87 | 0.55 | 0.06 |
| ILMN_2695964 | lep | Leptin | 9.06 | 8.26 | 0.57 | 0.44 |
| ILMN_2692723 | lpl | Lipoprotein lipase | 12.70 | 12.17 | 0.69 | 0.04 |
| ILMN_1222333 | slc2a4 | GLUT-4 | 8.46 | 7.94 | 0.69 | 0.01 |
| ILMN_2737480 | sirt3 | Sirtuin 3 | 8.75 | 8.27 | 0.72 | 0.77 |
| ILMN_1213531 | adipor1 | Adiponectin Receptor 1 | 11.85 | 11.53 | 0.80 | 0.82 |