Fig. S1. GW9662 treatment does not directly affect fibroblasts in vitro or keratinocytes in vivo. (A) In vitro analysis of primary fibroblasts shows that fibroblast proliferation and migration are the same when cultured with vehicle or GW9662 at the indicated concentrations. (B) The number of BrdU+ keratinocytes is the same in GW9662-injected mouse wounds and vehicle-injected controls at 3, 5 or 7 days after wounding. Additionally, the epidermal area is unchanged following GW9662 injection, indicating normal re-epithelialization.
Fig. S2. Analysis of macrophages in GW9662-treated mice after wounding. (A) FACS analysis of CD45+CD11b+/F4/80+ macrophages at 3 days after wounding. Percentage of CD45+CD11b+/F4/80+ cells is similar in vehicle-injected and GW9662-injected mouse wounds. (B) Immune cell populations are recruited in GW9662-injected wounds compared with vehicle controls at 3 days after wounding. Macrophage populations can be seen in skin sections of both wounds using F4/80, and neutrophils infiltrate wounded skin normally in both samples as seen by LY6G immunostaining. Dotted lines indicate the epidermal-dermal boundary. Asterisk indicates background staining in epidermis. (C) FACS analysis of CD45+ cells shows no difference in immune cell percentage of vehicle- and GW9662-injected mouse wounds at 5 or 7 days after wounding. (D) Fold changes of mRNA levels of CD45+CD11b+/F4/80+ macrophages compared with non-wounded CD45+ controls isolated from non-wounded skin, vehicle-injected wounds and GW9662-injected wounds are similar for several macrophage-produced cytokines.
Fig. S3. Effect of GW9662 treatment on adipocyte lineage cells during wounding. (A) Lack of perilipin*, mature adipocytes (green) in wounds of GW9662-injected and BADGE-injected mice 5 and 7 days after wounding compared with vehicle-injected mice as indicated. Dotted line indicates epidermal-dermal boundary. Asterisk indicates background staining. Scale bar: 100 μm. (B) H&E-stained skin sections of vehicle-injected and GW9662-injected wounds at 5 and 7 days after wounding showing abnormal dermal morphology. Dotted line outlines dermal wound bed. Scale bar: 200 μm. (C) The percentage of BrdU* adipocyte progenitor cells is the same in GW9662-injected mice compared with the vehicle-injected control mice at 5 and 7 days after wounding. The percentage of adipocyte progenitor cells within the Lin+, CD34+, CD29+ cell population is the same in GW9662-treated mice compared with vehicle-injected controls. (D) FACS histogram plots of dermal cells isolated from skin wounds at day 5 stained with IgG2a-FITC (isotype control) or α-SMA-FITC antibodies. Line indicates + gate for α-SMA staining used in Fig. 4C.