Supplemental Figure 1. Detailed explanation of the conducted assay techniques: Gene expression analysis with qPCR, immunofluorescent staining, and enzyme-linked immunosorbent assay (ELISA)

Gene expression analysis with qPCR

RNA was extracted from mouse skin wound tissue with a RNeasy Kit following manufacturer's instructions (Qiagen, Valencia, CA). Total RNA was quantified with a NanoDrop 2000c (NanoDrop, Wilmington, DE) and 2 µg of total RNA was retro-transcribed with a Superscript VILO cDNA Synthesis Kit (Life Technologies, Carlsbad, CA) for cDNA synthesis according to the manufacturer's protocol. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed and monitored using the SYBR Green PCR Mastermix (Life Technologies, Carlsbad, CA) on the StepOnePlus Real Time PCR System (Life Technologies, Carlsbad, CA). Reaction mixtures were incubated for 10 minutes at 95°C followed by 40 cycles of 15 seconds at 95°C, 1 minute at 60°C, and finally 15 seconds at 95°C, 1 minute at 60°C, and 15 seconds at 95°C. For each sample, gene expression was normalized to the levels of ACTB, HPRT and YWHAZ used as housekeeping genes. All samples were run in triplicate. The level of expression of the target gene was calculated as $2^{-\Delta\Delta Ct}$ as previously described.

Immunofluorescent Staining and Quantification

De-paraffinized sections of mouse skin wound were steamed in the antigen retrieval buffer (Abcam, Cambridge, UK) for 20 minutes, then washed with PBS, permeabilized with 0.1% Triton X (Sigma–Aldrich) and blocked with 10% goat serum albumin (Sigma–Aldrich) for 1 h. Sections were incubated with appropriately diluted primary antibodies: F4/80 (Abcam, Cambridge, UK), NOS2 (Abcam, Cambridge, UK) and ARG1 (Abcam, Cambridge, UK) overnight at 4 °C. After washing in PBST (3 times at 5 min), tissues were incubated with fluorescent secondary antibodies diluted in PBST for 1h at ambient temperature. After washing in PBST (3 times at 5 min), nuclei were stained with and DAPI (Akoya, Marlborough, MA) and diluted in PBST for 15 min at ambient temperature. Following the final washing procedure (PBST, 3 times at 5 min), glass cover slips were added to the slides using Fluoro-Gel (Electron Microscopy Sciences, Hatfield, PA). Immunostained samples were imaged with a Vectra Polaris microscope (Akoya, Marlborough, MA). HALO was used to quantify F4/80, NOS2 and ARG1 positivity in immunofluorescent staining of mouse skin wound samples.

Enzyme-linked immunosorbent assay (ELISA)

TNFα in mouse skin wound tissue was measured with ELISA kits (Abcam, Cambridge, UK) based on the manufacturer's protocol. Briefly, ~5 mg piece of tissue, add ~300 µL complete extraction buffer to the tube and homogenize with pestle. maintain constant agitation for 1 hr at 4°C (e.g. place on an orbital shaker in the cold room). Centrifuge for 20 min at 13,000 rpm at 4°C. The supernatant was then collected in a clean vial as the test sample. First, 100 uL samples and standard controls were added to each well in the antibody-coated plate and incubated for 2 hours at 37 °C in the incubator. After removing the liquid, 100 uL biotin antibody was added and incubated for another 1 hour at 37 °C in the incubator. After triple rinsing with washing buffer, 100 uL horseradish peroxidase avidin was added and incubated for another 1 hour at 37 °C. After five times rinsing with washing buffer, 90 uL TMB substrate was added and incubated for 15 minutes at 37 °C before adding 50 uL of the stop solution. The absorbance values were measured with a spectrometer at 450 nm (BioTek, Winooski, VT). The concentrations of growth factors were calculated based on the standard curve.