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Supplemental Information

Macrophages as a therapeutic target to promote diabetic wound healing

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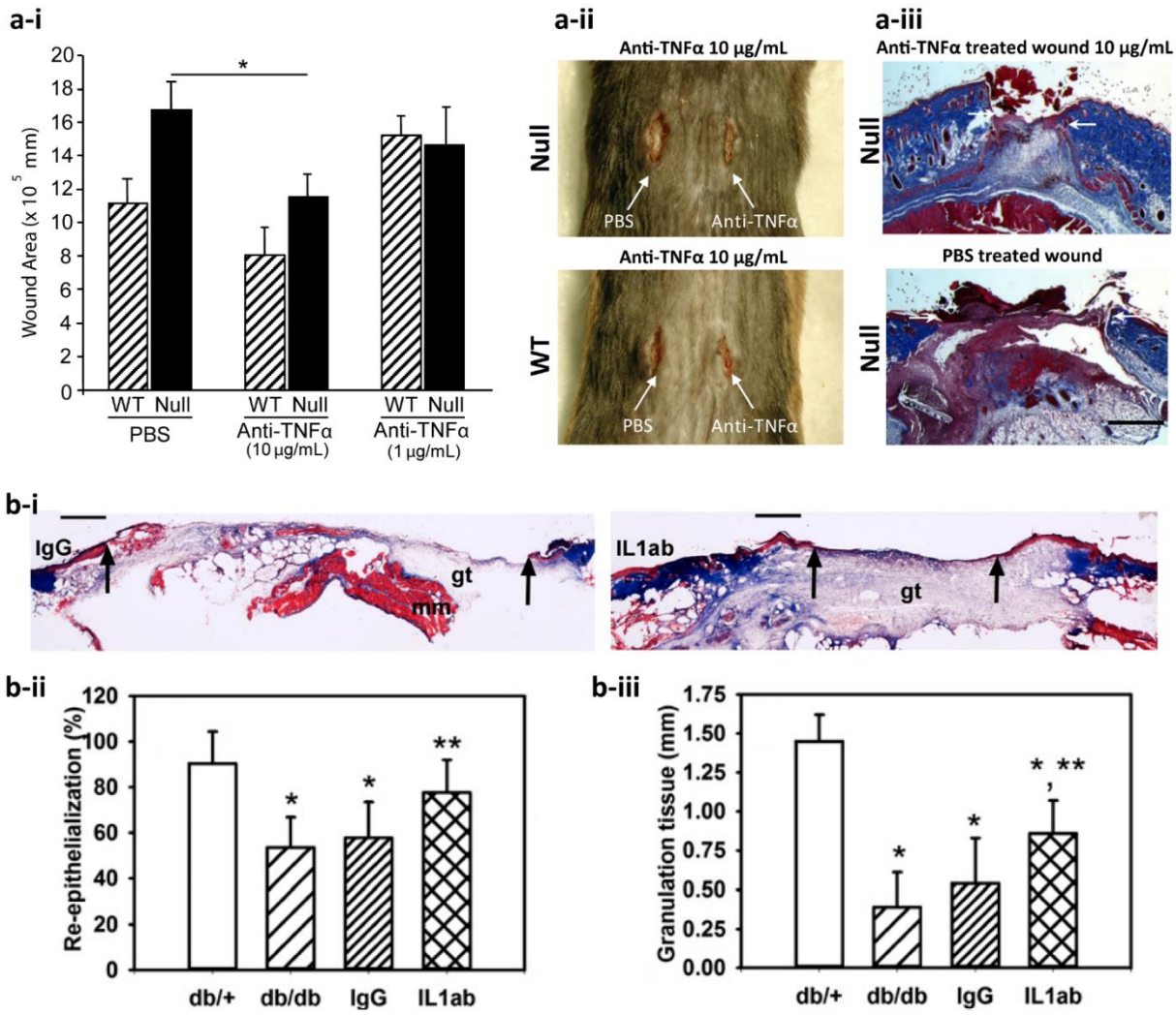

Figure S1. Inhibition of effects induced by inflammatory M1-like macrophages in the wound bed. **(a)** Accelerated wound healing by neutralization of tumor necrosis factor alpha (TNFα) in an SLPI null mice model, lacking anti-inflammatory and anti-proteolytic activity. i) Single administration of Anti-TNFα antibodies (10µg/mL) at the time of wounding significantly reduces cross-sectional wound areas compared to control (PBS treated) wounds at day 3 post-wounding. Data represent mean values and error bars the SEM. *$p < 0.05$. $n = 6$. null: secretory leukocyte protease inhibitor null mouse, WT: Wild-type. ii) The macroscopic images show accelerated healing after TNFα antibody treatment (Anti-TNFα) compared to control (PBS). iii) Masson's trichrome staining at day 3 post-wounding illustrates a marked reduction of wound size after treatment with anti-TNFα antibodies (10 µg/mL) compared to the PBS treated group. Arrows demarcate wound margins. Scale bar = 300 µm. Adopted from Ashcroft, G. S. et al.[1] Copyright (2011), with permission from Wiley. **(b)** Accelerated wound healing by Interleukin-1 beta (IL-1β) neutralization in diabetic mice. i) Accelerated re-epithelialization and granulation tissue formation is observed in the group treated by IL-1β antibody (IL1ab) in comparison to IgG-treated mice. Scale bar = 0.5 mm. Arrows indicate the ends of migrating epithelial tongues. gt, granulation tissue; mm, deep muscle. ii) Quantification of re-epithelization and iii) granulation tissue thickness of wounds in nondiabetic mice (db/+), untreated diabetic mice (db/db), IgG-treated diabetic mice (IgG), and IL1ab-treated diabetic mice (IL1ab), on day 10 postinjury. Adopted from Mirza, R. E. et al.[2]

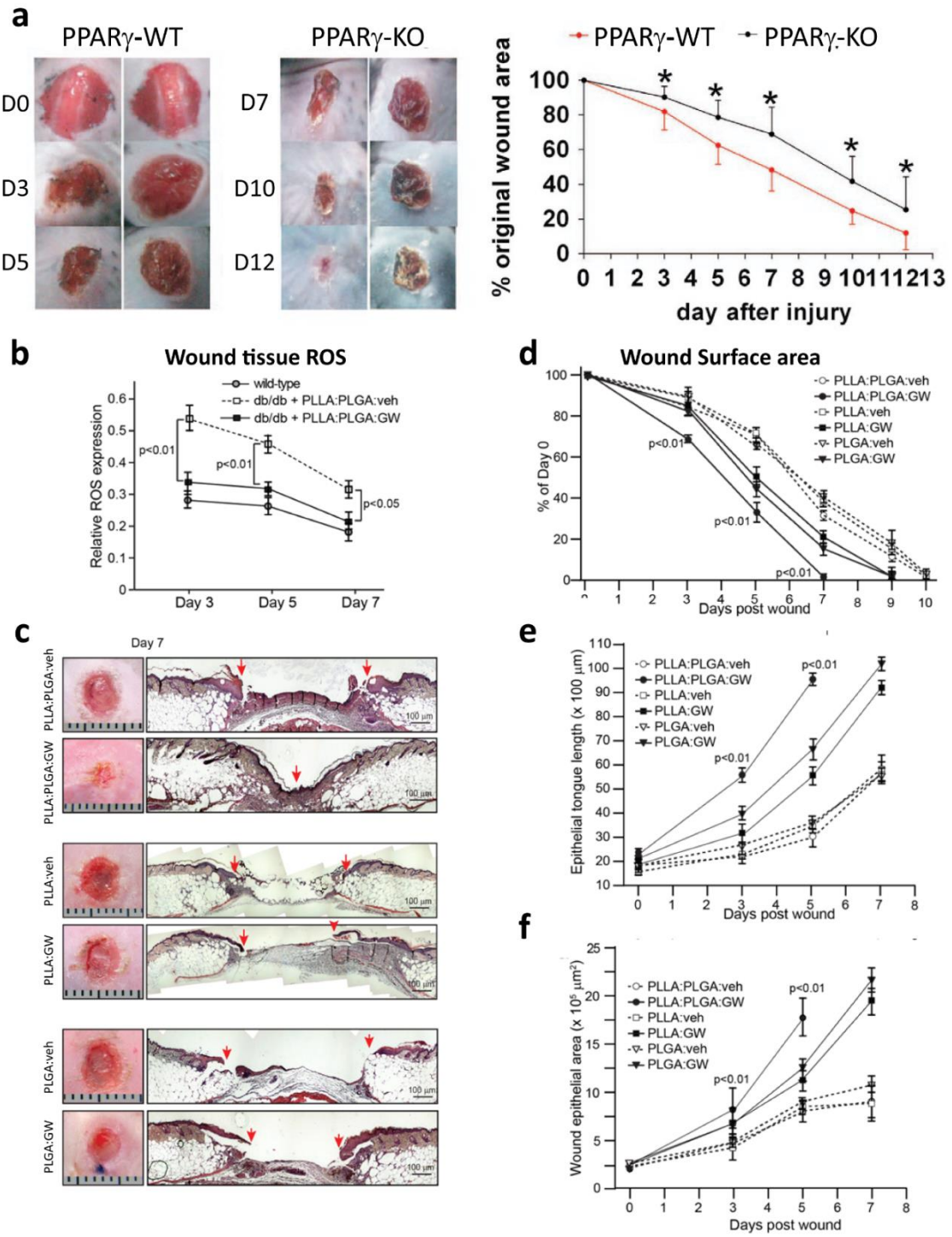

Figure S2. Diabetic wound interventions by application of peroxisome proliferator-activated receptors (PPARs) agonist. (a) Representative macroscopic images and statistical analysis of delayed wound healing in PPARγ-knock out (KO) mice versus PPARγ-wild type (WT) mice**.** Adapted from Chen, H. et al.[3] **(b)** Relative ROS levels of wounds treated with PPARβ/δ agonist GW501516 (GW) encapsulated in core-shell of Poly(L-lactide) and poly(D,L-lactide–co-glycolide) (PLLA:PLGA:GW) microparticles, and vehicle control microparticles without encapsulated GW (PLLA:PLGA:veh). **(c)** Representative macroscopic images (left) and H&E (right) sections of diabetic wounds (day 7) treated with PLLA:PLGA:veh, PLLA:PLGA:GW, PLLA:veh, PLLA:GW and PLGA:veh, PLGA:GW. Ruler units are in mm and red arrows show the epithelial wound edge. **(d)** statistical analysis of wound surface area expressed as percentage of the initial (day 0) wound size. Wound epithelial tongue length **(e)** and epithelial area **(f)** determined by histomorphometric analyses of wound biopsies treated with PLLA:PLGA:veh, PLLA:PLGA:GW, PLLA:veh, PLLA:GW and PLGA:veh, PLGA:GW at day 7 post-wounding. Data shown are mean values ± SD, P values are based on

comparisons between PLLA:PLGA:GW treatment and control PLLA:PLGA:veh. Reprinted from Wang, X. et al.[4] Copyright (2015), with permission from Elsevier.

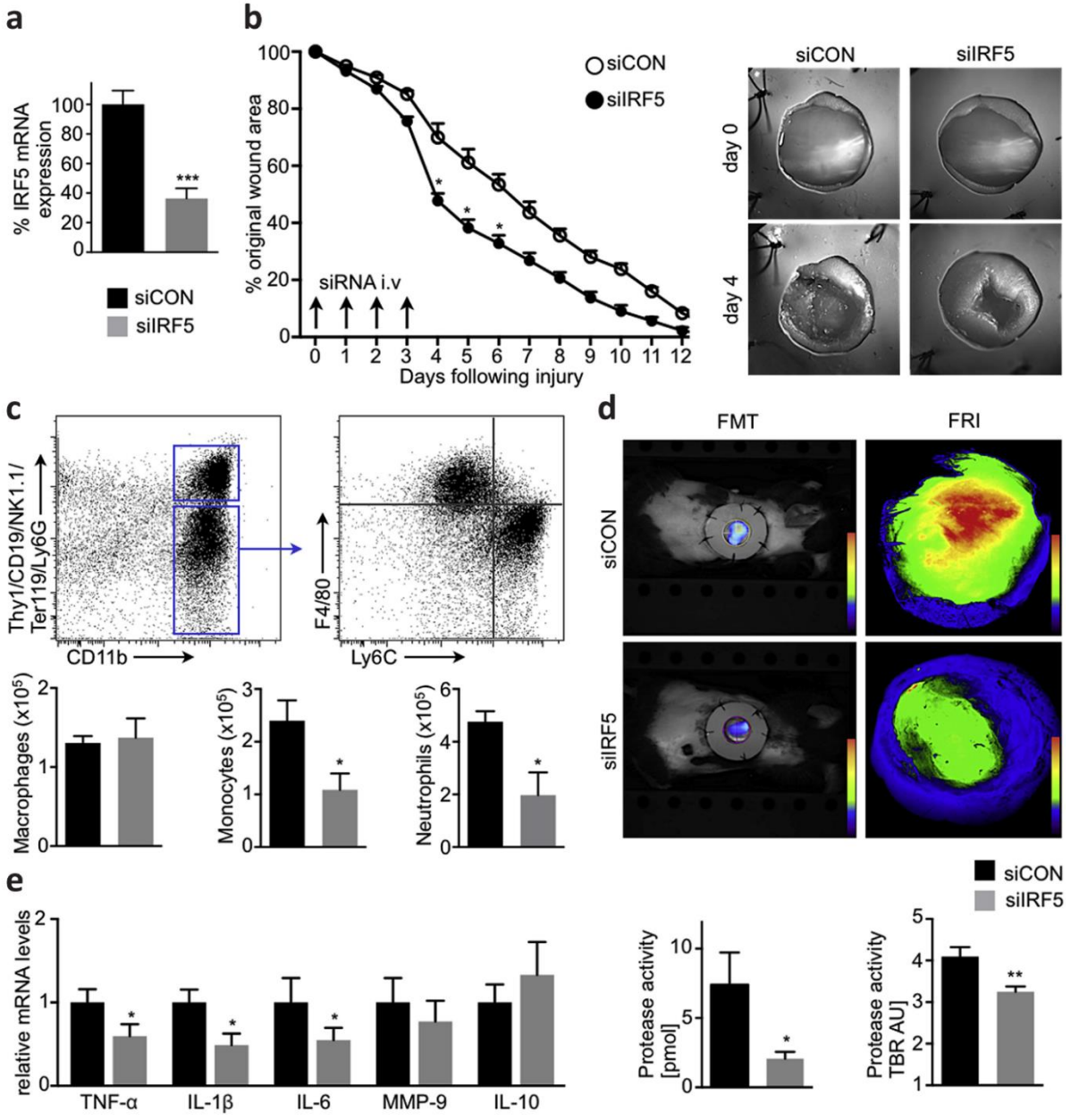

Figure S3. The effect of IRF5 silencing in skin wound healing. (a) IRF5 silencing in monocytes/macrophages isolated from excisional wounds of mice injected 4 days after wounding with siIRF5 or siCON (0.5 mg/kg) (n = 9 per group). **(b)** Wounds were imaged, and the open wound areas were measured daily in mice injected with siIRF5 and siCON (n = 7 to 8 per group). **(c)** Flowcytometry graph and analysis of wound leukocyte content on day 4 (n = 5 per group). **(d)** Wound protease activity on day 4, in mice treated i.v. with siIRF5 or siCON (0.5 mg/kg) by fluorescence molecular tomography–computed tomography (FMT-CT) and FRI (fluorescence reflectance imaging) (n = 8 per group) **(e)** Expression level of M1 and M2 mRNA in wound macrophages on day 4 (n = 10 to 12 per group). $*p < 0.05$. $**p < 0.01$. $***p < 0.001$. Reprinted from ourties, G. et al.[5] Copyright (2014), with permission from Elsevier.

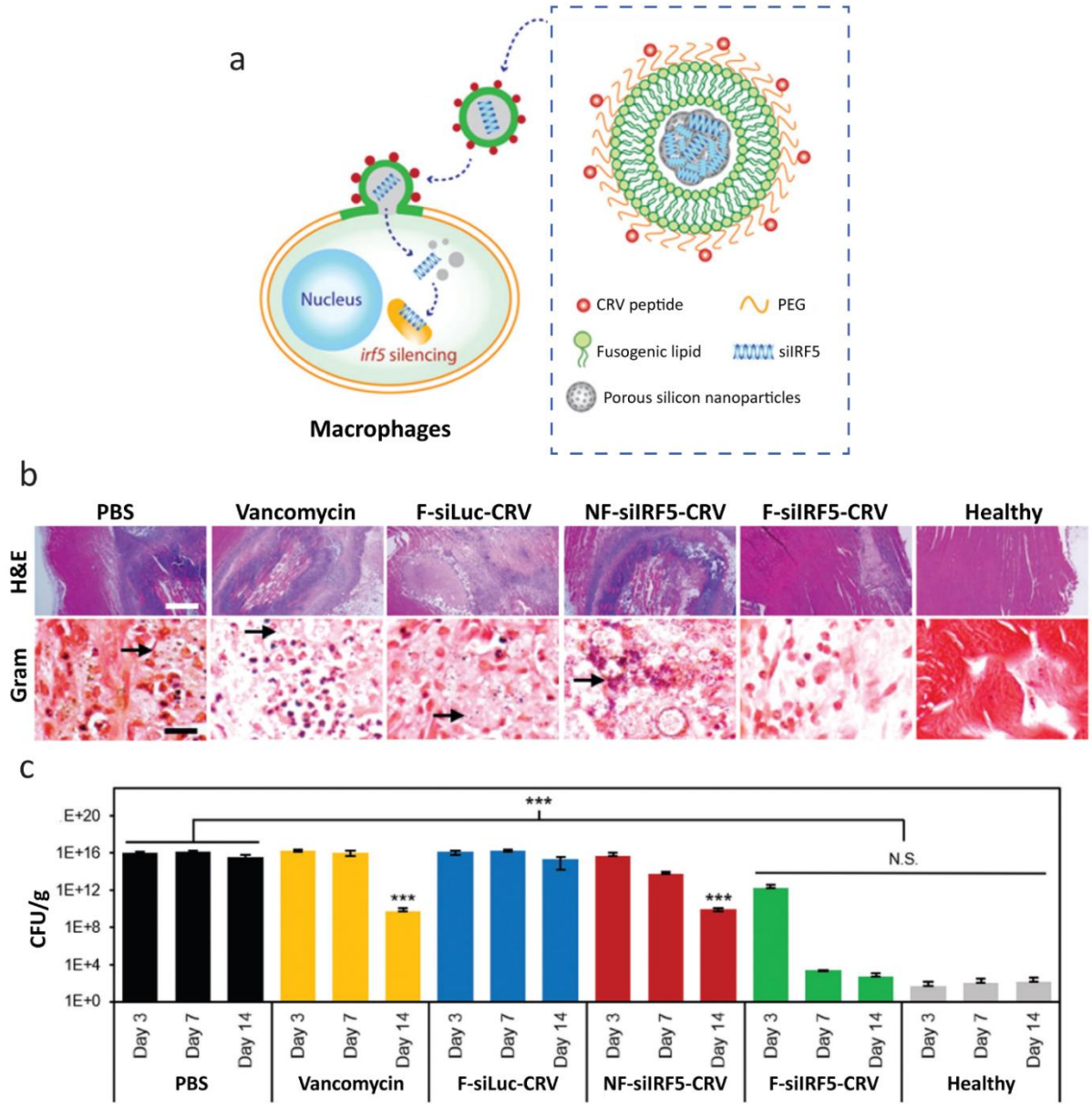

Figure S4. ***In vivo*** **therapeutic efficacy of siIRF5 in MRSA muscle infection. (a)** Schematic figure depicting the CRV peptide-targeted, fusogenic porous silicon nanoparticles (F-pSiNPs-CRV) for delivering and silencing the IRF5 gene in macrophages. **(b)** Stained infected muscle tissues (H&E and Gram) 7 days after treatment with different groups including: PBS, vancomycin, non-fusogenic, targeted pSiNPs containing siIRF5 (NF-siIRF5-CRV), fusogenic, CRV-targeted pSiNPs containing siRNA against luciferase, as a negative control for siIRF5 (F-siLuc-CRV), and fusogenic, CRV-targeted pSiNPs containing siIRF5 (F-siIRF5-CRV); scale bar represents 1 mm (top row) and 100 mm (bottom row); **(c)** Bacterial titer counts (CFU per mass of tissue) at days 3, 7, and 14 post-intravenous injection in healthy and methicillin-resistant Staphylococcus aureus (MRSA)-infected mice. Mice were infected on day 0 and injections were given on day 1. Bars indicate standard deviation with n = 6. N.S. represents no significance and ***represents $p < 0.01$ relative to the PBS group from one-way ANOVA with Tukey's HSD post hoc analyses. Reproduced from Ref.[6], with permission from the Royal Society of Chemistry.

References

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