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Supplemental information

Improved diabetic wound healing by LFcinB is associated with relevant changes in the skin immune response and microbiota

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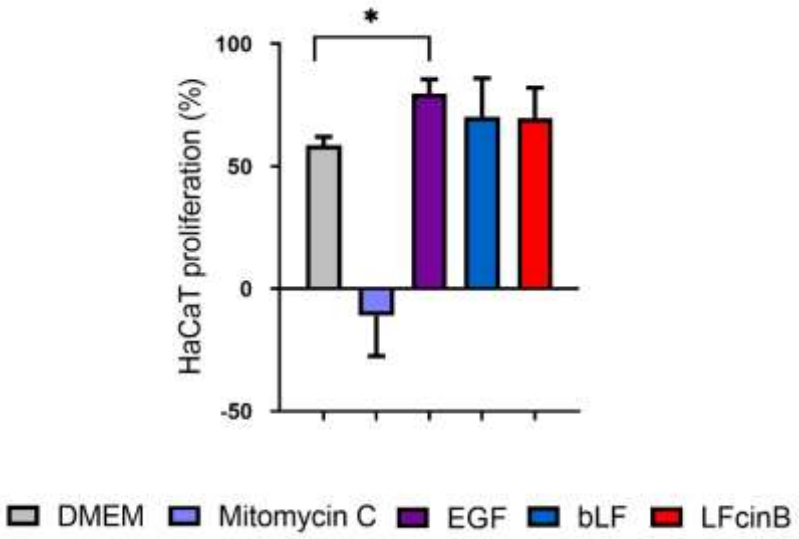
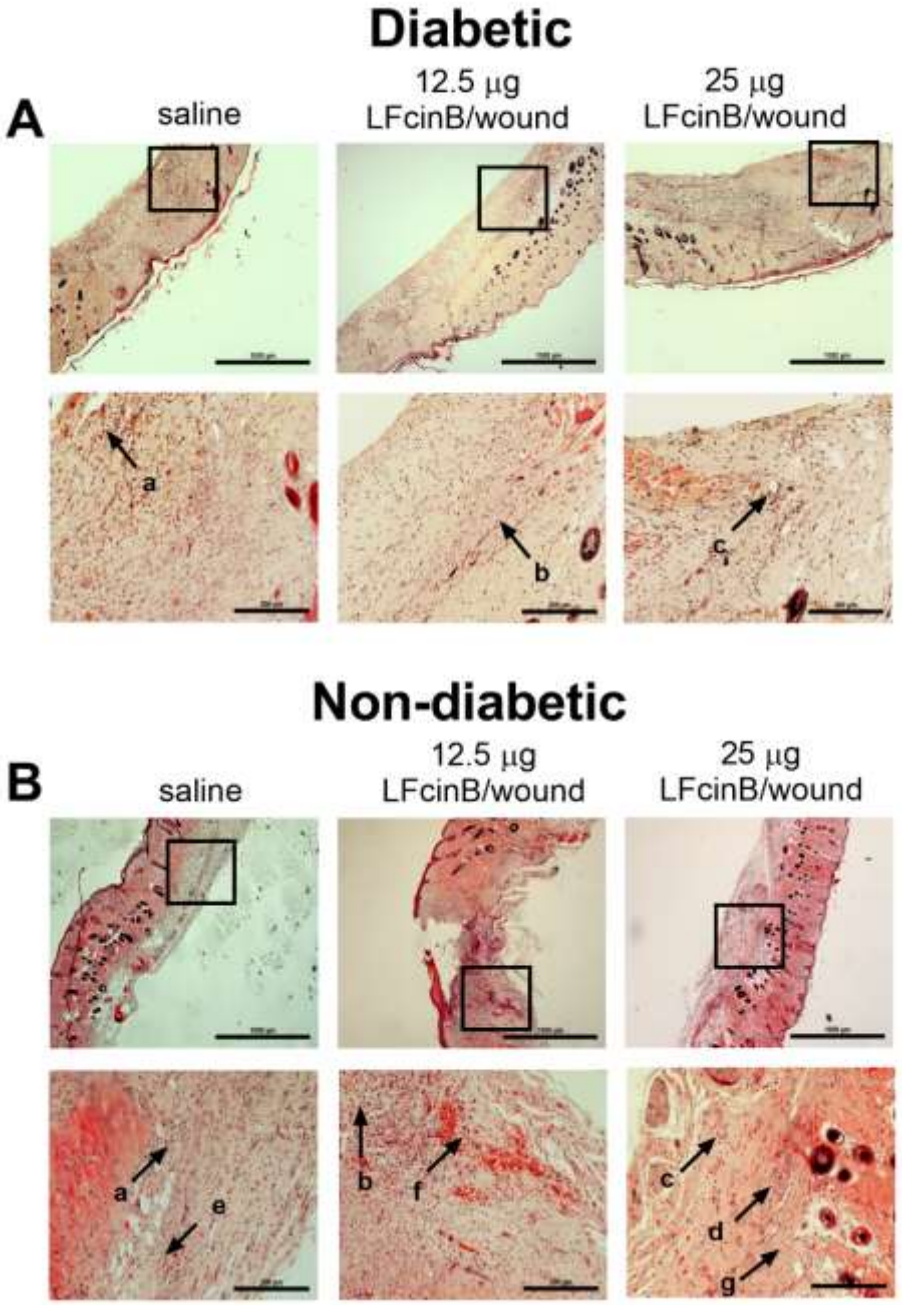
Supplementary Table 1: List of primer sequences and annealing temperatures

Primers	**Sequence (5'→3')**	**Annealing temperature**
RPLP0 forward	GCTTCCTGGAGGGTGTCC	52°C
RPLP0 reverse	GGACTCGTTTGTACCCGTTG	52°C
IL-6 forward	TACATCCTCGACGGCATCTC	60°C
IL-6 reverse	ACCAGGCAAGTCTCCTCATTG	60°C
IL-8 forward	CACCGGAAGGAACCATCTCA	60°C
IL-8 reverse	TTGGGGTGGAAAGGTTTGGAG	60°C
TNFα forward	TTCCTGATCGTGGCAGGC	60°C
TNFα reverse	GAGCTGCCCCTCAGCTTG	60°C



Supplementary Figure 1. Lactoferricin (LFcinB) and bovine lactoferrin (bLF) effect on HaCaT cell proliferation *in vitro.* The proliferation potential of LFcinB and bLF at 25 µg/ml were evaluated on the oCelloScope which was set to record the proliferation for 48 hours. Images acquired every 12th hour were used to quantify the proliferation rate. Epidermal growth factor (EGF), mitomycin C and DMEM were used as the positive and negative controls. * = $p<0.05$.

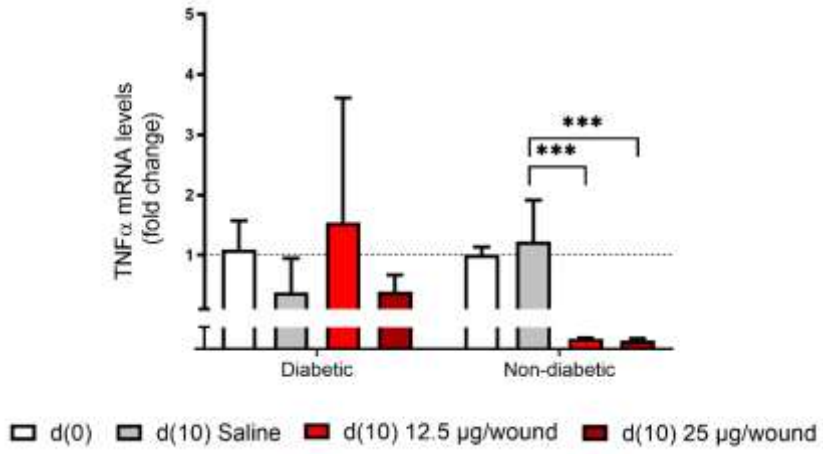


Supplementary Figure 2. Inflammatory response and tissue regeneration after dermal treatments with LFcinB. **A)** Representative photomicrographs of skin wound biopsies from diabetic animals following the treatment with saline and LFcinB, stained with standard hematoxylin and eosin.

B) Representative photomicrographs of skin wound biopsies from non-diabetic animals following the treatment with saline and LFcinB, stained with standard hematoxylin and eosin. **a)** Influx of

immune cells (fibroblasts and macrophages) **b)** Granulation tissue **c)** Blood vessels **d)** Inflammatory infiltrate **e)** Infiltrate enriched in neutrophils due to acute inflammation **f)** Hemorrhagic spots in the dermal area **g)** Visible granulation tissue and less inflammation.

Magnifications=50× and 200× for boxed areas. Scale bars=1000 μm and 200 μm for boxed areas. Black arrows indicate the morphological changes (n=4 animals per group).



Supplementary Figure 3. LFcinB alters mRNA cytokine levels in diabetic and healthy mouse skin wounds. The pro-inflammatory cytokine tumor necrosis α (TNFα) mRNA levels were measured by qPCR in skin wounds collected from diabetic and healthy mice. Skin wounds from three treatments groups: Low and high dose bovine lactoferricin (12.5 and 25 µg/wound) or saline were harvested before the wounding d (0) and 10 days post-wounding d (10). *** = $p<0.001$.