## Supporting information

### Mast cells are critical for controlling the bacterial burden and the healing of infected wounds

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## SI Materials and Methods

***Animals.*** All mice were obtained from breeding colonies of the animal facilities of the Charité - Universitätsmedizin Berlin. Female WBBF1-*$Kit^{W}$/$Kit^{W-v}$* and *$Kit^{+/+}$* mice (8 to 12 weeks old, telogen stage of hair follicle); C57BL/6J mice; C57BL/6 *Cpa3-Cre; $Mcl\text{-}1^{fl/fl}$* and C57BL/6 *Cpa3-Cre; $Mcl\text{-}1^{+/+}$* mice were used (*29*). B6.129S2-*Il6tm1Kopf*/J mice were kindly provided by Karen Gertz (Charité - Universitätsmedizin Berlin). C57BL/6 *Cpa3-Cre; $Il\text{-}6^{fl/fl}$* were generated by crossing *Cpa3-Cre;$Mcl\text{-}1^{+/+}$* mice with *$Il\text{-}6^{fl/fl}$* mice (*30*). The local ethics committee approved all animal experiments. The group size was determined by power analysis based on the differences between means (30%), standard deviation (20%), chosen α error (0.05) and chosen power (0.80). When working with only black-haired mice, groups were blinded during experimentation and analysis.

***Bacterial strain.*** *Pseudomonas aeruginosa* ATCC 27853 was kindly provided by Erich Gulbins (University Duisburg-Essen). Bacteria were cultured over night from DMSO (20%) frozen stocks and were grown in tryptic soy broth (TSB) to exponential growth phase and resuspended in Dulbecco's phosphate buffered saline (PBS, GIBCO). Bacterial concentrations were determined by optical spectrophotometer (600 nm). CFUs of the inoculum were verified by plate count after plating a serial dilution on TSB-Cetrimide agar plates (0.3 g pro L TSB agar).

***Model of skin wound infection with Pseudomonas aeruginosa.*** Back skin of mice was shaved, disinfected with ethanol 70% and one skin wound per mouse was created using a biopsy punch (Ø 6 mm) (Acuderm) on the lower back as described previously (*10*). Wounds were infected with an inoculum of $6.5 \times 10^5$ colony forming units (CFUs) *P. aeruginosa* or non-infected with sterile PBS as vehicle. Wounds were neither dressed nor sutured. In contrast to our previous study (*10*), all mice were housed individually and provided with Elizabethan collars for the first 24h to prevent cross contamination. Wound closure was photo-recorded daily and percentage of the initial wound area was calculated using Image64 free software. For IL-6 treatment, 200 ng recombinant IL-6 (recIL-6) (Biolegend) in 50 µl PBS or PBS only as vehicle control were applied topically with a pipette 1h prior to infection of the wounds. Assessment of IL-6

treatment and MC engraftment experiments (*Il-6*$^{-/-}$) was done by a researcher blinded to the identity of experimental groups.

***Determination of skin bacterial load.*** Half of a 12 mm circular wound biopsy was weighed and homogenized in 300 µl PBS. Serial dilutions of skin homogenates were plated on TSB-Cetrimide agar plates and the CFUs/0.1 gram of tissue was calculated from bacterial plate count after overnight incubation. For kinetic analyses, the bacterial load was measured daily by detecting fluorescent signal from XenoLight RediJect Bacterial Detection Probe using IVIS live imaging Spectrum Series imaging systems (Perkin Elmer). Substrate was diluted directly before use 1:100 and was added onto the wound. Imaging took place after 5 minutes. Radiant efficacy was calculated and auto-fluorescence was subtracted using Live Imaging Software 4.1 (Caliper Life Sciences).

***Mast-cell engraftment of Kit$^{W}$/Kit$^{W-v}$ back skin.*** MC engraftment of *Kit*$^{W}$/*Kit*$^{W-v}$ mice was carried out at 4 weeks of age. Engraftment of back skin was performed by intradermal injection of $10^6$ bone marrow-derived cultured MCs (BMCMCs) / 100 µl per $cm^2$ distributed over 4 $cm^2$ area (i.e., 8 injections of $5x10^5$ BMCMCs per site). Briefly, bone marrow-derived MCs from C57BL/6J mice or B6.129S2-*Il6tm1Kopf*/J mice were cultured for 4 weeks in Iscove's Modified Dulbecco's medium (IMDM, GIBCO) enriched with 10% heat inactivated fetal calf serum (Biochrom), 1% penicillin-streptomycin (P/S) and 10 ng/ml IL-3 (Biolegend) until MCs represented > 95% of the total cells according to FACS staining of c-Kit and FcεRI receptor expression. Engraftment was confirmed by histological analysis of 5 µm formalin-fixed and paraffin-embedded back skin sections using Giemsa staining. Briefly, skin sections were deparaffinized in Xylol and rehydrated using decreasing ethanol dilutions. Sections were stained for 15 minutes in Giemsa's Azure Eosin Methylene Blue (Merck Millipore), fixed in 0.1% acetic acid, dehydrated and mounted with Limonene mounting media (EMS). MCs from 3 high power fields of the skin were counted (at 200x magnification).

***In vitro bacterial infection assay.*** Pam212 mouse keratinocytes (KCs) were cultured in IMDM supplemented with 10% FCS, 1% P/S, 2 mM L-glutamine (Biochrom). KCs were washed with PBS, detached with 0.05% Trypsin-EDTA and, after centrifugation

(300 g, 8 min), seeded overnight. Human keratinocyte cell line HaCaT cells were cultured in DMEM supplemented with 10% FCS, 1% and P/S. Co-culture: A total of 50,000 cells per 96-well either single culture or BMCMC-KC co-culture (ratio 70:30), were washed twice with PBS and infected with a multiplicity of infection (MOI) of 5 *P. aeruginosa* in P/S-free, 1% FCS IMDM. After 1 min centrifugation at 300 g, cell cultures were incubated at 37°C in humidified 5% $CO_2$ incubator for 3h. CFUs were assessed by plate count after plating a serial dilution of the supernatant onto TSB-Cetrimide agar plates. For IL-6 treatment: 50 ng/ml (or as indicated) recIL-6 (Biolegend) in medium was added 1h prior to infection. Transwell experiments: Cells were separated using transwell inserts (0.4 µm). Supernatant transfer experiments: Cell culture supernatant from co-cultures either infected or non-infected were centrifuged (4000 g, 15 min), sterile filtered (0.2 µm), and transferred onto KCs. Infection was carried out as described above.

***Infection and treatment of human skin explants.*** Skin biopsies (Ø 4 mm) of healthy skin derived from reductive plastic surgery were disinfected with PBS + 5% P/S, washed twice in PBS, plated in a 96-well plate with epidermis side up and infected with $2.5 \times 10^5$ CFUs in P/S-free IMDM for 6h at 37°C. 50 ng/ml recIL-6 (Biolegend) in medium was added 1h prior to infection.

***Quantitative real-time PCR.*** Total RNA was isolated from cell cultures or back skin using Qiazol Lysis Reagent and further purified using the RNeasy Kit (Qiagen). Back skin was homogenized using Qiazol Lysis Reagent and GlycoBlue (Ambion). RNA was transcribed using High Capacity cDNA Reverse Transcription Kit (Invitrogen). All steps were carried out in accordance with the manufacturer's protocol. qRT-PCR was performed using the Light Cycler FastStart DNA Master SYBR Green I Kit (Roche) and sets of specific primers in a fluorescence temperature cycler (LightCycler; Roche). Samples were normalized to the β-actin transcript levels. Primers were designed using free software from https://www.roche-applied-science.com (Cambridge, MA, USA). *Il-6*: fw gctaccaaactggatataatcagga rev ccaggtagctatggtactccagaa, *Camp*: fw gccgctgattcttttgacat rev aatcttctccccacctttgc, *Defb14*: fw tgaggcttcattatctgctatttg rev tttcggagggtttttggtag, *S100A7:* fw gcctcgcttcatggacac rev cggaacagctctgtgatgtagt, *Actb*: fw ccgtgaaaagatgacccagat rev ctcagctgtggtggtgaagc.

***ELISA.*** Cell culture supernatants were analyzed using Multi-ELISA Mouse Th1 / Th2 / Th17 Cytokine Multi-Analyte ELISArray Kit (Qiagen), mouse mBD-14 and CAMP (Mybiosource) and mouse IL-6 Ready-SET-Go ELISA kit (e-bioscience). All procedures were carried out according to the manufacturer's instructions. For back skin analysis, skin was mechanically homogenized in PBS on ice and after centrifugation (4000 g, 15 min, 4°C) IL-6 was quantified in the skin supernatants and normalized to 0.1g skin.

***MPO Assay.*** MPO activity was determined by change in optical density (OD) at 460 nm resulting from the decomposition of $H_2O_2$ (Sigma) in the presence of o-dianisidine dihydrochloride. Skin biopsies were homogenized in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl-ammonium (Sigma). Samples were homogenized and twice sonicated using ice bath and freeze-thawed with liquid nitrogen. Supernatants were analyzed after centrifugation (4000 g, 20 min, 4°C). MPO content was calculated as units per 0.1 g skin by using a standard curve, which was established using recombinant MPO (R&D Systems).

***Statistics.*** Numeric data are presented as mean ± standard error of the mean (SEM). Statistical tests were carried out using Graph Pad Prism (5.0 for Mac OS X). All data sets with $n \geq 5$ were analyzed for Gaussian distribution using Kolmogorov Smirnov normality test. To compare two groups with $n < 5$, or non-parametric data sets, the rank-sum-based Mann-Whitney test for independent samples was applied. For the comparison of two groups derived from normally distributed data set, two-sided unpaired Student's *t* test for independent samples was used. For the comparison of multiple groups, the rank-sum-based Kruskal-Wallis test was used for nonparametric data sets followed by Dunn's post hoc test. For normally distributed data sets, the one-way ANOVA was used followed by Tukey's post hoc test. Repeatedly measured data sets were analyzed using two-way repeated measure ANOVA followed by Bonferroni post hoc test. Differences were considered statistically significant at *$p<0.05$, ** $p<0.01$ and *** $p<0.001$. Differences with $p>0.05$ were considered not statistically significant (n.s.).

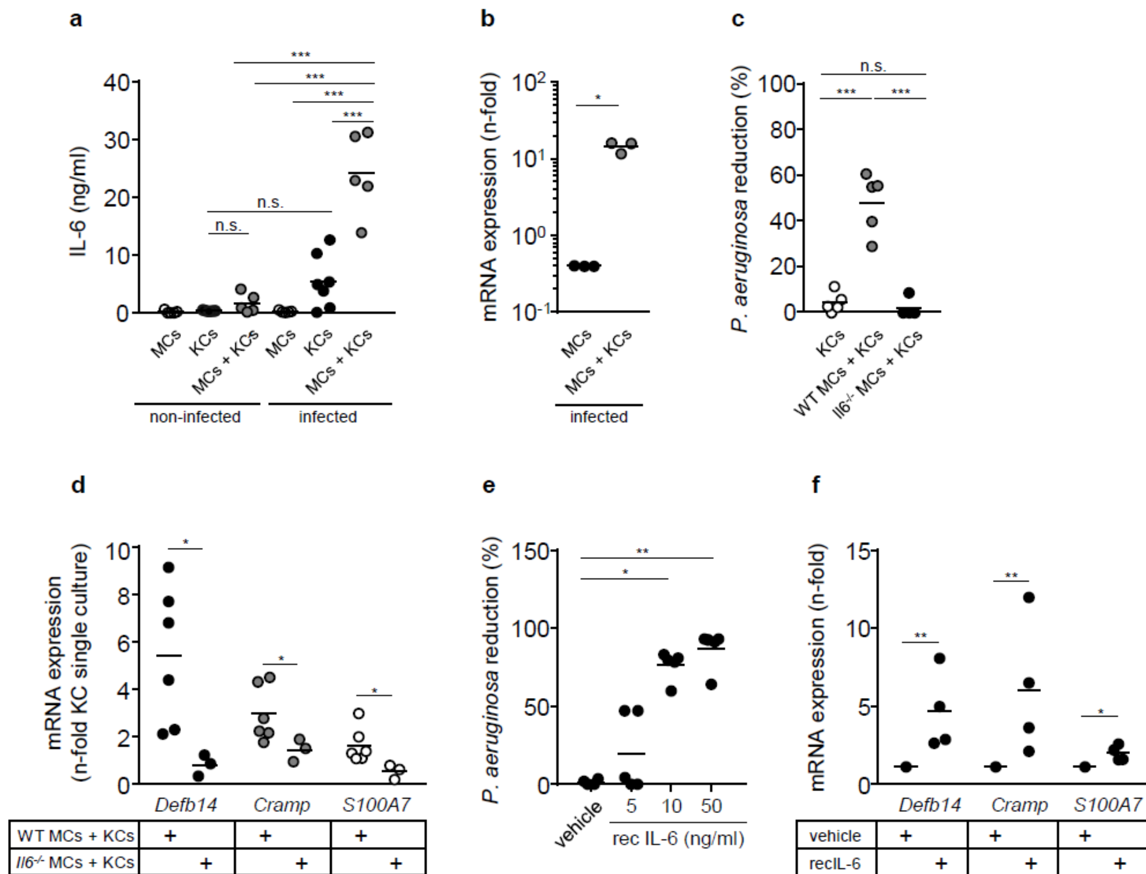


**Figure S1: Mast cell-derived IL-6 stimulates AMP response in keratinocytes.** (a) IL-6 protein levels in supernatants of infected or non-infected MC and KC single or co-cultures. Each data point represents an individual experiment, data pooled from 5 independent experiments and analyzed by one-way ANOVA followed by Tukey's post hoc test. (b) Relative *Il-6* mRNA expression in infected MCs compared to MCs in infected MC-KC co-cultures. Each data point represents an individual experiment, data pooled from 3 independent experiments and analyzed by Students *t* test. (c) CFU reduction of *P. aeruginosa* in supernatants of MC-KC co-cultures with either WT MCs (KCs + MCs) or IL-6 deficient MCs (KCs + *Il6*$^{-/-}$ MCs). Each data point represents an individual experiment, data pooled from 4 independent experiments and analyzed by one-way ANOVA followed by Tukey's post hoc test. (d) mRNA levels of AMPs expressed in KCs during co-culture with MCs from WT or *Il-6*$^{-/-}$ mice after 3h *P. aeruginosa* infection *in vitro*. Values are normalized to those of the internal control gene beta-actin and represented as fold change relative to that of non-infected KC controls, which was converted to 1. Each data point represents an individual experiment; data are expressed as means, pooled from at least 3 independent experiments and analyzed by Mann Whitney test. (e) CFU numbers of cell culture supernatants after *in vitro* stimulation of mouse KCs with recIL-6 or vehicle before infection with *P. aeruginosa*. Each data point represents an individual experiment; data are expressed as means, pooled from 5 independent experiments and analyzed by Kruskal-Wallis test followed by Dunn's post hoc test. (f) mRNA levels of AMPs expressed in mouse KCs stimulated with recIL-6 or vehicle before infection with *P. aeruginosa*. Values are normalized to those of the internal control gene beta-actin and represented as fold change relative to that of vehicle treated KC controls, which was converted to 1. Each data point represents an individual experiment; data are expressed as means, are pooled from 4 independent experiments and analyzed by Mann Whitney test. *p<0.05, **p<0.01, ***p<0.001, n.s. = not significant.

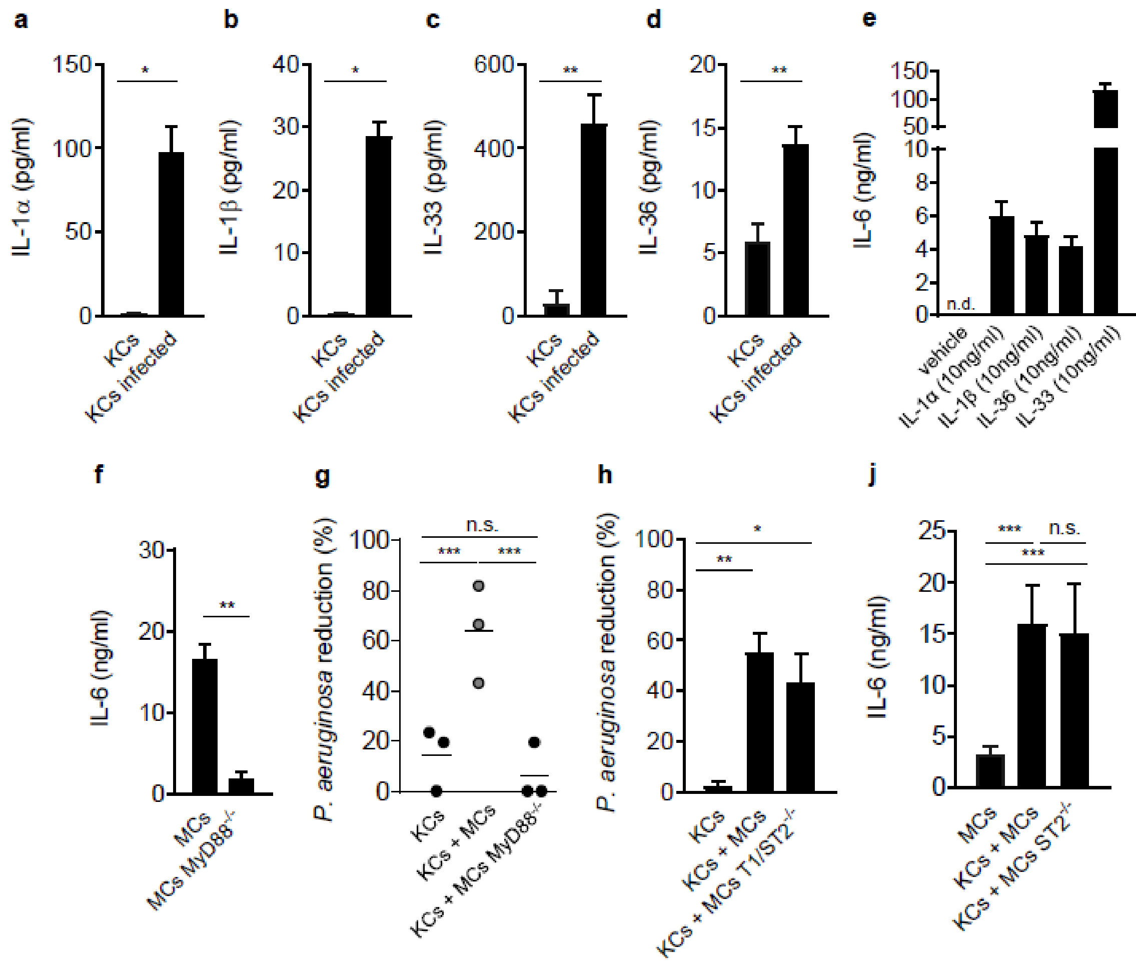


**Figure S2: IL-1 family members released by KCs induce IL-6 in MCs by a MyD88 dependent pathway and augment bacterial clearance.** (a-d) Cytokine levels in supernatants from infected and non-infected (PBS-treated) KCs measured by ELISA. (e) IL-6 release from MCs stimulated by IL-1 family members. (f) IL-6 release by MCs and MyD88-deficient MCs stimulated by supernatants from infected KCs. (g) Reduction of CFU from supernatants 3 h after *P. aeruginosa* infection of KCs, KCs + MCs co-culture and KCs co-cultured with MyD88-deficient MCs and (h) KCs co-cultured with T1/ST2-deficient MCs. (j) IL-6 in supernatants from MCs, KCs + MCs co-cultures and co-cultures of KCs and MCs deficient in expression of the IL-33 receptor (ST2). Data are expressed as mean ± SEM and analyzed using Mann Whitney test. *$p<0.05$, **$p<0.01$, ***$p<0.001$, n.s. = not significant, n.d. = not detectable.

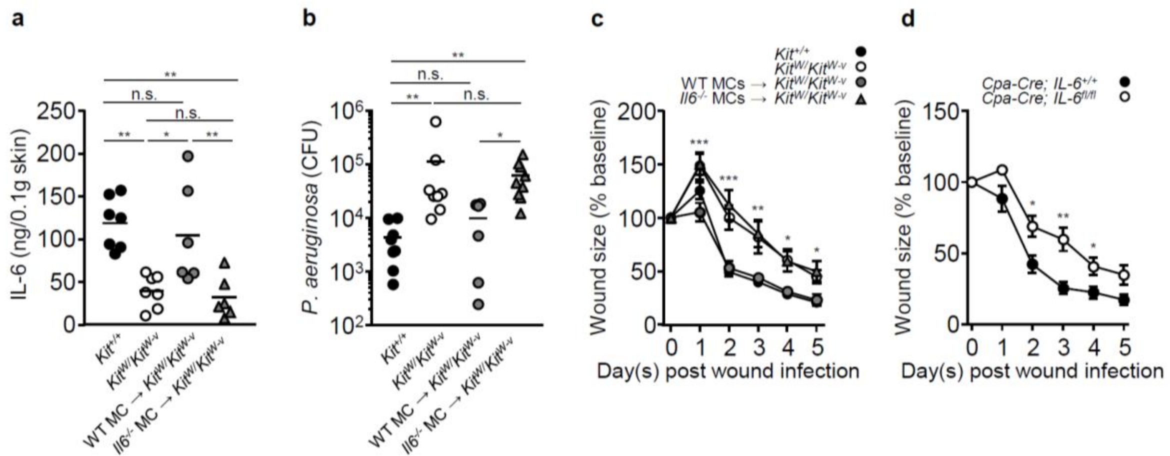


**Figure S3: Mast cell-derived interleukin-6 enhances skin antimicrobial capacity.** MC-derived IL-6 is crucial for optimal antimicrobial skin defense during *P. aeruginosa* infection. (a) IL-6 protein levels from infected skin wound supernatants 24 h post skin wound infection in $Kit^{+/+}$ mice (n=7), $Kit^{W}/Kit^{W\text{-}v}$ mice (n=7) and $Kit^{W}/Kit^{W\text{-}v}$ mice after intradermal engraftment of WT MCs (WT MCs → $Kit^{W}/Kit^{W\text{-}v}$) (n=6) or $Il\text{-}6^{-/-}$ MCs ($Il6^{-/-}$ MCs → $Kit^{W}/Kit^{W\text{-}v}$) (n=6). Data are expressed as mean per mouse, pooled from 3 independent experiments and analyzed by one-way ANOVA followed by Tukey's post hoc test. (b) *P. aeruginosa* CFUs from infected skin wound supernatants 24 h post skin wound infection of $Kit^{+/+}$ mice (n=8), $Kit^{W}/Kit^{W\text{-}v}$ mice (n=7) and MCs → $Kit^{W}/Kit^{W\text{-}v}$ mice engrafted with either WT (n=6) or $Il6^{-/-}$ MCs (n=9). Data are expressed as mean per mouse, pooled from 3 independent experiments and analyzed by Kruskal-Wallis test followed by Dunn's post hoc test. (c) Wound closure kinetics of infected skin wounds from (c) $Kit^{+/+}$ (n=7), $Kit^{W}/Kit^{W\text{-}v}$ mice (n=9), and $Kit^{W}/Kit^{W\text{-}v}$ engrafted with MCs from WT (WT MCs → $Kit^{W}/Kit^{W\text{-}v}$) (n=6) or $Il6^{-/-}$ mice ($Il6^{-/-}$ MCs → $Kit^{W}/Kit^{W\text{-}v}$) (n=7). Data are expressed as mean ± SEM, pooled from each of 3 independent experiments and analyzed using two-way repeated measure ANOVA followed by Bonferroni post hoc test. (d) Wound closure kinetics of infected skin wounds in *Cpa-Cre;* $Il6^{+/+}$ mice (n=7) and *Cpa-Cre;* $Il6^{fl/fl}$ mice (n=7). Data are expressed as mean ± SEM, pooled from each of 3 independent experiments and analyzed using two-way repeated measure ANOVA followed by Bonferroni post hoc test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, n.s. = not significant.

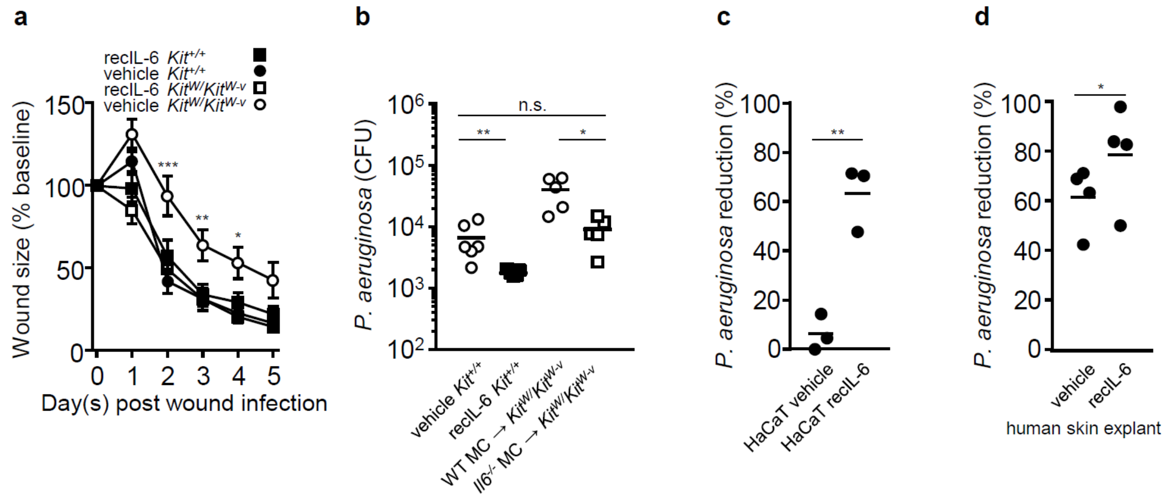


**Figure S4: Recombinant IL-6 stimulates mouse and human skin cells to combat *P. aeruginosa* infection.** (a-b) Mice have been pre-treated topically with recIL-6 or vehicle 1h prior infection. (a) Wound closure kinetics of infected skin wounds from $Kit^{+/+}$ mice (recIL6 n=10, PBS n=10) and $Kit^{W}/Kit^{W-v}$ mice (recIL6 n=6, PBS n=7). Data are expressed as mean ± SEM, pooled from each of 3 independent experiments and analyzed using two-way repeated measure ANOVA followed by Bonferroni post hoc test. (b) *P. aeruginosa* CFUs from skin wound supernatants 24 h after infection of $Kit^{+/+}$ (vehicle n=6, recIL6 n=5) mice, $Kit^{W}/Kit^{W-v}$ mice (vehicle n=5, recIL6 n=5) upon recIL-6 or vehicle treatment 1 h prior infection. (c-d) Reduction of CFU from supernatants 3 h after *P. aeruginosa* infection upon recIL-6 or vehicle treatment of (c) human HaCaT KCs, data are pooled from 3 independent experiments and analyzed using Students *t* test, and (d) human skin biopsies (n=4), data are pooled from 4 independent experiments and are analyzed using Mann Whitney test. All data shown as mean per experiment. *p<0.05, **p<0.01, ***p<0.001, n.s. = not significant.

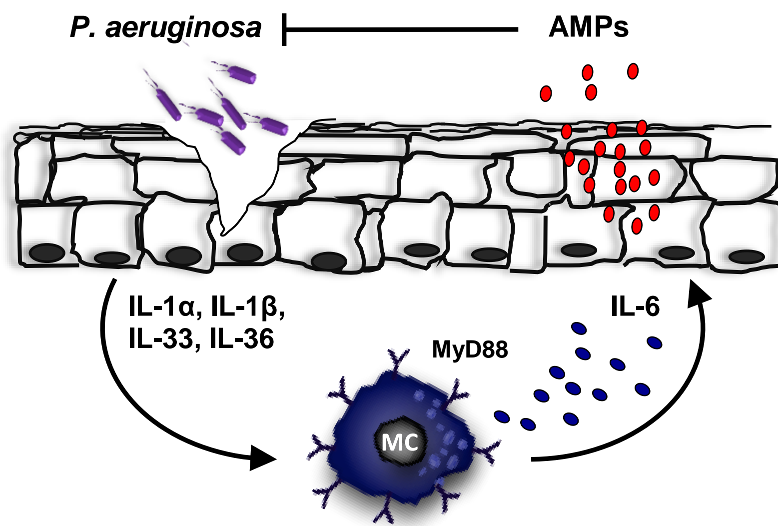


**Figure S5: The role of mast cells in the control of bacterial skin wound infection.**

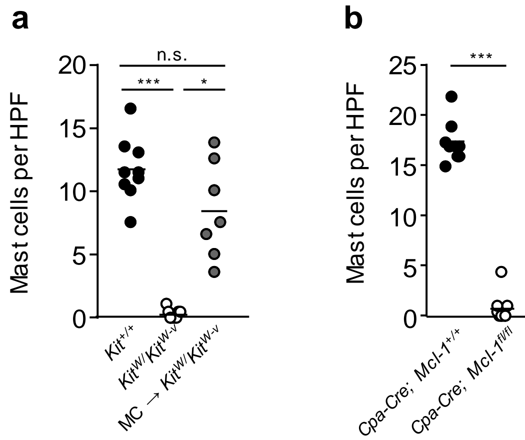


**Figure S6: Mast cell counts in engrafted and deficient mice.** Skin MC numbers per HPF from (d) *Kit*$^{+/+}$ mice, *Kit*$^{W}$/*Kit*$^{W\text{-}v}$ mice and *Kit*$^{W}$/*Kit*$^{W\text{-}v}$ mice after intradermal transfer of MCs (MCs → *Kit*$^{W}$/*Kit*$^{W\text{-}v}$) (e) *Cpa3-Cre; Mcl-1*$^{fl/fl}$ mice and *Cpa3-Cre; Mcl-1*$^{+/+}$ mice. Data are expressed as mean from 10 HPF counted per mouse. *p<0.05 **p<0.01 ***p<0.001 n.s. = not significant.