Supplemental Methods

Mice

C57BL/6 mice, B6.SJL-PtprcaPepcb/BoyJ, and TLR2-deficient mice were from The Jackson Laboratories. EGFP-lysozyme M (lys) knock in-mice (Lys-EGFP)\(^1\) (kind gift of Dr. Thomas Graf) and MyD88-deficient mice\(^2\) (kind gift of Dr. Richard Flavell) were bred in animal facilities at University of California at Davis. Lys-EGFP mice were crossed to MyD88-deficient mice (Lys-EGFP/MyD88\(~/-~\)) to obtain mice homozygous for both traits. All animal experiments were approved by Institutional Animal Care and Use Committee of the University of California at Davis and performed following the guidelines of Animal Welfare Act and Health Research Extension Act.

Non-invasive quantification of wound EGFP-PMN

We have shown that EGFP signal in the wound demonstrates linearity with PMN number from \(5 \times 10^4\) up to \(10^7\) in the wound and this non-invasive imaging method correlates closely to the number of PMN migrated into infected wounds\(^3,4\).

Mouse model of cutaneous S. aureus infection

Mice were premedicated with 0.2 mg/kg buprenorphine (Reckitt Benckiser Pharmaceuticals, Richmond, VA) intraperitoneally and anesthetized with 2% inhaled isoflurane (Phoenix Pharmaceuticals, Burlingame, CA). Back skin hair was shaved, and then antiseptically prepared with 10% w/v povidine-iodine and 70% ethanol. A 6 mm diameter full thickness wound was made in the skin over the dorsal thoracolumbar region using a skin biopsy punch (Miltex, York,
Wounds were inoculated immediately following injury with sterile saline, $2\times 10^6$, or $1\times 10^7$ CFU of a bioluminescent strain of *S. aureus*, all in a volume of 100 µl.

**Preparation of bioluminescent *S. aureus***

A bioluminescent SH1000 strain of *S. aureus* was streaked onto Tryptic soy agar (Tryptic soy broth [TSB] + 1.5% Bacto Agar). Two to 3 colonies were then transferred to liquid TSB culture and incubated overnight incubation at 37°C in a shaking incubator (210 rpm). Mid-logarithmic phase bacteria were obtained after a 3 hr subculture of 1:100 dilution of the overnight culture in the presence of the selection agent chloramphenicol (10 µg/mL). Bacterial cells were pelleted, resuspended, and washed twice in PBS. Bacterial concentrations were estimated with a spectrophotometer by determining the absorbance at 600 nm (A600). 100 µl of midlogarithmic growth phase *S. aureus* ($1\times 10^7$ CFU/100 mL) was used for wound inoculation.

**Isolation of blood cells from skin wounds***

To collect white blood cells and HSPCs from skin wounds, 8 mm diameter tissue biopsies were coarsely chopped and placed into 5 mL of RMPI 1640 (Invitrogen) with 10 mM HEPES, 1% penicillin-streptomycin, 1% collagenase, 0.025% DNase I (all from Sigma-Aldrich), and 1% fetal bovine serum (FBS) and incubated, rocking, for 1 hr at 37°C. After incubation, tissue digest was washed with cold 5 mM EDTA in DPBS and passed through 70 µm then 35 µm filters. Cells were pelleted at 1000 g for 10 minutes.

**Colony forming unit assay***
To quantify the capacity of cells isolated from BM and wounded skin to form mature blood cells, colony forming unit assays were performed. Three days post-wounding, BM cells and skin wounds were collected as described above. HSPCs were enriched by positive selection using CD117 magnetic beads (Stem Cell Technologies). Cells were resuspended in RMPI 1640 and 3x10^5/mL were plated in duplicate in Methocult GF M3434 (Stem Cell Technologies). Colony forming units were quantified 7 days later using a Leica DMIL inverted microscope (Leica Microsystems).

**Immuno-depletion of c-kit⁺ stem and progenitor cells**

C-kit⁺ cell depletion was performed as previously described⁶, with the following differences. One mg of the anti-c-kit antibody (clone ACK2) was given intraperitoneally 5 days prior to wounding and again 1 day after wounding. Control mice were given equivalent doses of rat anti-mouse IgG. All antibodies were from the University of California-San Francisco Monoclonal Antibody Core.

**Flow cytometric immunophenotyping**

Bone marrow, peripheral blood, and skin wound digest were collected and flow cytometric immunophenotyping was used to identify HSPCs (lineage-/c-kit⁺), LSK (lineage-/c-kit+/Sca-1⁺), CMPs, promyelocytes, and PMNs as described in Supplemental Methods and in Supplemental Figure 1. For BM collection, two whole femurs were crushed in 5 mM EDTA and 1% FBS in PBS using a mortar and pestle and passed through a 35 µm filter. Peripheral blood cells were drawn into a 3 ml syringe (25 g needle) filled with 100 ul of 20 mM EDTA immediately post-mortem via cardiac puncture. Total white blood cell number in the bone
marrow of two femurs and per ml of peripheral blood was determined by Coulter counter (Beckman Coulter). Cells were isolated from skin wounds as described above and cell number per 8 mm wound biopsy was determined using a hemacytometer. Then skin cell pellets were resuspended in 3 ml of FBS and gently pipetted for 5 minutes before washing. All cells were resuspended in 100 µl staining buffer (1% FBS + 1 mM EDTA in DPBS) at a concentration between 1 and 5 × 10^7/ml. SYTOX Blue dead stain (Invitrogen) was used for live-dead gating. The following anti-mouse antibodies were used for immunophenotyping (see Supplementary Figure 1 for gating scheme): Ter119 (Pacific Blue), B220 (Pacific Blue), CD3 (Pacific Blue), CD117 (APC), sca-1 (PE), CD16/32 (PE-Cy7), Ly6G (FITC), CD11b (APC-Cy7) from Biolegend; CD34 (Alexa Fluor 700) and F4/80 (PE-TR) from Invitrogen; biotin-Ly6G used in Lys-EGFP mice from University of California-San Francisco Hybridoma and Monoclonal Antibody Core used with streptavidin-Qdot 605 (Invitrogen). For evaluation of donor-derived PMN after adoptive transfer to congenic mice, anti-mouse CD45.1 (biotin) and CD45.2 (FITC) (Miltenyi Biotec) were used. Streptavidin PE (Invitrogen) was used as a secondary antibody. Events were acquired using a LSRII flow cytometer (Becton Dickinson) or FC500 flow cytometer (Beckman Coulter) and analyzed using FlowJo software (Treestar, Inc.). Positive and negative gates were based on fluorescence minus one (FMO) controls. Gating strategies for identification of LSK cells, CMP, promyelocytes, and PMN have been previously described^7-9^.

*In vitro proliferation of HSPCs*

Five hundred-thousand BM HSPCs per condition, enriched by lineage depletion using magnetic beads (Stem Cell Technologies), were incubated at 37°C with heat-killed (95°C for 5 minutes) *S. aureus* (1x10^8/mL) with or without indomethacin (0.5 µM; Sigma), PGE2 (10 µg/mL; Cayman
Chemical), or no stimulus for 48 h in 400 µl StemSpan media (Stem Cell Technologies) along with SCF (20 ng/ml) and Flt-3 ligand (100 ng/ml) (both from Peprotech, NJ) to promote cell viability. Bromodeoxyuridine (BrdU) was added at 10 mM for the final 12 hours of incubation. BrdU incorporation was evaluated via flow cytometry with FITC-conjugated anti-BrdU (BrdU Flow Kit, BD Pharmingen).

**Adoptive transfer of HSPCs**

Cells were washed once and resuspended in 0.9% saline at $5 \times 10^6$ cells/ml. 100 µl of cell suspension was injected directly into wounds 1 day after wounding to ensure delivery of equal numbers into wounds of respective strains. In some experiments a more pure population was required and lin\(^-/\)Sca-1\(^+\)/c-kit\(^+\) cells (LSK) were sorted via FACS as described below. For LSK cells, the injected dose was $2 \times 10^4$. Some HSPCs were incubated with 2 uM PGE\(_2\) for 2 hours on ice before adoptive transfer. PMNs derived from HSPCs were quantified by the intensity of EGFP signal in the wound 7 days after transfer. Alternatively, CD45.2\(^+\) HSPCs were transferred to CD45.1\(^+\) recipients. Wounds were collected, weighed and digested. Total WBC in digest was counted by Coulter counter (Beckman Coulter). PMNs were evaluated by flow cytometry as described above and enumerated using BD TruCount beads (BD Pharmingen).

**FACS sorting of LSK cells**

Bone marrow was isolated from naïve mice as described in Methods. Lineage depletion beads were used according to manufacturer’s instructions to enrich for HSPC prior to labeling for FACS sorting. HSPC were labeled with the lineage markers Ter119, B220, and CD3 as above (Pacific Blue), and biotin-Ly6G and Brilliant Violet 421 streptavidin secondary (Biolegend).
Cells were also labeled with c-kit (APC) and sca-1 (PE) as above. LSK cells negative for lineage markers and positive for c-kit and sca-1 were sorted using a MoFlo Cell Sorter (Beckman Coulter).

**Prostaglandin E$_2$ production by LSK cells in vitro**

Bone marrow LSK cells were obtained from naïve WT, TLR2- and MyD88-deficient mice as described above and 20,000 LSK cells per well were plated in 200 µl of StemSpan media. Either vehicle or the TLR2-specific agonist Pam$_3$CSK$_4$ (Invivogen) was added at 100 ng/ml and cells were incubated for 72 hours at 37°C. Culture supernatant was collected and PGE$_2$ production quantified using a PGE$_2$ EIA kit (Cayman Chemical) according to manufacturer’s instructions.

**In vitro differentiation of LSK cells to PMN**

FACS sorted LSK cells were plated at 5,000 cells per well in StemSpan media supplemented with Flt-3 ligand and SCF as described above. Cells were stimulated with Pam$_3$CSK$_4$ at 100 ng, 500 ng or 1 µg/ml for 4 to 7 days. Cells in each well were enumerated via hemacytometer and percent of PMN were evaluated by flow cytometry after labeling for Ly6G.

**Wound Prostaglandin E$_2$ measurement**

Wounds were collected using an 8 mm punch biopsy 3 days after wounding and infection. Wounds were snap frozen in liquid nitrogen and stored at -80°C until analysis. Frozen wounds were weighed and ground to a powder using a mortar and pestle placed on dry ice, and then resuspended in ethanol. Samples were purified using Isolute® C18 SPE columns (Biotage) exactly as described in the Cayman Chemical PGE$_2$ EIA kit with the exception of the use of
tritium-labeled PGE$_2$. Determination of PGE$_2$ production was made using a PGE$_2$ EIA kit (Cayman Chemical) according to manufacturer’s instructions.

**Statistical Analysis**

Statistical significance between two groups was determined by unpaired $t$ tests (using log transformation when appropriate), and between multiple groups by 2-way ANOVA. $P$ values of $<0.05$ were considered statistically significant.

**References**

Supplemental Figure 1. Immunophenotyping gating scheme. Cell singlets that were live and negative for the lineage markers CD3, B220, and Ter119 were evaluated. All gates were based upon fluorescence minus one controls. PMN were Ly6G<sup>hi</sup>/CD11b<sup>hi</sup>. Promyelocytes were Ly6G<sup>intermediate</sup>/CD11b<sup>intermediate</sup>. LSK cells were lineage<sup>-</sup> (including Ly6G<sup>-</sup>/CD11b<sup>-</sup>)/sca-1<sup>+</sup>/c-kit<sup>+</sup>. Common myeloid progenitors (CMP) were c-kit<sup>+</sup>/sca-1<sup>-</sup>/CD34<sup>+</sup>/CD16/32<sup>lo</sup>/lineage<sup>-</sup>/Ly6G<sup>-</sup>. 
Supplemental Figure 2. Peripheral Blood Counts of mice receiving anti-c-kit antibody and isotype antibody. Total white blood cell count (WBC). Number of leukocytes subtypes per µl blood were calculated using 100 cell count differentials from blood smears collected 4 days after antibody treatment (one day prior to wounding). There were no statistical differences between the two groups. Data represents 3 mice per group.
Supplemental Figure 3. *S. aureus* infection of cutaneous wounds causes minimal changes in PMN and their progenitors in the bone marrow. Populations of PMN and their progenitors in bone marrow of C57Bl/6 mice via flow cytometry on days 0, 1, 3, 5 post-wounding. LSK refers to the lineage negative, sca-1 and c-kit positive cell population; LKS− refers to the lineage negative, c-kit+, Sca-1 negative population; CMP, common myeloid progenitor cells; Bands and PMN, immature banded and mature segmented neutrophils. Data represents 8 mice per group and is presented as Mean +/- SEM. (* indicates significant difference compared to Day 0, p<0.05; # indicates significant difference compared to Saline, p<0.05)
Supplemental Figure 4. *S. aureus* infection of cutaneous wounds recruits HSC, committed progenitors and PMN to wounds. Populations of PMN and their progenitors skin wounds of C57Bl/6 mice via flow cytometry on days 0, 1, 3, 5 post-wounding. LSK refers to the lineage negative, sca-1 and c-kit positive cell population; LKS⁻ refers to the lineage negative, c-kit+, Sca-1 negative population; CMP, common myeloid progenitor cells; Bands and PMN, immature banded and mature segmented neutrophils. Data represents 8 mice per group and is presented as Mean +/- SEM. (* indicates significant difference compared to Day 0, p<0.05; # indicates significant difference compared to Saline, p<0.05)
Supplemental Figure 5. Granulocyte differentiation of LSK cells occurs in a dose-dependent manner after stimulation with the TLR2-specific agonist Pam$_3$CSK$_4$. A. 5000 LSK cells were stimulated with 0, 100, 500, or 1000 ng of Pam$_3$CSK$_4$ in vitro for 4 days. EGFP-expressing PMN were enumerated by flow cytometry. Data represents 4 mice. B. 5000 LSK cells were stimulated with 0, 500 ng or 1 ug of Pam$_3$CSK$_4$ in vitro for 7 days. EGFP-expressing PMN were enumerated by flow cytometry. Data represents two mice. All data presented as Mean +/- SEM.
**Supplementary Figure 6.** Mice lacking MyD88 or TLR2 do not enhance HSPC bone marrow number or peripheral blood mobilization in response to cutaneous *S. aureus* infection. C-kit+/lineage- HSPC number in bone marrow (A) and blood (B) was evaluated on day 3 post-wounding via flow cytometry in C57Bl/6, TLR2-/- and MyD88-/- mice. (*,p<0.05) Data represents 5 to 7 mice per group for A and 3 to 6 mice per group for B and is presented as Mean +/- SEM.
**Supplementary Figure 7. Bone marrow HSPC from TLR2 and MyD88 deficient mice form as many granulocyte colonies as wild-type.** Bone marrow HSPC collected 3 days post-wounding from C57BL/6, TLR2-/- and MyD88-/- mice with saline control or *S. aureus*-infected wounds were plated in equal number in methycellulose media supportive of granulocyte differentiation. Colonies were counted 7 days after plating. Data represents 4 to 6 mice per group and is presented as Mean +/- SEM. (*,p<0.05)
Supplementary Figure 8. MyD88-deficient mice have significantly decreased total wound PGE$_2$. *S. aureus*-inoculated wounds from WT and MyD88/- mice were collected 3 days post-wounding and total wound PGE$_2$ was measured by ELISA. Data represents 3 to 5 mice per group and is presented as Mean +/- SEM. (**,p<0.01)
Supplementary Figure 9. PGE\textsubscript{2}-induced PMN from MyD88\textsuperscript{-/-} and TLR2\textsuperscript{-/-} HSPC decrease bacterial burden. A. Wounds of MyD88-deficient mice were inoculated with 2x10\textsuperscript{6} CFU S. aureus and HSPC from WT mice, MyD88-deficient mice or HSPC from MyD88-deficient mice after pretreatment with PGE\textsubscript{2} were adoptively transferred into the wounds. Bacterial bioluminescent signals at day 7 is shown. B. Wounds of TLR2-deficient mice were inoculated with 1x10\textsuperscript{7} CFU S. aureus and HSPC from WT mice, TLR2-deficient mice or HSPC from TLR2-deficient mice after pretreatment with PGE\textsubscript{2} were adoptively transferred into the wounds. Bacterial bioluminescence at day 6 is shown. Data represents 5 mice per group for A and 4 mice per group for B and is presented as Mean +/- SEM. (*,p<0.05)