Supplementary Material:

Supplementary Material and Methods:

Preparation of ear cells

On day 1 or 2 post-infection, the ears were processed as described (*47*). In brief, ears were excised, washed with 70% ethanol, and allowed to dry for 5 min. The dorsal and ventral layers were separated and incubated at 37^{o} C for 90 min in RPMI medium containing Liberase, homogenized for 3.5 min in a Medimachine, flushed out of the Medicon, filtered, using a 50 μM strainer and centrifuged. The homogenate was then serially diluted 10-fold, plated on blood agar plates, and plates incubated at 37^{o} C for 18 h. Colonies were counted the following day to determine MRSA titers. The remaining ear homogenate was used for flow cytometric analysis using the indicated antibodies.

Antibodies for flow cytometric analysis

For mouse samples: anti-CD11b (M1/70), Gr-1, Ly6G (1A8), Ly6C (HK1.4), F4/80, CD127, and C5aR1 (20/70) antibodies and TruStain fcX were from Biolegend, and anti-TSLPR (FAB5461F) was from R&D Systems. The appropriate isotype controls from the corresponding company were used for all phenotyping antibodies. For intracellular staining, cells were fixed and permeabilized with Cytofix Cytoperm and Perm wash (BD Biosciences). For human samples: anti-CD16 (3G8), CD66b (G10F5), CD11b (M1/70) and Trustain were from BioLegend. Anti-TSLPR (1F11) was from BD biosciences and the cells were fixed and permeablized before staining with TSLPR. Samples were

collected using a FACS Canto II or Fortessa flow cytometer (BD Biosciences) and analyzed using Flow Jo analysis software (Treestar, Inc).

Phagocytosis assay

Mouse bone marrow neutrophils or human blood neutrophils were isolated as described above, stimulated with PBS or TSLP, and incubated with pHrodo® Green *S. aureus* bioparticles® (Life Technologies) for 5, 20, or 30 min, per the figure legends, and phagocytosis assessed by flow cytometry on a FACS Canto II. For inhibition of phagocytosis, neutrophils were pretreated with either DMSO or cytochalasin D (10 μg/ml) for 15 min.

RNA sequencing

Neutrophils were purified from 2 independent human donors on different days (in 2 independent experiments) and stimulated with either PBS or TSLP, with or without heat killed MRSA for 4 and 24 hr. The cells were washed, RNA purified, RNA-Seq libraries prepared using the KAPA Stranded mRNA-Seq kit (Kapa Biosystems), and sequencing performed using an Illumina HiSeq 2000 platform in the NHLBI DNA Sequencing core.

MAPK/ERK and PI3K inhibition experiments

For MAPK/ERK and PI3K inhibition, human neutrophils were pre-incubated for 20 min with 50 μM PD98059 or 20 μM Ly294002, respectively, and then either PBS or TSLP and MRSA were added for 2 h shaking. After the 2 hr incubation the samples were put on ice, 10-fold serial dilutions were made, spread on blood agar plates, incubated overnight

at 37^{o} C, and colonies counted to determine the CFU/tube. Each treatment was done in triplicate.

TSLP protein measurement

Mouse ears were excised, washed with 70% ethanol, allowed to dry for 5 min, and then the dorsal and ventral layers were separated, put into 1 ml of PBS with protease inhibitor, homogenized using a Minibead beater (Biospec), cleared by centrifugation, and samples were immediately frozen. TSLP protein was determined using the BioLegend Legendplex kit according to the manufacturer's protocol. Similar results were found with the mouse quantikine ELISA kit (R & D Systems).

Ear Pathology

Ears were excised, fixed with 3.7% formalin, and embedded in paraffin. Three segments of each ear were cut and the slides were stained with hematoxylin and eosin. Pathological scoring of the H&E stained sections was performed blinded.

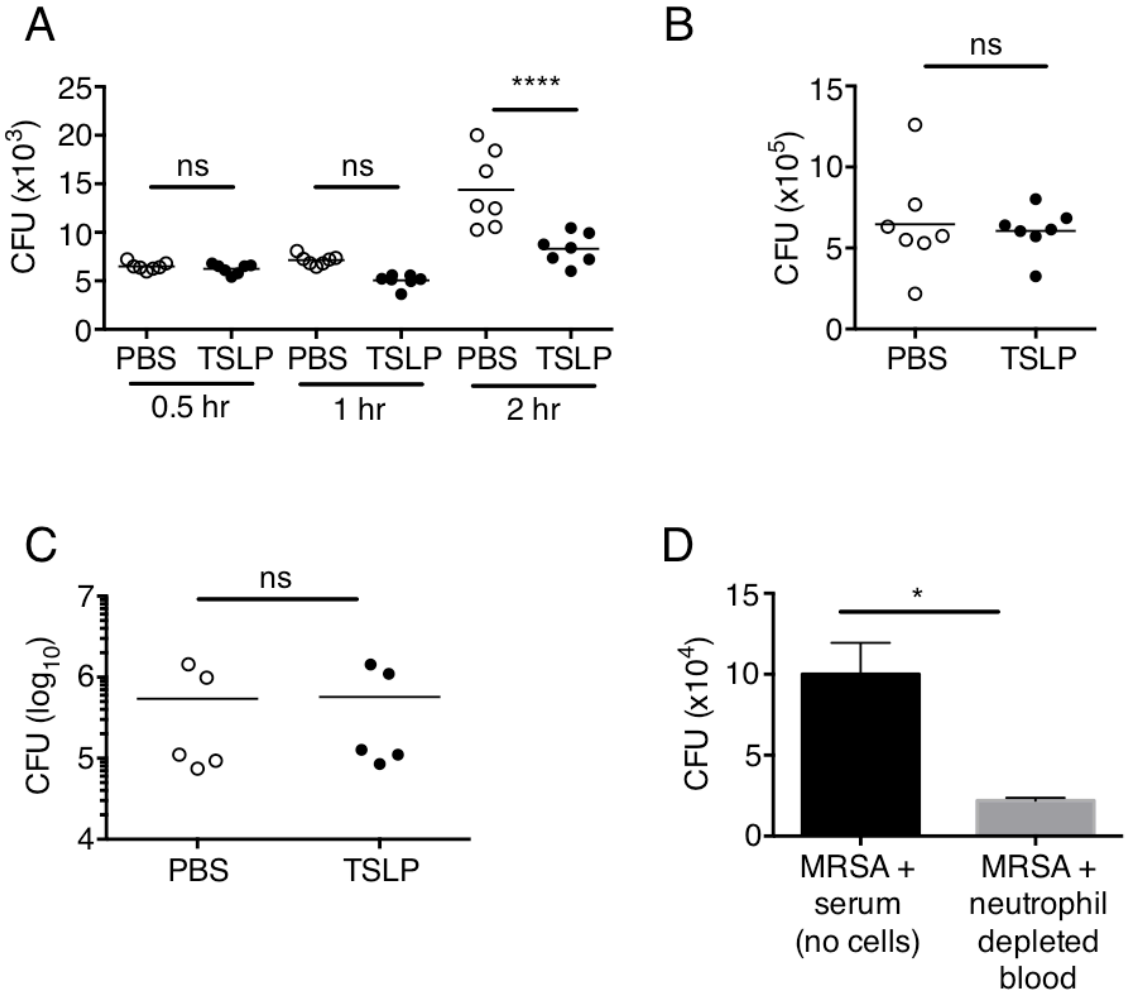

Figure S1. TSLP does not directly kill MRSA, and normal neutrophil-depleted blood can still reduce MRSA burden. (A, B) Mouse blood was incubated with PBS or TSLP and MRSA for **(A)** 0.5, 1 and 2 h or **(B)** 4 h and CFU determined (*n*=7, data combined from 2 experiments). **(C)** Mouse serum was incubated with MRSA and either PBS or TSLP for 3 h and CFU determined (*n*=5, data combined from 3 experiments). **(D)** CFU of MRSA incubated with serum (i.e., no blood cells were present) or after an *in vitro* whole blood killing assay was performed with blood from mice treated with anti-Ly6G antibodies. For each assay, blood was combined from 3 mice and assays were performed in triplicate. ns, not significant; ****, $p < 0.0001$ One way ANOVA (**A, B**); *, $p < 0.05$ using two-tailed Student's *t*-test (**C, D**).

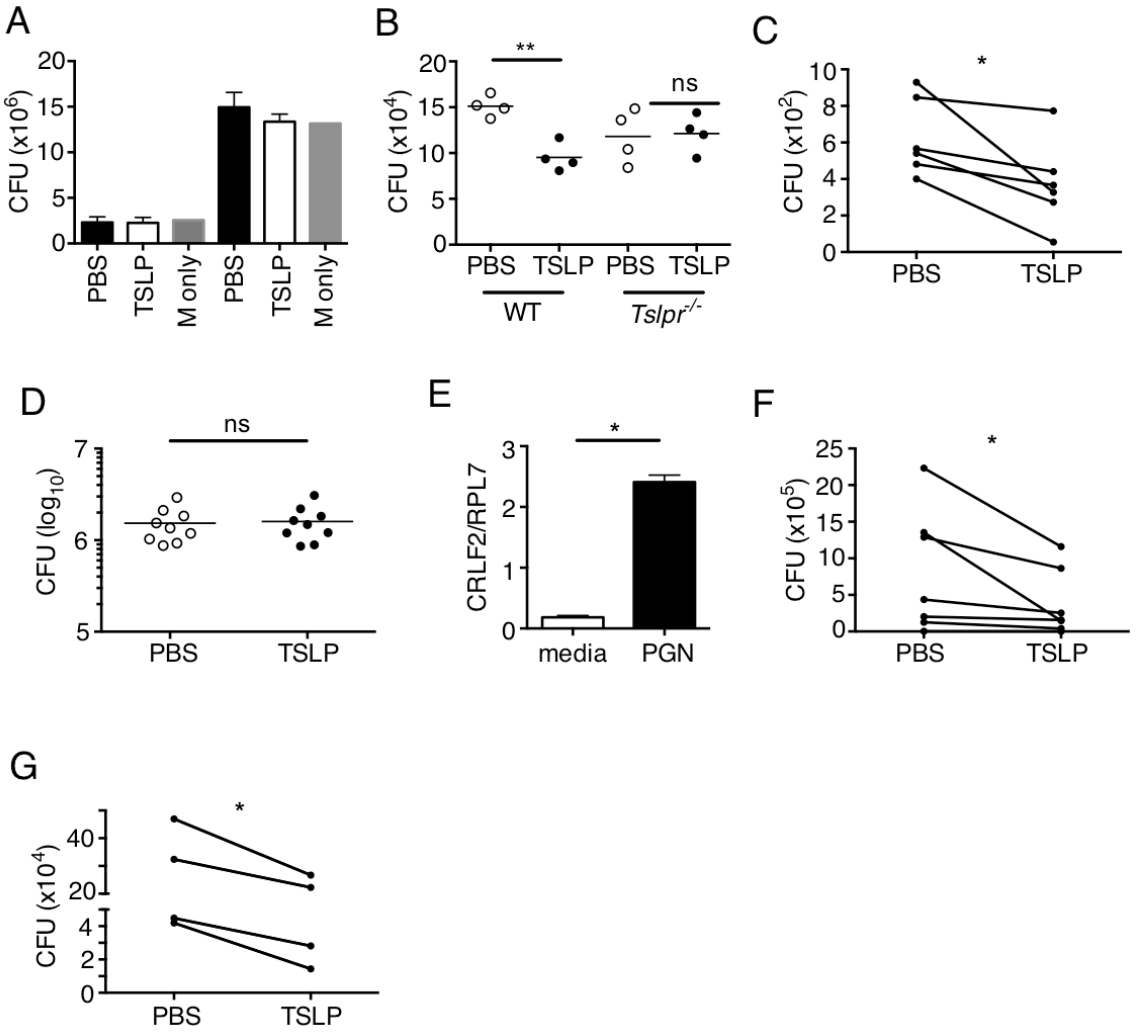

Figure S2. TSLP requires TSLPR and acts on human neutrophils to increase control of MRSA. (A) Bone marrow neutrophils were isolated from naïve mice and incubated with PBS or TSLP and MRSA for 2 or 4 hours, and CFU was enumerated. "M only" indicates tubes that only received MRSA (no cells). **(B)** CFU after thioglycollate-elicited purified neutrophils from WT or *Tslpr*$^{-/-}$ mice were incubated with PBS or TSLP and MRSA for 2 h. **(C)** Whole human blood was incubated with MRSA and either PBS or TSLP for 3 h and CFU determined (*n*=6). **(D)** MRSA was incubated with human serum (i.e., without cells) plus PBS or TSLP for 3 h and CFU determined (*n*=9). (**E**) *CRLF2* expression by purified human blood neutrophils determined by RT-PCR after 4 h treatment with medium alone or with peptidoglycan (PGN) and normalized to expression of *RPL7* (data from two individual donors combined). **(F, G)** Purified human neutrophils **(F)** or human neutrophils primed with HKSA plus either PBS or TSLP for 2 h **(G)** were incubated with MRSA and PBS or TSLP, and CFU assessed after 3 h (**F**) (*n*=7) or 2 h (**G**) (*n*=4). *****, $p < 0.05$; ns, not significant using the two-tailed paired Student's *t*-test (**C**, **E- G);** for **C**, **F** and **G** each line represents 1 donor. Two-tailed Student's *t*-test **(D)**. Data were representative of 3 individual mice (**A**) or from either 5 (**C, F**) or 3 (**D, G**) combined experiments.

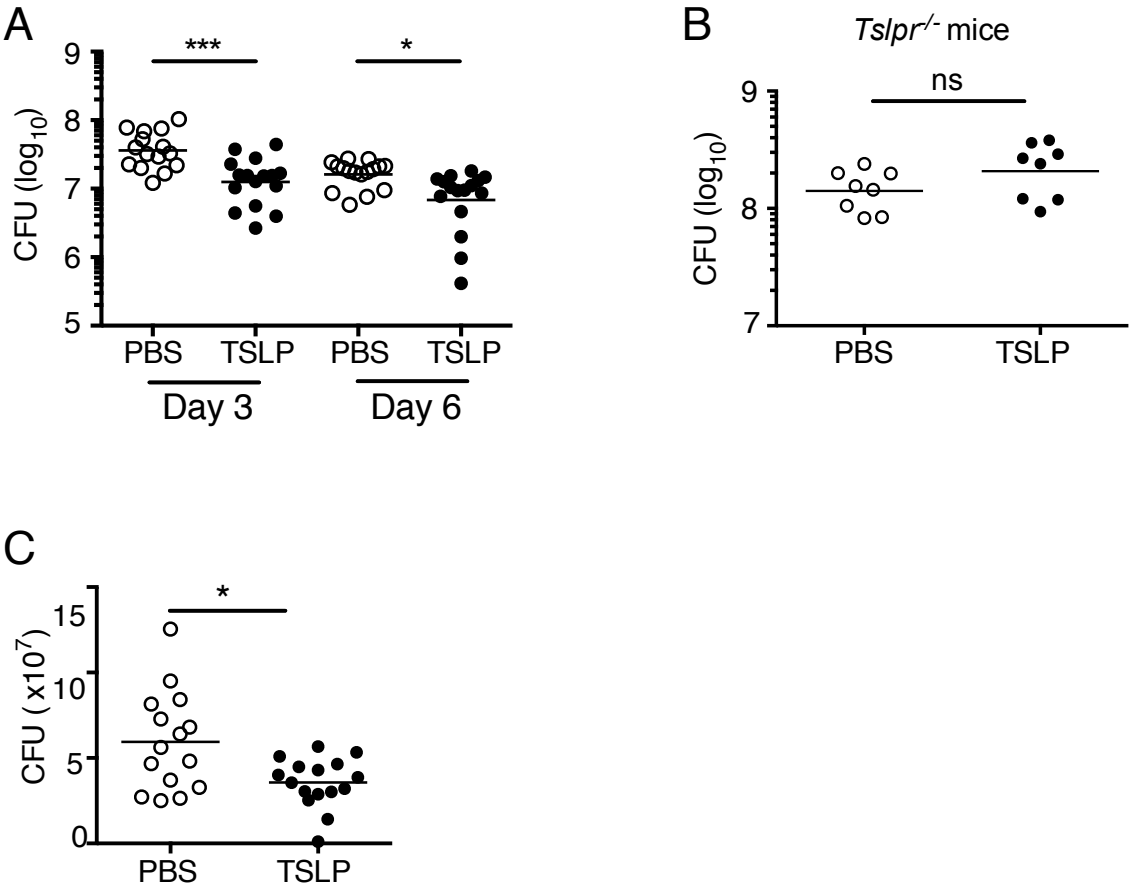

Figure S3. TSLP is TSLPR-dependent and enhances the killing of both MRSA and *S. aureus in vivo*. Mice were infected with MRSA i.d. in the ear. **(A)** CFU of MRSA at days 3 and 6 p.i. in the ears of WT mice treated with PBS or TSLP (Kruskal-Wallis ANOVA with Dunn's multiple comparison test). **(B)** *Tslpr*$^{-/-}$ mice were injected *in vivo* i.d. with MRSA plus either PBS or TSLP and CFU determined at day 1 p.i. (n=8, representative of 2 experiments, two-tailed Student's t-test). **(C)** CFU of *S. aureus* at day 2 p.i. in the ears of WT mice treated with PBS or TSLP and infected i.d. with *S. aureus* strain MW2 (Mann-Whitney test). ns, not significant. *, $p < 0.05$; **, $p < .01$; ****, $p \leq 0.0001$.

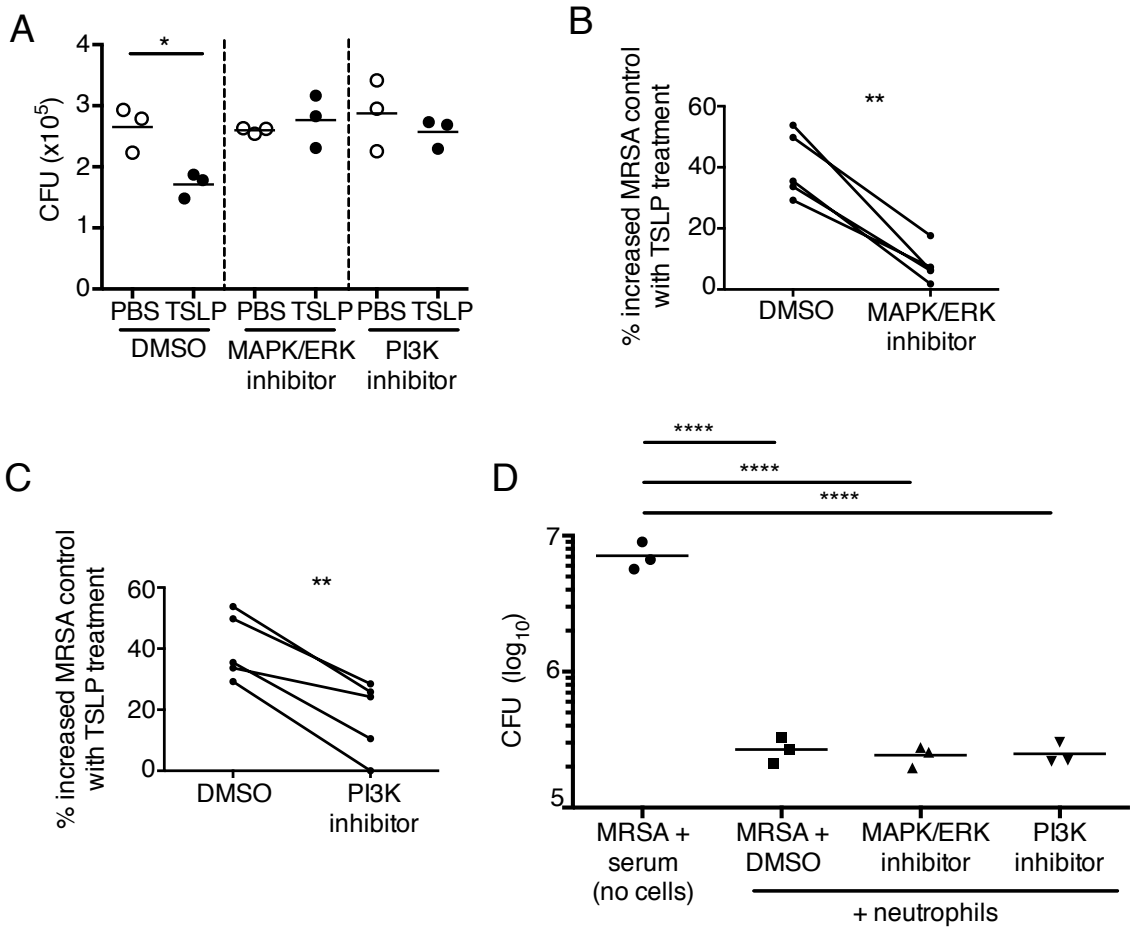

Figure S4. TSLP treatment increases killing of MRSA by human neutrophils in a PI3K- and MAPK/ERK-dependent manner. Purified human neutrophils were pretreated with MAPK/ERK kinase inhibitor (PD98059) or PI3K inhibitor (LY294002), incubated with MRSA plus either PBS or TSLP for 2 h, and CFU determined. **(A)** Representative donor shown (performed in triplicate). **(B and C)** Percent increased control of MRSA with TSLP shown for 5 individual donors. Treatment with MAPK/ERK kinase inhibitor **(B)** or PI3K inhibitor **(C)** each diminished control of MRSA. **(D)** Neutrophils were pretreated with DMSO, MAPK/ERK kinase inhibitor (PD98059), or PI3K inhibitor (LY294002), then incubated with MRSA and CFU determined, as compared to MRSA incubated with serum alone (i.e., no cells) for 2 h. Data are representative of 3 independent experiments. *, $p < 0.05$; **, $p < .01$; ****, $p \leq 0.0001$ using ANOVA (**A, D**) or two tailed paired Student's *t*-test (**B, C**).

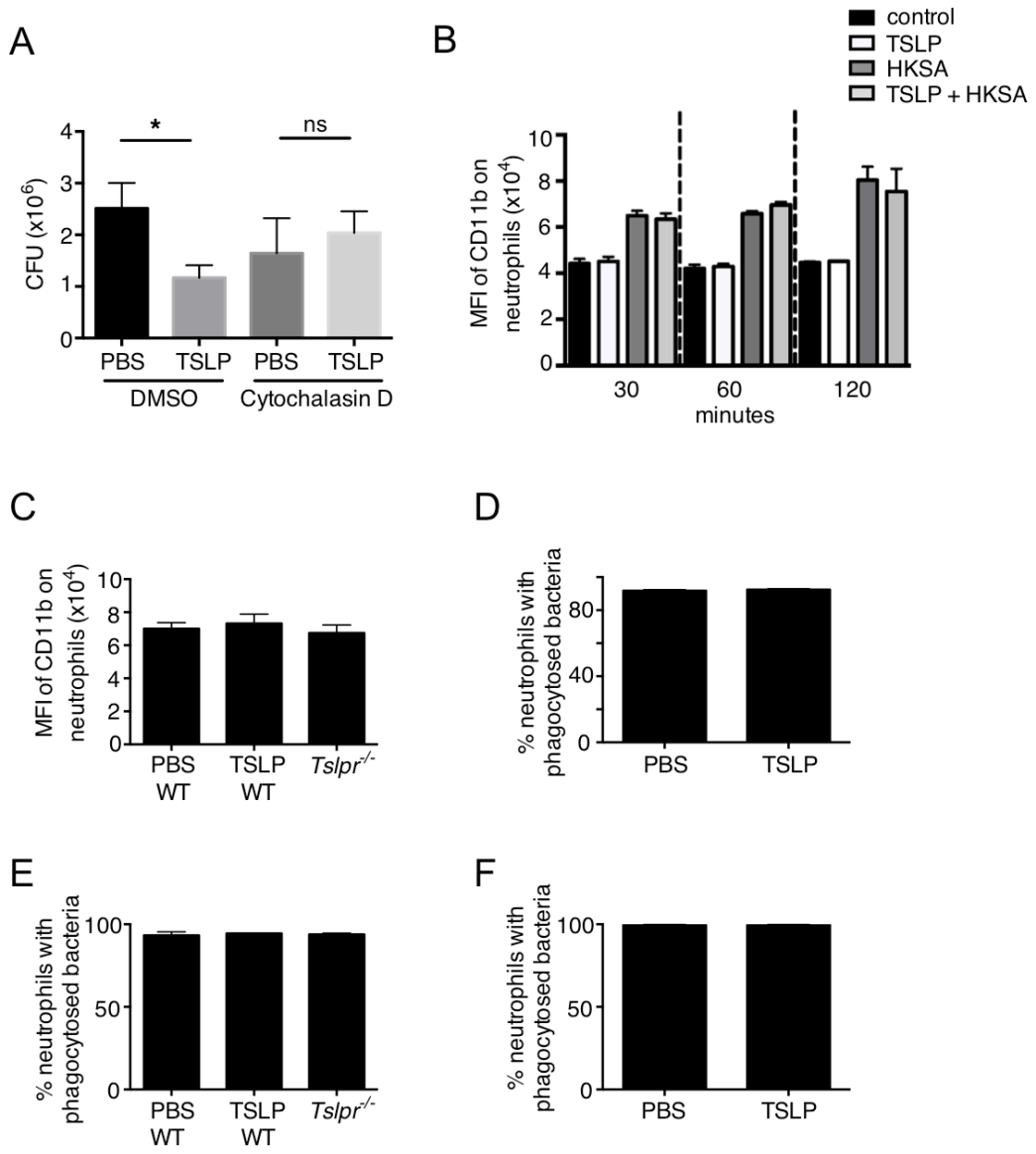

Figure S5. TSLP treatment of mouse or human neutrophils does not affect phagocytosis. (A) Purified thioglycollate-elicited neutrophils were pre-treated either with DMSO or cytochalasin D for 15 min and then incubated with PBS or TSLP and MRSA. CFU were enumerated 2 h later. **(B)** CD11b expression (MFI) on human blood neutrophils incubated for 30, 60, and 120 min with medium (control), TSLP, HKSA, or HKSA + TSLP (*n*=3). **(C)** CD11b expression (MFI) on mouse ear neutrophils from WT mice or *Tslpr*$^{-/-}$ mice 1 day p.i. with i.d. MRSA; WT animals were treated with PBS or TSLP as indicated (*n*=8). **(D)** Purified human neutrophils were incubated with pHrodo Green *S. aureus* BioParticles and the % phagocytosed bacteria was determined 30 min later by flow cytometry (*n*=2). **(E, F)** Neutrophils isolated from WT and *Tslpr*$^{-/-}$ bone marrow **(E)** or WT mouse bone marrow **(F)** were incubated with pHrodo Green *S. aureus* Bioparticles, and the % phagocytosed bacteria determined by flow cytometry after 20 (**E**) (*n*=2) or 5 (**F**) min (*n*=2). For WT BM, either PBS or TSLP was added as indicated.

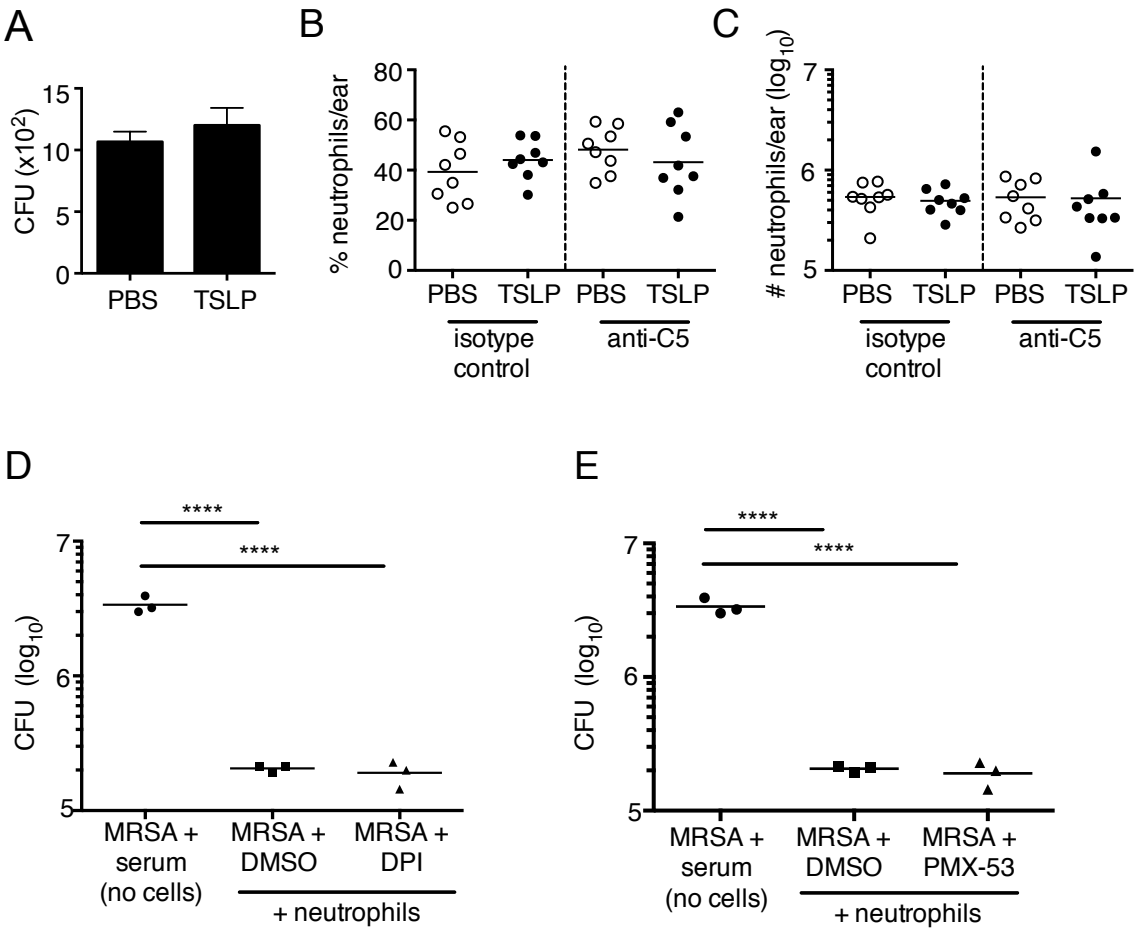

Figure S6. ROS- and complement-dependent TSLP-enhanced neutrophil killing. (A) Mouse blood was combined with MRSA and either PBS or TSLP in the presence of EDTA for 3 h, and CFU was then determined (*n*=3). (**B and C**) WT mice were injected i.d. in the ear with MRSA plus either PBS or TSLP and either isotype control or anti-C5 antibodies. Shown are percent **(B)** and total number **(C)** of neutrophils in the ear at day 1 p.i. (*n*=8 ears). **(D and E)** CFU determined after neutrophils were (**D**) pretreated with DMSO or DPI or (**E**) treated with DMSO or PMX-53 and then incubated with MRSA, compared to MRSA incubated in serum only (no cells) for 2 h. ****, $p \leq 0.0001$ using ANOVA. Representative of 3 independent experiments.

		Donor1	Donor2	Common
4hr	CTL vs. TSLP	0	8	0
	CTL vs. HKM	2664	1631	1394
	HKM vs. HKM+TSLP	1	2	0
24hr	CTL vs. TSLP	1	7	0
	CTL vs. HKM	1832	1702	1252
	HKM vs. HKM+TSLP	1	6	1*

*CCL22

Table S1. TSLP does not alter the transcriptional profile of human neutrophils. RNA-Seq performed on neutrophils after 4 or 24 h incubation with PBS (control) or TSLP with or without HKSA. Shown are the number of differentially expressed genes (Fold Change > 1.5, FDR < 0.05, RPKM > 4) from two different donors performed in two independent experiments and the number of common genes that were differentially expressed in both donors.