## SUPPLEMENTAL TEXT

### Animals

Foxp3-DTR mice express the human diphtheria receptor driven by Foxp3, allowing for selective depletion of Tregs through injection of DT. Treg depletion was performed using daily intraperitoneal injections of DT (50 ng/g) for two days, as previously described (1). In this model, Treg levels return to baseline after approximately ten days in the thymus and lymph nodes and two weeks in the spleen; this can be avoided with repeat DT injection, but we did not require this. Control animals received corresponding intraperitoneal injections of phosphate buffered saline (PBS).

All mice were maintained in a light- and temperature-controlled environment and fed *ad libitum*. When indicated, mice were anesthetized using isofluorane with the depth of anesthesia monitored at least every 15 minutes. Animals that ultimately developed wound infection or skin ulceration were excluded. At the conclusion of the experiments, mice were euthanized by carbon dioxide asphyxiation, as recommended by the American Veterinary Medication Association (AVMA) Guidelines on Euthanasia.

### Axillary lymph node dissection (ALND)

A 1 cm incision was made in the axilla, allowing for the axillary lymph node to be identified and excised with the surrounding fat pad. The incision was closed with 4-0 non-absorbable sutures. Control animals underwent anesthesia and axillary incision without lymphadenectomy, otherwise known as sham surgery. We have previously shown that ALND in this model results in modest but significant increases in arm volumes for as long as three weeks after surgery (2). Following this time, similar to the clinical scenario, arm volumes return to normal, but the tissues continue to have low-grade chronic accumulation of $CD4^+$ cells. Histologic and flow cytometry analysis was performed as described below, generally approximately six weeks after surgery.

### Histology

After antigen retrieval was completed using boiling citric acid and tissues were incubated overnight with primary antibody at $4^{\circ}C$, antibody staining was visualized using horseradish-peroxidase-conjugated secondary antibodies and developed with diaminobenzamine complex (DAB; Vector; Burlingame, CA). Primary antibodies included CD45 (R&D; Minneapolis, MN) and CD3 (Dako North America, Inc.; Carpinteria, CA). Secondary antibodies were obtained from Vector Laboratories (Burlingame, CA) and eBioscience (San Diego, CA). Foxp3 and *S. aureus* were identified through GFP and Alexa Flor® 594 expression, respectively.

### Flow cytometry

In one aspect of the study, mice were injected with thymidine analogue EdU one day prior to sacrifice to identify proliferating cells. Tissues were finely chopped, then digested with a mixture of collagenase IV, dispase, and DNaseI in an incubator shaker at $37^{\circ}C$. The digest was filtered using a 70-micron cell strainer and plated onto a 96-well plate. Cells were labeled for

EdU using a Click-iT$^{TM}$ reaction kit (Life Technologies, Grand Island, NY) and stained for TCR-β and Nrp-1 (eBioscience, San Diego, CA). Staining for CD45, CD4, CCR5, CXCR3, CCR4, CCR8, F4/80, CD11b, MHCII, CD11c, and Ly-6G (Biolegend; San Diego, CA) was performed in other experiments. Flow cytometry was performed using a BD Fortessa flow cytometry analyzer (BD Biosciences; San Jose, CA) with BD FACS Diva and analysis was conducted using FlowJo software (Tree Star; Ashland, OR).

**Contact hypersensitivity and non-specific inflammatory responses**

In the CHS model, 25 µL of 0.5% DNFB (Toronto Research Chemicals Inc.; Toronto, Ontario) dissolved in acetone with 20% olive oil was painted on the distal shaved forelimb six weeks after ALND or sham surgery and one day after injection of DT or PBS. Five days after sensitization, the opposite ear was challenged with 0.3% DNFB. Ears were harvested three days later (**Fig. 5a**).

In the non-specific inflammation model, 20 µl of 2% croton oil (Sigma-Aldrich) dissolved in 70% acetone (Fisher Scientific; Pittsburgh, PA) was applied to the inner ear surface of mice that had undergone ALND or sham surgery six weeks prior followed by DT or PBS injection one day prior. Ears were analyzed 18 hours later (**Fig. 5a**).

**Bacterial clearance**

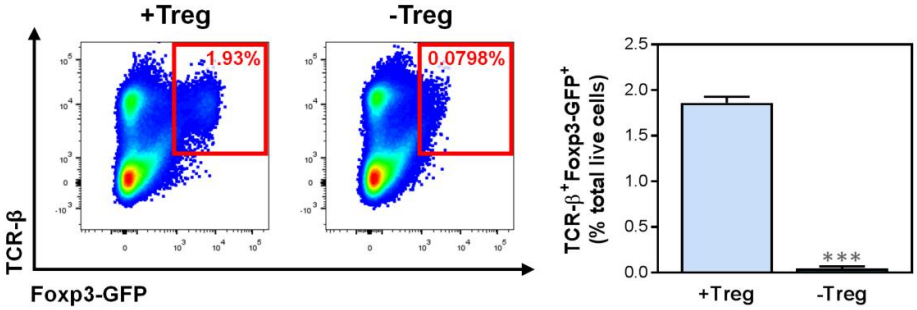
Six weeks after ALND or sham surgery, Foxp3-DTR mice were injected with DT or PBS as previously described. The following day, 1 x $10^7$ Alexa Flor® 594-conjugated *S. aureus* particles (Molecular Probes, Life Technologies; Eugene, OR) were injected into the distal ipsilateral forepaws. The distal forepaw skin was harvested one day later to analyze bacterial particle clearance and macrophage uptake of bacteria using flow cytometry and immunofluorescent staining (**Fig. 6a**).

**Humoral responses**

Five weeks after ALND or sham surgery, Foxp3-DTR mice were immunized intradermally in the ipsilateral forelimb with 40 µL of 0.1 mg/mL chicken ovalbumin (OVA) (Bachem, Inc.; Torrance, CA) in a 1:1 emulsion of PBS and alum adjuvant (Thermo Scientific; Waltham, MA) (**Fig. 6d**). One week later, DT or PBS was injected intraperitoneally as done previously. An OVA booster was then administered one day later in the distal forepaw of the affected limb (3). Serum anti-OVA IgG titers were analyzed after one week using enzyme-linked immunosorbent assays (ELISA; Alpha Diagnostic International; San Antonio, TX) (**Fig. 6e**).

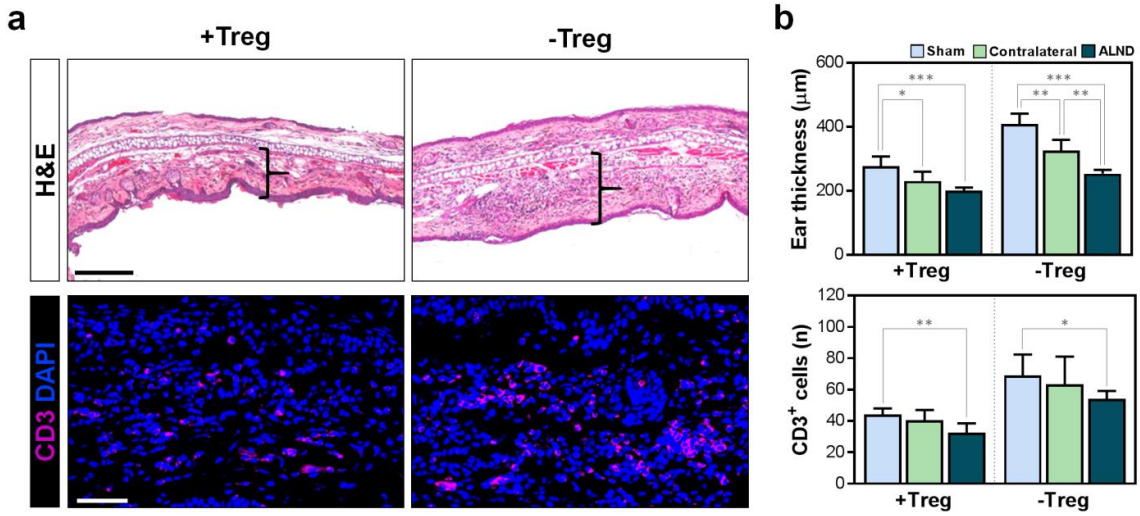
## SUPPLEMENTAL FIGURES

### Figure S1. DT injections result in Treg depletion in Foxp3-DTR mice




Representative FACS plots (*left*) and quantification (*right*) of TCR-$\beta^+$Foxp3-GFP$^+$ Tregs in the spleens of Foxp3-DTR mice injected with DT (-Treg) to activate Treg depletion or PBS as the control (+Treg) (n=4/group). Data expressed as mean ± SD. Statistically significant differences represented by **P*<0.05, ***P*<0.01, and ****P*<0.001. DT, diphtheria toxin.

### Figure S2. ALND results in mild immunosuppression in the contralateral limb

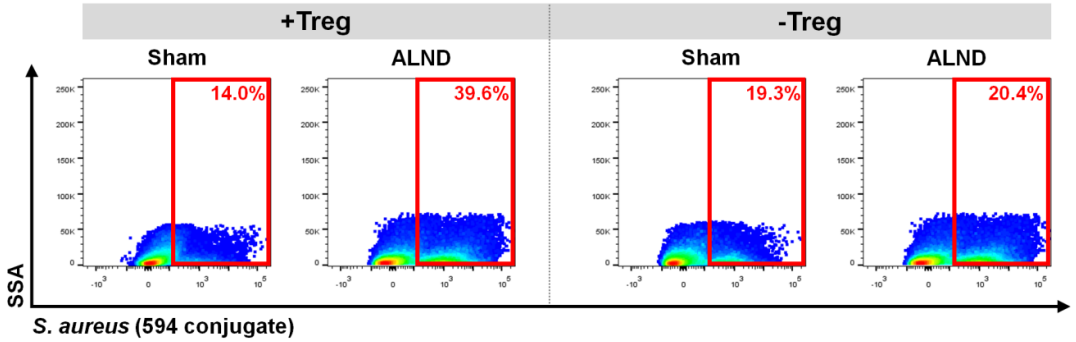



a) Representative H&E images with brackets indicating mouse ear dermal thickness (*upper*; scale bar 250 μm) and immunofluorescent images localizing mouse ear CD3$^+$ cells (*lower*; scale bar 50 μm) following the DNFB sensitization in the contralateral ALND-treated forelimb and challenge in the opposite ear to elicit T cell-mediate inflammation (CHS model).

b) Quantification of mouse ear thickness (*upper*) and mouse ear CD3$^+$ cells per 0.25 mm$^2$ (*lower*) in the CHS model (n=5/group, 3 HPF/mouse).

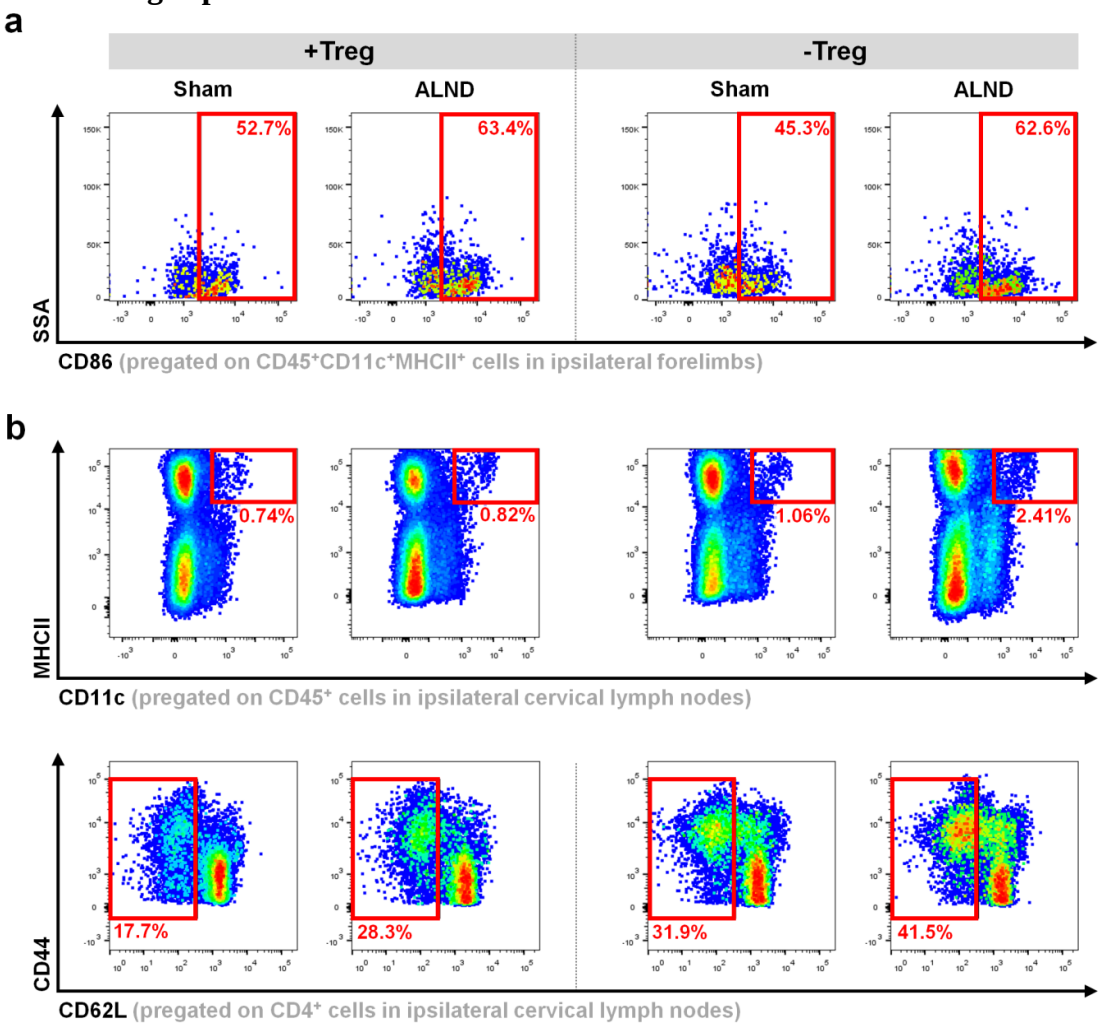
Data expressed as mean ± SD. Statistically significant differences represented by **P*<0.05, ***P*<0.01, and ****P*<0.001.

**Figure S3. Treg depletion after lymphatic injury improves bacterial phagocytosis**




Representative FACS plots of Alexa Flor® 594-conjugated *S. aureus* bacterial particles at dermal injection sites.

**Figure S4. Treg depletion increases DC activation with resultant increase in T cell activation**




a) Representative FACS plots of $CD45^+CD11c^+MHCII^+$ $CD86^+$ cells in mouse forelimbs.

b) Representative FACS plots of $CD45^+CD11c^+MHCII^+$ cells (*upper*) and $CD4^+CD44^+CD62L^-$ cells (*lower*) in mouse ipsilateral cervical lymph nodes.