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Supplemental Information

The IL-27 receptor regulates TIGIT on memory $CD4^+$ T cells during sepsis

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Supplemental Figures

Figure S1

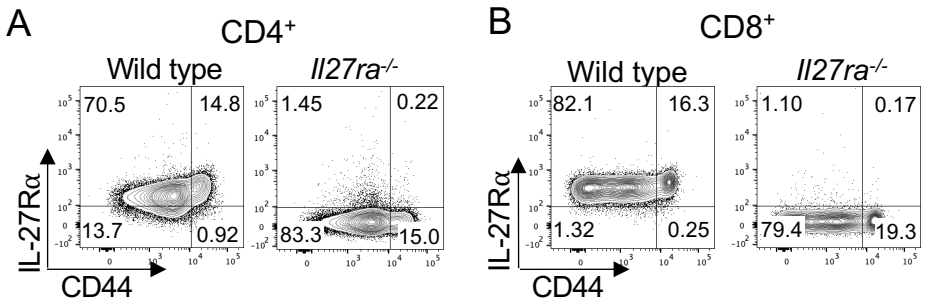

Figure S1: Gating strategy for IL-27Rα on CD4⁺ and CD8⁺ T cells (Related to Figure 1)
Splenocytes were obtained from wild type or *Il27ra*$^{-/-}$ mice and stained for IL-27Rα and CD44. (**A**) Representative flow cytometric plots showing IL-27Rα (y-axis) vs CD44 (x-axis) for CD4⁺ T cells in wild type (left) and *Il27ra*$^{-/-}$ (right) mice. (**B**) Representative flow cytometric plots showing IL-27Rα (y-axis) vs CD44 (x-axis) for CD4⁺ T cells in wild type (left) and *Il27ra*$^{-/-}$ (right) mice.

Figure S2

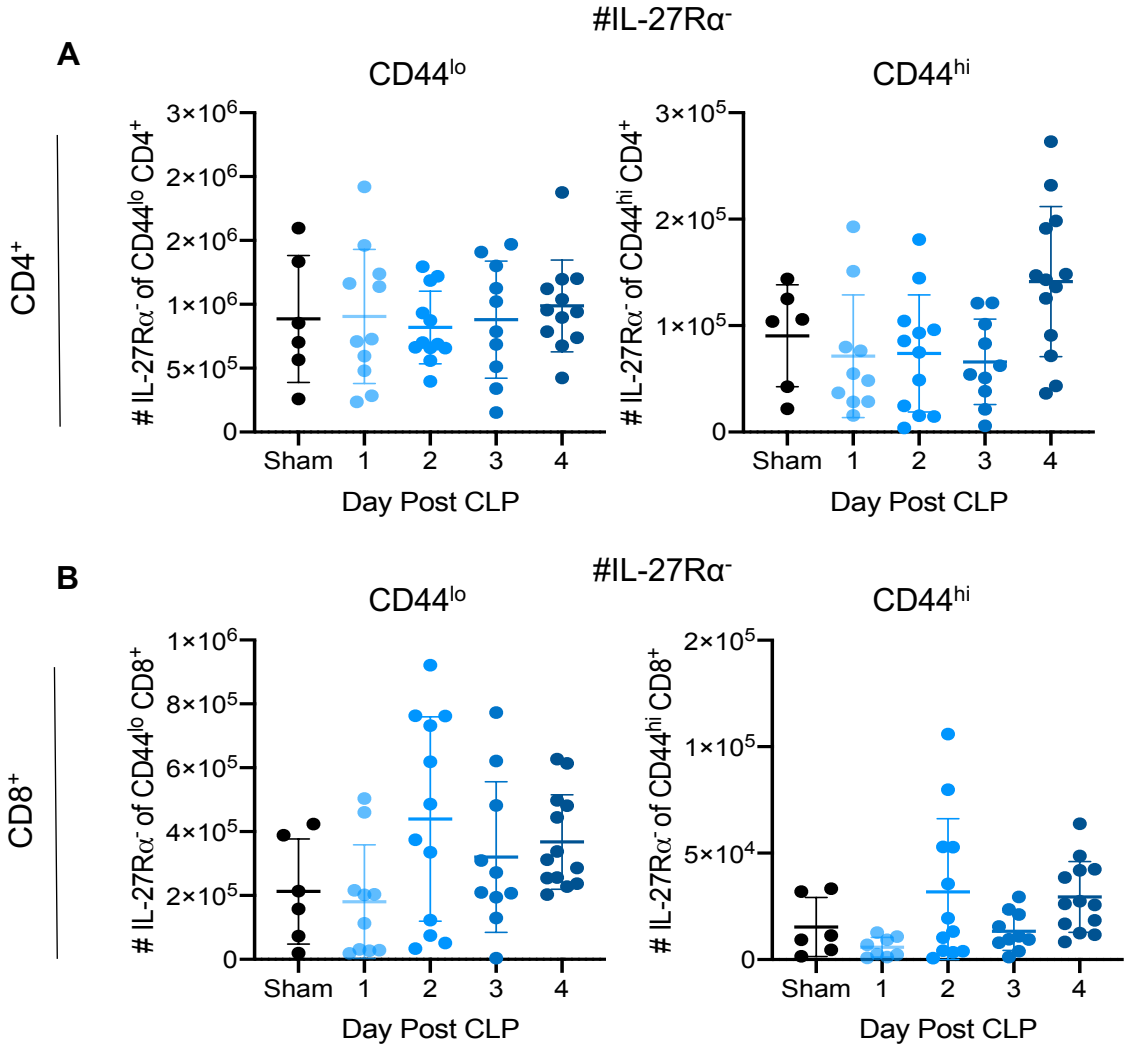

Figure S2: IL-27Ra- CD44lo and CD44hi numbers are unchanged following sepsis (Related to Figure 1). Following cecal ligation and puncture (CLP) or sham surgery (sham), animals were euthanized on the indicated days. Spleens were harvested for analysis by flow cytometry on days 1 through 4. **(A)** The absolute number of $CD4^+$ $CD44^{lo}$ naïve (left) and $CD4^+$ $CD44^{hi}$ memory (right) T cells *not* expressing IL-27Rα in sham and CLP mice on days 1-4 after surgery. **(B)** The absolute number of $CD8^+$ $CD44^{lo}$ naïve (left) and $CD8^+$ $CD44^{hi}$ memory (right) T cells *not* expressing IL-27Rα in sham and CLP mice on days 1-4 after surgery. All summary data was pooled from 3 independent experiments, with n=7-18 mice per group.

Figure S3

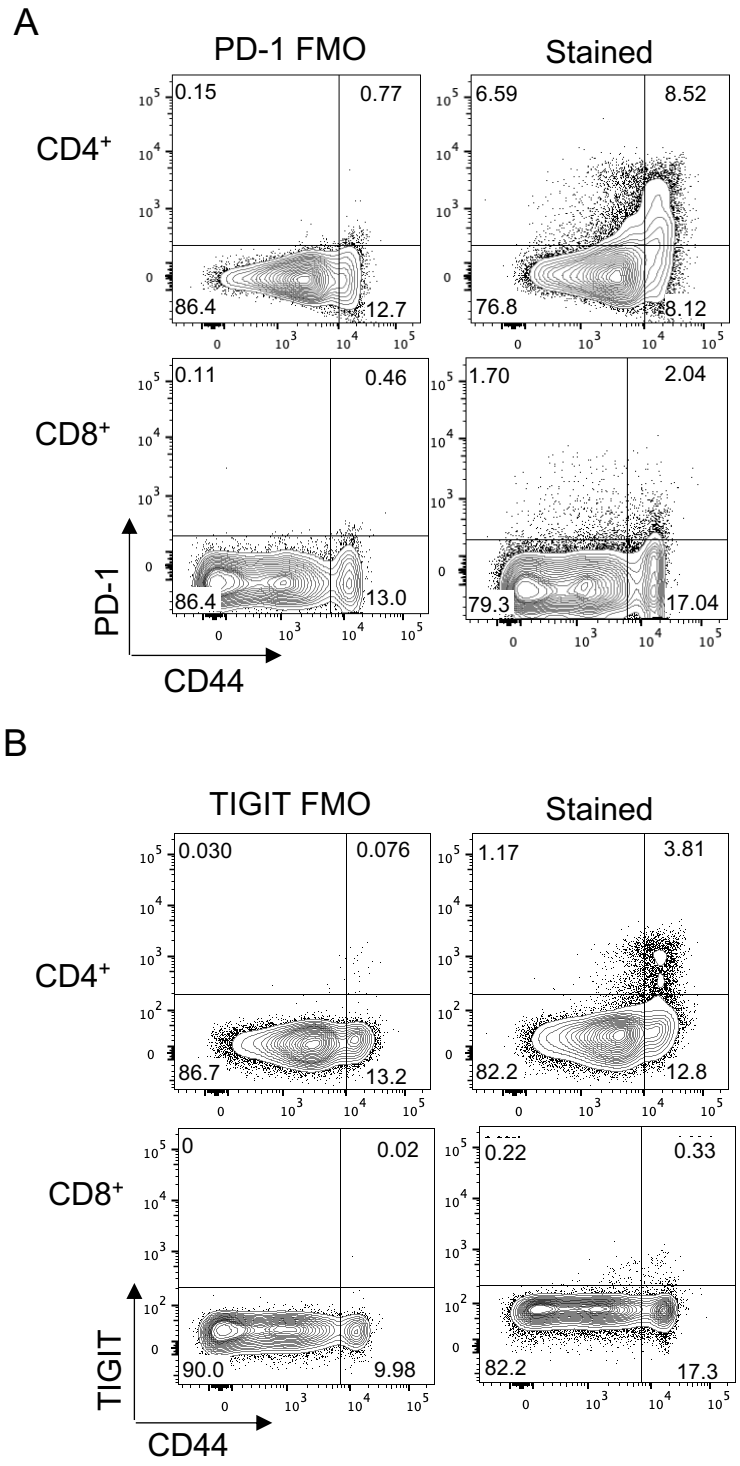

Figure S3: Gating strategy for PD-1 and TIGIT on CD4⁺ and CD8⁺ T cells (Related to Figure 2)
Splenocytes were obtained from wild type septic mice on day 4 following CLP and used for flow cytometric analysis. (**A**) Representative flow cytometric plots showing PD-1 (y-axis) vs CD44 (x-axis) using a PD-1 FMO (left) and stained (right) controls. $CD4^+$ T cells are shown in the top series and $CD8^+$ cells at bottom. **(B**) Representative flow cytometric plots showing TIGIT (y-axis) vs CD44 (x-axis) using a PD-1 FMO (left) and stained (right) controls. $CD4^+$ T cells are shown in the top series and $CD8^+$ cells at bottom.

Figure S4

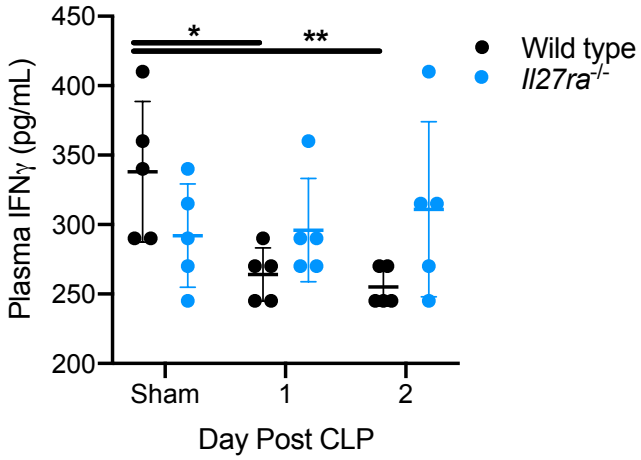

Figure S4: Circulating IFN*γ* is unchanged in *Il27ra*$^{-/-}$ vs wild type mice following CLP (Related to Figure 7)
Plasma was purified from the blood of wild type and *Il27ra*$^{-/-}$ mice that underwent sham ("sham") or CLP surgery on days 1 and 2 after surgery.

Transparent Methods

Animals

Six to 12-week-old male and female gender matched mice (mean weight 20g) were used for all experiments. The wild type mice used for T cell phenotyping experiments were either C57BL/6J or C57BL/6NJ mice obtained from Jackson Laboratories (Bar Harbor, ME). Transgenic *Il27ra*$^{-/-}$ mice on a mixed C57BL/6NJ and 6NTac background were a gift from Dr. Jacob Kohlmeier (Emory University, Atlanta, Georgia; animals were originally obtained from Jackson Laboratories, Bar Harbor, ME). C57BL/6NJ (Jackson Laboratories, Bar Harbor, ME) mice were used as controls in experiments with transgenic *Il27ra*$^{-/-}$ mice. Mice obtained from external sources were acclimated for at least 72 hours prior to being used in experiments. Mice were randomly allocated to receive either sham surgery or cecal ligation and puncture (CLP, details below) and were either sacrificed between 24 and 96 hours after surgery or followed for 7 days to determine survival. All experiments were performed in accordance with the National Institutes of Health Guidelines for the Use of Laboratory Animals and were approved by the Emory University Institutional Animal Care and Use Committee (Protocol 201700361.RM001-El-N). Mice were housed in specific pathogen free conditions with a 12-hour light cycle.

Cecal Ligation and Puncture (CLP)

Cecal ligation and puncture was performed as previously described (Chen et al., 2017, Chen et al., 2019). Surgeries were performed between the hours of 9am and 2pm to minimize the confounding effects of circadian rhythm. Prior to surgery, 0.1 mg/kg of buprenorphine (McKesson Medical, San Francisco, CA) was administered to each mouse subcutaneously (s.c.) to minimize suffering. Ophthalmic eye gel was administered to prevent corneal ulceration during surgery and abdominal fur was shaved to minimize risk of wound contamination. The anesthetic depth necessary for surgery was induced with inhaled isoflurane (3% in 100% O_2) and reduced to 2% after the surgical plane was reached. After skin disinfection, a midline incision was made, and the cecum was exteriorized. For mice in the control group (receiving sham surgery), the cecum was then replaced in the abdominal cavity, and the abdominal wall closed with 4-0 silk thread. If mice underwent cecal ligation and puncture, approximately 75% of the cecal length was ligated with nylon thread, before being punctured with a 25-gauge needle through and through. A small amount of stool was then gently expelled before the cecum was returned to the abdominal cavity. The abdominal wall was closed with 4-0 silk suture and the skin closed with veterinary glue. Immediately after surgery, 1mL of sterile saline was administered s.c. for fluid resuscitation in addition to 50 mg/kg ceftriaxone (Acros Organics, Morris Plains, NJ) and 35 mg/kg metronidazole (Sigma-Aldrich, St. Louis, MO) for pathogen control**.** Animals were then placed in a new cage on a warming pad and monitored for recovery. Following recovery, the cages were returned to their housing room. Antibiotics (same as above) were administered every 12 hours for the first 48 hours following surgery. All mice were monitored twice a day for the duration of the experiments and weighed every other day. Any mouse that lost 25% body weight or appeared moribund was humanely euthanized by asphyxiation with CO_2 or exsanguination after exposure to a high concentration of isoflurane followed by cervical dislocation. Moribund animals were defined by a) major organ failure or medical conditions unresponsive to treatment, b) surgical complications unresponsive to immediate intervention or c) clinical or behavioral signs unresponsive to appropriate intervention persisting for 24 hours. In experiments using neutralizing IL-27p28 antibody (clone: MM27.7B1, BioXCell), 500 µg was administered into the intraperitoneal cavity before abdominal wall closure following CLP or sham surgery.

ELISAs

Plasma samples were obtained via terminal heart puncture or saphenous vein collection into tubes containing EDTA. After centrifugation, the plasma layer was collected and cryopreserved until analysis. Samples were diluted using 1x PBS and assessed for IL-27 concentration using an anti-IL-27p28 ELISA kit (Invitrogen, Carlsbad, CA) or IFNγ concentration using an anti-IFNγ ELISA kit (Invitrogen, Carlsbad, CA) following manufacturer instructions. Results were analyzed using Four Parameter Logistic Regression.

Flow cytometry

On days 1-4 following CLP, mice from each group were randomly chosen for sacrifice. Spleens were harvested and strained through a 70 µM nylon filter before washing with cold 1x PBS through centrifugation. Splenocytes were subsequently resuspended in PBS and 2 million cells were used for staining. Prior to staining with target antibodies, all samples were stained with TruStain FcX anti-mouse CD16/32

(BioLegend, San Diego, CA) following manufacturer instructions. Cells were then stained for surface markers and incubated on ice for 25 minutes. After staining, samples were washed with MACS Buffer and resuspended in CountBright Absolute Counting Beads (Thermo Fisher Scientific, Waltham, MA) according to manufacturer instructions. The antibodies used for T cell exhaustion phenotyping are as follows: TIGIT-BV421 (Clone 1G9, BD), Live/Dead Aqua (Invitrogen), NK1.1-BV650 (clone PK136, BioLegend), CD44 on PerCP Cy 5.5 (clone IM7, BioLegend) or BUV737 (clone IM7, BD), IL-27Rα-PE (clone 2918, BD), PD-1-APC/Cy7 (clone 29F.1A12, BioLegend), CD4-BUV395 (clone GK1.5, BD), CD3e-BUV496 (clone 145-2C11, BD), and CD8a on BUV737 or BUV805 (both clone 53-6.7, BD). For caspase 3/7 staining, surface staining was done as described above with the following markers: CD4-BUV395 (clone GK1.5, BD), CD3e-BUV496 (clone 145-2C11, BD), CD8a on BUV737 (clone 53-6.7, BD), NK1.1-BV650 (clone PK136, BioLegend), and IL-27Rα-PE (clone 2918, BD).Cells were then resuspended in 1x PBS and Caspase-3/7 stain according to manufacturer instructions (CellEvent Caspase-3/7 Green Detection Reagent, Invitrogen, Carlsbad, CA). Samples were incubated for one hour at 37□ C and caspase 3/7 staining was immediately detected. For intracellular cytokine staining (ICCS), splenocytes were stimulated for 4 hours with 20 ng/mL PMA (Sigma-Aldrich) and 0.75 µg/mL Ionomycin (Sigma-Aldrich) in the presence of GolgiPlug (BD). Samples were subsequently surface stained as described above with CD4-BUV395 (clone GK1.5, BD), CD3e-BUV496 (clone 145-2C11, BD), CD8a on BUV737 (clone 53-6.7, BD), IL-27Rα-PE (clone 2918, BD). Following surface staining, the cells were fixed and permeabilized according to manufacturer's instructions (BD Fixation/Permeabilization Solution Kit). Cells were then stained with TNFα-APC and IFNγ-A700. For Treg and Ki67 (proliferation) staining, extracellular staining was done as above using CD4-BUV395 (clone GK1.5, BD), CD3e-BUV496 (clone 145-2C11, BD), CD8a on BUV737 (clone 53-6.7, BD), PD-1-APC/Cy7 (clone 29F.1A12, BioLegend), TIGIT-BV421 (Clone 1G9, BD), Live/Dead Aqua (Invitrogen), NK1.1-BV650 (clone PK136, BioLegend), and CD44-BUV737 (clone IM7, BD). Following extracellular staining, cells were fixed and permeabilized using the Foxp3/transcription staining buffer kit (eBioscience) according to manufacturer instructions. Cells were then stained with FoxP3-APC (clone FJK-16s, eBioscience) and Ki67-AF700 (clone 16A8, BioLegend). All samples were run on a LSRFortessa (BD Biosciences, San Jose, CA). All flow cytometric data was analyzed using FlowJo version 10.2 (BD, Ashland, OR). The FlowAI plugin (Monaco et al., 2016) found on FlowJo Exchange (www.flowjo.com/exchange) was used in sample pre-processing to eliminate artifacts caused by variable flow rate before analysis.

Statistics

All statistical analysis was performed using Prism 8.3.1 (GraphPad, San Diego, CA). Kruskal-Wallis tests with Dunn's multiple comparisons test was used when comparing within a single group longitudinally. When comparing between two groups at multiple time points, two-way ANOVA was used with Sidak's multiple comparison test. Survival curves were assessed using a Log-rank (Mantel-Cox) Test. Results are reported as the mean of each group ± SD. P-values ≤ 0.05 were considered statistically significant.

Supplemental References

MONACO, G., CHEN, H., POIDINGER, M., CHEN, J., DE MAGALHAES, J. P. & LARBI, A. 2016. flowAI: automatic and interactive anomaly discerning tools for flow cytometry data. *Bioinformatics,* 32**,** 2473-80.