

Supplemental material

Di Luccia et al., <https://doi.org/10.1084/jem.20180549>

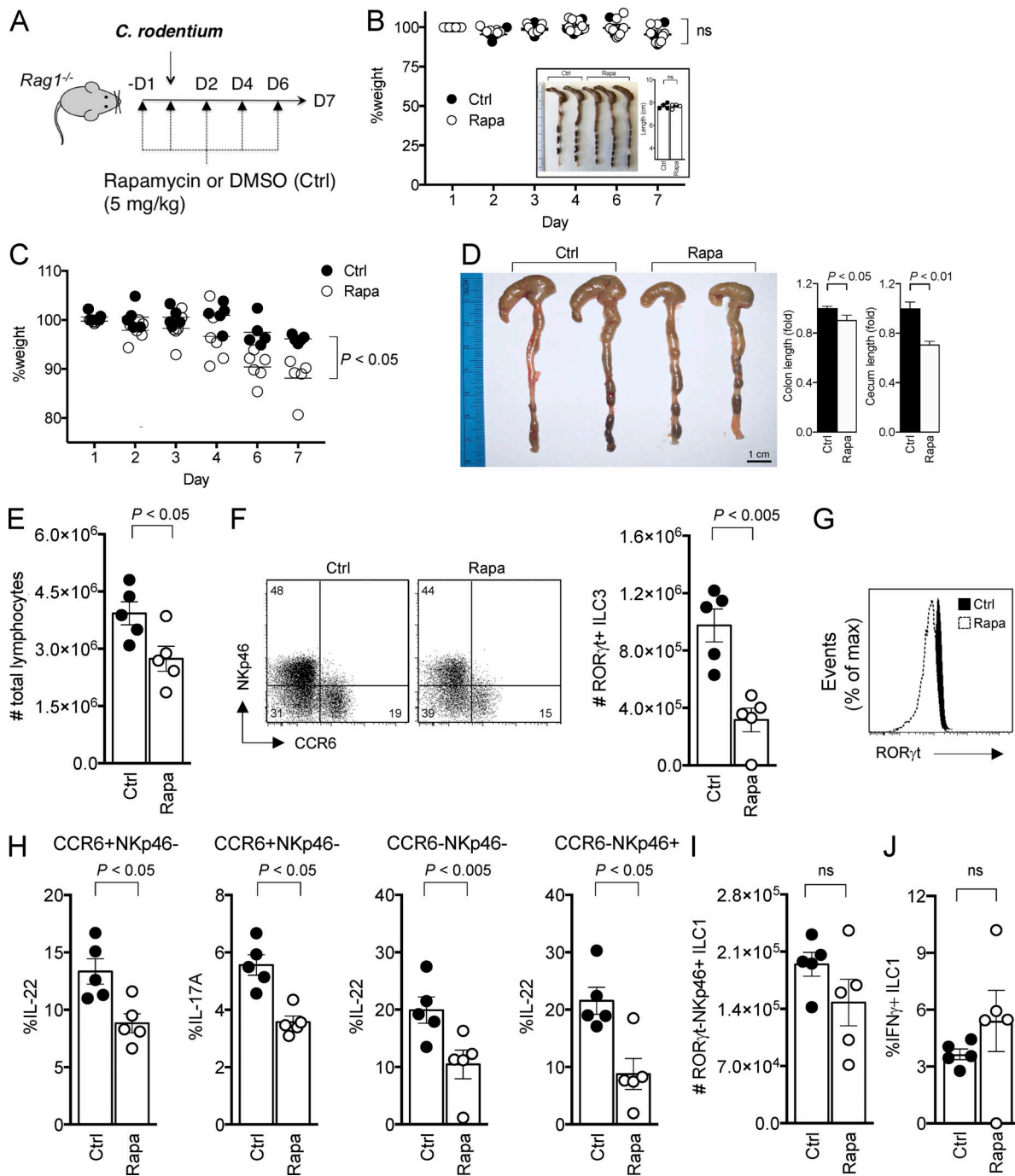


Figure S1. **Inhibition of mTORC1 signaling by Rapa impairs ILC3 response to bacterial infection.** (A) Schematic diagram of the experimental plan to assess the role of mTORC1 in ILC3 in vivo. (B) Changes (%) in body weights and colon length (cm) of naive *Rag1*^{-/-} mice administered Rapa (5 mg/kg) or control (Ctrl) DMSO vehicle (*n* = 7). (C) Changes (%) in body weights of *C. rodentium*-infected *Rag1*^{-/-} mice administered Rapa or DMSO (*n* = 5). (D) Images and lengths of colon and cecum from *C. rodentium*-infected mice administered DMSO or Rapa (day 7). (E) Total numbers of intestinal lymphocytes in *C. rodentium*-infected mice administered DMSO or Rapa (day 7, *n* = 5). (F and G) Expression of RORγt (F) and representative FACS plots and numbers of RORγt⁺ ILC3 cells (G) in *C. rodentium*-infected mice administered DMSO and Rapa (day 7, *n* = 5). (H) Percentages of cells expressing IL-17A and IL-22 within small intestine CCR6⁺NKp46⁻, CCR6⁻NKp46⁻, or CCR6⁻NKp46⁺ ILC3 in *C. rodentium*-infected mice administered DMSO or Rapa (day 7, *n* = 5). (I and J) Numbers of RORγt⁺NKp46⁺ ILC1 (I) and percentages of IFNγ⁺ ILC1 (J) in *C. rodentium*-infected mice administered DMSO or Rapa (day 7, *n* = 5). Data are pooled from two independent experiments (mean ± SEM of *n* = 5–7; Student's *t* test). ns, not significant.

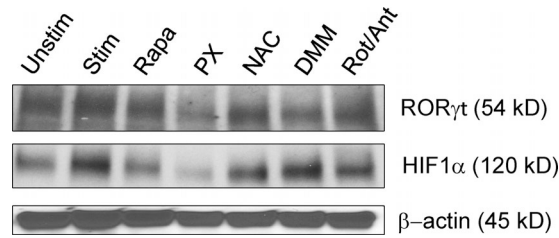


Figure S2. **Expression of RORγt and HIF1α in MNK3 by Western blotting.** Expression of RORγt, HIF1α, and β-actin from unstimulated (Unstim) MNK3 or MNK3 stimulated (Stim) with IL-1β + IL-23 for 3 h in the presence or absence of Rapa, PX, NAC, DMM, and Rot/Ant, assessed by immunoblot analysis. Data are from one experiment representative of two independent experiments.

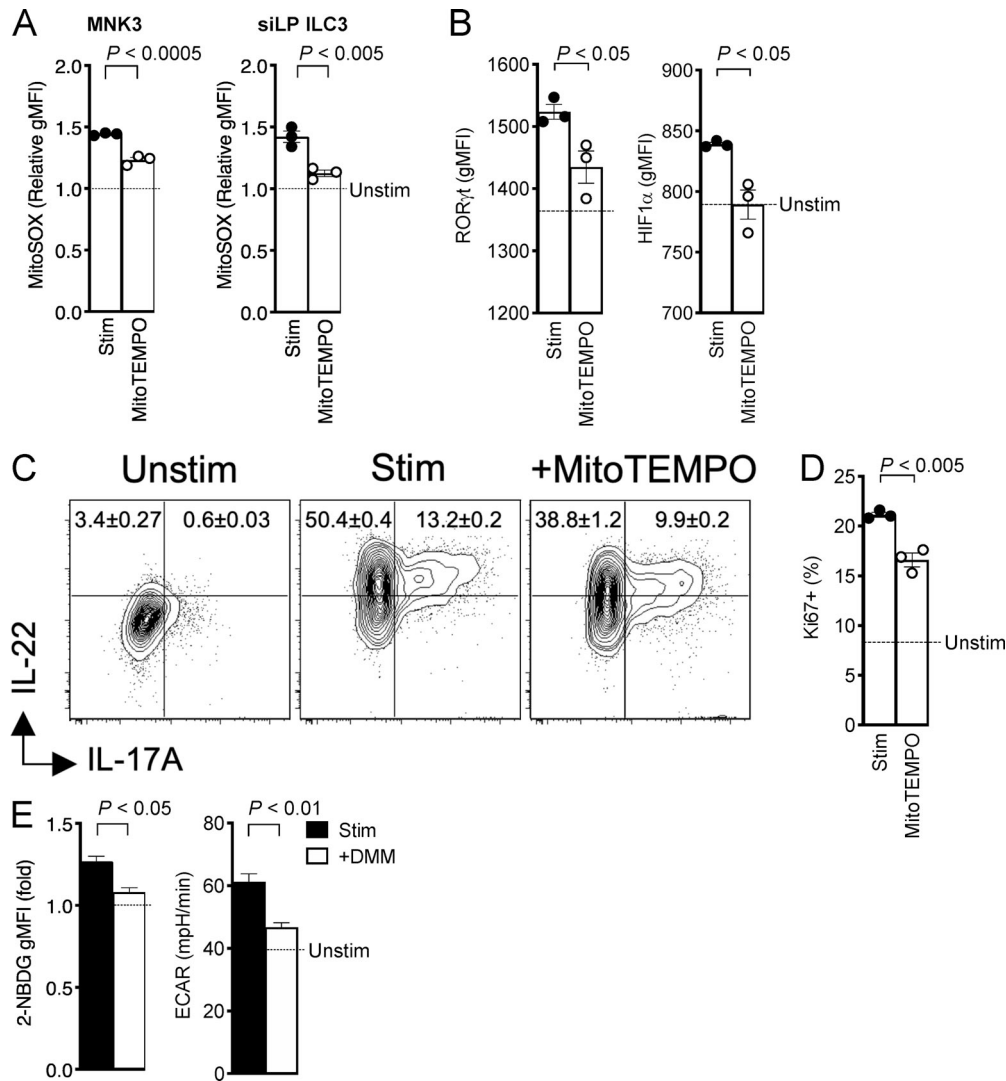


Figure S3. **Mitochondrial ROS supports ILC3 function.** (A) MitoSOX staining of MNK3 and small intestinal lamina propria (siLP) ILC3 stimulated (Stim) with IL-1β + IL-23 for 16 or 3 h with or without MitoTEMPO, respectively, assessed by flow cytometry. Bar graphs show MFI relative to that of naive cells. (B) Expression of RORγt and HIF1α in MNK3s stimulated with IL-1β + IL-23 for 6 h with or without MitoTEMPO, assessed by flow cytometry. (C) IL-17A and IL-22 intracellular content of MNK3, unstimulated or stimulated with IL-1β + IL-23 with or without MitoTEMPO, assessed by flow cytometry. (D) Percentage of Ki67⁺ MNK3 stimulated with IL-1β + IL-23 for 16 h with or without MitoTEMPO, assessed by flow cytometry. (E) 2-NBDG uptake and basal ECAR in MNK3 stimulated with IL-1β + IL-23 for 16 h with or without DMM. Data are from one experiment representative of two independent experiments (mean ± SEM of *n* = 2–5; Student's *t* test). gMFI, geometric MFI.