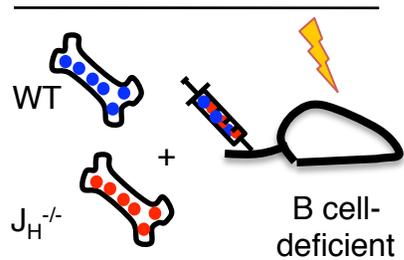


Supplementary Figure 5

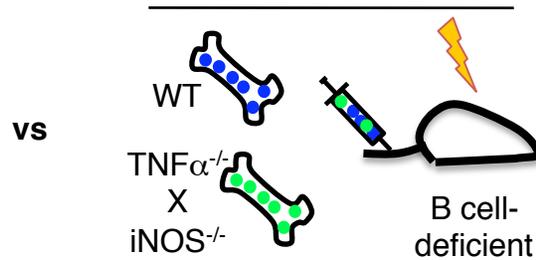
In vitro generation of iNOS-producing IgA⁺ plasma cells.

a, B220⁺ bone marrow (BM) cells derived from AID-YFP mice were co-cultured for up to 7 days with CD45.1⁺ intestinal lamina propria cells (“Gut stroma”) or the S17 bone marrow stromal cell line, in the presence of IL-7, TGFβ, IL-21 and αCD40 antibody. The expression of IgA, iNOS and YFP was analyzed by flow cytometry. IgA⁺iNOS⁺ cells = green rectangle, IgA⁺iNOS⁻ cells = blue rectangle and IgA⁻iNOS⁻ cells = red rectangle. Data are representative of at least three independent experiments. Representative flow cytometry plots are shown. **b**, B220⁺ bone marrow (BM) cells from CD45.2⁺ wild type mice were co-cultured for 7 days in the presence of IL-7, TGFβ, IL-21 and αCD40 antibody with: the BM-derived stromal cell line S17, CD45.1⁺ BM-derived cells (“BM stroma”), CD45.1⁺ intestinal lamina propria cells (“Gut stroma”), CD45.1⁺ intestinal lamina propria cells (“Gut stroma”) from LTβR^{-/-} animals (NB, LTβR^{-/-} mice have been back-crossed to the CD45.1 congenic background). To ensure selective analysis of BM-derived precursors, cells were pre-gated on the CD45.1⁺ population. Representative flow cytometry plots of cells analyzed for iNOS and IgA. IgA⁺iNOS⁺ cells are depicted by the red rectangle. Data are representative of at least three independent experiments.

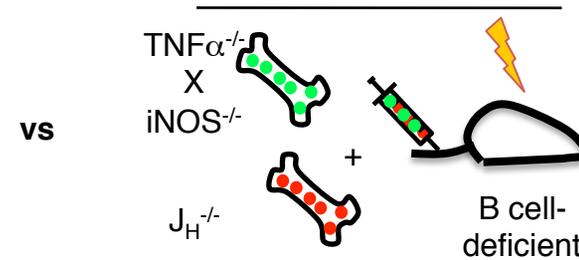
WT mixed chimeras:
All cells can make TNF/iNOS



Control mixed chimeras:
60% of all cells can make TNF/iNOS

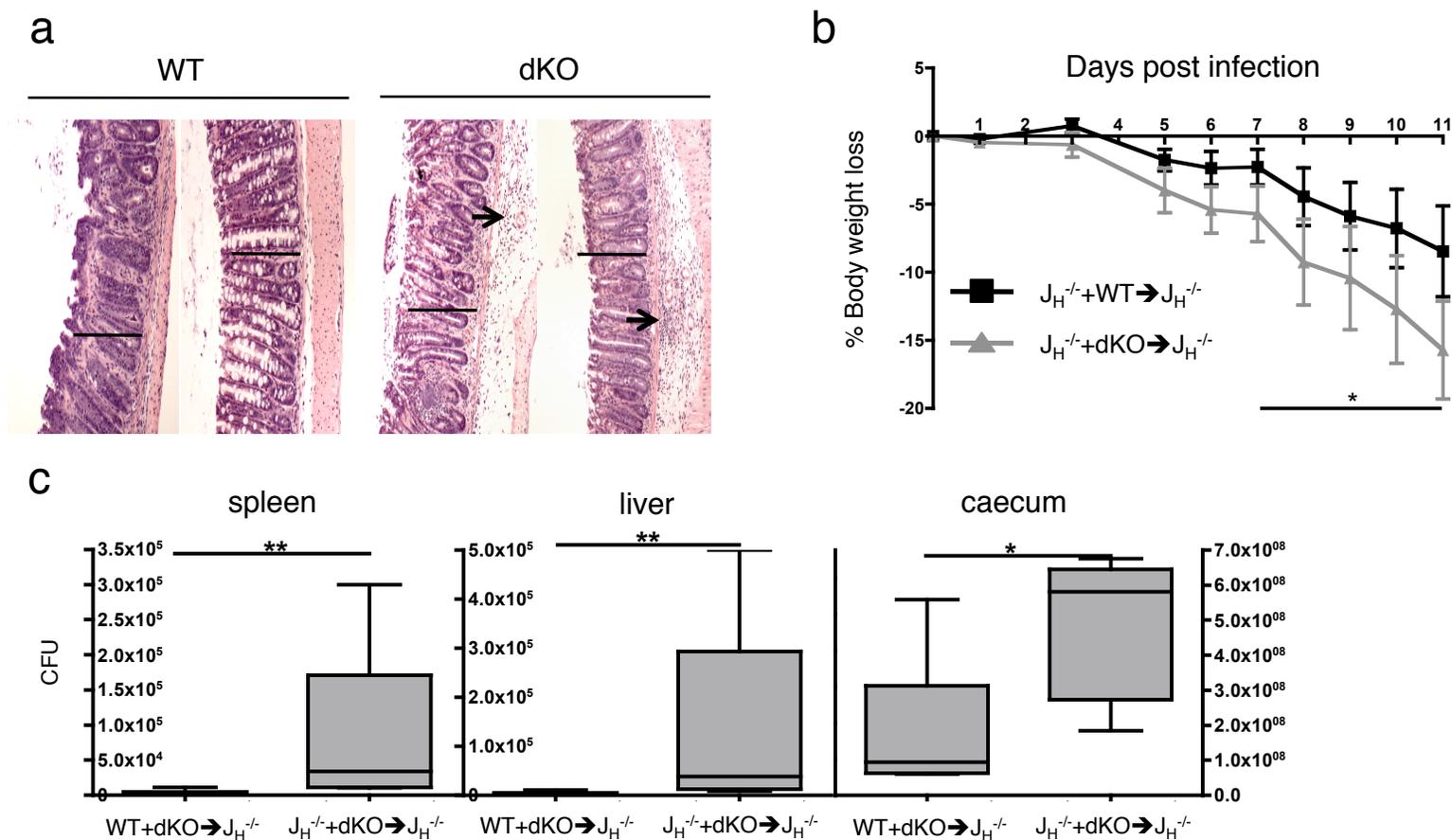


dKO mixed chimeras:
B cells cannot make TNF/iNOS



Generation of mixed bone marrow chimeric mice

Schematic representation of mixed bone marrow chimera generation. $Rag2^{-/-}$ or $J_H^{-/-}$ mice were irradiated to deplete radio-sensitive cells and animals were reconstituted with a mixture of 1) bone marrow from wild-type (WT) + $J_H^{-/-}$ animals (yielding mice where all B cells are $TNF\alpha^+iNOS^+$), 2) bone marrow from $TNF\alpha^-iNOS^-$ double-deficient + WT animals (yielding reconstituted mice where most B cells and other radio-sensitive cells are $TNF\alpha^+iNOS^+$) and 3) bone marrow from $TNF\alpha^-iNOS^-$ double-deficient + $J_H^{-/-}$ animals (yielding reconstituted mixed chimeras where all B cells are $TNF\alpha^-iNOS^-$).



iNOS/TNF α double-deficient mixed chimeras are more susceptible to infection with *Citrobacter rodentium*.

a, Large intestines (LI) from WT and dKO mixed chimeras mice were harvested 11 days post-infection and the pathological scores were analyzed by standard histological staining procedures using hematoxylin and eosin (H&E). LI's from dKO animals show more severe pathology. H&E staining of two representative dKO mice and WT mice are shown. Note the infiltrates in the dKO mice (arrows) and the shortened crypt length (scale bars). The panel shows the original magnification of 10x and scale bars represent 250 μ m. **b**, The percentage of body weight loss in WT + dKO \rightarrow $J_H^{-/-}$ versus $J_H^{-/-}$ + dKO \rightarrow $J_H^{-/-}$ mixed chimeras after *C. rodentium* infection over time is depicted. Significantly higher body weight loss in $J_H^{-/-}$ + dKO \rightarrow $J_H^{-/-}$ mice was observed from day 7 to day 11 post-infection (n = 7 per group). NB: One $J_H^{-/-}$ + dKO \rightarrow $J_H^{-/-}$ mouse was found dead at day 10 post-infection. Also note that in this experiment, *C. rodentium* was somewhat attenuated compared to prior experiments, thus resulting in later onset of weight loss and larger variability in weight loss. **c**, The colonization by *C. rodentium* was determined 11 days after infection by homogenizing spleens, livers and caecums followed by serial dilution plating on nalidixic acid-containing LB plates and counting of colonies. *C. rodentium* colonization of all 3 organs is significantly enhanced in dKO mice at day 11 post-infection. * p < 0.05, ** p < 0.01.