Supplementary Information

Goblet Cell Associated Antigen Passages are Inhibited During *Salmonella typhimurium* Infection to Prevent Pathogen Dissemination and Limit Responses to Dietary Antigens

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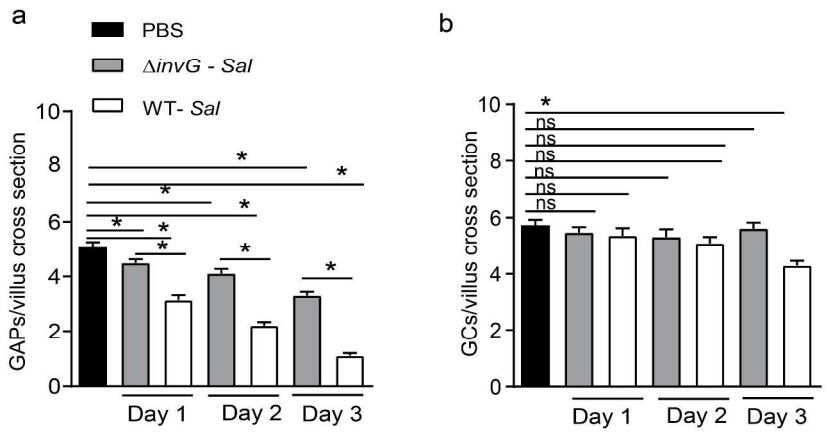


Figure S1

Supp Figure 1: *Salmonella inhibits GAPs independent of changes in GCs numbers*. (a) Density of SI GAPs and (b) GCs, identified by PAS staining, in mice infected with Δ*invG* or wildtype *Salmonella*. N=4 or more mice, ns= not significant, *=$p<0.05$, data presented as the mean ± SEM.

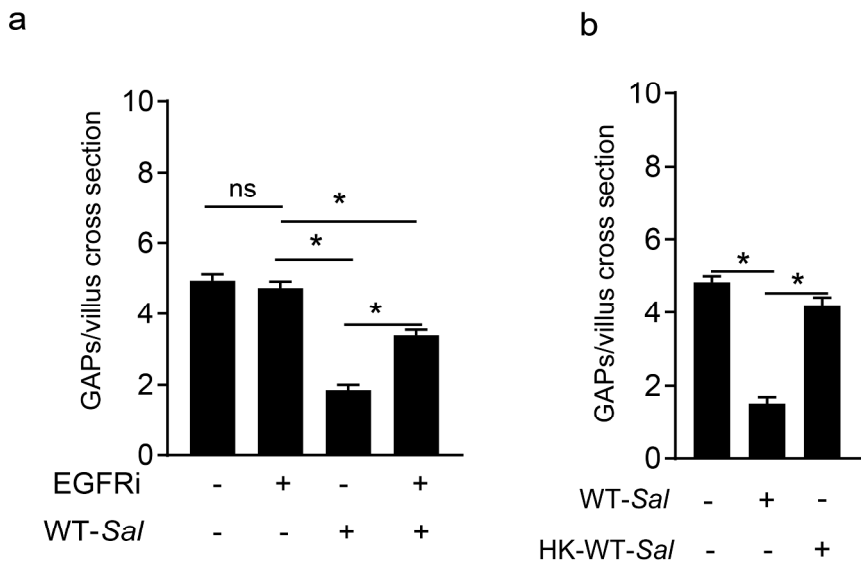


Figure S2

Supp Figure 2: *Blocking EGFR activation reverses GAP inhibition and heat-killed Salmonella is unable to inhibit GAPs.* Density of SI GAPs per villus cross section in C57BL/6 mice receiving vehicle or inhibition of EGFR activation (EGFRi) 2 days after oral administration of 5×10^7 CFU *Salmonella* or PBS. (b) Density of GAPs in C57BL/6 mice, given 5×10^8 CFU of wildtype or heat-killed wildtype *Salmonella* in the SI lumen 1 hr earlier. N=5 mice with 60 or more villus cross sections per mouse examined for each condition. ns= not significant, * = $p<0.05$, data presented as the mean ± SEM.

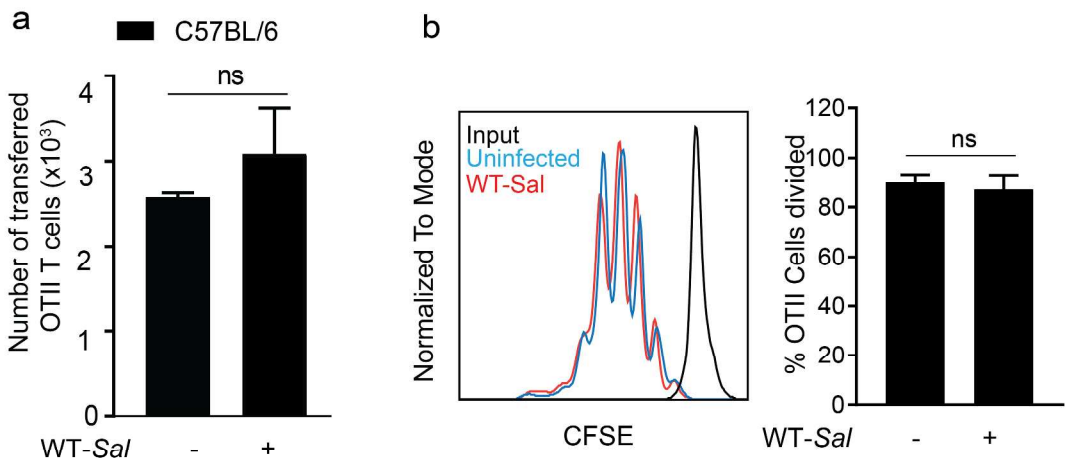


Figure S3

Supp Figure 3: Ova specific OTII T cell trafficking and proliferation in response to i.v antigen is not affected during infection. (a) Quantification of number of adoptively transferred Ova specific CD4+ OTII T cells in the MLN of C57BL/6 mice that were uninfected or infected with $5x10^7$ CFU *Salmonella.* (b) Flow cytometry plots of CFSE dilution and quantification of Ova specific CD4+ OTII T cells in the MLN of uninfected or infected C57BL/6 mice in response to systemic Ova. n=5 mice per group. ns= not significant, data presented as the mean ± SEM.

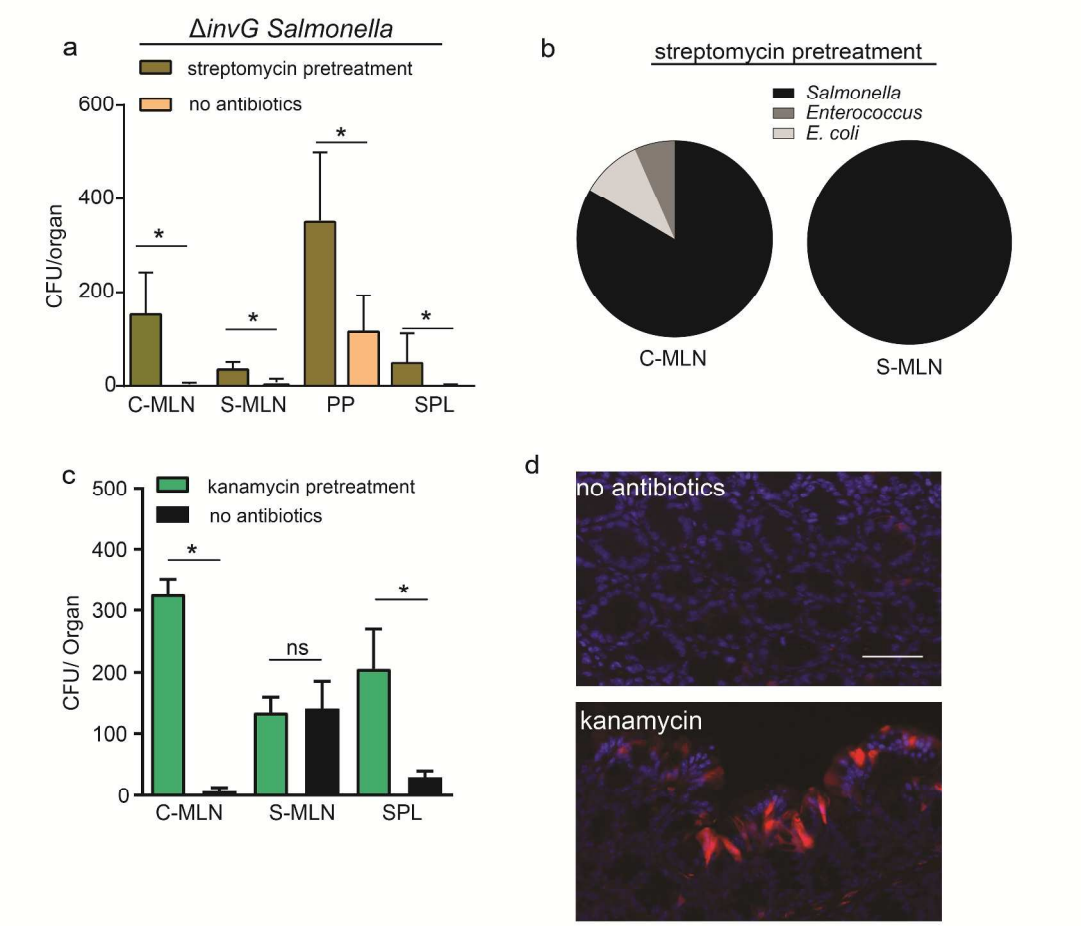
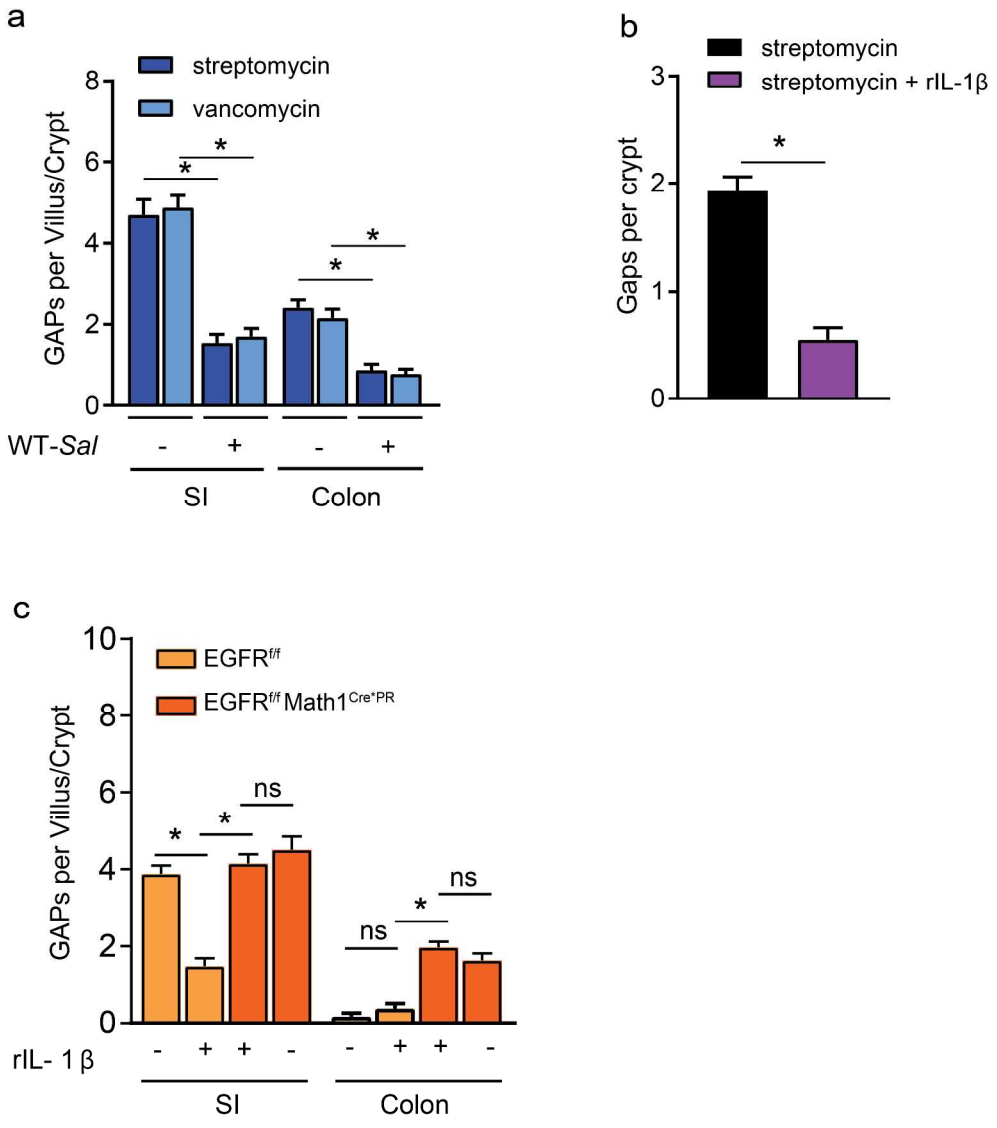


Figure S4

Supp Figure 4: Pretreatment with *streptomycin or kanamycin promotes dissemination of ΔinvG Salmonella and gut commensals to the colon draining MLN*. a) CFUs in the MLNs, PP, and spleen (SPL) two days after infection with 5×10^7 CFU *ΔinvG Salmonella* in untreated or streptomycin-pretreated C57BL/6 mice. b) Pie chart depicting live bacterial species identified by MALDI-TOF Biotyper isolated from the colon-draining MLN (C-MLN) or SI-draining MLN (S-MLN) of streptomycin-pretreated mice 2 days after infection with 5×10^7 CFU *Salmonella*. c) CFUs in the MLNs and SPL 2 days after infection with 5×10^7 CFU *Salmonella* in untreated mice or mice pretreated with kanamycin. d) Representative fluorescence image of colon from an untreated (no antibiotics) or kanamycin-treated mouse given luminal dextran for 45 min,

demonstrating colonic GAPs following kanamycin pretreatment. Data represented as the mean ±SEM,Scale bar in d= 50 μm. *p<0.05, ns- not significant. n=3 or more mice in each group.



Supp Figure 5: *Antibiotic-induced colonic GAPs are suppressed during Salmonella infection via IL-1β and EGFR signaling pathway*. a) GAP density in the SI villus or colonic crypt in untreated or antibiotic-treated mice that were uninfected or given 5×10^7 CFU wildtype *Salmonella* 2 d earlier. b) Density of colonic GAPs in C57BL/6 mice 3 days post streptomycin (500 µg) gavage and 1 hr after i.p. injection of vehicle or 100 ng recombinant IL-1β. c) SI and colonic GAP density in $EGFR^{f/f}$ and $EGFR^{f/f}$ $Math1^{Cre*PR}$ mice treated with RU486 for 5 d, and administered

with PBS or 100 ng recombinant IL-1β. Data represented as the mean ±SEM, *p<0.05, ns- not significant. n=3 or more mice per group.

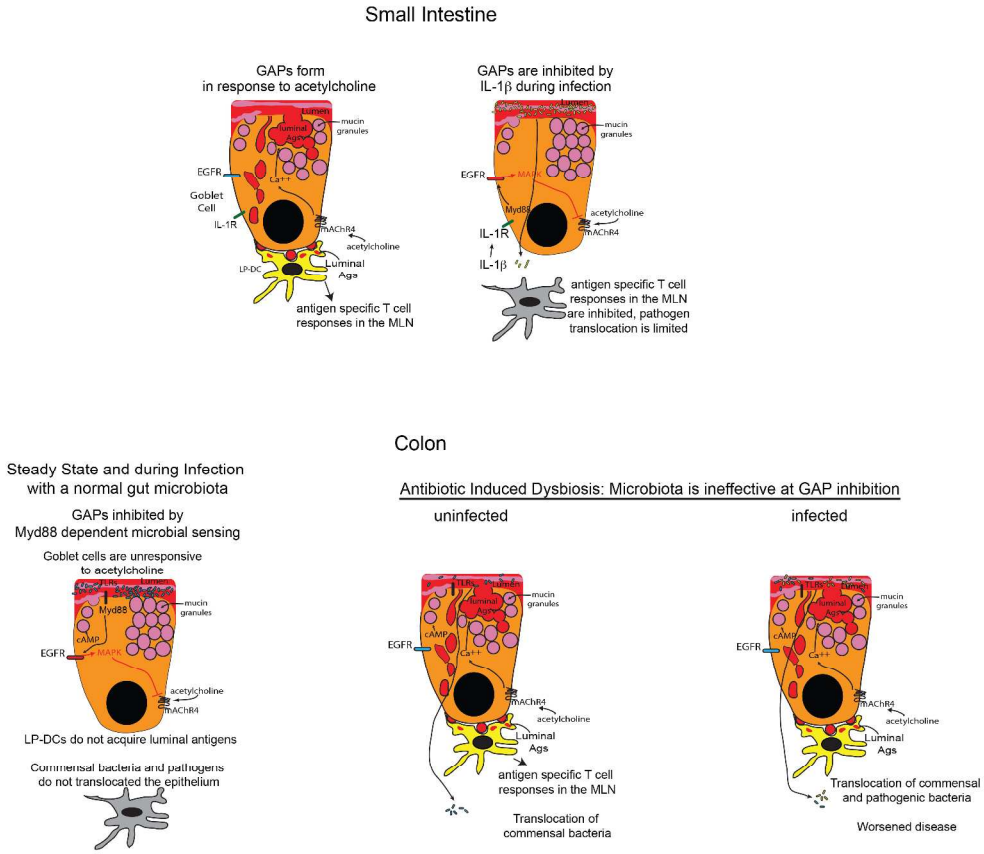


Figure S6

Supp Figure 6: *Inhibition of GAP formation to limit responses to dietary antigen and pathogen dissemination during enteric infection.* (upper panel) GAPs, dietary antigen delivery to the LP-DCs, and immune responses to dietary antigen in the SI draining MLN are acutely inhibited during enteric infection with *Salmonella* by IL-1β activating MyD88 and EGFR in GCs, suppressing the ability of GCs to respond to acetylcholine and form a GAP. Translocation of *Salmonella* to the SI draining MLN required GCs and correlated with GAP density. Overriding GAP inhibition during *Salmonella* infection resulted in inflammatory antigen specific T cell responses to dietary antigen in the SI draining MLN (not depicted). (lower panel) During the steady-state, colonic GCs do not form GAPs due to GC intrinsic sensing of the dense colonic microbitoa and *Salmonella* does not translocate across the colonic epithelium. However

dysbiosis following a single dose of some antibiotics, or deletion of MyD88 or EGFR withing GCs, results in formation of colonic GAPs, which allows *Salmonella* to translocate across the epithelium resulting in worsened disease.