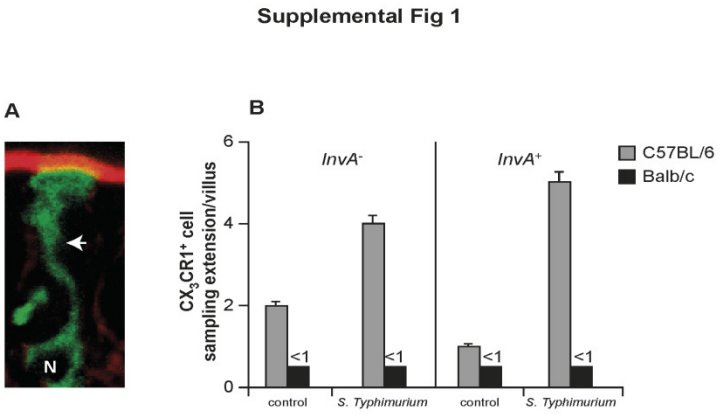
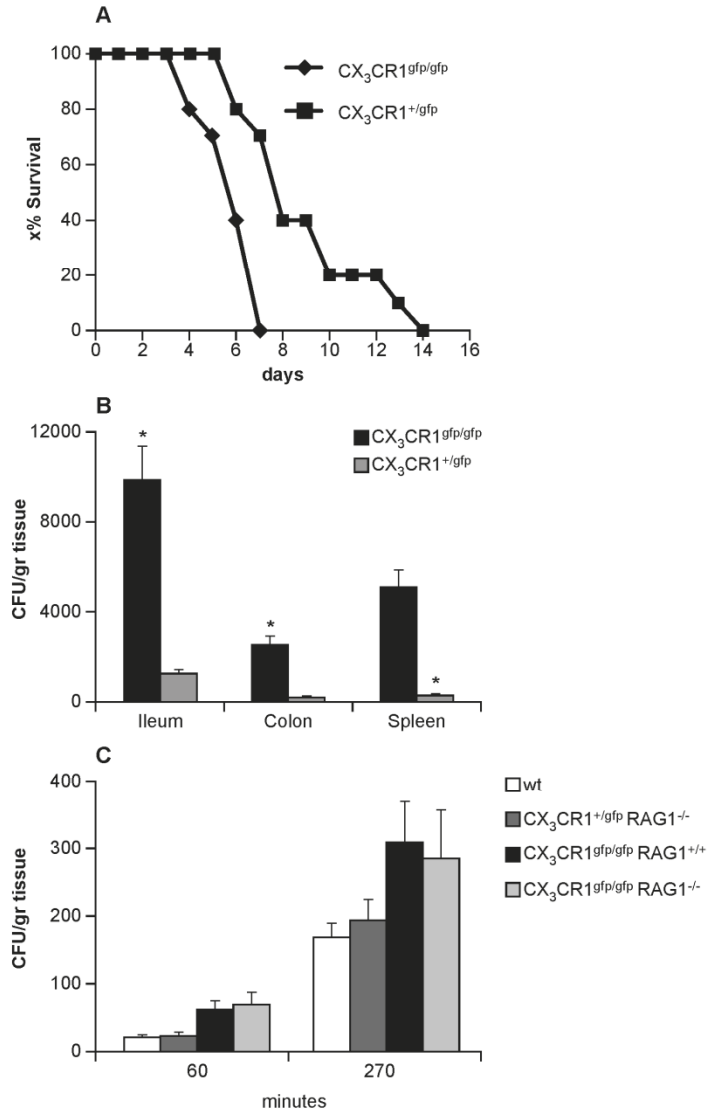
Supplemental Materials

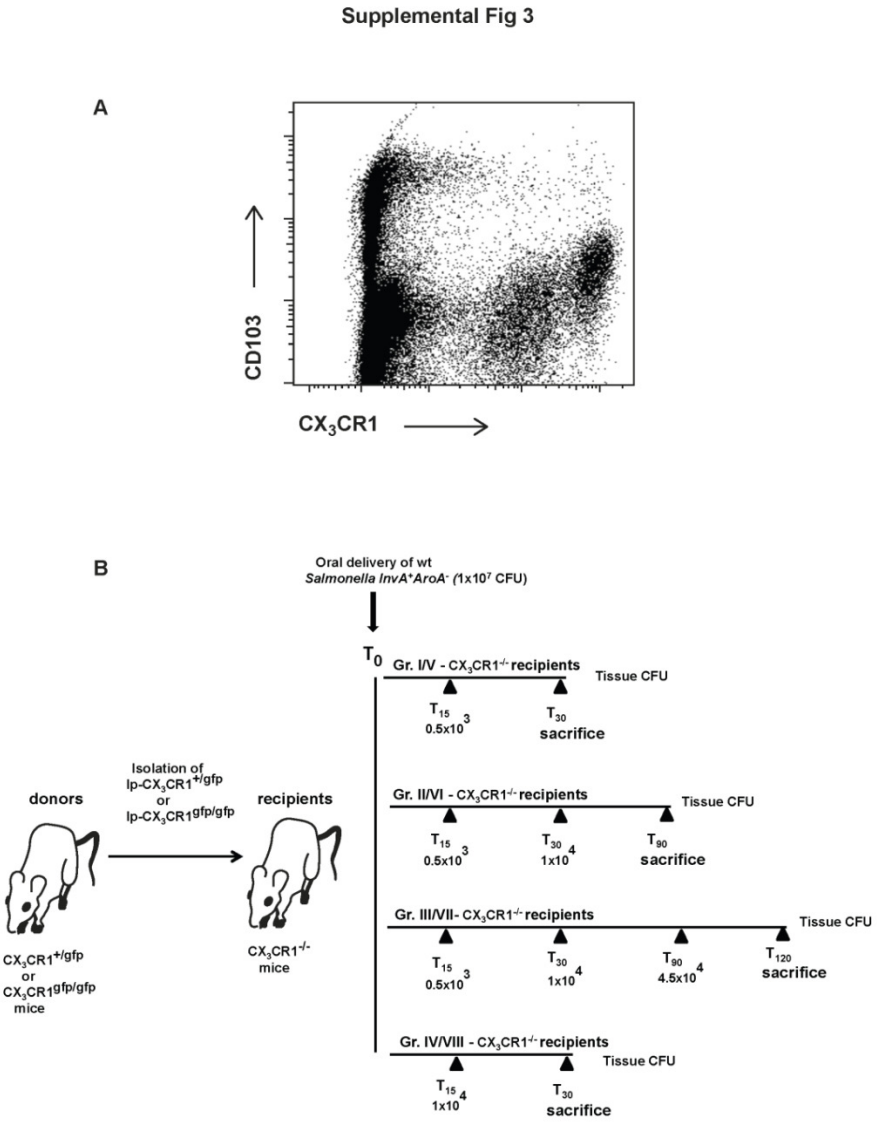


Supplemental Fig 1: *CX_3CR1-mediated antigen-sampling in mice of different genetic background*. Confocal microscopy was used to enumerate cellular extension, or dendrites (arrow head) considered being sampling devises of $CX_3CR1^{+/gfp}$ cells (1A) in the epithelium of the ileum of both C57BL/6 and Balb/c. The number of dendrites/villus increased significantly in C57BL/6, but not in Balb/c mice following challenge with both non-invasive (*InvA*$^-$) and invasive (*InvA*$^+$) Salmonella. As previously reported (8), the ability of *S.* Typhimurium to invade did not have a significant impact on the number of dendrites produced by $CX_3CR1^{+/gfp}$ cells.

Supplemental Fig 2



Supplemental Fig 2. *Role of CX_3CR1 and $RAG^{-/-}$ background on susceptiblity to salmonella infection and invasion of S. Typhimurium*. $CX_3CR1^{gfp/gfp}$ mice succumbed within 7 days from infection at a faster rate compared to $CX_3CR1^{+/gfp}$ mice (A) (12 mice/group). It appeared that in spite of lacking the ability to sample *S*. Typhimurium via the indirect route $CX_3CR1^{gfp/gfp}$ mice showed significantly higher bacterial load both locally (ileum and colon) and systemically (spleen) (B). Furthermore, increased susceptibility of these mice to infection could not be attributed to reduced antibody responses to *S*. Typhimurium as shown in Figures 6. Furthermore, to rule out that the impact of CX_3CR1 deficiency in the number of *S*. Typhimurium traversing the epithelium could be related to altered activation of adaptive immune responses we assessed bacterial translocation in $CX_3CR1^{+/-}$ and $CX3CR1^{-/-}$ on $RAG^{-/-}$ background (C). We observed that the $RAG^{-/-}$ background did not affect bacterial translocation



Supplemental Fig 3. *Protocol for Salmonella-exclusion assay*. Flow cytometry analysis was carried out to identify $CX_3CR1^-CD103^-$, $CX_3CR1^-CD103^+$ DC and CX_3CR1^+ $CD103^-$ cells in the lamina propria of $CX_3CR1^{+/gfp}$ mice (A). The latter population was introduced directly into the intestinal lumen at various intervals and increasing number after *Salmonella* infection as detailed in the pathogen exclusion assay protocol (B). Four groups of mice (6 mice/group) (I-IV) received a single oral delivery of $1x10^7$ invasive/non-replicating *InvA*$^+$*AroA*$^-$ *Salmonella* at T0; this was followed by the intraluminal injection of $CX_3CR1^{+/gfp}$ cells. The number of $CX_3CR1^{+/gfp}$ cells for the adoptive transfer of intraluminal cells in Gr I, II and III at any given time (T15, T30 and T90) was established according to the results of the time course experiment illustrated in Figure 3C. Group IV received a number of CX_3CR1^+ cells ($1x10^4$) that far exceeded the number of cells observed in the lumen 15 minutes (T15) after infection.
At the end of the experiments mice were sacrificed and CFU gr/tissue determined. The same protocol was repeated in four additional groups (V-VIII) to test the ability of $CX_3CR1^{gfp/gfp}$ cells to prevent *S*. Typhimurium from traversing the epithelial barrier.