

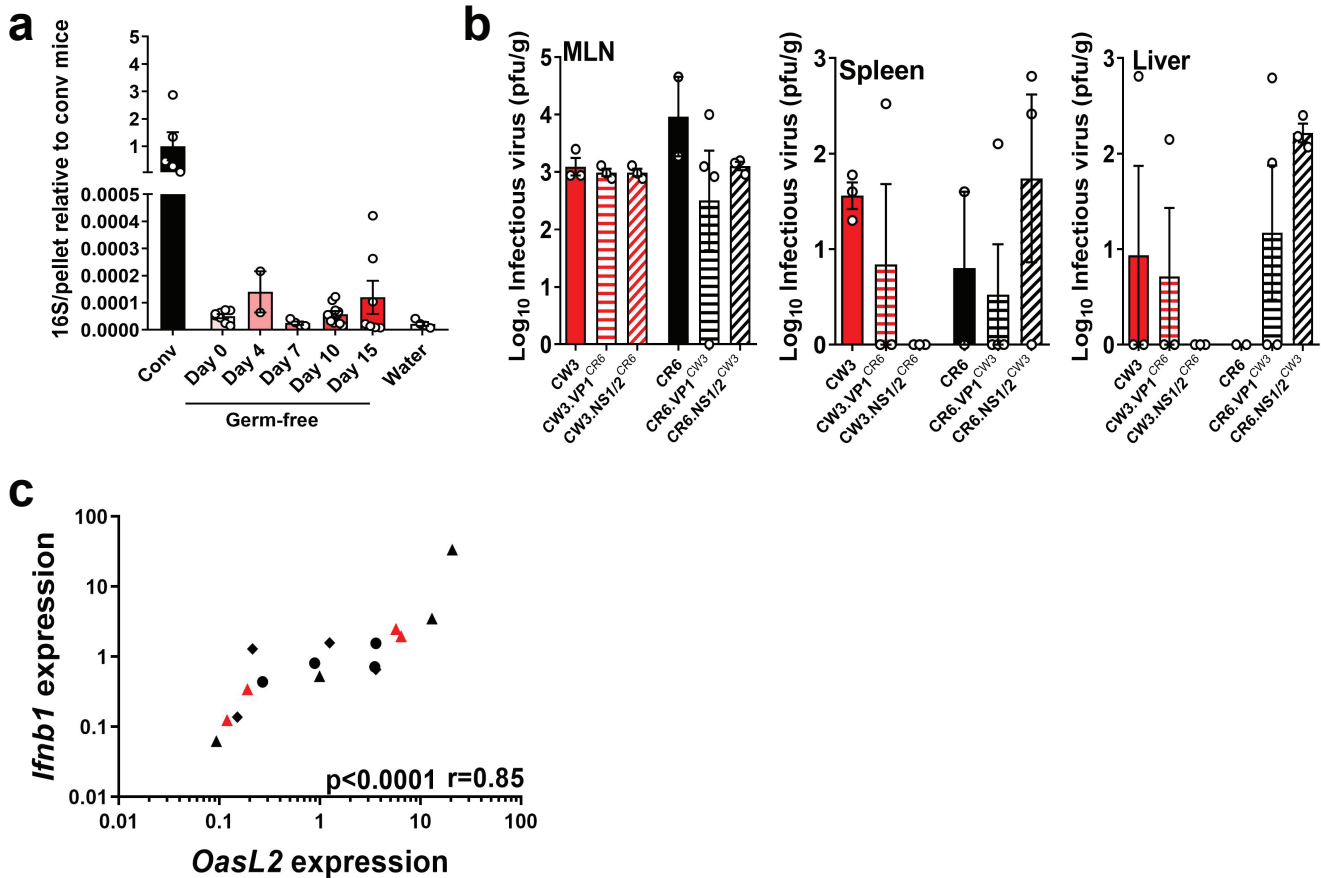
Supplementary Information for:

IFN-I and IL-22 mediate protective effects of intestinal viral infection

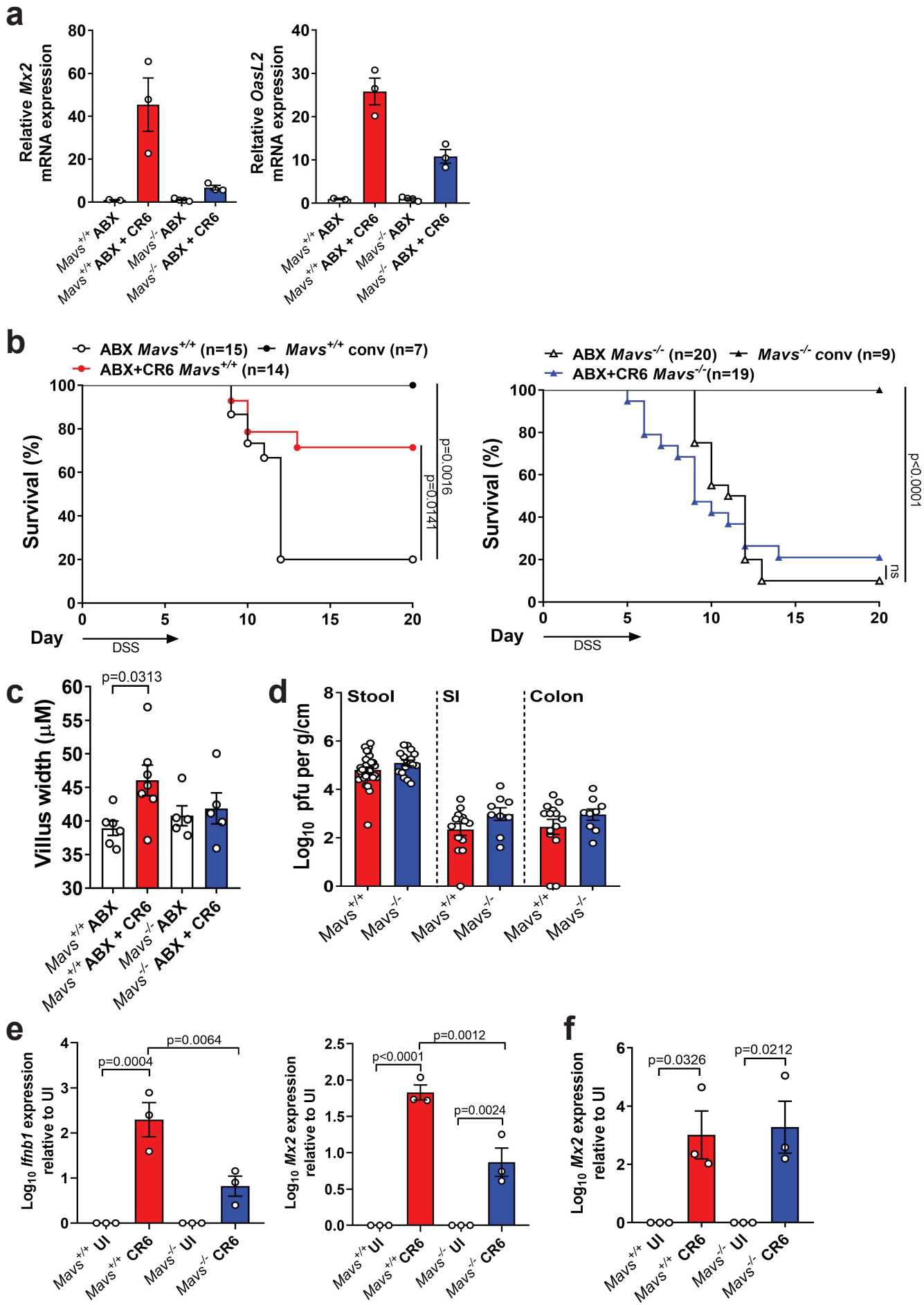
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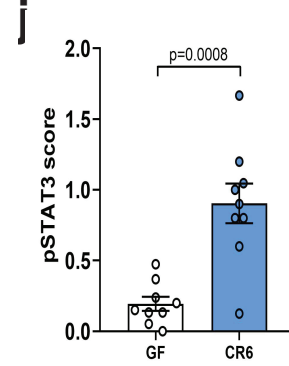
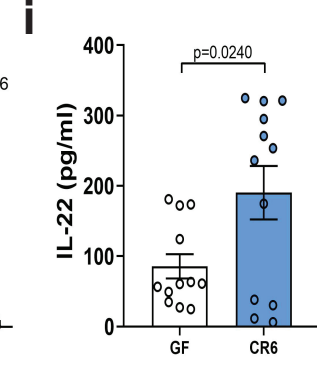
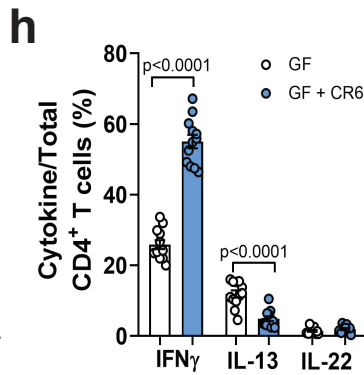
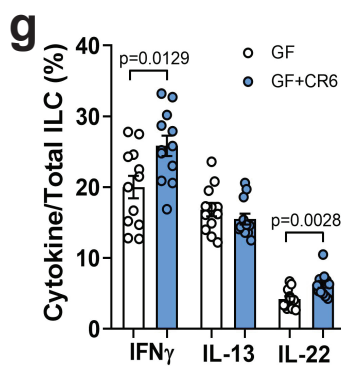
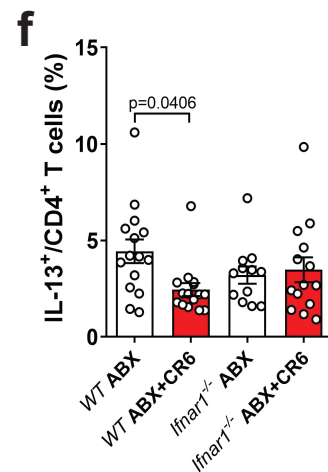
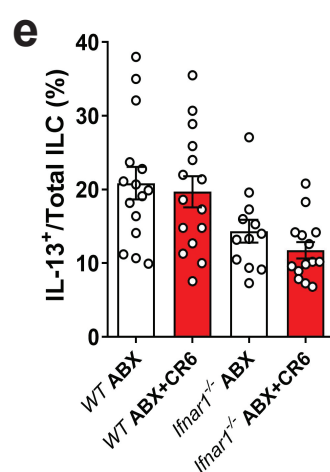
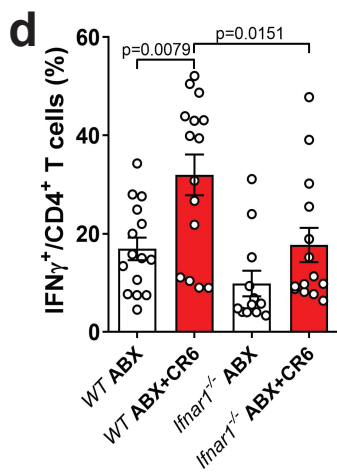
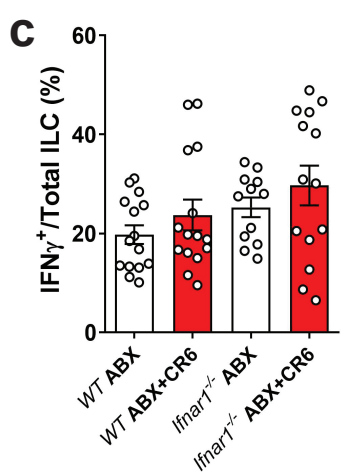
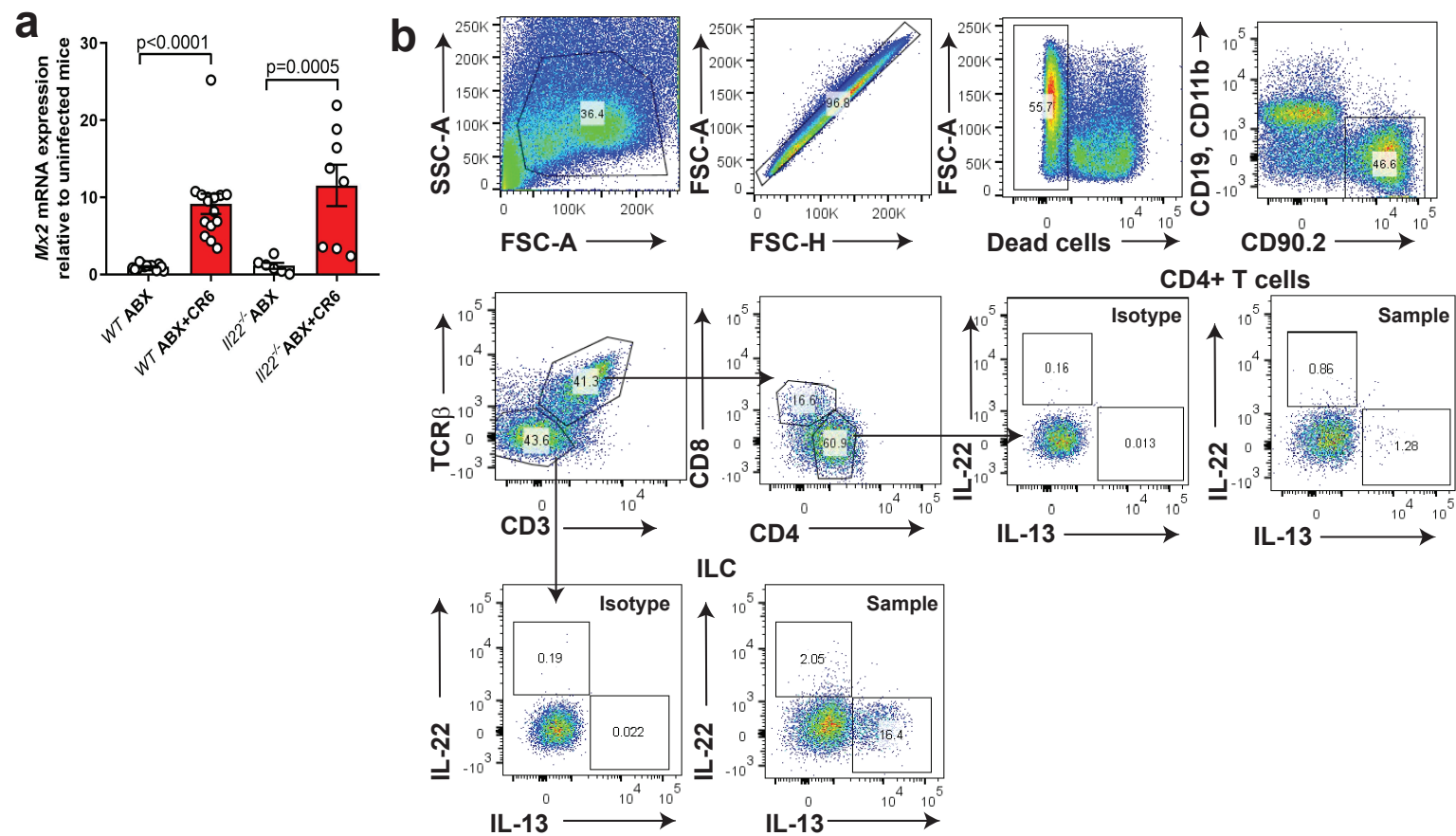
Supplementary Figure 1-6



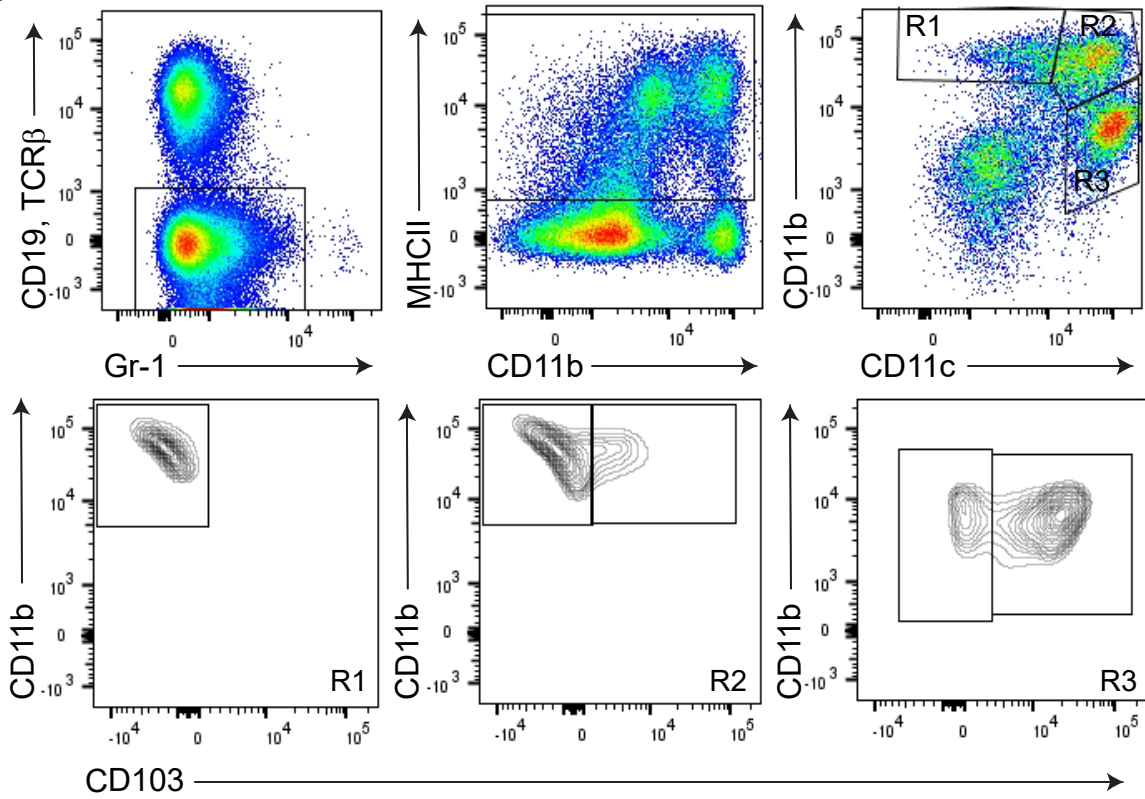
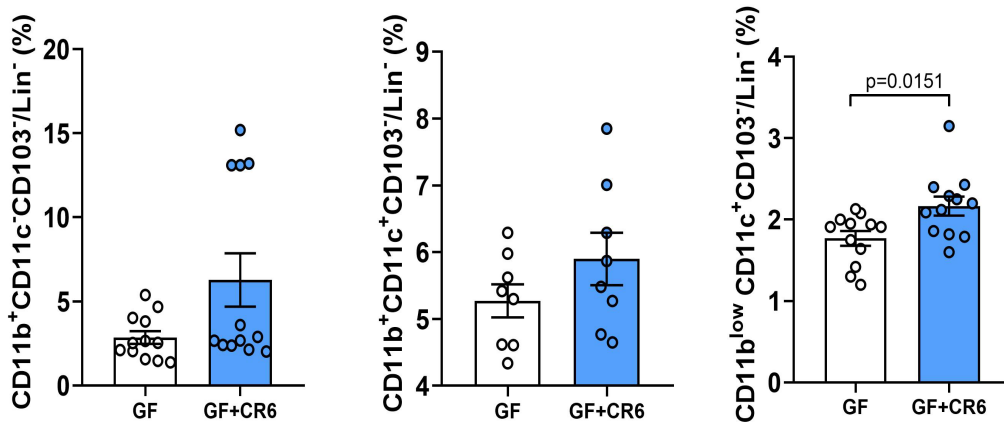
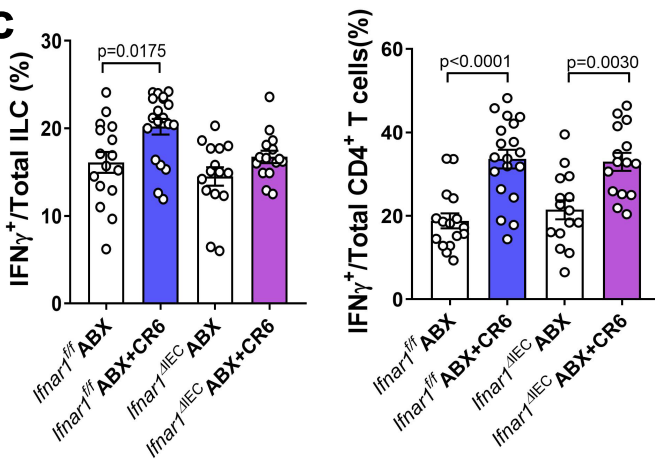
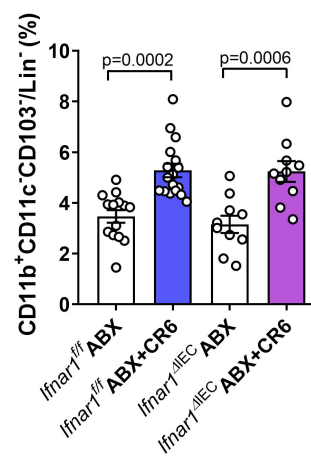
Supplementary Figure 1: Detection of bacteria and extra-intestinal spread of MNV in GF mice. (a) 16S in stool pellets of GF mice relative to Conv mice determined by qPCR. The number of samples analyzed in (a): Conv, n=5; Day 0, n=8; Day 4, n=2; Day 7, n=4; Day 10, n=10, Day 15, n=7 and water, n=4. (b) Infectious virus in the mesenteric lymph nodes (MLN), spleen and liver as determined by plaque assay at 10 dpi with MNV strains. The number of mice analyzed for virus titer: CW3, n=3; CW3.VP1^{CR6}, n=3; CW3.NS1/2^{CR6}, n=3; CR6, n=2; CR6.VP1^{CW3}, n=4 and CR6.NS1/2^{CW3}, n=3. All bars represent mean and error bars represent standard error of the mean. (c) *Ifnb1* mRNA expression over uninfected GF mice relative to *OasL2* expression in isolated LPLs. Data represents 2 independent experiments each with 2 mice per group. Correlation was analyzed using Pearson r with p-values and r-values as shown.



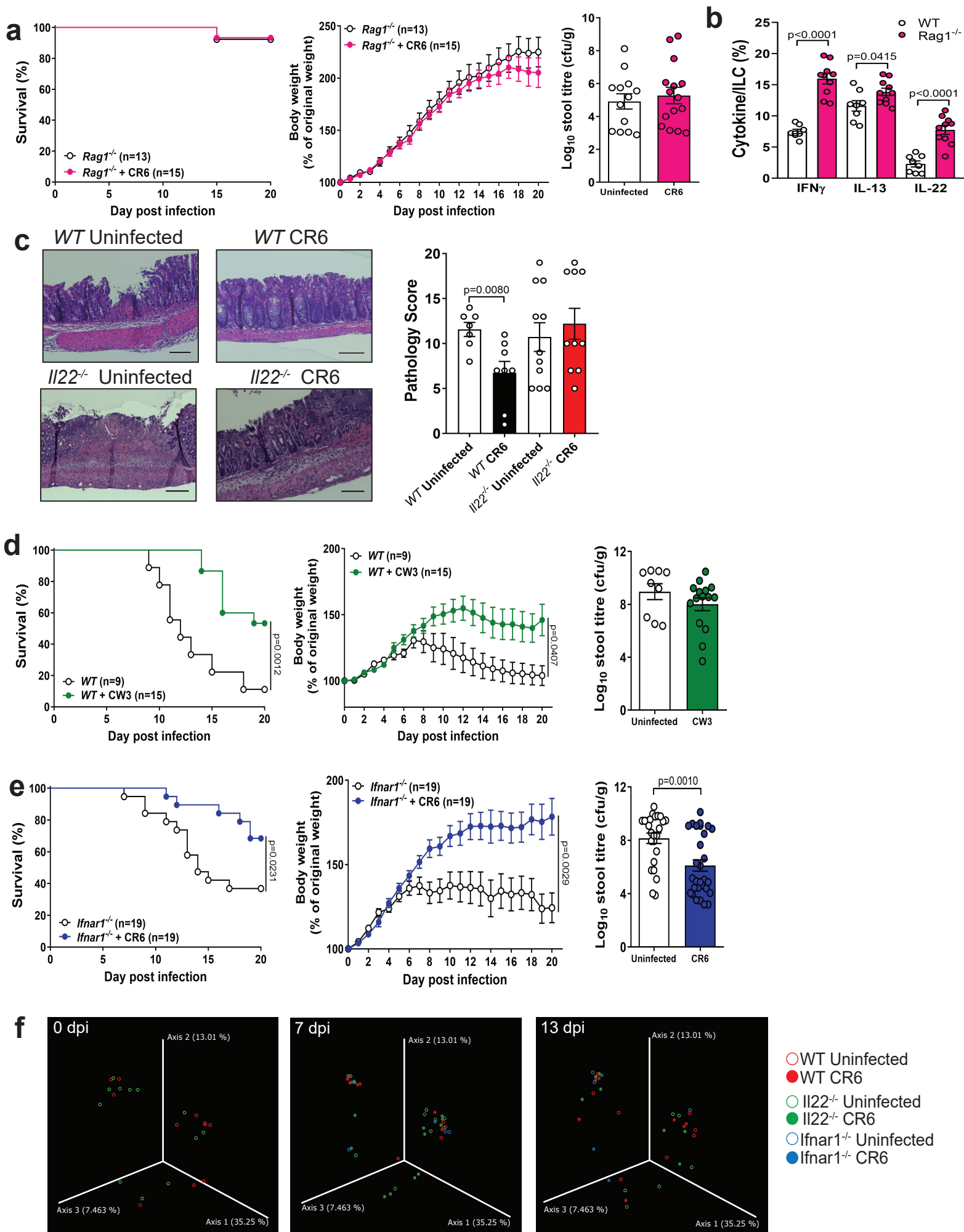
Supplementary Figure 2: Protection of ABX-treated mice from intestinal injury is dependent on MAVS signaling. (a) Expression of *Mx2* and *OasL2* mRNA in the colon following CR6 infection of ABX-treated *Mavs*^{+/+} and *Mavs*^{-/-} mice at day 6 post DSS compared to uninfected mice. Data shown is a representative of 2 independent experiments. The following mice were analyzed: *Mavs*^{+/+} ABX, n=2; *Mavs*^{+/+} ABX+CR6, n=3; *Mavs*^{-/-} ABX, n=4 and *Mavs*^{-/-} ABX+CR6, n=3. (b) Survival of *Mavs*^{+/+} and *Mavs*^{-/-} mice following DSS. Mice were treated with ABX for at least 10 days prior to CR6 infection and given DSS at 10 dpi for 6 days. Survival curves were analyzed using the log-rank Mantel–Cox test. Villus width (c) and infectious virus levels in SI, colon and stool (d) at day 10 dpi. The following mice were analyzed for villus width: *Mavs*^{+/+} ABX, n=6; *Mavs*^{+/+} ABX+CR6, n=7; *Mavs*^{-/-} ABX, n=5 and *Mavs*^{-/-} ABX+CR6, n=5. Villus width was analyzed using ANOVA with Dunnet’s multiple comparisons test. The following mice were analyzed for stool titer: *Mavs*^{+/+}, n=31 and *Mavs*^{-/-}, n=17; SI and colon titer: *Mavs*^{+/+}, n=14 and *Mavs*^{-/-}, n=9. (e) Relative *Ifnb1* and *Mx2* mRNA expression in BMDCs infected with MOI 1 of MNV CR6 for 16 h. Data shown represents 3 independent experiments. (f) Relative *Mx2* expression in SI organoids cultured in the presence of 3x10⁶ pfu/well of MNV CR6 for 16 h. Data represent 3 independent experiments. Gene expression was analyzed using ANOVA with Tukey’s multiple comparisons test. All bars represent mean and error bars represent standard error of the mean. All p-values are shown in the figure.



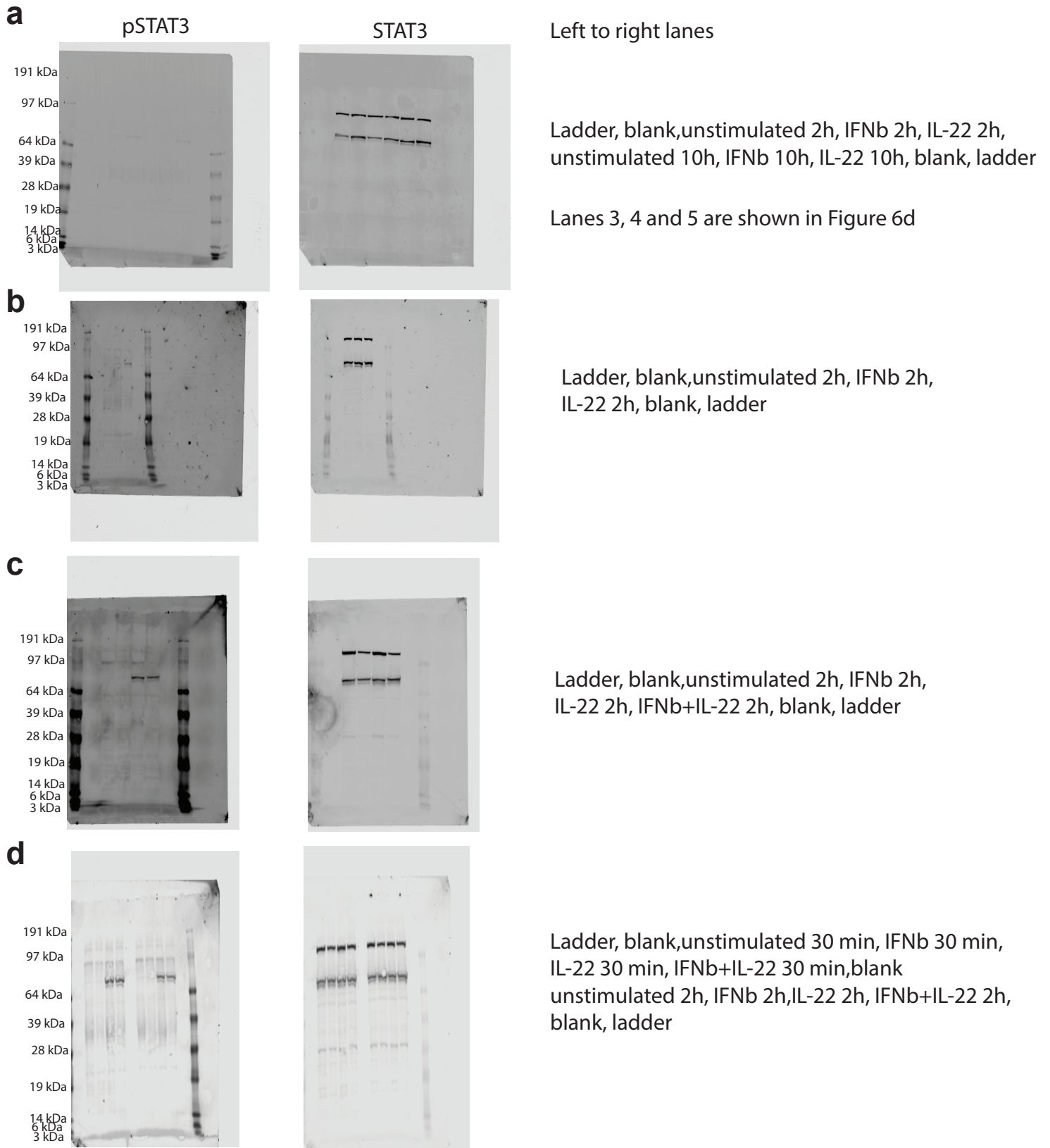
Supplementary Figure 3: Immune profiling of germ-free and antibiotics-treated mice following intestinal injury and MNV infection. (a) Expression of *Mx2* mRNA in the colon of *WT* and *I122^{-/-}* mice following DSS administration. The following number of mice were analyzed: *WT* ABX, n=14; *WT* ABX+CR6, n=15; *I122^{-/-}* ABX, n=6 and *I122^{-/-}* ABX+CR6, n=8. (b) Gating strategy of ILCs and CD4⁺ T cell populations in the colonic lamina propria. Proportion of IFN γ expressing ILC (c) and CD4⁺ T cells (d). Proportion of IL-13 expressing ILC (e) and CD4⁺ T cells (f) in the colon of ABX-treated *WT* and *Ifnar1^{-/-}* mice following DSS administration. The following mice were analyzed for cell proportions: *WT* ABX, n=15; *WT* ABX+CR6, n=15; *Ifnar1^{-/-}* ABX, n=12 and *Ifnar1^{-/-}* ABX+CR6, n=14. Cytokine expression by ILC (g) and CD4⁺ T cells (h) of GF and CR6-infected GF mice following DSS administration. (i) IL-22 levels in colon explants cultured for 48 h as measured by ELISA. A total of 12 mice/group were analyzed in (g), (h) and (i). (j) pSTAT3 expression score in the colon at day 5 post DSS. A total of 9 mice/group were analyzed in (j). Data were analyzed using ANOVA with Tukey's multiple comparisons test except for GF data which were analyzed by two-tailed t-test. All bars represent mean and error bars represent standard error of the mean. All p-values are shown in the figure.

a**b****c****d**

Supplementary Figure 4: MNV affects populations of MHCII⁺ cells in the colon following DSS. (a) Gating strategy for MHCII⁺ populations in the colonic lamina propria. (b) Proportion of MHCII⁺ populations in GF and CR6-infected GF mice following DSS administration. 12 mice were analyzed per group and data was analyzed by two-tailed t-test. Proportion of IFN γ expressing ILC and CD4⁺ T cells (c) and CD11b⁺CD11c⁻CD103⁻ (d) cells in *Ifnar1^{fl/fl}* and *Ifnar1 Δ IEC* mice at day 6 post DSS. The following number of mice were analyzed in (c): *Ifnar1^{fl/fl}* ABX, n=16; *Ifnar1^{fl/fl}* ABX+CR6, n=19; *Ifnar1 Δ IEC* ABX, n=15 and *Ifnar1 Δ IEC* ABX+CR6, n=15. The following number of mice were analyzed in (d): *Ifnar1^{fl/fl}* ABX, n=14; *Ifnar1^{fl/fl}* ABX+CR6, n=17; *Ifnar1 Δ IEC* ABX, n=10 and *Ifnar1 Δ IEC* ABX+CR6, n=10. Data were analyzed using ANOVA with Tukey's multiple comparisons test. All bars represent mean and error bars represent standard error of the mean. All p-values are shown in the figure.



Supplementary Figure 5: MNV protects young mice from *C. rodentium* infection in a strain-independent manner and does not alter the microbiome. (a) Survival, weights and stool bacterial titers in *Rag1*^{-/-} mice infected with CR6. 13 *Rag1*^{-/-} Uninfected and 15 *Rag1*^{-/-} CR6 mice were analyzed. (b) Proportion of ILCs expressing IFN γ , IL-13 and IL-22 in the colon lamina propria of uninfected 21-day-old *WT* and *Rag1*^{-/-} mice. 8 *WT* and 10 *Rag1*^{-/-} mice were analyzed. (c) Representative images and pathological score of the colon in *WT* and *IL22*^{-/-} mice at day 6 post *C. rodentium* infection. Scale bars represent 100 μ M. The following number of mice were analyzed: *WT* Uninfected, n=7; *WT* CR6, n=8; *IL22*^{-/-} Uninfected, n=11 and *IL22*^{-/-} CR6, n=10. Survival, weights and stool bacterial titers in *WT* mice infected with CW3 (d) and *Ifnar1*^{-/-} mice infected with CR6 (e) and challenged with *C. rodentium*. The following mice were analyzed for stool titer: *WT* uninfected, n=9; *WT* CW3, n=15; *Ifnar1*^{-/-} uninfected, n=25 and *Ifnar1*^{-/-} CR6, n=29. (f) Alpha diversity of uninfected and CR6-infected *WT*, *IL22*^{-/-} and *Ifnar1*^{-/-} mice at 0, 7 and 13 dpi with CR6. Each dot represents a mouse. *WT* and *IL22*^{-/-} mice were analyzed from 2-3 independent experiments and *Ifnar1*^{-/-} mice were from a single experiment. Survival curves were analyzed using the log-rank Mantel–Cox test. For weight curves, AUC was determined for each mouse and difference between uninfected and MNV-infected groups determined by two-tailed t-test. Log₁₀ transformed bacterial titers and pathology score were analyzed by two-tailed t-test. All bars represent mean and error bars represent standard error of the mean. All p-values are shown in the figure.



Supplementary Figure 6: Western Blots for pSTAT3 and STAT3 in murine small intestinal organoids. (a) Experiment 1, (b) Experiment 2, (c) Experiment 3 and (d) Experiment 4 analyzing pSTAT3 and STAT3 expression in murine small intestinal organoids left unstimulated or stimulated with IFN β or IL-22 for various lengths of time. Ladder and lane details are shown.