Figure S1

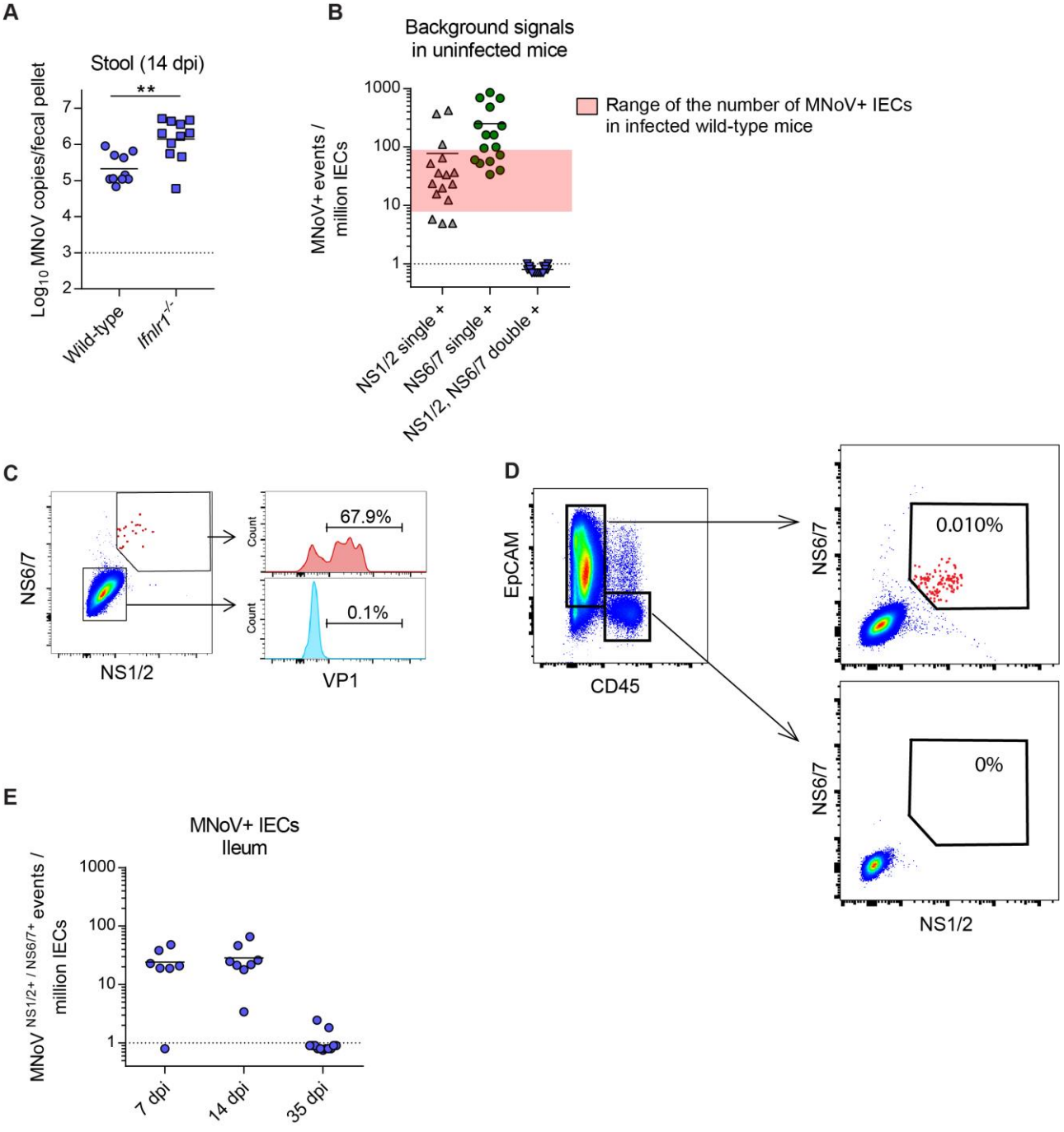

A
Stool (14 dpi)
**
Log10 MNoV copies/fecal pellet
Wild-type
Ifnlr1-/-
B
Background signals in uninfected mice
MNoV+ events / million IECs
Range of the number of MNoV+ IECs in infected wild-type mice
NS1/2 single +
NS6/7 single +
NS1/2, NS6/7 double +
C
NS6/7
NS1/2
Count
67.9%
0.1%
VP1
D
EpCAM
CD45
NS6/7
0.010%
0%
NS1/2
E
MNoV+ IECs Ileum
MNoV NS1/2+ / NS6/7+ events / million IECs
7 dpi
14 dpi
35 dpi

Figure S1. Detection and validation of MNoV-infected intestinal epithelial cells, related to Figure 1.

(A) Wild-type and *Ifnlr1*$^{-/-}$ mice were infected with CR6 and the viral shedding was analyzed at 14 dpi by qRT-PCR for MNoV genome copies. *N* = 10 to 12 per group, combined from two independent experiments. **(B)** Quantification of pseudo-positive signals of NS1/2 and NS6/7 single or double staining of IECs from uninfected wild-type mice. Pink box indicates the range of the number of MNoV+ IECs in wild-type mice at 7 to 35 days post infection. *N* = 16 per group, combined from three independent experiments. **(C)** Histogram of anti-VP1 capsid staining from NS1/2+NS6/7+ pre-gated population (red, upper panel) and NS1/2-NS6/7- pre-gated population (blue, bottom panel). IECs from CR6-infected *Ifnlr1*$^{-/-}$ mice were analyzed by flow cytometry at 35 dpi. **(D)** Flow cytometry for NS1/2 and NS6/7 double staining of EpCAM+/CD45- or EpCAM-/CD45+ cells from colonic epithelial cells. *Ifnlr1*$^{-/-}$ mice were infected with CR6 then analyzed at 7 dpi. **(E)** Quantification of MNoV+ IECs from ileum during CR6 infection in wild-type mice at 7, 14, and 35 dpi. *N* = 7 to 13 mice per group, combined from four independent experiments. Statistical significance was determined by Mann Whitney test. $^{**}P < 0.01$.

Figure S2

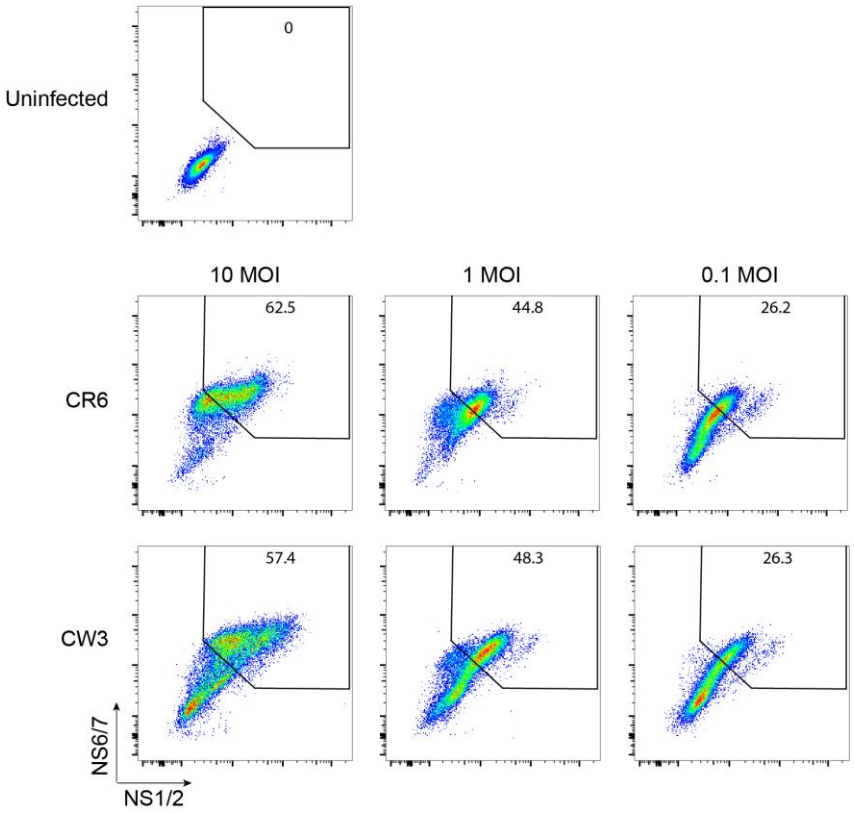
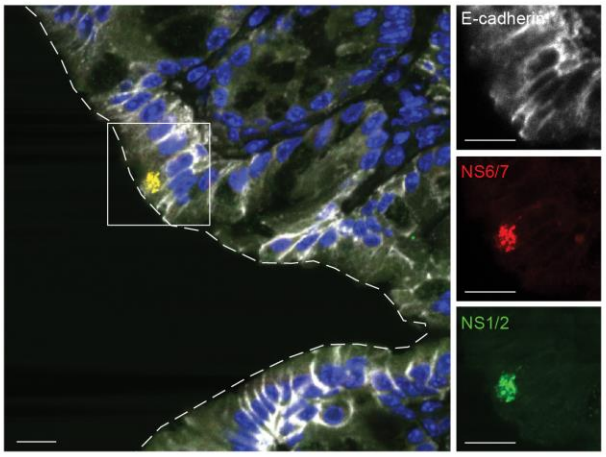


Figure S2. Comparison of viral protein antibody detection for CW3 and CR6, related to Figure 3.

BV2 cells were infected with CW3 or CR6 and flow cytometric analysis to detect NS1/2 and NS6/7 was performed at 16 hours post-infection.

Figure S3

A


E-cadherin
NS6/7
NS1/2

B

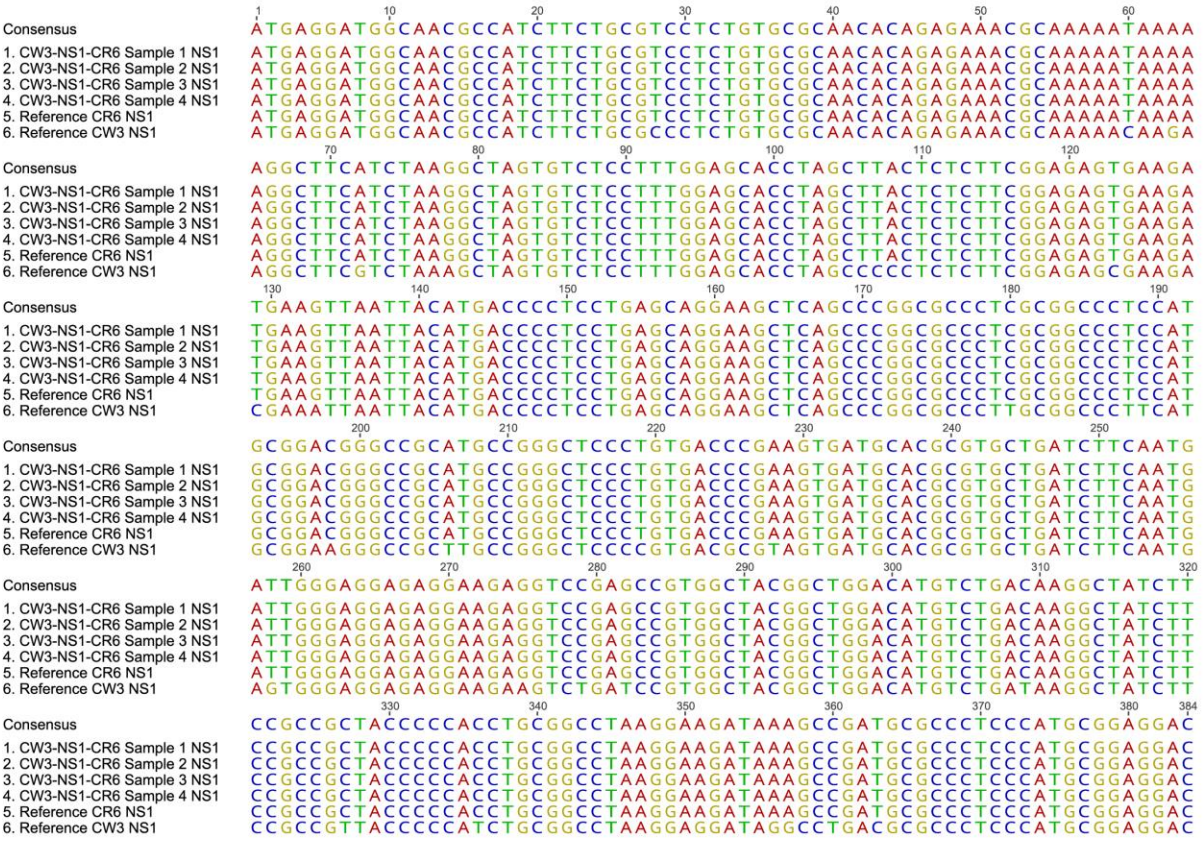

Consensus
1. CW3-NS1-CR6 Sample 1 NS1
2. CW3-NS1-CR6 Sample 2 NS1
3. CW3-NS1-CR6 Sample 3 NS1
4. CW3-NS1-CR6 Sample 4 NS1
5. Reference CR6 NS1
6. Reference CW3 NS1

Figure S3. Immunofluorescence and sequence analysis of CW3$^{NS1\text{-}CR6}$, related to Figure 4.

(A) Immunofluorescence microscopy for detection of NS1/2, NS6/7, DAPI and E-cadherin staining from 14 dpi of wild-type mice with CW3$^{NS1\text{-}CR6}$. **(B)** Sequencing results of NS1 gene from stool cDNA of CW3$^{NS1\text{-}CR6}$-infected wild-type mice at 7 dpi, compared to CR6 and CW3 reference sequences.

Figure S4

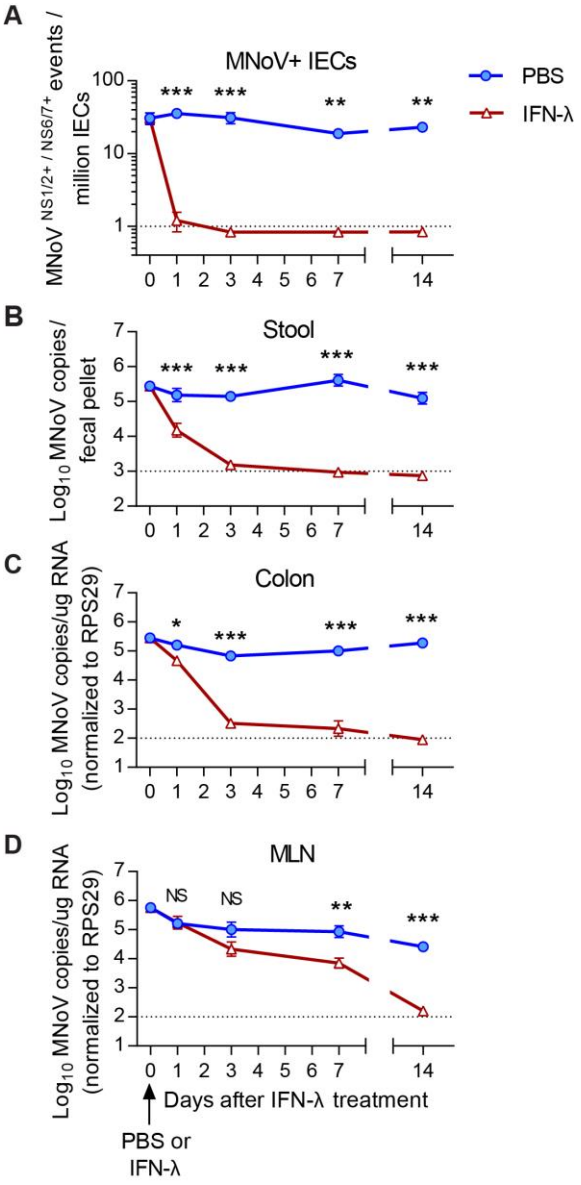

A
MNoV NS1/2+ / NS6/7+ events / million IECs
MNoV+ IECs
PBS
IFN-λ
B
Log10 MNoV copies / fecal pellet
Stool
C
Log10 MNoV copies/ug RNA (normalized to RPS29)
Colon
D
Log10 MNoV copies/ug RNA (normalized to RPS29)
MLN
Days after IFN-λ treatment
PBS or IFN-λ

Figure S4. Rapid clearance of MNoV-infected IECs by exogenous IFN-λ, related to Figure 5.

(A-D) Wild-type mice were infected with CR6. At 7 dpi, CR6-infected mice were treated with 3 µg of IFN-λ or PBS, and sacrificed at 0, 1, 3, 7 and 14 days after treatment. **(A)** IECs from proximal colons were analyzed by flow cytometry to quantify MNoV+ IECs. MNoV genomes from stool **(B)**, colon **(C)** and MLN **(D)** were quantified by qRT-PCR. *N* = 6 to 16 per group, combined from three independent experiments. Dashed lines represent limit of detection. Statistical significance was determined by two-way ANOVA followed by Sidak's multiple-comparisons test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, NS = not significant.

Figure S5

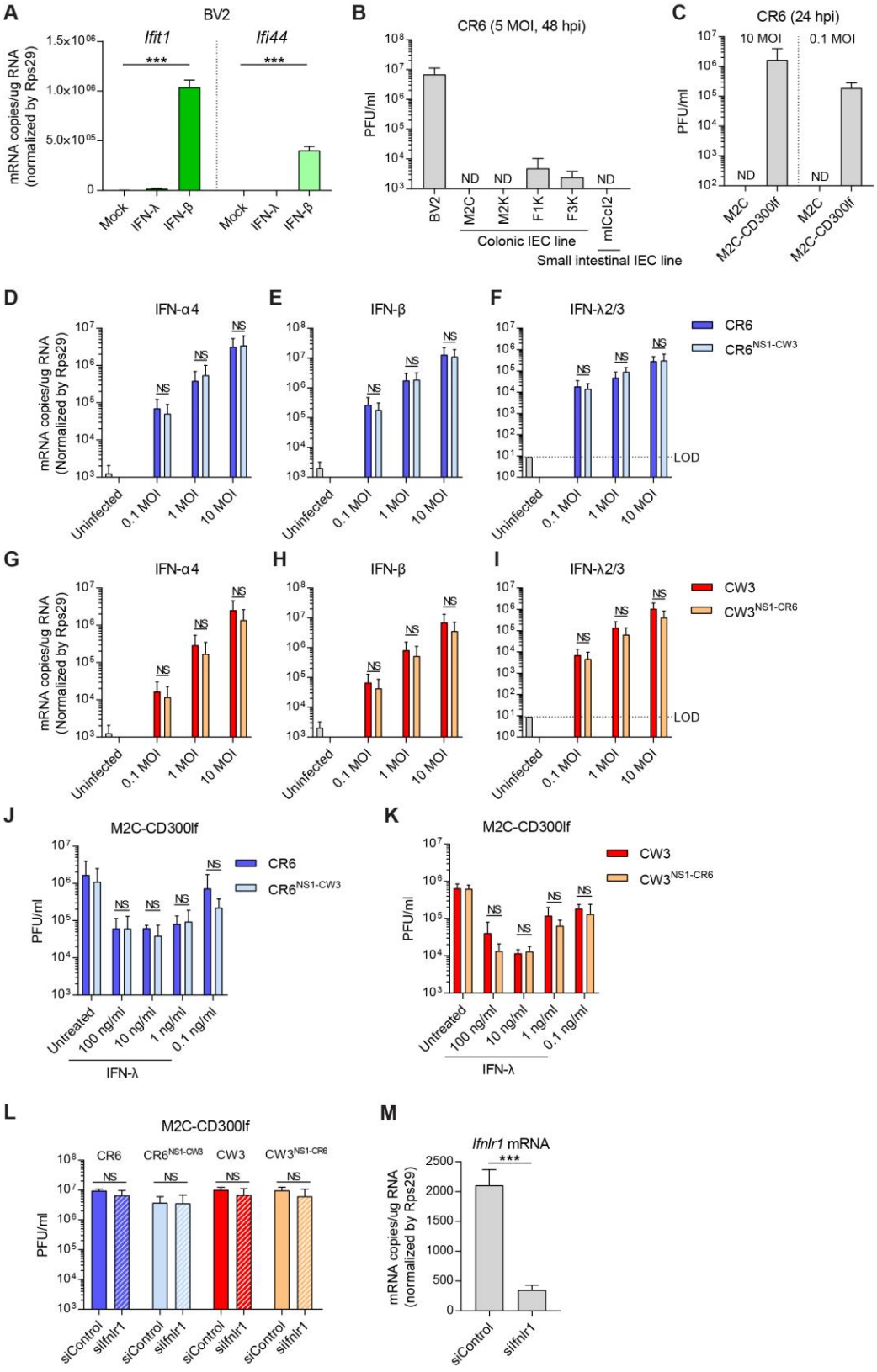

A
BV2
Ifit1
Ifi44
***
mRNA copies/ug RNA (normalized by Rps29)
Mock
IFN-λ
IFN-β
B
CR6 (5 MOI, 48 hpi)
PFU/ml
ND
BV2
M2C
M2K
F1K
F3K
mICcl2
Colonic IEC line
Small intestinal IEC line
C
CR6 (24 hpi)
10 MOI
0.1 MOI
M2C
M2C-CD300lf
D
IFN-α4
E
IFN-β
F
IFN-λ2/3
CR6
CR6NS1-CW3
NS
LOD
Uninfected
0.1 MOI
1 MOI
10 MOI
G
IFN-α4
H
IFN-β
I
IFN-λ2/3
CW3
CW3NS1-CR6
J
M2C-CD300lf
CR6
CR6NS1-CW3
Untreated
100 ng/ml
10 ng/ml
1 ng/ml
0.1 ng/ml
IFN-λ
K
M2C-CD300lf
CW3
CW3NS1-CR6
L
M2C-CD300lf
CR6
CR6NS1-CW3
CW3
CW3NS1-CR6
siControl
siIfnlr1
M
Ifnlr1 mRNA
***
siControl
siIfnlr1

Figure S5. *In vitro* assay development for exploration of IFN-λ interactions with MNoV, related to Figure 7.

(A) BV2 cells were treated with recombinant 100 ng/ml IFN-λ or 100 U/ml IFN-β for 24 hours and interferon-stimulated genes *Ifit1* and *Ifi44* were assessed by qPCR. **(B)** BV2 cells, colonic IEC lines M2C, M2K, F1K, and F3K, and small intestinal IEC line mICcl2 were inoculated with CR6 at MOI 5 and viral growth at 48 hpi was assessed by plaque assay. **(C)** M2C-CD300lf cells were developed by lentiviral transduction of M2C cells followed by puromycin selection. CR6 was inoculated at MOI 0.1 or 10 and viral growth at 24 hpi was assessed by plaque assay. **(D-I)** 12 hpi of M2C-CD300lf cells with **(D-F)** CR6 or $CR6^{NS1\text{-}CW3}$ or **(G-I)** CW3 or $CW3^{NS1\text{-}CR6}$ at MOI 0.1, 1, or 10, **(D, G)** IFN-α4, **(E, H)** IFN-β, or **(F, I)** IFN-λ2/3 levels were assessed by qPCR. **(J, K)** M2C-CD300lf cells were treated with 100, 10, 1, or 0.1 ng/mL recombinant IFN-λ for 24 hours then infected with **(J)** CR6 or $CR6^{NS1\text{-}CW3}$ or **(K)** CW3 or $CW3^{NS1\text{-}CR6}$ at MOI 10. **(L)** Growth of CR6, $CR6^{NS1\text{-}CW3}$, CW3 or $CW3^{NS1\text{-}CR6}$ was assessed in M2C-CD300lf cells administered either control or *Ifnlr1* siRNA. **(M)** Knockdown of *Ifnlr1* after siRNA treatment was confirmed by qPCR. Results shown represent mean ± SEM for combined data from three or four independent experiments, except **(L)** and **(M)**, which are representative of three independent experiments. Statistical significance was determined by Mann Whitney test. ***$P < 0.001$, NS = not significant. ND = not detected.

Figure S6

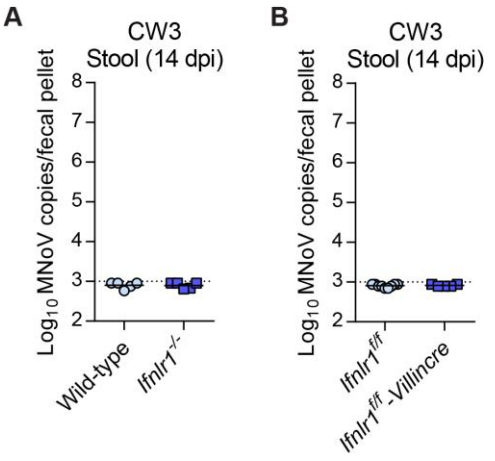


Figure S6. CW3 infection of *Ifnlr1*$^{f/f}$*-Villincre* mice, related to Figure 7.

(A and B) Wild-type and *Ifnlr1*$^{-/-}$ mice **(A)**, and *Ifnlr1*$^{f/f}$*-Villincre* mice and their *Ifnlr1*$^{f/f}$ littermate controls **(B)** were orally infected with CW3, and stool and intestinal tissues were analyzed for the presence of MNoV genome copies at 14 dpi. *N* = 5 to 9 mice per group, combined from two independent experiments. Statistical significance was determined by Mann Whitney test. NS = not significant.