Supplementary Material for

Enteric bacteria promote human and mouse norovirus infection of B cells

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Supplementary Materials

Materials and Methods

Cell lines. The M12 (*34*), WEHI-231 (*35*), and BJAB (*36*) B cell lines were cultured in RPMI with 10% fetal bovine serum; M12 and WEHI-231 media was supplemented with 50 µM β-mercaptoethanol. RAW264.7, CMT-93, 293T, and HT-29 cell lines were cultured in DMEM with 10% fetal bovine serum. Media for all experiments contained 100 U/mL penicillin and 100 µg/mL streptomycin. For co-culturing human IECs and B cells, HT-29 cells were grown to confluency on hanging wells. Polarization was confirmed prior to infections using fluorescein-conjugated dextran 3000 Da (FITC-dextran) exclusion. Specifically, 4 µg/mL FITC-dextran was loaded into the apical chamber. At 45 min. post-incubation, basal supernatants were collected and analyzed for fluorescent signal using a Synergy BioTek plate reader. Basal supernatants with fluorescence maxima less than 10% of the signal detected in a control well with no HT-29 cells were considered to be polarized. BJAB cells were then cultured in the basal chamber.

Viruses. Recombinant MNV-1 and MNV-3 that have been previously described (*14*) were used for all experiments. In brief, virus stocks were generated by transfecting 5 µg infectious clone per 10^6 293T cells, freeze-thawing cells after 1 d, and infecting RAW264.7 cells with 293T lysates at MOI 0.05. RAW264.7 lysates were generated when >90% of cells displayed CPE and were purified through a 25% sucrose cushion. We have previously described the generation of a neutral-red containing MNV-1 stock (*37*). In brief, RAW264.7 cells were infected with MNV-1 at MOI 0.05 in the presence of 10 µg/mL neutral red (Sigma) and a stock was generated as described above. All steps were carried out in the dark and labeling was confirmed by a minimum two-log reduction in stock titer upon light exposure. Titers of all MuNoV stocks were determined using a standard $TCID_{50}$ assay (*16*). For HuNoV infections, a stool sample that tested positive for the GII.4-Sydney strain was provided by the Centers for Disease Control (CDC); this stool sample tested negative for GI and GIV HuNoVs, sapovirus, astrovirus, rotavirus, and enteric adenovirus. We also performed pilot studies with two de-identified stool samples testing positive for the GII.4-Sydney strain that were obtained from Shands Hospital associated with the University of Florida. Stool samples were not processed prior to inoculation of cells unless otherwise indicated. Titers of HuNoV stocks were determined by quantitative RT-PCR (RT-qPCR) using established genogroup-specific primer sets (*38*) (see below for details).

MuNoV infections and growth curves. Cells were infected at MOI 5 unless otherwise indicated and incubated for 1 h on ice. Cells were then washed to remove unbound virus and incubated at 37°C. At the indicated times post-infection, culture supernatants were collected and virus titers determined using a standard $TCID_{50}$ assay (*16*).

Cell viability assays. Cells were incubated with propidium iodide (BD Pharmingen) at a final concentration of 2.5 µg/mL for 5 min. at room temperature. Flow cytometric analysis was performed on a FACSCalibur instrument (BD Biosciences) and the CellQuest Pro software was used to analyze data. Data are reported as the percentage of cells that did not incorporate dye (% viable cells).

Western blotting. Cells were lysed in 1x Laemmli sample buffer, and proteins were separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes. Previously described antibodies to the MNV-1 protease-polymerase (ProPol) nonstructural proteins (*39*), the VP1 major capsid protein (*40*), and the VP2 minor capsid protein (*14*) as well as a mouse anti-actin antibody (EMD Millipore) were used sequentially to probe the PVDF membranes for

MuNoV studies. The blots were stripped using Restore PLUS Western Blot Stripping Buffer (Thermo Scientific) in between antibody incubations. A previously described antibody to a genogroup II HuNoV NS6 peptide (*41*) was used to probe membranes for HuNoV studies.

MuNoV immunofluorescence assay. Cells were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) and deposited onto glass slides using a Cytopro Cytocentrifuge (Wescor Inc.). Cells were then permeabilized with 0.1% Triton X-100/4% PFA in PBS and stained with anti-ProPol antibody (*39*) at a 1:1000 dilution followed by an Alexa Fluor 594-conjugated secondary antibody (Invitrogen). Antibodies were diluted in 1% bovine serum albumin in PBS. Nuclei were stained with mounting media containing DAPI (Vector Laboratories, Inc.). Stained cells were imaged with a Zeiss Axioplan-2 Upright Fluorescent Microscope. To determine infectivity frequencies, three random 40X fields were counted per sample and the data averaged.

MuNoV persistence assays. M12 cells were infected at MOI 5 and plated at 3×10^5 cells/mL. At 2 dpi, cells were washed extensively to remove extracellular virus and re-plated at 3×10^5 cells/mL. This procedure was repeated every 2 d. The culture supernatant and cells were collected at every fifth passage for viral detection using the described $TCID_{50}$ assay and IFA, respectively.

Mice, virus infections, and *in vivo* tissue titer determination. Wild-type B6 mice (Jackson #00064) and *Ighm*^{tm1Cgn} mice (also known as µMT, Jackson #002288), which lack B cells, were bred and housed in animal facilities at the University of Florida under specific-pathogen-free conditions. 129S6/SvEv *Stat1*^{tm1Rds} (Taconic #2045; referred to as *Stat1*$^{-/-}$) mice were bred and housed in animal facilities at the University of Michigan under specific-pathogen-free conditions. Six- to ten-week old, sex-matched mice were used in all experiments. Virus infections were performed perorally (p.o.) with 25 µL inoculum, unless otherwise indicated. For neutral red-labeled MNV-1 infections, virus was administered through oral gavage with 100 µL inoculum and infections were carried out in the dark. For determining *in vivo* tissue titers, specified tissues were dissected from perfused mice, weighed, and homogenized in media by bead beating using 1.0 mm zirconia/silica beads (BioSpec Products, Inc.). Plaque assays of tissue samples have been described (*19*, *20*). For titering tissues from mice inoculated with neutral red-labeled MNV-1, tissues were homogenized in the dark. One portion of each homogenate was titered in the dark (total titer) while a second portion was exposed to light for 30 min. and titered in a parallel plaque assay (light-insensitive titer representing replicated virus).

Magnetic separation of cells. Groups of B6 mice ($n = 8$) were inoculated p.o. with mock inoculum or 10^7 $TCID_{50}$ units MNV-1 or MNV-3. At 1 dpi, Peyer's patches were dissected and pooled single cell suspensions were prepared by grinding through a 70-µm strainer. B cells were positively selected from bulk Peyer's patch cells using the EasySep Mouse CD19 Positive Selection Kit and EasySep magnet (STEMCELL Technologies Inc.). The purity of separated B cells was measured by flow cytometric analysis and determined to be >97% in each experiment.

MuNoV quantitative RT-PCR. RNA was extracted from purified Peyer's patch cells using the E.Z.N.A MicroElute Total RNA Kit (Omega Bio-Tek Inc.) and cDNA was synthesized using the ImProm-II Reverse Transcription System (Promega). Quantitative PCR was performed on each cDNA sample in triplicate. Primers for the MuNoV genome were sense 5'-CTTTGGAACAATGGATGCTG-3' and antisense 5'-CGCCATCACTCATCCTCAT-3'. Serial dilutions of a plasmid with the viral target sequence were used to generate a standard curve for the purpose of determining viral genome copy numbers in each sample.

MuNoV flow cytometric detection. STAT1$^{-/-}$ mice were inoculated with mock inoculum or 10^7 pfu MNV-1. At 1 dpi, Peyer's patches were dissected and single cell suspensions prepared. Cells were treated with 2.4G2 hybridoma supernatant to block Fc receptors (*42*), stained for the B cell surface markers CD19 (clone 6D5; Biolegend) or B220 (clone RA3-6B2; eBioscience), permeabilized, and stained with a rabbit polyclonal antibody raised against the MNV-1 nonstructural amino-terminal (N-term) protein (*43*) generously provided by Vernon Ward (Otago University). An Alexa Fluor 488-conjugated secondary goat anti-rabbit antibody was used to detect the primary anti-N-term antibody. Flow cytometric analysis was performed on a FACSCanto (Becton Dickinson) and data analyzed using FlowJo software (Treestar).

Antibiotic depletion of the murine intestinal microbiota. The intestinal microbiota was depleted by oral gavage of a standard antibiotic cocktail, as previously described (*30*, *44*). In brief, mice were orally gavaged daily for five days with 10 mg each of vancomycin (Fisher Scientific), ampicillin (Acros Organics), metronidazole (Acros Organics), and neomycin (Sigma). After the fifth day of gavage, antibiotics were added to the drinking water at a concentration of 1 g/L for ampicillin, metronidazole, and neomycin and 500 mg/L for vancomycin. Fresh fecal samples were collected from mice after the fifth day of oral gavage, homogenized, plated on brain-heart infusion (BHI) agar plates with 10% sheep blood, and incubated under anaerobic conditions at 37°C for 2 d followed by aerobic conditions at 37°C for 1 d to confirm efficient microbial depletion. Mice were maintained on the antibiotic- or PBS-containing water for the duration of the experiment, and infections were only performed after antibiotic-treated mice were verified to be free of detectable culturable bacteria.

HuNoV infections and genome copy number determination. The indicated numbers of viral genome copy numbers were applied directly to BJAB cells; or in the apical chamber of a hanging well containing polarized HT-29 cells grown on the membrane and BJAB cells in the lower chamber. Cultures were incubated at 37°C for the indicated times, at which point the contents of the entire well (direct infections) or the entire basal chamber contents (co-culture) were freeze/thawed two times and the cell lysate used for RNA extraction and RT-qPCR detection of viral genome copy number. Published primers NK2PF (5'-ATGTTYAGRTGGATGAGATTCTC) and NK2PR (5'-TCGACGCCATCTTCATTCAC) were used to amplify genogroup II viruses at a final concentration of 200nM each (*38*). Two µl of undiluted cDNA was added to each reaction and each sample was run in triplicate. Samples were amplified on a iCycler iQ (Bio-Rad) with SYBR Green Master Mix (Thermo Scientific) under the following cycling conditions: 95°C for 10 min. followed by 41 cycles of 95°C for 15s, 58°C for 30s, and 72°C for 30s. Serial dilutions of a plasmid with the viral target sequence provided by Park et al. (*38*) were used to generate a standard curve for the purpose of determining viral genome copy numbers in each sample. A representative standard curve **(Fig. S4A)** and RT-PCR products from one direct infection experiment run on an agarose gel **(Fig. S4B)** are presented. The 89-nucleotide products detected in this gel were purified and sequenced to confirm specificity of the reaction. In certain experiments, aliquots of HuNoV stocks were inactivated with 200,000 µJ cm^{-2} UV for 25 min. prior to inoculation; aliquots of HuNoV stocks were incubated with 10 µg/mL anti-VP1 antibody (Abcam, catalog # ab92976) for 1 h at 37°C prior to inoculation of BJAB cells to test for antibody-mediated virus neutralization; and stocks were filtered through a 0.2 µm membrane prior to inoculation.

HuNoV immunofluorescence assay. The protocol described for MuNoV IFAs was used with the following modifications. After permeabilization, cells were blocked with 5% normal goat

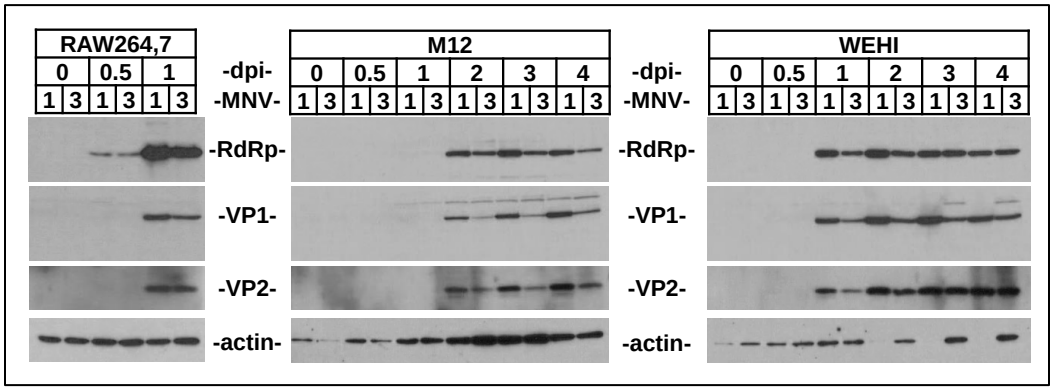
serum. A Tyramide Signal Amplification Kit (Invitrogen) was then used according to manufacturer's protocol. Briefly, cells were stained with anti-VP1 mouse monoclonal antibody (Abcam; catalog #ab80022) at a 1:25 dilution followed by anti-mouse IgG-HRP and Alexa Fluor 594-conjugated tyramide.

HuNoV passaging studies. A passage 0 (P0) virus stock was prepared by infecting BJAB cells with 5 x 10^5 viral genome equivalents of the GII.4-Sydney HuNoV-positive stool for 2 h, washing the cells extensively to remove unbound virus, subjecting 3 dpi cultures to two freeze-thaw cycles, pelleting and discarding cellular debris, and aliquoting supernatant. 5 x 10^5 genome equivalents of this P0 stock were then applied to naïve B cells and infections analyzed as described above. This passaging protocol designed to test whether infectious progeny virus is produced in BJAB cultures is depicted schematically in **Fig. S5** to differentiate it from experiments designed to assess whether BJAB cells become persistently infected.

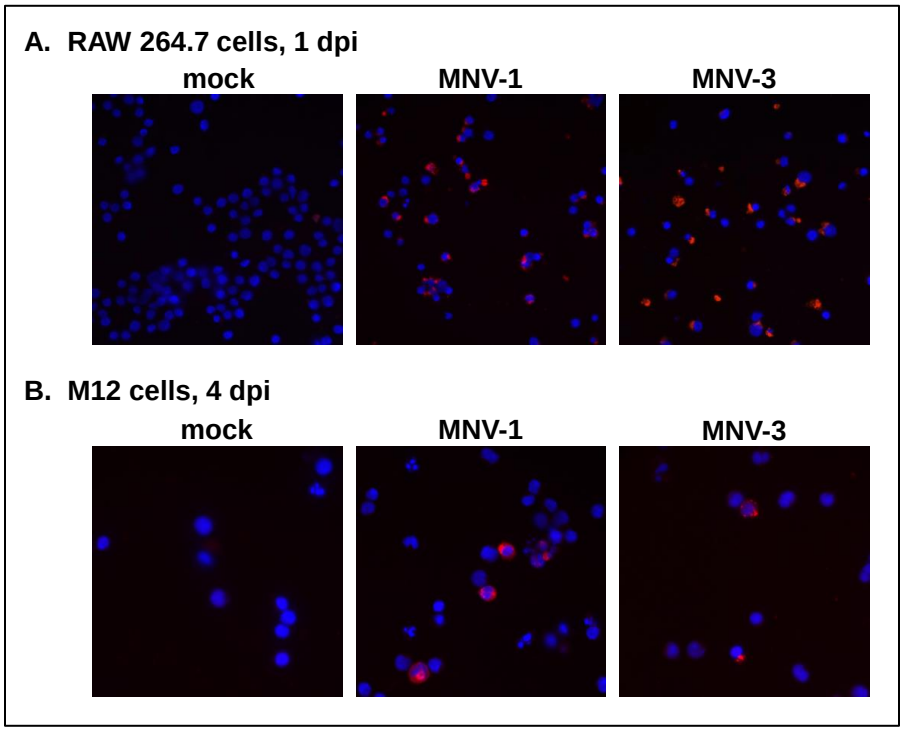
HuNoV persistence studies. BJAB cells were inoculated with 5 x 10^5 viral genome equivalents of the GII.4-Sydney HuNoV-positive stool. At 3 dpi, cells were washed three times to remove extracellular virus and re-plated at 5 x 10^5 cells/mL. This procedure was repeated every 3 d. At each passage, one well was harvested for viral genome copy number determination.

HuNoV:bacteria incubation studies. *Enterobacter cloacae* was purchased from ATCC (ATCC 13047). *Escherichia coli* DH5-alpha from our common laboratory stock was used. Bacteria were inoculated into nutrient broth (NB) and incubated overnight at 37°C. Overnight cultures were serially diluted in NB to achieve the indicated concentrations and incubated at 65°C for 40 min. for the purpose of heat inactivation. Inactivation was confirmed by plating on nutrient agar and inoculating into NB. For HuNoV studies, heat-inactivated bacteria were pre-incubated with virus for 1 h at 37°C prior to inoculation onto cells. Lysates of heat-killed *E. cloacae* and *E. coli* were probed for H-type HBGA expression by western blotting with anti-H IgM antibody (Santa Cruz). Synthetic blood type H HBGA (GlycoTech product number 08-019; expected size of 238 kDa) was run in parallel as a positive control. The H-type antigen expressed by *E. cloacae* is ca. 63 kDa.

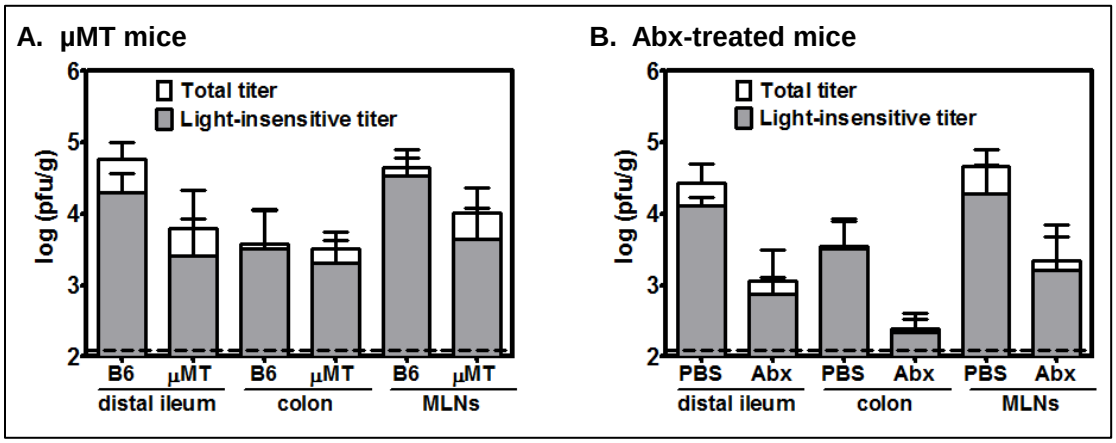
Figures S1-S7



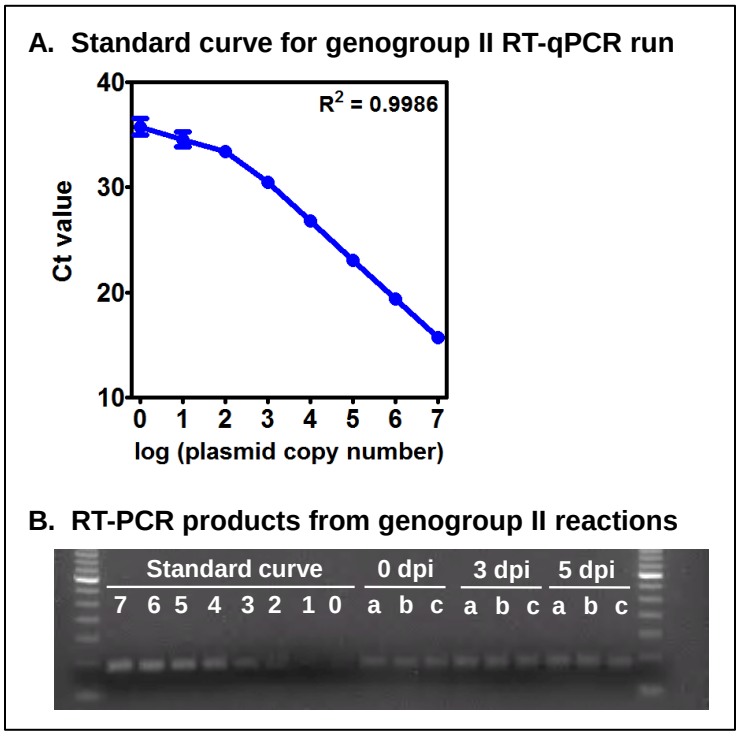
Suppl. Figure 1. MuNoV protein synthesis is delayed in B cells compared to macrophages. RAW264.7, M12, and WEHI-231 cells were infected with MNV-1 (1) or MNV-3 (3). At the indicated dpi, lysates were generated for the purpose of western blotting. Membranes were sequentially probed with anti-ProPol which recognizes the RNA-dependent RNA polymerase (RdRp), anti-VP1, anti-VP2 and anti-actin. The experiment was repeated three times in duplicate. One representative sample set is presented.



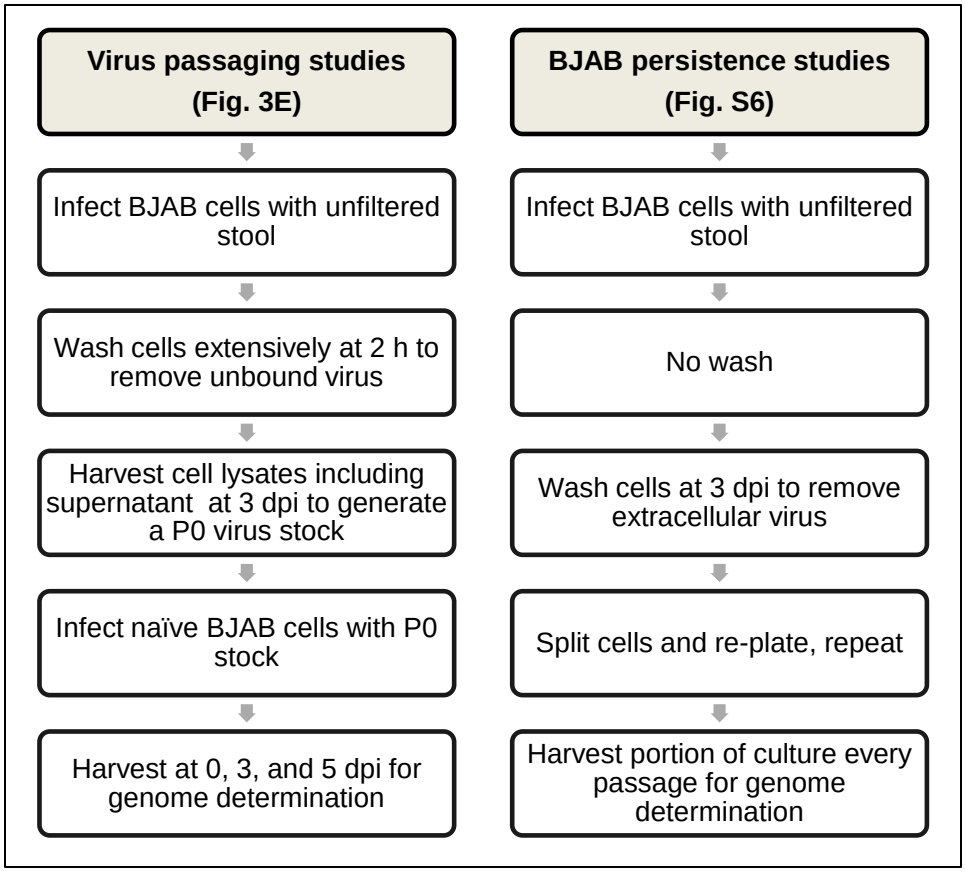
Suppl. Figure 2. The infectivity of MuNoVs is lower in mature B cells than in macrophages. **(A)** RAW264.7 cells were inoculated with mock inoculum or infected with MNV-1 or MNV-3 at MOI 5. At 1 dpi, cells were stained with anti-ProPol and DAPI and imaged on a fluorescent microscope. The ProPol antibody was visualized with an Alexa Fluor 594-conjugated anti-goat secondary antibody. **(B)** The same experiment was performed for M12 cells except that cells were infected with MNV-1 or MNV-3 at MOI 20 and cells were stained at 4 dpi. Multiple time points were examined for M12 and WEHI-231 cells and the data quantified, as presented in Fig. 1C.



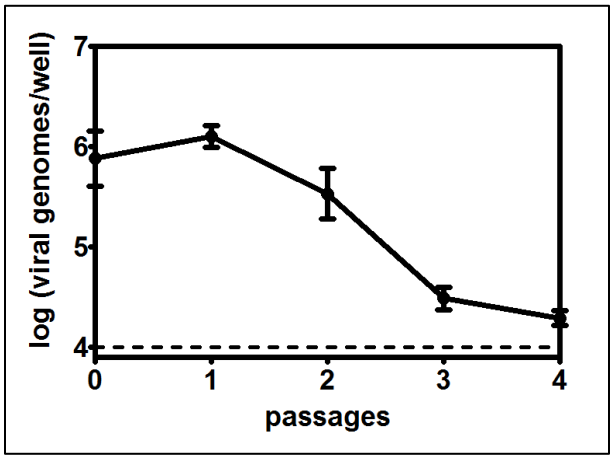
Suppl. Figure 3. Mice lacking B cells or depleted of their intestinal microbiota support reduced MuNoV replication. Groups (*n* = 2-3) of B6 and µMT mice **(A)**, and PBS- and Abx-treated B6 mice **(B)**, were infected with 6 x 10^5 pfu neutral red-labeled MNV-1 by oral gavage and harvested at 1 dpi. Virus titers were determined by performing parallel plaque assay on homogenates of the indicated tissues that were either unexposed (white bars; total titer) or light-exposed (gray bars; light-insensitive titer) prior to titering. All procedures were performed in the dark with only a red safelight. The data are presented as pfu per gram tissue on a logarithmic scale and data for all mice in each group were averaged (*N* = 2). Limits of detection are indicated by dashed lines. Error bars denote mean +/- SD.



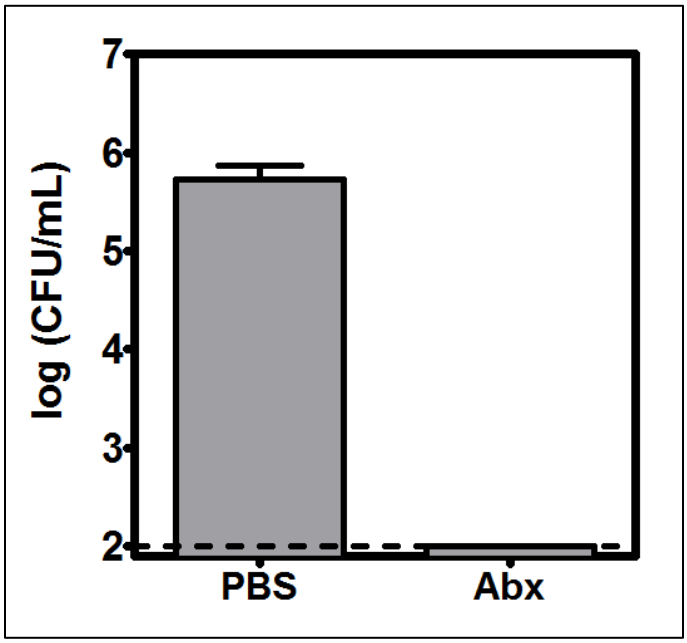
Suppl. Figure 4. Validation of the RT-qPCR assay to detect HuNoV genomic RNA. (A) The standard curve from a representative RT-qPCR run for detecting genogroup II HuNoV RNA is shown, with each plasmid dilution run in triplicate. Error bars denote mean +/- SD. **(B)** One reaction per plasmid concentration (e.g., 7 = 10^7 molecules of plasmid) was run on a 3% agarose gel (labeled standard curve). Triplicate samples from a direct infection at 0, 3, and 5 dpi are also shown. The expected product was 89 base pairs. The bands in this gel were sequenced and confirmed to be GII.4-Sydney HuNoV.



Suppl. Figure 5. Outline of GII.4-Sydney HuNoV passaging and persistence experimental design. The strategies used to test whether infectious virus is produced during HuNoV infection of BJAB cells via passaging (left flow chart), and to test whether BJAB cells become persistently HuNoV-infected (right flow chart), are presented in parallel to differentiate them from one another.



Suppl. Figure 6. GII.4-Sydney HuNoV does not persistently infect BJAB cells. 5 x 10^5 genome copy numbers of unfiltered GII.4-Sydney HuNoV-positive stool were inoculated onto BJAB cells. The cultures were passaged every 3 d. Viral RNA was measured from a fraction of cell lysates at each passage using virus-specific RT-qPCR.



Suppl. Figure 7. Validation of the antibiotic depletion protocol of the murine intestinal microbiota. Feces from PBS- and Abx-treated treated mice (n = 6 per group) were collected in 1 mL of PBS on the fifth day of oral gavage. Samples were homogenized and plated to BHI/BL Agar (BD#221843). Plates were incubated in anaerobic chambers at 37°C for 2 d, then incubated aerobically at 37°C for 1 d, and colonies counted (N = 3).

Reference and Notes

1. D. C. Payne, J. Vinjé, P. G. Szilagyi, K. M. Edwards, M. A. Staat, G. A. Weinberg, C. B. Hall, J. Chappell, D. I. Bernstein, A. T. Curns, M. Wikswo, S. H. Shirley, A. J. Hall, B. Lopman, U. D. Parashar, Norovirus and medically attended gastroenteritis in U.S. children. *N. Engl. J. Med.* **368**, 1121–1130 (2013). Medline doi:10.1056/NEJMsa1206589

2. H. L. Koo, N. Ajami, R. L. Atmar, H. L. DuPont, Noroviruses: The leading cause of gastroenteritis worldwide. *Discov. Med.* **10**, 61–70 (2010). Medline

3. M. M. Patel, M. A. Widdowson, R. I. Glass, K. Akazawa, J. Vinjé, U. D. Parashar, Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg. Infect. Dis.* **14**, 1224–1231 (2008). Medline doi:10.3201/eid1408.071114

4. R. I. Glass, U. D. Parashar, M. K. Estes, Norovirus gastroenteritis. *N. Engl. J. Med.* **361**, 1776–1785 (2009). Medline doi:10.1056/NEJMra0804575

5. J. J. Siebenga, H. Vennema, D. P. Zheng, J. Vinjé, B. E. Lee, X. L. Pang, E. C. Ho, W. Lim, A. Choudekar, S. Broor, T. Halperin, N. B. Rasool, J. Hewitt, G. E. Greening, M. Jin, Z. J. Duan, Y. Lucero, M. O'Ryan, M. Hoehne, E. Schreier, R. M. Ratcliff, P. A. White, N. Iritani, G. Reuter, M. Koopmans, Norovirus illness is a global problem: Emergence and spread of norovirus GII.4 variants, 2001-2007. *J. Infect. Dis.* **200**, 802–812 (2009). Medline doi:10.1086/605127

6. E. Duizer, K. J. Schwab, F. H. Neill, R. L. Atmar, M. P. Koopmans, M. K. Estes, Laboratory efforts to cultivate noroviruses. *J. Gen. Virol.* **85**, 79–87 (2004). Medline doi:10.1099/vir.0.19478-0

7. M. K. Lay, R. L. Atmar, S. Guix, U. Bharadwaj, H. He, F. H. Neill, K. J. Sastry, Q. Yao, M. K. Estes, Norwalk virus does not replicate in human macrophages or dendritic cells derived from the peripheral blood of susceptible humans. *Virology* **406**, 1–11 (2010). Medline doi:10.1016/j.virol.2010.07.001

8. E. Papafragkou, J. Hewitt, G. W. Park, G. Greening, J. Vinjé, Challenges of culturing human norovirus in three-dimensional organoid intestinal cell culture models. *PLOS ONE* **8**, e63485 (2013). Medline doi:10.1371/journal.pone.0063485

9. M. M. Herbst-Kralovetz, A. L. Radtke, M. K. Lay, B. E. Hjelm, A. N. Bolick, S. S. Sarker, R. L. Atmar, D. H. Kingsley, C. J. Arntzen, M. K. Estes, C. A. Nickerson, Lack of norovirus replication and histo-blood group antigen expression in 3-dimensional intestinal epithelial cells. *Emerg. Infect. Dis.* **19**, 431–438 (2013). Medline doi:10.3201/eid1903.121029

10. T. M. Straub, K. Höner zu Bentrup, P. Orosz-Coghlan, A. Dohnalkova, B. K. Mayer, R. A. Bartholomew, C. O. Valdez, C. J. Bruckner-Lea, C. P. Gerba, M. Abbaszadegan, C. A. Nickerson, In vitro cell culture infectivity assay for human noroviruses. *Emerg. Infect. Dis.* **13**, 396–403 (2007). Medline doi:10.3201/eid1303.060549

11. S. Takanashi, L. J. Saif, J. H. Hughes, T. Meulia, K. Jung, K. A. Scheuer, Q. Wang, Failure of propagation of human norovirus in intestinal epithelial cells with microvilli grown in three-dimensional cultures. *Arch. Virol.* **159**, 257–266 (2014). Medline doi:10.1007/s00705-013-1806-4

12. S. M. Mumphrey, H. Changotra, T. N. Moore, E. R. Heimann-Nichols, C. E. Wobus, M. J. Reilly, M. Moghadamfalahi, D. Shukla, S. M. Karst, Murine norovirus 1 infection is associated with histopathological changes in immunocompetent hosts, but clinical disease is prevented by STAT1-dependent interferon responses. *J. Virol.* **81**, 3251–3263 (2007). Medline doi:10.1128/JVI.02096-06

13. M. Basic, L. M. Keubler, M. Buettner, M. Achard, G. Breves, B. Schröder, A. Smoczek, A. Jörns, D. Wedekind, N. H. Zschemisch, C. Günther, D. Neumann, S. Lienenklaus, S. Weiss, M. W. Hornef, M. Mähler, A. Bleich, Norovirus triggered microbiota-driven mucosal inflammation in interleukin 10-deficient mice. *Inflamm. Bowel Dis.* **20**, 431–443 (2014). Medline doi:10.1097/01.MIB.0000441346.86827.ed

14. S. Zhu, D. Regev, M. Watanabe, D. Hickman, N. Moussatche, D. M. Jesus, S. M. Kahan, S. Napthine, I. Brierley, R. N. Hunter 3rd, D. Devabhaktuni, M. K. Jones, S. M. Karst, Identification of immune and viral correlates of norovirus protective immunity through comparative study of intra-cluster norovirus strains. *PLOS Pathog.* **9**, e1003592 (2013). Medline doi:10.1371/journal.ppat.1003592

15. K. Bok, G. I. Parra, T. Mitra, E. Abente, C. K. Shaver, D. Boon, R. Engle, C. Yu, A. Z. Kapikian, S. V. Sosnovtsev, R. H. Purcell, K. Y. Green, Chimpanzees as an animal model for human norovirus infection and vaccine development. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 325–330 (2011). Medline doi:10.1073/pnas.1014577107

16. L. B. Thackray, C. E. Wobus, K. A. Chachu, B. Liu, E. R. Alegre, K. S. Henderson, S. T. Kelley, H. W. Virgin 4th, Murine noroviruses comprising a single genogroup exhibit biological diversity despite limited sequence divergence. *J. Virol.* **81**, 10460–10473 (2007). Medline doi:10.1128/JVI.00783-07

17. A. Arias, D. Bailey, Y. Chaudhry, I. Goodfellow, Development of a reverse-genetics system for murine norovirus 3: Long-term persistence occurs in the caecum and colon. *J. Gen. Virol.* **93**, 1432–1441 (2012). Medline doi:10.1099/vir.0.042176-0

18. C. C. Hsu, L. K. Riley, H. M. Wills, R. S. Livingston, Persistent infection with and serologic cross-reactivity of three novel murine noroviruses. *Comp. Med.* **56**, 247–251 (2006). Medline

19. S. M. Kahan, G. Liu, M. K. Reinhard, C. C. Hsu, R. S. Livingston, S. M. Karst, Comparative murine norovirus studies reveal a lack of correlation between intestinal virus titers and enteric pathology. *Virology* **421**, 202–210 (2011). Medline doi:10.1016/j.virol.2011.09.030

20. C. E. Wobus, S. M. Karst, L. B. Thackray, K. O. Chang, S. V. Sosnovtsev, G. Belliot, A. Krug, J. M. Mackenzie, K. Y. Green, H. W. Virgin, Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages. *PLOS Biol.* **2**, e432 (2004). Medline doi:10.1371/journal.pbio.0020432

21. Materials and methods are available as supplementary materials on *Science* Online.

22. J. van Beek *et al*., Indications for worldwide increased norovirus activity associated with emergence of a new variant of genotype II.4, late 2012. *Eurosurveillance* (2013); available at www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20345.

23. J.-S. Eden, M. M. Tanaka, M. F. Boni, W. D. Rawlinson, P. A. White, Recombination within the pandemic norovirus GII.4 lineage. *J. Virol.* **87**, 6270–6282 (2013). Medline doi:10.1128/JVI.03464-12

24. S. Marionneau, N. Ruvoën, B. Le Moullac-Vaidye, M. Clement, A. Cailleau-Thomas, G. Ruiz-Palacois, P. Huang, X. Jiang, J. Le Pendu, Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. *Gastroenterology* **122**, 1967–1977 (2002). Medline doi:10.1053/gast.2002.33661

25. P. Huang, T. Farkas, S. Marionneau, W. Zhong, N. Ruvoën-Clouet, A. L. Morrow, M. Altaye, L. K. Pickering, D. S. Newburg, J. LePendu, X. Jiang, Noroviruses bind to human ABO, Lewis, and secretor histo-blood group antigens: Identification of 4 distinct strain-specific patterns. *J. Infect. Dis.* **188**, 19–31 (2003). Medline doi:10.1086/375742

26. G. F. Springer, P. Williamson, W. C. Brandes, Blood group activity of Gram-negative bacteria, *J. Exp. Med.* **113**, 1077–1093 (1961). Medline doi:10.1084/jem.113.6.1077

27. D. A. Rasko, G. Wang, M. A. Monteiro, M. M. Palcic, D. E. Taylor, Synthesis of mono- and di-fucosylated type I Lewis blood group antigens by *Helicobacter pylori*. *Eur. J. Biochem.* **267**, 6059–6066 (2000). Medline doi:10.1046/j.1432-1327.2000.01683.x

28. W. Yi, J. Shao, L. Zhu, M. Li, M. Singh, Y. Lu, S. Lin, H. Li, K. Ryu, J. Shen, H. Guo, Q. Yao, C. A. Bush, P. G. Wang, *Escherichia coli* O86 O-antigen biosynthetic gene cluster and stepwise enzymatic synthesis of human blood group B antigen tetrasaccharide. *J. Am. Chem. Soc.* **127**, 2040–2041 (2005). Medline doi:10.1021/ja045021y

29. T. Miura *et al*., Histo-blood group antigen-like substances of human enteric bacteria as specific adsorbents for human noroviruses, *J. Virol.* 10.1128/JVI.01060-13 (2013).

30. S. K. Kuss, G. T. Best, C. A. Etheredge, A. J. Pruijssers, J. M. Frierson, L. V. Hooper, T. S. Dermody, J. K. Pfeiffer, Intestinal microbiota promote enteric virus replication and systemic pathogenesis. *Science* **334**, 249–252 (2011). Medline doi:10.1126/science.1211057

31. M. Kane, L. K. Case, K. Kopaskie, A. Kozlova, C. MacDearmid, A. V. Chervonsky, T. V. Golovkina, Successful transmission of a retrovirus depends on the commensal microbiota. *Science* **334**, 245–249 (2011). Medline doi:10.1126/science.1210718

32. C. M. Robinson, P. R. Jesudhasan, J. K. Pfeiffer, Bacterial lipopolysaccharide binding enhances virion stability and promotes environmental fitness of an enteric virus. *Cell Host Microbe* **15**, 36–46 (2014). Medline doi:10.1016/j.chom.2013.12.004

33. J. May, B. Korba, A. Medvedev, P. Viswanathan, Enzyme kinetics of the human norovirus protease control virus polyprotein processing order. *Virology* **444**, 218–224 (2013). Medline doi:10.1016/j.virol.2013.06.013

34. K. J. Kim, C. Kanellopoulos-Langevin, R. M. Merwin, D. H. Sachs, R. Asofsky, Establishment and characterization of BALB/c lymphoma lines with B cell properties. *J. Immunol.* **122**, 549–554 (1979). Medline

35. L. L. Lanier, M. Lynes, G. Haughton, P. J. Wettstein, Novel type of murine B-cell lymphoma. *Nature* **271**, 554–555 (1978). Medline doi:10.1038/271554a0

36. G. Klein, T. Lindahl, M. Jondal, W. Leibold, J. Menézes, K. Nilsson, C. Sundström, Continuous lymphoid cell lines with characteristics of B cells (bone-marrow-derived), lacking the Epstein-Barr virus genome and derived from three human lymphomas. *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3283–3286 (1974). Medline doi:10.1073/pnas.71.8.3283

37. J. W. Perry, C. E. Wobus, Endocytosis of murine norovirus 1 into murine macrophages is dependent on dynamin II and cholesterol. *J. Virol.* **84**, 6163–6176 (2010). Medline doi:10.1128/JVI.00331-10

38. Y. Park, Y.-H. Cho, G. Ko, A duplex real-time RT-PCR assay for the simultaneous genogroup-specific detection of noroviruses in both clinical and environmental specimens. *Virus Genes* **43**, 192–200 (2011). 10.1007/s11262-011-0626-4 Medline doi:10.1007/s11262-011-0626-4

39. H. Changotra, Y. Jia, T. N. Moore, G. Liu, S. M. Kahan, S. V. Sosnovtsev, S. M. Karst, Type I and type II interferons inhibit the translation of murine norovirus proteins. *J. Virol.* **83**, 5683–5692 (2009). Medline doi:10.1128/JVI.00231-09

40. S. M. Karst, C. E. Wobus, M. Lay, J. Davidson, H. W. Virgin 4th, STAT1-dependent innate immunity to a Norwalk-like virus. *Science* **299**, 1575–1578 (2003). Medline doi:10.1126/science.1077905

41. S. Taube, A. O. Kolawole, M. Höhne, J. E. Wilkinson, S. A. Handley, J. W. Perry, L. B. Thackray, R. Akkina, C. E. Wobus, A mouse model for human norovirus. *MBio* **4**, e00450-13 (2013). Medline doi:10.1128/mBio.00450-13

42. J. C. Unkeless, Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. *J. Exp. Med.* **150**, 580–596 (1979). Medline doi:10.1084/jem.150.3.580

43. E. S. Baker, S. R. Luckner, K. L. Krause, P. R. Lambden, I. N. Clarke, V. K. Ward, Inherent structural disorder and dimerisation of murine norovirus NS1-2 protein. *PLOS One* **7**, e30534 (2012). Medline doi:10.1371/journal.pone.0030534

44. S. Rakoff-Nahoum, J. Paglino, F. Eslami-Varzaneh, S. Edberg, R. Medzhitov, Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* **118**, 229–241 (2004). Medline doi:10.1016/j.cell.2004.07.002