Supplemental Materials:

Material and Methods

Human intestinal enteroid (HIE) cultures. HIE cultures were derived from biopsies from adults during routine endoscopy or from surgical specimens obtained during bariatric surgery. The biopsies were taken from healthy regions of intestinal tissue as assessed by the physician who obtained the tissues. HIEs were grown as multilobular, 3-dimensional (3D) cultures in Matrigel and maintained as previously described (*14*). For all infections, monolayer cultures in 96 well plates or transwells (BD 3413) were prepared from the 3D cultures using a protocol modified from (*15*). Each well of a 96 well plate or transwell was coated with 2.5 µl of Matrigel diluted in 100 µl ice-cold PBS that was removed after 90 min incubation at 37°C. Multilobular, 3D HIEs (50 HIEs/well for a 96 well plate or 100 HIEs/transwell) were washed with 0.5 mM EDTA in ice cold PBS (without calcium chloride-magnesium chloride) and dissociated with 0.05% trypsin/0.5 mM EDTA for 4 min at 37°C. Trypsin was then inactivated by adding FBS diluted in complete medium without growth factors [CMGF(-)] to the cell suspension at a final concentration of 10%. Single cell suspensions were prepared from the 3D HIEs following dissociation of the cells by pipetting with a P1000 pipet and passing the cells through a 40 µm cell strainer. The cells were pelleted for 5 min at 400 x *g*, suspended in 100 µl of complete medium with growth factors [CMGF(+)] containing the ROCK inhibitor Y-27632 (10 µM, Sigma), and seeded in a single well of 96-well plate or a transwell. For transwells, 600 µl of CMGF(+) medium containing 10 µM Y-27632 was added to the lower compartment of the well. After 24 hours of growth, the culture media was changed to differentiation medium to differentiate the cells for 4 days. Differentiation medium consisted of the same components as those of CMGF(+) medium without the addition of Wnt3A, SB202190, and nicotinamide as well as 50% reductions in the concentrations of Noggin and R-spondin. The secretor status of each enteroid line was determined by genotyping and previously described (*14*) and expression of HBGAs was confirmed by staining with UEA-1 lectin. Enteroid cultures are available under a Materials Transfer Agreement.

Stool filtrates. To prepare 10% stool suspensions (see table S1), PBS was added to HuNoV-positive or -negative stools, which were then homogenized by vortexing and sonicated three times for 1 min. The sonicated suspensions were centrifuged at 1,500 x *g*, 10 min at 4°C. The supernatant was transferred to a new tube, centrifuged for a second time, and the resulting supernatant was passed serially through 5 µm, 1.2 µm, 0.8 µm, 0.45 µm and 0.22 µm filters. The filtered samples were aliquoted and frozen at -80°C until used. A 25% suspension of the GI.1 stool filtrate was prepared as previously described (*41*). To determine the effect of LPS on HuNoV infection, HIE monolayers were infected with $2.5x10^5$ genome equivalents of GII.4/2012-1 stool filtrate that was either treated or not treated with 100 µg/ml polymyxin B (Sigma) for 1 hour at room temperature (*12*). The endotoxin (LPS) level in each sample was determined by the Limulus amebocyte lysate (LAL) QCL1000 kit (Lonza) according to the manufacturer's instructions. To determine the effect of LPS on HIE monolayers, monolayers were cultured in the presence or absence of 10 µg/ml LPS for 4 days and then cytotoxicity was evaluated by trypan blue exclusion.

Cell lines. Vero, African green monkey kidney epithelial cells; Huh7, hepatocyte-derived cellular carcinoma cells; 293FT, human embryonic kidney cells that stably express the SV40 large T antigen; and Caco-2 BBe, human epithelial colorectal adenocarcinoma cells were grown in DMEM containing 10% FBS and penicillin and streptomycin. The Caco-2 BBe cell medium also contained 1% nonessential amino acids. Undifferentiated Caco-2 BBe cells were cultured for 3 days and differentiated Caco-2 BBe cells were cultured for 21 days.

Bile. Waste human bile, obtained from adults undergoing hepatobiliary surgery, was collected through the Texas Medical Center Digestive Diseases Center Study Design and Clinical Research Core. The study protocol was approved by the Baylor College of Medicine Institutional Review Board. Bile was collected from sows and piglets under a study protocol approved by the Animal Care and Use Committee of Baylor College of Medicine and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals [DHHS publication no. (NIH) 85–23, revised 1985, Office of Science and Health Reports,

DRR/NIH, Bethesda, MD 20205]. Bovine and porcine bile were commercially obtained (Sigma) and a 30% weight/volume solution was prepared in PBS. To determine if the active component of bile enhancing HuNoV infection was a protein, human bile was heated to 100^{0}C for 5 min and immediately chilled on ice. Alternatively, human bile was incubated with or without 167 µg/ml trypsin (Worthington Biochemical) for 24 hours at 37^{0}C, followed by the addition of soybean trypsin inhibitor [31.2 µg/ml final concentration (Merck Millipore)] to inactive the trypsin. Heat- or trypsin-treated bile was added to medium at the concentration of 5% during infection as described below.

Human norovirus (HuNoV) infection of HIE monolayers, transwells or other cell lines. All experiments were performed on monolayer cultures of HIEs in 96-well plates or transwells because of the ease of infecting monolayers rather than 3D HIEs that require additional centrifugation steps and manipulation for infecting. Some experiments (Fig. 1A-F, Fig. S2A and B, and Fig. S3A-C) were performed in the absence of bile. For other experiments monolayers were pretreated with bile for 48 hours prior to infection. For experiments in Fig. 2Fb-e, bile was added for various times before and after adsorption as indicated. For inoculation, the HIE cell monolayers or transwells were washed once with CMGF(-) medium and mock-inoculated or inoculated with HuNoV diluted in CMGF(-) medium containing bile for 1 hour at 37ºC. The inoculum was removed and monolayers were washed twice with CMGF(-) medium to remove unbound virus. Differentiation medium containing bile was then added and the cultures were incubated at 37°C for the indicated time points. The source of bile and concentration used is indicated in each figure. Due to the limited amount of human bile, this bile was primarily used for GII.3 infections, and bile from other sources (bovine, porcine, sow and piglet) was used for GII.4 infections. Because differences in cytopathic effects (CPE), assessed by trypan blue exclusion, or RT-qPCR signal were not observed between HuNoV-negative stool- or mock-inoculated cultures, most mock-inoculation experiments described were performed using CMGF(-) medium. CPE was not observed in HuNoV-negative stool or mock-inoculated cultures. Infected monolayers on 96 well plates were used for all RT-qPCR and growth studies while infected monolayers on transwells were used for high

resolution confocal microscopy studies. High multiplicities of infection (MOI) were used for confocal microscopy, flow cytometry and Western blotting to ensure that protein would be detected at early time points post infection. In contrast, lower MOIs were used for RT-qPCR experiments. The input virus used for each experiment is given in each figure legend.

GII.4/2009 HuNoV was passaged in jejunal HIE monolayers with 2% piglet bile as described above. After 5 days post-infection, cells and media were subjected to two freeze/thaw cycles and sonicated three times for 1 min. For each subsequent passage, 20 µl of virus stock from the previous passage was used to infect jejunal HIE monolayers as described above.

Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR). At the indicated time points, 300 µl of Ribozol (Amresco) was added to each mock- and HuNoV-inoculated HIE well containing cells and supernatant and RNA was extracted. RNA extracted at 1 hpi, used as a baseline to account for input virus that remained associated with cells, was from cultures where the inoculum was removed, the cells were washed twice to remove unbound virus and differentiation medium was added. For RT-qPCR, the primer pair p165/p166 and the probe p167 were used for GI (*22*) and the primer pair COG2R /QNIF2d and probe QNIFS were used for GII (*42, 43*) with qScript XLT One-Step RT-qPCR ToughMix reagent with ROX (Quanta Biosciences). Reactions were performed on an Applied Biosystems StepOnePlus thermocycler in a 15 µl reaction volume using the following cycling conditions: 50°C (15 min), 95°C (5 min), followed by 40 cycles of 95°C (15 sec) and 60°C (35 sec). A standard curve based on a recombinant HuNoV RNA transcript was used to quantitate viral genome equivalents in RNA samples (*44, 45*).

Electron Microscopy (EM). To visualize HuNoV particles, supernatant from infected jHIE monolayers (20 hpi) was clarified by centrifugation at 8,000 x *g* for 10 min. The particles were either directly visualized by EM or were pelleted through a 30% (w/v) sucrose cushion at 135,000 x *g* for 1.5 hours. After staining with 1% ammonium molybdate (pH 5.5), particles were visualized using a JEOL 1230

transmission electron microscope (JEOL USA) with a Gatan Ultrascan 1000 CCD and Digital Micrograph Software (Gatan).

Immunofluorescence. HIE monolayers on 96 well plates or transwells were fixed with methanol for 20 min at -20°C, 4% paraformaldehyde (PFA) at room temperature for 20 min or fixed in 10% formalin and embedded in paraffin. Transwell cultures were used to obtain high resolution confocal images to determine what cell types are infected by HuNoVs. Cells were blocked for 2 hours in 0.01 M PBS containing 5% BSA. Primary antibodies were incubated overnight at 4°C. Expression of the capsid protein (VP1) was detected using antisera to guinea pig anti-GII.4/2012 virus-like particles (VLPs) [(1:250) made by Covance, Princeton, NJ as previously described by (*46*)] or anti-GII.3 (TCH04-577) VLPs (1:250), or to non-structural proteins [rabbit anti-U201 polymerase (Pol, 1:100), anti-NTPase (1:100), or anti-VPg (1:100)] (*47*). Double-stranded RNA (dsRNA) was detected using the J2 monoclonal antibody (1:100, Scicons). Expression of differentiation markers was detected using antisera raised against sucrase isomaltase (1:100, Santa Cruz), mucin 2 (1:500, Santa Cruz), chromogranin A (1:100, Novus Biologicals), or villin (ready to use, Cell Marque). HBGAs and actin were stained with UEA-1 lectin (1:500, Sigma) and phalloidin (1:1000, Sigma), respectively, that were directly conjugated with Alexa 549. After washing with 0.01 M PBS, the cells were incubated for 2 hours at room temperature with the corresponding secondary antibody conjugated to Alexa Fluor 594 or 488 (1:1,500, Invitrogen). Nuclei were stained with 300 nM DAPI for 15 min at room temperature. After the final wash step, the monolayers in the 96 well plate were imaged in PBS while the monolayers on transwells were mounted in prolong gold (Thermo Fisher). The immunofluorescence was detected with an IX-70 inverted microscope (for HIE monolayers in 96-well plates) or a Nikon A1-Rs inverted laser-scanning microscope (for HIE monolayers on transwells or optical bottom 96 well plates).

Hematoxylin and eosin (H&E) staining was performed on deparaffinized sections. Sections were incubated in hematoxylin for 1 min, rinsed with tap water, immersed in 95% ethanol, counterstained with eosin for 30 seconds and then dehydrated and mounted in permount.

Western blot analysis. Proteins in mock- and HuNoV-inoculated HIEs were detected by Western blot analysis. Briefly, supernatants (100 µl per well) of mock- and HuNoV-infected monolayers in 96-well plates were collected at indicated time points and monolayers were lysed in 50 µl/well NP-40 lysis buffer [50 mM Tris-HCl pH7.5, 150 mM NaCl, 1% NP-40, 10% glycerol, 1 mM DTT, 1x protease inhibitor cocktail (CalBiochem)]. Each cell lysate was clarified by centrifugation and 10-15 µl lysate or supernatant was subjected to SDS-PAGE and Western blotting. Proteins were separated on 4-15% SDS-PAGE and then transferred to nitrocellulose membranes. VP1 expression was detected with guinea pig anti- GII.4/2012 VLP serum (1:2,000). VPg-containing polyprotein processing intermediates were detected using a rabbit polyclonal antiserum to HuNoV GI.1 strain Norwalk virus (NV) VPg (1:1000), which broadly detects VPg of HuNoV GII strains, as described previously (*47*). Actin was detected as a loading control with a mouse monoclonal anti-actin antibody (1:5,000, Abcam). Species-specific HRP-conjugated secondary antibodies (1:20,000, Sigma) were used to detect the primary antibodies. A lysate of HEK293FT cells transfected with $pHuNoV_{Saga1F}$, an expression plasmid of HuNoV GII.4 strain Saga1 full-length genome at 24 hours post transfection was used as a positive control for detection of VPg-containing polyprotein processing intermediates and the mature VPg, as described previously (*47*).

Flow cytometry. Human intestinal enteroid cells were harvested from monolayers on 96-well plates after mock-inoculation or infection with HuNoV (GII.4/2012-1, $9x10^7$ genome equivalents) by incubation with 100 µl of 0.05% trypsin-EDTA (Gibco) for 5 min at 37°C. Trypsin was inhibited by addition of 1 ml DMEM with 8% FBS. Cells were pelleted by centrifugation at 400 x g for 5 min and suspended in 500 µl of Cytofix (BD Biosciences) for 10 min at 4^0C. All washes were carried out twice with Stain Buffer (BD Biosciences) containing 0.5% BSA followed by pelleting each time. Cells were washed, suspended in 900 µl of -20^0C methanol, and incubated at 4^0C for 30 min. Cells were washed and suspended in Stain Buffer containing 5% BSA and guinea pig anti-GII.4/2012 VLP or preimmune antibody (1:20,000). The cells were incubated with antibody for 30 min at room temperature, pelleted and washed. The cell pellet was suspended in Stain Buffer containing 5% BSA and goat anti-guinea pig Alexa 488 antibody (1:500)

and incubated for 30 min at room temperature in the dark. The cells were pelleted and after the final wash, cells were suspended in 500 µl of Stain Buffer containing 0.5% BSA and analyzed using a LSR II flow cytometer (BD Biosciences). Control mock-inoculated cells were incubated with goat anti-guinea pig Alexa 488 antibody or with guinea pig anti-GII.4/2012 VLP or preimmune antibody, and then goat anti-guinea pig Alexa 488 antibody to assess non-specific binding.

Inactivation of HuNoV.

a) Virus neutralization assays. Serial dilutions of two serum samples were carried out in CMGF(-) medium. GII.4/2012-1 or GII.3 virus ($2.5x10^5$ genome equivalents) were mixed with an equal volume of media or dilutions of each serum sample and incubated at 37ºC for 1 hour. CMGF(-) containing 1% sow bile for GII.4 or 5% human bile for GII.3 was added to each sample and inoculated onto bile-treated HIEs for 1 hour as described above. To determine whether virus-induced CPE was neutralized by human serum, $5x10^7$ genome equivalents of GII.4/2012-1 was mixed with an equal volume of media or dilutions of each serum sample and incubated at for 1 hour at 37ºC. CMGF(-) containing 1% sow bile was added to each sample and inoculated onto HIEs for 1 hour as described above. CPE was assessed by trypan blue exclusion as described above.

b) Heat inactivation of human noroviruses. GII.4 and GII.3 HuNoV stool filtrates ($9x10^5$ and $4.3x10^5$ genome equivalents, respectively) were heat-treated in a digital dry heat block at 60°C for the indicated time periods or incubated at 25°C for 0 and 60 min. The treated virus was placed on ice after completion of incubation to stop the heat treatment. Each sample was inoculated onto a HIE monolayer treated with 1% sow bile for GII.4 or 5% human bile for GII.3 as described above.

c) Inactivation of HuNoVs by γ-irradiation. GII.4 and GII.3 HuNoV stool filtrates were exposed to 8 kilo Grays (kGy) of γ-irradiation at room temperature using a Gammacell-1000 Irradiator (Atomic Energy of Canada Ltd.) with a Cesium-137 source, at a dose rate of 0.5 kGy/hour. Unexposed HuNoVs were

incubated at room temperature for the same length of time as the exposed samples. Each sample was inoculated onto a HIE monolayer treated with 1% sow bile for GII.4 or 5% human bile for GII.3 as described above.

Statistical analysis. Each experiment was performed two or more times, with three technical replicates of each culture, condition and time point in each experiment. Data from 1 representative experiment is presented. All statistical analyses were performed on GraphPad Prism version 6.0 for Windows (GraphPad Software, La Jolla California USA). Samples with RNA levels below the limit of detection of the RT-qPCR assay were assigned a value that is one-half the limit of detection of the assay. Comparison between groups was performed using the Students t-test, with statistical significance determined using the Holm-Sidak method. P-values < 0.05 were considered statistically significant. A sigmoidal, 4-parameter logistic curve was used to calculate 50% neutralizing antibody titers.

Supplemental figures

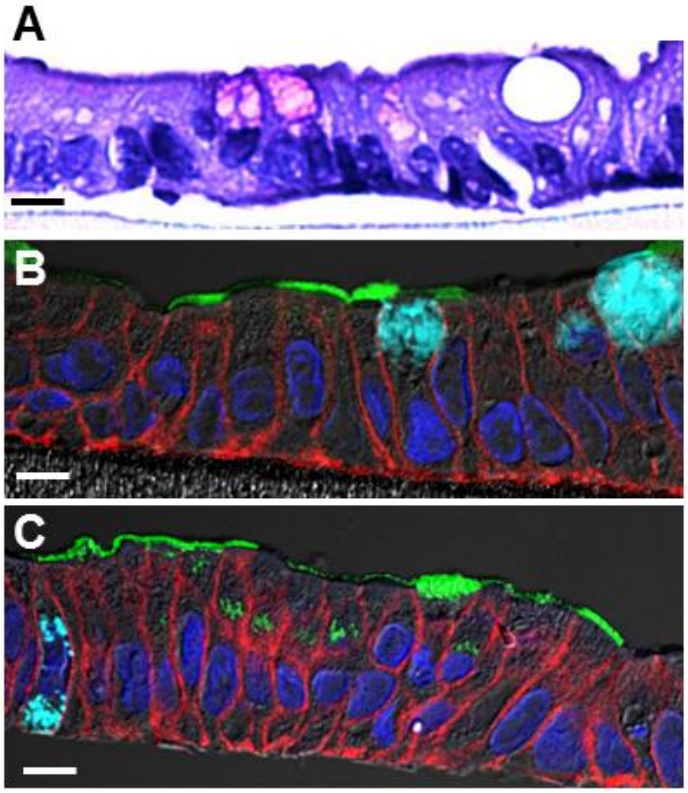

Fig. S1. Monolayer cultures of human intestinal enteroids contain enterocytes, goblet and enteroendocrine cells. Jejunal human intestinal enteroid monolayers grown on Matrigel-coated transwells were fixed with 10% formalin and embedded in paraffin. Thin sections were stained with (A) hematoxylin and eosin (H&E), and for expression of (B) the differentiation markers sucrase isomaltase (green) and Muc2 (cyan) that label enterocytes and goblet cells, respectively, or (C) chromogranin A (cyan) that labels enteroendocrine cells. (C) Expression of histoblood group antigens (HBGAs) was detected by UEA-1 lectin (green). In (B and C), adherens junctions are stained by E-cadherin (red) and nuclei by DAPI. Scale bar - 10 μm.

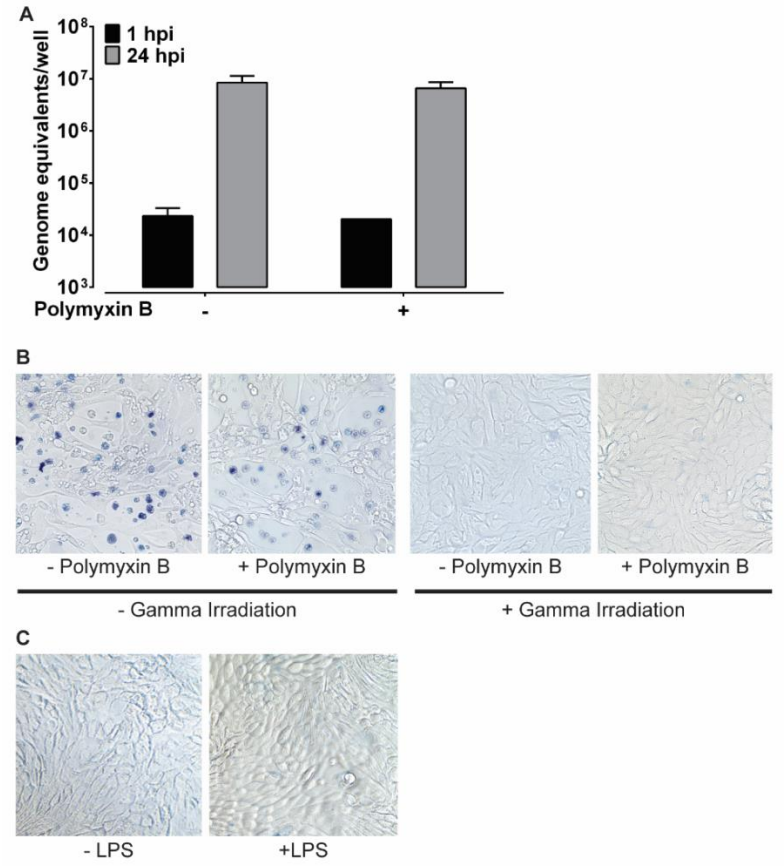

Fig. S2. Lipopolysaccharide does not enhance GII.4 replication and virus infection induces CPE. (A) GII.4/2012-1 stool filtrates were not treated (-) or treated (+) with 100 μg/ml polymyxin B for 24 hours. Then, $9x10^5$ genome equivalents of the filtrates were inoculated onto each jejunal HIE monolayer for 1 hour. The monolayers were washed twice with CMGF(-) medium and cultured in differentiation medium. RNA was extracted from the cells and media and viral genome equivalents quantified by RT-qPCR. Data represent the mean of three wells for each treatment and time point. Error bars denote standard deviation. (B) Gamma irradiated or non-irradiated GII.4/2012-1 stool filtrates were either not treated (-) or treated (+) with polymyxin B for 24 hours. Jejunal HIE monolayers were inoculated with $9x10^7$ genome equivalents for 1 hour as described above. (C) Jejunal HIE monolayers were either not treated (-) or treated (+) with LPS. (B and C) To assess cytotoxicity, trypan blue was added to the cultures at 3 dpi and imaged by bright field on an Olympus IX70 microscope with 20X magnification.

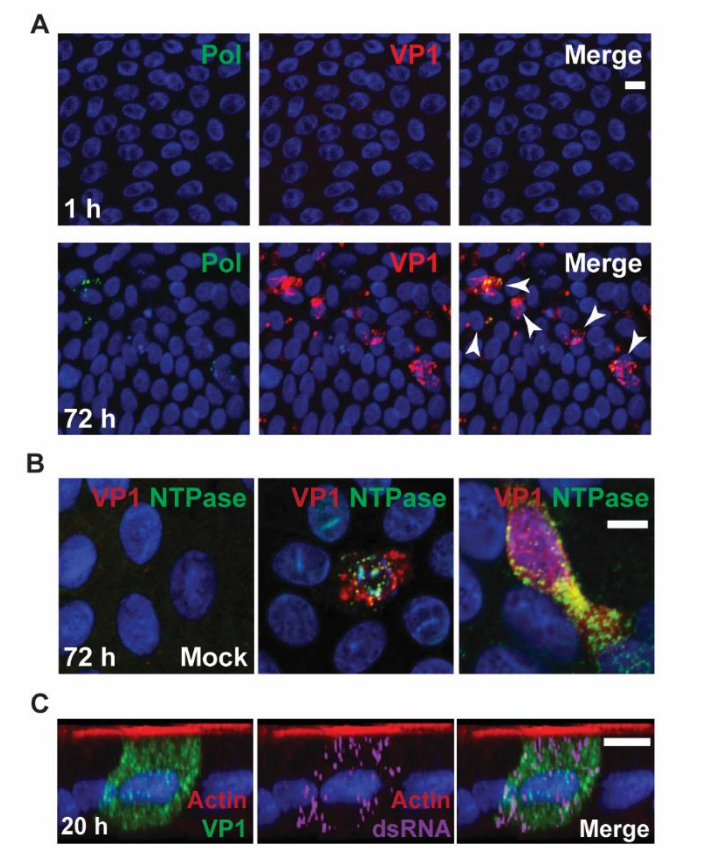

Fig. S3. Detection of GII.4 replication in human intestinal enteroids by immunofluorescence. Monolayers of jejunal human intestinal enteroids were mock-inoculated (B, left panel) or inoculated with $5.5x10^5$ genome equivalents of GII.4/2006b-3 (all panels in A, and middle and right panels in B) or $9x10^7$ genome equivalents of GII.4/2012-1 (C) for 1 hour at 37°C. The inoculated monolayers were washed twice with CMGF(-) media and cultured for 3 days (A and B) or 20 hours (C) in differentiation medium. (A-C) Expression of HuNoV proteins was detected in 4% PFA-fixed enteroid monolayers. VP1 was detected using guinea pig anti-GII.4/2012 VLP serum (red, A and B; green, C). Non-structural proteins were detected using (A) rabbit anti-GII.3 HuNoV polymerase (Pol, green) and (B) rabbit anti-GII.3 HuNoV NTPase (green), and double-stranded RNA (dsRNA) was detected (C) using J2 monoclonal anti-dsRNA (purple). DAPI detects nuclei (blue). (A) Arrowheads indicate cells expressing both VP1 and polymerase. (B) The middle and right panels represent two different cells expressing both VP1 and NTPase. Yellow represents colocalization of these two HuNoV proteins. (A-C) Scale bar - 10 μm.

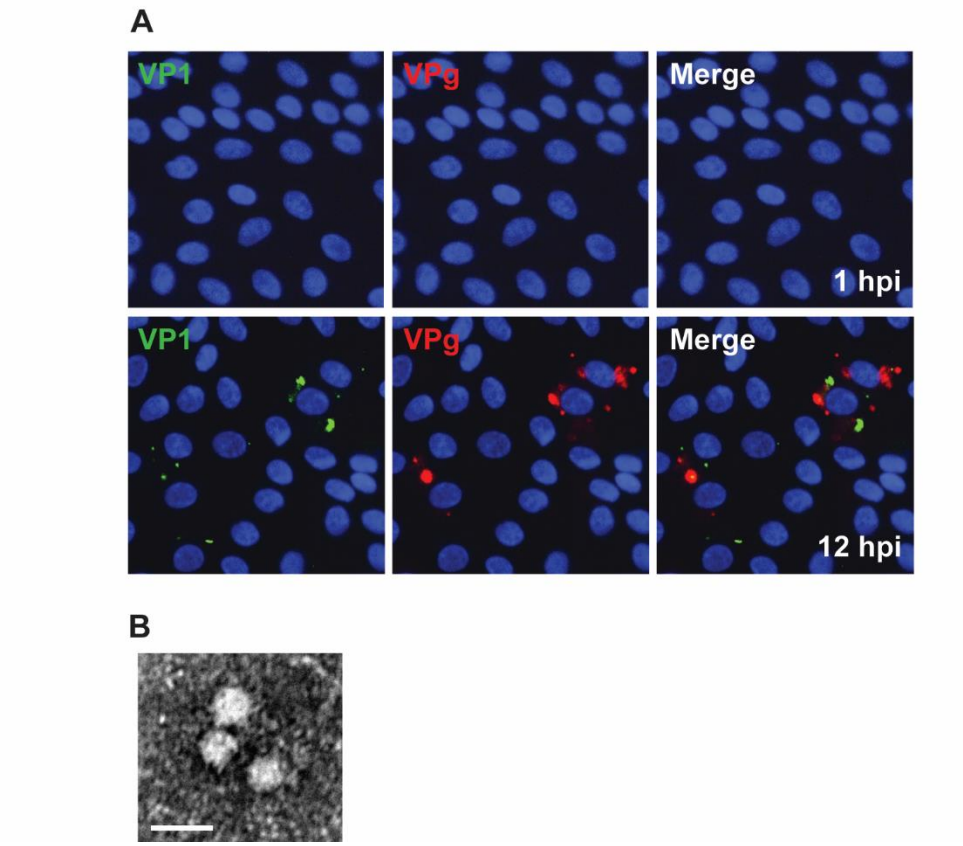

Fig. S4. Detection of HuNoV antigen and virus particles in passage 4 infections. Jejunal HIEs were infected with GII.4/2012-1 passage 3 virus. (A) Cells were fixed at 1 and 12 hpi and stained with antibody against GII.4/2012 VLPs (green) and VPg (red). (B) Media collected at 72 hpi, was clarified by centrifugation for 10 min at 8,000 x *g*, applied to grids, stained and imaged by EM as described in Fig. 1. Scale bar = 50 nm.

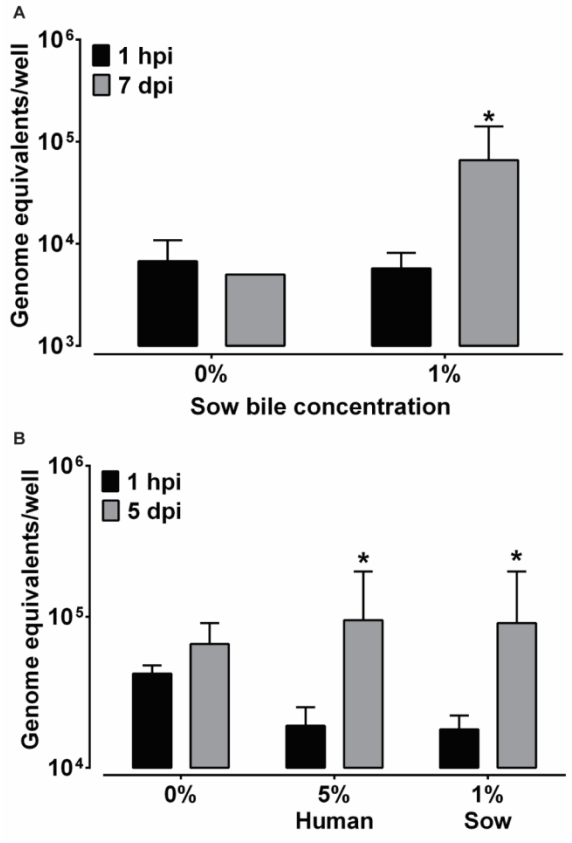

Fig. S5. GI.1 and GII.17 HuNoVs require bile for replication in human intestinal enteroids. Jejunal human intestinal enteroid monolayers were pretreated with the indicated concentrations of bile for 2 days, inoculated with (A) GI.1 or (B) GII.17 stool filtrates ($2.9x10^6$ or $9.7x10^6$ genome equivalents, respectively) in the presence of the indicated concentrations of bile for 1 hour at 37°C. The monolayers were washed three times with CMGF(-) medium and cultured for the indicated days in differentiation medium in the absence (0%) or presence of the indicated concentrations of bile. At 1 hour and the indicated time post-inoculation, the cells and medium were harvested, RNA extracted and viral genome equivalents quantified by RT-qPCR. Data represent the mean of three wells for each treatment and time point. Error bars denote standard deviation. *, $P<0.05$ comparing genome equivalents from 1 hpi to the indicated time points.

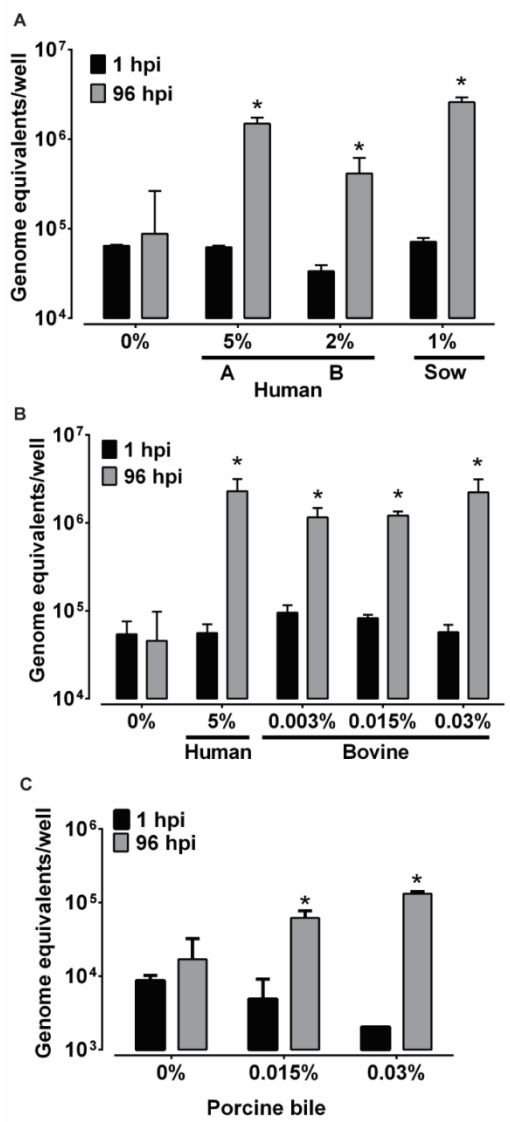

Fig. S6. Bile from different sources promotes GII.3 HuNoV replication. (A-C) Jejunal human intestinal enteroid monolayers were pretreated with the indicated concentrations of bile for 2 days, inoculated with $4.3x10^5$ genome equivalents of GII.3 stool filtrates in the presence or absence of bile for 1 hour at 37°C. The monolayers were washed twice with CMGF(-) medium and cultured for 96 hours in differentiation medium in the absence (0%) or presence of the indicated concentrations of bile. At 1 and 96 hpi, the cells and medium were harvested, RNA extracted and viral genome equivalents quantified by RT-qPCR. Data represent the mean of three wells for each treatment and time point. Error bars denote standard deviation. *, $P<0.05$ comparing genome equivalents from 1 to 96 hpi.

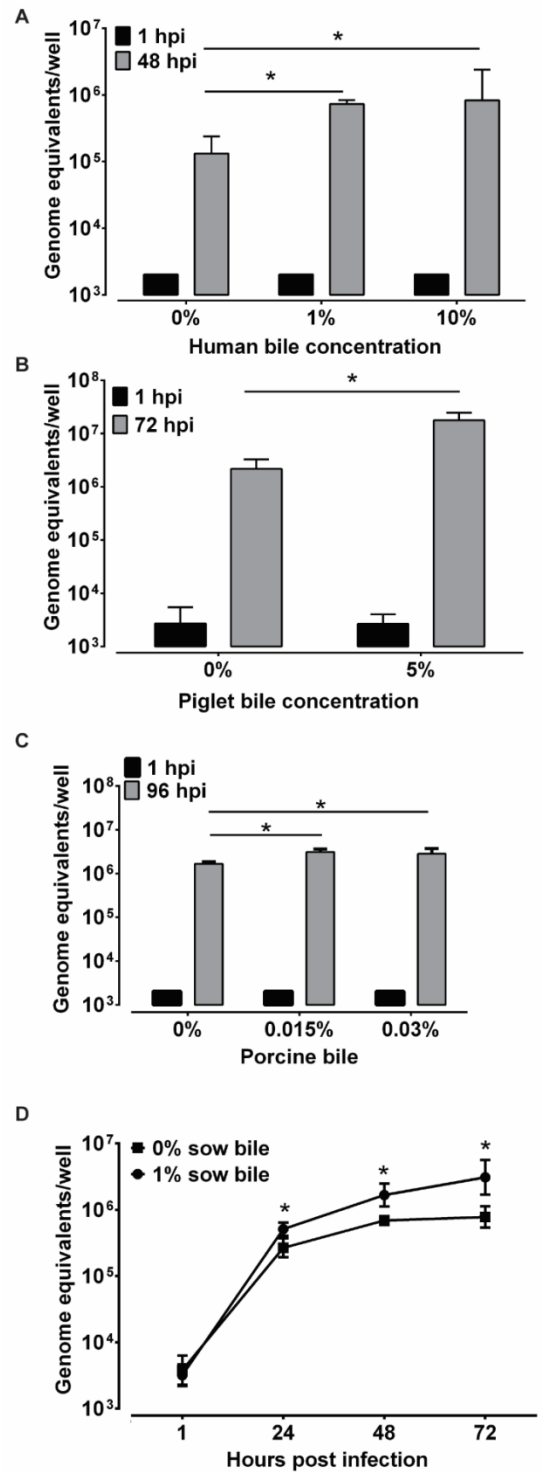

Fig. S7. **Bile is not required but enhances GII.4 HuNoV replication.** (A-D) Jejunal human intestinal enteroid monolayers were treated with the indicated concentrations of bile for 2 days during differentiation. The monolayers were inoculated with $5.5x10^5$ genome equivalents of (A) GII.4/2006b-3

or, (B) GII.4/ 2009, or (C and D) GII.4/2012-1 in the absence or presence of the indicated bile for 1 hour at 37^{o}C. The monolayers were washed twice with CMGF(-) medium and cultured in differentiation medium in the absence (0%) or presence of the indicated concentrations of bile. RNA was extracted from the cells and media and viral genome equivalents quantified by RT-qPCR. Data represent the mean of three wells for each treatment and time point. Error bars denote standard deviation. *, $P<0.05$ comparing genome equivalents between no bile and bile treatment for A-C, and between 0 and 1% sow bile for each time point for D.

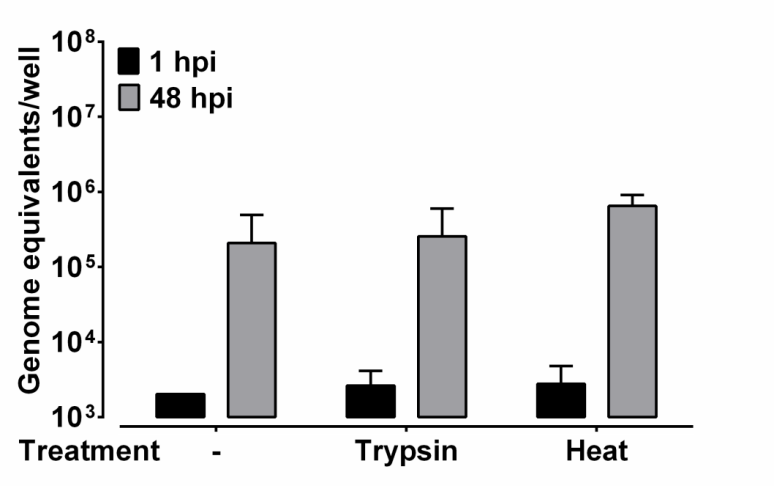

Fig. S8. The factor in bile required for GII.3 infection is not proteinaceous. Human bile was not treated (-) or treated with trypsin for 24 hours at 37^0C, followed by the addition of soybean trypsin inhibitor to inactivate the trypsin or bile was heated at 100ºC for 5 minutes. These bile preparations (5% final concentration) were used to pretreat HIE monolayers for 2 days during differentiation followed by infection with $4.3x10^5$ genome equivalents of GII.3 for 1 hour at 37ºC as previously described. RNA was extracted from the cells and media, and viral genome equivalents quantified by RT-qPCR. Data represent the mean of three wells for each treatment and time point. Error bars denote standard deviation.

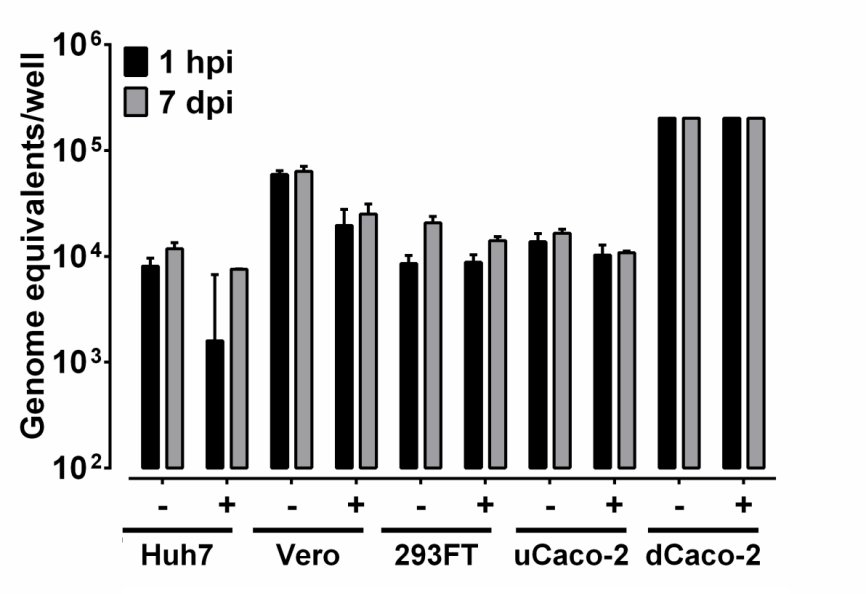

Fig. S9. GII.3 does not replicate in transformed cell lines treated with bile. Huh7, Vero, 293FT and undifferentiated Caco-2 BBe (uCaco-2) cells were pretreated with 0 (-) or 5% (+) human bile for 2 days. The pretreated cells were inoculated with GII.3 ($4.3x10^6$ genome equivalents of GII.3 stool filtrate) for 1 hour at 37^{o}C and cultured for 7 days. Differentiated Caco-2 BBe (dCaco-2) cells cultured in transwells for 21 days were pretreated as indicated with bile and inoculated with GII.3 stool filtrate. The human bile was added or not into medium during and post-inoculation. At 1 hour and 7 days post-inoculation, the cells and medium were harvested, RNA extracted and viral genome equivalents quantified by RT-qPCR. Data represent the mean of three wells for each treatment and time point. Error bars denote standard deviation.

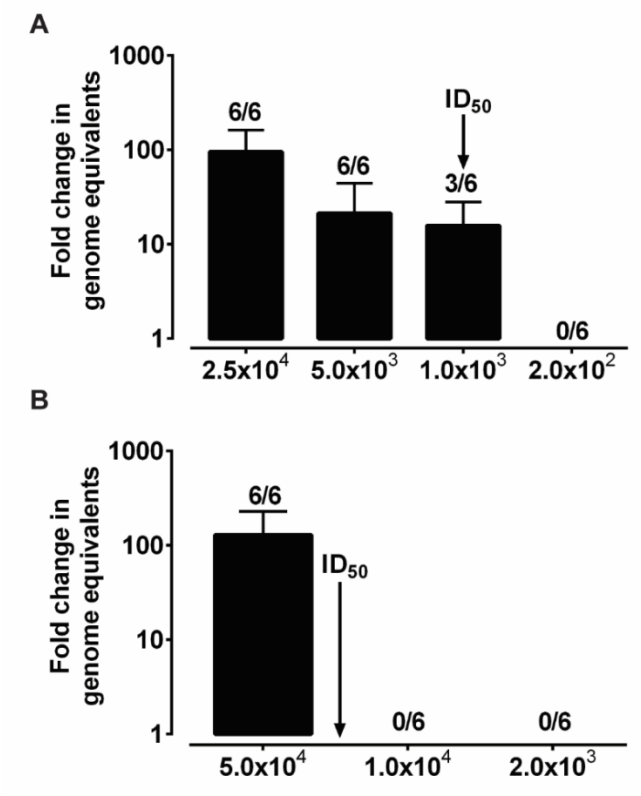

Fig. S10. Determination of ID_{50}. Jejunal human intestinal enteroid monolayers were treated with bile (see below) for 2 days during differentiation. The monolayers were inoculated with serial dilutions of (A) GII.4/2012-1 or (B) GII.3 HuNoV genome equivalents in the presence of 1% sow bile for GII.4 or 5% human bile for GII.3 for 1 hour at 37ºC. The monolayers were washed twice with CMGF(-) medium and cultured for 7 days in differentiation medium in the presence of bile. At 1 hpi and 7 dpi, the cells and medium were harvested, RNA extracted and viral genome equivalents quantified by RT-qPCR. Data represent the mean of three wells for each treatment and time point from one representative experiment. Error bars denote standard deviation. Infectious dose 50 (ID_{50}) was calculated by the Reed-Muench method using the geometric mean of two experiments.

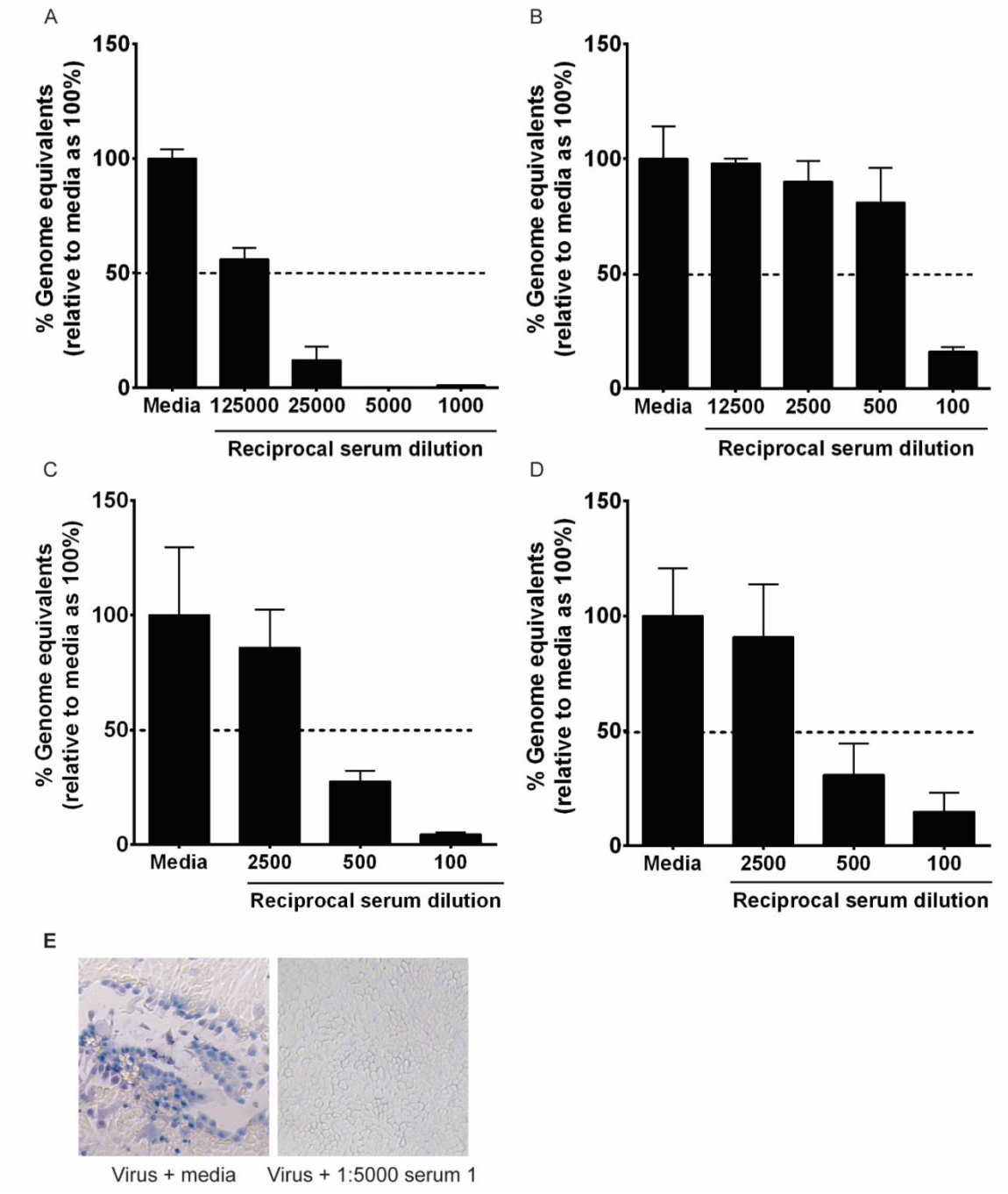

Fig. S11. Two human serum samples (1 and 2) neutralize GII.4/2012-1 and GII.3 viruses. GII.4 or GII.3 virus was mixed with an equal volume of media or dilutions of serum 1 and serum 2, and then incubated at 37ºC for 1 hr. Bile-treated human jejunal enteroids (1% sow bile for GII.4 and 5% human bile for GII.3 virus) were inoculated with each virus-serum mixture for 1 hr at 37ºC in the presence of bile. The monolayers were washed twice and then cultured in the presence of bile for 24 hr. The percent

reduction in genome equivalents compared to media was determined. (A) GII.4-serum 1; (B) GII.4-serum 2; (C) GII.3-serum 1; (D) GII.3-serum 2. The dotted line represents 50% neutralization. Data are from 3 wells for each treatment. (E) GII.4/2012-1 ($5x10^7$ genome equivalents) was mixed with an equal volume of media (left panel) or serial dilutions (1:250-1:5000) of serum 1, and then incubated for 1 hr at 37°C. Human jejunal enteroids were infected and CPE assessed by trypan blue exclusion at 72 hpi and imaged by bright field on an Olympus IX70 microscope with 20X magnification. CPE was observed in wells inoculated with virus and media (left panel) but CPE was not observed in any of the cultures treated with the virus-serum mixtures (1:5000 shown, right panel).

Table S1. Cultivatable HuNoV strains.

Reference Strain	Strain	Genotype_variant	Strain Designation	P-type[1]	Titer (genome equivalents/µl)[2]
GI/Hu/US/1968/GI.1/Norwalk	BCM723-02	GI.1	GI.1	GI.P1	5.7×10^6
GII/Hu/US/2004/GII.3/TCH04-577	TCH04-577	GII.3	GII.3	GII.P21	8.5×10^6
GII/Hu/US/2007/GII.4/TCH07-194	TCH07-194	GII.4_Yerseke_2006a	GII.4/2006a	GII.P4	7.0×10^7
GII/Hu/US/2007/GII.4/TCH07-882	TCH07-882	GII.4_Den Haag_2006b	GII.4/2006b-1	GII.P4	1.5×10^7
GII/Hu/US/2008/GII.4/TCH08-227	TCH08-227	GII.4_Den Haag_2006b	GII.4/2006b-2	GII.P4	5.3×10^6
GII/Hu/US/2009/GII.4/MDA09-01	MDA09-01	GII.4_Den Haag_2006b	GII.4/2006b-3	GII.P4	1.1×10^7
GII/Hu/US/2011/GII.4/TCH11-64	TCH11-64	GII.4_New Orleans_2009	GII.4/2009	GII.P4	3.0×10^7
GII/Hu/US/2012/GII.4/TCH12-580	TCH12-580	GII.4_Sydney_2012	GII.4/2012-1	GII.Pe	1.8×10^8
GII/Hu/US/2014/GII.4/TCH14-10	TCH14-10	GII.4_Sydney_2012	GII.4/2012-2	GII.Pe	3.0×10^6
GII/Hu/US/2014/GII.17/TCH14-38	TCH14-38	GII.17	GII.17	GII.Pn	1.4×10^7

[1] Polymerase type.

[2] Titer of the processed 10% stool suspension filtered through a 0.22 µm filter (25% for GI.1 and 10% for GII.17 filtered through a 0.45 µm filter).