SUPPLEMENTAL INFORMATION

MATERIALS AND METHODS

Growth and differentiation of enteroids. In brief, biopsy samples were obtained from adult patients undergoing routine endoscopy at Washington University School of Medicine with patients' consent and approval of the Institutional Review Board. Specimens were maintained at the Washington University Digestive Diseases Research Core Center BioSpecimens Core, and were prepared as previously described.[15] Purified crypt tissue was thawed and resuspended in Matrigel (BD Biosciences, San Jose, CA, 15 μL/well in 24 well plates), and incubated at 37°C with a 1:1 mixture of conditioned media from a cell line expressing Wnt, R-spondin and noggin (L-WRN) and primary culture media (Advanced DEM/F12, Invitrogen) supplemented with 20% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/mL penicillin, 0.1 mg/mL streptomycin, 10 μM Y-27632 (ROCK inhibitor; Tocris Bioscience, R and D Systems, Minneapolis, MN), and 10 μM SB 431542 (TGFBR1 inhibitor; Tocris Bioscience, R and D Systems).

Differentiation was induced by incubating the cells in differentiation media (1:20 mixture of L-WRN CM and primary culture media) lacking the transforming growth factor-β inhibitor SB 431542 and supplemented with 5 μM N-[N-(3,5-difluorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester (EMD Millipore, Billerica, MA) if grown on Transwells (Corning, Tewksbury, MA) for microscopy and polarization. Two wells of stem cells in matrigel were resuspended in 200 μL of differentiation media and plated in a 96-well plate unless otherwise stated. Cells were grown to confluence for 3 days in differentiation media before use.

Blood groups were previously determined from the patient record at the time of biopsy and confirmed by genotyping[16] and/or immunofluorescence. Samples from two different patients with blood group A and two with blood group O were used for both colonic and ileal lines. Several enteroid lines were used, including the group O colonic lines 39A and 130A, group A colonic lines 124A and 135A, as well as group O ileal lines 235D and 130D, and group A ileal lines 124D and 135D.

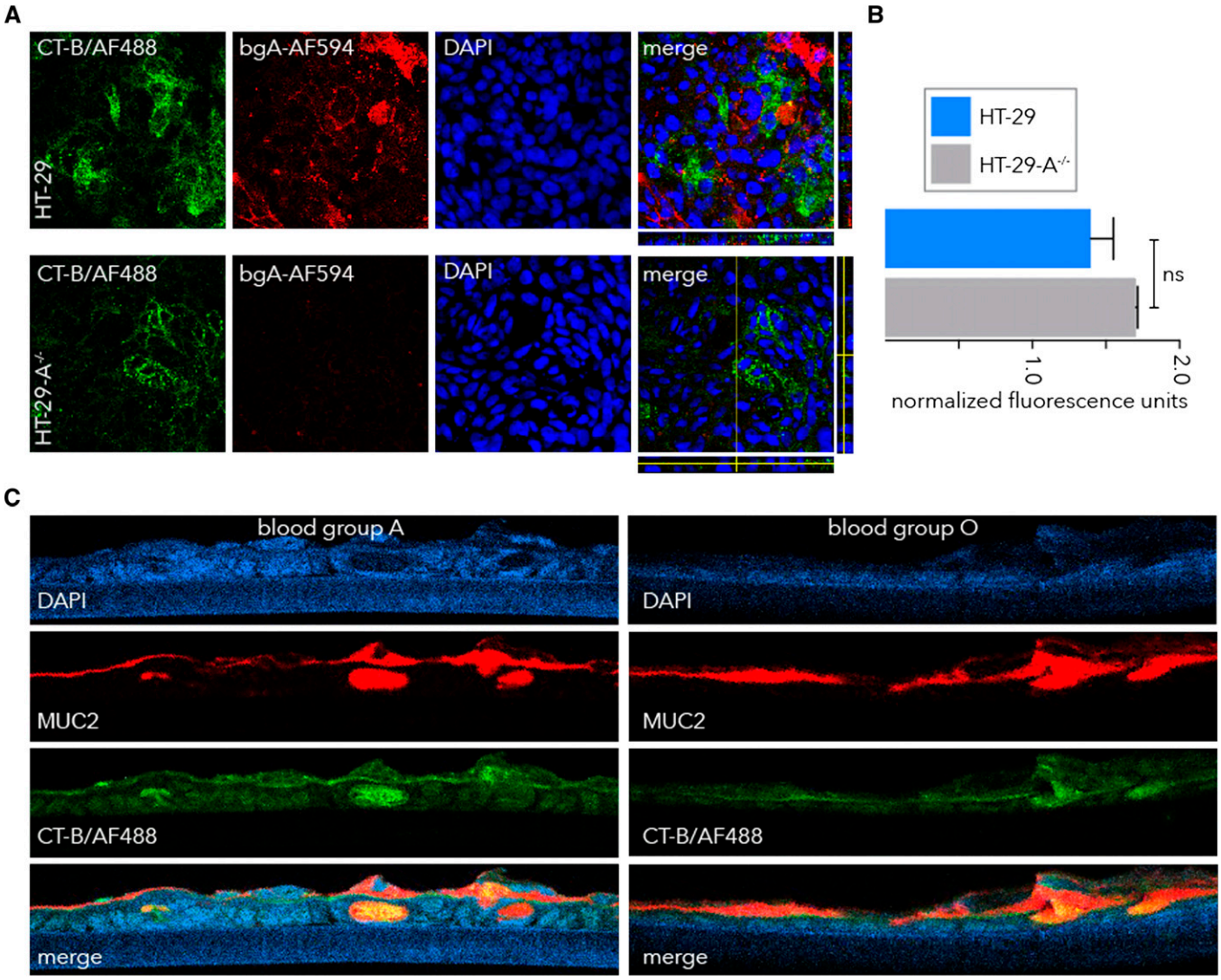
Construction of a glycosyltransferase-deficient cell line. HT-29 cells were obtained from American Type Culture Collection (ATCC HTB-38). Construction of a HT-29 derived cell line deficient in the α1-3-N-acetylgalactosaminyltransferase (accessions GI: 55773627, GenBank: D82843.2) responsible for blood group A, was performed using CRISPR/Cas9 genome editing in the Genome Engineering and iPSC Center at Washington University (http://geic.wustl.edu).

HT-29 cells were grown in McCoy's-5A medium (Gibco, Life Technologies, Grand Island, NY) supplemented with 10% FBS. Cells were plated at 2.5×10^5 cells/mL and cultured overnight before use.

cAMP determinations. Enteroids were incubated overnight with varying concentrations of CT. cAMP was detected by competitive enzyme immunoassay (DetectX® Cyclic AMP Direct Immunoassay kit Arbor Assays, Ann Arbor, MI). Enteroids were washed once in prewarmed phosphate-buffered saline (PBS), resuspended in 75 μL lysis buffer, and 50 μL was tested per enzyme-linked immunosorbent assay well. Absorbance values were obtained at 450 nm (Eon, microplate reader, BioTek, VT), and cAMP concentrations were determined against a reference standard curve. Total protein was assessed using Coomassie Plus Protein Assay Reagent (Thermo Scientific, Waltham, MA). 10 μL of the remaining cell lysate in sample buffer was added to 300 μL of Coomassie Plus Protein Reagent according to the provided protocol. The absorbance at 595 nm was assessed and total protein concentrations were determined against a reference standard curve generated in the same buffer. Data were analyzed using Gen5 Version 2.0 software (BioTek, Winooski, VT).

Immunofluorescence microscopy. To obtain polarized enteroids, organoids cells were grown as above on Transwells filters (Corning Costar No. 3470); likewise, HT-29 cells were grown on coverslips in 24-well plates (TPP No. 92024). Cells were washed in PBS and incubated with 200 μL of 4% paraformaldehyde for 15 minutes at room temperature or Carnoy's solution (60% ethanol, 30% chloroform, 10% glacial acetic acid) for 30 minutes at 4°C as indicated. Cells were washed thrice in PBS, and were blocked for 30 minutes with 200 μL of blocking buffer (2% bovine serum albumin in PBS). Primary antibodies used included anti–blood group antibodies (Santa Cruz Biotechnology, Dallas, TX) at 1:100 (mouse anti-A monoclonal IgM, Z2A), or 1:50 (mouse anti-H monoclonal IgM, 87-N), or rabbit polyclonal anti-MUC2 Ab at 1:20 (H-300), all for 1 hour at room temperature. Cell Mask Orange (Invitrogen, Carlsbad, CA) or Red was used for membrane staining at 1:2,000, 4′,6-Diamidino-2-Phenylindole, Dihydrochloride was used at 1:6,000, and Alexa Fluor 488-conjugated CT-B subunit (Invitrogen) at 1:200 for 1 hour with goat anti-mouse IgM 647 at a 1:200 dilution for blood group antigens or goat anti-rabbit IgG 594 at a 1:200 dilution for MUC2 detection (Invitrogen, Carlsbad, CA). All samples were washed in PBS before mounting with prolong gold overnight. Images were obtained using ZEISS LSM 880 confocal laser scanning microscope in the Molecular Microbiology Imaging Center at Washington University School of Medicine, and processed using the Fiji package[17] for ImageJ version 2.0.0-rc-43/1.50g (National Institutes of Health, Bethesda, MD).

To quantitate fluorophore-labeled CT-B binding, cells were fixed and labeled as described above in 96-well plates. Fluoresence was detected (Synergy H1 microplate reader; BioTek, Winooski, VT) using excitation and emission wavelengths of 485 nm and 528 nm, respectively, for detection of the CT-B fluorophore and 358 nm and 461 nm to detect DAPI. CT-B fluorescence detection was normalized to the DAPI signal.



Supplemental Figure 1 (**A**) Confocal imaging of recombinant cholera toxin B (CT-B) subunit Alexa Fluor 488 conjugate (CT-B/AF-488) to the surface of HT-29 or HT-29-A$^{-/-}$ cells. Merged panels at include yz and xz orthogonal views generated from confocal image stacks (Fiji/ImageJ 2.2.0). (**B**) Quantitative data showing CT-B/AF-488 bound to the HT-29 cells or the A glycosyl transferase mutant cells. (Data represent background-subtracted fluorescence signals normalized per the nuclear [DAPI] signal. Ns = non-significant difference by Mann–Whitney two-tailed nonparametric testing). (**C**) Confocal immunofluorescence microscopy images demonstrating colocalization of labeled CT-B subunit (CT-B/AF-488) with MUC2 mucin produced by both blood group A (left) and blood group O (right) enteroids.