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Supplementary Materials for

Rotavirus Induces Intercellular Calcium Waves through ADP Signaling

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Other Supplementary Materials for this manuscript includes the following:

Movies S1 to S11

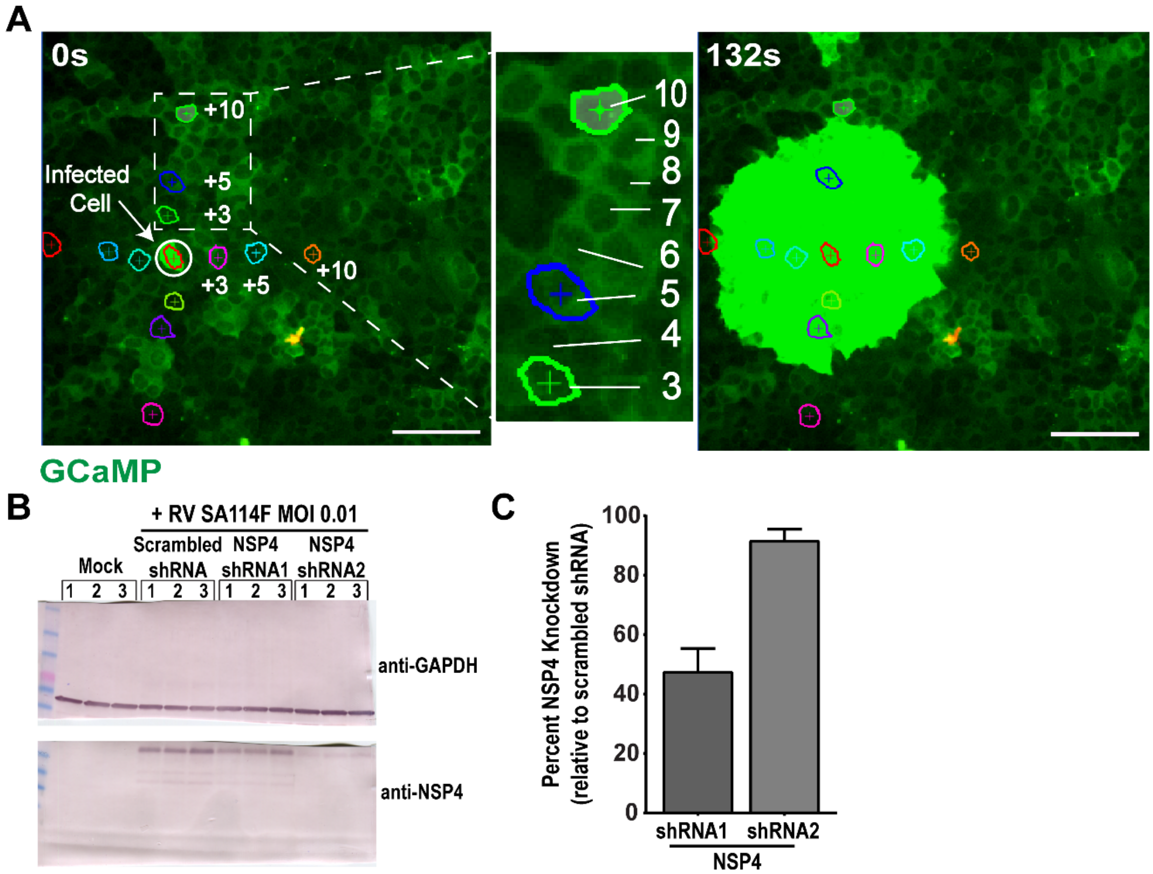

A
0s
+10
Infected Cell
+5
+3
+3
+5
+10
10
9
8
7
6
5
4
3
132s
GCaMP
B
+ RV SA114F MOI 0.01
Mock
Scrambled shRNA
NSP4 shRNA1
NSP4 shRNA2
1 2 3 1 2 3 1 2 3 1 2 3
anti-GAPDH
anti-NSP4
C
Percent NSP4 Knockdown (relative to scrambled shRNA)
100
80
60
40
20
0
shRNA1
shRNA2
NSP4

Figure S1. System for analyzing calcium signals in a rotavirus-infected cell and its neighboring, uninfected cells.

(A) Calcium signal analysis of RV-infected and neighboring, uninfected cells. MA104 cell monolayer infected with RV (SA114F) at MOI 0.01 at ~14 hpi. After identifying the central RV-infected cell, neighboring uninfected cells were designated by the number of cells away from the RV-infected cell (e.g. +3, +5, +10). scale bar = 100 µm. **(B)** Western blot for RV NSP4 expression in mock-infected or RV (SA114F)-infected (MOI 0.01) MA104-GCaMP/shRNA cell lines, expressing either scrambled, NSP4 shRNA1, or NSP4 shRNA2. Cells were harvested at 19 hpi. **(C)** Densitometry analysis of western blots for RV NSP4 expression levels, normalized to that of GAPDH expression, expressed as relative to scrambled shRNA cell NSP4 expression. Data shown are 3 infections/condition and representative of *N*=3 independent experiment. Data represented as mean ± SD.

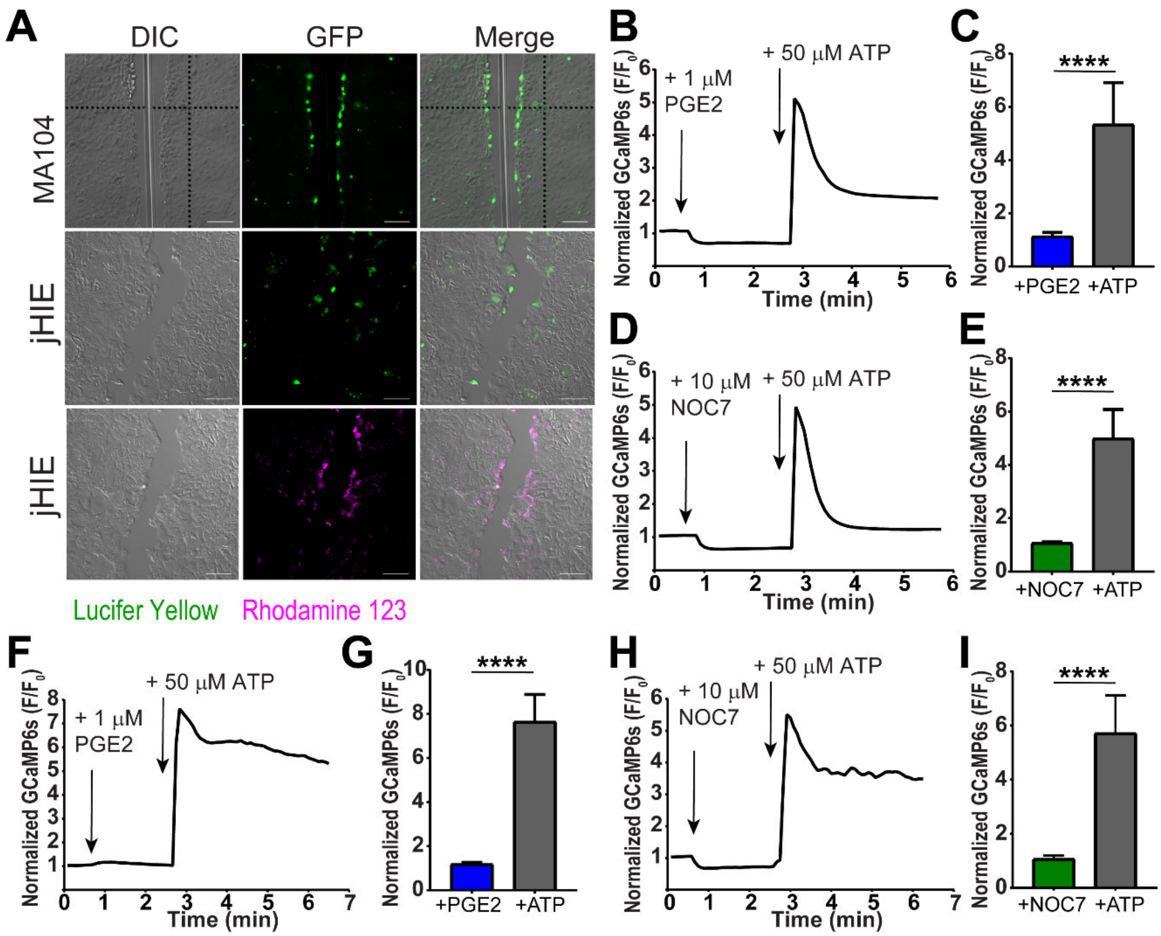

A
DIC
GFP
Merge
MA104
jHIE
jHIE
Lucifer Yellow
Rhodamine 123
B
+ 1 μM PGE2
+ 50 μM ATP
Normalized GCaMP6s (F/F0)
Time (min)
C
****
+PGE2
+ATP
D
+ 10 μM NOC7
+ 50 μM ATP
E
+NOC7
F
G
H
I

Figure S2. Rotavirus-induced calcium waves do not occur via gap junctions, prostaglandin E2, or nitric oxide.

(A) Scrape loading/dye transfer assay with Lucifer Yellow and Rhodamine 123 in MA104 cells and patient J3 jHIE monolayers. Images representative of *N*=3 independent experiments, scale bar = 100 µm. **(B-C)** MA104-GCaMP cells treated with 1 µM prostaglandin E2 (PGE2) followed by 50 µM ATP with **(B)** representative normalized GFP fluorescence average trace and **(C)** maximum normalized GFP fluorescence increase (n = 30 cells, data representative of *N*=4 experiments) **(D-E)** MA104-GCaMP cells treated with 10 µM NOC7 followed by 50 µM ATP with **(D)** representative normalized GFP fluorescence average trace and **(E)** maximum normalized GFP fluorescence increase (n = 30 cells, data representative of *N*=4 experiments). **(F-G)** Patient J2 jHIE-GCaMP6s cells treated with 1 µM PGE2 followed by 50 µM ATP with **(F)** representative normalized GFP fluorescence average trace and **(G)** maximum normalized GFP fluorescence increase (n = 30 cells, data representative of *N*=4 experiments). **(H-I)** jHIE-GCaMP6s cells (J2) treated with 10 µM NOC7 followed by 50 µM ATP with **(H)** representative normalized GFP fluorescence average trace and **(I)** maximum normalized GFP fluorescence increase (n = 30 cells, data representative of *N*=4 experiments) **(C,E,G,I)** Mann-Whitney test used. Data represented as mean ± SD, (****p<0.0001).

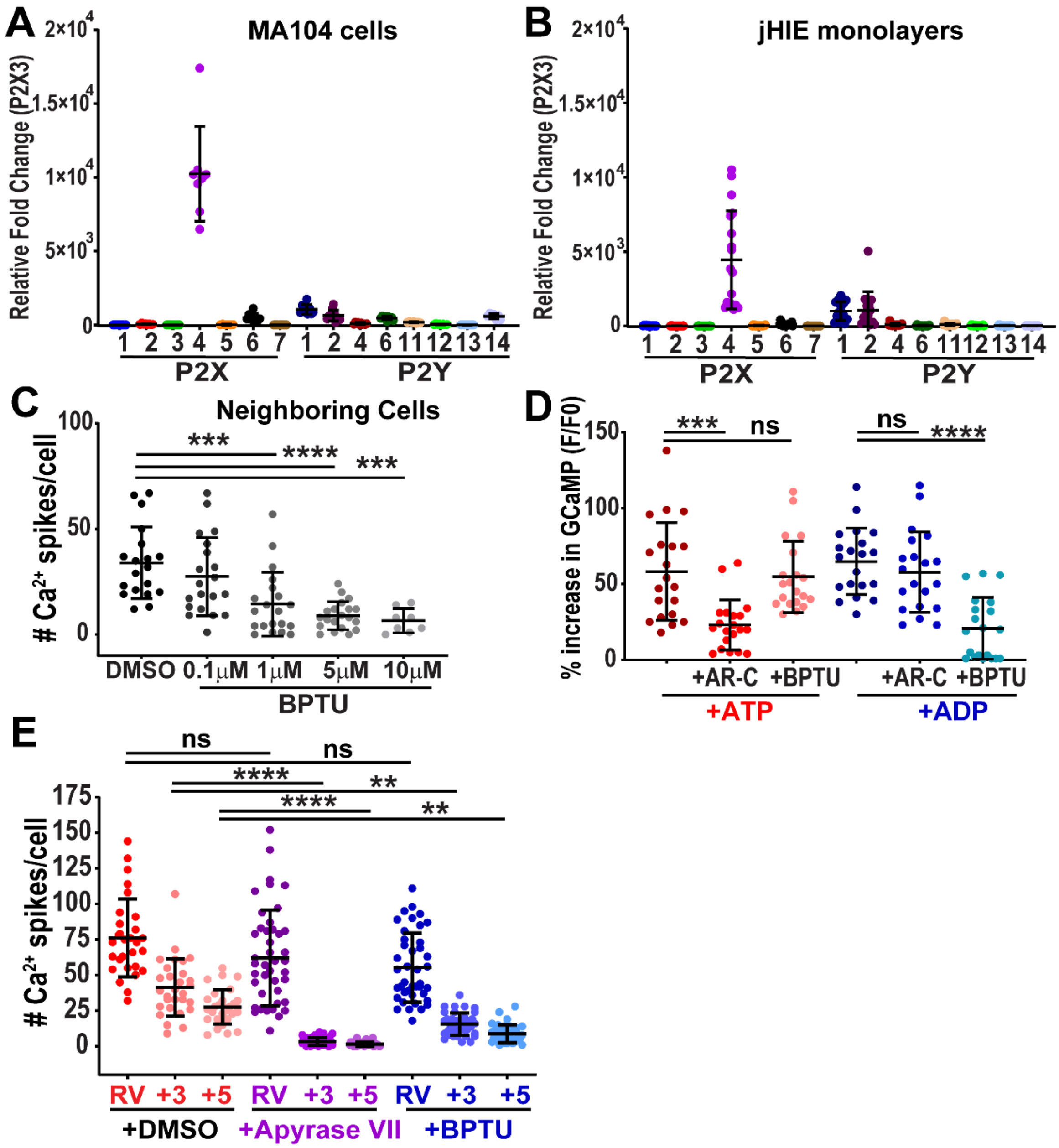
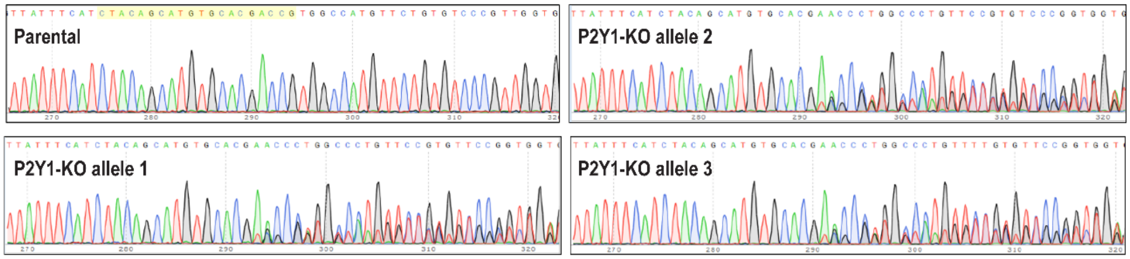

A
MA104 cells
Relative Fold Change (P2X3)
2×10⁴
1.5×10⁴
1×10⁴
5×10³
0
1 2 3 4 5 6 7 1 2 4 6 11 12 13 14
P2X
P2Y
B
jHIE monolayers
C
Neighboring Cells
Ca²⁺ spikes/cell
***
****
***
DMSO 0.1μM 1μM 5μM 10μM
BPTU
D
% increase in GCaMP (F/F0)
ns
****
+AR-C +BPTU
+ATP
+ADP
E
**
RV +3 +5
+DMSO
+Apyrase VII
+BPTU

Figure S3. Purinergic blockers of P2Y1 reduce rotavirus-induced calcium waves.

(A-B) qPCR of purinergic receptor mRNA normalized to 18S mRNA transcripts and fold change relative to P2X3 mRNA levels in MA104 cells infected with **(A)** rotavirus (RV) SA114F-infected MA104 cells or **(B)** RV (Ito)-infected jHIE monolayers, MOI 1, at 24 hpi (data combined from *N*=3 independent experiments). **(C**) Number of Ca^{2+} spikes in neighboring (NB) cells of RV (SA114F)-infected MA104-GCaMP cells treated with DMSO or BPTU. (n= 20 cells, data representative of *N*=3 independent experiments) **(D)** Normalized relative GFP fluorescence of MA104-GCaMP cells incubated with 10 µM AR-C 118925XX (AR-C) or 10 µM BPTU for 3.5 min before addition with 10 nM ADP or 1 µM ATP (n= 20 cells, data representative of *N*=3 independent experiments). **(E)** Ca^{2+} spikes/cell in RV-infected or NB+3 or NB+5 cell of MA104-GCaMP cells infected with RV (RRV) at MOI 0.05 and treated with DMSO, 10 U/mL apyrase VII, or 10 µM BPTU and imaged ~6-30 hpi (data combined from *N*=3 independent experiments). **(C-E)** Kruskal-Wallis with Dunn's comparisons test. Data represented as mean ± SD, (***p<0.001, ****p<0.0001).

A

MA104 cells



B

P2Y1-KO allele 1

```
              Y  F  I  Y  S  M  C  T   T  V  A  M  F  C  V  P  L  V  L  I  L  G  C  Y  G  L  I  V  R  A  L  I  Y
Parental      TATTTCATCTACAGCATGTGCACG-ACCGTGGCCATGTTCTGTGTCCCGTTGGTGCTGATTCTGGGCTGTTATGGATTAATTGTGAGAGCTTTGATTTA
P1D5_a1       TATTTCATCTACAGCATGTGCACGAACCCTGGCCCTGTTCCGTGTCCCGGTGGTGCTGATTCTGGGCTGGTATGGATTAATTGGGAAAGCTTTGATTTA
P2F2_a1       TATTTCATCTACAGCATGTGCACGAACCCTGGCCCTGTTCCGTGTCCCGGTGGTGCTTATTCTGGGCTGGTATGGATTAATTGGGAAAGCTTTGATTTA
P2F3_a1       TATTTCATCTACAGCATGTGCACGAACCCTGGCCCTGTTCCGTGTCCCGGTGGTGCTGATTCTGGGCTGGTATGGATTAAATGGGAAAGCTTTGATTTA
              Y  F  I  Y  S  M  C  T  N  P  G  P  V  P  C  P  G  G  A  D  S  G  L  V  W  I  N  W  E  S  F  D  L

               K  D  L  D  N  S  P  L  R  R  K  S  I  Y  L  V  I  I  V  L  T  V  F  A  V  S  Y  I  P  F  H  V  M  K  T  M.
Parental      CAAAGATCTGGACAACTCTCCTCTGAGGAGAAAATCGATATACCTGGTGATCATTGTACTGACTGTTTTTGCTGTGTCTTACATCCCTTTCCATGTGATGAAAACGATG
P1D5_a1       AAAAGAACTGGACAACTCTCCTCTGAAGAAAAAAACAATATACCTGGTGATCATTGGACTGACTGGTTTTGCTGTGTCTTACATCCCTTTCCATGAGAAGAAAAAAATG
P2F2_a1       AAAAGAACTGGACAACTCTCCTCTGAAGAAAAAAATCATATACCTGGTGATCATTGGACTGACTGGTTTTGCTGTGTCTTACATCCCTTTCCATGGGAAGAAAAAAATT
P2F3_a1       AAAAGAACTGGACAACTCTCCTCTGAAGAAAAAAACAATATACCTGGTGATCATTGGACTGACTGGTTTTGCTGTGTCTTACATCCCTTTCCATGTGAAGAAAAAGATT
              K  R  T  G  Q  L  S  S  E  E  K  N  N  I  P  G  D  H  W  T  D  W  F  C  C  V  L  H  P  F  P  W  E  E  K  N
```

P2Y1-KO allele 2

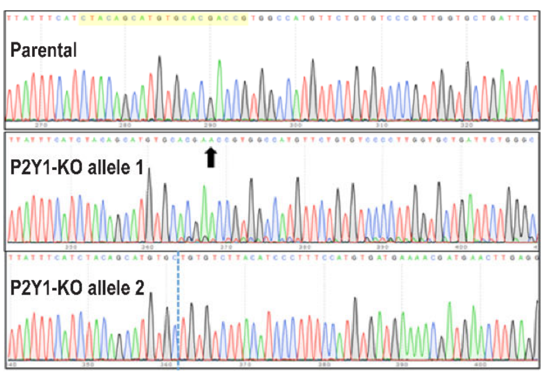
```
              Y  F  I  Y  S  M  C  T   T  V  A  M  F  C  V  P  L  V  L  I  L  G  C  Y  G
Parental      TATTTCATCTACAGCATGTGCACGA-CCGTGGCCATGTTCTGTGTCCCGTTGGTGCTGATTCTGGGCTGTTATGGA
P1D5_a2       TATTTCATCTACAGCATGTGCACGAGCCCAGGTCCTGTTCCCGGTCCCCGTGGAGGTGAATCTGGGATGGTATAG
P2F2_a2       TATTTCATCTACAGCATGTGCACGAGCCCAGGTCCTGTTCCCGGTCCCCGTGGAGGTTAATCTGGGATGGTATAG
P2F3_a2       TATTTCATCTACAGCATGTGCACGAGCCCAGGTCCTGTTCCCGGTCCCCGTGGAGGTGAATCTGGGATGGTATAG
              Y  F  I  Y  S  M  C  T  S  P  G  P  V  P  G  P  R  G  G  E  S  G  M  V  *
```

P2Y1-KO allele 3

```
              Y  F  I  Y  S  M  C  T  T     V  A
Parental      TATTTCATCTACAGCATGTGCACGAC--CGTGGCCA
P1D5_a3       TATTTCATCTACAGCATGTGCACGACGCGGTGGTAA
P2F2_a3       TATTTCATCTACAGCATGTGCACGACGCGGTGGTAA
P2F3_a3       TATTTCATCTACAGCATGTGCACGACGCGGTGGTAA
              Y  F  I  Y  S  M  C  T  T  R  W  *
```

C

Jejunum HIEs



D

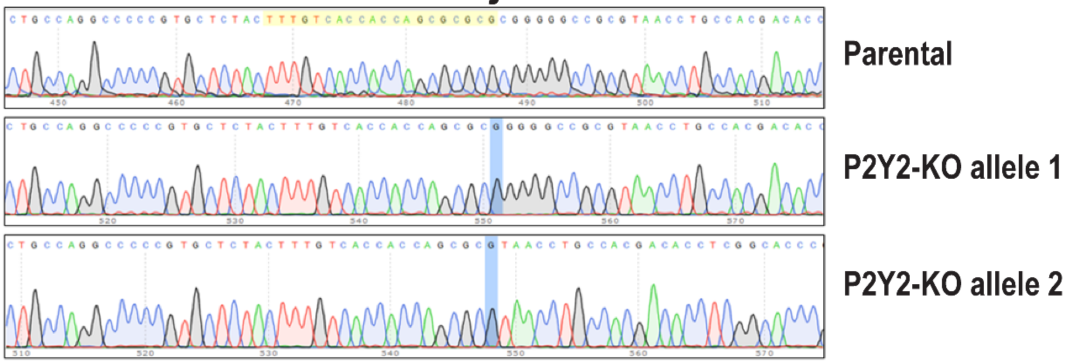
```
WT_J2G6s      648 CTACAGCATGTGCACGA-CCGTGGCCATGTTCTGTGTCCCCTTGGTGCTGATTCTGGGCTGTTACGGAT
KO_P2RY1g1    648 CTACAGCATGTGCACGAACCGTGGCCATGTTCTGTGTCCCCTTGGTGCTGATTCTGGGCTGTTACGGAT
KO_P2RY1g1    648 CTACAGCATGTGC---------------------------------------------------------

WT_J2G6s          TAATTGTGAGAGCTTTGATTTACAAAGATCTGGACAACTCTCCTCTGAGGAGAAAATCGATTTACCTGG
KO_P2RY1g1        TAATTGTGAGAGCTTTGATTTACAAAGATCTGGACAACTCTCCTCTGAGGAGAAAATCGATTTACCTGG
KO_P2RY1g1        ---------------------------------------------------------------------

WT_ J2G6s         TAATCATTGTACTGACTGTTTTTGCTGTGTCTTA
KO_P2RY1g1        TAATCATTGTACTGACTGTTTTTGCTGTGTCTTA
KO_P2RY1g1        -------------------------TGTGTCTTA
```

Figure S4. Genotyping of P2Y1 knockout MA104 cells and jejunum human intestinal enteroids.

(A) Sequencing chromatogram and **(B)** genotyping of P2Y1 receptor knockout in MA104-GCaMP-P2Y1ko cells. Parental P2Y1 receptor sequence compared to 3 alleles, mutations in red. **(C)** Sequence chromatogram and **(D)** genotyping of P2Y1 receptor knockout in jejunum HIE-GCaMP6s-P2Y1ko cells. Parental P2Y1 receptor sequence compared to 2 alleles of the P2Y1 receptor knockouts with an insertion (black arrow) and deletion (dashed line) indicated. The sequence shown is 648 nucleotides relative to the P2Y1 receptor start codon. Small guide RNA sequences highlighted in yellow.

A

Jejunum HIEs



B

```
WT_J2G6s     502GTGCTCTACTTTGTCACCACCAGCGCGCGCGGGGGCCGCGTAACCTGCCACGACAC
KO_P2RY2g1   502GTGCTCTACTTTGTCACCACCAGCGCG----GGGGCCGCGTAACCTGCCACGACAC
KO_P2RY2g1   502GTGCTCTACTTTGTCACCACCAGCG---------------TAACCTGCCACGACAC
```

Figure S5. Genotyping of P2Y2 knockout jejunum human intestinal enteroids.

(A) Sequence chromatogram and **(B)** genotyping of P2Y2 receptor knockout in jejunum HIE-GCaMP6s-P2Y2ko cells. Parental P2Y2 receptor sequence is compared to 2 alleles from the P2Y2 receptor knockouts with the sites of mutation highlighted in blue. Small guide RNA sequence highlighted in yellow. The sequence shown is 502 nucleotides relative to the P2Y2 receptor start codon.

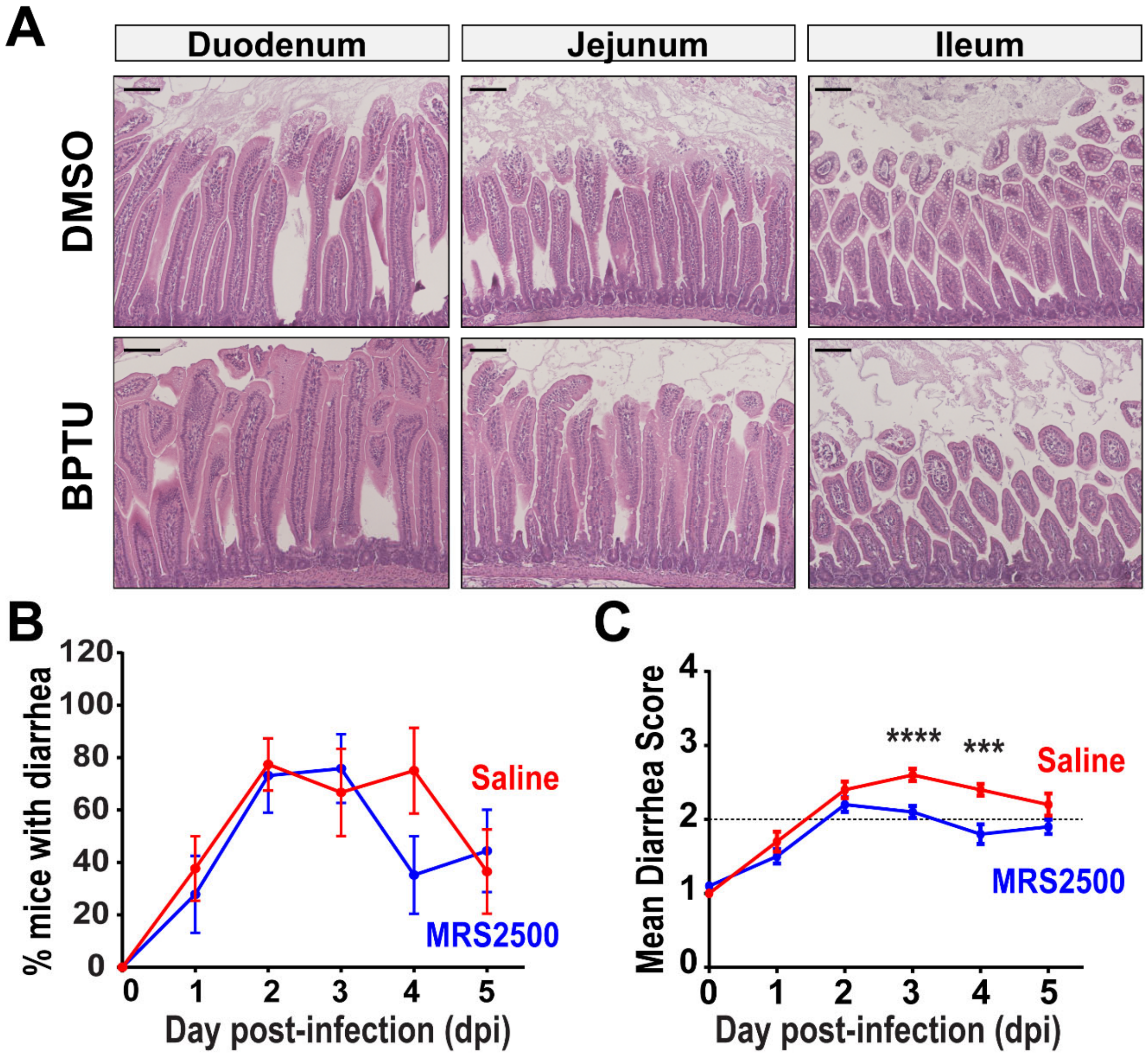

A
Duodenum
Jejunum
Ileum
DMSO
BPTU
B
% mice with diarrhea
120
100
80
60
40
20
0
0
1
2
3
4
5
Day post-infection (dpi)
Saline
MRS2500
C
Mean Diarrhea Score
4
3
2
1
0
0
1
2
3
4
5
Day post-infection (dpi)
****
***
Saline
MRS2500

Figure S6. P2Y1 receptor blockers attenuate rotavirus diarrhea in neonatal mice.

(A) Hematoxylin and eosin-stained intestinal sections from RV-infected mouse pups treated with DMSO or BPTU, 5 dpi. Scale bar = 100 µm. **(B)** Percentage of C57Bl/6J mouse pups with diarrhea infected with Rhesus RV and vehicle- (8 cages, n = 46 pups) or MRS2500-treated (4 mg/kg) (9 cages, n = 60 pups) and the **(C)** mean diarrhea score. Mann-Whitney test, data presented as mean ± SEM (*$p<0.05$, **$p<0.01$).

Table S1: Purinergic receptor expression comparison of mock- and rotavirus-infected cells

	MA104 cells			Jejunum Human Intestinal Enteroids		
	Mock	RV-Infected	Statistics	Mock	RV-Infected	Statistics
Receptor	Average ± Stdev	Average ± Stdev	p value	Average ± Stdev	Average ± Stdev	p value
P2X1	2.2 ± 2.8	4.6 ± 3.3	>0.9999	15.4 ± 10.4	18.6 ± 17.9	>0.9999
P2X2	45.5 ± 326.9	58.1 ± 35.1	>0.9999	4.4 ± 6.7	4.7 ± 7.9	>0.9999
P2X3	1.9 ± 2.1	1.5 ± 2.1	>0.9999	2.4 ± 2.1	2.4 ± 3.4	>0.9999
P2X4	12412.8 ± 1837.2	10818.4 ± 3229.8	>0.9999	8196.5 ± 876.3	4601.3 ± 3231.1	>0.9999
P2X5	6.6 ± 9.12	21.9 ± 17.0	>0.9999	35.9 ± 19.3	31.4 ± 25.6	>0.9999
P2X6	943.8 ± 377.3	551.5 ± 258.0	0.0709	357.3 ± 184.9	152.1 ± 129.4	0.9991
P2X7	0.4 ± 0.5	0.5 ± 0.9	>0.9999	3.6 ± 4.9	1.6 ± 2.3	>0.9999
P2Y1	1637.0 ± 585.7	1144.6 ± 321.5	0.06	2363.0 ± 1621.5	1117.2 ± 685.1	>0.9999
P2Y2	587.5 ± 278.5	638.9 ± 368.6	>0.9999	1791.4 ± 965.6	1348.3 ± 1309.5	0.4078
P2Y4	26.4 ± 21.7	93.7 ± 58.9	>0.9999	110.2 ± 73.7	97.5 ± 76.9	>0.9999
P2Y6	570.1 ± 99.7	465.8 ± 117.3	1.0	19.1 ± 11.5	19.7 ± 22.4	>0.9999
P2Y11	213.2 ± 119.9	183.7 ± 45.6	1.0	322.8 ± 197.9	165.9 ± 150.5	0.9944
P2Y12	32.1 ± 21.3	43.3 ± 27.6	>0.9999	41.9 ± 35.1	35.3 ± 27.3	>0.9999
P2Y13	6.4 ± 7.6	13.2 ± 12.3	>0.9999	16.4 ± 18.3	16.4 ± 20.3	>0.9999
P2Y14	308.0 ± 336.6	584.8 ± 171.8	>0.9999	28.9 ± 19.9	16.2 ± 12.0	>0.9999

Table S2: Genotyping primers for P2Y1 and P2Y2 receptors

Gene Target	Species	Product size	Forward (5') Sequence	Reverse (3') Sequence
P2RY1	Human	954 bp	CAGACTGGATCTTCGGGGATGCC	CCCGCCAAGAAATAGAGAATGGGG
P2RY2	Human	1025 bp	CCTGGAATGACACCATCAATGGC	CCTCTGCATGTCAGTTCTGTCG

Table S3: qPCR primer sequences

Gene	Species	Forward (5') Sequence	Reverse (3') Sequence
18S	Universal	CGCCTTCCTCTTCGAGTATGA	AGATAACGCCCACCTTCTTATTACG
IL-1α	Human	GAATGACGCCCTCAATCAAAGT	TCATCTTGGGCAGTCACATACA
COX2	Human	ATCATTCACCAGGCAAATTGC	GGCTTCAGCATAAAGCGTTTG
iNOS	Human	CAGCTCCACAAGCTGGCTCG	CAGGATGTCCTGAACGTAGACCTTG
P2X1	Human	CGCCTTCCTCTTCGAGTATGA	AGATAACGCCCACCTTCTTATTACG
P2X2	Human	GCCTACGGGATCCGCATT	TGGTGGGAATCAGGCTGAAC
P2X3	Human	GCTGGACCATCGGGATCA	GAAAACCCACCCTACAAAGTAGGA
P2X4	Human	CCTCTGCTTGCCCAGGTACTC	CCAGGAGATACGTTGTGCTCAA
P2X5	Human	CTGCCTGTCGCTGTTCGA	GCAGGCCCACCTTCTTGTT
P2X6	Human	AGGCCAGTGTGTGGTGTTCA	TCTCCACTGGGCACCAACTC
P2X7	Human	TCTTCGTGATGACAAACTTTCTCAA	GTCCTGCGGGTGGGATACT
P2Y1	Human	CGTGCTGGTGTGGCTCATT	GGACCCCGGTACCTGAGTAGA
P2Y2	Human	GAACTGACATGCAGAGGATAGAAGAT	GCCGGCGTGGACTCTGT
P2Y4	Human	CCGTCCTGTGCCATGACA	TGACCGCCGAGCTGAAGT
P2Y6	Human	GCCGGCGACCACATGA	GACCCTGCCTCTGCCATTT
P2Y11	Human	CTGGAGCGCTTCCTCTTCAC	GGTAGCGGTTGAGGCTGATG
P2Y12	Human	AGGTCCTCTTCCCACTGCTCTA	CATCGCCAGGCCATTTGT
P2Y13	Human	GAGACACTCGGATAGTACAGCTGGTA	GCAGGATGCCGGTCAAGA
P2Y14	Human	TTCCTTTCAAGATCCTTGGTGACT	GCAGAGACCCTGCACACAAA

Movie S1. Rotavirus infection induces calcium signaling beyond the infected cell.

MA104-GCaMP cells were mock- or rotavirus (strain SA114F)-infected and live time-lapse imaging was performed. GCaMP reports cytoplasmic Ca^{2+} as changes in fluorescence intensity (green) and images of RV-antigen positive cells by immunofluorescence (pink) were superimposed on the movies. Note the imbedded timer displays time from the beginning of acquisition not the time post-infection.

Movie S2. Rotavirus-infected cells elicit intercellular calcium waves.

MA104-GCaMP5G cells were mock-inoculated or rotavirus-infected with the recombinant SA11cl3-mRuby3 reporter virus and live time-lapse imaging was performed. GCaMP5G reports cytoplasmic Ca^{2+} as changes in fluorescence intensity (green) and rotavirus protein synthesis is reported by mRuby3 expression (pink) from the nonstructural protein 3 (NSP3) open-reading frame. Note the imbedded timer displays time from the beginning of acquisition not the time post-infection.

Movie S3. shRNA knockdown of NSP4 reduces intercellular calcium waves in rotavirus infection.

MA104-GCaMP cells expressing either a scrambled shRNA (left) or NSP4-specific shRNA (right) were rotavirus-infected with the recombinant SA11cl3-mRuby3 reporter virus and live time-lapse imaging was performed. GCaMP reports cytoplasmic Ca^{2+} changes in fluorescence intensity (green) and rotavirus protein synthesis is reported by mRuby3 expression (pink) from the NSP3 open-readying frame. Note the imbedded timer displays time from the beginning of acquisition not the time post-infection.

Movie S4. Blocking enterotoxin NSP4 signaling does not reduce intercellular calcium waves in rotavirus infection.

MA104-GCaMP cells were mock-inoculated or rotavirus-infected with the recombinant SA11cl3-mRuby3 reporter virus and live time-lapse imaging was performed. GCaMP5G reports cytoplasmic Ca2+ as changes in fluorescence intensity (green) and rotavirus protein synthesis is reported by mRuby3 expression (pink) from the nonstructural protein 3 (NSP3) open-reading frame. Cells were treated with anti-VP7 M60 MAb, anti-NSP4 MAb 622, or anti-NSP4 antisera 120-147 (Rb Ab) after infection. Note the imbedded timer displays time from the beginning of acquisition not the time post-infection.

Movie S5. Blocking purinergic signaling inhibits intercellular calcium waves in rotavirus infection.

MA104-GCaMP cells were mock-inoculated or rotavirus-infected with the recombinant SA11cl3-mRuby3 reporter virus and live time-lapse imaging was performed. GCaMP5G reports cytoplasmic Ca^{2+} as changes in fluorescence intensity (green) and rotavirus protein synthesis is reported by mRuby3 expression (pink) from the nonstructural protein 3 (NSP3) open-reading frame. Cells were treated with 10 U/mL apyrase after infection. Note the imbedded timer displays time from the beginning of acquisition not the time post-infection.

Movie S6. Blocking the P2Y1 receptor inhibits intercellular calcium waves in rotavirus infection.

MA104-GCaMP cells were mock-inoculated or rotavirus-infected with the recombinant SA11cl3-mRuby3 reporter virus and live time-lapse imaging was performed. GCaMP5G reports cytoplasmic Ca^{2+} as changes in fluorescence intensity (green) and rotavirus protein synthesis is reported by mRuby3 expression (pink) from the nonstructural protein 3 (NSP3) open-reading frame. Cells were treated with 10 μM BPTU after infection. Note the imbedded timer displays time from the beginning of acquisition not the time post-infection.

Movie S7. Rotavirus infection induces intercellular calcium waves in human intestinal enteroids.

Jejunum HIE-GCaMP6s enteroid monolayers were mock- or rotavirus (strain Ito)-infected, and imaged once per minute for ~7-22 hpi. GCaMP6s reports cytoplasmic Ca^{2+} as changes in fluorescence intensity (green). Note the imbedded timer displays time from the beginning of acquisition not the time post-infection.

Movie S8. Blocking purinergic signaling and P2Y1 receptor inhibits intercellular calcium waves in human intestinal enteroids.

Jejunum HIE-GCaMP6s enteroid monolayers were mock- or rotavirus (strain Ito)-infected, and treated with vehicle (DMSO), 100 U/mL apyrase, or 10 μM BPTU and imaged once per minute for ~7-22 hpi. GCaMP6s reports cytoplasmic Ca^{2+} as changes in fluorescence intensity (green). Note the imbedded timer displays time from the beginning of acquisition not the time post-infection.

Movie S9. CRISPR/Cas9 knockout of the P2Y1 receptor reduces intercellular calcium waves.

MA104-GCaMP6s or MA104-GCaMP6s-P2Y1ko cells were mock-inoculated or rotavirus-infected with the recombinant SA11cl3-mRuby3 reporter virus and live time-lapse imaging was performed. GCaMP reports cytoplasmic Ca^{2+} as changes in fluorescence intensity (green) and rotavirus protein synthesis is reported by mRuby3 expression (pink) from the nonstructural protein 3 (NSP3) open-reading frame. Note the imbedded timer displays time from the beginning of acquisition not the time post-infection.

Movie S10. CRISPR/Cas9 knockout of the P2Y1 receptor inhibits intercellular calcium waves in human intestinal enteroids.

Jejunum HIE-GCaMP6s, jHIE-GCaMP6s-P2Y1ko, and jHIE-GCaMP6s-P2Y2ko monolayers were mock- or rotavirus (strain Ito)-infected, imaged once per minute for ~7-22 hpi. GCaMP6s reports cytoplasmic Ca^{2+} as changes in fluorescence intensity (green). Note the imbedded timer displays time from the beginning of acquisition not the time post-infection.

Movie S11. Blocking the P2Y1 receptor decreases RV-induced human intestinal enteroid swelling.

3D jejunum HIE-GCaMP6s enteroids were mock- or rotavirus (strain Ito)-infected, treated with vehicle (DMSO) or 10 µM BPTU, and imaged once per 2-3 min in GFP and differential interference contrast on a widefield epifluorescence microscope for ~3-21 hpi. GCaMP6s reports cytoplasmic Ca^{2+} as changes in fluorescence intensity (green). Note the imbedded timer displays time from the beginning of acquisition not the time post-infection.