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Supplemental Information

Novel Chimeric Gene Therapy Vectors Based on Adeno-Associated Virus and Four Different Mammalian Bocaviruses

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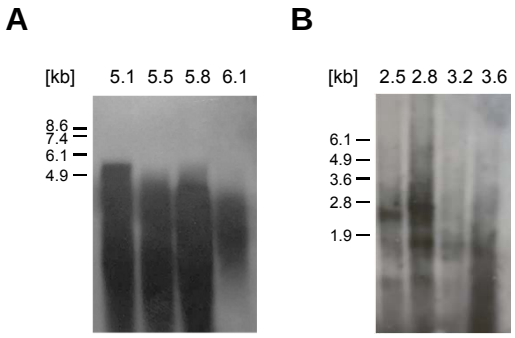

Figure S1. Southern blot analysis of rAAV genomes packaged into AAV2. (A) The oversized ssAAV-CRISPR genomes shown in Figure 1D were packaged into AAV2 capsids. The packaged viral DNA was isolated and resolved on a 0.7% alkaline agarose gel. The number above each lane indicates the size of the packaged genome. AAV vector genomes were labeled with a probe against the *Sp*Cas9 cassette. **(B)** Neutral gel electrophoresis of scAAV-YFP genomes packaged into AAV2 capsids (see Fig. 1G for schemes of all constructs). Southern blotting was performed using a probe against the *yfp* cassette.

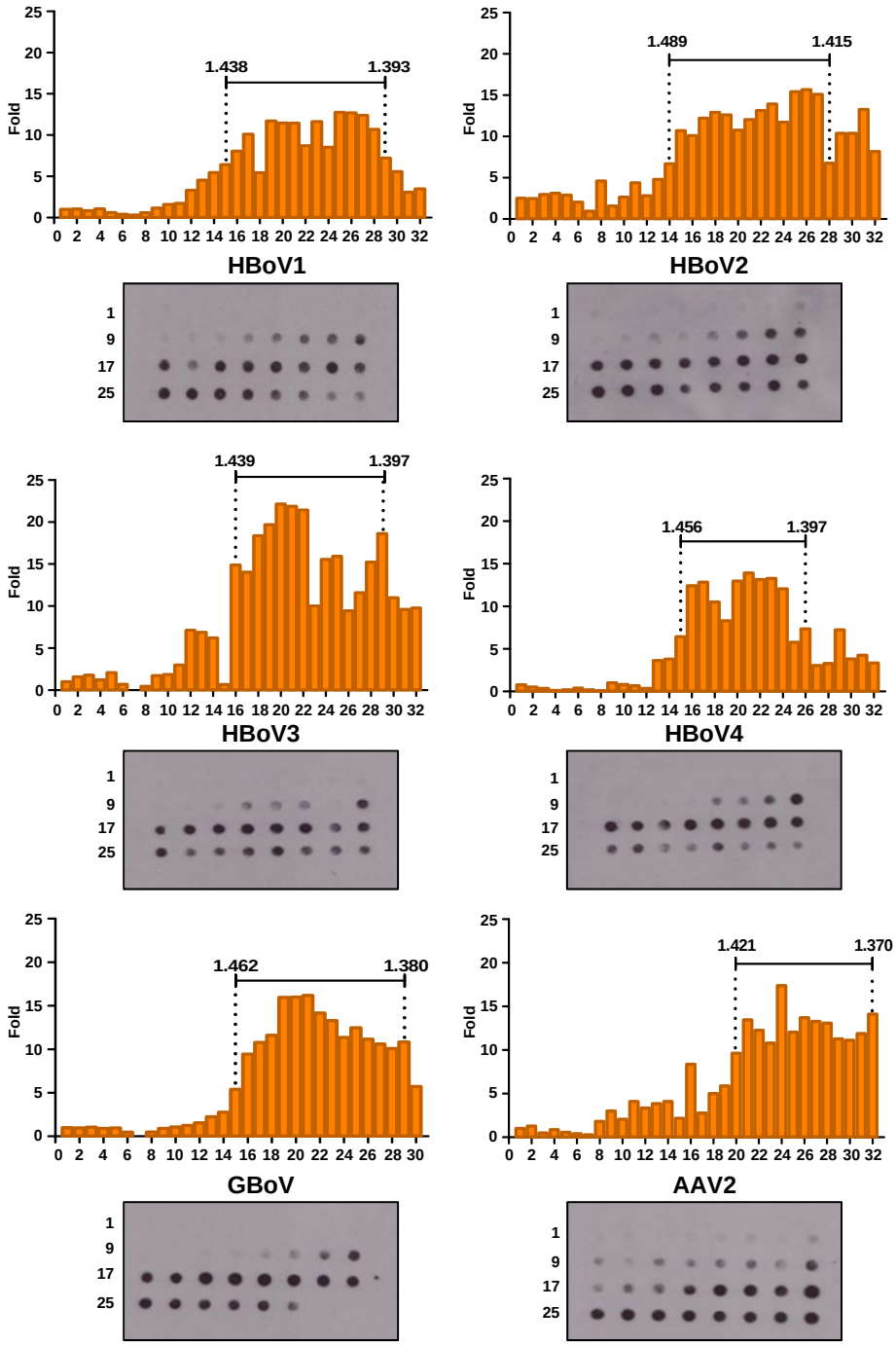

Figure S2. Purification of scAAV-YFP/BoV vectors using CsCl density centrifugation. scAAV-YFP genomes were packaged into HBoV1-4/GBoV or AAV2 as control using the triple transfection protocol. Viral particles were purified from the crude cell lysate using CsCl density centrifugation. To determine the virus-containing fractions, a total of 30 to 32 fractions was collected from each CsCl gradient. For detection of encapsidated AAV genomes, 10 to 15 µL of each fraction were loaded onto a dot blot apparatus followed by Southern blot analysis using a probe binding in the *yfp* cassette. Dot blots were analyzed in ImageJ and the optical density of each spot was determined after background subtraction. From these densities, the fold-change to fraction 1 was calculated and plotted on the y-axis. Brackets define the range with the highest viral content, and the numbers above refer to the density of the indicated fractions (g/ml).

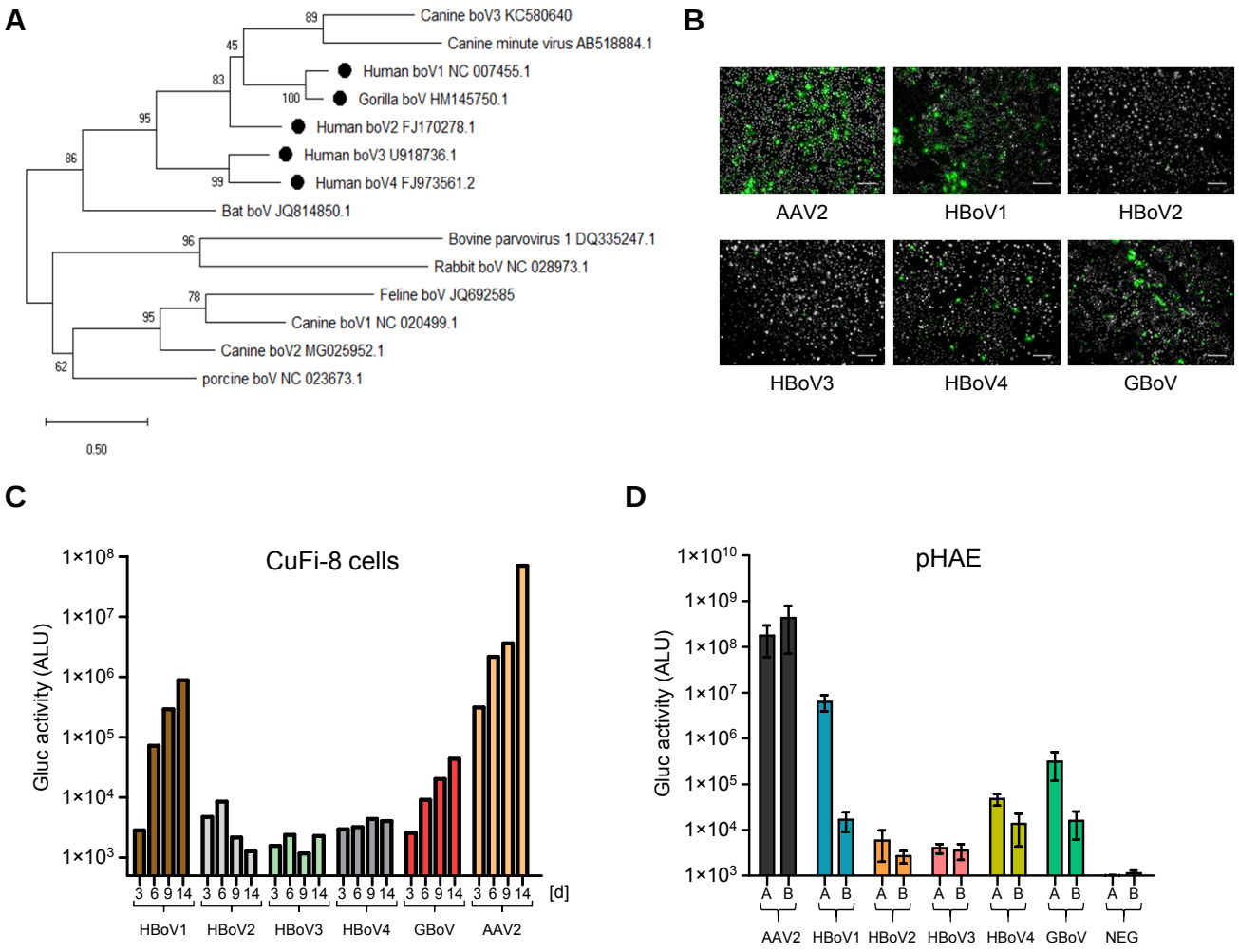

Figure S3. Phylogenetic analysis and initial testing of scAAV-YFP/BoV vectors on pHAE and CuFi-8 cell line. (A) Phylogenetic analysis of the BoV serotypes used in this work (marked with black circles). The evolutionary history was inferred in MEGAX by using the Maximum Likelihood method based on the Tamura-Nei model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The scale bar represents the average number of nucleotide substitutions per site. **(B)** Transduction of pHAE with scAAV-YFP/BoV or AAV2 as control (MOI of 4×10^4). Nuclei were stained with Hoechst. Images were taken 14 days post-transduction. Scale bar = 50 µm. **(C)** Transduction of CuFi-8 cell line with scAAV-Gluc/BoV vectors or AAV2 as control. A total of 2×10^{10} viral genomes was added to the apical side of each transwell overnight in the presence of doxorubicin and LLnL at final concentrations of 1 µM and 8 nM, respectively. Gluc activity was measured at time intervals of 3, 6, 9 and 14 days post-transduction. Shown is the average of two replicates, *i.e.*, two independent transwells. **(D)** Transduction of pHAE at a MOI of 2×10^4 with scAAV-Gluc/BoV variants from the apical (A) or basolateral (B) side of the transwell. Shown is the measured Gluc activity (mean ±SEM, n = 5) in the medium at day 9 post-transduction. For the transduction of pHAE, LLnL and doxorubicin were added at concentrations of 40 µM and 5 µM, respectively.

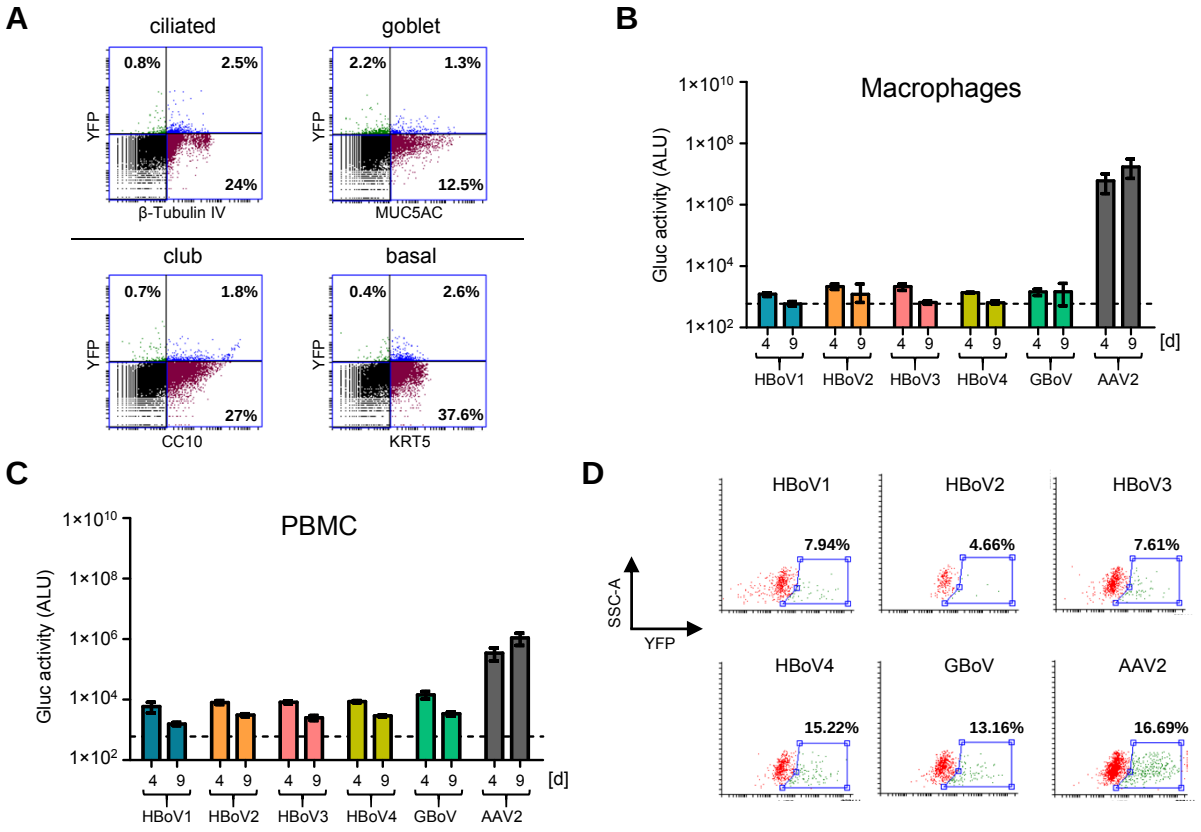

Figure S4. Transduction of primary human cells with scAAV/BoV vectors. (A) Flow cytometry analysis of pHAE transduced with scAAV-YFP/GBoV at a MOI of 5×10^4, n = 4 independent transwells. Cells were co-stained for YFP and a cell type-specific marker: ß-Tubulin IV (ciliated cells), MUC5AC (goblet cells), CC10 (club cells), or KRT5 (basal cells). Percentages of double-positive cells are shown in the upper right quarter. **(B)** Transduction of primary macrophages with 5×10^8 genomes of scAAV-Gluc/BoV vectors or AAV2 control. Gluc activity was measured at 4 and 9 days post-transduction. Shown is the mean plus range of two donors. **(C)** Transduction of primary PBMCs with scAAV-Gluc/BoV vectors or AAV2 control (MOI of 6×10^4). Gluc activity was measured at 4 and 9 days post-transduction. Shown is the mean plus range of two donors. **(D)** Flow cytometry analysis of primary T-cells transduced with scAAV-YFP/BoV vectors or AAV2 control (MOI of 6×10^4). Only living cells were analyzed. The percentage of YFP-positive cells is shown.

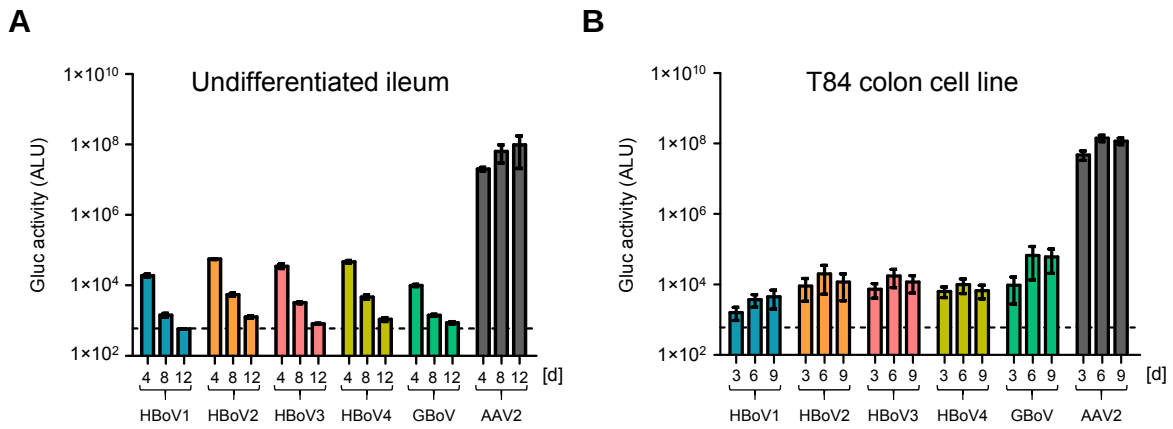

Figure S5. Transduction of primary intestinal cells and cell lines with scAAV-Gluc/BoV vectors. (A) Transduction of undifferentiated primary human ileum organoids which were maintained in basal medium. Organoids were transduced with 5×10^9 viral genomes of scAAV-Gluc/BoV, and the Gluc activity was measured at the indicated time points. Data represent the mean and range of two independent experiments (n = 2 donors). All transductions were performed in the presence of 1 µM doxorubicin. **(B)** T84 colon adenocarcinoma cells were transduced with scAAV-Gluc/BoV vectors or AAV2 control at MOI = 5×10^4. Secreted Gluc activity was measured in the medium at 3 to 9 days post-transduction. Shown is the mean (±SEM) of three independent experiments.

Table S1. Titers of rAAV-YFP vectors obtained using iodixanol versus CsCl purification.

	Iodixanol	CsCl
	Titer (gc / cell) [a,b]	Titer (gc / cell) [a,c]
HBoV1	4.6×10^3	1.5×10^3
HBoV2	5.7×10^3	2.0×10^3
HBoV3	4.9×10^3	2.6×10^3
HBoV4	1.0×10^4	3.9×10^3
GBoV	8.4×10^3	5.4×10^3
AAV2	2.4×10^4	1.1×10^4

[a] gc, genome copies.
[b] Data represent the average of four (BoV) or two (AAV2) independent productions and titrations.
[c] Data represent the average of two independent productions.

Table S2. Ratios of Gluc expression at day 9 post-transduction with the different BoV variants (or the AAV2 control) relative to HBoV1 (set to 1.0).

	SK	Cardio	Pul	Vein	T-cells	pHeps[a]	Colon	pHAE[b]	Lung (in)	Lung (br)	CuFi-8	T84
HBoV1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
HBoV2	5.1	1.1	1.1	0.5	3.9	1.3	4.1	0.0	0.3	0.1	0.01	2.7
HBoV3	3.0	0.1	1.2	0.5	18.5	0.4	2.9	0.0	0.4	0.1	0.0	2.6
HBoV4	2.4	0.7	0.6	0.5	56.9	1.0	4.0	0.1	0.2	0.2	0.02	1.5
GBoV	8.3	2.9	7.8	1.0	114.5	0.9	1.8	0.6	0.2	0.8	0.07	13.7
AAV2	8088.0	61458.0	64677.0	15839.0	435.0	2461.7	3907.0	n.d.	184.6	7667.2	12.4	26554.0

[a] Data were calculated based on Gluc expression at day 6 because of deteriorating cell vitality at day 9.
[b] Transduction from the apical side. Data were calculated from Fig. 2D.
n.d., not determined

Table S3. Primers used in this study.

No.	Primer Name	Primer sequence (5‘- 3‘)
#1	F1_HIND III	GACAATA**AAGCTT**TACAGCTTTTG
#2	R1_2xBsmbI	CAAAAAAGAGGCTTATAAGA**TGAGACG**CTGT**CGT CTCA**CTGCTTCCATGCTTTCAGC
#3	F2_2xBsmbI	TGCTGAAAGCATGGAAGCAG**TGAGACG**ACAG**CG TCTCA**TCTTATAAGCCTCTTTTTTGCTTCTGC
#4	R2_NotI	ATGA**GCGGCCGC**TCTAGATGTA
#5	Boca3_partI_notI_fwd	ATTAG**GCGGCCGC**ATGCCTCCAATTAAAAGGCAA C
#6	Boca3_partI_bsmbI_rev	GATC**CGTCTCG**CTTCCATTTCTGCAAGTTCATG
#7	Boca3_part2_BsmbI fwd	GATC**CGTCTCG**GAAGACTCCAATGCAGTAGAAAA AGCA
#8	Boca3_part2_claI_rev	GATC**ATCGAT**TTACAACACTTTATTGATGTTTGTTT TAACTGG
#9	Boca4_partI_notI_fwd	GATC**GCGGCCGC**ATGCCTCCAATTAAACGC
#10	Boca4_partI_bsmbI_rev	GATC**CGTCTCG**CTTCCATTTCAGCAAGTTCATG
#11	Boca4_part2_BsmbI_fwd	GATC**CGTCTCG**GAAGACTCAAATGCTGTAGAAAA AGCA
#12	Boca4_part2_claI_rev	GAT**CATCGAT**TTACAACACTTTATTGATGTTTGTTT TAACTGGAAAG
#13	BocaGo_partI_notI_fwd	GATC**GCGGCCGC**ATGCCTCCAATTAAAAGGCA
#14	BocaGo_partI_BsmbI_rev	GATC**CGTCTCG**TTCCATCAAGATCGGCAAGC
#15	BocaGo_part2_BsmbI_fwd	GATC**CGTCTCG**GGAACTACTGCTGGAGGAACTGC
#16	JF_BocaGo_part2.1_BsmbI_rev	GATC**CGTCTCG**CTCTGTAGAGGAGTTGGTCTCTA AGC
#17	JF_Goboca_part2.2_BsmbI_fwd	GATC**CGTCTCG**AGAGGAAACCAAACAACATAC
#18	BocaGo_part2_claI_rev	GATC**ATCGAT**TTACAACACTTTATTGATGTTTGTTT TTACAGGCATA
#19	Boca2_partI_notI_Fwd	ATTAG**GCGGCCGC**ATGCCTCCAATTAAACGC
#20	Boca2_sub1_BsmbI rev	GATC**CGTCTCG**CCAG ATGGTTGTTGGTCTTG
#21	Boca2_sub2_BsmbI_fwd	GATC**CGTCTCG**CTGG CTCCATGGAGGAGCGAGG
#22	Boca2_partI_BsmbI_rev	GATC**CGTCTCG**CGTCTTCCATTTCAGCCAGT
#23	Boca2_part2_BsmbI_Fwd	GATC**CGTCTCG**GACGCAAATGCTGTAGAAAAAGC TATAGC
#24	Boca2_part2_ClaI_rev	GATC**ATCGAT**TTACAACACTTTATTGATGTTTGTTT TGA
#25	JF_boca4_BsmbI_fwd	GTTA**CGTCTCT**GCAGATGCCTCCAATTAAACGC
#26	JF_boca4_BsmbI_rev	GTTA**CGTCTCT**AAGATTACAACACTTTATTGATGTT TGTTTTAAC
#27	JF_boca3_BsmbI_fwd	GTTA**CGTCTCT**GCAGATGCCTCCAATTAAAAGGCA AC
#28	JF_boca3_BsmbI_rev	GTTA**CGTCTCT**AAGATTACAACACTTTATTGATGTT TGTTTTAAC
#29	JF_Goboca_BsmbI_fwd	GTTA**CGTCTCT**GCAGATGCCTCCAATTAAAAGGCA
#30	JF_Goboca_bsmbI_rev	GTTA**CGTCTCT**AAGATTACAACACTTTATTGATGTT TGTTTTTAC
#31	JF_boca2_BsmbI_fwd	GTTA**CGTCTCT**GCAGATGCCTCCAATTAAACGC
#32	JF_boca2_BsmbI_rev	GTTA**CGTCTCT**AAGATTACAACACTTTATTGATGTT TGTTTTG

#33	Acceptor shuffling OE fwd1	GACAGAAG**AGAGACG**CGGAAAGTGAAGGGTGACTG
#34	Acceptor shuffling OE Rev1	GGCTAGGTT**CGAGACG**GTAAC
#35	Acceptor shuffling OE Rev2	CCCTTCACTTTCCG**CGTCTCT**CTTCTGTCTGTGAGGAAACA
#36	HboV1 wt genome EagI fwd	GATTC**CGGCCG**CCACAAGGAGGAGTGGTTATA
#37	HboV1 wt genome PacI rev	AGTTC**TTAATTAA**ATAAGCAAACAAAACAGCTCC
#38	HBoV1_BstBI fwd	CACTGC**TTCGAA**GACCTCA
#39	HBoV1 rev	CACAATGTACAAGGGCTGTC
#40	Fwd_chimBoV_BsmbI	AATGA**CGTCTCC**GAAGCAGACGAGATAACTGACGAGG
#41	LacZ fwd	CG**GAATTC**GTGCCGGAAAGCTGGCTGGAG
#42	LacZ 500 rev	ACTT**GAATTC**GTAGCGGCTGATGTTGAACTGG
#43	LacZ 800 rev	CG**GAATTC**GCAGACCATTTTCAATCCGCAC
#44	LacZ 1200 rev	CG**GAATTC**GATCCAGCGATACAGCGCGTC
#45	LacZ 1600 rev	CG**GAATTC**GCAAAGACCAGACCGTTCATAC
#46	Cas9_Nhe_fw	ATCAG**CGCTAG**CGCCACCATGGATTACAAAGAC
#47	Cas9_Cla_rv	GCTGAT**ATCGAT**TCATTTCTTTTTCTTAGCTTGACCAGC
#48	CMV_pacI_fwd	GTTAC**TTAATTAA**TTCGGTACCCGTTACATAACTTACGG
#49	SV40_Intron_NheI	GTTA**CGCTAG**CGGTGGCGACCGGTGCGG

Bold: restriction sites.

Table S4. Promoters used in this study.

Promoter	Sequence (5'- 3')
miCMV	GACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTT GTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCG CCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATAT AAGCAGAGCTCGTTTAGTGAACCGTCAGATC
CMV	CTGGATTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAAC GACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCC AATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTG CCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATT GACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGA CCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTA TTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCG GTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGA GTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACT CCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCT ATATAAGCAGAGCTGG
CMV+I	TTCGGTACCCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCG CCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGT AACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT AAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCC CCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTA CATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCAT CGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGAT AGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAAT GGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAA CAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGA GGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGA**TCGCCTGGAGA CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCC AGCCTCCGGACTCTAGAGGATCCGGTACTCGAGGAACTGAAAAACCAG AAAGTTAACTGGTAAGTTTAGTCTTTTTGTCTTTTATTTCAGGTCCCGGA TCCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGTGGATGTTGCCTT TACTTCTAGGCCTGTACGGAAGTGTTACTTCTGCTCTAAAAGCTGCGGA ATTGTACCCGCGGCCGCACCGGTCGCCACC**

Bold: SV40 intron.

Table S5. Basal and differentiation media used for cultivation of intestinal crypts.

Basal media	**Final concentration**
DMEM/F12 +GlutaMAX +HEPES +P/S	
Wnt3A	50% by volume
B27	1:50
N2	1:100
N-acetyl-cysteine	1 mM
R-spondin	10% by volume
Noggin	100 ng/ml
EGF	50 ng/ml
Gastrin	10 mM
Nicotinamide	10 mM
A83-01	500 nM
Sb202190	10 µM
Differentiation Media	**Final concentration**
DMEM/F12 +GlutaMAX +HEPES +P/S	
B27	1:50
N2	1:100
N-acetyl-cysteine	1 mM
R-spondin	5% by volume
Noggin	50 ng/ml
EGF	50 ng/ml
Gastrin	10 mM
A83-01	500 nM
Sb202190	10 µM

Table S6. Primer and probe sets used for titration of viral stocks.

Primer/Probes	Sequence 5'- 3'
NP1_fwd	GCACAGCCACGTGACGAA[1]
NP1_rev	TGGACTCCCTTTTCTTTTGTAGGA[1]
BoV_NP1_Probe	FAM-TGAGCTCAGGGAATATGAAAGACAAGCATCG-BHQ1[1]
CMVenh_fwd	AACGCCAATAGGGACTTTCC
CMVenh_rev	GGGCGTACTTGGCATATGAT
CMVenh_Probe	FAM-CGGTAAACTGCCCACTTGGCAGT-BHQ1
YFP_fwd	GAGCGCACCATCTTCTTCAAG
YFP rev	TGTCGCCCTCGAACTTCAC
YFP_probe	FAM-ACGACGGCAACTACA-BHQ1

1. Huang, Q, Deng, X, Yan, Z, Cheng, F, Luo, Y, Shen, W*, et al.* (2012). Establishment of a reverse genetics system for studying human bocavirus in human airway epithelia. *PLoS Pathog* **8**: e1002899.