CRISPR/Cas9-mediated Gene-knockout Screens and Target Identification via Whole Genome Sequencing Uncover Host Genes Required for Picornavirus Infection

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Supplementary Figure S1. IGV view of the *PVR* sgRNA target site in the RPV-1 and RPV-2 clones. The WGS data shows that both clones have mutations at the *PVR* target site: wild-type and mutant sequences are presented. Small indels were verified using IGV. The genomic location is chr19:45,150,636-45,150,704. The PAM sequences are indicated in red, and an insertion is indicated in blue.

Supplementary Figure S2. IGV view of the *ST3GAL4* sgRNA target site in the R68-1 and R68-2 clones. The WGS data reveal that both clones have mutations at the *ST3GAL4* target site. The genomic location is chr11:126,277,962-126,278,040. The PAM sequences are indicated in red, and an insertion is indicated in blue.

Supplementary Figure S3. Sequences of the sites containing sgRNA library-induced mutations in the R68-1 and R68-2 clones. IGV was used for the analysis. The PAM sequences are indicated in red, and the insertion sequences are indicated in blue. + indicates wild type.

Supplementary Figure S4. EV-D68-resistant colonies formed after transfection of sgRNAs targeting the *ST3GAL4* gene.

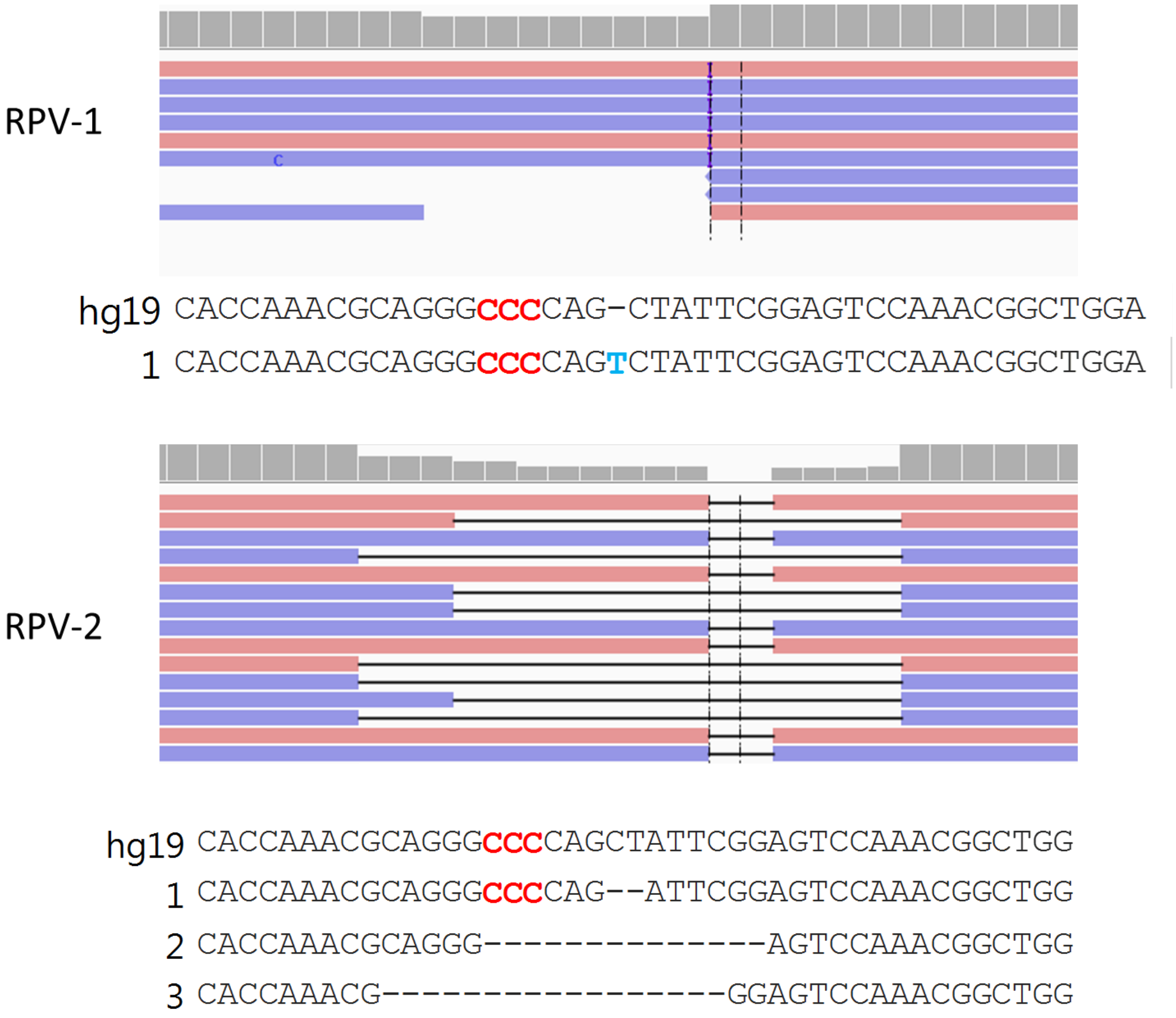
Supplementary Figure S5. IGV view of the genes that were knocked-out in clones R68-3, 4, 5, and R68-6. R68-3; MGAT5, chr2:135,012,121-135,012,263. R66-4, 5; COG1, chr17:71,189,364-71,189,404. R68-6; COG5, chr7:107,204,316-107,204,356. The PAM sequences are indicated in red, and the insertion sequences are indicated in blue.

Supplementary Figure S6. Sequences of the sites containing sgRNA library-induced mutations in the R68-3, 4, 5, and R68-6 clones. IGV was used for the analysis. The PAM sequences are indicated in red, and the insertion sequences are indicated in blue. + indicates wild type.

Supplementary Table S1. List of sgRNA sequence

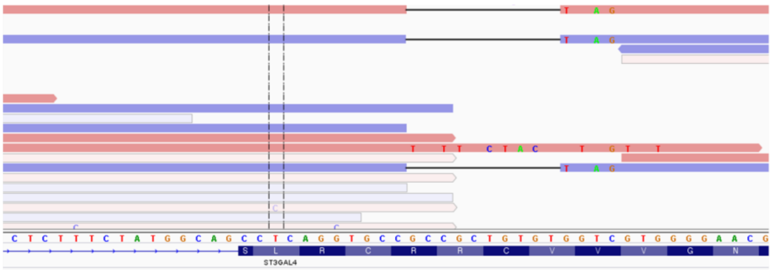
Supplementary Table S2. Sequence of RPV-1, 2 clones

Supplementary Figure S1. IGV view of the *PVR* sgRNA target site in the RPV-1 and RPV-2 clones. The WGS data shows that both clones have mutations at the *PVR* target site: wild-type and mutant sequences are presented. Small indels were verified using IGV. The genomic location is chr19:45,150,636-45,150,704. The PAM sequences are indicated in red, and an insertion is indicated in blue.



Supplementary Figure S2. IGV view of the *ST3GAL4* sgRNA target site in the R68-1 and R68-2 clones. The WGS data reveal that both clones have mutations at the *ST3GAL4* target site. The genomic location is chr11:126,277,962-126,278,040. The PAM sequences are indicated in red, and an insertion is indicated in blue.

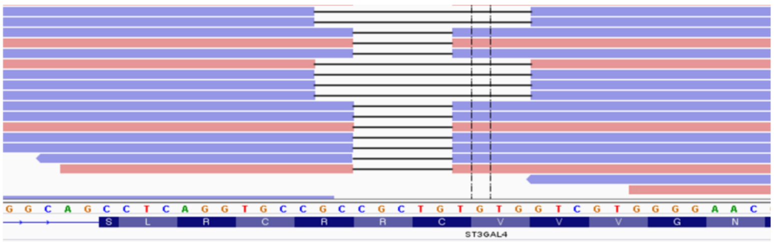
R68-1



6484

```
hg19 GCAGCCTCAGGTGCCGCCGCTGTGTGGTCGTGGGGAACGGGCACCGGCTGCGGAACAGCT
   1 GCAGCCTCAGGTGCCTGAG----------GTGGGGAACGGGCACCGGCTGCGGAACAGCT    10del
   2 GCAGCCTCAGGTGCCGCC------------------------------------------    1015del
```

R68-2



6484

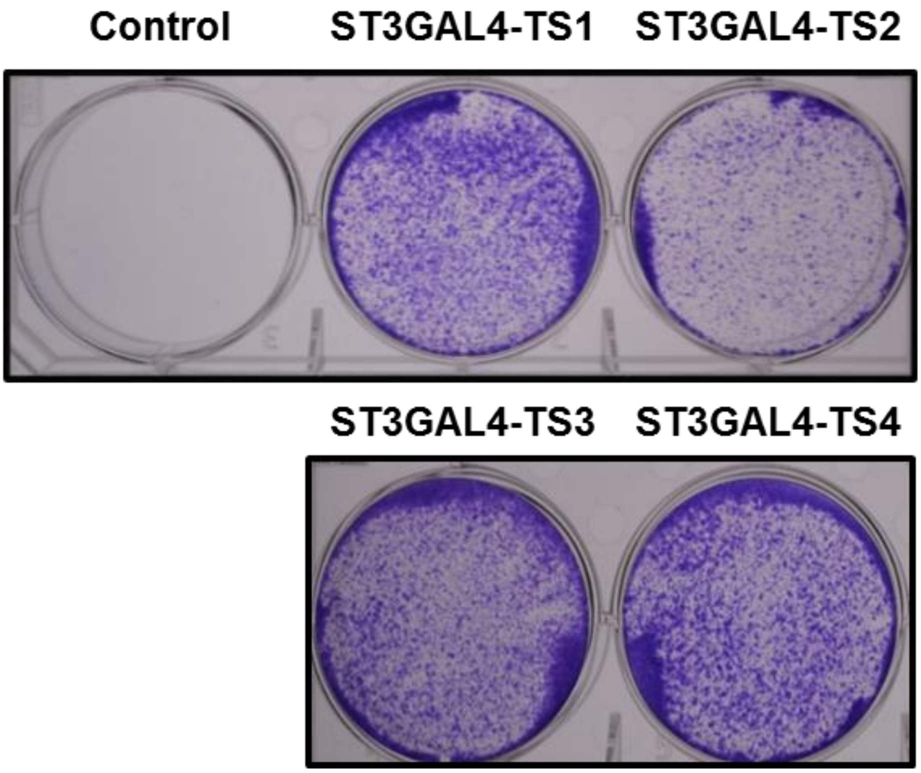
```
hg19 GACCACTCCTCTTTCTATGGCAGCCTCAGGTGCCGCCGCTGTGTGGTCGTGGGGAACGGG
   1 GACCACTCCTCTTTCTATGGCAGCCTCAGGTGCCGC-----TGTGGTCGTGGGGAACGGG    5del
   2 GACCACTCCTCTTTCTATGGCAGCCTCAGGTGCCG-----------TCGTGGGGAACGGG    11del
```

Supplementary Figure S3. Sequences of the sites containing sgRNA library-induced mutations in the R68-1 and R68-2 clones. IGV was used for the analysis. The PAM sequences are indicated in red, and the insertion sequences are indicated in blue. + indicates wild type.

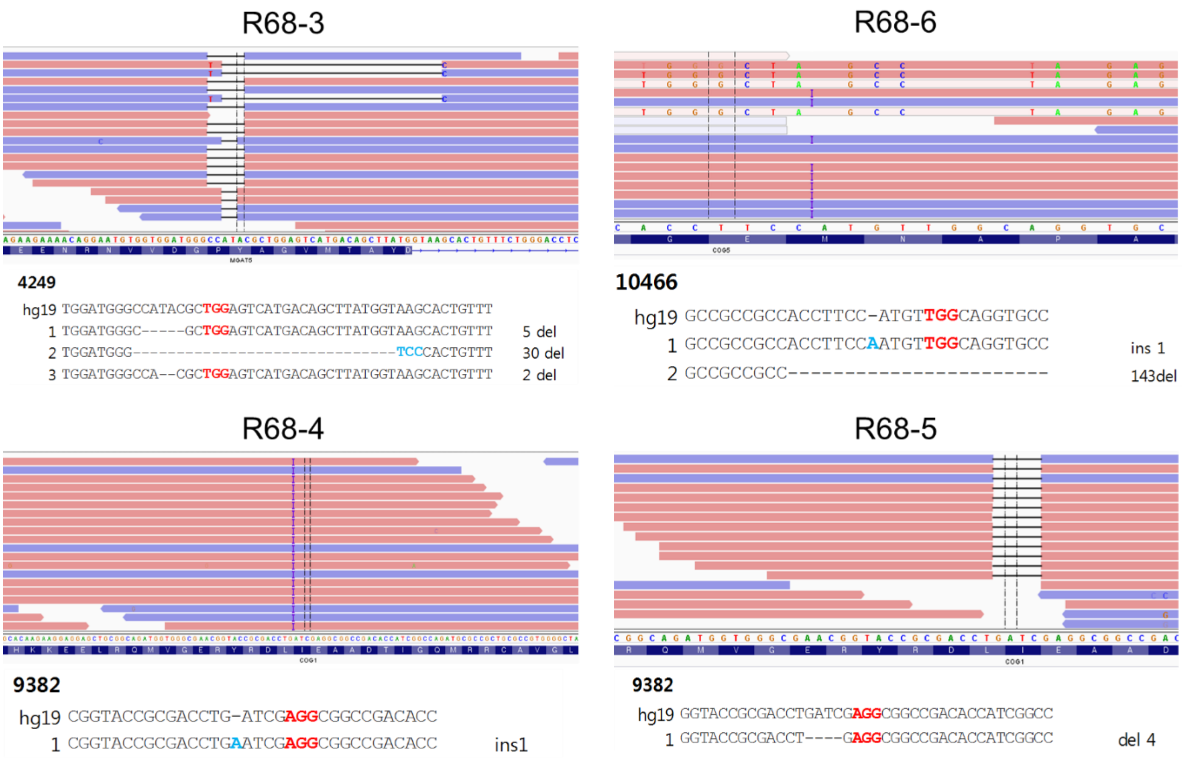
```
R68-1
  6484
  hg19 GCAGCCTCAGGTGCCGCCGCTGTGTGGTCGTGGGGAACGGGCACCGGCTGCGGAACAGCT
     1 GCAGCCTCAGGTGCCTGAG----------GTGGGGAACGGGCACCGGCTGCGGAACAGCT      10del
     2 GCAGCCTCAGGTGCCGCC------------------------------------------      1015del
  1606
  hg19 GCCTTGTGAAGTCAGCACCTATGCCAAGTCTCGGAAGGA-CATTGGTGTGAGTGATCTCA
     1 GCCTTGTGAAGTCAGCACCTATGCCAAGTCTCGGAAGGA-CATTGGTGTGAGTGATCTCA      +
     2 GCCTTGTGAAGTCAGCACCTATGCCAAGTCTCGGAAGGATCATTGGTGTGAGTGATCTCA      1 ins
 84432
  hg19 TACAGGCCTGTG-AGCGGGATGTCCAGTGTGGGGCAGGCACCTGCTGTGCCATCAGCCTG
     1 TACAGGCCTGTG-AGCGGGATGTCCAGTGTGGGGCAGGCACCTGCTGTGCCATCAGCCTG      +
     2 TACAGGCCTGTGAAGCGGGATGTCCAGTGTGGGGCAGGCACCTGCTGTGCCATCAGCCTG      1 ins

R68-2
  6484
  hg19 GACCACTCCTCTTTCTATGGCAGCCTCAGGTGCCGCCGCTGTGTGGTCGTGGGGAACGGG
     1 GACCACTCCTCTTTCTATGGCAGCCTCAGGTGCCGC-----TGTGGTCGTGGGGAACGGG      5del
     2 GACCACTCCTCTTTCTATGGCAGCCTCAGGTGCCG-----------TCGTGGGGAACGGG      11del
 79587
  hg19 ATGAAAGAAATTATCTGAGGAATATTTTCGGTTACCCTCAGGTACA-CCGTGGGTGGGA
     1 ATGAAAGAAATTATCTGAGGAATATTTTCGGTTACCCTCAGGTACA-CCGTGGGTGGGA       +
     2 ATGAAAGAAATTATCTGAGGAATATTTTCGGTTACCCTCAGGTACAACCGTGGGTGGGA       1 ins
 56140
  hg19 TTCCATGTGGACGTGGAGGTGAAGGATGTTAATGACAACCCGCC-AGTGTTCCGG
     1 TTCCATGTGGACGTGGAGGTGAAGGATGTTAATGACAACCCGCC-AGTGTTCCGG           +
     2 TTCCATGTGGACGTGGAGGTGAAGGATGTTAATGACAACCCGCCAAGTGTTCCGG           1 ins
```

Supplementary Figure S4. EV-D68-resistant colonies formed after transfection of sgRNAs targeting the *ST3GAL4* gene.



Supplementary Figure S5. IGV view of the genes that were knocked-out in clones R68-3, 4, 5, and R68-6. R68-3; MGAT5, chr2:135,012,121-135,012,263. R66-4, 5; COG1, chr17:71,189,364-71,189,404. R68-6; COG5, chr7:107,204,316-107,204,356. The PAM sequences are indicated in red, and the insertion sequences are indicated in blue.



Supplementary Figure S6. Sequences of the sites containing sgRNA library-induced mutations in the R68-3, 4, 5, and R68-6 clones. IGV was used for the analysis. The PAM sequences are indicated in red, and the insertion sequences are indicated in blue. + indicates wild type.

R68-3

4249

```
hg19 GGGCCATACGCTGGAGTCATGACAGCTTATGGTAAGCAC
   1 GGGC-----GCTGGAGTCATGACAGCTTATGGTAAGCAC      5 del
   2 GGG------------------------------TCCCAC      30 del
   3 GGGCCA--CGCTGGAGTCATGACAGCTTATGGTAAGCAC      2 del
```

2525

```
hg19 GGGTGGGGCGGGTGGGAGTGGTGTCCTGTCGGGAGGACC
   1 GGGTGGGGCGGGTGGGAGTGGTGTCCTGTCGGGAGGACC      +
   2 -------------GGGAGTGGTGTCCTGTCGGGAGGACC      Large del
```

R68-4

9382

```
hg19 CGGTACCGCGACCTG-ATCGAGGCGGCCGACACC
   1 CGGTACCGCGACCTGAATCGAGGCGGCCGACACC           ins1
```

9320

```
hg19 TTTTGAAGCCAGCTTGGTAGGTTTCGCAGATCT
   1 TTTTGAAGCCAGCTTGGTAGGTTTCGCAGATCT            +
   2 TTTTGAAGCCAGCTTC--AGGTTTCGCAGATCT            del 2
```

127665

```
hg19 GCCCGCCCCGCAGGCACCGGTAGGGA
   1 GCCCGCCCCGCAGGCACCGGTAGGGA                   +
   2 GCCCGCCCCG----TCCCTGTAGGGA                   del 4
```

201232

```
hg19 TCGCCTCCATAGGAA-TCGCGGTGCAGCA
   1 TCGCCTCCATAGGAA-TCGCGGTGCAGCA                +
   2 TCGCCTCCATAGGAAATCGCGGTGCAGCA                ins 1
   3 TCGCCTCCATAGGAATTCGCGGTGCAGCA                ins 1
```

57514

```
hg19 GGCGCTGAACATCTCCGAGCCCTTTGCGGTATCTGTG
   1 GGCGCTGAACATCTCCGAGCCCTTTGCGGTATCTGTG        +
   2 GGCGCTG----------------------TATCTGTG        del 22
   3 GGCGCTGAACATCTCCGATACTCCACCGGTATCTGTG        ins 8, del 8
```

7150

```
hg19 AAGATAGAGCCTCCTGGACTTTT-CCGTGGCCGCGG
   1 AAGATAGAGCCTCCTGGACTTTT-CCGTGGCCGCGG         +
   2 AAGATAGAGCCTCCTGGACTTTTTCCGTGGCCGCGG         ins 1
```

R68-6

10466

```
hg19 GCCGCCGCCACCTTCC-ATGTTGGCAGGTGCC
   1 GCCGCCGCCACCTTCCAATGTTGGCAGGTGCC             ins 1
   2 GCCGCCGCC-----------------------             143del
```

576

```
hg19 TGCGTGCCCGTGGCCTGGCACATGCGGAAGCGG
   1 TGCGTGCCCGTGGCCTGGCACATGCGGAAGCGG            +
   2 TGCGTGCCCGTGGCCTGGCAC------AAGCGG            6del
```

6860

```
hg19 CTGGCAAGCCACGG-GCTTCCTTGA
   1 CTGGCAAGCCACGG-GCTTCCTTGA                    +
   2 CTGGCAAGCCACGGGGCTTCCTTGA                    ins 1
```

R68-5

9382

```
hg19 GGTACCGCGACCTGATCGAGGCGGCCGACACCATCGGCC
   1 GGTACCGCGACCT----GAGGCGGCCGACACCATCGGCC      del 4
```

84699

```
hg19 CAGGACGGCATCCTGAGACACGTGGAGCTGGGCGAGGG
   1 CAGGACGGCATCCTGAGACACGTGGAGCTGGGCGAGGG       +
   2 CAGGACGG------------CGTGGAGCTGGGCGAGGG       del 12
```

2175

```
hg19 CTGGAGCCGTGC-AGATCTGTCCCACGCTA
   1 CTGGAGCCGTGC-AGATCTGTCCCACGCTA               +
   2 CTGGAGCCGTGCAAGATCTGTCCCACGCTA               ins 1
```

553115

```
hg19 GGAGATATAGCCACTG-TGATCTGAGTCCACCG
   1 GGAGATATAGCCACTG-TGATCTGAGTCCACCG            +
   2 GGAGATATAGCCACTGTTGATCTGAGTCCACCG            ins 1
```

7084

```
hg19 GGCTGCCTTTGATGAGCCACTCCTCATGGAGATGGTGAA
   1 GGCTGCCTTTGATGAGCCACTCCTCATGGAGATGGTGAA      +
   2 GGCT-----------------------------GGTGAA      del 29
```