Supplementary Figure 1. CRISPR Screen Workflow.

Flowchart illustrating the workflow and timeline to generate CRISPR library and necessary validation steps, including the generation of BV2-dCas9-VP64 cells and their validation.

Supplemental Figure 2. Comparison of MNV CRISPRa screens.

A) A heat map showing enrichment of genes from the current CRISPRa screens performed in BV2 cells and a previously conducted CRISPRa screen in HeLa-CD300lf with indicated MNV strains. Genes are color-coded based upon their z-score for comparison across differential screening conditions. The genes TBX19 and MROH3 (black boxes) were absent in the library Calabrese while present in Caprano. The genes Obox6 and Olfr290 (black boxes) are murine genes without human homologues.

B) Gene set enrichment analysis (GSEA) was performed on the top genes in MNV CRISPRa screens across the four conditions as indicated by color. Analysis of genes against GO terms is presented in a bubble plot where bubble size represents gene ratio.

Supplemental Figure 3. Coverage maps of deep sequencing of MNV populations.

Alignment of the sequencing reads to the MNVCW3 (left) or MNVCR6 (right) genome for the six individual passaging experiments as indicated. The y-axis depicts sequencing coverage per position. The x-axis denotes the nucleotide position in the genome a diagram of the protein coding regions below.

Supplemental Figure 4. The NS6-7 cleavage sequence is highly conserved amongst MNV strains

A) Sequence alignment of the NS6-7 junction from over 70 MNV strains in Genbank.

B) A sequence logo of the NS6-7 junction from the MNV sequences in **A.**

Supplemental Table 1. BV2 CRISPRa Read Counts

**Supplemental Table 2. Analysis output of BV2 CRISPRa screensSupplemental Table 3. GSEA analysis of MNV CRISPRa screens**

**Supplemental Table 4 . HeLa-CD300lf CRISPRa Read Counts**

**Supplemental Table 5. List of sgRNA constructs used in this study**

Supplemental Table 6. Mutational analysis of MNV passaging experiments in Trim7 expressing cells

Supplemental Figure 1

○ Transduce BV2 cells with dCas9

dCas9-VP64_Blast
Select with Blasticidin

○ Transduce BV2 cells with Reporter Assay

XPR_502 vector, Cd4 sgRNAs
or
XPR_502 vector, empty
Select with Puromycin

○ Cytometric assessment of dCas9 activity
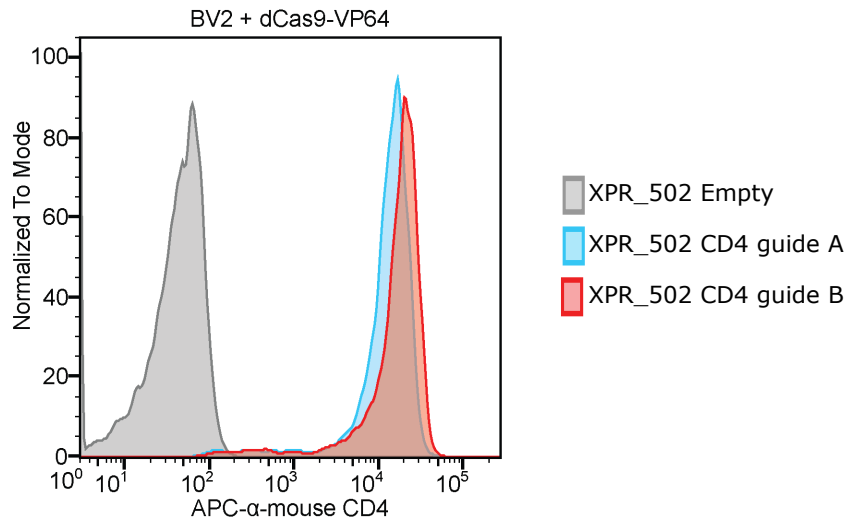
Day 0 ○ Transduce dCas9-VP64_Blast BV2 cells with Caprano

sgRNA library in XPR_502
Gene Set A
Gene Set B
Select with Puromycicn

Day 5 ○ Split to infection arms

MNV^{CW3}
MNV^{CR6}

Day 16+ ○ Pellet screen

Genomic DNA extraction
PCR of sgRNAs
NextGen Sequencing

Supplemental Figure 2
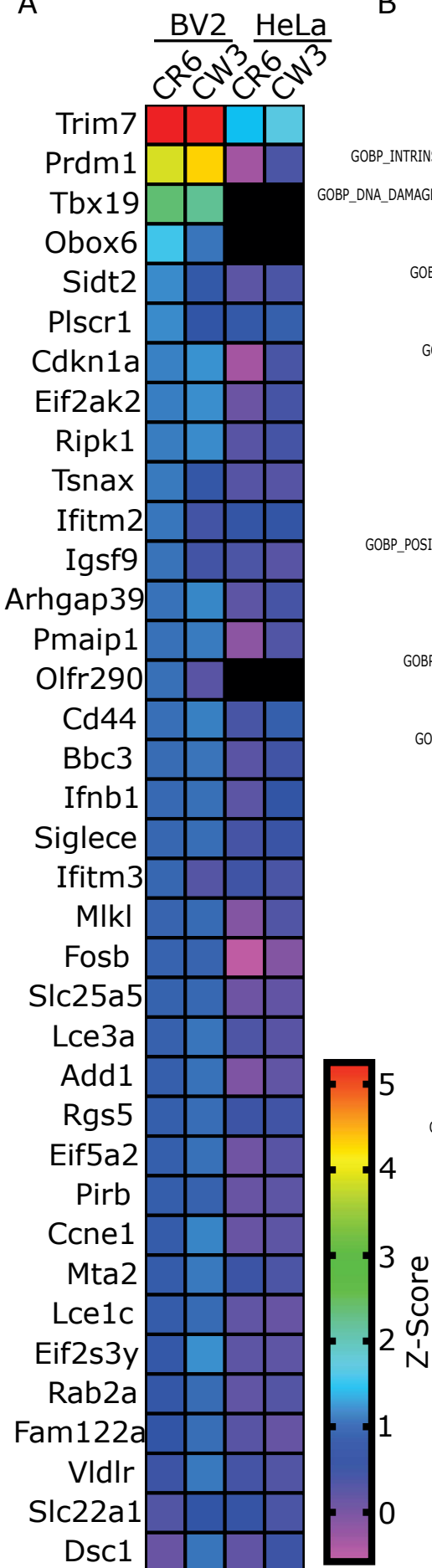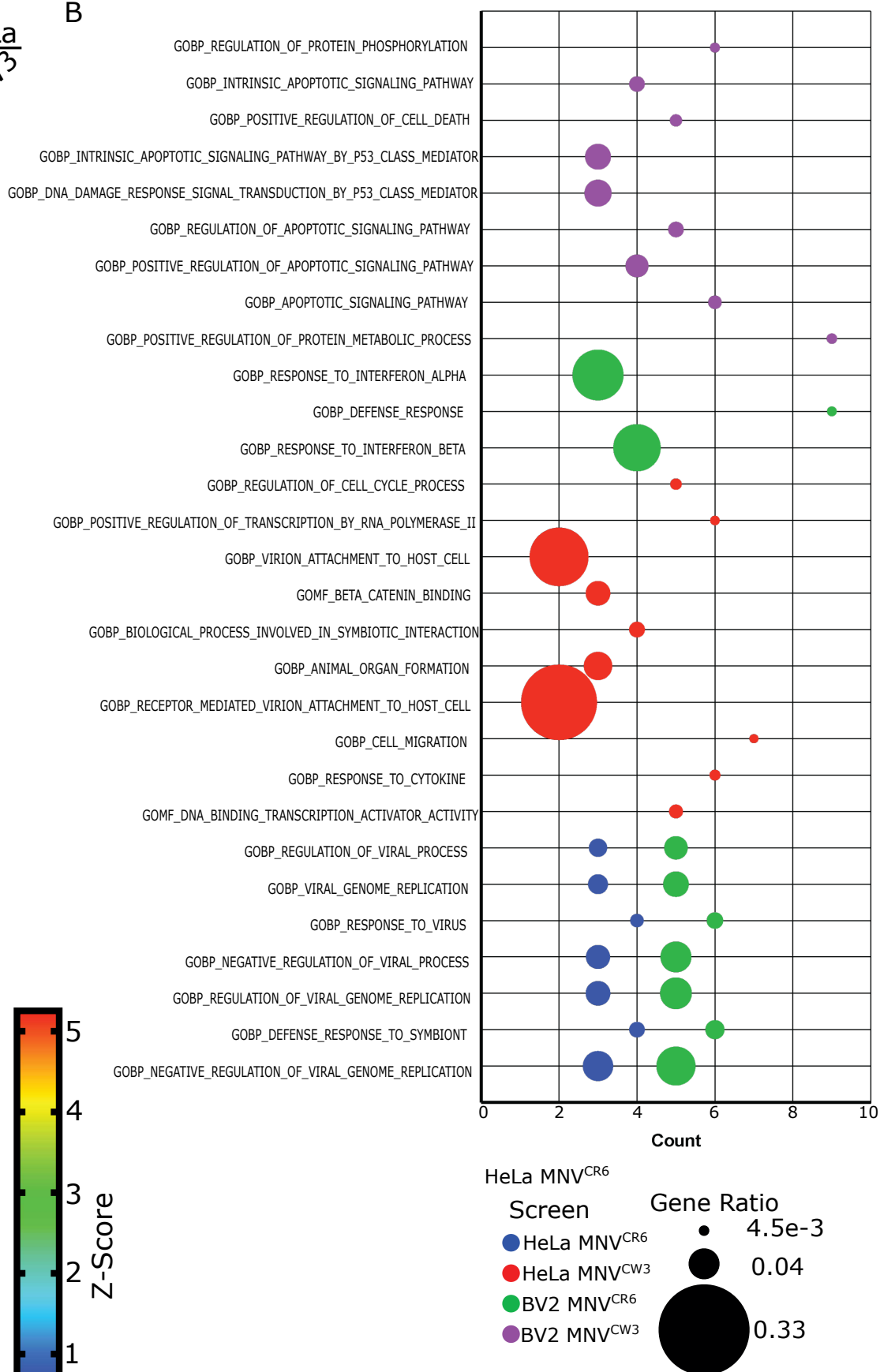
Supplemental Figure 3

MNV^CW3 + HeLa-CD300lf-Trim7 Replicate A
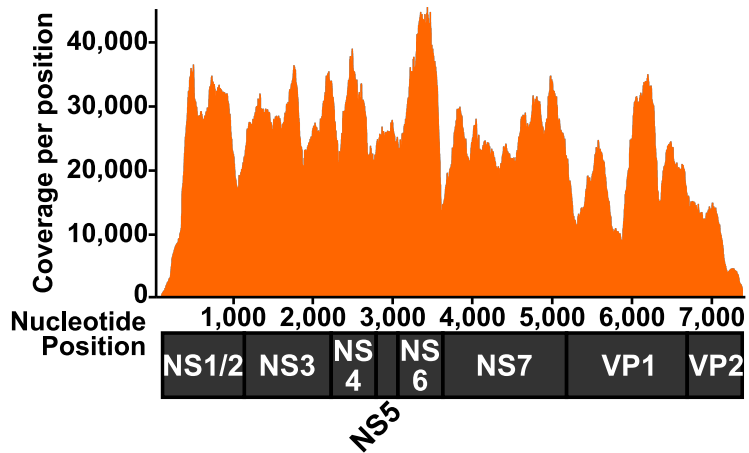
MNV^CR6 + HeLa-CD300lf-Trim7 Replicate A
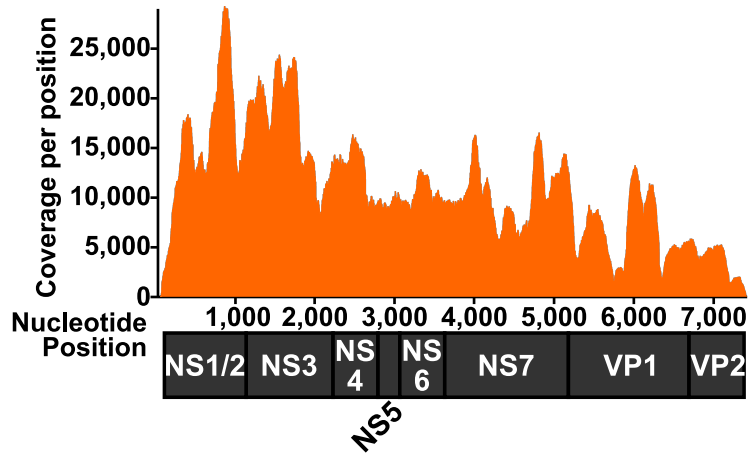
MNV^CW3 + HeLa-CD300lf-Trim7 Replicate B
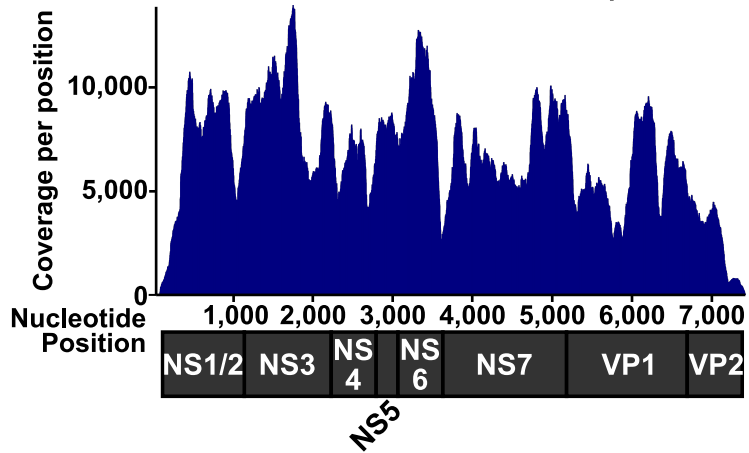
MNV^CR6 + HeLa-CD300lf-Trim7 Replicate B
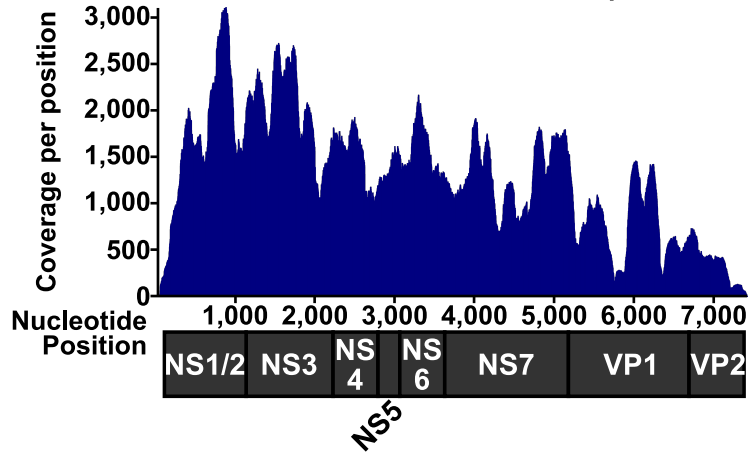
MNV^CW3 + BV2-Trim7
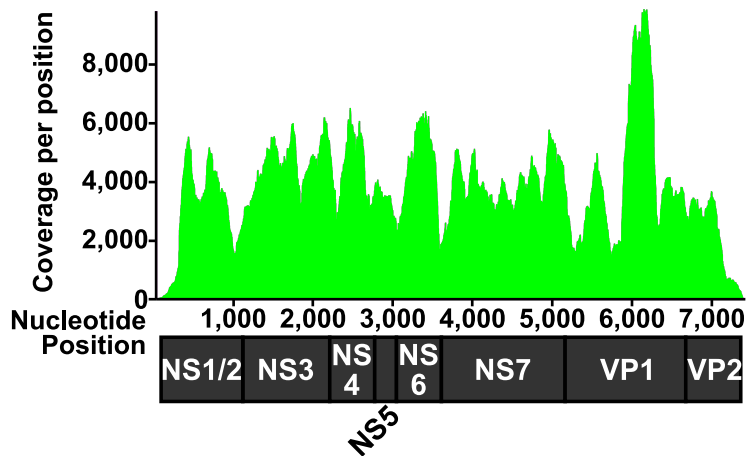
MNV^CR6 + BV2-Trim7

Supplemental Figure 4

A

B