Physiologic RNA Targets and Refined Sequence Specificity of Coronavirus EndoU

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Supplemental Figures and Tables
SUPPLEMENTAL FIGURES AND TABLES

Figure S1. Frequency and location of endoribonuclease cleavage sites in U6 snRNA and MHV negative-strand RNA in WT, IFNAR<sup>−/−</sup>, and RNase L<sup>−/−</sup> BMM. Normalized 2′-3′-cp cDNA reads captured at each position along the (A) U6 snRNA and (B) MHV negative-strand RNA at 9 and 12 hpi with MHV<sup>(S)</sup>, MHV<sup>(V)</sup>, PDE<sup>mut</sup>, and EndoU<sup>mut</sup> virus in IFNAR<sup>−/−</sup> BMM.

Figure S2. Frequency and location of endoribonuclease cleavage sites in MHV positive-strand RNA in IFNAR and RNase L KO BMM. (A and B) Normalized cyclic phosphate cDNA reads captured at each position along the MHV genomic RNA at 9 and 12 hpi with MHV<sup>(S)</sup>, MHV<sup>(V)</sup>, PDE<sup>mut</sup>, and EndoU<sup>mut</sup> virus in (A) IFNAR<sup>−/−</sup> and (B) RNase L<sup>−/−</sup> BMM. Putative cleavage sites attributed to EndoU or RNase L were calculated from RNase L- or EndoU-dependent signal generated by subtracting signal from each captured position that occurs in the absence of either enzyme (RNase L<sup>−/−</sup> BMM or during EndoU<sup>mut</sup> infection). These data were then filtered for sites with reads representing at least 0.01 % of total reads in the library. At each of these positions, the log<sub>2</sub> fold change in signal when either RNase L or EndoU were absent was calculated and sites with ≥2.5 fold change were designated putative RNase L or EndoU sites.

Figure S3. Dinucleotide cleavage pattern in MHV positive-strand RNA downstream of captured 3′ RNA end. Percent total 2′-3′-cp cDNA reads captured at each dinucleotide in WT BMM at 9 and 12 hpi for bases +1:+2 (A) and +2:+3 (B) from the captured cleavage position (0-base).

Figure S4. Endoribonuclease cleavage preferences in MHV RNA from IFNAR<sup>−/−</sup> and RNase L<sup>−/−</sup> BMM. (A) and (B) Dinucleotide specificity analysis for cleavage in MHV positive-strand RNA by percent total cyclic phosphate cDNA reads captured at each 3′-dinucleotide at 9 and 12 hpi in (A) IFNAR<sup>−/−</sup> BMM for positions -2:-1 and in (B) RNase L<sup>−/−</sup> BMM for positions -1:+1.
Figure S5. Interaction between RNase L and EndoU cleavage at UA sequences in MHV RNA. (A) UA cleavage scoring analysis. All UA sequences in the MHV positive-strand RNA with >= 30 cyclic phosphate counts in either the UA* or U*A cleavage position were compared by calculating the ratio of normalized counts (UA* counts / U*A counts). Ratios > 1 were scored as UA* (RNase L) sites and ratios < 1 were scored as U*A sites (EndoU) and total number of scored sites for either position are shown for each condition of viral infection in IFNAR−/− and RNase L−/− BMM. (B) Frequency and location of UA positions in WT BMM under all conditions of viral infection which had ≥ 50 counts at U*A positions and ≤ 1 counts at UA* positions. The top 5 of these positions by normalized count are labeled. (C) Frequency and location of UA positions in IFNAR−/− and RNase L−/− BMM under all conditions of viral infection which had ≥ 50 counts at U*A positions and ≤ 1 counts at UA* positions.

Figure S6. MHV RNA abundance and RNAseq reads across the MHV genome. (A) Total RNAseq normalized counts assigned to MHV genomic features for each library. (B) Coverage of RNAseq read density across the MHV genome in reads per million.

Figure S7. Frequency and location of endoribonuclease cleavage in MHV 3′-UTR. Nucleotide and sequence resolution graphs displaying normalized counts in (A) WT, (B) IFNAR−/−, (C) RNase L−/− BMM during infection with MHV(V) and EndoUmut MHV. Nucleotide resolution graphs of region directly upstream of poly-A tail with cyclic phosphate counts normalized by RNA abundance in (D) WT, (E) IFNAR−/− (F) RNase L−/− BMM.

Figure S8. Regional cleavage of MHV RNA and subgenomic mRNA abundance. A) Percent normalized cyclic phosphate signal captured per MHV genomic region and normalized to length of each region for WT, IFNAR−/−, RNase L−/− BMM across all conditions of viral infection at 12 hpi. Dotted line represents baseline percent of cleavage expected by cell type ([total number of cyclic phosphate counts / total genome size x 100 ]). (B) Cyclic phosphate counts normalized by viral RNA abundance displayed by region for WT, IFNAR−/−, RNase L−/− BMM across all
conditions of viral infection at 12 hpi. (C) Percent cyclic phosphate counts normalized by mRNA abundance and to the length of each region for WT, IFNAR\(^{-/-}\), RNase L\(^{-/-}\) BMM across all conditions of viral infection at 12 hpi.

**Figure S9. Cyclic phosphate sequencing analysis of experiment 2.** (A) Normalized cyclic phosphate cDNA reads \([\text{reads at each position} / \text{total reads in library}]\) aligning to host and viral RNAs at 9 and 12 hpi in WT and RNase L\(^{-/-}\) bone marrow macrophages (BMM). (B) Putative cleavage sites attributed to EndoU or RNase L were calculated from RNase L- or EndoU-dependent signal generated by subtracting signal from each captured position that occurs in the absence of either enzyme (RNase L\(^{-/-}\) BMM or during EndoU\(^{mut}\) infection). These data were then filtered for sites with reads representing at least 0.01 % of total reads in the library. At each of these positions, the log2 fold change in signal when either RNase L or EndoU were absent was calculated and sites with >= 2.5 fold change were called as putative RNase L or EndoU sites. (C and D) Dinucleotide specificity analysis for cleavage in MHV positive-strand RNA by percent total cDNA reads captured at each 3´-dinucleotide in WT BMM at 9 and 12 hpi for (C) Dinucleotide analysis for positions -2: -1 and (D) Dinucleotide analysis for positions -1:+1 from captured cleavage position (0 position). (E) Cumulative distribution of normalized counts by position of the MHV genome for every position with >= 10 cyclic phosphates counts across all cell types and infection conditions. (F) UA cleavage scoring analysis. All UA sequences in the MHV positive-strand RNA with ≥30 cyclic phosphate counts in either the UA\(^\downarrow\) or U\(^\downarrow\)A cleavage position were compared by calculating the ratio of normalized counts (UA\(^\downarrow\) counts / U\(^\downarrow\)A counts). Ratios > 1 were scored as UA\(^\downarrow\) (RNase L) sites and ratios <1 were scored as U\(^\downarrow\)A sites (EndoU) and total number of scored sites for either position are shown for each condition of viral infection in WT BMM at 9 and 12 hpi. (G) Nucleotide and sequence resolution graphs displaying normalized counts in WT BMM during infection with MHV\(^{(S)}\) and EndoU\(^{mut}\) MHV of region directly upstream of poly(A) tail.
Figure S10. Cyclic phosphate and RNAseq analysis of MHV RNA from experiment 2. (A) Sum of normalized counts displayed by region for WT and RNase L<sup>−/−</sup> BMM across all conditions of viral infection at 12 hpi. Transcription regulatory sequences (TRS) are numbered by their associated mRNA (2-7). Other genomic regions are labeled as shown in Figure 1A. (B) Sum of cyclic phosphate counts normalized by mRNA abundance and length of each genomic region [(sum per region (cyclic phosphate counts / RNAseq counts) / length of region (bp) *100 ) sum of cyclic phosphate abundance normalized counts per region)/length of region * 100] displayed by region for WT and RNase L<sup>−/−</sup> BMM across all conditions of viral infection at 12 hpi. (C) Normalized counts (sum of subgenomic mRNA / sum of all mRNAs) of subgenomic mRNAs detected in WT and RNase L<sup>−/−</sup> BMM across all conditions of viral infection at 9 and 12 hpi.

Figure S11. Endoribonuclease cleavage of rRNA during WT and mutant MHV infection. (A) RNase L-dependent cleavage sites in 18S rRNA by fold change in signal when comparing WT or IFNAR<sup>−/−</sup> BMM mock-infected or infected with MHV<sup>(S)</sup>, MHV<sup>(V)</sup>, PDE<sup>mut</sup>, and EndoU<sup>mut</sup> virus to RNase L<sup>−/−</sup> BMM. Log2 fold change in the absence of RNase L activity was calculated for each position in the rRNA. The distribution of the top 100 RNase L-dependent cleavage sites were compared in WT and IFNAR<sup>−/−</sup> BMM under conditions of mock infection or infection with MHV<sup>(S)</sup>, MHV<sup>(V)</sup>, PDE<sup>mut</sup>, and EndoU<sup>mut</sup> virus at 9 hpi. 18S rRNA was cleaved in an RNase L-dependent manner at UU<sup>542</sup>, UU<sup>543</sup>, UU<sup>771</sup> and UA<sup>772</sup>. (B) RNase L-dependent cleavage of 18S rRNA at UU<sup>771</sup> and UA<sup>772</sup> at 9 and 12 hpi, predominantly in MHV PDE<sup>mut</sup>- and EndoU<sup>mut</sup>-infected WT BMM. (C) Top 3 RNase L-dependent cleavage sites in 18S rRNA by fold change (log2WT BMM / RNase L<sup>−/−</sup> BMM) for all conditions of infection at 9 and 12 hpi in WT BMM. (D) Table of total RNase L- or EndoU-dependent cleavage sites in 18S, 28S, 5.8S, and 5S rRNA. RNase L- or EndoU-dependent cleavage were determined by identifying the top 1% of enzyme-dependent signal with a > 4 fold change in signal in the absence of RNase L or EndoU that match the RNase L ("UA", "UU", "UG", "UC") or EndoU ("UA", "CA") sequence preferences.
Figure S12. Correlation between cyclic phosphate counts and viral RNA abundance. (A and B). Correlation between viral RNA abundance and 2’-3’-cp counts at each base captured in the MHV positive-strand RNA (all comparisons significant (p < 10^-50) for experiment 1 (A) and experiment 2 (B). (C and D) Distribution of sites in MHV by normalized cyclic phosphates counts for sense and antisense RNAs from experiment 1 (C) and experiment 2 (D) across all conditions of infection and cell types. Positions are only shown if there was > 1 read in either the sense and antisense RNA.

Figure S13. Effect of WT and mutant MHV infection on other cellular endoribonucleases. Expression (log10 normalized RNAseq counts) of (A) RNase A, (B) angiogenin, (C) RNaseT2A, and (D) RNase T2B transcripts.

Figure S14. Differential expression of host RNAs involved in innate and inflammatory pathways during MHV infection. (A) Results of differential expression analysis within samples comparing 9 and 12 hpi using only samples with at least 2 replicates. (B and C) Gene ontology (GO) analysis of host gene expression during MHV infection. Categories of biological processes (BP) enriched with significantly upregulated genes (p < 0.01, log2FC > 2) during infection with (B) MHV(s) or (C) EndoUmut in WT BMM. All significantly enriched (weightFisher < 0.01) results from the BP category are shown. (D and E) Heatmaps showing the expression of transcripts from the enriched GO categories involving (D) “cytokine enrichment” and (E) “positive regulation of NF-kappaB” ordered by hierarchical clustering for experiments 1 and 2 at 12 hpi.

Figure S15. Relationship between cyclic phosphate and RNAseq abundance in host mRNAs. (A and B) An Enrichment score was calculated for mRNAs using the total sum of cyclic phosphate or RNAseq normalized counts in mock/wt MHV/PDEmut samples / total sum of cyclic phosphate or RNAseq normalized counts in EndoUmut samples at 9 and 12 hpi in WT and RNase L-/- BMM (enrichment score = [sample] / [EndoUmut]) for experiment 1 (A) and experiment 2 (B). Genes were assigned to bins as follows: Bin1 = cyclic phosphate and
RNAseq enrichment scores were < 1, Bin 2 = cyclic phosphate and RNAseq enrichment scores >=1, Bin 3 = cyclic phosphate score < 1 and RNAseq score > 1=, Bin 4 = cyclic phosphate score >= 1 and RNAseq score < 1. Each bin includes genes assigned from 9 and 12 hpi and highlighted genes include those identified as part of the dsRNA response (Fig. 7C) and those involved in innate and inflammatory signaling (Figs, 7D and 7E).

Figure S16. Endoribonuclease cleavage specificity of host mRNAs during MHV infection. (A-D) Dinucleotide specificity analysis for cleavage of transcripts involved in the dsRNA response (Fig. 7C) during infection with mock, MHV\textsuperscript{(v)} and EndoU\textsuperscript{mut} for positions -2:-1 (A) or mock, MHV\textsuperscript{(v)} and PDE\textsuperscript{mut} for positions -1:+1 (B) from experiment 1. For experiment 2, analysis included mock, MHV\textsuperscript{(s)}, PDE\textsuperscript{mut}, and EndoU\textsuperscript{mut} for positions -2:-1 (C) and positions -1:+1 (D) in WT and WT and RNase L \textsuperscript{-/-} BMM. Percent of cleavage at each 3’-dinucleotide was calculated relative to the total cDNA reads aligned to the mm10 transcriptome per library at 9 and 12 hpi. (E and F) Dinucleotide specificity analysis for all mRNAs during infection with mock, MHV\textsuperscript{(s)}, MHV\textsuperscript{(v)}, PDE\textsuperscript{mut}, and EndoU\textsuperscript{mut} viruses for positions -2:-1 (E) and positions -1:+1 (F) in WT BMM from experiment 1.

Table S1 and S2. Dinucleotide enrichment and de-enrichment analysis at 9 and 12 hpi in WT BMM. Complete tables of dinucleotide enrichment and de-enrichment for -2 base: +2 base (table 1) or -1 base: -1 base (table 2) from the captured RNA end (0-base position) for each condition of viral infection at 9 and 12 hpi in WT BMM by comparing the frequency of dinucleotide capture in experimental conditions to the frequency of occurrence for each dinucleotide in the MHV genomic RNA sequence (control). Significant enrichment was determined by adjusted p-value (q) for fold change (log2(experiment / control)). (<0.02\textsuperscript{*}, <0.0001\textsuperscript{**}, <1x10\textsuperscript{8}\textsuperscript{***}).

Table S3: SNP variants in MHV genome related to endoribonuclease cleavage. Table of all single nucleotide variants (SNPs) identify from alignments of RNAseq libraries to the MHV
genome. The SNPs in green are sites where the mutation generated a “CA” dinucleotide that was cleaved by EndoU. The SNPs in red and yellow are the inactivating mutations in the EndoU and PDE domains of MHV respectively.

Table S4: Coronavirus RNA sequences adjacent to poly(A) tail.
A  **Position +1 to +2 dinucleotide analysis in WT BMM**

![Graphs showing dinucleotide cleavage analysis for MHV(V) and PDE](image)

**Figure S3**

B  **Position +2 to +3 dinucleotide analysis in WT BMM**

![Graphs showing dinucleotide cleavage analysis for MHV(V) and PDE](image)
A  Dinucleotide analysis in IFNAR\(^{-}\) BMM

Position -2 to -1 dinucleotide analysis

Position -1 to +1 dinucleotide analysis

Position +1 to +2 dinucleotide analysis

B  Dinucleotide analysis in RNase L\(^{-}\) BMM

Position -2 to -1 dinucleotide analysis

Position -1 to +1 dinucleotide analysis

Position +1 to +2 dinucleotide analysis

Figure S4
Figure S6

A

B

Position (Kb)
A WT BMM

B IFNAR<sup>-/-</sup> BMM

C RNase L<sup>-/-</sup> BMM

D

E

F

Figure S7
Figure S9
C

| Position | time | mock | MHV(V) | MHV(S) | PDEmt | EndoUmt |
|----------|------|------|--------|--------|-------|---------|
| UA772    | 9    | -1.09| 3.47   | 4.48   | 6.58  | 8.66    |
| UU771    | 9    | -0.65| 1.32   | 2.65   | 3.77  | 7.30    |
| UU543    | 9    | -2.05| -0.07  | 1.00   | 1.94  | 4.29    |
| UA772    | 12   | 2.21 | 3.29   | 1.02   | 5.52  | 4.88    |
| UU542    | 12   | 4.32 | 2.01   | 2.17   | 3.37  | 4.26    |
| UU771    | 12   | -0.64| -0.33  | 1.55   | 1.57  | 3.94    |

D

|           | RNase L sites | EndoU sites |
|-----------|---------------|-------------|
| 18S rRNA  | 8             | 0           |
| 28S rRNA  | 26            | 0           |
| 5.8S rRNA | 3             | 0           |
| 5S rRNA   | 1             | 0           |

Figure S11
A

| Cell   | Virus   | Corr. estimate |
|--------|---------|----------------|
| WT     | MHV(S)  | 0.151          |
| WT     | MHV(V)  | 0.109          |
| WT     | PDEmut  | 0.084          |
| WT     | EndoUmut| 0.139          |
| IFNAR- | MHV(S)  | 0.194          |
| IFNAR- | MHV(V)  | 0.103          |
| IFNAR- | PDEmut  | 0.154          |
| IFNAR- | EndoUmut| 0.252          |
| RNaseL-| MHV(S)  | 0.210          |
| RNaseL-| MHV(V)  | 0.172          |
| RNaseL-| PDEmut  | 0.174          |

B

| Cell   | Virus   | Corr. estimate |
|--------|---------|----------------|
| WT     | MHV(S)  | 0.151          |
| WT     | PDEmut  | 0.084          |
| WT     | EndoUmut| 0.139          |
| RNaseL-| MHV(S)  | 0.194          |
| RNaseL-| MHV(V)  | 0.103          |
| RNaseL-| PDEmut  | 0.154          |

C

D
Figure S13
### Differential expressed genes within samples at 9 vs. 12 hpi

| Cell type   | Virus  | Upregulated genes | Downregulated genes |
|-------------|--------|-------------------|---------------------|
| WT          | mock   | 0                 | 1                   |
| WT          | MHV<sup>(S)</sup> | 0                 | 0                   |
| WT          | MHV<sup>(V)</sup> | 2                 | 2                   |
| WT          | PDE<sup>mut</sup> | 0                 | 0                   |
| WT          | EndoU<sup>mut</sup> | 1               | 0                   |
| RNase L KO  | mock   | 1                 | 0                   |
| RNase L KO  | MHV<sup>(S)</sup> | 0                 | 1                   |
| RNase L KO  | MHV<sup>(V)</sup> | 1                 | 2                   |
| RNase L KO  | PDE<sup>mut</sup> | 0                 | 2                   |
| RNase L KO  | EndoU<sup>mut</sup> | 0               | 0                   |

### GO terms in Biological Process: MHV<sup>(S)</sup> vs. Mock in WT BMM

- Cellular response to drug
- Positive regulation of cytokine production
- Positive regulation of cell death
- Positive regulation of endopeptidase activity
- Cellular response to interferon-gamma
- Cellular response to lipopolysaccharide
- Cellular response to NF-kappaB
- Cellular response to interferon-gamma

### Top 5 GO terms in Biological Process: EndoU<sup>mut</sup> vs. Mock in WT BMM

1. Positive regulation of tumor necrosis factor
2. Positive regulation of cytokine production
3. Positive regulation of cell death
4. Positive regulation of endopeptidase activity
5. Cellular response to interferon-gamma

### E

![Heatmap of gene expression](image)

**Mock**
- MHV<sup>(S)</sup>
- PDE<sup>mut</sup>
- EndoU<sup>mut</sup>

**MHV<sup>(V)</sup>**
- PDE<sup>mut</sup>
- EndoU<sup>mut</sup>

**RNase L KO**
- MHV<sup>(S)</sup>
- PDE<sup>mut</sup>
- EndoU<sup>mut</sup>

**RNase L KO**
- MHV<sup>(V)</sup>
- PDE<sup>mut</sup>
- EndoU<sup>mut</sup>

**WT BMM**
- MHV<sup>(S)</sup>
- PDE<sup>mut</sup>
- EndoU<sup>mut</sup>

**RNase L <sup>-</sup> BMM**
- MHV<sup>(S)</sup>
- PDE<sup>mut</sup>
- EndoU<sup>mut</sup>
Figure S15

Cyclic Phosphate and RNAseq Enrichment: mock / EndoU^mut

Enrichment Bins

- Bin1
  RNAseq & cyclic phosphate enrichment < 1
- Bin2
  RNAseq & cyclic phosphate enrichment >= 1
- Bin3
  RNAseq >= 1 & cyclic phosphate enrichment < 1
- Bin4
  RNAseq < 1 & cyclic phosphate enrichment >= 1

Cyclic Phosphate and RNAseq Enrichment: MHV^30 / EndoU^mut

Cyclic Phosphate and RNAseq Enrichment: PDE^mut / EndoU^mut
Table 1A. Dinucleotide enrichment and de-enrichment in WT BMM at 12 hpi (position -2 to -1)

| Dinucleotide | MHV(S) Fold change | qval | MHV(V) Fold change | qval | PDE mut Fold change | qval | EndoU mut Fold change | qval |
|--------------|-------------------|------|--------------------|------|---------------------|------|-----------------------|------|
| UA           | -0.57             | 1    | -0.59              | 1    | 1.36                | 0.0E-1000 | 1.71                | 0.0E-1000 |
| UU           | 1.01              | 0.0E-1000 | 0.97              | 0.0E-1000 | 1.22                | 0.0E-1000 | 1.35                | 0.0E-1000 |
| UG           | -1.87             | 1    | -1.98              | 1    | -0.69               | 1    | -0.29                | 1    |
| UC           | 1.80              | 0.0E-1000 | 1.99              | 0.0E-1000 | 0.87                | 4.81E-123 | 0.15                | 0.0018  |
| GA           | -1.65             | 1    | -1.79              | 1    | -1.24               | 1    | -1.05                | 1    |
| GU           | 0.29              | 1.19E-23 | 0.15              | 6.35E-07  | -0.23               | 1    | -0.94                | 1    |
| GG           | -2.67             | 1    | -2.83              | 1    | -2.65               | 1    | -2.70                | 1    |
| GC           | 0.69              | 5.32E-99 | 0.78              | 1.22E-120 | -0.30               | 1    | -1.01                | 1    |
| CA           | -0.89             | 1    | -1.00              | 1    | -0.42               | 1    | 0.20                 | 6.52E-07 |
| CU           | -0.11             | 1    | -0.27              | 1    | -0.55               | 1    | -0.88                | 1    |
| CG           | -1.94             | 1    | -2.05              | 1    | -1.98               | 1    | -1.86                | 1    |
| CC           | 0.52              | 3.96E-38 | 0.62              | 4.42E-52  | -0.45               | 1    | -1.03                | 1    |
| AA           | -1.60             | 1    | -1.77              | 1    | -1.01               | 1    | -0.74                | 1    |
| AU           | 0.10              | 0.0005 | 0.00              | 1    | -0.34               | 1    | -0.90                | 1    |
| AG           | -2.20             | 1    | -2.44              | 1    | -2.11               | 1    | -2.08                | 1    |
| AC           | 0.98              | 3.21E-174 | 1.11              | 7.50E-221 | 0.10                | 0.0195 | -0.61                | 1    |

Table 1B. Dinucleotide enrichment and de-enrichment in WT BMM at 9 hpi (position -2 to -1)

| Dinucleotide | MHV(S) Fold change | qval | MHV(V) Fold change | qval | PDE mut Fold change | qval | EndoU mut Fold change | qval |
|--------------|-------------------|------|--------------------|------|---------------------|------|-----------------------|------|
| UA           | -0.20             | 1    | 0.13               | 0.00135 | 0.19                | 0.0071 | 1.43                | 0.0E-1000 |
| UU           | 1.04              | 0.0E-1000 | 1.21              | 0.0E-1000 | 0.94                | 9.92E-88 | 1.27                | 0.0E-1000 |
| UG           | -1.71             | 1    | -1.43              | 1    | -1.21               | 1    | -0.45                | 1    |
| UC           | 1.57              | 0.0E-1000 | 0.92              | 4.41E-84  | 0.70                | 8.77E-17 | 0.68                | 5.39E-59  |
| GA           | -1.22             | 1    | -0.94              | 1    | -0.88               | 1    | -1.03                | 1    |
| GU           | 0.41              | 4.91E-41 | 0.50              | 8.87E-43  | 0.65                | 2.73E-27 | -0.60                | 1    |
| GG           | -2.46             | 1    | -2.11              | 1    | -1.51               | 1    | -2.78                | 1    |
| GC           | 0.50              | 1.95E-41 | 0.01              | 0.981    | 0.26                | 0.00181 | -0.41                | 1    |
| CA           | -0.71             | 1    | -0.53              | 1    | -0.15               | 1    | 0.21                 | 2.18E-07 |
| CU           | -0.03             | 1    | 0.08               | 0.111    | 0.29                | 0.00019 | -0.81                | 1    |
| CG           | -1.72             | 1    | -1.40              | 1    | -1.27               | 1    | -1.93                | 1    |
| CC           | 0.29              | 1.19E-10 | -0.11             | 1    | -0.09               | 1    | -0.49                | 1    |
| AA           | -1.22             | 1    | -0.89              | 1    | -0.98               | 1    | -0.77                | 1    |
| AU           | 0.12              | 0.00019 | 0.31              | 2.33E-16 | 0.32                | 6.74E-07 | -0.79                | 1    |
| AG           | -1.95             | 1    | -1.76              | 1    | -1.97               | 1    | -2.28                | 1    |
| AC           | 0.66              | 6.51E-63 | 0.17              | 0.0014   | -0.03               | 1    | -0.12                | 1    |

Table S1
Table 2A. Dinucleotide enrichment and de-enrichment in WT BMM at 12 hpi (position -1 to +1)

| Dinucleotide | MHV<sup>(S)</sup> Fold change | MHV<sup>(V)</sup> Fold change | PDE<sup>mut</sup> Fold change | EndoU<sup>mut</sup> Fold change |
|--------------|-------------------------------|-------------------------------|-----------------------------|--------------------------------|
| UA           | 2.00 0.0E-1000                | 1.93 0.0E-1000                | 1.43 0.0E-1000              | 1.02 1.05E-281                  |
| UU           | -0.85 1                       | -1.06 1                       | -0.19 1                     | -0.18 1                         |
| UG           | -0.82 1                       | -0.79 1                       | -0.06 1                     | 0.15 3.42E-07                   |
| UC           | -0.67 1                       | -0.94 1                       | -0.99 1                     | -0.84 1                         |
| GA           | -1.94 1                       | -2.07 1                       | -1.17 1                     | -0.93 1                         |
| GU           | -1.81 1                       | -1.97 1                       | -1.04 1                     | -0.84 1                         |
| GG           | -2.60 1                       | -2.75 1                       | -1.44 1                     | -0.85 1                         |
| GC           | -2.39 1                       | -2.59 1                       | -2.50 1                     | -2.34 1                         |
| CA           | 2.46 0.0E-1000                | 2.60 0.0E-1000                | 1.40 0.0E-1000              | 0.56 8.72E-50                   |
| CU           | -1.01 1                       | -0.95 1                       | -1.31 1                     | -1.57 1                         |
| CG           | -0.58 1                       | -0.28 1                       | -1.00 1                     | -1.38 1                         |
| CC           | -0.49 1                       | -0.35 1                       | -1.32 1                     | -1.82 1                         |
| AA           | -0.83 1                       | -0.89 1                       | 0.51 1.78E-68               | 0.98 2.08E-239                  |
| AU           | -0.88 1                       | -1.00 1                       | 0.37 3.80E-39               | 0.62 5.98E-95                   |
| AG           | -1.36 1                       | -1.44 1                       | 0.04 0.670003               | 0.41 8.99E-31                   |
| AC           | -1.64 1                       | -1.73 1                       | -0.72 1                     | -0.37 1                         |

Table 2B. Dinucleotide enrichment and de-enrichment in WT BMM at 9 hpi (position -1 to -1)

| Dinucleotide | MHV<sup>(S)</sup> Fold change | MHV<sup>(V)</sup> Fold change | PDE<sup>mut</sup> Fold change | EndoU<sup>mut</sup> Fold change |
|--------------|-------------------------------|-------------------------------|-----------------------------|--------------------------------|
| UA           | 2.01 0.0E-1000                | 2.06 0.0E-1000                | 2.08 0.0E-1000              | 1.28 0.0E-1000                  |
| UU           | -0.91 1                       | -0.52 1                       | -0.76 1                     | -0.46 1                         |
| UG           | -0.41 1                       | -0.04 1                       | -0.28 1                     | -0.02 1                         |
| UC           | -0.72 1                       | -0.79 1                       | -0.59 1                     | -0.84 1                         |
| GA           | -1.61 1                       | -1.27 1                       | -1.59 1                     | -1.05 1                         |
| GU           | -1.70 1                       | -1.48 1                       | -1.24 1                     | -1.10 1                         |
| GG           | -2.24 1                       | -2.00 1                       | -1.73 1                     | -1.00 1                         |
| GC           | -2.36 1                       | -2.03 1                       | -1.38 1                     | -2.23 1                         |
| CA           | 2.18 0.0E-1000                | 1.49 0.0E-1000                | 1.38 3.23E-112              | 1.24 2.27E-302                  |
| CU           | -1.11 1                       | -1.17 1                       | -0.97 1                     | -1.61 1                         |
| CG           | -0.41 1                       | -0.35 1                       | -0.42 1                     | -0.96 1                         |
| CC           | -0.70 1                       | -0.98 1                       | -0.90 1                     | -1.40 1                         |
| AA           | -0.35 1                       | -0.06 1                       | 0.03 1                      | 0.86 3.03E-182                  |
| AU           | -0.69 1                       | -0.40 1                       | -0.42 1                     | 0.32 2.80E-24                   |
| AG           | -0.99 1                       | -0.66 1                       | -0.59 1                     | 0.27 1.19E-13                   |
| AC           | -1.40 1                       | -1.15 1                       | -0.65 1                     | -0.45 1                         |
| Position (bp) | Reference | Variant |
|--------------|-----------|---------|
| 67           | T         | C       |
| 256          | A         | T       |
| 2471         | T         | C       |
| 5304         | T         | C       |
| 6265         | C         | T       |
| 6796         | A         | T       |
| 7475         | G         | A       |
| 9245         | C         | T       |
| 10136        | A         | C       |
| 12140        | G         | A       |
| 13604        | G         | A       |
| 16624        | G         | C       |
| 16990        | C         | T       |
| 17533        | T         | G       |
| 18646        | A         | C       |
| 18681        | C         | T       |
| 20552        | C         | G       |
| 20553        | A         | C       |
| 20554        | T         | G       |
| 22147        | A         | G       |
| 22739        | T         | G       |
| 22742        | T         | C       |
| 23393        | C         | T       |
| 23860        | T         | C       |
| 24404        | T         | A       |
| 24447        | G         | A       |
| 24448        | C         | T       |
| 24450        | T         | A       |
| 26074        | G         | C       |
| 28960        | T         | C       |
| 29012        | G         | C       |
| 29650        | T         | C       |

Table S3
| Genus         | Virus                | 3' NTR / poly(A) junction       | Accession # |
|---------------|----------------------|---------------------------------|-------------|
| Alpha CoVs    | HCoV NL63            | auggauacacaaaaa                 | AY567487    |
|               | HCoV 229E            | auggauacacaaaaa                 | AF304460    |
| Beta CoVs     | MHV A59              | gaagaauacacaaaaa                | NC_001846.1 |
|               | HCoV OC43            | gaagaauacacaaaaa                | NC_006213.1 |
|               | HCoV HKU1            | gaggaauuacacaaaaa               | AY597011    |
|               | MERS-CoV             | gaugauuuggcaaaaaaaa             | KJ556336.1  |
|               | SARS-CoV             | ggagaaugacaaaaa                 | AY274119.3  |
|               | SARS-CoV-2           | ggagaaugacaaaaa                 | MN908947.3  |
|               | Bat CoV HKU9         | gaggaauuugcaaaaaaaa             | MG762674.1  |
| Gamma CoVs    | IBV                  | gaguuagagcaaaaaaaa              | MN987231.1  |
|               | Beluga Whale CoV     | aaauuguagcaaaaaa                | NC_010646.1 |
| Delta CoVs    | Porcine CoV HKU15    | gggauggagcaaaaaaaa              | KJ569769.1  |
|               | Bulbul CoV HKU11     | gggagggagcaaaaaaaa              | NC_011547.1 |