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Supplemental information

An eIF3d-dependent switch regulates HCMV replication by remodeling the infected cell translation landscape to mimic chronic ER stress

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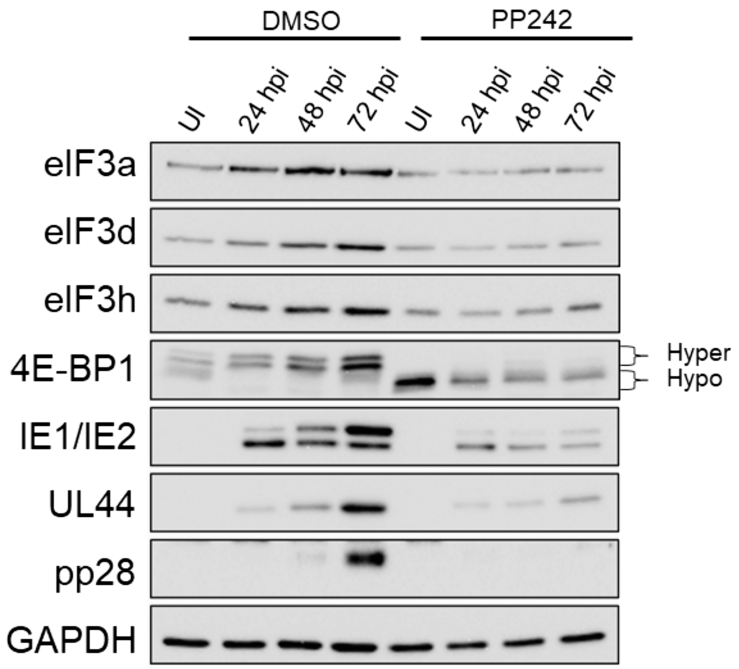
SUPPLEMENTAL FIGURE 1



<u>Figure S1</u>. Accumulation of eIF3 subunits in HCMV-infected cells is mTOR-dependent. Related to Figure 1. NHDFs were mock-(uninfected [UI]) or HCMV-infected (TB40/E strain; MOI = 3) and treated either with DMSO or the mTOR active site inhibitor, PP242 (added at 1.5 hpi; used at 2.5 μM). At each indicated time post-infection (*hpi*) total protein was collected, fractionated by SDS-PAGE, and analyzed by immunoblotting using the antibodies shown. GAPDH served as the loading control. PP242 activity was confirmed by monitoring the change in 4E-BP1 migration from slow-migrating, hyper-phosphorylated (*hyper*) to fast-migrating hypo-phosphorylated (*hypo*).

SUPPLEMENTAL FIGURE 2

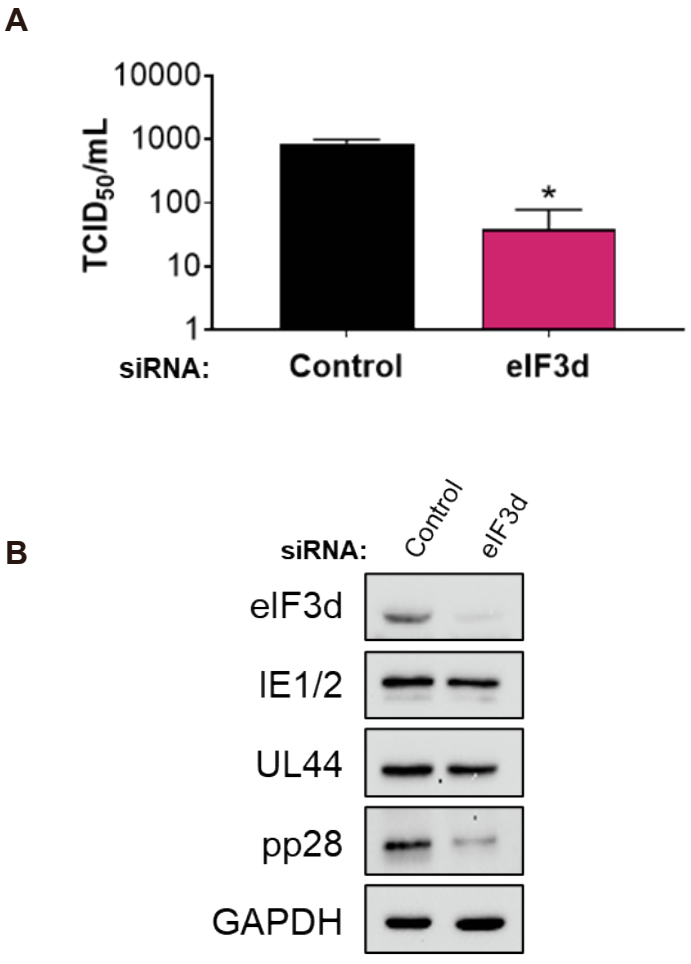


Figure S2. **Depletion of eIF3d interferes with HCMV replication and protein accumulation in epithelial cells. Related to Figure 2.** A) ARPE-19 cells transfected with non-silencing control or eIF3d-specific siRNA#2 were infected with HCMV (TB40/E strain, MOI=0.1). Infectious virus was quantified from supernatants collected 5 days post-infection (dpi) using a TCID50 assay (n=3). Error bars indicate SEM. (*) $P \leq 0.05$ by Student's *t*-test. B) As in A except cells were infected at MOI=3. At 72 hpi, total protein was collected, fractionated by SDS-PAGE, and the accumulation of viral proteins encoded by representative immediate early (IE1/2), early (UL44), and late (pp28) genes was analyzed by immunoblotting. GAPDH served as the loading control.

SUPPLEMENTAL FIGURE 3

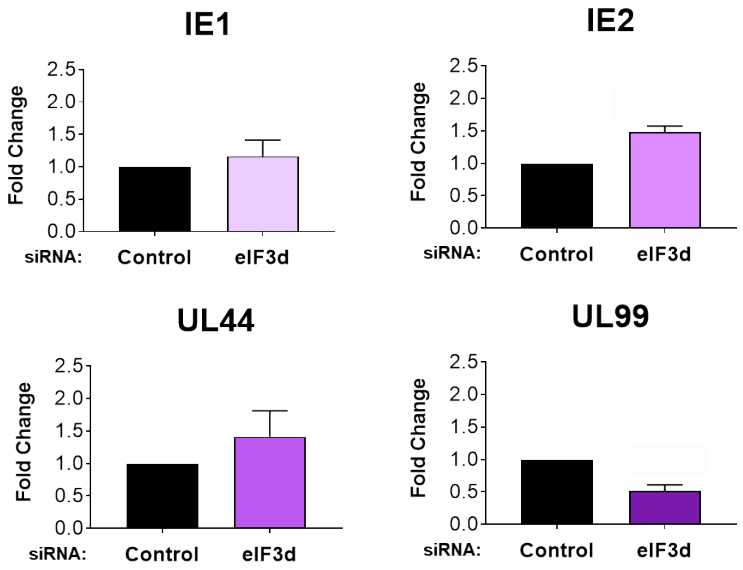


Figure S3. Depletion of eIF3d regulates HCMV gene expression post-transcriptionally and selectively interferes with HCMV replication. Related to Figure 2. Total RNA isolated at 72 hpi from HCMV-infected (AD169 strain; MOI=3). NHDFs transfected with the indicated siRNA (control non-silencing or eIF3d siRNA #2), was analyzed by RT-qPCR using primers specific for each specified virus gene (n=3). Error bars indicate SEM.

SUPPLEMENTAL FIGURE 4

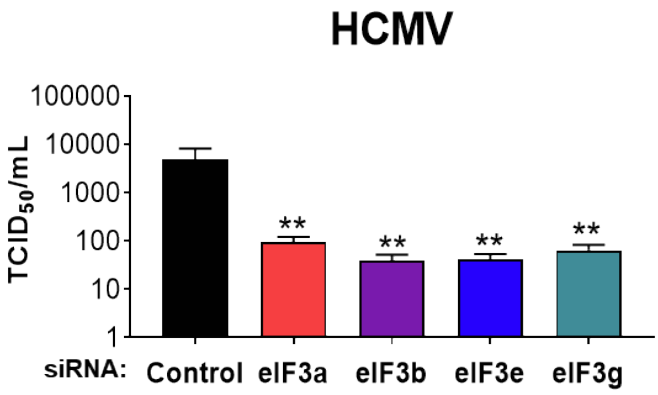


Figure S4. Depletion of select individual eIF3 complex subunits impacts HCMV replication. Related to Figure 3. NHDFs transfected with non-silencing control siRNA or siRNAs specific for the indicated eIF3 subunits were infected HCMV (AD169 strain; MOI = 0.1). After 5d, the infectious virus in collected supernatants was quantified using a TCID50 assay (n=3). Error bars indicate SEM. (**) P ≤0.01 by Student's t-test.

SUPPLEMENTAL FIGURE 5

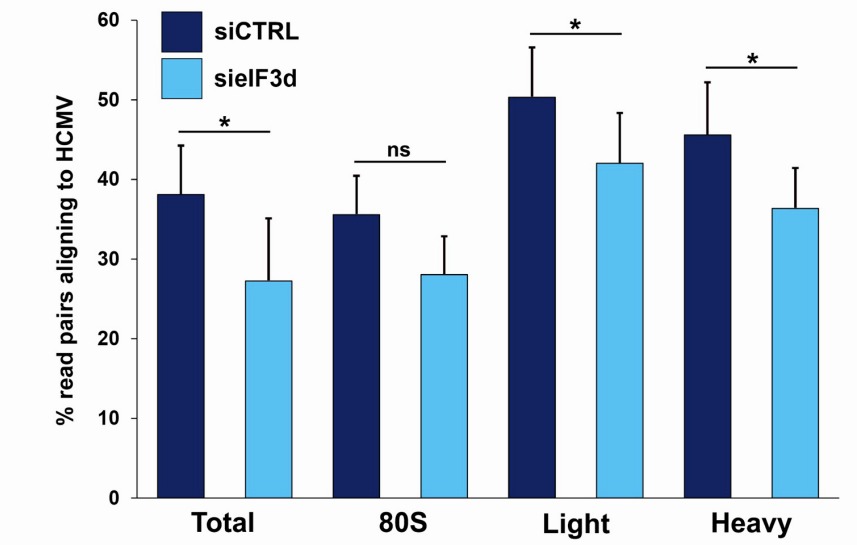
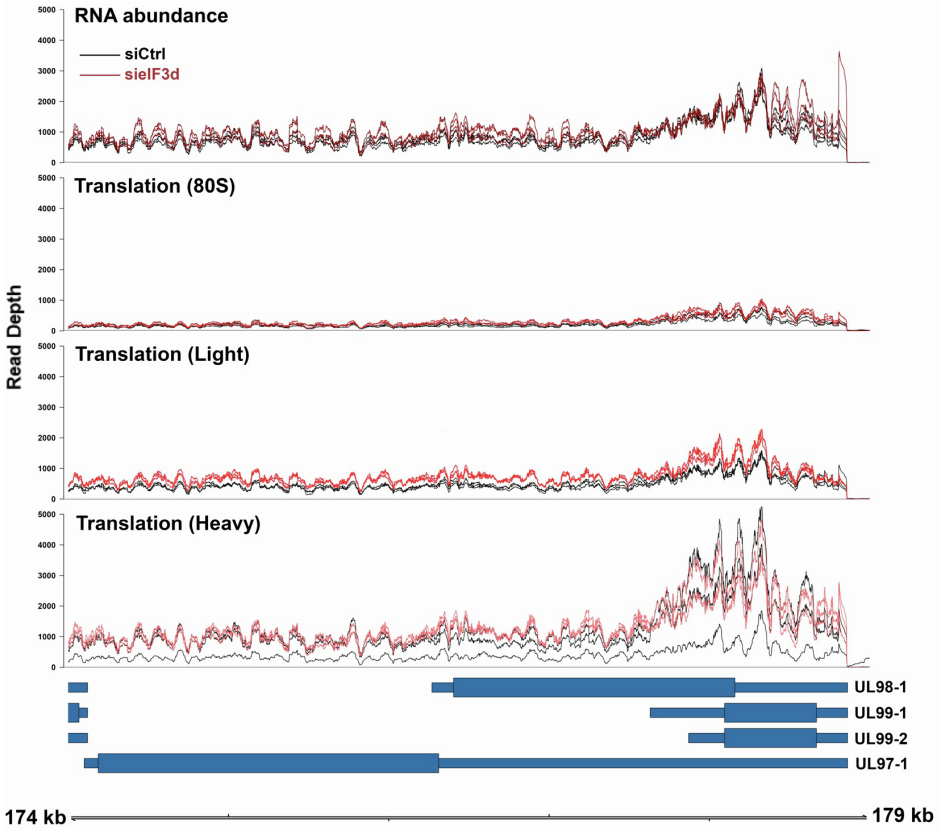


Figure S5. Characteristics of sequencing data. **Related to Figure 5.** Twenty-four sequencing libraries were generated with three biological replicates generated per condition (Supplementary Table S4). Sequence reads were subsequently aligned against a hybrid reference comprising the human genome and HCMV strain TB40/E genome using STAR to determine the proportion of HCMV reads in each dataset. In all cases, the proportion of HCMV reads was reduced in eIF3d-depleted datasets relative to their matched controls. These reductions were statistically significant in the Heavy, Light and Total fractions according to a Student's paired t-test. ns – not significant, * $p < 0.05$.

SUPPLEMENTAL FIGURE 6



<u>Figure S6</u>. UL99 RNAs overlap with UL97 and UL98 RNAs. Related to Figure 5. Coverage map of RNA-seq read depths across the UL97/UL98/UL99 locus representing transcript abundance (total RNA) or translation from the pooled 80S, light polysome, or heavy polysome peak fractions (*identified in Figure 5 and described in main text*). RNAs transcribed from this locus include two UL99 isoforms that differ in their 5'-UTR lengths but not their encoded ORFs, and single RNA isoforms encoding the UL97 and UL98 ORFs. The UL98 and UL99 RNAs overlap with the 3'-UTR of the UL97 RNA, preventing assignment of individual sequence reads to a specific isoform.

SUPPLEMENTAL FIGURE 7

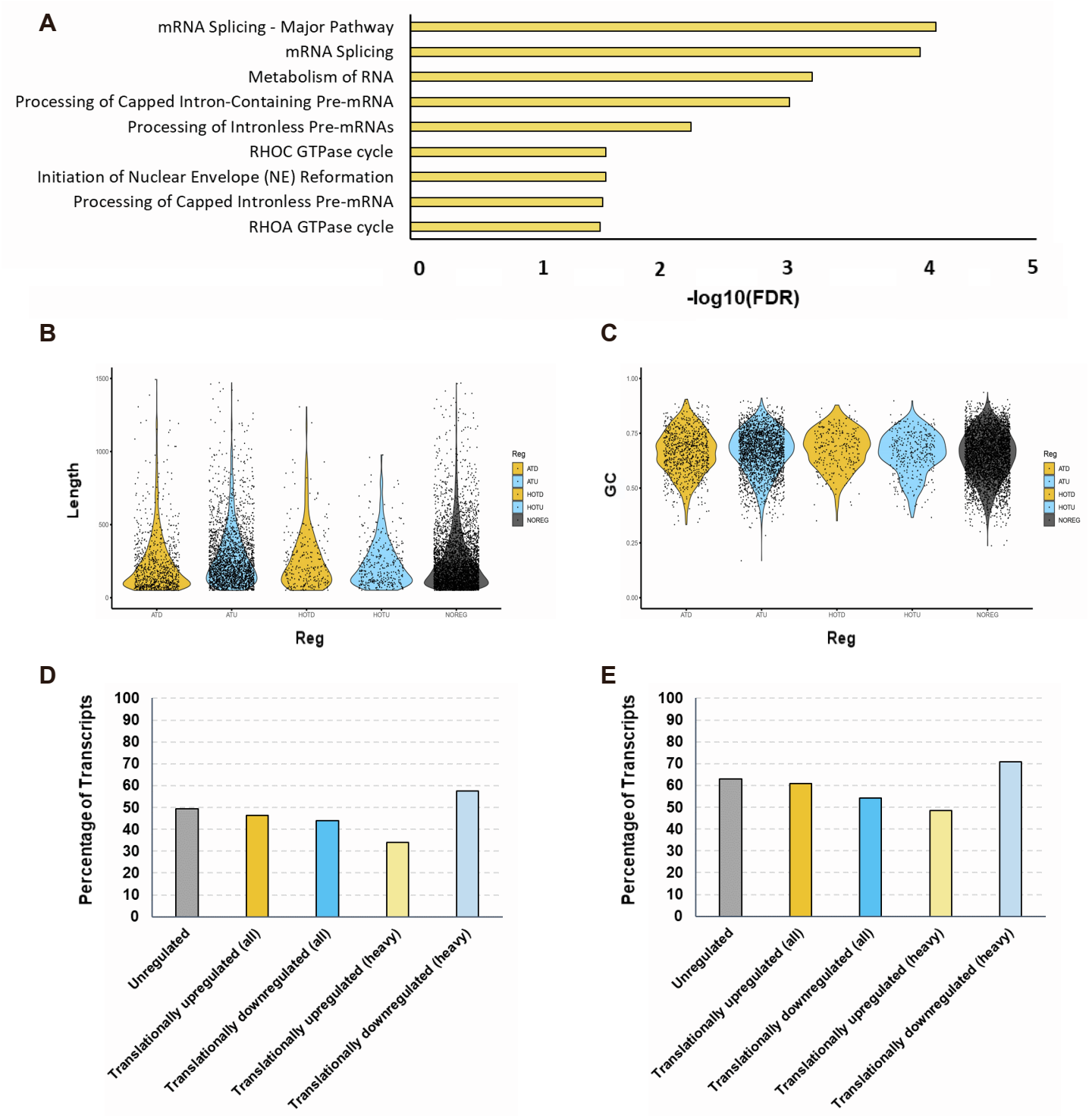


Figure S7. Characteristics of host mRNAs translationally-regulated by eIF3d in response to HCMV infection. Related to Figure 6. (A) Gene set enrichment analysis was performed using REACTOME (Jassal et al., 2020) for genes encoding translationally upregulated RNAs in the heavy polysome pooled fraction. (B) Distribution of 5'-UTR lengths among host eIF3d-responsive genes showing translationally downregulated in all fractions [pooled 80S monosomes, light polysome and heavy polysome peak fractions] (ATD), translationally upregulated in all fractions (ATU), translationally downregulated in the heavy polysome fraction only (HOTD), translationally upregulated in the heavy polysome fraction only (HOTU), or random sampling of 5000 unregulated transcripts (NOREG). (C) As in *B* except showing the distribution of GC content. (D-E) For each group of transcripts outlined above, the 5' UTRs were examined using EMBOSS (Rice et al., 2000) for the presence of small upstream ORFs (uORFs) at least (D) 10 amino acids or (E) 2 amino acids in length. Fisher's Exact Tests with Bonferroni correction were used to determine whether individual groups were enriched or depleted for transcripts with uORFs relative to unregulated transcripts.

SUPPLEMENTAL FIGURE 8

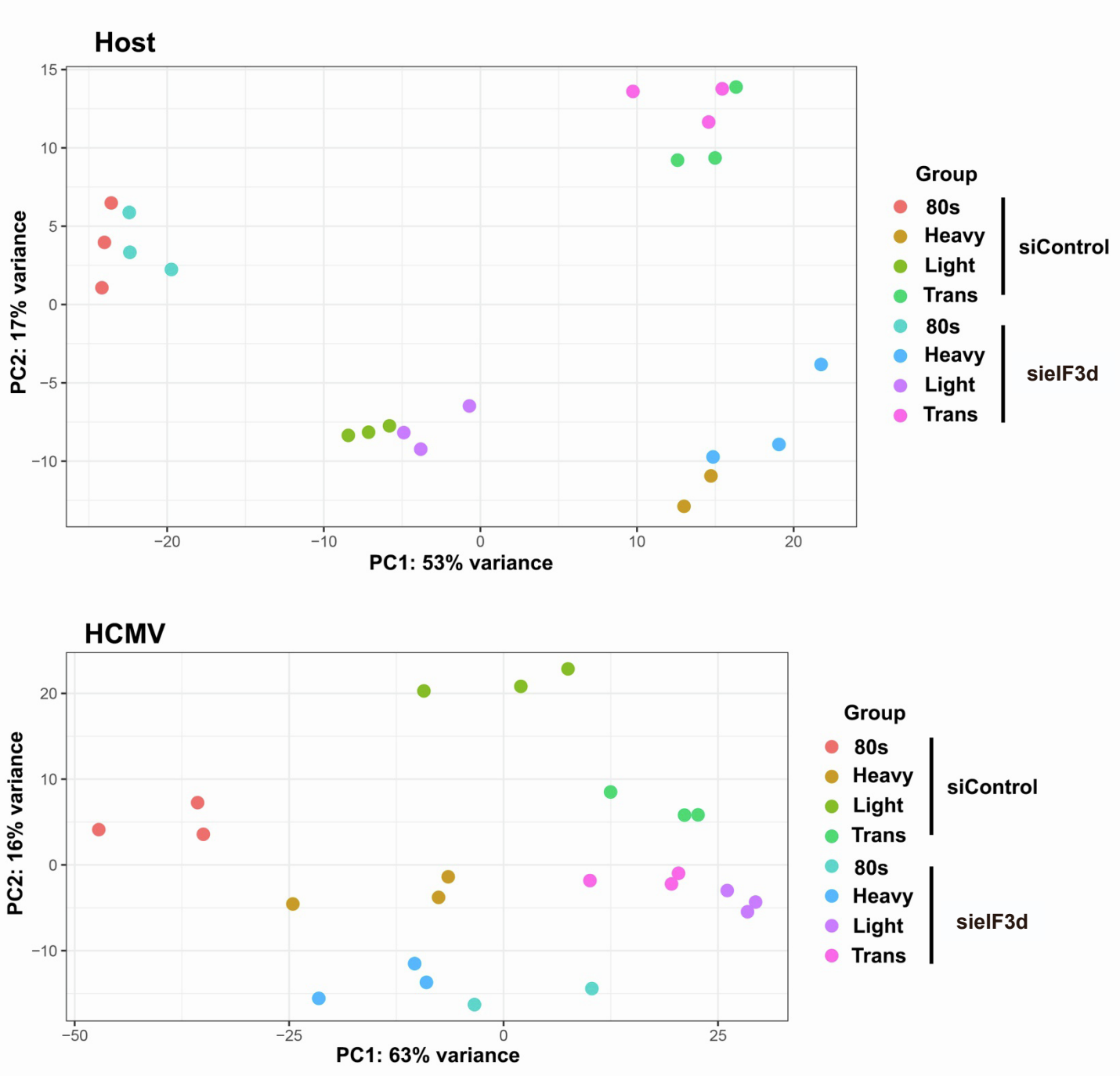


Figure S8. Clustering of sequence datasets by principal component analysis. Related to STAR Methods. Sequence reads were either pseudoaligned against the host transcriptome or aligned against the HCMV genome with raw abundance counts generated according to sliding windows. Batch correction was performed using ComBat-Seq (Zhang et al, 2020) and results visualized using principal component analysis plots.

Supplemental references

Rice, P., Longden, I., Bleasby, A., 2000. EMBOSS: the European molecular biology open software suite. Trends Genet. 16, 276–277.