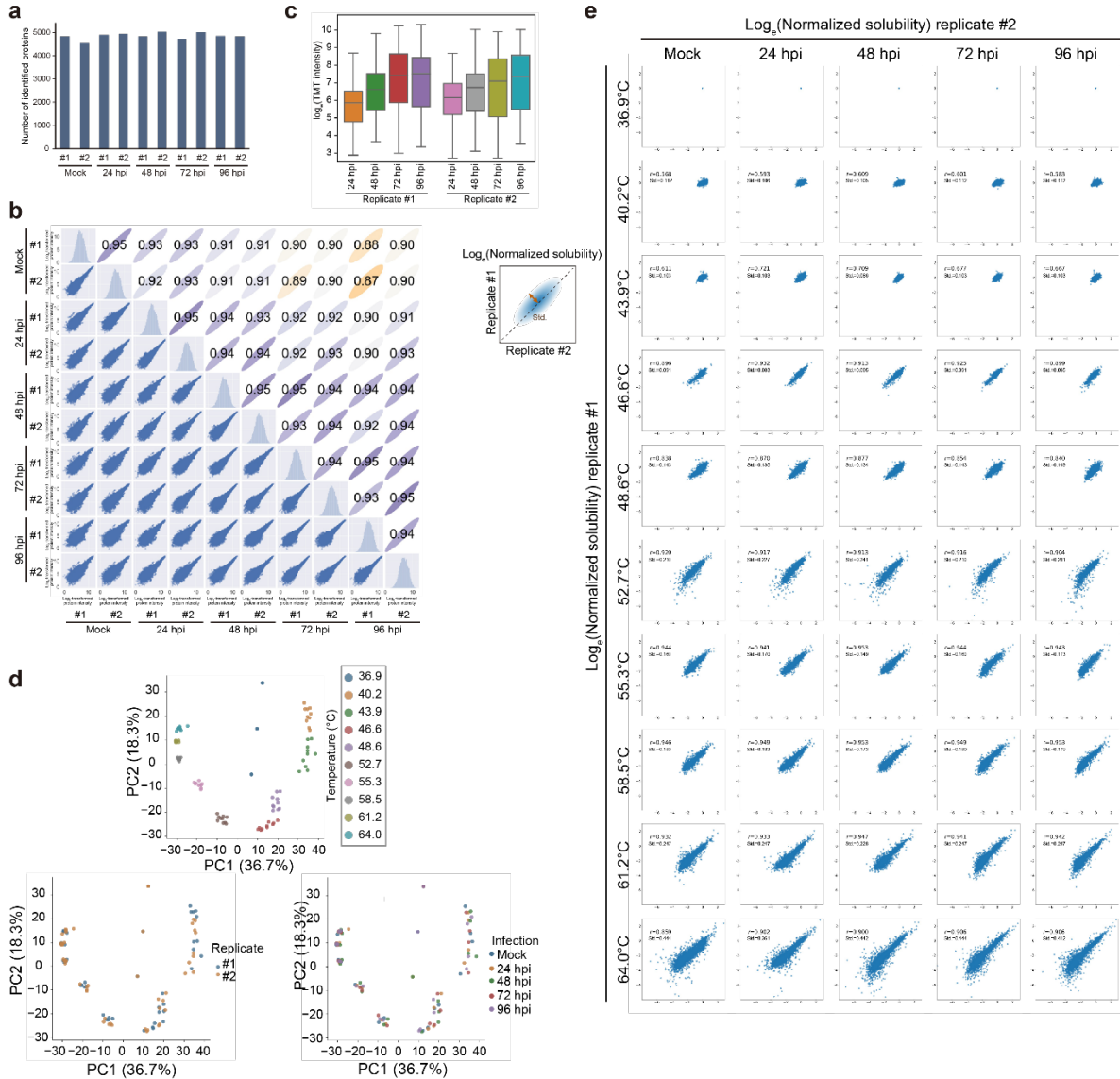


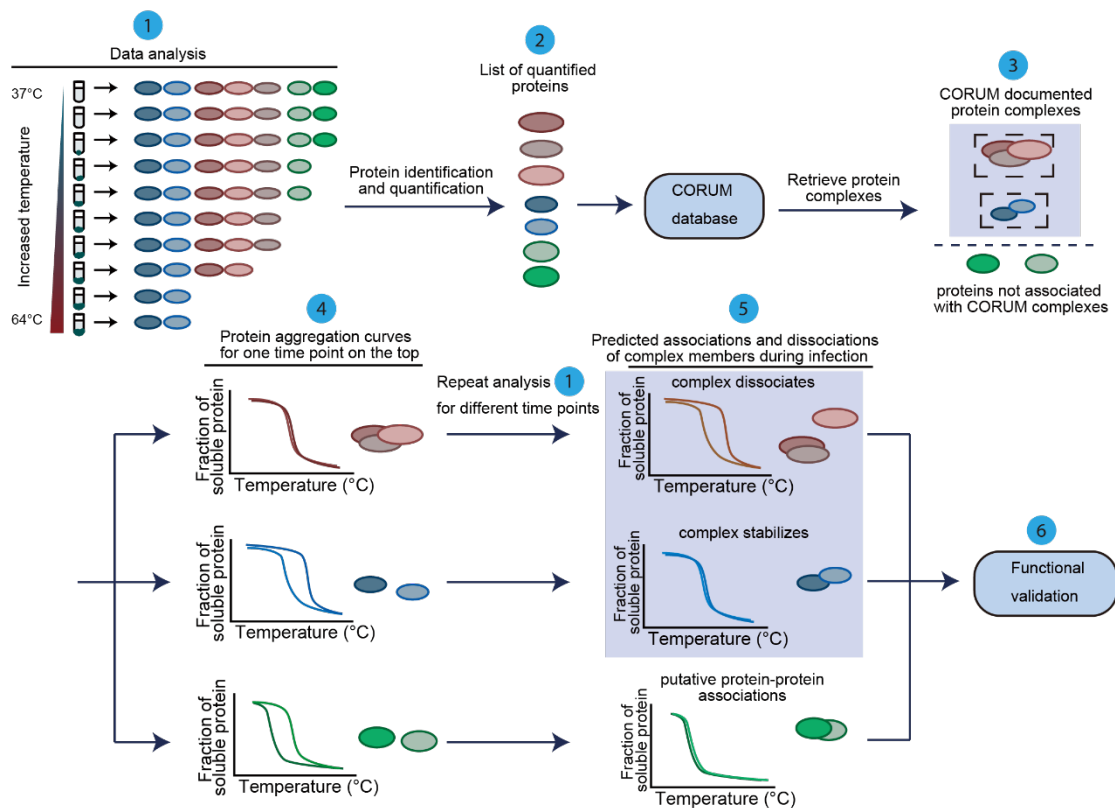
Temporal dynamics of protein complex formation and dissociation during human cytomegalovirus infection

Hashimoto, et al.



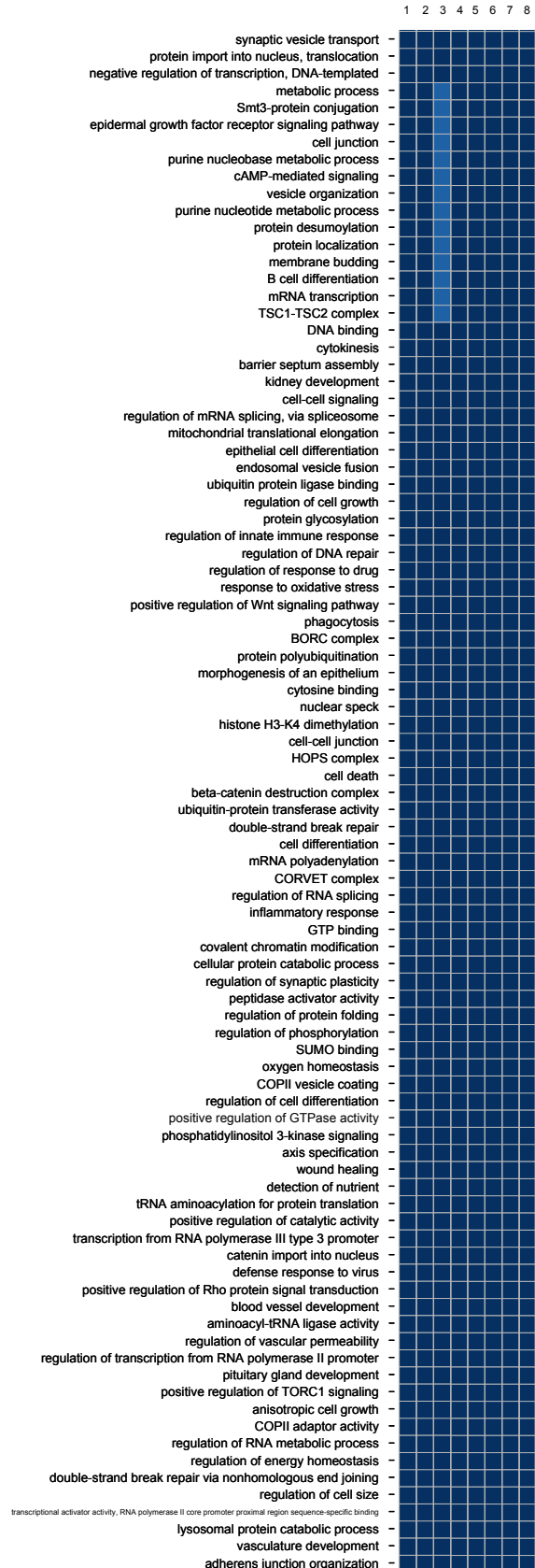
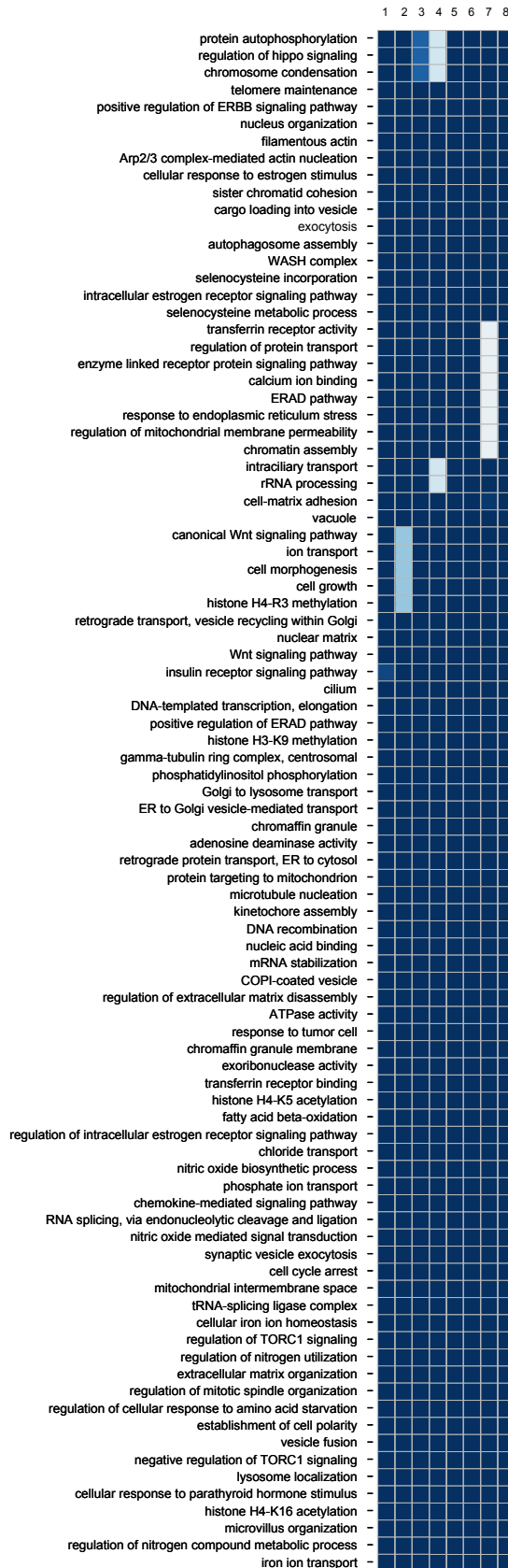
Supplementary Figure 1. Assessment of reproducibility. **a** Number of identified proteins in each replicate at all time points. **b** Correlation matrix of all proteins between the samples. Correlation coefficients were calculated based on the $\log_e$ transformed abundances, estimated from the sum of the peptide reporter ion intensities for peptides shared across time points for the indicated proteins. Pearson's r -values are shown on the top triangle, and the dots for individual protein abundances are plotted on the lower triangle. **c** Comparison of TMT intensities for viral protein abundance detected at different infection time points. Centre line, bounds of box, and

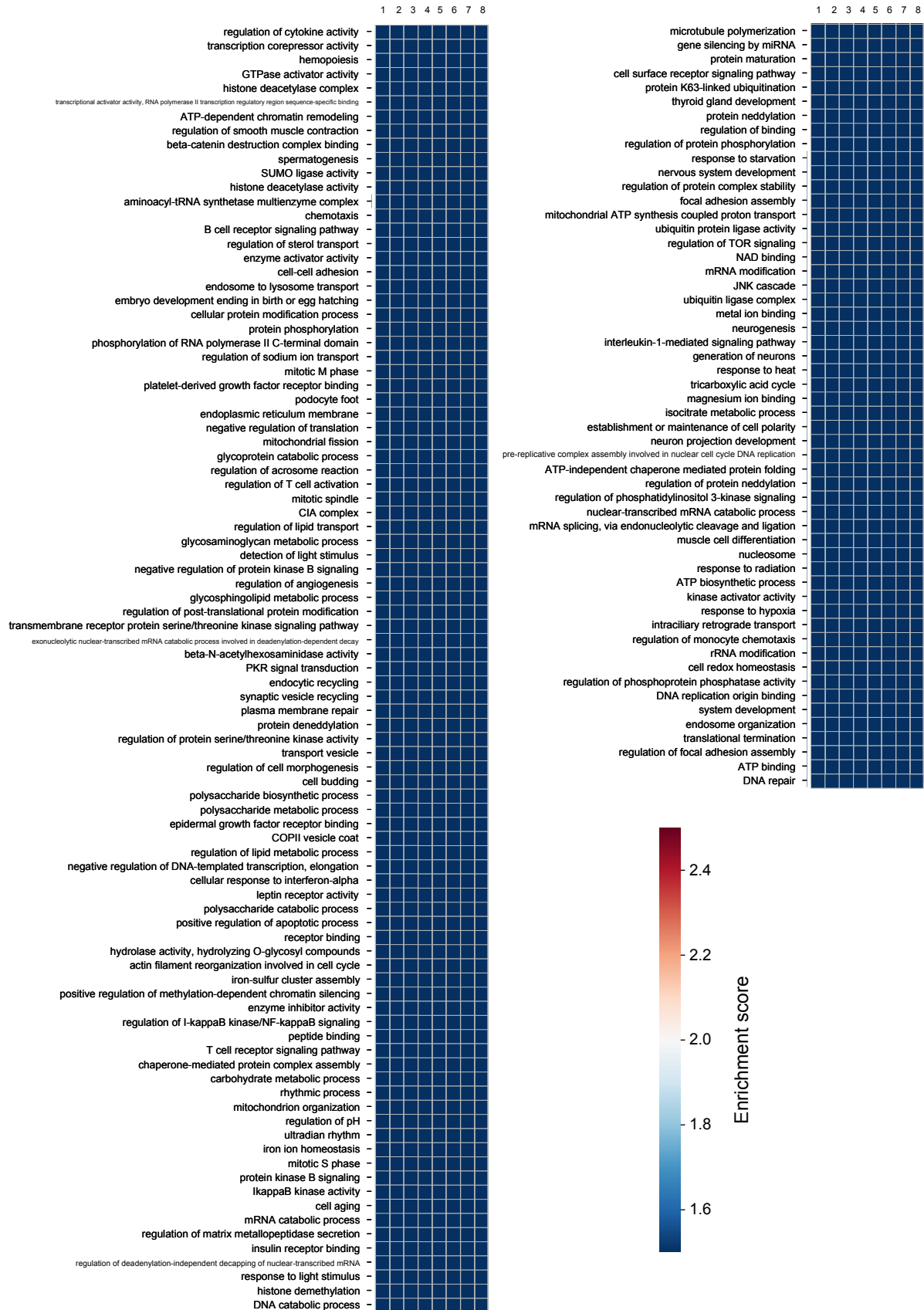
bounds of whiskers represent, respectively, median, quartile, and sample minimum and maximum that are not outliers. **d** PCA plots of all normalized solubilities for temperatures, replicates, and HCMV infection time points. **e** Scatterplots of $\log_e$ transformed normalized adjusted solubility depicting the Pearson correlation coefficient and the standard deviation of the distance to the midline of the data points.



Supplementary Figure 2. Workflow for protein complex analysis. Workflow for analyzing the presence of putative protein complexes and predicting the maintenance, association, and dissociation of members of CORUM protein complexes.

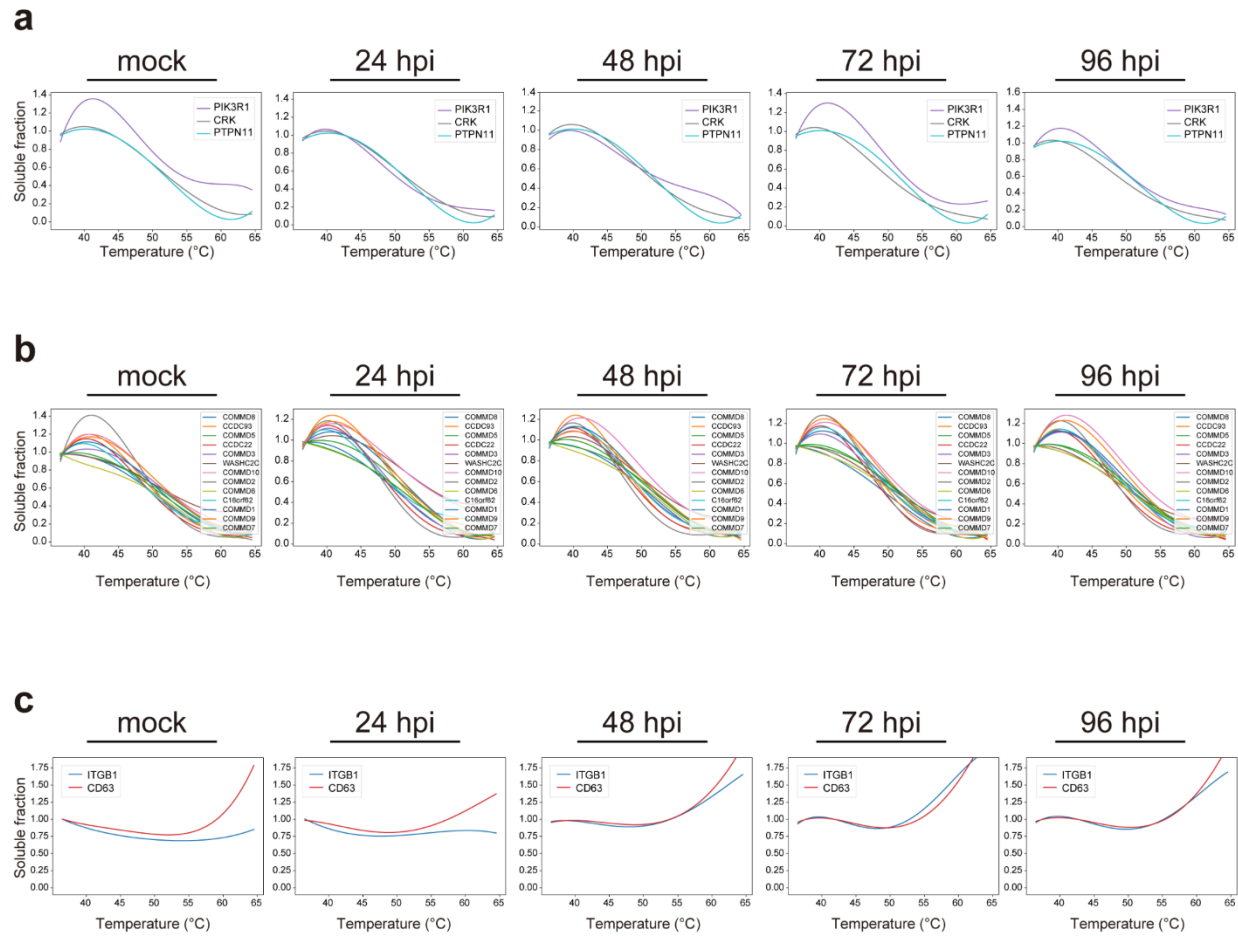




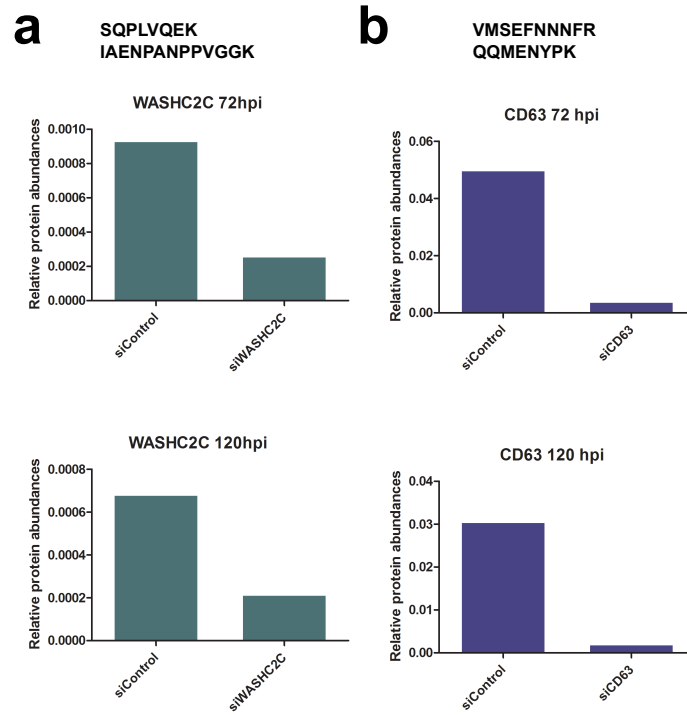


Supplementary Figure 3. GO enrichment analysis highlights protein complexes enriched in distinct temporal profiles (clusters 1-8). a GO enrichment analysis of protein clusters in Fig.

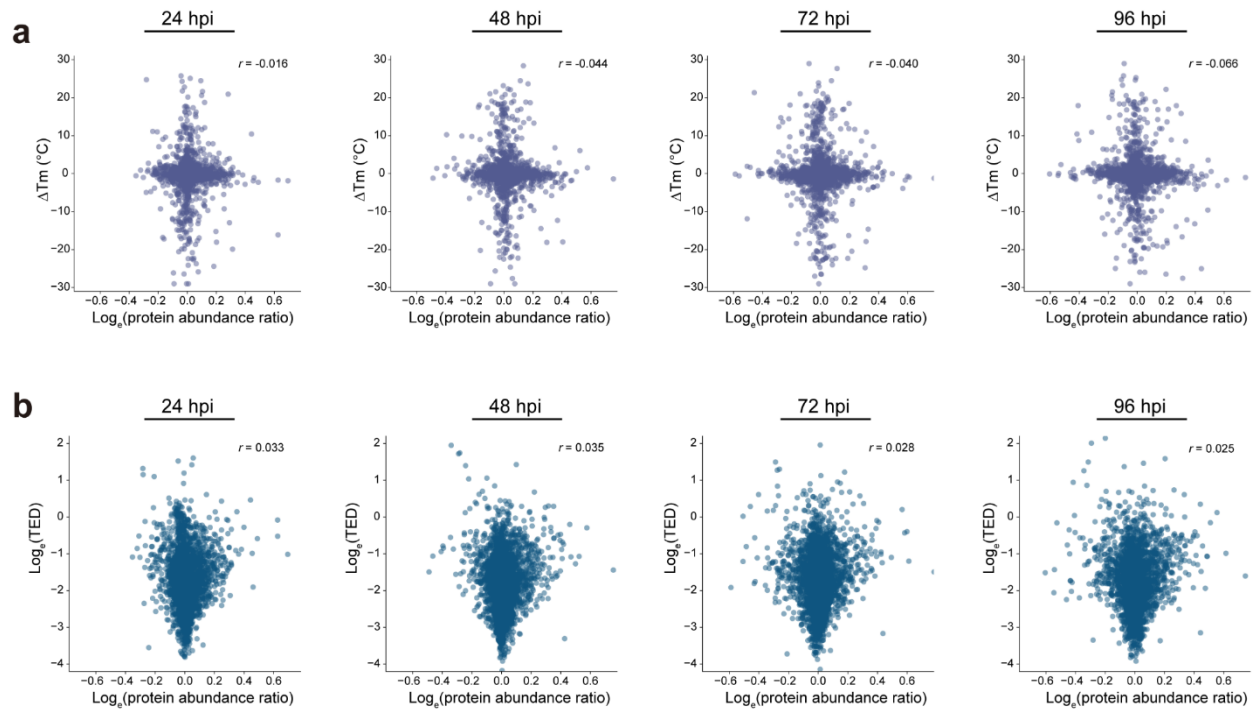
2a. Enriched GO terms without a limit to the number of complexes assigned to a GO term are shown. Number at the top corresponds to the cluster illustrated in Fig. 2a. Color of bins indicates enrichment scores for each GO term during infection. The enrichment scores were based on the p-values of the Fisher's exact test, being calculated as $-\log_{10}$ p-value.



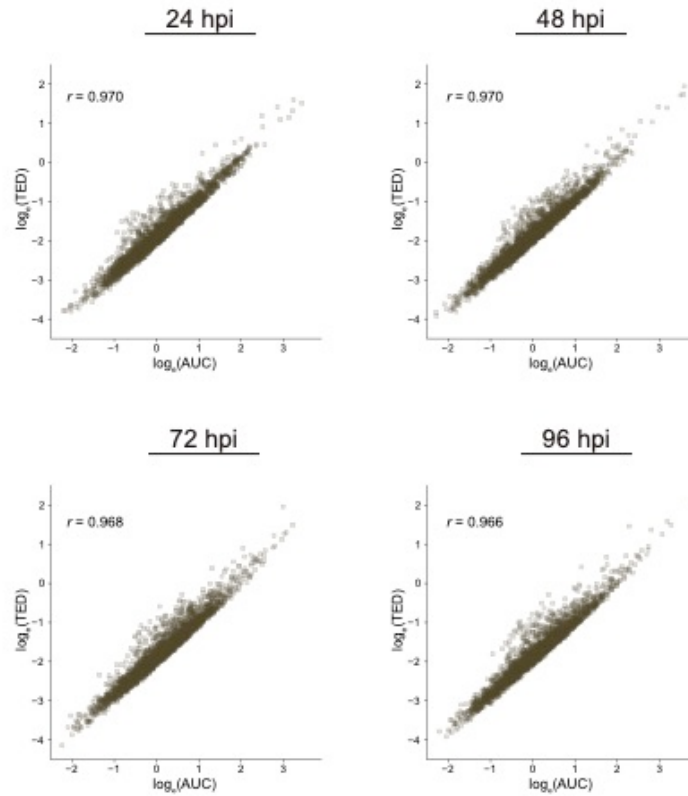
Supplementary Figure 4. Melting curves for selected cellular proteins of interest at different time points of HCMV infection. Melting curves for **a** PI3K pathway proteins, **b** WASHC2C and members of the CCC complex, and **c** ITGB1 and CD63 over the time course of HCMV infection.



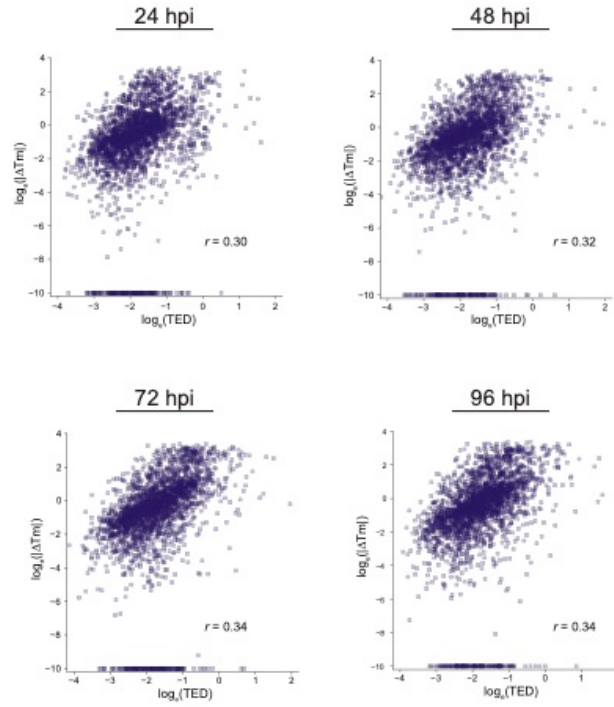
Supplementary Figure 5. siRNA-mediated knockdowns are maintained throughout the HCMV infection. PRM analysis demonstrates that siRNA-mediated knockdowns of **a** WASHC2C and of **b** CD63 are maintained at 72 and 120 hpi. Peptides targeted by PRM analysis for quantification are shown above the plots for each protein.



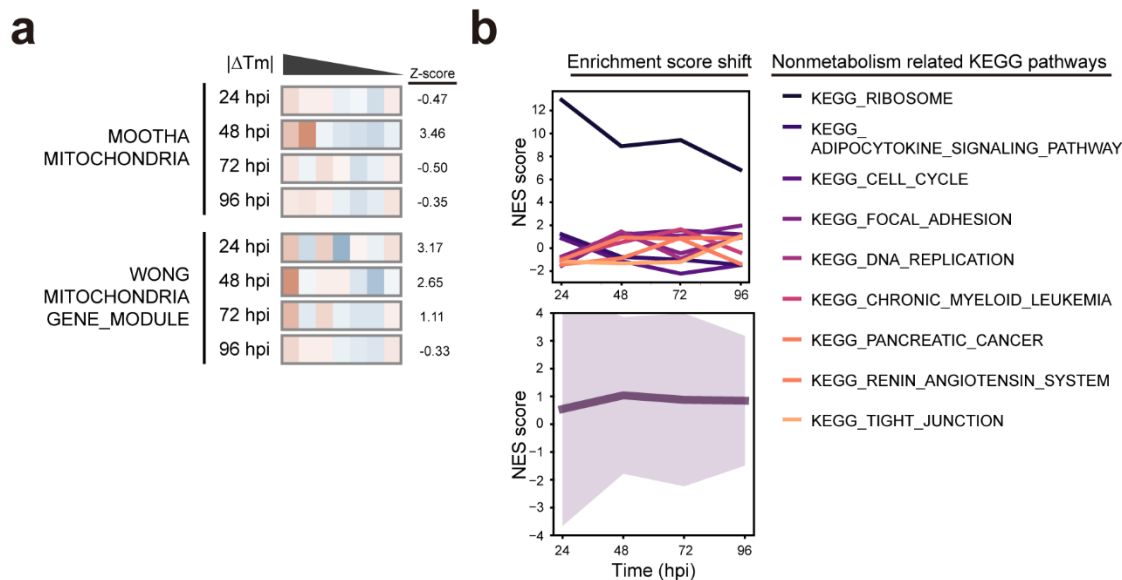
Supplementary Figure 6. Alterations in protein abundance are not the primary driver of changes in ΔT_m or TED. **a** Scatter plot of ΔT_m values versus $\log_e(\text{protein abundance at the indicated time point/protein abundance at mock})$. Pearson's r -value is shown for each infection time point. **b** Scatter plot of $\log_e(\text{TED})$ values versus $\log_e(\text{protein abundance at the indicated time point/protein abundance at mock})$. Pearson's r -value is shown for each infection time point.



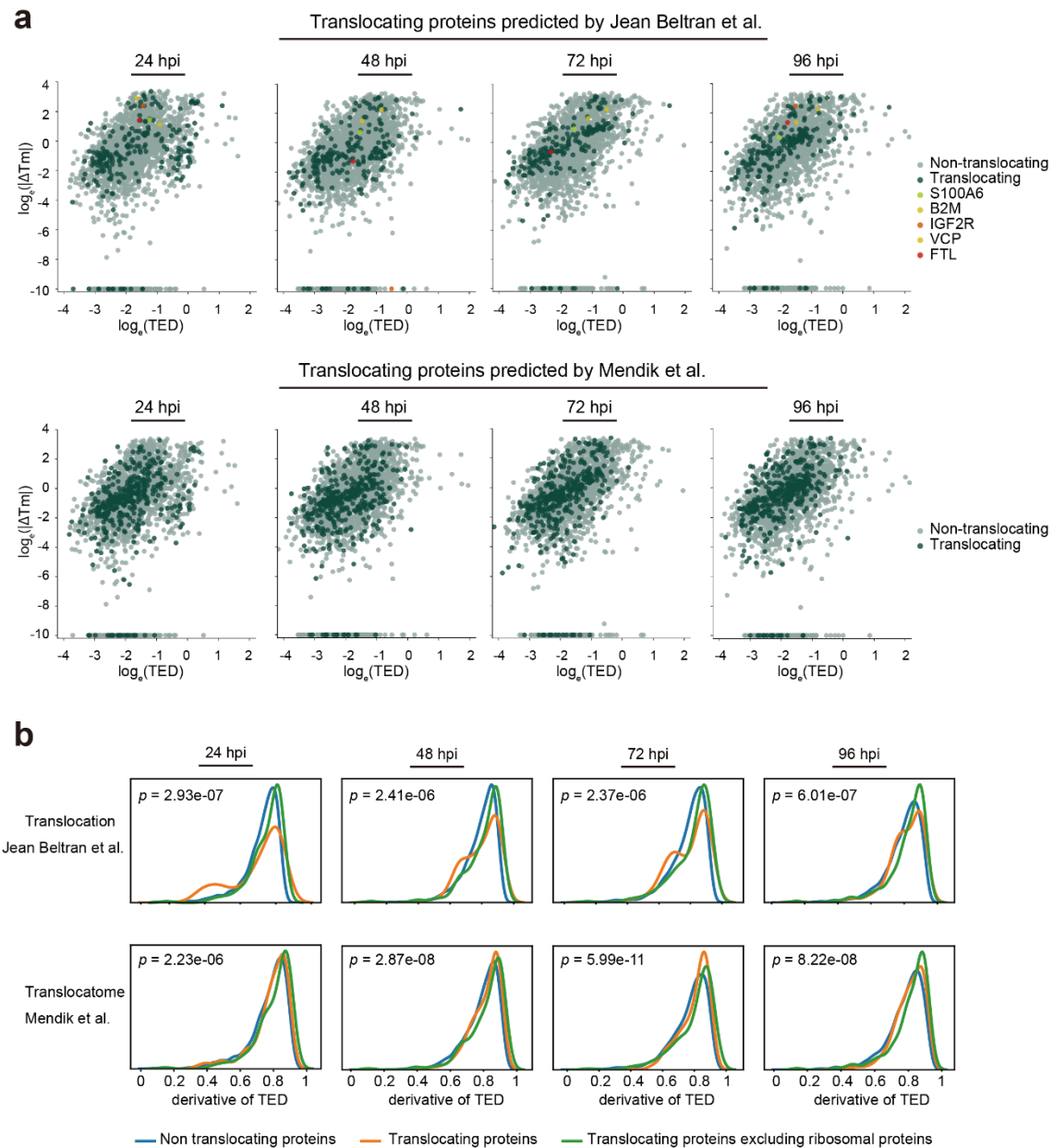
Supplementary Figure 7. Temporal Euclidean distance (TED) correlates with area under the curve (AUC) at every infection time point. $\log_e(\text{TED})$ versus $\log_e(\text{AUC})$ is depicted for each time point during HCMV infection. Pearson's r -value is shown for each infection time point.



Supplementary Figure 8. TED and ΔT_m display positive correlation. $\log_e(\text{TED})$ versus $\log_e(|\Delta T_m|)$ values are depicted for each time point during HCMV infection. Pearson's r -value is shown for each infection time point. Data points that fall at $-10 \log_e|\Delta T_m|$ mainly reflect proteins with T_m values greater than 65°C .



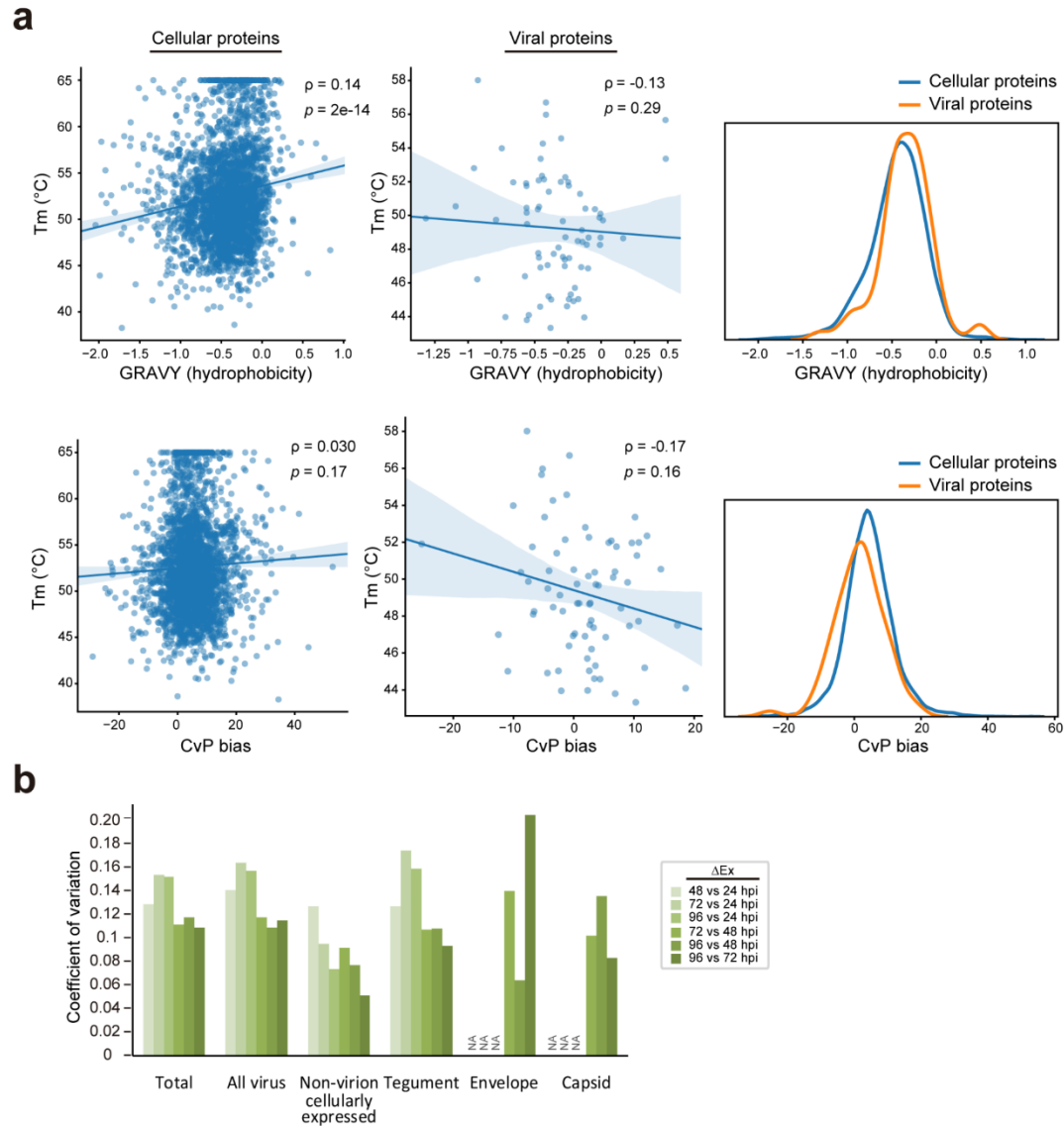
Supplementary Figure 9. Non-mitochondrial gene sets and pathways display varying disruption patterns across infection time. **a** Enrichment analysis based on $|\Delta T_m|$ for MOOTHA_MITOCHONDRIA, and WONG_MITOCHONDRIA_GENE_MODULE. Z-scores are shown for each time point. **b** NES score plots for the enriched non-metabolism related pathways. Pathways that display significant changes between early and late stages during infection are selected, among which non-metabolic pathways are plotted. Data represent individual NES scores (top) and the averaged NES scores (bottom) $\pm$ SD.



Supplementary Figure 10. TED and Tm values for the majority of translocating proteins are not changed during infection. a Scatter plots of $\log_e(TED)$ versus $\log_e(|\Delta T_m|)$ values at all infection time points are shown, where each dot represents one protein and the dark dots indicate high-confidence translocating proteins documented by the Jean Beltran, et al. (upper) and the translocatome study (lower). Several specific proteins of interest are also indicated. **b**

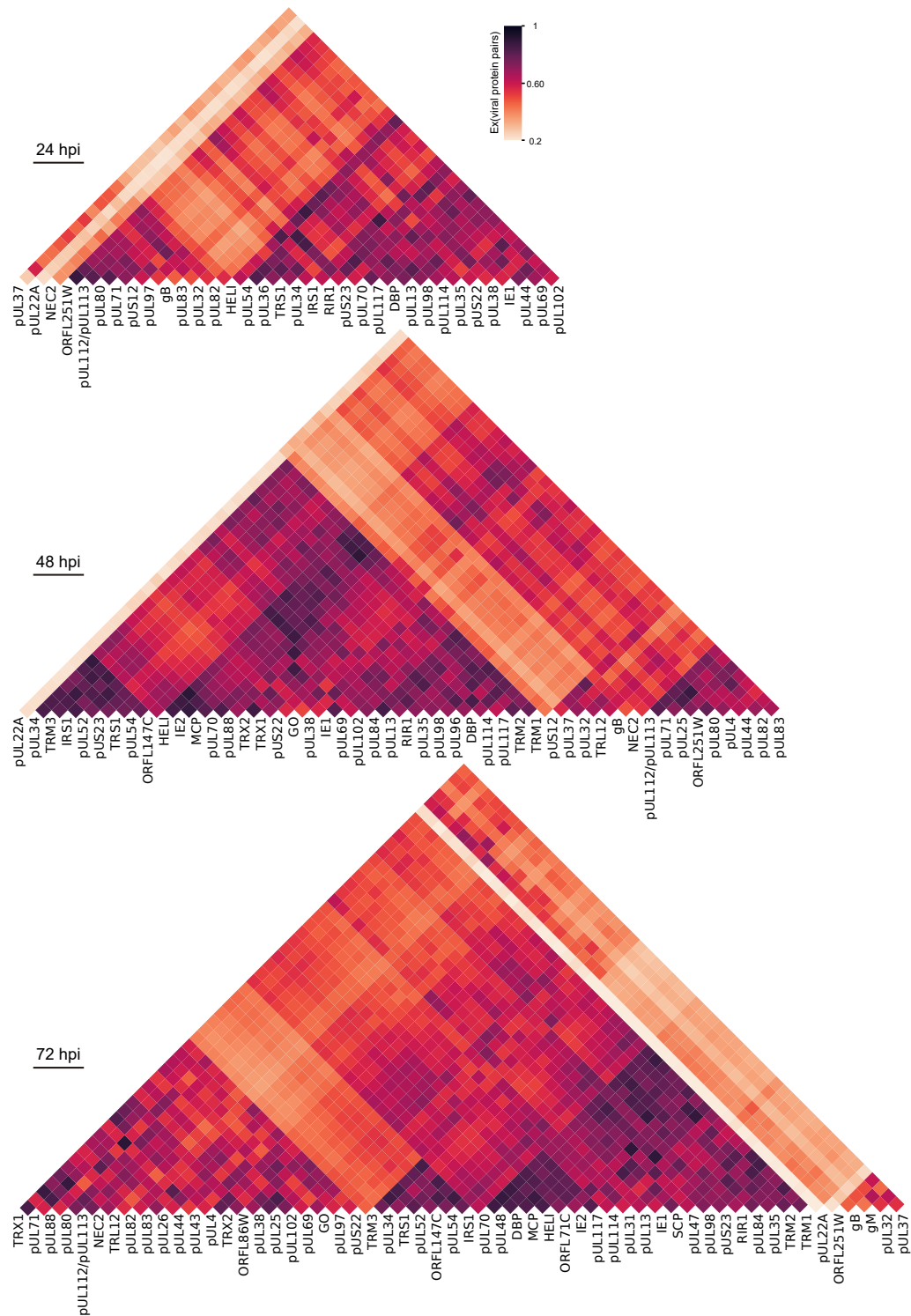
Distribution of the derivative of TED for non-translocating proteins, all translocating proteins, and translocating proteins excluding ribosomal proteins at each infection time point.

Translocating proteins are predicted from Jean Beltran et al. (upper) and the translocatome by Mendik et al. (lower). P-values calculated by a Mann-Whitney U-test on the distribution of TED values between non-translocating proteins and translocating proteins excluding ribosomal proteins are shown.



Supplementary Figure 11. Viral proteins have no significant differences from cellular proteins with regard to hydrophobicity or amino acid composition but show variation based on virion compartment. a Top panels: Correlation of average T_m values and GRAVY scores that indicate hydrophobicity for cellular proteins (left) and viral proteins (middle). Spearman's ρ and p values are also shown. The distribution of GRAVY scores of cellular proteins and viral proteins are displayed (right). Bottom panels: Correlation of average T_m values and CvP bias scores for cellular proteins (left) and viral proteins (middle). Spearman's ρ and p values

are also shown. The distribution of CvP bias scores of cellular proteins and viral proteins are displayed (right). **b** Coefficient of variation (CV) of Ex between different infection time points for all proteins, all viral proteins, and viral proteins assigned to specific virion compartments. CVs were not calculated (missing values, NA) for time points with less than 2 proteins.



Supplementary Figure 12. Distance matrices delineate potential virus-virus protein

associations at 24 hpi, 48 hpi, and 72 hpi. Ex values per pair of viral proteins at 24 hpi (top), 48

hpi (middle), and 72 hpi (bottom). The colors of the bins indicate Ex values for each protein pair at the indicated time point. Viral proteins were clustered by average (UPGMA) method.