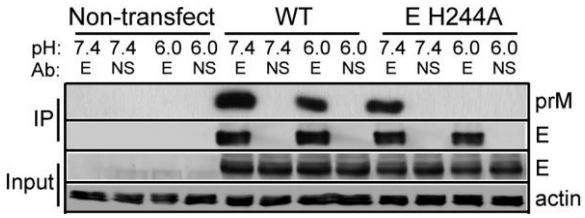
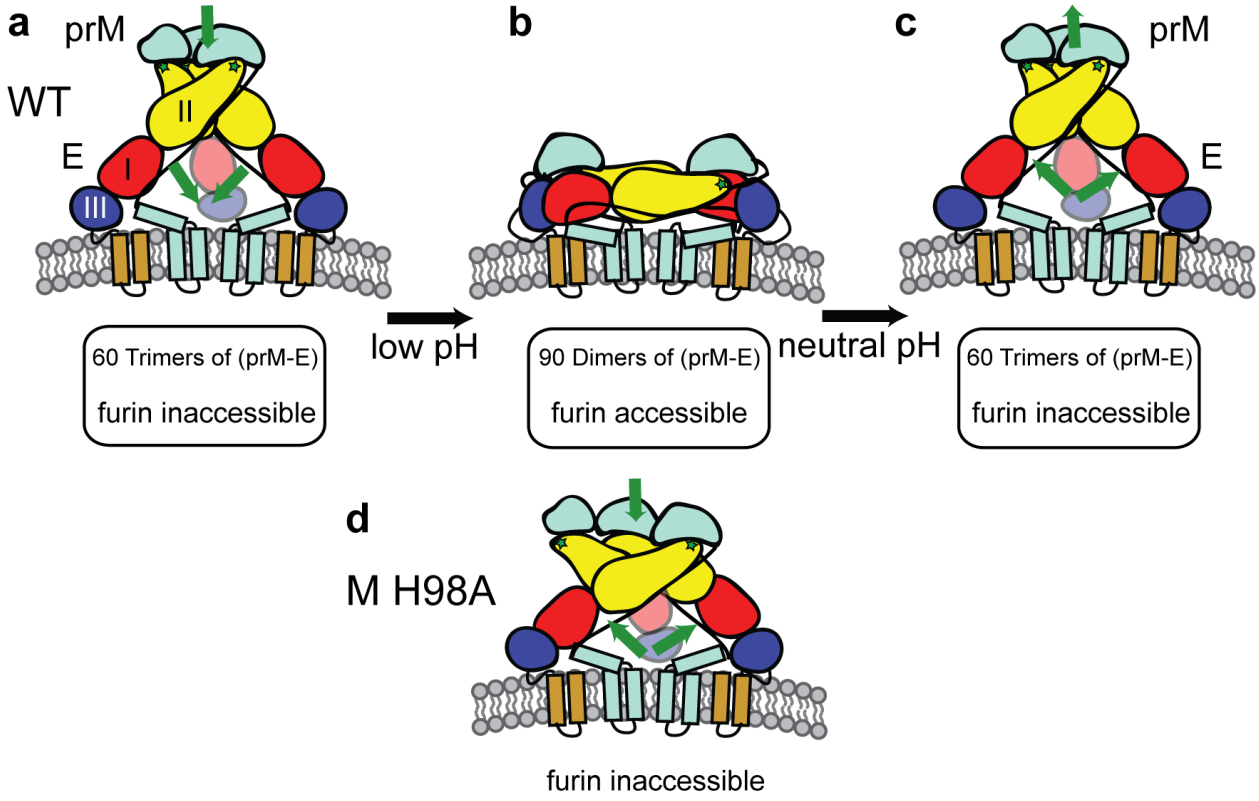
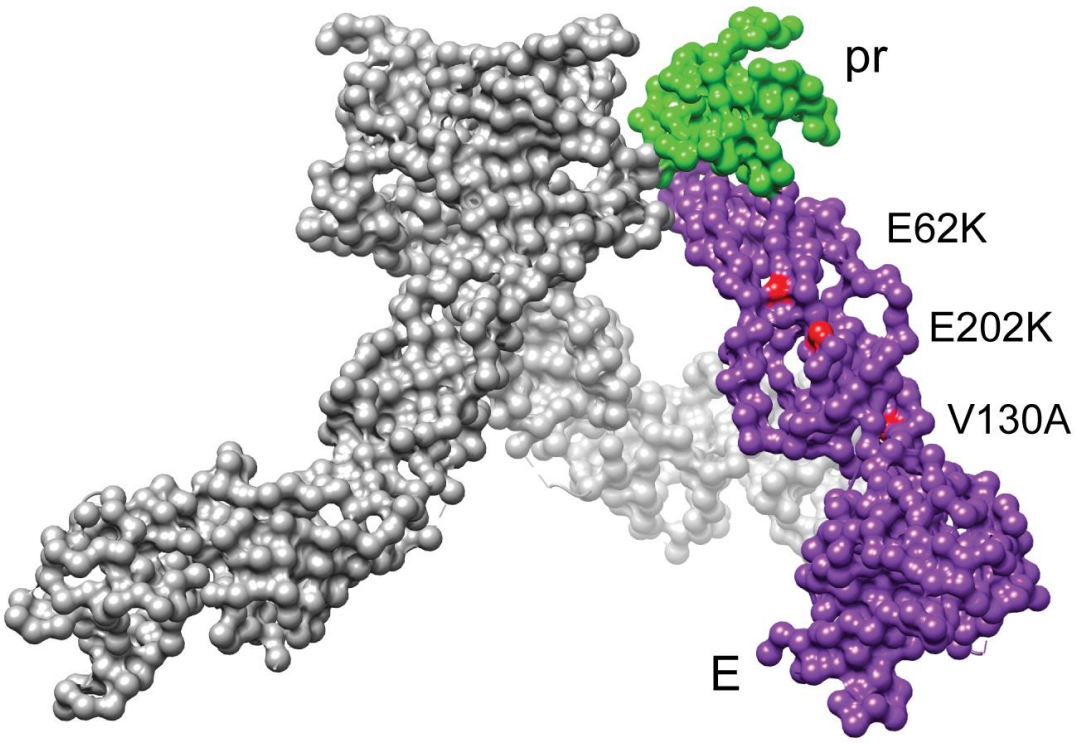
Supplementary Information



Supplementary Figure 1. The M region of DENV prM protein binds E protein at neutral pH. 293T cells were transfected with plasmids encoding WT or mutant prME. Two days post-transfection, the cells were lysed in buffers at the indicated pH. Aliquots of the lysates were immunoprecipitated at the indicated pH with anti-E mAb 4E11 (E) or a non-specific mAb (NS). The eluted proteins were analyzed by SDS-PAGE and western blot to detect prM or E. Expression levels in the lysates were evaluated by western blot (input). Data are a representative example of two independent experiments. Full scans of the blots are in Supplementary Fig. 4.



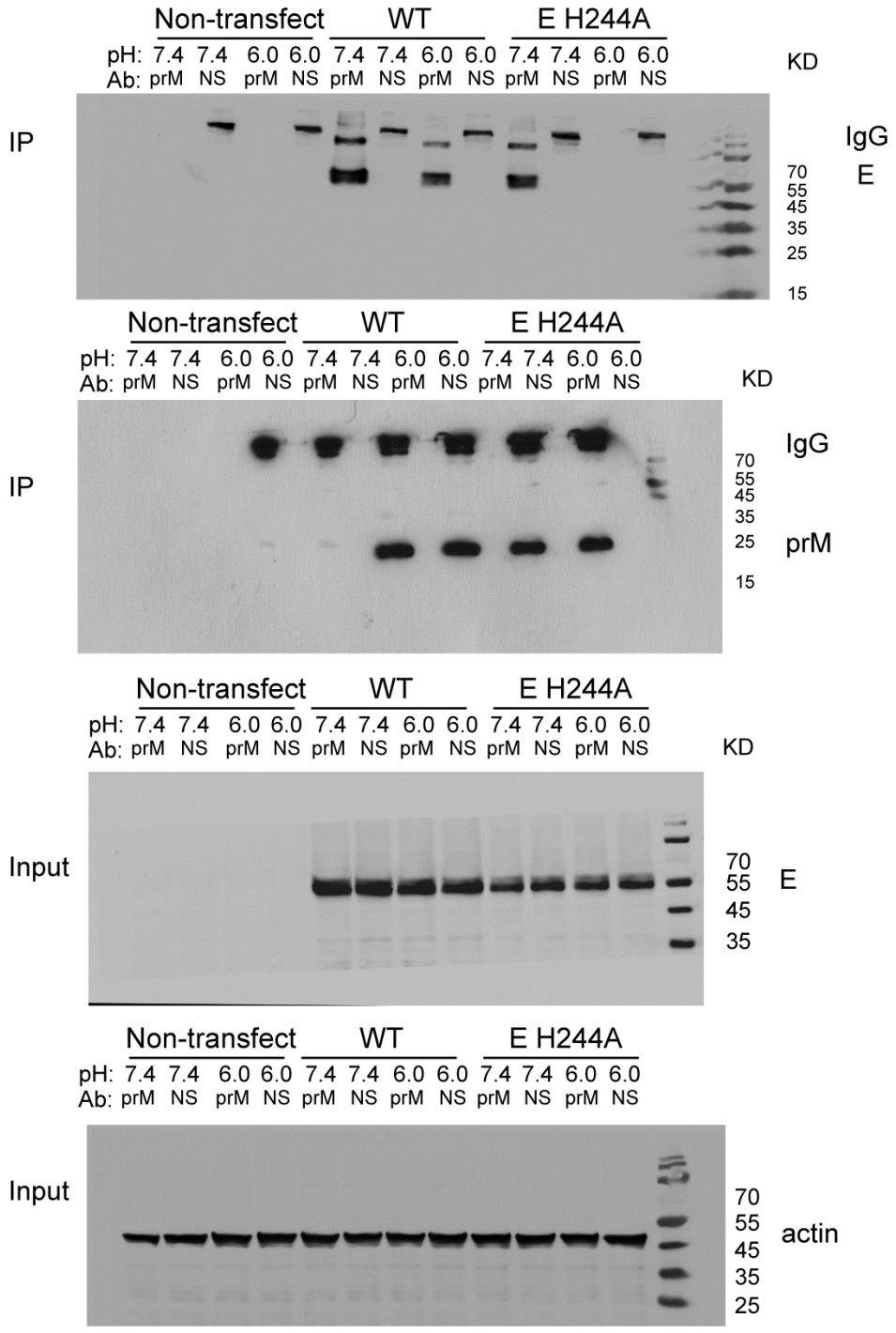
Supplementary Figure 2. Model for the reversible pH-triggered transition of immature DENV particles. DI, DII and DIII of E protein are colored red, yellow and blue, and the fusion loop at the DII tip is indicated by a star. The prM and E TM domains are displayed in cyan and brown, respectively. The pr region is shown in cyan, and the black line indicates the N-terminal region of M. a. The immature trimer of prM-E is shown. The interactions between prM and E triggered by low pH are highlighted by the green arrows, which indicate pr-E binding and M-E repulsion. b. Low pH conformation of the immature virus. c. Return to the immature trimer conformation. The interactions between prM and E triggered by neutral pH are highlighted by the green arrows, which indicate pr-E repulsion and M-E binding. d. Proposed intermediate conformation of prM H98A virus at low pH. The interactions between prM and E triggered by low pH are highlighted by the green arrows, which indicate pr-E binding and M-E binding. Modified from Trends in Microbiology, vol. 17 (11), C. Sánchez-San Martín, C. Y. Liu, M. Kielian, Dealing with low pH: entry and exit of alphaviruses and flaviviruses, p. 514-521, Copyright (2009), with permission from Elsevier.



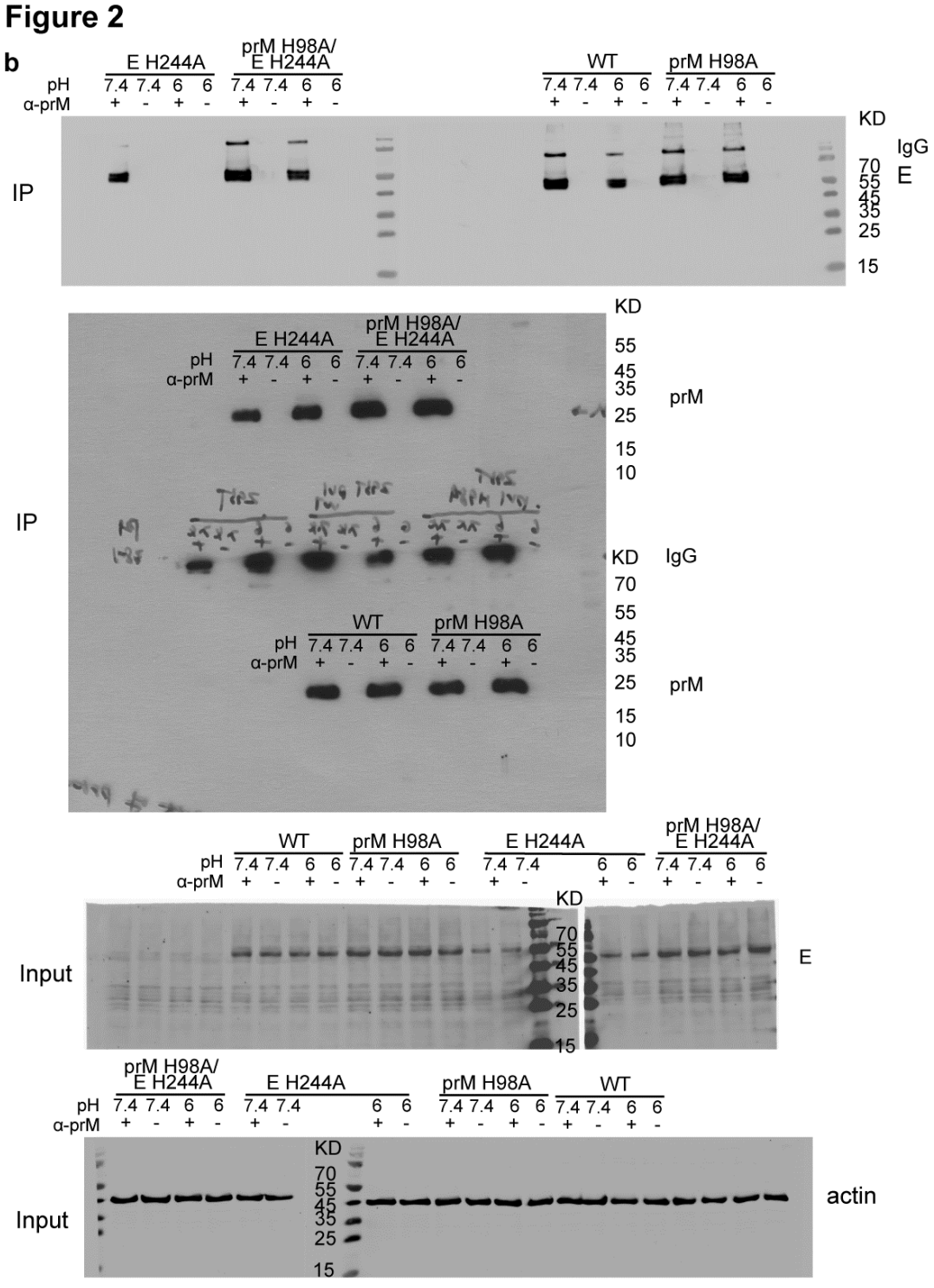
Supplementary Figure 3. Locations of the E revertant mutations in the immature prM-E trimer structure. Surface view of the immature trimer of pr-E is shown, as derived by the fit of the prM-E heterodimer structure into the cryoEM reconstruction of the dengue immature virus at neutral pH (PDB accession number 3C6D). One pr peptide is indicated in green and one E protein in purple, with the other proteins in the trimer shown in dark or light grey. The positions of the E protein mutations at residues 62, 130 and 202 are indicated. Figure prepared using PyMOL (The PyMOL Molecular Graphics System, Version 1.5.0.4, Schrödinger, LLC).

Supplementary Figure 4. Full scans of the western blots and phosphorimages used in this study. Panels are labeled to correspond with the number of the original figure.

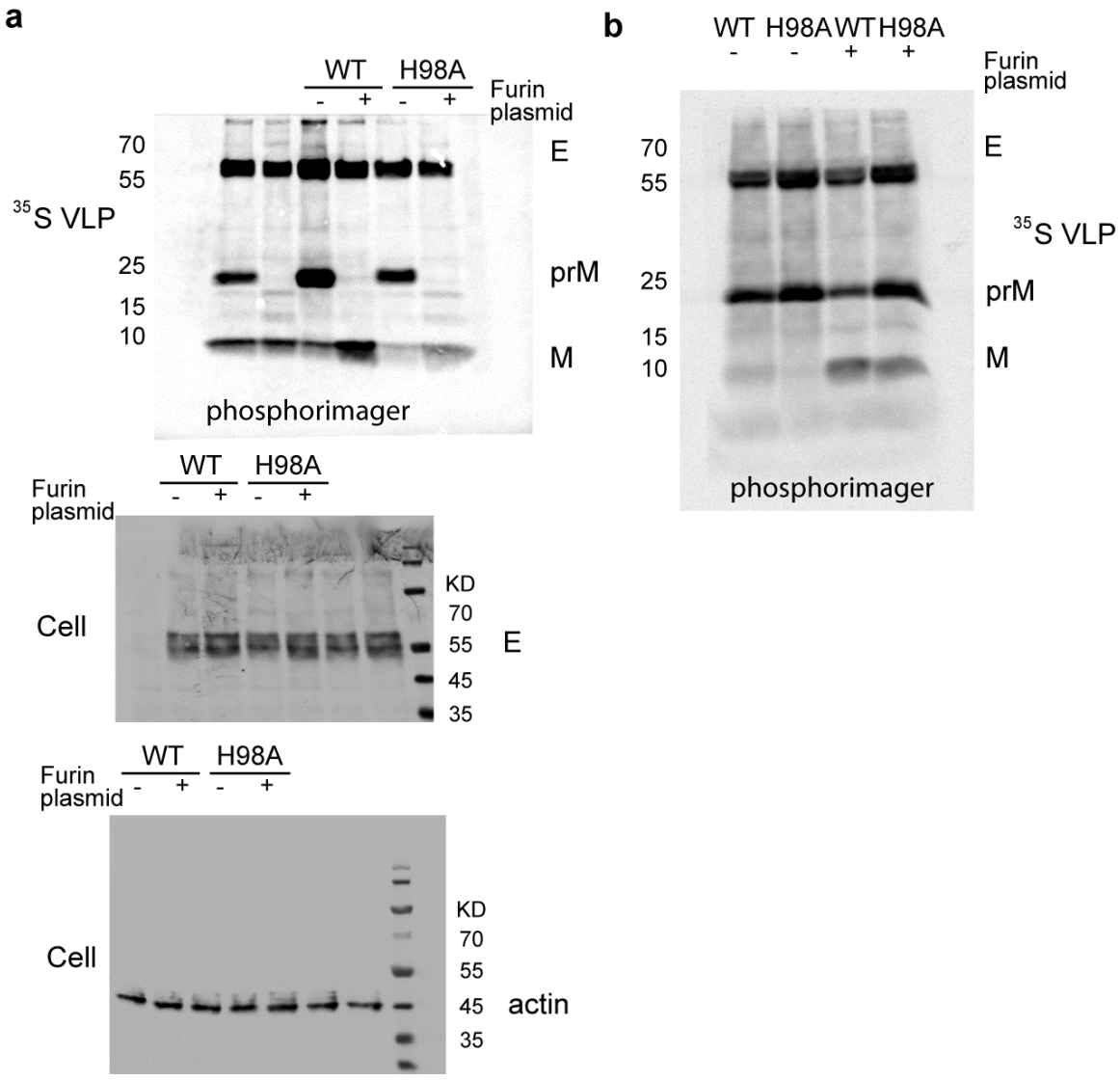
Figure 1



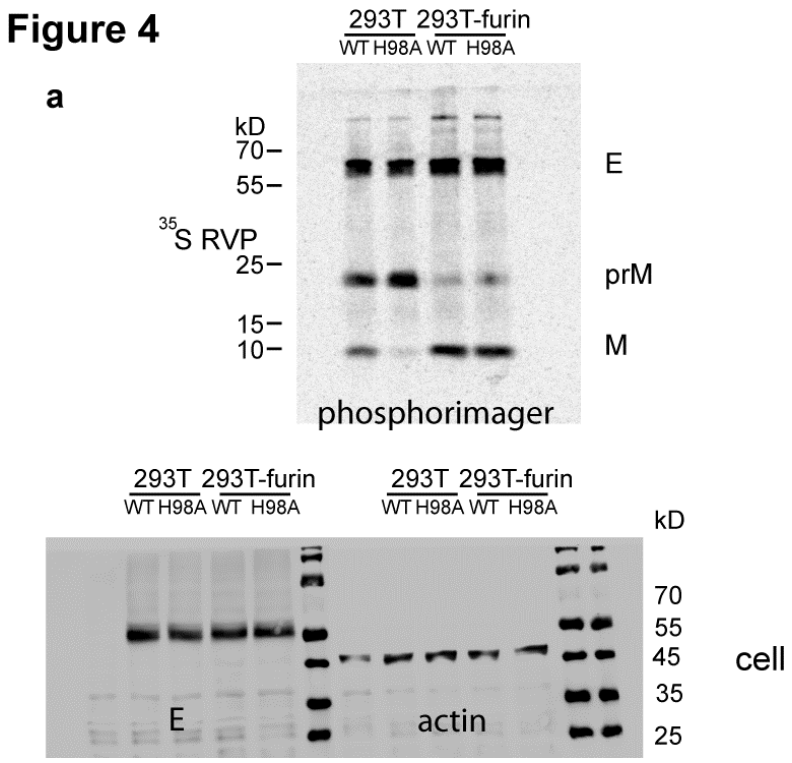
Supplementary Figure 4 Continued



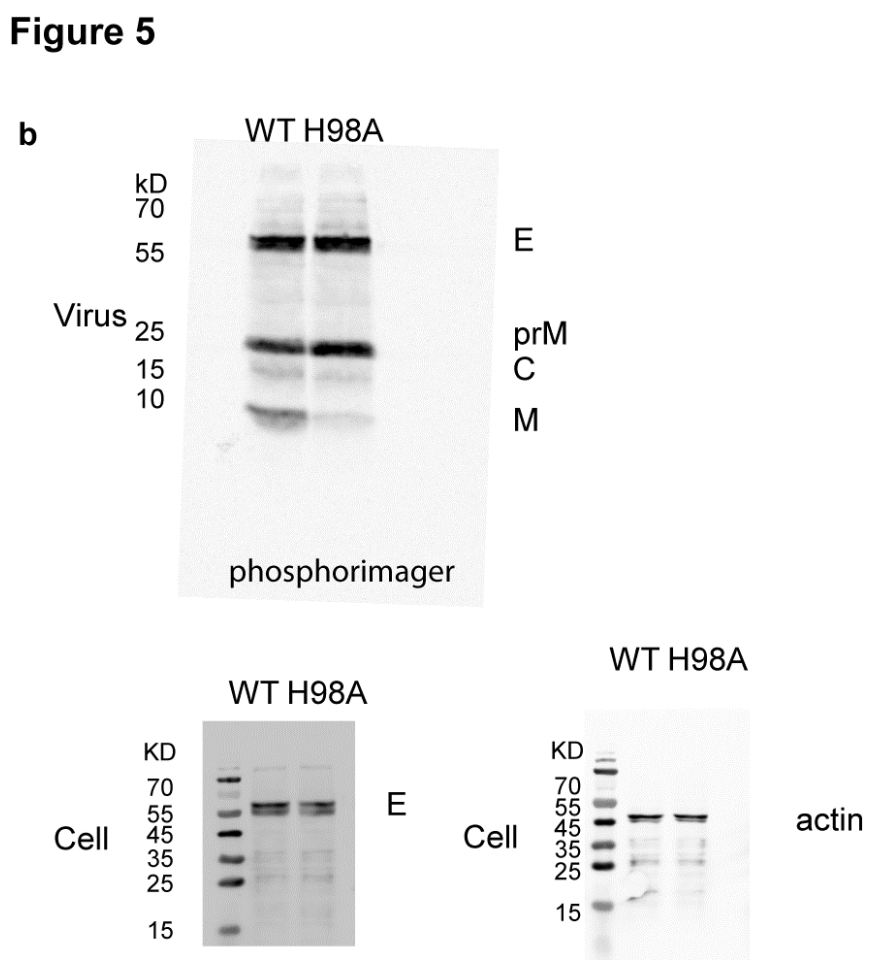
Supplementary Figure 4 Continued



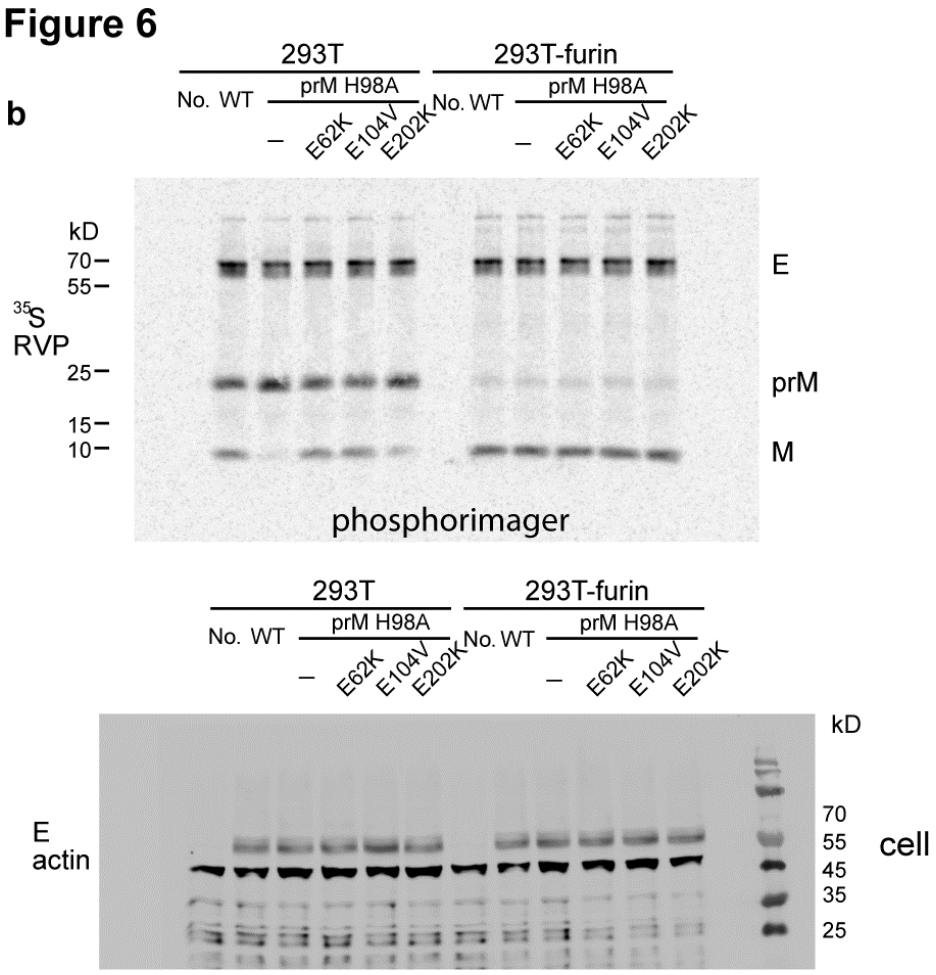
Supplementary Figure 4 Continued



Supplementary Figure 4 Continued



Supplementary Figure 4 Continued



Supplementary Figure 4 Continued