Supplementary Figure 4. Translation reporter screen for 5'leaders that enhance translation during HSV1 infection. (A) Agarose gel visualization of other HSV1 5'leaders (including the rest of the 5' leader in Figure 2) amplified from total RNA of HSV1 infected cells. Negative controls (RNA from uninfected cells) confirmed the specificity of the PCR products for HSV1 transcripts only. (*): unspecific band. (B) Translation reporter assay to screen for HSV1 leader sequences that enhance translation during HSV-1 infection. 4T1 cells were transfected with the CAT plasmid and a β-GAL expression plasmid that serve as a transfection control. 8 hours post transfection, cells were infected with HSV-1716-GFP at an MOI of 5. Cells were lysed 18 hours post infection and CAT expression was quantified by ELISA, while β-GAL activity was quantified by colorimetric assay using ONPG substrate. (C) Predicted secondary structure and folding free energy of US11 (left panel) and UL27 (right panel) leaders using Vienna RNAfold[33]. Color scale bars represent base pairing probabilities. (D) Heatmap representing folding free energy of candidate HSV1 leaders, calculated using Vienna RNAfold.