

Supplementary Figure 5. Characterizing expression enhancement by US11 5' Leader in oncolytic HSV1. (A) . Monolayer of Vero cells was infected with the indicated virus at a MOI of 5, and the culture supernatant was collected 24 hours post infection. GM-CSF concentration was quantified by ELISA. One-way ANOVA with Dunnett's post-hoc test was performed. $n = 3$ biological replicates. Error bars: \pm sd. **** $p < 0.0001$. (B) Representative GFP fluorescence of HSV1 expressing Leaderless CSF2-GFP or US11 5'leader-CSF2-GFP. Monolayer of CT26 was infected with the indicated virus at an MOI of 5, then fluorescence microscopy images were taken at 24 hours post-infection. (C) Time course of GFP fluorescence of HSV1 expressing Leaderless CSF2-GFP or US11 5'leader-CSF2-GFP. Monolayer of CT26 was infected with the indicated virus at an MOI of 0.2, 1 or 5, then GFP fluorescence was monitored over 2 days post-infection using the Incucyte live cell imaging system. (D) Dose dependency analysis of secreted GM-CSF. CT26 cells were infected with the indicated virus at an MOI of 0.2, 1 or 5, then culture supernatant was collected 24 hours post-infection. GM-CSF concentration was quantified using ELISA. Two-way ANOVA with Sidak post-hoc test was performed. $n = 3$ biological replicates. Error bars: \pm sd. **** $p < 0.0001$.