Genetics Day Poster Submission

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Title: PROXIMITY LABELING REVEALS HUMAN CYTOMEGALOVIRUS UL26 PROTEIN INTERACTS WITH INNATE IMMUNE SYSTEM REGULATOR PIAS1 TO SUPPORT VIRAL REPLICATION

Abstract: Human Cytomegalovirus (HCMV) is a ubiquitous β-Herpesvirus that latently infects 60-90% of the adult population worldwide. HCMV causes severe morbidity and mortality in immunocompromised individuals, particularly neonates. Currently there is no FDA-approved vaccine for HCMV and anti-viral therapeutics are lacking. Elucidating the mechanisms involved in successful viral replication could provide novel points for therapeutic intervention. Like many viruses, HCMV must evade or suppress the host innate immune system for successful replication. Our laboratory previously found that the HCMV tegument protein UL26 is necessary for high titer viral replication and sufficient to block cytokine-induced NFκB innate immune activation. The underlying mechanisms through which UL26 the innate immune response and contribute to successful viral replication remain unclear. To address this, we sought to identify host proteins that UL26 interacts with during infection by creating a recombinant virus containing UL26 fused to a promiscuous biotin ligase, TurboID. The addition of biotin during infection facilitated biotin-tagging of proteins proximal to UL26. Biotinylated proteins were isolated and identified by LC-MS/MS to generate a list of putative UL26-interacting proteins that were subsequently interrogated for their role in HCMV infection and replication. Our results indicate that UL26 interacts with several innate immune response factors including transcription factors STAT1-3 and protein inhibitor of activated STAT (PIAS) proteins. Consistent with these interactions, we found that UL26 is sufficient to block IFNα-induced interferon stimulated response element (ISRE) activation. We next hypothesized that UL26 interacts with PIAS1, a regulator of diverse transcription factors, to explain the ability of UL26 to antagonize both NFκB and ISRE activation. We used CRISPR Cas9 to generate a PIAS1 knockout cell line and found that HCMV spread was significantly reduced in cells lacking PIAS1. We find PIAS1KO cells are more sensitive to cytokines pretreatment, indicating cells lacking PIAS1 have lost the ability to negatively regulate gene expression triggered by the innate immune response. Further, PIAS1 deletion did not restore UL26-mutant viral growth defect. Together, these data indicate a cooperative interaction between UL26 and PIAS1 that supports viral replication.