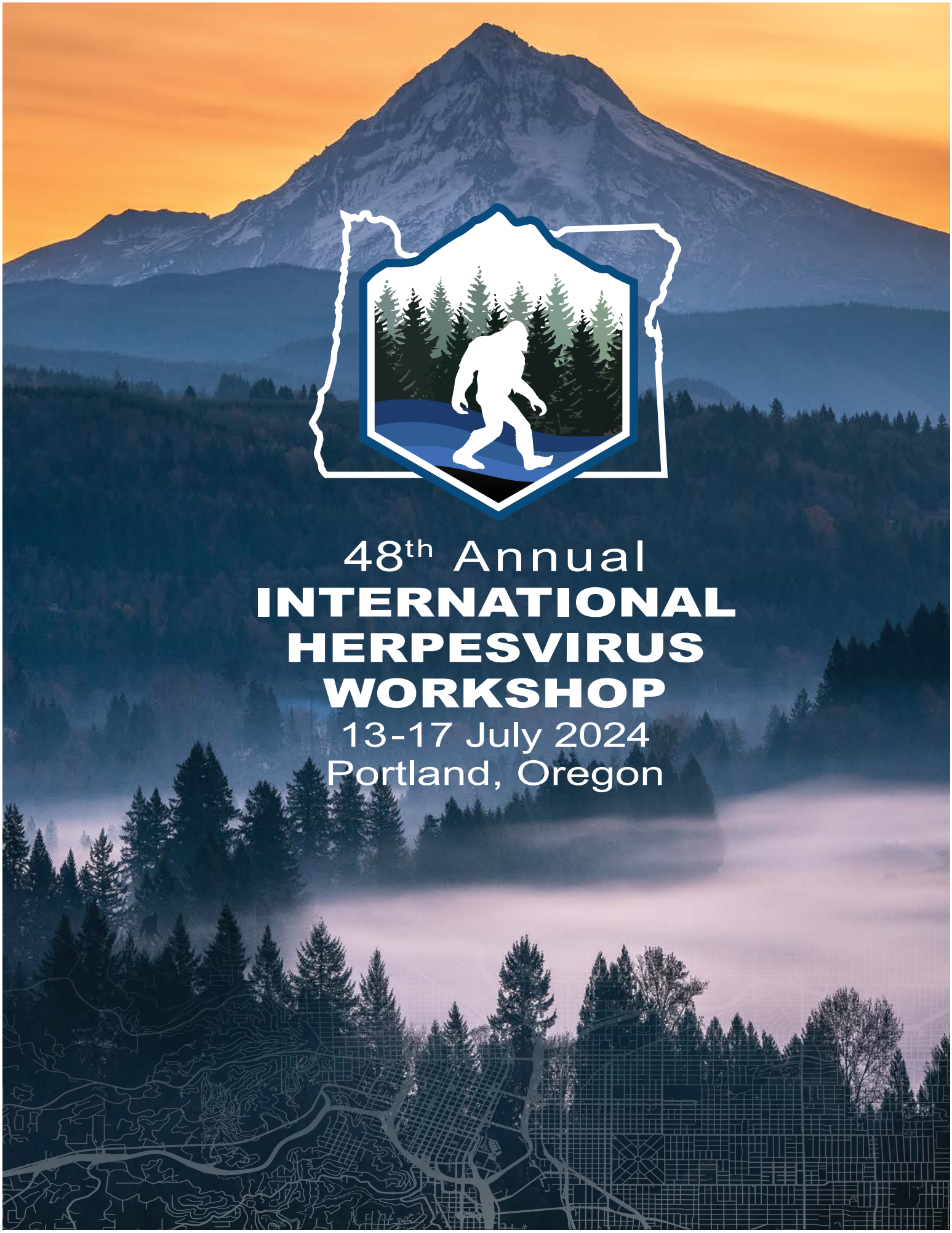
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8.75

Feline Gammaherpesvirus Protein F10, a Novel E3 Ubiquitin Ligase, Downregulates MHC-I from the Cell Surface via a Proteasome and ERAD-Dependent Mechanism.

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Gammaherpesviruses (GHVs) encode many proteins that manipulate the host environment through virus-host protein interactions. *Felis catus* gammaherpesvirus 1 (FcaGHV1), is a recently discovered GHV that infects millions of domestic cats worldwide. No *in vitro* infection model exists, and little is known about its gene expression and protein function.

FcaGHV1 protein F10 is a novel E3 ubiquitin ligase homologous to host animal and viral E3 ligases, including the KSHV protein K3. F10 encodes an N-terminal RING domain likely responsible for E2 ubiquitin conjugating enzyme recruitment, and a C-terminal tyrosine endocytosis domain likely responsible for target protein internalization. We used immunofluorescence microscopy to identify F10 as a likely ER-resident protein. We established through flow cytometry experiments in transfection and lentiviral pseudovirus-based systems that Crandell-Rees Feline Kidney (CRFK) cells expressing F10 have significantly less surface MHC-I compared to a negative control. Antibody internalization assays indicate that F10 enhances the rate of MHC-I endocytosis. Point mutations in either the RING or tyrosine endocytosis domains also cause a significant rescue of MHC-I surface expression, suggesting the importance of each domain to F10's function. We found that treatment with the proteasome inhibitor MG-132 resulted in significant rescue of CRFK MHC-I surface expression in the presence of F10, suggesting a proteasome-dependent mechanism of action. Interestingly, treatment of CRFKs with the E1 enzyme inhibitor PYR-41 caused no significant reduction of F10's function, contrasted to significant inhibition of K3's MHC-I downregulation, implying a difference in mechanism between the two homologues. Treatment of F10-expressing cells with the ER-associated degradation (ERAD) pathway inhibitor kifunensine also significantly inhibited F10's downregulation of host MHC-I. Investigation into other host targets of F10, as well as the effect of other small molecule inhibitors is ongoing. Our findings detail a potentially unique mechanism used by a herpesvirus protein to downregulate MHC-I.

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Interplay between HCMV Protein pp65 and cGAS-STING Pathway: Modulating Viral Infectivity via Lipid Metabolism

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The phosphoprotein 65 (pp65) constitutes a major component of the virion tegument in human cytomegalovirus (HCMV), despite its nonessential role in virion assembly. Nevertheless, pp65 plays a pivotal role in modulating and dampening host antiviral responses. This study seeks to elucidate the impact of pp65 on viral infectivity. We initially assessed the replication and infectivity of a mutant strain of HCMV TB40-BAC4, unable to express UL83-encoded pp65 (v65Stop), in comparison to its pp65 revertant counterpart (v65Rev), using primary human foreskin fibroblasts (HFFs), under multiplicity of infection (MOI) condition. No differences in replication were observed between v65Stop and v65Rev, consistent with existing literature. However, infecting HFFs with an equivalent number of v65Stop or v65Rev virions per cell revealed a significant reduction in v65Stop infectivity relative to v65Rev, underscoring the involvement of pp65 in viral infectivity. Viral infectivity is a multifaceted process, where lipid metabolism plays a crucial role. Hence, our focus turned to exploring how pp65 impacts fatty acid synthase (FASN), the rate-limiting enzyme of *de novo* lipogenesis. Our results demonstrated that HCMV infection significantly upregulates the FASN expression and lipid concentrations highlighting a regulatory role of pp65 in de novo lipogenesis. Furthermore, given the relevance of the cGAS-STING pathway in maintaining metabolic homeostasis, we generated cell lines with silenced cGAS and STING genes. Remarkably, we observed a restoration of v65Stop infectivity in silenced cells, supporting the relevance of the cGAS-STING pathway in lipid metabolism. Understanding the intricate connections between pp65, lipid metabolism, and the interferon pathway provides valuable insights into the molecular mechanisms driving HCMV infectivity and unveils potential targets for therapeutic interventions against HCMV infections.