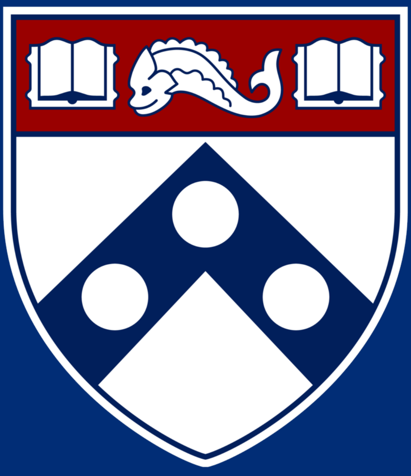
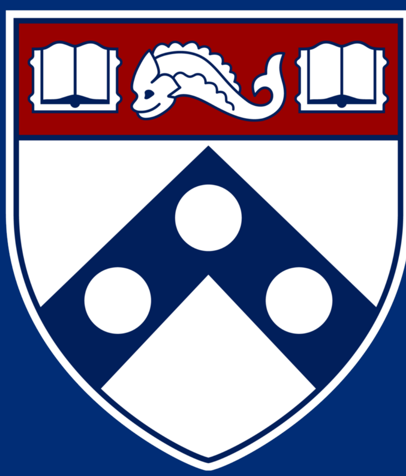


Developing Tools to Study the Immune Antagonistic C15 Virulence Protein



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Introduction

The orthopoxvirus (OPXV) genus is home to many severe mammalian pathogens, including monkeypox (MPXV), variola (VARV), cowpox (CPXV), and ectromelia (ECTV). This project focuses on C15, a 1900 amino acid surface glycoprotein encoded within ECTV, the cause of mousepox. The Eisenlohr lab has demonstrated that C15 is necessary for the lethality of ECTV in a BALB/c mouse infection model as well as efficient replication in C57BL/6 mice, suggesting that the protein is an essential virulence factor. Notably, previous work has shown the capacity for C15 to inhibit both the activation of CD4+ T cells and the degranulation of natural killer (NK) cells. However, relatively little is known about the molecular mechanisms that mediate this immunomodulation by C15 at both the innate and adaptive immune levels.

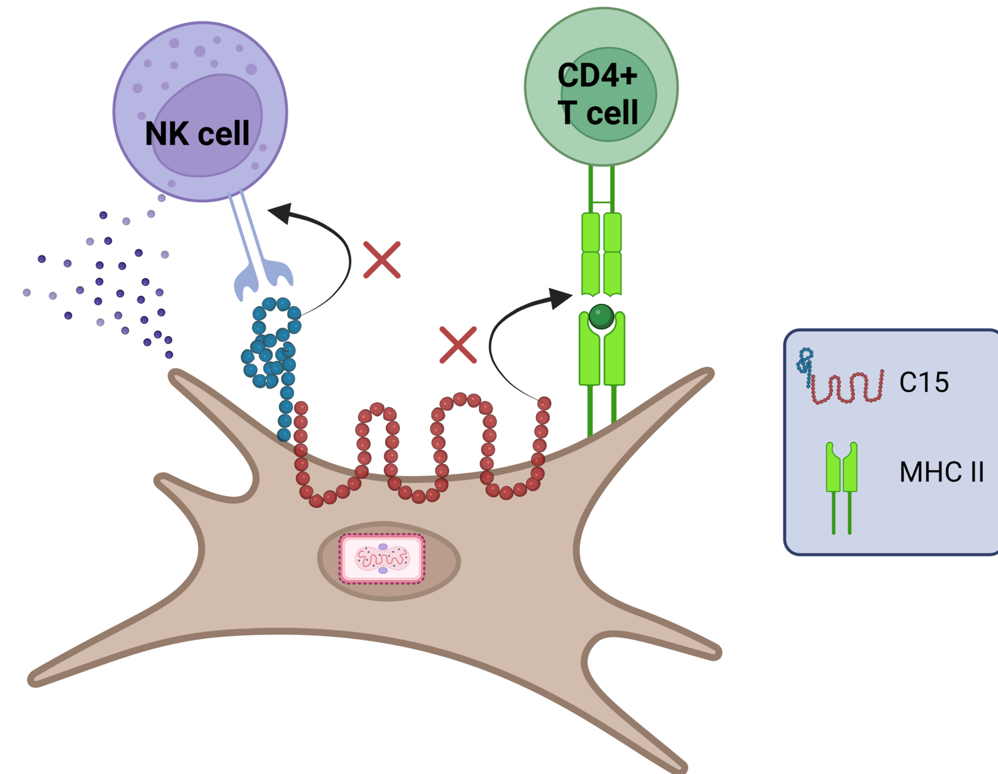

Figure 1. **Model figure of C15 function.** C15 inhibits the innate and adaptive immune systems. Different protein regions are hypothesized to antagonize these functions as shown.

Methods

1) We generated a stable cell line expressing mouse MHC II molecules (IAb and IEd) and their transactivator (CIITA) as well as C15-HA using the Sleeping Beauty transposon system. We incorporated a Tet-on expression system, which allowed for the inducible expression of C15 through overnight incubation with doxycycline.

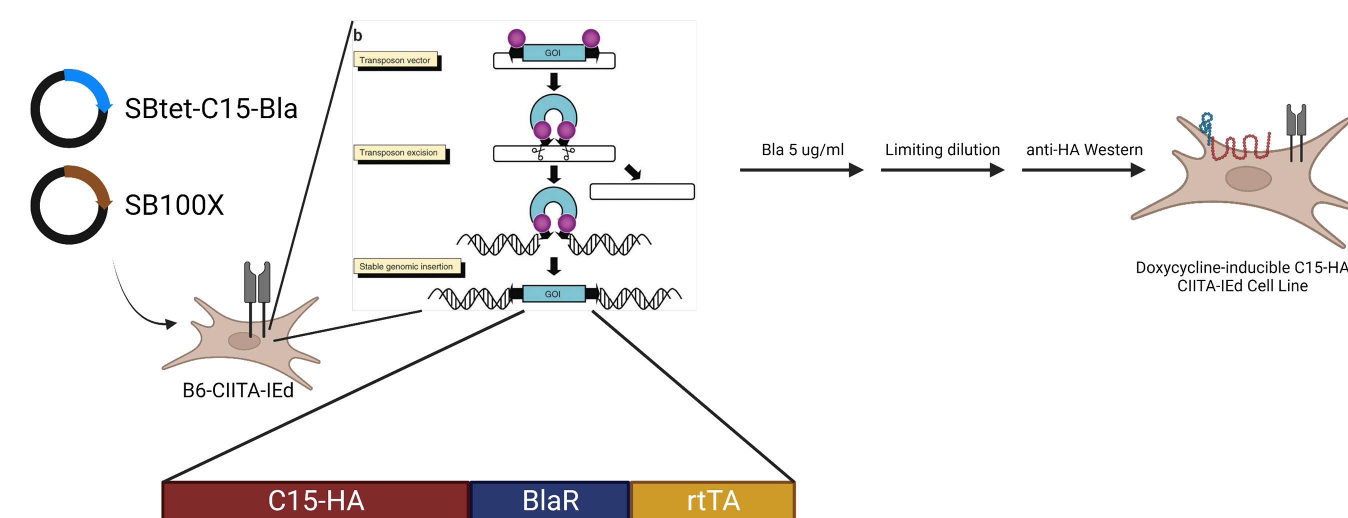

Figure 2. **Overview of the scheme to generate the B6-CIITA-IEd-C15-HA cell line.** We first co-transfected B6-CIITA-IEd cells with a C15 expression vector and the Sleeping Beauty transposon. We then selected positive clones with blasticidin and performed clonal expansion through limiting dilution.

1.1) We used the cell line in an immunoprecipitation to isolate the C15 protein and its binding partners with an HA-specific agarose column. The eluates were then stained with anti-mouse MHC II via Western blot.

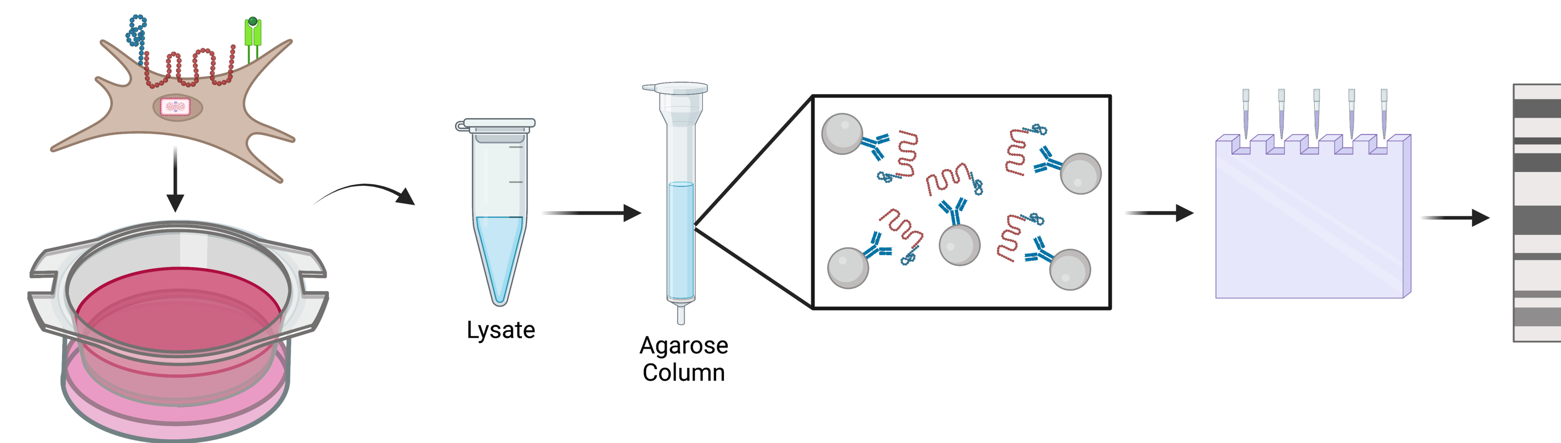

Figure 3. Anti-HA immunoprecipitation procedure. We induced B6-CIITA-IEd-C15-HA cells overnight. After, we collected cell lysates, clarified, and incubated them on an anti-HA agarose column for two hours. We then eluted proteins off the column and analyzed by Western blot.

2) A recombinant ECTV including the N-terminus of C15 (amino acids 1-456) was generated using a CRISPR/Cas9 selection system with sgRNA GFP.

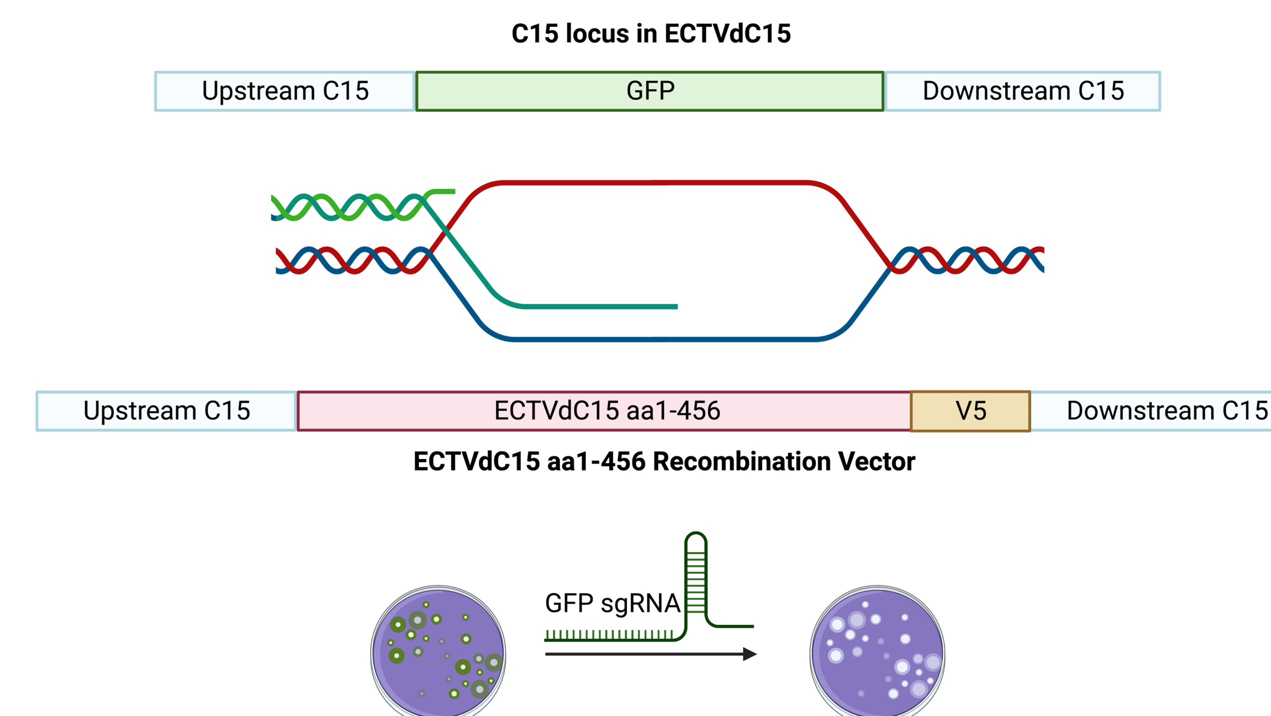

Figure 4. CRISPR/Cas9 selection system using GFP sgRNA. We transfected ECTV-infected cells with a recombination plasmid and selected against unrecombined viruses by transfecting with a sgRNA that targets the GFP locus.

Results

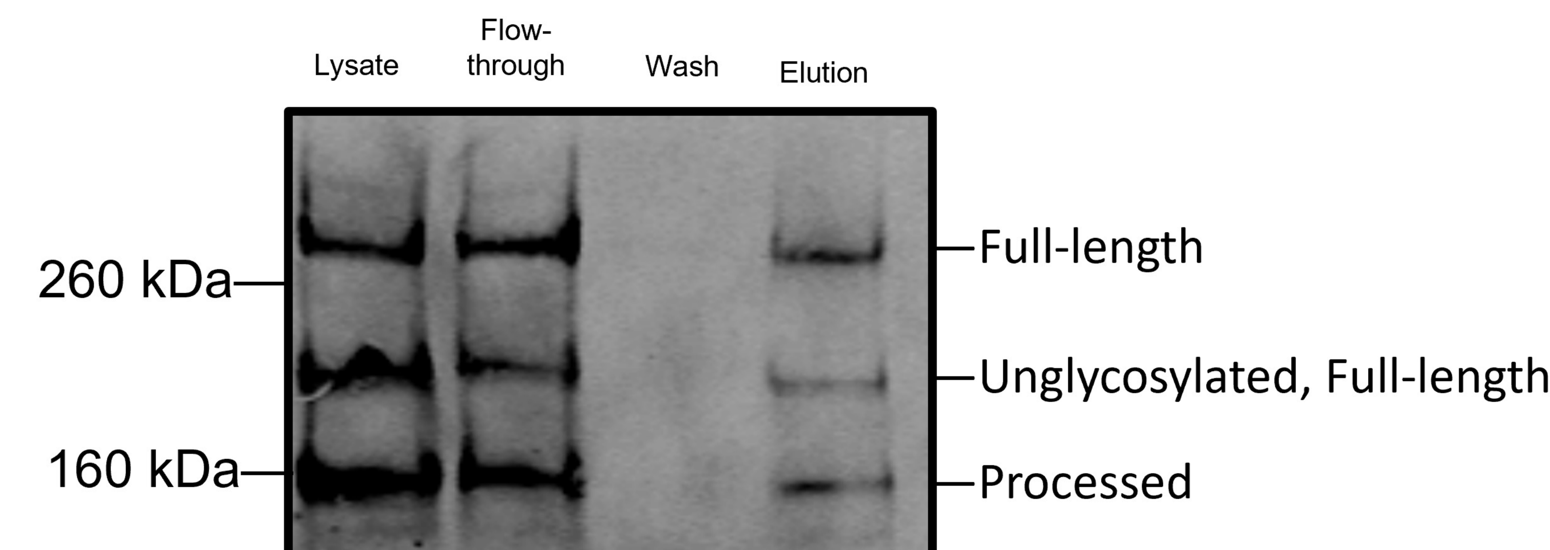

Figure 5. **C15-HA immunoprecipitation reactions.** Lysate, flow-through, wash, and elution samples were collected using an anti-HA immunoprecipitation kit with B6-CIITA-IEd-C15-HA cell lysates. Samples were run via SDS-PAGE under reducing conditions and immunoblotted with anti-HA.

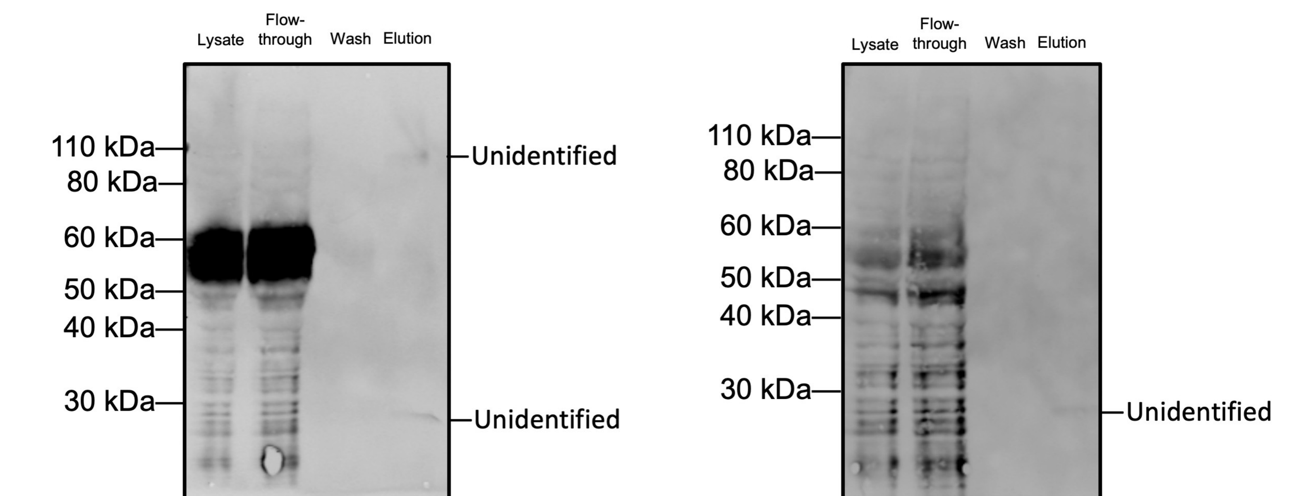

Figure 6. **MHCII co-immunoprecipitation reactions.** Lysate, flow-through, wash, and elution samples were collected using an anti-HA immunoprecipitation kit with B6-CIITA-IEd-C15-HA cell lysates. Samples were run via SDS-PAGE under reducing conditions and immunoblotted with anti-mouse MHC II.

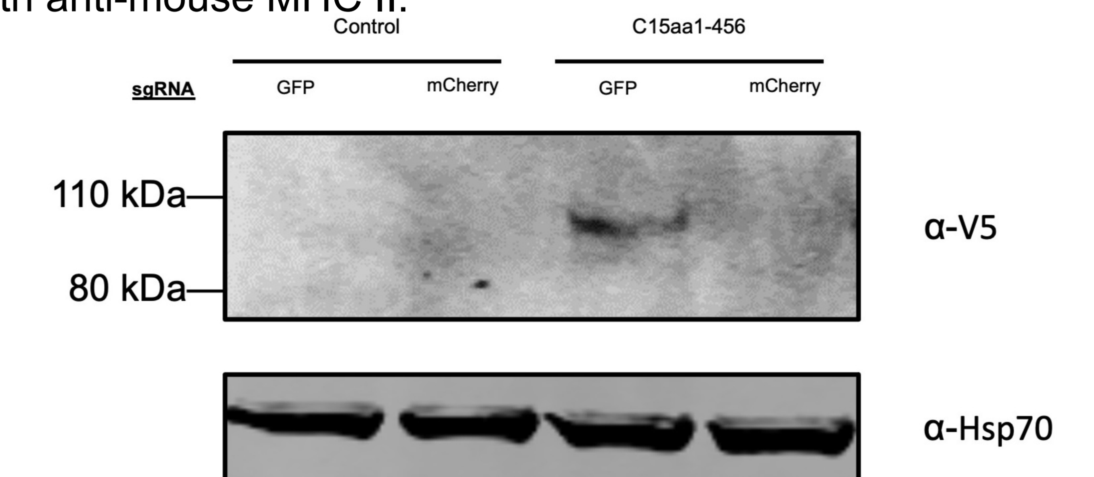

Figure 7. **Generation of rECTV-C15aa1-456.** Three rounds of selection with GFP and mCherry sgRNA were performed on BSC-1 cells infected with either ECTV GFP, ECTVC15KO, or ECTVC15KO + transfected recombination plasmid. Following selection, cells were lysed and run via SDS-PAGE under reducing conditions. The nitrocellulose membrane was cut and blotted with antibodies against V5 and Hsp70.

Conclusions

- We successfully generated an inducible cell line that expresses C15-HA
- C15 is able to be immunoprecipitated from our cell line using an anti-HA agarose purification column
- We created a recombinant ECTV that expresses aa1-456 of C15 through GFP sgRNA selection

Future Directions

- Perform an MHCII immunoprecipitation followed by blot for C15-HA
- Identify binding partners of C15 through mass spectrometric analysis
- Screen and purify rECTV-C15aa1-456
- Test the ability of rECTV-C15aa1-456 to i) inhibit CD4+ T cell activation *in vitro* and ii) NK cell function *in vivo*

Acknowledgements

- Funding sources: UPenn CURF, 1R21AI160063-01
- Eisenlohr lab

PennCURF
CENTER *for* UNDERGRADUATE RESEARCH & FELLOWSHIPS