Interferon (IFN) vs. HIV

Which IFN-stimulated genes block infection in primary CD4⁺ T cells?

Hannah Itell[1,2], Daryl Humes[2], Julie Overbaugh[2]

[1] Molecular and Cellular Biology PhD Program, University of Washington, Seattle, Washington
[2] Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, Washington

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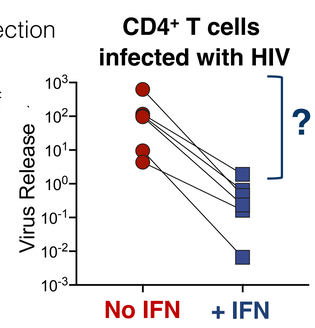
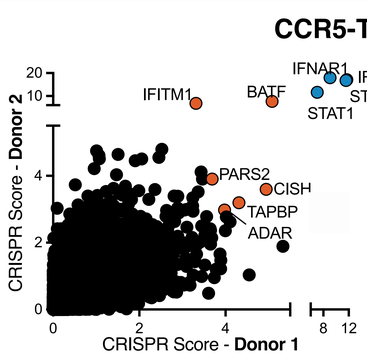
1. Background

- Innate immune sensing of viral infection triggers interferon (**IFN**) secretion, which upregulates of thousands of IFN-stimulated genes (**ISGs**) with antiviral functions
- HIV is sensitive to IFN *in vitro* and *in vivo*, suggesting that IFN may shape early stages of infection
- However, there are limited data on the ISGs that act as HIV antiviral factors in the main target cells of HIV infection, primary human CD4⁺ T cells

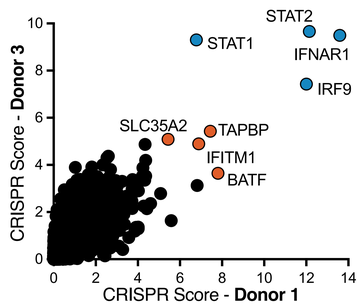


<u>Project Goal:</u> To identify the ISGs that contribute to IFN-mediated restriction of HIV in primary CD4⁺ T cells

2. ISG CRISPR Screens in CD4⁺ T Cells



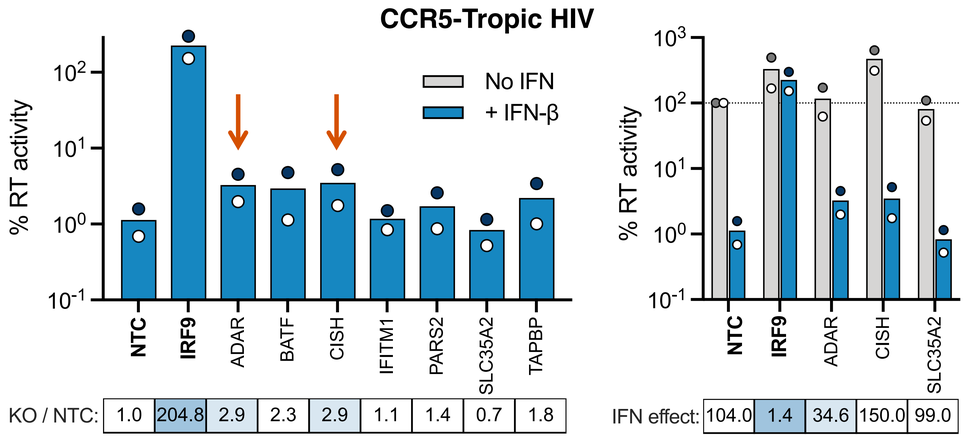
Gene	Average Score
IRF9	14.4
BATF	6.4
IFITM1	5.1
CISH	4.3
PARS2	3.8
TAPBP	3.8
ADAR	3.5
SLC35A2	1.1

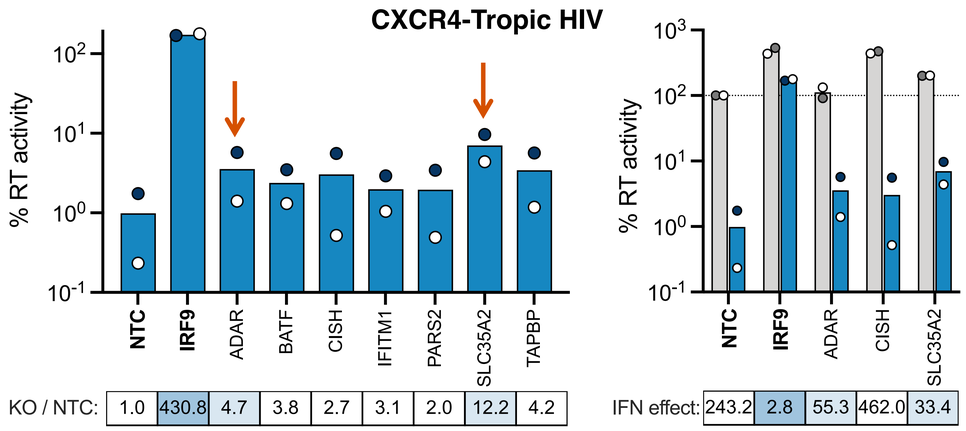


Gene	Average Score
IRF9	9.7
TAPBP	6.4
IFITM1	5.9
BATF	5.7
SLC35A2	5.3
ADAR	2.8
CISH	2.0
PARS2	1.6

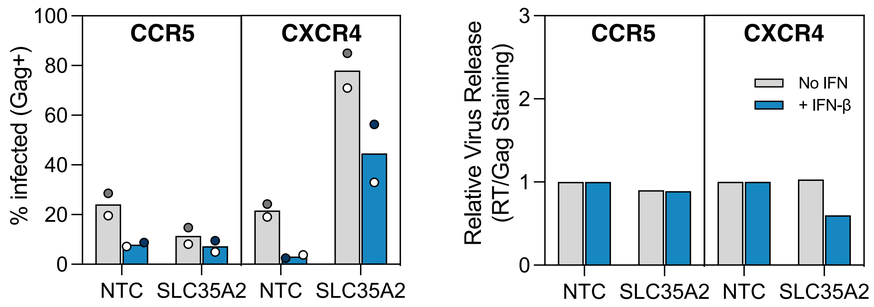
✓ Screens enriched for IFN pathway genes
Validate top hits (7 genes) by knocking out (KO) each gene individually in CD4s from two donors

3. Single KOs of ISG Screen Hits in CD4⁺ T Cells





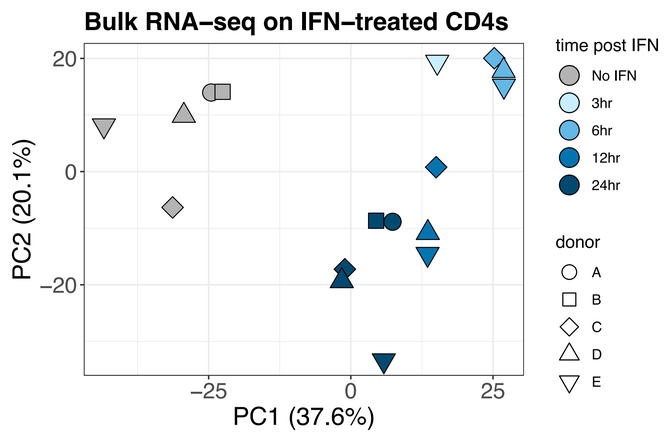
Caveat: **ADAR** KO also influenced cell growth and viability, so results are inconclusive
CISH KO consistently increases HIV levels for both viruses, with <u>and</u> without IFN
SLC35A2 KO seems to be specific to the CXCR4-tropic virus – let's take a closer look!



SLC35A2 KO increases RT levels *and* percent of infected cells only for the CXCR4 virus. This does not impact virus release, suggesting a role during viral entry.

4. New CD4-specific ISG CRISPR Library

Top hits from the previous CRISPR screen do not explain the entire IFN effect – is the library missing important ISGs?



43% of the top 555 IFN-upregulated hits are <u>not</u> present in the previous CRISPR library.

Solution? Build new CRISPR library with these genes!

5. Conclusions

- Our CRISPR screen approach is functional in primary CD4⁺ T cells, yet the library was missing many ISGs in CD4s
- **CISH** knockout increases HIV replication in CD4s regardless of IFN treatment or HIV tropism
- **SLC35A2** seems to restrict CXCR4-tropic HIV exclusively, via a mechanism independent of viral release

6. Future Directions

- Infect **SLC32A2** KO cells with LAI and LAIΔenv+VSV-G to determine whether the mechanism is coreceptor-dependent
- I have generated the new **CD4-specific ISG CRISPR library** – ready to perform duplicate screens with each virus!

7. Acknowledgments

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Please email me if you have any questions/comments: haitell@uw.edu