Comprehensive synergy mapping links a BAF- and MOF/NSL-containing "supercomplex" to the transcriptional silencing of HIV-1

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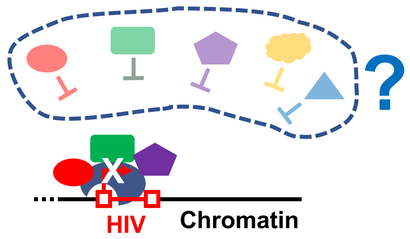
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HOPE HIV Obstruction by Programmed Epigenetics

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What Are the Host Repressors That Target HIV?

HIV persists in chronically-infected people, largely because its transcription is temporarily silenced in most of the infected cells. This gives us hope that if we can identify the host repressors and study their mechanisms, we may have a chance to prolong and even permanently silence HIV, by enhancing those HIV silencing mechanisms that are already in the cells.

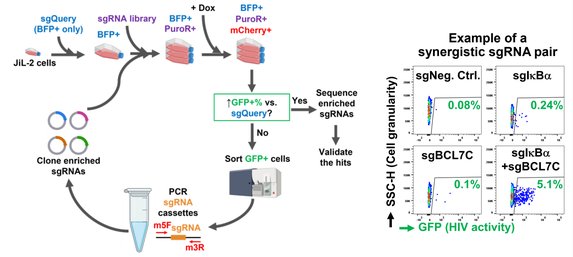


Hypothesis:
Hidden repressors synergizing with known repressors

Method:
Iterative genome wide screening

Systematic Mapping of Genetic Interaction Networks for HIV Latency

Reiterative Enrichment and Authentication of CRISPRi Targets for **Synergy** (REACTS)



- 7 sgRNAs targeting known repressors as Query
- A genome-wide sgRNA library as Input
- Enriching sgRNAs in Input that synergize with Query

Bliss Independence Test for judging synergy:

GFP+% in cells w/ sgRNAx — GFP+% in cells w/ sgRNAy

Predicted Additive Effect: $fa_{xyP} = fa_x + fa_y - (fa_x)(fa_y)$

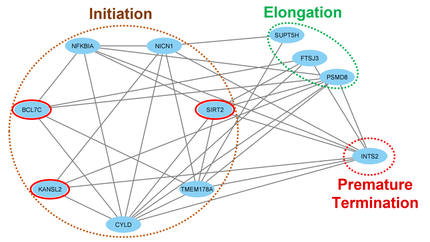
Observed Effect – Predicted Additive: $\Delta fa_{xy} = fa_{xyO} - fa_{xyP}$

Observed effect in cells w/ sgRNAx + sgRNAy — Predicted additive effect in cells w/ sgRNAx + sgRNAy

Heuristic Threshold: fa_{xyO} *vs.* fa_{xyP} $p < 0.05$, AND $\Delta fa_{xy} > 2.5\%$ or $fa_{xyO} / fa_{xyP} > 2$

Synergistic Pairs of Repressors Identified

A network of 32 synergies among 11 repressors of latent HIV:



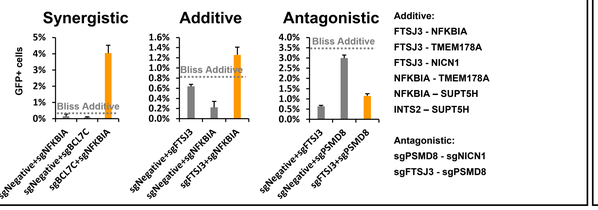
3 new repressors identified by synergy with known repressors:

BCL7C: A key subunit in SWI/SNF chromatin-remodeling complexes

KANSL2: In MOF (KAT8) histone acetylation complex, increasing its activity

SIRT2: A deacetylase targeting both histone and tubulin

6 additive and 2 antagonistic pairs also found, for example:

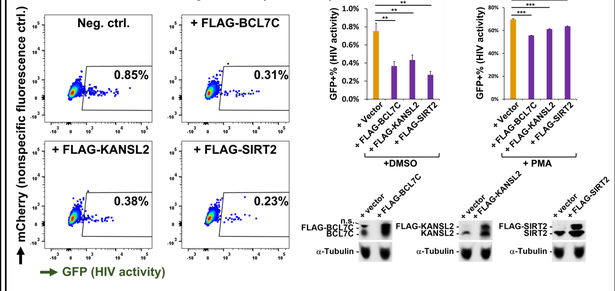


Additive:
FTSJ3 - NFKBIA
FTSJ3 - TMEM178A
FTSJ3 - NICN1
NFKBIA - TMEM178A
NFKBIA – SUPT5H
INTS2 – SUPT5H

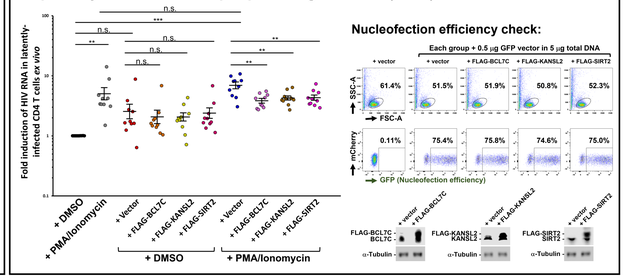
Antagonistic:
sgPSMD8 - sgNICN1
sgFTSJ3 - sgPSMD8

Overexpressing BCL7C, KANSL2 & SIRT2 Enhanced HIV Latency

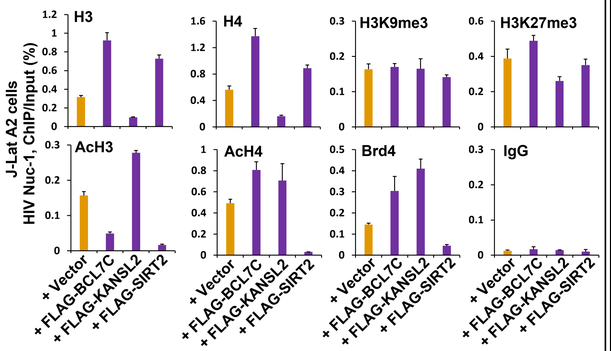
In cell line-based latency model (J-Lat A2):



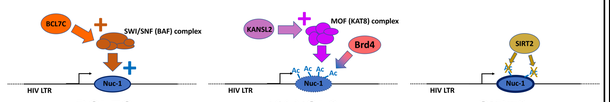
In primary CD4 cells from people living with HIV (n=10):



Overexpression of BCL7C and KANSL2 Increased Ac-Histone and Brd4 on HIV LTR



Indicated mechanisms:

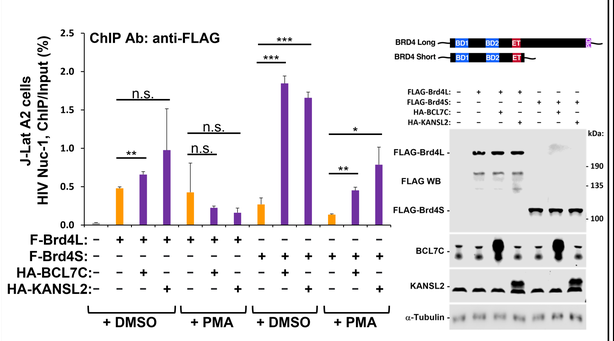


BCL7C:
Increases Nucleosome Occupancy on HIV promoter.

KANSL2:
Increases Brd4 on HIV LTR by hyper acetylating histones.

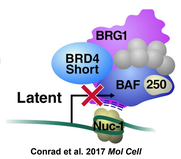
SIRT2:
Deacetylate histones to make HIV promoter more accessible.

BCL7C and KANSL2 Increased the Short Form Brd4 on HIV LTR



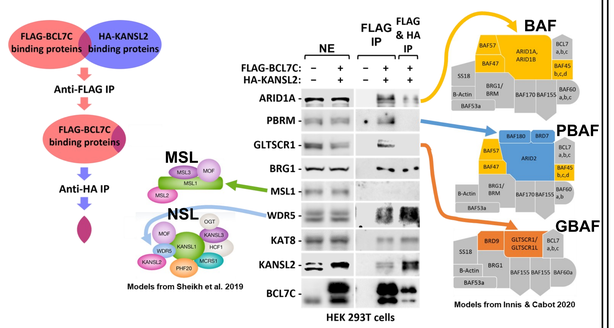
Upon overexpression of BCL7C and KANSL2, Brd4S, rather than Brd4L, more significantly increased on HIV LTR, and a significant amount of Brd4S retained on LTR even after PMA treatment.

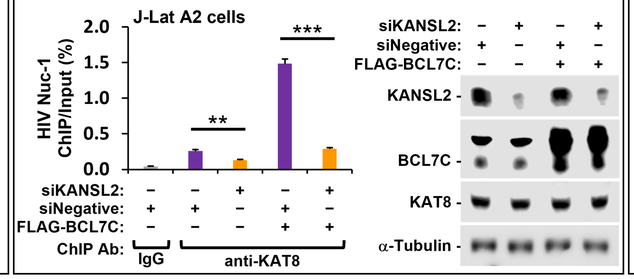
According to Conrad et al. (*Mol Cell* 2017), Brd4S is a corepressor of HIV transcription by binding BRG1 and tethering SWI/SNF (BAF) to LTR.



Conrad et al. 2017 *Mol Cell*

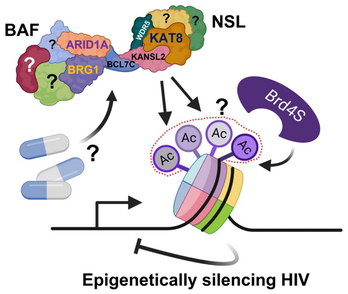
A BAF-NSL Complex Delivers KAT8 to the Nucleosome on HIV LTR





Conclusion and Future

- Systematic mapping of genetic interaction networks for HIV latency
- New host repressors identified by synergies of their sgRNAs
- A BAF-NSL super complex inhibits HIV by Brd4S-BAF pathway



- Broad mapping of both core and condition-specific repressors
- In-depth characterization of the BAF-NSL complex
- Permanent HIV-silencing by chromatin inhibition