Identifying novel regulators of LINE-1 expression through CRISPR/Cas9 screens

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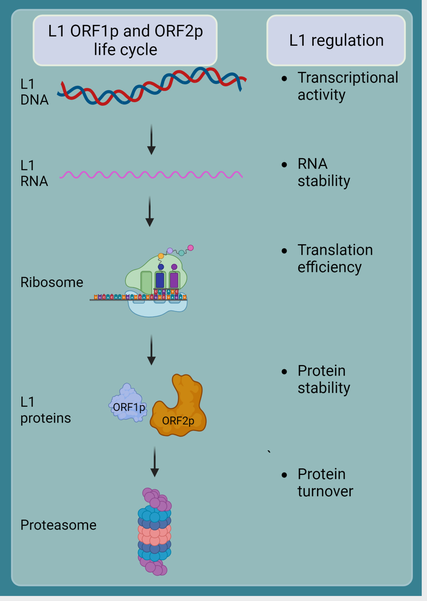
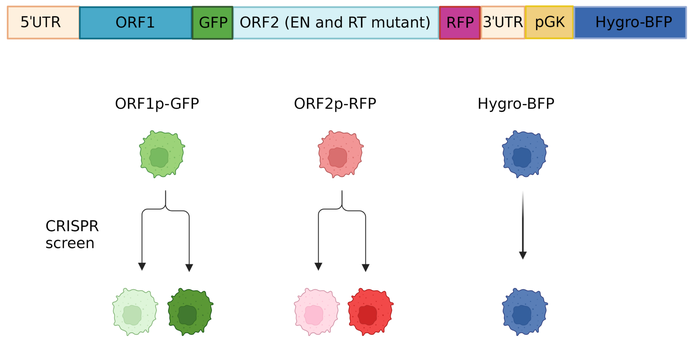
Abstract

Long Interspersed Elements-1 (LINE-1, L1) are transposable elements that make up roughly 17% of the human genome. These elements can copy and insert themselves into new genomic locations. Typically, they are kept silent in healthy tissues but are expressed in various human diseases. LINE-1 expression has been associated with aging, neurodegenerative disorders, cancer, and autoimmune diseases. Despite the correlation of LINE-1 expression with disease, little is understood of how LINE-1 expression is regulated. To explore this, we developed a cellular reporter system to monitor the protein levels of LINE-1 encoded ORF1p and ORF2p simultaneously. Using genome-wide CRISPR/Cas9-based screens with this reporter system, we identified genes that control LINE-1 expression at both the RNA and protein levels. Besides known regulators like the HUSH complex, our screening uncovered previously unknown regulators of ORF1p and ORF2p, many of which appear to be involved in key molecular pathways implicated in human disease. These findings may enhance our understanding of the molecular mechanisms regulating LINE-1 and provide insights into potential therapeutic targets for diseases linked to LINE-1 dysregulation.

What are the upstream regulators of L1?



1. Design of the screen



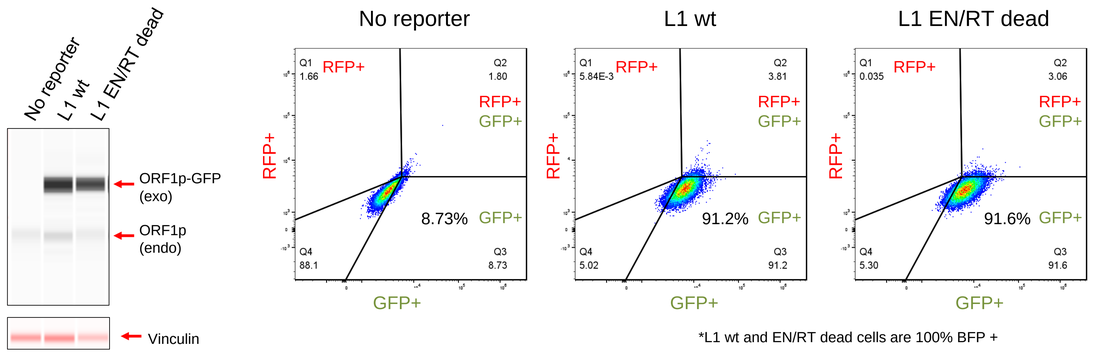
Engineer HCT-116 cells to express ORF1p-GFP and ORF2p-RFP (wt or EN/RT dead mutant)

EN: Endonuclease, RT: Reverse Transcriptase

Primary screen: Knock out 19,000 genes individually using CRISPR screening strategy (4 gRNAs per gene)
Secondary screen: Select 1,500 genes from the top hits in the primary screen, along with additional literature targets, and employ the same screen (10 gRNAs per gene)

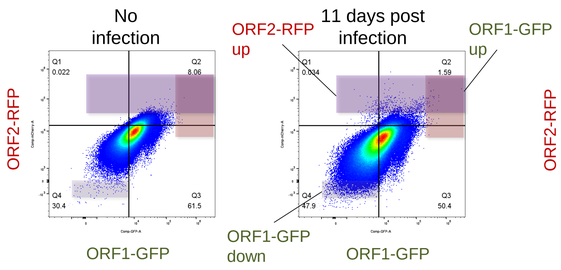
Identify proteins that regulate ORF1p or ORF2p levels in cells.
Sort and sequence cell populations that have increased/decreased GFP or RFP signal and no change in BFP signal

2. Validation of the L1 reporter system by Western blot and flow cytometry

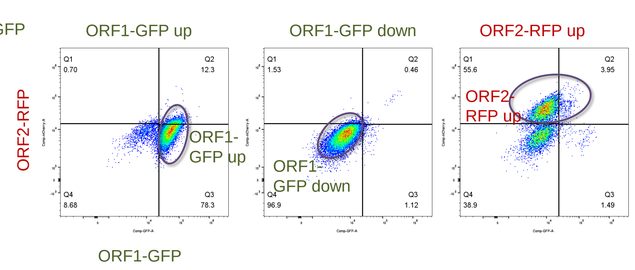


*L1 wt and EN/RT dead cells are 100% BFP +

3. Infection with the gRNA library led to the emergence of cell populations with higher ORF1p or ORF2p levels, or reduced ORF1p levels



4. Sorted cells maintain expression of ORF1p and ORF2p for over 10 days in culture



5. Screening identified known L1 regulators, validating the high quality of the screen

A. Genome-wide primary screen identified both positive and negative regulators of LINE-1

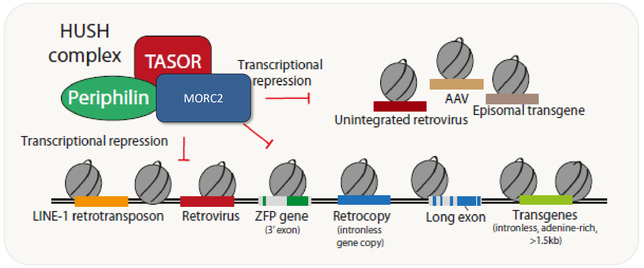
Positive regulators

Protein A knockdown → LINE-1 downregulation

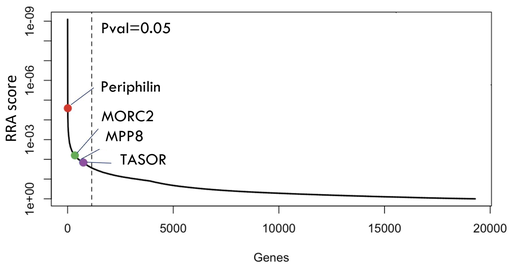
Negative regulators

Protein B knockdown → LINE-1 upregulation

B. The HUSH complex safeguards the genome against retroelement invasion through epigenetic repression of LINE-1

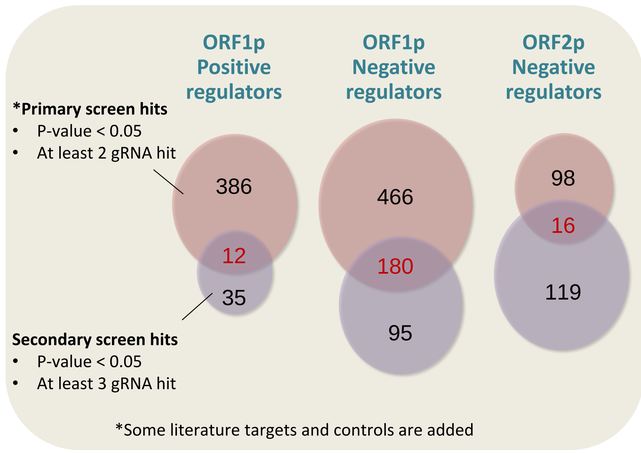


C. The screen identified all core HUSH complex members as negative regulators of L1



Seczynska and Lehner Trends in Genetics 2023; Seczynska Nature 2022; Tunbak Nat. Comm. 2020; Liu Nature 2017; Robbez-Masson Genome Res 2017;

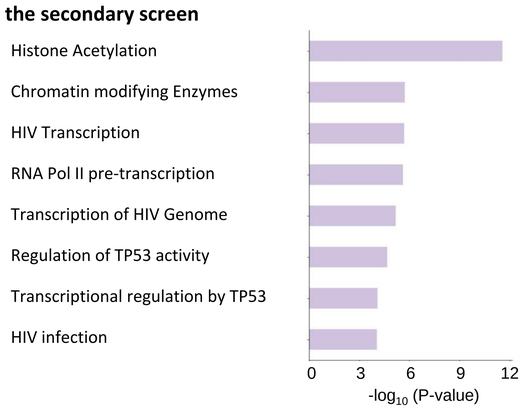
6. Screening identified many unknown Positive and Negative Regulators of ORF1p and ORF2p that are validated in a secondary screen



*Some literature targets and controls are added

7. Potential LINE-1 regulators are involved in molecular pathways implicated in human disease

Pathway analysis of all genes that are validated by the secondary screen



8. Discussion

- Uncovering upstream regulators of LINE-1 may help us understand how LINE-1 is upregulated in human diseases such as autoimmune, neurodegenerative, and cancer
- Targeting upstream regulators of LINE-1 may provide a new therapeutic approach for human diseases linked to LINE-1 dysregulation

Opportunities to target upstream regulators of LINE-1