

Silence on LINE-1

- We identify functionally diverse genes that either promote or restrict L1 retrotransposition.
- These genes, which are often associated with human diseases, control the L1 life cycle at the transcriptional or the post-transcriptional level in a manner that can depend on the endogenous L1 nucleotide sequence

Nature. 2018 Jan 11;553(7687):228-232.

Silence on LINE-1

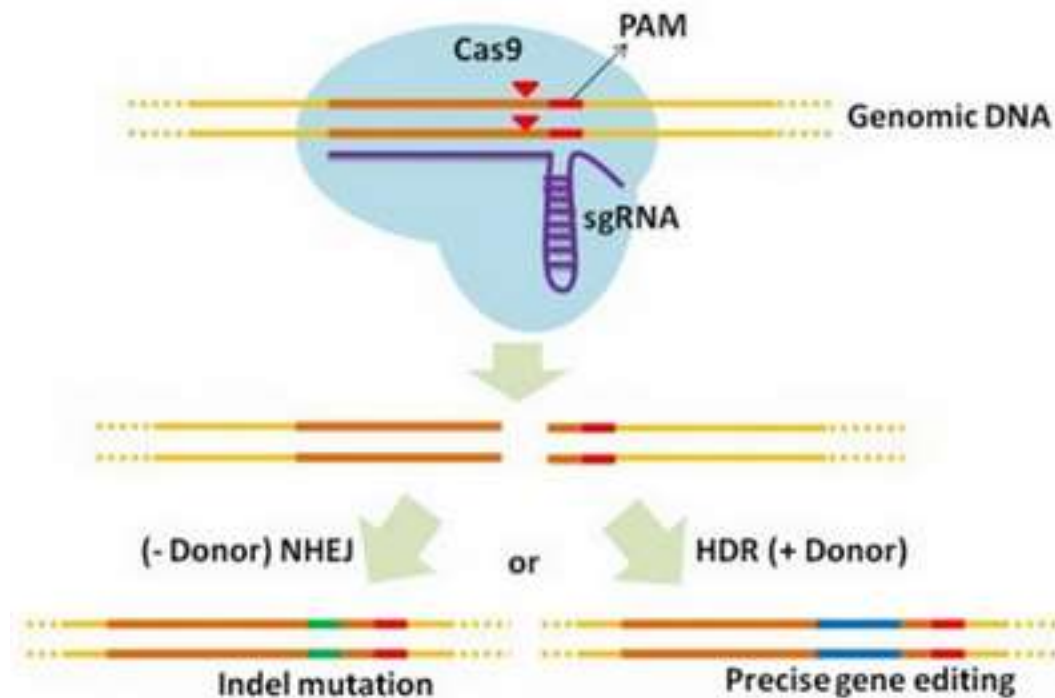
1 systematic CRISPR–Cas9 screen in human cell lines for factors that control L1 retrotransposition

Lentiviral delivery of Cas9 and sgRNA provides efficient depletion of target genes

synthetic single-guide RNA (sgRNA) targeted to specific coding regions of genes

programming the CRISPR (clustered regularly interspaced short palindromic repeats)–associated nuclease Cas9 to modify specific genomic loci

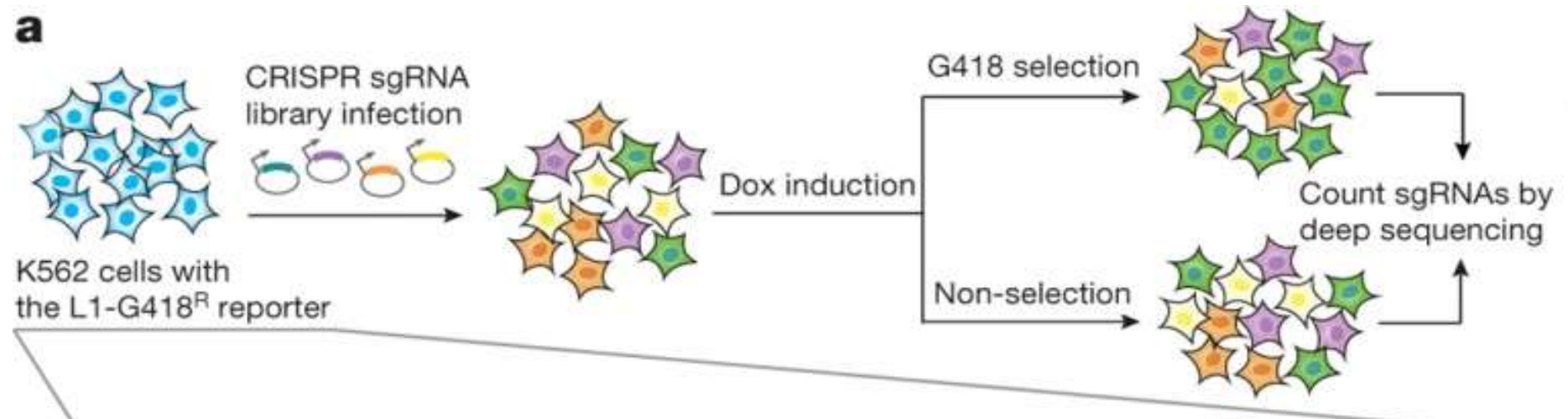
Programming the CRISPR (clustered regularly interspaced short palindromic repeats)– associated nuclease Cas9 to modify specific genomic loci



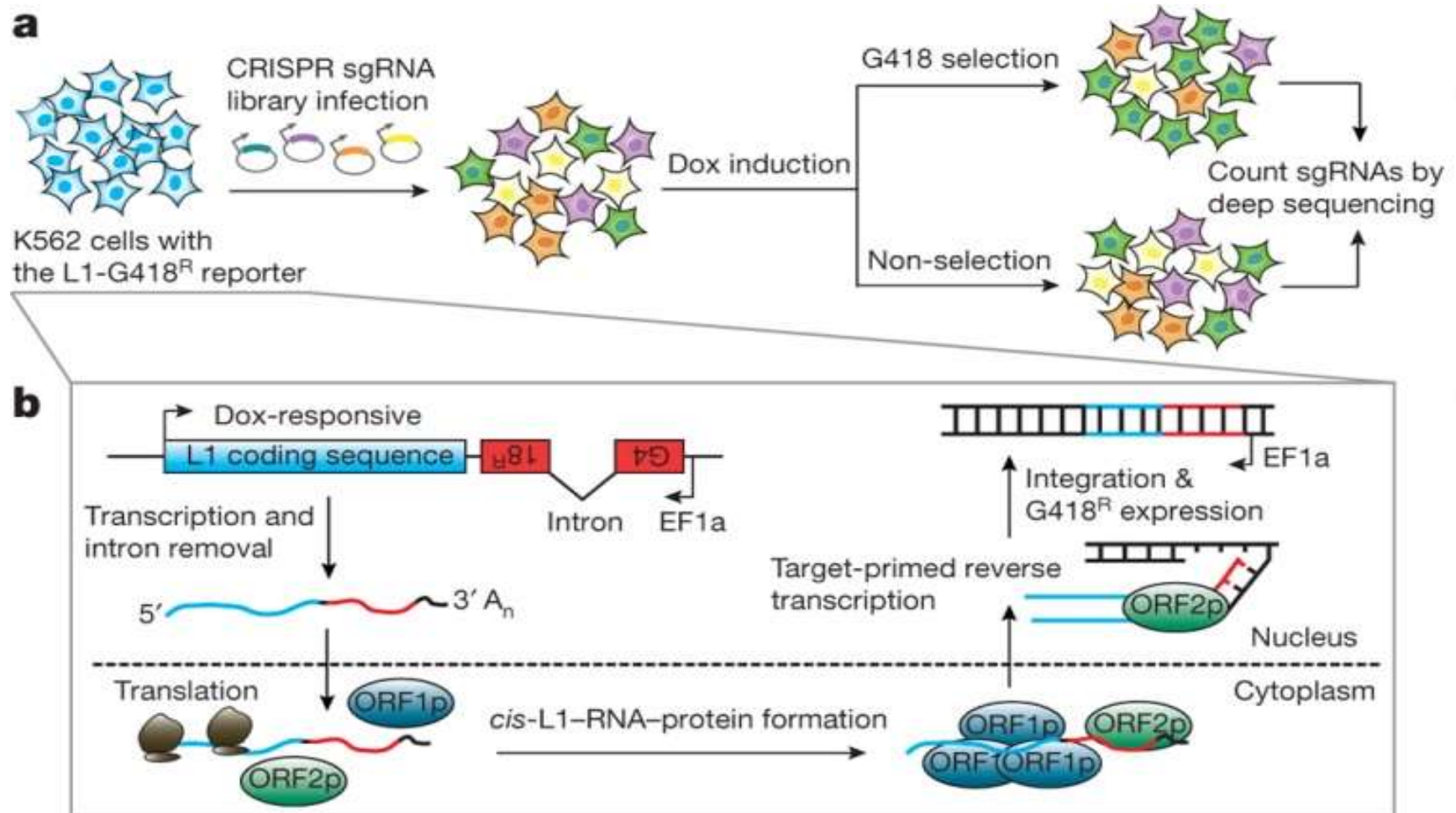
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Ophir Shalem et al. Science 2014;343:84-87

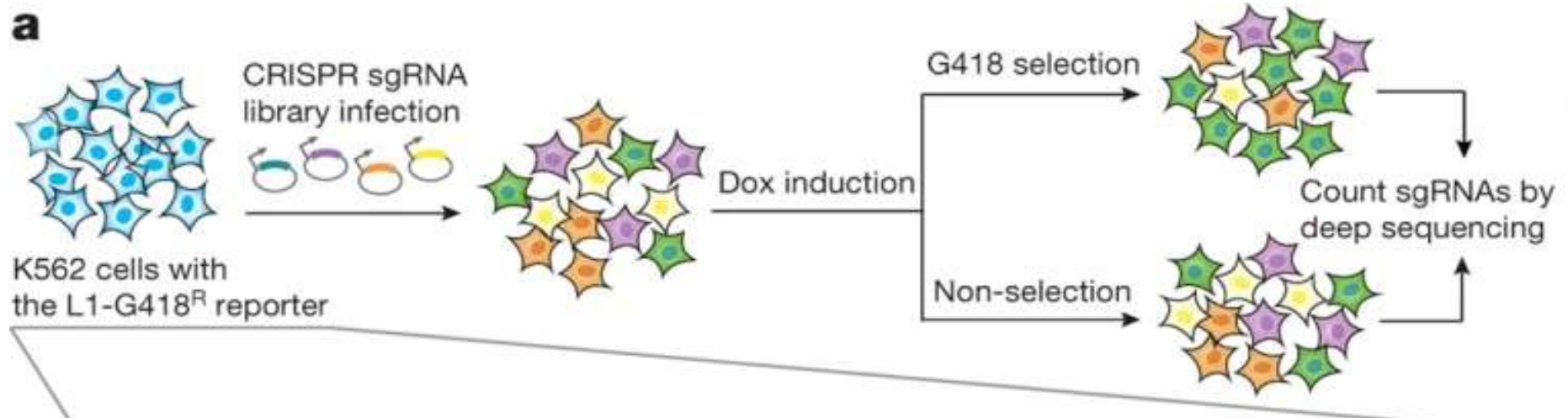
Genome-wide screen for L1 activators and suppressors in cells



Genome-wide screen for L1 activators and suppressors in K562 cells

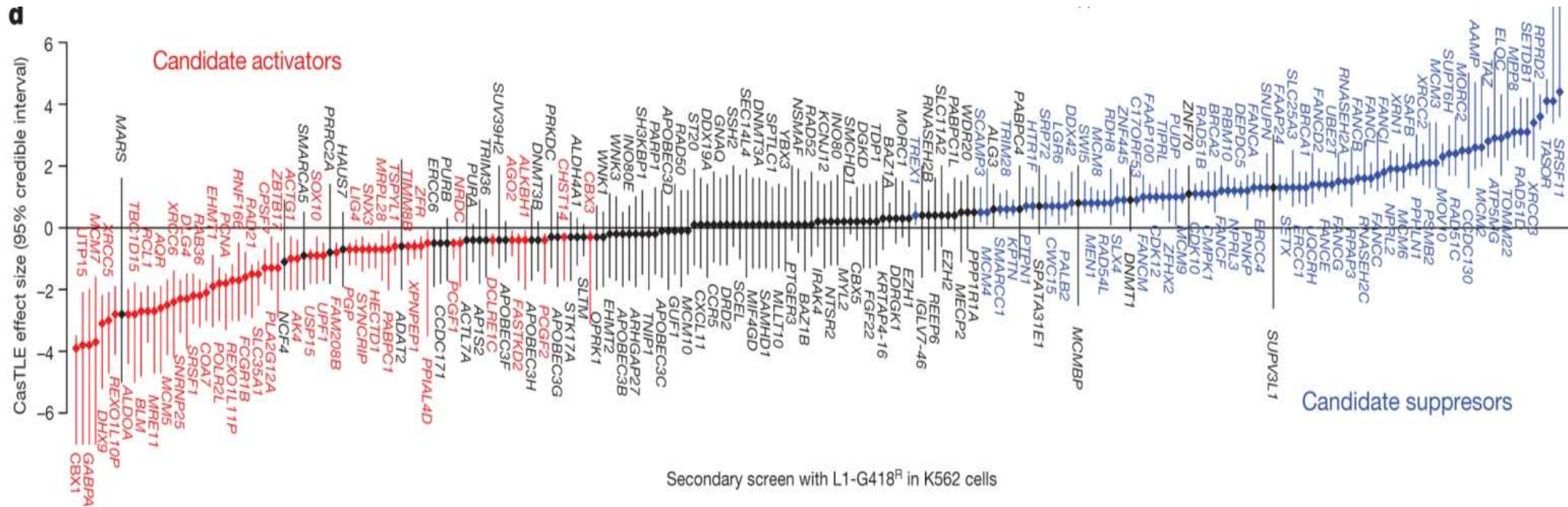


Genome-wide screen for L1 activators and suppressors in cells



cells transduced with sgRNAs targeting L1 suppressors would have more retrotransposition events than negative control cells and would be enriched through the G418 selection; conversely, cells transduced with sgRNAs targeting L1 activators would be depleted

Genome-wide screen for L1 activators and suppressors in K562 cells

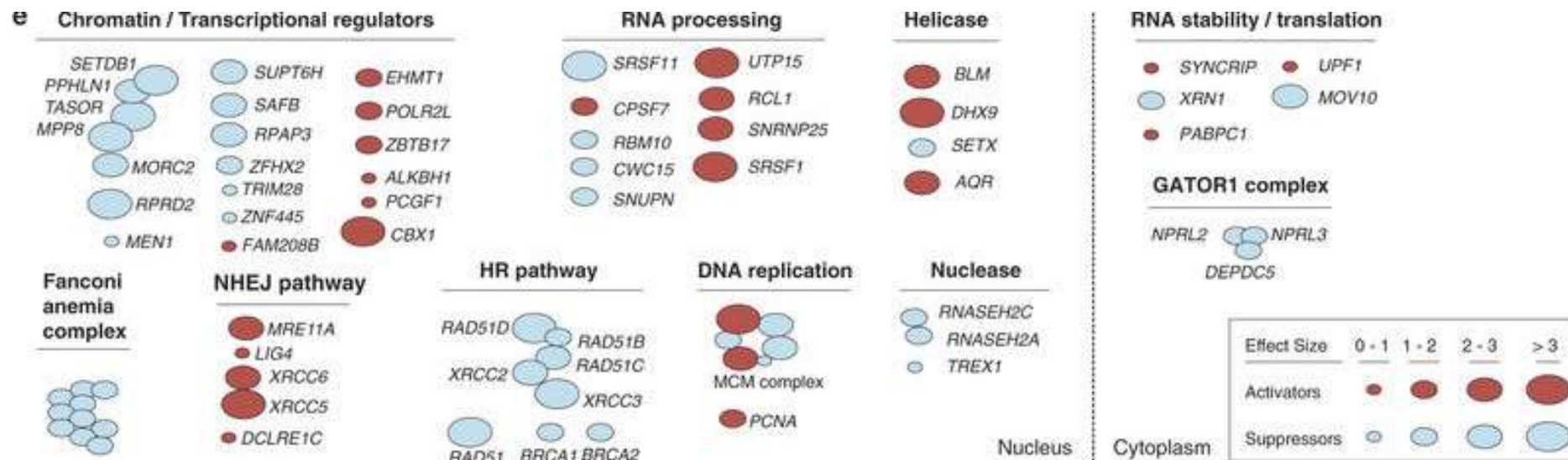


L1 activators are shown in red; L1 suppressors are shown in blue; and insignificant genes for which the credible interval includes zero are shown in grey

Silence on LINE-1

- 1 systematic CRISPR–Cas9 screen in human cell lines for factors that control L1 retrotransposition
- 2 functionally diverse factors and pathways that control L1 activity at transcriptional or post-transcriptional levels.

functionally diverse L1 regulators



functionally diverse L1 regulators

e Chromatin / Transcriptional regulators



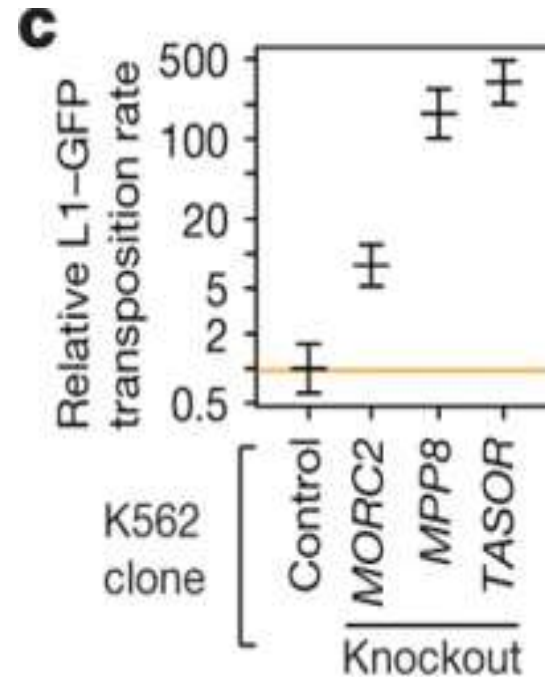
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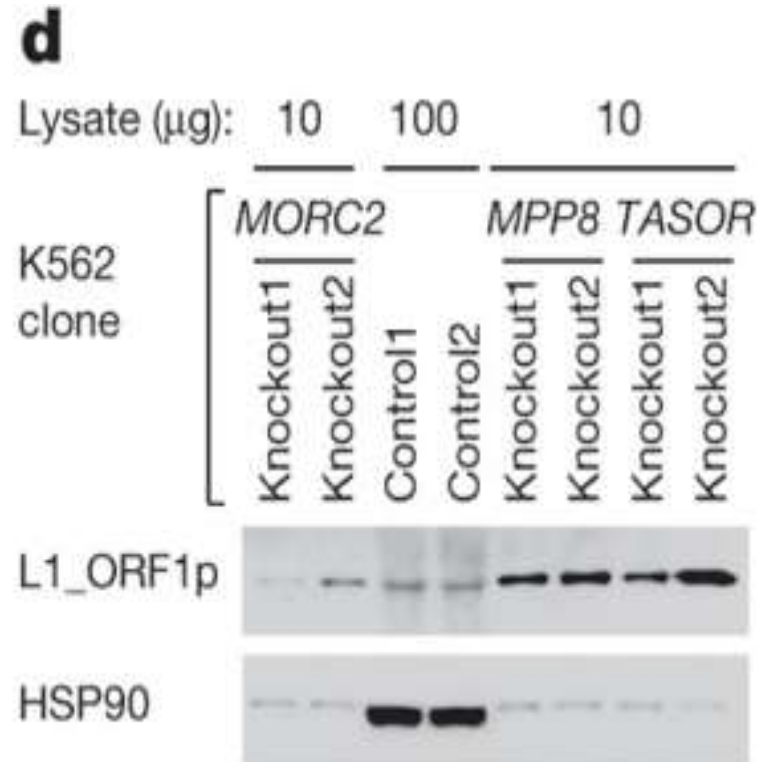
3 transcriptional silencing of L1 retrotransposons by MORC2 and HUSH complex subunits can occur within introns of transcriptionally active genes

MORC2 knockout, MPP8 knockout and TASOR knockout increase L1Transposition



HUSH and MORC2 silence L1 transcription to inhibit retrotransposition

MORC2 knockout, MPP8 knockout and TASOR knockout increase L1 ORF1p expression



Endogenous L1_ORF1p levels in K562 clones shown by western blotting with HSP90 as a loading control

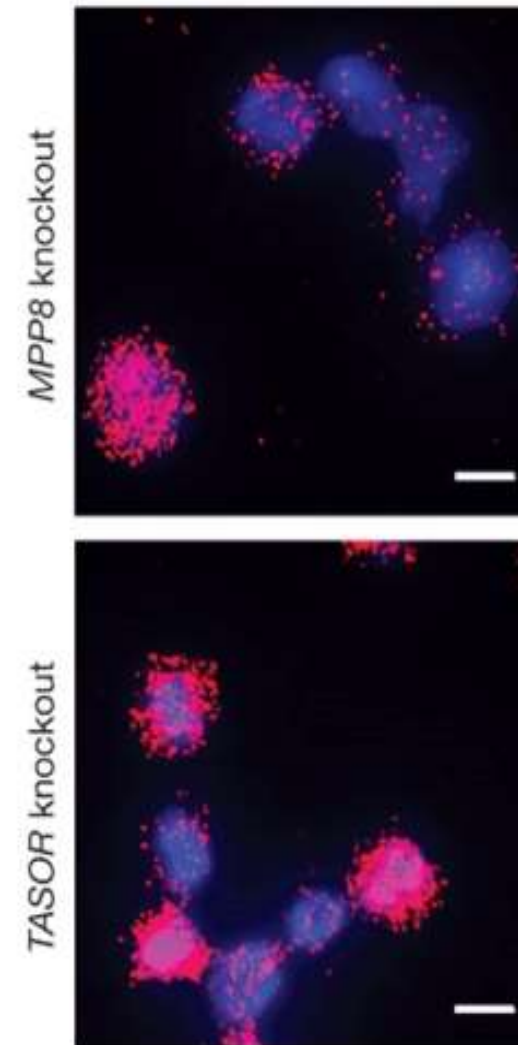
HUSH and MORC2 silence L1 transcription to inhibit retrotransposition

nature

MPP8 knockout and TASOR knockout increase L1 expression

L1–GFP mRNAs in dox-induced K562 clones

single single-molecule fluorescent in situ hybridization (smFISH)



HUSH and MORC2 silence L1 transcription to inhibit retrotransposition

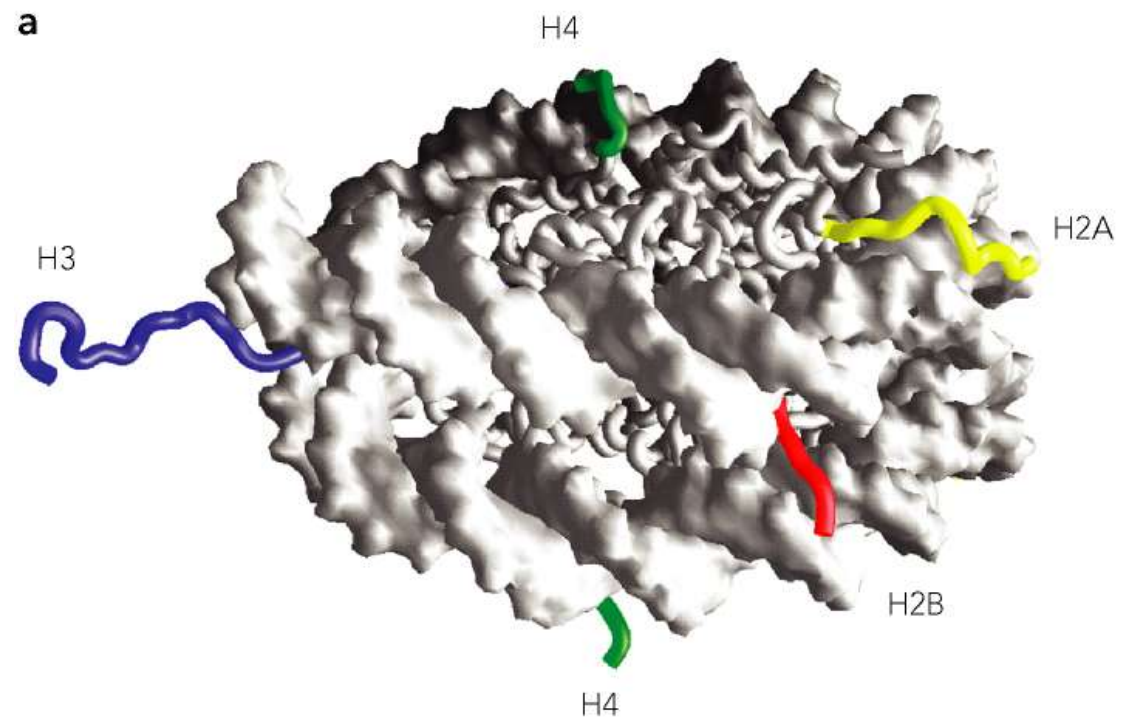
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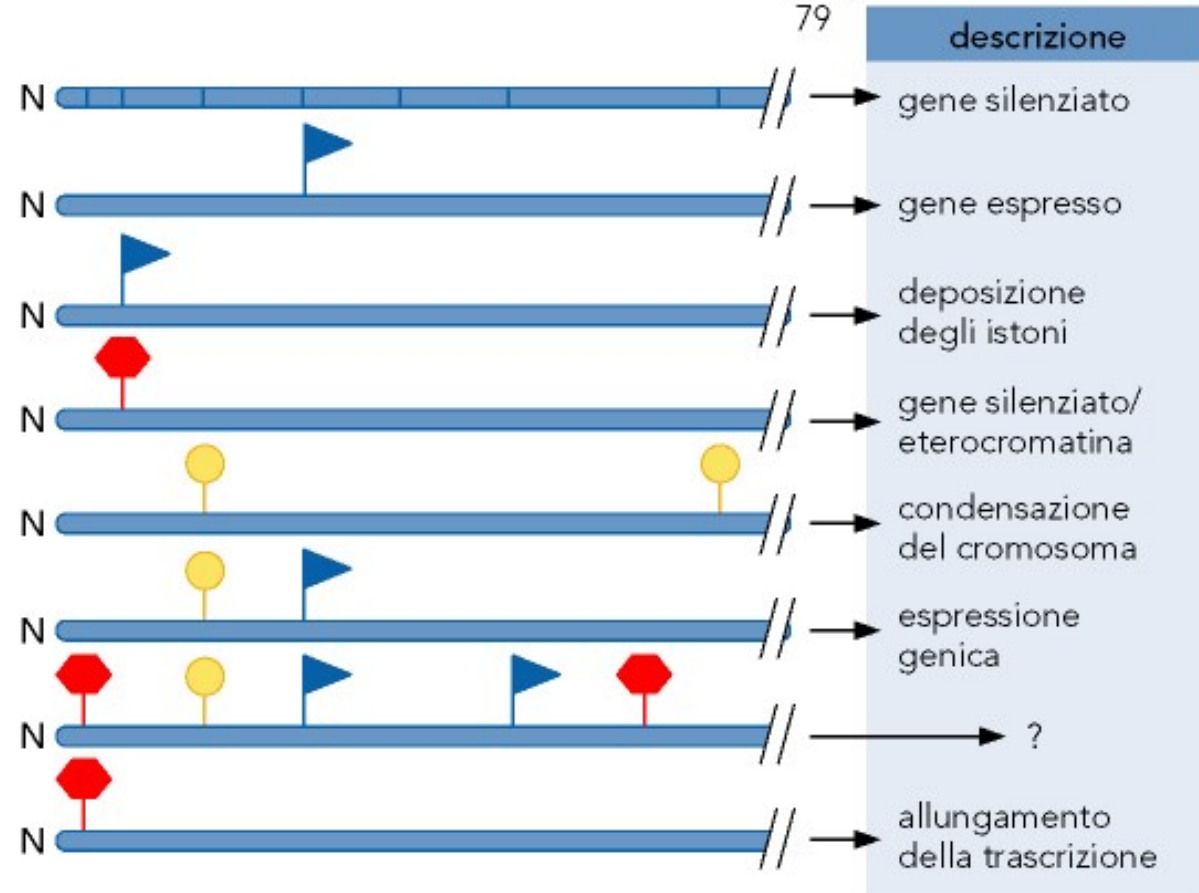
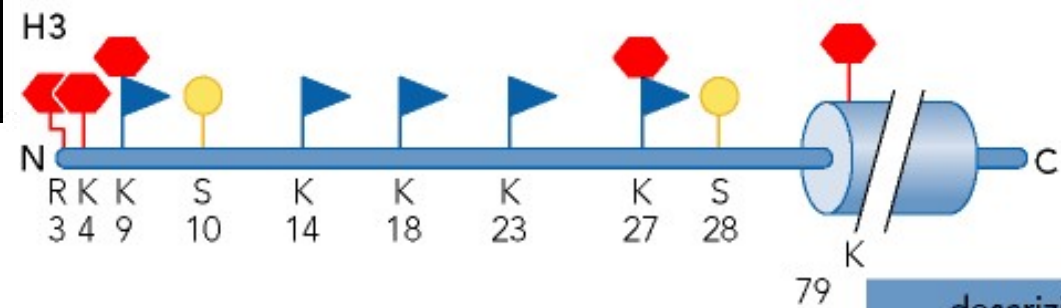
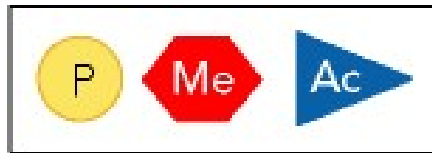
Silence on LINE-1

- 1 systematic CRISPR–Cas9 screen in human cell lines for factors that control L1 retrotransposition
- 2 functionally diverse factors and pathways that control L1 activity at transcriptional or post-transcriptional levels.
- 3 transcriptional silencing of L1 retrotransposons by MORC2 and HUSH complex subunits can occur within introns of transcriptionally active genes
- 4 transcriptional silencing of L1 can dampen expression of these genes and can influence host gene expression

epigenetic repression in human cells.

- the HUSH (human silencing hub) complex comprise TASOR, MPP8
- this complex is absent from *Drosophila* but is conserved from fish to humans.
- Loss of HUSH components resulted in decreased H3K9me3 both at endogenous genomic loci and at retroviruses integrated into heterochromatin.
- The HUSH complex is recruited to genomic loci rich in H3K9me3, where subsequent recruitment of the methyltransferase SETDB1 is required for further H3K9me3 deposition to maintain transcriptional silencing.





Heterochromatin (inactive/condensed)



Euchromatin (active/open)



Figure 6-33b
Molecular Cell Biology, Sixth Edition
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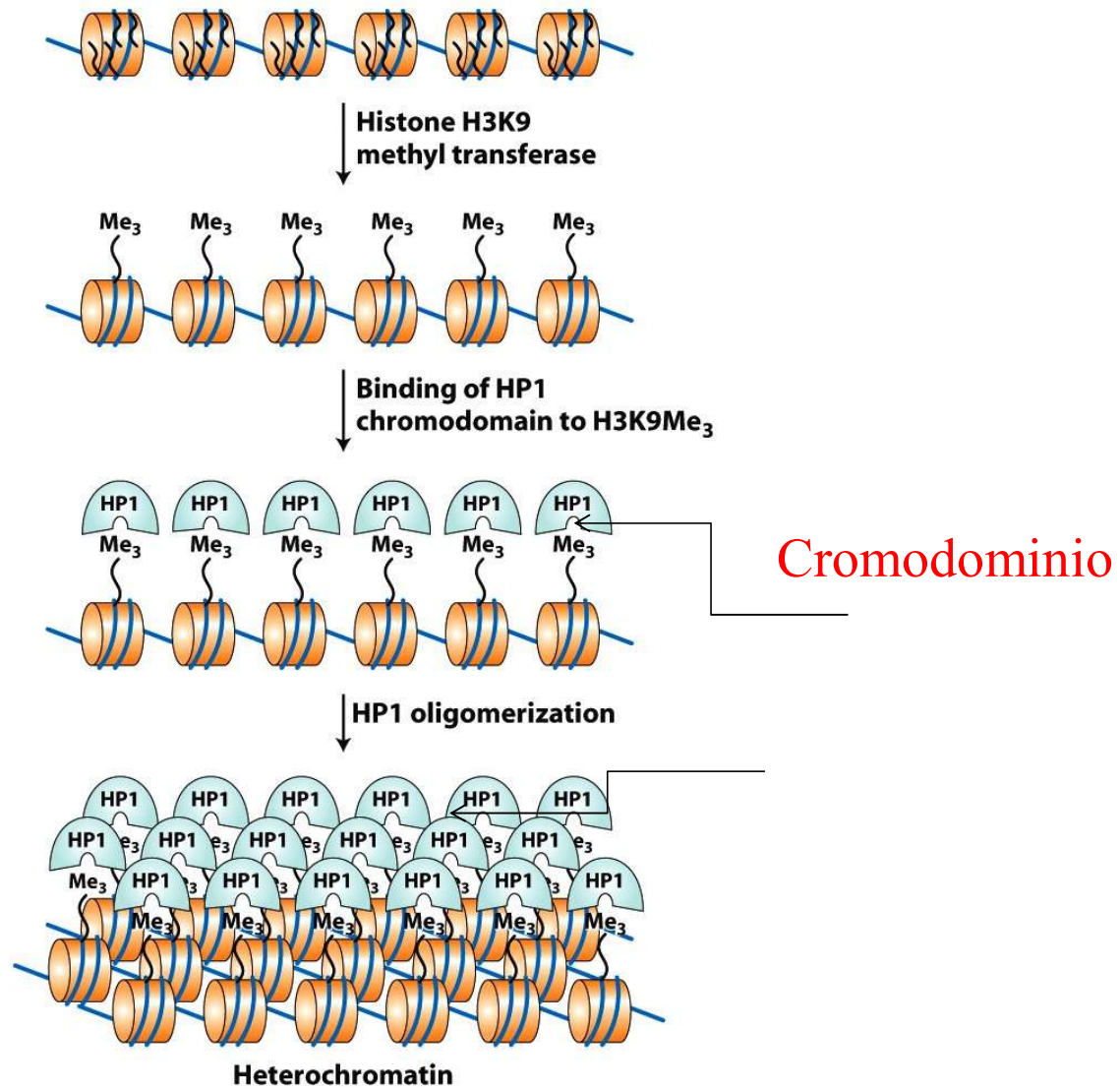
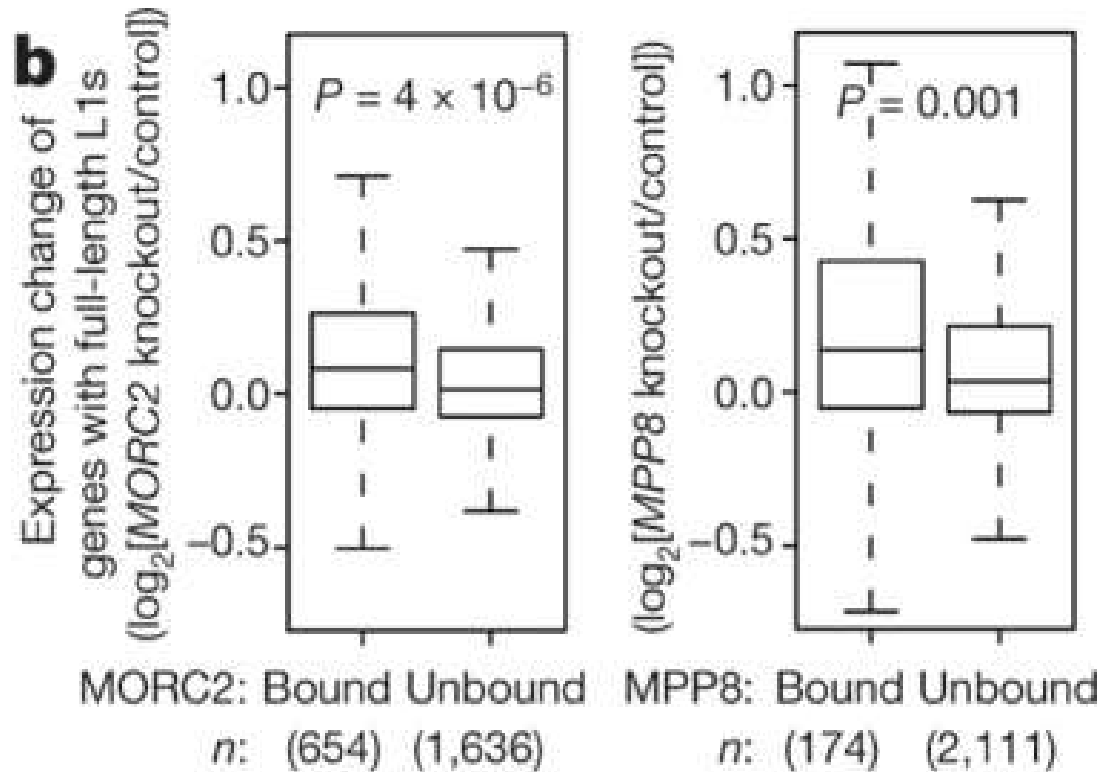


Figure 6-34a
Molecular Cell Biology, Sixth Edition
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Silence on LINE-1

- We investigate the restriction of L1 by the protein MORC2 and by the human silencing hub (HUSH) complex subunits MPP8 and TASOR
- HUSH and MORC2 can selectively bind evolutionarily young, full-length L1s located within euchromatic environments, and promote deposition of histone H3 Lys9 trimethylation (H3K9me3) for transcriptional silencing
- Silencing events often occur within introns of transcriptionally active genes, and lead to the downregulation of host gene expression.
epigenetic silencing of transposable elements rewires host gene expression programs.

HUSH or MORC2 binding at L1s decreases active host gene expression



Gene expression changes with intronic full-length L1s that are bound or unbound by MORC2 or MPP8 (RNA-seq reads from knockout K562 clones compared to control).

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Actively transcribed genes

Permissive chromatin marks

Exon

Young full-length L1

Exon

ON

ON

L1 mRNA

RNA

HUSH or MORC2 binding

Retrotransposition

Inhibit

Decreased expression of host genes

H3K9me3

HUSH or MORC2

Exon

Young full-length L1

Exon

ON

OFF

New L1 copy

The diagram illustrates the HUSH/MORC2 pathway for silencing young L1 elements. It is divided into two main states: an active state (top) and a silenced state (bottom).

Active State (Top): An actively transcribed gene with permissive chromatin marks (green) contains a young full-length L1 element (white box). The L1 element is transcribed into L1 mRNA (blue wavy line). The host gene is also transcribed into RNA (blue wavy line). The L1 mRNA is labeled "ON" and "L1 mRNA".

Silenced State (Bottom): The HUSH or MORC2 complex (orange) binds to the L1 element, leading to H3K9me3 methylation (red) and silencing of the L1 element (OFF). This process also leads to decreased expression of host genes (ON) and retrotransposition of the L1 element (New L1 copy). The L1 element is labeled "OFF" and "Young full-length L1". The host gene is labeled "ON" and "Exon".

Key components and processes shown:

- Actively transcribed genes**: The top part of the diagram.
- Permissive chromatin marks**: Indicated by green shapes above the DNA.
- Exon**: Labeled on the DNA segments.
- Young full-length L1**: The L1 element being transcribed.
- ON**: Indicates active transcription of the L1 element and host gene.
- L1 mRNA**: The transcript of the L1 element.
- RNA**: The transcript of the host gene.
- HUSH or MORC2 binding**: The process of silencing the L1 element.
- Retrotransposition**: The process of creating a new L1 copy.
- Inhibit**: A red arrow indicating inhibition of the L1 element.
- Decreased expression of host genes**: The result of HUSH/MORC2 binding.
- H3K9me3**: The repressive histone mark associated with silencing.
- HUSH or MORC2**: The silencing complex.
- ON** and **OFF**: Indicators of gene expression status.
- New L1 copy**: The result of retrotransposition.

N Liu *et al.* *Nature* **553**, 228–232 (2018) doi:10.1038/nature25179