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

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

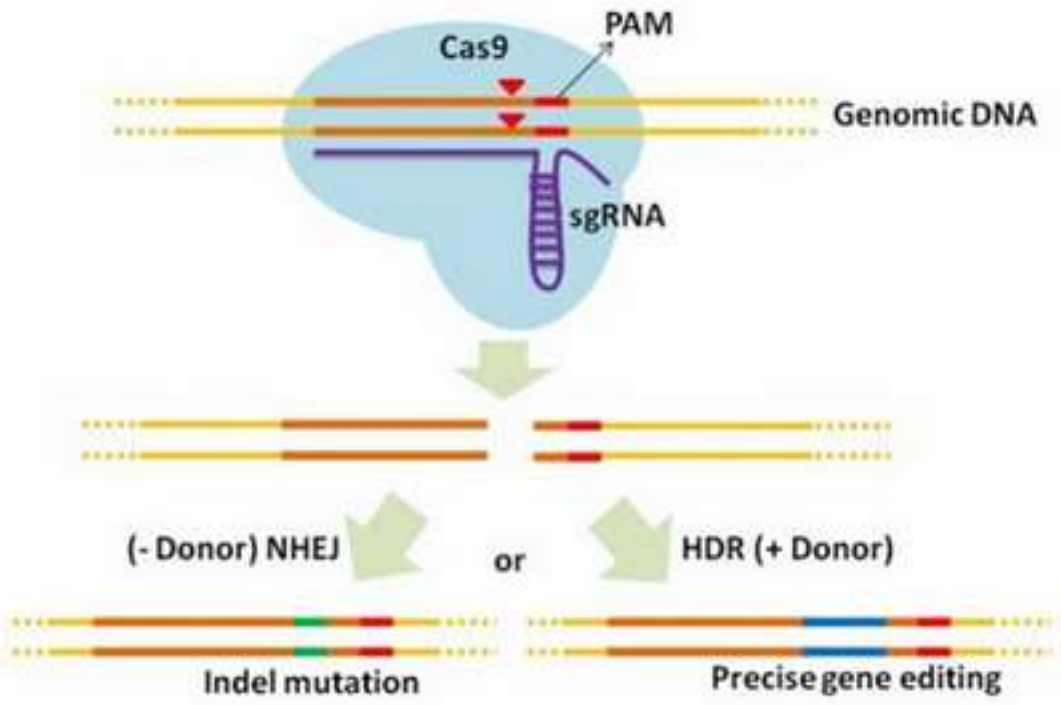
Lentiviral delivery of Cas9 and sgRNA provides efficient depletion of target genes

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

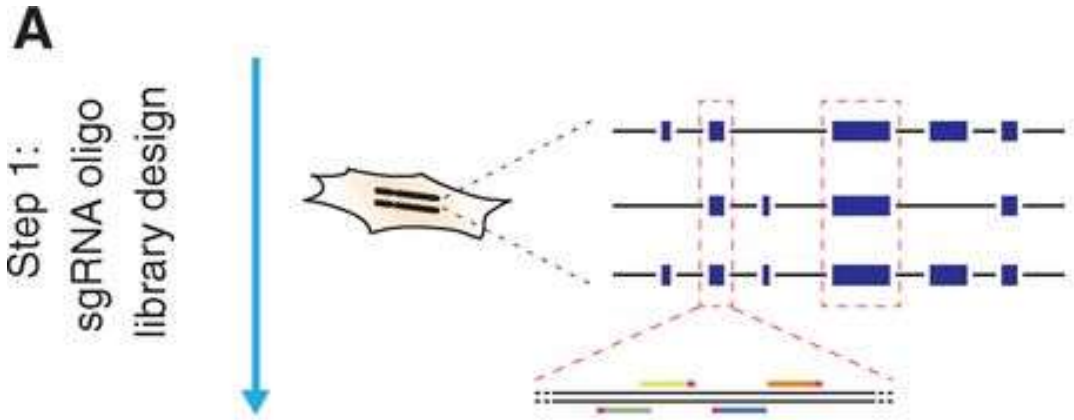
Ophir Shalem et al. Science 2014;343:84-87

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



Doudna et al., 2014

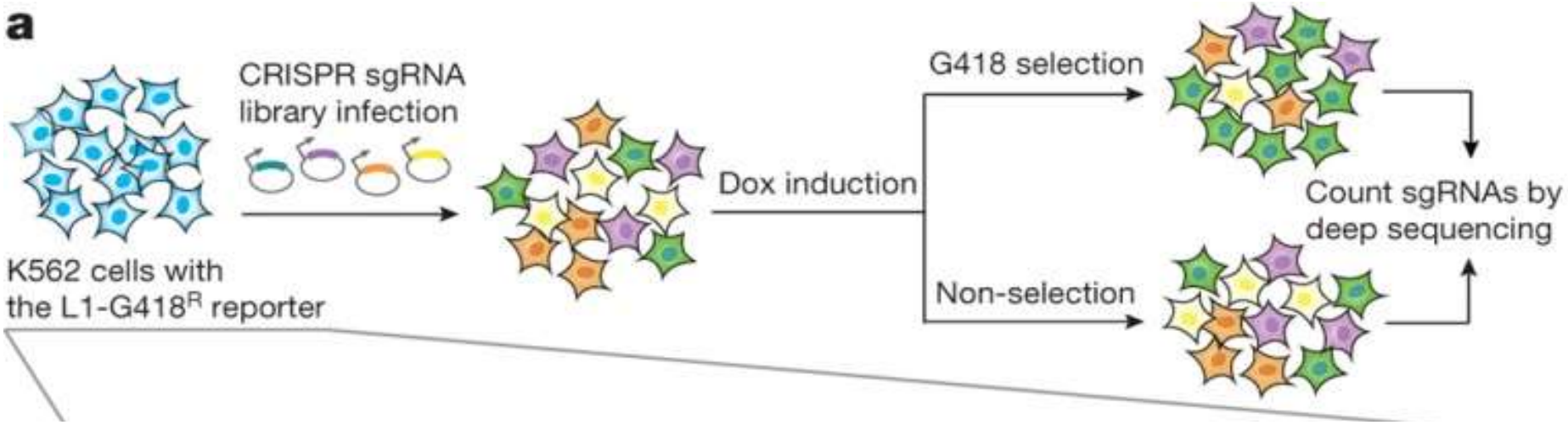
Design of sgRNA library for genome-scale knockout of coding sequences in human cells



CRISPR library sgRNAs targeting exons of 18,080 genes in the human genome with an average coverage of 3 to 4 sgRNAs per gene

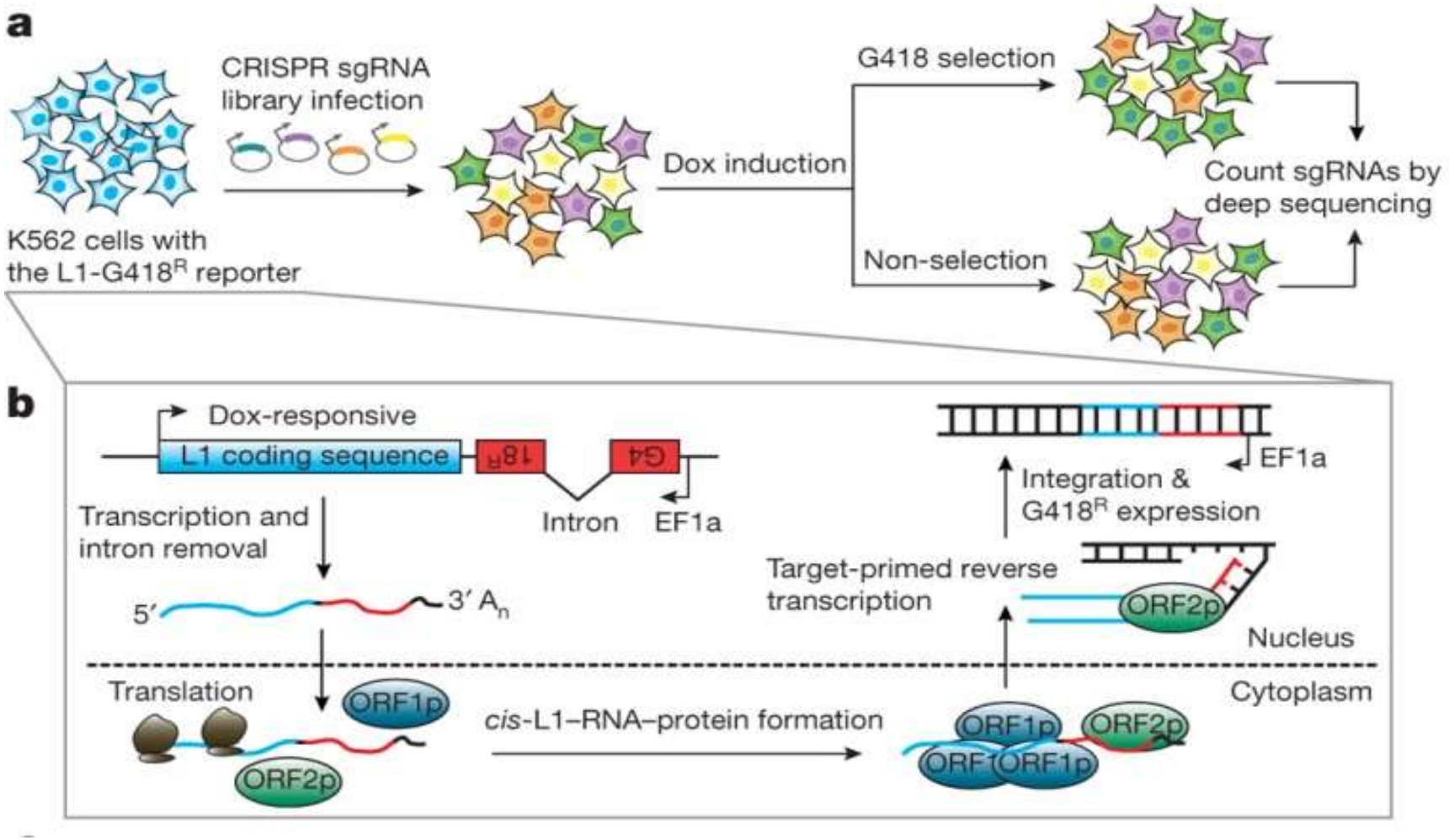
Ophir Shalem et al. Science 2014;343:84-87

Genome-wide screen for L1 activators and suppressors in cells



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

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



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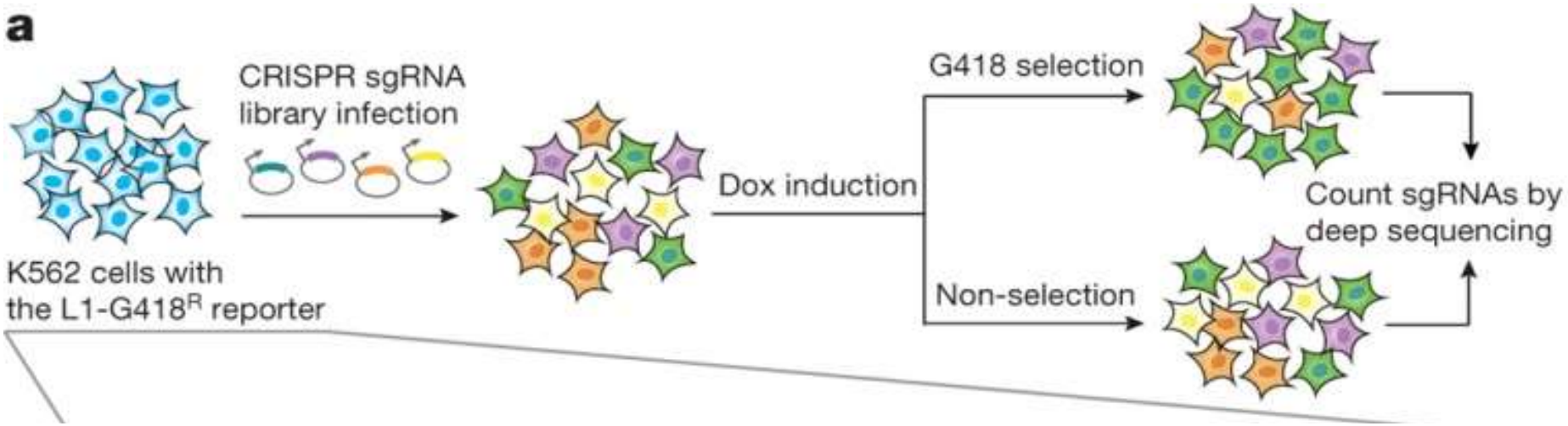
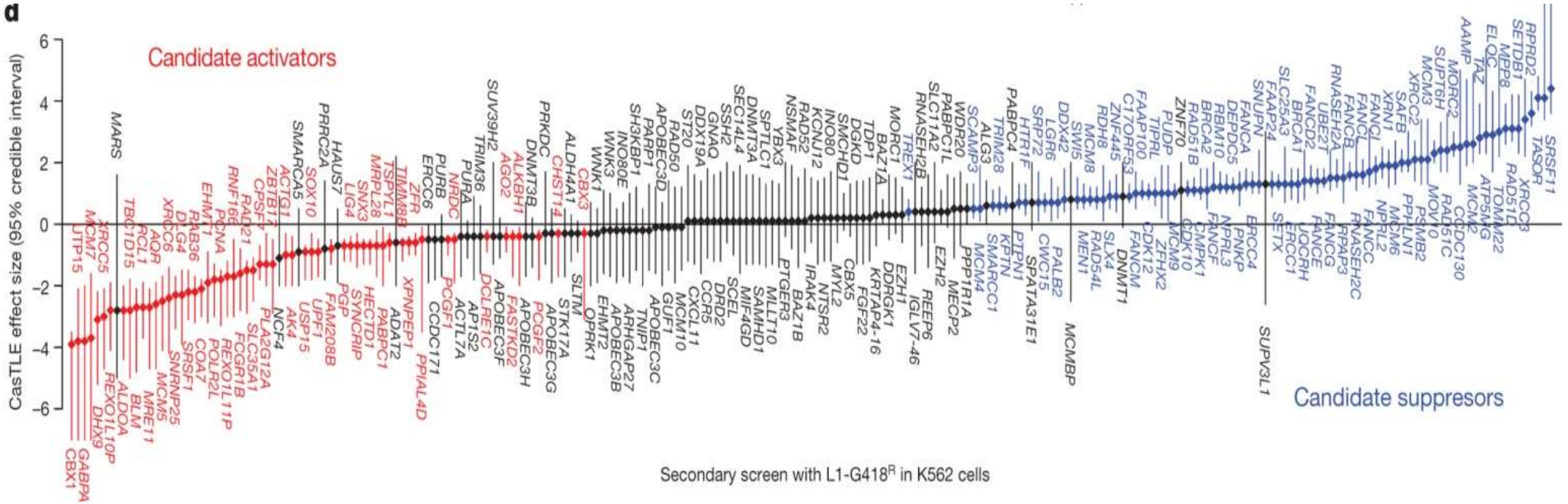
Genome-wide screen for L1 activators and suppressors in cells



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

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

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

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

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.

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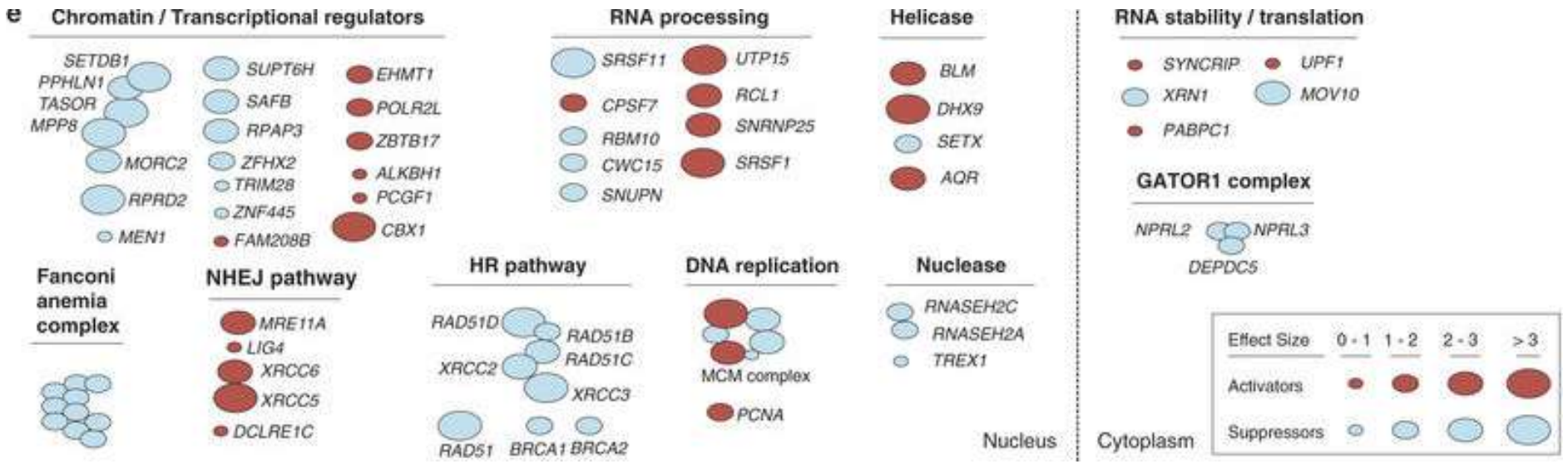
functionally diverse L1 regulators



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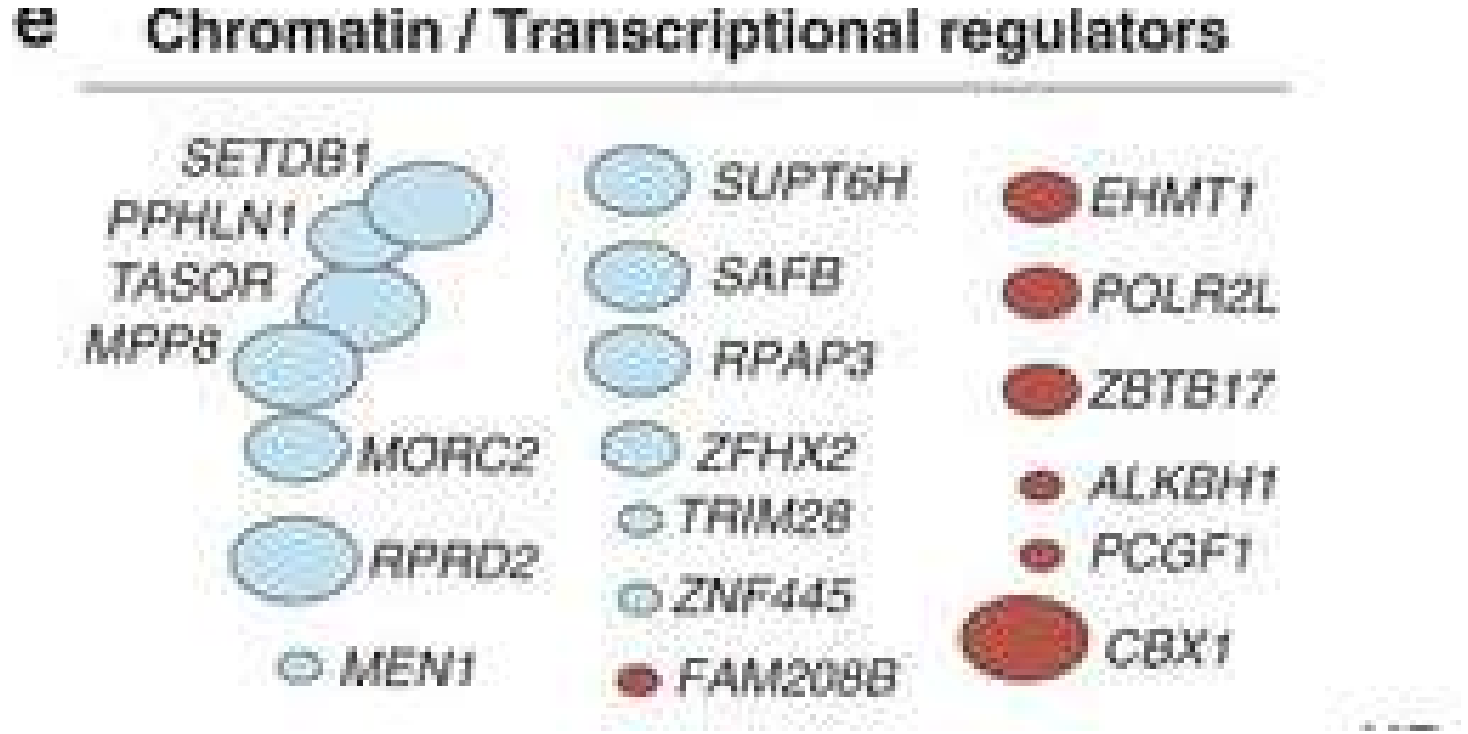

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Silence on LINE-1

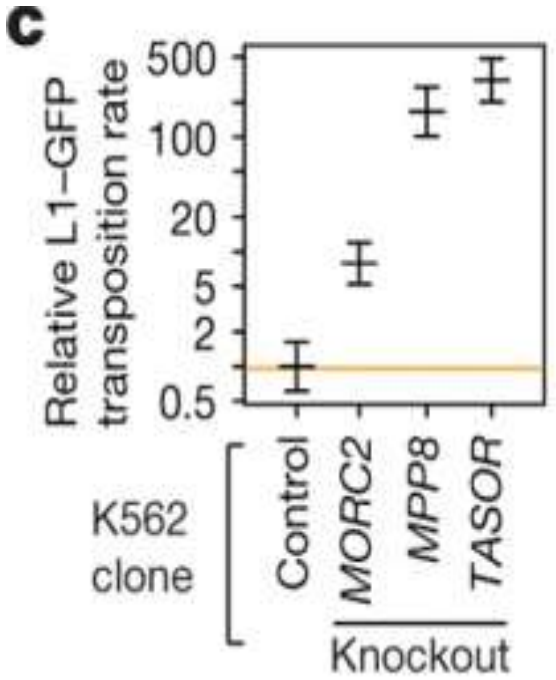
1 systematic CRISPR–Cas9 screen in human cell lines for factors that control L1 retrotransposition

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

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

MORC2 knockout, MPP8 knockout and TASOR knockout increase L1Transposition

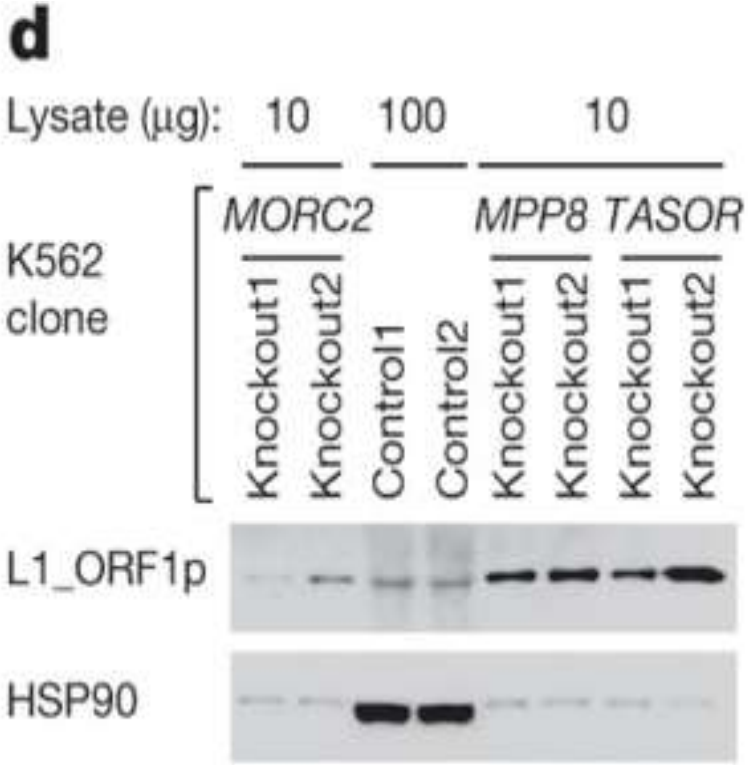


HUSH and MORC2 silence L1 transcription to inhibit retrotransposition

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

nature

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

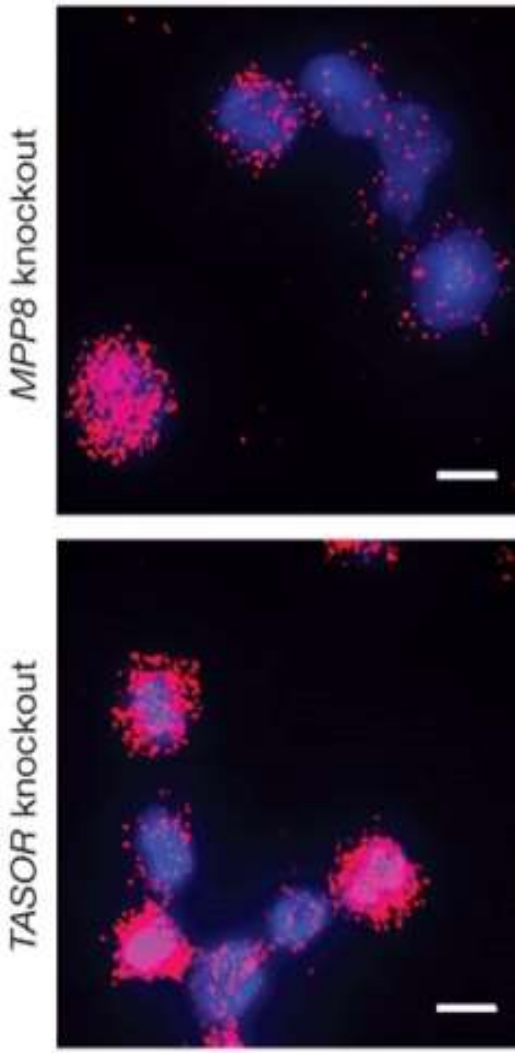
N Liu *et al. Nature* **553,** 228–232 (2018) doi:10.1038/nature25179

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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nature

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.

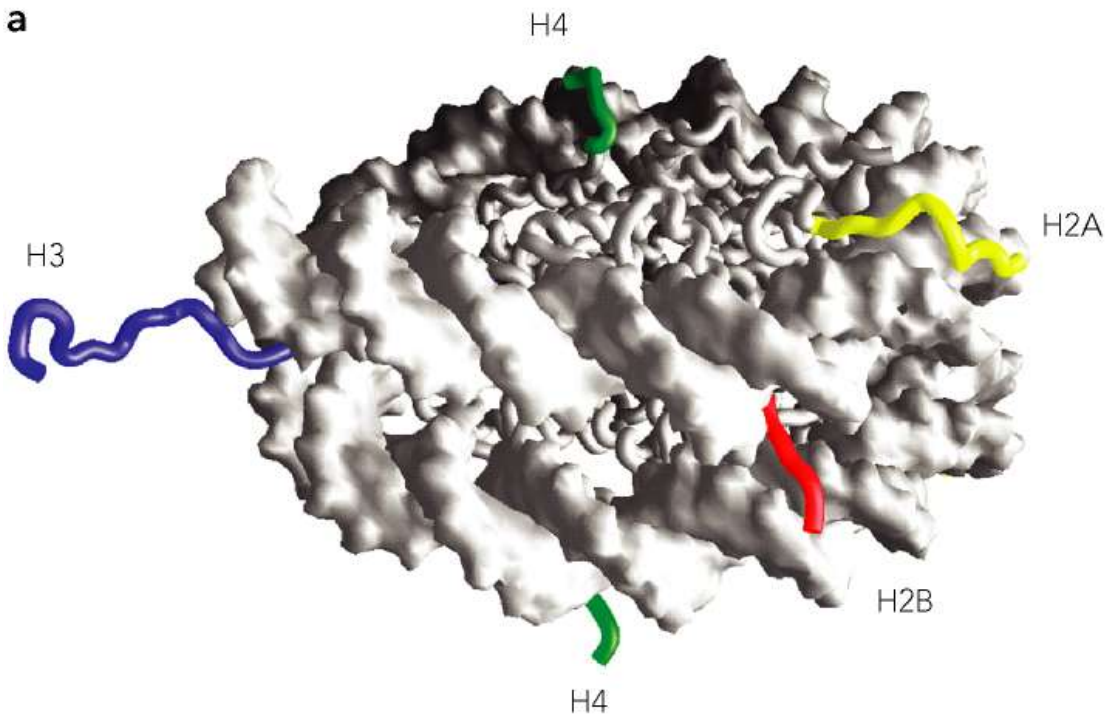
3 transcriptional silencing of L1 retrotransposons by MORC2 and HUSH complex subunits can occur within introns of transcriptionally active genes

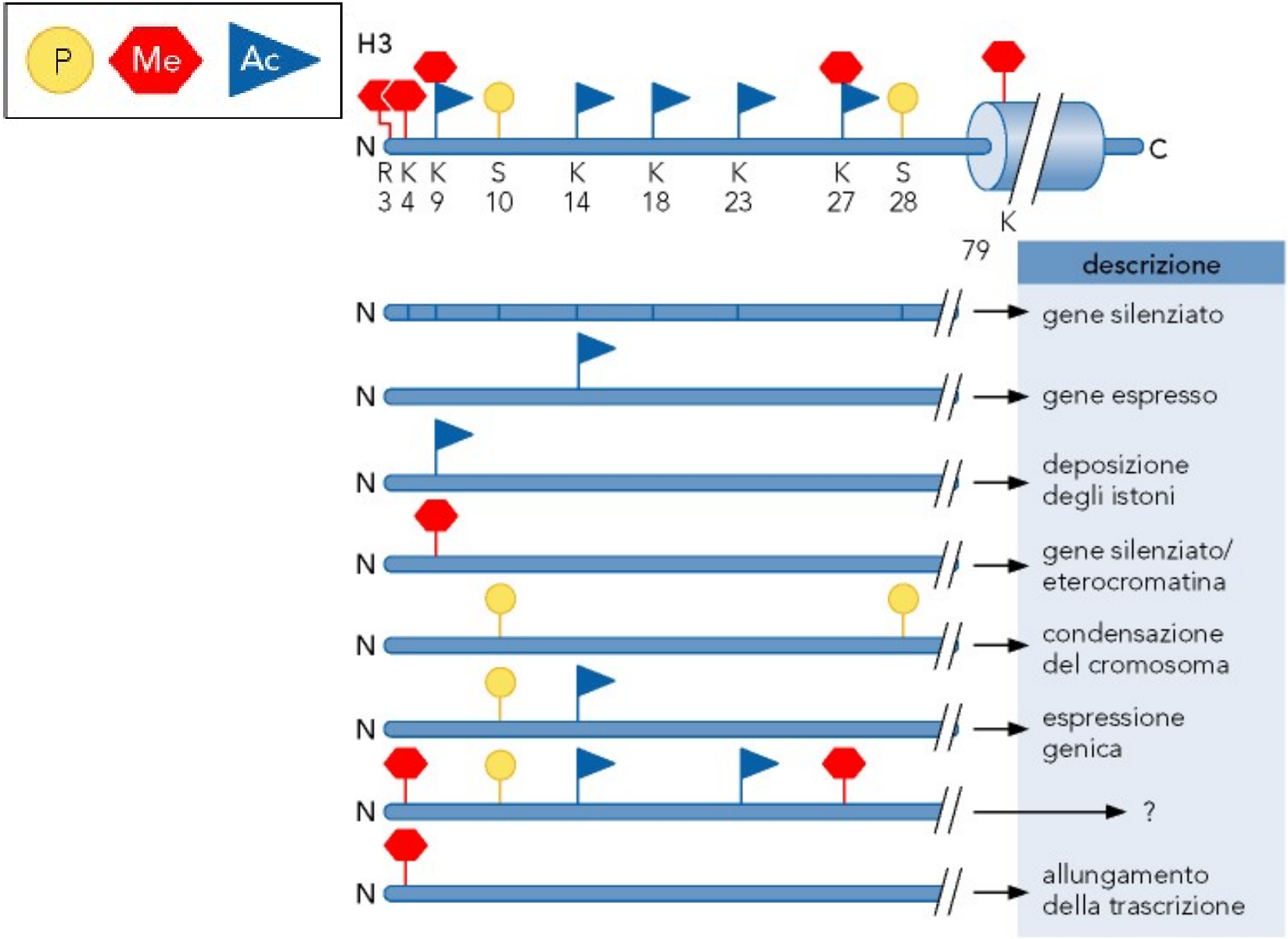
4 transcriptional silencing of L1 can dampen expression of these genes and can influence host gene expression

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

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.





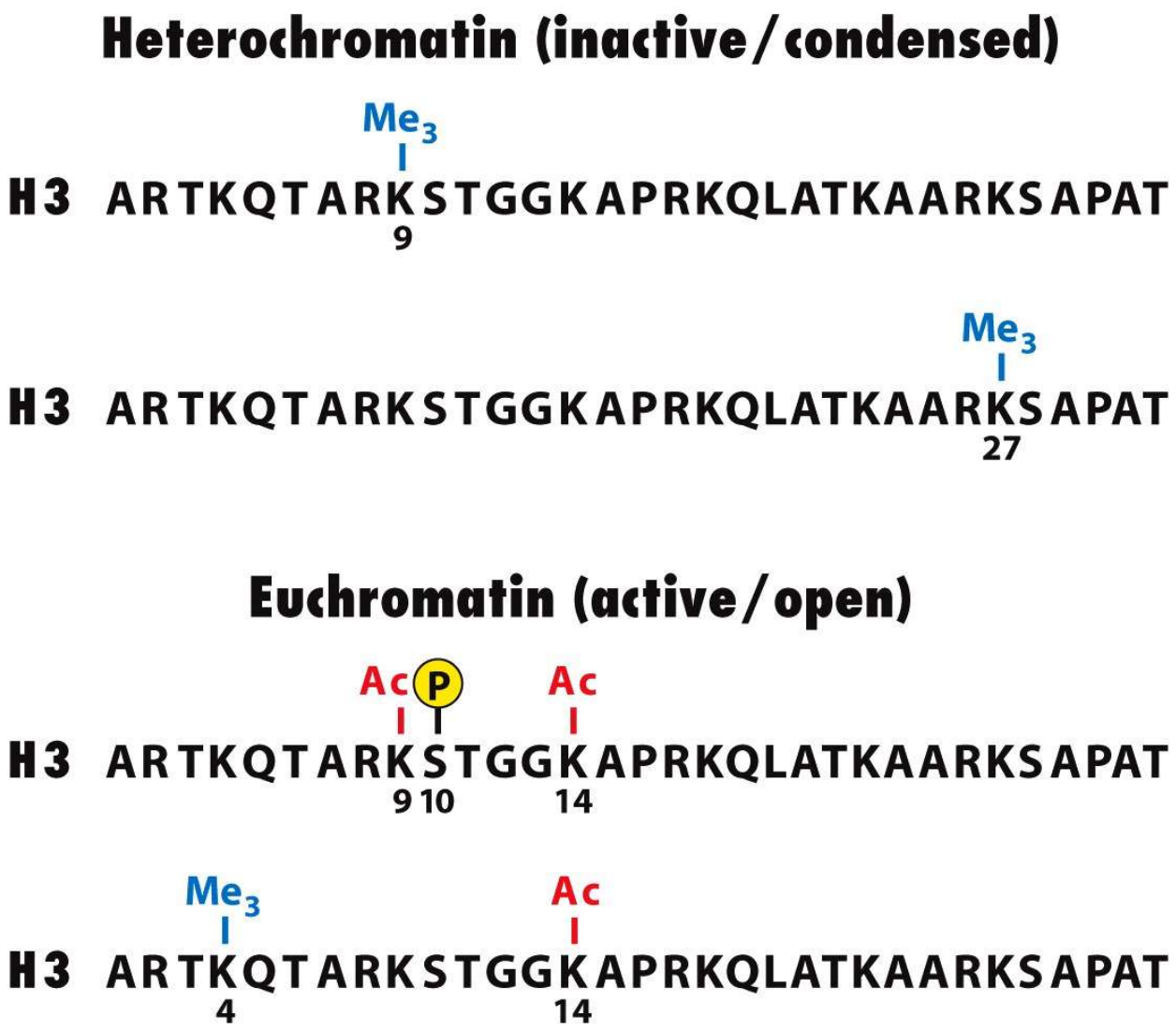
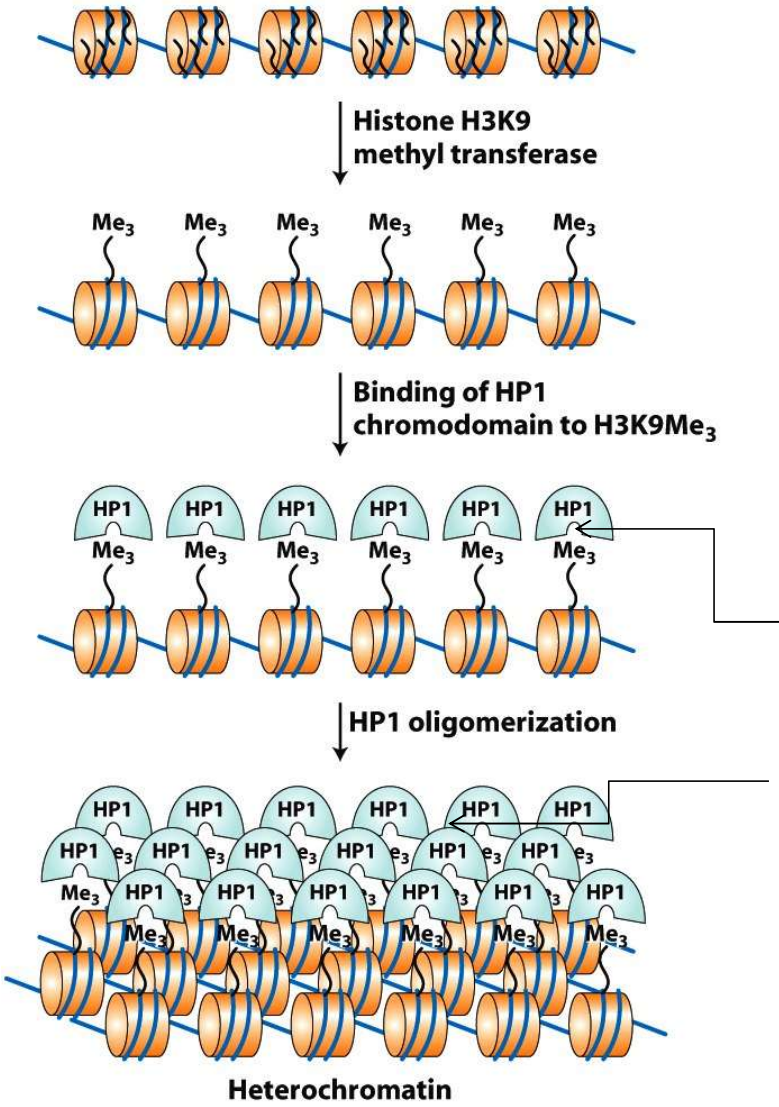


Figure 6-33b
Molecular Cell Biology, Sixth Edition



Cromodominio

Figure 6-34a
Molecular Cell Biology, Sixth Edition

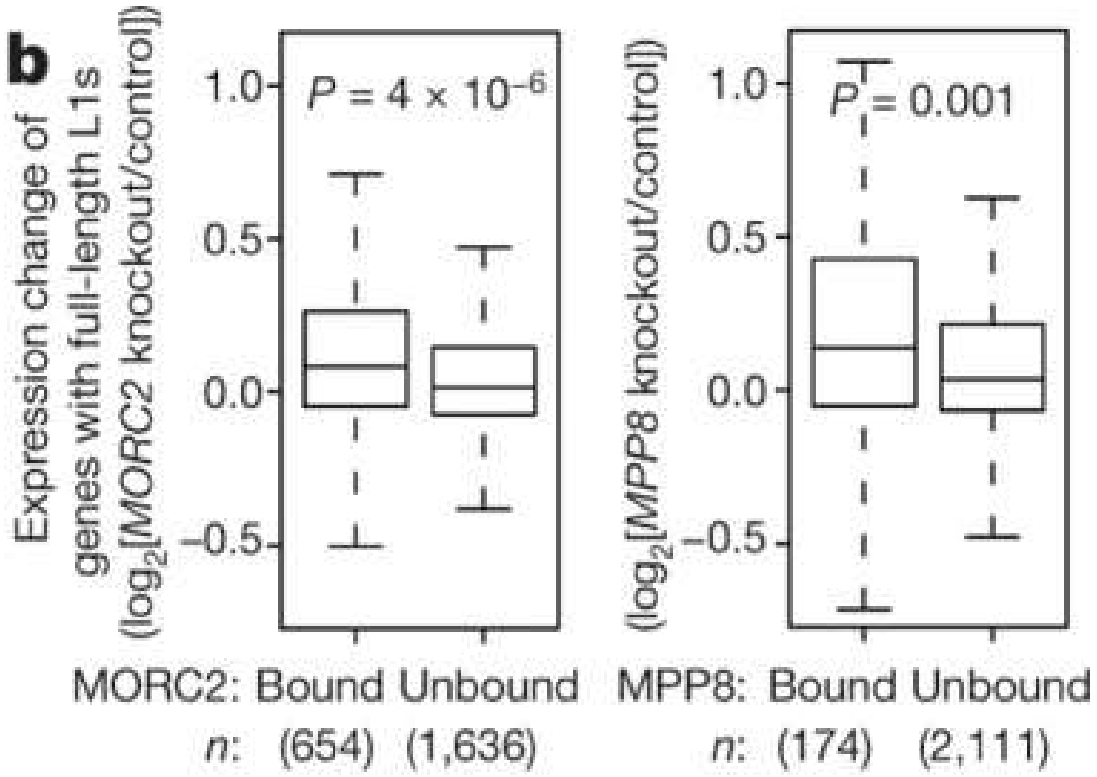
Silence on LINE-1

- 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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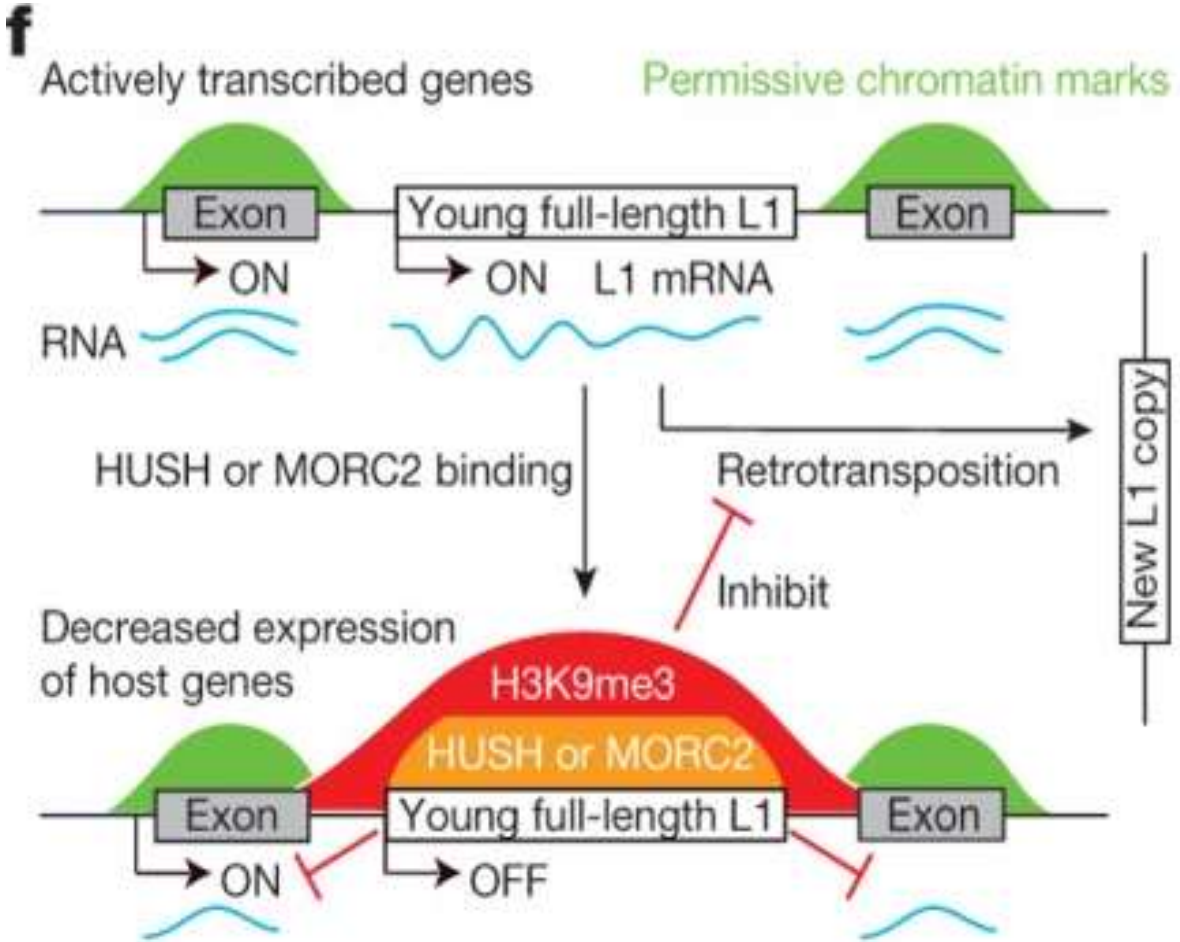
nature

HUSH or MORC2 binding at L1s decreases active host gene expression



HUSH and MORC2 bind young full-length L1s within transcriptionally active genes, and promote H3K9me3 deposition at target L1s to silence L1 transcription and thus decreases host gene expression.

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