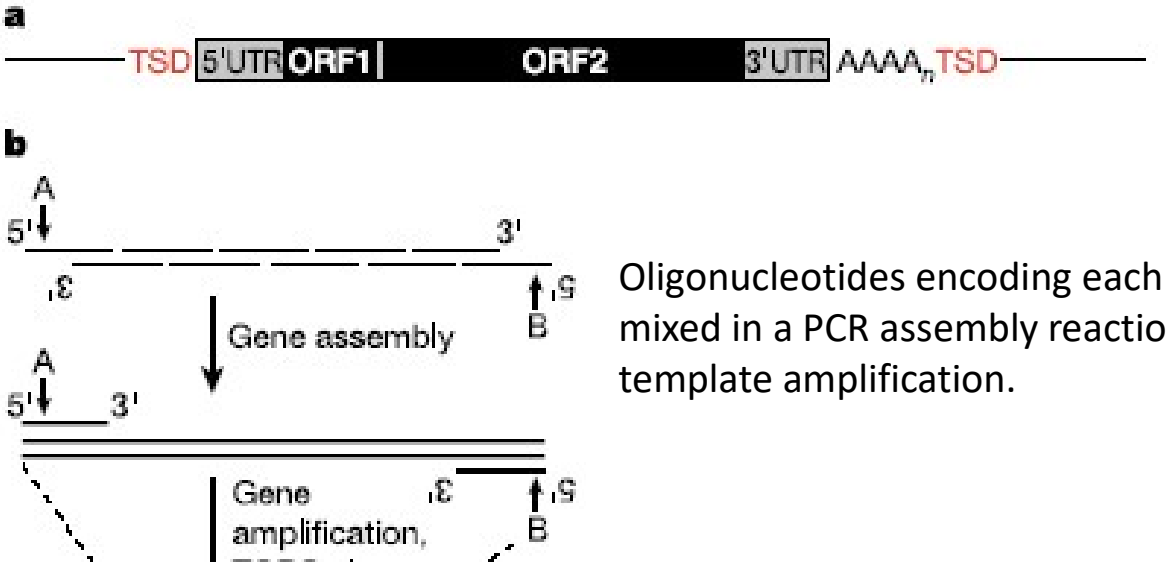
Corso di laurea in Scienze Biologiche
Corso di laurea magistrale in Scienze Biomolecolari e dell'Evoluzione

Materiale didattico di supporto

Tutto il materiale fornito a supporto delle lezioni e reperibile nel minisito dell'insegnamento o sulla piattaforma online UniFE deve essere inteso come traccia degli argomenti svolti e non sostituisce il libro di testo.

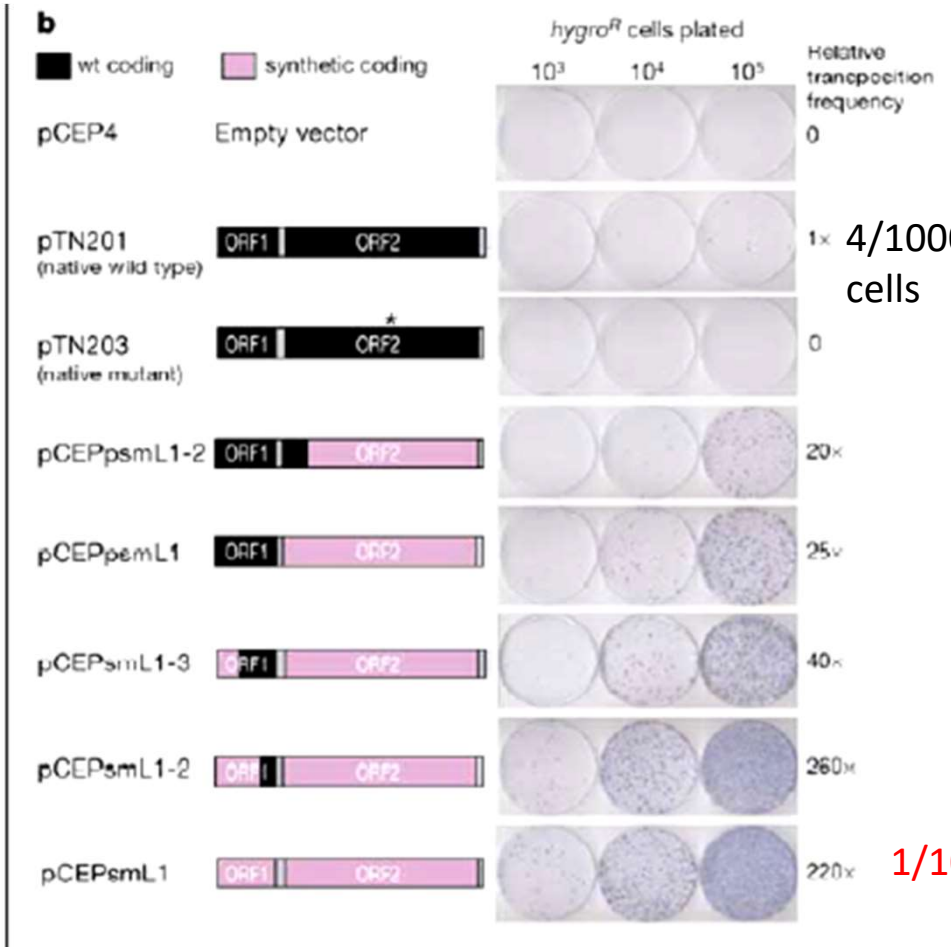
A highly active synthetic mammalian retrotransposon.

Han JS Nature. 429:314 2004



Oligonucleotides encoding each fragment were mixed in a PCR assembly reaction and used as template amplification.

Synthesis cloning and expression of synthetic mouse ORFs



selezione di cellule resistenti aseguito di retrotrasposizione

4/10000 cells

1/10 cells!

Figure 2 Retrotransposition of synthetic mL1. **a**, The retrotransposition assay. The L1 element contains an intron-interrupted *neo* reporter in the 3′ untranslated region with its own promoter and polyadenylation signal. Only when *neo* is transcribed from the L1 promoter, spliced, reverse transcribed and integrated into the genome does a cell become G418-resistant[4]. Blue lines represent probes for RNA analysis (Fig. 4). SD, splice donor; SA, splice acceptor. **b**, Retrotransposition was assayed in HeLa cells ($N = 3$). pTN201 contains only wild-type native mouse L1 sequence, and pTN203 contains wild-type native mouse L1 sequence with a D709Y reverse transcriptase point mutation[22]. The average absolute number of colonies for pTN201 was 440 events per 10^6 transfected cells.

A highly active synthetic mammalian retrotransposon.

Han JS Nature. 429:314

Transcription through L1 open reading frames is inefficient owing to an elongation defect
This elongation defect probably controls L1 transposition frequency in mammalian cells.
We report bypassing this transcriptional defect by synthesizing the open reading frames of L1 from synthetic oligonucleotides, altering 24% of the nucleic acid sequence without changing the amino acid sequence.
When the synthetic open reading frames were substituted for the wild-type open reading frames in an established retrotransposition assay, transposition levels increased more than 200-fold.
These synthetic retrotransposons are also the most highly active L1 elements known so far and have potential as practical tools for manipulating mammalian genomes

Come contrastare l’attività di trasposizione «silenziare i trasposoni»

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

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

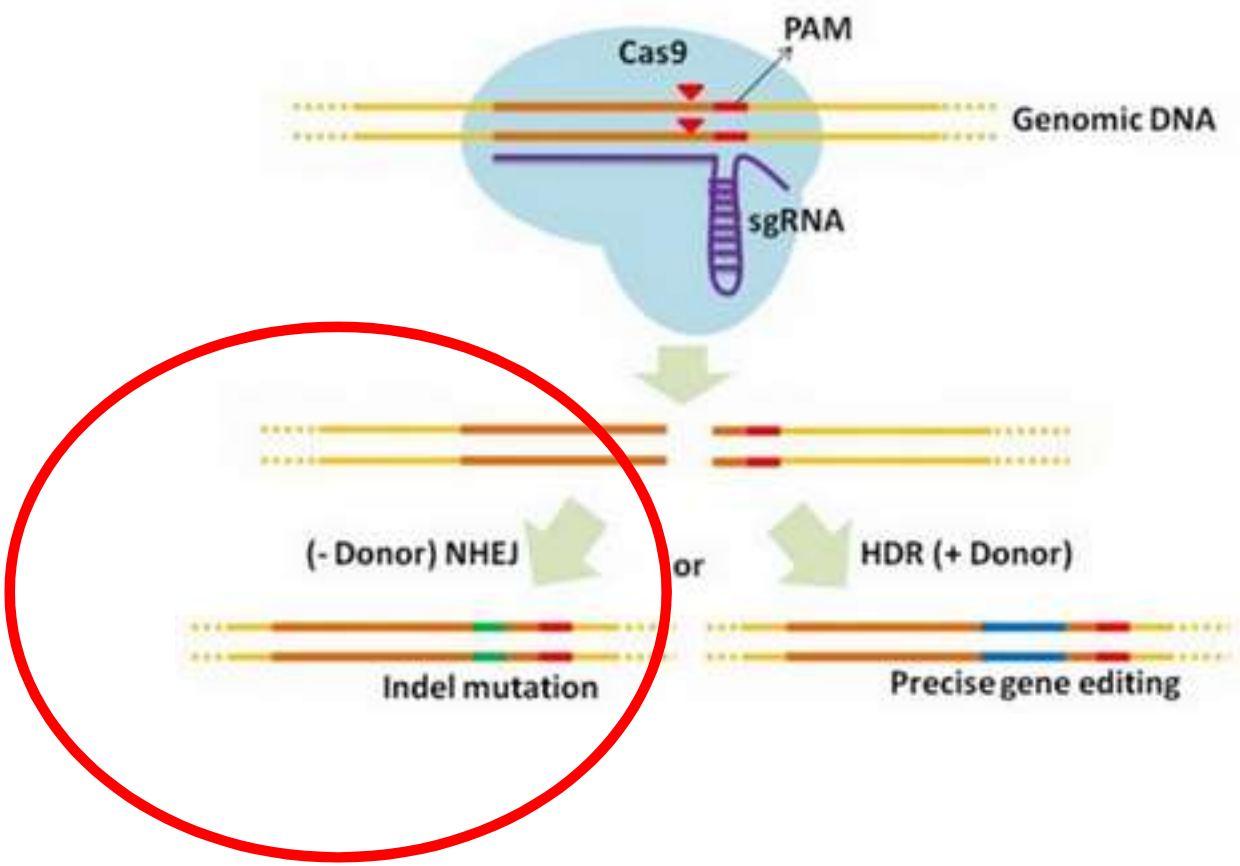
Silence on LINE-1

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

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



Doudna et al., 2014

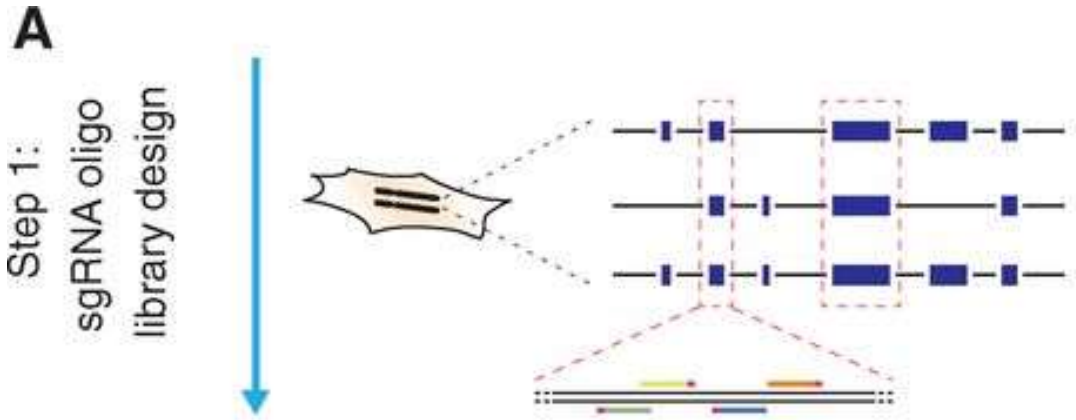
Delivery of Cas9 and sgRNA provides efficient depletion of target genes

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

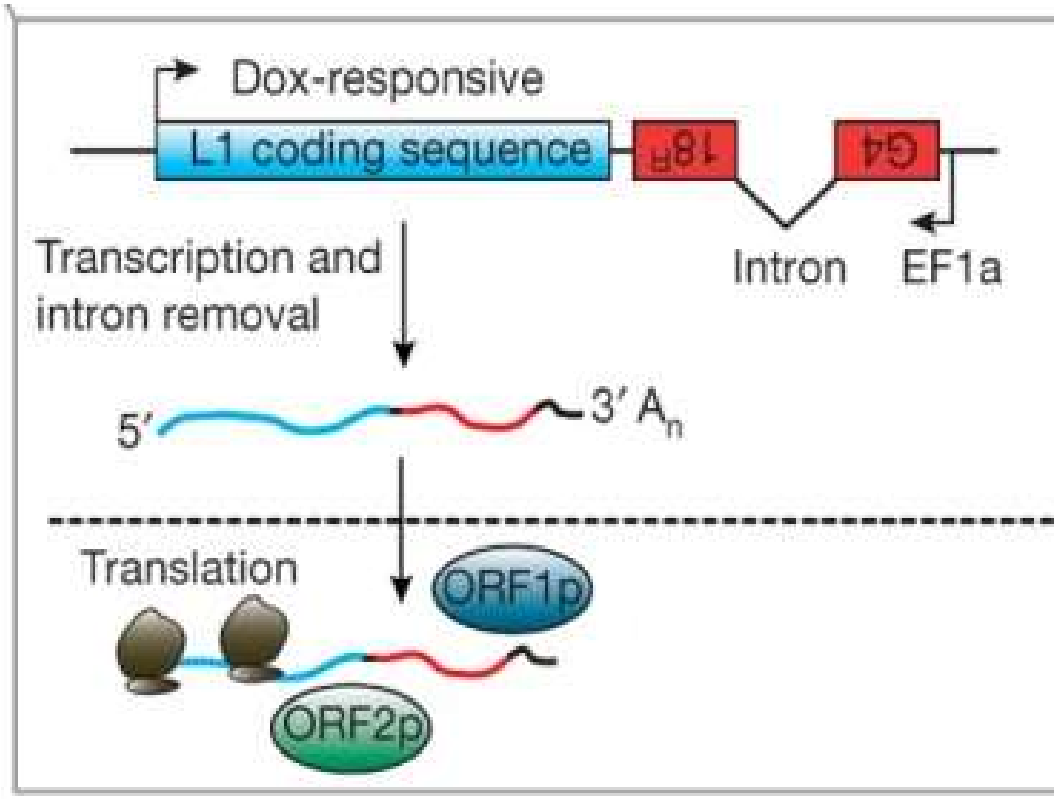
Design of sgRNA library for genome-scale knockout of coding sequences in human cells



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

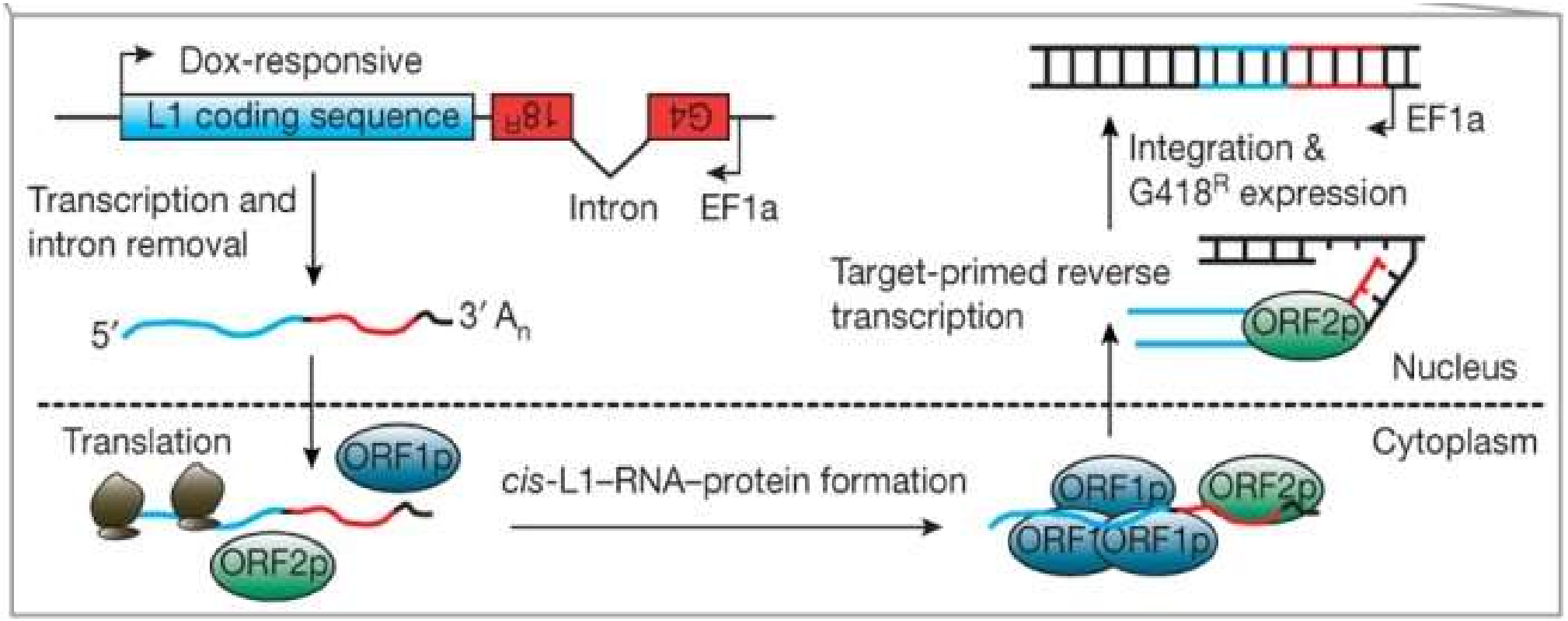
Genome-wide screen for L1 activators and suppressors in K562 cells



L1-G418^{R} retrotransposition

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

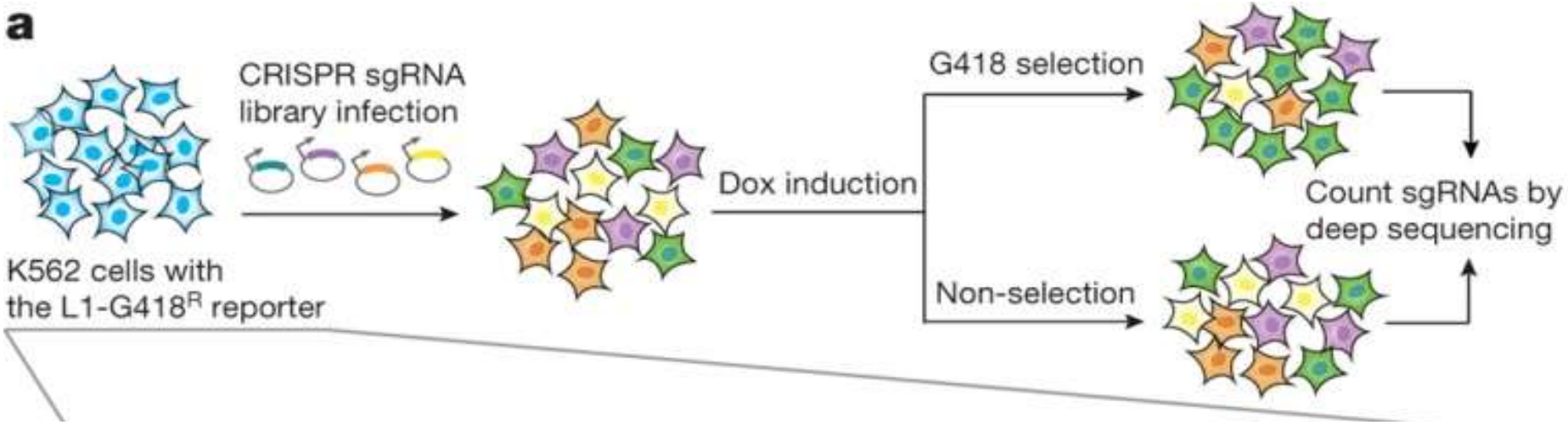
Genome-wide screen for L1 activators and suppressors in K562 cells



L1-G418^{R} retrotransposition

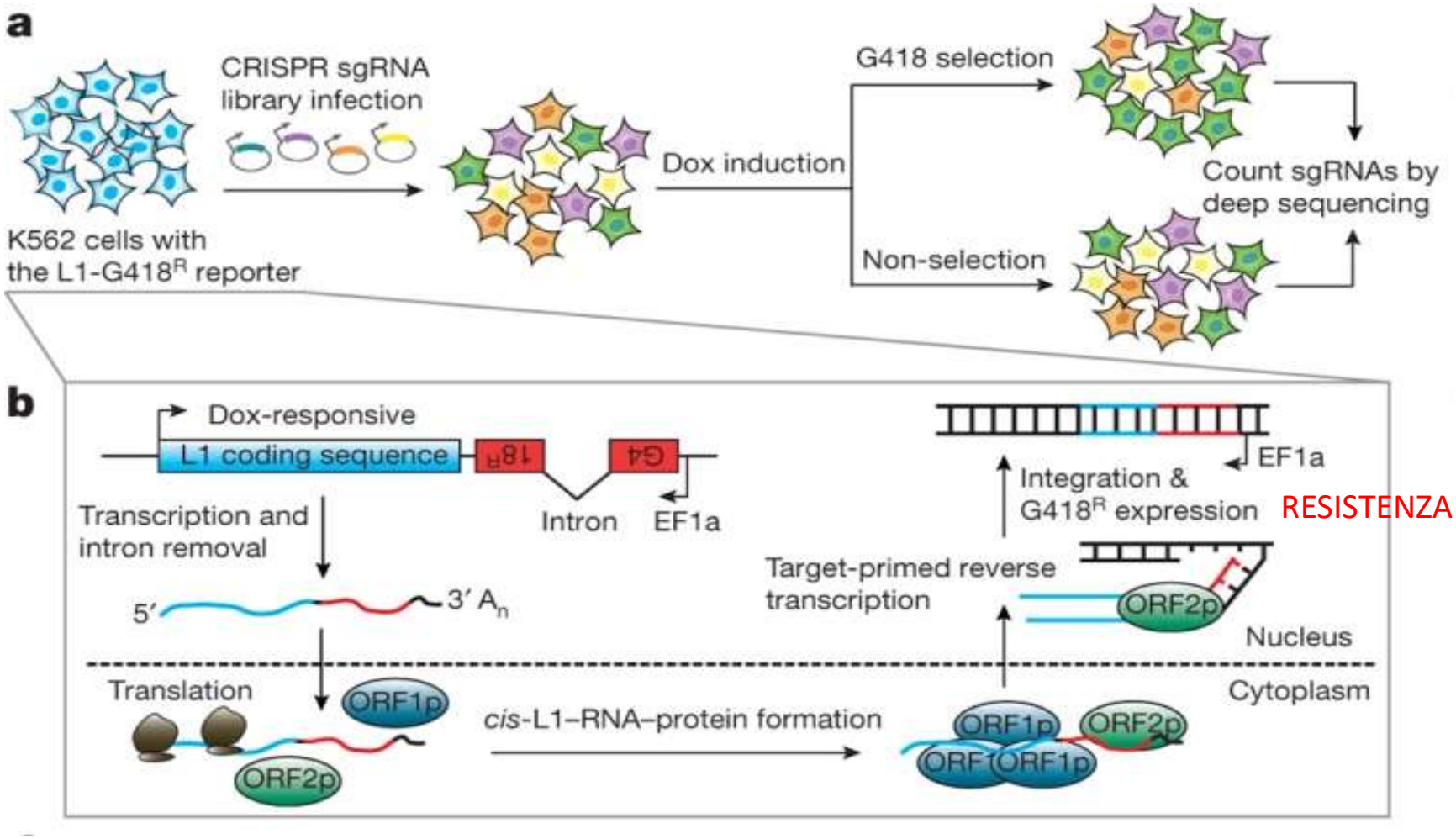
N Liu *et al. Nature* **553,** 228–232 (2018) doi:10.1038/nature25179

Genome-wide screen for L1 activators and suppressors in cells



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

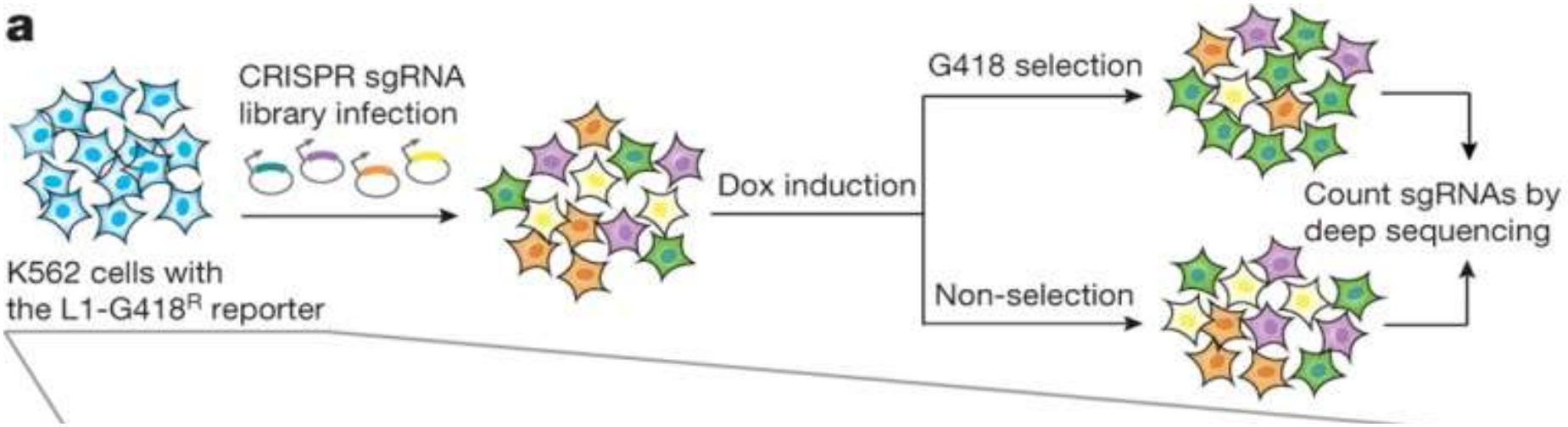
Genome-wide screen for L1 activators and suppressors in K562 cells



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

«PIU TRASPOSIZIONE PIU RESISTENZA»

Genome-wide screen for L1 activators and suppressors in cells



..cells transduced with sgRNAs targeting L1 activators would be depleted

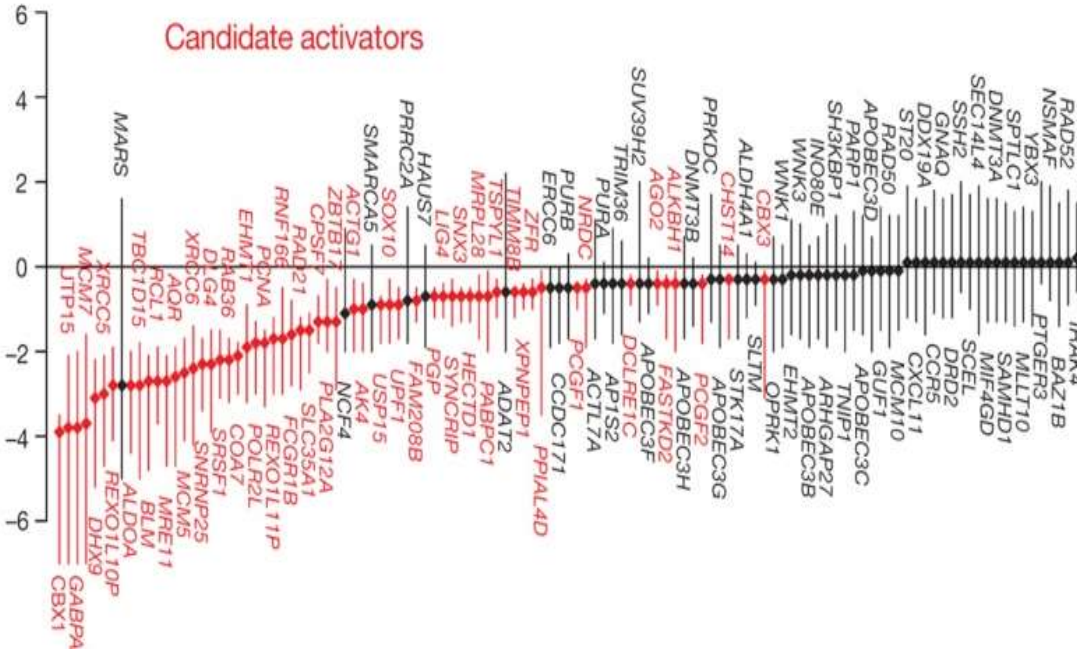
Meno geni attivatori espressi

MENO TRASPOSIZIONE MENO RESISTENZA MENO cellule

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

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

Attivatori trasposizione inattivati

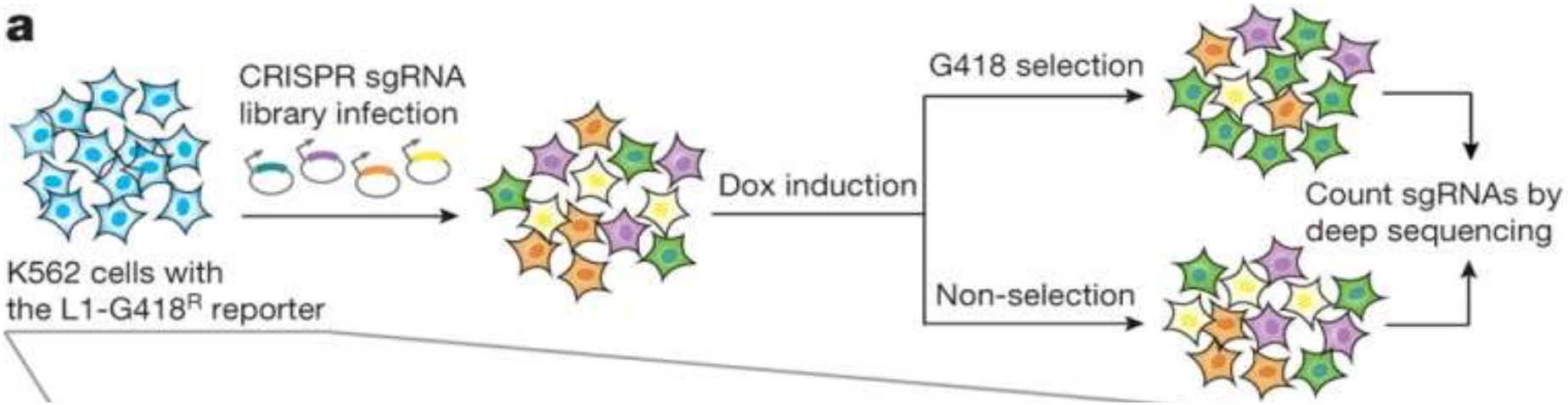


Meno CELLULEMENO sgRNA

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

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

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

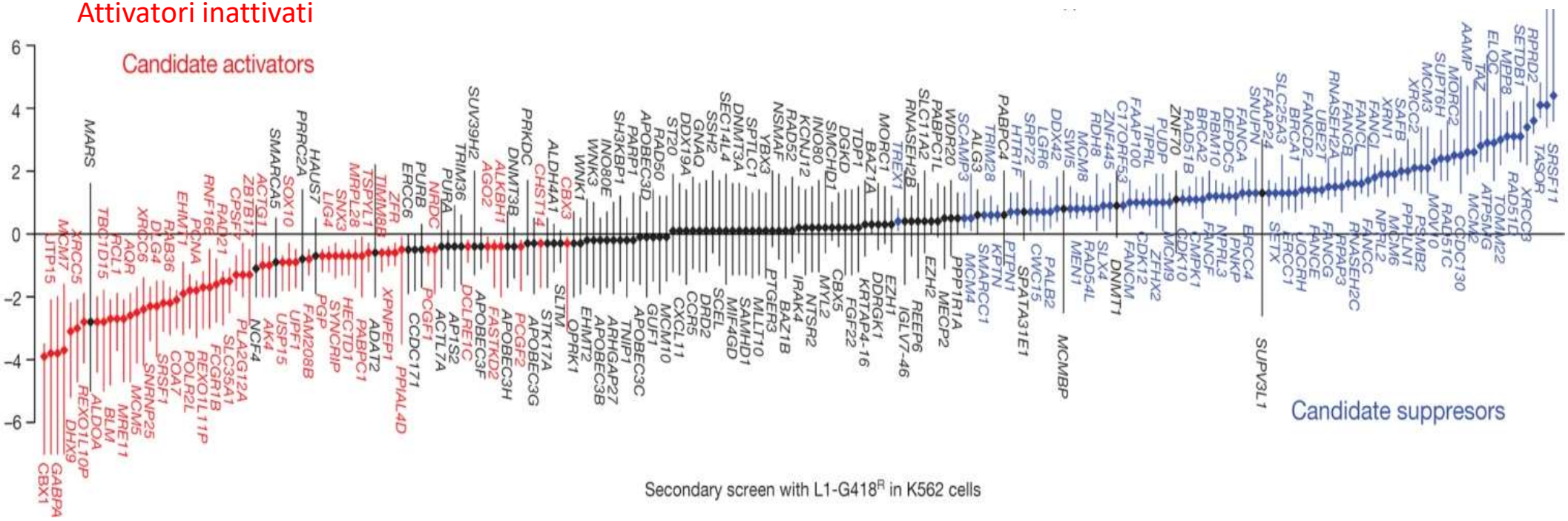
Meno geni soppressori espressi PIU TRASPOSIZIONE PIU RESISTENZA piu cellule

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

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

Soppressori trasposizione inattivati
PIU sgRNA -CELLULE

Attivatori inattivati

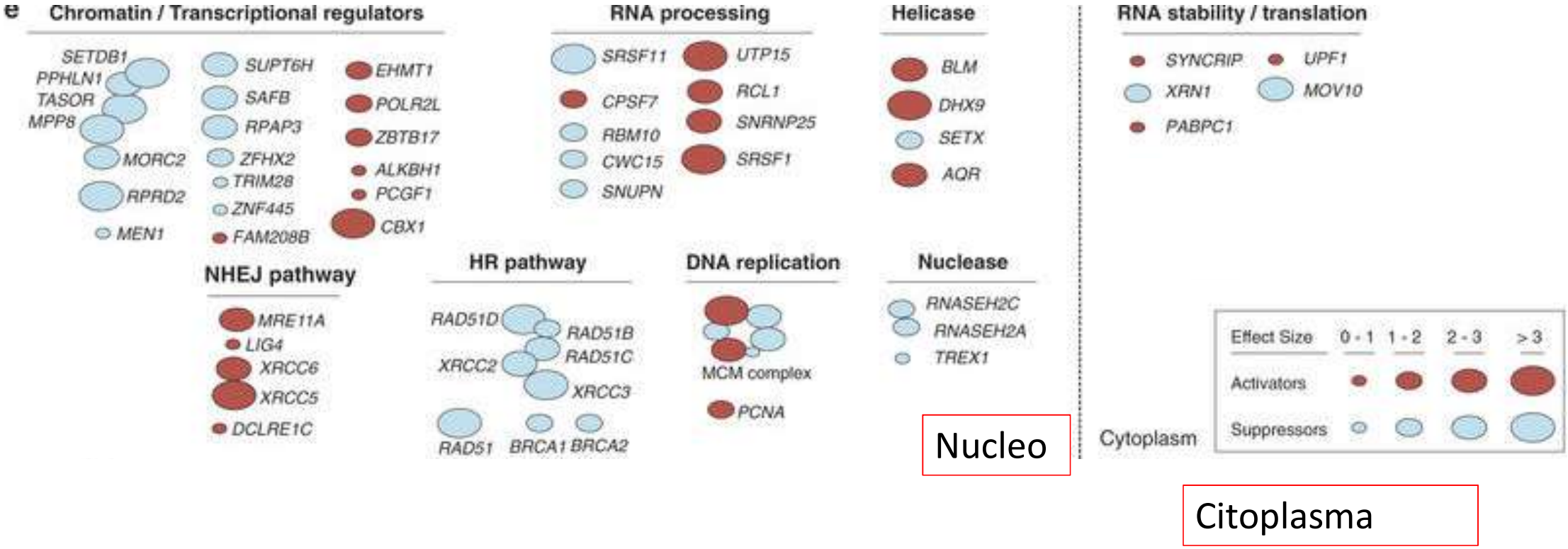


MENO sgRNA -CELLULE

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

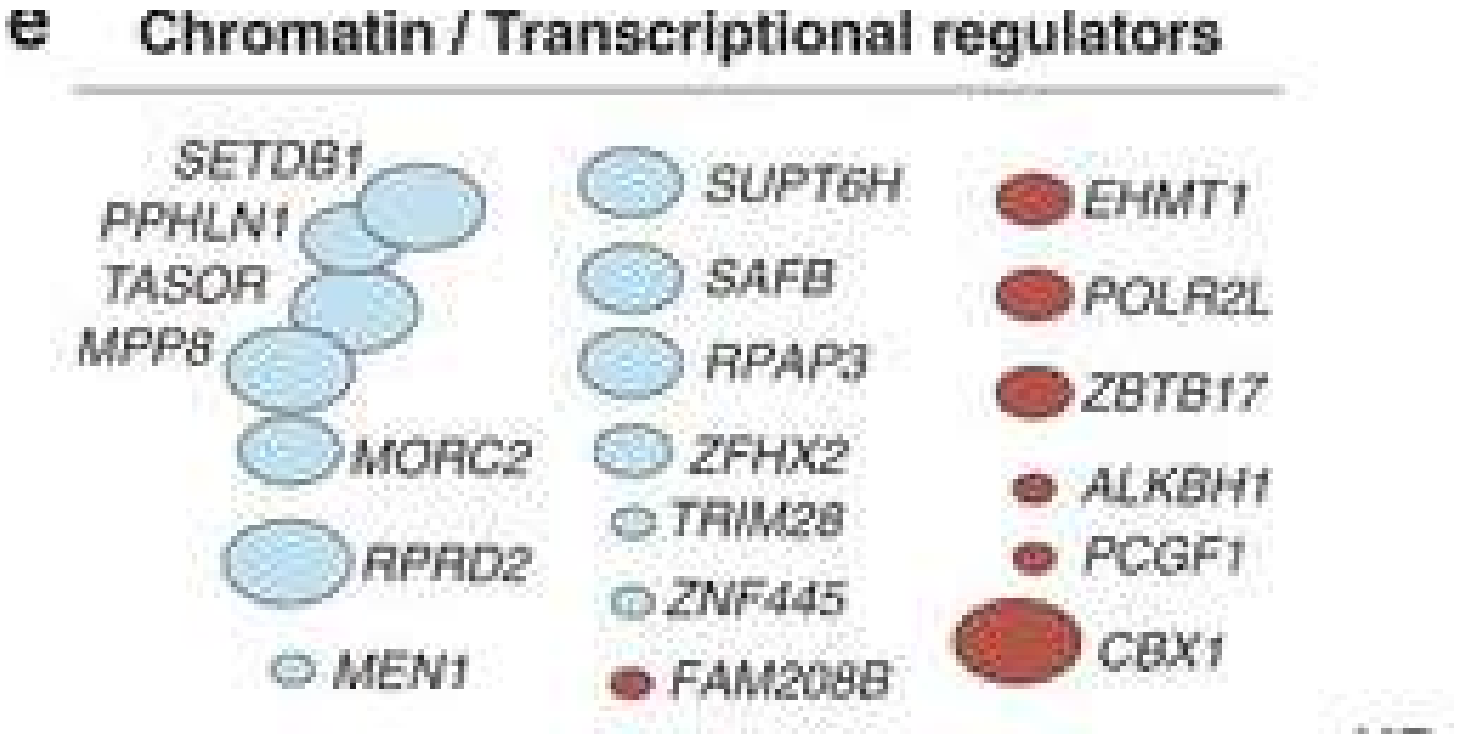
N Liu *et al. Nature* **553,** 228–232 (2018) doi:10.1038/nature25179

Classificazione funzionale **Attivatori** ed **Inibitori** L1



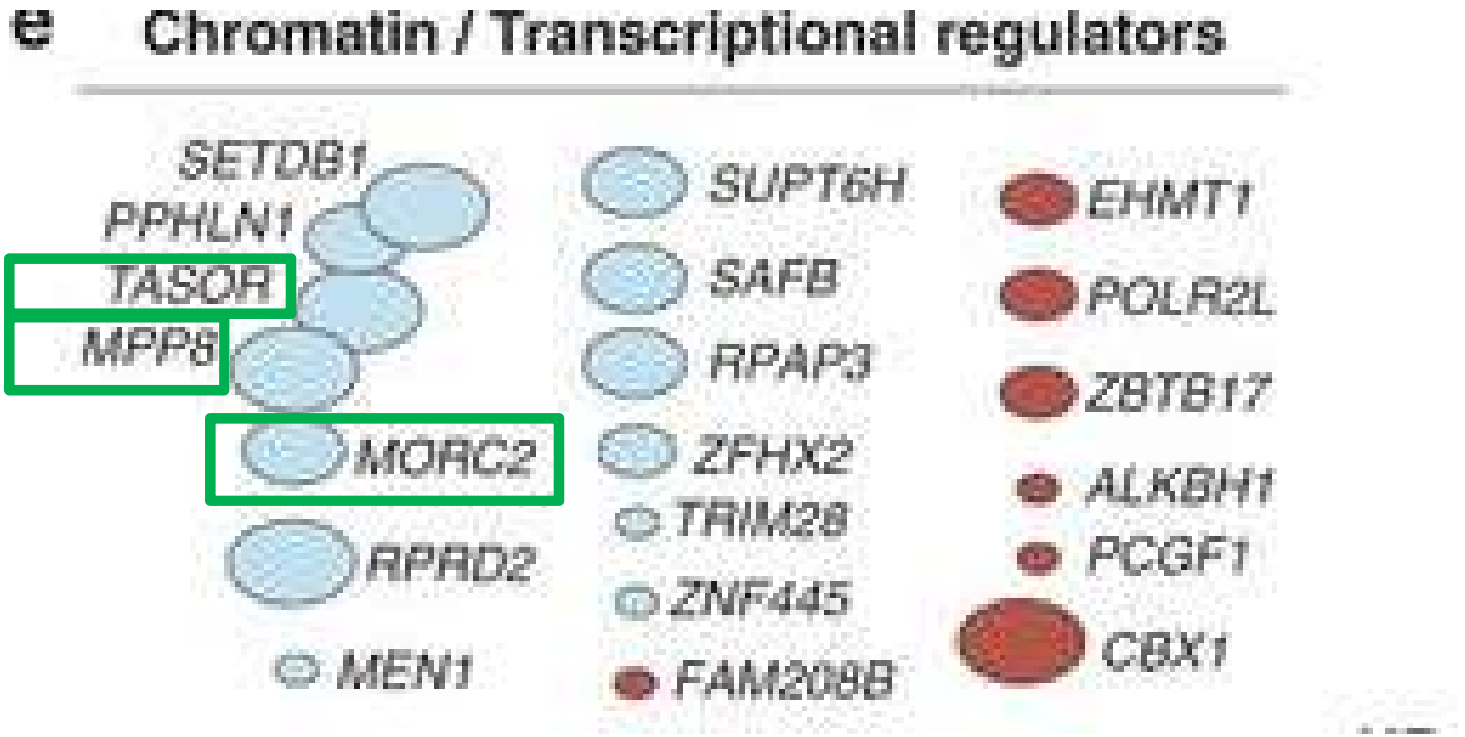
Citoplasma

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



Inibitori trasposizione **Attivatori trasposizione**

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

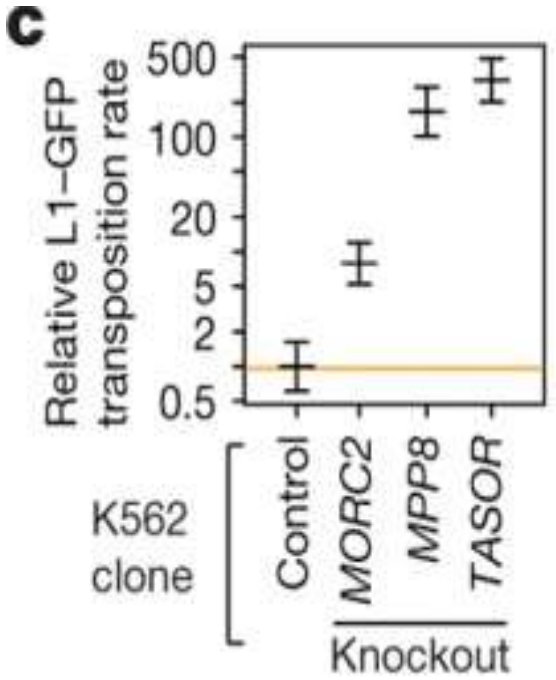


Inibitori trasposizione

Attivatori trasposizione

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

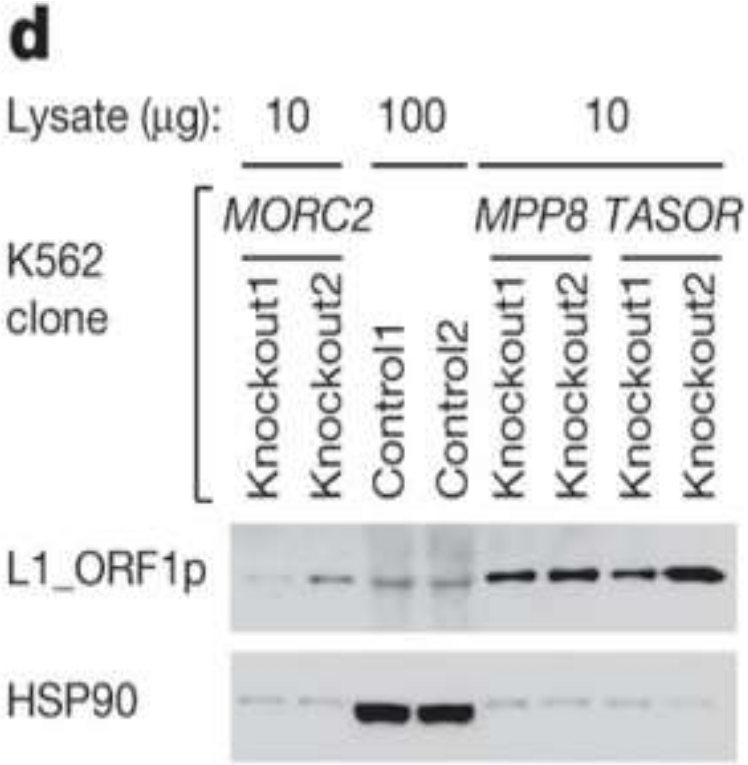
Knockout of MORC2 MPP8 and TASOR increase L1Transposition



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

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

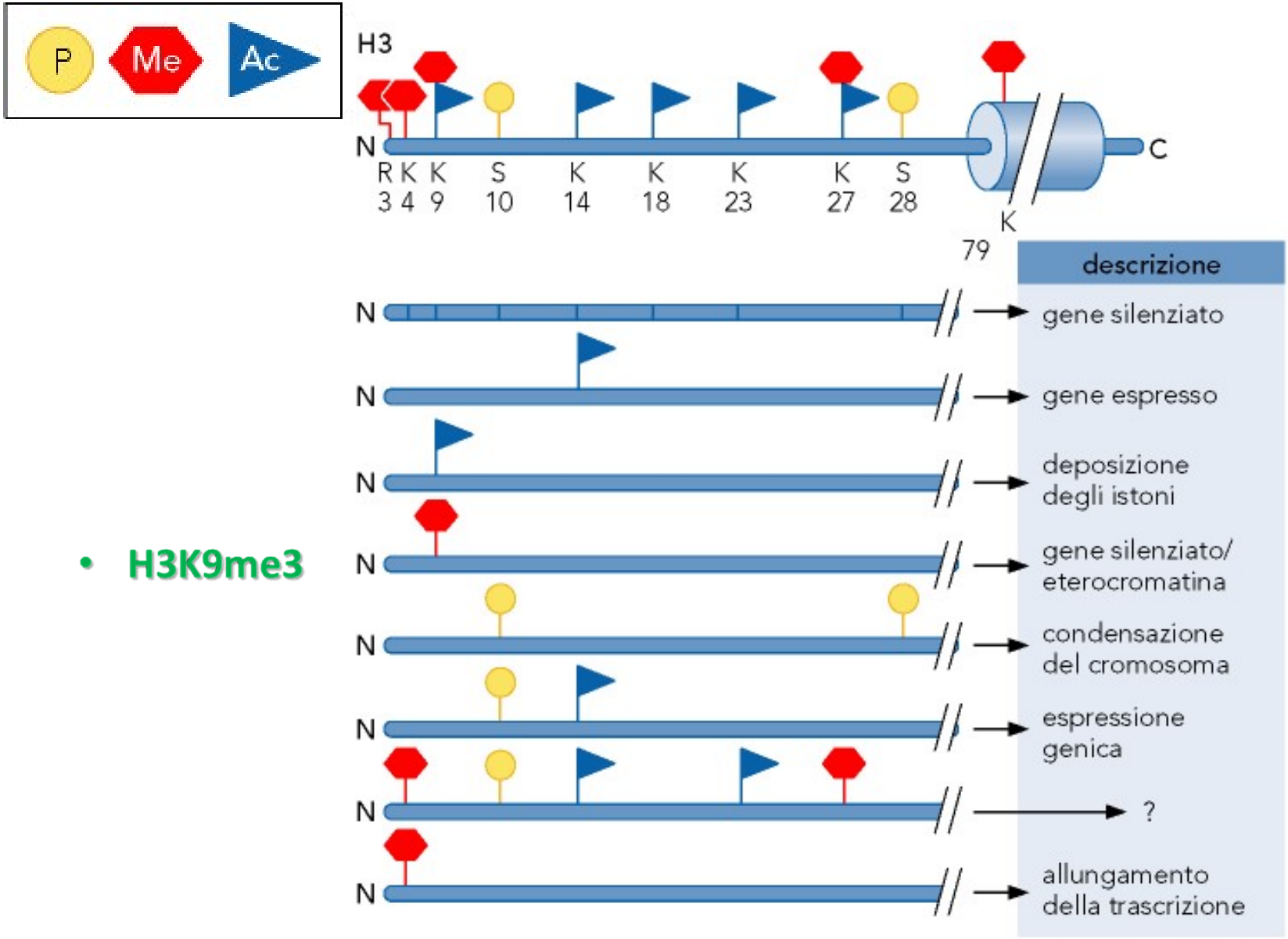
nature

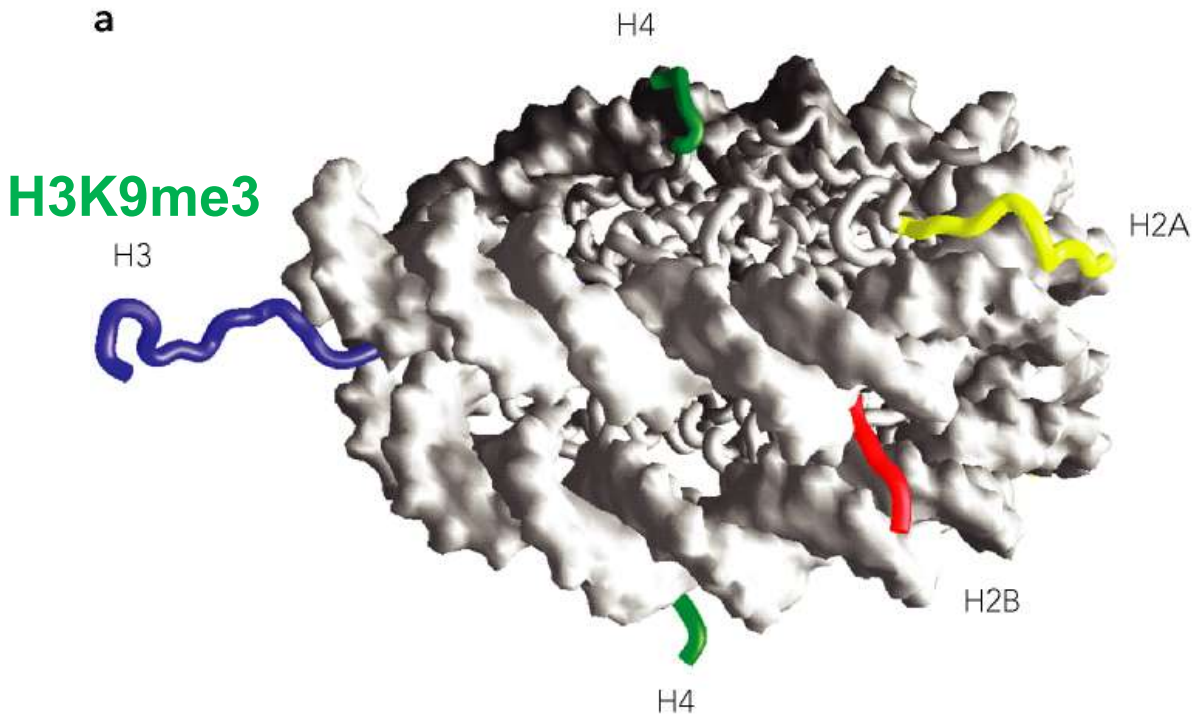
epigenetic repression in human cells.

- TASOR, MPP8 are components of the **HUSH (human silencing hub)** complex
- This complex is conserved from fish to humans.

- The HUSH complex is recruited to methylated chromatin rich in **H3K9me3.**
- Recruitment of the **methyltransferase SETDB1** is required for further H3K9me3 deposition to maintain transcriptional silencing

- **H3K9me3**





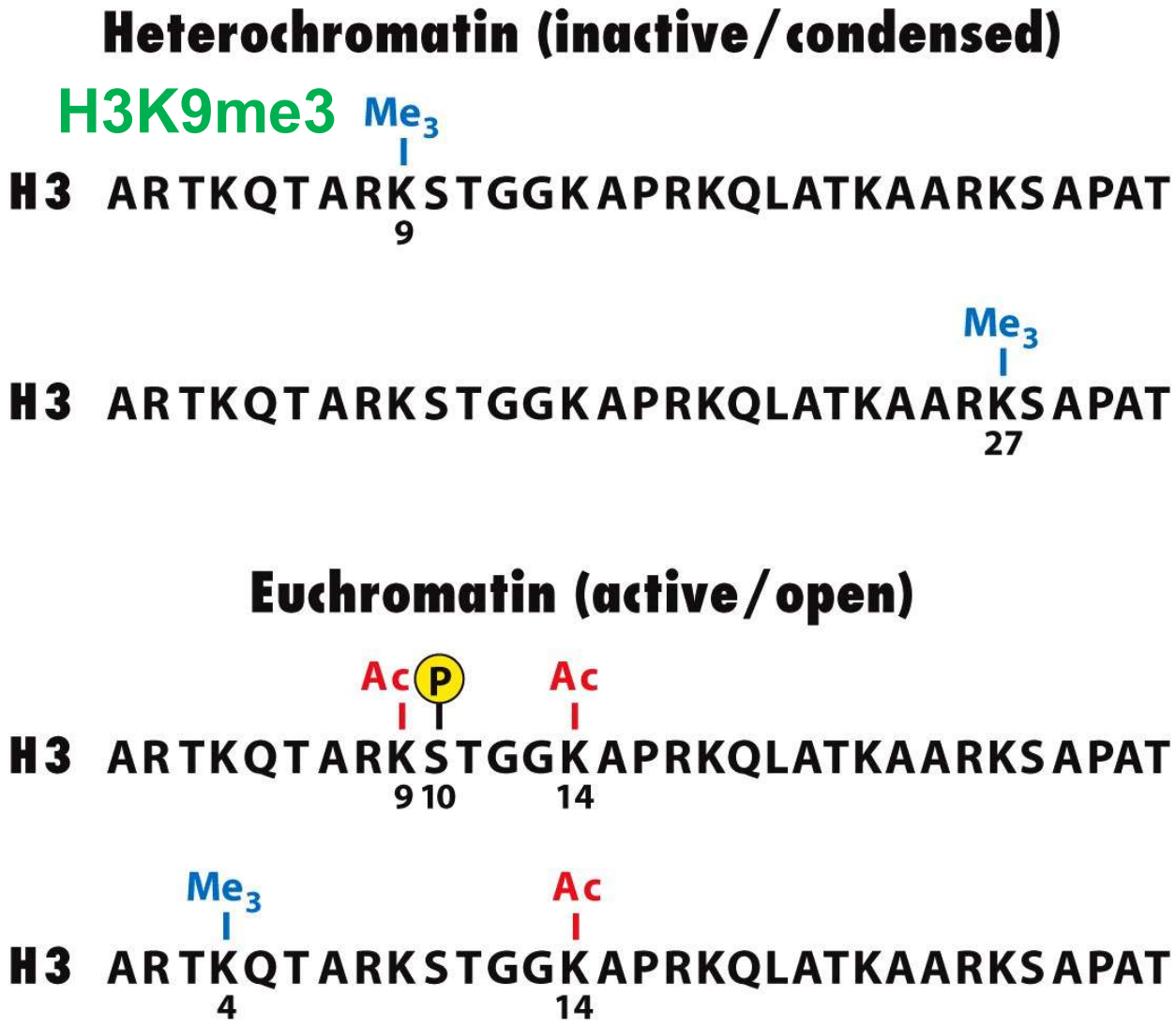


Figure 6-33b
Molecular Cell Biology, Sixth Edition

H3K9me3

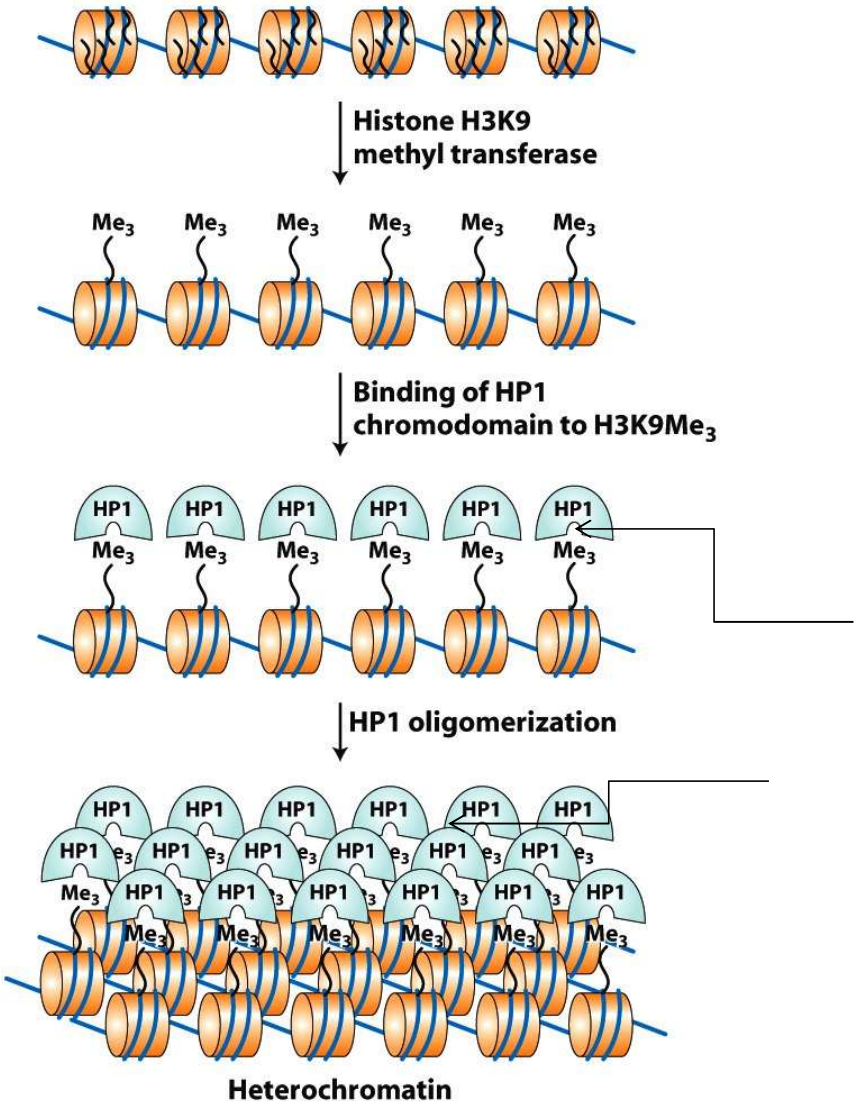
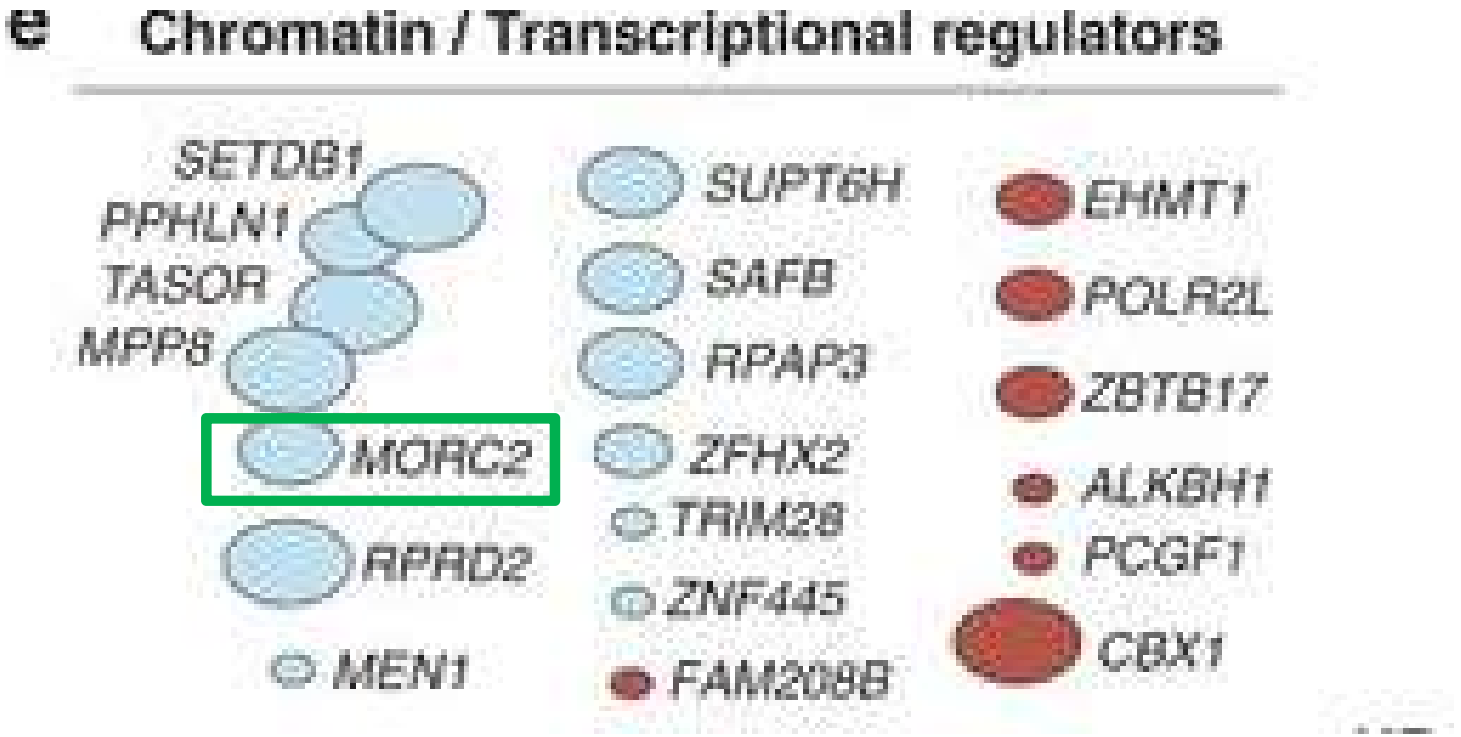


Figure 6-34a
Molecular Cell Biology, Sixth Edition

Conseguenze per l’espressione di geni che ospitano sequenze L1?



Inibitori trasposizione

Attivatori trasposizione

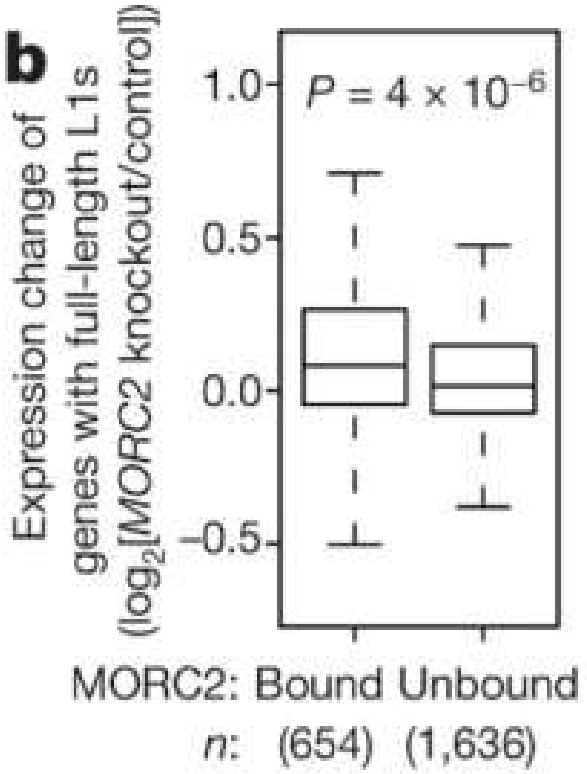
N Liu *et al. Nature* **553,** 228–232 (2018) doi:10.1038/nature25179

Silence on LINE-1 (MORC2)

- MORC2 ATPase activity and ability to bind methylated chromatin (chromatin-remodeling enzyme) is required to mediate silencing

- Mutations in the ATPase domain of MORC2 have recently been implicated in frequently inherited neurological disorders

MORC2 binding at L1s **decreases** active host gene expression

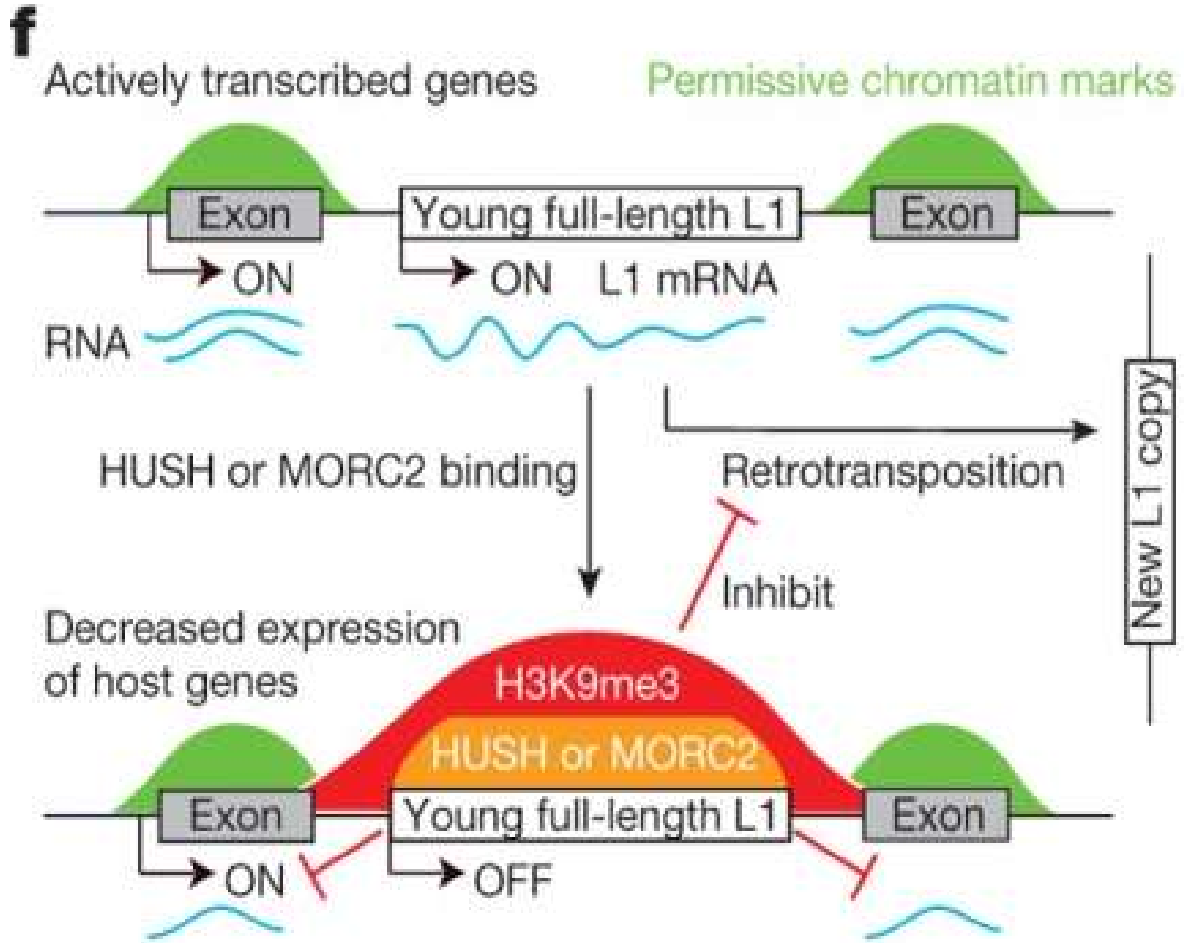


Gene expression changes with intronic full-length L1s that are bound or unbound by MORC2 (RNA-seq reads from knockout K562 clones compared to control).

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

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

Silence on LINE-1

- HUSH/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

- Loss of HUSH/MORC2 results in chromatin decompaction with a loss of H3K9me3 and transcriptional derepression.

Silence on LINE-1

L1 expression is restricted by the protein MORC2 and by the human silencing hub (HUSH) complex subunits MPP8 and TASOR

- 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**.