Locus-specific editing of histone modifications at endogenous enhancers

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Article

Locus-specific editing of histone modifications at endogenous enhancers

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Study

Develop a strategy for testing the functions of genomic elements and associated chromatin states in their endogenous context

Focus on active enhancers, which are marked by histone marks:

- H3K4me1
- H3K4me2
- H3K27ac

Study plan

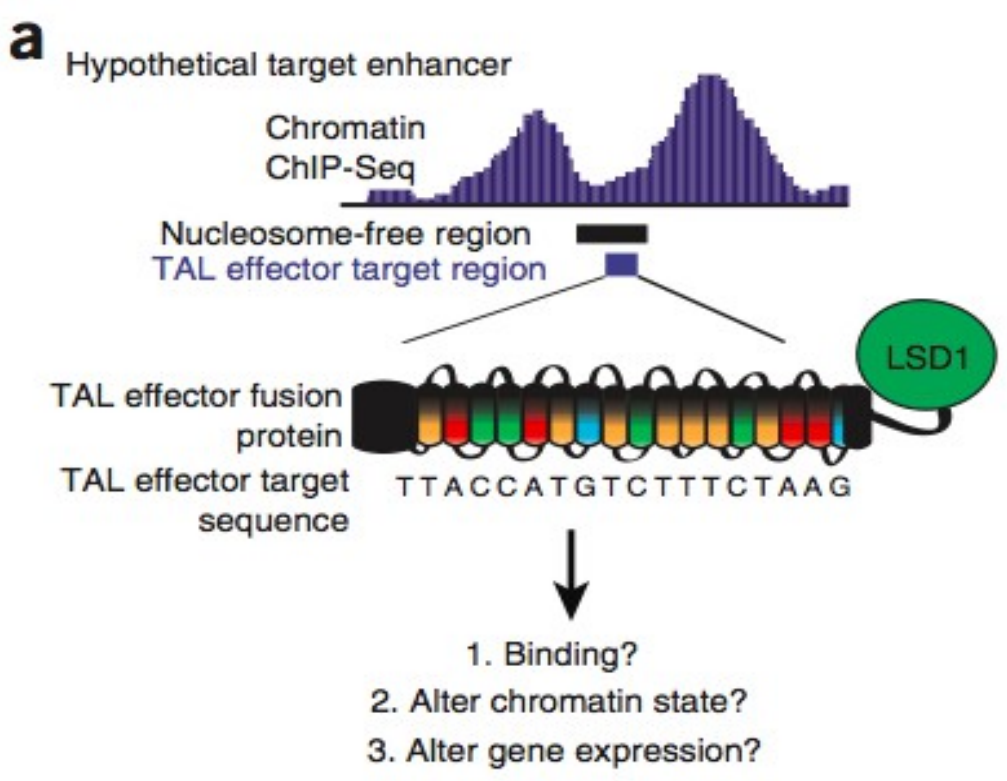
- Cell K562 erythroleukemia cells
- Enhancer could be inactivated by removal of these chromatin marks by TAL-LSD1
- Eliminate the possibility that the chromatin changes reflect displacement of other transcription factors by the TAL effector
- Expand to larger set of candidate enhancers with active chromatin in K562 cells
- Consider whether reduced chromatin activity at specific enhancers affects the transcriptional output of nearby genes

Stem Cell Leukemia Locus (SCL)

- SCL encodes a developmental transcription factor with critical functions in hematopoiesis that is expressed in K562 cells
- TAL effector repeats are modular DNA-binding domains that can be designed to bind essentially any genomic sequence of interest

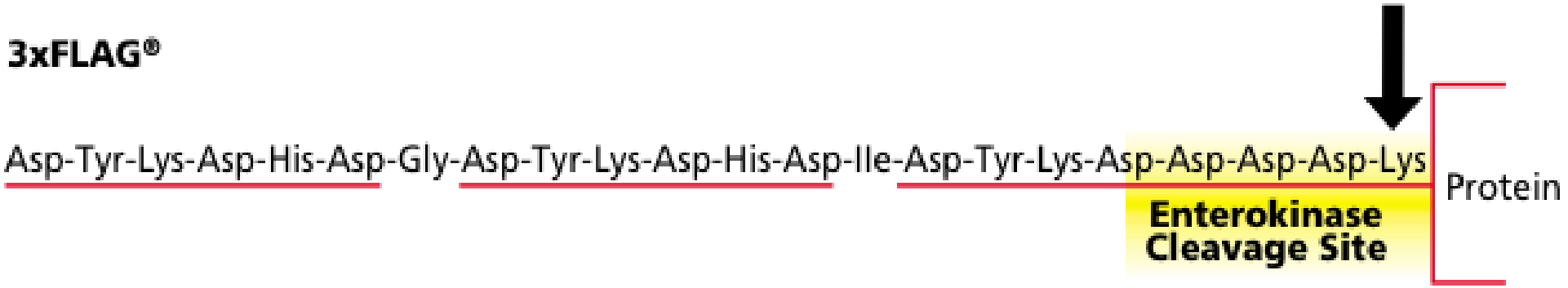
TAL effector design

- We designed a TAL effector array to bind an 18-base sequence in a segment of LCS enhancer predicted to be nucleosome-free based on DNase hypersensitivity.



TAL effector design

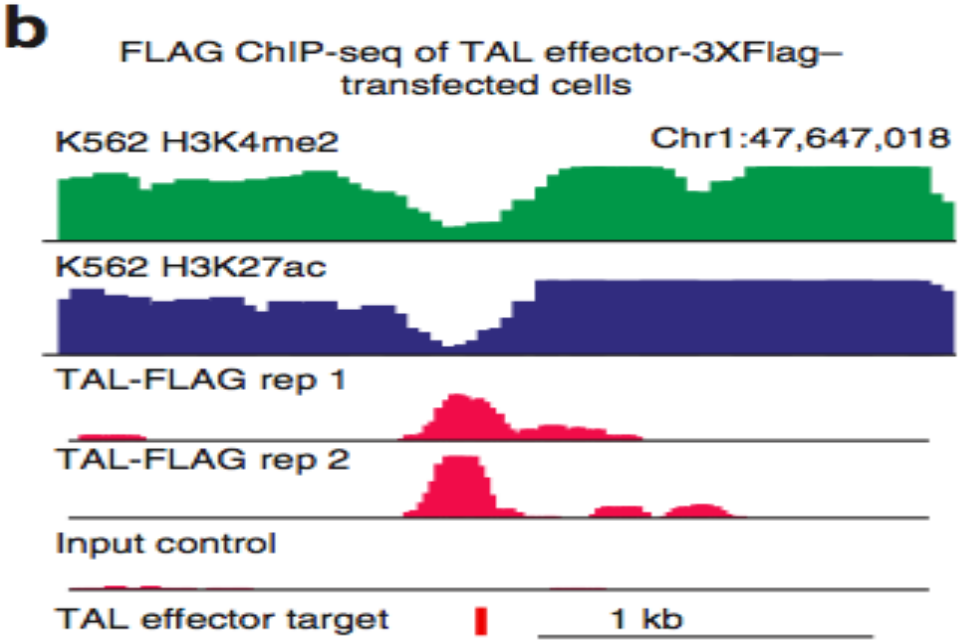
- As the binding specificity of monomeric TAL effectors has yet to be thoroughly characterized, we created an expression construct encoding this TAL effector array fused to a 3X FLAG epitope.
- 3XFlag – small hydrophilic tags facilitate superior detection and purification of recombinant fusion proteins when using our highly specific and sensitive ANTI-FLAG® antibodies



TAL effector design

- Transfected this construct into K562 cells, confirmed expression by western blot analysis, and mapped genome-wide binding by ChIP-seq.
- Top-ranked binding site corresponds precisely to the target sequence within the SCL locus.

- Picture: ChIP-seq signal tracks show H3K4me2, H3K27ac and TAL effector binding in K562 cells across a targeted enhancer in the SCL locus. Control track shows input chromatin.

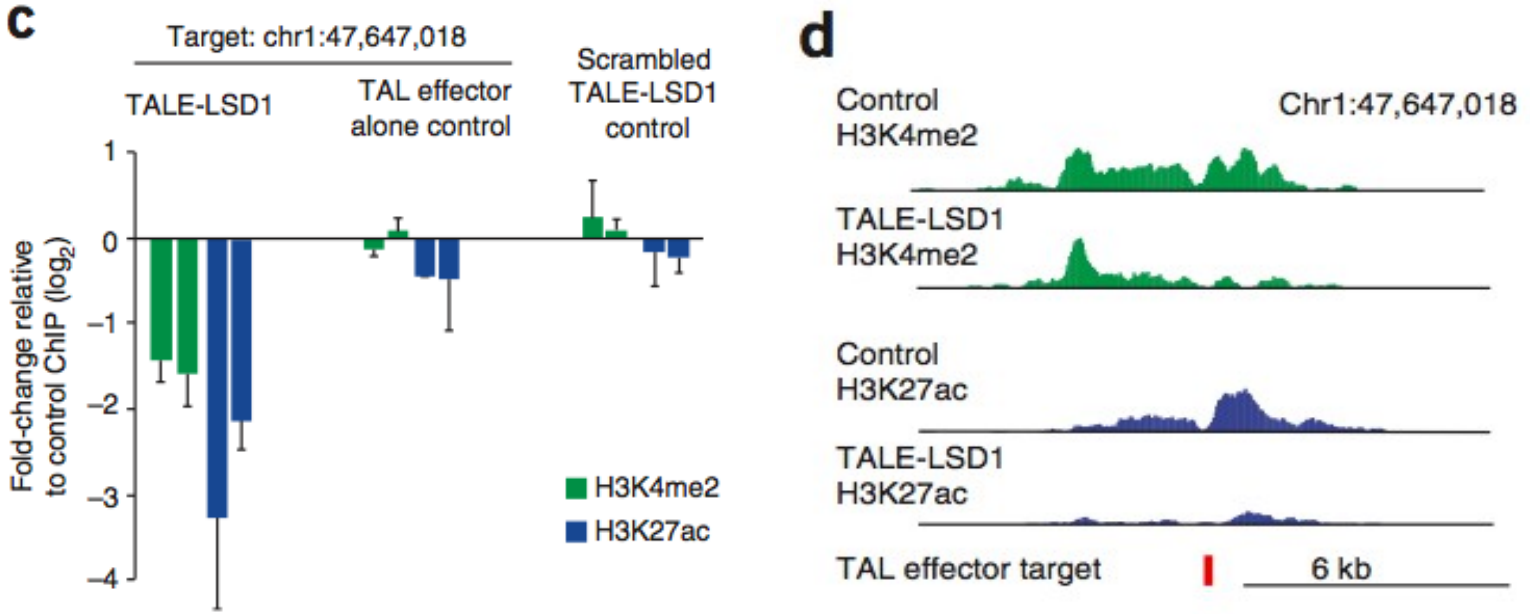


Experiment1

- Combined the corresponding TAL effector with the LSD1 demethylase.
- Transfected K562 cells with a construct encoding this TAL effector-LSD1 (TALE-LSD1) fusion or a control mCherry vector
- Also transfecting with TALE only, TALE-LSD with scrumbled sequence
- Cultured the cells for 3 days
- LSD1 has enzymathic activity and physically interacts with other chromatin-modifying enzymes, including histone deacetylases
- Measured histone modification levels by ChIP-qPCR H3K4me2, H3K27ac

Experiment1 results

- Fusion reduced H3K4me2 signals at the target locus by about threefold relative to control, but had no effect at several nontarget control enhancers
- Fusion reduced H3K27ac levels by more than fourfold, suggesting that LSD1 recruitment leads to generalized chromatin inactivation at the target enhancer
- These data confirmed loss of H3K4me2 and H3K27ac across a 2-kb region surrounding the target sequence within the SCL



Experiment2

Expanded study to investigate a larger set of candidate enhancers with active chromatin in K562 cells.

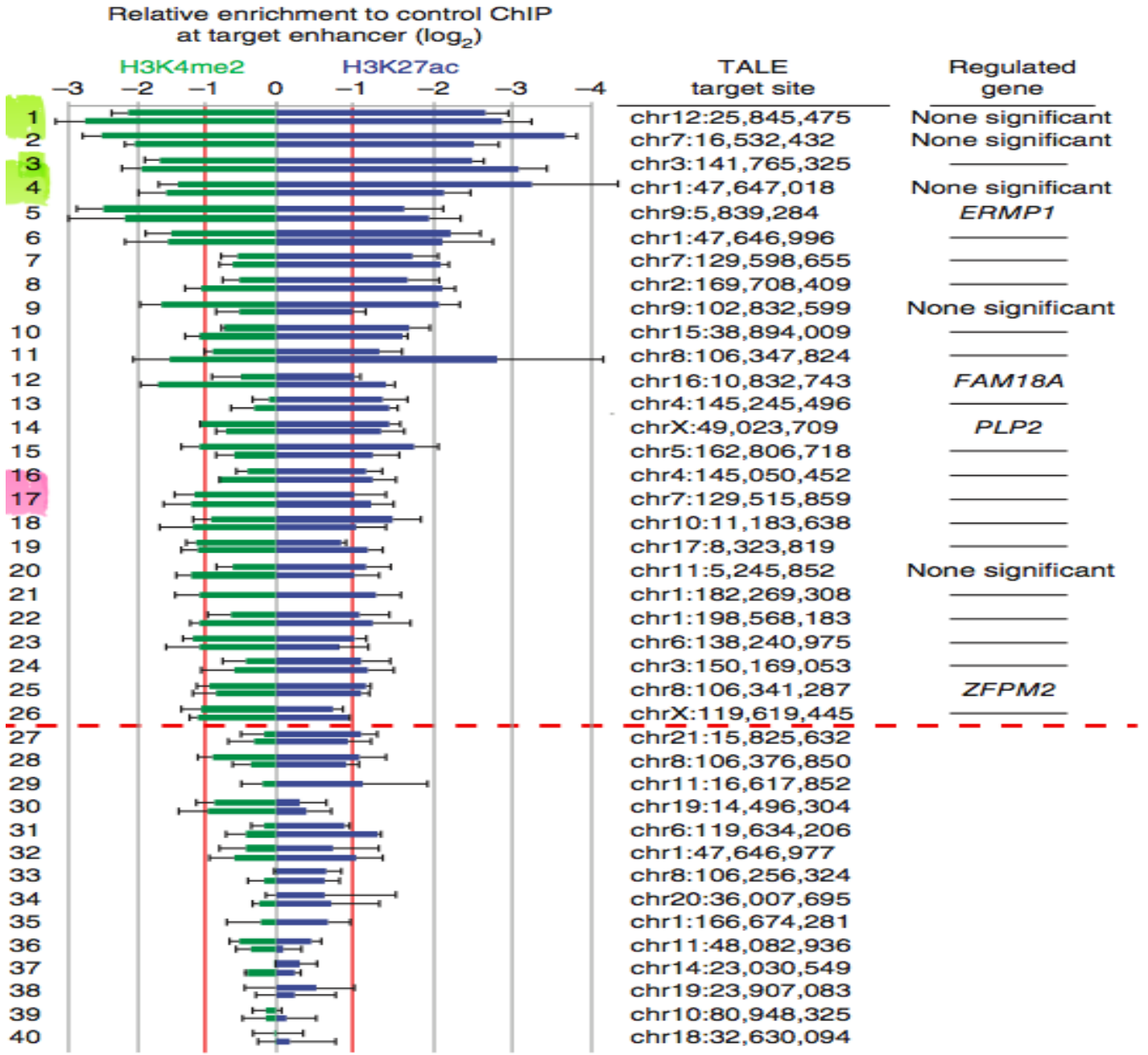
- 9 elements in developmental loci
- 16 additional highly cell typespecific elements
- 15 intergenic elements

The same workflow and conditions as in Experiment1.

Experiment2 results

- 26 of the 40 TALE- LSD1 constructs (65%) substantially reduced levels of these modifications at their target loci, relative to control-transfected cells
- 8 constructs caused more modest reductions

Experiment2 results



Influence on transcription

- 9 TALE-LSD1 fusions that robustly altered chromatin state (Fig. 2) and systematically screened for regulated genes using a modified RNA-seq procedure termed 3digital gene expression (3DGE).
- By only sequencing the 3ends of mRNAs, this procedure enables quantitative analysis of transcript levels at modest sequencing depths

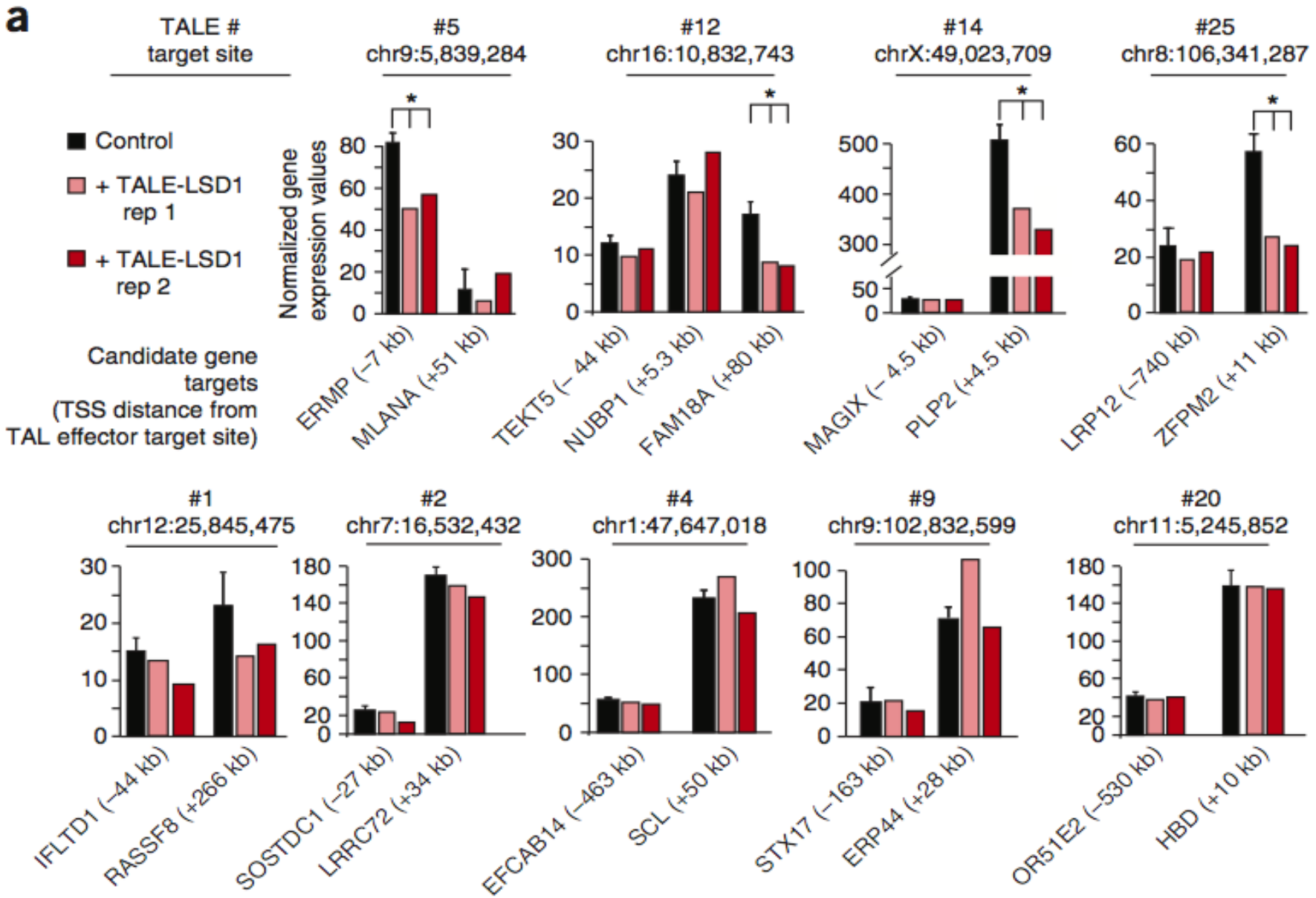
Experiment3

- Fusion construction
- Transfection
- 3days
- 3` DGE rna seq

Experiment3 results

- Normalization each 3DGE data set based on a negative binomial distribution and excluded any libraries that did not satisfy quality controls
- Four of the nine tested fusions (44%) caused a nearby gene to be downregulated by at least 1.5-fold, with both biological replicates
- The significance of these transcriptional changes was supported by a simulated analysis of a random sampling of 1,000 genomic locations that did not yield any false positives in which an adjacent gene scored as a regulated gene (false-discovery rate < 0.1%)
- The high prevalence of putative enhancers in the genome suggests that many act redundantly or function only in specific contexts, which could explain our inability to assign target genes to roughly half of the tested elements.

Experiment3 results



Summary

- These results indicate the potential of pro- grammable TALE-LSD1 fusions to shed light on complex regulatory interactions among multiple enhancers and genes in a locus.
- Study presents epigenome editing tools to modulate the activity of a poorly characterized class of functional genomic elements in their native contexts. The approach should also be useful for directing alterations of other epigenomic features, including repressive chromatin states and potentially with temporal control

Methods

T-TESTs

RNeasy Mini kit for 3` sequencing

3DGE analysis pipeline (M. Garber, University of Massachusetts Medical School, personal communication)

- Estimates gene expression based on the maximum number of reads in any 500-base-pair window within 10 kb of the annotated 3gene end.
- This approach compensates for the fact that annotated ends for some genes are imprecise and may be cell type dependent, and yields accurate quantifications.
- Normalized the gene expression levels, scaling samples by the median gene intersample variation. (DESeq)
- This approach controls for differences in sequencing depth between libraries and in the overall transcript abundance distribution. We excluded libraries with extreme normalization coefficients <0.7 or >1.5.

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