

2311 TALE-based epigenetic modulators show sustained knock-down of target genes in T-cells and HEPG2 via a high-throughput multiplex screening platform

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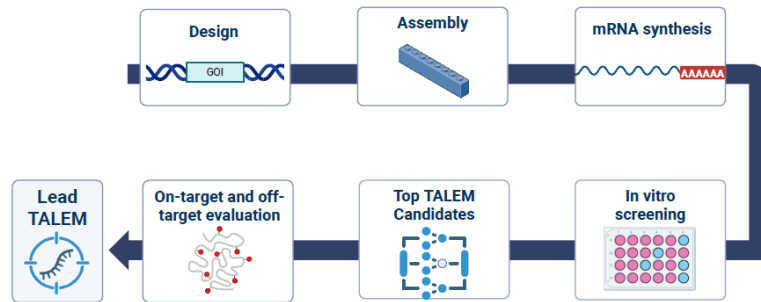
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Abstract

Transcription activator-like effector-based epigenetic modulators (TALEM) are engineered fusion proteins consisting of a TALE DNA-binding domain with catalytic domains that mediate epigenetic modifications. The recent advancement of epigenetic editors, which do not induce double-strand breaks or permanent alterations to the target DNA sequence, has provided a new avenue for safe genome editing. To overcome the limitations associated with individual TALE synthesis and testing, we developed a high-throughput strategy to synthesize and screen hundreds of TALEM constructs targeting a defined genomic locus. This approach allowed us to leverage the modular architecture and flexible targeting capacity of the TALE system to saturate gene regions of interest. By iterating this process, optimal individual or pairs of TALEM are deduced from the screening pools, to identify modulators that are both highly active and specific. Additionally, the genomic regions underlying these epigenetic changes could be precisely mapped, to indicate key regions that influence the desired gene expression outcome. In proof-of-concept studies, we applied this strategy to two distinct genes: one highly expressed in hepatocytes and another implicated in T-cell dysfunction and exhaustion. By probing the expression of the target gene or protein using various orthogonal assays, a robust (~10-fold) and stable knockdown was sustained throughout the experimental end points for both target genes. Collectively, these findings establish a multiplex screening framework that enables the identification of lead TALEM capable of inducing sustained epigenetic alterations, for precise and durable gene modulation.

#1 A high-throughput screening system for TALEM

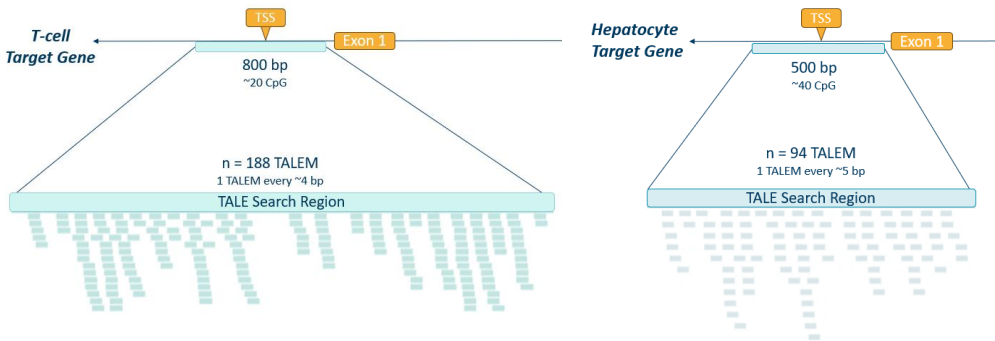
We developed a high-throughput screening platform to evaluate large and diverse collections of TALE-based epigenetic modulators (TALEM).



The workflow includes:

- A computational design phase, where TALE DNA-binding domains are generated and selected to generate hundreds of unique TALEM
- TALEM are assembled, synthesized, and co-transfected into a cell type of interest in a defined multiplexed pool
- Read-outs from the primary screen are used to identify top-performing multiplex groups based on their ability to modulate target gene expression.
- TALEM are de-multiplexed and re-combined to identify lead candidate TALEM with optimal activity and potential synergistic effects

#2 Multiplex screening allows for saturation of TALEM targeting sites



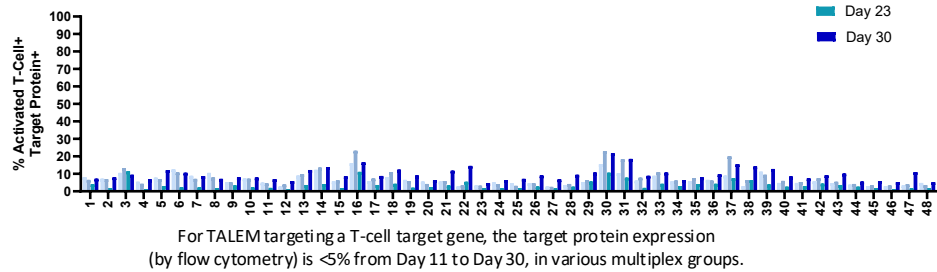
TALEM sites, which can be targeted to the positive or negative strand of a given locus and are not constrained by PAM sequences, are tiled around a defined region around the transcription start site (TSS). For the T-cell target gene, n = 188 target sites within a 800 bp region were selected. For the hepatocyte target gene, n = 94 target sites within a 500 bp region were selected. This results in a TALEM placed at approximately every 4 bp and 5 bp within each target locus, respectively.

#3 De-multiplexing of screening groups enables identification of top-performing TALEM

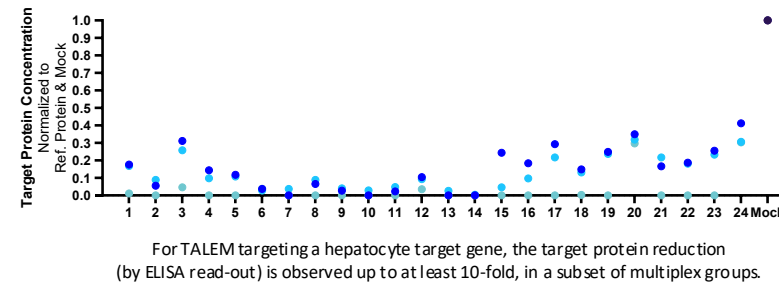
By selecting the top-performing multiplex groups from the primary screening process, individual lead candidate (LC) TALEM are defined. These TALEM can be transfected individually, or in new combinations, to identify which TALEM are most effective.

At the DNA level, methylation (up to ~90%) is observable at CpG sites that are unmethylated in Mock electroplated cells. By RT-PCR, knock-down is both observable and well-correlated with ELISA or flow cytometry, at the same timepoint. At the protein level, we show highly effective knock-down from the first sampling day through the corresponding experimental end-point.

T-cell target gene in primary T-cells



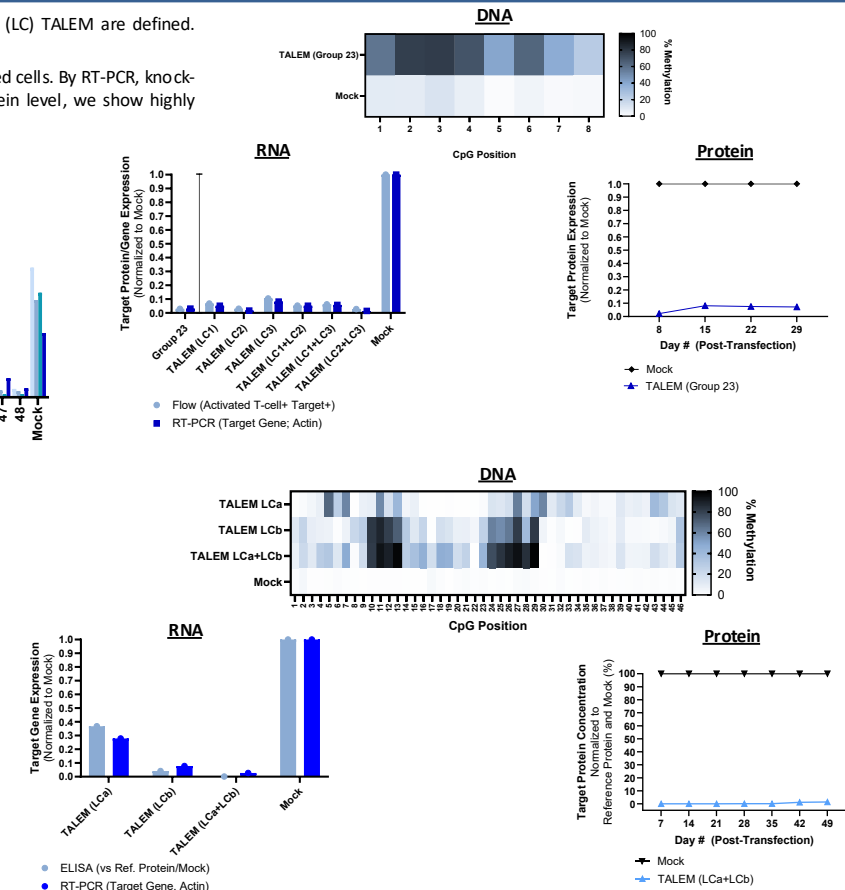
Hepatocyte target gene in liver-derived HEPG2 cells



#4 Conclusions

- We have established a scalable and high-throughput TALEM screening platform
- Our multiplex approach allows for saturation of target loci and detection of optimal target sites
- De-multiplexing allows for identification of top-performing TALEM and potential synergistic effects

Collectis' TALEM platform expands our toolbox of gene editors and generates new opportunities for T-cell and in-vivo gene engineering in future applications



- Efficient and stable knock-down can be achieved in primary T-cells and liver-derived HepG2 cells
- We demonstrate a stable ~10-fold reduction in the target protein for at least 29 days in T-cells
- Evidence of up to ~10-fold reduction in the target protein secretion was achieved for at least 49 days in HepG2 cells