

Mapping the functional impact of non-coding regulatory elements in primary T cells through single-cell CRISPR screens

1. Background & Motivation

- **Problem:** Many genetic variants linked to diseases (especially autoimmune and immune-related diseases) are found in **non-coding regions** of DNA-areas that don't directly code for proteins but often act as regulatory elements like enhancers or silencers.
- **Challenge:** It's hard to figure out which genes these non-coding elements control, especially in primary human cells (like T cells), which are more relevant to disease than cell lines.
- **Goal:** Develop a method to systematically map which non-coding regulatory elements control which genes in **primary human T cells**.

2. The Method: Primary T Cell crisprQTL

- **CRISPRi:** Uses a modified CRISPR system (CRISPR interference) that can “turn down” (but not cut) gene expression by targeting specific DNA sequences with a dead Cas9 (dCas9) fused to a repressor domain (ZIM3).
- **Targeting Regulatory Elements:** The researchers designed guide RNAs (gRNAs) to target both gene promoters (where transcription starts) and distant regulatory elements (like enhancers).
- **Single-Cell RNA-seq:** After introducing these gRNAs into primary CD4+ T cells, they used single-cell RNA sequencing (scRNA-seq) to measure the expression of all genes in each cell and identify which gRNA was present in each cell.
- **Pooled Screening:** By pooling many gRNAs and analyzing thousands of single cells, they could see the effect of silencing each regulatory element on gene expression, one cell at a time.

3. What Did They Actually Do?

- **Library Design:** Created a library of 355 gRNAs targeting 45 non-coding elements and 35 gene promoters, plus controls.
- **Transduction:** Introduced these into primary human CD4+ T cells expressing the CRISPRi machinery.
- **scRNA-seq:** Collected transcriptome data from about 250,000 single cells, each tagged with its gRNA identity.
- **Analysis:** Used custom computational pipelines to link changes in gene expression to specific regulatory element perturbations.

4. Key Findings

- **Efficient Silencing:** CRISPRi efficiently reduced activity at both promoters and distal regulatory elements in primary T cells.
- **Element-to-Gene Mapping:** They could map which regulatory elements control which genes (element-to-gene, or E2G, links), including both known and novel connections.
- **Effect Size:** The strength of the effect depended on how active the regulatory element was and how much the target gene was normally expressed.
- **Reproducibility:** Effects were robust-supported by multiple gRNAs per element.
- **Relevance to Disease:** Many of the elements studied overlapped with regions implicated in immune-related diseases by GWAS, showing the method's potential for disease research.

5. Why Is This Important?

- **Bridging the Variant-to-Function Gap:** This method helps connect non-coding genetic variants (found in GWAS) to their functional gene targets-a major challenge in human genetics.

- **Drug Discovery:** By pinpointing which genes are controlled by disease-associated regulatory elements, researchers can better prioritize drug targets.
- **Primary Cells:** Doing this in primary human T cells (not cell lines) makes the findings more relevant for understanding and treating human disease.

6. How to Dive Deeper (in 30 Minutes)

1. **Read the Abstract and Introduction:**
 - Get the big picture and motivation.
2. **Skim the Methods:**
 - Focus on how CRISPRi and scRNA-seq are combined in primary T cells.
3. **Look at Figures 1 and 2:**
 - These usually show the experimental workflow and main results.
4. **Read the Results and Discussion:**
 - See what they found and why it matters.
5. **Check the Conclusion:**
 - Understand the broader impact and future directions.

Figure 1: Establishing Efficient CRISPRi in Primary T Cells

- **Panel A:**
Schematic diagram showing the workflow for CRISPR interference (CRISPRi) in primary CD4⁺ T cells. T cells are activated, transduced with dCas9-repressor constructs, selected, and then transduced with guide RNAs (gRNAs) targeting specific genes or regulatory elements. This sets up the system for targeted gene repression.
- **Panel B:**
Flow cytometry scatterplot demonstrating that targeting the transcription start site (TSS) of the CD4 gene with CRISPRi leads to a ~75% reduction in CD4 protein expression 10 days after gRNA transduction, confirming effective knockdown.
- **Panel C/D/E:**
Comparison of different dCas9-repressor constructs and multiple gRNAs targeting various cell surface genes (CD4, CD81, BST2, ATP1B3). Flow cytometry histograms show reduced surface protein levels for each target, validating the efficiency and versatility of the method.
- **Panel F:**
Single-cell RNA-seq data (10X Genomics) showing that the reduction in gene expression at the RNA level mirrors the reduction seen at the protein level for the same targets, confirming that CRISPRi effects can be robustly detected by scRNA-seq.

Takeaway:

Figure 1 demonstrates the successful implementation and validation of efficient CRISPRi-mediated gene silencing in primary human CD4⁺ T cells, both at the protein and RNA level¹.

Figure 2: Design and Implementation of the crisprQTL Screen

- **Panel A:**
Schematic summarizing the types of genomic loci targeted in the screen:
 - CD2 locus control region (LCR) elements,
 - Enhancers previously linked to genes in K562 cells (“Gasparini enhancers”),
 - Candidate cis-regulatory elements (cCREs) from ENCODE (both intronic and intergenic),
 - Transcription start sites (TSSs) for technical controls.
- **Panel B:**
Schematic of the experimental workflow for the primary T cell crisprQTL screen:
T cells expressing ZIM3-dCas9 are transduced with a pooled gRNA library, then analyzed by single-cell RNA-seq to link perturbations to transcriptomic changes.

- **Panel C:**
Pie chart or bar plot showing the proportion of single cells that received one gRNA, multiple gRNAs, or none. This confirms that most cells receive only one perturbation, which is important for clean interpretation of results.
- **Panel D:**
Distribution plot of the number of cells recovered per gRNA, indicating good representation of each perturbation in the dataset.

Takeaway:
Figure 2 describes the design of the pooled CRISPRi screen and confirms that the experimental system allows robust, single-perturbation analysis in thousands of primary T cells¹.

Figure 3: Sensitivity and Specificity of crisprQTL for Element-to-Gene Mapping

- **Panel A:**
Bar graph showing that for the majority of significant element-to-gene (E2G) links, the effect is supported by at least two different gRNAs, demonstrating reproducibility.
- **Panel B:**
Scatterplot or violin plot illustrating that the magnitude of gene expression change upon perturbation depends on both the strength of the regulatory element and the baseline expression level of the target gene.
- **Panel C:**
Comparison of the number of significant E2G links detected for TSS perturbations versus non-coding element perturbations, showing higher sensitivity for TSSs (which have stronger effects).
- **Panel D:**
Plot showing that genes with higher baseline expression are more likely to yield statistically significant effects when their regulatory elements are perturbed.

Takeaway:
Figure 3 demonstrates that the crisprQTL platform can reliably detect regulatory element-to-gene connections, especially for genes with higher expression and for perturbations with strong effects, and that results are reproducible across multiple gRNAs¹.

Summary Table

Figure	Main Focus	Key Message
1	CRISPRi validation in primary T cells	Efficient, robust gene silencing detectable by flow cytometry and scRNA-seq
2	Screen design and single-cell assignment	Pooled, single-perturbation screen in primary T cells is feasible
3	Sensitivity and reproducibility of mapping	Reliable E2G mapping, especially for strong elements/high-expression genes

In summary:
Each figure builds on the last: Figure 1 validates the CRISPRi system in primary T cells; Figure 2 shows the design and execution of the pooled screen; Figure 3 demonstrates the platform’s power and limitations for mapping non-coding regulatory elements to their gene targets at single-cell resolution¹².