Perturb-seq analysis of TNFα-induced transcriptional response

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Abstract

We performed a single-cell Perturb-Seq screen using a 10X Genomics instrument on HEK293-Cas9 cells with an 84-sgRNA pooled library, aimed at identifying genes involved in TNFα-induced transcriptional response. As a reference, a parallel screen was performed with cells transduced in arrayed format with the same set of sgRNAs as the pooled library. The 10X Perturb-Seq screen was carried out in triplicate with increasing amounts of cells per 10X reaction, in order to determine (1) the minimum number of cells per sgRNA needed to cover the full complexity of a pooled library, and (2) the maximum number of cells/10X reaction before single-cell resolution degrades because of cell doublet encapsulation.

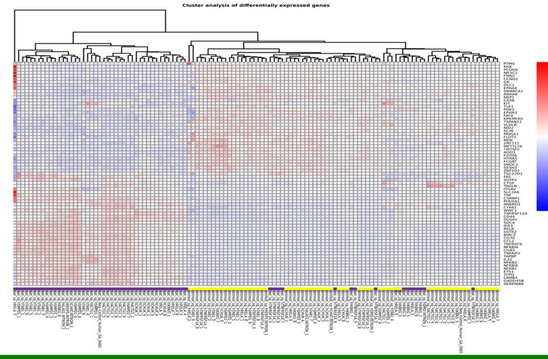
For the Perturb-Seq screen, cells were transduced with the pooled sgRNA library, puromycin selected and expanded for 10 days, then split into 2 samples for 72h TNFα treatment/mock-treatment. After treatment, cell samples were loaded onto the 10X Genomics instrument according to manufacturer's specifications at 2,000, 7,500 and 15,000 cells/reaction.

For the arrayed screen, cells were transduced with individual sgRNAs, puromycin selected and expanded for 10 days, then split into 2 samples for 72h TNFα treatment/mock-treatment. Genome-wide transcriptome analysis was performed using the DriverMap™ Human Genome-Wide Gene Expression Profiling Assay for the arrayed screen. For the Perturb-Seq screen, 10X Genomics Cell Ranger software was used. Both screens identified TNFRSF1A, IKBKG, CHUK and RELA as mediators of TFNα transcriptional response. Increasing the number of cells/10X reaction from 2,000 to 7,500 and 15,000 showed a positive effect on sgRNA library coverage (more sgRNAs were detected).

Arrayed CRISPR-sgRNA screen

Fig. 1

Arrayed CRISPR-sgRNA screen. HEK293-Cas9 cells transduced with individual sgRNA constructs (60 sgRNAs), followed by TNFα treatment/mock-treatment. DriverMap genome-wide transcriptional profiling identified TNFRSF1A, IKBKG, RELA, CHUK as key mediators of TNFα transcriptional response.



Purple: TNFα-treated cells
Yellow: mock-treated cells

TNFRSF1A
IKBKG
CHUK
RELA

Perturb-Seq, pooled CRISPR-sgRNA library screen

Perturb-Seq, pooled CRISPR-sgRNA library screen. HEK293-Cas9 cells transduced with pooled sgRNA library (84 sgRNAs), followed by TNFα treatment/mock-treatment. Single-cell partitioning was performed on (a) 2000 cells/10X reaction (2 reactions), (b) 7,500 cells/10X reaction, (c) 15,000 cells/10X reaction. Similarly to the arrayed screen, the Perturb-Seq screen identified TNFRSF1A, IKBKG, RELA, CHUK as key mediators of TNFα transcriptional response. Higher numbers of loaded cells/10X reaction increased the number of detected sgRNAs.

Fig. 2a

2,000 cells/10X reaction

2-10X reactions

4,000 cells total

1- Illumina NextSeq 500 (NS500) flow cell

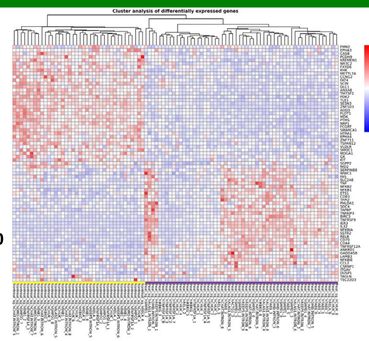


Fig. 2b

7,500 cells/10X reaction

1-10X reaction

7,500 cells total

1/2 NS500 flow cell

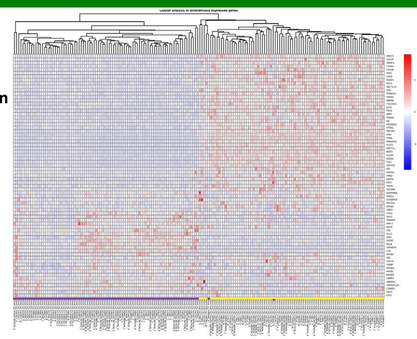


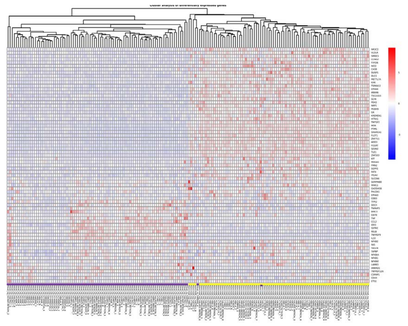
Fig. 2c

15,000 cells/10X reaction

1-10X reaction

15,000 cells total

1/2 NS500 flow cell



Purple: TNFα-treated cells
Yellow: mock-treated cells

TNFRSF1A
IKBKG
CHUK
RELA

Effects of gene knockout on TNFα-induced transcriptional activation of IL32 and RELB genes

Effects of gene knockout on TNFα-induced transcriptional activation of IL32. IL32 and RELB were identified as top transcriptionally activated genes upon TNFα treatment in parental HEK293 cells (data not shown) and control-sgRNA expressing HEK293-Cas9 cells in both the arrayed and the pooled library Perturb-Seq screens. We therefore used the detected activation-fold of these 2 genes to measure gene-knockout perturbation of TNFα transcriptional response. (a) TNFα-induced transcriptional activation of IL32. (b) TNFα-induced transcriptional activation of RELB.

Fig. 3a TNFα-induced transcriptional activation of IL32

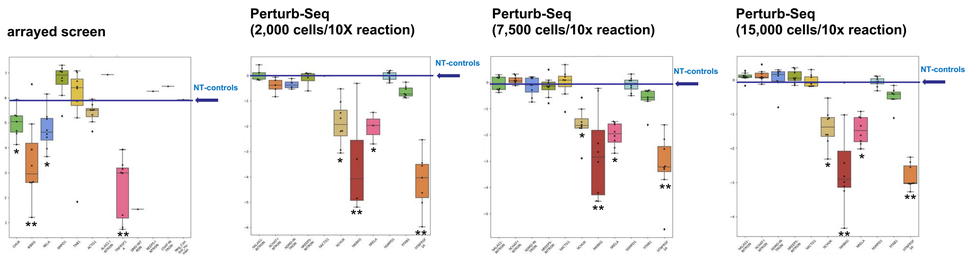
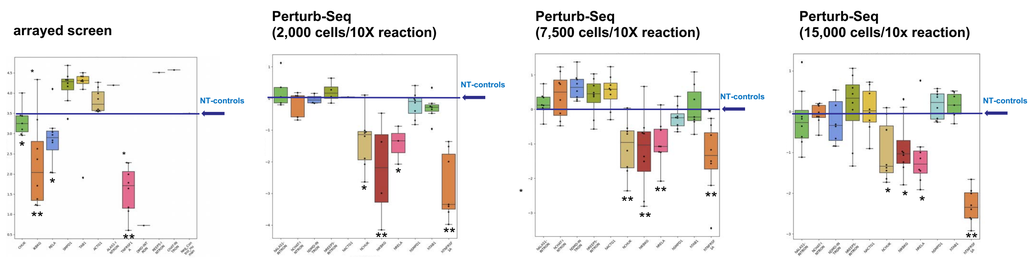


Fig. 3b TNFα-induced transcriptional activation of RELB



Conclusion

The 84 sgRNA library 10X Perturb-Seq screens were carried out at 2,000 cells, 7,500 cells, 15,000 cells/10X reaction, corresponding to 23 cells/sgRNA, 90 cells/sgRNA, 180 cells/sgRNA ratios, respectively. The 23 cells/sgRNA ratio appeared to be too low, while 90 cells/sgRNA turned out to be adequate to cover the full complexity of the library. At the same time, raising the number of cells/10X reaction from 2000 to 7,500 and 15,000 appeared to reduce the magnitude of the measured effect of TNFRSF1A, IKBKG, RELA, CHUK knockout on TNFα mediated transcriptional activation. Because the NGS sequencing depth of the 7,500/10X and 15,000/10X samples was ~4-fold and ~8-fold lower than the 2,000/10X sample, it is still unclear at this point if the reduced Perturb-Seq sensitivity was due to a decreased efficiency of single-cell partitioning in the 10X reaction, or to the lower NGS sequencing depth. To discriminate between the two possibilities, the 7,500/10X and 15,000/10X samples will be sequenced again to match the NGS sequencing depth of the 2,000/10X sample.