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Q1 2026

Large-Scale CROP-Seq Datasets Available at Myllia Biotechnology

www.myllia.com

Datasets available for licensing at Myllia

1. Genome-wide CRISPRi CROP-Seq screen in THP-1 cells

Genome-wide sgRNA library
Targeted sequencing panel of 357 mRNAs

2. Genome-wide CRISPRi CROP-Seq screen in Jurkat cells

Genome-wide sgRNA library
Targeted sequencing panel of 374 mRNA

3. CRISPRn CROP-Seq screen in primary human pan-T cells

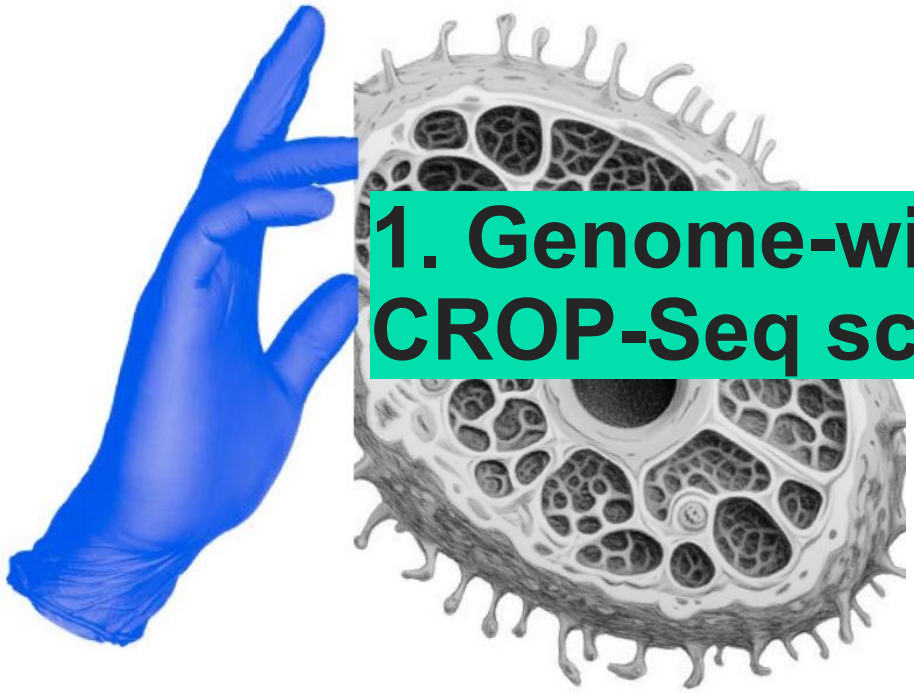
Custom sgRNA library targeting 42 genes
Targeted sequencing panel of 300 mRNAs

4. CRISPRn CROP-Seq screen in primary human $CD4^+$ Th2 cells

Custom sgRNA library targeting 102 genes
Targeted sequencing panel of 300 mRNAs

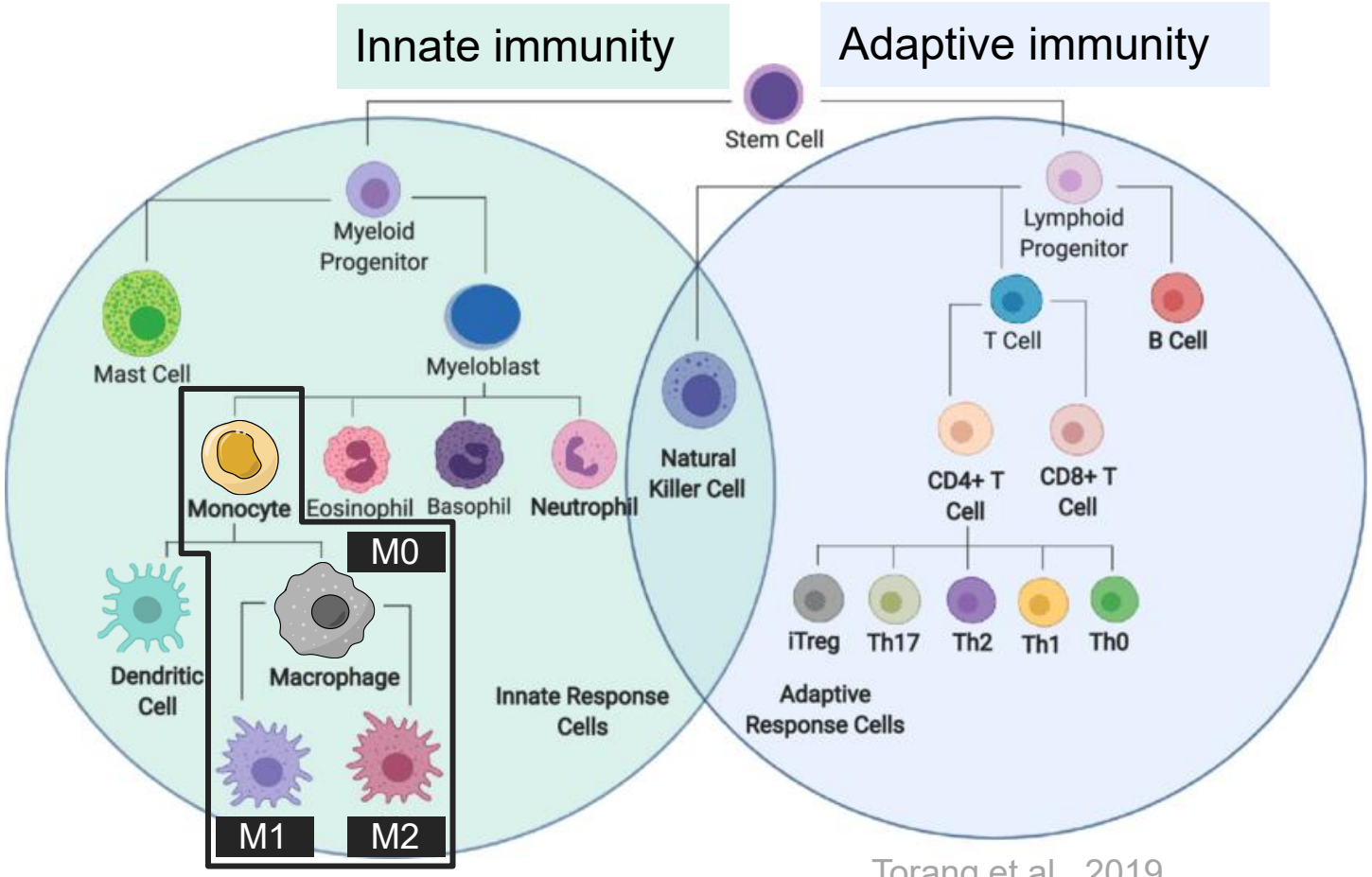
5. Comparative CROP-Seq screen in 8 human cell lines

Custom sgRNA library targeting 218 genes
Whole-transcriptome analysis (WTA)

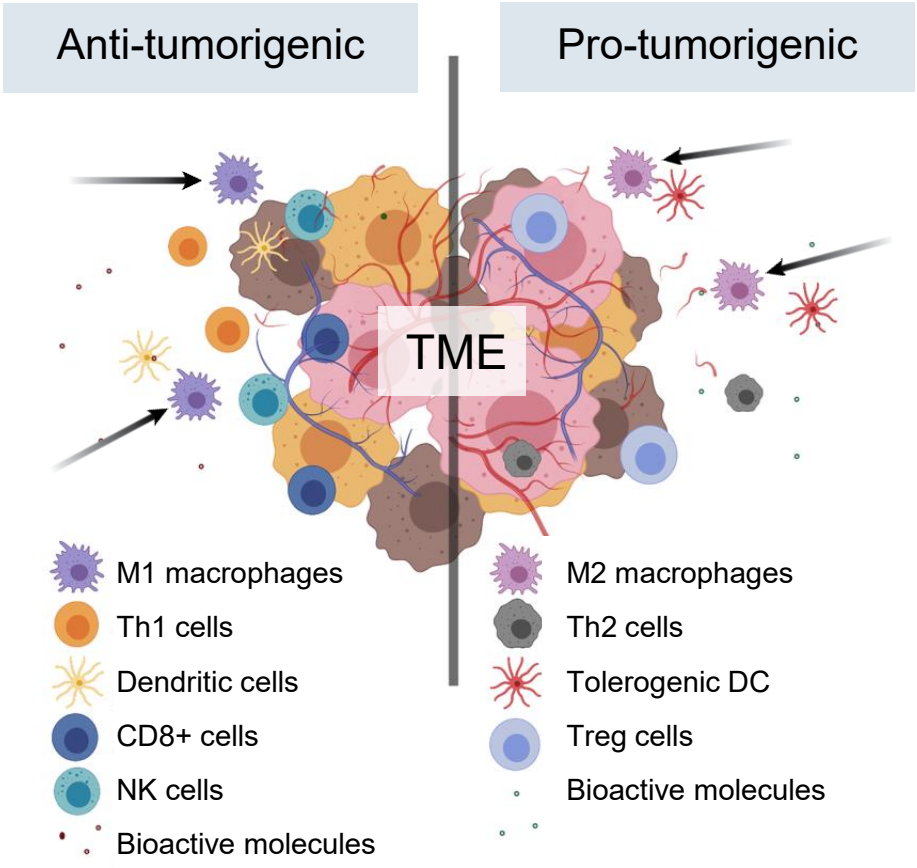


1. Genome-wide CRISPRi CROP-Seq screen in THP-1 cells

Macrophages are key players of the innate immune system

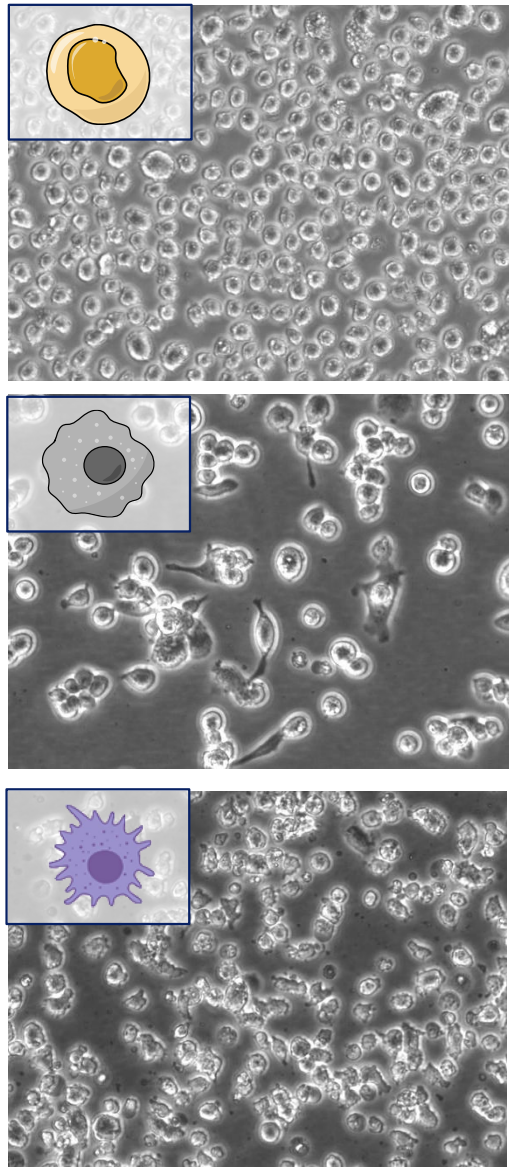


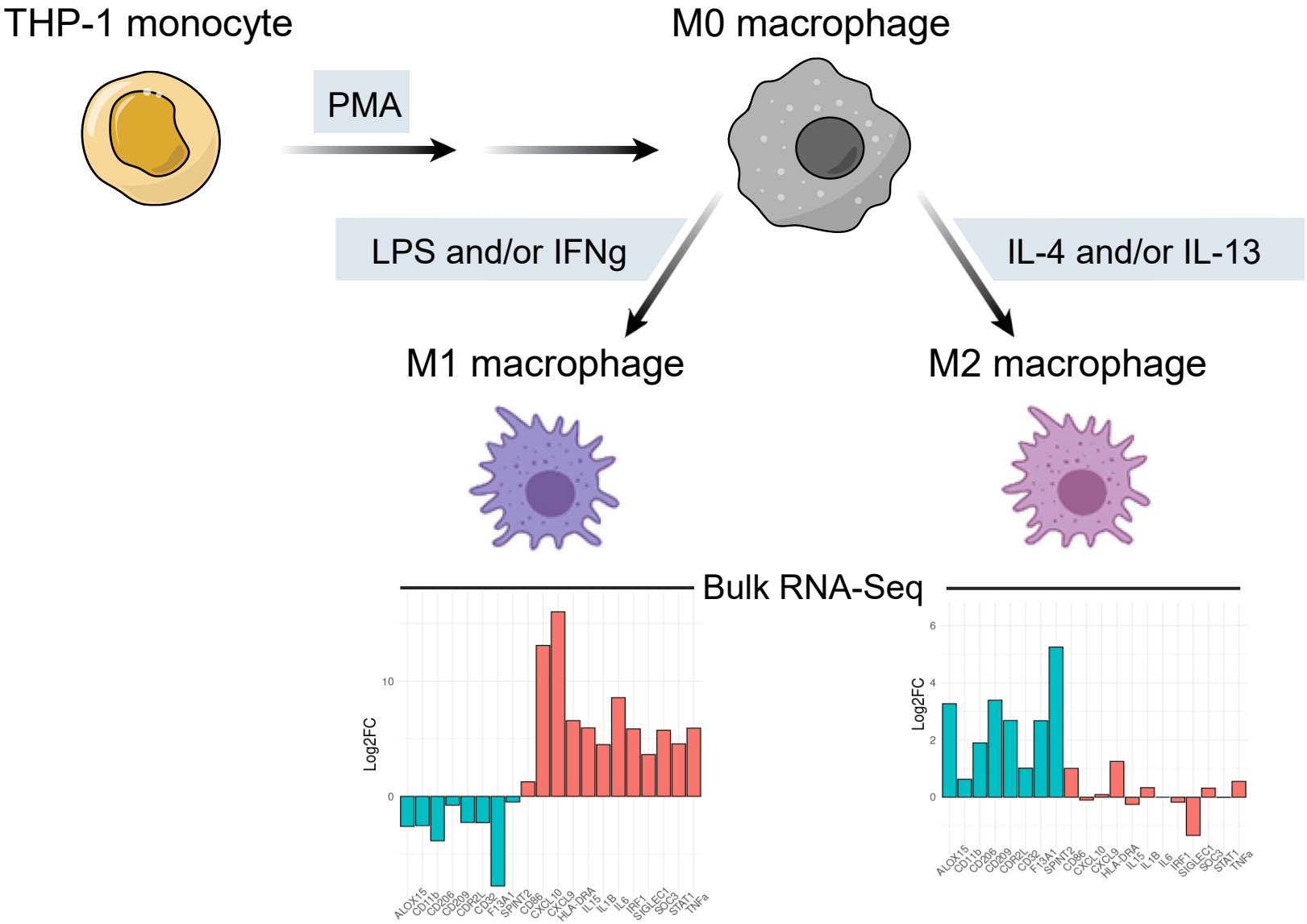
Torang et al., 2019



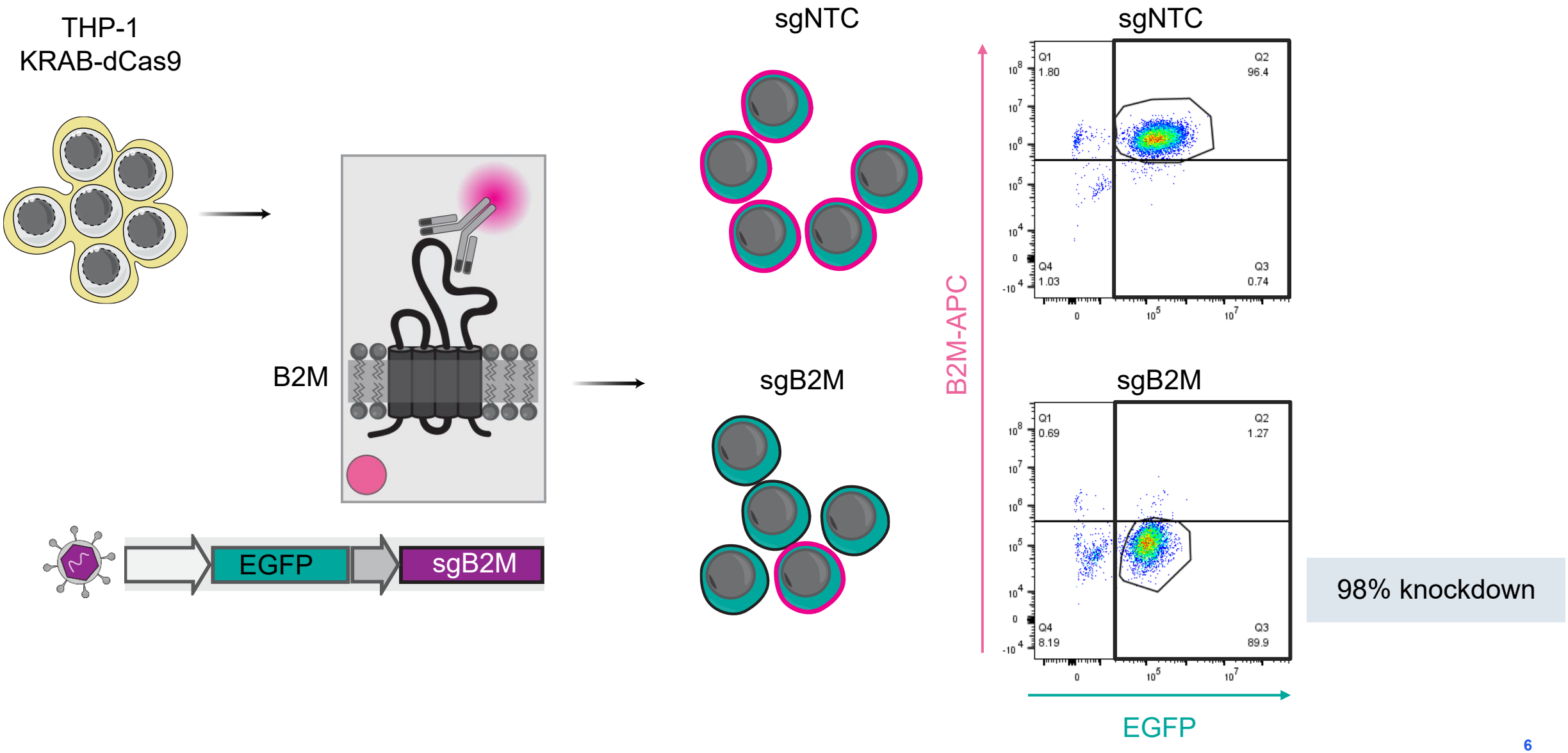
Hourani et al., 2019

Bulk RNA sequencing establishes transcriptomic signature for M1/ M2



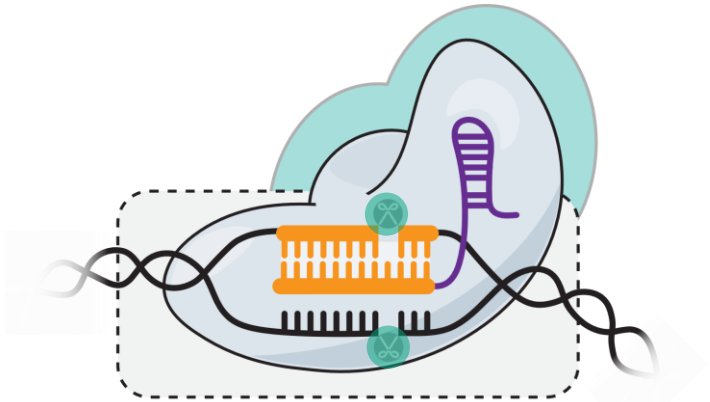


Validation of CRISPRi activity in engineered THP-1 cells



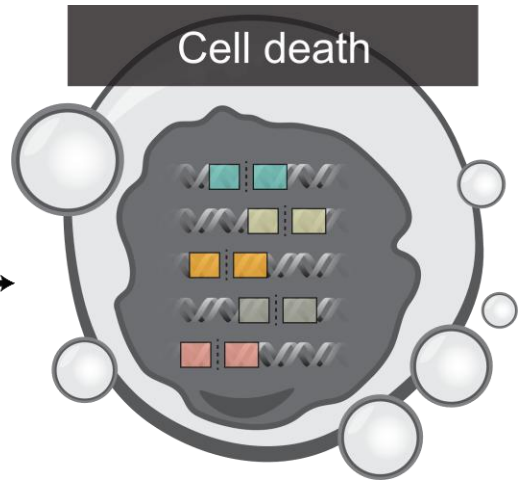
98% knockdown

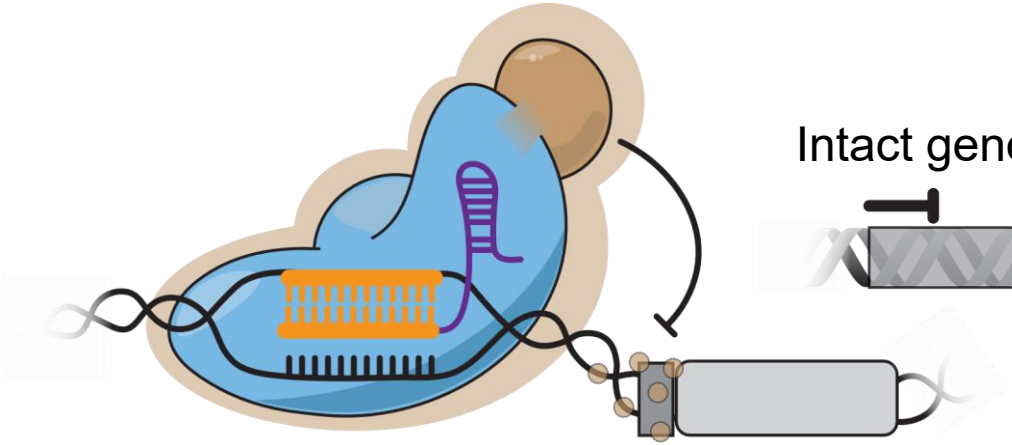
CRISPR interference allows the delivery of multiple sgRNAs per cell



Indel formation
Gene knockout

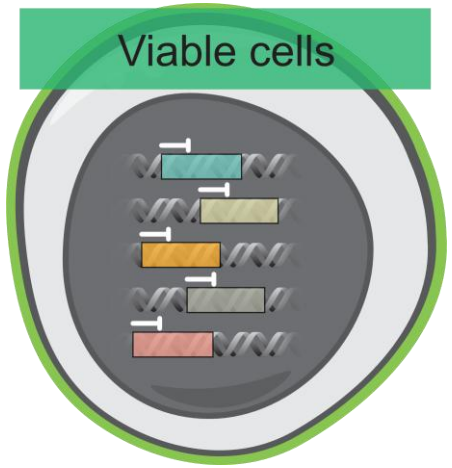
Multiple DSBs





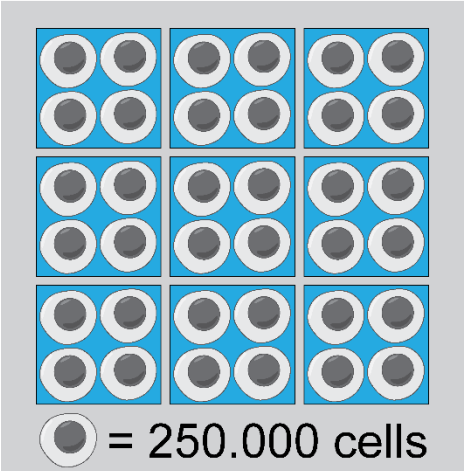
Repression
Gene knockdown

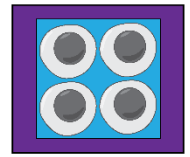
Intact genome



Compressed screens

9 million cells





1 million cells

Overview of the experimental steps

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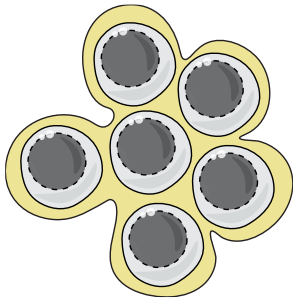
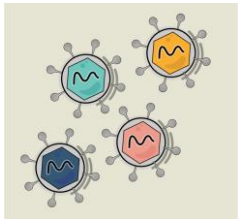
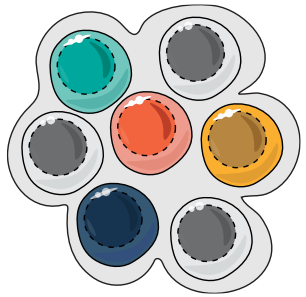
THP-1
KRAB-dCas9



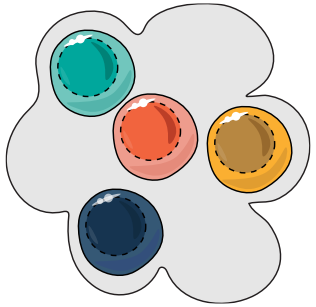
Genome-wide pooled
sgRNA library



Lentiviral transduction
(High MOI)



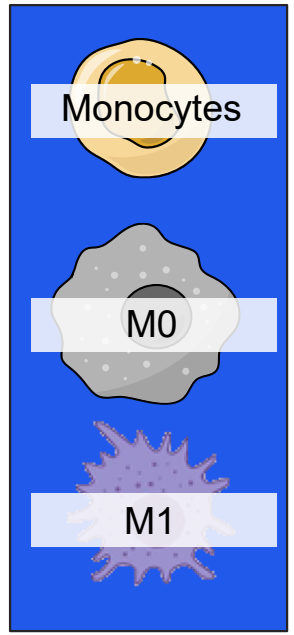
Puromycin
selection



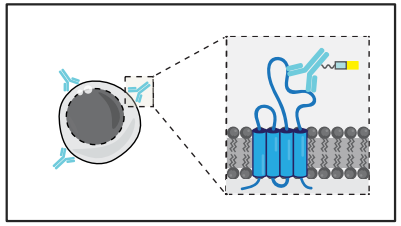
1 Monocytes 5%

2 PMA → M0 5%

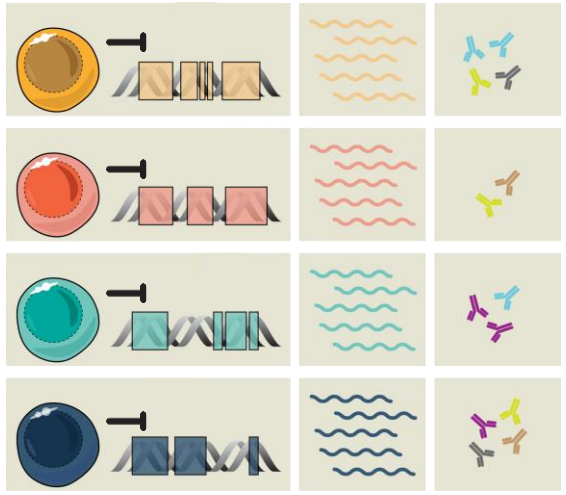
3 PMA → LPS / IFNg → M1 90%



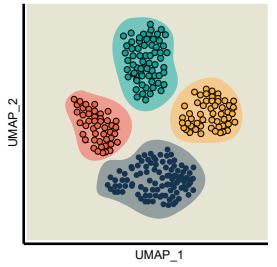
CITE-Seq



Single-cell capture
BD Rhapsody-HT Xpress



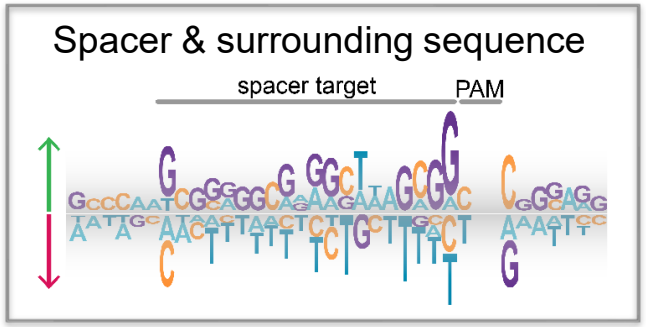
RNA Protein

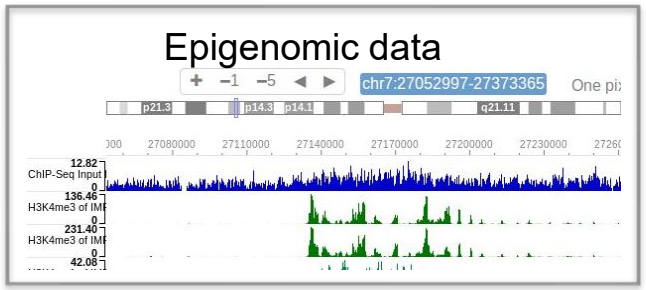


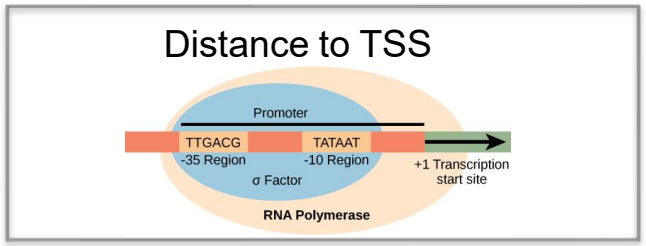
Bioinformatics

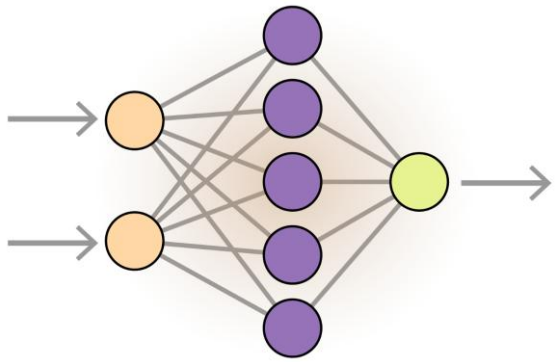
Myllia's proprietary genome-wide CRISPRi sgRNA library

- Convolutional neural network model
- Trained on ~31.000 sgRNAs
- Tested on ~4.100 sgRNAs

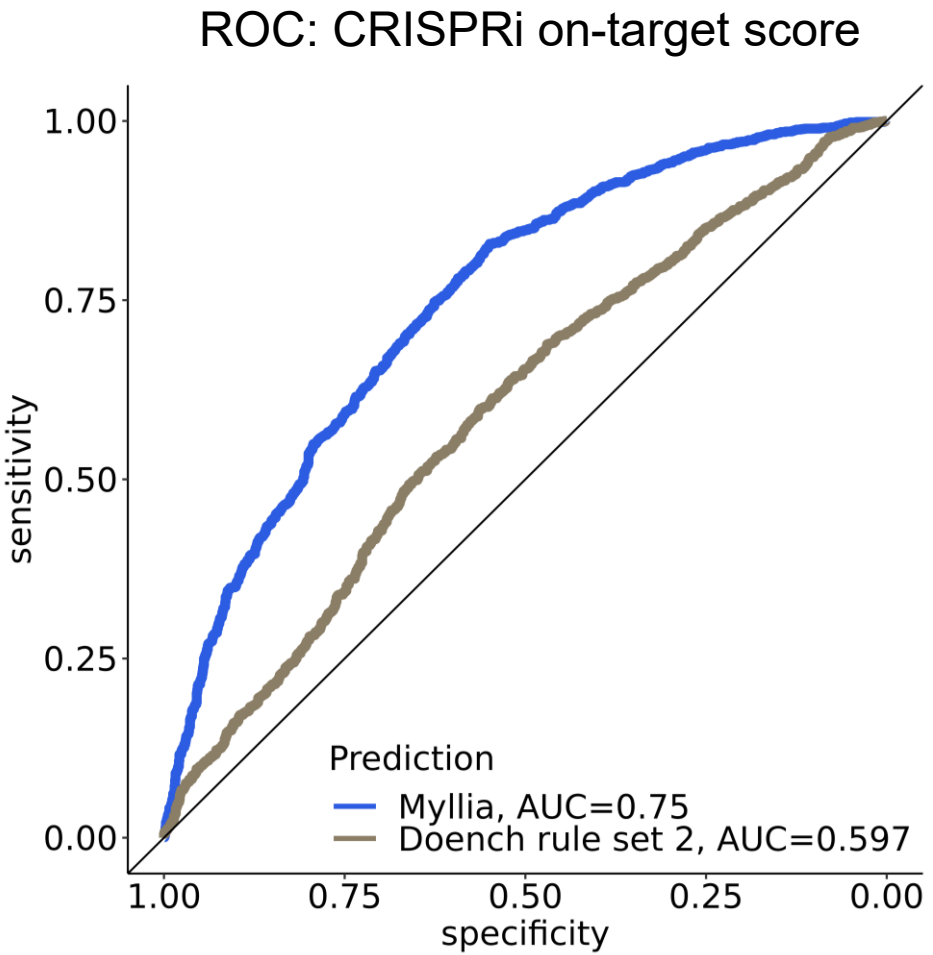






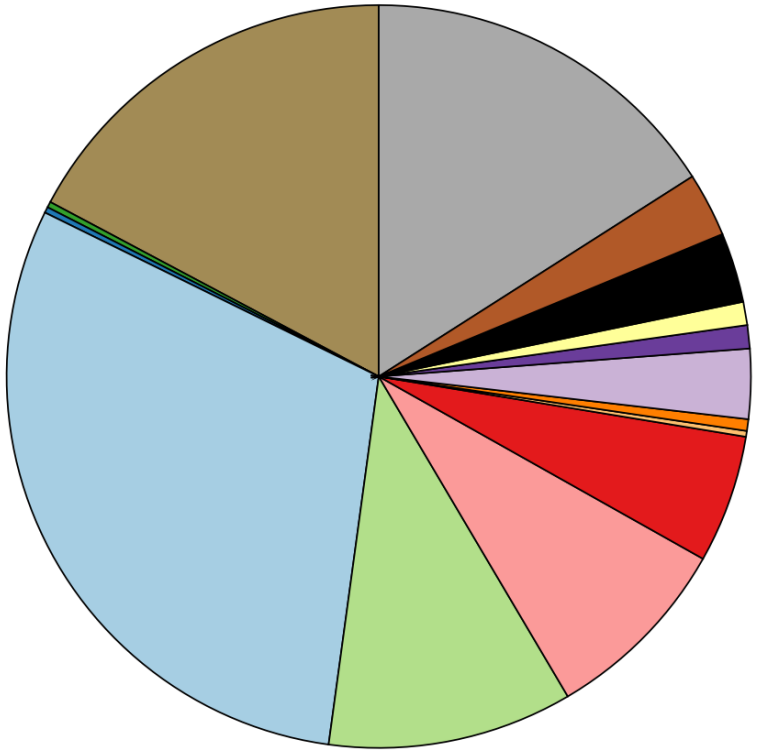


... and other features



Design of the primer panel for targeted RNA-Seq

Composed of 357 marker genes



- Upregulated from bulk RNA-Seq
- Downregulated from bulk RNA-Seq
- CITE-seq panel
- M1 markers derived from literature
- M2 markers derived from literature
- Monocyte markers
- Cholesterol homeostasis
- Glycolysis
- Pentose phosphate pathway
- TCA cycle
- Key transcription factors
- Cell cycle
- Miscellaneous

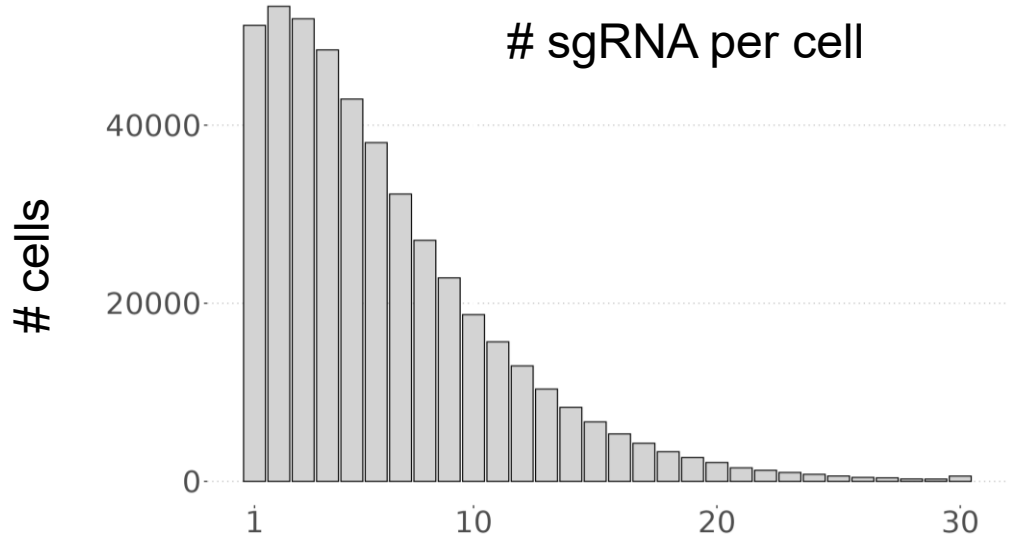
Quality control of the screen and sequencing metrics

BD Rhapsody-HT Xpress

Targeted NGS library preparation and NGS

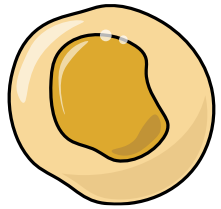
# of loaded cells on BD Rhapsody-HT	1.674.112
# of cells retrieved	936.449
# of cells with ≥1 sgRNA called	465.828
# of sgRNAs per cell (median)	5
# of cells per knockdown (median)	160
NovaSeq X (1/2)	12 billion reads
# of reads/cell	9.023
# of UMIs/cell (mean)	1.687

THP-1 M1 cells

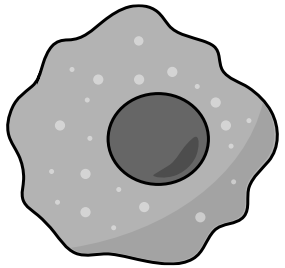


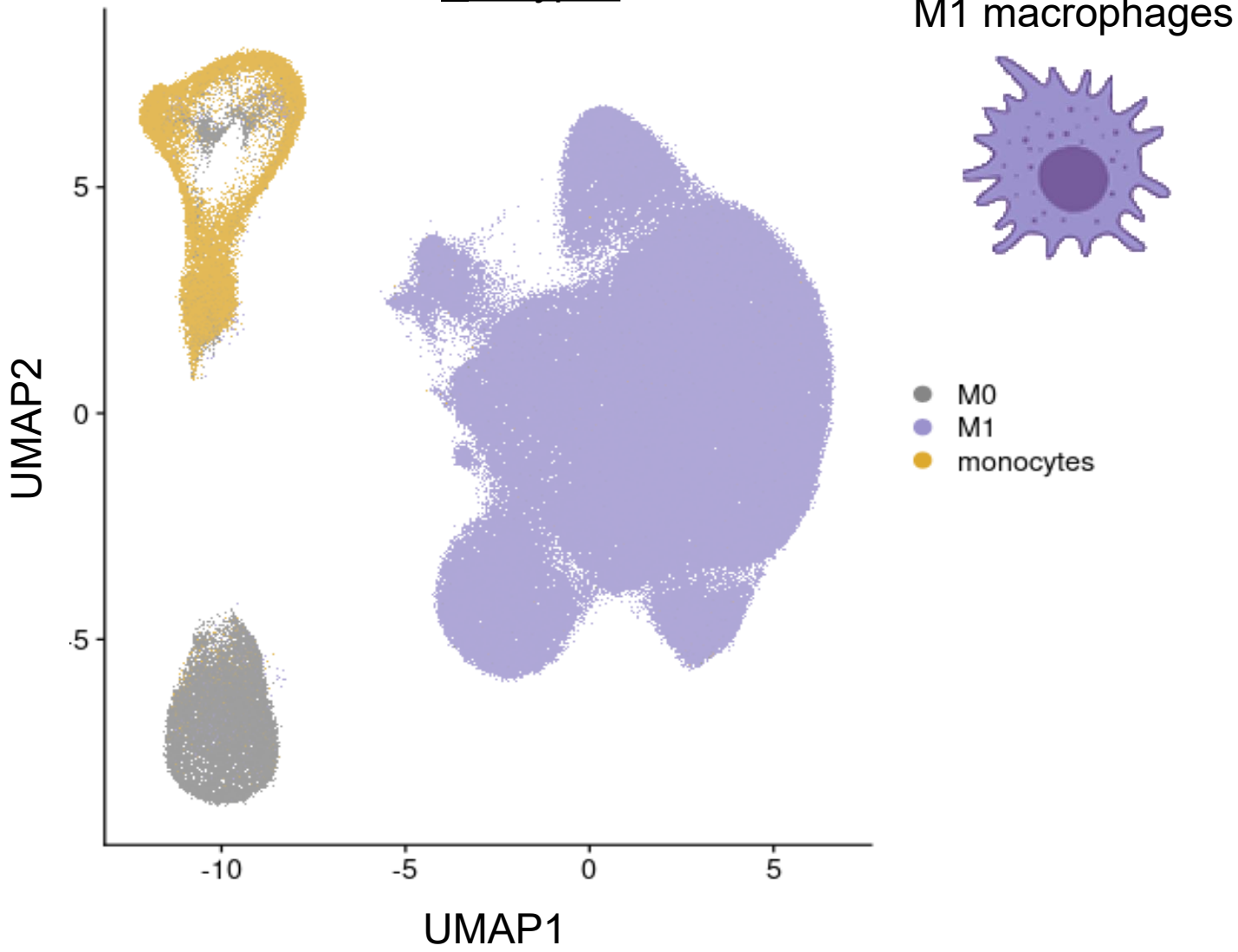
Different cell types can be separated in the UMAP

Monocytes



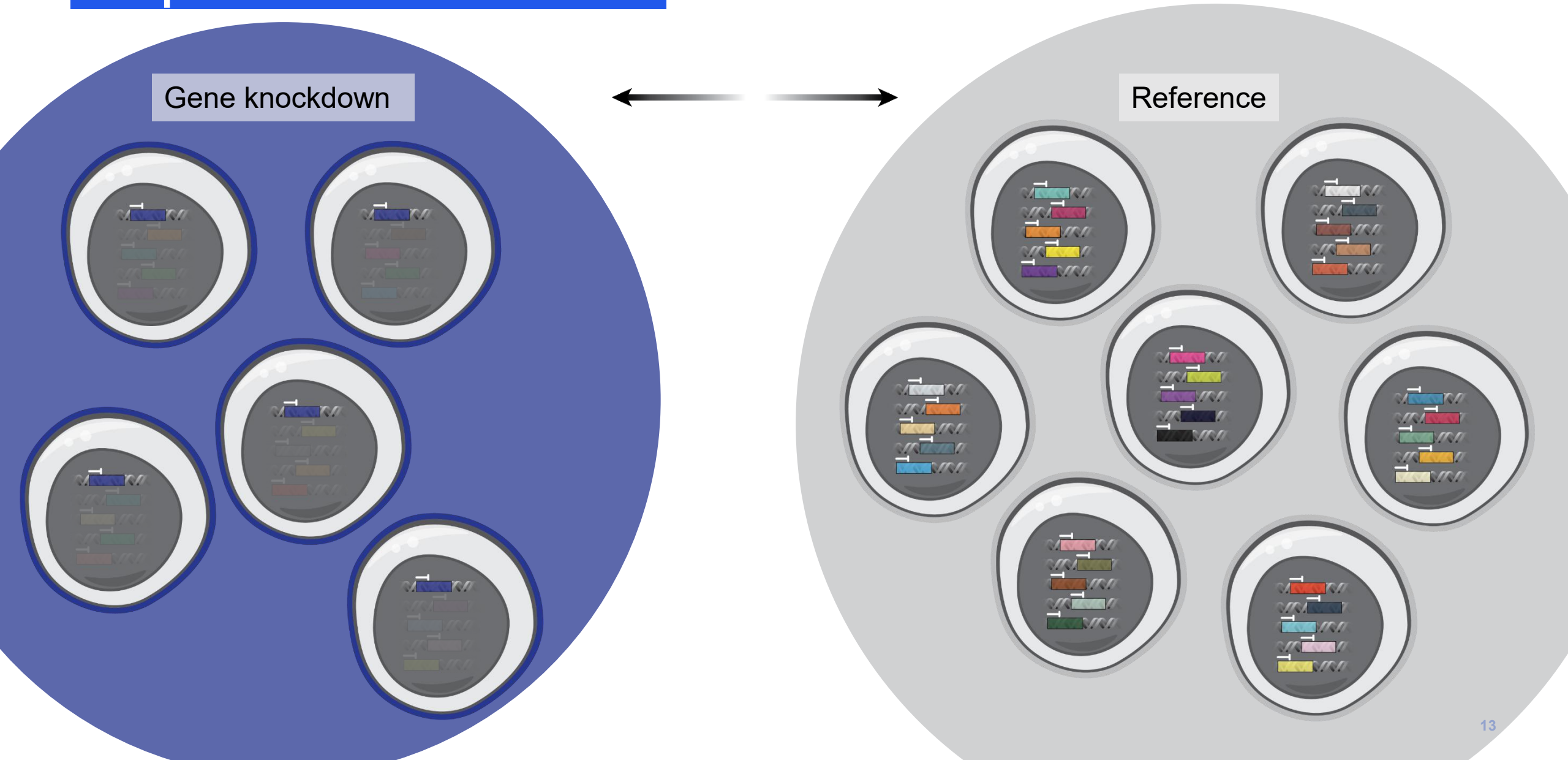
M0 macrophages



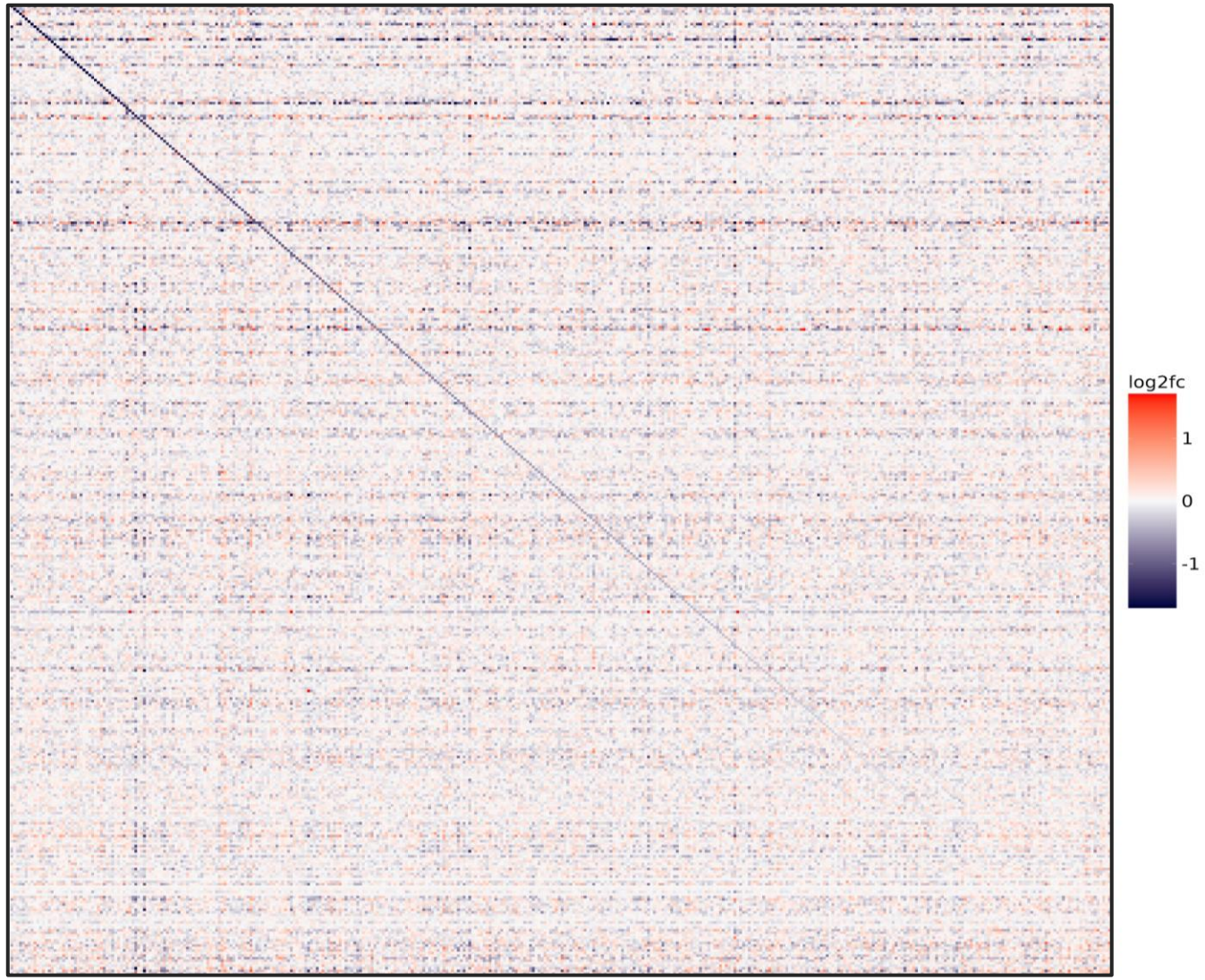


M1 macrophages

Analysis of differentially expressed genes in a compressed CRISPR screen



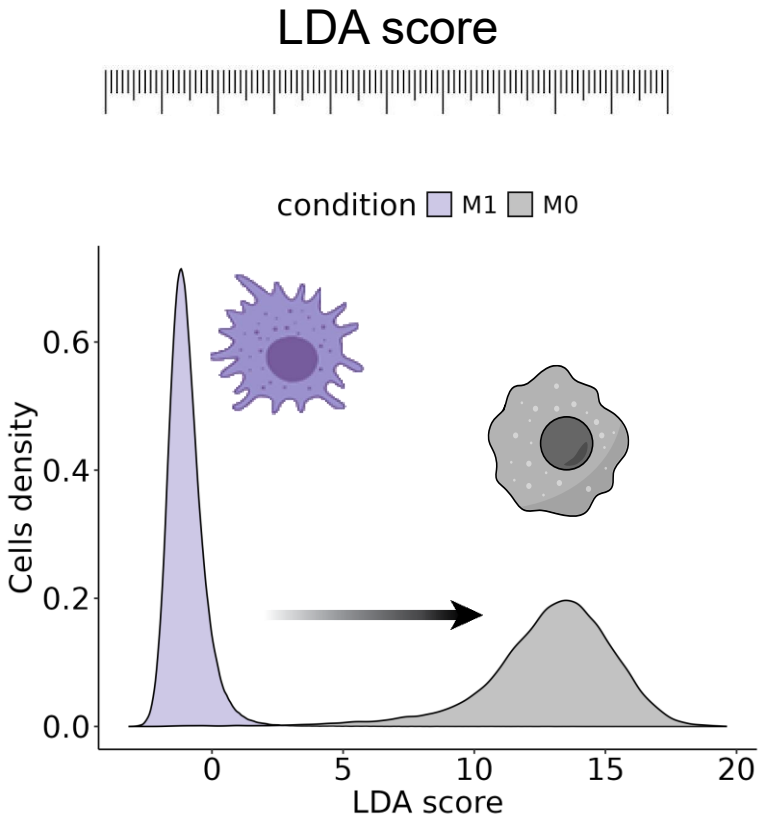
Strong downregulation of most genes from the target panel

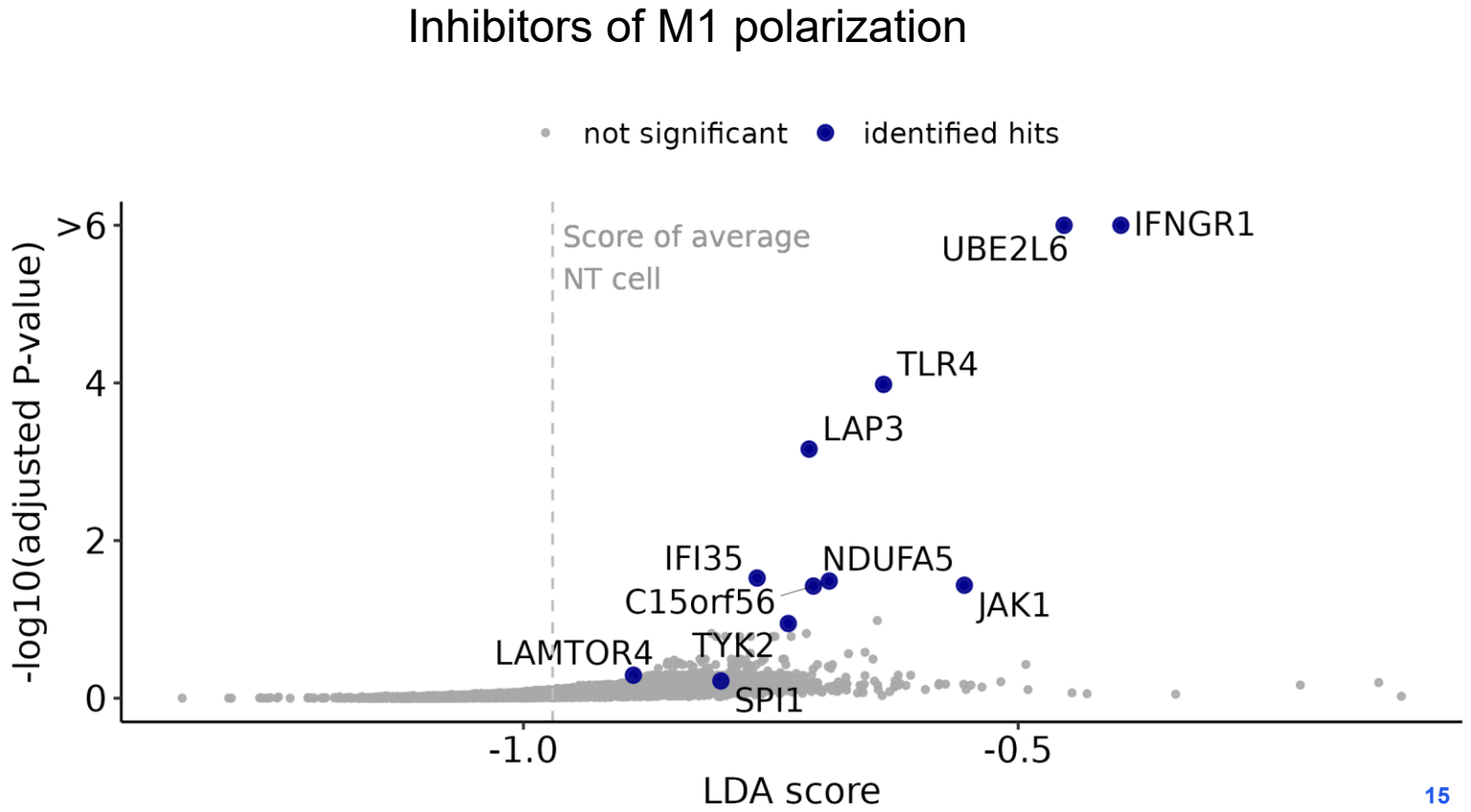


- **238/357 genes** significantly downregulated (p-value < 0.05)
- **53/119 genes** where CRISPRi KD is not significant have TPM<5

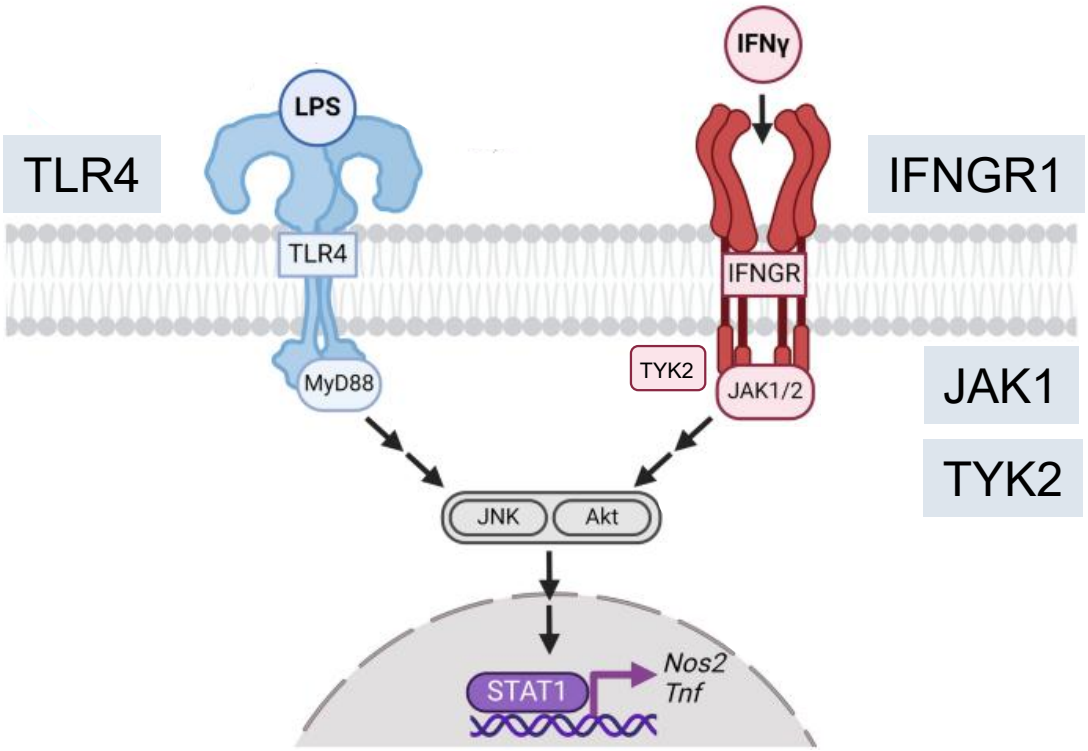
Linear Discriminant Analysis – a measure of phenotype intensity

- Are there any knockdowns in M1 macrophages pushing cells towards M0, i.e., inhibiting M1 polarization?
- LDA (***L**inear **D**iscriminant **A**nalysis*) approach: find best separation between M1 and M0 cells, calculate "LDA score" for each cell and then find knockdowns that move more towards M0





Analysis of genes that impair M1 polarization



TLR4

IFNGR1

JAK1

TYK2

UBE2L6

Research Article

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Ube2L6 Promotes M1 Macrophage Polarization in High-Fat Diet-Fed Obese Mice via ISGylation of STAT1 to Trigger STAT1 Activation

LAMTOR4

FEBS Letters

FEBS PRESS
science publishing by scientists

LAMTOR/Ragulator regulates lipid metabolism in macrophages and foam cell differentiation

SPI1

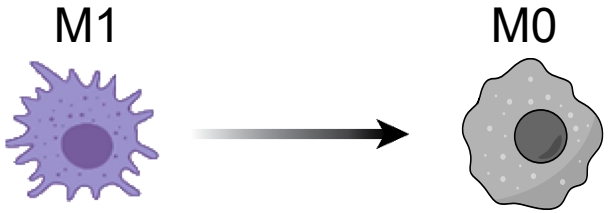
blood®

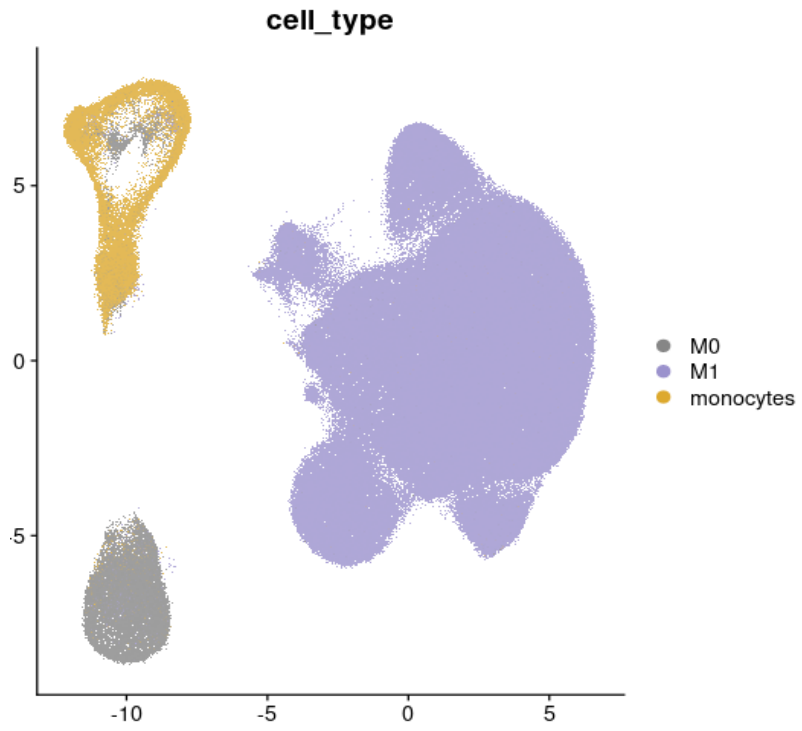
Volume 116, Issue 3, 22 July 2010, Pages e1-e11

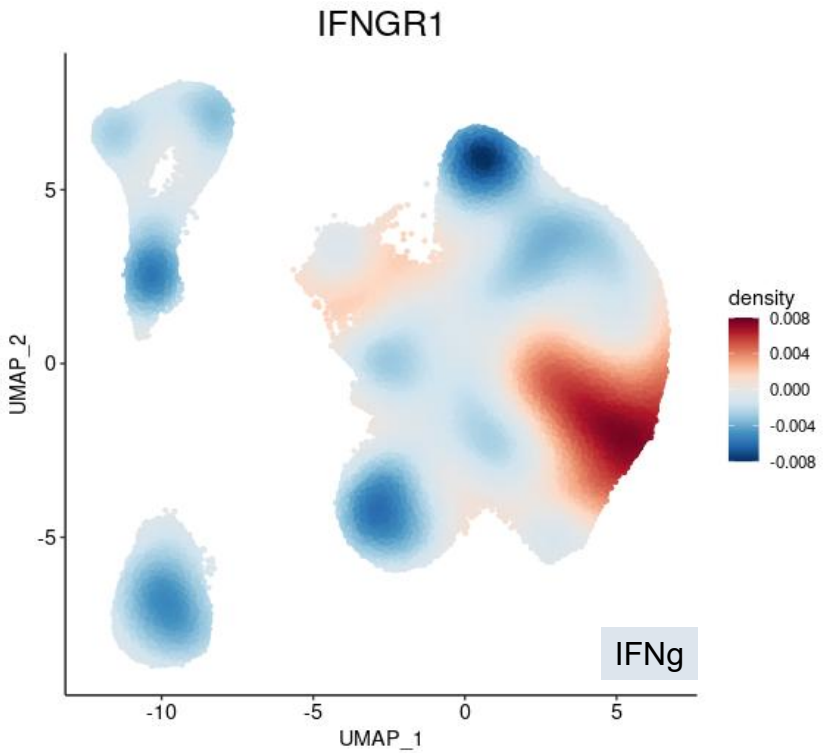
E-Blood

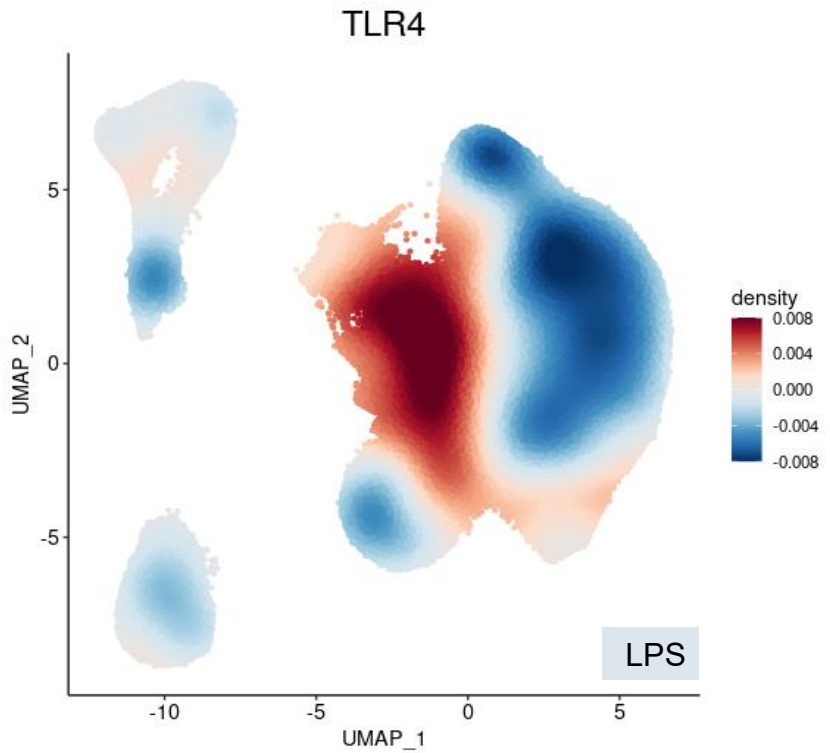
Macrophage-specific gene functions in Spi1-directed innate immunity

UMAP for selected CRISPR perturbations affecting M1 polarization



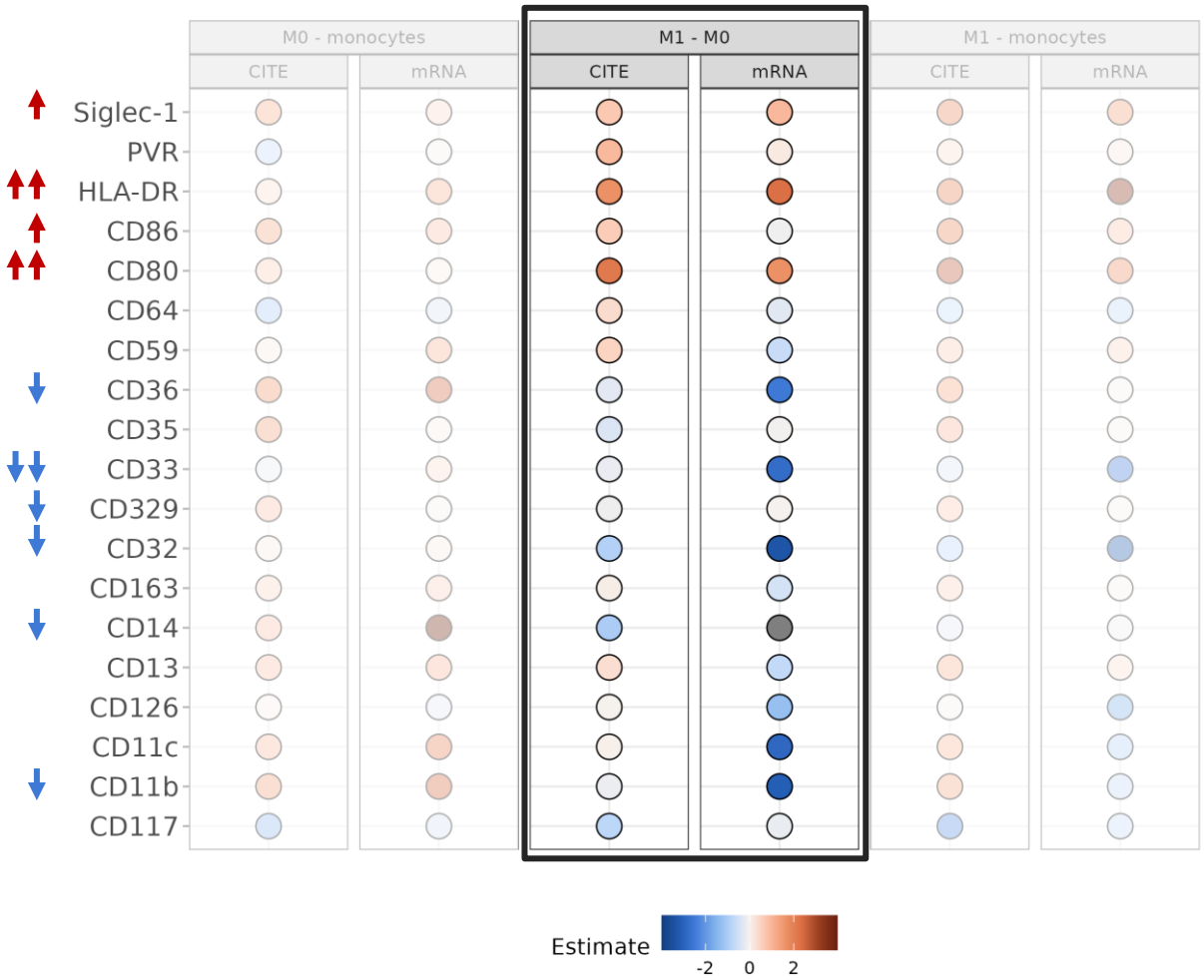






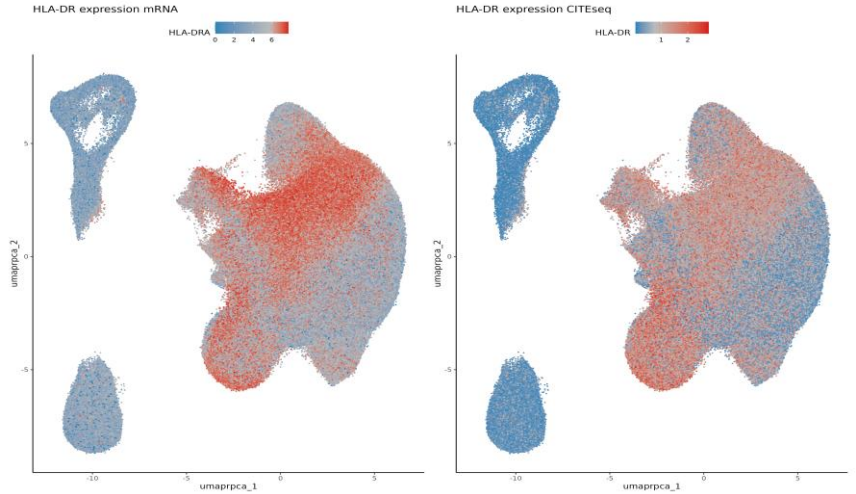
Comparison of RNA and protein markers (CITE-Seq) allows separation of M1/M0

Macrophages orchestrate immune responses to microenvironmental stimuli sensed by a complex set of surface receptors.

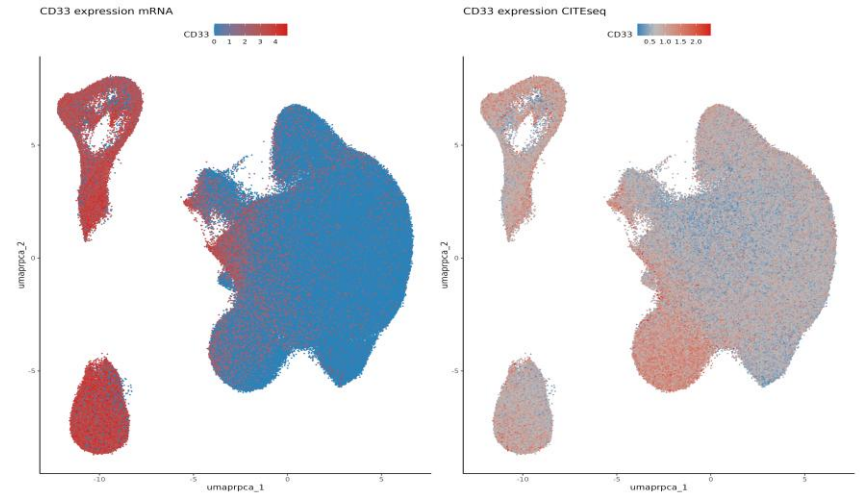


mRNA

Protein

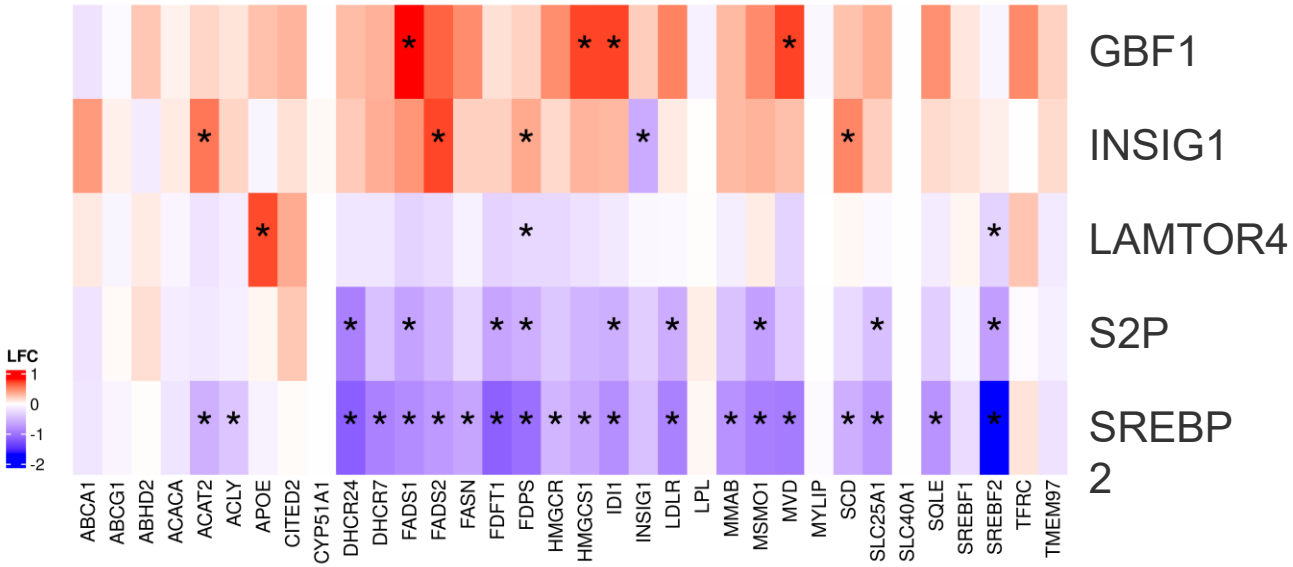


HLA-DR

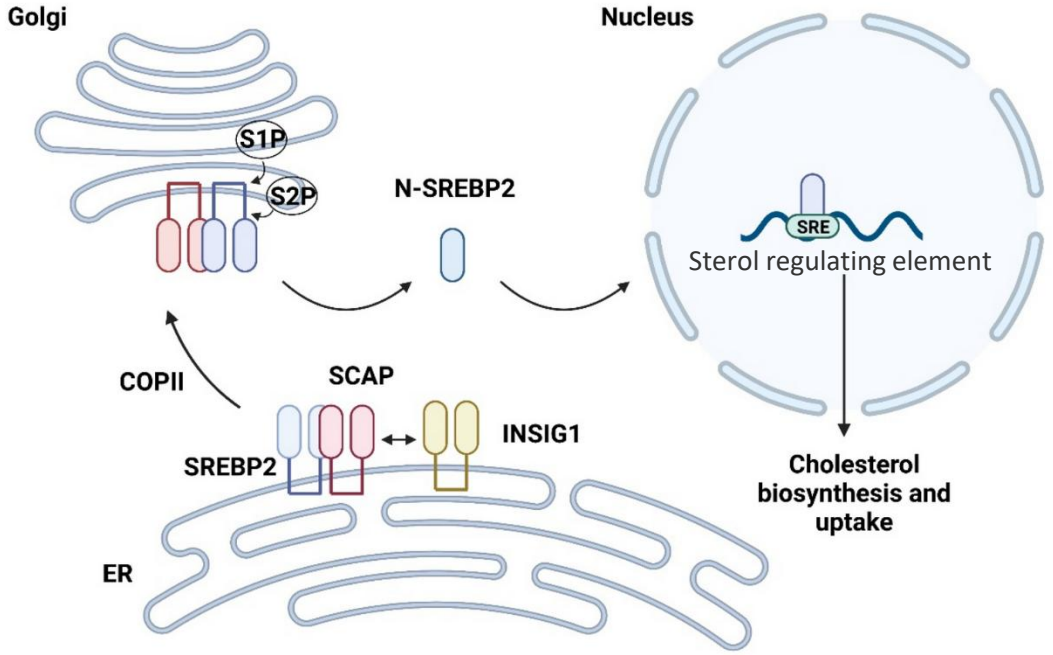


CD33

Knockdown of S2P and SREBP2 modulates lipid metabolism in M1 cells



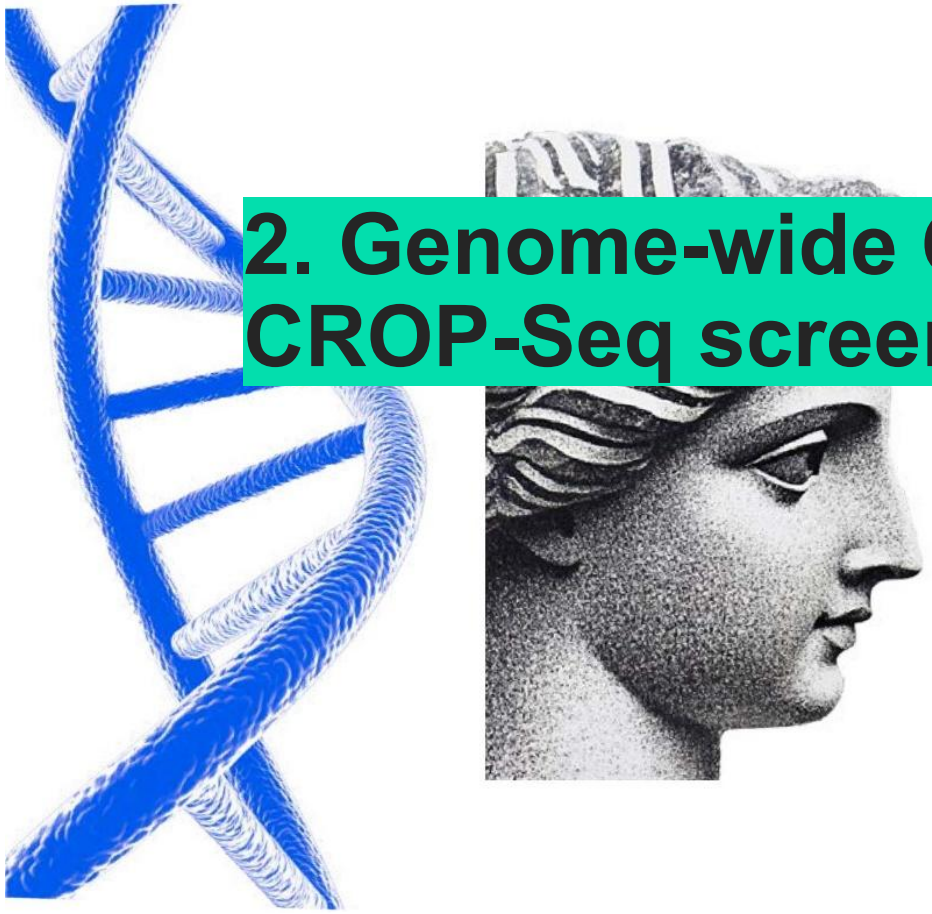
Cholesterol Biosynthesis and Uptake Genes



Itkonen et al., 2023

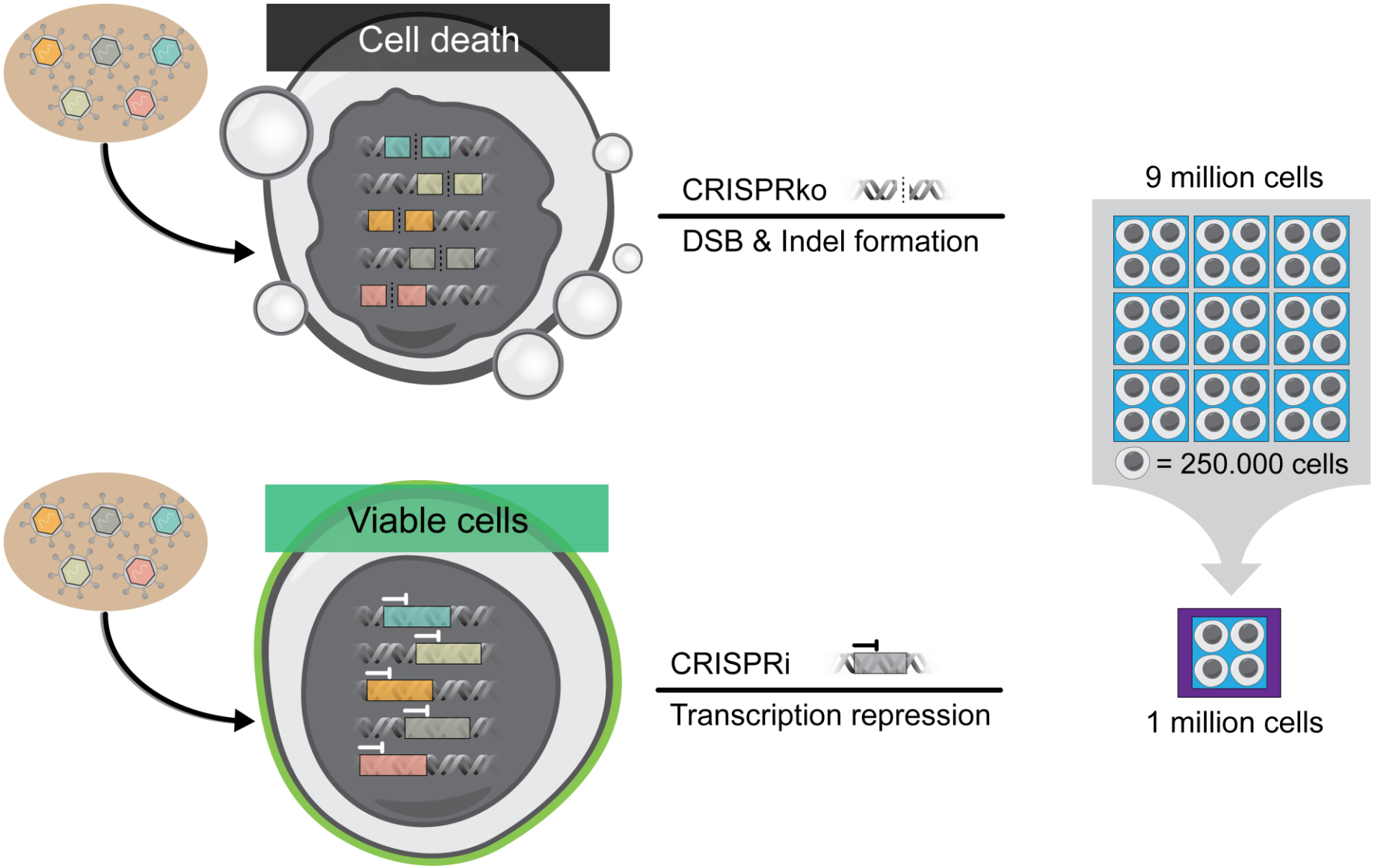
Conclusions

- Genome-wide compressed Perturb-seq/CROP-seq screens can be “squeezed” into 1 million cells
- Targeted sequencing leads to significant NGS cost reduction and, at the same time, increases the depth of the transcriptomic data
- The “main screen” identified key factors involved in LPS- and IFNg-induced M1 macrophage polarization
- A “side screen” for regulators of cholesterol biosynthesis identifies S2P and SREBP1
- AbSeq/CITE-Seq enriches and complements single-cell RNA-Seq data

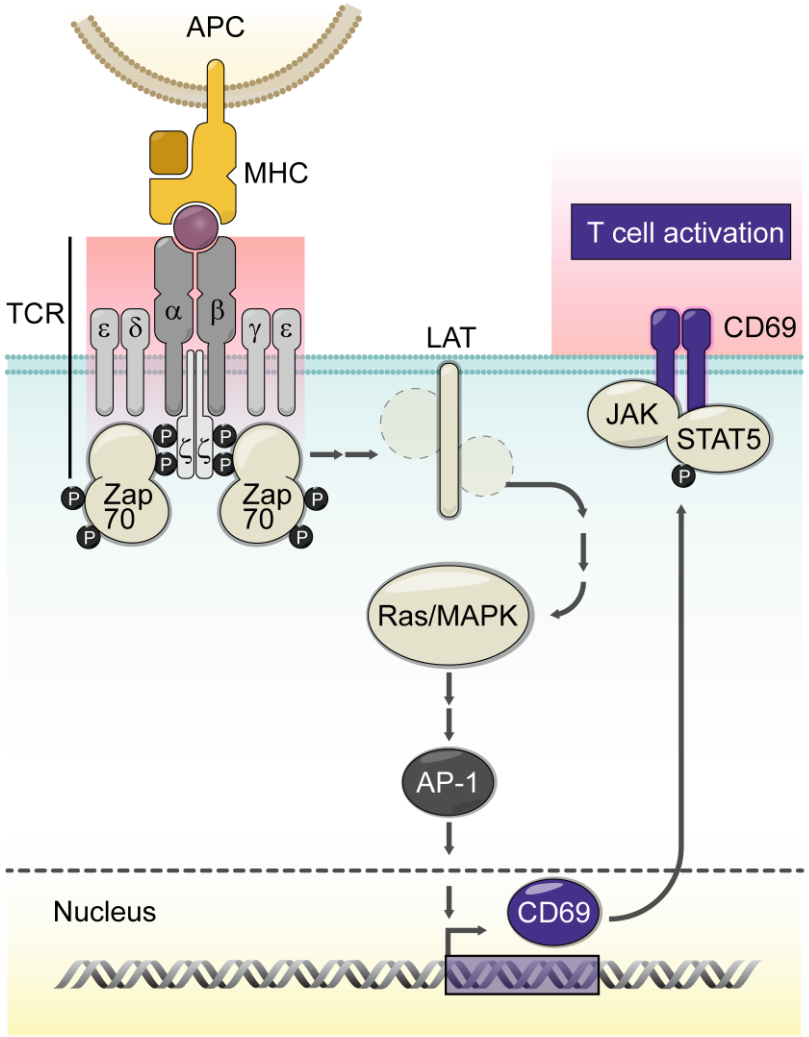


2. Genome-wide CRISPRi CROP-Seq screen in Jurkat cells

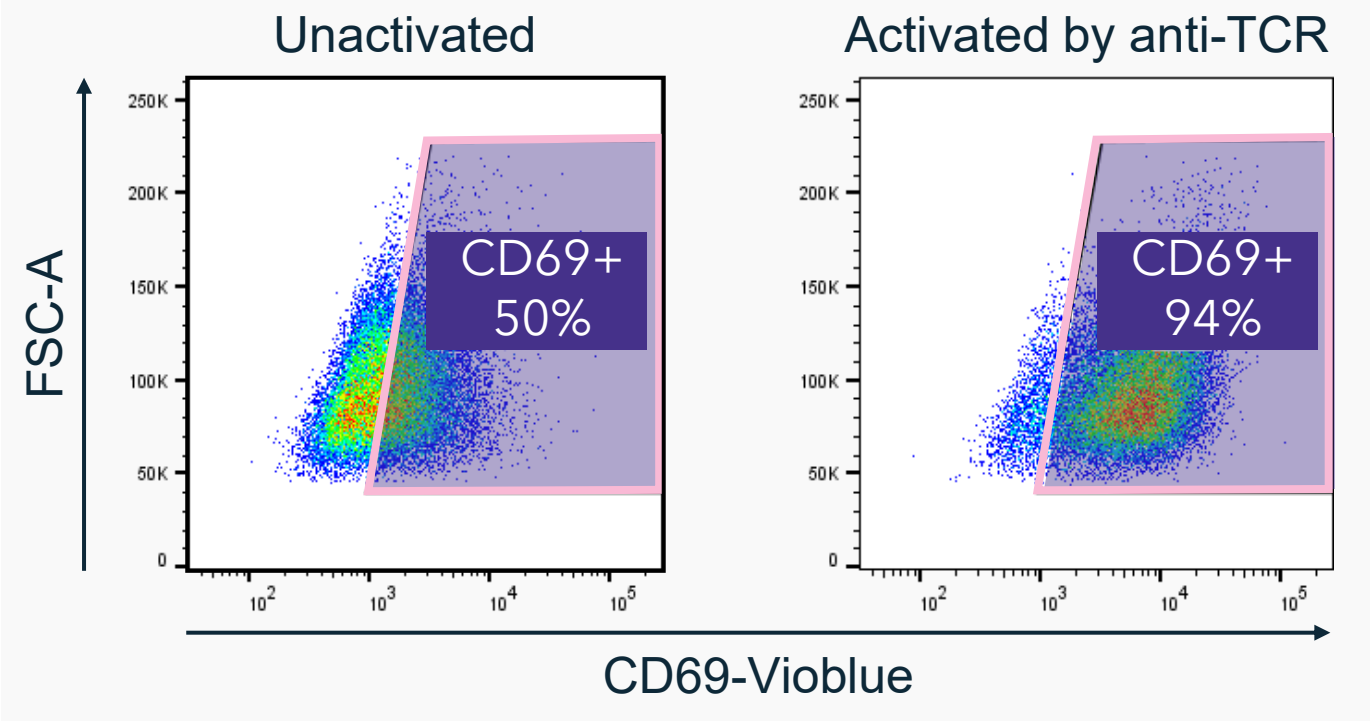
CRISPR interference allows the delivery of multiple sgRNAs per cell



TCR signaling can be studied in human Jurkat T cells

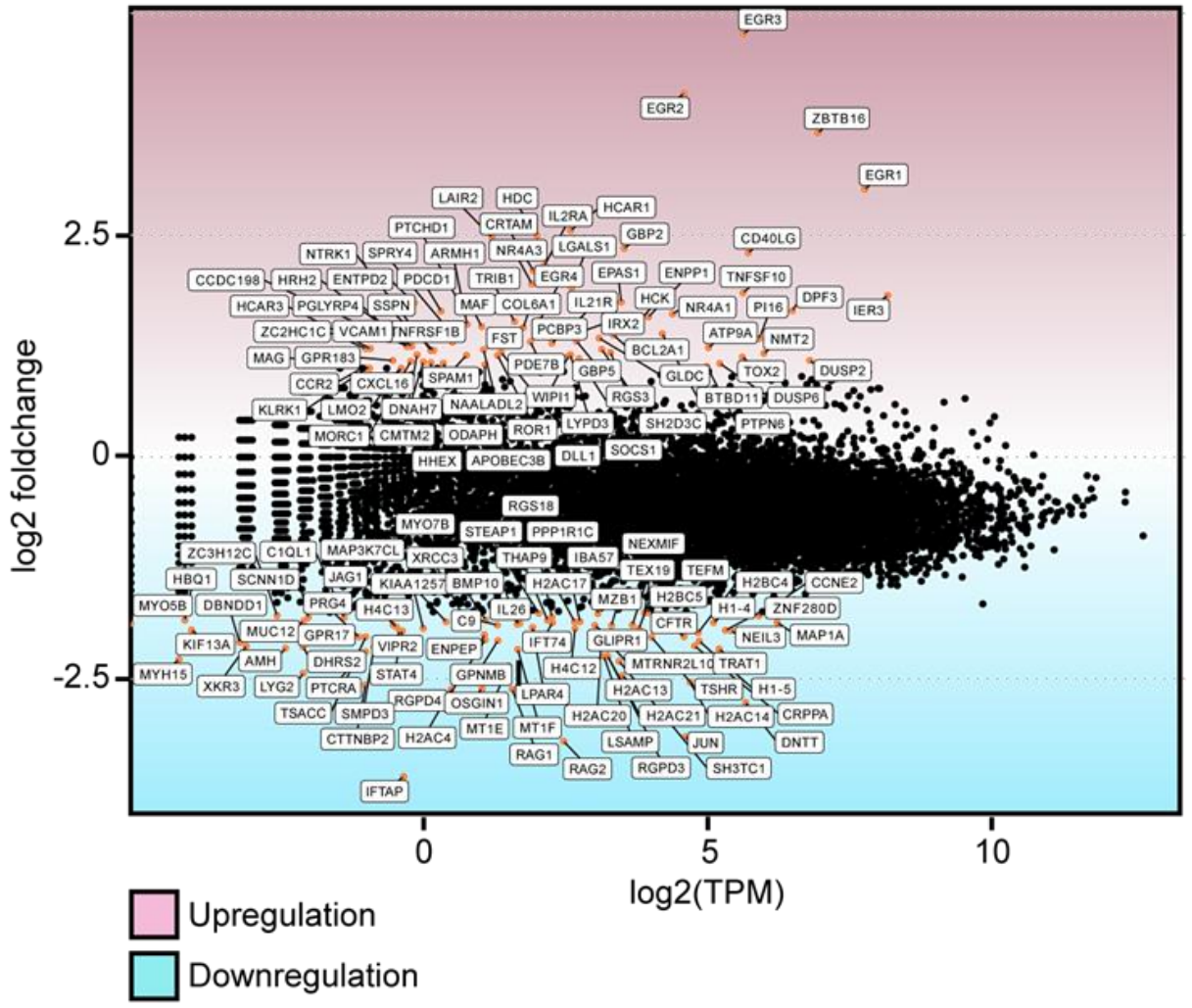


Jurkat = immortalized human T lymphocytes

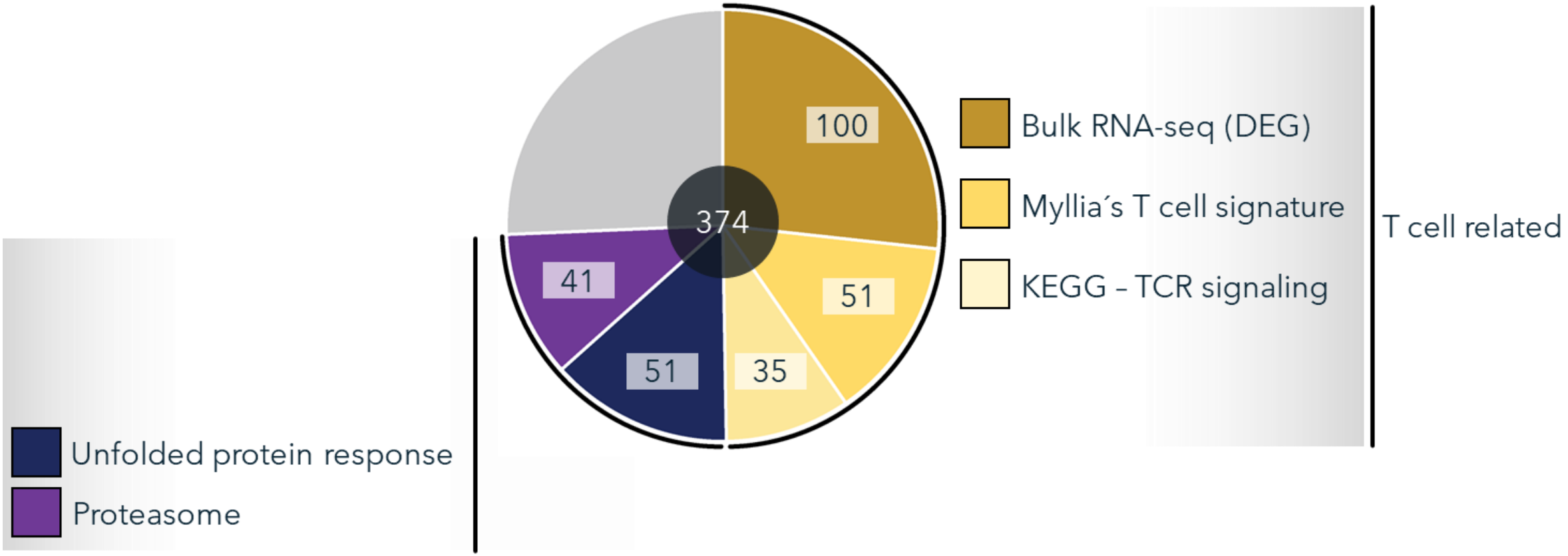


Stimulation of Jurkat cells triggers a distinct transcriptomic signature

- Stimulation of Jurkat cells with anti-TCR antibodies
- Capture changes in the transcriptome by bulk RNA sequencing
- Total number of differentially expressed genes: 3.321 genes
  - 1.663 genes upregulated
  - 1.568 genes downregulated

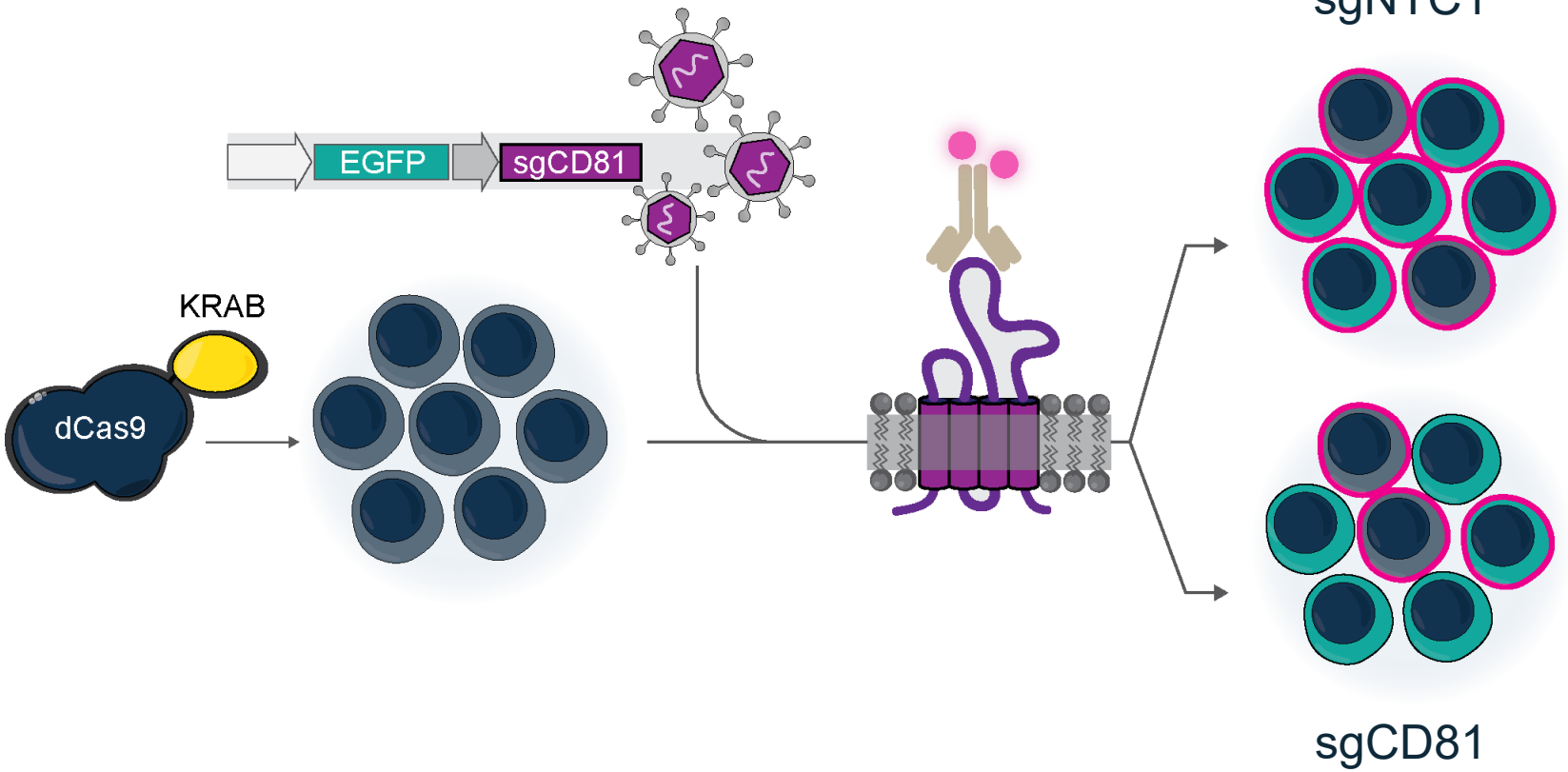


Selected signatures for the multiplexed primer panel for targeted sequencing

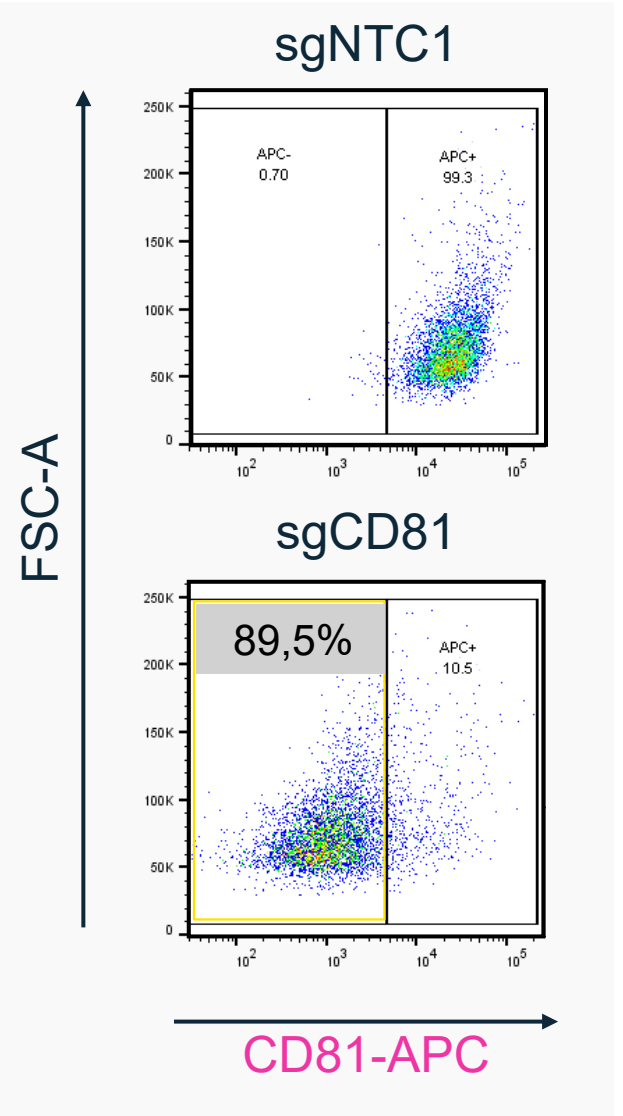


Replogle JM, Saunders RA, Pogson AN, Hussmann JA, Lenail A, Guna A, Mascibroda L, Wagner EJ, Adelman K, Lithwick-Yanai G, Iremadze N, Oberstrass F, Lipson D, Bonnar JL, Jost M, Norman TM, Weissman JS. Mapping information-rich genotype-phenotype landscapes with genome-scale Perturb-seq. Cell. 2022 Jul 7;185(14):2559-2575.e28. doi: 10.1016/j.cell.2022.05.013. Epub 2022 Jun 9. PMID: 35688146; PMCID: PMC9380471.

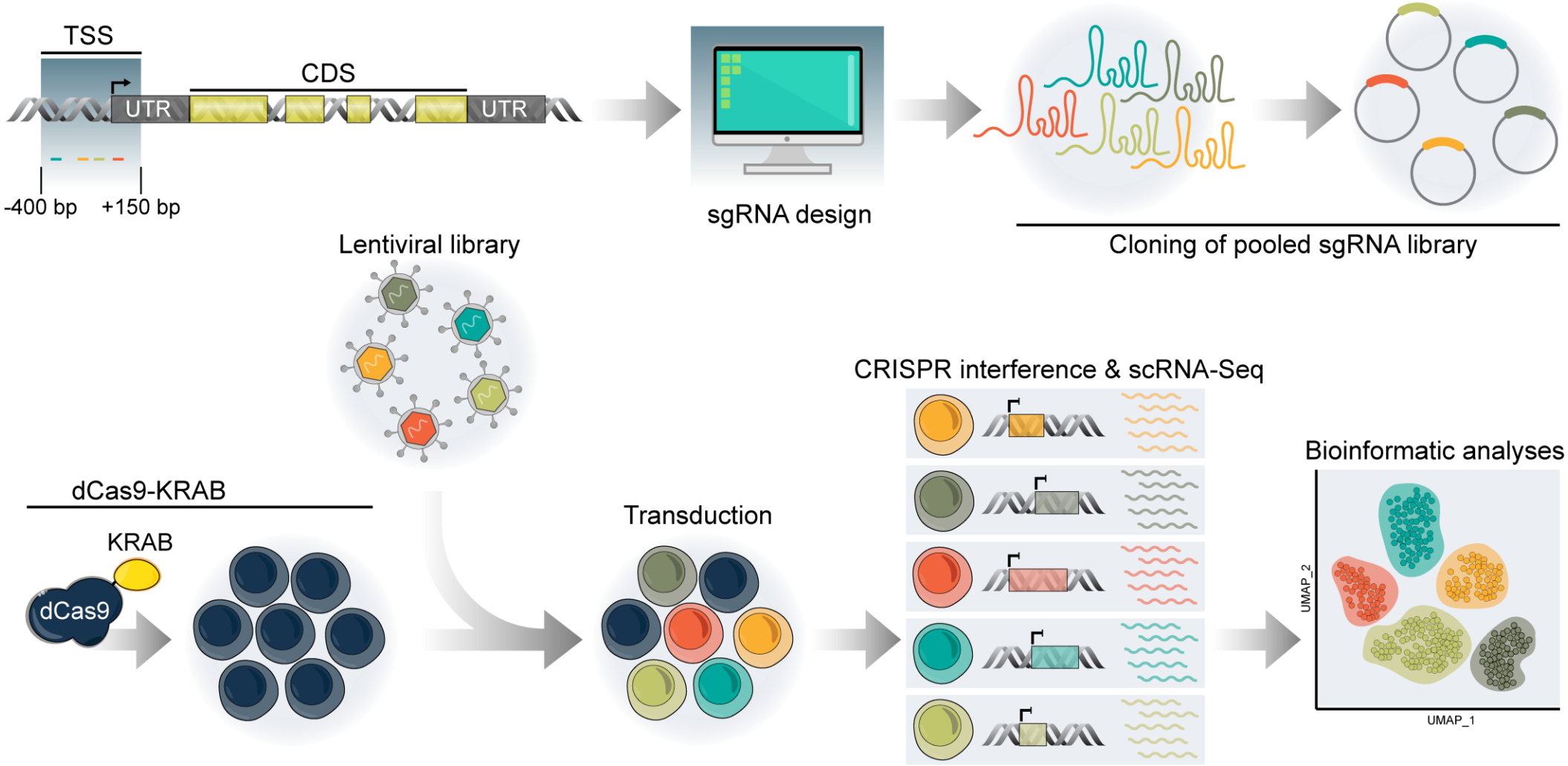
Validation of CRISPRi in dCas9-KRAB-engineered Jurkat cells



Gated for EGFP+

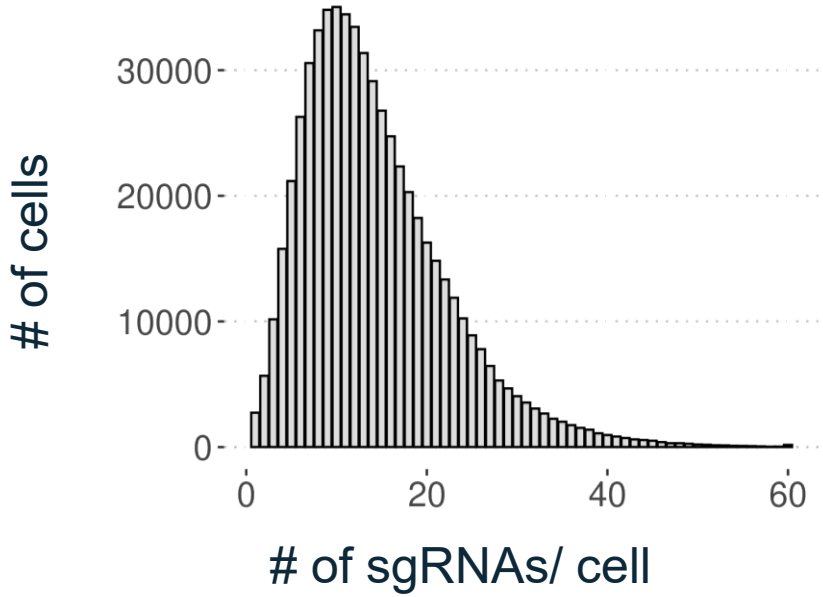


A genome-wide CRISPRi CROP-Seq screen



Metrics of the 10x Chromium X run (3’ chemistry)

Targeted NGS library preparation and NGS



# of loaded cells on 10x Chromium X	1.840.000
# of cells retrieved from 16 channels	930.000
# of cells after filtering out multiplets	716.000
# of cells after filtering out damaged cells	586.000
# of guides per cell (median)	13
# of cells per knockdown	400
# of NovaSeq S4 runs	1
# of reads/cell	10.000
# UMIs/cell	2.000

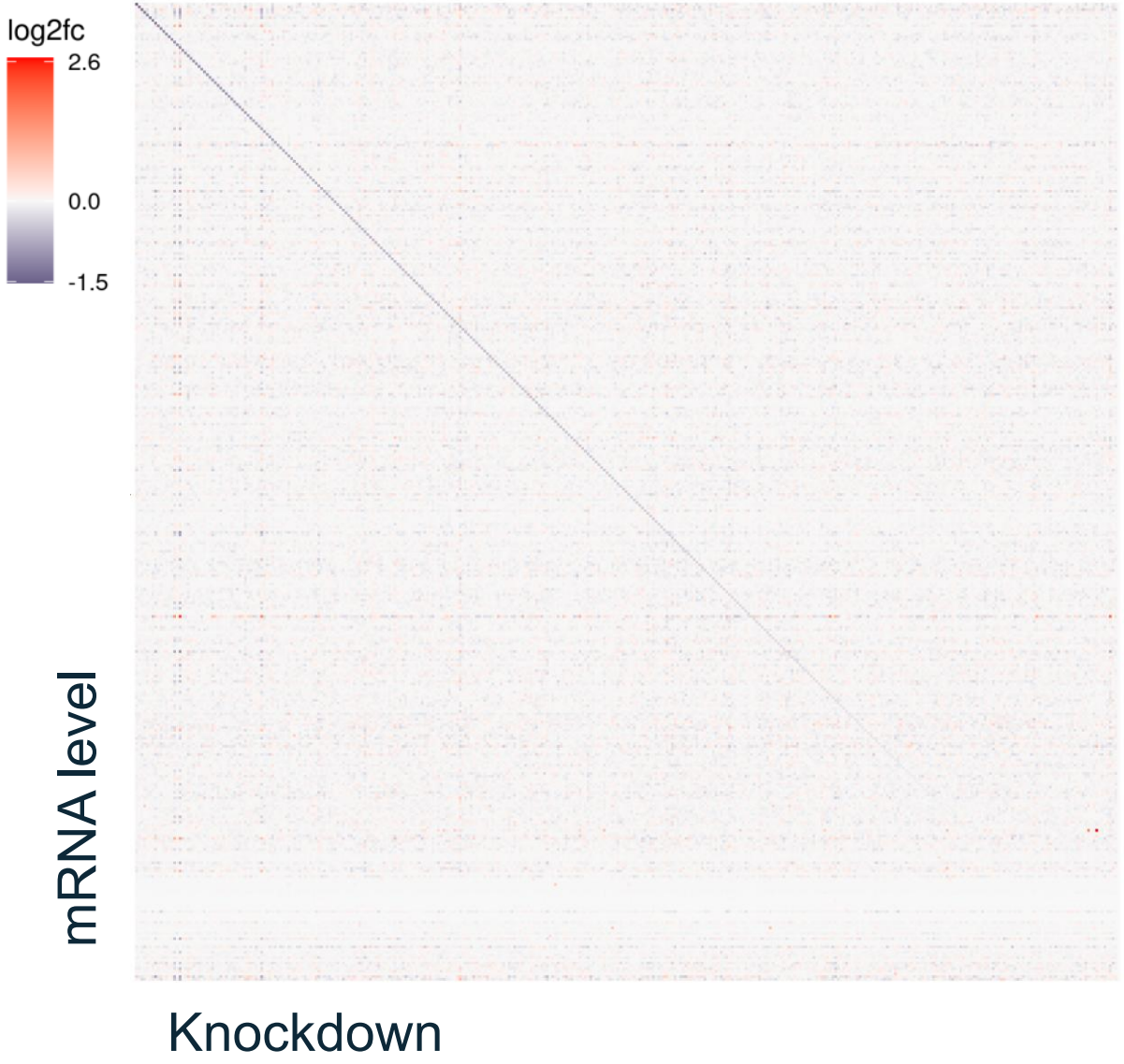
CRISPRi reduces the expression of target genes

– sgRNA target genes from the primer panel (374)

– 94% (350/374 marker genes) successfully downregulated by dCas9-KRAB

– 6% (24/374 marker genes) downregulation by dCas9-KRAB is not detectable

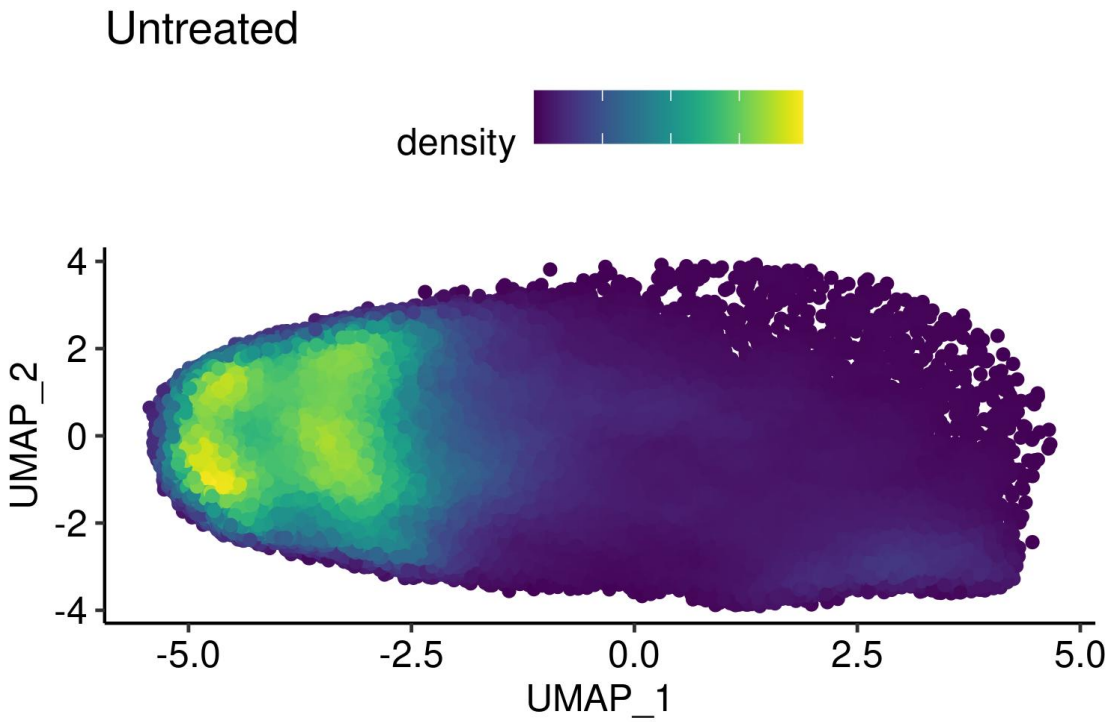
- 17/24 show very low expression
- 7/24 potentially have a suboptimal sgRNA design

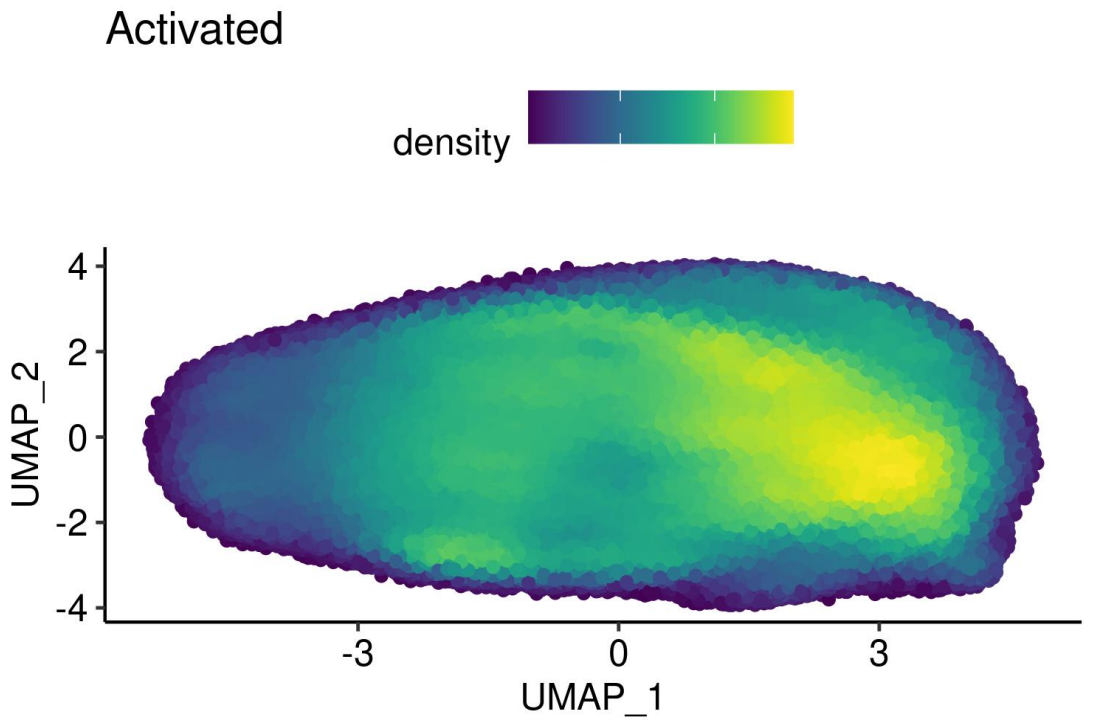


Untreated cells are different from activated cells

myllia.

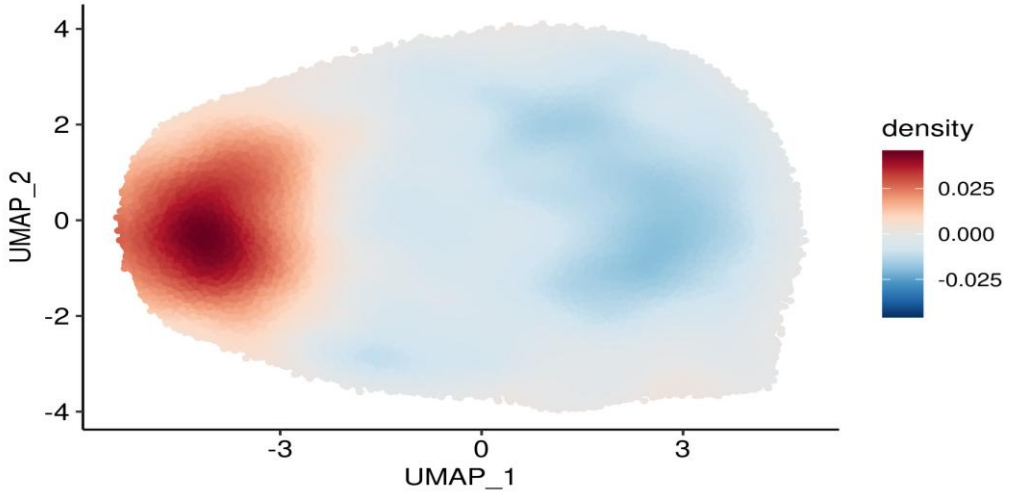
UMAPs can be used to visualize transcriptomic differences between untreated and activated Jurkat cells



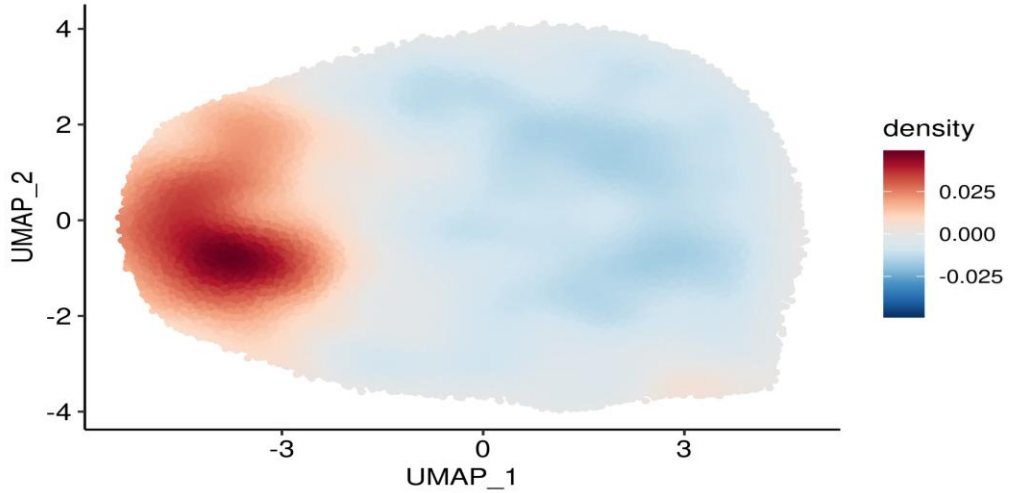


Knockdown of CD247 and LAT diminished T cell activation

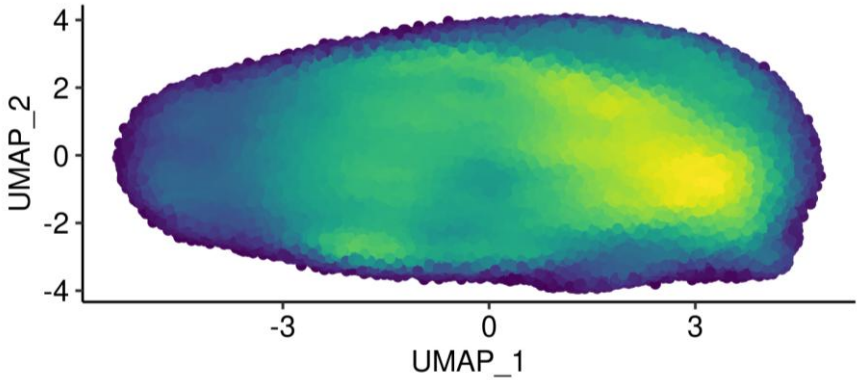
CD247 knockdown



LAT knockdown

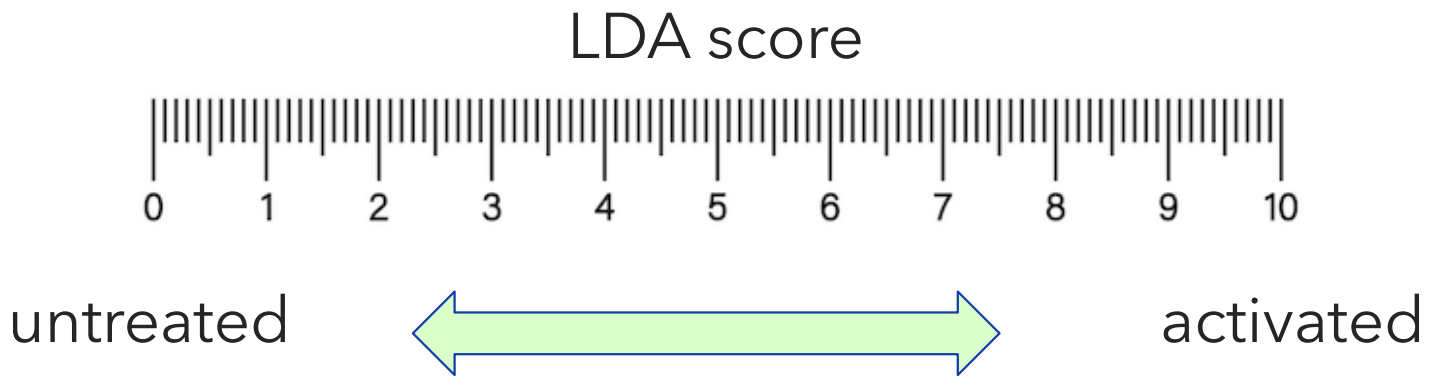


Activated

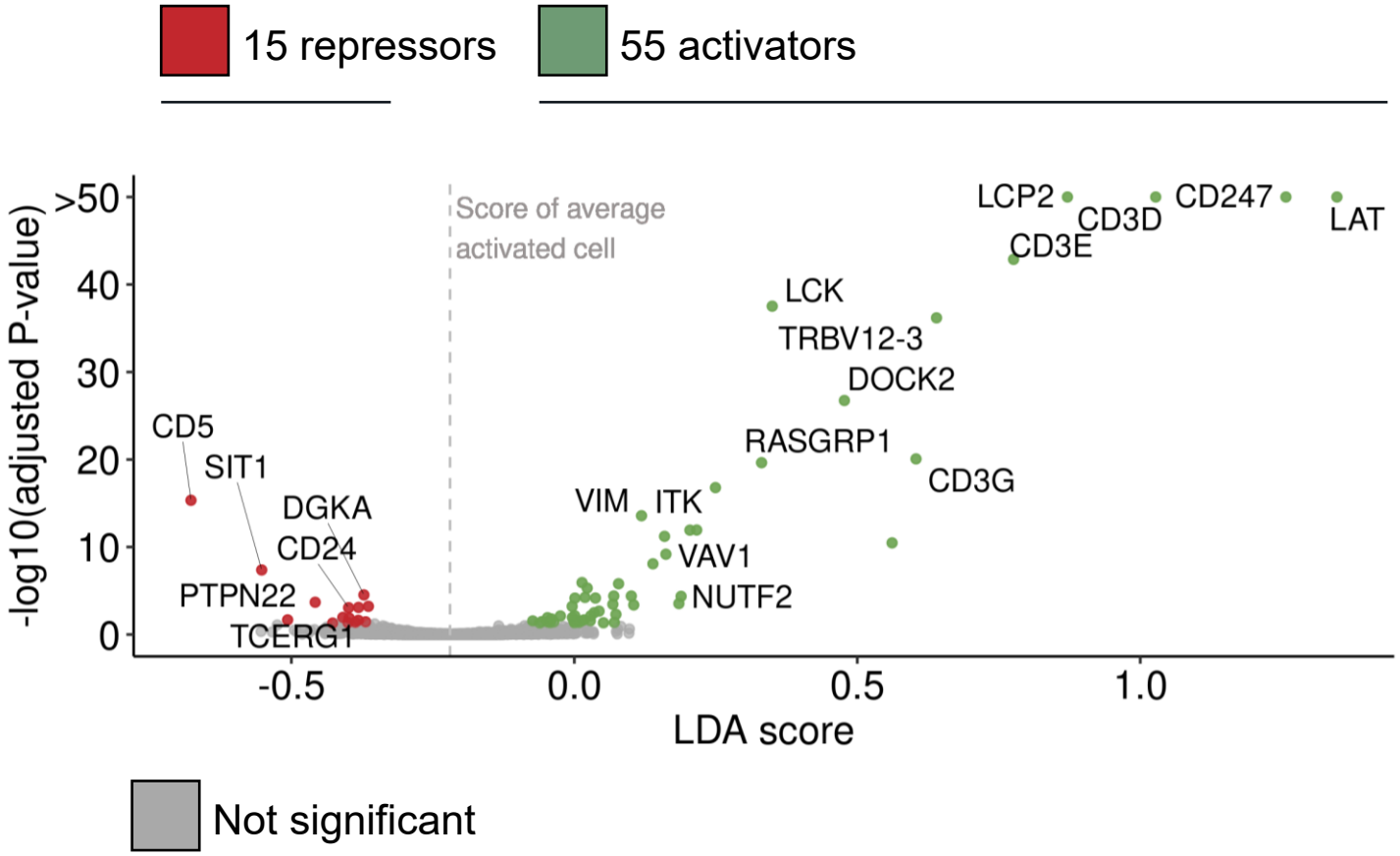


Do we find knockdowns that promote or prevent T cell activation?

– Measure "activation" using LDA (Linear Discriminant Analysis)
– Each cell gets a single number, a "score" which is a measurement of activation
– Test if cells with a knockdown are significantly pushed towards one direction



Genes that regulate TCR signaling in Jurkat cells were identified



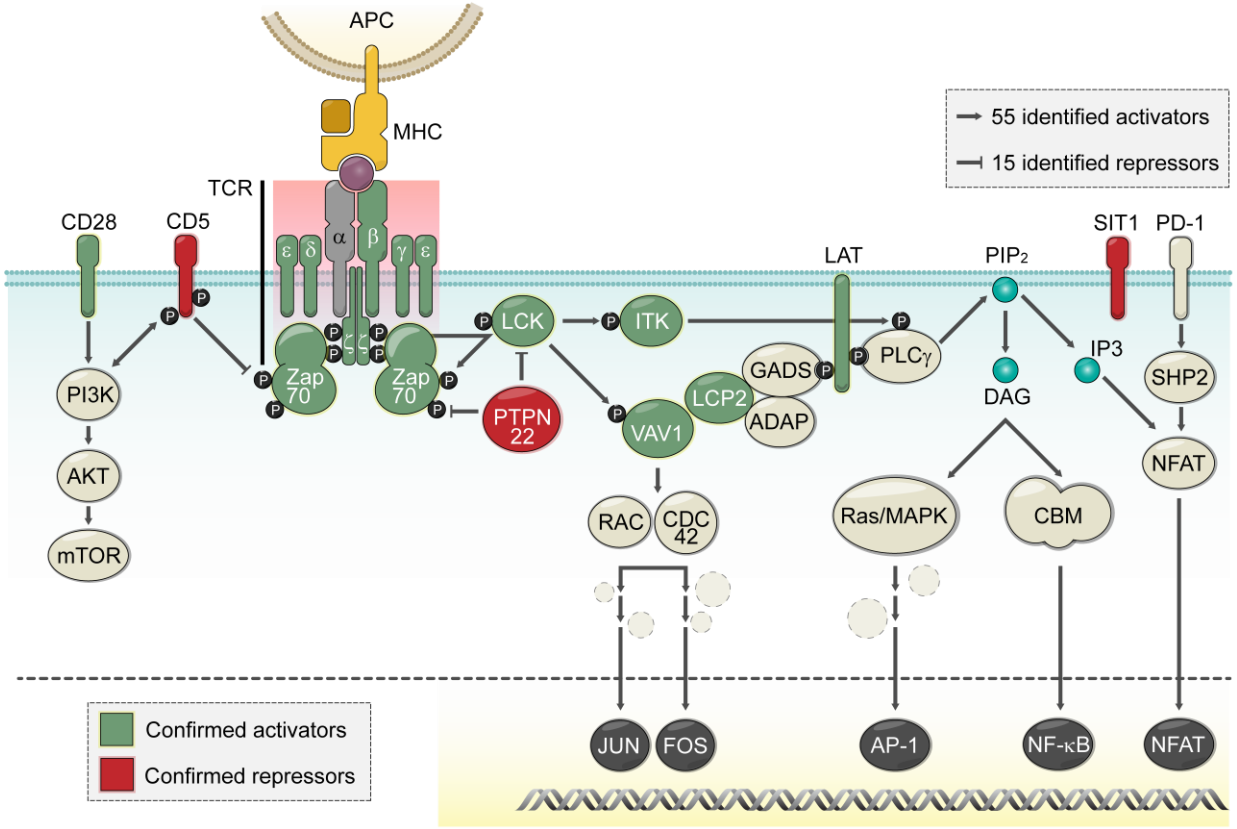
LAT	BEND4	**PGGT1B**	BZW1
CD247	**NUTF2**	MRGBP	**RHOH**
CD3D	SHOC2	JPH4	**SASH3**
LCP2	**VAV1**	**GIT2**	**ACLY**
CD3E	SPCS2	XRRA1	**ZAP70**
TRBV12-3	**VIM**	**EHD3**	**RHOA**
CD3G	CNBD2	SLC35B1	CCNC
GPR35	SPCS3	**SRF**	ANKEF1
DOCK2	RGP1	SEC63	**ETS1**
LCK	HSPA5	THG1L	**GRAP2**
RASGRP1	HYAL4	UBA3	**CD28**
ITK	MED25	INTS14	BAHD1
SMC3	EMX2	STRIP1	PHB
TMX1	SERPINB2	SLC24A1	

CD5	GFY	TMEM126A	**DGKA**
SIT1	DNAJC3	FGF23	TFAP4
PTPN22	**TCERG1**	LEPROTL1	SELL
TARDBP	**CD24**	ISL2	

Previously identified repressors/activators are highlighted in bold

CROP-Seq screens in Jurkat cells recapitulate known T cell biology

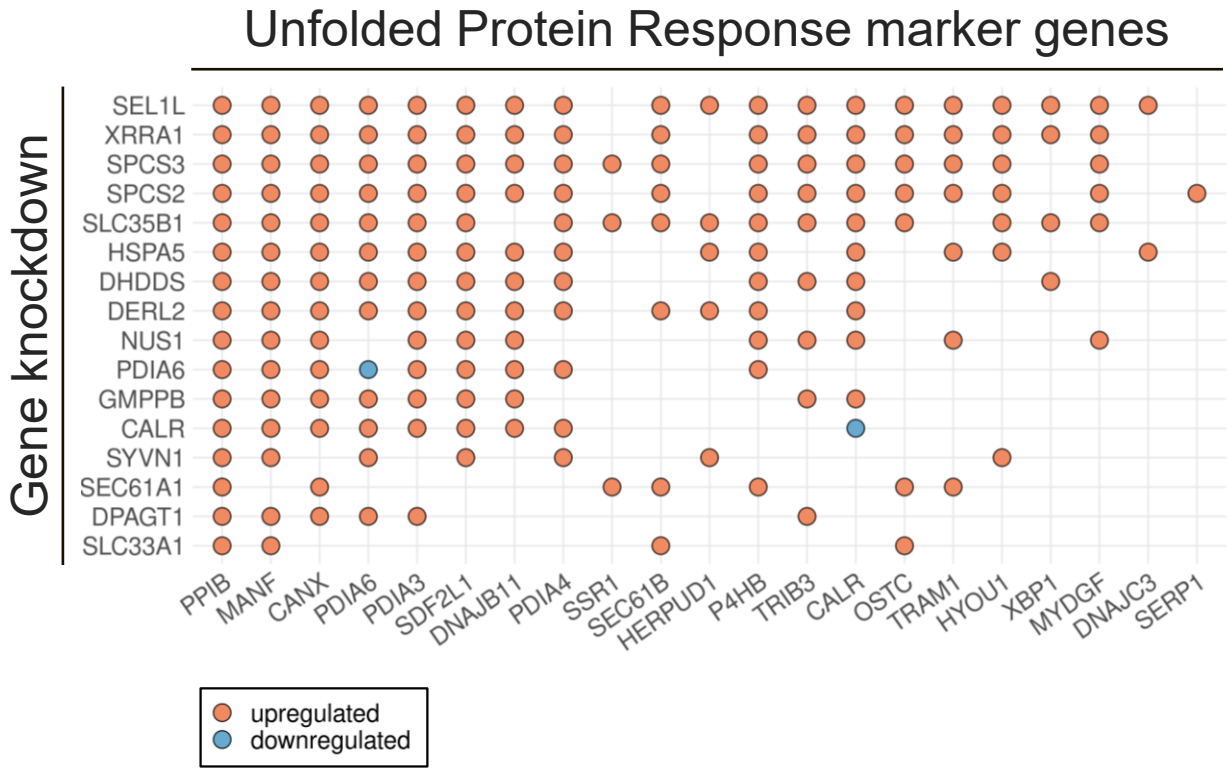
- We successfully performed a cost-effective genome-wide single-cell CRISPRi screen in Jurkat cells
- We identified 15 repressors and 55 activators of T cell signaling encompassing known and novel regulators
- A comparable screening workflow will be utilized to investigate T cell signaling in primary cells

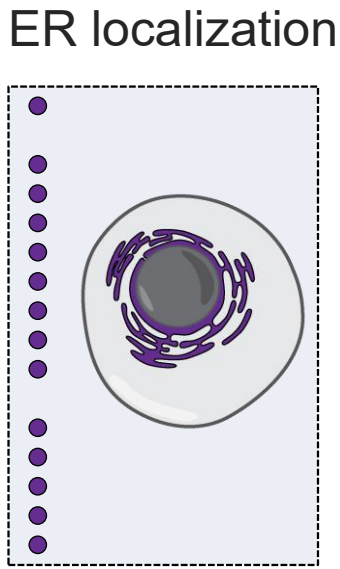


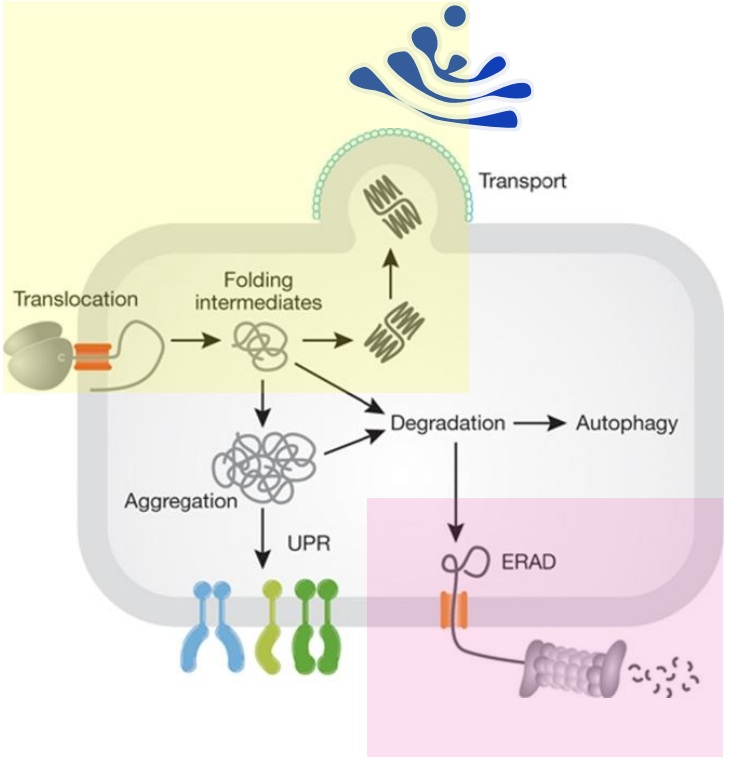
55 activators

15 repressors

Genes involved in the Unfolded Protein Response are ER-localized

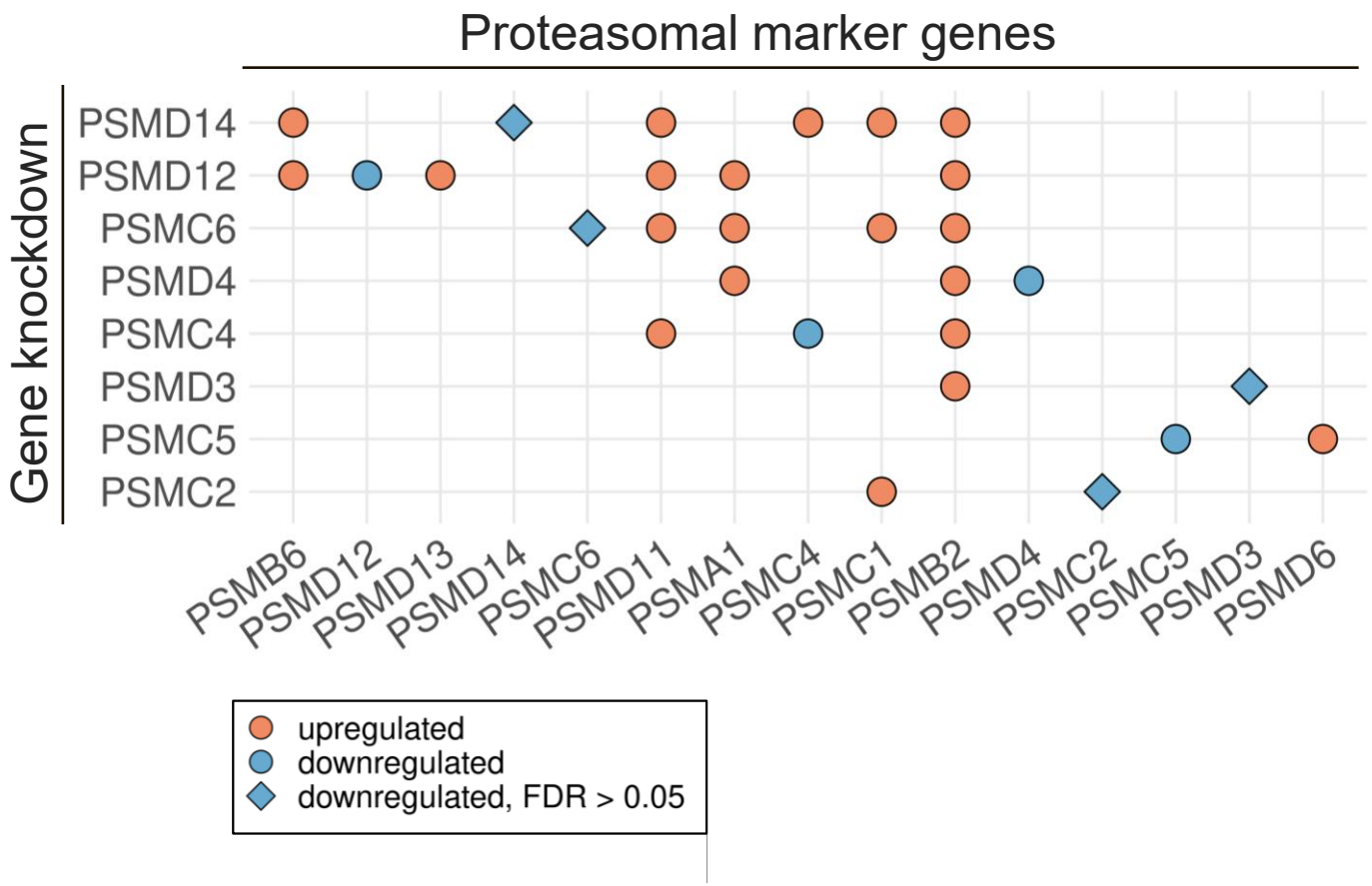






doi:10.1038/embor.2009.224

CRISPRi of proteasomal subunits lead to compensatory upregulations



Molecular Cell
Article

Cell PRESS

Transcription Factor Nrf1 Mediates the Proteasome Recovery Pathway after Proteasome Inhibition in Mammalian Cells

Senthil K. Radhakrishnan,[1] Candy S. Lee,[3] Patrick Young,[4] Anne Beskow,[4] Jefferson Y. Chan,[3] and Raymond J. Deshaies[1,2,*]

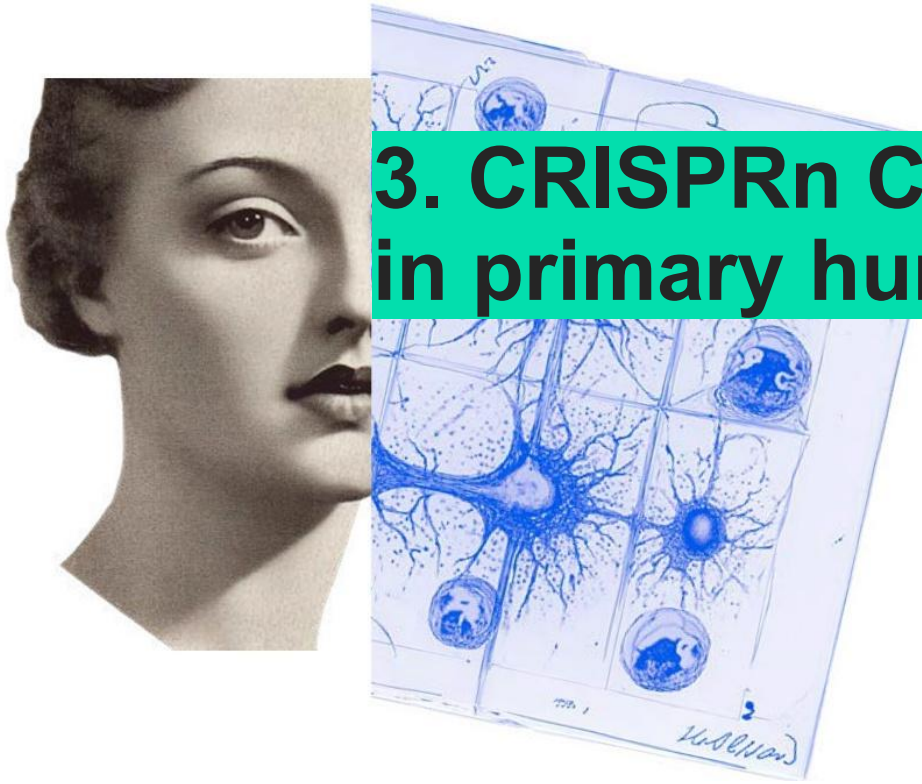
Conclusions

– The genome-wide CRISPRi CROP-Seq screen was completed successfully

– Recovery of both known and new regulators of T cell signaling

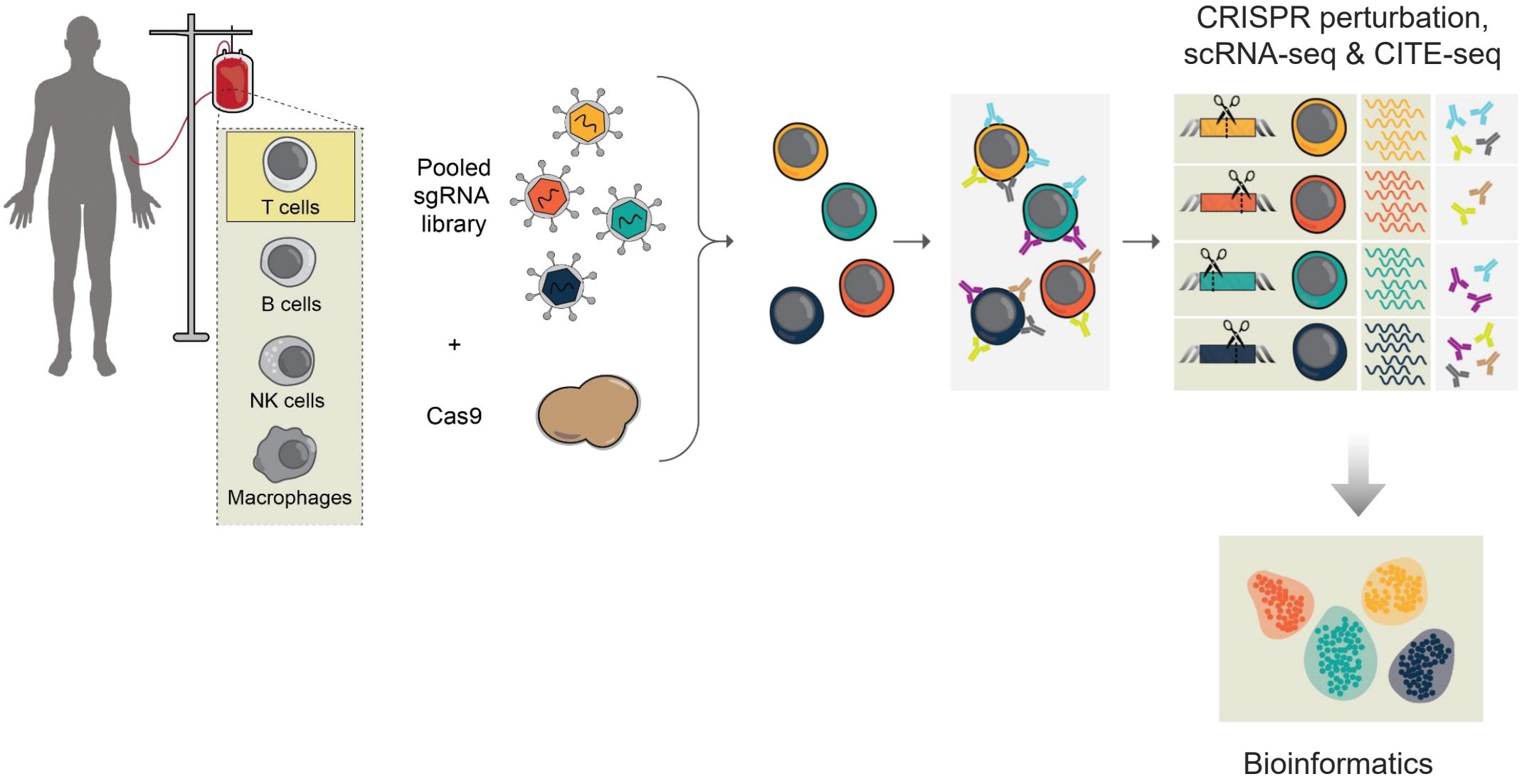
– Reasonable “economics” through
  - Multiplexed CRISPRi perturbations (~13 sgRNAs per cell)
  - Targeted sequencing (TA-Seq) of 374 marker genes
  - 1 million cells processed on 16 channels and sequenced on a single NovaSeq S4 flow cell

– CROP-Seq exits the niche of drug target validation and is ready for drug target identification

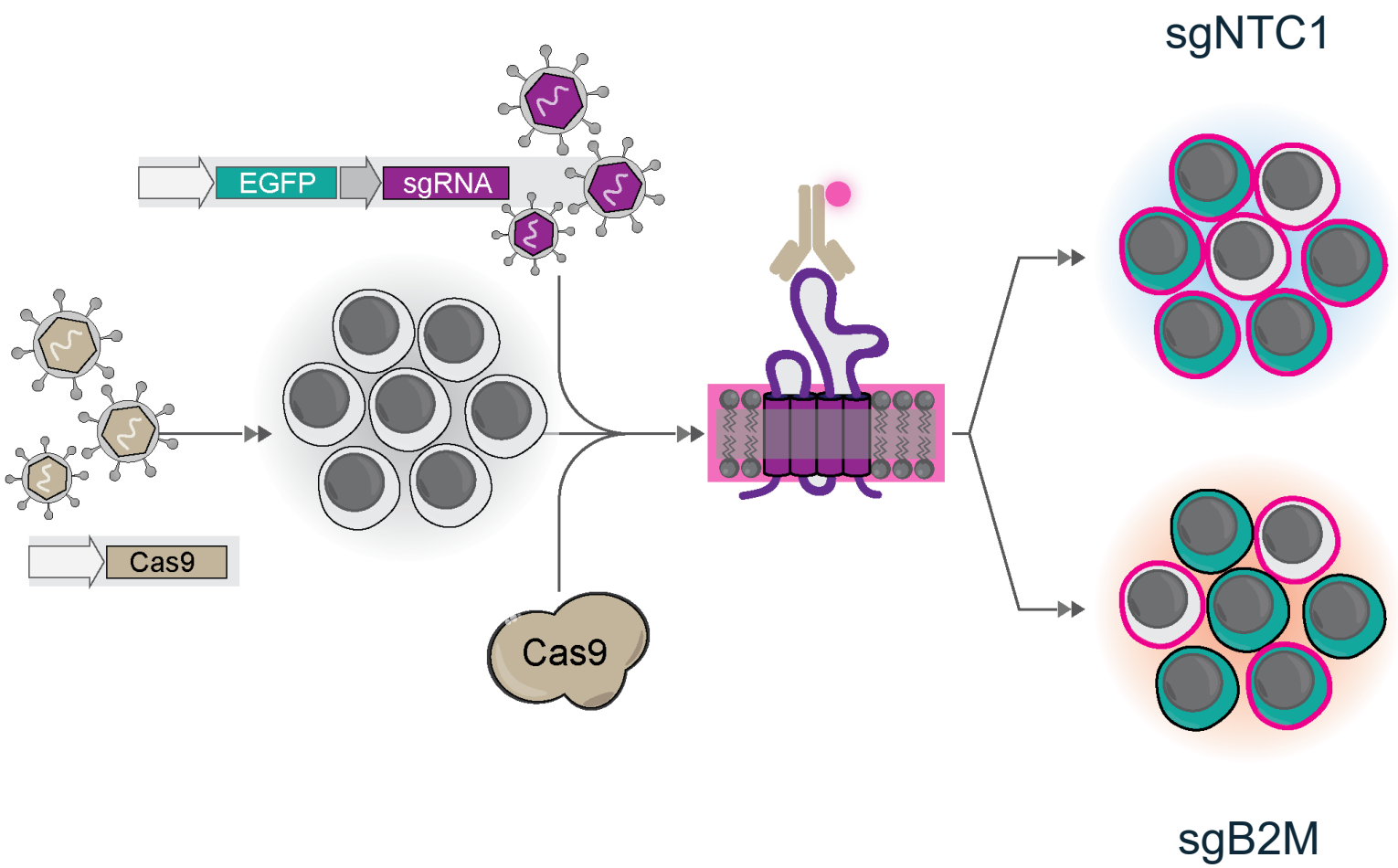


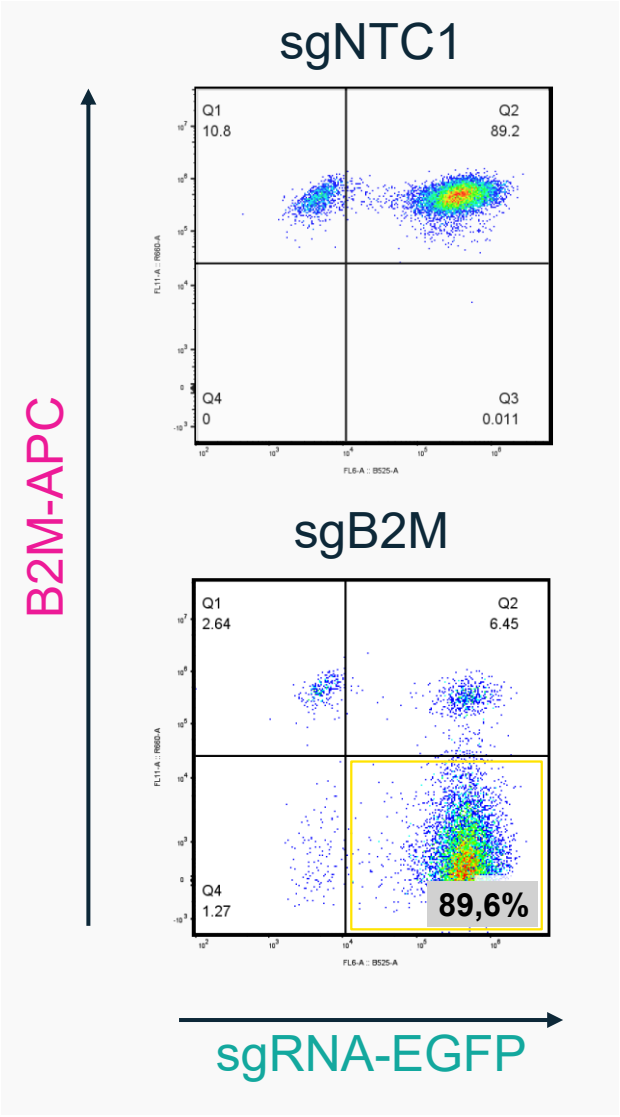
3. CRISPRn CROP-Seq screen in primary human pan-T cells

CRISPR screens in primary human T cells



Flow cytometry confirms a successful knockout of B2M receptors





A CROP-Seq screen in 100,000 primary human T cells

CROP-Seq experiment in T cells

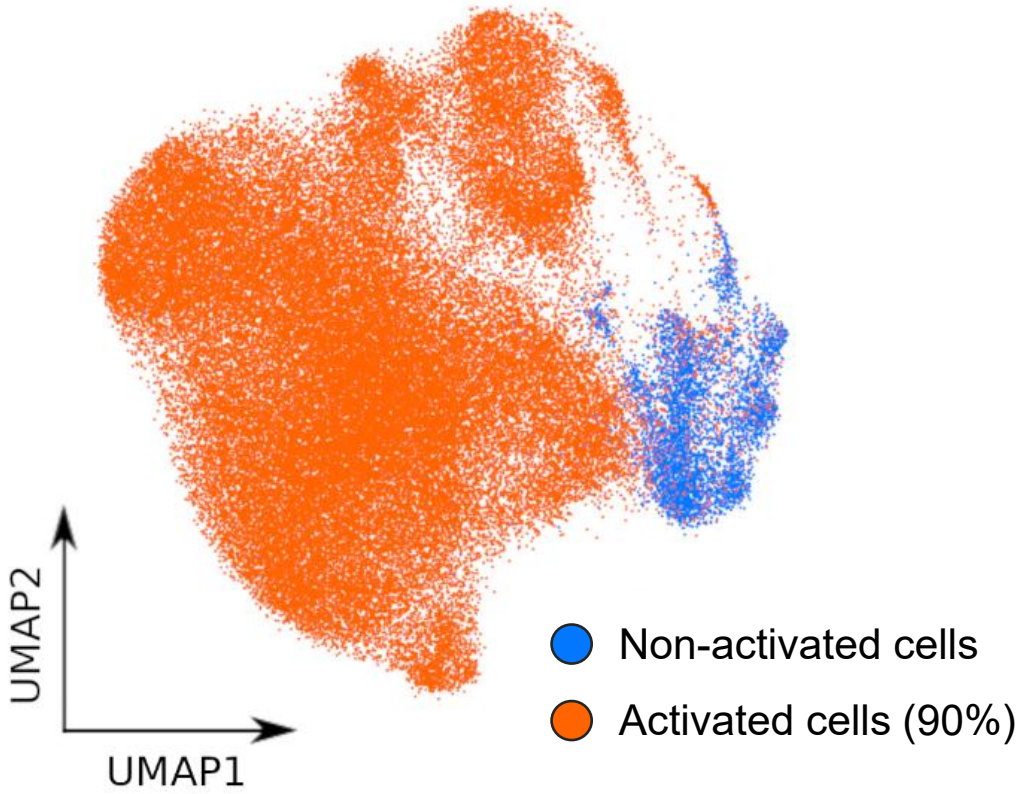
- Non-activated cells
- Cells activated with CD3/CD28-Dynabeads

Number of perturbed genes

- **42 genes**
- 188 sgRNAs (4 sgRNAs/ gene)

Targeted single-cell RNA sequencing of 300 mRNAs

- T cell activation markers
- Markers for cell types
- Cell cycle markers
- Immune checkpoint genes



T cell subsets and effector phenotypes at single-cell resolution

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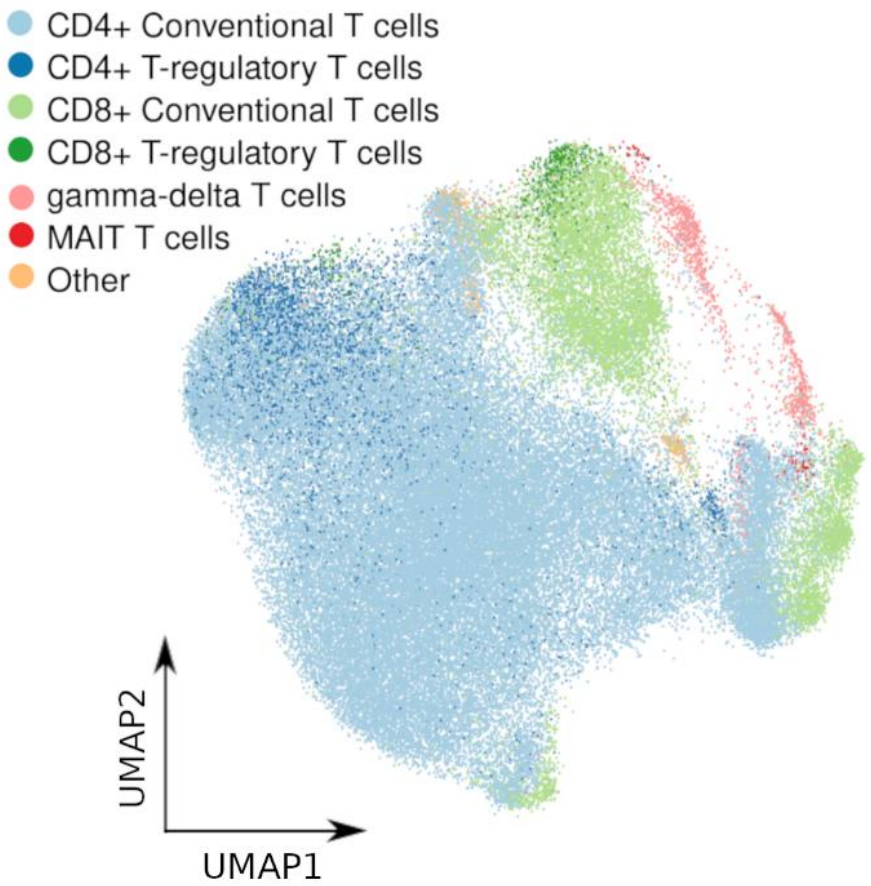
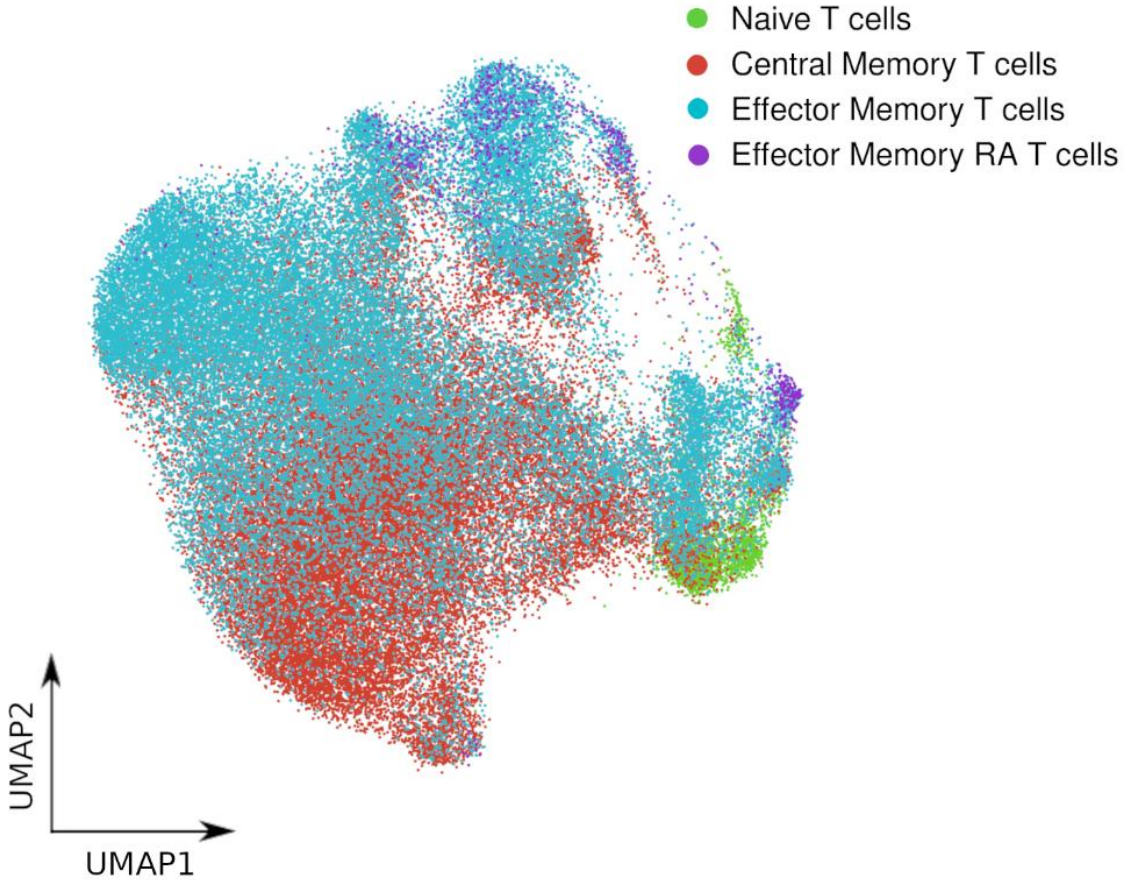
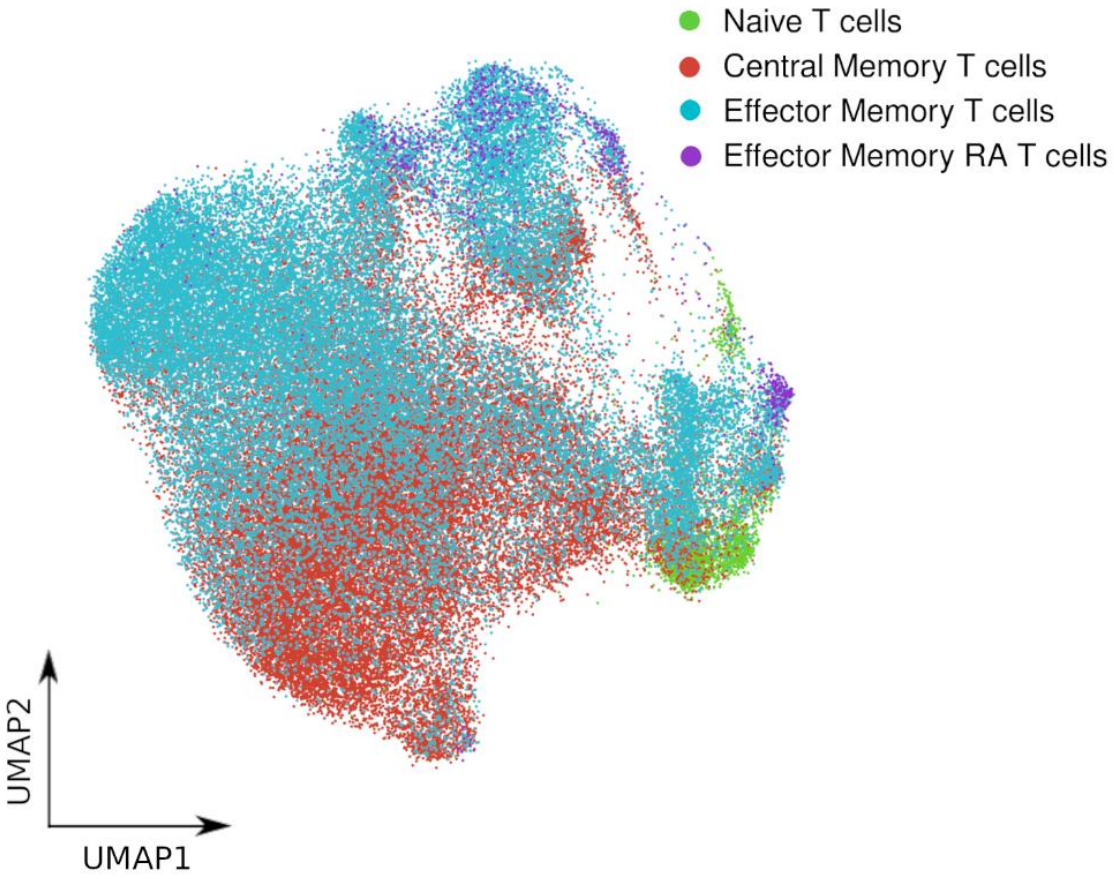
Which T cell subsets do we see?

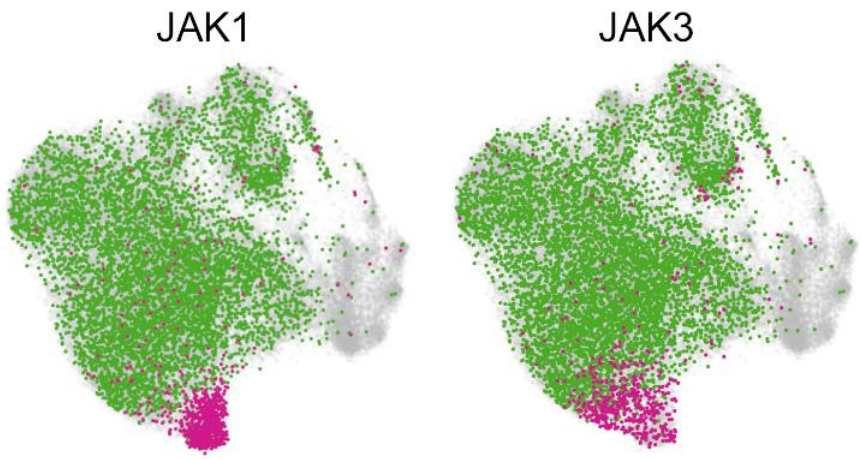


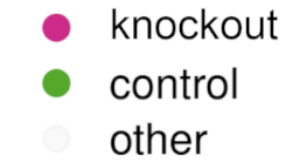
Which effector types do we see?

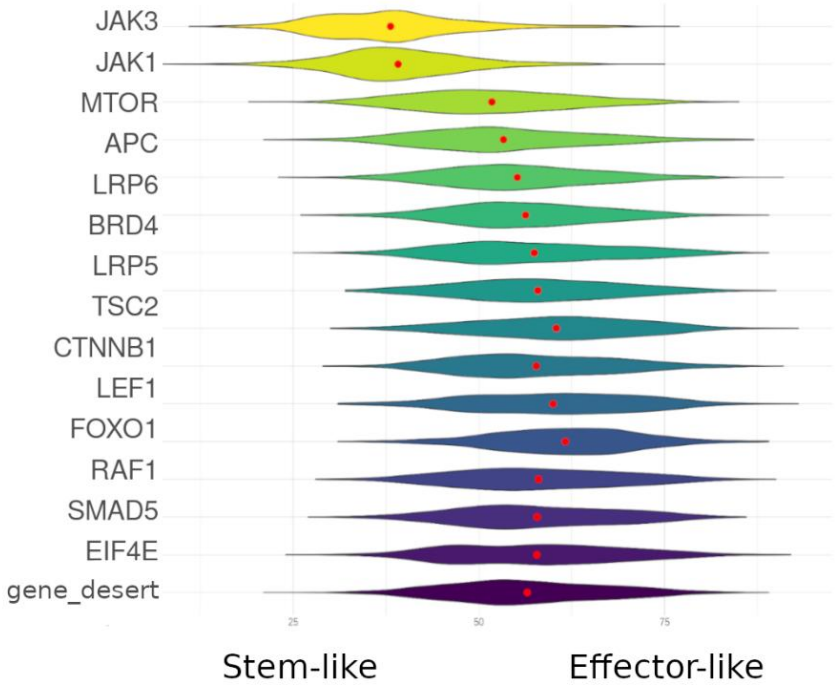


Gene knockouts affecting T cell stemness and effector phenotypes

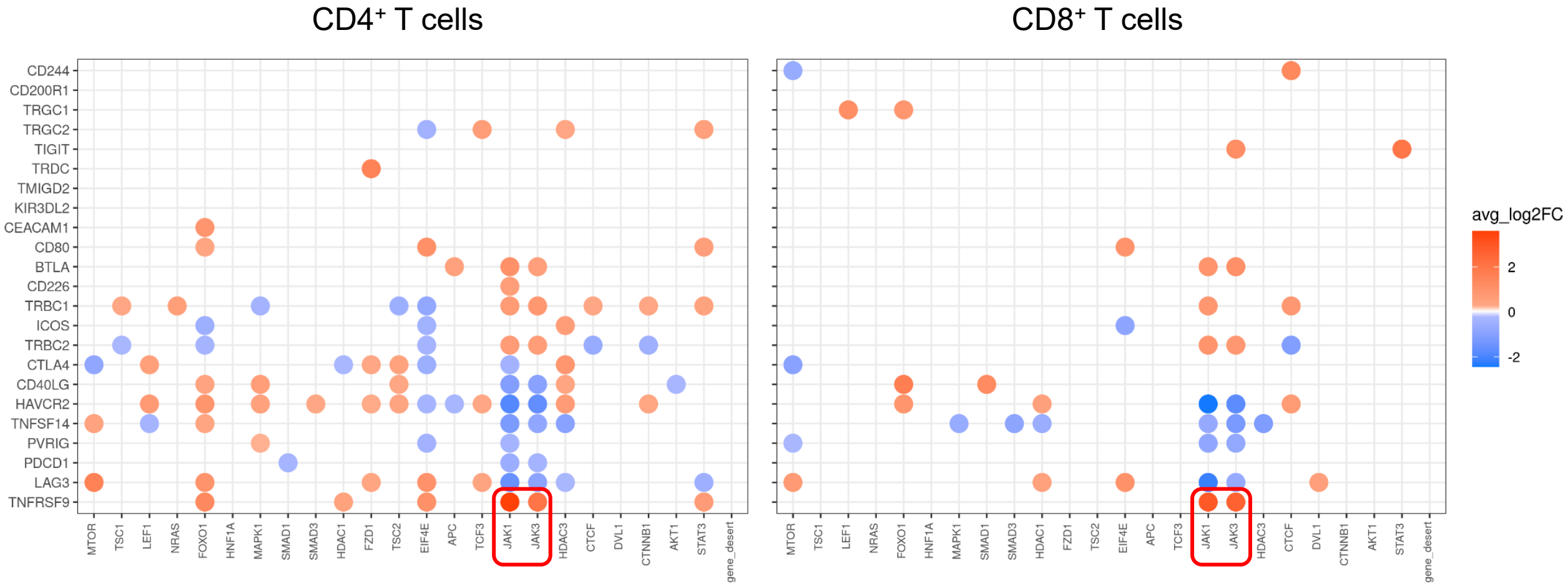




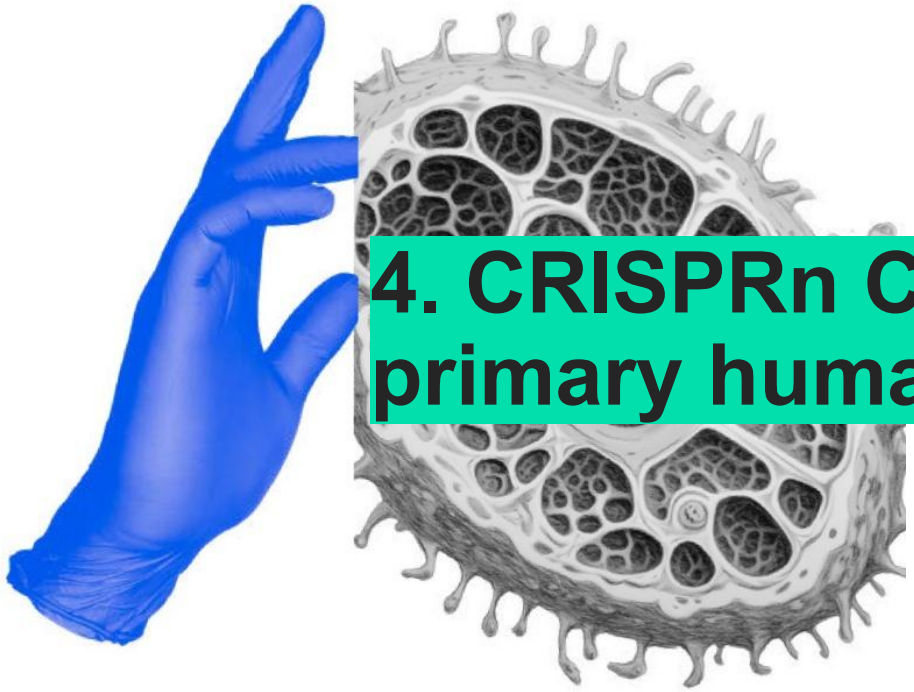




Impact of gene knockouts on expression levels of checkpoint genes

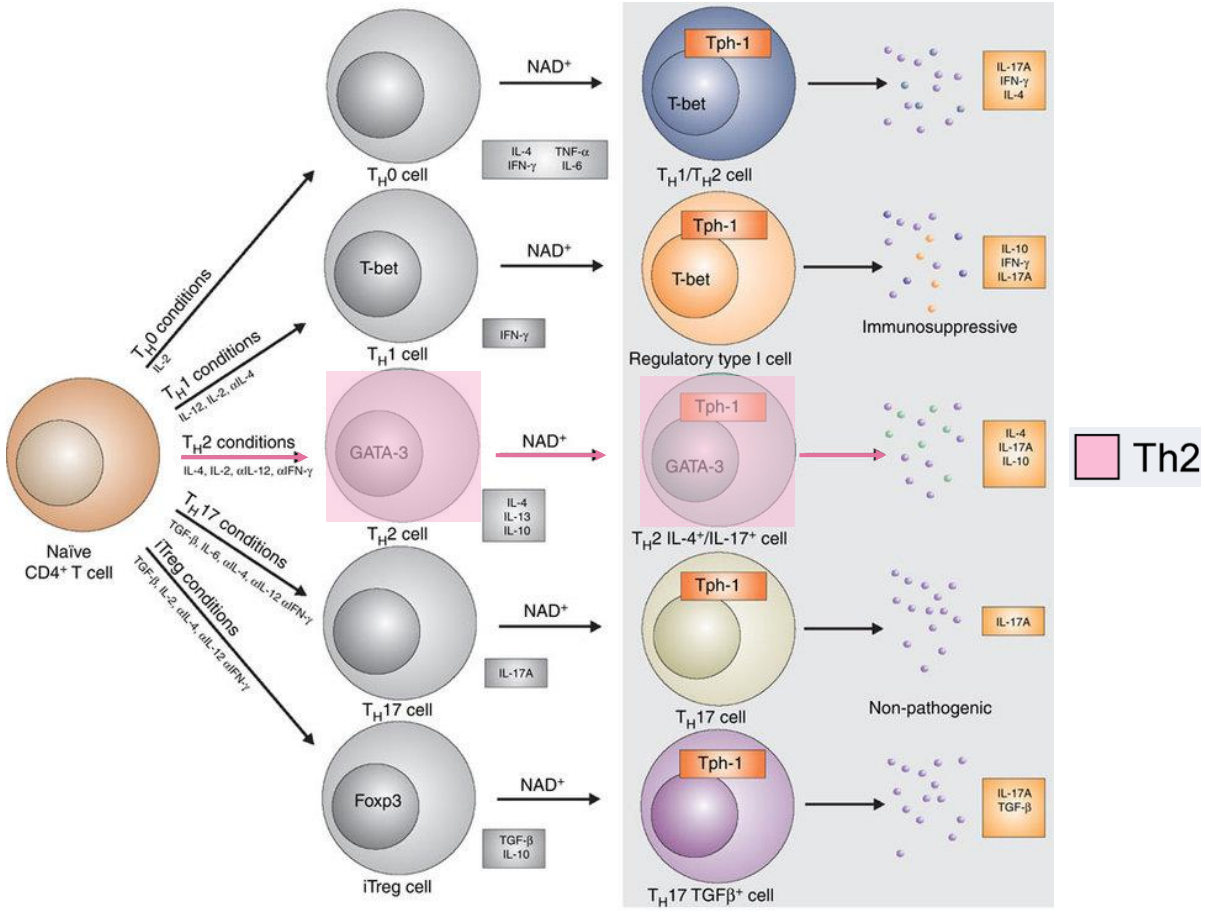


- Knockouts of JAK1 and JAK3 lead to a strong upregulation of TNFRSF9 (4-1BB)



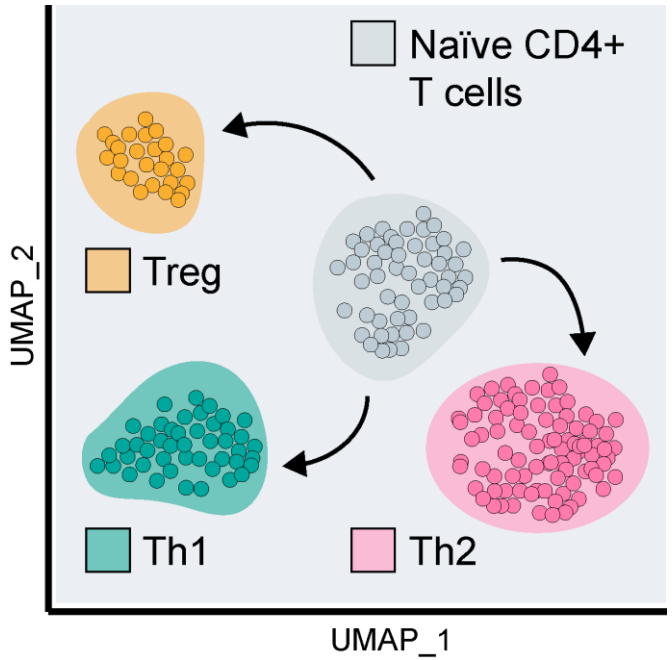
4. CRISPRn CROP-Seq screen in primary human CD4^{+} Th2 cells

CROP-Seq in primary human T cells to elucidate Th2 differentiation



Tullius et al., 2014

Can we identify genes involved in skewing of CD4⁺ T cells towards the Th2 subset?



A CROP-Seq screen in 150,000 primary human T cells

CROP-Seq experiment in CD4⁺ T cells

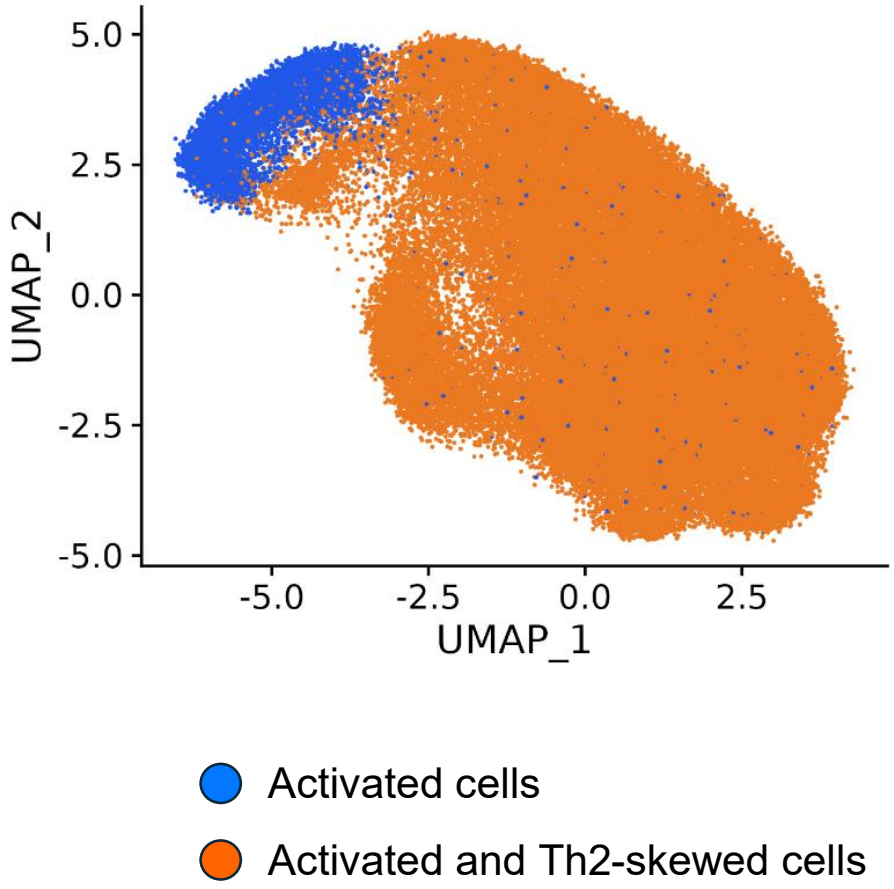
- Activated cells
- Activated and Th2-skewed cells

Number of perturbed genes

- **102 genes**
- 415 sgRNAs (4 sgRNAs per gene)

Targeted single-cell RNA sequencing of 300 mRNAs

- T cell activation markers
- Curated markers for T helper cell subsets
- Cell cycle markers



Th2 cells can be defined by transcriptomic signatures

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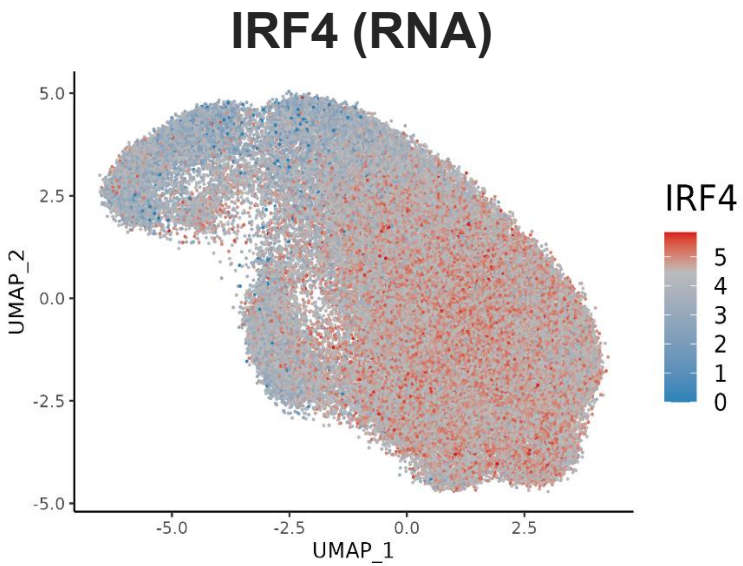
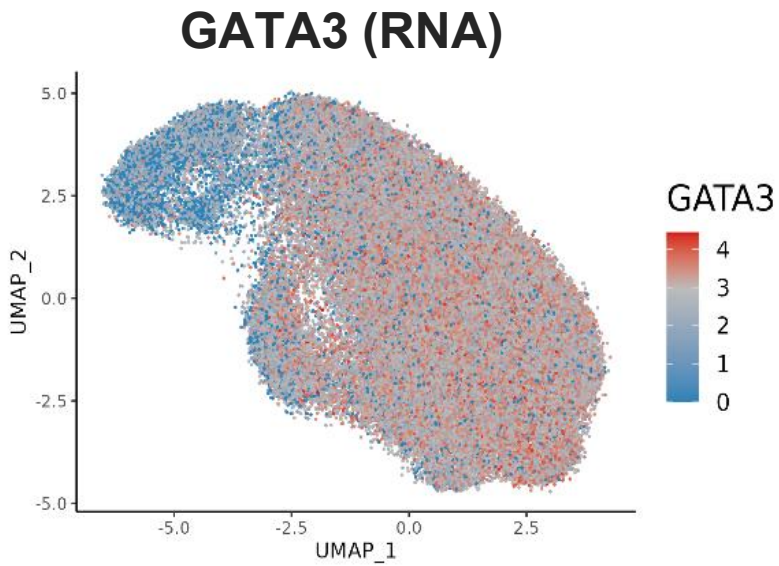
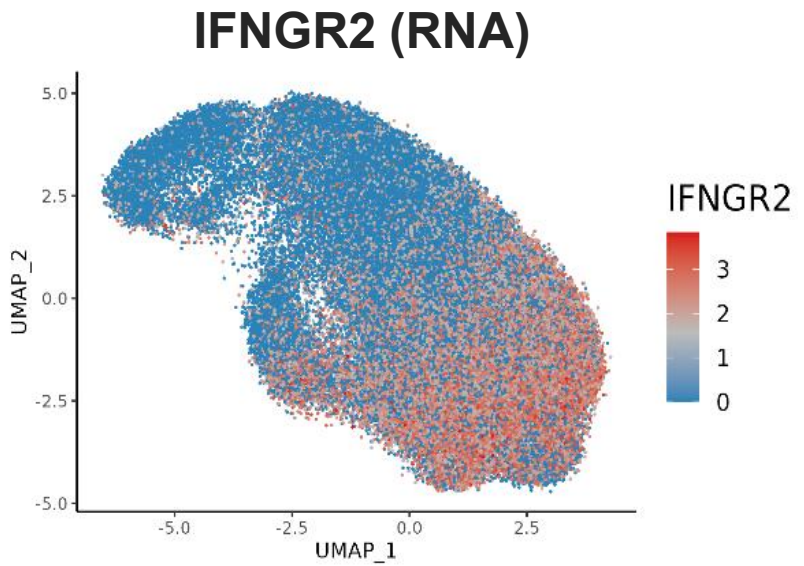
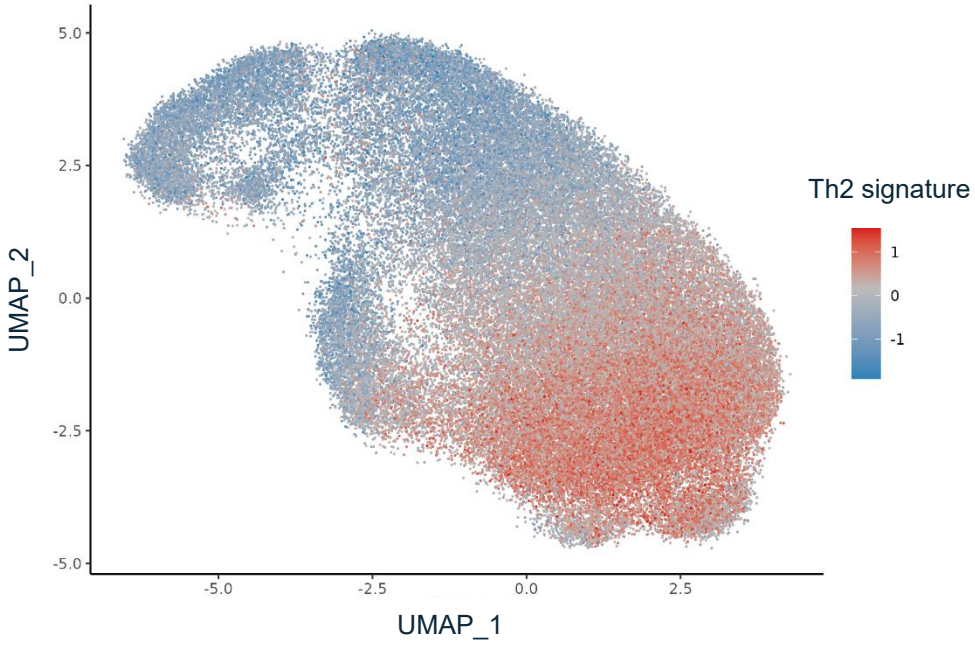




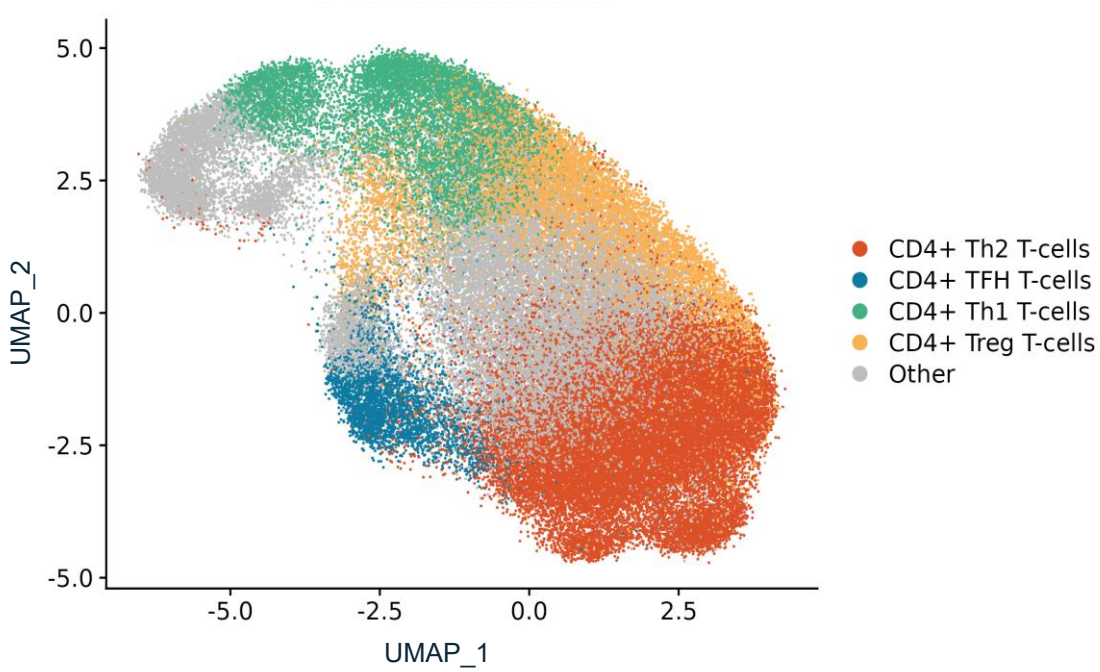


T cell activation and Th2 skewing affect transcriptomic signatures

Th2 subset annotation



T helper subset annotation

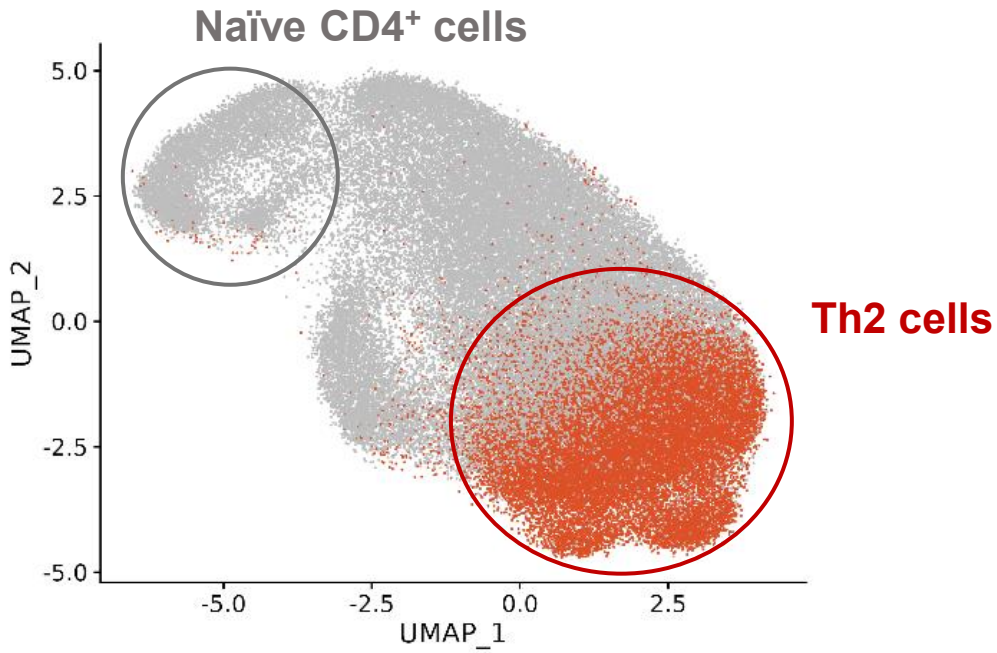


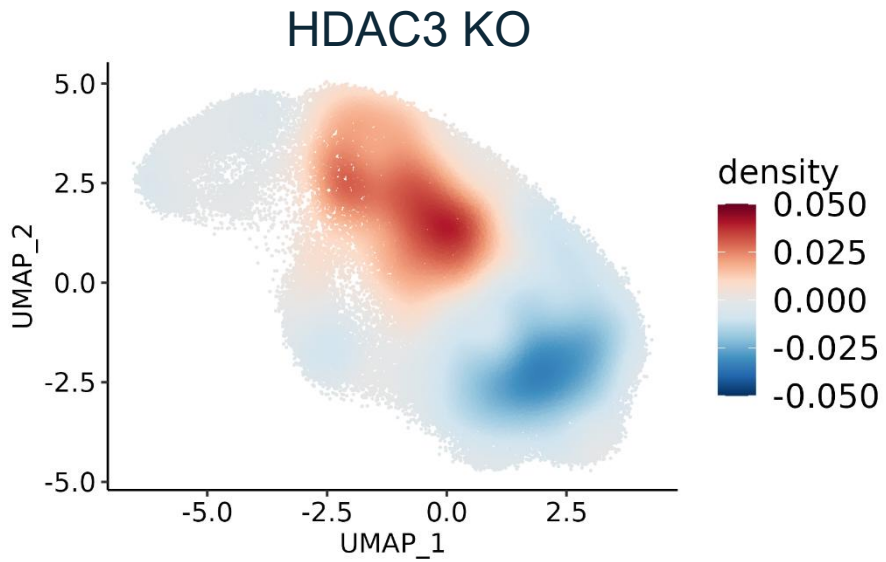
- Additional transcriptomic markers of T helper subset annotation
- Gradients of transcriptomic phenotypes help identify Th1, Th2, Tfh and Treg subsets
- Up to 40 individual markers considered for plasticity signatures

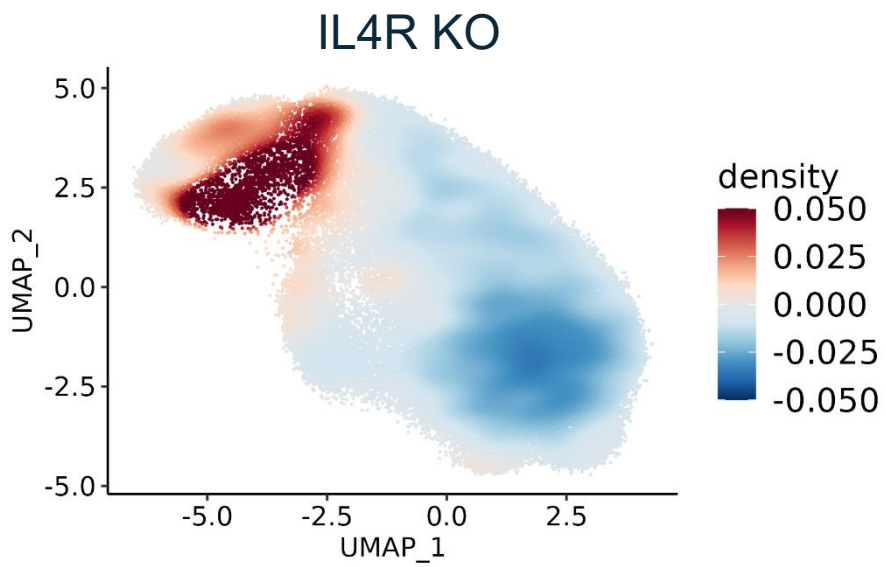
Gene knockouts depleted in differentiated Th2 cells

myllia.

1. Isolate primary human CD4⁺ T cells from healthy donors
2. Transduce with lentiviral sgRNA library and Cas9
3. Skew T cells towards the Th2 lineage
4. Perform single-cell RNA sequencing (scRNA-Seq)
5. Identify Th2 cells
6. Assess knockout (KO) phenotypes





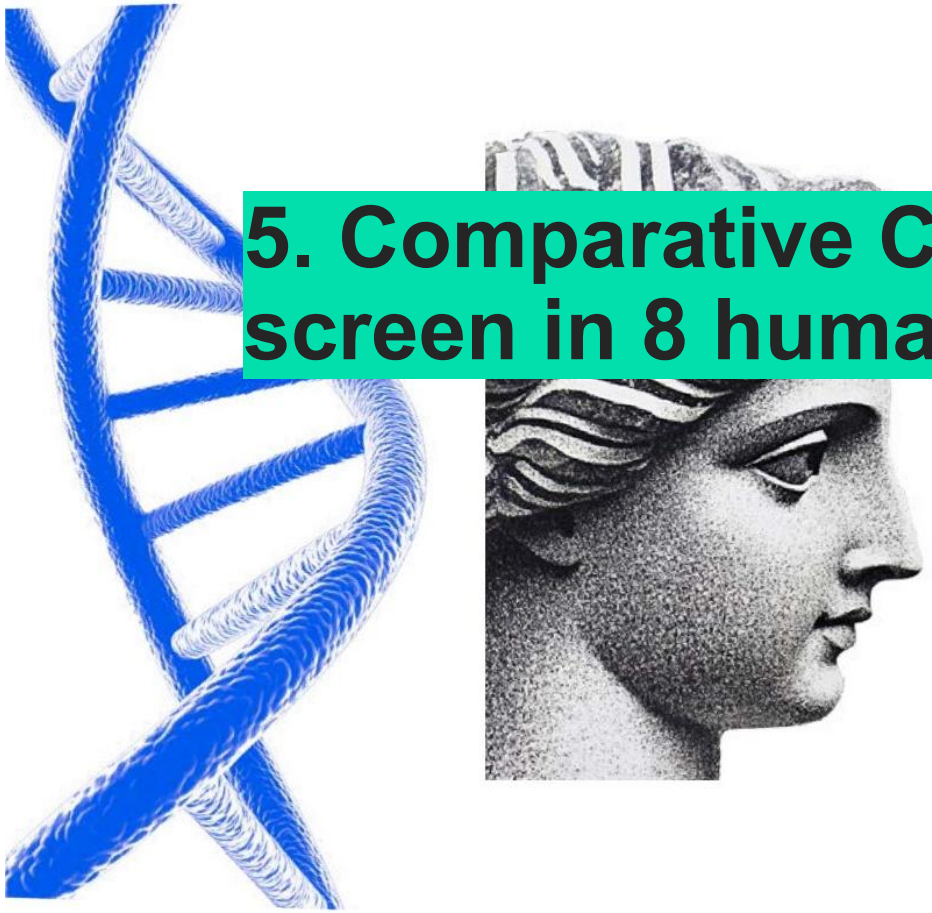


Conclusions: CRISPR screens in primary human immune cells

– Proven track record for single-cell CRISPR screens in primary human immune cells

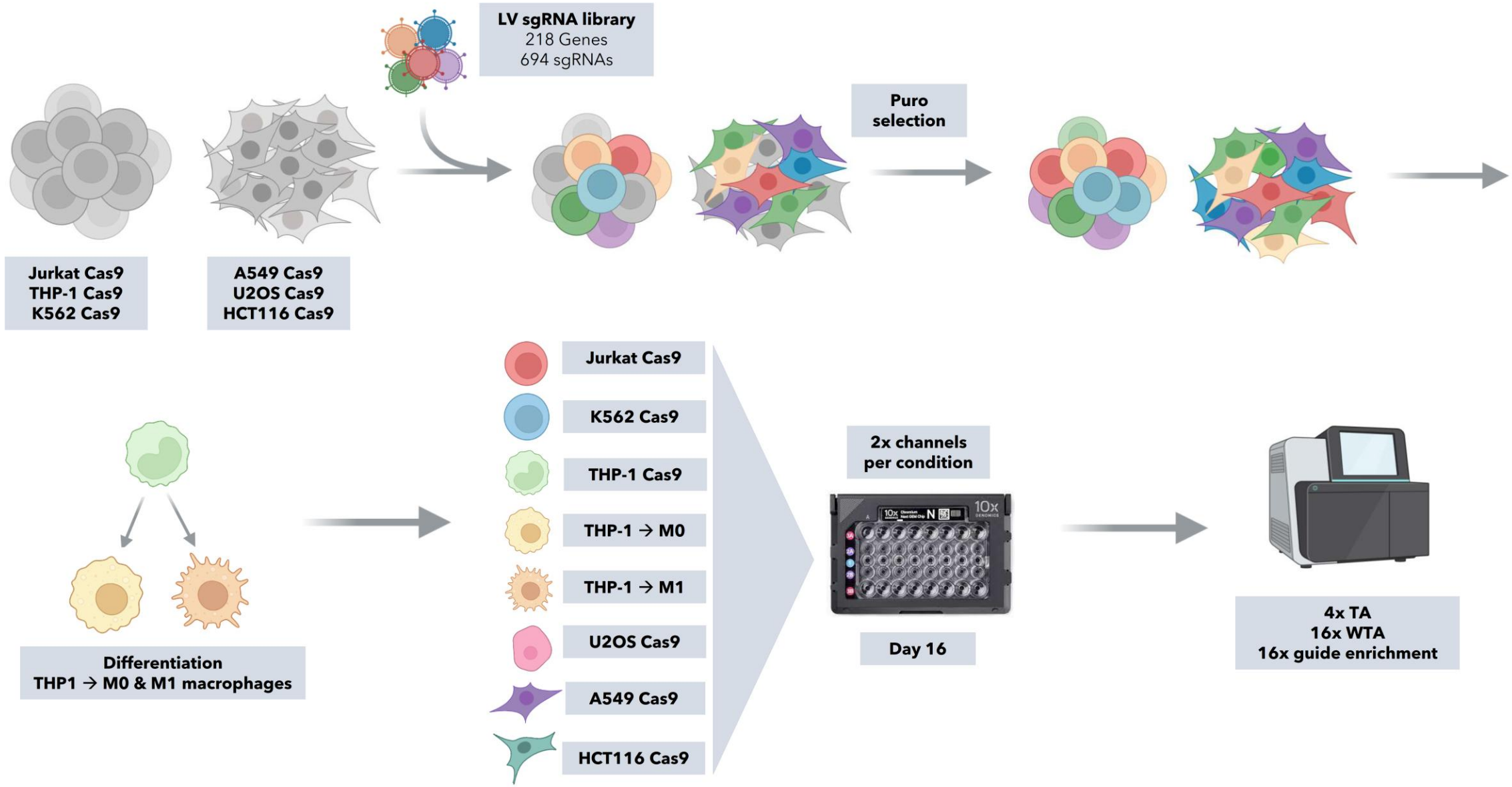
- Proprietary algorithms for sgRNA design
- Proprietary algorithms for design of multiplex primer panels for targeted sequencing
- CROP-Seq is ready for genome-scale in cell lines
- CROP-Seq in primary human T cells and myeloid cells (DCs and macrophages)

– Identify or validate drug targets in cancer cells and primary human T cells
– Identify modulators of CAR-T or TCR-T cell therapies to enhance T cell function
– Unravel the mechanism of disease-associated genes and immune pathways
– Decipher the mechanism of action (MoA) of drugs



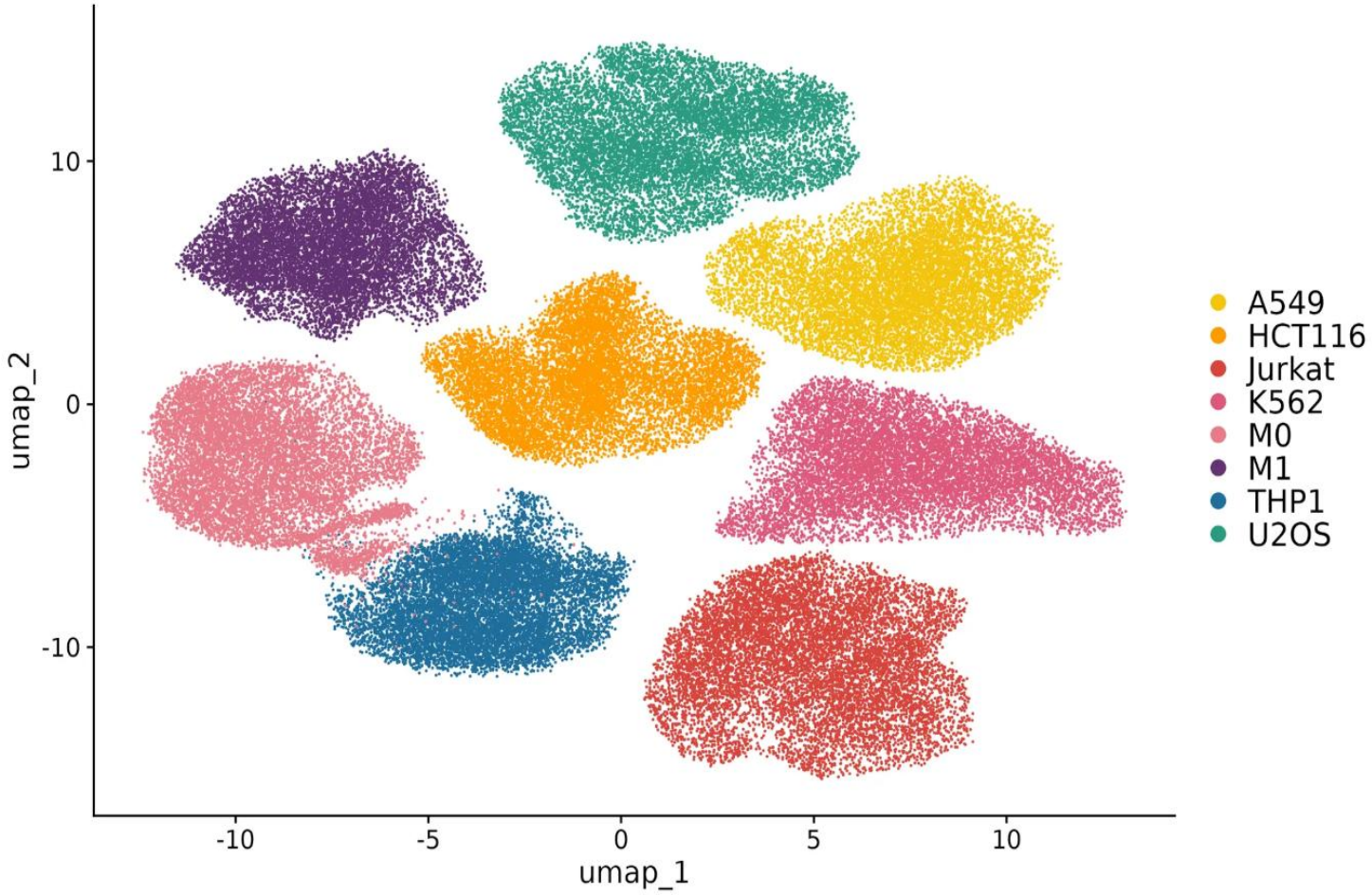
5. Comparative CROP-Seq screen in 8 human cell lines

A sizeable CROP-Seq screen performed across 8 human cell lines

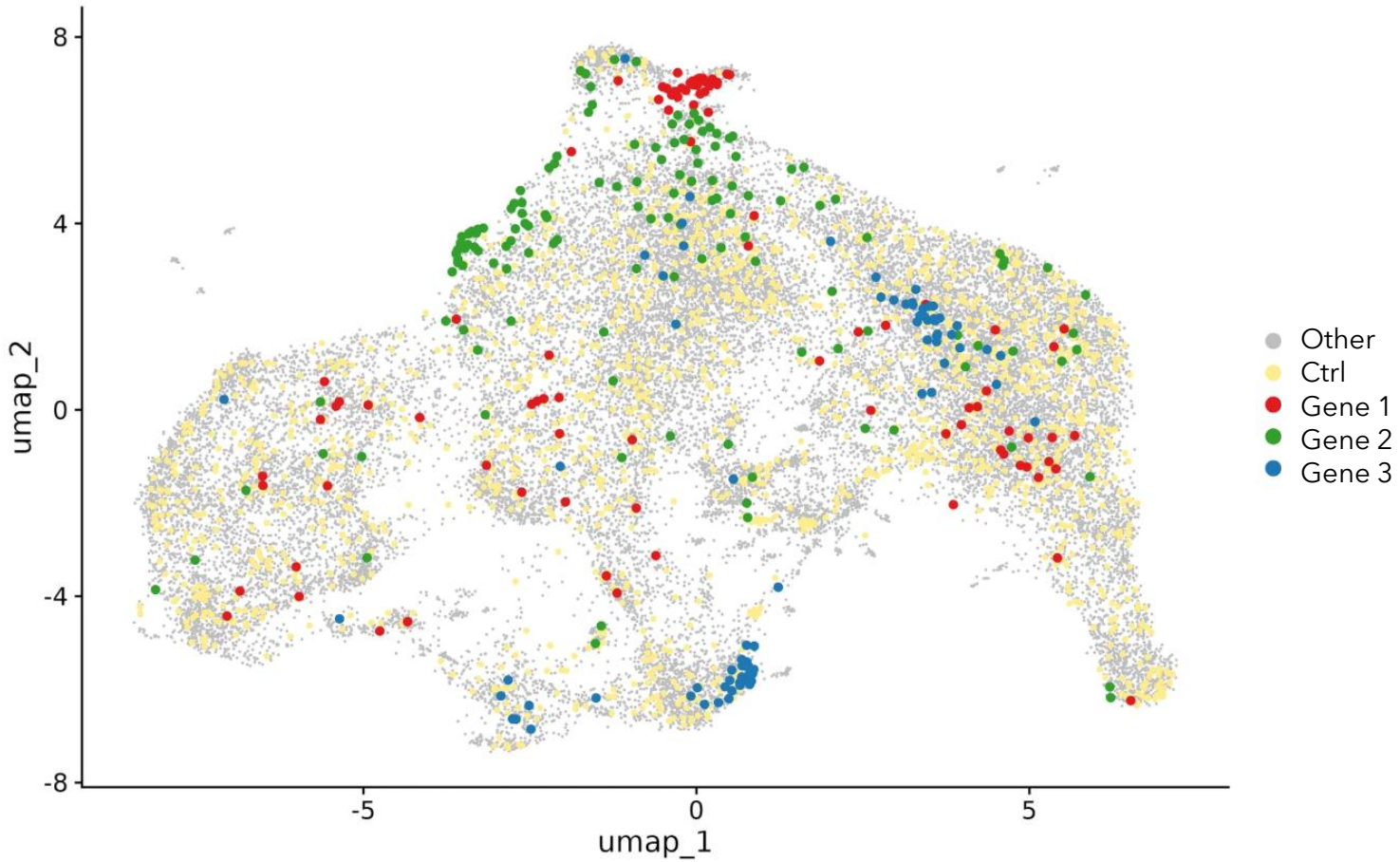


UMAP plot of all experimental conditions across cell lines

- Comparative CROP-Seq screen in 6 cell lines:
  - A549
  - HCT116
  - Jurkat
  - K562
  - THP-1
  - U2OS
- One cell line in 3 conditions: THP-1
  - THP-1 monocytes,
  - THP-1 M0 macrophages,
  - THP-1 M1 macrophages
- 218 target genes perturbed using Cas9
- Unbiased whole transcriptome amplification
- Almost 300.000 single-cell transcriptomes
- Queried phenotypes:
  Cell-type specific perturbation responses and differential gene expression



Transcriptomic phenotypes of distinct gene knockouts



Data sharing and CROP-Seq documentation

– Details about cell type

- Monoclonal cells? Polyclonal cells? Primary cells?

– Experimental details

- Low MOI screen? / high MOI screen?
- Modality
- # of target genes
- # of sgRNAs per gene
- # of NT/gene desert controls

– Details of the single cell experiment

- Which chemistry was used?
- # of cells that were loaded and retrieved
- # of good quality cells after the analysis
- Cell hashing?

– Which libraries were prepared for NGS?

- NGS details: Which flow cell was used? Which read mode?

– Files to be transferred

- Raw data (fastq files)
- Count matrices and/or Seurat objects (including sgRNAs assigned to cells)