JEM Journal of Experimental Medicine

PERSPECTIVE

Next-generation CRISPR screens enable causal systems immunology

Hao Shi[1] and Hongbo Chi[1]

Mapping the causal circuits that shape the phenotypic and functional landscape of immune cells remains a formidable challenge. Recent advances in pooled CRISPR-based screens, coupled with multiplexed single-cell profiling and imaging-based spatial readouts, make this goal increasingly attainable. In this Perspective, we discuss how CRISPR-based genetic screens will fundamentally transform our understanding of immunobiology. We highlight the applications of state-of-the-art, high-throughput pooled perturbation approaches, including emerging methodologies for bulk, single-cell, and spatial CRISPR screens, to advance our understanding of immunity and in vivo biology. Additionally, we summarize new strategies to address the complexity of combinatorial perturbations to uncover genetic interactions and mechanistic drivers of immunity at unprecedented scale and resolution. By integrating CRISPR screening data with experimental insights, we advocate a new framework in immunology research that leverages perturbation-driven regulatory effects and networks to discover new therapeutic targets and establish causal systems biology and immunology for advancing immunological knowledge and therapeutic application.

Introduction

CRISPR screens have emerged as powerful tools for functional genomics, enabling the dissection of mechanisms governing immune cell differentiation and function, as well as the identification of regulators mediating cell–cell interactions, particularly in physiologically relevant settings in vivo (Shi et al., 2023). However, the intrinsic heterogeneity of immune cell populations and the context-dependent rewiring of gene regulatory networks present challenges for traditional bulk CRISPR screens. Single-cell CRISPR (scCRISPR) and spatial CRISPR approaches help overcome these limitations by generating rich, multimodal datasets with transcriptomics, chromatin accessibility, proteomics, or spatial information following genetic perturbations at scale. Altogether, we propose that these technologies will facilitate the discovery of master regulator genes, elucidation of downstream mechanisms, mapping of causal biological circuits, and exploration of non–cell-autonomous effects, thereby accelerating therapeutic engineering and clinical translation (Fig. 1 A).

New platforms for bulk CRISPR screens in immune cells

New CRISPR technologies uncover regulators of T cell–mediated immunity

CRISPR/Cas-based technologies have allowed for precise perturbations across the genome (Anzalone et al., 2020), epigenome (Nakamura et al., 2021), and transcriptome (Villiger et al., 2024), including in immune cells (Shi et al., 2023). These tools were effectively applied to primary T cells in vitro (Shi et al., 2023), where bulk CRISPR knockout (KO) screens focused on robust readouts, such as T cell activation, proliferation, mTORC1 signaling, cytokine release, and lineage-defining factor (e.g., Foxp3) expression (Arce et al., 2024; Carnevale et al., 2022; Chen et al., 2025b; Long et al., 2021; Shifrut et al., 2018; Umhoefer et al., 2025) (Fig. 1 B). In contrast to KO screens, gene activation or repression via CRISPR activation (CRISPRa) or CRISPR interference (CRISPRi) in primary T cells, which requires stable expression of endonuclease-dead Cas9 (dCas9) that binds DNA without cleaving it, has proven more challenging due to limitations in efficient lentiviral delivery (Schmidt et al., 2022). One study addressed this bottleneck by optimizing protocols for high-titer lentiviral production, enabling the effective delivery of the molecular machinery permissive for CRISPRi or CRISPRa screens in T cells to identify regulators of cytokine production (Schmidt et al., 2022). Another study used a more compact dCas9 variant from *Staphylococcus aureus* to improve its packaging into adeno-associated virus and subsequent delivery into human T cells, compared with the *Streptococcus pyogenes*–derived dCas9 commonly used for CRISPRi/a screens (McCutcheon et al., 2023). Furthermore, catalytically dead guide RNAs have been employed in Cas9-expressing primary T cells to allow for high-

[1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN, USA.

Correspondence to Hongbo Chi: hongbo.chi@stjude.org.

https://doi.org/10.1084/jem.20241266

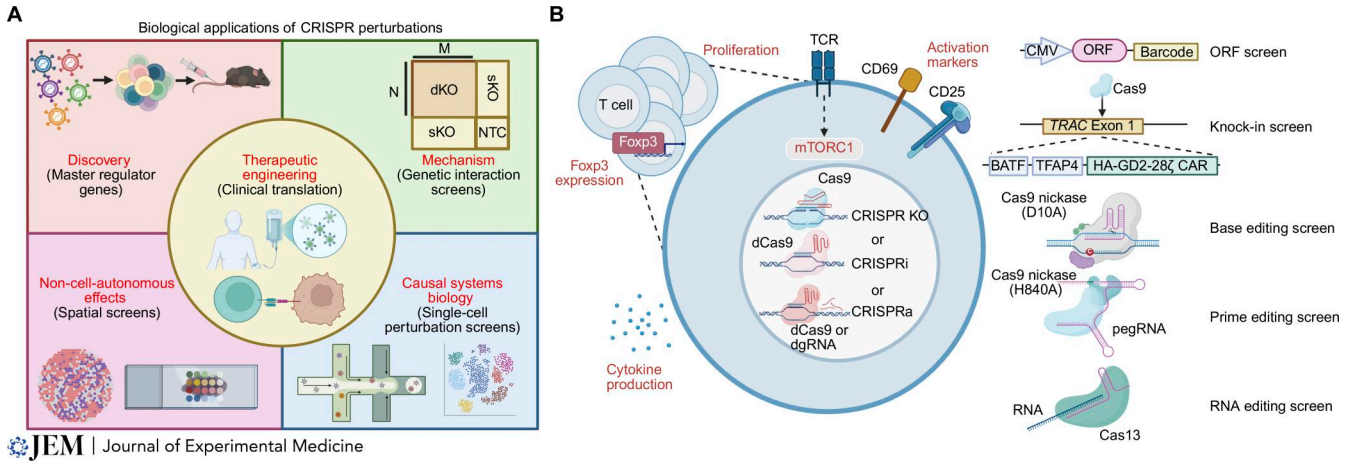

Figure 1. **Biological applications of CRISPR perturbations and expanded modalities of bulk CRISPR screens in T cells. (A)** Biological applications of CRISPR perturbations include the discovery of master regulator genes, identification of downstream mechanisms through genetic interaction screens, mapping of causal biological circuits via single-cell perturbation screens, and investigation of non–cell-autonomous effects through spatial screening approaches, which collectively help facilitate therapeutic engineering. **(B)** Bulk CRISPR-based screening approaches, including CRISPR KO, CRISPRi, and CRISPRa screens, have been widely used in T cells to probe key functional readouts, such as activation (e.g., CD69 and CD25 expression), proliferation, mTORC1 signaling, cytokine production, and Foxp3 expression. Emerging screening strategies, including ORF overexpression, targeted knock-in, base editing, prime editing, and RNA editing, are adding new layers of functional interrogation to these platforms. sKO, single knockout; NTC, nontargeting control; dgRNA, catalytically dead guide RNA; pegRNA, prime editing guide RNA; HA-GD2-28ζ CAR, GD2-targeting CAR with HA tag and CD28-CD3ζ signaling domain.

throughput, gain-of-function screening for factors that enhance their effector functions (Ye et al., 2022). Collectively, these innovations have expanded the CRISPR toolkit for functional interrogation of primary T cells via loss- and gain-of-function approaches that uncover key regulators of T cell activation and function.

For gain-of-function screens, besides CRISPRa approaches, large-scale open reading frame (ORF) and modular knock-in (KI) libraries have been applied in primary T cells. For instance, the overexpression of ∼12,000 barcoded human ORFs in T cells identified lymphotoxin-β receptor, a receptor typically absent in lymphocytes, as a potent driver of transcriptional and epigenomic remodeling to enhance T cell effector function (Legut et al., 2022). Furthermore, a study introduced a modular pooled KI screening platform that employs two libraries—one targeting 100 transcription factors and the other targeting 129 surface receptors—allowing for the combinatorial screening of thousands of synthetic constructs integrated at defined genomic loci alongside clinically relevant T cell receptor (TCR) or chimeric antigen receptor (CAR) genes. These screens identified multigene pairs, such as TFAP4 and BATF, whose combined KI enhances T cell fitness (Blaeschke et al., 2023). Overall, these studies highlight large-scale, gain-of-function screens as powerful methods to discover key drivers capable of reprogramming T cell states with enhanced durability and therapeutic functions.

Recently, pooled base editor and prime editor screens have emerged as tools for in situ mutational scanning (Anzalone et al., 2019; Lue and Liau, 2023; Rees and Liu, 2018; Ren et al., 2023). These approaches allow for precise nucleotide substitutions at gRNA-targeted loci without introducing double-strand breaks or relying on homology-directed repair (HDR). In human T cells, base editing is permissive for multiplexed gene deletion while minimizing the risk of chromosomal translocations associated with double-strand break-based methods, advancing their utility in clinical-stage CAR T cell products (Diorio et al., 2022). A recent CRISPR base editor screen targeting protein-coding regions in 385 genes revealed functional insights into specific protein domains and amino acid residues that regulate T cell activation and cytokine production (Schmidt et al., 2024). In parallel, advances in prime editing have improved editing efficiency in immune cells. For instance, fusing the prime editor protein PE7 to the RNA-binding domain of the exonuclease protection factor La markedly enhances prime editing mediated by gRNAs in human T cells (Yan et al., 2024). Moreover, ongoing efforts include the use of base editing (Katti et al., 2024) and prime editing (Ely et al., 2024) to introduce somatic mutations in mouse models, laying the groundwork for future in vivo functional screening in immune cells.

Compared with DNA-targeted CRISPR screens, gRNA-based tools for manipulating RNAs have emerged more recently (Abudayyeh et al., 2017). Unlike CRISPR-mediated genome editing, Cas13d-mediated transcriptome perturbation, which is achieved without introducing DNA breaks, offers a potentially safer and more precise alternative. Recently, screens using the multiplexed effector guide array screening platform, which allows for programmable and scalable regulation of the transcriptome via CRISPR/Cas13d, uncovered paired regulators of T cell function (Tieu et al., 2024). Of note, T cells can produce pro-inflammatory cytokines upon recognition of Cas13d, which should be taken into consideration when applying Cas13d-based therapeutic strategies (Tang et al., 2022).

Unleashing the full potential of in vivo CRISPR screening

In vivo CRISPR screens face additional challenges beyond those encountered in vitro, including bottlenecks imposed by engraftment efficiency and clonal representation, which can

substantially limit effective library coverage (Shi et al., 2023). These constraints are particularly pronounced in hematopoietic stem and progenitor cell–based systems, where only a small fraction of transplanted cells successfully engraft, self-renew, and contribute to long-term hematopoiesis, amplifying stochastic loss of gRNAs early in the experiment. Furthermore, biological variability is often increased in vivo due to heterogeneous microenvironments, niche competition, immune interactions, and host-dependent factors, complicating quantitative interpretation. The development of Cas9 KI mice was highly instrumental for performing in vivo KO screens (Chu et al., 2016; Platt et al., 2014). Among immune cells, the majority of in vivo CRISPR screens have been performed in T cells, often leveraging adoptive transfer models of antigen-specific T cells followed by antigen stimulation to induce robust expansion and selection of donor-derived T cells (Shi et al., 2023). In $CD8^+$ T cells, bulk CRISPR screens have been employed to uncover regulators of T cell accumulation and antitumor activity (Baxter et al., 2023; Belk et al., 2022; Chen et al., 2021; Dong et al., 2019; Guo et al., 2022; Huang et al., 2021; LaFleur et al., 2025; Raynor et al., 2024; Wei et al., 2019; Ye et al., 2019). Similar approaches have been used to study $CD4^+$ T cell differentiation (Fu et al., 2021; Huang et al., 2022; Sugiura et al., 2022; Sutra Del Galy et al., 2021) and migration (Kendirli et al., 2023). More recently, an in vivo CRISPR screen in primary human T cells utilized tumor cells overexpressing a CD3 scFv, which facilitates efficient expansion and recovery of adoptively transferred human T cells from immunodeficient mice, thereby achieving high-cell-coverage screening with genome-scale libraries (Liu et al., 2025a, *Preprint*). Beyond T cells, in vivo CRISPR screens have been extended to natural killer (NK) cells, dendritic cells (DCs), and macrophages. CAR-NK therapies have demonstrated clinical success against hematological malignancies and show efficacy in preclinical solid tumor models (Peng et al., 2024b), with genome-scale CRISPR screening already performed in primary human NK cells in vitro to identify targets for enhancing antitumor potency (Biederstadt et al., 2025). Moreover, in vivo CRISPR screens uncover key genetic checkpoints (e.g., CALHM2) that modulate CAR-NK cell activity against tumors (Peng et al., 2024a). In addition, genome-wide, in vivo CRISPR screening in bone marrow–derived DCs transferred into tumors has revealed key modulators (e.g., PDE5) of DC migration to boost antitumor function (Tang et al., 2025a). Moreover, in vivo CRISPR screening targeting cytokine signaling pathways has uncovered genes that regulate the migration and polarization of macrophages in mouse models of multiple sclerosis (de la Rosa et al., 2026). Together, these studies highlight the growing utility of in vivo CRISPR screening in adoptive cell transfer models to uncover key regulators of immune cell function and therapeutic efficacy.

Beyond CRISPR screens via adoptive transfer systems, direct in vivo CRISPR screens offer opportunities to establish gene function in immune cell types that are challenging to manipulate ex vivo. A major limitation of this approach, however, lies in the efficient and cell type–specific delivery of genetic materials, including Cas9 and gRNAs, as no single delivery system is universally applicable. To expand the range of immune cell types amenable to in vivo CRISPR screens, the chimeric immune editing (CHIME) approach was developed using bone marrow–derived hematopoietic stem cells from Cas9-expressing mice to facilitate gene deletion in both innate and adaptive immune cell lineages (LaFleur et al., 2019). For example, CHIME has identified master regulators in tumor-infiltrating Treg cells, which are typically rare and challenging to study (Obradovic et al., 2023). The new X-CHIME platforms, including L-CHIME for lineage-specific gene deletion and I-CHIME for inducible temporal gene perturbation, further extend the original strategy (LaFleur et al., 2024). In addition, in vivo CRISPR delivery, such as inducible mosaic animal for perturbation (iMAP), streamlines the gRNA preparation and delivery steps and allows for simultaneous assessment of genetic perturbations in vivo under the homeostatic state (Liu et al., 2022). These tools will be highly instrumental for expanding the applications of in vivo CRISPR screens to endogenous T and B cells, along with macrophages and neutrophils.

scCRISPR screens to uncover immune heterogeneity and causal systems immunology

The immune system is characterized by cellular diversity and heterogeneity governed by a multitude of extrinsic and intrinsic factors in vivo (Chi et al., 2024). scCRISPR screening technologies now facilitate systematic dissection of cellular heterogeneity, allowing researchers to map gene regulatory networks and uncover how distinct perturbations influence diverse cellular states at high resolution (Fig. 2).

scCRISPR screens using transcriptome changes as readouts

Pioneering scCRISPR screening methods—such as Perturb-seq (Adamson et al., 2016; Dixit et al., 2016), CRISP-seq (Jaitin et al., 2016), and Mosaic-seq (Xie et al., 2017)—expanded screening readouts beyond simple measurements (e.g., cell fitness or reporter expression) to high-resolution profiling of transcriptional states. These methods typically utilize a separate barcode sequence with a poly(A) tail to indirectly capture the gRNAs. However, one major obstacle was undesirable recombination during lentiviral packaging and delivery, which could uncouple gRNA sequences from their corresponding barcodes (Hill et al., 2018; Xie et al., 2018). To circumvent this constraint, CROP-seq was developed as an alternative strategy; it allows gRNA expression and polyadenylated transcript detection by 3′-based single-cell RNA-sequencing (scRNA-seq) (Datlinger et al., 2017). However, the long terminal repeat modifications in CROP-seq may reduce viral titers, potentially requiring further optimization to perform effective screening in biological applications (Liscovitch-Brauer et al., 2021). Furthermore, gRNA capture efficiency remains a major limitation, with recent studies reporting capture rates of ∼30%, increasing to ∼50% with optimization in mouse T cells using droplet-based platforms (Enk et al., 2024). To overcome these challenges, newer strategies such as ECCITE-seq (Mimitou et al., 2019) and direct-capture Perturb-seq (Replogle et al., 2020) have been developed. These methods conduct direct sequencing of gRNAs together with transcriptome profiling via multiple

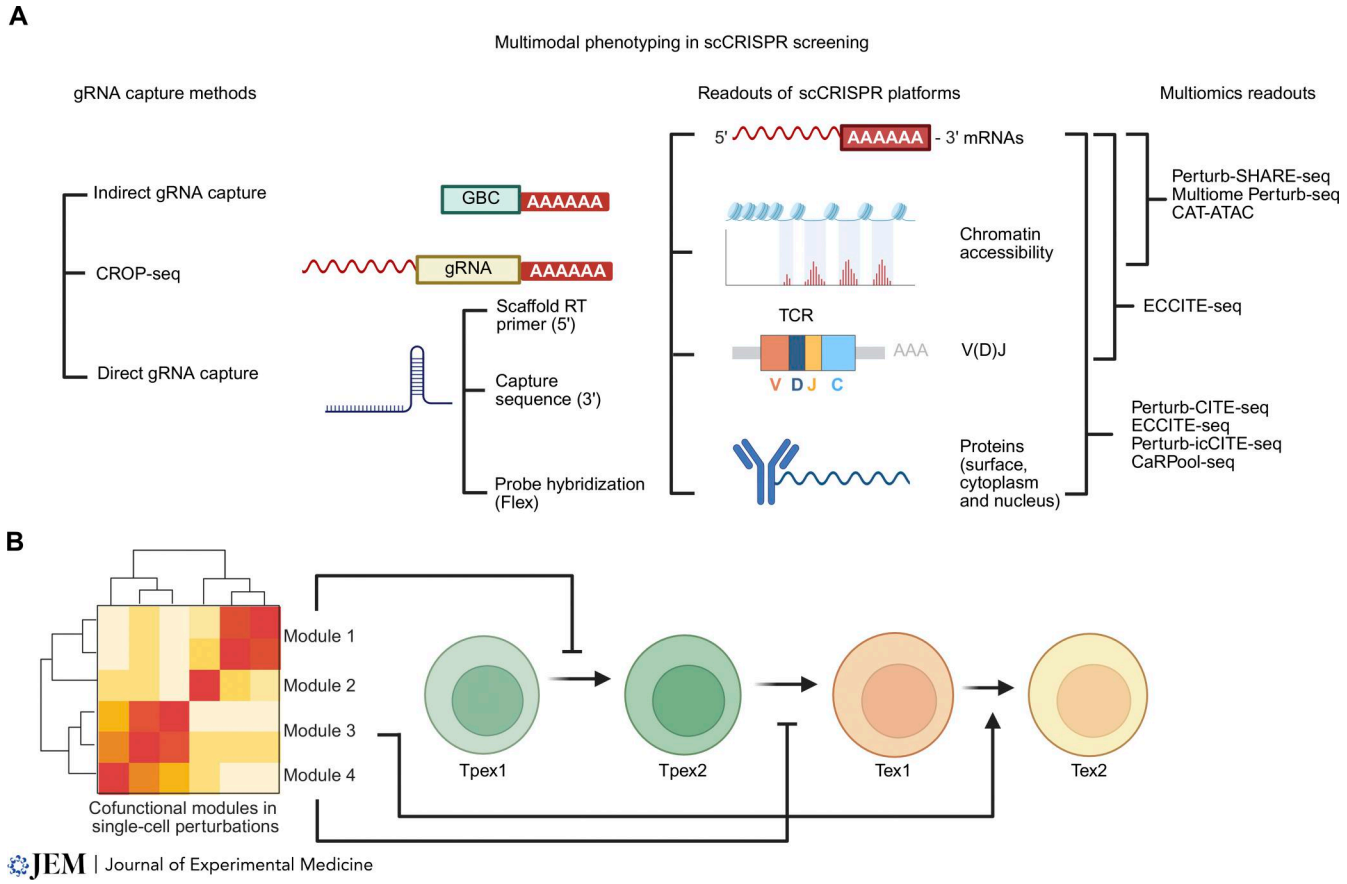

Figure 2. **Multimodal phenotyping in scCRISPR screening and analysis. (A)** scCRISPR screening technologies differ in their strategies for capturing gRNAs. Indirect gRNA capture methods use a separate barcode sequence with a poly(A) tail to infer gRNA identity. CROP-seq improves upon this method by integrating the U6 promoter and gRNA cassette into the 3′ long terminal repeat of the lentiviral vector, which is duplicated upon integration, enabling both functional gRNA expression and direct detection of a polyadenylated transcript. In contrast, direct gRNA capture methods allow simultaneous sequencing of gRNAs and transcriptomes using (1) specific primers codelivered with capture beads (5′ strategies), (2) capture sequences linked to oligo-dT beads (3′ strategies), or (3) probe hybridization, as used in the Flex assay. scCRISPR platforms can incorporate diverse readouts, including mRNA expression, chromatin accessibility, V(D)J sequencing of TCRs, and protein abundance. Platforms with multiomic readouts are highlighted. **(B)** scCRISPR screening of transcription factors paired with transcriptome profiling identifies cofunctional gene modules that regulate differentiation trajectories of CD8⁺ T cell subsets in cancer, including precursor exhausted-like state 1 (Tpex1), Tpex2, terminal exhausted-like state 1 (Tex1), and Tex2 populations. GBC, guide barcode; RT, reverse transcription.

strategies, including incorporating specific capture primers alongside the beads (5′ strategies), linking capture sequences to oligo-dT beads (3′ strategies), or probe hybridization in the 10X Genomics Flex assay (Saunders et al., 2025). These direct-capture approaches greatly enhance gRNA detection efficiency (Zhou et al., 2023), improving the scalability and resolution of scCRISPR screens (Fig. 2 A).

scCRISPR screens decode immune cell heterogeneity and causal networks

In innate immunity, Perturb-seq and CRISP-seq studies were performed to map transcriptional regulatory circuits underlying bone marrow–derived myeloid cell responses upon LPS stimulation and in vivo myeloid cell development. By grouping genetic perturbations based on their global effects on downstream gene programs rather than relying on predefined markers, these studies revealed how transcription factors drive myeloid cell heterogeneity (Dixit et al., 2016; Jaitin et al., 2016). In addition, an arrayed scCRISPR screening platform in primary mouse macrophages was established to reveal causal regulatory circuits and master switches (e.g., Zeb2) of tumor-associated macrophages (Sheban et al., 2025). Recently, integrated CROP-seq and CITE-seq assays in a mouse macrophage cell line enabled in-depth dissection of gene regulatory programs in response to *Listeria* infection (Traxler et al., 2025). In human cells, a CROP-seq screen using an iPSC-derived microglial differentiation model identified distinct regulators of microglial developmental states (Drager et al., 2022), highlighting that scCRISPR-based approaches unravel complexities of innate immune responses.

CROP-seq has been successfully applied for scCRISPR screens in human T cells (Datlinger et al., 2017; Datlinger et al., 2025; Shifrut et al., 2018; Tsuchida et al., 2023; Chen et al., 2025b) and mouse CD4⁺ T (Enk et al., 2024) and CD8⁺ T cells (Pretto et al., 2025). More recently, direct gRNA capture methods have become increasingly prevalent in T cell studies (Belk et al., 2022; Schmidt et al., 2022; Zhou et al., 2023; Liu et al., 2024; Fagerberg et al., 2025; Knudsen et al., 2025). These single-cell perturbation approaches have primarily focused on identifying regulators of

T cell activation and Foxp3 expression in vitro, as well as exhaustion and effector/memory differentiation in vivo. For example, targeting negative regulators of T cell proliferation, such as CD5, RASA2, and SOCS1, enhances expression of activation markers, cell cycle genes, and effector molecules in response to TCR stimulation (Shifrut et al., 2018). To improve gRNA delivery in mouse T cells for in vivo screening, we modified the standard lentiviral system to a retroviral vector, enabling efficient gRNA transduction and direct capture in mouse $CD8^+$ T cells. This platform was used to dissect transcription factor–mediated regulatory networks underlying stemness, proliferation, effector differentiation, and exhaustion of intratumoral $CD8^+$ T cells, as well as key transcriptional checkpoints for $CD8^+$ T cell differentiation in tumors (Zhou et al., 2023). Our findings highlight two central strategies for enhancing antitumor immunity: promoting the exit of precursor exhausted cells from quiescence and enriching the proliferative exhausted cell state. Our study also provides a causal systems biological framework to integrate cell fate regulomes with reprogrammable determinants of T cell function in cancer (Fig. 2 B). Beyond tumor models, scCRISPR screens have also been used to generate a "differentiation space map" of individual $CD8^+$ T cells in acute infection. This approach elucidated relationships between T cell states and identified key regulators of the memory-to-effector lineage trajectory, such as KLF2 that maintains effector lineage fidelity (Fagerberg et al., 2025). Notably, current scCRISPR studies have been largely limited to T cells. There continues to be strong potential to extend these approaches to other lymphocyte subsets and myeloid populations to further elucidate immune regulation across diverse contexts.

Due to the high cost of scCRISPR screens, many studies have applied these approaches in a limited scale, following bulk CRISPR screens to validate the transcriptomics effects of top candidate genes in T cells (Belk et al., 2022; Chen et al., 2025b; Knudsen et al., 2025; Schmidt et al., 2022; Shifrut et al., 2018). Recent advances in scCRISPR technologies have expanded the scale of these experiments to screening thousands of genes (Geiger-Schuller et al., 2023, *Preprint*) or the entire genome (Replogle et al., 2022). Experimental scalability is further enhanced through new strategies, such as targeted Perturb-seq (Schraivogel et al., 2020; Song et al., 2025), and by incorporating dual gRNAs within a single vector to improve gene perturbation efficiency (Replogle et al., 2020; Zhou et al., 2023). In parallel, recent innovations in combinatorial indexing and next-generation sequencing have allowed for the study of combinatorial perturbations at single-cell resolution (Datlinger et al., 2021; Jiang et al., 2025). Other approaches introduce multiple gRNAs into individual cells via increased multiplicity of infection (Gasperini et al., 2019; Yao et al., 2024). Whether these approaches can be effectively used in primary immune cell screening remains to be determined.

scCRISPR screens using epigenome or proteome changes as readouts

Beyond scRNA-seq, the functional readouts for scCRISPR screens include changes in chromatin accessibility—such as Perturb-ATAC (Rubin et al., 2019), CRISPR-sciATAC (Liscovitch-Brauer et al., 2021), and Spear-ATAC (Pierce et al., 2021)—as well as protein abundance, as demonstrated by CyTOF-based screens (Wroblewska et al., 2018). Recent advances have permitted joint profiling of transcriptomes with additional modalities for multiomic readouts of cell states, such as protein expression via Perturb-CITE-seq (Frangieh et al., 2021), ECCITE-seq (Mimitou et al., 2019; Papalexi et al., 2021), Perturb-icCITE-seq (Chen et al., 2025b), and CaRPool-seq (Wessels et al., 2023), or chromatin accessibility via Perturb-SHARE-seq (Otto et al., 2023), Multiome Perturb-seq (Metzner et al., 2025), or CAT-ATAC (Shevade et al., 2025). These integrative approaches provide deeper insights into how genetic perturbations influence gene regulation, chromatin remodeling, and protein expression at single-cell resolution, thereby advancing our understanding of cellular responses to perturbation across regulatory layers (Fig. 2 A).

Multimodal scCRISPR approaches are powerful tools to study the interplay between epigenetic regulation, protein expression, and immune function in different contexts. For example, Perturb-CITE-seq, which integrates transcriptomics and surface protein profiling, uncovered regulatory circuits underlying tumor cell responses to IFN-γ treatment and T cell–mediated killing (Frangieh et al., 2021). This study revealed both IFN-γ–JAK/STAT-dependent and IFN-γ–JAK/STAT-independent cofunctional modules, including a notable role of CD58 in mediating immune resistance independently of IFN-γ signaling (Frangieh et al., 2021). In addition, the recently developed Perturb-icCITE-seq platform was applied in primary human T cells to target candidate genes while simultaneously profiling >300 surface and intracellular epitopes to identify regulators of FOXP3 and other Treg cell–associated proteins (Chen et al., 2025b). Further, Perturb-ATAC identified key regulators of chromatin accessibility, transcription factor occupancy, and nucleosome positioning in human B cells, uncovering the hierarchical network of trans-factors that control B cell state transitions (Rubin et al., 2019). To expand the accessibility and resolution of scCRISPR using ATAC-seq as the readout, two studies leveraging the 10X Genomics Multiome platform have substantially improved gRNA transcript recovery, including Multiome Perturb-seq, which uses an optimized CROP-seq vector (Metzner et al., 2025), and CAT-ATAC, which employs a direct-capture Perturb-seq dual-guide vector (Shevade et al., 2025). These innovations hold promise for the systematic discovery of novel epigenetic regulators of immune cell function.

In summary, these scCRISPR methods support perturbation mapping of genotype–phenotype relationships at large scale and single-cell resolution, thereby enabling causal systems biology and immunology. Nonetheless, their performance in immune cells and in vivo settings is influenced by several technical considerations. For example, these platforms are limited by data sparsity arising from less optimal mRNA (or chromatin accessibility) capture, gRNA barcode loss, inefficient perturbation–barcode linkage (Hill et al., 2018; Xie et al., 2018), and variable editing efficiency across targets. In addition, size effects (systematic differences in total molecular readouts, e.g., total RNA counts, per cell), library complexity, and cell state–dependent transcriptional variability introduce structured noise that increases false negatives and reduces sensitivity for detecting regulators with moderate effects. These factors may collectively

contribute to systematic biases that complicate perturbation assignment and hit identification. Recent experimental (Replogle et al., 2020; Replogle et al., 2022; Zheng et al., 2025; Zhou et al., 2023; Zhu et al., 2025, *Preprint*) and computational (Braunger and Velten, 2024; Heumos et al., 2025; Liu et al., 2025b) advances continue to improve perturbation assignment, noise modeling, and analytical robustness, supporting scalable and more reliable application of scCRISPR-based frameworks.

Spatial CRISPR screens to identify non–cell-autonomous regulators in the TME

The above-described scCRISPR assays can reveal causal gene regulatory networks, albeit without spatial resolution, thereby limiting the ability to decipher non–cell-autonomous regulation within tissues in vivo. For example, inter- or intratumor heterogeneity in cancer cell clones and/or TME composition is likely a major factor for immunotherapy resistance in lung and ovarian cancers (Chen et al., 2025a; Vazquez-Garcia et al., 2022; Wu et al., 2021; Zhang et al., 2018), yet the mechanisms controlling such heterogeneity or tumor–immune interactions are not fully understood. Building on Pro-Code, a combinatorial protein barcode system for identifying gRNA expression in individual cells (Wroblewska et al., 2018), the spatial functional genomics platform Perturb-map was developed to interrogate how tumor clonality influences the spatial organization of the TME in a lung cancer model. This approach revealed that *Tgfbr2* deficiency in cancer cells promotes TME remodeling and immune exclusion (Dhainaut et al., 2022) (Fig. 3). Beyond TGF-β and its receptor, cancer cells also interplay with the TME via additional ligand–receptor interactions. A Perturb-map screen focused on putative ligands and receptors involved in tumor–macrophage communication revealed that interleukin-4 promotes resistance to anti-PD-1 therapy (Mollaoglu et al., 2024). Therefore, heterogeneous TMEs emerge from localized changes in the expression of specific cancer-derived extracellular factors that establish distinct immune neighborhoods.

While Perturb-map successfully integrates transcriptomics, CRISPR perturbation, and spatial information, the use of protein barcodes reduces compatibility with large-scale pooled screening workflows, and the limited resolution of the 10X Genomics Visium platform may restrict its applicability to spatial CRISPR screens that require higher resolution. There remains a need for scalable tools that combine single-cell-resolution spatial transcriptomics or other spatial readouts with in situ gRNA detection. A recently developed technology, Perturb-FISH, couples MERFISH with localized amplification of the gRNA region, enabling spatial decoding of both perturbations and transcriptome changes (Binan et al., 2025) (Fig. 3). When applied in a human melanoma xenograft model with engrafted peripheral blood mononuclear cells, Perturb-FISH revealed how tumor cell–intrinsic perturbations influence gene expression in tumor-infiltrating lymphocytes, offering insights into mechanisms of cancer cell–mediated T cell suppression (Binan et al., 2025). Several additional approaches—including CRISPRmap (Gu et al., 2024), PerturbView (Kudo et al., 2024), NIS-Seq (Fandrey et al., 2025), and the Perturb-DBiT (Fan et al., 2025, *Preprint*) and RCA-MERFISH (Saunders et al., 2025) platforms—conduct simultaneous spatial transcriptome or multiplexed optical phenotyping and gRNA detection on the same tissue section for spatial CRISPR screening. These methods differ in how they capture gRNAs: through engineered expression constructs with barcode positioning (Gu et al., 2024), T7 promoter–driven in situ amplification (Binan et al., 2025; Fandrey et al., 2025; Kudo et al., 2024), or direct detection following in situ polyadenylation (Fan et al., 2025, *Preprint*). Among spatial CRISPR methods with transcriptomics readouts, FISH-based approaches, such as Perturb-FISH and RCA-MERFISH, achieve subcellular resolution and provide higher spatial precision than Perturb-DBiT, which can be applied at single-cell pixel resolution (10 μm). Moreover, the spatial resolution of PerturbView is dictated by the underlying spatial transcriptomics platform (e.g., Xenium). Importantly, although Perturb-DBiT does not achieve subcellular resolution, it offers distinct advantages, including whole-transcriptome and noncoding RNA detection, compatibility with both formalin-fixed paraffin-embedded and fresh-frozen tissues, and scalability to genome-wide CRISPR libraries. Notably, Perturb-DBiT using a genome-scale CRISPR library identified enrichment of Regnase-1 gRNAs in lymphoid structure-adjacent tumor regions with low PD-L1 expression, suggesting that Regnase-1 loss not only boosts $CD8^+$ T cell function (Wei et al., 2019) but may also impair tumor immune evasion (Fan et al., 2025, *Preprint*). These emerging spatial screening tools illustrate the cellular and molecular architecture of the TME, and will be insightful for advancing next-generation immunotherapies. While spatial CRISPR screening platforms allow for unprecedented integration of genetic perturbation, spatial context, and cellular state, they also face technical challenges, including ambiguous mapping between perturbations and cellular phenotypes, spatial mixing and misassignment artifacts, limited temporal resolution from static snapshots, and the inherent difficulty of disentangling direct cell-intrinsic genetic effects from non–cell-autonomous influences. Resolving these limitations will improve accurate mapping between genetic perturbations and spatial phenotypes and also facilitate systematic discovery of spatially encoded regulatory mechanisms that shape immune responses and therapeutic outcomes in vivo.

Combinatory screens for multiplexed perturbations in immunotherapy

The immunosuppressive nature of the TME necessitates combinatorial strategies to achieve effective immunotherapy (Havel et al., 2019); however, identifying optimal cotargets within tumor or immune cells remains a major challenge. For instance, uncovering synergistic interactions involving regulators of tumor immune evasion or T cell effector function often requires large- or genome-scale genetic interaction screens performed in both wild-type and "query" mutant tumor cells to reveal context-specific vulnerabilities (Aregger et al., 2020; Lawson et al., 2020; Raynor et al., 2024; Wei et al., 2019; Yuan et al., 2025). While such query-based CRISPR screens have unbiasedly

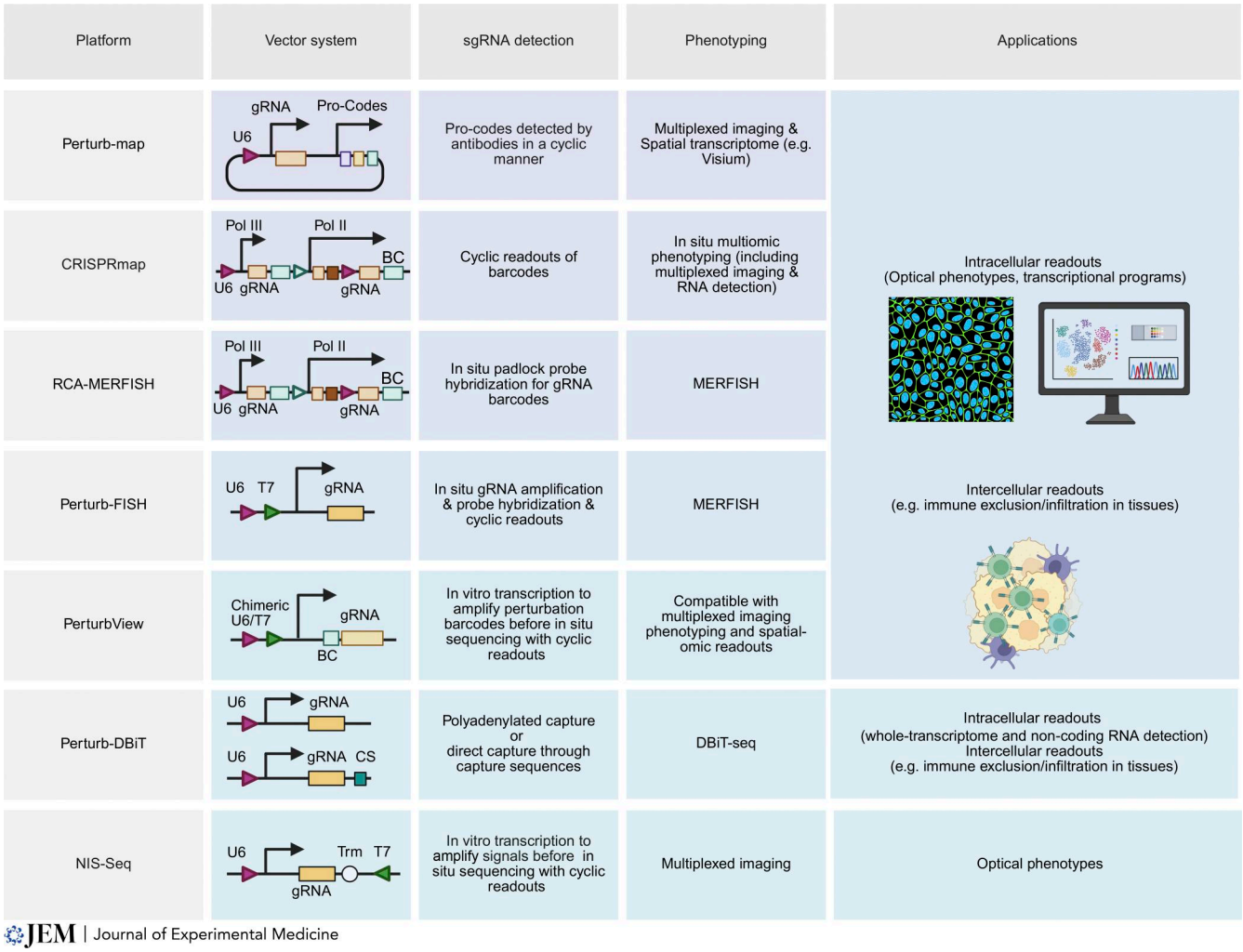

Figure 3. **Spatial CRISPR screens reveal intracellular and intercellular immune regulatory mechanisms.** Building upon the Pro-Code method, Perturb-map was developed to integrate spatial information with CRISPR perturbations, uncovering how tumor clonality shapes the composition of the TME. CRISPRmap introduces a library of barcoded gRNAs into cells and couples in situ multiomic phenotyping (multiplexed immunofluorescence and RNA detection) with simultaneous cyclic in situ barcode detection and amplification. RCA-MERFISH first hybridizes probes to mRNAs or gRNA barcodes in fixed cells or tissues, ligates and performs RCA of those probes to generate localized DNA amplicons, then conducts sequential MERFISH readout cycles to image and decode multiplexed RNA identities in situ. Perturb-FISH combines MERFISH with localized amplification of gRNA sequences, allowing spatially resolved profiling of both perturbations and transcriptomes. PerturbView leverages IVT to amplify perturbation barcodes before ISS. Perturb-DBiT employs two methods for capturing gRNAs. The first is polyadenylation-based capture, where enzyme-mediated in situ polyadenylation adds a poly(A) tail to the 3′ end of the gRNA, enabling its detection. The second is direct capture, which uses a "capture" sequence complementary to the constant region of the gRNA scaffold to selectively bind and retrieve gRNAs. NIS-Seq produces bright sequencing signals directly from nuclear genomic DNA by leveraging T7 IVT to generate multiple RNA copies within the nucleus, followed by a cyclic ISS for readout. These spatial screening platforms support intracellular readouts in primary immune cells (optical phenotypes and transcriptional programs) and intercellular readouts (e.g., immune cell exclusion or infiltration within tissues), offering a powerful framework for dissecting spatially organized immune regulation and revealing biological insights in vivo. RT, reverse transcription; BC, barcode; CS, capture sequence; Trm, polymerase III terminator of a gRNA expression cassette; DBiT-seq, deterministic barcoding in tissue for spatial omics sequencing; NIS-Seq, nuclear in situ sequencing; RCA, rolling circle amplification; IVT, in vitro transcription; ISS, in situ sequencing.

identified synergistic or alleviating cotargets, their scalability is limited, as each query gene necessitates a separate screen to identify its corresponding partners.

To allow for high-throughput genetic interaction mapping, CRISPR/Cas9 double-KO (dKO) technologies have recently emerged, leveraging dual-guide RNA libraries designed to simultaneously target gene–gene pairs. Such a dual-guide CRISPR library targeting combinations of tumor suppressor and immune resistance genes was used to systematically probe genetic interactions involved in CD8$^+$ T cell–mediated tumor cell killing (Park et al., 2022). These combinatorial perturbation strategies are also being extended to scCRISPR screens, where advances in computational frameworks now make it possible to decode complex gene regulatory networks and identify genetic interactions at single-cell resolution (Adamson et al., 2016; Norman et al., 2019; Replogle et al., 2020). However, as the order of combinatorial complexity increases, the number of cells required for optimal coverage in CRISPR screens grows exponentially. Therefore, in vivo applications remain challenging due to limited cell coverage, which restricts the ability of dKO

screens to comprehensively map genetic interactions across large gene sets. Future applications of these combinatorial approaches in immune cells are essential to systematically uncover the mechanisms underlying genetic interactions.

Cas12a is an RNA-programmable DNA endonuclease with intrinsic RNase activity, enabling it to process gRNA arrays for multiplexed gene perturbation (Zetsche et al., 2015). Building on this capability, the CRISPR-based Library-scale AAV Perturbation with Simultaneous HDR KI (CLASH) system was recently developed to facilitate high-efficiency, high-throughput KI engineering in human T cells. In CLASH, Cas12a mRNA is delivered alongside AAVs to mediate simultaneous gene editing and precise transgene insertion via massively parallel HDR (Dai et al., 2023). Cas12a has also been used to generate transgenic mice with conditional and constitutive LbCas12a KI, enabling efficient multiplexed gene editing within $CD4^+$ or $CD8^+$ T cells, B cells, and bone marrow–derived DCs (Tang et al., 2025b). Beyond DNA-targeting approaches, RNA-level perturbation strategies have also been developed. For example, CaRPool-seq leverages a cleavable CRISPR array and an associated barcode sequence to simultaneously target multiple genes at the RNA level using Cas13d in tumor cells (Wessels et al., 2023). Similarly, the CRISPR/Cas13d-based Multiplex Universal Combinatorial Immunotherapy via Gene silencing system facilitates the silencing of multiple endogenous immunosuppressive genes in the TME. In one recent application, delivery of an AAV-PGGC gRNA pool targeting PD-L1, galectin-9, galectin-3, and CD47 significantly reduced tumor growth in four distinct mouse models that exhibit varied responses to immune checkpoint blockade therapy (Zhang et al., 2025). These emerging combinatorial CRISPR platforms offer improved efficiency and precision in assigning multiple perturbations to individual cells, outperforming Cas9-based systems and facilitating the identification of candidates for combination therapies (Wessels et al., 2023). Further optimization, such as the identification of a DjCas13d variant (Wei et al., 2023), is needed to minimize cellular toxicity caused by Cas13-mediated RNA trans-cleavage activity, thereby facilitating their broader use for comprehensive genetic interaction studies.

Conclusion

As CRISPR screening technologies continue to advance and become more cost-effective, future efforts of applying functional genomics in immunity will likely evolve to generate comprehensive perturbation atlases at genome-wide scale for immune cells in homeostatic and disease contexts (Rood et al., 2024), together with integrative computational analysis of CRISPR screens to identify dual-action immunotherapeutic targets in different cell types, such as tumor and T cells (Luo et al., 2025). Bulk and single-cell-level genetic interaction screens will be foundational for designing multiplexed genetic perturbation strategies aimed at improving combinatorial therapeutic approaches for complex diseases. Although spatial CRISPR screens in tumor models have illustrated how local ligand–receptor interactions between tumor and adjacent immune cells influence therapeutic responses, immune cells also engage in highly dynamic interactions across diverse tissue niches, and current methodologies to systematically identify regulators of such intertissue communication remain underdeveloped. A recent study using in vivo CRISPRa screen and niche labeling highlighted the essential role of liver-derived signals in supporting the seeding of disseminated tumor cells for metastasis (Borrelli et al., 2024). Emerging technologies such as niche-labeling systems (Nakandakari-Higa et al., 2024; Ombrato et al., 2019) offer promising avenues for uncovering the key regulators of immune cell interactions beyond the tumor settings. Collectively, these next-generation CRISPR screening strategies—spanning bulk, single-cell, spatial and combinatorial modalities—hold tremendous potential to advance our understanding of causal systems biology and immunology and to guide the rational design of more precise and effective immunotherapies.

Acknowledgments

The authors acknowledge N. Chapman for insightful scientific discussions and editing of the manuscript. Figures were created using BioRender.com.

Research in the Chi Lab is supported by American Lebanese Syrian Associated Charities, National Institutes of Health (NIH) grants CA253188, CA281868, AI105887, AI131703, AI140761, AI150241, and AI150514, and the Lupus Research Alliance. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Author contributions: Hao Shi: conceptualization, investigation, validation, visualization, and writing—original draft, review, and editing. Hongbo Chi: conceptualization, funding acquisition, investigation, resources, supervision, and writing—review and editing.

Disclosures: H. Chi reported personal fees from Kumquat Biosciences and TCura Bioscience outside the submitted work; in addition, H. Chi had a patent to the field of immunotherapy pending. No other disclosures were reported.

Submitted: 19 December 2025
Revised: 2 February 2026
Accepted: 3 February 2026

References

Abudayyeh, O.O., J.S. Gootenberg, P. Essletzbichler, S. Han, J. Joung, J.J. Belanto, V. Verdine, D.B.T. Cox, M.J. Kellner, A. Regev, et al. 2017. RNA targeting with CRISPR-Cas13. *Nature*. 550:280–284. https://doi.org/10.1038/nature24049

Adamson, B., T.M. Norman, M. Jost, M.Y. Cho, J.K. Nunez, Y. Chen, J.E. Villalta, L.A. Gilbert, M.A. Horlbeck, M.Y. Hein, et al. 2016. A multiplexed single-cell CRISPR screening platform enables systematic dissection of the unfolded protein response. *Cell*. 167:1867–1882.e21. https://doi.org/10.1016/j.cell.2016.11.048

Anzalone, A.V., L.W. Koblan, and D.R. Liu. 2020. Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. *Nat. Biotechnol.* 38:824–844. https://doi.org/10.1038/s41587-020-0561-9

Anzalone, A.V., P.B. Randolph, J.R. Davis, A.A. Sousa, L.W. Koblan, J.M. Levy, P.J. Chen, C. Wilson, G.A. Newby, A. Raguram, and D.R. Liu. 2019. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*. 576:149–157. https://doi.org/10.1038/s41586-019-1711-4

Arce, M.M., J.M. Umhoefer, N. Arang, S. Kasinathan, J.W. Freimer, Z. Steinhart, H. Shen, M.T.N. Pham, M. Ota, A. Wadhera, et al. 2024. Central control of dynamic gene circuits governs T cell rest and activation. *Nature*. 637:930–939. https://doi.org/10.1038/s41586-024-08314-y

Aregger, M., K.A. Lawson, M. Billmann, M. Costanzo, A.H.Y. Tong, K. Chan, M. Rahman, K.R. Brown, C. Ross, M. Usaj, et al. 2020. Systematic mapping of genetic interactions for de novo fatty acid synthesis identifies C12orf49 as a regulator of lipid metabolism. *Nat. Metab.* 2:499–513. https://doi.org/10.1038/s42255-020-0211-z

Baxter, A.E., H. Huang, J.R. Giles, Z. Chen, J.E. Wu, S. Drury, K. Dalton, S.L. Park, L. Torres, B.W. Simone, et al. 2023. The SWI/SNF chromatin remodeling complexes BAF and PBAF differentially regulate epigenetic transitions in exhausted CD8(+) T cells. *Immunity*. 56:1320–1340.e10. https://doi.org/10.1016/j.immuni.2023.05.008

Belk, J.A., W. Yao, N. Ly, K.A. Freitas, Y.T. Chen, Q. Shi, A.M. Valencia, E. Shifrut, N. Kale, K.E. Yost, et al. 2022. Genome-wide CRISPR screens of T cell exhaustion identify chromatin remodeling factors that limit T cell persistence. *Cancer Cell*. 40:768–786.e7. https://doi.org/10.1016/j.ccell.2022.06.001

Biederstadt, A., R. Basar, J.M. Park, N. Uprety, R. Shrestha, F. Reyes Silva, M. Dede, J. Watts, S. Acharya, D. Xiong, et al. 2025. Genome-wide CRISPR screens identify critical targets to enhance CAR-NK cell antitumor potency. *Cancer Cell*. 43:2069–2088.e11. https://doi.org/10.1016/j.ccell.2025.07.021

Binan, L., A. Jiang, S.A. Danquah, V. Valakh, B. Simonton, J. Bezney, R.T. Manguso, K.B. Yates, R. Nehme, B. Cleary, and S.L. Farhi. 2025. Simultaneous CRISPR screening and spatial transcriptomics reveal intracellular, intercellular, and functional transcriptional circuits. *Cell*. 188:2141–2158.e18. https://doi.org/10.1016/j.cell.2025.02.012

Blaeschke, F., Y.Y. Chen, R. Apathy, B. Daniel, A.Y. Chen, P.A. Chen, K. Sandor, W. Zhang, Z. Li, C.T. Mowery, et al. 2023. Modular pooled discovery of synthetic knockin sequences to program durable cell therapies. *Cell*. 186:4216–4234.e33. https://doi.org/10.1016/j.cell.2023.08.013

Borrelli, C., M. Roberts, D. Eletto, M.D. Hussherr, H. Fazilaty, T. Valenta, A. Lafzi, J.A. Kretz, E. Guido Vinzoni, A. Karakatsani, et al. 2024. In vivo interaction screening reveals liver-derived constraints to metastasis. *Nature*. 632:411–418. https://doi.org/10.1038/s41586-024-07715-3

Braunger, J.M., and B. Velten. 2024. Guide assignment in single-cell CRISPR screens using crispat. *Bioinformatics*. 40:btae535. https://doi.org/10.1093/bioinformatics/btae535

Carnevale, J., E. Shifrut, N. Kale, W.A. Nyberg, F. Blaeschke, Y.Y. Chen, Z. Li, S.P. Bapat, M.E. Diolaiti, P. O'Leary, et al. 2022. RASA2 ablation in T cells boosts antigen sensitivity and long-term function. *Nature*. 609:174–182. https://doi.org/10.1038/s41586-022-05126-w

Chen, H., C. Deng, J. Gao, J. Wang, F. Fu, Y. Wang, Q. Wang, M. Zhang, S. Zhang, F. Fan, et al. 2025a. Integrative spatial analysis reveals tumor heterogeneity and immune colony niche related to clinical outcomes in small cell lung cancer. *Cancer Cell*. 43:519–536.e5. https://doi.org/10.1016/j.ccell.2025.01.012

Chen, K.Y., T. Kibayashi, A. Giguelay, M. Hata, S. Nakajima, N. Mikami, Y. Takeshima, K. Ichiyama, R. Omiya, L.S. Ludwig, et al. 2025b. Genome-wide CRISPR screen in human T cells reveals regulators of FOXP3. *Nature*. 642:191–200. https://doi.org/10.1038/s41586-025-08795-5

Chen, Z., E. Arai, O. Khan, Z. Zhang, S.F. Ngiow, Y. He, H. Huang, S. Manne, Z. Cao, A.E. Baxter, et al. 2021. In vivo CD8(+) T cell CRISPR screening reveals control by Fli1 in infection and cancer. *Cell*. 184:1262–1280.e22. https://doi.org/10.1016/j.cell.2021.02.019

Chi, H., M. Pepper, and P.G. Thomas. 2024. Principles and therapeutic applications of adaptive immunity. *Cell*. 187:2052–2078. https://doi.org/10.1016/j.cell.2024.03.037

Chu, V.T., T. Weber, R. Graf, T. Sommermann, K. Petsch, U. Sack, P. Volchkov, K. Rajewsky, and R. Kuhn. 2016. Efficient generation of Rosa26 knock-in mice using CRISPR/Cas9 in C57BL/6 zygotes. *BMC Biotechnol.* 16:4. https://doi.org/10.1186/s12896-016-0234-4

Dai, X., J.J. Park, Y. Du, Z. Na, S.Z. Lam, R.D. Chow, P.A. Renauer, J. Gu, S. Xin, Z. Chu, et al. 2023. Massively parallel knock-in engineering of human T cells. *Nat. Biotechnol.* 41:1239–1255. https://doi.org/10.1038/s41587-022-01639-x

Datlinger, P., E.V. Pankevich, C.D. Arnold, N. Pranckevicius, J. Lin, D. Romanovskaia, M. Schaefer, F. Piras, A.C. Orts, A. Nemc, et al. 2025. Systematic discovery of CRISPR-boosted CAR T cell immunotherapies. *Nature*. 646:963–972. https://doi.org/10.1038/s41586-025-09507-9

Datlinger, P., A.F. Rendeiro, T. Boenke, M. Senekowitsch, T. Krausgruber, D. Barreca, and C. Bock. 2021. Ultra-high-throughput single-cell RNA sequencing and perturbation screening with combinatorial fluidic indexing. *Nat. Methods*. 18:635–642. https://doi.org/10.1038/s41592-021-01153-z

Datlinger, P., A.F. Rendeiro, C. Schmidl, T. Krausgruber, P. Traxler, J. Klughammer, L.C. Schuster, A. Kuchler, D. Alpar, and C. Bock. 2017. Pooled CRISPR screening with single-cell transcriptome readout. *Nat. Methods*. 14:297–301. https://doi.org/10.1038/nmeth.4177

Dhainaut, M., S.A. Rose, G. Akturk, A. Wroblewska, S.R. Nielsen, E.S. Park, M. Buckup, V. Roudko, L. Pia, R. Sweeney, et al. 2022. Spatial CRISPR genomics identifies regulators of the tumor microenvironment. *Cell*. 185:1223–1239.e20. https://doi.org/10.1016/j.cell.2022.02.015

Diorio, C., R. Murray, M. Naniong, L. Barrera, A. Camblin, J. Chukinas, L. Coholan, A. Edwards, T. Fuller, C. Gonzales, et al. 2022. Cytosine base editing enables quadruple-edited allogeneic CART cells for T-ALL. *Blood*. 140:619–629. https://doi.org/10.1182/blood.2022015825

Dixit, A., O. Parnas, B. Li, J. Chen, C.P. Fulco, L. Jerby-Arnon, N.D. Marjanovic, D. Dionne, T. Burks, R. Raychowdhury, et al. 2016. Perturb-Seq: Dissecting molecular circuits with scalable single-cell RNA profiling of pooled genetic screens. *Cell*. 167:1853–1866.e17. https://doi.org/10.1016/j.cell.2016.11.038

Dong, M.B., G. Wang, R.D. Chow, L. Ye, L. Zhu, X. Dai, J.J. Park, H.R. Kim, Y. Errami, C.D. Guzman, et al. 2019. Systematic immunotherapy target discovery using genome-scale in vivo CRISPR screens in CD8 T cells. *Cell*. 178:1189–1204.e23. https://doi.org/10.1016/j.cell.2019.07.044

Drager, N.M., S.M. Sattler, C.T. Huang, O.M. Teter, K. Leng, S.H. Hashemi, J. Hong, G. Aviles, C.D. Clelland, L. Zhan, et al. 2022. A CRISPRi/a platform in human iPSC-derived microglia uncovers regulators of disease states. *Nat. Neurosci.* 25:1149–1162. https://doi.org/10.1038/s41593-022-01131-4

Ely, Z.A., N. Mathey-Andrews, S. Naranjo, S.I. Gould, K.L. Mercer, G.A. Newby, C.M. Cabana, W.M. Rideout 3rd, G.C. Jaramillo, J.M. Khirallah, et al. 2024. A prime editor mouse to model a broad spectrum of somatic mutations in vivo. *Nat. Biotechnol.* 42:424–436. https://doi.org/10.1038/s41587-023-01783-y

Enk, L.U.B., M. Hellmig, K. Riecken, C. Kilian, P. Datlinger, S.L. Jauch-Speer, T. Neben, Z. Sultana, V. Sivayoganathan, A. Borchers, et al. 2024. Targeting T cell plasticity in kidney and gut inflammation by pooled single-cell CRISPR screening. *Sci. Immunol.* 9:eadd6774. https://doi.org/10.1126/sciimmunol.add6774

Fagerberg, E., J. Attanasio, C. Dien, J. Singh, E.A. Kessler, L. Abdullah, J. Shen, B.G. Hunt, K.A. Connolly, E. De Brouwer, et al. 2025. KLF2 maintains lineage fidelity and suppresses CD8 T cell exhaustion during acute LCMV infection. *Science*. 387:eadn2337. https://doi.org/10.1126/science.adn2337

Fan, R., A. Baysoy, X. Tian, F. Zhang, P. Renauer, Z. Bai, H. Shi, D. Zhang, H. Li, B. Tao, et al. 2025. Spatially resolved panoramic in vivo CRISPR screen via perturb-DBiT. *Res. Sq.* https://doi.org/10.21203/rs.3.rs-6481967/v1 (Preprint posted May 8, 2025).

Fandrey, C.I., M. Jentzsch, P. Konopka, A. Hoch, K. Blumenstock, A. Zackria, S. Maasewerd, M. Lovotti, D.J. Lapp, F.N. Gohr, et al. 2025. NIS-Seq enables cell-type-agnostic optical perturbation screening. *Nat. Biotechnol.* 43:1337–1347. https://doi.org/10.1038/s41587-024-02516-5

Frangieh, C.J., J.C. Melms, P.I. Thakore, K.R. Geiger-Schuller, P. Ho, A.M. Luoma, B. Cleary, L. Jerby-Arnon, S. Malu, M.S. Cuoco, et al. 2021. Multimodal pooled perturb-CITE-seq screens in patient models define mechanisms of cancer immune evasion. *Nat. Genet.* 53:332–341. https://doi.org/10.1038/s41588-021-00779-1

Fu, G., C.S. Guy, N.M. Chapman, G. Palacios, J. Wei, P. Zhou, L. Long, Y.D. Wang, C. Qian, Y. Dhungana, et al. 2021. Metabolic control of T(FH) cells and humoral immunity by phosphatidylethanolamine. *Nature*. 595:724–729. https://doi.org/10.1038/s41586-021-03692-z

Gasperini, M., A.J. Hill, J.L. McFaline-Figueroa, B. Martin, S. Kim, M.D. Zhang, D. Jackson, A. Leith, J. Schreiber, W.S. Noble, et al. 2019. A genome-wide framework for mapping gene regulation via cellular genetic screens. *Cell*. 176:377–390.e19. https://doi.org/10.1016/j.cell.2019.02.027

Geiger-Schuller, K., B. Eraslan, O. Kuksenko, K.K. Dey, K.A. Jagadeesh, P.I. Thakore, O. Karayel, A.R. Yung, A. Rajagopalan, A.M. Meireles, et al. 2023. Systematically characterizing the roles of E3-ligase family members in inflammatory responses with massively parallel perturb-seq. *bioRxiv*. https://doi.org/10.1101/2023.01.23.525198 (Preprint posted January 24, 2023).

Gu, J., A. Iyer, B. Wesley, A. Taglialatela, G. Leuzzi, S. Hangai, A. Decker, R. Gu, N. Klickstein, Y. Shuai, et al. 2024. Mapping multimodal phenotypes to

perturbations in cells and tissue with CRISPRmap. *Nat. Biotechnol.* 43: 1101–1115. https://doi.org/10.1038/s41587-024-02386-x

Guo, A., H. Huang, Z. Zhu, M.J. Chen, H. Shi, S. Yuan, P. Sharma, J.P. Connelly, S. Liedmann, Y. Dhungana, et al. 2022. cBAF complex components and MYC cooperate early in CD8(+) T cell fate. *Nature*. 607:135–141. https://doi.org/10.1038/s41586-022-04849-0

Havel, J.J., D. Chowell, and T.A. Chan. 2019. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. *Nat. Rev. Cancer*. 19: 133–150. https://doi.org/10.1038/s41568-019-0116-x

Heumos, L., Y. Ji, L. May, T.D. Green, S. Peidli, X. Zhang, X. Wu, J. Ostner, A. Schumacher, K. Hrovatin, et al. 2025. Pertpy: An end-to-end framework for perturbation analysis. *Nat. Methods*. 23:350–359. https://doi.org/10.1038/s41592-025-02909-7

Hill, A.J., J.L. McFaline-Figueroa, L.M. Starita, M.J. Gasperini, K.A. Matreyek, J. Packer, D. Jackson, J. Shendure, and C. Trapnell. 2018. On the design of CRISPR-based single-cell molecular screens. *Nat. Methods*. 15:271–274. https://doi.org/10.1038/nmeth.4604

Huang, B., J.D. Phelan, S. Preite, J. Gomez-Rodriguez, K.H. Johansen, H. Shibata, A.L. Shaffer 3rd, Q. Xu, B. Jeffrey, M. Kirby, et al. 2022. In vivo CRISPR screens reveal a HIF-1alpha-mTOR-network regulates T follicular helper versus Th1 cells. *Nat. Commun.* 13:805. https://doi.org/10.1038/s41467-022-28378-6

Huang, H., P. Zhou, J. Wei, L. Long, H. Shi, Y. Dhungana, N.M. Chapman, G. Fu, J. Saravia, J.L. Raynor, et al. 2021. In vivo CRISPR screening reveals nutrient signaling processes underpinning CD8(+) T cell fate decisions. *Cell*. 184:1245–1261.e21. https://doi.org/10.1016/j.cell.2021.02.021

Jaitin, D.A., A. Weiner, I. Yofe, D. Lara-Astiaso, H. Keren-Shaul, E. David, T.M. Salame, A. Tanay, A. van Oudenaarden, and I. Amit. 2016. Dissecting immune circuits by linking CRISPR-pooled screens with single-cell RNA-seq. *Cell*. 167:1883–1896.e15. https://doi.org/10.1016/j.cell.2016.11.039

Jiang, L., C. Dalgarno, E. Papalexi, I. Mascio, H.H. Wessels, H. Yun, N. Iremadze, G. Lithwick-Yanai, D. Lipson, and R. Satija. 2025. Systematic reconstruction of molecular pathway signatures using scalable single-cell perturbation screens. *Nat. Cell Biol.* 27:505–517. https://doi.org/10.1038/s41556-025-01622-z

Katti, A., A. Vega-Perez, M. Foronda, J. Zimmerman, M.P. Zafra, E. Granowsky, S. Goswami, E.E. Gardner, B.J. Diaz, J.M. Simon, et al. 2024. Generation of precision preclinical cancer models using regulated in vivo base editing. *Nat. Biotechnol.* 42:437–447. https://doi.org/10.1038/s41587-023-01900-x

Kendirli, A., C. de la Rosa, K.F. Lammle, K. Eglseer, I.J. Bauer, V. Kavaka, S. Winklmeier, L. Zhuo, C. Wichmann, L.A. Gerdes, et al. 2023. A genome-wide in vivo CRISPR screen identifies essential regulators of T cell migration to the CNS in a multiple sclerosis model. *Nat. Neurosci.* 26: 1713–1725. https://doi.org/10.1038/s41593-023-01432-2

Knudsen, N.H., G. Escobar, F. Korell, T. Kienka, C. Nobrega, S. Anderson, A.Y. Cheng, M. Zschummel, A. Armstrong, A. Bouffard, et al. 2025. In vivo CRISPR screens identify modifiers of CAR T cell function in myeloma. *Nature*. 646:953–962. https://doi.org/10.1038/s41586-025-09489-8

Kudo, T., A.M. Meireles, R. Moncada, Y. Chen, P. Wu, J. Gould, X. Hu, O. Kornfeld, R. Jesudason, C. Foo, et al. 2024. Multiplexed, image-based pooled screens in primary cells and tissues with PerturbView. *Nat. Biotechnol.* 43:1091–1100. https://doi.org/10.1038/s41587-024-02391-0

LaFleur, M.W., A.M. Lemmen, I.S.L. Streeter, T.H. Nguyen, L.E. Milling, N.M. Derosia, Z.M. Hoffman, J.E. Gillis, Q. Tjokrosurjo, S.C. Markson, et al. 2024. X-CHIME enables combinatorial, inducible, lineage-specific and sequential knockout of genes in the immune system. *Nat. Immunol.* 25: 178–188. https://doi.org/10.1038/s41590-023-01689-6

LaFleur, M.W., L.E. Milling, P. Prathima, V. Li, A.M. Lemmen, I.S.L. Streeter, P.K.S. Heisig, N.M. Derosia, E. Riffo, H. Xu, et al. 2025. A STUB1-CHIC2 complex inhibits CD8(+) T cells to restrain tumor immunity. *Nat. Immunol.* 26:1476–1487. https://doi.org/10.1038/s41590-025-02231-6

LaFleur, M.W., T.H. Nguyen, M.A. Coxe, K.B. Yates, J.D. Trombley, S.A. Weiss, F.D. Brown, J.E. Gillis, D.J. Coxe, J.G. Doench, et al. 2019. A CRISPR-Cas9 delivery system for in vivo screening of genes in the immune system. *Nat. Commun.* 10:1668. https://doi.org/10.1038/s41467-019-09656-2

Lawson, K.A., C.M. Sousa, X. Zhang, E. Kim, R. Akthar, J.J. Caumanns, Y. Yao, N. Mikolajewicz, C. Ross, K.R. Brown, et al. 2020. Functional genomic landscape of cancer-intrinsic evasion of killing by T cells. *Nature*. 586: 120–126. https://doi.org/10.1038/s41586-020-2746-2

Legut, M., Z. Gajic, M. Guarino, Z. Daniloski, J.A. Rahman, X. Xue, C. Lu, L. Lu, E.P. Mimitou, S. Hao, et al. 2022. A genome-scale screen for synthetic drivers of T cell proliferation. *Nature*. 603:728–735. https://doi.org/10.1038/s41586-022-04494-7

Liscovitch-Brauer, N., A. Montalbano, J. Deng, A. Mendez-Mancilla, H.H. Wessels, N.G. Moss, C.Y. Kung, A. Sookdeo, X. Guo, E. Geller, et al. 2021. Profiling the genetic determinants of chromatin accessibility with scalable single-cell CRISPR screens. *Nat. Biotechnol.* 39:1270–1277. https://doi.org/10.1038/s41587-021-00902-x

Liu, B., Z. Jing, X. Zhang, Y. Chen, S. Mao, R. Kaundal, Y. Zou, G. Wei, Y. Zang, X. Wang, et al. 2022. Large-scale multiplexed mosaic CRISPR perturbation in the whole organism. *Cell*. 185:3008–3024.e16. https://doi.org/10.1016/j.cell.2022.06.039

Liu, Q., P.A. Chen, E. Urs, S. Zhang, M.M. Arce, C.H. Wang, Z. Li, J. Seo, N. Kale, T.N. LaFlam, et al. 2025a. In vivo genome-wide CRISPR screens in human T cells to enhance T cell therapy for solid tumors. *bioRxiv*. https://doi.org/10.1101/2025.09.23.678127 (Preprint posted September 24, 2025).

Liu, S., M.C. Hamilton, T. Cowart, A. Barrera, L.R. Bounds, A.C. Nelson, S.F. Dornbaum, J.W. Riley, R.W. Doty, A.S. Allen, et al. 2025b. Characterization and bioinformatic filtering of ambient gRNAs in single-cell CRISPR screens using CLEANSER. *Cell Genom.* 5:100766. https://doi.org/10.1016/j.xgen.2025.100766

Liu, S.J., C. Zou, J. Pak, A. Morse, D. Pang, T. Casey-Clyde, A.A. Borah, D. Wu, K. Seo, T. O'Loughlin, et al. 2024. In vivo perturb-seq of cancer and microenvironment cells dissects oncologic drivers and radiotherapy responses in glioblastoma. *Genome Biol.* 25:256. https://doi.org/10.1186/s13059-024-03404-6

Long, L., J. Wei, S.A. Lim, J.L. Raynor, H. Shi, J.P. Connelly, H. Wang, C. Guy, B. Xie, N.M. Chapman, et al. 2021. CRISPR screens unveil signal hubs for nutrient licensing of T cell immunity. *Nature*. 600:308–313. https://doi.org/10.1038/s41586-021-04109-7

Lue, N.Z., and B.B. Liau. 2023. Base editor screens for in situ mutational scanning at scale. *Mol. Cell*. 83:2167–2187. https://doi.org/10.1016/j.molcel.2023.06.009

Luo, C., R. Zhang, R. Guo, L. Wu, T. Xue, Y. He, Y. Jin, Y. Zhao, Z. Zhang, P. Zhang, et al. 2025. Integrated computational analysis identifies therapeutic targets with dual action in cancer cells and T cells. *Immunity*. 58: 745–765.e9. https://doi.org/10.1016/j.immuni.2025.02.007

McCutcheon, S.R., A.M. Swartz, M.C. Brown, A. Barrera, C. McRoberts Amador, K. Siklenka, L. Humayun, M.A. Ter Weele, J.M. Isaacs, T.E. Reddy, et al. 2023. Transcriptional and epigenetic regulators of human CD8(+) T cell function identified through orthogonal CRISPR screens. *Nat. Genet.* 55:2211–2223. https://doi.org/10.1038/s41588-023-01554-0

Metzner, E., K.M. Southard, and T.M. Norman. 2025. Multiome perturb-seq unlocks scalable discovery of integrated perturbation effects on the transcriptome and epigenome. *Cell Syst.* 16:101161. https://doi.org/10.1016/j.cels.2024.12.002

Mimitou, E.P., A. Cheng, A. Montalbano, S. Hao, M. Stoeckius, M. Legut, T. Roush, A. Herrera, E. Papalexi, Z. Ouyang, et al. 2019. Multiplexed detection of proteins, transcriptomes, clonotypes and CRISPR perturbations in single cells. *Nat. Methods*. 16:409–412. https://doi.org/10.1038/s41592-019-0392-0

Mollaoglu, G., A. Tepper, C. Falcomata, H.T. Potak, L. Pia, A. Amabile, J. Mateus-Tique, N. Rabinovich, M.D. Park, N.M. LaMarche, et al. 2024. Ovarian cancer-derived IL-4 promotes immunotherapy resistance. *Cell*. 187:7492–7510.e22. https://doi.org/10.1016/j.cell.2024.10.006

Nakamura, M., Y. Gao, A.A. Dominguez, and L.S. Qi. 2021. CRISPR technologies for precise epigenome editing. *Nat. Cell Biol.* 23:11–22. https://doi.org/10.1038/s41556-020-00620-7

Nakandakari-Higa, S., S. Walker, M.C.C. Canesso, V. van der Heide, A. Chudnovskiy, D.Y. Kim, J.T. Jacobsen, R. Parsa, J. Bilanovic, S.M. Parigi, et al. 2024. Universal recording of immune cell interactions in vivo. *Nature*. 627:399–406. https://doi.org/10.1038/s41586-024-07134-4

Norman, T.M., M.A. Horlbeck, J.M. Replogle, A.Y. Ge, A. Xu, M. Jost, L.A. Gilbert, and J.S. Weissman. 2019. Exploring genetic interaction manifolds constructed from rich single-cell phenotypes. *Science*. 365:786–793. https://doi.org/10.1126/science.aax4438

Obradovic, A., C. Ager, M. Turunen, T. Nirschl, M. Khosravi-Maharlooei, A. Iuga, C.M. Jackson, S. Yegnasubramanian, L. Tomassoni, E.C. Fernandez, et al. 2023. Systematic elucidation and pharmacological targeting of tumor-infiltrating regulatory T cell master regulators. *Cancer Cell*. 41:933–949.e11. https://doi.org/10.1016/j.ccell.2023.04.003

Ombrato, L., E. Nolan, I. Kurelac, A. Mavousian, V.L. Bridgeman, I. Heinze, P. Chakravarty, S. Horswell, E. Gonzalez-Gualda, G. Matacchione, et al. 2019. Metastatic-niche labelling reveals parenchymal cells with stem features. *Nature*. 572:603–608. https://doi.org/10.1038/s41586-019-1487-6

Otto, J.E., O. Ursu, A.P. Wu, E.B. Winter, M.S. Cuoco, S. Ma, K. Qian, B.C. Michel, J.D. Buenrostro, B. Berger, et al. 2023. Structural and functional properties of mSWI/SNF chromatin remodeling complexes revealed through single-cell perturbation screens. *Mol. Cell*. 83:1350–1367.e7. https://doi.org/10.1016/j.molcel.2023.03.013

Papalexi, E., E.P. Mimitou, A.W. Butler, S. Foster, B. Bracken, W.M. Mauck 3rd, H.H. Wessels, Y. Hao, B.Z. Yeung, P. Smibert, and R. Satija. 2021. Characterizing the molecular regulation of inhibitory immune checkpoints with multimodal single-cell screens. *Nat. Genet.* 53:322–331. https://doi.org/10.1038/s41588-021-00778-2

Park, J.J., A. Codina, L. Ye, S. Lam, J. Guo, P. Clark, X. Zhou, L. Peng, and S. Chen. 2022. Double knockout CRISPR screen for cancer resistance to T cell cytotoxicity. *J. Hematol. Oncol.* 15:172. https://doi.org/10.1186/s13045-022-01389-y

Peng, L., P.A. Renauer, G. Sferruzza, L. Yang, Y. Zou, Z. Fang, J.J. Park, R.D. Chow, Y. Zhang, Q. Lin, et al. 2024a. In vivo AAV-SB-CRISPR screens of tumor-infiltrating primary NK cells identify genetic checkpoints of CAR-NK therapy. *Nat. Biotechnol.* 43:752–761. https://doi.org/10.1038/s41587-024-02282-4

Peng, L., G. Sferruzza, L. Yang, L. Zhou, and S. Chen. 2024b. CAR-T and CAR-NK as cellular cancer immunotherapy for solid tumors. *Cell Mol. Immunol.* 21:1089–1108. https://doi.org/10.1038/s41423-024-01207-0

Pierce, S.E., J.M. Granja, and W.J. Greenleaf. 2021. High-throughput single-cell chromatin accessibility CRISPR screens enable unbiased identification of regulatory networks in cancer. *Nat. Commun.* 12:2969. https://doi.org/10.1038/s41467-021-23213-w

Platt, R.J., S. Chen, Y. Zhou, M.J. Yim, L. Swiech, H.R. Kempton, J.E. Dahlman, O. Parnas, T.M. Eisenhaure, M. Jovanovic, et al. 2014. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. *Cell*. 159:440–455. https://doi.org/10.1016/j.cell.2014.09.014

Pretto, S., Q. Yu, P. Bourdely, S. Trusso Cafarello, H.H. Van Acker, J. Verelst, E. Richiardone, L. Vanheer, A. Roshanzadeh, F. Schneppenheim, et al. 2025. A functional single-cell metabolic survey identifies Elovl1 as a target to enhance CD8(+) T cell fitness in solid tumours. *Nat. Metab.* 7:508–530. https://doi.org/10.1038/s42255-025-01233-w

Raynor, J.L., N. Collins, H. Shi, C. Guy, J. Saravia, S. Ah Lim, N.M. Chapman, P. Zhou, Y. Wang, Y. Sun, et al. 2024. CRISPR screens unveil nutrient-dependent lysosomal and mitochondrial nodes impacting intestinal tissue-resident memory CD8(+) T cell formation. *Immunity*. 57:2597–2614.e13. https://doi.org/10.1016/j.immuni.2024.09.013

Rees, H.A., and D.R. Liu. 2018. Base editing: Precision chemistry on the genome and transcriptome of living cells. *Nat. Rev. Genet.* 19:770–788. https://doi.org/10.1038/s41576-018-0059-1

Ren, X., H. Yang, J.L. Nierenberg, Y. Sun, J. Chen, C. Beaman, T. Pham, M. Nobuhara, M.A. Takagi, V. Narayan, et al. 2023. High-throughput PRIME-editing screens identify functional DNA variants in the human genome. *Mol. Cell*. 83:4633–4645.e9. https://doi.org/10.1016/j.molcel.2023.11.021

Replogle, J.M., T.M. Norman, A. Xu, J.A. Hussmann, J. Chen, J.Z. Cogan, E.J. Meer, J.M. Terry, D.P. Riordan, N. Srinivas, et al. 2020. Combinatorial single-cell CRISPR screens by direct guide RNA capture and targeted sequencing. *Nat. Biotechnol.* 38:954–961. https://doi.org/10.1038/s41587-020-0470-y

Replogle, J.M., R.A. Saunders, A.N. Pogson, J.A. Hussmann, A. Lenail, A. Guna, L. Mascibroda, E.J. Wagner, K. Adelman, G. Lithwick-Yanai, et al. 2022. Mapping information-rich genotype-phenotype landscapes with genome-scale perturb-seq. *Cell*. 185:2559–2575.e28. https://doi.org/10.1016/j.cell.2022.05.013

Rood, J.E., A. Hupalowska, and A. Regev. 2024. Toward a foundation model of causal cell and tissue biology with a perturbation cell and tissue atlas. *Cell*. 187:4520–4545. https://doi.org/10.1016/j.cell.2024.07.035

de la Rosa, C., A. Kendirli, S. Baygun, F. Bauernschmitt, A.S. Thomann, I. Kisioglu, D. Beckmann, Y. Carpentier Solorio, V. Pfaffenstaller, Y.H. Tai, et al. 2026. In vivo CRISPR screen reveals regulation of macrophage states in neuroinflammation. *Nat. Neurosci.* 29:493–509. https://doi.org/10.1038/s41593-025-02151-6

Rubin, A.J., K.R. Parker, A.T. Satpathy, Y. Qi, B. Wu, A.J. Ong, M.R. Mumbach, A.L. Ji, D.S. Kim, S.W. Cho, et al. 2019. Coupled single-cell CRISPR screening and epigenomic profiling reveals causal gene regulatory networks. *Cell*. 176:361–376.e17. https://doi.org/10.1016/j.cell.2018.11.022

Saunders, R.A., W.E. Allen, X. Pan, J. Sandhu, J. Lu, T.K. Lau, K. Smolyar, Z.A. Sullivan, C. Dulac, J.S. Weissman, and X. Zhuang. 2025. Perturb-multimodal: A platform for pooled genetic screens with imaging and sequencing in intact mammalian tissue. *Cell*. 188:4790–4809.e22. https://doi.org/10.1016/j.cell.2025.05.022

Schmidt, R., Z. Steinhart, M. Layeghi, J.W. Freimer, R. Bueno, V.Q. Nguyen, F. Blaeschke, C.J. Ye, and A. Marson. 2022. CRISPR activation and interference screens decode stimulation responses in primary human T cells. *Science*. 375:eabj4008. https://doi.org/10.1126/science.abj4008

Schmidt, R., C.C. Ward, R. Dajani, Z. Armour-Garb, M. Ota, V. Allain, R. Hernandez, M. Layeghi, G. Xing, L. Goudy, et al. 2024. Base-editing mutagenesis maps alleles to tune human T cell functions. *Nature*. 625:805–812. https://doi.org/10.1038/s41586-023-06835-6

Schraivogel, D., A.R. Gschwind, J.H. Milbank, D.R. Leonce, P. Jakob, L. Mathur, J.O. Korbel, C.A. Merten, L. Velten, and L.M. Steinmetz. 2020. Targeted perturb-seq enables genome-scale genetic screens in single cells. *Nat. Methods*. 17:629–635. https://doi.org/10.1038/s41592-020-0837-5

Sheban, F., T.S. Phan, K. Xie, F. Ingelfinger, C. Gur, Y. Shapir Itai, R. Blecher-Gonen, C. Yu, R. Avellino, P. Chalan, et al. 2025. ZEB2 is a master switch controlling the tumor-associated macrophage program. *Cancer Cell*. 43:1227–1241.e11. https://doi.org/10.1016/j.ccell.2025.03.021

Shevade, K., Y.A. Yang, K. Feng, K. Mader, V. Sevim, J. Parsons, G. Arora, H. Elfawy, R. Mace, S. Federman, et al. 2025. Simultaneous capture of single cell RNA-seq, ATAC-seq, and CRISPR perturbation enables multiomic screens to identify gene regulatory relationships. *Cell Rep. Methods*. 5:101222. https://doi.org/10.1016/j.crmeth.2025.101222

Shi, H., J.G. Doench, and H. Chi. 2023. CRISPR screens for functional interrogation of immunity. *Nat. Rev. Immunol.* 23:363–380. https://doi.org/10.1038/s41577-022-00802-4

Shifrut, E., J. Carnevale, V. Tobin, T.L. Roth, J.M. Woo, C.T. Bui, P.J. Li, M.E. Diolaiti, A. Ashworth, and A. Marson. 2018. Genome-wide CRISPR screens in primary human T cells reveal key regulators of immune function. *Cell*. 175:1958–1971.e15. https://doi.org/10.1016/j.cell.2018.10.024

Song, B., D. Liu, W. Dai, N.F. McMyn, Q. Wang, D. Yang, A. Krejci, A. Vasilyev, N. Untermoser, A. Loregger, et al. 2025. Decoding heterogeneous single-cell perturbation responses. *Nat. Cell Biol.* 27:493–504. https://doi.org/10.1038/s41556-025-01626-9

Sugiura, A., G. Andrejeva, K. Voss, D.R. Heintzman, X. Xu, M.Z. Madden, X. Ye, K.L. Beier, N.U. Chowdhury, M.M. Wolf, et al. 2022. MTHFD2 is a metabolic checkpoint controlling effector and regulatory T cell fate and function. *Immunity*. 55:65–81.e9. https://doi.org/10.1016/j.immuni.2021.10.011

Sutra Del Galy, A., S. Menegatti, J. Fuentealba, F. Lucibello, L. Perrin, J. Helft, A. Darbois, M. Saitakis, J. Tosello, D. Rookhuizen, et al. 2021. In vivo genome-wide CRISPR screens identify SOCS1 as intrinsic checkpoint of CD4(+) T(H)1 cell response. *Sci. Immunol.* 6:eabe8219. https://doi.org/10.1126/sciimmunol.abe8219

Tang, H., Z. Wei, B. Zheng, Y. Cai, P. Wu, L. Wu, X. Ma, Y. Chen, S. Su, J. Xu, et al. 2025a. Rescuing dendritic cell interstitial motility sustains anti-tumour immunity. *Nature*. 645:244–253. https://doi.org/10.1038/s41586-025-09202-9

Tang, K., L. Zhou, X. Tian, S.Y. Fang, E. Vandenbulcke, A. Du, J. Shen, H. Cao, J. Zhou, K. Chen, et al. 2025b. Cas12a-knock-in mice for multiplexed genome editing, disease modelling and immune-cell engineering. *Nat. Biomed. Eng.* 9:1290–1308. https://doi.org/10.1038/s41551-025-01371-2

Tang, X.E., S.X. Tan, S. Hoon, and G.W. Yeo. 2022. Pre-existing adaptive immunity to the RNA-editing enzyme Cas13d in humans. *Nat. Med.* 28:1372–1376. https://doi.org/10.1038/s41591-022-01848-6

Tieu, V., E. Sotillo, J.R. Bjelajac, C. Chen, M. Malipatlolla, J.A. Guerrero, P. Xu, P.J. Quinn, C. Fisher, D. Klysz, et al. 2024. A versatile CRISPR-Cas13d platform for multiplexed transcriptomic regulation and metabolic engineering in primary human T cells. *Cell*. 187:1278–1295.e20. https://doi.org/10.1016/j.cell.2024.01.035

Traxler, P., S. Reichl, L. Folkman, L. Shaw, V. Fife, A. Nemc, D. Pasajlic, A. Kusienicka, D. Barreca, N. Fortelny, et al. 2025. Integrated time-series analysis and high-content CRISPR screening delineate the dynamics of macrophage immune regulation. *Cell Syst.* 16:101346. https://doi.org/10.1016/j.cels.2025.101346

Tsuchida, C.A., N. Brandes, R. Bueno, M. Trinidad, T. Mazumder, B. Yu, B. Hwang, C. Chang, J. Liu, Y. Sun, et al. 2023. Mitigation of chromosome loss in clinical CRISPR-Cas9-engineered T cells. *Cell*. 186:4567–4582.e20. https://doi.org/10.1016/j.cell.2023.08.041

Umhoefer, J.M., M.M. Arce, S. Kasinathan, S. Whalen, R. Dajani, S. Subramanya, L. Goudy, J.A. Belk, R. Zhou, M.T.N. Pham, et al. 2025. FOXP3 expression depends on cell-type-specific cis-regulatory elements and

transcription factor circuitry. *Immunity*. 59:129–144.e13. https://doi.org/10.1016/j.immuni.2025.10.020

Vazquez-Garcia, I., F. Uhlitz, N. Ceglia, J.L.P. Lim, M. Wu, N. Mohibullah, J. Niyazov, A.E.B. Ruiz, K.M. Boehm, V. Bojilova, et al. 2022. Ovarian cancer mutational processes drive site-specific immune evasion. *Nature*. 612:778–786. https://doi.org/10.1038/s41586-022-05496-1

Villiger, L., J. Joung, L. Koblan, J. Weissman, O.O. Abudayyeh, and J.S. Gootenberg. 2024. CRISPR technologies for genome, epigenome and transcriptome editing. *Nat. Rev. Mol. Cell Biol.* 25:464–487. https://doi.org/10.1038/s41580-023-00697-6

Wei, J., L. Long, W. Zheng, Y. Dhungana, S.A. Lim, C. Guy, Y. Wang, Y.D. Wang, C. Qian, B. Xu, et al. 2019. Targeting REGNASE-1 programs long-lived effector T cells for cancer therapy. *Nature*. 576:471–476. https://doi.org/10.1038/s41586-019-1821-z

Wei, J., P. Lotfy, K. Faizi, S. Baungaard, E. Gibson, E. Wang, H. Slabodkin, E. Kinnaman, S. Chandrasekaran, H. Kitano, et al. 2023. Deep learning and CRISPR-Cas13d ortholog discovery for optimized RNA targeting. *Cell Syst.* 14:1087–1102.e13. https://doi.org/10.1016/j.cels.2023.11.006

Wessels, H.H., A. Mendez-Mancilla, Y. Hao, E. Papalexi, W.M. Mauck 3rd, L. Lu, J.A. Morris, E.P. Mimitou, P. Smibert, N.E. Sanjana, and R. Satija. 2023. Efficient combinatorial targeting of RNA transcripts in single cells with Cas13 RNA perturb-seq. *Nat. Methods*. 20:86–94. https://doi.org/10.1038/s41592-022-01705-x

Wroblewska, A., M. Dhainaut, B. Ben-Zvi, S.A. Rose, E.S. Park, E.D. Amir, A. Bektesevic, A. Baccarini, M. Merad, A.H. Rahman, and B.D. Brown. 2018. Protein barcodes enable high-dimensional single-cell CRISPR screens. *Cell*. 175:1141–1155.e16. https://doi.org/10.1016/j.cell.2018.09.022

Wu, F., J. Fan, Y. He, A. Xiong, J. Yu, Y. Li, Y. Zhang, W. Zhao, F. Zhou, W. Li, et al. 2021. Single-cell profiling of tumor heterogeneity and the microenvironment in advanced non-small cell lung cancer. *Nat. Commun.* 12:2540. https://doi.org/10.1038/s41467-021-22801-0

Xie, S., A. Cooley, D. Armendariz, P. Zhou, and G.C. Hon. 2018. Frequent sgRNA-barcode recombination in single-cell perturbation assays. *PLoS One*. 13:e0198635. https://doi.org/10.1371/journal.pone.0198635

Xie, S., J. Duan, B. Li, P. Zhou, and G.C. Hon. 2017. Multiplexed engineering and analysis of combinatorial enhancer activity in single cells. *Mol. Cell*. 66:285–299.e5. https://doi.org/10.1016/j.molcel.2017.03.007

Yan, J., P. Oyler-Castrillo, P. Ravisankar, C.C. Ward, S. Levesque, Y. Jing, D. Simpson, A. Zhao, H. Li, W. Yan, et al. 2024. Improving prime editing with an endogenous small RNA-binding protein. *Nature*. 628:639–647. https://doi.org/10.1038/s41586-024-07259-6

Yao, D., L. Binan, J. Bezney, B. Simonton, J. Freedman, C.J. Frangieh, K. Dey, K. Geiger-Schuller, B. Eraslan, A. Gusev, et al. 2024. Scalable genetic screening for regulatory circuits using compressed perturb-seq. *Nat. Biotechnol.* 42:1282–1295. https://doi.org/10.1038/s41587-023-01964-9

Ye, L., J.J. Park, M.B. Dong, Q. Yang, R.D. Chow, L. Peng, Y. Du, J. Guo, X. Dai, G. Wang, et al. 2019. In vivo CRISPR screening in CD8 T cells with AAV-sleeping beauty hybrid vectors identifies membrane targets for improving immunotherapy for glioblastoma. *Nat. Biotechnol.* 37:1302–1313. https://doi.org/10.1038/s41587-019-0246-4

Ye, L., J.J. Park, L. Peng, Q. Yang, R.D. Chow, M.B. Dong, S.Z. Lam, J. Guo, E. Tang, Y. Zhang, et al. 2022. A genome-scale gain-of-function CRISPR screen in CD8 T cells identifies proline metabolism as a means to enhance CAR-T therapy. *Cell Metab.* 34:595–614.e14. https://doi.org/10.1016/j.cmet.2022.02.009

Yuan, S., R. Sun, H. Shi, N.M. Chapman, H. Hu, C. Guy, S. Rankin, A. Kc, G. Palacios, X. Meng, et al. 2025. VDAC2 loss elicits tumour destruction and inflammation for cancer therapy. *Nature*. 640:1062–1071. https://doi.org/10.1038/s41586-025-08732-6

Zetsche, B., J.S. Gootenberg, O.O. Abudayyeh, I.M. Slaymaker, K.S. Makarova, P. Essletzbichler, S.E. Volz, J. Joung, J. van der Oost, A. Regev, et al. 2015. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*. 163:759–771. https://doi.org/10.1016/j.cell.2015.09.038

Zhang, A.W., A. McPherson, K. Milne, D.R. Kroeger, P.T. Hamilton, A. Miranda, T. Funnell, N. Little, C.P.E. de Souza, S. Laan, et al. 2018. Interfaces of malignant and immunologic clonal dynamics in ovarian cancer. *Cell*. 173:1755–1769.e22. https://doi.org/10.1016/j.cell.2018.03.073

Zhang, F., R.D. Chow, E. He, C. Dong, S. Xin, D. Mirza, Y. Feng, X. Tian, N. Verma, M. Majety, et al. 2025. Multiplexed inhibition of immunosuppressive genes with Cas13d for combinatorial cancer immunotherapy. *Nat. Biotechnol.* 43:2054–2067. https://doi.org/10.1038/s41587-024-02535-2

Zheng, X., P.C. Thompson, C.M. White, and X. Jin. 2025. Massively parallel in vivo perturb-seq screening. *Nat. Protoc.* 20:1733–1767. https://doi.org/10.1038/s41596-024-01119-3

Zhou, P., H. Shi, H. Huang, X. Sun, S. Yuan, N.M. Chapman, J.P. Connelly, S.A. Lim, J. Saravia, A. Kc, et al. 2023. Single-cell CRISPR screens in vivo map T cell fate regulomes in cancer. *Nature*. 624:154–163. https://doi.org/10.1038/s41586-023-06733-x

Zhu, R., E. Dann, J. Yan, J.R. Retana, R. Goto, R.C. Guitche, L.K. Petersen, M. Ota, J.K. Pritchard, and A. Marson. 2025. Genome-scale perturb-seq in primary human CD4+ T cells maps context-specific regulators of T cell programs and human immune traits. *bioRxiv*. https://doi.org/10.64898/2025.12.23.696273 (Preprint posted December 24, 2025).