Supplementary Material

Tumor Immunology CRISPR Screening: Present, Past, and Future

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Table S1. Exhaustive list of cancer-immune CRISPR screens.

Citation	Cell Type	Species & Condition (in vitro/in vivo)	CRISPR Library	Brief Description	Read-out
[S1]	K562 monocytic cell line	Human in vitro	Whole genome CRISPRi library	To identify Siglec-7 ligands, dCas9-KRAB K562 cells were stained with recombinant Siglec-7-Fc protein	FACS sorting on K562 cells with Siglec-7-Fc low staining
[S2]	B16F10 melanoma cell line	Murine in vivo	Integrated computational analysis of 8 different whole genome screens and 12 published ICB clinical studies	Integrated computation analysis to identify N-linked glycoproteins that are depleted in ICB responsive tumors, identifying MAN2A1	Tumor growth kinetics and survival of *MAN2A1* perturbation in the presence or absence of ICB therapy compared to control
[S3]	Ramos: Burkitt's lymphoma cell line	Human in vitro	Whole genome CRISPR-ko library; whole genome CRISPRa library	To identify intrinsic regulators of cancer cell phagocytosis, Cas9-Ramos cells were incubated with anti-CD20 (rituximab) antibody and J774 macrophages in the presence or absence of anti-CD47 antibodies.	crRNA libraries were prepared from suviving cells and compared to untreated Ramos cell controls. For validate gene, authors specifically looked at genes depleted in CRISPR-ko cells to identify mutated genes that sensitize cancer cells to macrophage-mediated phagocytosis
[S4]	THP-1 monocytic cell line	Human in vitro	Whole genome CRISPR-ko library	To identify genes involved with monocyte/macrophage activation, Cas9-THP-1 cells were sorted based on inducible CD14 in the presence or absence of PMA stimulation	Identified druggable kinases that were selectively enriched in CD14-low expressing THP-1 cells that were stimulated with PMA
[S5]	U937 monocytic cell line	Human in vitro	Whole genome CRISPR-ko library	To enrich for genes involved with LPS-induced cell death, Cas9-U937 cells were serially electroporated in the presence of LPS four times	Identified genes enriched in LPS-treated U937 cells compared to untreated U937 control

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[S6]	RAW 264.7 monocytic cell line	Murine in vitro	Whole genome CRISPR-ko library	To identify genes required for NLRP1B-mediated pyroptosis, Cas9-RAW 264.7 cells were treated with either nonselective-NLRP1B inflammasome inducer (DPP8/9 inhibitor) or selective-NLRP1B inflammasome inducer (anthrax lethal factor)	Genes enriched in response to both treatments were broadly involved with inflammasome activation. Gene enriched in lethal factor treatment only group identified genes involved selectively with NLRP1B-mediated pyroptosis
[S7]	Cas1-/-Casp11-/- immortal macrophages (iMacs)	Murine in vitro	Whole genome CRISPR-ko library	To delinate genes involved with different inflammasome-specific processes, Cas9-iMacs were created from Cas1-/-; Cas11-/- mice and electroporated with or without flagellin	Identified genes enriched in flagellin-treated samples compared to untreated controls
[S8]	Tlr4 −/− iMacs	Murine in vitro	Whole genome CRISPR-ko library	To identify genes with NLRP1B-mediated pyroptosis, Cas9-iMacs cells were treated with lethal toxin.	Surviving cells were sequenced for crRNA enrichment compared to untreated controls
[S9]	iMacs	Murine in vitro	crRNAs targeting upregulated lncRNAs	To identify the role of lncRNAs in NF-kB regulation, iMacs were transfected with a synthetic NF-kB promoter to drive GFP expression and used fur CRISPR screen in the context of different TLR stimulation	Small library of guides allowed researchers to study each crRNA individually using NF-kB reporter, as previously discussed
[S10]	iMacs	Murine in vitro	Whole genome CRISPR-ko library and library of essential genes targeting 3' UTR	Performed multiple screens: To identify genes involved with cell viability, Cas9-iMacs were taken on day 0 and day 21 to look for crRNAs enriched in later time point. To identify regulatory regions of essential genes, they designed select library targeting 3' UTR of genes. To identify modulator of NF-kB, they stimulated NF-kB-GFP-reporter iMacs with various TLR stimuli.	Negative regulators of essential genes were positively enriched for while positive regulators of essential genes were negatively selected. Positive regulators of NF-kB resulted in low GFP expression in response to stimulation while negative regulators were enriched in GFP-high expressing cells.

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[S11]	Ba/F3 pro-B cell line	Murine in vitro	Whole genome CRISPR-ko library	To identify positive regulators of NF-kB, they stimulated NF-kB-GFP reporter B cell lines with TLR5, TLR7 and TLR9 agonist and serially sorted on GFP low cells	Identified genes enriched in GFP low cells to identify positive regulators of TLR signaling
[S12]	KBM7 myeloid cell line (near haploid)	Human in vitro	Whole genome CRISPR-ko library	To identify regulators of TLR3 pathway, Cas9-KBM7 cell lines expressing NF-kB-GFP reporter were sequentially stimulated with poly(I:C) and sorted on GFP-negative cells	Positive regulators were identified by sorting on GFP-low KBM7 cells after sequential rounds of stimluation and sorting
[S13]	THP-1 monocytic cell line	Human in vitro	Whole genome CRISPRi library	Cyclic dinucleotides (CDN) are potent inducers of the cGas-STING pathway. To identify genes involved with CDN uptake and metabolism, Cas9-THP-1 were transduced with a CDN-inducible reporter and cocultured with various CDNs	Genes involved with CDN uptake/metabolism were enriched in reporter low cells compared to reporter high cells.
[S14]	U937 monocytic cell line	Human in vitro	Whole genome CRISPR-ko library	To identify genes involved with CDN uptake and metabolism, Cas9-U937 cells were serially treated with lethal doses of cGAMP	Genes involved with CDN uptake/metabolism were enriched in surviving cells compared to untreated controls
[S15]	THP-1 monocytic cell line	Human in vitro	Whole genome CRISPR-ko library	To identify modulators of phagocytosis in monocytes, Cas9-THP-1 cells were incubated with *S. aureus* labeled with pHrodo red, which increases in intensity with decreasing pH levels	Positive regulators of phagocytosis were identified in the pHrodo red-low group and negative regulators of phagocytosis were identified in the pHrodo-red high group
[S16]	U937 monocytic cell line	Human in vitro	crRNAs targeting solute carriers (SLCs)	To identify SLC that modulate phagocytosis in monocytes, Cas9-U937 cells were incubated with latex beads labeled with pHrodo red, which increases in intensity with decreasing pH levels, and pH-insensitive YG dye	Positive regulators of phagocytosis were depleted in pHrodo-red+; YG+ cells but enriched in pHrodo-red-; YG- cells

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[S17]	U937 monocytic cell line	Human in vitro	Whole genome CRISPR-ko library	To identify modulators of phagocytosis, Cas9-U937 were treated with different antigens conjugated to different iron reagents and sorted through magnetic separation	Genes enriched in the unbound magnetic fraction were determined to be positive regulators of phagocytosis and genes enriched in the magnetically bound fraction were consider to be negative regulators of phagocytosis
[S18]	KPCY+ pancreatic ductal adenocarcinoma (PDA) tumor cell line	Mouse in vivo	crRNAs targeting 850 epigenetic factors	To identify epigenetic factors modulating antitumor immunity, the author subcutaneously or orthotopically transplanted Cas9-PDA cells and treated the mice with gemcitabine, abraxane, CD40-agonist, CTLA4-inhibition, and PD-1-inhibition (GAFCP)	Genes depleted in GAFCP-treated tumors conferred sensitivity to antitumor immunity
[S19]	E0771 Triple Negative Breast Cancer cell line, Pan02 PDA cell line, B16F10 melanoma cell line	Mouse in vivo	Whole genome CRISPRa library	Demonstrating the utility of gene activation via CRISPRa to elicit antitumor immunity	Tumor growth curves to test efficacy of gene activation
[S20]	Juso melanoma cell line	Mouse in vitro	Whole genome CRISPR-ko library	To identify regulators of MHCII, Cas9-Juso cells were transduced with SteD-GFP, which is a bacterial pathogenic factor that downregulates MHCII, and stained for MHCII	MHCII-high expressing cells were serially sorted to identify negative regulators of MHCII expression
[S21]	Primary conventional dendritic cells	Mouse in vitro	Pathway specific, transcriptome-based focused CRISPR-ko library	To identify regulators of cross presentation, sorted cDC2s were individually infected with crRNAs and then cocultured with antigen-specific CD4 T cells.	Regulators of cross presentation were identified by crRNAs that attenuated T cell proliferation in the presence of antigen via CFSE cell proliferation assay.

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[S22]	Daudi: Burkitt's lymphoma cell line	Human in vitro	Whole genome CRISPR-ko library	To identify positive and negative regulators of CD40 responses, Cas9-Daudi cells were stimulated with CD40L, which induced Fas surface expression. Cells were sorted on their ability or inability to induce high levels of Fas expression.	Negative regulators of CD40 expression were identified as inducing high levels of Fas expression. Positive regulators of CD40 expression failed to induce high levels of CD40 expression.
[S23]	OCI-Ly-7: diffuse large B-cell lymphoma (DLBCL) line	Human in vitro	Whole genome CRISPR-ko library	To identify genes conferring resistance to anti-CD20 (rituximab) treatment, OCI-Ly-7 cells were treated with short-term (24-72 hours followed by 14-days rest) and long-term exposure (21-day continuous treatment) to rituximab treatment. Cell death was induced by complement activation; complement is found in human serum	crRNAs enriched with rituximab treatment represents genes involved with treatment sensitivity. crRNAs depleted in the rituximab treatment group represent genes that confer resistance to anti-CD20 therapy
[S24]	Ramos: Burkitt's lymphoma cell line	Human in vitro	Whole genome CRISPR-ko library	To identify regulators of antibody-drug conjugated toxicity, Cas9-Ramos cells were serially treated with anti-CD22 maytansine to induce cell cytotoxcity in vitro	crRNAs enriched in surviving cells revealed genes involved endolyosomal regulators that when knocked out prevented the endocytosis and subsequent activation of anti-CD22 maytansine
[S25]	JeKo-1: Mantle cell lymphoma line	Human in vitro	Whole genome CRISPRa library	To identify genes that confer resistance to bispecific antibody therapy (CD20xCD3), dCas9-MS2-JeKo-1 cells were serially treated with bispecific antibody and coincubated with cytotoxic CD8 T cells to induce JeKo-1 cell killing	Because this is CRISPRa, enriched crRNAs represent genes that confer resistance to bispecific T cell killing compared to untreated cell.

Citation	Cell Type	Species & Condition (in vitro/in vivo)	CRISPR Library	Brief Description	Read-out
[S26]	MOLM-13: acute myeloid leukemia cell line	Human in vitro	Whole genome CRISPR-ko library	To identify genes that confer resistance to bispecific antibody therapy (CD123xCD3), Cas9-MOLM13 cells were treated with anti-CD123 antibodies followed by the addition of CD8 T cells. Surviving cells were allowed to expand for 2 weeks before NGS sequencing	Resistant hits are enriched crRNAs of genes that normally confer sensitivity to bispecific antibody treatment. Sensitization hits are depleted crRNAs of normal genes that would confer resistance to bispecific antibody treatment
[S27]	Jurkat: T acute lymphoblastic leukemia cell line	Human in vitro	Whole genome CRISPR-ko library	To identify genes involved with proximal T cell antigen receptor signaling, Cas9-Jurkat T cells were stimulated with anti-TCR antibody to induce TCR crosslinking and subsequent upregulation of CD69.	crRNAs enriched in CD69 high cells respresent negative regulators of TCR signaling whereas crRNAs enriched in CD69 low cells respresent positive regulator of TCR signaling
[S28]	68-41 T cell line	Mouse in vitro	Whole genome CRISPR-ko library	To identify genes involved with PD-1 expression, Cas9-68-41 T cells were serially sorted on PD-1 negative to low expression	Enriched crRNAs in PD-1 negative to low expressing cells represent genes augment PD-1 expression
[S29]	Primary CD8 T cells	Human in vitro	Whole genome CRISPR-ko library	To identify genes involved with T cell proliferation, Cas9-CD8 T cells were stained with the cytoplasmic CSFE proliferation dye prior to being stimulated with anti-CD3/CD28 bead to induce T cell activation and proliferation. Nonproliferating cells were sorted on CFSE-hi expression and Proliferating cells were sorted on CFSE-lo expression	crRNAs enriched in nonproliferating T cells represent positive regulators of TCR signaling. crRNAs enriched in proliferating T cells represent regulatory genes that suppresses TCR signaling and proliferation.

Citation	Cell Type	Species & Condition (in vitro/in vivo)	CRISPR Library	Brief Description	Read-out
[S30]	Primary CD8 T cells	Mouse in vitro and in vivo	Whole genome CRISPR-ko library	To identify genes involved with tumor killing, Cas9-CD8 T cells were coincubated with cancer cells expressing their cognate antigen and stained with anti-CD107a antibody to capture TCR-induced degranulation/killing. To identify genes involved with tumor infiltration, Cas9-CD8 T cells were injected into antigen-expressing tumor-bearing mice and after several days tumors were extracted to identify T cells that were enriched in tumors	crRNAs enriched in CD107a-high cells represent negative regulators of T cell degranulation/killing. crRNAs enriched in tumors represent negative regulators of T cell infiltration
[S31]	Primary CD8 T cells	Mouse in vivo	Whole genome CRISPR-ko library and library targeting metabolic genes	To identify metabolic genes involved with antitumor activity, Cas9-CD8 T cells were injected into antigen-expressing tumor-bearing mice and after several days tumors were extracted to identify T cells that were enriched in tumors	crRNAs enriched in tumors represent metabolic regulators that suppress T cell activity within tumors
[S32]	Primary CD8 T cells	Mouse in vitro	CRISPR-ko library targeting kinases	Multiple flow cytometric assays were designed to identify genes involved with cell proliferation (CFSE), memory formation (CD62L), oxidative stress (DCFDA), and genomic stability (gH2AX).	Favorable crRNAs were enriched in cell that induced high cell expansion and increased memory formation while limiting oxidative stress and genome instability. These crRNAs represent genes that normally inhibit these functions due to the ability of the crRNAs to induce silencing frameshift mutations
[S33]	NALM6: B acute lymphoblastic leukemia	Human in vitro	Whole genome CRISPR-ko library	To identify genes essential for CAR-T cytotoxicity, Cas9-NALM 6 cells were either treated with anti-CD19 CAR-T cells alone or treated with anti-CD19 CAR-T plus an apoptotic sensitizer (SMAC)	Enriched crRNAs represent mutated genes that confer resistance to CAR-T cell killing in the presence or absence of SMACs

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[S34]	ID8: murine serous ovarian carcinoma cell line	Mouse in vitro	Curated CRISPR-ko library of control genes, immune modulators, epigenetic regulators, and MHC genes	To identify mutated genes that confer sensitivity to T cell killing, Cas9-ID8 cells were coincubated with T cells	crRNAs depleted in surviving cells reveal mutated genes that confer sensitization to T cell killing, which was confirmed by drug inhibition
[S35]	B16F10 melanoma cell line	Mouse in vitro	Whole genome CRISPR-ko library	To identify genes that modulate PD-1 and MHC-I upregulation in response to IFN-gamma, Cas9-B16F10 cells were treated with high- and low-dose IFN-gamma and stained with anti-PD-1 and anti-MHC-I antibodies	The readout for this screen is quite complex and is better illustrated in the primary text. Briefly, regulatory genes involved with MHC-I expression, PD-1 expression, and MHC-I; PD-1 double expression were identified in B16 cells that behave contrary to what was expected with each treatment
[S36]	THP-1, K562, KG-1, and U937 monocytic cell lines	Human in vitro	Curated CRISPR-ko library of transcription factors, chromatin regulators, signaling regulators, and modifiers of protein stability	This study was designed to generate a platform for single cell RNA-sequencing that simultaneously allows researchers to perform CRISPR screening, transcriptional analysis, and protein analysis	The readout is deconvolution of NGS data to identify crRNAs that downregulate PD-L1 expression at both the transcript and protein level
[S37]	ALK+ anaplastic large cell lymphoma	Human in vitro	Whole genome CRISPR-ko library	To identify genetic modifiers involved with PD-L1 expression, Cas9-ALK+ ALCL cells were serially sorted on PD-L1-low expression	Enriched crRNAs are regulatory genes involved with repressing PD-L1 expression
[S38]	GIMEN: neuroblastoma cell line	Human in vitro	Whole genome CRISPR-ko library	To identify gene modulating MHC-I expression and NF-kB activity, Cas9-GIMEN cells were transduced with NF-kB-GFP reporter and stained with anti-MHC-I antibody	Positive regulators of MHC-I and NF-kB activity were identified in MHC-I; GFP double negative cells whereas negative regulators were enriched in MHC-I; GFP double positive cells.

Citation	Cell Type	Species & Condition (in vitro/in vivo)	CRISPR Library	Brief Description	Read-out
[S39]	p53-/-; Myc+ heptocellular carcinoma cell line	Mouse in vivo	Whole genome CRISPR-ko library	To identify tumor-intrinsic factors that modulate antitumor immunity in vivo, Cas-HCC cell lines were subcutaneously transferred to immunocompetent and immunoincompetent mice. WT Cas9-HCC cells were rejected in immunocompetent mice while library-infected Cas9-HCC cells grew.	crRNAs in tumors that grew in immunocompetent mice were sequenced and compared to initital cell input. Enriched crRNAs represent genes that confer immune resistance in immunocompetent mice that when mutated allow HCCs to grow.
[S40]	B16F10 melanoma cell line	Mouse in vitro and in vivo	Whole genome CRISPR-ko library	To identify genes involved with T cell evasion, Cas9-B16F10-OVA cells were either subcutaneously transplanted into C57B6 mice or treated with OT-1 T cells. Resistance cells were obtained and placed under another round of T cell selection	Surviving cells were sequenced to determine crRNA enrichment compared to untreated controls, which identifies mutated genes that confer T cell resistance
[S41]	MC38 colon adenocarcinoma cell line	Mouse in vitro	Whole genome CRISPR-ko library	Cas9-MC38-OVA cells were treated with OT-1 T cells isolated from different genetic backgrouds in order to delinate genes involved with specific types of evasion mechanisms	Surviving cells were sequenced to determine crRNA enrichment compared to untreated controls, which identifies mutated genes that confer T cell resistance
[S42]	B16F10 melanoma cell line	Mouse in vivo	Whole genome CRISPR-ko library	Cas9-B16F10 melanoma cell lines were subcutaneously transplanted into Tcra-/- mice, C57BL6 mice treated with GM-CSF + irradiated B16F10 (GVAX), or C57BL6 mice treated with GVAX + anti-PD-1	Surviving cells from different treatment groups were sequenced. Enriched crRNAs in GVAX or GVAX + anti-PD-1 treatment compared to Tcra-/- represent mutated genes that confer immune escape.
[S43]	B16F10 melanoma cell line; 4T1 and EMT6 breast cancer cell lines; CT26 and MC38 colon adenocarcinoma cell line; RenCa renal cell carcinoma cell line	Mouse in vitro	Whole genome CRISPR-ko library	To identify genes involved with T cell evasion, Cas9-expressing cell lines were treated with OT-1 T cells	Sensitizer genes are mutated genes that were depleted under T cell selection; resistor genes are mutated genes that were enriched under T cell selection

Citation	Cell Type	Species & Condition (in vitro/in vivo)	CRISPR Library	Brief Description	Read-out
[S44]	B16F10 melanoma cell line	Mouse in vitro	Whole genome CRISPR-ko library	To identify genes involved with T cell evasion, Cas9-B16F10-OVA cell lines were treated with OT-1 T cells	Sensitive cell represent cells bearing mutated genes that were depleted under T cell selection; Resistant cells possess mutated genes that were enriched under T cell selection
[S45]	K562 monocytic cell line	Human in vitro	Whole genome CRISPR-ko library	To identify mutated genes that confer sensitivity to NK cell-mediated killing, Cas9-K562 cells were coincubated with primary NK cells	Surviving cells were sequenced to determine crRNA enrichment compared to untreated controls, which identifies mutated genes that confer NK cell resistance
[S46]	K562 monocytic cell line	Human in vitro	Whole genome CRISPR-ko library	To identify mutated genes that confer sensitivity to NK cell-mediated killing, Cas9-K562 cells were coincubated with primary NK cells. To identify mutated genes that affect MHC-I processing and presentation, Cas9-K562 cells were treated with IFN-gamma	Depleted mutated genes after NK-92 challenge represent genes that confer sensitivity to NK cells; Enriched mutated genes after NK-92 challenge represent genes that confer NK cell resistance; Genes enriched in MHC-I-low cells are reflected in the impaired MHC-I response and those from the MHC-I-high cells are represented in exaggerated MHC-I response
[S47]	B16F10 melanoma cell line	Mouse in vitro	Whole genome CRISPR-ko library	To identify mutated genes that confer sensitivity to NK cell-mediated killing, Cas9-B16F10 cells were coincubated with primary NK cells. To identify mutated genes that affect MHC-I processing and presentation, Cas9-B16F10 cells were treated with IFN-gamma	Depleted mutated genes after NK cell challenge represent genes that confer sensitivity to NK cells; Enriched mutated genes after NK cell challenge represent genes that confer NK cell resistance; Genes enriched in MHC-I-low cells are reflected in the impaired MHC-I response and those from the MHC-I-high cells are represented in exaggerated MHC-I response

Citation	Cell Type	Species & Condition (in vitro/in vivo)	CRISPR Library	Brief Description	Read-out
[S48]	Patient-derived GBM cells and primary CAR-T cells	Human in vitro	Whole genome CRISPR-ko library	To identify genes that negatively regulate CAR-T activity, primary CAR-T cells were incubated with GBM cells and stained for PD-1 expression. To identify mutated genes that confer resistance to CAR-T cell therapy, Cas9-GBM cells were incubated with CAR-T cells	crRNAs in PD-1-negative expressing cells represent genes that negatively regulate T cell activity; crRNAs enriched in surviving GBM cells reflect mutated genes that confer GMB survival in response to CAR-T cell therapy
[S49]	Primary CD8 T cells	Human in vitro	CAR-T cell library	Performed various functional flow cytometric assays	Sorted on cells that express the higheset favorable phenotype or express the lowest unwanted phenotype
[S51]	HEK293T: human embryonic kidney cell line	Human in vitro	Whole genome CRISPR-ko library	To identify the ligand of non-MHCI binding CD8 TCR, Cas9-HEK293T cells were coincubated with MC.7.G5-expressing T cells	crRNAs enriched in surviving cells reveal genes encoding potential ligand for MC.7.G5 TCR
[S51]	Primary CD8 T cells	Mouse in vivo	Library targeting surface receptor proteins	To identify surface protein regulators of CD8 T cell activity, CD8 T cells were injected into GMB bearing mice	crRNAs enriched in GBM tumors encode for potential genes that negatively regulate CD8 T cell activity

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