# CRISPR Library Screen to Identify Driver Mutations of Tumor Growth and Metastasis

## **Oncology & Immunology Unit**







Yong Cang, PhD Webinar, April 23, 2020

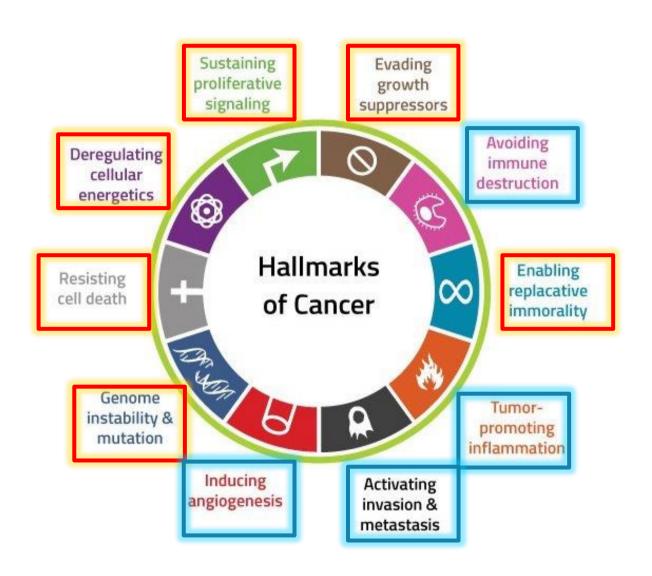


## **TOPICS**

- 1. CRISPR library screen to discover novel oncology and oncoimmunology targets
- 2. Technical considerations for successful CRISPR screens in vitro and in vivo

## Somatic mutations overcome cell-intrinsic and environmental restraints on tumor development





**Tumor intrinsic:** 

**Enabling self proliferation** 

**Tumor extrinsic:** 

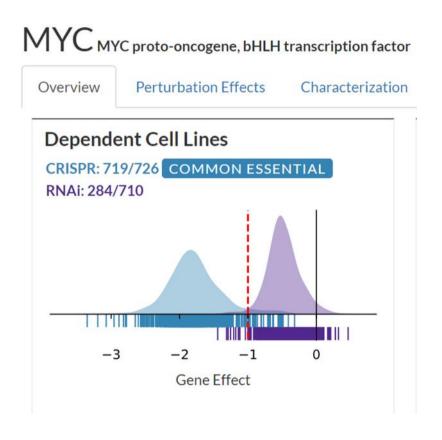
Escaping environmental constraint

## Identification of Essential Genes in Cancer Cell Lines by Project Achilles





"Project Achilles is a systematic effort aimed at identifying and cataloging gene essentiality across hundreds of genomically characterized cancer cell lines."



## Identification of Essential Genes under Different Growth Conditions by CRISPR Knockout Library Screen



**Article** 

## CRISPR screens in cancer spheroids identify 3D growth-specific vulnerabilities

Han, et al. Nature 2020

LETTER

https://doi.org/10.1038/s41586-018-0291-z

CRISPR screens identify genomic ribonucleotides as a source of PARP-trapping lesions

Zimmermann, et al. Nature 2018

**CANCER** 

A major chromatin regulator determines resistance of tumor cells to T cell-mediated killing

Pan, et al. Science 2018

**ARTICLE** 

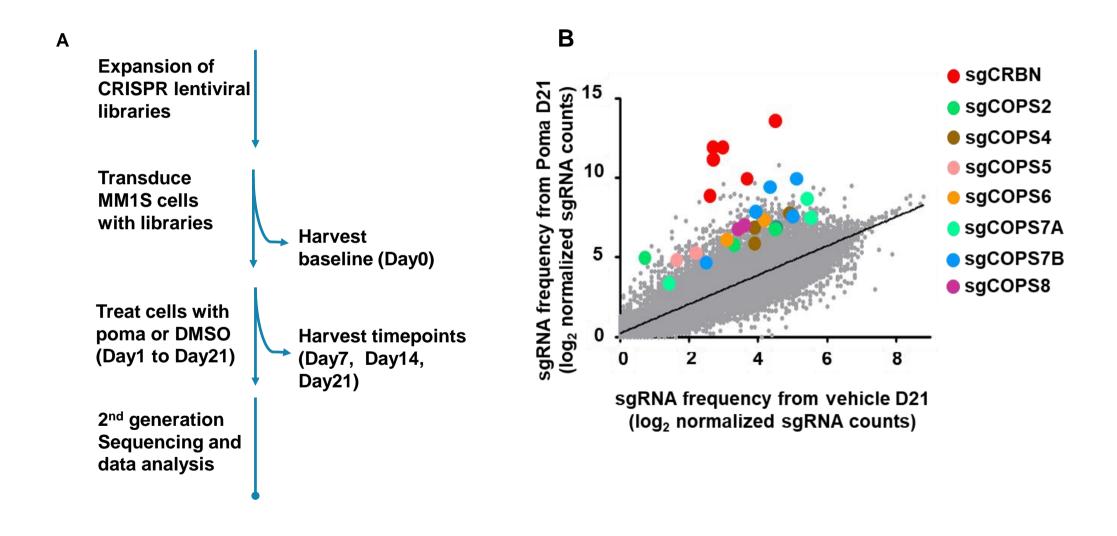
doi:10.1038/nature23270

In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target

Manguso, et al. Nature 2017

## Interrogation of drug resistant mechanism by in vitro CRISPR Knockout Screen

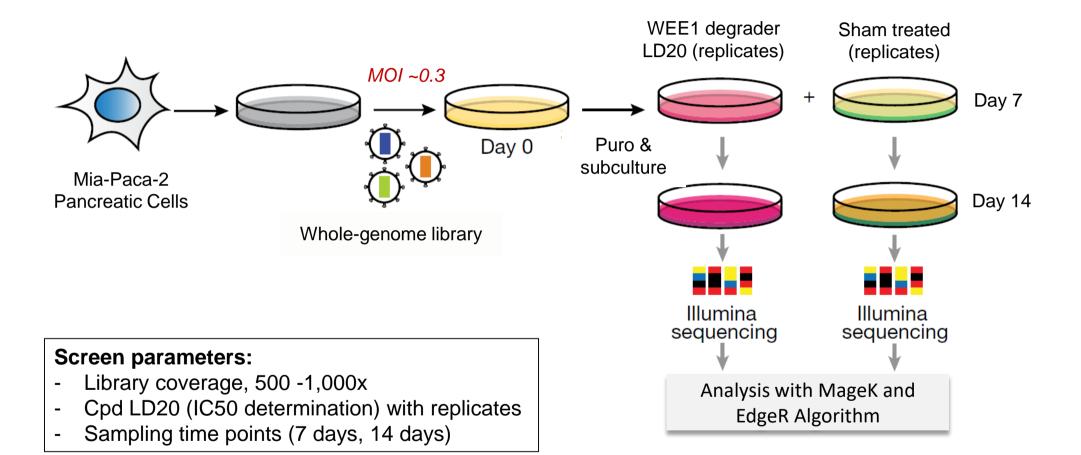




## Synthetic lethal screen with a cell cycle checkpoint inhibitor P Null Annited



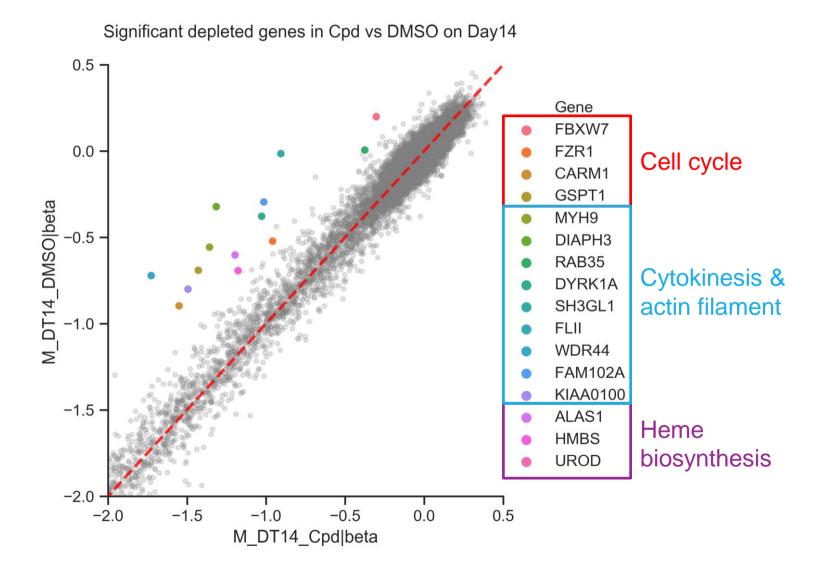
Workflow to identify sensitizing mutations



## Synthetic lethal screen with a cell cycle checkpoint inhibitor \*\* WUXI ADD Tec



Hits include regulators of cell cycle progression and heme biosynthesis



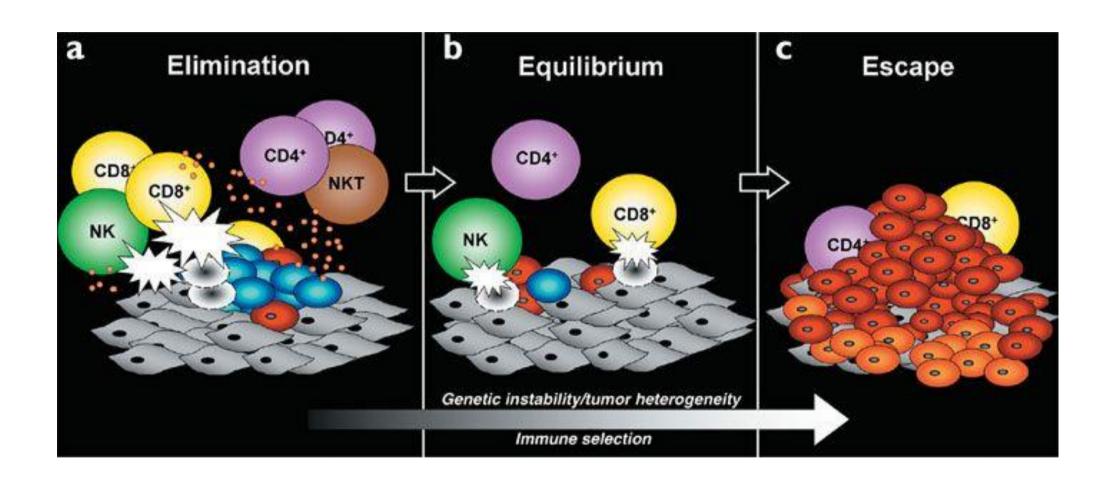
#### selection parameters:

- Fold change .1.5
- FDR<0.05 each guide
- At least 3 guides each gene

### **Cancer Immunoediting**

Elimination, equilibrium, escape





Dunn, et al, Nature Immunology 2002



## What mutations enable cancer evasion from immune restraint?

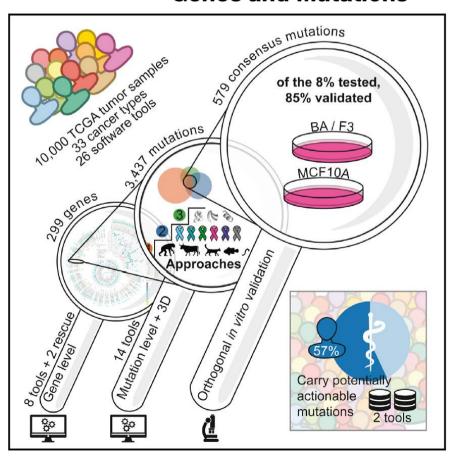
## **Customized CRISPR library targeting cancer drivers**

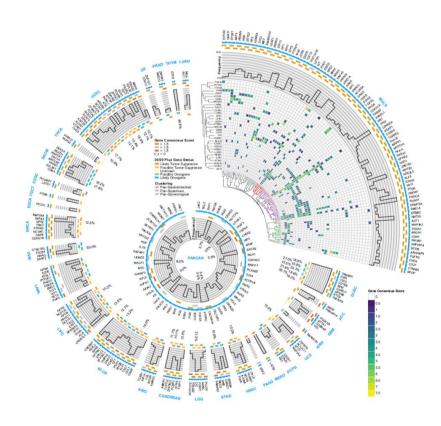


- 1978 sgRNAs (including 100 nontarget sgRNAs)
- 313 genes highly mutated in human cancers (6 sgRNAs each gene)



## **Comprehensive Characterization of Cancer Driver Genes and Mutations**

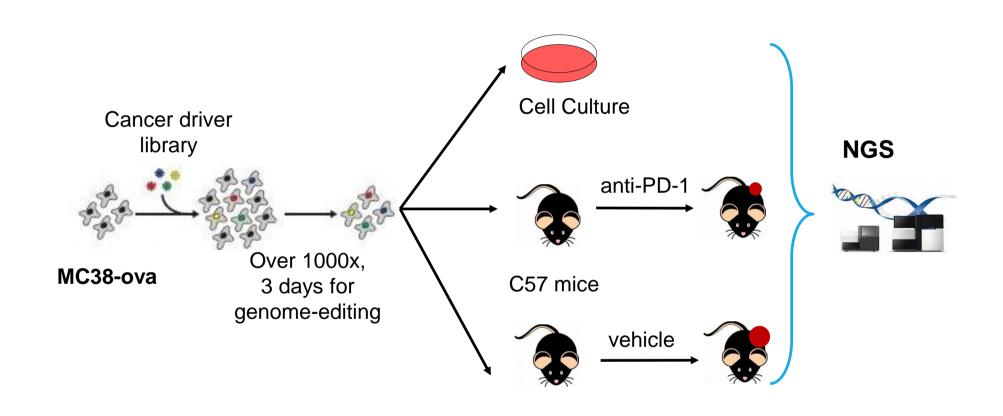




## **CRISPR** library screen in syngeneic tumor models



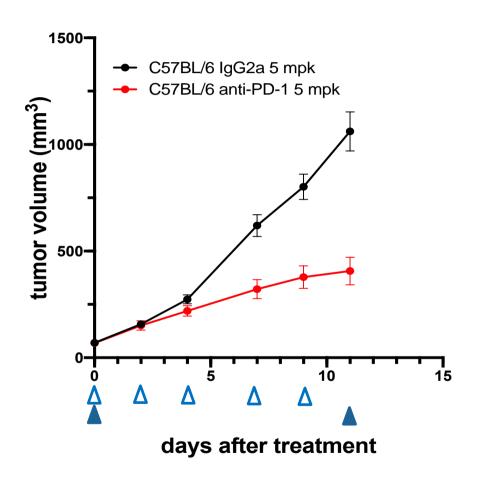
Workflow with MC38-Ova in C57B6 host



## **CRISPR** library screen in syngeneic tumor models



Tumor growth, dosing schedule, sample collection

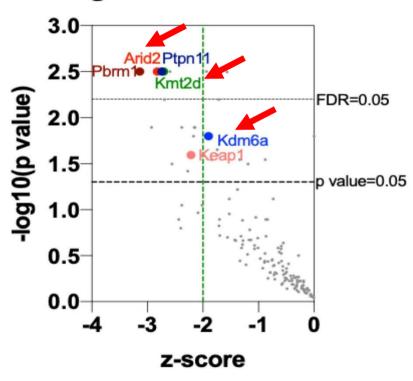


- △ Dosing time
- ▲ Tumor collection time

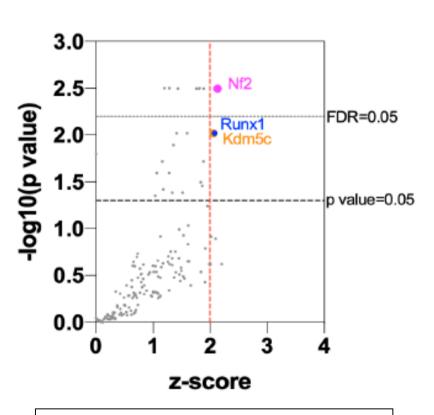


## Mutations sensitizing or antagonizing PD-1 blocker in vivo

#### B6-IgG vs B6-anti-PD-1



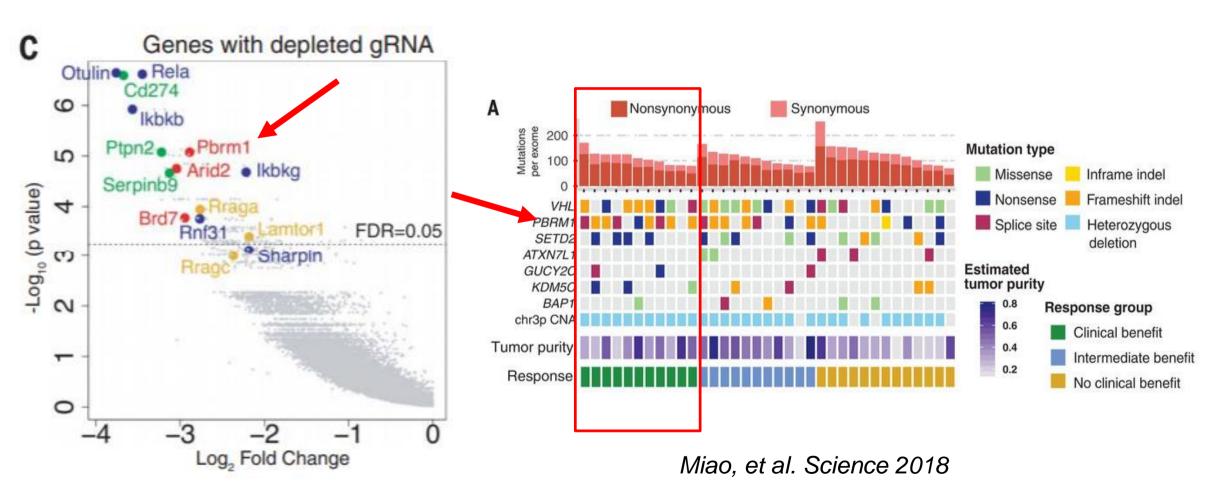




**Genes with enriched sgRNAs** 

## PBAF mutations sensitize to T cell killing and anti-PD1 immunotherapy



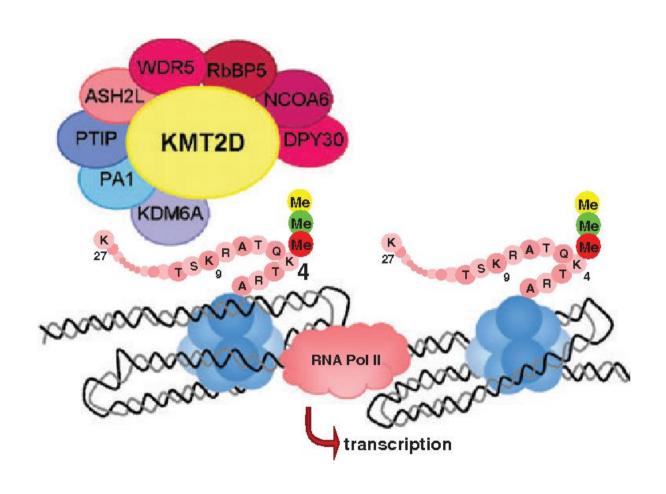


Pan, et al. Science 2018

Private and Confidential 15

## KMT2D and KDM6A are frequently mutated in cancer and Kabuki Syndromes



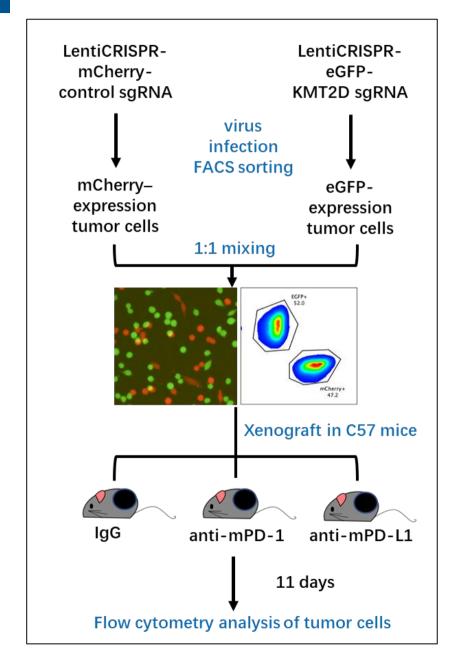


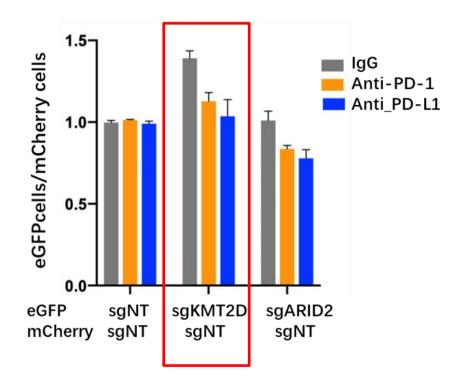
#### KMT2D/MLL2

- A major H3K4 mono-methyltransferase
- Enhancer activation and cell type-specific gene expression.
- Mutations associated with developmental diseases and various cancers.



## Validation of KMT2D by in vivo competition assays



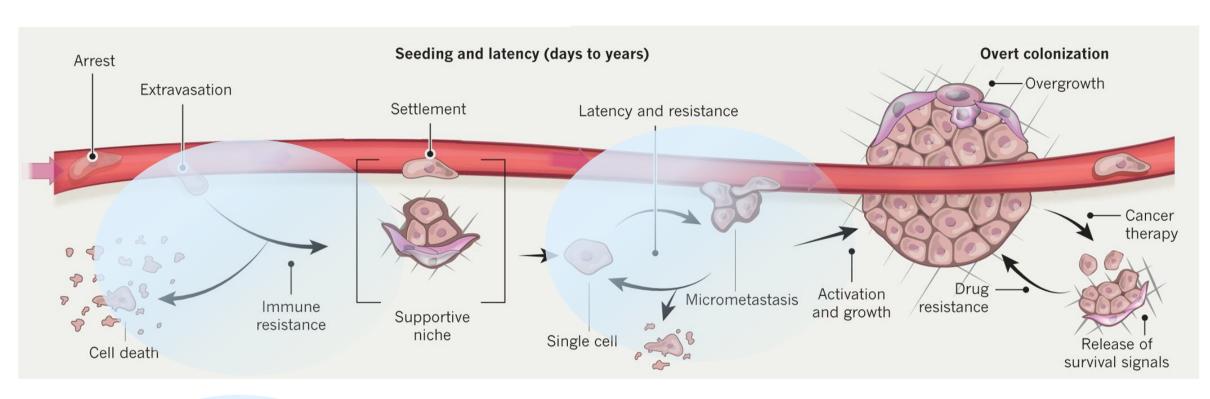


#### **Working Hypothesis:**

loss of KMT2D enables PD-1-dependent immune escape

## **Immune Control of Metastatic Seeding and Colonization**



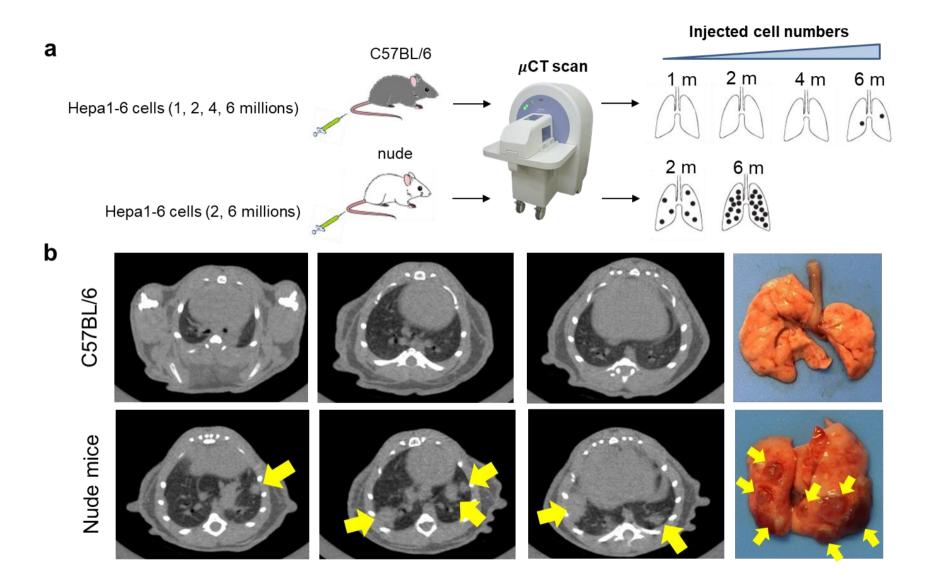


Immune elimination step

Adapted from Massague & Obenauf, Nature 2016

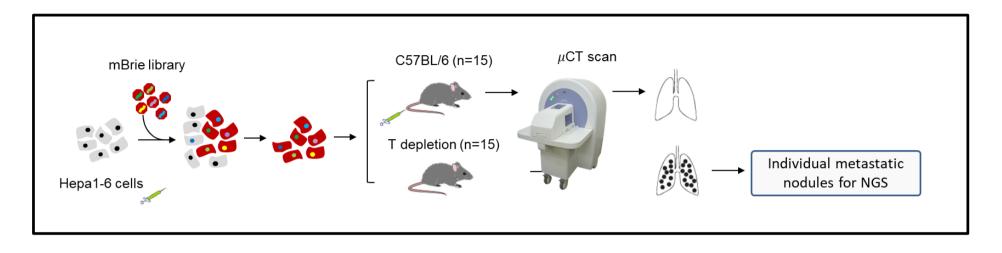
## Syngeneic liver cancer model for T cell-controlled pulmonary dissemination

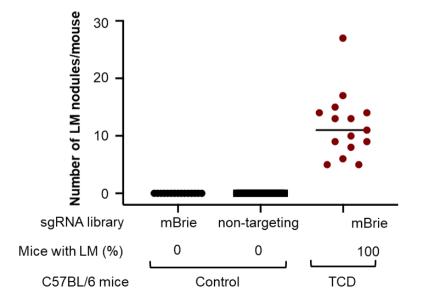


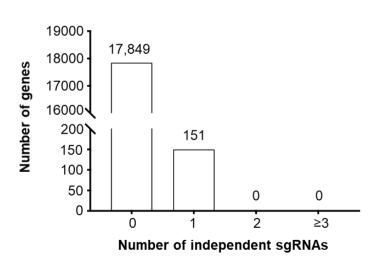


## Failed CRISPR screen with whole-genome library





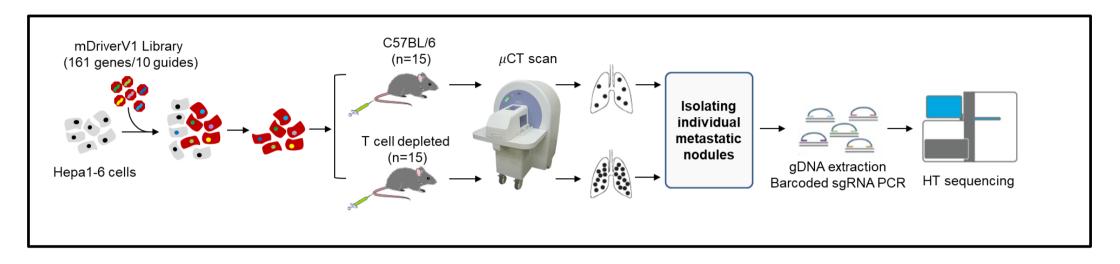


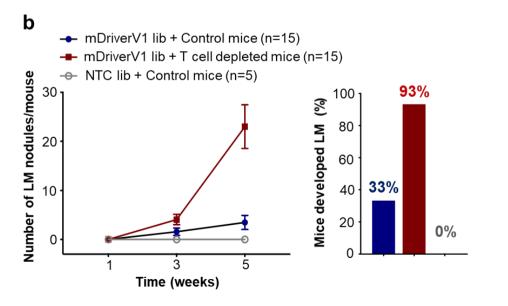


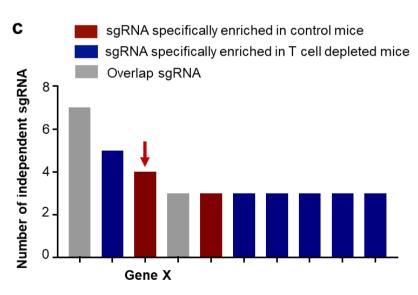
## Identification of a novel gene controlling T cell killing



A much smaller focused library to increase coverage

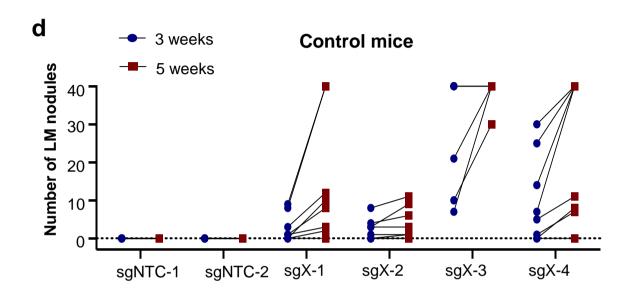


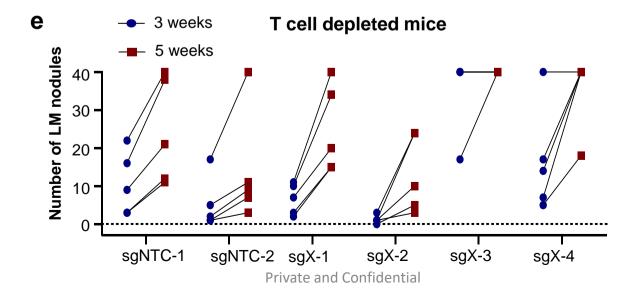




## Validation of the hit







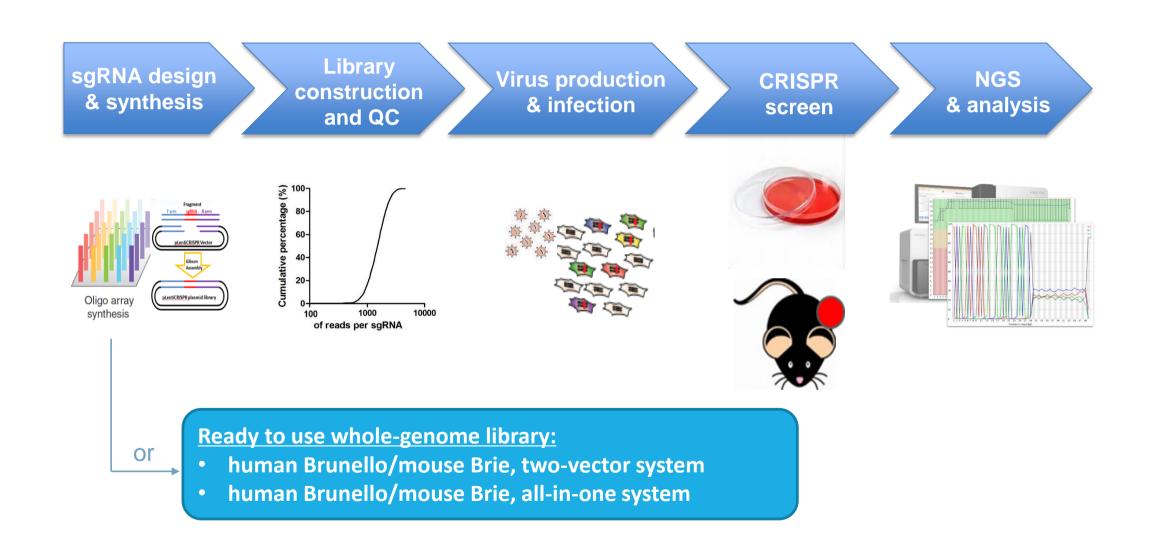


### Technical considerations for a successful CRISPR screen

## **Overview of CRISPR Library Screen Workflow**



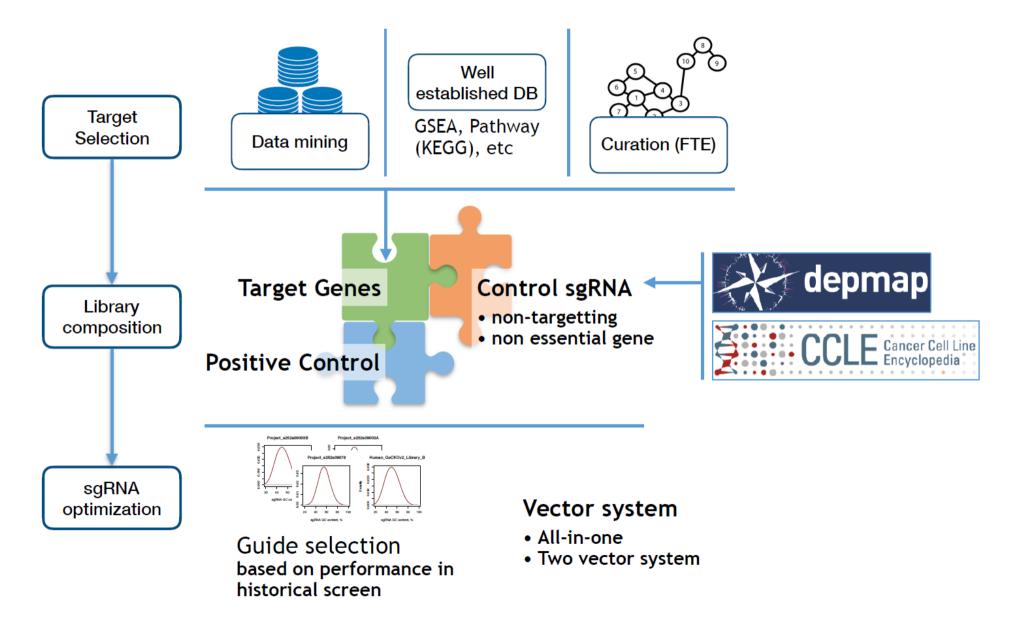
For in vitro and in vivo screens



### **Library Design**



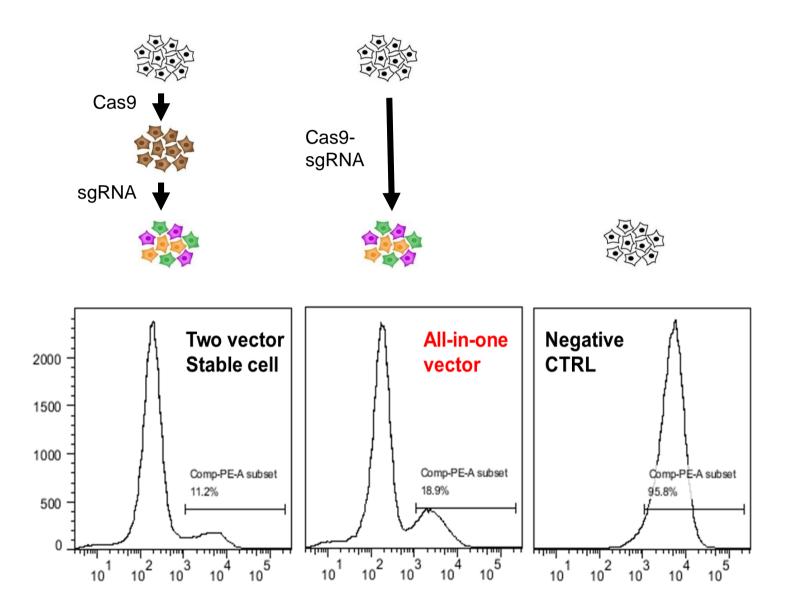
In-house pipeline for customized library design



### **Validated Lentivirus Vector Efficiency**



Two vector system v.s. all-in-one system



## In vitro Screens Performed at Wuxi

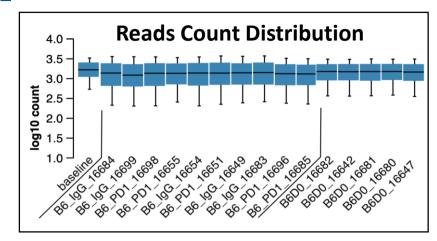


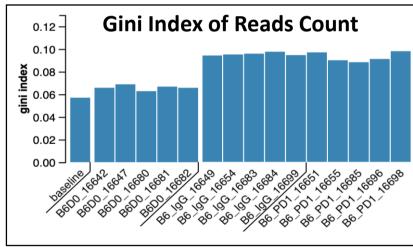
## In-house validated screen-friendly human cell lines, growing

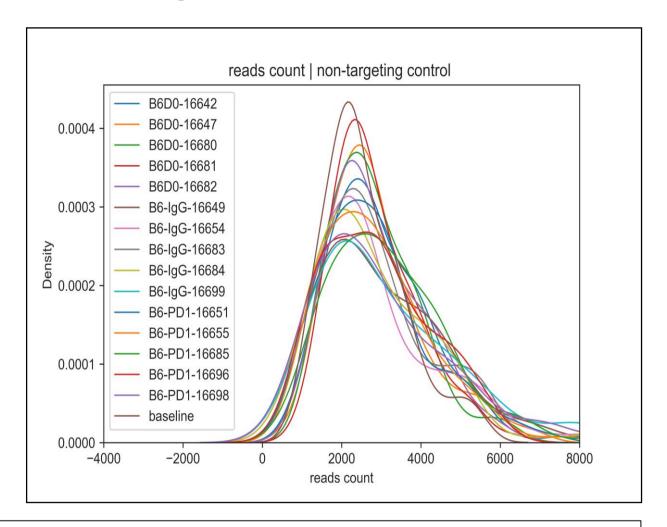
Cell Line Name	Cancer Type	Approx. Doubling Time	Infections efficiency	Screen Validated
A2780	Human Ovarian cancer	<=24h	Good	Yes
A549	Human Lung (carcinoma; non-small cell lung cancer)	24-36h	OK	Yes
Caki-1	Human Kidney, Clear Cell Carcinoma	36-48h	Good	Yes
ES-2	Human Ovarian cancer	<=24h	Good	Yes
HCC1187	Human Breast cancer	>=48h	Good	Yes
HCC1395	Human Breast cancer	>=48h	Good	Yes
HCC1599	Human Breast cancer	>=48h	Good	Yes
HCC1937	Human Breast cancer	36-48h	Good	Yes
HCT116	Human Colorectal cancer	<=24h	Good	Yes
HK-2	Human Kidney epithelial, transformed normal PTC	>=48h	Good	Yes
HT-29	Human Colorectal cancer	<=24h	Good	Yes
KURAMOCHI	Human Ovarian Carcinoma	36-48h	Good	Yes
MCF-10A	Human Breast epithlial, spontaneously immortilized	>=48h	Good	Yes
MCF-7	Human Breast cancer	36-48h	Good	Yes
MDA-MB-157	Human Breast cancer	>=48h	Good	Yes
MDA-MB-436	Human Breast cancer	>=48h	Good	Yes
MDA-MB-468	Human Breast cancer	>=48h	Good	Yes
MM.1S	Human Hematopoietic (multiple myeloma)	>>48h	Good	Yes
NCI-H1299	Human Lung (carcinoma; non-small cell lung cancer )	<=24	Good	Yes
NCI-H1563	Human Lung (adenocarcinoma; non-small cell lung cancer)	>=48h	Good	Yes
NCI-H1975	Human Lung (adenocarcinoma; non-small cell lung cancer)	36-48h	Good	Yes
NCI-H2172	Human Lung (non-small cell lung cancer)	36-48h	Good	Yes
NCI-H23	Human Lung (adenocarcinoma; non-small cell lung cancer)	36-48h	Good	Yes
NCI-H441	Human Lung (papillary adenocarcinoma )	36-48h	Good	Yes
NCI-H460	Human Lung (carcinoma; large cell lung cancer)	<=24h	Good	Yes
OV-90	Human Ovarian Carcinoma	36-48h	Good	Yes
OVCAR.3	Human Ovarian cancer	>=48h	Good	Yes
OVMANA	Human Ovarian Carcinoma	36-48h	Good	Yes
PEO1	Human Ovarian Carcinoma	>=48h	Good	Yes
PEO4	Human Ovarian Carcinoma	>=48h	Good	Yes
RWPE-1	Human Prostate epithelial	>=48h	Good	Yes
SW480	Human Colorectal cancer	24-36h	Good	Yes

## Distribution and representation coverage





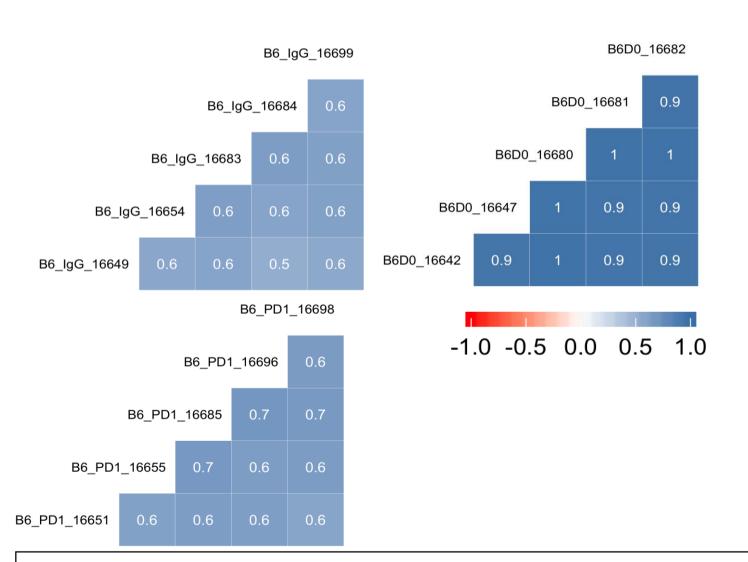




Distribution Pattern is similar within treatment group. Gini index revealed limited unbalance in read count distribution. No distribution or representation bias of non-targeting control sgRNAs was observed in D0 samples (in vivo tumor baseline).







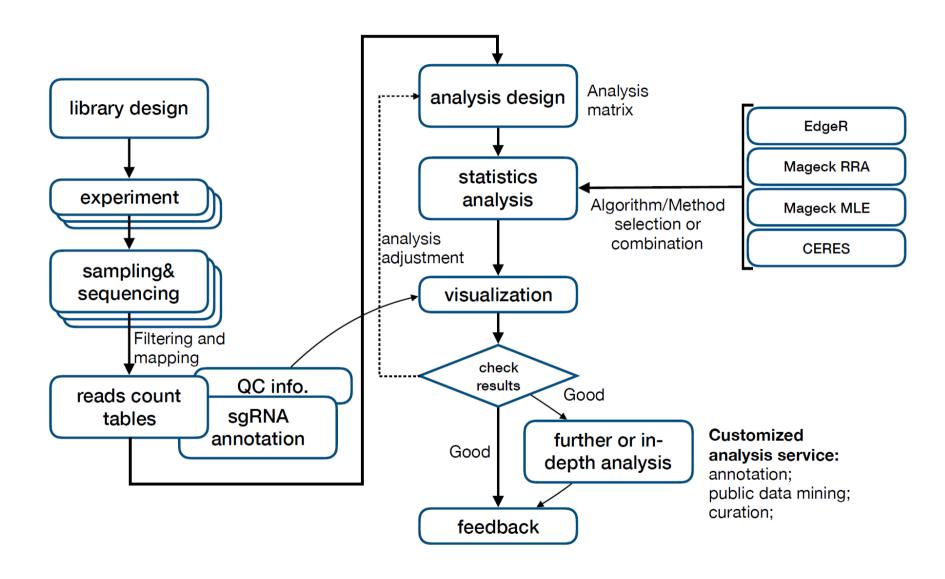
GROUP	SAMPLE	Zero Count(%)
Baseline	baseline	0
	B6D0-16642	0.05%
	B6D0-16647	0.1%
B6-D0	B6D0-16680	0.05%
	B6D0-16681	0
	B6D0-16682	0.05%
	B6-IgG-16649	0.61%
	B6-IgG-16654	0.51%
IgG	B6-IgG-16683	0.61%
	B6-IgG-16684	0.61%
	B6-IgG-16699	0.46%
	B6-PD1-16651	0.71%
	B6-PD1-16655	0.81%
Anti-PD-1	B6-PD1-16685	0.3%
	B6-PD1-16696	0.76%
	B6-PD1-16698	0.91%

Read count is used to calculate correlation value (Pearson) between repeats within each group. Proportion of zero count shows good representation of sgRNA within each sample

## **Bioinformatics Workflow for CRISPR Screen Data Analysis**



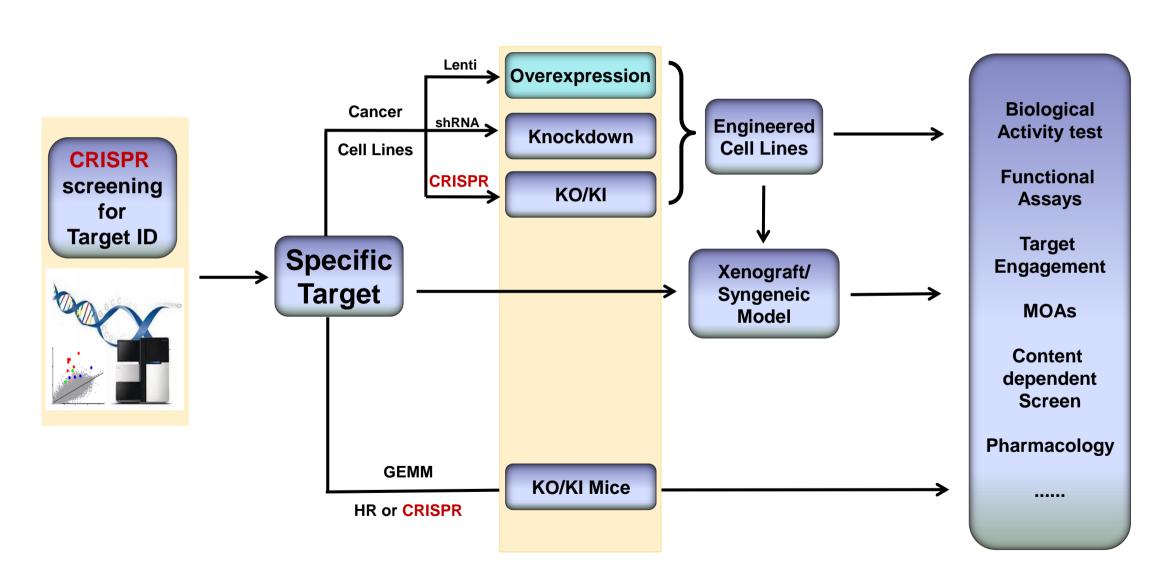
From library design to data feedback



### Target Identification and Validation Platform at WX



Combining genome-wide screen and gene editing





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