

CRISPR/CAS9 based high-throughput screening

Journal club

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High Throughput Screening (HTS)

HTS is a method for scientific experimentation especially used in drug discovery and relevant to the fields of biology and chemistry. Using robotics, data processing and control software, liquid handling devices, and sensitive detectors, High-Throughput Screening allows a researcher to quickly conduct millions of chemical, genetic, or pharmacological tests. Through this process one can rapidly identify active compounds, antibodies, or genes that modulate a particular biomolecular pathway. The results of these experiments provide starting points for drug design and for understanding the interaction or role of a particular biochemical process in biology.

wikipedia

High Throughput Screening (HTS)

Chemical compounds - ready to be druggable

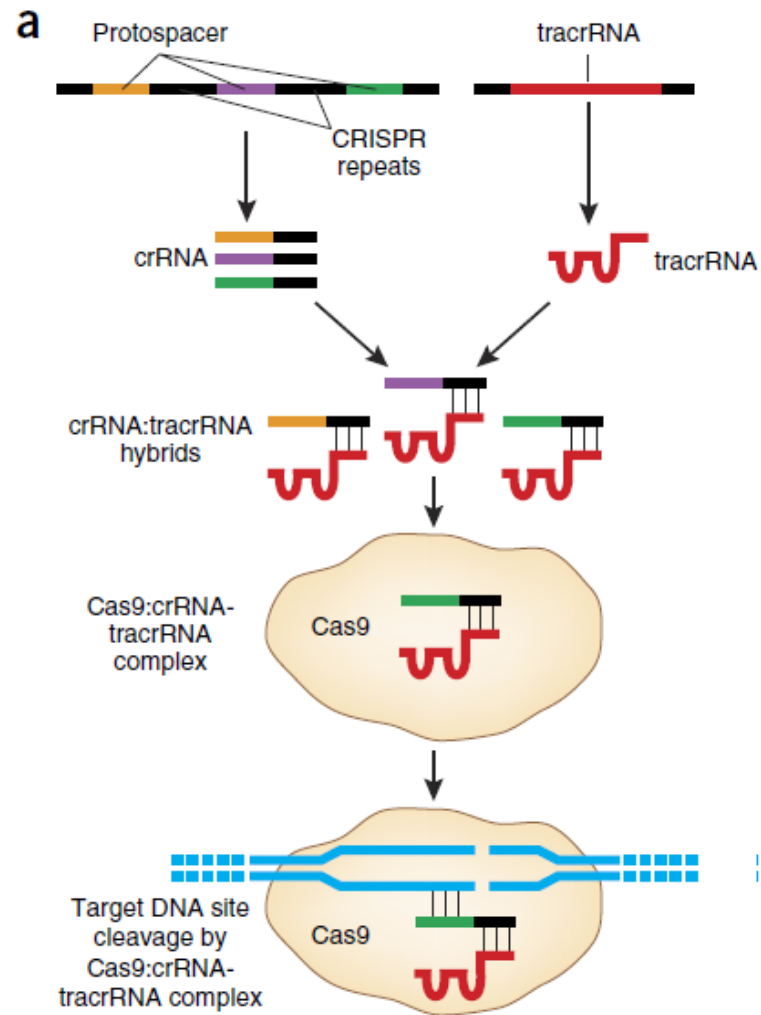
Genetic screening - to interrogate gene functions in genome-wide scale, is hypothesis-free approach to discover genes and pathways that underlie biological processes

- **Insertion mutagenesis** - Haploid cell only, integration biases
- **RNAi** – mRNA based, not complete silencing, off-target
- **Nucleases** based gene manipulation
 - *Meganucleases*
 - *ZFN*
 - *TALENs*
 - **CRISPR-CAS9**

Genome editing nucleases for HTS

Name	Components	Mechanism of action	Specificity/off-target effects	Possibility to rapidly generate large-scale libraries
Genome editing				
Zinc finger nucleases (ZFNs)	Fok1 restriction nuclease fused to multiple zinc finger peptides; each targeting 3 bp of genomic sequence	Induces double-strand breaks in target DNA	Can have off-target effects	No – requires customization of protein component for each gene
Transcription activator-like effector nucleases (TALENs)	Non-specific DNA-cleaving nuclease fused to a DNA-binding domain specific for a genomic locus	Induces double-strand breaks in target DNA	Highly specific	Feasible, but technically challenging (Reyon et al., 2012)
Homing meganucleases	Endonuclease with a large recognition site for DNA (12–40 base pairs)	Induces double-strand breaks in target DNA	Highly specific	No – limited target sequence specificity available
<u>CRISPR/Cas</u>	20 nt crRNA fused to tracrRNA and Cas9 endonuclease	Induces double-strand breaks in target DNA (wt Cas9) or single-strand DNA nicks (Cas9 nickase)	Some off-target effects that can be minimized by selection of unique crRNA sequences	<u>Yes</u> – requires simple adapter cloning of 20 nt Oligos targeting each gene into a plasmid

CRISPR-CAS9



CRISPR-CAS9 for targeted genome editing

RNA-guided editing of bacterial genomes using CRISPR-Cas systems

Wenyan Jiang^{1,4}, David Bikard^{1,4}, David Cox^{2,3}, Feng Zhang^{2,3} & Luciano A Marraffini¹

Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems

James E. DiCarlo^{1,2}, Julie E. Norville², Prashant Mali², Xavier Rios², John Aach² and George M. Church^{2,*}

Heritable genome editing in C. elegans via a CRISPR-Cas9 system

Ari E Friedland¹, Yonatan B Tzur¹, Kevin M Esvelt², Monica P Colaiácovo¹, George M Church^{1,2} & John A Calarco³

Highly Efficient Targeted Mutagenesis of Drosophila with the CRISPR/Cas9 System

Andrew R. Bassett^{1,*} Charlotte Tibbit¹ Chris P. Ponting¹ and Ji-Long Liu^{1,*}

Efficient genome editing in zebrafish using a CRISPR-Cas system

Woong Y Hwang^{1,7}, Yanfang Fu^{2,3,7}, Deepak Reyon^{2,3}, Morgan L Maeder^{2,4}, Shengdar Q Tsai^{2,3}, Jeffrey D Sander^{2,3}, Randall T Peterson^{1,5,6}, I-R Joanna Yeh^{1,5} & J Keith Joung²⁻⁴

Simple and Efficient CRISPR/Cas9-Mediated Targeted Mutagenesis in Xenopus tropicalis

Takuya Nakayama¹, Margaret B. Fish¹, Marilyn Fisher¹, Jamina Oomen-Hajagos², Gerald H. Thomsen² and Robert M. Grainger^{1,*}

Multiplex and homologous recombination-mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9

Jian-Feng Li^{1,2}, Julie E Norville^{2,3}, John Aach^{2,3}, Matthew McCormack^{1,2}, Dandan Zhang^{1,2}, Jenifer Bush^{1,2}, George M Church^{2,3} & Jen Sheen^{1,2}

One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering

Haoyi Wang^{1,6}, Hui Yang^{1,6}, Chikdu S. Shivalila^{1,2,6}, Meelad M. Dawlaty¹, Albert W. Cheng^{1,3}, Feng Zhang^{4,5} and Rudolf Jaenisch^{1,3,*}

Heritable gene targeting in the mouse and rat using a CRISPR-Cas system

Dali Li^{1,4}, Zhongwei Qiu^{1,4}, Yanjiao Shao¹, Yuting Chen¹, Yuting Guan¹, Meizhen Liu¹, Yongmei Li¹, Na Gao¹, Liren Wang¹, Xiaoling Lu², Yongxiang Zhao² & Mingyao Liu^{1,3}

One-step generation of knockout pigs by zygote injection of CRISPR/Cas system

Tang Hai^{1,*}, Fei Teng^{1,2,*}, Runfa Guo¹, Wei Li¹, Qi Zhou¹

Effective gene targeting in rabbits using RNA-guided Cas9 nucleases

Dongshan Yang^{1,*}, Jie Xu^{1,*}, Tianqing Zhu¹, Jianglin Fan², Liangxue Lai³, Jifeng Zhang^{1,*}, and Y. Eugene Chen^{1,*}

Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos

Yuyu Niu^{1,5,7}, Bin Shen^{2,7}, Yiqiang Cui^{3,7}, Yongchang Chen^{1,5,7}, Jianying Wang², Lei Wang³, Yu Kang^{1,5}, Xiaoyang Zhao⁴, Wei Si^{1,5}, Wei Li⁴, Andy Peng Xiang⁶, Jiankui Zhou², Xuejiang Guo³, Ye Bi³, Chenyang Si^{1,5}, Bian Hu², Guoying Dong³, Hong Wang^{1,5}, Zuomin Zhou³, Tianqing Li^{1,5}, Tao Tan^{1,5}, Xiuqiong Pu^{1,5}, Fang Wang^{1,5}, Shaohui Ji^{1,5}, Qi Zhou⁴, Xingxu Huang^{2,*}, Weizhi Ji^{1,5,*} and Jiahao Sha^{3,*}

CRISPR-CAS9 based HTS

- Library design and construction
- Delivery of sgRNAs and selection strategy
- Pooled screen, sgRNA as barcode for deep sequencing
- KO efficiency (biallelic inactivation and in-frame indels)
- Specificity, off-target
- Hits validation

CRISPR-CAS9 based HTS

SCIENCE VOL 343 3 JANUARY 2014

Genetic Screens in Human Cells Using the CRISPR-Cas9 System

Tim Wang,^{1,2,3,4} Jenny J. Wei,^{1,2} David M. Sabatini,^{1,2,3,4,5*}† Eric S. Lander^{1,3,6*}†

Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells

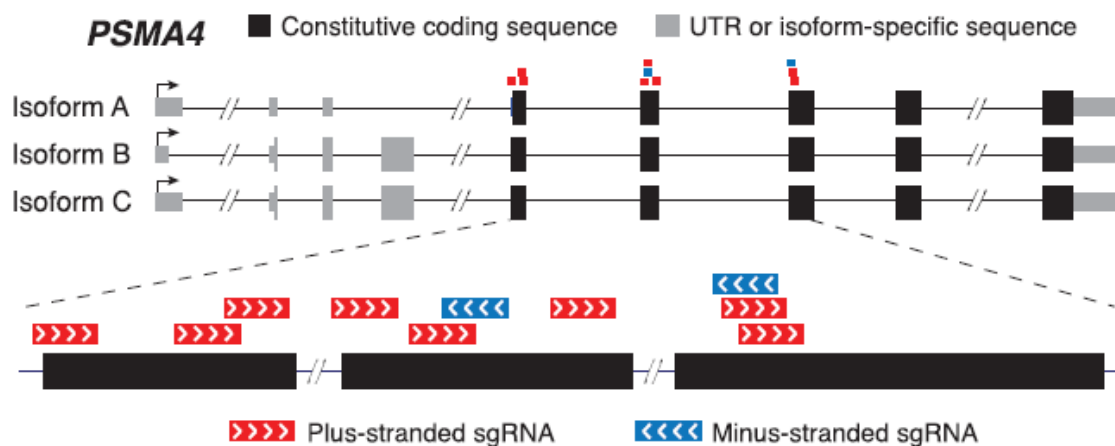
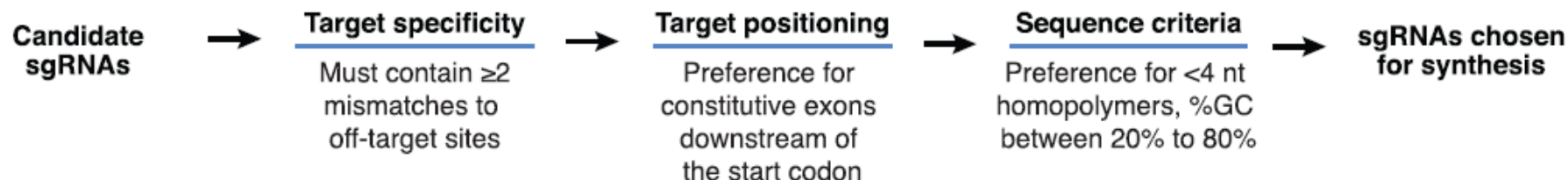
Ophir Shalem,^{1,2*} Neville E. Sanjana,^{1,2*} Ella Hartenian,¹ Xi Shi,^{1,3}
David A. Scott,^{1,2} Tarjei S. Mikkelsen,¹ Dirk Heckl,⁴ Benjamin L. Ebert,⁴ David E. Root,¹
John G. Doench,¹ Feng Zhang^{1,2}†

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Library design and construction

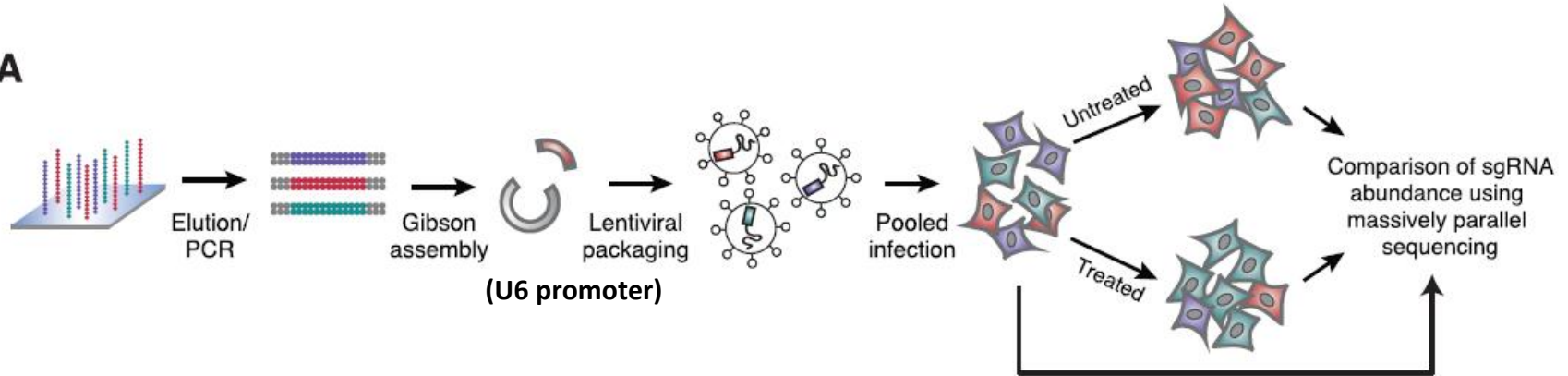
PAMs in coding exons near the beginning of each gene



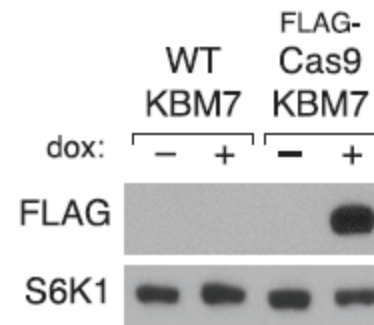
Pool	Genes	sgRNAs
Ribosomal protein	83	2741
Kinase	507	5070
Cell cycle	983	9830
Nuclear	3733	37330
Unknown function	1808	18080
Non-targeting	NA	100
Total	7114	73151

Library construction and delivery

A



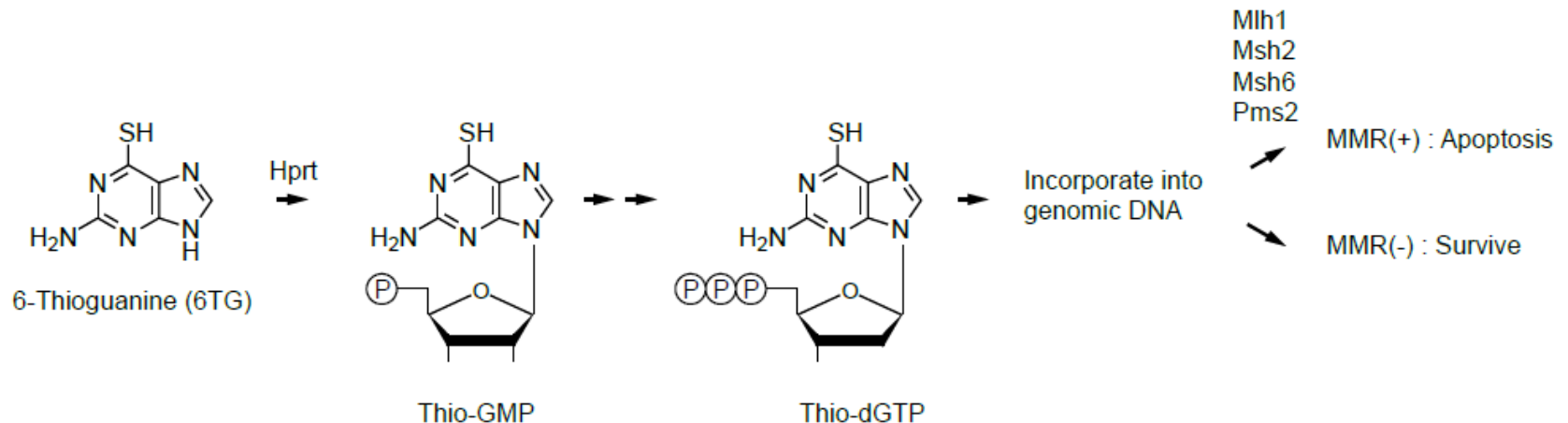
- Lentiviral vector expressing Cas9 nuclease (with a FLAG-tag at its N terminus) under a doxycycline inducible promoter



- Established cell lines Cas9-KBM7 (haploid) and Cas9-HL60 (diploid) cells expressing dox-inducible FLAG-Cas9
- Infected Cas9-cells with pooled sgRNA library

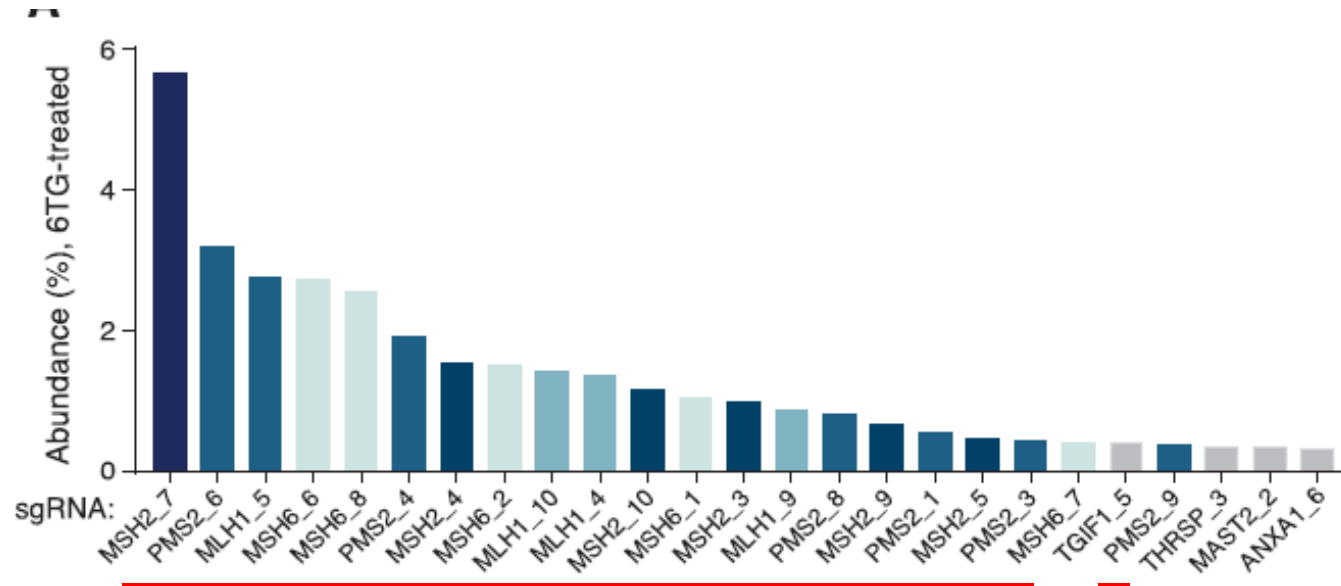
Positive selection 1

- CRISPR-CAS9 screening for genes involved in DNA mismatching repair (MMR)
- 6-thioguanine (6-TG) induced lesion leads to cell death, but with MMR gene mutations, cells do not recognize the lesion and continue divide
- Cas9-KBM7 (haploid) cells were infected with sgRNA library, and selected with 6-TG (12d). sgRNA barcodes are sequenced in the survival cells (deep sequencing).

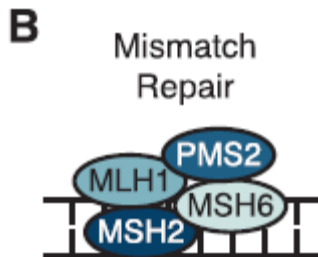


Positive selection 1

CRISPR-CAS9 screening for genes involved in DNA mismatching repair (MMR)

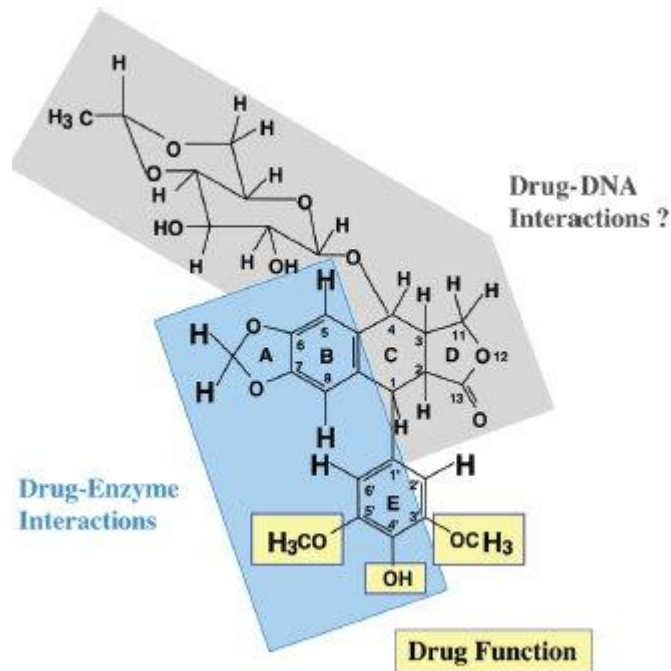


- sgRNAs targeting genes encoding the 4 components for MMR pathway were dramatically enriched (>30% of all barcodes): **MSH2**, **MSH6**, **MLH1** and **PMS2**



Positive selection 2

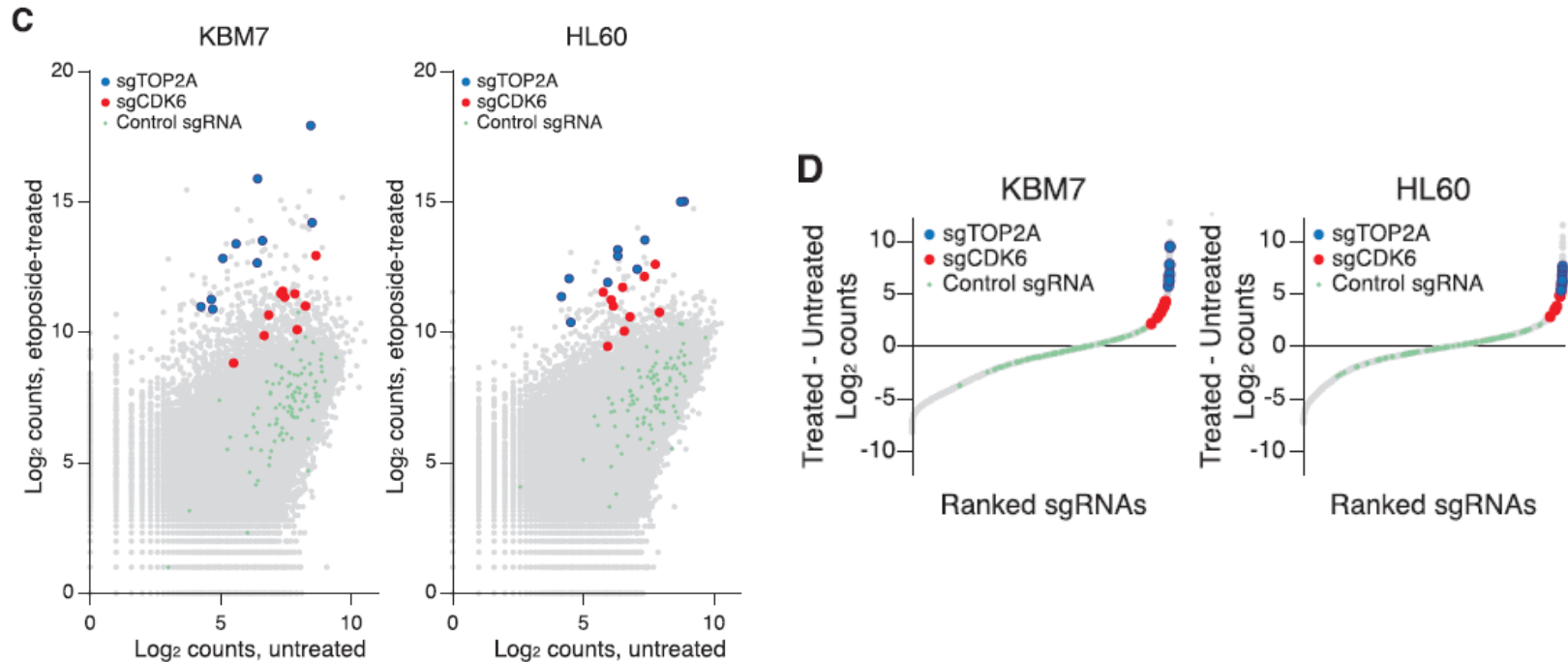
- CRISPR-CAS9 screening for genes important for etoposide toxicity
- Etoposide: a chemotherapeutic agent inhibits DNA topoisomerase IIA, TOP2A
- Cas9-KBM7 (haploid) and Cas9-HL60 (diploid) cells were infected with sgRNA library, and treated with etoposide(12d). Compare the sgRNA reads between treated and untreated one.



Etoposide is lethal to cells by stabilizing the normally transient covalent enzyme-cleaved DNA complex (the cleavage complex), leading to cell death.

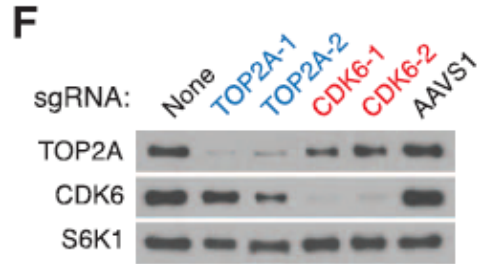
Positive selection 2

- CRISPR-CAS9 screening for genes important for etoposide toxicity.



- sgRNAs targeting identical genes were detected in KBM7 and HL60 screening: **TOP2A, CDK6.**
- Specificity of the inactivation
- Efficiency in inactivating both alleles in diploid cells

Validation of the hits

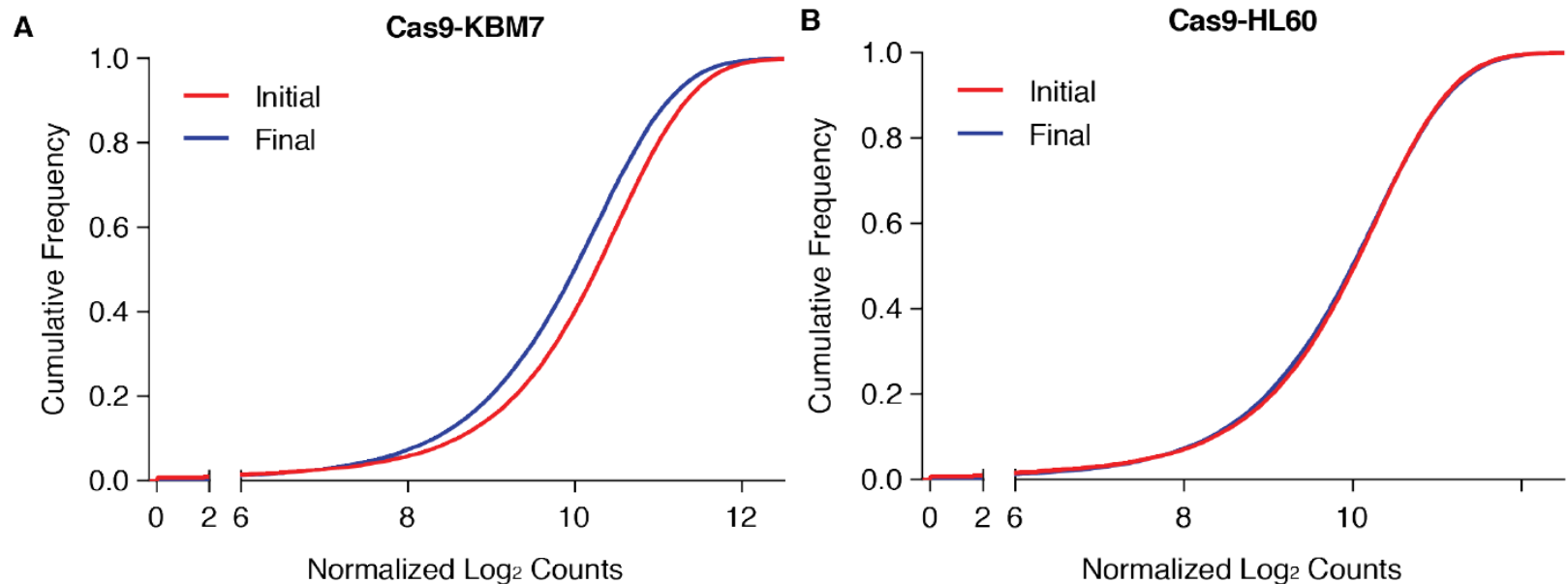


- Individual sgRNAs targeting TOP2A or CDK6 for HL60 cell line.

➤ **CRISPR-CAS9 system enable large-scale positive selection loss-of-function screens**

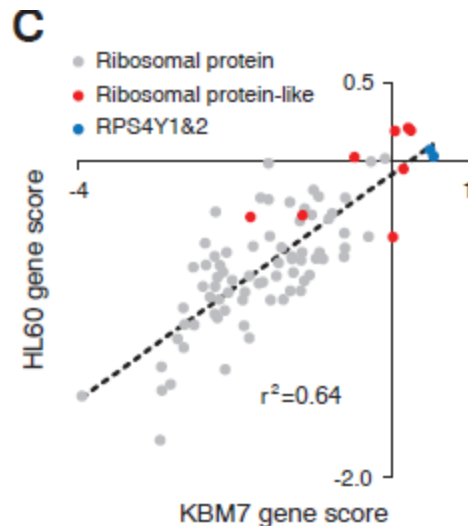
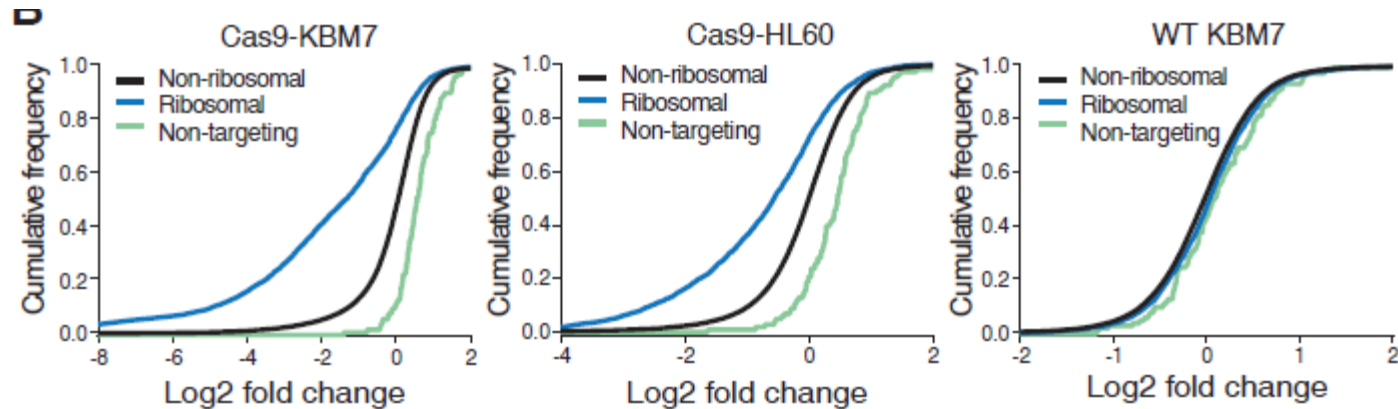
Negative selection

- CRISPR-CAS9 screening for genes important for cellular proliferation.
- Cas9-KBM7 (Haploid), Cas9-HL60 (diploid) and WT-KBM7 cells were infected with sgRNA library. Deep sequence the gDNA and compare the sgRNA reads between the initial seeding population and final population after 12 cell-doublings (24h).



Negative selection

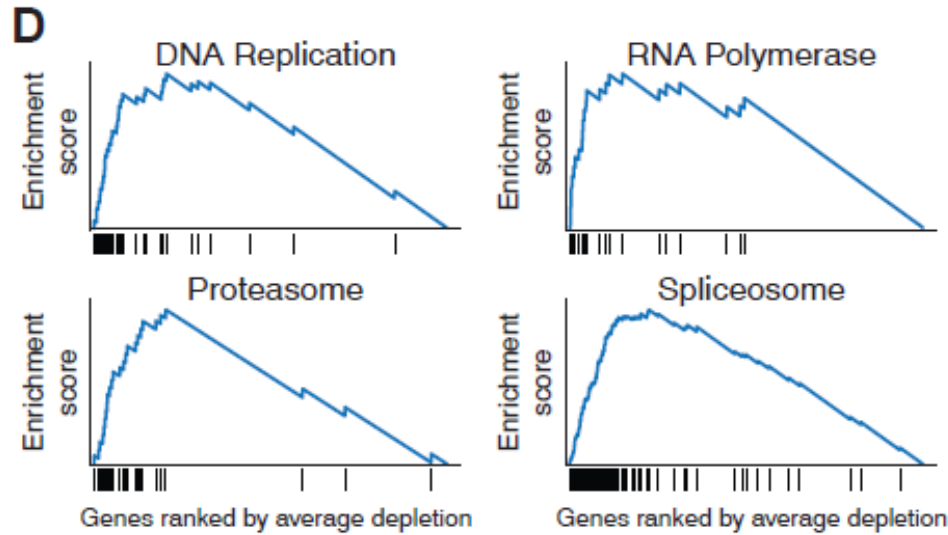
- CRISPR-CAS9 screening for genes important for cellular proliferation.



- Ribosomal proteins are essential for cell proliferation
- Good concordance between KBM7 and HL60 screenings
- Ribosomal protein like or RPS4Y1 and 2 may be required in select tissues and lowly expressed in KBM7 cells

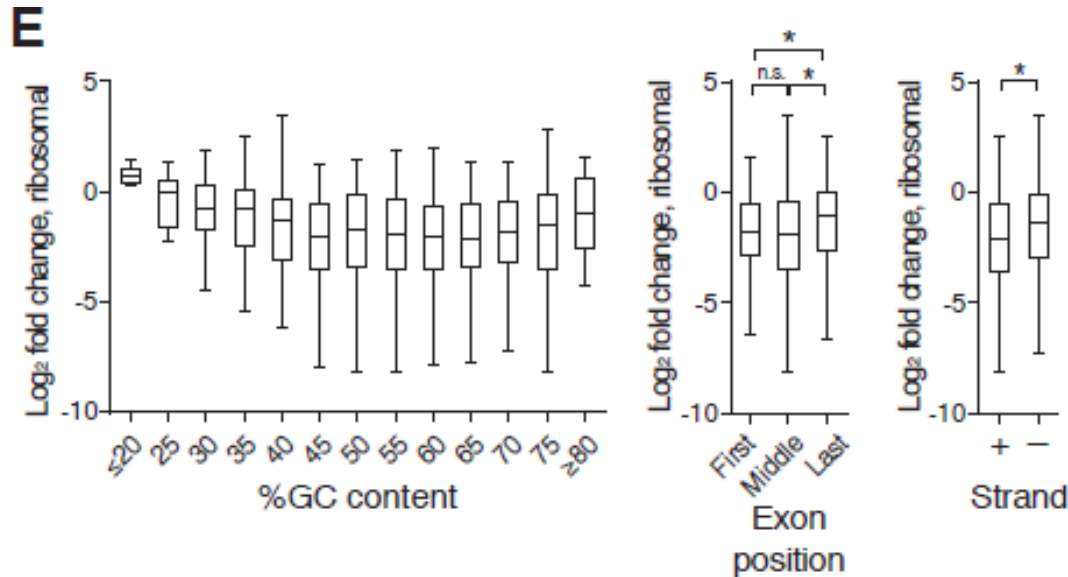
Negative selection

- CRISPR-CAS9 screening for genes important for cellular proliferation.



- Gene set enrichment analysis (GSEA)
- sgRNA targeting genes involved in fundamental biological processes were also depleted

sgRNA targeting efficacy (sgRNA targeting ribosomal proteins)



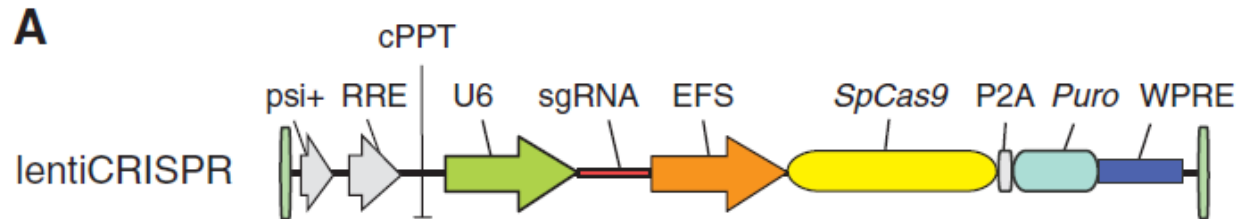
- sgRNAs with very low or high GC content were less effective
- sgRNAs targeting last exons were less effective
- sgRNAs targeting transcribed strand (-) were less effective

Conclusion 1

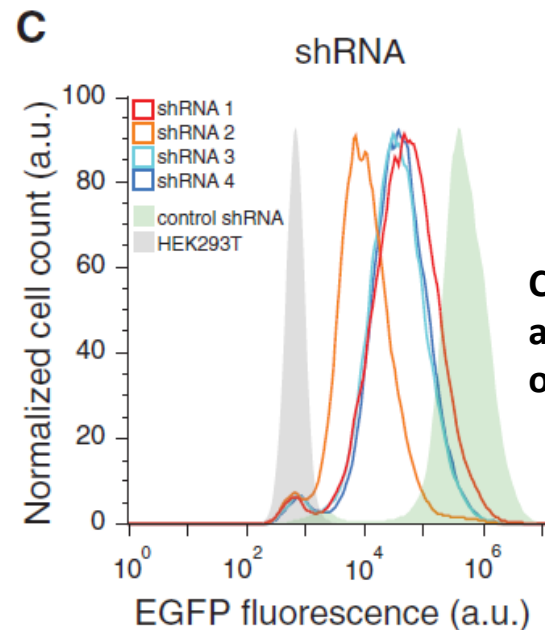
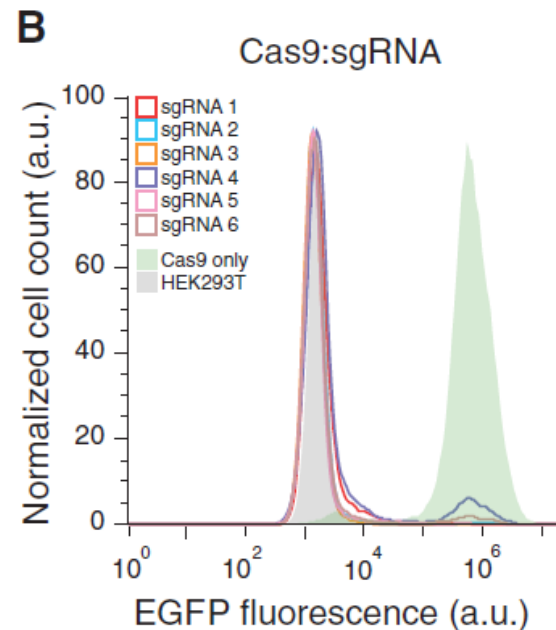
- CRISPR-CAS9 system can be used to establish sgRNA library (73151 sgRNAs targeting 7114 genes) and functional screens (positive and negative selection)
- Effective coverage of sgRNA library in large-scale screen
- Off-target effects do not appear to seriously hamper the screens, most of off-target sites are in noncoding regions

Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells

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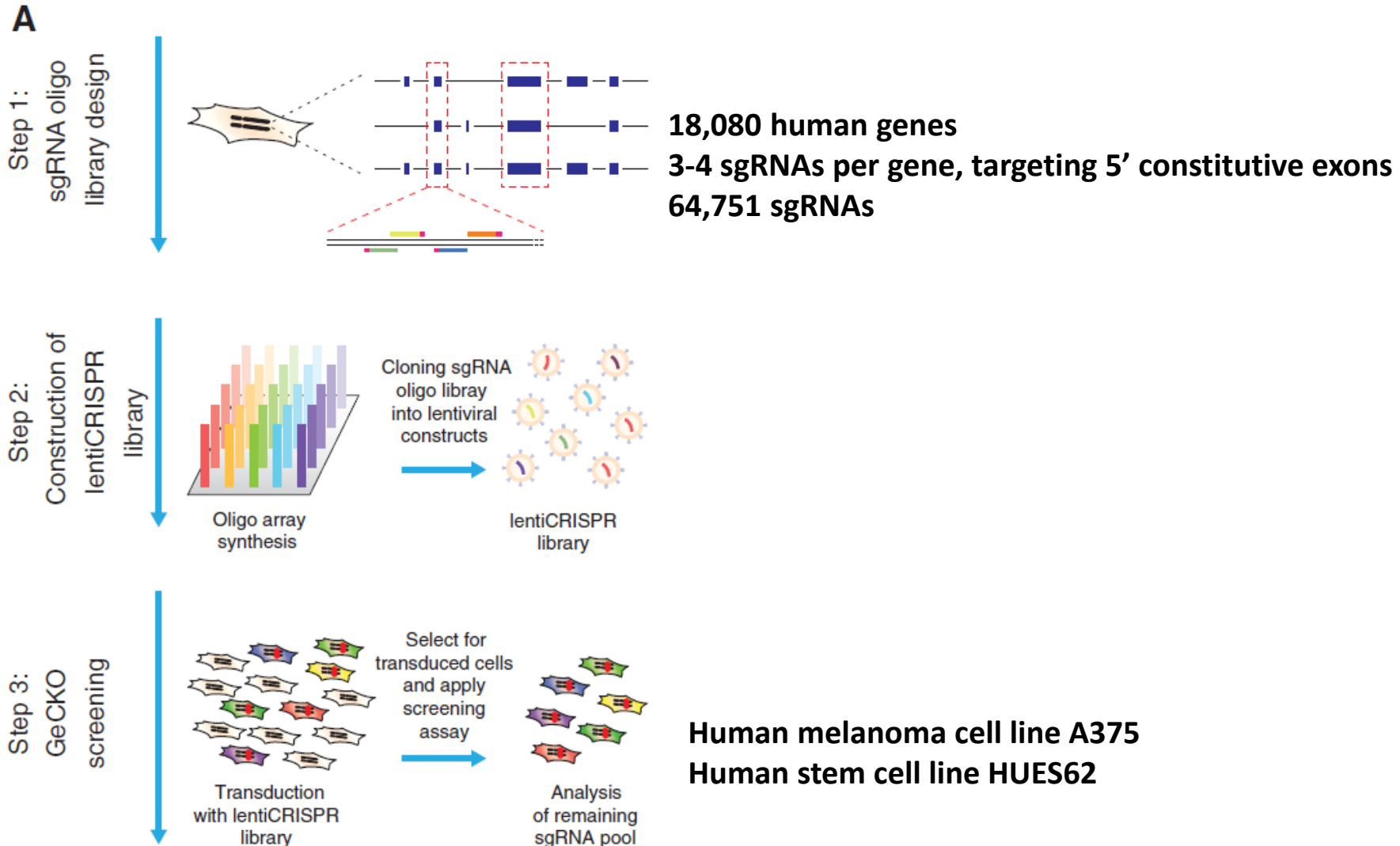


Single element for CRISPR-CAS9

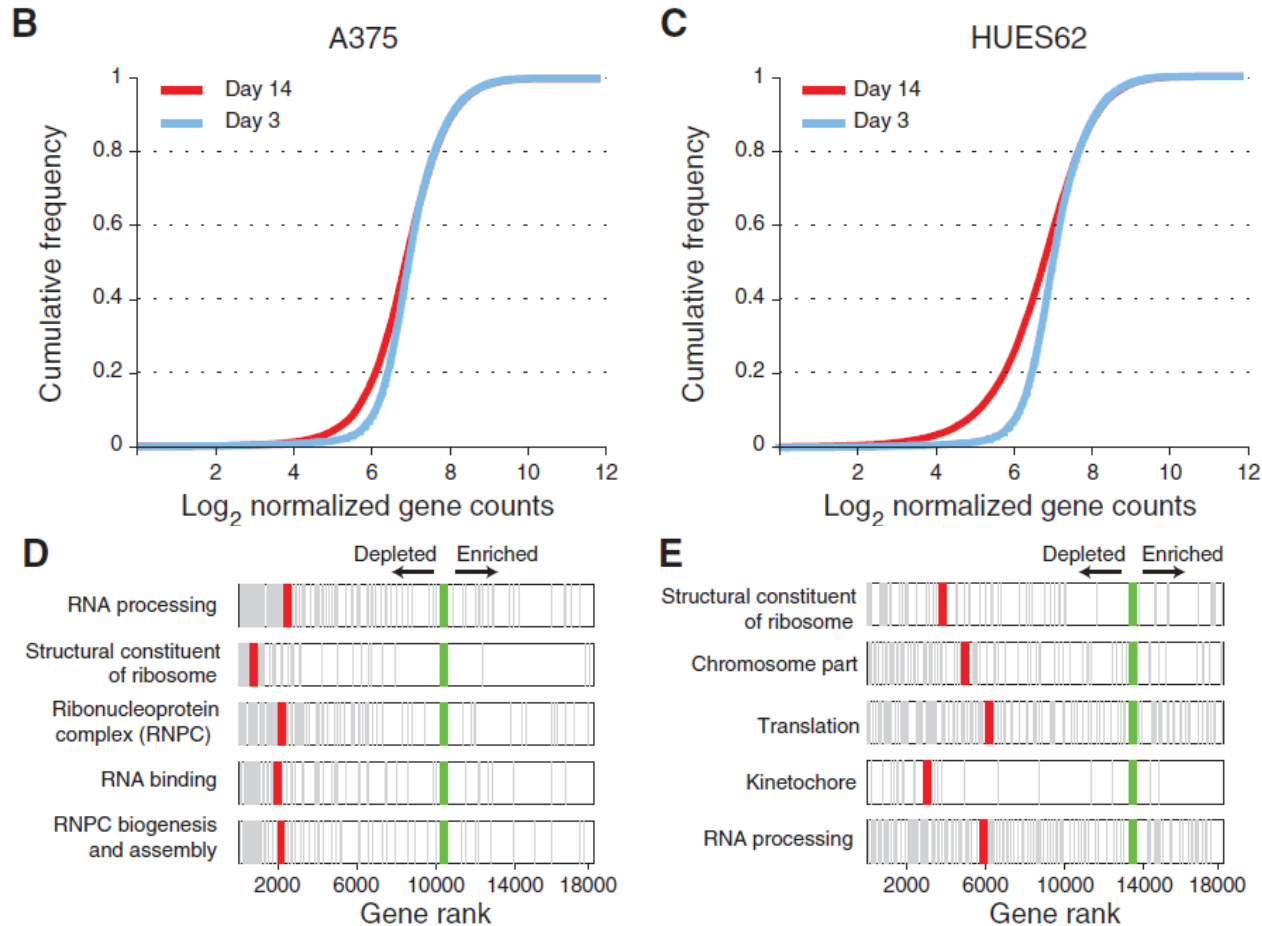


Comparison of CRISPR-CAS9 and RNAi (targeting single copy of EGFP in HEK 293 cells)

Library design and construction



Negative selection

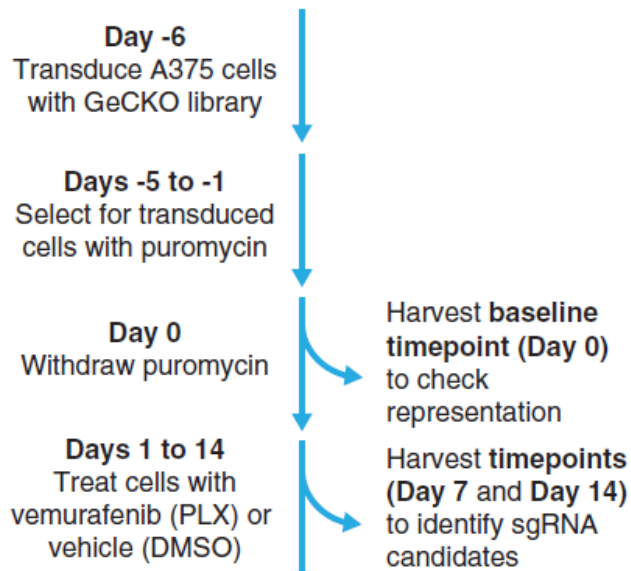


- Significant reduction of sgRNA after 14d culture
- Gene set element analysis (GSEA) indicated most of depleted sgRNAs targeting ribosomal structural constituent and other essential biological processes
- Overlapping categories between the two cell lines

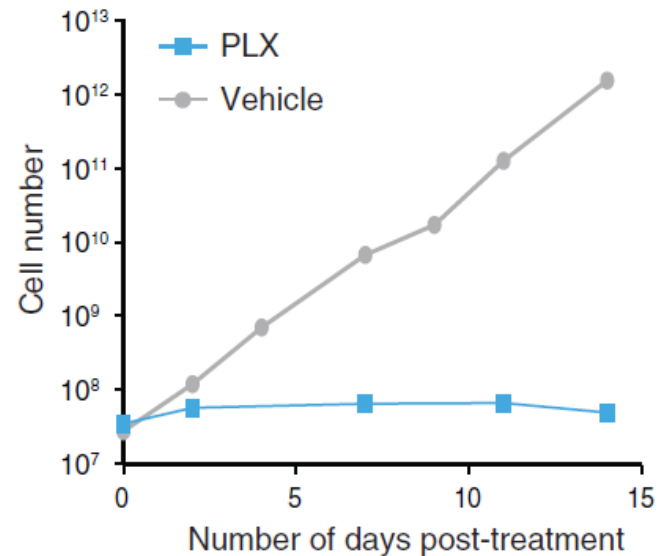
Positive selection

- CRISPR-CAS9 screening for genes involved in BRAF protein kinase inhibitor vemurafenib (PLX)
- PLX is a drug kills melanoma cells, but with short-lived effect and develop resistance

A



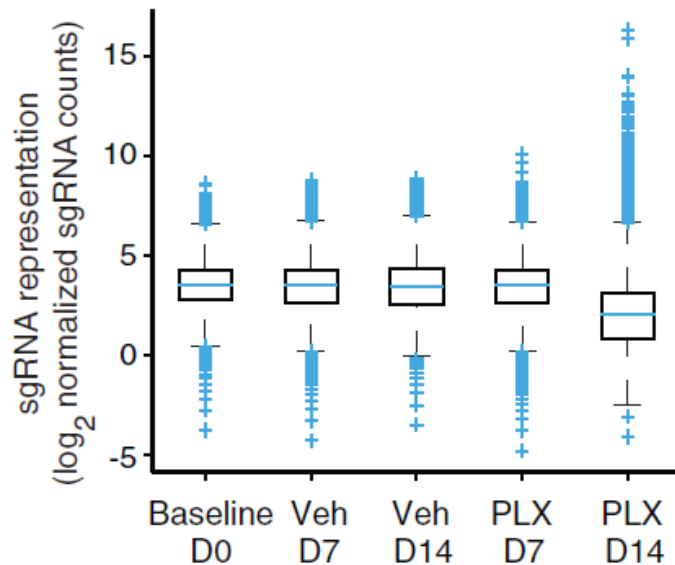
B



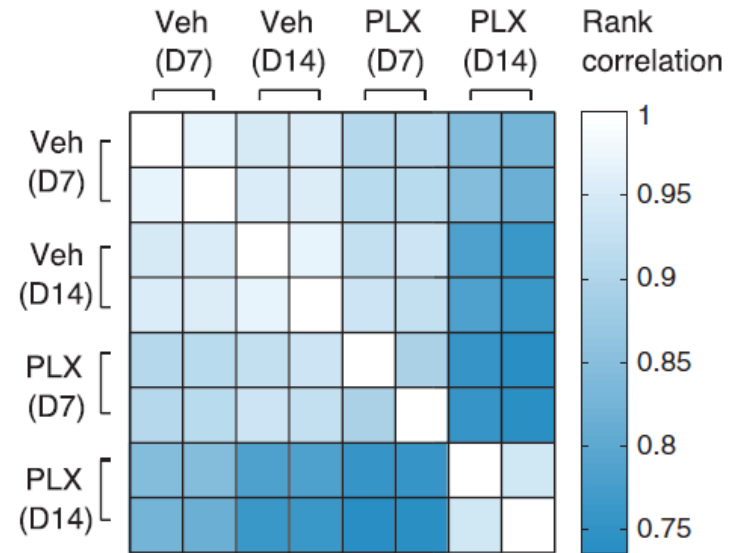
Positive selection

- CRISPR-CAS9 screening for genes involved in BRAF protein kinase inhibitor vemurafenib (PLX)

C



D

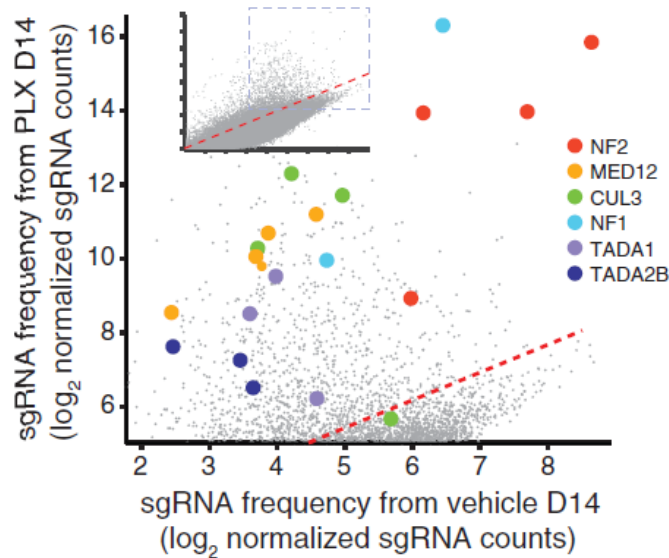


- Significant change of sgRNA distribution after 14d culture with PLX treatment

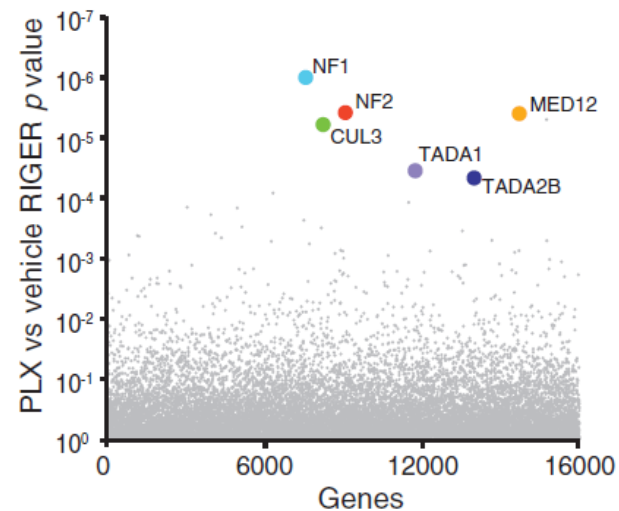
Positive selection

- CRISPR-CAS9 screening for genes involved in BRAF protein kinase inhibitor vemurafenib (PLX)

E



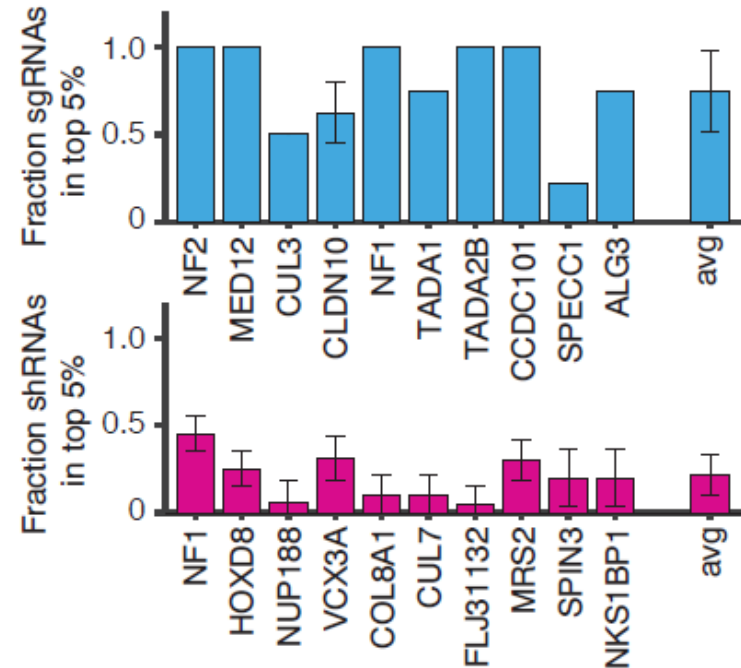
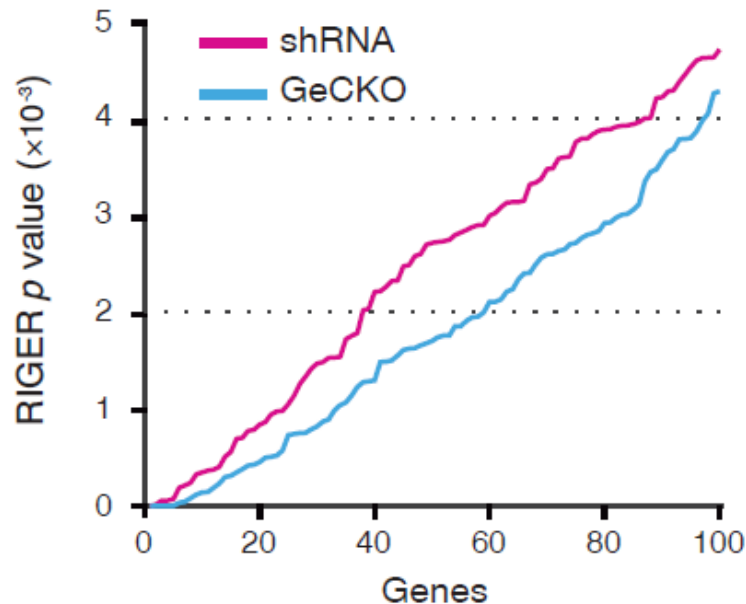
F



- Enrichment of sgRNAs targeting genes involved in PLX resistance
- **Known hits:** NF1 and MED12; **Novel hits:** NF2, CUL3, TADA1 and TADA2
- RNAi Gene Enrichment Ranking (RIGER) algorithm to rank the hits consistency

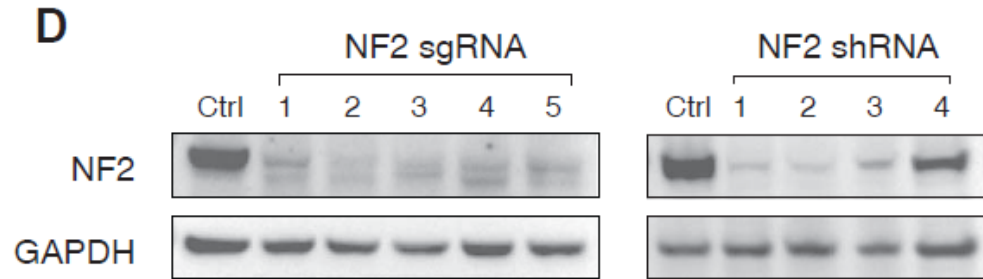
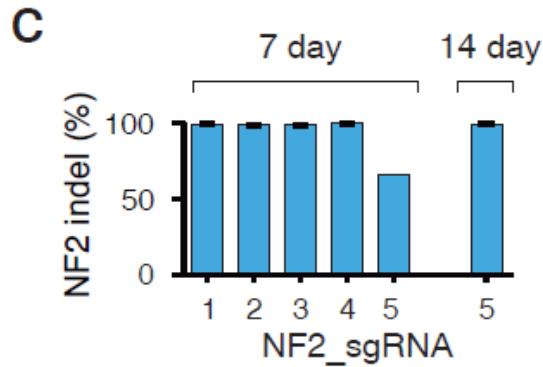
Comparison of shRNA and sgRNA screens

- Previously conducted shRNA screen (90,000 shRNAs)



- Lower p value/better scoring consistency for sgRNA (top 100 genes)
- $78 \pm 27\%$ of sgRNA targeting top 10 genes rank among top 5%, $20 \pm 12\%$ of shRNA rank among top 5%

Validation of sgRNA hits and comparison to shRNA



- High efficiency of sgRNA KO
- Complete KO of NF2 is required to increase the PLX resistance

Conclusion 2

- Second human sgRNA (64751 sgRNAs targeting 18080 genes)
- CAS9 can be delivered together with sgRNA for gene inactivation, and for genome wide genetic screening (both negative and positive selection)
- High validation rate of top hits
- sgRNA screen has better performance compared to shRNA screen

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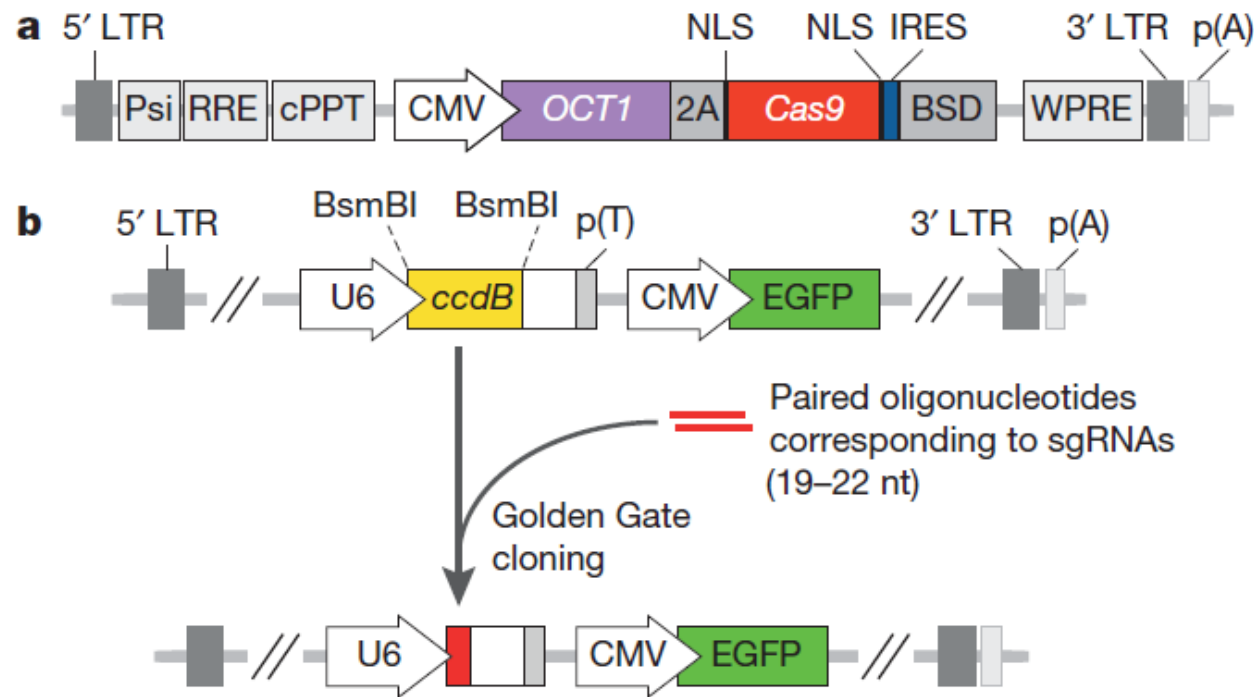
doi:10.1038/nature13166

High-throughput screening of a CRISPR/Cas9 library for functional genomics in human cells

Yuexin Zhou^{1*}, Shiyu Zhu^{1*}, Changzu Cai^{1*}, Pengfei Yuan¹, Chunmei Li², Yanyi Huang² & Wensheng Wei¹

High-throughput screening of a CRISPR/Cas9 library for functional genomics in human cells

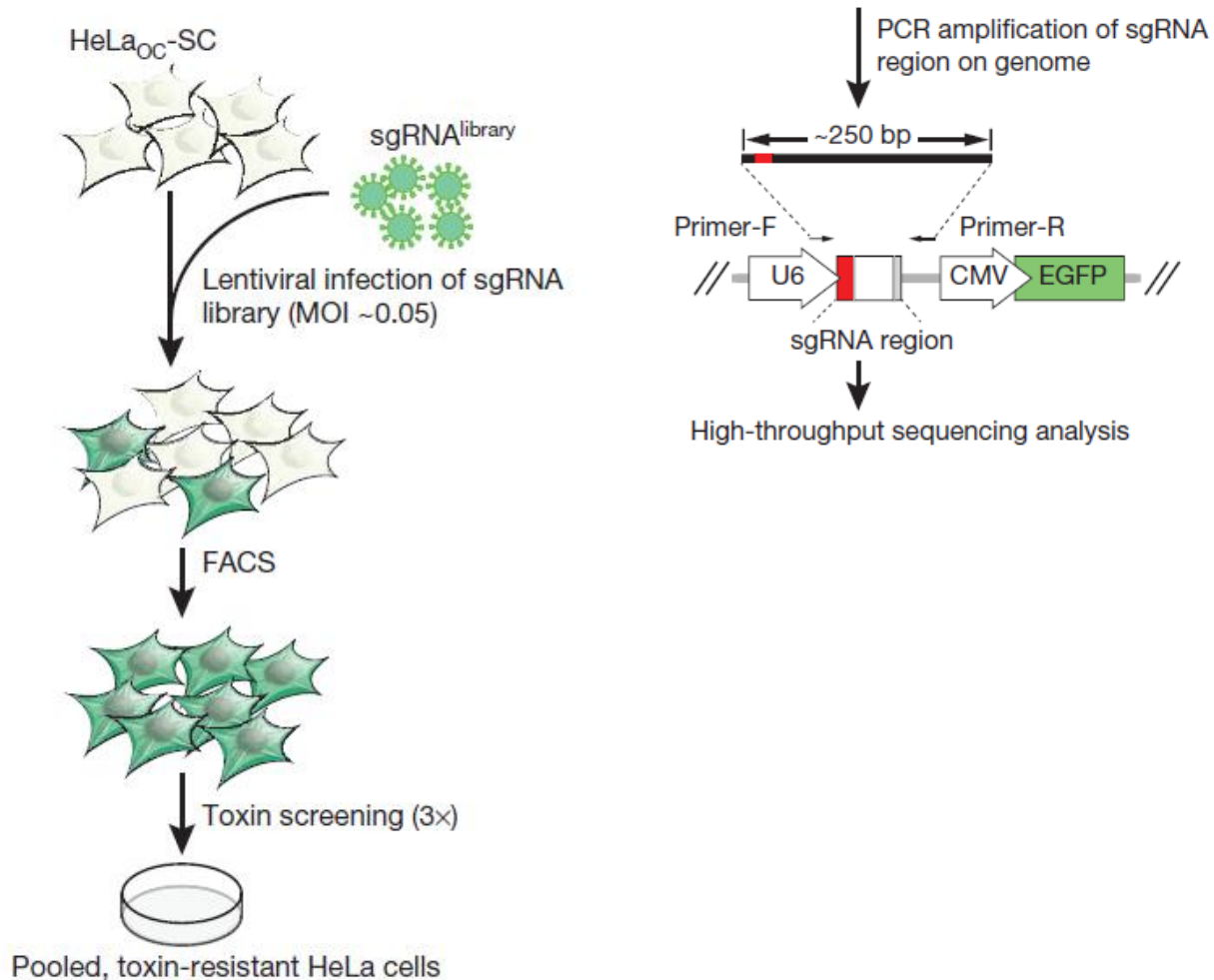
Yuxin Zhou^{1*}, Shiyu Zhu^{1*}, Changzu Cai^{1*}, Pengfei Yuan¹, Chunmei Li², Yanyi Huang² & Wensheng Wei¹



Library design and construction

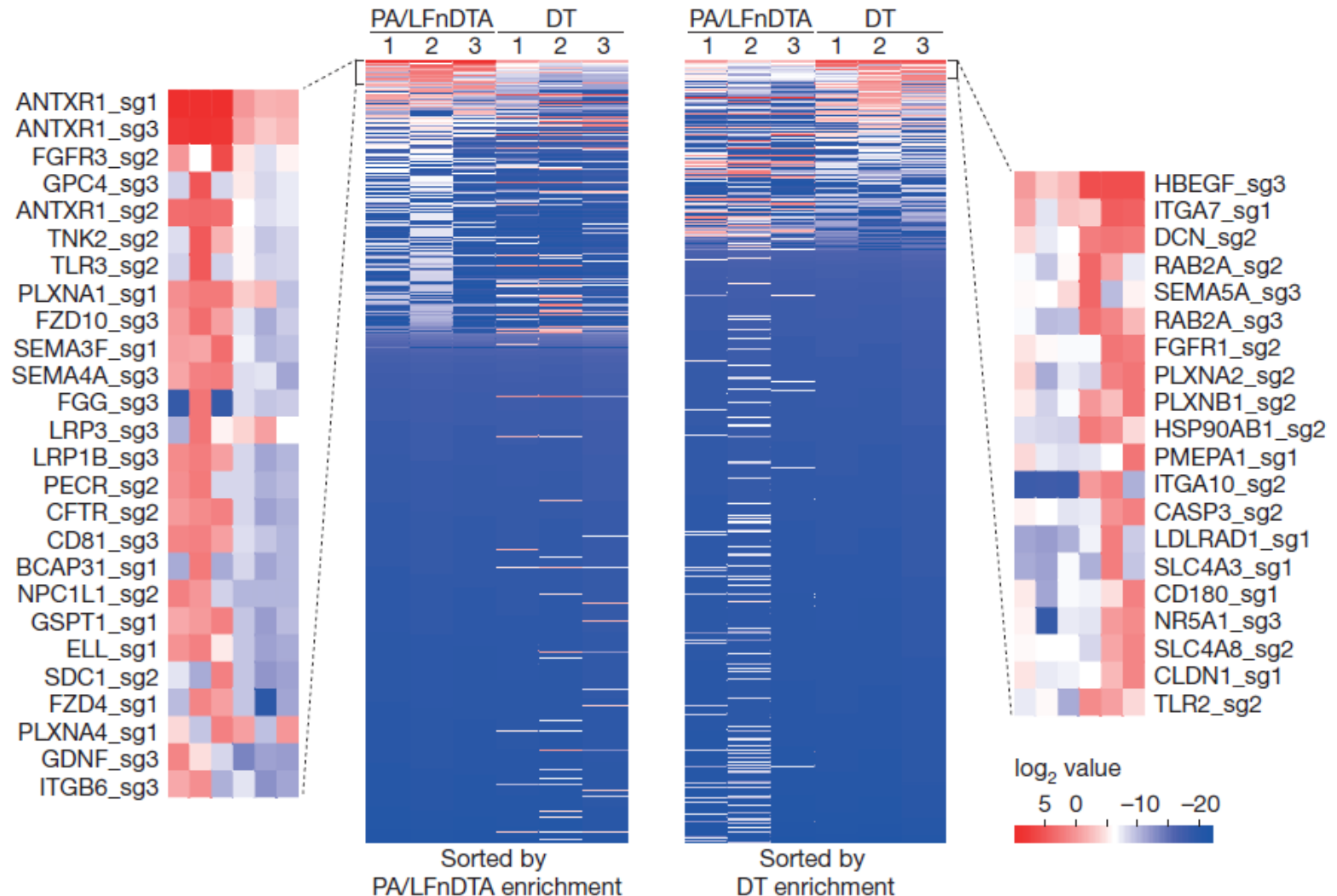
Library: 291 genes, 3 sgRNAs/gene, 869 sgRNA

Genes encoding cell surface proteins, and important for endocytosis, trafficking and cell death.



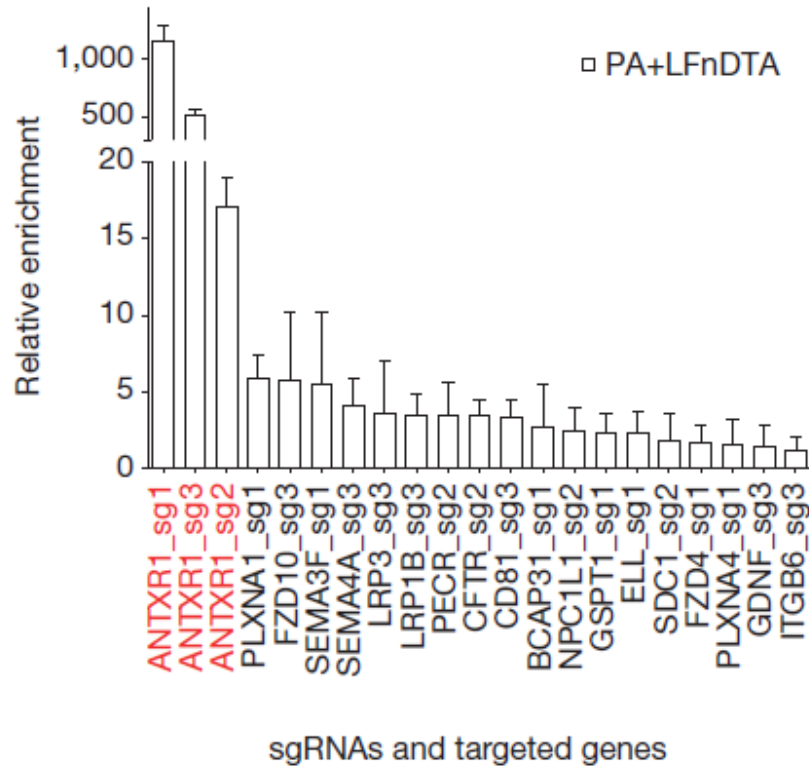
Positive selection

Genes essential for diphtheria toxin (DT) and chimaeric anthrax toxin (PA/LFnDTA, protective antigen (PA)/N-terminal domain of lethal factor (LF) fused to catalytic subunit of DT) induced cell death



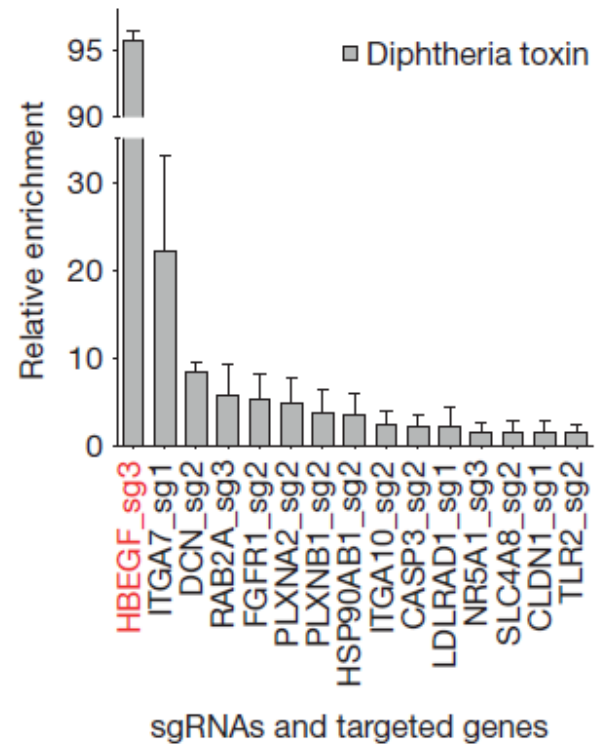
Positive selection

b



ANTXR: receptor for Anthrax

c

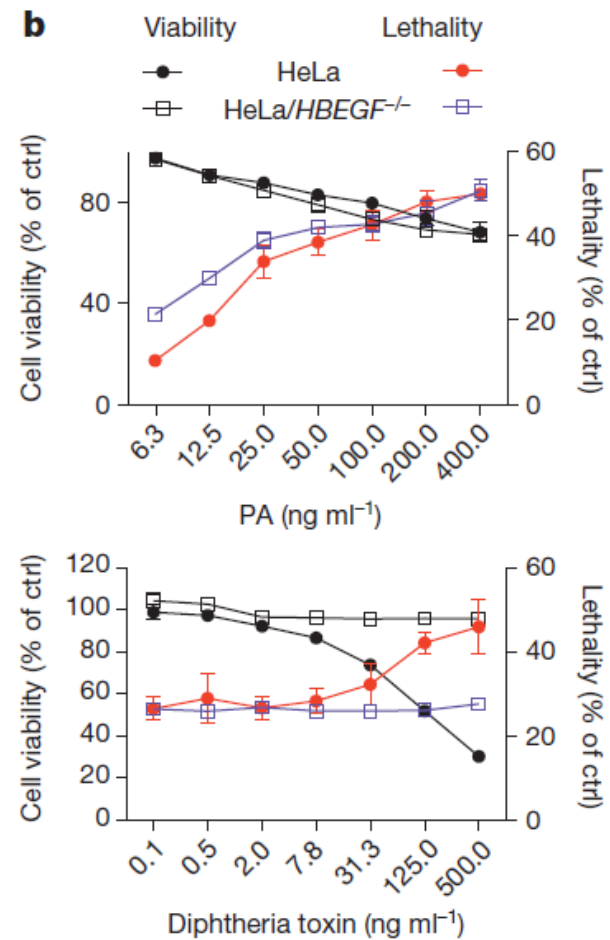
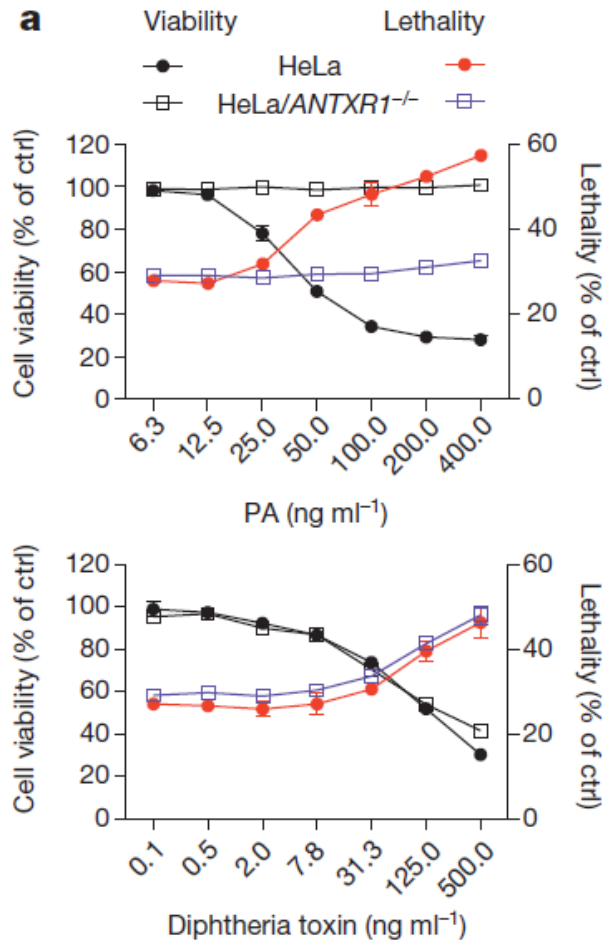


HBEGF: unique receptor for DT

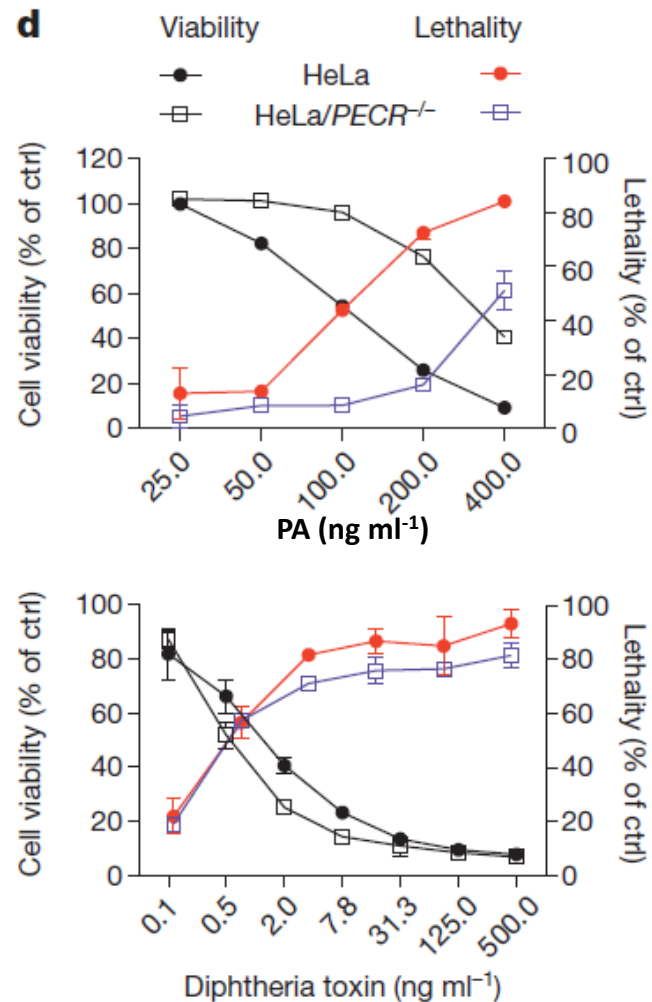
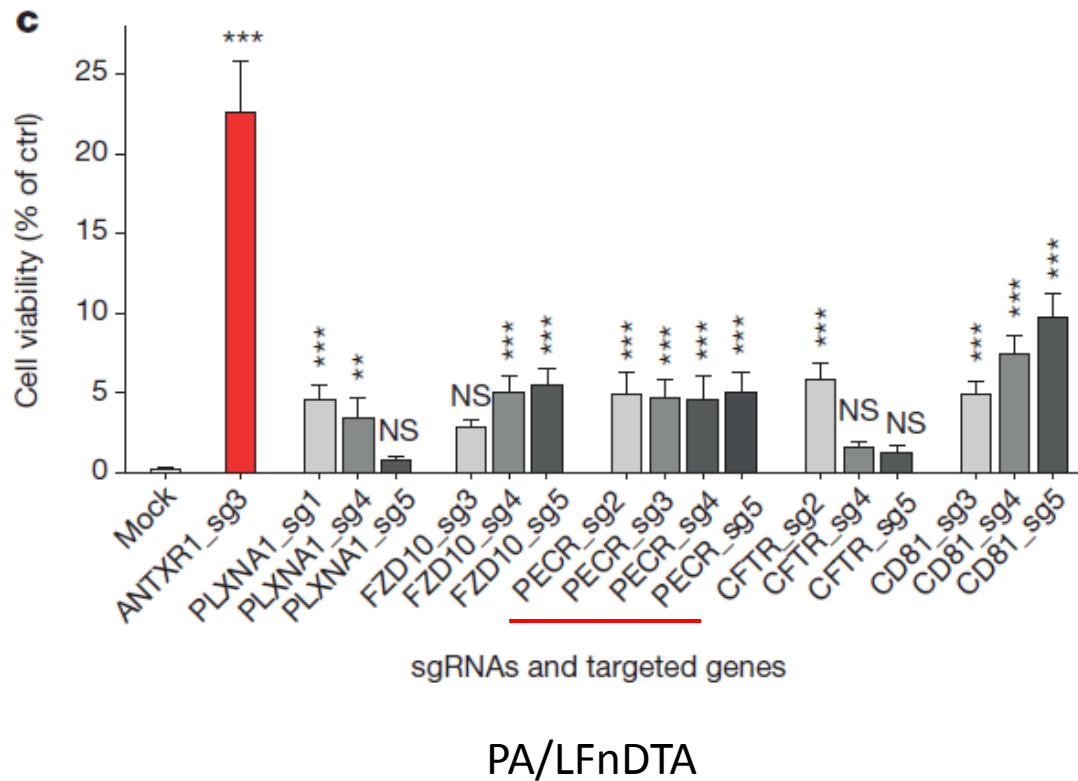
Validation of the hits

Viability: XTT

Lethality: LDH



Validation of the hits



Conclusion 3

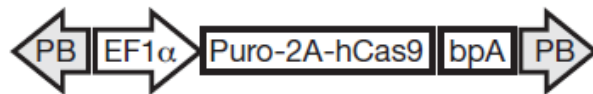
- CRISPR-CAS9 based screening with focused library (869 sgRNAs targeting 291 genes) instead of genome wide library
- Knowledgee-based screening

Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library

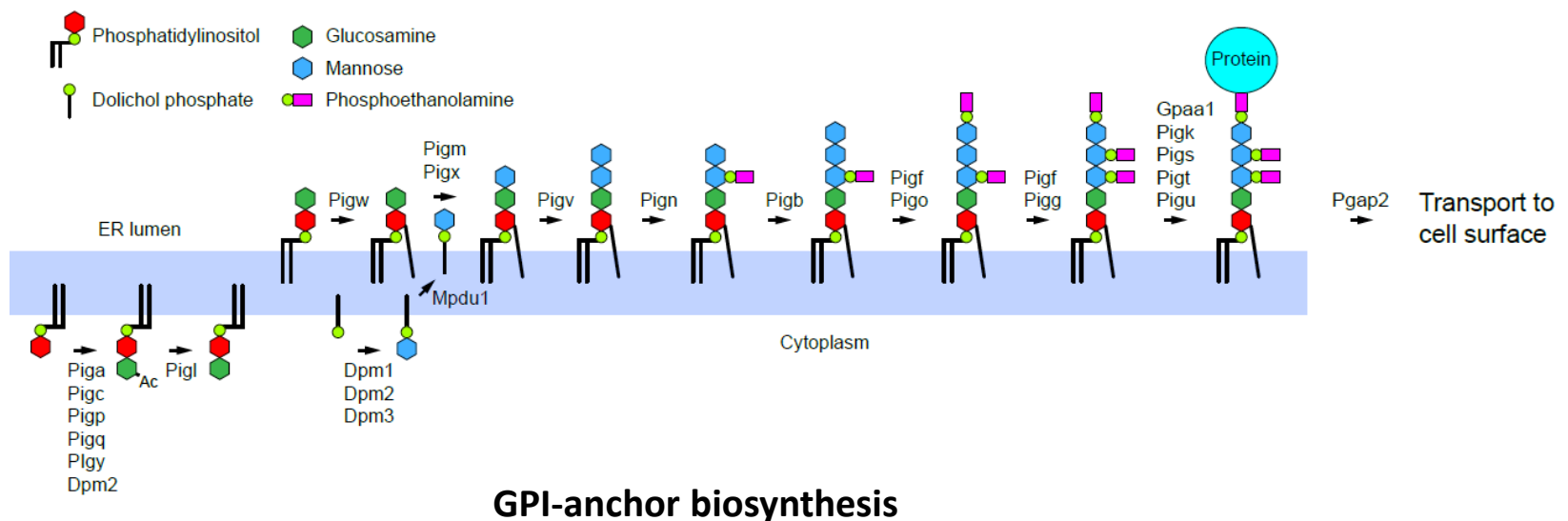
Hiroko Koike-Yusa^{1,2}, Yilong Li^{1,2}, E-Pien Tan¹, Martin Del Castillo Velasco-Herrera¹ & Kosuke Yusa¹

Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library

Hiroko Koike-Yusa^{1,2}, Yilong Li^{1,2}, E-Pien Tan¹, Martin Del Castillo Velasco-Herrera¹ & Kosuke Yusa¹



Target cell: **Male B6 Mouse ESCs** - JM8 transfected single copy *piggyBac* transposon expressing hCas9



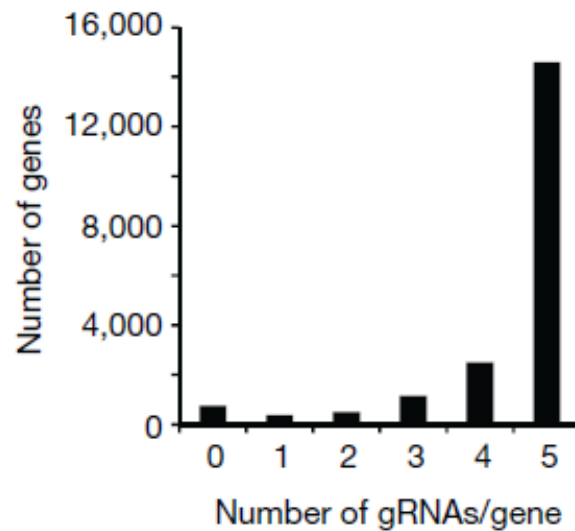
- **Alpha toxin** binds to GPI anchors to elicit toxicity
- **FLAER**: fluorescently labeled aerolysin, binds to mammalian GPI anchors

Library design and construction



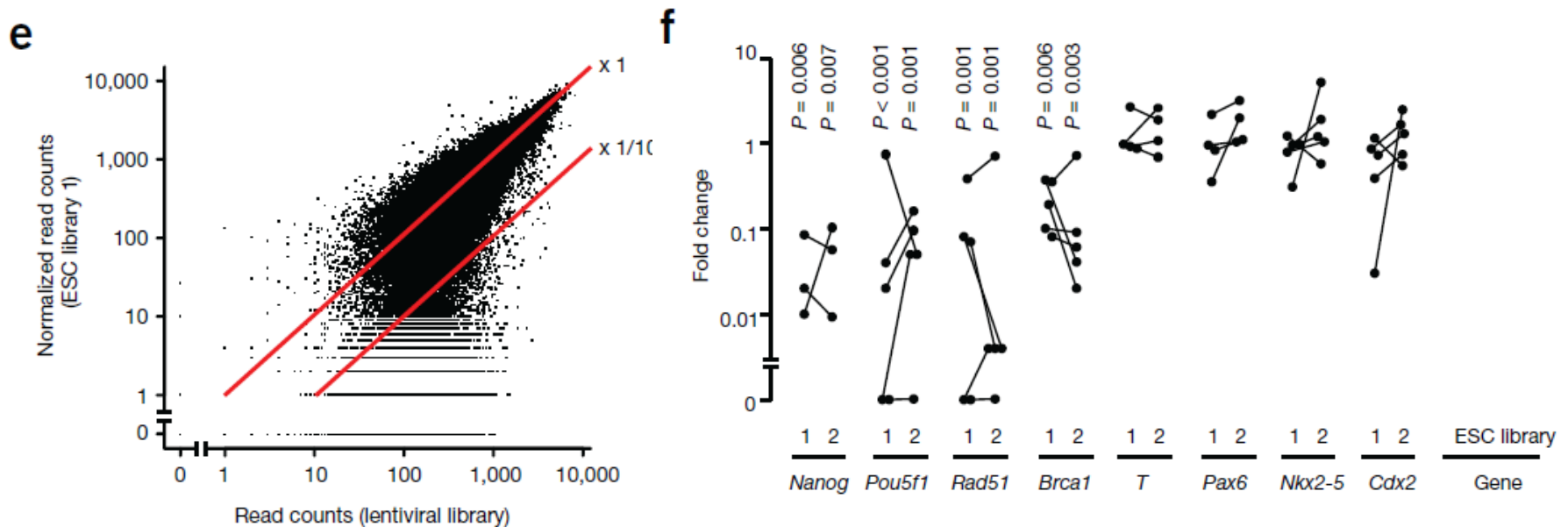
U6 promoter, G+N19+NGG

Mouse gRNA library: 87,897 gRNAs targeting 19150 genes, 94.3% of genes have at least two gRNAs per gene



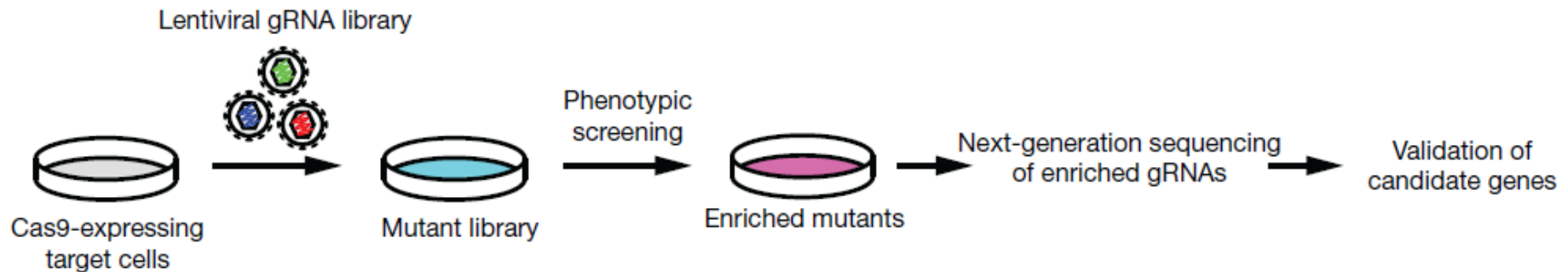
sgRNA screen for ESC proliferation

Lentiviral library vs ESC library (ESC infected with sgRNA lentiviral library)



- Pluripotency genes (*Nanog*, *Pou5f1*), DNA repair genes (*Rad51*, *Brca1*) are depleted, while lineage-specification genes are preserved.

sgRNA screen for GPI anchor biosynthesis and MMR (positive selection)

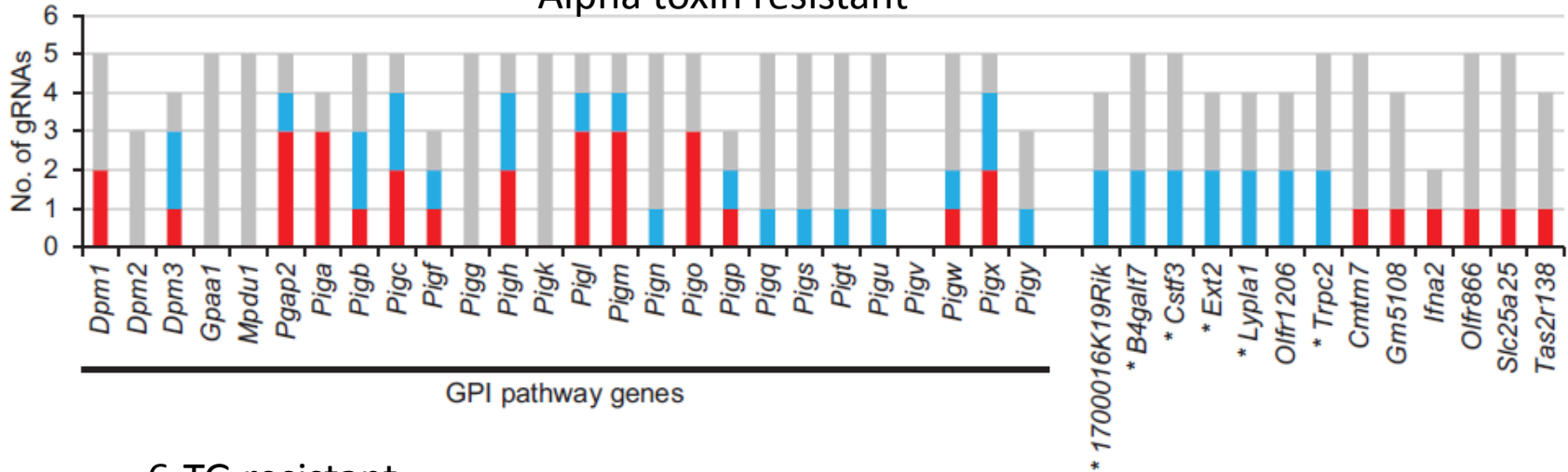


- Alpha toxin treatment
- 6-TG treatment

Hits identification

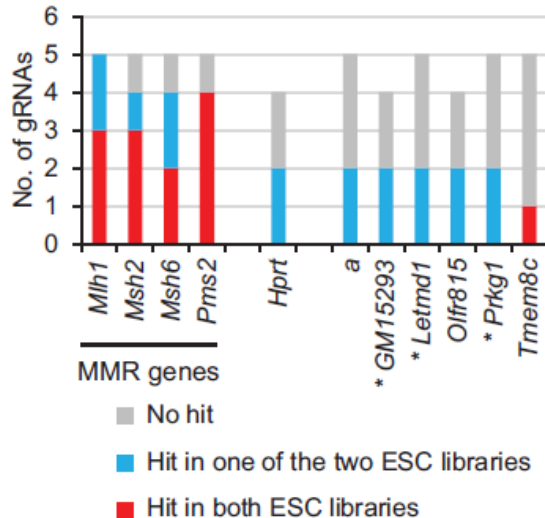
a

Alpha toxin resistant



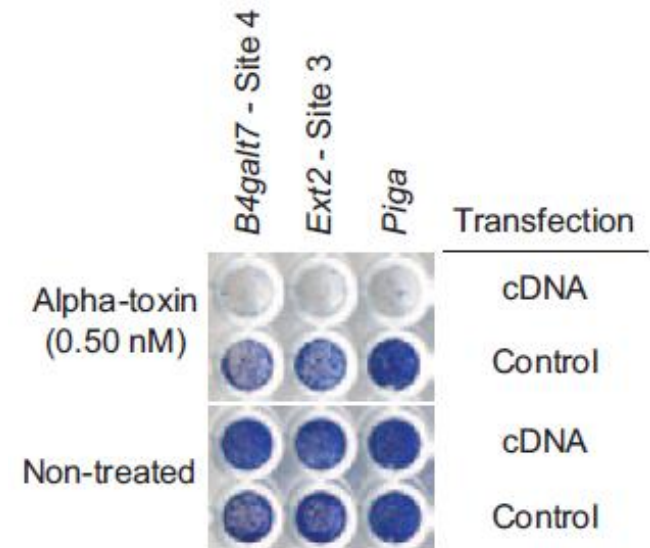
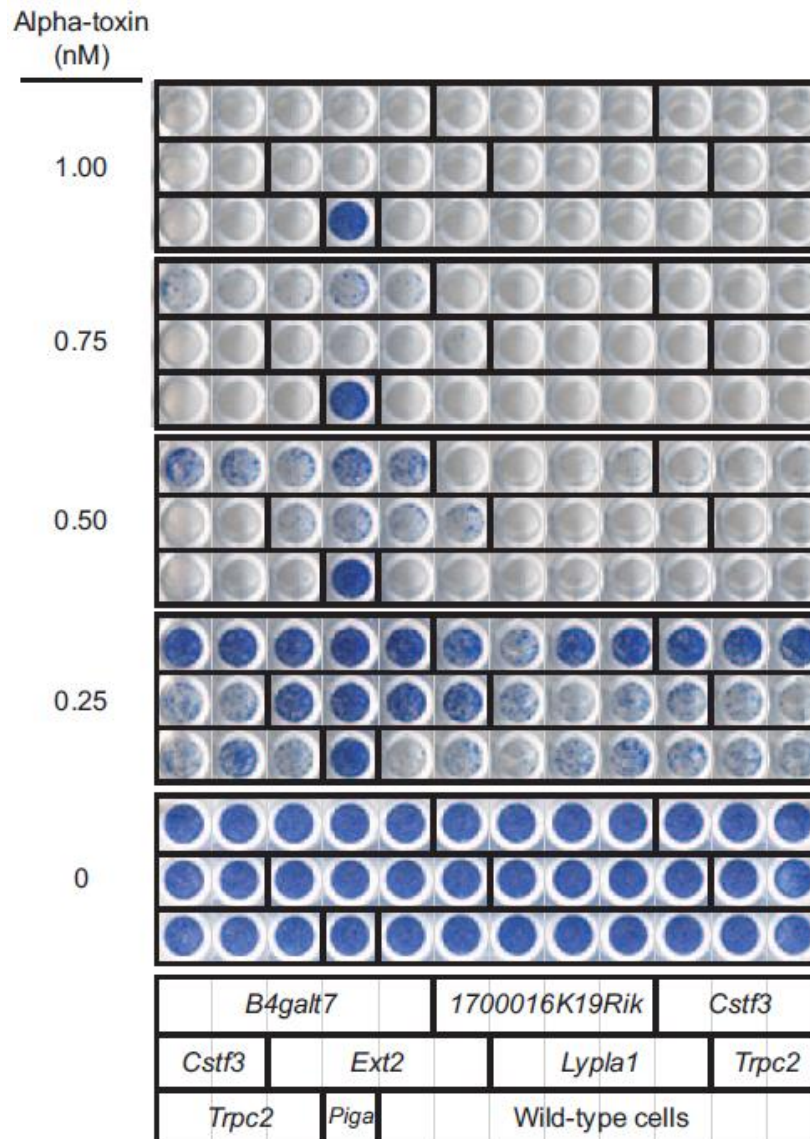
b

6-TG resistant

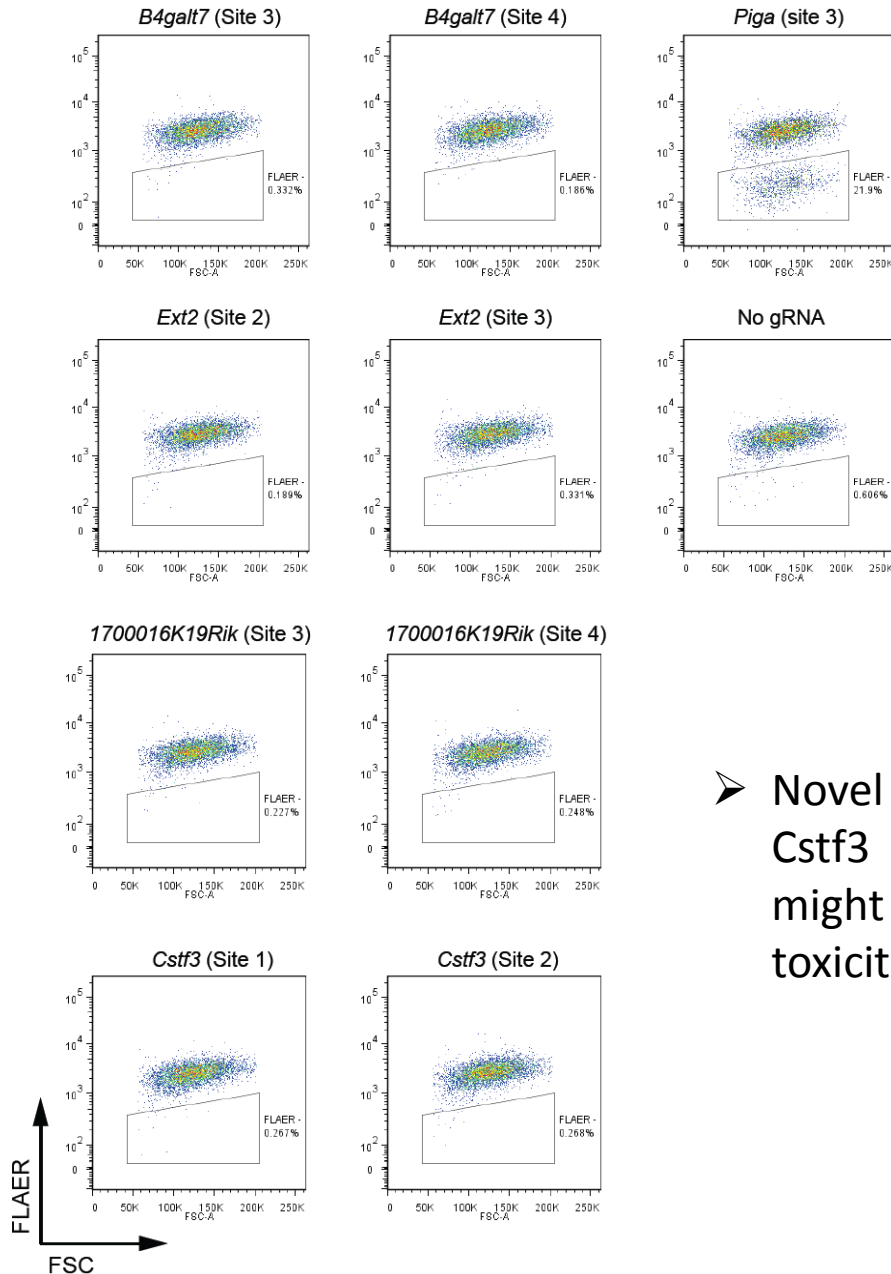


- sgRNAs targeting GPI anchor biosynthesis genes were enriched in Alpha toxin resistant cells
- sgRNA targeting MMR were enriched in 6-TG resistant cells
- Novel hits

Hits validation (methylene blue stain)

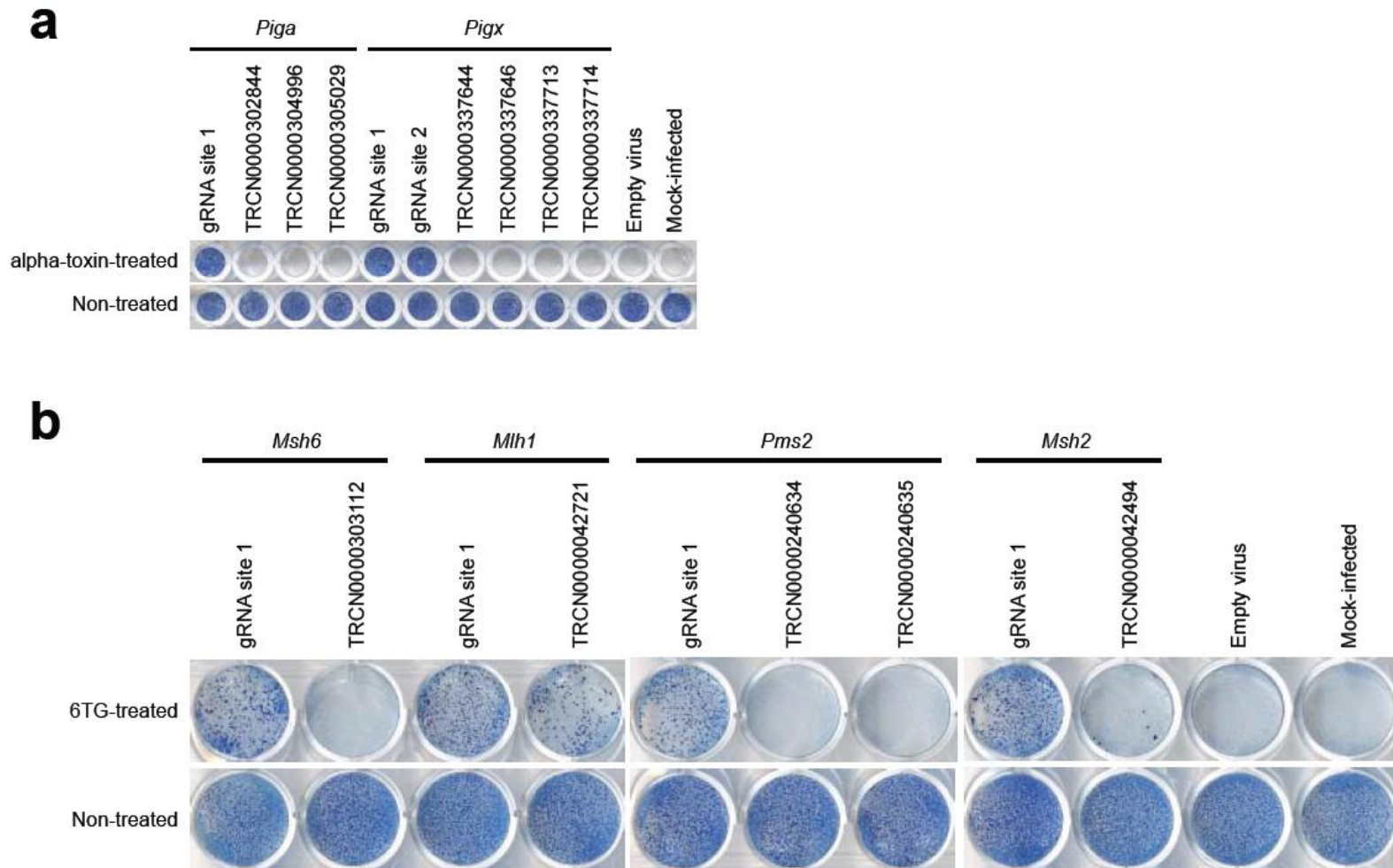


Hits validation



- Novel hits *B4galt7*, *Ext2*, *1700016K19Rik* and *Cstf3* did not affect anchorbiosynthesis, they might be involved in Alpha toxin induced cell toxicity in alternative pathway.

Comparison of shRNA and sgRNA screens



➤ Complete inactivation of genes is required to observe phenotype

Conclusion 4

- Mouse sgRNA library (87897 sgRNAs targeting 19150 genes)
- Function screening in both negative (cell proliferation) and positive (Alpha tocin and 6-TG) selection
- Better performance than shRNA, especially for phenotypes that can only be observed when the genes are completely inactivated
- CRISPR-CAS9 inactivates genes at DNA level, enable complete loss-of-function of genes, and also possible to functional interrogate the nontranscribed elements which are inaccessible by RNAi
- New version CAS9 or other CAS could enhance the power of HTS

CRISPR-CAS9 based pooled screen in prion research?

- 2 human shRNA libraries and 1 mouse sgRNA library are available from Addgene
- LentiCRISPR infected cells + prion/NBH (Ab/IgG) --- Reads enrichment? (susceptibility, replication, toxicity...)
- LentiCRISPR infected cells + recPrP (FT-PrP) --- Reads enrichment? (PrP receptor, PrP induced signaling...)

Thank you!

