Applications of CRISPR/Cas9 based genome-wide screening in mammalian cell cultures

Buket Baddal, PhD

Senior Lecturer in Microbiology

Department of Pharmaceutical Microbiology

Faculty of Pharmacy, Near East University

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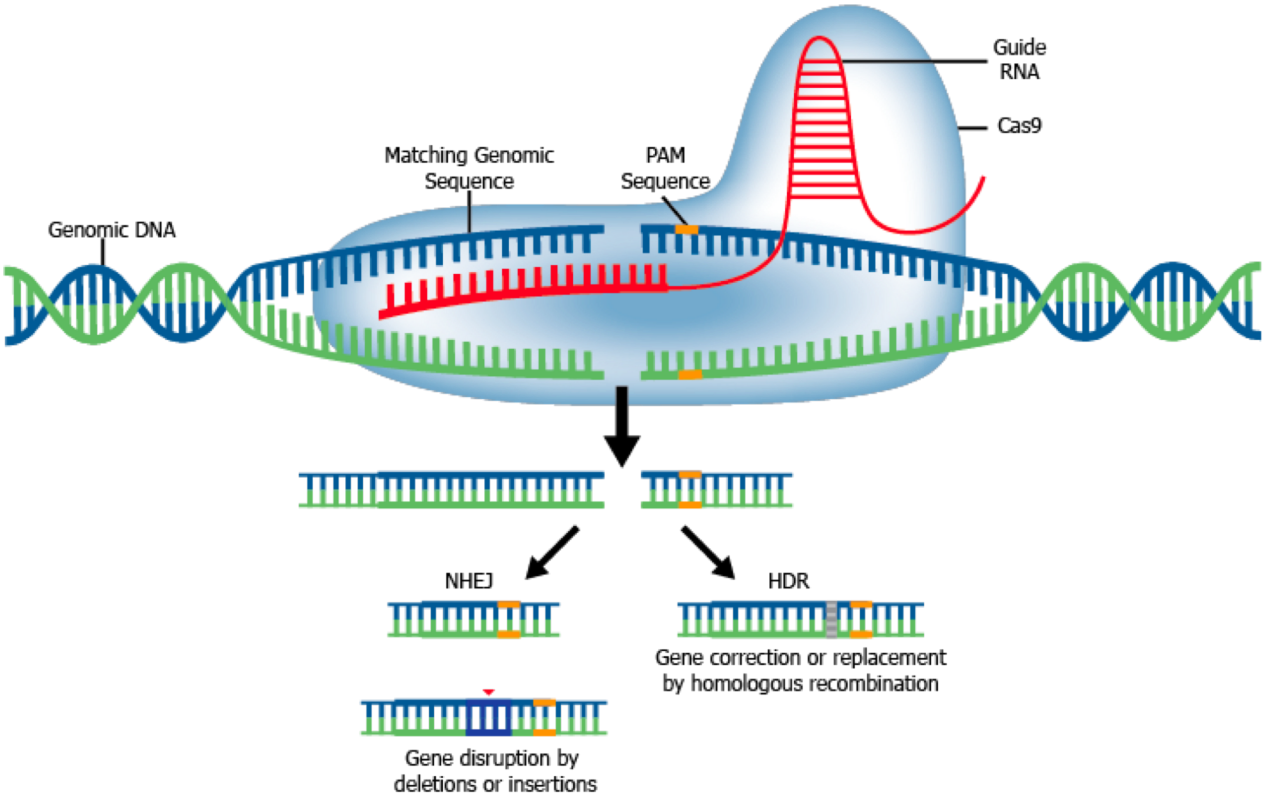
Outline

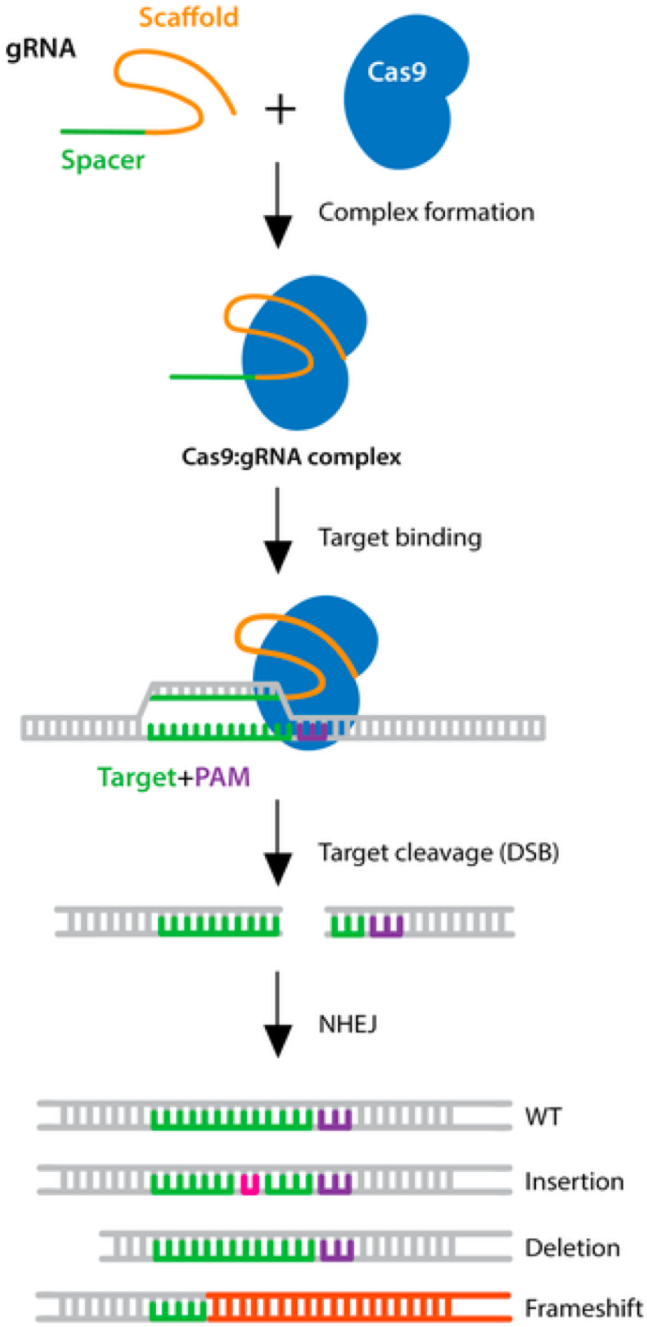
- CRISPR-Cas9 targeting system
- Genome-wide CRISPR-Cas9 library screening
- Applications in mammalian cell cultures
- Applications in microbiology & microbial pathogenesis

CRISPR-Cas9 targeting system

- CRISPR (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats) is a microbial nuclease system

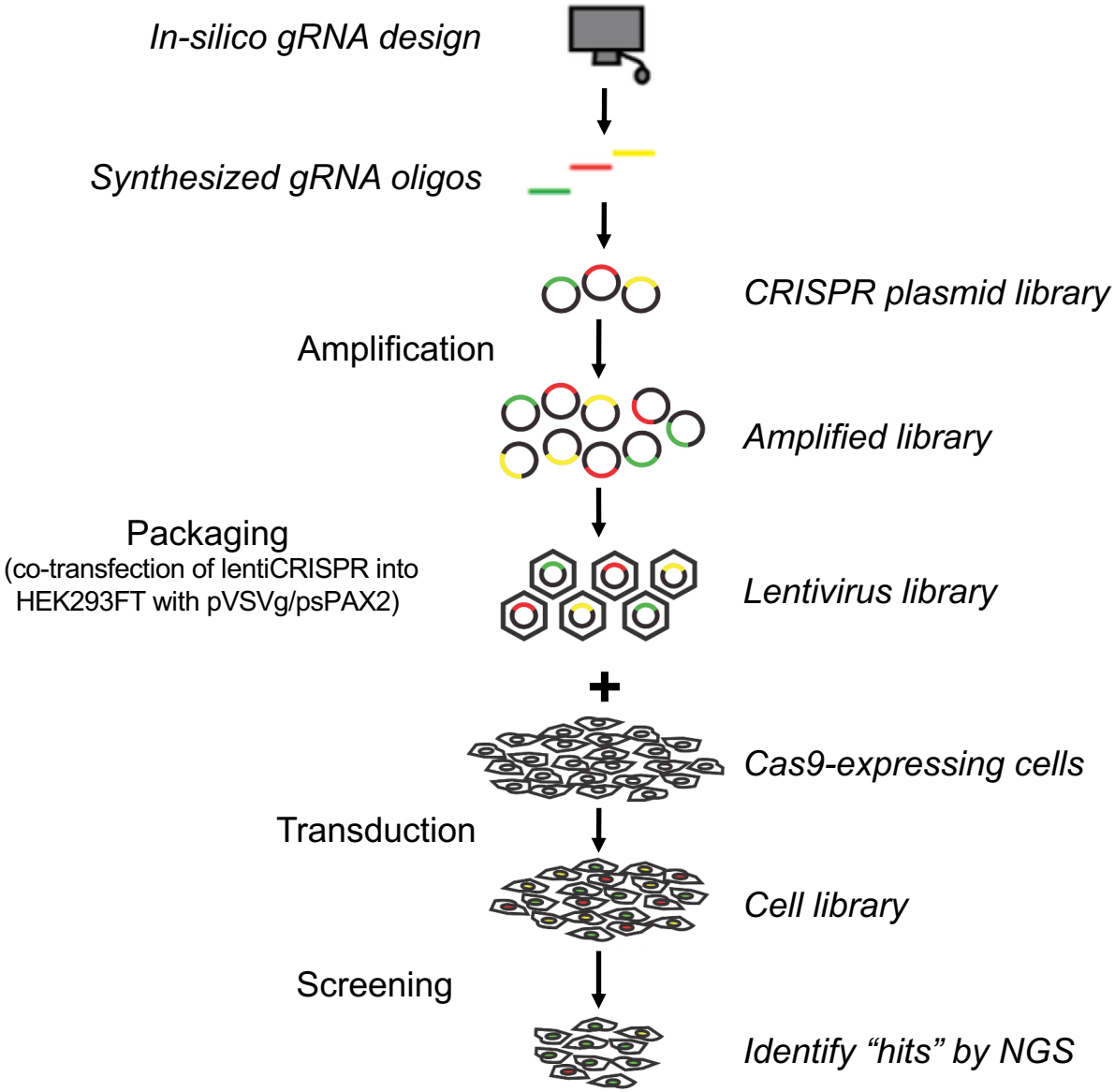
- CRISPR contains two components: a nonspecific CRISPR-associated endonuclease (Cas9) and a “guide” RNA (gRNA) capable of programming the specificity of the CRISPR-mediated nucleic acid cleavage





Creating a Loss-of-Function Mutation using CRISPR/Cas9

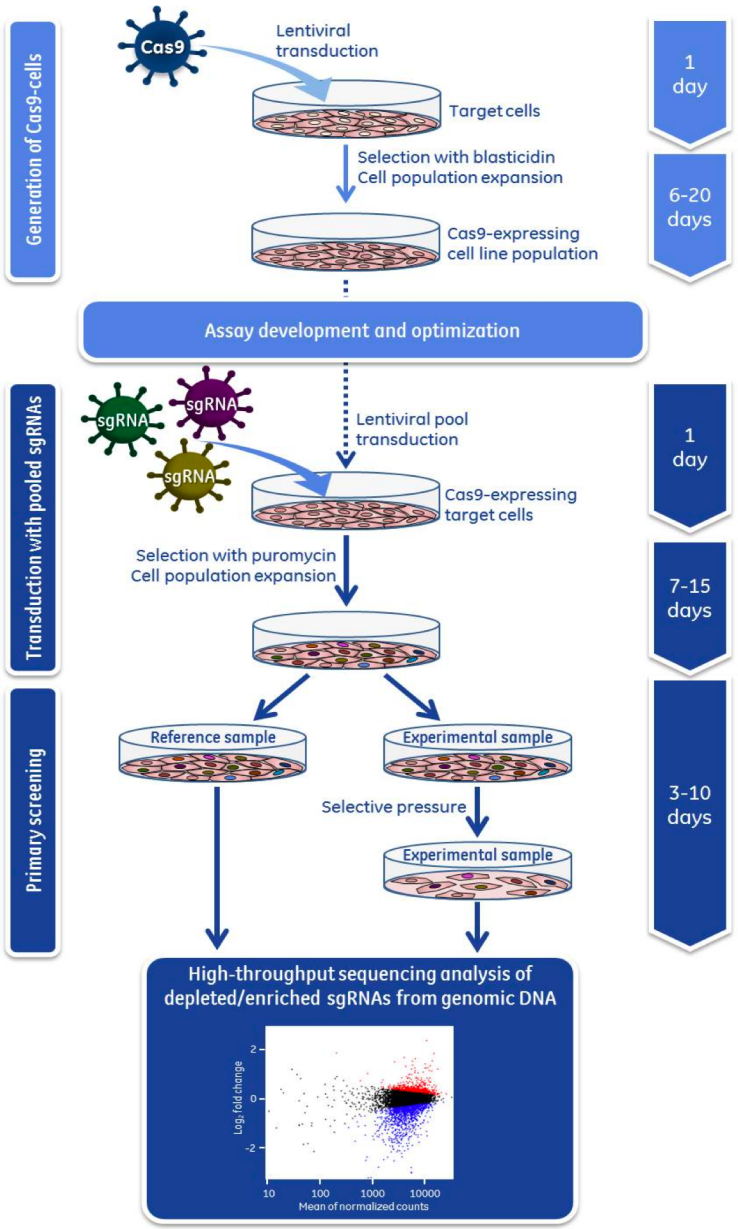
Genome-wide CRISPR/Cas9 screening



Genome-wide CRISPR/Cas9 screening

- Genome-wide scale screens involve lentiviral delivery of a pooled **genome-scale CRISPR-Cas9 knockout (GeCKO)** library targeting **19,050 genes** in human cells

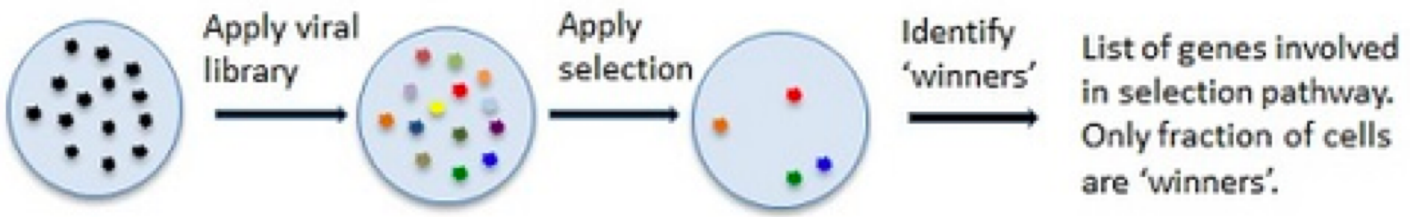
- The goal of a CRISPR screen is to use Cas9 and a pool of gRNAs to identify **genes essential for a given phenotype**



Genome-wide CRISPR/Cas9 screening

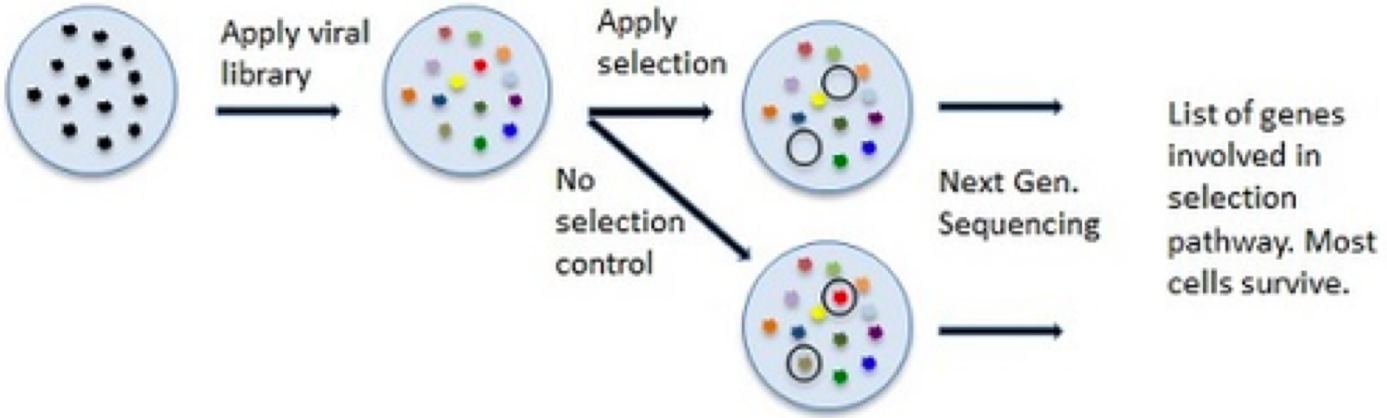
Positive screen

- e.g. genes essential for cell viability



Negative screen

- e.g. genes whose loss is involved in resistance to a drug

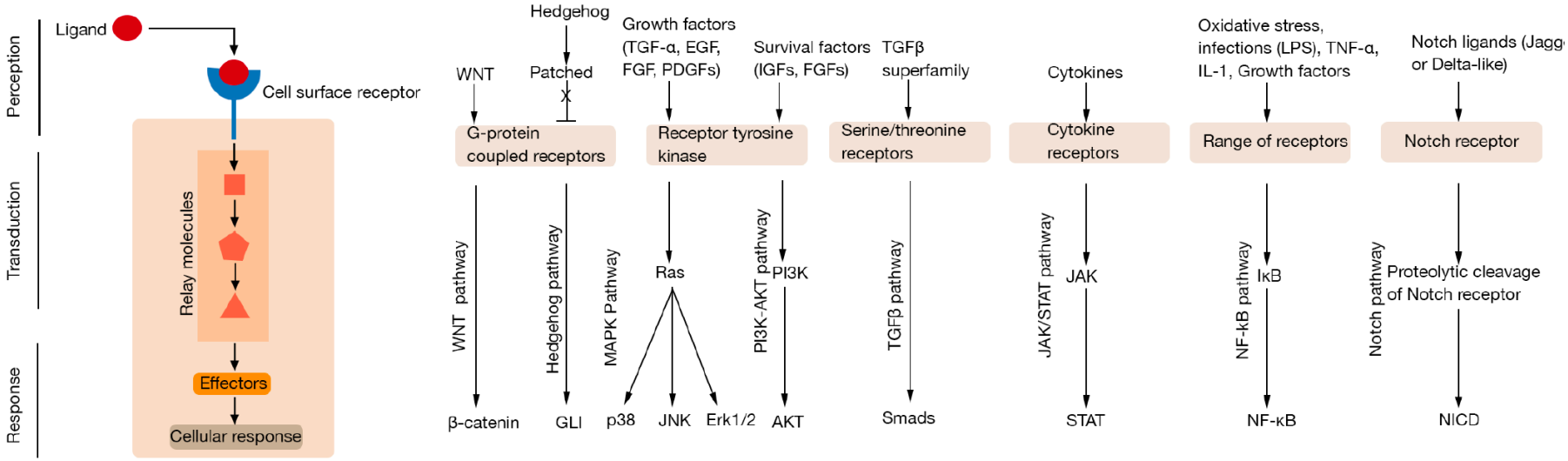


Applications of CRISPR library screening:

- Cellular signaling
- Drug discovery
- Bacterial/viral host target discovery
- Microbial toxin receptor discovery

Discovery/investigation of cellular signalling networks using CRISPR/Cas9 screens

- A pooled screening approach provides an opportunity to interrogate thousands of genetic perturbations in a single experiment



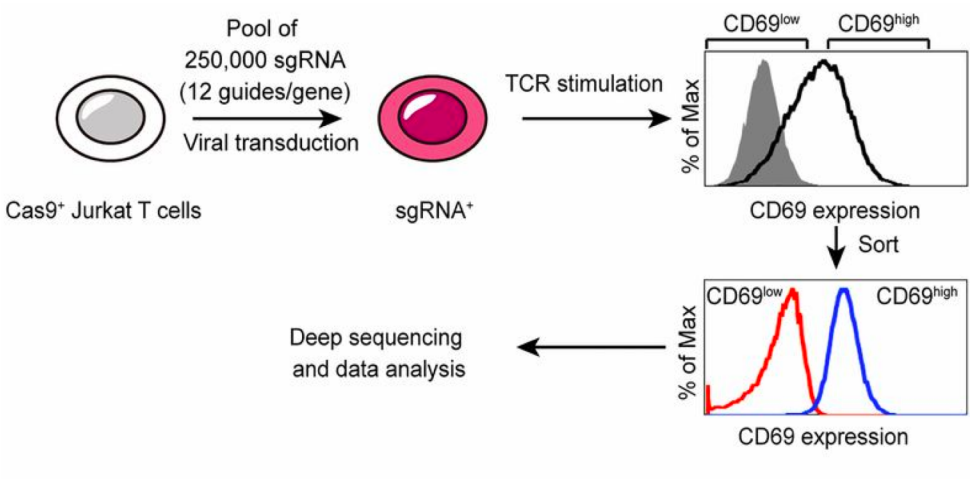
PNAS

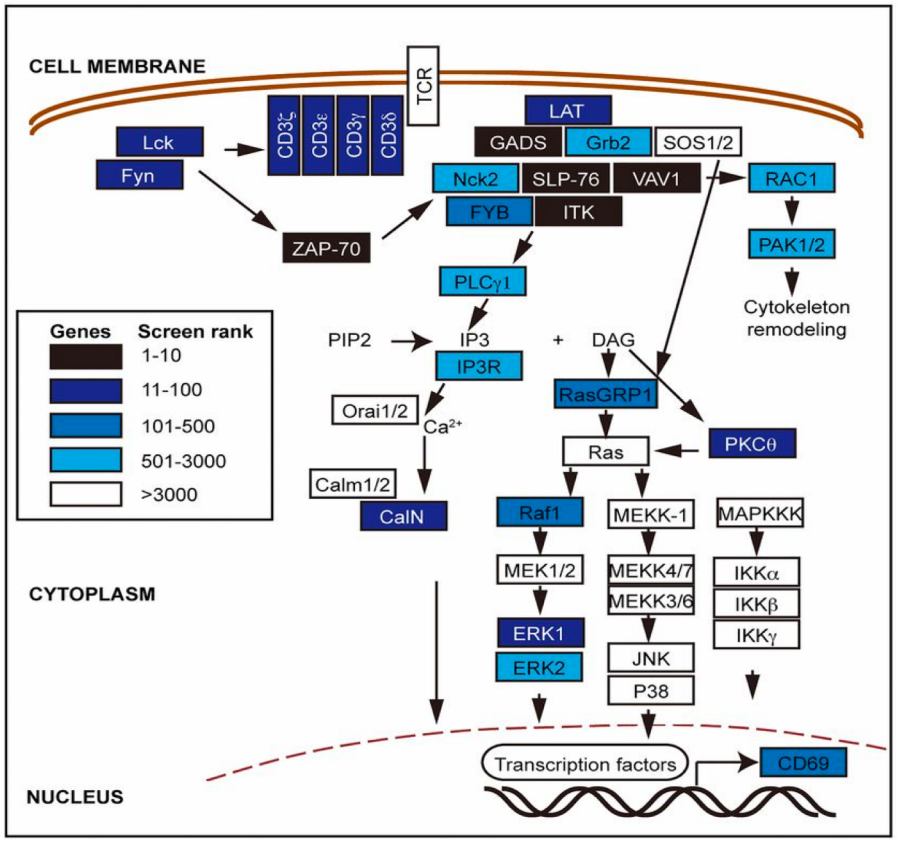
Genome-wide CRISPR screen identifies FAM49B as a key regulator of actin dynamics and T cell activation

Wanjing Shang[a,b,c,1], Yong Jiang[a,b,c,1], Michael Boettcher[d,1], Kang Ding[a,c,e], Marianne Mollenauer[f,g], Zhongyi Liu[a,b,c], Xiaofeng Wen[h], Chang Liu[a], Piliang Hao[a], Suwen Zhao[a,e], Michael T. McManus[d], Lai Wei[h,2], Arthur Weiss[f,g,2], and Haopeng Wang[a,2]

[a]School of Life Science and Technology, ShanghaiTech University, 201210 Shanghai, China; [b]Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 200031 Shanghai, China; [c]University of Chinese Academy of Sciences, 100049 Beijing, China; [d]W. M. Keck Center for Noncoding RNAs, Diabetes Center, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143; [e]iHuman Institute, ShanghaiTech University, 201210 Shanghai, China; [f]Division of Rheumatology, Department of Medicine, Rosalind Russell and Ephraim P. Engleman Rheumatology Research Center, University of California, San Francisco, CA 94143; [g]Howard Hughes Medical Institute, University of California, San Francisco, CA 94143; and [h]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 510060 Guangzhou, China

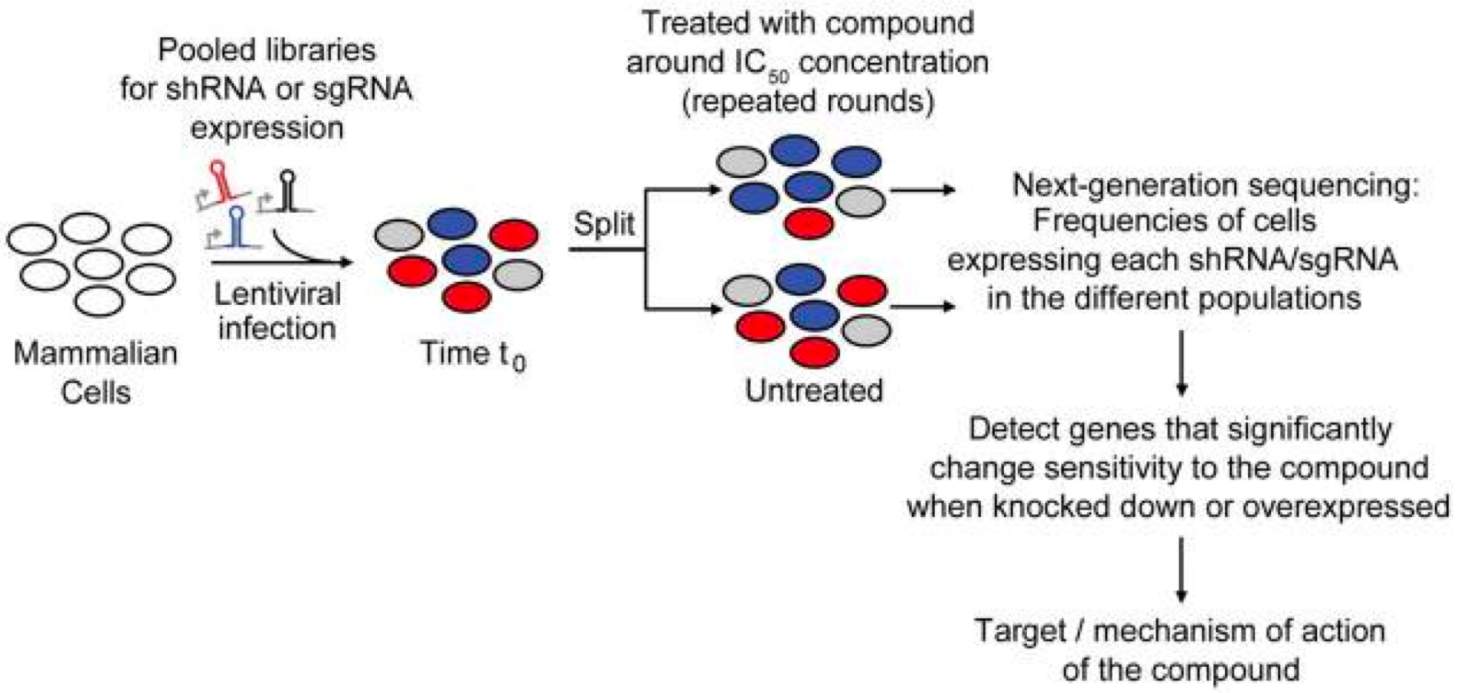
- T cells play an essential role in maintaining human health against cancer, infection, and autoimmune diseases
- mechanisms controlling T cell activation are only partially known
- important for T cell-based immune cancer therapies
- a genome-wide CRISPR screen to search for genes that regulate T cell activation



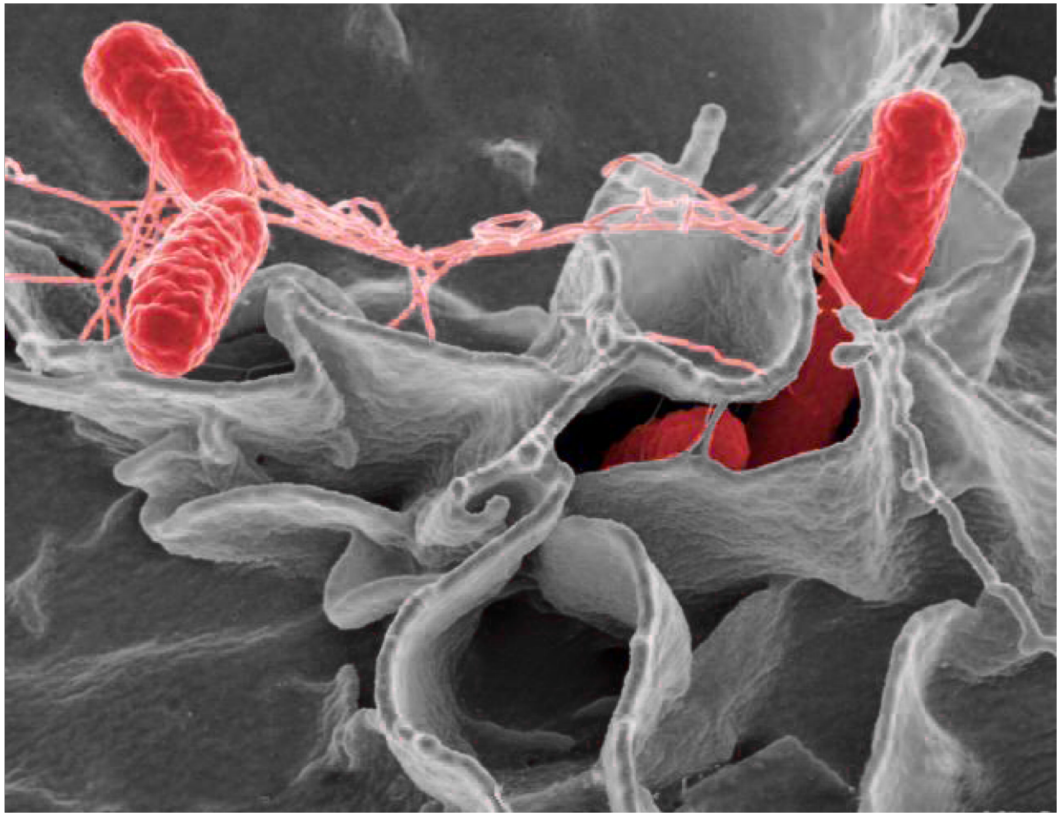


Discovery of new cancer drug targets using CRISPR/Cas9 screens

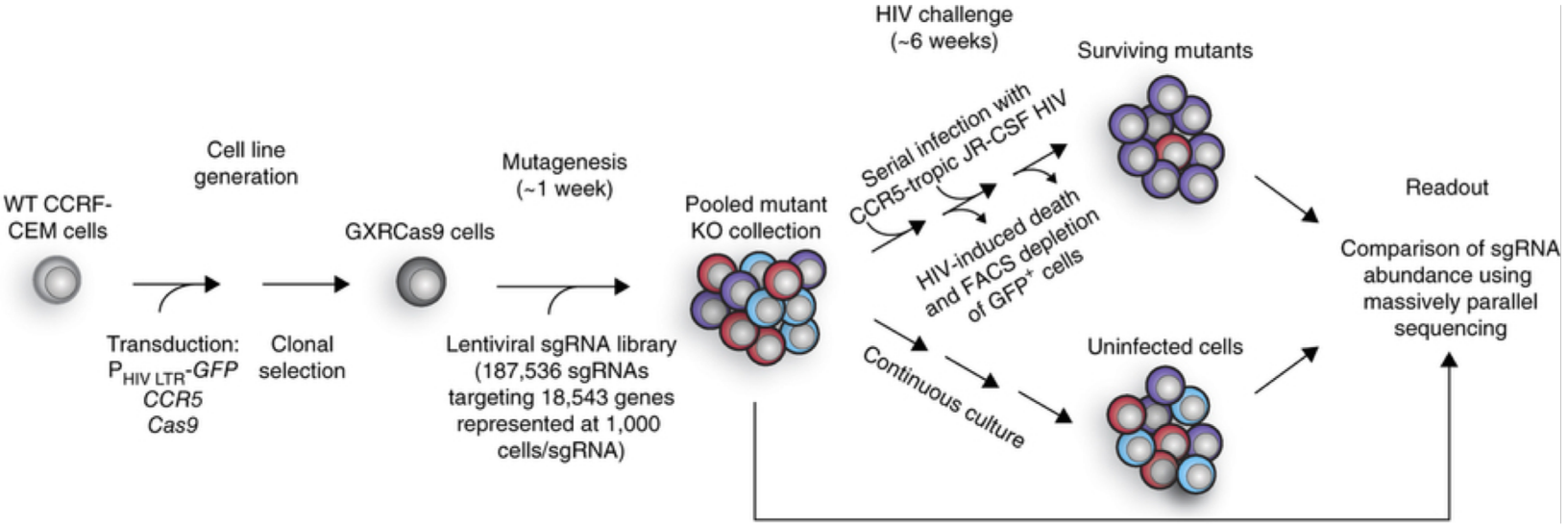
- Biochemical approaches rely on physical binding of the compound to its protein target
- RNAi-based screens are noisy due to off-target effects of siRNAs

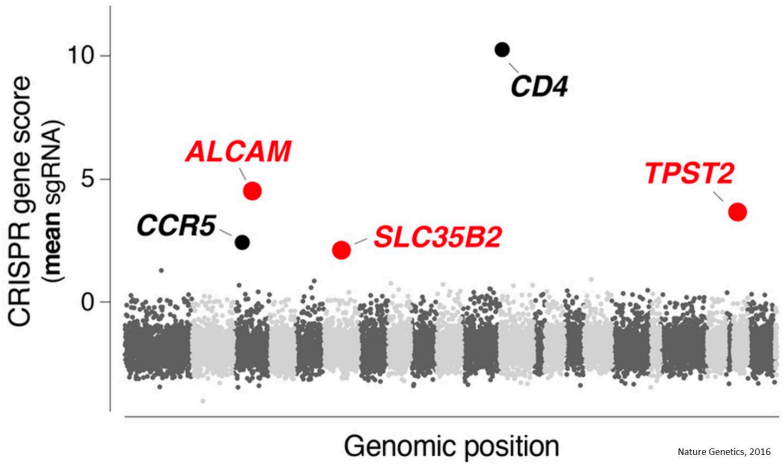


Host-Pathogen Interactions



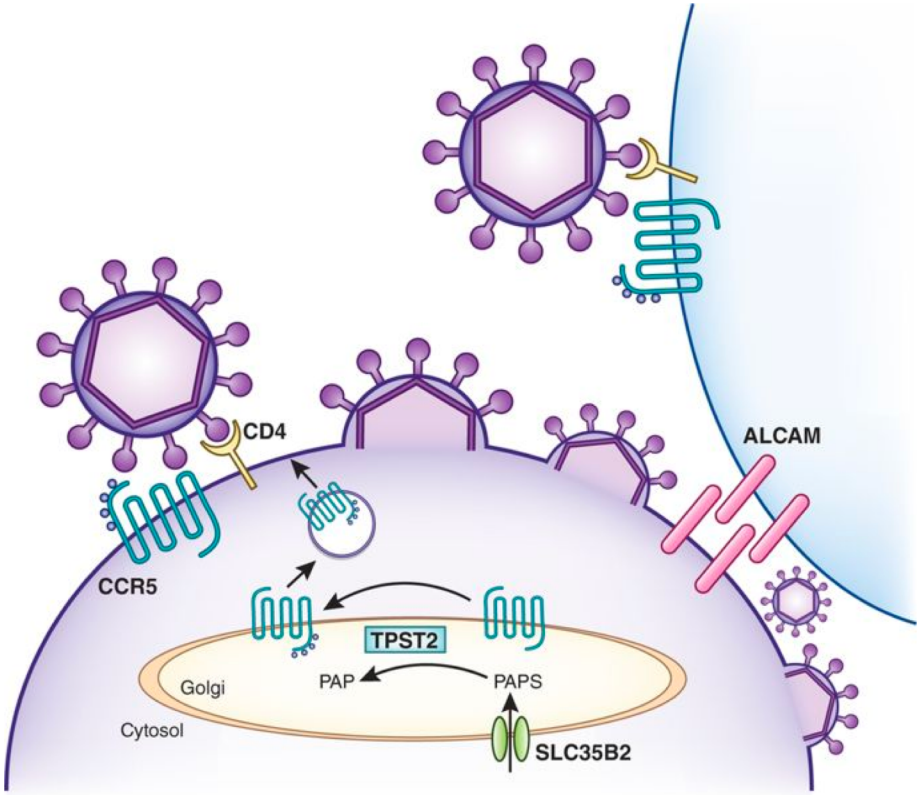
A CRISPR screen for novel host targets for HIV infection





Park et al., Nature Genetics 2016

Potential drug targets for anti-HIV therapy



- This screen was performed in CD4 + T-cells and was designed to find candidate genes required for successful HIV infection, but whose inactivation did not affect cell viability.
- Two known (*CCR5* and *CD4*) and three novel **(*ALCAM*, *SLC35B2* and *TPST2*)** cellular factors were discovered, which upon abrogation, prevented HIV infection.
- These new genes are involved in sulfation and cell aggregation pathways and represent **candidate targets for interventional HIV therapy.**

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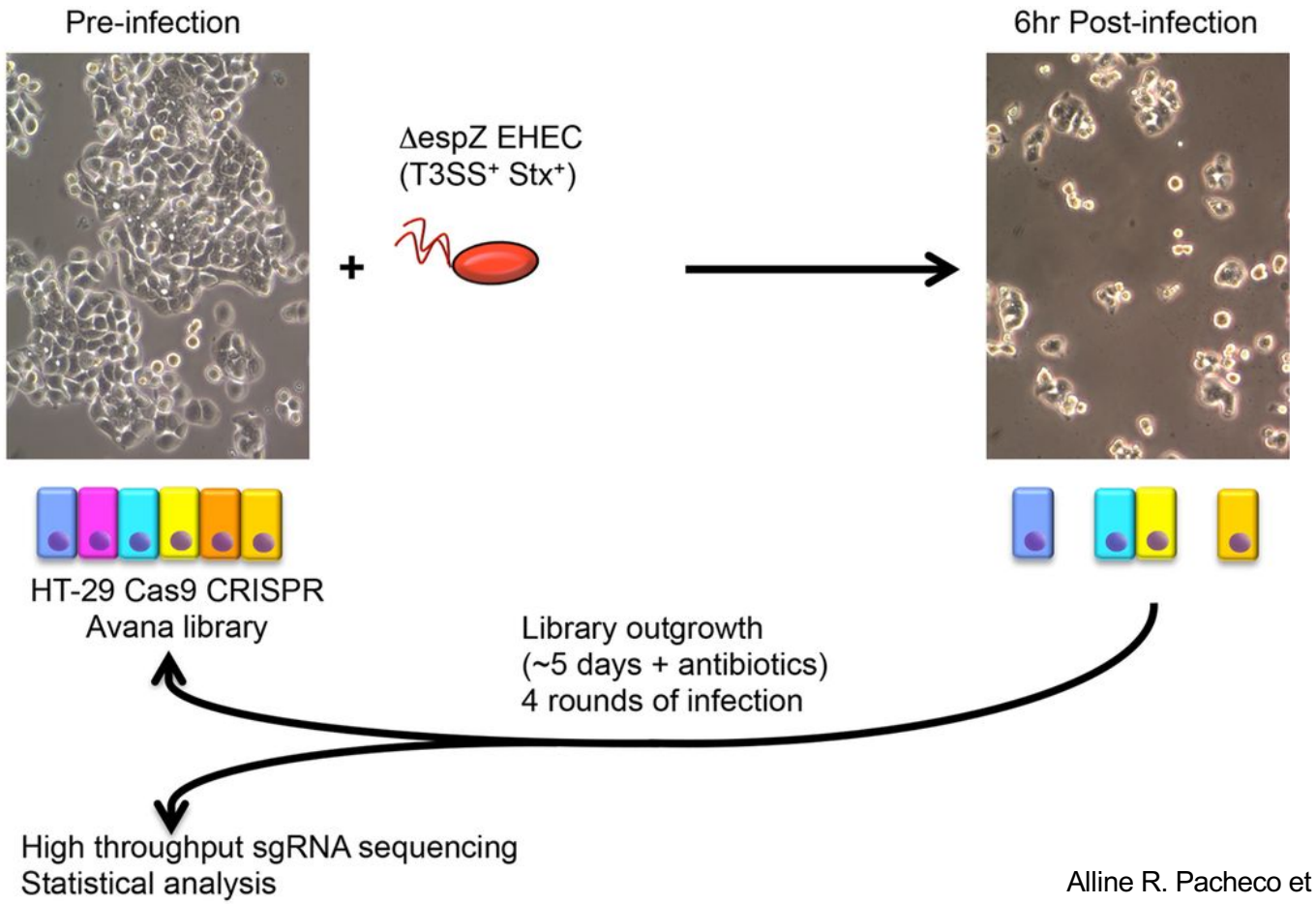
RESEARCH ARTICLE

CRISPR Screen Reveals that EHEC's T3SS and Shiga Toxin Rely on Shared Host Factors for Infection

Alline R. Pacheco,[a,b] Jacob E. Lazarus,[a,b,c] Brandon Sit,[a,b] Stefanie Schmieder,[d,e,f] Wayne I. Lencer,[d,e,f] Carlos J. Blondel,[a,b]* John G. Doench,[g] Brigid M. Davis,[a,b] Matthew K. Waldor[a,b,g,h]

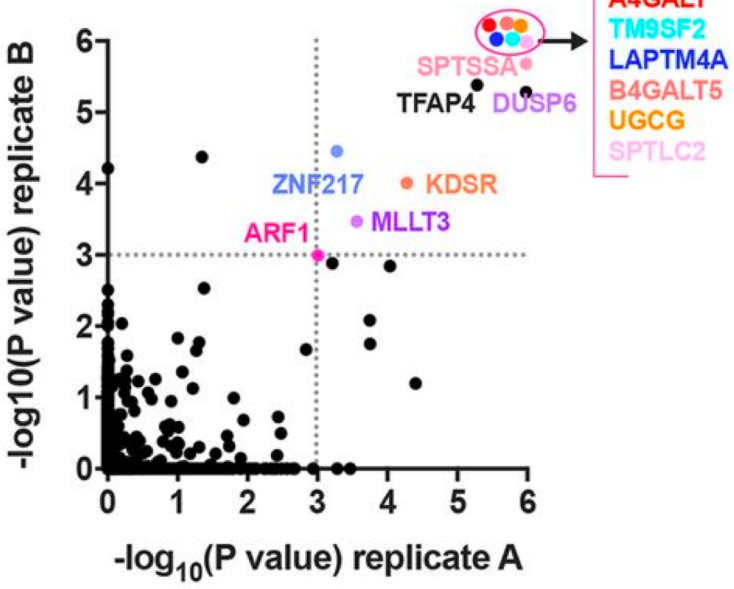
[a]Division of Infectious Diseases, Brigham and Women's Hospital, Boston, Massachusetts, USA
[b]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, USA
[c]Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts, USA
[d]Division of Gastroenterology, Boston Children's Hospital, Boston, Massachusetts, USA
[e]Department of Pediatrics, Harvard Medical School, Boston, Massachusetts, USA
[f]Department of Pediatrics, Harvard Digestive Diseases Center, Boston, Massachusetts, USA
[g]Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA
[h]Howard Hughes Medical Institute, Boston, Massachusetts, USA

Design of a CRISPR/Cas9 screen to identify host factors underlying susceptibility to enterohemorrhagic *Escherichia coli* (EHEC) infection



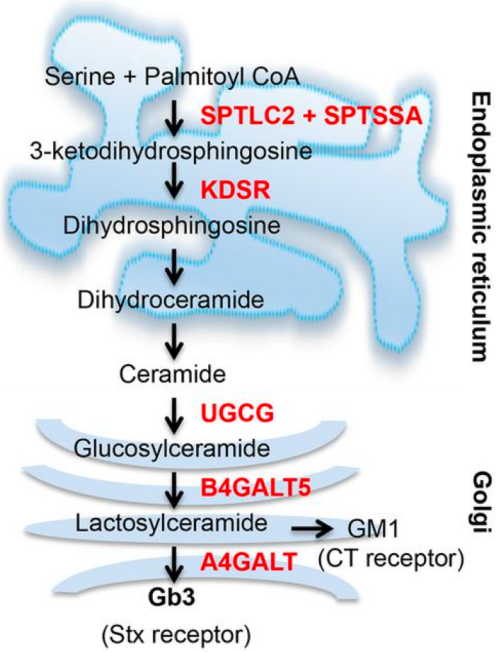
Alline R. Pacheco et al. mBio 2018

A



B

	Hit	Gene name
Sphingolipid biosynthesis	A4GALT	α-1,4-galactosyltransferase; Gb3 synthase
	B4GALT5	β-1,4-galactosyltransferase; major lactosylceramide synthase
	UGCG	UDP-glucose:ceramide glucosyltransferase; synthesize glucosylceramide
	KDSR	3-ketodihydrosphingosine reductase
	SPTLC2	serine palmitoyltransferase long chain base subunit 2
	SPTSSA	serine palmitoyltransferase small subunit A
	ARF1	ADP-ribosylation factor 1
Unknown	LAPTM4A	Lysosomal Protein Transmembrane 4 Alpha
	TM9SF2	Transmembrane 9 Superfamily Member 2
Cell proliferation	DUSP6	Dual Specificity Phosphatase 6; inactivates specifically ERK1/2
	TFAP4	Transcription Factor AP-4
	ZNF217	Zinc Finger Protein 217
	MLLT3	Myeloid/Lymphoid Or Mixed-Lineage Leukemia; Translocated To 3



Alline R. Pacheco et al. mBio 2018

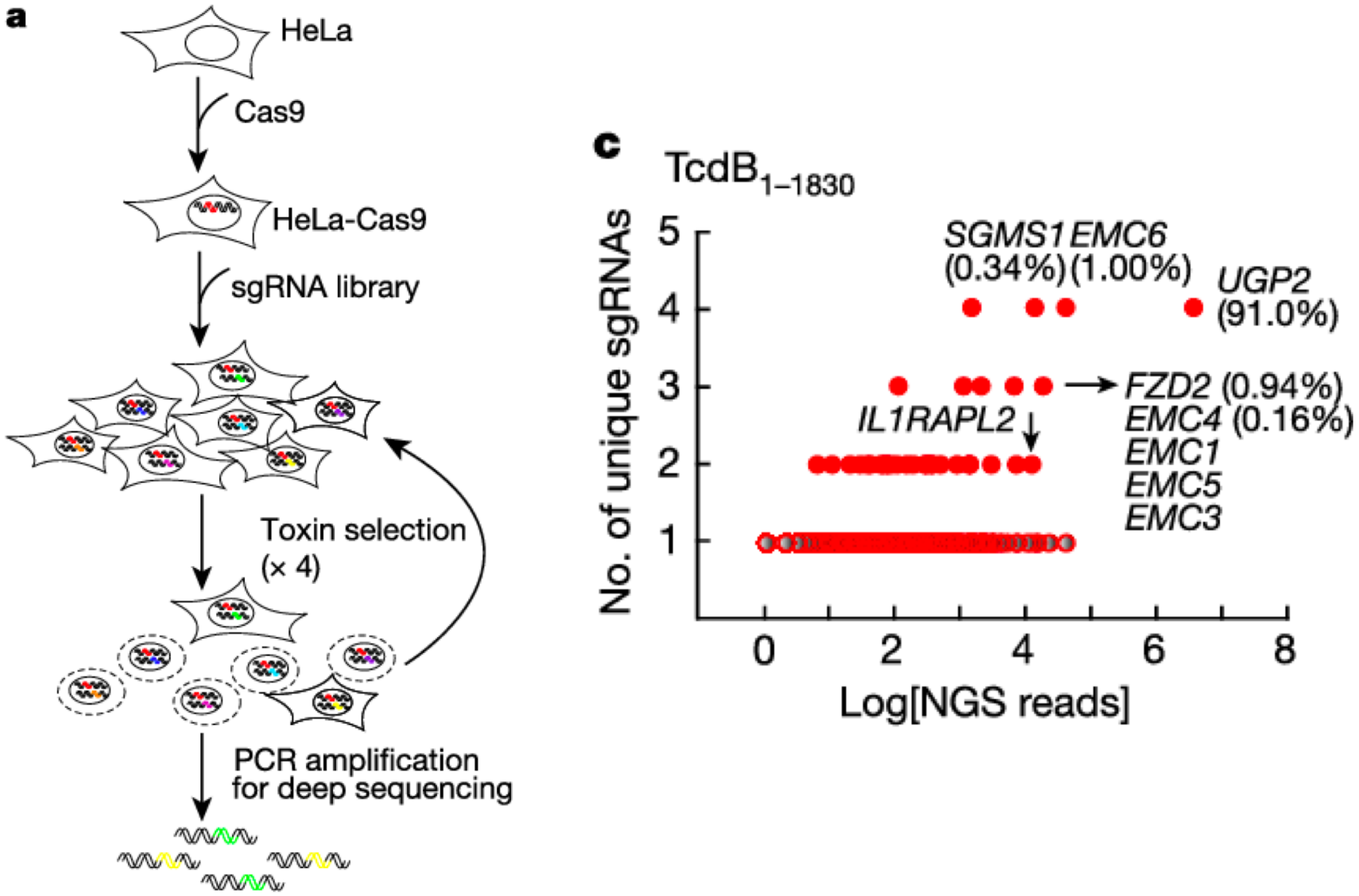
ARTICLE

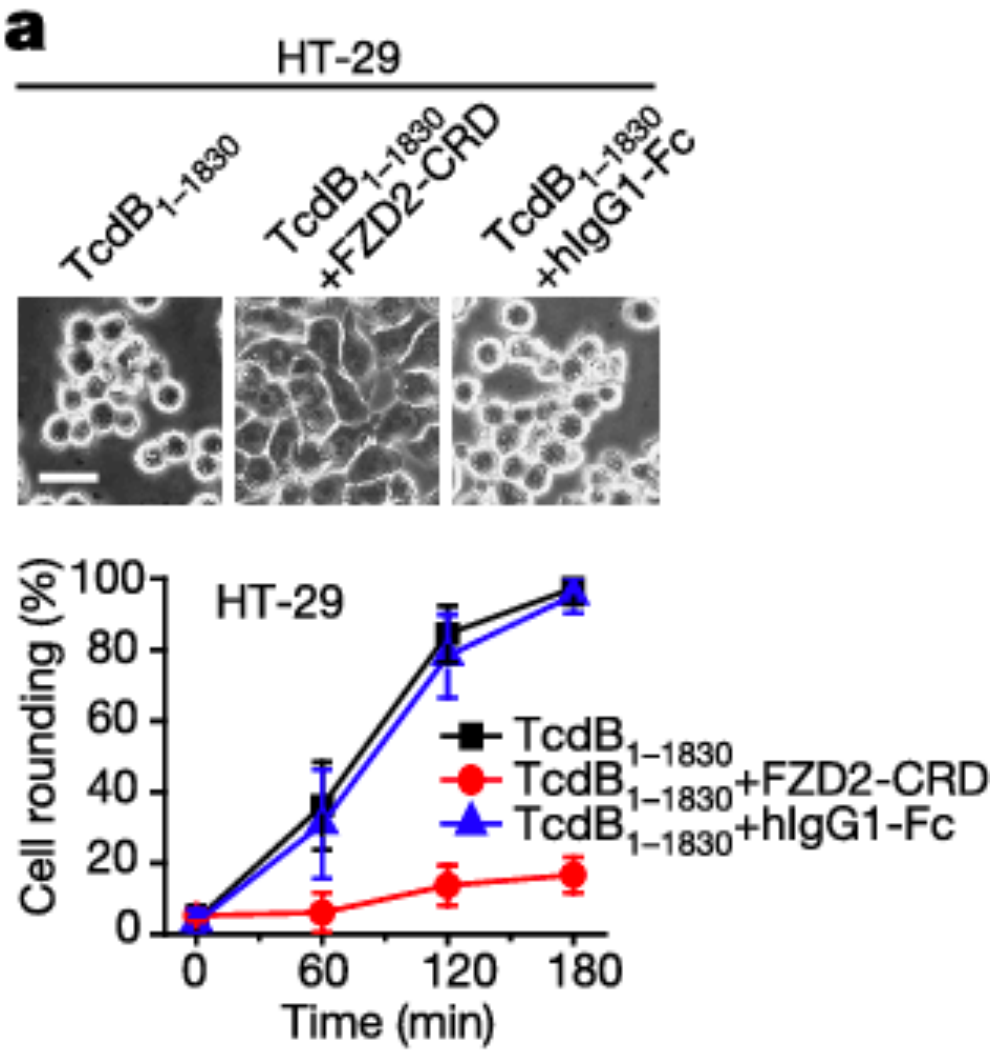
doi:10.1038/nature19799

Frizzled proteins are colonic epithelial receptors for *C. difficile* toxin B

Liang Tao[1,2], Jie Zhang[1,2]*, Paul Meraner[3]*, Alessio Tovaglieri[4], Xiaoqian Wu[5], Ralf Gerhard[6], Xinjun Zhang[7,8], William B. Stallcup[9], Ji Miao[4,10], Xi He[7,8], Julian G. Hurdle[5], David T. Breault[4,10,11], Abraham L. Brass[3,12] & Min Dong[1,2]

Clostridium difficile **toxin B (TcdB) is a critical virulence factor that causes diseases associated with *C. difficile* infection. Here we carried out CRISPR–Cas9–mediated genome–wide screens and identified the members of the Wnt receptor frizzled family (FZDs) as TcdB receptors. TcdB binds to the conserved Wnt–binding site known as the cysteine–rich domain (CRD), with the highest affinity towards FZD1, 2 and 7. TcdB competes with Wnt for binding to FZDs, and its binding blocks Wnt signalling. FZD1/2/7 triple–knockout cells are highly resistant to TcdB, and recombinant FZD2–CRD prevented TcdB binding to the colonic epithelium. Colonic organoids cultured from FZD7–knockout mice, combined with knockdown of FZD1 and 2, showed increased resistance to TcdB. The colonic epithelium in FZD7–knockout mice was less susceptible to TcdB–induced tissue damage *in vivo*. These findings establish FZDs as physiologically relevant receptors for TcdB in the colonic epithelium.**

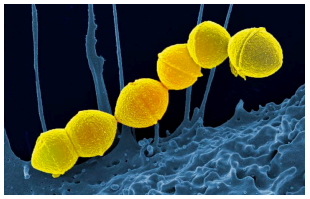

a
HeLa
Cas9
HeLa-Cas9
sgRNA library
Toxin selection
(× 4)
PCR amplification
for deep sequencing
c
TcdB1–1830
No. of unique sgRNAs
SGMS1 (0.34%)
EMC6 (1.00%)
UGP2 (91.0%)
FZD2 (0.94%)
EMC4 (0.16%)
EMC1
EMC5
EMC3
IL1RAPL2
Log[NGS reads]



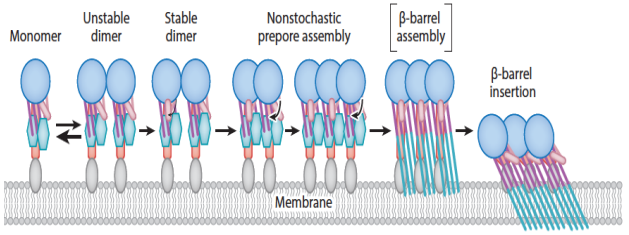
FZDs are functional receptors for TcdB

Streptolysin O (SLO)

- secreted toxin of Group A Streptococcus



- member of cholesterol-dependent cytolysins

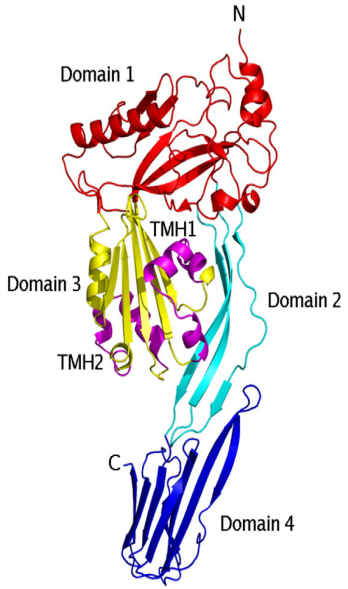


Tweten et al. 2015

- binds to cholesterol-containing membranes, oligomerizes and inserts into the lipid bilayer to form large pores

- domain 4 contains cholesterol recognition motif and a putative carbohydrate-binding site

- mutation of SLO cholesterol recognition motif or chemical depletion of host cell cholesterol does not diminish SLO membrane binding



Feil et al. 2013
Meehl et al. 2004

Glycan microarray analysis reveals SLO has glycan-binding activity

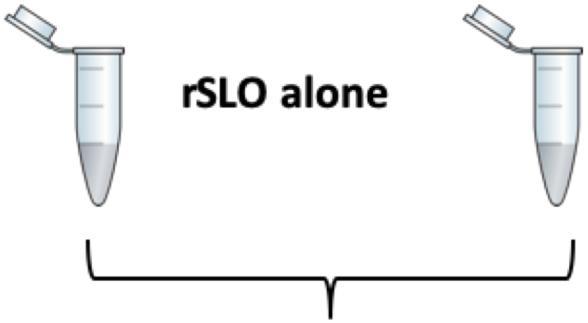
SLO binding partners:

Code	Name	Formula	Structure	K_D (M)
N/A	Lactose	Galβ1-4Glc		2.92×10^{-8} ($\pm 8.72 \times 10^{-9}$)
1A	Lacto-*N*-Biose I	Galβ1-3Glc*N*Ac		n.i.*
1B	*N*-Acetyllactosamine	Galβ1-4Glc*N*Ac		1.73×10^{-7} ($\pm 9.1 \times 10^{-9}$)
1E	β1-3galactosyl-*N*-acetylgalactosamine	Galβ1-3Gal*N*Ac		1.73×10^{-7} ($\pm 8.70 \times 10^{-10}$)
1G	Lacto-*N*-tetraose	Galβ1-3Glc*N*Acβ1-3Galβ1-4Glc		1.31×10^{-8} ($\pm 5.50 \times 10^{-10}$)
1H	Lacto-*N*-neotetraose	Galβ1-4Glc*N*Acβ1-3Galβ1-4Glc		6.44×10^{-10} ($\pm 1.82 \times 10^{-10}$)
1O	Linear B-2 Trisaccharide	Galα1-3Galβ1-4Glc*N*Ac		n. i.*
2C	Terminal disaccharide of globotetraose	Gal*N*Acβ1-3Gal		n. i.*

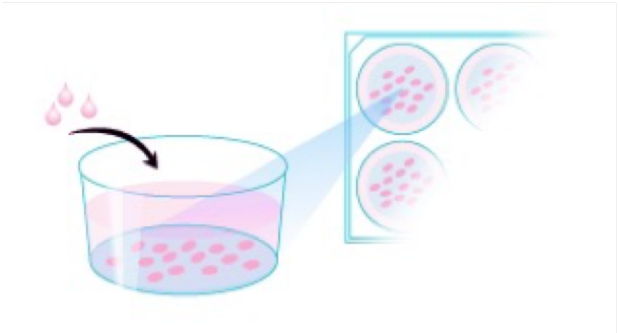
Symbol nomenclature

*n.i. = No interaction (K_D observed was greater than 1×10^{-3} M and/or the chi-square value was greater than 50% of the R_{max})

Inhibition assay for rSLO host surface binding



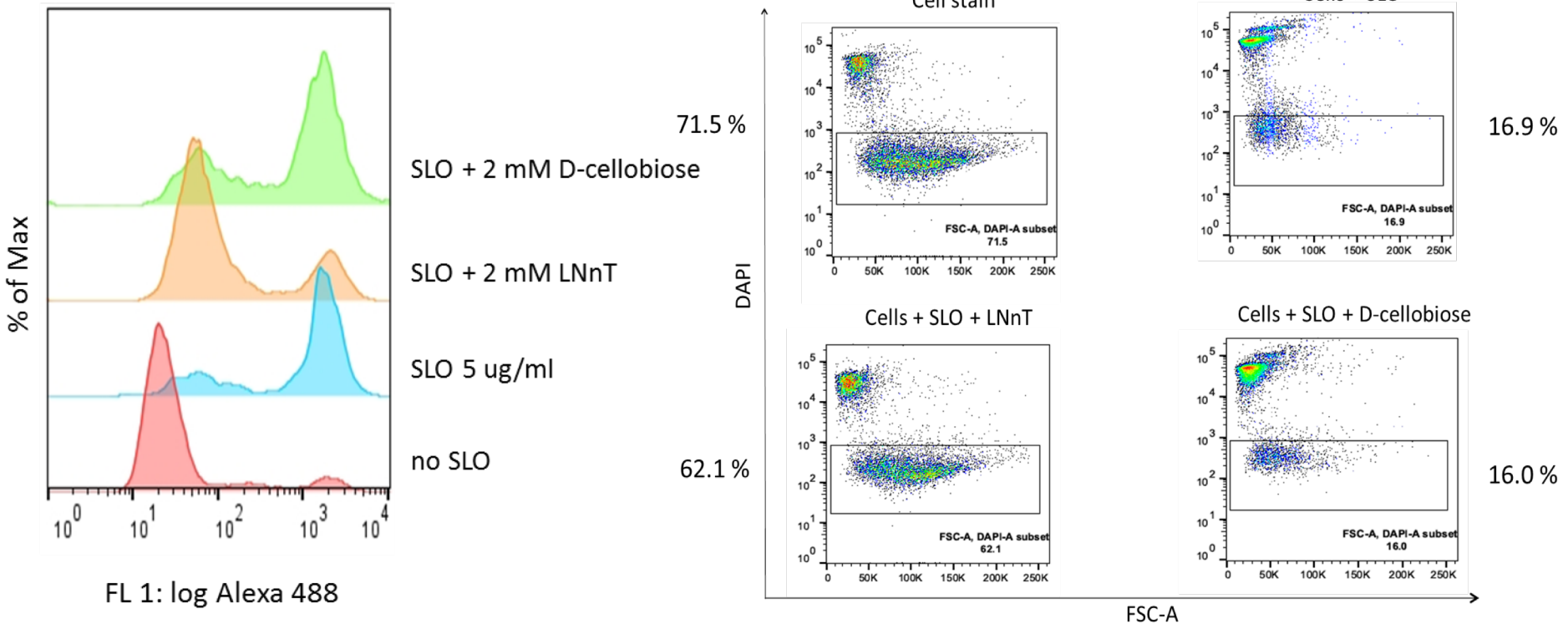
rSLO preincubated with 2mM glycan inhibitor (LNnT/D-cellobiose) for 15 mins at RT



Treat the OKP7 cells with rSLO for 45 mins on ice to allow membrane binding

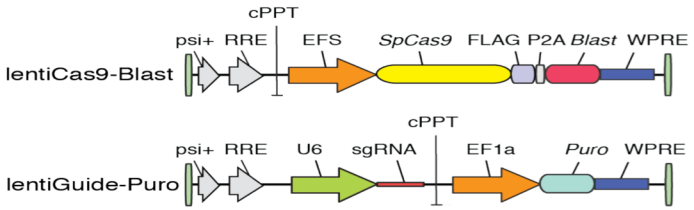
Stain & analyze for Alexa 488 expression

Preincubation with oligosaccharide LNnT prevents SLO binding to human oropharyngeal keratinocytes, blocks pore formation and increases cell viability



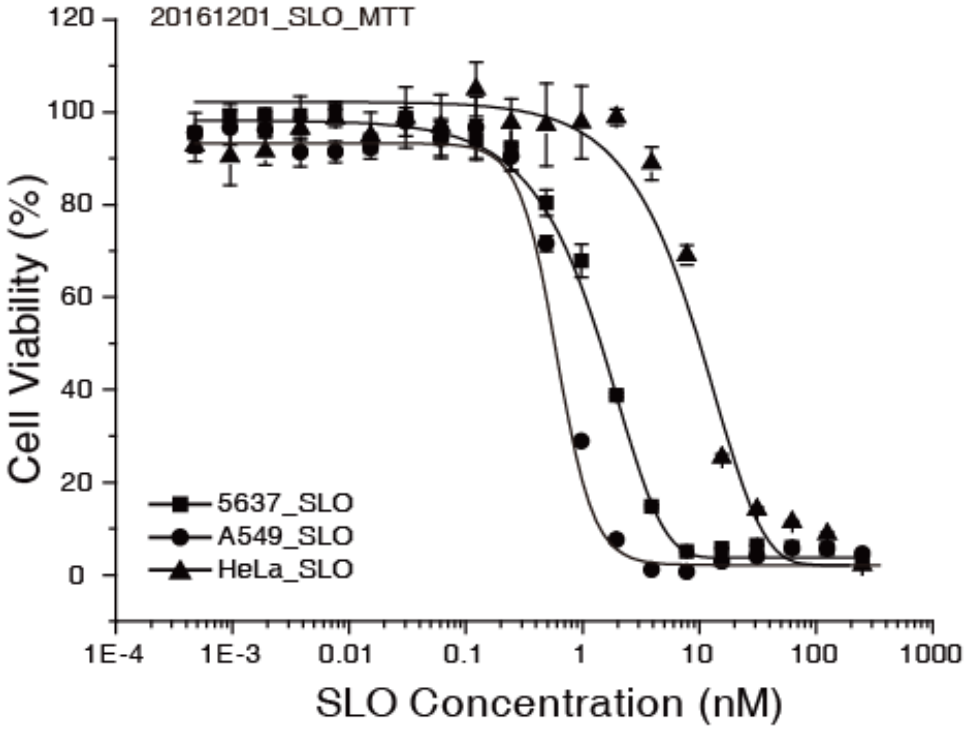
CRISPR/Cas9 knockout screening strategy

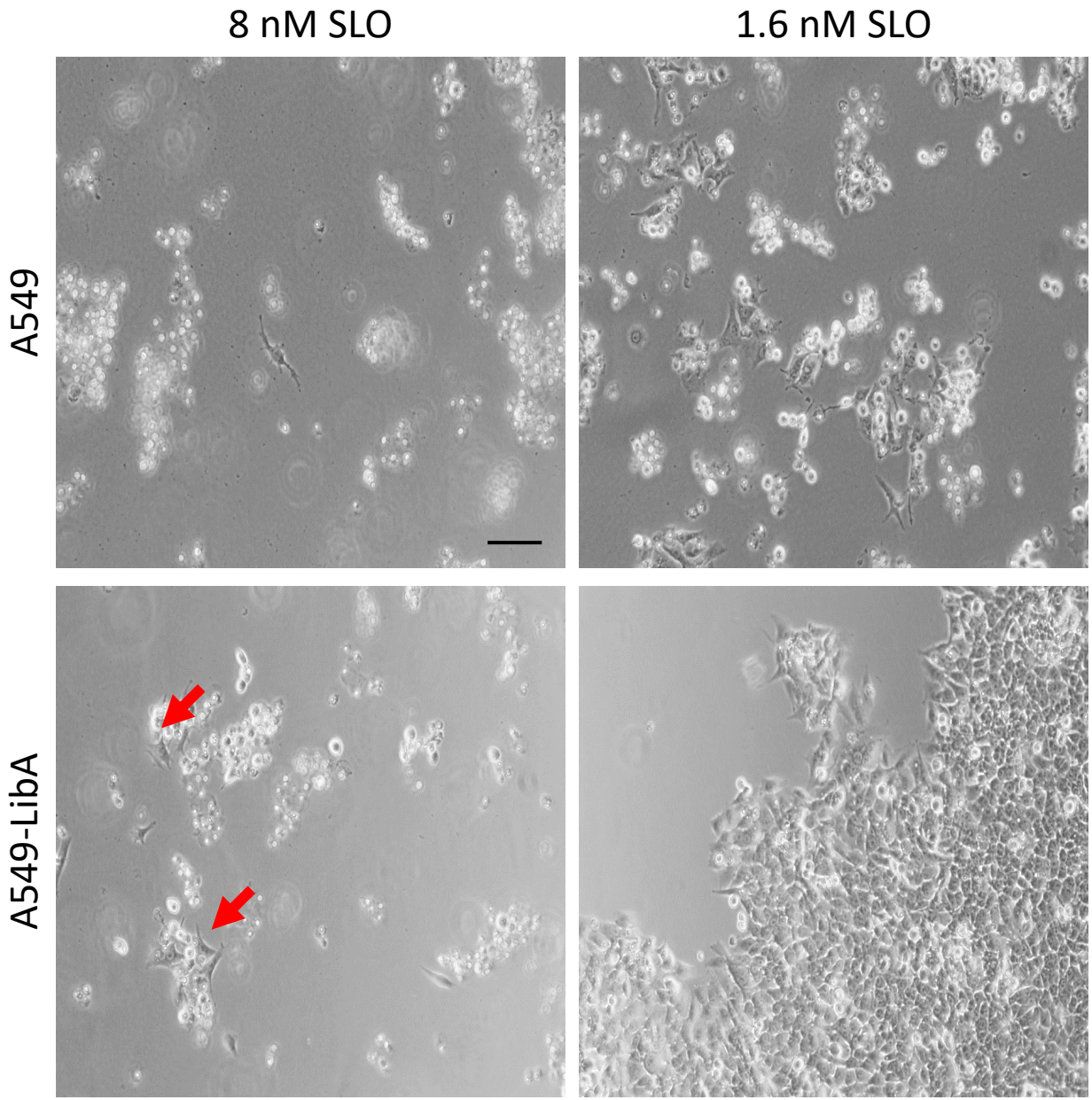
GeCKO v2.0 two vector system



	GeCKO v2 human library
Species	human
Number of genes targeted	19,050
Targeting constructs per gene	6 per gene (3 in Library A, 3 in Library B)
Number of miRNA targeted	1,864
Targeting constructs per miRNA	4 per miRNA
Control (non-targeting) sgRNAs	1,000
Total sgRNA constructs	122,411 (65,383 in Library A, 58,028 in Library B)

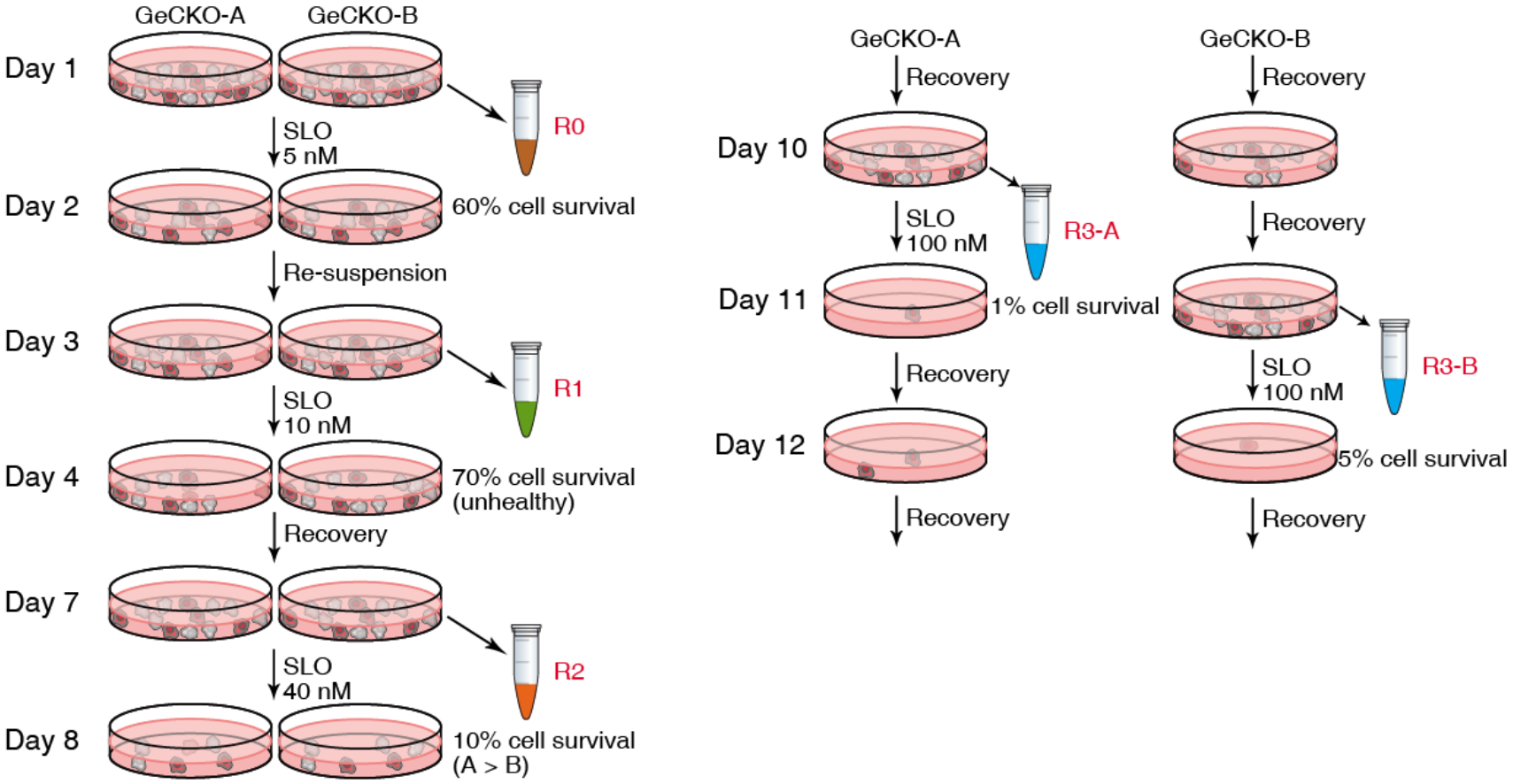
Sensitivity: A549 ≈ 5637 > HeLa

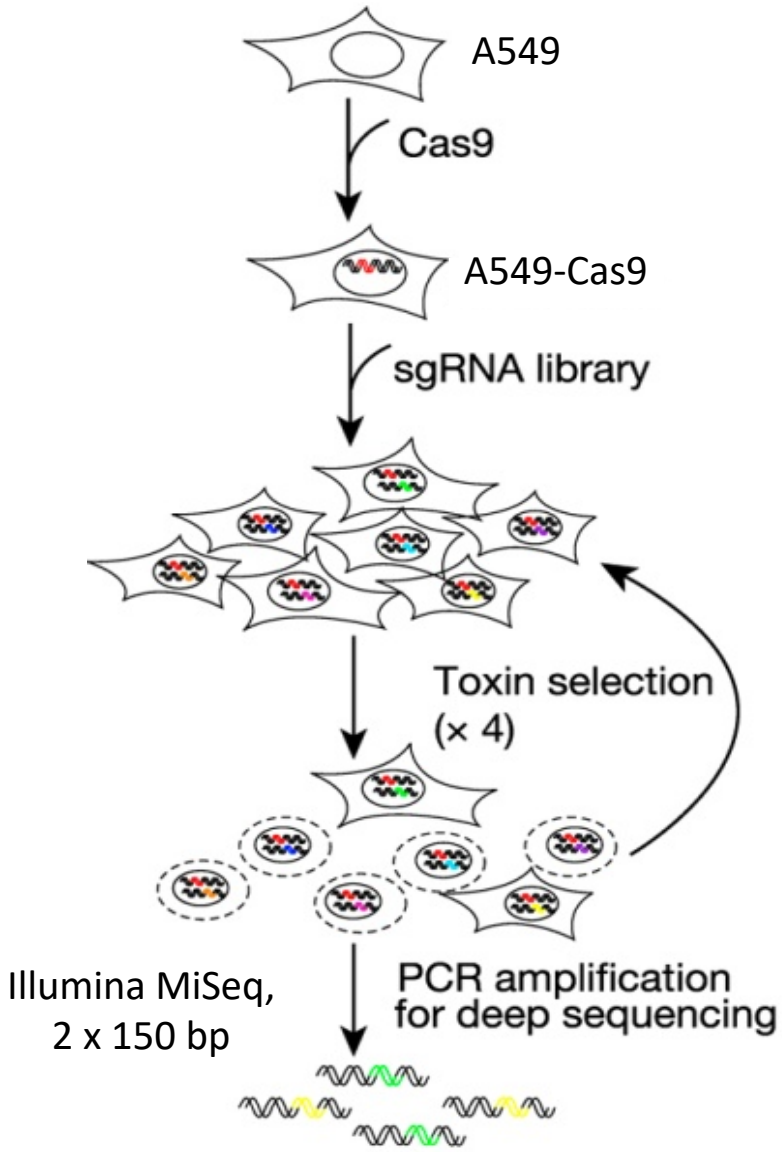




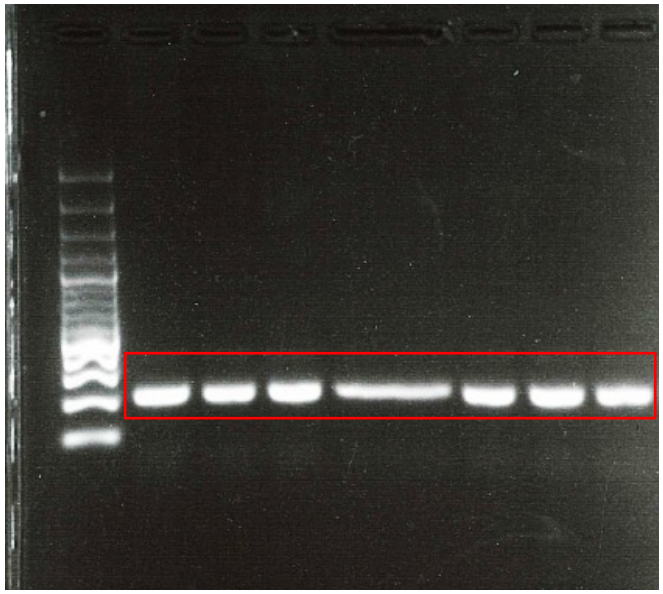
Bar: 100 μm
Arrow head: “normal cell”

SLO screen


GeCKO-A
GeCKO-B
Day 1
R0
SLO
5 nM
Day 2
60% cell survival
Re-suspension
Day 3
R1
SLO
10 nM
Day 4
70% cell survival
(unhealthy)
Recovery
Day 7
R2
SLO
40 nM
Day 8
10% cell survival
(A > B)
GeCKO-A
Recovery
Day 10
R3-A
SLO
100 nM
Day 11
1% cell survival
Recovery
Day 12
Recovery
GeCKO-B
Recovery
Recovery
R3-B
SLO
100 nM
5% cell survival
Recovery



PCR for sgRNA amplicon enrichment



All sgRNA sequences have universal 3’ and 5’ flanking sequence

Conclusions

- CRISPR/Cas9 genome-wide library screening is advantageous over existing techniques
- More precise (less off-targets), easy to use
- Applicable to multiple areas of research (drug discovery, signalling, pathogenesis, immunology)
- Allows identification of bacterial toxins, viral receptors
- Allows discovery of novel drug targets for new therapeutic drug design

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  - Meredith Benson PhD
  - Nicola Lynskey PhD
- Dong Lab
  - Min Dong PhD
  - Songhai Tian PhD
- Bioinformatics
  - Joann Arce PhD
  - George Fagogenis PhD

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