VIRUS-FREE (VF) CRISPR SCREENS

An overview of the current strategies

Technical Journal Club
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Dalila Vena

JBC ARTICLE

Author's Choice

CRISPR/Cas9-mediated gene knockout screens and target identification via whole-genome sequencing uncover host genes required for picornavirus infection

Received for publication, February 21, 2017, and in revised form, April 21, 2017 Published, Papers in Press, April 26, 2017, DOI 10.1074/jbc.M117.782425

Heon Seok Kim[‡§1], Kyungjin Lee[¶1], Sangsu Bae[‖], Jeongbin Park[‖], Chong-Kyo Lee[¶], Meehyein Kim[**], Eunji Kim[‡‡], Minju Kim[‡‡], Seokjoong Kim[‡‡], Chonsaeng Kim[¶2], and Jin-Soo Kim[‡§3]

2017
Transient CRISPR-Cas9 system expression

ARTICLES

nature biotechnology

High-throughput mapping of regulatory DNA

Nisha Rajagopal[1], Sharanya Srinivasan[1,2], Kameron Kooshesh[2,3], Yuchun Guo[1], Matthew D Edwards[1], Budhaditya Banerjee[2], Tahin Syed[1], Bart J M Emons[2,4], David K Gifford[1] & Richard I Sherwood[2]

2016
Construct with dummy gRNA

Cell Reports Methods

CellPress
OPEN ACCESS

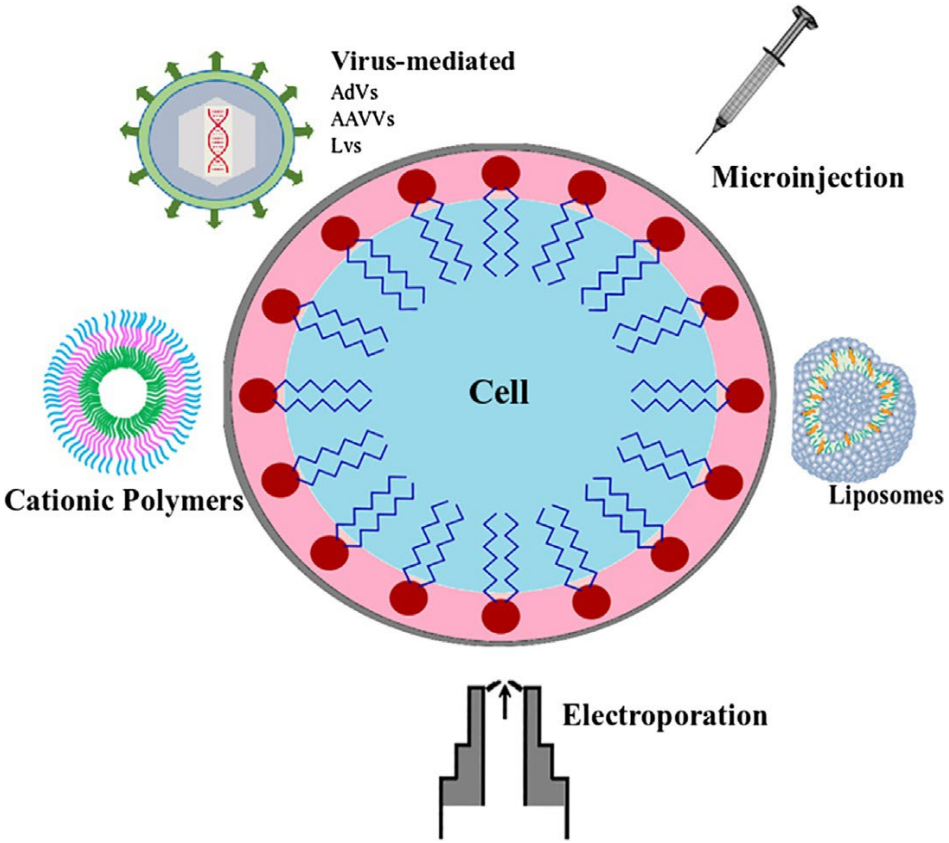
Article

An optimized genome-wide, virus-free CRISPR screen for mammalian cells

Kai Xiong,[1] Karen Julie la Cour Karottki,[1] Hooman Hefzi,[2,5] Songyuan Li,[1] Lise Marie Grav,[1] Shangzhong Li,[2,6] Philipp Spahn,[2,5] Jae Seong Lee,[3] Ildze Ventina,[1] Gyun Min Lee,[1,4] Nathan E. Lewis,[2,5,6] and Lasse Ebdrup Pedersen[1,7,8,*]

August 2021
Recombinase-based approach

Delivery strategies of the CRISPR-Cas9 gene-editing system



Non-viral delivery

Physical delivery (Electroporation, microinjection, etc.)	**Chemical delivery** (Liposomes, Cationic vectors, etc.)	**Viral-mediated delivery** (Adenoviruses, AAV, Lentiviruses)

Chandrasekaran, A.P., et al., 2018. *Different methods of delivering CRISPR/Cas9 into cells*

Table 2
Summary of different delivery systems for CRISPR-Cas9.

Delivery system	Advantages	Disadvantages
Electroporation	• Suitable for any cell type • High transfection efficiency • Can be used *in vitro* and *in vivo* • Suitable for all strategies of CRISPR-Cas9	• Induce significant cell death • Nonspecific transfection
Microinjection	• Highly specific and reproducible • Suitable for all strategies of CRISPR-Cas9	• Induce cell damage • Require a high level of sophistication and manual skills • Low-throughput
iTOP (Induced transduction by osmocytosis and propanebetaine)	• Effective for the delivery of Cas9 protein and sgRNA	• Lower efficiency in primary cells • Not suitable for *in vivo* applications
Mechanical cell deformation	• High delivery efficiency • Low cell death	• Limited to *in vitro* use
Hydrodynamic injection	• Simple and efficient method for *in vivo* transfection in small animals • Highly efficient for transfecting the liver • Suitable for all strategies of CRISPR-Cas9	• May cause cardiac dysfunction, liver expansion, and even animal death • Not suitable for large animals and clinical applications • It is highly efficient for the liver but not for other organs
Lipid nanoparticles	• Easy to prepare • Safe • Suitable for all strategies of CRISPR-Cas9	• Low delivery efficiency
Polymer nanoparticles	• Easy to prepare • Safe • Suitable for all strategies of CRISPR-Cas9	• Low delivery efficiency
Cell-penetrating peptide (CPP) delivery	• Safe • Small in size	• Chemical conjugation is needed
DNA nanostructure	• Controllable size and architecture	• Assembly is complicated • Poor stability of the DNA carrier
Gold nanoparticles	• High delivery efficiency	• Potential toxicity *in vivo* at high concentrations
Adeno-associated virus (AAV)	• High infection efficiency • Safe • Broad cell tropism	• Limited packaging size • Difficulty in production
Lentivirus	• High infection efficiency • Large packing size • Long-term gene expression	• Potential for insertional mutagenesis

Liu, C. et al., 2017. *Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications.*

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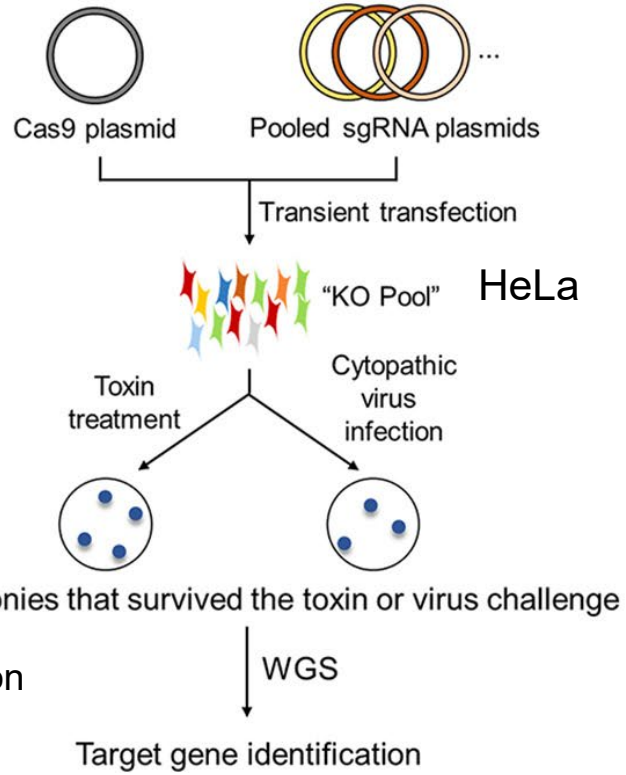
VF approach: Transient CRISPR-Cas9 system expression

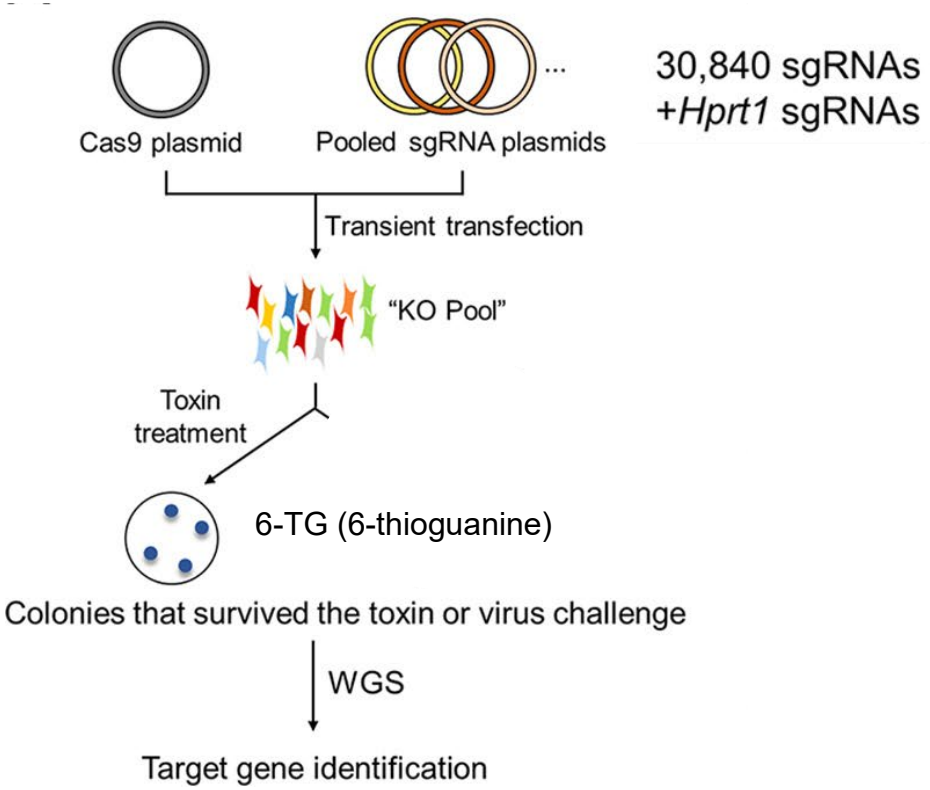


Whole-genome sequencing to detect mutations (rather than statistical estimation through targeted amplicon sequencing)

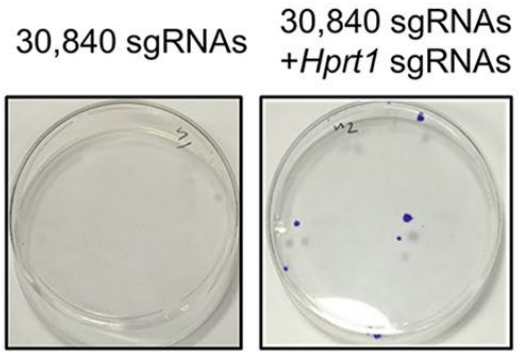
→ Identification of known and novel genes essential for viral infection in human cells

Gene knockout screens using pooled sgRNA libraries

30,840 pairs of individually synthesized oligos to construct genome-scale sgRNA library → 10,280 human genes targeted → 3 sgRNAs per gene



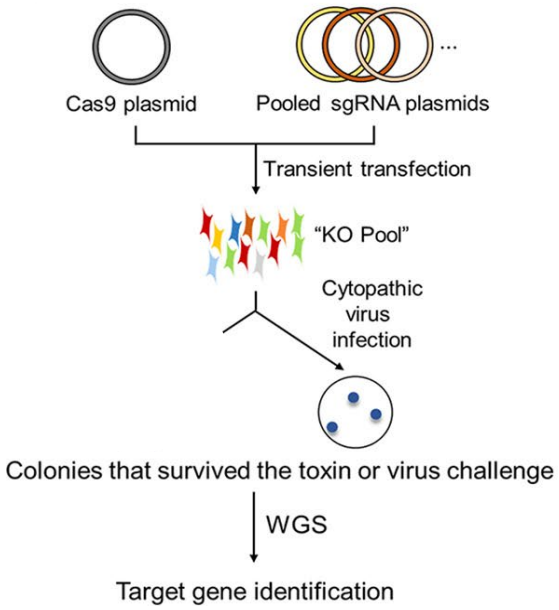
Hprt1:hypoxanthine phosphoribosyl transferase 1
- purine salvage pathway
- converts 6-TG (6-thioguanine) into an active compound that can kill cells



Sanger sequencing → each resistant colony had mutations at sgRNA target

An sgRNA plasmid in a pool of tens of thousands of sgRNAs can still direct Cas9 to induce complete KO of a target gene in human cell lines

Pooled sgRNA screens for enterovirus EV-D68



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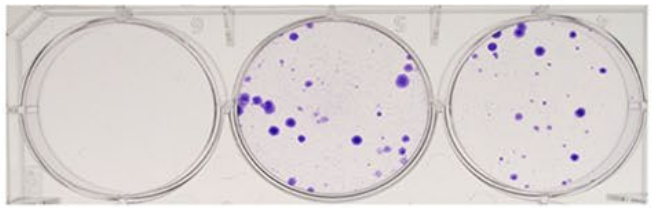
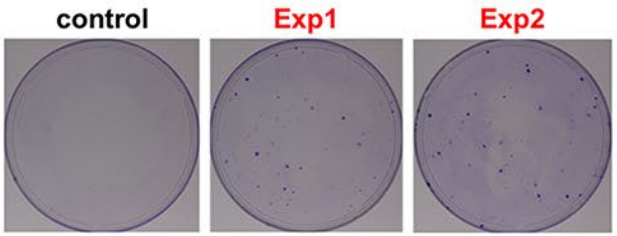


C

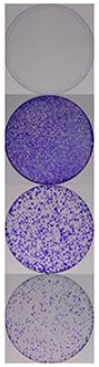
Gene	R68-1	Gene	R68-2
ST3GAL4	Δ10 / Δ1075	ST3GAL4	Δ5 / Δ11
DGKA	+ / ins1	CARS2	+ / ins1
PROK1	+ / ins1	PCDHA8	+ / ins1

ST3GAL4: ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 4

Dropout library
in which the three sgRNAs specific to ST3GAL4 were excluded



Gene	R68-3	Gene	R68-6
MGAT5	Δ2 / Δ5 / Δ30	COG5	ins1 / Δ143
FUT3	+ / Large del	BAI2	+ / Δ6
		SYT4	+ / ins1
Gene	R68-4	Gene	R68-5
COG1	ins1	COG1	Δ4
TRIP12	+ / Δ2	CREBL3L3	+ / Δ12
ZNF648	+ / Δ4	FANCA	+ / ins1
SLC16A13	+ / ins1 / ins1	PEF1	+ / ins1
ARHGAP31	+ / Δ22 / Δ8, ins8	TK2	+ / Δ29
TOP1	+ / ins1		



Mock

MGAT5 mannosyl (α-1,6-)-glycoprotein B-1,6-N-acetyl-glucosaminyltransferase

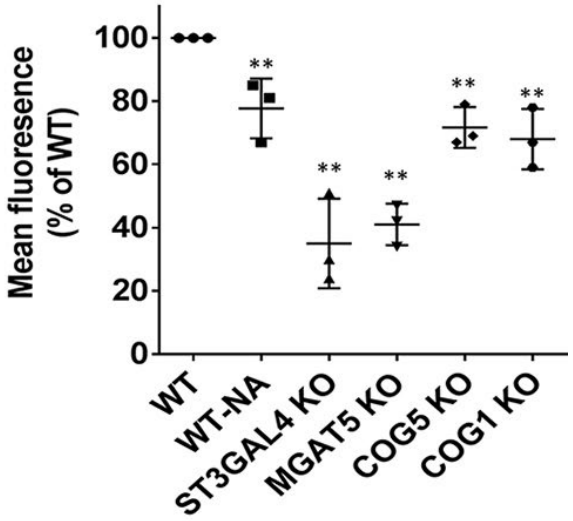
COG1 component of oligomeric Golgi complex 1

COG5 component of oligomeric Golgi complex 5

Hits characterization

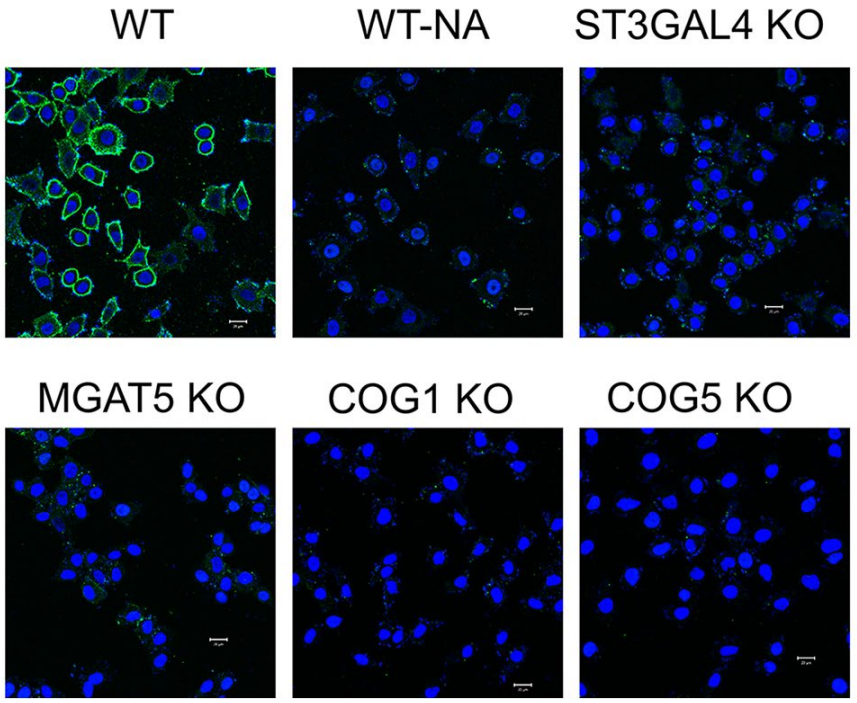
EV-D68 entry into cells is dependent on cell-surface sialic acid
ST3GAL4 and *MGAT5* genes as essential for EV-D68 infection via their role in sialic acid conjugation in the Golgi
→ *COG1* and *COG5* genes associated with sialic acid conjugation?

Flow cytometry
Surface sialic acid presentation



Fluorescein-labeled Maackia amurensis lectin I (MALI), which binds selectively to 2,3-linked sialic acid

WT-NA: neuraminidase, which removes sialic acid residues from glycoproteins on the cell surface



DAPI
Green: anti-enterovirus D68 VP1 antibody

COG1 and COG5 play an important role in presenting sialic acid on the cell surface and disruption of these genes leads to resistance to EVD68 entry into cells

Advantages

- Transient transfection of sgRNA and Cas9 plasmids into human cells gives rise to high-level expression of these components, resulting in efficient disruption of the target genes
- Few days to disrupt genes compared to the 2–3 weeks required by lentiviral sgRNA

Disadvantages

- Cell lines in which transfection with cationic lipids is not efficient
- Mutation-phenotype reliable link: difficult for genomically unstable cell lines (HEK, HeLa, CHO)
- Genes exerting mild phenotypes could be missed due to the stringent selection performed in this experiment in comparison with lentivirus-based screening (high depth sequencing required)

ARTICLES

nature biotechnology

High-throughput mapping of regulatory DNA

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VF approach: replacing an integrated dummy gRNA with a pooled library by homologous recombination (electroporation)

MERA: Multiplexed Editing Regulatory assay

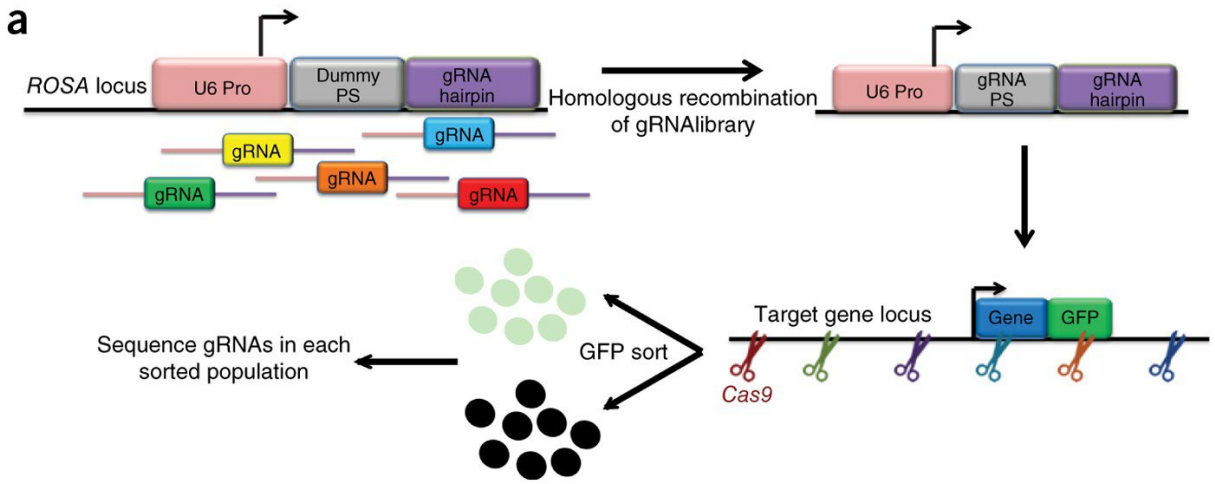
- High-throughput CRISPR-Cas9-based approach that tiles thousands of mutations across *cis*-regulatory regions
- Knock-in GFP reporters to read out gene activity (four embrionic stem cell-specific genes)

→ Identification and quantification of the effects of *cis*-regulatory DNA on gene expression

Developing the MERA assay

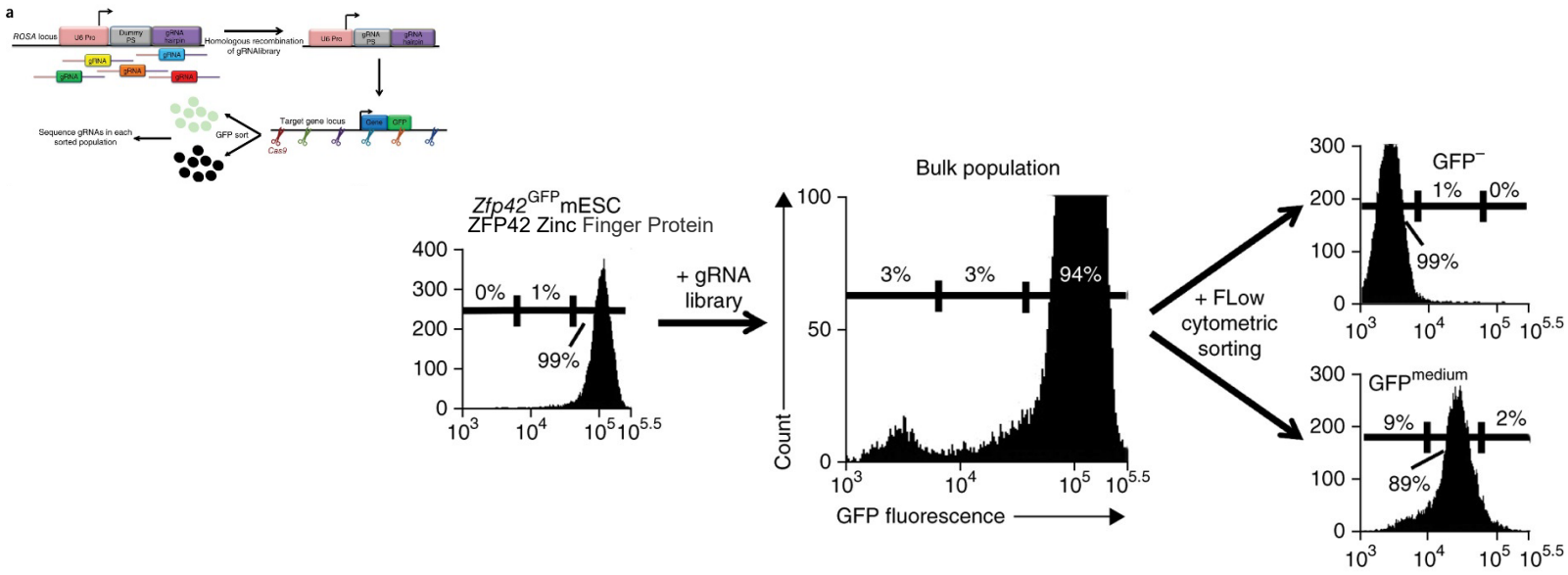
- Crucial to have only one gRNA per cell
- Each of the 4 genes requires a different gRNA library (3,908 gRNAs tiling *cis*-regulatory regions)

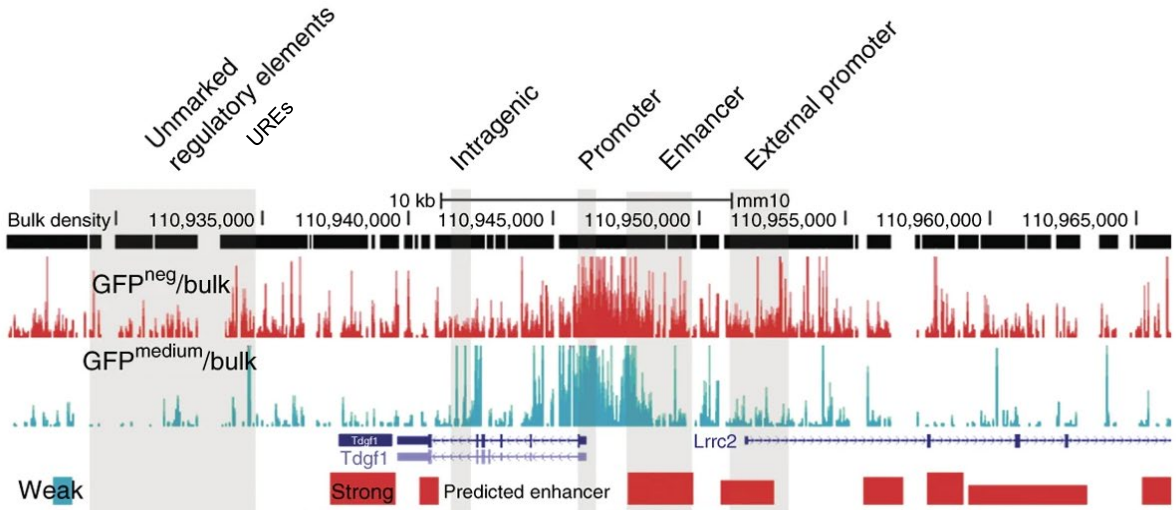
→ Lentiviral library production would be time-consuming and expensive



- Integration of a single copy of the gRNA expression construct (U6 promoter driving expression of a dummy gRNA hairpin) into the ROSA locus of mouse embryonic stem cells
- The gRNA library has homology arms to the expression construct
- CRISPR-Cas9 mediated Homologous Recombination to replace the dummy gRNA with the library, such that each cells receives a single gRNA (30% efficiency)

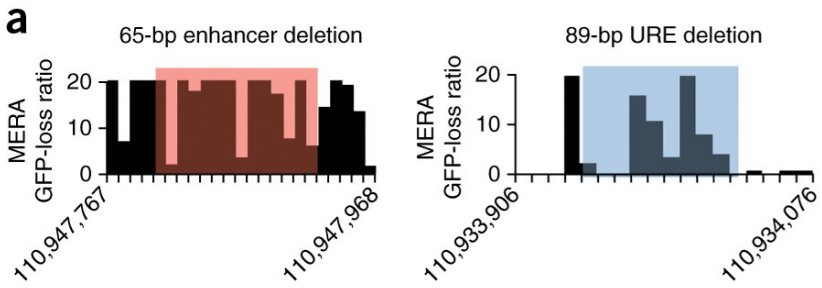
MERA screens identify required regulatory regions



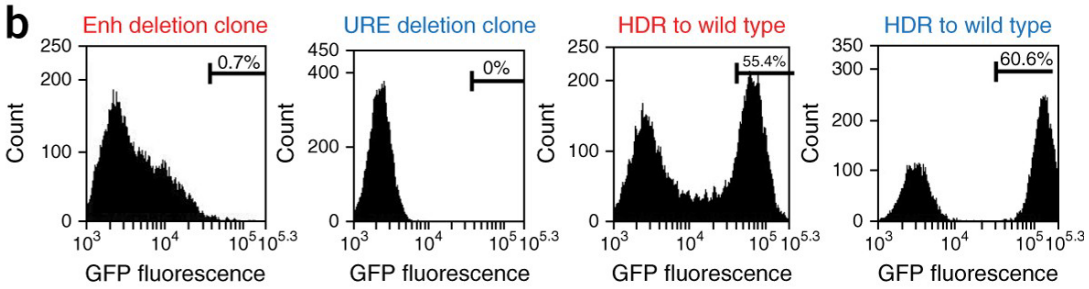


Tdgf1
Teratocarcinoma-Derived Growth Factor 1

Hits validation

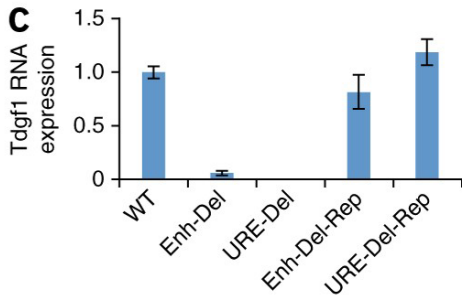


gRNAs flanking two regions (enhancer and URE) predicted to induce GFP loss by the MERA screen



GFP^{neg} contain the deletion genotype

HDR to wt: partial recover of GFP expression



HDR to wt: recover of Tdgf1 expression

This robust and straightforward relationship between local genotype and GFP expression provides compelling evidence that the local DNA sequence at a URE is required for Tdgf1 expression.

Advantages

- Single gRNA integration
- Reduced time, cost and effort compared to lentiviral library production

Disadvantages

- Lower efficiency (30%) compared to lentiviral libraries
- Large number of cells needed
- Not ideal for cells with limited homologous recombination

Article

An optimized genome-wide, virus-free CRISPR screen for mammalian cells

Kai Xiong,[1] Karen Julie la Cour Karottki,[1] Hooman Hefzi,[2,5] Songyuan Li,[1] Lise Marie Grav,[1] Shangzhong Li,[2,6] Philipp Spahn,[2,5] Jae Seong Lee,[3] Ildze Ventina,[1] Gyun Min Lee,[1,4] Nathan E. Lewis,[2,5,6] and Lasse Ebdrup Pedersen[1,7,8,*]

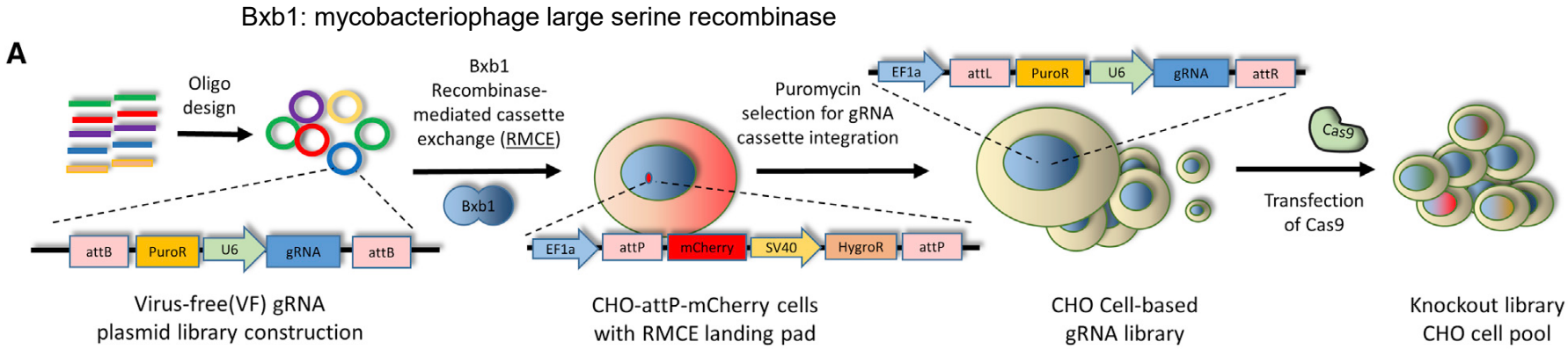
MOTIVATION Although lentivirus-based delivery of genome-wide CRISPR screen components has proven successful, there are situations in, e.g., industry and hospitals where working with live viruses is difficult or simply not an option. For those situations we have developed an alternative to virus-based, genome-wide CRISPR screens that retains compatibility with the software tools developed for analyzing the results, takes a similar amount of time, and offers improved signal-to-noise ratio.

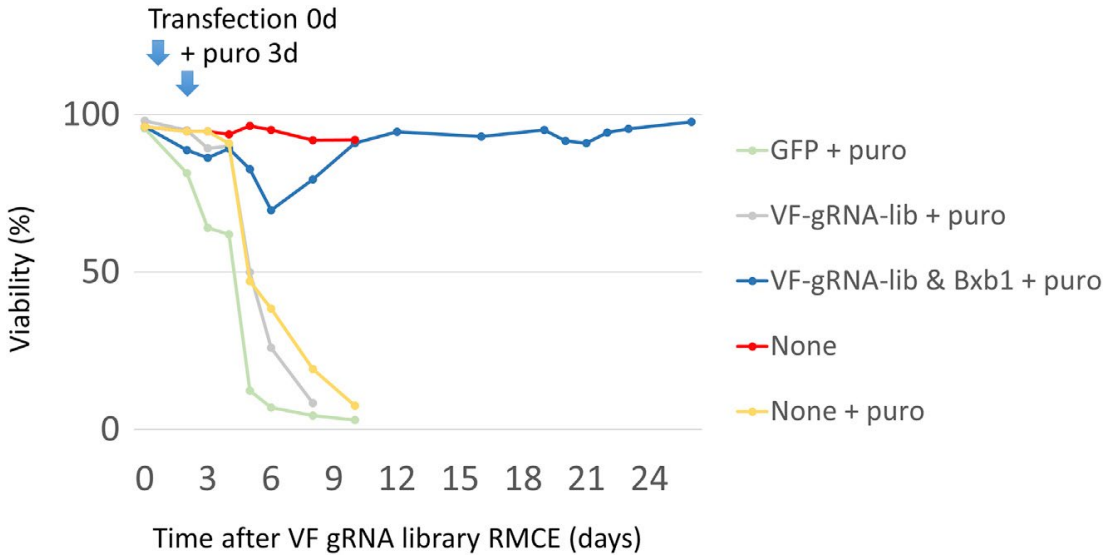
VF approach: Recombinase-based method (transfection)

CHO: Chinese Hamster Ovary cells
75,488 gRNAs targeting 15,028 expressed genes

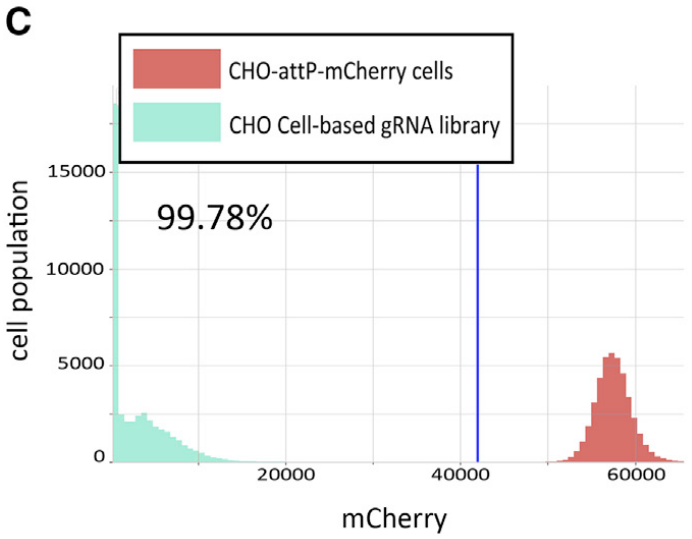
→ Negative selection (cell proliferation) and positive selection (improve survivability in stress context) screen

Design of the VF, genome-wide CRISPR pooled screening platform



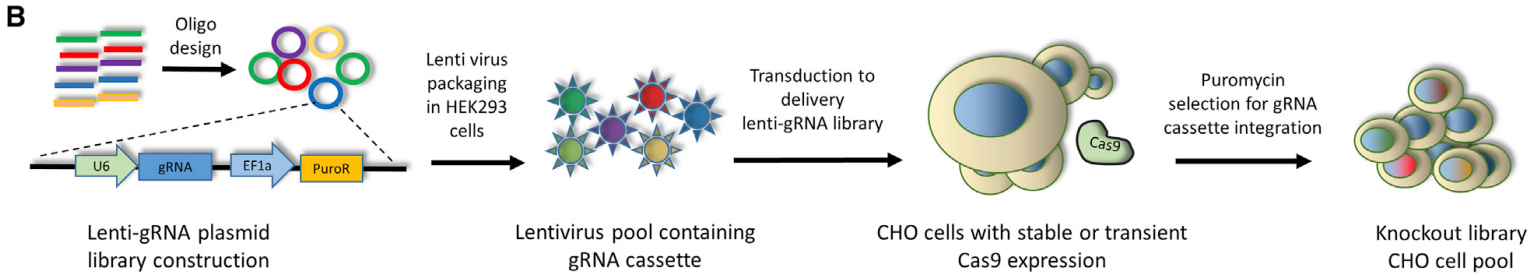


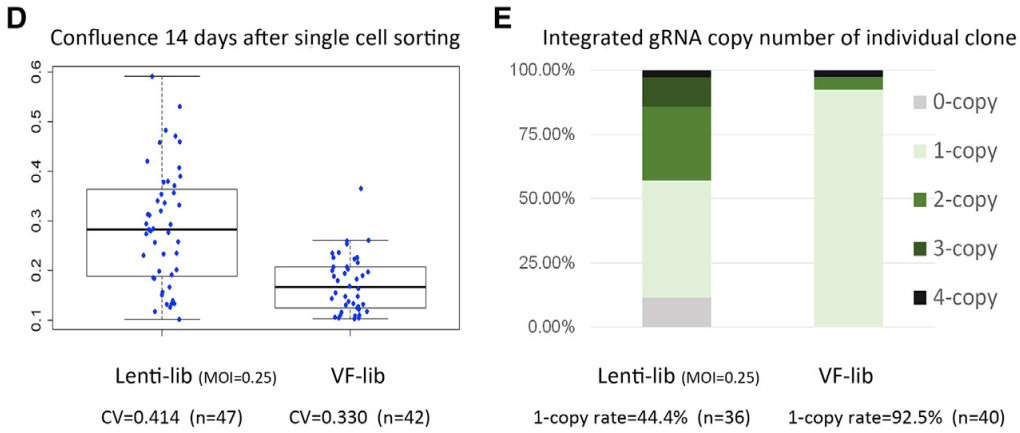
The RMCE-positive cells were then further enriched by puromycin selection for 14–20 days

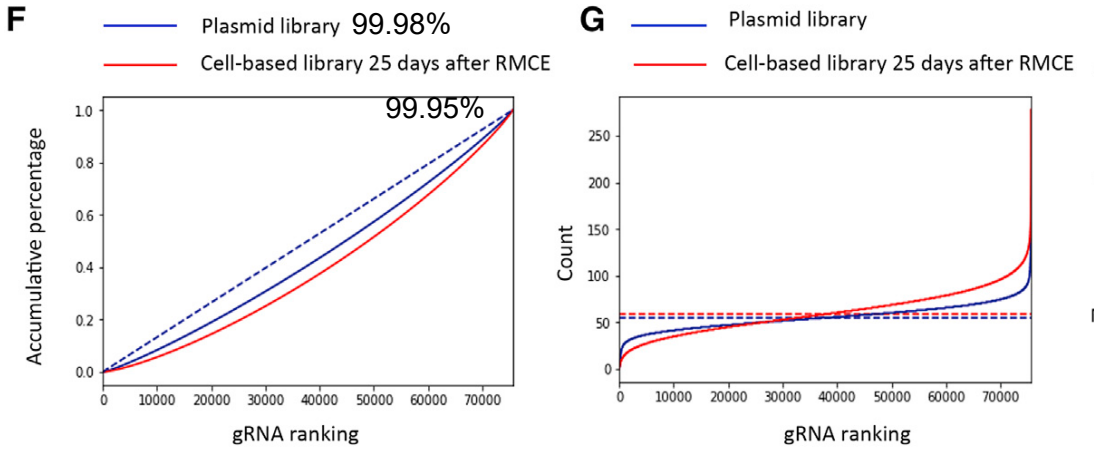


Twenty-five days after gRNA RMCE, the RMCE cell population was fully enriched

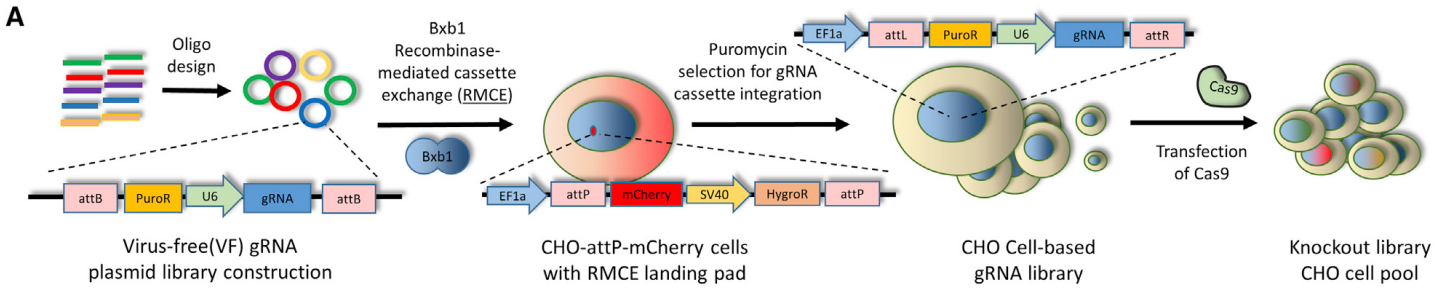
Precise integration of gRNA expression cassette results in lower clonal variance in cell population and achieves high library coverage



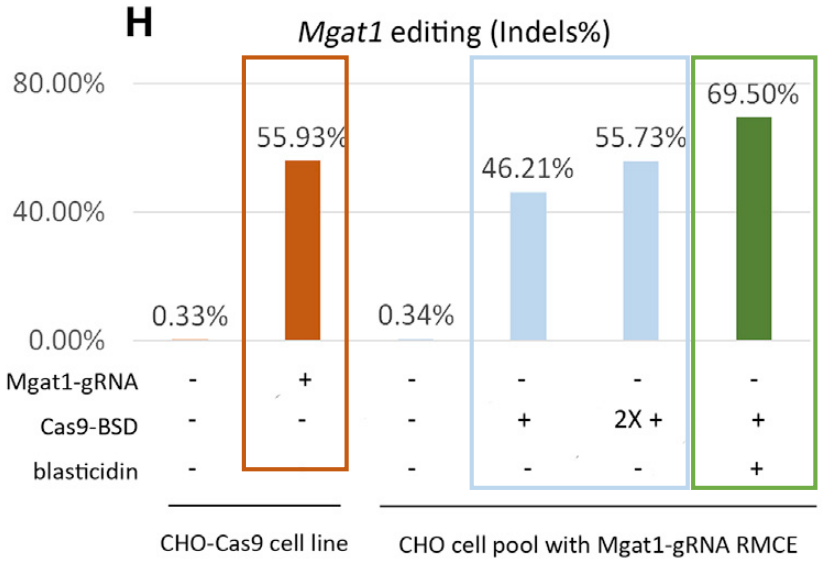




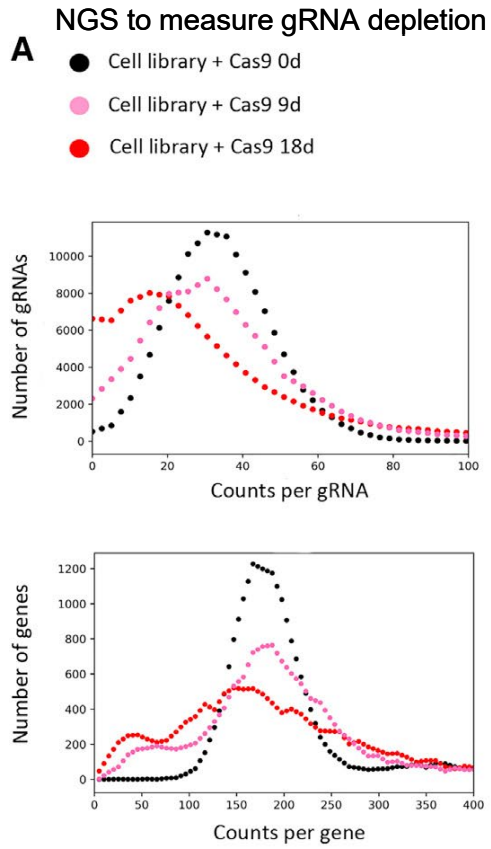
Enriching for Cas9-transfected cells results in high gene editing rate

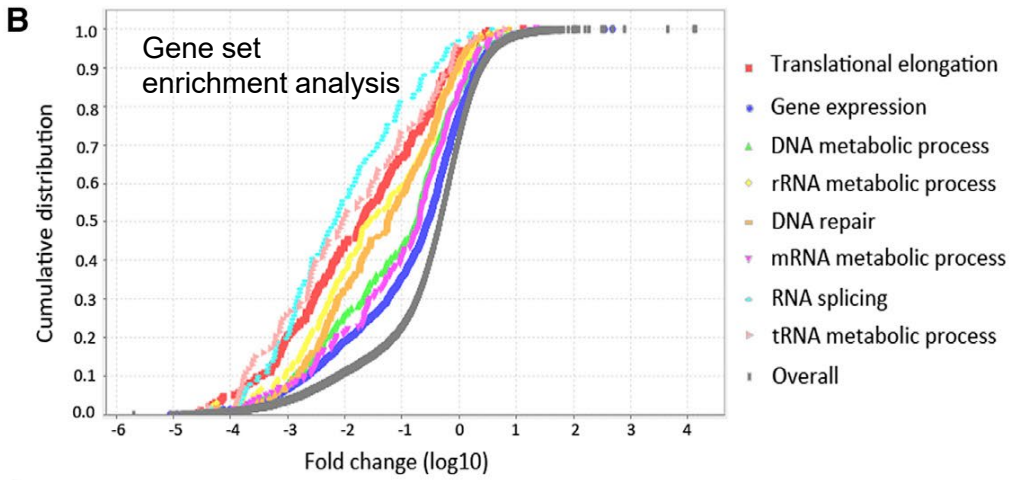


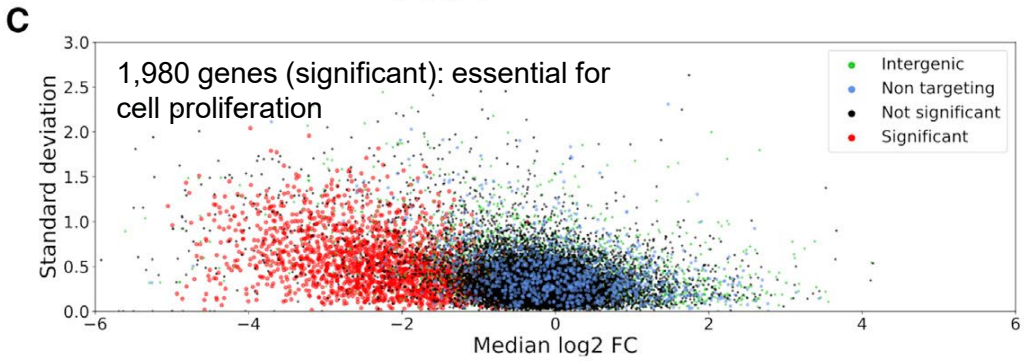
1. Blasticidin-selected Cas9-transfected CHO cells
2. Cas9-BSD without blasticidin selection (1 or 2 rounds)
3. Transient transfection of the gRNA into a CHO cell line with constitutive Cas9 expression

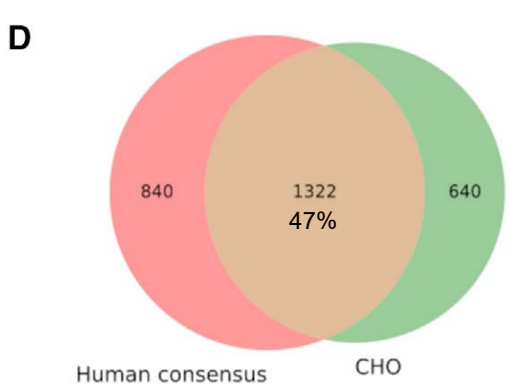


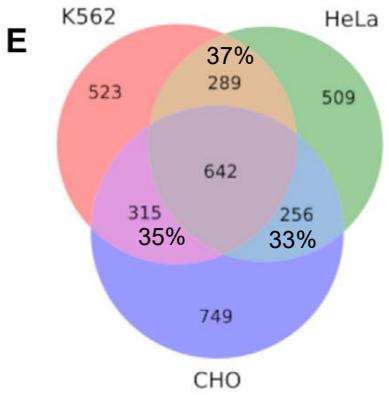
Genes essential for cell proliferation are identified in the VF CRISPR screen

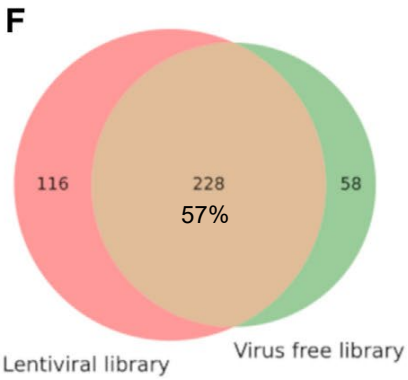








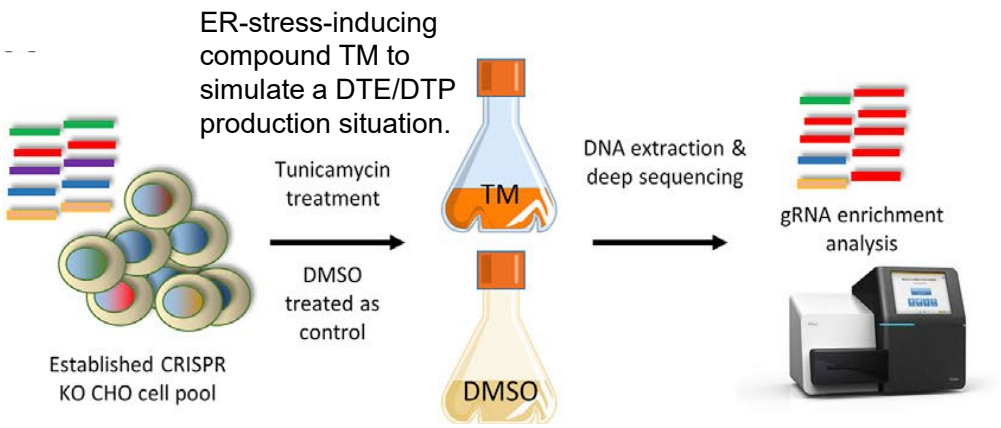


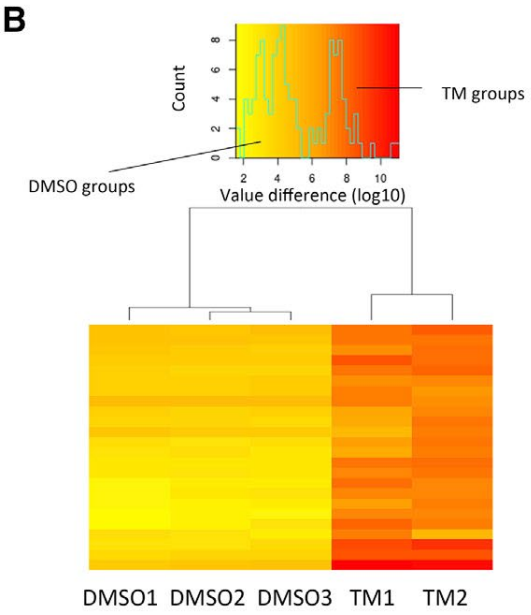


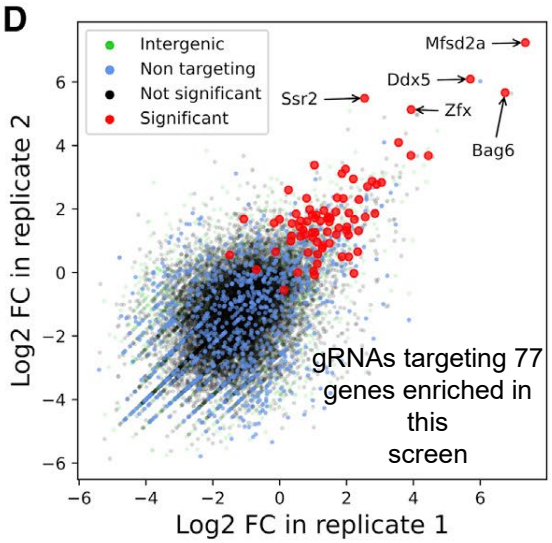
Genes sensitive to induced ER stress are identified in VF CRISPR screen

‘‘difficult-to-express‘‘(DTE)/"difficult-to-produce" (DTP) proteins → unfolding → unfolded protein response (UPR) → apoptosis

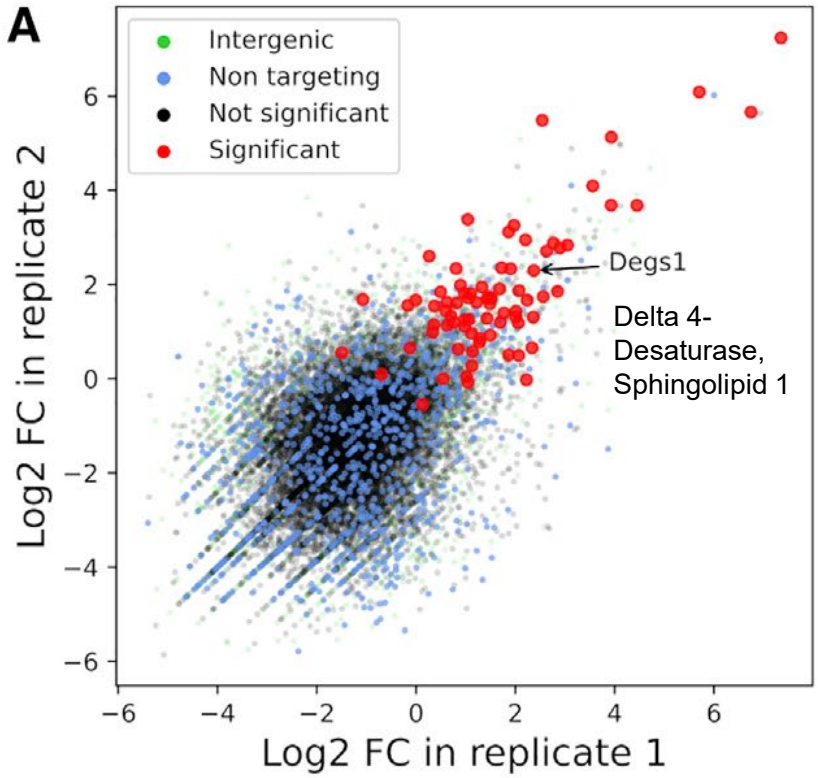
Reducing UPR-mediated ER stress to improve protein productivity?

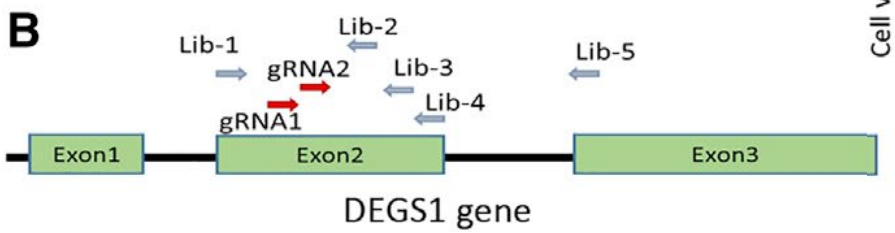


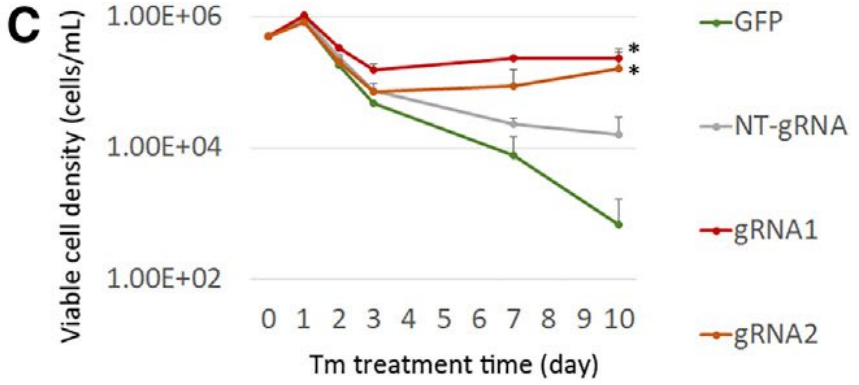


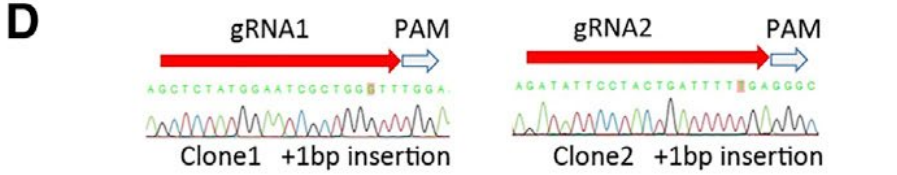


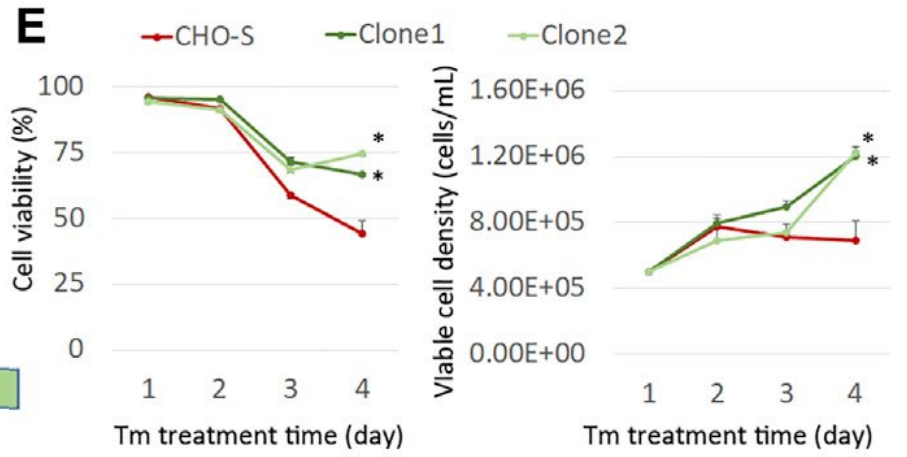
Validation of candidate genes





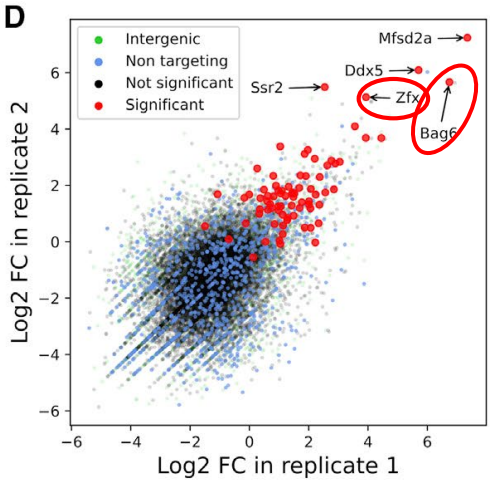




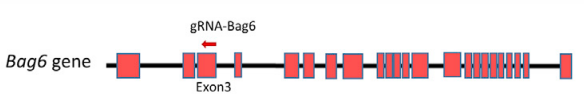


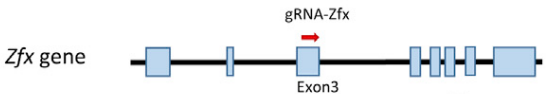
Cas9-CHO cells with stable Cas9 expression were transfected with these two gRNAs, independently. Seven days after transfection, we treated the cell pools with TM (20 ng/mL) for an additional 7 days.

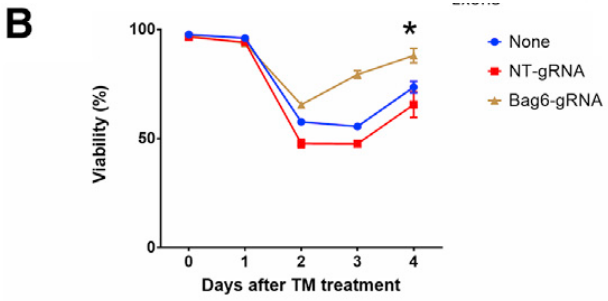
Validation of candidate genes

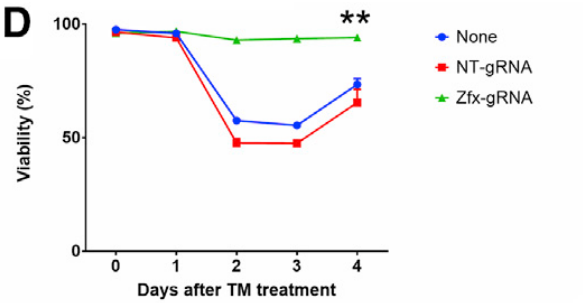


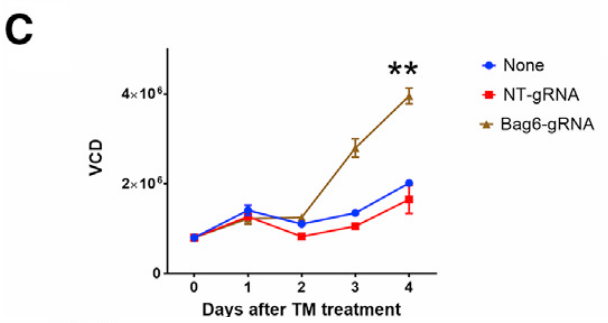
Bag6 (BCL2-Associated Athanogene)
Zfx (Zinc-Finger Protein X-Linked)

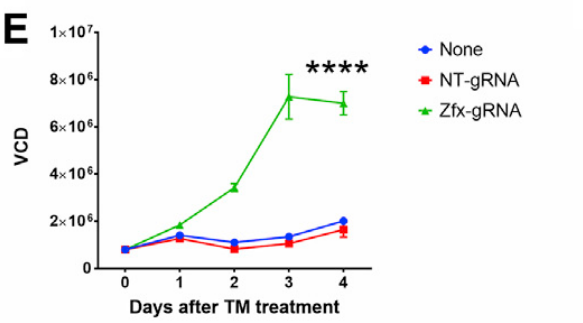


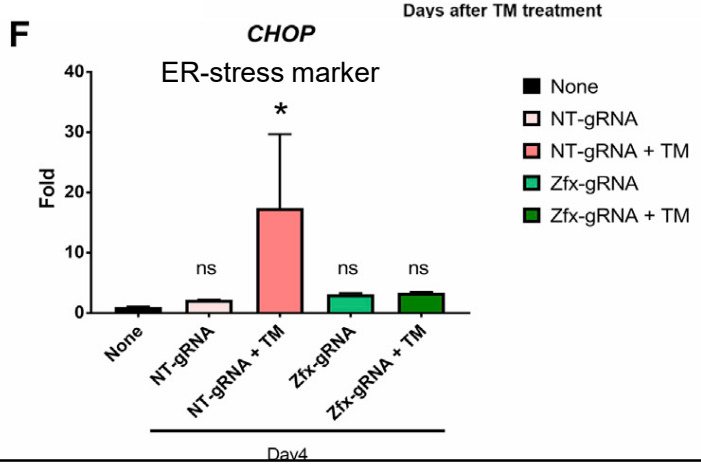


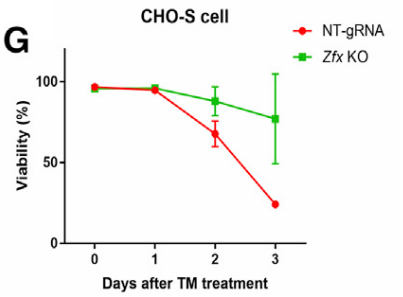












VF CRISPR screen can successfully identify candidate genes via gRNA enrichment analysis

Advantages

- Works with CRISPR screen analysis tools
- Single integration in 92.5% of cells → decreased bias
- Targeted integration

  → decreased clone-to-clone variance compared with random insertion
- Coverage: 99.95% (comparable to lentiviral)
- Stability of the platform→ no cassette loss
- Higher efficiency than paper #2

Limitations

- Pre-engineer cells to have the RMCE landing pad integrated in the genome (primary cells, cells difficult to trasfect)

Thank you for your attention.

Questions?

No?

Great!