Recent advances in CRISPR-Cas9 based genome-wide screen

Technical Journal Club – Special Series on Laboratory Animal Science

Caihong Zhu

05.03.2019

CRISPR screen

Library format

I. Pooled library: cell growth competition (essential genes, synthetic lethality, **selective pressure**, **cell sorting based phenotype**)

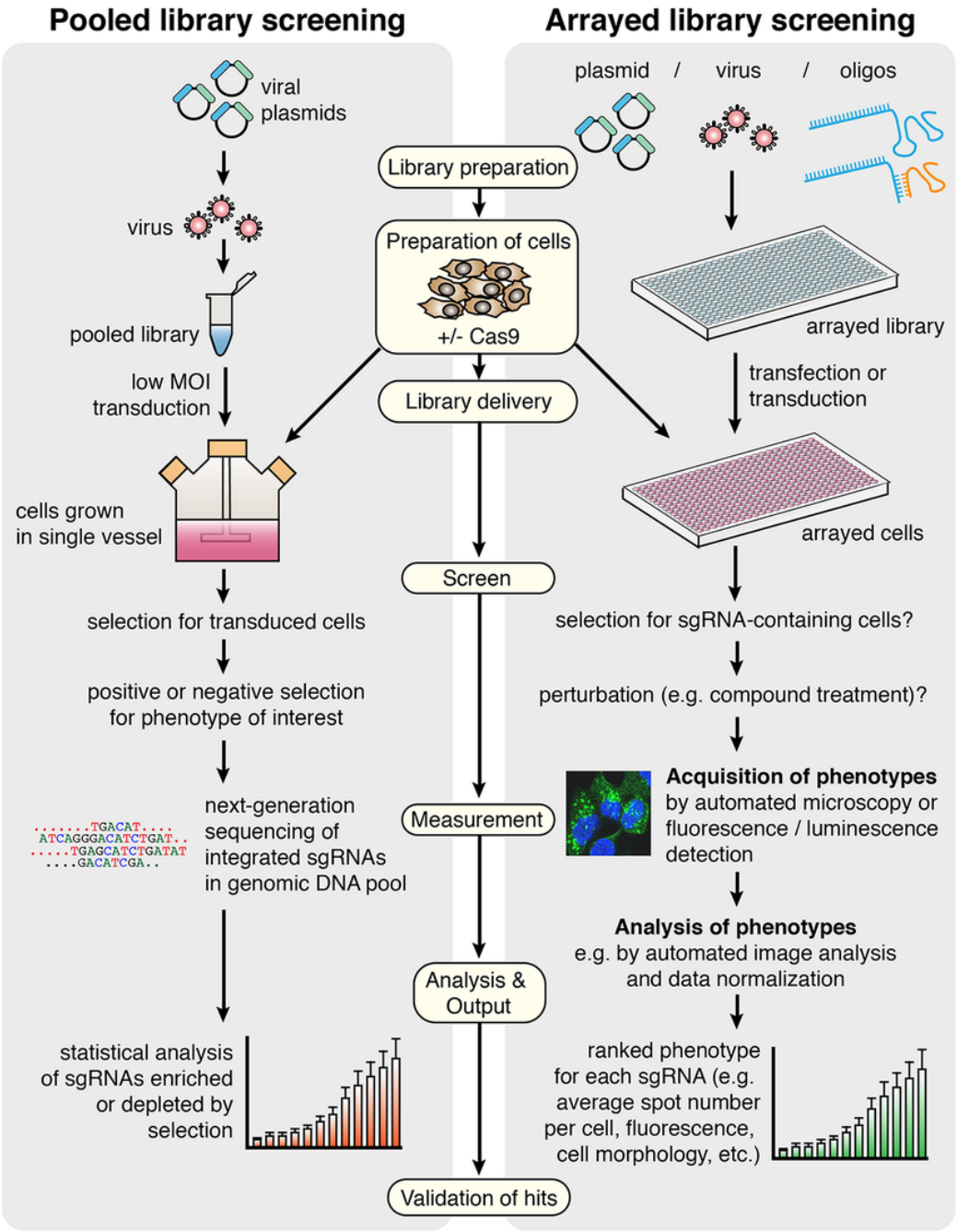
II. Arrayed library: cell morphology, protein translocation within cells, low-level analytes

Editing methods

I. CRISPR-Cas9 KO screen

II. CRISPR-Cas9 activation/inhibition screen

Workflow for screening using CRISPR/Cas9 in pooled versus arrayed approaches



Agrotis & Ketteler, 2015

Science 2014

Genetic Screens in Human Cells Using the CRISPR-Cas9 System

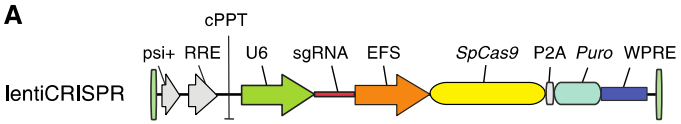
Tim Wang,[1,2,3,4] Jenny J. Wei,[1,2] David M. Sabatini,[1,2,3,4,5]*† Eric S. Lander[1,3,6]*†

- Human KBM7 CML cell line (near haploid) and HL60 (pseudo-diploid)
- 73151sgRNAs/7114 human genes
- 6-thioguianine (6-TG), mismatch repair (MMR) – positive selection;
- Etoposide, poisons DNA topoisomerase IIA - positive selection;
- Essential gene for cell proliferation – negative selection

Science 2014

Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells

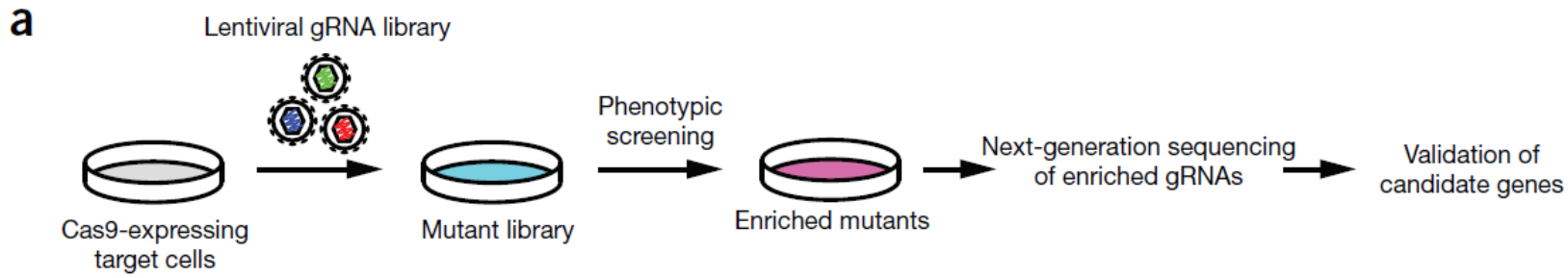
Ophir Shalem,[1,2]* Neville E. Sanjana,[1,2]* Ella Hartenian,[1] Xi Shi,[1,3]
David A. Scott,[1,2] Tarjei S. Mikkelsen,[1] Dirk Heckl,[4] Benjamin L. Ebert,[4] David E. Root,[1]
John G. Doench,[1] Feng Zhang[1,2]†



- Human melanoma cell line A375 and human stem cell line HUES62
- 64751sgRNAs/18080 human genes
- Essential genes
- BRAF protein kinase inhibitor vemurafenib (PLX).

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 Tan[1], Martin Del Castillo Velasco-Herrera[1] & Kosuke Yusa[1]



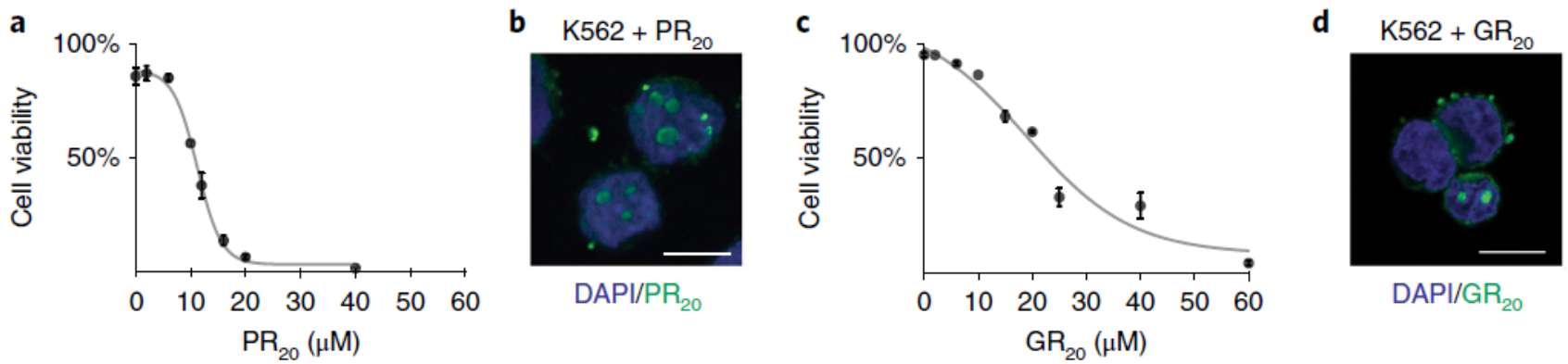
- Mouse ESC cell line stably express Cas9
- 87897sgRNAs/19150 mouse genes
- Essential genes for ESC proliferation (Nanog, Pou5f1) -depleted
- Alpha-toxin and 6-thiouanine: 27 known genes and 4 novel hits (**4** out of **7** alpha-toxin hits, **0** out of **3** 6-TG hits).

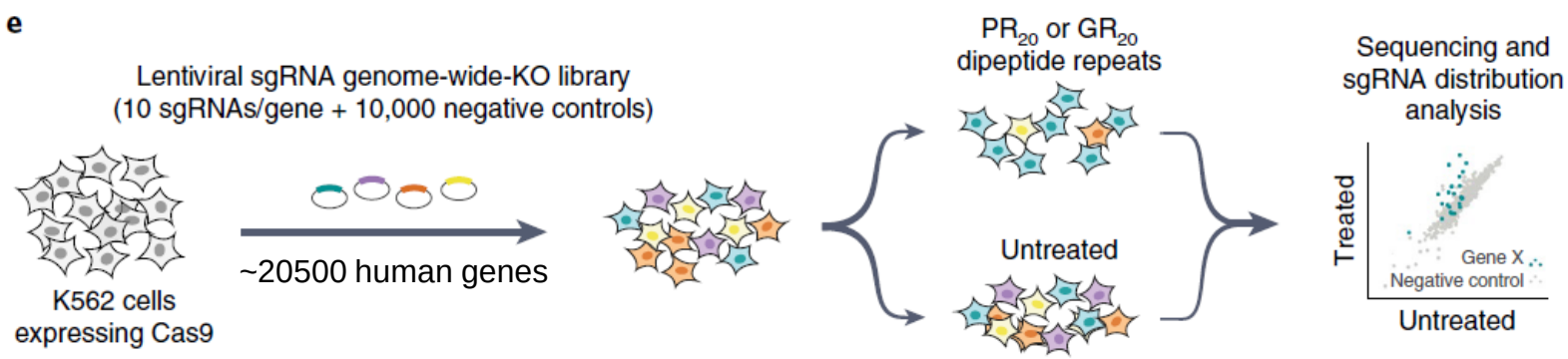
nature biotechnology 2014

CRISPR–Cas9 screens in human cells and primary neurons identify modifiers of *C9ORF72* dipeptide-repeat-protein toxicity

nature genetics 2018

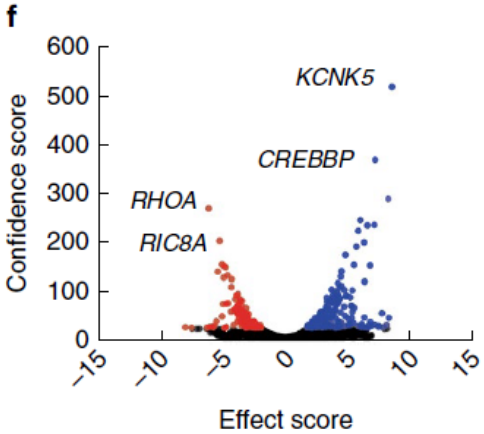
Nicholas J. Kramer[1,2,6], Michael S. Haney[1,6], David W. Morgens[1], Ana Jovičić[1,5], Julien Couthouis[1], Amy Li[1], James Ousey[1], Rosanna Ma[1], Gregor Bieri[1,2], C. Kimberly Tsui[1], Yingxiao Shi[3], Nicholas T. Hertz[4], Marc Tessier-Lavigne[4], Justin K. Ichida[3], Michael C. Bassik[1*] and Aaron D. Gitler[1*]

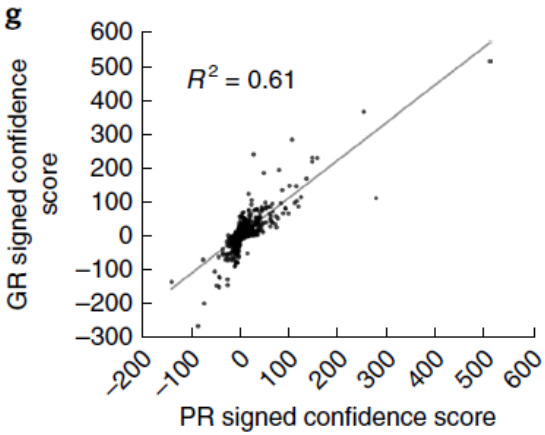


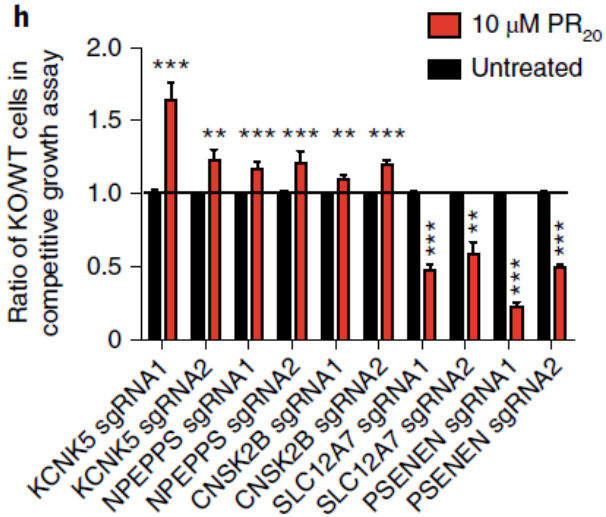


Human myelogenous leukemia cell line

CRISPR KO screen on K562 cells



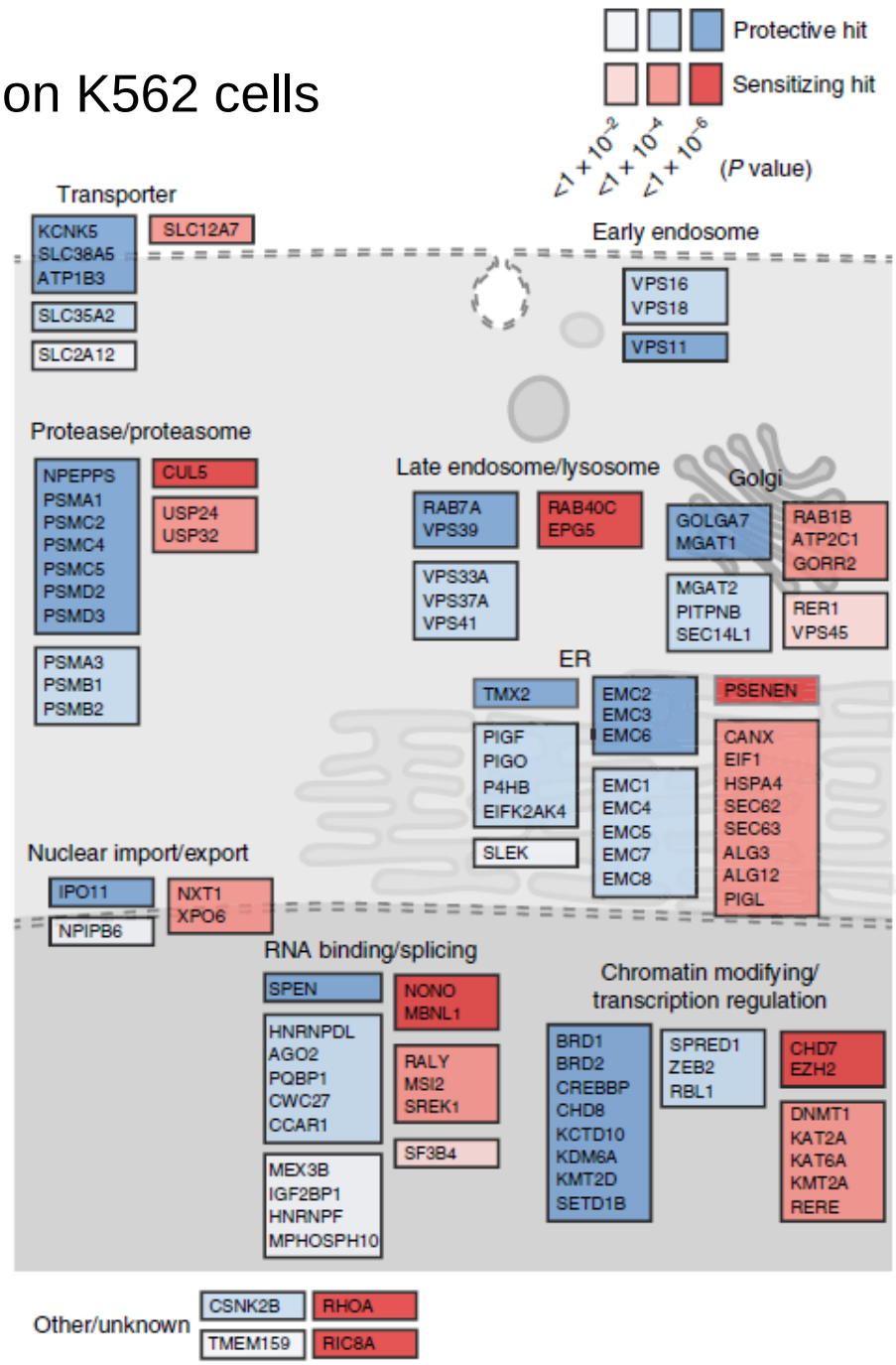




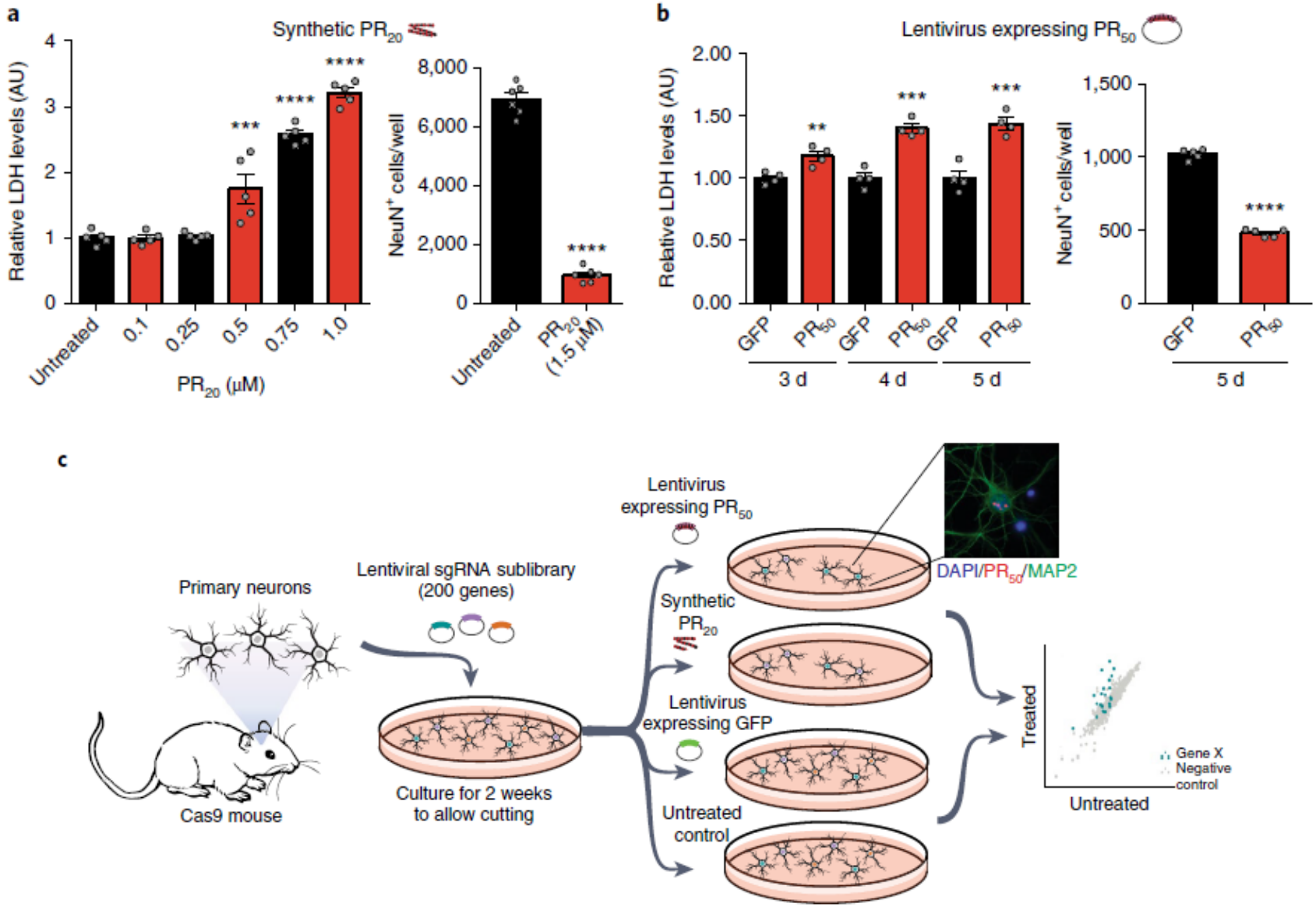
215 modifier for PR_{20}
(387 modifier for GR_{20})

KO (GFP+) + wt (GFP-) cells coculture
Percentage of GFP+ cells

CRISPR KO screen on K562 cells



CRISPR KO screen on primary neurons (200 top hits)



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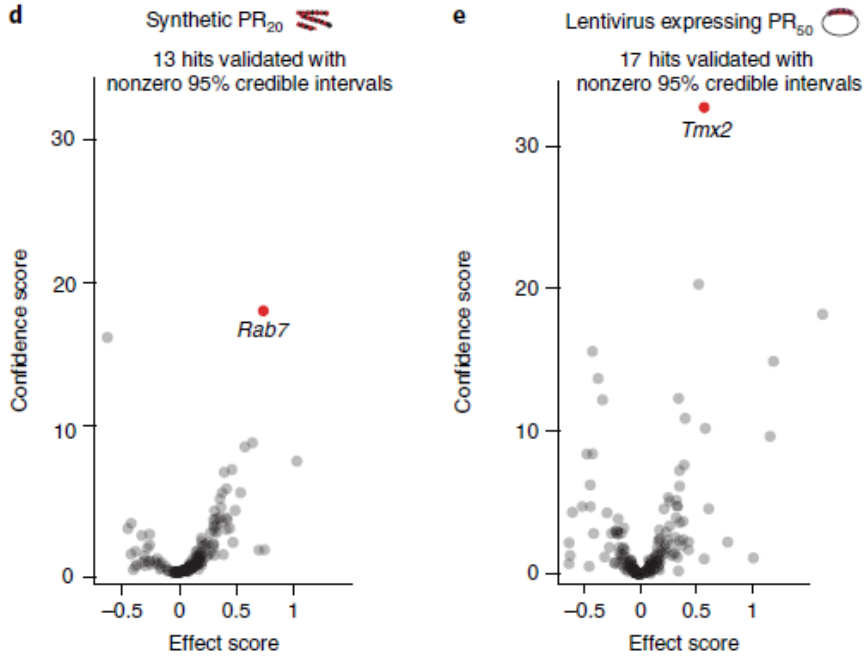
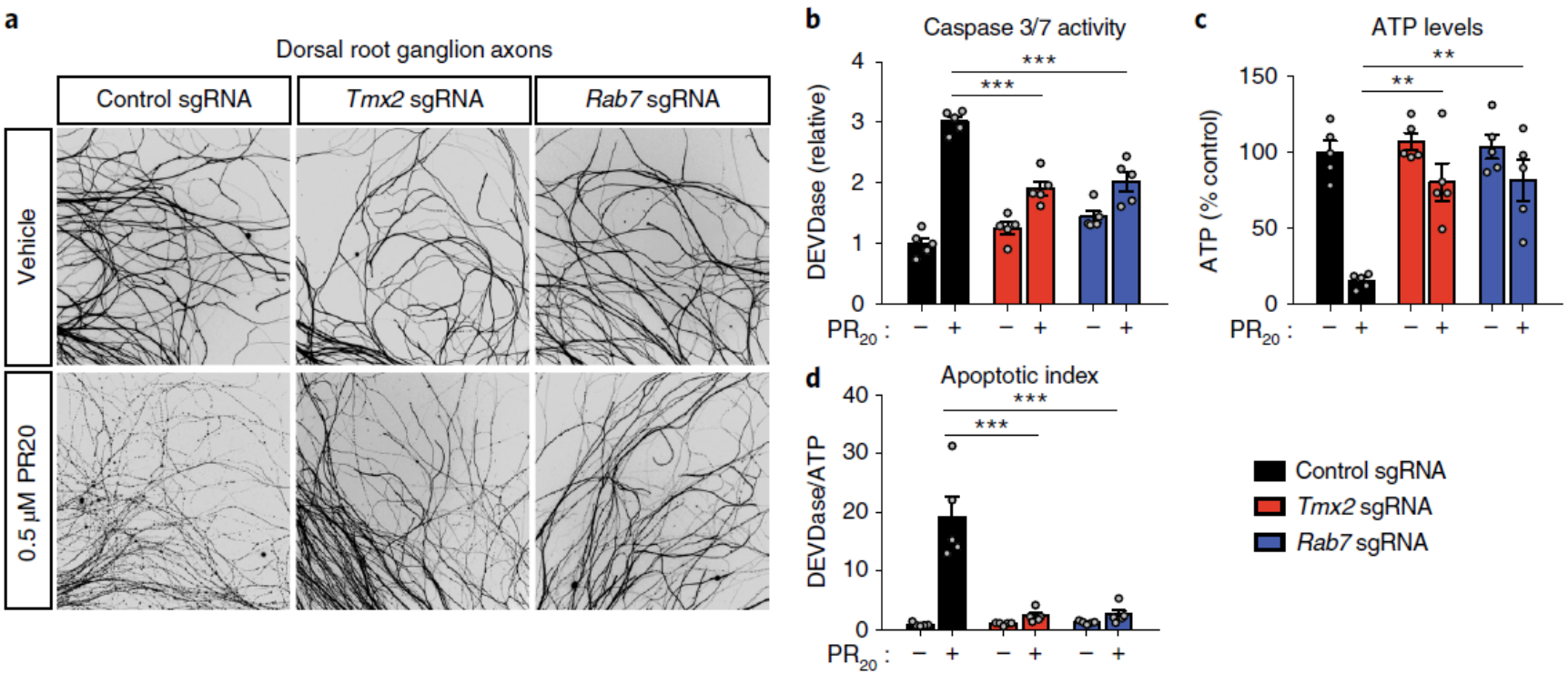


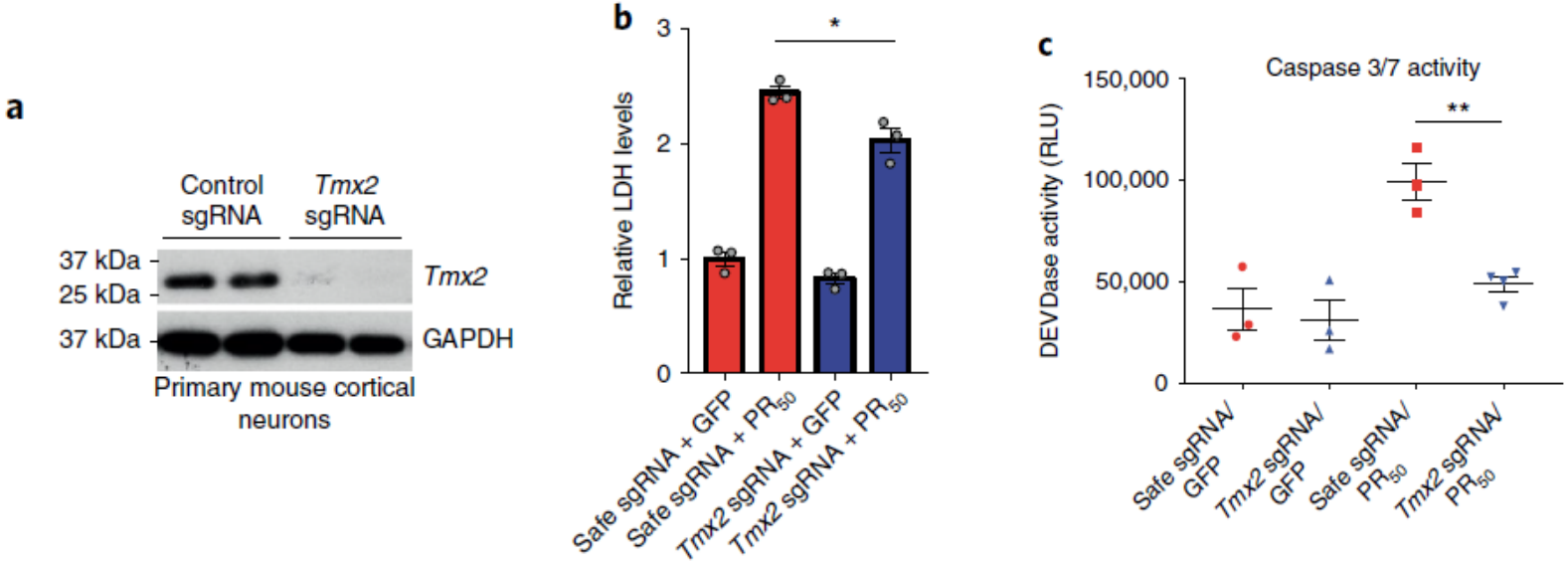
Uptake/trafficking vs. cell toxicity after entry

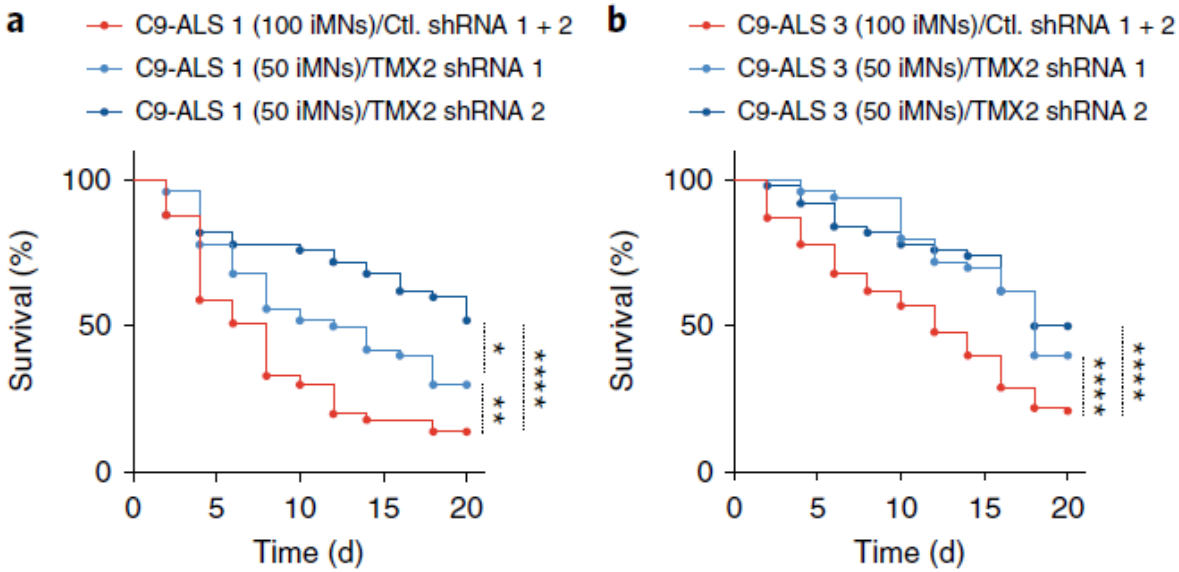
- Rab7: intracellular transport of PR20 is important for the DPR toxicity
- Tmx2: ER resident transmembrane thioredoxin protein. ER stress is involved in DPR induced cell death.

Validation of 2 strongest hits



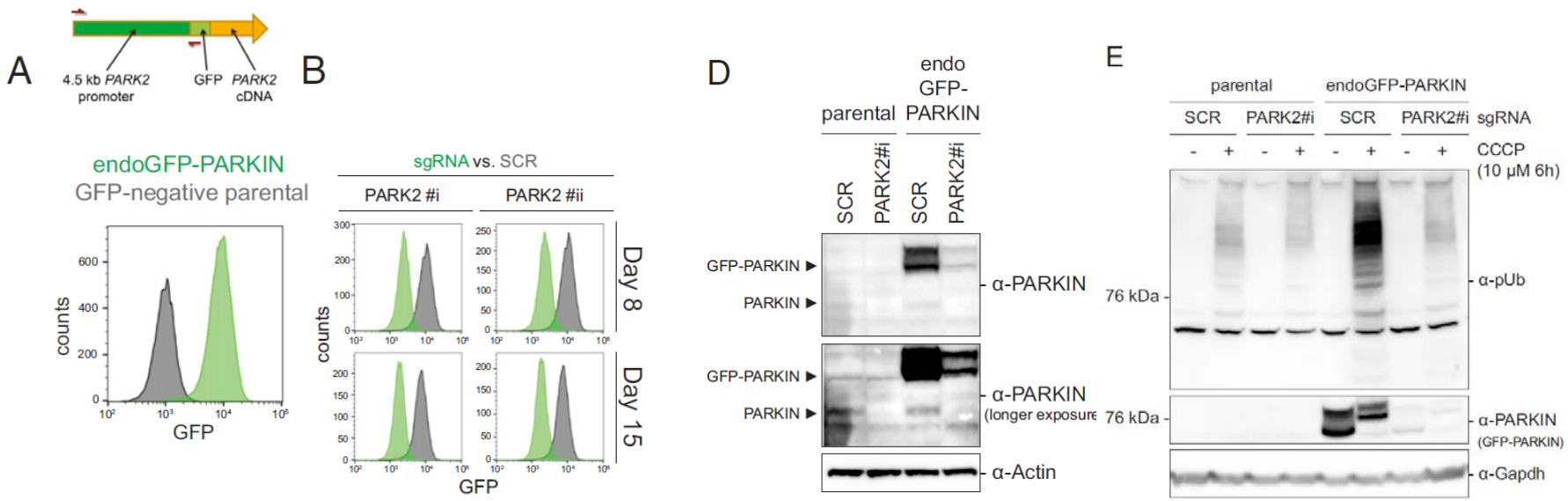
Validation of 2 strongest hits





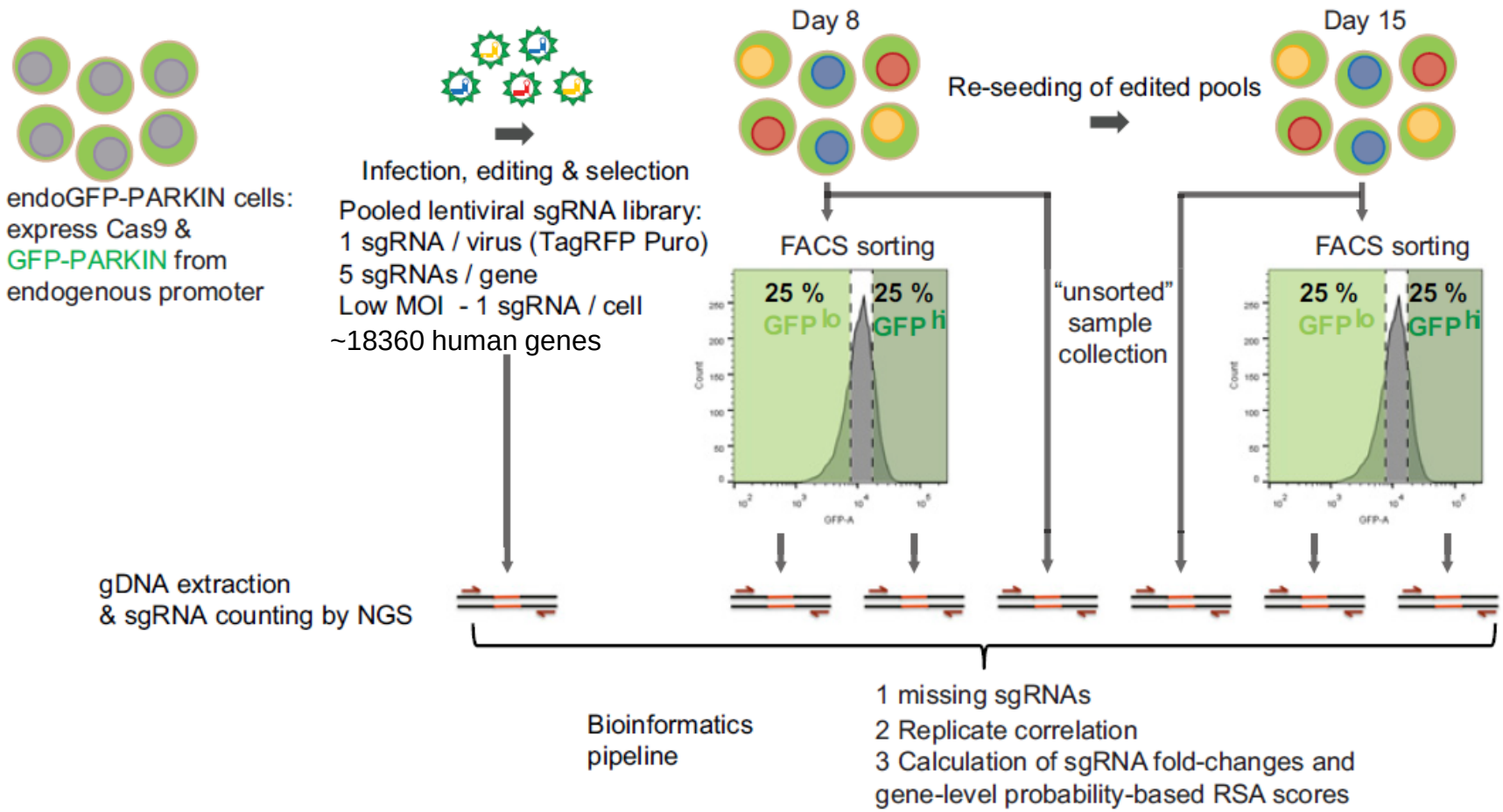
Genome-wide CRISPR screen for PARKIN regulators reveals transcriptional repression as a determinant of mitophagy

Christoph Potting[a], Christophe Crochemore[a], Francesca Moretti[a], Florian Nigsch[a], Isabel Schmidt[a], Carole Manneville[a], Walter Carbone[a], Judith Knehr[a], Rowena DeJesus[b], Alicia Lindeman[b], Rob Maher[b], Carsten Russ[b], Gregory McAllister[b], John S. Reece-Hoyes[b], Gregory R. Hoffman[b], Guglielmo Roma[a], Matthias Müller[a], Andreas W. Sailer[a], and Stephen B. Helliwell[a,1]

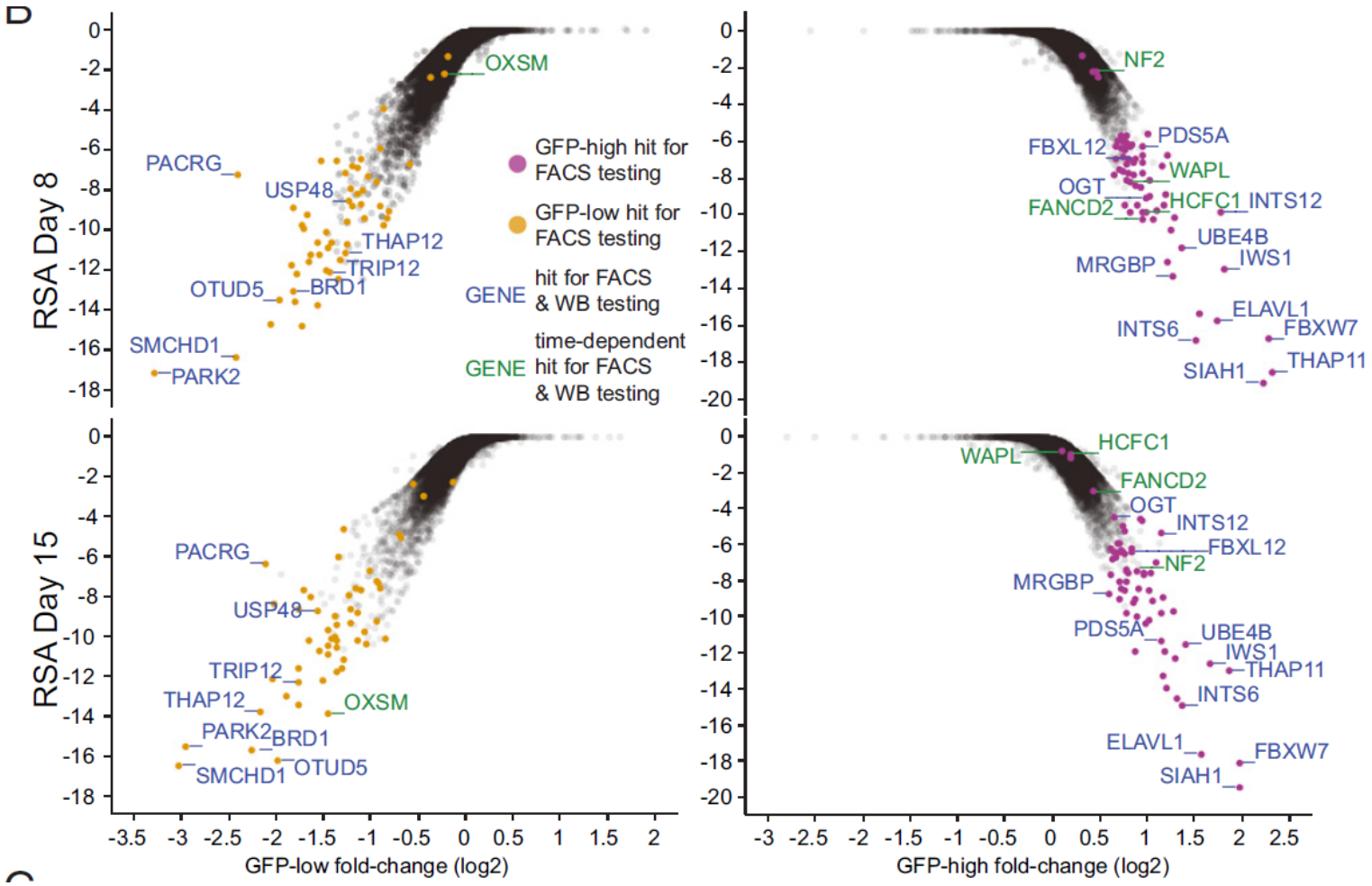


- PARK2-endoGFP-PARKIN
- *PARK2 sgRNA -> GFP?*
- GFP signal correlates with PARKIN expression level
- PARKIN expression level correlates with the pUb accumulation and mitophage

CRISPR KO screen on endoGFP-PARKIN cells

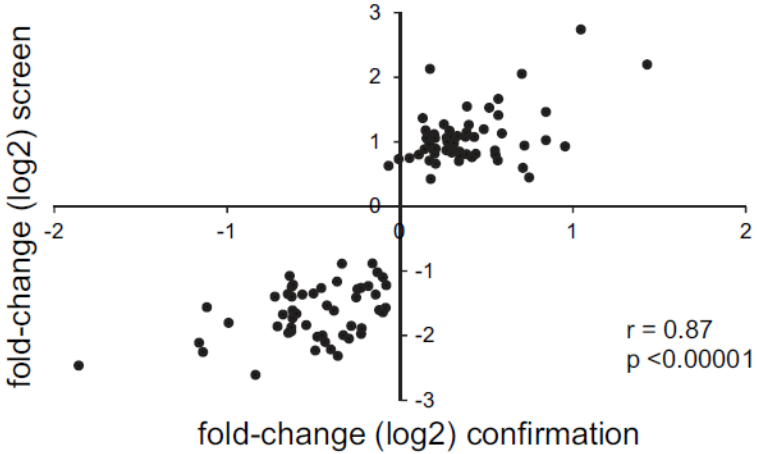


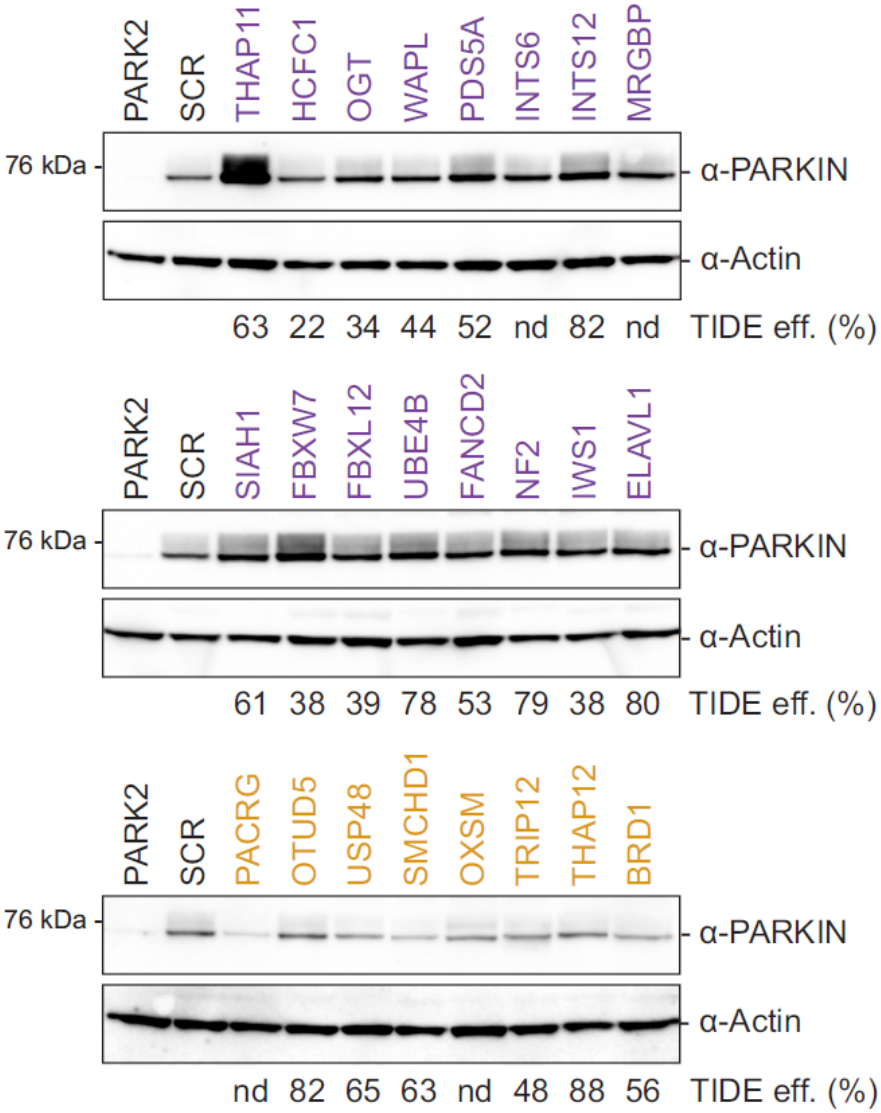
CRISPR KO screen on endoGFP-PARKIN cells



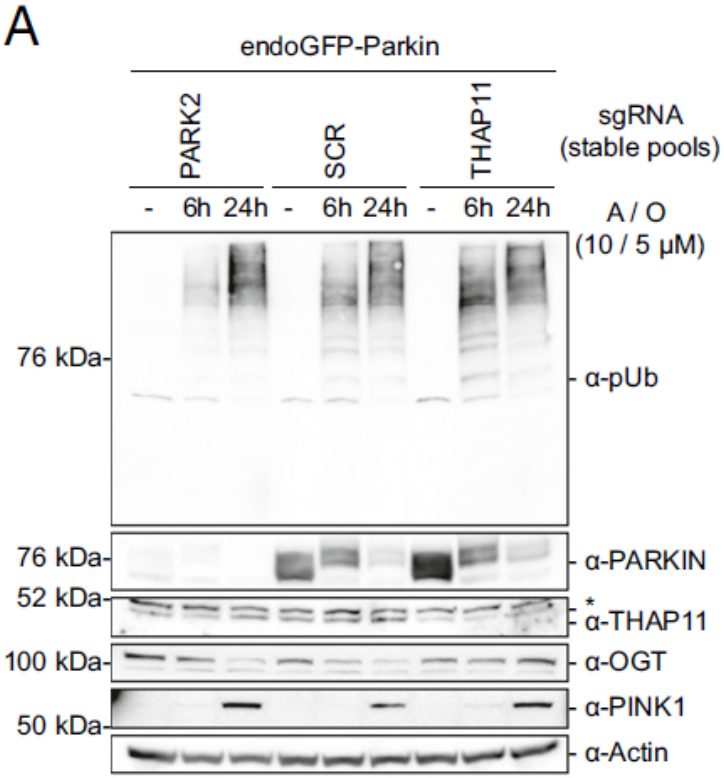
- 53 positive and negative regulators of GFP-PARKIN expression

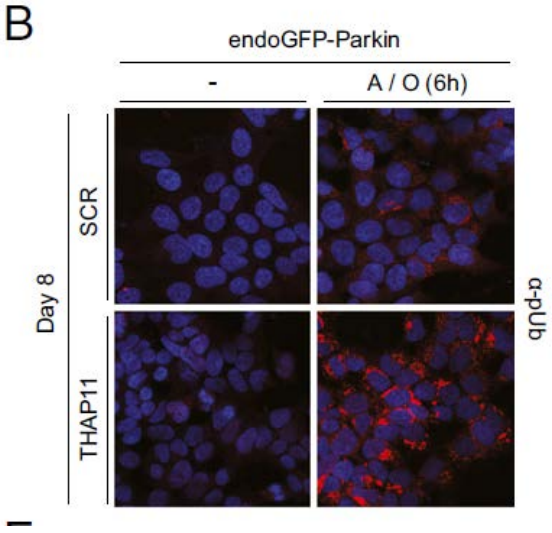
Validation of the top 24 hits





THAP11 is a regulator of PARKIN expression and pUb level





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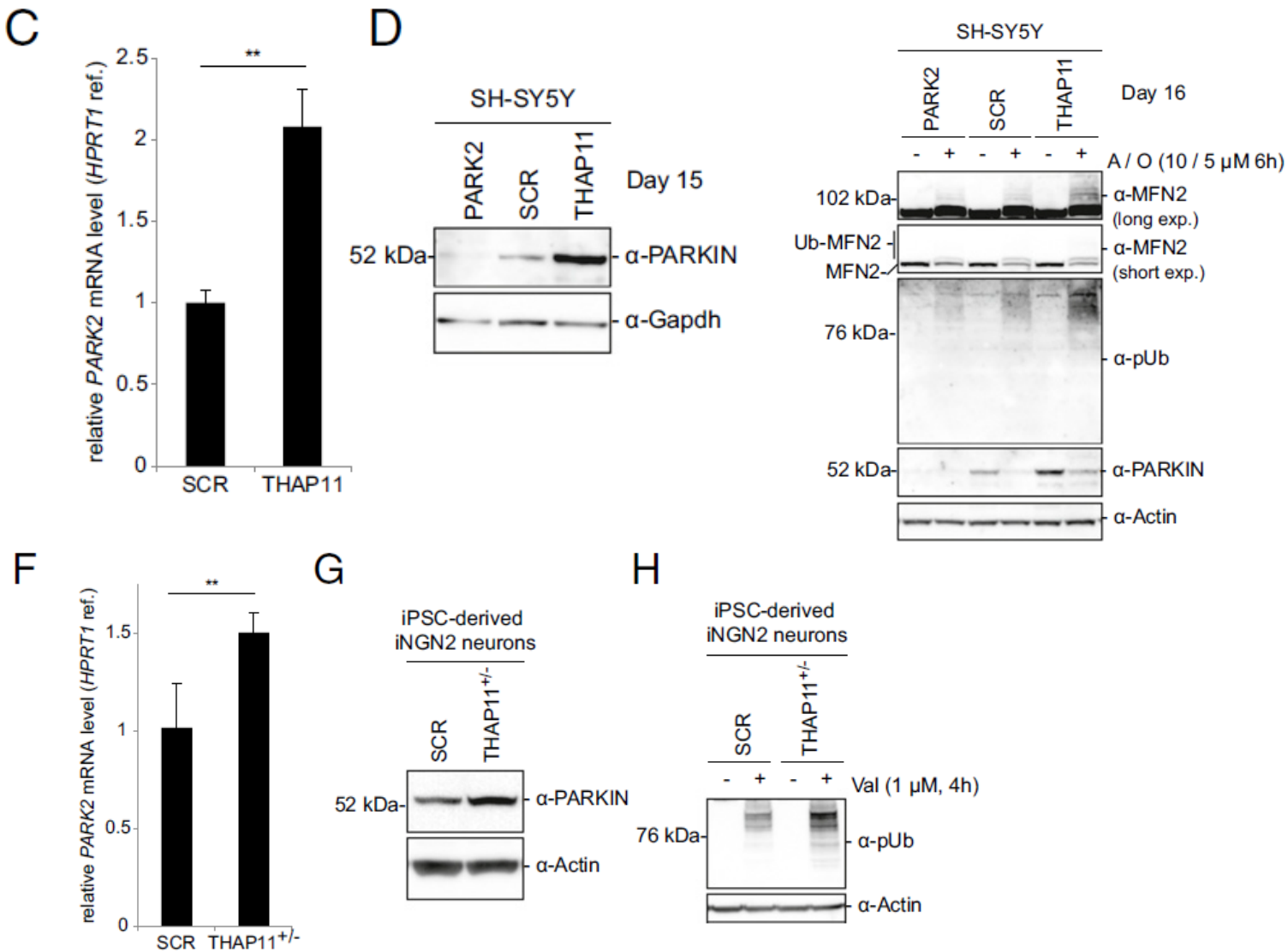

Relevance to our work

- ❖ PrP^{Sc} induced cell phenotype screen?
- ❖ PrP^{C} expression screen?

- ➢ Good cell model
- ➢ Robust readout

CRISPR screen

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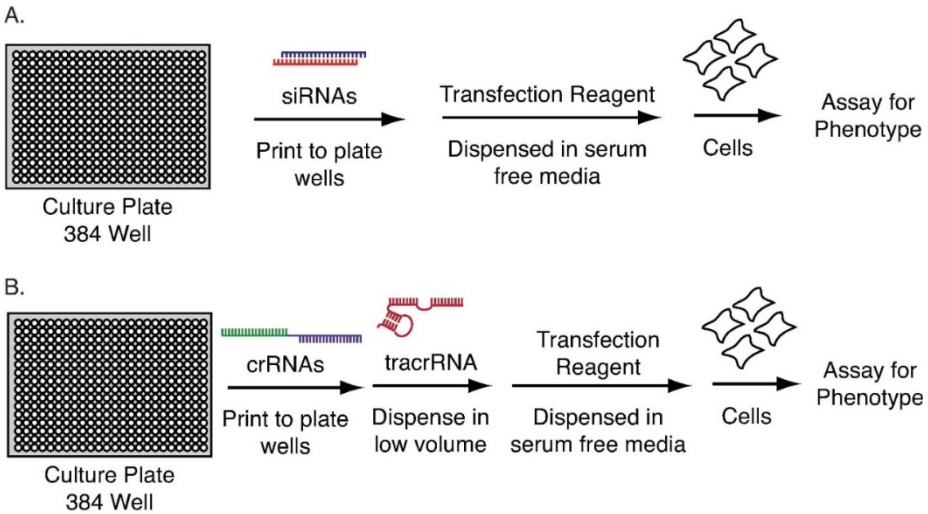
RESEARCH ARTICLE

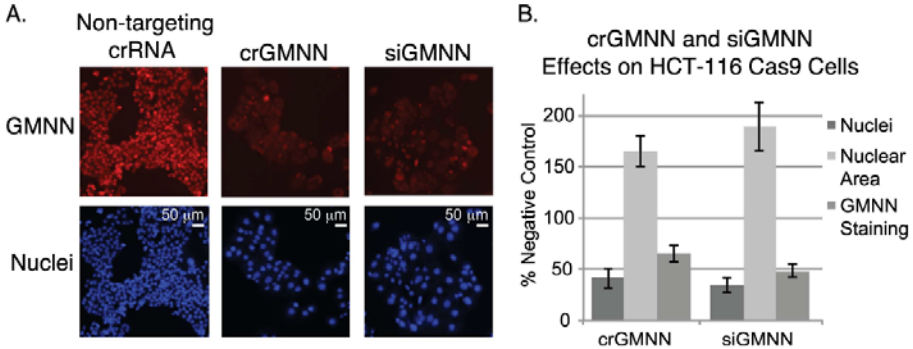
Validation of Synthetic CRISPR Reagents as a Tool for Arrayed Functional Genomic Screening

Jenille Tan, Scott E. Martin*

PLOS ONE

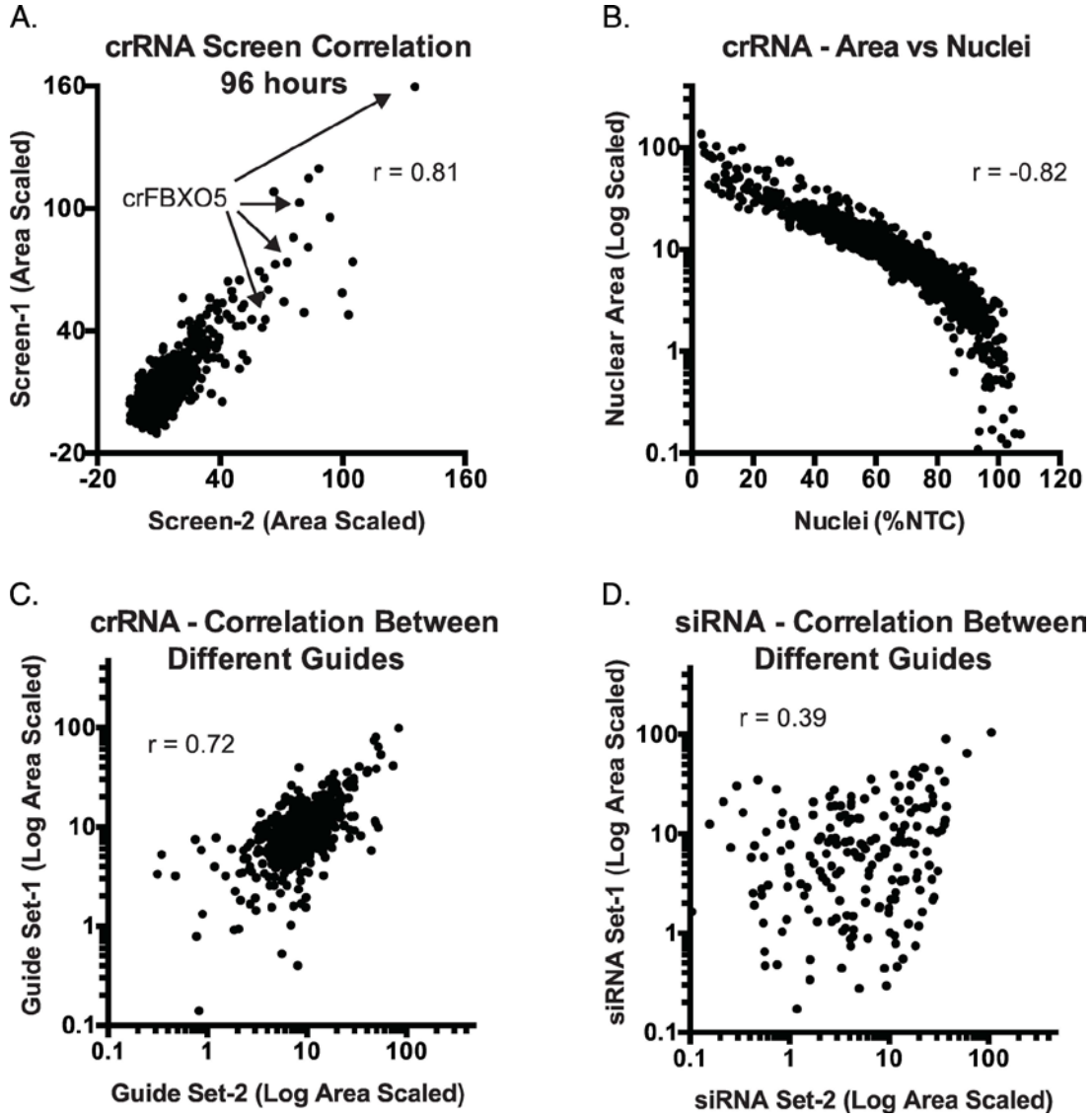
2016





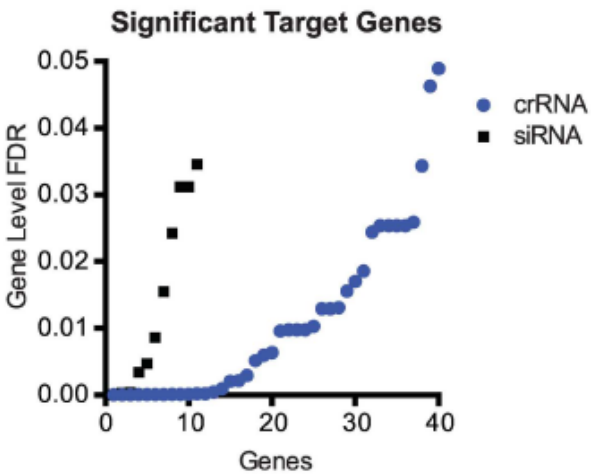
- HCT-116 (human colon cancer cell line) stably express Cas9
- 640 ubiquitin-related genes, 4crRNA per gene vs. 4 siRNA per gene
- 1 crRNA or 1 siRNA per well
- 384-well plates
- 96-hours
- Image-based readout: nuclei numbers and area

crRNA and siRNA screen



crRNA and siRNA screen

A.



B.

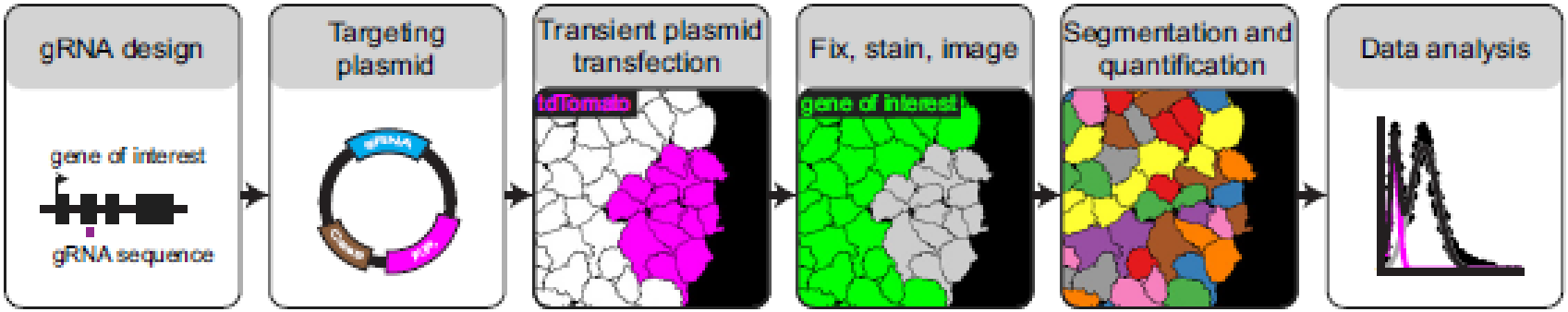
		crRNA		OTP siRNA		SilSelect siRNA		All siRNA	
Gene Symbol	Gene ID	RSA p-value	FDR	RSA p-value	FDR	RSA p-value	FDR	RSA p-value	FDR
FBXO5*	26271	4.16E-09	2.30E-06	4.82E-08	3.09E-05	1.31E-08	7.24E-06	3.78E-12	2.42E-09
UBE2I*	7329	7.19E-09	2.30E-06	1.62E-02	2.53E-01	2.38E-04	2.64E-02	7.87E-04	3.60E-02
PSMD14*	10213	1.11E-07	2.37E-05	8.01E-07	2.56E-04	7.20E-03	2.38E-01	1.09E-06	1.75E-04
UBA1*	7317	4.91E-07	6.59E-05	3.62E-03	1.10E-01	2.64E-02	3.63E-01	3.48E-04	2.03E-02
ARIH1	25820	5.21E-07	6.59E-05	5.73E-02	3.34E-01	1.20E-02	3.17E-01	3.18E-02	2.17E-01
RBX1*	9978	6.18E-07	6.59E-05	1.83E-06	3.89E-04	3.40E-06	9.41E-04	8.33E-10	2.66E-07
PRPF19*	27339	7.29E-07	6.67E-05	7.81E-03	1.79E-01	3.35E-02	3.79E-01	7.31E-04	3.60E-02
CUL1*	8454	9.80E-07	7.36E-05	3.66E-05	4.69E-03	2.24E-04	2.64E-02	2.63E-07	5.61E-05
USP37	57695	1.04E-06	7.36E-05	2.47E-02	2.70E-01	8.79E-02	4.31E-01	1.54E-02	1.72E-01
PRPF8*	10594	1.15E-06	7.36E-05	8.06E-05	8.60E-03	2.62E-02	3.63E-01	2.09E-05	2.68E-03
PHF5A*	84844	3.16E-06	1.84E-04	2.11E-05	3.37E-03	N/A	N/A	1.04E-04	8.34E-03
TRAIP*	10293	3.74E-06	1.99E-04	3.94E-02	3.06E-01	7.79E-05	1.44E-02	1.62E-04	1.16E-02
RNF113A*	7737	8.96E-06	4.41E-04	1.58E-03	6.74E-02	7.90E-03	2.43E-01	9.39E-05	8.34E-03
CUL2*	8453	2.00E-05	9.14E-04	1.69E-04	1.55E-02	2.65E-01	5.57E-01	1.18E-03	5.05E-02

molecular systems biology

Large-scale image-based profiling of single-cell phenotypes in arrayed CRISPR-Cas9 gene perturbation screens

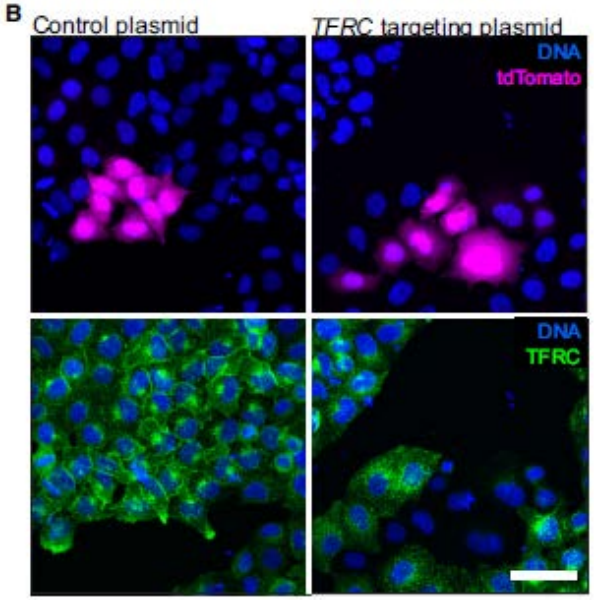
Reinoud de Groot[1], Joel Lüthi[1,2], Helen Lindsay[1], René Holtackers[1] & Lucas Pelkmans[1,*]

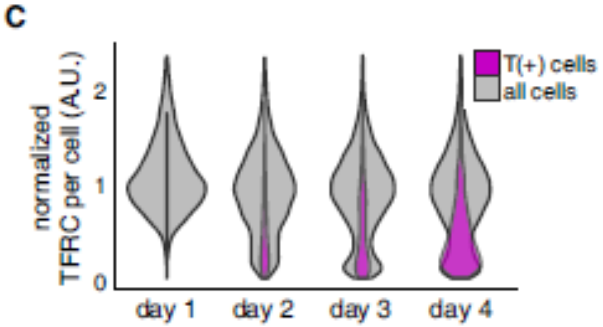
A

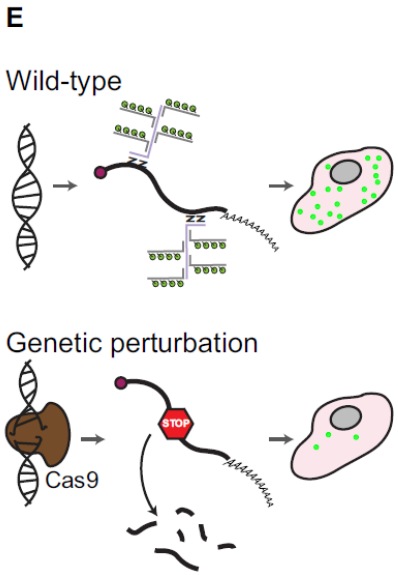


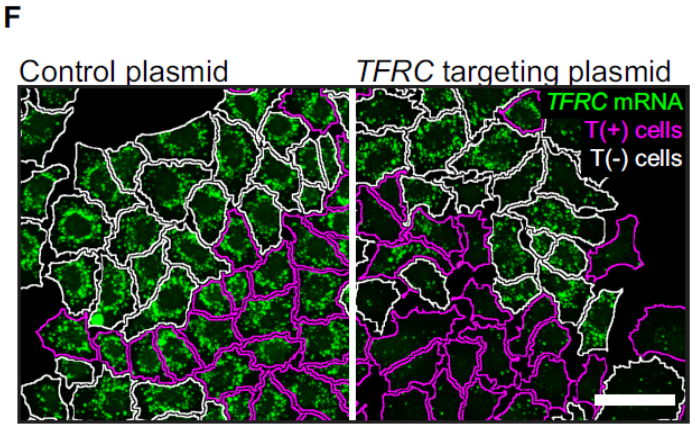
CRISPR KO of TFRC

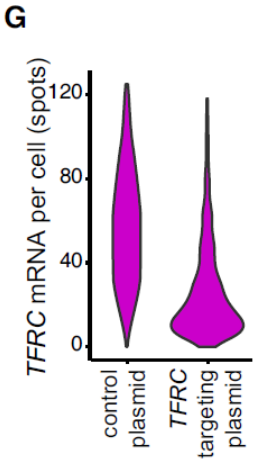
Hela











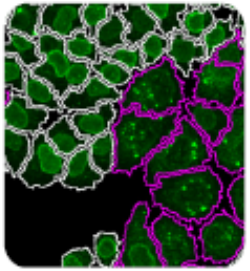
CRISPR library and screen

- 2281 sgRNA/1457 genes
- Arrayed in 384-well plates
- Transient transfection
- Stain DNA and mAb414, monoclonal Ab against phenylalanine –glycine repeats which presents in several subunits of nuclear pore complex (NPC)
- Image 4000 cells per well, shape and size of the cells, fluorescence intensity and texture of subregions of every cells

Gene perturbation characterization

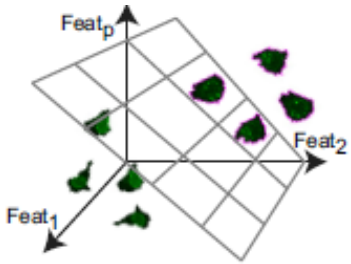
A

1. Mosaic population of wild-type and genetically perturbed cells



2. Standardize single cell features to T(-) cells

$$\mathrm{Cell}_x\ \mathrm{Feat}_y' = \frac{\mathrm{Cell}_x\ \mathrm{Feat}_y - \mu(\mathrm{Feat}_y(T-))}{\sigma\ (\mathrm{Feat}_y(T-))}$$



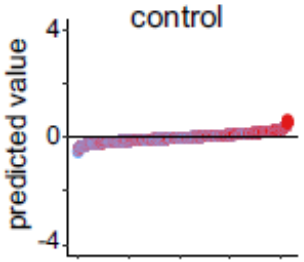
3. Logistic regression model to classify wild-type and transfected cells

$$\ln\left(\frac{F(x)}{1-F(x)}\right) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_p X_p$$

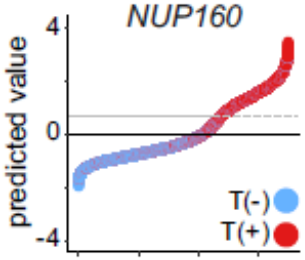
↓

Misclassification error
Single cell prediction

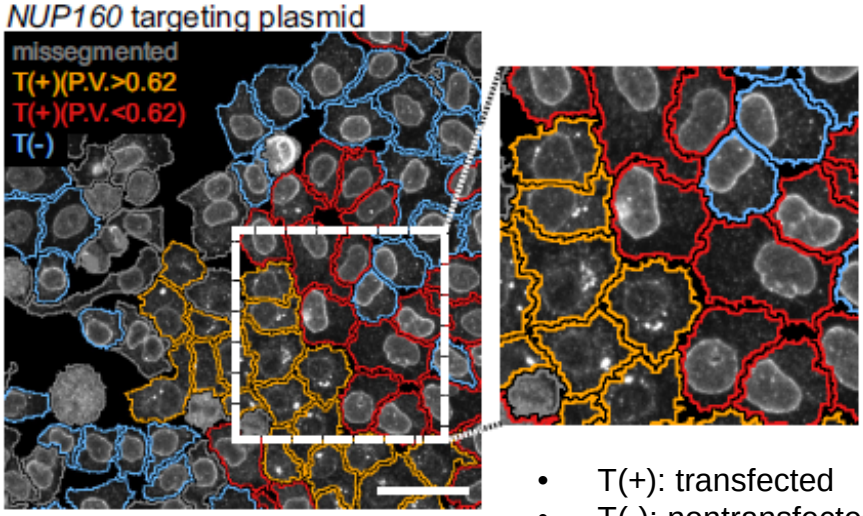
B



C

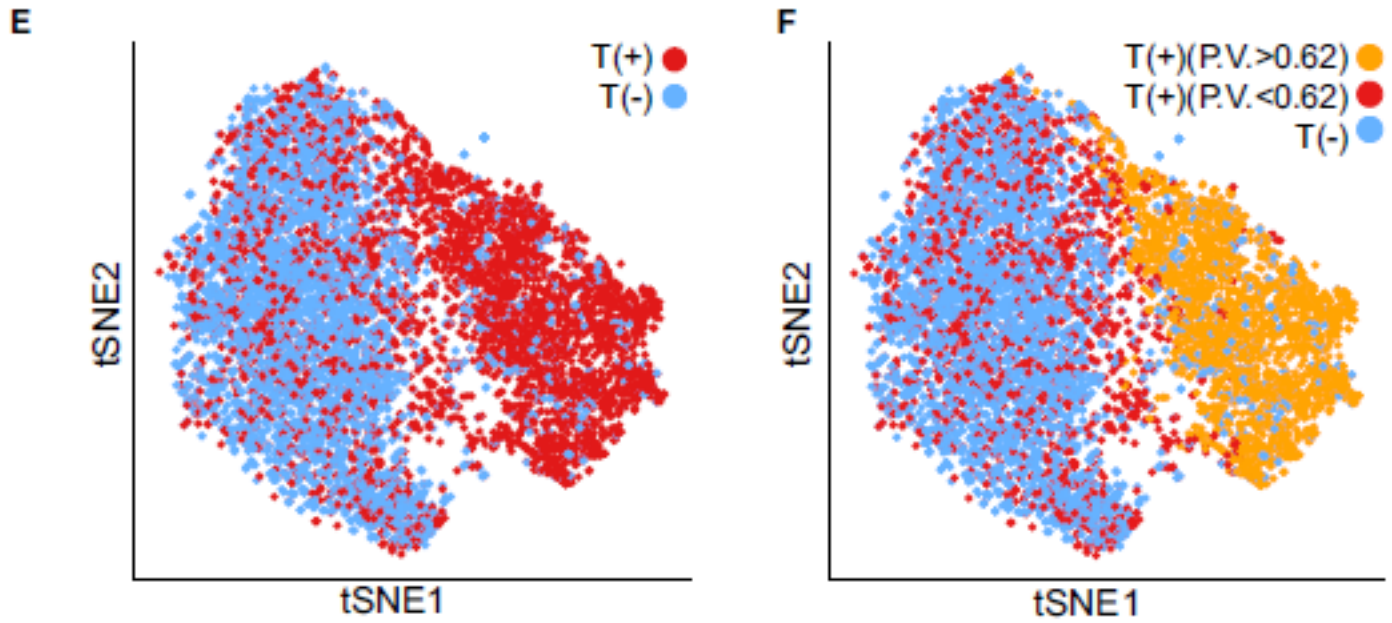


D

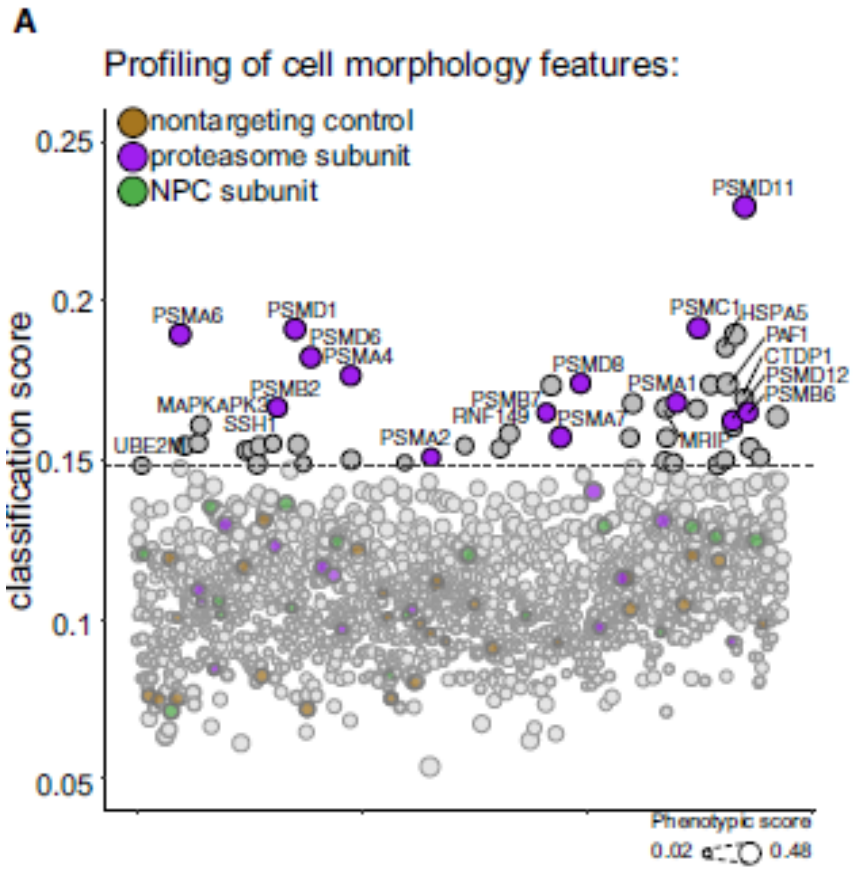


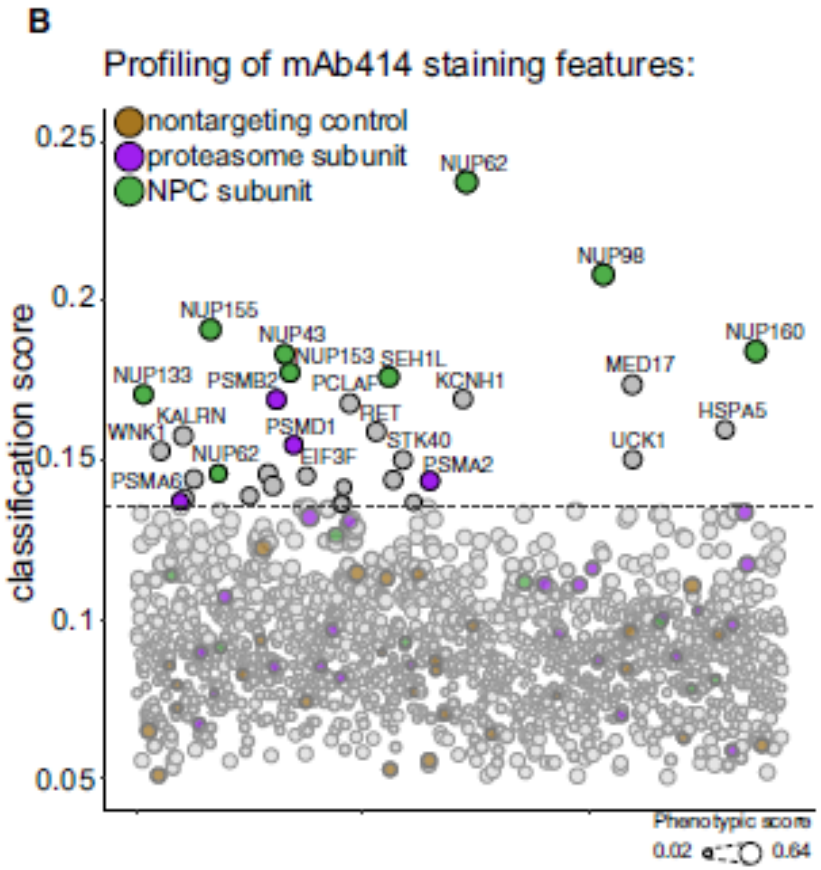
- T(+): transfected
- T(-): nontransfected
- PV: predicted value

Gene perturbation characterization



Arrayed CRISPR library screen





86 features of cellular morphology and intensity and texture of the total protein stain

- 49 perturbations

118 features of the mAb414 staining pattern

- 9 perturbations targeting NPC

Genome Research
www.genome.org

Arrayed CRISPR screen with image-based assay reliably uncovers host genes required for coxsackievirus infection

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sgRNA plasmid array in 96-well plate
(1514 genes, 3 sgRNAs / gene)

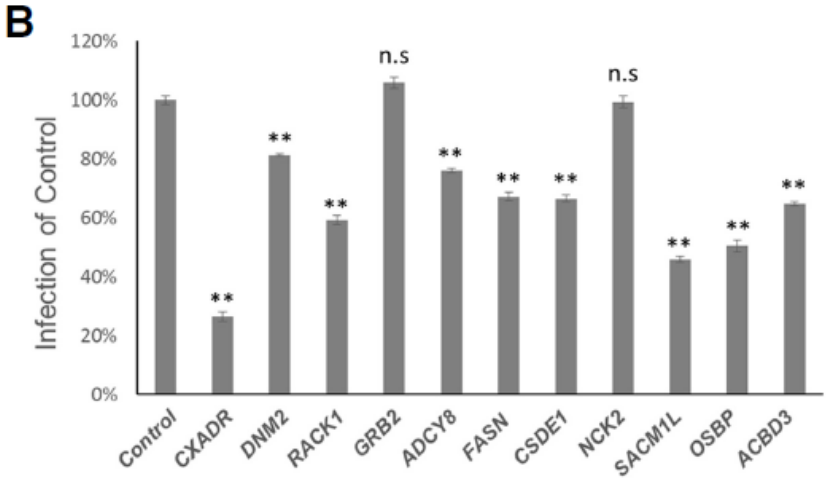
Transfect HeLa with Cas9 expression plasmids

Infect CVB3 (MOI = 5) in duplicate

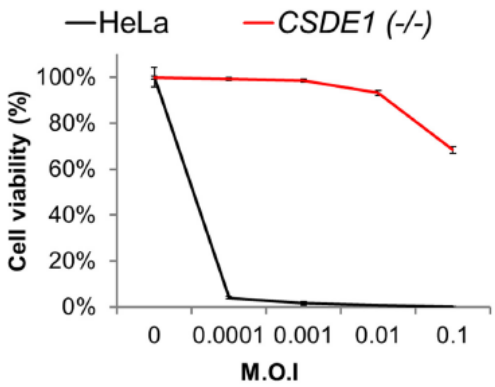
coxsackievirus B3 (CVB3)

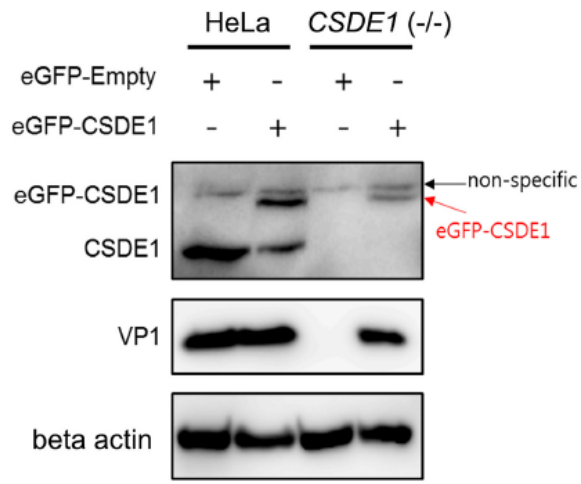
Immunostaining & Image acquisition

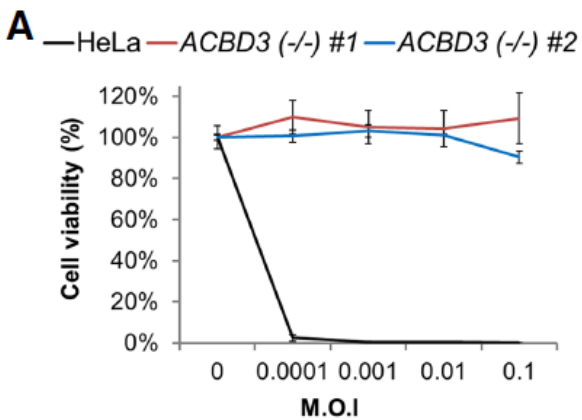
Analysis & Target identification

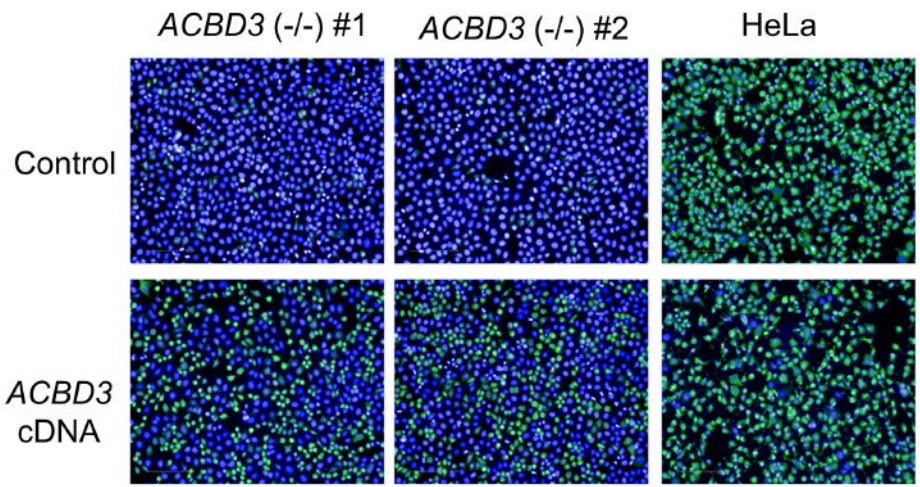


CSDE1 and ACBD3 are required for CVB3 infection









Pooled vs. Arrayed CRISPR library

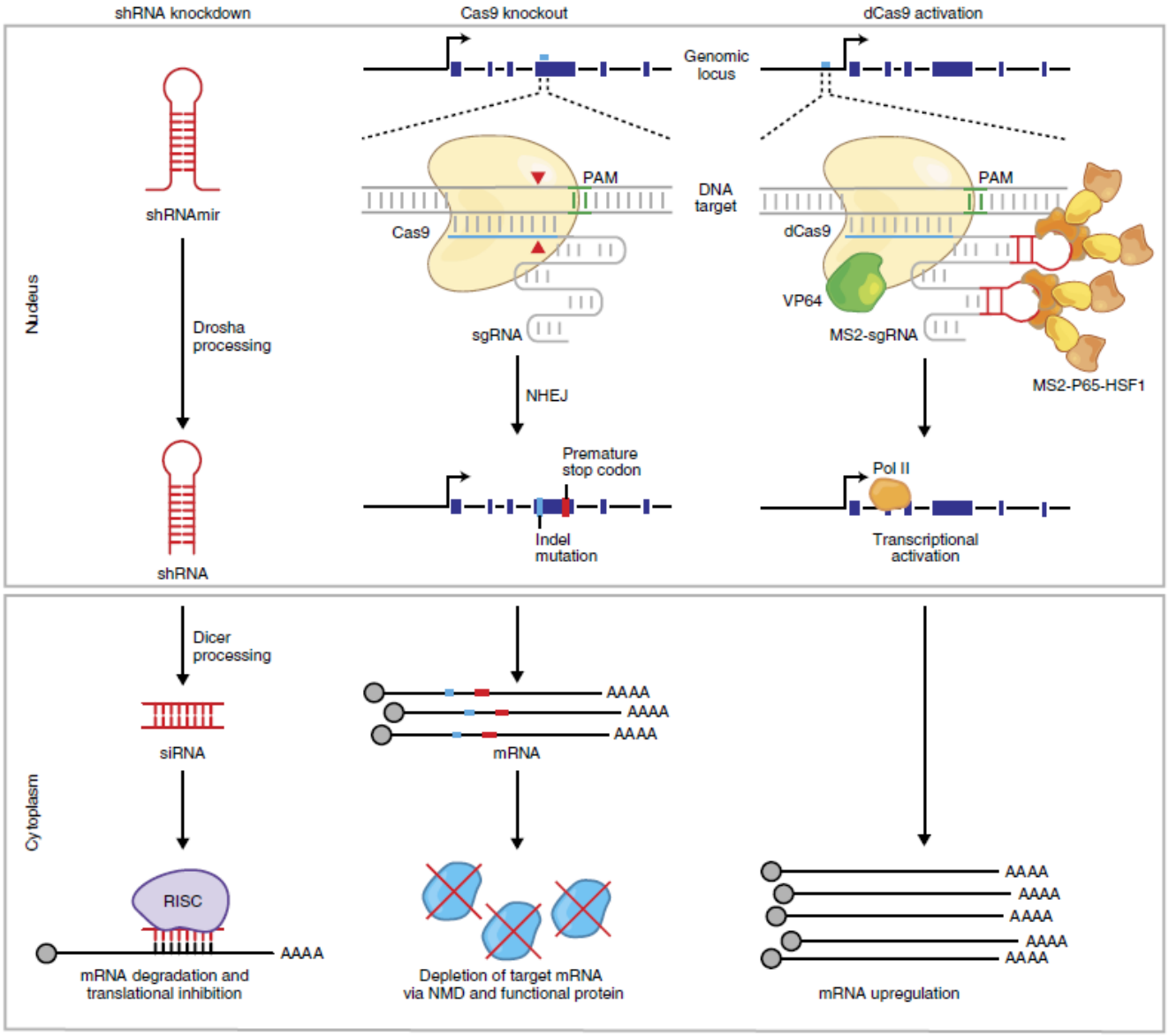
- Pooled CRISPR library: relatively easy to perform, cheap, sgRNA can be used barcode, different sgRNA targeting same gene can be cross-validating; need NGS for readout, require strong selection
- Arrayed CRISPR library: used for more subtle phenotype, compatible to siRNA screen platform; more expensive, heterogeneity, sophisticated data analysis

- Depends on the biological questions to be asked, choose an appropriate library format!

TABLE 1 | Previously published screens using Cas9.

Type of screen	Selection	Organism	Cas9 variant	References
Knockout	Vemurafenib resistance (positive)	*In vitro*; A375 (human melanoma cell line)	Wild-type Cas9	37
Knockout	6-Thioguanine/etoposide resistance (positive); gene essentiality (negative)	*In vitro*; HL60, KBM70 (human leukemic cell line)	Wild-type Cas9	38
Knockout	6-Thioguanine/*Clostridium septicum* α-toxin resistance (positive)	*In vitro*; mouse embryonic stem cells	Wild-type Cas9	39
Knockout	Anthrax/diphtheria toxin resistance (positive)	*In vitro*; HeLa (human adenocarcinoma cell line)	Wild-type Cas9	40
Knockout	Surface receptor expression (negative)	*In vitro*; EL4 (mouse thymic cell line) and MOLM13/NB4/TF1 (human acute myeloid leukemia cell lines)	Wild-type Cas9	41
Knockout	Metastasis (positive)	*In vivo*; mouse	Wild-type Cas9	42
Knockout	Chromatin regulatory domain dependence (negative)	*In vitro*; RN2 (murine acute myeloid leukemia cell line)	Wild-type Cas9	43
Knockout	Bacterial lipopolysaccharide response (marker gene)	*Ex vivo*; bone-marrow-derived dendritic cells (mouse)	Wild-type Cas9	44
Knockout	Fetal hemoglobin regulation by *BCL11A* enhancer (marker gene)	*In vitro*; HUDEP-2 (human erythroid progenitor cell line)	Wild-type Cas9	53
Knockout	Gene dependency for essential genes (negative)	*In vitro*; KBM7 (human chronic myelogenous leukemia cell line)	Wild-type Cas9	45
Knockout	p53-binding sites (positive); ESR1-binding sites (negative)	*In vitro*; BJ (human fibroblast cell line); MCF-7, T47D, and MDA-MB-231 (human breast cancer cell line)	Wild-type Cas9	54
Knockout	*POU5F1* regulation (marker gene)	*In vitro*; H1(human embryonic stem cell line)	Wild-type Cas9	55
Knockout	Combinatorial gene dependency (negative)	*In vitro*; OVCAR8-ADR (human ovarian cancer cell line)	Wild-type Cas9	46
Knockout	Vemurafenib resistance (positive); essential genes (melanoma cell line); (negative); 6-thioguanine resistance (positive); interferon survival (positive)	*In vitro*; A375 (human HT29 (human colorectal adenocarcinoma cell line); HEK293T (human embryonic kidney cell line); BV2 (mouse cell line)	Wild-type Cas9	47
Knockout	Survival under oxidative stress (positive)	*In vitro*; K562 (human leukemic cell line)	Wild-type Cas9	48
Knockout	Dengue virus resistance/hepatitis C virus resistance	*In vitro*; Huh7.5.1 (human hepatocyte cell line)	Wild-type Cas9	49
Knockout	West Nile virus resistance	*In vitro*; 293T (human embryonic kidney cell line)	Wild-type Cas9	50
Knockout	Type III secretion system resistance	*In vitro*; HT29 (human colorectal adenocarcinoma cell line)	Wild-type Cas9	51
Knockout	Norovirus resistance	*In vitro*; BV2 (mouse microglial cells)	Wild-type Cas9	52
Knockout	*CUL3* regulation and Vemurafenib resistance (positive)	*In vitro*; A375 (human melanoma cell line)	Wild-type Cas9	56
Activation	Ricin sensitivity (both); cell growth (both)	*In vitro*; K562 (human leukemic cell line)	sunCas9-VP64	28
Activation	Vemurafenib resistance (positive)	*In vitro*; A375 (human melanoma cell line)	dCas9- VP64/ P65/HSF 1	30
Knockdown	Ricin resistance (positive)/essential genes (negative)/cholera sensitivity (both)	*In vitro*; K562 (human leukemic cell line)	dCas9 or dCas9-KRAB	28
Knockdown	Gene essentiality	*In vitro*; K562 (human leukemic cell line)	KRAB-dCas9	57

Joung et al, 2017

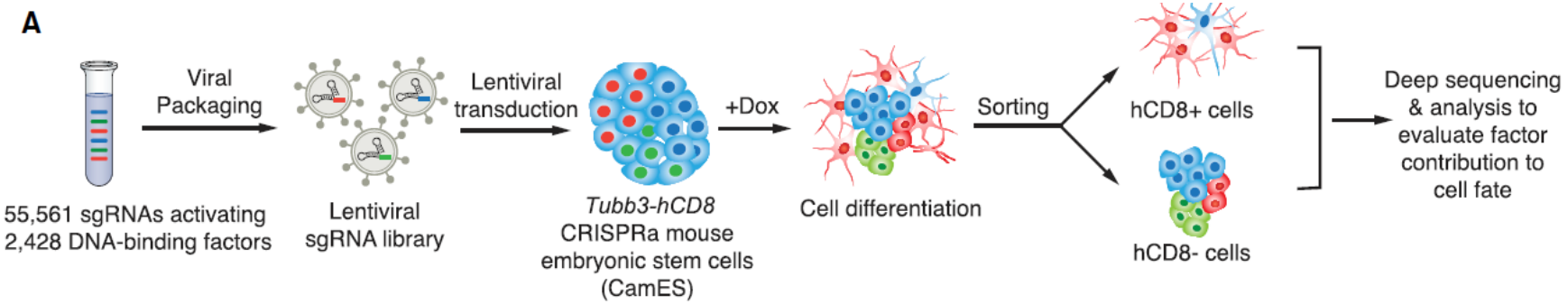


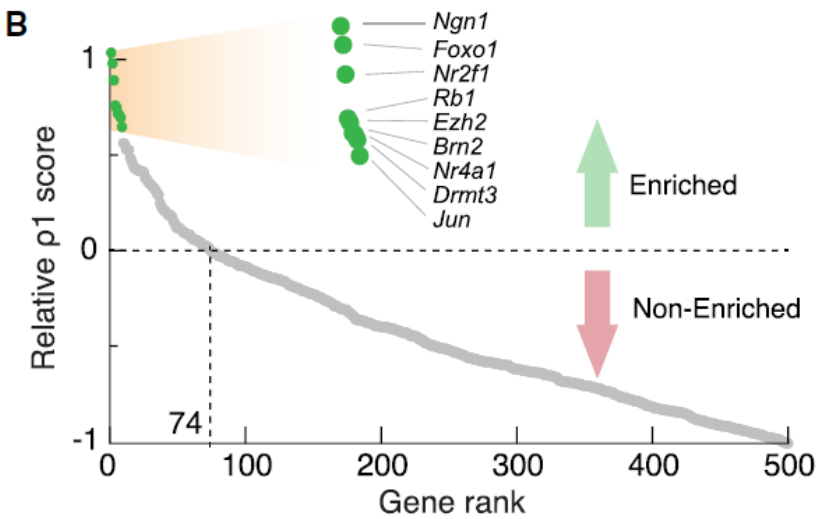
Joung et al, 2017

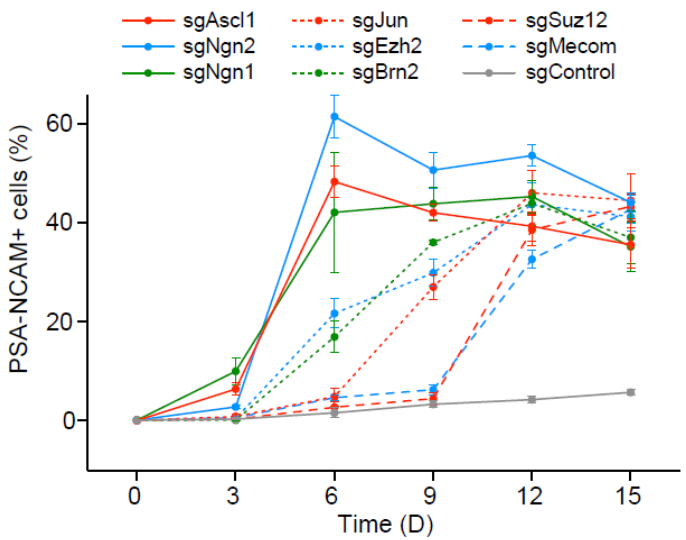
Resource 2018

Cell Stem Cell

CRISPR Activation Screens Systematically Identify Factors that Drive Neuronal Fate and Reprogramming







Up, down, and out: optimized libraries for CRISPRa, CRISPRi, and CRISPR-knockout genetic screens

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https://www.biorxiv.org/content/biorxiv/early/2018/07/02/356626.full.pdf

Advances in CRISPR-Cas9 technology have enabled the flexible modulation of gene expression at large scale. In particular, the creation of genome-wide libraries for CRISPR knockout (CRISPRko), CRISPR interference (CRISPRi), and CRISPR activation (CRISPRa) has allowed gene function to be systematically interrogated. Here, we evaluate numerous CRISPRko libraries and show that our recently-described CRISPRko library (Brunello) is more effective than previously published libraries at distinguishing essential and non-essential genes, providing approximately the same perturbation-level performance improvement over GeCKO libraries as GeCKO provided over RNAi. Additionally, we developed genome-wide libraries for CRISPRi (Dolcetto) and CRISPRa (Calabrese). Negative selection screens showed that Dolcetto substantially outperforms existing CRISPRi libraries with fewer sgRNAs per gene and achieves comparable performance to CRISPRko in the detection of gold-standard essential genes. We also conducted positive selection CRISPRa screens and show that Calabrese outperforms the SAM library approach at detecting vemurafenib resistance genes. We further compare CRISPRa to genome-scale libraries of open reading frames (ORFs). Together, these libraries represent a suite of genome-wide tools to efficiently interrogate gene function with multiple modalities.tracr

CRISPR libraries available from Addgene

Name	Addgene ID	Library Type	Species	PI	Lentiviral Generation	gRNAs per gene	Total gRNAs
Bassik Human CRISPR Knockout Library	101926 — 101934	Knockout	Human	Bassik	3rd	10	Varies
Bassik Mouse CRISPR Knockout Library	1000000121 — 100000	Knockout	Mouse	Bassik	3rd	10	Varies
Activity-optimized genome-wide library	Discontinued	Knockout	Human	Sabatini and Lander	3rd	10	178,896
Activity-optimized genome-wide library	1000000100	Knockout	Human	Sabatini and Lander	3rd	10	187,535
Broad GPP genome-wide Brunello	73179 (1 plasmid)	Knockout	Human	Doench and Root	3rd	4	76,441
	73178 (2 plasmid)						
Broad GPP genome-wide Brie	73632 (1 plasmid)	Knockout	Mouse	Doench and Root	3rd	4	78,637
	73633 (2 plasmid)						
Broad GPP kinome Brunello	75314, 75315	Knockout	Human	Doench and Root	3rd	4	3,052
	(1 plasmid)						
	75312, 75313						
	(2 plasmid)						
Broad GPP kinome Brie	75317 (1 plasmid)	Knockout	Mouse	Doench and Root	3rd	4	2,852
	75316 (2 plasmid)						
Broad GPP activation Calabrese p65-HSF	92379 (Set A)	Activation	Human	Doench and Root	3rd	3–6	56,762 (Set A)
	92380 (Set B)						56,476 (Set B)
Broad GPP activation Caprano p65-HSF	92383 (Set A)	Activation	Mouse	Doench and Root	3rd	3–6	67,187 (Set A)
	92384 (Set B)						66,889 (Set B)
Broad GPP inhibition Dolcetto	92385 (Set A)	Inhibition	Human	Doench and Root	3rd	3–6	57,050 (Set A)
	92386 (Set B)						57,011 (Set B)
Broad GPP inhibition Dolomiti	104090 (Set A)	Inhibition	Mouse	Doench and Root	3rd	3–6	67,366 (Set A)
	104091 (Set B)						67,194 (Set B)
Cas13a/C2c2 Protospacer flanking site (PFS) Library	79153	Knockout	E. coli	Zhang	N/A	N/A - The protospa	N/A
CRiNCL - Human CRISPRi Non-coding Libraries	86538 — 86550	Inhibition	Human	Weissman	3rd	10	Varies
CRISPR/Cas9-assisted Removal of Mitochondrial DNA (CARM) Library	82480	Knockout	Mouse	Xie	N/A	N/A	395
CRISPRa	Discontinued	Activation	Human	Weissman	3rd	10	198,810
CRISPRa-v2	83978	Activation	Human	Weissman	3rd	5	104,540
	1000000091					10	209,080
CRISPRa-v2	83996	Activation	Mouse	Weissman	3rd	5	107,105
	1000000093					10	214,210
CRISPRi	Discontinued	Inhibition	Human	Weissman	3rd	10	206,421
CRISPRi-v2	83969	Inhibition	Human	Weissman	3rd	5	104,535
	1000000090					10	209,070
CRISPRi-v2	83987	Inhibition	Mouse	Weissman	3rd	5	107,415
	1000000092					10	214,830
Enriched subpools (kinase, nuclear, ribosomal, cell cycle)	51043 — 51048	Knockout	Human	Sabatini and Lander	3rd	10	Varies
Focused Ras Synthetic Lethal Human CRISPR Knockout Library	92352	Knockout	Human	Sabatini and Lander	3rd	50	6,661
hCRISPRa-v2 subpooled libraries	83980 — 83986	Activation	Human	Weissman	3rd	5	Varies
hCRISPRi-v2 subpooled libraries	83971 — 83977	Inhibition	Human	Weissman	3rd	5	Varies
mCRISPRa-v2 subpooled libraries	83998 — 84004	Activation	Mouse	Weissman	3rd	5	Varies
mCRISPRi-v2 subpooled libraries	83989 — 83995	Inhibition	Mouse	Weissman	3rd	5	Varies
Human CRISPR Knockout Library	1000000132	Knockout	Human	X.S. Liu	3rd	10	185,634
Human GeCKO v2	1000000048	Knockout	Human	Zhang	3rd	6	123,411
	(1 plasmid)						
	1000000049						
	(2 plasmid)						
Human genome-wide library v1	69763	Knockout	Human	Wu	3rd	4	77,406
Human improved genome-wide library v1	67989	Knockout	Human	Yusa	3rd	5	90,709
Human CRISPR lncRNA Activation Pooled Library	1000000106	Activation	Human	Zhang	3rd	10	96,458
Human CRISPR Metabolic Gene Knockout Library	110066	Knockout	Human	Sabatini	3rd	10	30,290
Human miRNA CRISPR Knockout Library	112200	Knockout	Human	Lin	3rd	04-May	8,382
Human Paired-guide RNA (pgRNA) Library for Long Non-coding RNAs (lncRNAs)	89640	Knockout	Human	Wei	3rd	Varies	12,472 pairs
Mouse GeCKO v2	1000000052	Knockout	Mouse	Zhang	3rd	6	130,209
	(1 plasmid)						
	1000000053						
	(2 plasmid)						
Mouse genome-wide library v1	Discontinued	Knockout	Mouse	Yusa	3rd	5	87,897
Mouse improved genome-wide library v2	67988	Knockout	Mouse	Yusa	3rd	5	90,230
Oxford Fly	64750	Knockout	D. melano	Liu	N/A	3	40,279
Perturb-seq Guide Barcodes (GBC)	85968	Barcode	Human	Weissman	3rd	N/A	N/A
SAM v1 - 3 plasmid system	1000000057 (Zeocin)	Activation	Human	Zhang	3rd	3	70,290
	1000000074 (Puromycin)						
SAM v1 - 3 plasmid system	1000000075 (Puromyci	Activation	Mouse	Zhang	3rd	3	69,716
SAM v2 - 2 plasmid system	1000000078 (Blasticidi	Activation	Human	Zhang	3rd	3	70,290
Toronto KnockOut - Version 1	1000000069	Knockout	Human	Moffat	3rd	12	176,500
Toronto KnockOut - Version 3	90294	Knockout	Human	Moffat	3rd	4	70,948
Toxoplasma Knockout	80636	Knockout	T. gondii	Lourido	N/A	10	8,158
Two plasmid human activity-optimized genome-wide library	1000000095	Knockout	Human	Sabatini and Lander	3rd	10	187,536
Two plasmid mouse activity-optimized genome-wide library	1000000096	Knockout	Mouse	Sabatini and Lander	3rd	10	188,509

Thank you!