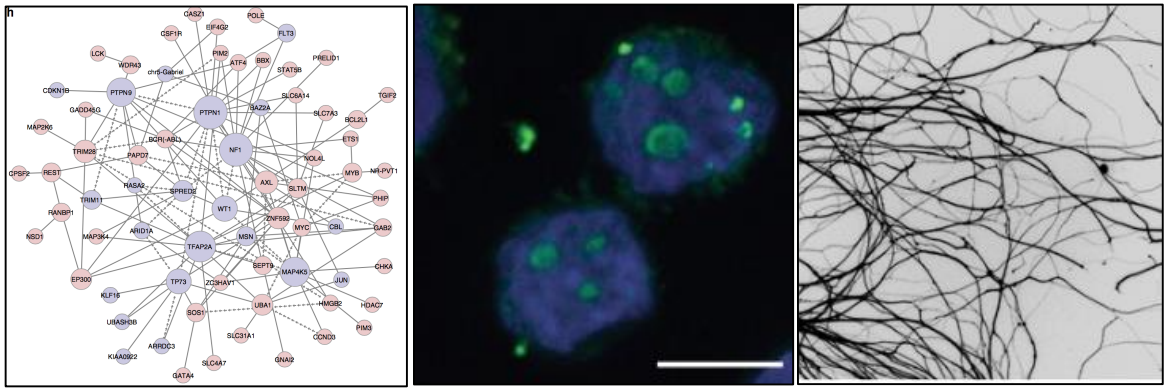
How CRISPR-Cas9 screens unlock novel ways of genetic interactions and disease mechanisms

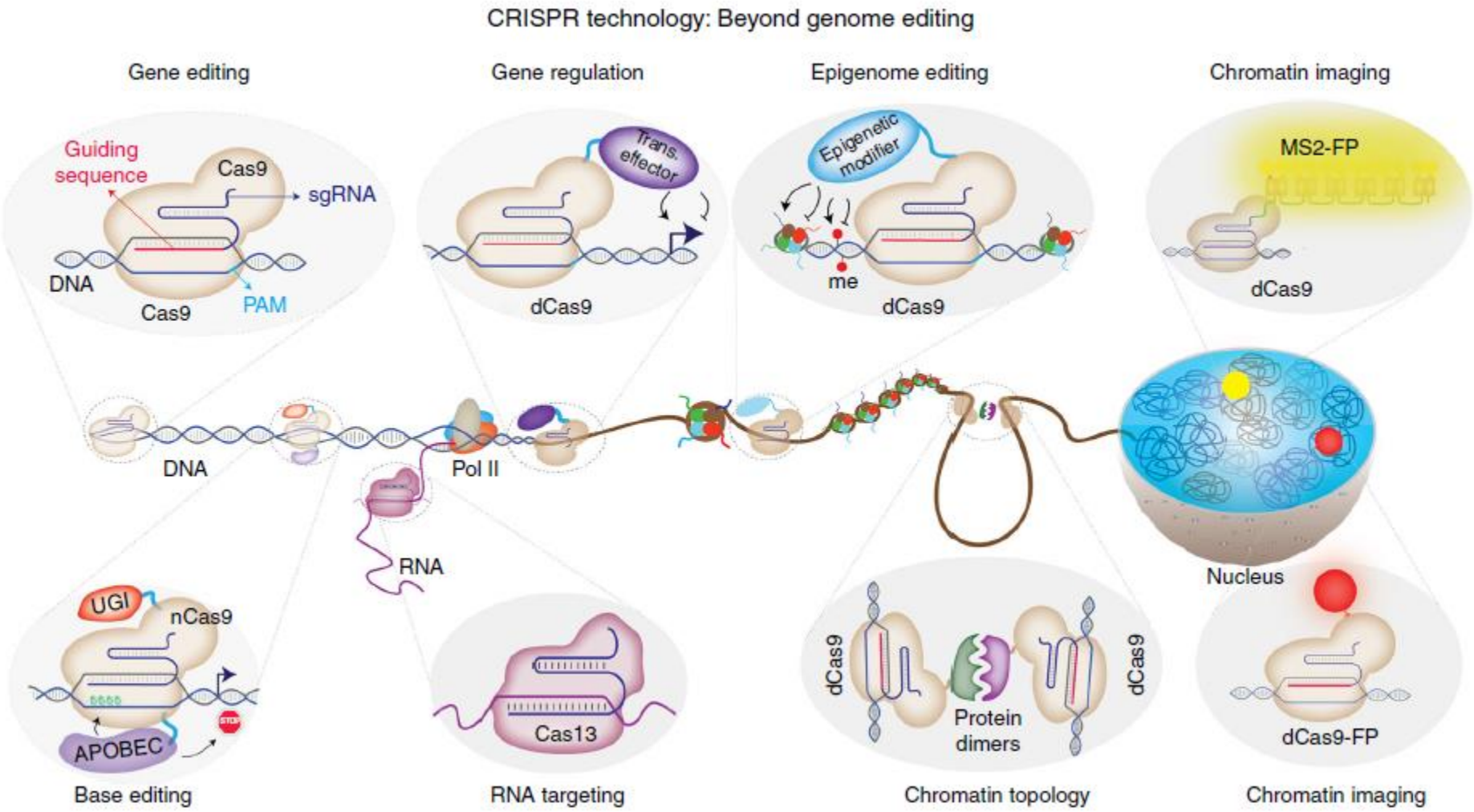


Francesca D. Franzoso, PhD

UniversitätsSpital Zürich

Context

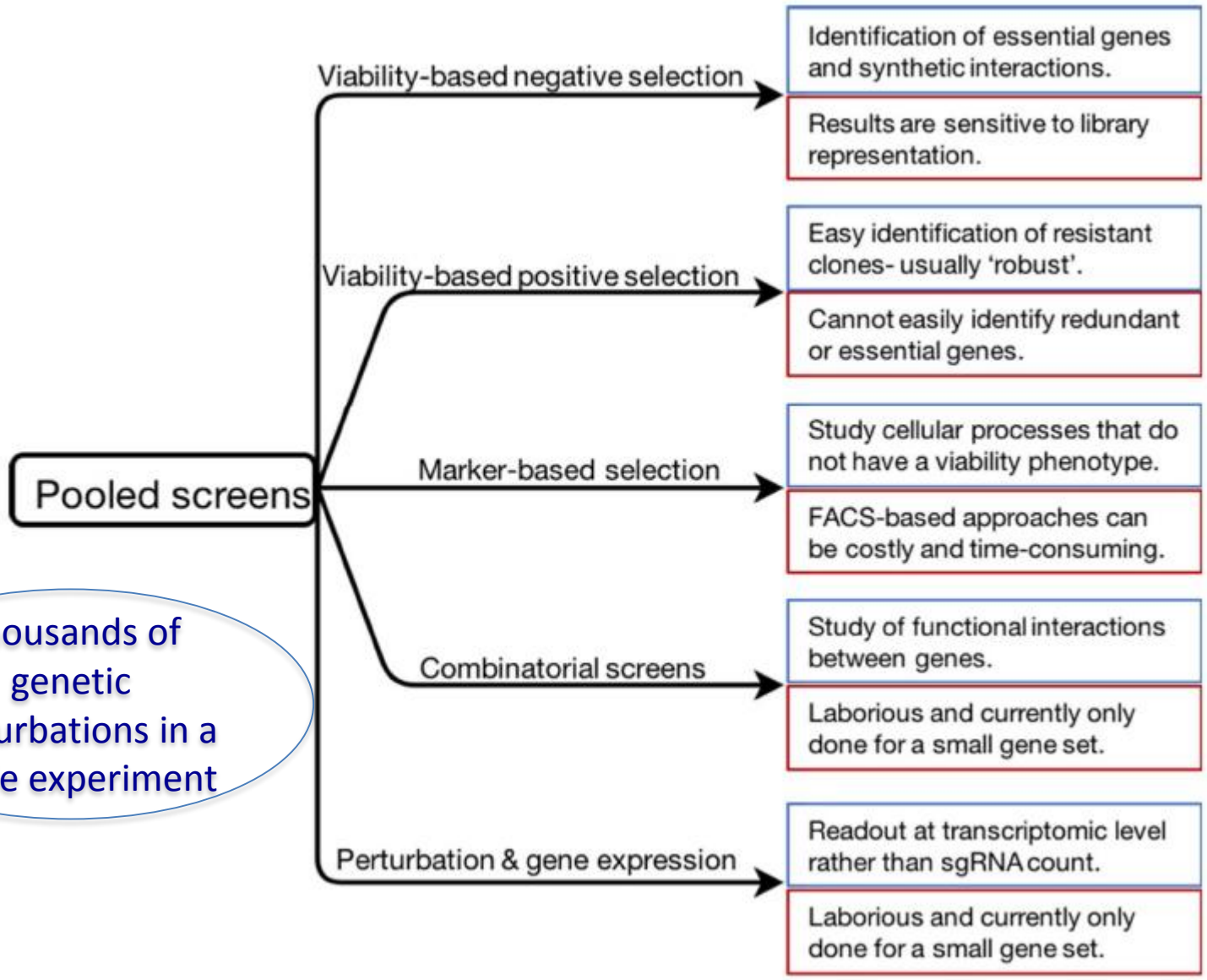
Applications of CRISPR-Cas-based technologies



Adli M, Nature Communications 2018

Context

CRISPR Screening Approaches



Thousands of genetic perturbations in a single experiment

Sharma S, Int J Mol Sci 2018

Overview

CRISPR-Cas9 screens


nature biotechnology

Orthologous CRISPR–Cas9 enzymes for combinatorial genetic screens

Fadi J Najm[1–3,5], Christine Strand[1,5], Katherine F Donovan[1,5], Mudra Hegde[1,5], Kendall R Sanson[1,5], Emma W Vaimberg[1], Meagan E Sullender[1], Ella Hartenian[1], Zohra Kalani[1], Nicolo Fusi[4], Jennifer Listgarten[4], Scott T Younger[1], Bradley E Bernstein[1–3], David E Root[1] & John G Doench[1]

nature biotechnology

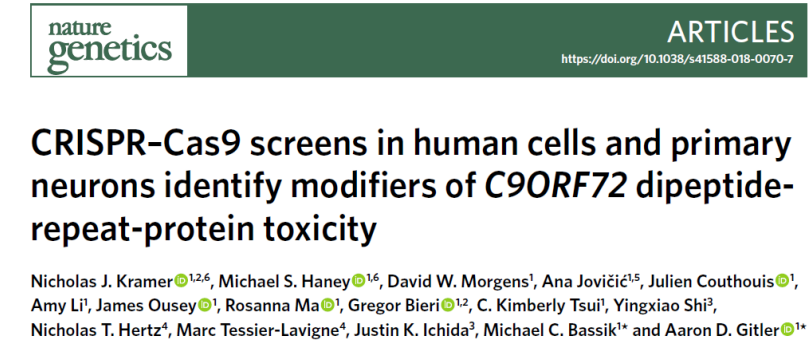
Dual-Cas9 system to identify genetic interactions - orthogonal Cas9 enzymes paired *S. aureus* and *S. pyogenes* for interactions – "Big Papi" screens


Dual gene activation and knockout screen reveals directional dependencies in genetic networks

Michael Boettcher[1], Ruilin Tian[2], James A Blau[1], Evan Markegard[3], Ryan T Wagner[1], David Wu[1], Xiulei Mo[4], Anne Biton[5,6], Noah Zaitlen[5], Haian Fu[4], Frank McCormick[3], Martin Kampmann[2] & Michael T McManus[1]

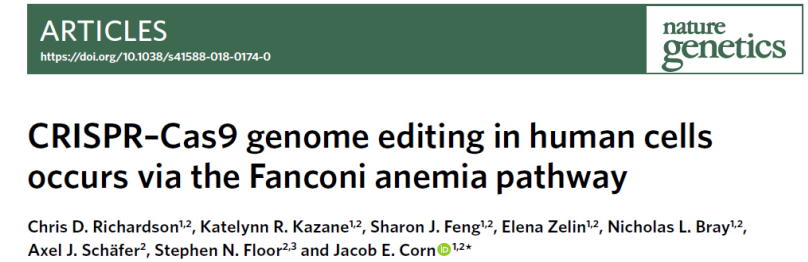
nature genetics ARTICLES https://doi.org/10.1038/s41588-018-0070-7

Orthogonal screening to identify directional genetic interactions between genes after activation/ablation in cancer CML cells


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

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*]

Comprehensive genome-wide KO screens in human cells and targeted screens in mouse primary neurons – genetic modifiers of C9ORF72 DPR toxicity


ARTICLES https://doi.org/10.1038/s41588-018-0174-0 nature genetics

CRISPR-Cas9 genome editing in human cells occurs via the Fanconi anemia pathway

Chris D. Richardson[1,2], Katelynn R. Kazane[1,2], Sharon J. Feng[1,2], Elena Zelin[1,2], Nicholas L. Bray[1,2], Axel J. Schäfer[2], Stephen N. Floor[2,3] and Jacob E. Corn[1,2*]

Coupled inhibition-editing screening platform Human Cas9-induced single-strand template repair (SSTR) requires FA pathway

Combinatorial genetic screens

- ✓ To explore complex gene networks
- ✓ To deconvolute complex cellular signaling pathways
- **Current limitations**: interference between the sgRNAs; limited gene targeting activity

Solution:

- ➢ orthogonal Cas9 enzymes from *S. aureus* and *S. pyogenes* & paired *S. aureus* Cas9 with *S. pyogenes* Cas9 to achieve dual targeting in a high fraction of cells
- ➢ «Big Papi» approach: **p**aired **a**ureus and **p**yogenes for **i**nteractions

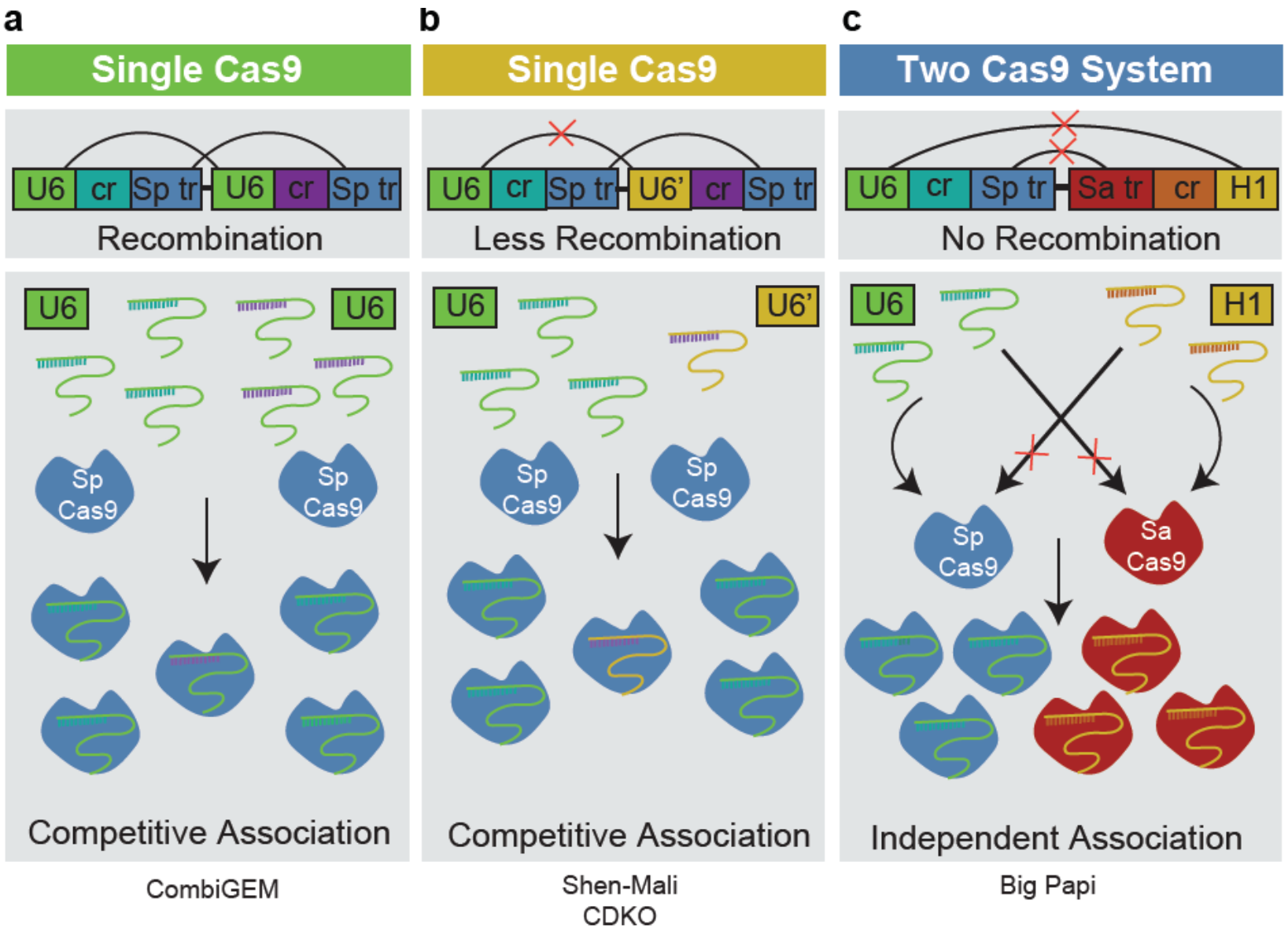
Study Rationale

To develop an approach using orthogonal Cas9 enzymes **SpCas9** and **SaCas9** → dual knockout efficiencies → robust screening

↓

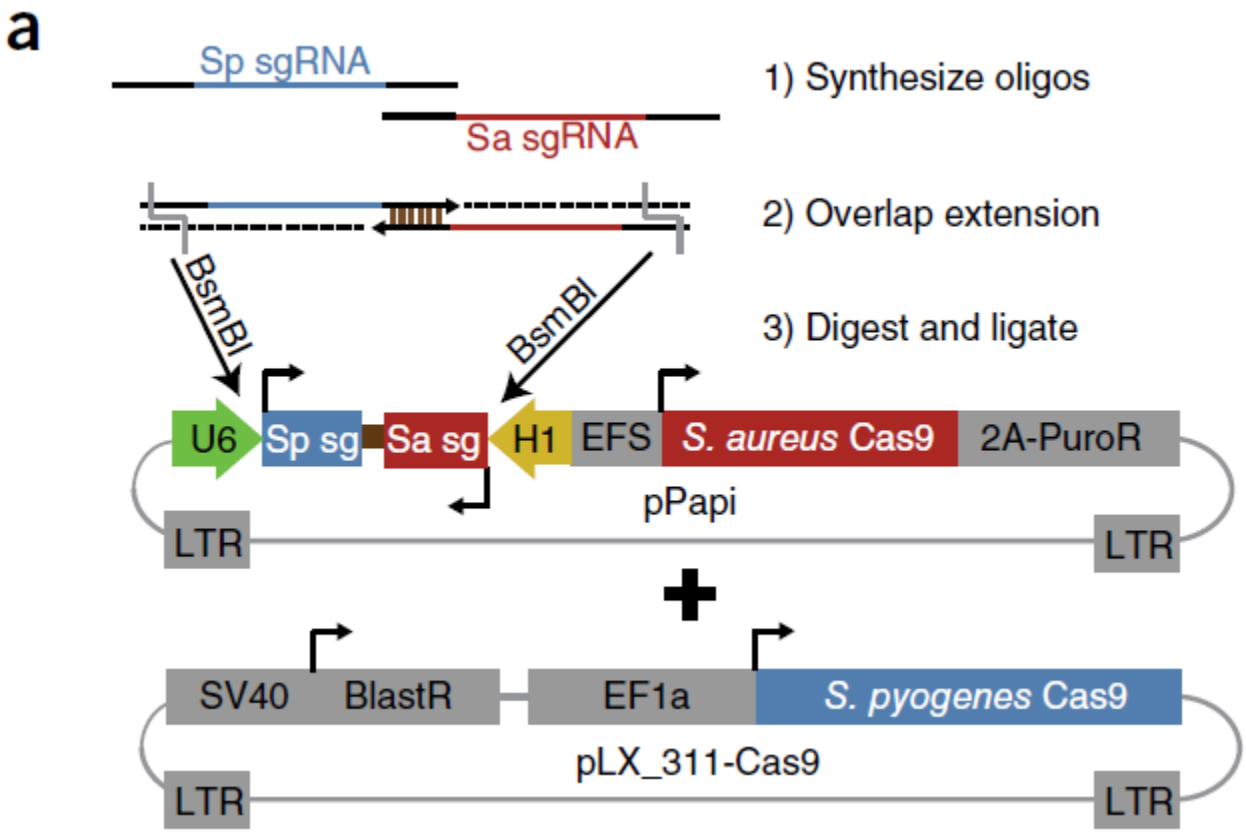
Combine knockout and overexpression (CRISPRa) in the same screen
Dubbed Big Papi screens

Aim: to develop a system with maximal on-target efficiency at two independent genomic sites



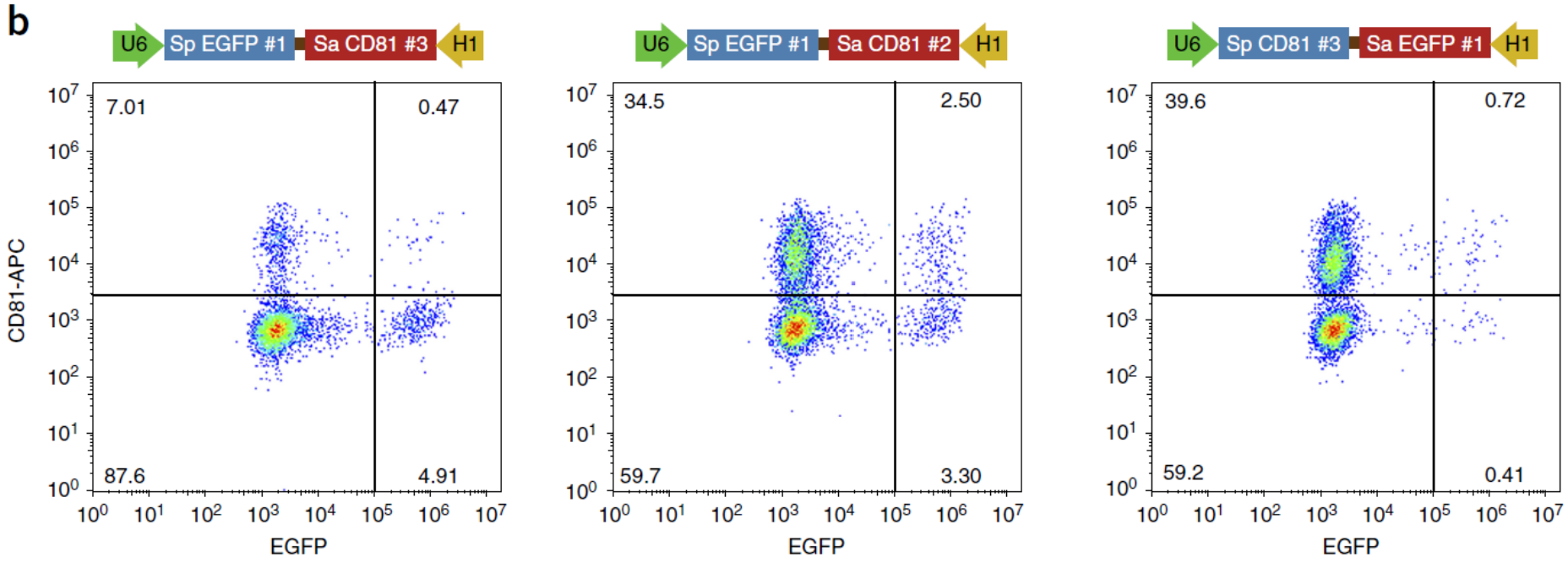
The two Cas9 system

Design a lentiviral construct pPapi to express SaCas9 and two sgRNAs from the U6 and H1 promoters



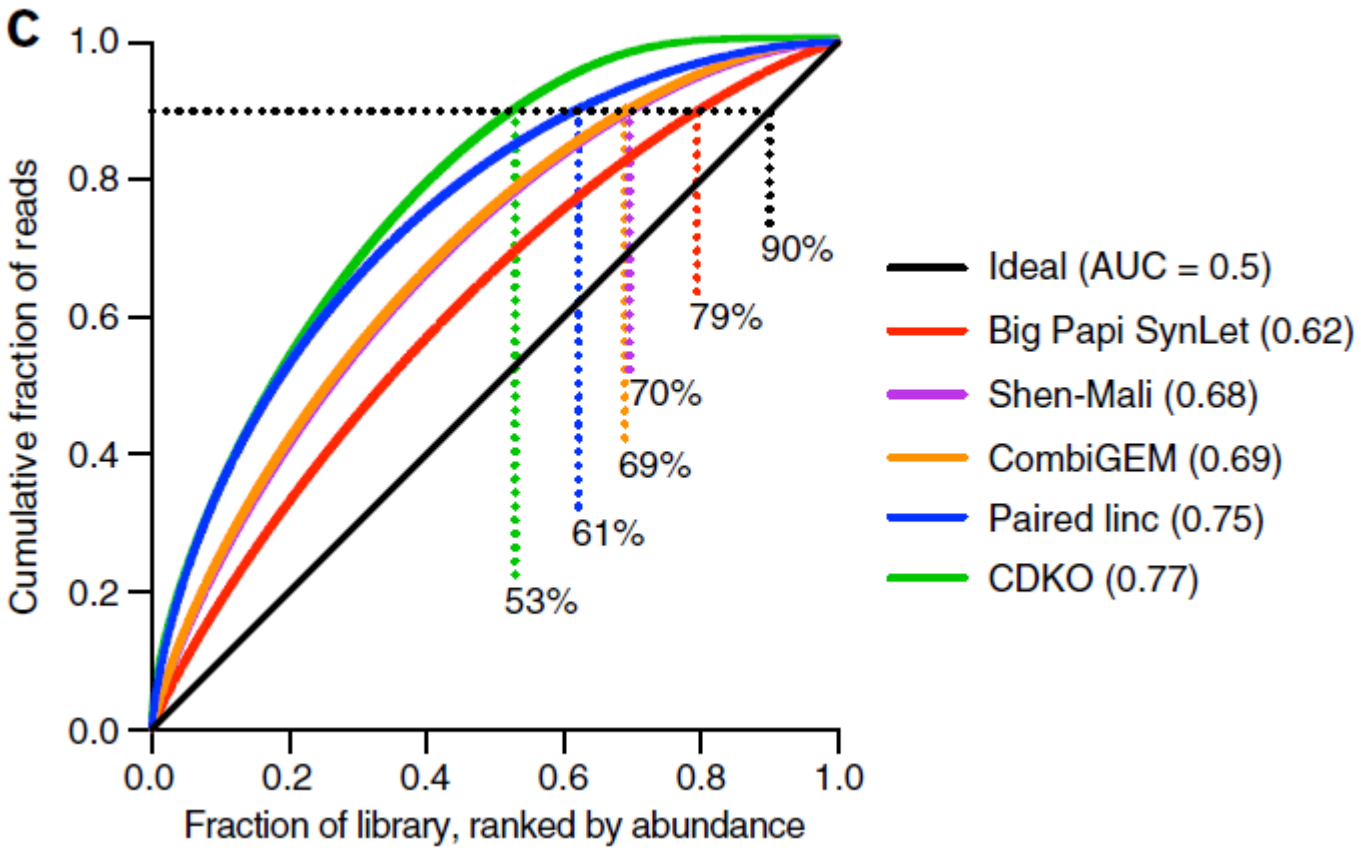
The two Cas9 system

Double knockout efficiency by partenering SaCas9 and SpCas9 sgRNAs



The two Cas9 system

Comparing Big Papi SynLet library to four other published libraries



79% of sgRNA pairs were found in the top 90% of the reads

BROAD INSTITUTE | GENETIC PERTURBATION PLATFORM

GPP Web Portal

Design sgRNAs for CRISPRko (*S. pyogenes* and *S. aureus*)

For notes about revisions, updates, and bug fixes please see our sgRNA Design Tool Changelog.

This tool ranks and picks candidate sgRNA sequences for the targets provided, while attempting to maximize on-target activity and minimizing off-target activity. For more information about the inputs and outputs of this tool, see How to use the sgRNA Designer (CRISPRko).

On-target scoring is performed using the "Rule Set 2" method described in Doench, Fusi et al., Nature Biotechnology 2016. The current Microsoft implementation of this scoring model is Azimuth 2.0. Off-target sites are evaluated using the CFD (**C**utting **F**requency **D**etermination) score. Please see How the sgRNA Designer Works for more details on these annotation strategies. For general discussion on sgRNA design, see Addgene. The Brunello and Brie human and mouse libraries using this design methodology and an earlier version of the Rule Set 2 model are available from Addgene.

Looking for a downloadable tool to compute CFD scores for existing sgRNA designs? Go here.

Select CRISPR Enzyme: S. pyogenes (NGG)

The scope of this tool is currently limited to the *S. pyogenes* (**NGG** PAM) and *S. aureus* (**NNGRR** PAM) CRISPR Cas9 enzyme families; i.e. only on-target sites that include the appropriate PAM are considered, and off-target CFD scores are reduced for sites that depart from this PAM.

Select Target Genome: human GRCh38

Input Transcript IDs, Gene IDs/Symbols, or raw DNA sequence:

Enter up to 100 Human or Mouse **RefSeq** Transcript IDs (e.g., NM_014911, NM_014911.3, etc.), **NCBI** Gene IDs or Gene Symbols (e.g., 988, CDC5L, etc.), *or* a **single** nucleotide sequence of at least 30 bases.

Please refer to our sgRNA Designer Help Page for details on how a transcript is chosen for a gene input.

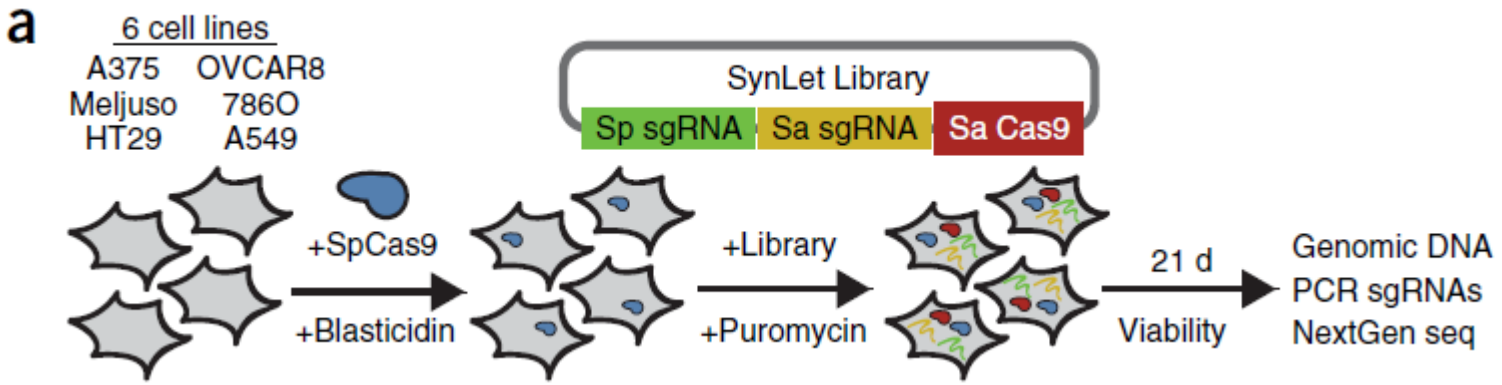
Or Upload a list of Transcript IDs, Gene IDs/Symbols, or a FASTA file of DNA sequences:

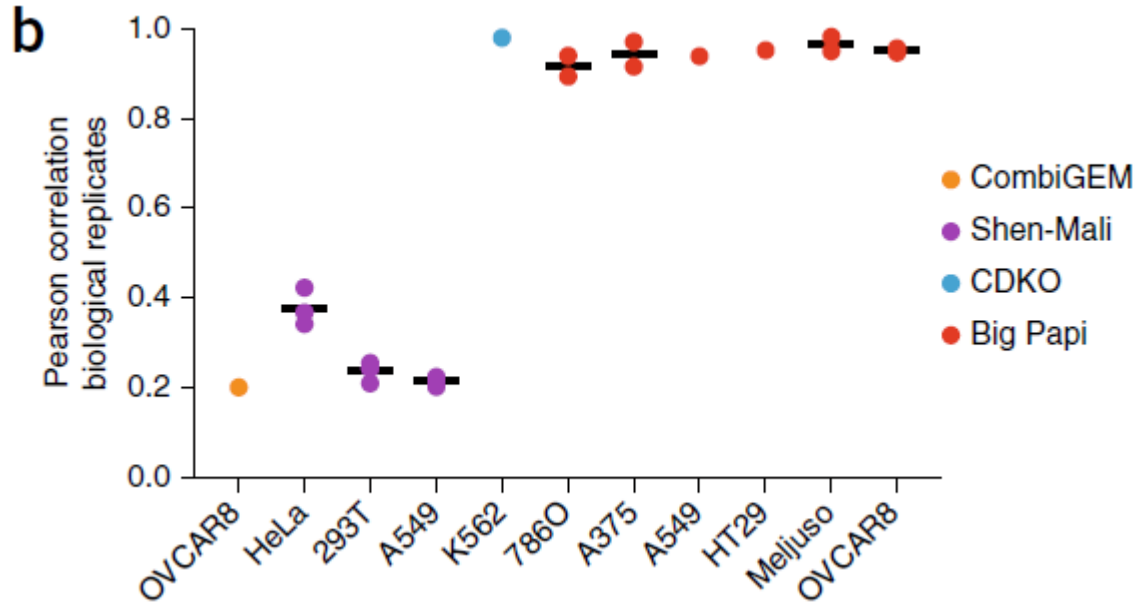
Next Steps

Combinatorial gene targeting

- To test the Big Papi approach by screening for **synthetic lethal gene combinations**
- Selected gene families with known or potential redundancy in their function, including **MAPKs, AKTs, and ubiquitins**
- Each gene pair was assessed with **18 unique sgRNA combinations** (2 Cas9s × 3 gene A sgRNAs × 3 gene B sgRNAs)
- Targeted two control genes: **EEF2** (3 sgRNAs), a core essential gene, and **CD81** (10 sgRNAs), a cell surface marker with two sets of negative controls, sgRNAs that target introns of **HPRT1** (5 sgRNAs)
- The resulting 96 × 96 = 9,216 member **SynLet library** was packaged into lentivirus for use in six diverse tumor cell lines engineered to express SpCas9: A375 (skin); Meljuso (skin); HT29 (colon); A549 (lung); 786O (kidney) and OVCAR8 (ovary)

Improvement in model performance

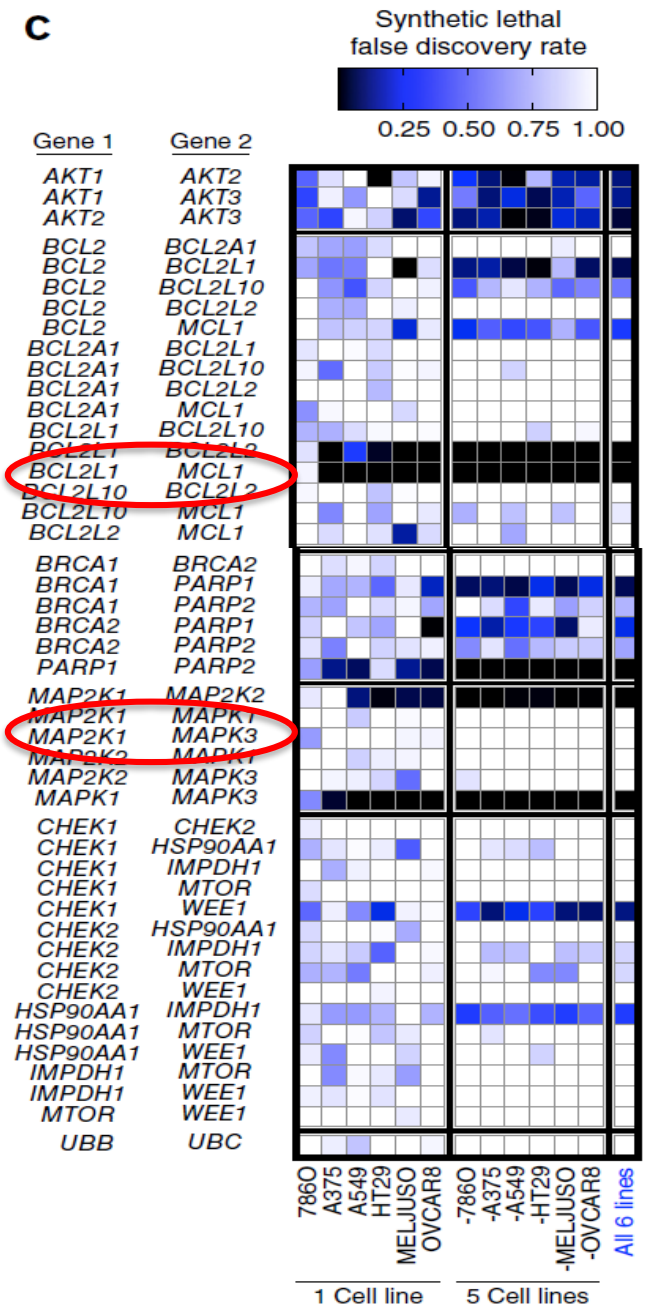


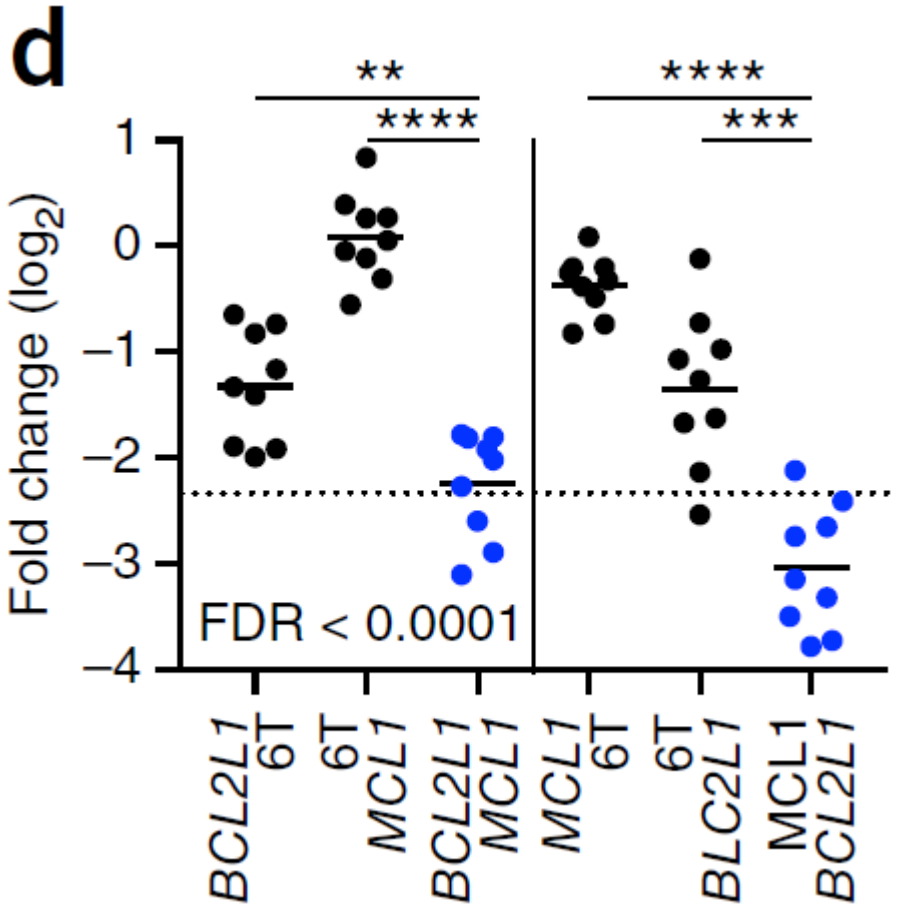


Independent infection replicates well correlated for all six cell lines

Primary screen data

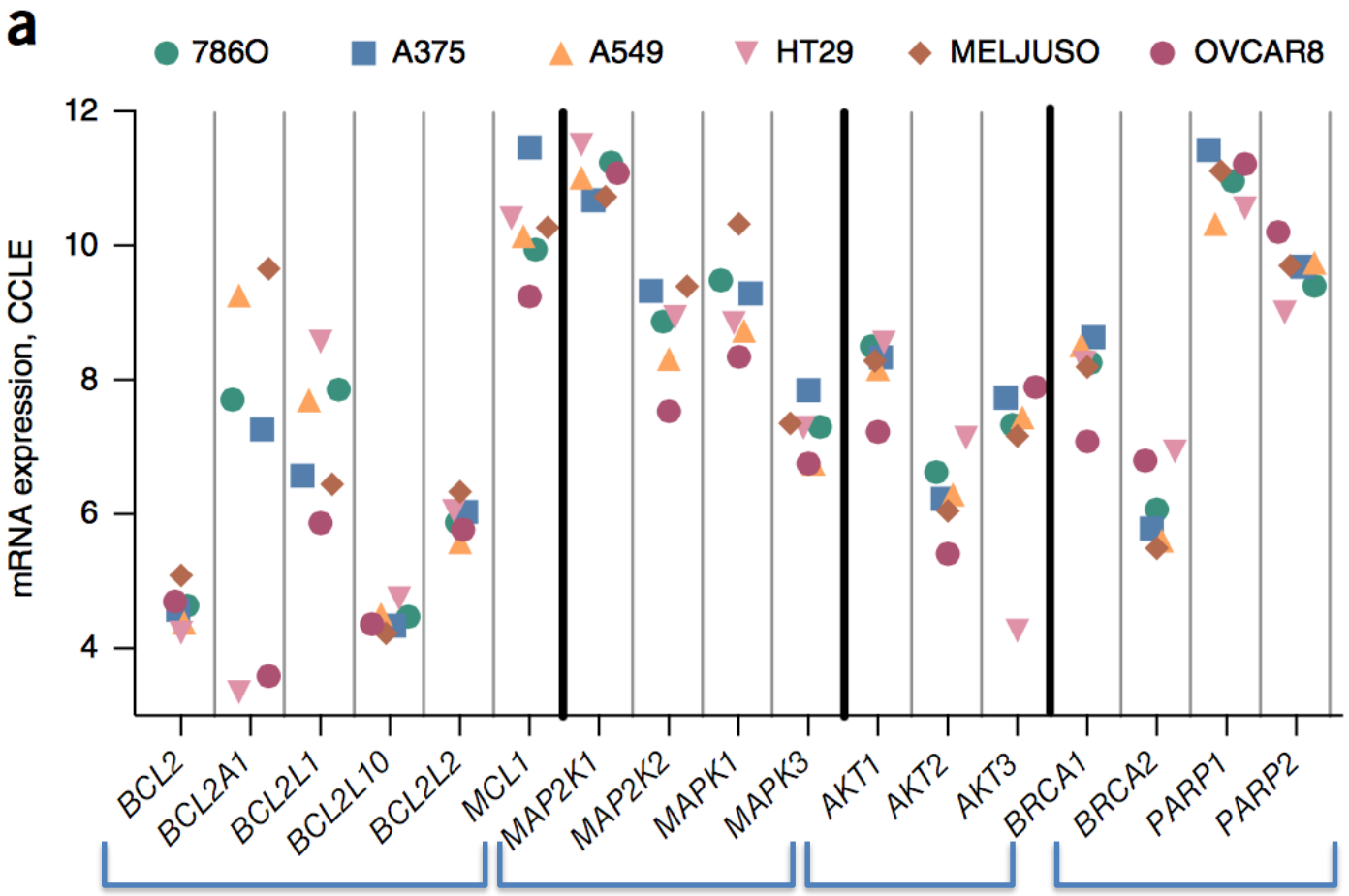
Performance of sgRNAs for BCL2L1 & MCL1





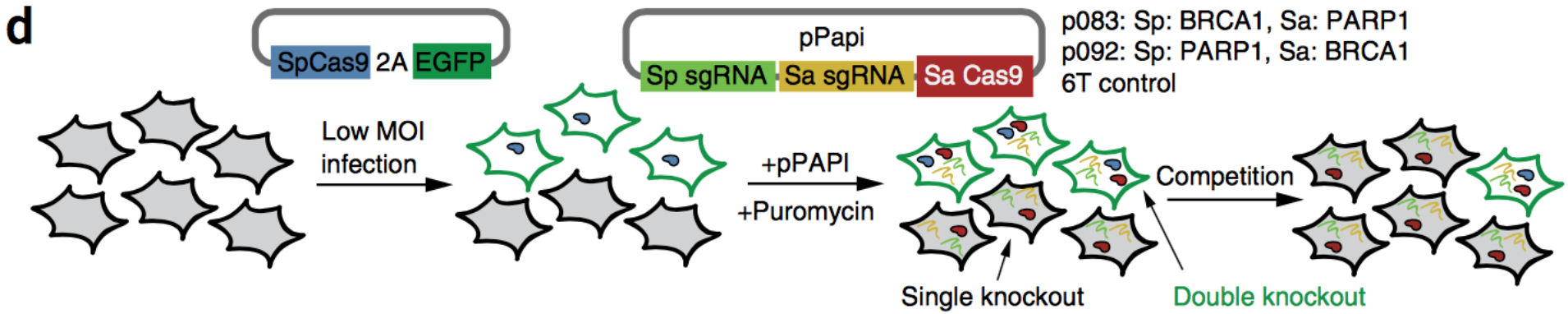
Genetic interactions & Gene expression values

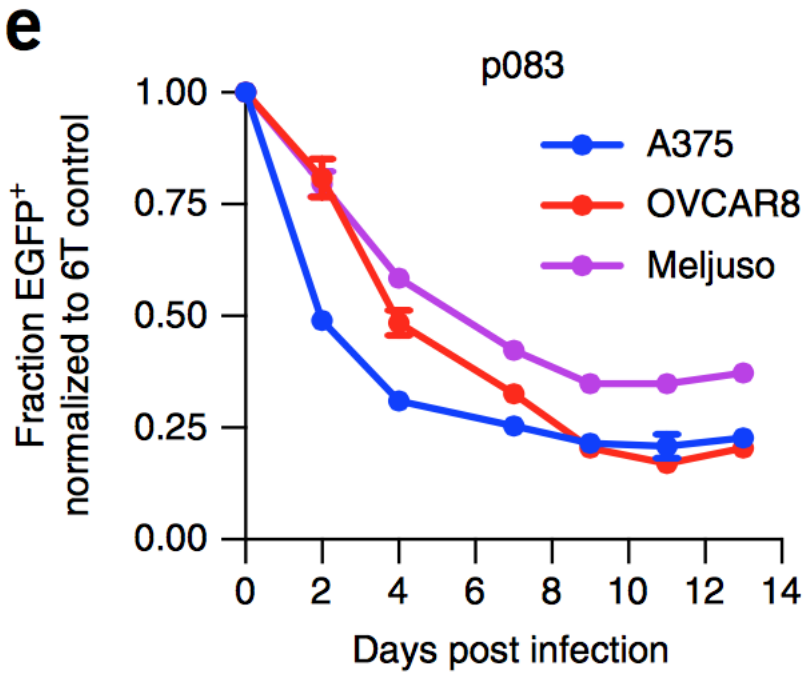
6 tumor cell lines engineered to express SpCas9 (kidney, skin, lung, colon, skin, ovary)

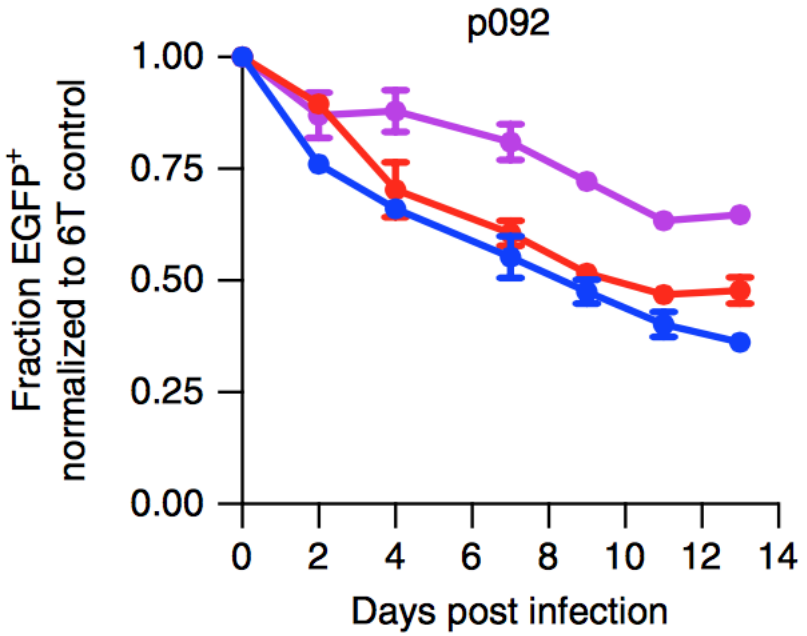


Competition assay in three cell types

To compare cell viability of single vs. double KO of BRCA1 and PARP1

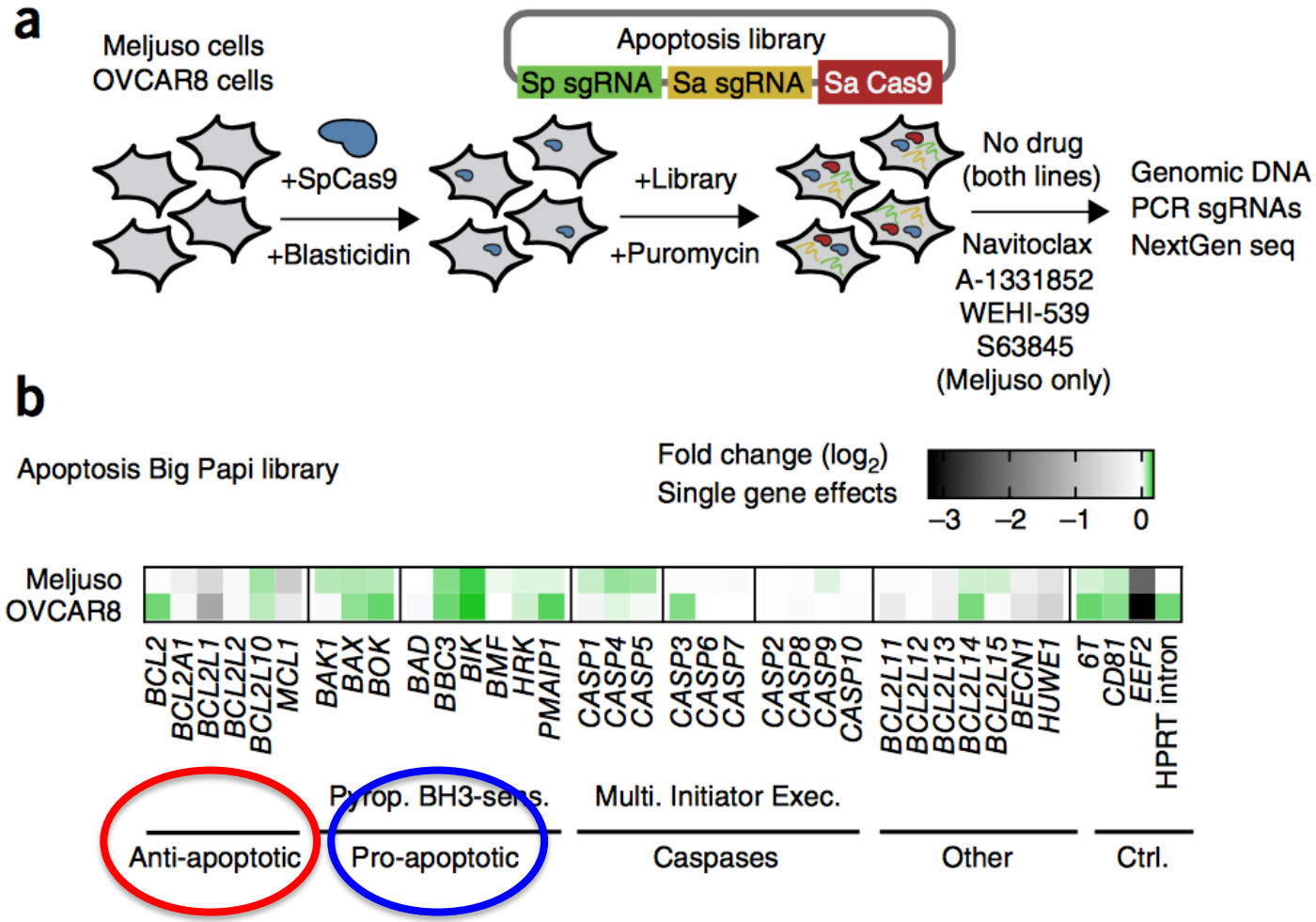




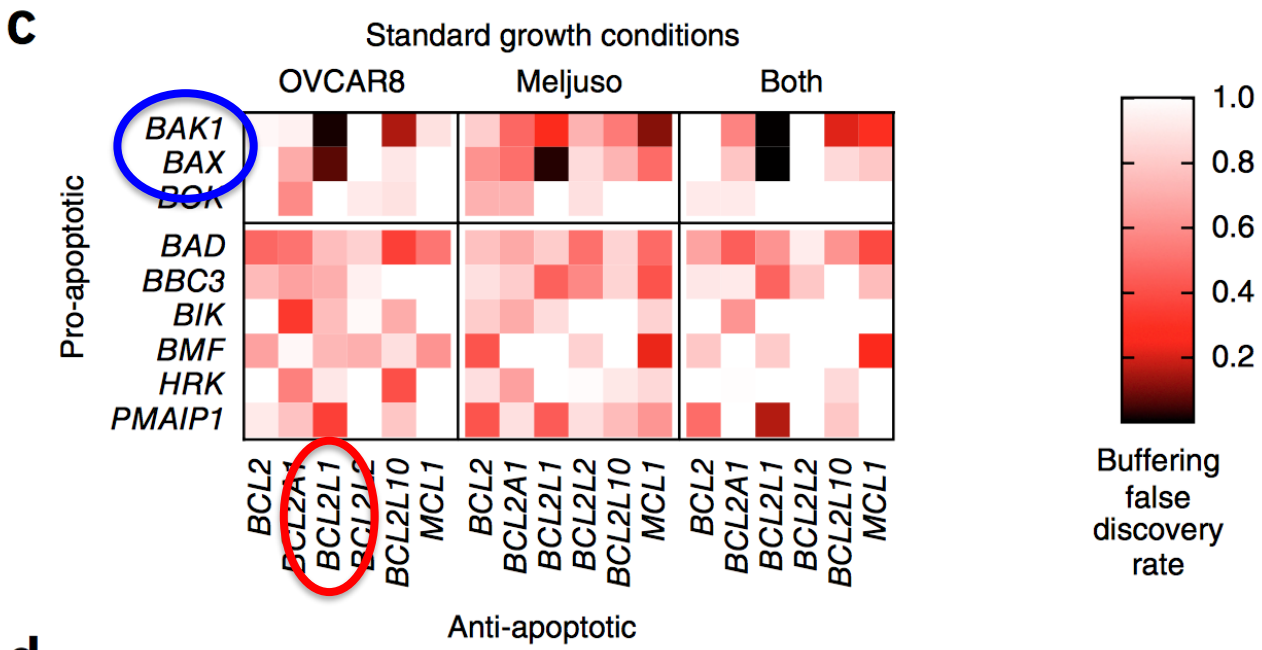


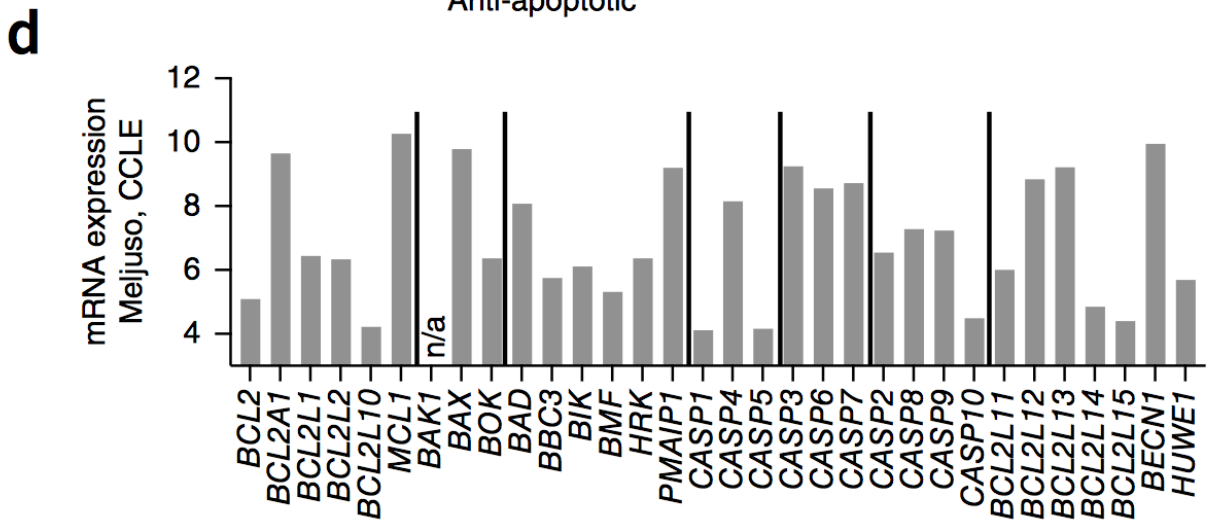
Apoptosis screen

Genes targeted by the Apoptosis library and cell viability effects



Apoptosis screen





Dual Cas9 System to identify genetic interactions

Applications / Advantages

- efficient, cost effective, and supports pooled library generation and screening
- identified interactions within several groups of functionally related genes with high statistical confidence
- to map buffering interactions between genes involved in apoptosis, both in standard growth conditions, revealing additional genetic interactions
- combined CRISPR-mediated knockout and overexpression to uncover interactions with *TP53*
- reasonable performance with only two sgRNAs per gene
- **Big Papi approach: powerful mean to map genetic interactions in mammalian cells**

Dual Cas9 System to identify genetic interactions

Open questions / Disadvantages

- The importance of cell context in detecting interactions
- **SynLet Library**: no gene pair scored strongly (FDR < 0.01) in all six cell lines
- Some interactions detected more strongly in some lines compared to others: influence of mutation status and mRNA expression?
- Combining information across cell lines – useful strategy for detecting generalizable interactions?

nature biotechnology

Dual gene activation and knockout screen reveals directional dependencies in genetic networks

Michael Boettcher[1], Ruilin Tian[2], James A Blau[1], Evan Markegard[3], Ryan T Wagner[1], David Wu[1], Xiulei Mo[4], Anne Biton[5,6], Noah Zaitlen[5], Haian Fu[4], Frank McCormick[3], Martin Kampmann[2] & Michael T McManus[1]

[1]Department of Microbiology and Immunology, University of California San Francisco Diabetes Center, WM Keck Center for Noncoding RNAs, University of California, San Francisco, San Francisco, California, USA. [2]Institute for Neurodegenerative Diseases, Department of Biochemistry and Biophysics, University of California, San Francisco and Chan Zuckerberg Biohub, San Francisco, California, USA. [3]Helen Diller Family Comprehensive Cancer Center, Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, California, USA. [4]Department of Pharmacology and Emory Chemical Biology Discovery Center, Emory University School of Medicine, Atlanta, Georgia, USA. [5]Department of Medicine, Lung Biology Center, University of California, San Francisco, San Francisco, California, USA. [6]Centre de Bioinformatique, Biostatistique et Biologie Intégrative (C3BI, USR 3756 Institut Pasteur et CNRS), Paris, France. Correspondence should be addressed to M.T.M. (michael.mcmanus@ucsf.edu).

Study Rationale

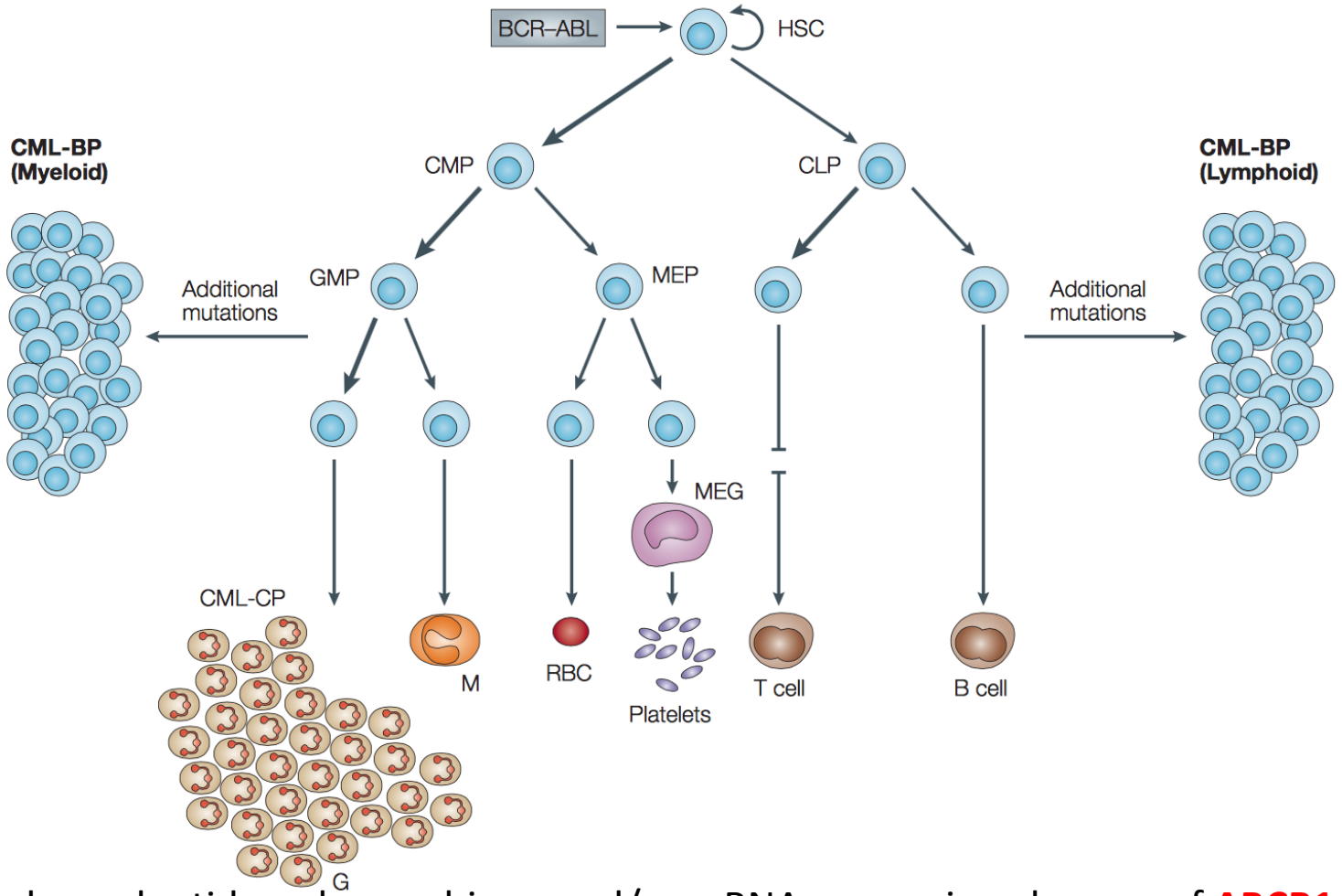
→ To identify directional genetic interactions between genes whose activation or ablation alter the fitness of human chronic myeloid leukemia (CML) cells

↓

- ✓ Orthogonal screening approach can quantify loss- and gain-of-function phenotypes from the same cell
- ✓ To systematically identify genetic interactions between cancer-relevant genes

Context

Development of chronic myeloid leukemia



Three single-nucleotide polymorphisms and/or mRNA expression changes of **ABCB1** gene were demonstrated to be associated with inter- individual variability of **imatinib** response in CML patients

Ren R, Nature Reviews Cancer 2005

CRISPR screening conditions

➢ Ultra-complex, genome-scale sgRNA library consisting of over 260,000 total sgRNAs targeting every coding and over 4,000 non-coding Refseq annotated (hg19) transcripts in the human genome

✓ Characterize K562 cell response to a broad range of imatinib drug concentrations

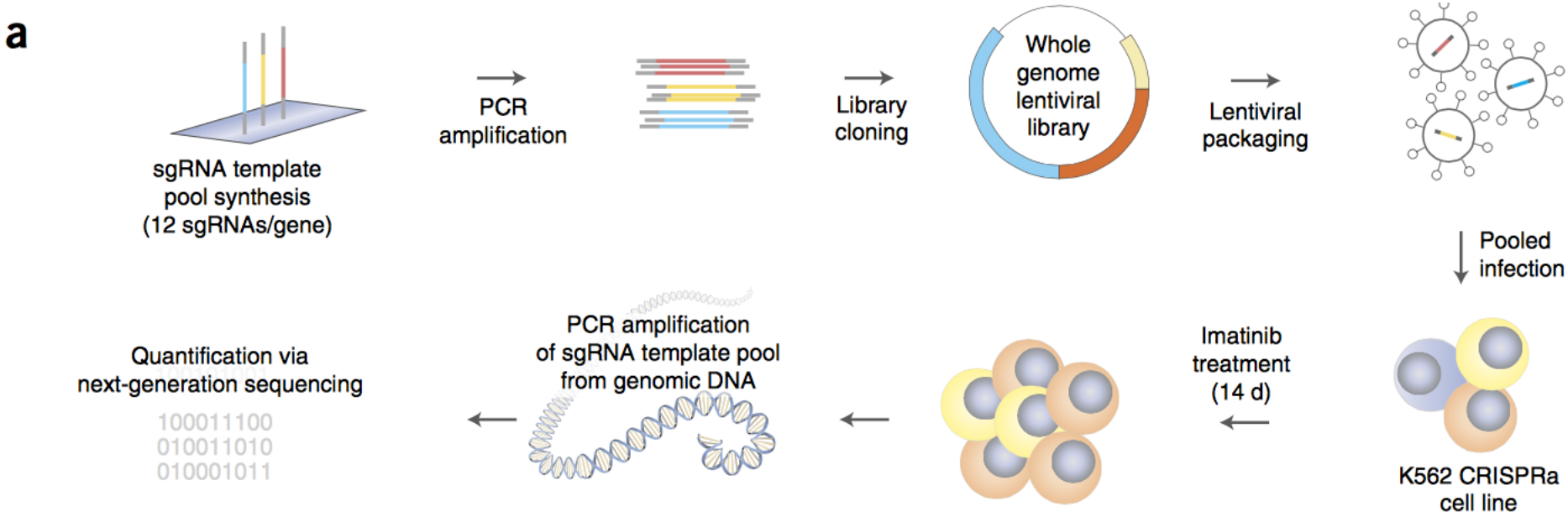
✓ Quality-controlled sgRNA libraries

✓ Abundance of sgRNA-encoding sequences via NGS: comparing the beginning (baseline) and endpoint (day 14) of the screen

✓ Normalized to define an enrichment score (τ) for each gene

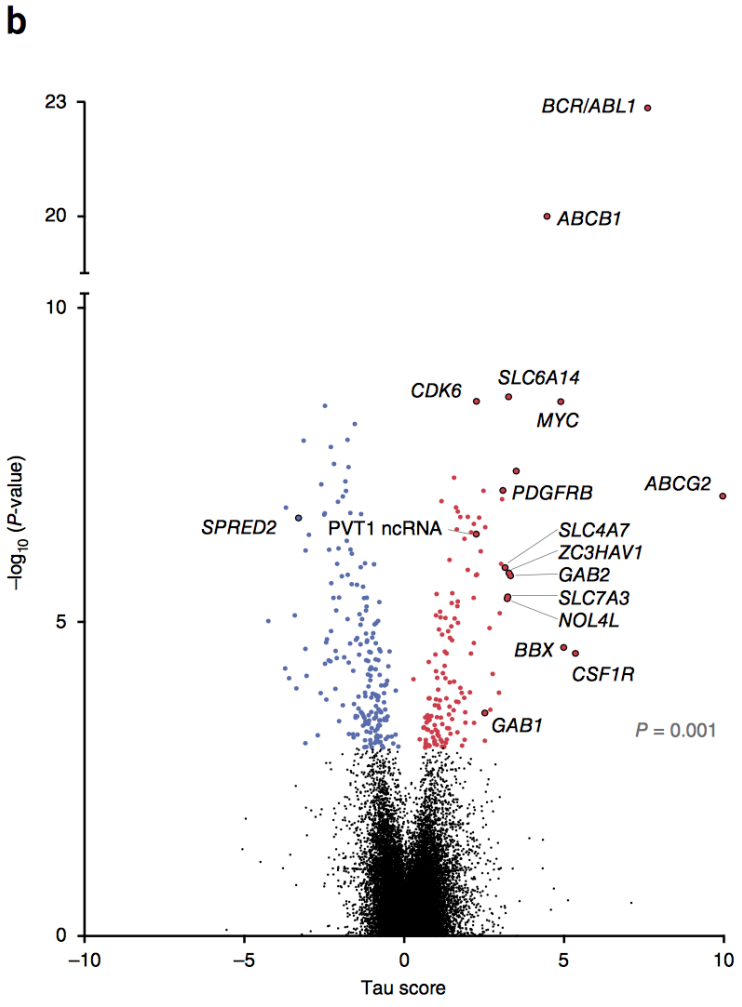
Schematic CRISPRa screening approach

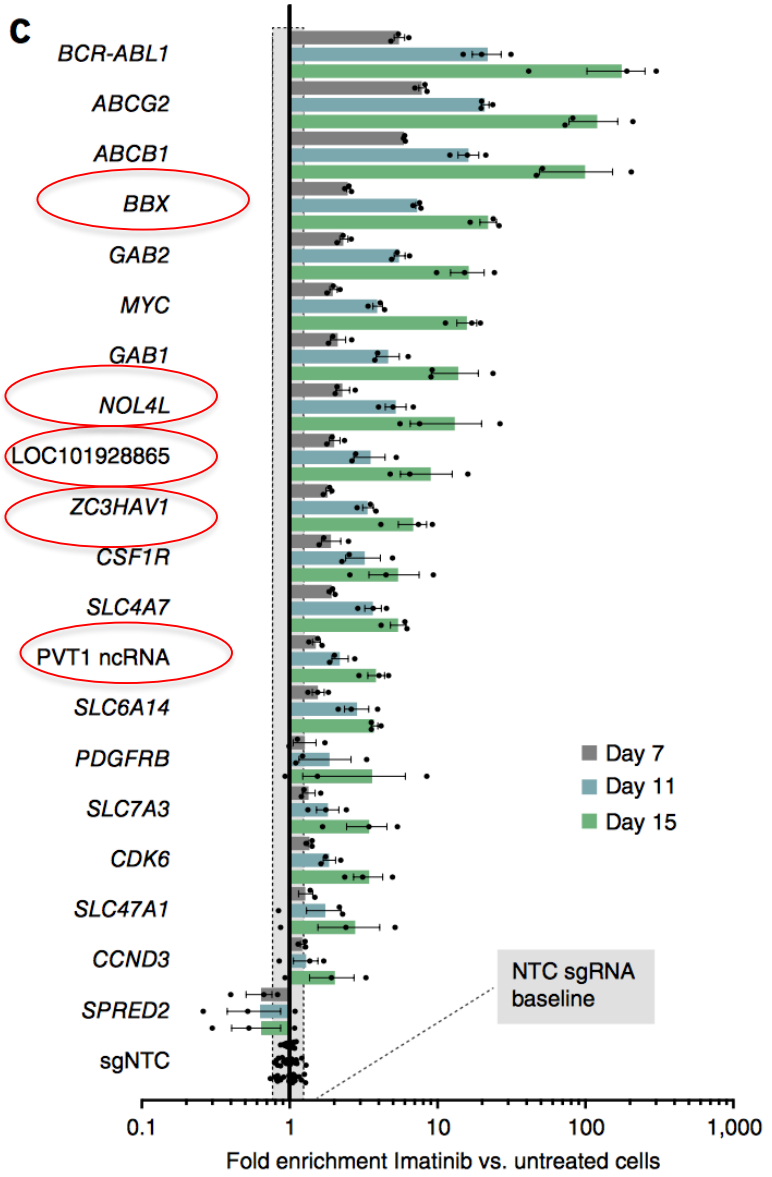
To systematically identify genes whose activation can alter imatinib drug response



Candidate genes of CRISPRa screen

Red: cell enrichment (43%)
Blue: depletion (57%)





GSEA & pathway reconstruction

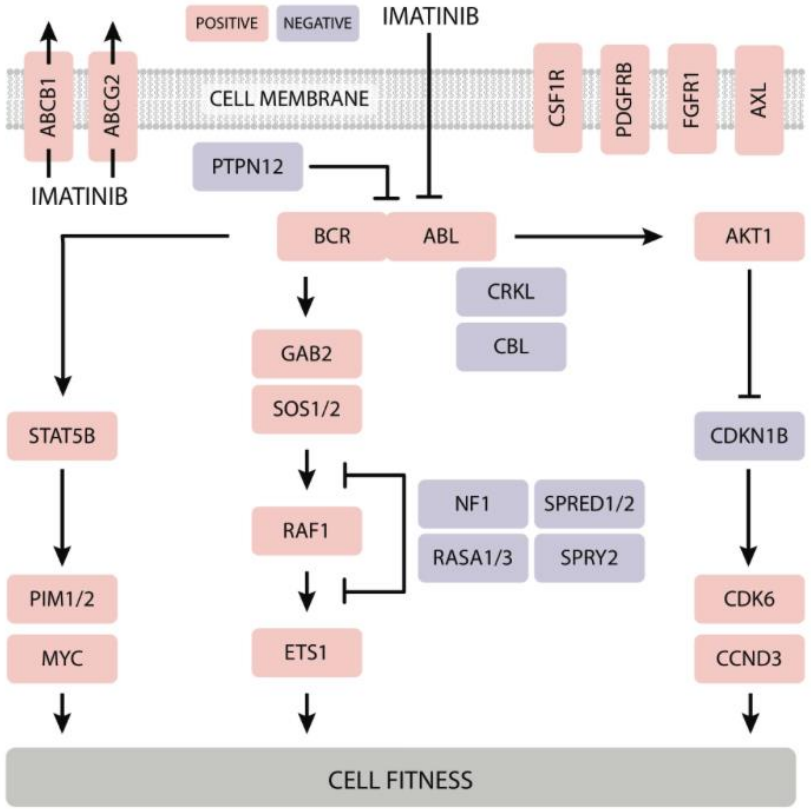
332 target genes identified
in the imatinib CRISPRa screen

Quality of the screening data:
Gene Set Enrichment Analysis
(GSEA)

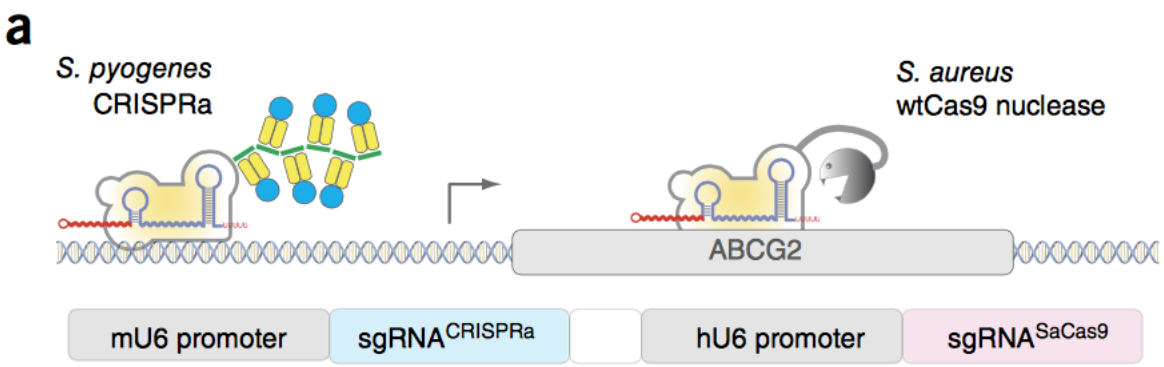
a

KEGG Pathway	Enrichment	p	FDR
Acute myeloid leukemia	11.54	6.6E-10	8.08E-07
Chronic myeloid leukemia	9.67	1.2E-09	1.45E-06
ErbB signaling pathway	8.00	1.3E-08	1.63E-05
Pathways in cancer	3.42	4.5E-08	5.49E-05
Transcriptional misregulation in cancer	5.03	1.9E-07	2.30E-04
MAPK signaling pathway	3.90	6.0E-07	7.45E-04

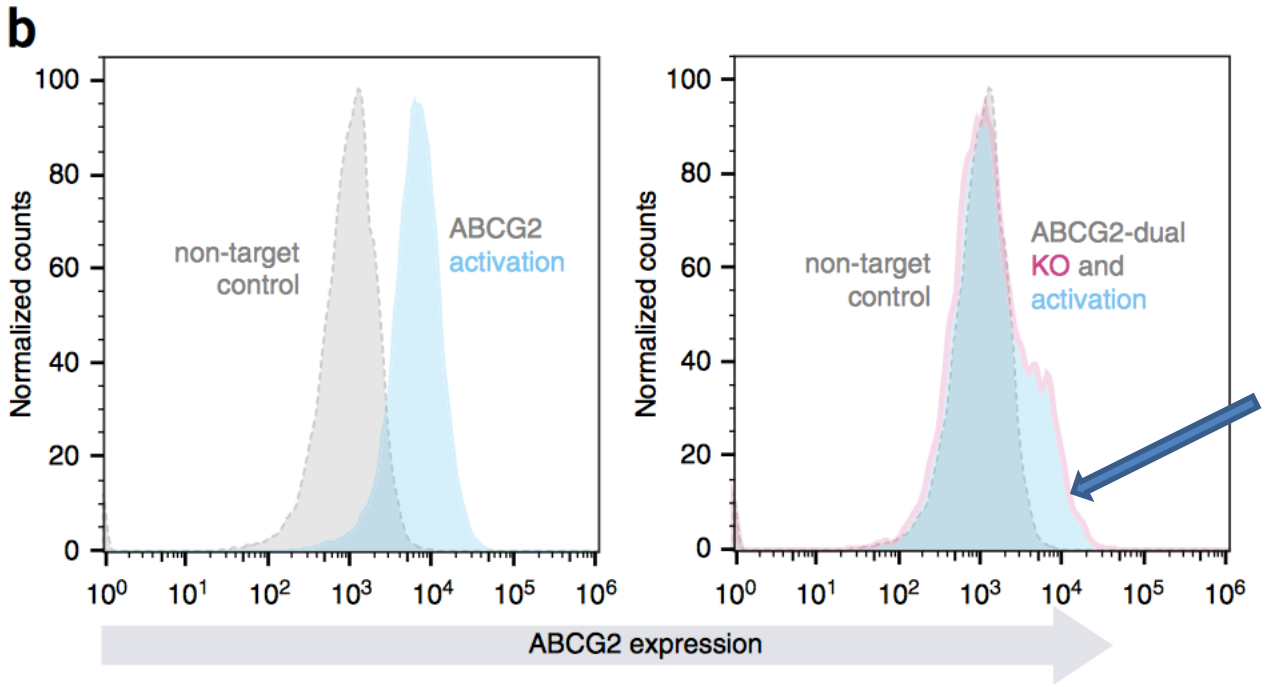
b



The orthogonal CRISPR system



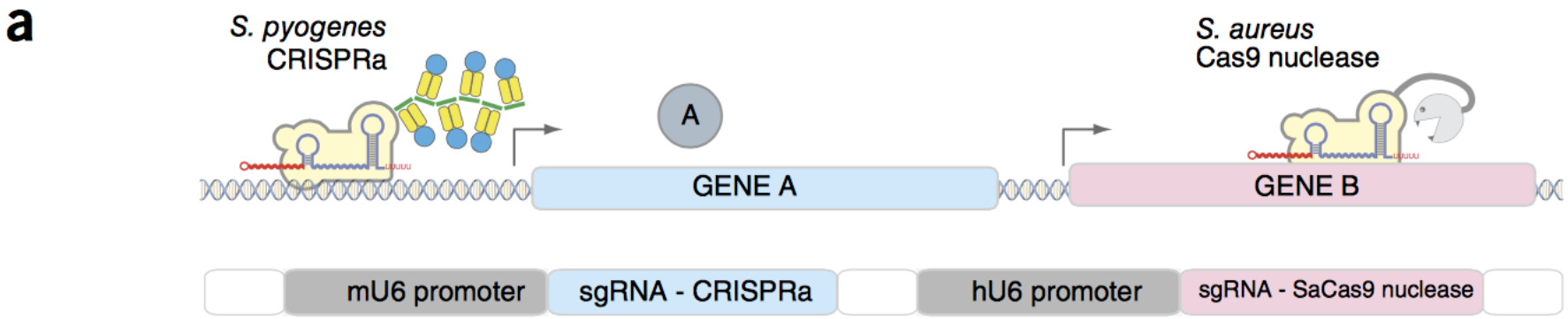
Simultaneous activation and KO of genes in the same cell by expressing two appropriate sgRNAs

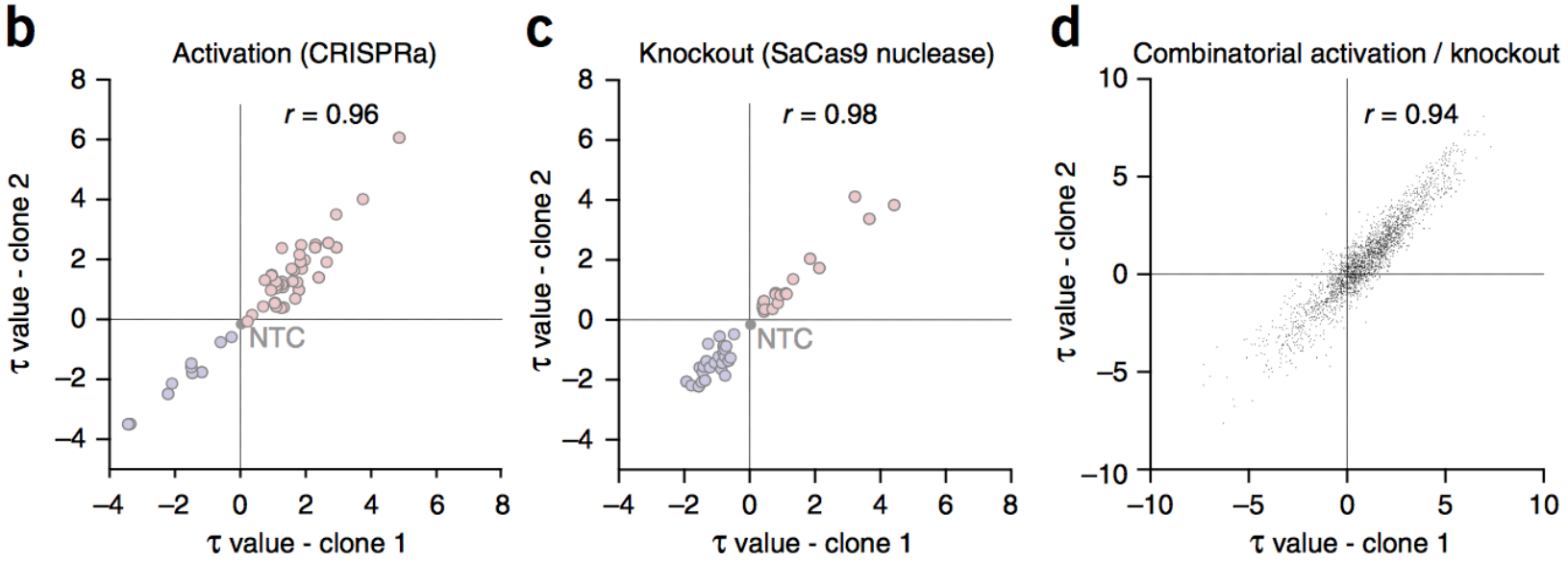


non-edited/in-frame indels of the ABCG2 gene

Directional gene interaction

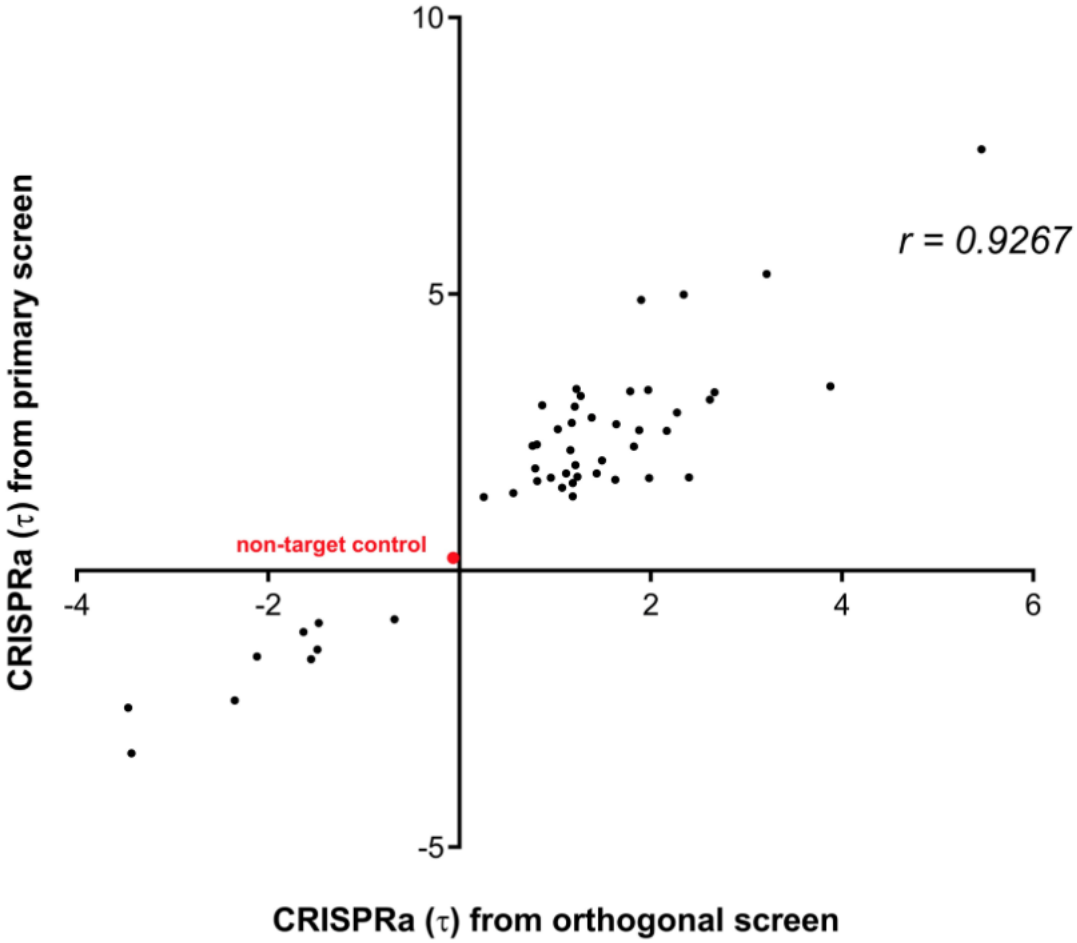
Concept of the application of the orthogonal system for DGI





Correlation of T values from two clonal cell line replicates – replicate performance

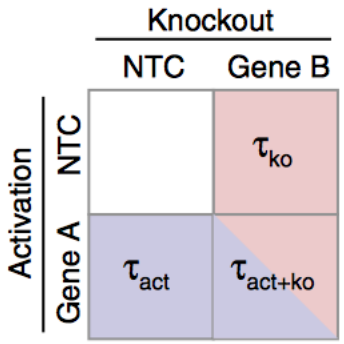
Screen reproducibility



Candidate genes from primary CRISPRa screen reproduce in orthogonal screen

Deducing directional dependencies

e



Blue: depleted
Red: enriched
NTC: non-target control sgRNA

f

Directionality score

$\Psi = \tau_{act} \times \tau_{ko} \times GI^2$

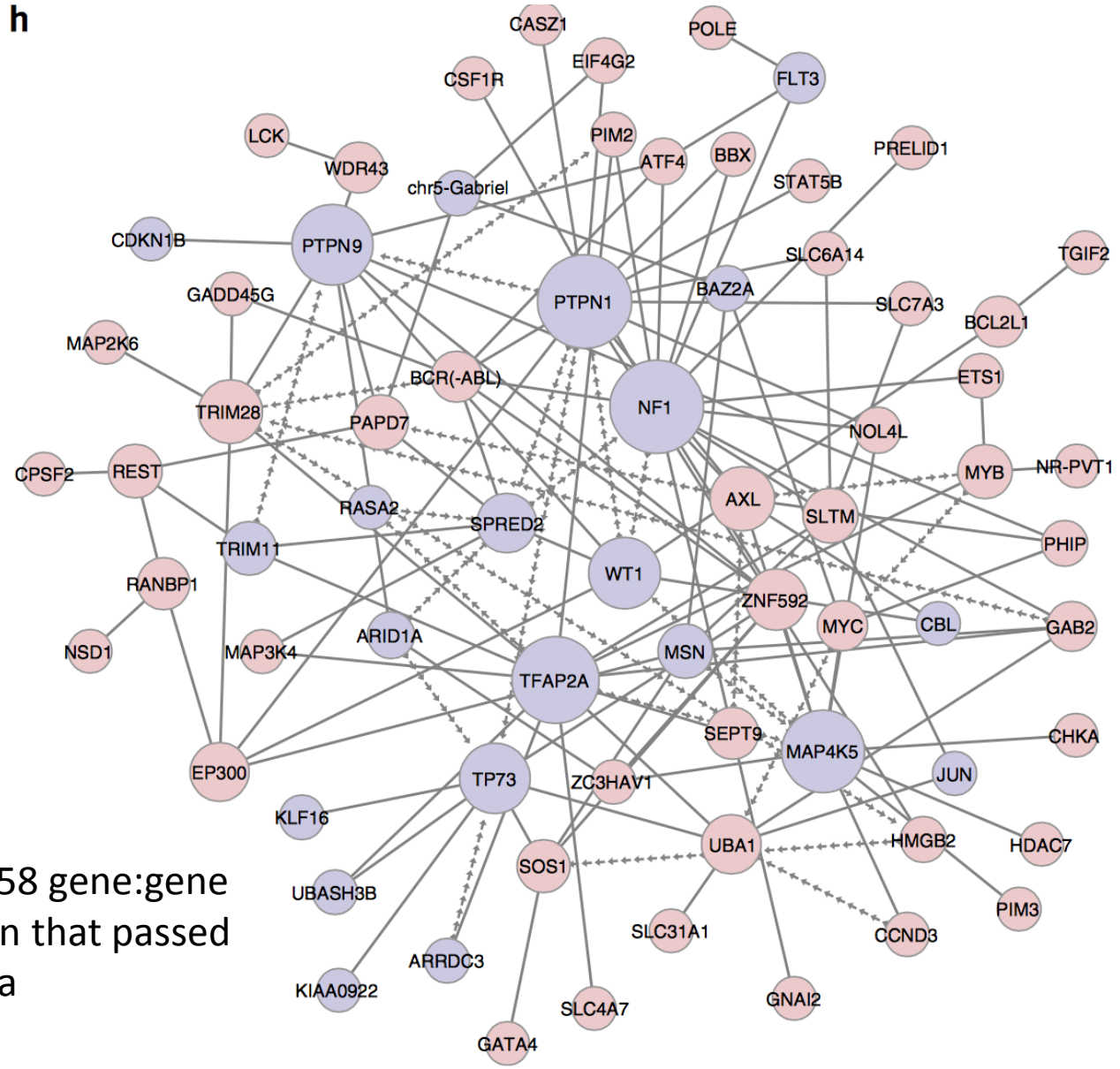
GI: genetic interaction score

g

Activated gene functions **downstream** of the deleted gene

Activated gene functions **upstream** of the deleted gene

Genetic interaction model

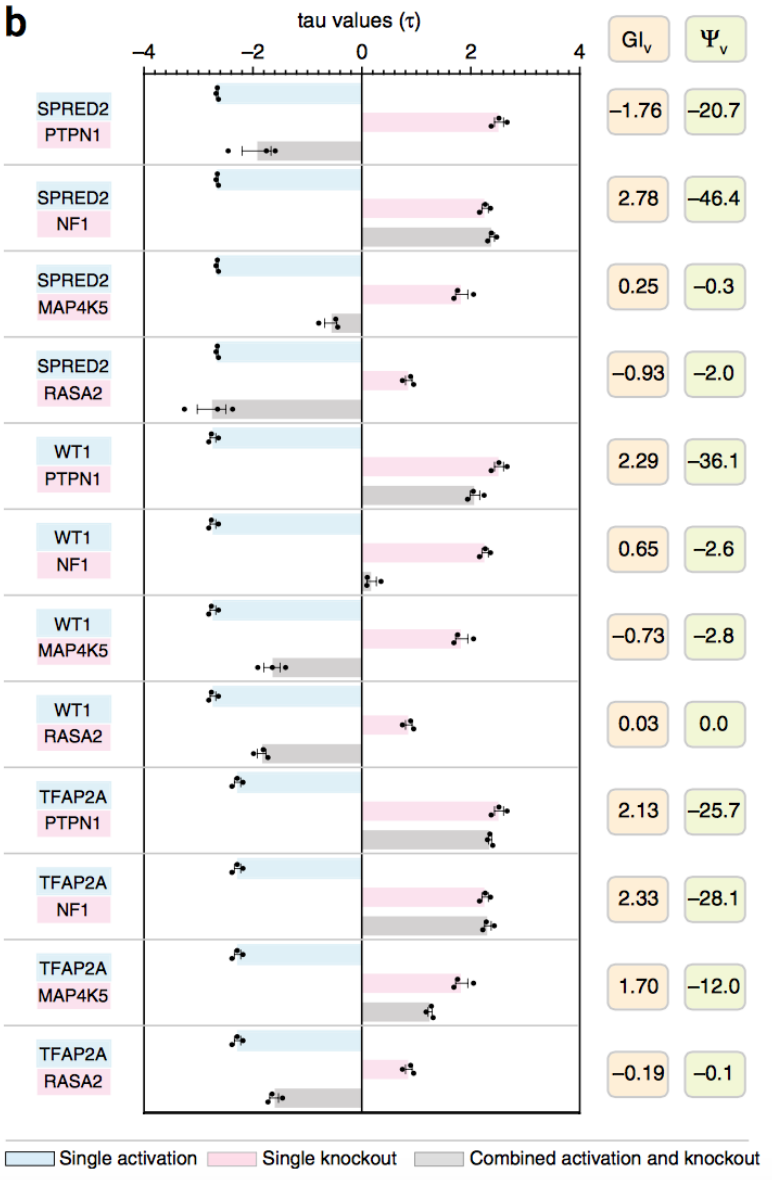


Red: positive
Blue: negative regulators

Total of 2,258 gene:gene combination that passed filter criteria

Validated directional genetic interaction

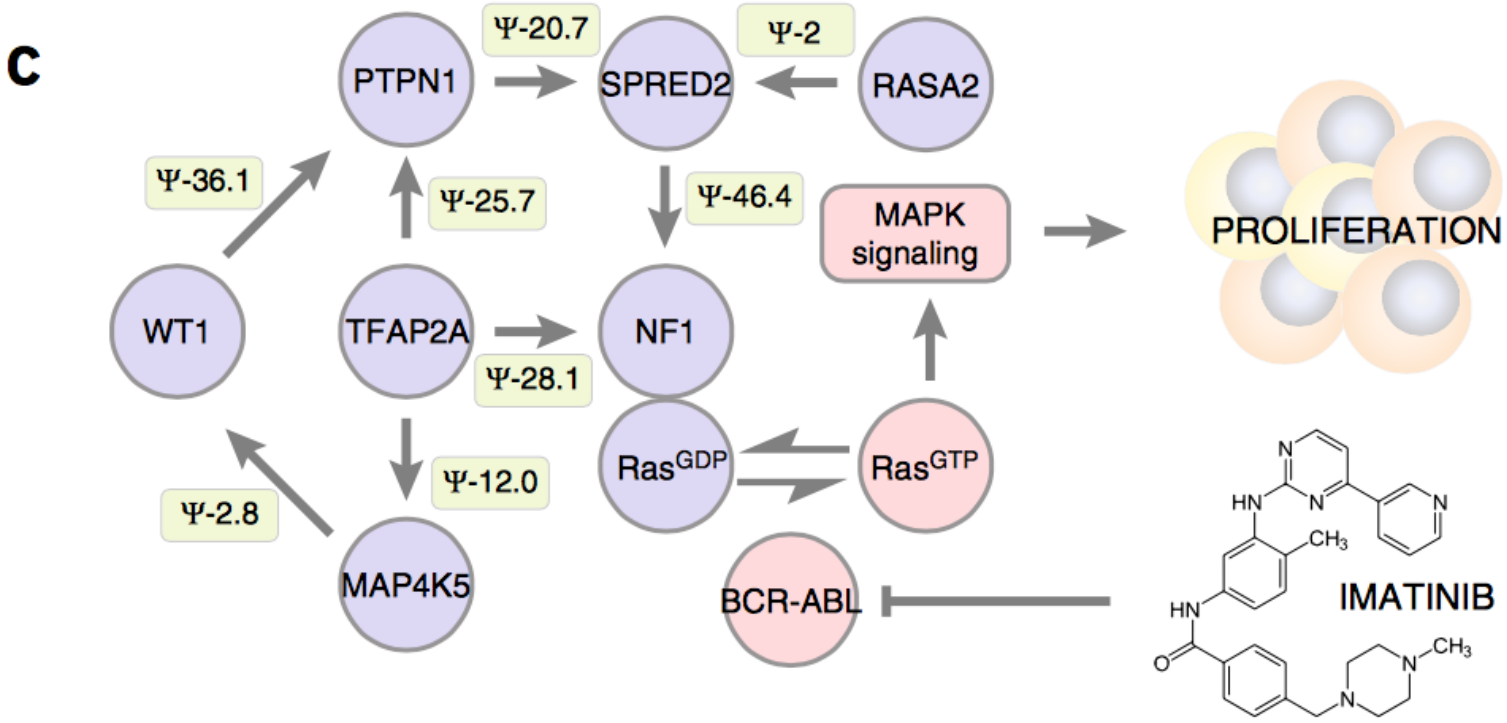
12 activation/KO combinations retested



Activation of *SPRED2, WT1, TFAP2A* – sensitizing effect to imatinib

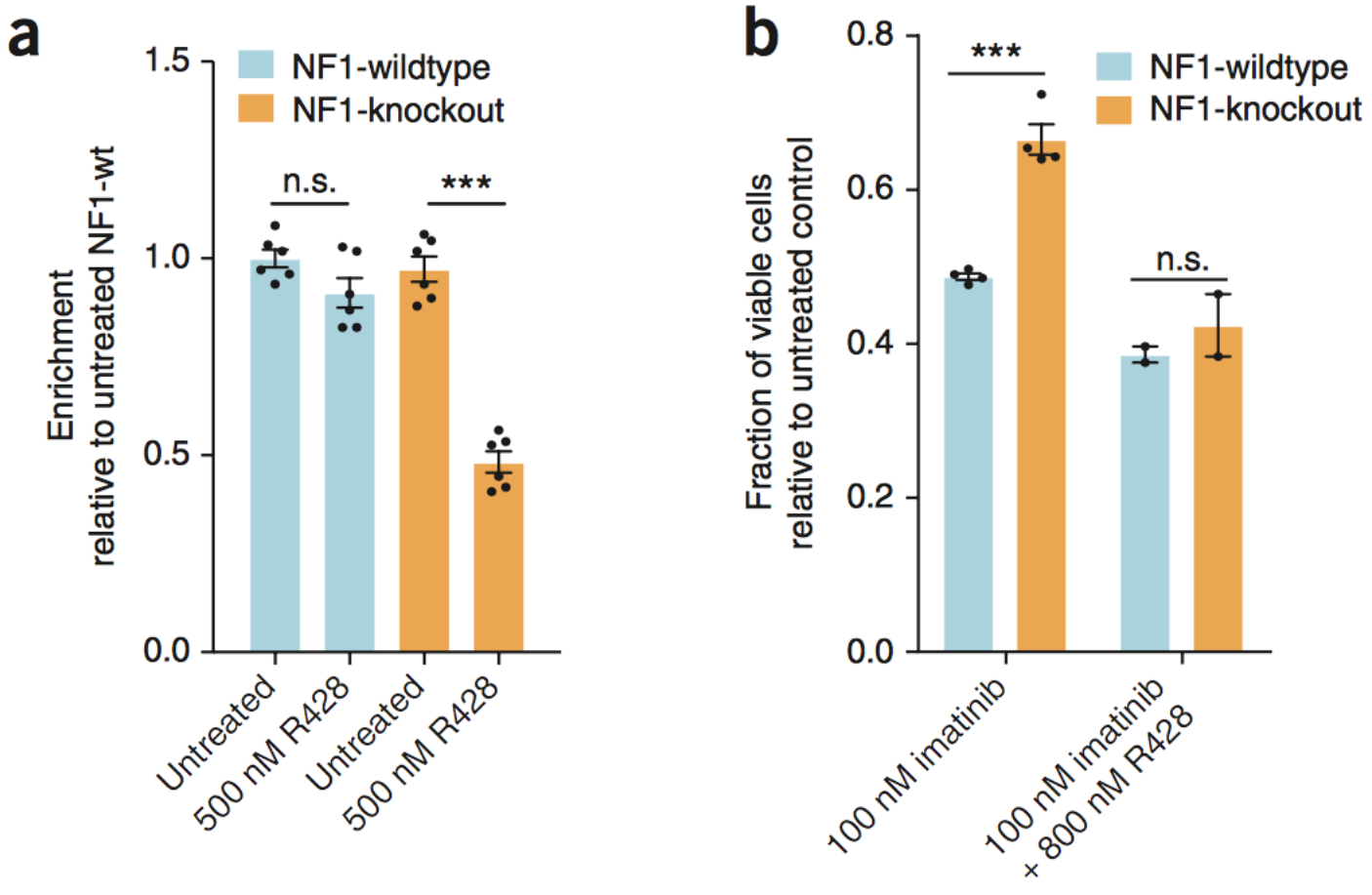
Deletion of of *PTPN1, NF1, MAP4K5, RASA2* – enriched cells in the culture over time

Directional genetic interaction model



Arrows: direction of functional dependencies

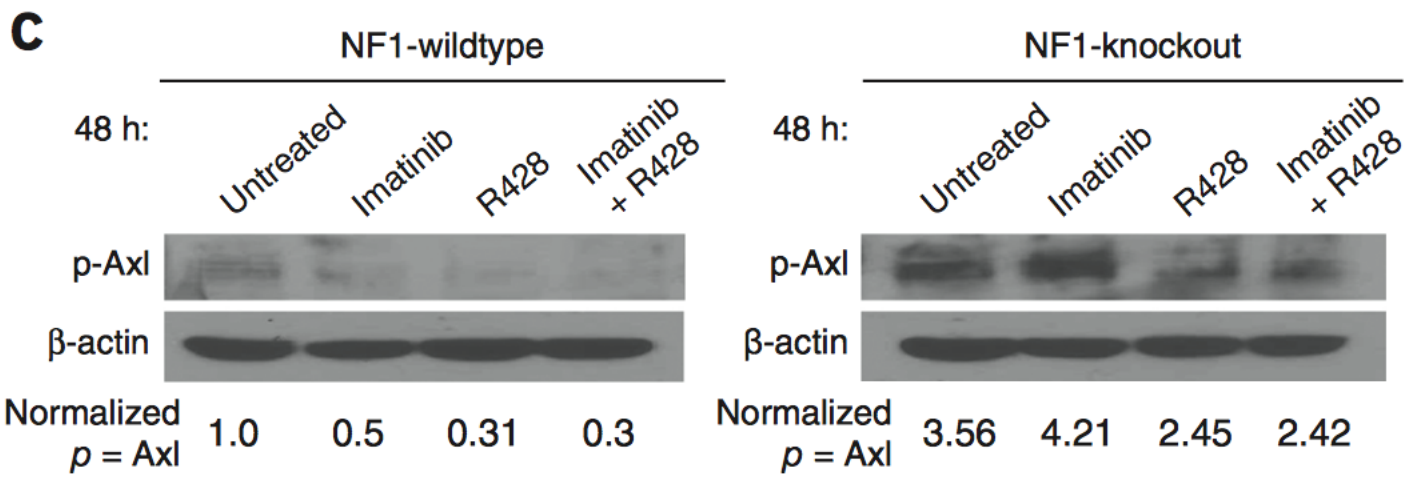
Targeting AXL-mediated pathway

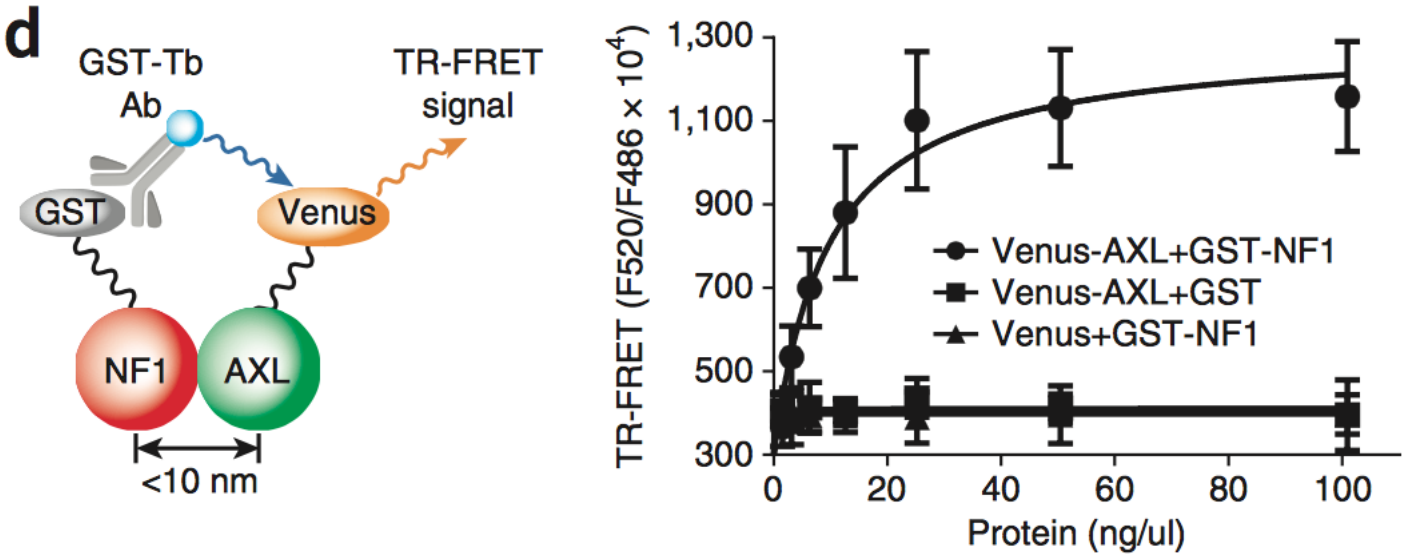


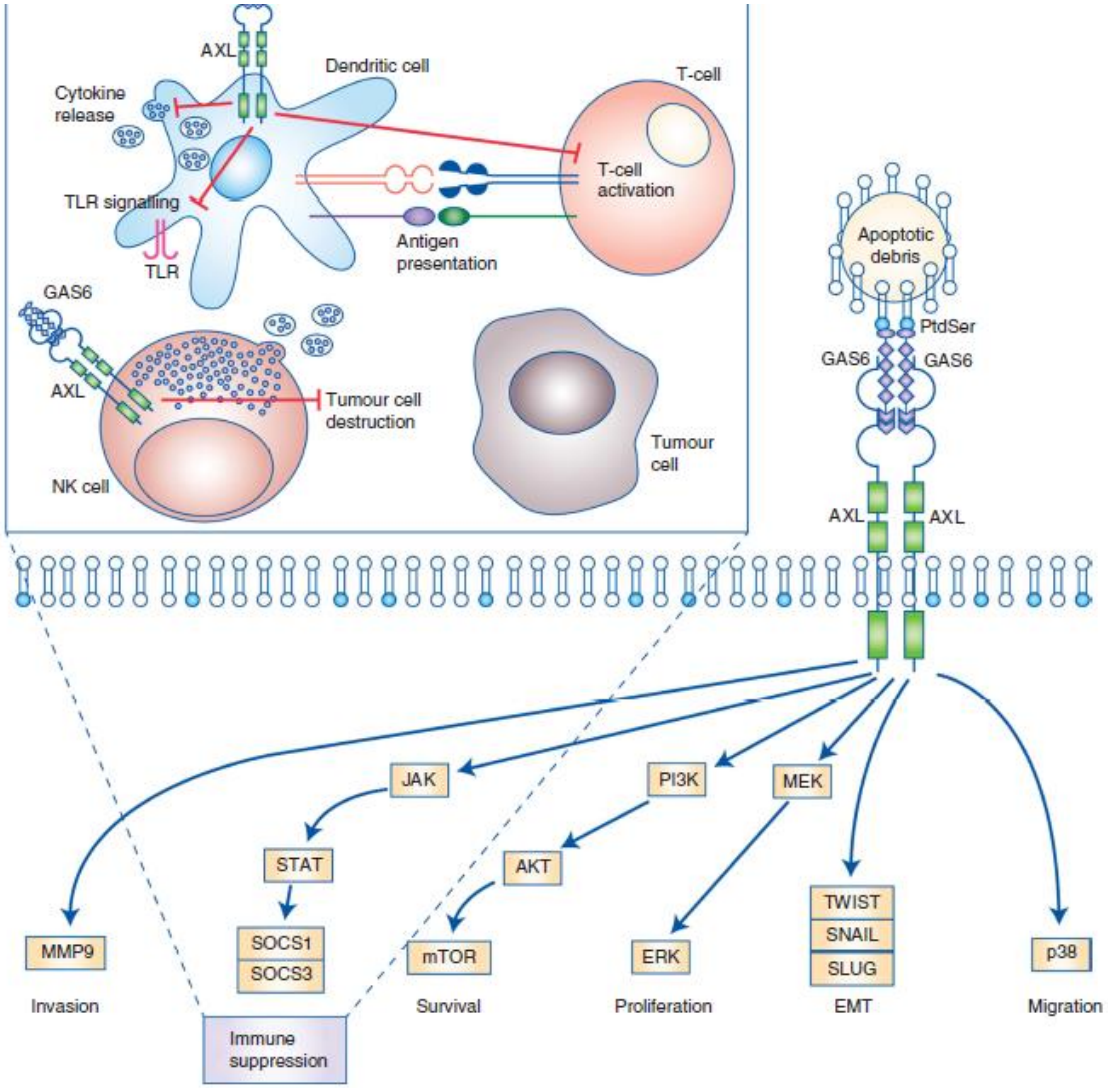
R428: AXL inhibitor

AXL – promising target for cancer therapeutics

Targeting AXL-mediated pathway

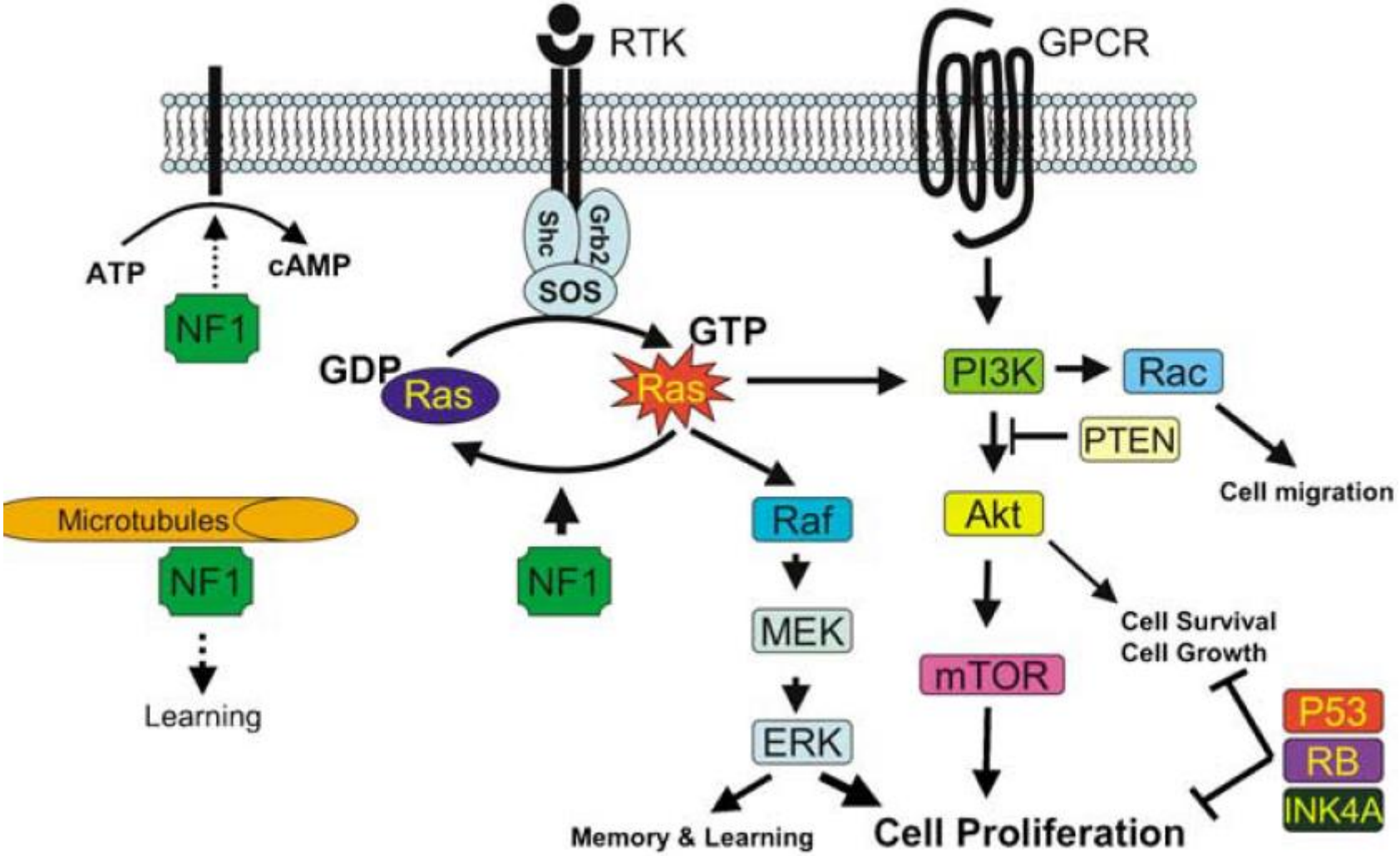






Cellular processes regulated by AXL activity

Gay et al, BJC 2017



NF1 interactions with the Ras and PI3K pathways

Le et al, Oncogene 2017

Discussion

Interferring the direction of genetic interactions

➢ The ability for both Cas9 systems to work in parallel to produce activation and knockout phenotypes in the same cell

✓ Suitability of NGS analysis pipeline to accurately quantify phenotypes from combinatorial gene perturbations

✓ Conduct highly parallel directional CRISPR screens in human cancer cells

✓ Inference of directionality strategy is readily applicable to numerous other dual activation/inhibition expression embodiments

✓ High-confidence network of genetic interactions to further understand why some patients respond well to tyrosine kinase inhibitors like imatinib

✓ Directional dependencies need to be considered when designing a treatment plan for patients harboring multiple genetic lesions

nature genetics

ARTICLES

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

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Study Rationale

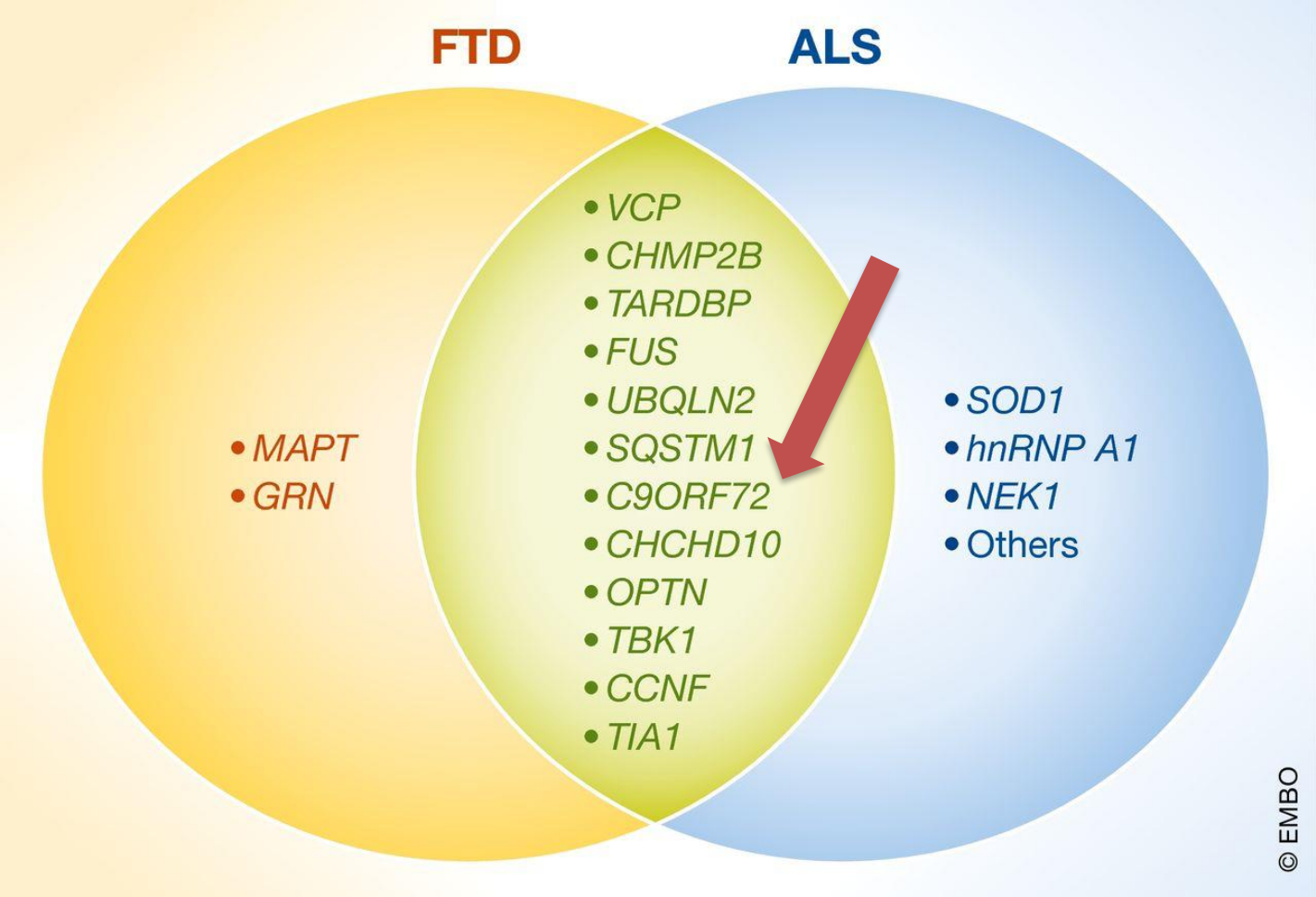
→ CRISPR–Cas9 system to perform comprehensive genome-wide KO screens in human cells and targeted screens in mouse primary neurons to identify genetic modifiers of *C9ORF72* DPR toxicity.

↓

- ✓ Orthogonal screening approach can quantify loss- and gain-of-function phenotypes from the same cell
- ✓ To systematically identify genetic interactions between cancer-relevant genes

Context

Causing genetic mutations of ALS & FTD

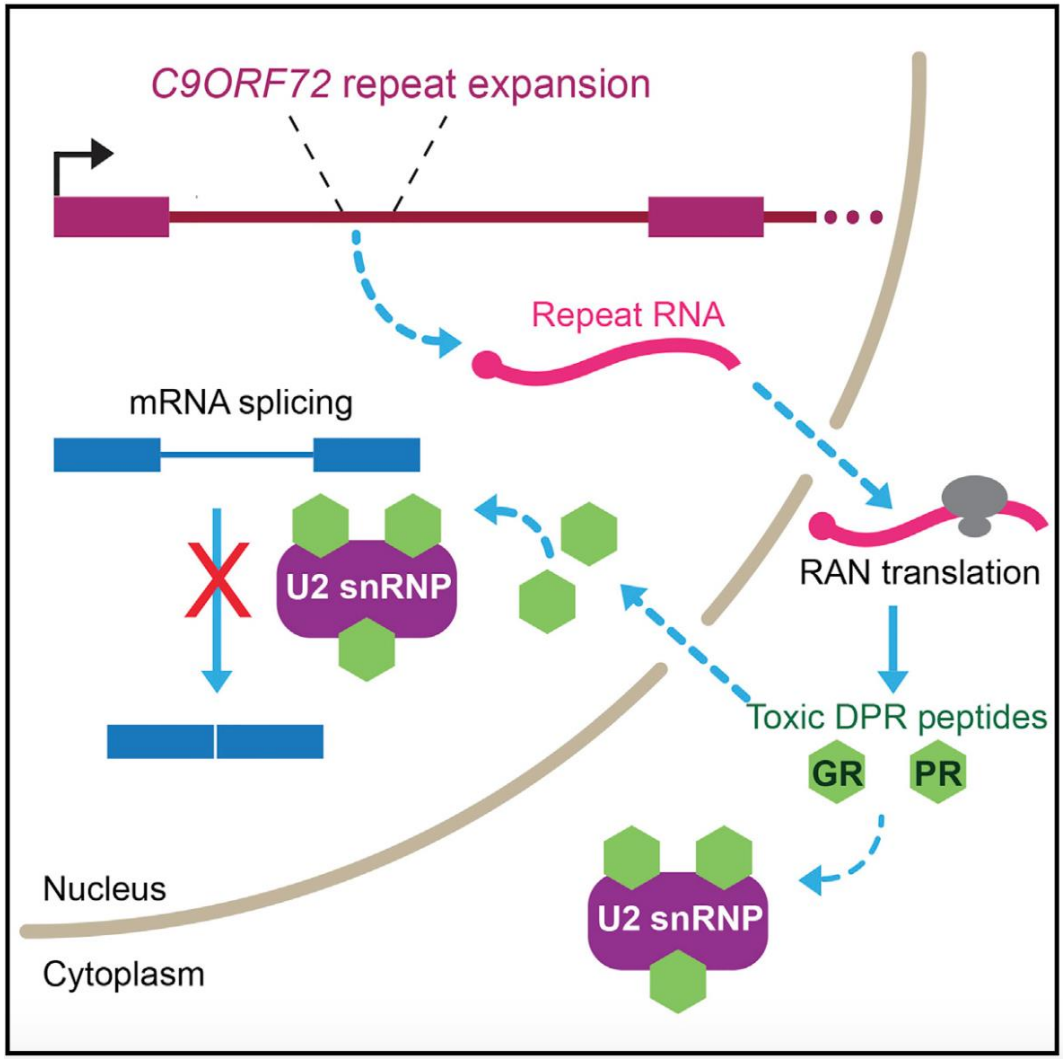


ALS and FTD are related and represent two ends of a spectrum disorder as supported by a large body of recent pathological and genetic evidence

Ren R, Nature Reviews Cancer 2005

Context

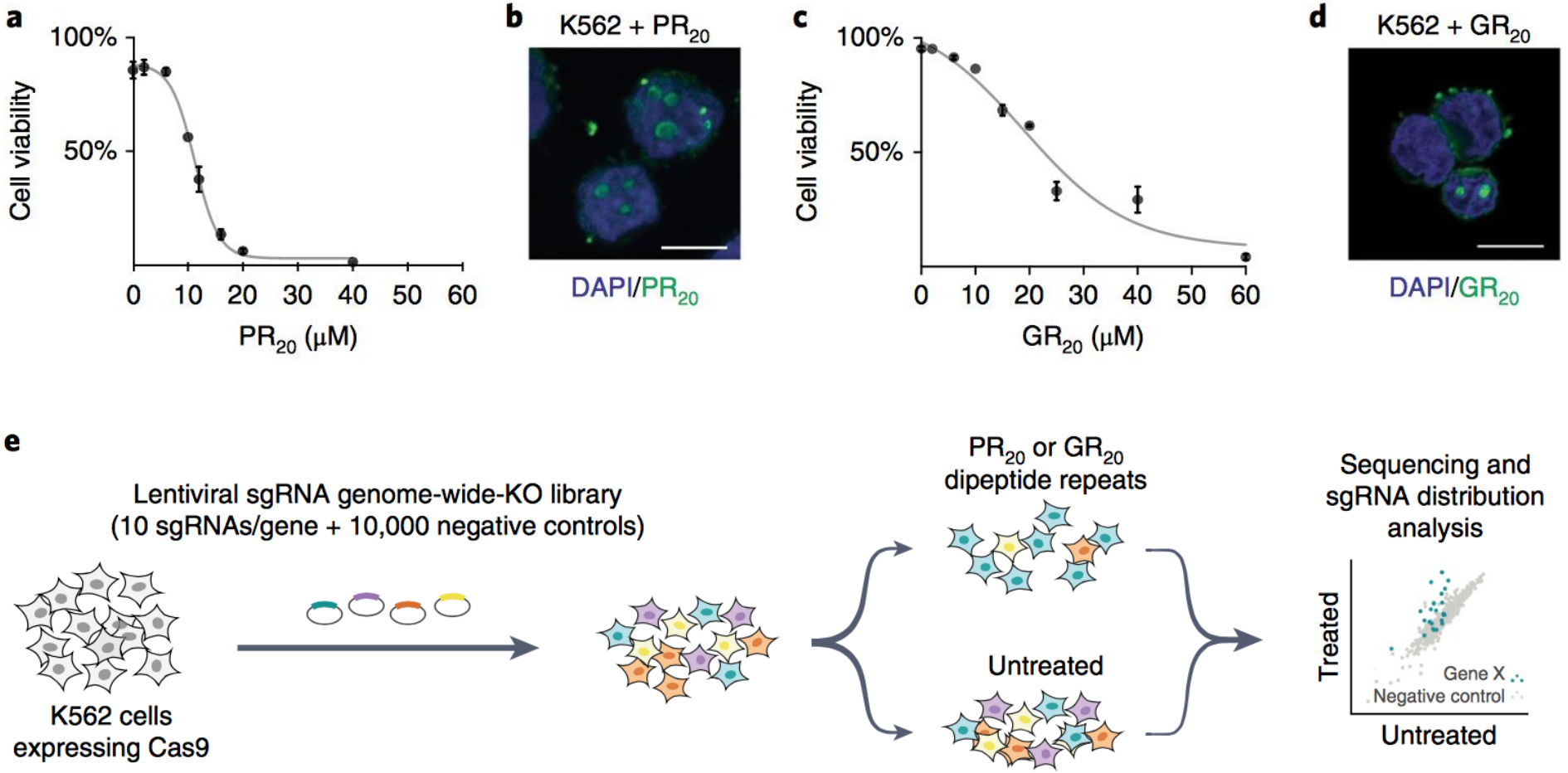
Causing genetic mutations of ALS & FTD



Hexanucleotide repeat expansion in C9ORF72 gene results in production of DPR proteins that may disrupt pre-mRNA splicing in ALS and FTD patients

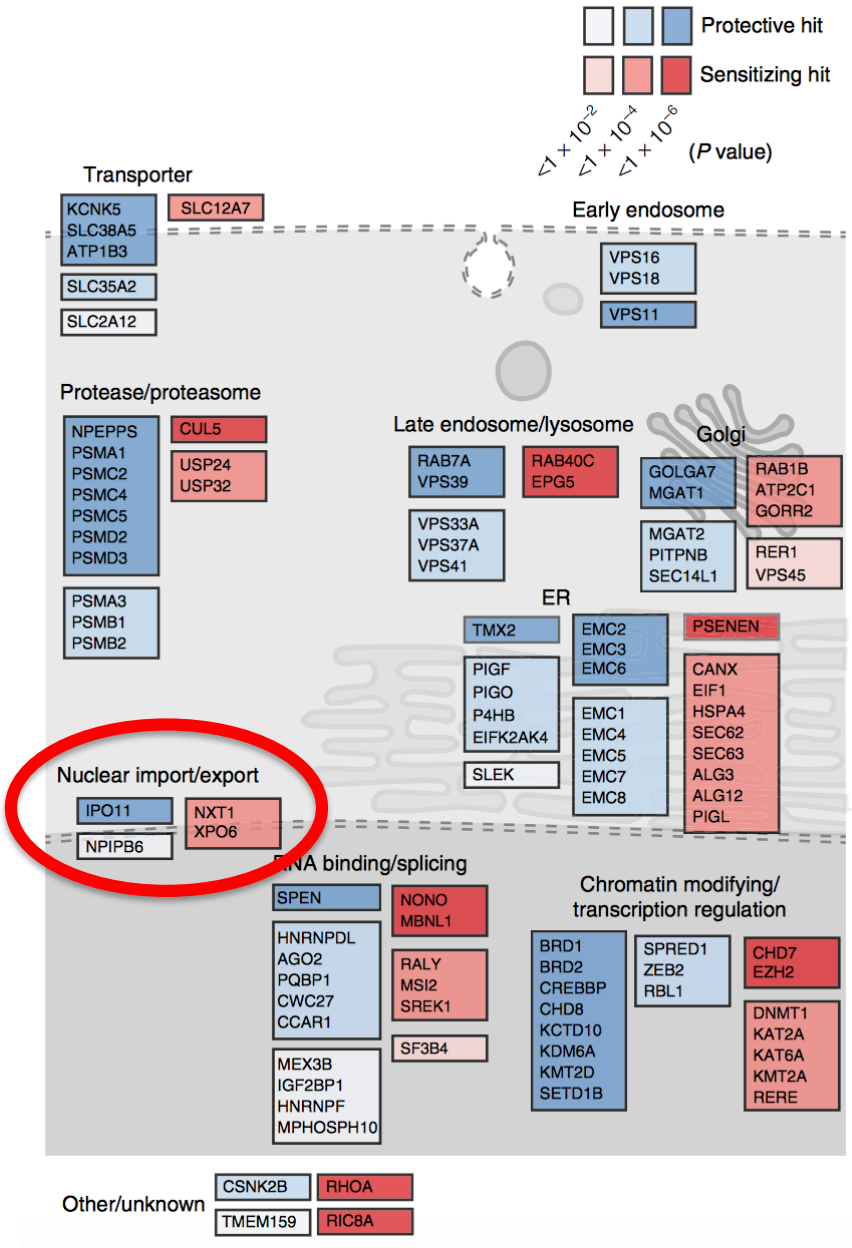
Yin et al, Cell Reports 2017

Pooled CRISPR screening paradigm

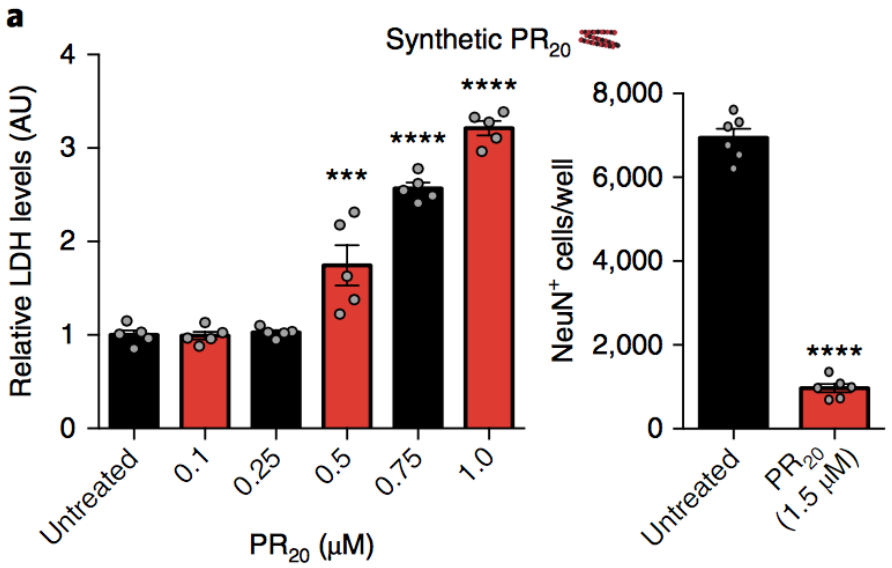


Identified modifiers in genome-wide KO

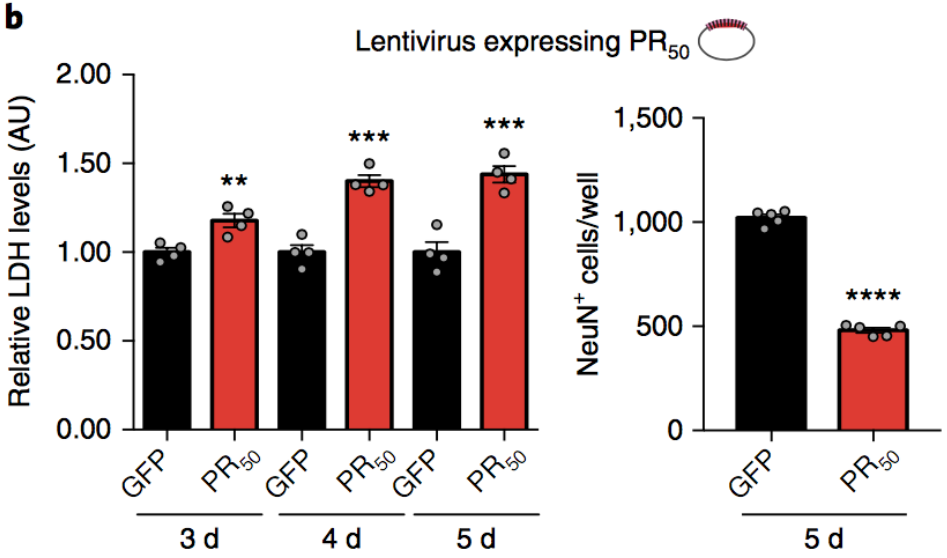
Schematic of proteins encoded by selected hits from both PR20 and GR20 screens



PR_{20} toxicity in primary mouse neurons

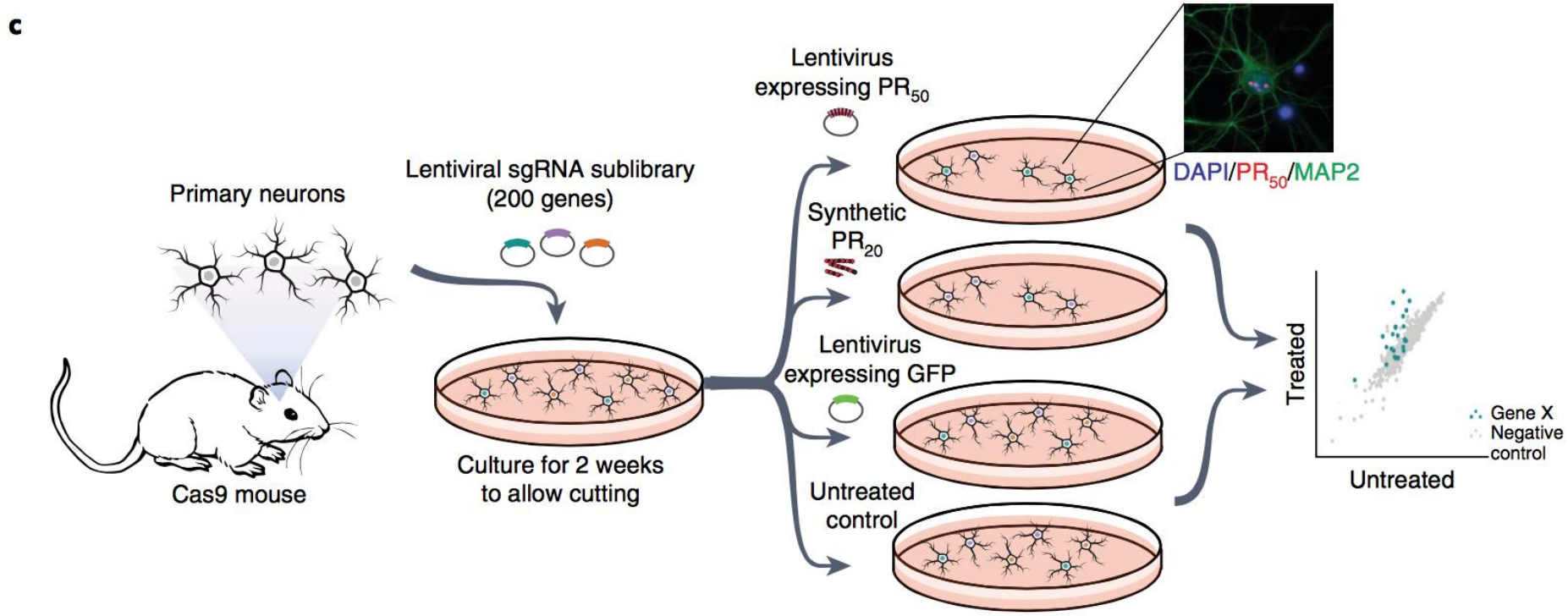


Dose-depended cytotoxicity



Time-depended cytotoxicity

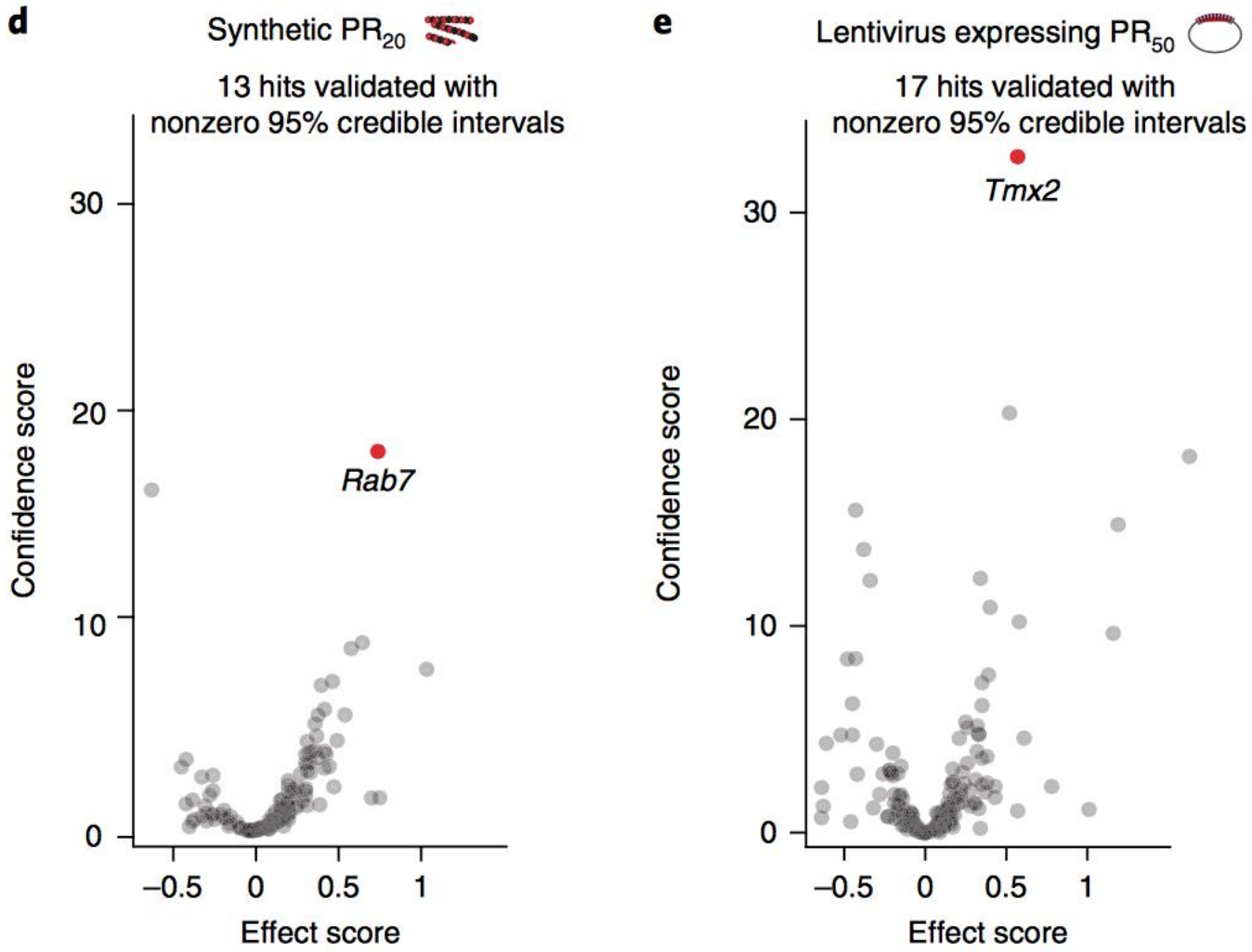
CRISPR screen in primary mouse neurons



The abundance of sgRNAs in surviving cells – measured by sgRNA deep sequencing

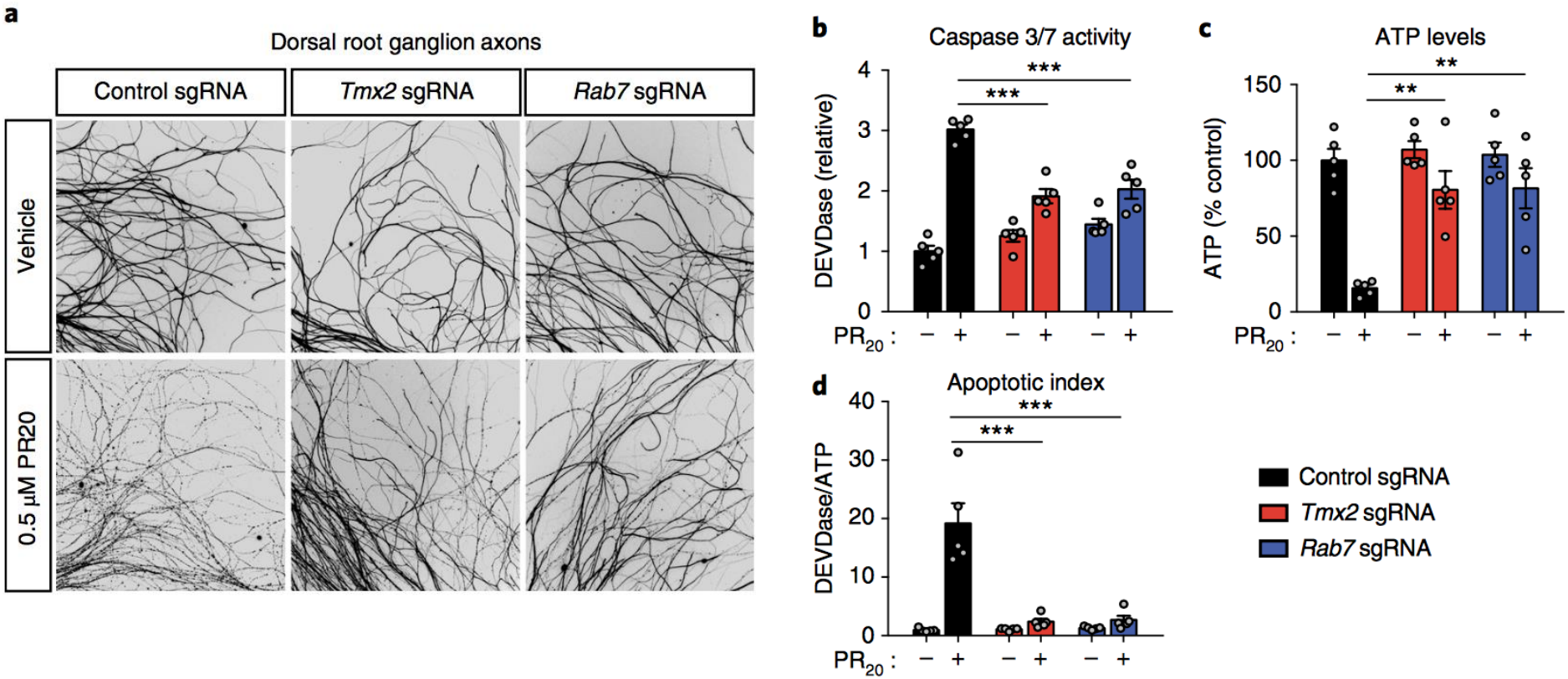
PR_{50} localized to the nuclei in cultured neurons

Identified modifiers in genome-wide KO



Effect score: gene-level summary of how protective or sensitizing the KO is
Confidence score: log-likelihood ratio describing the significance of the effect

Validation of *Rab7* and *Tmx2*



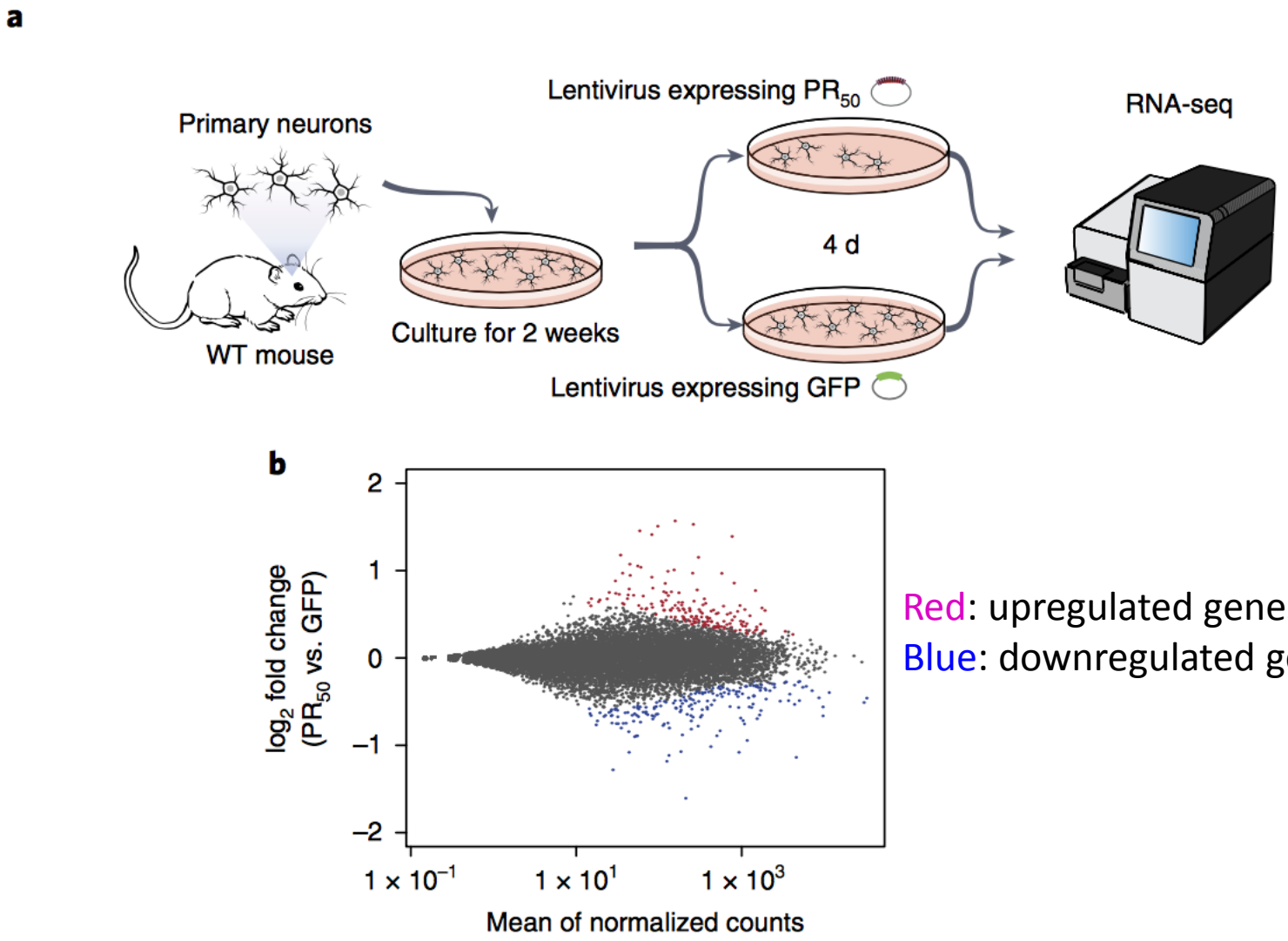
The strongest genetic modifiers: genes encoding proteins localized to the **nucleus and ER**

- Top protective KO: ER-resident transmembrane thioredoxin protein **Tmx2**
- Top hit modifier of synthetic PR20-induced toxicity: the endolysosomal trafficking gene ***RAB7***

KO of either *Rab7* or *Tmx2* protected against PR toxicity in primary neurons

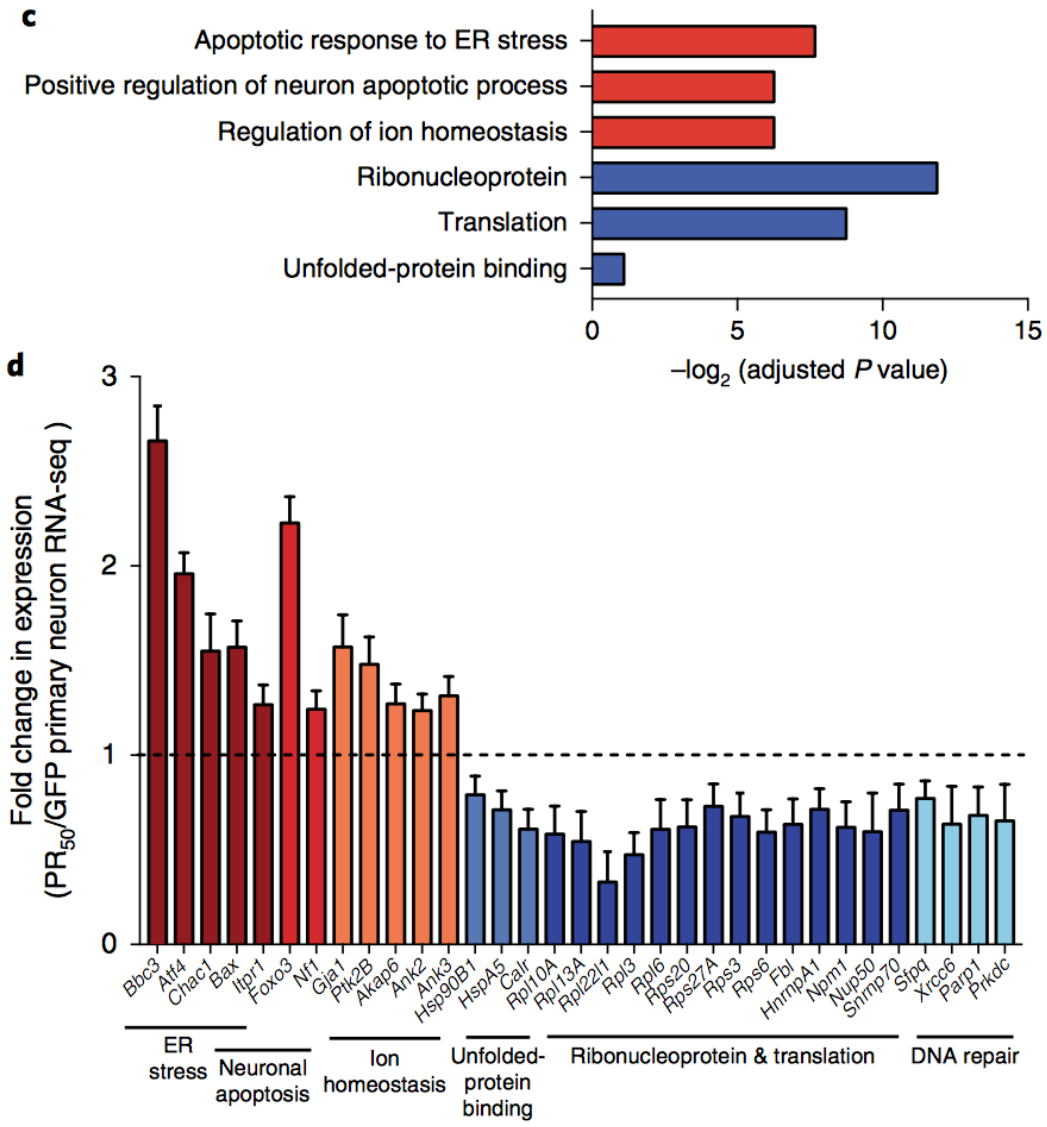
Transcriptional analysis

Hypothesis: DPR accumulation might induce an ER-stress response



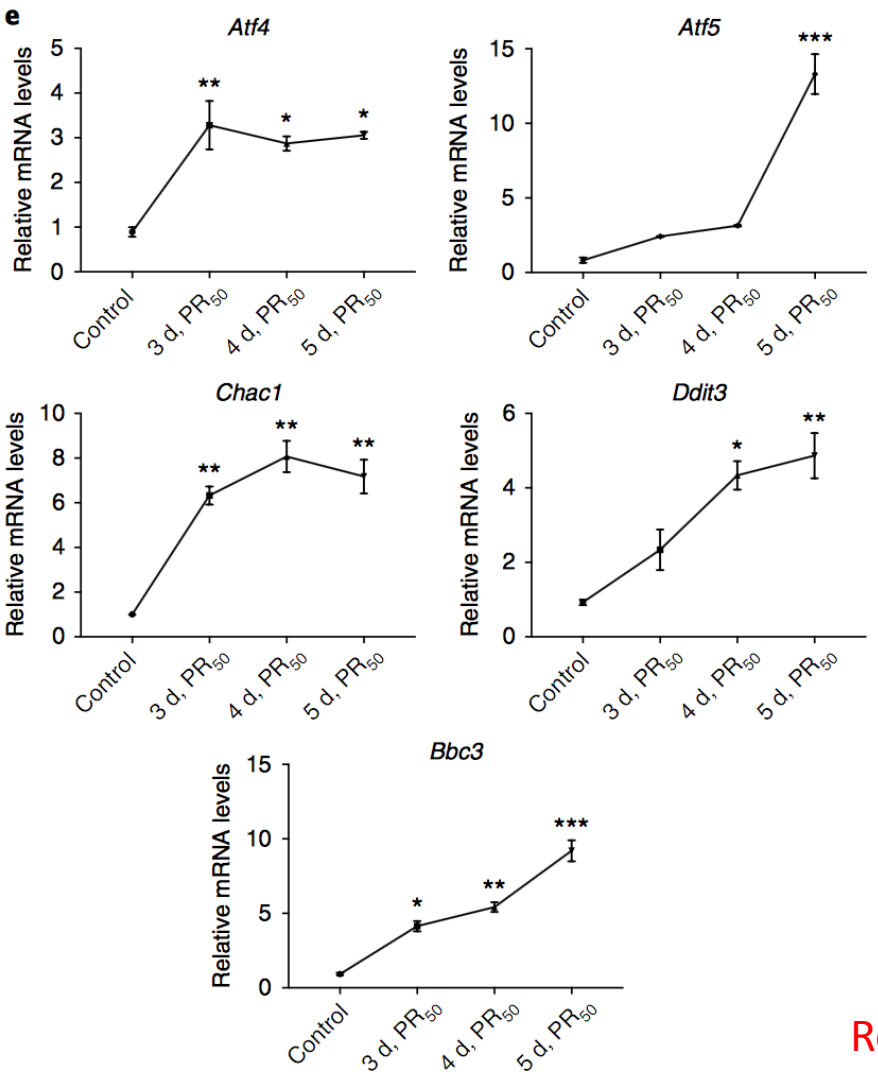
Red: upregulated genes (126)
Blue: downregulated genes (133)

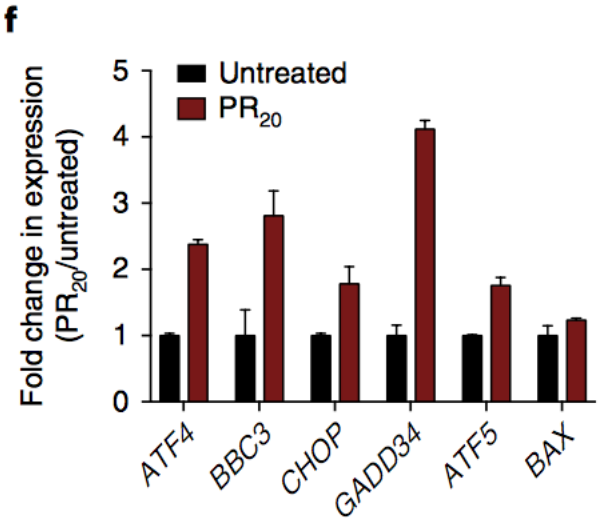
Differentially expressed genes

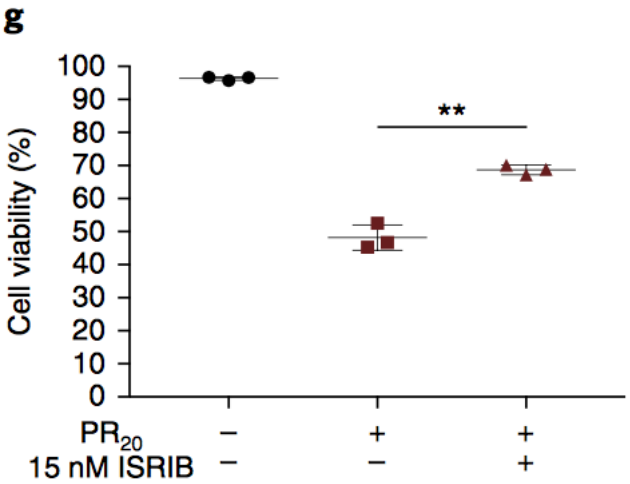


Validation assays

Time-dependent induction of ER-stress-related genes





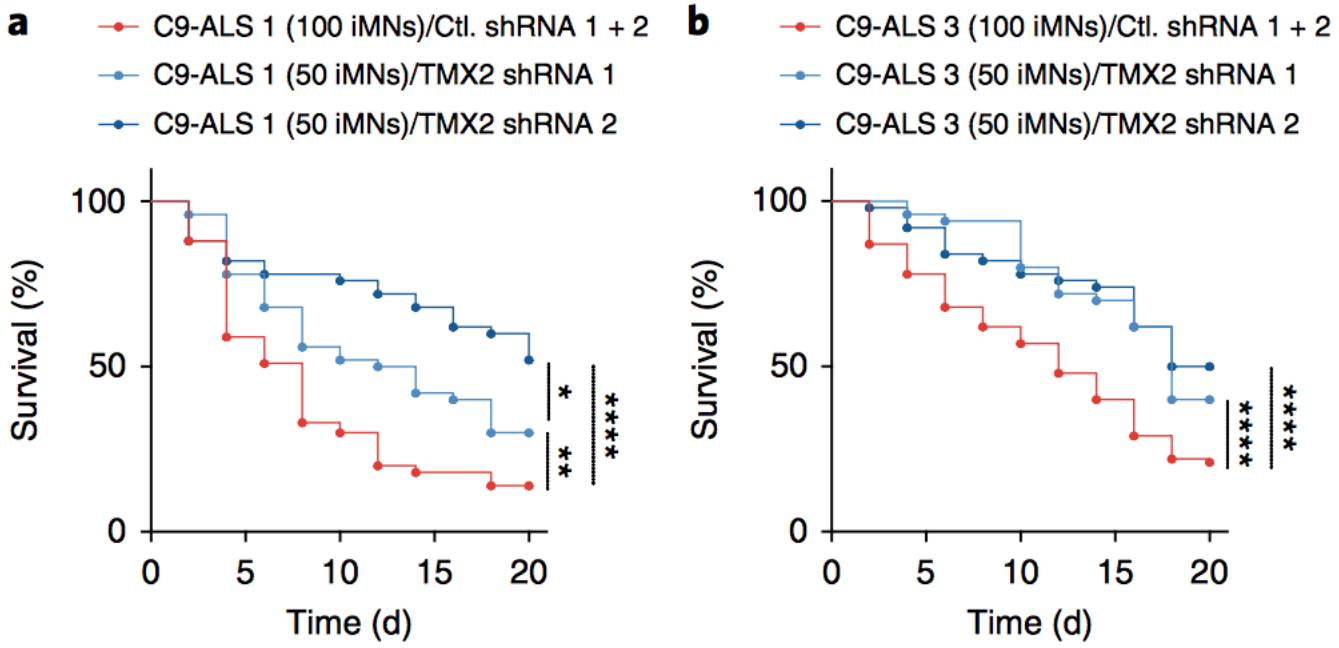


ISRIB: inhibitor of the cellular response to ER stress

Role of ER stress in DPR-mediated toxicity

Results

Decreased TMX2 improves survival of hiMNs



- Induced motor neurons (iMNs) from iPSCs from ALS patients
- 2 independent shRNAs targeting *TMX2* – increased proportion of surviving iMNs

TMX2 as potential therapeutic target

Identified modifiers in genome-wide KO

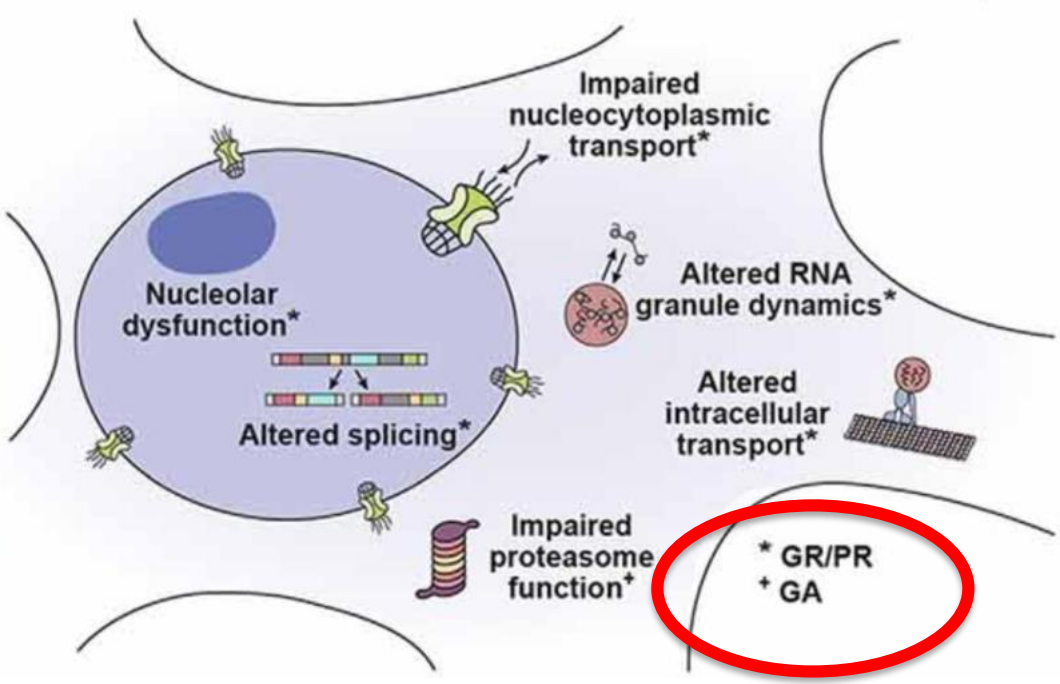
➢ Comprehensive CRISPR–Cas9 KO screens in human cells with further validation screens in primary neurons to discover modifiers of *C9ORF72* DPR toxicity

✓ Identified nucleocytoplasmic-transport machinery and new genes that suggest that ER function and ER stress are important in FTD/ALS pathogenesis

✓ Decreased *TMX2* expression conferred protection from PR toxicity also in primary rodent neurons

✓ *TMX2* may serve as future therapeutic target

Fresh targets for C9orf72 ALS?



DPRs may contribute to motor neuron toxicity in part by **increasing ER stress** and by **disrupting nucleocytoplasmic transport**

Conlusions

Summary of the 3 CRISPR screens