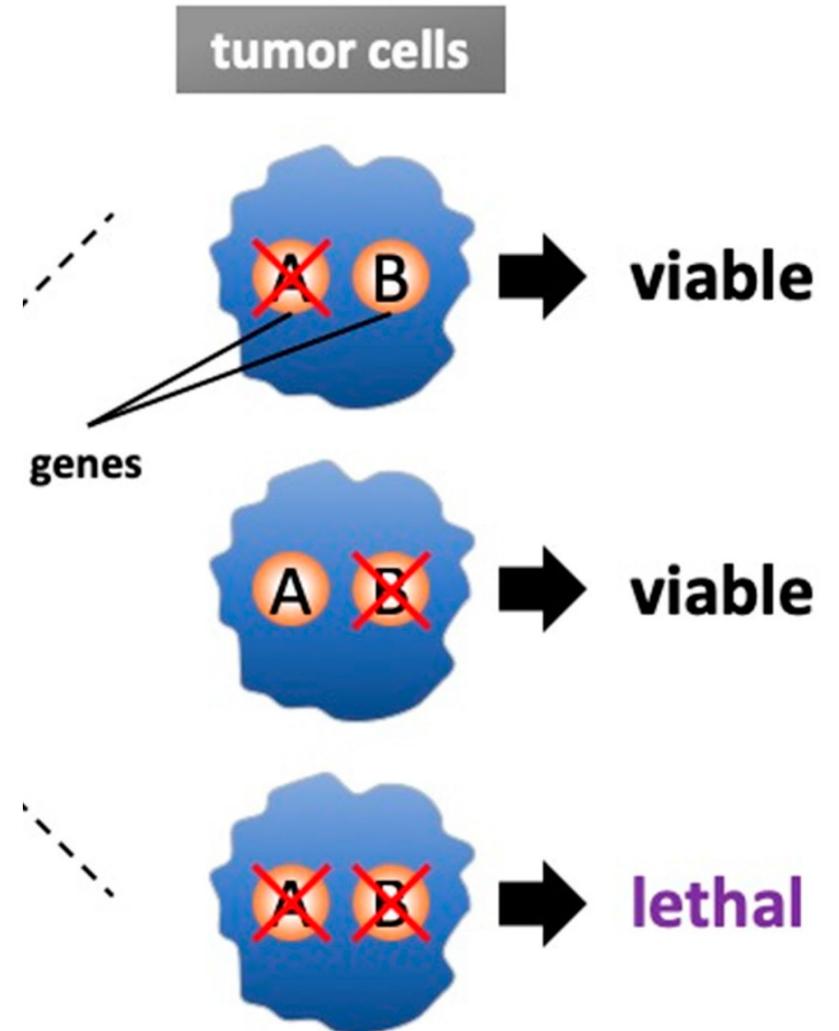
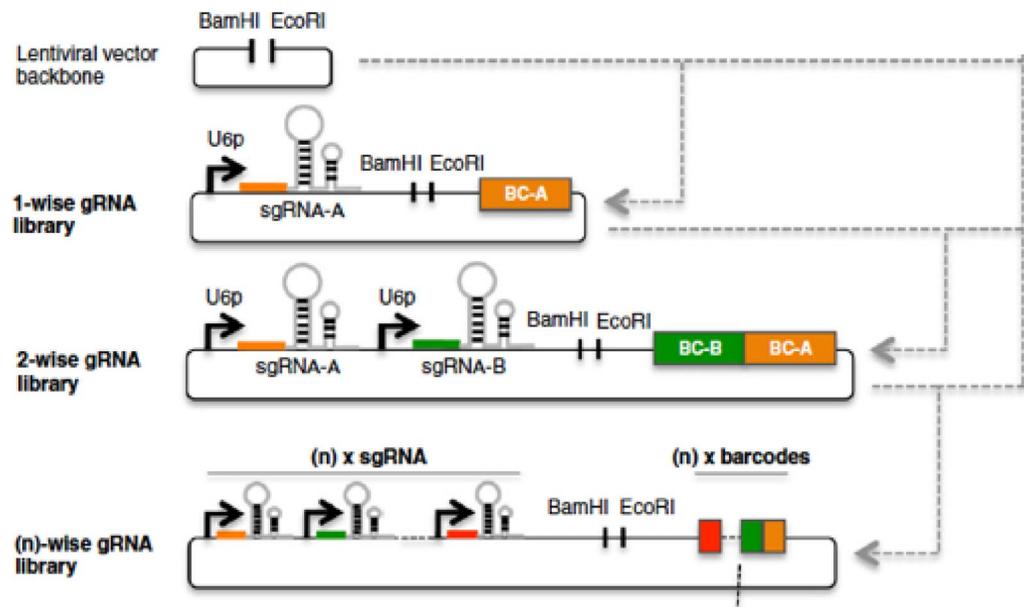


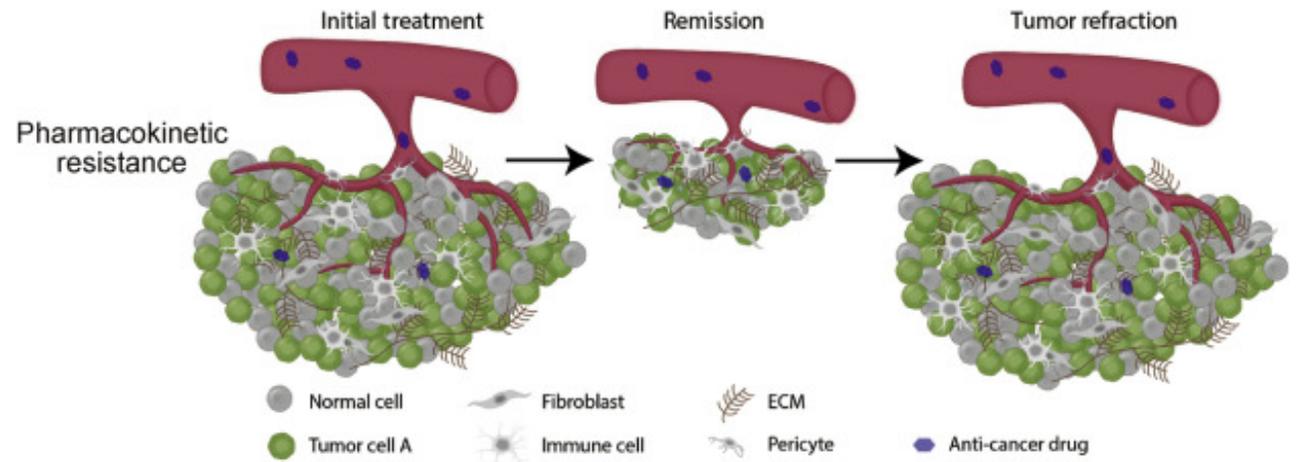
Combinatorial CRISPR screens

Asvin Lakkaraju
29.06.2021



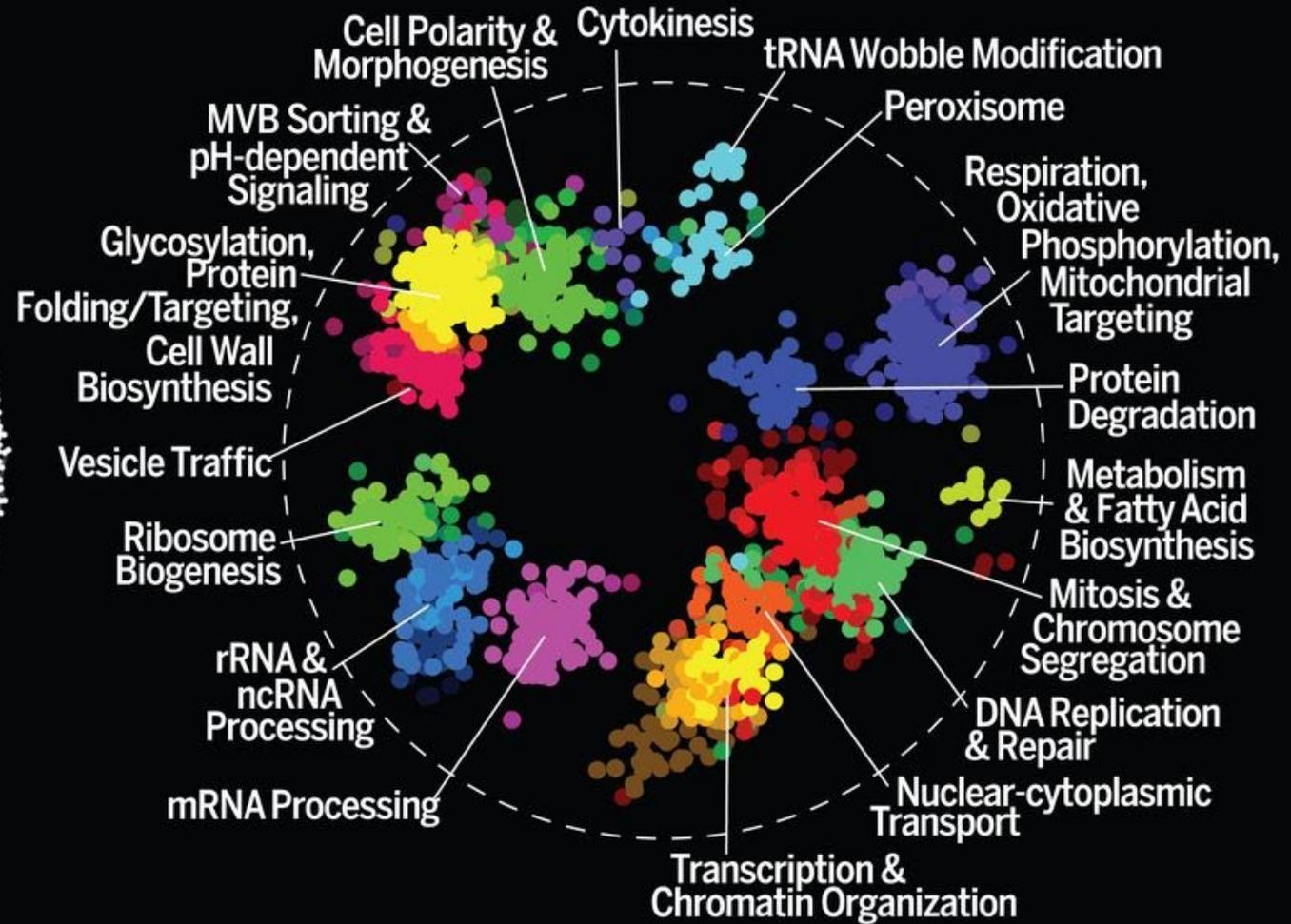
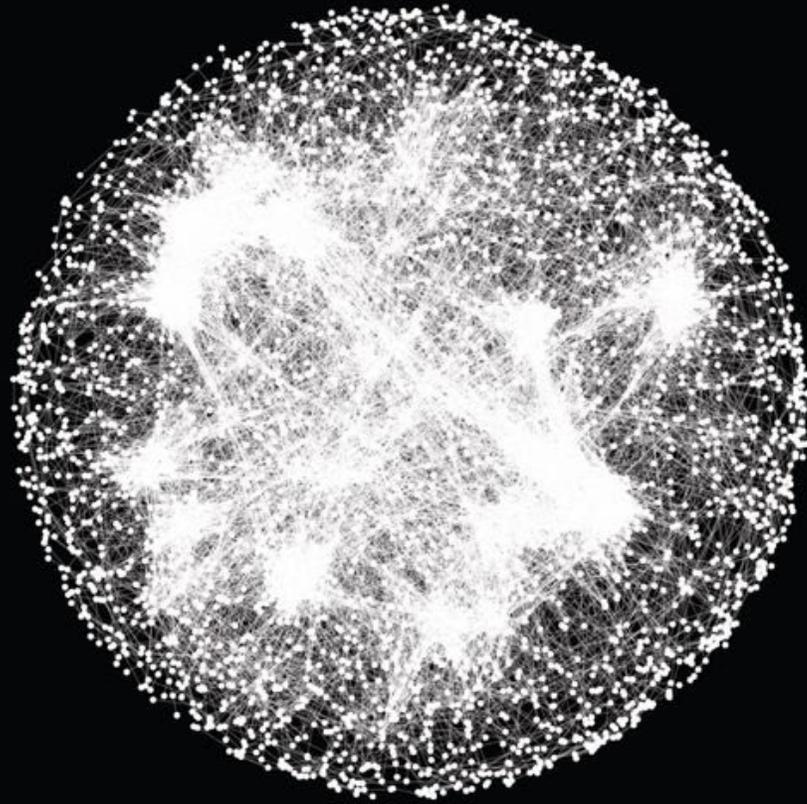


Combinatorial CRISPR strategy



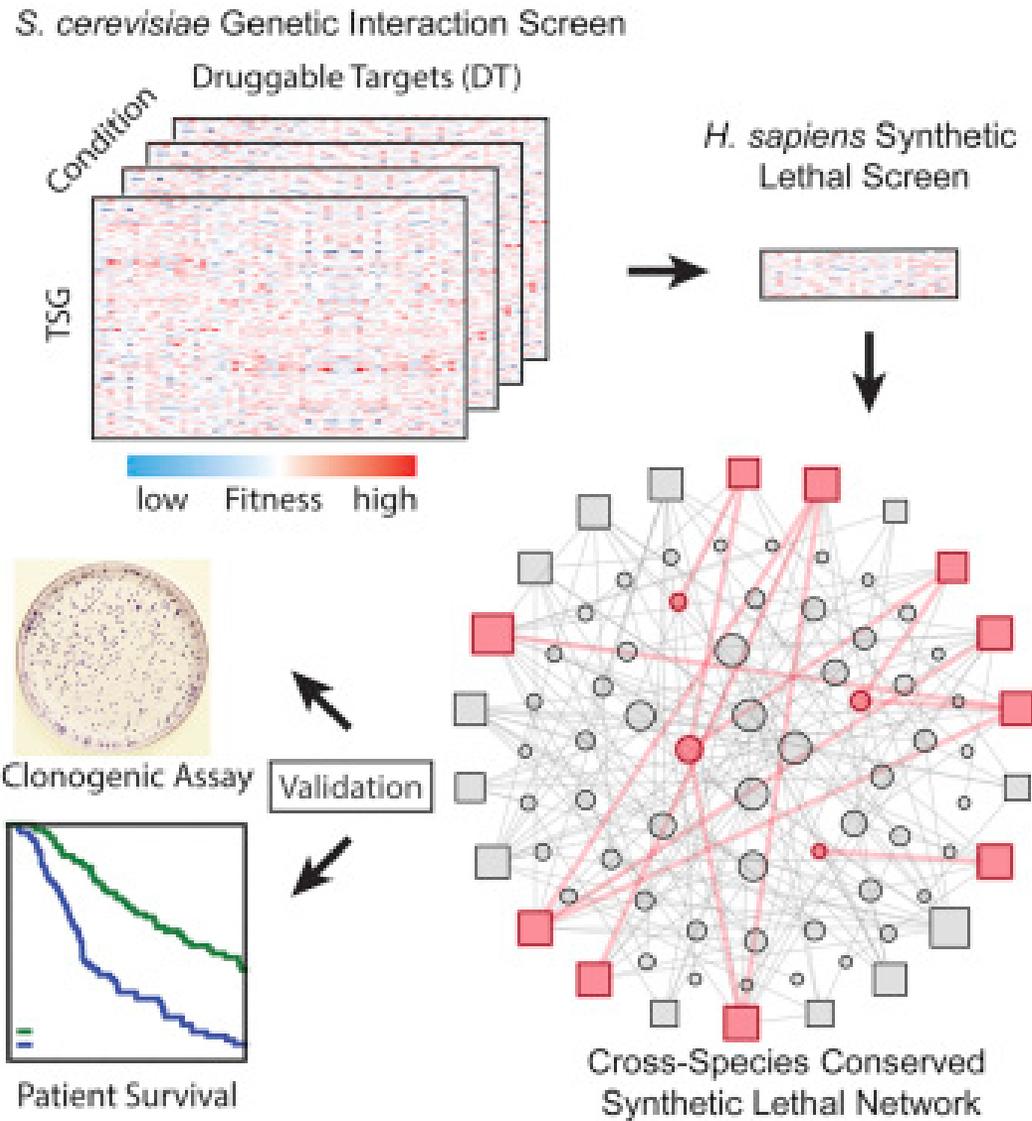
Resistance to treatment

Synthetic lethality in Yeast

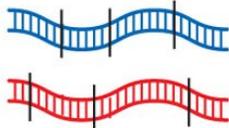
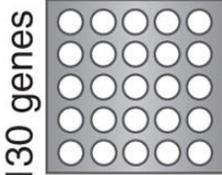
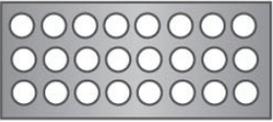
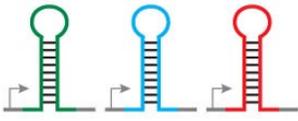
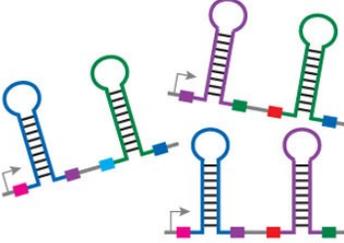


23 million double mutants, identifying about 550,000 negative and about 350,000 positive genetic interactions.

Genetic Interaction maps in mammalian cells

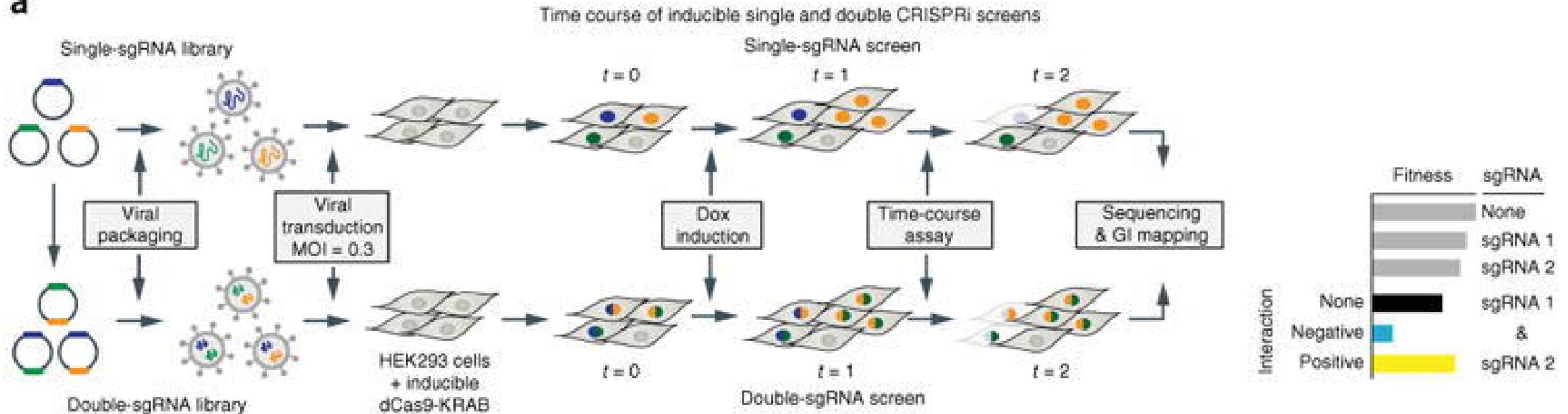


Combinatorial screens

	Select genes	Generate library	Perform experiment	Assay	Pros	Cons
Roguev <i>et al.</i> ³	130 genes (chromatin modifiers)	 esiRNA	 130 genes 130 genes	Cell count	Reagent design	Reproducibility
Laufer <i>et al.</i> ²	323 genes (chromatin modifiers)	 siRNA	 20 genes 323 genes	High-content microscopy	Reproducibility; deep phenotyping may improve GI calls	Labor-intensive reagent design
Bassik <i>et al.</i> ¹	 60 genes (hits from primary screen)	 Dual shRNA	 Pooled library	Sequence depth	Reproducibility; pooled libraries and sequencing most scalable	Labor-intensive reagent design; paired hairpins limit scale

Mammalian Cells: CRISPR based screens

a



Mammalian cells: Complexities

1. High off target effects and low KD efficiency with RNAi and shRNA
2. Variability among the cell types chosen.
3. Problems with CRISPR methodologies so far: multiple plasmid transfection or cloning steps, large constructs and complex combinations of promoters, which limit its usage in genome-wide genetic screening due to high risk of losing library components during library construction.
4. The application of barcode to label multiple gRNAs has been found result in half of the mismatch of gRNA-barcodes due to lentiviral template switching

3 papers using different CRISPR based screens to map genetic interactions

nature > nature communications > articles > article

Article | [Open Access](#) | Published: 26 February 2021

Combinatorial CRISPR screen identifies fitness effects of gene paralogues

Nicola A. Thompson, [Marco Ranzani](#), Louise van der Weyden, Vivek Iyer, Victoria Offord, Alastair Droop, Fiona Behan, Emanuel Gonçalves, Anneliese Speak, Francesco Iorio, James Hewinson, Victoria Harle, Holly Robertson, Elizabeth Anderson, Beiyuan Fu, Fengtang Yang, Guido Zagnoli-Vieira, Phil Chapman, Martin Del Castillo Velasco-Herrera, Mathew J. Garnett, Stephen P. Jackson & David J. Adams 

Nature Communications **12**, Article number: 1302 (2021) | [Cite this article](#)

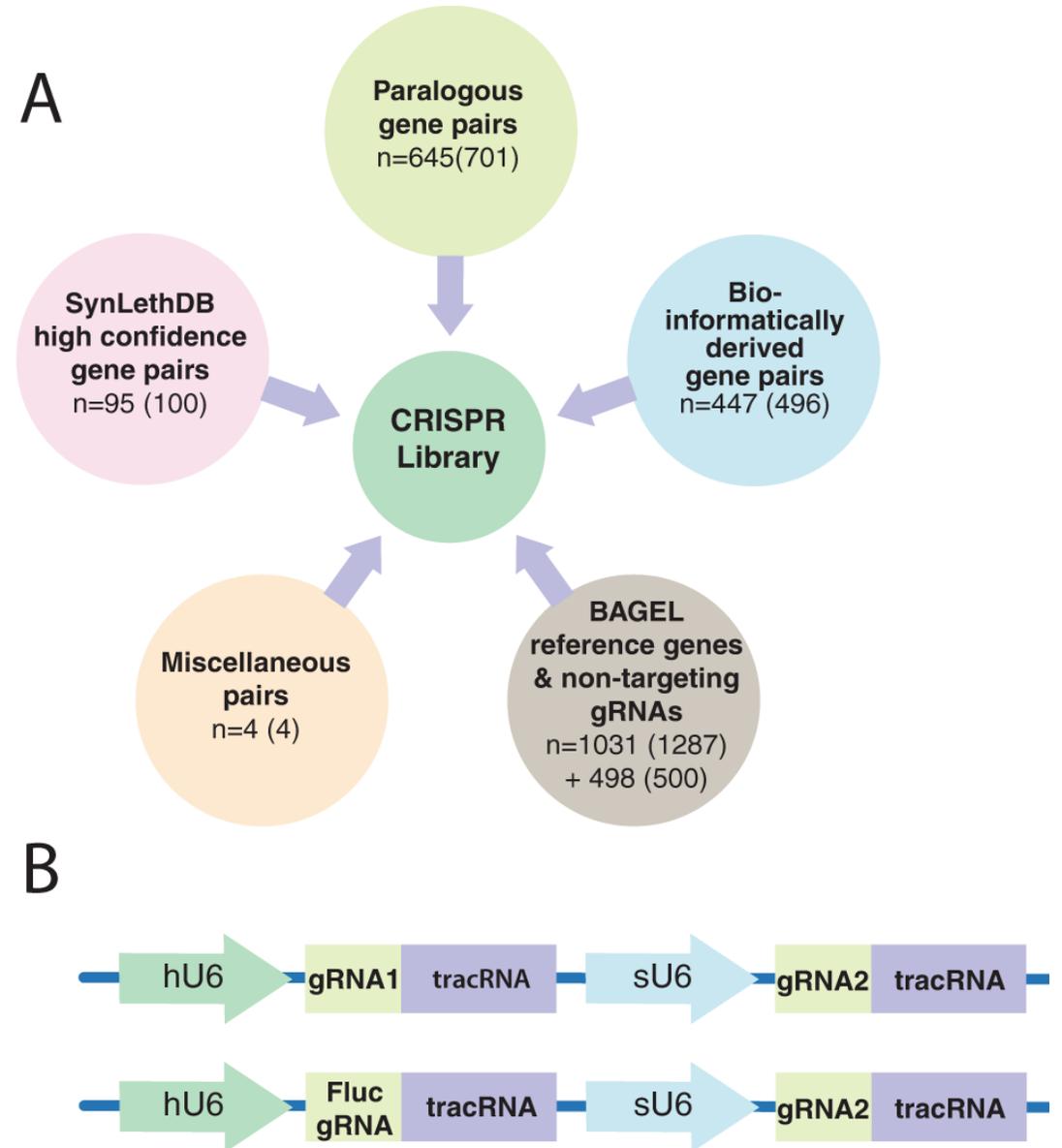
6791 Accesses | **2** Citations | **70** Altmetric | [Metrics](#)

Aim: Identify gene pairs that are synthetic lethals and possibly new therapeutic targets.

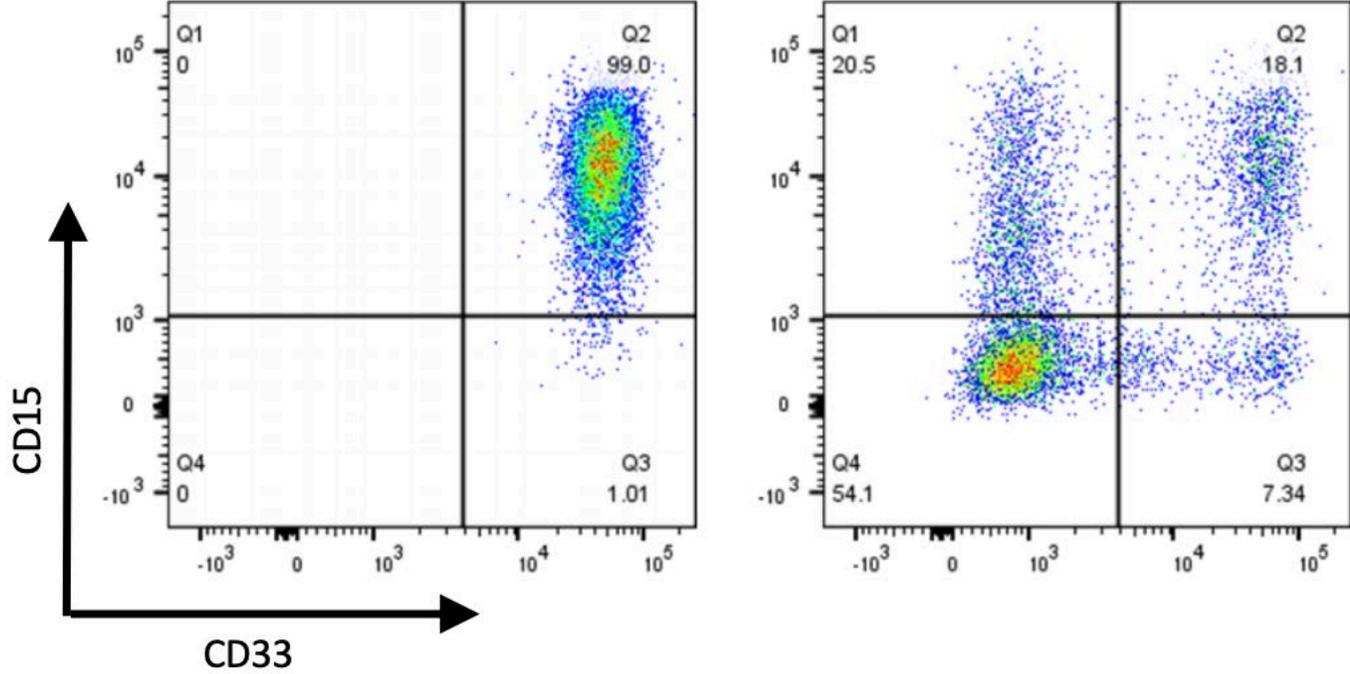
Create genome wide map of cancer dependancies

Generation of Synthetic lethal pairs

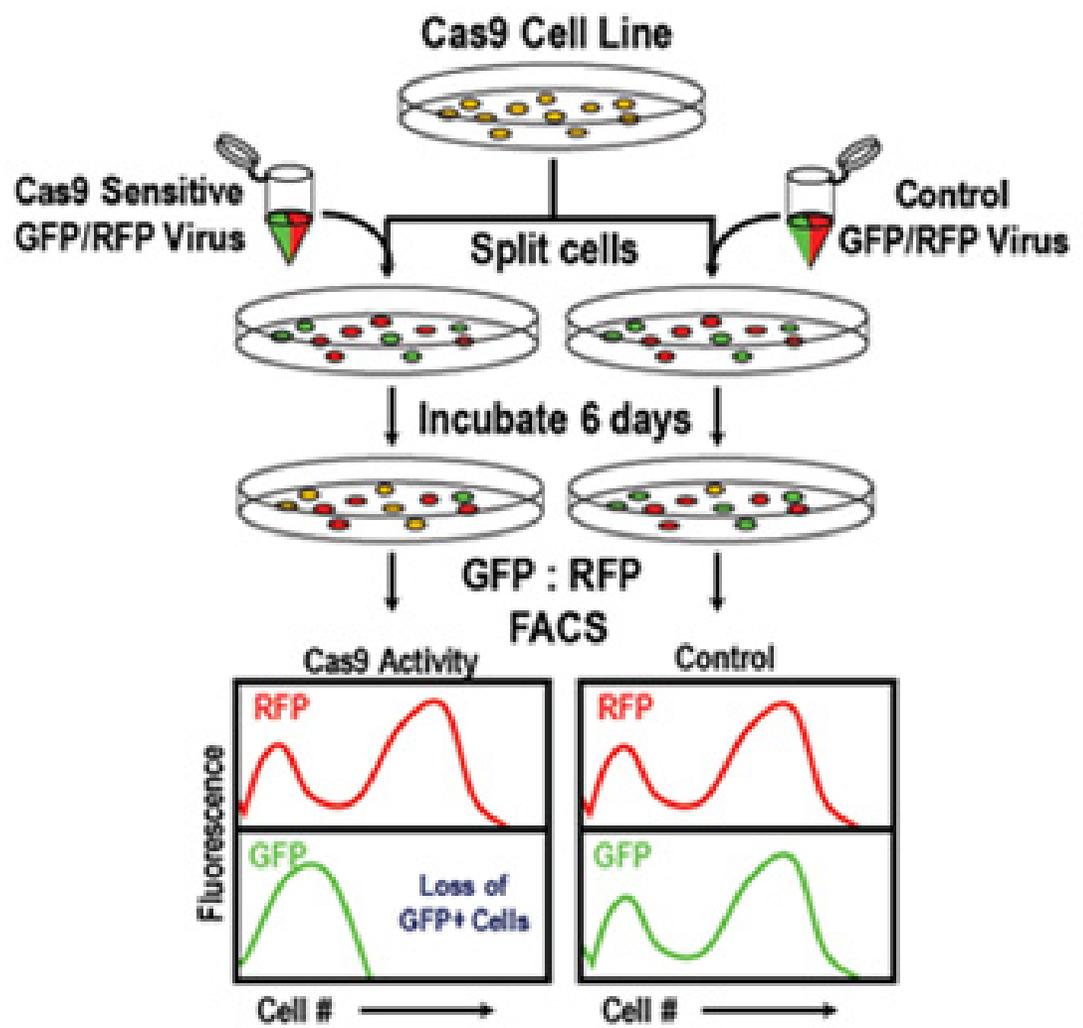
Strategy to identify pairs of genes



Does the two guide RNA strategy works in downregulating expression?



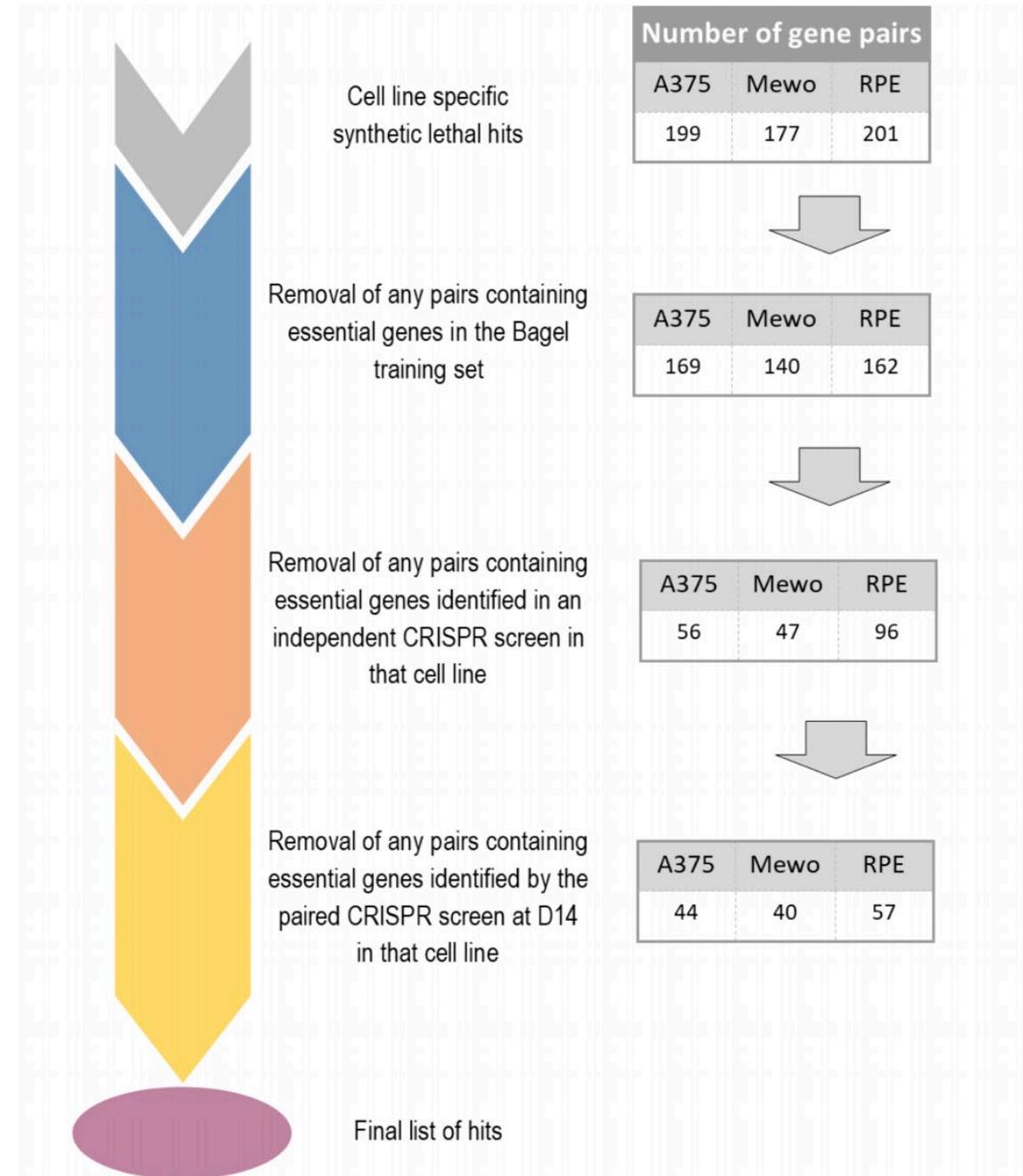
Measuring Cas9 activity



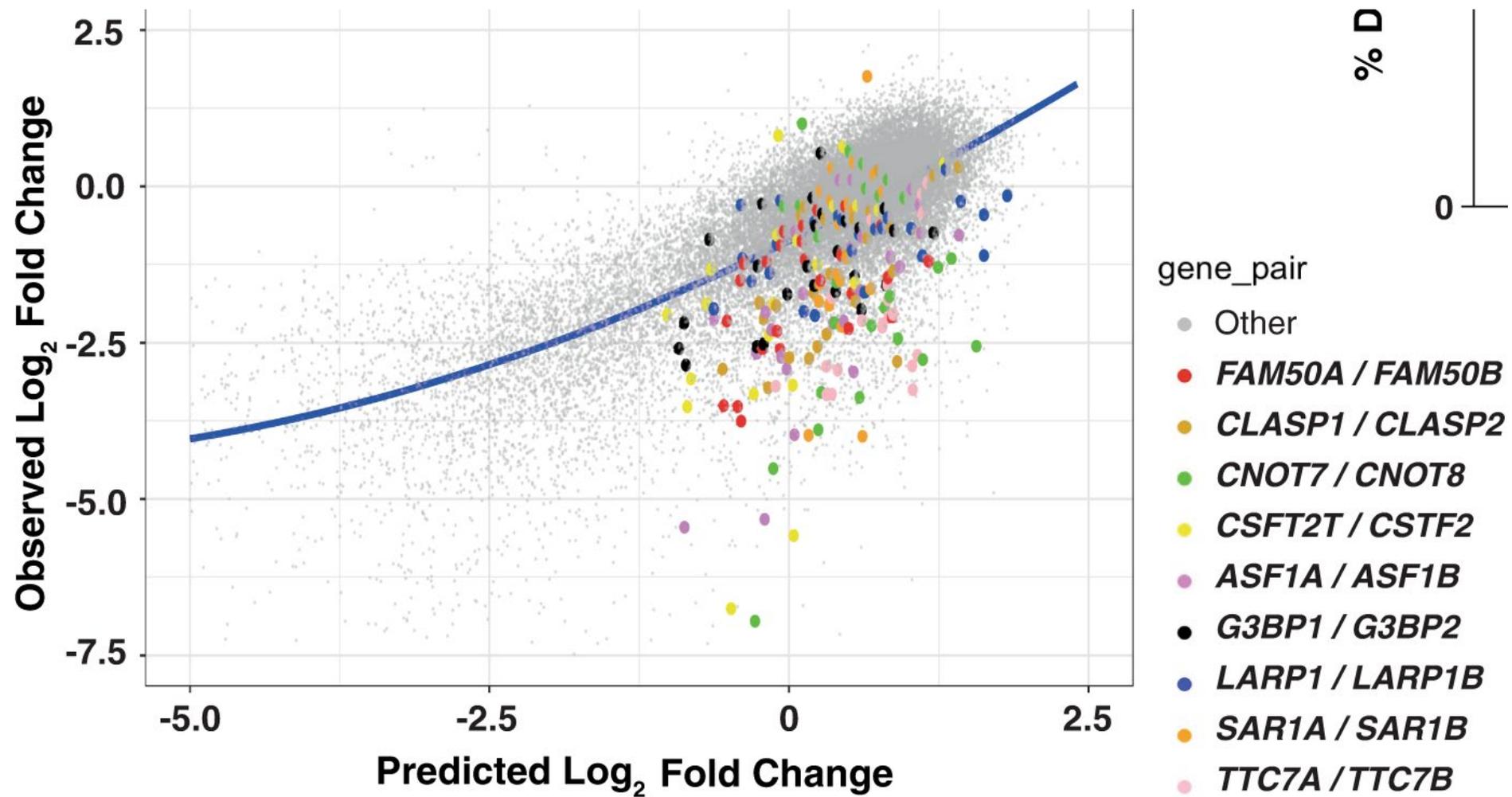
Cell Line	Cas9 Activity
A375	90.0%
Mewo	95.8%
Molm-13	77.3%
RPE	93.7%

Work flow for hit selection

A total of 701 gene pairs were used for screening

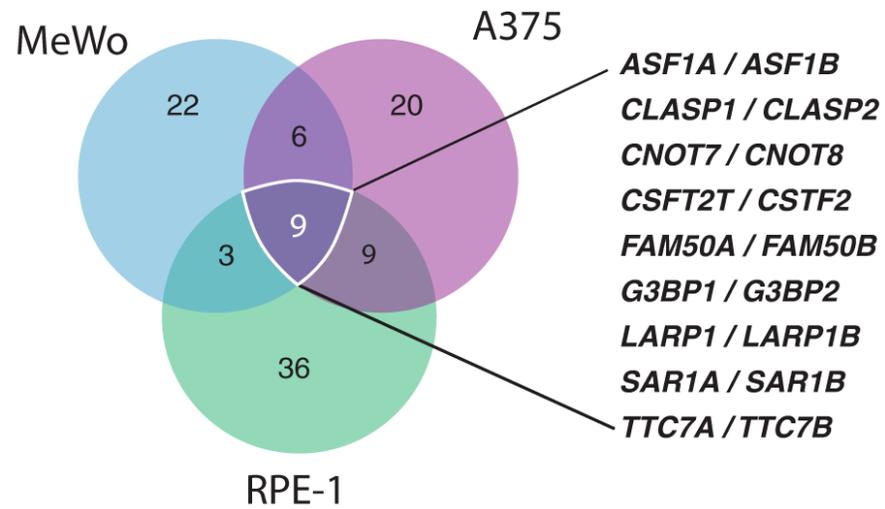


Comparison of predicted vs observed fitness values

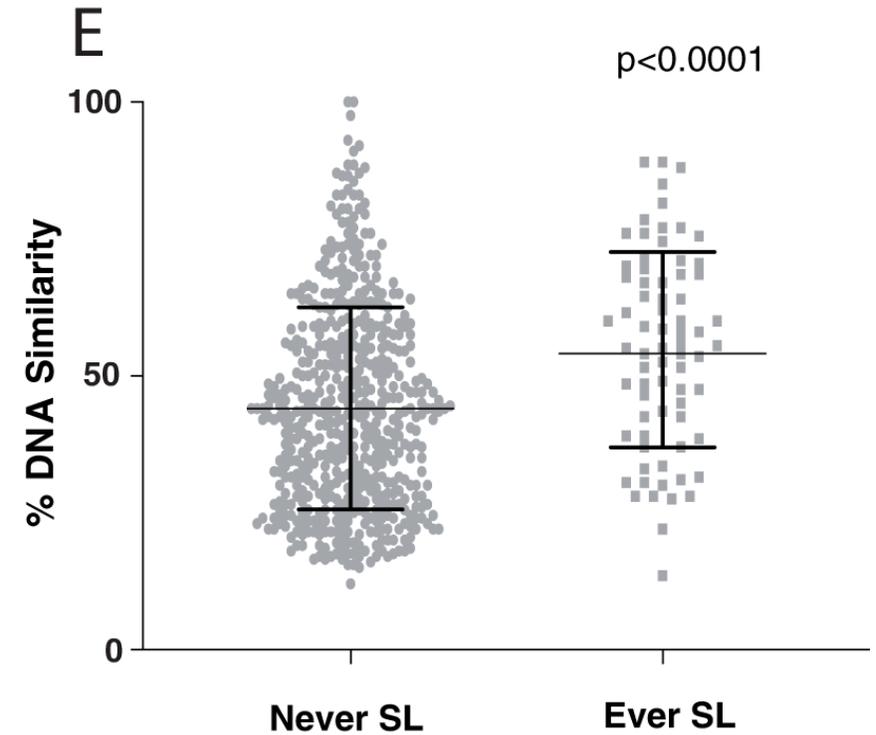


Hit overlap in all the cell lines

D



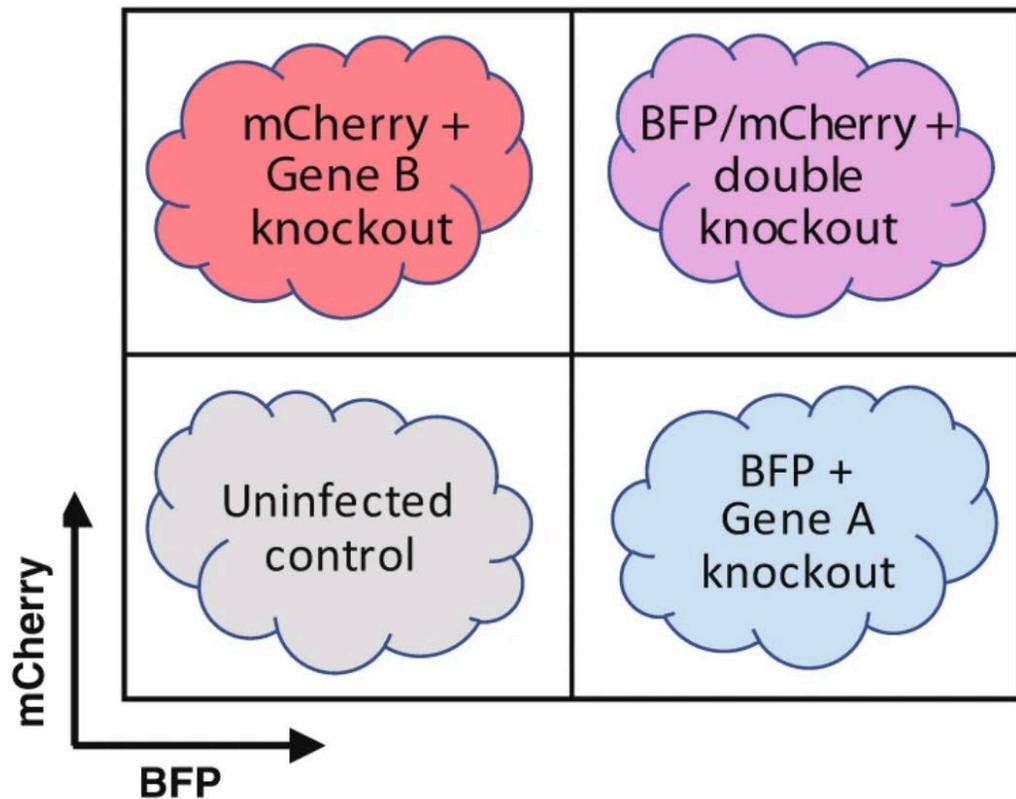
Paralogs in synthetic lethality



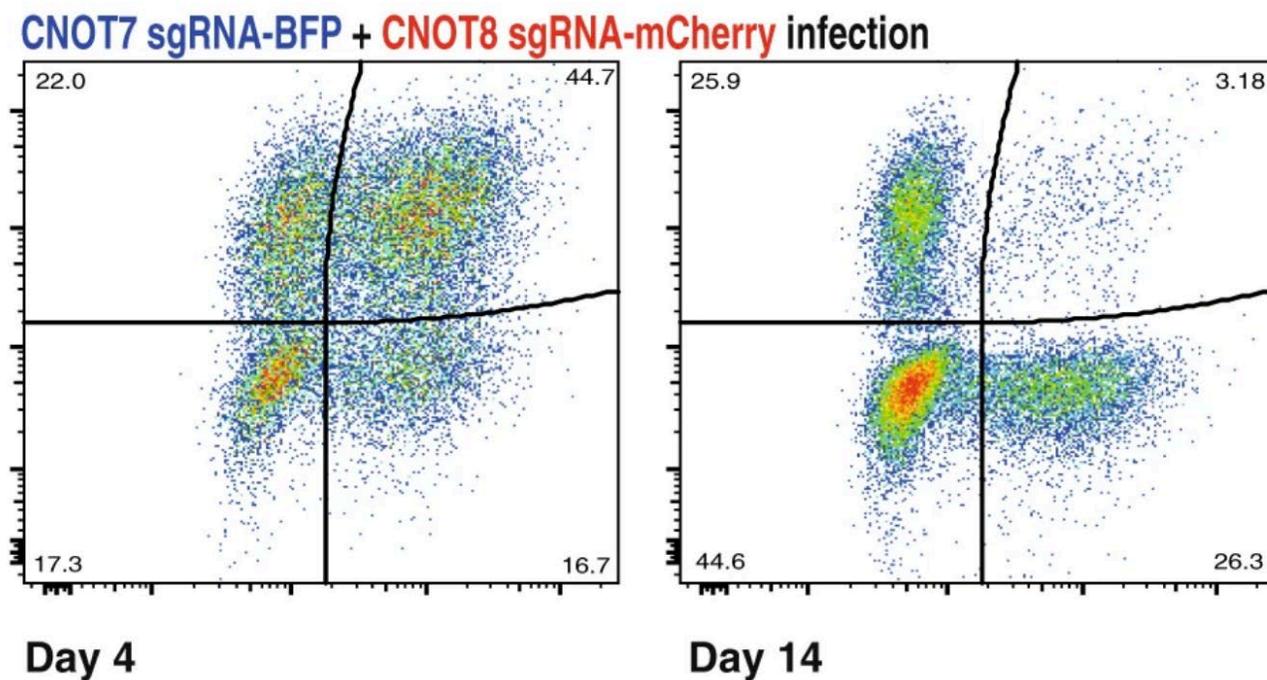
Most of the hits are gene paralogs

Hit validation in an orthogonal manner

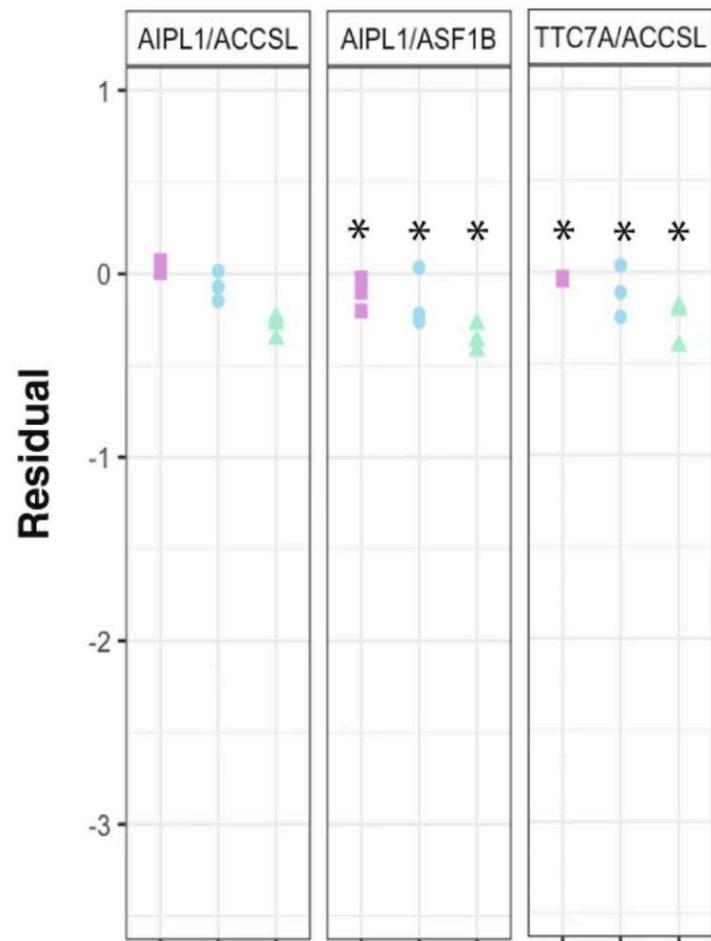
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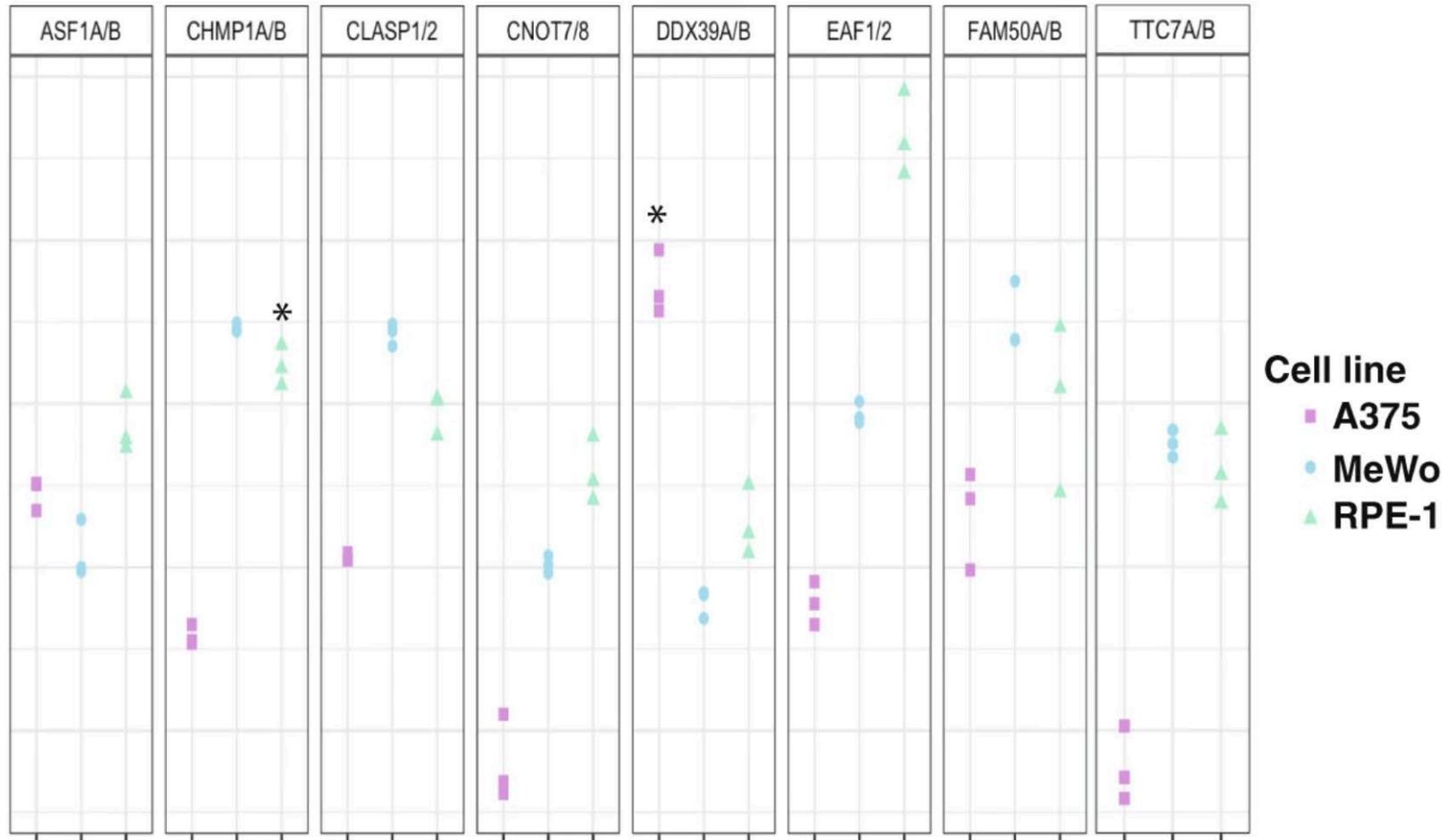
B



C Controls (neutral interactions)



Interactions

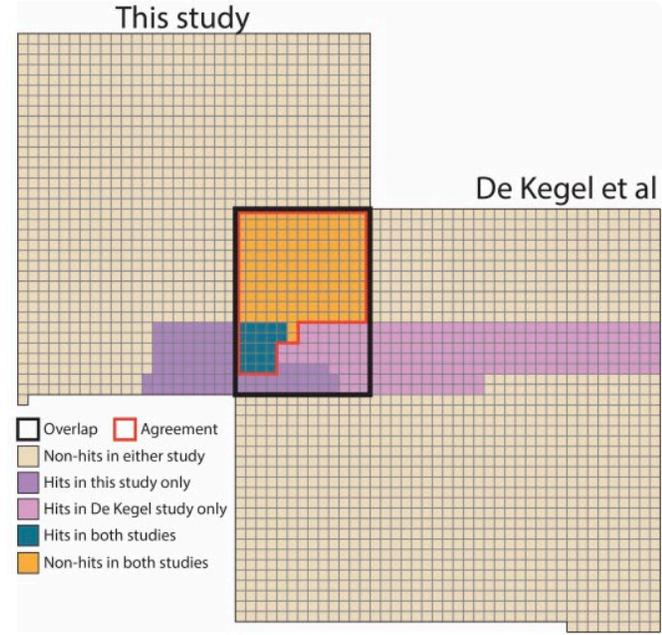


Cell line
■ A375
● MeWo
▲ RPE-1

*Non-significant interactions. Note: Statistical analysis was performed relative to the *AIPL1/ACCSL* pair.

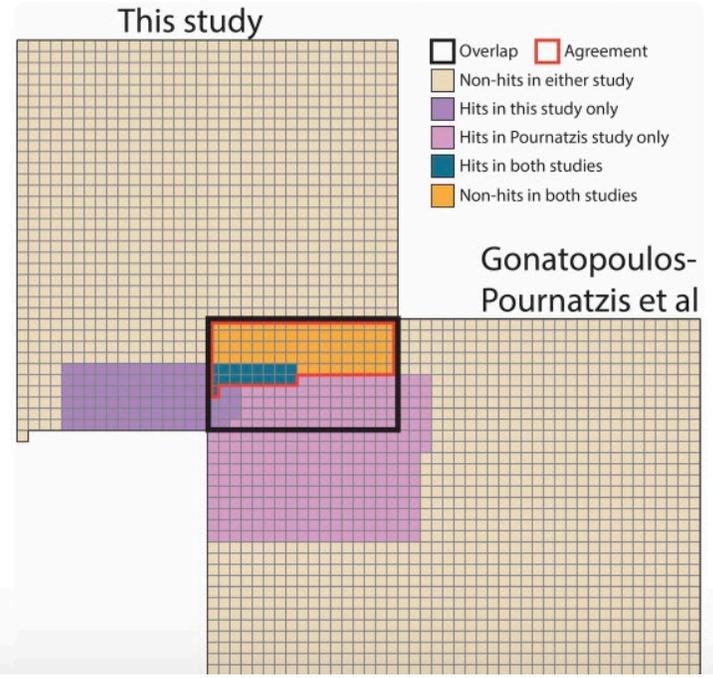
Overlap of gene pairs with De Kegel et al.,

Overlap of the hit list with previously published screens on synthetic lethality

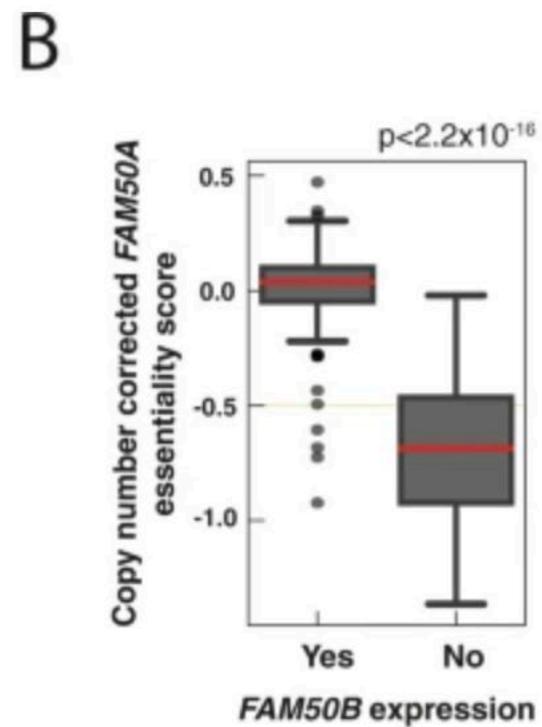
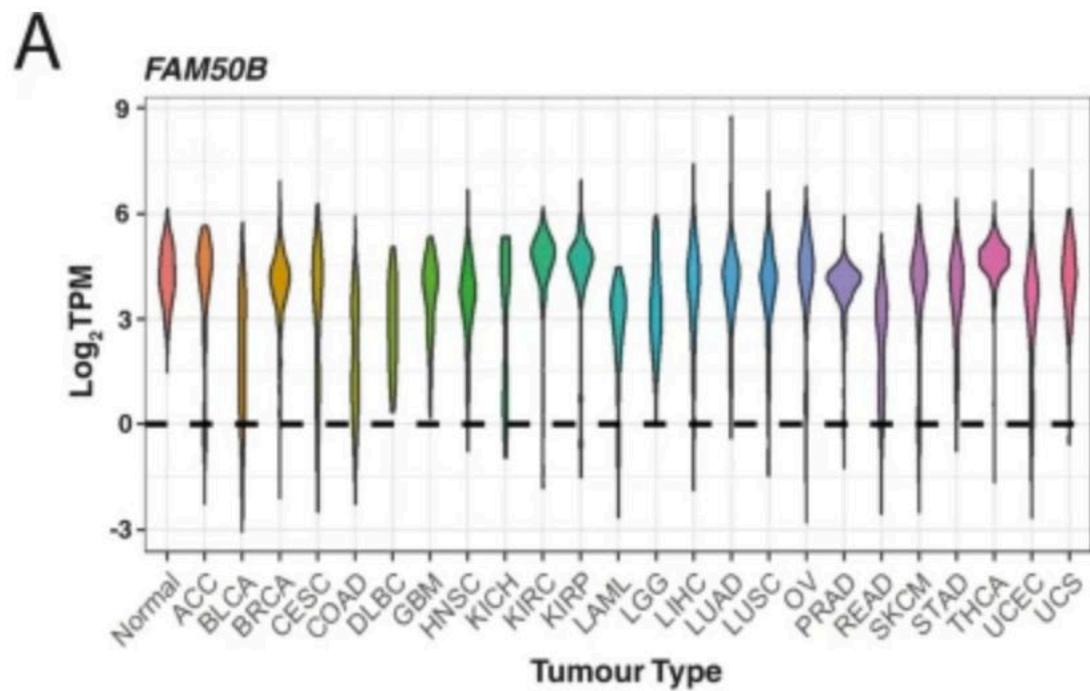


What is new?

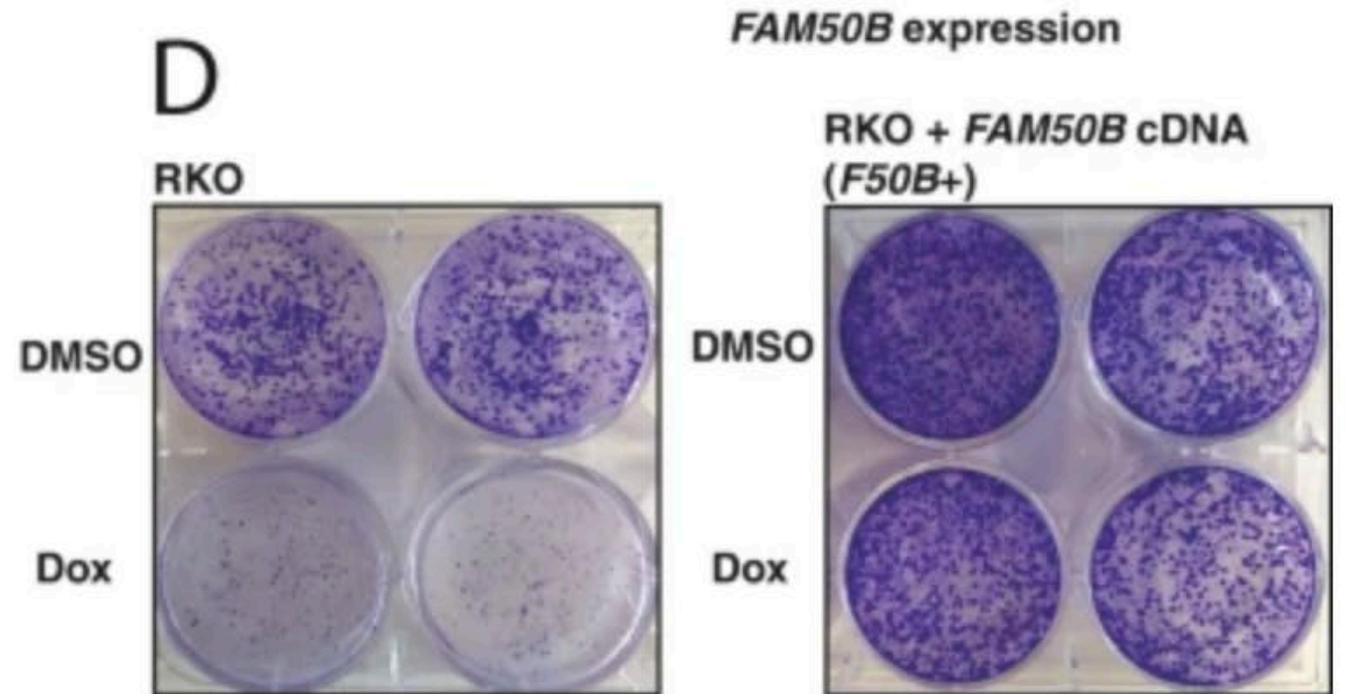
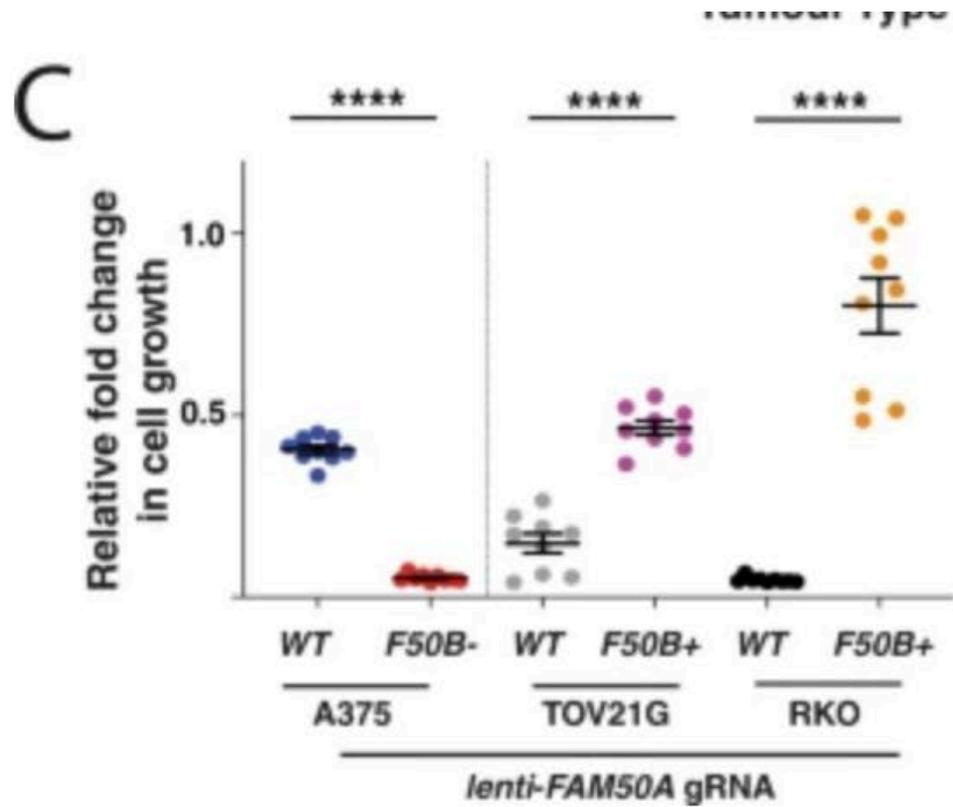
Overlap of gene pairs with Gonatopoulos-Pournatzis et al.,



FAM50A-FAM50B axis

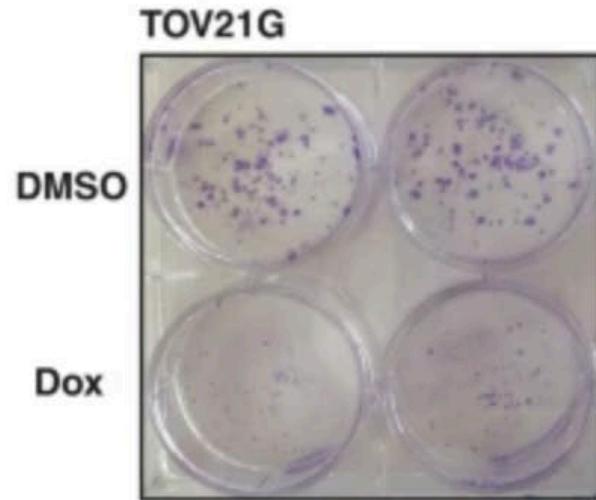


Are FAM50A and B involved in cell proliferation?

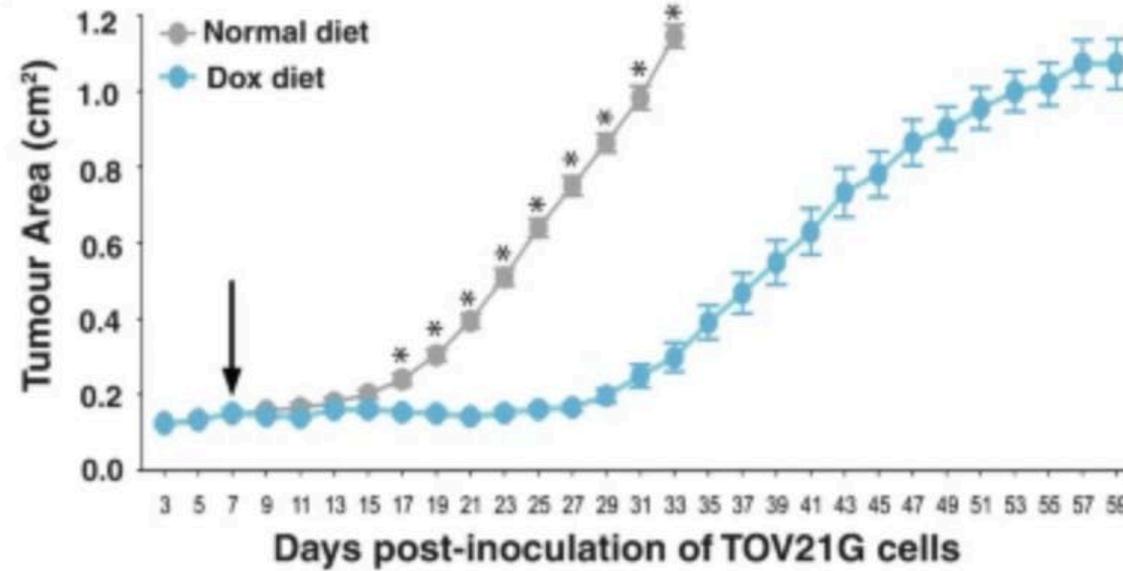


Tumor expansion in mice after interfering with the FAM axis

A

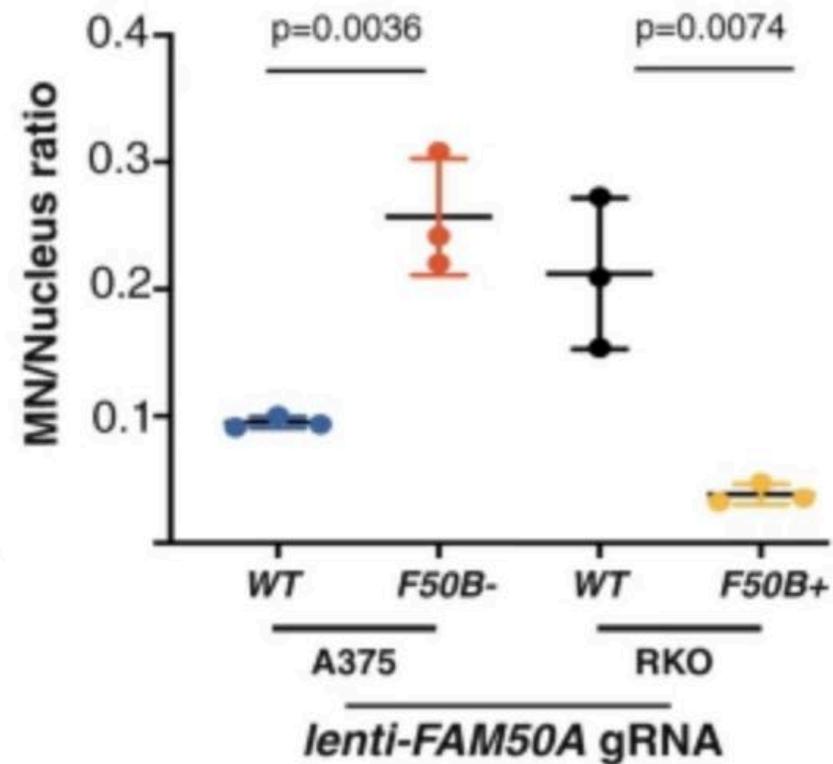
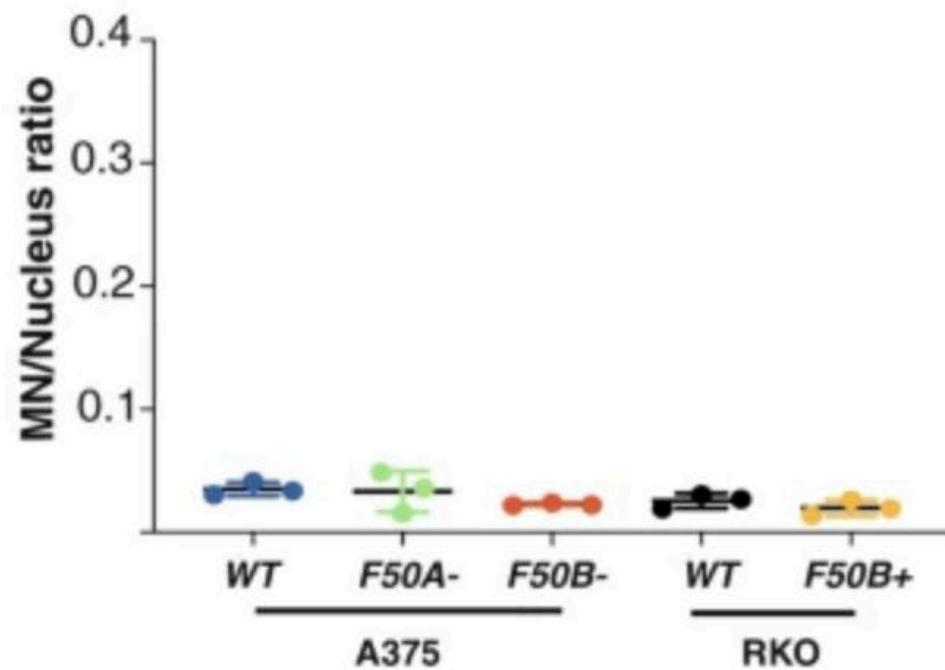


B



How does FAM reintroduction induce apoptosis

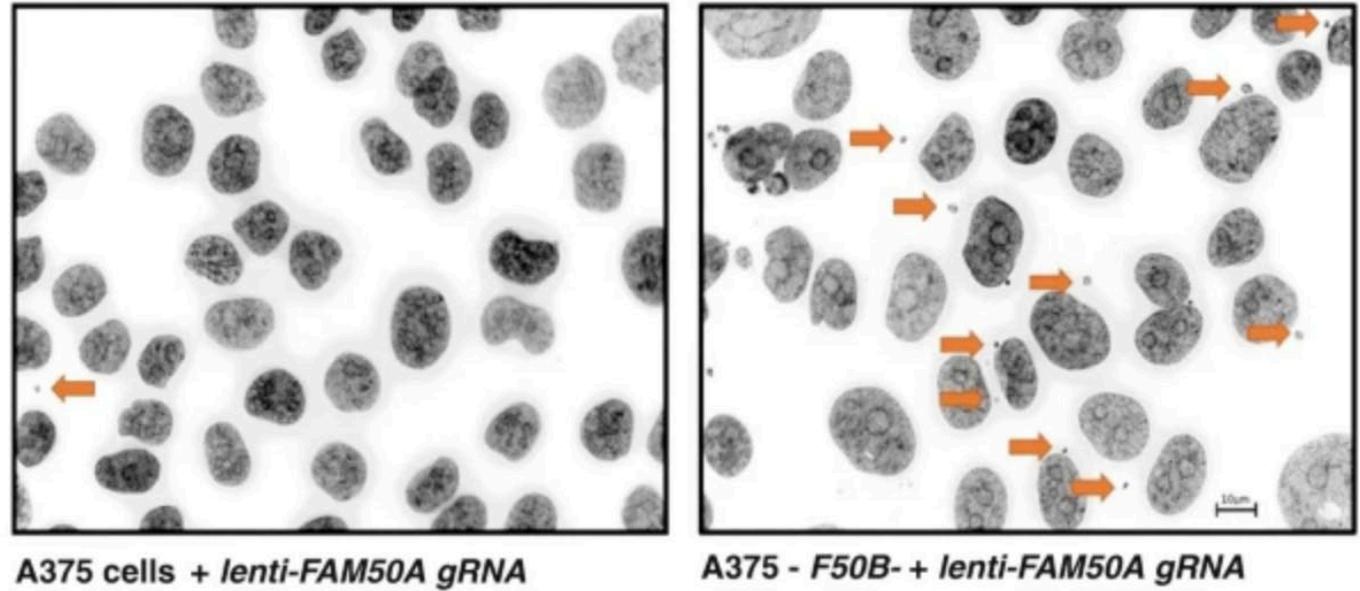
A



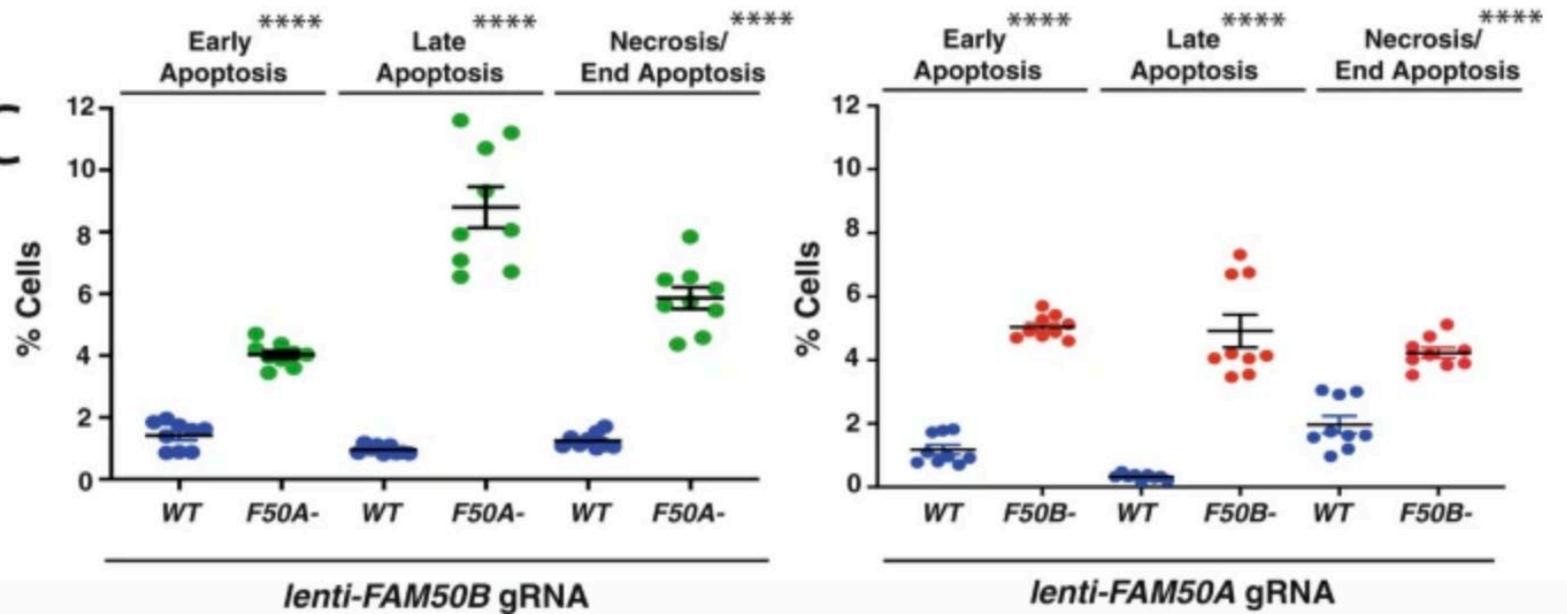
D

Induction with Apoptosis

B



C



Summary

1. New synthetic lethal pairs have been identified that are involved in ascertaining fitness of cells.
2. Most of the hits turned out to be gene paralogs.
3. New therapeutic modules are potentially discovered.

Drawbacks:

1. Limited number of genes tested.
2. Not all hits will be additive effects.



SOURCE
DATA



TRANSPARENT
PROCESS

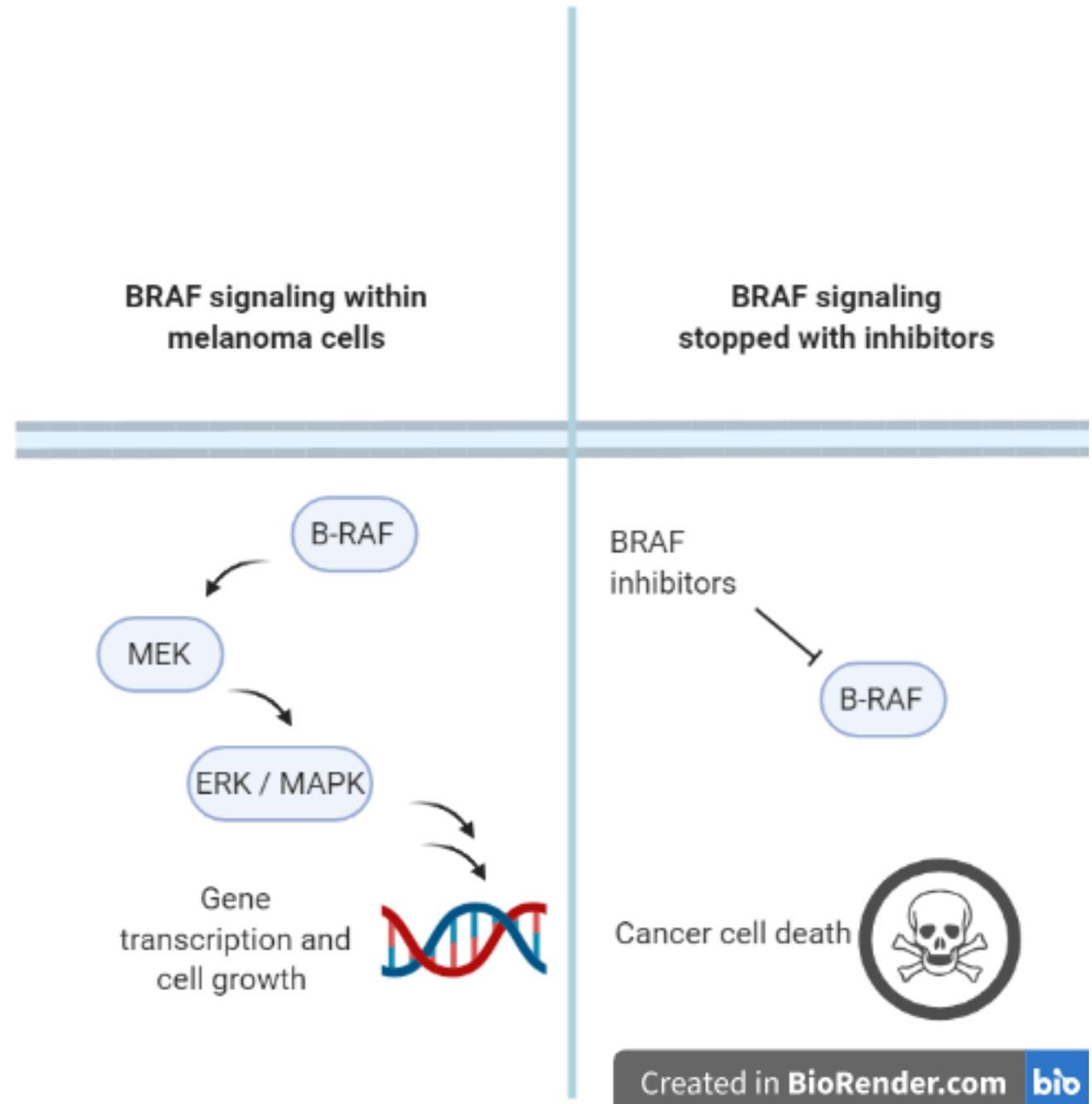


OPEN
ACCESS

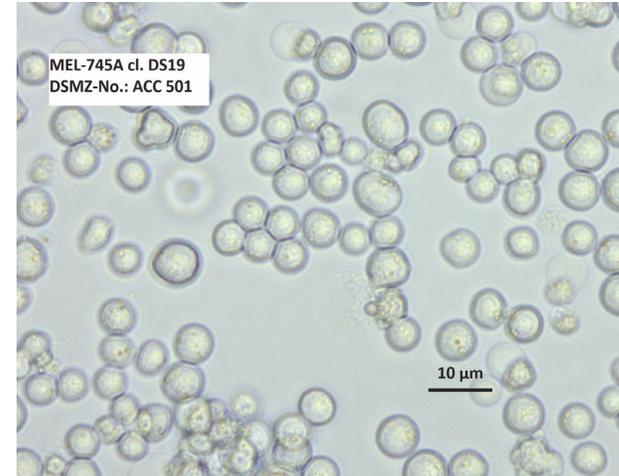
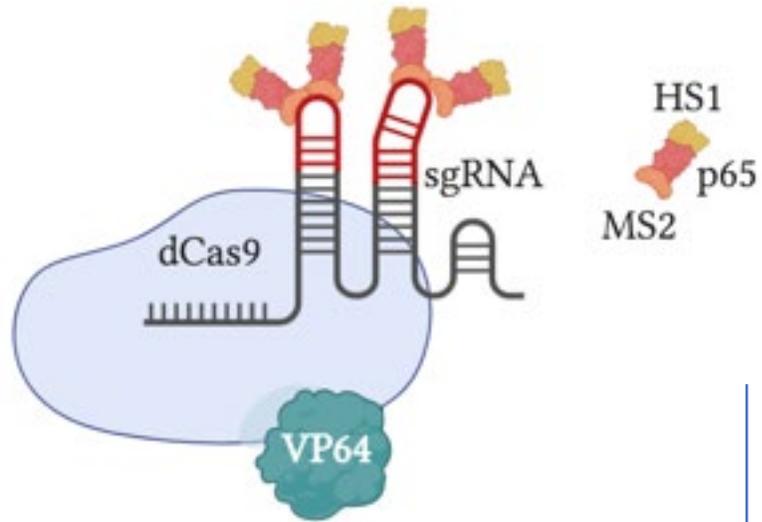
CRISPR screens identify tumor-promoting genes conferring melanoma cell plasticity and resistance

Arthur Gautron¹, Laura Bachelot¹, Marc Aubry^{1,2,†} , Delphine Leclerc^{3,†}, Anaïs M Quéméner^{1,†}, Sébastien Corre^{1,†} , Florian Rambow^{4,5}, Anaïs Paris¹, Nina Tardif¹, Héloïse M Leclair¹, Oskar Marin-Bejar^{4,5}, Cédric Coulouarn³, Jean-Christophe Marine^{4,5}, Marie-Dominique Galibert^{1,6,*}  & David Gilot^{1,‡,**} 

Aim: Identify signaling mechanisms
Conferring resistance to BRAF inhibition



Methodology

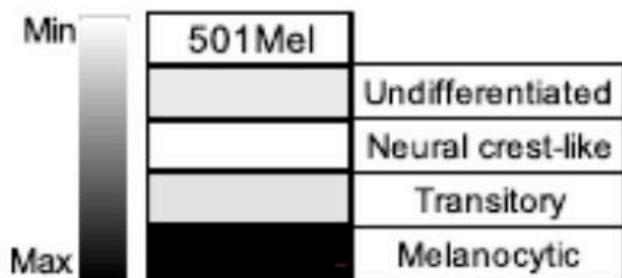
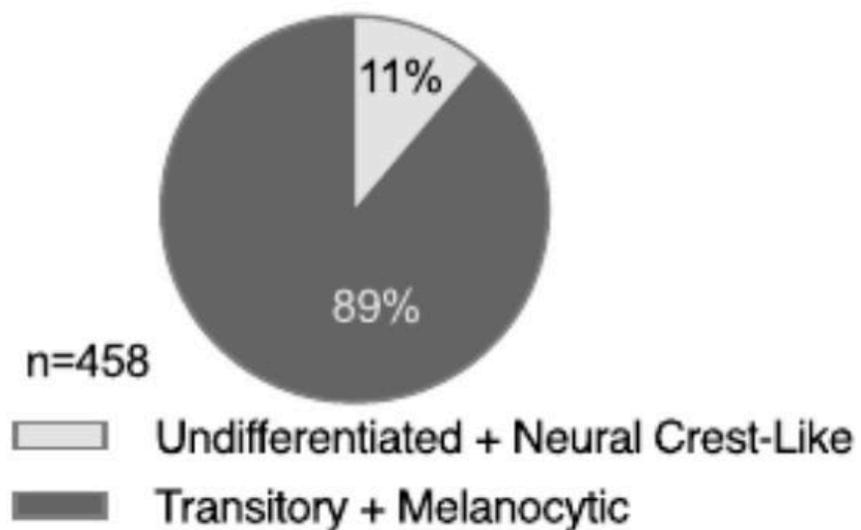


Pooled sgRNA library

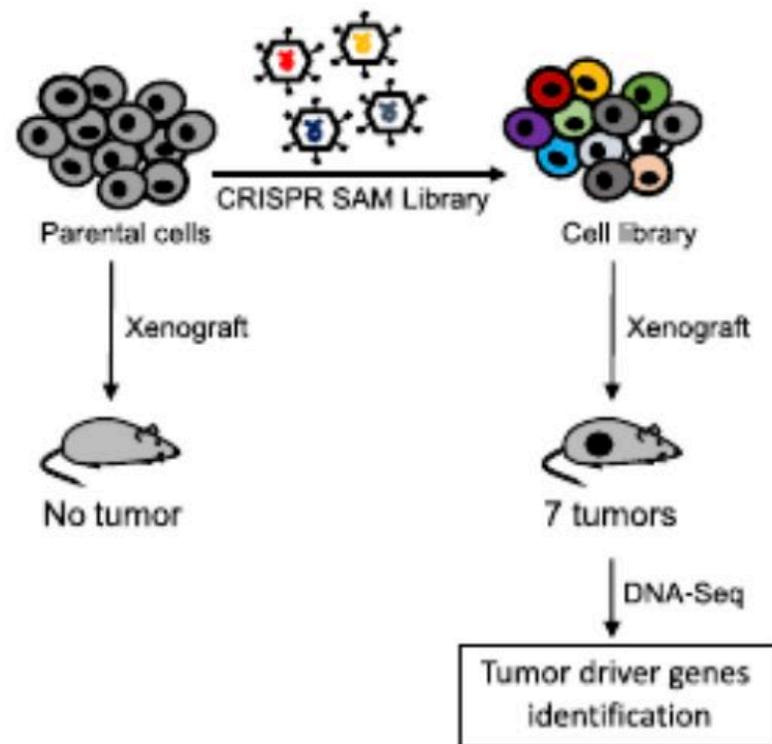
Xenograft into mice

Screen

A

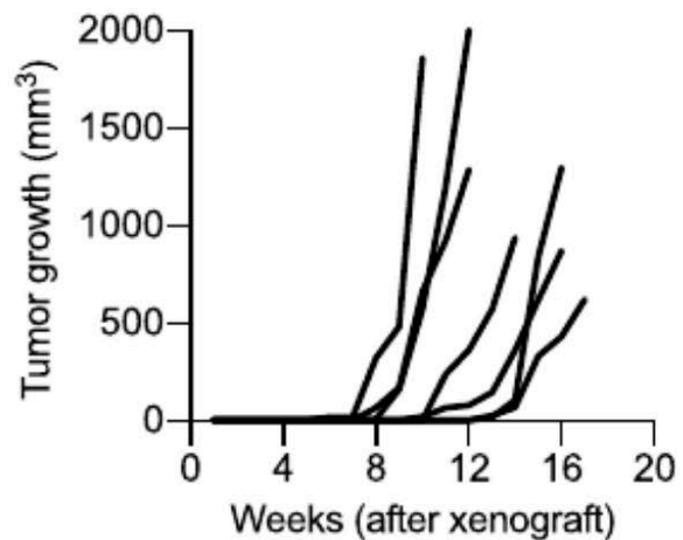


B

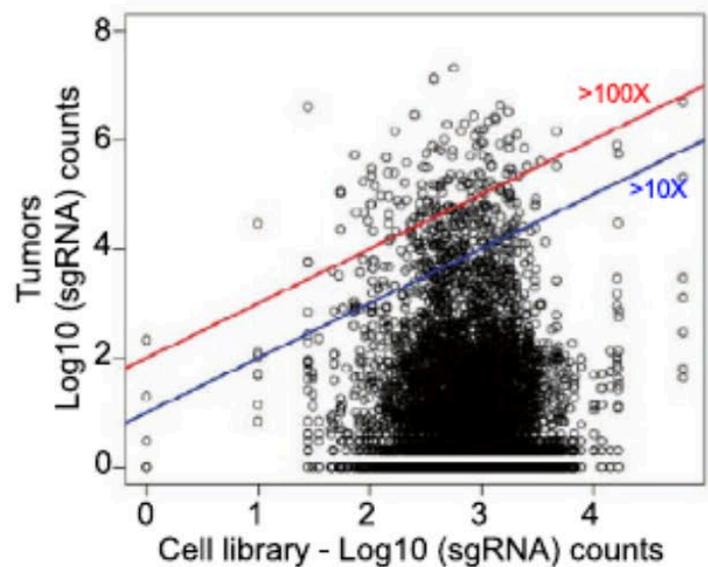


Hit identification from the tumors

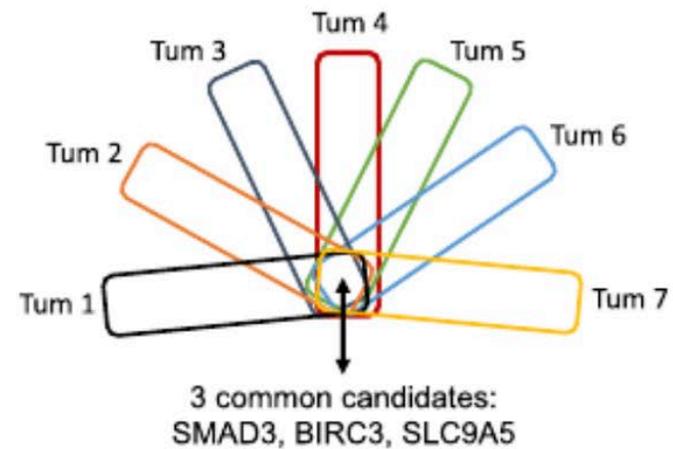
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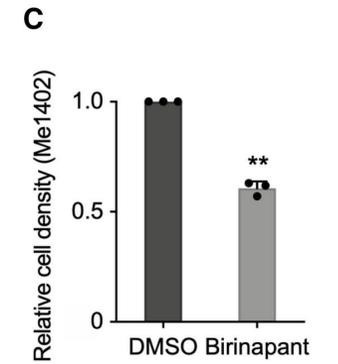
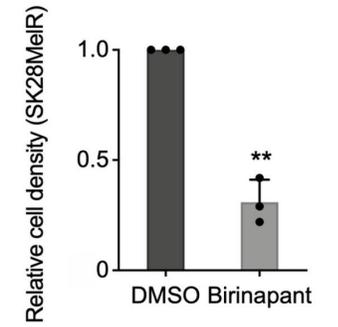
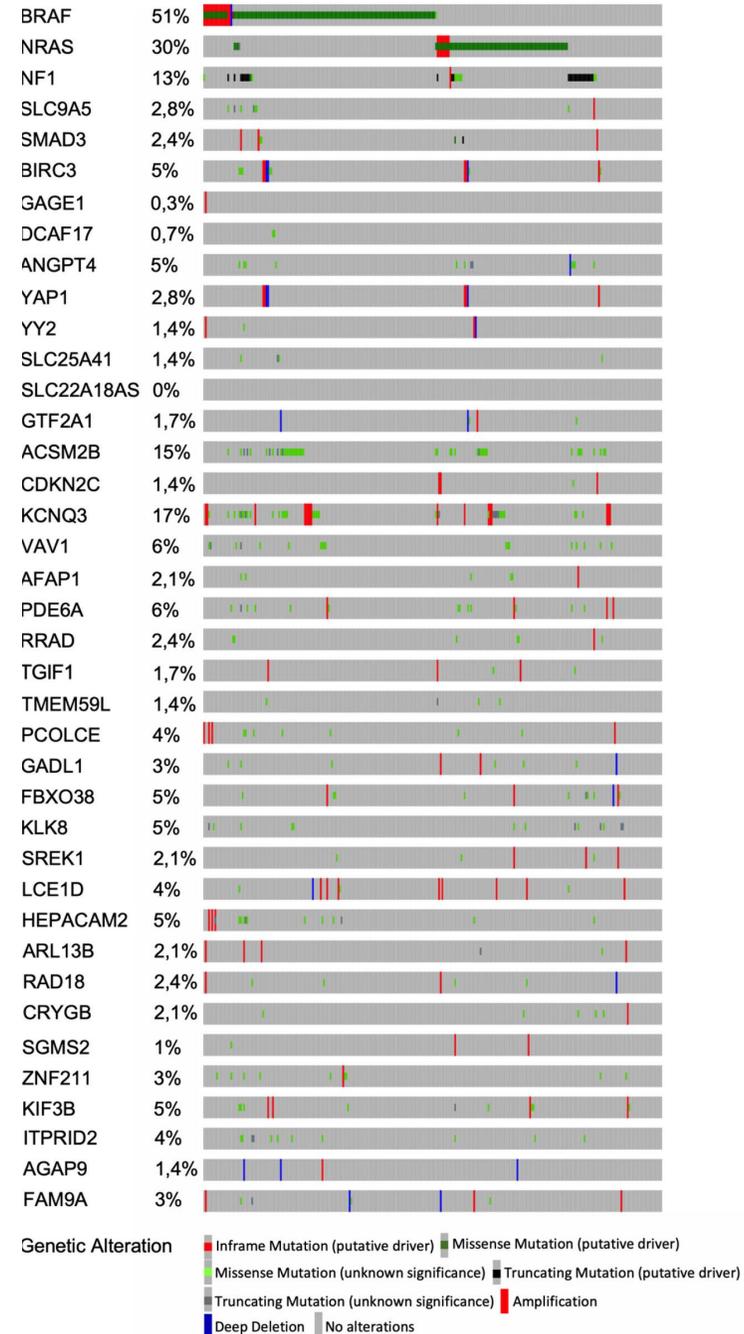
E



F

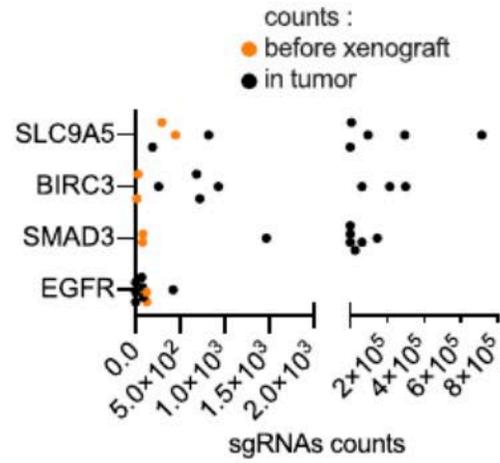


List of All Hits

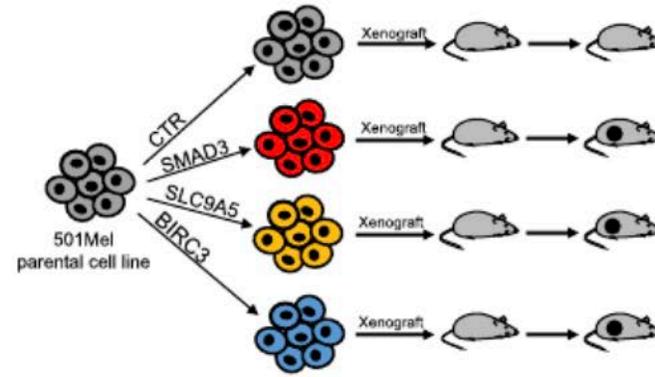


Validation of the Hits

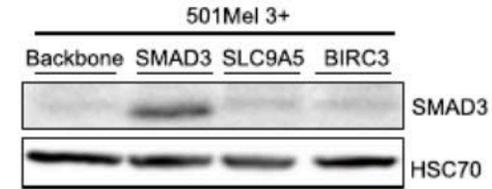
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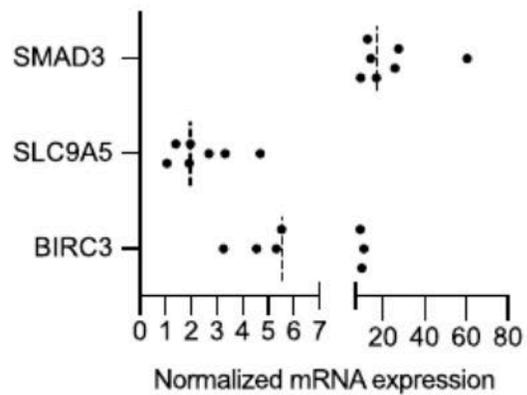
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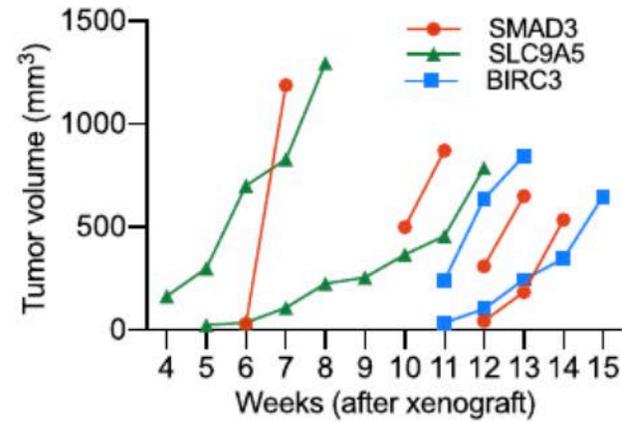
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J

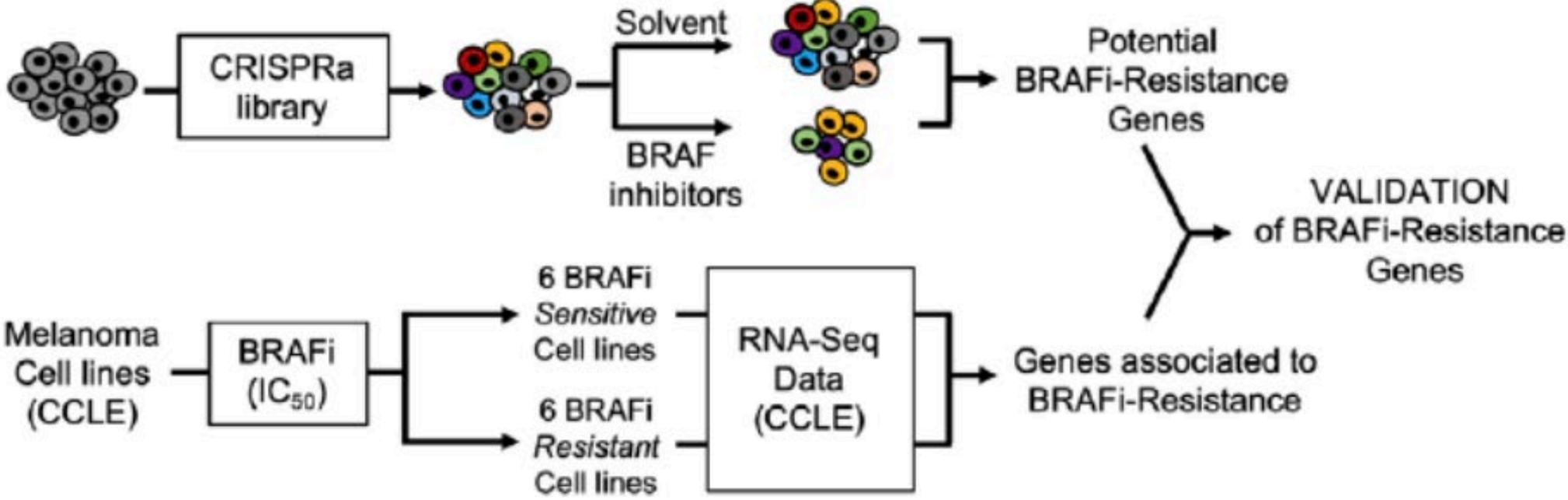


K



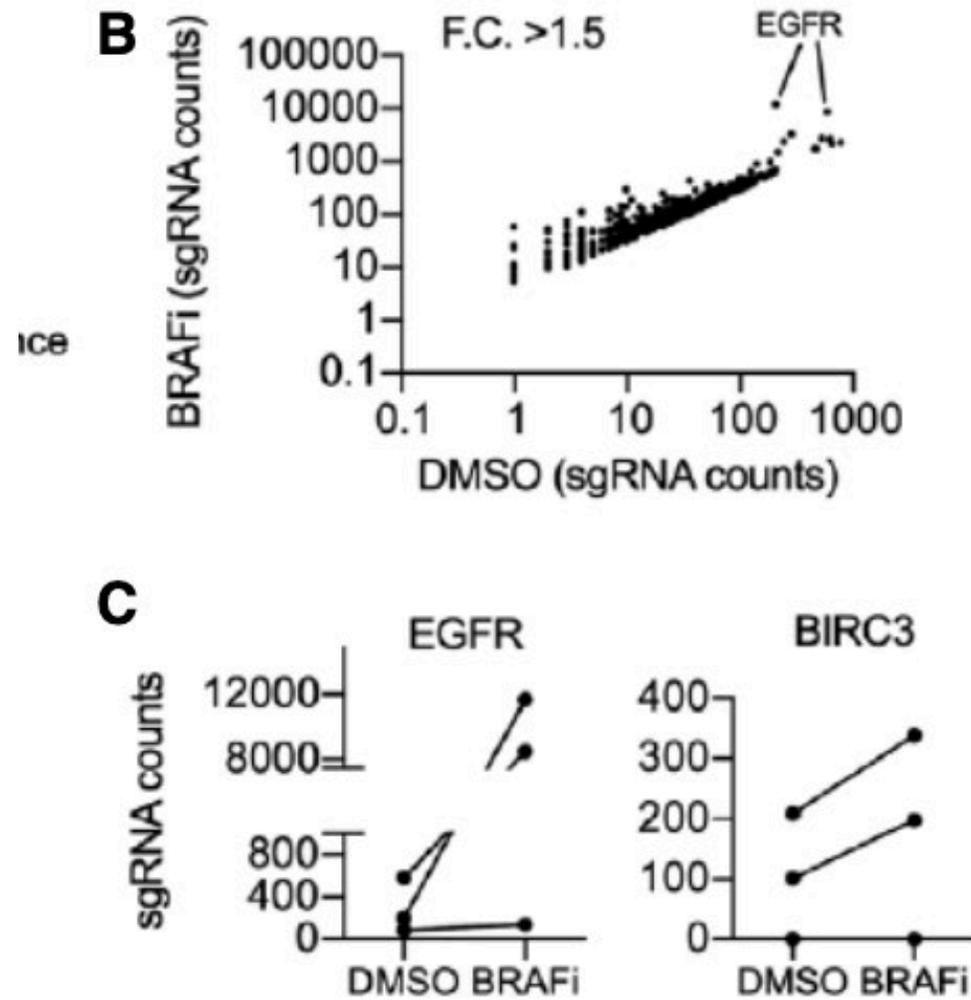
A complementary screen to compare the hits

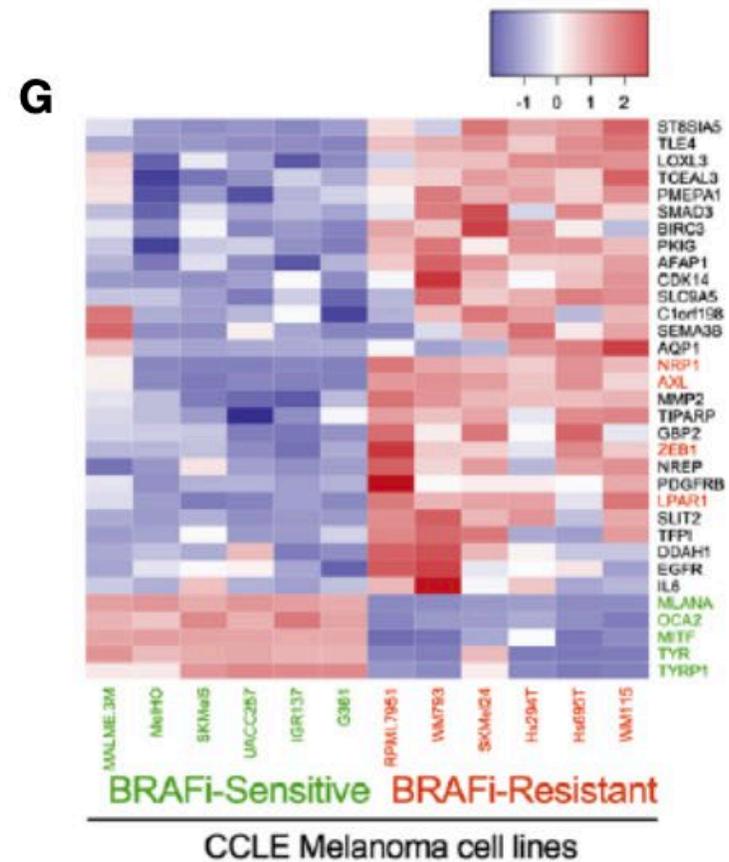
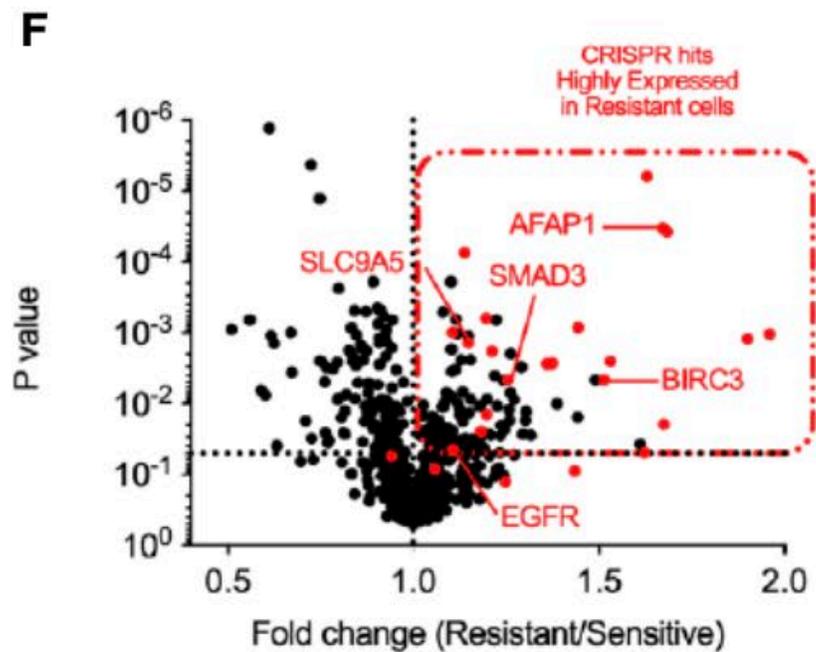
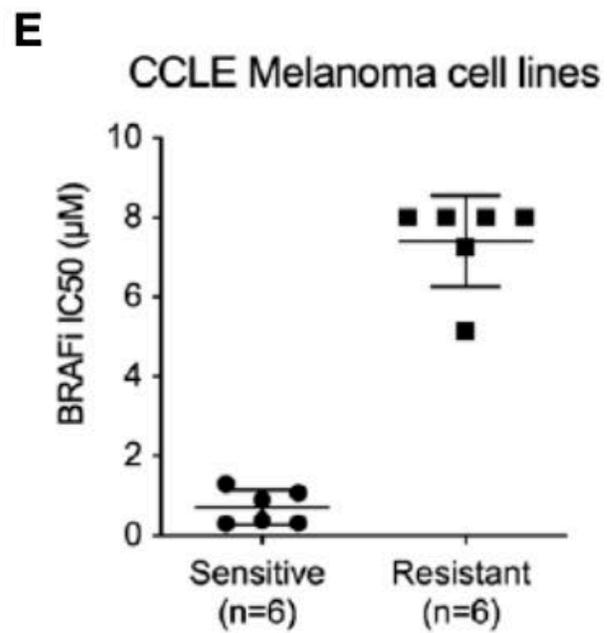
A



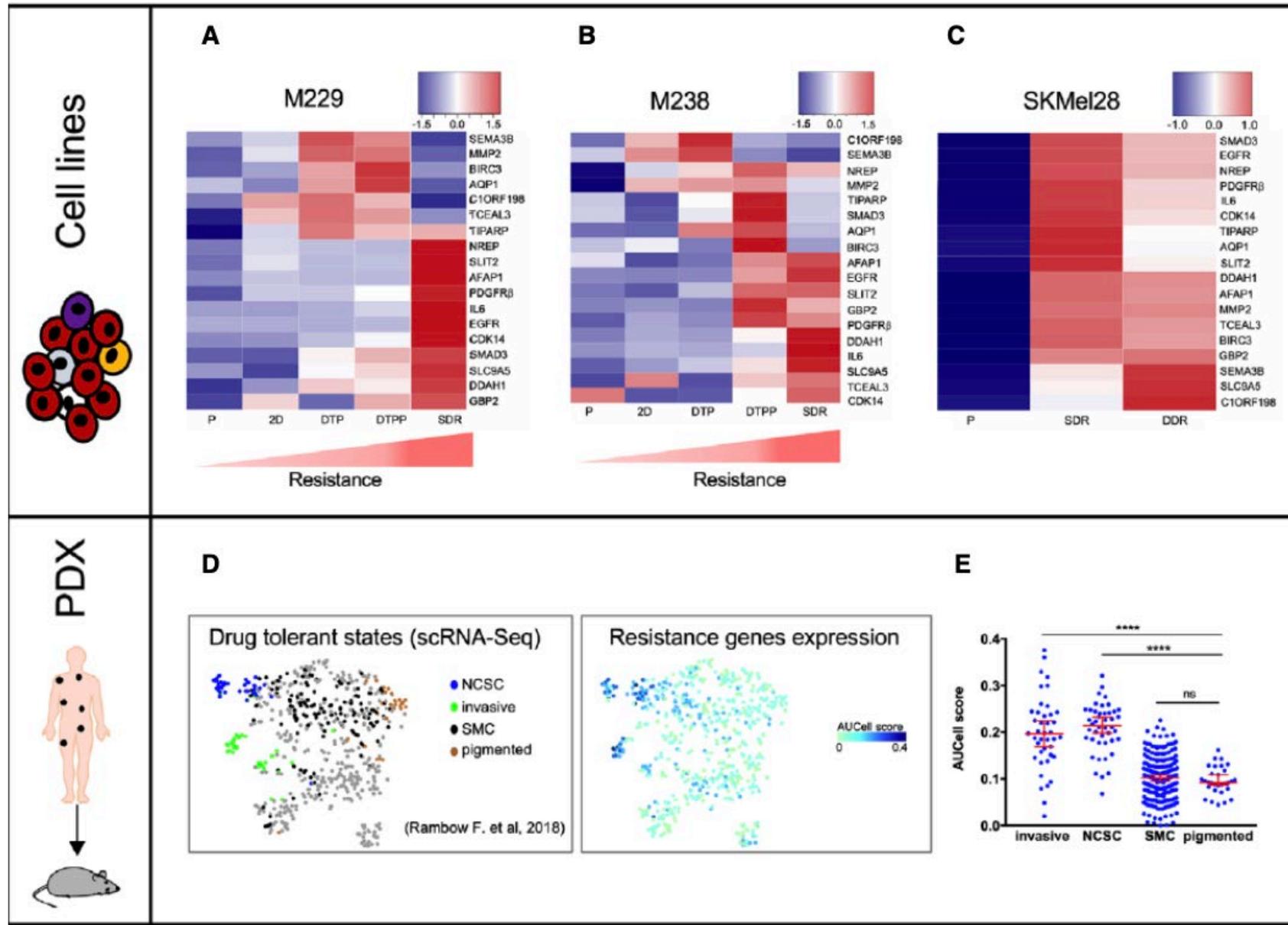
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Hit List

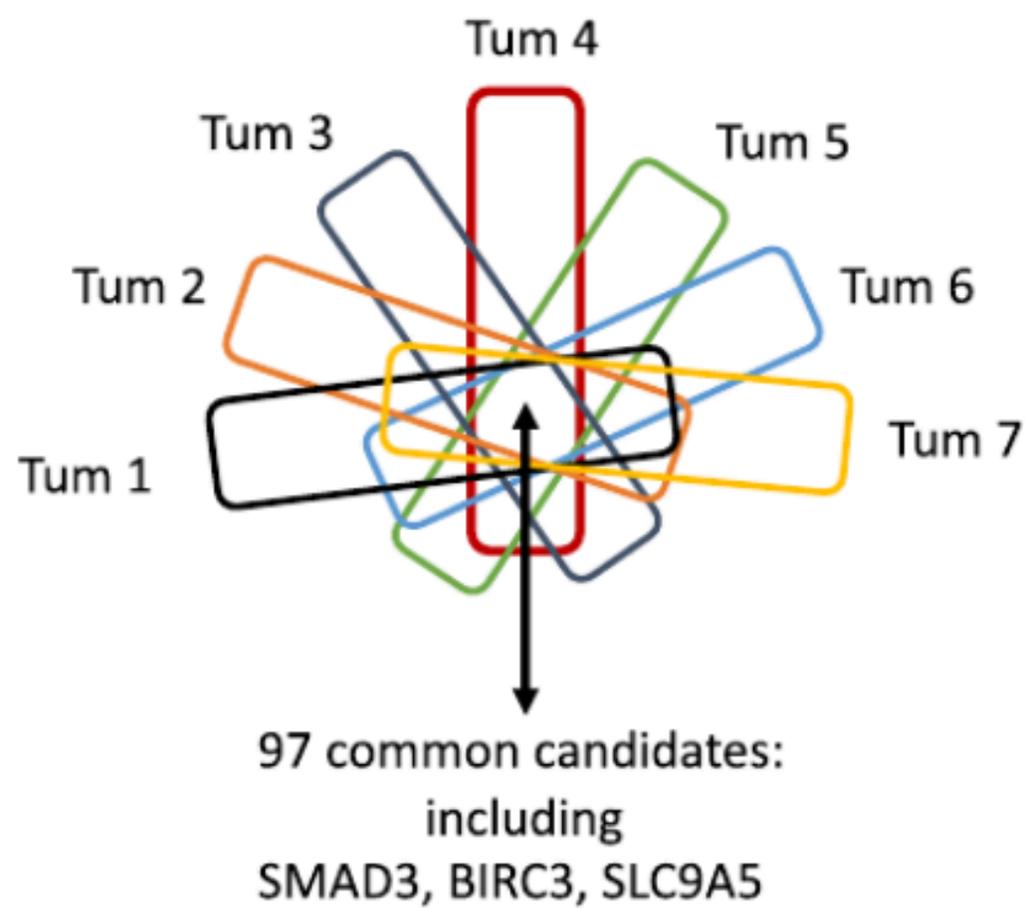




Validation in model systems

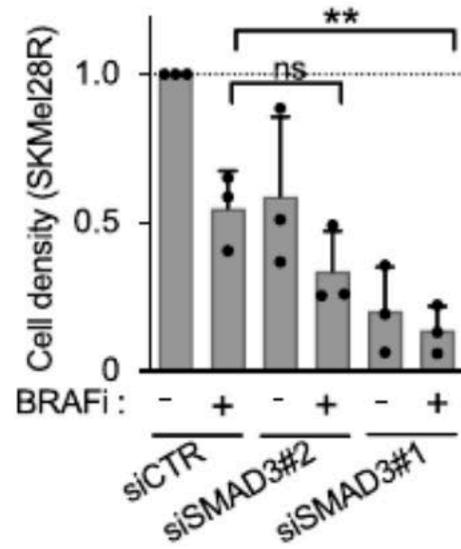


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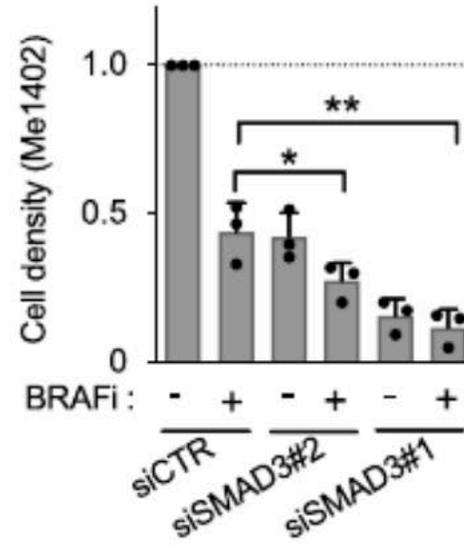


Validation of SMAD3 as a hit

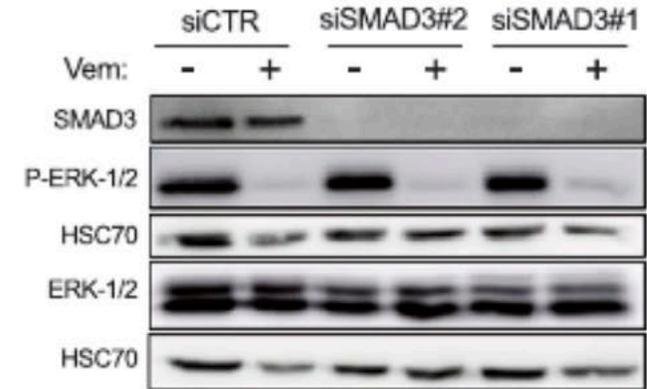
G



H

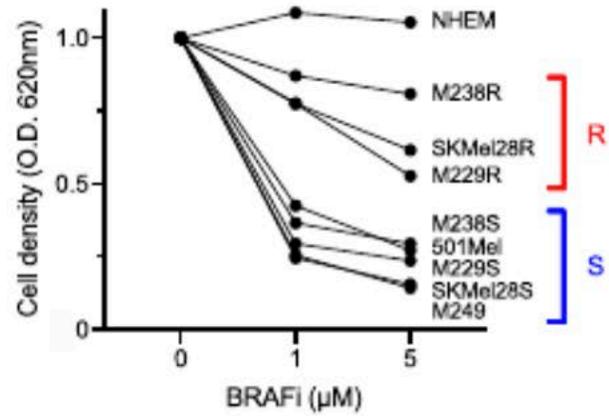


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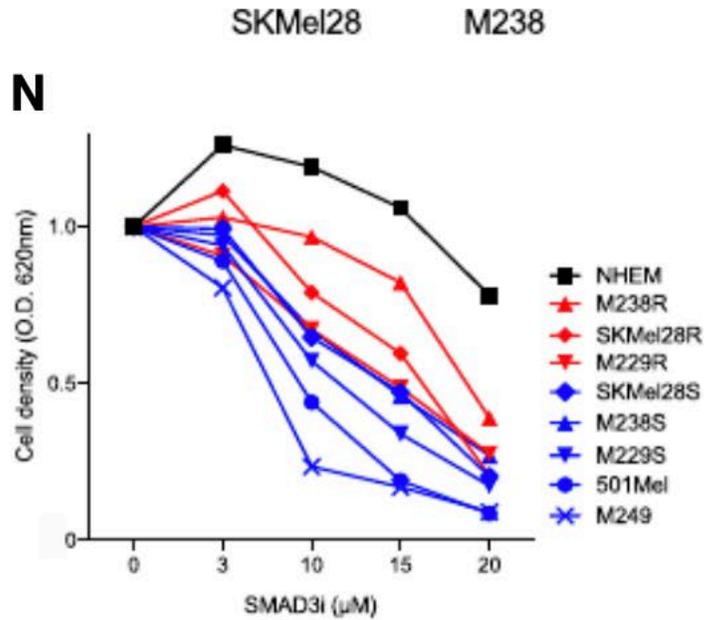


Inhibitors against SMAD3 affects tumor formations

M



N



O

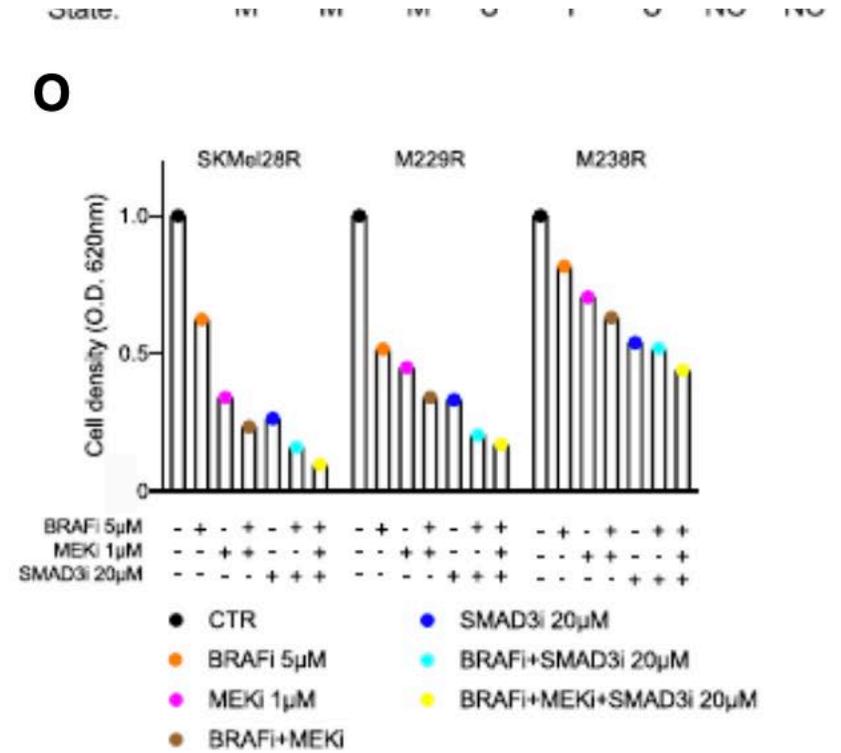
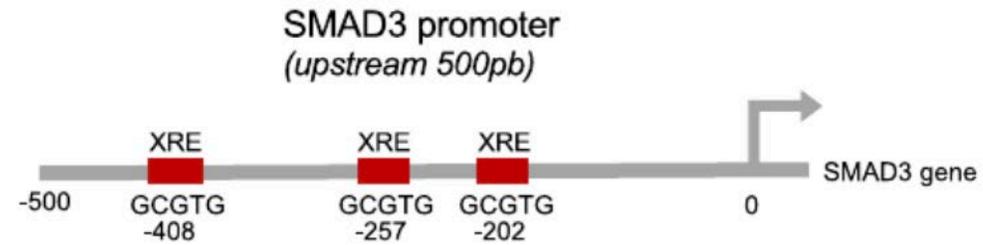


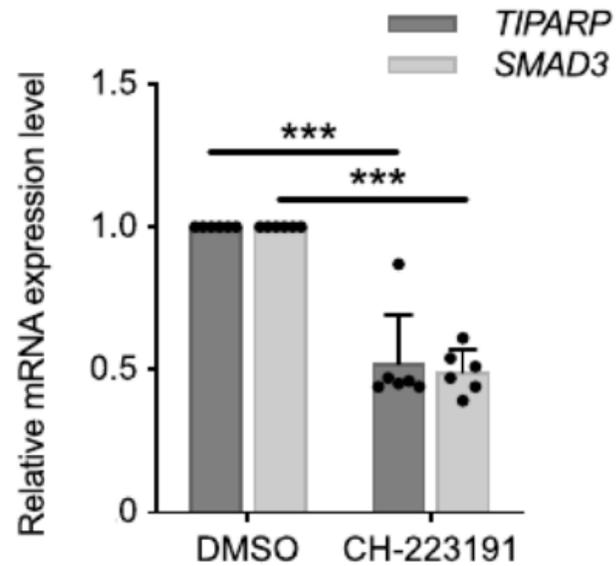
Figure 5.

Identification of the pathway driving SMAD3 expression

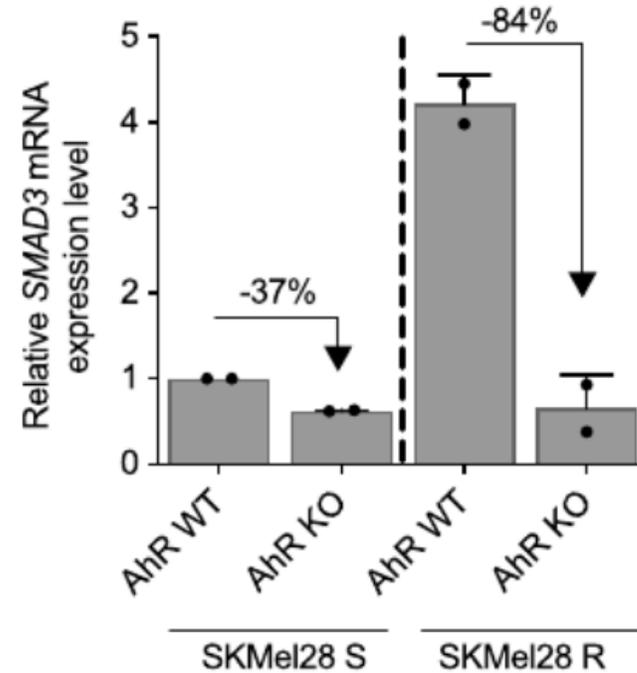
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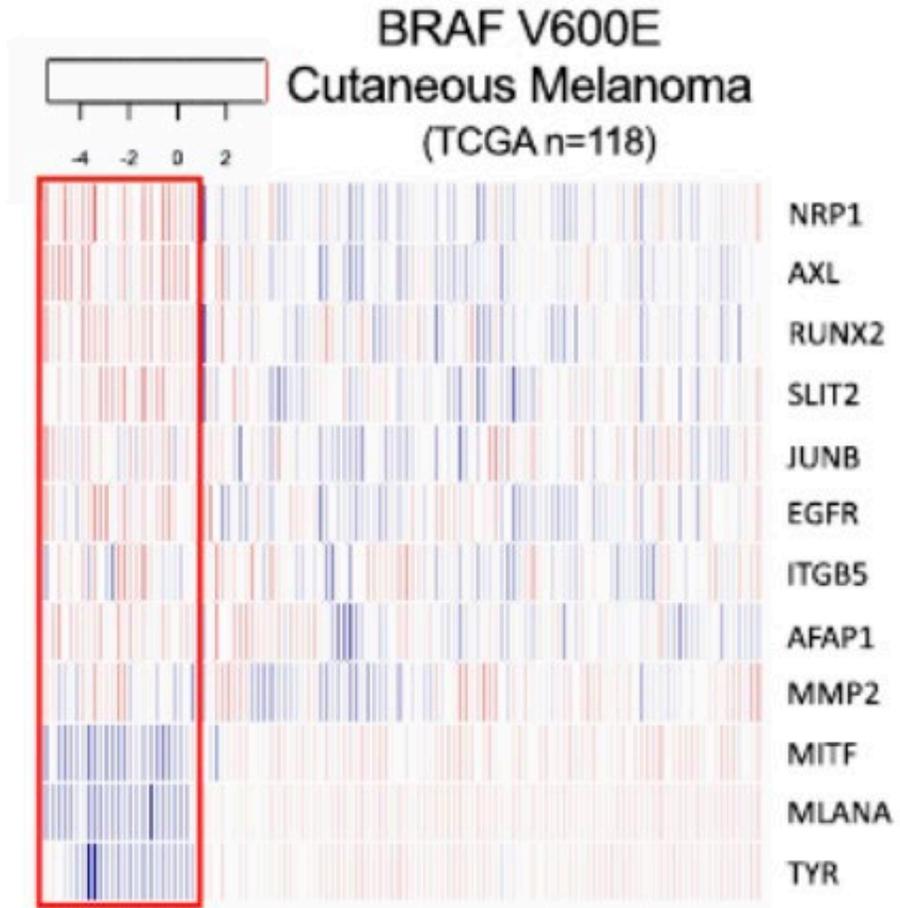
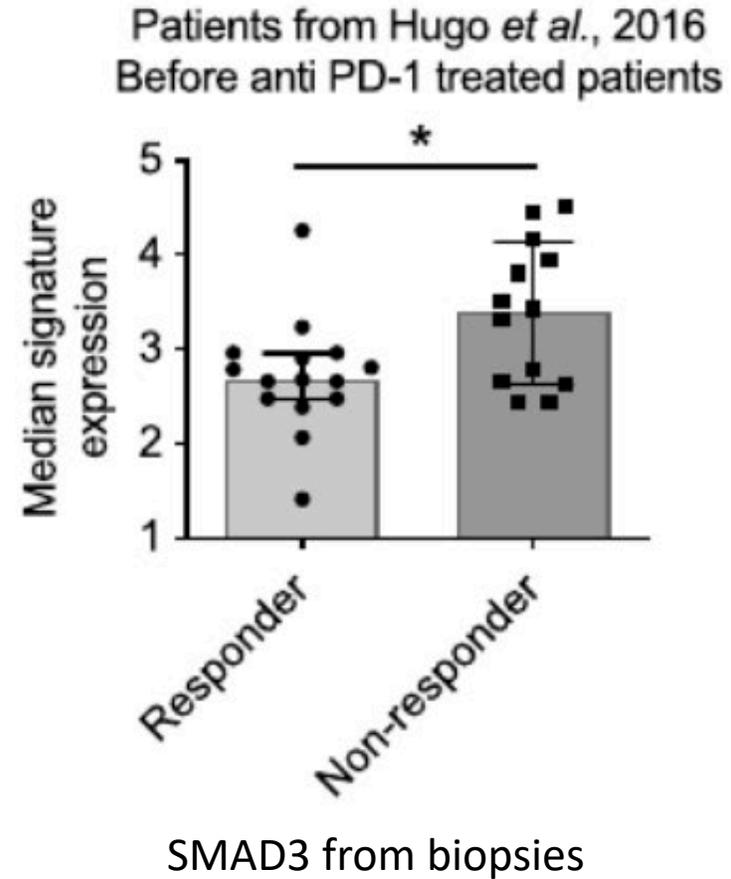


D



E



E**F**

Summary

1. The study highlights the pathways associated with persister cells
2. New pathways involved in persister cells are identified

Drawbacks

1. Functionality of CRISPRa plasmids used in the current study is unclear
2. Reprogramming transcriptional profiles would be a complicated approach for therapy.



Targeted CRISPR screening identifies PRMT5 as synthetic lethality combinatorial target with gemcitabine in pancreatic cancer cells

Xiaolong Wei^{a,1}, Jiekun Yang^{a,1}, Sara J. Adair^b , Harun Ozturk^c, Cem Kuscu^d, Kyung Yong Lee^a , William J. Kane^b, Patrick E. O'Hara^b , Denis Liu^b, Yusuf Mert Demirlenk^a , Alaa Hamdi Habieb^a, Ebru Yilmaz^a , Anindya Dutta^a , Todd W. Bauer^b , and Mazhar Adli^{a,c,2} 

^aDepartment of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, VA 22903; ^bDepartment of Surgery, University of Virginia School of Medicine, Charlottesville, VA 22903; ^cFeinberg School of Medicine, Robert Lurie Comprehensive Cancer Center, Department of Obstetrics and Gynecology, The Northwestern University, Chicago, IL 60611; and ^dTransplant Research Institute, University of Tennessee Health Science Center, Memphis, TN 38163

Paper -3

Cell Reports

 CellPress
OPEN ACCESS

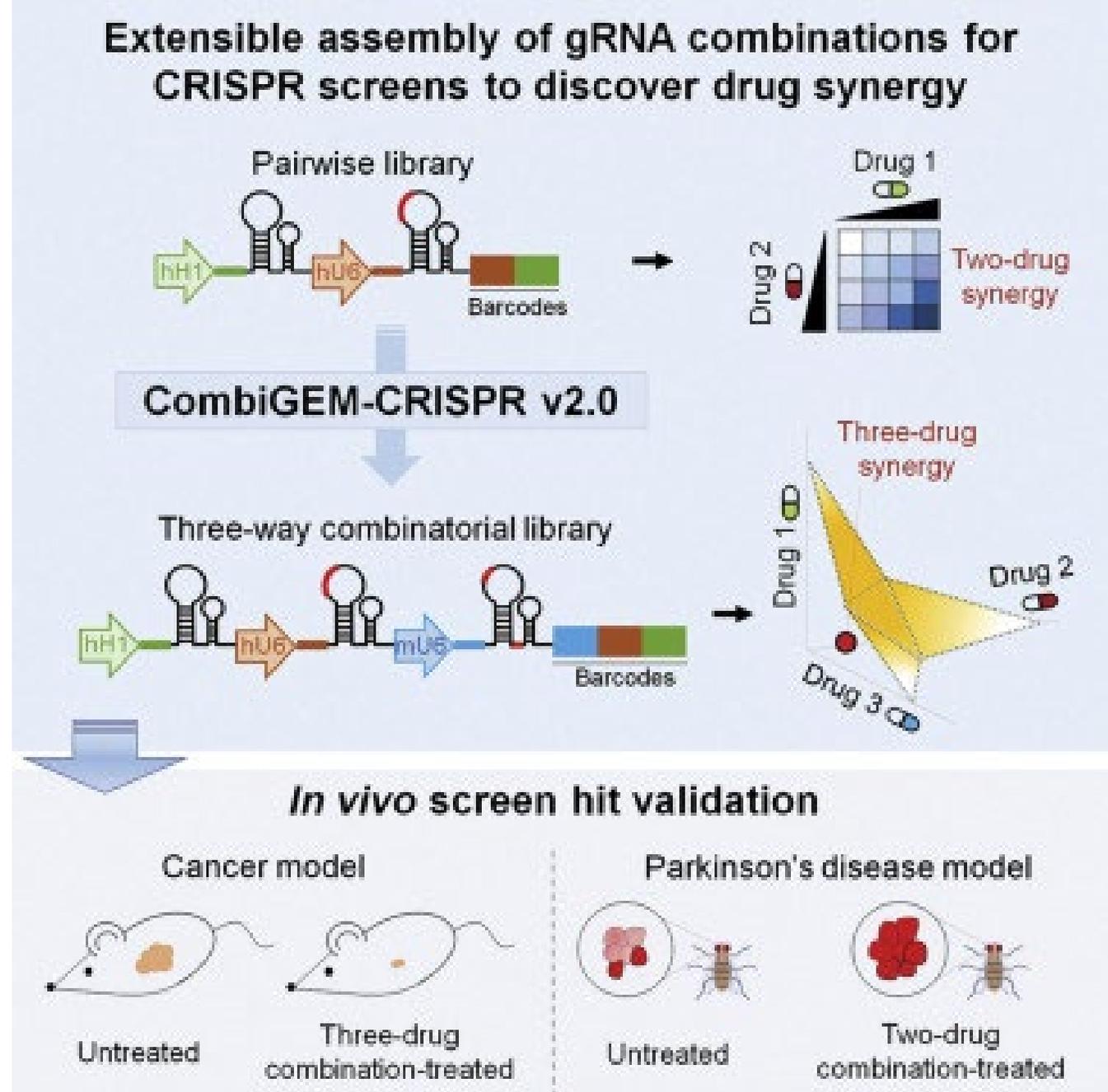


Article

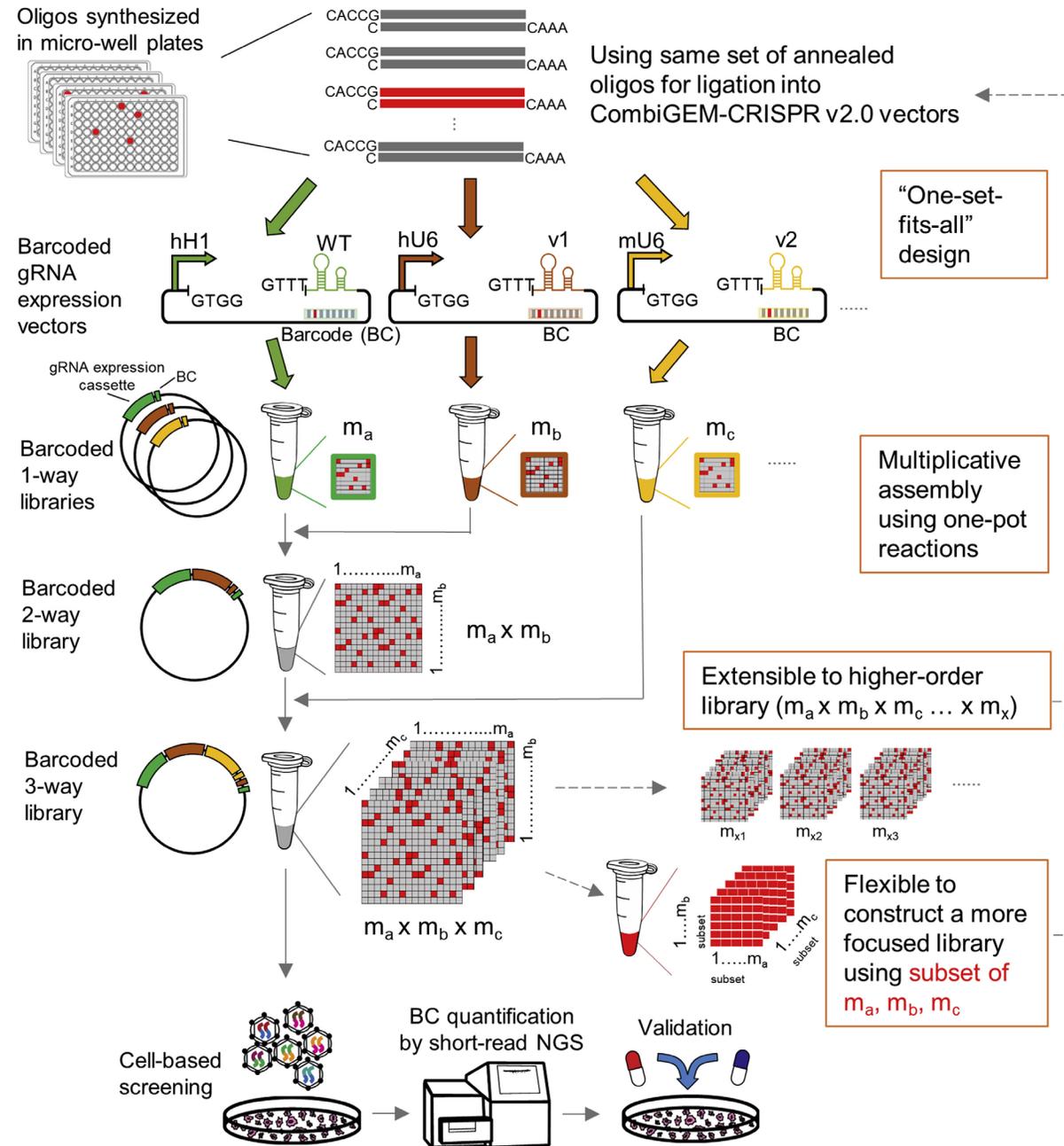
A Three-Way Combinatorial CRISPR Screen for Analyzing Interactions among Druggable Targets

Peng Zhou,¹ Becky K.C. Chan,¹ Yuk Kei Wan,¹ Chaya T.L. Yuen,¹ Gigi C.G. Choi,¹ Xinran Li,² Cindy S.W. Tong,¹ Sophia S.W. Zhong,¹ Jieran Sun,¹ Yufan Bao,^{3,4} Silvia Y.L. Mak,³ Maggie Z.Y. Chow,³ Jien Vei Khaw,¹ Suet Yi Leung,^{5,6,7} Zongli Zheng,^{3,4,8} Lydia W.T. Cheung,² Kaeling Tan,^{9,10} Koon Ho Wong,^{9,11} H.Y. Edwin Chan,^{12,13} and Alan S.L. Wong^{1,14,15,*}

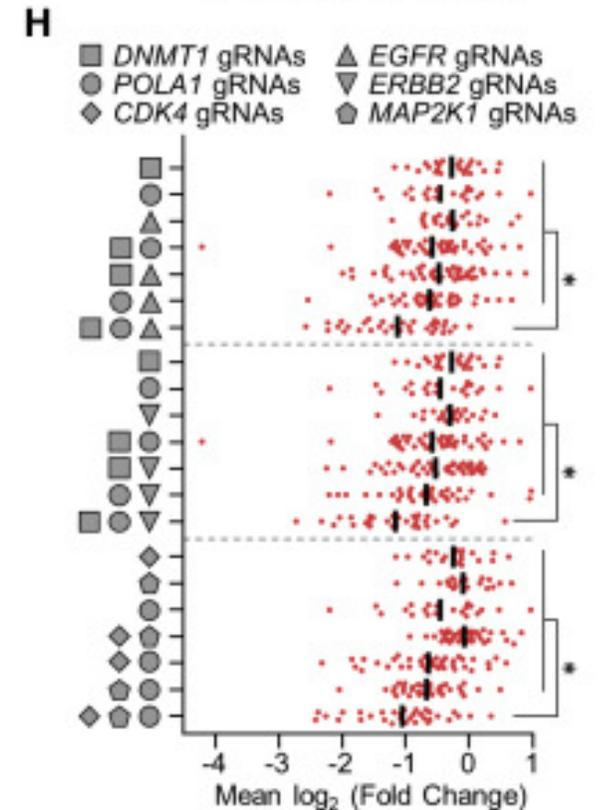
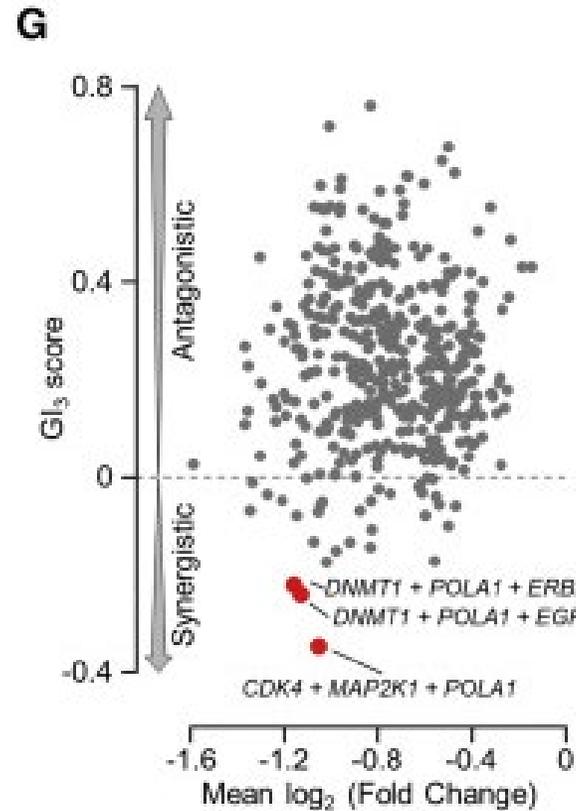
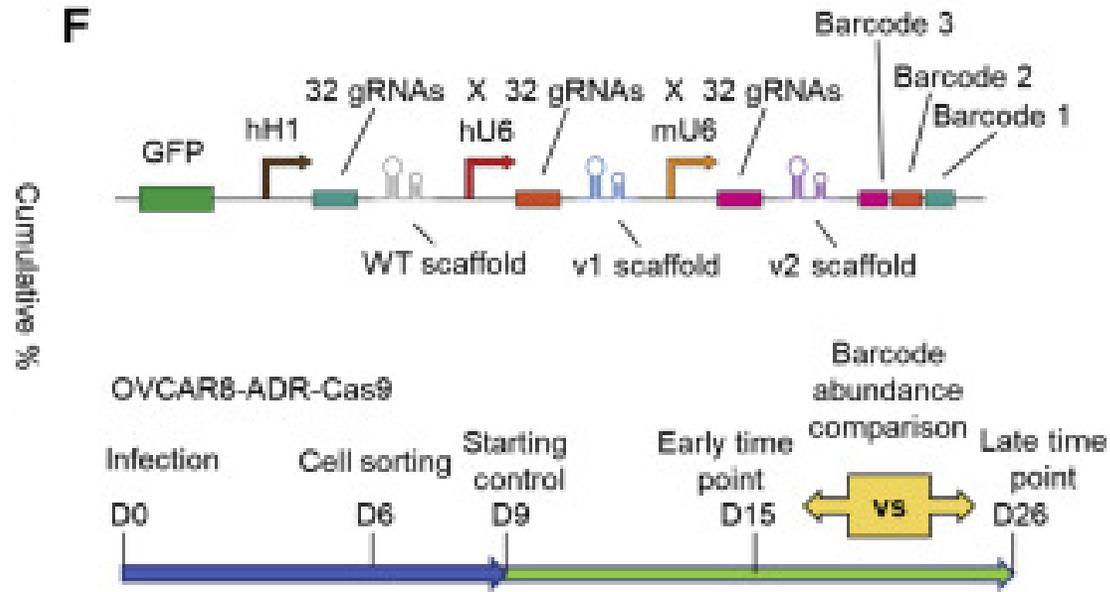
Combigen Strategies



Screen Outlay



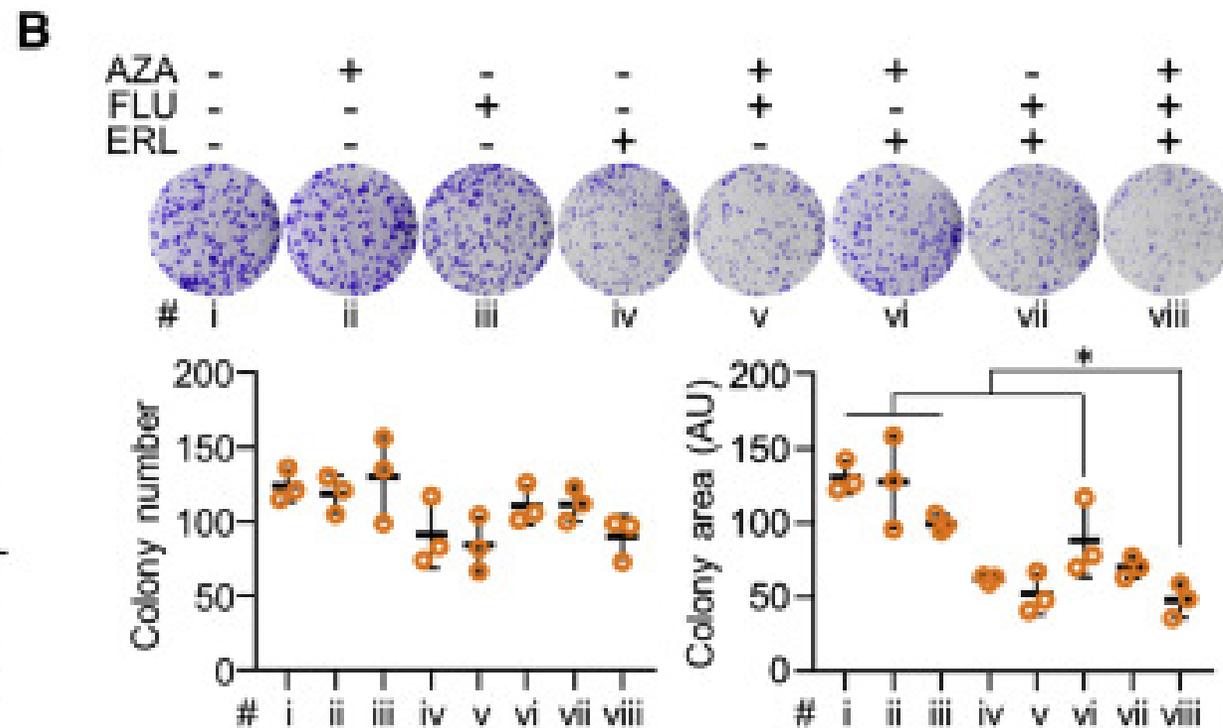
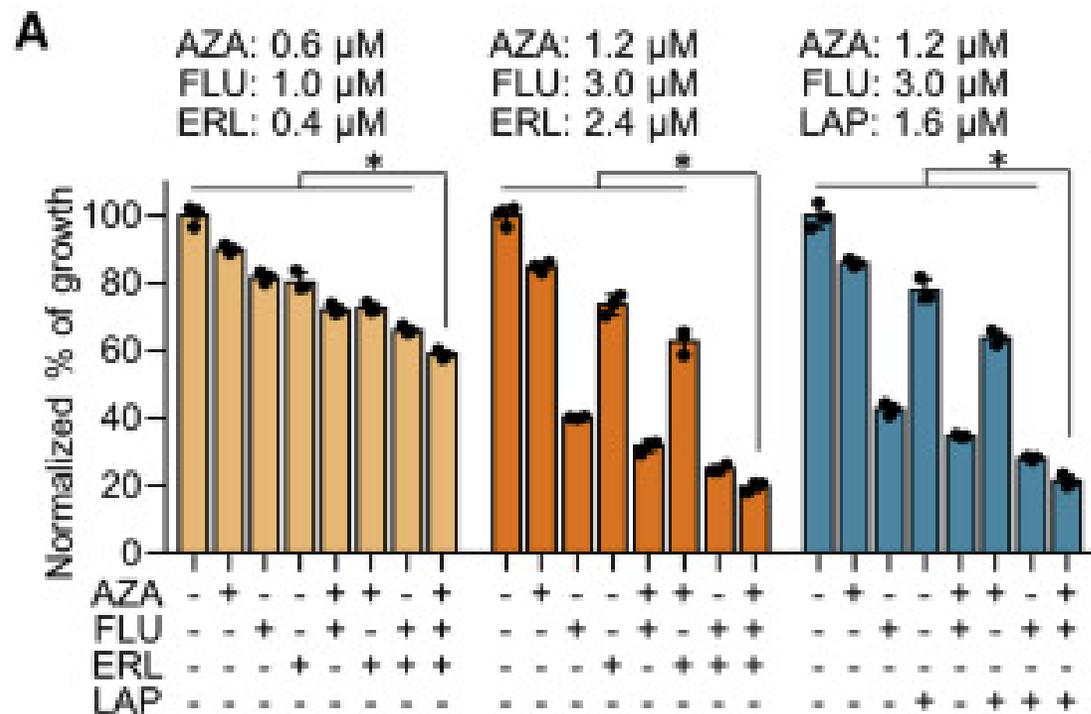
Triple gene knock out-Ovarian cancer



15 druggable genes were chosen

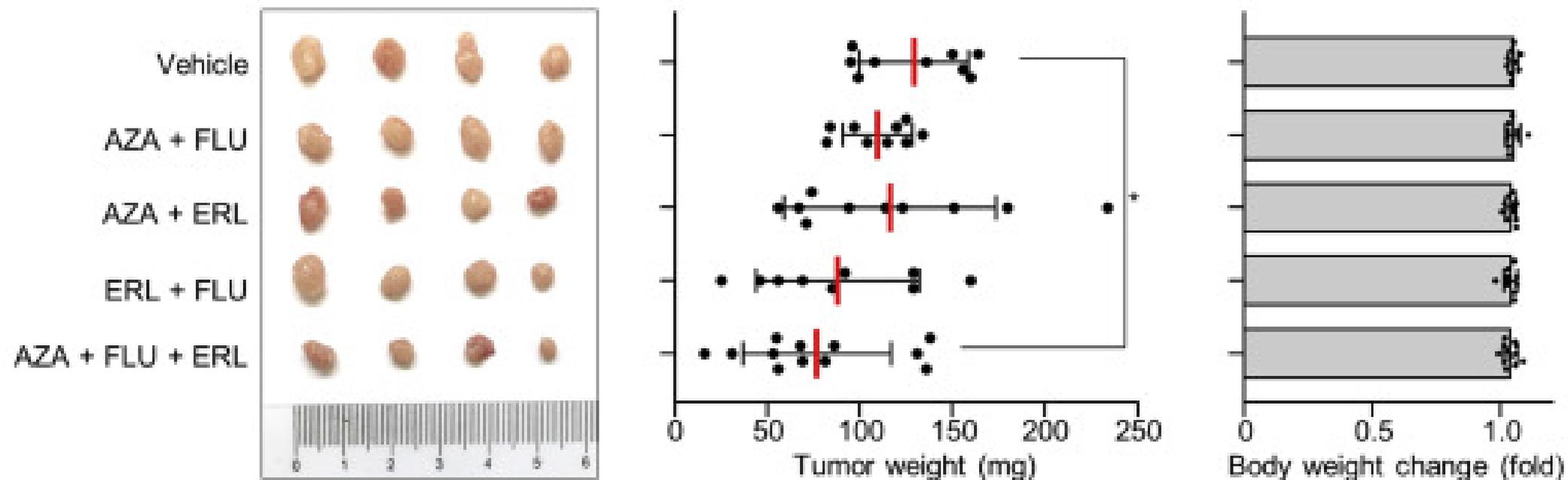
32x32x32gRNA were used

Validation of the hits in the screens using pharmacological inhibitors

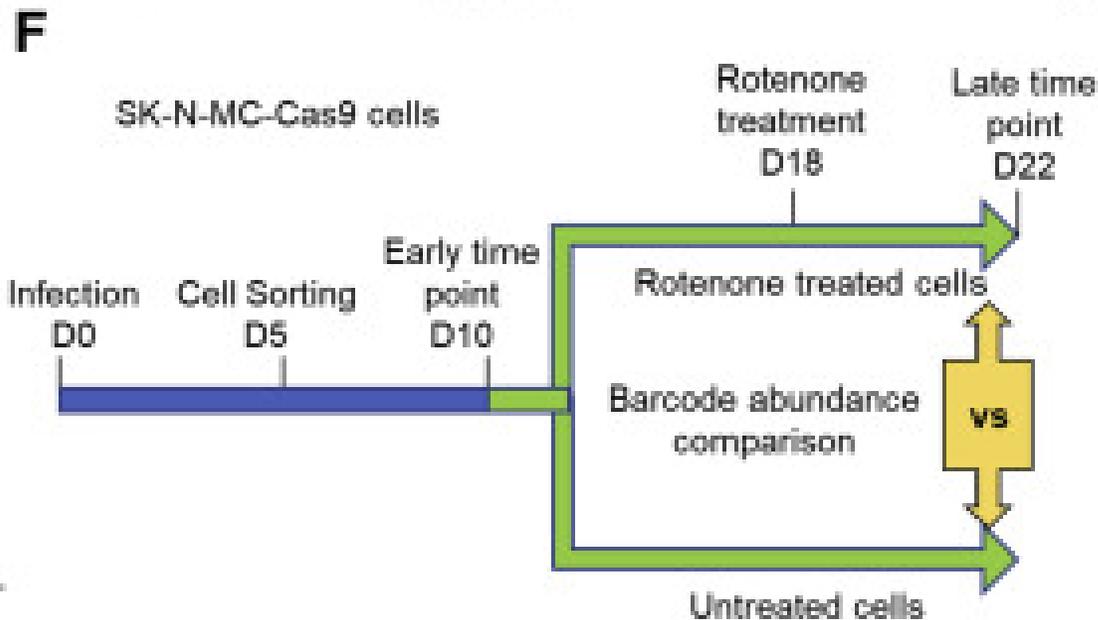


New combination therapy of drugs alters the tumor size in vivo

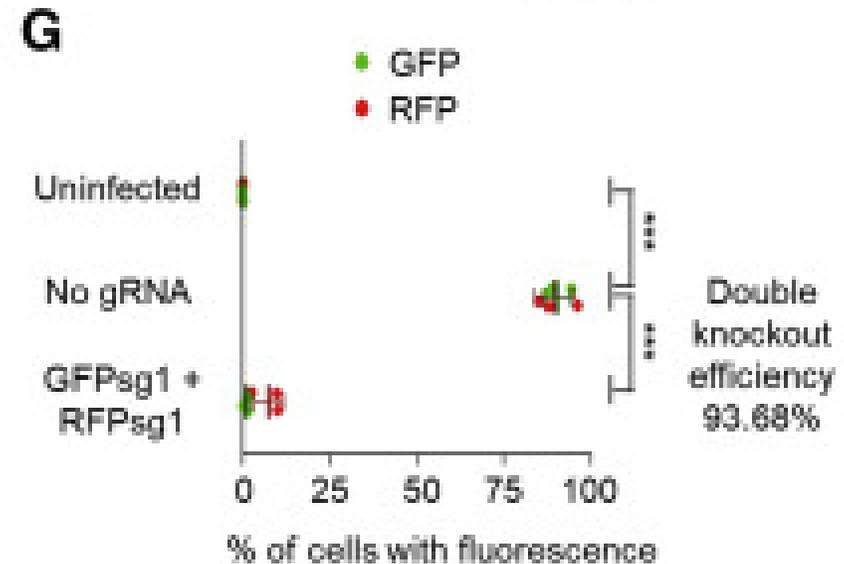
D



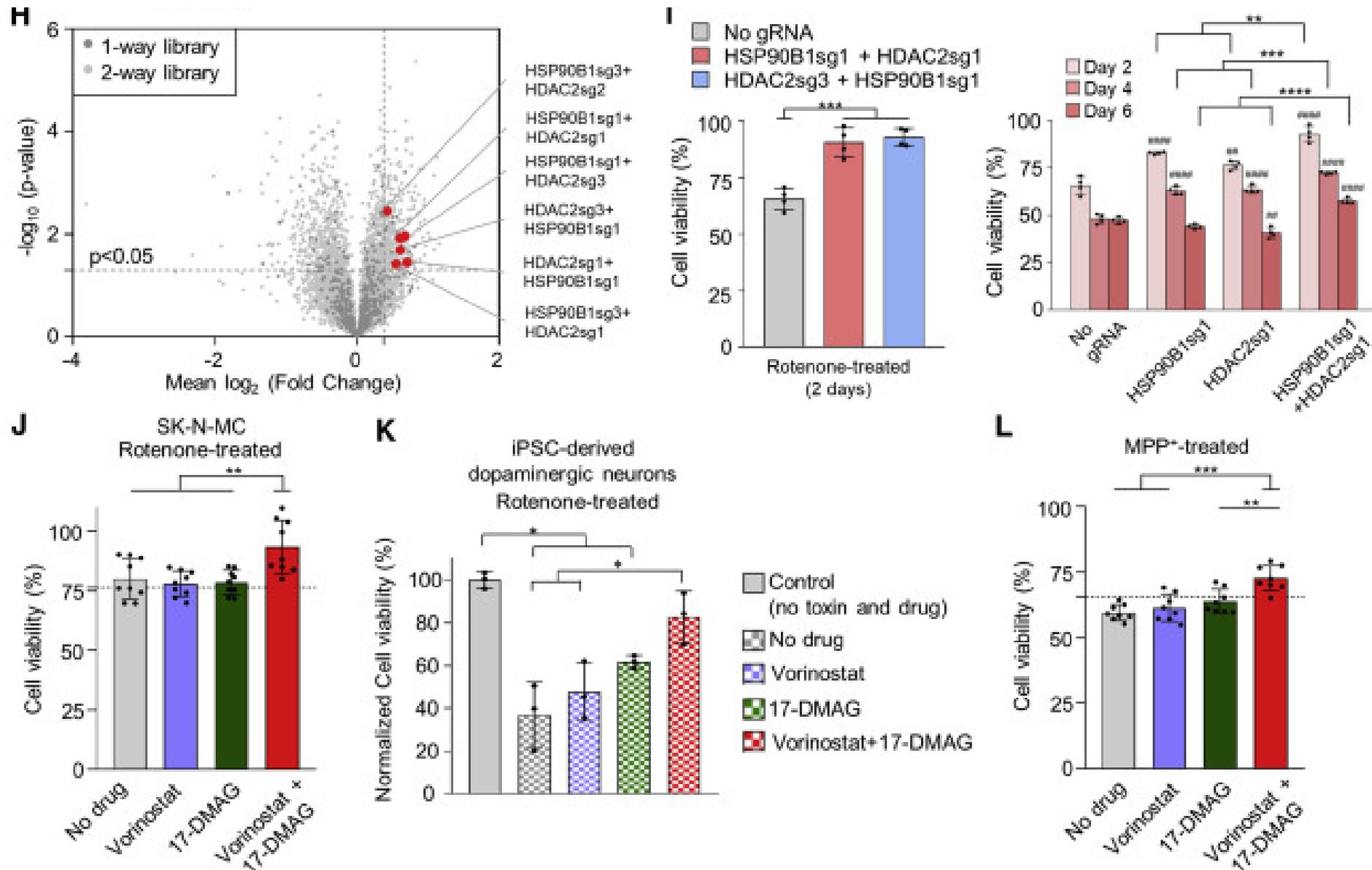
Dual-Gene Knockout Screen Identifies a Drug Combination that Enhances Protection against PD Toxicity



28 druggable genes were used



Validation of the Hits



Conclusions

1. A nice strategy to identify novel combination of genes to identify new therapeutic targets.
2. Platform can be combined with CRISPRi to mimic drugs.
3. Platform can be combined with single cell RNA seq.

Questions:

1. Patient derived cell lines?
2. Efficiency of the guide RNAs