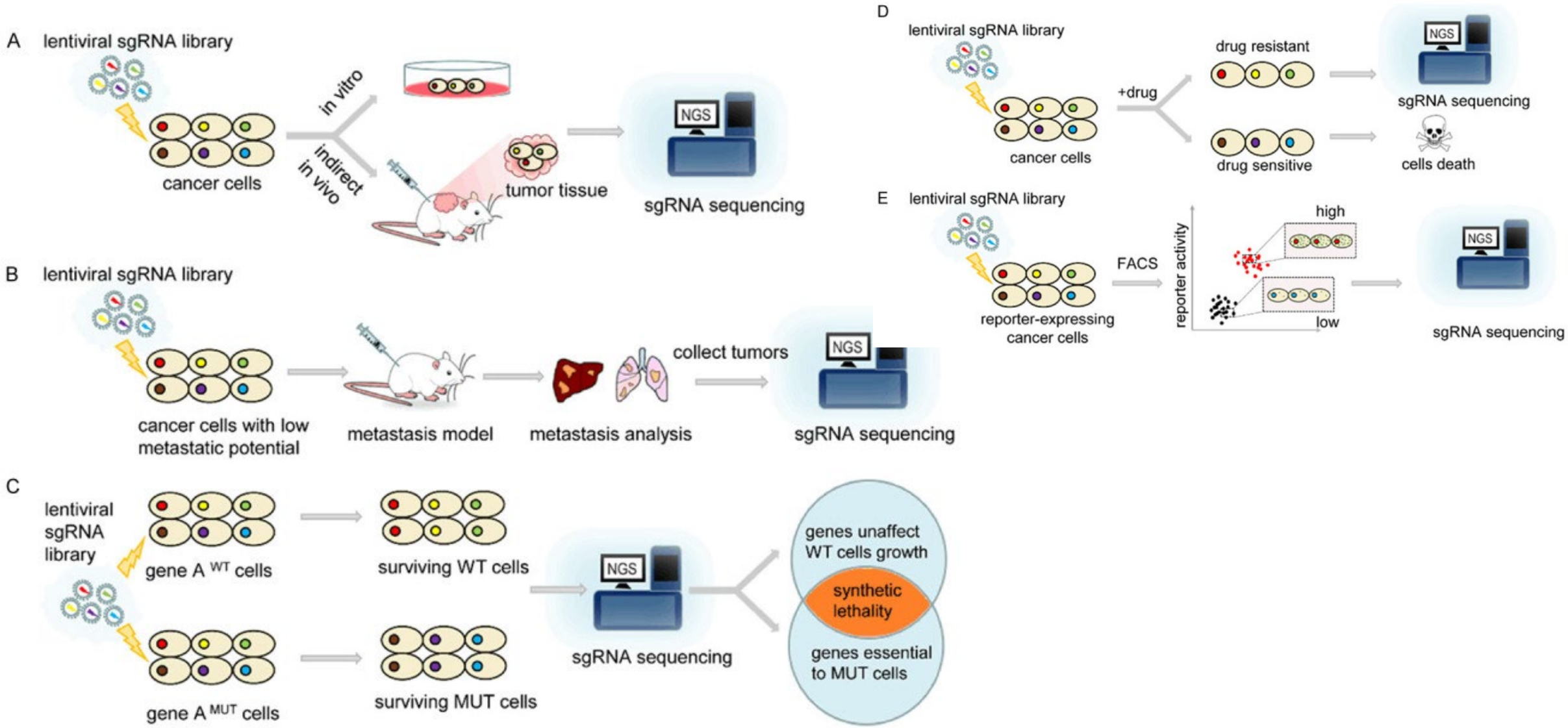
In vivo CRISPR Screens

Asvin Lakkaraju
02.11.2021

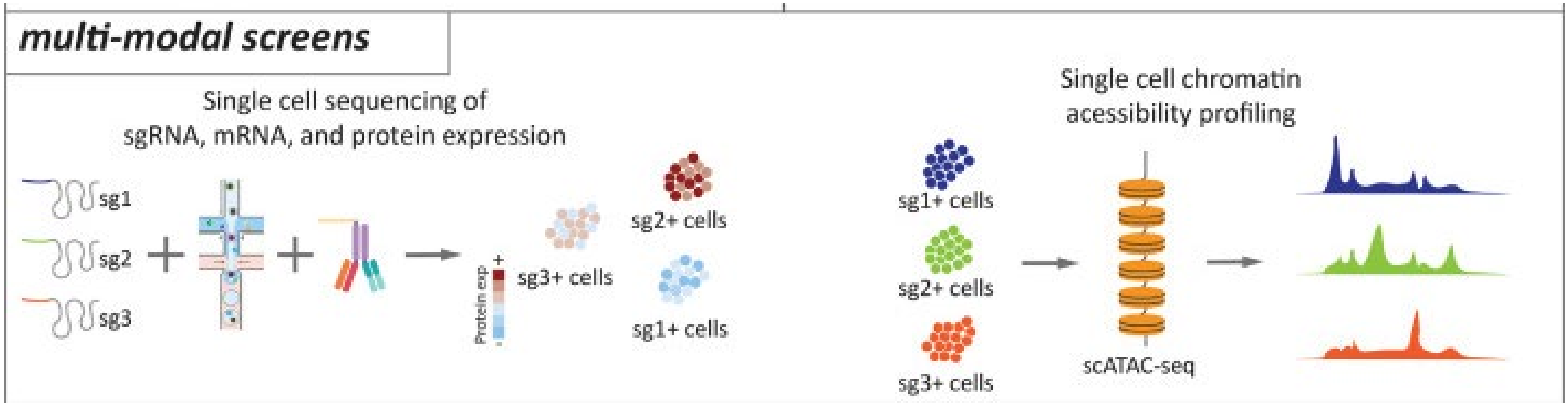
Continious Education on Laboratory Animal Sciences

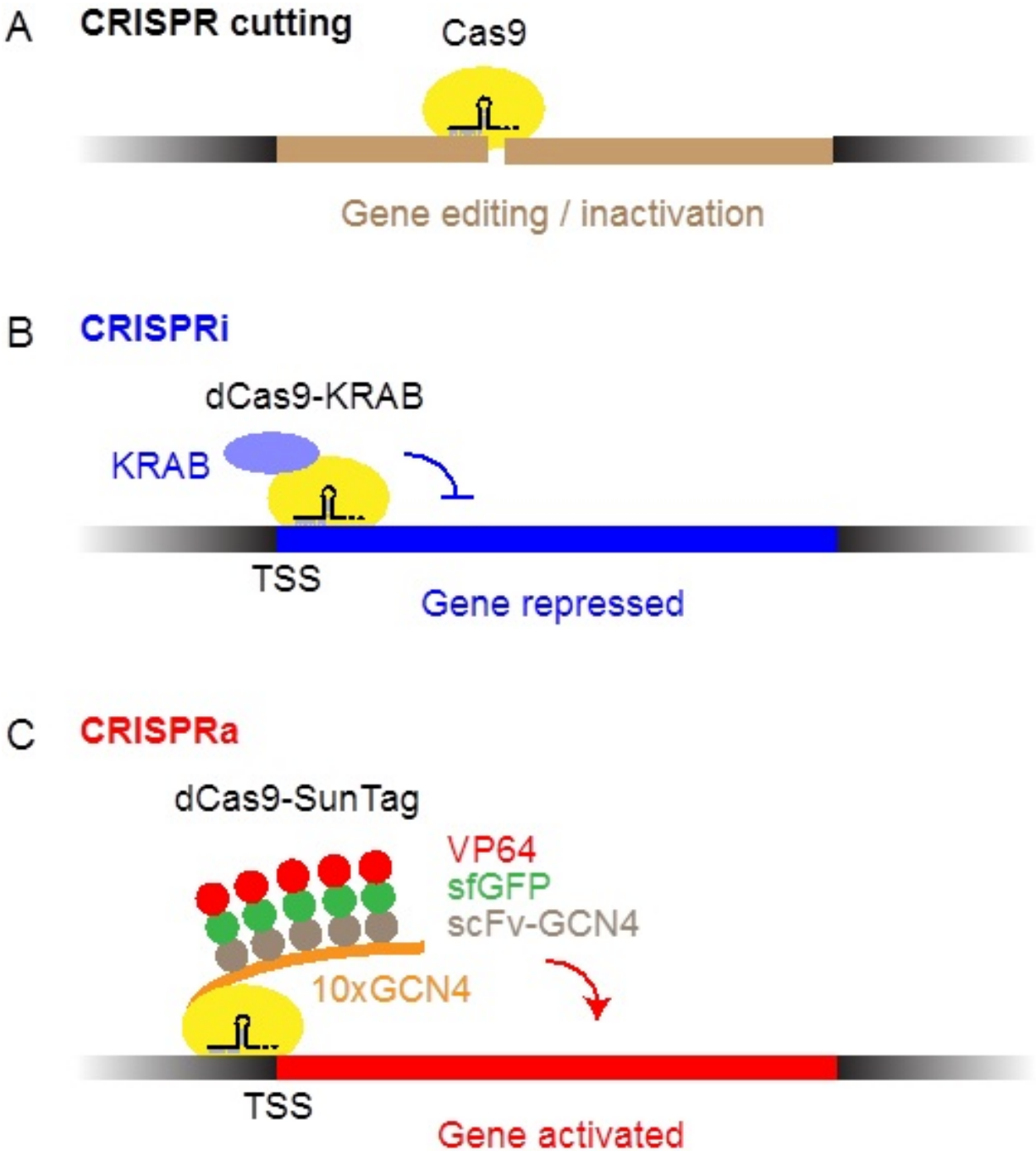
Types of CRISPR Screens

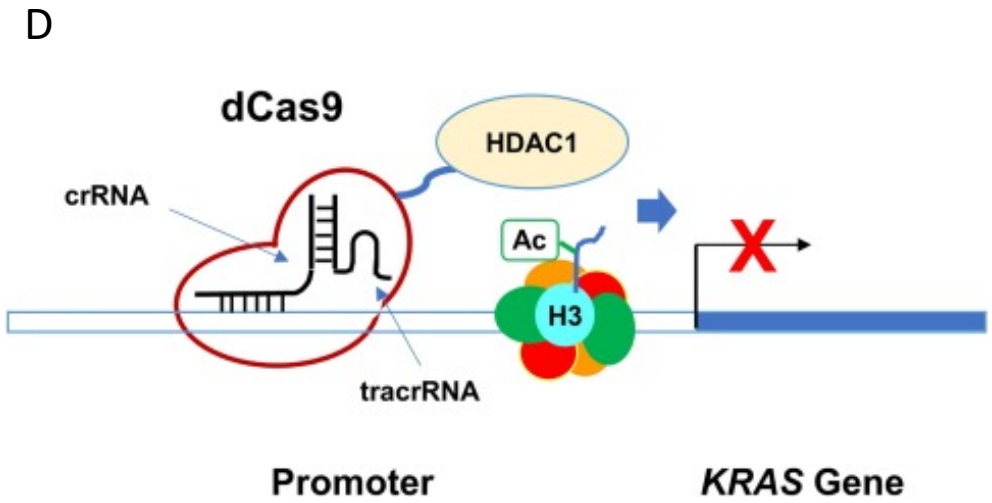


Am J Cancer Res. 2021; 11(4): 1031–1050.

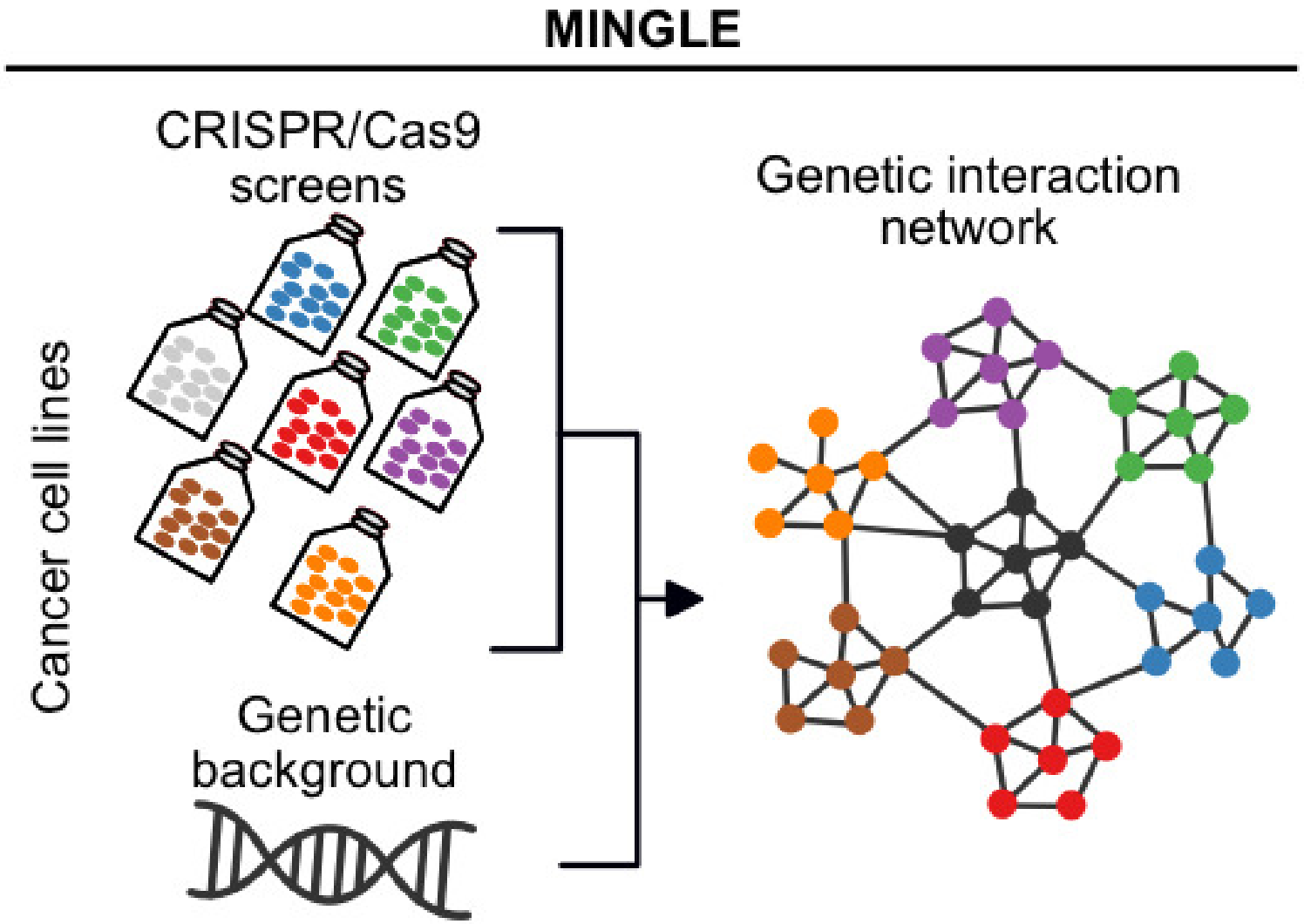
Increasing complexity of CRISPR Screens




A
CRISPR cutting
Cas9
Gene editing / inactivation
B
CRISPRi
dCas9-KRAB
KRAB
TSS
Gene repressed
C
CRISPRa
dCas9-SunTag
VP64
sfGFP
scFv-GCN4
10xGCN4
TSS
Gene activated

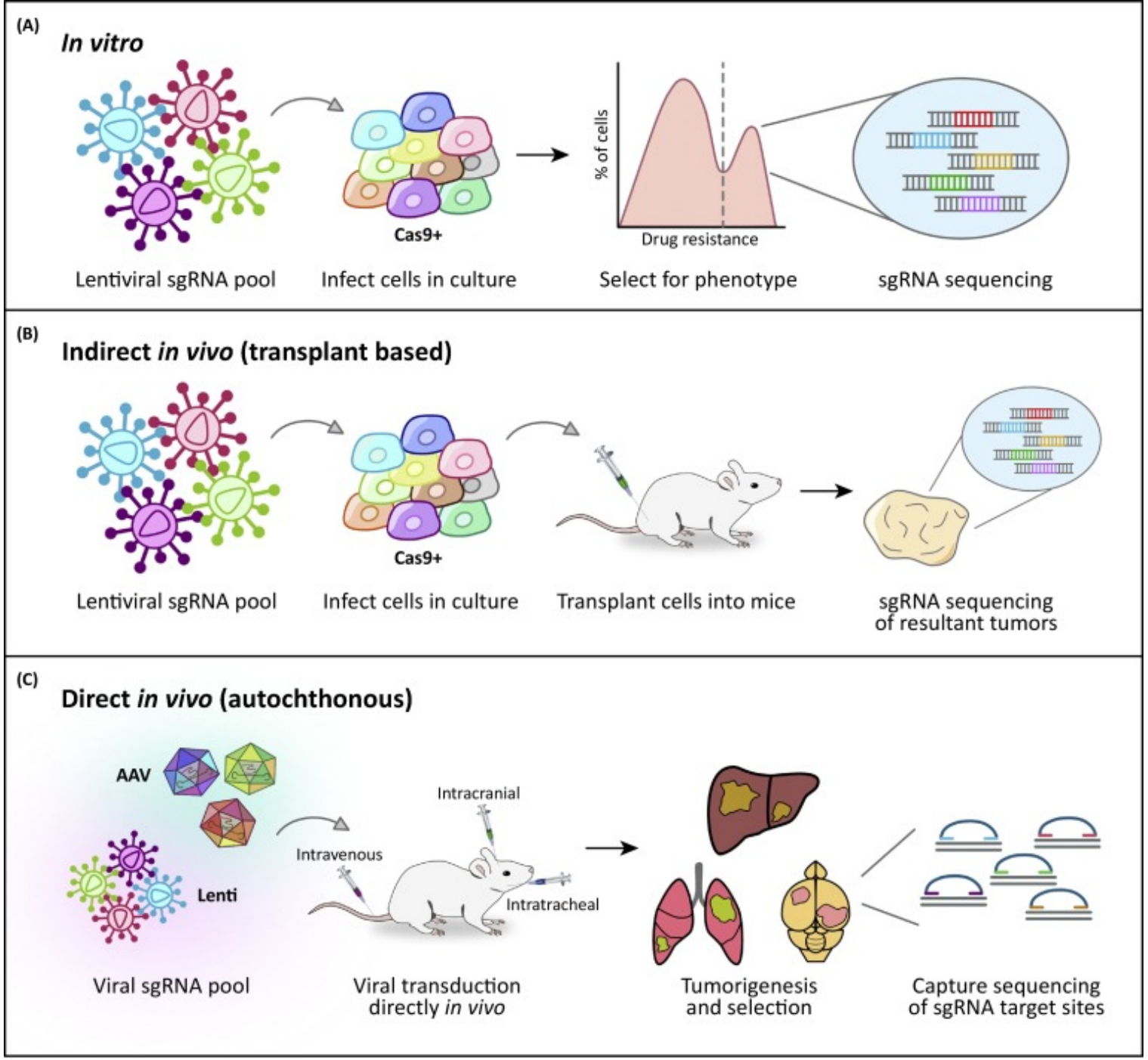

D
dCas9
HDAC1
crRNA
Ac
H3
tracrRNA
Promoter
KRAS Gene

Genetic Interaction maps in mammalian cells



Rauscher et al; Mol Syst Biol (2018)14:e7656

In vivo screens



Chow et al; https://doi.org/10.1016/j.trecan.2018.03.002

Immunity

CellPress

Resource

In vivo screens using a selective CRISPR antigen removal lentiviral vector system reveal immune dependencies in renal cell carcinoma

Juan Dubrot,[1,5] Sarah Kate Lane-Reticker,[1,5] Emily A. Kessler,[1] Austin Ayer,[1] Gargi Mishra,[1] Clara H. Wolfe,[1] Margaret D. Zimmer,[1] Peter P. Du,[1] Animesh Mahapatra,[1] Kyle M. Ockerman,[1] Thomas G.R. Davis,[1] Ian C. Kohnle,[1] Hans W. Pope,[1] Peter M. Allen,[1] Kira E. Olander,[1] Arvin Iracheta-Vellve,[1] John G. Doench,[1] W. Nicholas Haining,[1,2,3] Kathleen B. Yates,[1,4,*] and Robert T. Manguso[1,4,6,*]

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[5]These authors contributed equally

[6]Lead contact

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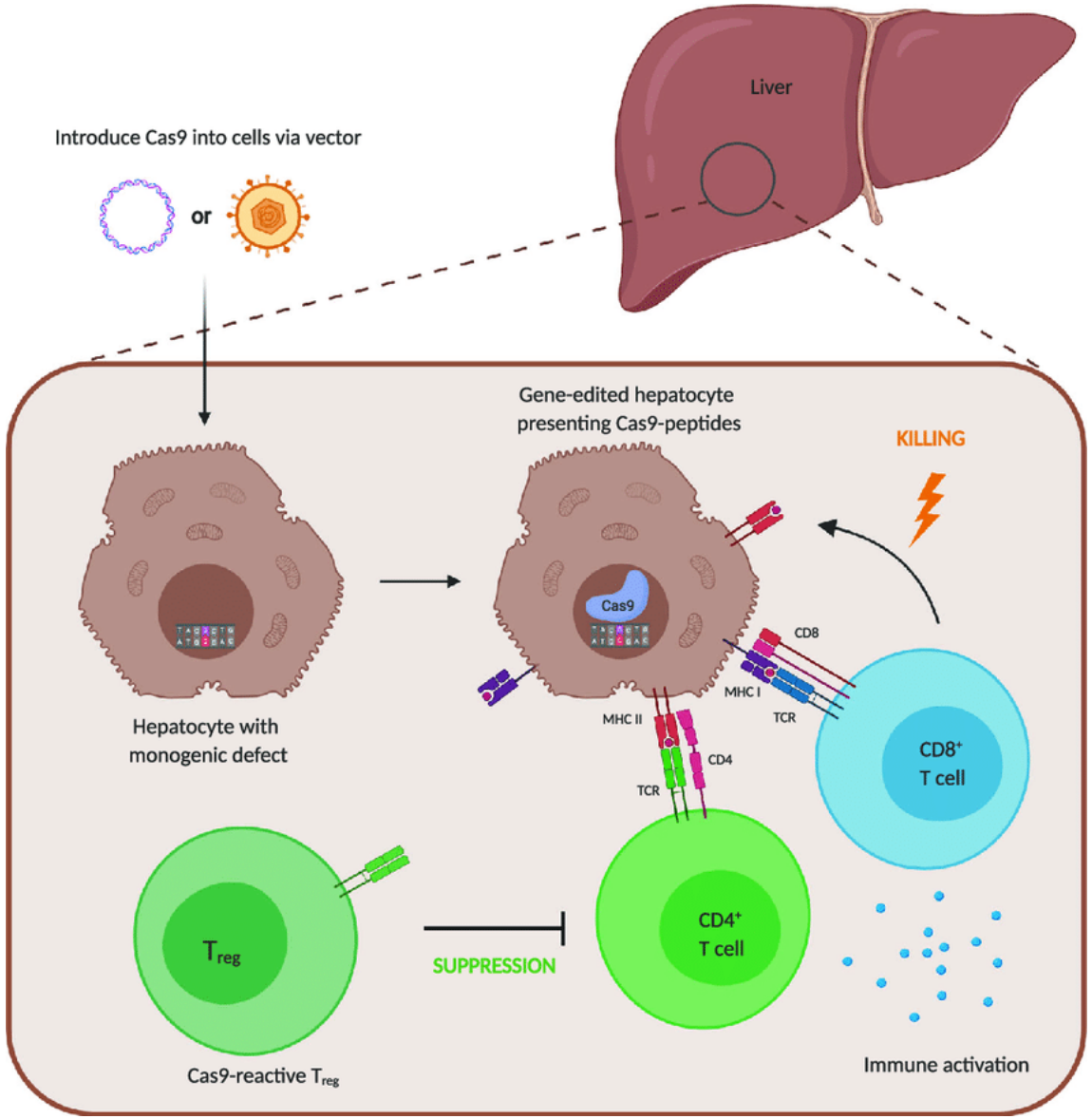
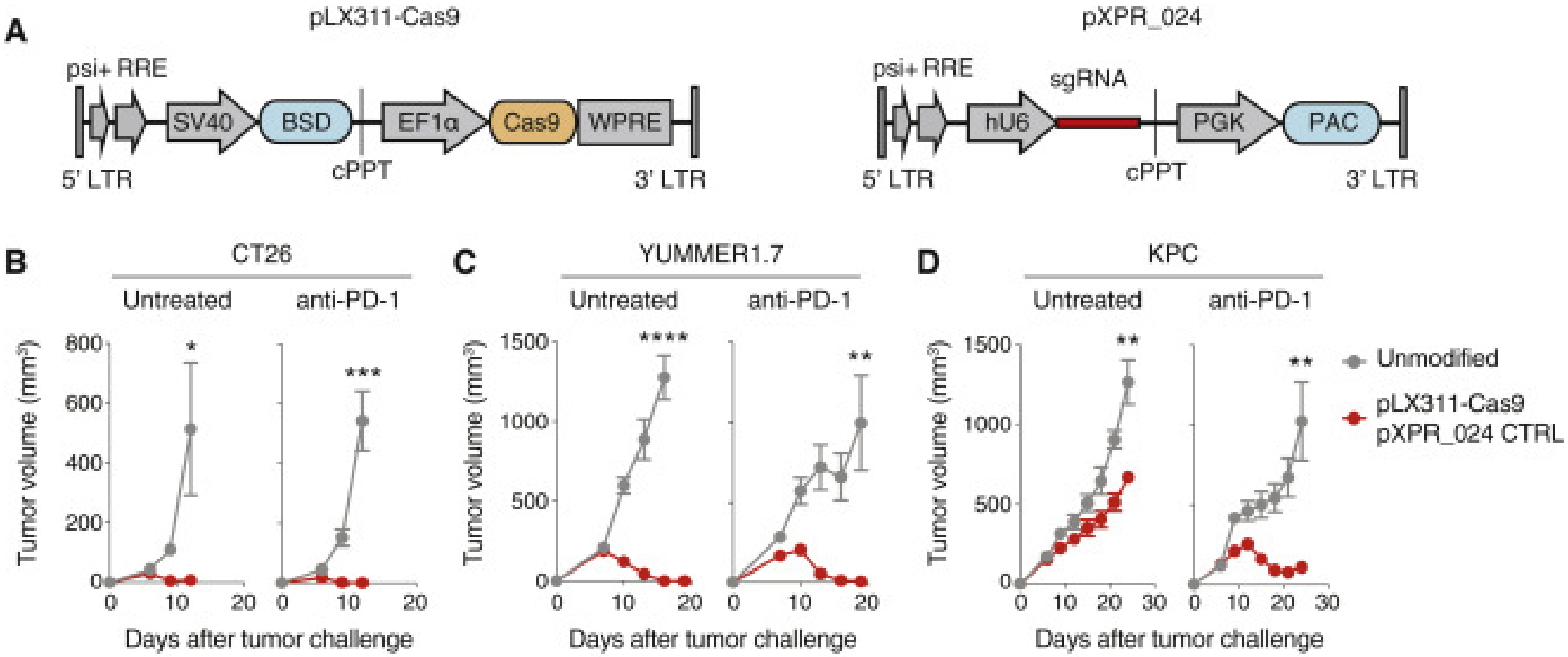
https://doi.org/10.1016/j.immuni.2021.01.001

CRISPR-Cas9 Components: Immune rejection

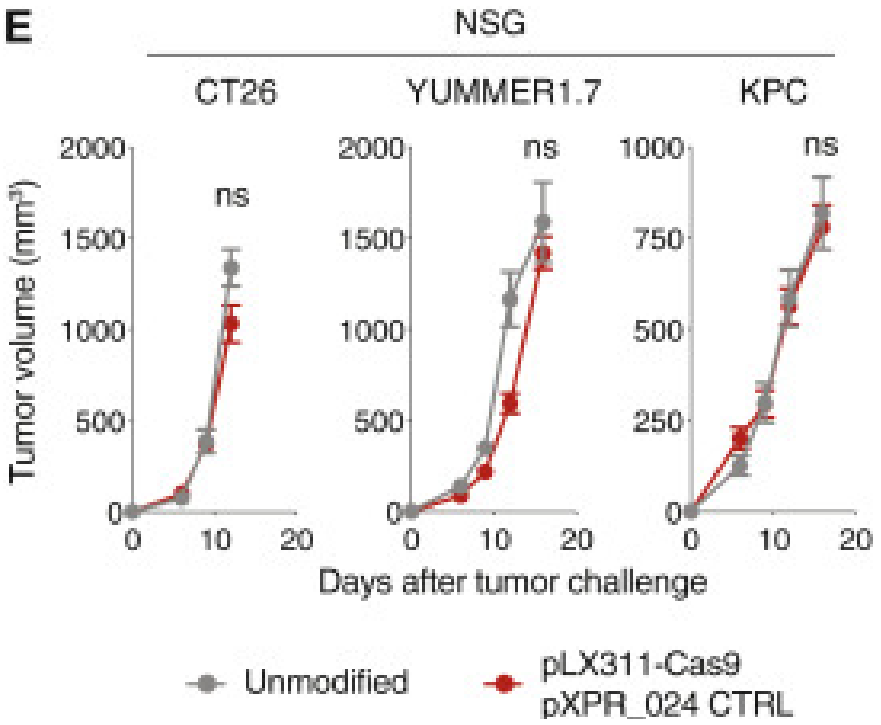


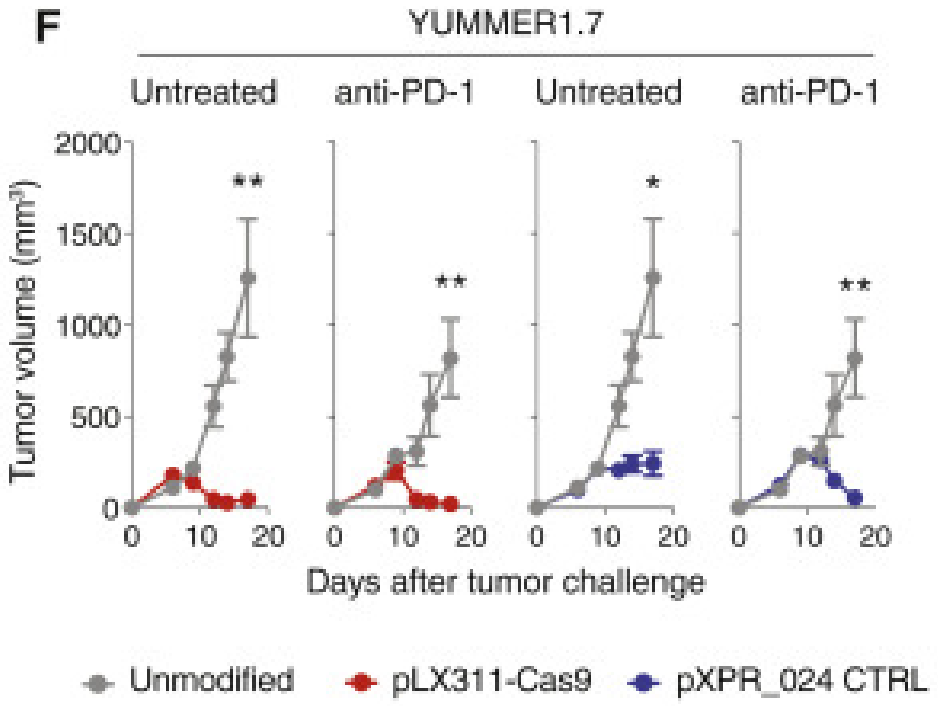
CRISPR-Cas9 Components: Immune rejection



Immune rejections result from Cas9, Antibiotic resistance genes

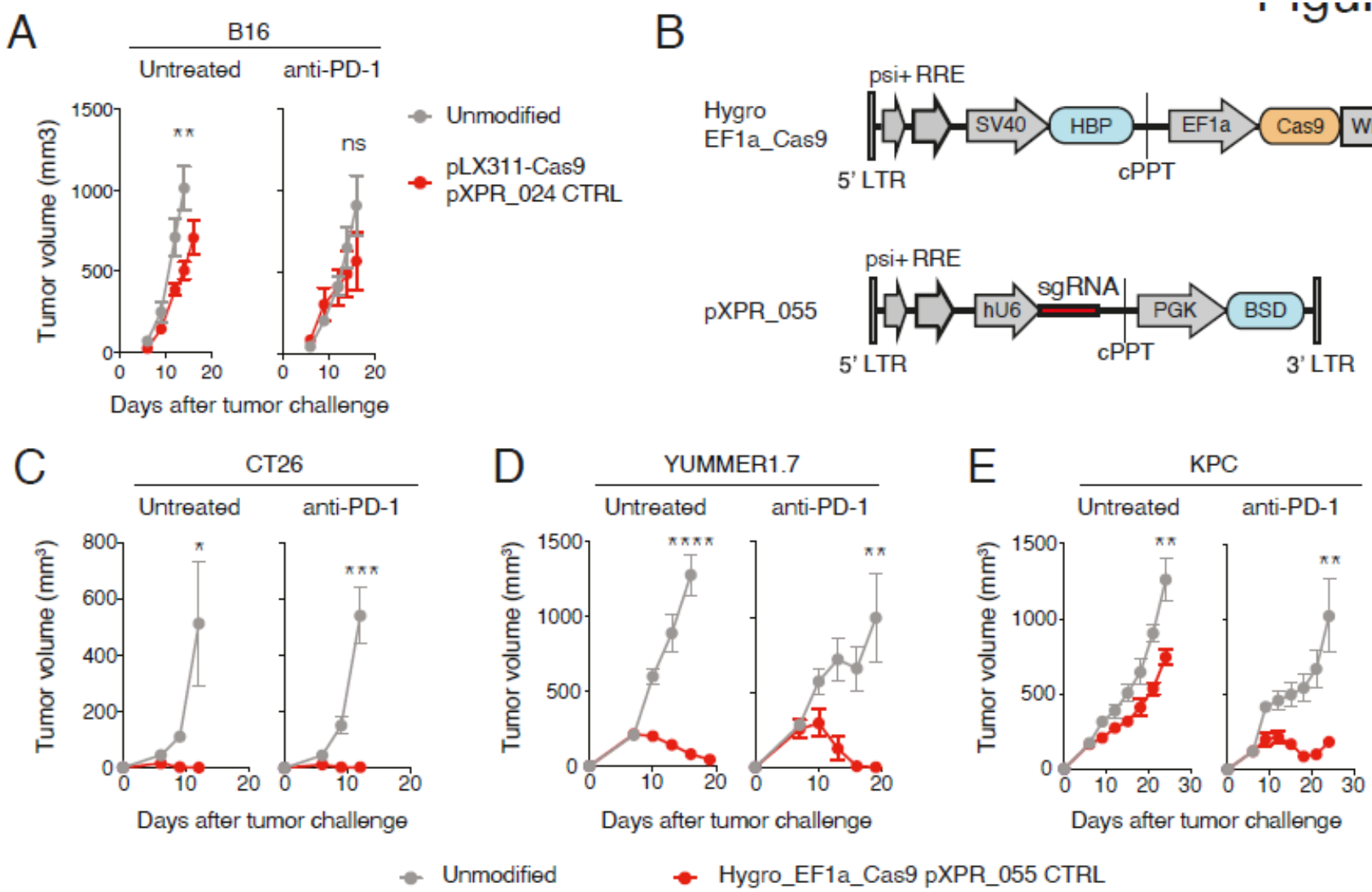
Mice reject the tumor formation



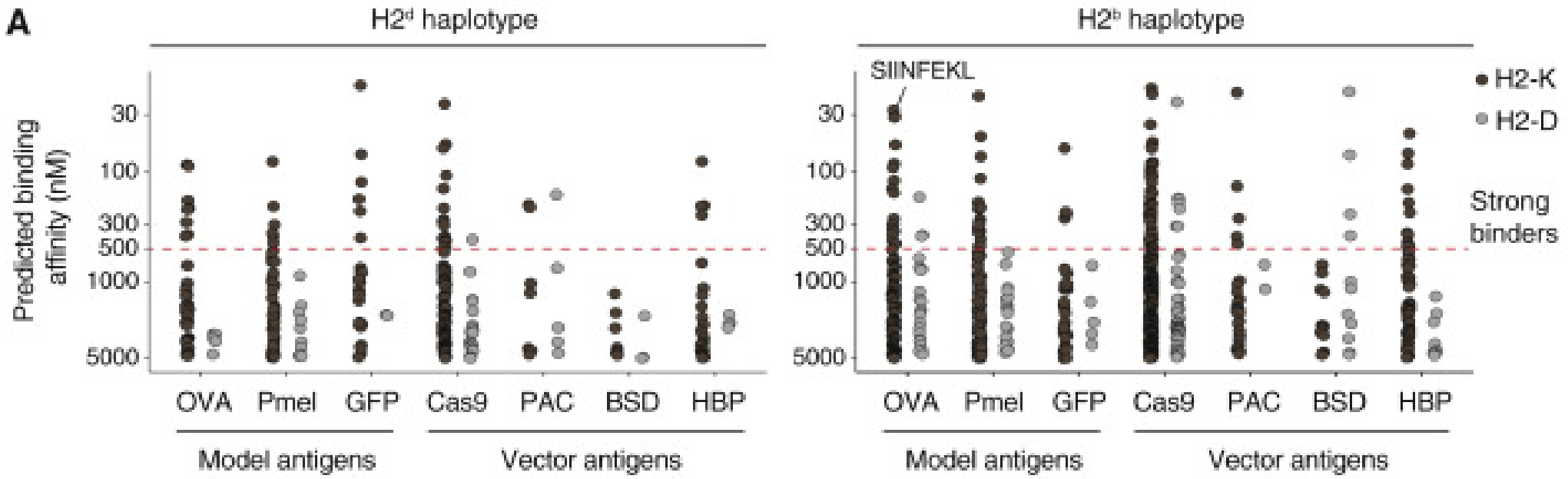


Immuno-compromised mice

Rejection is independent of antibiotic gene

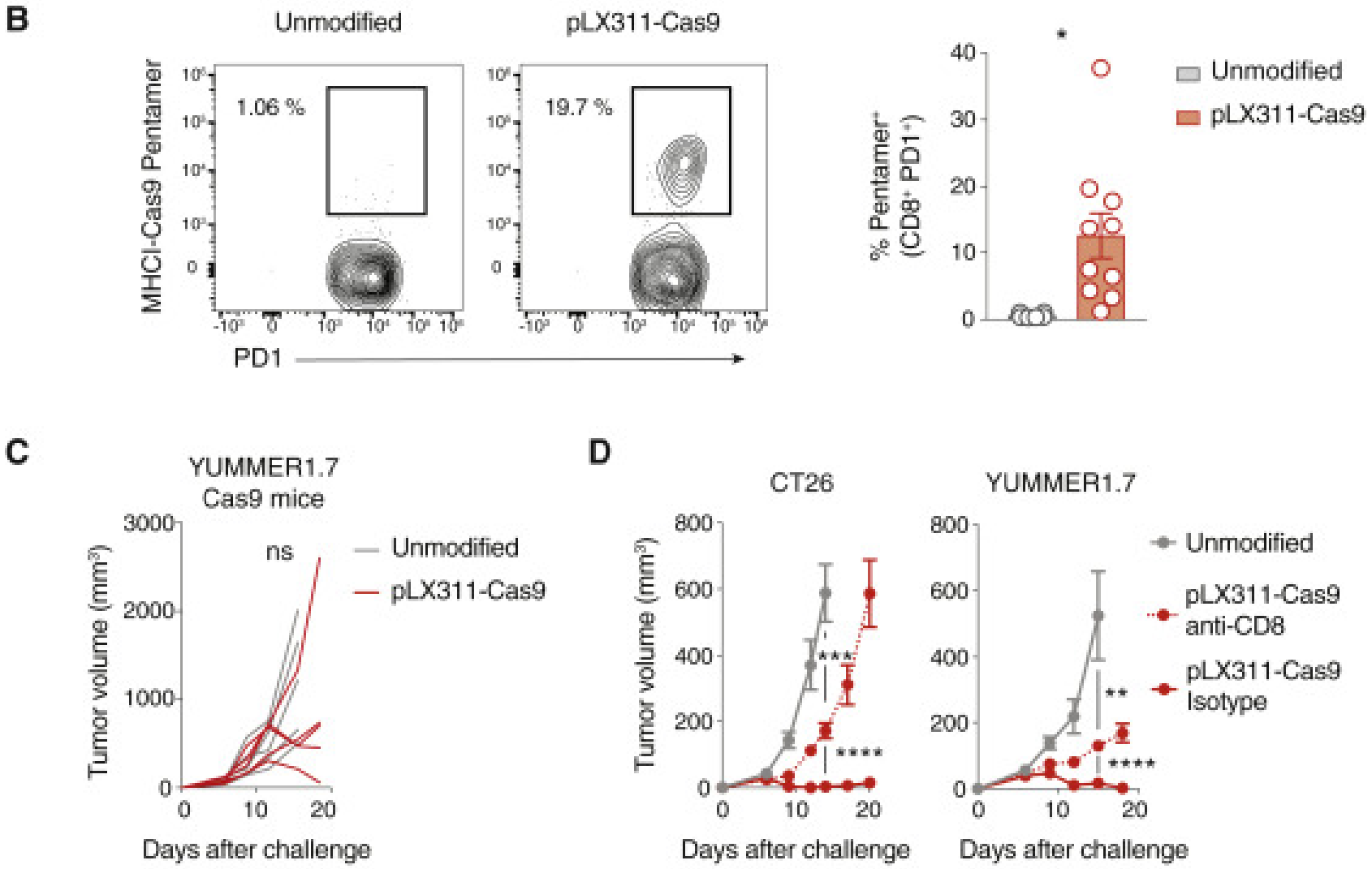


Is Cas9 Immunogenic?

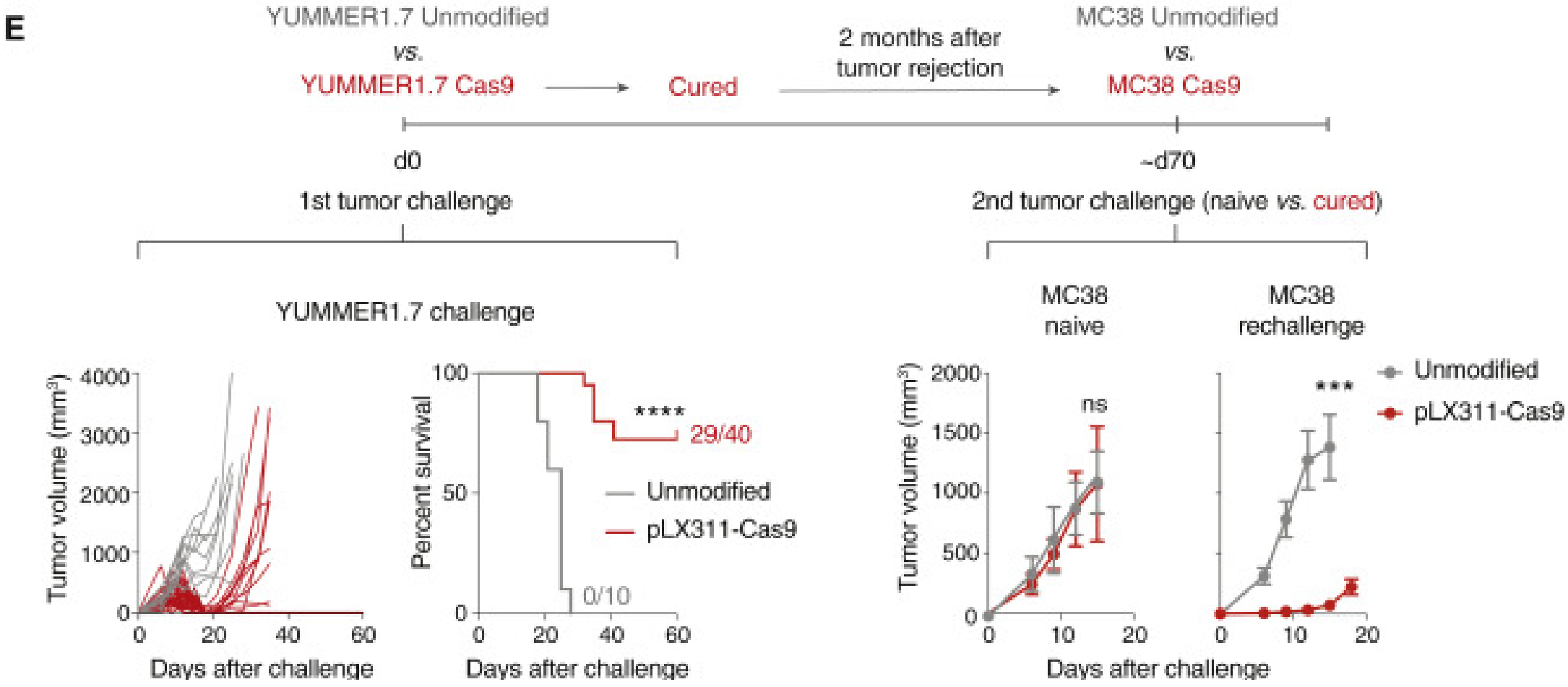


NetMHcpan: Prediction software for the MHC class 1 binding peptides

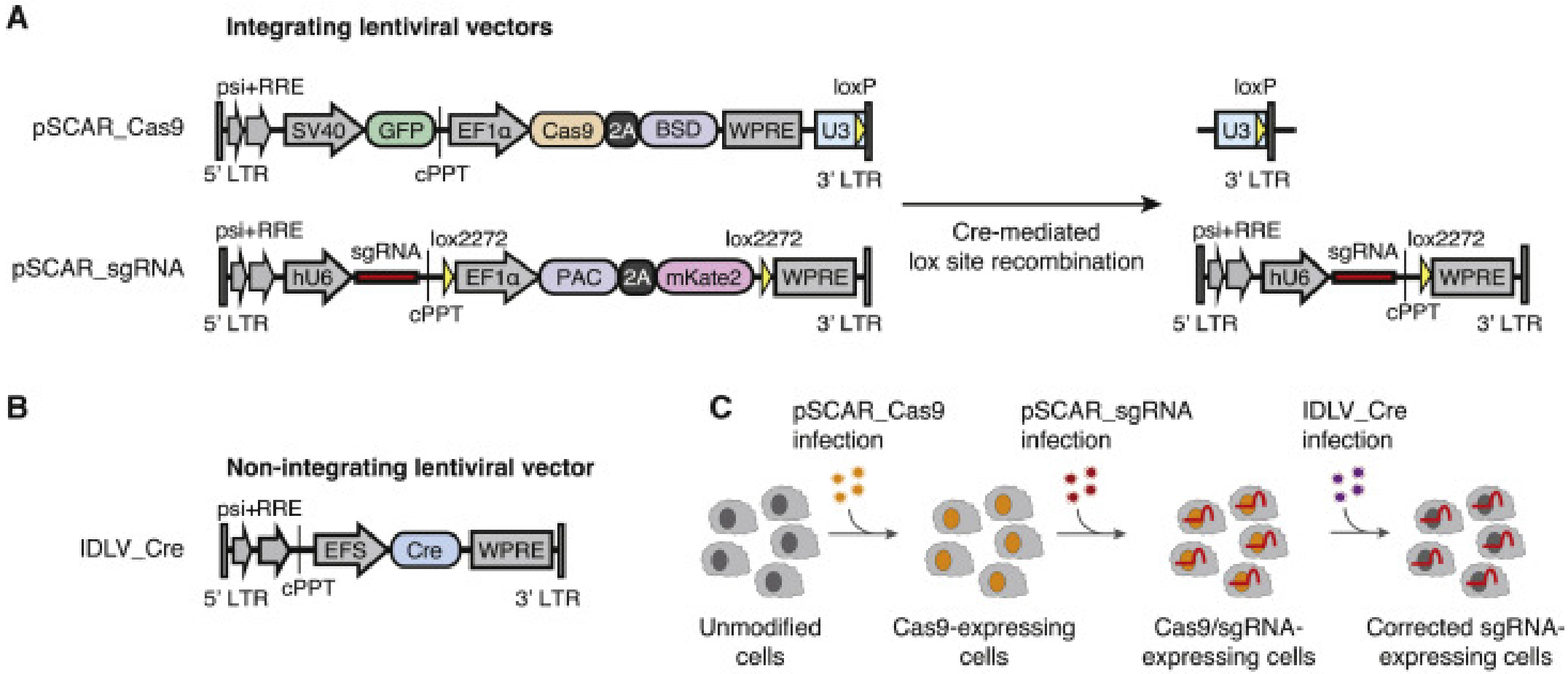
Cas9 peptides induce immune response



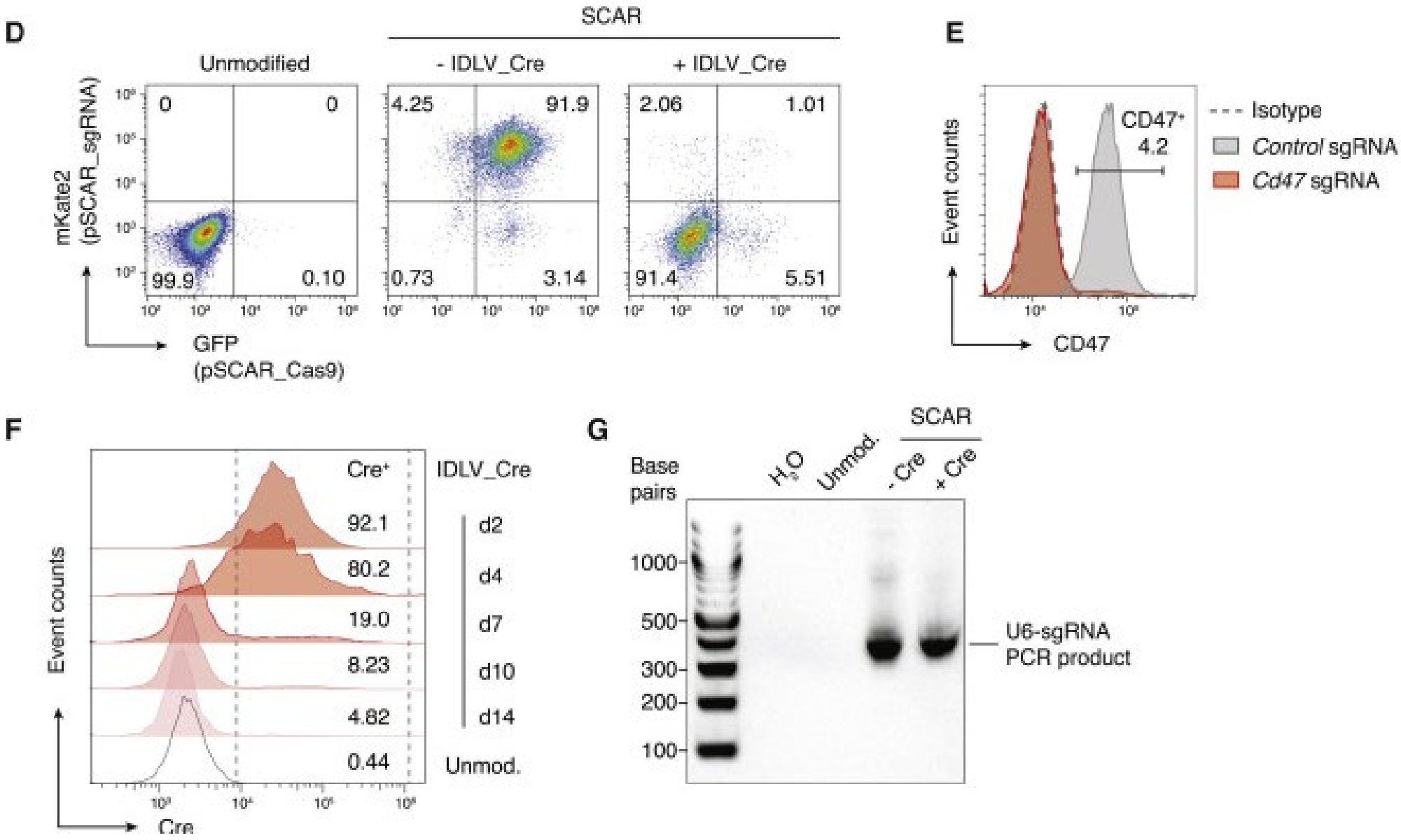
Cas9 peptides induce memory of immune response



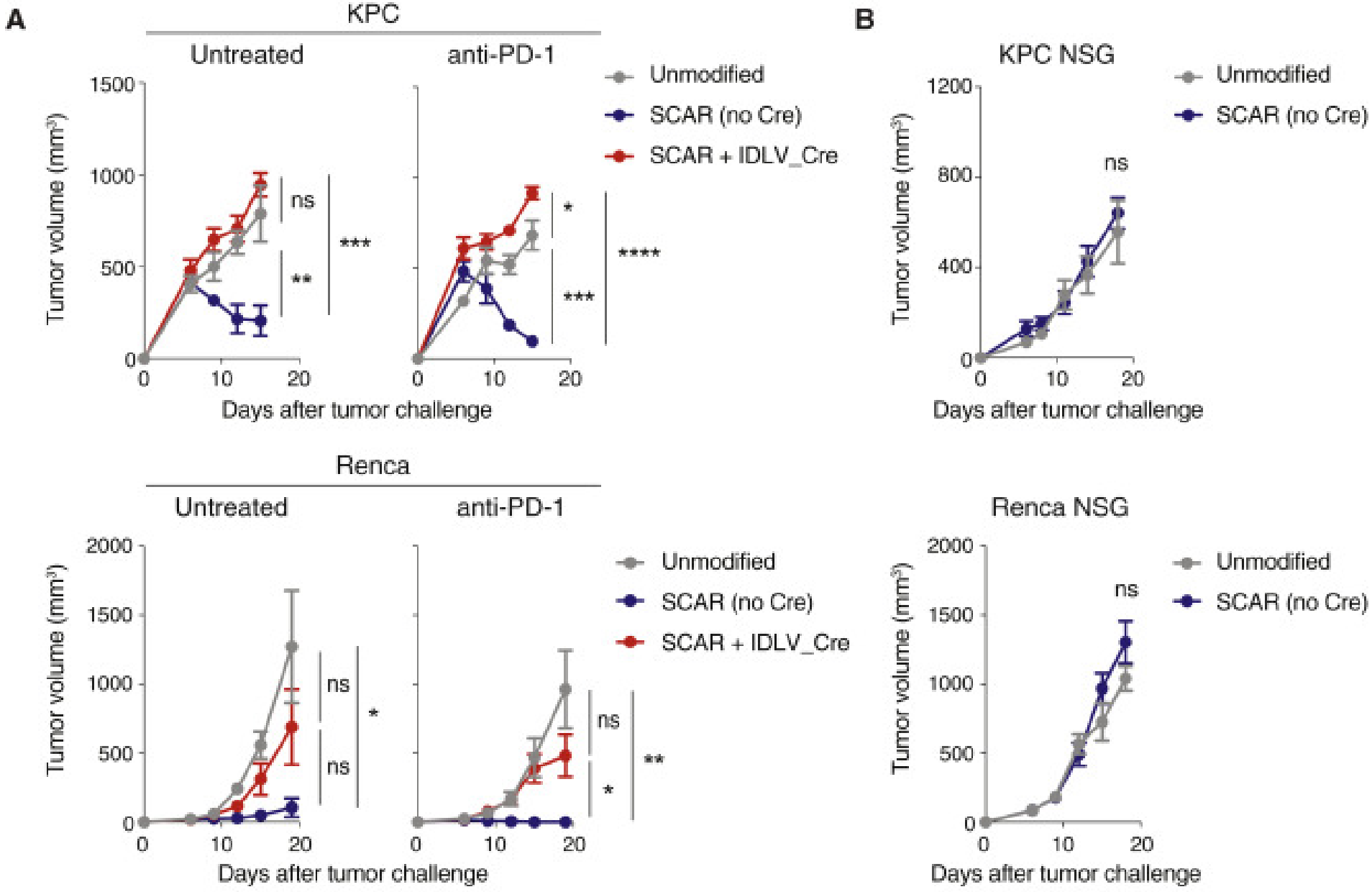
SCAR lentiviral vector system



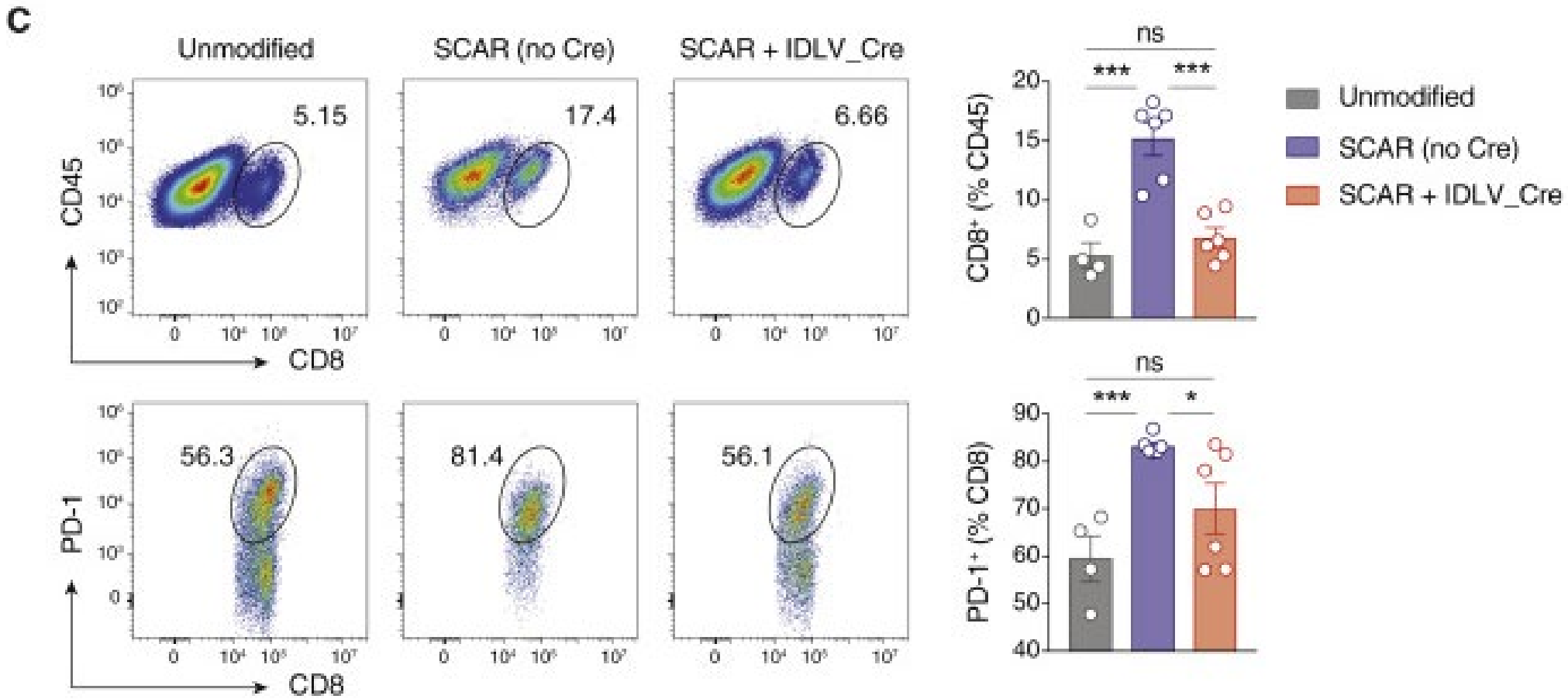
SCAR lentiviral vector system efficients dowregulates genes



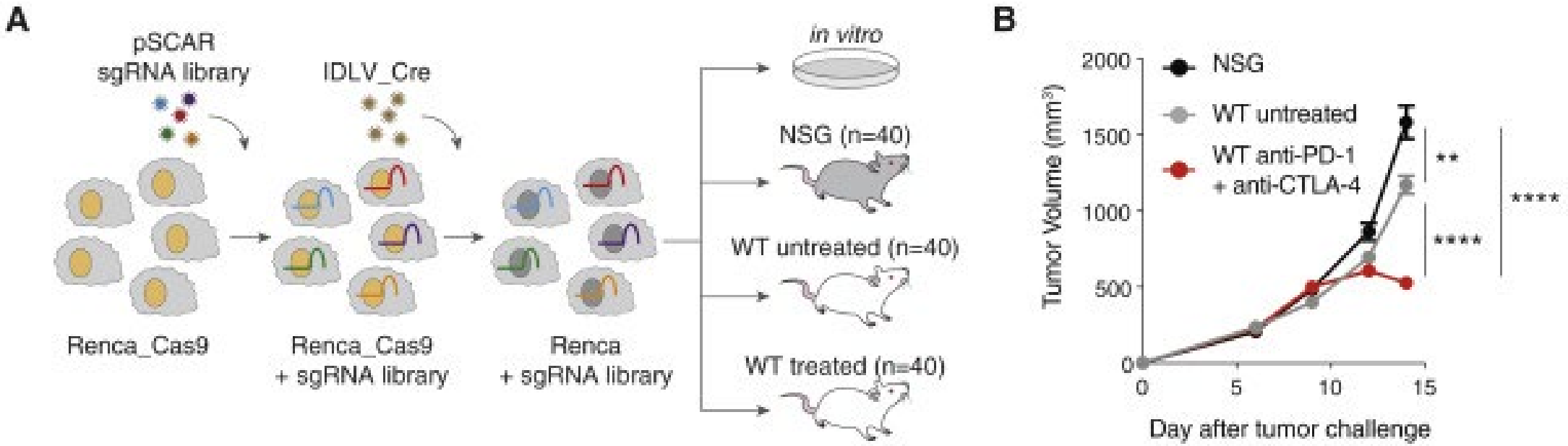
SCAR lentiviral vector system overcomes immune response against Cas9



SCAR prevents activation of Immune response cells



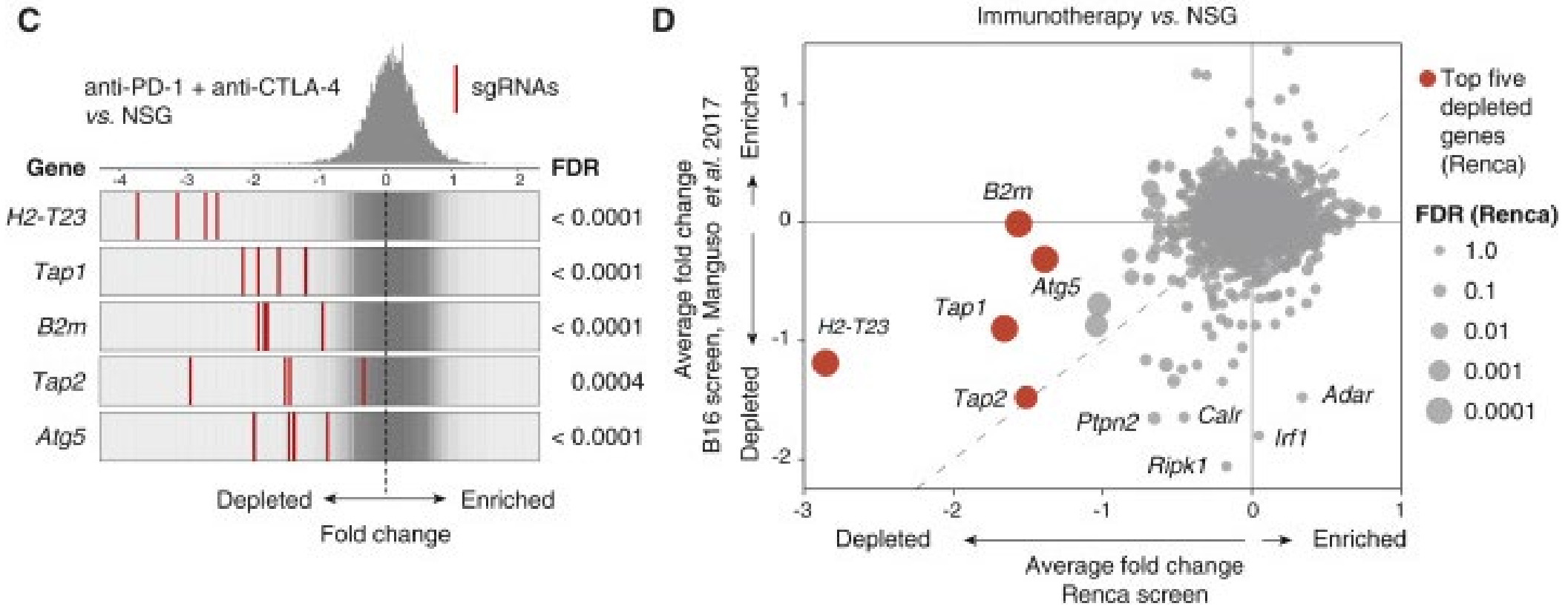
In vivo screen



Hits:

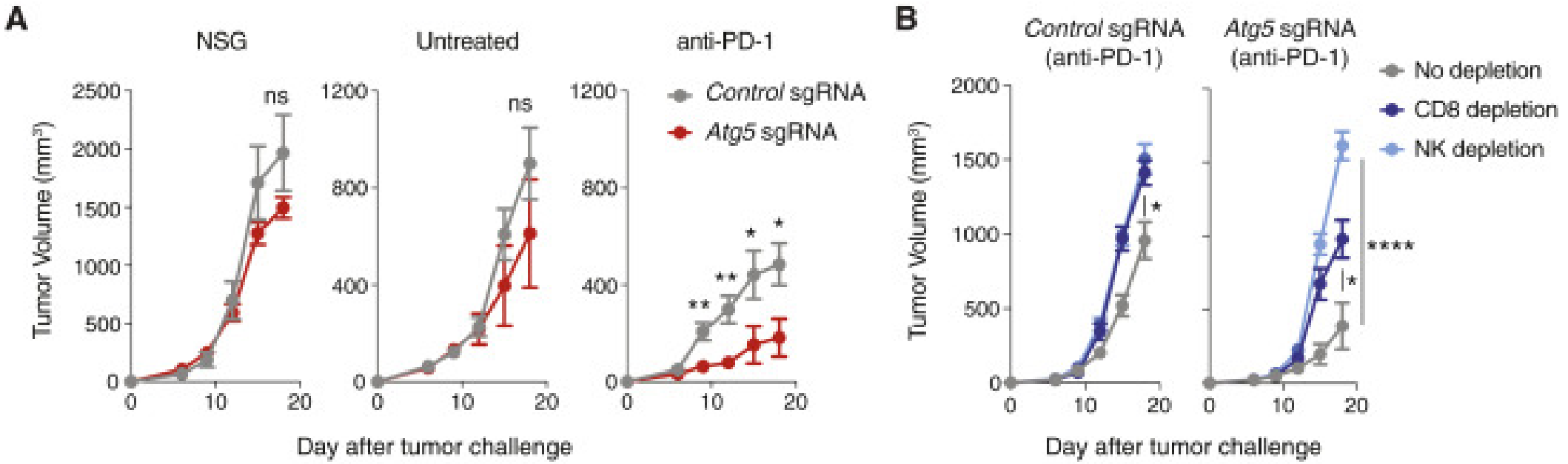
1. sgRNA enrichment: Genes that upon knock down enhance tumor growth (Tumor suppressors)
2. sgRNA depletion: Genes whose depletion result in tumor cell mortality / Tumor promoters

Volcano Plot of the differentially enriched sgRNAs



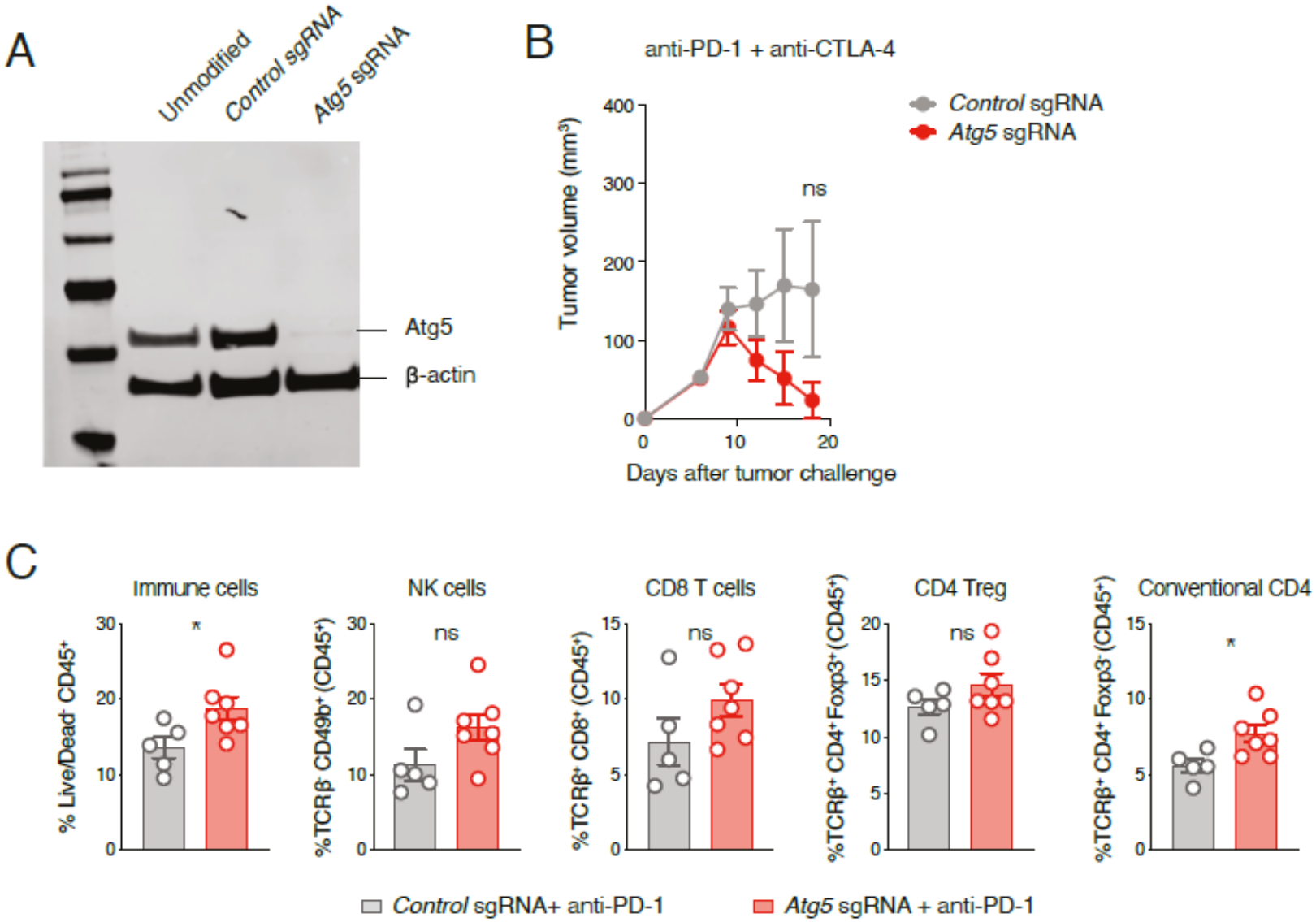
Genes whose sgRNA are depleted: Antigen presenting fctors, Atg5 and B2M

Role of Autophagy in tumor progression

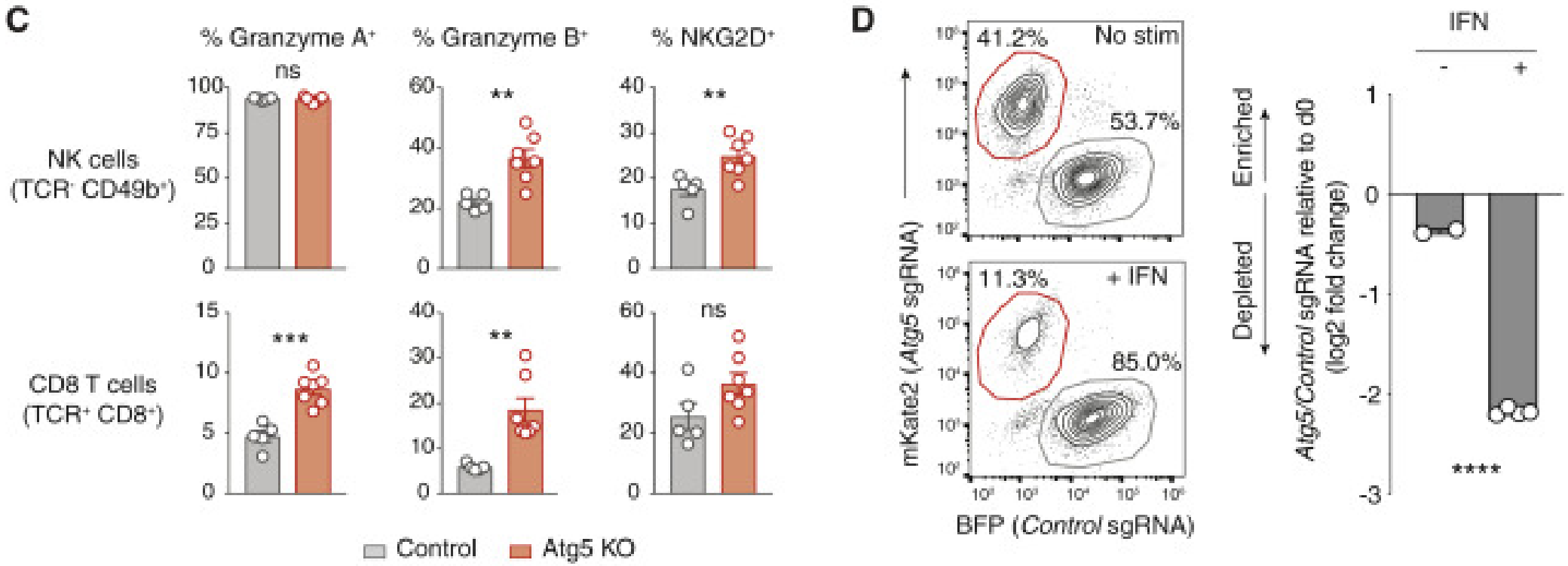


In the absence of Atg5, NK cells proliferate

Role of Autophagy in tumor progression

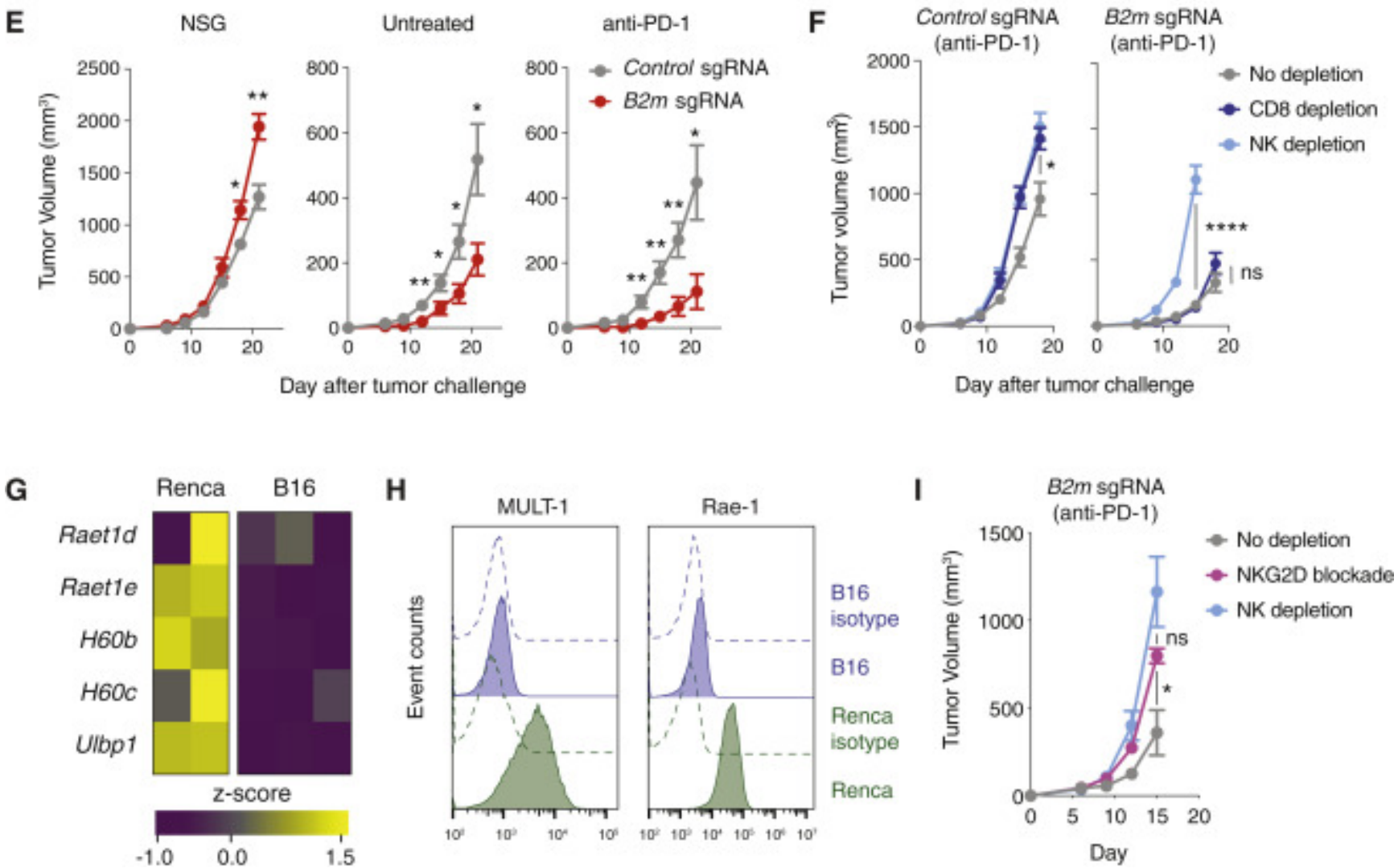


Ligands and products of NK cells get activated in the absence of Atg5



In the absence of Atg5, cells are more sensitive to apoptosis by IFN gamma.

Role of B2M in tumor progression



Summary

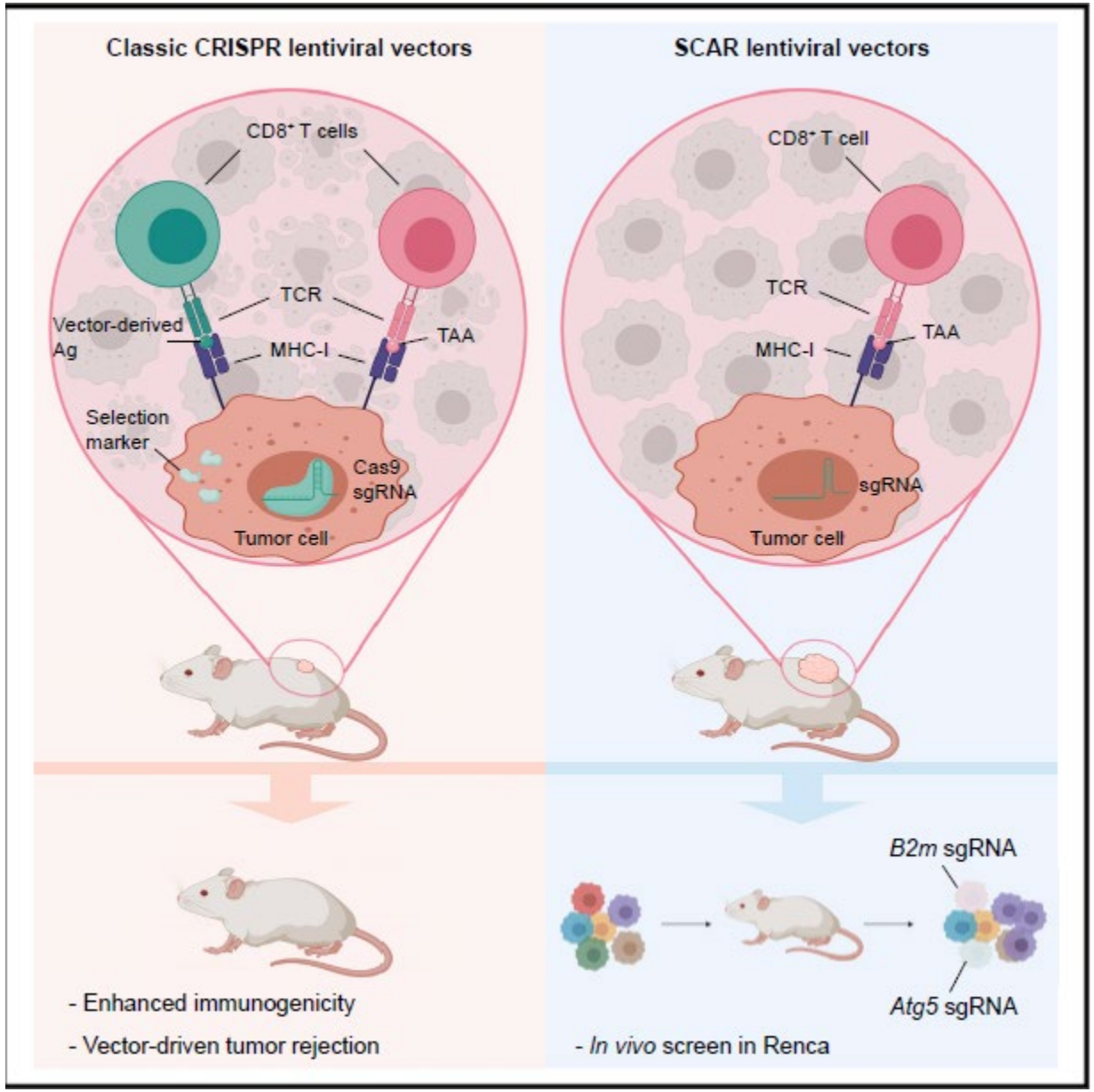
1. Immune rejection associated with Cas9 can be solved using SCAR system.

2. Identified new targets to target diseases.

Questions:

1. Reproducibility of the screens in different Mice?

2. What about the stage of the tumors?



nature COMMUNICATIONS

ARTICLE

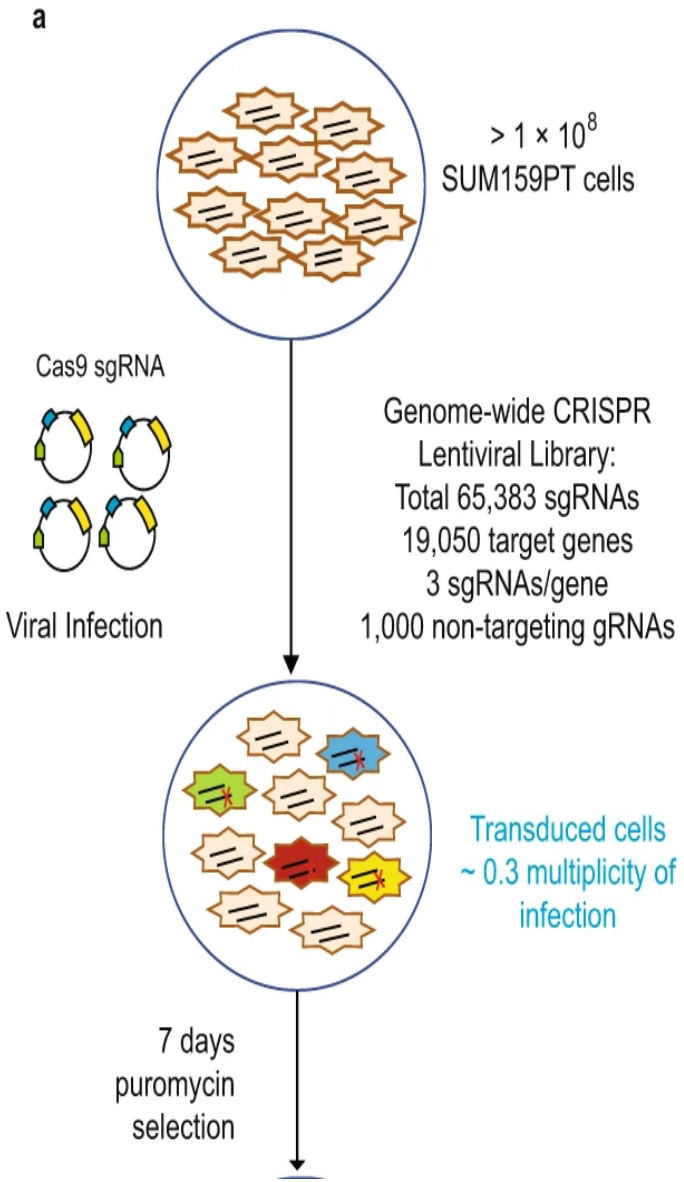
https://doi.org/10.1038/s41467-021-23316-4 OPEN

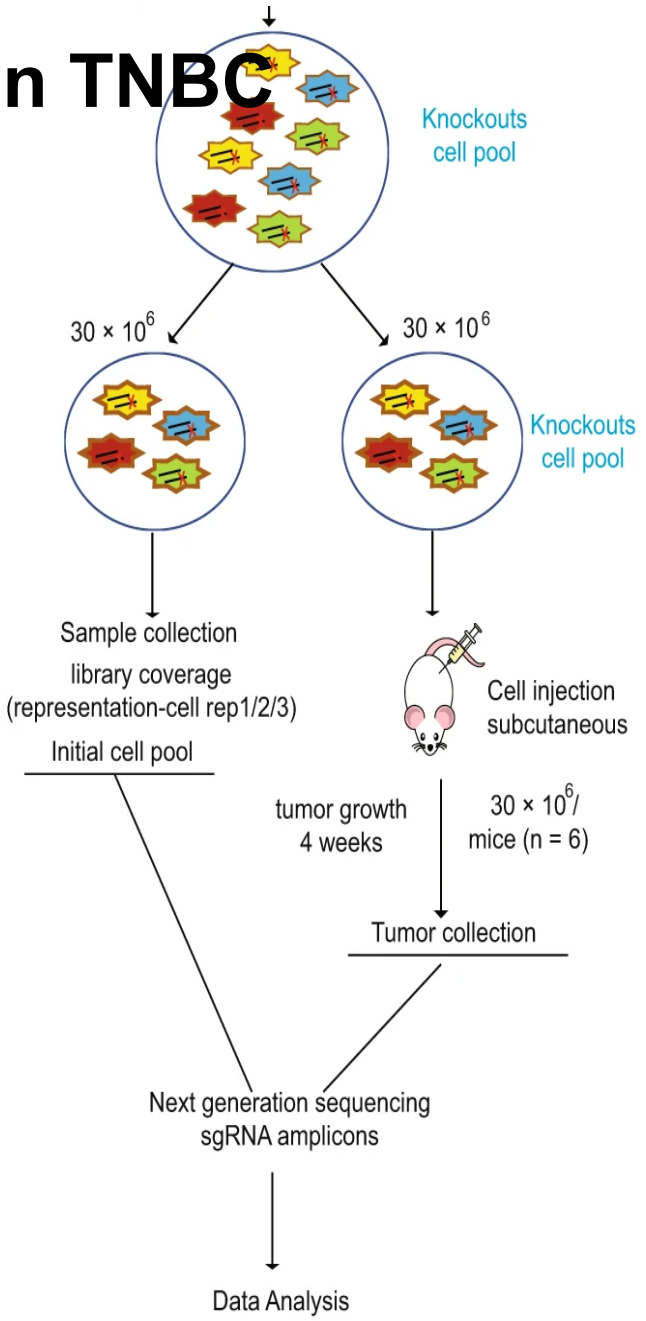
In vivo genome-wide CRISPR screen reveals breast cancer vulnerabilities and synergistic mTOR/Hippo targeted combination therapy

Meiou Dai[1], Gang Yan[1], Ni Wang[1], Girija Daliah[1], Ashlin M. Edick[2], Sophie Poulet[1], Julien Boudreault[1], Suhad Ali[1], Sergio A. Burgos[2,3] & Jean-Jacques Lebrun[1✉]

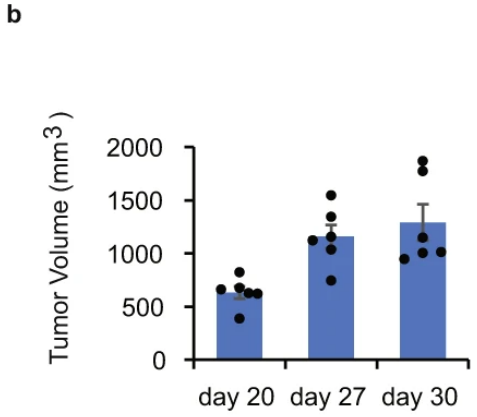
In vivo genome-wide CRISPR knockout screen in TNBC

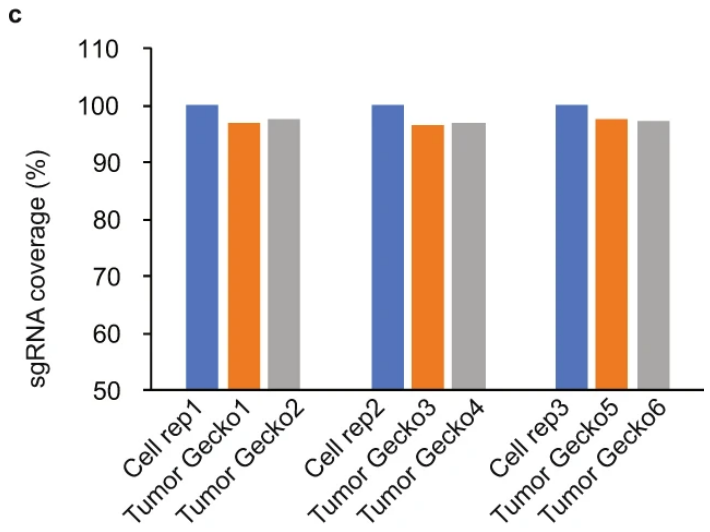
Aim:

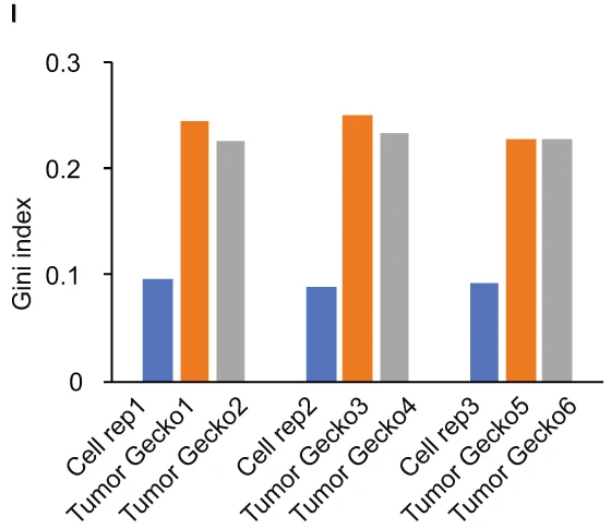


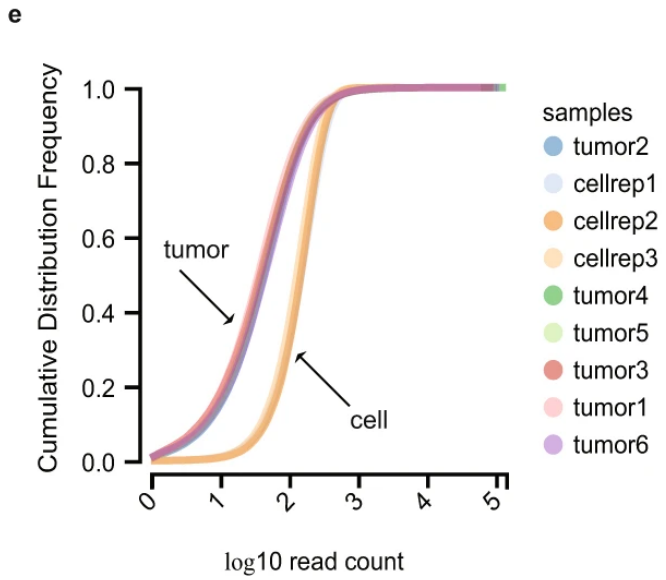


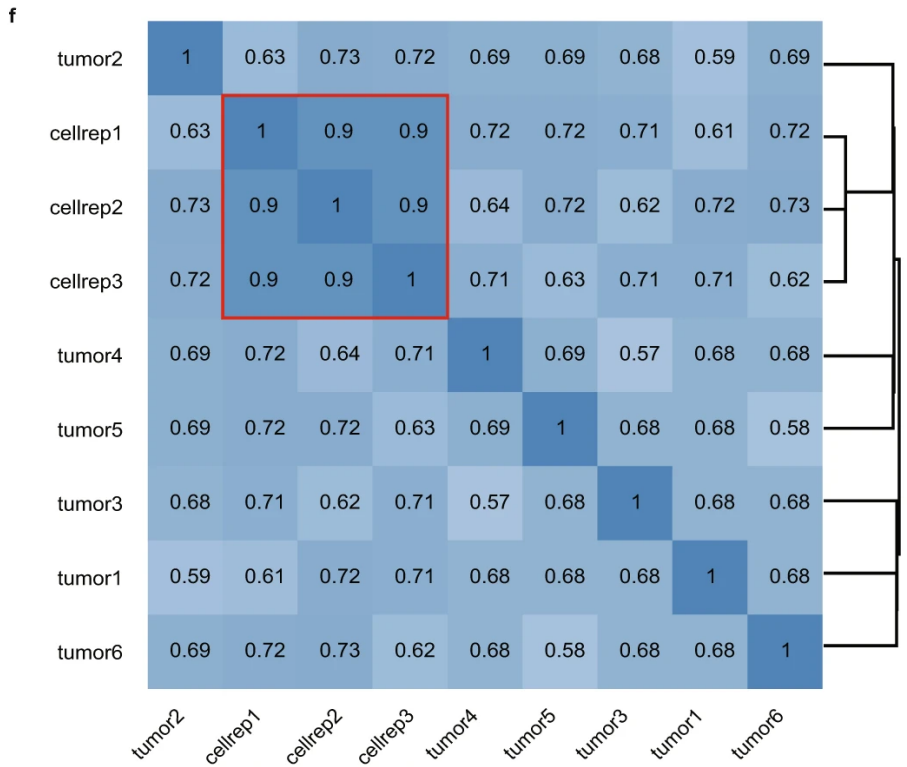
Quality control of the screen



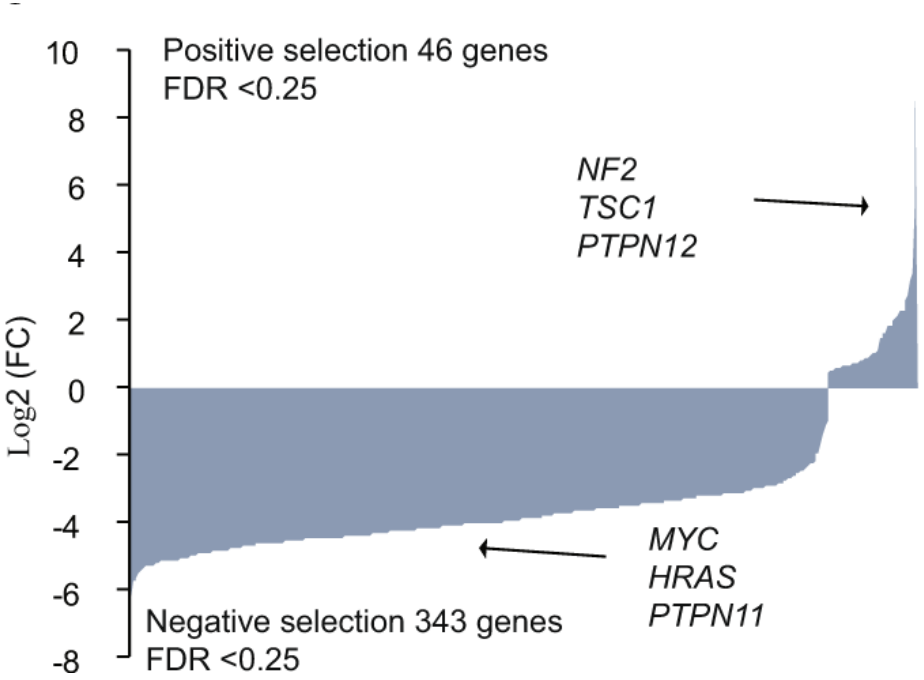


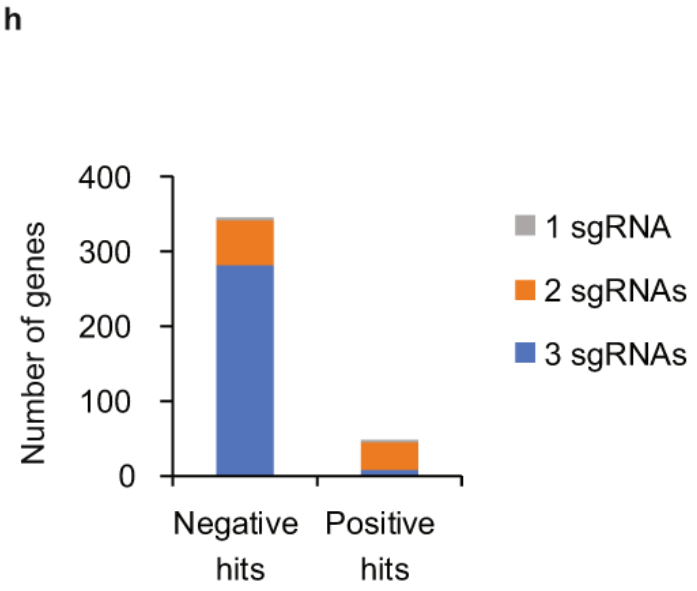




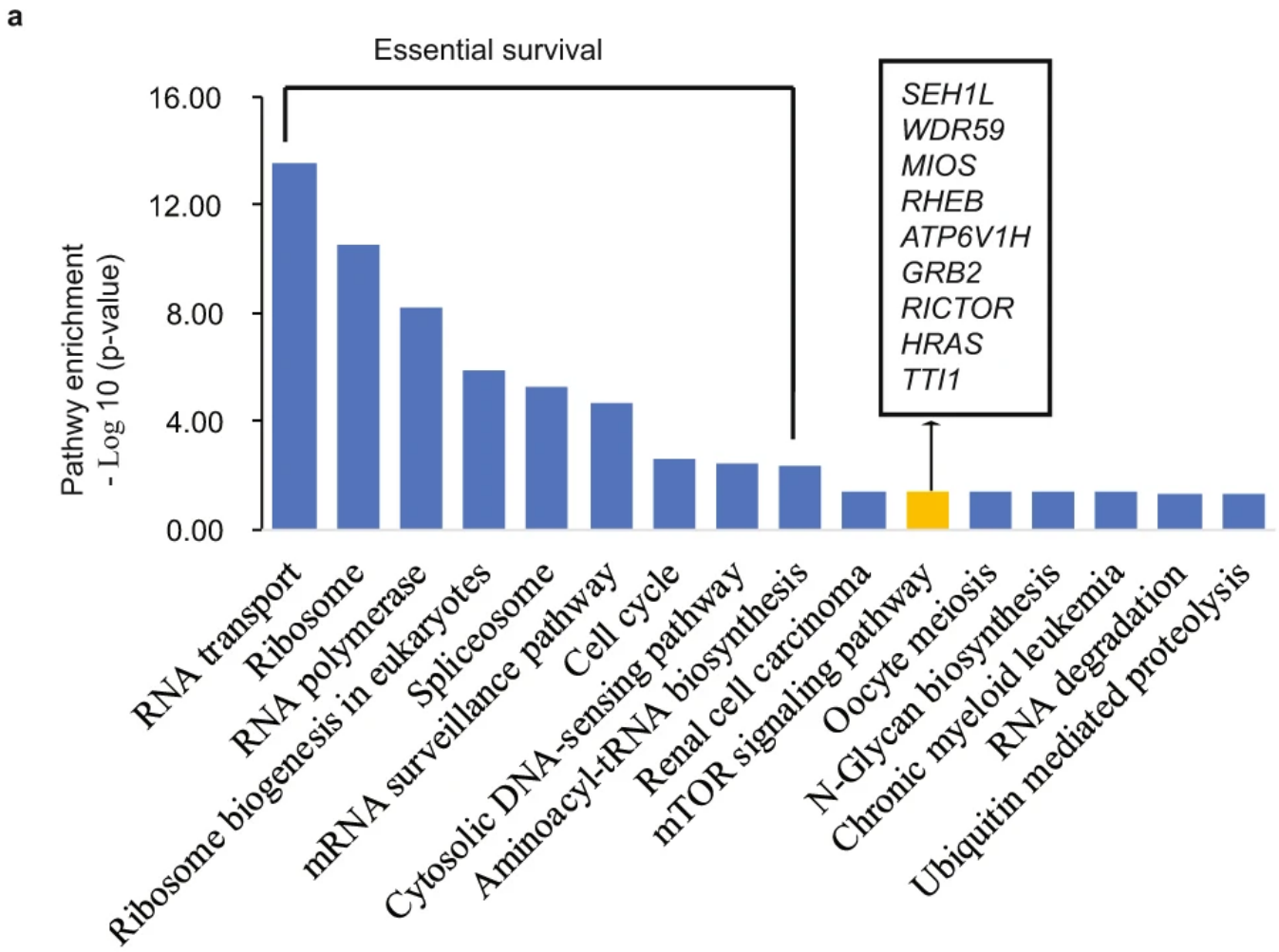


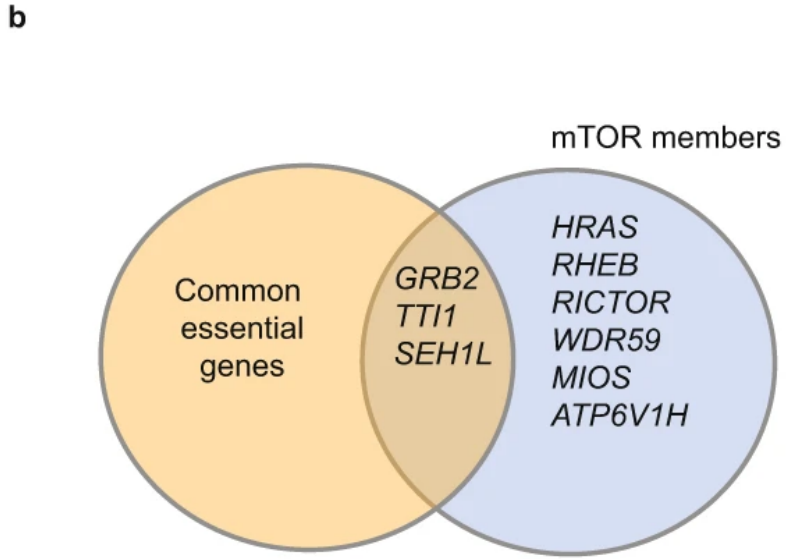
Hits in the screen



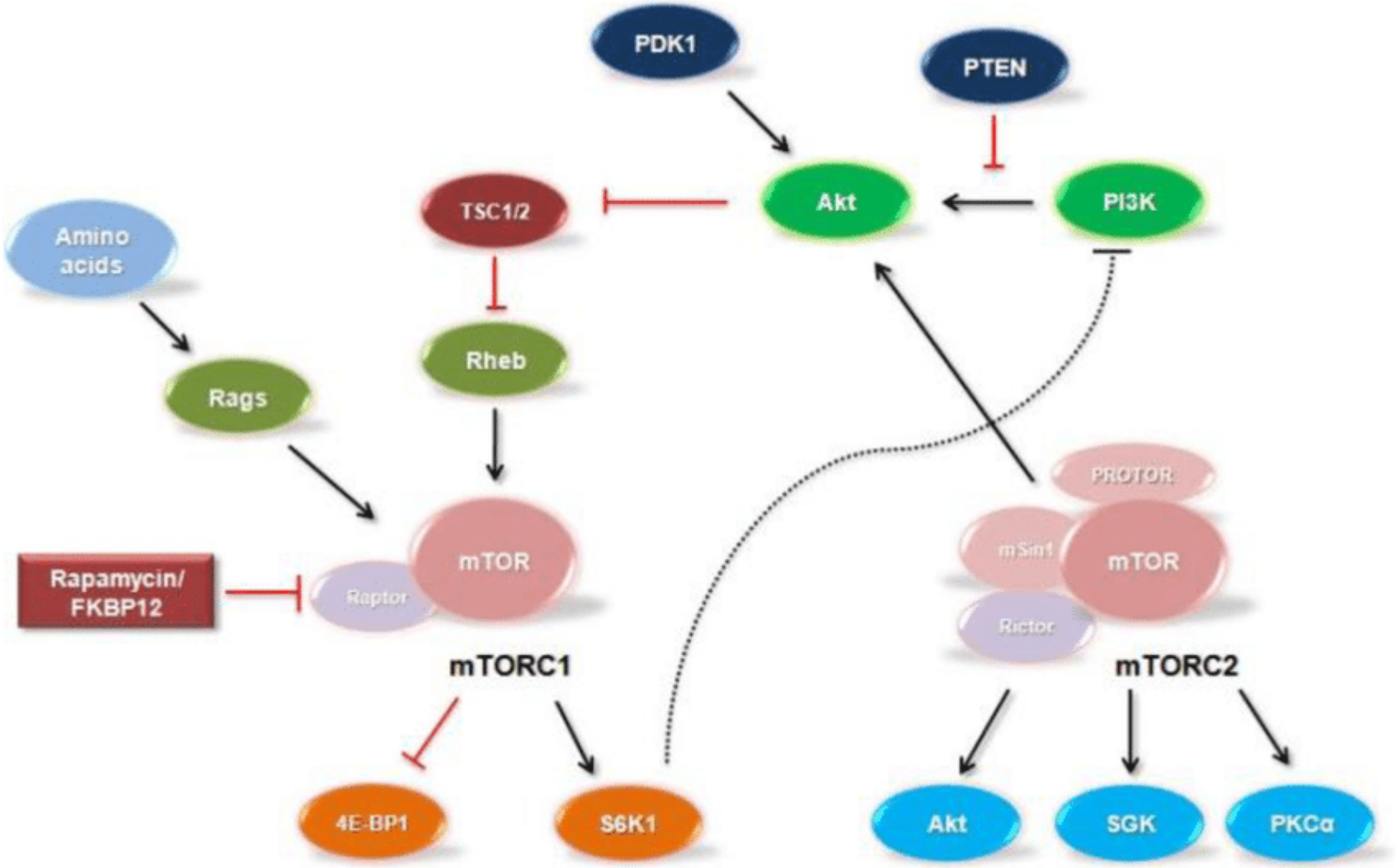


Pathway analysis on the screen hits

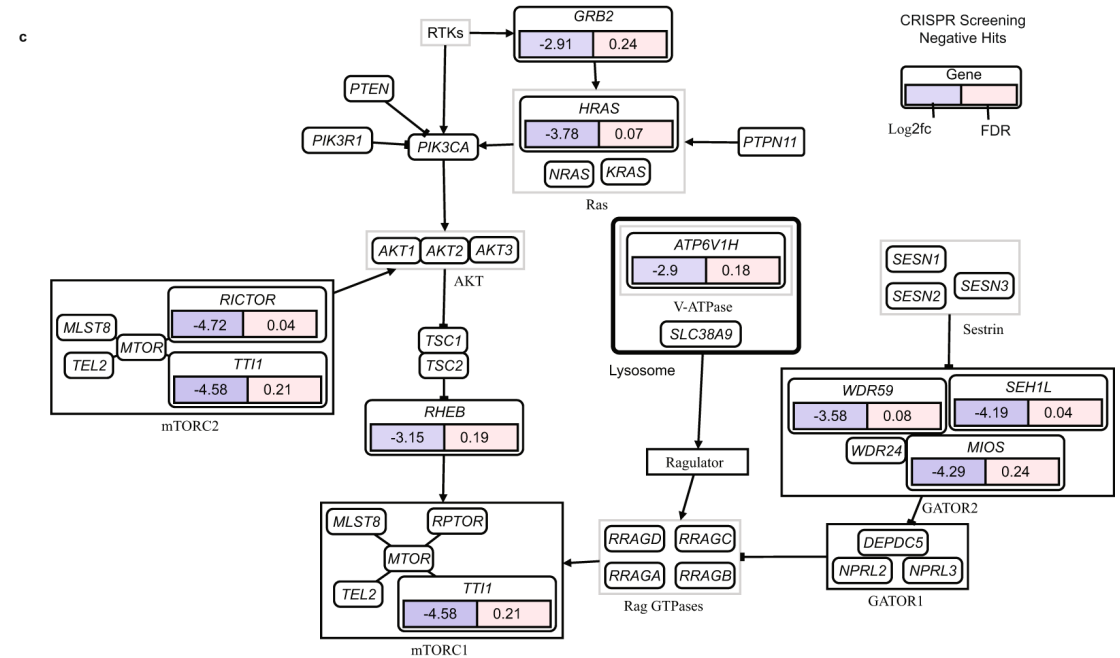




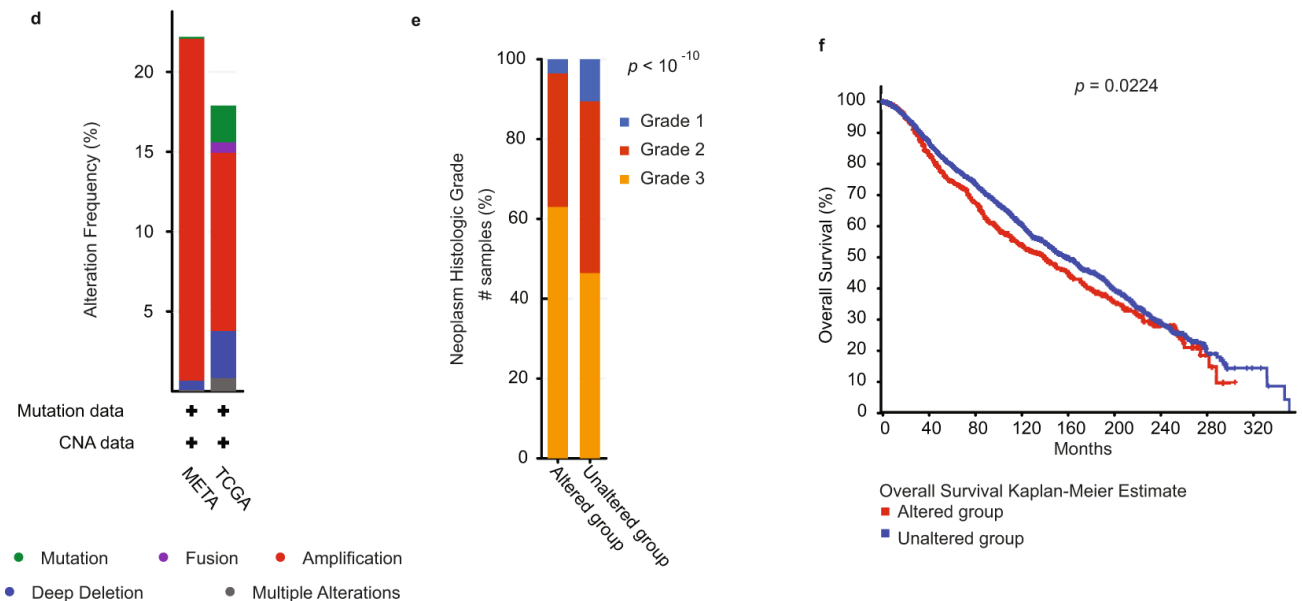
mTOR pathway



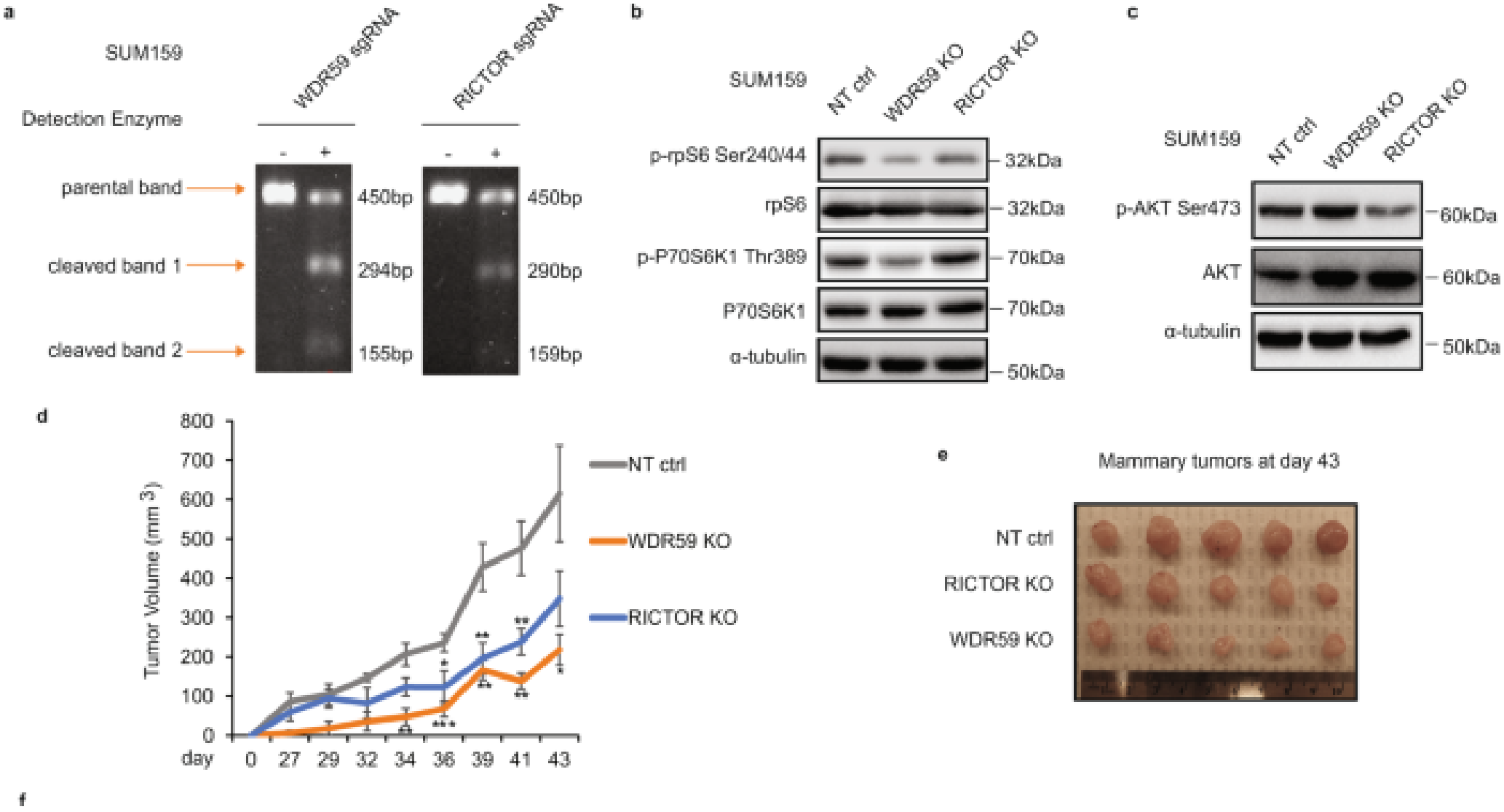
Genes in the mTOR pathway in the screen



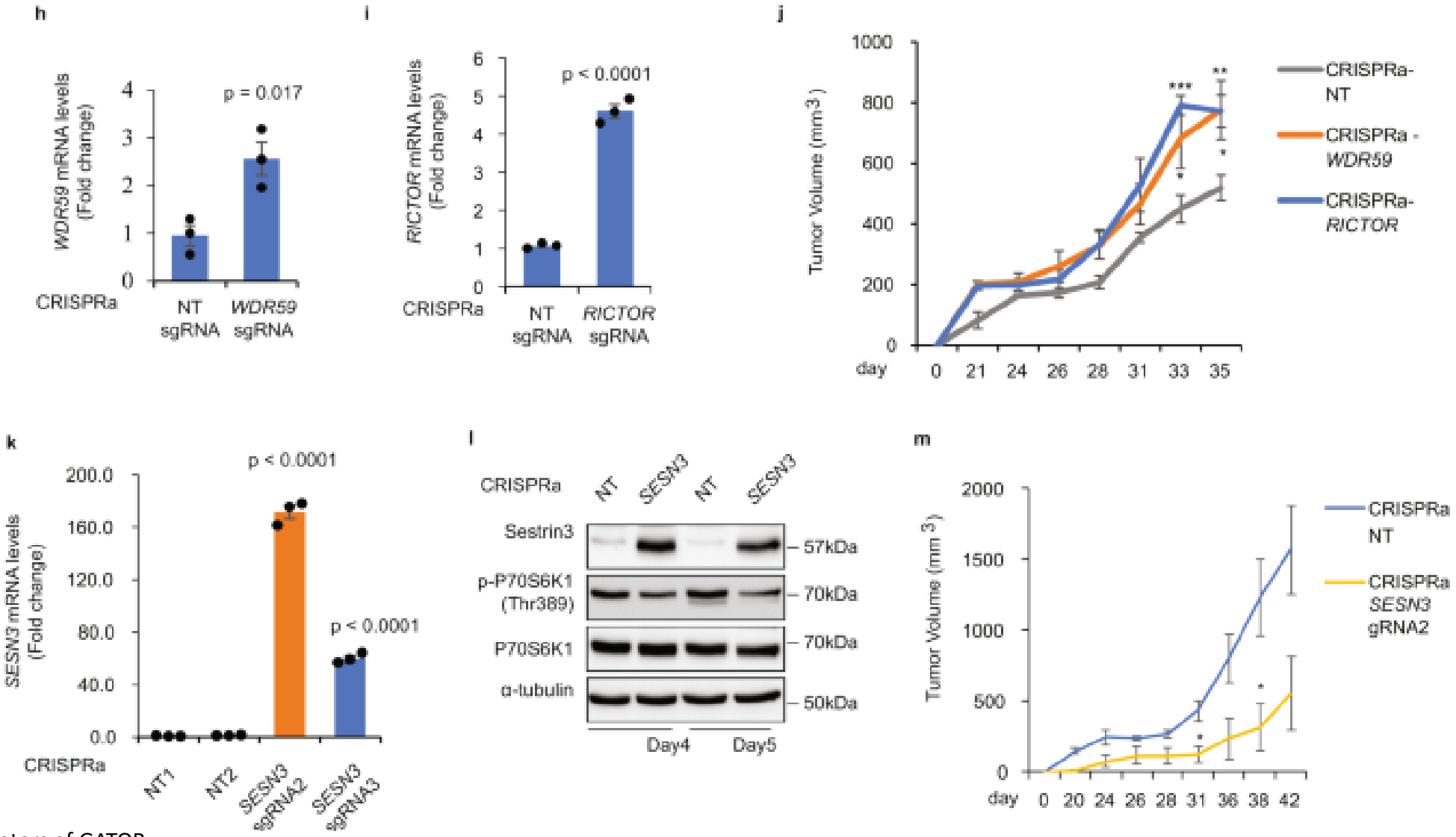
Genetic alterations of the 9 combined TOR hits in 3953 patients



Characterization of mTORC2 and GATOR2 function on TNBC tumor growth.

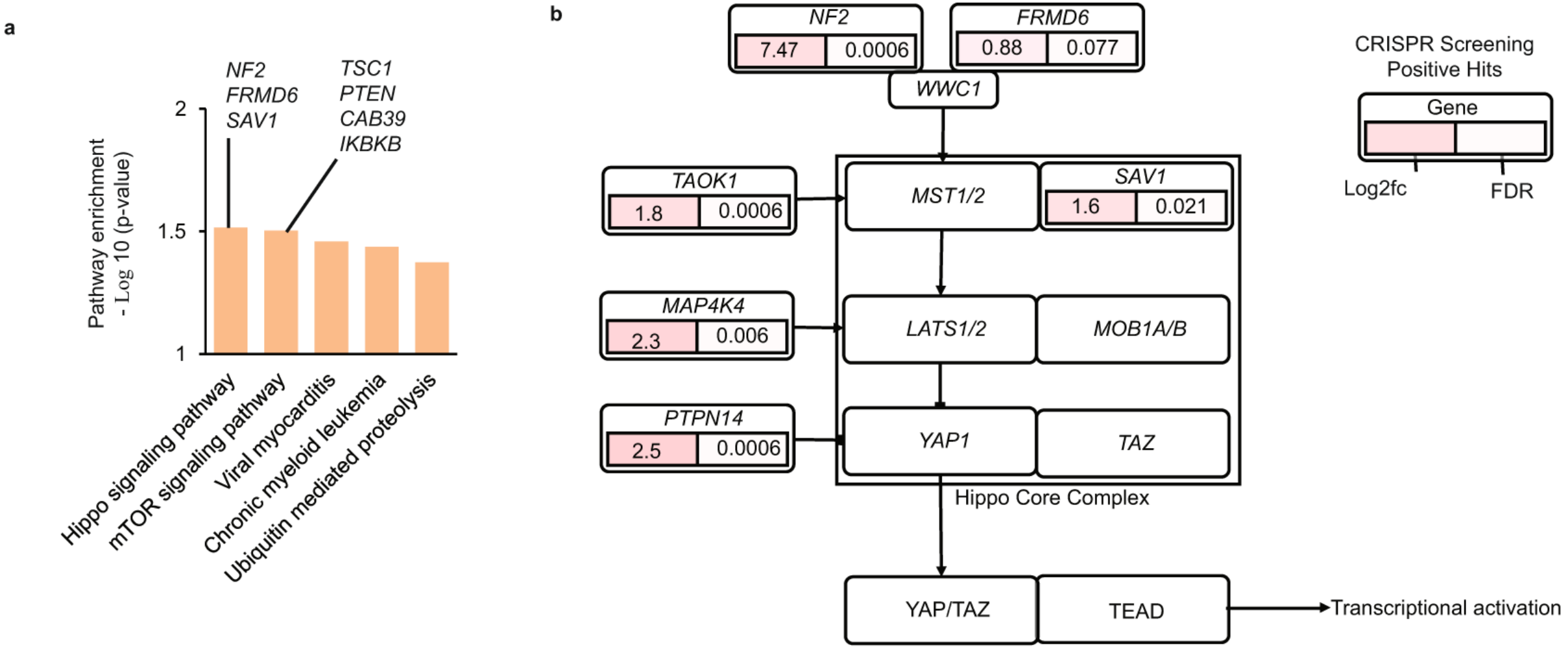


CRISPR activation of targeted hits

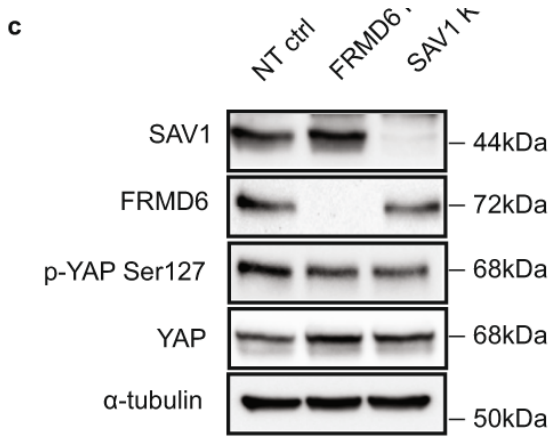


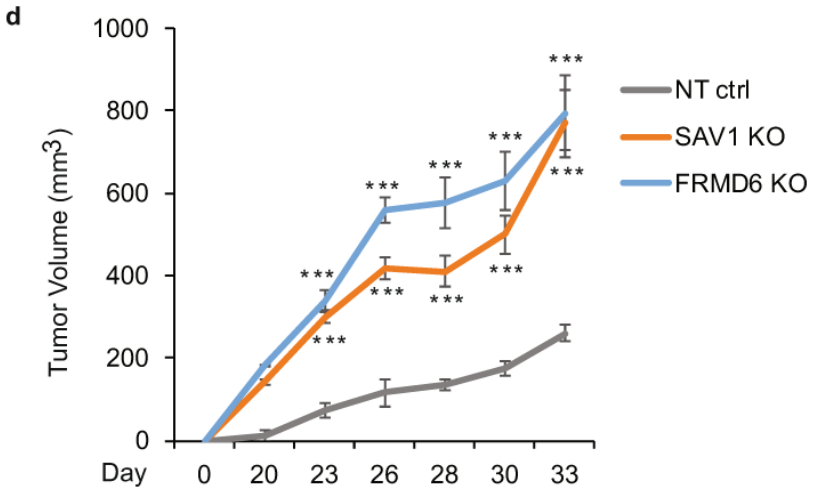
Sestrins are negative Regulators of GATOR proteins.

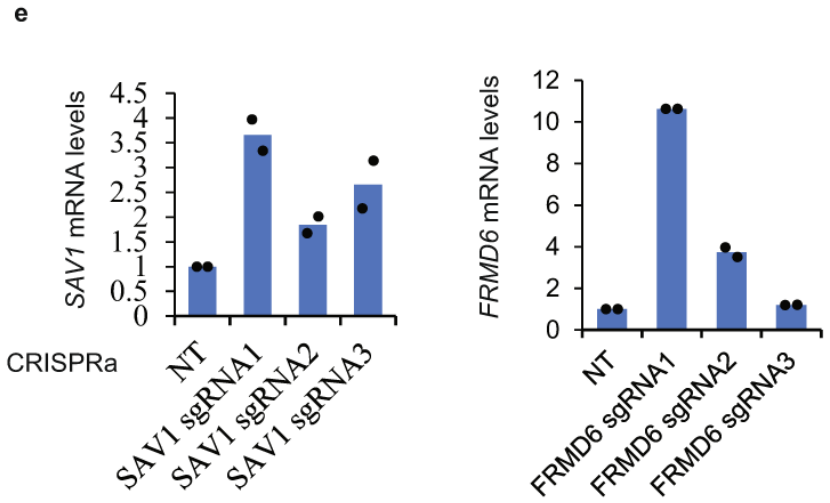
Characterization of Hippo pathway on TNBC tumor growth.

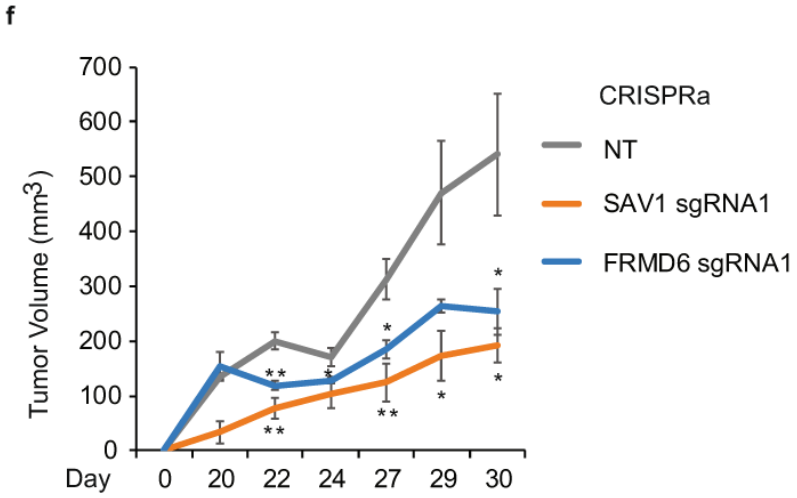


Characterization of Hippo pathway on TNBC tumor growth.

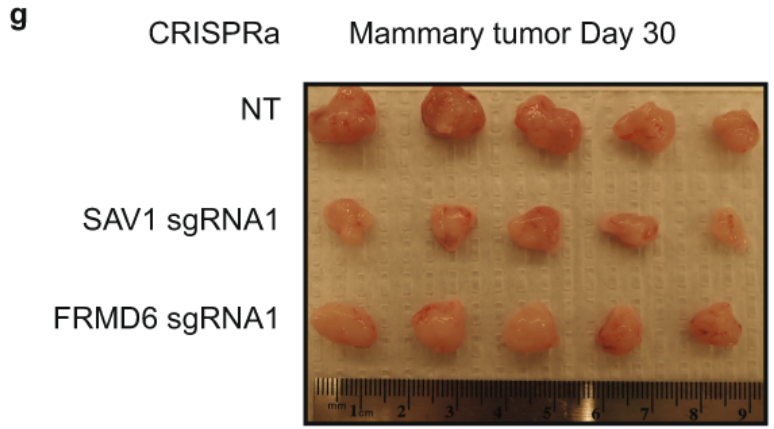


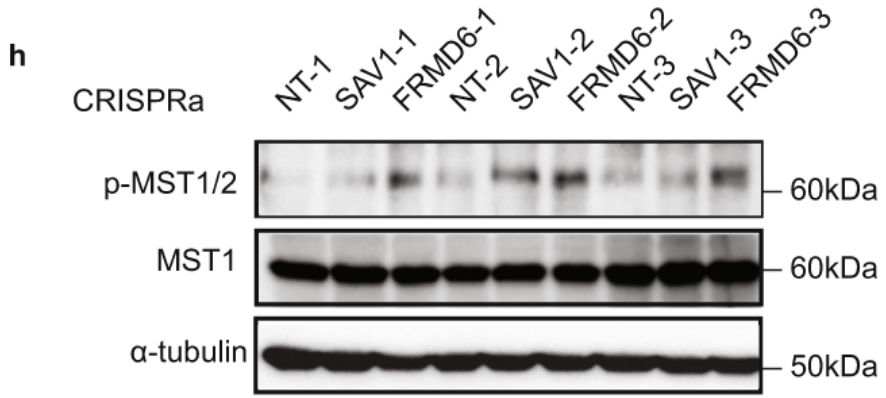


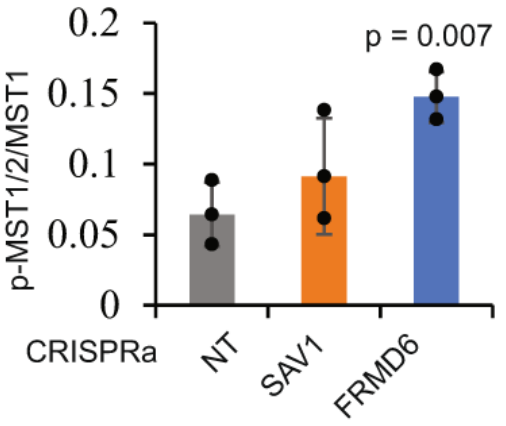


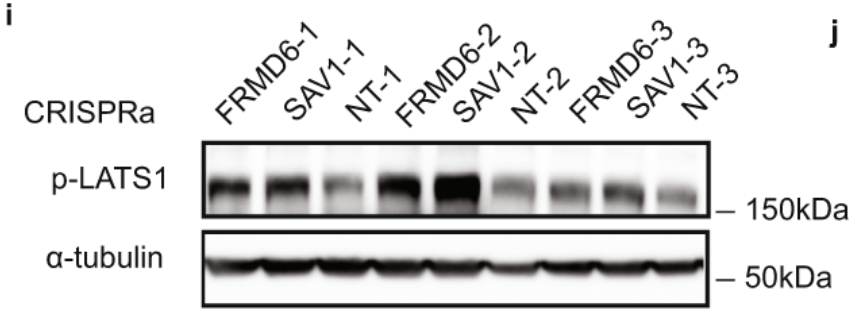


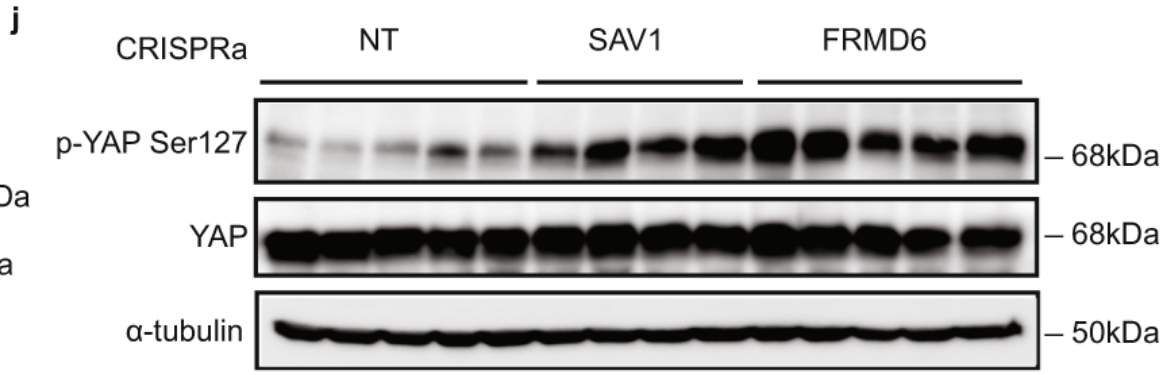
CRISPR activation of HIPPO pathway leads to reduction in tumor size

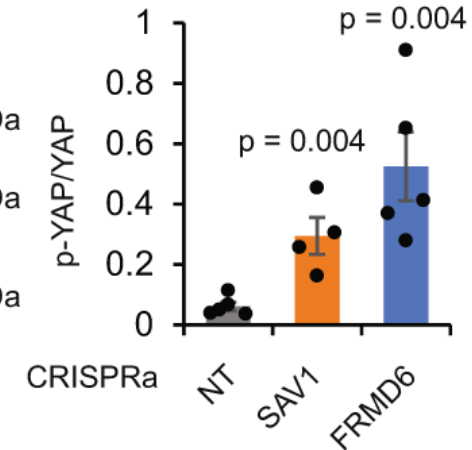




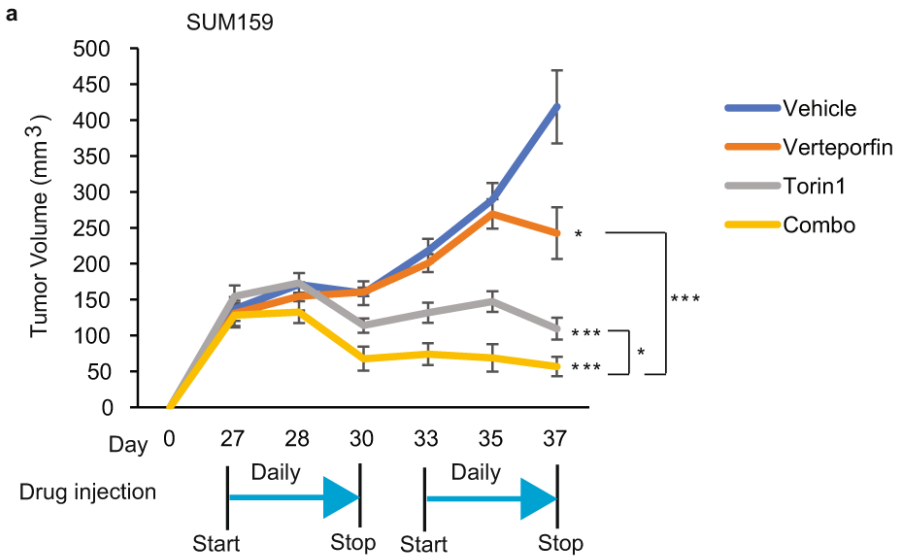


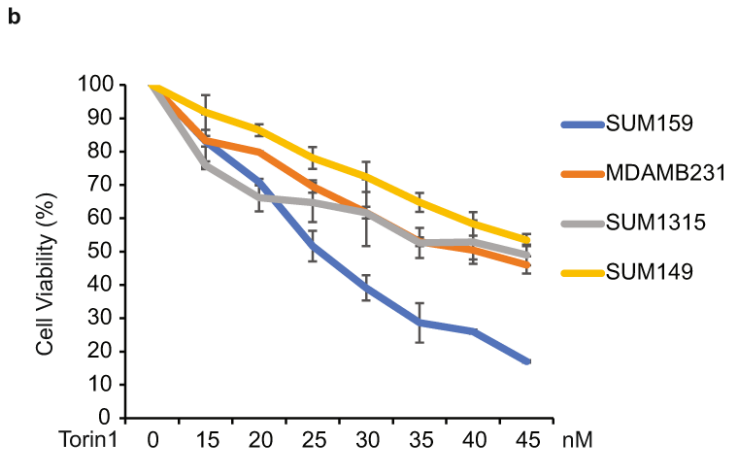


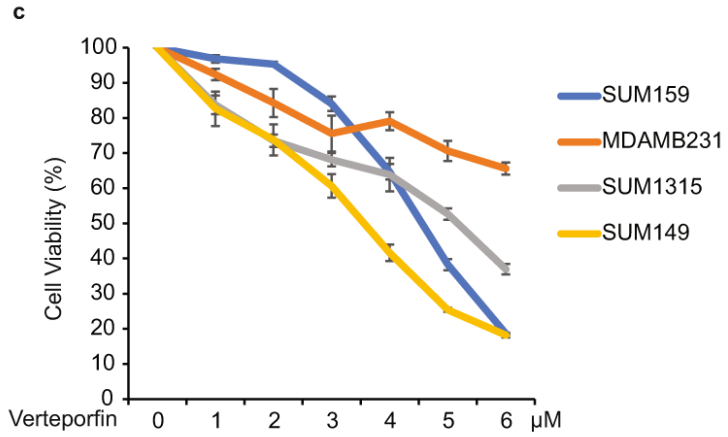




Synergistic activity of drugs tageting mTOR and Hippo pathway on TNBC

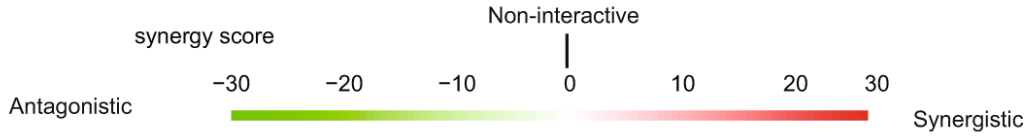


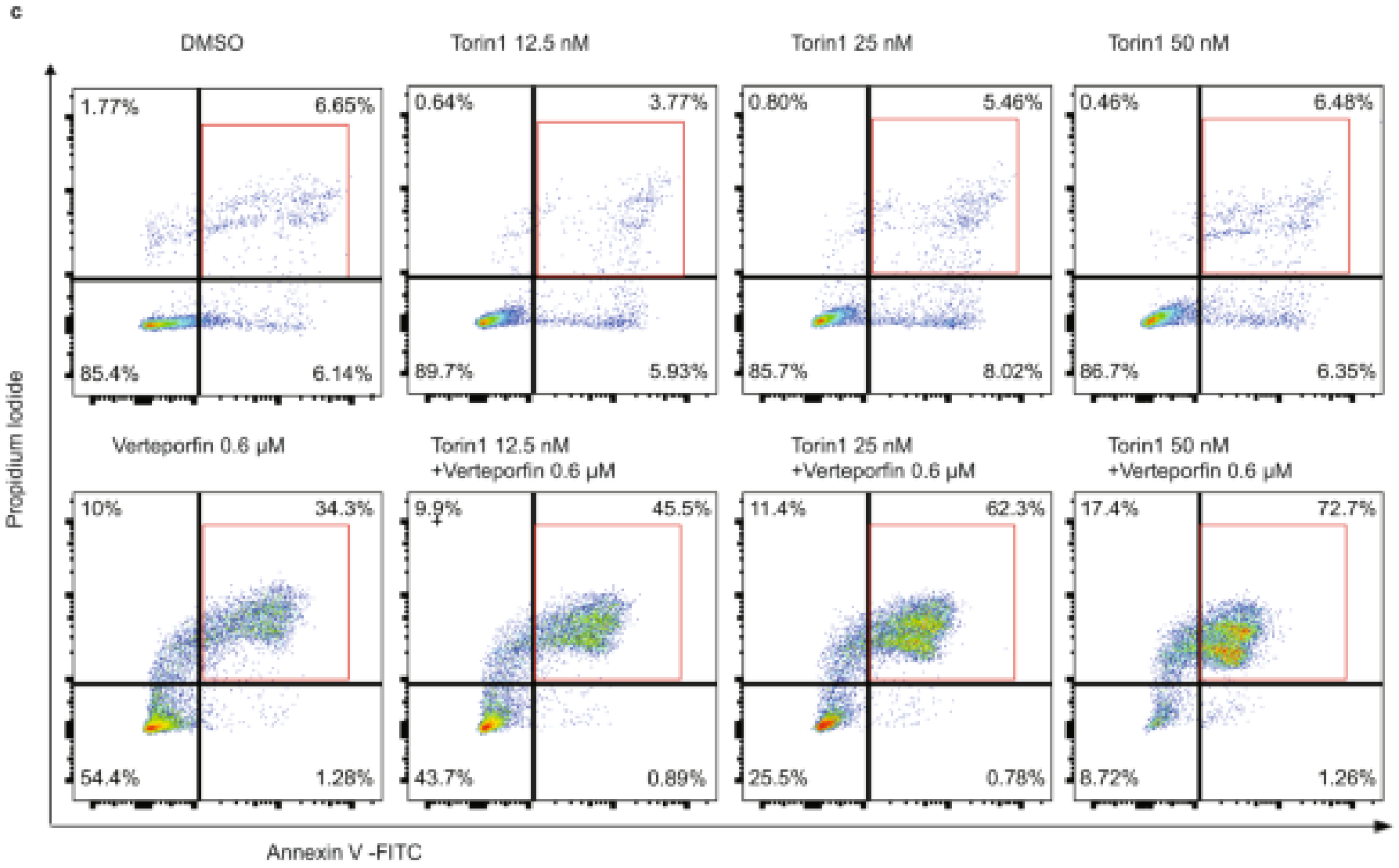




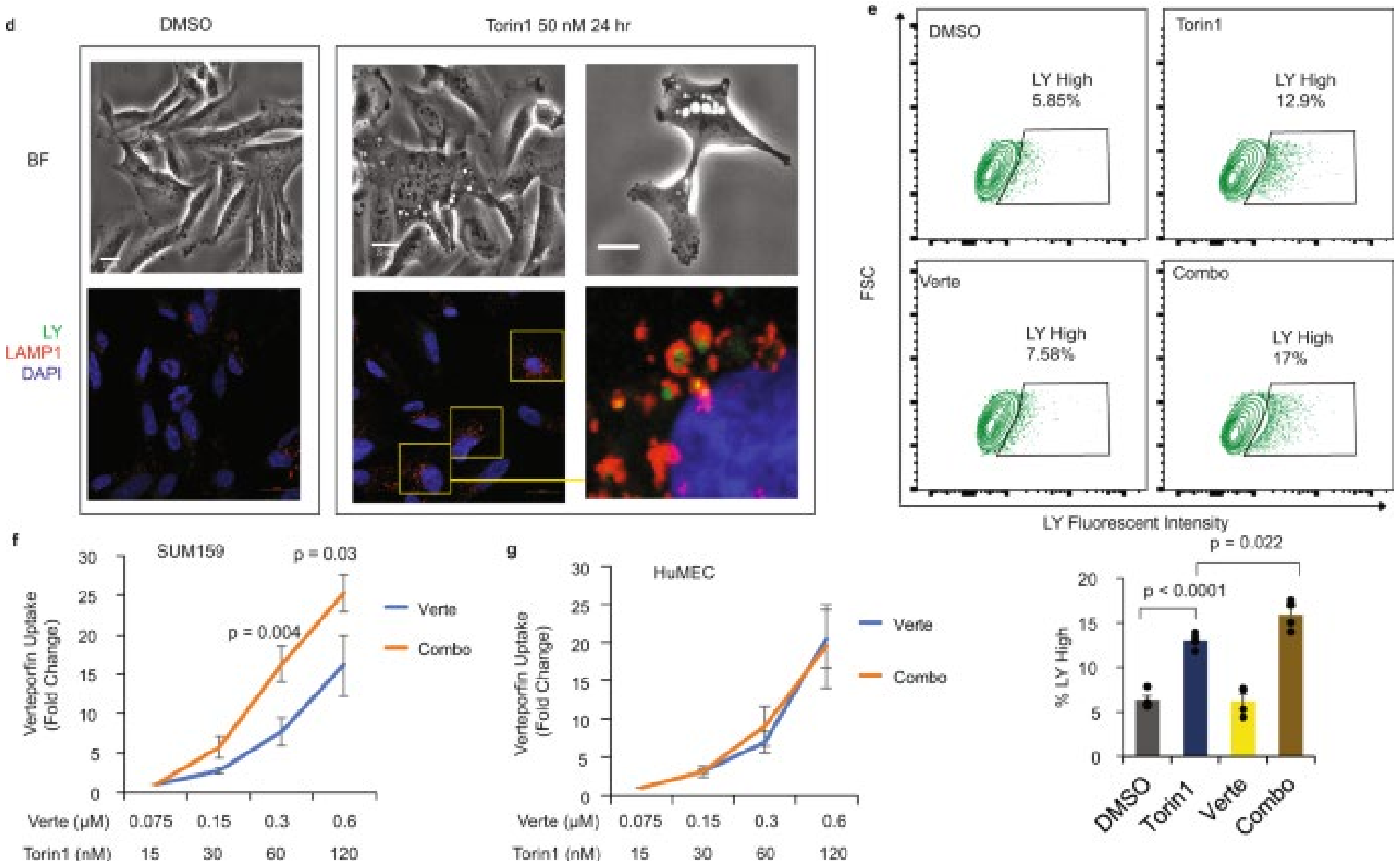
d

Drug combination (Torin1/Verteporfin)	Synergy score (HSA)	Most synergistic score (HSA)	Synergy score (Bliss)	Most synergistic score (Bliss)	Synergy score (ZIP)	Most synergistic score (ZIP)
SUM159	8.05	16.12	1.89	9.91	1.97	11.83
MDAMB231	10.33	17	3.38	6.79	3.22	6.38
SUM1315	11.28	20.74	2.90	14.45	2.85	16.03
SUM149	9.62	19	3.87	9.98	3.94	9.69

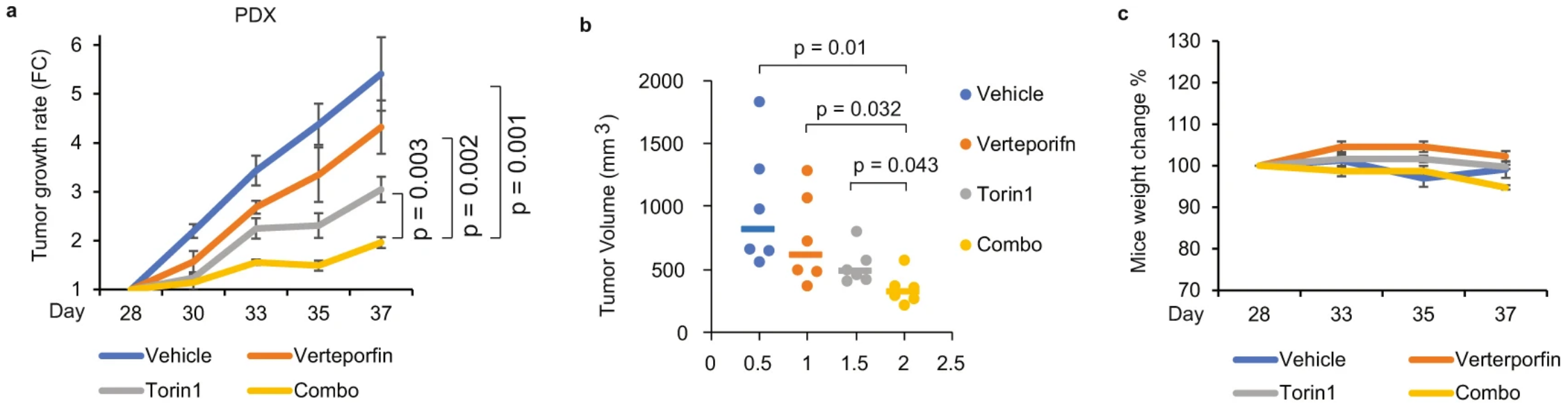



c
DMSO
Torin1 12.5 nM
Torin1 25 nM
Torin1 50 nM
1.77%
6.65%
85.4%
6.14%
0.64%
3.77%
89.7%
5.93%
0.80%
5.46%
85.7%
8.02%
0.46%
6.48%
86.7%
6.35%
Verteporfin 0.6 μM
Torin1 12.5 nM
+Verteporfin 0.6 μM
Torin1 25 nM
+Verteporfin 0.6 μM
Torin1 50 nM
+Verteporfin 0.6 μM
10%
34.3%
54.4%
1.28%
9.9%
45.5%
43.7%
0.89%
11.4%
62.3%
25.5%
0.78%
17.4%
72.7%
8.72%
1.26%
Propidium Iodide
Annexin V -FITC

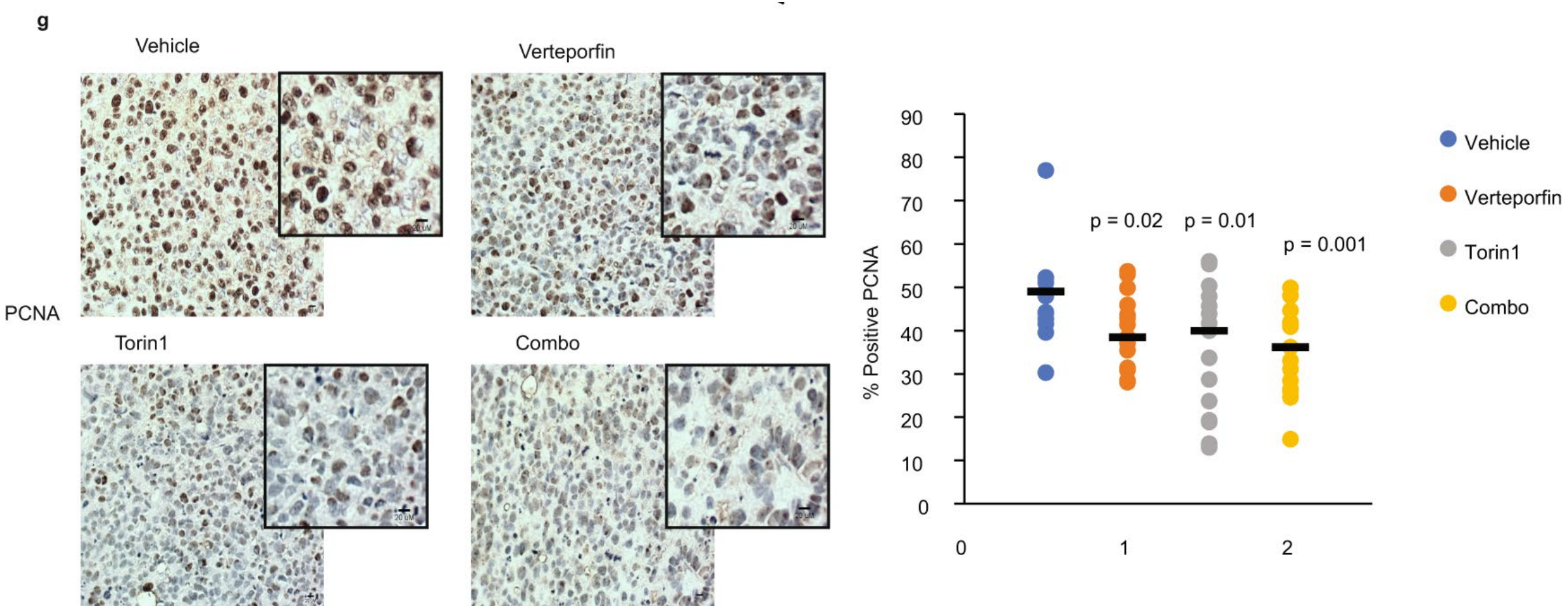
Mechanism of how the drugs work



The combo therapy is efficient in Patient derived xenografts



The combo therapy leads to depletion of proliferating cells in Patient derived xenografts



Summary

1. New therapeutic targets can be identified using CRISPR screens.
2. New pathways involved in TNBC proliferation are identified:mTOR and Hippo pathways
3. Drugs targeting the pathways can be used as a therapeutic option

Questions:

1. Are the identified pathways universal?
2. Do we need to move towards custom sgRNA screens for the patients to identified suitable therapy?