UT Southwestern Harold C. Simmons Comprehensive Cancer Center

NCI Designated Comprehensive Cancer Center

SAN ANTONIO BREAST CANCER SYMPOSIUM

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Loss of *ASXL1* tumor suppressor promotes resistance to CDK4/6 inhibitors in ER+ breast cancer

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Disclosure statement

- AstraZeneca employee since October 2021
- All data were generated during my tenure at the Simmons Comprehensive Cancer Center, in the laboratory of Dr. Carlos Arteaga

Novel approaches to unravel the CDK4/6 inhibitor resistance landscape

Genomic profiling of MBCs progressing on CDK4/6i has identified several resistance-associated somatic alterations, albeit at low frequencies –

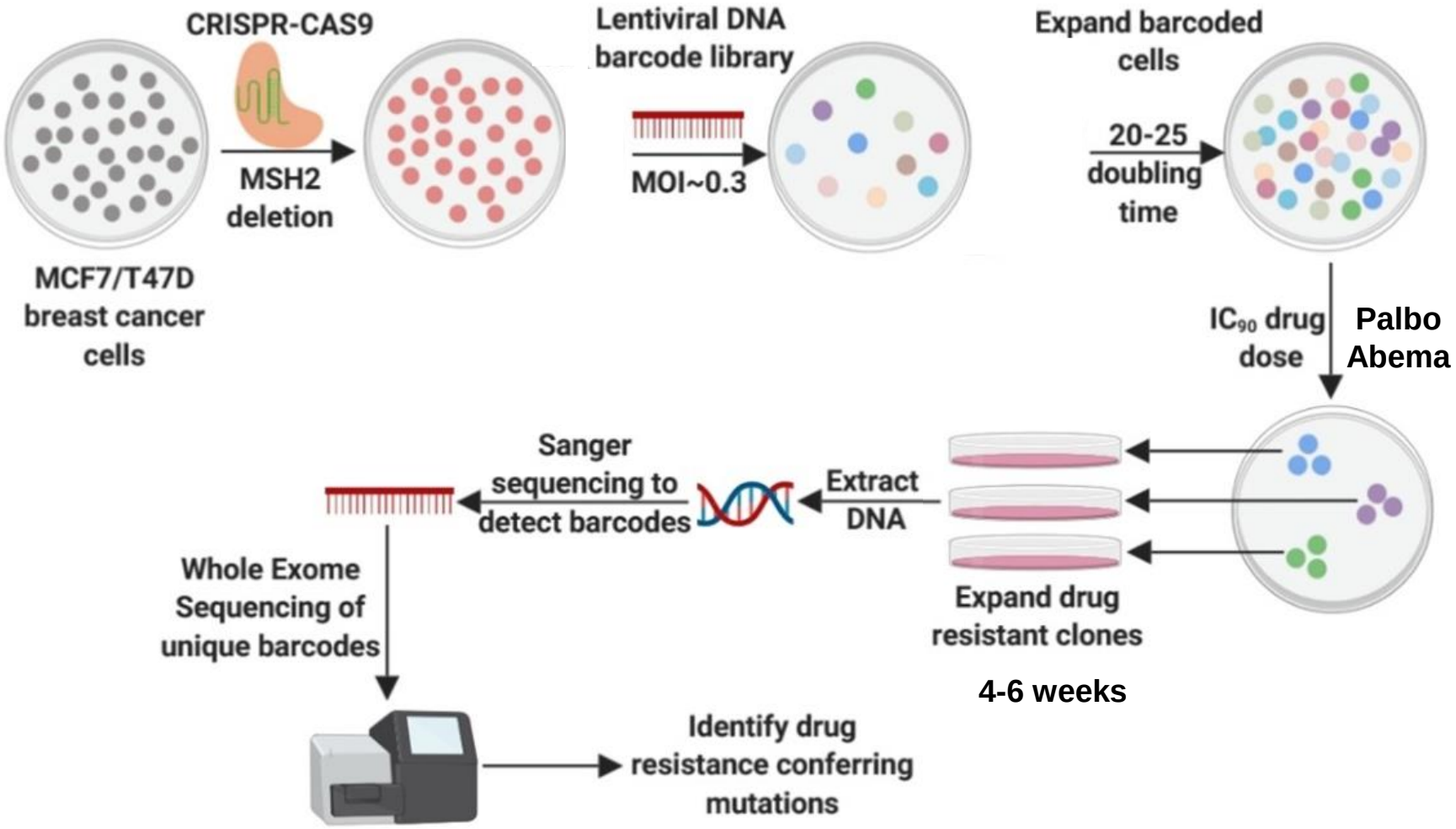
- *RB1* mutations (6/127; **4.7%**) (O'Leary et al, Cancer Discovery, 2018)
- *FGFR1* amplification (Formisano et al, Nature Communications, 2019)
- *FAT1* loss-of-function alterations (6/348; **1.7%**) (Li et al, Cancer Cell, 2018)
- *FGFR2* (3/41), *AKT1* (5/41), *AURKA* (11/41), *KRAS* (2/41), and *HRAS* (1/41) alterations (Wander et al, Cancer Discovery, 2020)

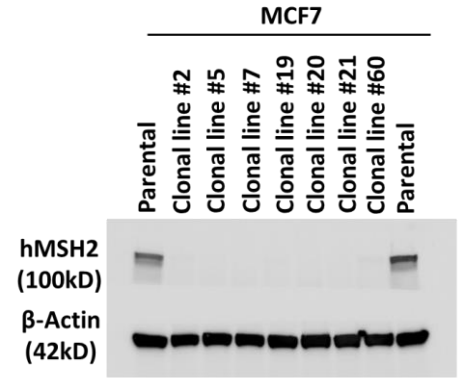
There is an unmet need to uncover the full spectrum of genomic drivers of resistance to CDK4/6 inhibitors

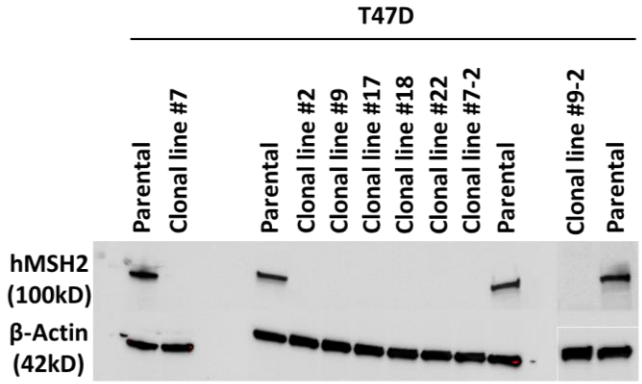
Accelerated mutagenesis approaches provide a robust and unbiased platform for the discovery of novel resistance mechanisms

Accelerated mutagenesis screen to discover a spectrum of novel mutations causally associated with resistance to CDK4/6 blockade

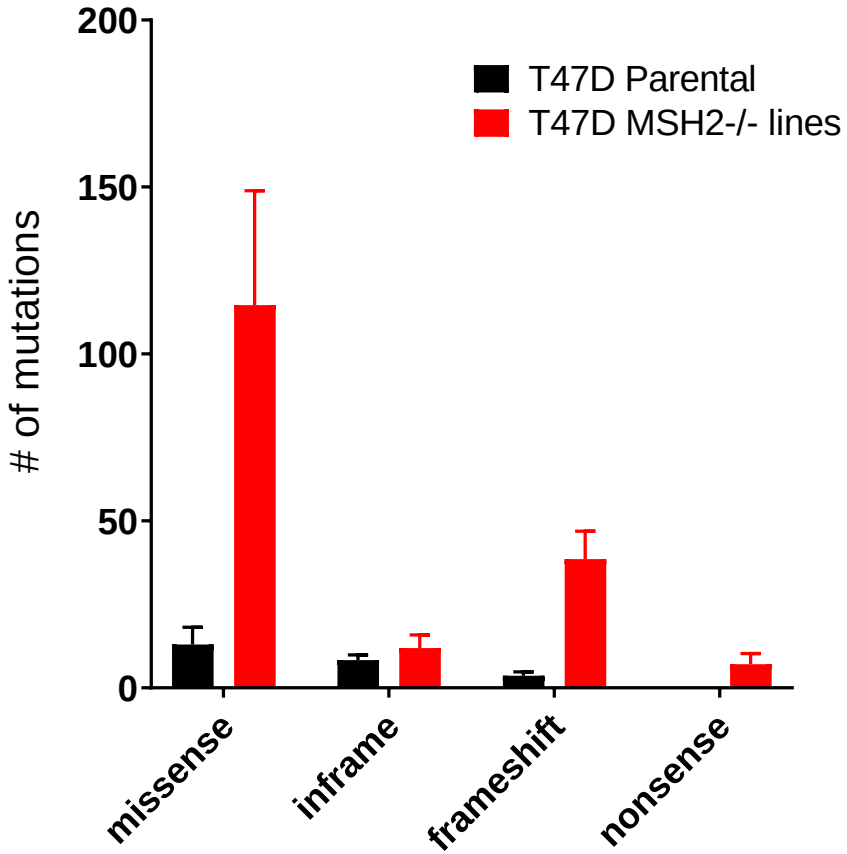
Impaired DNA repair accelerates the rate of nucleotide substitution leading to rapid accumulation of new mutations



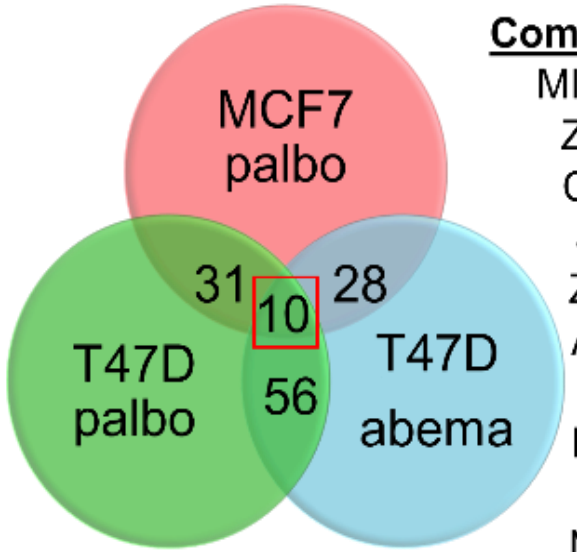




Accelerated mutagenesis screen to discover a spectrum of novel mutations causally associated with resistance to CDK4/6 blockade



Number of missense and frameshift mutations accumulated by the MSH2$^{-/-}$ clones are higher compared to the parental cells



Common genes	Types of mutation
MIS18BP1*	frameshift+missense
ZNF469*	frameshift
C8orf34*	missense
SYNE2	missense
ZNF541	missense+frameshift
ASXL1*	missense+frameshift
ATR*	missense+frameshift
BICRA*	frameshift
MYRF*	missense+frameshift
NGLY1*	splice junction

*cross-resistant to fulvestrant

Majority of alterations noted in these 10 candidate genes were frameshift or truncating mutations, suggesting loss of function.

Top hits are associated with clinical resistance to CDK4/6 inhibitors

DFCI CCPM / MBC Project

Hugo Symbol	Protein Change	Drug	Resistance Category
ASXL1	p.RGGE65fs	Palbociclib	Acquired
ASXL1	p.E484K	Palbociclib	Intrinsic
ASXL1	p.E824Q	Palbociclib	Intrinsic
ASXL1	p.G949D	Palbociclib	Putative intrinsic
ASXL1	p.E513*	Ribociclib	Intrinsic
ASXL1	p.R235Q	Palbociclib	Intrinsic
ASXL1	p.R549C	Palbociclib	Acquired
ATR	p.M2551I	Palbociclib	Putative intrinsic
ATR	p.K899T	Palbociclib	Intrinsic
ATR	p.E2405Q	Abemaciclib	Intrinsic
MIS18BP1	p.K845N	Palbociclib	Putative intrinsic

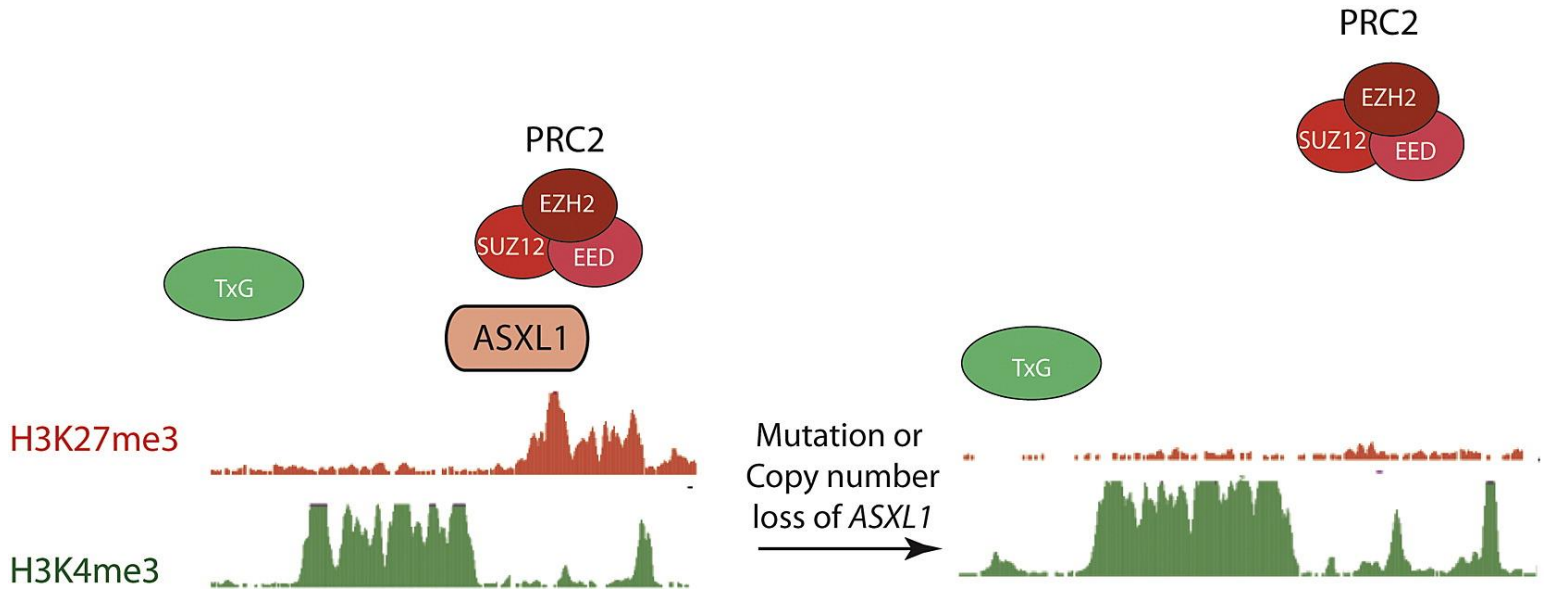
TEMPUS

Hugo Symbol	Incidence	Alteration Frequency
ASXL1	37/1796	2.06%
ATR	49/1796	2.72%
MIS18BP1	3/1796	0.17%

Additional Sex Combs Like Transcriptional Regulator 1 (ASXL1)

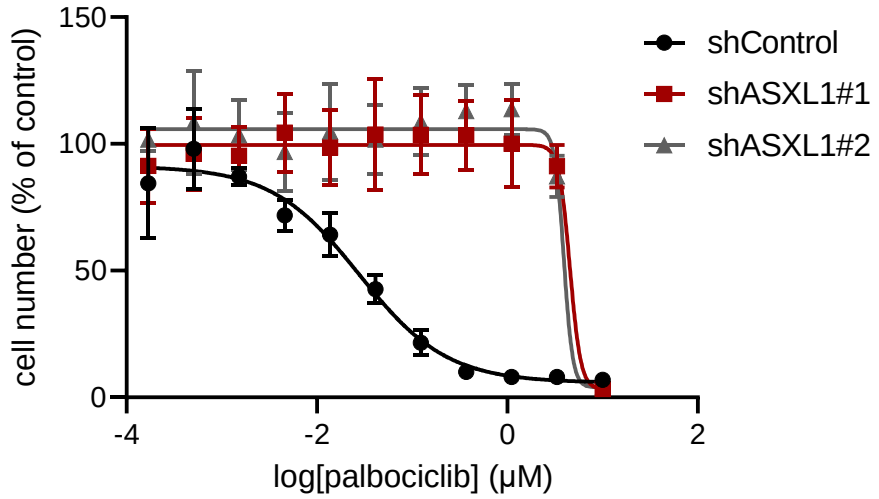
Heterozygous truncating mutations in the *ASXL1* gene is a common event in myeloid malignancies

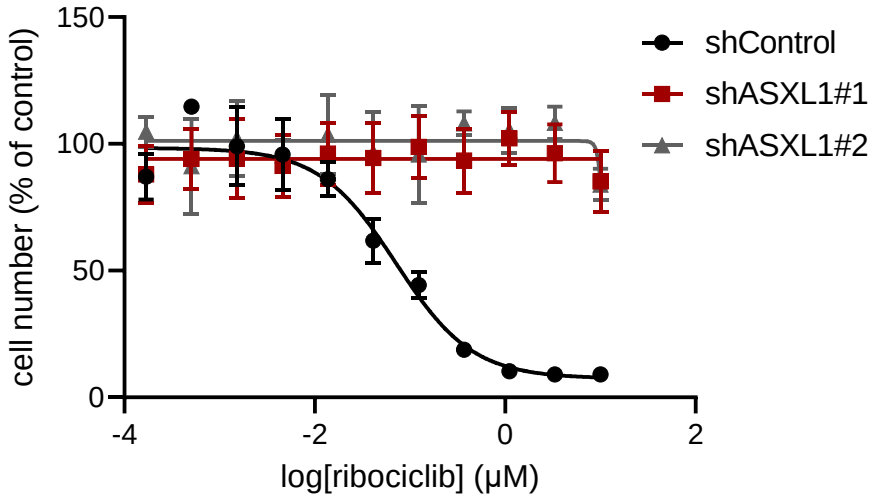
ASXL1 inactivation induces epigenetic and transcriptional reprogramming through global loss of H3K27me3 chromatin repressive marks.

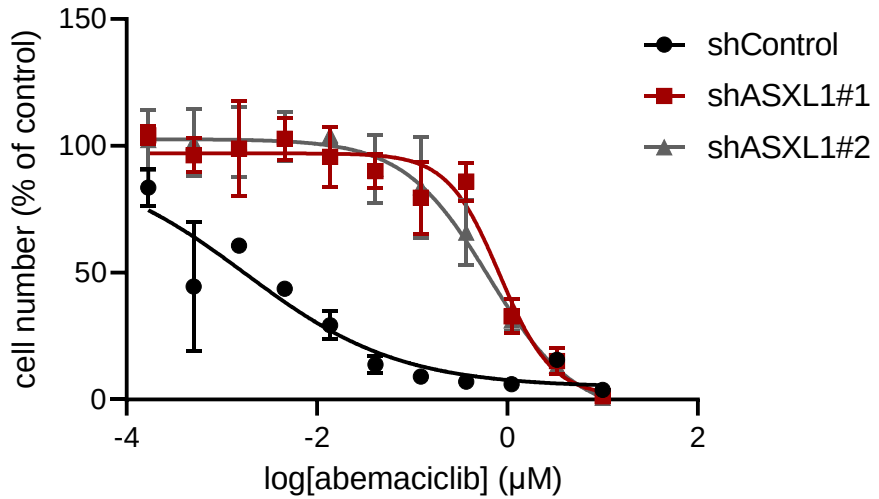


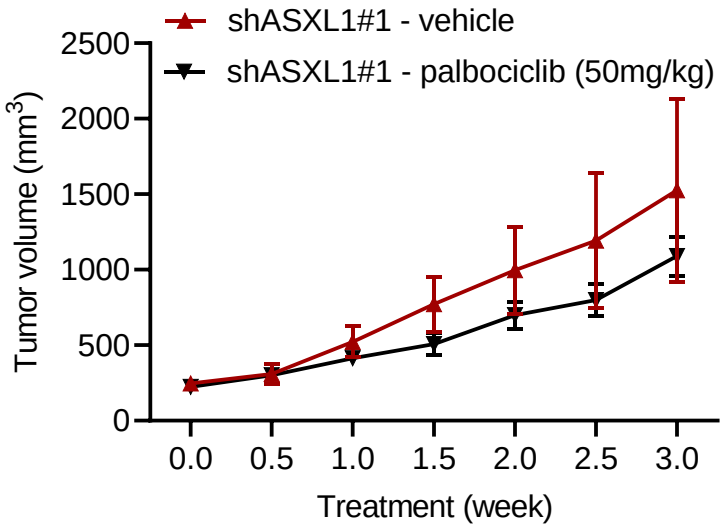
Abdel-Wahab et al. Cancer Cell, 2012

ASXL1 deficient ER+ breast cancer cells are resistant to CDK4/6 inhibition

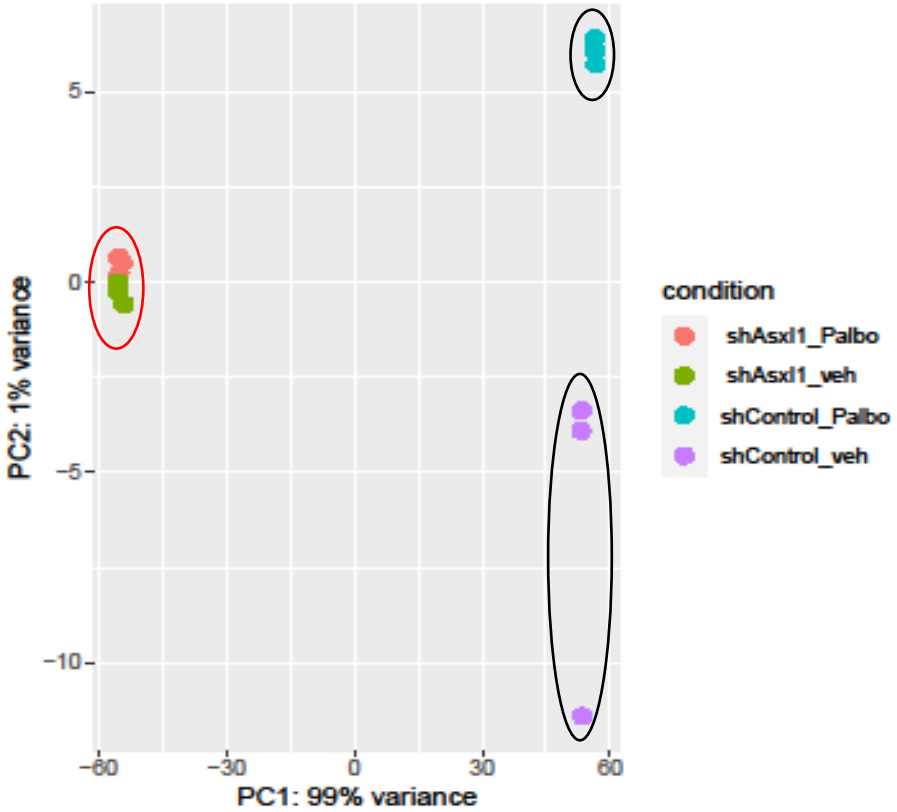


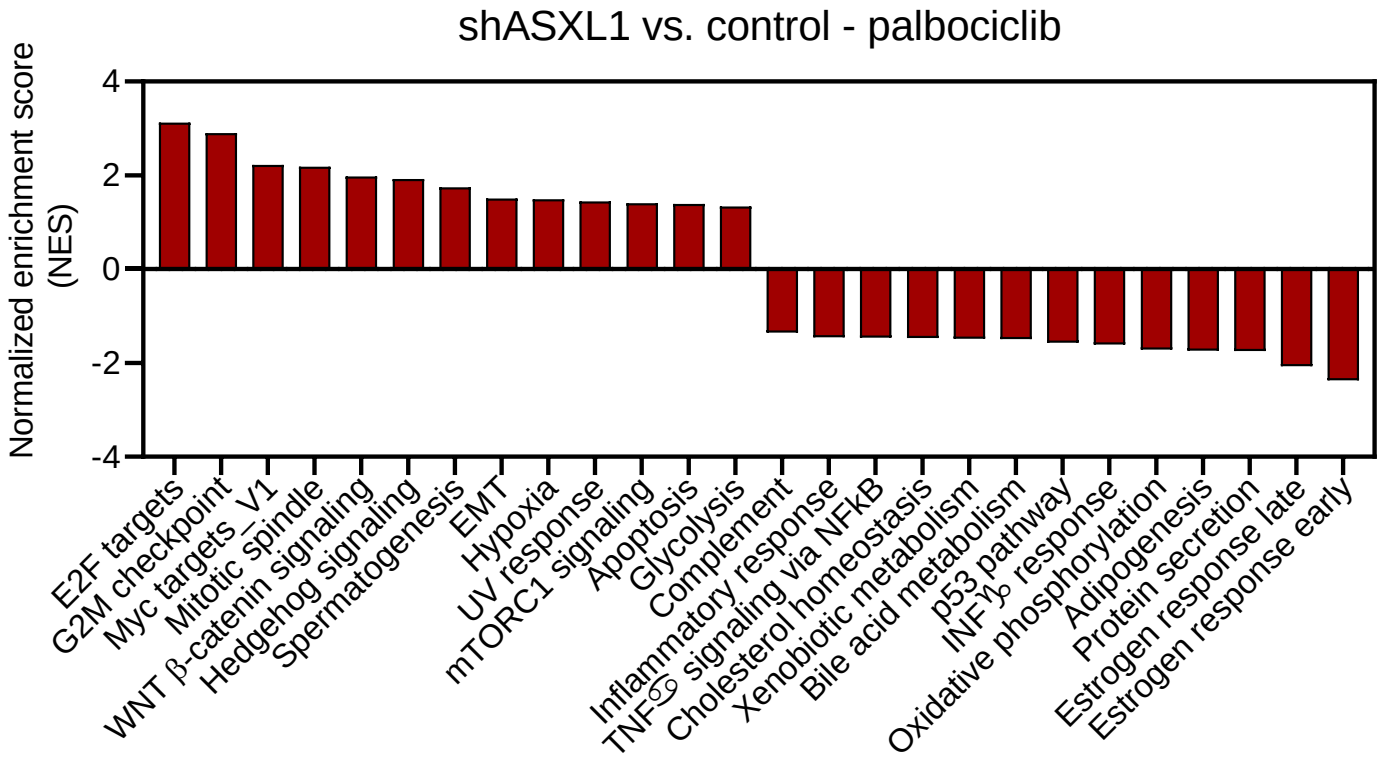






ASXL1 deficiency leads to robust induction of E2F target genes

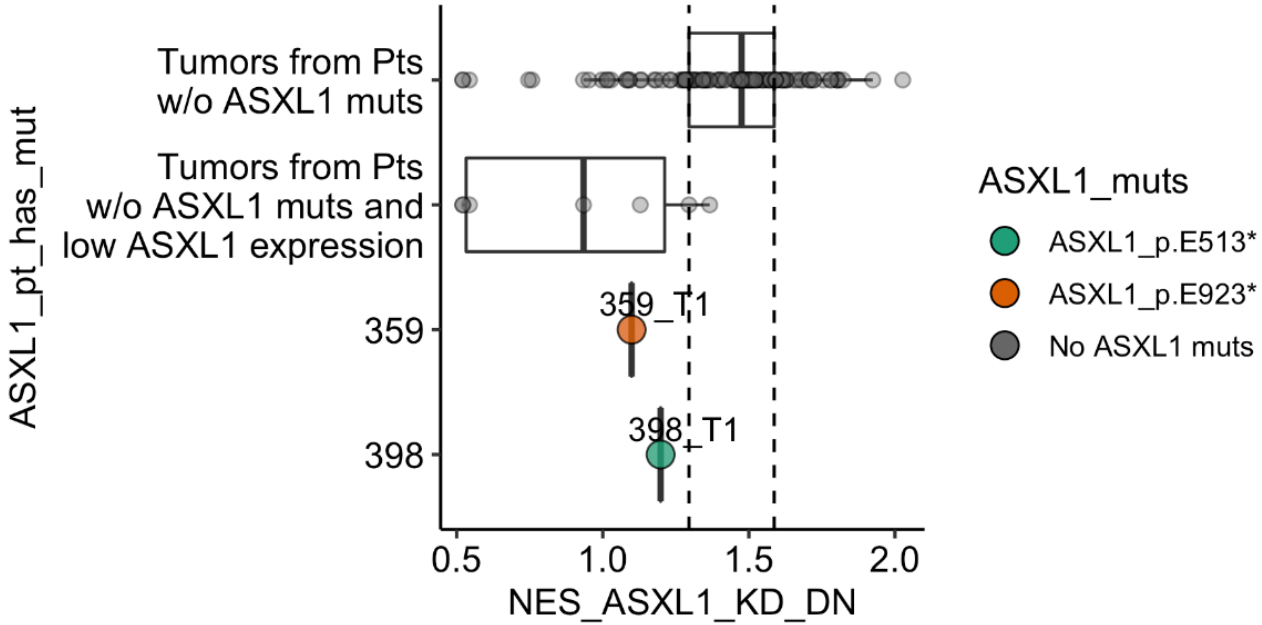




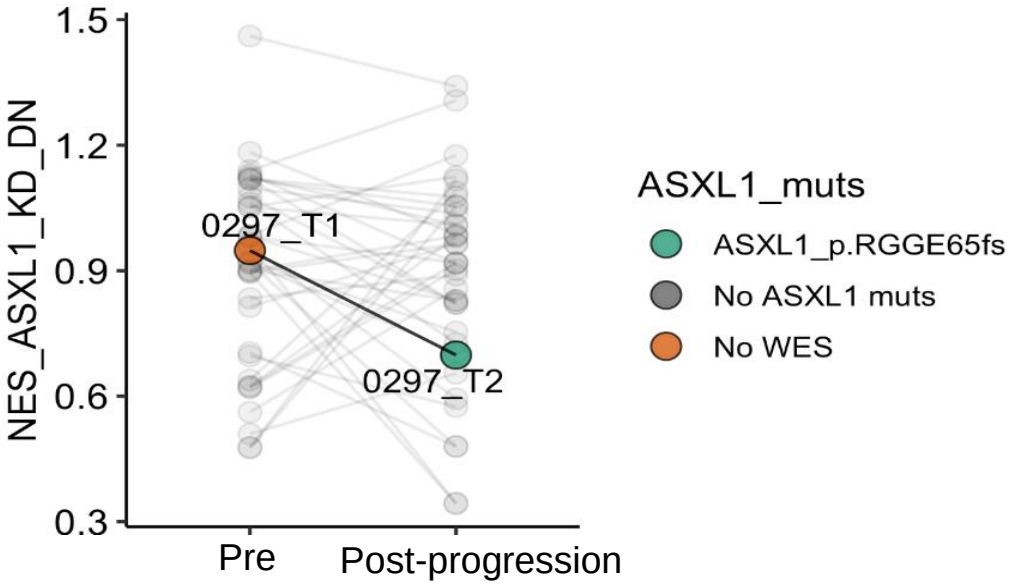
Loss of function ASXL1 mutations are acquired in CDK4/6 inhibitor resistant ER+ MBCs

ASXL1 loss gene signature queried in clinical datasets

CCPM RNAseq dataset

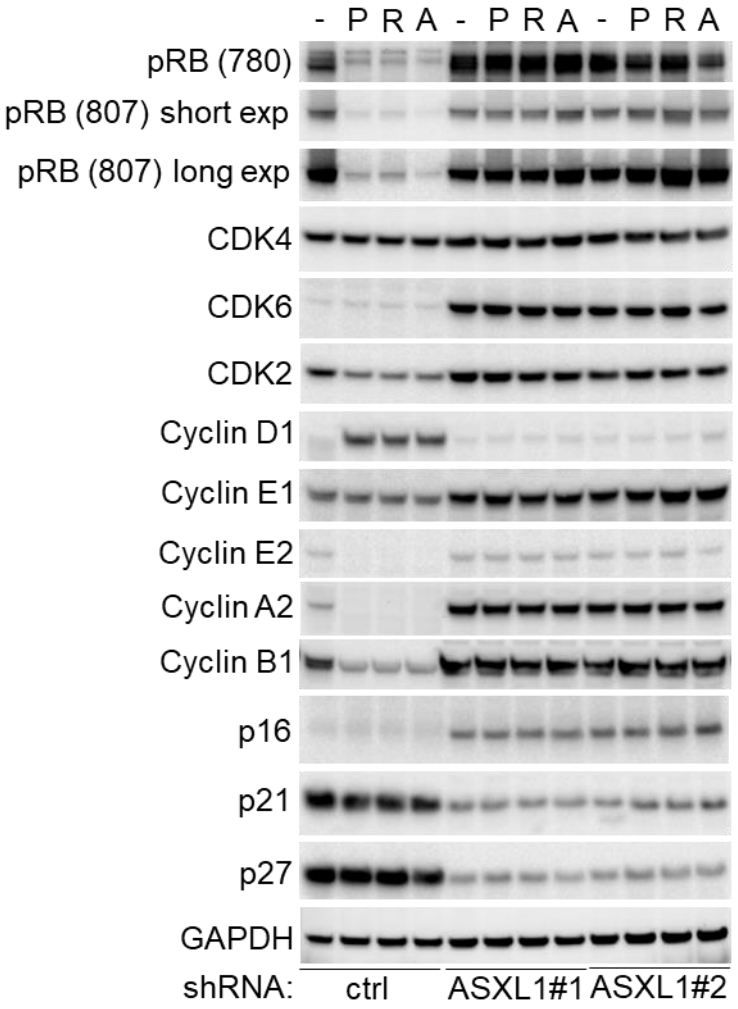


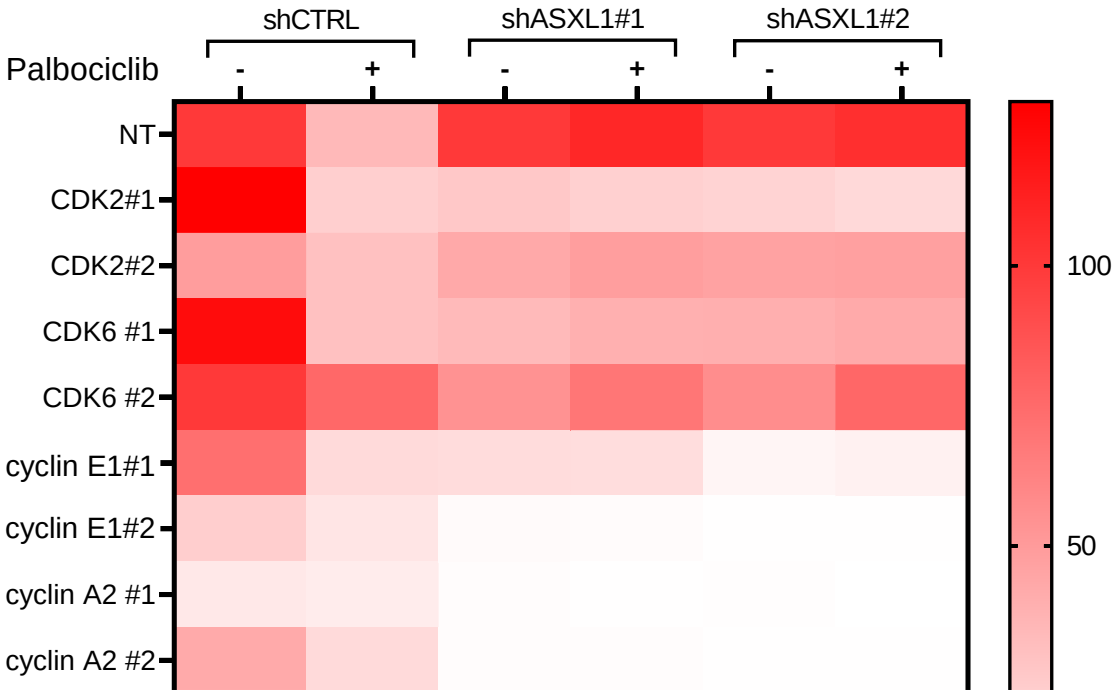
MBC Project RNAseq dataset

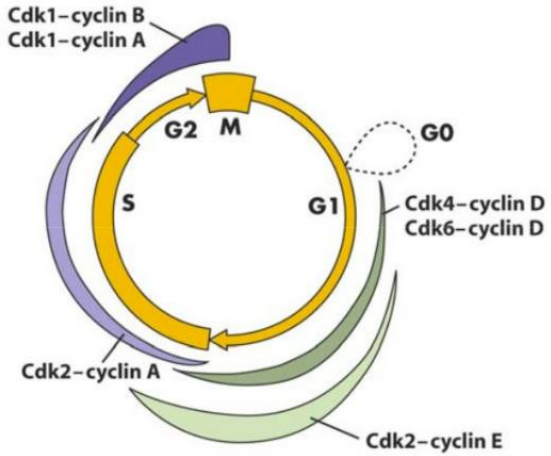


ASXL1 deficiency leads to robust induction of cell cycle genes

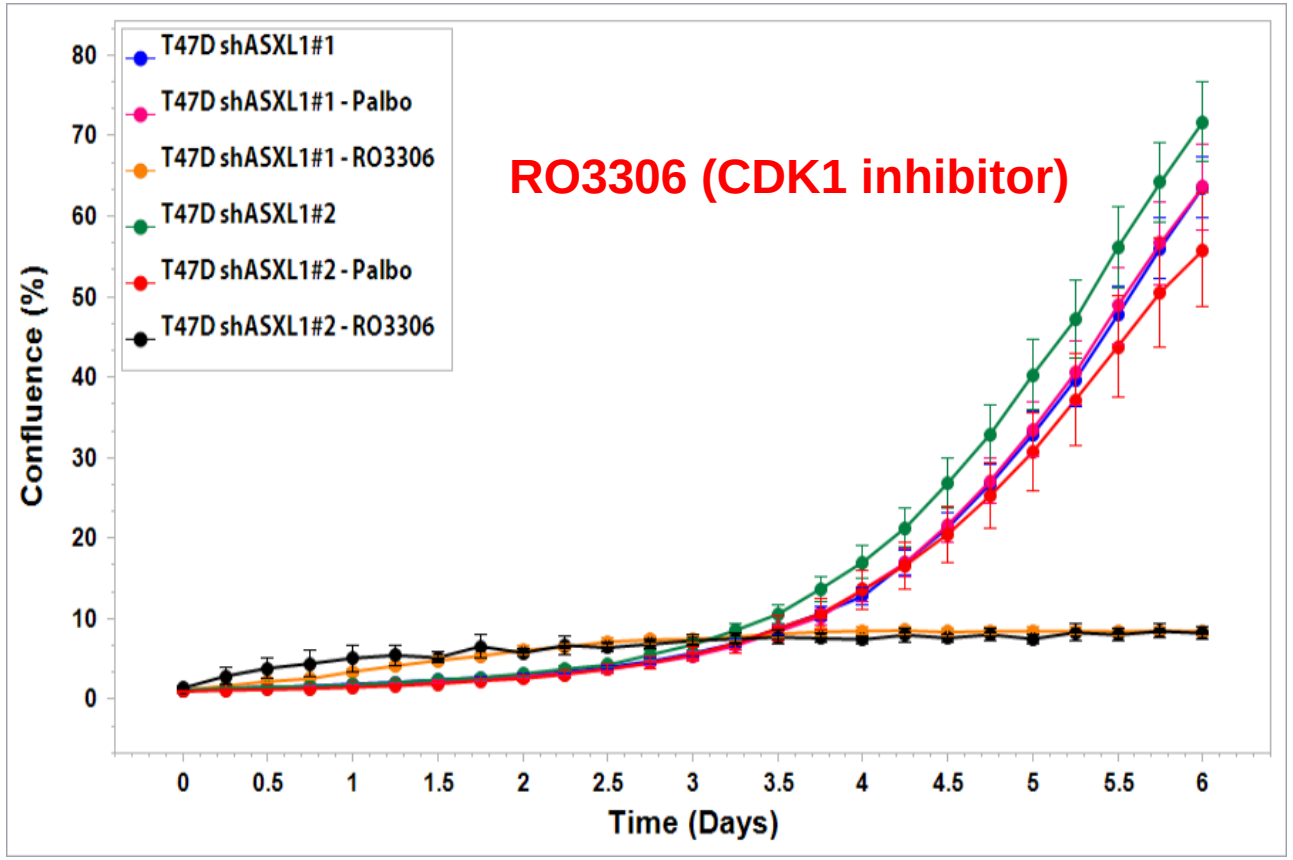
Palbociclib (P); Ribociclib (R); Abemaciclib (A)

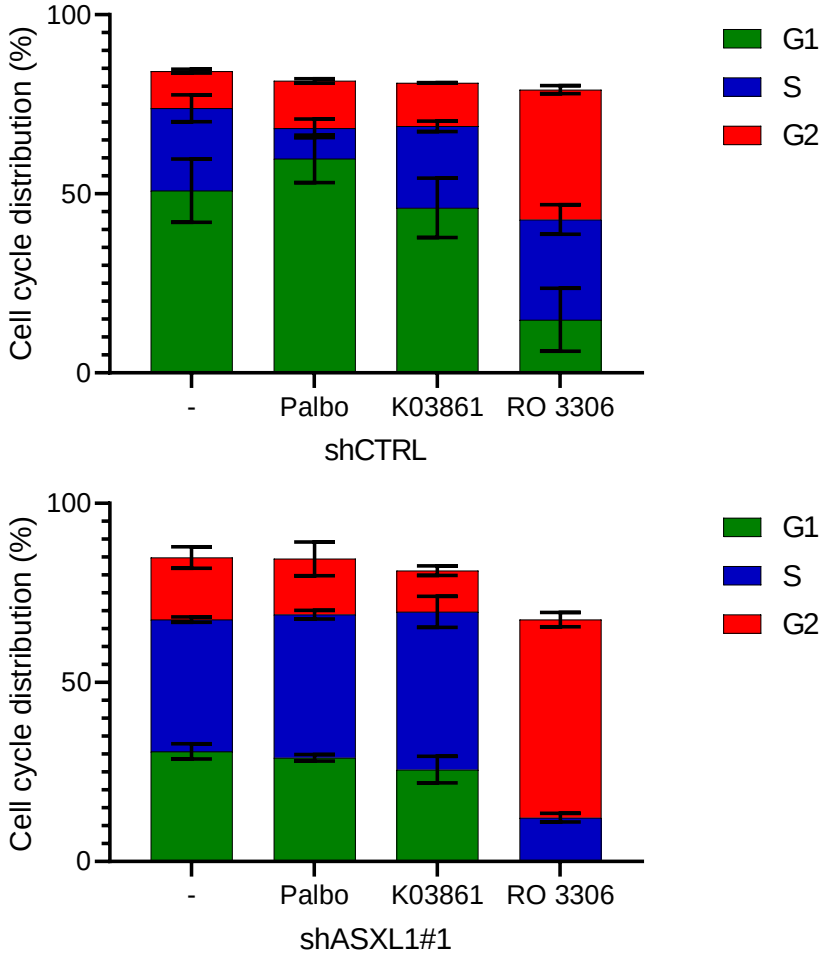






ASXL1 deficient cells are sensitive to CDK1/CCNA2 targeting





CDK2 inhibitor: K03861
CDK1 inhibitor: RO3306

Summary

Accelerated mutagenesis screening platform discovered a spectrum of novel mutations associated with resistance to CDK4/6 blockade

ASXL1 deficiency leads to robust induction of several cell cycle genes thus bypassing G1 arrest induced by cell cycle inhibitors

Knockdown of cyclin A or CDK1, but not CDK2, completely inhibited growth of ASXL1 deficient cells

CDK1 may present a clinically actionable vulnerability of ASXL1-deficient, CDK4/6i resistant cells

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