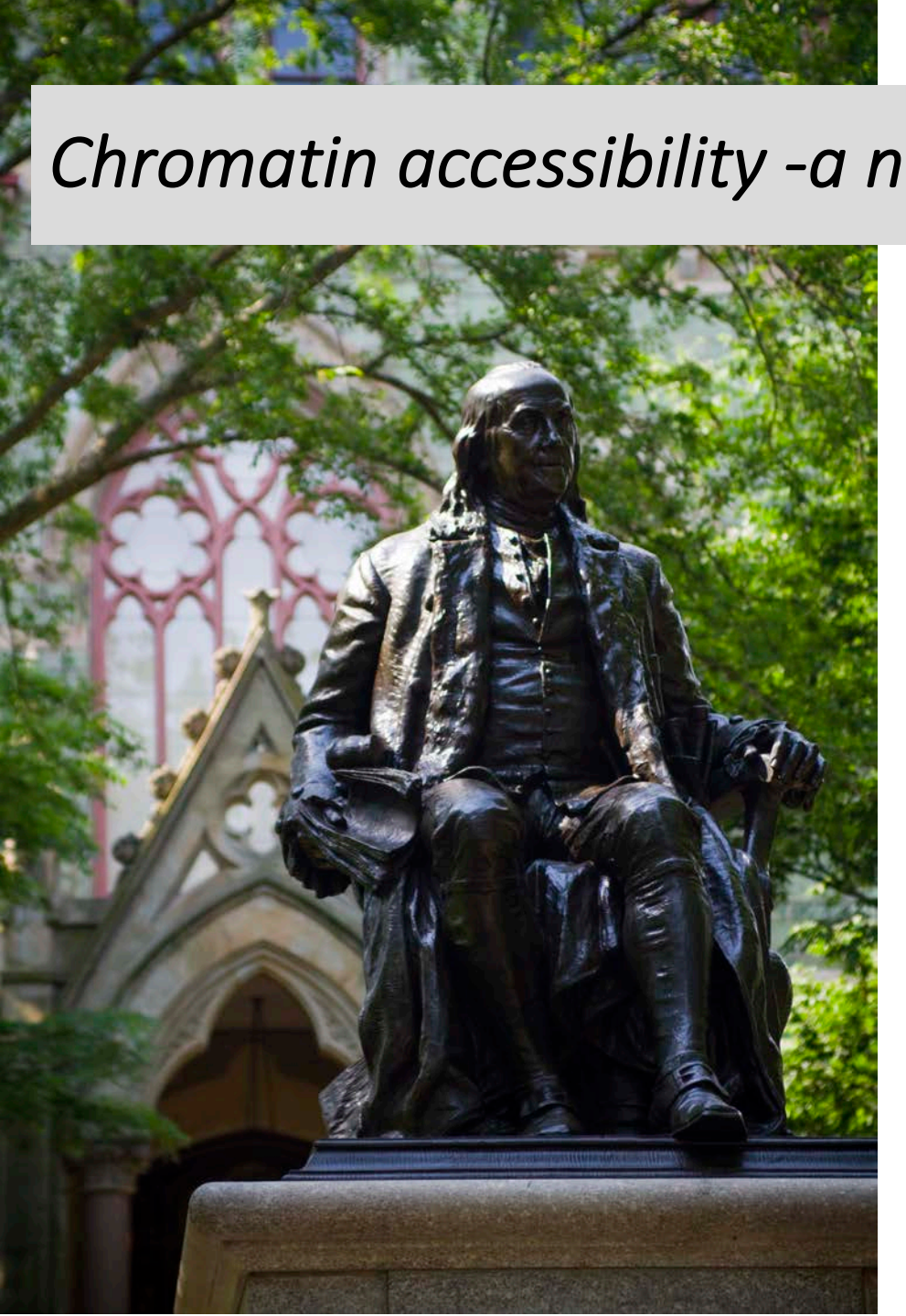
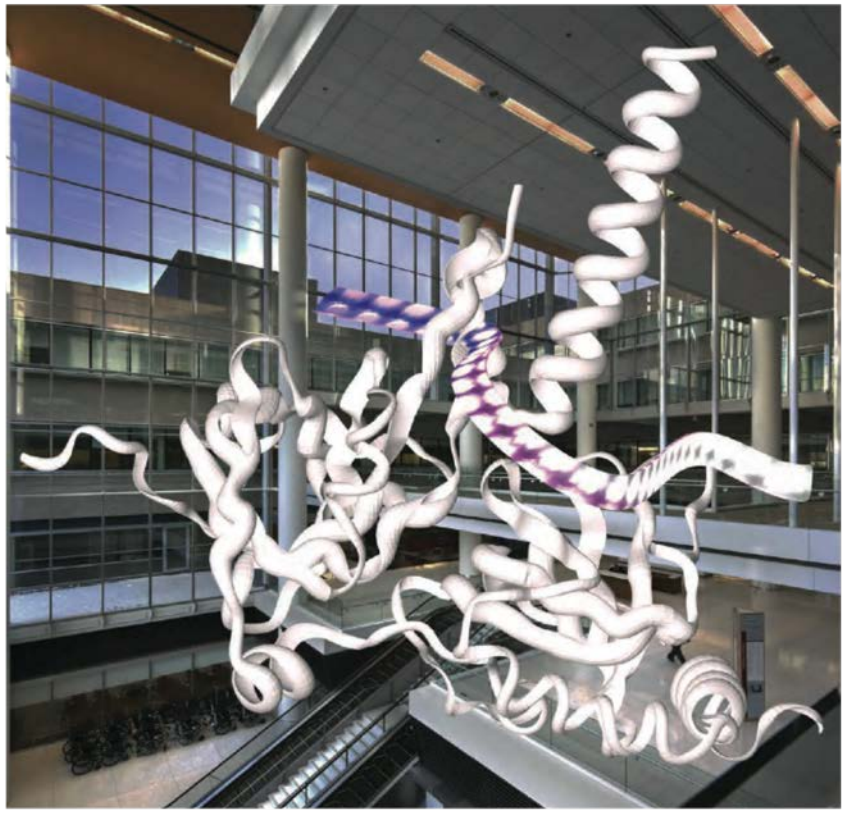
Chromatin accessibility -a new vulnerability in BRCA mutant cancers





Roger Greenberg

Penn Center for Genome Integrity

Basser Center for BRCA

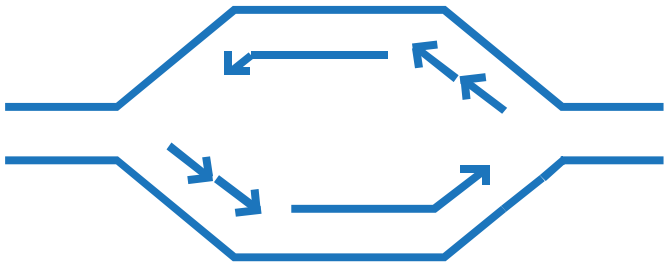
University of Pennsylvania

Priyanka Verma

Junwei Shi

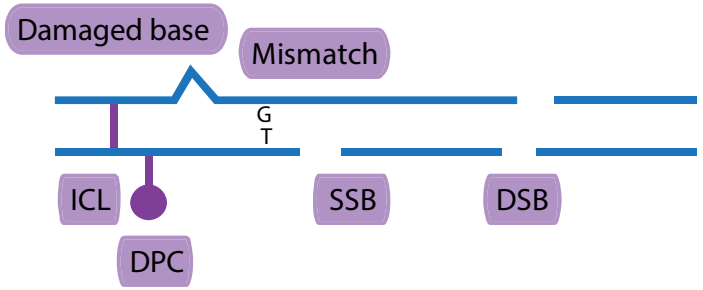
Challenges to DNA replication

DNA replication

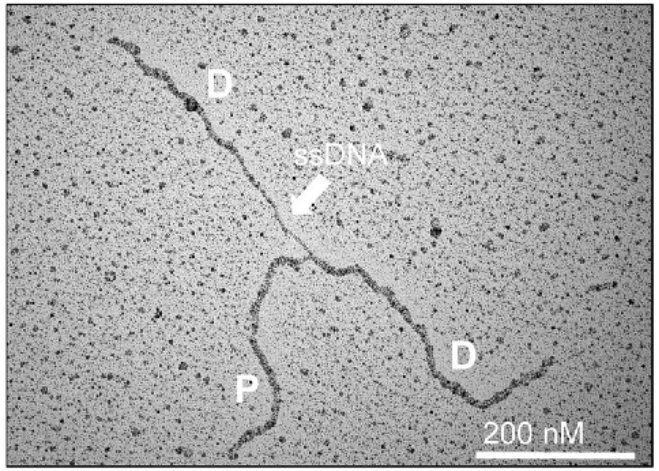


S-phase

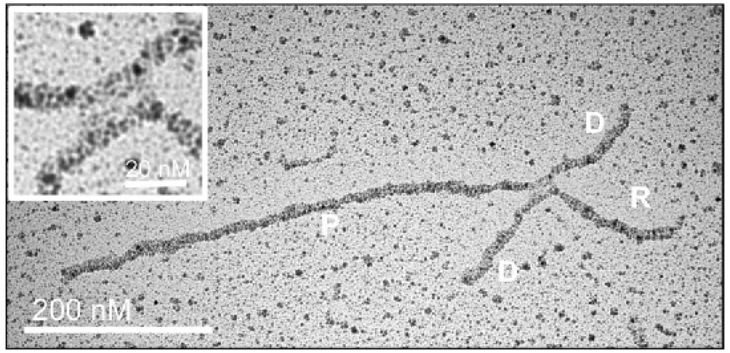
DNA damage



Replication fork with ssDNA at the junction



Reversed replication fork

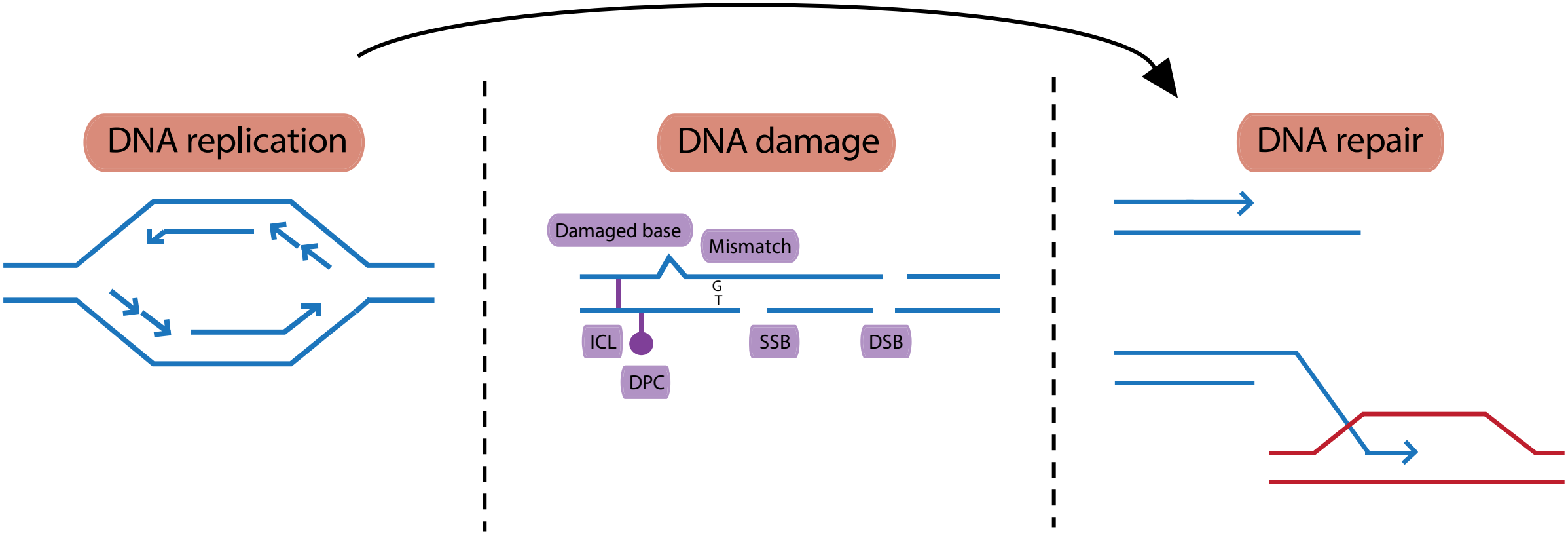


-Ribonucleotide incorporation ~10^6 per day

-Base alkylation, abasic sites, single stranded breaks >10,000 per day

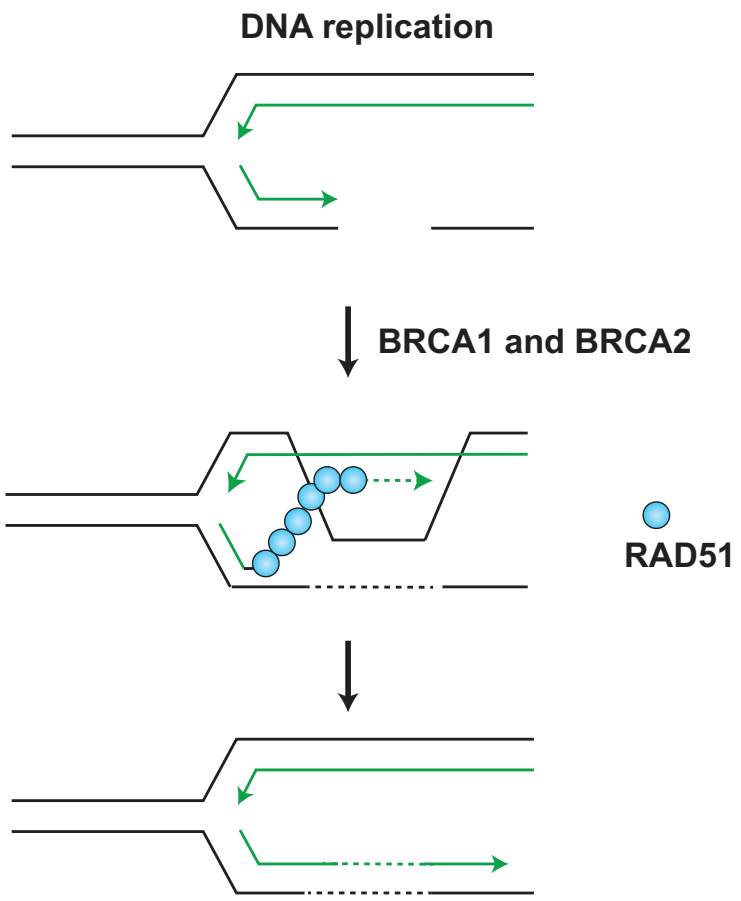
Vindigni & Lopes, 2017

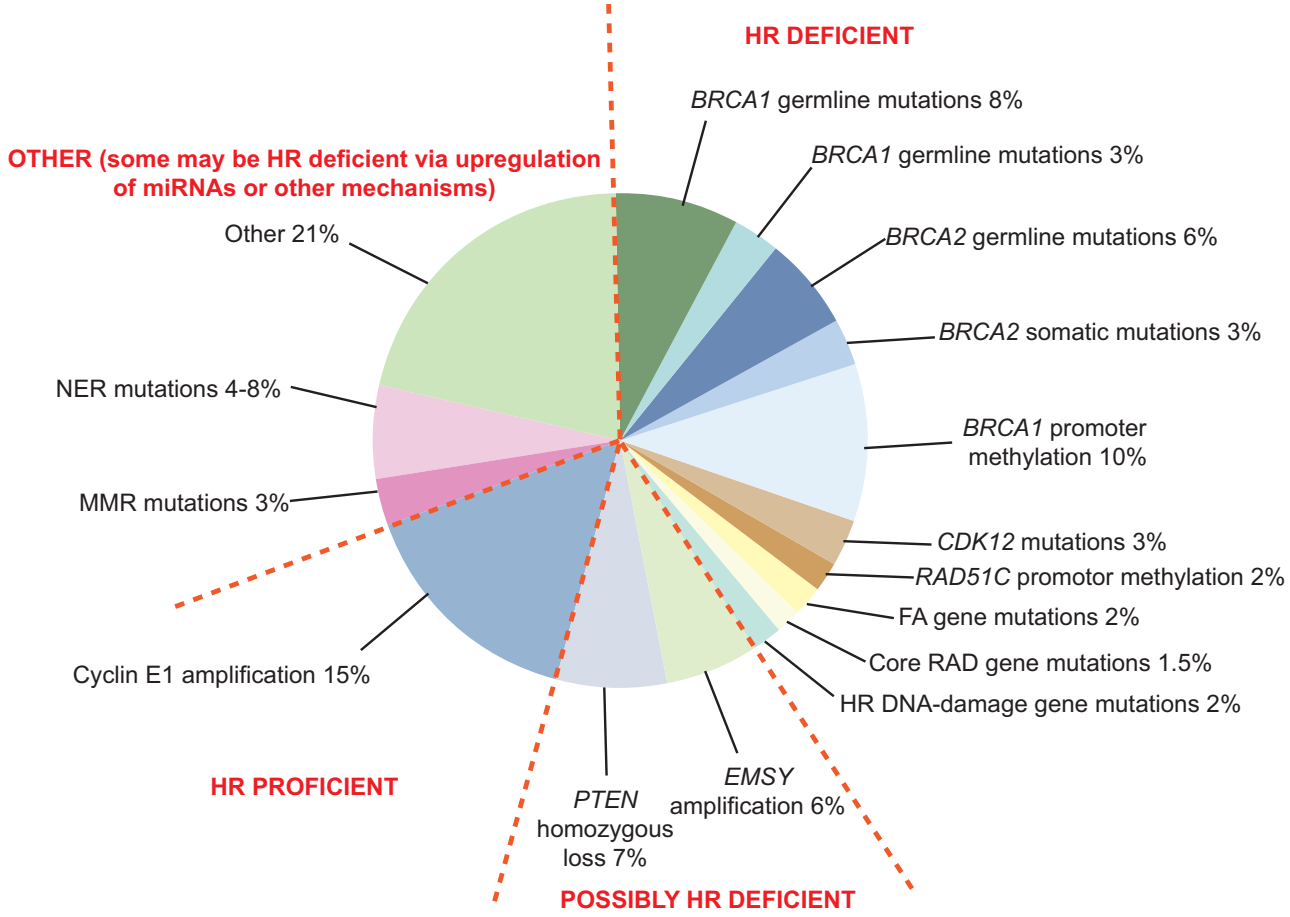
Challenges to DNA replication



The BRCA Tumor Suppressor Network

HOMOLOGOUS RECOMBINATION (HR)





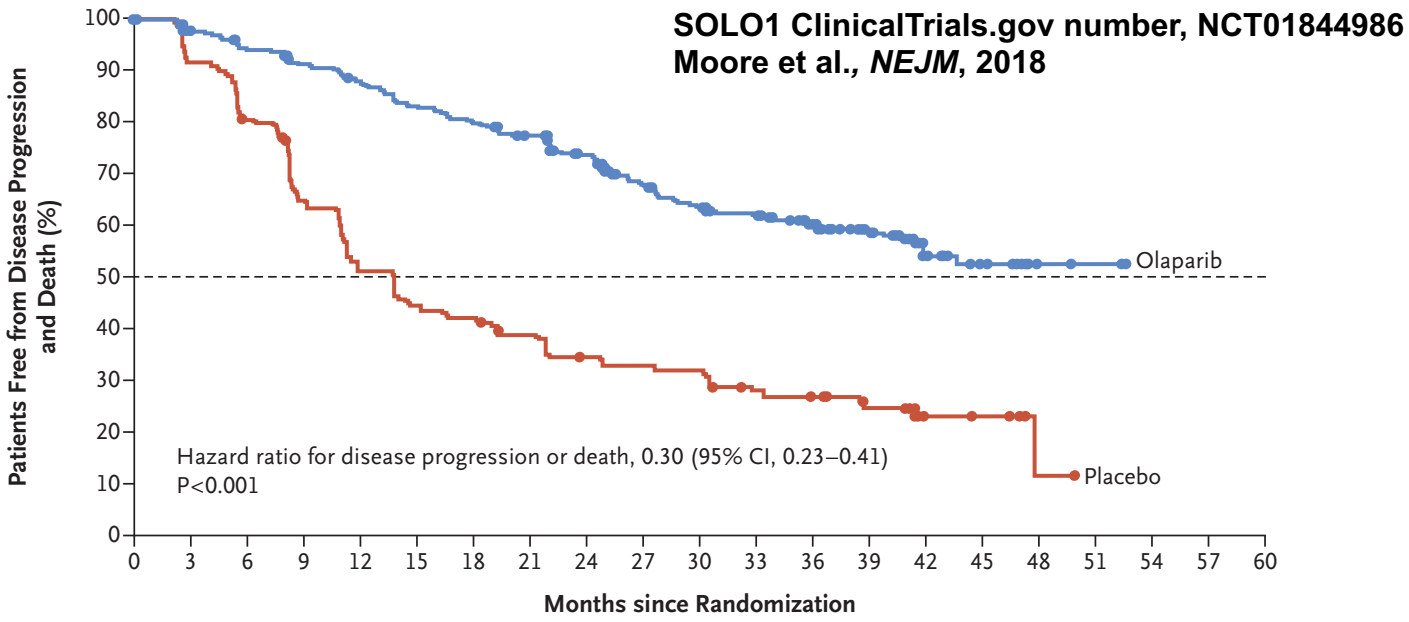
Vulnerabilities in the DNA Damage Response

"Synthetic lethality arises when a combination of mutations in two or more genes leads to cell death, whereas a mutation in only one of these genes does not, and by itself is said to be viable"

Hartman, Garvik, Hartwell Science 2001

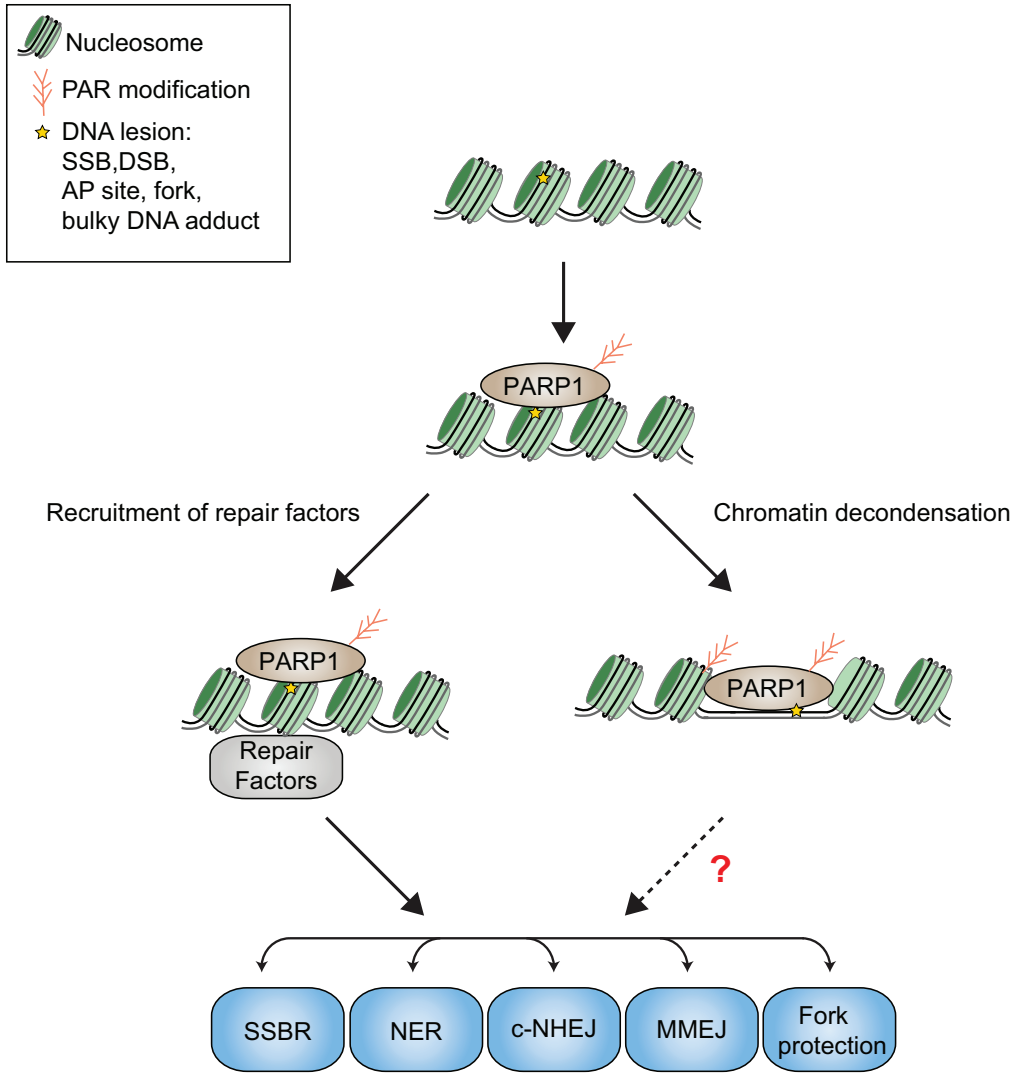
BRCA mutation carrier +/- HR intact
BRCA mutant tumor -/- HR deficient

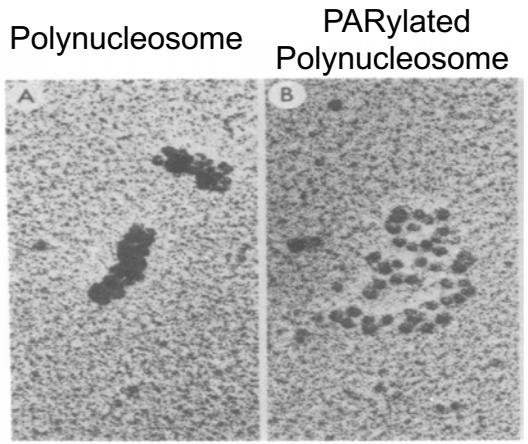
PARP inhibitors kill BRCA-/- >100X BRCA+/-



- 4 FDA approved PARPi for HR deficient ovarian, breast, prostate Ca, active in pancreatic
- ~50% of patients don't respond; acquired resistance in all patients
- ***Need for agents that overcome resistance.***

Contributions of PARP to DNA repair





Poirier *et al*, PNAS,1982
Lee Krauss and others

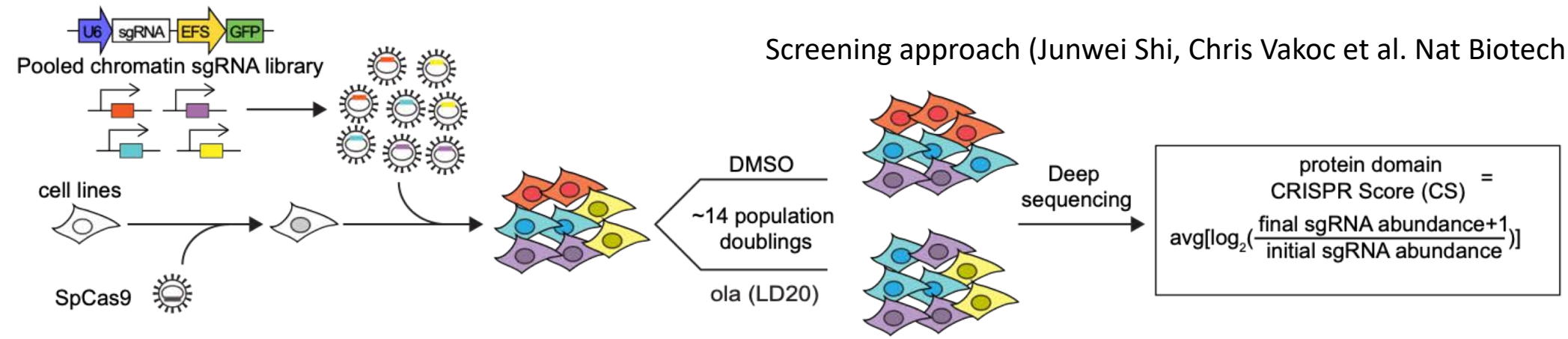
Is PARP dependent chromatin remodeling required for viability of BRCA mutant cells?

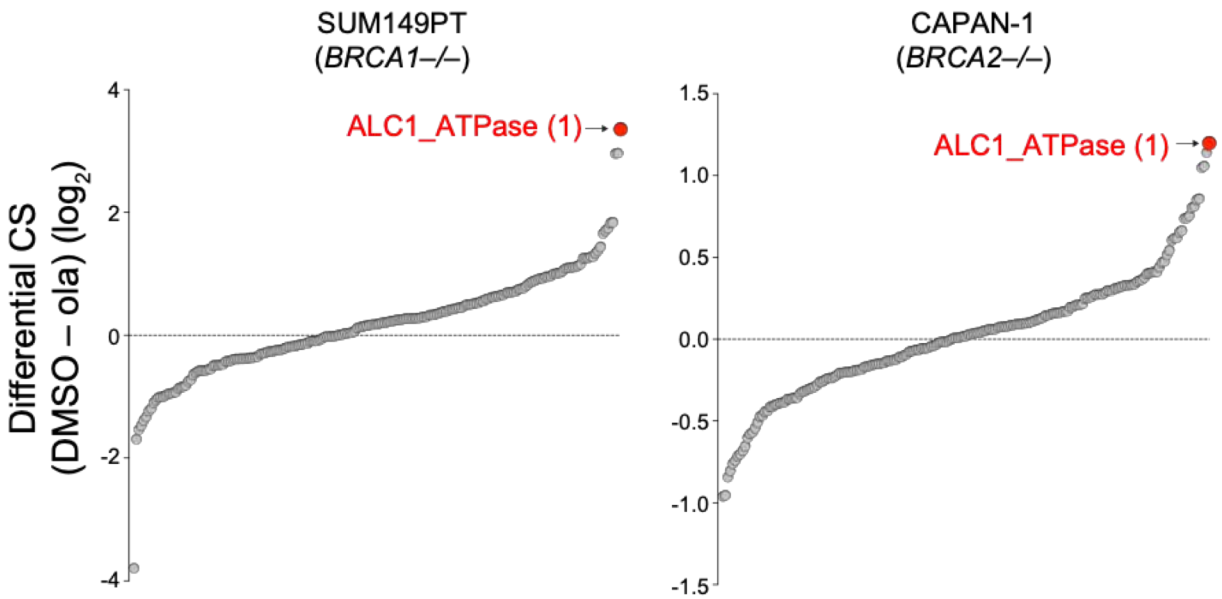
Can it be targeted to enhance PARPi respones and overcome resistance?

What are the endogenous lesions that PARP repairs to sustain viability in BRCA mutant cells?

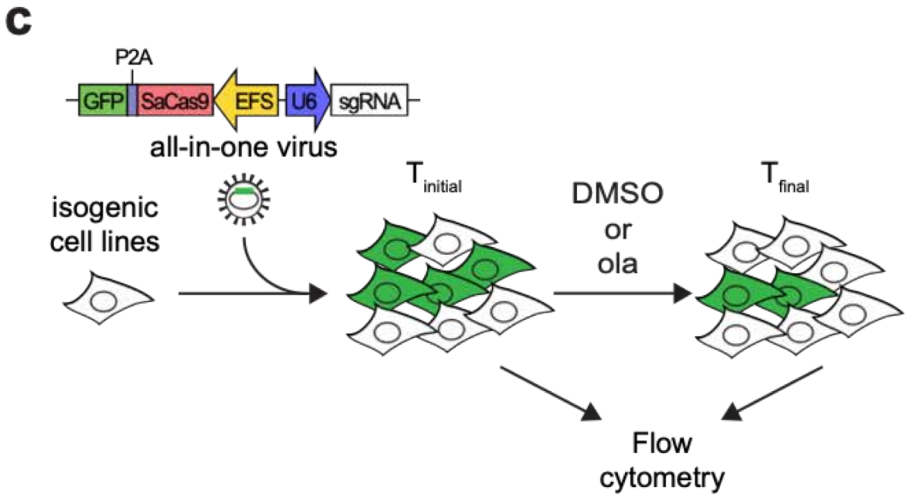
Search for chromatin vulnerabilities in BRCA-mutant cancers

Screening approach (Junwei Shi, Chris Vakoc et al. Nat Biotech 2015)

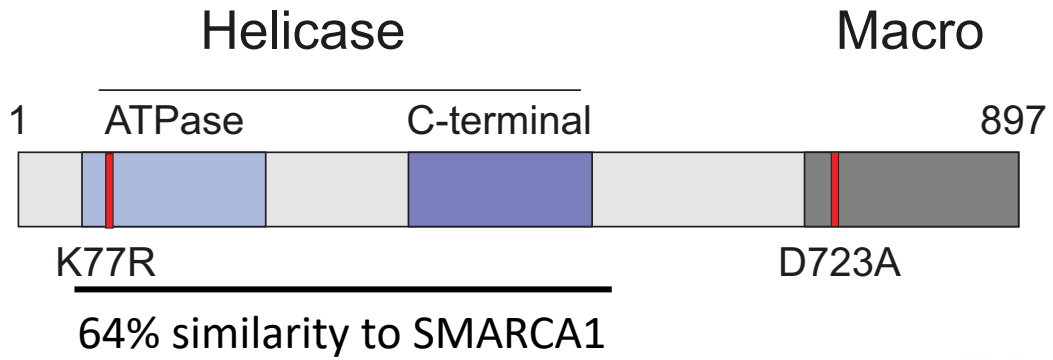


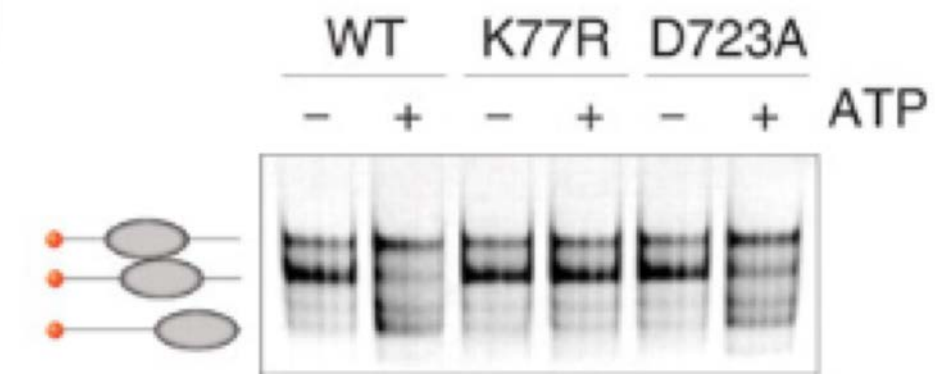


197 protein domains ranked by differential senstivity to ola

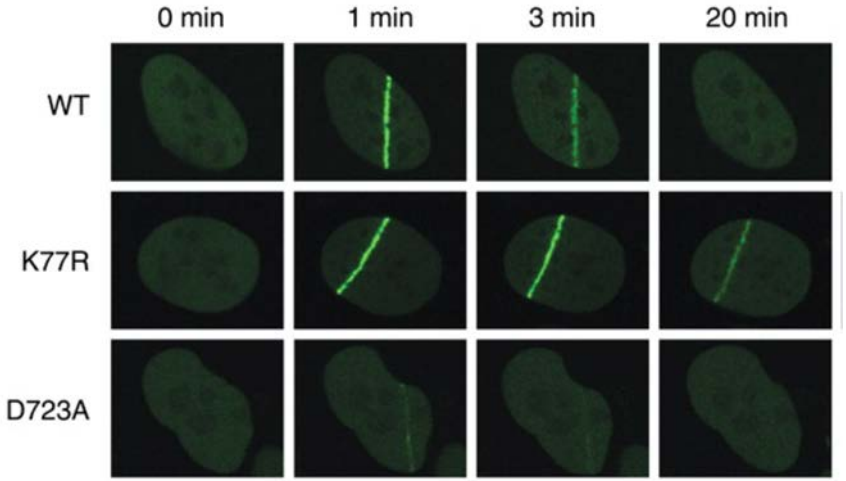


ALC1/CHD1L (Amplified in Liver Cancer1)





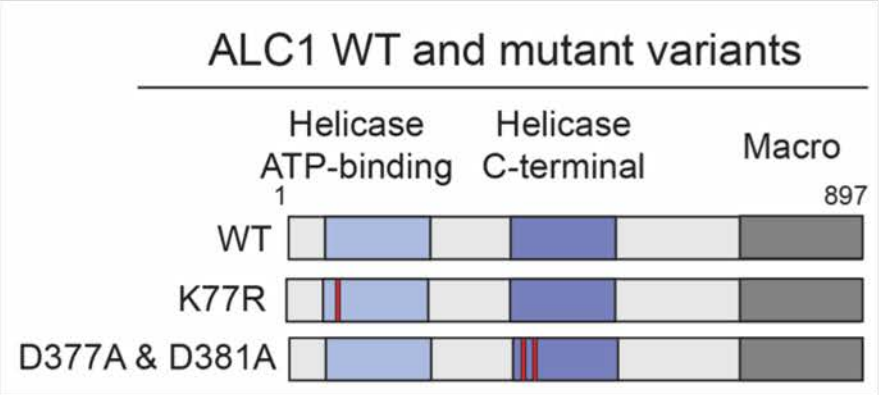
- **ATPase dependent nucleosome sliding**
- **Enhanced by PARP1 and macrodomain**



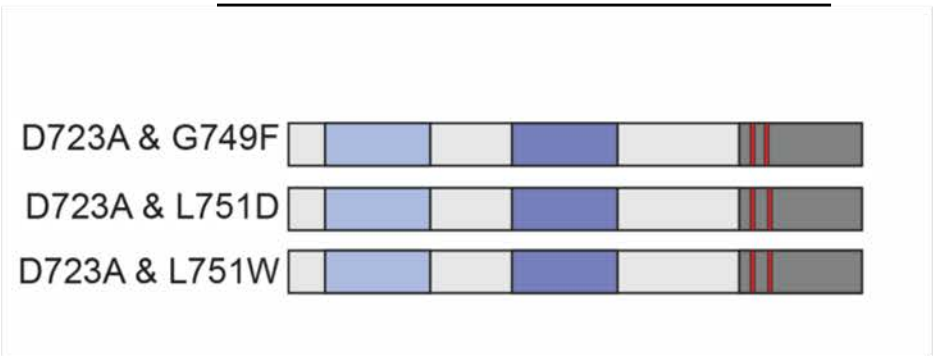
- **Macro domain damage recruitment**
- **Kd 10nM for tri-ADP-ribose**

Ahel, Boutlon et al., Science, 2009
Gottschalk, Connaways et al., PNAS, 2009
Lehmann, Deindl et al. Mol Cell 2019
Singh, Ladurner et al. Mol Cel 2019

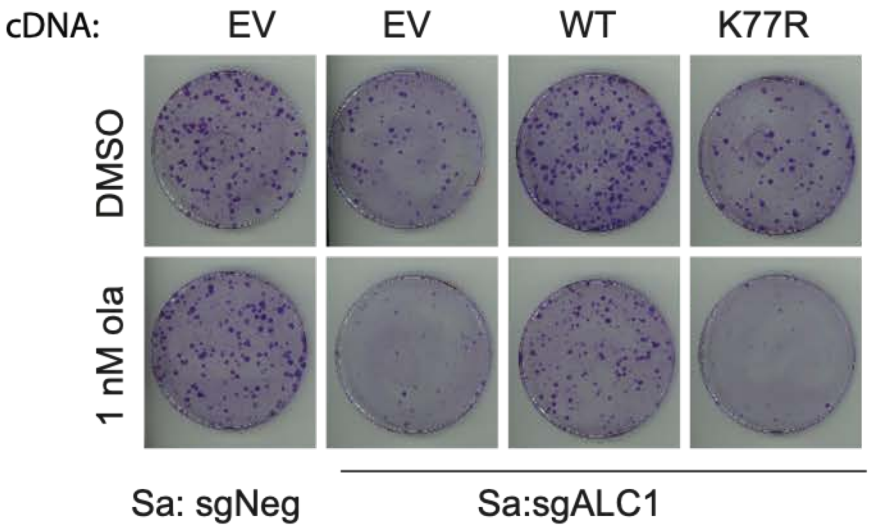
Chromatin remodeling and PAR-binding are required for PARPi response



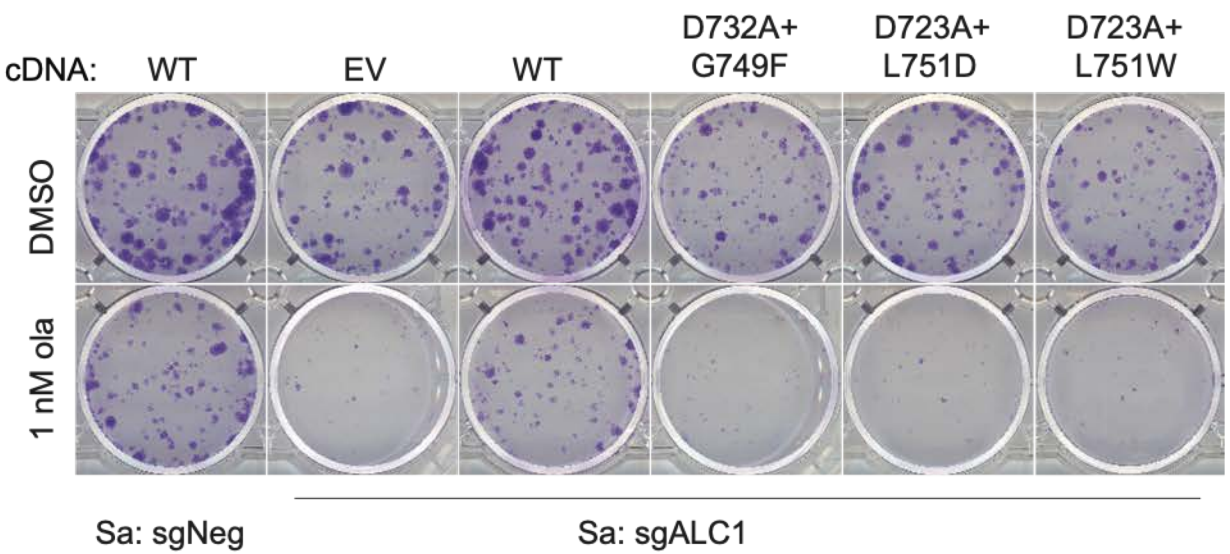
ALC1 WT and Macrodomain mutants



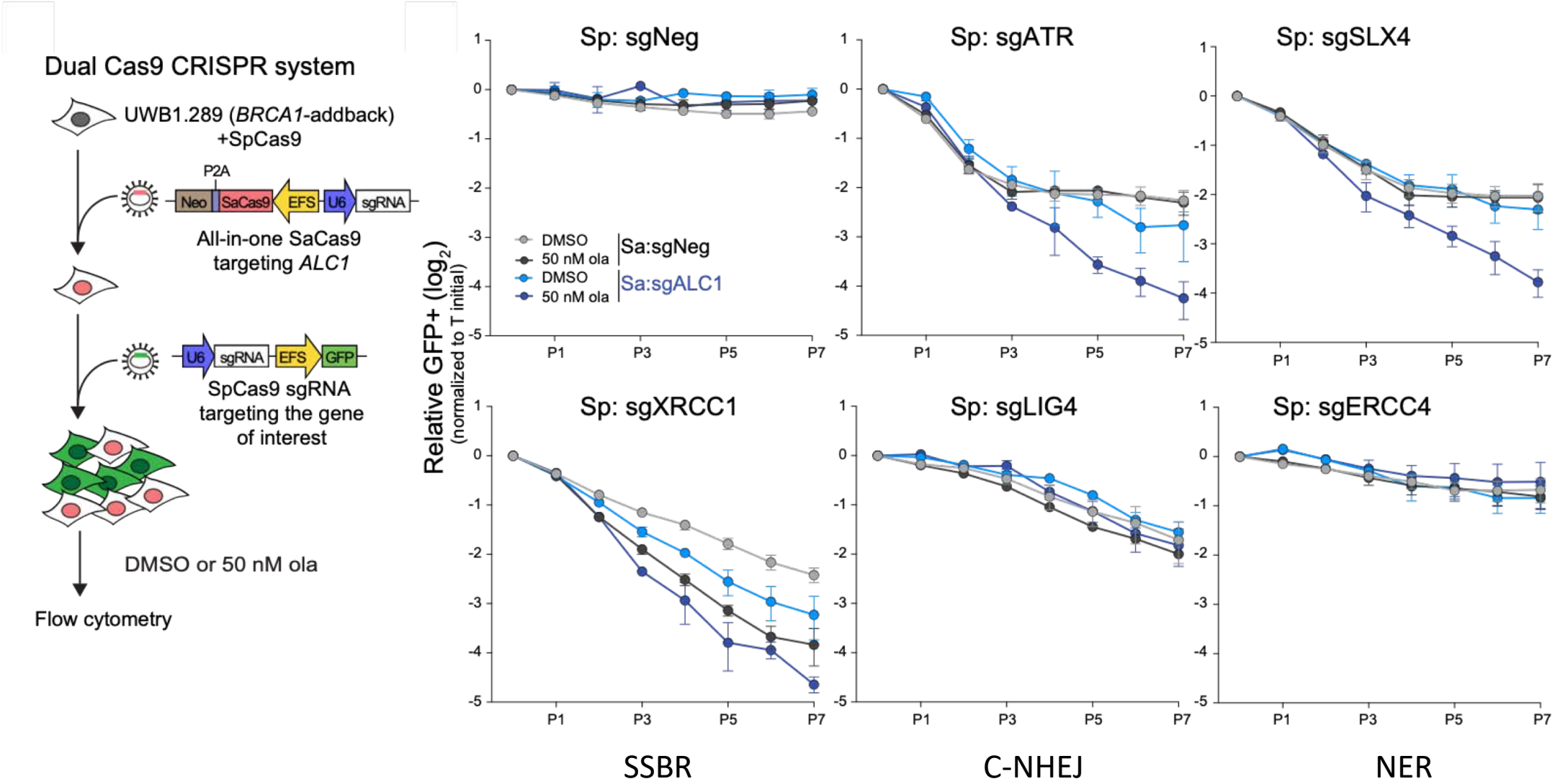
hTERT-RPE1 (*BRCA1–/–*)



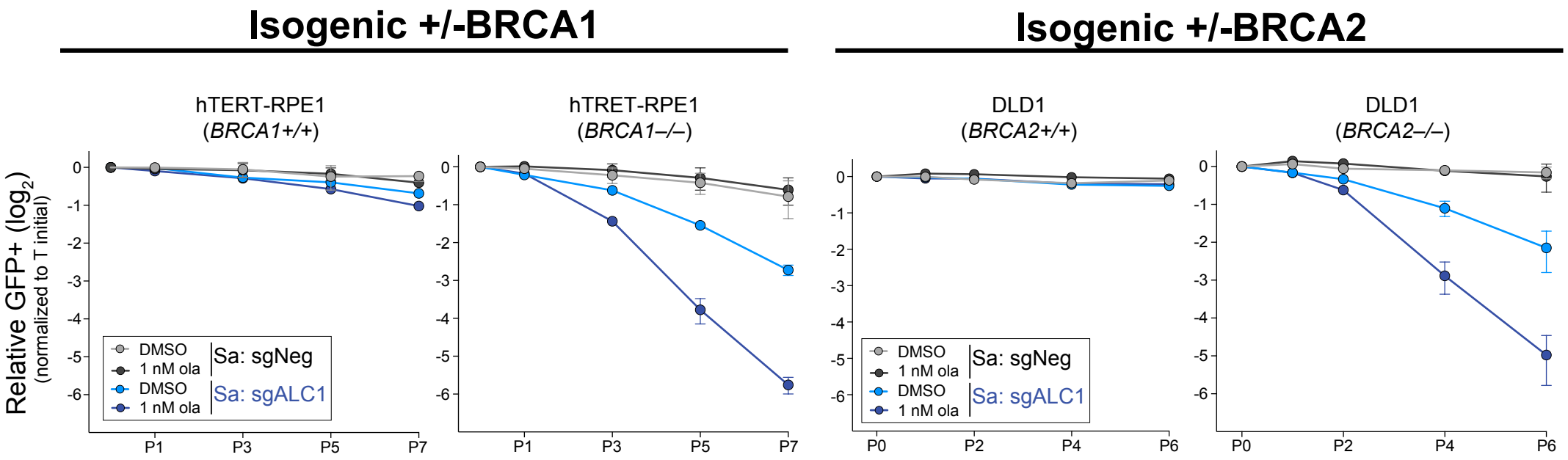
SUM149PT (*BRCA1–/–*)



ALC1 loss selectively sensitizes HR and SSBR defective cells to PARPi

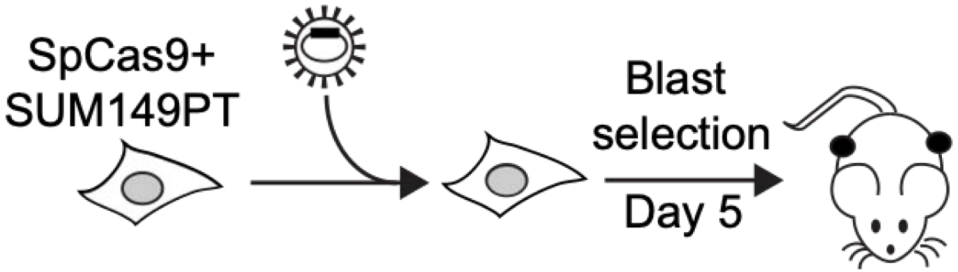


ALC1 deficiency enhances PARPi therapeutic window

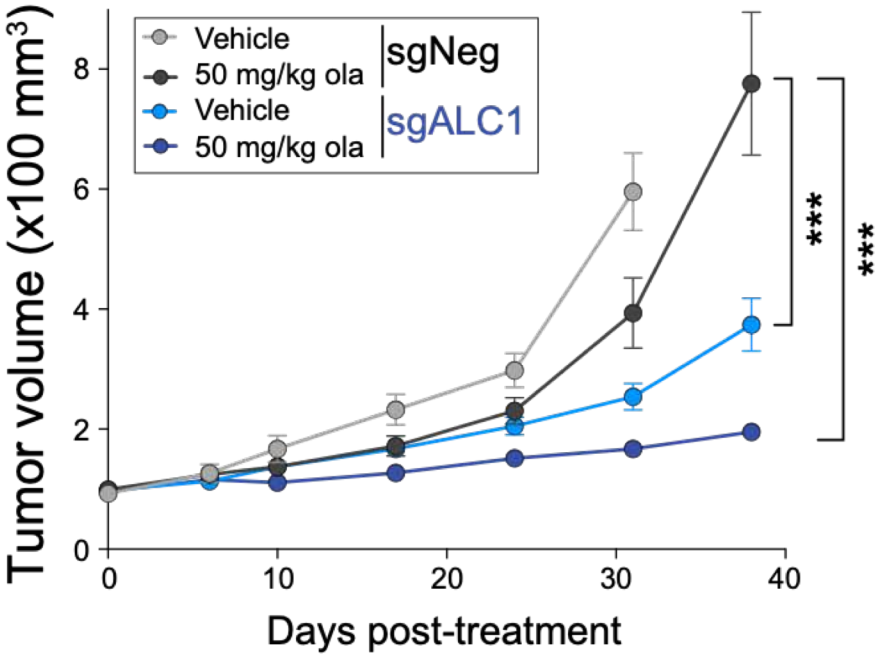


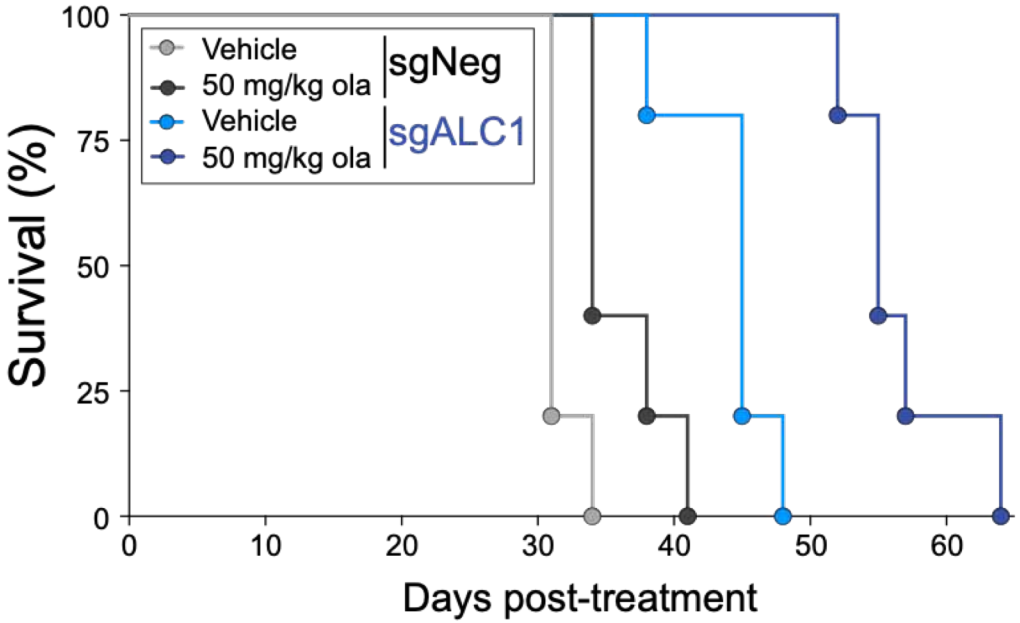
- *ALC1 loss caused up to 250 fold increases in PARPi sensitivity in BRCA mutant cells (Cell titre Glo assay)*
- *ALC1 does not affect responses to cisplatin in BRCA wildtype or null cells*

ALC1 loss enhances PARPi response in BRCA mutant tumors

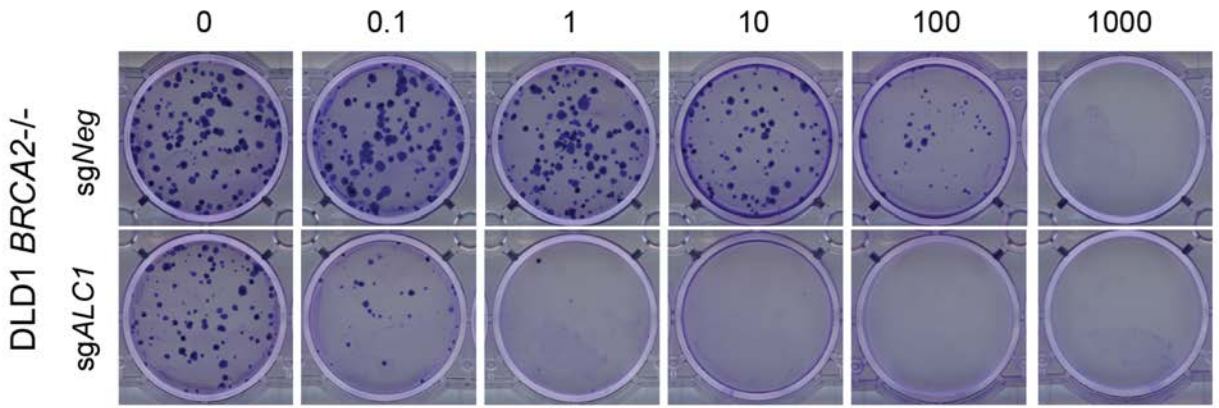


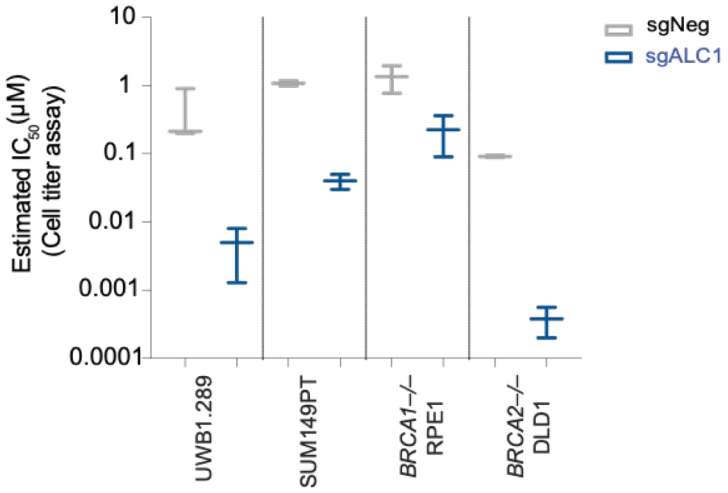
- SUM149PT BRCA1 mutant TNBC
- Olaparib 50mg/kg oral gavage 5 days/ week

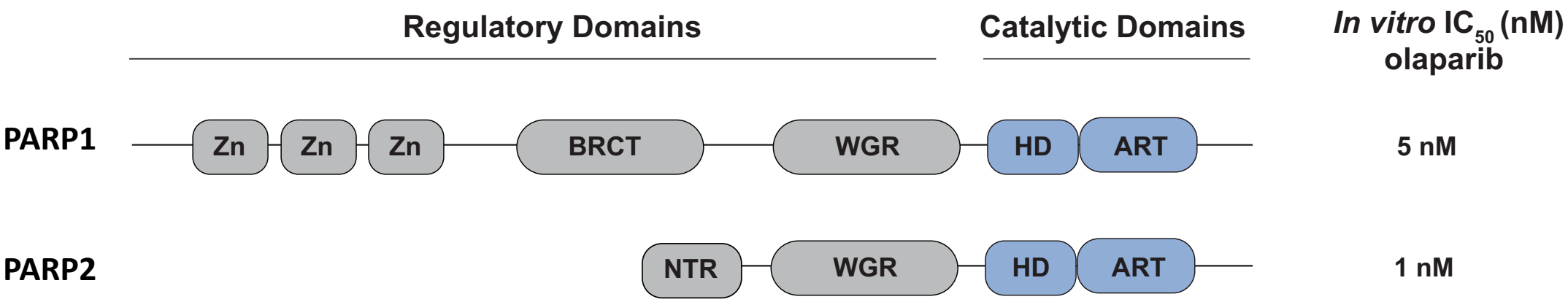




ALC1 deficiency allows PARPi efficacy at very low doses In HR-deficient cells

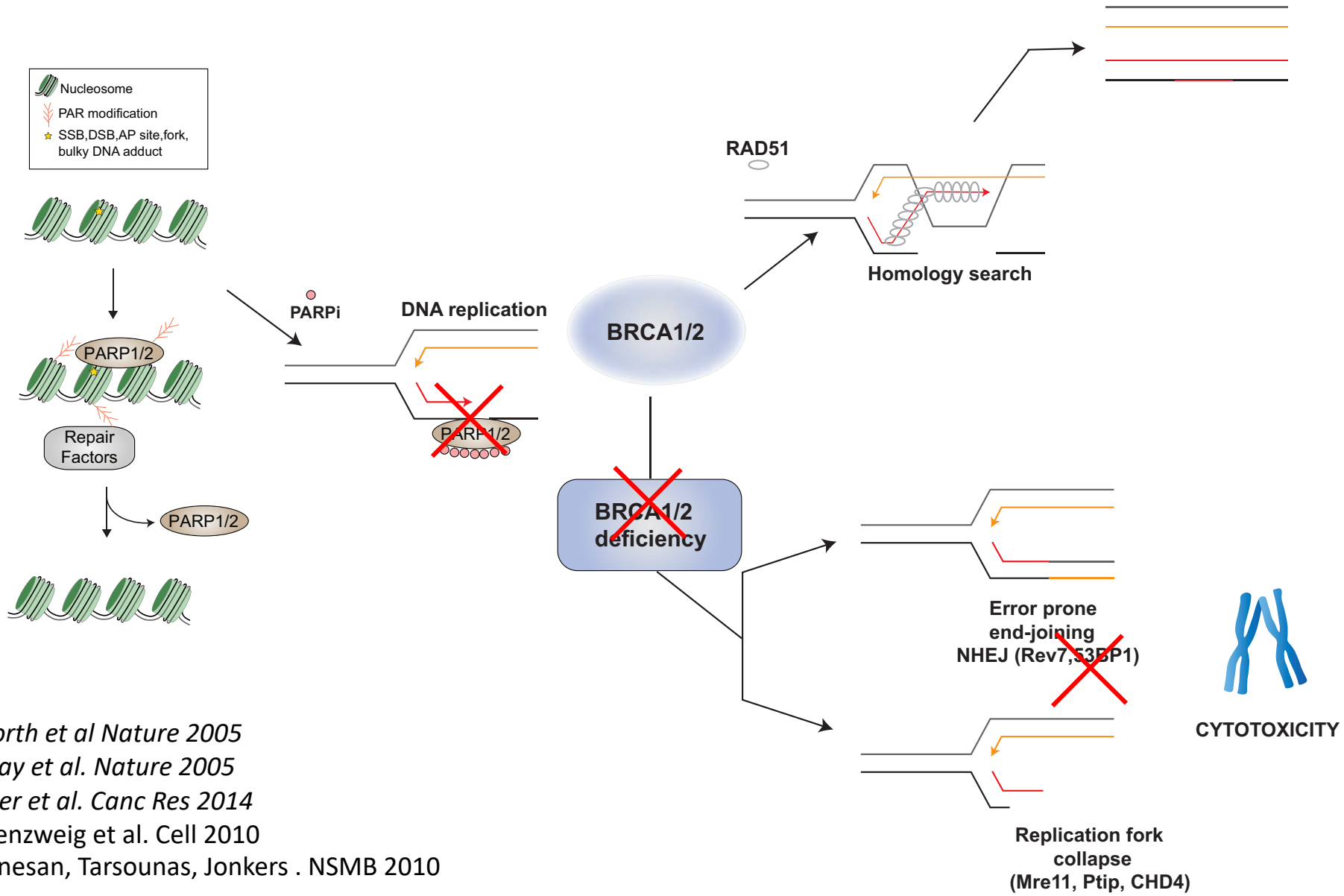






IC50 (nM) of ola in BRCA mutant cells ~10-100nM

Framework for understanding PARP inhibitor response

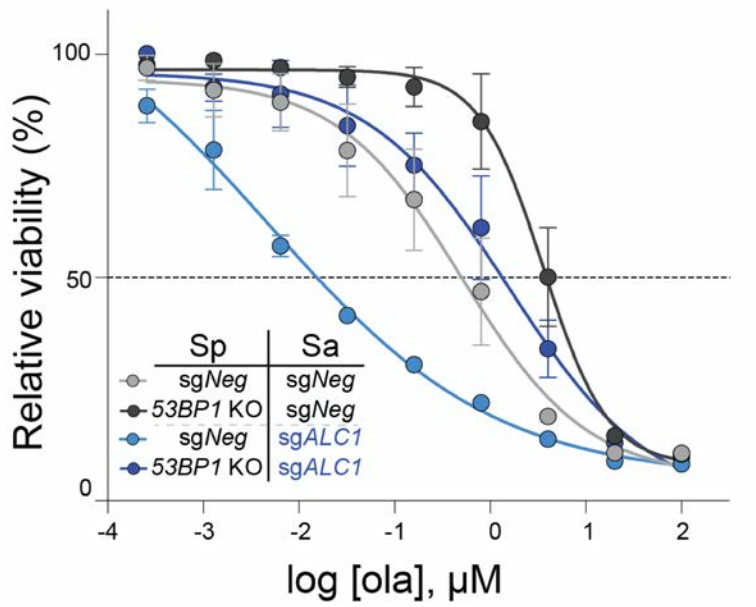


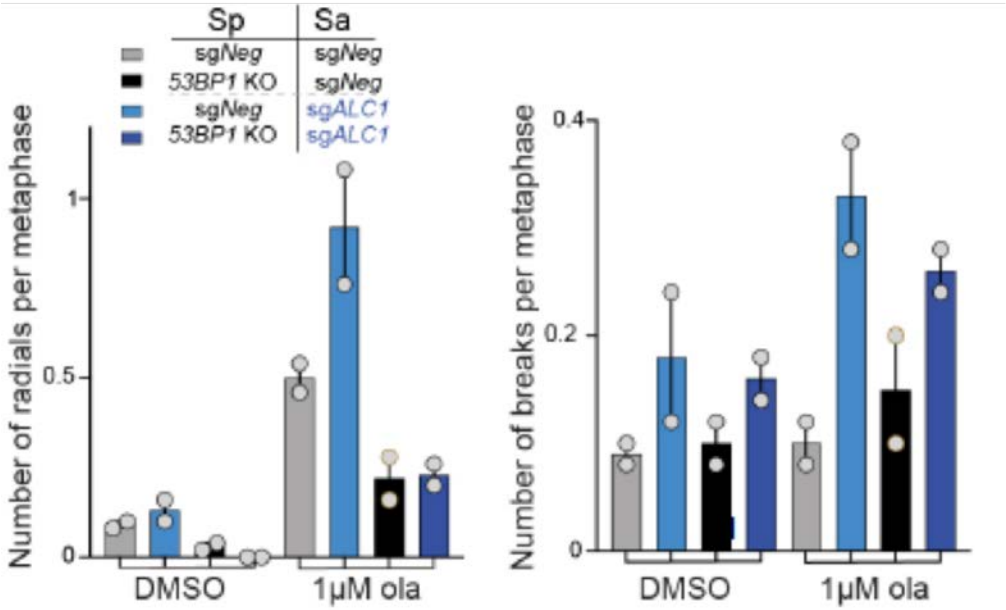
Farmer, Ashworth et al Nature 2005
Bryant, Helleday et al. Nature 2005
Murai, Pommier et al. Canc Res 2014
Bunting, Nussenzweig et al. Cell 2010
Bouwman, Ganesan, Tarsounas, Jonkers . NSMB 2010

ALC1 loss maintains PARPi sensitivity in cells with engineered resistance

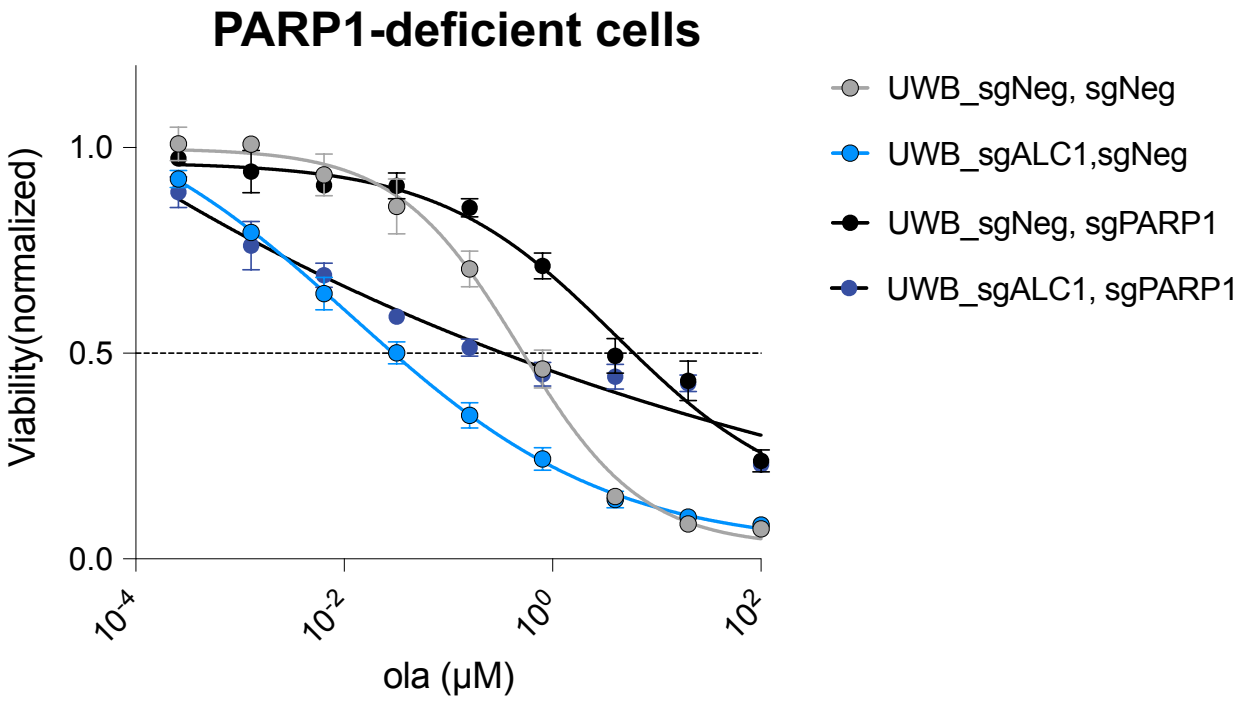
UWB1.289 BRCA1 mutant

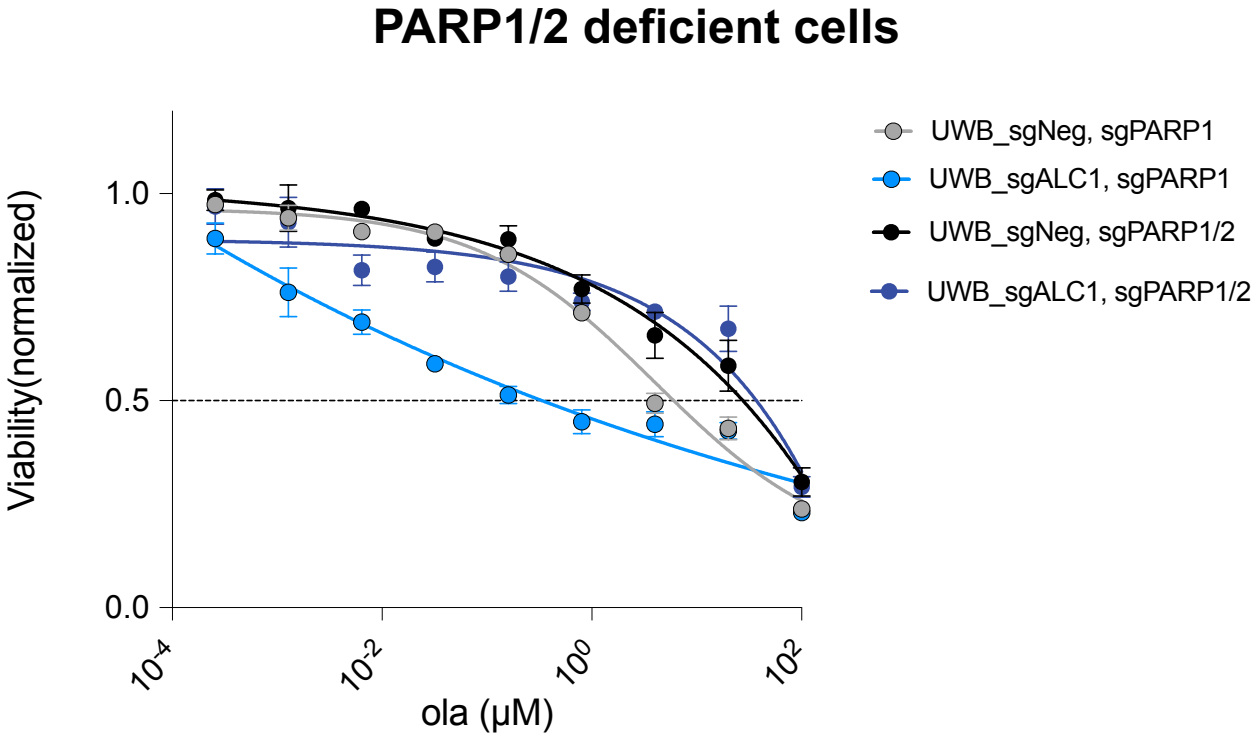
53BP1-Shieldin deficient





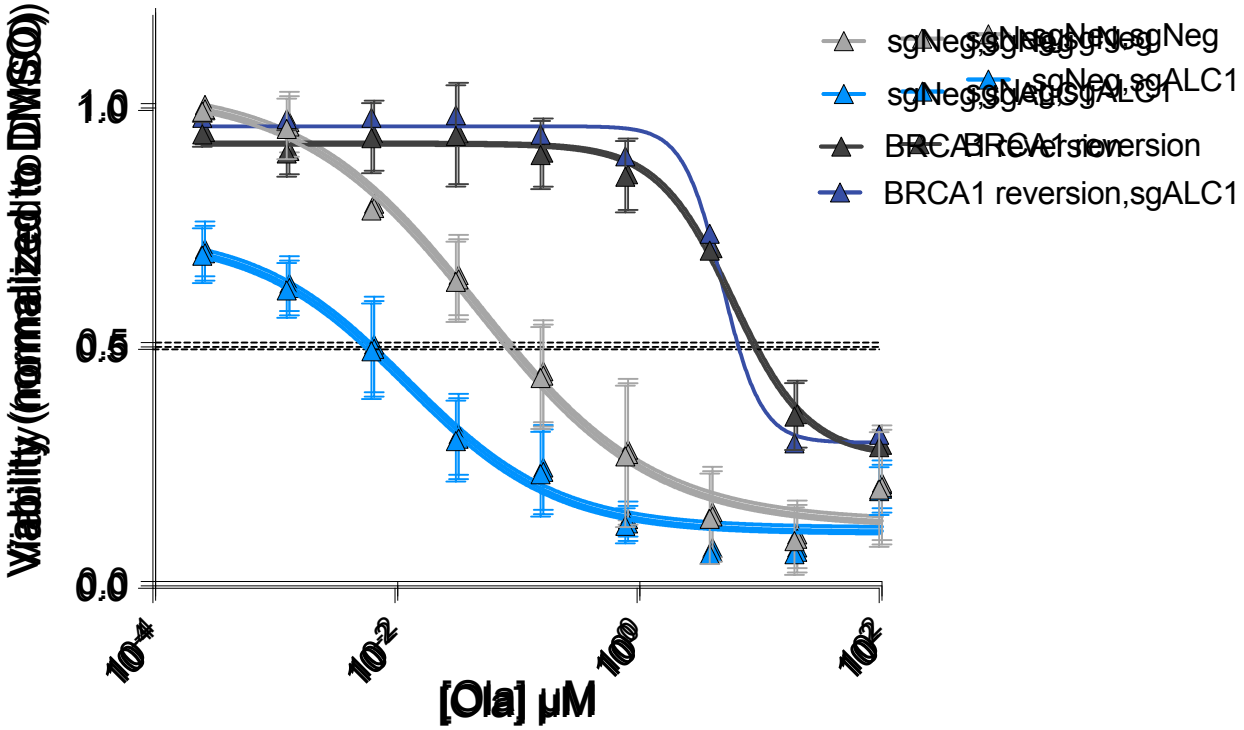
ALC1 loss exerts toxicity through PARP1 and PARP2 trapping

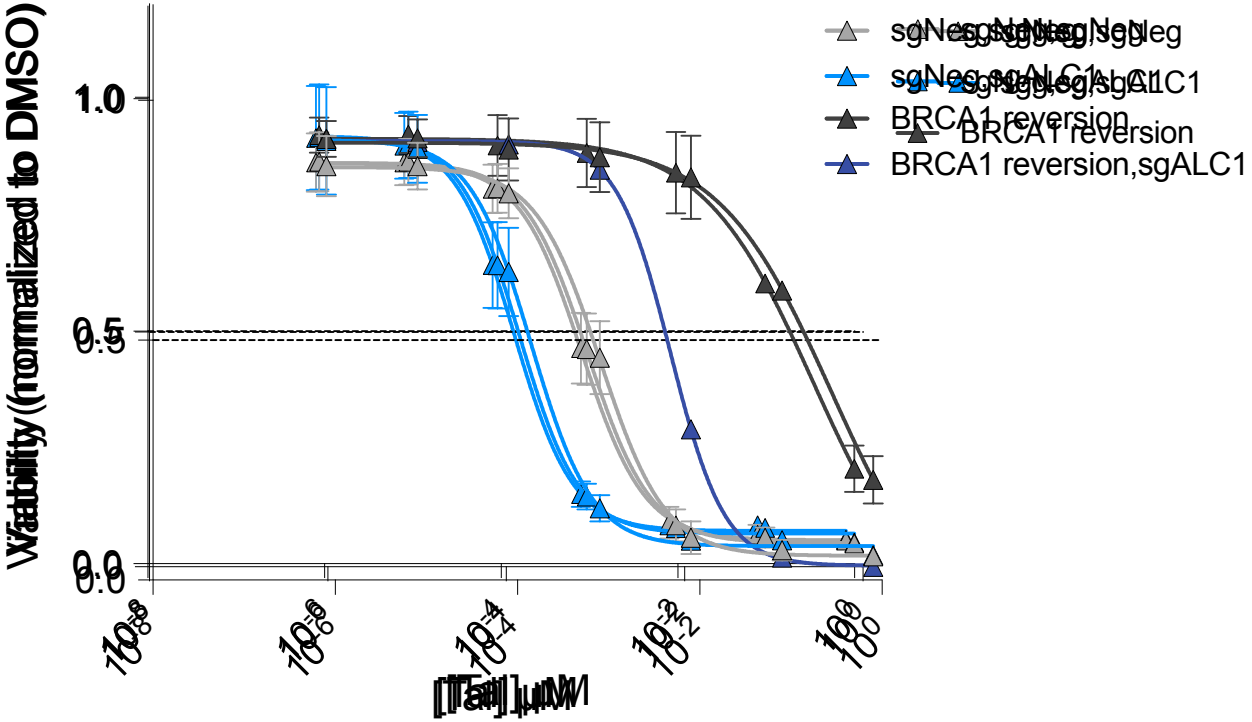




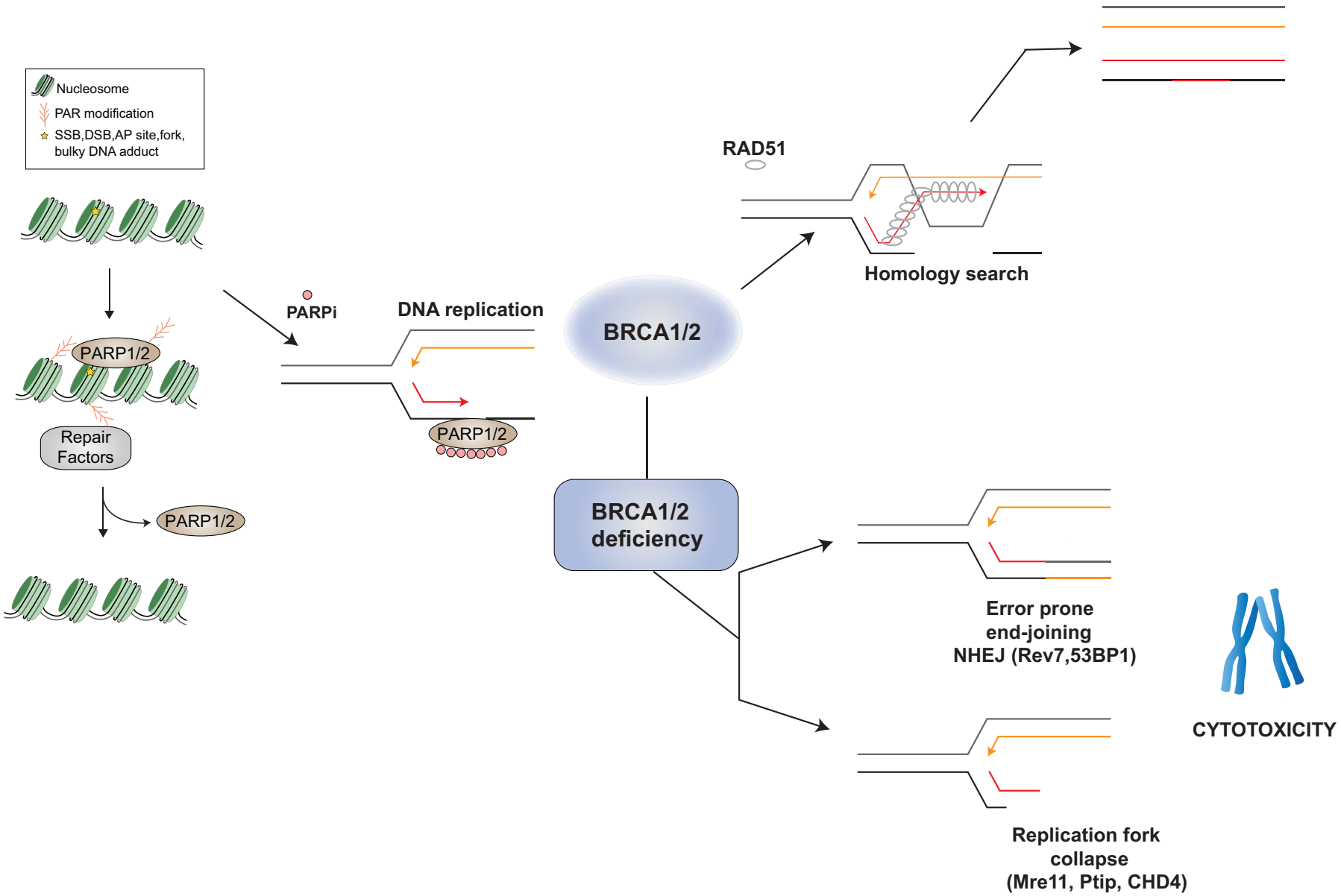
PARPi sensitivity upon ALC1 loss is reliant on the HR status of cancer cells

(Wang, Johnson 2016)

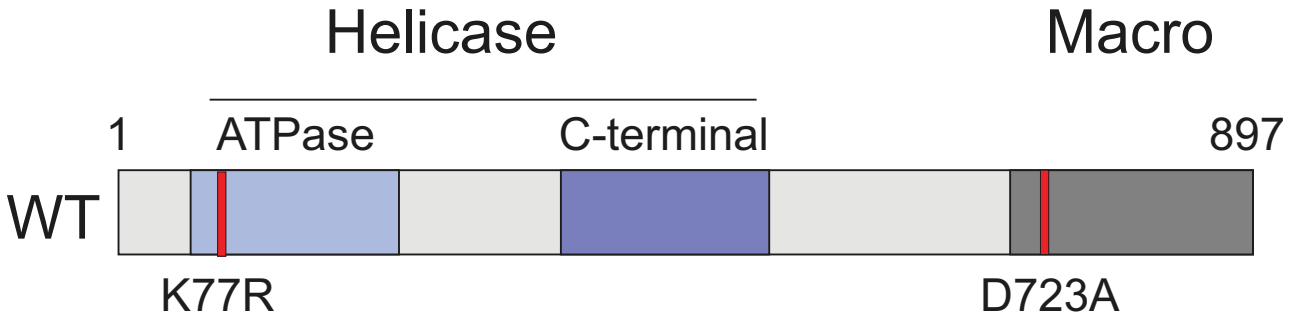




Framework for understanding PARP inhibitor response



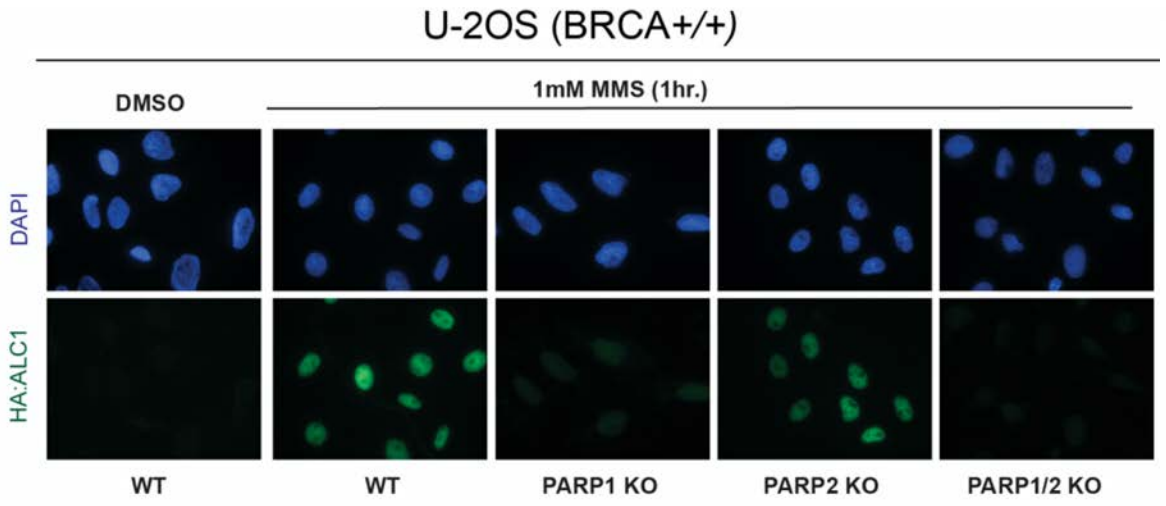
How does a loss of a PAR dependent chromatin remodeler cause hypersensitivity to PARPi?

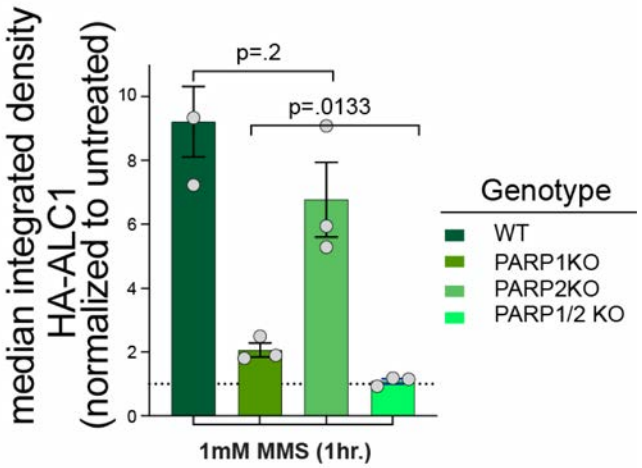


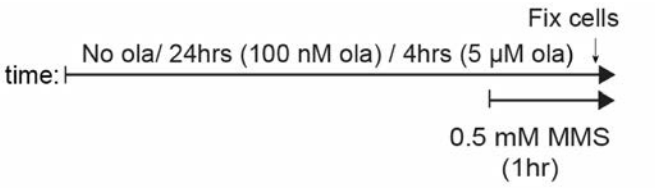
- Macrodomain binds tri-ADP ribose with Kd of ~10nM
- ATP dependent nucleosome sliding requires PAR binding

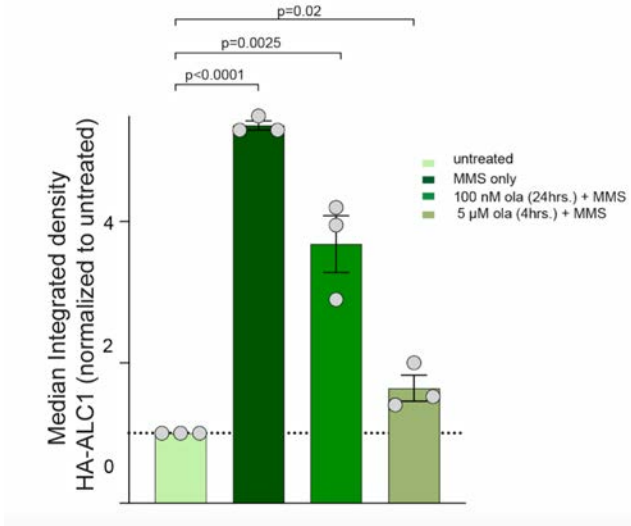
Ahel, Boutlon et al., Science, 2009
Gottschalk, Connaways et al., PNAS, 2009
Lehmann, Deindl et al. Mol Cell 2019
Singh, Ladurner et al. Mol Cel 2019

Is PARPi equivalent to combined loss of PARP1 and PARP2



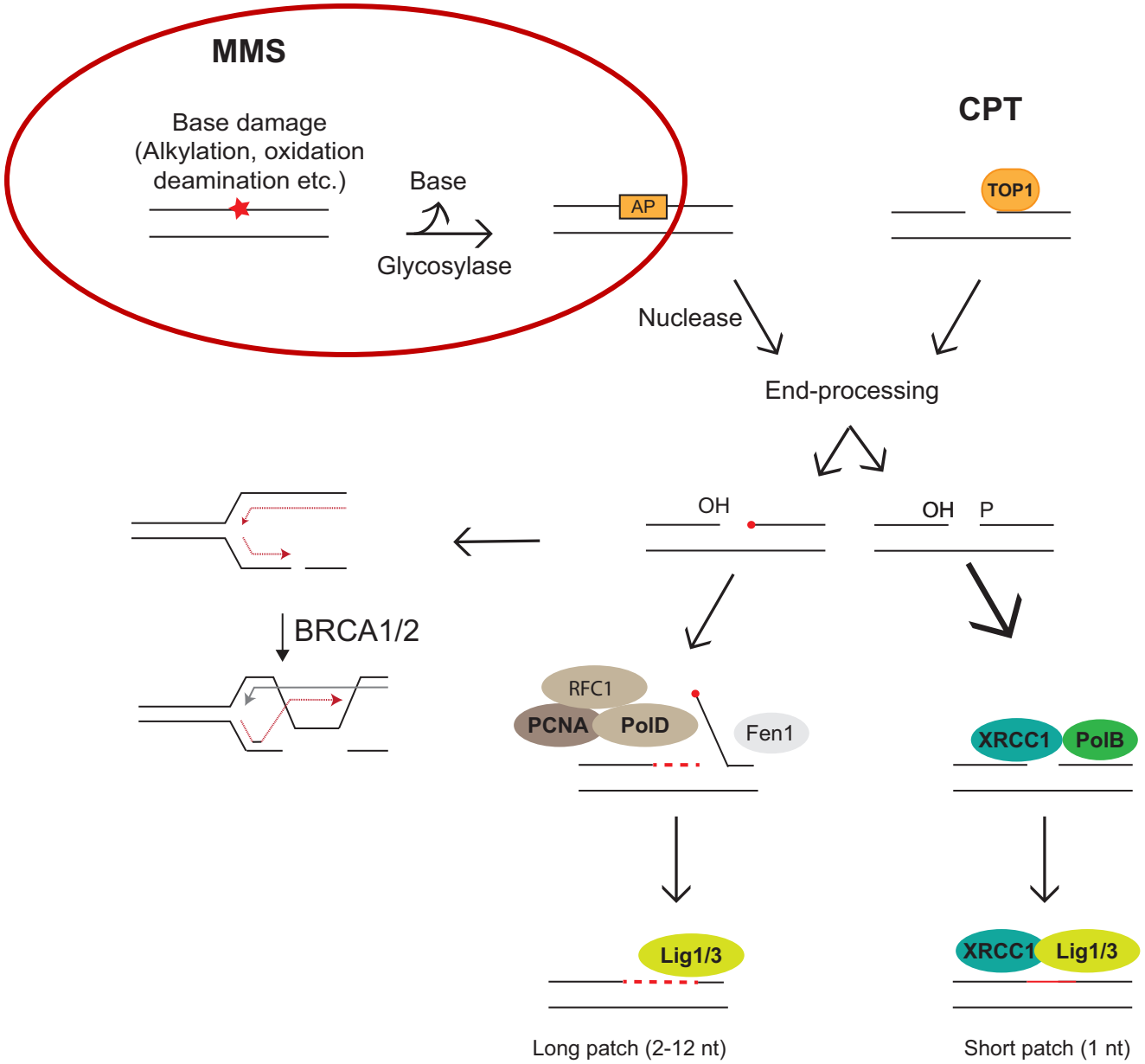


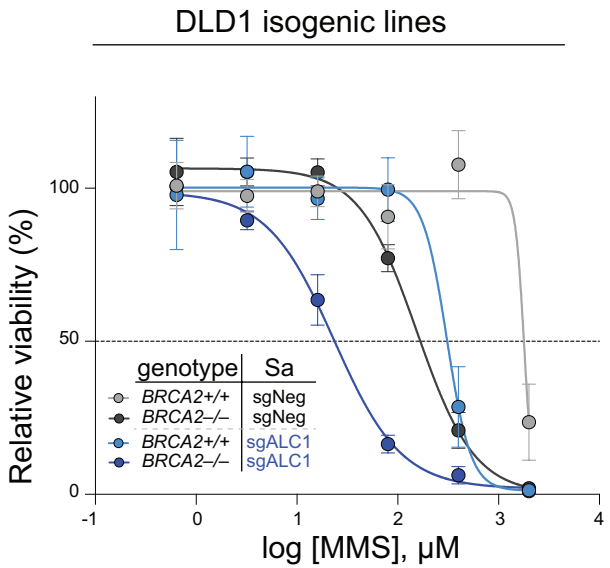


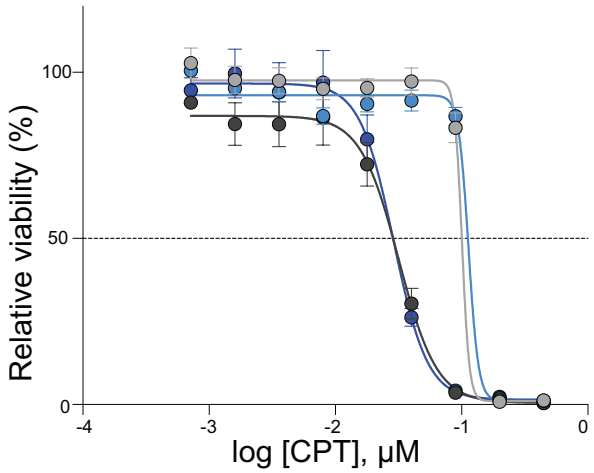


- Required 20uM olaparib to eliminate ALC1 from chromatin
- 10uM olaparib was needed to recapitulate PARP1/2 dKO in reducing XRCC1 chromatin association (Hanzlikova et al, NAR,2017). PARylated chromatin is detected at 10uM PARPi (Michelena Nat Comm 2018).

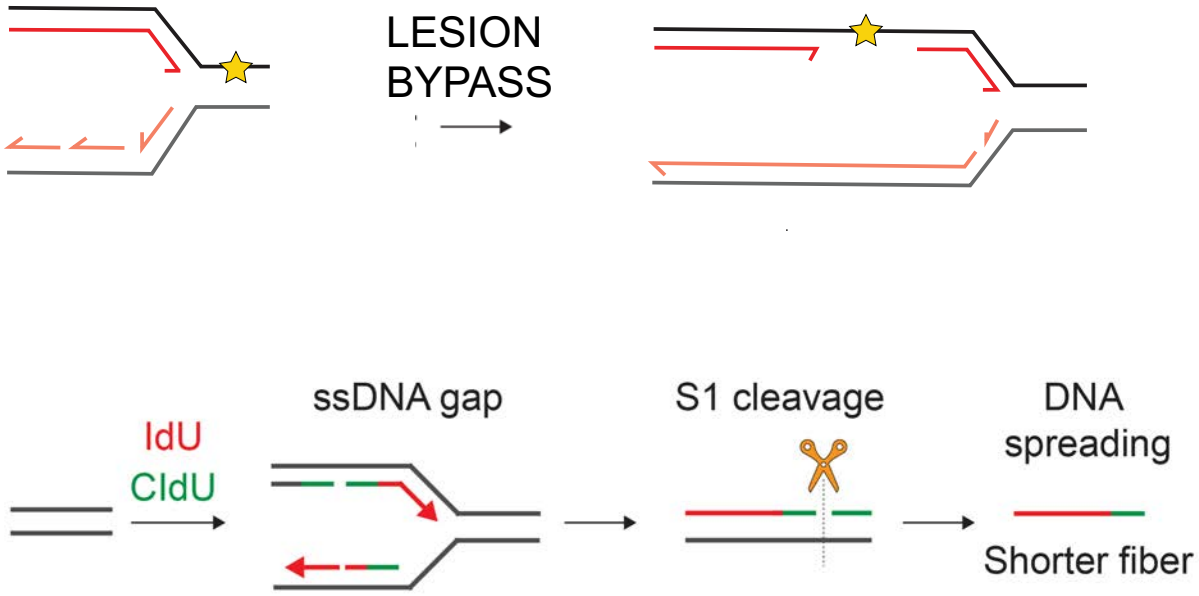
Which subset of PAR-activating lesions require ALC1?

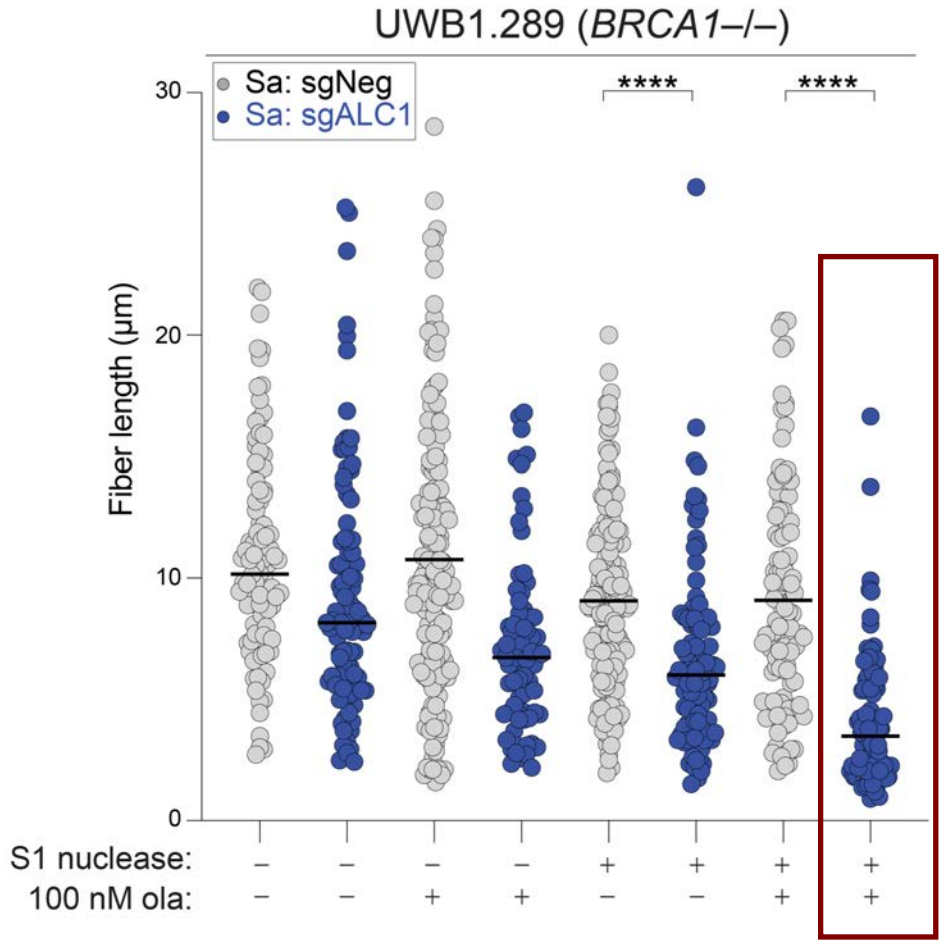






ALC1 loss increases replication associated ss-gaps





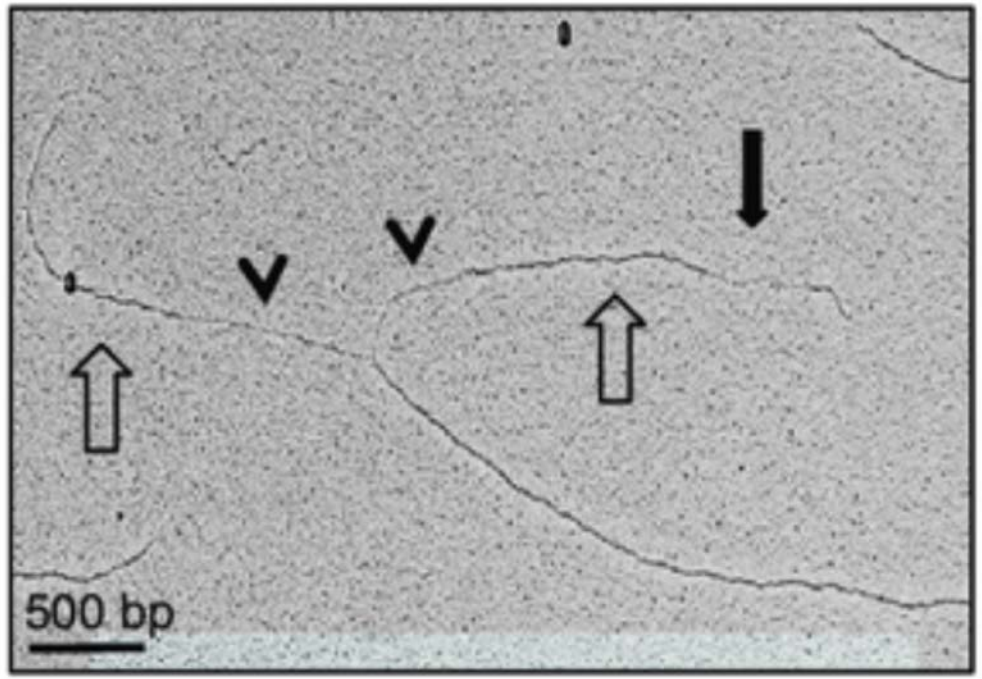
Base damage, replication gaps and HR

Discontinuities in the DNA synthesized in an Excision-defective Strain of *Escherichia coli* following Ultraviolet Irradiation

W. Dean Rupp and Paul Howard-Flanders

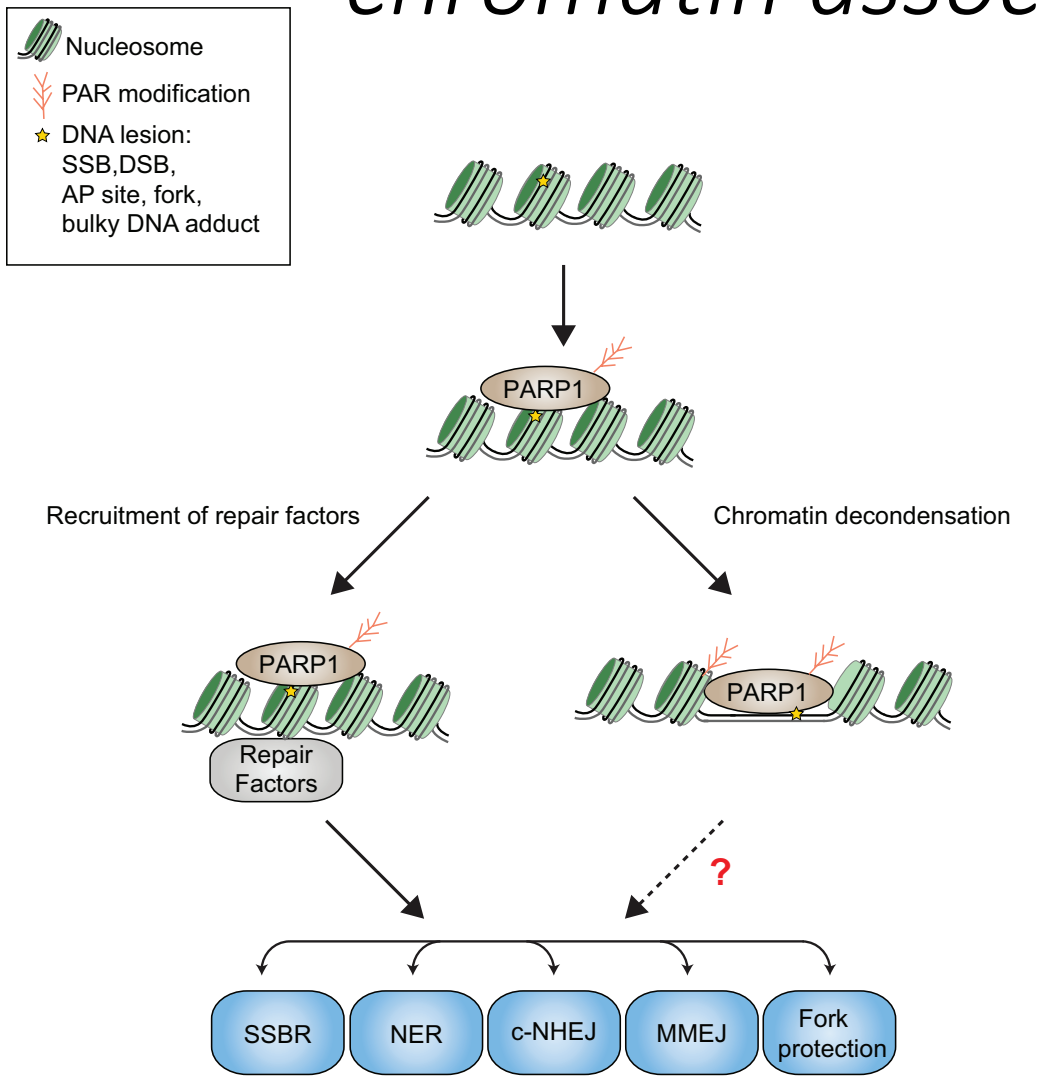
Radiobiology Laboratories
Yale University School of Medicine
New Haven, Connecticut, U.S.A.

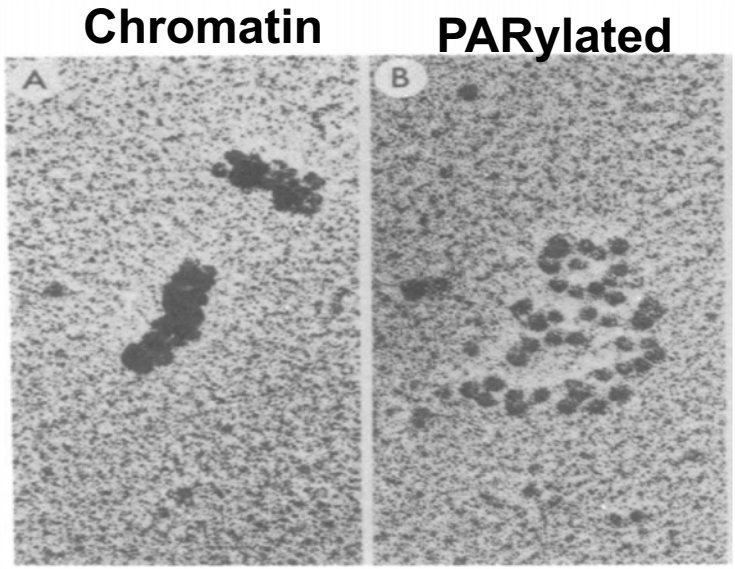
(*Received 11 April 1967, and in revised form 18 October 1967*)



Kolinjivadi, Costanzo et al. Mol Cell 2017

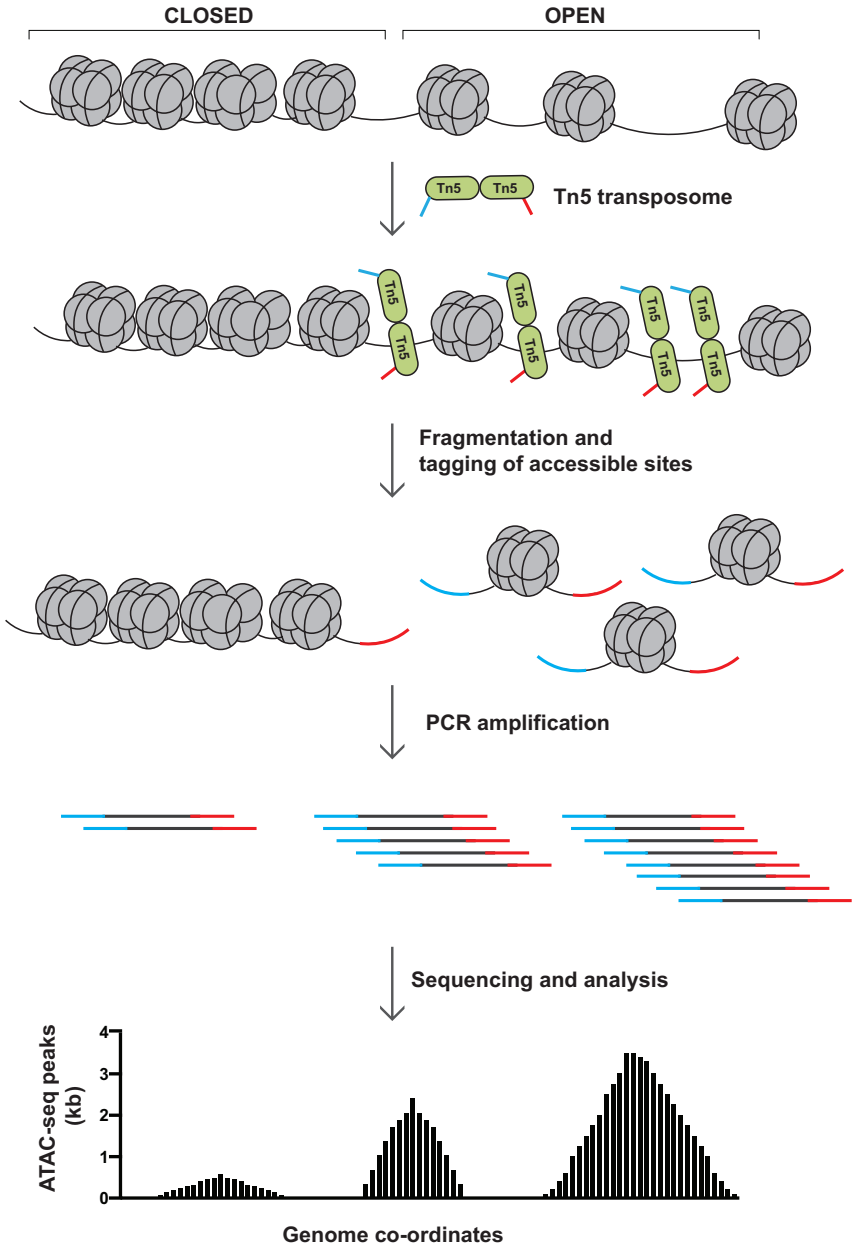
How do ALC1 and PARP co-operate to resolve chromatin associated base lesions?





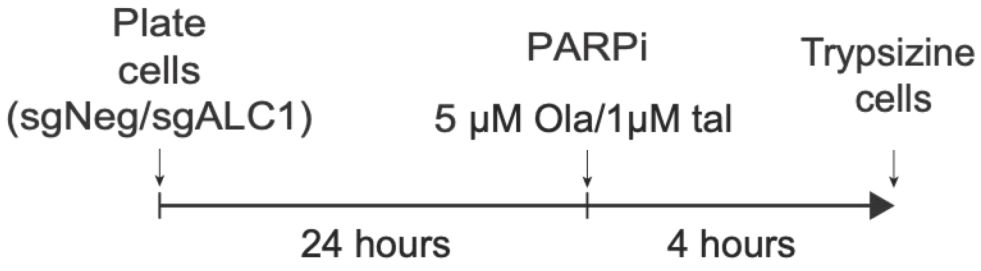
Poirier *et al*, PNAS,1982

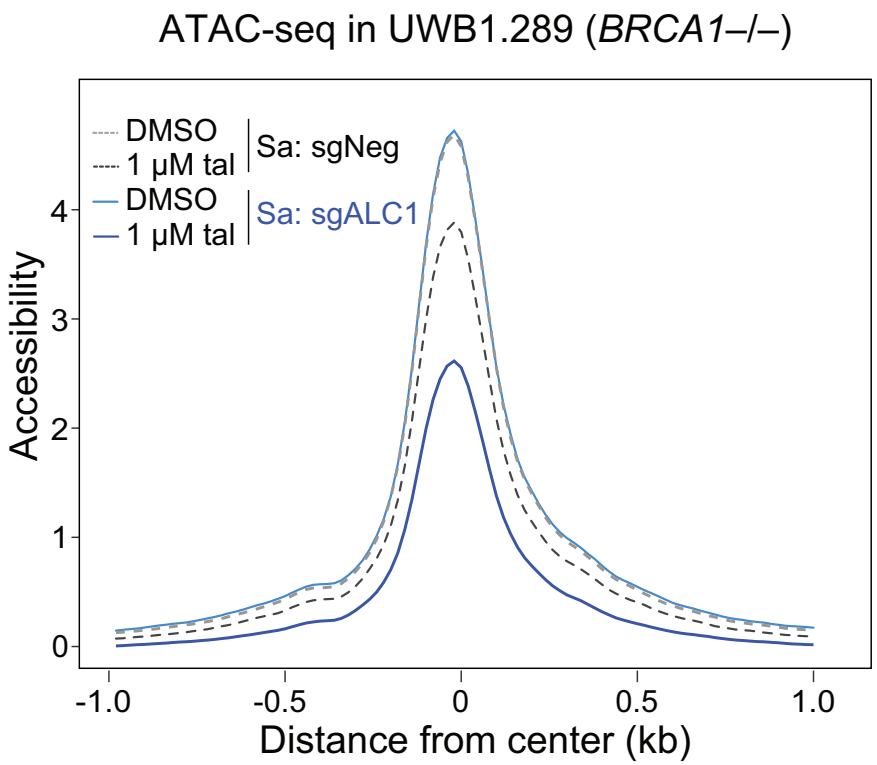
ATAC-Seq

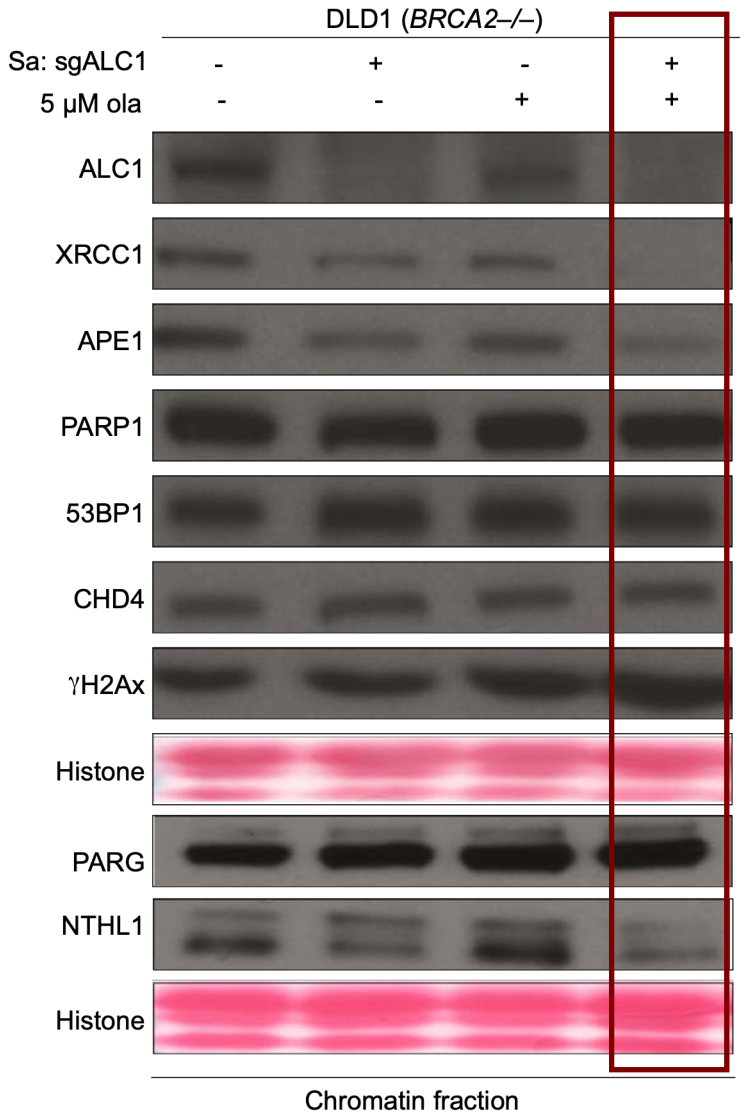


With Yeqiao Zhou and Babak Faryabi

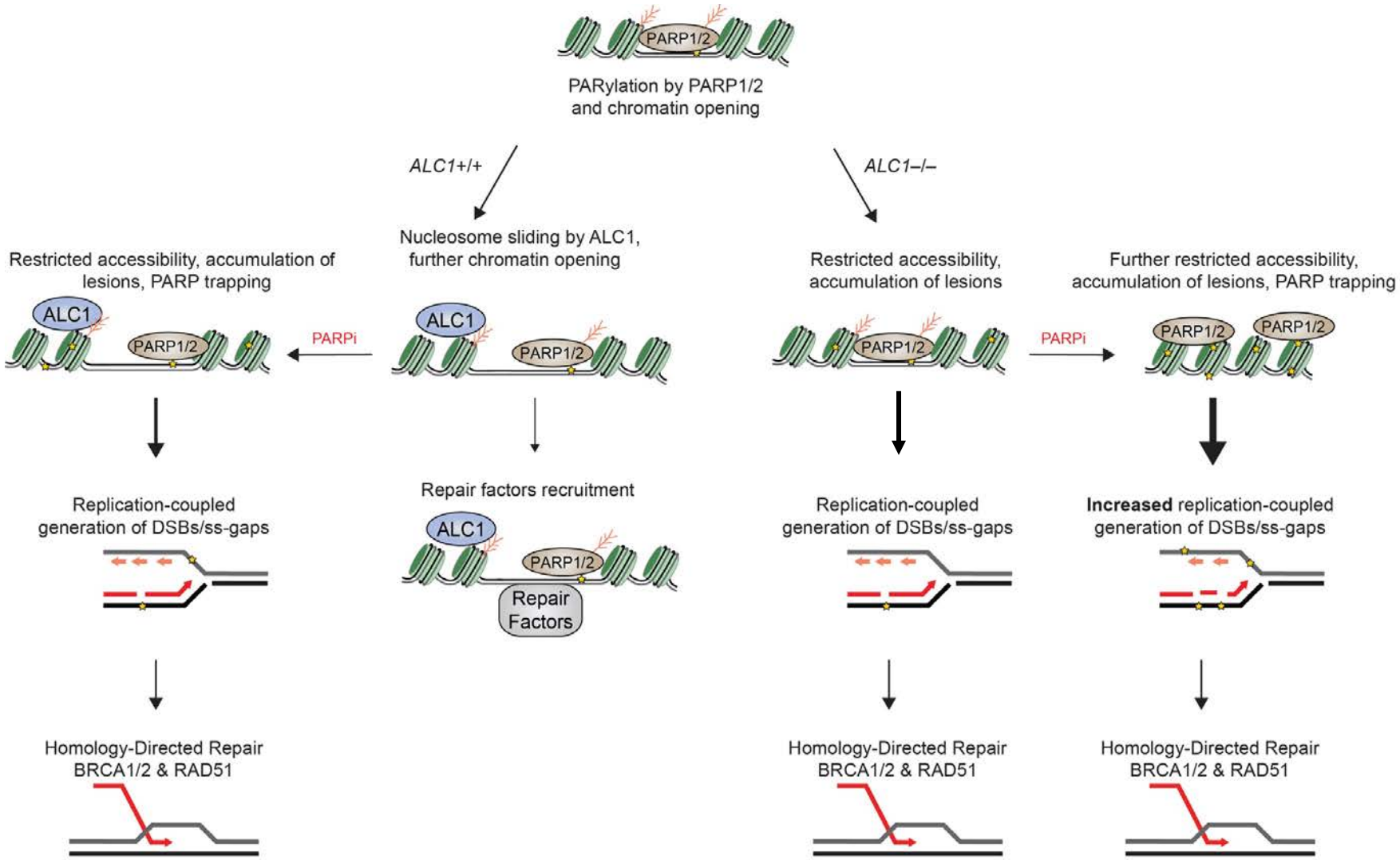
ALC1 and PARP activities promote access to base damage





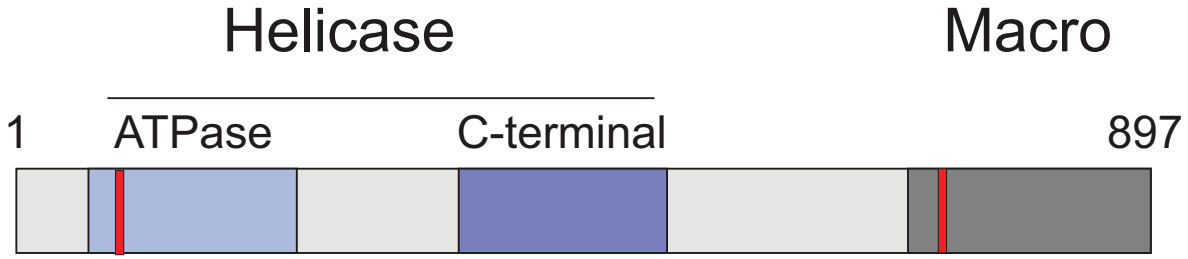


PARP dependent chromatin accessibility and homologous recombination



Verma et al. Nature Cell Biology in press

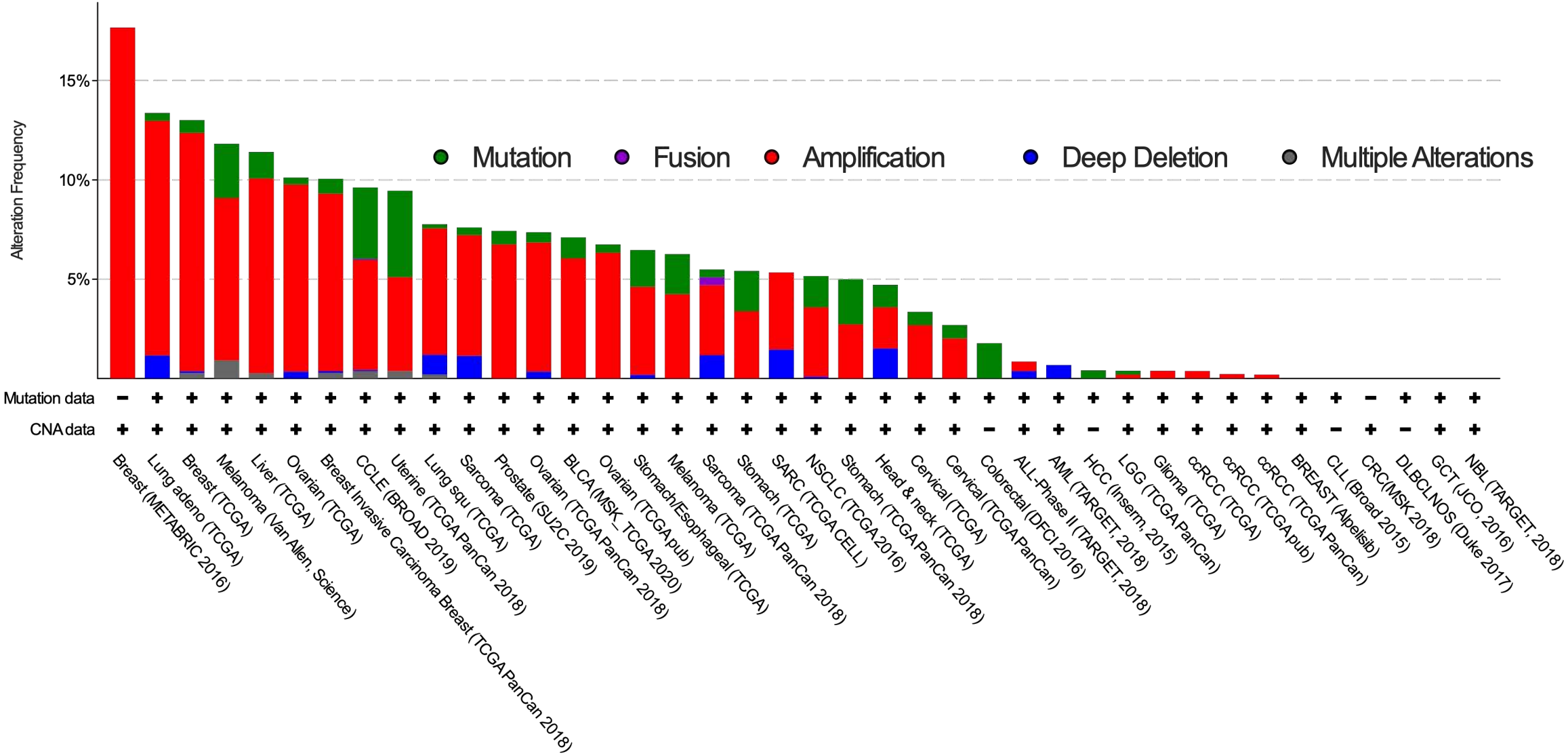
Chromatin remodeling and PARP inhibitor response



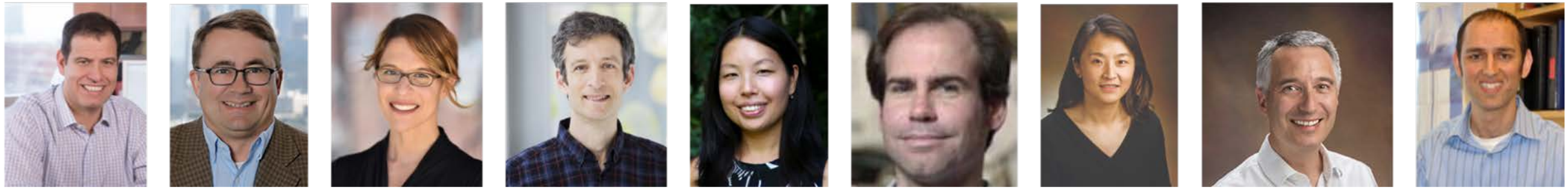
- *Chromatin accessibility to base damage is essential for the survival of HR deficient cells and confers extreme sensitivity to PARPi*
- *ALC1 loss is **not epistatic** with other determinants of PARPi sensitivity (except PARP1 + PARP2)*
- *What endogenous base lesions accumulate in ALC1 null cells and how are they toxic to HR deficient cells?*
- *Is this PAR dependent chromatin remodeling required at specific genomic locations?*

Cancer as a stress response

Requirement for PAR dependent chromatin remodeling



The Penn Center for Genome Integrity



Advance fundamental understanding of genome integrity and its contributions to human biology

Integrate cutting edge research from **DNA Repair and Replication, Mitosis, Innate Immune Signaling, Virology, Epigenetics**, **Stem Cell Biology** and access to new approaches (**Chemical biology, engineering, human artificial chromosomes, CRISPR Screening, Evolutionary biology**)

Promote **multi-PI grants, shared resources**, intellectual property and translation of basic science

Become as a world class center for genome integrity research

https://www.med.upenn.edu/pcgi/

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