# LIG1 inactivation selectively inhibits growth of BRCA1 mutant cells in vitro and in vivo

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Abstract #3363

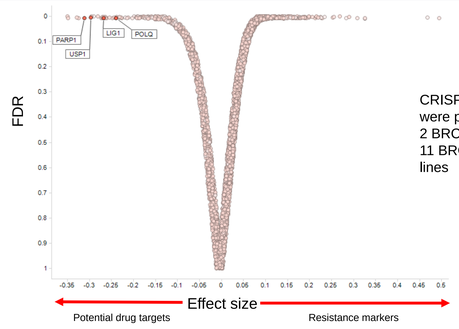
## Introduction

Women harboring a mutation in the *BRCA1* gene have a >7X increased risk of developing breast cancer and >35X increased risk of developing ovarian cancer in their lifetime compared to women lacking such mutations. While PARP1/2 inhibitors are efficacious in the *BRCA* mutant setting, this efficacy is limited and resistance develops readily. Additional therapeutic targets beyond PARP1/2 are needed to treat cancer patients with *BRCA* mutations.

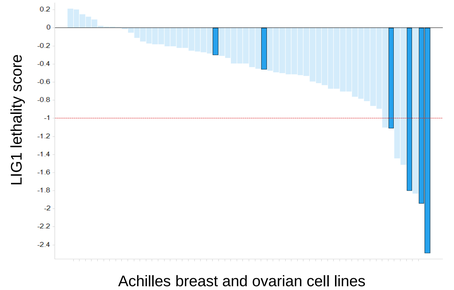
We performed CRISPR screens in 11 BRCA1/2 wild-type and 2 BRCA1 mutant cancer cell lines to identify targets that are synthetic lethal with BRCA1 mutations. In addition to USP1, PARP1 and POLQ, we identified the DNA ligase gene LIG1 as a novel target that when knocked out, kills BRCA1 mutant cells selectively. Internal analysis of BRCA mutations in breast and ovarian cancer cell lines in the Project Achilles database further validated the hyperdependence of BRCA1 mutant cells on LIG1. Single-gene perturbation of LIG1 using CRISPRn, CRISPRi, and RNAi confirmed the lethal effect of LIG1 inactivation in BRCA1 mutant cell lines, but not BRCA1/2 wild-type cell lines. This loss of viability could be rescued by complementing with an exogenous wild-type LIG1 cDNA, demonstrating the on-target nature of the genetic tools. Using a degradable DNA Ligase I fusion protein, we demonstrated a strong correlation between DNA Ligase I protein level and viability in BRCA1 mutant cells. Enzymatically inactive DNA Ligase I mutant protein (LIG1$^{K568A}$) was unable to rescue the loss of viability caused by endogenous LIG1 depletion supporting the tractability of this target from a small molecule inhibitor perspective. These data were reproduced in vivo using BRCA1 mutant MDA-MB-436 derived tumors, in which tumor growth was inhibited >80% upon loss of LIG1.

Mechanistically, DNA ligase I plays a critical role in DNA replication and damage repair by sealing nicks in the phosphodiester backbone of DNA. When these nicks are not repaired, they are marked by the addition of PAR chains. Consistent with this mechanism, we demonstrated that inactivation of *LIG1* leads to increased PARylation. The induction of PARylation was proportional to the level of inactivation of LIG1 protein, supporting a correlation between LIG1 activity and DNA nick repair. Together, these data support DNA ligase I as a synthetic lethal target in the context of *BRCA1* mutations.

## CRISPR screens identify LIG1 as a top dependency in BRCA mutant cell lines

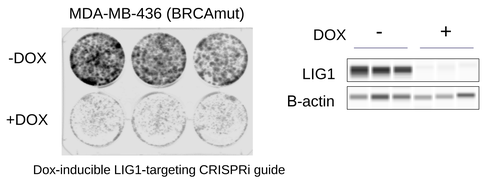



CRISPRn screens were performed in 2 BRCA mutant and 11 BRCA WT cell lines



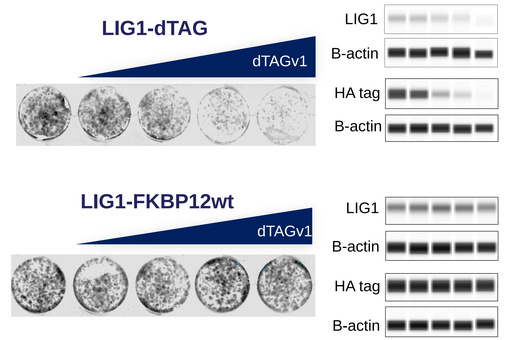

## LIG1 inactivation is lethal in BRCA mutant cells across multiple perturbation platforms

### CRISPRi knockdown




Dox-inducible LIG1-targeting CRISPRi guide

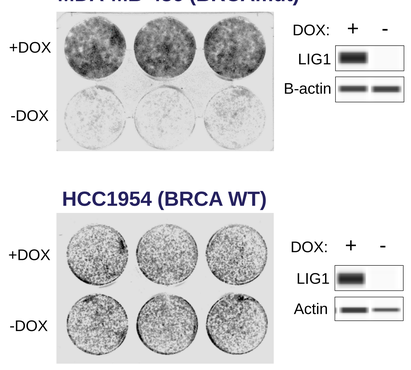
### dTAG-based protein degradation




MDA-MB-436 cells expressing a CRISPR-resistant (cr) LIG1 cDNA fused to either the FKBP12wt degron (non-degradable) or FKBP12mut (dTAG) degron (degradable), with constitutive knockout of endogenous LIG1

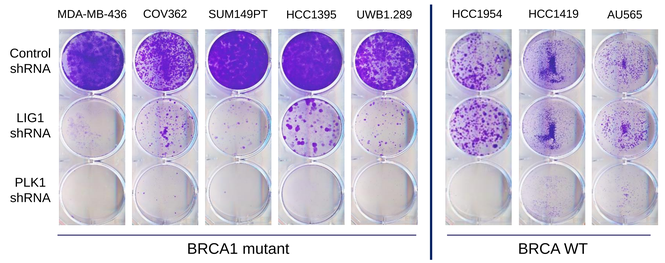
## LIG1 inactivation represses BRCA mutant cell growth in an on-target and selective manner

### CRISPRn knockout

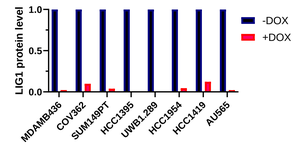



DOX-inducible CRISPR-resistant LIG1 cDNA with knockout of endogenous LIG1

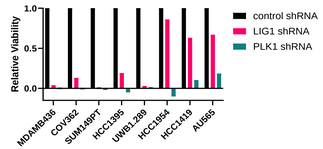
## LIG1 inactivation represses colony formation of BRCA mutant, but not BRCA WT cells



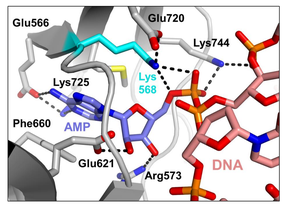

### Quantification of LIG1 Knockdown



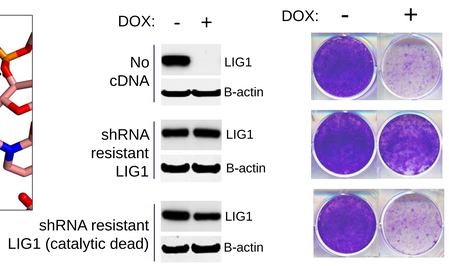

### Quantification of Colony Formation




## Catalytic activity of LIG1 is required for viability in BRCA mutant cells

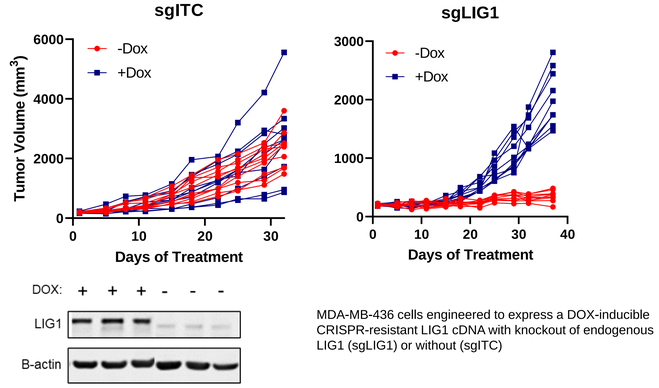



1.73 Å resolution crystal structure of LIG1 with nicked dsDNA



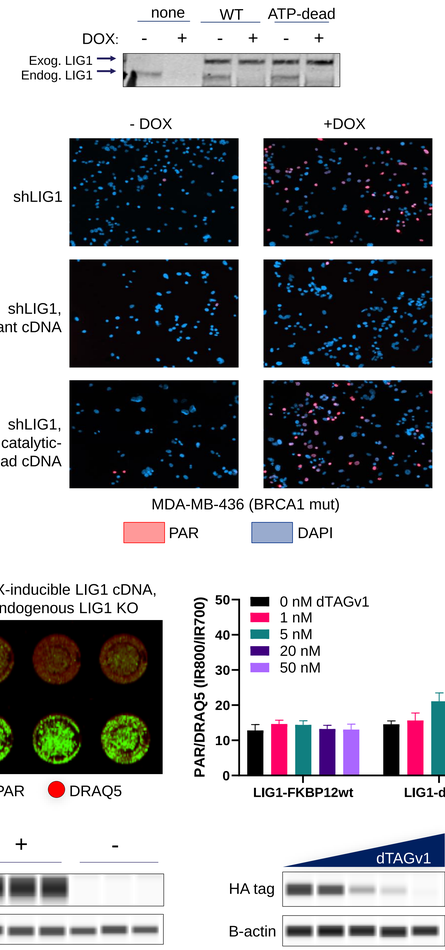

shRNA-resistant LIG1 WT or K568A mutant cDNA with DOX-inducible shRNA targeting LIG1

## LIG1 inactivation inhibits BRCA mutant tumor growth in vivo




MDA-MB-436 cells engineered to express a DOX-inducible CRISPR-resistant LIG1 cDNA with knockout of endogenous LIG1 (sgLIG1) or without (sgITC)

## Inactivation of LIG1 causes accumulation of PAR chains at DNA strand breaks










## Summary

- **Inactivation of LIG1 is synthetic lethal with BRCA1 loss in a variety of cell lines**
- **Inactivation of LIG1 does not significantly impair viability of BRCA WT cells**
- **Defects in cell viability resultant from LIG1 inactivation in BRCA1 mutant cells are on-target and dependent on the catalytic activity of LIG1**
- **LIG1 loss impairs growth of BRCA1 mutant xenografts resulting in tumor stasis**
- **Loss of LIG1 leads to increased PARylation in cells, a readout of accumulation of DNA nicks**

**Acknowledgements**

ChemPartner, WuXi AppTec, Pharmaron