Study of DYNLL1 in double-strand break repair and synthetic lethality in BRCA1-deficient breast cancer

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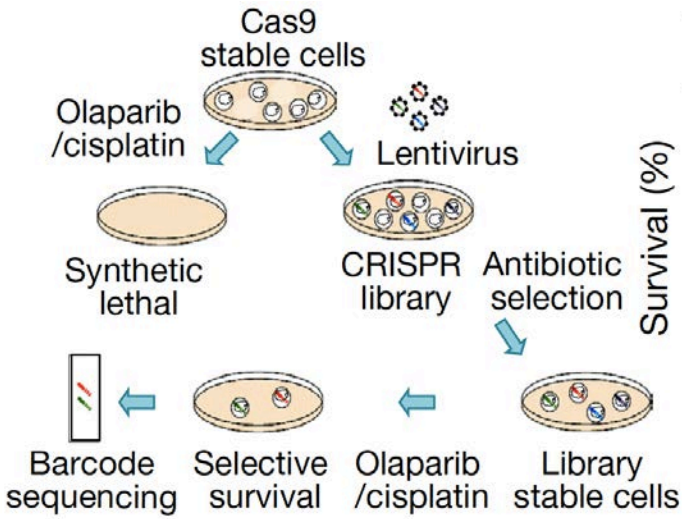
A CRISPR-screen to find new genes involved in drug resistance in BRCA1-mutated cells

UWB1.289, COV362 and JHOS-2 cells : BRCA1-mut



Gene Symbol	STARS Score	p-value
DYNLL1	9,117519799	0
C20orf196	8,947371836	0
TP53BP1	8,895261185	0
ATMIN	7,155399415	6,80E-06
IFNAR1	6,188073532	2,04E-05
IFNAR2	6,159698481	2,04E-05
TEN1	4,886833622	0,000373931
DHX29	4,748660891	0,000482711
NHEJ1	4,431478718	0,000951824

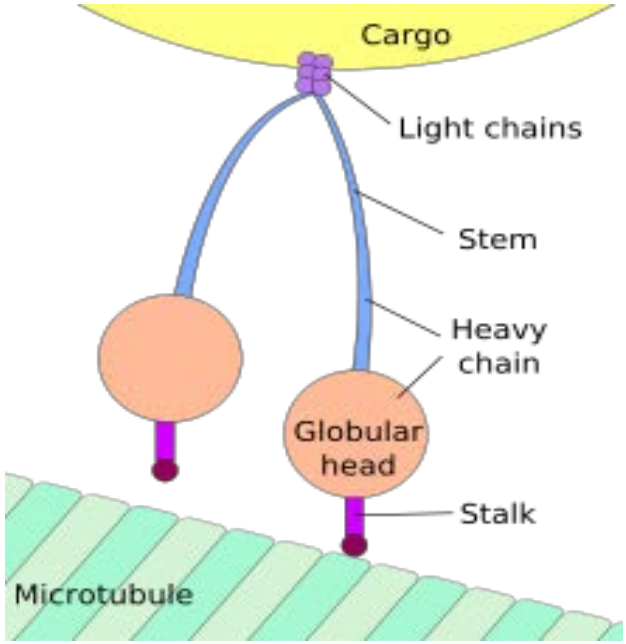
Olaparib screen

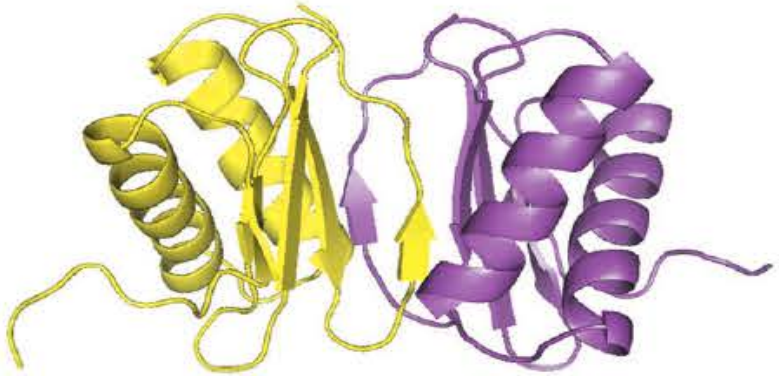
Gene Symbol	STARS Score	p-value
TAF5L	10,0310281	0
LRRC8D	6,94863567	4,5298E-06
EP300	5,6462175	3,6238E-05
TADA2B	4,92430952	0,00020082
DYNLL1	4,81555163	0,00026877
PPFIA4	4,74196526	0,00031256
SUPT20H	4,72601053	0,00032313
DYRK1A	4,43030584	0,00063266
MYBL1	4,41369903	0,00065531
MED12	4,33048023	0,00078366

Cisplatin screen

He *et al.*, *Nature*, 2018

DYNLL1: light chain component of cytoplasmic dynein



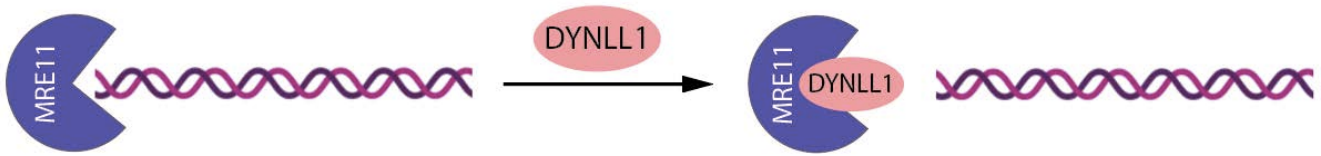


- Hub protein of **10 kDa**
- Thought to be involved in linking dynein to cargos
- May play a role in changing or maintaining the cytoskeletal structures
- Promotes dimerization of various targets
- Promotes apoptosis by interacting with BCL2L2
- **Inhibits resection via its interaction with MRE11**

He *et al.*, *Nature*, 2018 ; King, 2008 ; Becker *et al.*,2018 ; Song, 2008

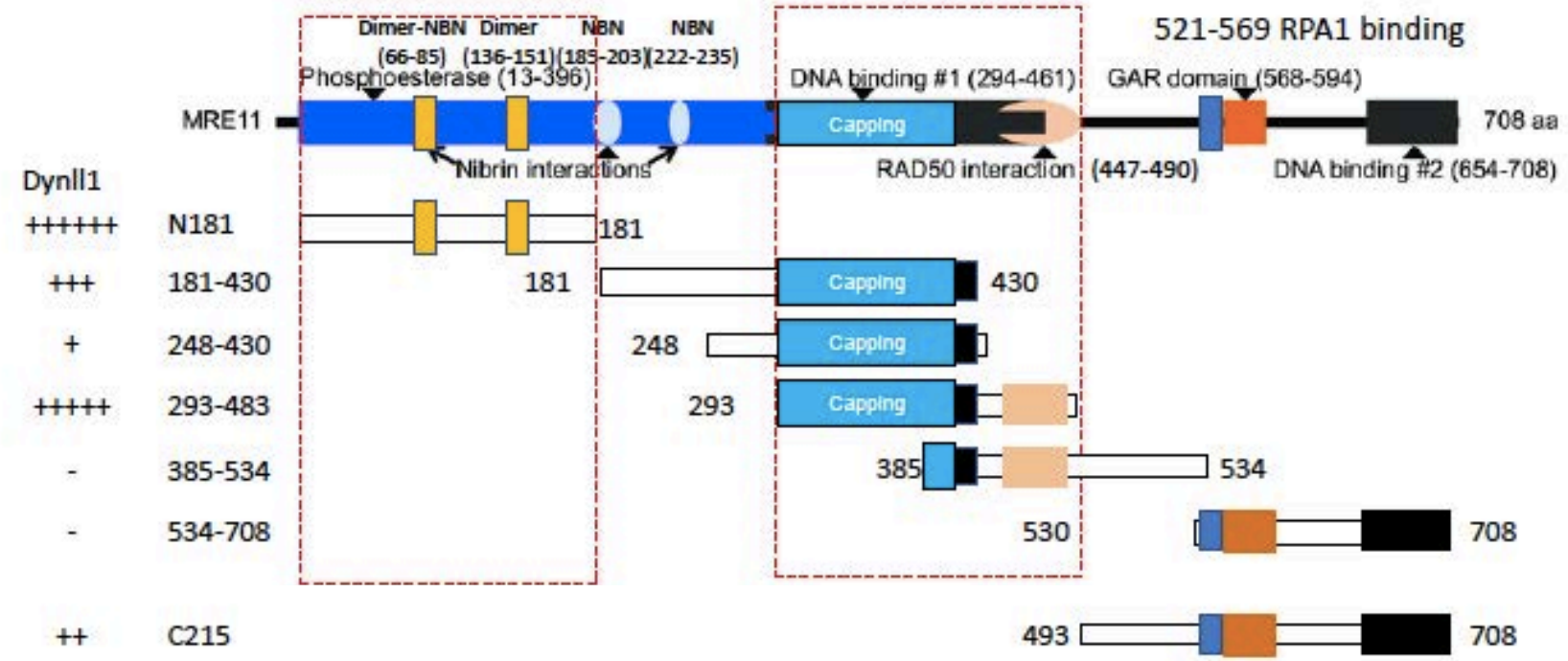
Objectives

- To understand the biochemical mechanism by which DYNLL1 prevents DNA-resection, *in vitro*

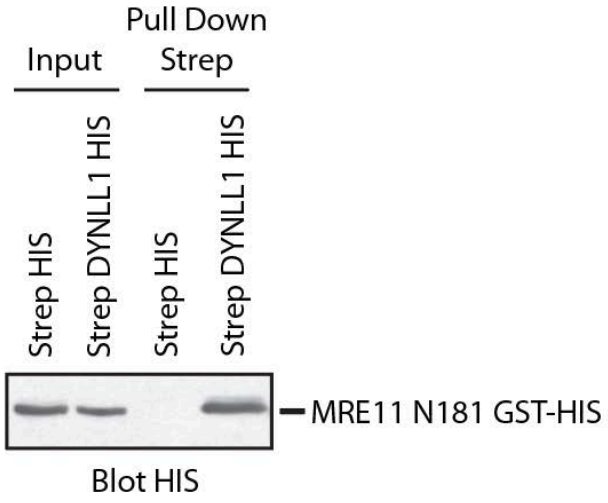


- To determine whether DYNLL1 is a good prognosis marker and a good biomarker for BRCA1-deficient breast cancer therapeutic decision, *in cellulo* and *in vivo*

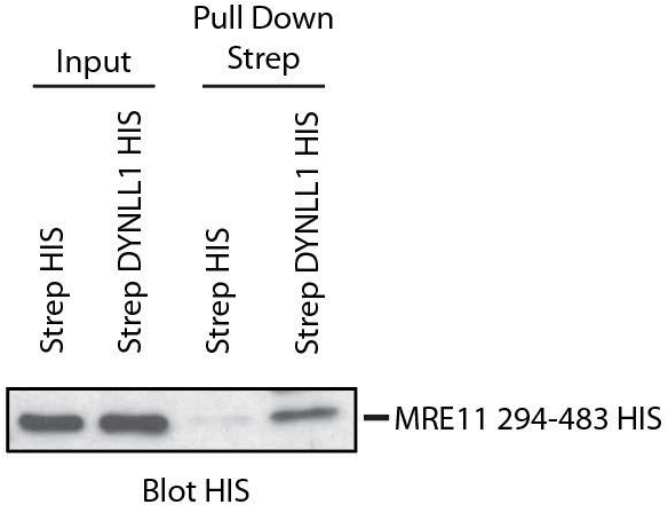
Fragments of MRE11 bind preferentially DYNLL1 *in cellulo* and *in vitro*



a)

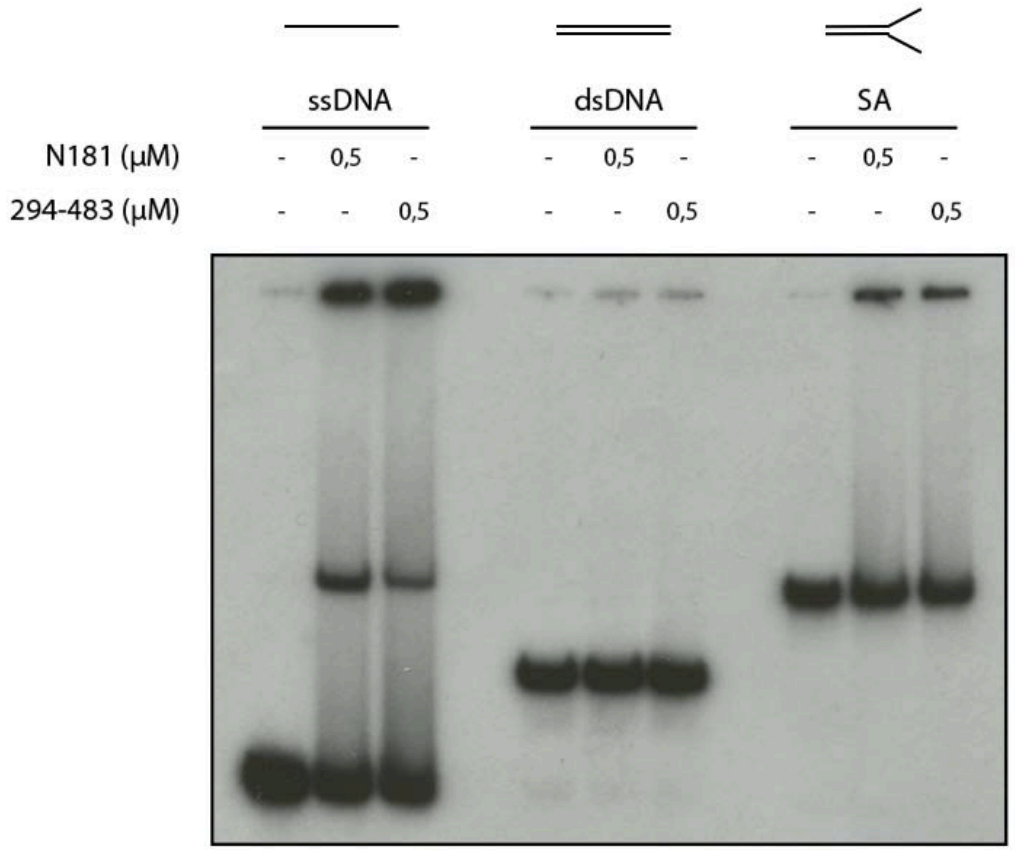


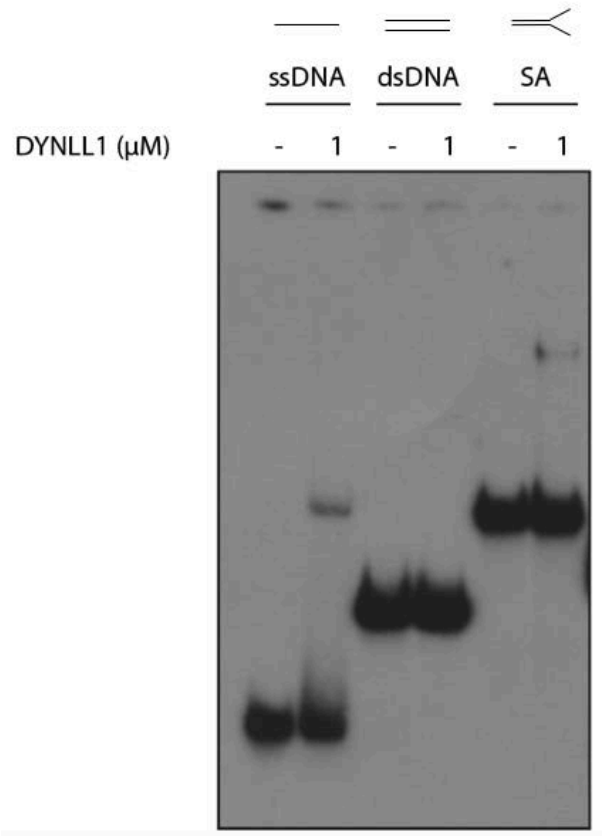
b)

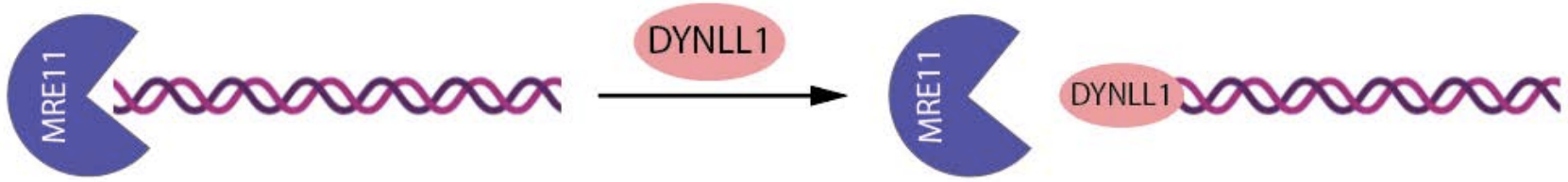


- Pulldown *in cellulo* showed that the N181 region and the 294-483 region of MRE11 bind preferentially DYNLL1
- Pulldown *in vitro* (co-infection (a) and with purified proteins (b)) show the specific binding of those MRE11 regions to DYNLL1

Fragments of MRE11 and DYNLL1 bind to DNA without fixation



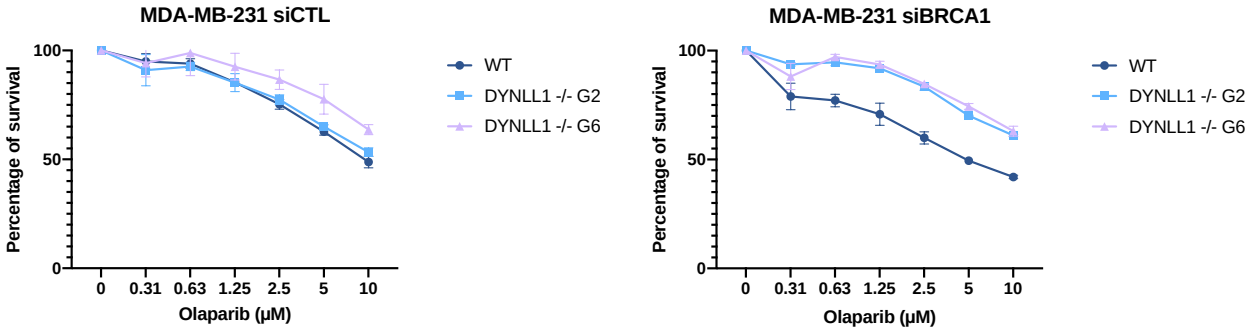


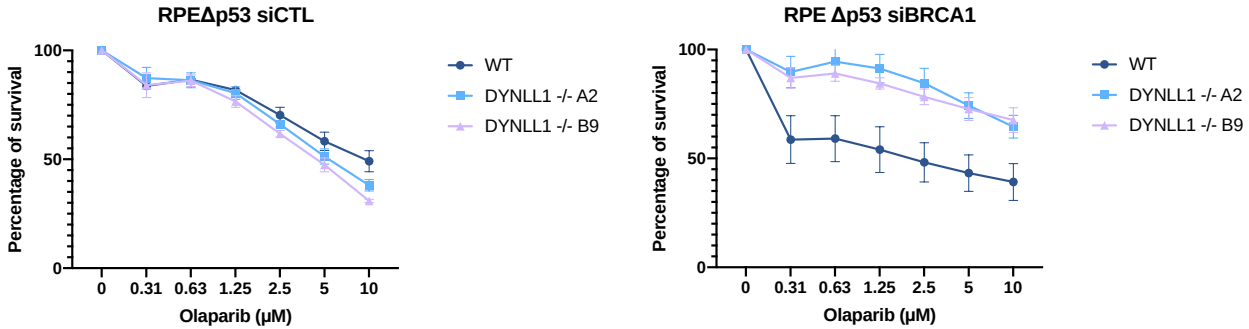


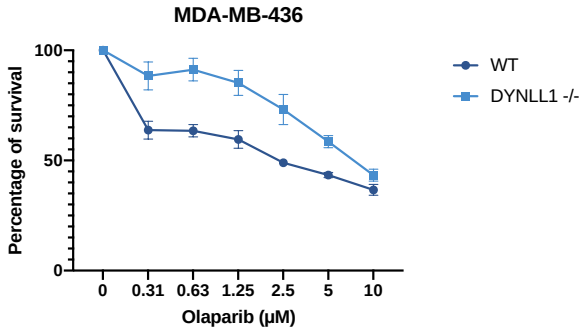
- DYNLL1 binds DNA to inhibit resection by MRE11

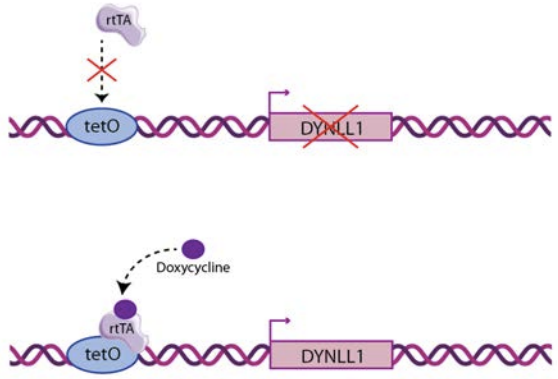
DYNLL1 knockout leads to PARPi resistance in BRCA1-deficient cells

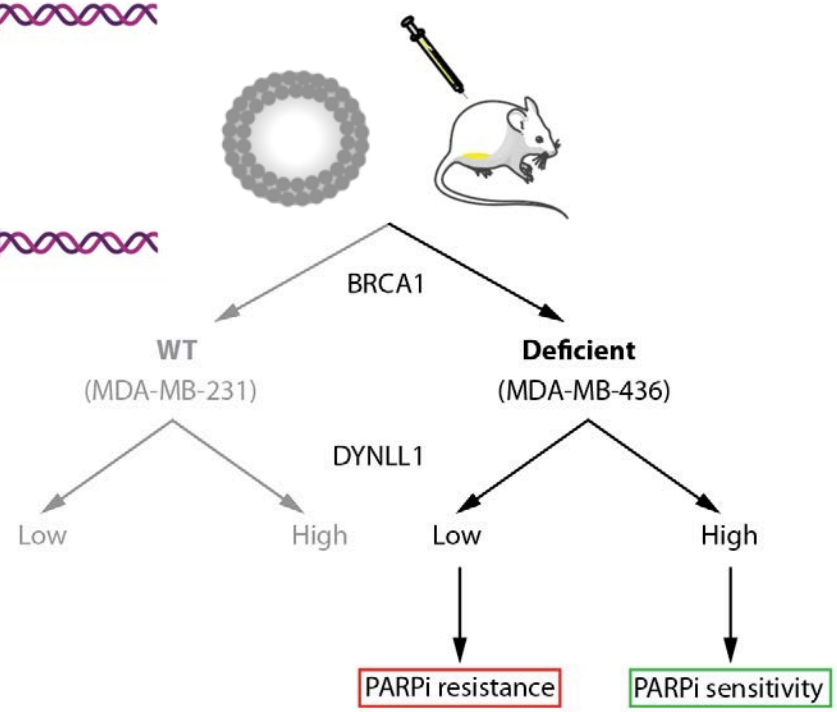
MDA-MB-231 : BRCA1 WT
MDA-MB-436 : BRCA1-deficient





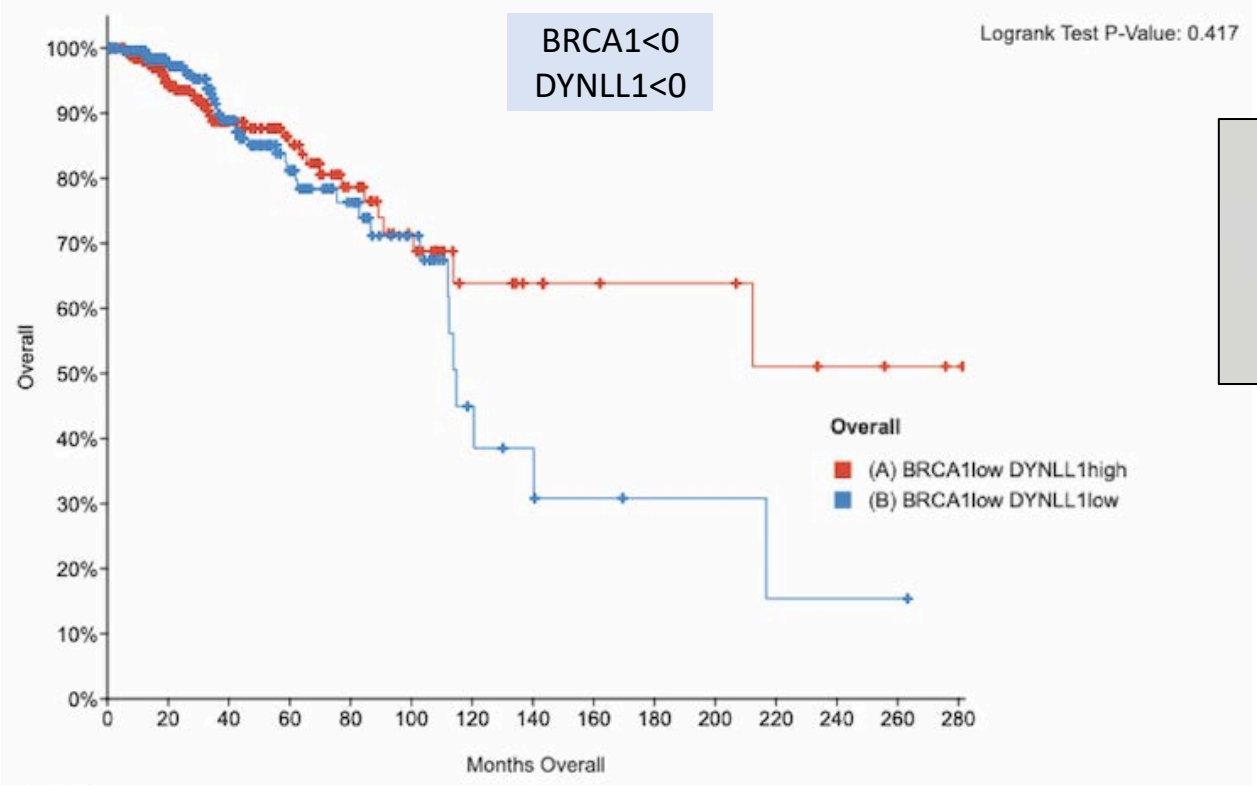




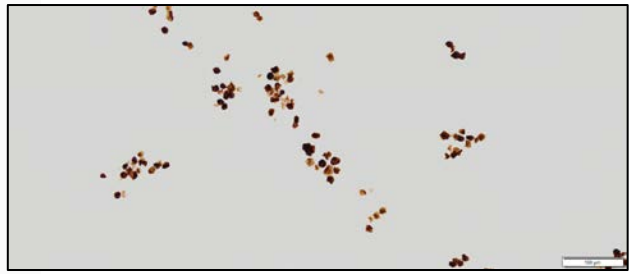


- Cell survival assays show a resistance to the PARPi treatment (Olaparib) only when cells are BRCA1-deficient and DYNLL1-/-
- We want to induce different levels of DYNLL1 expression in tumours and test the sensitivity to PARPi treatment

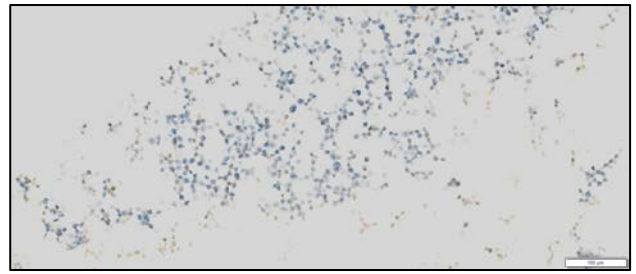
DYNLL1 is a potential good prognosis marker in BRCA1-deficient cells and tumours



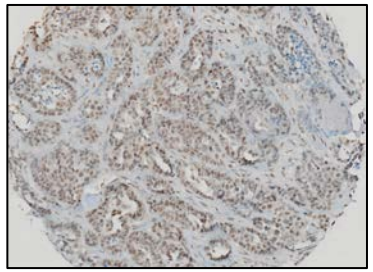
Breast Invasive Carcinoma (TCGA, Firehose Legacy)



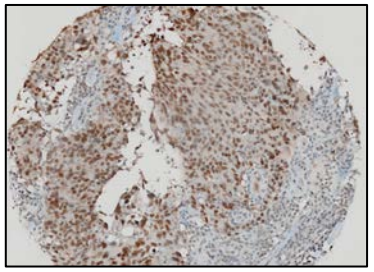
RPE Δp53 WT



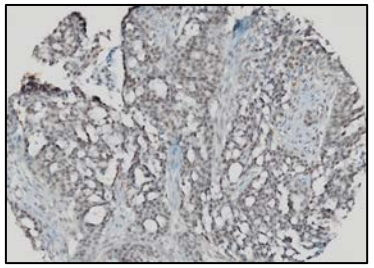
RPE Δp53 DYNLL1 -/-



Ovarian tumour 1



Ovarian tumour 2



Ovarian tumour 3

- Patient data show a better overall survival when DYNLL1 is more expressed in a BRCA1^{low} context
- DYNLL1 antibody is very specific in immunohistochemistry and can be used further on TMA

➢ BRCA1^{low} DYNLL1^{low} → HR reactivation → PARPi resistance

Summary

- We aim to determine how DYNLL1 regulates DNA resection via understanding biochemically its interaction with MRE11 ;

- Understanding the role of DYNLL1 in PARPi resistance using different models (2D, 3D, in *vivo)* will allow us to find better ways to counteract this resistance ;

- Establishing DYNLL1 as a biomarker for the therapeutic choice of *BRCA1*-deficient cancers could provide a more precise approach for personalized treatment with PARPi

Acknowledgment & Literature

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He YJ, Meghani K, Caron M-C, Yang C, Ronato DA, Bian J, et al. DYNLL1 binds to MRE11 to limit DNA end resection in BRCA1-deficient cells. Nature. nov 2018;563(7732):522-6.

Song C, Wen W, Rayala SK, Chen M, Ma J, Zhang M, et al. Serine 88 Phosphorylation of the 8-kDa Dynein Light Chain 1 Is a Molecular Switch for Its Dimerization Status and Functions. J Biol Chem. 15 févr 2008;283(7):4004-13.

Becker JR, Cuella-Martin R, Barazas M, Liu R, Oliveira C, Oliver AW, et al. The ASCIZ-DYNLL1 axis promotes 53BP1-dependent non-homologous end joining and PARP inhibitor sensitivity. Nat Commun. déc 2018;9(1):5406.

King SM. Dynein-independent functions of DYNLL1/LC8: redox state sensing and transcriptional control. Sci Signal. 2008 Nov 25;1(47):pe51. doi: 10.1126/scisignal.147pe51. PMID: 19036713.

Thank you for your time!