MASSIVELY PARALLEL FUNCTIONAL ANALYSIS OF MISSENSE MUTATIONS IN THE PALB2 TUMOR SUPPRESSOR GENE

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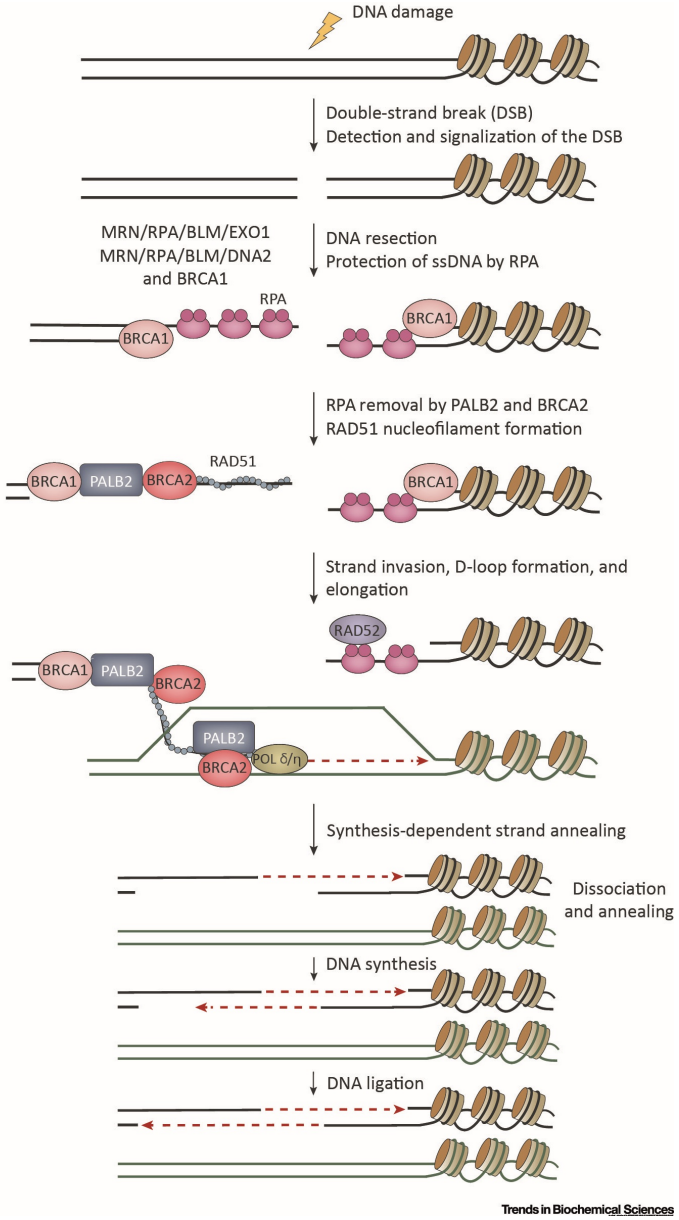
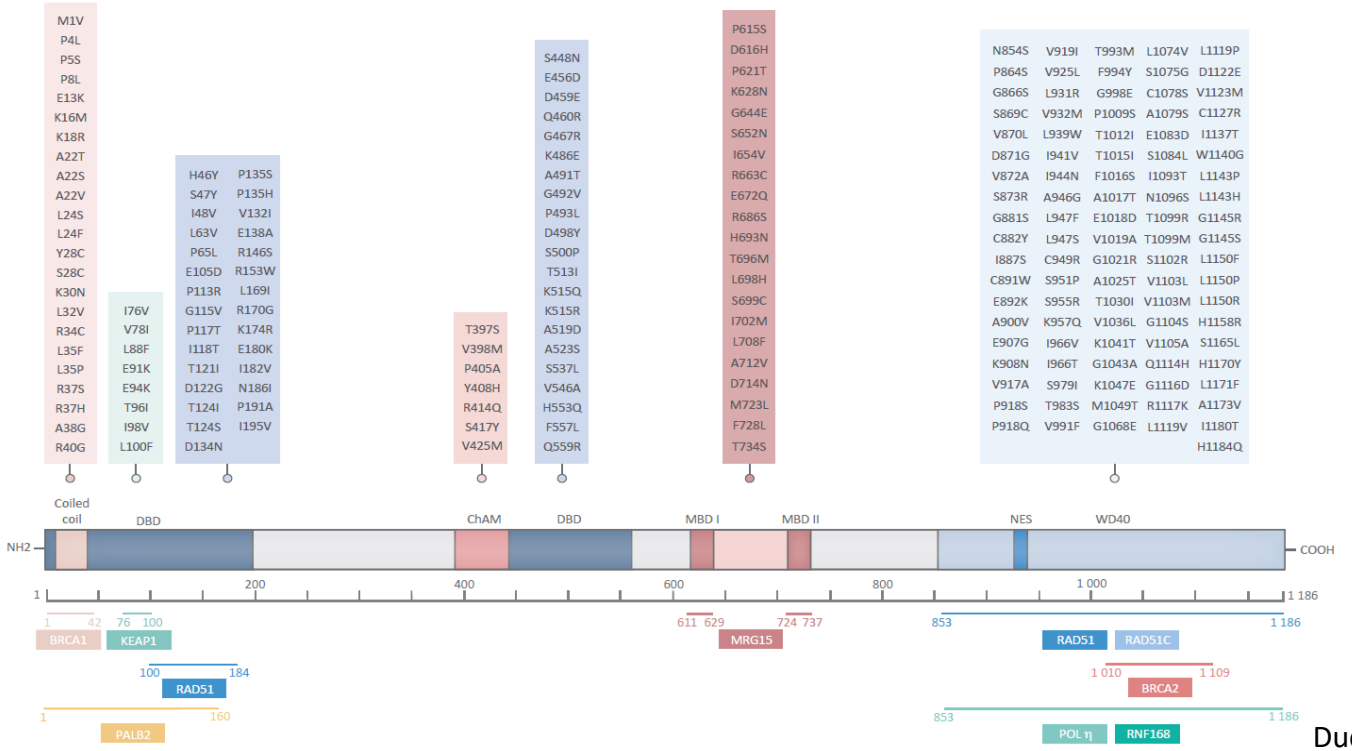
PALB2 (partner and localizer of BRCA2)



- Essential player DNA repair by homologous recombination (HR) , by maintaining genome integrity and tumorigenesis suppression;
- Monoallelic mutations in *PALB2* predispose to breast and familial pancreatic cancer;
- A large number of sequence alterations in PALB2, mostly missense variations, have been uncovered. However, only a few have been characterized;
- Whether or not these missense variants are associated with increased breast cancer (BC) risks and HR deficit remains unknown for the most part, posing a challenge for genetic counselling.



Ducy *et al*, 2019

A SYSTEMATIC APPROACH TO IDENTIFY VARIANTS WITH DELETERIOUS EFFECTS ON PALB2 FUNCTIONS

- Using a combination of CRISPR/Cas based homologous recombination assay, biochemical, and cellular assays, we established the landscape of HR functionality and vulnerabilities to PARP inhibitors for a number of *PALB2* variants of unknown significance (VUS) observed in BC patients.

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A global functional analysis of missense mutations reveals two major hotspots in the *PALB2* tumor suppressor

Amélie Rodrigue[1,2], Guillaume Margaillan[3], Thiago Torres Gomes[4,5], Yan Coulombe[1,2], Gemma Montalban[1,2,3], Simone da Costa e Silva Carvalho[1,4,6], Larissa Milano[1,2], Mandy Ducy[1,2,3], Giuliana De-Gregoriis[4,5], Graham Dellaire[7], Wilson Araújo da Silva Jr[6], Alvaro N. Monteiro[8], Marcelo A. Carvalho[4,5,*], Jacques Simard[3,*] and Jean-Yves Masson[1,2,*]

ARTICLE Genetics inMedicine
Open

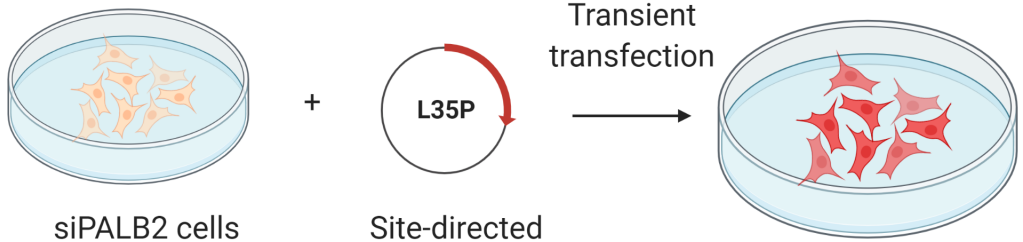
Functional characterization of 84 *PALB2* variants of uncertain significance

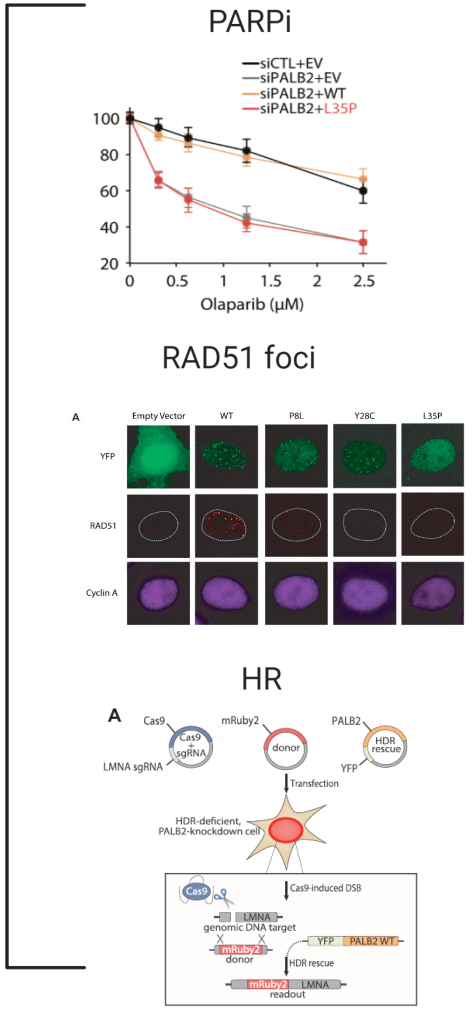
Timothy Wiltshire, PhD[1], Mandy Ducy, PhD[2,3,4], Tzeh Keong Foo, PhD[5], Chunling Hu, PhD[1], Kun Y. Lee, PhD[1], Anil Belur Nagaraj, PhD[1], Amélie Rodrigue, BS[2,4], Thiago T. Gomes, BS[6], Jacques Simard, PhD[3], Alvaro N. A. Monteiro, PhD[7], Bing Xia, PhD[5], Marcelo A. Carvalho, PhD[6], Jean-Yves Masson, PhD[2,4] and Fergus J. Couch, PhD[1]

nature COMMUNICATIONS
ARTICLE
https://doi.org/10.1038/s41467-019-13194-2 OPEN

Functional analysis of genetic variants in the high-risk breast cancer susceptibility gene *PALB2*

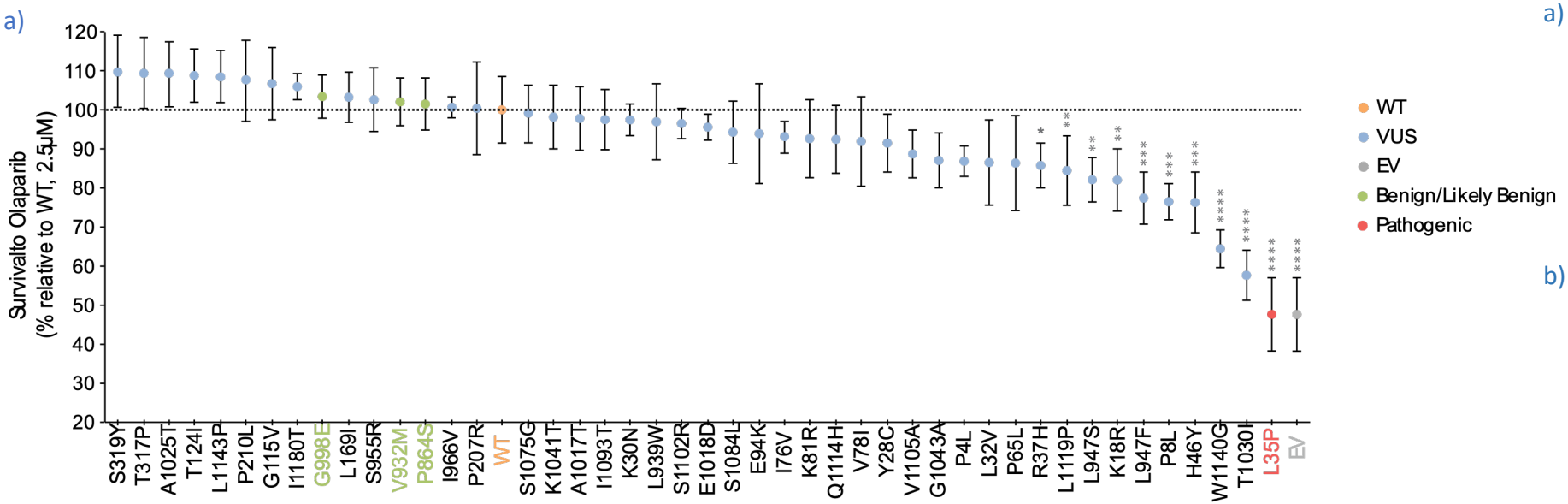
Rick A.C.M. Boonen[1], Amélie Rodrigue[2,3], Chantal Stoepker[1], Wouter W. Wiegant[1], Bas Vroling[4], Milan Sharma[1], Magdalena B. Rother[1], Nandi Celosse[1], Maaike P.G. Vreeswijk[1], Fergus Couch[5], Jacques Simard[2,6], Peter Devilee[1,7], Jean-Yves Masson[2,3] & Haico van Attikum[1*]





SENSITIVITY OF PALB2 VARIANTS TO PARPI OLAPARIB AND RAD51 FOCI FORMATION

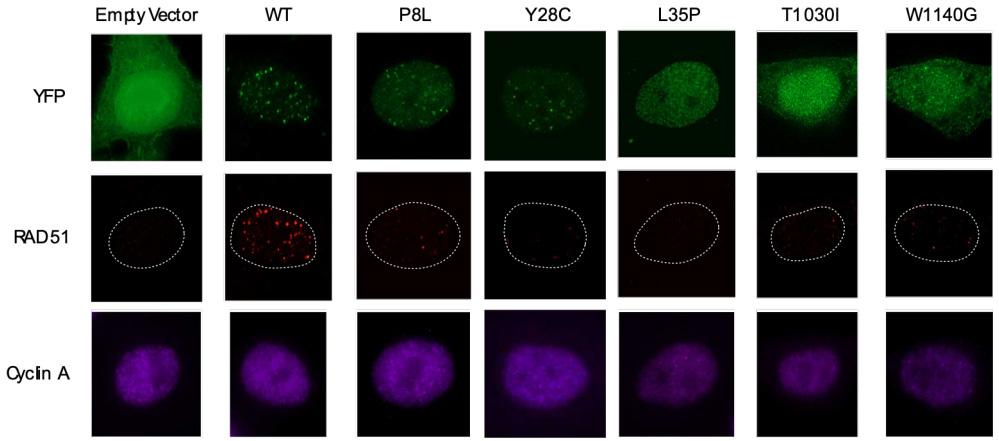
a)

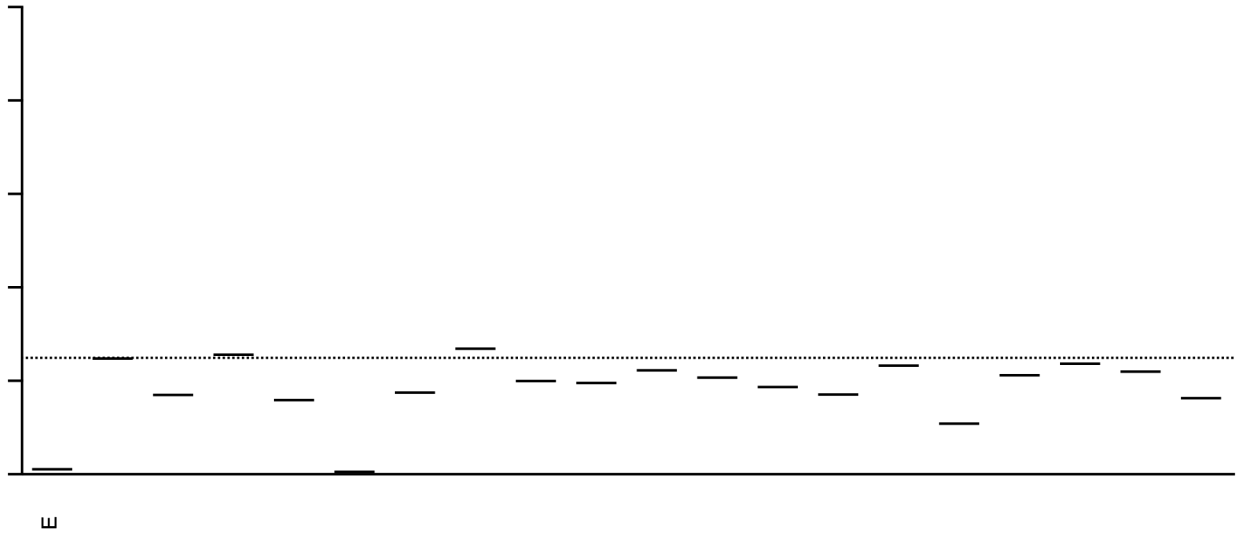


a) Olaparib sensitivity profiles for the complete set of variants at a concentration of 2.5 µM, with the WT condition set at 100%. Survival data are presented as the mean (± SD) from at least 3 independent experiments. Statistical significance was determined by Kruskal–Wallis test with Dunn's multiple comparison post-test. (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$ and (****) $P < 0.0001$

b) Immunofluorescence images of RAD51 foci (red) in siPALB2 HeLa cells complemented with the empty vector or the indicated siRNA-resistant YFP-PALB2 construct (green). The scatter dot plot shows the number of RAD51 foci in cyclin A-positive (purple) cells expressing the indicated YFP construct, with the horizontal lines designating the mean values of at least three independent trials. The percentage change relative to the WT mean is also indicated for each variant. (*) $P < 0.05$; (****) $P < 0.0001$.

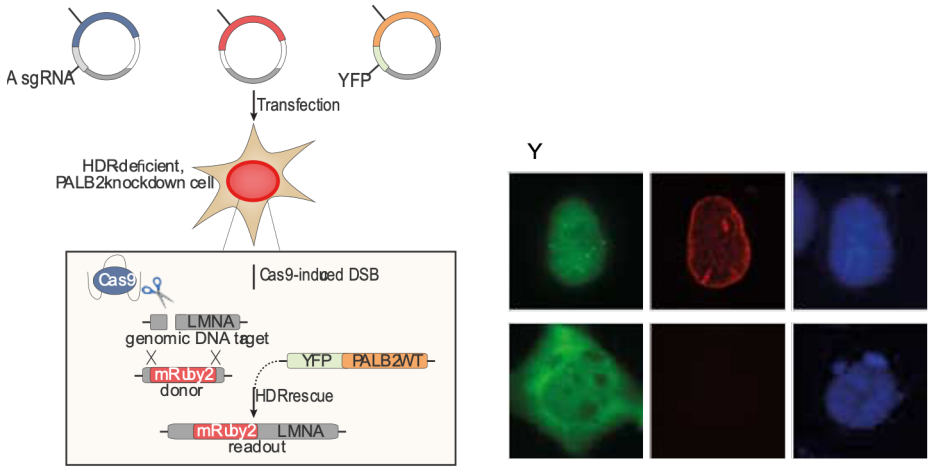
b)

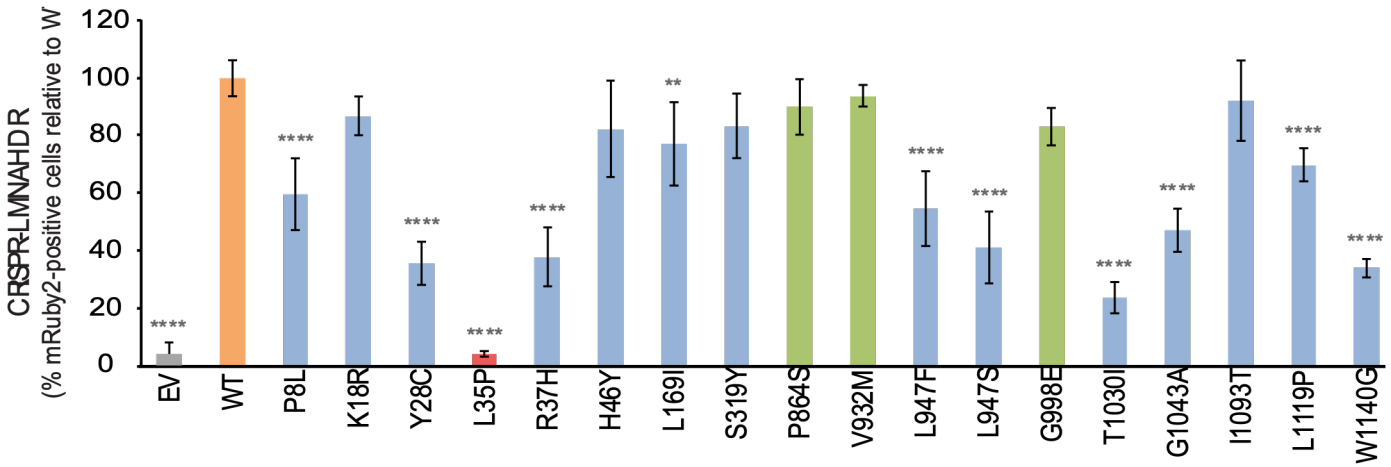




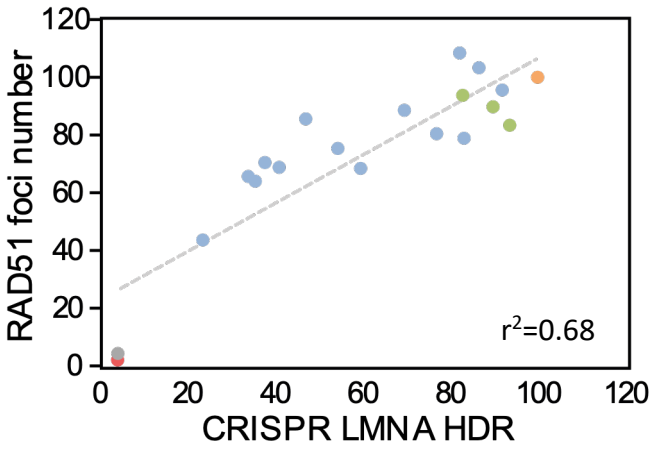
CRISPR-LMNA HDR assay and HR correlations

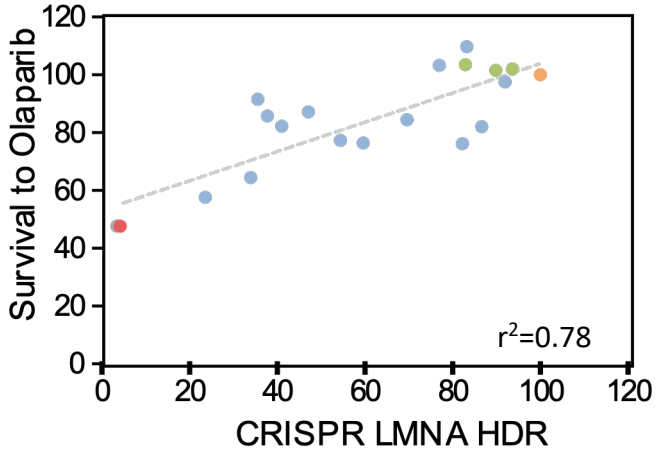
a)





b)





a) Schematic representation of HDR activity using the CRISPR-LMNA system. The CRISPR-LMNA system measures the HDR-dependent insertion of mRuby2 into a Cas9-mediated DSB in the LMNA gene, resulting in cellular expression of mRuby2-tagged lamin A/C (LMNA) that serves as readout for HDR activity. Representative fluorescence microscopy image of a cell expressing mRuby2-LMNA after HDR. Quantification of HDR activity after complementation of siPALB2 cells with indicated siRNA-resistant PALB2 construct. Data represents mean relative percentages (± SD) of mRuby2-positive cells among the YFP-positive population (n > 300 YFP-positive cells per condition) relative to the WT condition. (***) P < 0.001 and (****) P < 0.0001.

b) Scatter graphs with regression lines (in gray) correlating HDR activity with cell survival to olaparib or the mean number of RAD51 foci per cell. Values are expressed in percentage relative to WT (set to 100%). *R*2 values are shown.

INTERROGATE THOUSANDS OF VARIANTS IN THE SAME POOLED EXPERIMENT

VALIDITY

THROUGHPUT

Individual functional and genetic analysis

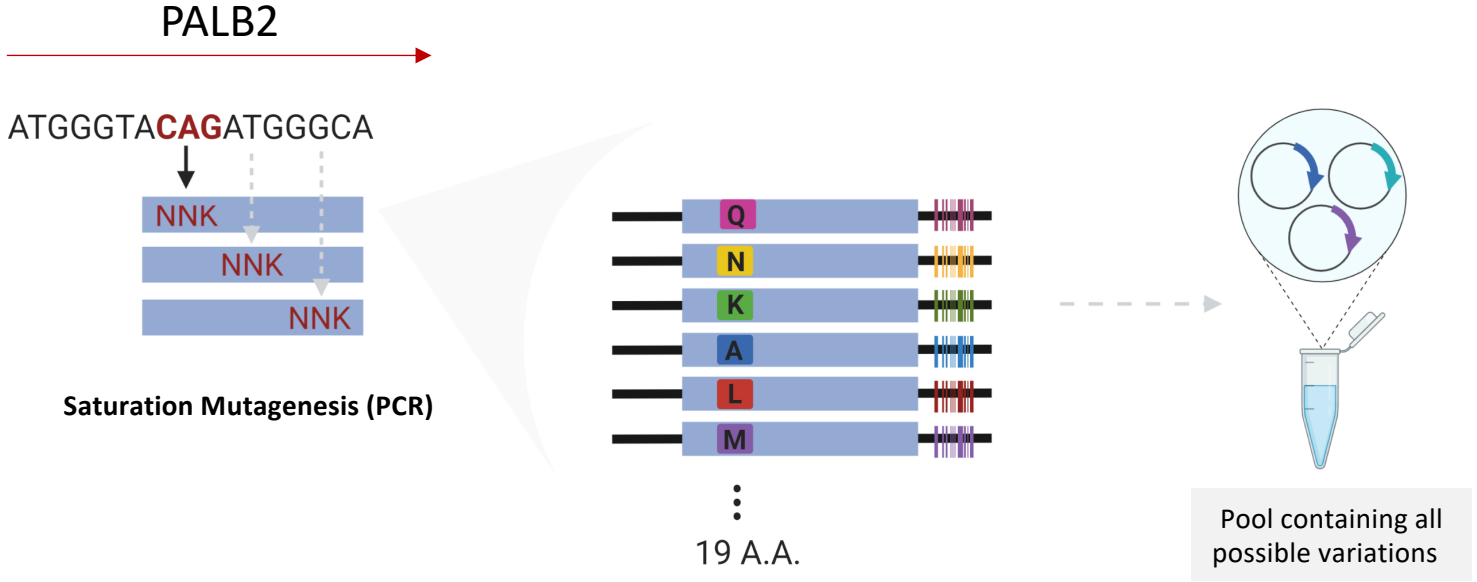
In Silico tools

MASSIVE PARALEL FUNCTIONAL ANALYSIS
MULTIPLEX ASSAYS OF VARIANT EFFECTS (MAVES)

HOW TO DO IT IN LARGE SCALE?

- Oligonucleotide libraries generated by massively parallel DNA synthesis - within a single experiment, program every single-nucleotide change or every possible amino acid change in a protein;
- Next-generation DNA sequencing - to track and quantify the functional effects of all these variants within a single experiment.

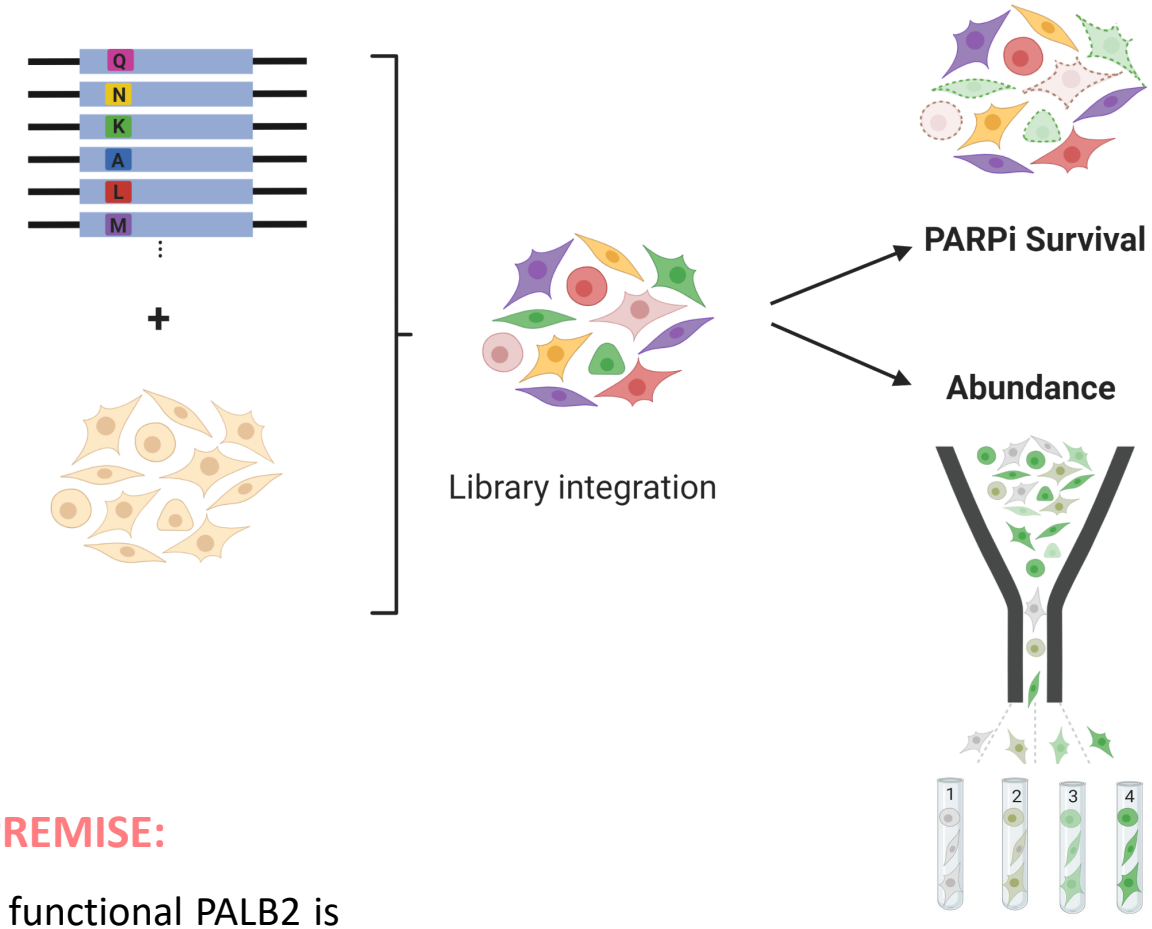
PALB2 SITE-SATURATION VARIANT LIBRARY



PALB2 LIBRARY - QUALITY CONTROL

Number of sites:	1186 amino acid positions
Requested # of variants:	23720 (20 aa/position)
Sites passed:	1133
Sites failed:	52
Variants passed QC:	95.4%

SCREENING STRATEGY



PREMISE:

- Loss of functional PALB2 is synthetic lethal with PARP inhibitors;
- PALB2 C-terminal presents a WD40 domain which is of great importance for PALB2 stability.

PARPi SENSITIVITY

Olap/BMN673 selection

Neutral variant = Enriched
Deleterious variant = Depleted

STABILITY OF VARIANTS (VAMP-Seq)

FACS ANALYSIS

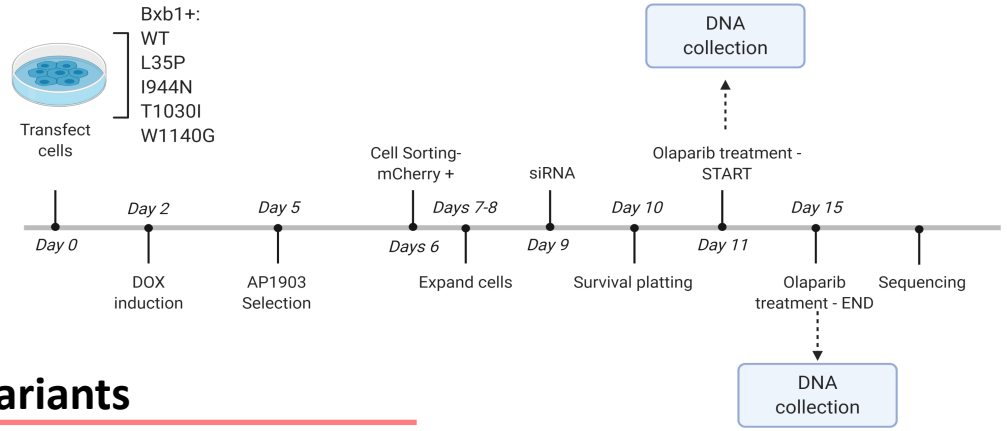
Neutral variant = High GFP signal
Deleterious variant = Low GFP signal

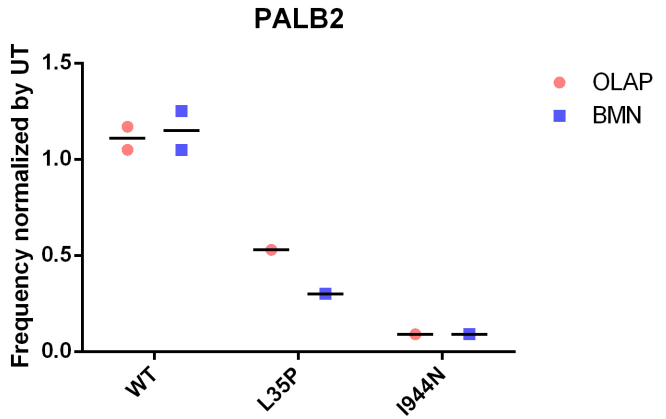
MINI-SCREEN WITH PALB2 VARIANTS- VALIDATION

PALB2 VARIANTS CHOSEN

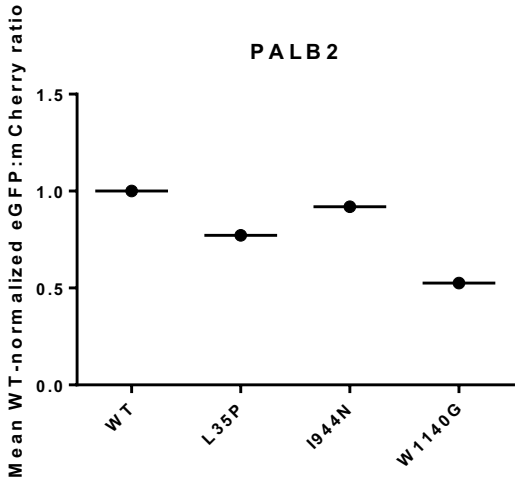
- L35P: inactivate the interaction with BRCA1
- W1140G: variants destabilizing PALB2 and affect nuclear localisation

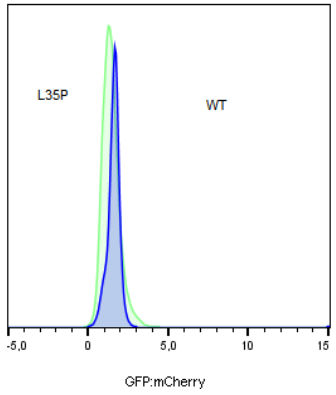
PARPi sensitivity

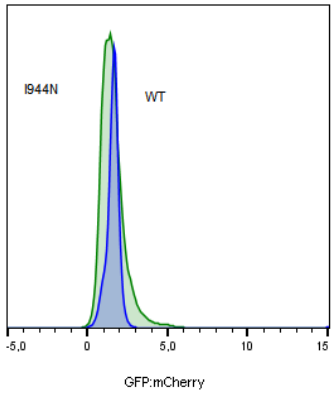


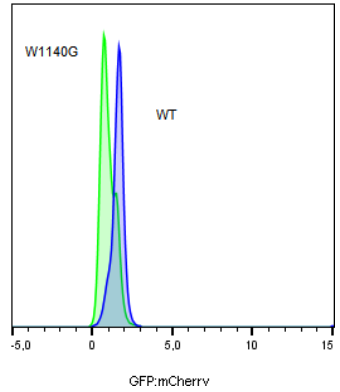


Stability of variants









SUMMARY

- Our comprehensive functional analysis reveals two hotspots for potentially deleterious variations within PALB2;
- Performing functional analysis for each newly identified variant is a very time-consuming strategy considering the increasing number of variants in public databases;
- Deleteriousness of PALB2 variants is being addressed by *multiplex* assays of variant effect (MAVEs) measuring the functional impact of a large library of variants simultaneously. The results generated from this approach will produce a variant effect map displaying the functional outcomes of all possible single variants in PALB2.
- Systematic functional assessment is necessary to comprehensively assess the impact of germline missense variants on PALB2 function, in order to guide proper classification of their deleteriousness and facilitate clinical and therapeutic management of carriers.

Thank you for your time!