

# FORX-06-428 IS A NOVEL PARG INHIBITOR WITH POTENT ANTI-TUMOR EFFICACY IN PRECLINICAL CANCER MODELS

Olivier Querolle<sup>1</sup>, Ulrich Lücking<sup>1</sup>, Luca Iacovino<sup>1</sup>, Alena Freudenmann<sup>1</sup>, Giacomo Rossetti<sup>1</sup>, Marina Gysin<sup>1</sup>, Serena Bologna<sup>1</sup>, Vasilis Dionellis<sup>1</sup>, Marta Malattia<sup>1</sup>, Alessandro Potenza<sup>1</sup>, Nicolas Bocquet<sup>1</sup>, Irena Konstantinova<sup>1</sup>, Andreas Goutopoulos<sup>1</sup>, Sotirios Sotiriou<sup>1</sup>, Thanos Halazonetis<sup>2</sup>, Tarig Bashir<sup>1</sup>, Frank T. Zenke<sup>1</sup>

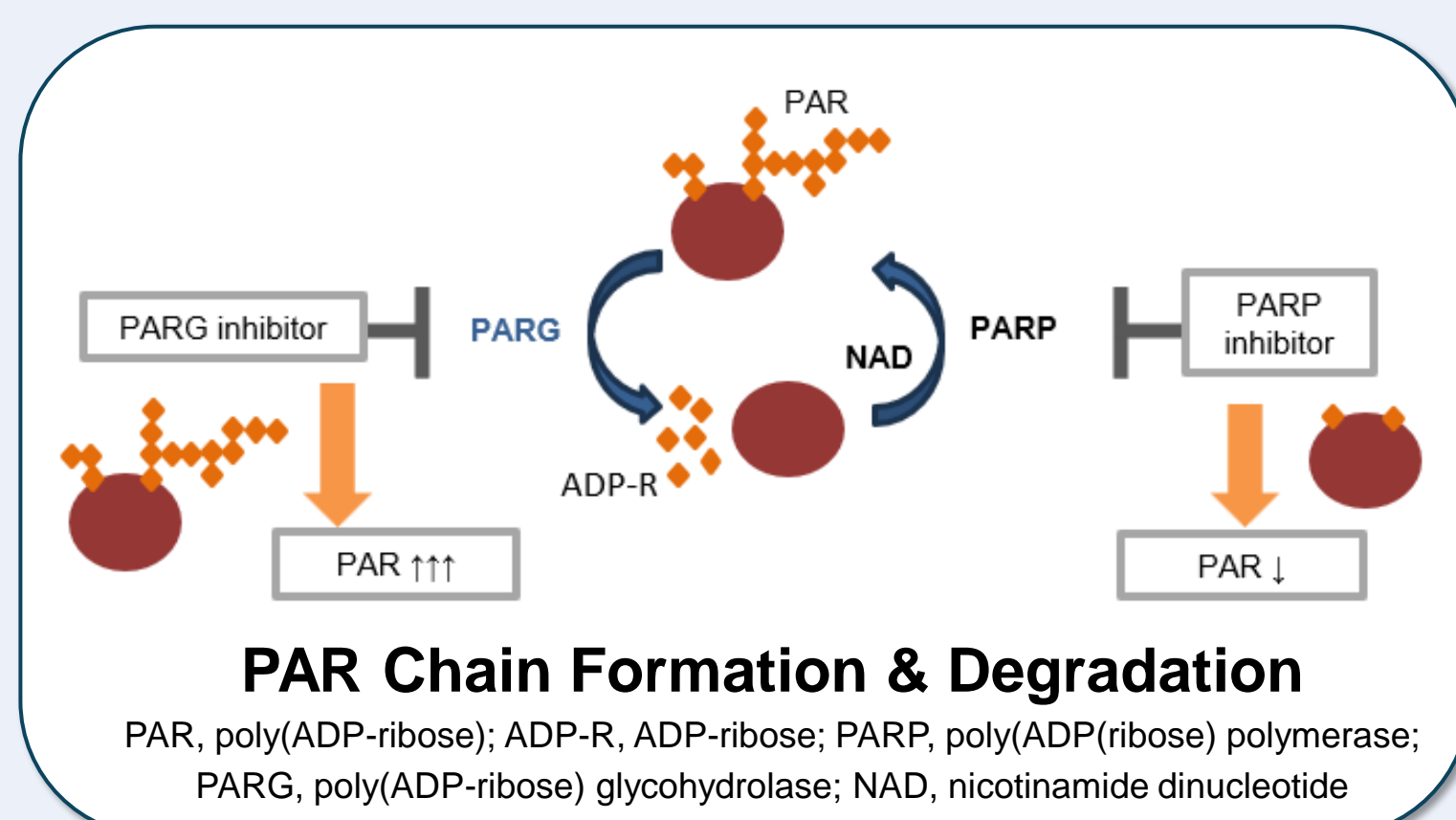
<sup>1</sup>FoRx Therapeutics AG, Lichtstrasse 35, CH-4056 Basel, Switzerland

<sup>2</sup>University of Geneva, Department of Molecular and Cellular Biology, 30 quai Ernest-Ansermet, CH-1211 Geneva, Switzerland

Poster # 3366

## INTRODUCTION:

- Formation of poly(ADP-ribose) (PAR) chains by PARP enzymes is a characteristic post-translational protein modification during repair of certain types of DNA damage
- PAR chains serve as docking platforms for DNA repair proteins that are recruited to sites of damage and resolve DNA lesions
- Equally important is the subsequent removal of PAR chains to conclude the repair process and restore genomic integrity
- Poly(ADP-ribose) glycohydrolase (PARG) is a key enzyme in this process<sup>1,2</sup> and has recently attracted significant attention as a novel cancer target triggering the search and discovery for novel small molecule inhibitors<sup>3,4</sup>
- Pharmacological inhibition of PARG blocks DNA repair; however, the mechanism of action of PARG inhibition is clearly different from PARP inhibition, opening a path to novel therapeutic opportunities



## KEY HIGHLIGHTS:

- FoRx-06-428** is a novel, orally bioavailable and highly potent, low molecular weight PARG inhibitor
- FoRx-06-428** shows selective reduction of viability in a panel of cancer cell lines, highlighting therapeutic potential in multiple cancer indications
- BRCA2 knockout strongly sensitized DLD1 to PARG inhibition suggesting that **homologous recombination repair deficiency** could be used as **predictive biomarker**
- FoRx-06-428** induces a dose-dependent accumulation of PAR chains *in vivo* which constitutes a tractable target engagement biomarker for clinical exploration
- FoRx-06-428** demonstrates compelling anti-tumor efficacy as single agent in several xenograft models (breast & ovarian cancer) with excellent tolerability

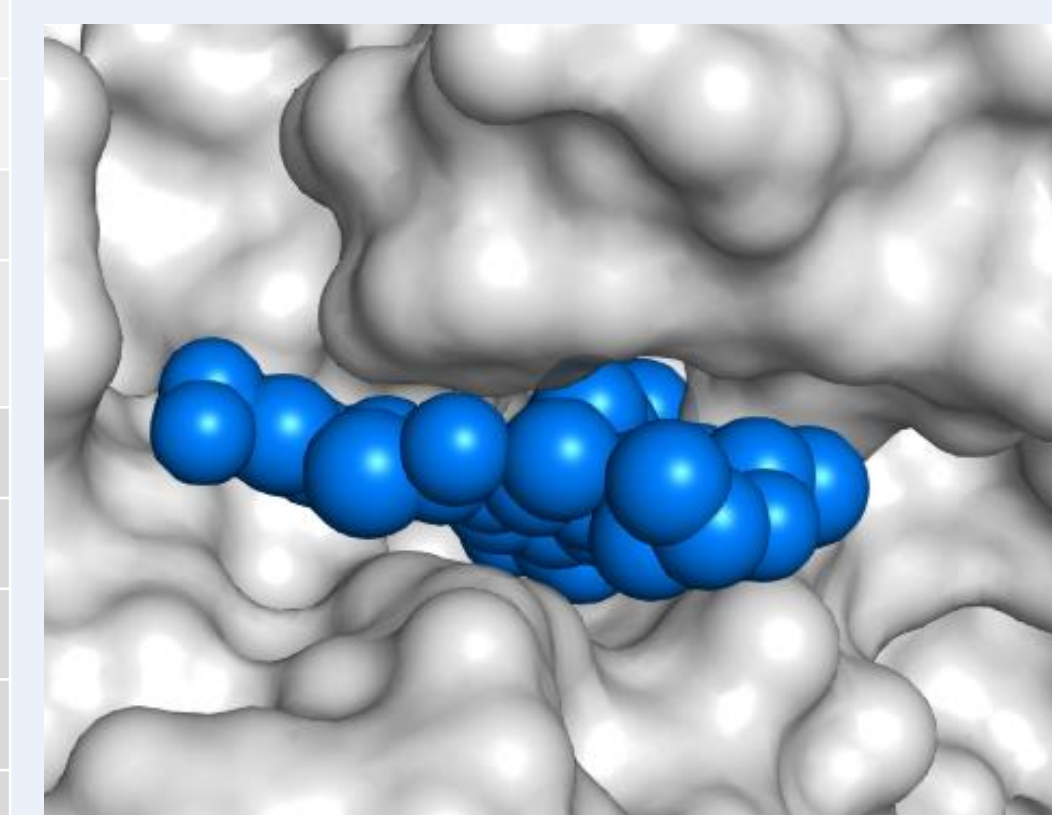
## RESULTS:

### FoRx-06-428 is a Potent and Selective PARG Inhibitor Showing DNA Damage Response in Cancer Cells

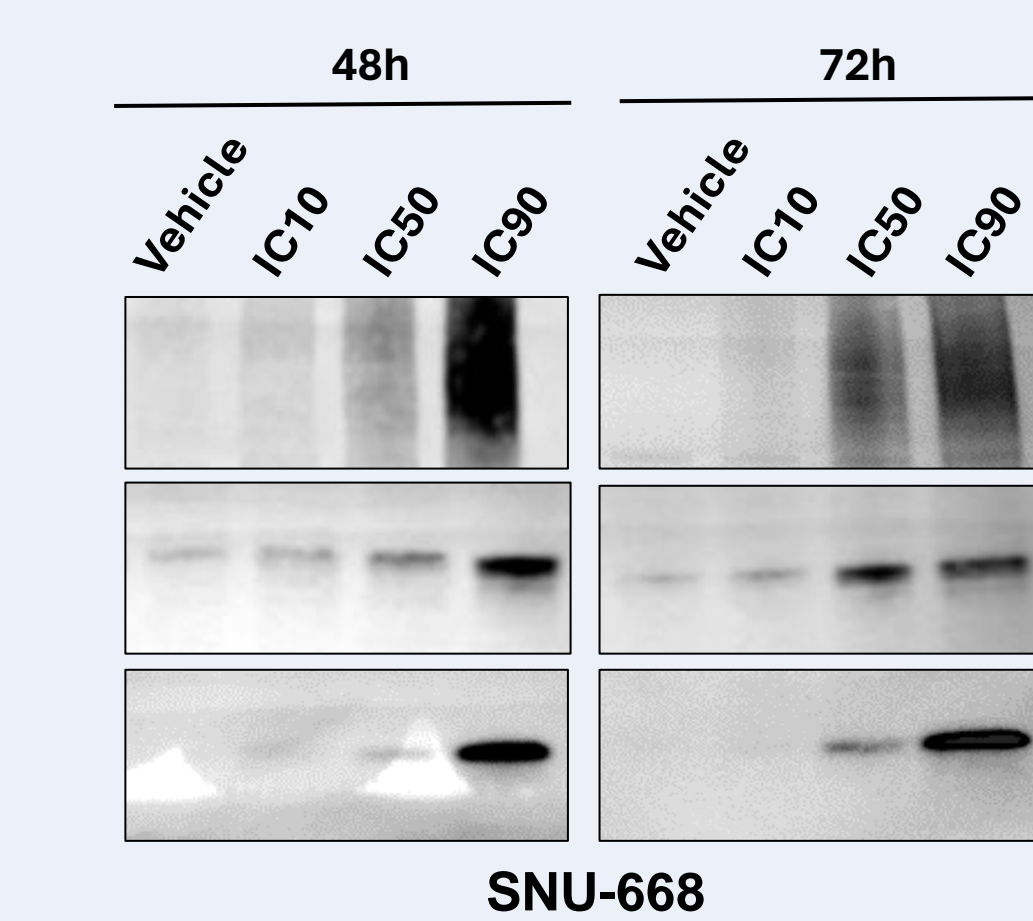
#### A) Pharmacology

Enzyme, IC <sub>50</sub> [nM]	PARG WT human	0.25
	PARG WT mouse	0.29
Binding	SPR (37°C) K <sub>d</sub> [nM]	0.09
	Target Residence Time [min]	> 90
Viability Assay IC <sub>50</sub> [nM]	MDA-MB-436 (breast)	19
	RMUG-S (ovarian)	0.60
	Kuramochi (ovarian)	0.57
	U2OS (osteosarcoma)	> 5,000
	HS68 (fibroblast)	> 10,000

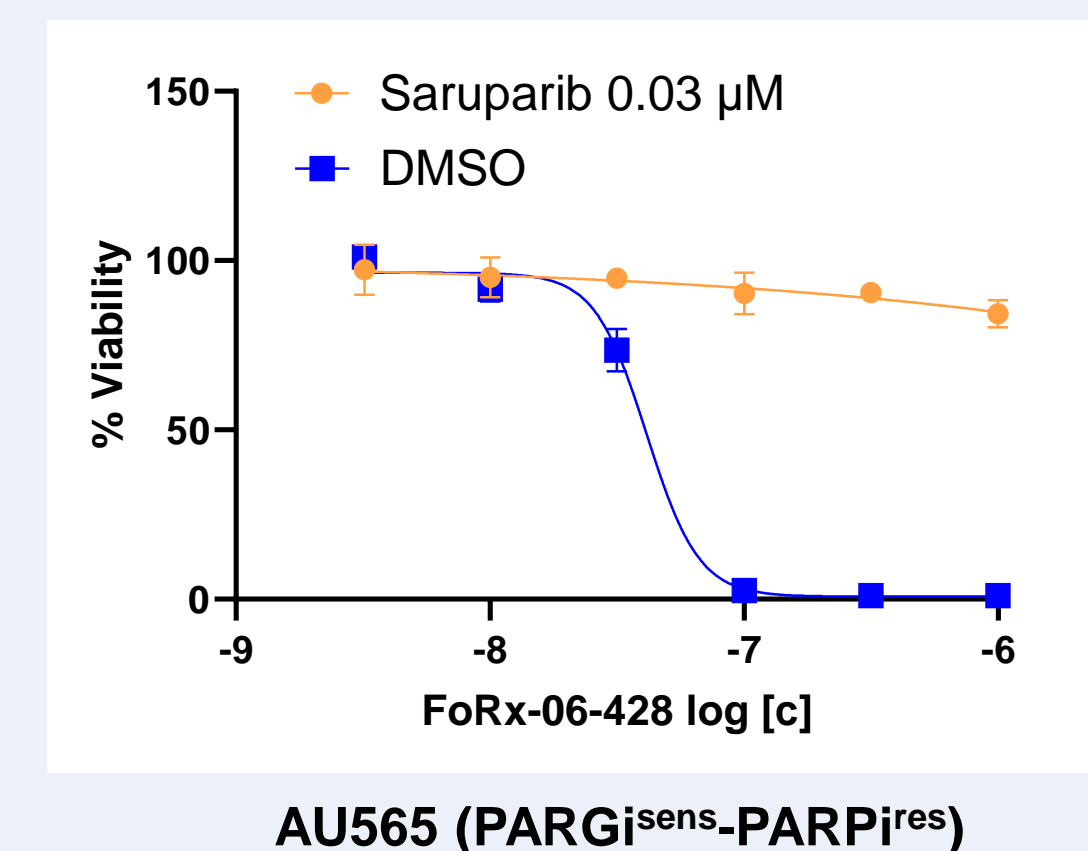
#### B) Co-Crystal Structure (2.5Å)



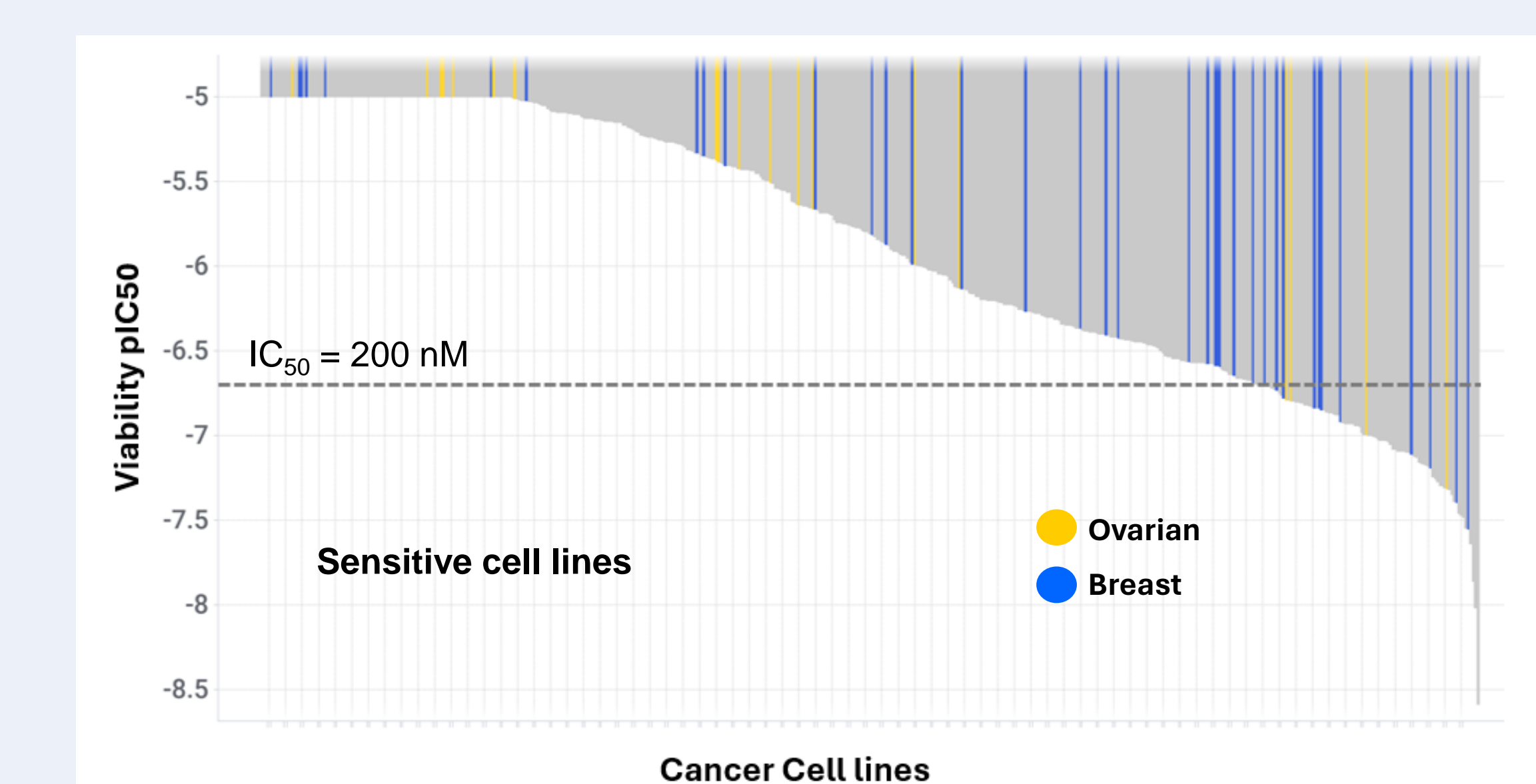
#### C) DNA Damage Response



#### D) Inhibition of Viability & Reversal by Saruparib



#### E) Cancer Cell Line Profiling



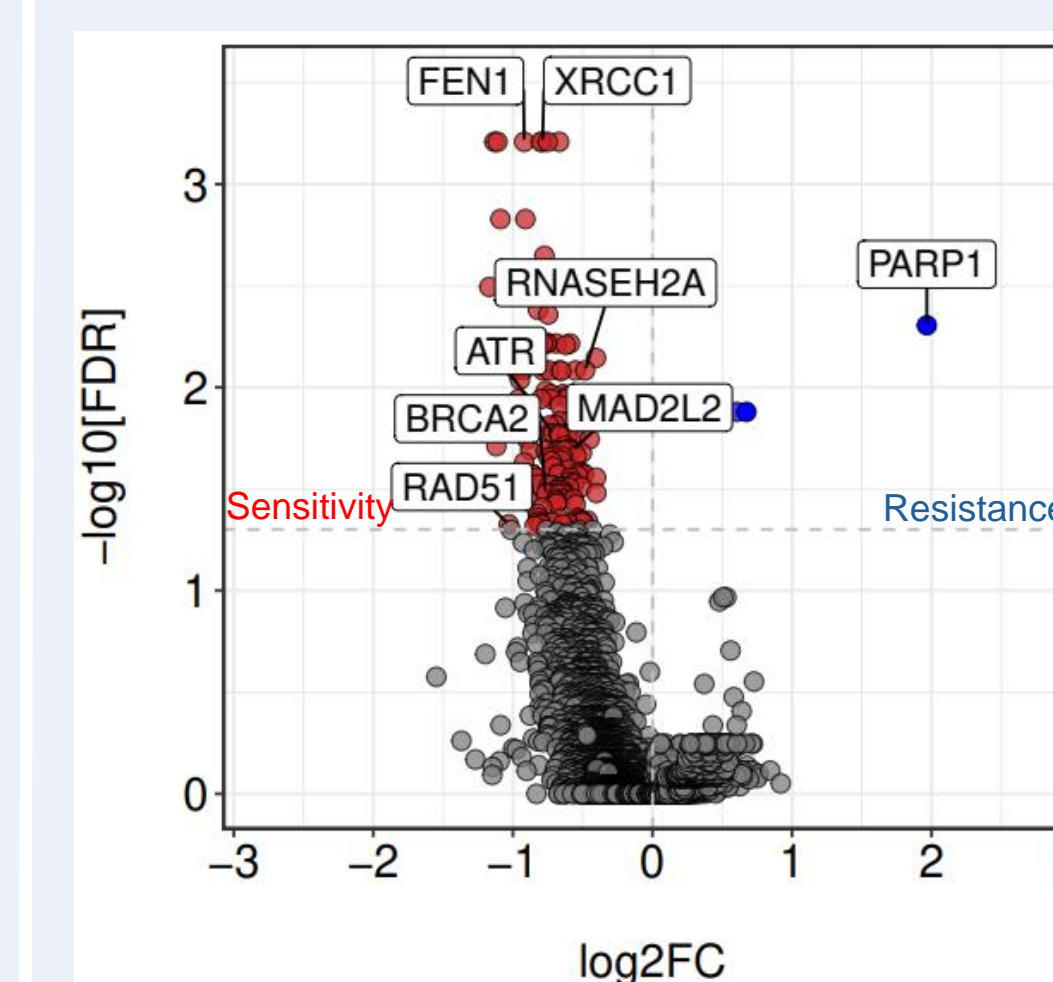
FoRx-06-428 was tested against 515 cancer cell lines in a Cell Titer Glo assay (5-day incubation, concentration range: 10 μM – 0.15 nM). 18% of cancer cell lines are inhibited at IC<sub>50</sub> ≤ 200 nM. Ovarian and breast cancer cell lines are marked in yellow and blue, respectively.

## BIBLIOGRAPHY:

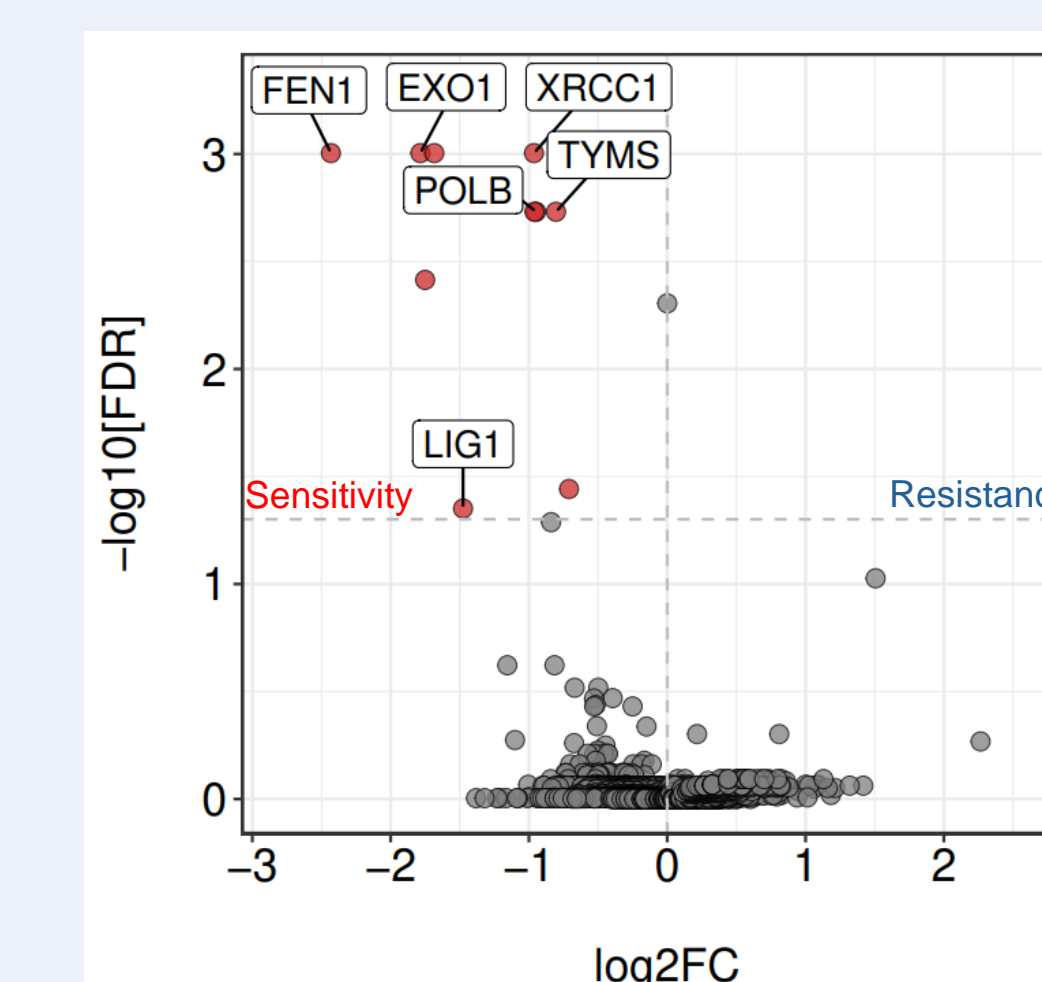
- 1) Pillay et al., *Cancer Cell* **2019**, 35, 519–533; 2) McDermott et al., *Cancer Cell* **2019**, 35, 344–346; 3) James et al., *ACS Chem. Biol.* **2016**, 11, 11, 3179–3190; 4) Waszkowycz et al., *J. Med. Chem.* **2018**, 61, 23, 10767–10792

### Identification and Validation of Predictive Biomarkers

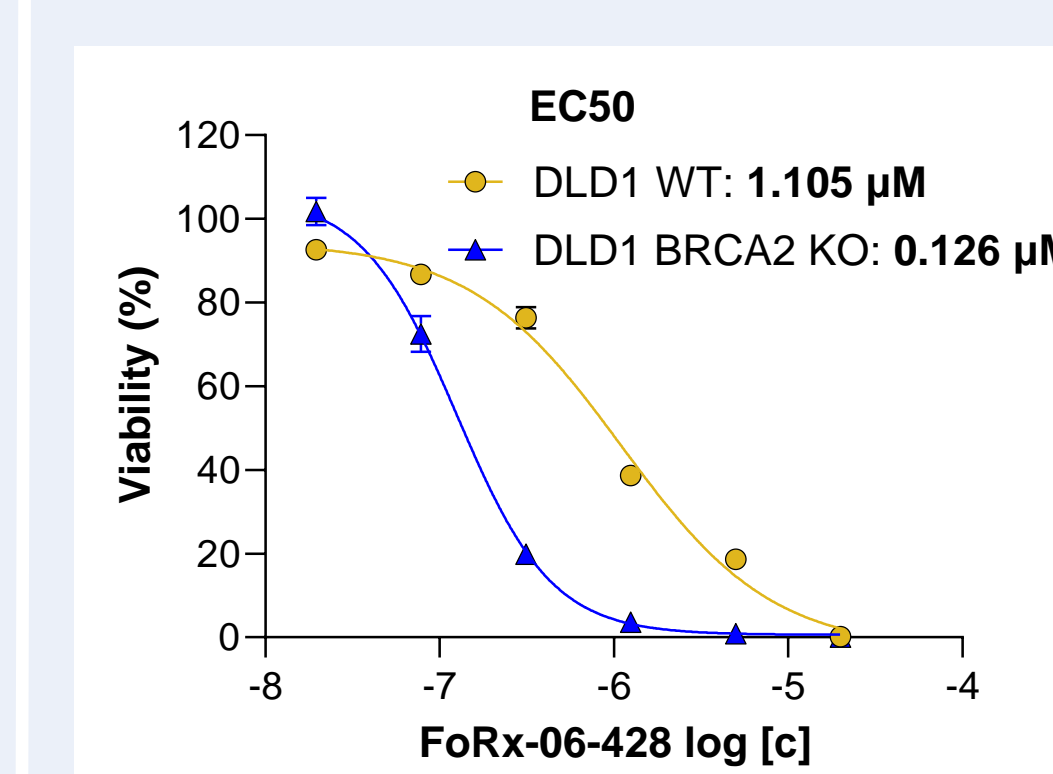
#### A) CRISPR screen, U87MG



#### B) CRISPR screen, U2OS



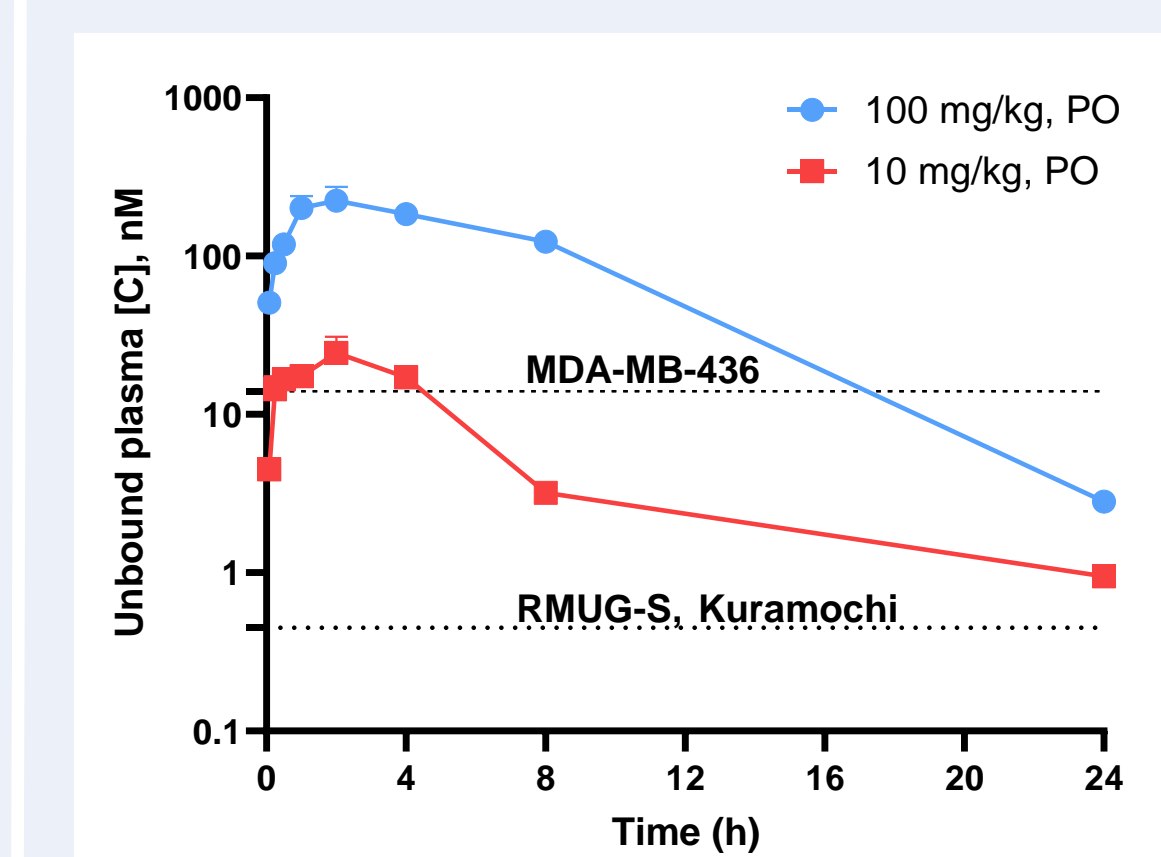
#### C) Viability, DLD1 WT & DLD1 BRCA2 KO



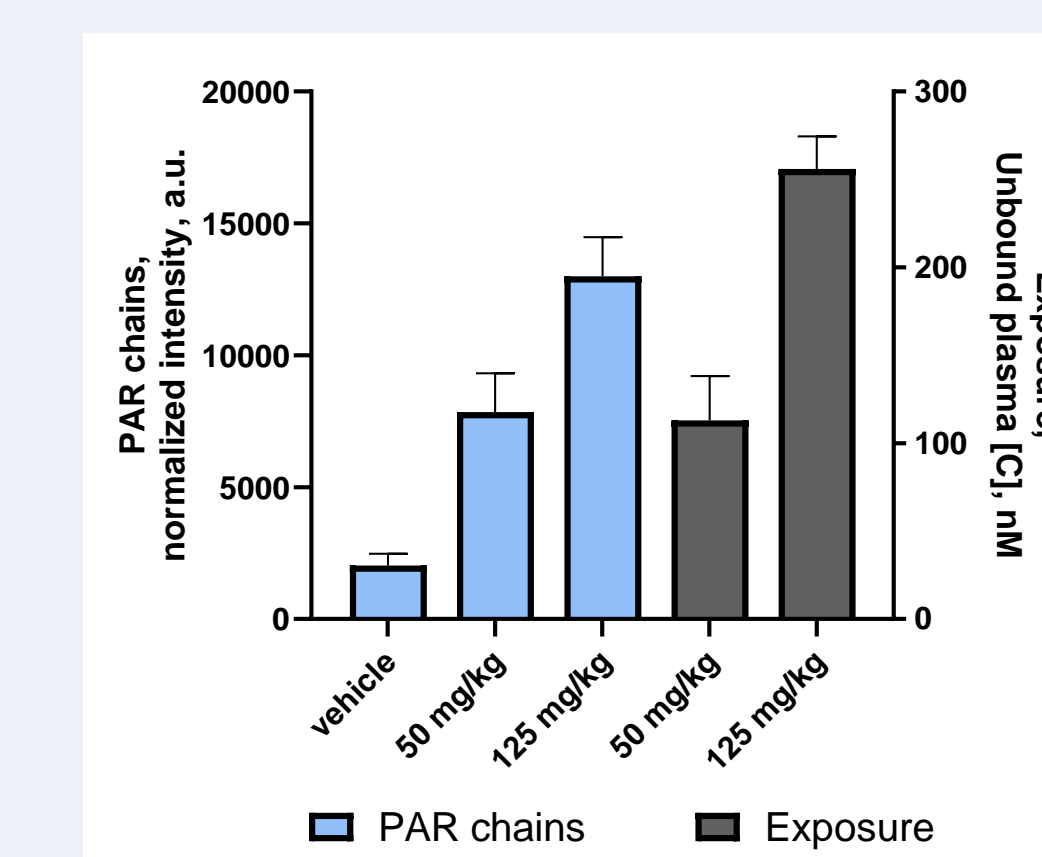
**A, B)** Results from two genome-wide CRISPR sensitizer screens. PARG inhibitors were used at 0.5 μM (PARGi A) for the glioblastoma line U87MG and at 2.5 μM (PARGi B) for the osteosarcoma line U2OS. Statistically significant genetic drop-out and enriched hits are colored in red and blue, respectively. **C)** Inhibition of viability by FoRx-06-428 in DLD1 wildtype cells and DLD1 BRCA2 KO cells (7 days incubation) validating BRCA2 loss-of-function as a sensitizer to PARG inhibition.

### Exposure Evaluation & Target Engagement

#### A) Pharmacokinetics



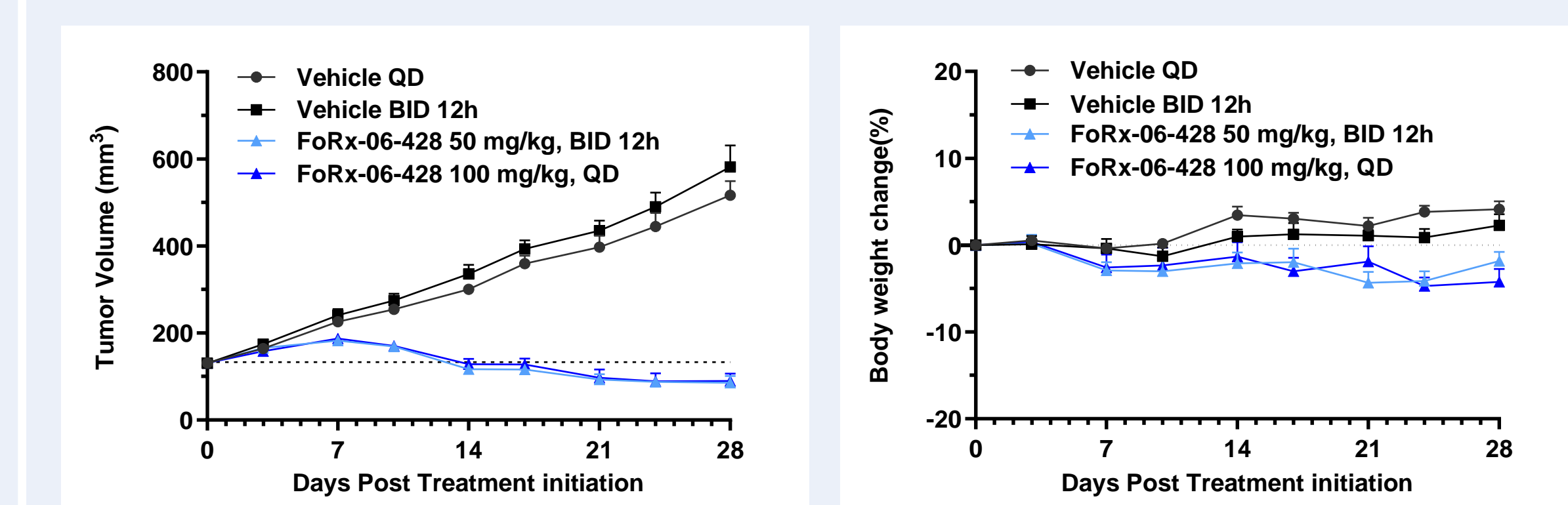
#### B) Target Engagement



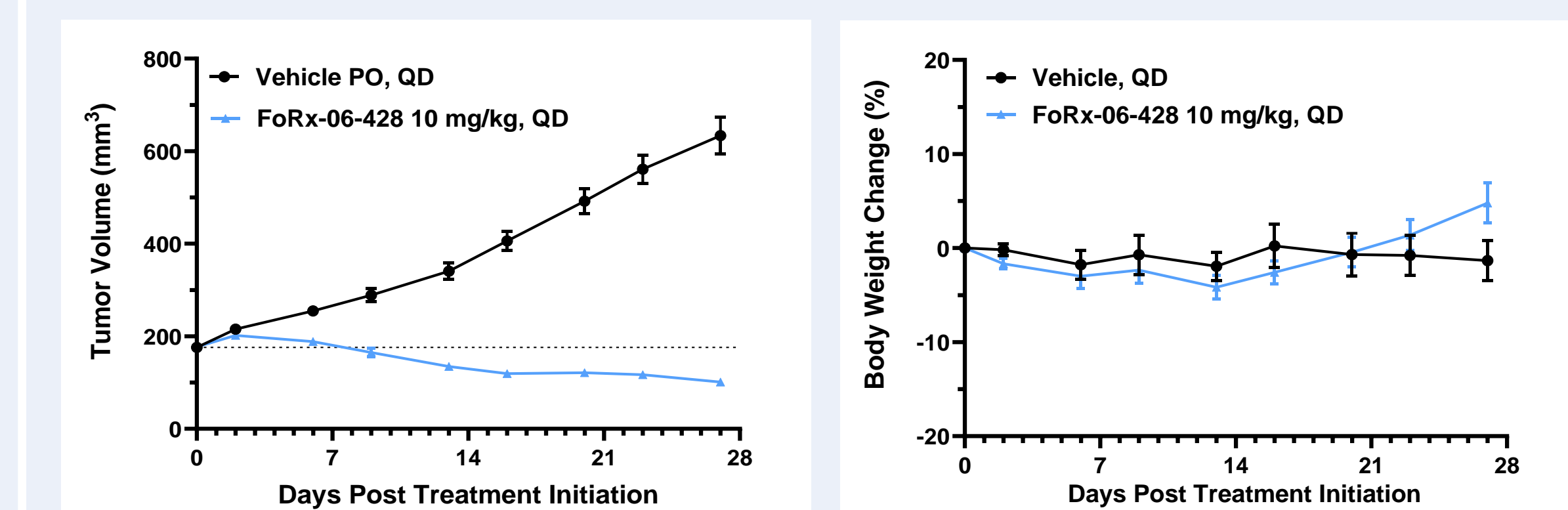
**A)** Plasma pharmacokinetics of FoRx-06-428 in BALB/c nude female mice (n=3 per group) following single dose administration by oral gavage of 10 and 100 mg/kg (All data points are the mean of 3 measurements except 10 mg/kg, 24h for which plasma exposure was detected in only one mouse). Breast and ovarian cancer cell lines IC<sub>50</sub> (unbound) represented by dotted lines. **B)** Dose-dependent PAR chain accumulation in MDA-MB-436 xenograft tumors and plasma exposure (unbound) on day 3 at 2 h following QD dosing with FoRx-06-428

### Anti-Tumor Efficacy in Human Breast and Ovarian Cancer Xenograft Models

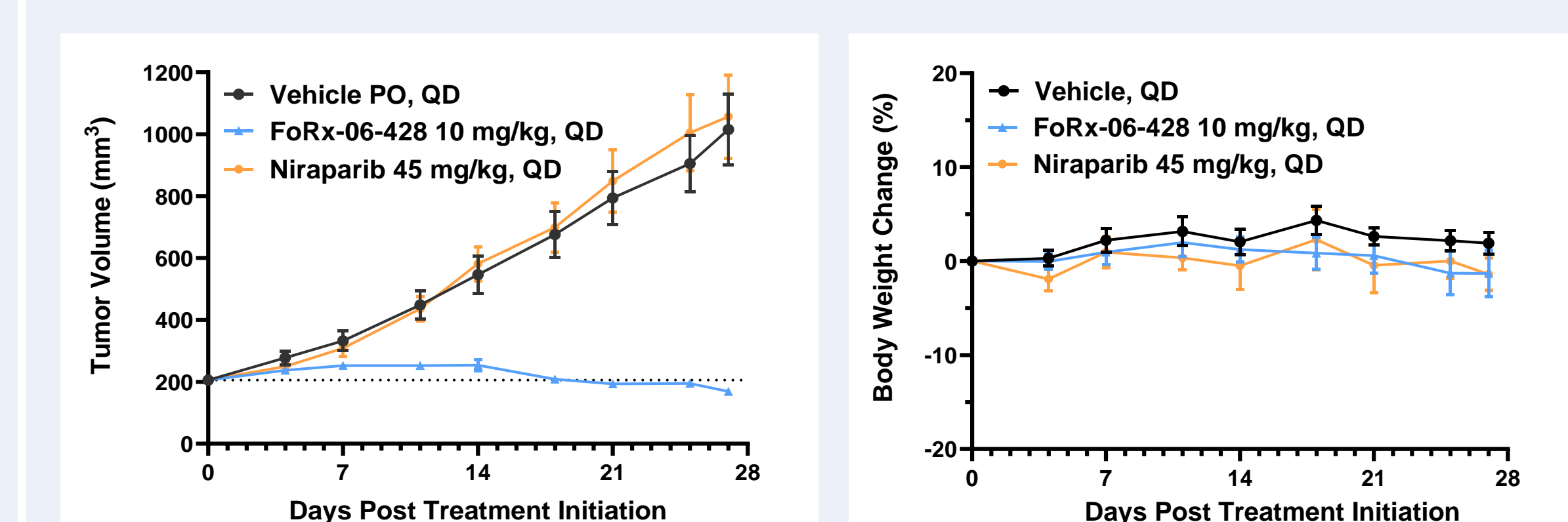
#### A) MDA-MB-436



#### B) RMUG-S



#### C) Kuramochi



**A, B, C)** MDA-MB-436, RMUG-S or Kuramochi tumor-bearing mice were treated orally daily with FoRx-06-428 and/or Niraparib for 28 days. Tumor volume & body weight curves are shown.

## CONCLUSIONS:

Collectively, our data show that FoRx-06-428 is a highly potent PARG inhibitor with an excellent pharmacology profile combined with robust efficacy in multiple xenograft models, which warrants further development of this compound.

AACR annual meeting 2024, San Diego

