

FORX-428: A Novel, Potent PARG Inhibitor Demonstrating Strong Anti-Tumor Activity in Preclinical Cancer Models

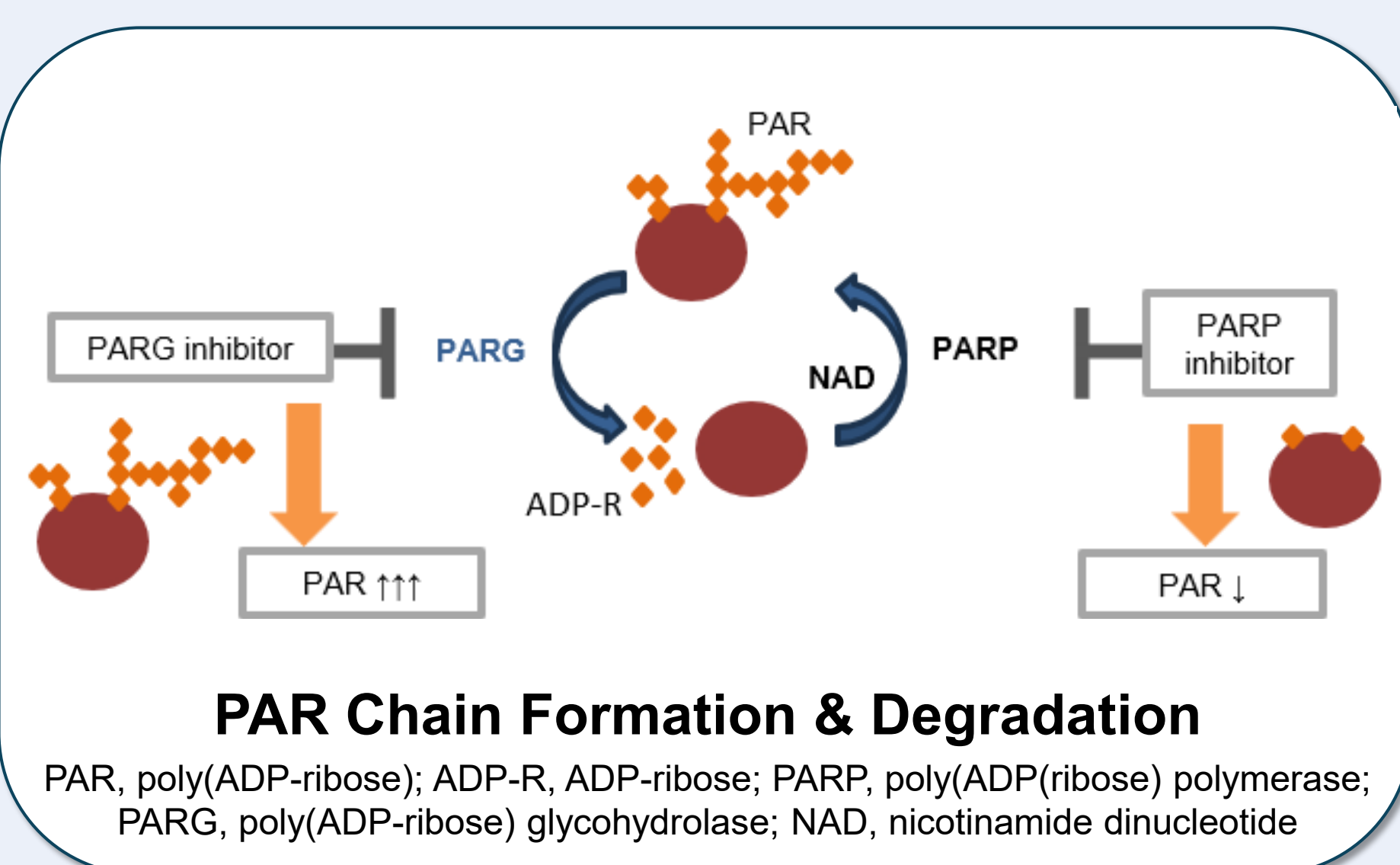
Olivier Querolle¹, Ulrich Lücking¹, Luca Iacovino¹, Alena Freudenmann¹, Giacomo Rossetti¹, Marina Gysin¹, Serena Bologna¹, Hanna Kok¹, Vasilis Dionellis¹, Marta Malattia¹, Alessandro Potenza¹, Nicolas Bocquet¹, Irena Konstantinova¹, Andreas Goutopoulos¹, Sotirios Sotiriou¹, Thanos Halazonetis², Tarig Bashir¹, Frank T. Zenke¹

¹FoRx Therapeutics AG, Lichtstrasse 35, CH-4056 Basel, Switzerland

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

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^{1,2} and has recently attracted significant attention as a novel cancer target triggering the search and discovery for novel small molecule inhibitors^{3,4}
- 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-428** is a novel, orally bioavailable and highly potent, low molecular weight PARG inhibitor
- FORX-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-428** induces a dose-dependent accumulation of PAR chains *in vivo* which constitutes a tractable target engagement biomarker for clinical exploration
- FORX-428** demonstrates compelling anti-tumor efficacy as single agent in several xenograft models (breast & ovarian cancer) with excellent tolerability

BIBLIOGRAPHY

- 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

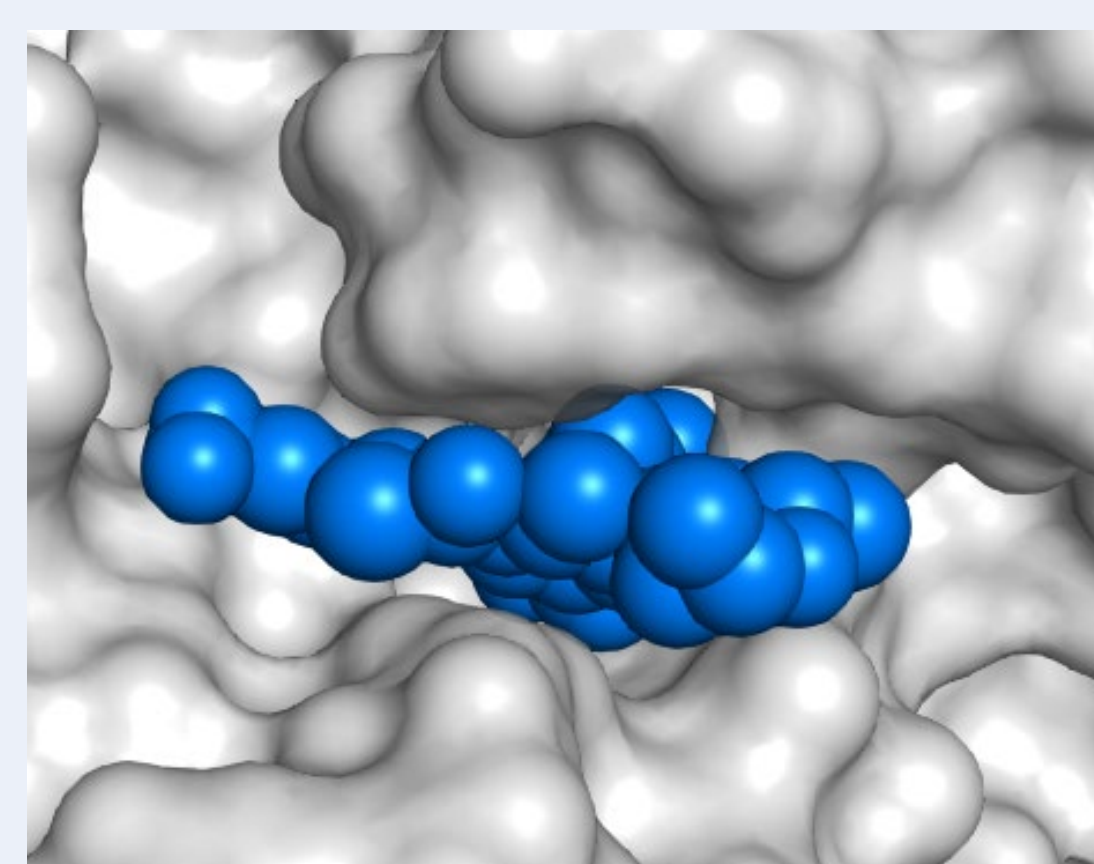
RESULTS

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

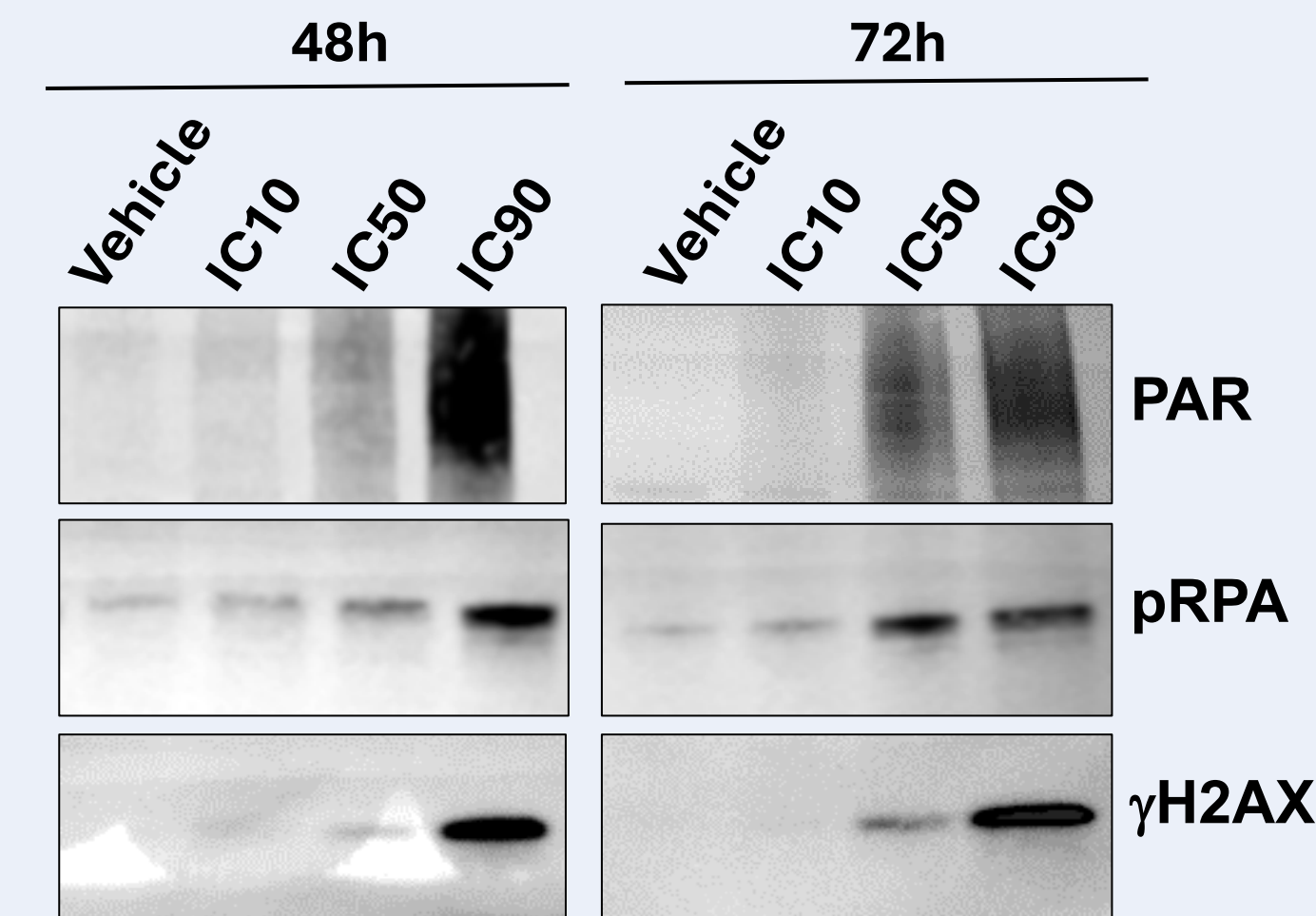
A) Pharmacology

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

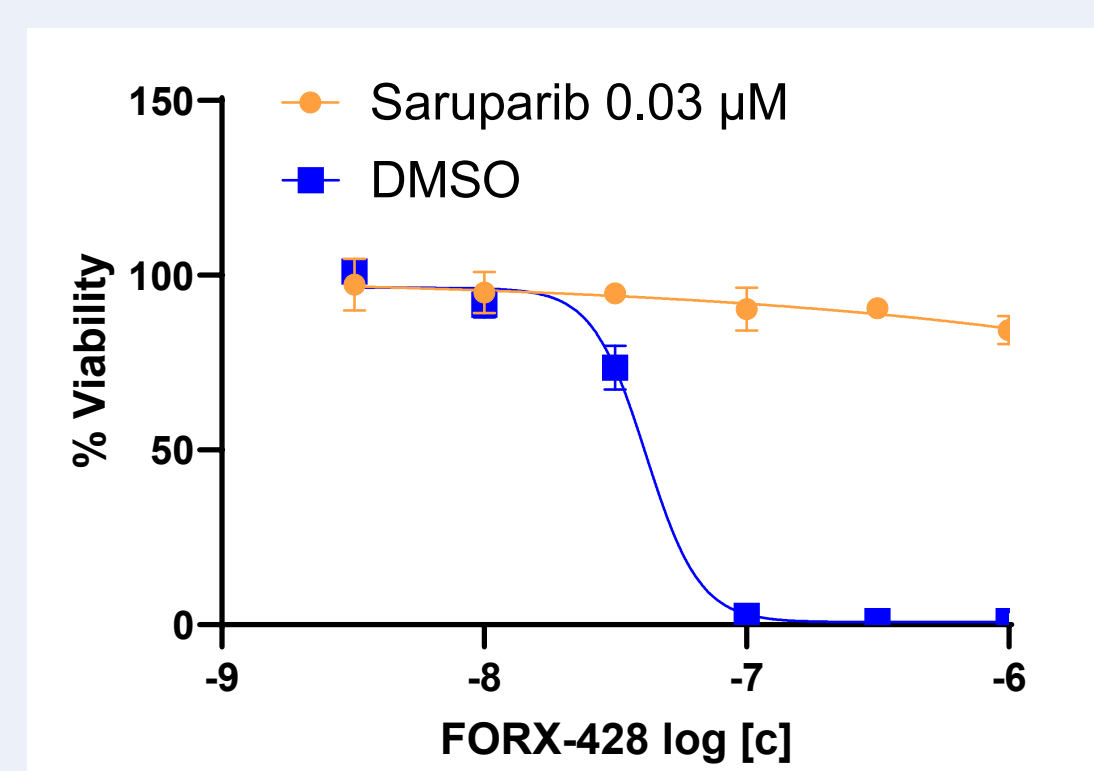
B) Co-Crystal Structure (2.5Å)



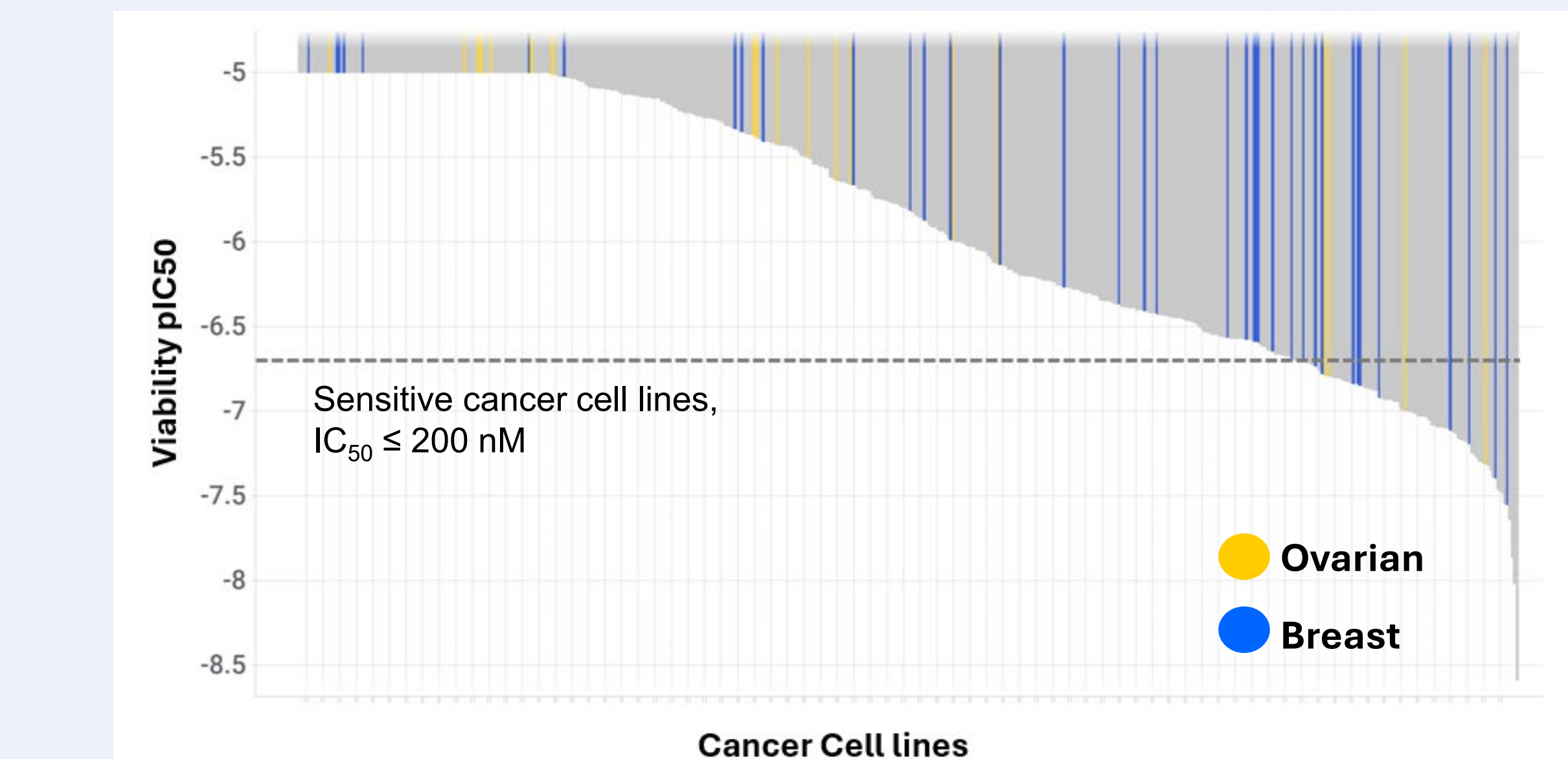
C) DNA Damage Response



D) Inhibition of Viability & Reversal by Saruparib



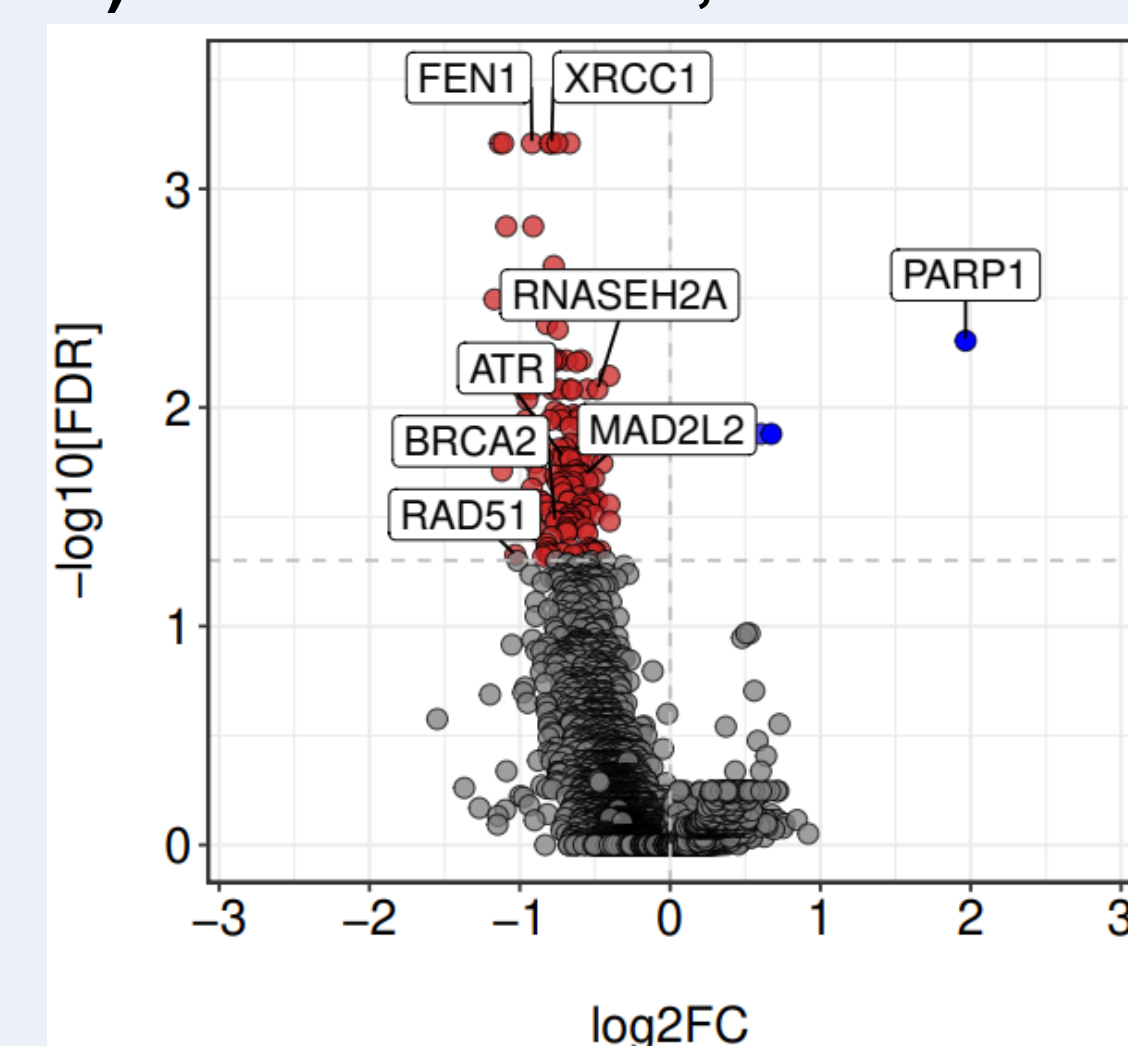
E) Cancer Cell Line Profiling



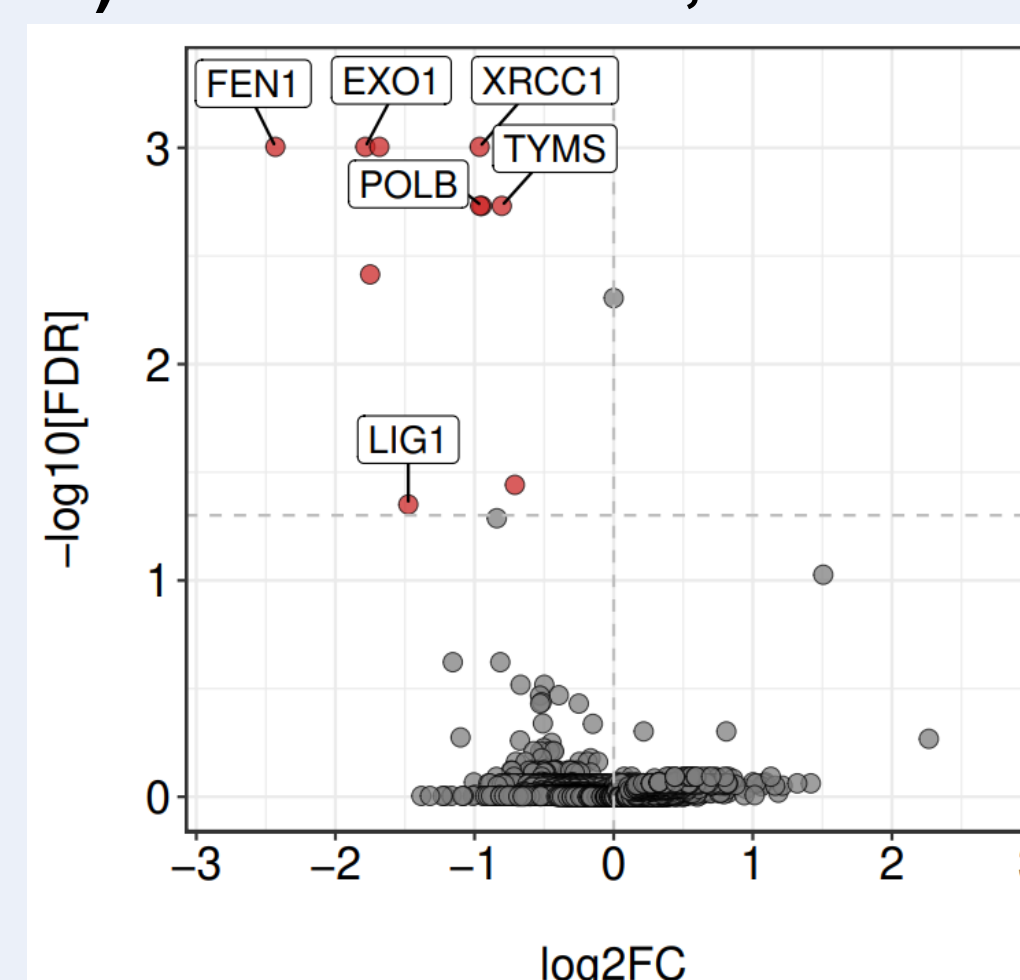
FORX-428 was tested against 515 cancer cell lines in a Cell Titer Glo assay (5-day incubation, concentration range: 10 μM – 0.15 nM). 18% of cancer cell lines are inhibited at IC₅₀ ≤ 200 nM. Ovarian and breast cancer cell lines are marked in yellow and blue, respectively.

Identification and Validation of Predictive Biomarkers

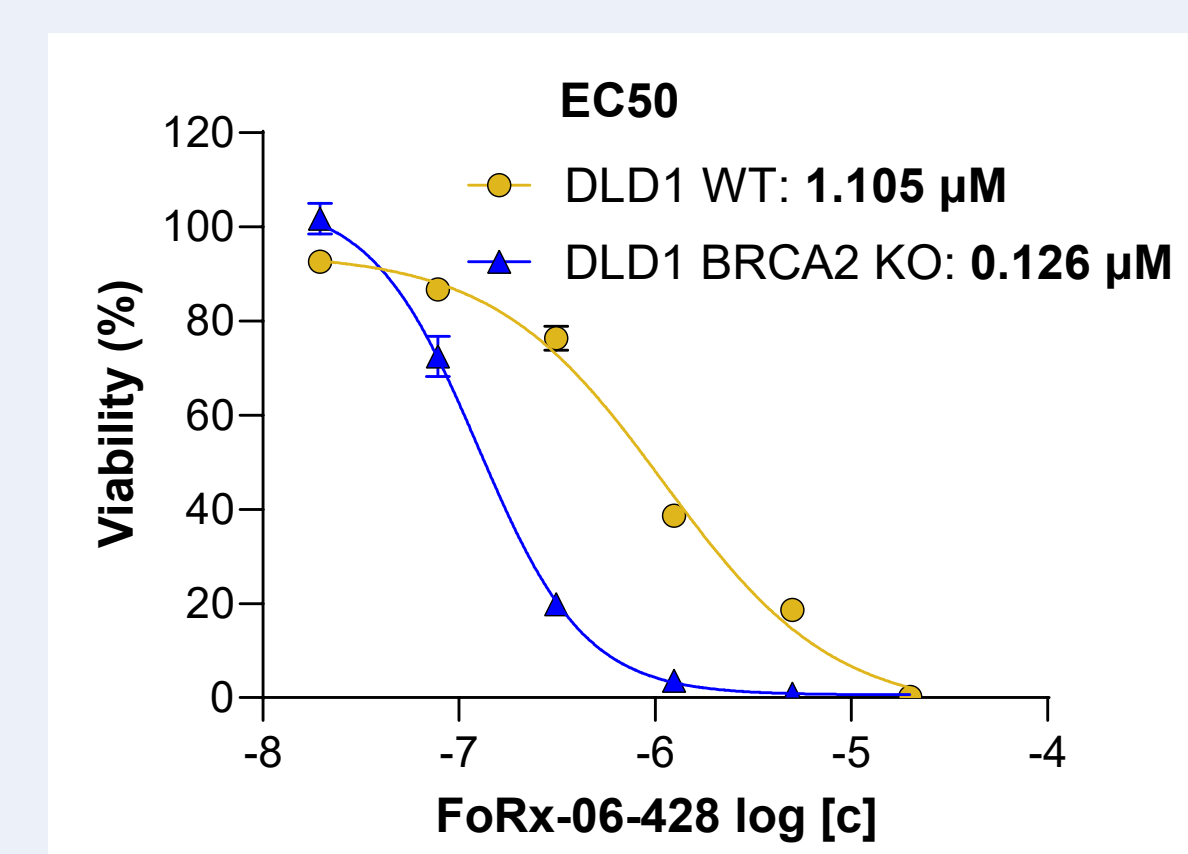
A) CRISPR screen, U87MG



B) CRISPR screen, U2OS



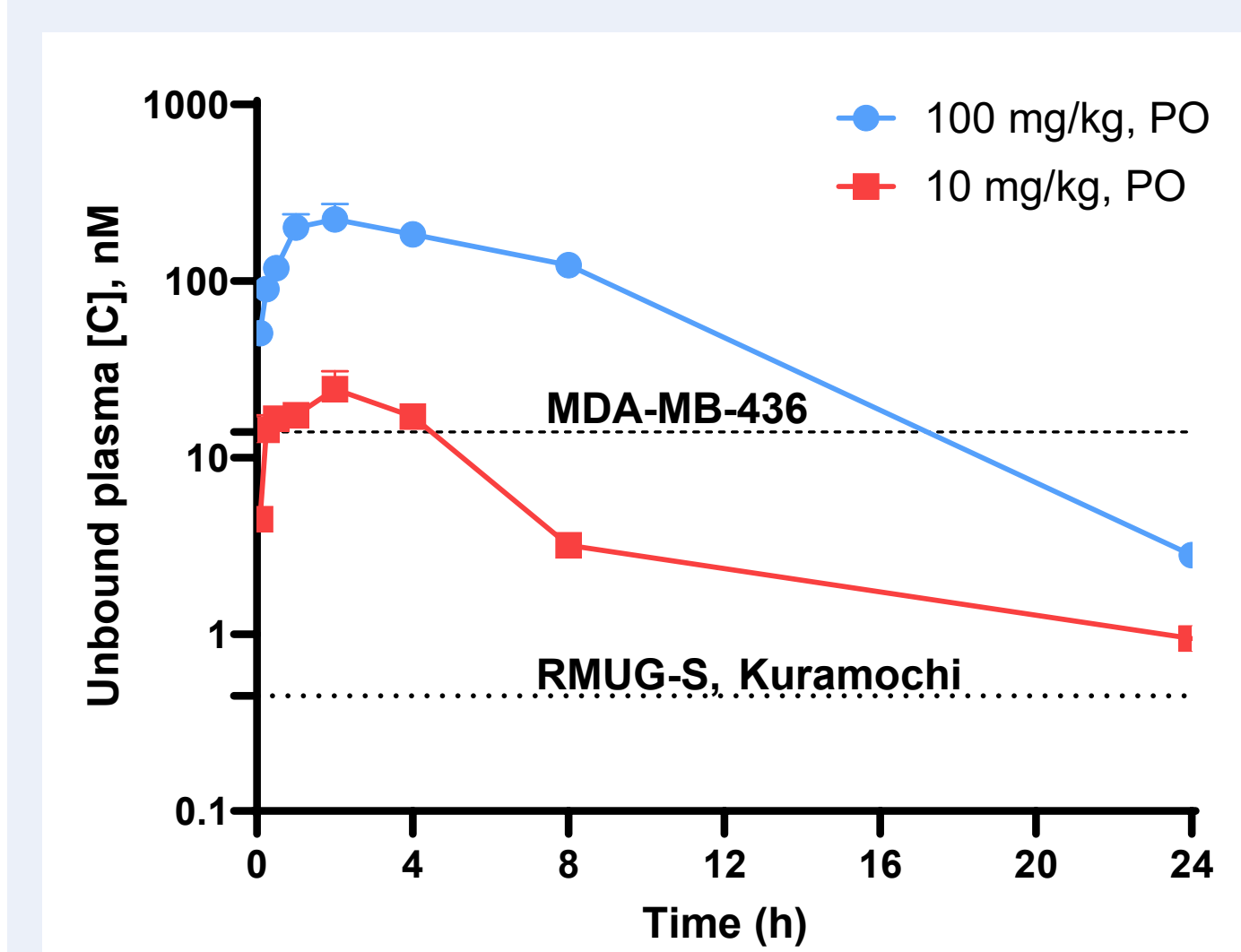
C) Viability, DLD1 WT & DLD1 BRCA2 KO



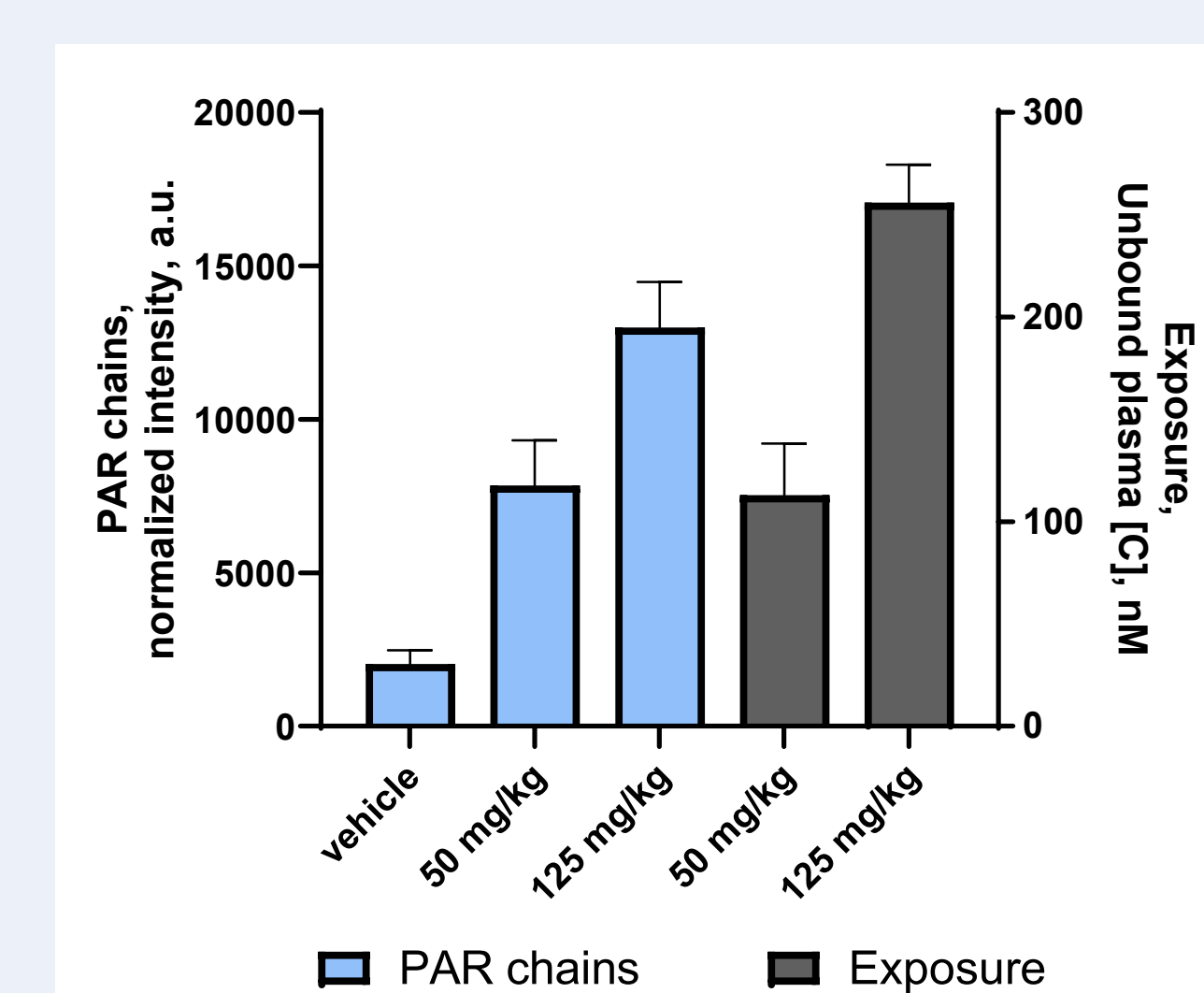
A, B) Results from two genome-wide CRISPR sensitizer screens. PARG inhibitors were used at 0.5 μM (PARGi A) for the glioblastoma line U87MG and at 2.5 μM (PARGi B) for the osteosarcoma line U2OS. Statistically significant genetic drop-out and enriched hits are colored in red and blue, respectively. C) Inhibition of viability by FORX-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



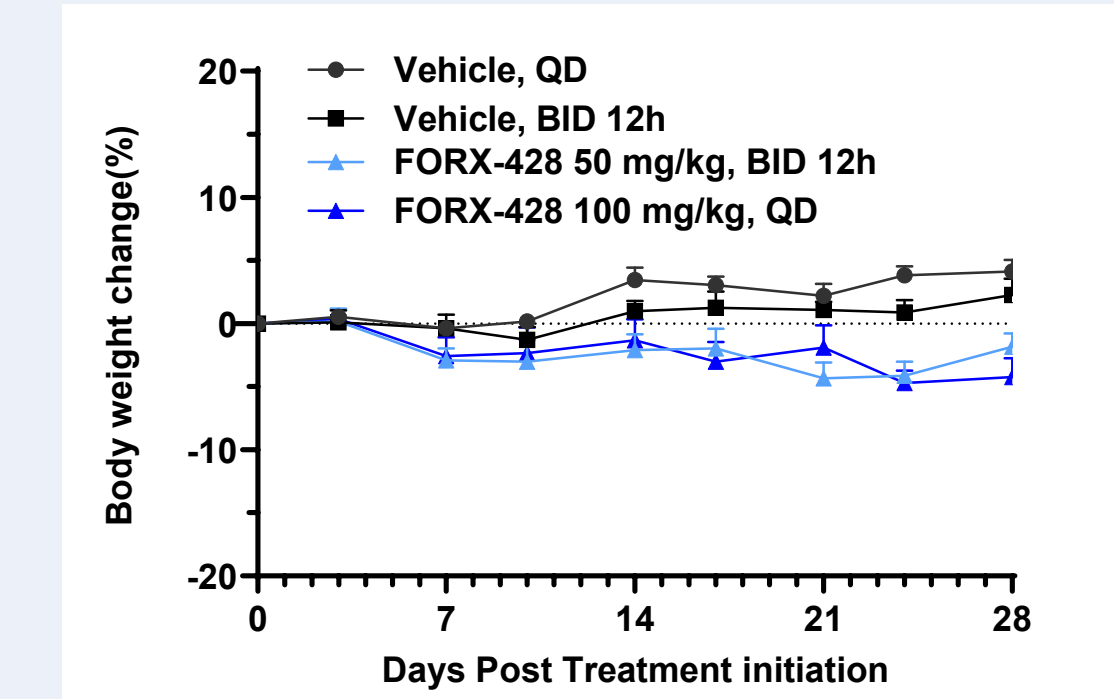
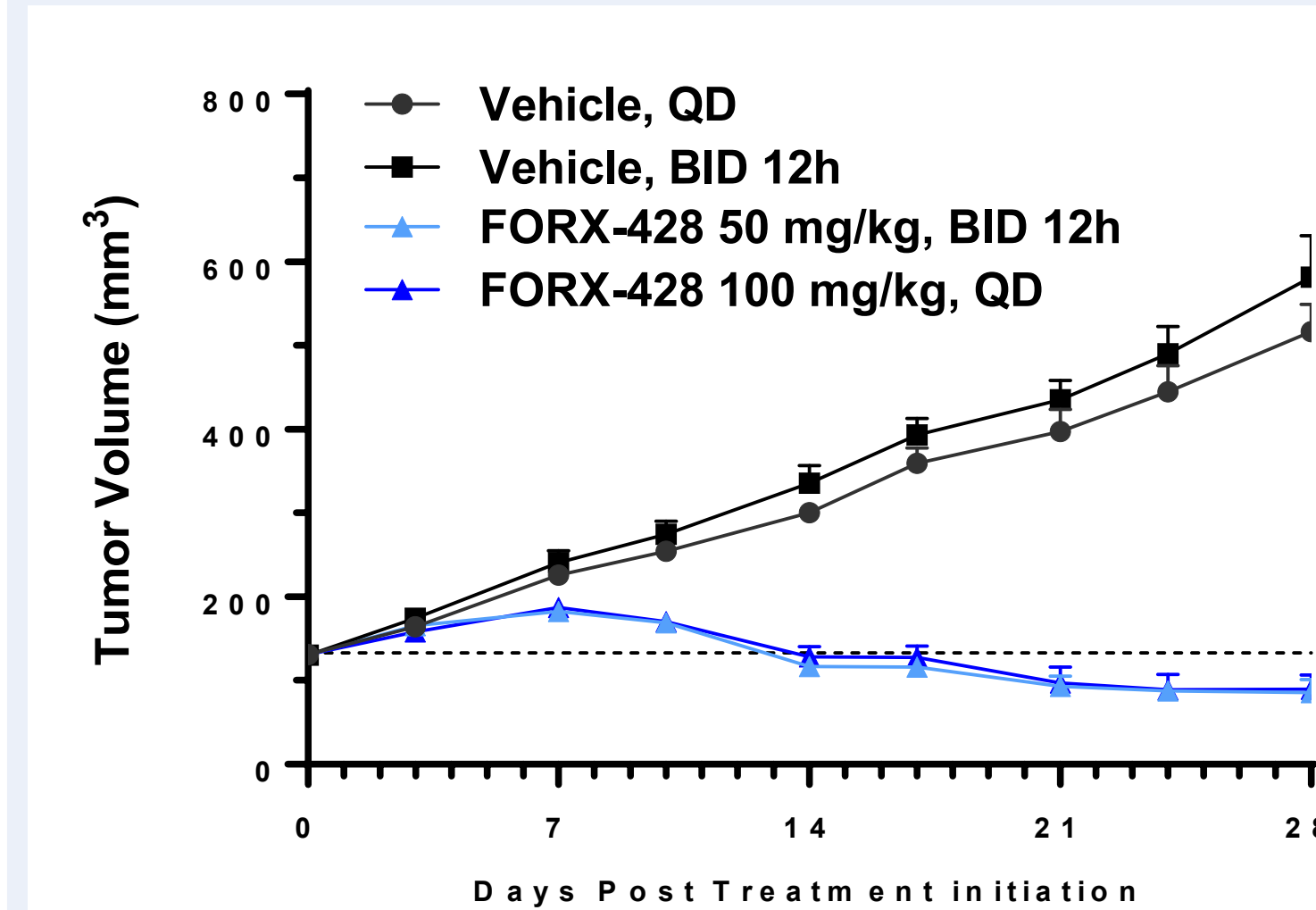
B) Target Engagement



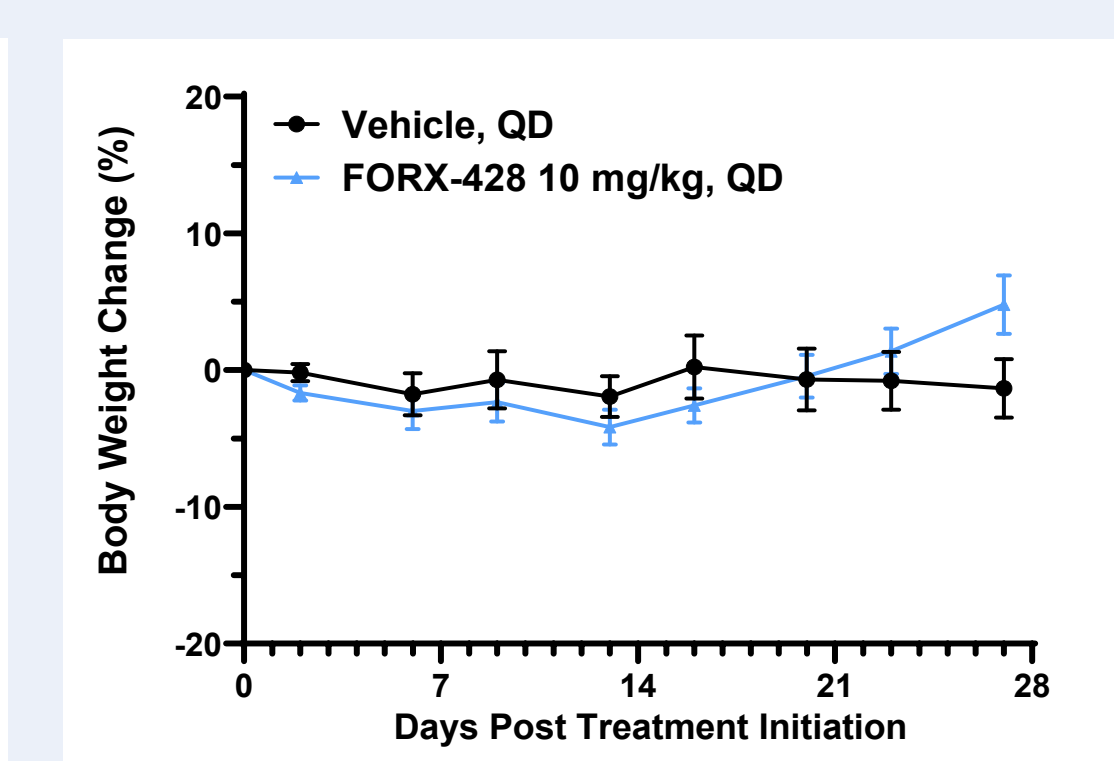
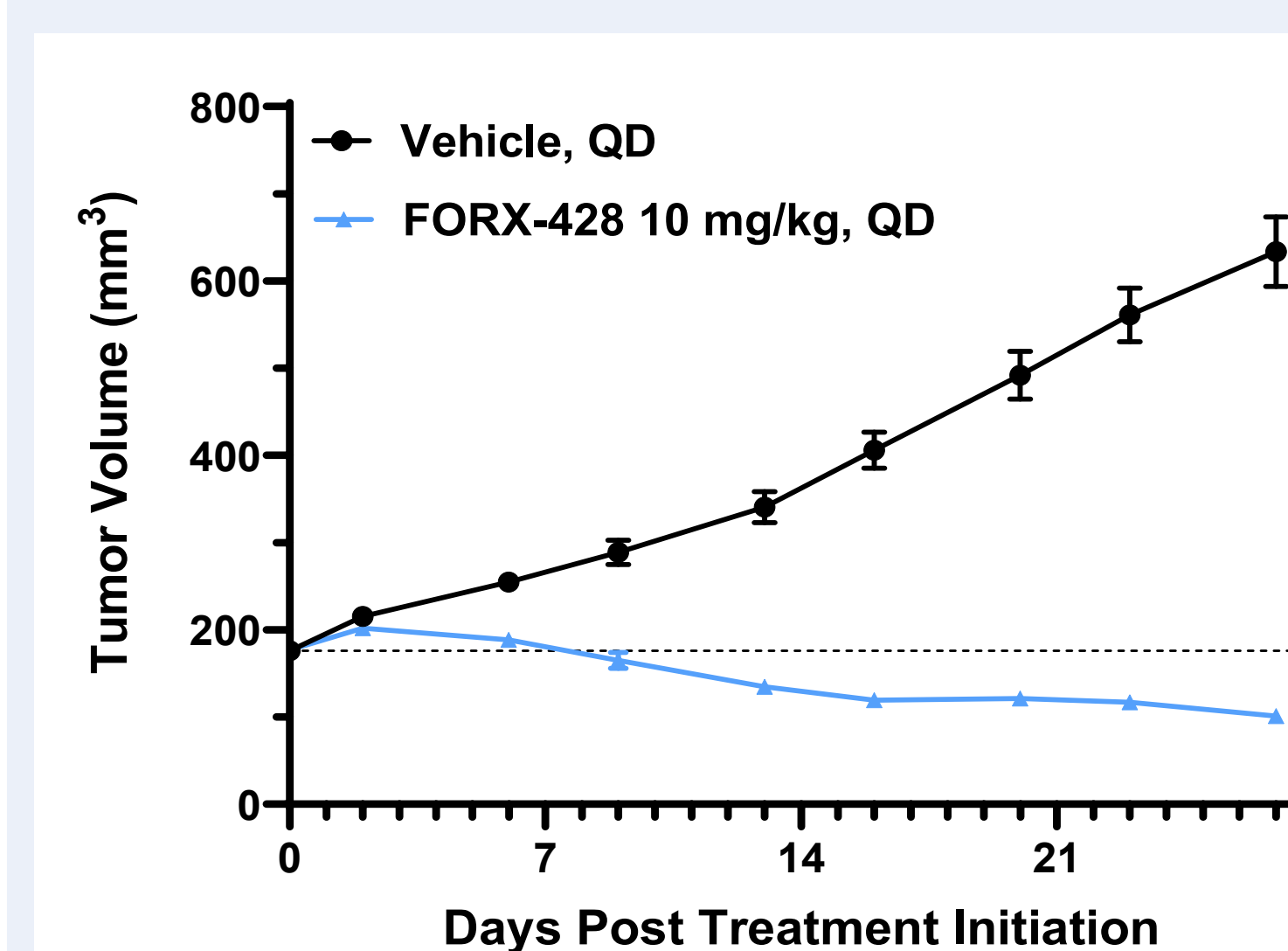
A) Plasma pharmacokinetics of FORX-428 in BALB/c nude female mice (n=3 per group) following single dose administration by oral gavage of 10 and 100 mg/kg (all data points are the mean of 3 measurements except 10 mpk, 24h for which plasma exposure was detected in only one mouse). Breast and ovarian cancer cell lines IC₅₀ (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-428

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

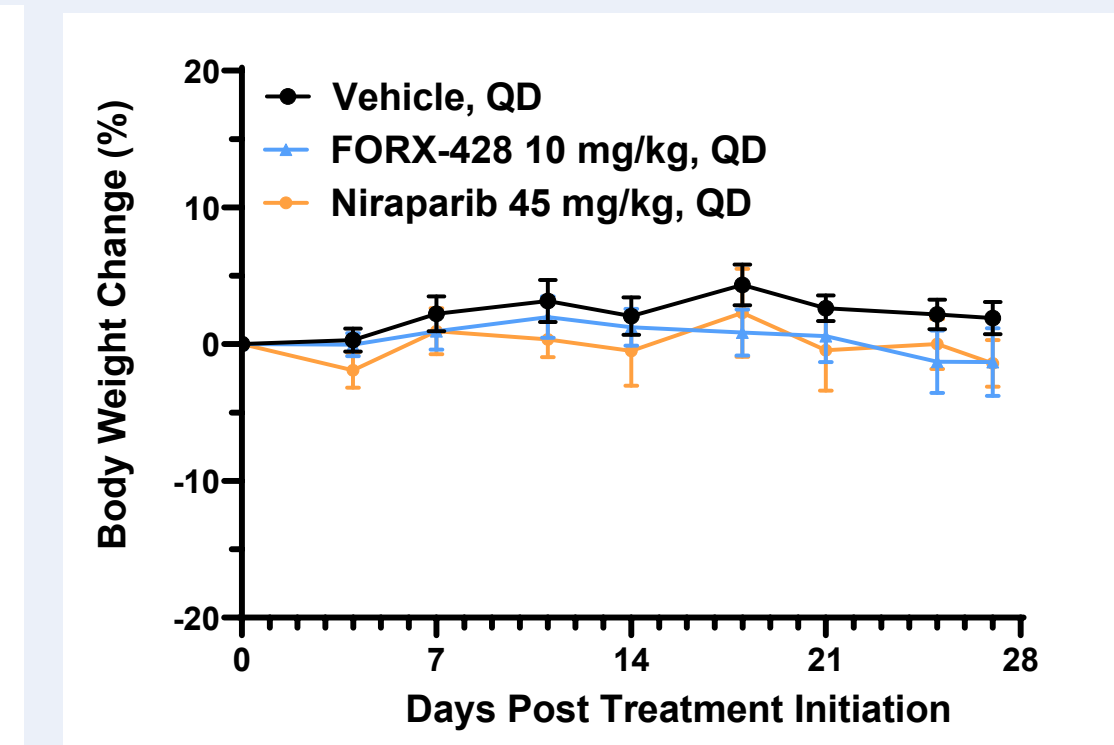
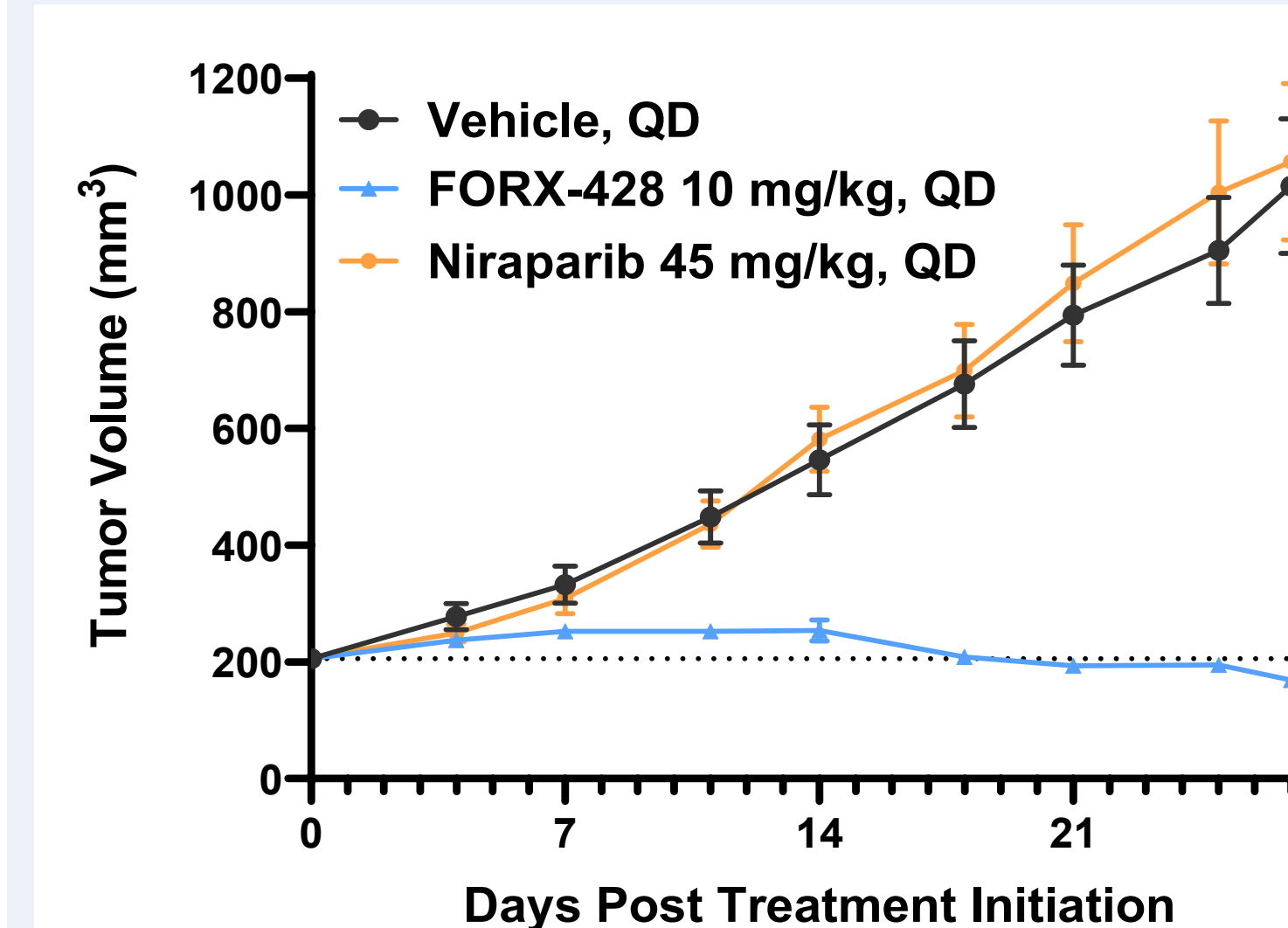
A) MDA-MB-436



B) RMUG-S



C) Kuramochi



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

CONCLUSIONS

Collectively, our data show that FORX-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.

The DNA Damage Response in cell physiology and diseases
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