AACR American Association for Cancer Research®
ANNUAL MEETING 2022 New Orleans
APRIL 8-13, 2022 • #AACR22

KSQ-4279: A first-in-class USP1 inhibitor for the treatment of cancers with homologous recombination deficiency

Andrew Wylie
KSQ Therapeutics, Cambridge, MA

Disclosure Information

Andrew Wylie

I have the following relevant financial relationships to disclose:

I am an Employee and Stockholder of KSQ Therapeutics

CRISPR screen identifies targets that are selectively essential in cancer cell line subsets

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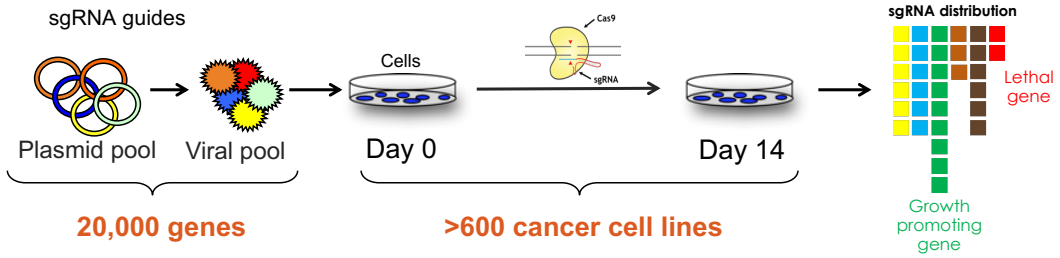
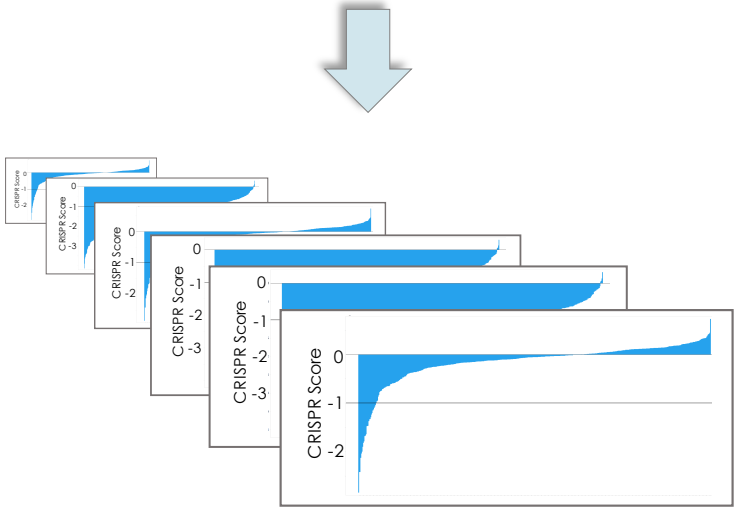
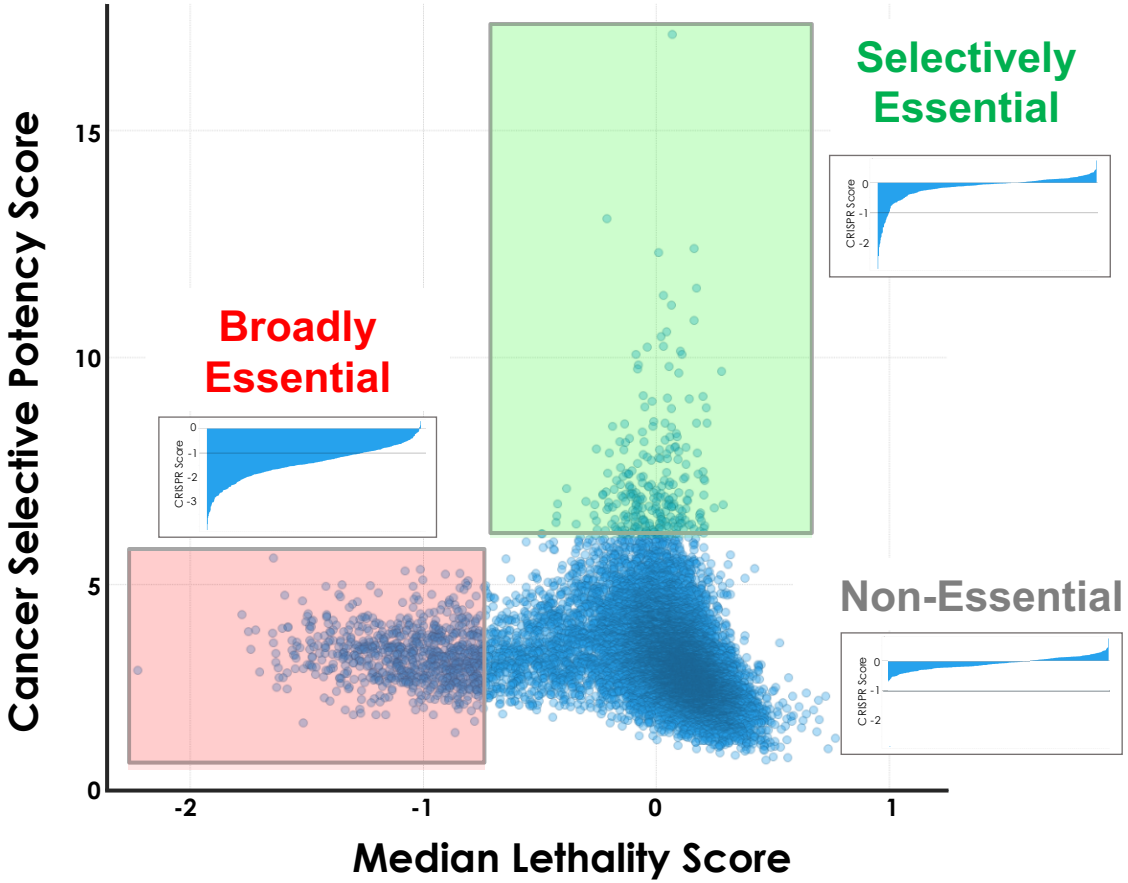
CRISPRomics® database





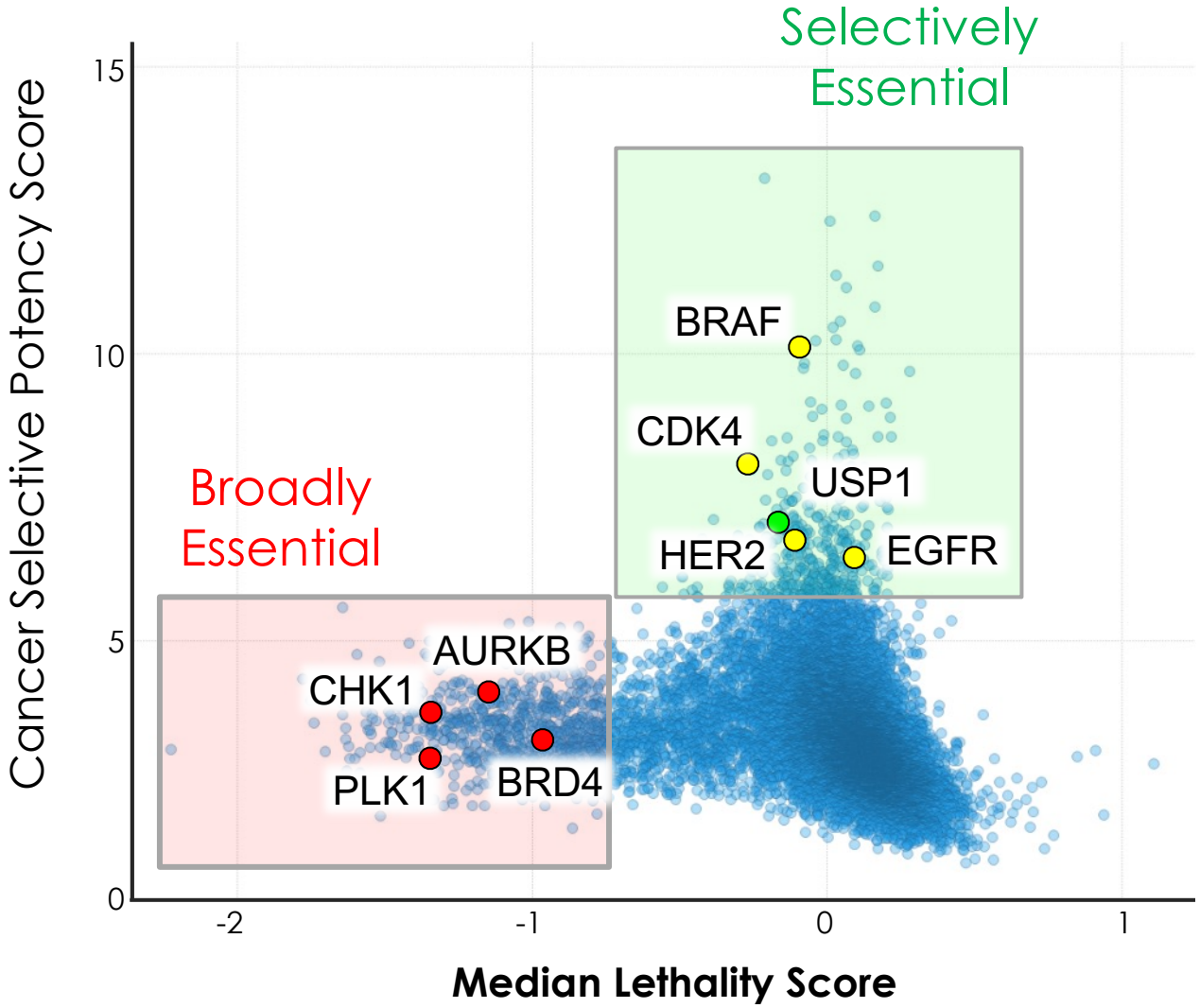
20,000 drop-out profiles

Genome-wide view



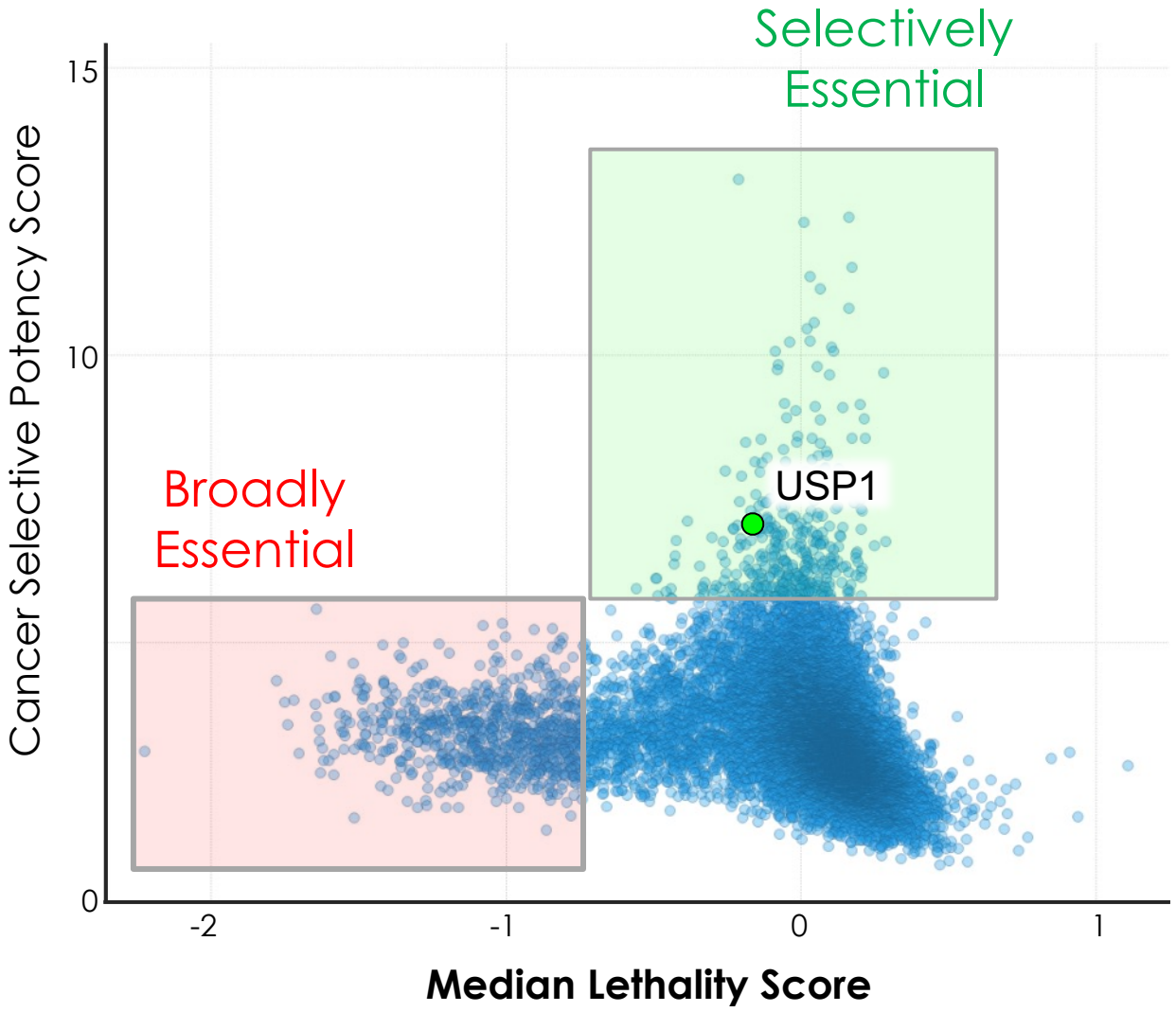
KSQ Knowledgebase: Identification of Cancer-Selective Targets

Database highlights targets with potential for rapid clinical development

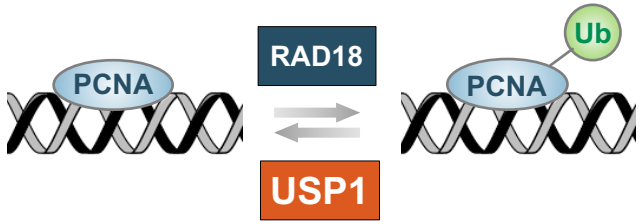


CRISPRomics® Identifies USP1 as a novel, DDR pathway target

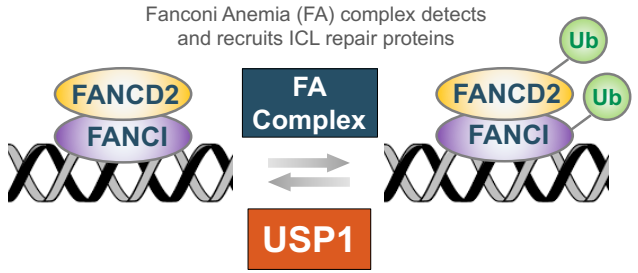
USP1 is a de-ubiquitinase that regulates DNA damage repair pathways



USP1 regulates key DDR pathways



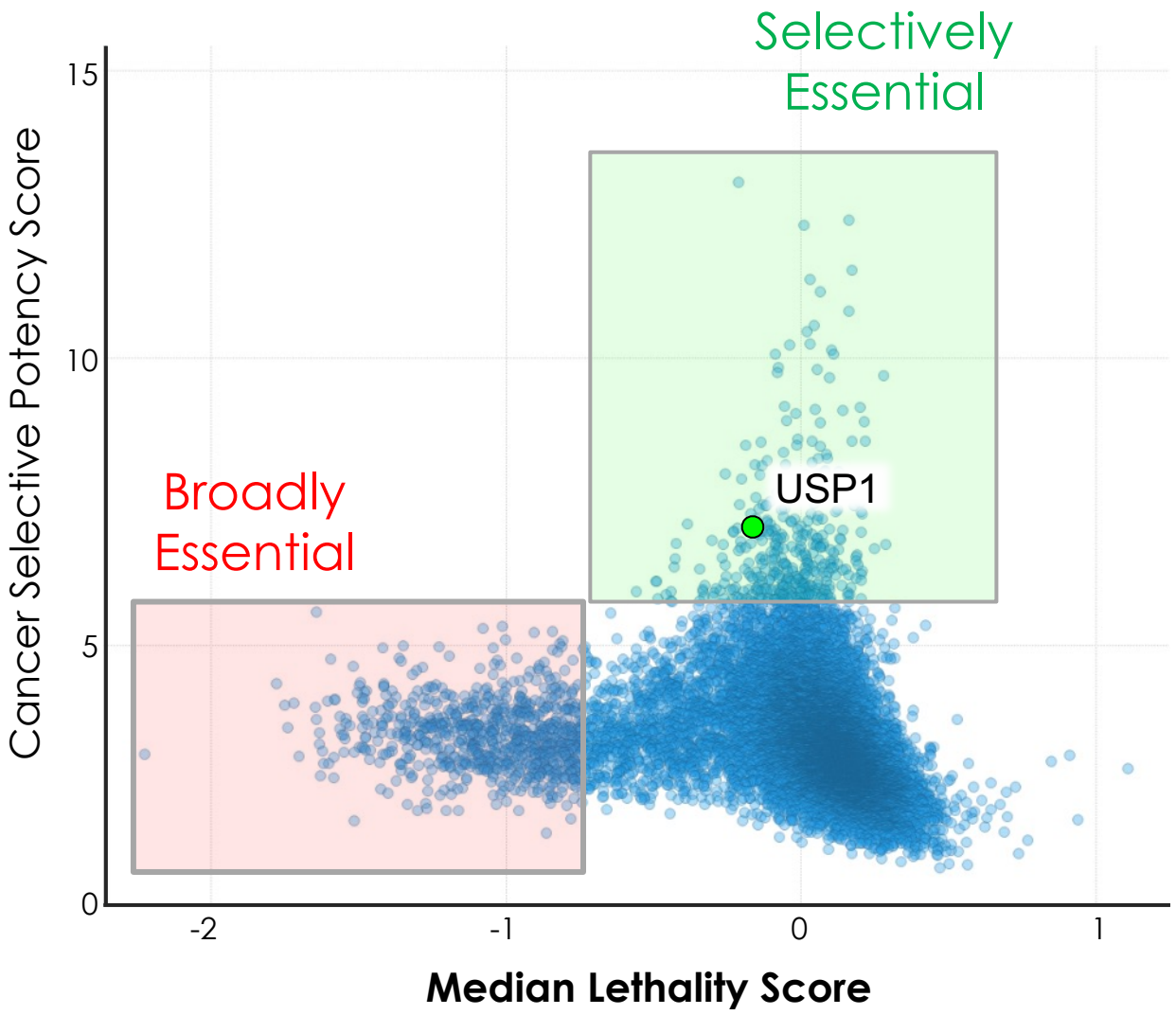
Translesion synthesis



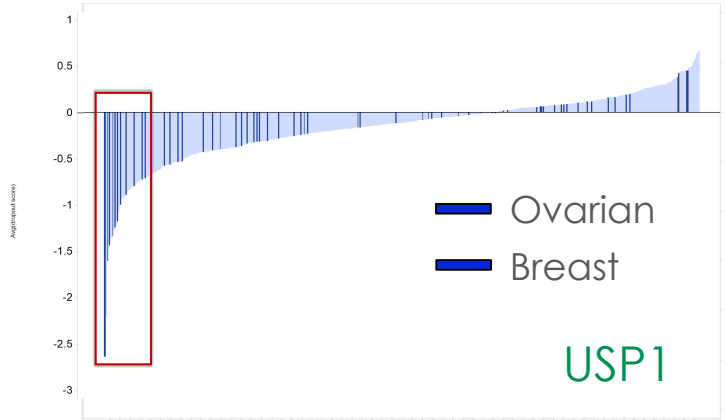
Intra-strand Crosslink Repair

CRISPRomics® Identifies USP1 as a novel, DDR pathway target

Ovarian and Breast cancer lineages are enriched in USP1 sensitive cells



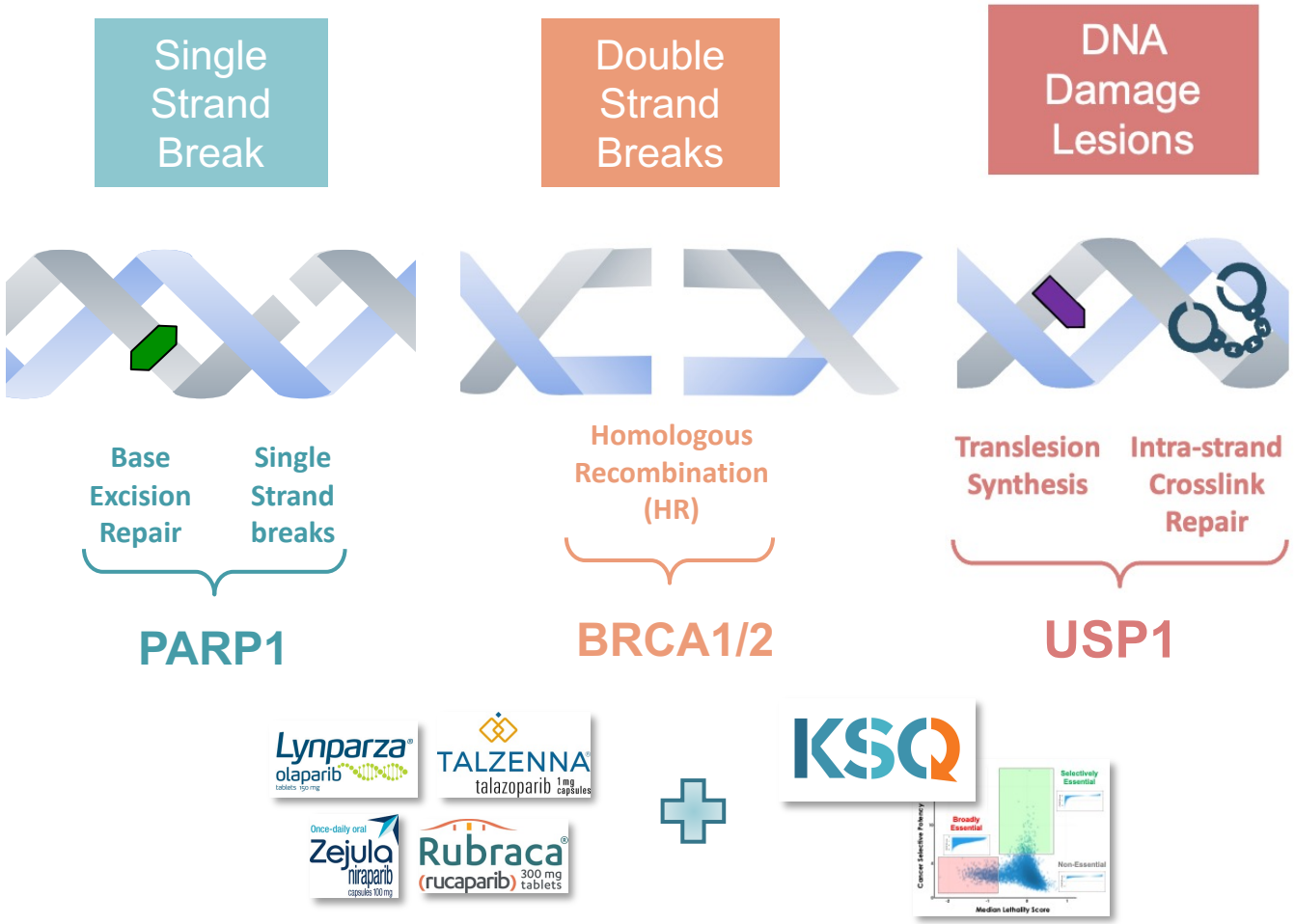
Cell line enrichment profile



- Ovarian/Breast often associated with BRCA1/2^{MUT} and other defects in Homologous Recombination
- 6 of 9 Ovarian/Breast lines with USP1 dependency are HRD+/BRCAmut
- Despite activity of PARP inhibitors, HRD+/ BRCA^{MUT} tumors remain a high unmet clinical need

*HRD+ = homologous recombination deficiency

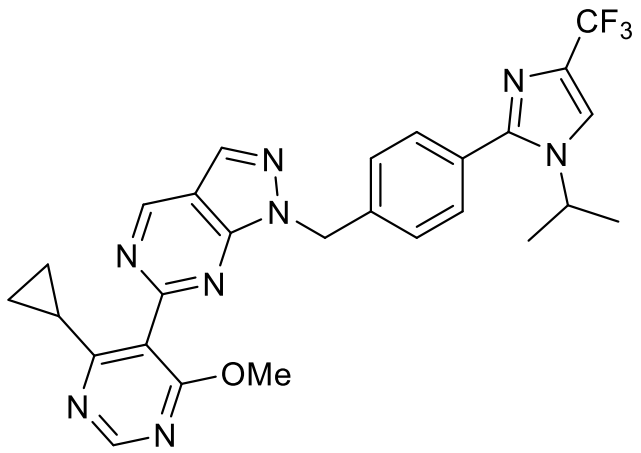
USP1 regulates a distinct set of DNA Damage response pathways from PARP inhibitors

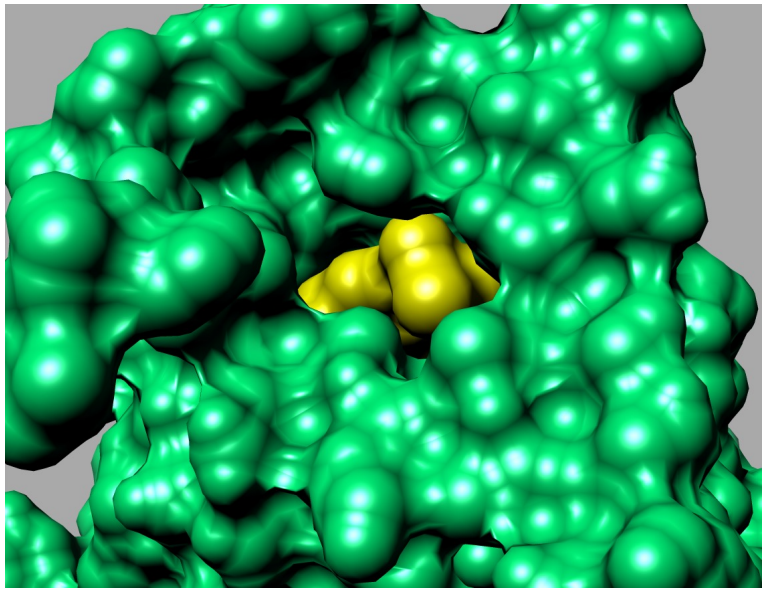


Development of KSQ-4279, a potent, selective USP1 inhibitor

KSQ-4279, a potent, selective USP1 inhibitor

KSQ-4279

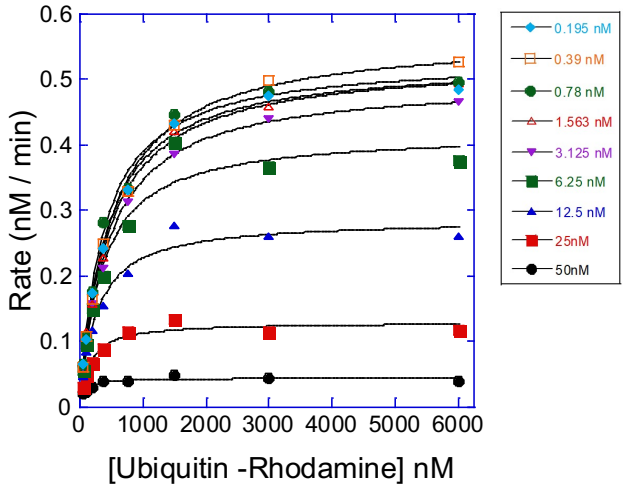


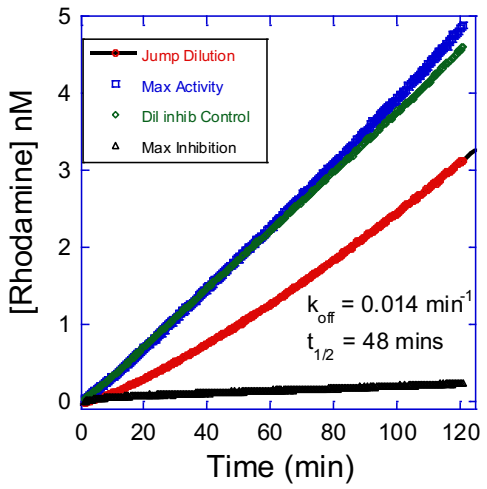


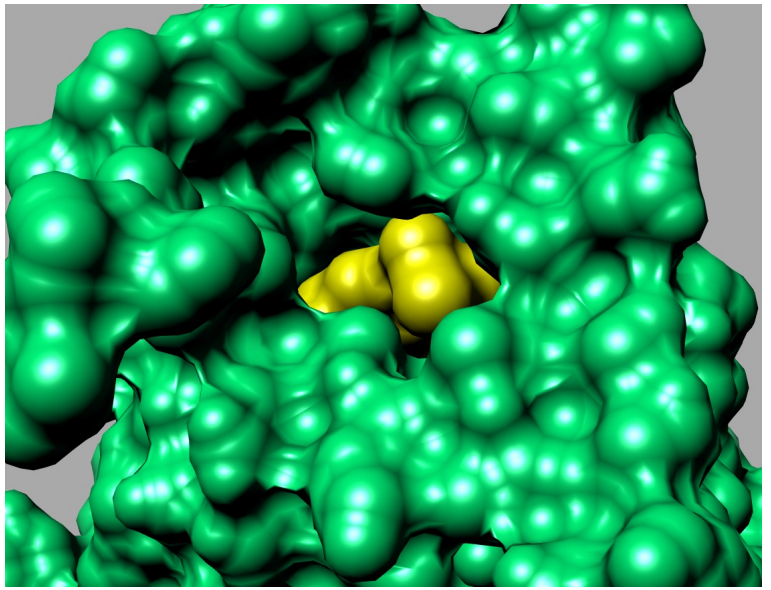
- KSQ-4279 is a reversible, allosteric inhibitor of USP1 with a **Ki = 1.2nM**
- Both KSQ-4279-bound and unbound USP1 structures solved revealing an induced fit mechanism

KSQ-4279 is a potent, allosteric inhibitor of USP1 with excellent selectivity

Enzyme kinetics and mode of inhibition

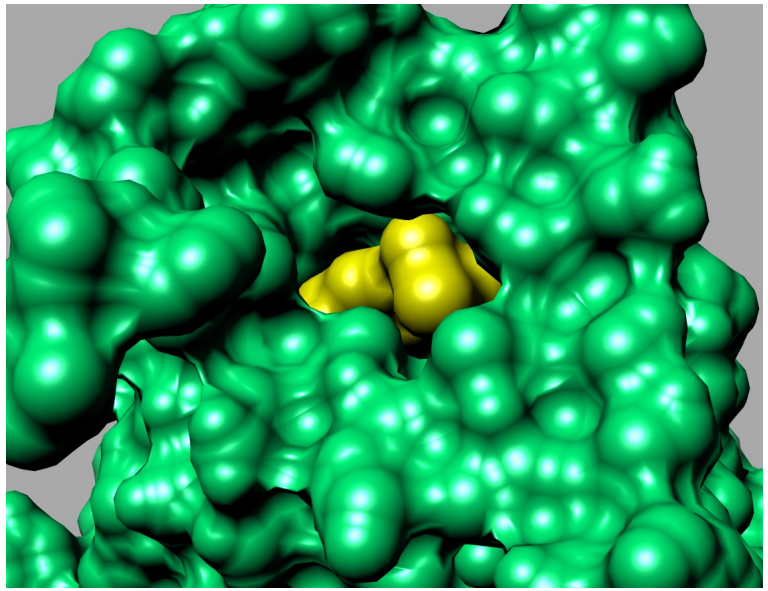


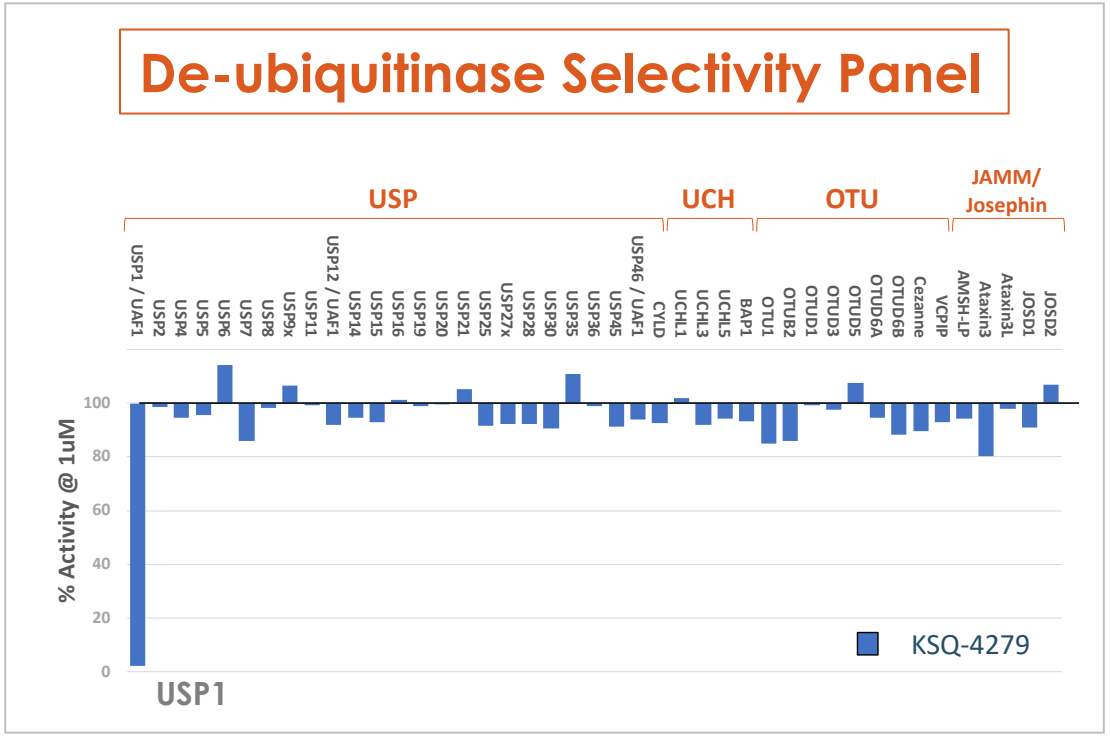




- Both V_{max} and K_m decrease indicating uncompetitive mode of inhibition
- Reversible binding with a slow k_{off} and a $t_{1/2}$ = 48 mins

KSQ-4279 is a potent, allosteric inhibitor of USP1 with excellent selectivity





- DUB family profiling indicates KSQ-4279 is a selective inhibitor of USP1

KSQ-4279 leads to accumulation of mono-Ub substrates and anti-proliferative activity correlates with genetic knock-out

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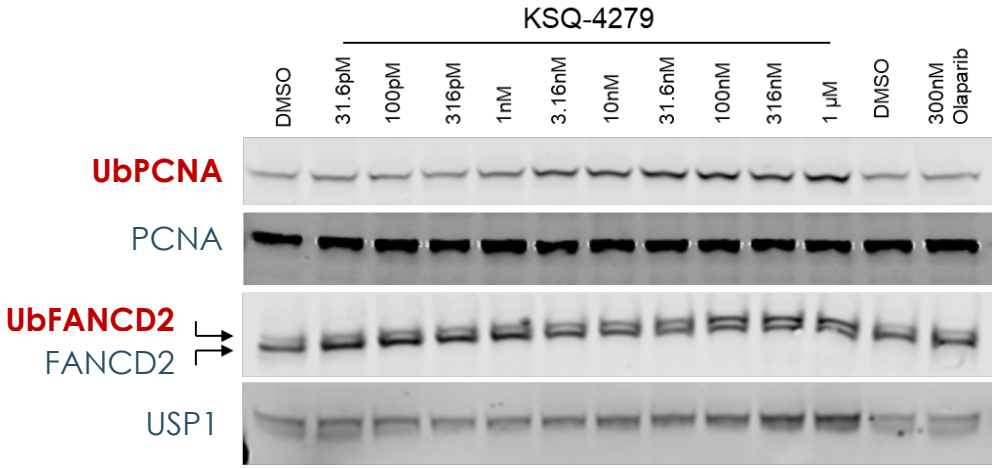
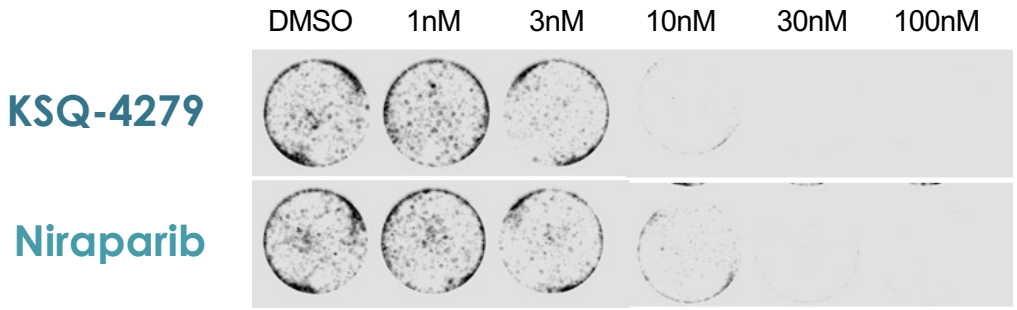
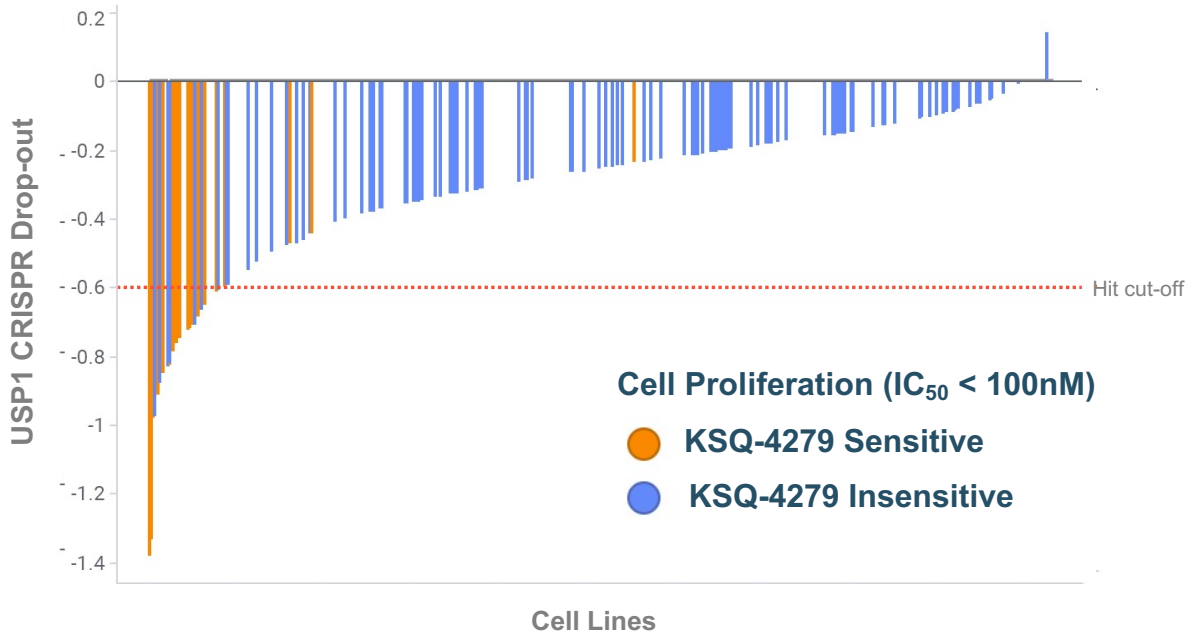
PD changes in MDA-MB-436 (BRCA1^{MT}, p53^{MT})



Colony formation assay in MDA-MB-436 (BRCA1^{MT}, p53^{MT})



Comparison between CRISPR knockout and KSQ-4279 sensitivity



What is the mechanism of action of KSQ-4279?

KSQ-4279 Induces DNA Damage and S/G2-Phase Arrest in BRCA1^{MT} Cells

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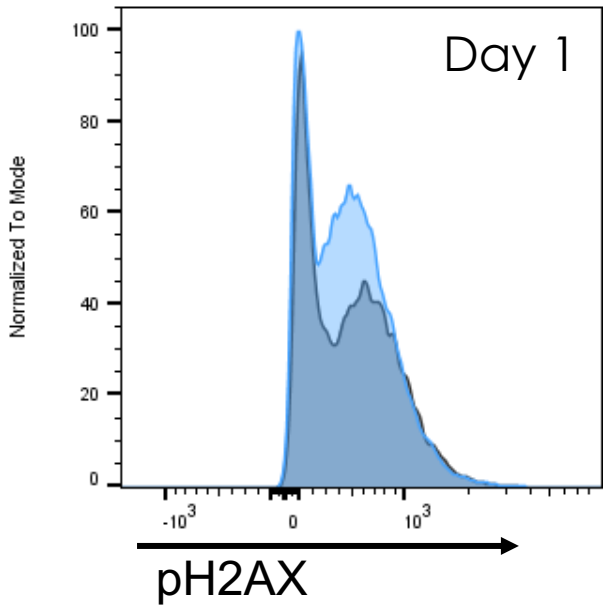
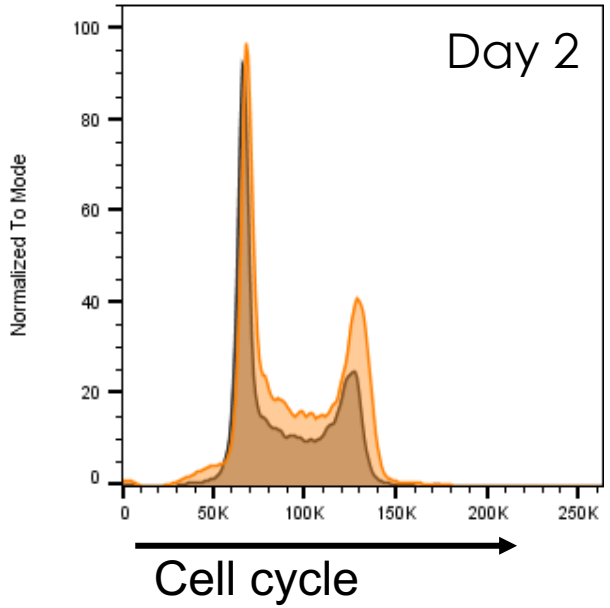
UWB1.289 cells (BRCA1^{MT}, p53^{MT})

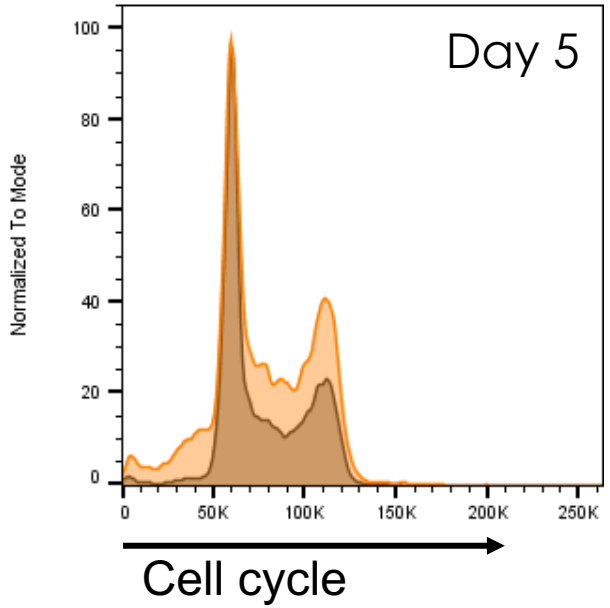
pH2AX levels



DMSO
KSQ-4279

Cell Cycle Analysis

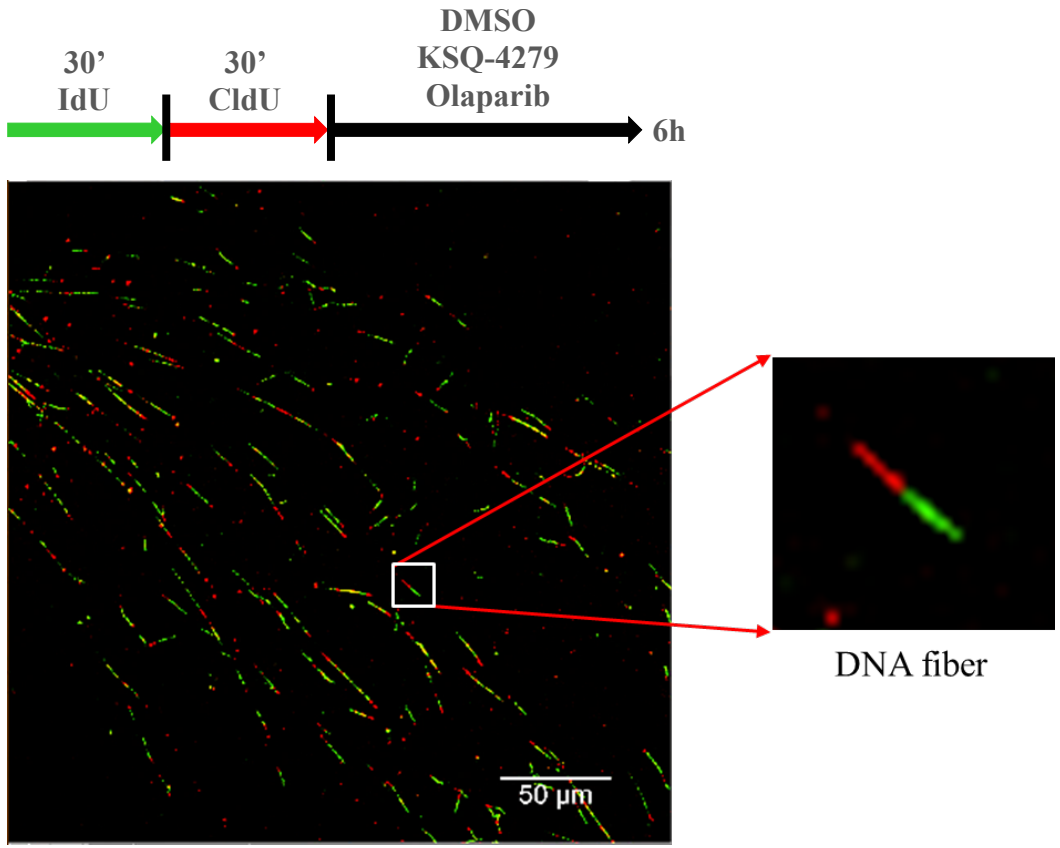




DMSO
KSQ-4279

USP1 reported to be required for replication fork protection in BRCA1-deficient tumors

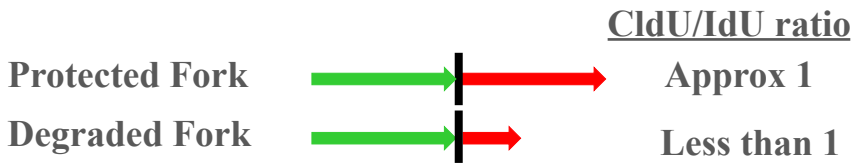
DNA Fiber Fork Protection Assay



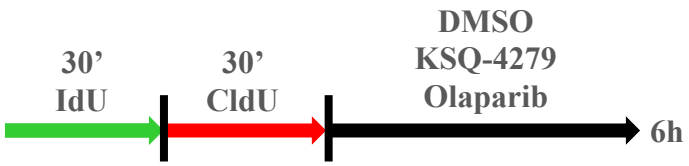
USP1 Is Required for Replication Fork Protection in BRCA1-Deficient Tumors

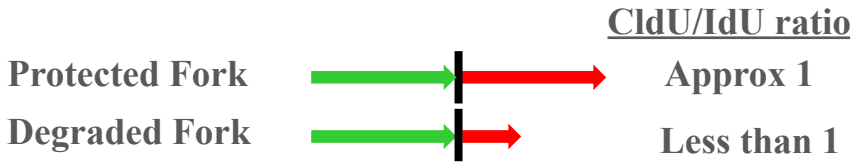
Kah Suan Lim,[1] Heng Li,[3] Emma A. Roberts,[1] Emily F. Gaudiano,[1] Connor Clairmont,[1] Larissa Alina Sambel,[1,2] Karthikeyan Ponnienselvan,[1] Jessica C. Liu,[1] Chunyu Yang,[1,2] David Kozono,[1] Kalindi Parmar,[1,2] Timur Yusufzai,[1] Ning Zheng,[3,4] and Alan D. D'Andrea[1,2,5,*]

Molecular Cell, 72, 925-941 (2018)

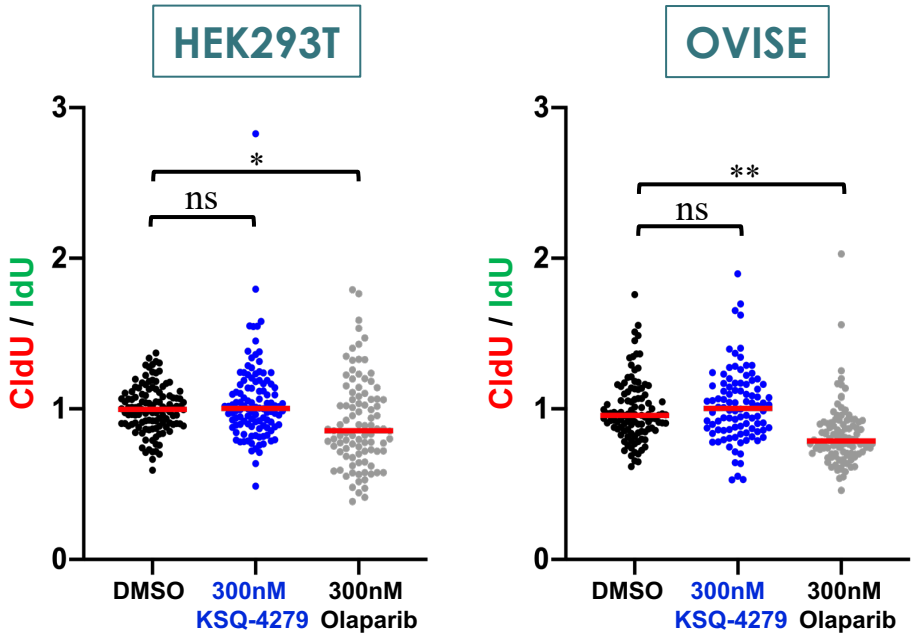


Sensitivity to KSQ-4279 correlates with degradation of the replication fork

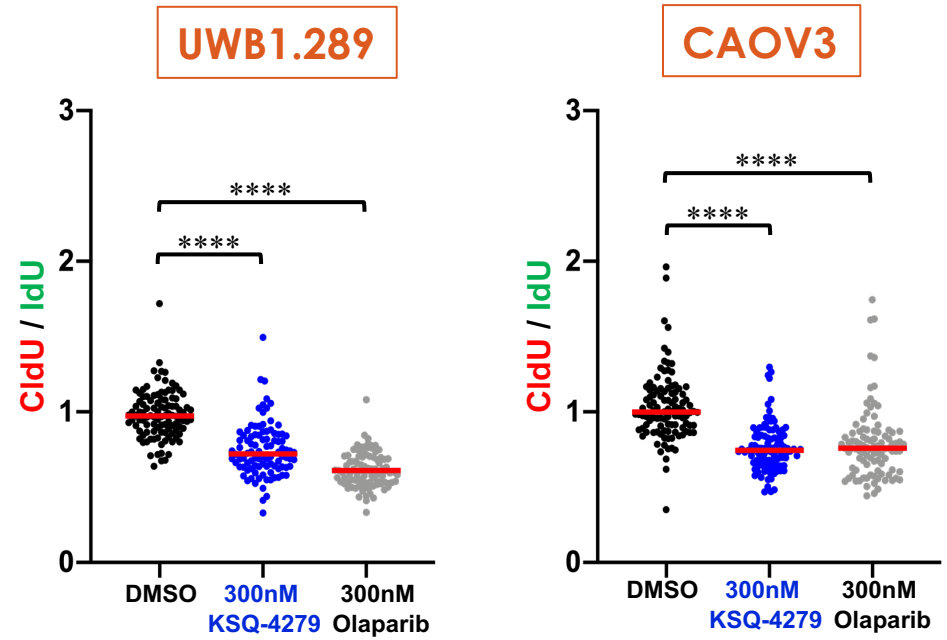




Insensitive cell lines

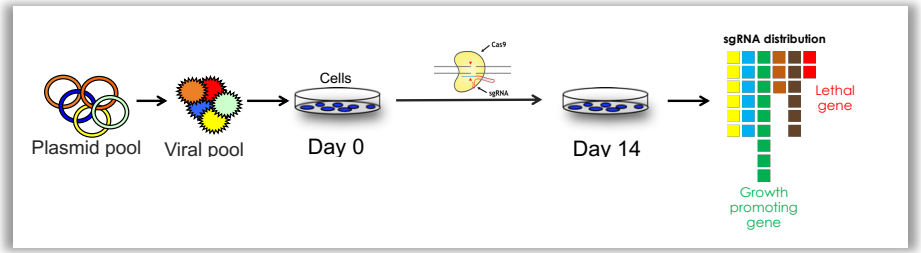


Sensitive cell lines

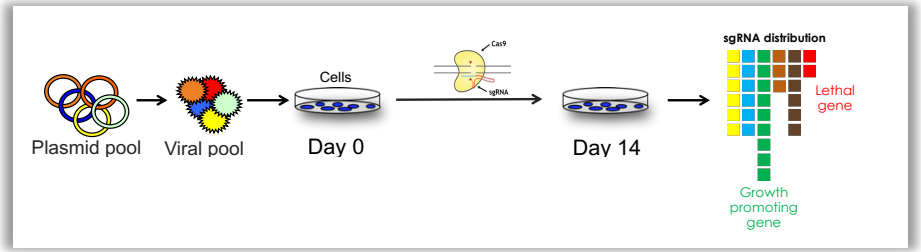


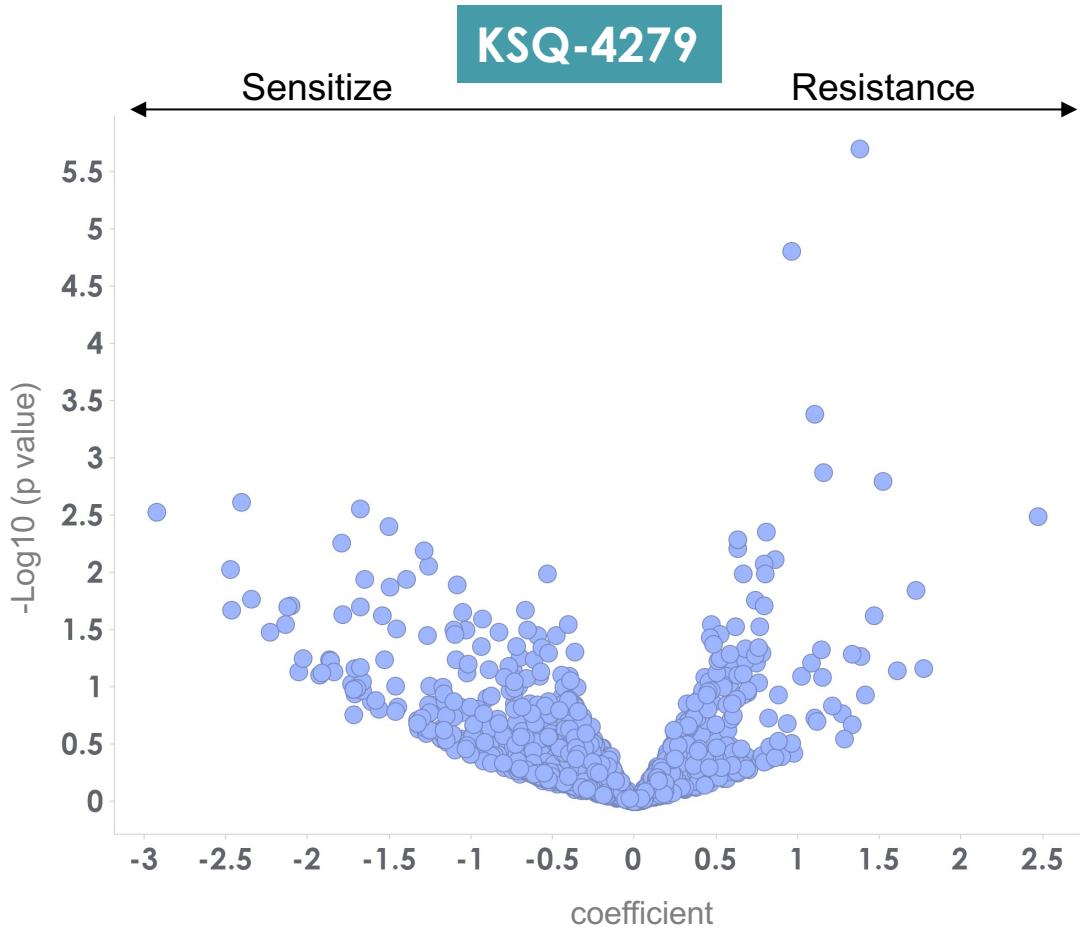
CRISPR screening reveals pathways that influence sensitivity to KSQ-4279

DMSO

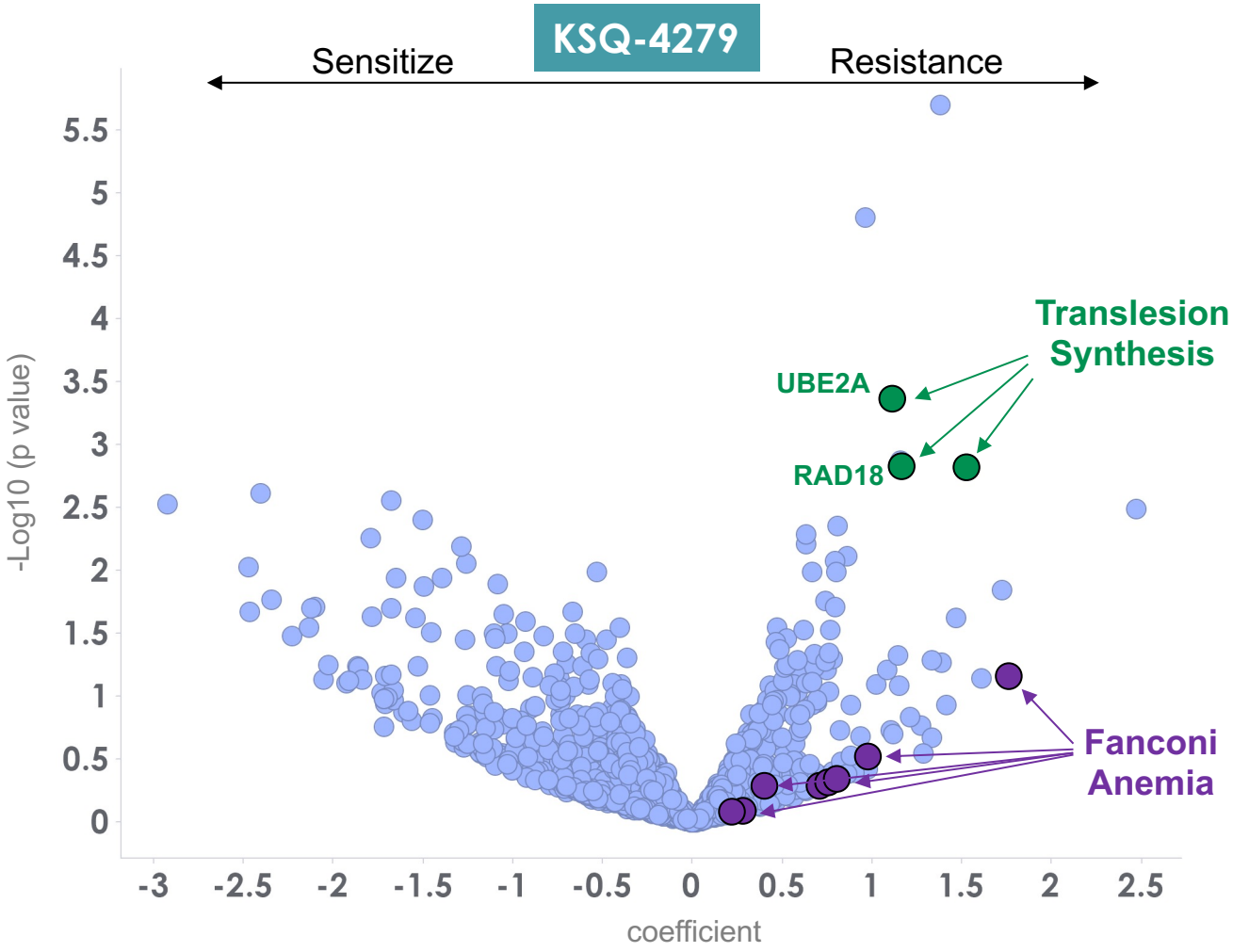


USP1i

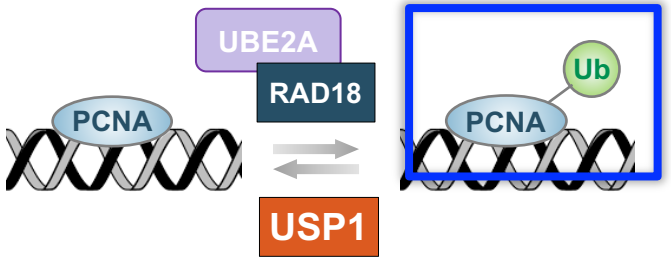




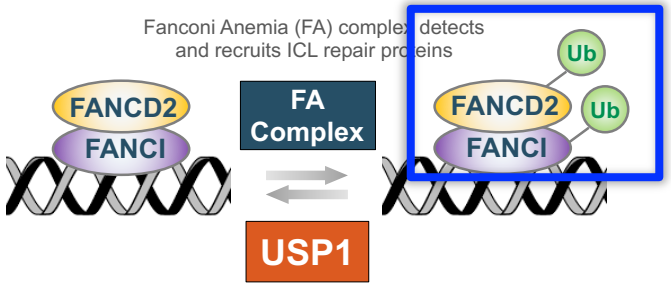
CRISPR screening reveals pathways that influence sensitivity to KSQ-4279



USP1 regulates TLS & FA Pathway



Translesion synthesis



Intra-strand Crosslink Repair

CRISPR screening reveals pathways that influence sensitivity to KSQ-4279

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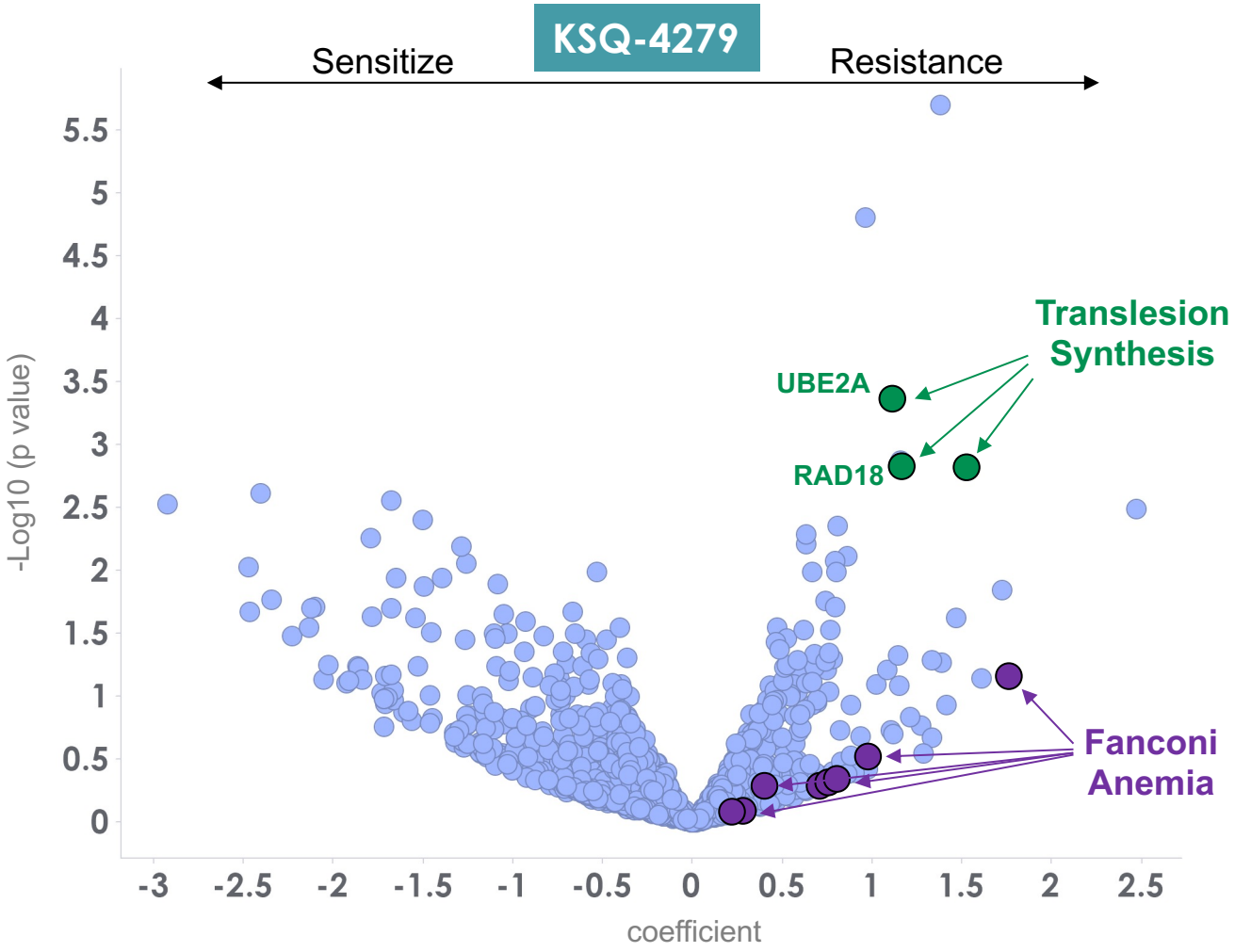
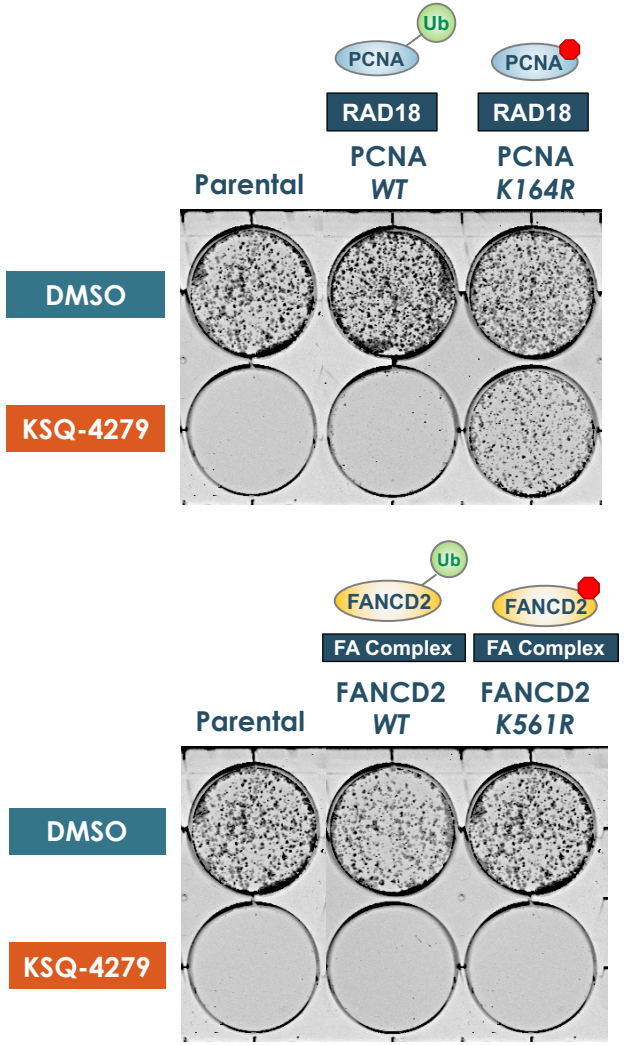
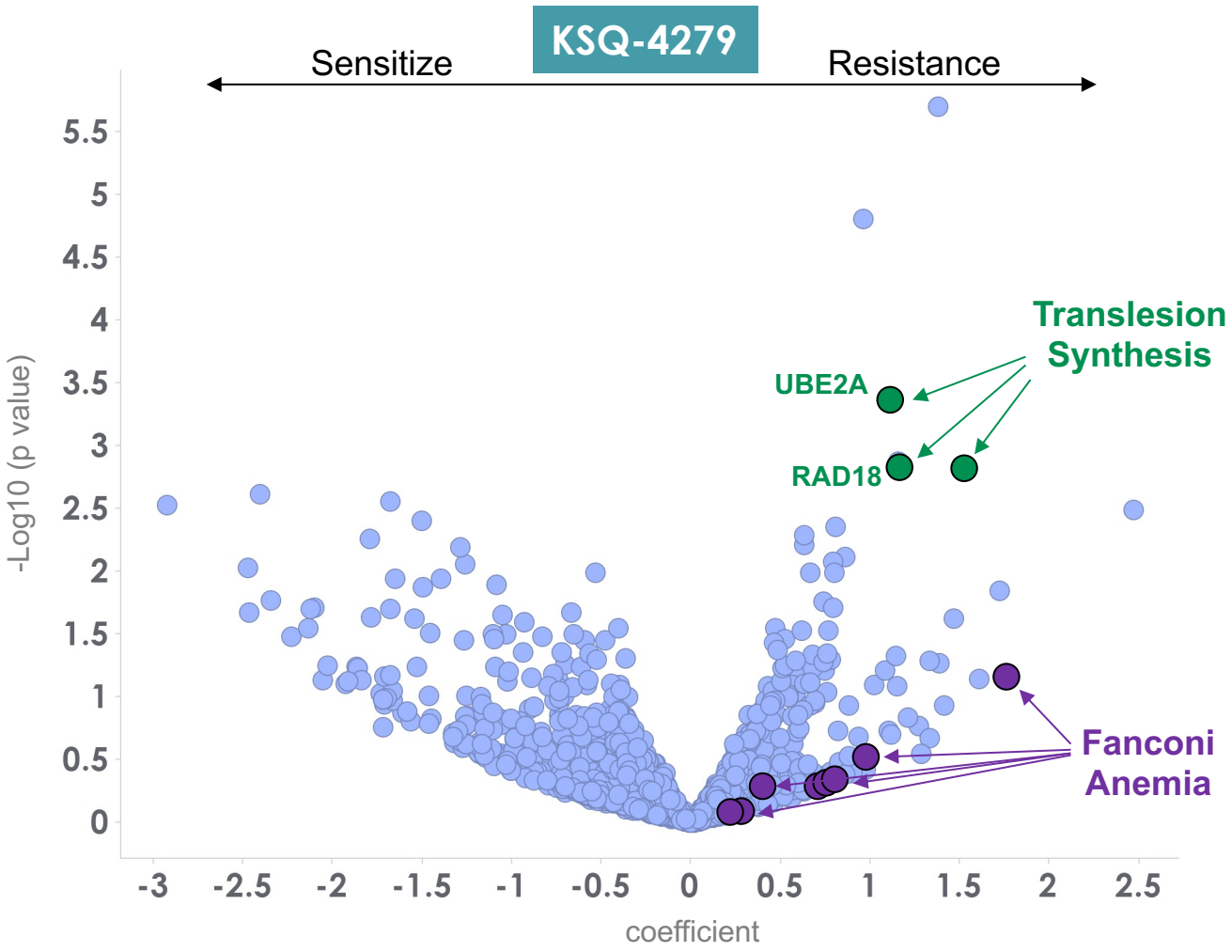
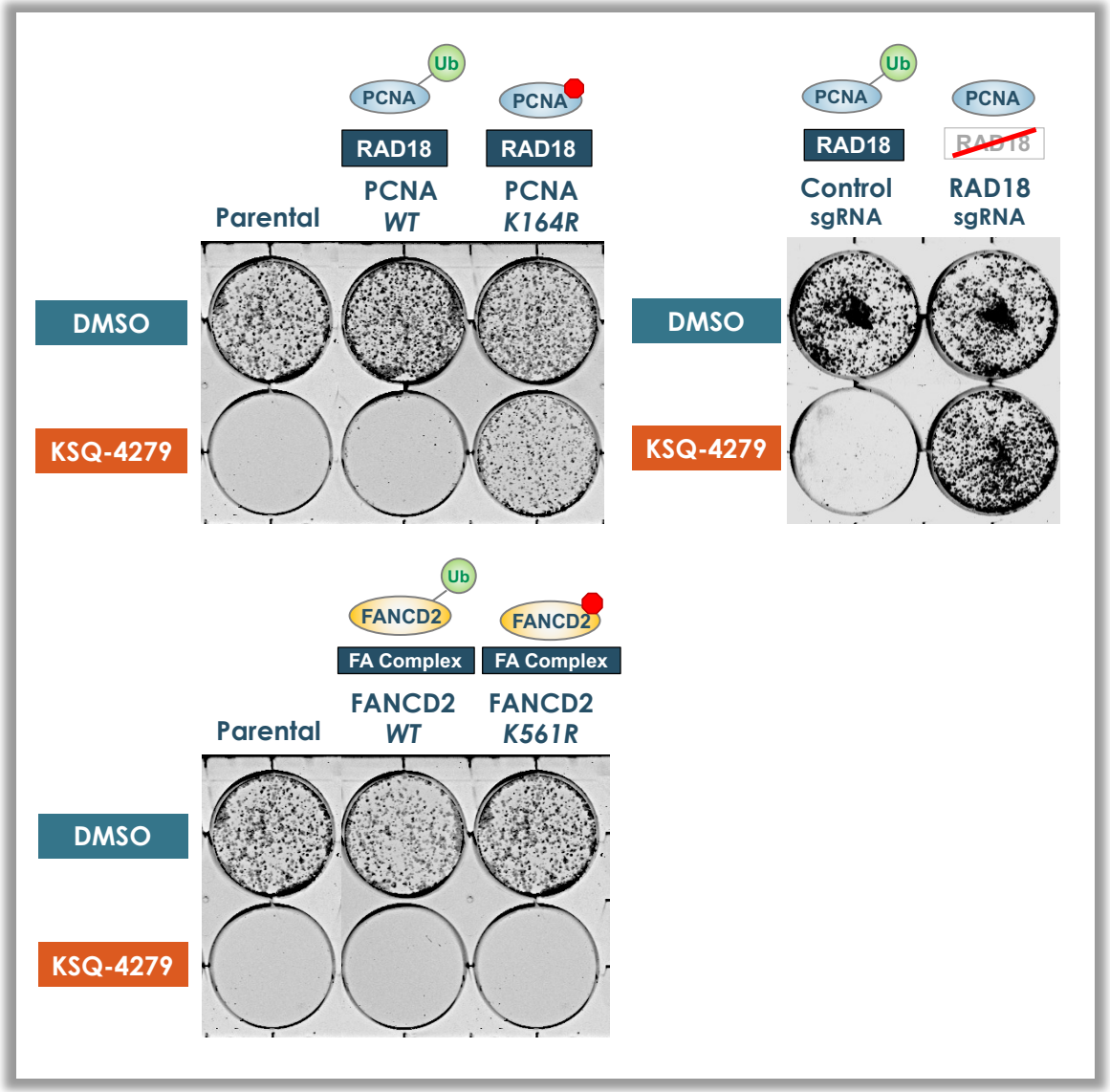




CRISPR screening reveals pathways that influence sensitivity to KSQ-4279





CRISPR screening reveals pathways that influence sensitivity to KSQ-4279

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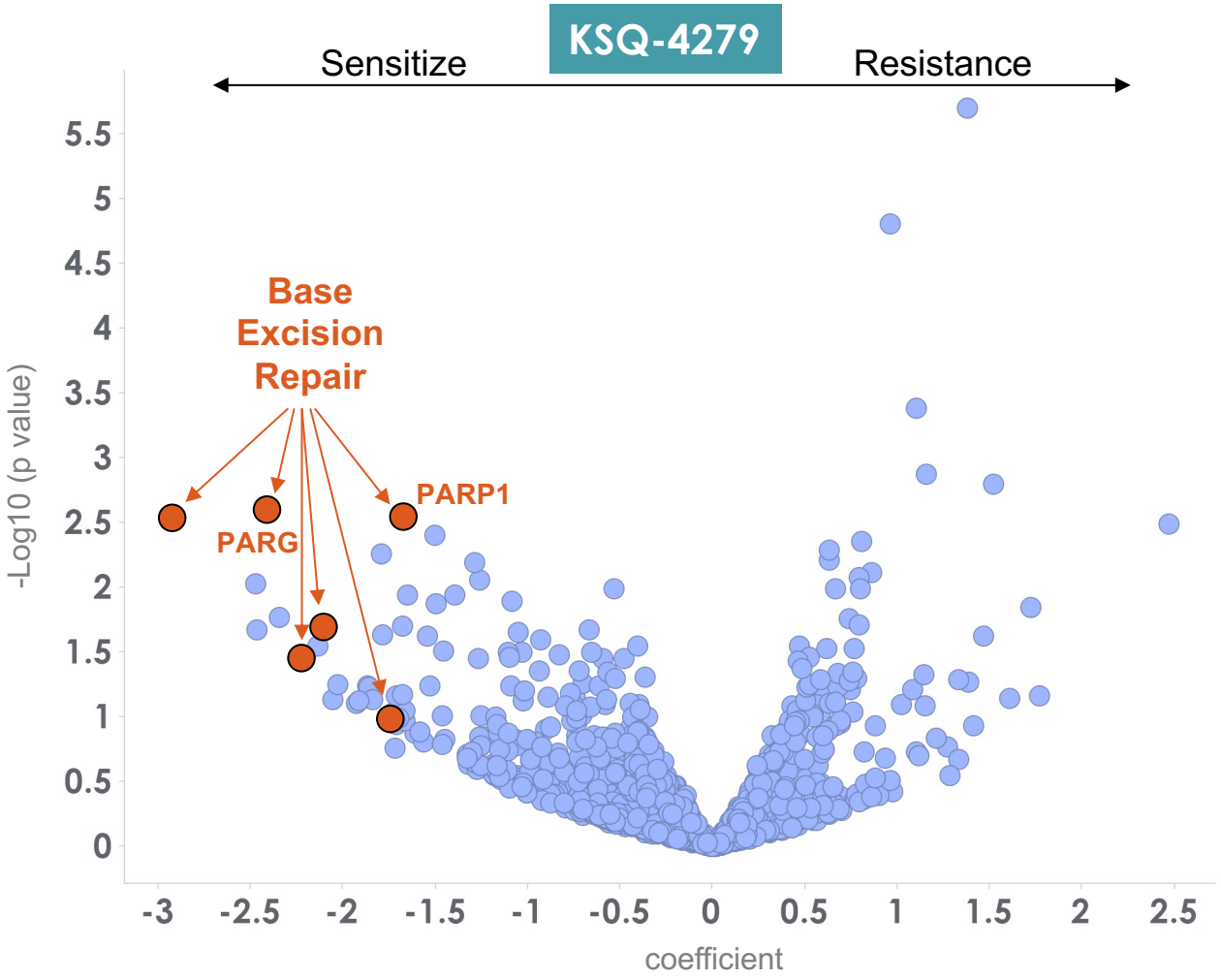
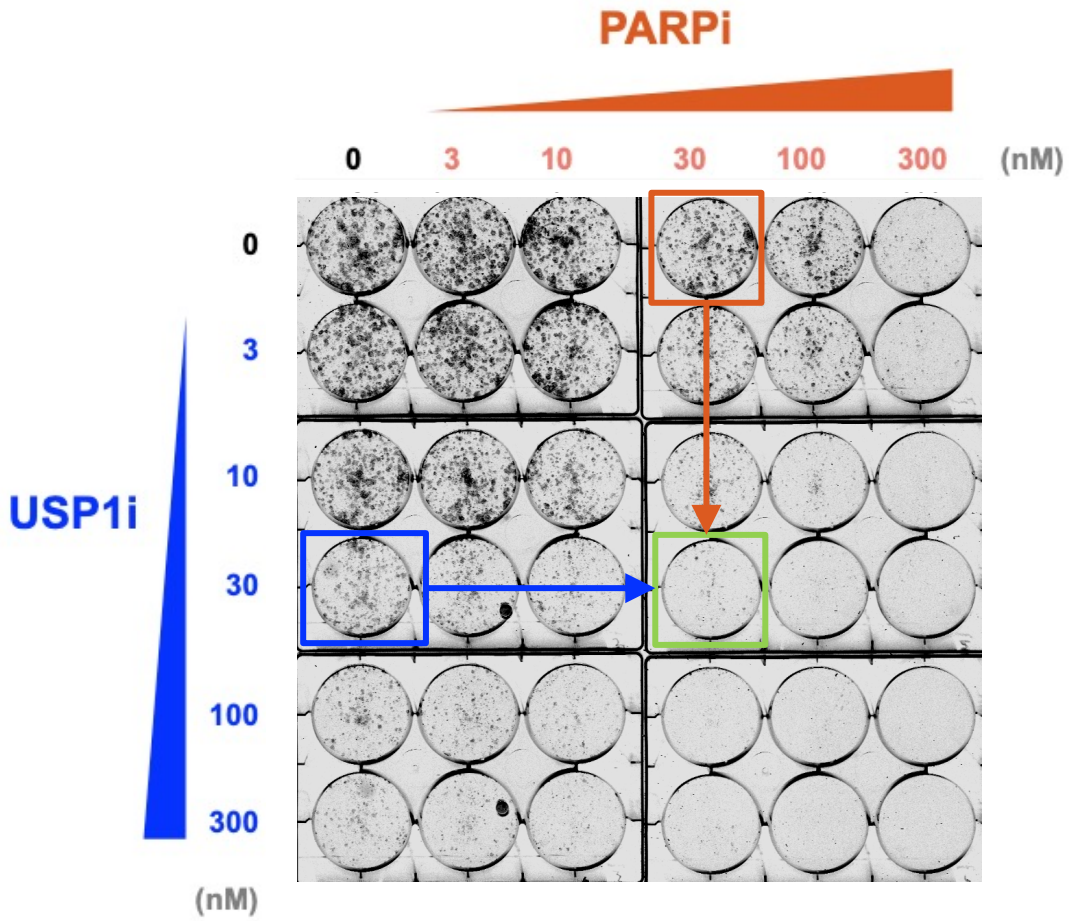
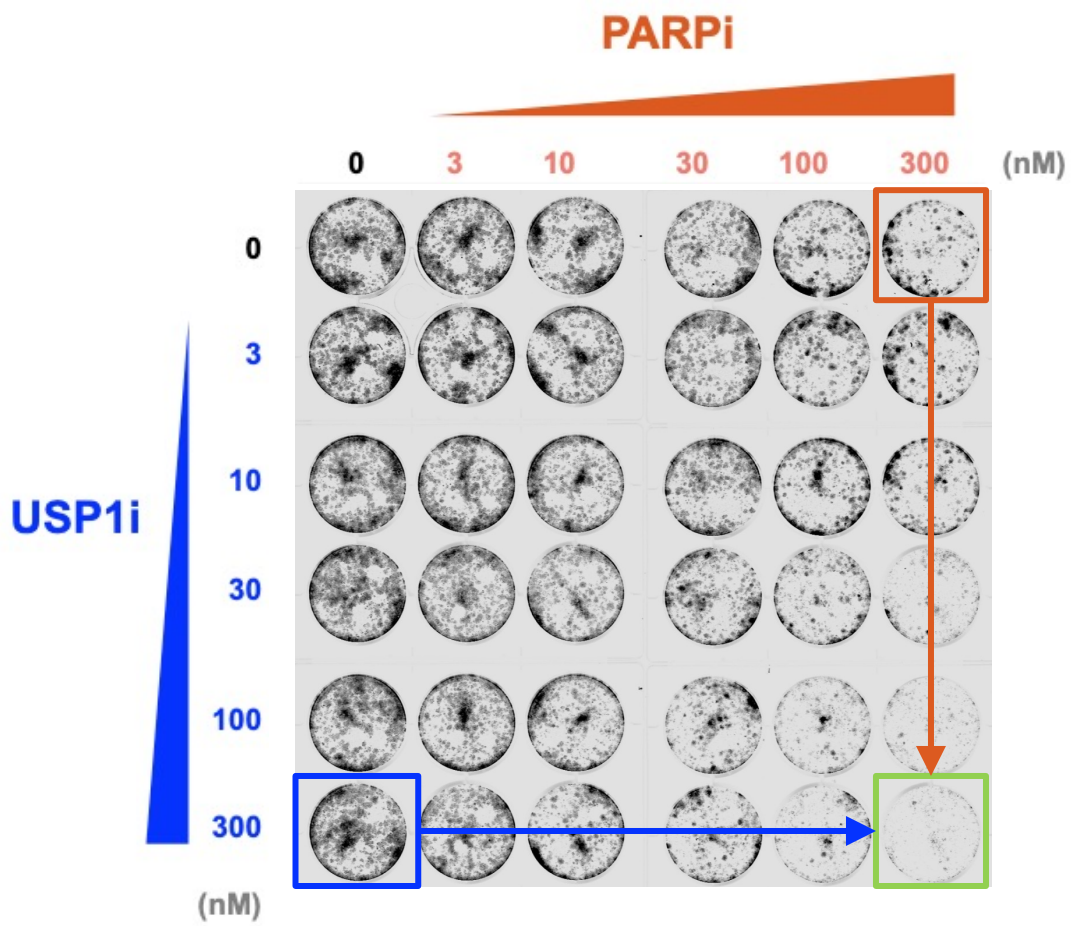


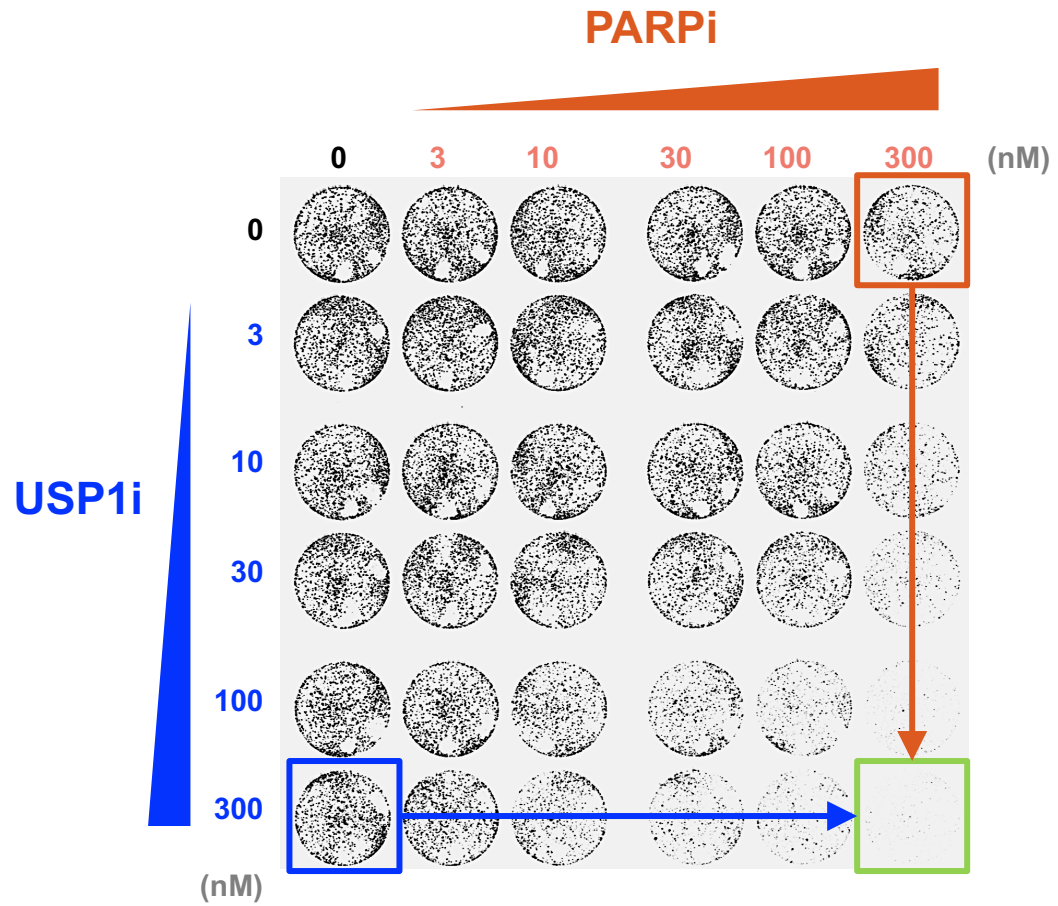


UWB1.289 – TP53^{MUT}, BRCA1^{MUT}
Ovarian Cancer

USP1i and PARPi have synergistic activity across different lineages

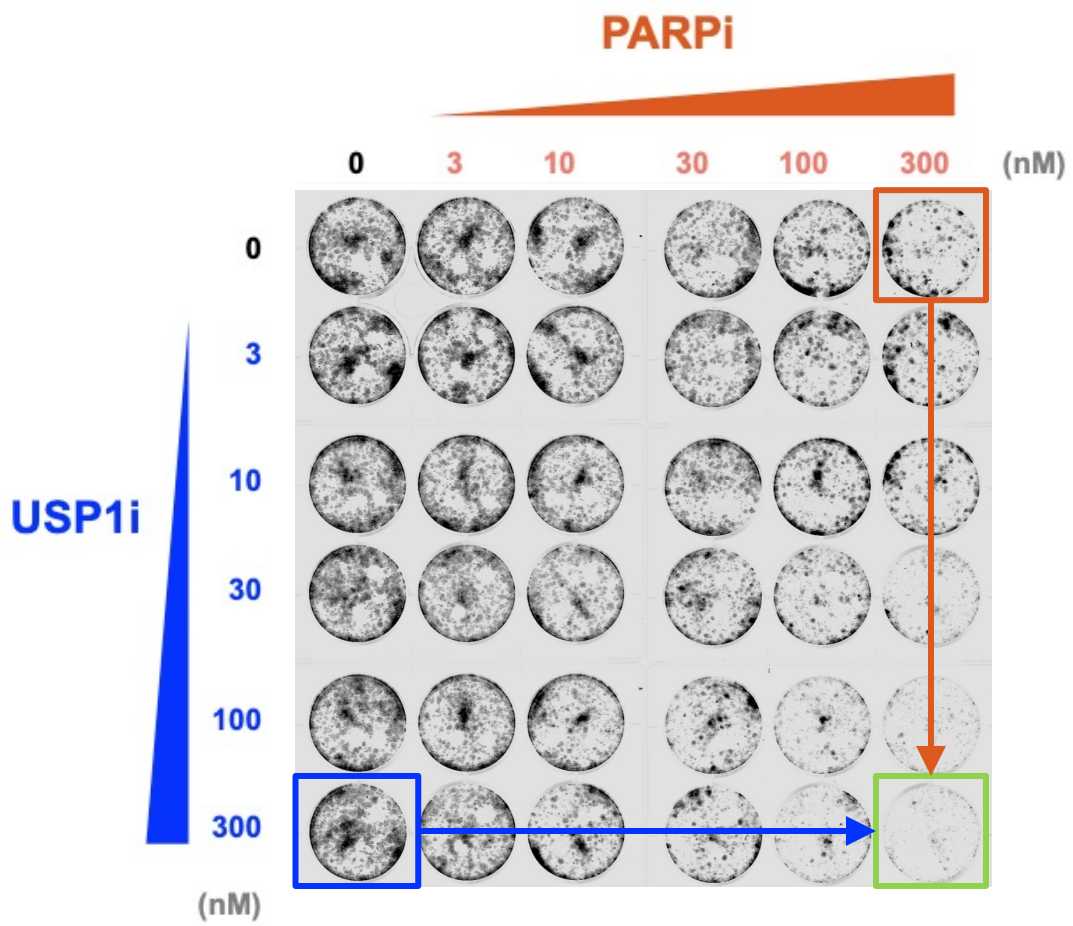


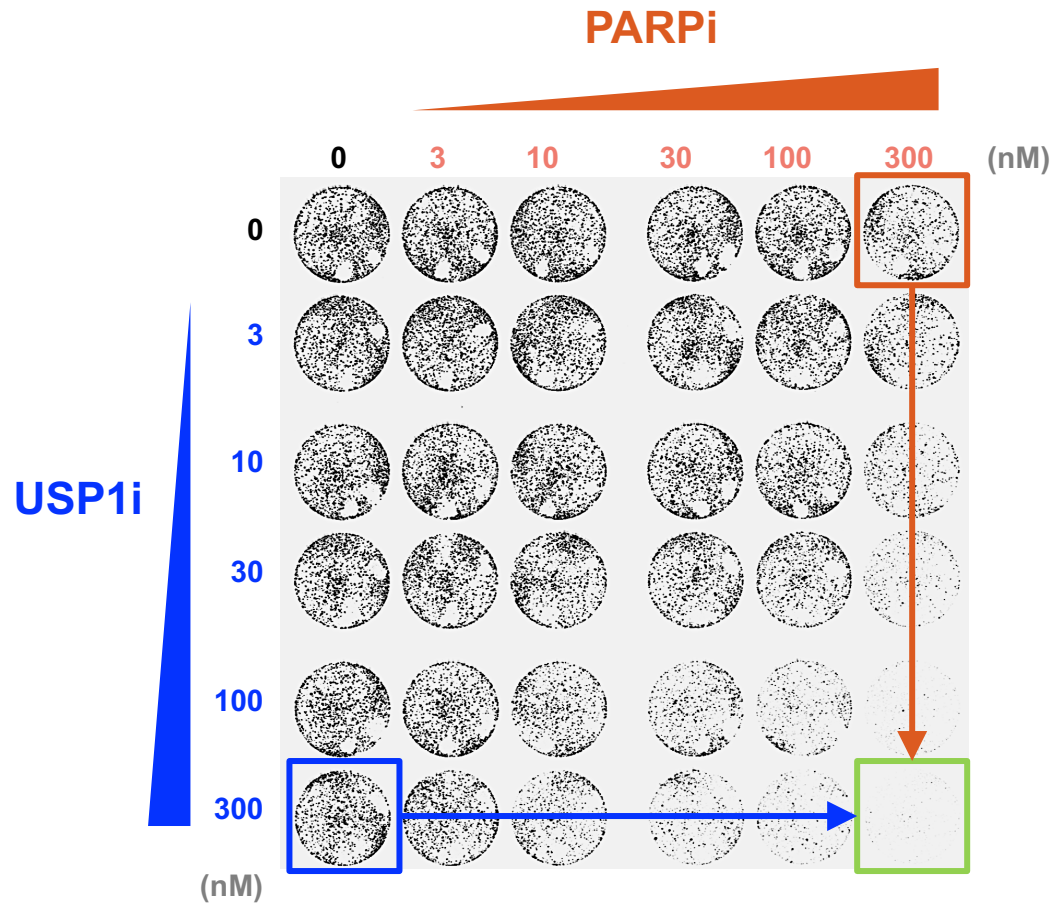
SUM149PT – TP53^{MUT}, BRCA1^{MUT}
Triple-Negative Breast Cancer



NCI-H520 – TP53^{MUT}, ATM^{MUT}
Lung squamous cell carcinoma

USP1i and PARPi have synergistic activity across different lineages





- Of ~160 cell lines evaluated using USP1i + PARPi combination, strong synergy observed in ~10-15% lines

What is the in vivo activity of KSQ-4279 as a single agent and in combination?

KSQ-4279 dose dependent efficacy correlates with induction of Ub-PCNA in Ovarian PDX model

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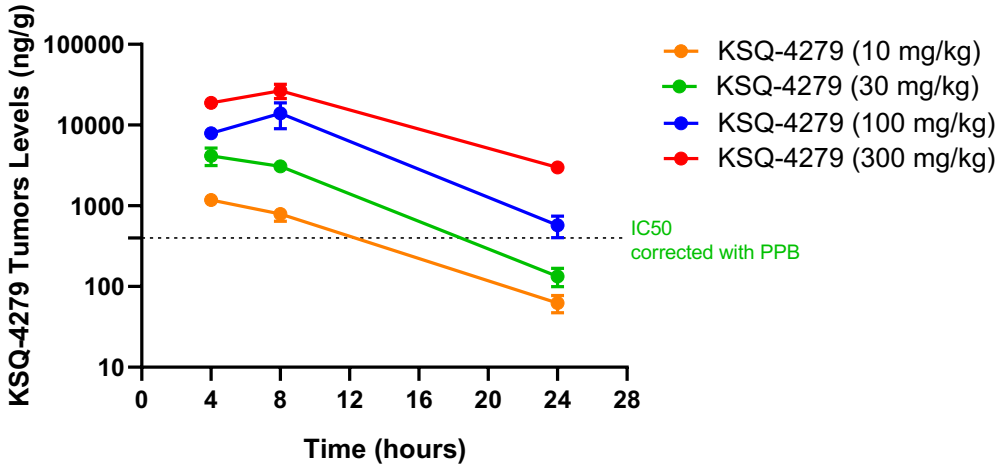
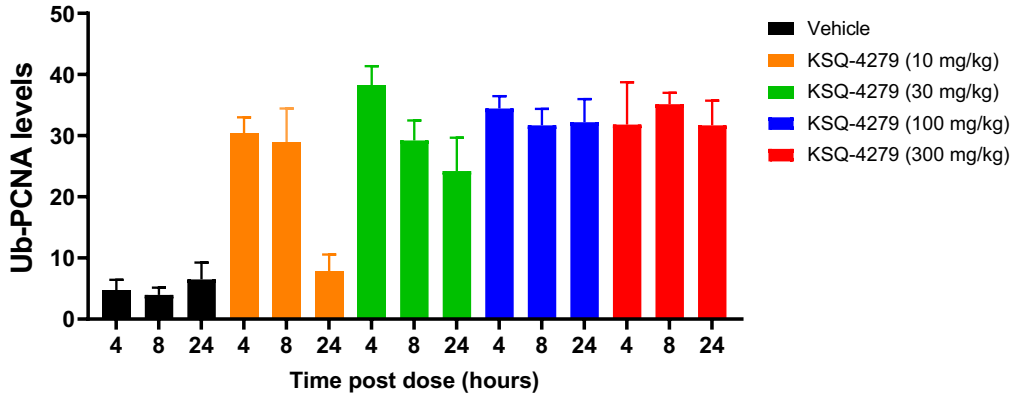
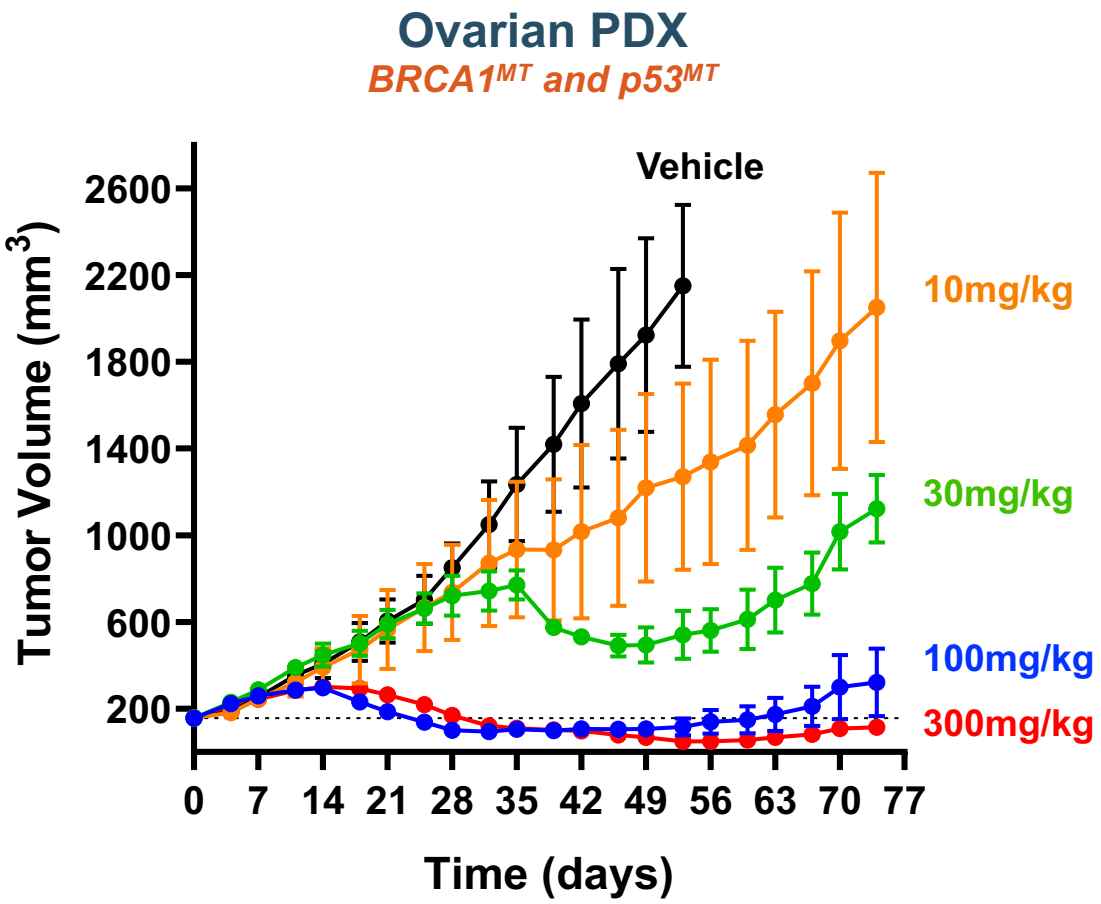
KSQ-4279 Tumor Exposure



Ub-PCNA levels in Tumor



KSQ-4279 PDX Tumor Efficacy



KSQ-4279 in combination with Olaparib leads to durable tumor regression in Ovarian PDX's

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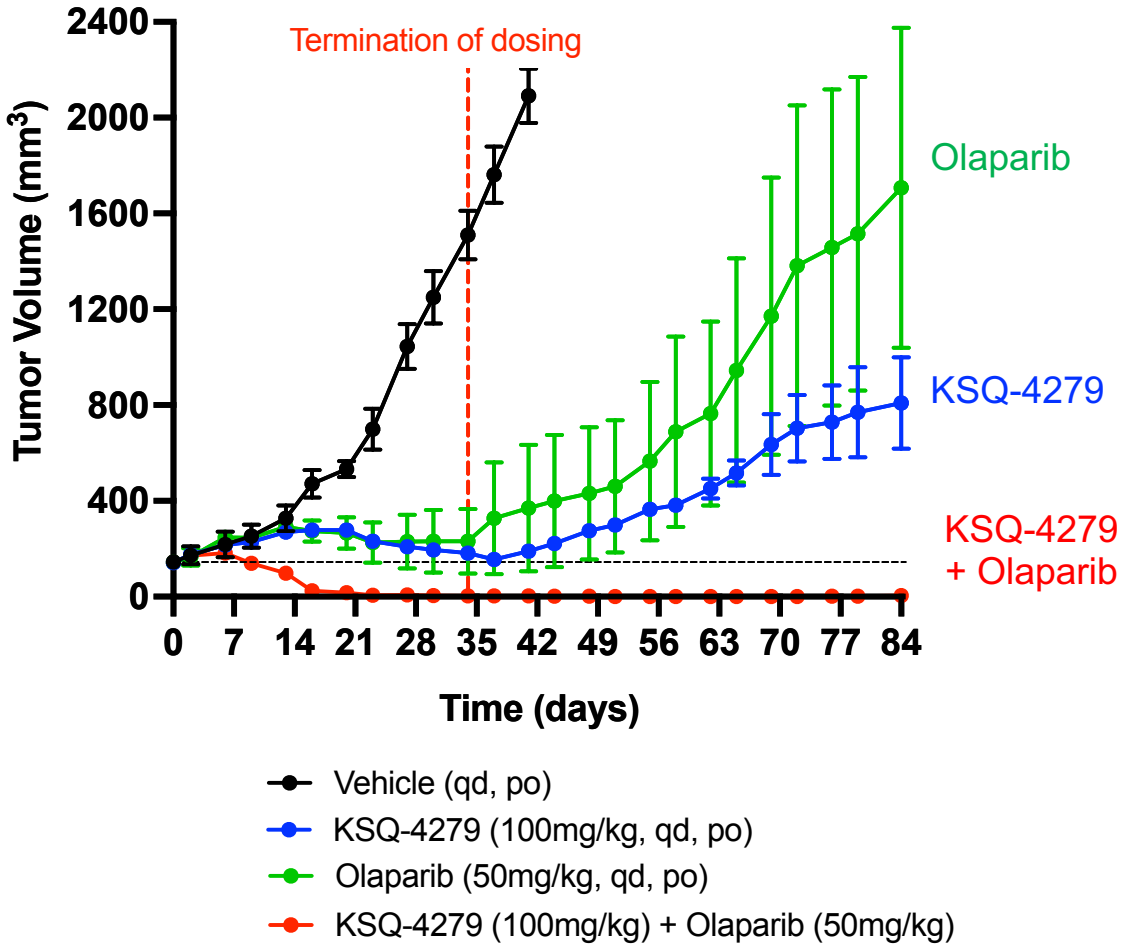
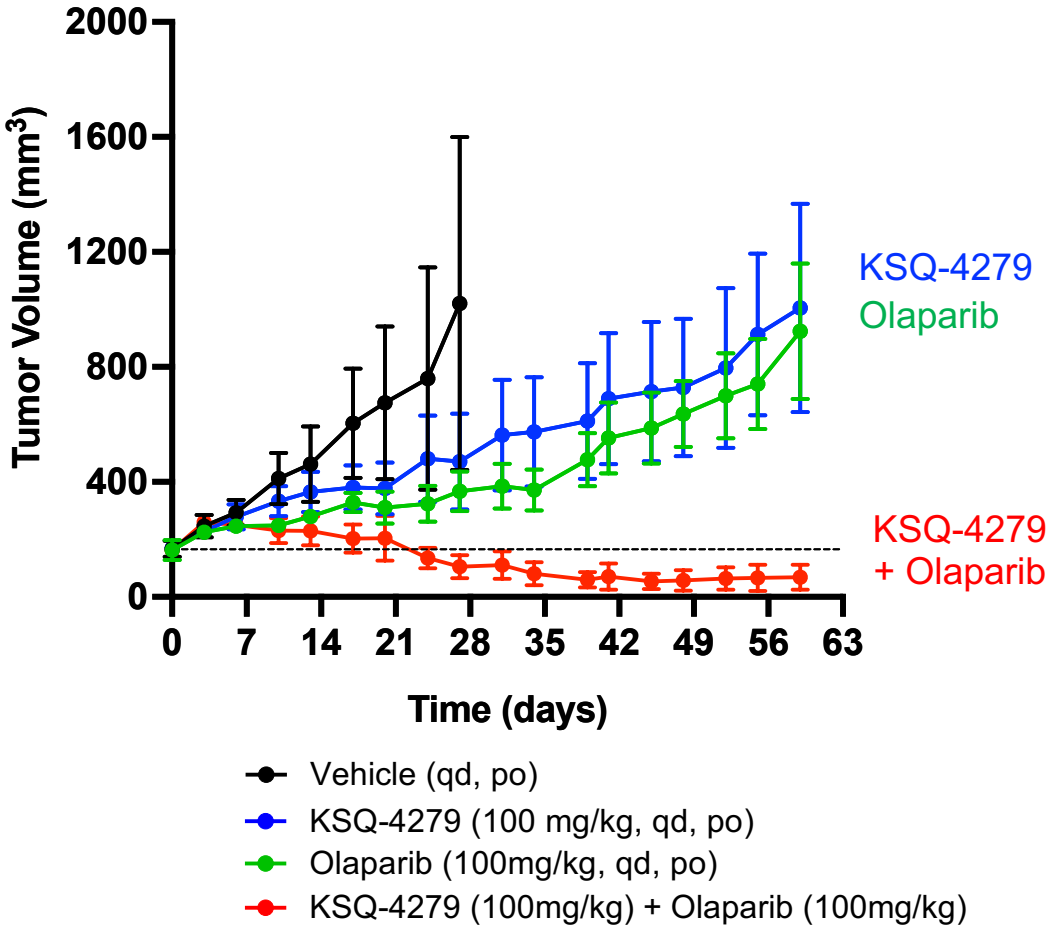
Ovarian PDX #1

$BRCA1^{MT}$, $p53^{MT}$



Ovarian PDX #2

$BRCA1^{MT}$, $p53^{MT}$



KSQ-4279 in combination with Olaparib leads to durable tumor control in Olaparib-resistant TNBC PDX models

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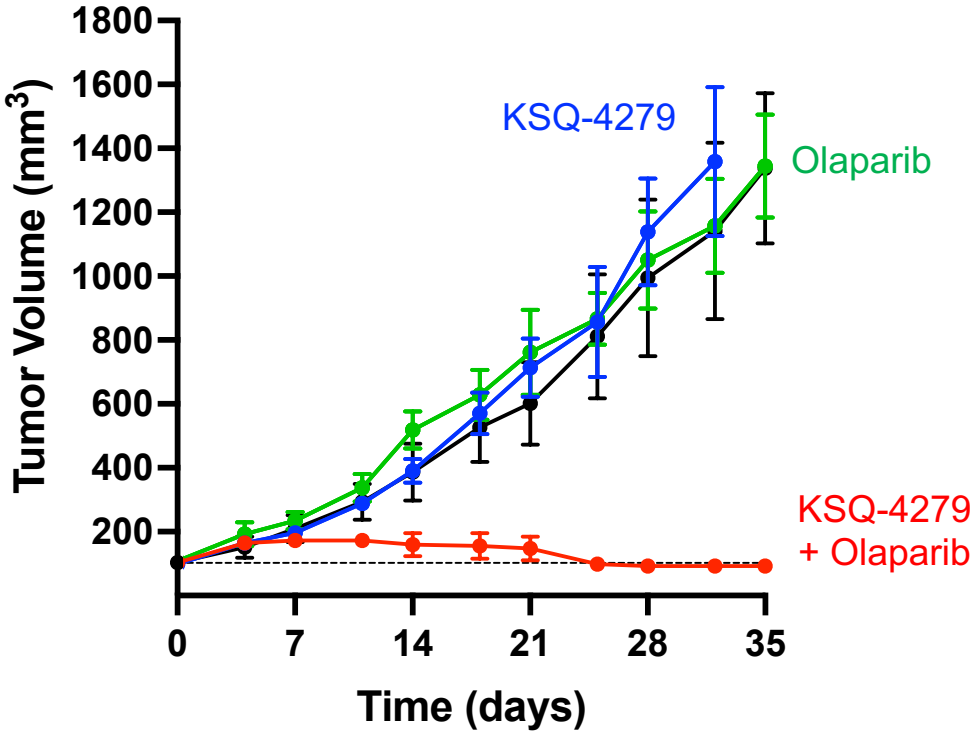
TNBC PDX #1

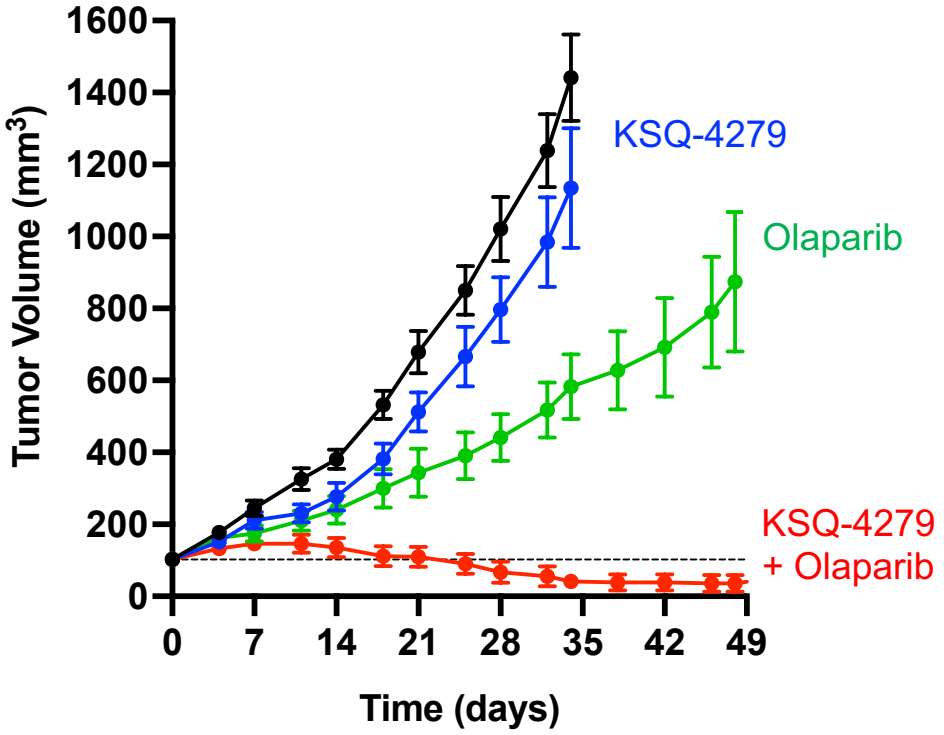
BRCA1^{MT}, p53^{MT}



- Vehicle (qd, po)
- KSQ-4279 (100 mg/kg, qd, po)
- Olaparib (100mg/kg, qd, po)
- KSQ-4279 (100mg/kg) + Olaparib (100mg/kg)

TNBC PDX #2

BRCA1^{MT}, p53^{MT}



- Vehicle (qd, po)
- KSQ-4279 (100mg/kg, qd, po)
- Olaparib (50mg/kg, qd, po)
- KSQ-4279 (100mg/kg) + Olaparib (50mg/kg)

KSQ-4279 – A First-in-Class USP1 Inhibitor for the Treatment of cancers with homologous recombination deficiency

- **KSQ CRISPRomics® platform identified USP1 as an attractive cancer target**
  - USP1 regulates DNA damage repair pathways distinct from PARP inhibitors
- **KSQ-4279 is a potent, selective, allosteric inhibitor of USP1**
- **Efficacy observed both as a single agent and in combination with PARPi across multiple BRCA/HRD xenograft models**
- **CRISPRomics® resistance screens indicate that KSQ-4279 has a complimentary resistance profile to PARP inhibitors**
- **KSQ-4279 Phase I trial ongoing**

Acknowledgements

Back-up slides

Are Distinct Resistance Profiles Associated With USP1 vs PARP Inhibitors?

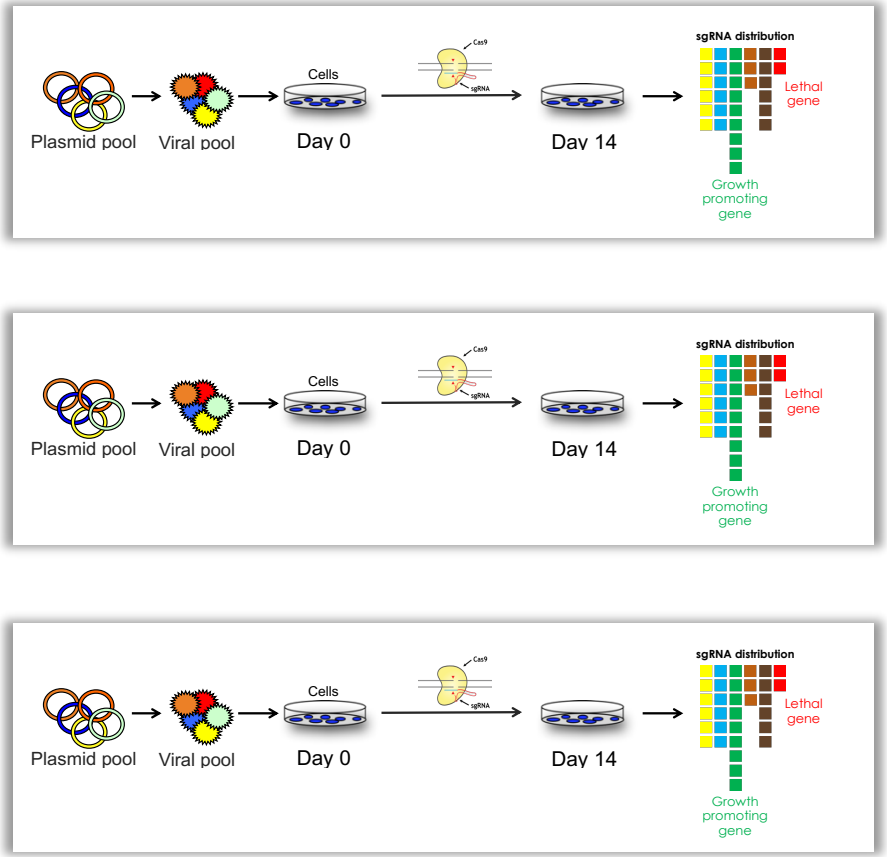
CRISPR screens used to investigate primary mechanisms of resistance to USP1i and PARPi

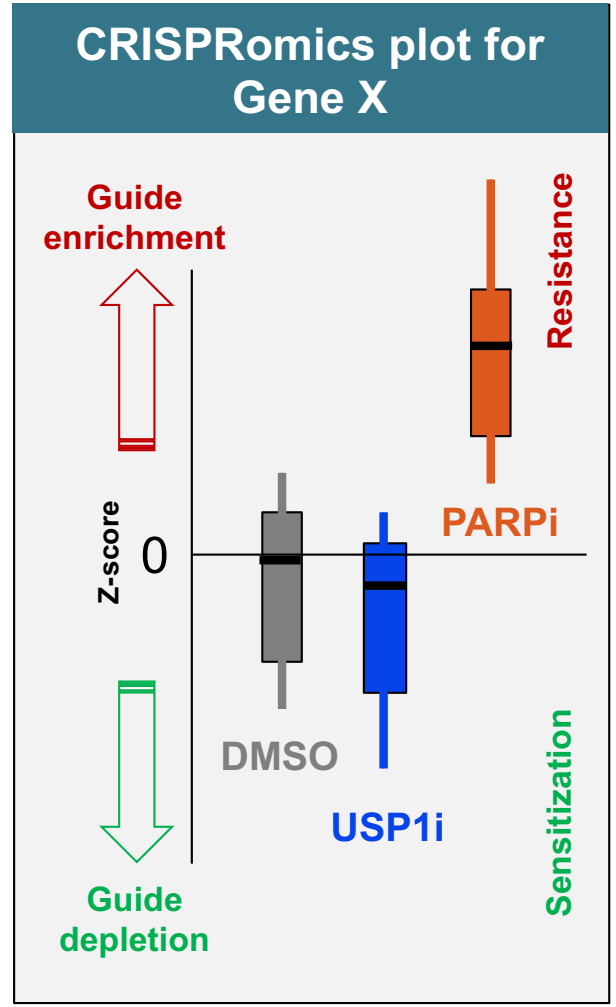
Treatment

DMSO

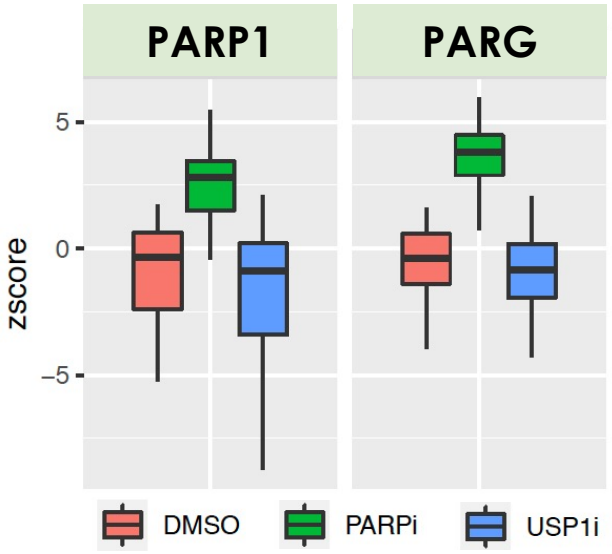
USP1i

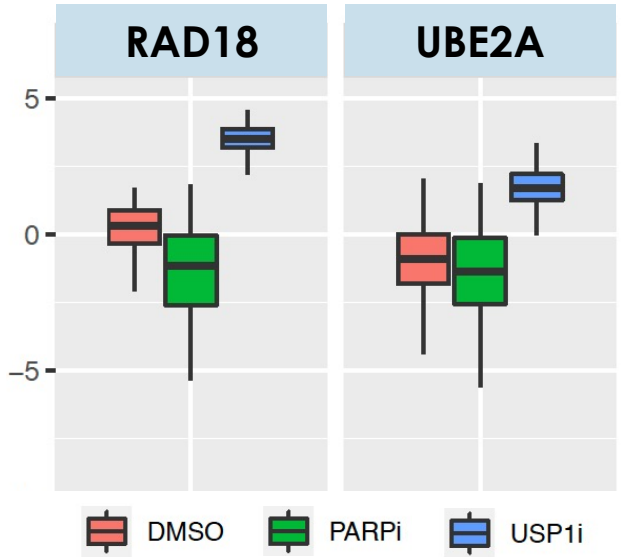
PARPi

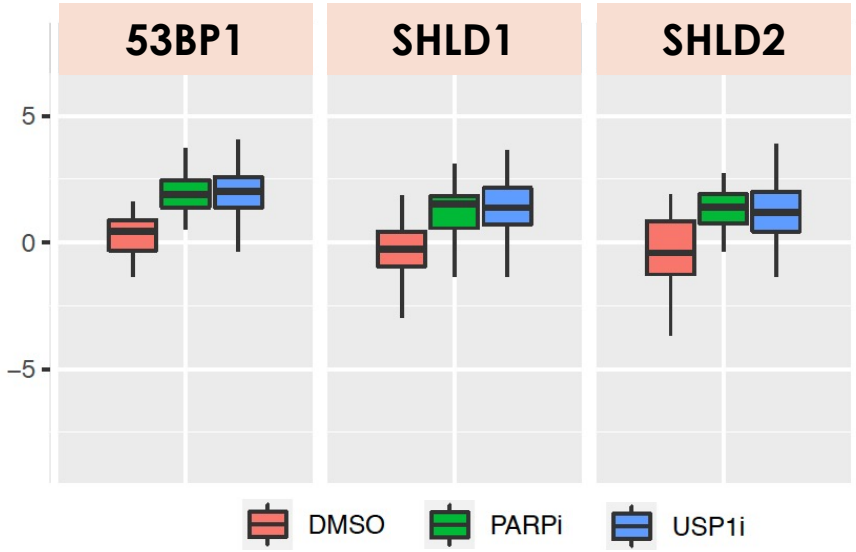




CRISPR screening highlights different mechanisms of resistance to USP1i and PARPi



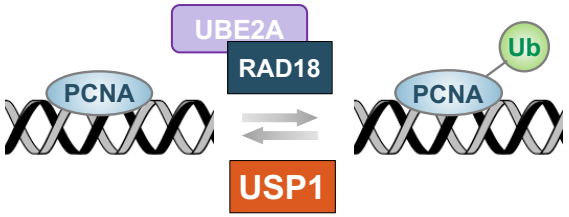




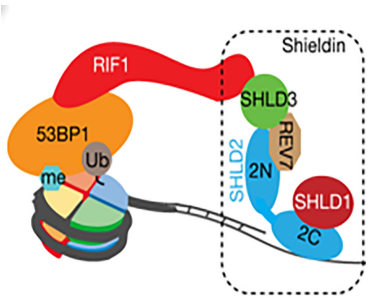
Selective Loss of PARG Restores PARylation and Counteracts PARP Inhibitor-Mediated Synthetic Lethality

Genome-wide and high-density CRISPR-Cas9 screens identify point mutations in *PARP1* causing PARP inhibitor resistance

Stephen J. Pettitt[1,2], Dragomir B. Krastev[1,2], Inger Brandsma[1,2], Amy Dréan[1,2], Feifei Song[1,2], Radoslav Aleksandrov[3], Maria I. Harrell[4], Malini Menon[1,2], Rachel Brough[1,2], James Campbell[1,2], Jessica Frankum[1,2], Michael Ranes[5], Helen N. Pemberton[1,2], Rumana Rafiq[1,2], Kerry Fenwick[6], Amanda Swain[6], Sebastian Guettler[5], Jung-Min Lee[7], Elizabeth M. Swisher[4], Stoyno Stoynov[3], Kosuke Yusa[8], Alan Ashworth[9] & Christopher J. Lord[1,2]



Translesion synthesis



Non-Homology End Joining (NHEJ)