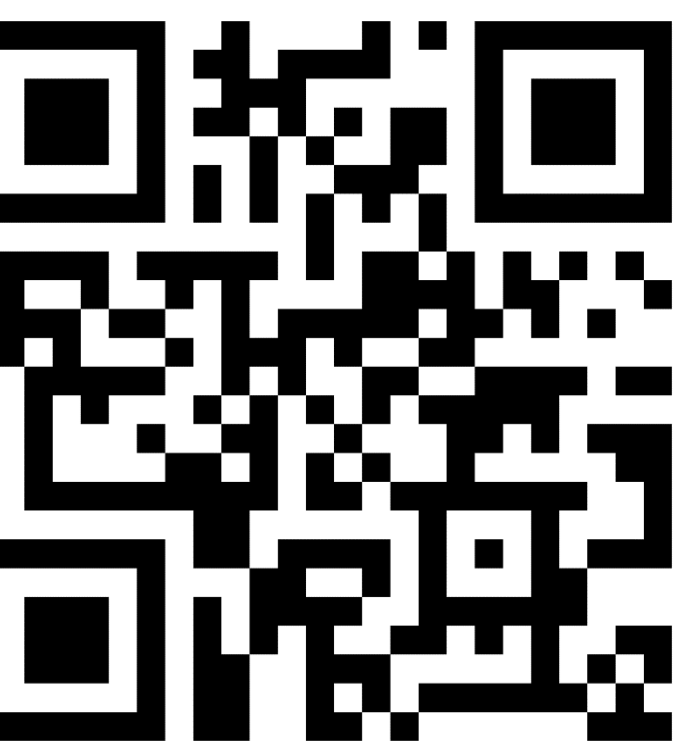


VRK1 inhibition leverages paralog synthetic lethality to selectively target VRK2-deficient cancer cells

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Poster #3090

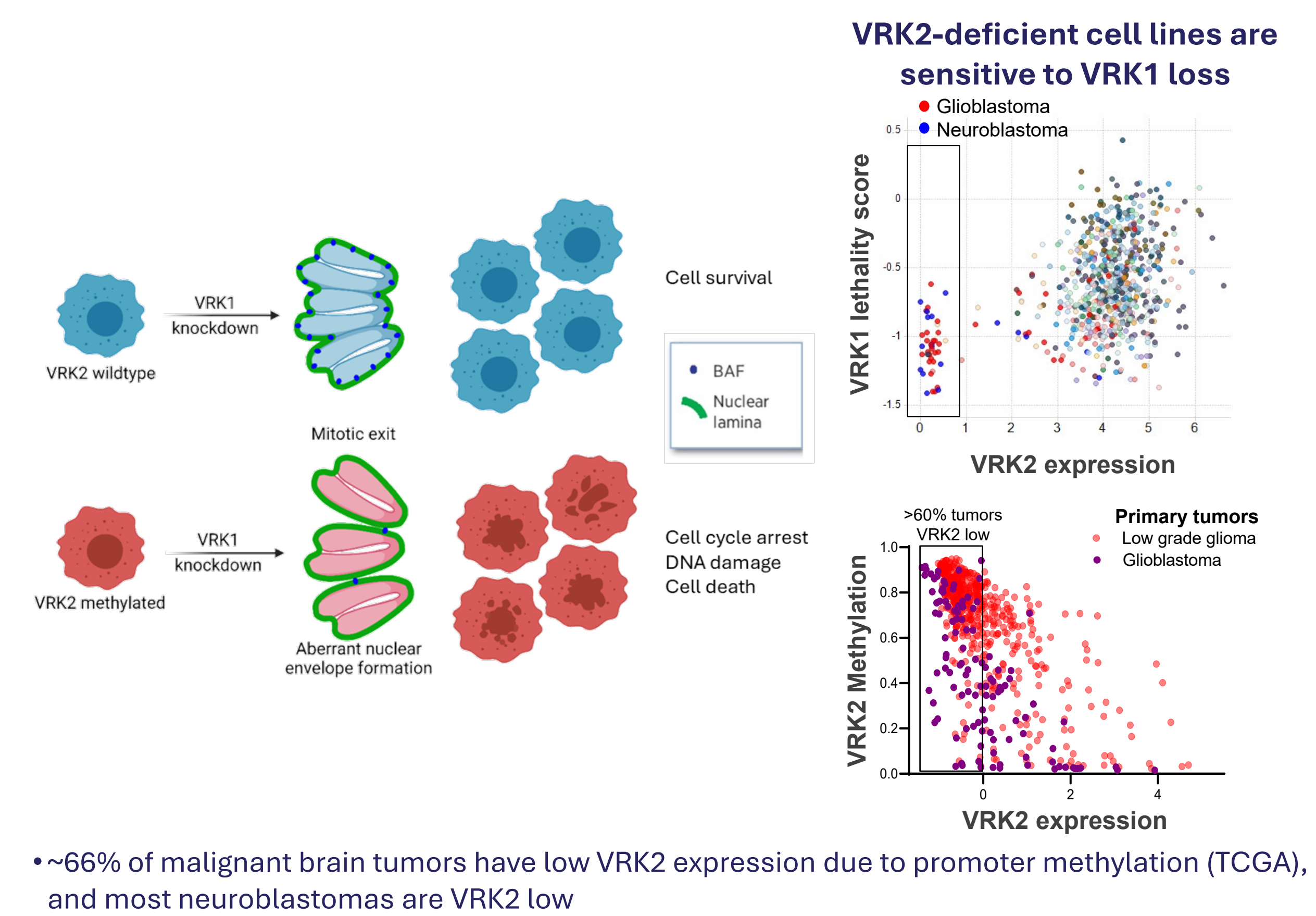
Tango Therapeutics, Boston, MA USA



INTRODUCTION

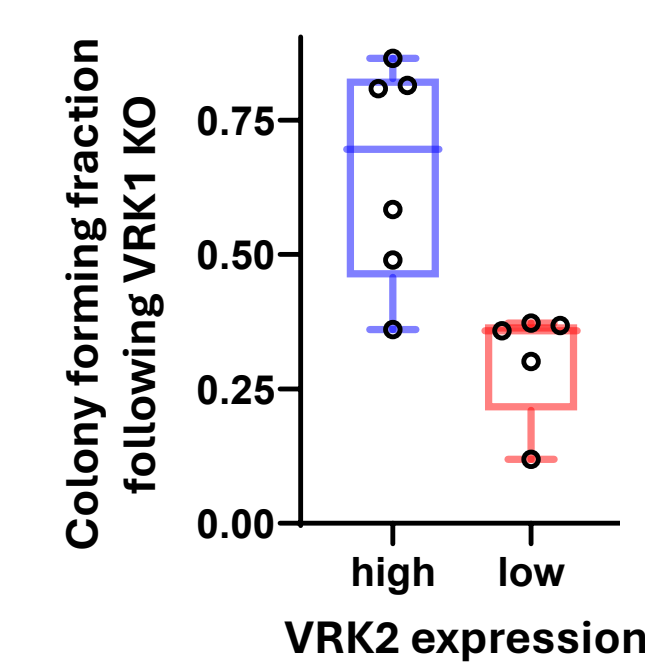
Vaccinia-related kinases (VRKs) are a family of serine/threonine kinases involved in a variety of cellular processes, including cell signaling, chromatin modification, nuclear envelope dynamics, and cell cycle progression. More than 60% of glioblastomas and nearly all neuroblastomas have low expression of the VRK2 gene resulting in deficient VRK2 activity. VRK1 has been identified as a paralog synthetic lethal target in these VRK2-deficient cancers, with the potential for indication expansion into additional cancer types. Here, we show that inhibition of VRK1 leads to a concomitant loss of Barrier to Autointegration Nuclear Assembly Factor 1 (BANF1 or BAF) phosphorylation leading to aberrant nuclear envelope formation and downstream loss of cellular viability. As a target, VRK1 is both tractable and structurally-enabled, with chemical series capable of achieving >4000-fold biochemical selectivity against its paralog VRK2 and >70-fold viability selectivity in VRK2 isogenic cell line pairs. Compounds show strong correlations between biochemical, cellular target engagement, pharmacodynamic, and functional viability assays. Furthermore, in vivo tolerability studies suggest that VRK1 inhibitors are well-tolerated in immunocompromised mice. Preclinical validation studies support the development of VRK1 inhibitors for the treatment of patients with VRK2-deficient tumors such as glioblastoma and neuroblastoma.

VRK1 INHIBITION IS SYNTHETIC LETHAL WITH VRK2-DEFICIENCY¹



CRISPRn validation

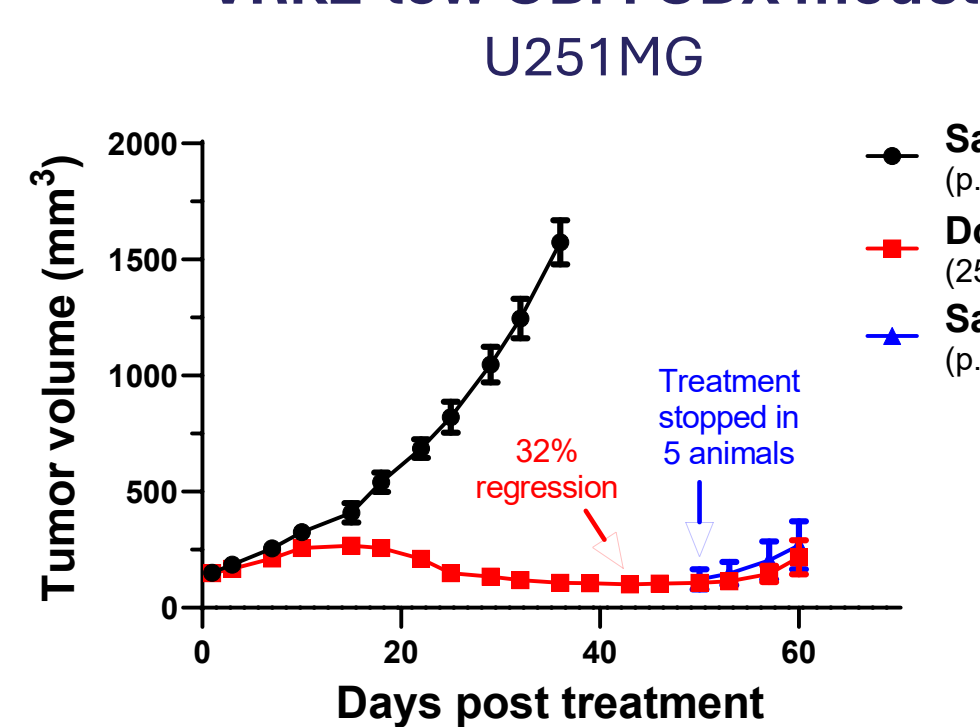
14-day colony formation assays



Paralog synthetic lethality is maintained in a GBM cell line panel

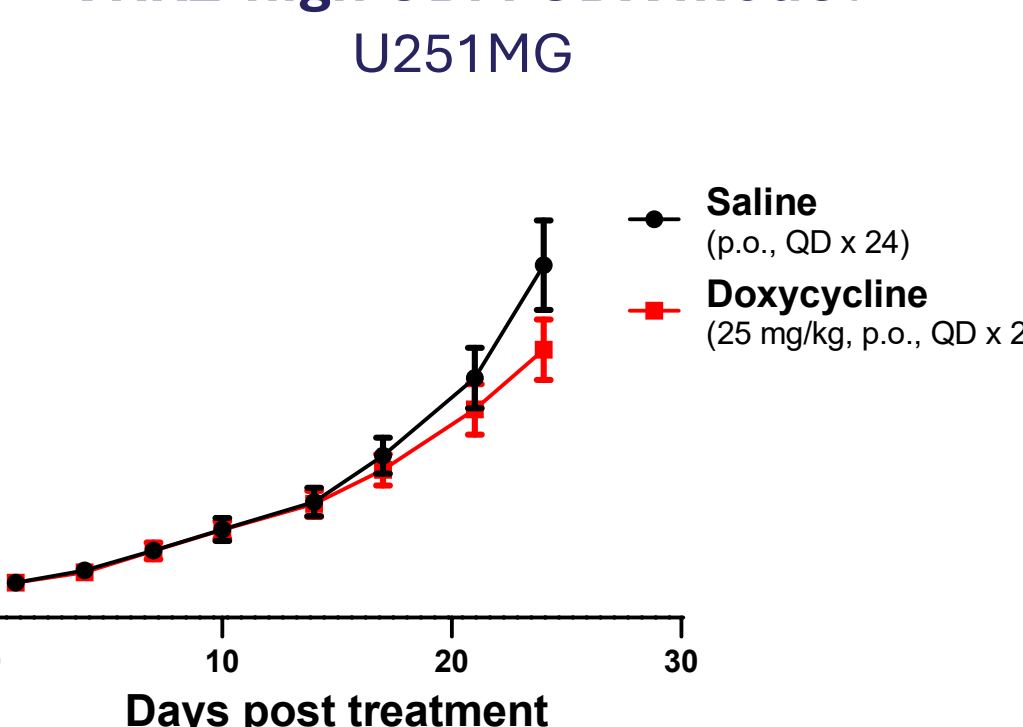
VRK1 kinase activity is necessary for VRK2-low cell survival

VRK2-low GBM CDX model

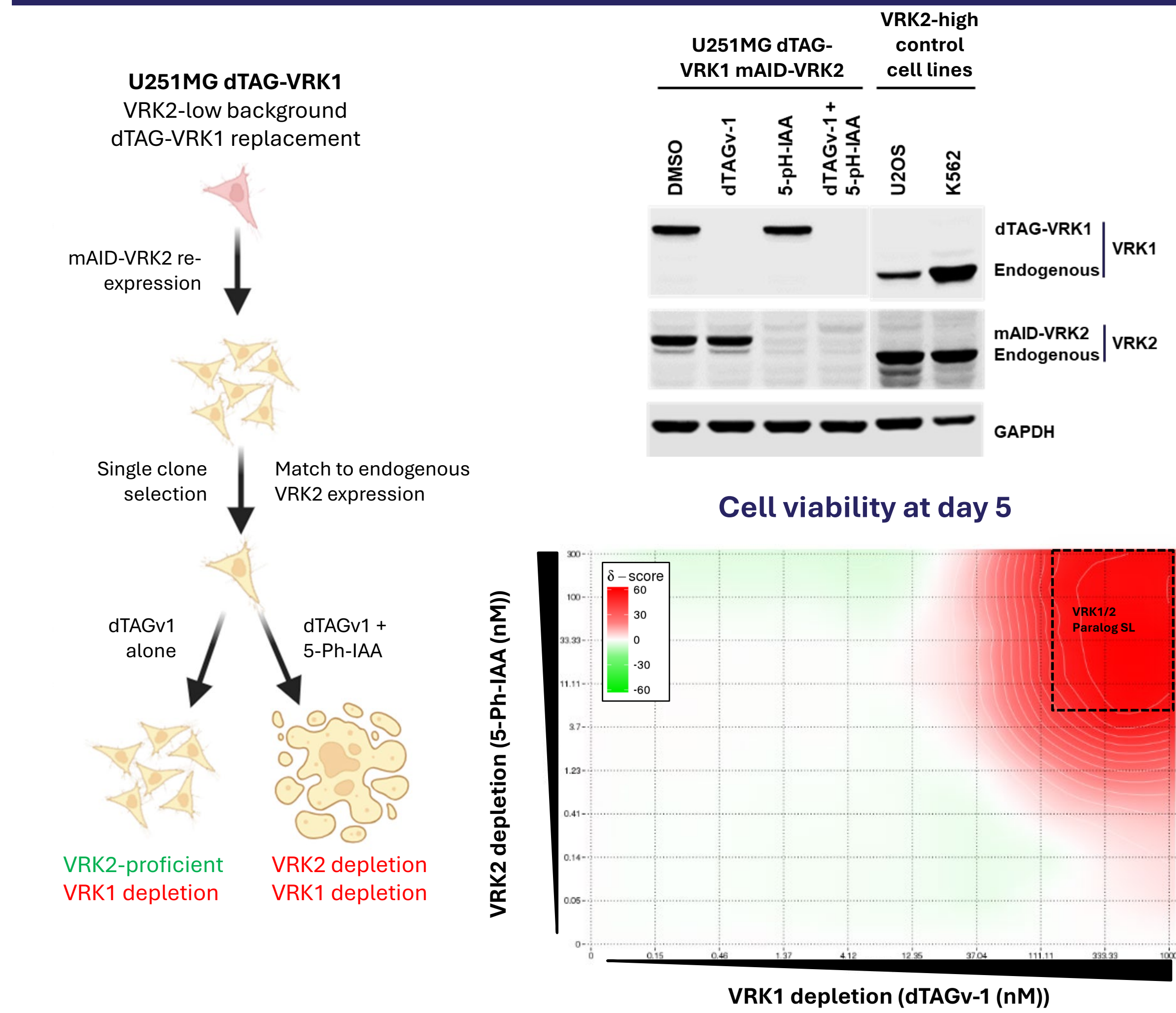


VRK2-low xenograft model is selectively dependent on VRK1

VRK2-high GBM CDX model

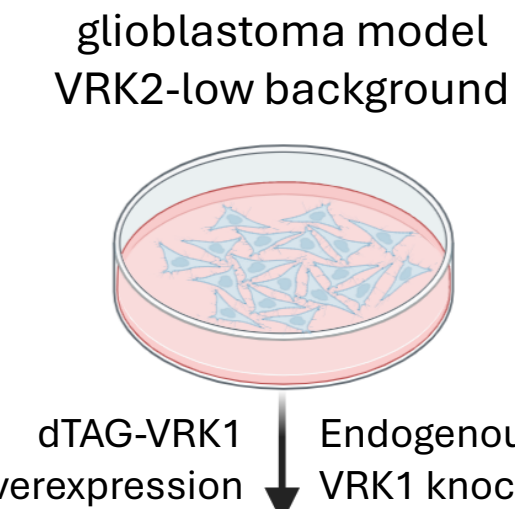


TARGETED PROTEIN DEGRADATION SYSTEMS PHARMACOLOGICALLY VALIDATE PARALOG SYNTHETIC LETHALITY

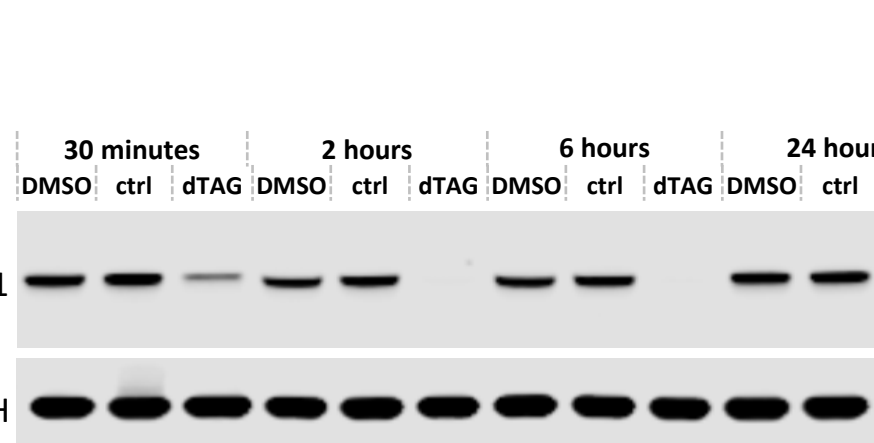


Dual degradation system shows strong synergy between VRK1 and VRK2 degradation (ZIP synergy score: 10.06)

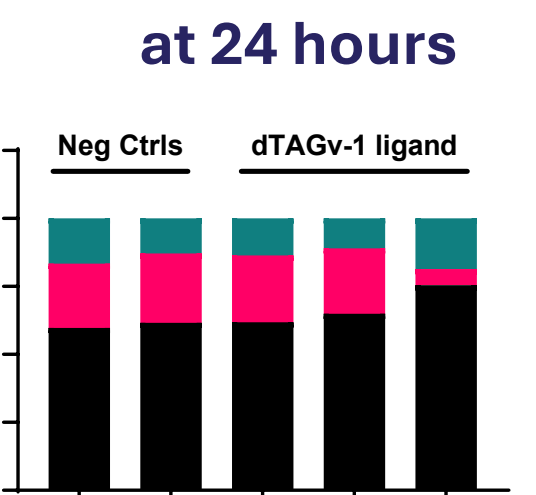
U251MG glioblastoma model



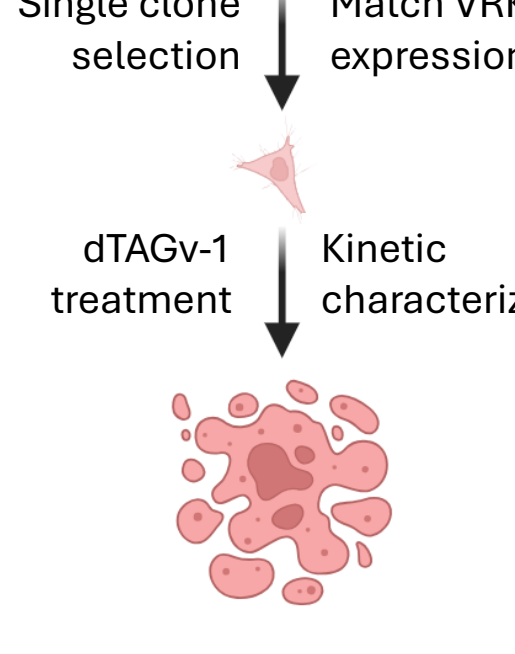
VRK1 degradation in 2 hours



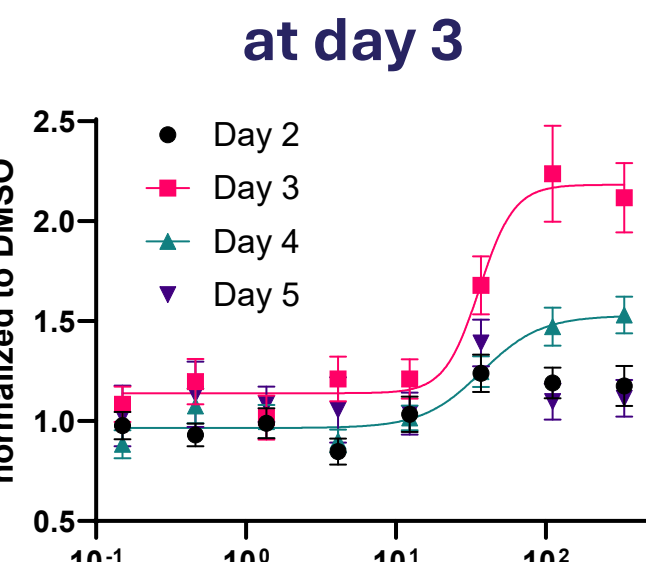
Cell cycle arrest at 24 hours



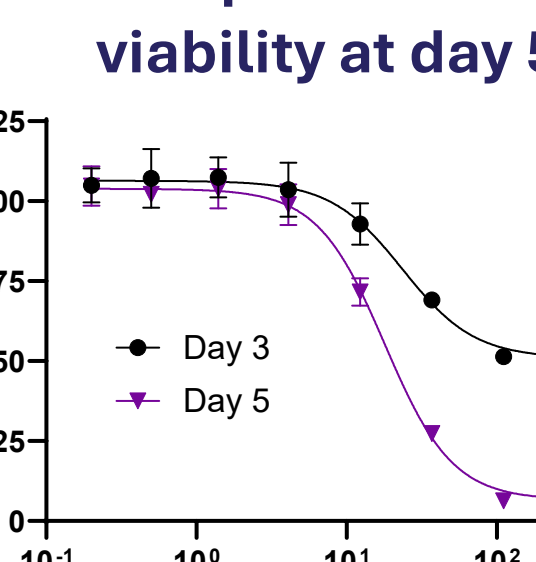
Single clone selection



Apoptosis induction at day 3



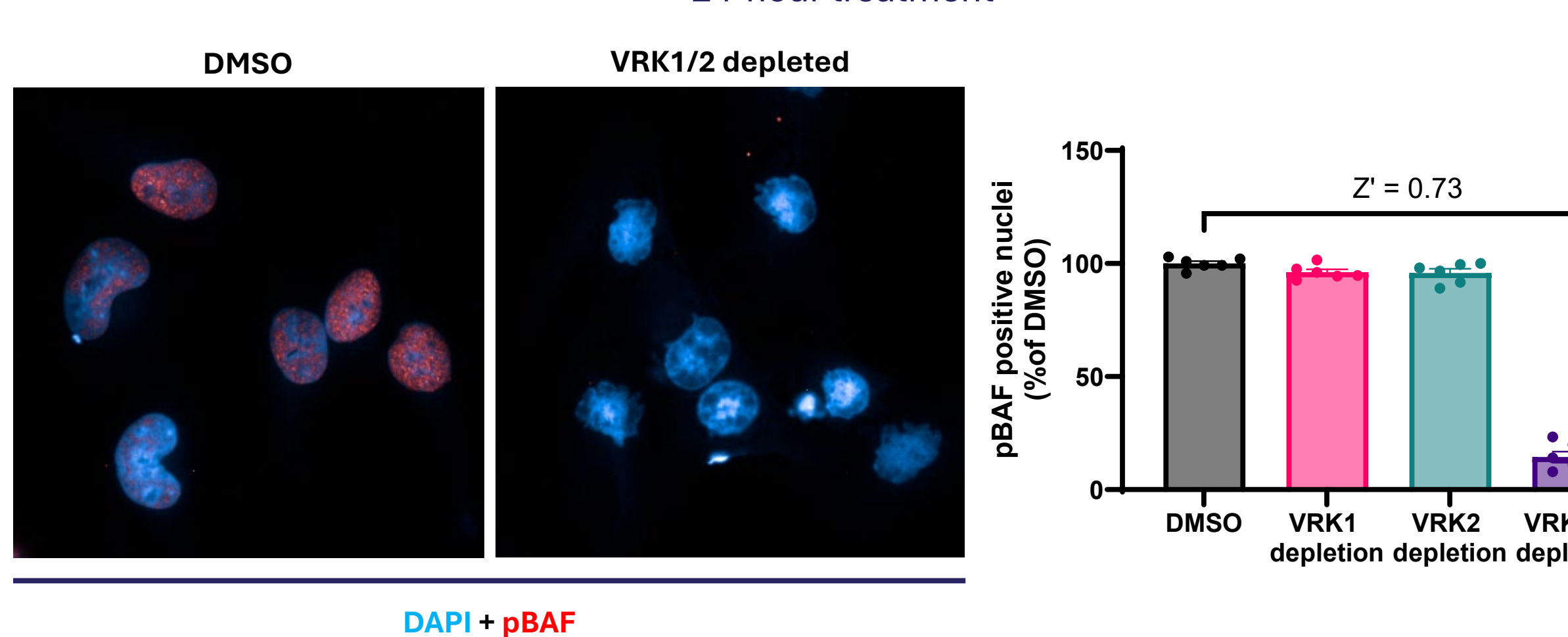
Complete loss of viability at day 5



Targeted protein degradation using dTAG confirms synthetic lethality

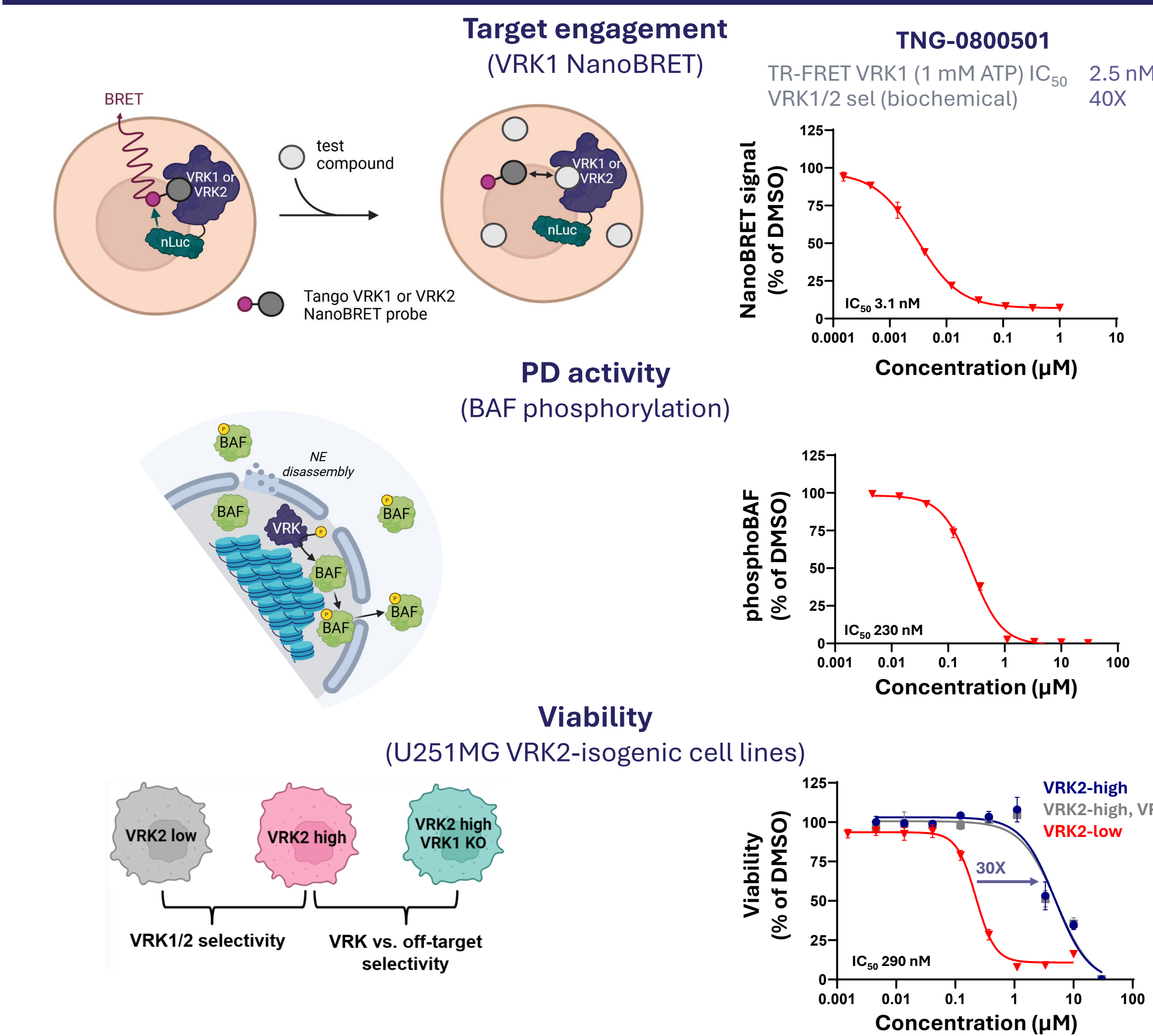
TANGO pBAF PD ASSAY REPORTS VRK1/2 PERTURBATION

High content screening assay 24-hour treatment

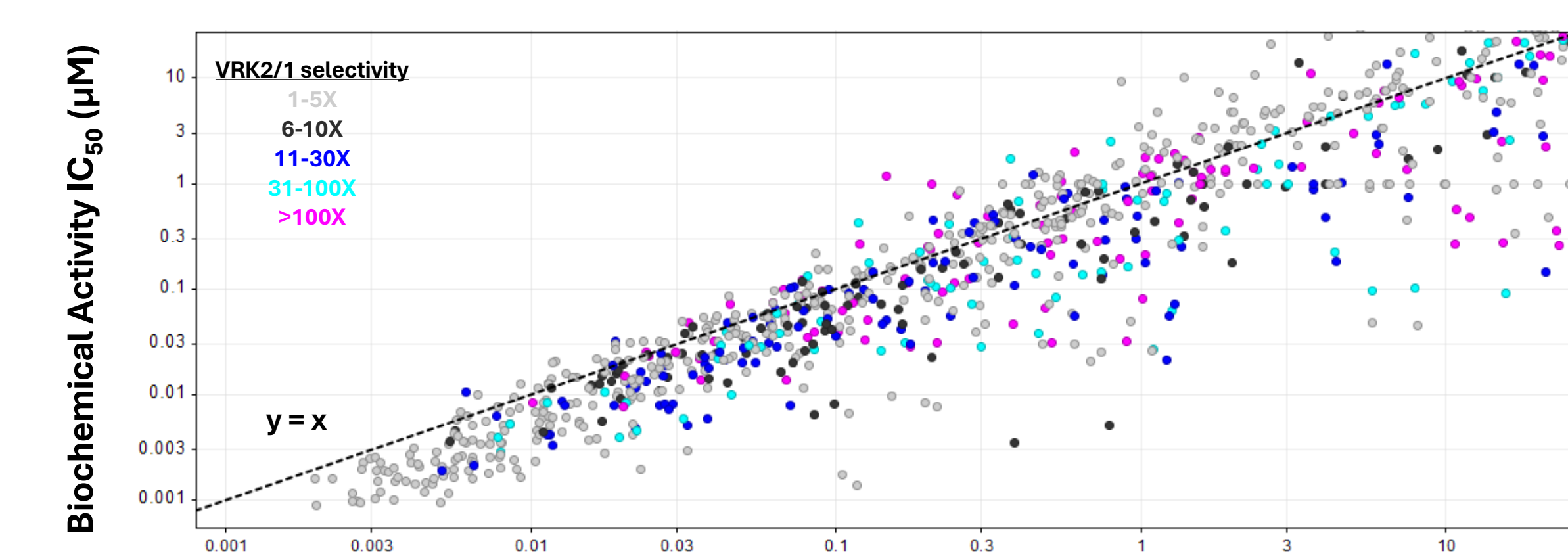


Tango-developed pBAF antibody detects shared VRK1/2 phosphorylation site

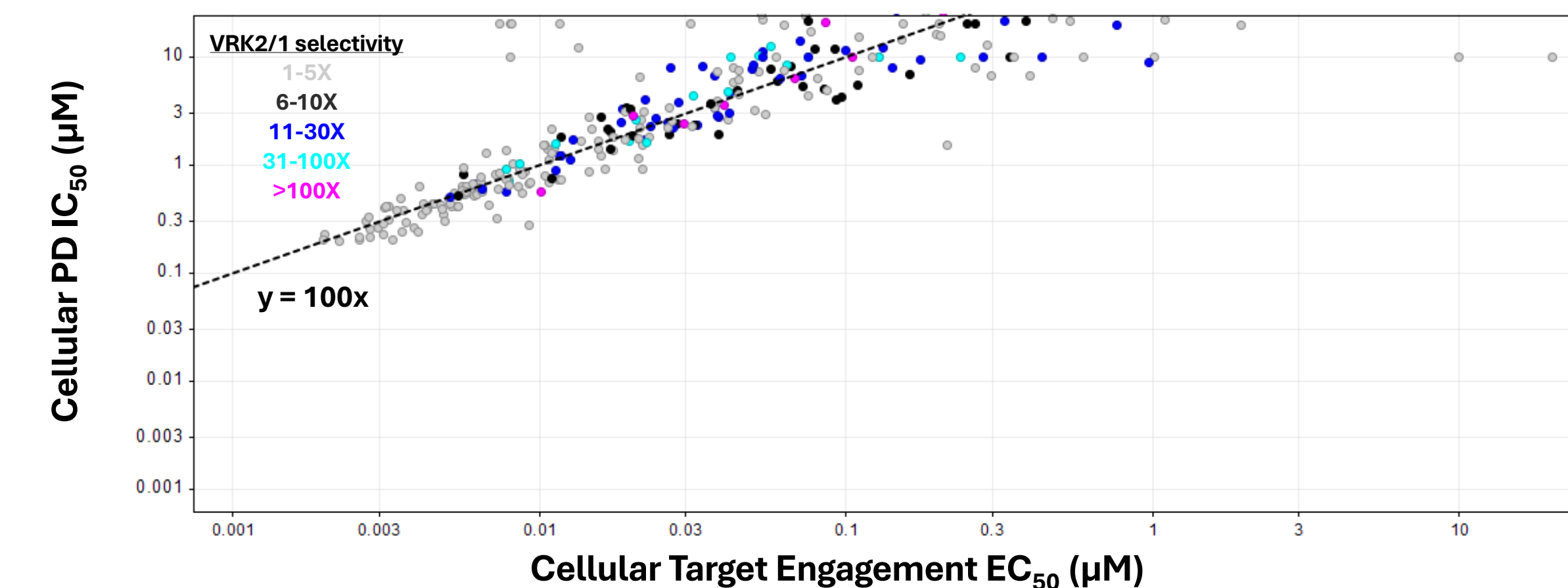
TANGO VRK1 SMALL MOLECULE INHIBITORS GENERATE PROOF-OF-CONCEPT FOR CELLULAR SELECTIVITY AND EFFICACY



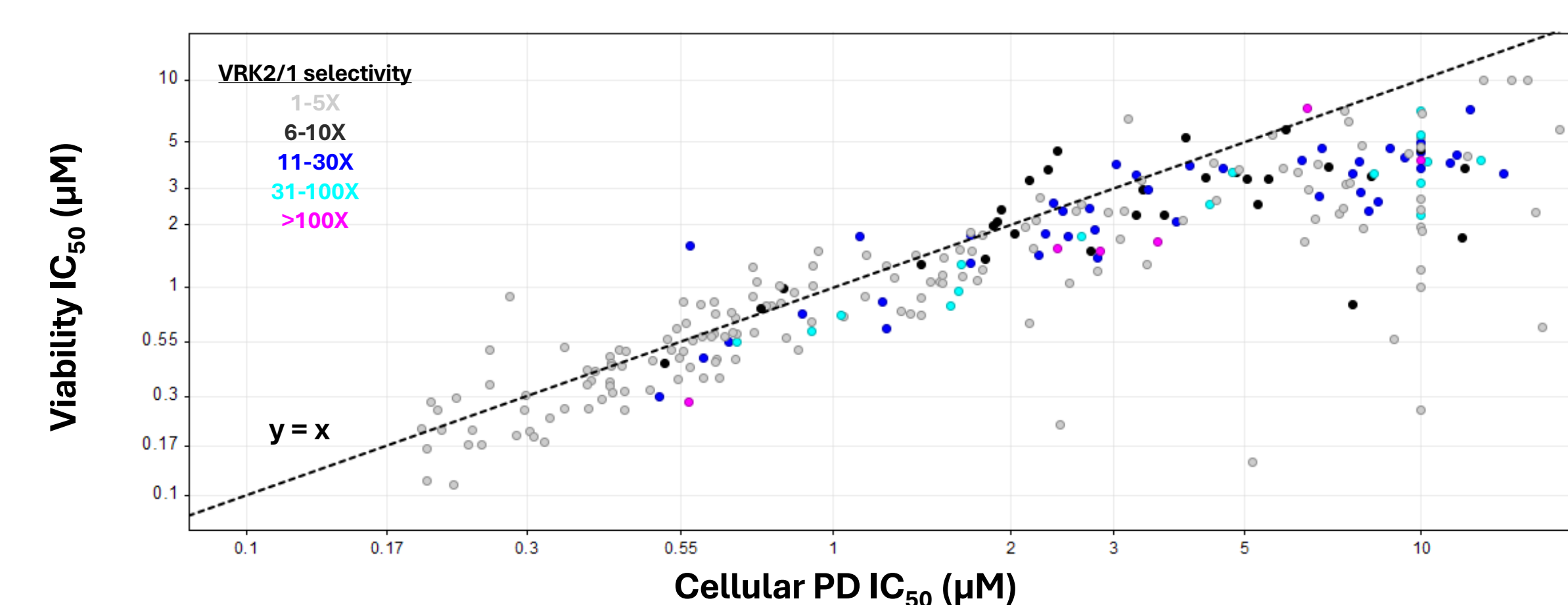
Cellular drug discovery funnel characterizes compounds for target engagement, PD, and VRK2-selective viability



Cellular target engagement (NanoBRET) correlates with physiologically-relevant biochemical activity

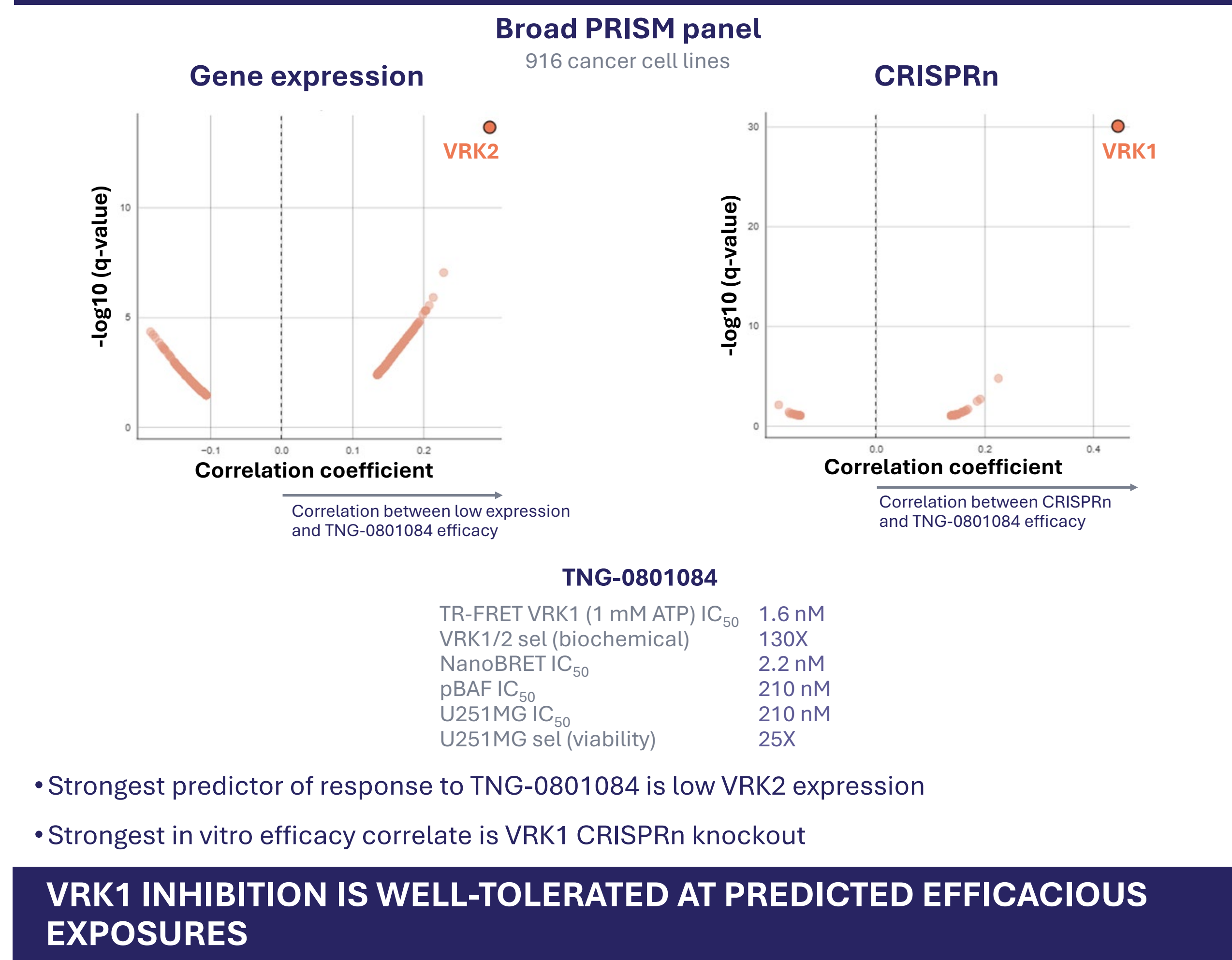


Cellular target engagement (NanoBRET) correlates with BAF phosphorylation PD assay



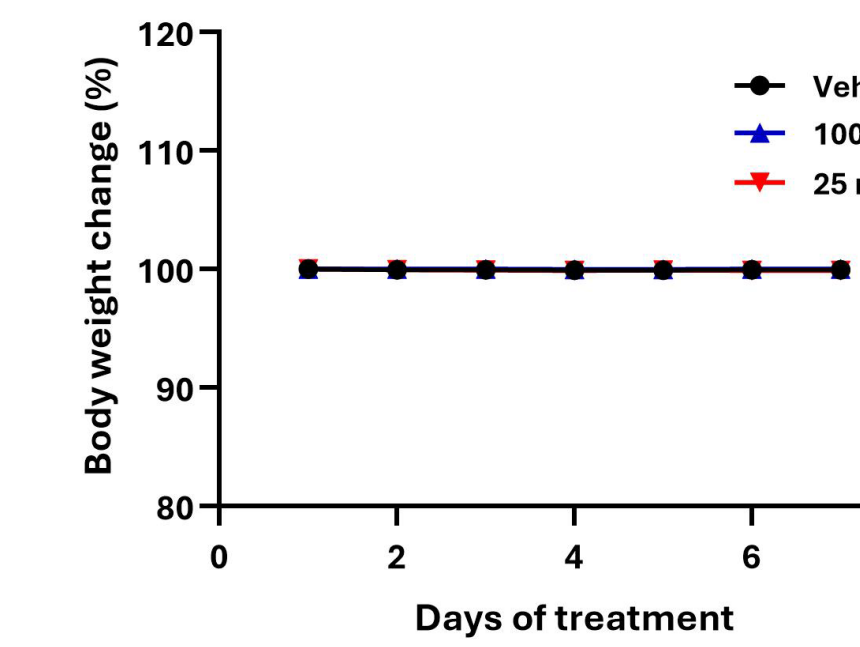
BAF phosphorylation PD assay correlates with cellular viability in VRK2-low cells

EXEMPLAR MOLECULE CONFIRMS THERAPEUTIC RATIONALE IN LARGE PANEL OF CANCER CELL LINES



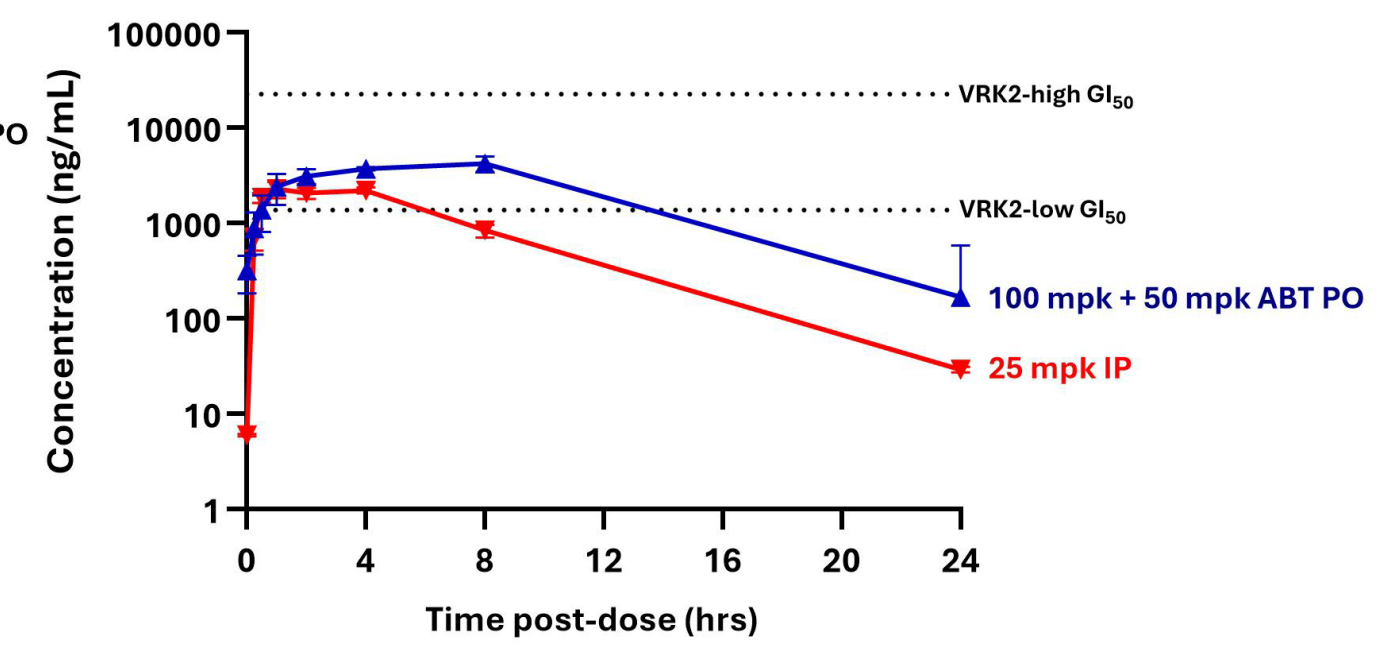
Tolerability study

BALB/c nude mice



Terminal PK

BALB/c nude mice



TNG-0803263

TR-FRET VRK1 (1 mM ATP) IC ₅₀	1.6 nM
VRK1/2 sel (biochemical)	90X
NanoBRET IC ₅₀	2.0 nM
pBAF IC ₅₀	210 nM
U251MG IC ₅₀	120 nM
U251MG sel (viability)	16X

Predicted efficacious exposures are extremely well-tolerated in BALB/c nude mice

SUMMARY

- VRK1 is a novel therapeutic target in tumors with low VRK2 expression, particularly brain cancers and neuroblastoma.
- Drug discovery program is fully enabled with excellent correlation between biochemical and cellular functional and target engagement assays.
- Excellent VRK1/2 biochemical selectivity translates into strong selectivity for VRK2-low cancer cells in viability assays.
- There is a large addressable patient population, including low-grade glioma, glioblastoma, and neuroblastoma.
- Biochemical data complement biological validation (see AACR poster #484).
- VRK1 has been enabled for drug discovery and is available for partnering.

Acknowledgements

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References

¹ Shields JA, Meier SR, Bandi M, et al. VRK1 Is a Synthetic-Lethal Target in VRK2-Deficient Glioblastoma. Cancer Res. 2022;82(21):4044-4057. doi:10.1158/0008-5472.CAN-21-4443