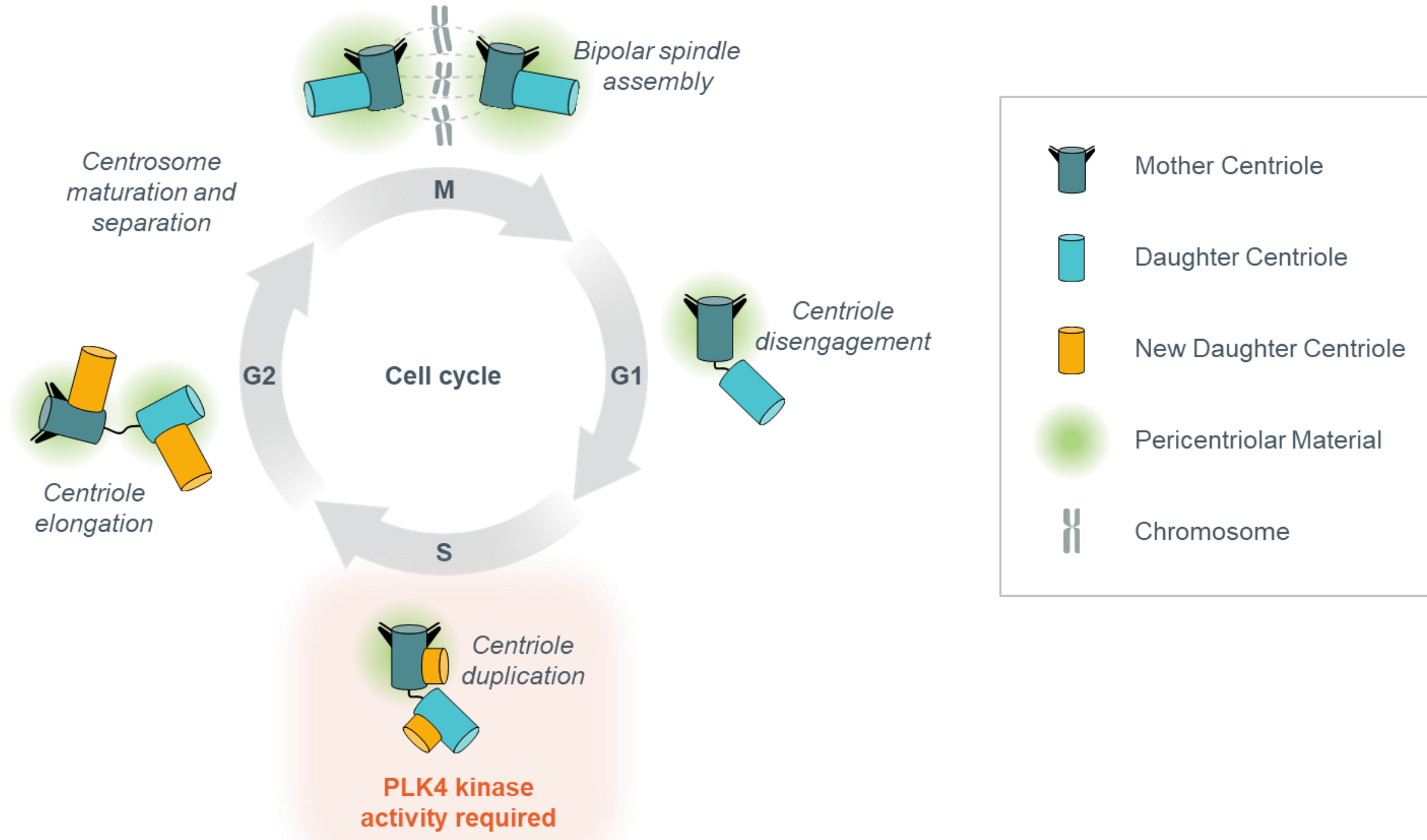
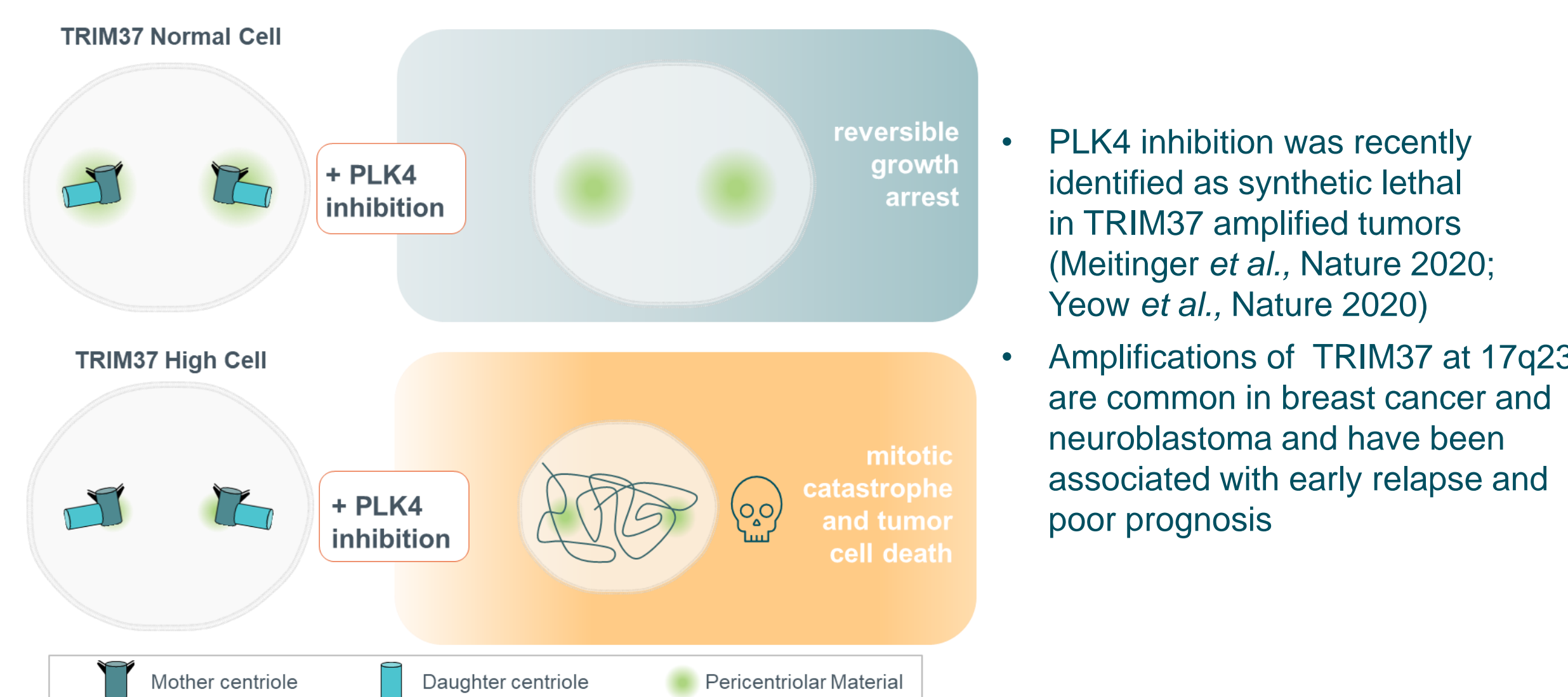


Background

Polo-like kinase 4 (PLK4) is a Cell Cycle Kinase That Controls Centrosome Duplication



Synthetic Lethality of PLK4 Inhibition in TRIM37 Amplified Tumor Cells



- PLK4 inhibition was recently identified as synthetic lethal in TRIM37 amplified tumors (Meitinger *et al.*, Nature 2020; Yeow *et al.*, Nature 2020)
- Amplifications of TRIM37 at 17q23 are common in breast cancer and neuroblastoma and have been associated with early relapse and poor prognosis

1. ORIC PLK4 Inhibitors are Potent and Selective

Biochemical Potency of PLK4 Inhibitors

	ORIC Compound X	ORIC Compound Y	ORIC Compound Z	Centrinone	CFI-400945
PLK4 IC ₅₀ (nM)	0.434	1.58	1.79	0.493	0.496
Fold AurA/PLK4	3,304x	973x	8,849x	156x	86x
Fold AurB/PLK4	535x	134x	969x	1,582x	12x
Inhibition of PLK1@1uM	-	19%	1%	-	1%
Inhibition of PLK2@1uM	-	4%	0	-	14%
Inhibition of PLK3@1uM	-	3%	1%	-	31%

Figure 1. Biochemical IC₅₀ values were determined measuring luminescence generated from ADP-Glo. ORIC compounds X, Y, and Z are novel inhibitors of PLK4 synthesized by ORIC. Aurora A and B are closely related kinases. Reference structures: Centrinone, Wong *et al.*, Science 2015; CFI-400945, Mason *et al.*, Cancer Cell 2014.

2. ORIC PLK4 Inhibitors Have Excellent Kinome Selectivity

Kinome Profiles of PLK4 Inhibitors

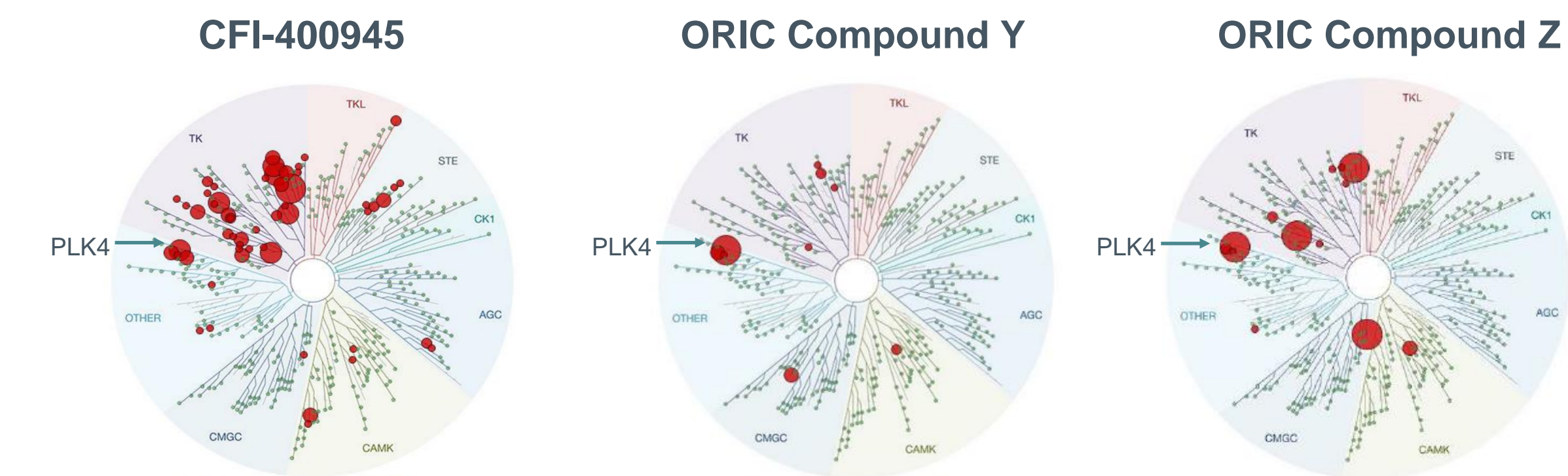
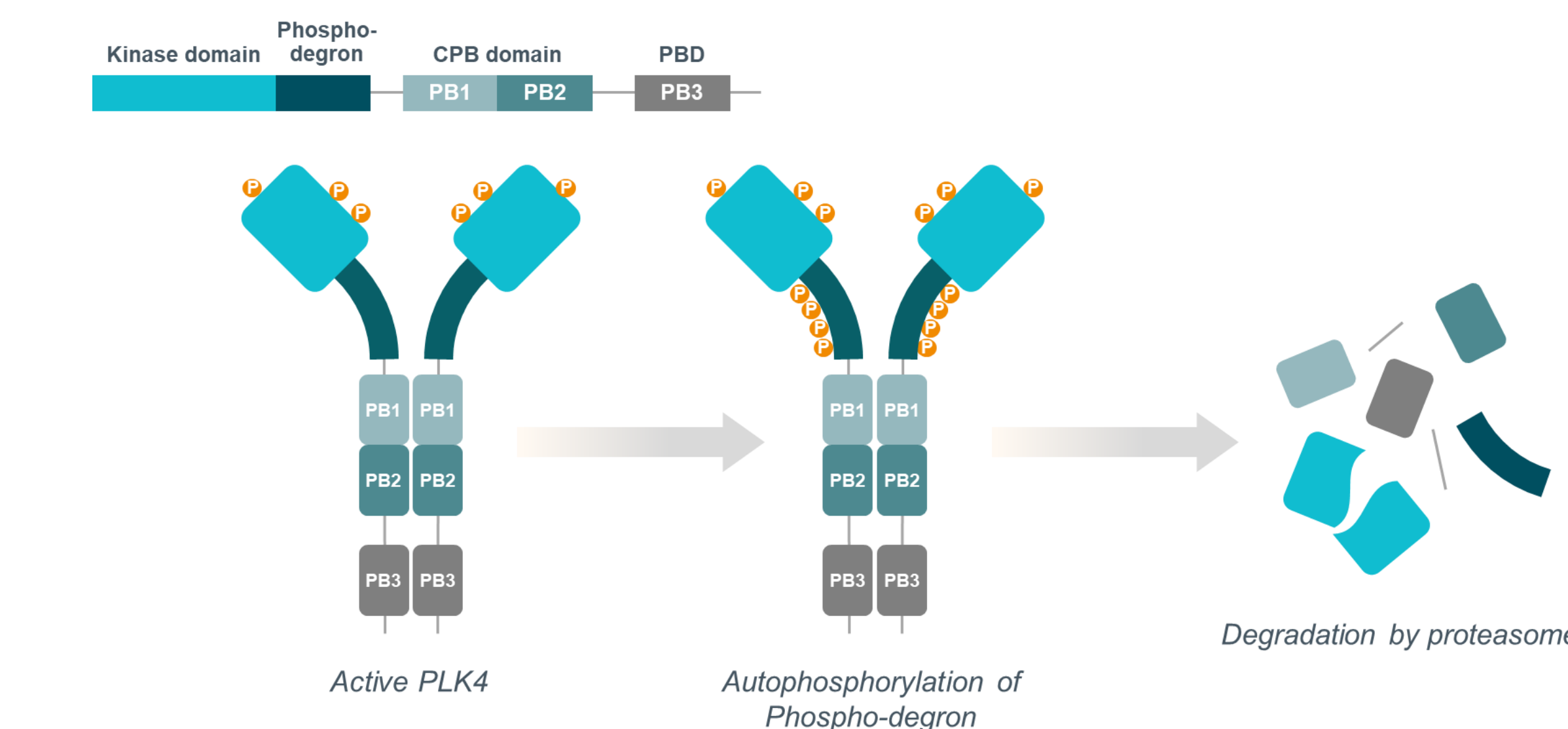


Figure 2. Kinase binding profiles across 489 kinases at 1uM assessed using KINOMEScan. Individual kinome trees are depicted with red circles indicating the kinases impacted within 10% of control.

3. PLK4 Inhibition Leads to Protein Stabilization

PLK4 Activity Results in Trans-Autophosphorylation and PLK4 Degradation



Selective PLK4 Inhibitors Block Autophosphorylation Leading to Stabilization of PLK4

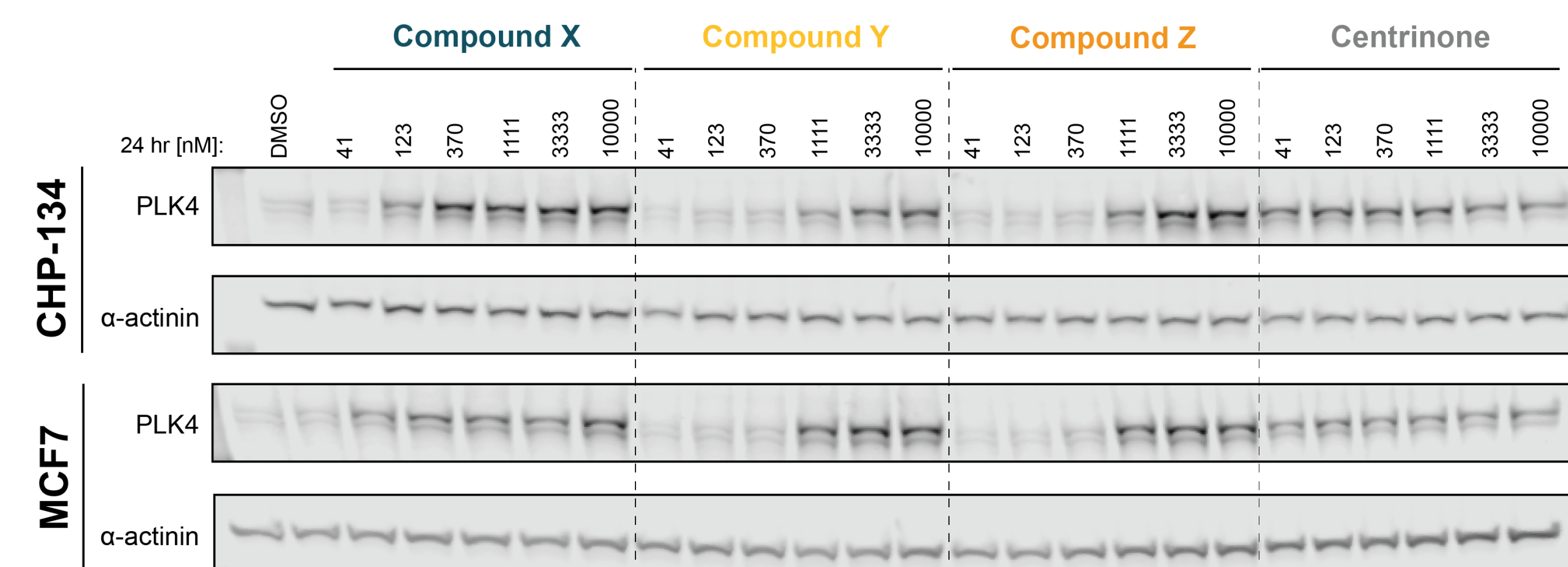


Figure 3. TRIM37 high CHP-134 neuroblastoma cells or MCF7 breast cancer cells were treated for 24 hours with dose-titrations of centrinone, ORIC compounds X, Y, and Z and cell lysates collected. Protein extracts from lysates were run on 3-8% Tris-Acetate gel and transferred to nitrocellulose. Blots were probed with antibodies to PLK4 or alpha-actinin and analyzed on LI-COR Odyssey. TRIM37 high/low as defined in Meitinger *et al.*, Nature 2020.

4. Dependence on PLK4 Correlates with TRIM37 Levels in Breast Cancer Cell Lines

a. Breast Cancer Cell Lines 14 Day siRNA Screen (DepMap)

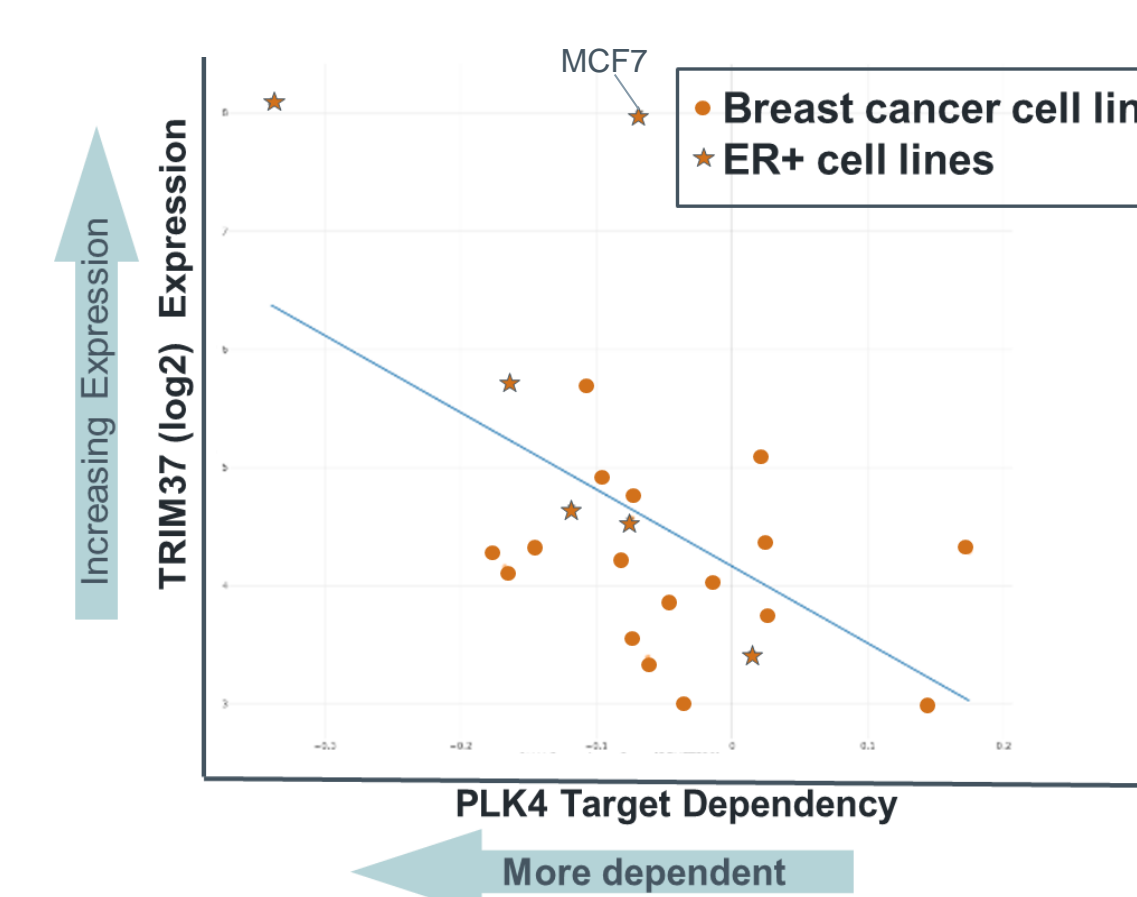
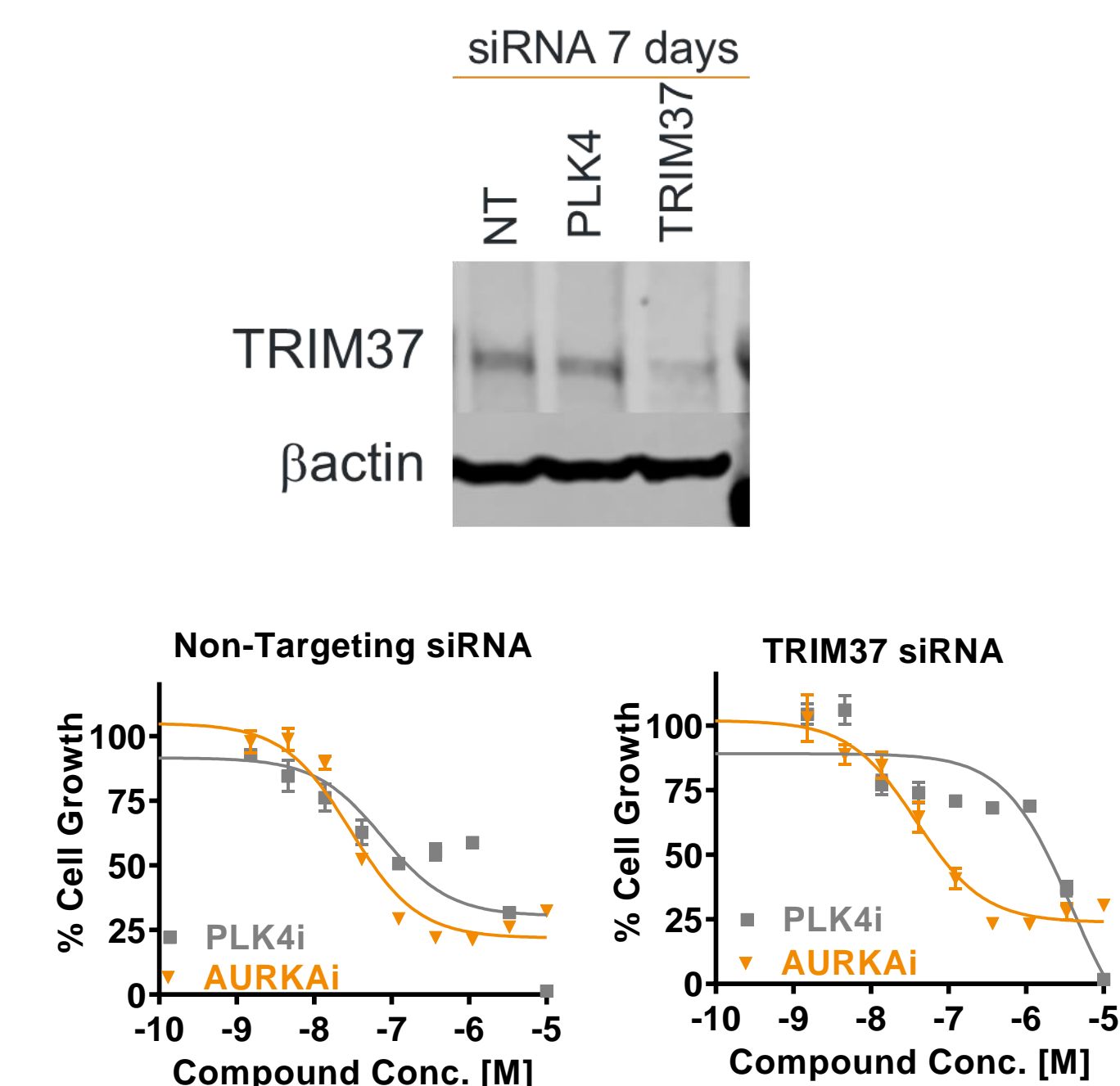


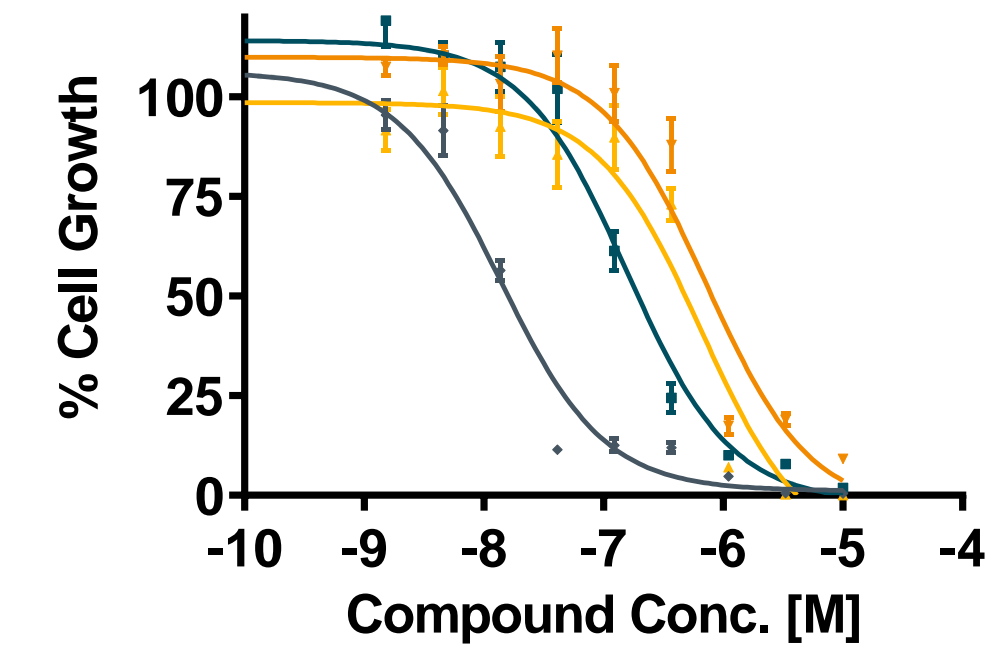
Figure 4a. Source: <https://depmap.org/portal/> 14 day siRNAi (Drive, DEMETER2) vs. Expression 22Q1 Public 4b. 7 day CellTiter-Glo assay in TRIM37 high MCF7 breast cancer cell line. NT, non-targeting siRNA; PLK4i=Centrinone; AURKAIi= alisertib, Sells *et al.*, ACS Med. Chem. Lett. 2015.

b. Knock Down of TRIM37 Reduces Sensitivity to PLK4 Inhibition

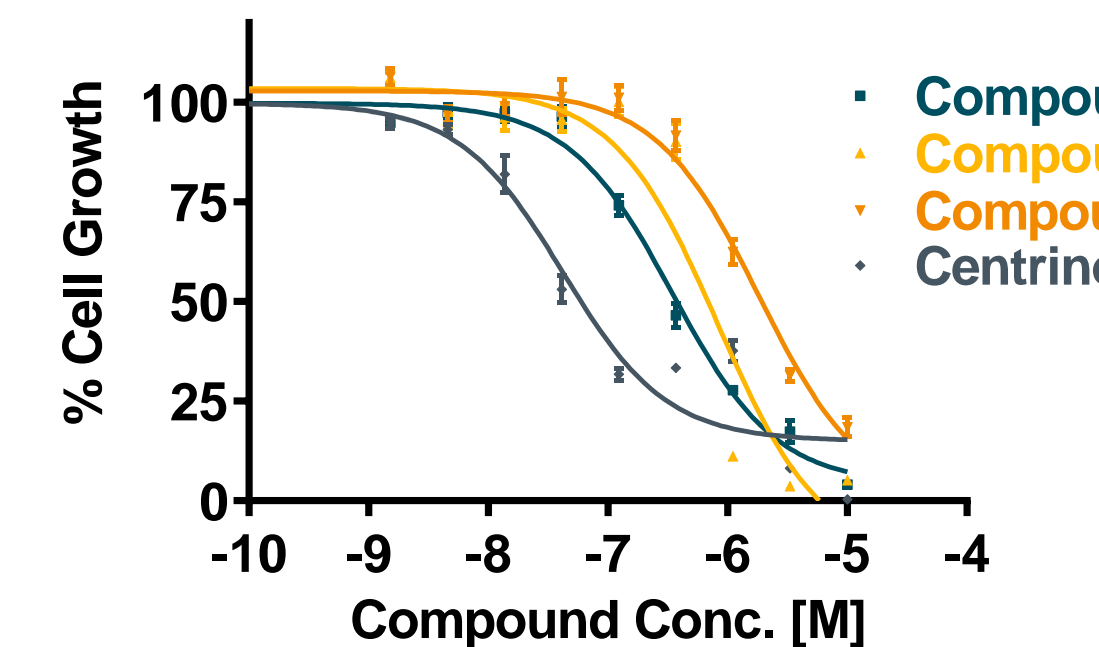


5. ORIC PLK4 Inhibitors Block Growth of TRIM37 High Tumor Cell Lines

Cell Growth Inhibited in TRIM37 High CHP-134 Neuroblastoma Cell Line



Cell Growth Inhibited in TRIM37 High MCF7 Breast Cancer Cell Line



Selective PLK4 Inhibitors Show Greater Potency in TRIM37 High vs. Low Cell Lines; Differential Not Observed for Non-Selective Inhibition

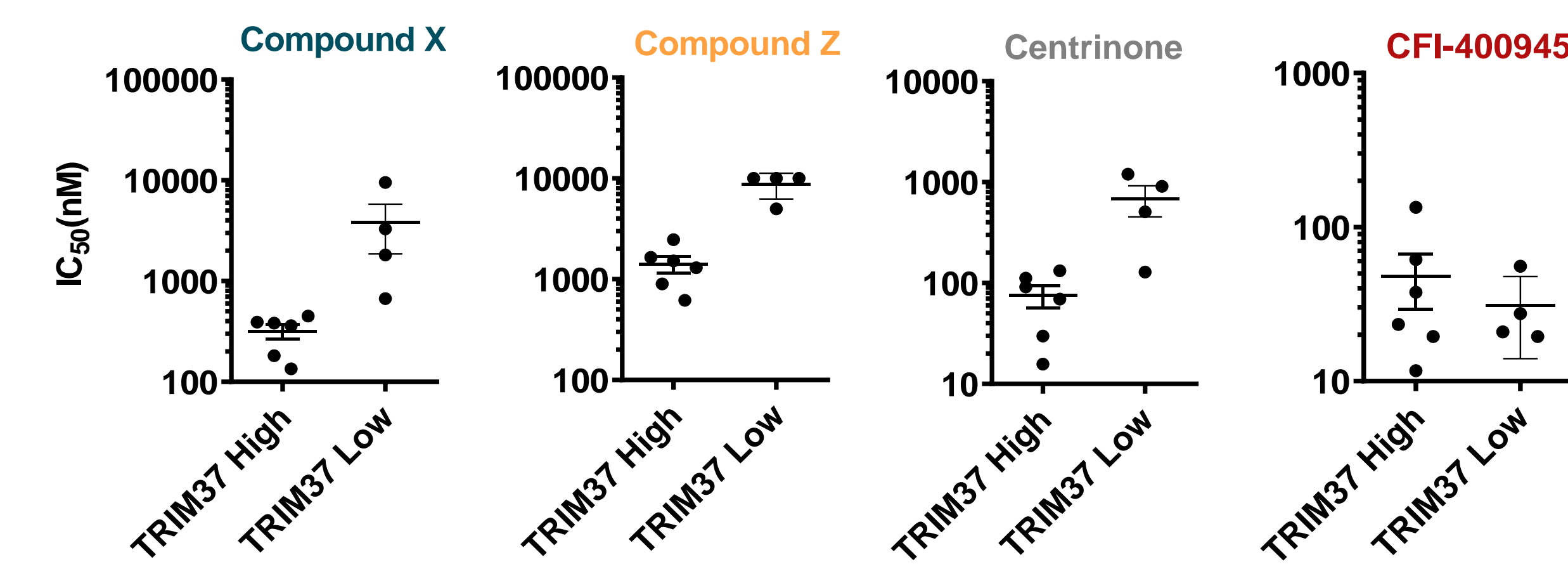
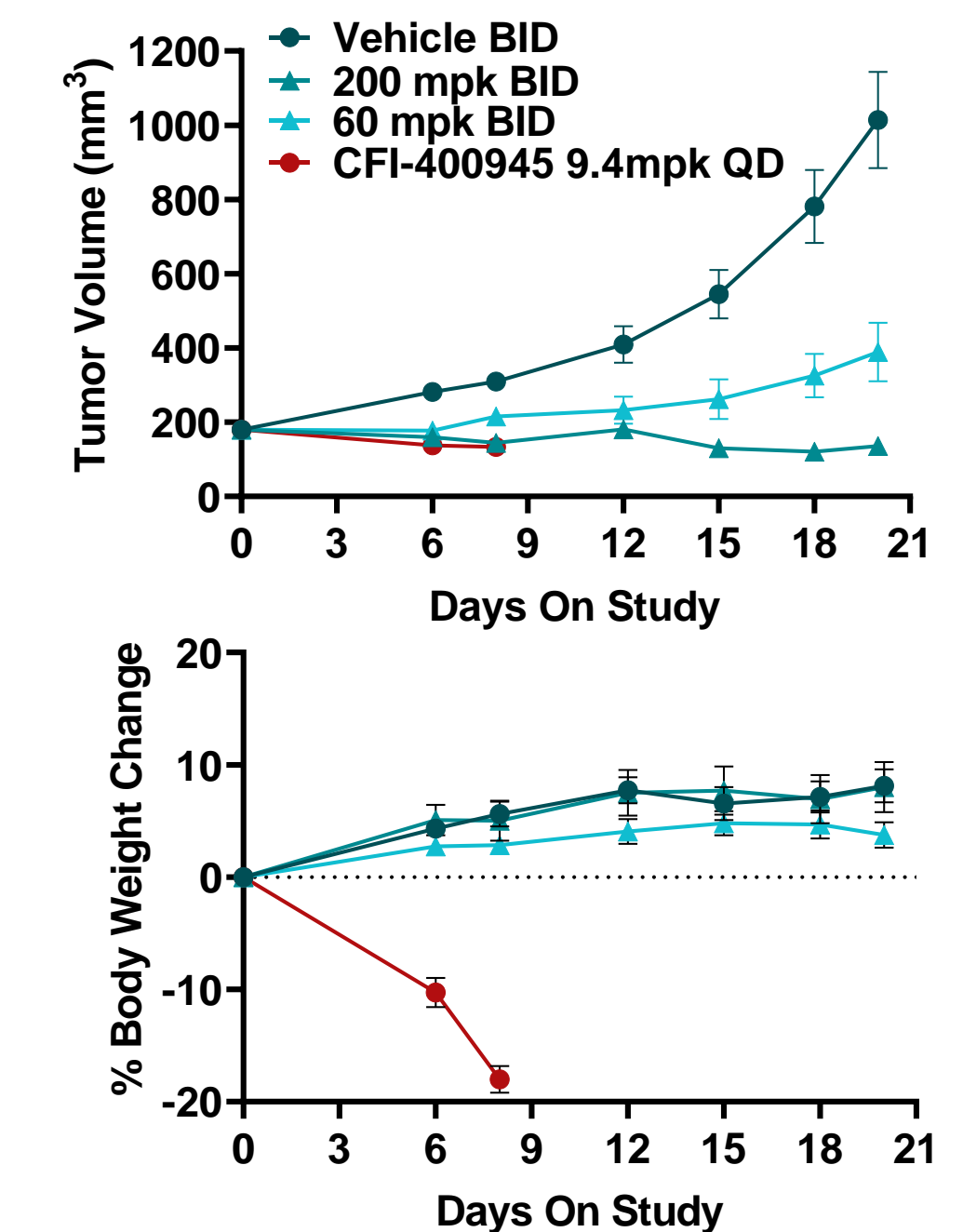


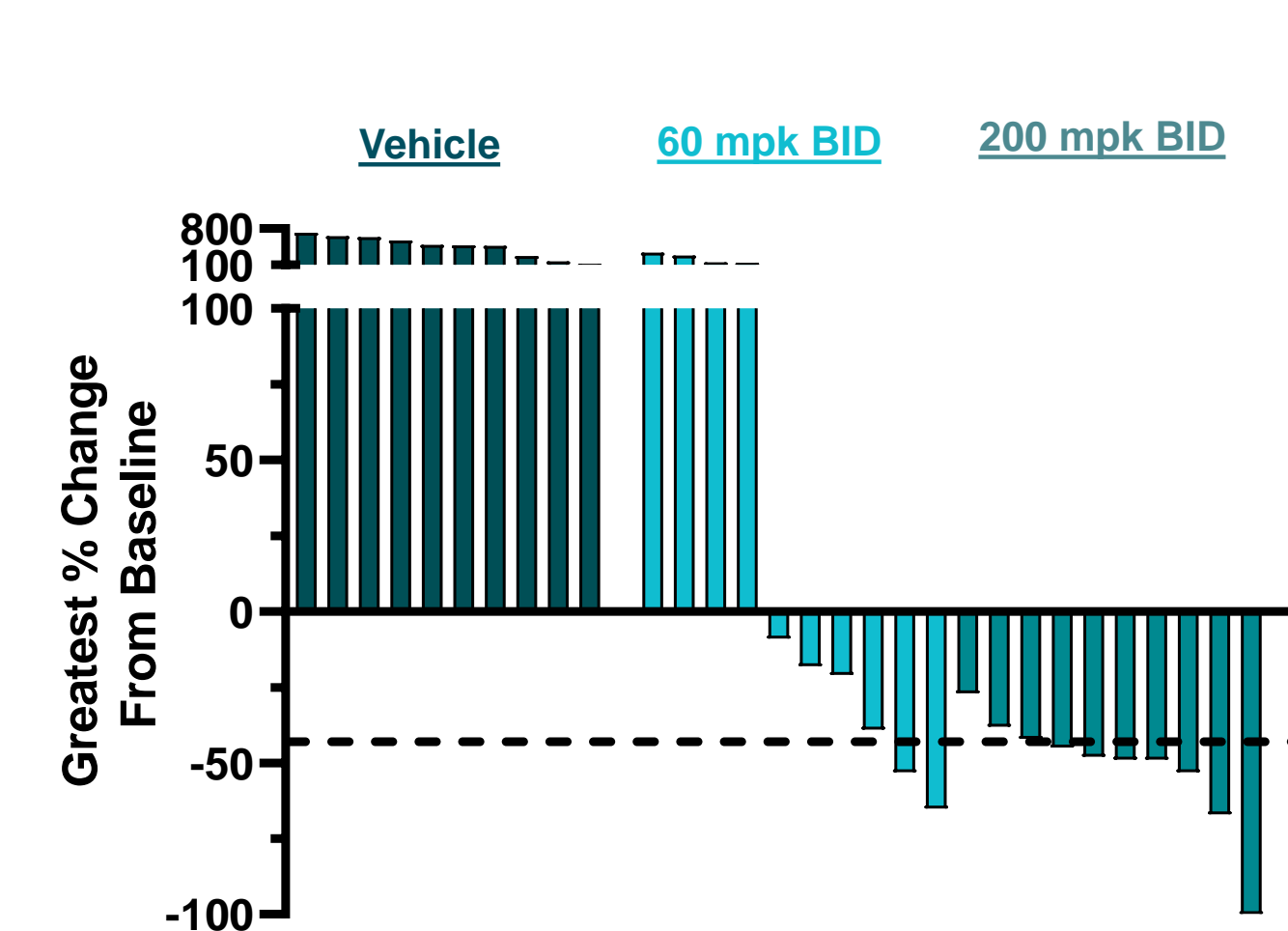
Figure 5. Cell panel consists of breast cancer and neuroblastoma cell lines assessed after 3-4 cell doublings using a CellTiter-Glo assay. TRIM37 high/low as defined in Meitinger *et al.*, Nature 2020.

6. Oral Dosing of ORIC PLK4 Inhibitors Induced Tumor Regressions in TRIM37 High Models

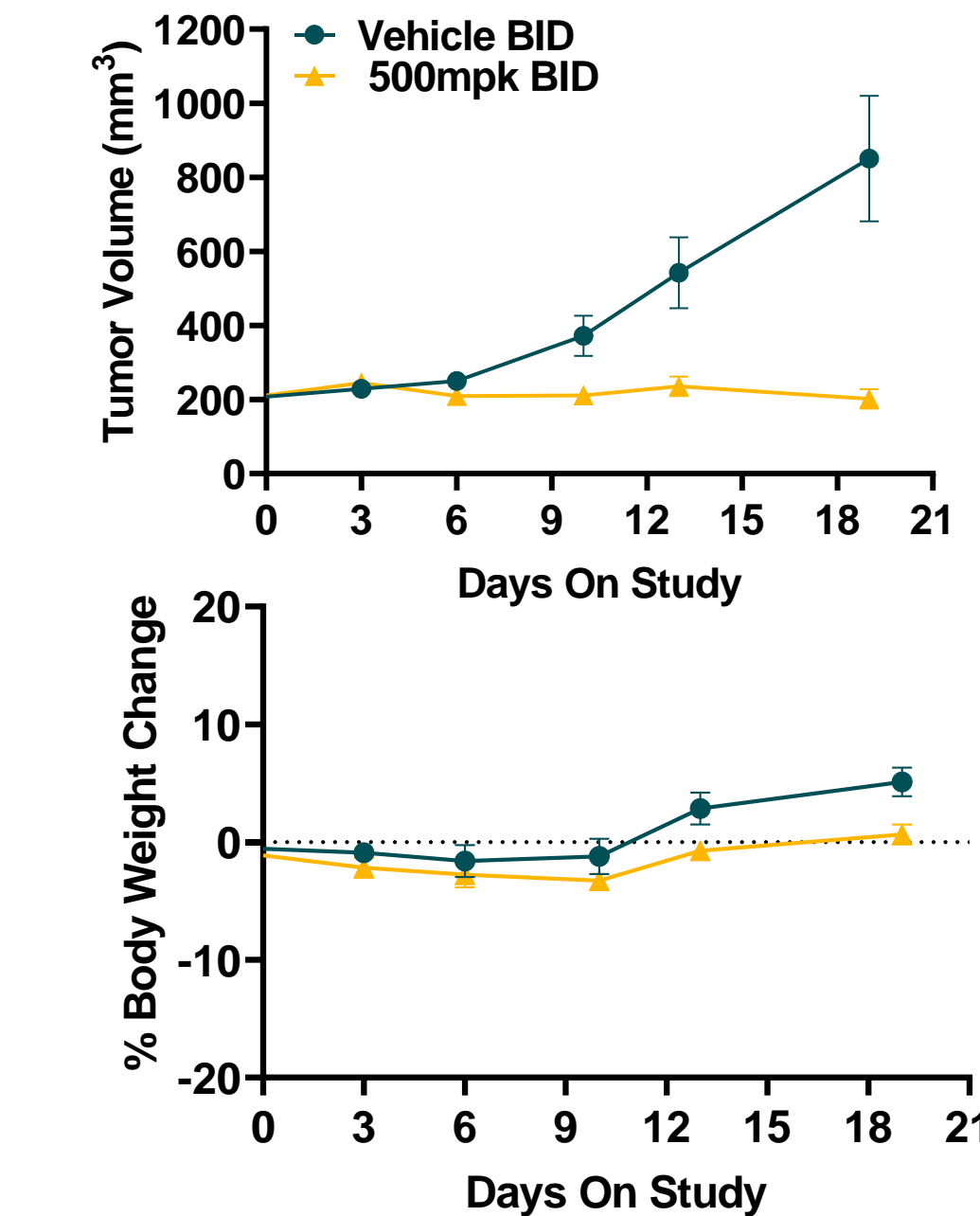
Compound Y: Efficacy and Body Weight



Compound Y: Tumor Regressions



Compound Z: Efficacy and Body Weight



Compound Z: In Vivo PD

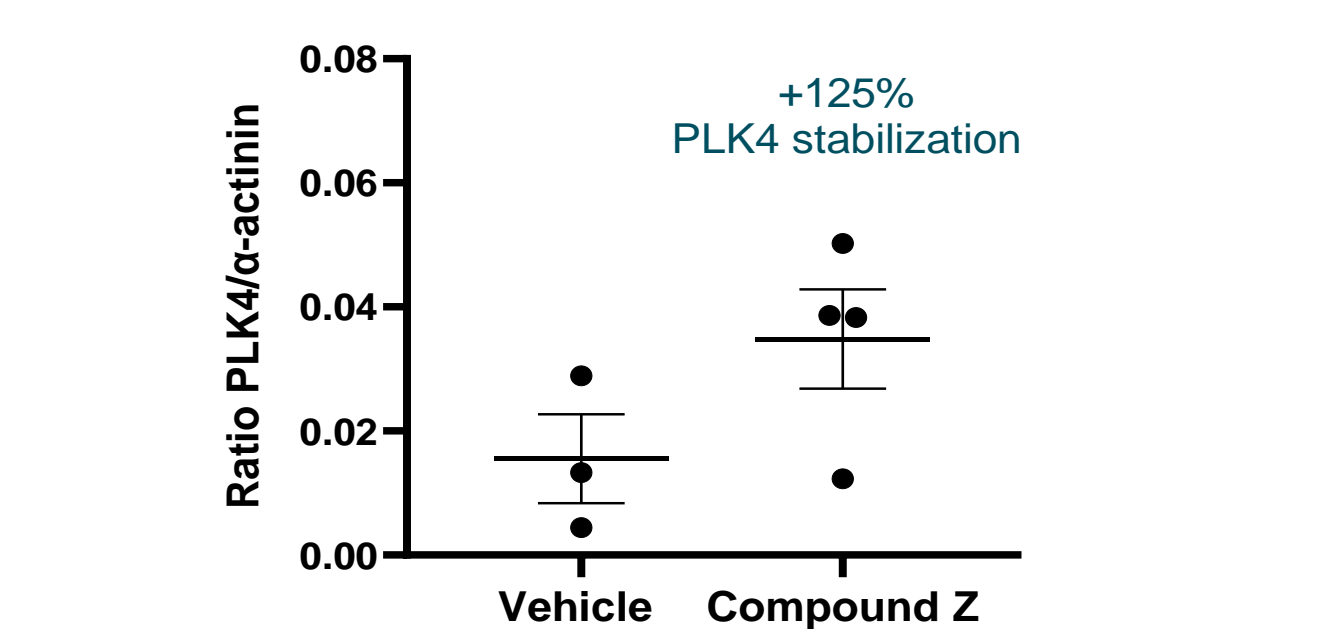


Figure 6. Oral dosing of ORIC PLK4 inhibitor compound Y (upper panels) and compound Z (lower panels) in TRIM37 High CHP-134 neuroblastoma xenograft model leads to tumor regressions (n=10 per cohort; mean +/- SEM). CFI-400945 was not tolerated. Tumors were collected and lysed 1hr post second 2nd dose of vehicle or 500mpk of Compound Z. Protein extracts from lysates were run on 3-8% Tris-Acetate gel and transferred to nitrocellulose. Blots were probed with antibodies to PLK4 or alpha-actinin and quantified on LI-COR Odyssey.

CONCLUSIONS

- We have discovered potent small molecule inhibitors of PLK4 that are highly selective, including against the closely related aurora kinases
- ORIC PLK4 inhibitors blocked kinase activity leading to stabilization of PLK4
- Cell viability assessment across a cancer cell line panel revealed that the highly selective ORIC PLK4 inhibitors showed greater potency in TRIM37 high cancer cell lines as compared to TRIM37 low cell lines
- Oral administration of ORIC PLK4 inhibitors resulted in regressions of TRIM37 high xenograft tumors, with corresponding PD effects and no body weight loss

Acknowledgments

- Thanks to our colleagues at Paraza Pharma Inc.