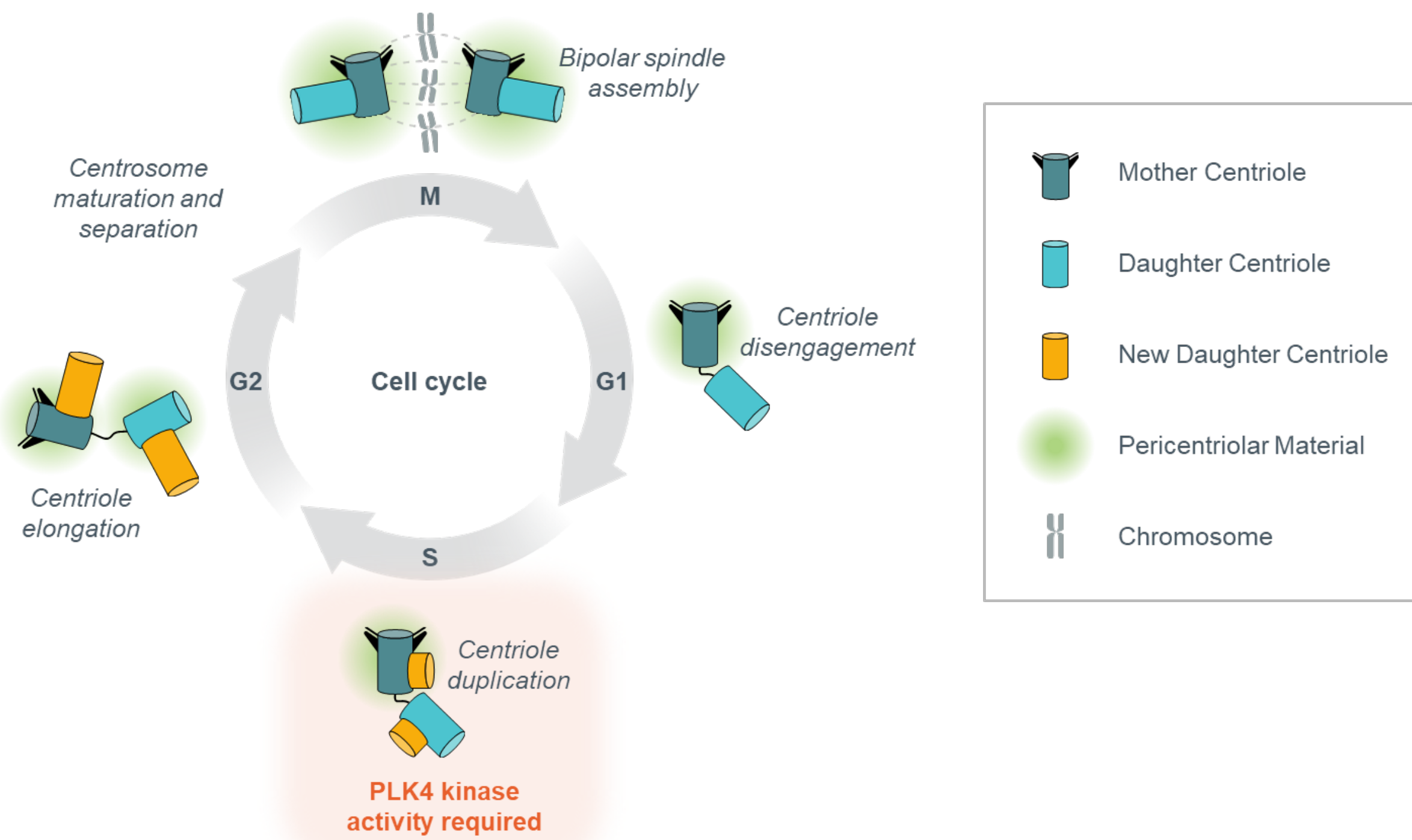
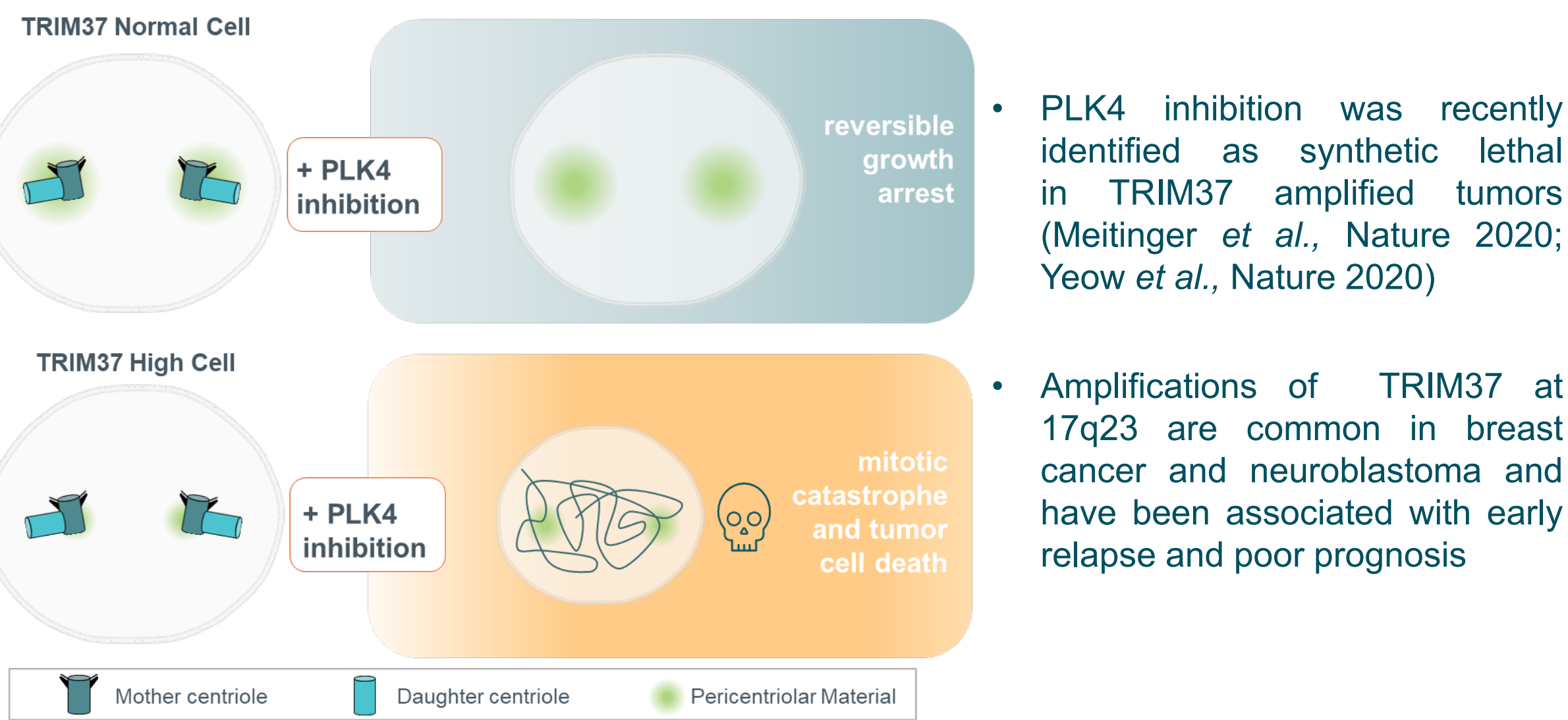


BACKGROUND

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



Synthetic Lethality of PLK4 Inhibition in TRIM37 Amplified Tumor Cells



1. ORIC PLK4 Inhibitors are Potent and Selective

Biochemical Potency of PLK4 and Aurora Kinase Inhibitors

	ORIC Compound X	ORIC Compound Y	Centrinone	CFI-400945	Alisertib	Barasertib
PLK4 IC ₅₀ (nM)	1.66	0.69	0.55	0.50	103	4836
Fold PLK1/PLK4	>30,000x	>30,000x	>30,000x	>30,000x	>30,000	>30,000
Fold AurA/PLK4	7,000x	3,842x	92x	85x	0.008x	0.012x
Fold AurB/PLK4	816x	975x	1,582x	12x	0.039x	0.004x

Figure 1. Biochemical IC₅₀ values were determined measuring luminescence generated from ADP-Glo. ORIC compounds X and Y are novel inhibitors of PLK4 synthesized by ORIC. Aurora A and B are closely related kinases. Reference structures: Centrinone, a highly selective *in vitro* tool compound lacking *in vivo* exposure (Wong *et al.*, Science 2015); CFI-400945, (Mason *et al.*, Cancer Cell 2014); Alisertib, an Aurora A kinase inhibitor (Manfredi *et al.* Clin. Cancer Res, 2011); and Barasertib, an Aurora kinase inhibitor (Mortlock *et al.*, J. Med. Chem 2007).

2. ORIC PLK4 inhibitors Block the Growth of TRIM37 High Cancer Cell Lines

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

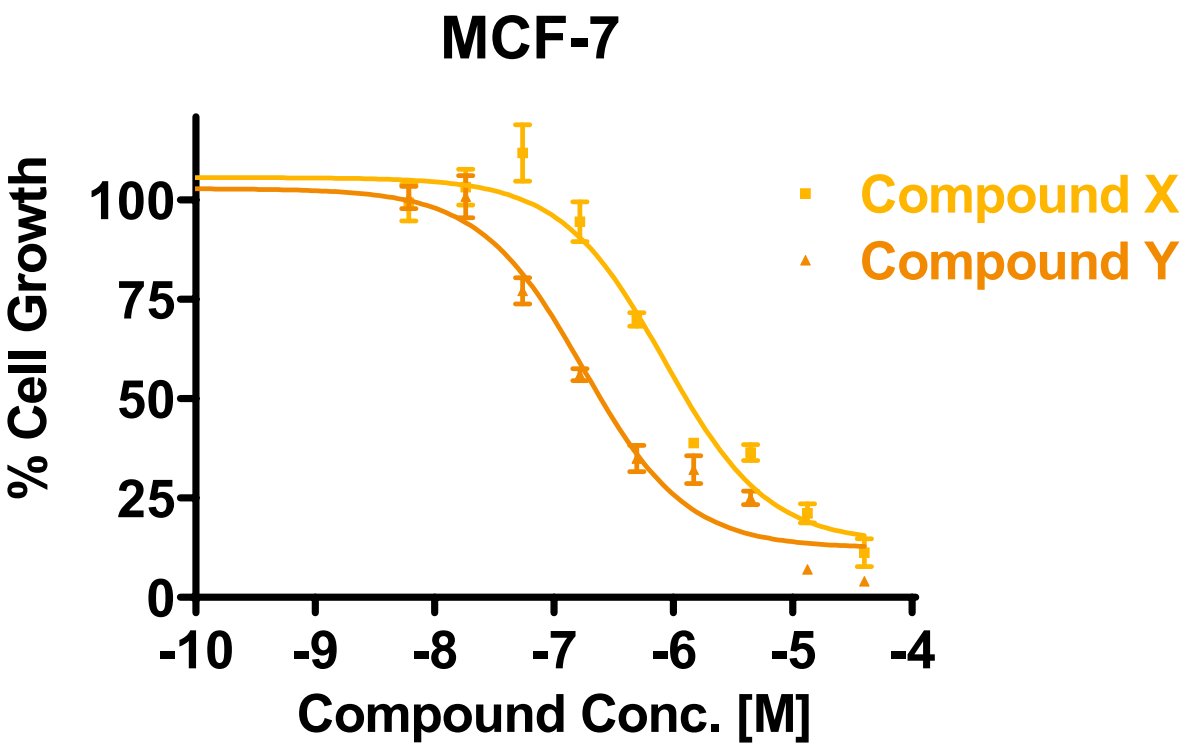
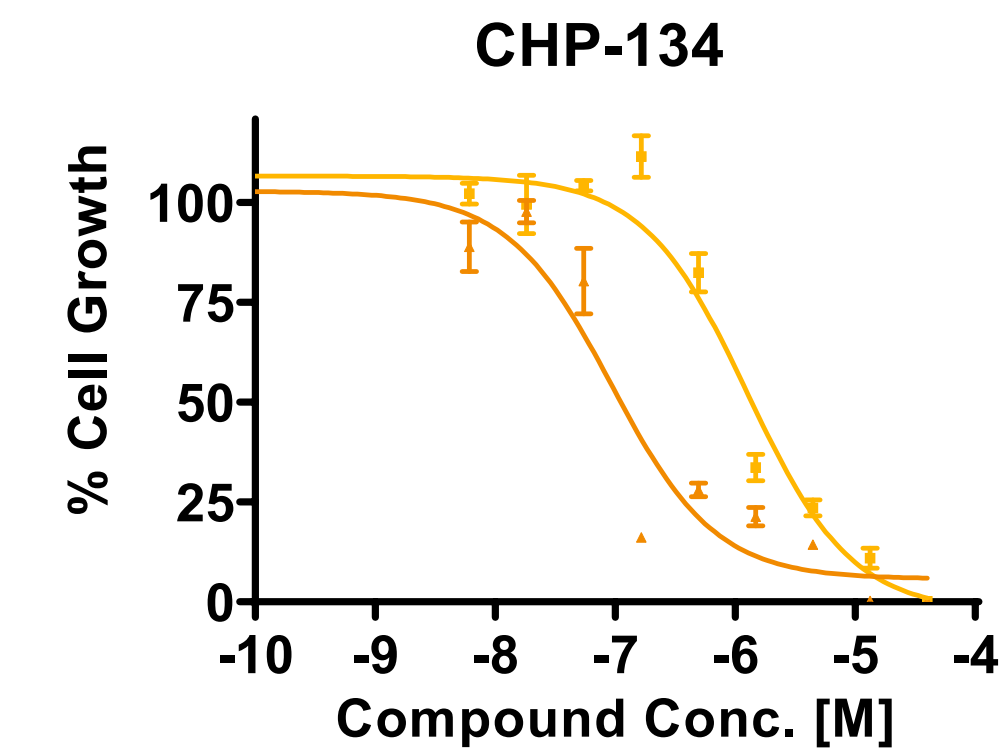


Figure 2. Neuroblastoma and breast cancer cell lines assessed after 3-4 cell doublings using a CellTiter-Glo assay. TRIM37 high as defined in Meitinger *et al.*, Nature 2020.

3. ORIC PLK4 Inhibitors are Synthetic Lethal in TRIM37 High Cancer Cell Lines

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

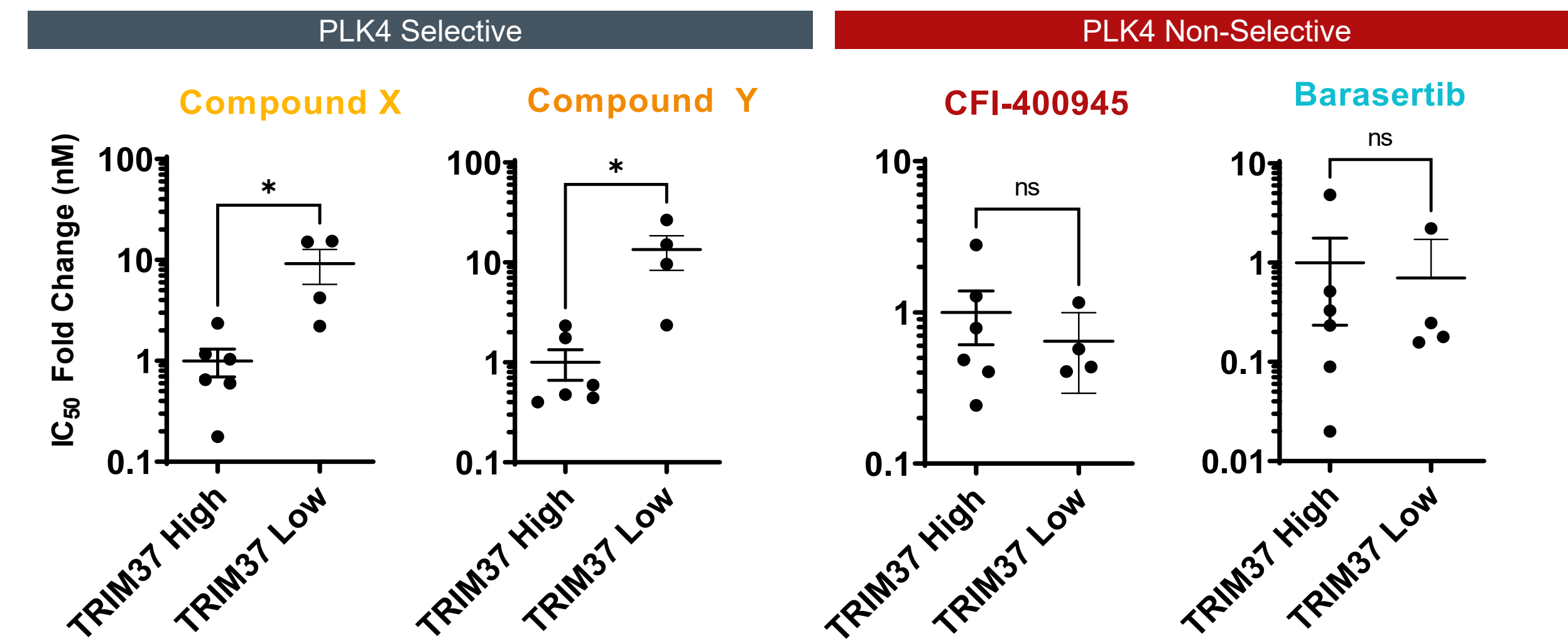


Figure 3A. Cell panel consists of breast cancer and neuroblastoma cell lines assessed after 3-4 cell doublings using a CellTiter-Glo assay and expressed as cellular IC₅₀/TRIM37 high mean IC₅₀. TRIM37 high/low as defined in Meitinger *et al.*, Nature 2020. TRIM high cell lines: CHP-134, MCF7, SK-N-FI, IMR-32, BT474, CHP-212. TRIM low cell lines: MDA-MB-231, HEPG2, BT549, and KPNNY.

Apoptotic Cell Death Solely in TRIM37 High Cancer Cells for ORIC Compounds X & Y; Not Observed for Non-Selective Inhibitors

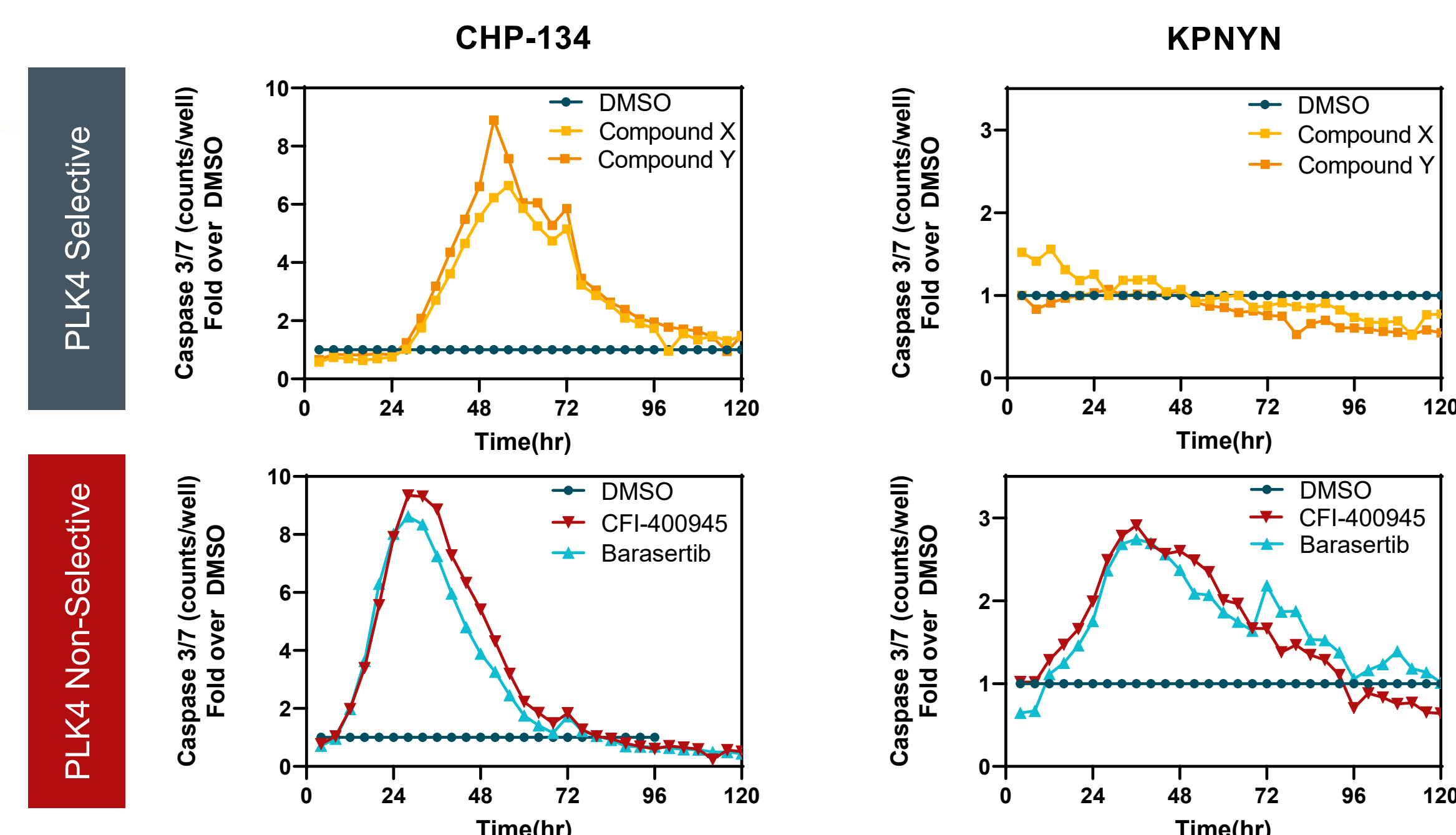


Figure 3B. Human neuroblastoma cell lines were assessed using a Caspase 3/7 assay. TRIM37 high cell line, CHP-134; TRIM37 low cell line, KPNNY.

4. PLK4 G95L Active Site Mutation is a Tool to Assess On-Target Selectivity of PLK4 Inhibitors

Steric Hindrance Observed in Model of PLK4 G95L with Centrinone

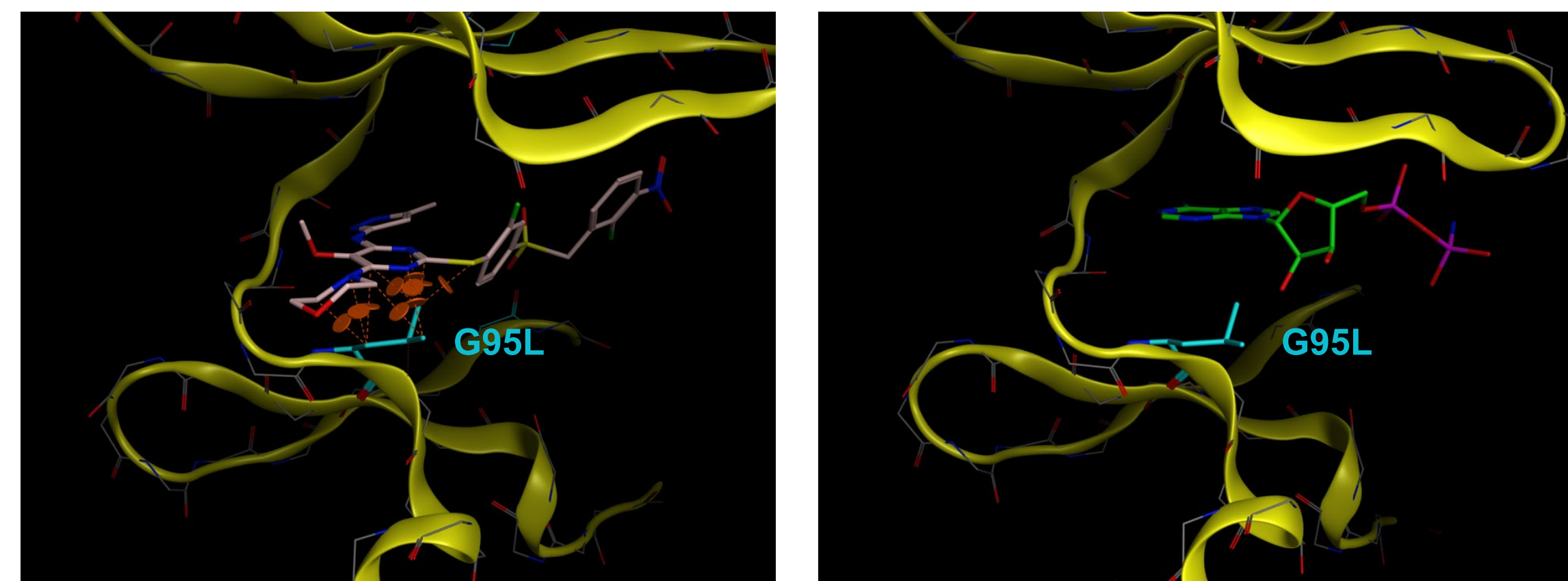


Figure 4. Centrinone (left) or an ADP analog (right) is shown binding in the PLK4 active site. G95L residue is shown in teal. PLK4 protein in yellow.

5. Activity in Engineered PLK4 G95L Reveals Selectivity and Off-Target Activity of Compounds

Selective PLK4 Inhibitors Lose Activity in TRIM37 High G95L cells, Indicating On-Target Cell Activity Compared with Less Selective Inhibitors that Retain Potency

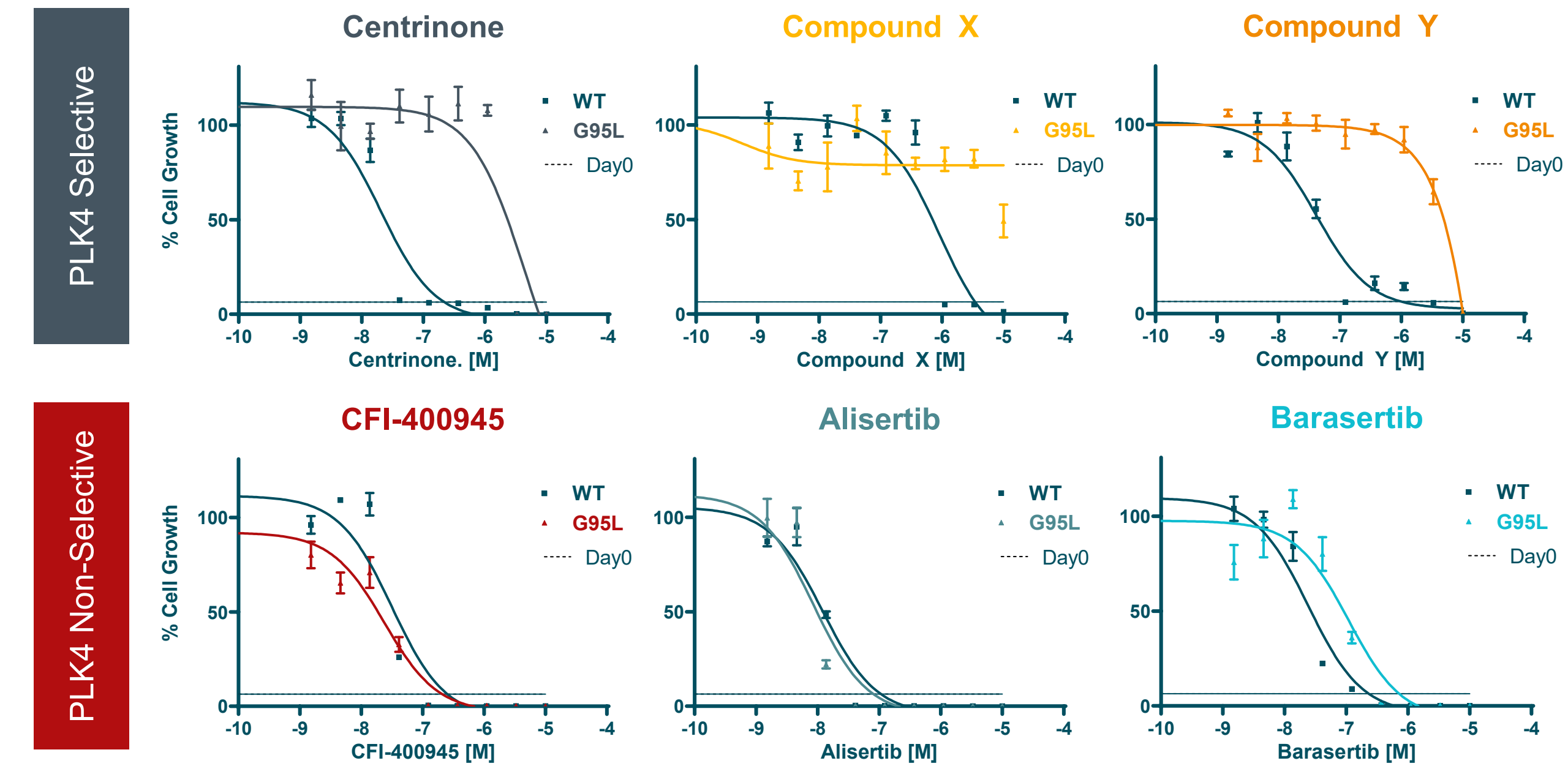


Figure 5A. CHP-134 cells with knock-in of PLK4 G95L were generated by CRISPR/Cas9 and validated by sequencing. CHP-134 parental or G95L K1 neuroblastoma cell lines were assessed after 3-4 cell doublings using a CellTiter-Glo assay.

All Compounds Lose Binding Against PLK4 G95L Protein in Biochemical Assays; Selective PLK4 Inhibitors Lose Potency in G95L, Indicating Cell Activity is On-Target for PLK4

	AssayQuant with 1mM ATP	PLK4 G95L IC ₅₀ (nM)	PLK4 WT IC ₅₀ (nM)	Fold G95L/WT Cell IC ₅₀ (nM)
PLK4 Selective	Compound X	>50,000	137	95X
	Compound Y	>50,000	30	87X
	Centrinone	>50,000	71	70X
PLK4 Non-Selective	CFI-400945	>50,000	48	1.6X
	Alisertib	>50,000	2044	0.8X
	Barasertib	>50,000	>50,000	3x

Figure 5B. Table 1 (left/central) shows biochemical IC₅₀'s obtained using Assay Quant in the presence of 1mM ATP. Table 2 (right) shows cellular IC₅₀'s assessed after 3-4 cell doublings using a CellTiter-Glo assay and expressed as a ratio of PLK4 G95L/WT cellular IC₅₀.

6. PLK4 Selective Inhibition Leads to Protein Stabilization Which Correlates to Cell Activity

PLK4 Activity Results in Trans-Autophosphorylation and PLK4 Degradation that is Specific to PLK4 Inhibition and Correlates with Cell Viability for Selective Compounds

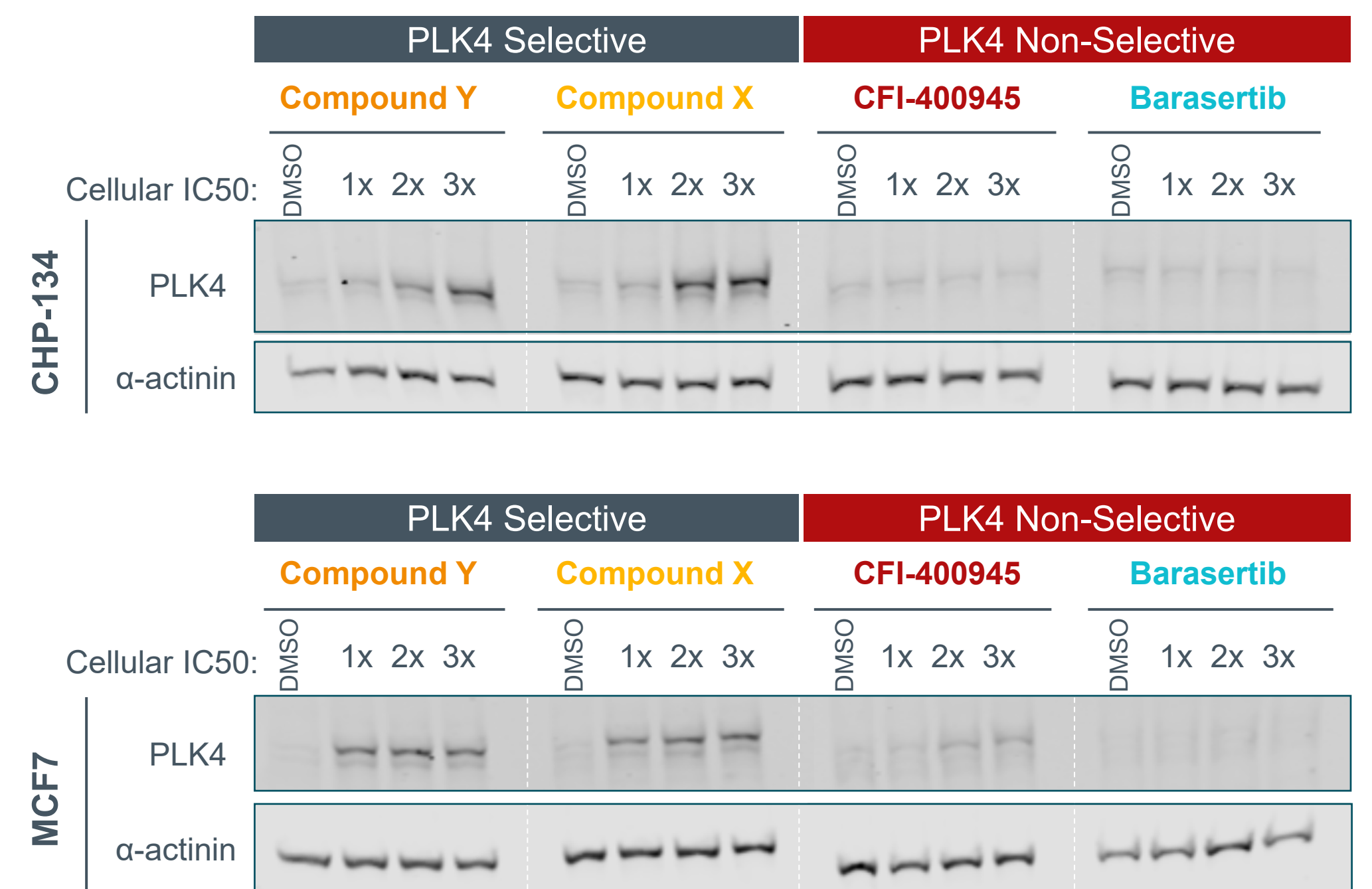
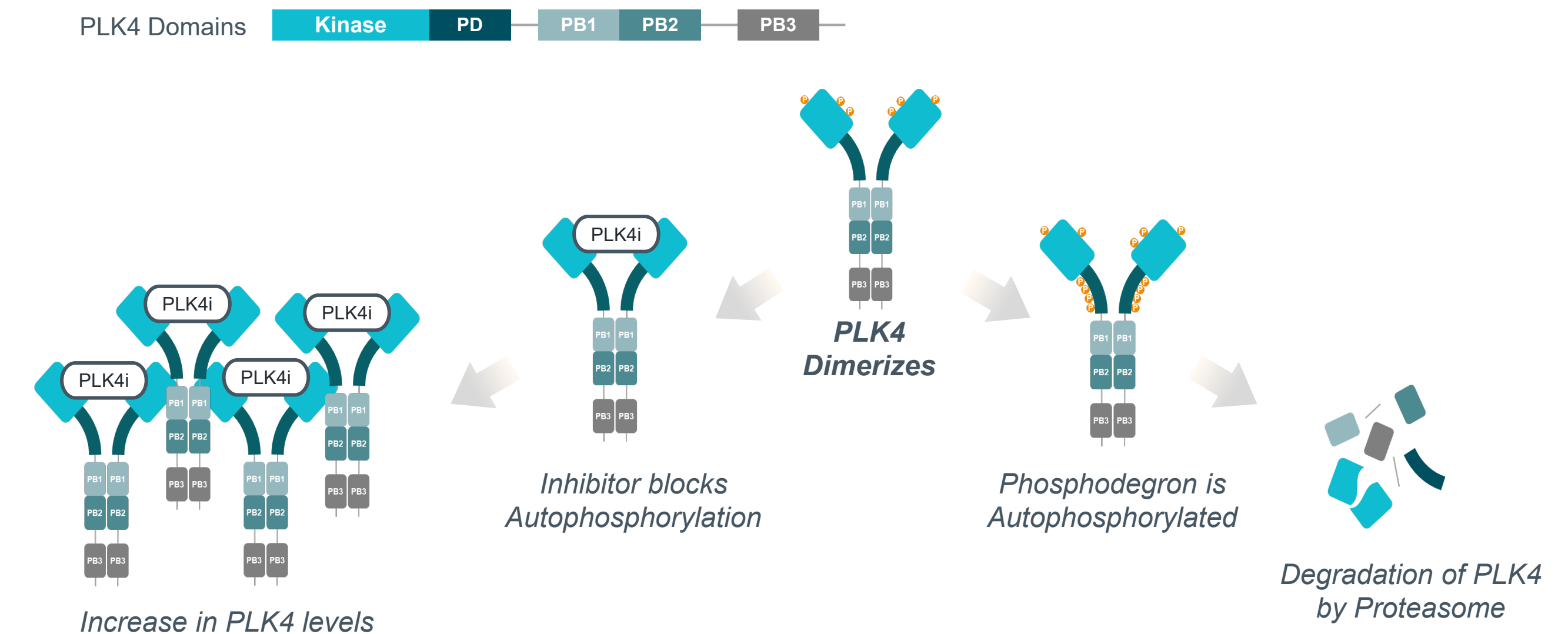


Figure 6. PLK4 stabilization occurs when autophosphorylation of the phosphodegron is inhibited, and PLK4 cannot be degraded by the proteasome. Protein extracts from CHP-134 and MCF7 lysates were run on 3-8% Tris-Acetate gels and transferred to nitrocellulose. Blots were probed with antibodies to PLK4 or α -actinin and quantified on LICOR Odyssey to assess PLK4 stabilization.

CONCLUSIONS

- We have discovered potent small molecule inhibitors of PLK4 that are highly selective, including against the closely related aurora kinases
- 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
- Apoptotic cell death was induced specifically in TRIM37 high cells, confirming the synthetic lethal interaction of selective PLK4 inhibitors
- PLK4 G95L revealed that binding and inhibition of PLK4 drives the cellular activity of selective ORIC inhibitors, demonstrating their efficacy is on-target
- ORIC PLK4 inhibitors blocked kinase activity leading to stabilization of PLK4, correlating with cellular IC₅₀'s for selective compounds

ACKNOWLEDGEMENTS

- Thanks to our ORIC PLK4 team, and colleagues at Paraza Pharma

