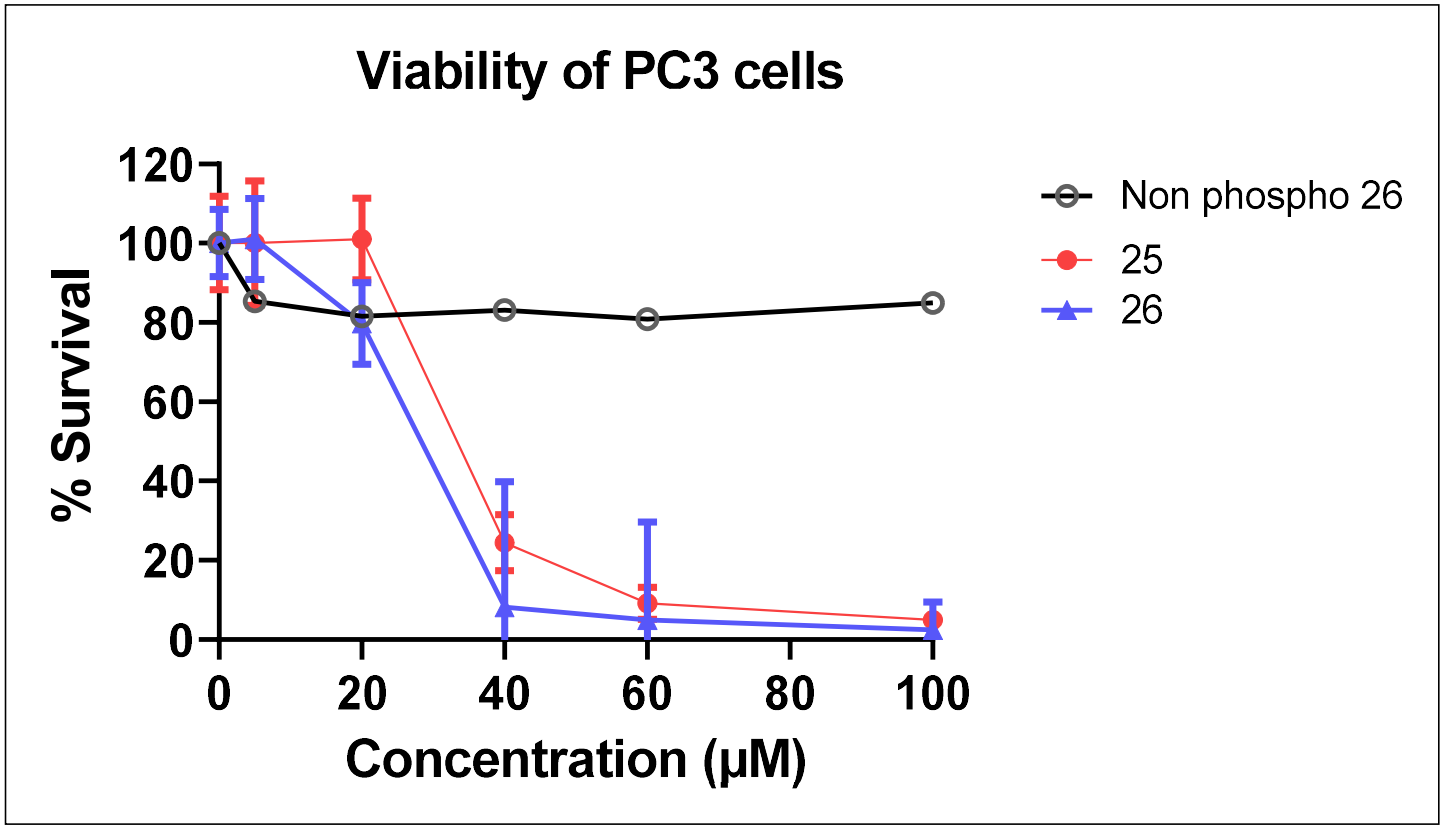
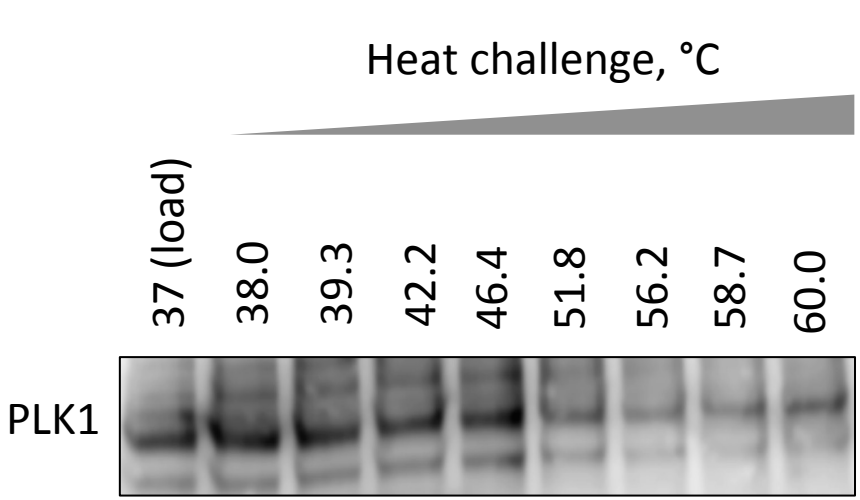
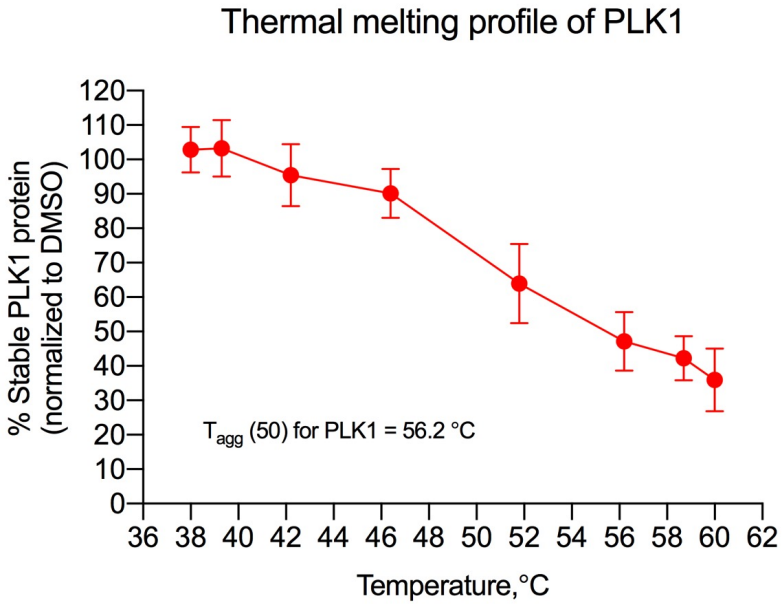


Supplemental Figure 1. Anti-proliferative activity of FLIPs

Determination of melting profile of PLK1



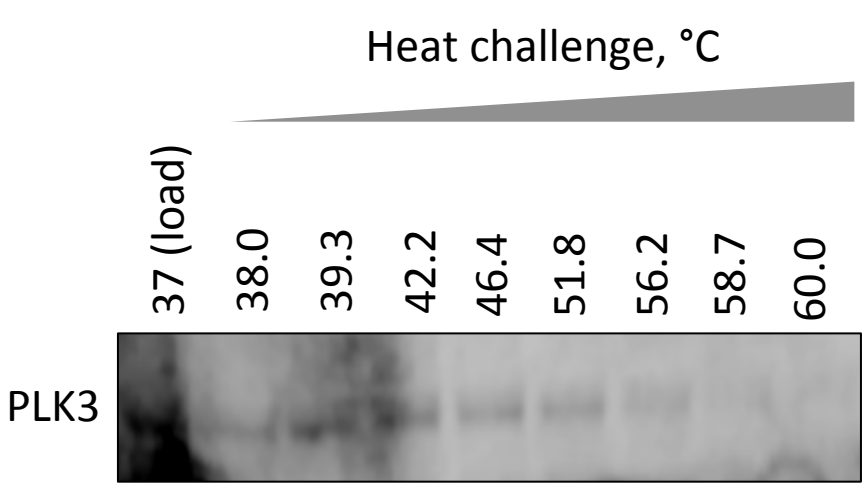
Treatment: DMSO

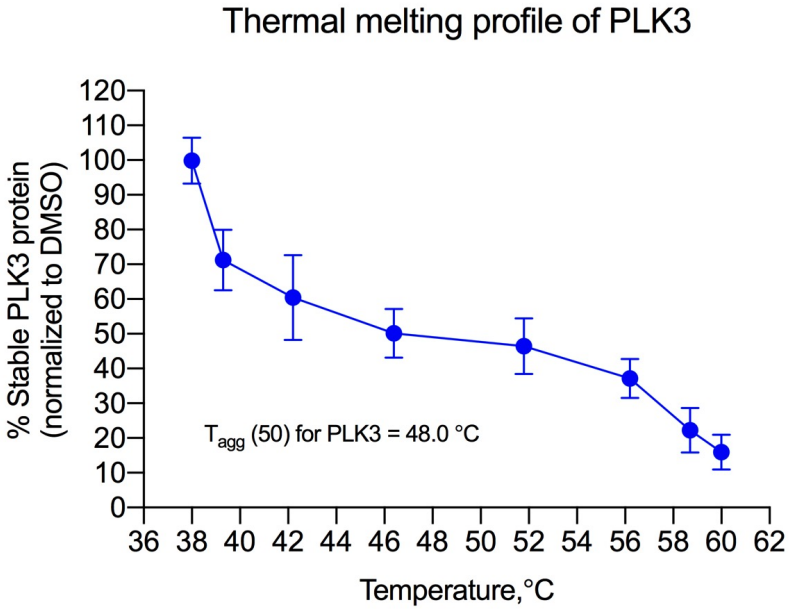


Dose-response measurements with inhibitors were done at $T_{agg}(50)$

Supplemental Figure 2. Cellular Thermal Stability of PLK1

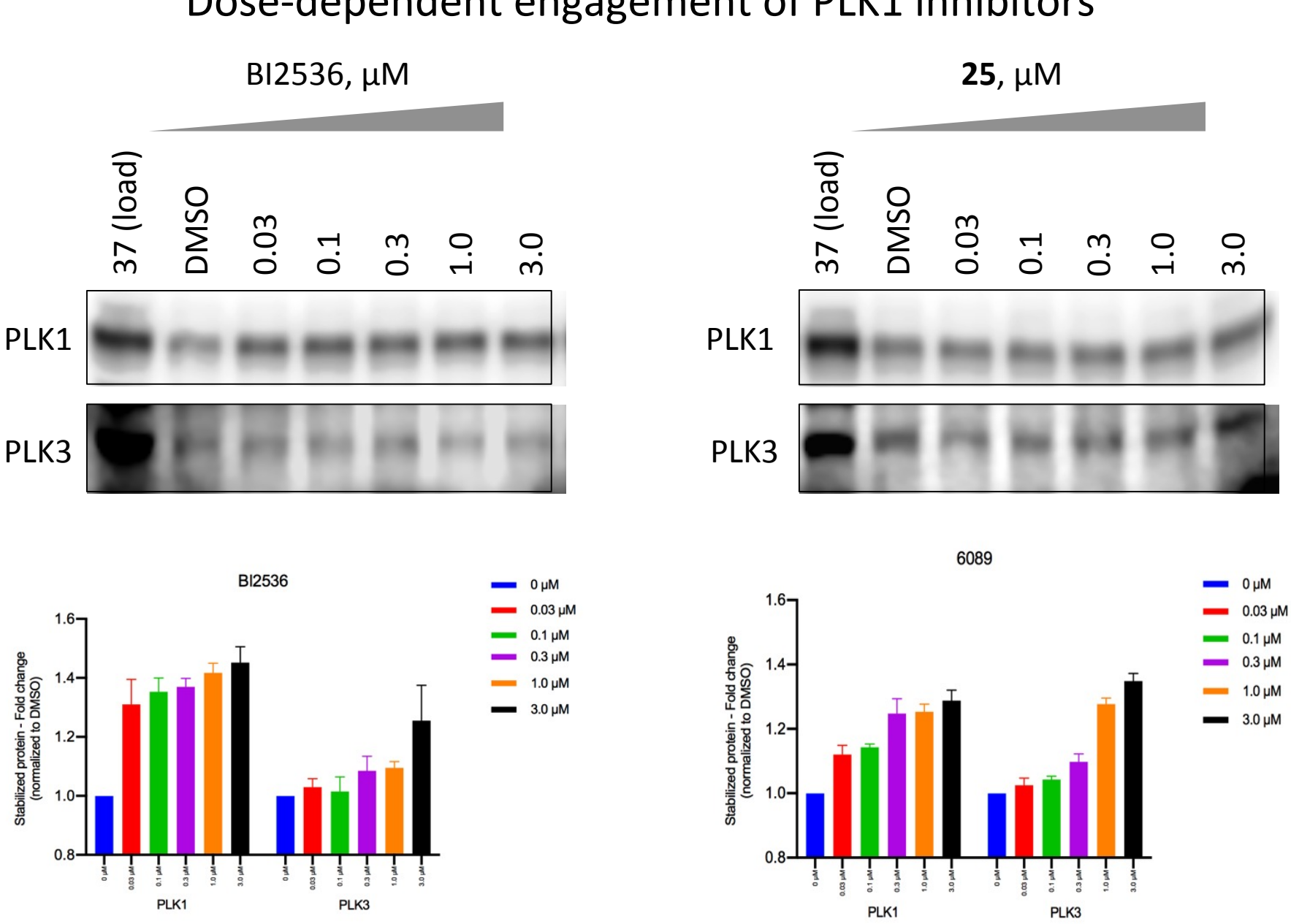
Determination of melting profile of PLK3



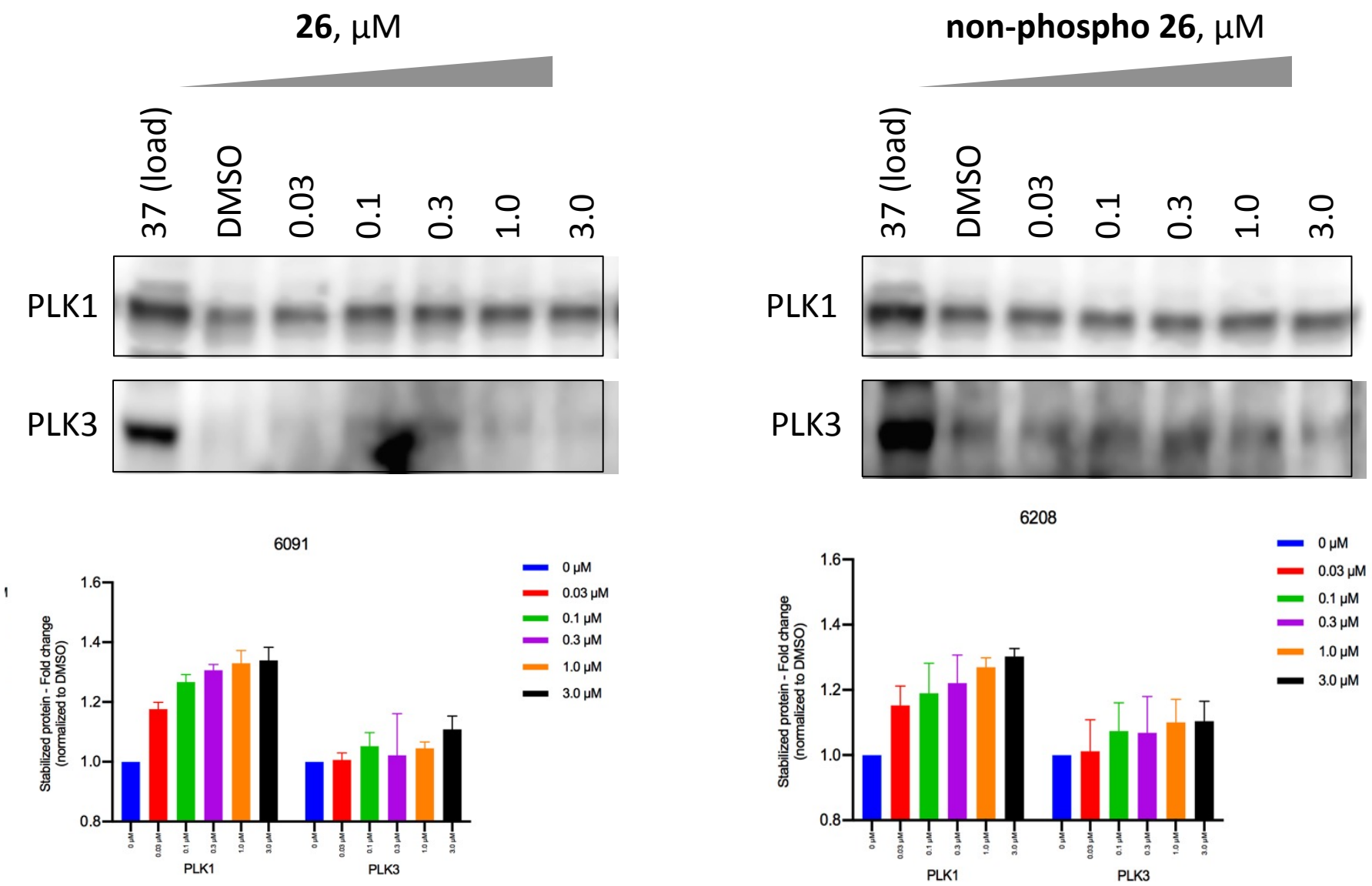


Dose-response measurements with inhibitors were done at $T_{agg}(50)$

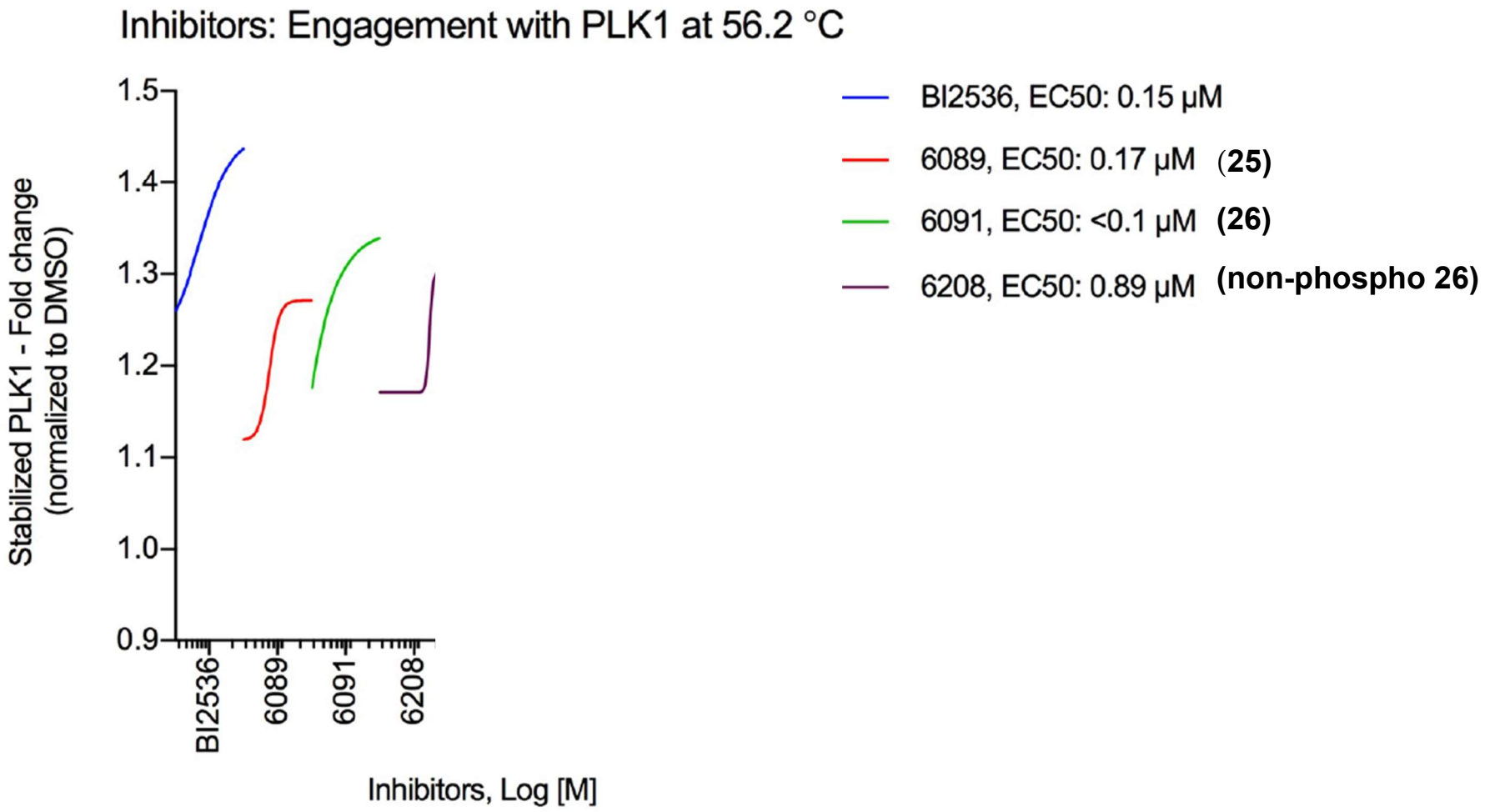
Supplemental Figure 3. Cellular Thermal Stability of PLK3



Supplemental Figure 4. CETSA EC50 Values for treatment of PC3 cells with PLK1 Inhibitors

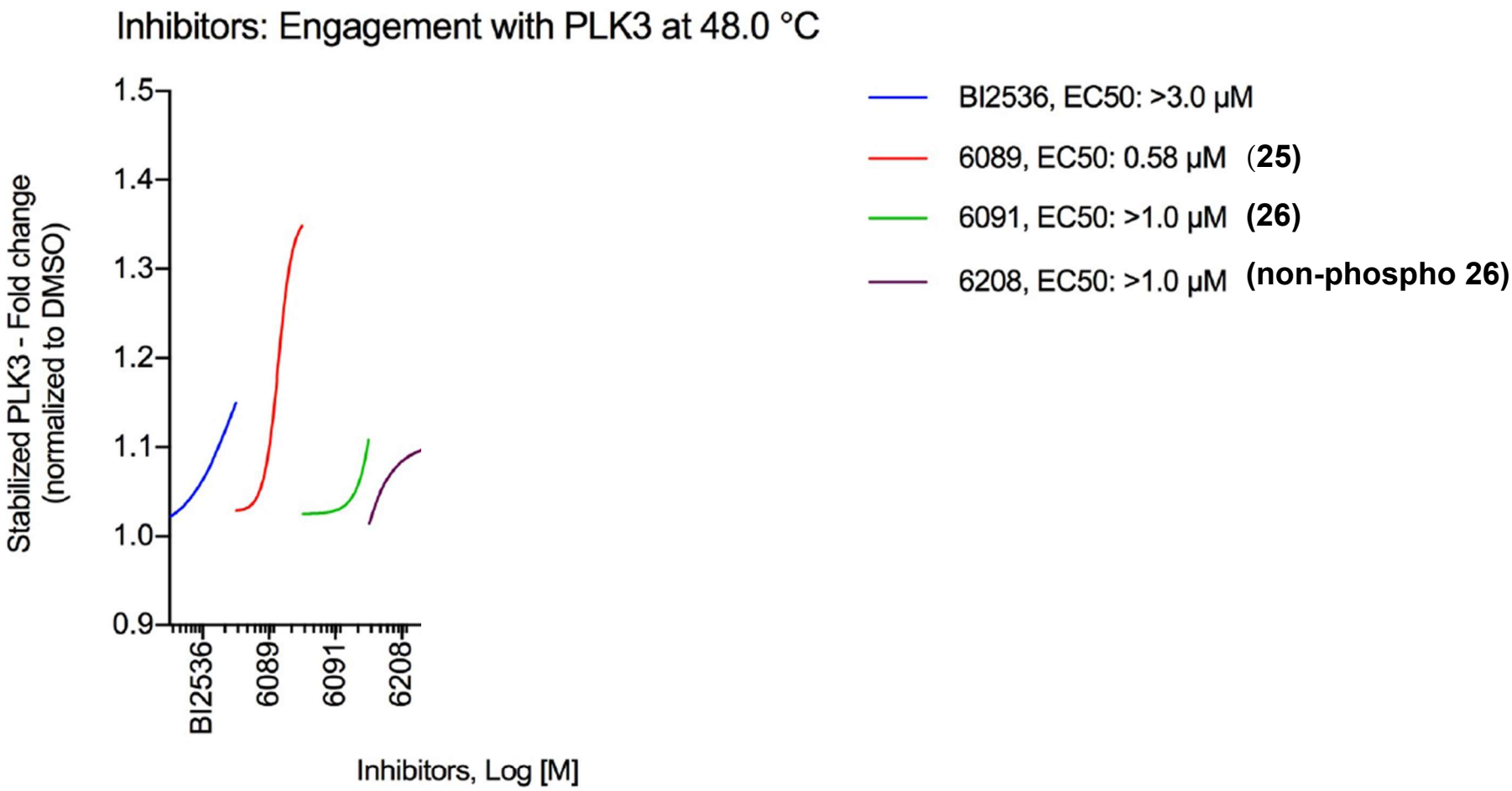


Supplemental Figure 5. CETSA EC50 Values for treatment of PC3 cells with PLK1 Inhibitors



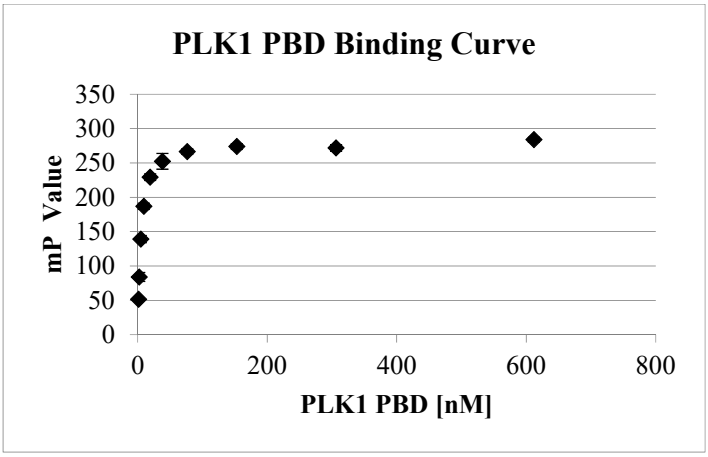
Dose-dependent saturation of inhibitors indicate target selectivity.
Maximum stabilized target levels indicate potency.

Supplemental Figure 6. CETSA EC50 Curves for PLK1

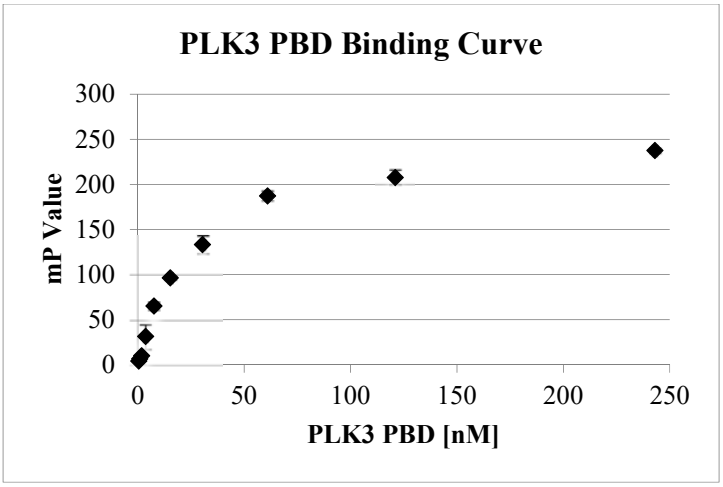


Dose-dependent saturation of inhibitors indicate target selectivity.
Maximum stabilized target levels indicate potency.

Supplemental Figure 7. CETSA EC50 Curves for PLK3

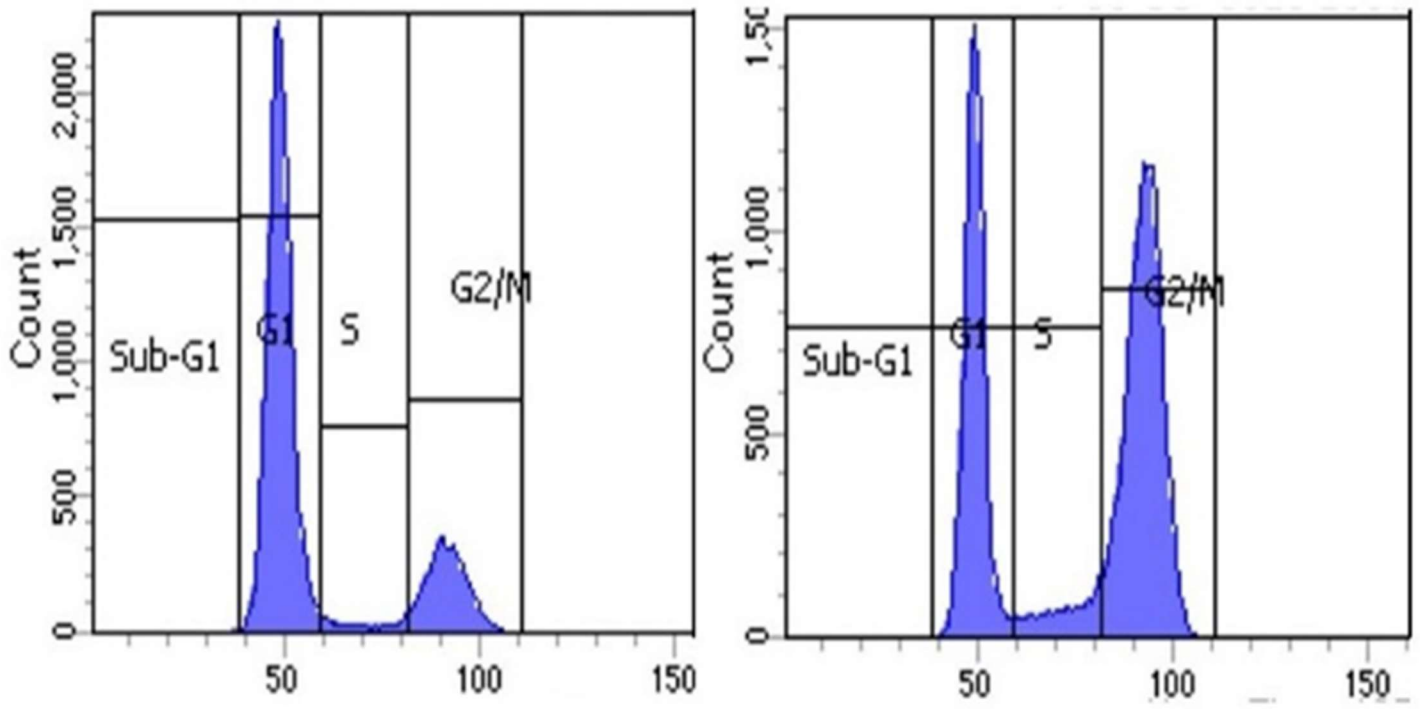


Supplemental Figure 8. PLK1 PBD dilution curve. The dissociation constant (Kd = 4.6 nM) and the EC90 (41.1 nM) was determined from the curve.



Supplemental Figure 9. PLK3 PBD dilution curve. The dissociation constant (Kd = 27.2 nM) and the EC90 (245 nM) was determined from the curve.

NOTE: The EC90 for the tracer-PBD interaction is calculated then used in all subsequent experiments so that the polarization value is achieved that provides the highest signal to noise ratio. Displacement of the tracer from the PBD by increasing concentration of peptide or FLIP queried then reduces the polarization value. The IC50 values of compounds binding to the tracer is therefore not a direct Kd measurement, but a reflection of competition with the tracer and a relative affinity.



Supplemental Figure 10. Figure 3. Cell cycle analysis of PC3 cells following 24-hour treatment with **24**. Cells were synchronizedby serum starvation for 72 hours prior to treatment. Untreated cells were serum starved then released into drug free media for 24 hours (left panel) or treated with 200 μM **24** for 24 hours.

Supplementary Table 1. Analytical Data for PBD Binding Peptides

	Purity (%)	Column Dimensions	Method	FlowRate	Retention Time	Theoretical MW	Observed MW
1	91.9	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	14.6	997.1	996.7
2	98.1	4.6 × 250 nm	15-75% acetonitrile/water/0.1%TFA	1ml/min	17.1	917.1	917.6
3	99	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	16.1	1057.1	1056.3
4	97.2	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	14.9	859.9	859.5
5	90	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	14.4	762.8	762.5
6	95.5	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	12.2	745.7	746.4
7	97.5	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	11.7	1122.1	1122.1
8	90	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	14.7	1107.1	1106.6
9	99.3	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	12.4	1072.1	1073.2
10	90.8	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	13.4	787.6	787.4
11	98.9	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	15.2	801.6	801.5
12	91.5	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	16.9	815.6	815.4
13	90.1	4.6 × 250 nm	15-75% acetonitrile/water/0.1%TFA	1ml/min	16.5	829.6	829.4
14	94.9	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	21.8	895.0	895.4
15	96.5	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	21.3	891.0	891.5
16	98.8	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	19.2	817.6	817.3
17	95	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	13.3	800.6	800.4
18	95.3	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	17.2	829.0	828.5
19	97.9	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	21.3	827.8	828.4
20	98.8	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	24.6	855.6	855.8
21	91	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	26.9	883.6	883.5
22	94.3	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	27.2	573.3	573.2
23	96.1	4.6 × 250 nm	5-65% acetonitrile/water/0.1%TFA	1ml/min	8.2	686.4	686.4
24	91.3	4.6 × 250 nm	35-95% acetonitrile/water/0.1%TFA	1ml/min	17.0	712.8	712.3
25	100	4.6 × 250 nm	5-95% acetonitrile/water	1ml/min	15.9	685.8	686.0
26	98.2	4.6 × 250 nm	5-95% acetonitrile/water	1ml/min	16.9	699.8	700.0
27	95.3	4.6 × 250 nm	40-100% acetonitrile/water/0.05% TFA	1ml/min	15.6	619.8	619.4

Supplementary Table 2. MTT assay results for WT and C67V Retinal Pigment Epithelial Cells.

Compound ID#	WT PLK1 RPE Cells IC_{50} [µM]	C67V RPE Cells IC_{50} [µM]
BI-2536 (control)	0.021	>2.5
23	298.6	136.8
24	158.5 ± 8.8	86.8 ± 33.8