Supplementary section

Structural basis for small molecule targeting of Doublecortin Like Kinase 1 DCLK1

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Running title: Structural basis for DCLK1-IN-1 inhibition

Table S1. Data collection and refinement statistics

	DCLK1-IN-1	XMD-8-85	FMF-03-055-1
Data collection			
Temperature	100K	100K	100K
Space group	C2	I2	P21
Cell dimensions			
a, *b*, *c* (Å)	143.95, 61.71, 65.31	66.18, 63.33, 152.49	65.92, 62.43, 72.08
α, β, γ (°)	90.00, 103.04, 90.00	90.00, 100.47, 90.00	90.00, 96.07, 90.00
Resolution (Å)	44.29-3.00 (3.18-3.00)	74.97-2.50 (2.60-2.50)	71.7-3.1 (3.31-3.10)
R_{pim}[1]	6.4 (31.6)	6.0 (34.5)	11.5 (44.4)
I/σ_1	11.4 (3.6)	10.8 (2.9)	7.3 (2.6)
Completeness (%)	98.9 (93.0)	99.1 (99.7)	99.4 (99.8)
Total N°. observations	76277 (11337)	101629 (11767)	50639 (9282)
N°. unique observations	11200 (1669)	21500 (2436)	10702 (1926)
Multiplicity	6.8 (6.8)	4.7 (4.8)	4.7 (4.8)
$CC_{1/2}$ (%)	99.6 (81.9)	99.3 (70.2)	98 (70.9)
Refinement statistics			
R_{factor}[2] (%)	20.3	19.4	19.6
R_{free}[3] (%)	25.9	22.5	25.1
No. atoms			
• Protein	3935	3919	3943
• Ligand	96	68	70
• Water	4	92	-
Ramachandran plot (%)			
• Most favoured	97.5	98.6	97.4
• Allowed region	2.5	1.4	2.4
B-factors (Å^2)			
• Protein	56.6	36.9	37.8
• ligand	60.7	36.9	42.8
rmsd bonds (Å)	0.007	0.004	0.658
rmsd angles (°)	0.967	0.707	0.006

[1] $R_{p.i.m} = \Sigma_{hkl} [1/(N-1)]^{1/2} \Sigma_i | I_{hkl,i} - \langle I_{hkl} \rangle | / \Sigma_{hkl} \langle I_{hkl} \rangle$

[2] $R_{factor} = (\Sigma | |F_o| - |F_c| |) / (\Sigma |F_o|)$ - for all data except as indicated in footnote 3.

[3] 5% of data was used for the R_{free} calculation

Values in parentheses refer to the highest resolution bin.

Table S2. SPR binding of inhibitors to DCLK1 – summary of fitted values

Inhibitor	Immobilised protein	Fitted values					
		K_D (nM)	k_{on} (1/Ms) (x 10^5)	k_{off} (1/s)	$t_{1/2}$ (sec)	R_{max} (RU)	N =
DCLK1-IN-1	**DCLK1 FL1Δ D511N**	**53 ± 5**	5.1 ± 0.3	0.027 ± 0.001	26 ± 1	11 ± 1	4
FMF-03-055-01	**DCLK1 FL1Δ D511N**	**13 ± 2**	11 ± 4	0.014 ± 0.006	50 ± 20	10 ± 1	3
XMD8-85	**DCLK1 FL1Δ D511N**	**8 ± 1**	12.8 ± 0.5	0.010 ± 0.001	67 ± 6	8 ± 1	3
DCLK1-NEG	**DCLK1 FL1Δ D511N**	**> 10000**					3
DCLK1-IN-1	**DCLK1 FL1Δ WT**	**83 ± 22**	6 ± 2	0.04 ± 0.01	17 ± 4	6 ± 1	4
FMF-03-055-01	**DCLK1 FL1Δ WT**	**12 ± 2**	9.6 ± 0.6	0.011 ± 0.001	60 ± 1	4 ± 1	3
XMD8-85	**DCLK1 FL1Δ WT**	**7.5 ± 0.3**	22 ± 2	0.016 ± 0.001	44 ± 2	3 ± 1	3
DCLK1-NEG	**DCLK1 FL1Δ WT**	**> 10000**					3

Errors are SEM, ND = not determined
K_D, dissociation constant; k_{on}, on-rate; k_{off}, off-rate; $t_{1/2}$, dissociative half-life for the protein/inhibitor complex (calculated from the fitted dissociation rate constant (k_{off}), according the equation $t_{1/2} = \ln 2/k_{off}$); N is the number of independent experiments.

Figure S1

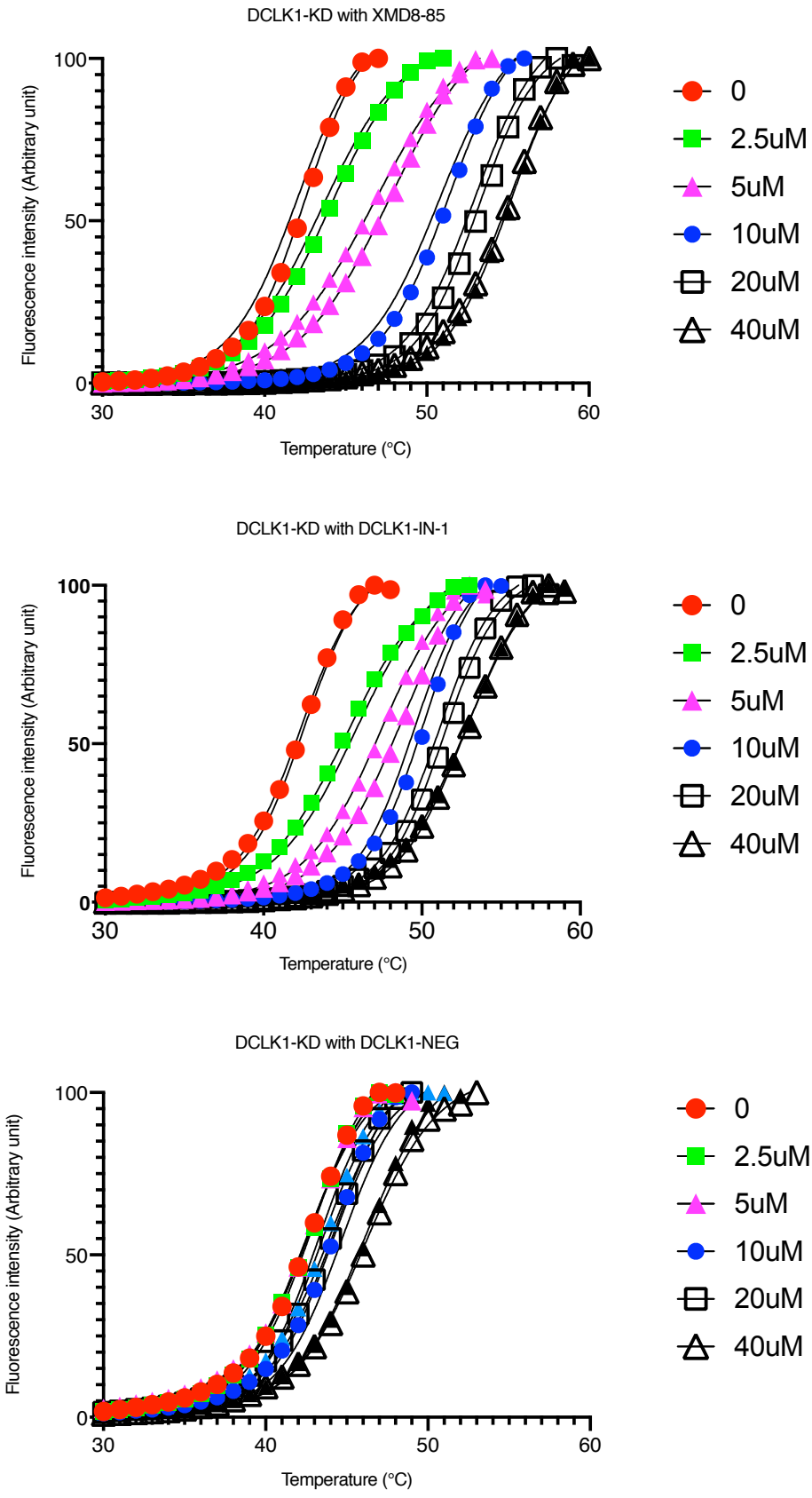


Figure S1. Thermal shift assay comparing melting profile of XMD8-85, DCLK1-IN-1 and DCLK1-NEG when bound to DCLK1-KD. Inhibitor concentration tested is as shown.

Figure S2

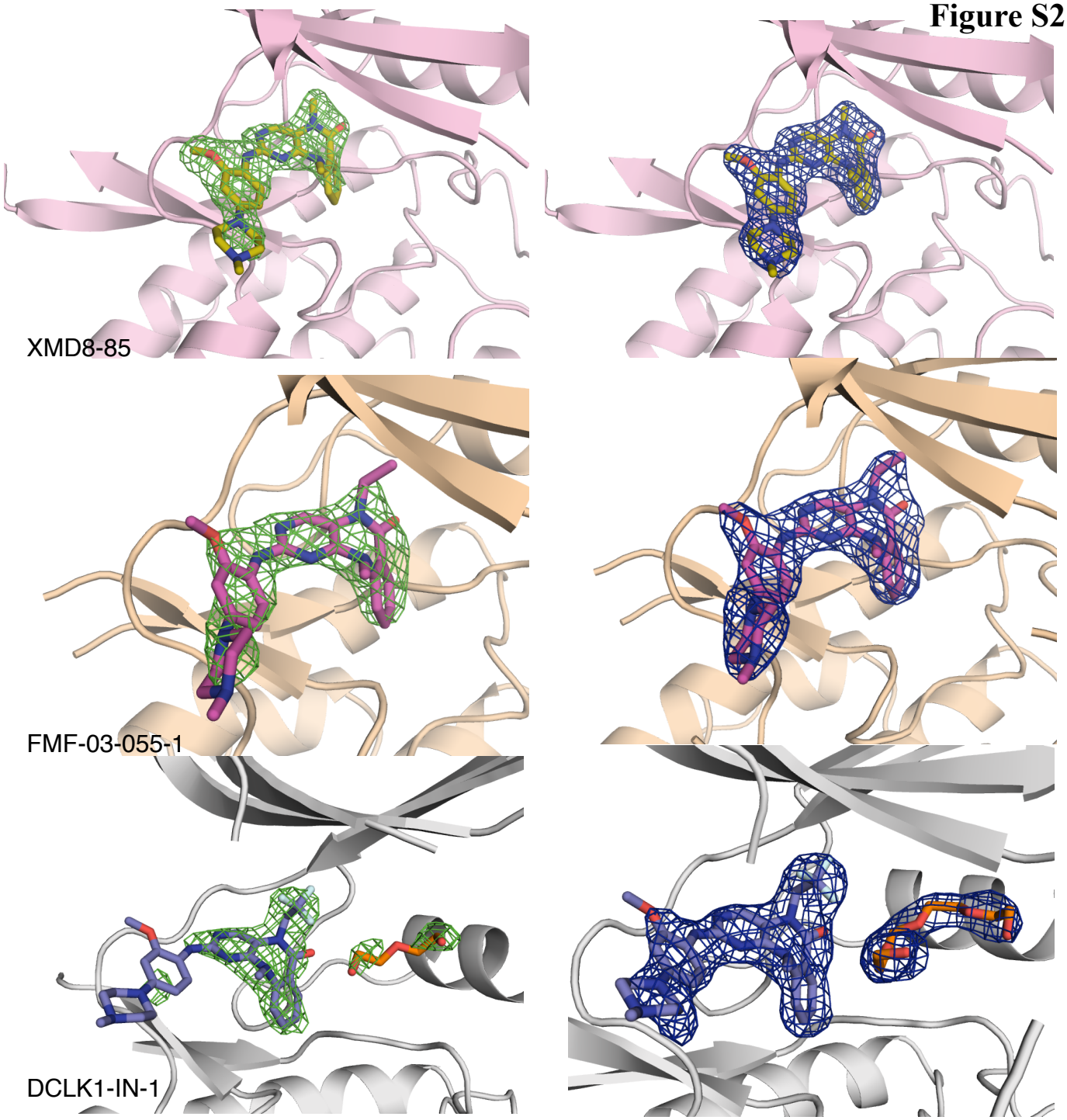


Figure S2. (A) Unbiased Fo-Fc density map contoured at 3σ (left) and 2Fo-Fc map contoured at 1σ (right) for XMD8-85, FMF-03-055-1 and DCLK1-IN-1 bound to DCLK1.

Figure S3

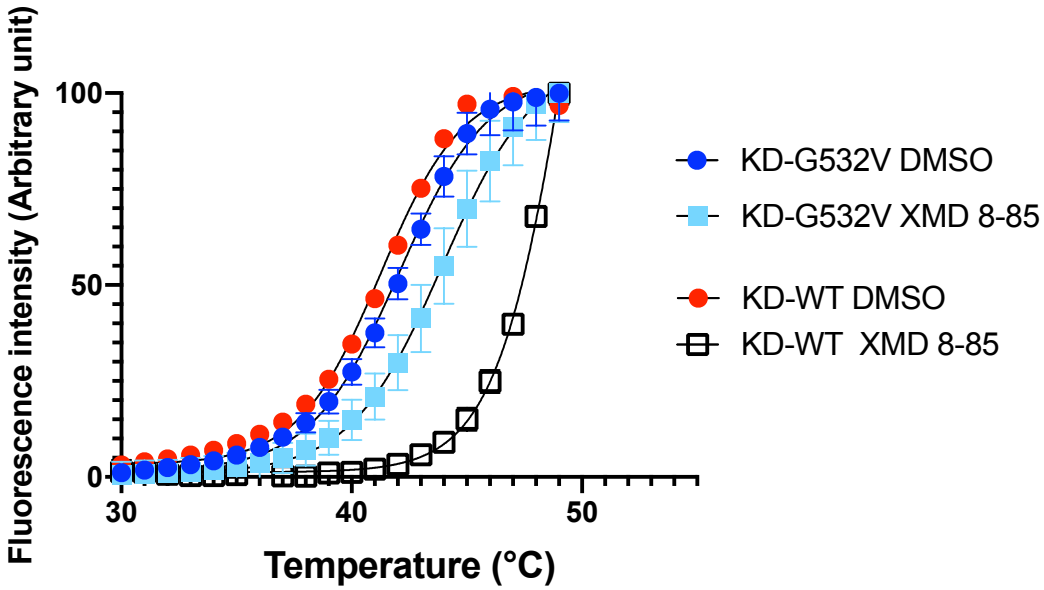


Figure S3. Thermal shift assay comparing melting profile of DCLK1-KD WT and DCLK1-KD G532V with XMD8-85.

Figure S4

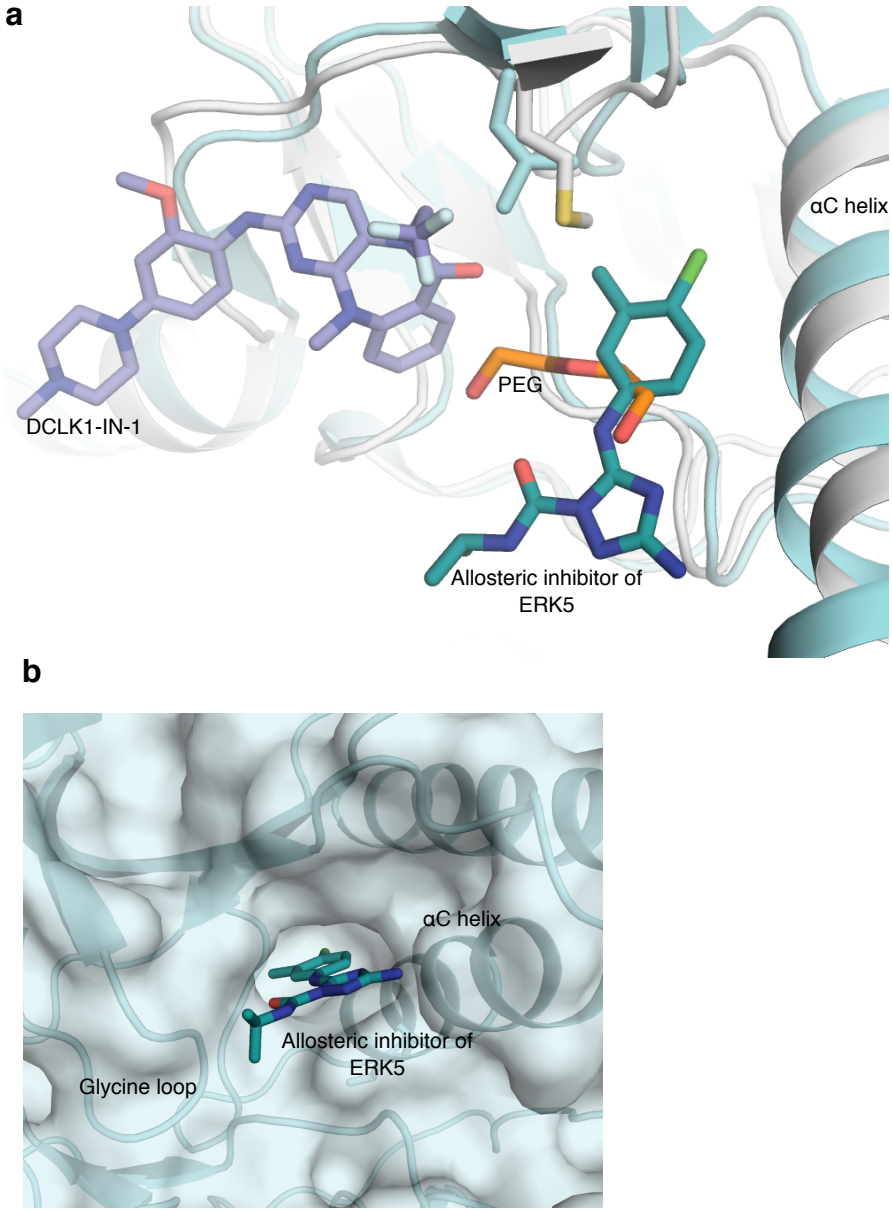


Figure S4. **a** Overlay of DCLK1-KD:DCLK1-IN-1 with ERK5 structure bound to an allosteric inhibitor (PDB 4ZSJ). The position of the PEG molecule in DCLK1-KD:DCLK1-IN-1 aligns with the position of the allosteric inhibitor in ERK5. **b** The crystal structure of ERK5 with an allosteric inhibitor (PDB 4ZSJ) showing the allosteric pocket stabilised in between the glycine loop and the αC helix.

Figure S5

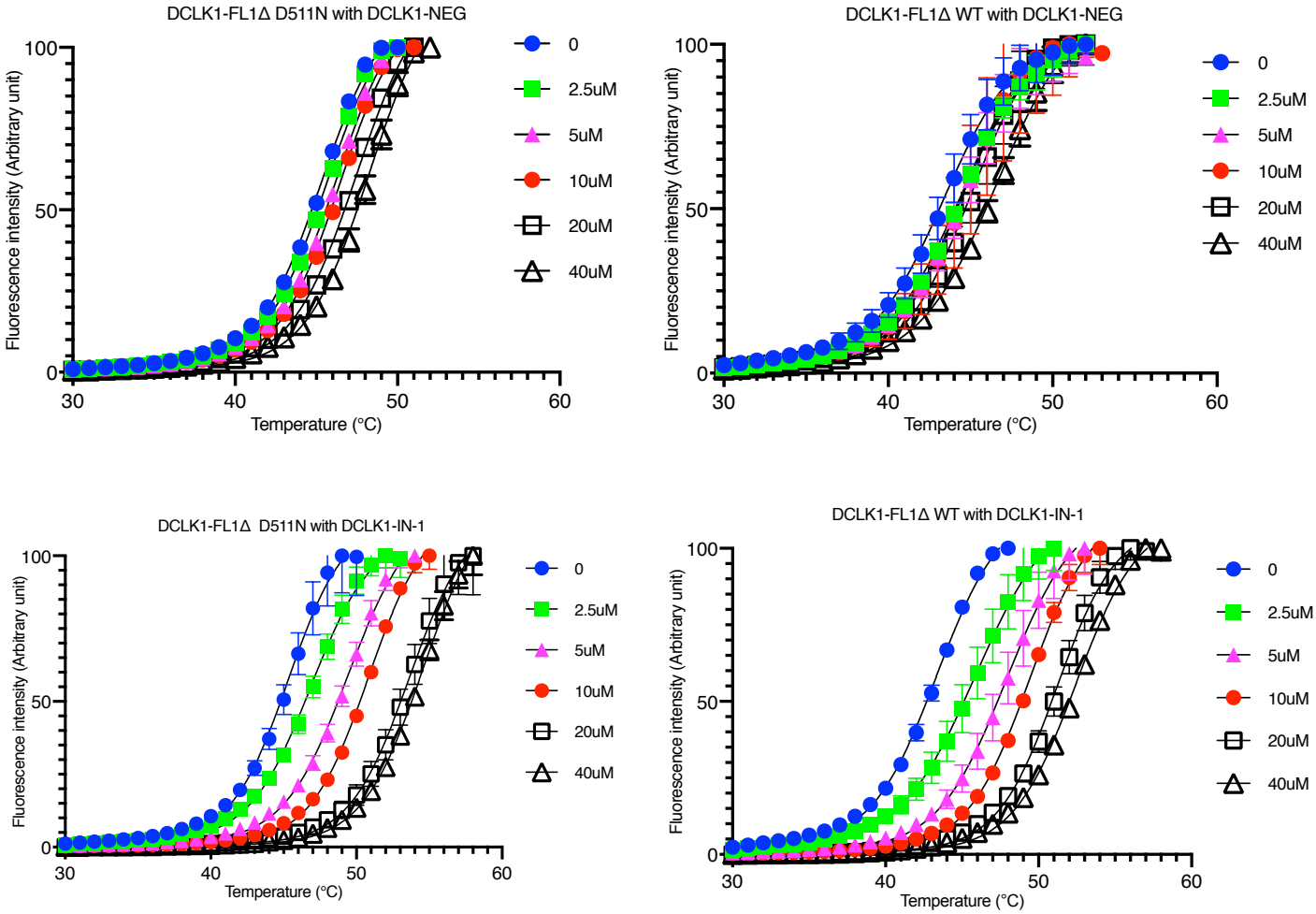


Figure S5. Thermal shift assay comparing melting profile of DCLK1-IN-1 and DCLK1-NEG with DCLK1-FL1Δ WT and DCLK1-FL1Δ D511N. Inhibitor concentration tested is as shown.

Figure S6

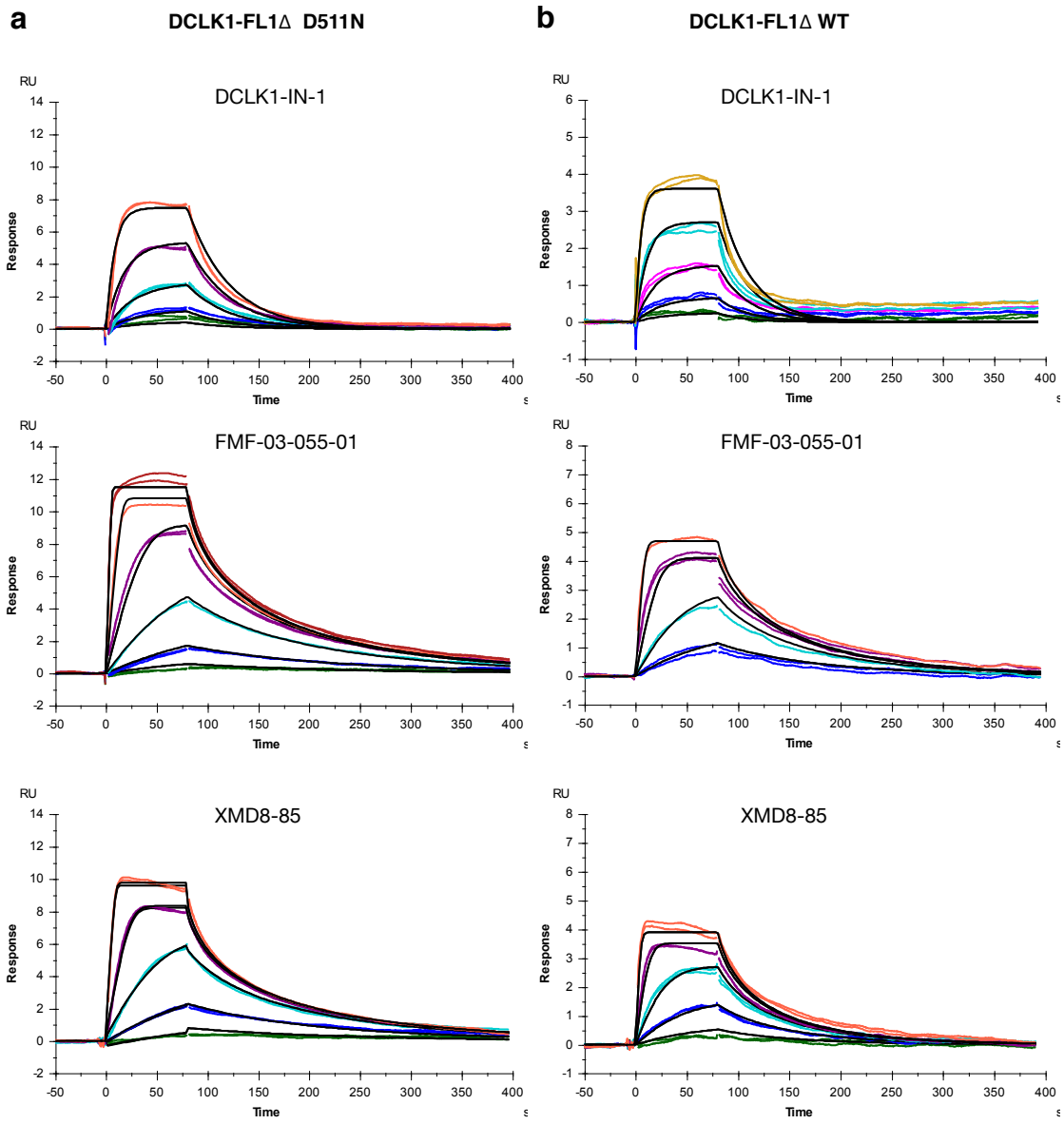
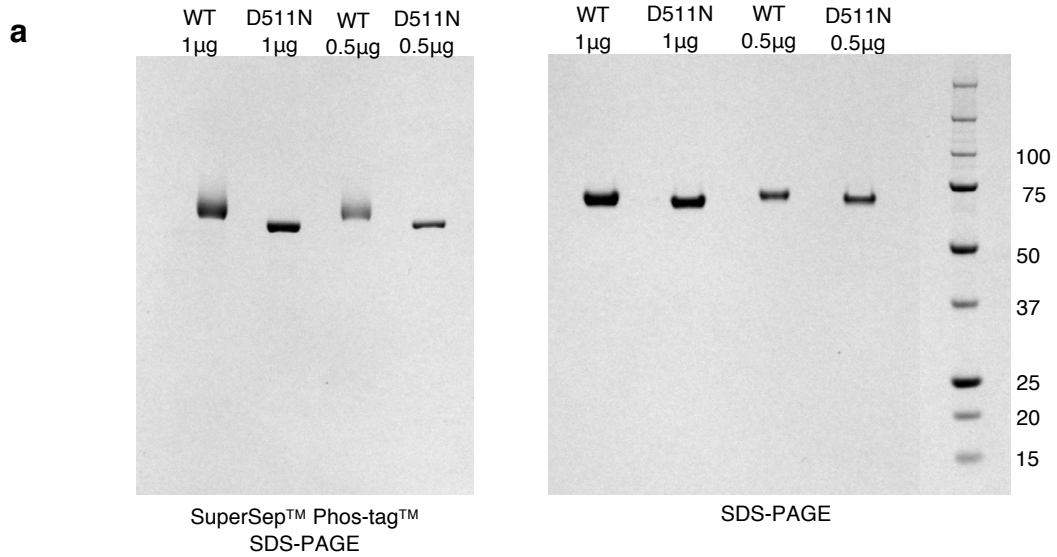
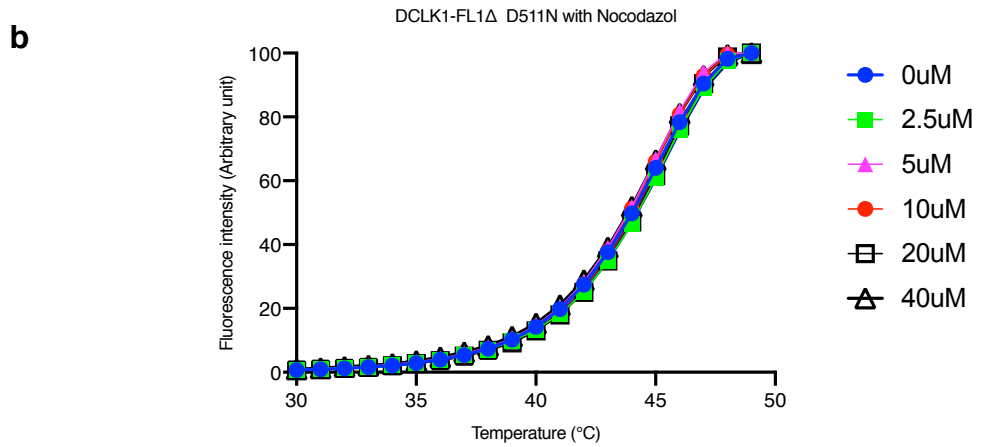


Figure S6. SPR kinetic fitting. Representative fitted SPR sensorgrams for DCLK1-IN-1, FMF-03-055-1 or XMD8-85 binding to immobilised DCLK1 FL1Δ D511N (a) or DCLK1 FL1Δ WT (b). Black lines represent kinetic fitting using a 1:1 binding model. Data represents an average of either four (DCLK1-IN-1) or three (FMF-03-0550-1 and XMD8-85) independent experiments. Mean fitted values are listed in Table S2.

Figure S7





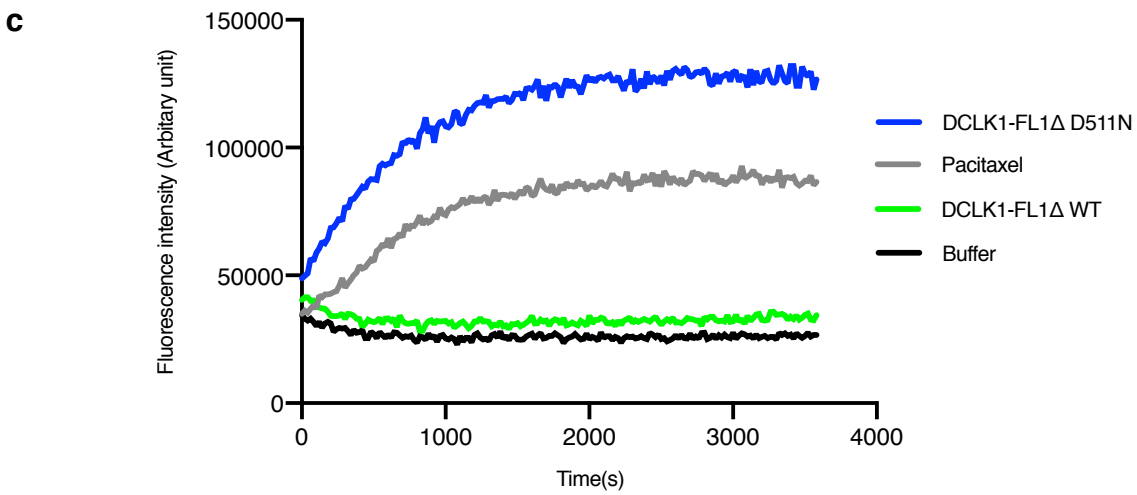


Figure S7. a. SuperSep™ Phos-tag™ 12.5% SDS-PAGE gel (left) and SDS-PAGE analysis (right) of DCLK1-FLΔ WT and DCLK1- FLΔ D511N. 1 µg and 0.5 µg samples were loaded on the gel. SDS-PAGE gel with molecular weight markers was run in parallel to make sure the proteins for the phos-tag analysis were not degraded. **b** Thermal shift assay to show that DCLK1- FLΔ D511N does not bind tubulin destabilisation drug, nocodazole (40 µM). This curve is a representation of samples tested in duplicates.

c Tubulin polymerisatioin assay. Tubulin was incubated alone (control buffer), with paclitaxel (3 µM), or with DCLK1-FLΔ WT (4 µM) and DCLK1- FLΔ D511N (4 µM). This curve is a representation of samples tested in duplicates and in two independent experiments.