SUPPLEMENTAL INFORMATION

Analyzing resistance to design selective chemical inhibitors for AAA proteins

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spastin-AAA:	N527C	T692A	WT	T692A	T692A	
ligand:	compound 1	compound 1	compound 4	compound 4 form A	compound 4 form B 6P14	
pdbID:	6P10	6P11	6P12	6P13		
Data collection						
Space group	P65	P65	P65	P65	P65	
Cell dimensions						
<i>a</i> , <i>b</i> , <i>c</i> (Å)	79.4 79.4 97.3	79.5 79.5 97.2	80.9 80.9 96.3	79.7 79.7 96.9	105.3 105.3 65.4	
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120	
Resolution (Å)	50.0-2.30	50.0-2.15	50.0-1.94	50.0-2.10	50.0-1.93	
	(2.34 - 2.30)	(2.19-2.15)	(1.97-1.94)	(2.14 - 2.10)	(1.96 - 1.93)	
Wavelength (Å)	0.9181	0.9201	0.9197	0.9201	0.9201	
R _{merge}	0.044 (0.468)	0.035 (0.391)	0.031 (0.450)	0.043 (0.448)	0.039 (0.439)	
< <u>I</u> >/σI	32.2 (1.8)	37.4 (2.7)	52.5 (2.8)	42.0 (2.7)	40.7 (2.9)	
Completeness (%)	99.0 (99.5)	99.8 (99.9)	99.0 (99.2)	99.9 (100.0)	99.9 (100.0)	
Multiplicity	4.7 (4.1)	5.5 (5.6)	6.5 (6.7)	5.8 (6.0)	5.8 (6.0)	
Total reflections	71736	103752	168901	118576	180192	
Unique reflections	15364 (783)	18985 (940)	26138 (1323)	20435 (1015)	31137 (1549)	
CC1/2	100 (81.9)	99.39 (89.6)	99.6 (91.3)	99.4 (86.9)	99.8 (90.6)	
Refinement						
Resolution $(Å)$	39 7-2 30	39 7-2 15	40 34 - 1 94	39 85 - 2 10	34 47 - 1 93	
Resolution (A)	(2.38-2.30)	$(2, 23_2, 2, 15)$	(2 01 - 1 94)	(2 18-2 10)	(1.99 - 1.93)	
No reflections	(2.36 - 2.36) 15360 (1546)	(2.23 - 2.13) 18944 (1878)	26123 (2613)	(2.10-2.10) 20389 (2020)	(1.99 - 1.93) 31108 (3101)	
No refl for $R_{\rm for}$	1548 (147)	1889 (189)	26123 (2613)	20309(2020) 2048(203)	3091 (306)	
Runda / Rena	0 206/0 252	0 213/0 241	0.193 / 0.230	0.207 / 0.245	0 179 / 0 200	
No atoms	2120	2291	2354	2208	2199	
Protein	2044	2291	2005	2208	1070	
Ligands/ions	20 44 40	20 4 0 67	2095 A1	2039 A1	1979	
Water	36	178	218	108	171	
R-factors	50	170	210	100	1/1	
Protein	61 51	55 31	48.00	50 54	43 94	
Ligand/ion	64.08	52.86	40.00	15 QA	42.07 42.02	
Water	67 46	63 29	57 1 <i>4</i>		46.83	
R m s deviations	02.40	05.29	57.14	55.78	+0.05	
Rond lengths $(Å)$	0.003	0.002	0.003	0.002	0.019	
Bond angles (°)	0.64	0.002	0.58	0.002	1.58	
Clashaaara	4.40	4.12	0.38	1.04	2.51	
Dataman Outliana	4.40	4.12	4.//	1.94	2.31	
Rotanier Outliers	06.24	0.00	0.20	0.00	2.05 07.67	
Kamachandran	96.34	90.09	98.90	97.00	97.07	
ravored (%)	2 66	2 21	1 10	2.04	2.22	
Kamachandran	3.00	3.31	1.10	2.94	2.33	
Allowed (%)	0.00	0.00	0.00	0.00	0.00	
Kamachandran	0.00	0.00	0.00	0.00	0.00	
Outliers (%)						

Table S1.	Crystallography	data collection	and refinement	statistics. Re	elated to Figur	res 2 and 4

*Data in brackets indicate the high-resolution shell.

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[compound 1], (µM)	k _{cat}	Hill coeff.	K _{1/2}
40	n.d.	n.d.	n.d.
20	n.d.	n.d.	n.d.
10	3.1 (0.6)	2.5 (0.2)	1.4 (0.1)
5	3.0 (0.3)	2.8 (0.2)	0.8 (0.1)
2.5	3.5 (0.4)	2.6 (0.2)	0.5 (0.1)
1.25	3.6 (0.6)	2.2 (0.1)	0.4 (0.1)
DMSO	3.5 (0.03)	2.2 (0.2)	0.2 (0.01)



Figure S1. Related to Figure 1.

(A) ATP concentration-dependence of the steady-state ATPase activity of spastin-WT (*Drosophila melanogaster* spastin, aa 209-758, see Methods for details) at different concentrations of compound **1**, analyzed using a steady-state NADH-coupled assay. To calculate enzyme parameters, rates were plotted against ATP concentration and data were fit to the Michaelis-Menten equation for cooperative enzymes.

Range of ATP concentrations tested was 0.04 to 2.5 mM. The data from each experiment were fit separately and the values determined by the fitting were averaged. Values for apparent enzyme activity parameters including k_{cat} , Hill coefficient and $K_{1/2}$ are provided (average, s.d. in parentheses, n=3). n.d. = not determined. (B) Schematic shows the AAA domain (gray box) of the truncated recombinant construct (*Drosophila melanogaster* spastin-AAA, aa 445-758, see Methods for details). The positions of three variability hot-spot residues are highlighted (arrows). The first and last residues of the construct are also numbered. (C) SDS-PAGE analysis (Coomassie blue) of purified spastin-AAA-WT and constructs with mutations in variability hot-spot residues (Q488V, N527C, and T692A). (D-G) Differential scanning fluorimetry analysis of the melting temperature of spastin-AAA constructs in the presence of compound 1 (Supporting data for Figure 1E). Data from one representative experiment are shown for each construct (n=2; spastin-AAA-WT, -Q488V, -N527C and T692A).



Figure S2. Related to Figure 1.

(A-J) Computational docking models for compound 1 bound to spastin. Five top scoring poses for compound 1 docked in the ATP site of spastin are shown (A, C, E, G and I). See Methods for details how these models were generated. Interaction maps for each pose are also shown (B, D, F, H and J; for details see panel K). The expected positions of the variability hotspot residues are highlighted by circles (Q488 – green, N527 – orange, T692 – blue; residue-compound distance <4 Å: full circle, >4 Å: dotted circle). Pose 5 (Figure S2I, J) is also shown in Figure 1G.



Figure S3. Related to Figure 2.

(A) Melting temperature of spastin-AAA-WT, -Q488V, -N527C and T692A constructs analyzed using differential scanning fluorimetry (n = 3; observed T_m for spastin-AAA-WT: ~38.5°C; -N527C: ~42°C; -Q488V: ~34.5°C; -T692A: ~39°C). (B) Crystal structure of spastin-AAA-N527C-compound 1 complex. Spastin's AAA domain (ribbon representation, gray) and compound 1 (stick representation, orange) are shown. Composite omit electron density around the compound is also shown (contoured at 3 σ , green mesh). (C) Two views of spastin-AAA-N527C-compound 1 complex. Simulated annealing omit electron density map around compound 1 contoured at 3 σ is shown (green mesh). Predicted hydrogen-bonding network between compound 1 and spastin's N-loop is also shown (black dashed lines).



Figure S4. Related to Figure 3.

(A) Schematic for the synthesis of compound **1** analogs (adapted from Malerich et al, 2010). (B-E) Differential scanning fluorimetry analysis of the melting temperature of spastin-AAA constructs (spastin-AAA-WT, -Q488V, -N527C and -T692A) in the presence of compound **4** (Supporting data for Figure 3D, n=2). Data from one representative experiment are shown for each construct.

		N-loop
Dm_Spastin_Q8l0P1 Hs_KTNA1_O75449 Hs_FIGL1_Q6PIW4 Hs_Vps4B_O75351	469 195 387 120	ILDEIVEGGAKVEWTDIAGQDVAKQALQEMVILPSVRPE 507 LERDIISQNPNVRWDDIADLVEAKKLLKEAVVLPMWMPE 233 IMNEIMDHGPPVNWEDIAGVEFAKATIKEIVVWPMLRPD 425 LQGAIVIERPNVKWSDVAGLEGAKEALKEAVILPIKFPH 158
Dm_Spastin_Q8l0P1 Hs_KTNA1_O75449 Hs_FIGL1_Q6PlW4 Hs_Vps4B_O75351	508 234 426 159	P-loop LFTGLRAPAKGLLLFGPPGNGKTLLARAVATECS-ATFL 545 FFKGIRRPWKGVLMVGPPGTGKTLLAKAVATECK-TTFF 271 IFTGLRGPPKGILLFGPPGTGKTLIGKCIASQSG-ATFF 463 LFTGKRTPWRGILLFGPPGTGKSYLAKAVATEANNSTFF 197
Dm_Spastin_Q8I0P1 Hs_KTNA1_O75449 Hs_FIGL1_Q6PIW4 Hs_Vps4B_O75351	546 272 464 198	NISAASLTSKYVGDGEKLVRALFAVARHMQPSIIFIDEV 584 NVSSSTLTSKYRGESEKLVRLLFEMARFYSPATIFIDEI 310 SISASSLTSKWVGEGEKMVRALFAVARCQQPAVIFIDEI 502 SISSSDLVSKWLGESEKLVKNLFQLARENKPSIIFIDEI 236
Dm_Spastin_Q8I0P1 Hs_KTNA1_O75449 Hs_FIGL1_Q6PIW4 Hs_Vps4B_O75351	585 311 503 237	DSLLSER-SSSEHEASRRLKTEFLVEFDGLPGNPDGDR- 621 DSICSRRGTSEEHEASRRVKAELVQMDGVGGTSENDDP 349 DSLLSQR-GDGEHESSRRIKTEFLVQLDGATTSSEDR- 538 DSLCGSR-SENESEAARRIKTEFLVQMQGVGVDNDG- 271
Dm_Spastin_Q8I0P1 Hs_KTNA1_O75449 Hs_FIGL1_Q6PIW4 Hs_Vps4B_O75351	622 350 539 272	hinge IVVLAATNRPQELDEAALRRFTKRVYVSLPDEQTRE 657 SKMVMVLAATNFPWDIDEALRRRLEKRIYIPLPSAKGRE 388 ILVVGATNRPQEIDEAARRRLVKRLYIPLPEASARK 574 ILVLGATNIPWVLDSAIRRRFEKRIYIPLPEPHARA 307
Dm_Spastin_Q8I0P1 Hs_KTNA1_O75449 Hs_FIGL1_Q6PIW4 Hs_Vps4B_O75351	658 389 575 308	Sensor-II LLINRLLQKQGSPLDTEALRRLAKITDGYSGSDLTALAK 696 ELLRISLR - ELELADDVDLASIAENMEGYSGADITNVCR 426 QIVINLMSKEQCCLSEEEIEQIVQQSDAFSGADMTQLCR 613 AMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVR 346

Figure S5. Related to Figure 3.

Α

(A) Sequence alignment of the AAA domains of *D. melanogaster* spastin, human katanin, human FIGL1 and human VPS4B proteins (supporting data for Figure 3F). Conserved ATP binding motifs are highlighted (N-loop, P-loop, hinge and sensor-II). Residues at the entrance of the ATP pocket are indicated (red box). Variability hot-spot residues in the N-loop (green), P-loop (orange), hinge (gray) and sensor-II motifs (blue) are also indicated by colored boxes.



Figure S6. Two views of compound **1** in the active site of spastin-AAA-T692A. Related to Figure 5. (A) View of compound **1**, the N-loop motif and the side chains of variability hot-spot residues in the active site of spastin (Gln-488, green; Asn-527, orange; T692A, blue). (B) View of compound **1** from the entrance of the ATP binding site. Potential interaction of the compound **1**'s phenylsulfonamide moiety (conformer 2) with the residue in helix 4 of the small AAA subdomain (Arg-662) is highlighted (black dashed line).