Supplementary Information

Analysis of the Structural Mechanism of ATP Inhibition at the AAA1 Subunit of Cytoplasmic Dynein-1 Using a Chemical "Toolkit"

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Figure S1

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P36022 DYHC_YEAST   1749  ISQSGYLLQYKFEYIGIPERLIYTPLLLIGFATLTDSLHQKYGGCFFGPAGTGKTETVKA   1808
P34036 DYHC_DICDI   1927  IHMANATFYYGFEYLGIGERLVQTPLTDRCYLTLTQALESRMGGNPFGPAGTGKTETVKA   1986
                          *  :.  : * ***:** ***: ***    : ***::*..: **  **************

P36022 DYHC_YEAST   1809  FGQNLGRVVVVFNCDDSFDYQVLSRLLVGITQIGAWGCFDEFNRLDEKVLSAVSANIQQI   1868
P34036 DYHC_DICDI   1987  LGSQLGRFVLVFCCDEGFDLQAMSRIFVGLCQCGAWGCFDEFNRLEERILSAVSQQIQTI   2046
                          :*.:***.*:** **:.** *.:**::**: * ************:*::***** :** *

P36022 DYHC_YEAST   1869  QNGLQVGKSHITLLEE-ETPLSPHTAVFITLNPGYNGRSELPENLKKSFREFSMKSPQSG   1927
P34036 DYHC_DICDI   2047  QVALKENSKEVELLGGKNISLHQDMGIFVTMNPGYAGRSNLPDNLKKLFRSMAMIKPDRE   2106
                          * .*: ....: **   :  *  . .:*:*:**** ***:**:**** **.::* .*:

P36022 DYHC_YEAST   1928  TIAEMILQIMGFEDSKSLASKIVHFLELLSSKCSSMNHYHFGLRTLKGVLRN-------C   1980
P34036 DYHC_DICDI   2107  MIAQVMLYSQGFKTAEVLAGKIVPLFKLCQEQLSAQSHYDFGLRALKSVLVSAGGIKRKC   2166
                           **::::   **: :: **.*** :::* ..: *: .**.****:**.** .       *

P36022 DYHC_YEAST   1981  SPLIS-EFGEGEK--------------TVVESLKRVILPSLGDTDELVFKDELSKIFDS-   2024
P34036 DYHC_DICDI   2167  QPPQLPPITDAESKTKADQIYCQYEIGVLLNSINDTMIPKLVADDIPLIQSLLLDVFPGS   2226
                          .*     : :.*.                .:::*:: .::*.*   *  :::. * .:* .

P36022 DYHC_YEAST   2025  AGTPLNSKAIVQCLKDAGQRSGFSMSEEFLKKCMQFYYMQKTQQALILVGKAGCGKTATW   2084
P34036 DYHC_DICDI   2227  QLQPIQMDQLRKKIQEIAKQRHLVTKQEWVEKILQLHQILNINHGVMMVGPSGGGKTTSW   2286
                             *:: . : : ::: .::  :  .:*:::* :*:: : : ::.:::** :* ***::*

P36022 DYHC_YEAST   2085  KTVIDAMAIFDGHANVVYVIDTKVLTKESLYGSMLKATLEWRDGLFTSILRRVNDDITGT   2144
P34036 DYHC_DICDI   2287  EVYLEAIEQVDNIKSEAHVMDPKAITKDQLFGSLDLTTREWTDGLFTATLRRIIDNVRGE   2346
                          :. ::*:  .*.  . .:*:* *.:**:.*:**:  :* ** *****: ***: *:: *

P36022 DYHC_YEAST   2145  FKNSRIWVVFDSDLDPEYVEAMNSVLDDNKILTLPNGERLPIPPNFRILFETDNLDHTTP   2204
P34036 DYHC_DICDI   2347  S-TKRHWIIFDGDVDPEWVENLNSLLDDNKLLTLPNGERLALPNNVRVMFEVQDLKYATL   2405
                            ..* *::**.*:***:** :**:*****:********* :* *.*::**.::*.::*

P36022 DYHC_YEAST   2205  ATITRCGLLWFSTDVCSISSKIDHL----LNKSYEALDNKLSM------FELDKLKDLIS   2254
P34036 DYHC_DICDI   2406  ATISRCGMVWFSEEILTTQMIFQNYLDTLSNEPFDPQEKEQQKRNENAQLQQQQQTTITS   2465
                          ***:***::*** :: : .  :::      *: ::  ::: .       :: :: . : *

P36022 DYHC_YEAST   2255  D-----------SFD--------MASLTNIFTCSN---------DLV-----------HI   2275
P34036 DYHC_DICDI   2466  PILTSPPTTSSSSRSTTSTTSMIPAGLKVQKECAAIISQYFEPGGLVHKVLEDAGQRPHI   2525
                                       * .         *.*.    *:         .**          **

P36022 DYHC_YEAST   2276  LGVRTFNKLETAV---QLAVHLISSYRQWFQNLD--DKSLKDVITLLIKRSLLYALAGDS   2330
P34036 DYHC_DICDI   2526  MDFTRLRVLNSFFSLMNRSIVNVIEYNQLHSDFPMSPENQSNYITNRLLYSLMWGLGGSM   2585
                          :..  :. *:: .   : ::  : .*.* ..::    :. .: **  :  **::.*.*.

P36022 DYHC_YEAST   2331  TG---ESQRAFIQTINTY-FGHDSQELSDYSTIVIANDKLSFSSFCSEIPSVSLEAHEVM   2386
P34036 DYHC_DICDI   2586  GLVERENFSKFIQTIAITPVPANTIPLLDYS---VSIDDANWSLWKNKVPSVEVETHKVA   2642
                               *.   *****    .  ::  * ***   :: *. .:* : .::***.:*:*:*
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Sequence conflict
Turn
Coiled coil
Helix
Beta strand
Nucleotide binding

Figure S1: Amino acids sequence alignment of the AAA1 and AAA2 subunits of dynein corresponding to *S. cerevisiae* (1758-2273) and *D. discoideum* (1936-2531) with the ClustalW program. The W-A motif is highlighted in blue. The grey highlights represent sequence similarity. A singular dot (.) indicates that residues belong to a different group of amino acids, while a colon (:) Residues belong to the same group of amino acids and asterisk (*) indicates sequence identity.

Figure S2

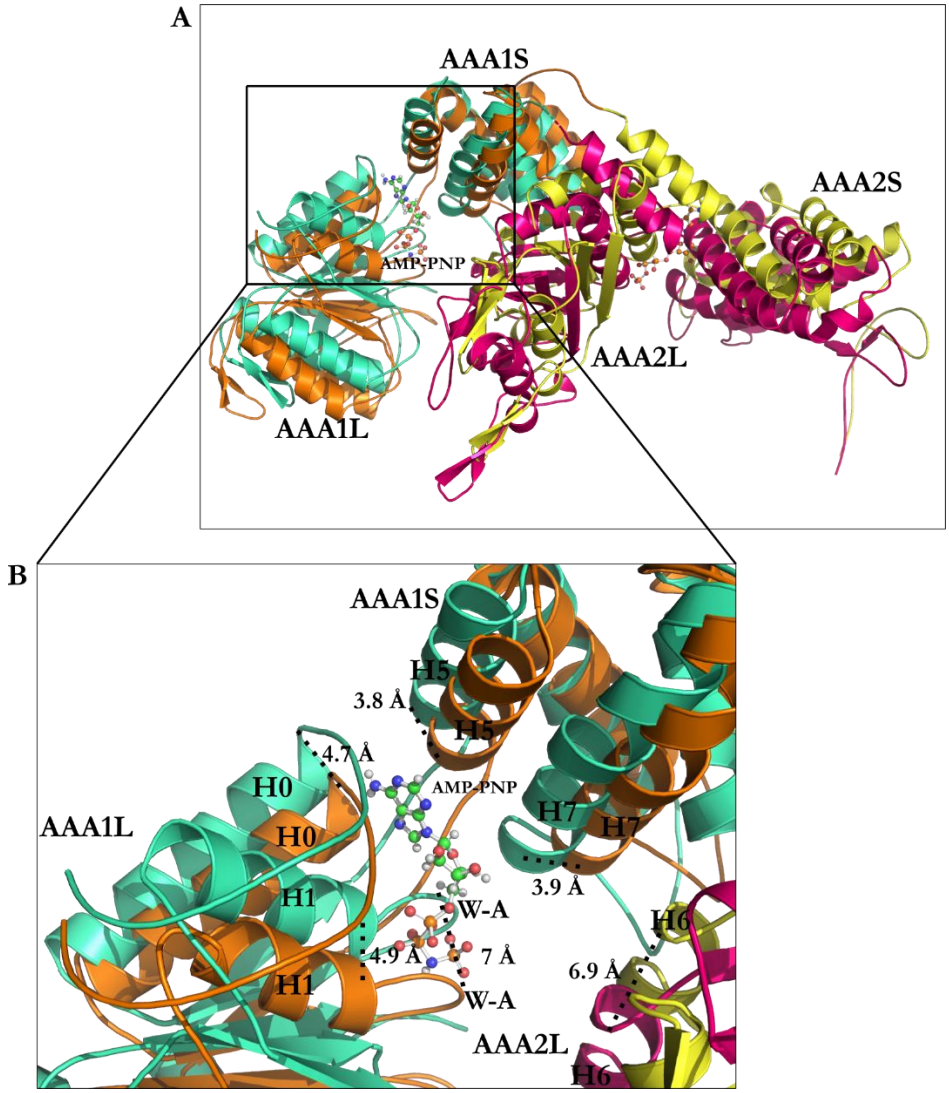

Figure S2: A) Structural alignment of the AAA1 and AAA2 of yeast-AMPPNP (4W8F) and yeast-apo (4AKG), **B)** A close-up of the AAA1 binding site. Helices (H0, H1, H5, and H7) of the AAA1 subdomain, the Walker-A (W-A) loops with the AMP-PNP in the ball and stick representation in green located at the AAA1 nucleotide-binding site and the H6 of the AAA2 subdomains of the yeast-AMPPNP (4W8F) and yeast-apo (4AKG). Color code: yeast-AMPPNP (AAA1 in green cyan and AAA2 in sharp pink) and yeast-apo (AAA1 in orange and AAA2 in yellow)

Figure S3

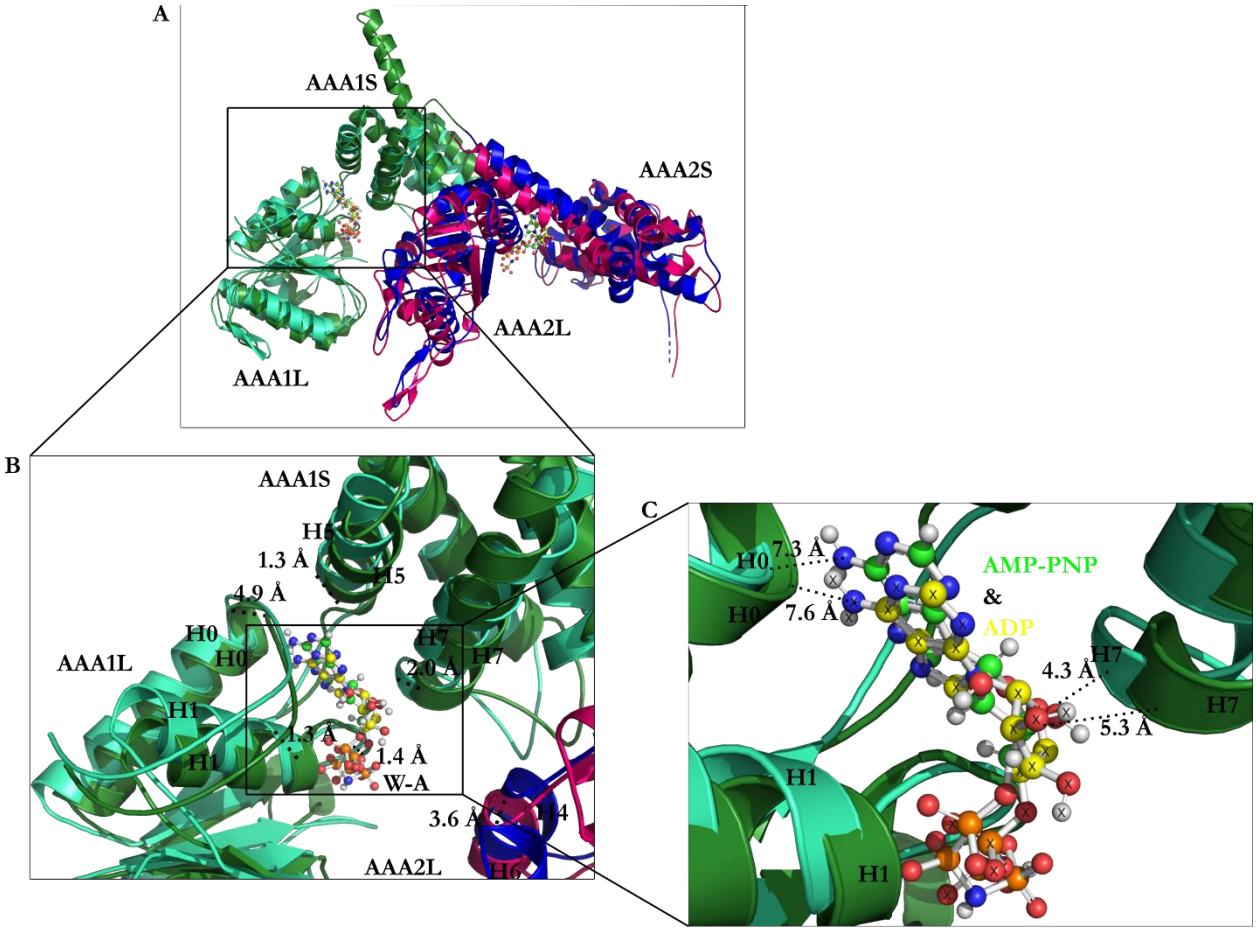

Figure S3: A) Structural alignment of the AAA1 and AAA2 subunit of the *Dictyostelium-ADP* (3VKG) and yeast-AMPPNP (4W8F), **B)** A close-up of the AAA1 binding site. Helices (H0, H1, H5, and H7) of the AAA1 subdomain, the Walker-A (W-A) loops with the AMP-PNP in the ball and stick representation in green and ADP in the ball and stick representation in yellow located at the AAA1 nucleotide-binding site and the H4 of the AAA2 subdomain of the *Dictyostelium-ADP* (3VKG) and the H6 of the AAA2 subdomain of the yeast-apo (4AKG), **C)** Structural alignment of ligands at the AAA1 nucleotide-binding site of the *Dictyostelium-ADP* (3VKG) and yeast-AMPPNP (4W8F) with AMP-PNP in the ball and stick representation in green and ADP in the ball and stick representation in yellow with a cross (x) on its atoms to distinguish them from the atoms of AMP-PNP. Color code: *Dictyostelium*-ADP (AAA1 in green and AAA2 in blue) and yeast-AMPPNP (AAA1 in green cyan and AAA2 in sharp pink)

Figure S4

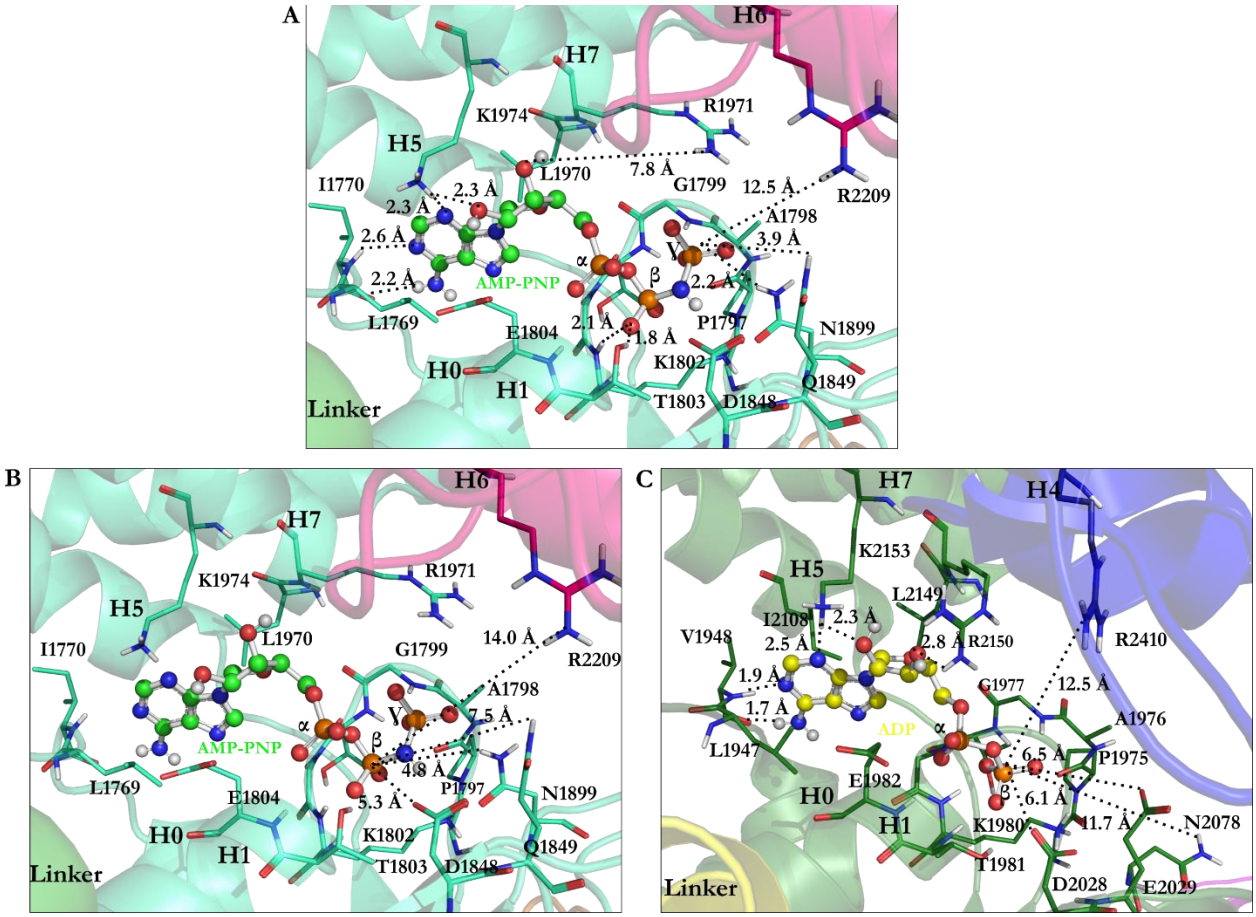

Figure S4: A) AMP-PNP interacting and residues of the AAA1 nucleotide-binding site. Distances between catalytic residues of the AAA1 nucleotide-binding site of dynein and the β-phosphate of **B)** AMP-PNP and **C)** ADP.

Figure S5

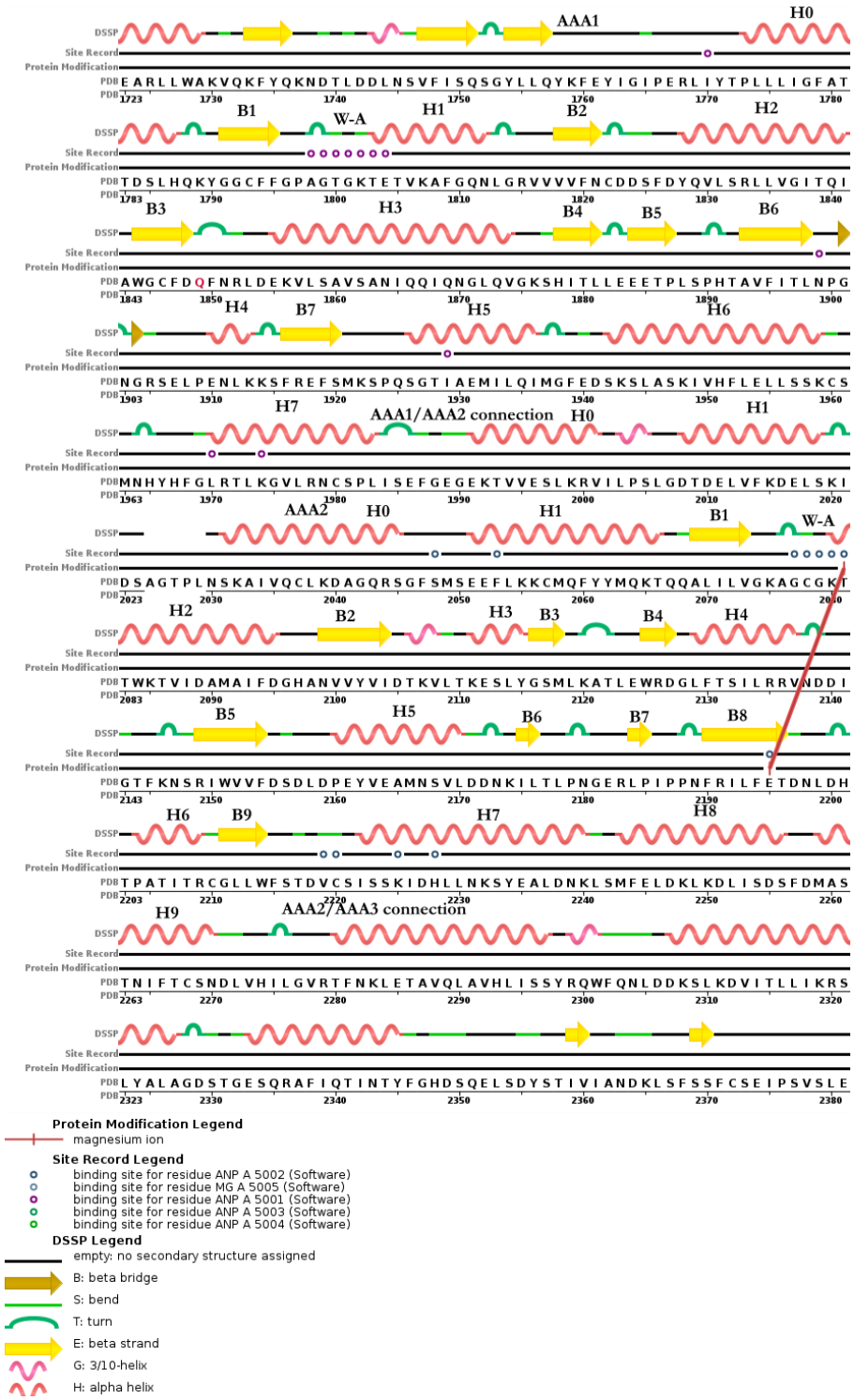

Figure S5: Annotation of the secondary structure of AAA1 and AAA2 domains of cytoplasmic dynein according to the 4W8F crystal structure retrieved from the PDB.

Figure S6

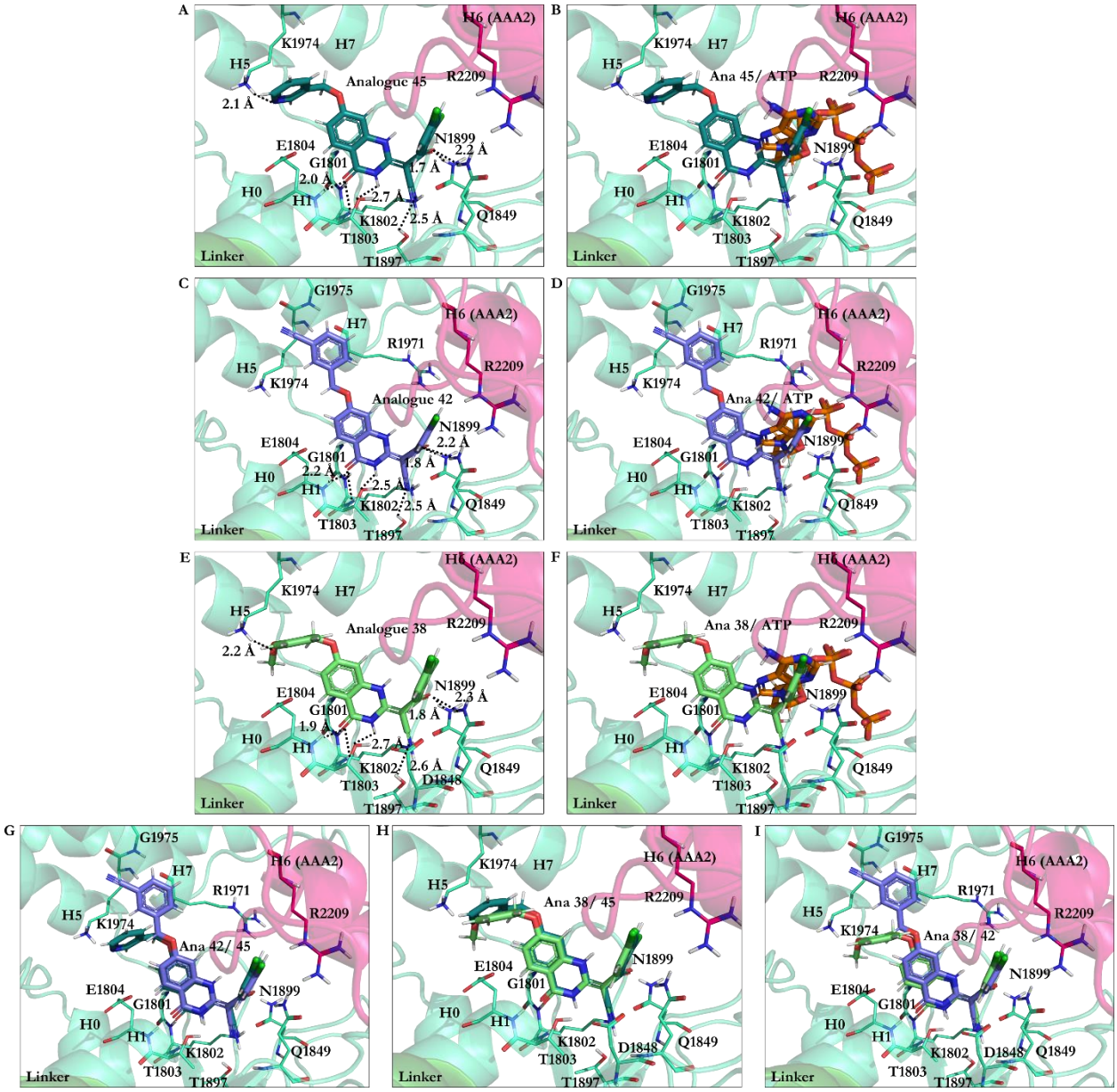

Figure S6: Binding modes of ciliobrevin analogs at the AAA1 binding site of dynein 1. **A)** Analog 45. **B)** Analog 45 superimposed on ATP, **C)** Analog 42, **D)** Analog 42 superimposed on ATP, **E)** Analog 38, **F)** Analog 38 superimposed on ATP. Superimposition of **G)** Analogs 42 and 45, **H)** Analogs 38 and 45, **I)** Analogs 38 and 42.

Figure S7

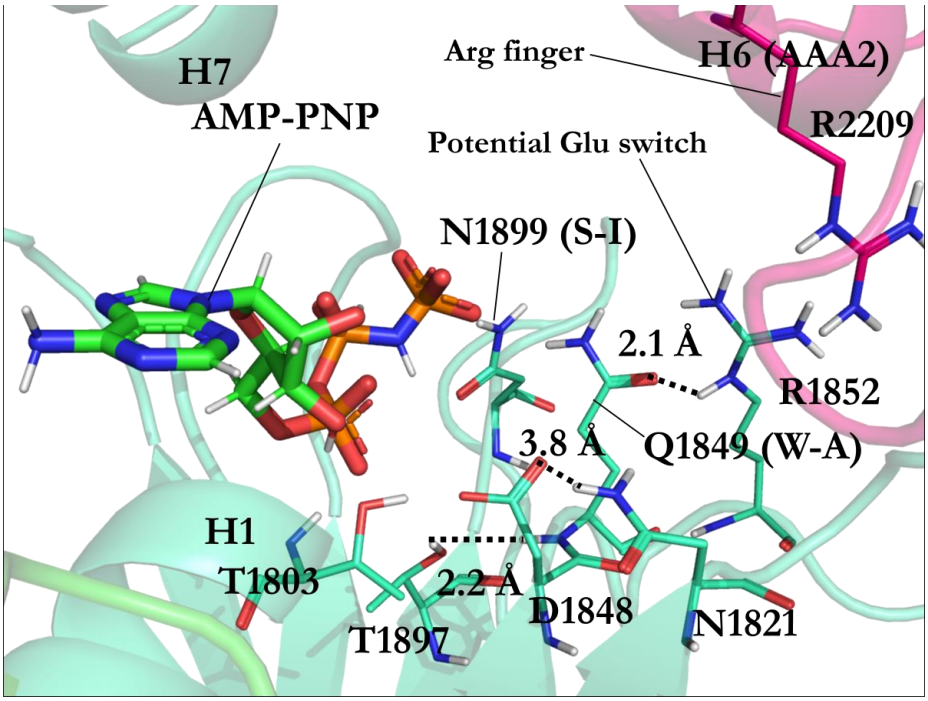

Figure S7: Intramolecular interactions between the catalytic residues at the AAA1 binding site and Arg1852 involving a potential glutamate switch. The docking solution of AMP-PNP is represented in green and stick. Thr1803 and Asp1848 accommodate Mg^{2+} during ATP hydrolysis. Sensor I (N1899), W-B (E1849Q), R-finger (R2209) play catalytic roles during ATP hydrolysis.

Figure S8

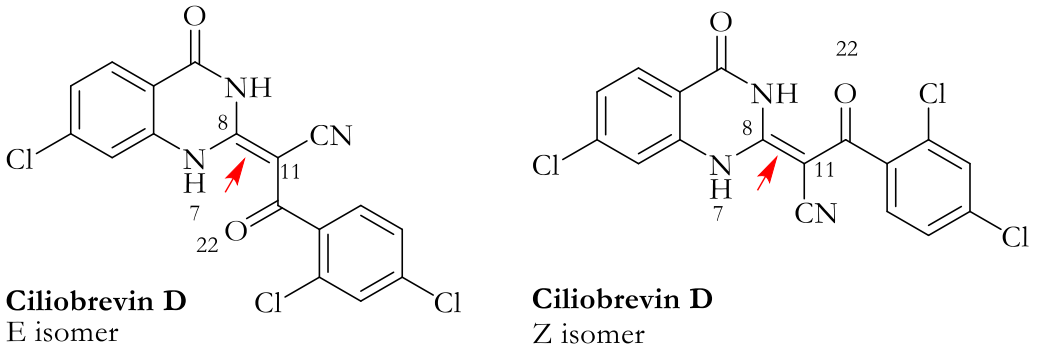

Figure S8: *E* and *Z* configurations of ciliobrevin D. Red arrows point to the double bond between the C8 and C11 atoms.

Figure S9

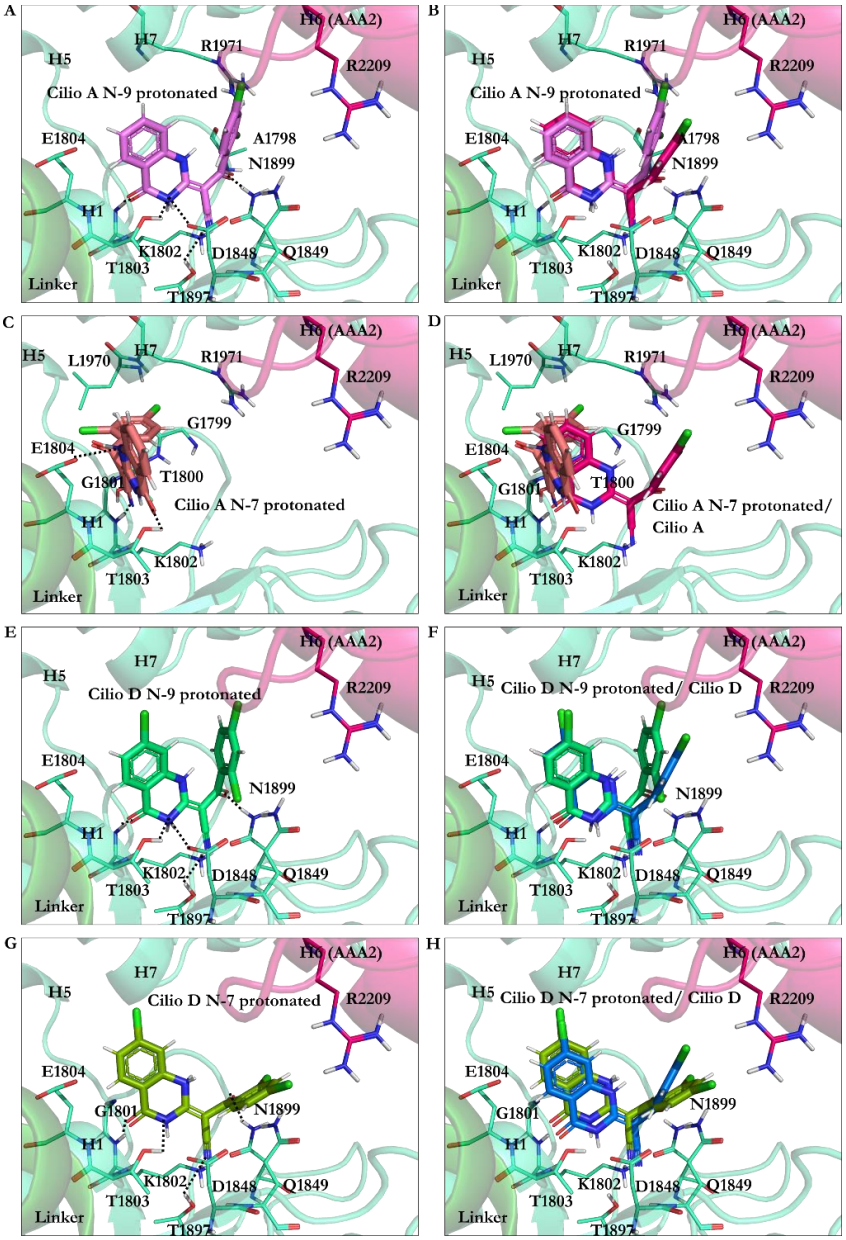

Figure S9: Binding modes of the protonated forms of ciliobrevin at the AAA1 binding site of dynein 1. **A)** Ciliobrevin A protonated at the N9 atom, **B)** Ciliobrevin A protonated at the N9 atom superimposed on ciliobrevin A, **C)** Ciliobrevin A protonated at the N7 atom, **D)** Ciliobrevin A protonated at the N7 atom superimposed on ciliobrevin A, **E)** Ciliobrevin D protonated at the N9 atom, **F)** Ciliobrevin D protonated at the N9 atom superimposed on ciliobrevin D, **G)** Ciliobrevin D protonated at the N7 atom, **H)** Ciliobrevin D protonated at the N7 atom superimposed on ciliobrevin D.

Figure S10

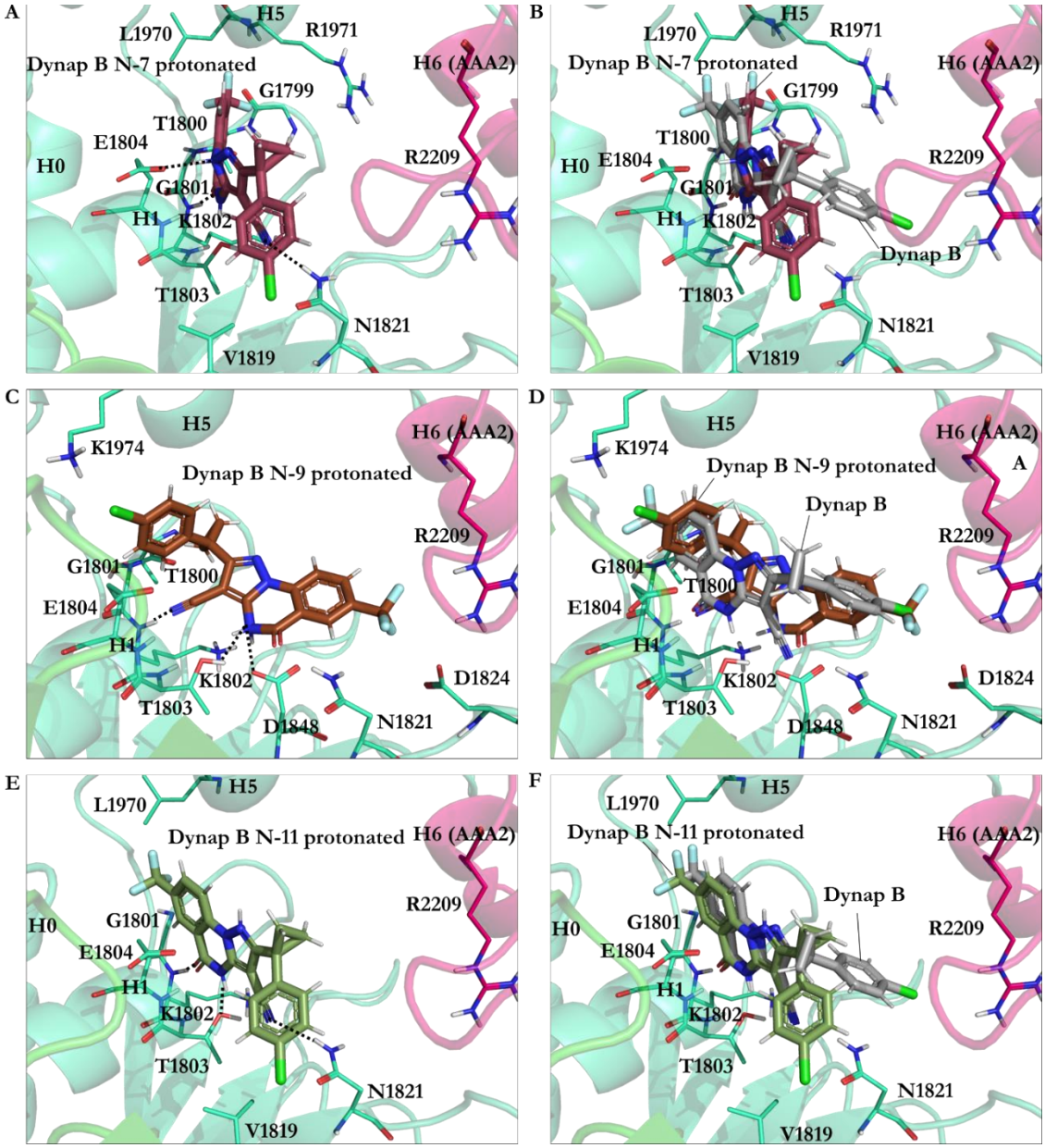

Figure S10: Main binding interactions of the protonated forms of dynapyrazole B at the AAA1 binding site of dynein 1. **A)** Dynapyrazole B protonated at the N7 atom, **B)** Dynapyrazole A protonated at the N7 atom superimposed on dynapyrazole B, **C)** Dynapyrazole B protonated at the N9 atom, **D)** Dynapyrazole B protonated at the N9 atom superimposed on dynapyrazole A, **E)** Dynapyrazole B protonated at the N11 atom, **F)** Dynapyrazole B protonated at the N11 atom superimposed on dynapyrazole B.

Table S1

Compound	Binding Energy (kJ/mol)	Residues interacting with the compound
Analog 30	−27.87	Gly1801, Lys 1802, Thr1803, Glu1804, Asp1848, Gln1849, Thr1897, Asn1899
Analog 29	−27.37	Gly1801, Lys1802, Thr1803, Glu1804, Asp1848, Gln1849, Thr1897, Asn1899
Analog 28	−27.27	Gly1801, Lys1802, Thr1803, Glu1804, Asp1848, Gln1849, Thr1897, Asn1899

Table S1: Networking interactions of analogs 28, 29, and 30 with AAA1 of dynein 1.

Table S2

Compound	Binding energy (kJ/mol)	Residues interacting with the ligand
Analog 45	−29.72	Gly1801, Lys1802, Thr1803, Glu1804, Gln1849, Thr1897, Asn1899, Lys1974
Analog 42	−27.43	Gly1801, Lys1802, Thr1803, Glu1804, Gln1849, Thr1897, Asn1899, Lys1974, Gly1975, Arg1971
Analog 38	−27.27	Gly1801, Lys1802, Thr1803, Glu1804, Asp1848, Gln1849, Thr1897, Asn1899, Lys1974

Table S2: Interacting amino acids with analogs 38, 42, and 45.

Table S3

Compound	Binding energy (kJ/mol)	Residues interacting with the docked ligand
Ciliobrevin A *E*-isomer	−26.23	Gly1801, Lys1802, Thr1803, Glu1804, Asp1848, Gln1849, Thr1897, Asn1899
Ciliobrevin A *Z*-isomer	−19.41	Thr1803, Asn1821, Asp1848, Gln1849, Arg 1852, Asn1899, Arg1852, Arg1971
Ciliobrevin D *E*-isomer	−23.92	Gly1801, Lys1802, Thr1803, Glu1804, Asp1848, Gln1849, Thr1897, Asn1899
Ciliobrevin D *Z*-isomer	−19.78	Gly1799, Thr1800, Gly1801, Lys1802, Thr1803, Glu1804, Asp1848, Gln1849, Leu1970, Arg1971, Lys1974
***E*-Analog 22**	−16.83	Ala1798, Gly1969, Tyr1902, Pro1900, Arg1971, His1967
***Z*-Analog 22**	−17.49	Leu1769, Gly1801, Lys1802, Glu1804, Leu1970, Lys1974

Table S3: The binding energy of *E* and *Z* isomers of ciliobrevin A and ciliobrevin D.

Table S4

Compounds	Binding energy (kJ/mol)	Residues interacting with the compound
Dynapyrazole B N-7 protonated	−26.95	Gly1799, Thr1800, Gly1801, Lys1802, Thr1803, Glu 1804, Val1819, Asn1821, Leu1970, Arg1971
Dynapyrazole A N-7protonated	−25.99	Gly1801, Lys1802, Thr1803, Glu1804, Asn1821
Dynapyrazole A N-9 protonated	−19.81	Glu1586, Lys1696, Glu1699, Pro1766, Glu1767, Arg1978
Dynapyrazole B	−18.49	Thr1800, Gly1801, Lys1802, Thr1803, Glu1804, Asn1821, Leu1970
Dynapyrazole B N-9 protonated	−18.47	Thr1800, Gly1801, Lys1802, Thr1803, Glu1804, Asn1821, Asp1824, Asp1848, Lys1974
Dynapyrazole A	−18.17	Gly1801, Lys1802, Thr1803, Glu1804, Asn1821
Dynapyrazole A N-11 protonated	−17.49	Lys 1802, Thr1803, Glu1804, Asp1848, Gln1849, Asn1899
Dynapyrazole B N-11 protonated	−17.13	Gly1801, Lys1802, Thr1803, Glu1804, Val1819, Asn1821, Leu1970

Table S4: Interacting amino acids with dynapyrazole A and B in the protonated and deprotonated states.