dark grey) and causing gap opening between AAA1/AAA2 and AAA5/AAA6. The gap at AAA1 leads to a low nucleotide affinity state for this site. The AAA2 site contains a tightly bound ATP and is likely to play a purely structural role. Sites AAA3 and AAA4 appear to adopt a semi-closed conformation and can bind and release nucleotide. While the AAA3 ATP hydrolysis site seems to be in a catalytically competent conformation, important catalytic residues of the AAA4 site might not be able to support ATP hydrolysis.

## Online Methods

### Crystallization constructs

Crystals were produced with construct GST-Dyn1-314kD$_{\Delta 3039\text{-}3291}$, which is identical to the previously reported construct[7], that lacks the MTBD and has a truncated stalk region fused with the peptide PKAPPEEKEA. Crystals were also obtained with the construct GST-Dyn1-314kD$_{\Delta 3039\text{-}3289}$ that was produced by modifying GST-Dyn1-314kD $_{\Delta 3039\text{-}3291}$. The truncated stalk region in this case was fused with the peptide GD. All genomic manipulations were carried out using standard molecular biology techniques.

### Crystallization and Data Collection

Crystals of constructs GST-Dyn1-314kD$_{\Delta 3039\text{-}3291}$ or Dyn1-Dyn1-314kD$_{\Delta 3039\text{-}3289}$ were obtained by under-oil batch crystallization. 3 µl of protein solution (6 – 15 mg ml$^{-1}$ in 20 mM Tris-HCl 8.0, 100 mM KAc, 2mM MgAc, 1 mM EGTA, 10% (v/v) glycerol and 1 mM DTT) were mixed with an equal amount of seeding solution produced from crystals that were resuspended in 12% (v/v) PEG3350, 100 mM NaCitrate pH 5.7 and 200 mM ammonium sulfate and covered with 10 µl of Al's Oil (Hampton

Research). The crystals for the seeding solution were produced with methods described previously[7]. Crystallization employing the under-oil batch approach was carried out at 4°C and crystals grew to maximal dimension of 0.8 x 0.4 x 0.2 $mm^3$. To obtain LuAc-derivatives, crystals were soaked overnight in solutions consisting of 5 mM LuAc, 100 mM Tris-HCl 7.5, 200 mM ammonium sulfate, 20% (v/v) PEG3350 and 15% (v/v) glycerol. We also tried to obtain LuAc-derivatives by co-crystallization trials in the presence of 1 mM LuAc. $WO_4$-derivatization was carried out by transferring crystals into solutions containing 20 mM $Na_2WO_4$, 100 mM Tris-HCl 7.0, 200 mM ammonium sulfate, 20% (v/v) PEG3350 and 15% (v/v) glycerol for 4 h. Data set LuAc-1 originated from LuAc soaking experiments and in terms of nucleotide binding we refer to this data set as “unsoaked” or “nucleotide-free”. The ADP-complex was produced by soaking crystals in 20 mM ADP, 100 mM NaCitrate pH 5.7, 200 mM ammonium sulfate, 20% (v/v) PEG3350 and 20% (v/v) glycerol for 15 h. An alternative soaking condition also included 2 mM $MgCl_2$. However, both conditions produced similar results in terms of ligand binding. The ATP and AMPPNP complexes were obtained by transferring crystals for 12 h into solutions consisting of 100 mM Tris-HCl 8.0, 200 mM ammonium sulfate, 20% (v/v) PEG 3350, 20% (v/v) glycerol, 1 mM MgAc and 10 mM ATP or AMPPNP, respectively. Using higher $MgCl_2$ and ATP concentrations (25 mM) in the soaking experiments did not significantly change the results in terms of ligand binding. Crystals obtained from heavy-atom and nucleotide soaking experiments were directly plunge-frozen in liquid nitrogen prior to data collection, whereas the crystals obtained from the LuAc co-crystallization experiments were first quickly soaked in cryo-protectant (100 mM NaCitrate pH 5.7, 200 mM ammonium sulfate, 20% (v/v) PEG3350 and 15-20% (v/v) glycerol) and subsequently cryo-cooled. SAD data on the LuAc and $Na_2WO_4$

derivatives were collected at 100 K on beamline I02 at the Diamond Light source (DLS) at a wavelength of 1.3408 Å or 1.21471 Å, respectively, and data collections on the nucleotide soaked crystals were carried out at the European Synchrotron Radiation Facility (ESRF) or at the DLS. All data sets collected from single or multiple crystals were integrated and scaled using HKL2000[25], XDS[26] or MOSFLM[27] and AIMLESS[28].

**Structure Determination**

The location of heavy-atom positions was determined from anomalous difference fourier electron density maps calculated with FFT[29] with phases obtained by molecular replacement using PHASER[30] employing the recently published low-resolution structure as a search model[7]. The derivative data alongside with the respective heavy-atom positions were fed into the AutoSol wizard of PHENIX[31] using the SAD approach to produce experimentally phased electron density maps. The overall figures of merit after heavy atom phasing for the LuAc-1 and $Na_2WO_4$ derivative were 0.30 or 0.37, respectively. In addition, the LuAc-2 data set was combined with recently obtained W12-cluster low-resolution phases[7]. The quality of electron density maps calculated from the LuAc-2 data set was improved in combination with the other derivative data using multi-crystal-averaging and phase extension in DMMULTI[32], which allowed for manual model building in COOT[33]. This preliminary model was used in a molecular-replacement approach against the data-set obtained from the ADP-soaked crystals using PHASER[30]. The low-resolution data of the latter approach were incorporated in the multi-crystal-averaging procedure described above to obtain an updated model. Further improvements could be achieved by iterative rounds of refinement with REFMAC[34] or CNS[35] against the

ADP-data set and manual rebuilding in COOT[33]. This final model was used as a search template in molecular replacement approaches with PHASER[30] against the data sets obtained from the ATP- and AMPPNP-soaked crystals as well as the LuAc-1 derivative. The obtained models were updated by iterative rounds of refinement in REFMAC[34] and manual rebuilding in COOT[33]. The data and refinement statistics are summarized in table 1. Figures were prepared using PYMOL (W.L. DeLano, 2002, www.pymol.org). Alignments were carried out with CEALIGN[36] and surface area calculation were done with AREAIMOL[29] or SURFACE[29].

**ATP.vanadate-mediated UV cleavage**

10 µl of purified GST-Dyn1-314kD or GST-Dyn1-314kD$_{\Delta 3039\text{-}3291}$ (2 – 6 mg ml$^{-1}$) were diluted into 38 µL of buffer (50 mM Hepes pH 7.5, 100 mM KAc, 5mM MgAc, 1 mM DTT) followed by the addition of 1 µl of ATP-Mg (100 mM) and 1 µl of Na-orthovanadate (100 mM) solution. The Na-orthovanadate was incubated at 95 °C for 10 min prior to usage. The samples were incubated on ice for 30 min then, still being incubated on ice, UV-irradiated for another 60 min and subsequently analyzed by SDS-PAGE.

**Microtubule Binding Assay**

The assay was carried out with a microtubule binding assay kit (Cytoskeleton). Protein and microtubules were mixed and incubated with and without 10 mM ATP. The samples were centrifuged at 265000 g for 10 minutes. The supernatant was decanted and the pellet was suspended in buffer (50 mM Tris-HCl pH 8.0, 150 mM KAc, 2 mM MgAc, 1 mM EGTA, 10% (v/v) glycerol, 1 mM DTT, 10 µM taxol) to an

equal volume as supernatant. Input, supernatant and pellet samples were analyzed by SDS-PAGE.

**Motility- and ATPase assays**

The Motility and ATPase assays were carried out as described in Reck-Perterson *et. al*.[14].

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