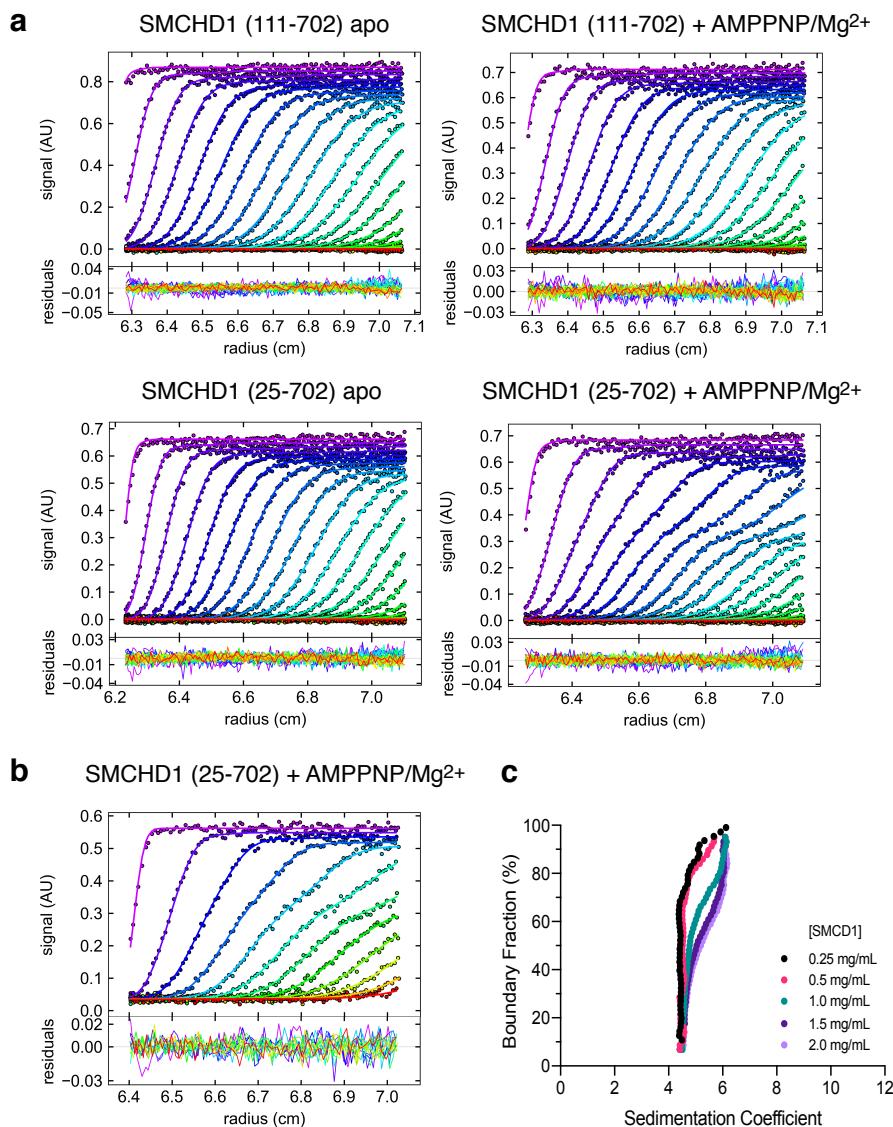


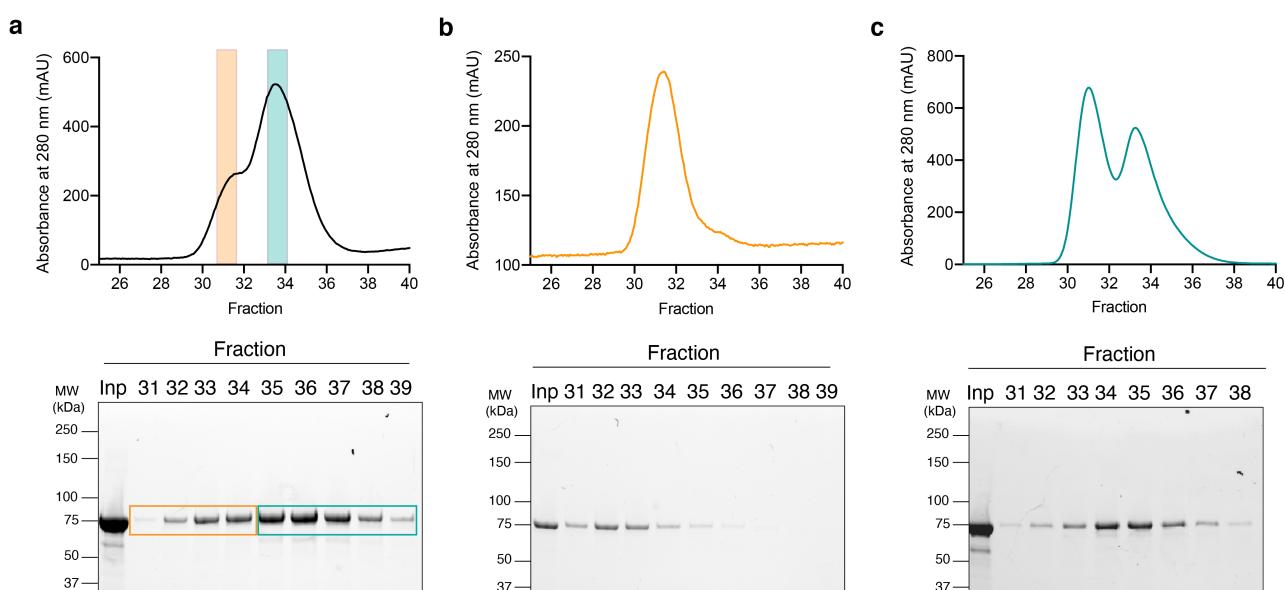
SUPPLEMENTARY FIGURES

Supplementary Figure 1. Supporting sedimentation velocity analytical ultracentrifugation (AUC) data. **a-b.** Raw sedimentation profiles of absorbance at 290 nm versus cell radius for initial sedimentation velocity analyses performed at 1.0 mg/mL protein, for the outlined samples described above each panel (**a**); and for additional sedimentation velocity analyses examining UBL-containing SMCHD1 ATPase (residues 25-702) in the presence of AMPPNP/Mg²⁺ at varying protein concentrations, as exemplified with a representative profile for the 0.5 mg/mL protein concentration sample (**b**). For both **a** and **b**, the bottom panels represent the residuals for the continuous size, c(s), distribution best fits plotted as a function of radial position (cm) from the axis of rotation. Analyses were performed using the program SEDFIT [1]. **c** van-Holde Weischet analysis [2] of UBL-containing SMCHD1 ATPase (residues 25-702) in the presence of AMPPNP/Mg²⁺ at varying protein concentrations.



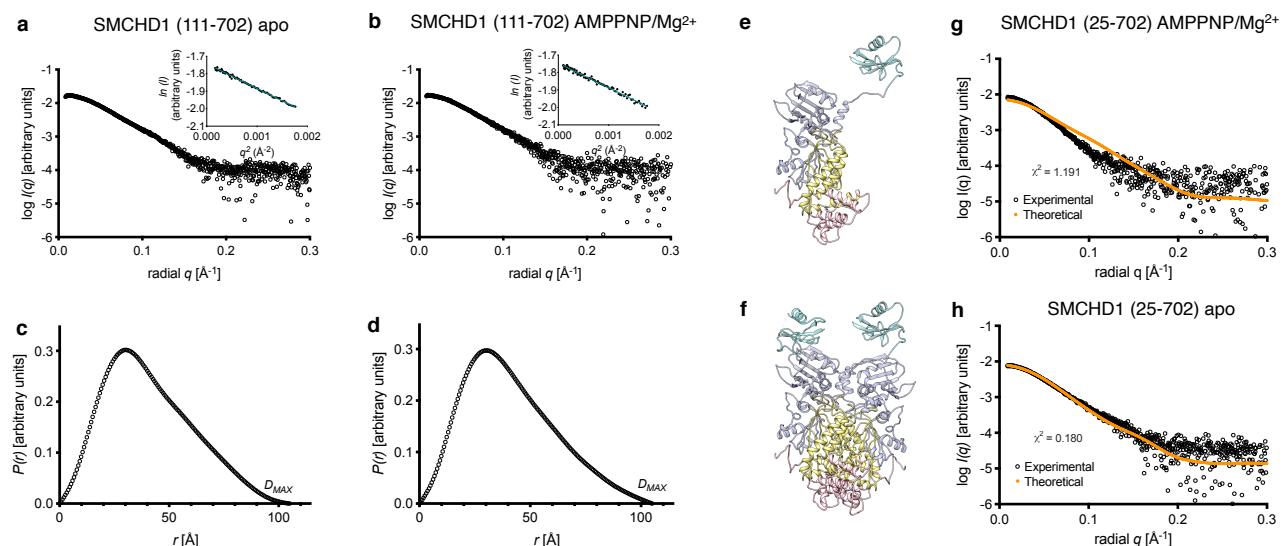
Supplementary Figure 2. Analytical size exclusion chromatography (SEC) of SMCHD1 (residues 25-702) reveals stable dimer formation in solution in the presence of AMPPNP/Mg²⁺.

a-c (Top panels) SEC profiles obtained using a Superdex-200 Increase 10/300 GL column for **a** SMCHD1 (residues 25-702) upon 30 min incubation on ice with 5 mM AMPPNP/Mg²⁺ showing **b** the re-injection of the pooled dimer peak from **a**, and **c** the re-injection of the pooled monomer peak from **a** after incubation with 5 mM AMPPNP/Mg²⁺. The SMCHD1 (residues 25-702) dimer peak is highlighted in orange, and the monomeric peak is depicted in green. **a-c (Bottom panels)** Reducing Stain-Free SDS-PAGE analyses of selected fractions following SEC analyses of the corresponding samples above, and the injected sample (Inp) showing molecular weight marker position on the left, in kilodaltons (kDa).



Supplementary Figure 3. SAXS analyses of the ΔUBL SMCHD1 ATPase (residues 111-702) reveals a stable monomer in solution in the presence or absence of ligand, AMPPNP/Mg²⁺.

a-b SAXS scattering profiles, with the log intensity of scattered X-rays $I(q)$, as a function of momentum transfer, q , in Å⁻¹ for the ΔUBL SMCHD1 ATPase (residues 111-702) under apo conditions (**a**) and in the presence of AMPPNP/Mg²⁺ (**b**). The insets show Guinier plots, where linearity indicates the presence of monodisperse particles. **c-d** The corresponding real space pairwise-distribution functions, $P(r)$, for apo ΔUBL SMCHD1 ATPase (residues 111-702) (**c**) and in the presence of AMPPNP/Mg²⁺ (**d**), obtained via Fourier transformations of the scattering intensity using the software GNOM. The D_{MAX} (maximum dimension) values for each occur where the curve bisects the x-axis. **e-f** Three-dimensional models of the monomeric (**e**) and dimeric (**f**) SMCHD1 ATPase (residues 25-702) were obtained by merging a previously developed model of the ΔUBL SMCHD1 ATPase (residues 111-702) based on the high-resolution structure of the GHKL protein Hsp90 (PDB:1CG9) [3], and the SMCHD1 ATPase (residues 25-580) crystal structure (PDB:6MW7) [4]. Structure cartoons were generated using PyMOL. **(g-h)** SAXS scattering profiles for UBL-containing SMCHD1 ATPase (residues 25-702) in the presence of AMPPNP/Mg²⁺ (**g**) or in the absence of AMPPNP/Mg²⁺ (**h**), displaying the log intensity of scattered X-rays, $I(q)$, as a function of momentum transfer, q , in Å⁻¹. Experimental scatter is shown in black, whereas the theoretical scatter, which was obtained using CRYSTAL from the models in **e** and **f** is shown in orange, with the quality of fit estimated by a chi-squared (χ^2) value.



Supplementary Table 1. Data collection and scattering parameters for SAXS analyses

Data collection parameters				
Instrument	Australian Synchrotron SAXS/WAXS beamline			
Beam geometry	120 μm point source			
Beam wavelength (\AA)	1.033			
q range (\AA^{-1})	0.0114-0.4			
Exposure time (seconds)	1			
Protein concentration	\sim 5 mg/ml sample injected via in-line size exclusion chromatography			
Temperature ($^{\circ}\text{C}$)	8			
Structural parameters				
Protein sample	SMCHD1 (111-702 apo)	SMCHD1 (111-702 AMPPNP/Mg $^{2+}$)	SMCHD1 (25-702 apo)	SMCHD1 (25-702 AMPPNP/ Mg $^{2+}$)
$I(0)(\text{cm}^{-1})$ [from Guinier]	0.01806 \pm 0.00013	0.01791 \pm 0.00022	0.08244 \pm 0.00082	0.08785 \pm 0.00132
Rg (\AA) [from Guinier]	31.2 \pm 0.344	31.1 \pm 0.630	34.6 \pm 0.546	37.6 \pm 0.878
$I(0)(\text{cm}^{-1})$ [from $P(r)$]	0.01825 \pm 0.00009	0.01812 \pm 0.00016	0.00837 \pm 0.00005	0.00894 \pm 0.00011
Rg (\AA) [from $P(r)$]	32.17 \pm 0.206	32.33 \pm 0.383	36.10 \pm 0.244	39.20 \pm 0.515
D_{\max} (\AA) [from $P(r)$]	105	105	115	125
Rg (\AA) [from CRYSTOL]	29.3	29.4	34.4	36.3
D_{\max} (\AA) [from CRYSTOL]	103	103	117	128
MW (kDa) [from SAXSMoW]	68.4 (68 kDa expected)	70.7 (68 kDa expected)	95.7 (78 kDa expected)	139.7 (156 kDa expected)
Software				
Primary data reduction	ScatterBrain (Australia Synchrotron)			
Data processing	PRIMUS [5], GNOM [6], CRYSTOL [7], SAXSMoW [8]			

SUPPLEMENTARY REFERENCES

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