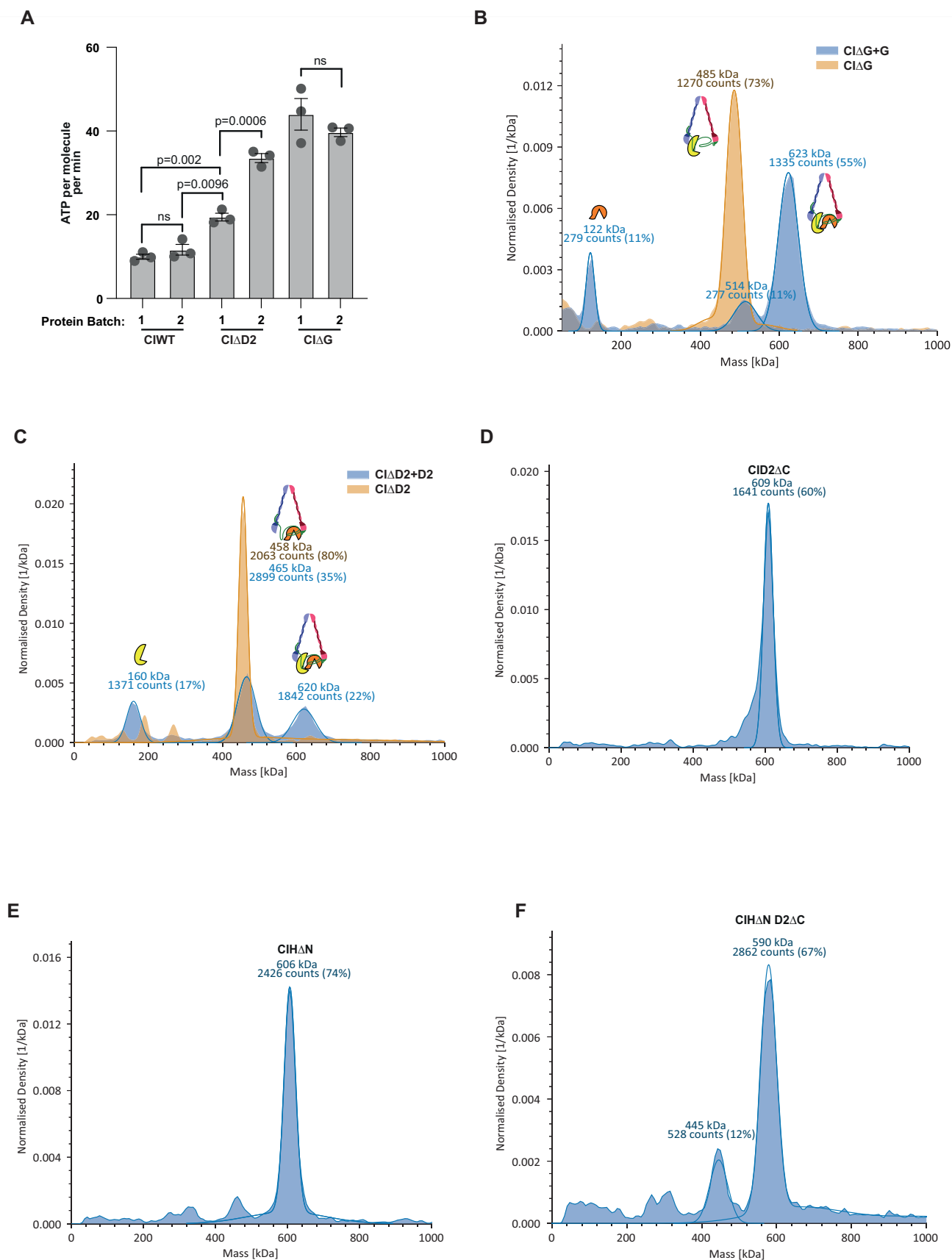


Expanded View Figures

Figure EV1. Condensin I mutant complex quality control.

(A) Comparison of two different batches of recombinant CIWT pentamer, and CIΔG and CIΔD2 tetramers. Trend of tetrameric complexes being significantly more active than pentameric condensin I is consistent. Data from three technical replicates. Error bars represent s.e.m. *P* values indicated are from unpaired, two-tailed *t* test with Welch's correction. (B) Mass photometry data of condensin I tetramers, CIΔG, with and without NCAPG being added to reconstitute the pentamer. (C) Mass photometry data of condensin I tetramers, CIΔD2, with and without NCAPD2 being added to reconstitute the pentamer. (D-F) Mass photometry data of condensin I pentamers, CID2ΔC, CIHΔN and CIHΔN,D2ΔC, respectively, consistent with pentameric mass and confirming they are the major species present. Source data are available online for this figure.



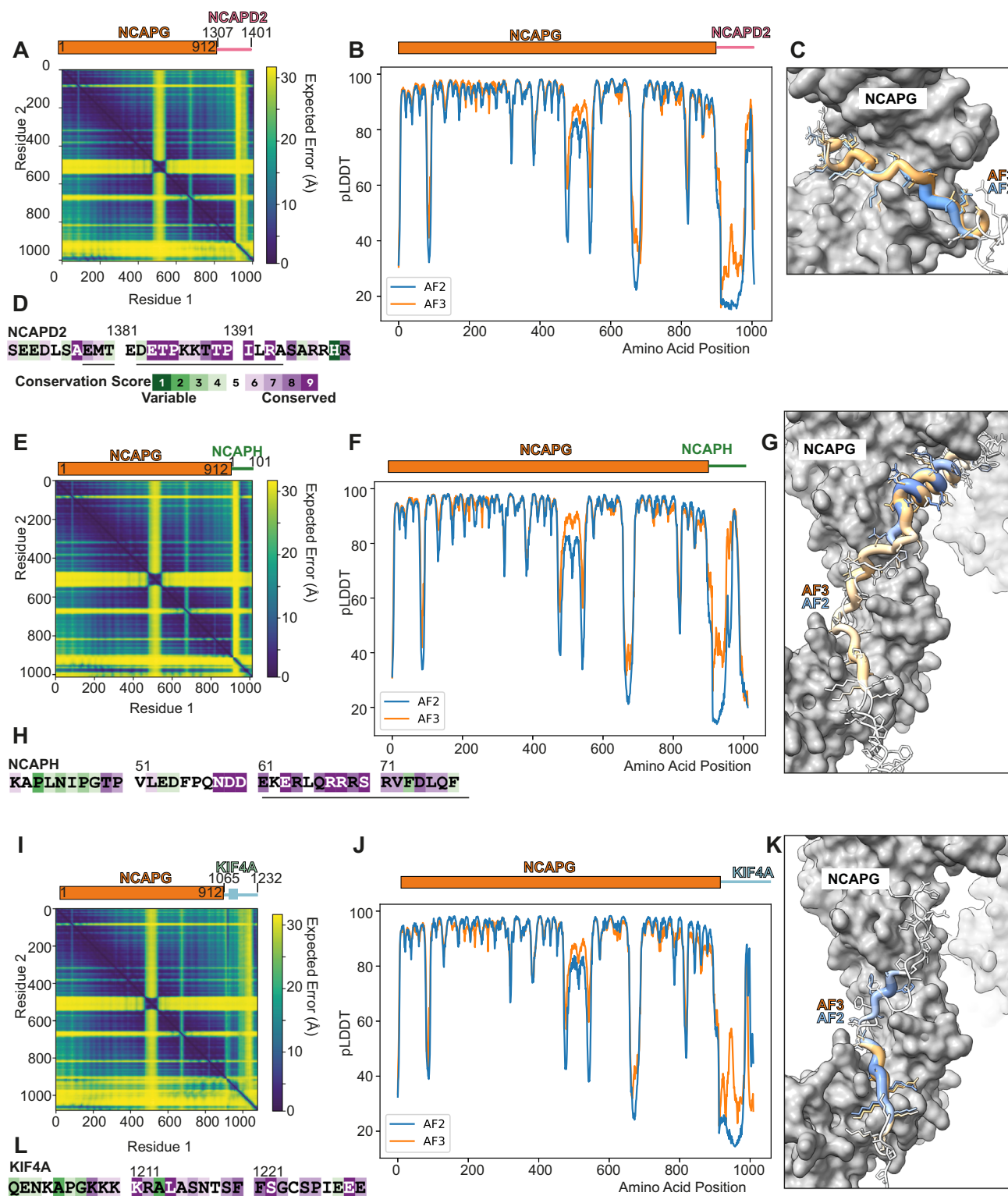


Figure EV2. NCAPG SLiM interaction AlphaFold2 and 3 confidence.

(A) AlphaFold2 predicted alignment error plot for interaction between NCAPG and NCAPD2₁₃₀₇₋₁₄₀₁. (B) Predicted local distance difference test (pLDDT) score per residue of AlphaFold2 and 3 (AF2 and AF3, respectively) predictions of NCAPG and NCAPD2₁₃₀₇₋₁₄₀₁. (C) Comparison of AF2 (blue) and AF3 (orange) predictions of NCAPG and NCAPD2₁₃₀₇₋₁₄₀₁. AlphaFold pLDDT score is represented in thickness of NCAPD2 backbone and colour intensity. (D) SLiM region with conservation colouring from ConSurf (Ashkenazy et al, 2016). (E-H) Equivalent data to (A-D) for prediction of NCAPG with NCAPH₁₋₁₀₁. (I) AlphaFold2 predicted alignment error plot for interaction between NCAPG and KIF4A₁₀₆₅₋₁₄₀₁. (J-L) Equivalent data to (B-D) for prediction of NCAPG with KIF4A₁₁₃₆₋₁₂₃₂.

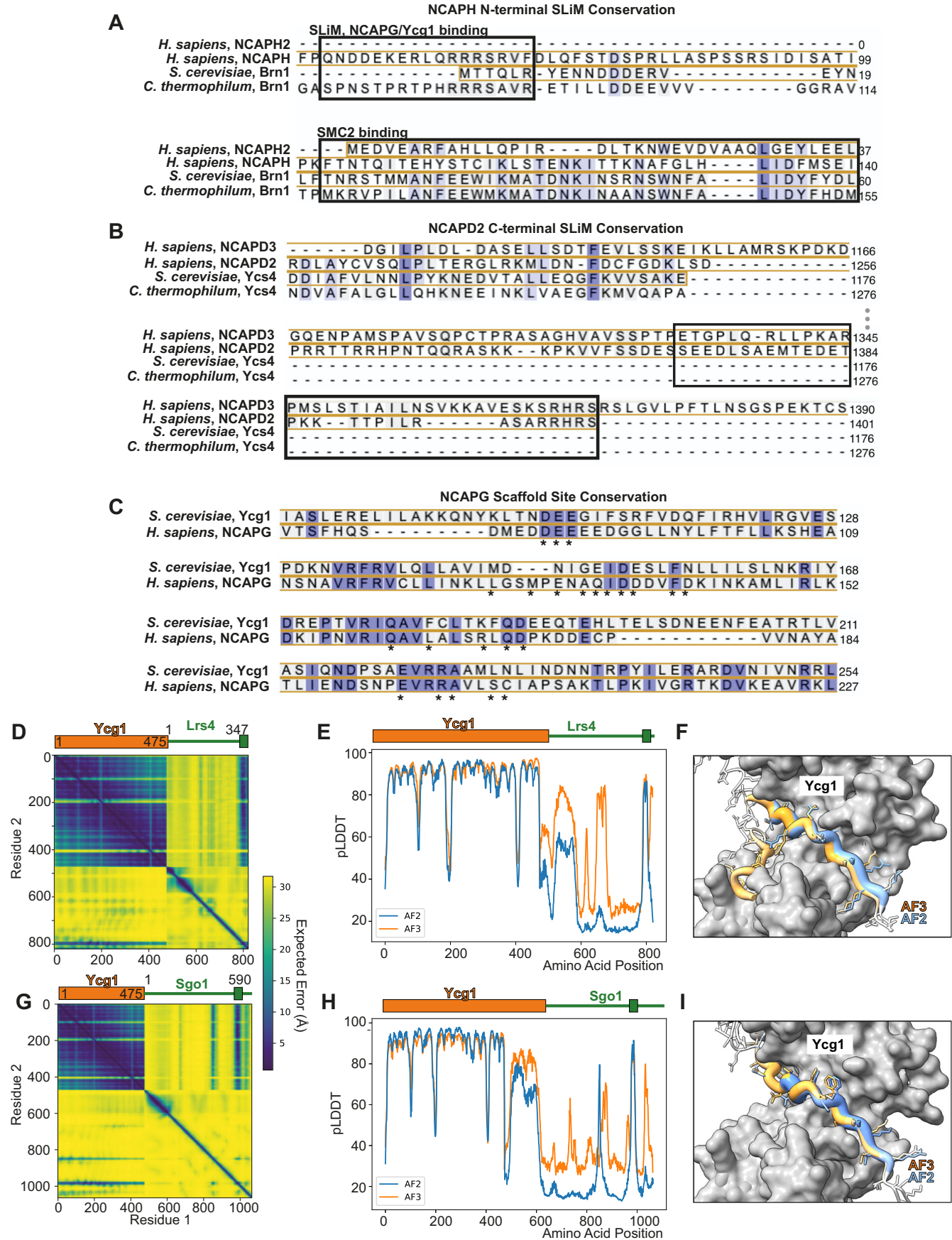
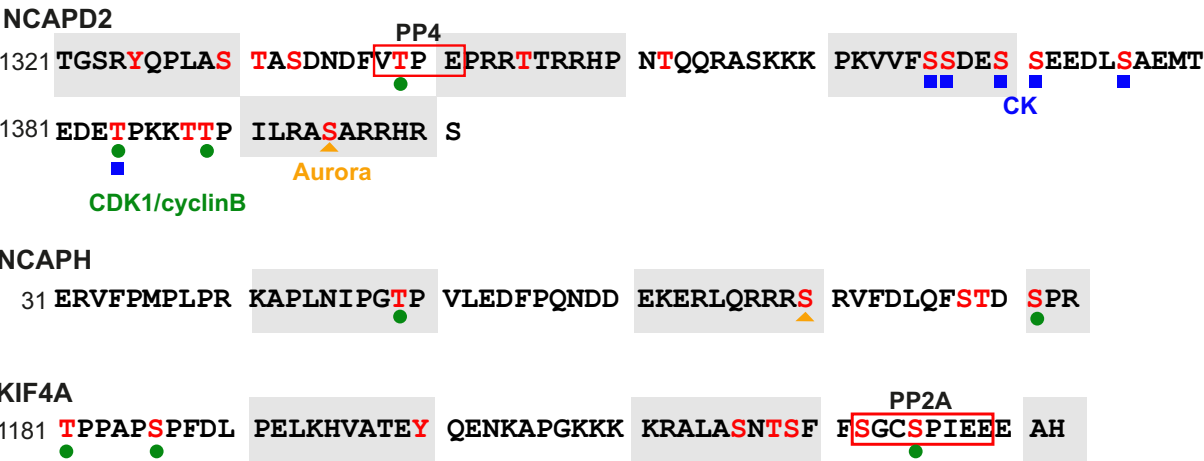


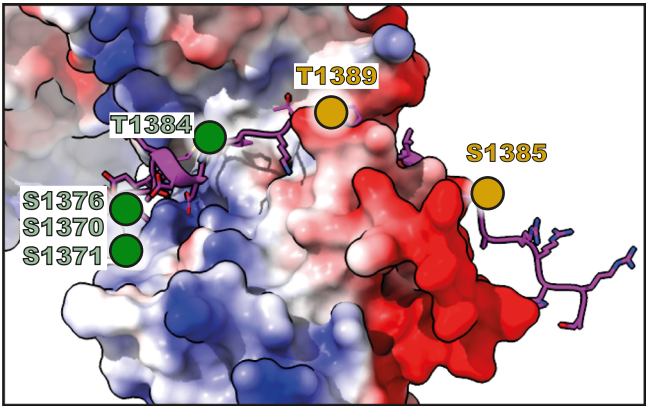

Figure EV3. Condensin disordered sequence conservation.

(A) Alignment of N-terminal region of NCAPH/H2 and Brn1, in *H. sapiens*, *S. cerevisiae* and *C. thermophilum*, with NCAPG/Ycg1 and SMC2 binding regions indicated. (B) Alignment of C-terminal region of NCAPD2/3 and Ycs4 in *H. sapiens*, *S. cerevisiae* and *C. thermophilum*. (C) Alignment of SLiM docking region in NCAPG and Ycg1, from *H. sapiens* and *S. cerevisiae*, (*) indicates residues within 4 Å of predicted bound SLiM. (D) AlphaFold2 predicted alignment error of Ycg1₁₋₄₇₅ and Lrs4. (E) Predicted local distance difference test (pLDDT) score per residue of AlphaFold2 and 3 (AF2 and AF3, respectively) predictions of Ycg1₁₋₄₇₅ and Lrs4. (F) Comparison of AF2 (blue) and AF3 (orange) predictions of Ycg1₁₋₄₇₅ and Lrs4. AlphaFold pLDDT score is represented in thickness of Lrs4 backbone and colour intensity. (G-I) Equivalent data as (D-F) for Ycg1₁₋₄₇₅ and Sgo1. Sequence data sourced from Uniprot (Bateman et al, 2023).

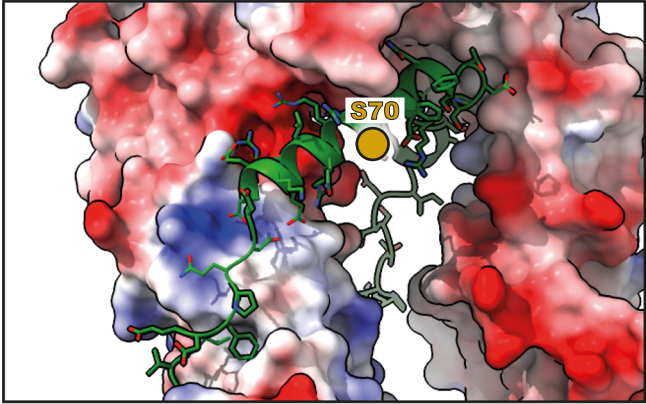
A



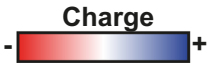
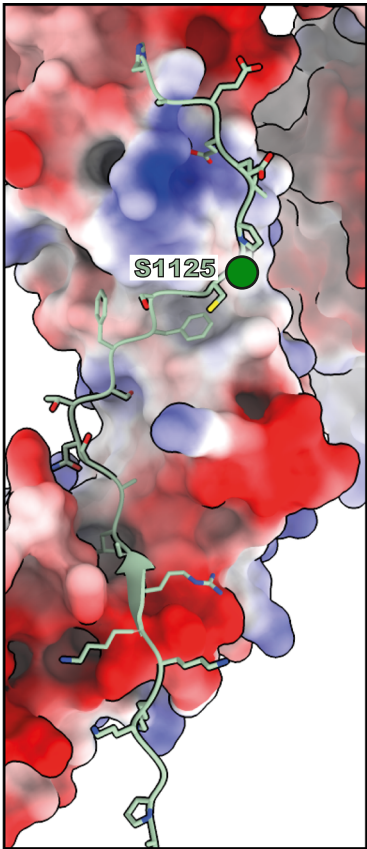
B NCAPD2



C NCAPH



D KIF4A



**Figure EV4. Phosphorylation and phosphatase sites in SLiMs.**

(A) Theoretical sites of phosphorylation by Aurora (orange triangle), CDK1/cyclin B (green circle) and casein (blue square) kinases in SLiM of NCAPD2, NCAPH and KIF4A. Red rectangles indicate phosphatase binding sites. (B–D) The location of phosphorylation sites indicated in (A) in NCAPD2, NCAPH and KIF4A, respectively. Based off of electrostatic surface charge, the effect of the phosphorylation is coloured either green, for potentially enhancing the interaction or orange, for resulting in a potential charge clash.