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Supplementary Information for

Roles of singleton tryptophan motifs in COPI coat stability and vesicle tethering

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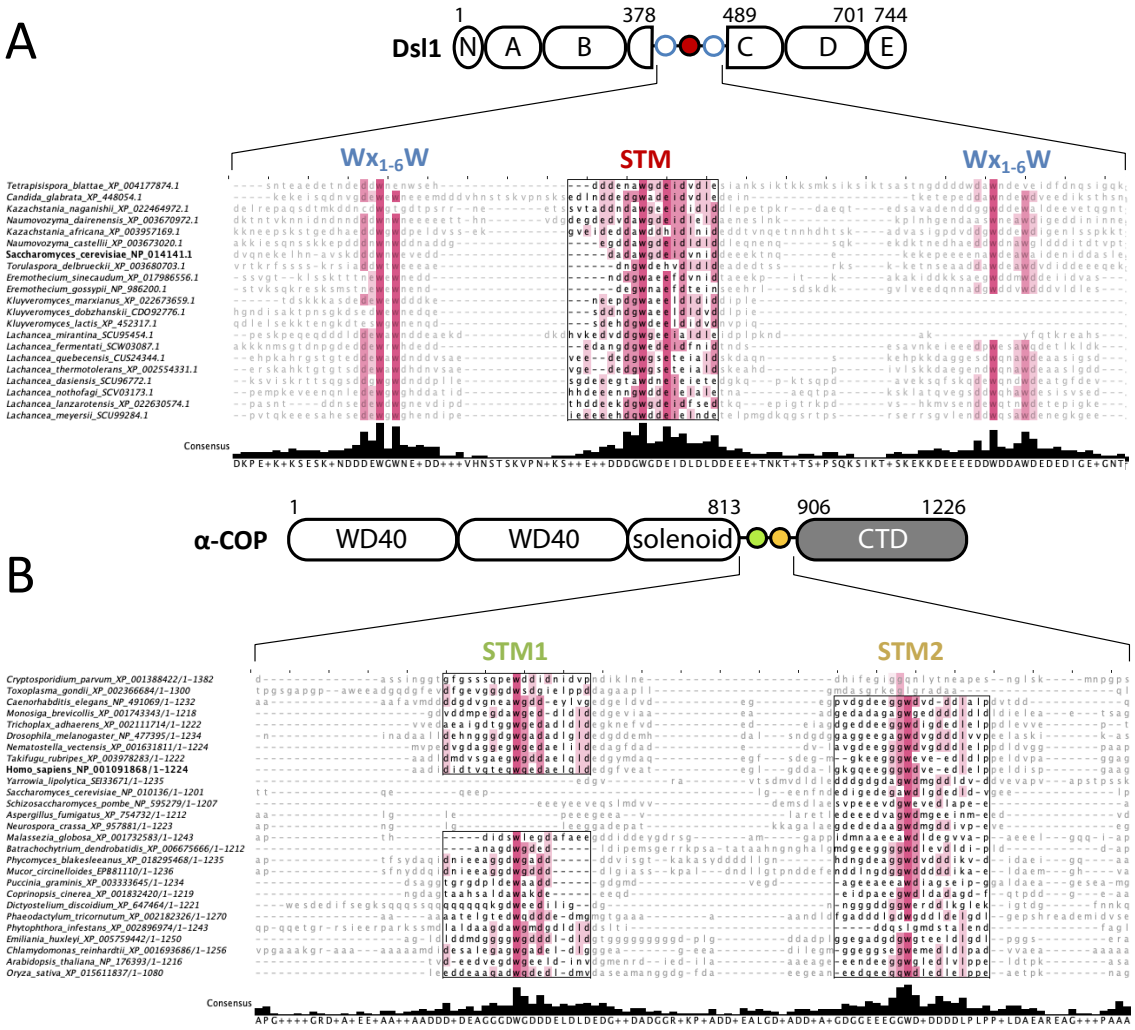


Fig. S1. Dsl1 and α-COP contain STM sequences within a low complexity acidic loop

(A) Alignment of *Ascomycota* Dsl1 lasso sequences shows moderate conservation (where a purple background indicates degree of conservation) of tryptophan residues surrounded by low complexity polyacidic sequence. Two distinct patterns of tryptophan motifs emerge. $Wx_{1\text{-}6}W$ motifs (blue) flank a singleton tryptophan-containing sequence (STM; boxed, red).

(B) Alignment of α-COP Dsl1-like sequences shows absolute conservation of at least one tryptophan in the low complexity acidic loop found between the solenoid and α_{CTD}. Most species have two copies of this motif (boxed). The C-terminal STM2 motif (yellow) has higher sequence conservation than the N-terminal STM1 motif (green).

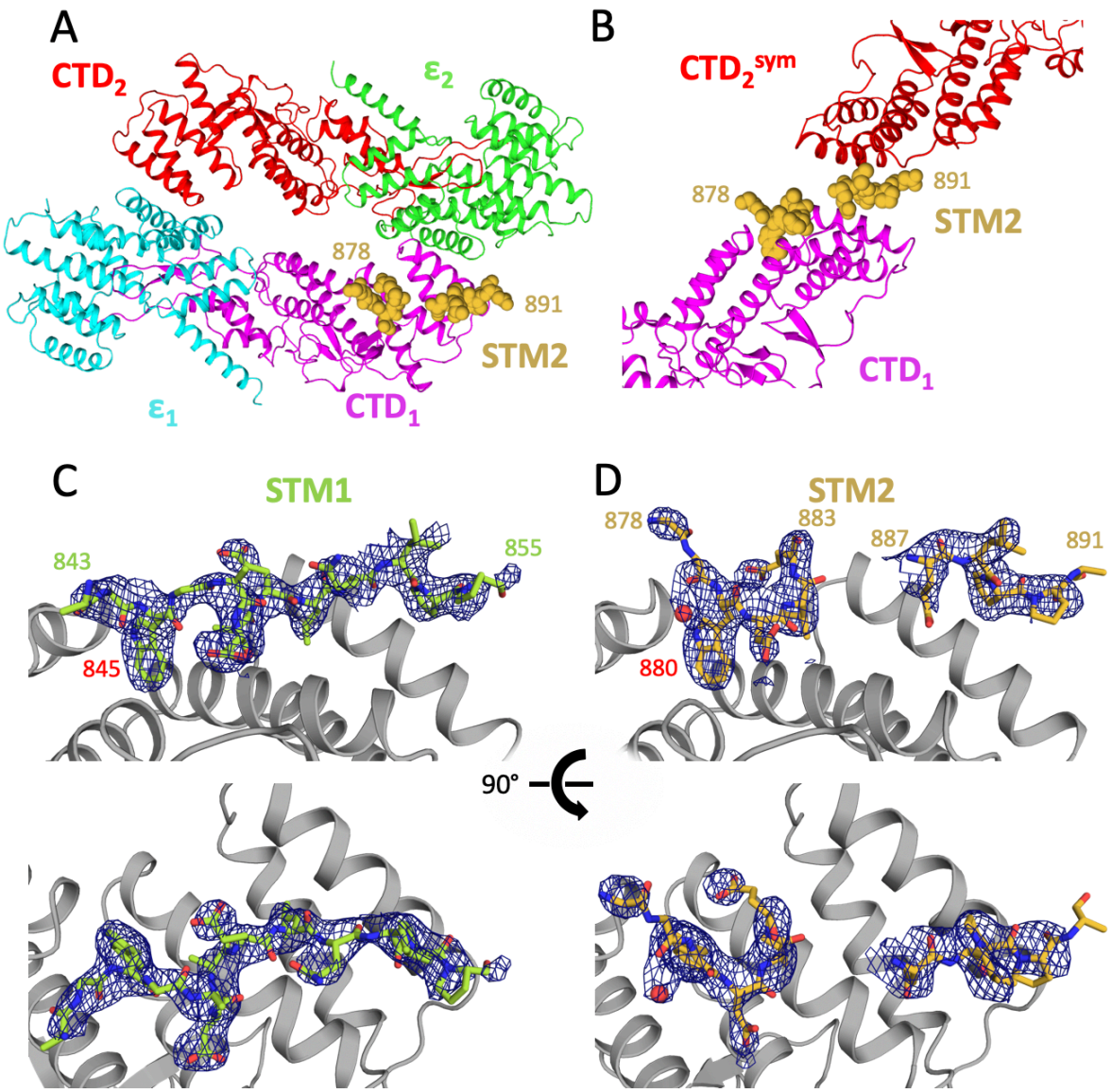


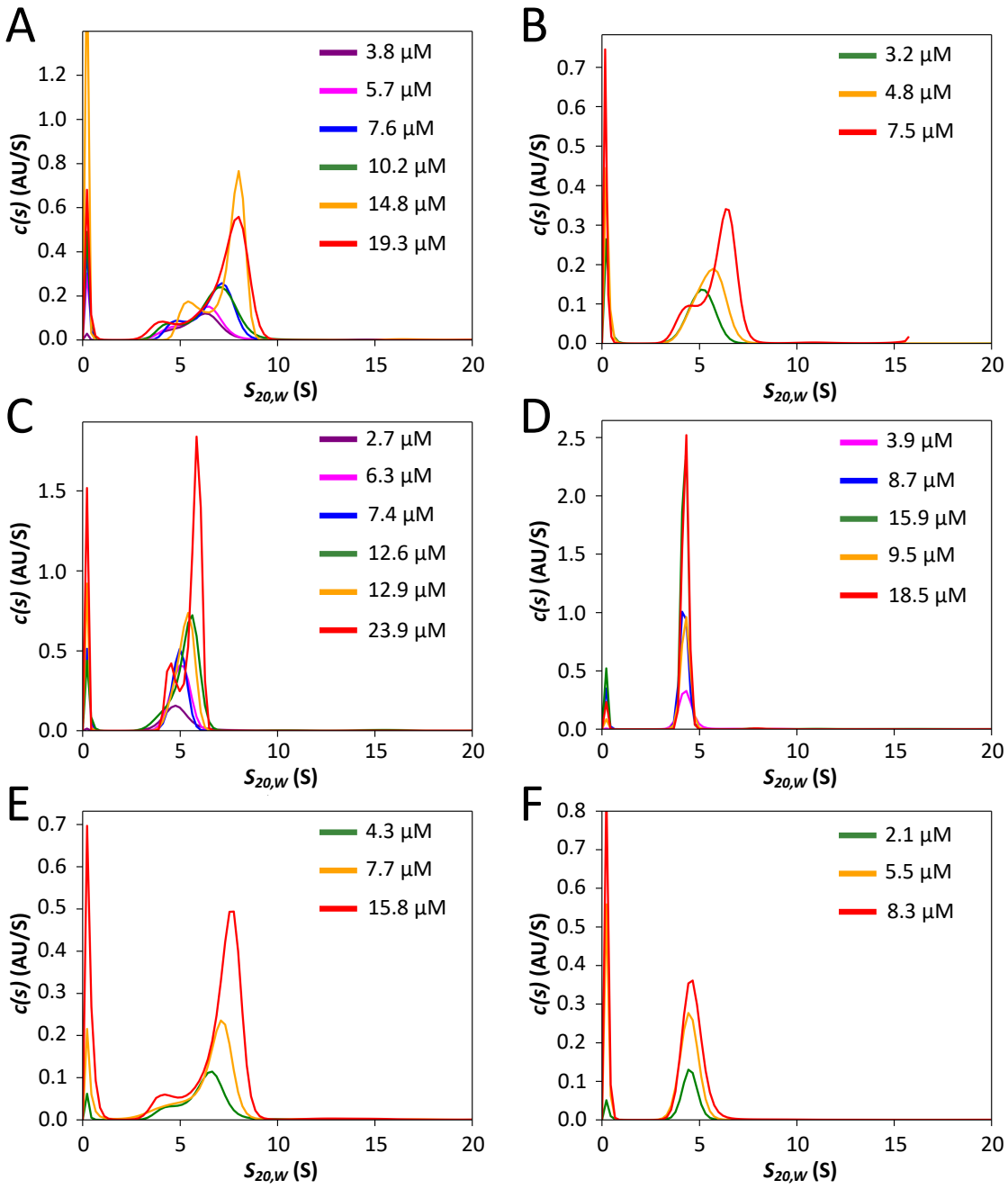
Fig. S2. Crystal packing and electron density

(A) The asymmetric unit of $\alpha_{STM2\text{-}CTD}$•ε crystals contains two non-equivalent copies of α_{CTD}•ε. Only CTD1 (magenta) is bound to STM2 (yellow).

(B) The C-terminal portion of STM2 (yellow) bound to CTD1 (magenta) interacts at a crystal contact with a symmetry-related copy of CTD2 (red).

(C) In the $\alpha_{STM1\text{-}CTD}$•ε structure, 12 residues of STM1 (green) could be visualized in the composite omit 2Fo-Fc map (blue mesh, contoured at 1.0 σ). The N-terminal portion of STM1, containing the singleton tryptophan, is better ordered than the C-terminal portion.

(D) In the $\alpha_{STM2\text{-}CTD}$•ε structure, two discontinuous fragments of STM2 (yellow), comprising nine residues in total, could be visualized in the composite omit 2Fo-Fc map (blue mesh, contoured at 1.0 σ). The C-terminal portion of STM2 is only partially ordered.



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Stoichiometry	s-value (S)
α•ε monomer	4.3
α•ε dimer	6.8
α•ε trimer	8.6
α•ε tetramer	10.7

Fig. S3. STM-dependent α-COP homo-oligomerization

(A) By sedimentation velocity analytical ultracentrifugation, the apparent sedimentation of human $\alpha_{STM1\text{-}STM2\text{-}CTD}$•ε varied depending on sample concentration (inset, μM concentrations). At the highest concentrations, the peak approached an asymptotic value of 8 S, close to that expected for a trimer.

(B) Mutation of STM1 (W845A) resulted in complex that formed homo-oligomers over low micromolar concentrations. At all concentrations tested, the mutant complex sedimented more slowly than wild-type.

(C) Mutation of STM2 (W880A) resulted in a complex that formed homo-oligomers at mid-micromolar concentrations. However, the apparent sedimentation of the largest species formed was closer to that expected for a dimer than a trimer.

(D) Mutation to alanine of both STM tryptophans abolished homo-oligomerization at all concentrations tested.

(E) The apparent sedimentation of yeast $\alpha_{STM\text{-}CTD}$•ε varied with concentration, converging on a value of 8 S at higher concentrations, consistent with a trimer.

(F) Mutation of the STM tryptophan to alanine (W870A) abolished homo-oligomerization at all concentrations.

(G) Theoretical sedimentation coefficients for different human α•ε stoichiometries.

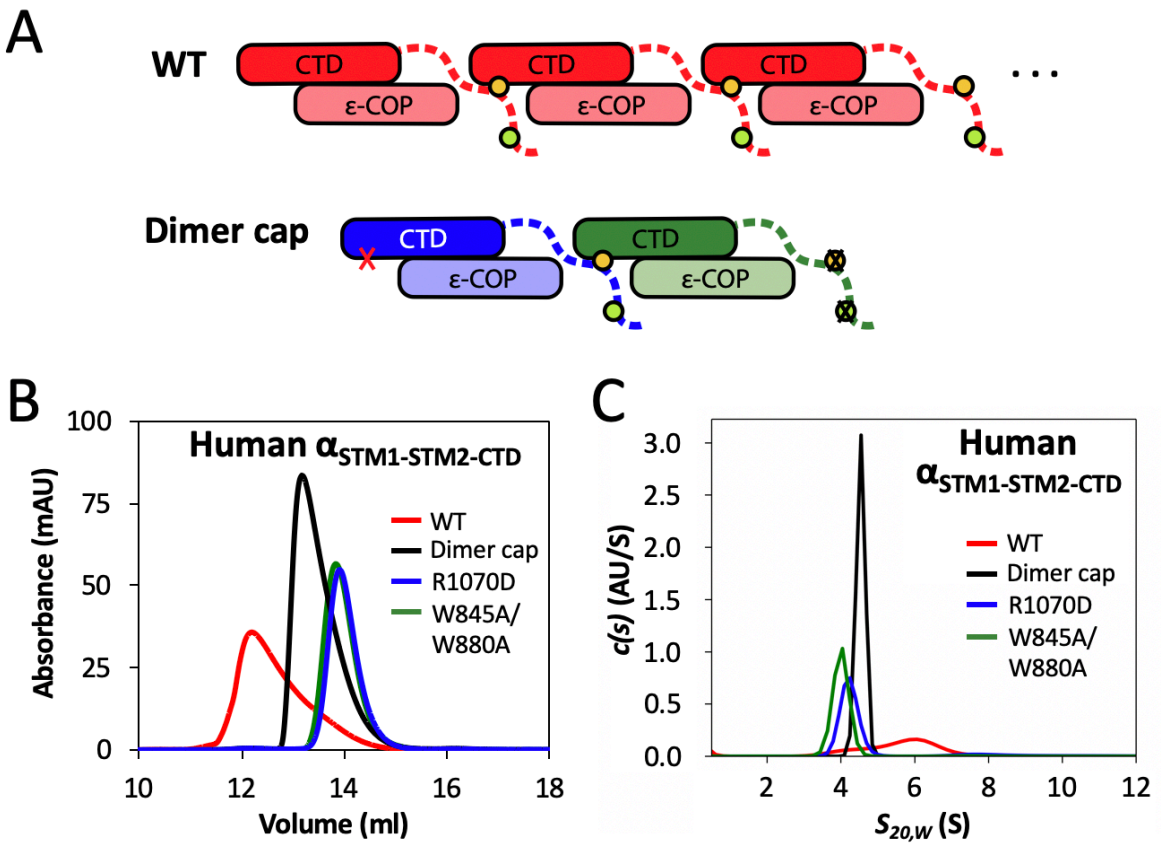


Fig. S4. Dimer capping experiment

(A) Experimental set-up. Wild-type human αSTM1-STM2-CTD•ε can form open-chain oligomers of arbitrary length. Although W845A/W880A and R1070D are both monomeric in isolation, a stoichiometric mixture of the two mutants can result in assembly, capped at a dimer.

(B) Size exclusion chromatography was used to analyze the dimer-capped mixture, which ran intermediate between the wild-type protein and either mutant alone.

(C) By sedimentation velocity analytical ultracentrifugation, the dimer-capped mixture sedimented more rapidly than either mutant alone, but significantly more slowly than the wild-type protein.

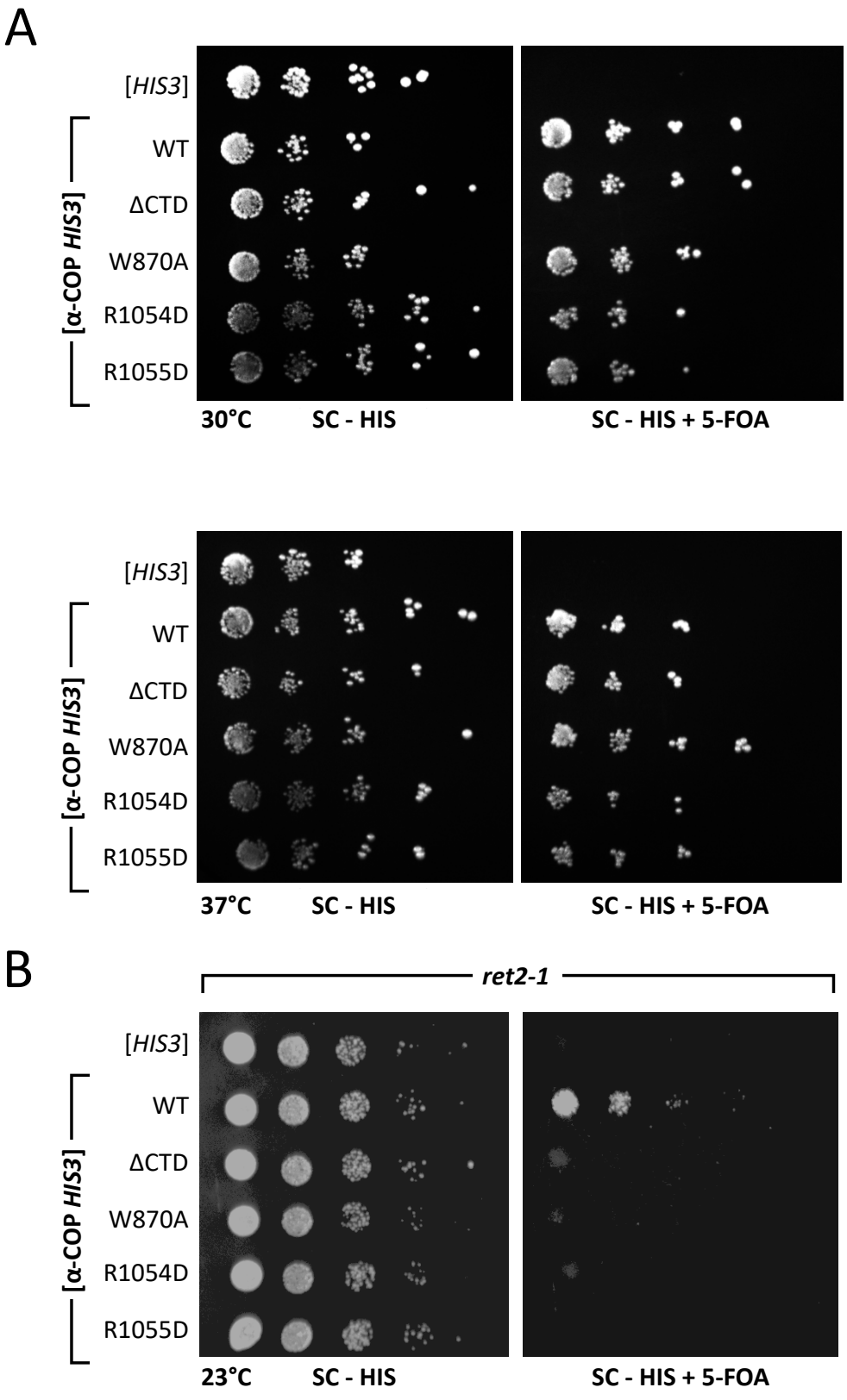
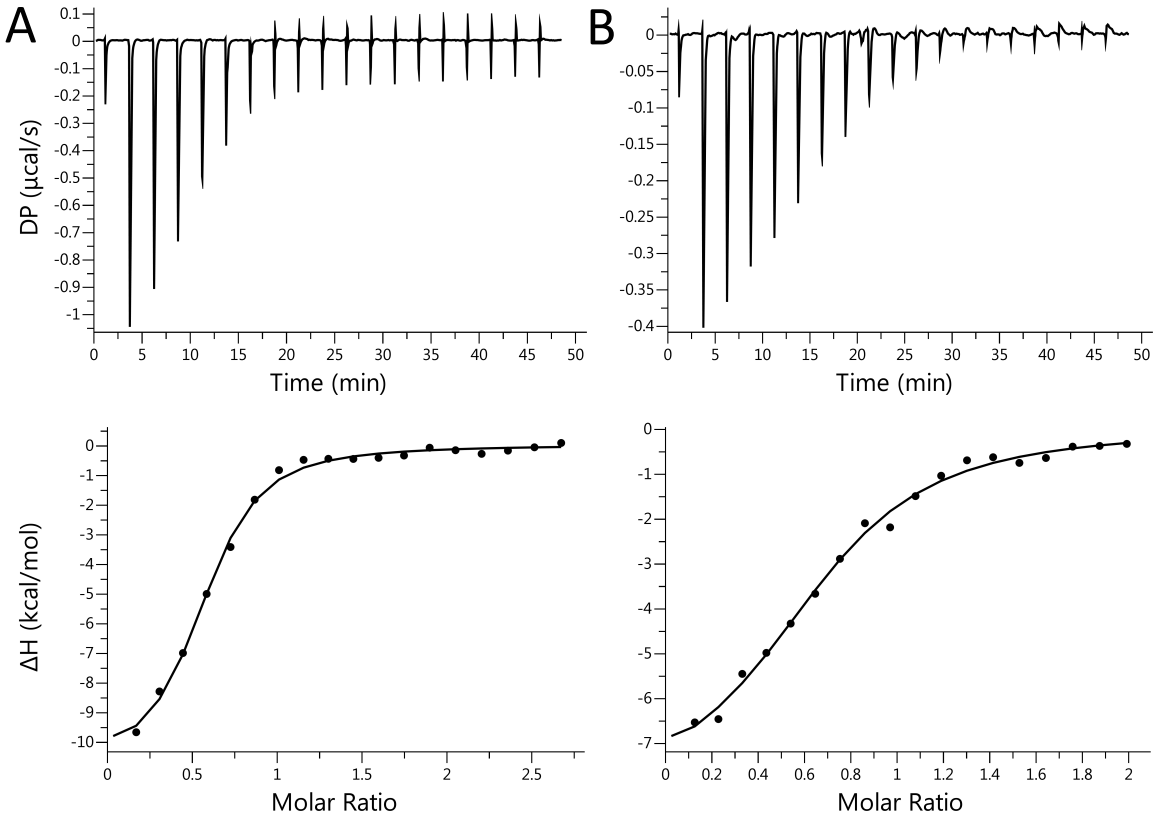

A
[HIS3]
WT
ΔCTD
W870A
R1054D
R1055D
[α-COP HIS3]
30°C
SC - HIS
SC - HIS + 5-FOA
37°C
SC - HIS
SC - HIS + 5-FOA
B
ret2-1
23°C
SC - HIS
SC - HIS + 5-FOA

Fig. S5. Eliminating α-COP•α-COP binding does not lead to temperature sensitivity

(A) In otherwise wild-type yeast, α-COP mutants that abolish the interaction between STM and CTD displayed normal viability at both 30°C and 37°C.

(B) In a *ret2-1* background, α-COP mutants that abolish the interaction between STM and CTD were lethal at 23°C.



Cell	Syringe	N	K_D (µM)	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
α_{CTD}•ε (32 µM)	*MBP-Dsl1*$_{410-440}$ (490 µM)	0.54 ± 0.01[a]	1.8 ± 0.3	-10.9 ± 0.4	-7.8	3.1
α_{CTD}•ε (31 µM)	*MBP-α-COP*$_{855-885}$ (330 µM)	0.66 ± 0.02	4.2 ± 1.0	-8.3 ± 0.7	-7.3	1.0

[a] Fitting parameter errors

Fig. S6. Isothermal titration calorimetry

(A) Titration of yeast MBP-Dsl1$_{410-440}$ into yeast α_{CTD}•ε.

(B) Titration of yeast MBP-α-COP$_{855-885}$ into yeast α_{CTD}•ε.

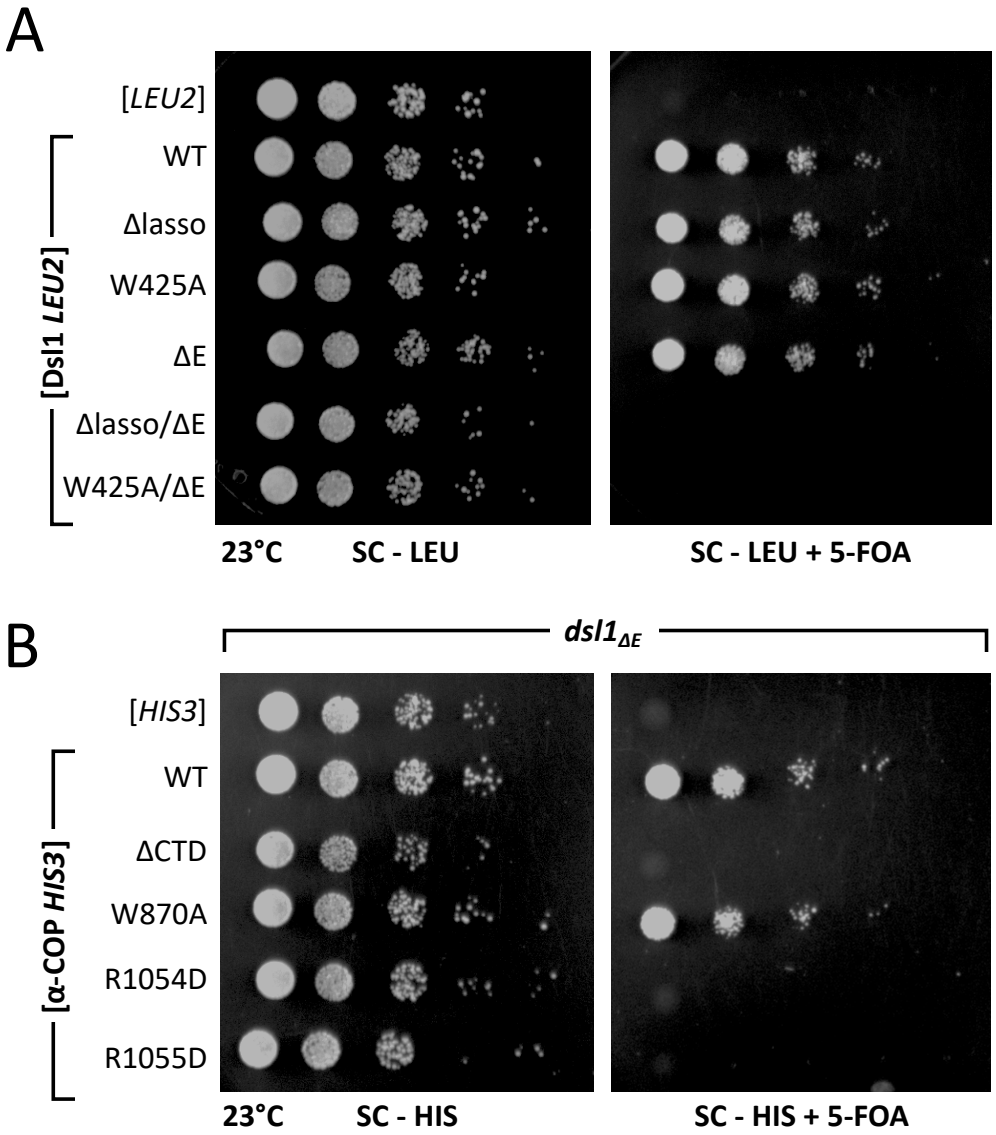


Fig. S7. Eliminating lasso•α-COP binding does not lead to temperature sensitivity

(A) The panel of Dsl1 alleles described in Fig. 6A was assayed for viability at 23°C.

(B) The panel of α-COP mutants described in Fig. 6B, in the presence of the Dsl1$_{\Delta E}$ allele, were assayed for viability at 23°C.

Table S1. Summary of crystallographic parameters

	S. cerevisiae α_{CTD}•ε	*Homo sapiens* $\alpha_{STM1\text{-}CTD}$•ε	*Homo sapiens* $\alpha_{STM2\text{-}CTD}$•ε
PDB accession	6U3W	6U3V	6TZT
Data collection			
Beamline	CHESS F1	NSLS-II FMX	NSLS-II AMX
Wavelength (Å)	0.97820	0.97933	0.92009
Space group	$P2_12_12_1$	$P3_121$	$P3_121$
Cell dimensions			
a, b, c (Å)	72.26, 84.59, 117.84	138.10, 138.10, 192.94	138.47, 138.47, 192.87
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 120.0	90.0, 90.0, 120.0
Resolution (Å)	29.46-2.39 (2.45-2.39)	29.48-2.96 (3.01-2.96)	29.62-3.07 (3.12-3.07)
Total reflections	175339	454381	413421
Unique reflections	29047	44992	40625
Completeness (%)	99.3 (95.0)	99.7 (98.0)	99.7 (98.5)
Multiplicity	6.4 (5.5)	10.1 (10.1)	10.2 (9.8)
R_{merge} (%)	10.4 (67.3)	11.4 (91.8)	8.9 (76.7)
R_{meas} (%)	11.4 (76.7)	12.0 (96.7)	9.4 (80.9)
$\langle I/\sigma_I \rangle$	15.2 (1.6)	16.6 (2.4)	20.7 (2.9)
$CC_{1/2}$	0.932 (0.724)	0.999 (0.787)	0.999 (0.841)
Refinement			
Resolution (Å)	29.46-2.39	29.5-2.96	29.6-3.07
No. reflections	28927	44987	40615
R_{work}/R_{free} (%)	0.178/0.238	0.176/0.229	0.178/0.223
No. atoms	4915	9801	9801
Protein	4727	9801	9800
Water	182	0	1
Average B-factor ($Å^2$)	45.0	71.2	74.5
RMSD			
Bond lengths (Å)	0.007	0.010	0.010
Bond angles (°)	0.877	1.39	1.38
Clashscore	4.44	4.73	5.09
Ramachandran			
Favored (%)	96.8	94.9	95.0
Allowed (%)	3.1	5.0	4.7
Outliers (%)	0.2	0.1	0.3

Table S2. Distance constraints based on (20) and this work

Model	Connector	N (aa)	Distance (Å)	Extension (Å/aa)
Linkage Ia	Solenoid to STM2	58	111±4[a]	1.9±0.1
	STM2 to CTD	15	17±4	1.1±0.3
Linkage Ib	Solenoid to STM2	58	59±8	1.0±0.1
	STM2 to CTD	15	17±4	1.1±0.3
Linkage IVa	Central solenoid to STM2	58	127	2.2
	STM2 to central CTD	15	22	1.4
Linkage IVb	Central solenoid to STM2	58	67	0.9
	STM2 to central CTD	15	22	1.4
Linkage IVc	Central solenoid to STM1	29	>35[b]	>1.2
	STM1 to STM2	22	36	1.6
	STM2 to central CTD	15	22	1.4
Linkage IV (20)	Peripheral solenoid to CTD	77	204	2.6
Linkage IV (this work)	Peripheral solenoid[c] to CTD	77	85	1.1
Crystal structure	STM2 to CTD	15	21	1.5
Triad	β-COP: solenoid to appendage	103	100	1.0
Triad	γ-COP: solenoid to appendage	57	45	0.8

[a] Since Linkage I possesses only approximate three-fold symmetry (20), the average and standard deviation of distances were computed across the three α-COP chains.
[b] The shortest through-space distance, 35 Å, passes through a domain
[c] The solenoid modeled originates from a different, closer COPI heteroheptamer in the peripheral triad than that modeled in (20).