Supporting Information

Lin et al. 10.1073/pnas.1115025109

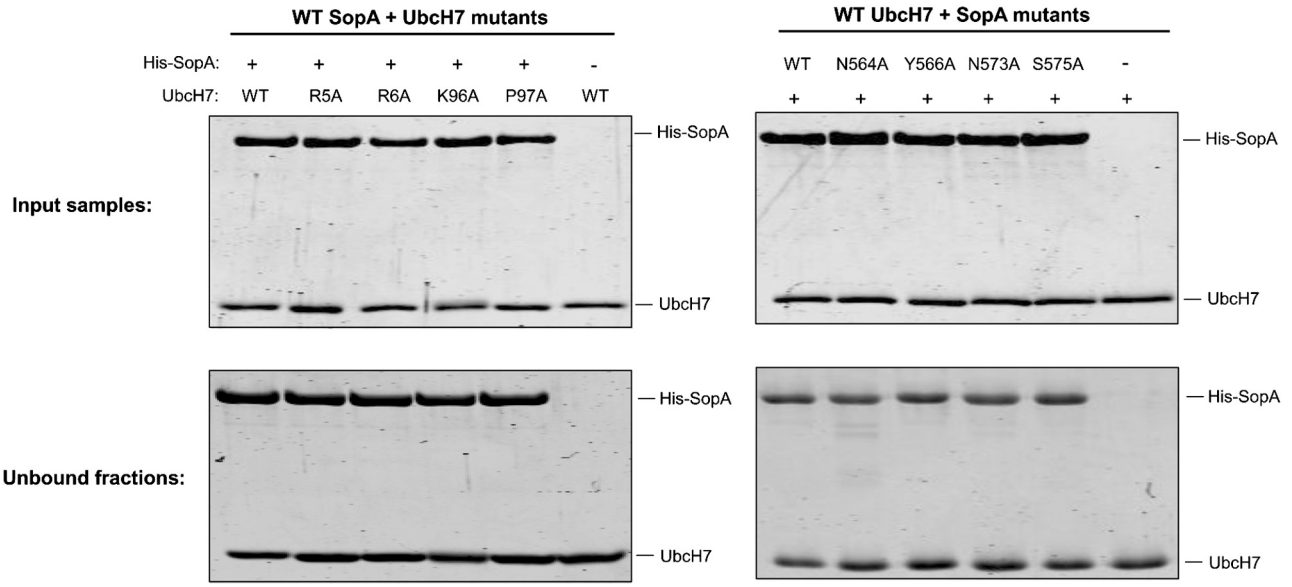
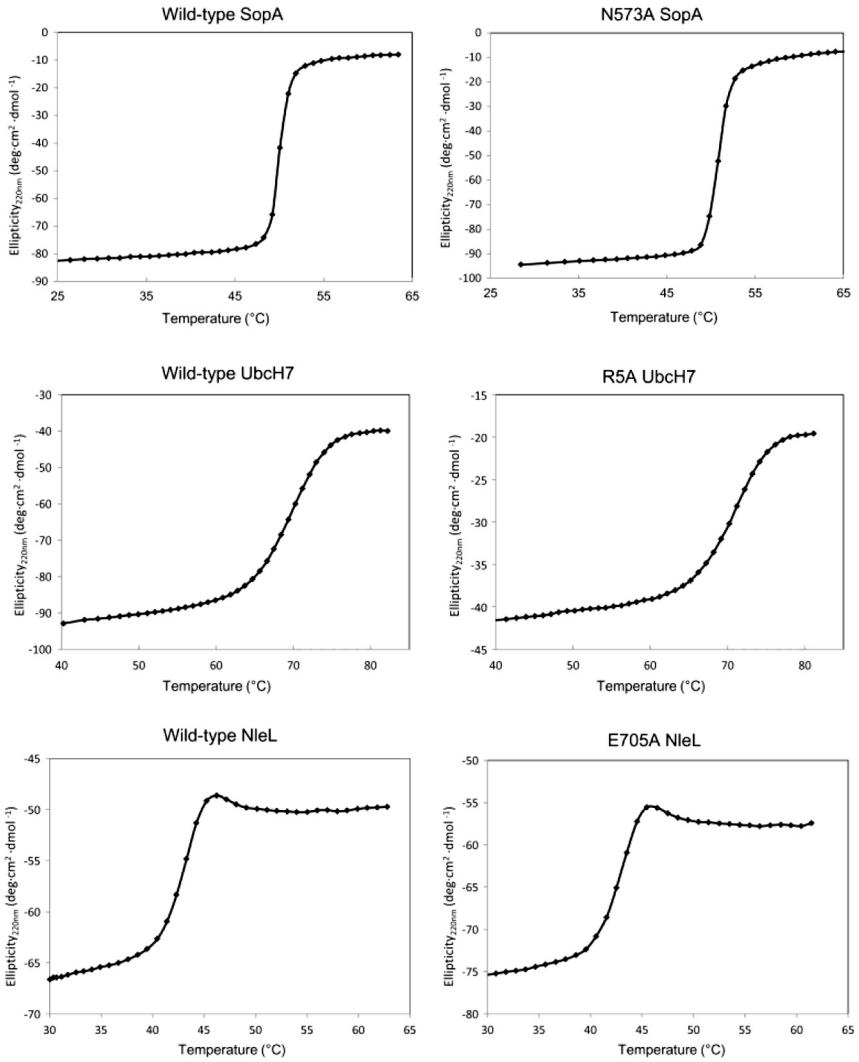


Fig. S1. SopA/UbcH7 pulldown experiment. His-SopA was incubated with UbcH7 in a 1:1 molar ratio for 30 min at 4 °C. Co^{2+} beads were then added to the mixture and incubated for 10 min before centrifugation. After extensive washing, proteins bound to the beads were analyzed on SDS-PAGE with Coomassie blue staining and shown in Fig. 2*B* in the main text. The input samples and unbound fractions are shown here per request of a reviewer.



SopA	T_m (°C)
Wild-type	50
N564A	52
Y566A	51
N573A	52
S575A	51

UbcH7	T_m (°C)
Wild-type	69
R5A	70
R6A	65
K96A	66
P97A	67

NleL	T_m (°C)
Wild-type	43
E705H	42
E705Q	42
E705A	42
F569A	41

Fig. S2. Thermal stability measurements. The thermal denaturation curves of the wild-type and a representative mutant are shown, together with the determined melting temperatures (T_m) of all protein samples used in this work. Circular dichroism (CD) measurements were conducted using a Chirascan™ CD Spectrometer (Applied Photophysics). Thermal denaturation curves were obtained by monitoring the CD signal at 220 nm with increasing temperature at a rate of 1 °C/ min. Melting temperatures (T_m) were calculated from two melting curve baselines based on a two-state folding model. The thermal denaturation of UbcH7 was reversible; SopA and NleL were both irreversible.

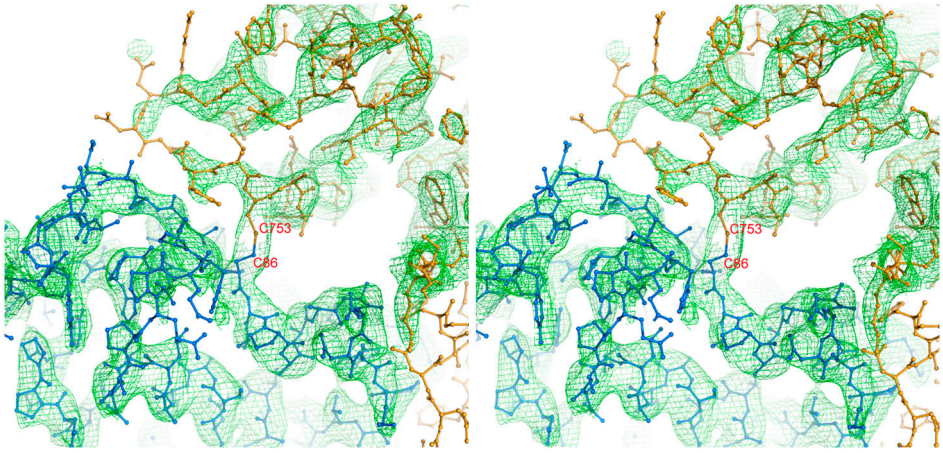


Fig. S3. Stereo view of the interface between UbcH7 (blue) and NleL C-lobe (yellow). Atoms with poor electron density as shown in the $2F_o - F_c$ map (green mesh) were removed in the final model (PDB code: 3SQV).

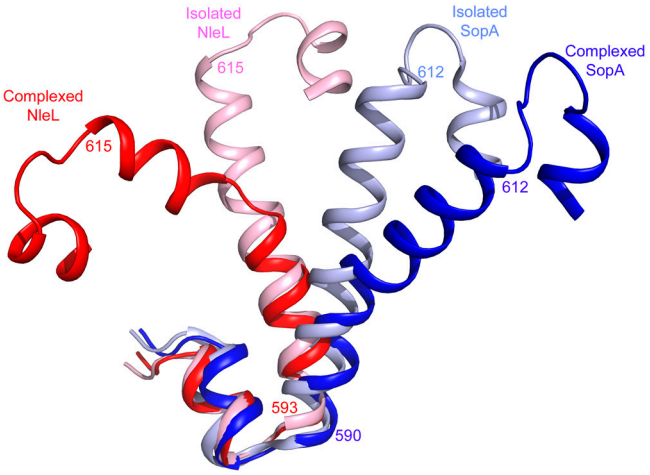


Fig. S4. Conformational flexibility of the hinge helix. Structures UbcH7/NleL, UbcH7/SopA, and two representative structures of isolated SopA (PDB code: 2QYU) and NleL (PDB code: 3NB2 chain C) are superimposed via the N-lobes and the β-helix domains. The structures of hinge helix are shown to demonstrate the different conformations of this region observed in these structures.

Table S1. Crystallographic and refinement statistics

	UbcH7/SopA	UbcH7/NleL
Data collection		
Space group	$P2_12_12_1$	C2
Cell dimensions		
$a \times b \times c$ (Å)	74.1, 118.4, 241.7	302.3, 72.0, 125.7
$\alpha \times \beta \times \gamma$ (°)	90.0, 90.0, 90.0	90.0, 109.2, 90.0
Resolution (Å)*	50.0-3.2 (3.3-3.2)	50-3.3 (3.4-3.3)
R_{sym}*	15.7 (57.5)	13.8 (63.5)
$I/\sigma I$*	12.0 (2.4)	20.3 (1.9)
Completeness (%)*	95.2 (94.9)	99.0 (95.2)
Redundancy*	3.1 (2.7)	4.9 (4.2)
No. reflections	31,667	38,818
Refinement Statistics		
Resolution (Å)*	45.4-3.3 (3.4-3.3)	50-3.3 (3.4-3.3)
R_{work}/R_{free} (%)*†	21.1/27.5 (30.1/39.6)	26.6/29.8 (37.0/38.7)
Number of atoms	11,660	10,988
Protein	11,640	10,911
H_2O	15	14
SO_4	5	45
Glycerol	0	18
Rms deviations		
bond length (Å)	0.005	0.005
bond angle (°)	0.864	0.870
B-factors (Å^2)‡		
Protein	85.3	128.1
Ligand/Ion	138.0	138.9
Water	46.8	90.3
ProCheck Ramachandran statistics		
Most favored (%)	80.0	74.6
Additional allowed (%)	19.4	24.7
Generously allowed (%)	0.6	0.7
Disallowed (%)	0.0	0.0
MolProbity		
Clashscore, all atoms§	27.68 [86th]	40.99 [67th]
MolProbity score§¶	3.18 [77th]	3.45 [62nd]

*Numbers in brackets refer to the highest resolution shell.

†A random 5% of the reflection data was omitted in the refinement and used to calculate R_{free}.

‡At this resolution, the atomic B factors cannot be accurately determined and should only be used to analyze which parts of the structure are relatively ordered.

§Numbers in square brackets are the percentiles among structures of comparable resolution. 100th percentile is the best; 0th percentile is the worst.

¶MolProbity score is defined as the following: 0.42574*log(1+clashscore) + 0.32996*log(1 +max(0,pctRotOut-1)) + 0.24979*log(1+max(0,100-pctRamaFavored-2)) + 0.5