Expanded View Figures

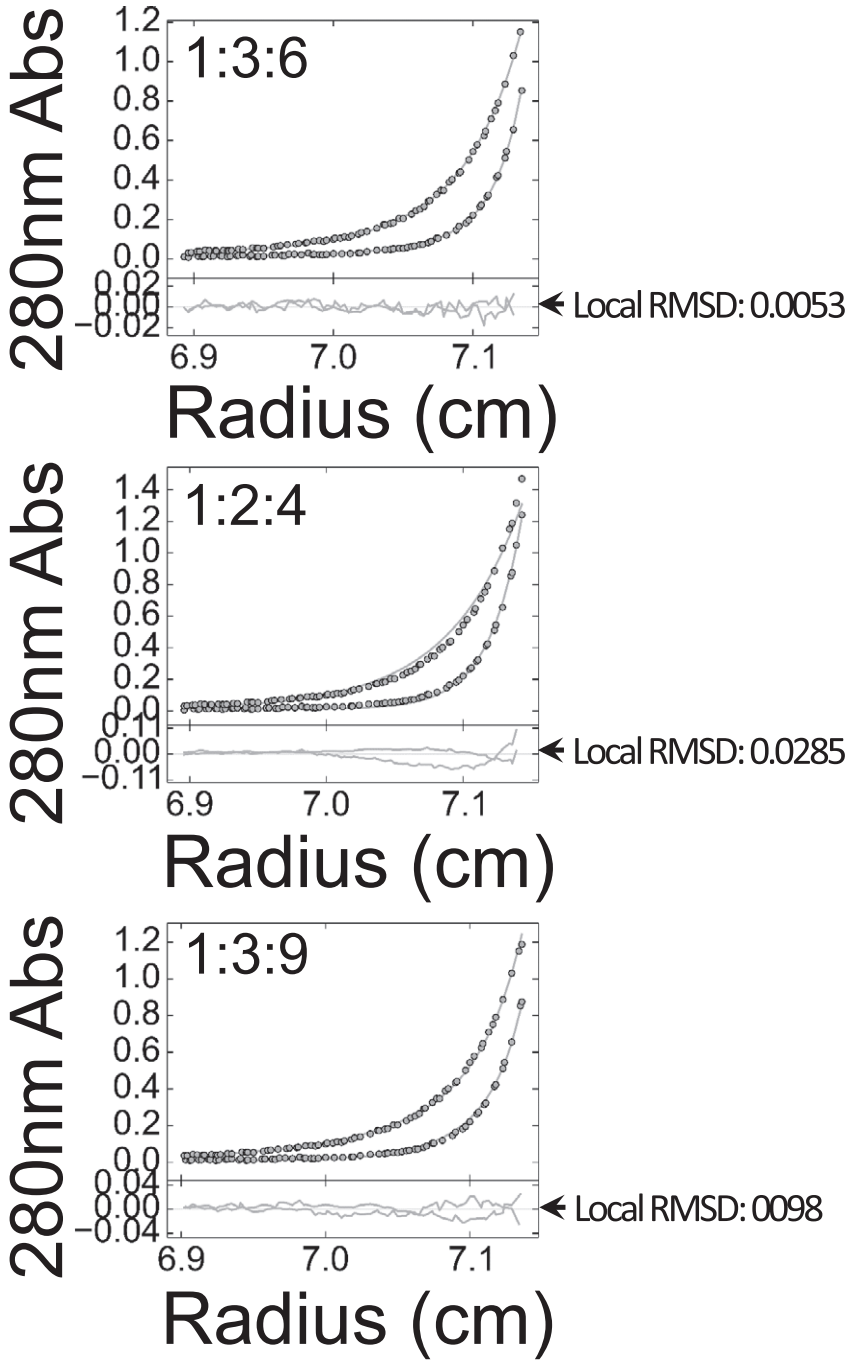

Figure EV1. Comparing alternative models for Δα1 self-association in SE analysis.
Interpretation of SE data from analytical ultracentrifugation of Δα1 in terms of self-assembly with orders of 1:3:6, 1:2:4 or 1:3:9. A_{280} was collected at 8,000 and 12,000 rpm. Data points, best fits, residuals and local RMSD values of each analysis are shown.

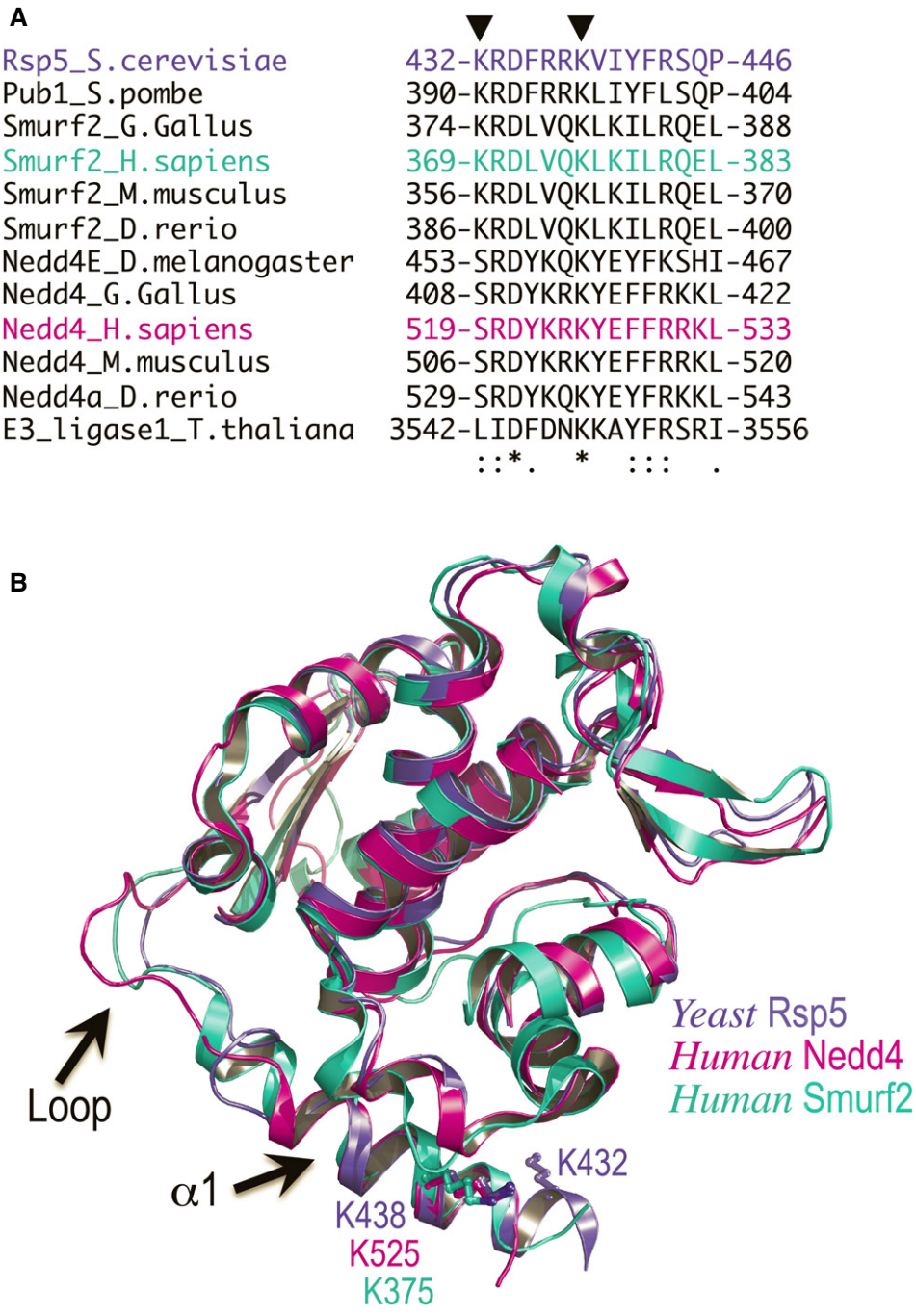

Figure EV2. Sequence and structural conservation of the α1 helix.

A Sequence alignment of α1 showing that K525 of human Nedd4 is fully conserved in the indicated organisms.

B The conserved Rsp5 K432 and the Nedd4 K525 are shown as ball and sticks. The loop connecting a1 to the HECT domain is highlighted. The significantly high B-factor values of the loop atoms and the different conformations assumed in each of the structures indicate that the loop is highly flexible.

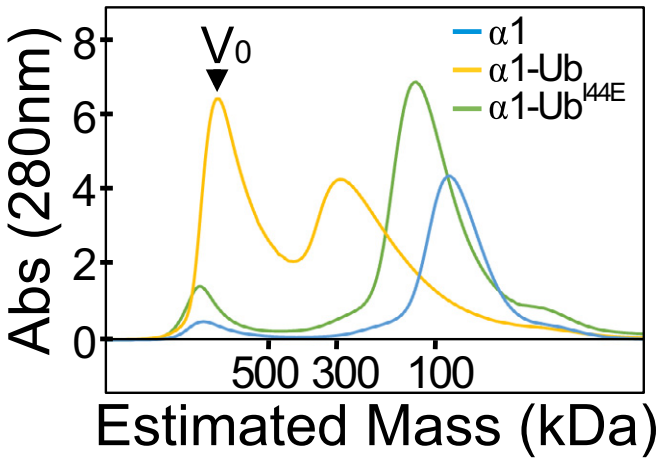

Figure EV3. Size-exclusion chromatography of His_6-MBP-Nedd4 fusion proteins.
Full-length protein and WT or I44E mutant of Ub-fused HECT were separated on a Superdex 200 16/60 column, and elution was monitored by A_{280} detection.

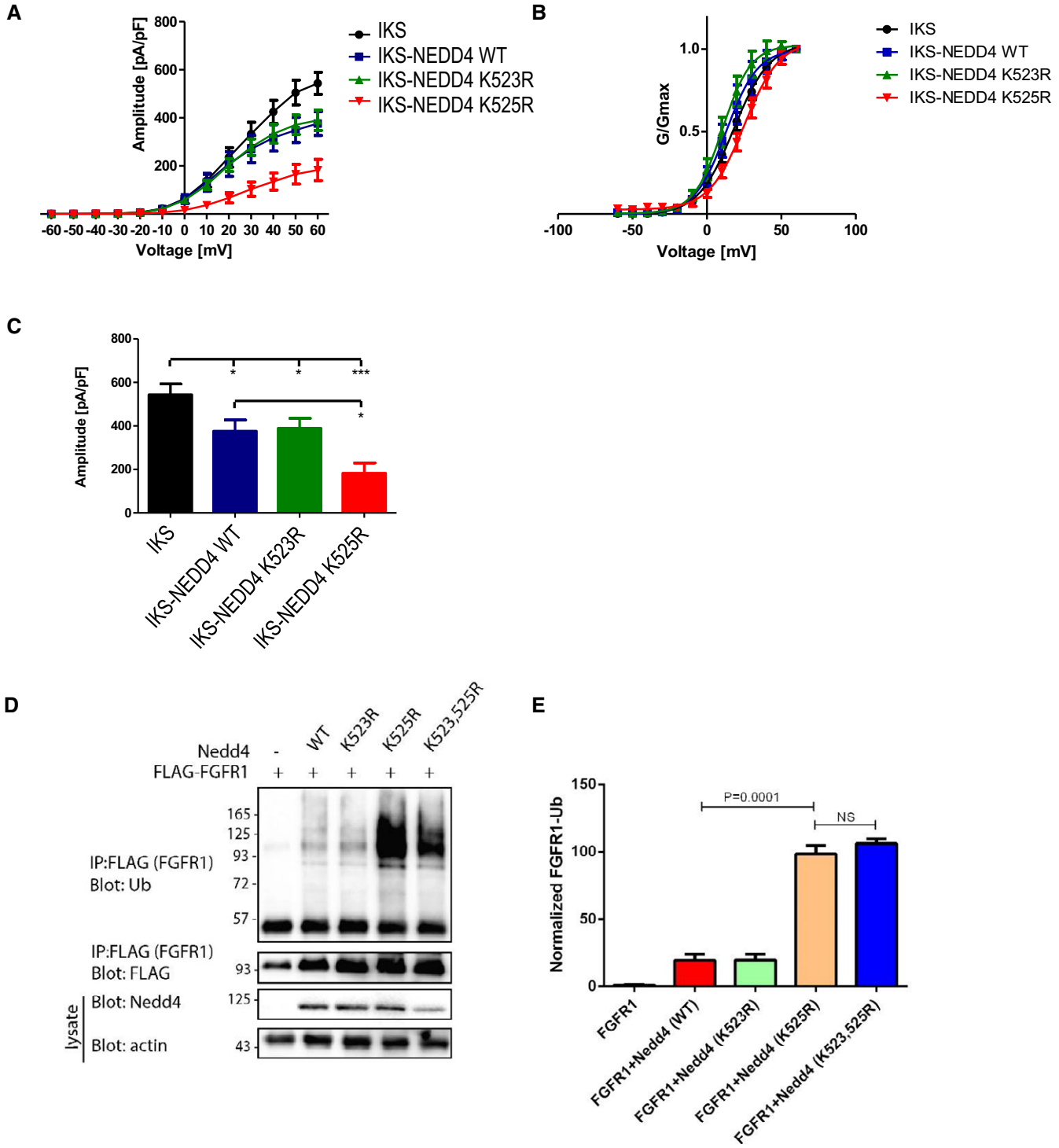

Figure EV4. Dissecting the role of individual lysine residues in Nedd4 regulation.

A, B Quantification (mean ± SEM) of (A) current-voltage and (B) conductance-voltage relationships of the recorded cells (n = 8–12), following co-expression of I_{KS} with Nedd4^{WT} or mutants bearing individual lysine mutations, that is Nedd4^{K523R} or Nedd4^{K525R}. Cells were held at −90 mV. Membrane voltage was stepped for 3 s from −60 mV to +60 mV in 10 mV increments followed by repolarization to −60 mV for 1.5 s. Normalized conductance curves were fitted to a single Boltzmann function.

C Quantified (mean ± SEM) amplitude at −60 mV, for each cell type (n = 8–12). One-way ANOVA; and Bonferroni's multiple comparison test; *$P < 0.05$, ***$P < 0.001$.

D Representative immunoblot of FGFR1 ubiquitylation (in the presence of serum) upon transfection of the indicated wild-type and mutant human Nedd4 constructs.

E Quantified (mean ± SEM) ubiquitylated/total FGFR1 ratio from three separate experiments. P-values are from Student's t-test.

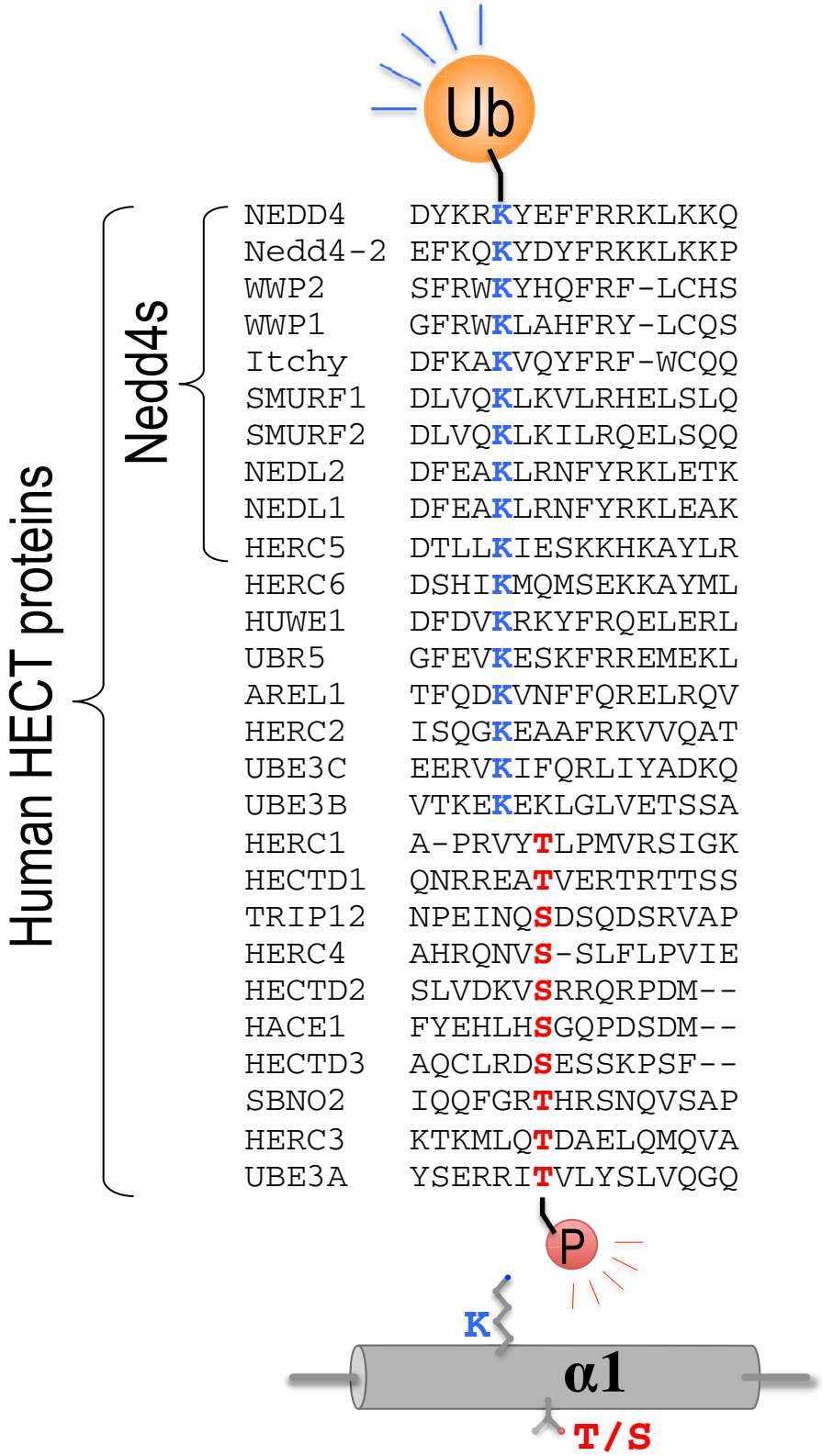

Figure EV5. Multiple sequence alignment of α1 sequences and post-translation modification sites.

The α1 sequences of human Nedd4, Rsp5 and other HECT ligases were aligned with ClustalW. The conserved lysine residue of the Nedd4 family and the threonine reside of E6AP that undergo ubiquitylation and phosphorylation are indicated.