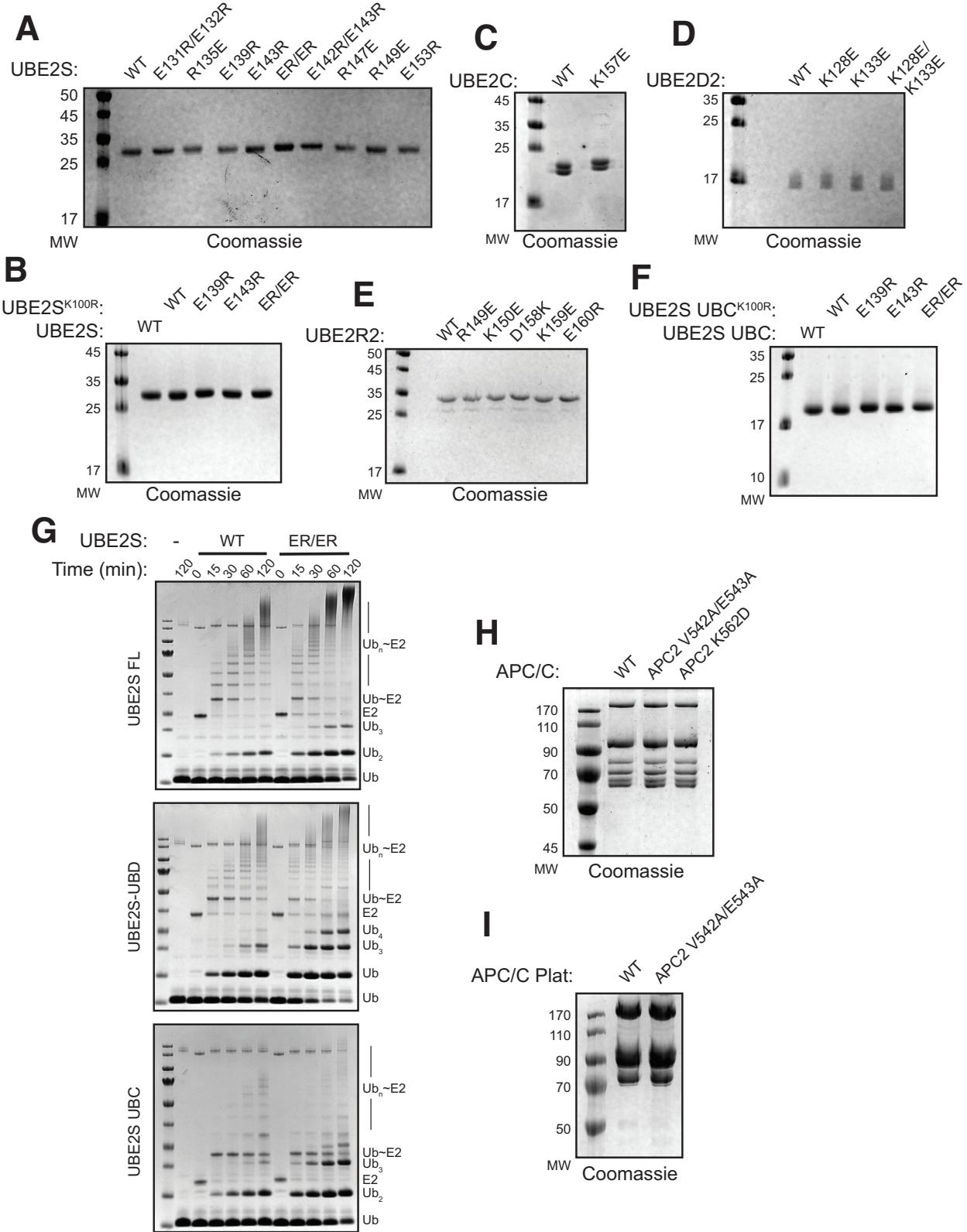


Functional conservation and divergence of the helix-turn-helix of E2 ubiquitin-conjugating enzymes

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Appendix Figure 1 – Coomassie-stained SDS-PAGE gels of proteins used in enzyme assays.

A Coomassie-stained SDS-PAGE gel of the panel of UBE2S charge-swap variants.

B Coomassie-stained SDS-PAGE gel of UBE2S WT and its variants used in pulse-chase diUb assays for [Fig 2E](#) and [EV1B](#).

C Coomassie-stained SDS-PAGE gel of UBE2C WT and K157E used in [Fig 2F](#), [6C](#), and [EV5B](#).

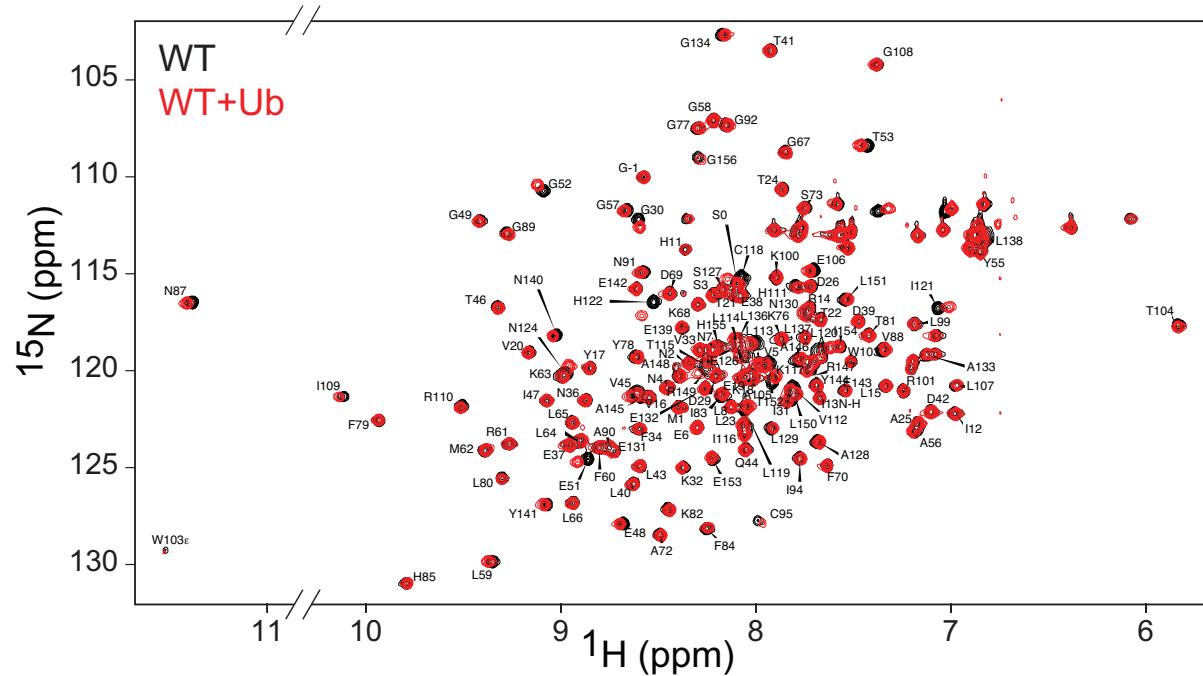
D Coomassie-stained SDS-PAGE gel of UBE2D2 WT and its variants used in [Fig 2G](#) and [6D](#).

E Coomassie-stained SDS-PAGE gel of UBE2R2 WT and its variants used in [Fig 2-4](#).

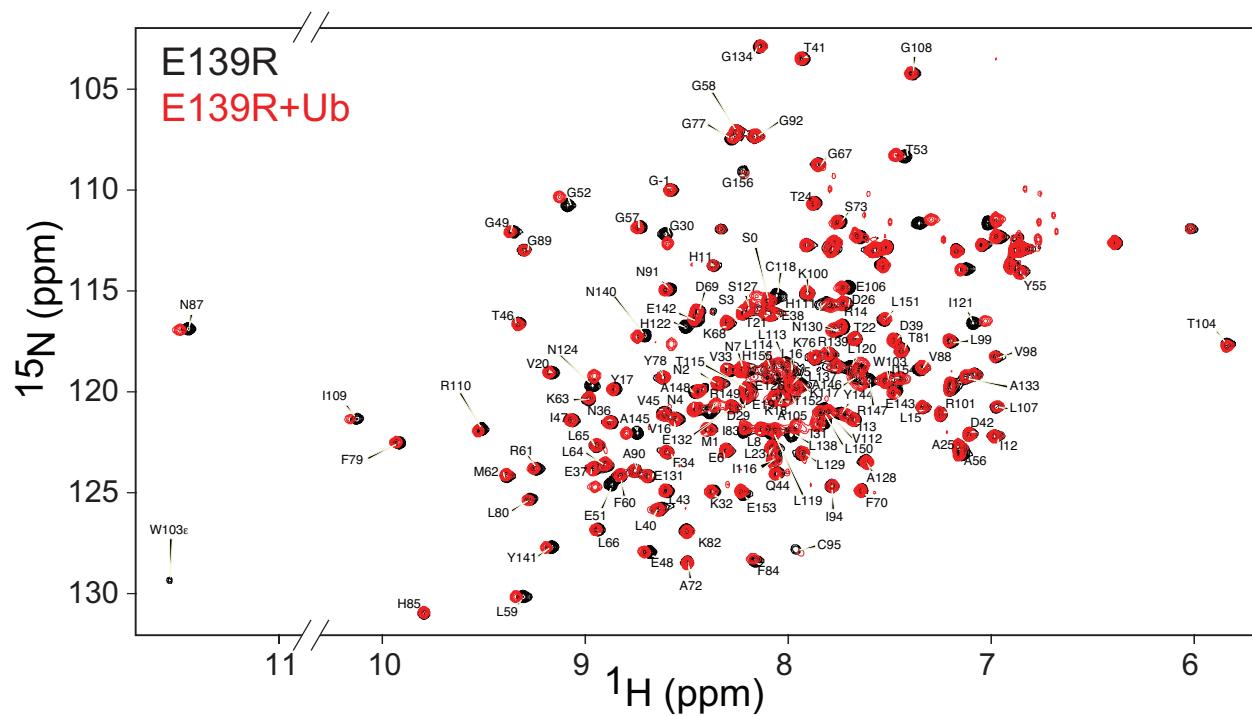
F Coomassie-stained SDS-PAGE gel of the UBE2S UBC WT and its variants used in pulse-chase lysine assays for [Fig 3C](#) and [Fig EV3A](#).

G Full Coomassie-stained SDS-PAGE gels of UBE2S-mediated diUb synthesis and automodification presented in [Fig 1D](#).

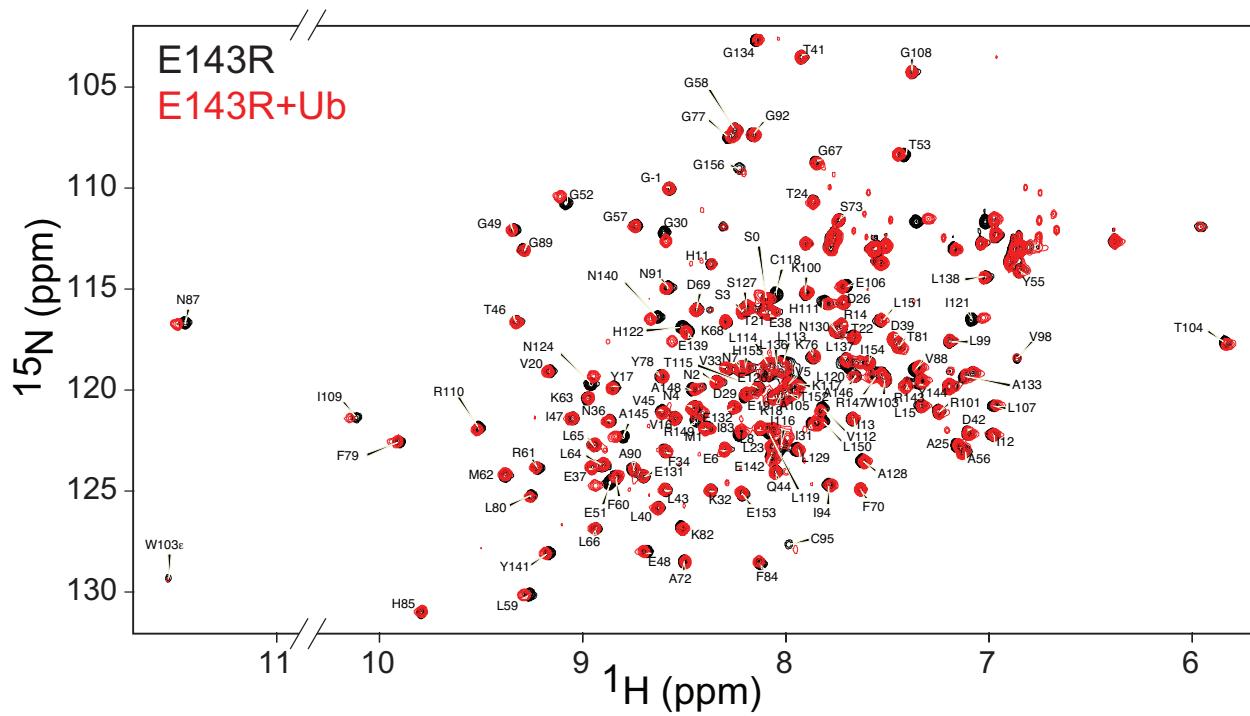
H, I Coomassie-stained SDS-PAGE gels of APC/C (G) and the Platform subcomplexes (H) containing substitutions in APC2 used in [Fig 7B-D](#).



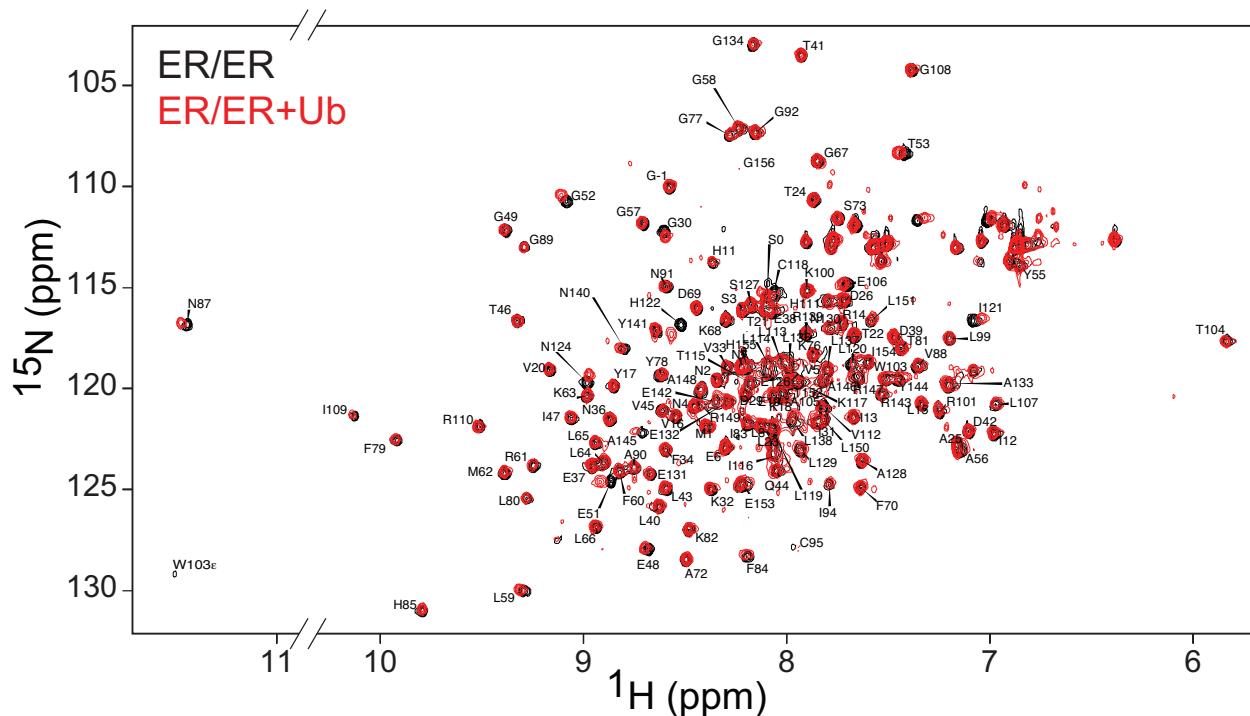
Appendix Figure S2 - HSQC spectra with the residue assignments of UBE2S WT in the presence and absence of Ub.



Appendix Figure S3 - HSQC spectra with the residue assignments of the UBE2S E139R variant in the presence and absence of Ub.



Appendix Figure S4 - HSQC spectra with the residue assignments of the UBE2S E143R variant in the presence and absence of Ub.



Appendix Figure S5 - HSQC spectra with the residue assignments of UBE2S ER/ER variant in the presence and absence of Ub.