Expanded View Figures

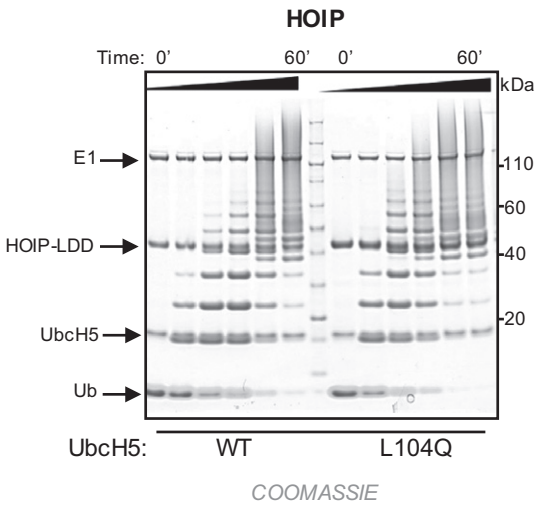

Figure EV1. HOIP does not require closed states of E2~Ub for linear chain formation.
Linear Ub chain building reactions were performed using $HOIP_{RBR\text{-}LDD}$ and either $UbcH5^{WT}$ or $UbcH5^{L104Q}$. Product formation (free linear Ub chains) was visualized by Coomassie blue staining.

Figure EV2. NMR mapping of HHARI RING1 binding to UbcH7.

A Spectral overlay of ^{15}N-UbcH7 in the absence (black) and presence (red) of 0.5 mol equiv. HHARI RING1. Affected resonances show peak doubling, characteristic of a high-affinity complex, where a new set of resonances that correspond to resonances from the complex appear simultaneously with resonances of the unbound species.

B Histogram displays the ratio of peak intensity of UbcH7 residues (bound/free) measured from the spectra shown above. The average intensity ratio is indicated with a gray dashed line.

C Left: Surface (top) and cartoon (bottom) representation of UbcH7 (PDB 1fbv). Residues that exhibit loss of intensity > 1 stdv from the average upon RING1 binding (intensity < 0.448) are colored in pink (residues 6, 11, 13, 14, 28, 40, 57, 61, 64, 78, 87, 89, 90, 96, 98–100, 102–106, 108). Right: For comparison, UbcH5 residues (PDB 2fuh) that are perturbed (either CSP or intensity loss) upon RING1 binding are indicated in pink (CSPs: > 0.028 ppm and intensity < 0.44).

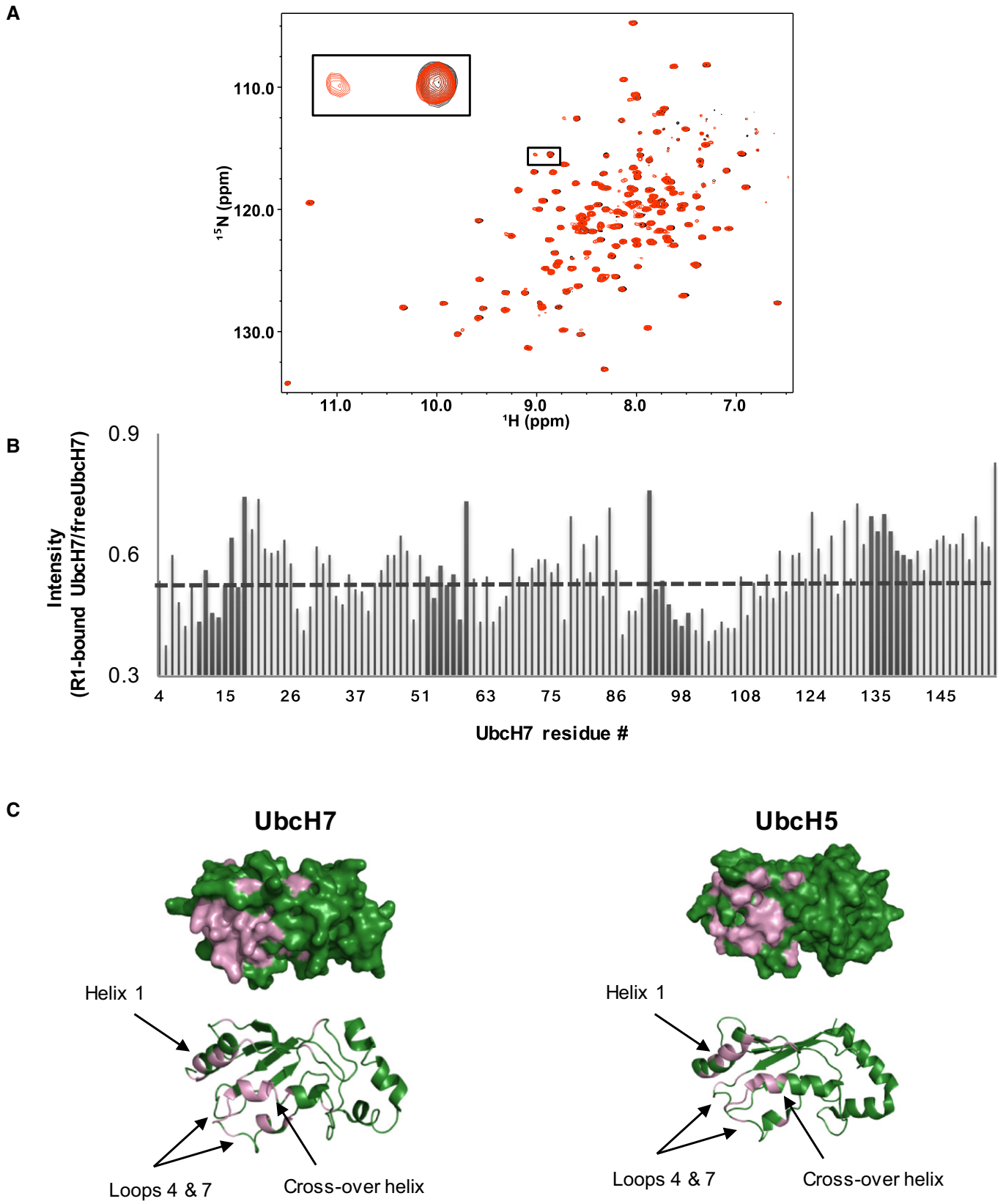

Figure EV2.

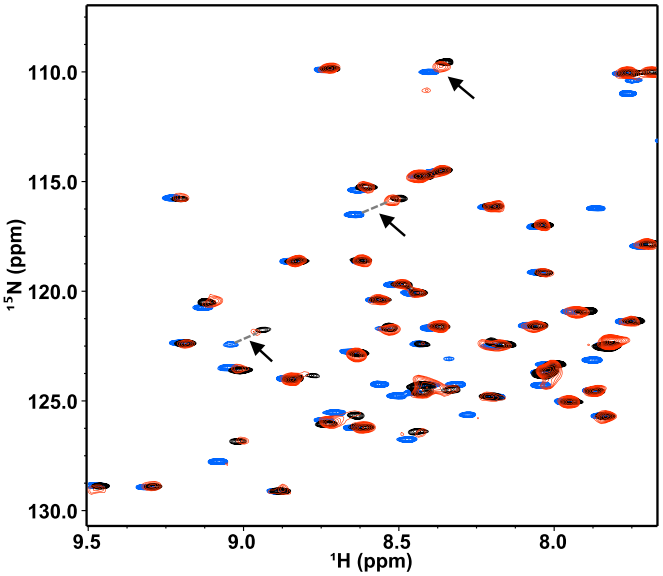

Figure EV3. HHARI RING1 disruption of (E3-independent) closed E2~Ub conformations.

Similar to UbcH7, Ubc13~Ub populates closed states in the absence of an E3 [35]. Shown here are three ^{1}H-^{15}N-HSQC-TROSY spectra of Ub overlaid: free ^{15}N-Ub (blue), Ubc13-O-^{15}N-Ub (black), and Ubc13-O-^{15}N-Ub in the presence of 3 mol equiv. HHARI RING1 (red). Due to the weak affinity binding of Ubc13 to HHARI RING1, the complex is far from saturation and the spectrum exhibits fast-to-intermediate exchange behavior. Therefore, the observed CSPs for the effects of RING1 binding to Ubc13-O-^{15}N-Ub are quite small (black versus red). Black arrows highlight several Ub resonances that shift along the same trajectory (but opposite direction) upon binding HHARI RING1 as experienced when Ub is conjugated to Ubc13, consistent with Ubc13~Ub populating more open-like states when in complex with HHARI RING1.

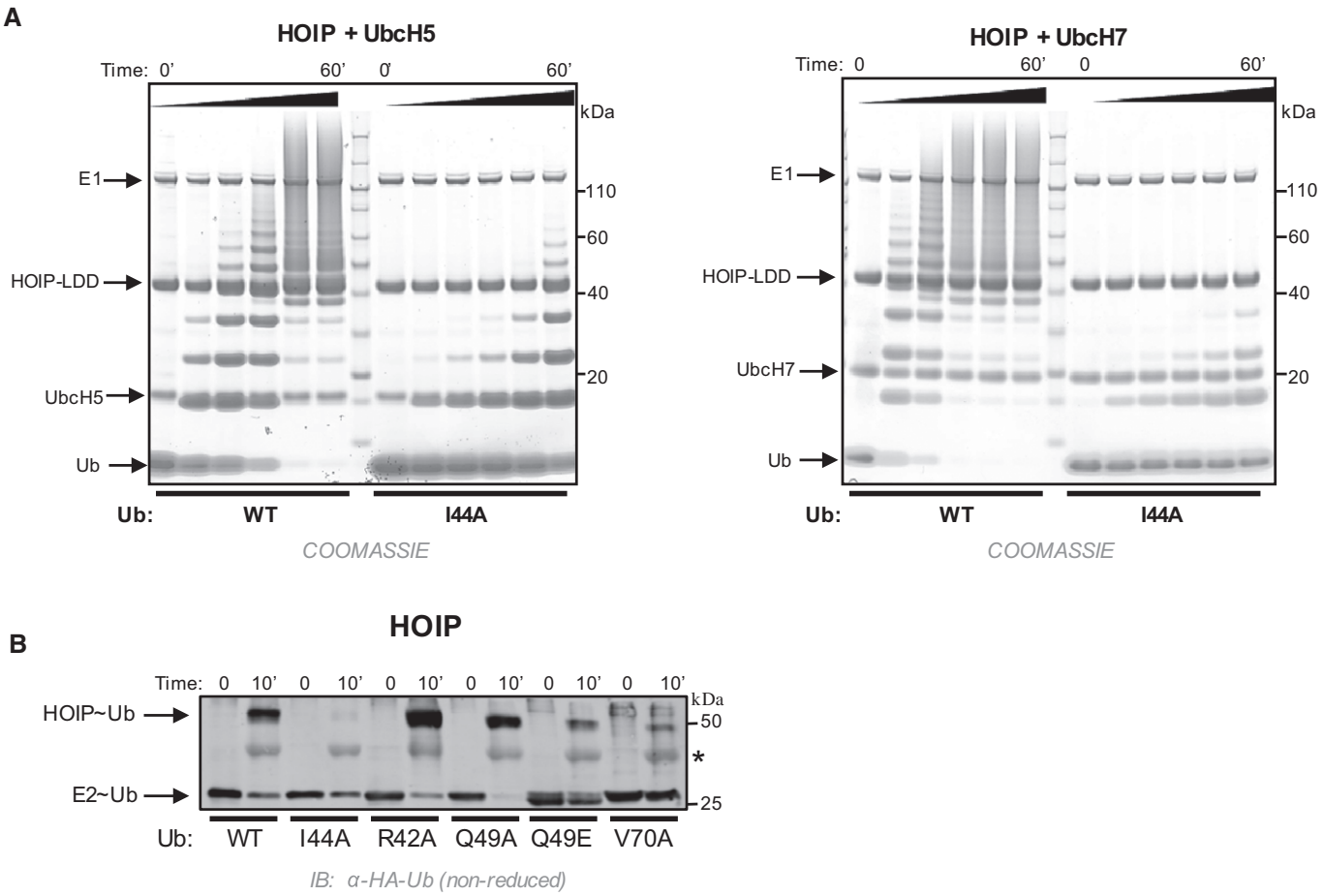

Figure EV4. Ub hydrophobic patch involvement in linear chain building by the RBR E3 HOIP.

A Time courses of linear chain building assays using $HOIP_{RBR\text{-}LDD}$ and either UbcH5 (left) or UbcH7 (right) conjugated with WT-Ub or I44A-Ub as followed by Coomassie-stained gels. The I44A mutation in the donor Ub does not impair the formation of di-Ub [9], yet I44A-Ub significantly reduces linear chain building ability by $HOIP_{RBR\text{-}LDD}$.

B UbcH7 was pre-conjugated with either WT-Ub or mutant Ub (as indicated) and the reaction was quenched with apyrase. Each UbcH7~Ub species was incubated with H887A-$HOIP_{RBR\text{-}LDD}$ to enable detection of the E3~Ub intermediate [41]. Samples were analyzed on SDS–PAGE under non-reducing conditions and subsequently visualized by Western blotting for the HA-tag on Ub. Time was recorded post-addition of H887A-$HOIP_{RBR\text{-}LDD}$. Note: The HA antibody cross-reacts with free HOIP (see *).

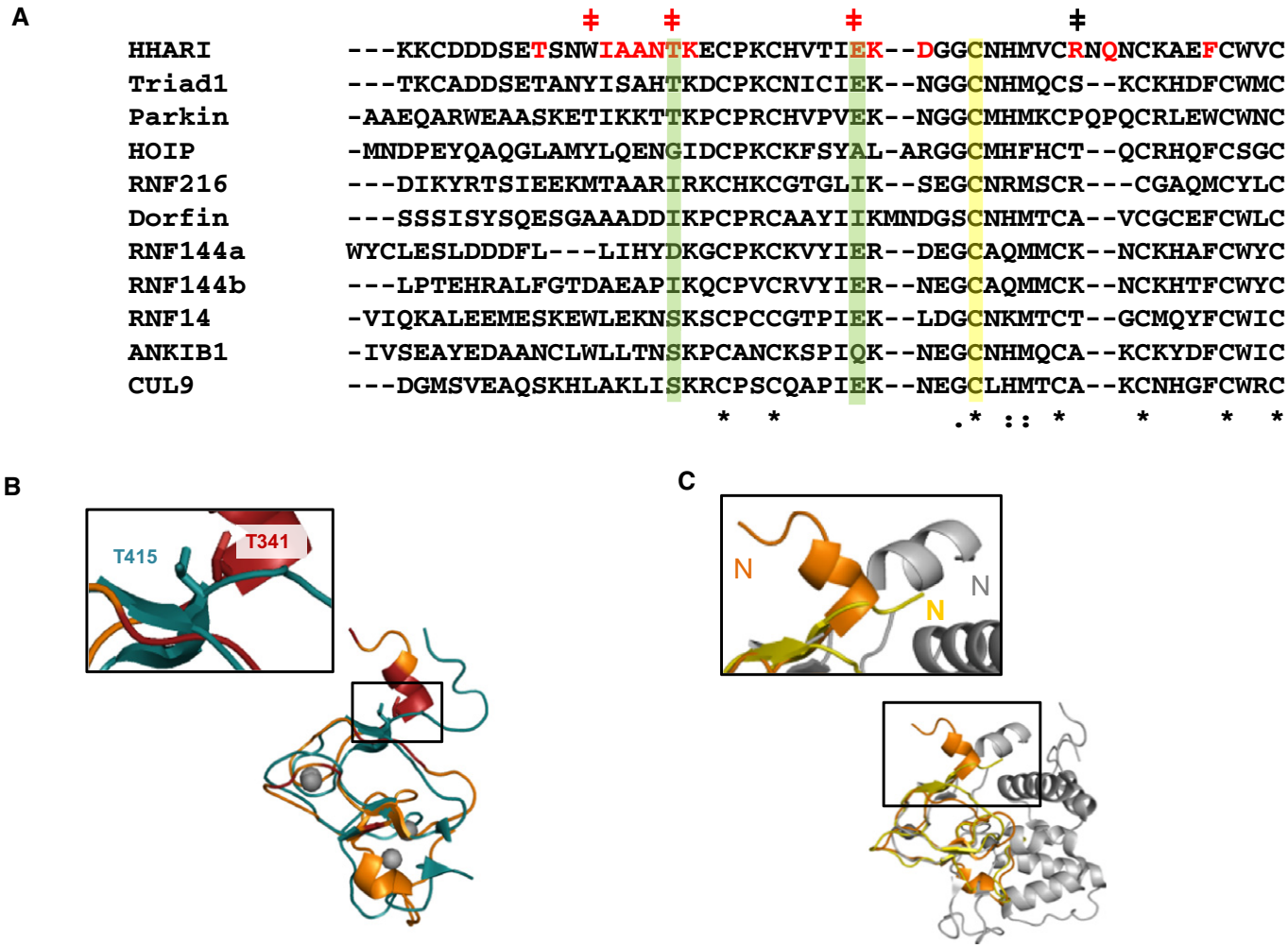

Figure EV5. Comparison of RBR RING2 domains.

A RING2 sequence alignments were generated using CLUSTAL OMEGA. Residues observed to be perturbed upon Ub binding to HHARI RING2 in NMR titration experiments are highlighted in red. HHARI residues that were mutated and tested for change in activity are marked with a (ǂ) with red indicating decreased and black indicating no observable change in activity (see Fig 6C). Green bars highlight residues that are important for Ub binding of HHARI RING2 that are well conserved among RBR RING2s. The active site Cys is marked with a yellow bar. 7th and 8th Zn^{2+} coordinating residues are not shown.

B Structural overlay of NMR solution structures of Parkin (teal, PDB 2lwr) and HHARI (orange, PDB 2m9y) RING2 domains. RING2 residues perturbed upon Ub binding (Fig 6A) are colored dark red on HHARI RING2. The HHARI RING2 mutation T341N abrogates Ub binding and decreases ligase activity (Fig 6D and E). Inset: The analogous mutation in Parkin, T415N, has been linked to early-onset Parkinson's disease [49].

C Structural overlay of three RING2 domains: a HHARI NMR solution structure (orange, PDB 2m9y), a HHARI crystal structure (yellow, PDB 4kc9), and a HOIP crystal structure (gray, PDB 4ljq). An enlargement of the N-terminal region that is part of the IBR-RING2 linker shows a helical disposition of the linker in the HOIP and HHARI NMR, but not in the HHARI crystal structure.