

Expanded View Figures

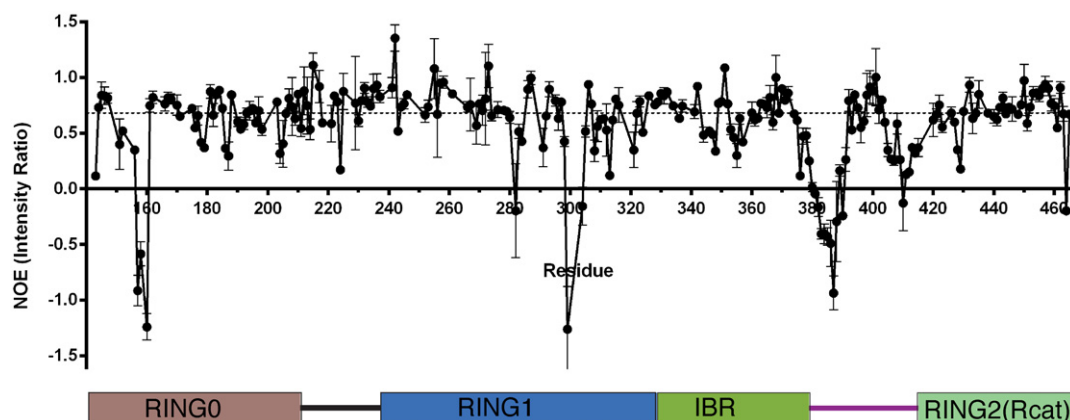


Figure EV1. Backbone flexibility of RORBR parkin in the RORBR:pUb complex.

The nOe intensity ratio measured from ^1H - ^{15}N heteronuclear nOe experiments is plotted per residue. A schematic of RORBR parkin is shown below the data. The average nOe for the protein is shown (dotted line), and nOes below this indicate increased flexibility. Experiments were measured at 600 MHz using ^2H , ^{13}C , ^{15}N -labelled RORBR parkin bound to pUb in 25 mM HEPES, 100 mM NaCl and 500 μM TCEP (pH 7.0) at 25°C. Saturation and non-saturation experiments were done in duplicate and averaged. Error bars denote the $\pm$ standard deviation from duplicate pairs of experiments.

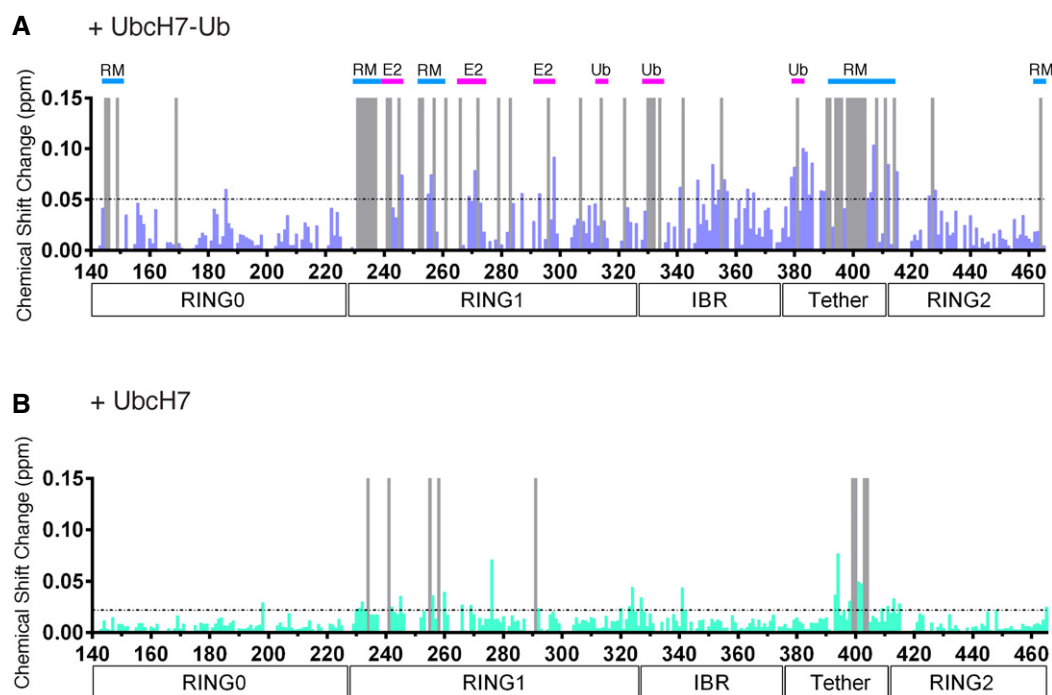


Figure EV2. Chemical shift changes used to map the UbchH7 and UbchH7-Ub binding sites with the RORBR:pUb complex.

A, B Chemical shift changes were measured in ^1H - ^{15}N TROSY NMR spectra of ^2H , ^{13}C , ^{15}N -labelled RORBR parkin in complex with ^2H -labelled pUb upon addition of (A) ^2H -labelled UbchH7-Ub or (B) ^2H -labelled UbchH7. Grey bars indicate that the resonance was undetectable in spectra with UbchH7-Ub or UbchH7 added due to a large change in position and/or broadening. Horizontal dashed lines indicate the average chemical shift change + one standard deviation. In (A), regions are identified either as UbchH7 (E2) and Ub binding (magenta bars) or as a result from re-modelling of the RING0/RING1/RING2 interface (RM, blue bars). Data were collected at 25°C in a buffer containing 25 mM HEPES, 50 mM NaCl and 500 μM TCEP (pH 7.0).

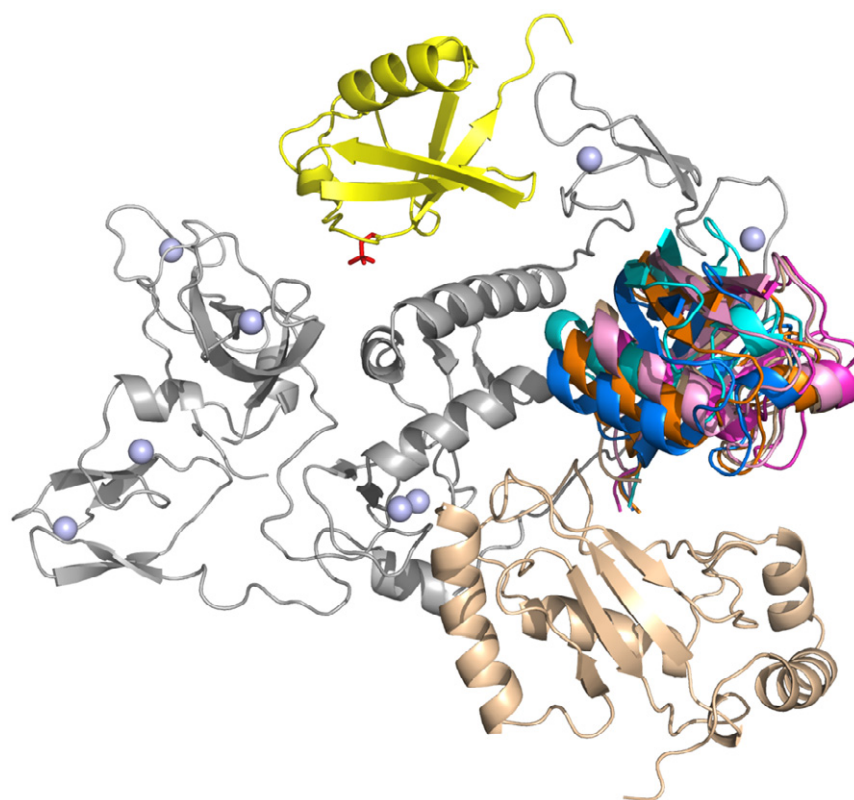


Figure EV3. HADDOCK structures show variable Ub orientations in Ubch7-Ub bound to RORBR: pUb.

The Ub position in the E2-Ub conjugate is shown for a selection of five HADDOCK models along with the lowest energy model (cyan). The superposition was prepared by superimposing the parkin (grey) and Ubch7 (wheat) portions of the models which show excellent agreement. The orientation of the complex is similar to that for Fig 2C. In the figure, only a single parkin, Ubch7 and pUb (yellow) molecule is shown for clarity.

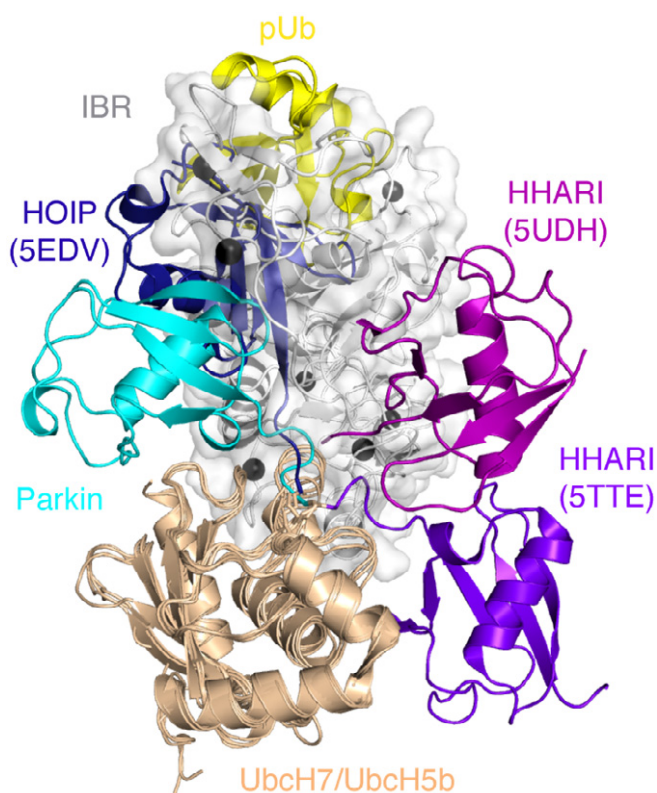


Figure EV4. Comparison of E2-Ub conjugate conformations when bound to different RBR E3 ligases.

The structure of the Ubch7-Ub conjugate (cyan Ub) bound to RORBR parkin (grey surface) in complex with pUb (yellow ribbon) is shown as determined from HADDOCK calculations in this work. Cartoon diagrams of the E2-Ub conjugates in complex with HOIP (Ubch5b-Ub, navy Ub; PDB 5EDV), HHARI (Ubch7-Ub, magenta Ub; PDB 5UDH) and HHARI (Ubch7-Ub, purple Ub; PDB 5TTE) are shown. The relative arrangement of the Ub molecules within the E2-Ub conjugates was created by superimposing the backbone atoms for the E2 proteins (beige).