Appendix

Synergistic Recruitment of UbcH7~Ub and Phosphorylated Ubl Domain Triggers Parkin Activation

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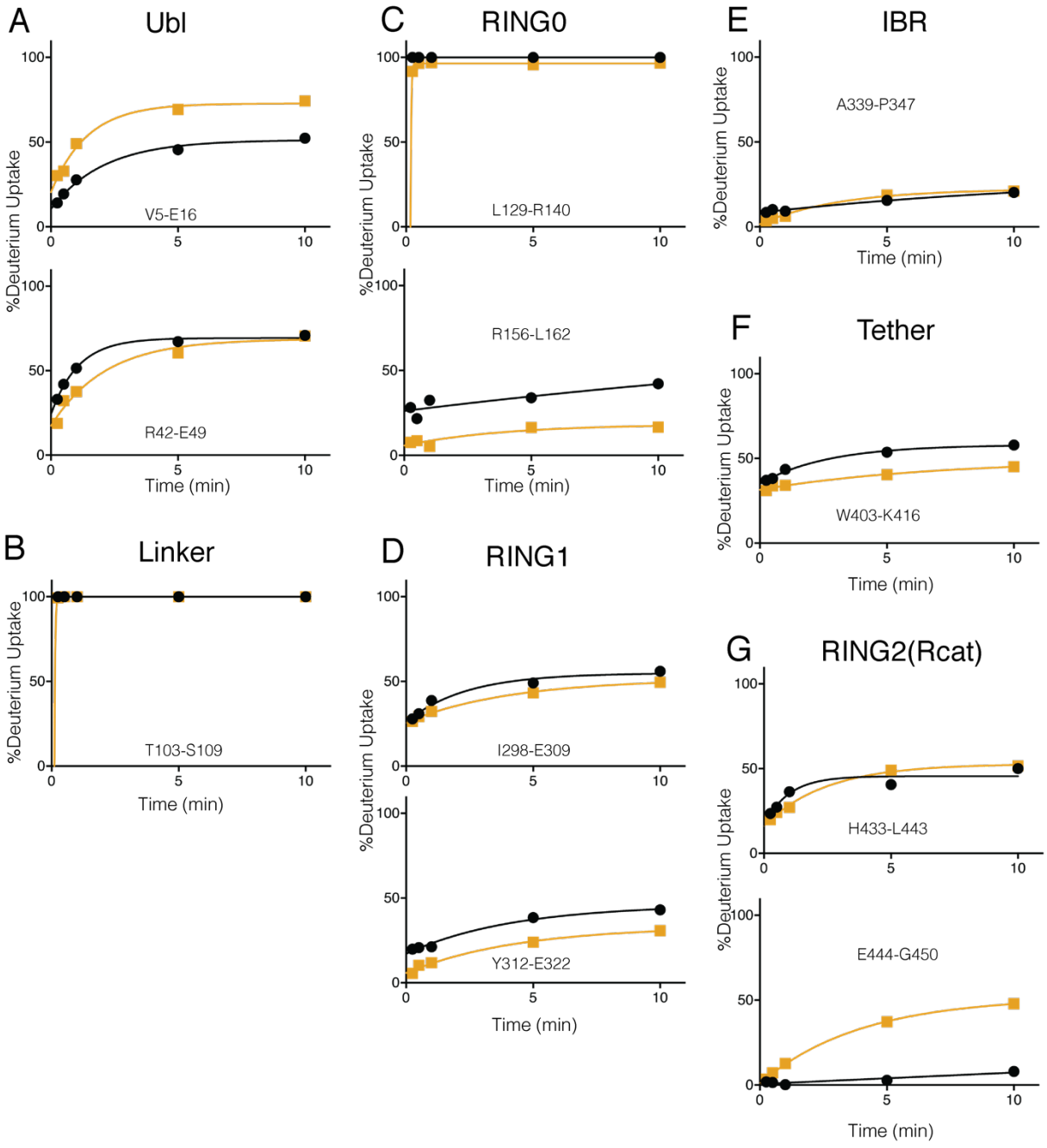

Figure S1. Selected deuterium uptake curves measured from HDX experiments using mass spectrometry. The curves show the % deuterium incorporation for a given peptide as a function of time (minutes) for parkin (●) and pParkin:pUb (■) in (A) Ubl/pUbl, (B) linker, (C) RING0, (D) RING1, (E) IBR, (F) tether and (G) RING2/Rcat domains.

Figure S2. Parkin peptide coverage map. The parkin peptide map, which shows the subset of contiguous peptides of parkin used for all MS-HDX experiments. This consistent group of peptides was used for all measurements involving parkin, the pParkin:pUb complex and the pParkin:pUb-UbcH7-Ub complex. Coverage is indicated at 91.3% coverage.

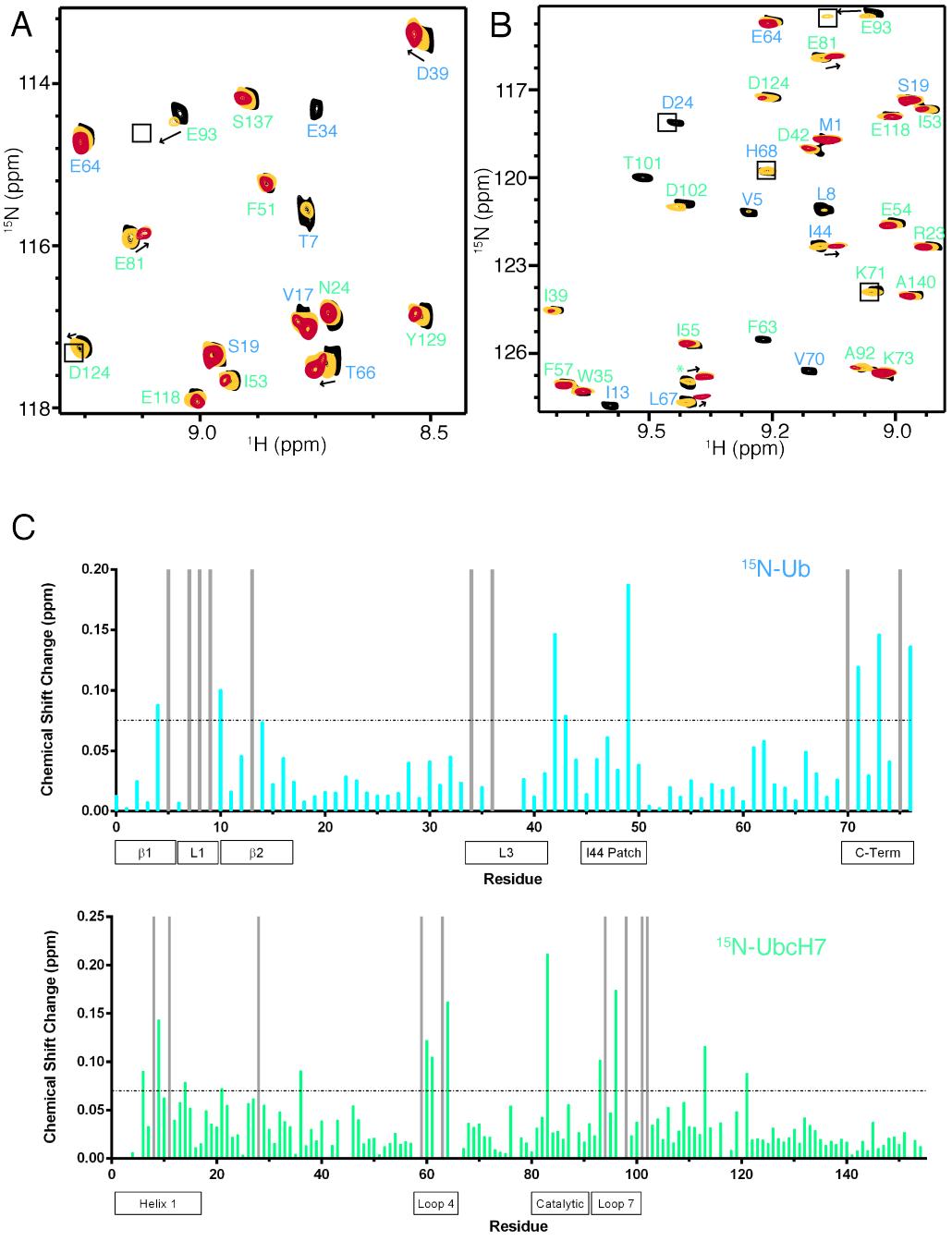

Figure S3. UbcH7-Ub binding interface with R0RBR:pUb. (A, B) Selected regions from ^{1}H-^{15}N TROSY NMR spectra of ^{2}H,^{12}C,^{15}N-labelled UbcH7-Ub in the absence (black contours) and presence of 0.5 equivalents (yellow contours) or 1 equivalent (red contours) ^{2}H-labelled R0RBR:pUb. The spectra are labelled for UbcH7 (green) or Ub (blue) in the UbcH7-Ub conjugate. (C) Measured chemical shift changes in either UbcH7 or Ub in the UbcH7-Ub conjugate upon R0RBR:pUb binding at 1 equivalent R0RBR:pUb. Grey bars indicate that the resonance was undetectable. The horizontal dashed line indicates the average CSP +1 standard deviation. Data was collected in 25 mM HEPES, 50 mM NaCl, 500 µM TCEP, pH 7.0 buffer at 25°C.

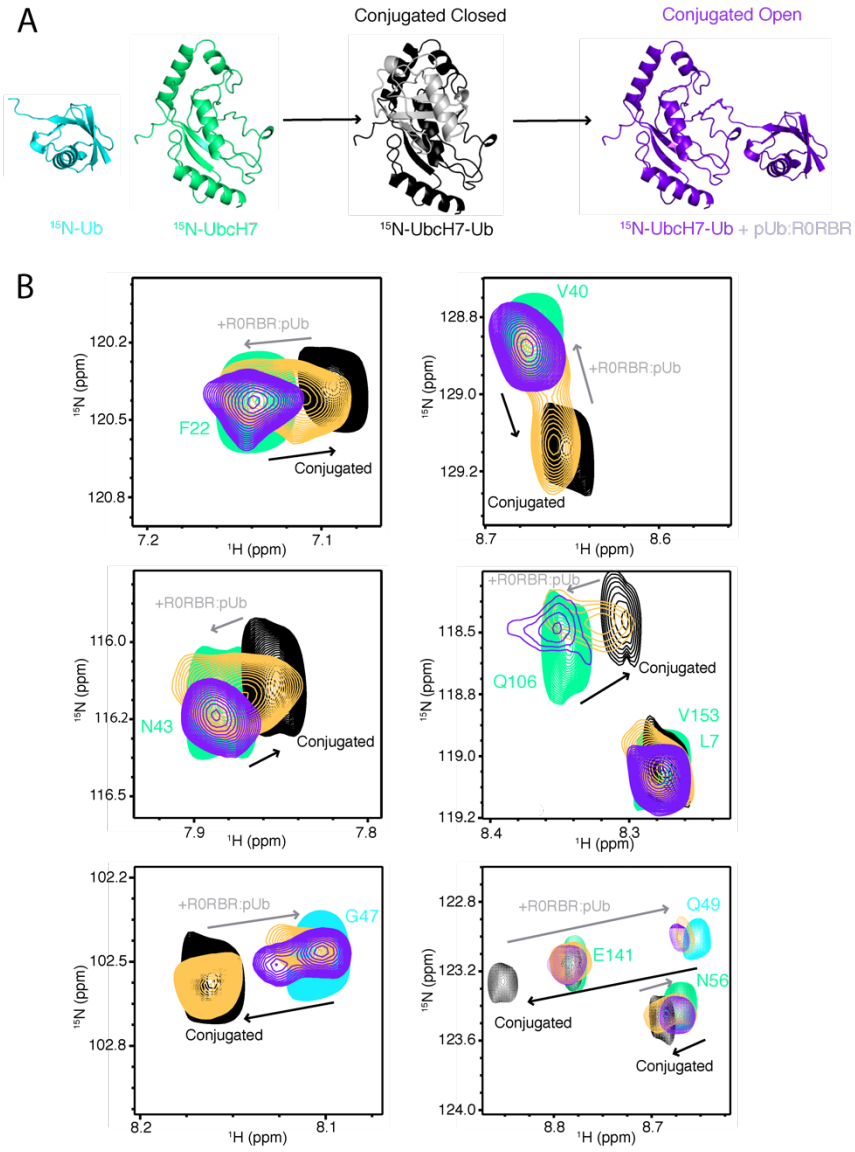

Figure S4. The closed UbcH7-Ub state is released upon binding to R0RBR:pUb. (A) Ribbon diagram representations of UbcH7 and Ub and the "closed" and "open" UbcH7-Ub states formed upon conjugation. (B) ^{1}H-^{15}N TROSY NMR spectra of unconjugated ^{2}H,^{12}C,^{15}N-labelled UbcH7 (green contours, top four panels) or ^{2}H,^{12}C,^{15}N-labelled Ub (cyan contours, bottom two panels) along with corresponding residue assignments. These two labelled moieties were then conjugated to form the UbcH7-Ub product (black contours). UbcH7 resonances for F22, V40, N43 and Q106 and Ub resonances for G47 and Q49 are perturbed upon conjugate formation representative of the "closed" UbcH7-Ub state. ^{2}H,^{12}C,^{15}N-labelled UbcH7-Ub was titrated with 0.5 equivalents (yellow contours) and 1 equivalent (purple contours) of ^{2}H-labelled R0RBR:pUb. In all cases the UbcH7 and Ub resonances revert to a position near that for the unconjugated forms of UbcH7 and Ub indicative of "open" conformation. All resonances exhibit slow or slow-intermediate exchange indicating the UbcH7-Ub conjugate interacts tightly R0RBR:pUb.

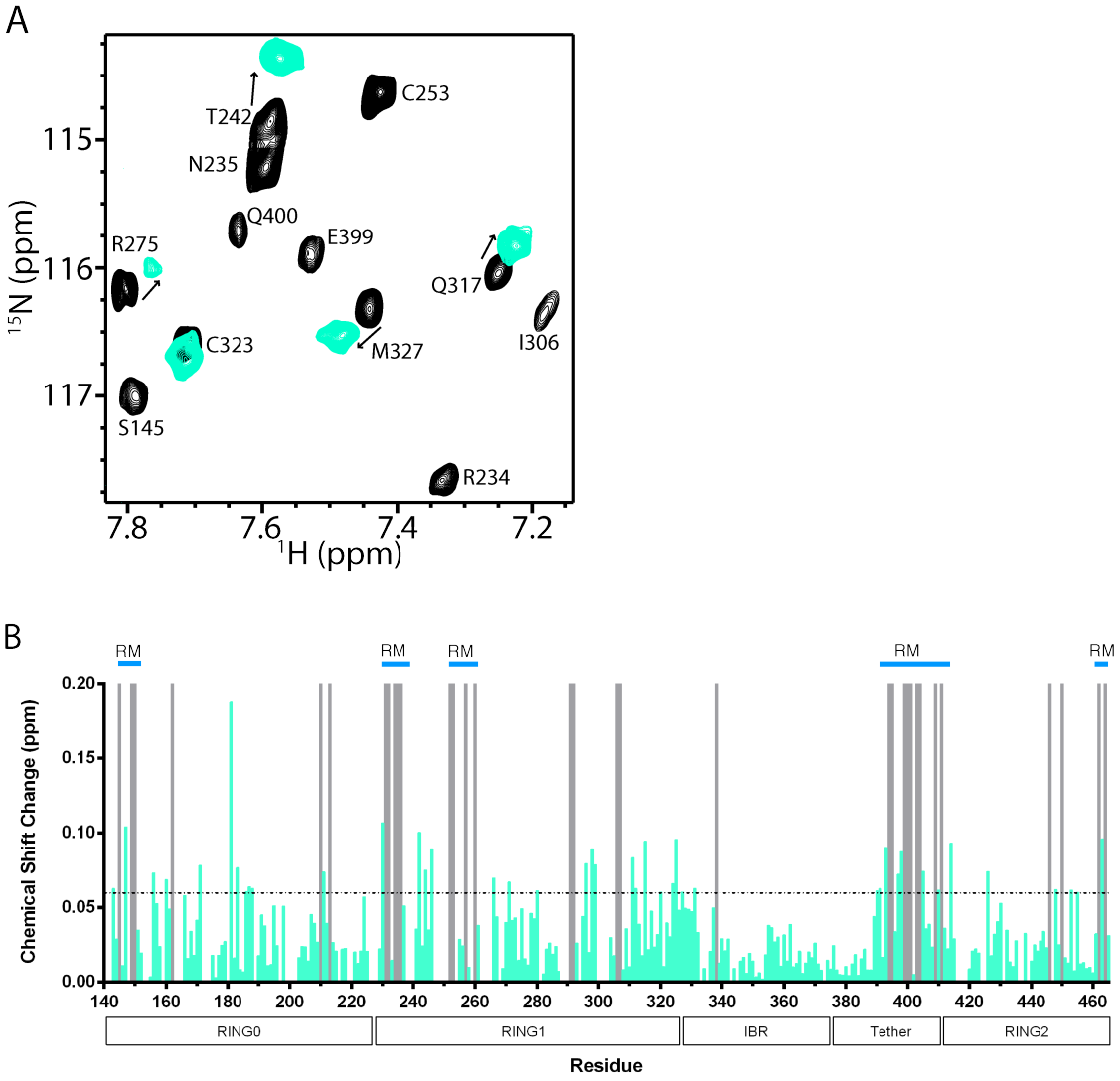

Figure S5. Effect of W403A substitution in R0RBR parkin. (A) Selected region of ^{1}H-^{15}N TROSY NMR spectra for ^{15}N-labelled R0RBR parkin (black contours) and R0RBR^{W403A} parkin (aquamarine contours) are shown labelled with assignments from R0RBR parkin. The spectra show that several signals for R0RBR^{W403A} are shifted due to a change in environment from the W403A substitution. (B) The chemical shift changes between R0RBR parkin and R0RBR^{W403A} parkin were calculated and plotted. Grey bars indicate that the resonance in R0RBR^{W403A} parkin was shifted significantly and could not be assigned. Above the plot regions identified from UbcH7-Ub binding experiments to the R0RBR:pUb complex due to remodelling of the RING0/RING1/RING2 interface are indicated (RM, blue bars).

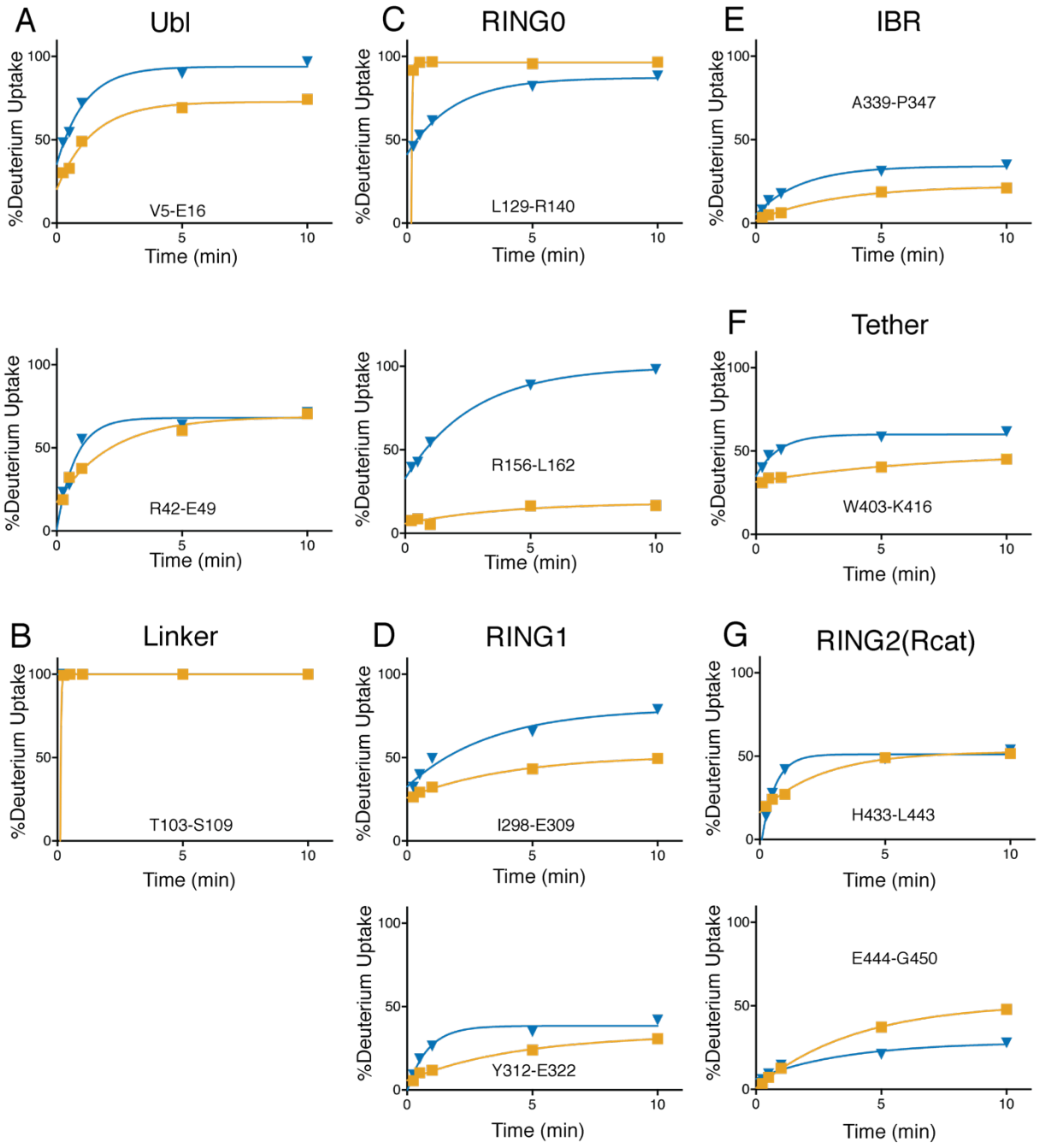

Figure S6. Selected deuterium uptake curves measured from HDX experiments using mass spectrometry. The curves show the % deuterium incorporation for a given peptide as a function of time (minutes) for pParkin:pUb (■) and pParkin:pUb in complex with UbcH7-Ub (▼) in (A) Ubl/pUbl, (B) linker, (C) RING0, (D) RING1, (E) IBR, (F) tether and (G) RING2/Rcat domains.

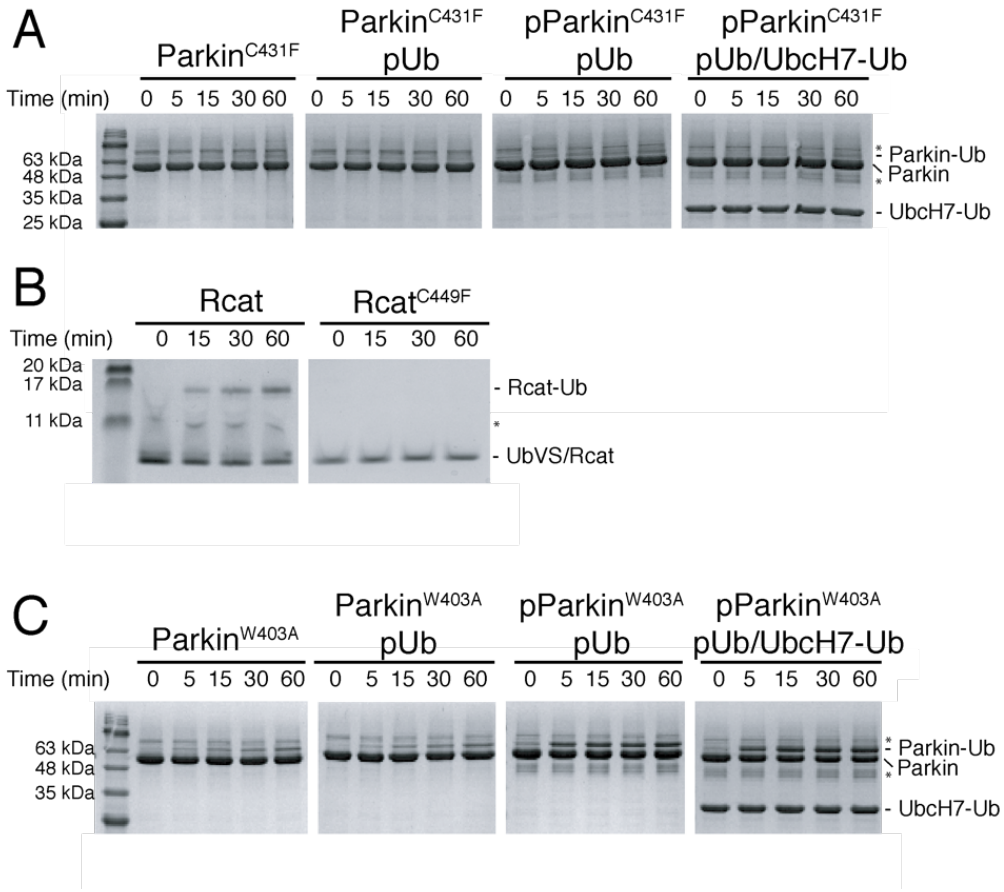

Figure S7. Reaction of the catalytic C431 residue in parkin, parkin^{W403A} and the RING2(Rcat) with a UbVS probe. (A) Experiments utilizing the ARJP substituted parkin^{C431F} show no formation of a UbVS adduct. (B) Reactivity of RING2(Rcat) or Rcat^{C431F} show reactivity is dependent of C431 in the RING2(Rcat) domain only. (C) Experiments with parkin^{W403A} show some reactivity of C431 is noted in the absence of phosphorylation or inclusion of pUb. All samples were taken at the times indicated (0-60 minutes) following addition of UbVS and visualized by SDS-PAGE.