Expanded View Figures

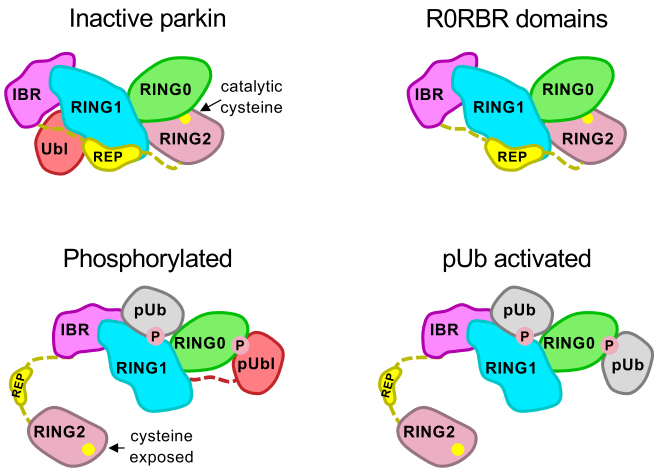

Figure EV1. Conformational changes in parkin upon phosphorylation or pUb binding.

Full-length parkin and the R0RBR module have low basal ubiquitin ligase activity due to masking of the RING2 catalytic site (cysteine 431) by the RING0 domain. Parkin is recruited to damaged mitochondria by a high-affinity site on the RING1 domain that binds phospho-ubiquitin (pUb). Upon phosphorylation of the Ubl domain or the binding of a second pUb, parkin becomes activated as the phosphorylated Ubl domain (pUbl) or pUb binds to a site on RING0 releasing and exposing the RING2 catalytic site.

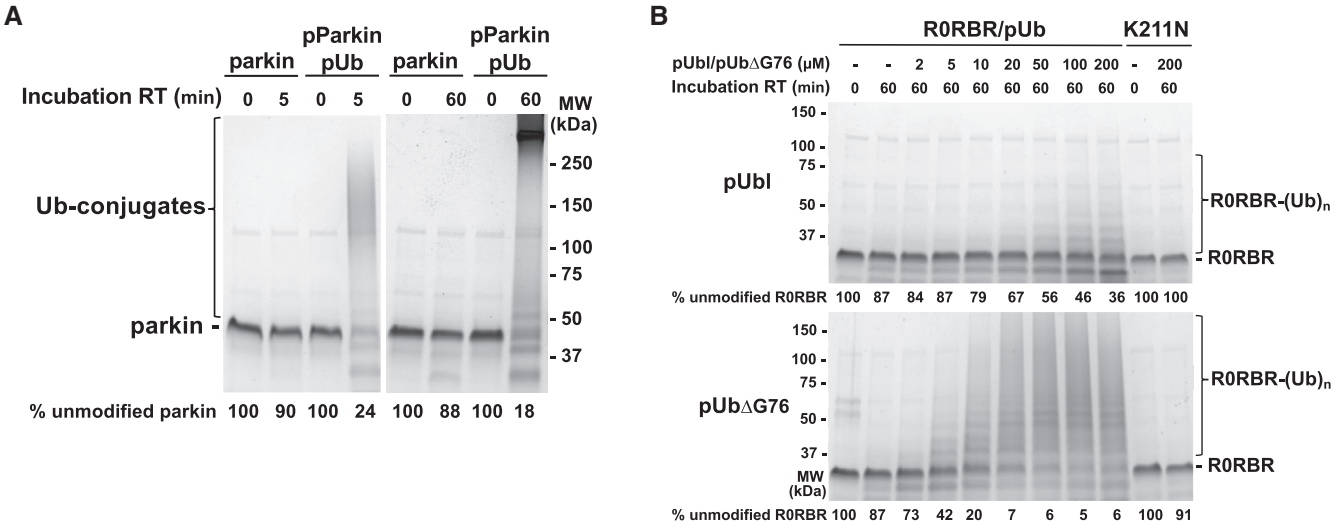

Figure EV2. Activation of parkin by pUbl or pUb binding.

A Autoubiquitination assays of parkin ligase activity of inactive and activated full-length parkin. Assays were performed with incubation of 2 μM of parkin or phosphorylated parkin purified in a 1:1 complex with pUb under the same conditions as autoubiquitination assays with GST-R0RBR/pUb (Fig 2D) and R0RBR/pUb.

B Autoubiquitination assays of parkin ligase activity induced by pUbl or pUbΔG76 *in trans*. 2 μM R0RBR in complex with pUb was incubated with 50 nM E1, 2 μM UbcH7, 100 μM ubiquitin, and 4 mM ATP. The percentage of unmodified parkin band in each lane is indicated under the gel.

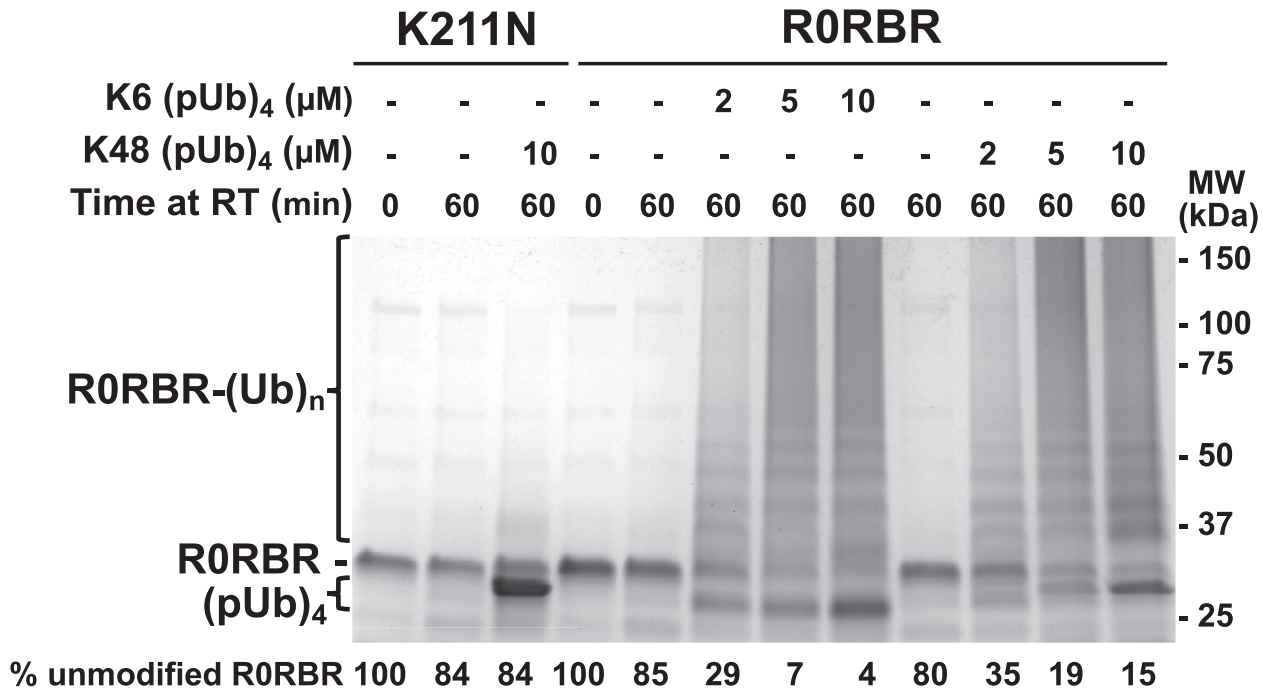

Figure EV3. Autoubiquitination assays of parkin activation by K6(pUb)$_4$ or K48(pUb)$_4$.

Assays with 2 μM R0RBR, 50 nM E1, 2 μM UbcH7, and 100 μM ubiquitin were performed in the presence of increasing amount of K6(pUb)$_4$ or K48(pUb)$_4$ chains. The percentage of unmodified parkin band in each lane is indicated under the gel.

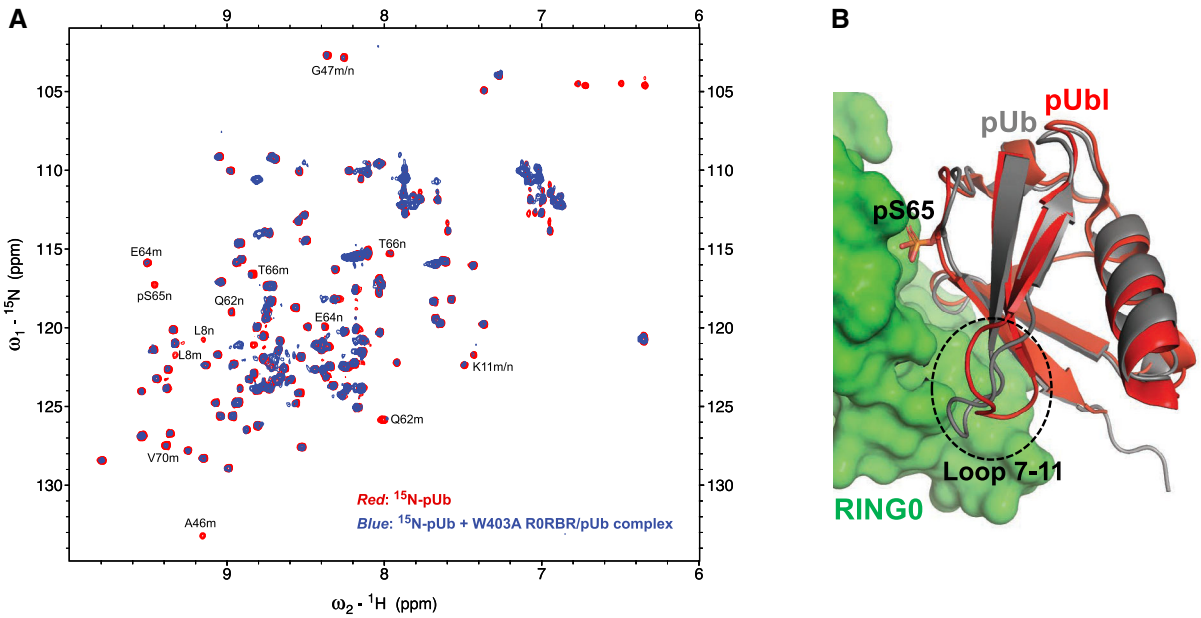

Figure EV4. pUb binds to the parkin RING0 domain.

A The NMR correlation spectrum (*red*) of ^{15}N-pUb alone (50 μM) overlaid with the spectrum (*blue*) of ^{15}N-pUb (50 μM) in the presence of the parkin W403A R0RBR and pUb complex (100 μM). Signals that are strongly attenuated by binding are labeled by residue number and *m* for the major or *n* for minor pUb conformation.

B pUb was overlaid on pUbl in the pParkinΔREP-RING2-pUb structure (PDB 6GLC) (Gladkova *et al*, 2018). RING0 is displayed in green, pUbl in red, and pUb in gray.

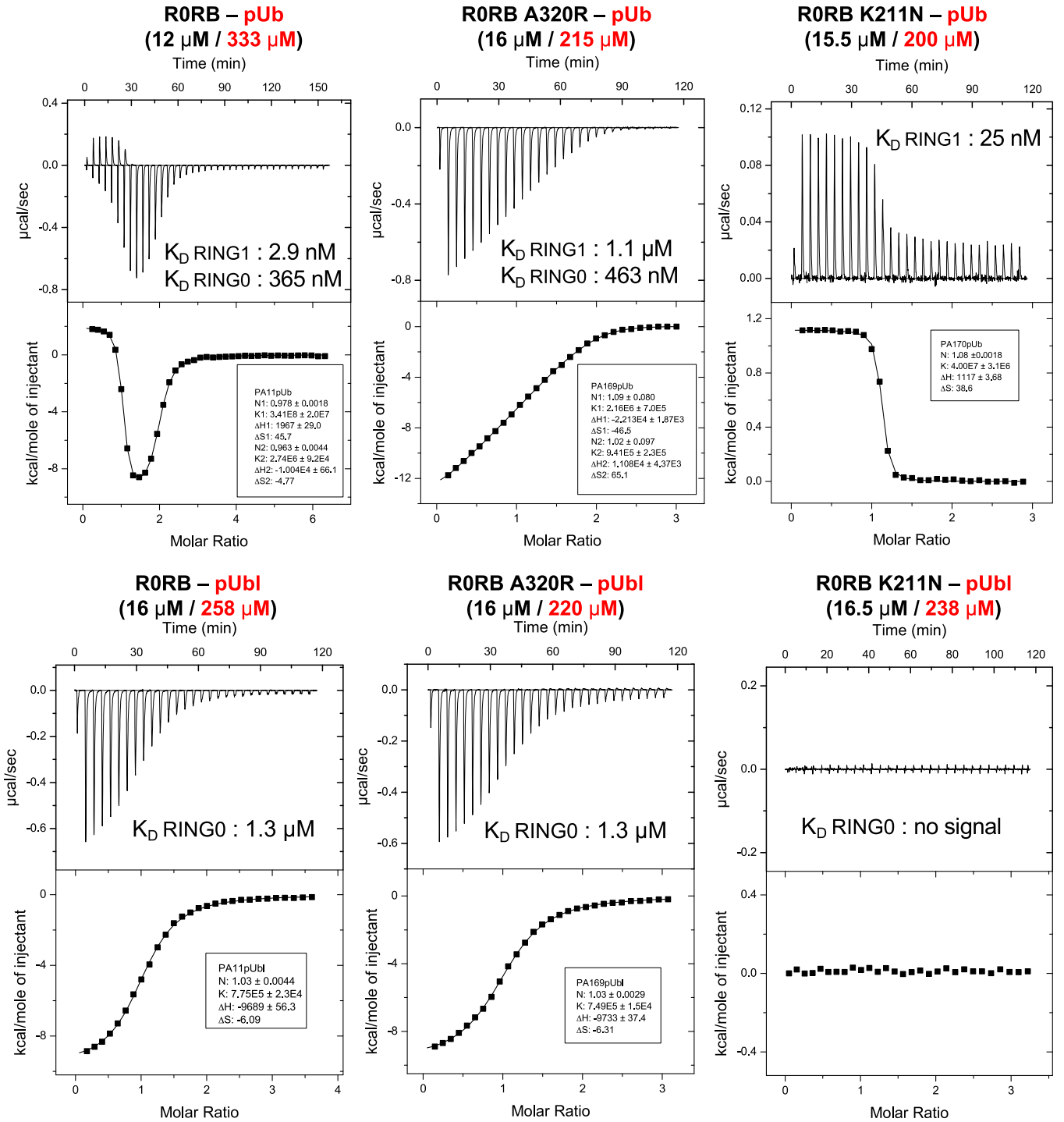

Figure EV5. Affinity of pUb and pUbl for RING0-pUbl-binding site.

ITC data and fits for the titration of rat R0RB wild-type, A320R or K211N with pUb and rat pUbl. Protein concentrations in the cell and the syringe and calculated affinities are indicated.