

## Supporting information:

### Identification and structural characterization of small molecule inhibitors of PINK1

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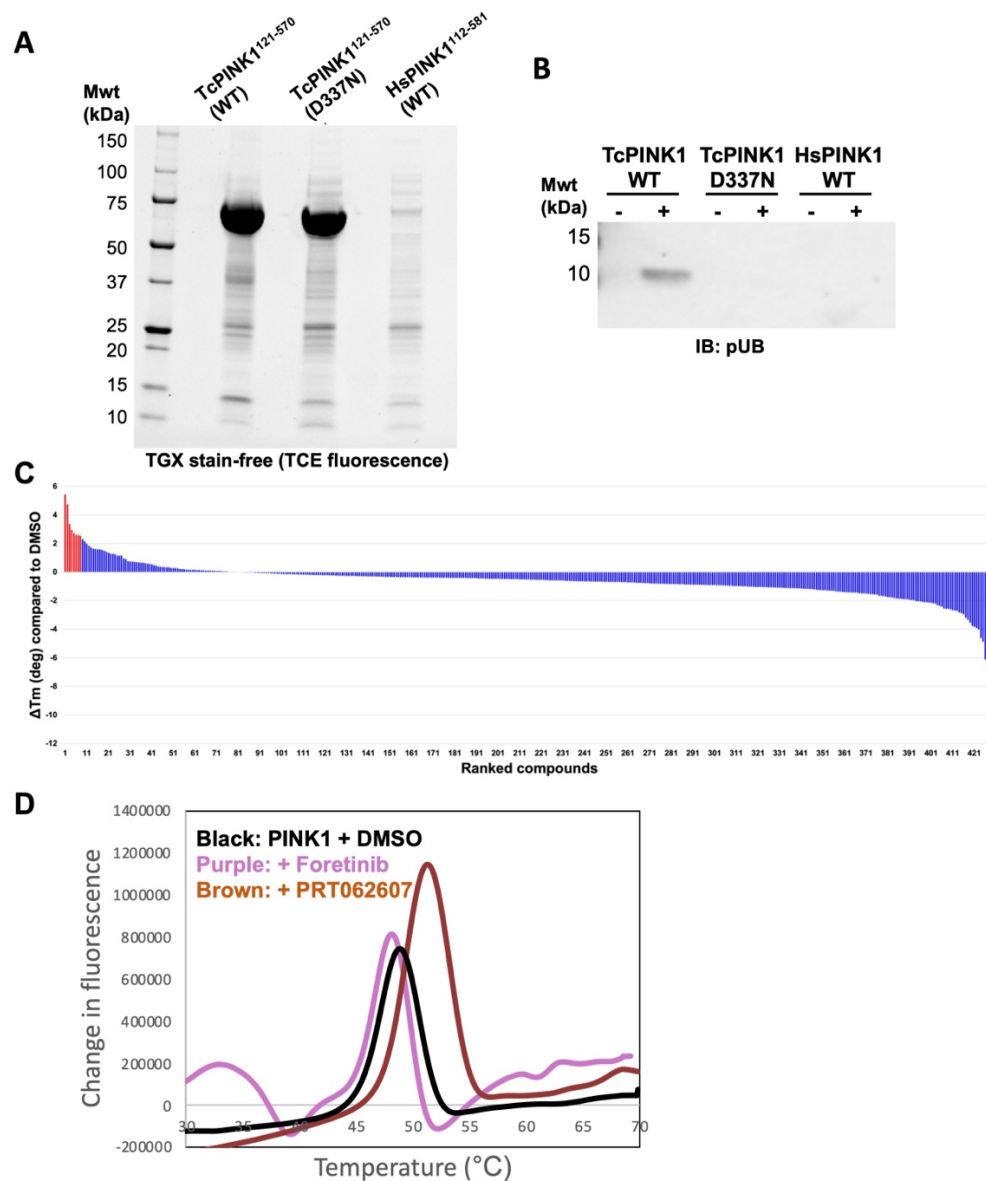
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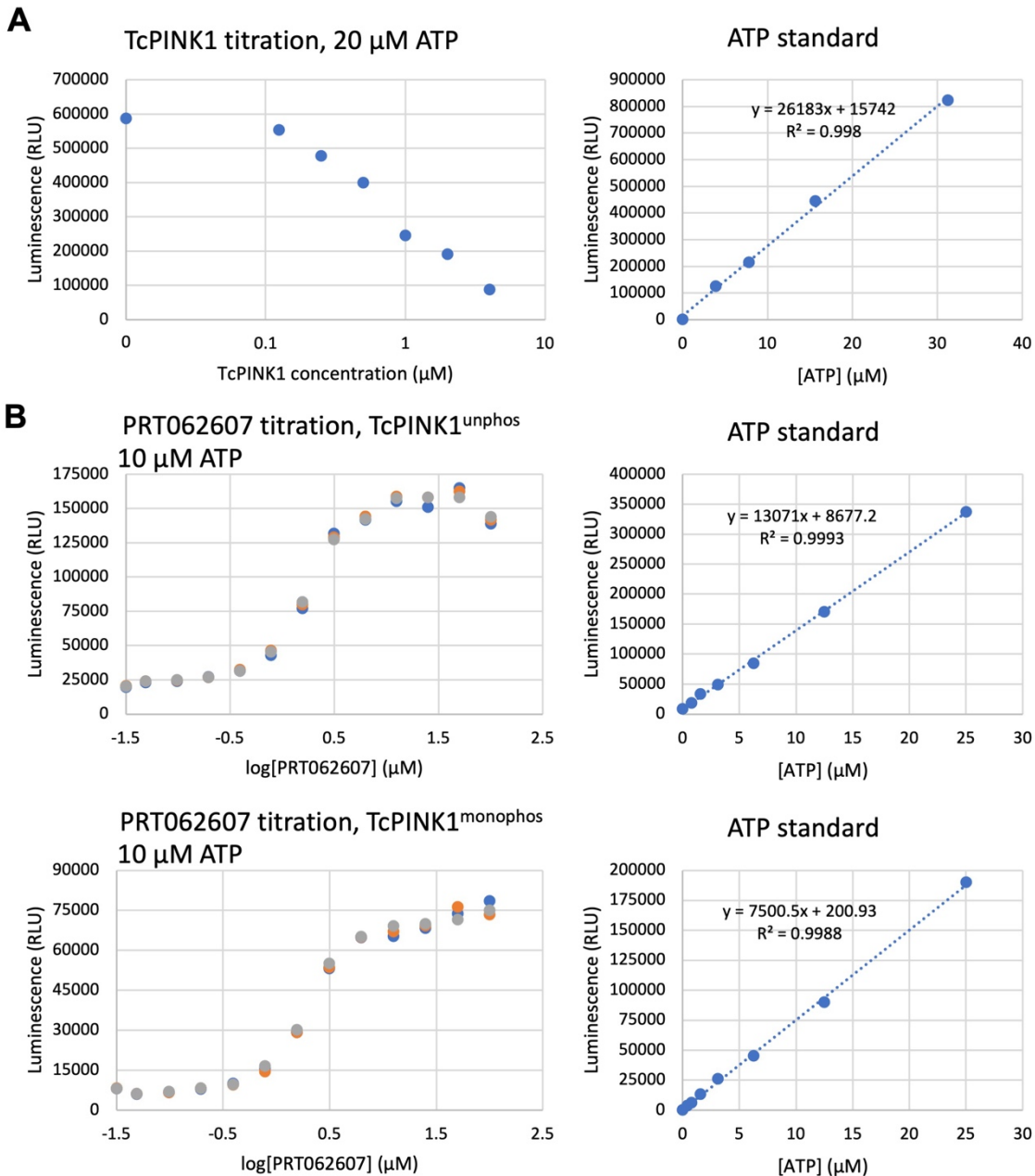
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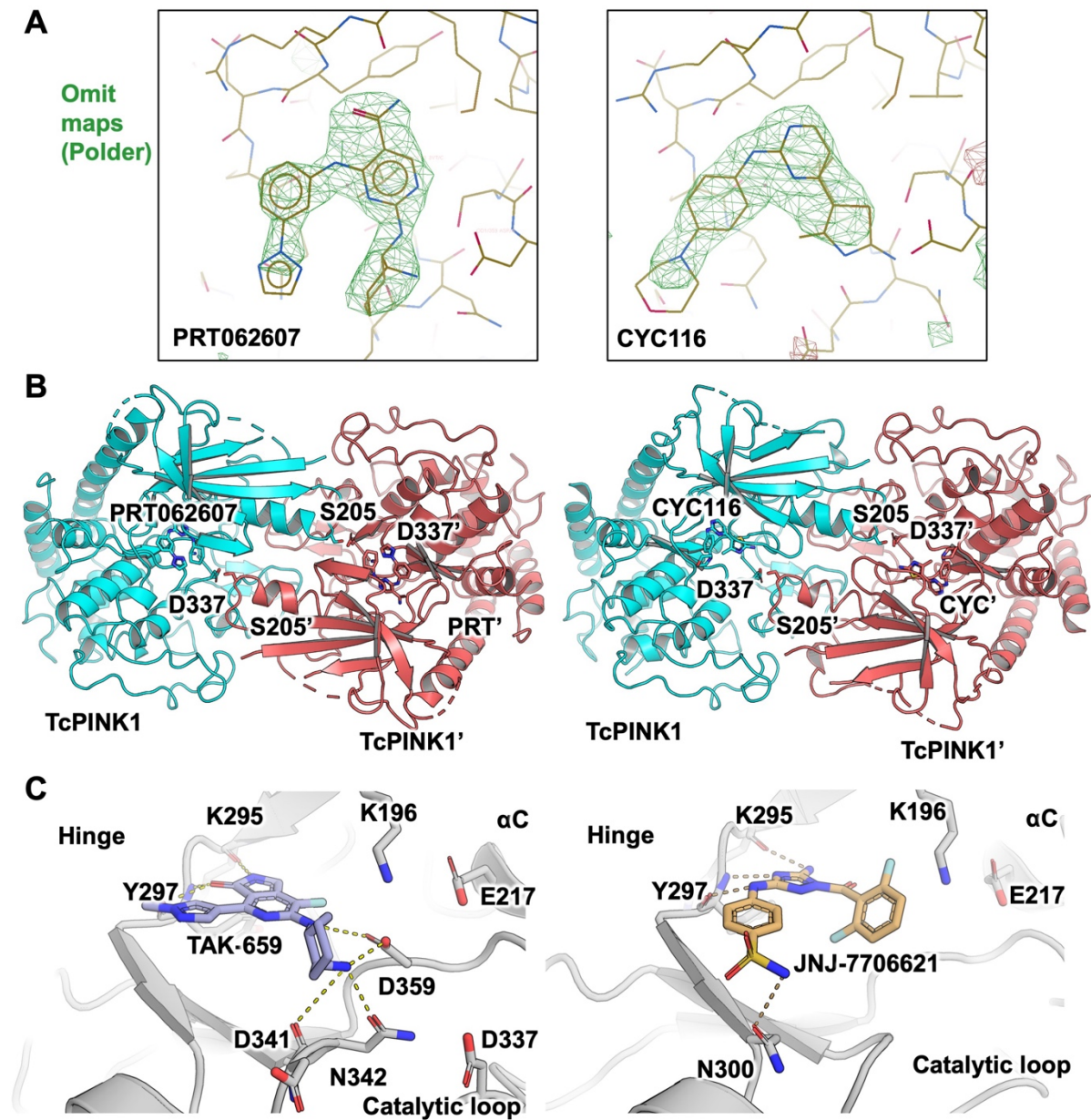
Running title: Structural characterization of PINK1 inhibitors



**Supplemental Figure S1. Thermal shift assay screening of TcPINK1 targeting molecules.** (A) Affinity purification of GST-PINK1 constructs expressed from same amount of E. coli culture. All constructs were eluted with equal volumes of elution buffer and equal volume was loaded on SDS-PAGE. D337N is a kinase-dead variant of TcPINK1. (B) Kinase assays were performed with 2  $\mu$ M of GST-PINK1 constructs and 20  $\mu$ M of ubiquitin. Products were resolved by SDS-PAGE and immunoblotted for phospho-UbS65. Results show that TcPINK1 WT phosphorylates ubiquitin, whereas TcPINK1-D337N and HsPINK1 do not. (C) Thermal shift ( $\Delta T_m$ ) values obtained for TcPINK1 incubated with Sypro Orange and 100  $\mu$ M compound derived from a subset of a SelleckChem kinase small molecule library (430 compounds). Compounds were ranked by  $\Delta T_m$  value. Top ranked compounds are shown in red. (D) Example of thermal denaturation data obtained for TcPINK1 incubated with DMSO (baseline), Foretinib (negative control) and PRT062607 (hit, positive  $\Delta T_m$ ). The first derivative of the change in fluorescence is plotted as a function of the temperature. The  $T_m$  corresponds to the peak of the change in fluorescence (inflection point).

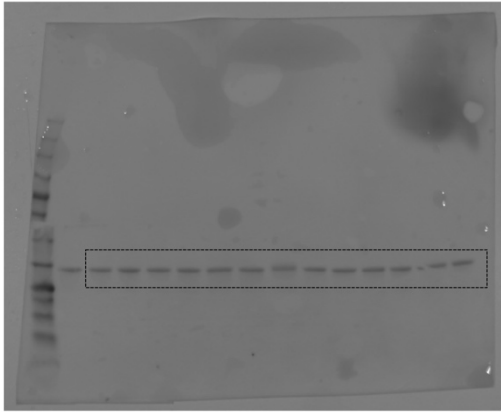


**Supplemental Figure S2. Optimization and analysis of the Kinase Glo assay for  $IC_{50}$  determination.** (A) Luminescence values observed after incubating 20  $\mu\text{M}$  ATP for 5 min in the presence of different TcPINK1 concentrations (left). The midpoint  $EC_{50}$  is around 1  $\mu\text{M}$  TcPINK1. The ATP standard curve used in this assay is shown to the right and shows linearity for the entire range of luminescence observed. (B) Raw luminescence data used for  $IC_{50}$  determination (see Figure 2D). Different concentrations of PRT062607 were incubated with 1  $\mu\text{M}$  TcPINK1<sup>121-570</sup> (unphosphorylated or mono-phosphorylated) and 10  $\mu\text{M}$  ATP for 5 min. Reactions were performed in triplicates. The ATP standard curve for each experiment is shown on the right.

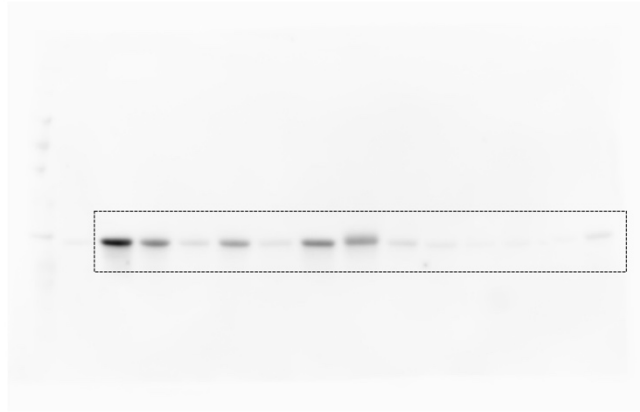


**Supplemental Figure S3. Structural analysis of TcPINK1 binding to different inhibitors. (A)** Polder ligand omit maps ( $3.5 \sigma$ ) calculated from the crystal structures TcPINK1<sup>121-570</sup> bound to PRT062607 (left) and CYC116 (right). **(B)** Crystallographic symmetry reveals face-to-face trans autophosphorylation complex in both PRT062607 and CYC116-bound TcPINK1, as observed previously for the apo and AMP-PN bound structures (PDB: 7MP8 and 7MP9). **(C)** Docking models of TcPINK1 bound to TAK-659 (left) or JNJ-7706621 (right), showing their interactions with the hinge, A-loop and catalytic loop. The apo structure of TcPINK1<sup>121-570</sup> (PDB 7MP8) was used for docking the small molecules using the software *DiffDock*.

**B**

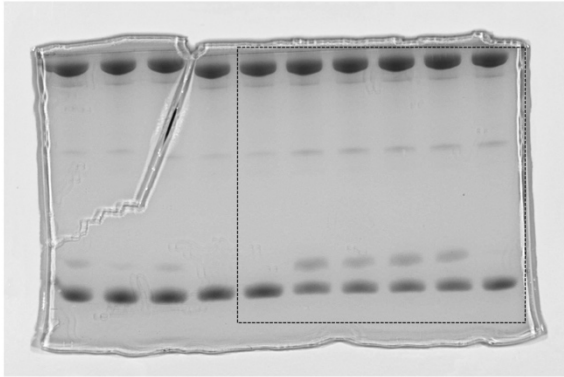


**Ponceau**



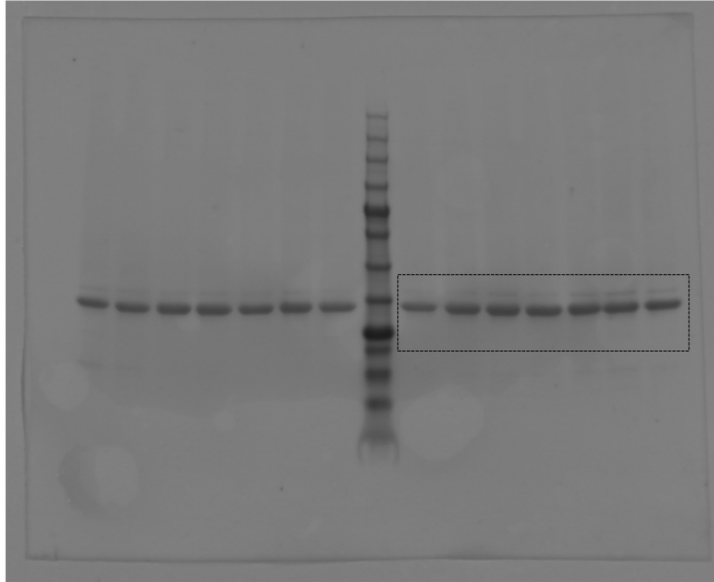
**IB: pUb**

**C**

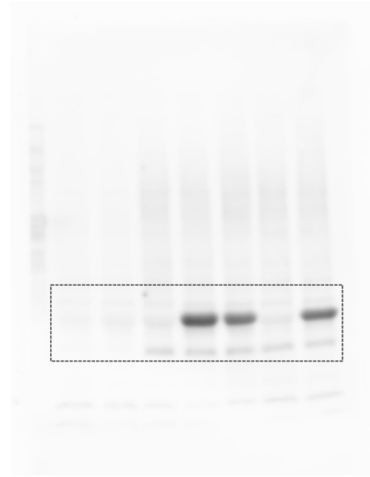


**Coomassie Blue (Phos-tag gel)**

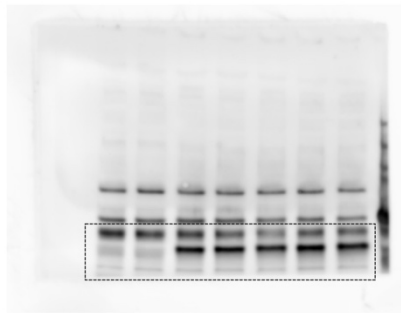
**Supplemental Figure S4. Uncropped images for the immunoblots shown in Figure 1B,C.**  
All lanes are loaded in the same order as the corresponding figure in the paper.



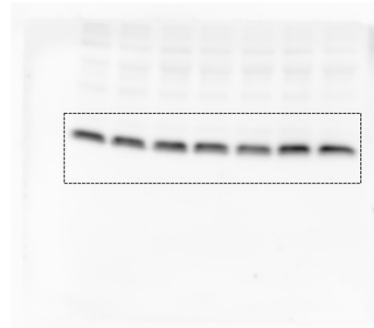
Ponceau



IB: pUb



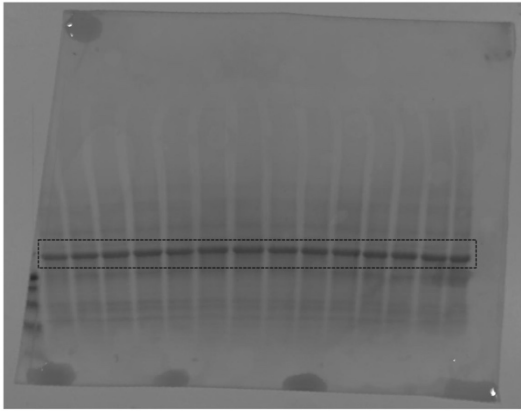
IB: PINK1



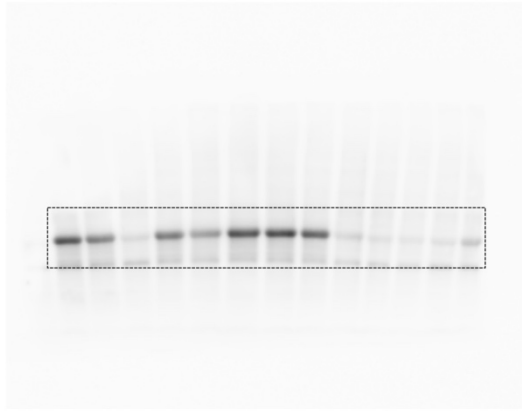
IB: CytC

**Supplemental Figure S5. Uncropped images for the immunoblots shown in Figure 3A. All lanes are presented in the same order as the corresponding figure in the paper.**

**B**

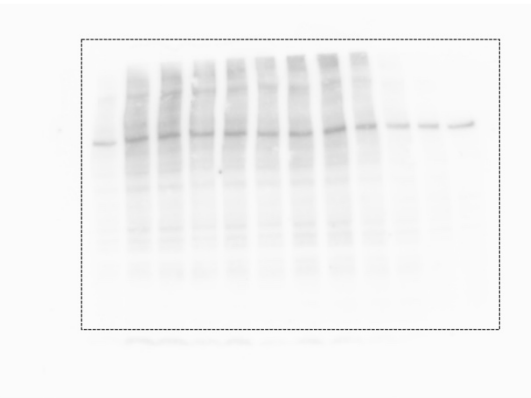


**Ponceau**

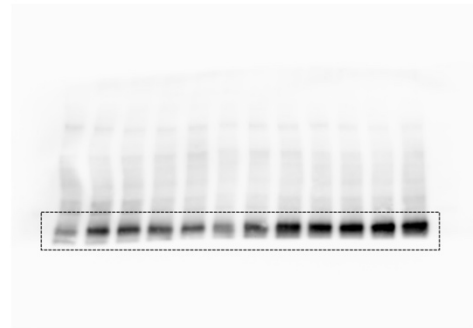


**IB: pUb**

**C**



**IB: pUb**

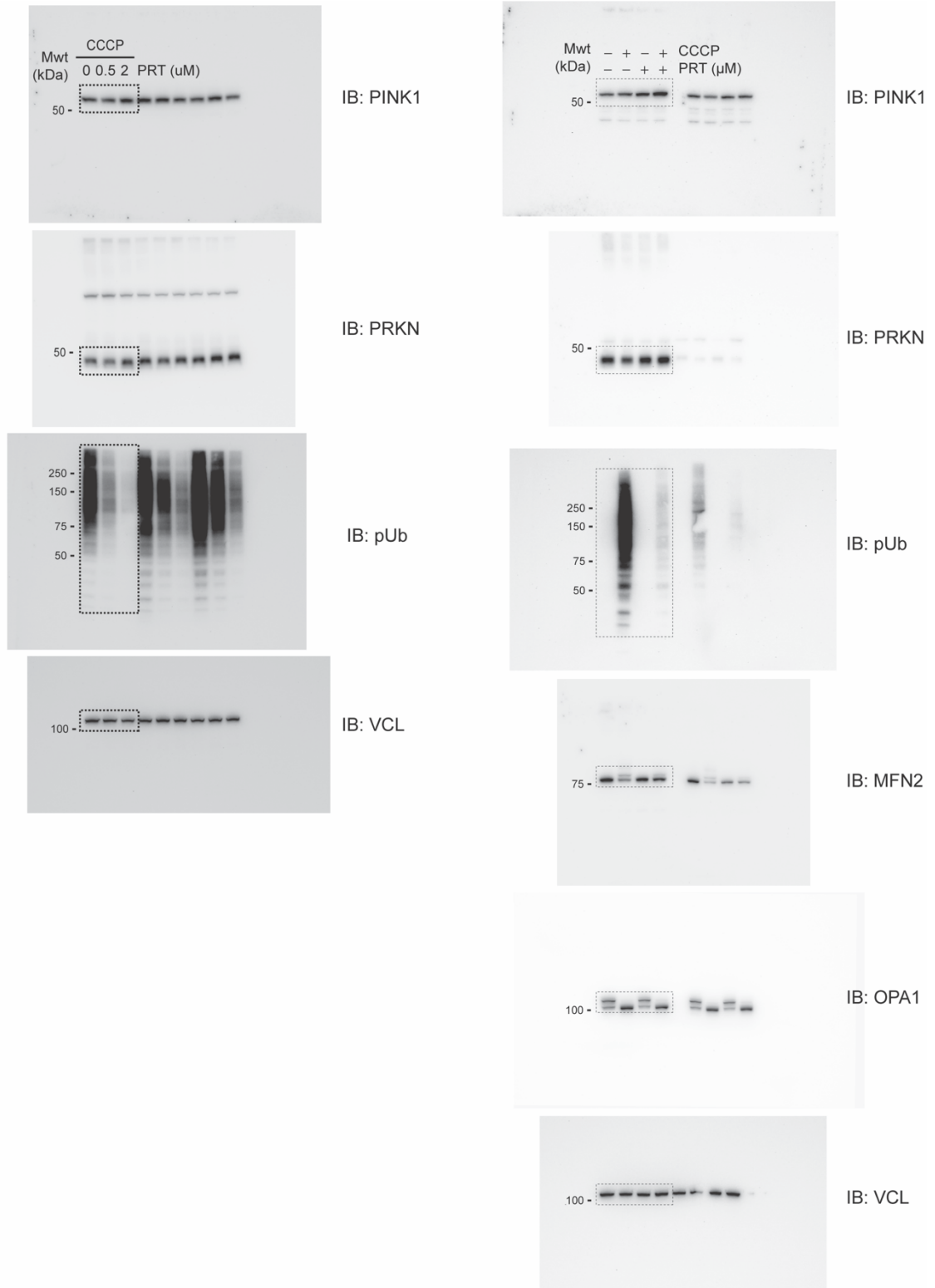


**IB: HA (PINK1)**



**IB: TOM40**

**Supplemental Figure S6. Uncropped images for the immunoblots shown in Figure 3B,C.**  
All lanes are presented in the same order as the corresponding figure in the paper.



Supplemental Figure S7. Uncropped images for the immunoblots shown in Figure 3D.