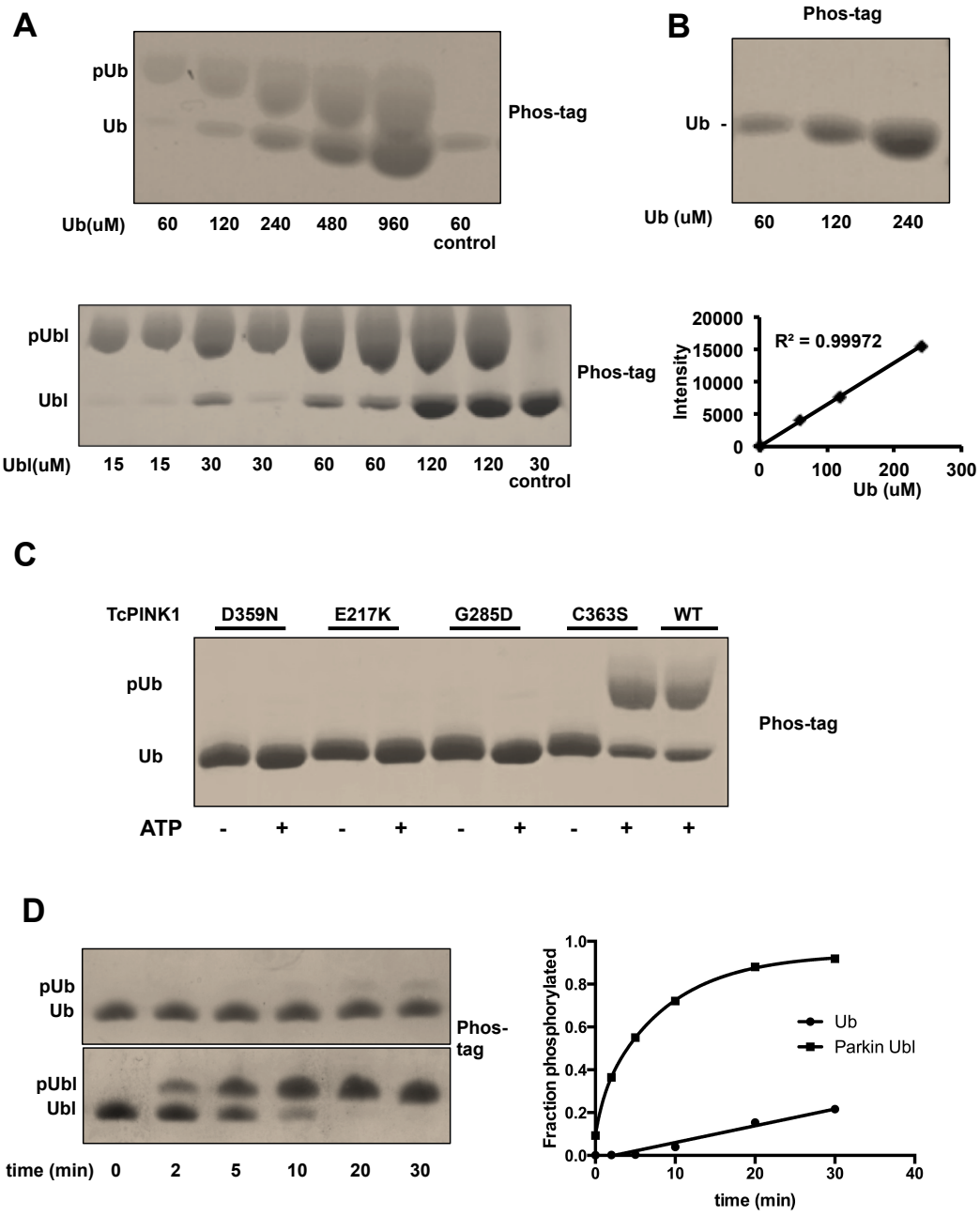
Appendix for:

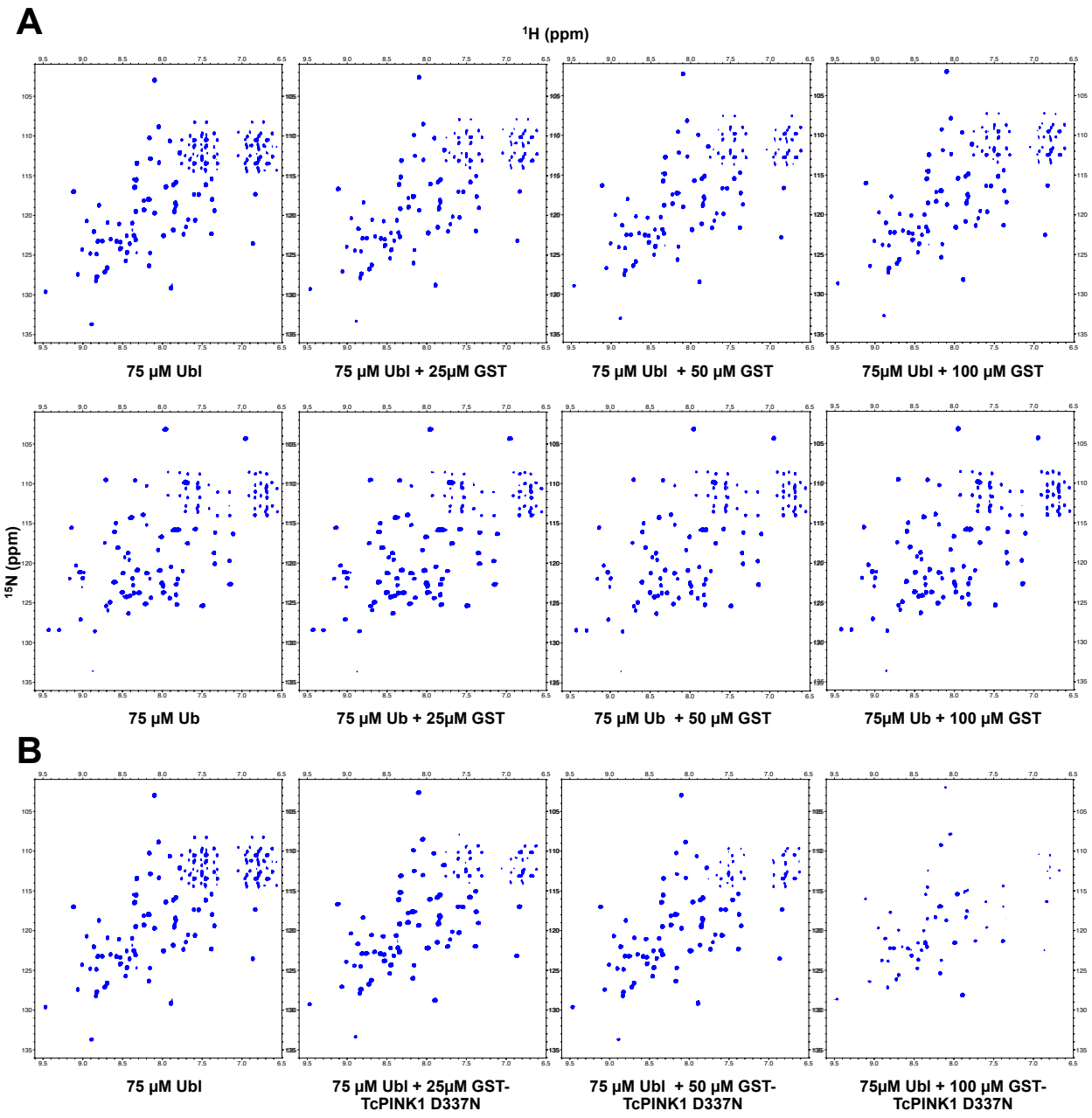
PINK1 autophosphorylation is required for ubiquitin recognition

Shafqat Rasool[1,2], Naoto Soya[3,#], Luc Truong[1,#], Nathalie Croteau[1], Gergely L Lukacs[2,3] & Jean-François Trempe[1,*]

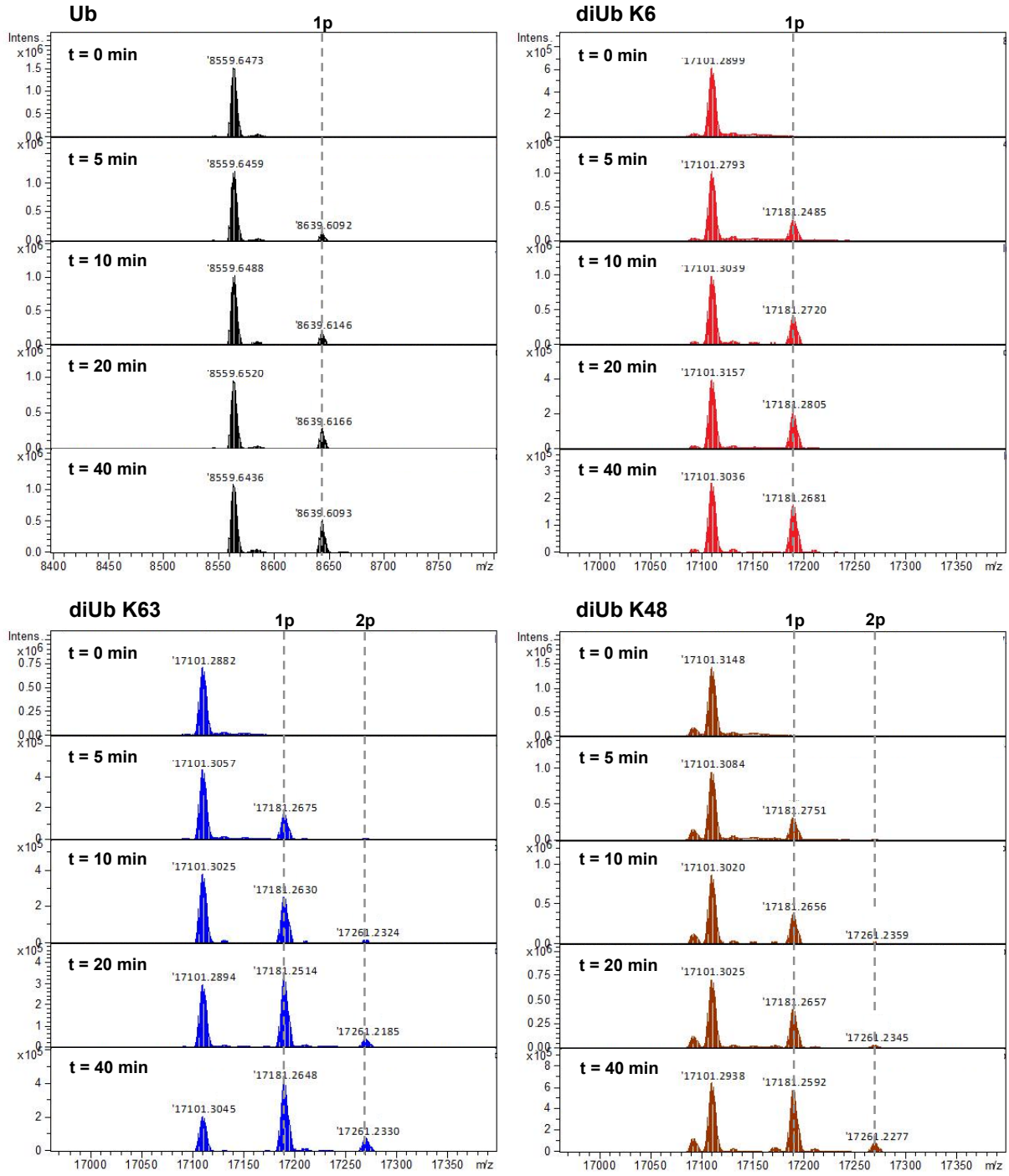
Table of Content



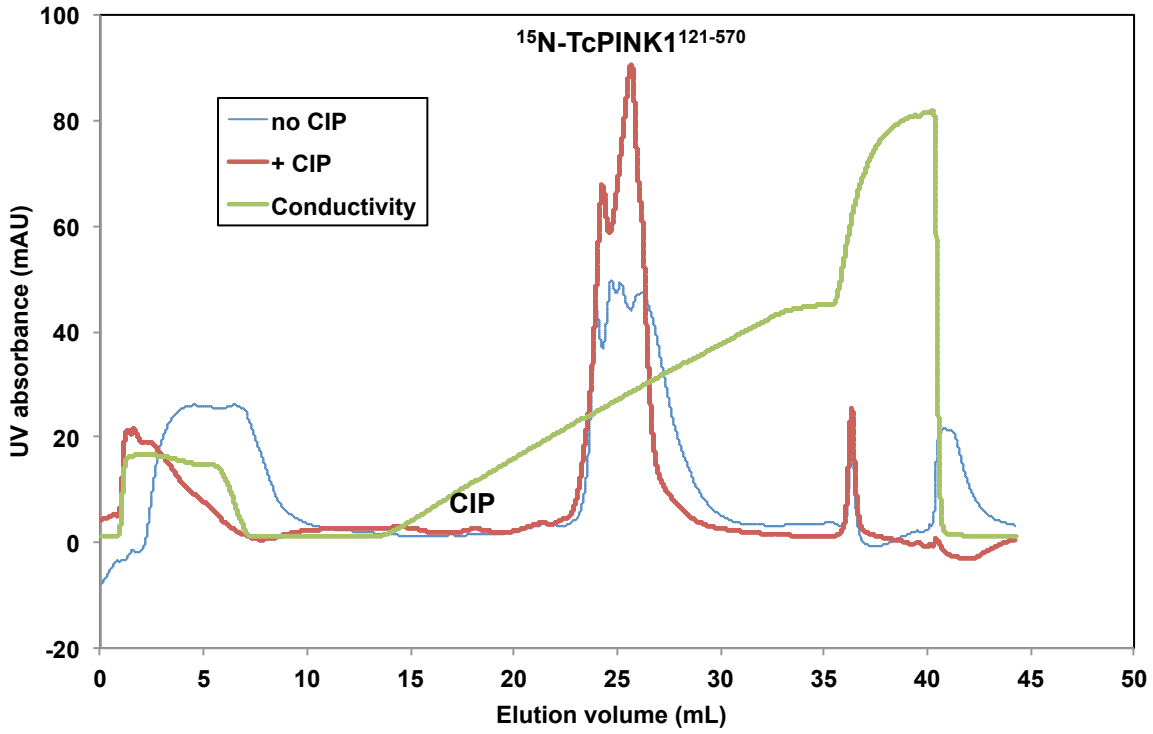
Appendix Figure S1 - Enzyme kinetics and phosphorylation assays with PD mutants.
A Phosphorylation assays with different concentrations of Ub and Parkin Ubl domain were conducted for 5 min and analyzed using phos-tag gels corresponding to the kinetics data shown in Fig 1A. Only one of the reaction sets for Ub is shown in top panel.
B (Above) Phos-tag gel loaded with different concentrations of Ub and (below) the band intensities analyzed graphically. The R^2 value represents the fit of the intensities to the linear line.
C Phosphorylation of Ub by PD mutants of PINK1. 30 min kinase assays were conducted with 2.5 μM GST-TcPINK1 (WT or mutant) and 120 μM His-Ub observed and loaded on phos-tag gels.
D (Left) Phos-tag gels and (right) graphical analysis of time course phosphorylation experiments conducted with 30 μM Parkin Ubl or Ub and 0.2 μM Full-length TcPINK1.



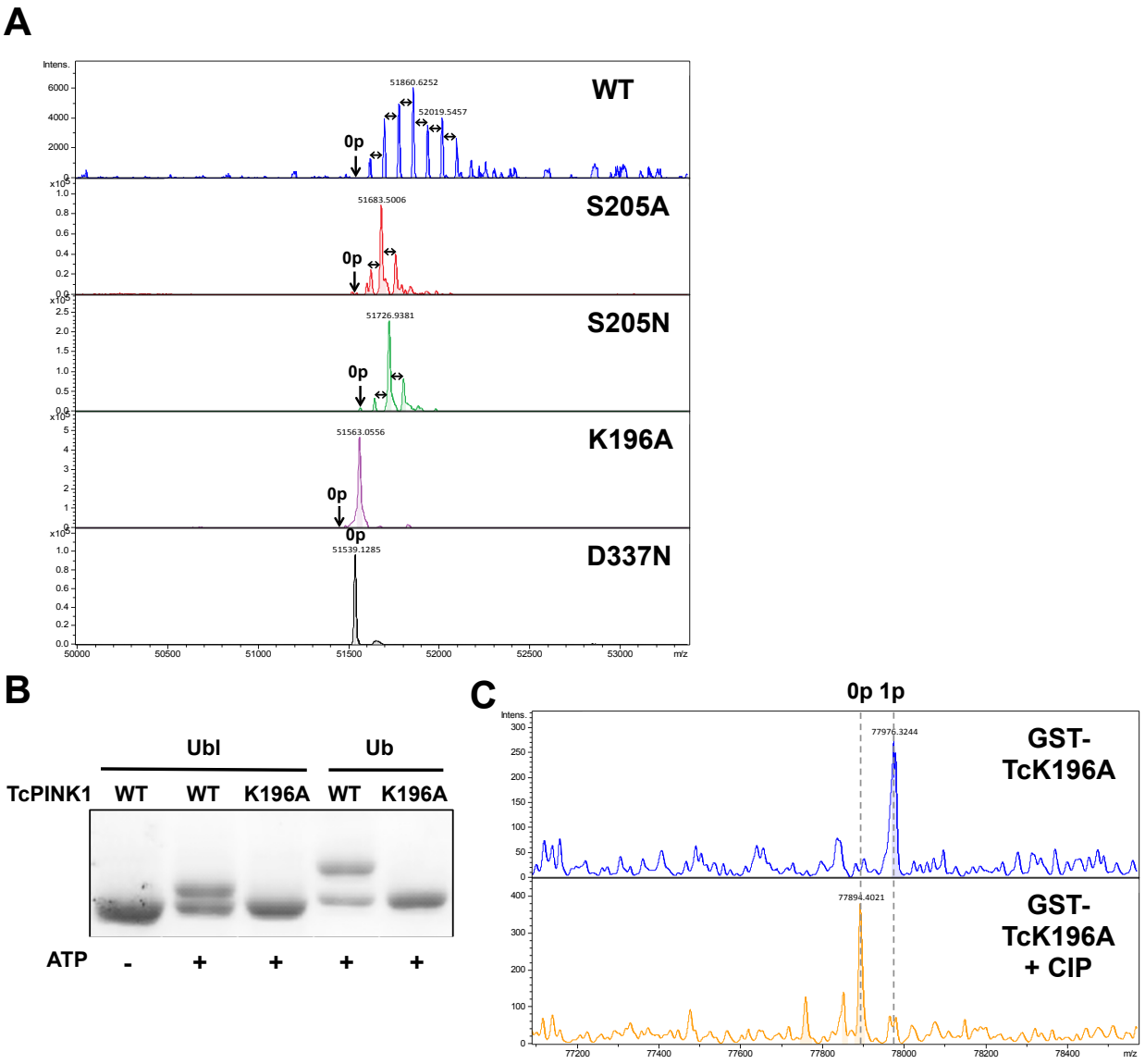
Appendix Figure S2 - Full TROSY NMR for GST and GST-TcPINK1 D337N titrations.
A Full ^{1}H-^{15}N TROSY NMR spectra of ^{2}H,^{15}N-labeled Ubl or Ub with different concentrations of GST.
B Full ^{1}H-^{15}N TROSY NMR spectra of ^{2}H,^{15}N-labeled Ubl with different concentrations of GST-TcPINK1 D337N.



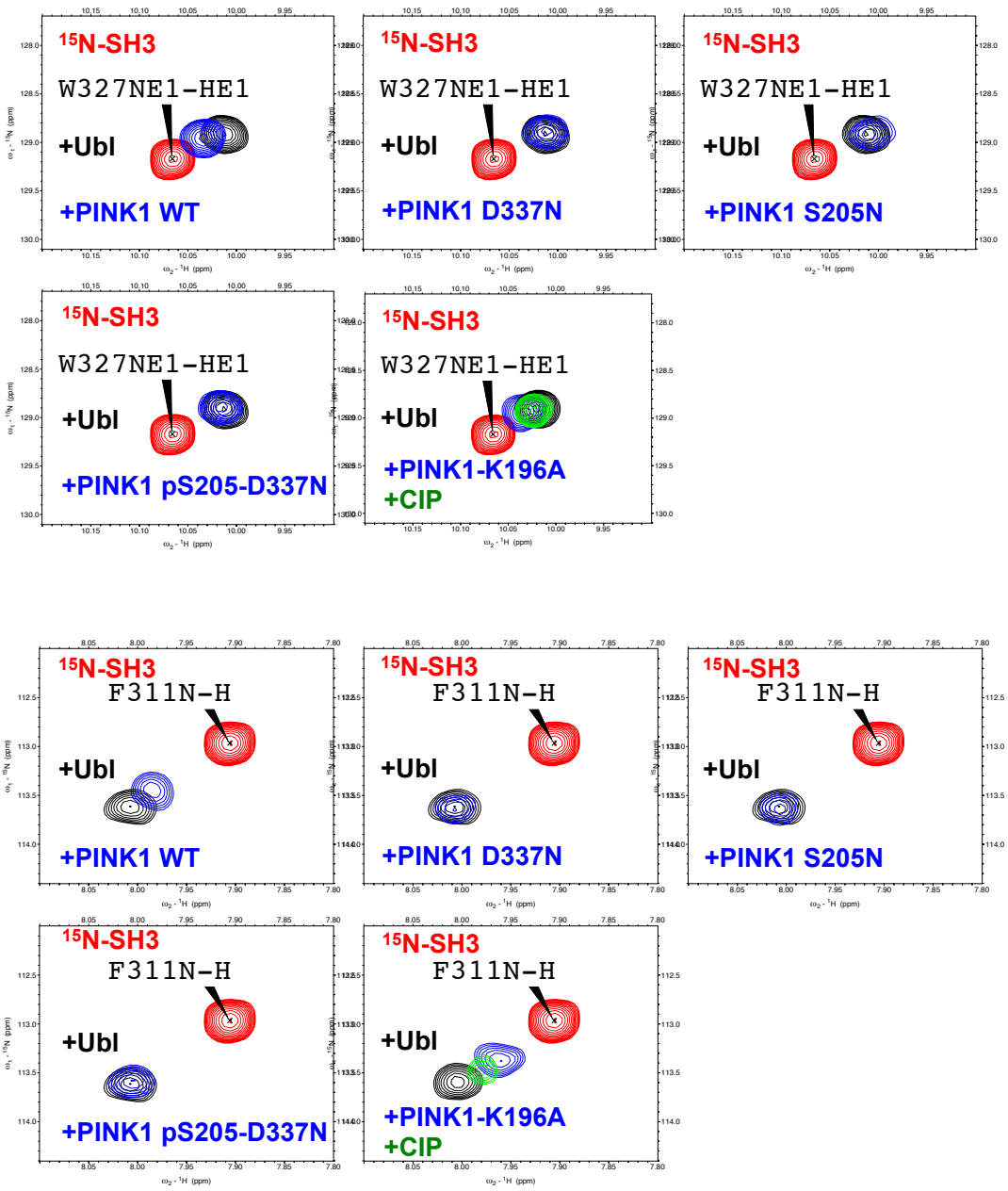
Appendix Figure S3 - Intact mass spectrometry analysis of mono Ub and Ub_2 phosphorylation. Intact mass spectra of the time course phosphorylation experiments of mono and Ub_2 (K6-, K48- and K63-linked) corresponding to Figure 1E. The masses shown on the figures represent the monoisotopic masses, calculated from the isotopomer peaks.



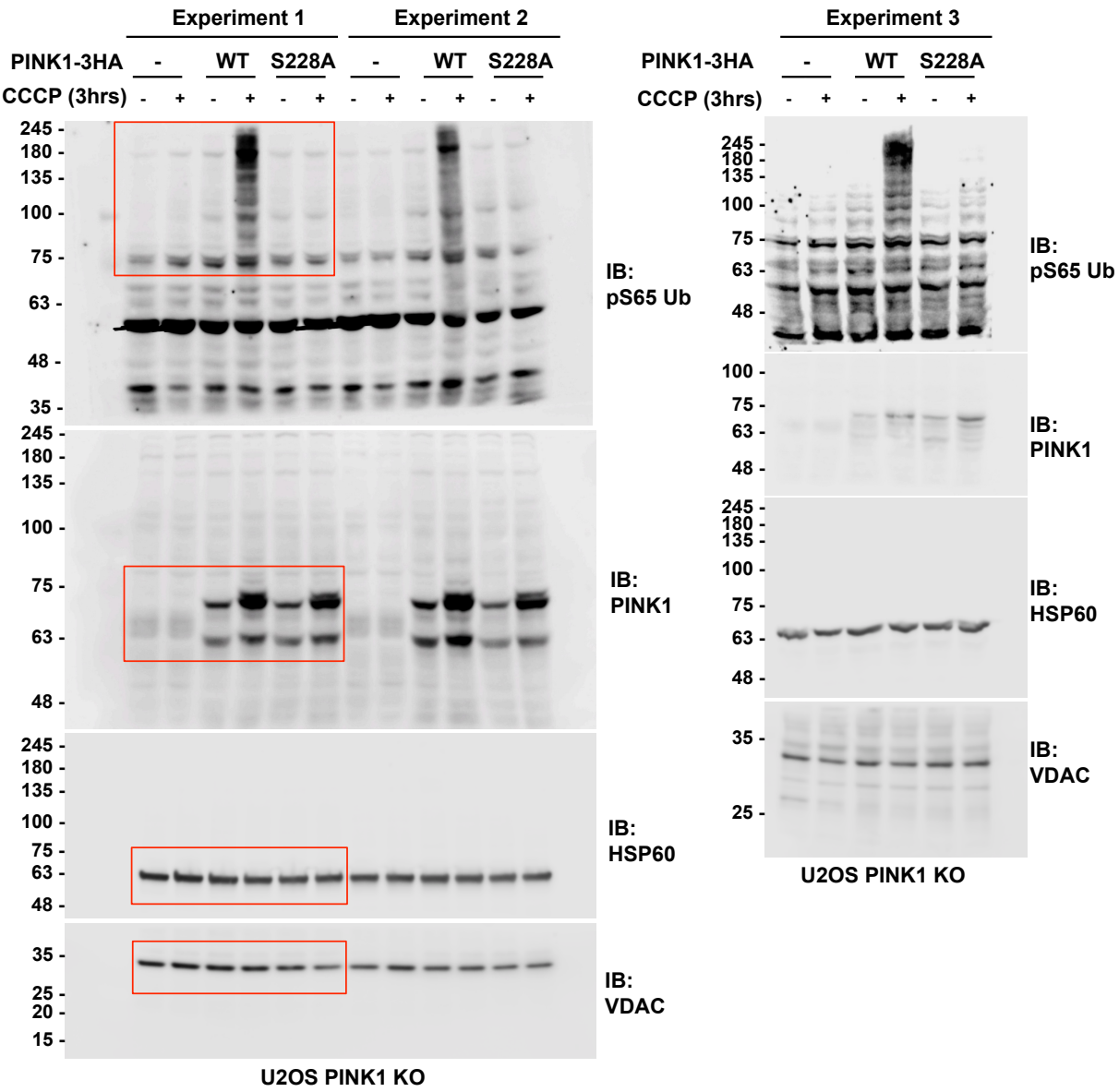
Appendix Figure S4 - Purification of CIP-treated TcPINK1.
^{15}N-labeled TcPINK1 incubated with CIP (or not) was loaded on a monoQ 5/50 column and eluted with a 0-0.5M NaCl gradient over 20 min. CIP elutes at 17 min, whereas TcPINK1 elutes later at 25 min. Protein elution was monitored by UV at 280 nm.



Appendix Figure S5 - Phosphorylation analysis of TcPINK1 WT and mutants.
A Intact mass spectra of TcPINK1 WT and mutants expressed in *E. coli* for 20 h. The double-edged arrows on the spectra are used to indicate a difference of 80 Da (1 phosphorylation) between peaks. '0p' indicates the theoretical mass of the unphosphorylated protein.
B Phos-tag gels of phosphorylation assays of 30 µM GST-tagged Ubl (5 min) or Ub (30 min) with 0.5 µM GST-TcPINK1 WT or K196A.
C Intact mass spectra of GST-TcPINK1 K196A before and after 30 min CIP treatment.

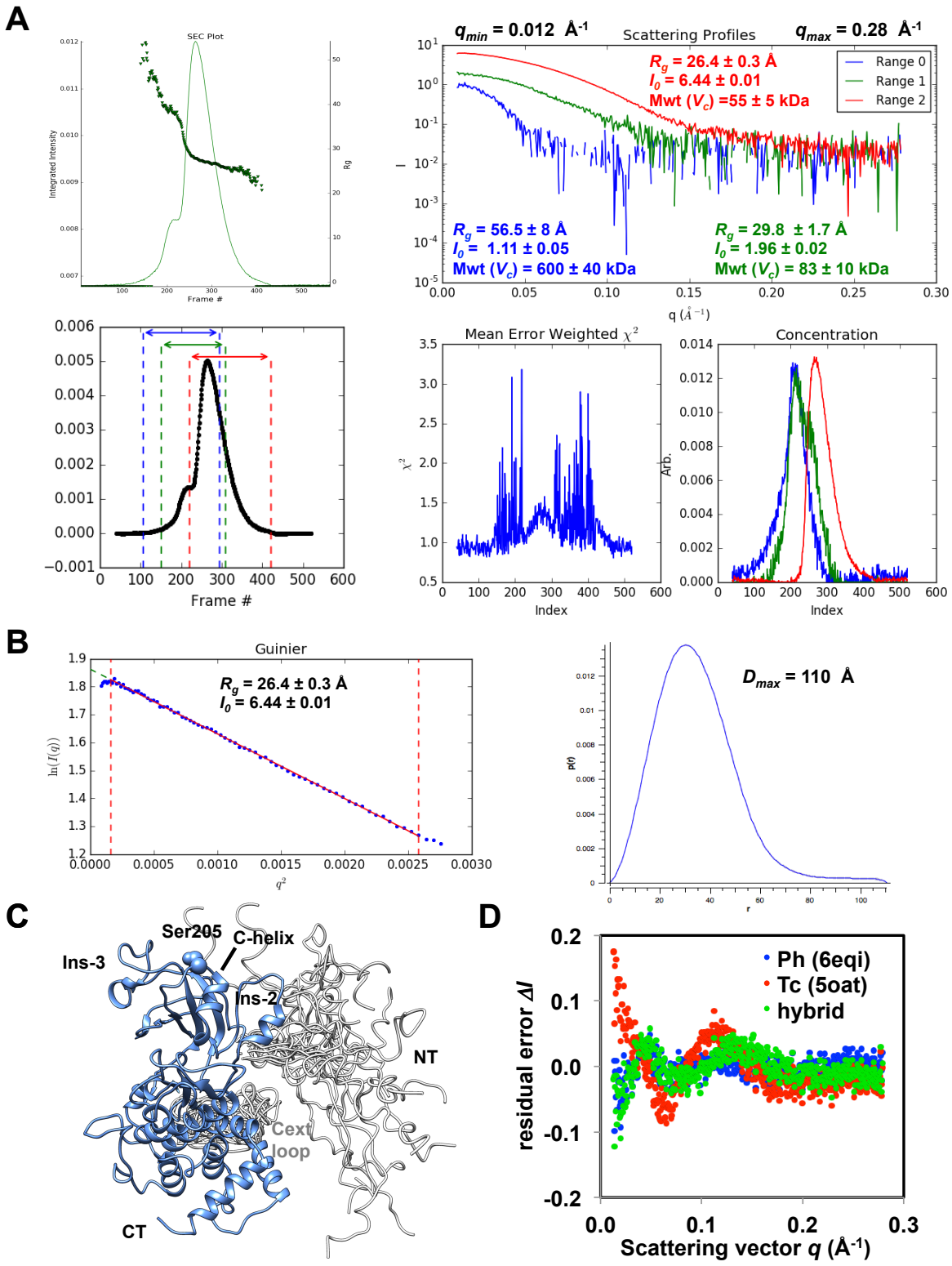


Appendix Figure S6 - NMR competition assays of TcPINK1 mutants with ^{15}N-SH3 for Ubl binding. ^{1}H-^{15}N HSQC NMR spectra of competition assays between ^{15}N-labeled Endophilin-A1 SH3 domain and different forms of GST-TcPINK1 for Ubl. The peaks represent the signals from two different peaks from the spectra of 40 μM SH3 alone (red), following the addition of 48 μM Ubl (black), 48 μM Ubl and 100 μM GST-TcPINK1 (blue), or 48 μM Ubl, 100 μM GST-TcPINK1 and CIP (green). The average relative chemical shifts from 9 peaks from each of these experiments was used for quantification (Fig 4C).



Appendix Figure S7- Immunoblots for the experiments with U2OS cell lines

Full immunoblots of PINK1, phospho-Ser65 Ub, HSP60 and VDAC from all 3 experimental replicates. The red squares indicate regions of the blots used in Fig 4D. The blots for phospho-Ser65 Ub and HSP60 were used for the quantification.



Appendix Figure S8 - SAXS analysis and model fitting of TcPINK1$^{121-570}$.
A Evolving Factor Analysis (EFA) of TcPINK1$^{121-570}$ D337N resolved on size-exclusion chromatography coupled to SAXS. Top-left : Integrated scattering intensity plotted as function of elution volume. The green triangles correspond to the Guinier-derived R_g values. Bottom-left : EFA analysis with three components,

showing the elution volume range (index #) for each species. Top-right : scattering curves for the three EFA components, with corresponding R_g and molecular weight (V_c-derived) values. Bottom-right : Mean-error weighted χ^2 values between experimental data and the EFA model as a function of frame #, and intensity of each component.
B Primus-analysis of the EFA component corresponding to the monomer of TcPINK$^{121-570}$. Left: Guinier analysis showing a straight line, indicating monodispersity. Right: Pair-distance distribution function, showing a symmetrical pattern characteristic of globular proteins.
C Ensemble of twenty TcPINK$^{121-570}$ models derived from the PhPINK1 crystal structure (pdb 6eqi) computed with the software CORAL, which fit best the SAXS data. The rigid-components (a.a. 151-182, 191-262, 287-515, 536-570) are colored in blue, and the flexible linkers are colored in white.
D Residual error for the three scattering profiles shown in Fig 5B.