Supporting Information

Structural and kinetic analysis of prolyl-isomerization/phosphorylation cross-talk in the CTD code

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Running Title: Complex structures of human Pin1 and substrate analogue inhibitors

Supplementary Figure Legends

Supplementary Figure 1. Overall structure of Pin1 represented by cartoon and surface. The PPIase domain is colored light pink, and the WW domain is colored light blue. The linker in between of the two domains is missing due to its high flexibility. The residues, Lys63, Arg68 and Arg69, that bind phosphate group is shown as sticks. The red arrow indicates the pocket that recognizes proline. PEG molecule is shown in green and red as sticks.

Supplementary Figure 2. Superimposition of multiple Pin1 structures published in PDB shows “open” and “closed” conformations of the loop containing Arg68. The ones that have ligand bound at the phosphate-binding pocket (red arrow) include 1pin (green), 2itk (cyan), 1nmw (magenta), and 3jyj (yellow). The structure with PDB code 1f8a (pink) has no ligand at the

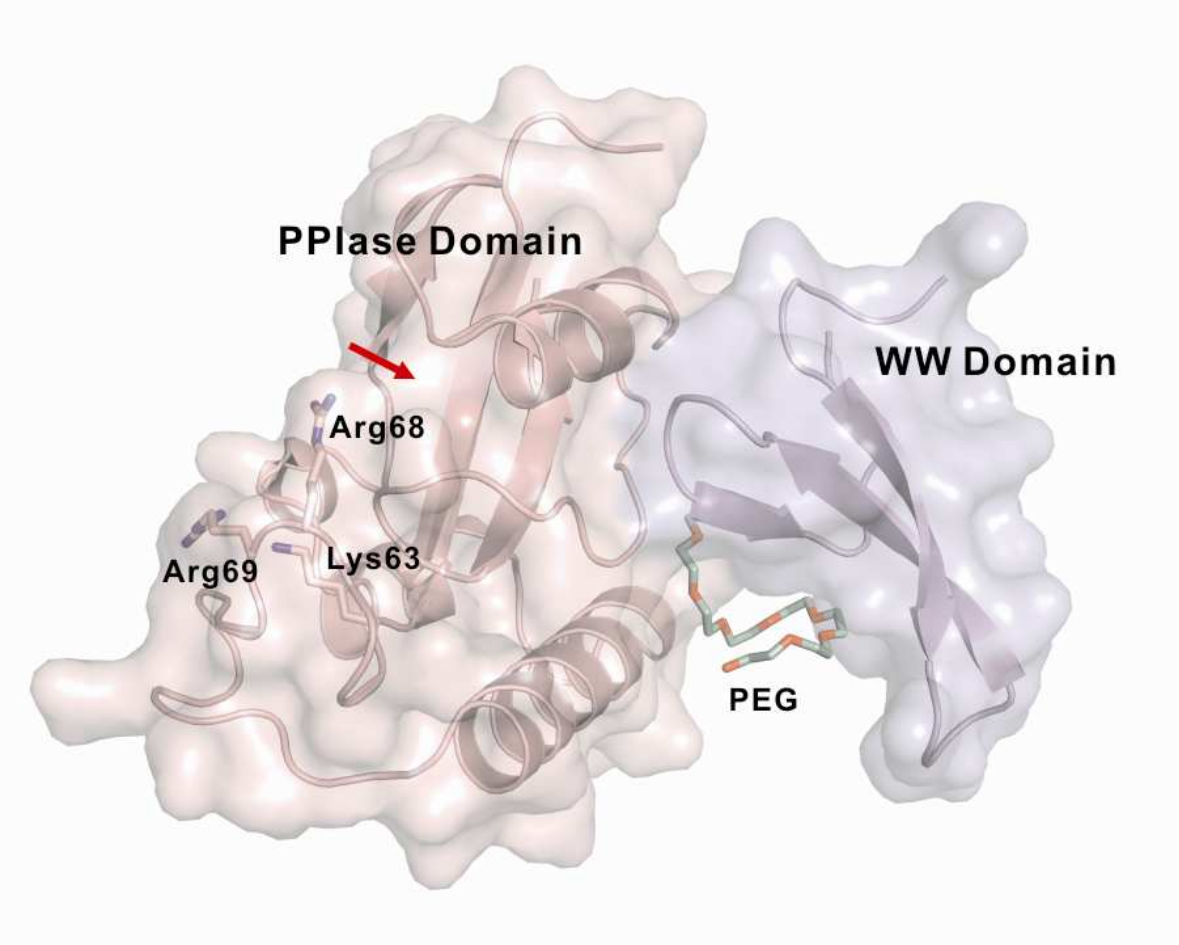
phosphate-binding pocket, possessing the loop swung out about 23.5 Å from the “closed” conformation.

Supplementary Figure 3. Superimposition of the active sites of the human (light blue, PDB code: 3o2q) and *Drosophila* (light orange, PDB code: 3omw) Ssu72. The three key residues at the active site are shown as sticks. The nucleophile Cys12 was mutated to Ser in the human Ssu72 in order to obtain the complex structure with the peptide (shown as yellow sticks).

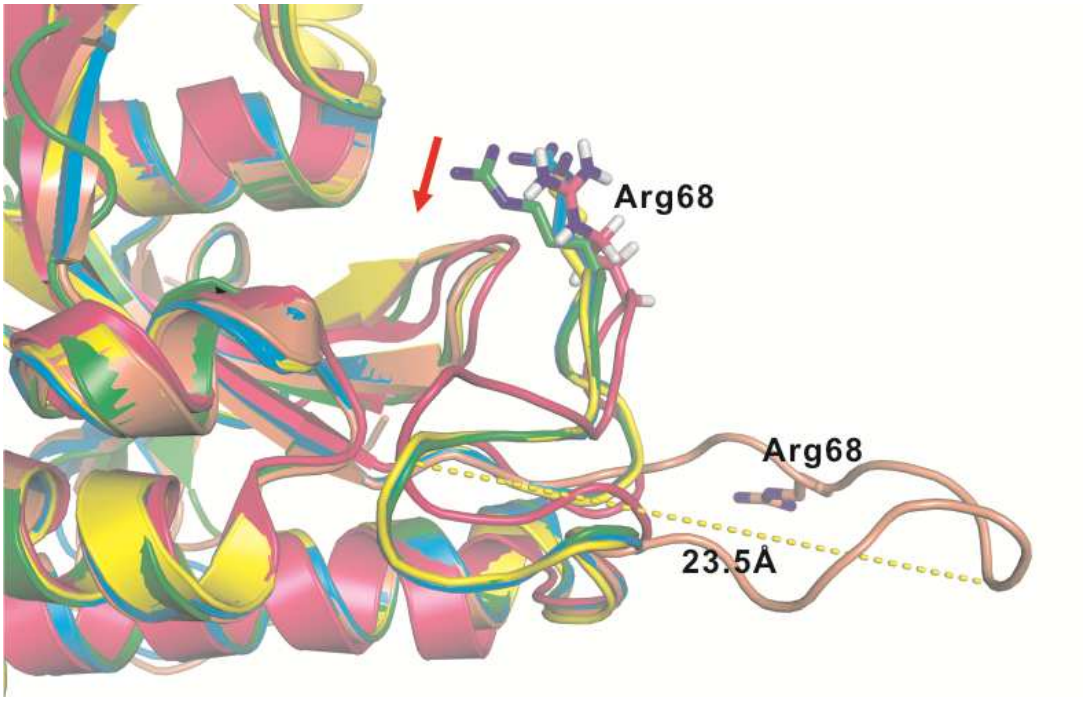
Supplementary Figure 4. Gel filtration traces of Ssu72 (~23 kDa), Pin1 (~18 kDa) and Ssu72-Pin1 mixture generated by Superdex 75 column (GE Healthcare). The wild-type *Drosophila* Ssu72 and human Pin1 were purified by following the same procedure described in **Methods**. Ssu72-Pin1 mixture was prepared by mixing nearly equal molar concentration (~300 μM) of Ssu72 and Pin1 in 4 °C and incubated for 6 hr. The arrows indicate where the standard 40 kDa and 20 kDa protein should be.

Supplementary Figure 5. Hydrogen bond (green dashed line) formed between the carbonyl of proline (or proline analogue) and the amide of Gln131. Shown here is an example from complex structure of Pin1 and L-PEPTIDE (PDB code: 2q5a).

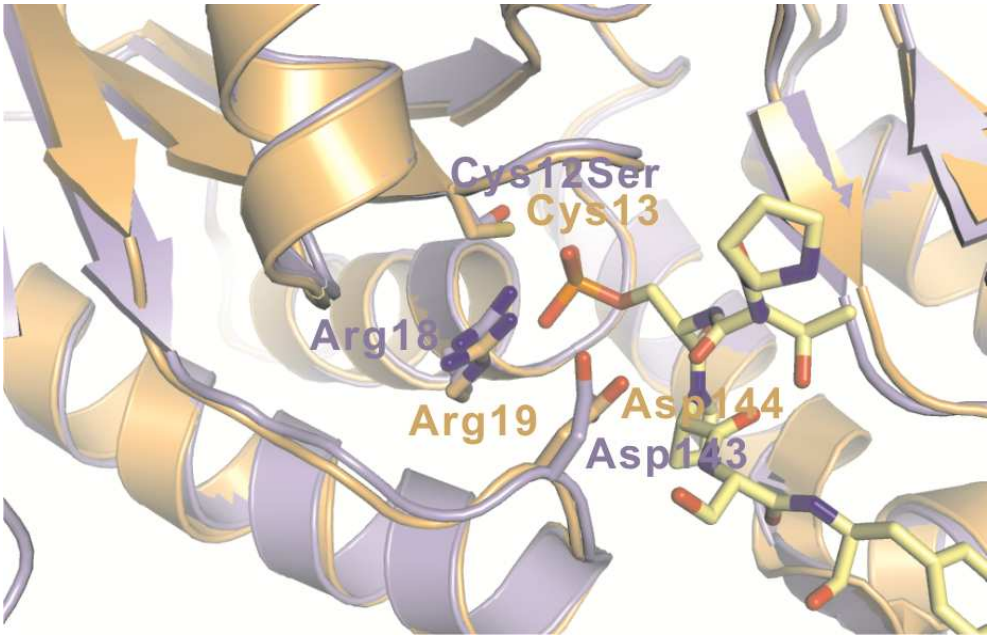
Supplementary Figure 1



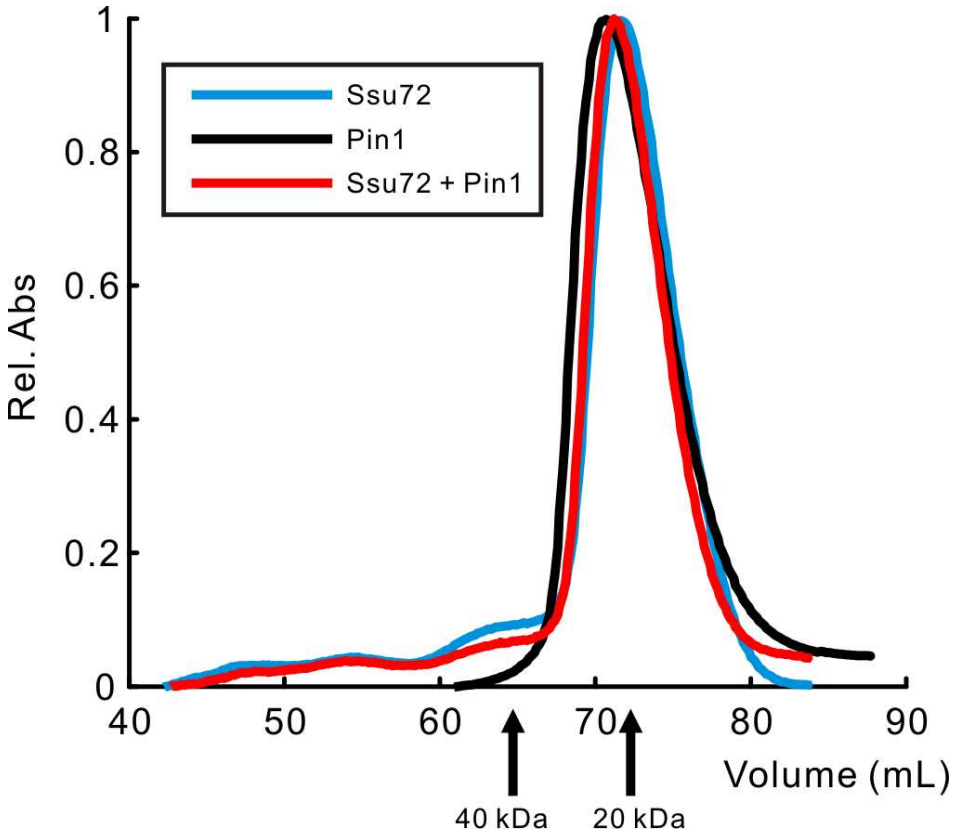
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5