



# Structure of the PUB domain from Ubiquitin Regulatory X domain protein 1 (UBXD1) and its interaction with the p97 AAA+ ATPase

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## **Supplemental Material**

- Figure S1: <sup>15</sup>N-HSQC-NMR titrations of UBXD1-PUB with p97 PIM peptides
- **Figure S2**: Fluorescence anisotropy titrations for all UBXD1-PUB mutants.
- **Figure S3**: Superposition of UBXD1-PUB and PNGase-PUB apo structures showing the H-bonding network around UBXD1-Y194 that is not present in PNGase.
- **Figure S4**: Superposition of the UBXD1-PUB/p97-PIM model and the crystal structures of PNGase and HOIP in complex with p97-PIM showing the position of the PIM peptide.
- Table S1. Oligonucleotides used as primers for PCR-amplification of the UBXD1-PUB (150-264) construct and for site-directed mutagenesis following the Quikchange protocol.

Figure S1



**Figure S1.** <sup>15</sup>N-HSQC-NMR titrations of UBXD1-PUB with p97 PIM peptides. (**A**) <sup>15</sup>N-HSQC titration of <sup>15</sup>N-labelled UBXD1-PUB with p97-C10 PIM peptide. Peptide concentrations range from 0  $\mu$ M (black) to 1.1 mM (red). Peaks shift during the titration, indicating intermediate-to-fast-exchange. The trajectories of signals which experience line broadening due to intermediate exchange are indicated with an arrow; (B) <sup>15</sup>N-HSQC titration of <sup>15</sup>N-labelled UBXD1-PUB with phosphorylated p97-C13 PIM peptide shows no binding. Peptide concentrations range from 0  $\mu$ M (black) to 1.1 mM (red).





**Figure S2.** Fluorescence anisotropy binding curves of FAM-labeled p97-C10 peptide with UBXD1-PUB mutants and of UBXD1-PUB with full length p97. Error bars represent the standard deviation of three experiments.

Figure S3



**Figure S3**. Superposition of the UBXD1-PUB (blue) and PNGase-PUB apo (gray, pdb # 2CCQ, [29]) structures showing the intramolecular H-bonding network (blue dashed lines) around UBXD1-Y194 that is not present in PNGase.

## Figure S4



**Figure S4**. Superposition of the UBXD1-PUB/p97 complex model (UBXD1-PUB backbone in blue, p97-PIM in pink) with the crystal structures of PNGase/p97-PIM (PNGase-PUB backbone in gray, p97-PIM in black) and HOIP-PUB/p97-PIM (HOIP-PUB backbone in bright green, p97-PIM in mint green).

## Table S1

| Construct              | Oligonucleotides                |
|------------------------|---------------------------------|
| UBXD1-PUB (150-264) wt | CAGTCATATGTCCACCGACCCAG         |
|                        | TCACAAGCTTTTATCACTCCGCAGCCAGCAG |
| Mutant                 |                                 |
| Y181A                  | CCATTGCCAAGGCGCTGGACAACATCC     |
|                        | GGATGTTGTCCAGCGCCTTGGCAATGG     |
| Y181F                  | CCATTGCCAAGTTCCTGGACAACATCC     |
|                        | GGATGTTGTCCAGGAACTTGGCAATGG     |
| N184A                  | CAAGTACCTGGACGCCATCCACCTGCAC    |
|                        | GTGCAGGTGGATGGCGTCCAGGTACTTG    |
| N184D                  | CAAGTACCTGGACGACATCCACCTGCAC    |
|                        | GTGCAGGTGGATGTCGTCCAGGTACTTG    |
| K193E                  | CCGAGGAGGAGGAGTACCGGAAGATC      |
|                        | GATCTTCCGGTACTCCTCCTCCTCGG      |
| Y194A                  | CGAGGAGGAGAAGGCACGGAAGATCAAGC   |
|                        | CGAGGAGGAGAAGGCACGGAAGATCAAGC   |
| Y194F                  | GAGGAGGAGAAGTTTCGGAAGATCAAGC    |
|                        | GCTTGATCTTCCGAAACTTCTCCTCCTC    |
| K198E                  | GTACCGGAAGATCGAGCTGCAGAAC       |
|                        | GTTCTGCAGCTCGATCTTCCGGTAC       |
| Q200A                  | GGAAGATCAAGCTGGCGAACAAGGTGTTTC  |
|                        | GAAACACCTTGTTCGCCAGCTTGATCTTCC  |
| N201A                  | GATCAAGCTGCAGGCCAAGGTGTTTCAGG   |
|                        | CCTGAAACACCTTGGCCTGCAGCTTGATC   |
| N201D                  | GATCAAGCTGCAGGATAAGGTGTTTCAGG   |
|                        | CCTGAAACACCTTATCCTGCAGCTTGATC   |
| V203A                  | CTGCAGAACAAGGCGTTTCAGGAGCG      |
|                        | CGCTCCTGAAACGCCTTGTTCTGCAG      |
| E206R                  | CAAGGTGTTTCAGCGCCGCATTAACTGCC   |
|                        | GGCAGTTAATGCGGCGCTGAAACACCTTG   |
| R207E                  | GGTGTTTCAGGAGGAGATTAACTGCCTGG   |
|                        | CCAGGCAGTTAATCTCCTCCTGAAACACC   |

**Table S1.** Oligonucleotides used as primers for PCR-amplification of the UBXD1-PUB (150-264) construct and for site-directed mutagenesis following the Quikchange protocol.