Supplementary Material

Stabilization of an unusual salt bridge in ubiquitin by the extra C-terminal domain of the proteasome-associated deubiquitinase UCH37 as a mechanism of its exo specificity

Marie E. Morrow^{1,#}, MyungIl Kim^{1,#}, Judith A. Ronau¹, Michael J. Sheedlo¹, Rhiannon R. White², Joseph Chaney¹, Lake N. Paul³, Markus A. Lill⁴, Katerina Artavanis-Tsakonas², Chittaranjan Das^{1,*}

Supplementary Figures

Supplementary Figure 1.



Figure S1: Superposition of all human UCH37 structures (PDB IDs 3IHR, 3RIS, 3RII, 3A7S, 3TB3) with TsUCH37^{cat}-UbVME (teal) and TsUCH37^{$\Delta C46$}-UbVME (olive). Residues 57-71 are missing in TsUCH37^{$\Delta C46$}-UbVME, as highlighted by the orange boxes.

Supplementary Figure 2.



Figure S2: Dynamics of Trp55 from the structures of the TsUCH37-UbVME constructs. TsUCH37 $^{\Delta C46}$ -UbVME is shown in olive, TsUCH37 cat -UbVME is shown in teal, UbVME is orange, and also shown is the analogous residue in the UCHL3-UbVME structure (PDB ID 1XD3), Ile58, in yellow.

Supplementary Figure 3.



Figure S3: Full sequence alignment of TsUCH37 and other UCH37 homologs. Completely conserved residues are highlighted in red.

Supplementary Figure 4.



Figure S4: NEDD8-AMC hydrolysis assay using TsUCH37, the same assay was used to investigate PfUCHL3, a dual ubiquitin and NEDD8 C-terminal hydrolase.



Supplementary Figure 5.

Figure S5: Conservation of ubiquitin binding residues in UCH family. Sequence alignment of TsUCH37 with human UCHL1 and UCHL3. Residues that make contact with UbVME in the

TsUCH37^{cat}–UbVME structure are marked as follows: red for contacts conserved in both UCHL1-UbVME and UCHL3-UbVME structures (PDB IDs 3KW5 and 1XD3), green for contacts conserved only in UCHL1-UbVME, pink for contacts conserved only in UCHL3-UbVME, and blue for contacts unique to TsUCH37-UbVME. Stars indicate catalytic residues (catalytic aspartate does not make direct contact in any of these structures and is colored black).