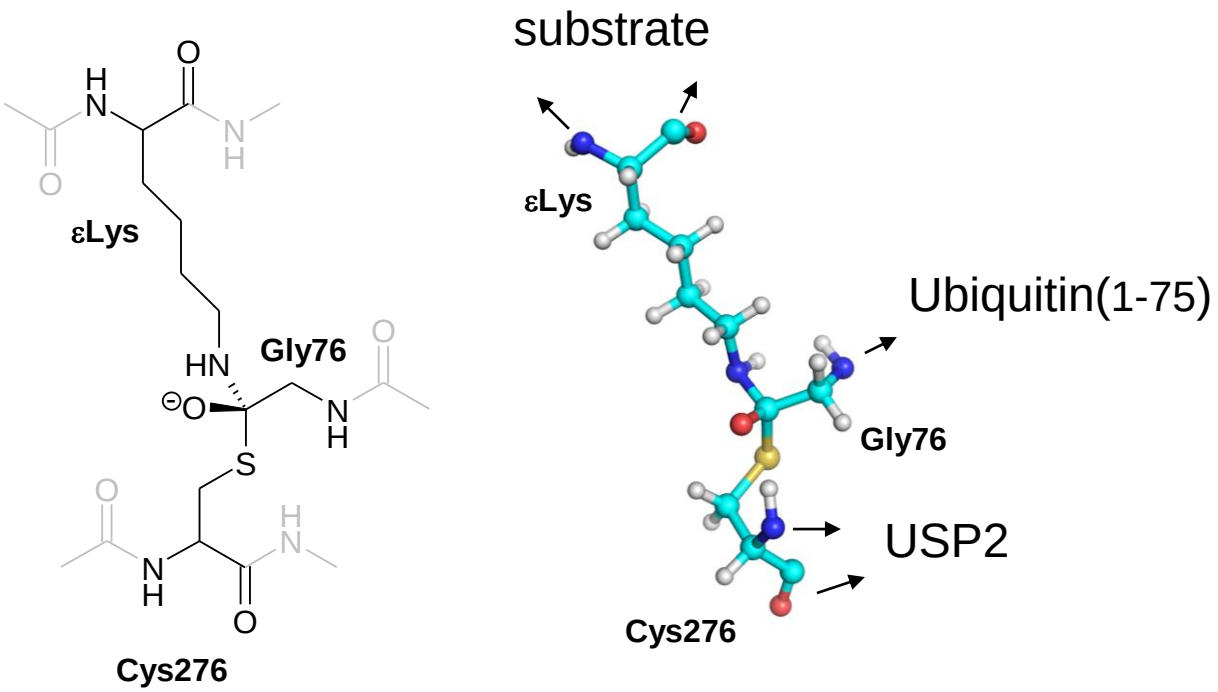
SUPPLEMENTARY MATERIAL

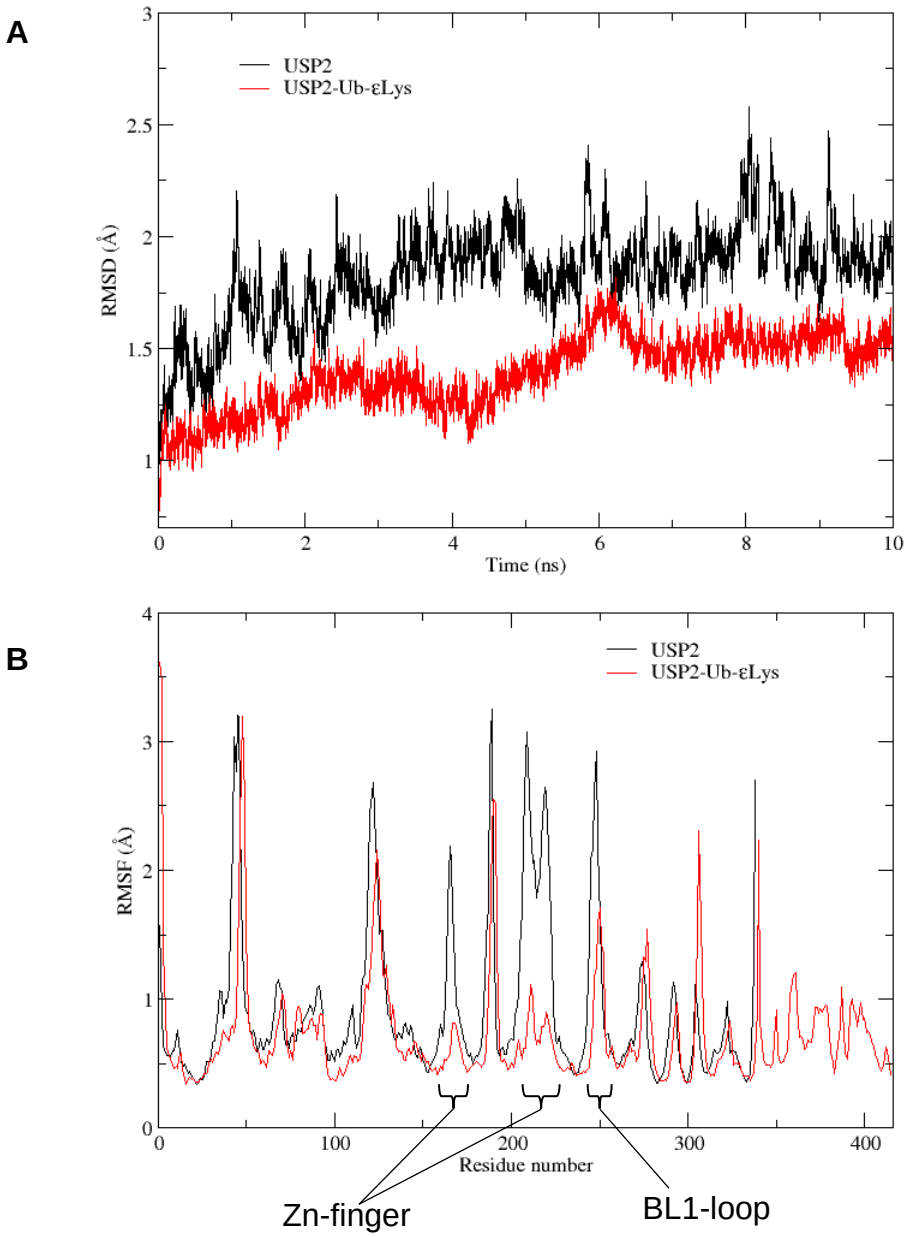
Supporting Information Table 1. Primers for site-direct mutagenesis of USP2

Mutation	Oligonucleotide sequence (5' to 3')
Oxyanion hole	
N271A	CTGGCTGGTCTTCGAGCCCTTGGGAACACGTGC
	GCACGTGTTCCCAAGGGCTCGAAGACCAGCCAG
D575A	GAATGGCACACTTTCAACGCGTCCAGCGTCACTCCC
	GGGAGTGACGCTGGACGCGTTGAAAGTGTGCCATTC
N271A / D575A	CTGGCTGGTCTTCGAGCCCTTGGGAACACGTGC
	GCACGTGTTCCCAAGGGCTCGAAGACCAGCCAG
	GAATGGCACACTTTCAACGCGTCCAGCGTCACTCCC
	GGGAGTGACGCTGGACGCGTTGAAAGTGTGCCATTC
Catalytic triad	
H557A	GAACCACCATGGGTGGCGCCTATACAGCCTACTGTCG
	CGACAGTAGGCTGTATAGGCGCCACCCATGGTGGTTC
N574A	GGAGAATGGCACACTTTCGCCGACTCCAGCGTCAC
	GTGACGCTGGAGTCGGCGAAAGTGTGCCATTCTCC
C276A	CGAAACCTTGGGAACACGGCCTTCATGAACTCAATTCTGC
	GCAGAATTGAGTTCATGAAGGCCGTGTTCCCAAGGTTTCG
N574A / D575A	GGAGAATGGCACACTTTCGCCGACTCCAGCGTCAC
	GTGACGCTGGAGTCGGCGAAAGTGTGCCATTCTCC
	GAATGGCACACTTTCAACGCGTCCAGCGTCACTCCC
	GGGAGTGACGCTGGACGCGTTGAAAGTGTGCCATTC

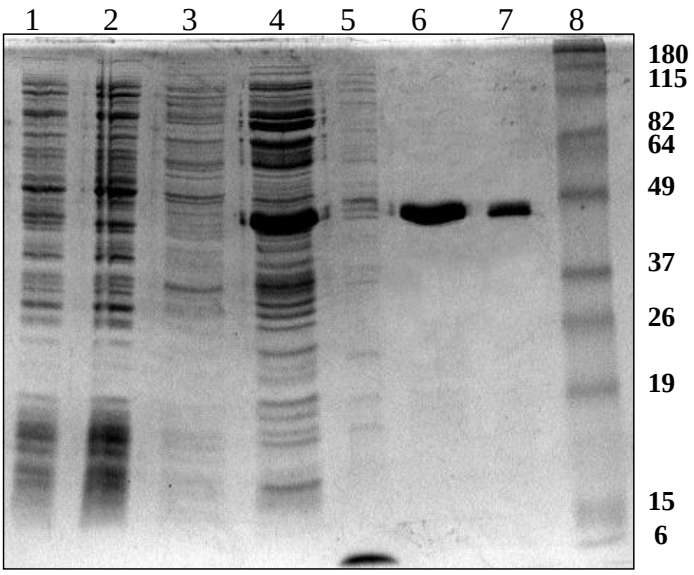
The modified nucleotides are underlined.



Supporting Information Figure 1. Cysteine tetrahedral intermediate (CTI) aminoacid residue parametrized for MD simulations. Panel on the left shows a 2D-drawing of the residue (black line) blocked with acetyl and methylamino groups (gray line) at 5 positions. RESP2 partial charges were calculated for the blocked CTI and renormalized to –1*e* for the unblocked CTI residue. Panel on the right shows a 3D conformation of the CTI residue with arrows indicating its 5 attachment points towards USP2, ubiquitin and substrate.

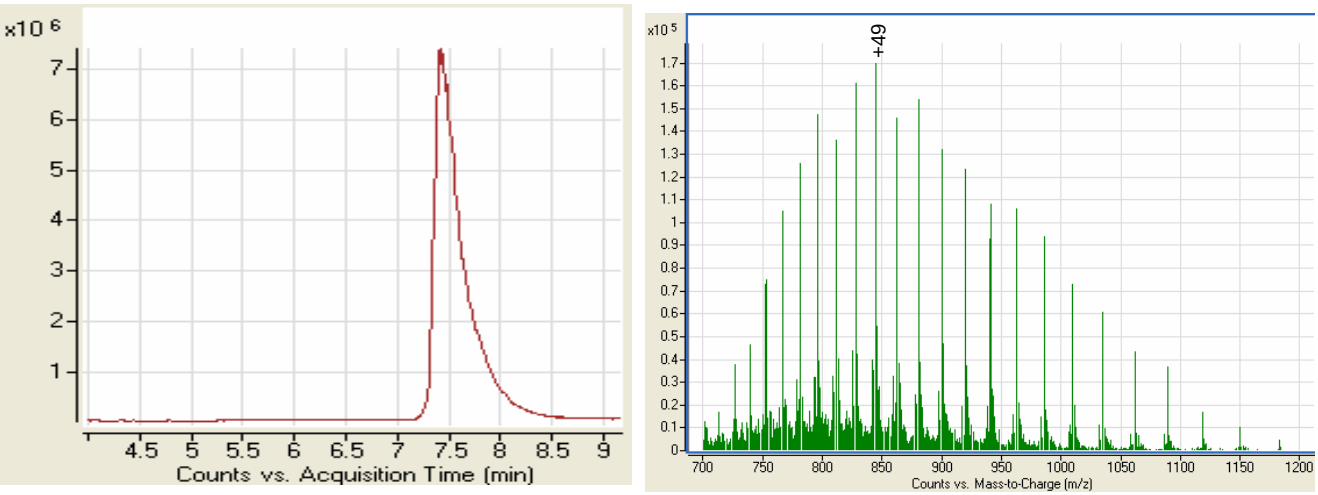


Supporting Information Figure 2. Structural dynamics of the USP2 free and complexed with the Ub-εLys substrate. (A) RMSD of backbone atoms during the course of simulation with respect to the initial structure. (B) Per-residue RMS fluctuations of backbone atoms over the 10-ns MD trajectory. Regions in the USP2 catalytic core domain that are stabilized upon ubiquitin binding are indicated.



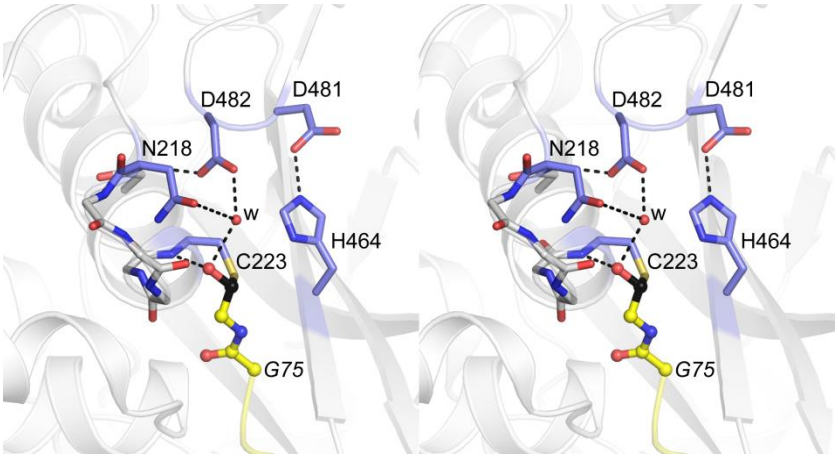
Supporting Information Figure 3. Purification of recombinant USP2. Coomassie blue-stained SDS-PAGE gel from a Ni-NTA purification of USP2-HIS construct. Lane 1, total cell extracts; lane 2, flow through; lanes 3-4, washes; lane 5, Ni-beads; lanes 6-7, 7 μg and 2 μg of purified protein, respectively; lane 8, molecular mass standards (in kDa). The calculated size of the recombinant protein is 41 kDa.

A **B**

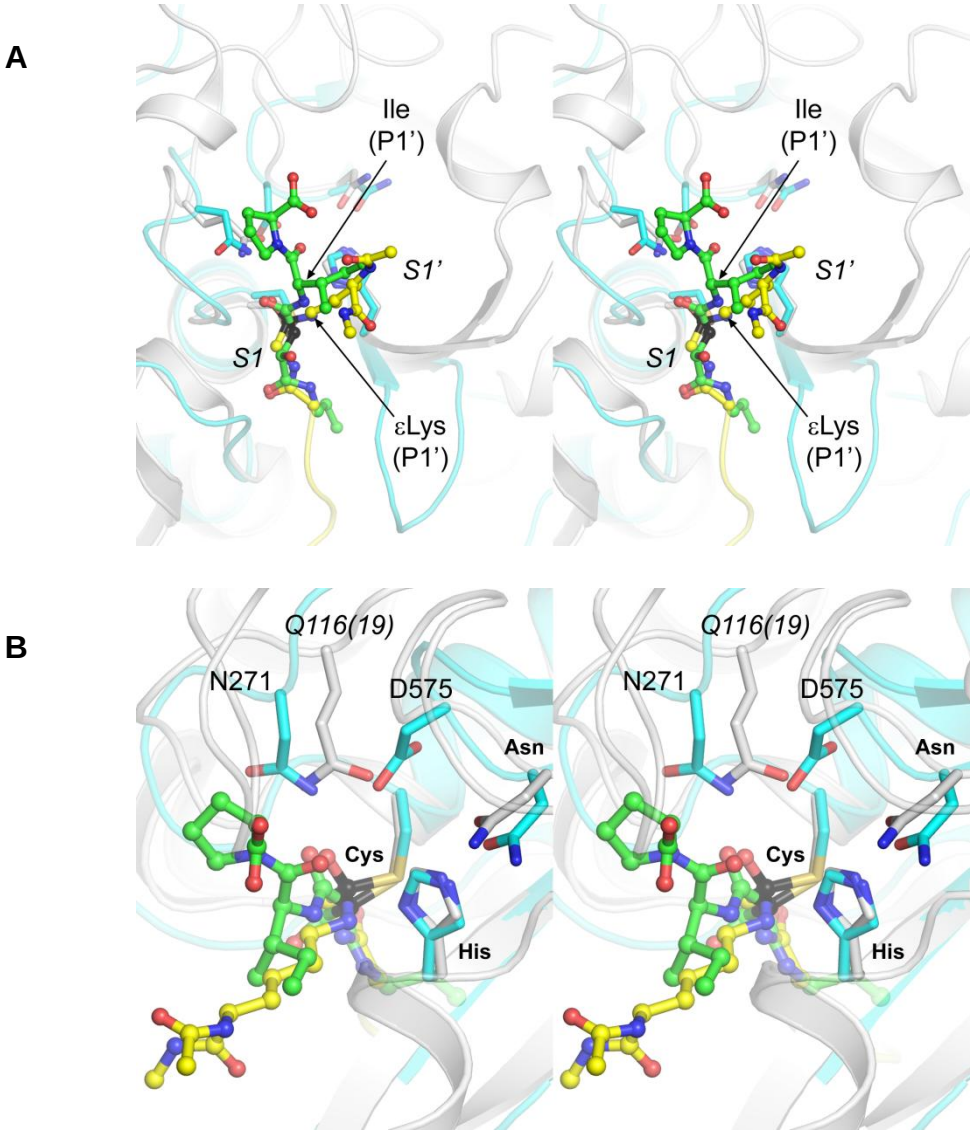


Enzyme	Observed MW (Da)	Theoretical MW (Da)	Difference (Da)
Wild-type	41362.1	41361.8	+0.3
N271A	41320.7	41318.8	+1.9
D575A	41317.9	41317.8	+0.1
N271A / D575A	41275.2	41274.8	+0.4
N574A	41319.8	41318.8	+1.0
N574A / D575A	41277.1	41274.8	+2.3
C276A	41328.6	41329.8	-1.2
H557A	41298.1	41295.7	+2.4

Supporting Information Figure 4. MW determination by mass spectrometry for wild-type and mutant USP2. Panel A. Representative LC-MS chromatograph of wild-type USP2. Panel B. Representative mass spectrum of wild-type USP2. The Table reports the observed vs theoretical MW for wild-type and mutant USP2 enzymes.

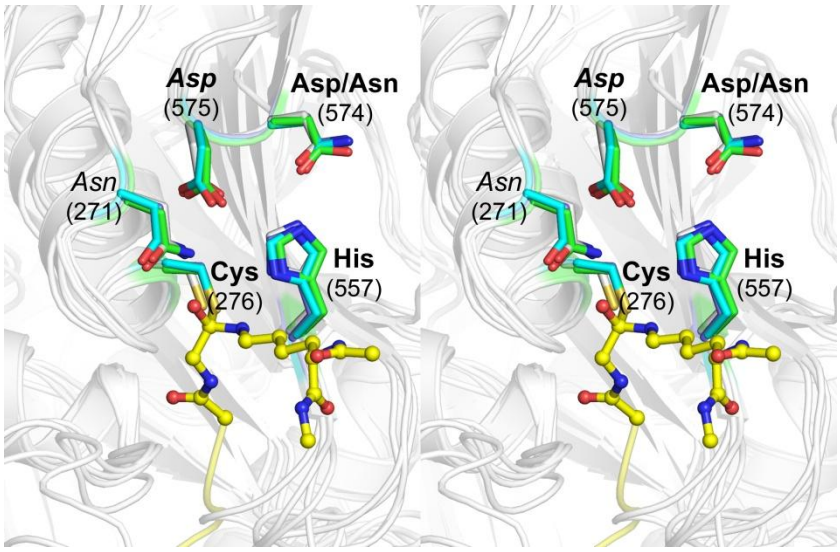


Supporting Information Figure 5. Oxyanion hole interactions in the X-ray structure of USP7 in complex with Ubal adduct. USP catalytic core is shown as gray ribbon, Ubal as yellow ribbon. Select USP7 side chains are shown as sticks, with the Gly75-glycinealdehyde fragment of Ubal adduct as ball-and-stick. Hydrogen bonds are indicated by dashed lines.



Supporting Information Figure 6. Comparison of USP and papain-like topologies. Overlay of the simulated USP2-Ub-εLys complex with the papain-like *T. brucei* cathepsin B complexed with CA-074 inhibitor (PDB code 3HHI). Shown is the USP2-Ub-εLys minimized MD average structure over the 10th ns of the MD production run. USP2 is shown as cyan ribbon, cathepsin B as gray ribbon. Select side chains are shown as sticks, with C atoms colored in cyan for USP2 and gray for cathepsin B. The Ub-εLys adduct bound to USP2 is shown as yellow ribbon with

the Gly75-Gly76-εLys portion as ball-and stick with yellow C atoms. The CA074 inhibitor bound to cathepsin B is shown as ball-and-stick with green C atoms. (A) The S1'-P1' interactions. (B) Oxyanion hole stabilization by the conserved Gln19 residue in papain-like cysteine proteases (Gln116 in *T. brucei* cathepsin B) is mimicked by the conserved Asn/Asp tandem in USPs (Asn271/Asp575 in USP2). The overlaid residues forming the catalytic triad Cys-His-Asn are indicated.



Supporting Information Figure 7. Oxyanion interaction-poised conformation of the USP active site. Overlay between the MD average structure USP2 complexed with Ub-εLys tetrahedral intermediate adduct, the MD average structure of free USP2 and crystal structures of free USP8 and USP14. Minimized MD average structures are calculated over the 10th ns of the MD production runs. Ubal-εLys bound to USP2 is render in yellow, with the Gly75-Gly76-εLys portion as ball-and-stick. Catalytic triad and oxyanion hole side chains have C atoms coloured as: complexed USP2 – cyan, free USP2 – purple, free USP8 – green, and free USP14 – gray.