SUPPORTING INFORMATION

Biochemical characterization of a multidomain deubiquitinating enzyme Ubp15 and the regulatory role of its terminal domains

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Figure S1. (A) Representative fluorescence traces of Ubp15 catalyzed hydrolysis of 200 nM (blue), 400 nM (red), and 800 nM (black) Ub-AMC. (B) Initial velocity analysis of hydrolysis of Ub-AMC catalyzed by truncated Ubp15 proteins. The initial rates are plotted against Ub-AMC concentration and fitted to the Michaelis-Menten equation.
Figure S2. Gel filtration analysis of truncated Ubp15 proteins. (A) Approximately 1.4 mg of Ubp15 (196-585) and Ubp15 (1-585) was injected into a Superose 6 gel filtration column (GE Healthcare). Protein elution was detected based on UV-vis absorbance at 280 nm. The calculated extinction coefficients of Ubp15 (196-585) and Ubp15 (1-585) are 59,375 M⁻¹ cm⁻¹ and 98,460 M⁻¹ cm⁻¹ at 280 nm respectively. (B) The identity and purity of the eluted protein in the peak fraction were further analyzed by SDS-PAGE and Coomassie Blue staining.
Figure S3. The Ubp15 N-terminal TRAF-like domain does not stimulate the activity of the Ubp15 catalytic core in trans. 20 μM N-terminal TRAF-like domain was incubated with 2 μM Ubp15 core for 30 minutes before the protein mixture was diluted into Ub-AMC assay solution, as described in Materials and Methods. 10 nM Ubp15 (196-585) alone hydrolyzed 100 nM Ub-AMC at 0.22 nM/s when incubated with 10-fold more N-terminal TRAF-like domain, while the Ubp15 catalytic core hydrolyzed 100 nm Ub-AMC at 0.15 nM/s.
Figure S4. Guanidine hydrochloride denaturation of the truncated Ubp15 core domains. (A) Far UV CD spectra of the Ubp15 (209-585) and Ubp15 (196-585) after treatment with different concentrations of guanidine hydrochloride (GdnHCl). (B) The percent decrease of alpha helix in response to GdnHCl treatment.
Figure S5. Multiple sequence alignment of Ubp15 and several human USPs with solved crystal structures. The alignment was generated using ClustalW2. The active site residues are strictly conserved (highlighted in yellow). Ubp15 residues that form potential hydrogen bonds with the N-terminal face of ubiquitin are marked with green squares and residues that form potential hydrogen bond to the C-terminal tail of ubiquitin are marked with purple squares. The secondary structural elements in the USP core domains are indicated above the sequence alignment. Both α-helix (red boxes) and β-sheet (blue boxes) positioning are highly conserved throughout the USP catalytic core.

Figure S6. A zoomed-in view of the model of the Ubp15 catalytic core (a.a. 196-554) in complex with ubiquitin. The C-terminal peptide of ubiquitin is colored in orange. The finger subdomain of Ubp15 is colored in green while the rest of the catalytic core is in grey. Residues D286, T283, H457, and Y466 that form potential hydrogen bond to the C-terminal peptide of ubiquitin are colored in magenta.
Figure S7. Cdh1 does not stimulate Ubp15’s activity. Individually purified Cdh1 was incubated with the full-length Ubp15 to determine its effect on Ubp15’s activity. Activity was determined using the Ub-AMC assay, as described in Materials and Methods. We observed no significant stimulation of Ubp15’s activity, even with addition of 20-fold more Cdh1.
Figure S8. Initial velocity analysis of hydrolysis of Ub-AMC catalyzed by full-length and truncated Ubp15 fused with GST tag. The initial rates are plotted against Ub-AMC concentration and fitted to the Michaelis-Menten equation.