

## **SUPPLEMENTARY FIGURE LEGENDS**

### **SUPPLEMENTARY FIGURE S1**

Crystal packing of the OTUB1- $\Delta$ N•UBC13•MMS2 complex. The asymmetric unit of the OTUB1- $\Delta$ N•UBC13•MMS2 complex. The structural unit OTUB1•(UBC13•MMS2)<sub>3</sub>, parallel aligned units and pseudo 2<sub>1</sub>-fold symmetry units are highlighted by orange, cyan and black dotted ellipses, respectively. See also the main text.

### **SUPPLEMENTARY FIGURE S2**

Electron density map of the OTUB1- $\Delta$ N•UBC13•MMS2 ternary complex.  $2F_o-F_c$  electron density map around Met64 of UBC13, contoured at 1.5 $\sigma$ . Drawing schemes are the same as Fig.1b.

### **SUPPLEMENTARY FIGURE S3**

Comparison of the amino acid sequences of E2s. 100% and more than 70% identical residues among human E2s are highlighted by red background and red letters, respectively. Residues that hydrogen bonds to OTUB1 are marked with orange circles. Residues that are involved in hydrophobic interactions with OTUB1 are marked with red triangles. The secondary structure and residue numbers of human UBC13 are shown above the alignment.

### **SUPPLEMENTARY FIGURE S4**

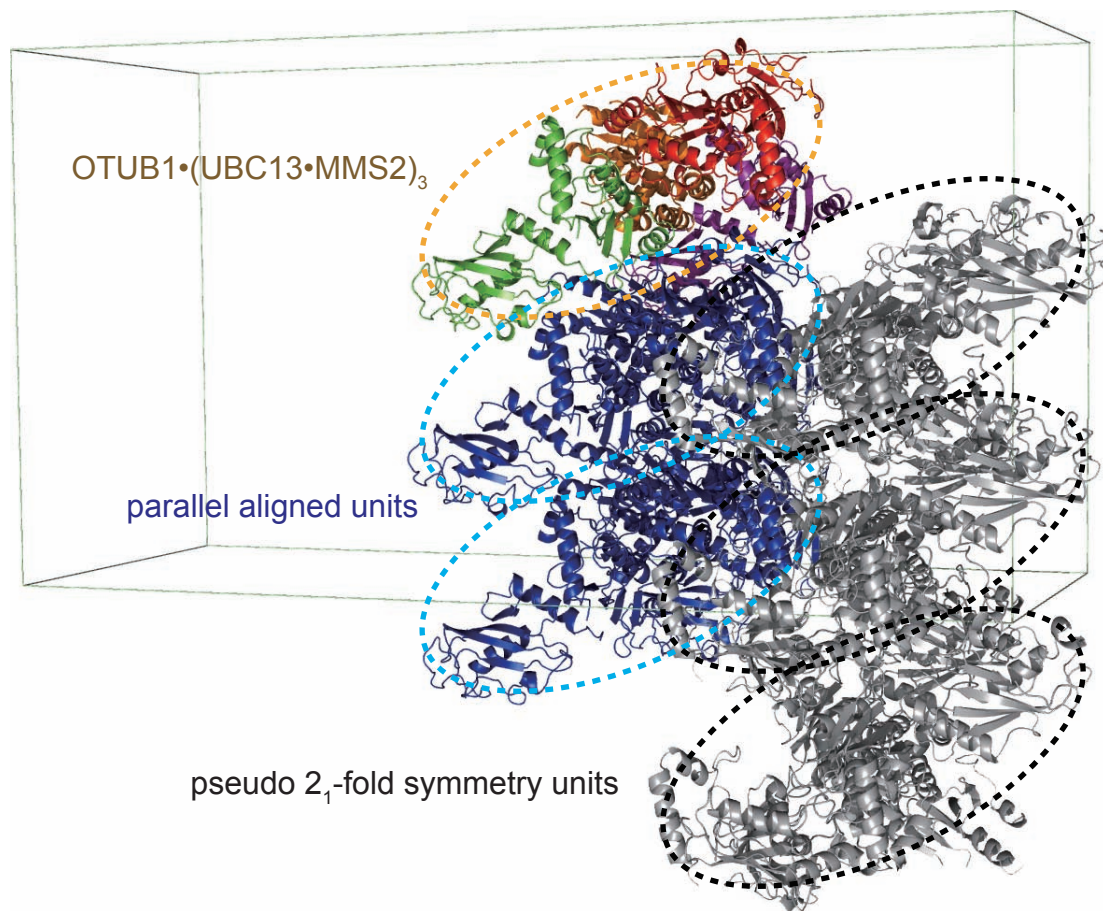
Sensorgrams of SPR spectrometry for analyzing the OTUB-UBC13 interaction.

### **SUPPLEMENTARY FIGURE S5**

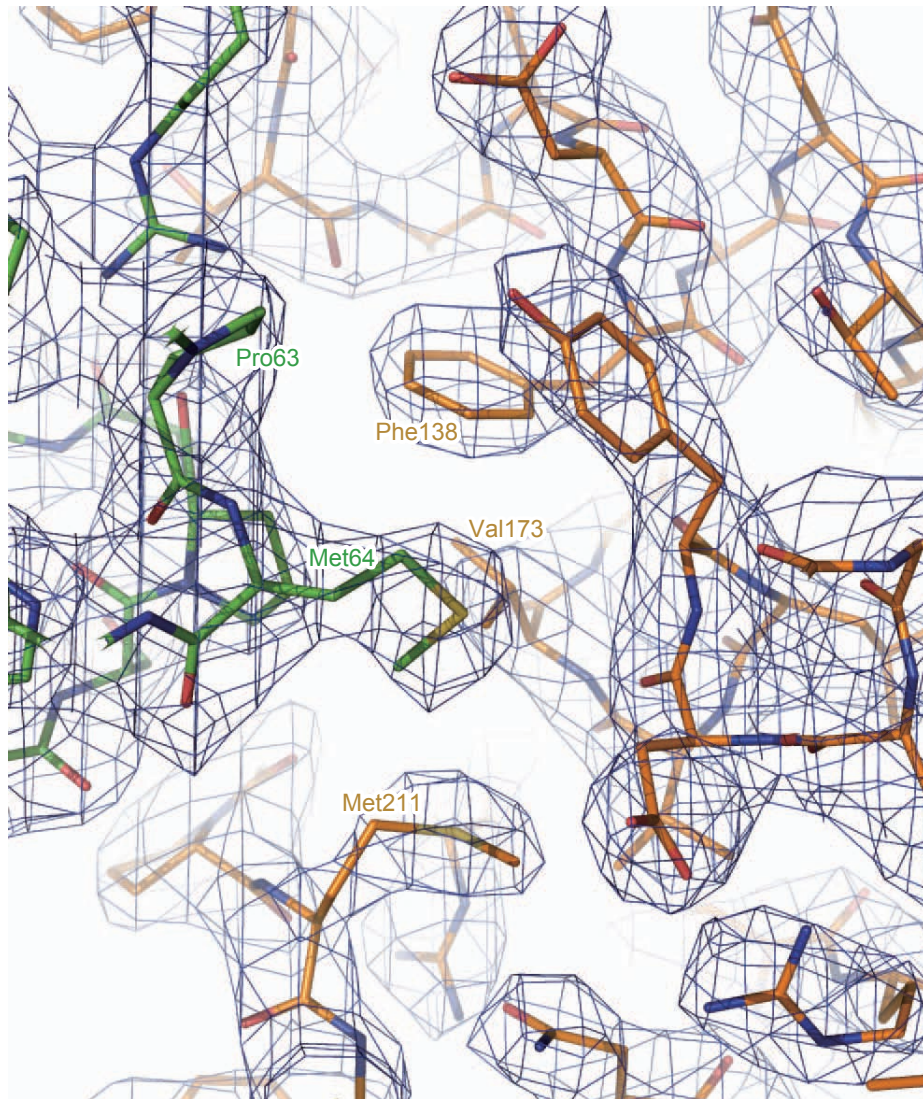
Comparison of the amino acid sequences of OTUB1 and OTUB2. Identical residues among only OTUB1 and OTUB1 and OTUB2 from representative organisms are highlighted by red and yellow backgrounds, respectively. Residues that hydrogen bond to UBC13 are marked with orange circles. Residues that are involved in hydrophobic interactions with UBC13 are marked with red triangles. The secondary structure and residue numbers of human OTUB1 are shown above the alignment. Abbreviations are as follows: *Hs*, Homo sapience; *Mm*, Mus musculus; *Md*, Monodelphis domestica; *Gg*, Gallus gallus; *Xl*, Xenopus laevis; *Ss*, Salmo salar.

### **SUPPLEMENTARY FIGURE S6**

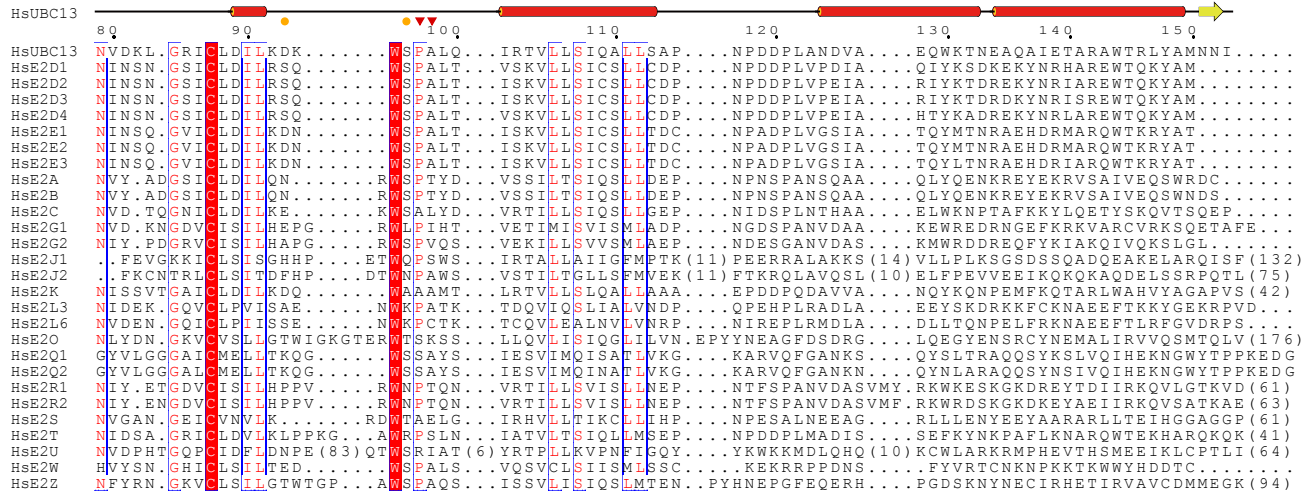
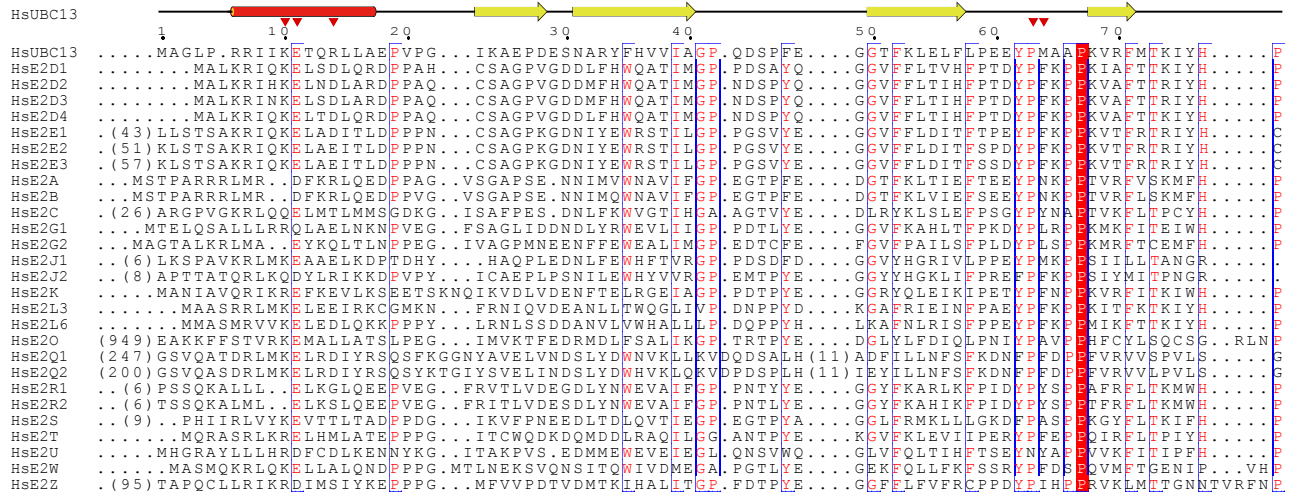
In vitro K63 chain synthesis by UBC13-MMS2 was performed in the presence or absence of the indicated recombinant OTUB1. Reaction products were analyzed by Western blotting for Ub.



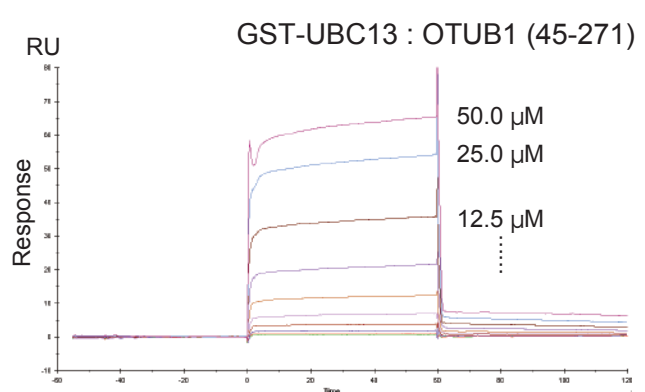
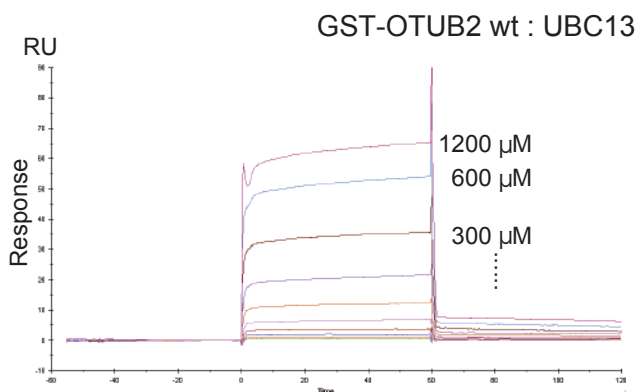
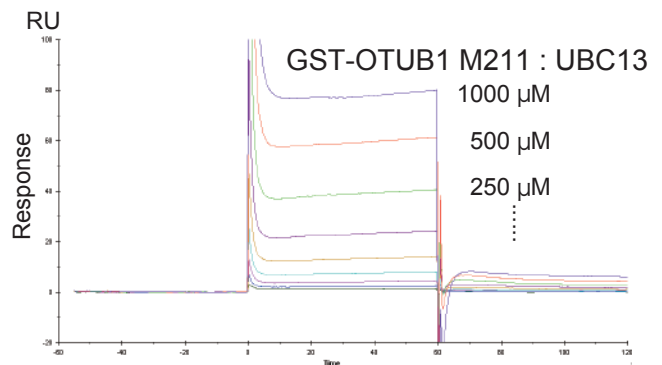
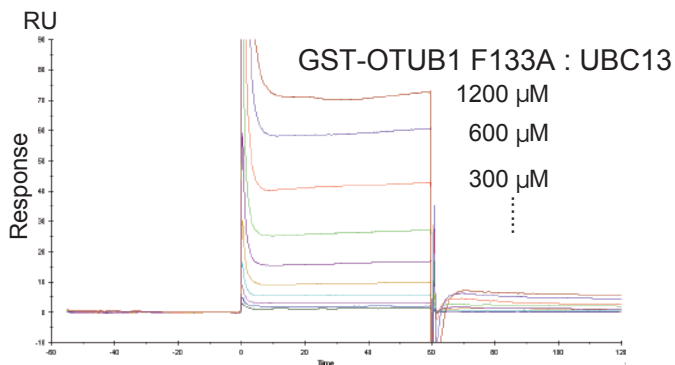
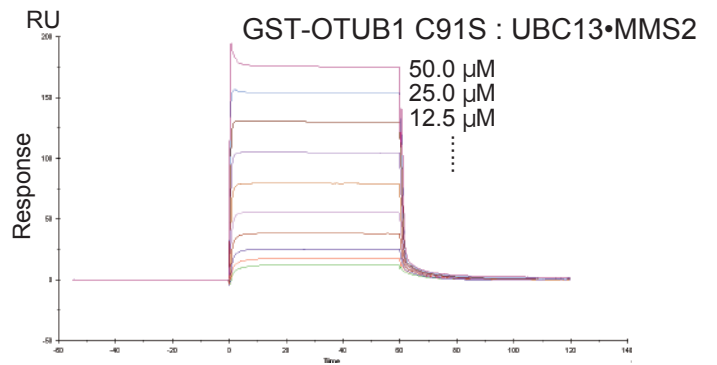
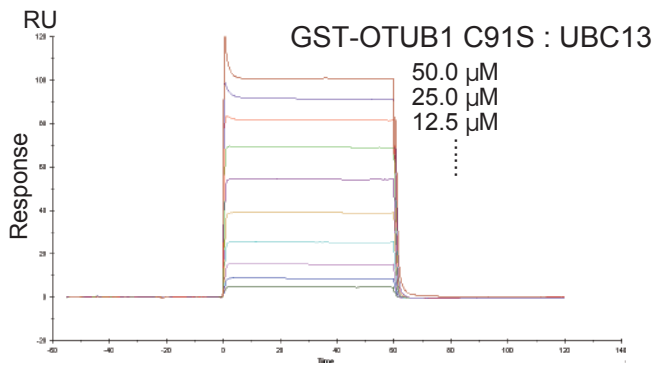
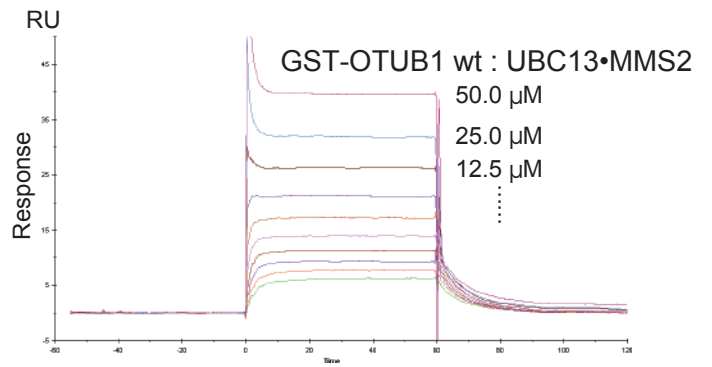
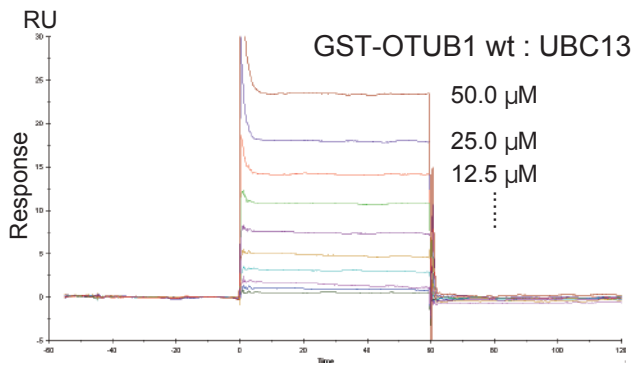
Fukai, S. Supplementary Figure S1



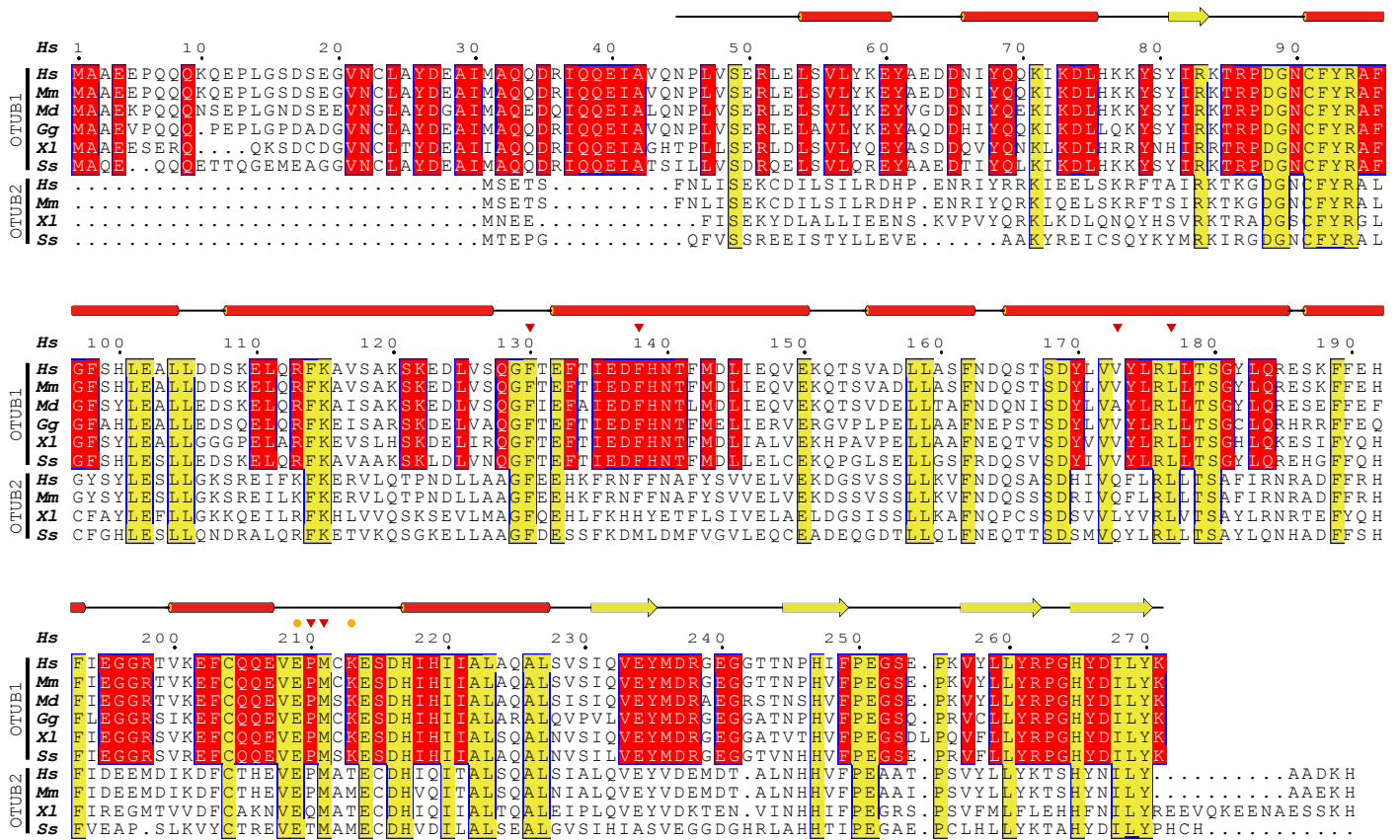
Fukai, S. Supplementary Figure S2



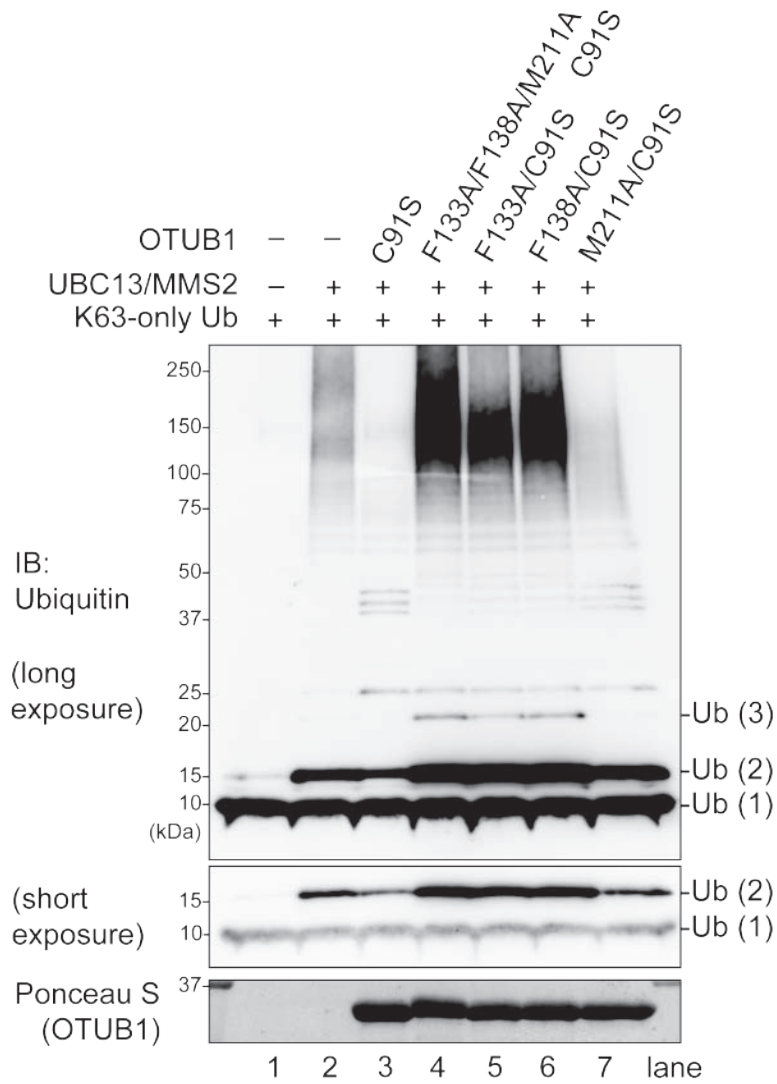
# Fukai, S. Supplementary Figure S3



Fukai, S. Supplementary Figure S4



Fukai, S. Supplementary Figure S5



Fukai, S. Supplementary Figure S6