

Figure S1

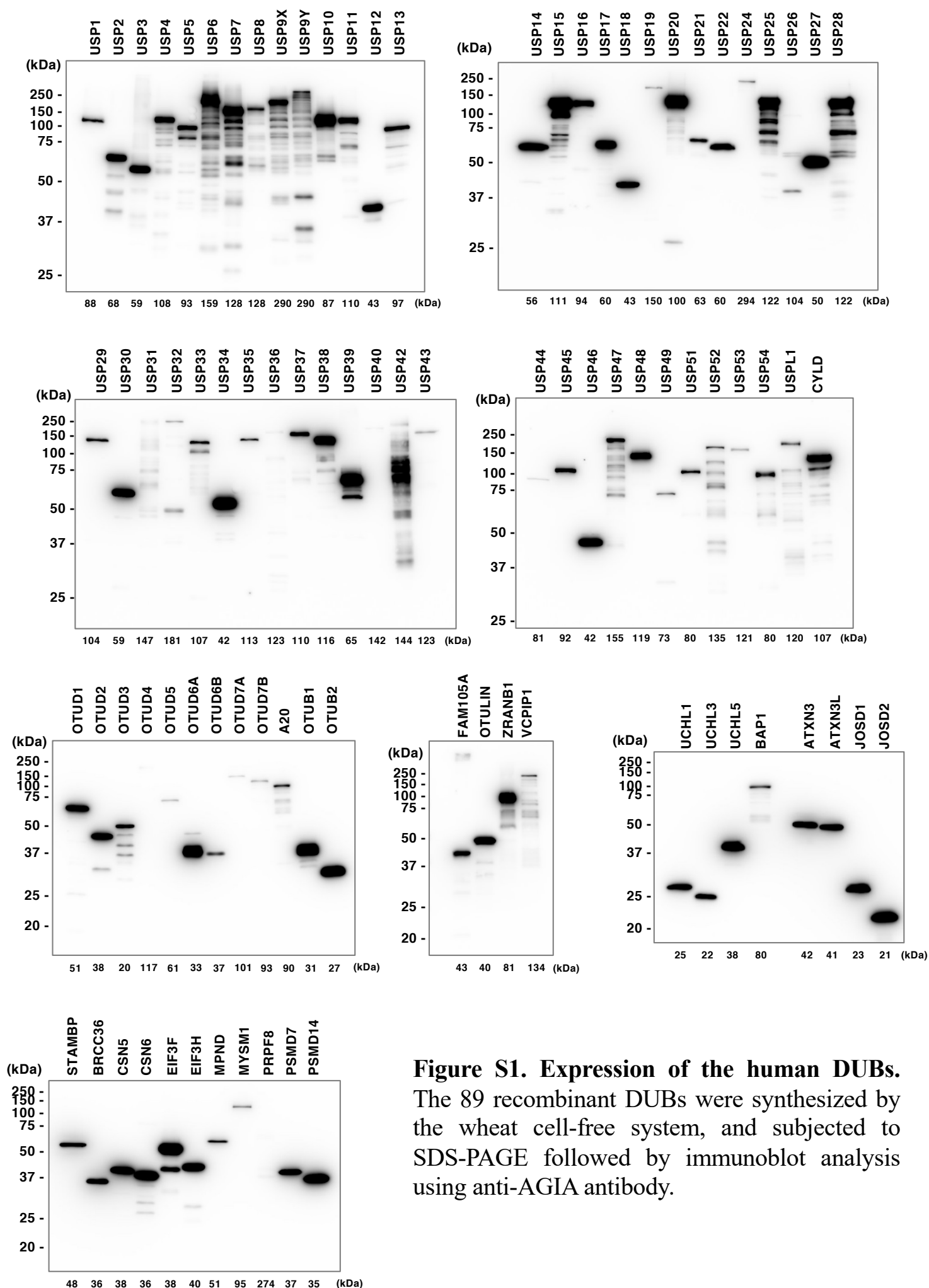
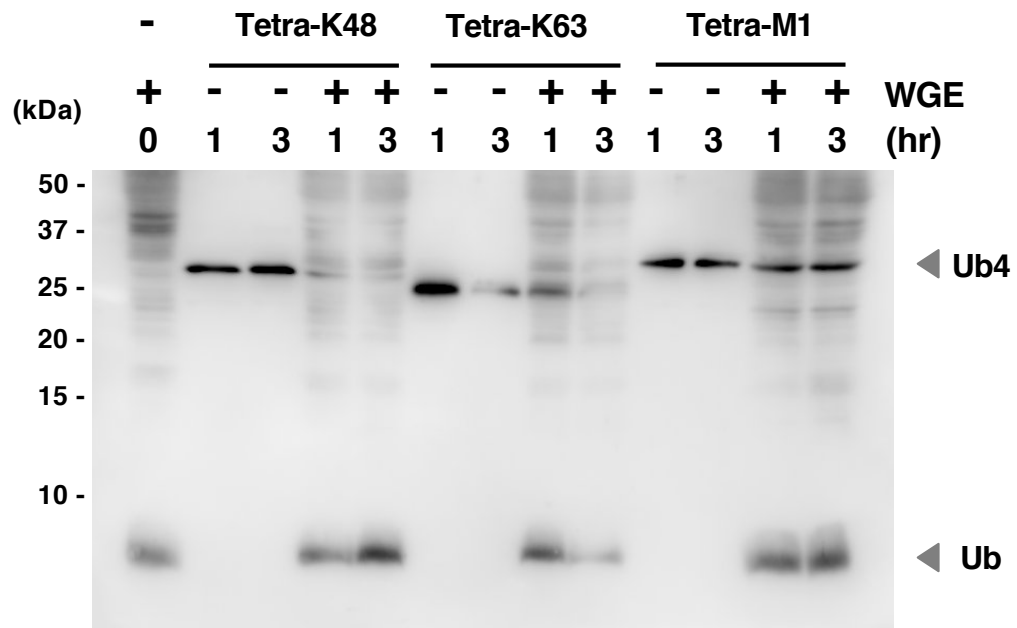


Figure S1. Expression of the human DUBs.
The 89 recombinant DUBs were synthesized by the wheat cell-free system, and subjected to SDS-PAGE followed by immunoblot analysis using anti-AGIA antibody.



IB: anti-Ub antibody

Figure S2. The wheat germ extract has distinct DUB activity. K48-, K63-, and M1-tetraubiquitins were incubated with 0.75 μ L of the wheat germ extract (WGE) for indicated time. The reaction products were separated by SDS-PAGE followed by immunoblot analysis using anti-ubiquitin antibody.

Figure S3

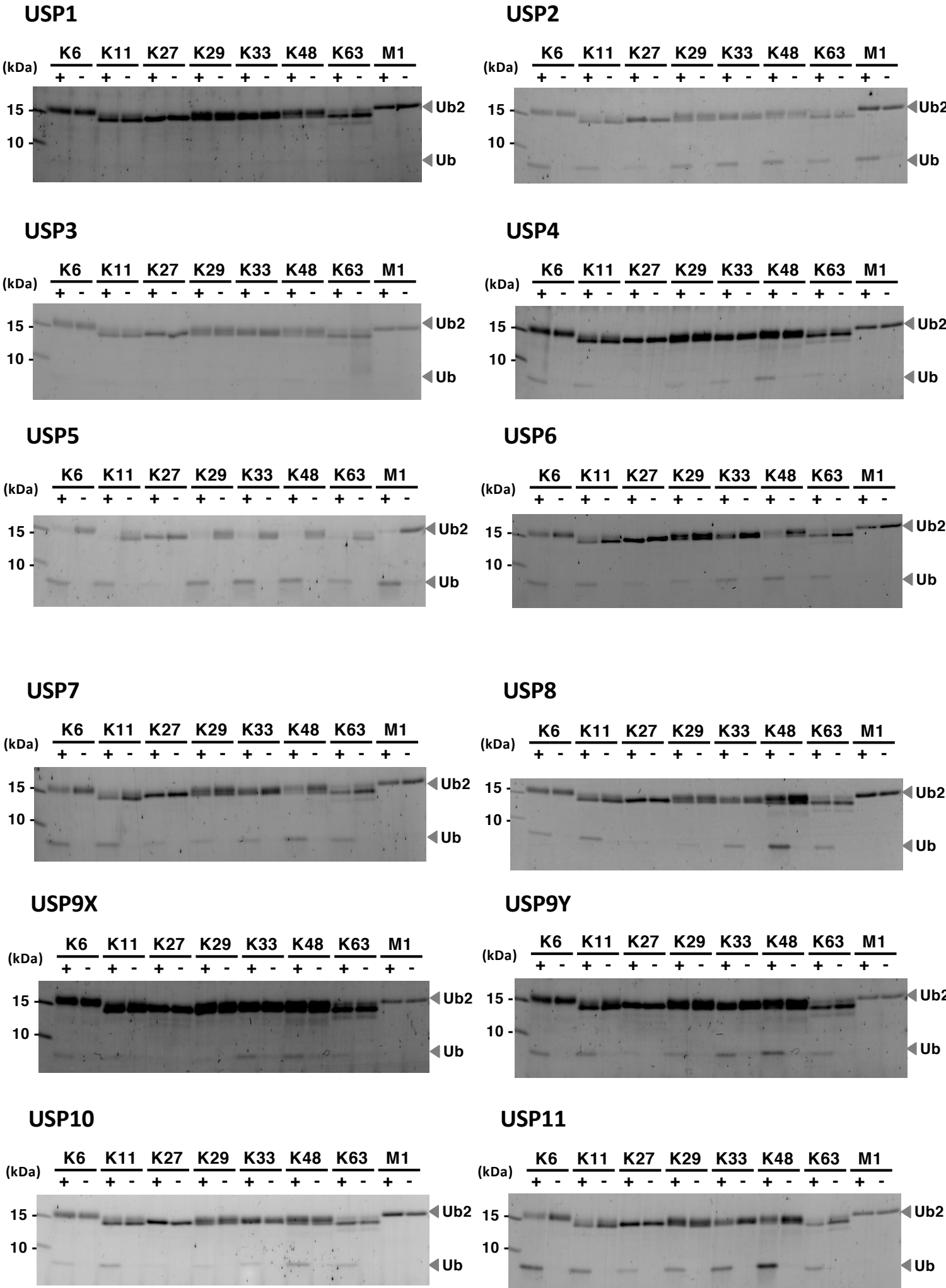
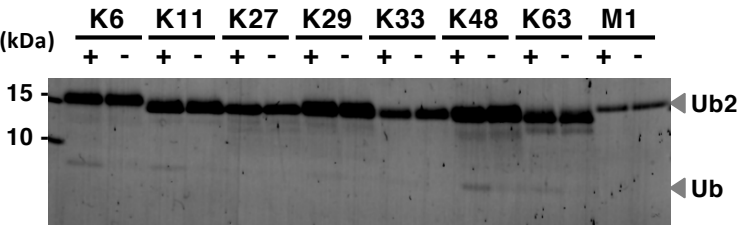
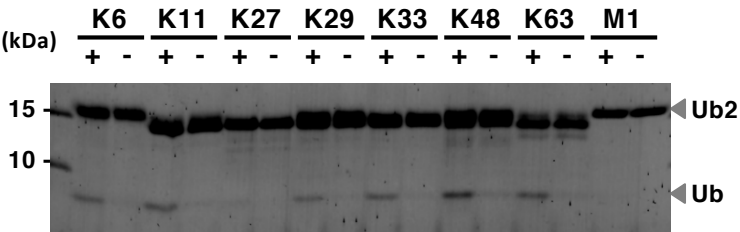


Figure S3

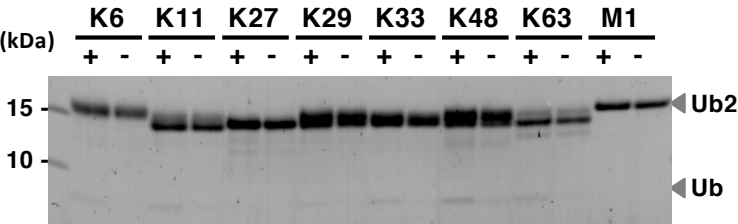
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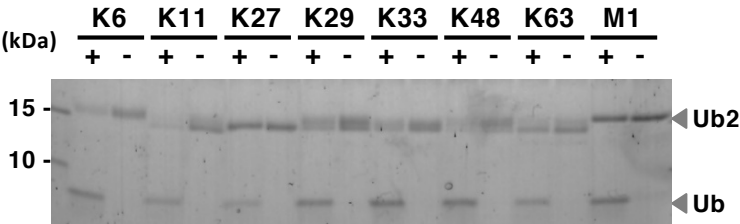
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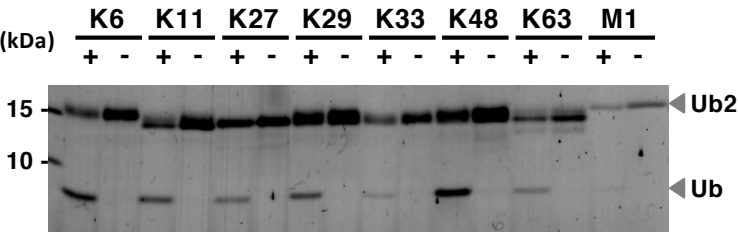
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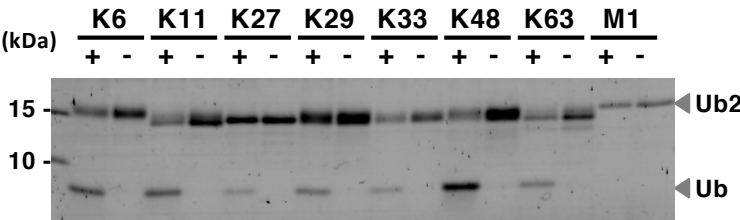
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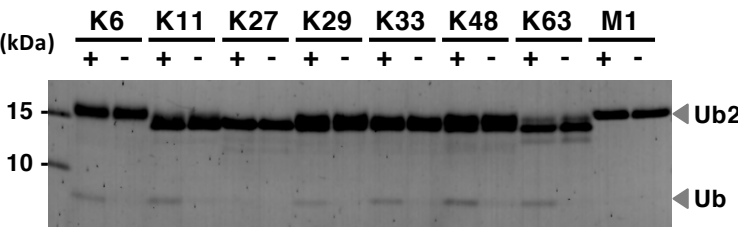
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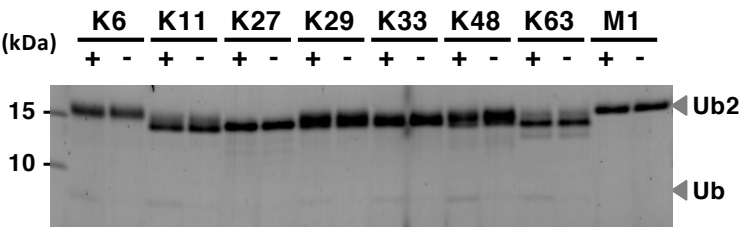
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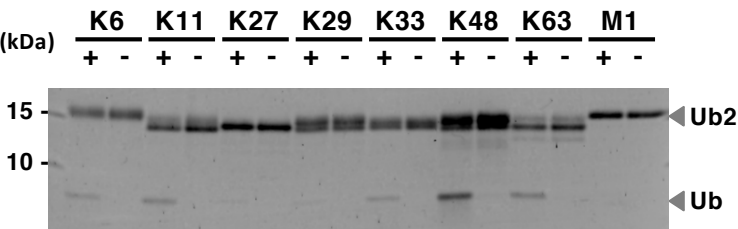
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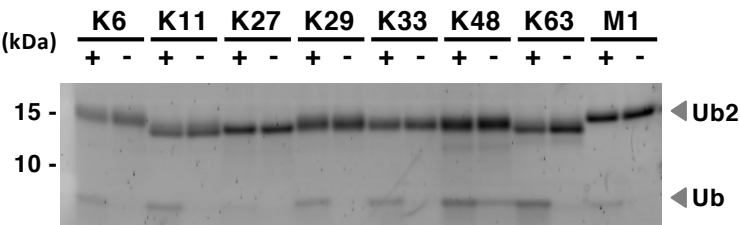
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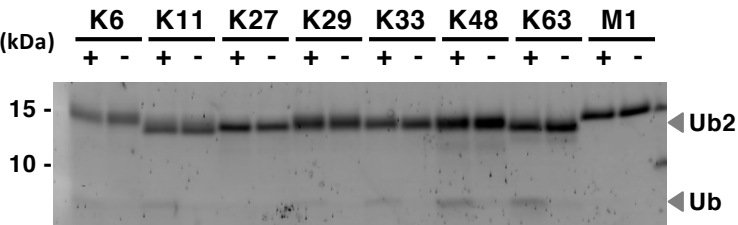
USP20



USP21



USP22



USP24

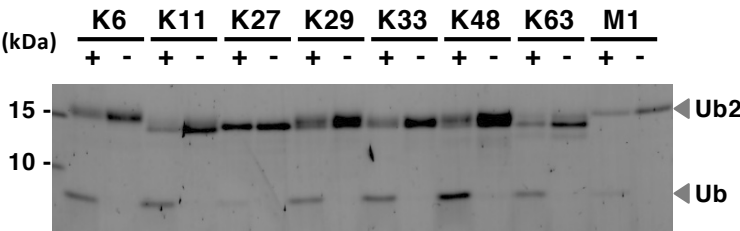


Figure S3

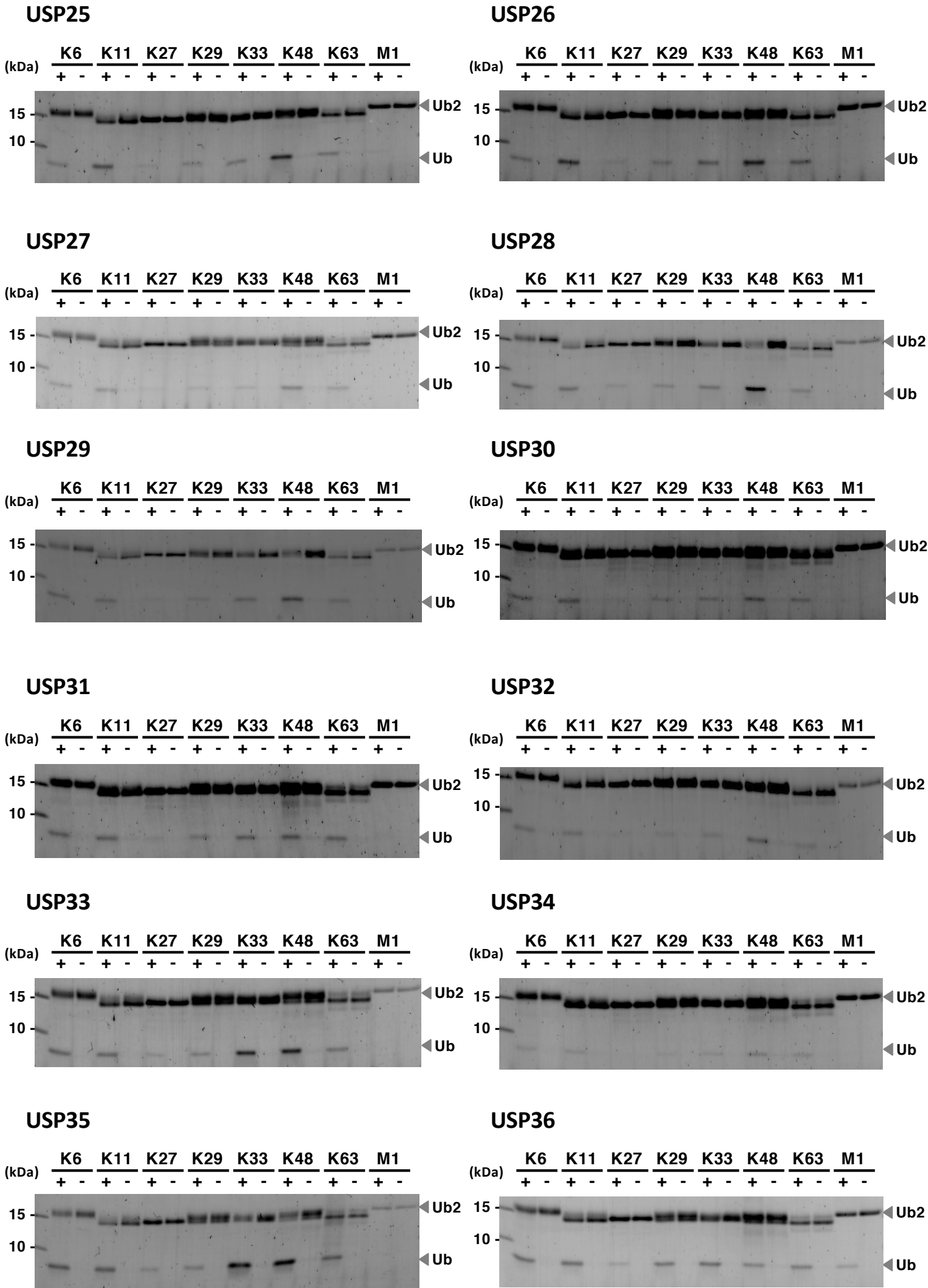


Figure S3

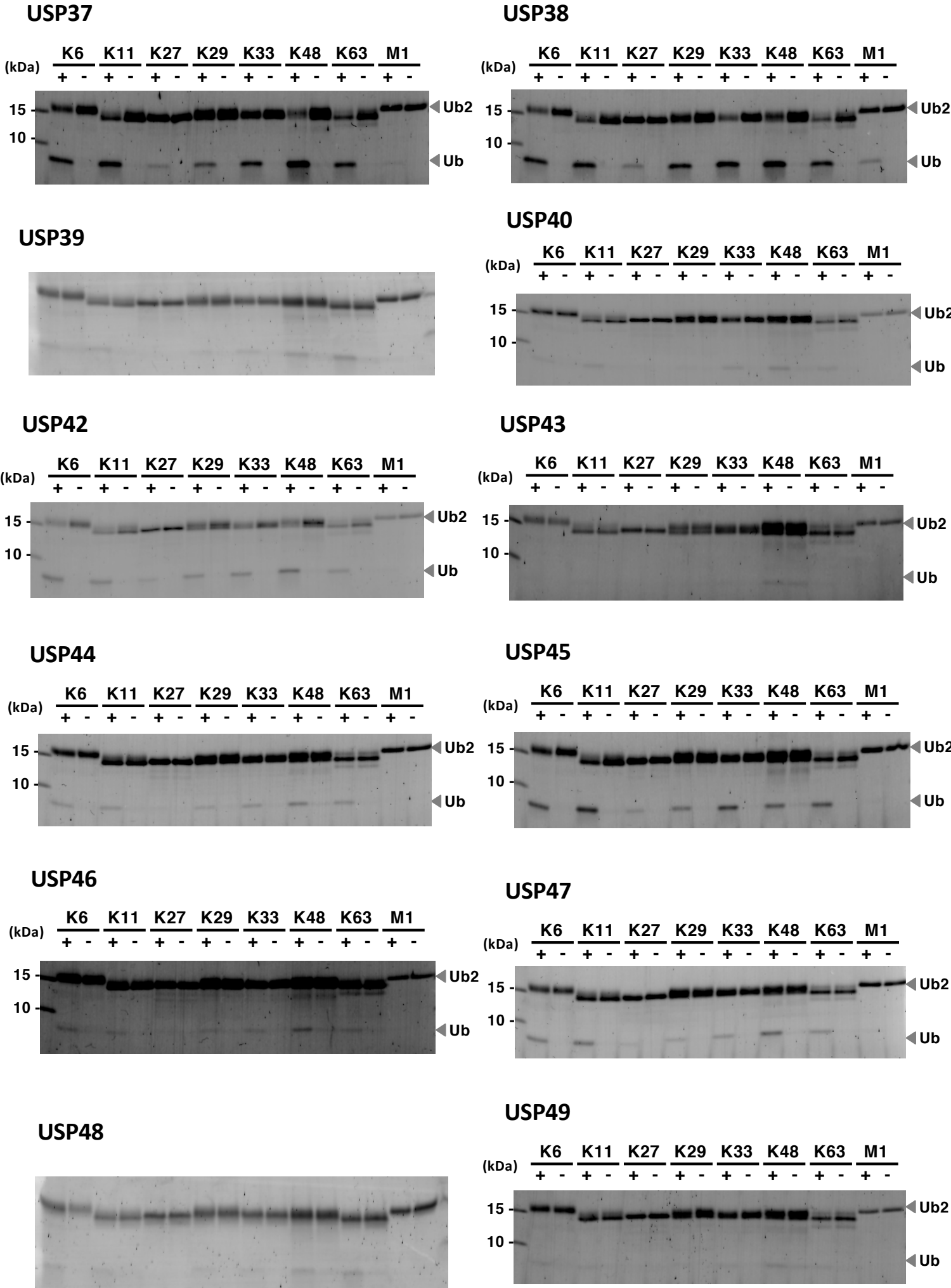
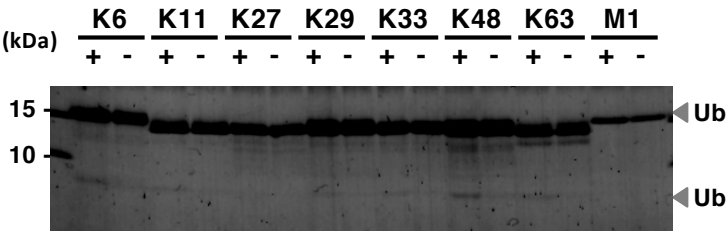
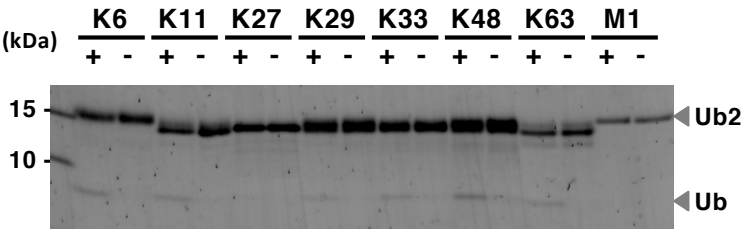


Figure S3

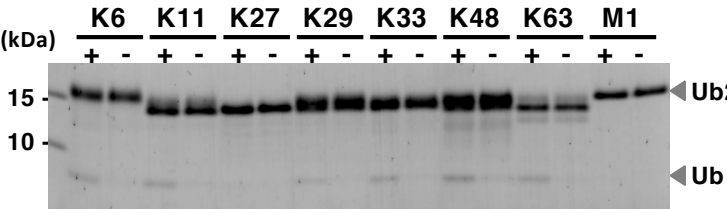
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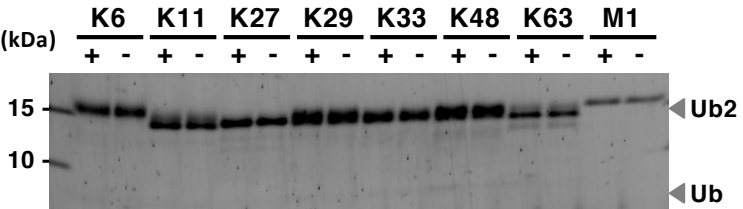
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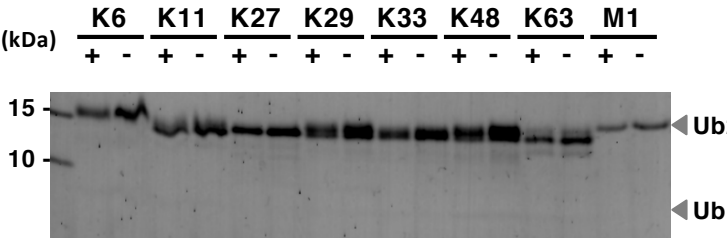
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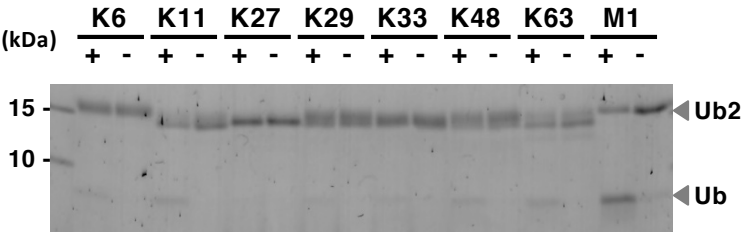
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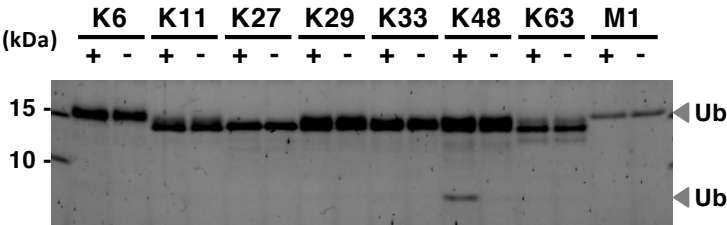
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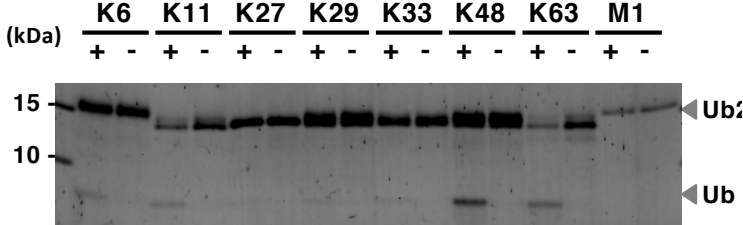
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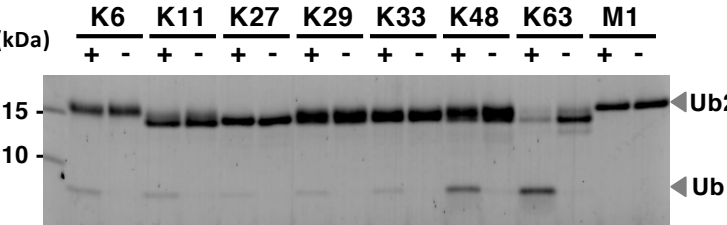
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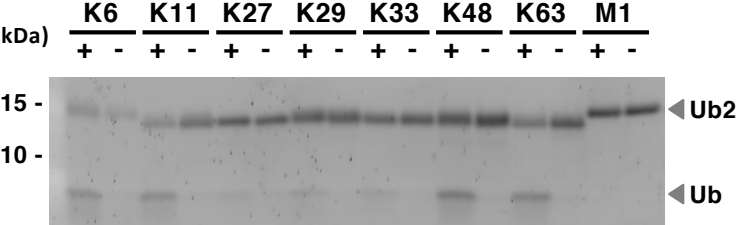
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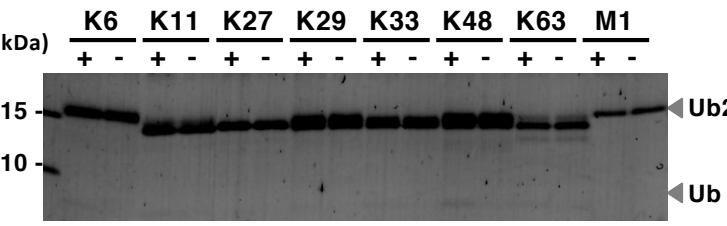
OTUD1



OTUD3



OTUD4



OTUD5

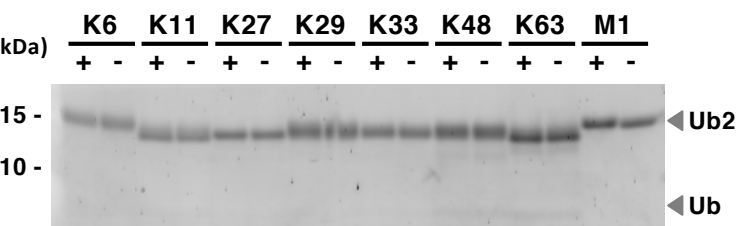


Figure S3

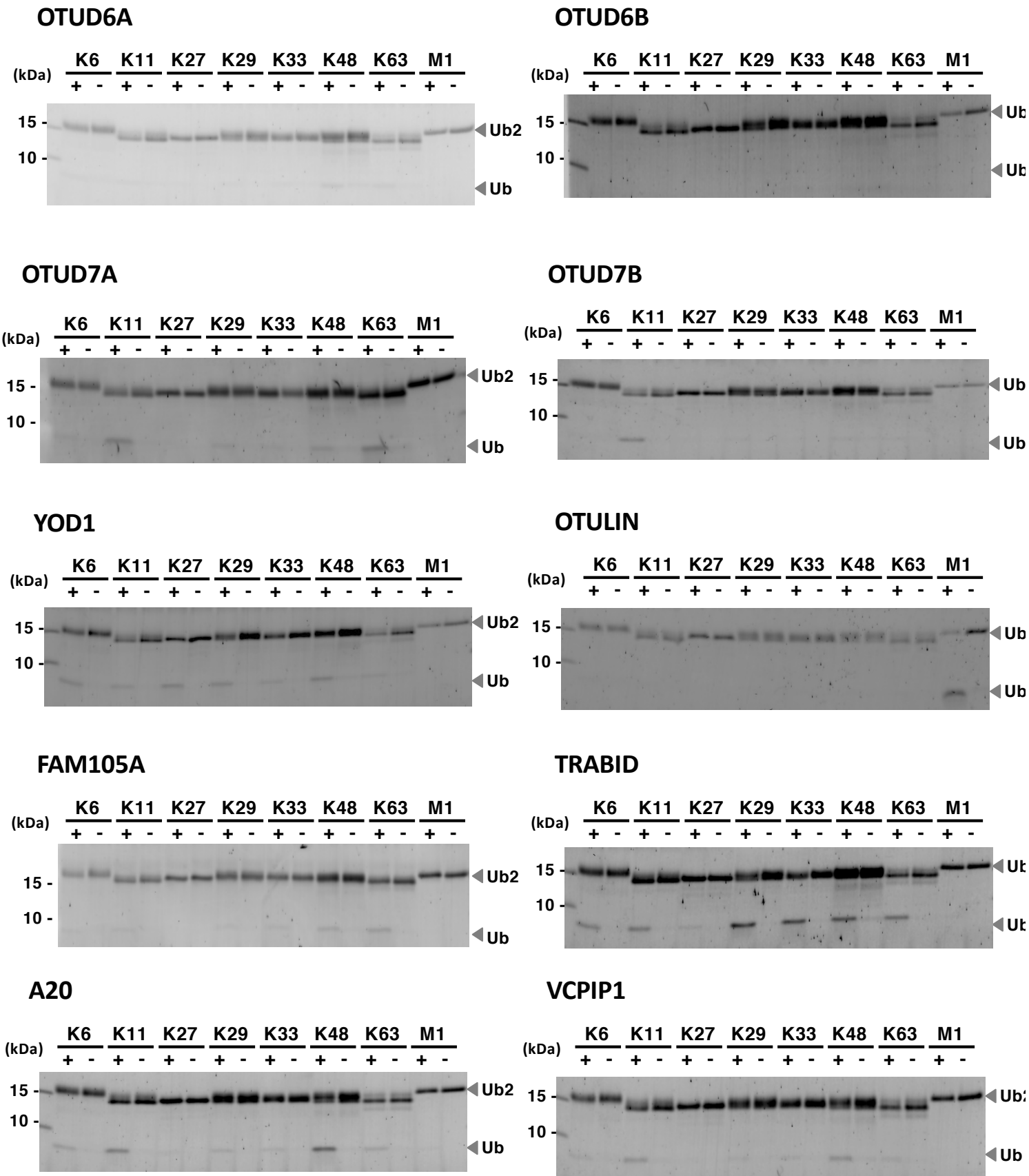
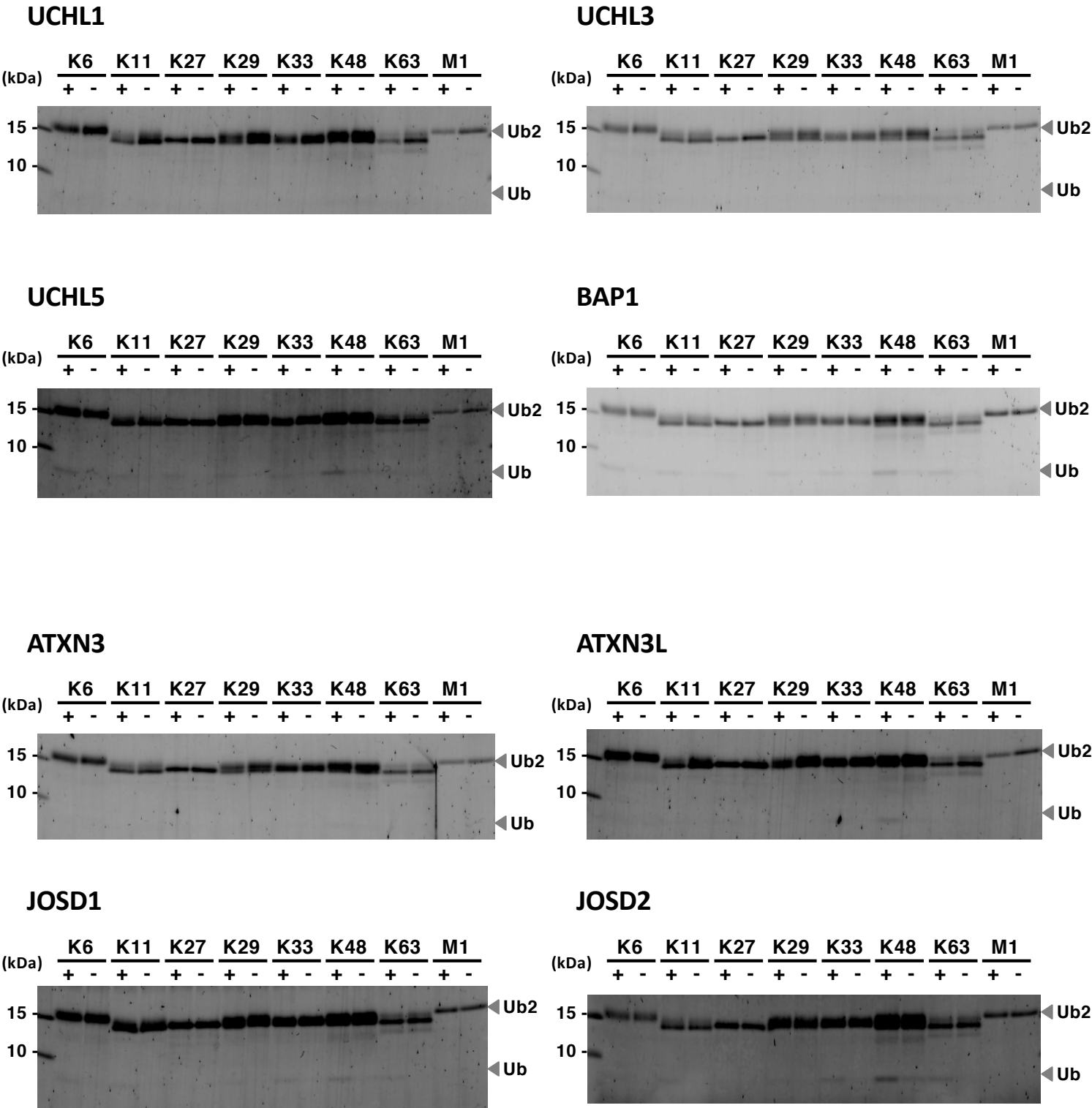


Figure S3



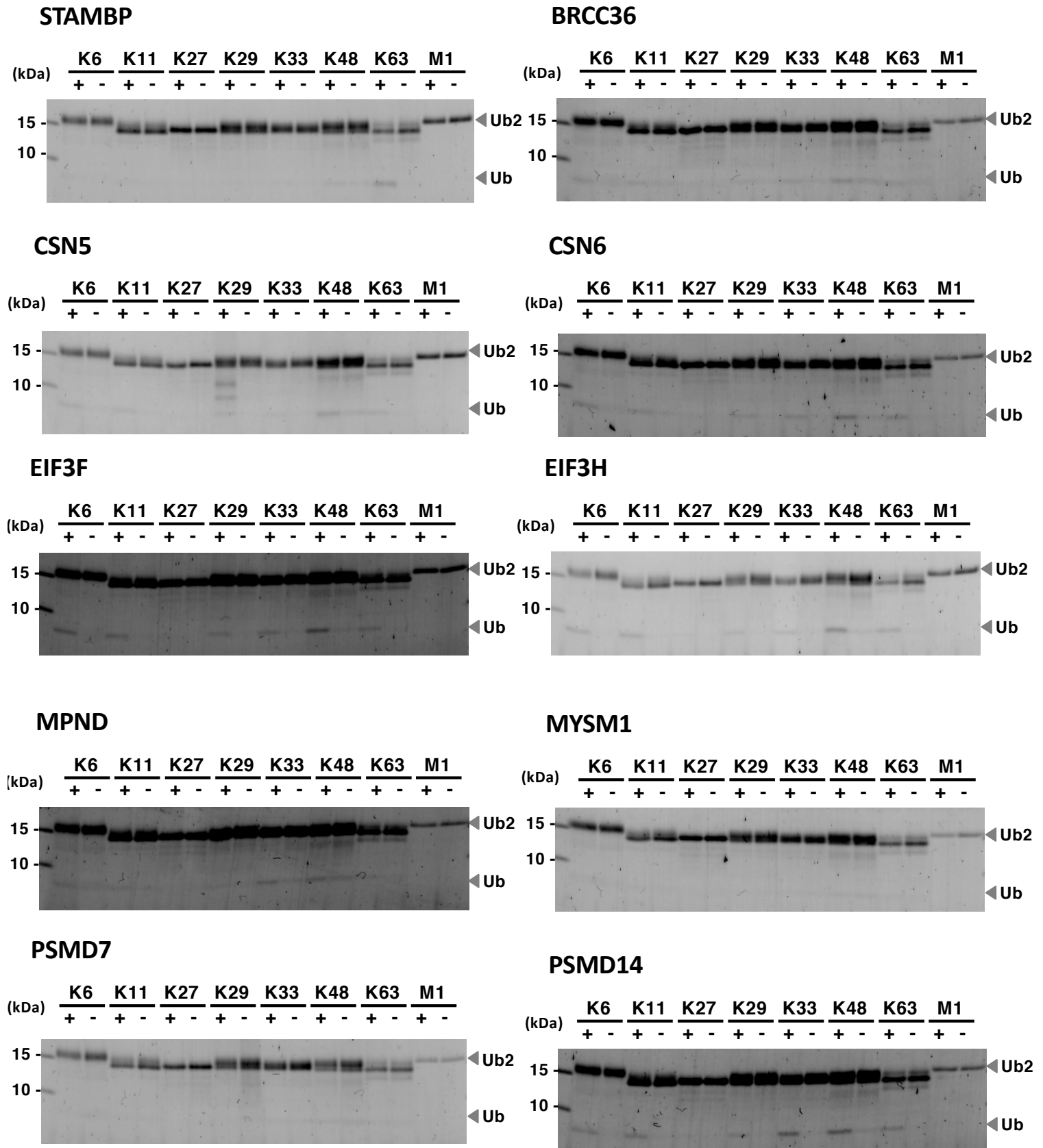


Figure S3. The result of in vitro DUB assay in Figure 3. All of the SYPRO Ruby staining gels after the DUB assay were shown.

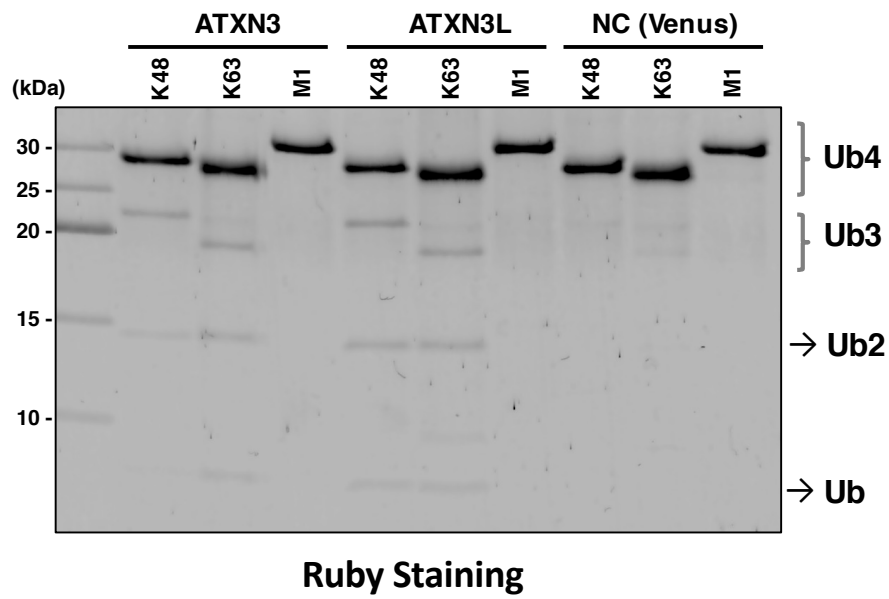


Figure S4. The result of DUB assay of ATXN3 and ATXN3L using tetraubiquitins as substrate. The in vitro DUB assay was carried out as same procedure as Figure 2, except for using K48-, K63-, and M1-tetraubiquitins were used.

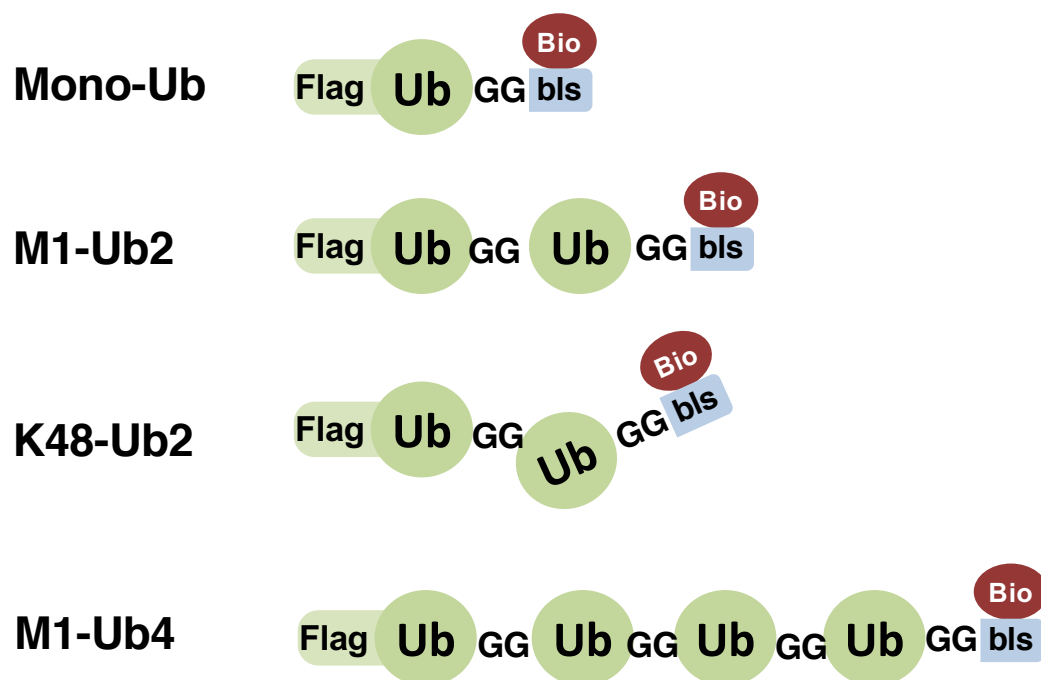


Figure S5. The schematic diagram of substrate ubiquitins for AlphaScreen-based DUB assay. Abbreviations are indicated as follows: Flag, FLAG-tag; Bio, biotin; bls, biotin ligase recognition sequence; Ub, ubiquitin.

Table S1. The list of all human DUBs. The other names, and predicted molecular weight and amino acid sequence of each DUB used in this study were indicated. DUBs that were not analyzed in this study are indicated in gray characters.

Table S2. The results of DUB assay using diubiquitins. The actual ratio of cleaved diubiquitins in Figure 3 are shown in column B to I. The concentration of each DUB used in the assay is determined by CBB staining (see detail in the materials and methods), and indicated in column J. N.D indicates the DUBs that we failed to determine the concentration, because the band of DUB staining with CBB was not obtained (*1) or was overlapped with the band of heavy chain of anti-AGIA antibody (*2). The comparison of the result of DUB assay between our result and previously published data is shown with following definition; The results between our assay and previous result are totally identical (Circle), partially agree (Triangle), and totally different (Cross mark).

Table S3. The results of the AlphaScreen-based evaluation of DUB inhibitors in Figure 4B. For individual DUB, substrates ubiquitin used and reaction time are shown in column C and D. The average of inhibition rate and its standard deviation from three independent assay were shown in E and H.