Identification of *Salmonella* Typhimurium deubiquitinase SseL substrates by immunoaffinity enrichment and quantitative proteomic analysis

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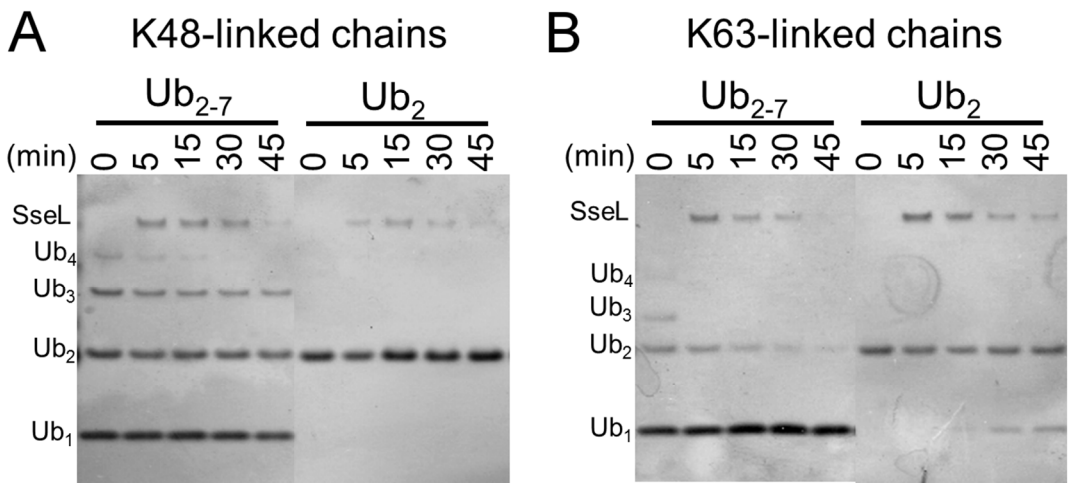
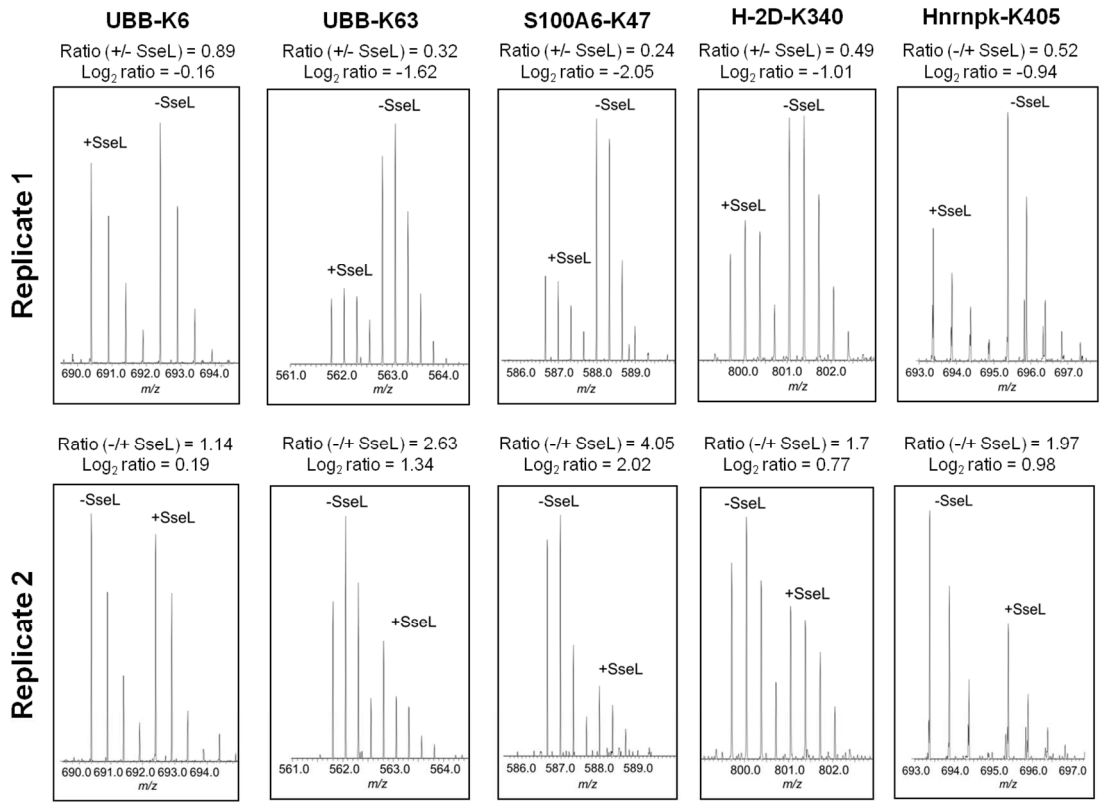
Supporting Information Figure S1



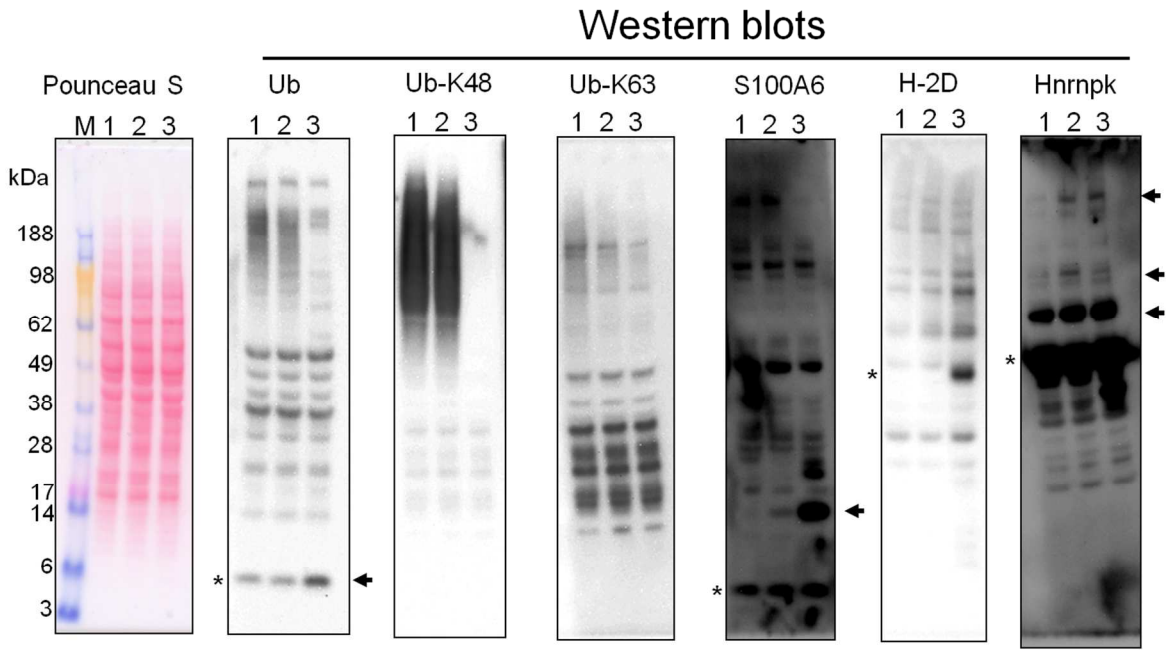
Supporting Information Figure S1 – SseL activity on diubiquitin (Ub_2) and oligoubiquitin (Ub_{2-7}) chains. SseL activity was assayed using K48- (A) and K63-linked (B) diubiquitin (Ub_2) and polyubiquitin (Ub_{2-7}) chains and products were separated on a SDS-PAGE gel and visualized with silver stain. Abbreviations: Ub_1 – ubiquitin monomer, Ub_2 – ubiquitin dimer, Ub_3 – ubiquitin trimer, Ub_4 – ubiquitin tetramer.

Supporting Information Figure S2



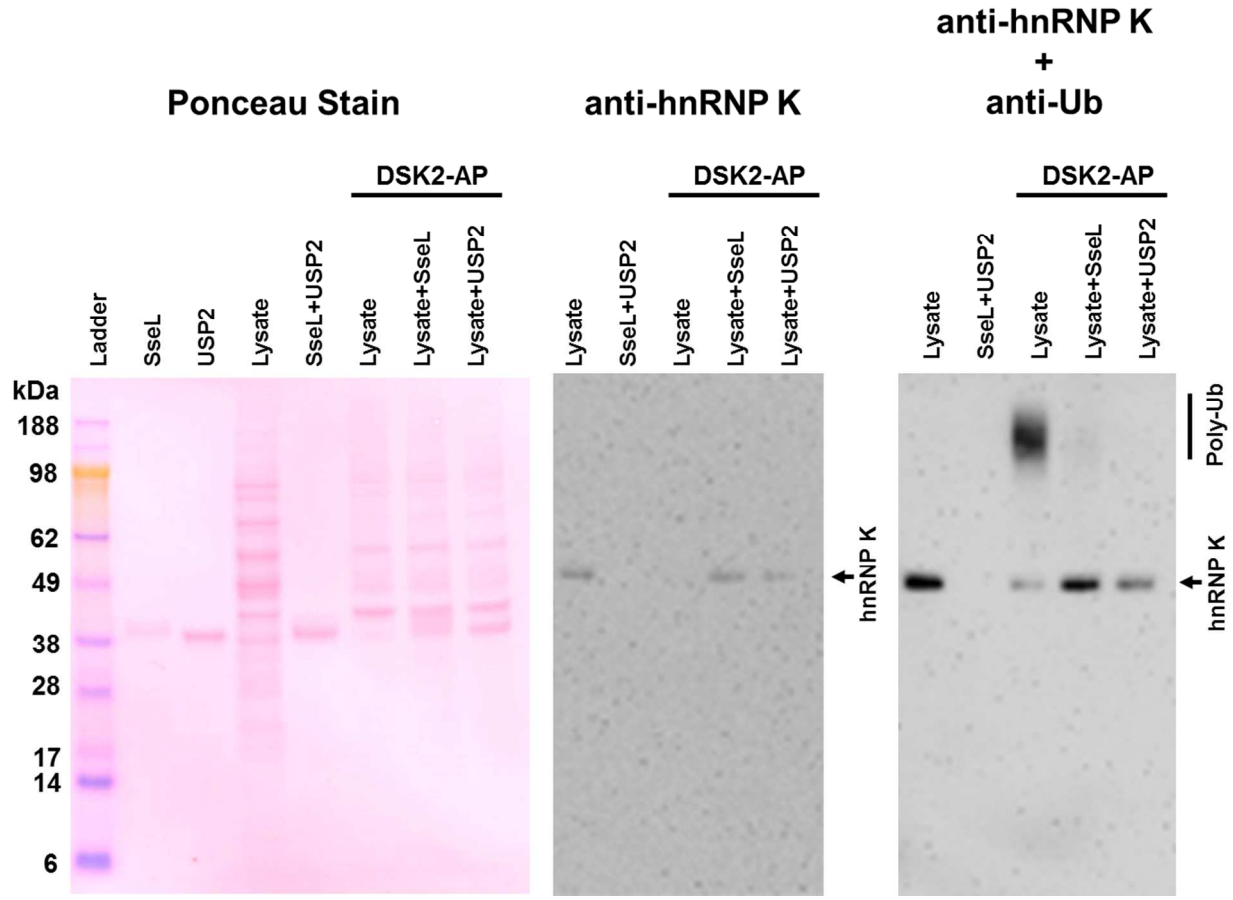
Supporting Information Figure S2 – Mass spectra for all SseL substrate candidates. Ubiquitination at lysine 63 of ubiquitin – UBB-K63, S100A6 at lysine 47 - S100A6-K47, MHC class I at lysine 340 – H-2D-K340, heterogeneous ribonucleoprotein K at lysine 405 – Hnrnpk-K405 were verified manually, along with a ubiquitination site unaffected by SseL treatment (ubiquitination at lysine 6 of ubiquitin – UBB-K6).

Supporting Information Figure S3



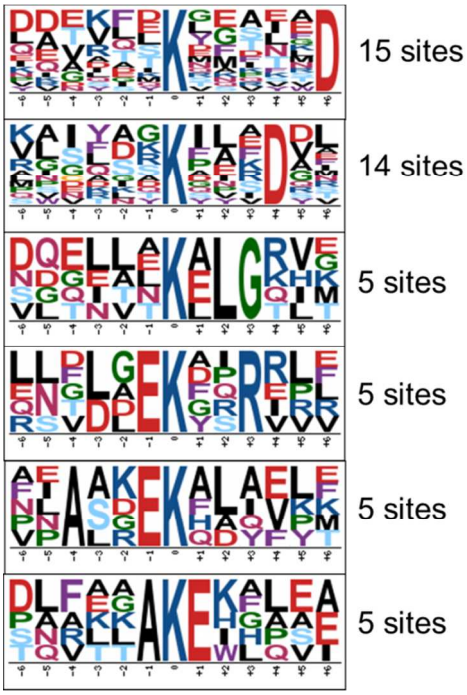
Supporting Information Figure S3 – Validation of SseL substrates in RAW 264.7 cells with over exposed versions of anti-S100A6 and Hnrnpk Western blots. RAW 264.7 cell lysates were incubated with no enzyme (lane 1) or SseL (lane 2) or USP2 (lane 3) and analyzed by western blots with specific antibodies. The asterisks show the mass of the unmodified protein, whereas the arrows show the protein bands that increase with the enzymatic treatment.

Supporting Information Figure S4



Supporting Information Figure S4 – Digestion of ubiquitinated hnRNP K protein by SseL. Ubiquitinated proteins were capture from RAW 264.7 cell lysates by affinity purification with the ubiquitin-binding domain of yeast DSK2 protein immobilized to agarose beads and then treated with SseL or USP2. Treatment product was then analyzed by western blot using anti-hnRNP K antibody alone or in combination with anti-ubiquitin antibody.

Supporting Information Figure S5



Supporting Information Figure S5 – Overrepresented motifs in the complete ubiquitinome dataset. All identified ubiquitination sites from RAW 264.7 cells were analyzed using a motif finder tool, Motif X.