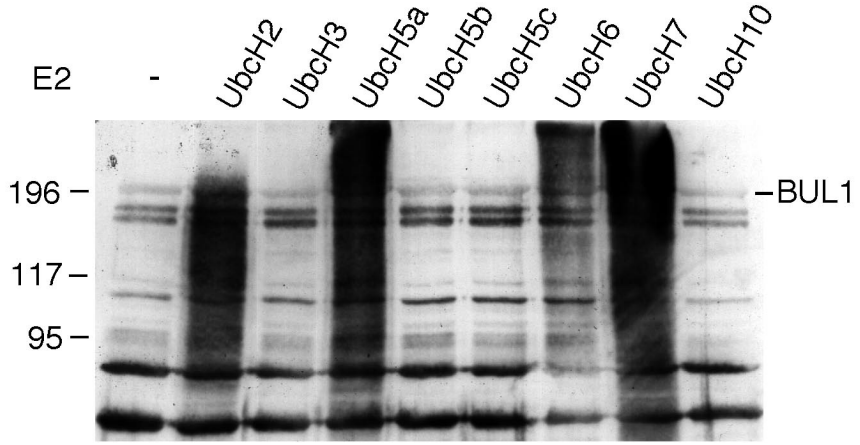
Supplementary data: Ubiquitin ligase activity of BUL1

METHOD

In vitro ubiquitination assay. Reaction mixtures (30μl) containing 0.11μg of E1 (Boston Biochem), 0.6μg of each E2 (UbcH2, H3, H5a, H5b, H5c, H6, H7 or H10; Boston Biochem), 0.8μg of recombinant BUL1, and 1μg of ubiquitin (Sigma) in 25 mM Tris-HCl (pH8.0), 120 mM NaCl, 1mM MgCl2, 2 mM ATP, 2 mM ATP-γ-S, and 1 mM DTT were incubated for 3 h at 30 °C. The reaction was terminated by the addition of SDS sample buffer and heating at 95 °C for 5 min. Samples were resolved by SDS/PAGE on 7.5% gel and then subjected to Western blot analysis with a rabbit anti-ubiquitin antibody (Santa Cruz).



Supplementary figure 1. Ubiquitin ligase activity of BUL1 *in vitro*. Ubiquitin thiol ester formation of the recombinant BUL1 is observed in the presence of specific E2, UbcH2, UbcH5a, UbcH6 and UbcH7. Ubiquitination of the proteins derived from bacterial extracts is also observed in this assay.