



**Supplementary Figure 2. The 600K complex assembles polyubiquitin chains.** (A, B) Gel filtration analysis of the recombinant 600K complex prepared from insect cells (A) or *E. coli* (B). The 600K complex was separated by Superdex 200 HR and subjected to SDS-PAGE. His<sub>6</sub>-HOIP (top) and HOIL-1L (bottom) in the complex were detected by immunoblotting with anti-HOIP and anti-HOIL-1L antibodies, respectively. \* indicates degradation products of HOIP. (C) The 600K complex prepared in bacterial cells is also able to polymerize ubiquitin. Ubiquitin was incubated with E1, UbcH5C and ATP in the presence of the 600K complex prepared in bacteria (lane 1, 2) or that prepared from insect cells (lane 3) at 37 °C (lane 2, 3) or on ice (lane 1) for 2 h. Samples were separated by SDS-PAGE and subjected to immunoblotting with anti-ubiquitin, anti-HOIP or anti-HOIL-1 antibodies.