



**Supplementary Figure 5. The NZFs of the 600K complex are involved in binding of ubiquitinated proteins, but dispensable for linear polyubiquitin conjugation.** (A) Deletion of NZFs from the 600K complex had little effect on the ubiquitin conjugation activity. Ubiquitin was incubated with E1, ATP and 10 (lanes 1 and 5), 5 (lanes 2 and 6), 2.5 (lanes 3 and 7) or 1.25 μg/ml of Ubch5C (lanes 4 and 8) together with the WT (lanes 1-4) or ΔNZF 600K complex (lanes 5-8), which is composed of HOIL-1L(ΔNZF)-His<sub>6</sub>-HA and Flag-HOIP(ΔNZF1&2), at 37 °C for 2 h. Samples were separated by SDS-PAGE and subjected to immunoblotting with anti-ubiquitin, anti-HOIP or anti-HOIL-1 antibodies. (B) 293T cells co-expressing HOIL-1L-His<sub>6</sub>-HA and Flag-HOIP (lanes 1-5) or HOIL-1L(ΔNZF)-His<sub>6</sub>-HA and Flag-HOIP(ΔNZF1&2) (lanes 6-10) were lysed and subjected to sequential affinity purification using Nickel (Ni) and anti-Flag antibody (M2) beads. The lysate (T), and the fractions that remained unbound (UB), or bound (B), to Nickel or anti-Flag antibody beads, were immunoblotted with anti-ubiquitin (top), anti-Flag (middle) or anti-HA antibodies (bottom). Of the collected samples, 2, 2, 5, 5 or 100 % (v/v) was loaded in T, UB (Ni), B (Ni), UB (M2) or B (M2), respectively.