





Supplementary Figure 1. Mass-spectrometry analyses of purified ubiquitylated-Ent1 derivatives from *E. coli* based expression system.



Supplementary Figure 2. Structural model of ENTH:Ub complex. We superimposed the yeast ENTH on the STAM-VHS:Ub complex (3LDZ) and then use Fiberdoc for flexible induced-fit backbone refinement [39]. The residues at the binding interface and the lysine residues that undergo ubiquitylation are shown as sticks.



Supplementary Figure 3. structure of yeast Ent2 homo dimer super imposed with rat Epsin1 ENHT domains. Top shows the overall structure of the proteins domain. The changes in the orientation of H_0 is shown. Bottom, is a zoom-in into the ligand binding site, shows the different location and orientation of the PIP₂ molecules. The orange PIP₂ is from the rat-ENTH complex and the green molecule is from the Ent2 complex.