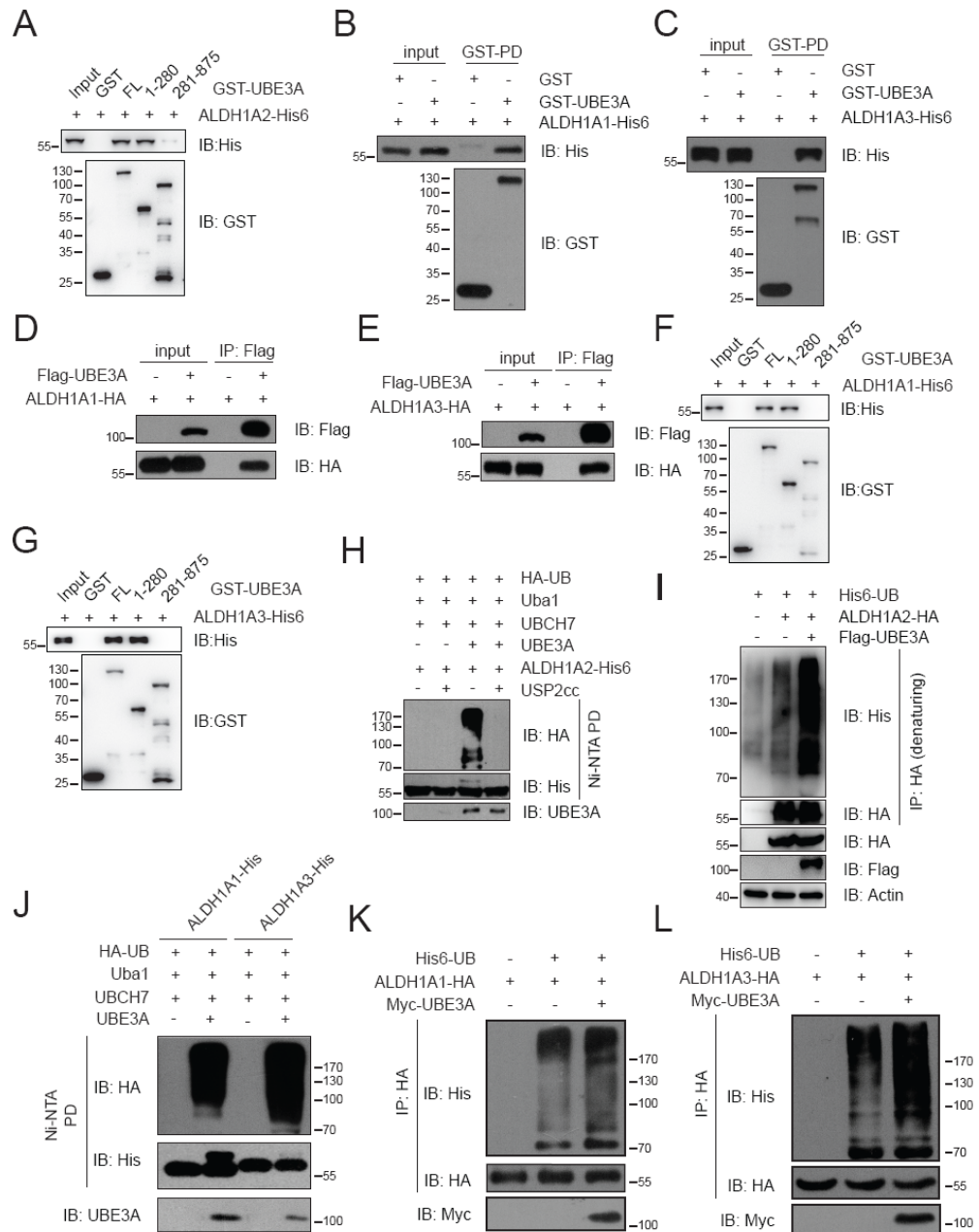


## Supplementary information, Figure S1



**Figure S1 UBE3A interacts with, and ubiquitylates other members of human ALDH1A family.** (A-C) Truncation or full-length UBE3A interacted with human ALDH1A family proteins *in vitro*. In GST pull-down assay, GST or GST-fused UBE3A

truncating proteins were incubated with purified ALDH1A2-His6 (A) *in vitro*, while GST-fused UBE3A full-length proteins were incubated with purified ALDH1A1-His6 proteins (B) or ALDH1A3-His6 proteins (C) *in vitro*, followed by incubation with glutathione-agarose beads, pulled-down and subjected to IB assay using anti-His and anti-GST antibodies. (D, E) Human UBE3A formed complex with the ALDH1A family proteins in intact cells. HEK-293FT cells expressing Flag-UBE3A and ALDH1A1-HA (D) or ALDH1A3-HA (E) were subjected to immunoprecipitation and IB analysis. (F, G) Residues 1-280 of UBE3A interacted with human ALDH1A family proteins *in vitro*. GST or GST-fused UBE3A truncating proteins were incubated with purified ALDH1A1-His6 (F), or ALDH1A3-His6 proteins (G) *in vitro*, followed by glutathione-agarose beads pulled-down and IB assay. (H) The bacterial reconstituted ubiquitylation system identified the conjugation of poly-Ub onto ALDH1A2 by UBE3A. (I) The poly-Ub chain on ALDH1A2 conjugated by UBE3A was detected after immunoprecipitation in denaturing condition. (J) UBE3A also ubiquitylated the other members of human ALDH1A family proteins in the reconstituted ubiquitylation system in BL21 bacterial strain. (K, L) UBE3A ubiquitylated other human ALDH1A family proteins in intact cells. HEK-293FT cells were co-transfected with constructs encoding His6-UB, Myc-UBE3A and ALDH1A1-HA (K) or ALDH1A3-HA (L), followed by immunoprecipitation and IB analysis. All experiments were conducted at least three times.