Supplementary information, Figure S6

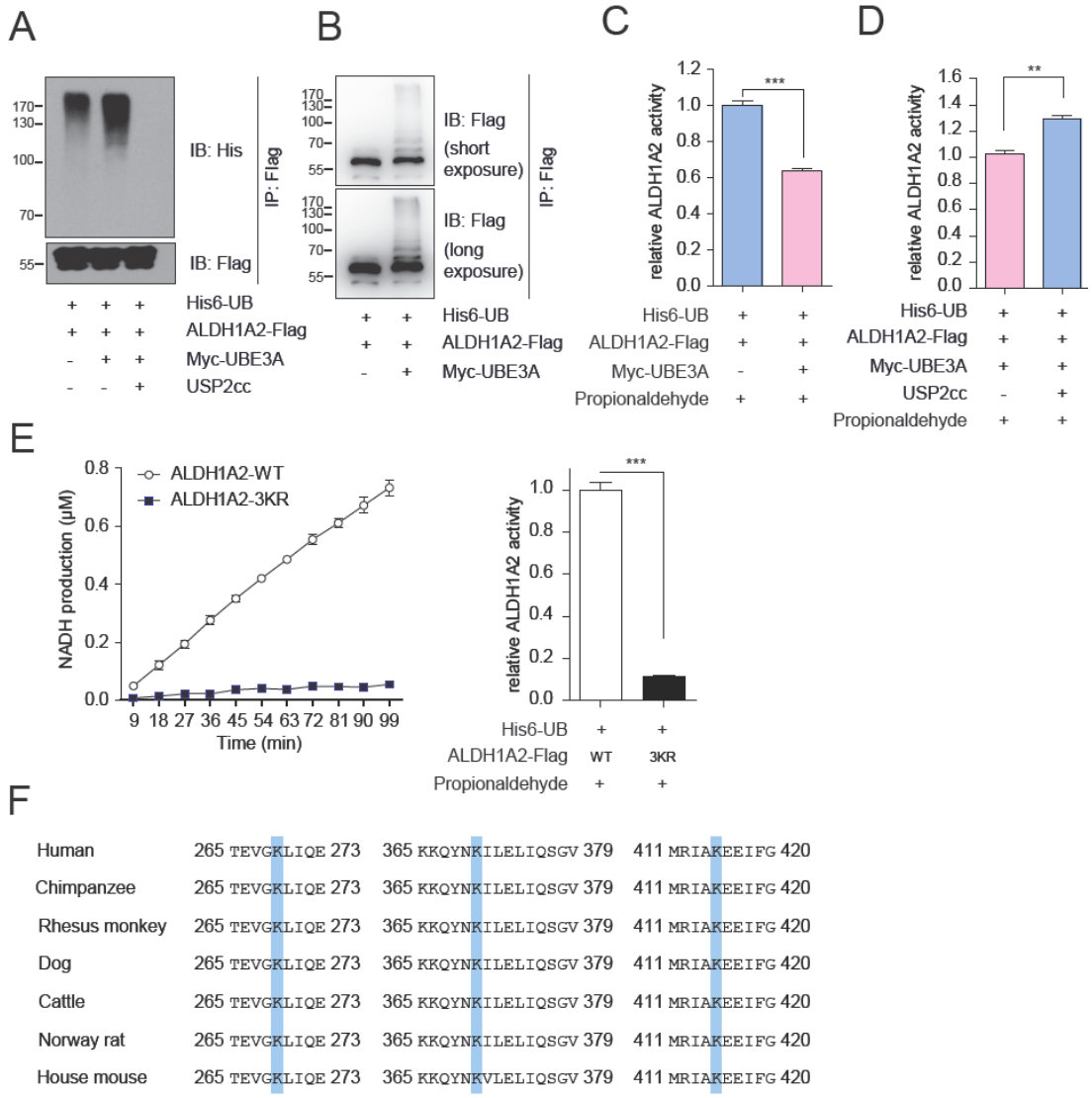

Figure S6 Ubiquitylation of ALDH1A2 compromises its dehydrogenase activity, using propionaldehyde as substrate. (**A, B**) HEK-293FT cells were co-transfected with the indicated plasmids, and ALDH1A2-Flag protein was enriched with anti-Flag antibody followed by elution with Flag peptide in enzymatic activity assay buffer. The hereby recovered ALDH1A2-Flag protein was then treated with USP2cc protein or BSA at 4 °C

overnight to remove the poly-Ub chains on ALDH1A2-Flag. The ubiquitylation states of ALDH1A2-Flag protein was examined through IB using anti-His antibody **(A)** or anti-Flag antibody **(B)**. The results were repeated three times. (**C**) The enzymatic activity of the recovered ALDH1A2-Flag protein upon ectopic UBE3A expression or not, was assessed using propionaldehyde as substrate. Relative enzymatic activity of ALDH1A2 protein was shown after normalization to that of the control group in means ± SEM; ***$P < 0.001$, two-tailed *t*-test, n = 4. (**D**) The enzymatic activity of ALDH1A2 protein, with and without USP2cc enzyme treatment, was assayed towards the substrate propionaldehyde. Relative ALDH1A2 activity was shown after normalization, and presented in means ± SEM; **$P < 0.01$, two-tailed *t*-test, n = 3. (**E**) The enzymatic activities of wild-type (WT) or K269/370/415-to-R (3KR) mutant of ALDH1A2 proteins were examined using propionaldehyde as substrate. The data for NADH production (left panel) and the relative enzymatic activities (right panel) were shown after normalization, and presented in means ± SEM; ***$P < 0.0001$, two-tailed *t*-test, n = 3. (**F**) An alignment of the amino acid sequences flanking the three main ubiquitylation sites (K269, K370, and K415) in ALDH1A2 proteins from different species.