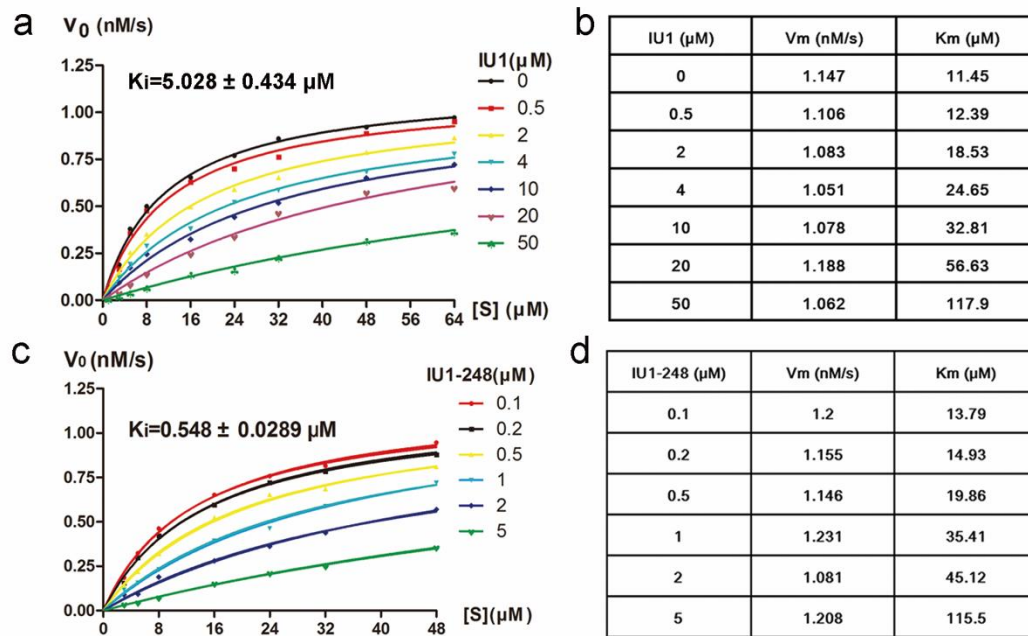




Supplementary information, Fig. S5 Kinetic analysis of the inhibitory activity of IU1 and IU1-248 inhibition of USP14 (a) Michaelis-Menten plot of the concentration-dependence of Ub-AMC (1-64 μM) hydrolysis in the presence of 50 nM USP14 and IU1 (0.5-50 μM). The data were fit to a curve by nonlinear regression ($R^2 > 0.99$) using the GraphPad Prism program. (b) Summary of approximate V_m and K_m values at different concentration of IU1. K_m but not V_m varied with IU1 concentration, indicating that IU1 acts as a competitive inhibitor. (c) Michaelis-Menten plot of the concentration-dependence of Ub-AMC (1-48 μM) hydrolysis in the presence of 120 nM USP14 and IU1-248 (0.1-5 μM). The data were fit to a curve by nonlinear regression ($R^2 > 0.99$) using the GraphPad Prism program. (d) Summary of approximate V_m and K_m values at different concentrations of IU1-248. K_m but not V_m varied with IU1-248 concentration, indicating that IU1-248 acts as a competitive inhibitor.