Supplemental data

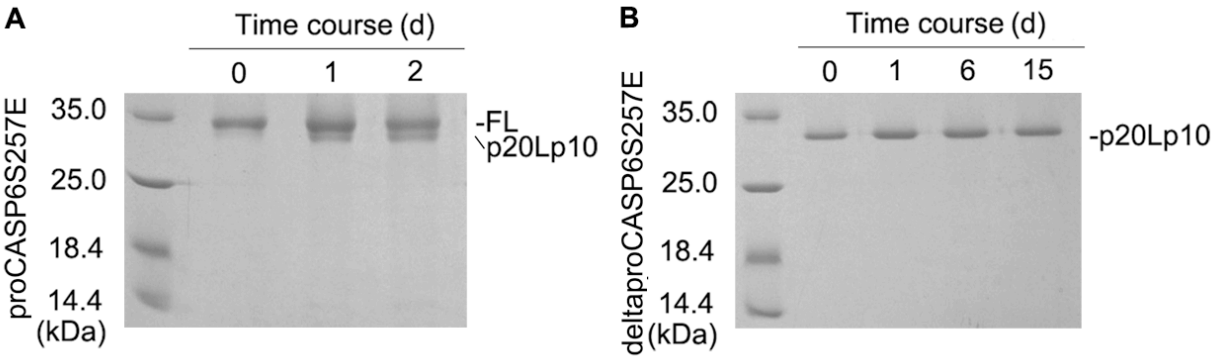

Figure S1: The S257E mutation inhibited CASP6 self-activation Stability assay of (**A**) proCASP6S257E and (**B**) ΔproCASP6S257E. FL, full length; p20, large subunit; L, intersubunit linker.

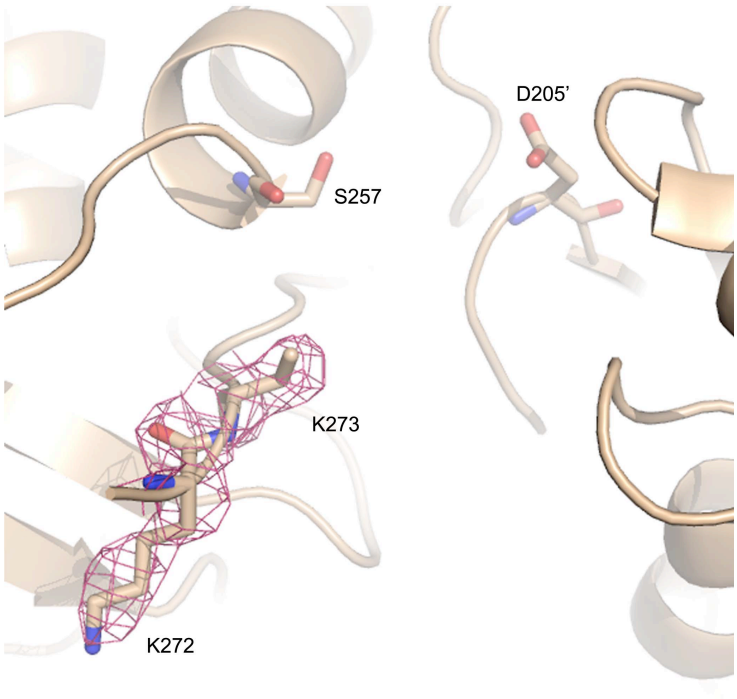

Figure S2: The electron density map of Lys272 and Lys273 in CASP6 zymogen The electron density map (2fo-fc maps) was shown at 1.0 σ, calculated by PHENIX refine.

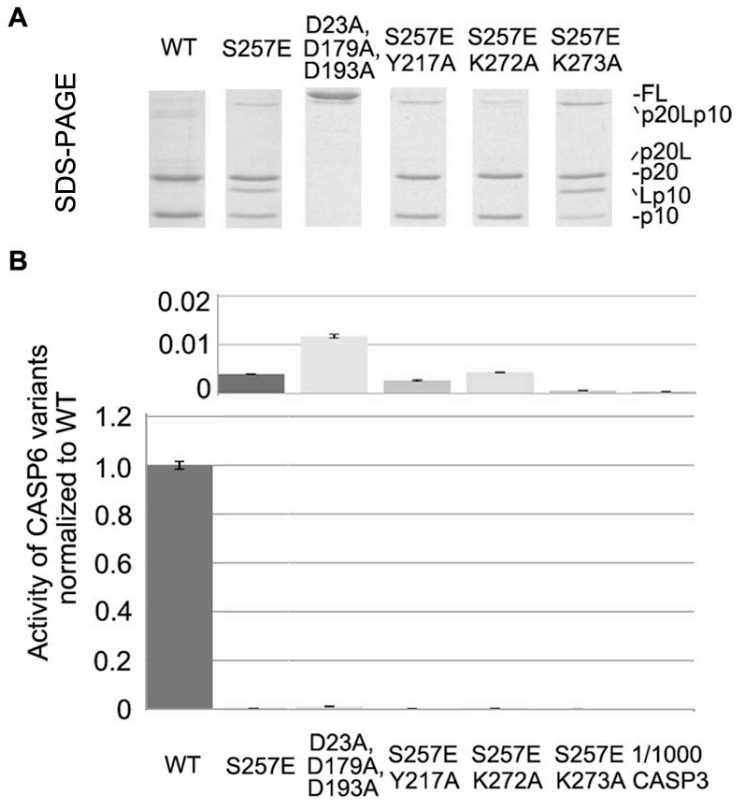

Figure S3: The S257E mutation inhibited CASP6 activity

Coomassie-blue-stained SDS-PAGE gels (**A**) and the VEIDase activity (**B**) of 1/1000 CASP3 processed CASP6 variants, normalized to WT CASP6. The activity of 1/1000 CASP3 is shown as a negative control. The activities with low values are also shown in a zoom-in view. The assays were done in triplicate and error bars represent s.d. FL, full length; p20, large subunit; L, intersubunit linker; p10, small subunit; WT, wild type.

Table S1 : The quantitative data of Thrombin proteolytic processing assay.

ΔproCASP6D179Th variants	Time course (h)					
	0	1	2	4	6	24
S257E,C163A	-	0	0	0	0	0
S257E	-	0	0	46.6	91.7	100
S257E,H168A	-	0	0	0	12.6	20.3
S257E,H219A	-	0	0	0	28.1	49.3
S257E,Y217A	-	0	72.8	100	100	100
S257E,K272A	-	0	76.0	100	100	100

The number shown in table are the percentage of Asp193 cleavage in Thrombin cleavage assay, which was calculated by scanning the SDS-PAGE in Fig.4C-4H and using intensity of p10 band divide by the sum of p10 band intensity plus Lp10 band intensity. The "-" symbols in 0 h column mean at 0 h time point no Lp10 nor p10 band were shown in the SDS-PAGE.