Supplemental Table

Table S1: The mutants used in this study. (Cter, C-terminal)

Name of the Mutant	Truncation Detail /N-terminal sequence	Mutation Detail	Generated in Which Study
proCASP6C163A	full length	C163A	(Wang *et al.*, 2010)
proCASP6H121A	full length	H121A	generated in this study
proCASP6(D23A,H121A)	full length	D23A, H121A	generated in this study
ΔproCASP6H121A	Ala24-Cter (delta-pro)	H121A	generated in this study
ΔproCASP6(S257E,K273A)	Ala24-Cter (delta-pro)	S257E, K273A	(Cao *et al.*, 2012)
ΔproCASP6S257K	Ala24-Cter (delta-pro)	S257K	(Cao *et al.*, 2012)
proCASP6(D23A,S257E,K273A)	full length	D23A, S257E, K273A	generated in this study
proCASP6(D23A,S257K)	full length	D23A, S257K	generated in this study
proCASP6(C163A,RE)	full length	R64E, C163A, R220E	(Wang *et al.*, 2010)
proCASP6(T22V,V192T,C163A,RE)	full length	T22V, R64E, C163A, V192T, R220E	generated in this study
N10CASP6(C163A,RE)	Gly10-Cter	R64E, C163A, R220E	generated in this study
N15CASP6(C163A,RE)	Gly15-Cter	R64E, C163A, R220E	generated in this study
N10CASP6(M19A,C163A,RE)	Gly10-Cter	M19A, R64E, C163A, R220E	generated in this study
N15CASP6(M19A,C163A,RE)	Gly15-Cter /GEENATETD	M19A, R64E, C163A, R220E	generated in this study
N16CASP6(M19A,C163A,RE)	Glu16-Cter /MEENATETD	M19A, R64E, C163A, R220E	generated in this study
N17CASP6(M19A,C163A,RE)	Glu17-Cter /MENATETD	M19A, R64E, C163A, R220E	generated in this study
N18CASP6(M19A,C163A,RE)	Asn18-Cter /MNATETD	M19A, R64E, C163A, R220E	generated in this study
N19CASP6(M19A,C163A,RE)	Met19-Cter /ATETD	M19A, R64E, C163A, R220E	generated in this study
N19CASP6(M19F,C163A,RE)	Met19-Cter /MFTETD	M19F, R64E, C163A, R220E	generated in this study
N20CASP6(C163A,RE)	Thr20-Cter /TETD	R64E, C163A, R220E	generated in this study
proCASP6(E17A,C163A,RE)	full length	E17A, R64E, C163A, R220E	generated in this study
proCASP6(N18A,C163A,RE)	full length	N18A, R64E, C163A, R220E	generated in this study
proCASP6(N18L,C163A,RE)	full length	N18L, R64E, C163A, R220E	generated in this study

Supplemental Figure

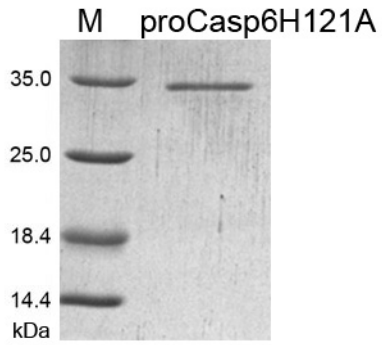

Figure S1: SDS-PAGE of proCASP6H121A crystal The results showed that the crystal was formed by a homogenous protein with the molecular weight around 34 kD , which represent the full-length CASP6.

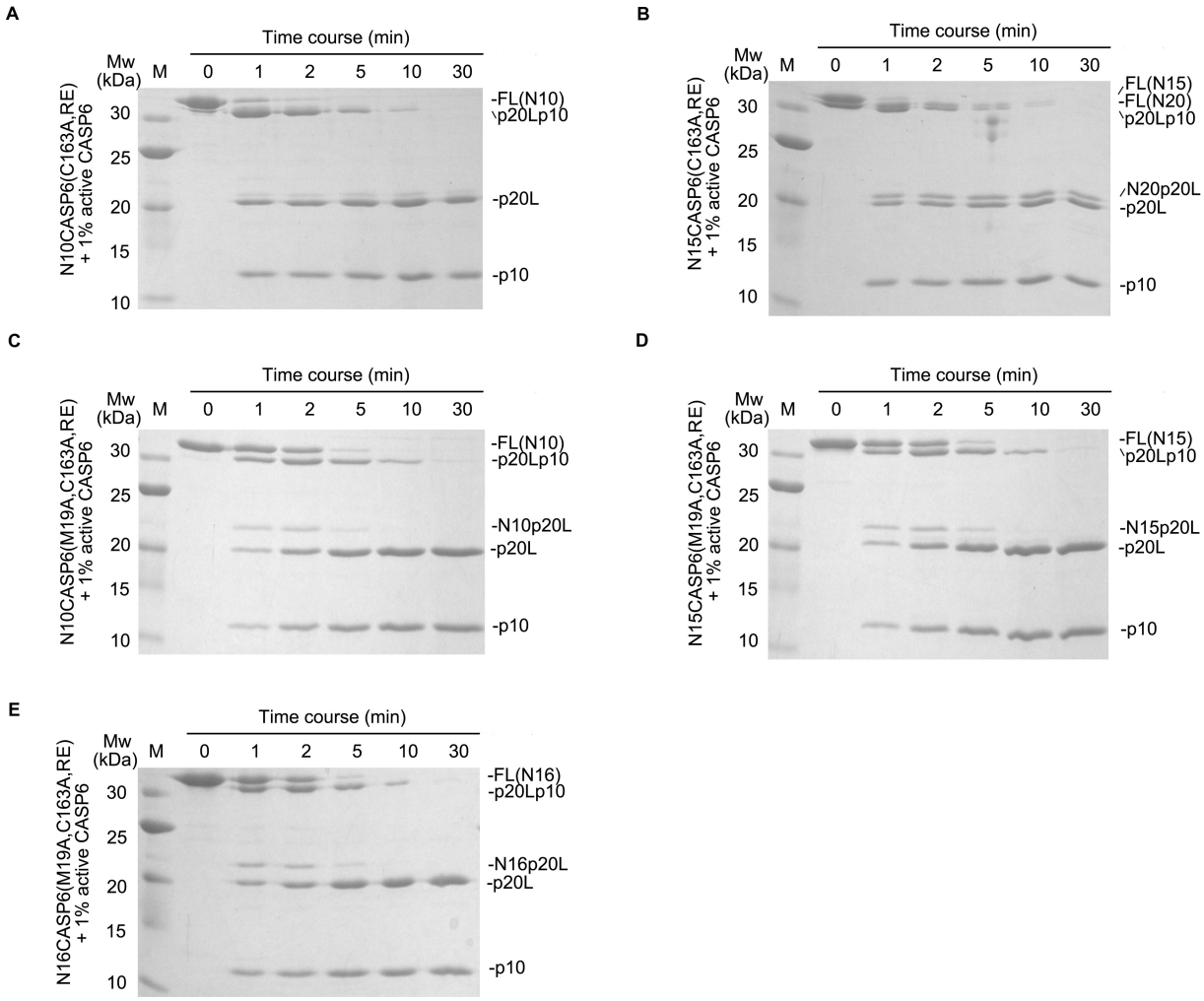

Figure S2: The detailed CASP6 truncates cleavage assays The coomassie blue stained SDS-PAGE of CASP6 truncates cleaved by active CASP6. (**A**) The result of N10CASP6(C163A,RE) showed the priority of TETD^{23} site to the TEVD^{193} site during inter-molecular cleavage was still exist when deleted 9 residues in N-terminal. (**B**) When truncated to the 15th residue, Met19 acted as a second translation initiator and the resulting truncate N15CASP6(C163A,RE) was expressed as N15CASP6(C163,RE) and N20(CASP6,RE) mixture (see lane '0'), and made the result of the cleavage assay too complicated. (**C**)-(**E**) The M19A mutation was induced to exclude the influence of double start point and (**C**) N10CASP6(M19A,C163A,RE) was cleaved by active CASP6 to investigate if M19A would influence the TETD^{23} site priority. The result

showed M19A made no influence and the results of (**D**) N15CASP6(M19A,C163A,RE) and (**E**) N16CASP6(M19A,C163A,RE) showed the priority of the TETD[23] site was exist when truncated to the 16th residues in N-terminal.

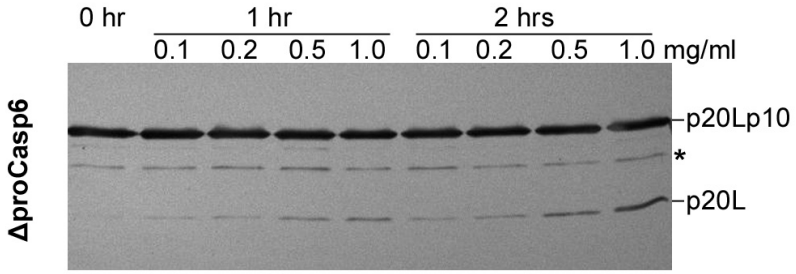

Figure S3: The concentration dependence assay of CASP6 auto-activation The purified ΔproCASP6 was diluted to the indicated concentration and incubated at 37 ºC for 2 hours. The speed of auto-activation was represented by the amount of p20L band appeared on SDS-PAGE. The results showed that the speed of CASP6 auto-activation depended on protein concentration. The asterisks * labeled bands were a bacterial contamination protein. p20, large subunit; L, intersubunit linker; p10, small subunit.

Supplemental Reference

Cao, Q., Wang, X. J., Liu, C. W., Liu, D. F., Li, L. F., Gao, Y. Q. & Su, X. D. (2012). *Journal of Biological Chemistry* **287**, 15371-15379.

Wang, X. J., Cao, Q., Liu, X., Wang, K. T., Mi, W., Zhang, Y., Li, L. F., LeBlanc, A. C. & Su, X. D. (2010). *EMBO Rep* **11**, 841-847.