INDUCIBLE DIMERIZATION AND INDUCIBLE CLEAVAGE REVEAL A REQUIREMENT FOR BOTH PROCESSES IN CASPASE-8 ACTIVATION

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SUPPLEMENTARY DATA

Figure S1: Purification of FKBP-Caspase-8. FKBP-Caspase-8-his fusion proteins were expressed in the bacteria using the pET28b vector, then purified according to published protocols. Purified proteins were resolved on SDS-PAGE gels and revealed by coomassie staining.

Figure S2: FKBP-Caspase-8 constructs containing a TEV cleavage site behave similarly to constructs with cleavage-abrogating asp>ala mutations. FKBP-Caspase-8 bearing the indicated mutations was expressed an purified as previously described. These proteins were activated using the dimerizer AP20187 or 1M sodium citrate as indicated. Enzymatic activity was measured using the fluorogenic substrate IETD-afc.

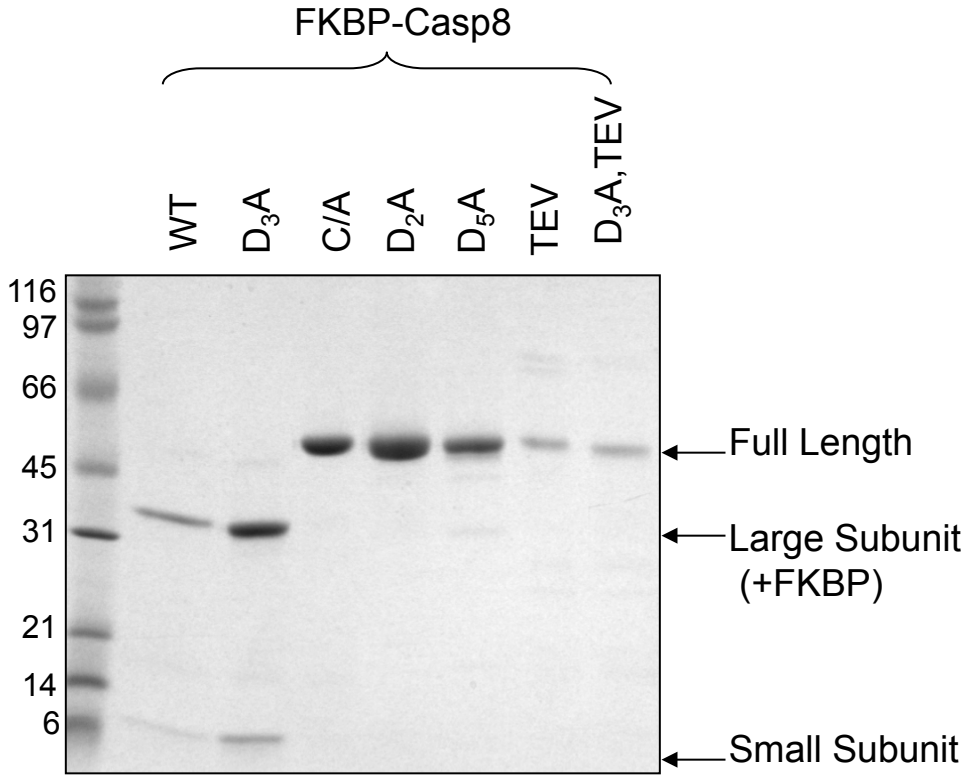
Figure S3: Codon optimization of TEV protease is required for efficient expression and substrate cleavage in cells. HeLa cells were transfected with the indicated plasmids. Twenty-four hours post-transfection, cells were lysed and lysates were resolved by western blotting using the indicated antibodies.

Figure S4: Co-expression of Caspase-7(TEV) and optimized TEV protease causes efficient cleavage of caspase-7. HeLa cells were transfected as indicated, in the presence or absence of 100uM zVAD-fmk. Forty-eight hours post-transfection, cells were harvested and analyzed by western blot as indicated.

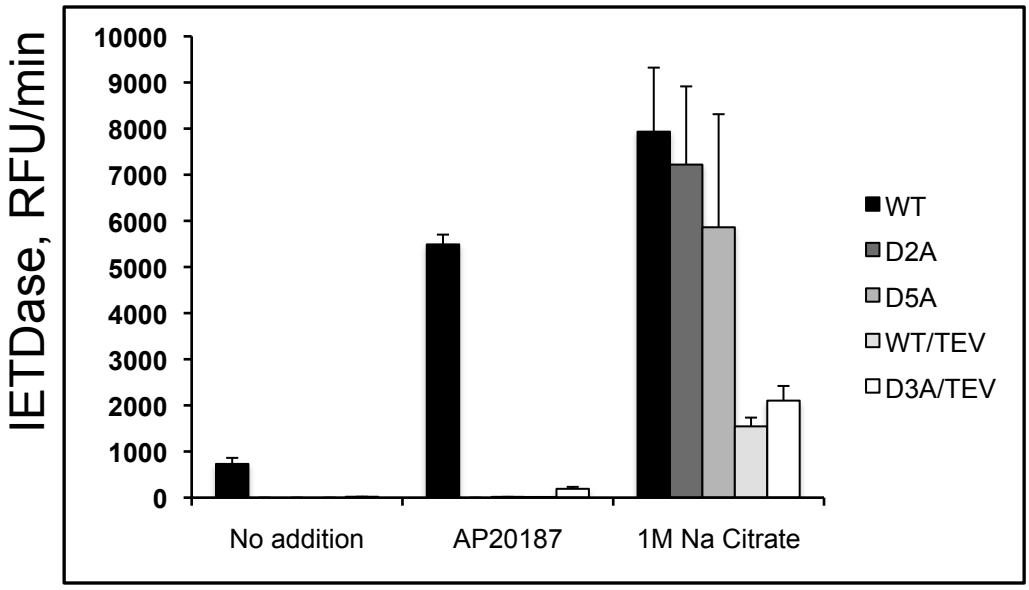
Figure S5: Purification and TEV protease-mediated cleavage of FKBP-Caspase-8. The indicated FKBP-Caspase-8 constructs were purified as previously described, then incubated with recombinant TEV protease. The products of these cleavage reactions were resolved by SDS-PAGE and revealed by coomassie staining.

Supplementary Table 1: Quantification of enzymatic activity presented in Fig. 6A. The enzymatic reactions presented in Fig. 6A are quantified; numbers represent averages of at least 3 independent reactions, with standard deviations presented in parenthesis.

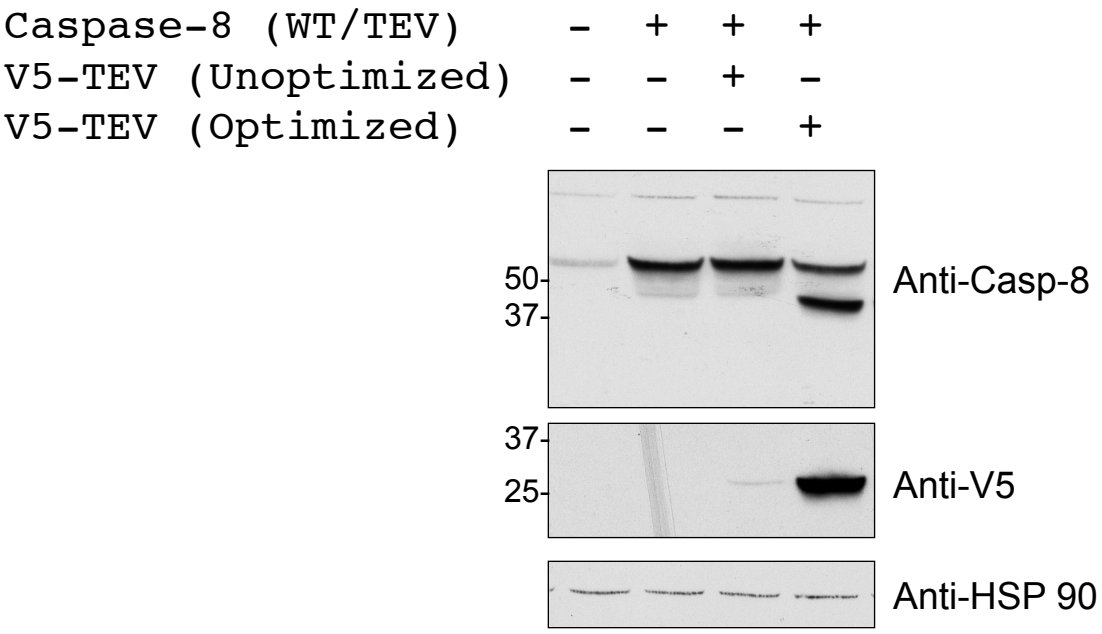
Oberst et al., Supplemental Figure 1



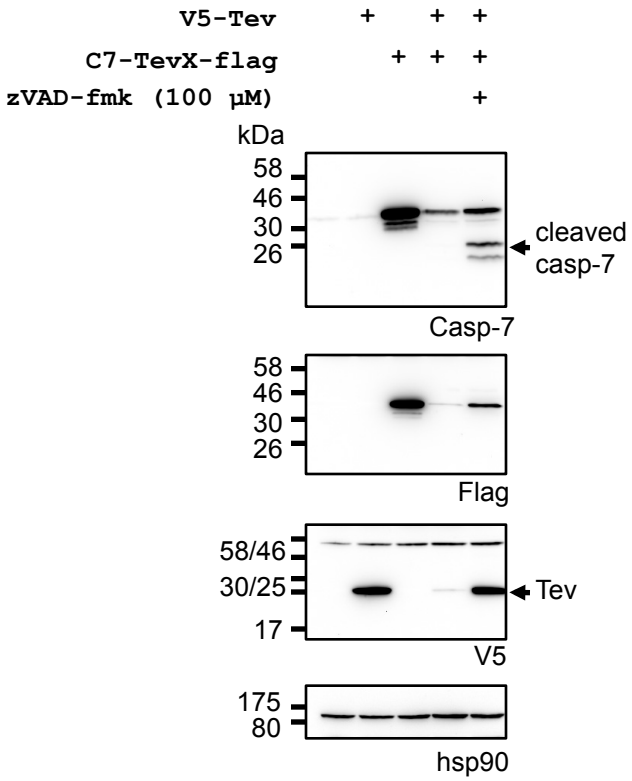
Oberst et al., Supplementary Fig. 2



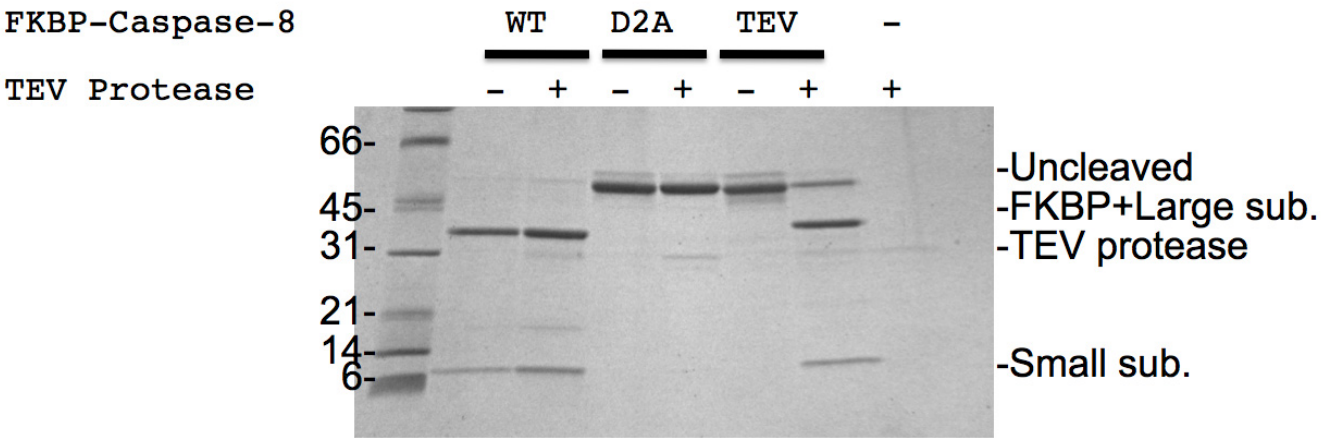
Oberst et al., Supplementary Fig. 3



Oberst et al. Supplementary Figure 4



Oberst et al., Supplementary Figure 5



Oberst et al., Supplementary Table 1

Supplementary Table 1: AcIETD-afc-ase activity of the indicated FKBP-caspase8 constructs under the indicated conditions

	No Addition	**TEV protease**	**AP20187**	**AP20187 +TEV**	**1M Na-Citrate**
FKBP-Casp8(D3A)	1887 (196)	964 (309)	17900 (2044)	18175 (1723)	17377 (3707)
FKBP-Casp8(D5A)	19 (24)	43 (38)	91 (6)	88 (4)	1548 (398)
FKBP-Casp8 (D3A/TEV)	48 (22)	606 (49)	71 (1)	9301 (61)	1556 (662)