Supporting Information

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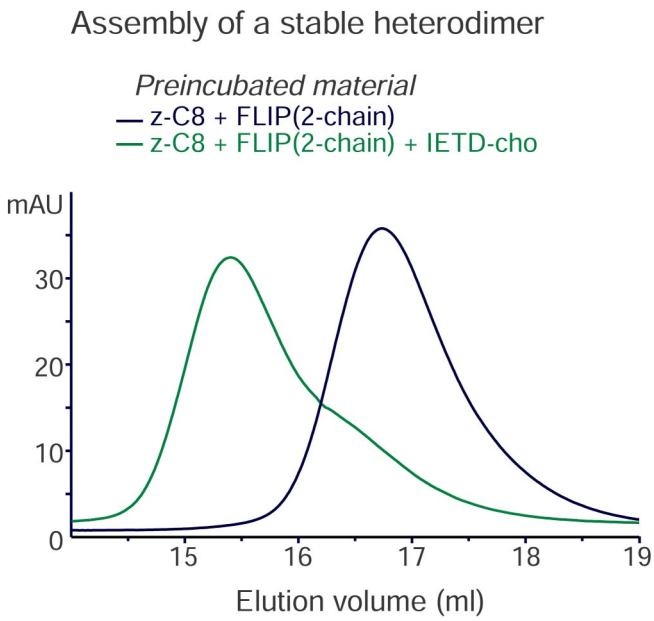

Fig. S1. Assembly of a stable heterodimer between zymogen caspase-8 (C8) and processed FLICE-inhibitory protein (FLIP) in the presence of the inhibitor Ac-IETD-cho, as assessed by gel filtration.

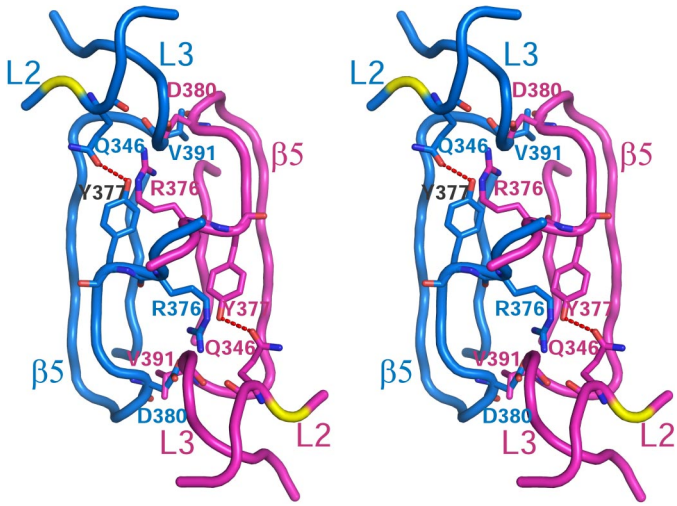

Fig. S2. Shown in stereo, the closed L2′ loop conformation causes steric clashes in the modeled zymogen C8 homodimer.

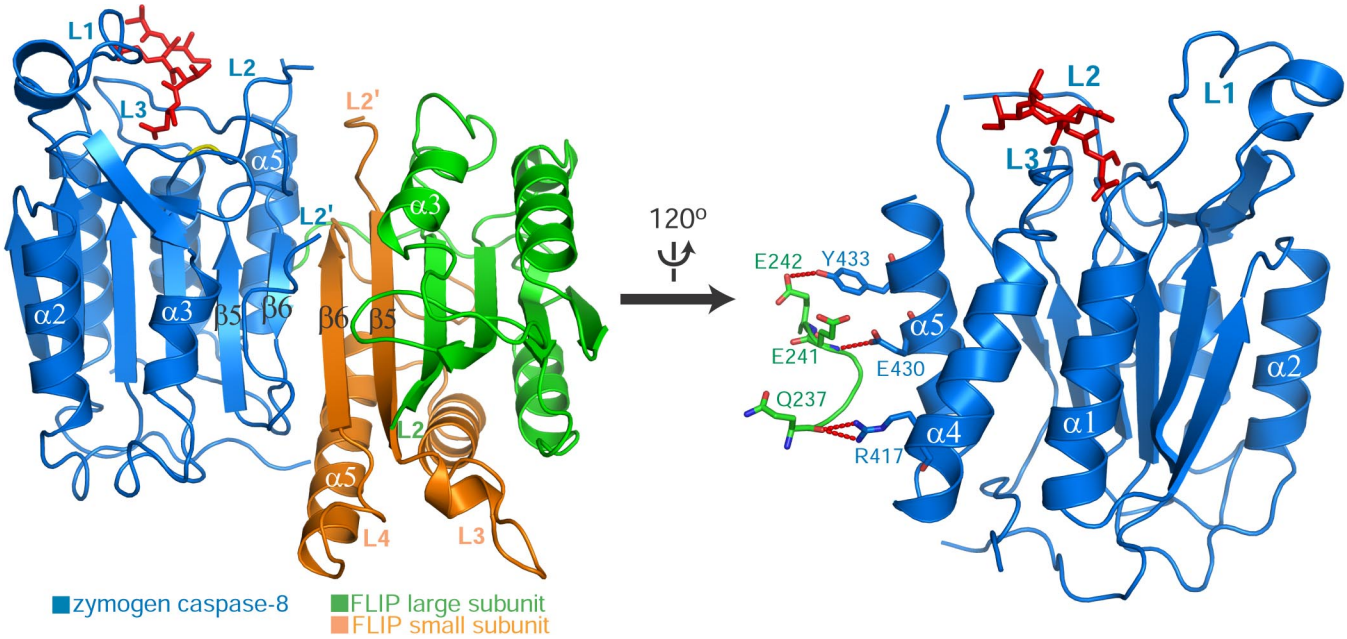

Fig. S3. The protease domain of FLIP contacts the α4/α5 helices of z-C8. These interactions are visible on rotation of the left view or Fig. 4*A* as indicated. For clarity, only a small portion of FLIP is shown on the right view.

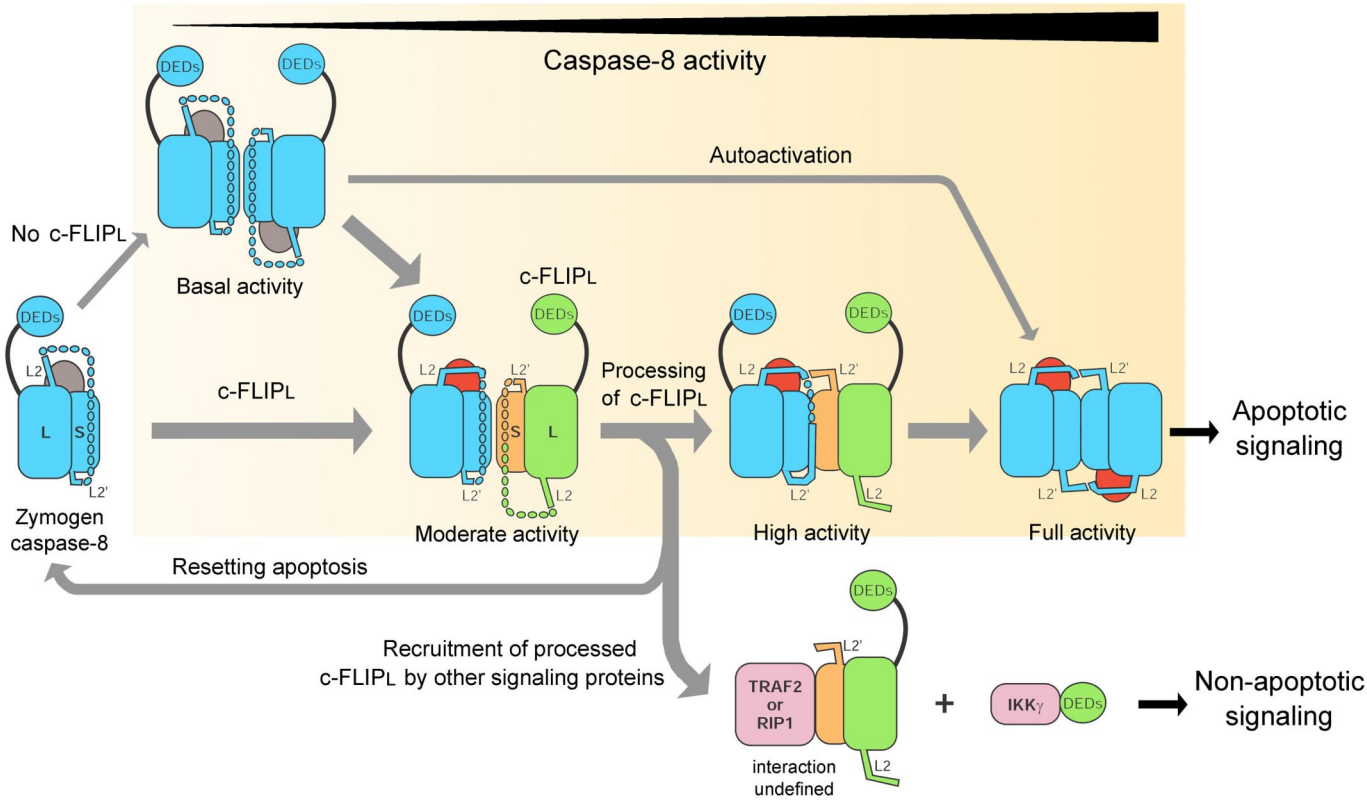

Fig. S4. A working model of c-FLIP$_L$-mediated multifaceted function. Upon ligand binding to death receptors, procaspase-8 is recruited to the death-inducing signaling complex (DISC) by its prodomain to drive the energetically unfavorable process of protease domain homodimerization and caspase activation. When present, FLIP is also recruited to the DISC, where it dramatically enhances this activation process through protease domain heterodimerization. In our model, proteolytic processing of FLIP by zymogen C8 is a central event. FLIP cleavage enhances procaspase-8 activation to a greater degree through improved heterodimerization. This would effectively accelerate zymogen C8 autoproteolysis toward full activation and hasten the induction of apoptosis. Under nonapoptotic conditions (i.e., up-regulation of FLIP$_S$ or FLIP$_L$ expression levels), the extent of procaspase-8 activation is restricted to a nonapoptotic level. In this setting, FLIP cleavage promotes recruitment of other signaling proteins and is sequestered from zymogen C8 to prevent further caspase activation. Consequently, apoptotic signaling would be reset to a quiescent stage and nonapoptotic responses will be initiated.

Table S1. Data collection and structure refinement statistics

Dataset	FLIP	FLIP (2-chain)/z-C8
Data collection		
Wavelength, Å	1.04	1.10
Resolution, Å	100–2.20	100–1.9
Outer shell, Å	2.28–2.20	1.97–1.9
Unique reflections	19,006	37,398
Redundancy	6.4 (6.4)	4.7 (4.7)
Completeness (%)	99.9 (100.0)	99.6 (99.7)
R_{sym}	0.068 (0.395)	0.073 (0.468)
Refinement		
Resolution, Å	50–2.2	50–1.9
Reflections, work/free	17,327/956	35,427/1,844
Completeness (%, work + free)	96.4	99.5
No. of atoms	1,781	3,719
No. of waters	76	123
R_{work}	0.187	0.212
R_{free}	0.220	0.250
rmsd bond lengths, Å	0.012	0.010
rmsd bond angles, °	1.49	1.54
rmsd *B* factors over bonds, Å^2	2.5	2.8
Average *B* factor, Å^2	36.4	36.9
Ramachandran plot		
Within favored, %	97.1	95.4
Within allowed, %	100.0	99.5
Outliers, %	0.0	.5

$R_{sym} = \Sigma_h \Sigma_i |I_{h,i} - I_h| / \Sigma_h \Sigma_i I_{h,i}$, where I_h *is the mean intensity of the i* observations of symmetry-related reflections of *h*. $R = \Sigma ||F_{obs}| - |F_{calc}|| / \Sigma |F_{obs}|$, where $F_{obs} = F_P$, and F_{calc} is the calculated protein structure factor from the atomic model (R_{free} was calculated with 5% of the reflections); rmsd in bond lengths and angles are the deviations from ideal values, and the rmsd in *B* factors is calculated between bonded atoms.