

Description of Additional Supplementary Files

File Name: Supplementary Data 1

Description: Contains all primers in this study.

File Name: Supplementary Movie 1

Description: An overview of the overall structure of the binary DED complex

Shows the side, top, and bottom views of the cryo-EM structure and the corresponding atomic coordinates of the 6:4 cFLIP_H7G-Casp-8_FGLG complex, in which DED molecules are colored as those in the protein ID color scheme of Fig. 1.

File Name: Supplementary Movie 2

Description: A breakdown of the three DED layers that compose the 6:4 complex

Introducing the Casp-8 (C8) tetramer/layer, the 1st cFLIP (CF) trimer/layer, and the 2nd cFLIP (CF) trimer/layer in the 6:4 cFLIP_H7G-Casp-8_FGLG complex. The molecules are colored as their counterparts in Fig. 3g

File Name: Supplementary Movie 3

Description: A demonstration of how the Caspase-8 DED layer exhibits an incomplete or impaired CBS

Shows that, after removing the cFLIP DED layers in the 6:4 cFLIP_H7G-Casp-8_FGLG complex, remaining Casp-8 DED layer lacks FADD to complete the CBS for cFLIP recruitment when compared to the CBS identified in our previous structures of the 3:3 or 1:5 FADD-Casp-8 intermediate complexes (Yang et al. (10.1038/s41467-024-47990-2)). The molecules are colored as their counterparts in Fig. 3g.

File Name: Supplementary Movie 4

Description: A visualization of how two layers of cFLIP DED form a CBS to recruit Caspase-8 DED, constructing the Caspase-8 DED layer at the top end

Shows that, after removing the Casp-8 DED molecules in the 6:4 cFLIP_H7G-Casp-8_FGLG complex, remaining cFLIP DED layers assemble the CBS to recruit the Casp-8 (the 1st C8 molecule), which subsequently assemble the CBS for recruiting the next Casp-8 (the 2nd C8 molecule). By repeating this process, a total of four Casp-8 DED molecules were recruited by the cFLIP DED double-layer complex. Please note there is a space between the 1st C8 molecule (green) and 4th C8 molecule (blue), indicating that the CBS for recruiting the 4th C8 molecule is impaired because it lacks the molecule that could provide the type Ib surface. The recruitment of the 4th C8 molecule terminates the assembly of the CBS for recruiting additional C8 DED molecules. The molecules are colored as their counterparts in Fig. 3g

File Name: Supplementary Movie 5

Description: A demonstration of how two layers of cFLIP DED form another CBS to recruit cFLIP DED, assembling the third cFLIP DED layer at the bottom end

Shows the cFLIP double layer in the 6:4 cFLIP-Casp-8 complex could assemble the CBS for sequentially recruiting three cFLIP DED molecules, resulting in the third CF layer in the 9:4 cFLIP_WT-Casp-8_FGLG complex. The molecules are colored as their counterparts in Fig. 3g.

File Name: Supplementary Movie 6

Description: A model illustrating the 9:4 cFLIP-Caspase-8 binary complex recruits FADD to the top end, leading to structural jamming and the formation of the 4:3:3 cFLIP-Caspase-8-FADD complex

Shows that, by comparing the 9:4 cFLIP-Casp-8 structure and our previous 3:3:4 FADD-Casp-8-cFLIP structure (Yang et al. (10.1038/s41467-024-47990-2)), a significant FADD-recruitment-induced structural jamming within the 4:3 cFLIP-Casp-8 segment could be observed, which could be used to reason why the recruitment of FADD to the top end of the 9:4 cFLIP-Casp-8 complex would result in the release of cFLIP molecules from the bottom end, leading to the formation of 4:3:3 cFLIP-Casp-8-FADD complex. The molecules are colored as their counterparts in Fig. 3g. FADD molecules are colored as their counterparts in Fig. 1d

File Name: Supplementary Movie 7

Description: A depiction of the structural morphing within the 4:3 cFLIP-Caspase-8 segment during the transition from the 9:4 cFLIP-Caspase-8 binary complex to the 4:3:3 cFLIP-Caspase-8-FADD complex

Shows the structural morphing within the 4:3 cFLIP-Casp-8 segment of the complexes in Supplementary Movie 6. The molecules are colored as their counterparts in Fig. 3g.