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Supplemental Information

A Death Effector Domain Chain DISC Model Reveals a Crucial Role for Caspase-8 Chain Assembly in Mediating Apoptotic Cell Death

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Inventory of Supplemental Information

Figure S1, related to Fig. 1
TRAIL DISC components are exclusively localized in the cleared cell lysate

Figure S2, related to Fig. 1 and Fig. 4
Known components of the TRAIL DISC were identified by mass spectrometry by multiple peptides and spectra

Figure S3, related to Fig. 2
Lipid raft markers were not associated with soluble TRAIL DISC (S-DISC)

Figure S4, related to Fig. 5 and Fig. 7
Putative models for the recruitment of procaspase-8 and other DED-only proteins to the DISC

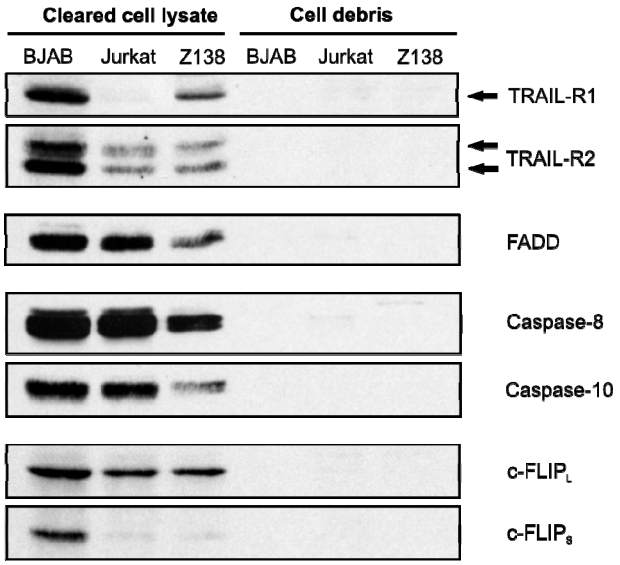
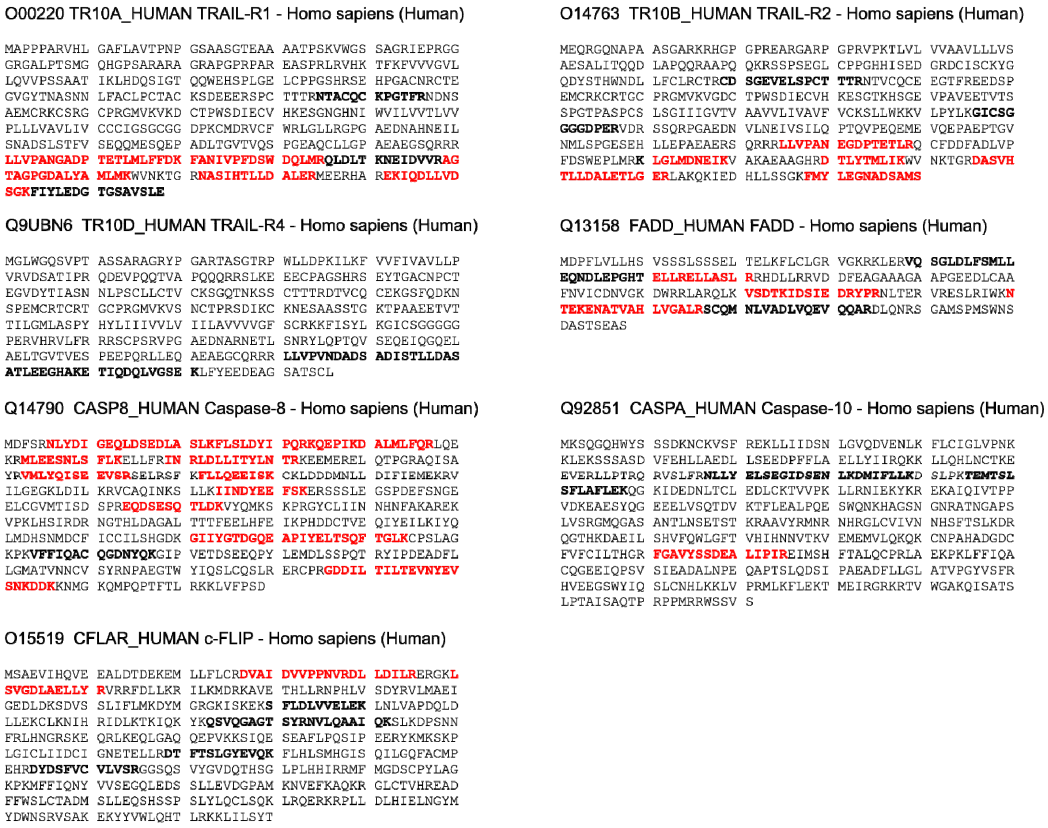


Figure S1. TRAIL DISC components are exclusively localized in the cleared cell lysate. BJAB, Jurkat or Z138 cells were stimulated with biotin-labelled (bTRAIL) as described in Experimental Procedures. Following TRAIL DISC formation and subsequent cell lysis, matched volumes of cleared cell lysate and cell debris were separated by SDS-PAGE and analyzed by western blotting for the presence of TRAIL-Rs, FADD, Caspase-8, Caspase-10 and c-FLIP($_L$ and $_S$). In matched samples of cleared cell lysate and cell debris, all core DISC components were exclusively localized in the cleared cell lysate across all three cell lines.

A



B

	High molecular weight BJAB TRAIL DISC	
	Experiment 1	Experiment 2
TRAIL	22	16
TRAIL-R1	18	8
TRAIL-R2	15	6
FADD	4	2
Caspase-8	81	40
Caspase-10	2	0
c-FLIP	7	0

Figure S2. Known components of the TRAIL DISC were identified by mass spectrometry by multiple peptides and spectra.

(A) Known components were identified by good peptide coverage. Peptides, corresponding to the known components of the TRAIL DISC, identified by mass spectrometry are depicted in bold. Red bold peptides were identified following mass spectrometry of the HMW BJAB TRAIL DISC (50 % peptide and protein probabilities, MudPit analysis). Black bold peptides are additional peptides that were identified by mass spectrometry of unfractionated TRAIL DISC isolated from BJAB, Jurkat or Z138 cells (50 % peptide and protein probabilities).

(B) Mass spectrometry of HMW weight BJAB TRAIL DISC resulted in the identification of multiple spectra for the known components. Numbers are the total assigned spectra for each known component across two experiments.

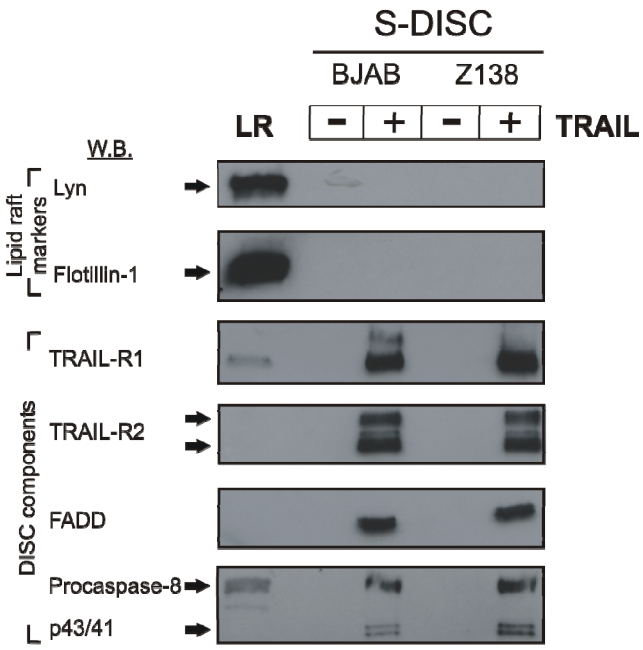


Figure S3 Lipid raft markers were not associated with soluble TRAIL DISC (S-DISC). Lipid raft markers are not associated with TRAIL DISC isolated from the soluble fraction of cells. TRAIL DISC was isolated from the soluble fraction of TRAIL treated BJAB or Z138 cells (S-DISC) and the presence of Lyn and Flotillin-1 (lipid raft markers) was investigated by western blotting. LR, Lipid raft fraction.

Figure S4

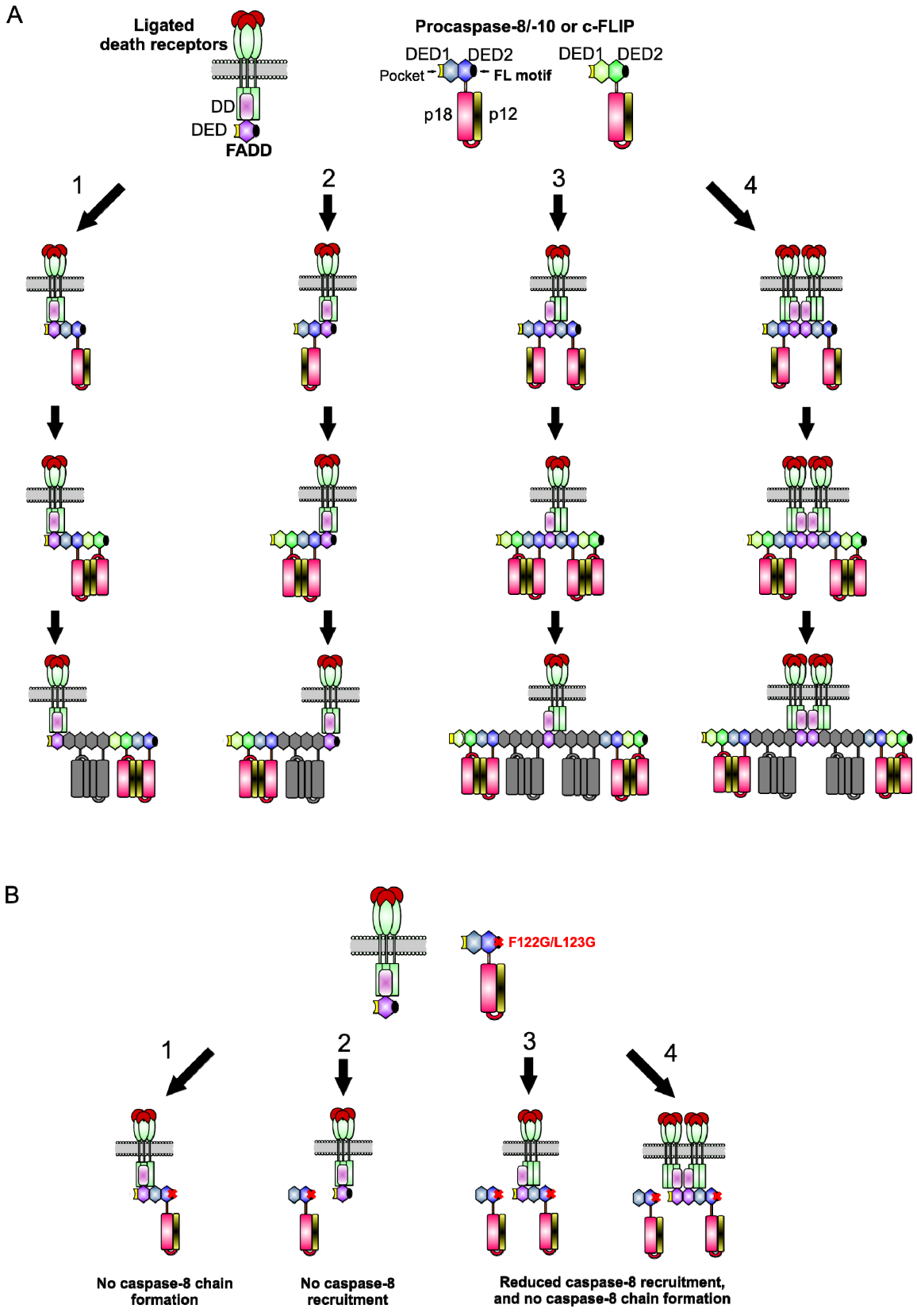

A
Ligated death receptors
DD
DED
FADD
Procaspase-8/-10 or c-FLIP
DED1
DED2
Pocket
FL motif
p18
p12
DED1
DED2
1
2
3
4
B
F122G/L123G
1
2
3
4
No caspase-8 chain formation
No caspase-8 recruitment
Reduced caspase-8 recruitment, and no caspase-8 chain formation

Figure S4 Putative models for the recruitment of procaspase-8 and other DED-only proteins to the DISC.

(A) Upon stimulation by the appropriate death ligand, death receptors recruit FADD via their death domains (DDs). FADD in turn recruits procaspase-8 (or procaspase-10 or c-FLIP) through a death effector domain (DED)-mediated interaction. FADD may initially recruit one molecule of procaspase-8 via a single interaction with either DED1 (model 1; see also Figure 7A) or DED2 (model 2). Alternatively, FADD may recruit two DED-only proteins, one via DED1 and the other via DED2 (model 3). In a final variation, FADD from different receptor complexes may self-associate and then caspase-8 chain formation could proceed from each FADD molecule (model 4). We propose that, once bound to the DISC, DED-only proteins are then able to use their exposed binding sites (pocket or FL motif) to recruit additional molecules to produce a chain enabling dimerization and full activation of caspase-8/10. (B) Mutation of the FL motif in caspase-8 DED2 (F122G/L123G) will affect each model differently. In model 1, DED2 mutant caspase-8 is still recruited to FADD via DED1 but is unable to form a chain, whilst in model 2, DED2 mutant caspase-8 cannot be recruited to FADD. In models 3 or 4, DED2 caspase-8 recruitment to FADD is reduced, as only DED1 can interact with FADD, and caspase-8 chains cannot form through mutated DED2.