

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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TRAIL DISC components are exclusively localized in the cleared cell lysate

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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

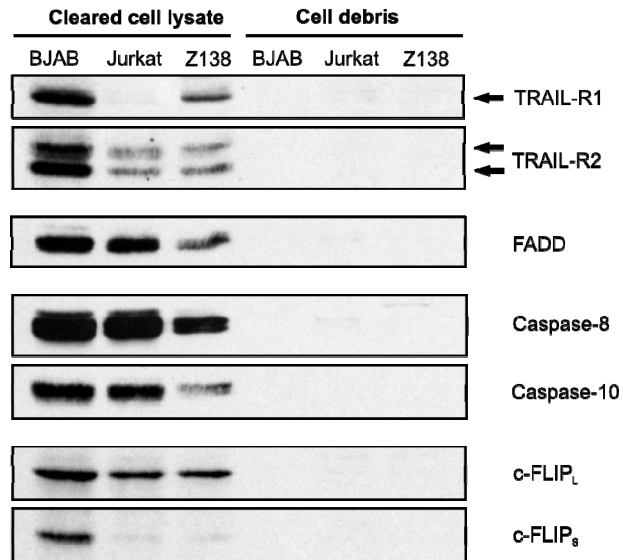


Figure S1. TRAIL DISC components are exclusively localized in the cleared cell lysate. BJAB, Jurkat or Z138 cells were stimulated with biotin-labelled (bTRAIL) as described in Experimental Procedures. Following TRAIL DISC formation and subsequent cell lysis, matched volumes of cleared cell lysate and 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 c-FLIP_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

O00220 TR10A_HUMAN TRAIL-R1 - Homo sapiens (Human)

MAPPFARVHL GAFLAVTPNP GSAASGTEAA AATPSKVWGS SAGRIEPRGG
GRGALPTSMG QHGFSARARA GRAPGPRPAR EASPRLRVHK TFKFVVVVGL
LQVVPSSAAT IKLHDQSIGT QQWEHSPLGE LCPGSGHRSE HPGACNRCTE
GVGYTNASNN LFACLPCTAC KSDEEERSPC TTTTNTACQC **KPGTFRNDNS**
AEMCRKCRSG CPRGMVKVKD CTPWSDIECV HKESGNGHNI WVILVVTVLV
PLLLVAVLIV CCGIGSGCGP DPKCMDRVCF WRLGLLRGP AEDNAHNEIL
SNADSLSTFV SEQMESQEP ADLTGVTVQS PGEAQCLLGP AEAEGSQRRR
LLVPANGADP TETIMLFFDK FANIVPFDSW DQLMRQLDIT KNEIDVVRAG
TAGPGDALYA MLMKWNKKTG RNASIHTELD ALEMERERHA REKIQDLIVD
SKKFIYLEDG TGSAVSLE

Q9UBN6 TR10D_HUMAN TRAIL-R4 - Homo sapiens (Human)

MGLWGQSVPT ASSARAGRYP GARTASGTRP WLLDPKILKF VVFIVAVLLP
VRVDSATIPR QDEVPQTVA PQQRRLSKE EECFAGSHRS EYTGACNFC
EGVDYTIASN NLFSCCLCTV CKSGQTNKSS CTTTRDTVCQ CEKGSFQDKN
SPEMCRCTCT CPRGMVKVVS NCTPRSDIKC KNEAASSTG RTPAAEETVT
TILGMLASYP HYLLIIVVLV ILLAVVVVGF SCRKKFISYL KIGICSGGGG
PERVHRVLFRR RSCFSPRVCP AEDNARNETL SNRYLQPTQV SEQEIQGQEL
AELTGVTVES PEEPQRLLQG AEAEGCQRRR **LLVFNVDADS ADISTLLDAS**
ATLEEGHAKI TIQDQVGESE KLFYEDEEAG SATSCL

Q14790 CASP8_HUMAN Caspase-8 - Homo sapiens (Human)

MDFSRLNYDI **GEQLDSEDLA SLKFLSLDYI PQRQSPFKD AMLPQRLQE**
KRMLEESNLS FLKELLERIN RLDELLITYLN TRKEEMEREL QTPGRAQISA
YRVMLEKQISE EVRSSELRSF KFLQSELSK CKLDDDMMLL DTFIEMEKRV
ILGQKLDLIL KRVCAQINKS **ILKIINDVYE FSKERSSLE GSPPEFSNGE**
ELGCVMTISD **SREQDSBQ TLDKVVQMS KRCYCLLIN NHPAKAREK**
VPKLSHTRDR NCHLDAGAL TTTPELHFE IKPHDCTIVE QIVYELLKIQ
LMDHNSMDFC ICCILSHGDK **GLIIVTDCQE APIYELTSQV GNLKCPSLAG**
KPKVFFQAC **QCDNYQKIP VETDSSEQPY LEMDLSSPT RYIPDEADF**
LGMATVNNCV SYRNPAGETW YIQSLCQLSR ERCPGR**DDIL TILTEVNYEV**
SNKDKKNGM KMQPQPTFL RKKLVPFSD

O15519 CFLAR_HUMAN c-FLIP - Homo sapiens (Human)

MSAEVIHQVE EALDTDEKEM LLFLCRDVAI **DVVFPNVDL LDILREGRKL**
SVGDLAELLY RVRRFDLKR ILKMDRKA VE THLLRNPHLV SDYRVLMAEI
GEDLDKSDVS SLIFLMKDYM GRGKISKEKS **FIDLIVVELEK LNLVAPDQLD**
LLEKCLKNIH RIDLKTQIK **YKQSVQAGT SYRNVLAQAI QKSLKDPNSN**
FRLNHRGSK EQLKEQLGAQ QEPVKKSIE SEAFLPQSIP EERYKMSKFP
LGCILIDICI GNETELLRDT **FTSLGYEVQK FLHLSMHGIS QILGQFACMP**
EHRDYDSFVC **VLVSRGGSQS VYGVDTQHS LPLHHIRMF MGDSCPYLAG**
KPKMFFIQNY VVSEGLQEDS **SLEVDGPAM KNVEFKAQKR GLCTVHREAD**
FFWNSLCTADM SLLEQSHSP SLYLQCLSQY LRQERKRPLL DLHIELNGYM
YDWNRSVSAK ERYVWLOHT LRKKLILSYT

O14763 TR10B_HUMAN TRAIL-R2 - Homo sapiens (Human)

MEQRGQNAFA ASGARKRHGP GPREARGARP GPRVPKTLVL VVAIVLLLV
AESALITQDQ LAPQORAAPO QKRSSPSEGL CPPGHHSIED GRDCISCKY
QDYSTHWNLD LFLRCTRCD **SGEVLSPECT TTRNTVCQCE ECTFREEDSP**
EMCRKCRCTC PRGMVKVGD C TPWSDIECVH KESGTHKSGE VPAVEETVTS
SPGTPASPCS LSGIIGVTV AAVVLIVAVE VCKSLLWKKV LPYLK**GICSG**
GGDPERVDR SSQRPGAEDN VLNVLIVLQ PTOVPEQEME VQEPABPTGV
NMLSPGESH LLEPAEAERS **QRRRLIVPAN EGDPTETLRQ CFDDFADLVP**
FDSWEPLMRK **LGLMDNEIKV AKAEAAGHRD TLYTMLIKW NKTGFDASVH**
TLLDALETLG ERLAKQKIED HLLSSGKFMV LEGNADSAMS

Q13158 FADD_HUMAN FADD - Homo sapiens (Human)

MDPFLVLLHS VSSLSLSEEL TELKFLCLGR VGKRLKERVQ **SGLDLFSMLL**
EQNDLEFGHT ELLRELLASL RRHDLRRVD DFEAGAAAGA APGEEDLCAA
FNVICDNVGH DWRLRLARQLK **VSDTKIDSIE DRVYRNTER VRESLRIWKN**
TEKENATVAK LVGALRSCQM NLIVDLVQEV QQARLDQNRV GAMS PMSWNS
DASTSEAS

Q92851 CASPA_HUMAN Caspase-10 - Homo sapiens (Human)

MKSQQQHWYS SSKNKCVSF REKLLIDSN LGVQDVENLK FLCIGLVPNK
KLEKSSASD VFEHLAEDL LSEEDPFFLA ELLYIIRQK LQHLNCTKE
EVERLLPFRQ **RVSFLRNLLY ELSGSDSEN LKMIFPLAK** **SLKREMTSL**
SFLAFLEKQG KIDEDNLICL EDLCKTVVPK LLRNLEKYNR EKAIGIVTTP
VDKBAESYQG EELVSDTDV KTFLEALPQE SWQNKHAGSN GNRATNGAPS
LVSRMGGAS ANTLNSETST KRAAVYRMMR NHRGLQVIVN NHPSTSLKDR
QCTHKDAEIL SHVFQWLGFT VHIHNNVTKV HEMVWLQKQ CNPAHADGDC
FVFCILTHGR **FCAVYSDEA LPIREINSH FALQCPRLA EKPILFFIOA**
CQCEEQSPV SIEADALNPE QAPTSLQDSI PAEAEFLGL ATVPYGVYFR
HYEGSWYIQ SLCNHLKLV FRMLKFEKI MEIRGRKRVG WGAQISATS
LPTAISQTF RPPRRRNVSSV S

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 unfractonated 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.

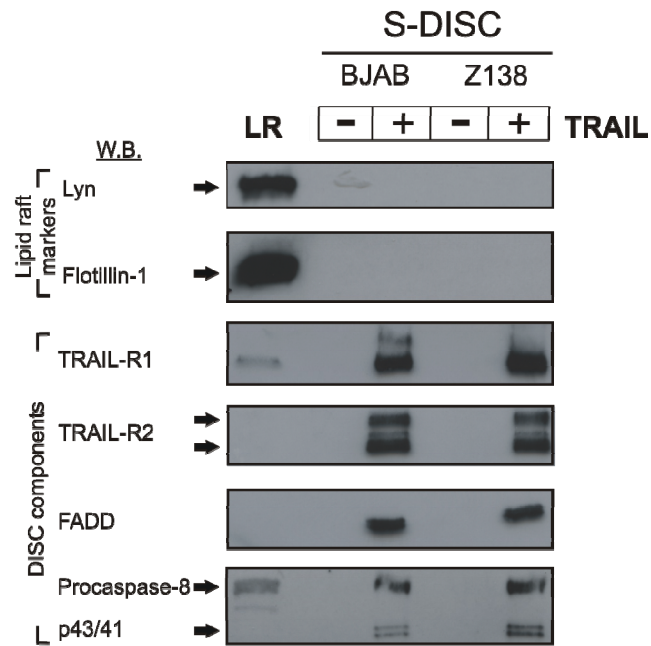


Figure S3 Lipid raft markers were not associated with soluble TRAIL DISC (S-DISC).

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

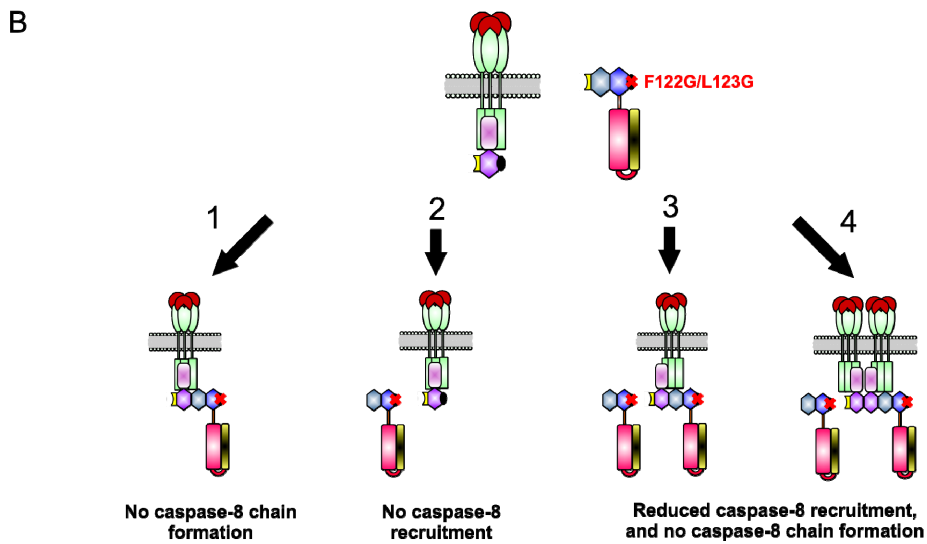
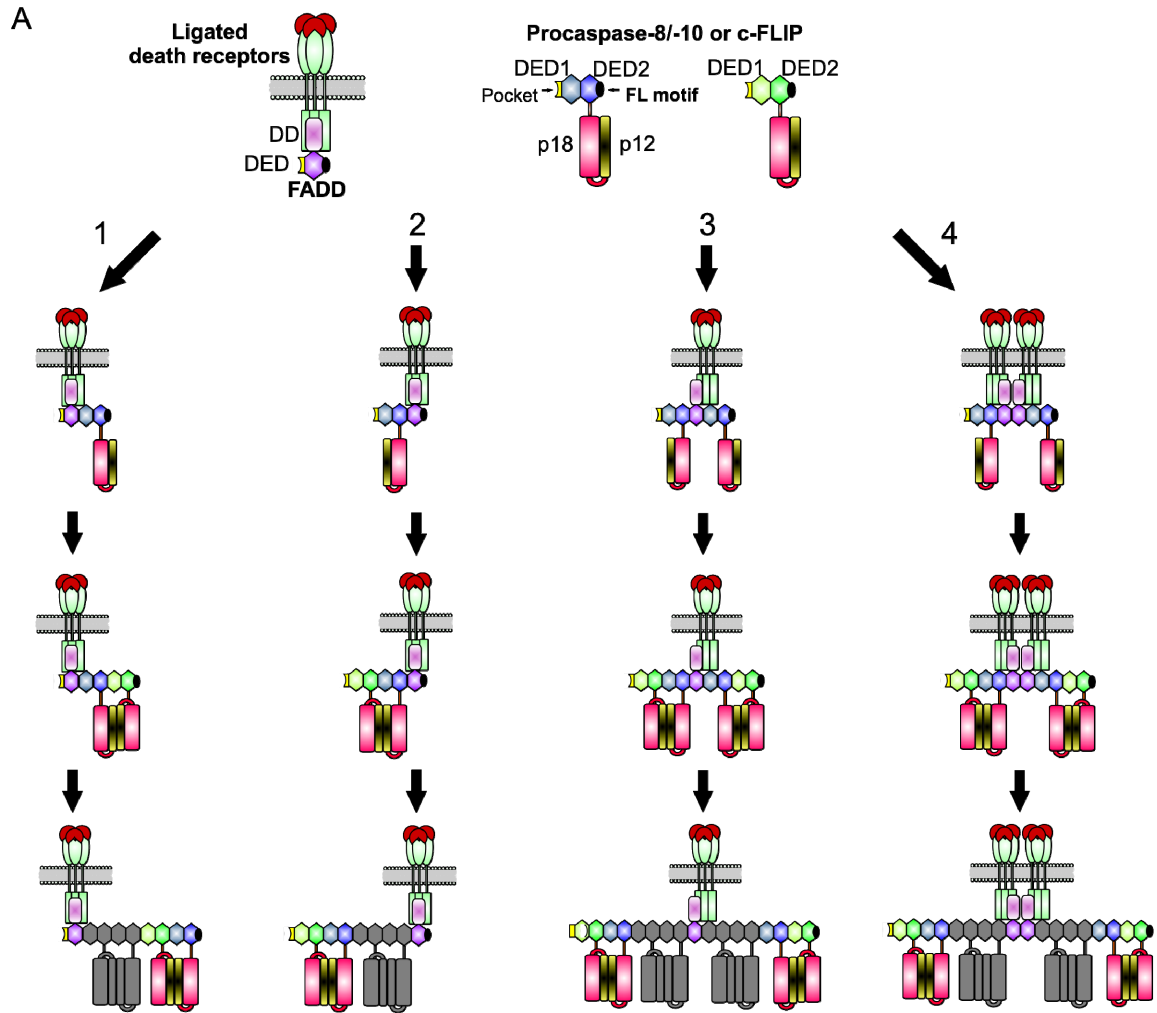


Figure S4 Putative models for the recruitment of procaspase-8 and other DED-only proteins to the DISC.

(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.