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Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors — [Source link](#)

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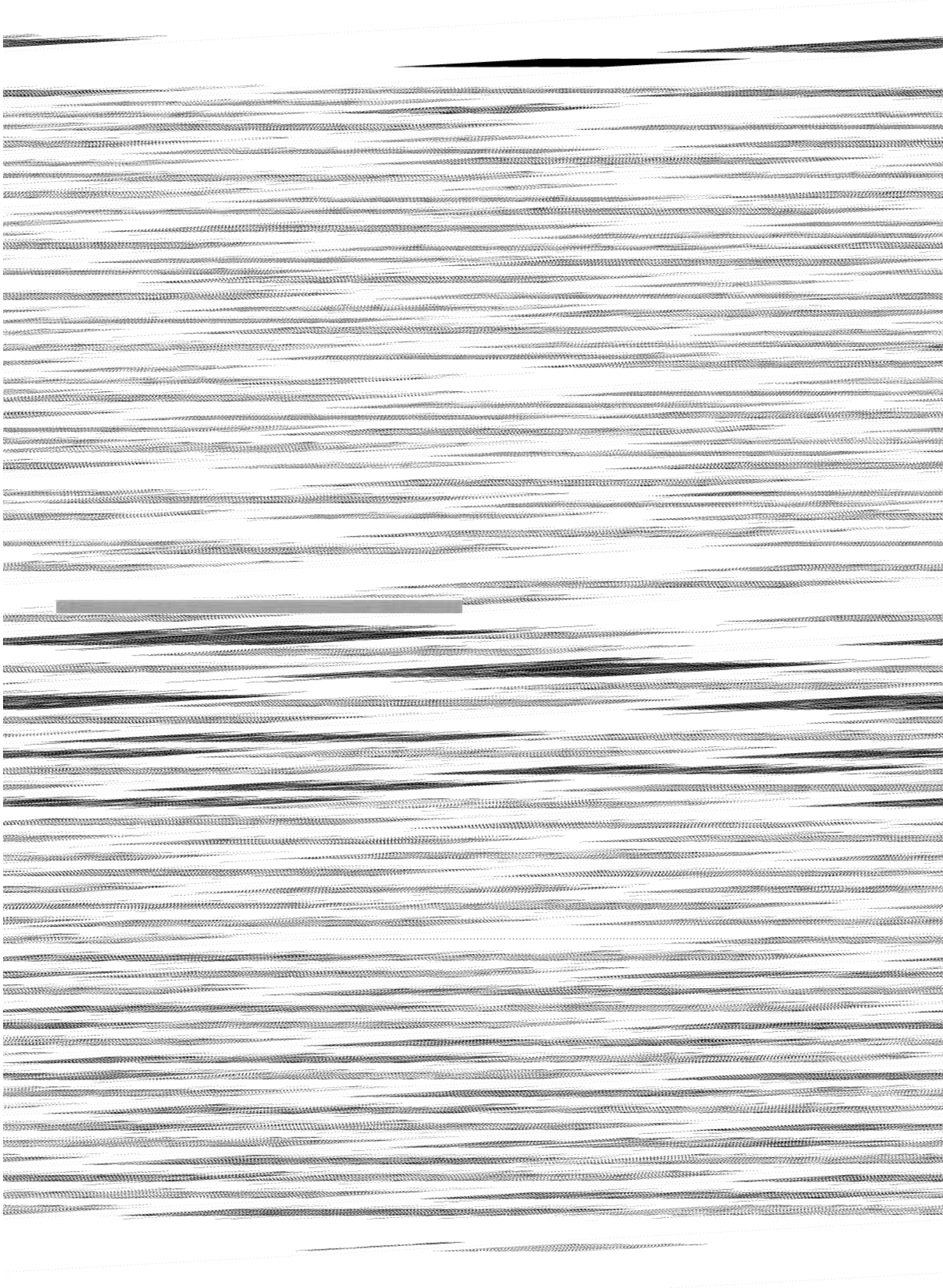
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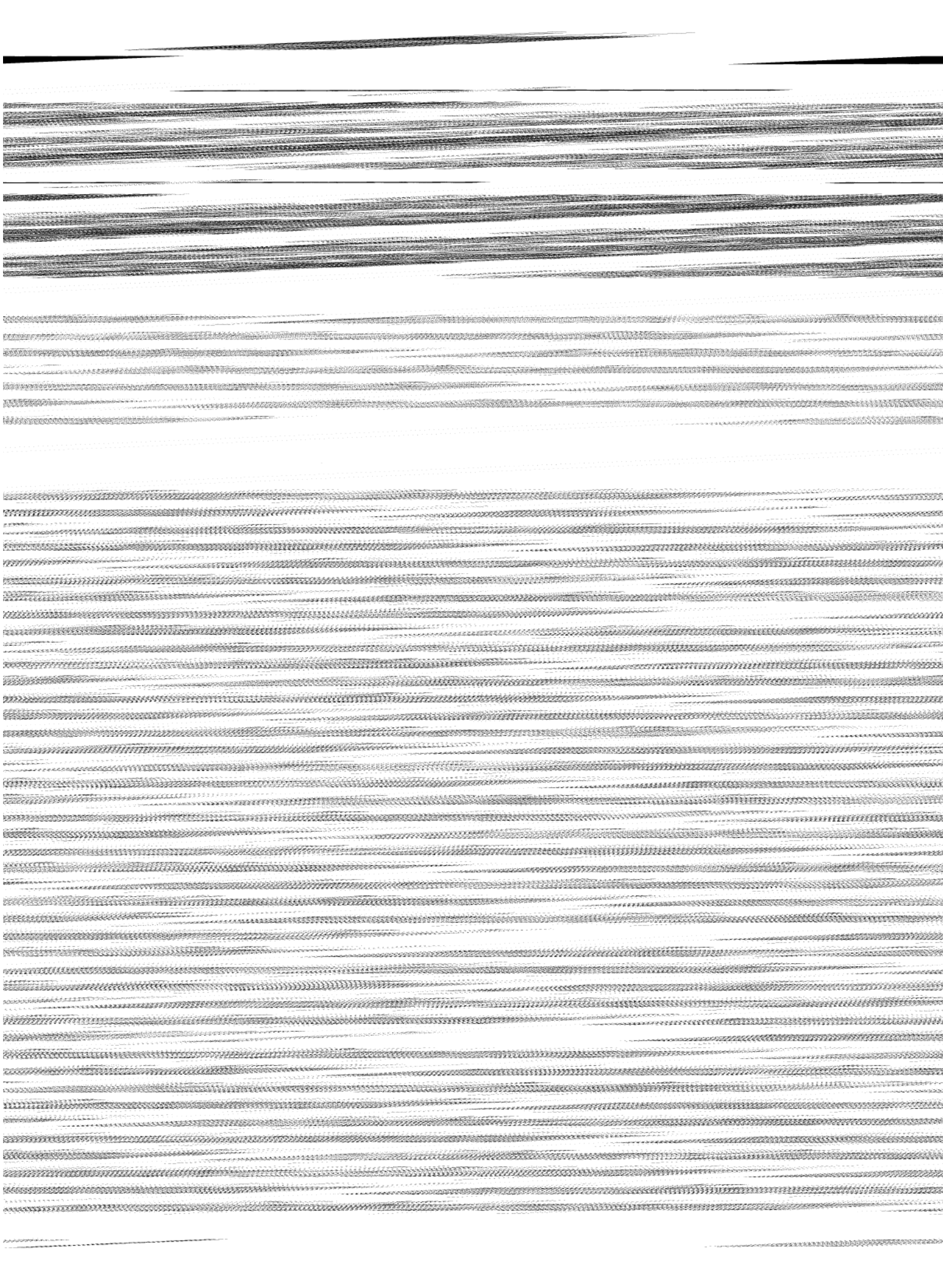
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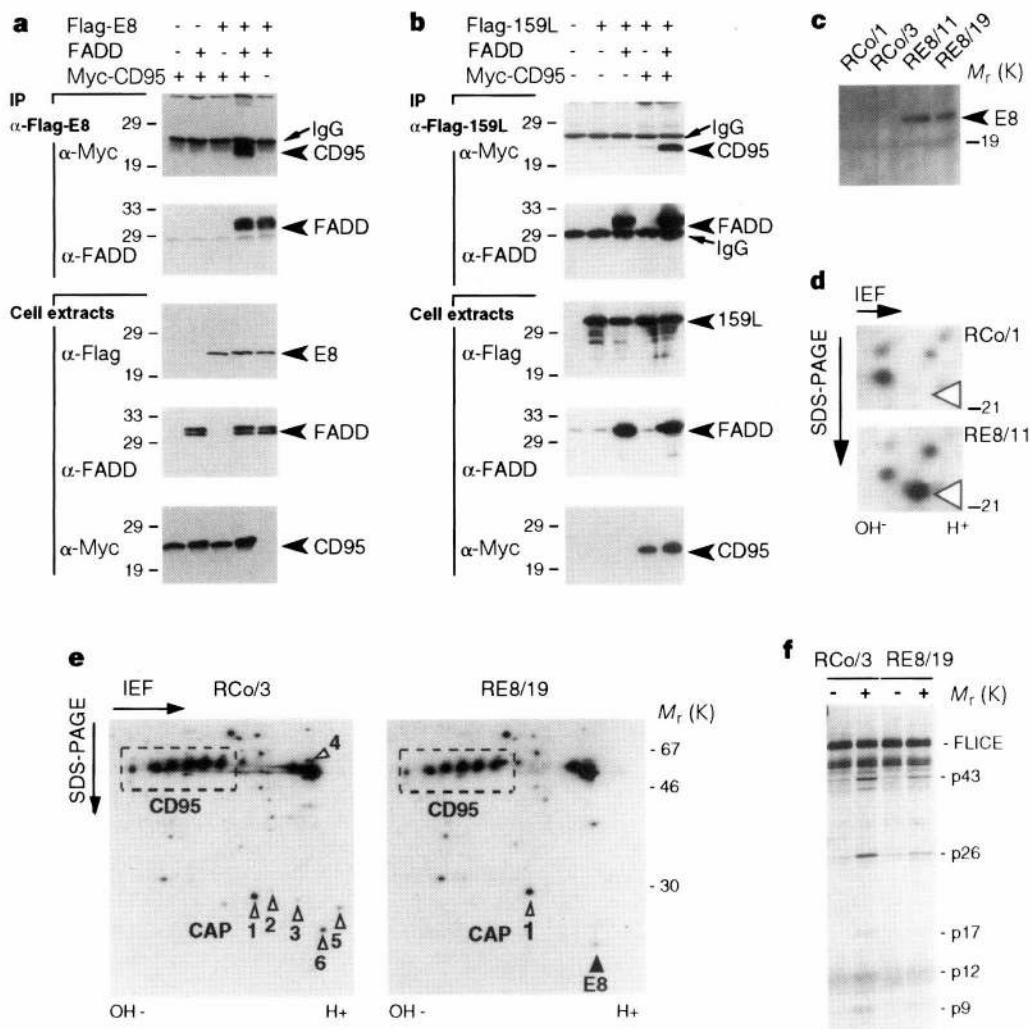


Figure 2 v-FLIPs interfere with CD95 signaling by binding to FADD. **a**, 293T cells were transiently transfected with 4 μ g of each expression vector encoding Flag-E8-FLIP, FADD and Myc-CD95 as indicated, or empty vector to keep the total amount of plasmid constant. Cells were lysed 30 h after transfection, and anti-Flag immunoprecipitates or total cell extracts were analysed for the presence of FADD, Flag-E8 or Myc-CD95 by western blotting as indicated. **b**, The association of MCV-159L-FLIP with FADD and CD95 was analysed as described in **a**. **c**, Flag-E8-FLIP expressing Raji clones RE8/11 and RE8/19, as well as the control clones RCo/1 and RCo/3 were tested for E8 expression by western blotting using an anti-Flag antibody. **d**, Analysis of anti-Apo-1 immunoprecipitates from 35 S-labelled control (upper panel) or Flag-E8-FLIP-expressing (lower panel) Raji clones by 2D gel electrophoresis and autoradiography. The arrow indicates the migration

position of a 23K protein of pI 5.0 found in the E8-FLIP-expressing clone RE8/11 and absent in the control clone RCo/1. **e**, Apo-1 immunoprecipitates from 35 S-labelled, anti-Apo-1-treated clones RCo/3 and RE8/19 were analysed by 2D gel electrophoresis and autoradiography. Numbers refer to CAP proteins: CAP1 and CAP2 (FADD), CAP3, CAP4 (FLICE), CAP5 and CAP6 (the prodomains of FLICE as a result of proteolytic cleavage). A filled arrowhead indicates the position of E8. **f**, FLICE-processing activity associated with the DISC in E8 expressing and control Raji cells. Cells were treated with anti-Apo-1 for 5 min, and Apo-1 immunoprecipitates from treated (+) or untreated (-) cells were analysed for FLICE processing activity by incubation with *in vitro* translated 35 S-labelled FLICE. FLICE-specific cleavage products (p43, p26, p17, p12 and p9)¹⁷ were detected by SDS-PAGE and autoradiography.

Interestingly, all FLIP encoding γ -herpesviruses also have a Bcl-2 homologue. The anti-apoptotic Bcl-2 family members block cell death induced by growth factor deprivation, γ -irradiation and cytotoxic drugs^{23,24}. However, in contrast to the v-FLIPs, these proteins have a less potent effect on CD95-mediated apoptosis of lymphoid cell lines. Some viruses may thus take advantage of two complementary anti-apoptotic functions provided on the one hand by a Bcl-2 homologue and on the other by v-FLIP.

Tissue homeostasis is maintained through the delicate balance of cell growth and apoptosis. Recent evidence indicates that tumour cells such as melanomas and hepatomas do not respond to CD95L-mediated apoptosis owing either to the downregulation of CD95 expression or to a blockade in the CD95 signalling pathway^{25,26}.

Thus, by interference with CD95 and other death-receptor-mediated signals, we propose that v-FLIPs may not only facilitate viral spread and persistence, but that they also contribute to the transforming capacity of some herpesviruses. This possibility is supported by the fact that MCV produces slow-growing epidermal neoplasms that persist for long periods with little immune response. HVS causes tumours in New World primates, and tight epidemiological links indicate that HHV-8 is an infectious cofactor for Kaposi's sarcoma and primary effusion lymphoma¹³. Future investigations should address the possible presence of v-FLIPs in tumours caused by viruses.

Note added in proof: Related findings have been reported by J. Bertin *et al.*³⁰ □

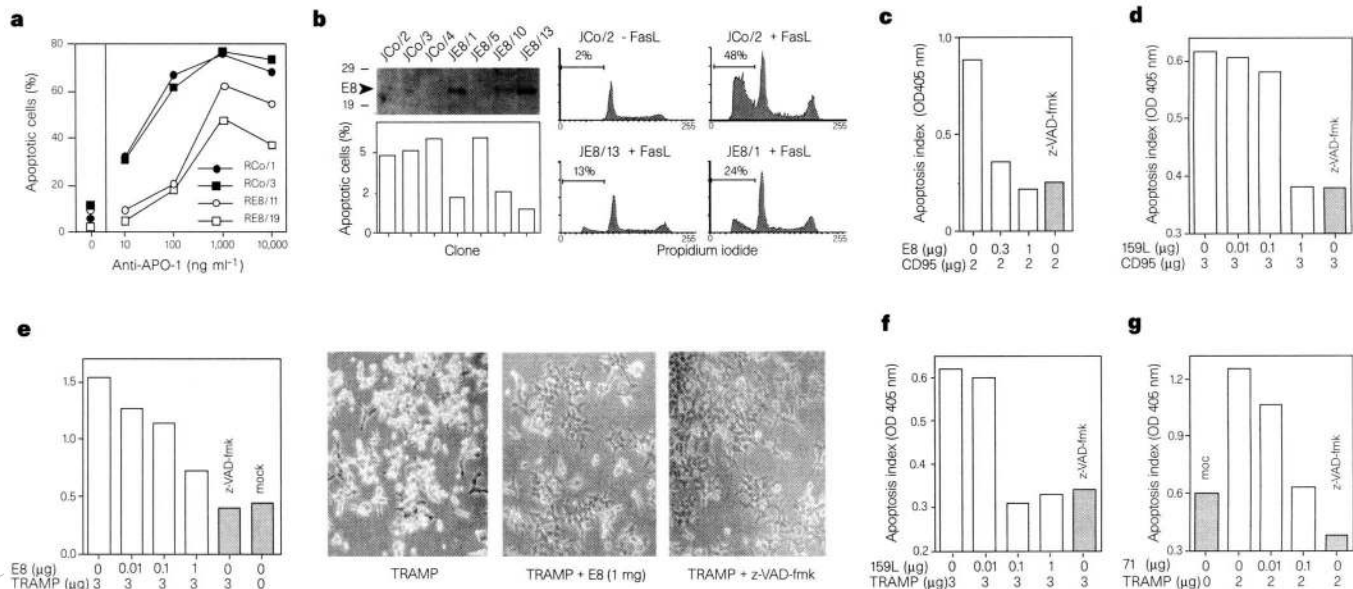


Figure 1 E8/E8 underlines kill non-death receptor-inducible apoptotic cells. The susceptibility of E8/E8-expressing RC0/1 and RE8/11 (control) RC0/2 and RC0/3 (Fig. 1) clones to anti-APO-1-mediated apoptosis was determined by the incubation of cells with the indicated concentration of anti-APO-1 in medium for 60 h at 37°C. Population viable surviving cell numbers were used to determine the percentage of apoptotic nuclei. Cellular contents of E8-transfected JCo/1, JE8/5, JE8/10 and JE8/13 (control) JCo/2, JCo/3 and JCo/4 underlines were analysed for Fig. 1 E8/E8 expression by anti-Fig. 1 western blotting. The susceptibility of the E8/E8-transfected control clones to CD95-induced apoptosis was determined by incubating cells for 24 h at 37°C with supernatants of recombinant cells expressing or not CD95. The fraction of apoptotic cells was determined as in Fig. 1. RC0/2 cells were transfected with the indicated amounts of an expression vector for the human CD95 with a minimal 5'UTR (E8/E8) or a MCV (CD95) E8/E8 expression vectors. Where indicated, 25 µM z-VAD-fmk was added to the cell culture after transfection. Quantitative analysis of cell death induced by the overexpression of CD95 in the absence of CD95 was done using a MTT reduction assay as described in Materials and Methods. In brief, 100 µl of 0.5% crystal violet solution was added into the preparation. The optical density (OD) of each underlined apoptotic cell transfection in various amounts of expression vector for the indicated amount of expression vector for the control vector during an assay and analysed after the assay of transfection. Control cells were transfected with the indicated amounts of an expression vector together with CD95 and/or E8/E8 and analysed together with CD95 and/or E8/E8. E8/E8 and JE8/10 and control E8/E8 (JE8/1) as a comparison expression control clones, JCo/2 and JCo/4 were incubated with the indicated concentrations of recombinant Fig. 1 supernatant for 24 h. Cell viability was determined using a cell proliferation assay.

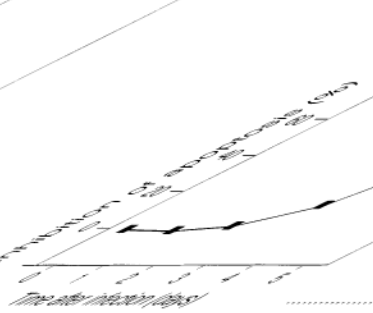
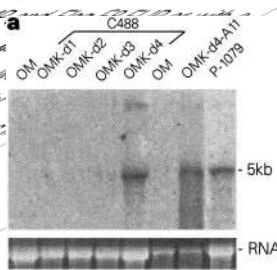


Figure 2 Northern blot analysis of permissive cell lines and regions. Human embryonic 293T cells, the human leukemic Jurkat T cell line and the human Burkitt lymphoma B cell line Raji were grown as described. Monoclonal antibodies used in immunoprecipitation and for western blotting in double anti-Fig. 1 antibody and anti-Fig. 1-garose. Rabbit anti-Fig. 1 antibody (1:1000) was used for immunoprecipitation. Transfection efficiency was compared. Leukotaxin and an antibody directed against the Fig. 1-garose (Fig. 1) were used. Specific soluble human TRAIL amino acids 8–20 was generated by PCR on a cDNA from the E8/E8 clone 1120 (Genbank accession number U9422) using the oligonucleotides 5'-TCTGCTGGCAGGCTGTGGGAGAG-3' and 5'-TGGTCTGGCAGGCTGTGGGAGAG-3' and was cloned into a modified E8/E8 vector (Fig. 1) containing the Fig. 1-garose and a linker.

CD95/E8/E8 is expressed during the life cycle and in **Methods**
permissive cells. Northern blot analysis of permissive cell lines and regions. Human embryonic 293T cells, the human leukemic Jurkat T cell line and the human Burkitt lymphoma B cell line Raji were grown as described. Monoclonal antibodies used in immunoprecipitation and for western blotting in double anti-Fig. 1 antibody and anti-Fig. 1-garose. Rabbit anti-Fig. 1 antibody (1:1000) was used for immunoprecipitation. Transfection efficiency was compared. Leukotaxin and an antibody directed against the Fig. 1-garose (Fig. 1) were used. Specific soluble human TRAIL amino acids 8–20 was generated by PCR on a cDNA from the E8/E8 clone 1120 (Genbank accession number U9422) using the oligonucleotides 5'-TCTGCTGGCAGGCTGTGGGAGAG-3' and 5'-TGGTCTGGCAGGCTGTGGGAGAG-3' and was cloned into a modified E8/E8 vector (Fig. 1) containing the Fig. 1-garose and a linker.

Figure 3 Cell viability assay. Human embryonic 293T cells, the human leukemic Jurkat T cell line and the human Burkitt lymphoma B cell line Raji were grown as described. Monoclonal antibodies used in immunoprecipitation and for western blotting in double anti-Fig. 1 antibody and anti-Fig. 1-garose. Rabbit anti-Fig. 1 antibody (1:1000) was used for immunoprecipitation. Transfection efficiency was compared. Leukotaxin and an antibody directed against the Fig. 1-garose (Fig. 1) were used. Specific soluble human TRAIL amino acids 8–20 was generated by PCR on a cDNA from the E8/E8 clone 1120 (Genbank accession number U9422) using the oligonucleotides 5'-TCTGCTGGCAGGCTGTGGGAGAG-3' and 5'-TGGTCTGGCAGGCTGTGGGAGAG-3' and was cloned into a modified E8/E8 vector (Fig. 1) containing the Fig. 1-garose and a linker.

