

The molecular basis for apoptotic defects  
in patients with CD95 (Fas/Apo-1) mutations

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J. Clin. Invest. 103:355–363 (1999)

In Figure 4b, lanes 1 and 6 in the bottom two blots did not  
reveal the proper data. The entire figure with corrected  
resolution appears here.

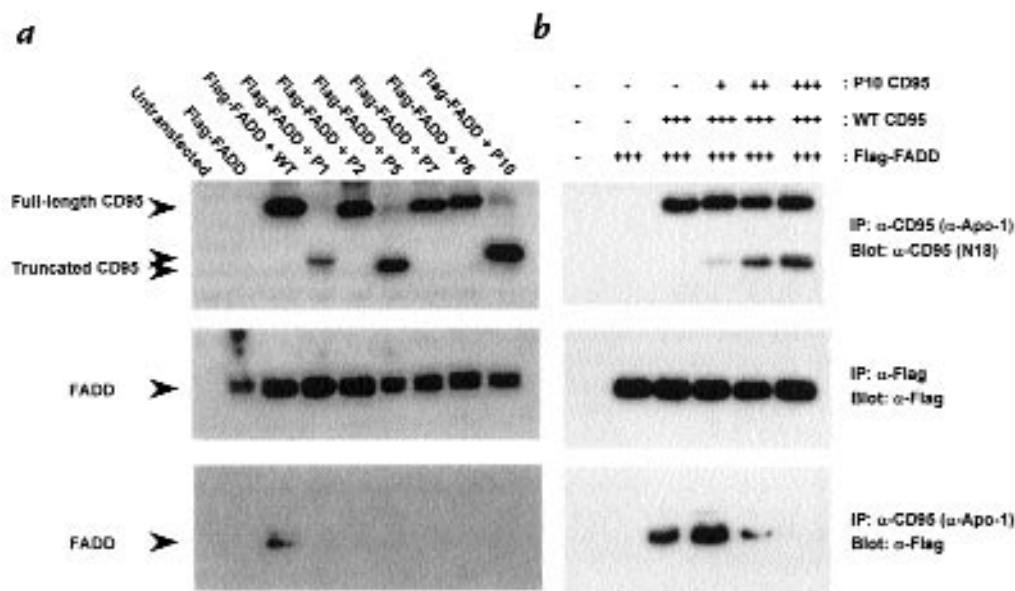


Figure 4

Defective protein-protein interactions of ICD CD95 mutant alleles. (a) ICD CD95 mutant alleles fail to recruit FADD to the CD95 death domain. 293T cells were cotransfected with pCDNA3 expression vectors for WT or mutant CD95 and Flag-FADD in pFlag-CMV-2. Transfected cells were lysed and subjected to IP-Western blot using the antibodies indicated in the figure. The trace signals of WT CD95 in lanes P1, P5, and P10 are due to slight spillover from adjacent lanes and were not seen when these mutants were run separately. (b) The dominant-negative action of ICD mutant CD95 is dose-dependent. 293T cells were transiently cotransfected with expression vectors for Flag-FADD and WT or P10 mutant CD95. Transfections were carried out using constant amounts of total DNA (4  $\mu$ g) using the pCDNA vector DNA, WT CD95 (0.5  $\mu$ g) and Flag-FADD (0.5  $\mu$ g) DNA, but the amount of DNA for the ICD mutant P10, was increased between experiments (lanes 4–6; + [0.5  $\mu$ g] to +++ [1.5  $\mu$ g]). The total amount of DNA transfected was kept constant. Transfected cells were lysed and analyzed by immunoprecipitation followed by Western blotting using the antibodies indicated. IP, immunoprecipitation.