



Supplementary figure 1: Effect of caspases inhibition on DEX-induced thymocyte apoptosis. The four histograms indicate apoptosis level of untreated thymocytes (labelled “untreated”) or treated with dexamethasone alone (“DEX”), plus Z-IETD-FMK (“DEX + Casp-8 In”) or plus Z-LEDHL-FMK (“DEX + Casp-9 In”). Cells were an aliquot of the groups utilized for western blot of figure 1A. Numbers indicate the percentage of apoptotic thymocytes. Data are representative of 3 independent experiments with similar results.

Apoptosis evaluation: Apoptosis was measured by flow cytometry. After 24 hours of culture, thymocytes were centrifuged, and the pellets were gently resuspended in 1.5 mL hypotonic propidium iodide (PI) solution (50 µg/mL, in 0.1 % sodium citrate plus 0.1 % Triton X-100). The tubes were kept in the dark at 4°C for 1 hour. The PI fluorescence of individual nuclei was measured by flow cytometry using standard FACScan equipment (Becton Dickinson, Franklin Lakes, NJ). The nuclei traversed a 488-nm Argon laser light beam. A 560-nm dichroid mirror and a 600-nm band pass filter (band width 35 nm) were used to collect the red fluorescence because of PI DNA staining. The data were recorded in logarithmic scale in a Hewlett Packard (HP 9000, model 310; Palo Alto, CA) computer. The percentage of apoptotic cell nuclei (subdiploid DNA peak in the DNA fluorescence histogram) was calculated with specific FACScan research software (Lysis II).