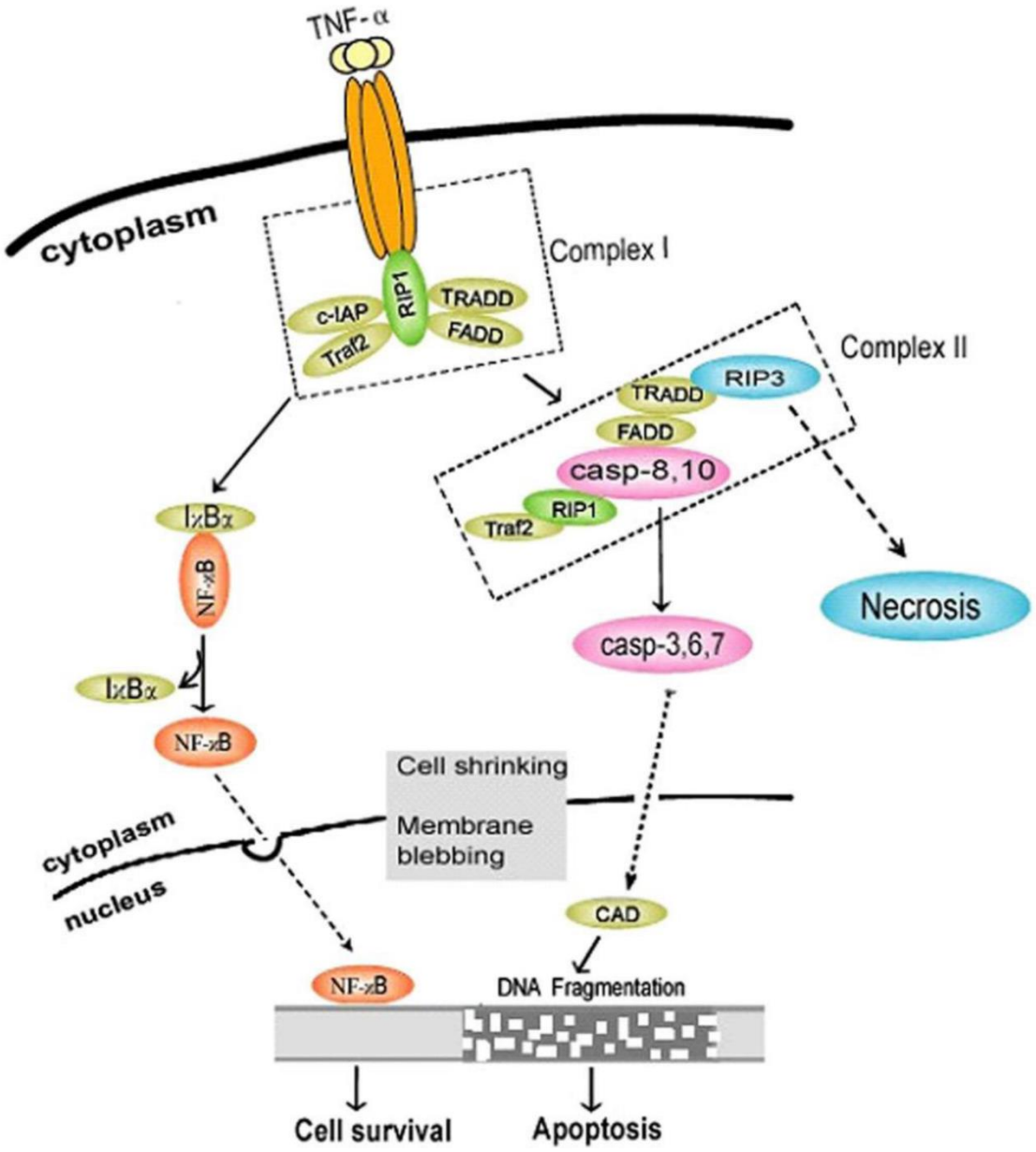

Fig. S1. Schematic illustration of TNF signal transduction pathways. Binding of trimeric TNF to TNFR1 triggers formation of complex I (RIP1, cIAP, Traf2 TRADD and FADD). This induces degradation of NF-κB inhibitors such as IκBα which results in activation of NF-κB and cell survival. Alternatively, triggered formation of complex II (RIP1, Traf2, caspase-8/10, FADD, TRADD and RIP3) leads to either apoptosis or necrosis.

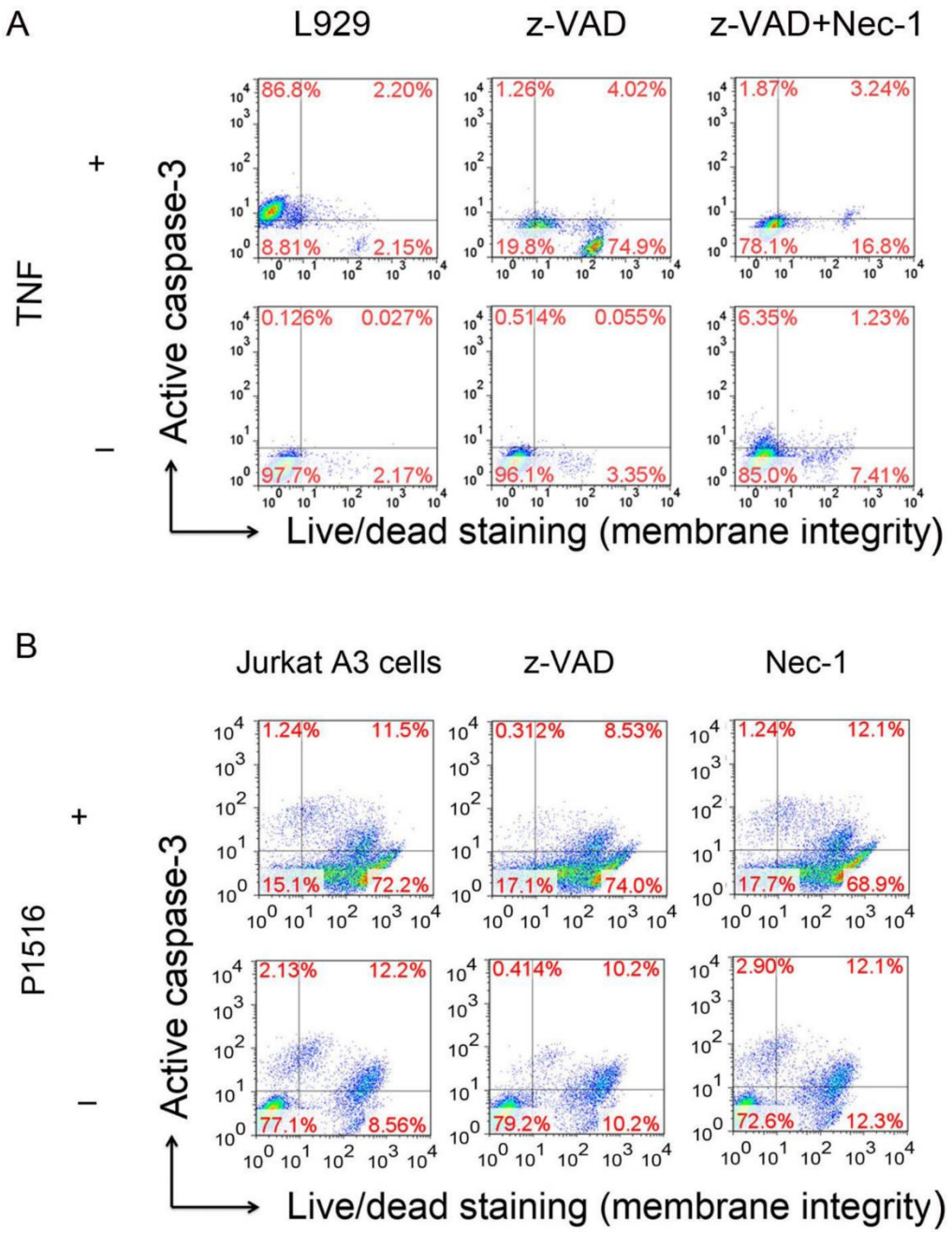

Fig. S2. P1516 induced necrosis cannot be inhibited by either z-VAD or Nec-1. (A) Z-VAD turns TNF induced apoptosis in L929 cells into necroptosis which can be inhibited by Nec-1. TNF induced apoptosis in L929 cells (upper left panel) becomes necrosis in the presence of z-VAD (upper middle panel). This process can be inhibited when add nec-1 to the system (upper right panel). The lower panels are control cells without the addition of TNF.

(B) Jurkat A3 cells were incubated with the 30-mer peptide P1516 at 30 μM overnight in the presence or absence of z-VAD or Nec-1. Apoptosis and necrosis were measured by flow cytometry of active caspase-3 expression and membrane integrity (live/dead cell staining), respectively.

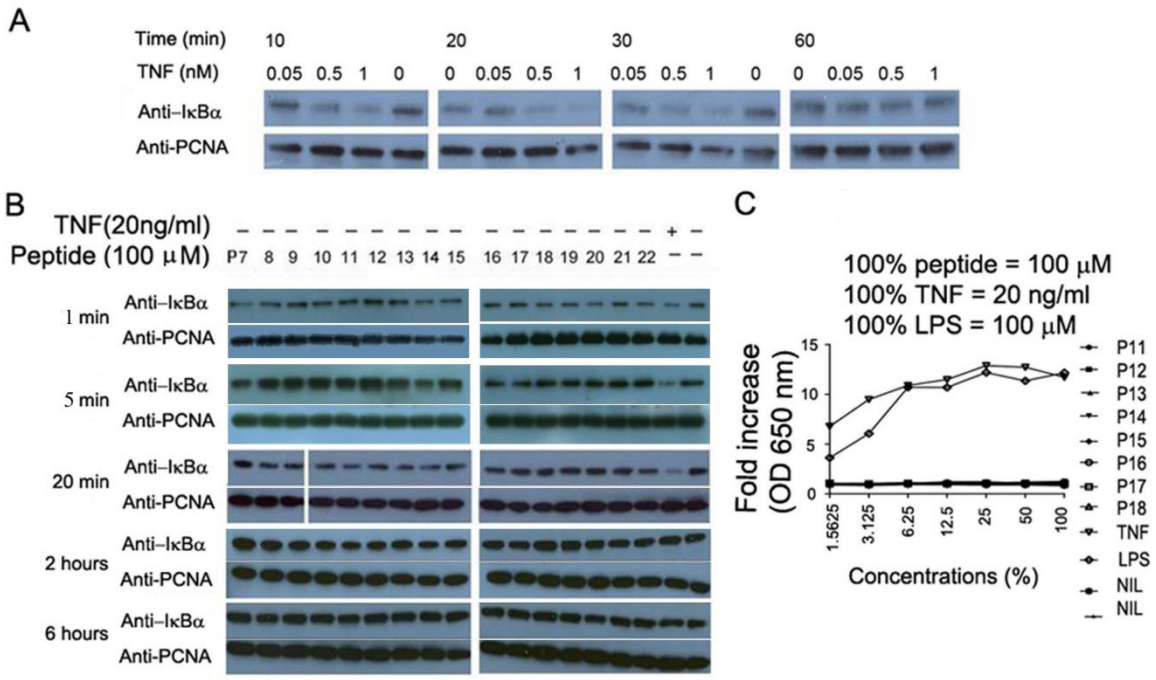

Fig. S3. TNF stimulates NF-κB but peptides derived from TNF do not activate NF-κB. (A) Jurkat A3 cells (6 x105) were incubated with various concentrations of TNF (0.05-1 ng/ml) for different times (10-60 min). Cell lysates were western blotted to nitrocellulose membranes and NF-κb activation was detected by IκB αdegradation with an IκB αspecific antibody. Proliferating cell nuclear antigen (PCNA) was measured as a loading control.

(B) Jurkat A3 cells (6 x10^5) were incubated with 1.2 nM TNF (20 ng/ml) or its peptides (P7-P22, 100 μM) for 1 min, 5 min, 20 min, 2 hours and 6 hours. Representative data from three experiments are shown.

(C) NF-κB indication cell line THP-1 cells (stably transfected with alkaline phosphatase) were incubated with peptides P11-P18 at the concentrations indicated. Soluble TNF and LPS were positive controls. NF-κB activation was shown by TNF and LPS stimulation but not by the peptides. The experiments were repeated three times with similar results.

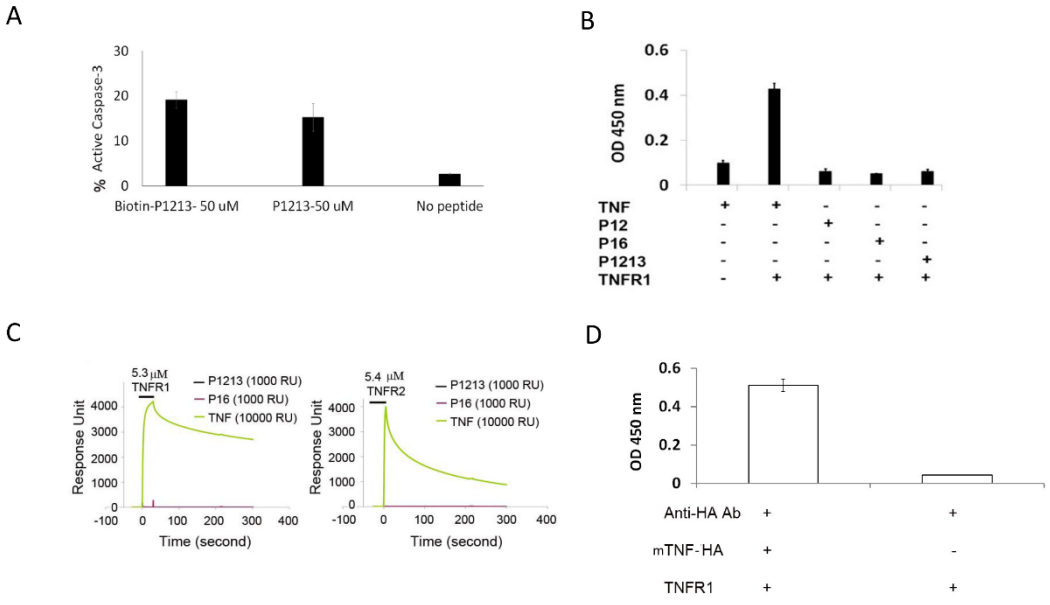

Fig. S4. TNF derived peptides do not bind to TNF1 and TNF2.

(A)Biotinylation of P1213 does not affect its function. Biotinylated- and wildtype- P1213 at 50 mM were incubated with Jurkat A3 cells overnight and active caspase-3 was measured for apoptosis.

(B) P12, P16 and P1213 do not bind to TNFR1. Biotinylated peptides or TNF were incubated with avidin-coated plates followed by incubating with soluble TNFR1. The binding of TNFR1 was detected by an anti-TNFR1 antibody conjugated with HRP.

(C) Biacore evidence of P1213 and P16 not binding to TNFR1 and TNFR2. Biacore experiment shows that these peptides do not bind to either TNFR1 (left panel) or TNFR2 (right panel)

(D) Mutant TNF binds to TNFR1. The ELISA plate was coated with TNFR1 followed by incubation of mTNF-HA. The binding was detected by anti-HA antibody and the second Ab conjugated with HR.