Supplemental Figure Legends

Supplemental Figure 1: Substitution of serines at position 348 and 350 does not affect the ability of caspase-8 to process caspase-9 in vitro. [^{35}S]-labeled murine caspase-9 and mutants AEPD, LDGD and LDVD proteins were incubated in the absence or presence of active human recombinant caspase-8 *in vitro*. Cleavage reactions were resolved by SDS-PAGE, and the gels fixed, dried and autoradiographed.

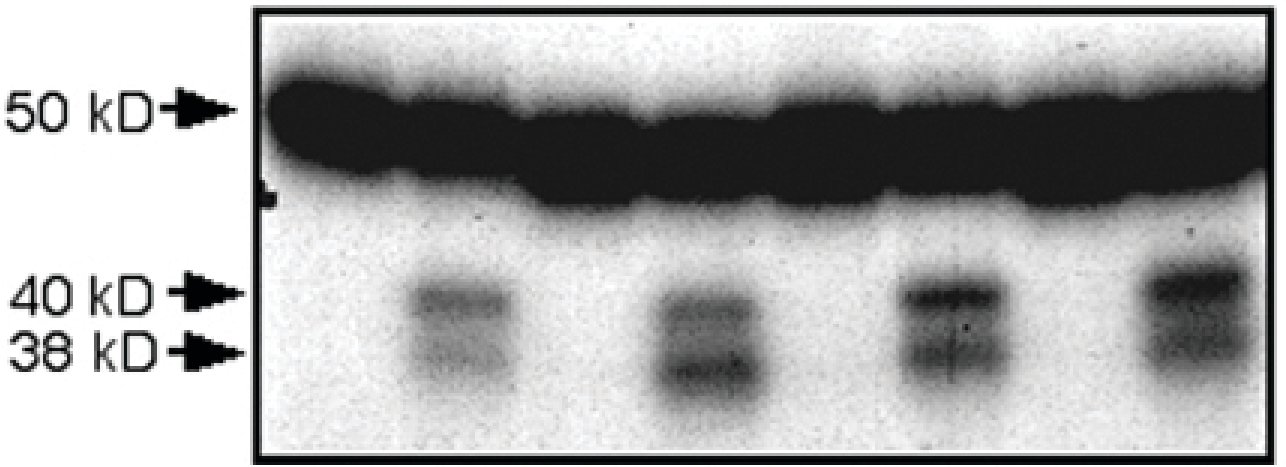
Supplemental Figure 2: Autoprocessing of murine Caspase-9 is not affected by CK2 phosphorylation. A. Western blot of cold *in vitro* translated caspase-9 incubated with or without CK2 in kinase buffer (details in Materials and Methods) probed with polyclonal phosphoserine-specific antibodies (top panel), stripped and reprobed with a monoclonal caspase-9 antibody. B. Caspase-9 (5μl from 25μl of the cold kinase reaction) were incubated alone, with 50μg caspase-9 -/- cytosolic S100 extract (2), or with extract, plus 1mM ATP and 0.2μg cyt c in Buffer A (details in Materials and Methods) in a total volume of 35μl. Reactions were carried out for 1 hour at 30^{o} C. Attenuated reactions were resolved by SDS/PAGE and western blotted using anti-caspase-9 antibody (Stressgen).

Supplemental Figure 3. Cells were exposed to 1 μM okadaic acid or DMSO for 30 minutes prior to TNF addition and cell death was determined by flow cytometric analysis of Annexin V and propidium iodide uptake at the indicated times.

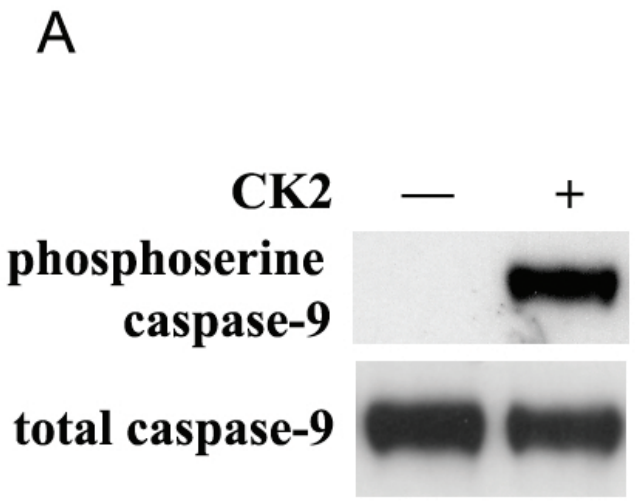
Supplemental Figure 4: A. Purified recombinant CK2 phosphorylates murine but not human caspase-9 in vitro. *In vitro* translated mC-9 protein was immunoprecipitated with anti-caspase-9 antibody and incubated in kinase buffer in the presence or absence of purified CK2 (top panel). Auto-phosphorylation of the CK2 b-subunit is evident in both lanes. Lower panel shows IP/western of cold *in vitro* translated murine and human caspase-9 proteins. B. Alignment of mouse, rat, dog and human residues in the vicinity of the CK2 consensus motif (underlined). The absence of a negatively charged (acidic) residue at the putative +1 position makes the human sequence a poor CK2 substrate.

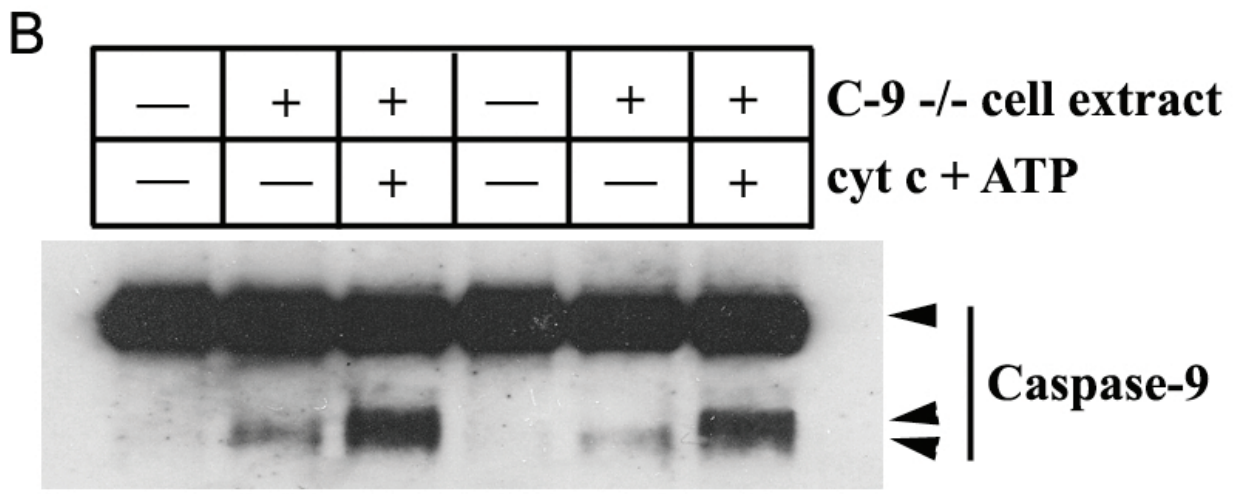
Supplemental Figure 1

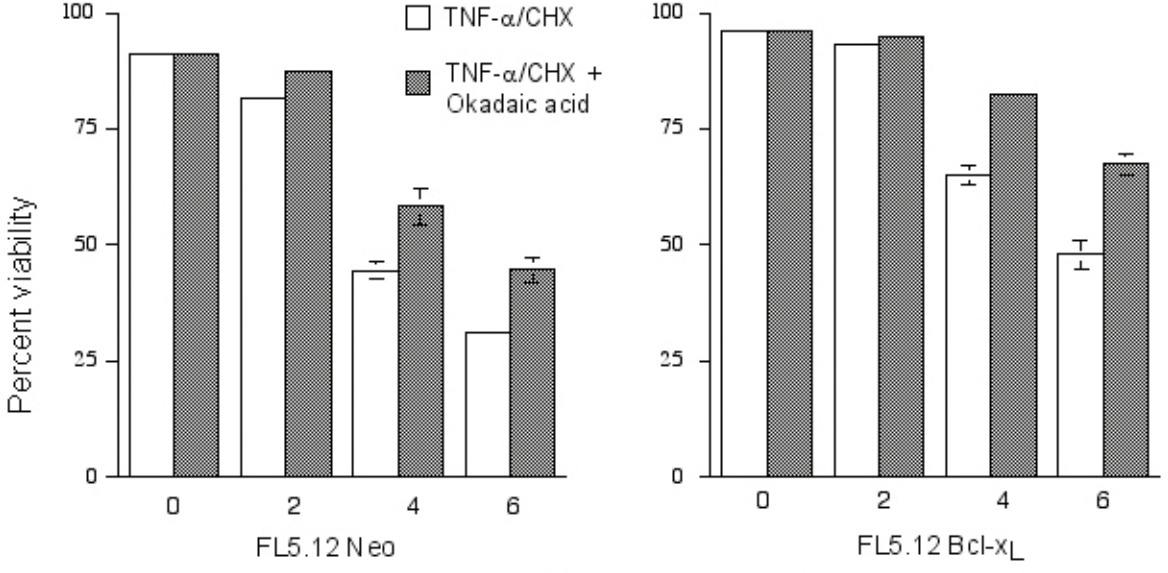
mC-9 or mutant	mC-9		AEPD		LDGD		LDVD	
active casp-8	–	+	–	+	–	+	–	+

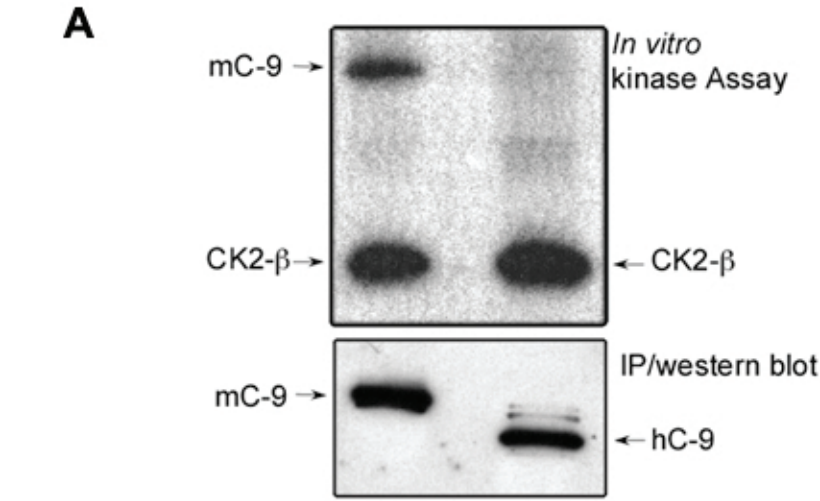


Supplemental Figure 2






TNF-α/CHX
TNF-α/CHX + Okadaic acid
Percent viability
100
75
50
25
0
0
2
4
6
FL5.12 Neo
FL5.12 Bcl-xL
Hours following treatment


A
mC-9
In vitro
kinase Assay
CK2-β
CK2-β
IP/western blot
mC-9
hC-9
B
AA 340 S S Q G R T L D S D S E P D A V P Y Q E mouse
AA 340 S S Q D K A F D S D S E P D A V P Y Q E rat
AA 332 S P E D R S P G S D S E P D A V P F Q E dog
AA 302 S P E D E S P G S N P E P D A T P F Q E human