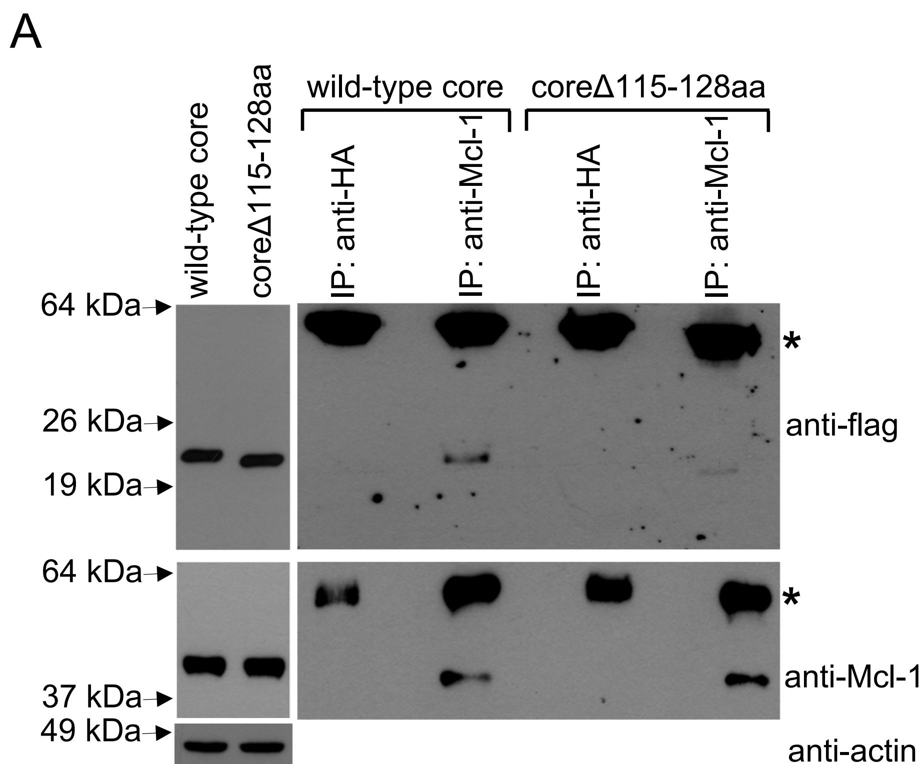
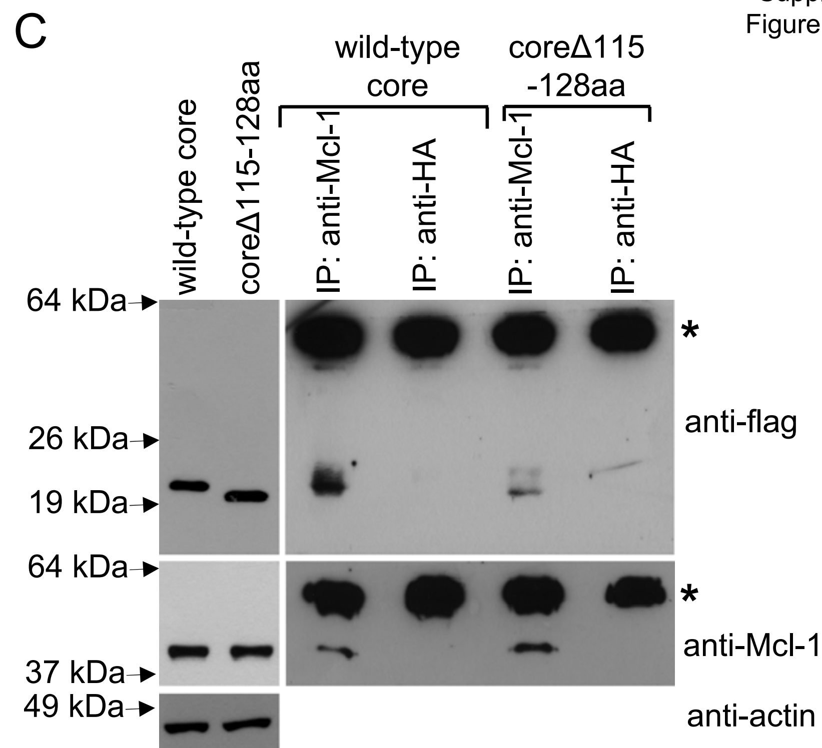
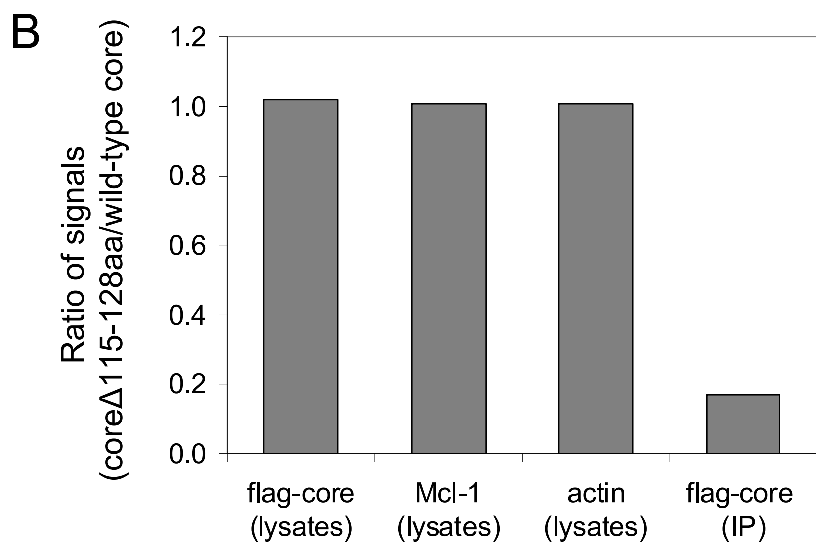
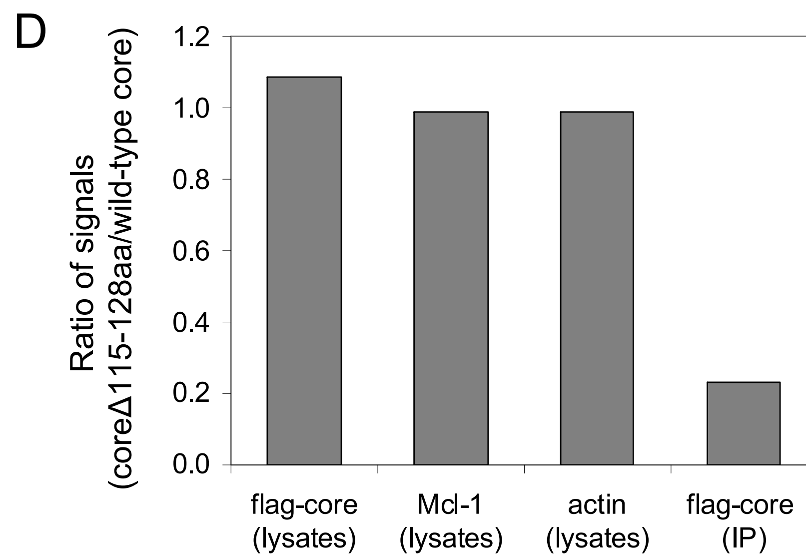
Supplementary Figure S1, A to D

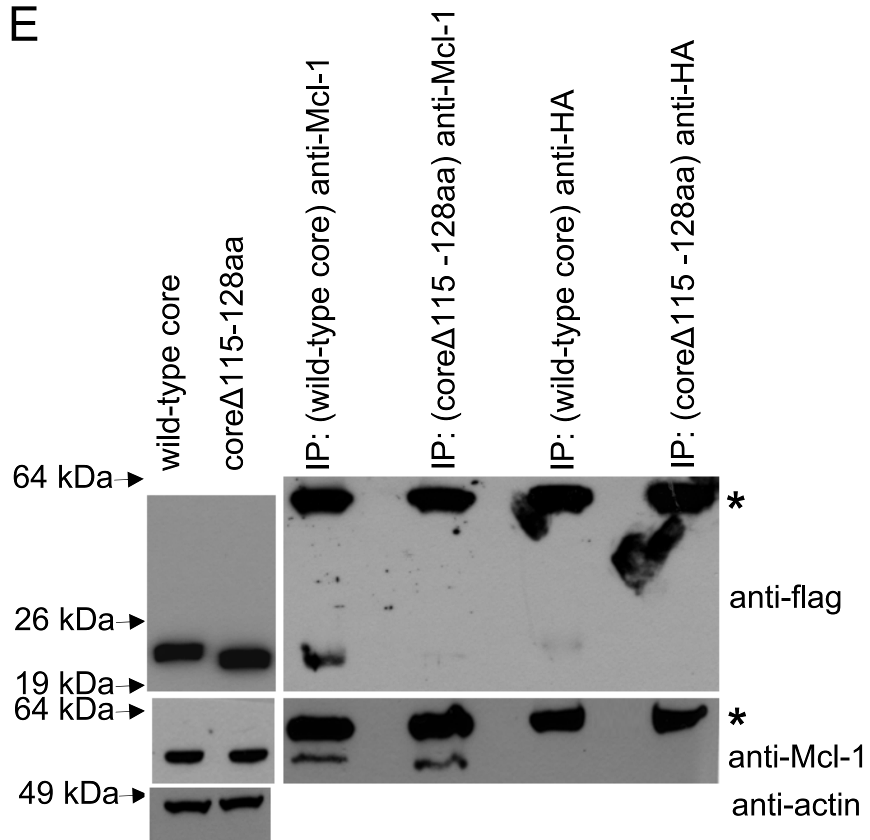




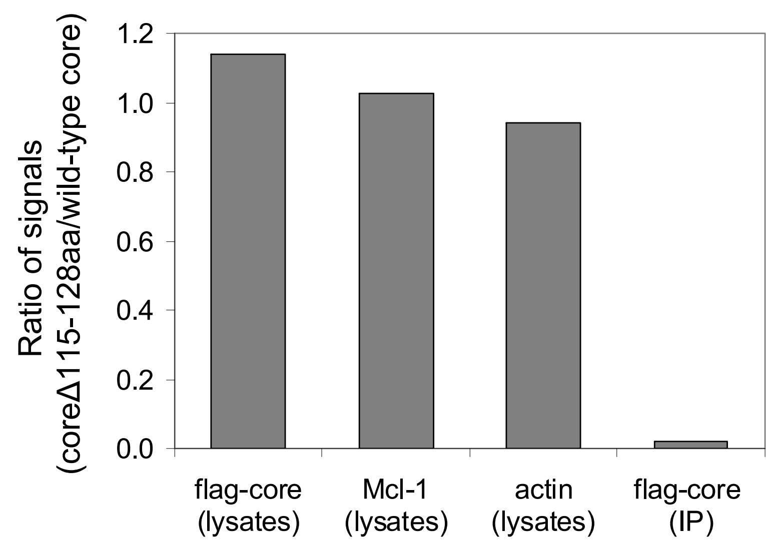




E



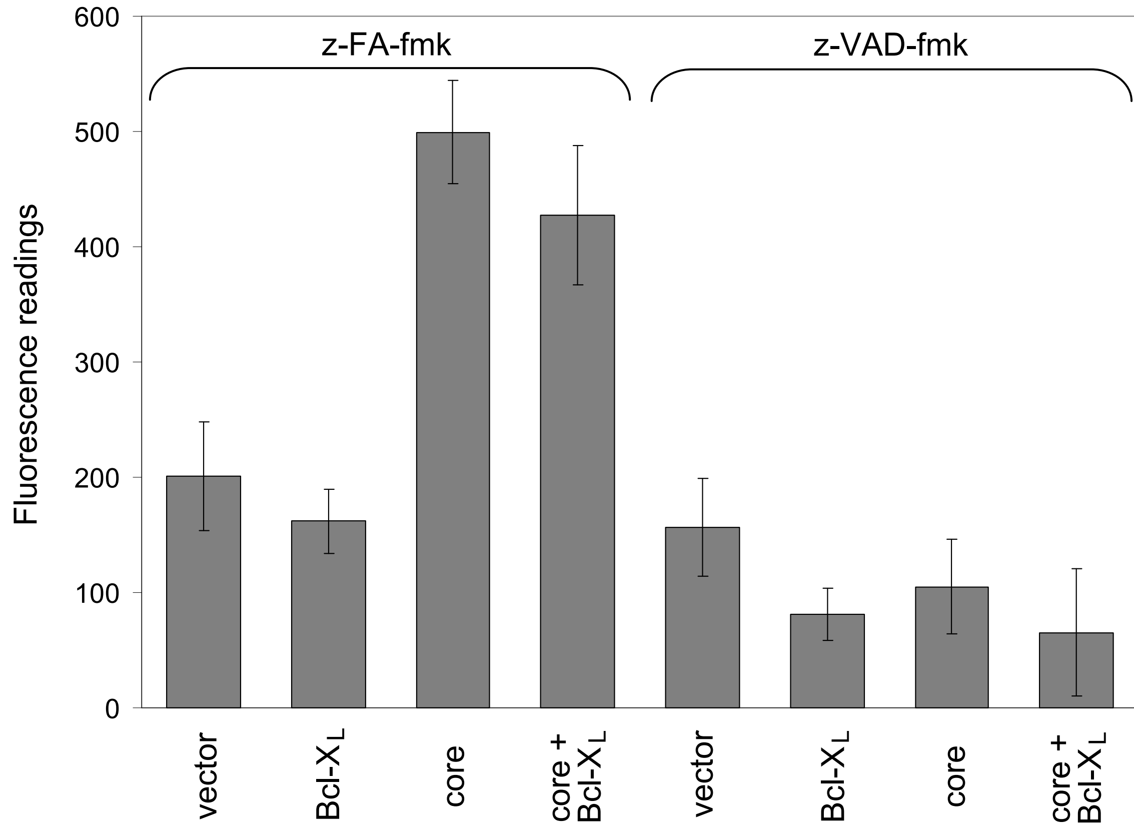
F



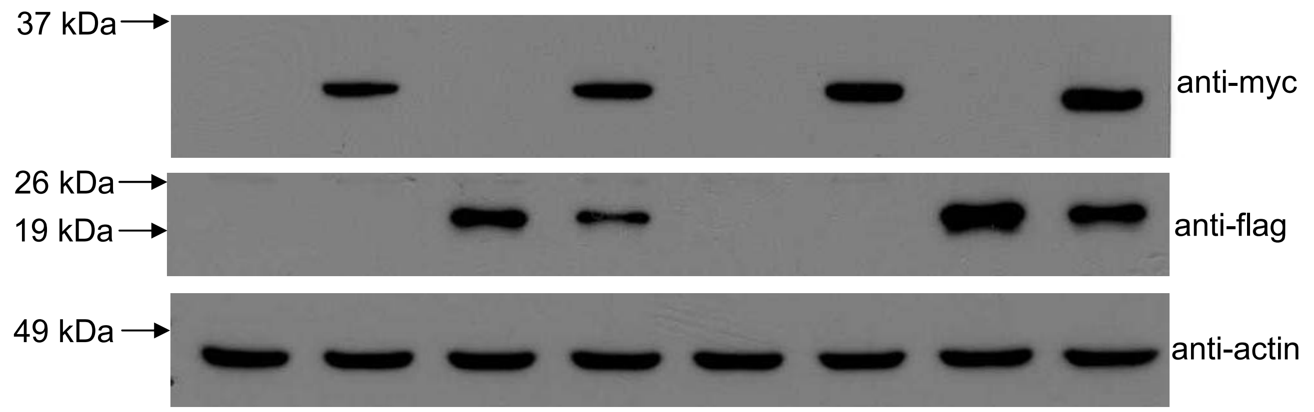
Legend for supplementary figure S1. Three independent co-immnunoprecipitation experiments were performed to determine the interaction between wild-type core or coreΔ115-128aa and endogenous Mcl-1 in Huh7 cells.

A, C and E. Huh7 cells were transfected with cDNA constructs for expressing flag-core or flag-coreΔ115-128aa. Immunoprecipitation was then performed using anti-Mcl-1 or anti-HA rabbit polyclonal antibodies and protein A agarose beads. The amounts of flag-tagged core protein in the lysates before immunoprecipitation (lanes 1 and 2) or co-immunoprecipitated (lanes 3 to 6) were determined by Western blot analysis with an anti-flag monoclonal antibody (top panel). Similarly, the amounts of endogenous Mcl-1 in these samples were detected using an anti-Mcl-1 monoclonal antibody (bottom panel). The protein marked with an asterisk represents the heavy chain of the antibody used for immunoprecipitation and the amounts of total cell lysates loaded were verified by measuring the levels of endogenous actin (lowest panel). *B, D and F.* An imaging densitometer was used to quantify the intensities of specific bands on the autoradiographs shown in *A, C and E.* For the amount of proteins in the lysates before immunoprecipitation (lanes 1 and 2), the ratio of the signals for samples transfected with coreΔ115-128aa to that for samples transfected with wild-type core is plotted. For the amounts of flag-tagged core protein co-immunoprecipitated (lanes 3 to 6), the signal for samples co-immunoprecipitated by HA antibody (unspecific binding) was subtracted from the signal for samples co-immunoprecipitated by Mcl-1 antibody. Then, the ratio of subtracted signals for samples transfected with coreΔ115-128aa to that for samples transfected with wild-type core is plotted.

A



B

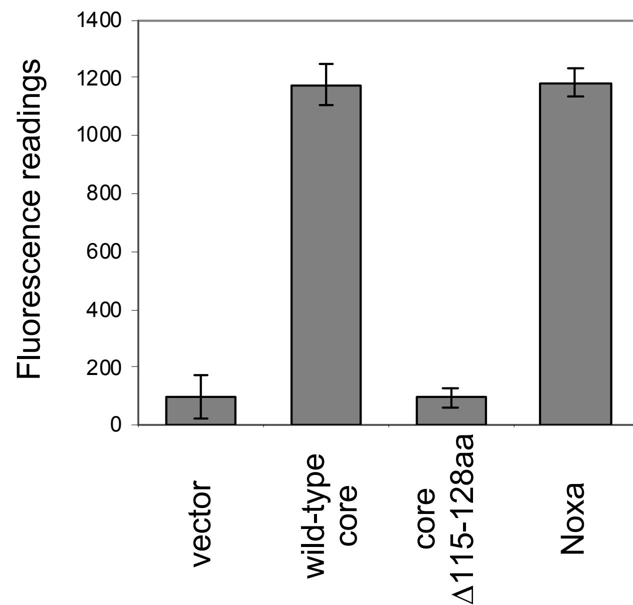


Legend for supplementary figure S2. Effects of apoptosis inhibition on the protein expression level of HCV core

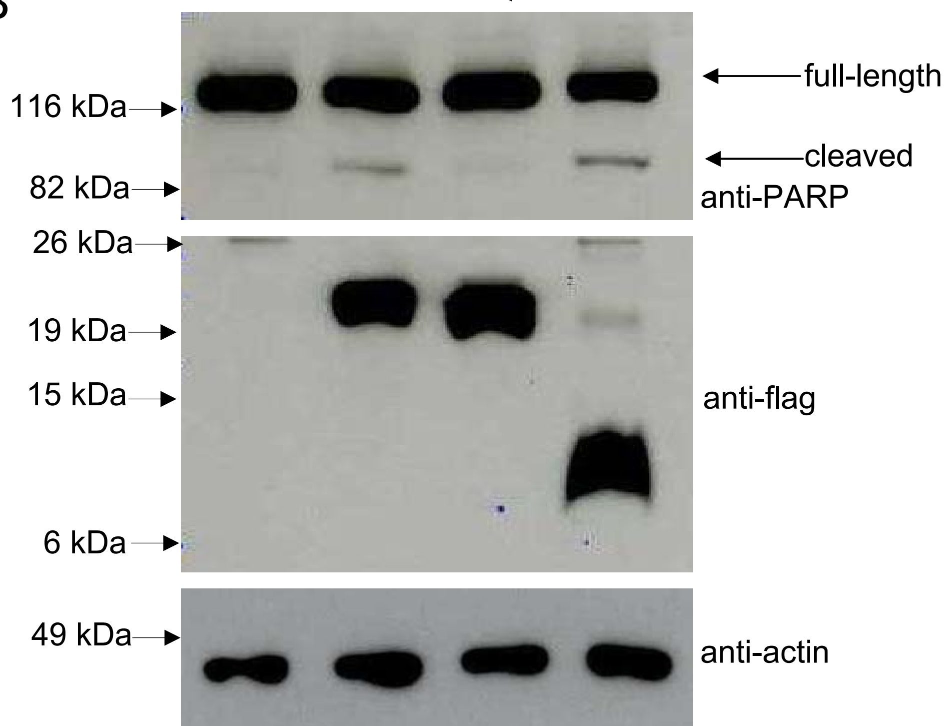
(A) A CaspACE fluorometric assay system from Promega Corporation (Madison, WI) was used to measure the activation of caspase-3 in Huh7 cells that were singly transfected with vector, Bcl-XL or wild-type core or co-transfected with wild-type core and Bcl-X_L in the presence of z-FA-fmk (an irrelevant peptide) or z-VAD-fmk (pan-caspase inhibitor). All experiments were performed in triplicates and the average values with standard deviations are plotted. (B) Western blot analysis was performed to determine the expression levels of the myc-tagged Bcl-X_L and flag-tagged core (upper and middle panels). The amounts of total cell lysates loaded were verified by measuring the levels of endogenous actin (lower panel).

Supplementary
Figure S3

A



B

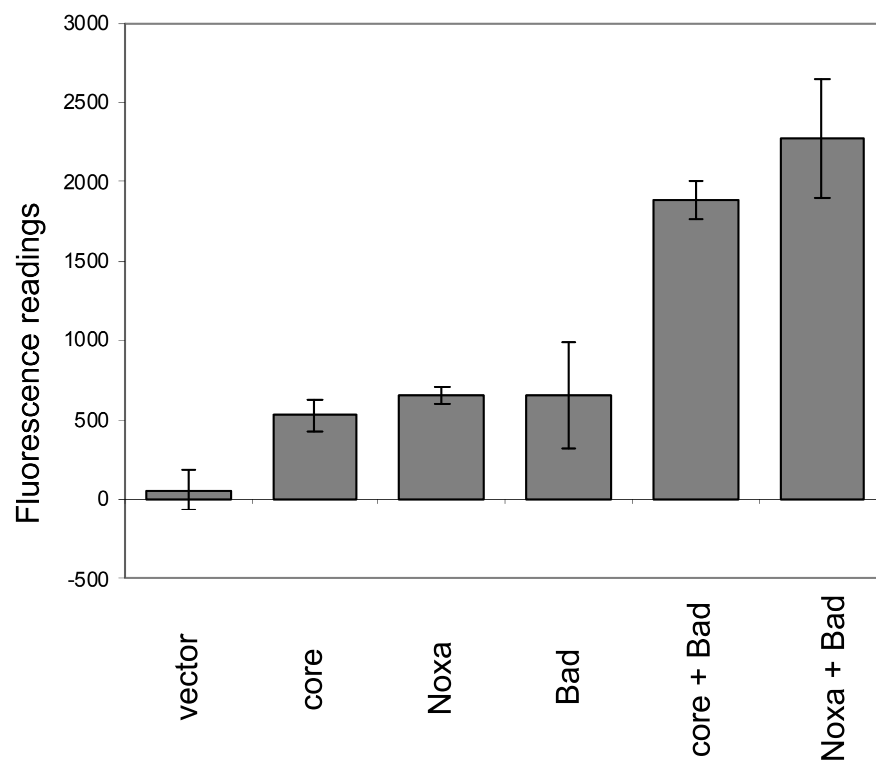


Legend for supplementary figure S3. Induction of apoptosis by the overexpression of core and Noxa in 293T cells.

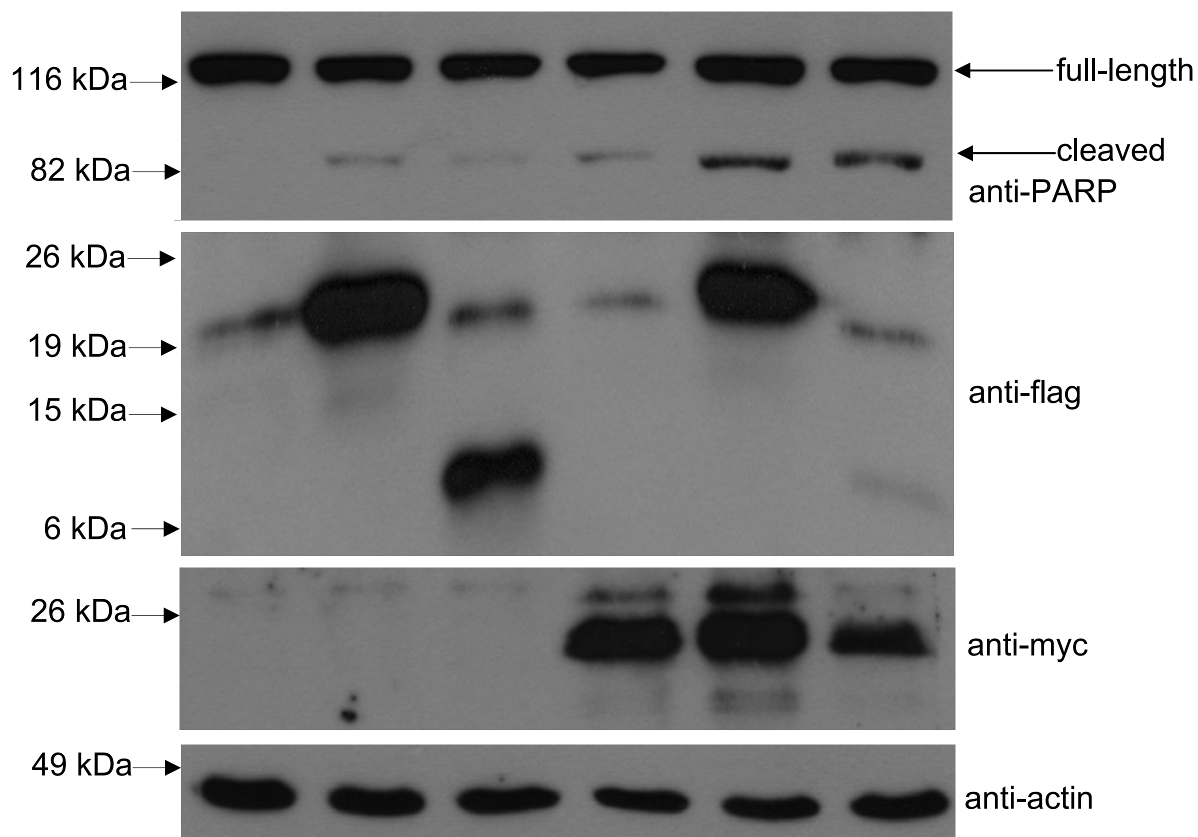
A. A CaspACE fluorometric assay system from Promega Corporation (Madison, WI) was used to measure the activation of caspase-3, which is a hallmark of apoptosis, in 293T cells that were transfected with vector only, wild-type core, a core mutant lacking the putative BH3 domian (coreΔ115-128aa) and human Noxa. A flag eptitope is fused to the N termini of the proteins as this allows comparison of protein expression levels with an anti-flag antibody. All experiments were performed in triplicates and the average values with standard deviations are plotted.

B. Western blot analysis was also performed to determine the cleavage of endogenous PARP, which is a substrate of activated caspase-3, from 116 kDa to 83 kDa (upper panel). Similarly, the expression levels of the different proteins were determined using anti-flag antibody (middle panel). The amounts of total cell lysates loaded were verified by measuring the levels of endogenous actin (lower panel).

A



B

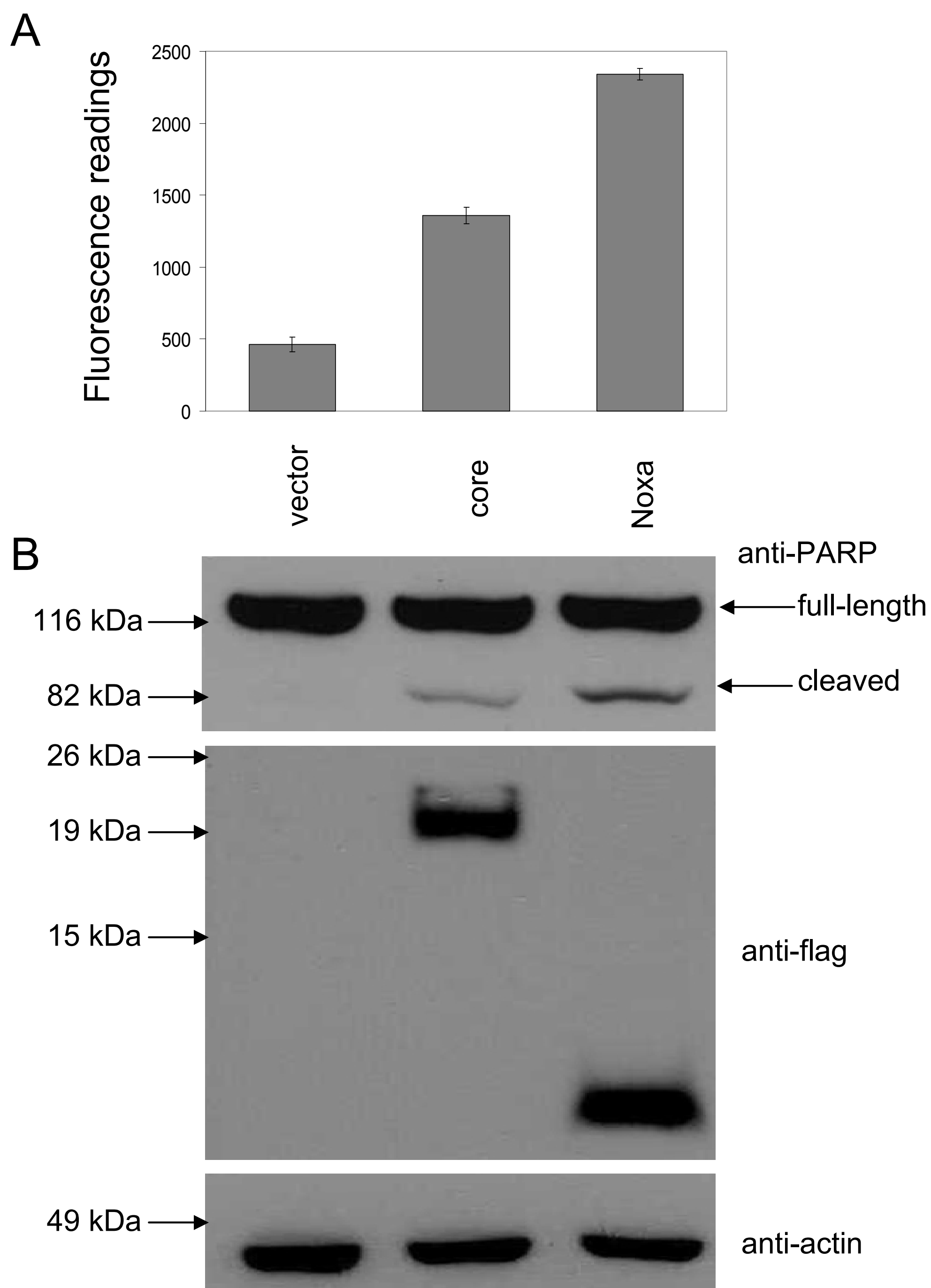


Legend for supplementary figure S4. Enhanced apoptosis by the coexpression of core and Bad in Huh7 cells.

A. A CaspACE fluorometric assay system from Promega Corporation (Madison, WI) was used to measure the activation of caspase-3, which is a hallmark of apoptosis, in Huh7 cells that were transfected with vector only, core, Noxa, Bad or a combination of core and Bad or Noxa and Bad. In each transfection, the total amount of DNA was normalized to 0.95 μg with the addition of empty vector if necessary. A flag eptitope is fused to the N termini of core and Noxa while a myc eptiotpe is fused to that of Bad. All experiments were performed in triplicates and the average values with standard deviations are plotted.

B. Western blot analysis was also performed to determine the cleavage of endogenous PARP, which is a substrate of activated caspase-3, from 116 kDa to 83 kDa (upper panel). Similarly, the expression levels of the different proteins were determined using anti-flag or anti-myc antibody (middle two panels). The amounts of total cell lysates loaded were verified by measuring the levels of endogenous actin (lower panel).

Supplementary
Figure S5



Legend for supplementary figure S5. Induction of apoptosis by the overexpression of core and Noxa in Huh7 cells.

A. A CaspACE fluorometric assay system from Promega Corporation (Madison, WI) was used to measure the activation of caspase-3, which is a hallmark of apoptosis, in Huh7 cells that were transfected with vector only, core and human Noxa. A flag eptitope is fused to the N termini of the proteins as this allows comparison of protein expression levels with an anti-flag antibody. All experiments were performed in triplicates and the average values with standard deviations are plotted.

B. Western blot analysis was also performed to determine the cleavage of endogenous PARP, which is a substrate of activated caspase-3, from 116 kDa to 83 kDa (upper panel). Similarly, the expression levels of the different proteins were determined using anti-flag antibody (middle panel). The amounts of total cell lysates loaded were verified by measuring the levels of endogenous actin (lower panel).

Supplementary Table 1. Effects of alanine substitutions on the binding of core to Mcl-1.

Mutant	% reduction in binding[a]				Average[b]	*p*-value[c]
	Exp 1	Exp 2	Exp 3	Exp 4		
L119A	26	21	40	43	33 (± 11)	0.001
V122A	64	63	66	55	62 (± 5)	0.001
L126A	3	3	10	18	9 (± 7)	0.0001

[a]The percentage in binding to Mcl-1 for each of the mutants when compared to wild-type core was estimated by using an imaging densitometer to measure the intensity of core signals after co-immunoprecipitation (Figure 7A). 4 independent experiments were performed (Exp1 to Exp 4). For each experiment, 3 different autoradiographs (with different exposure times) were used and the signals were normalized to the signal for wild-type core. Then, the average value was computed and shown.

[b]The mean value of the results from 4 independent experiments was computed and shown. The standard deviations are shown in parentheses.

[c]The difference between mutant and wild-type core in binding to Mcl-1 was compared using the 2-tailed Student's t test and the *p*-value is shown.