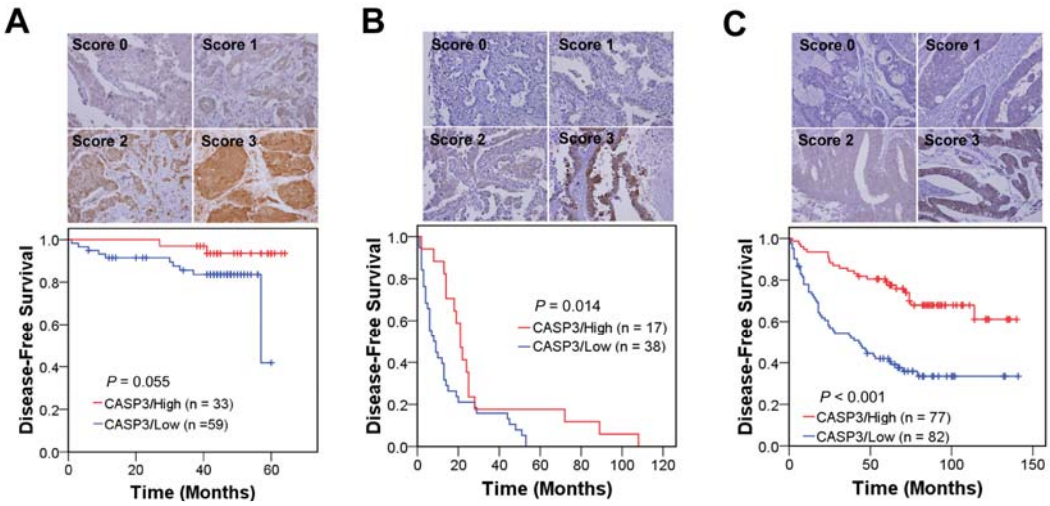
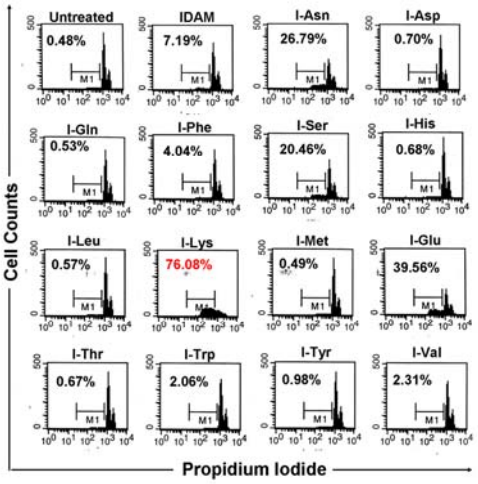
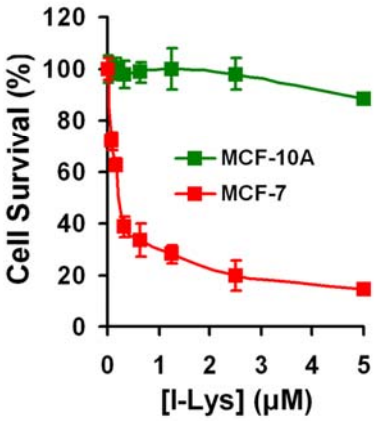


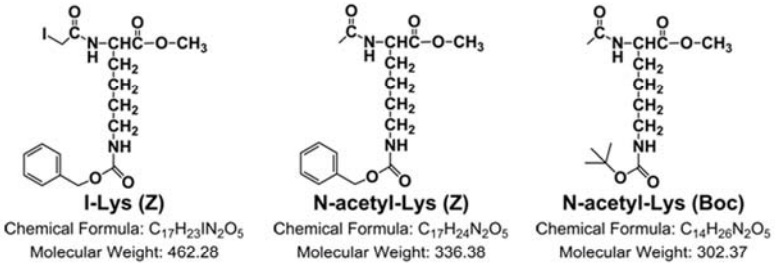
Supplementary Figure 1: (A, B and C) Scoring results of IHC staining (upper) and Kaplan-Meier analyses (lower) of CASP3 expression in a cohort of subjects with breast (A), lung (B) or colon (C) cancers. Log-rank tests were used for analysis of statistical significance.



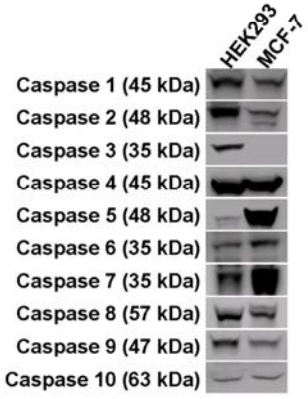
Supplementary Figure 2: (A) Screening of I-AAs against MCF-7 cells. Propidium iodide (PI)-based flow cytometric analysis was used to determine $SubG_0$ accumulation in MCF-7 cells treated without or with the indicated compounds at 1 μM for 24 hours. DMSO (0.1%) was used as a solvent control, and IDAM (1 μM) was used as a negative control. The minor cytotoxicity of I-Asn against MCF-7 cells is probably due to its weaker capability in disrupting XIAP:p19/p12-casp7 complex as shown later in Supplementary Figure 14C and that of I-Glu may be due to its incorporation into β-tubulin thereby destroying the β-tubulin:CCTβ complex (Lin et al, Cancer Research 2009).



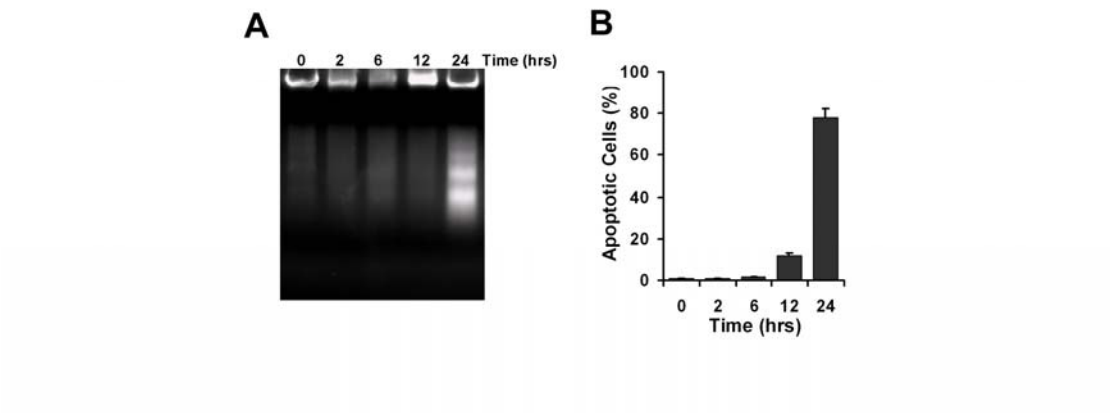
Supplementary Figure 3: Long-term cytotoxicity assay. Cells (10^4/ml) treated with I-Lys at concentrations ranging from 0 to 10 µM for 6 days. The cell viability was determined by MTT assay. Data from three independent experiments are presented in the mean ± SEM.



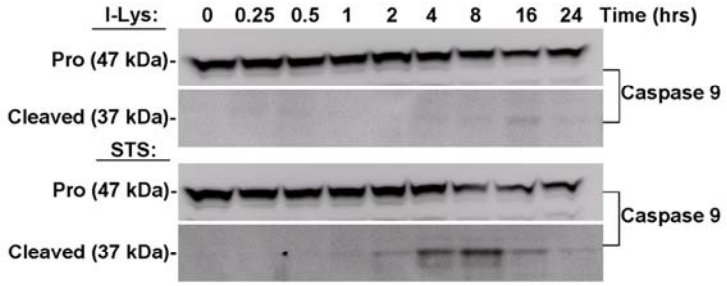
Supplementary Figure 4: Chemical structure of I-Lys analogues with differently protection groups or without iodo-group.



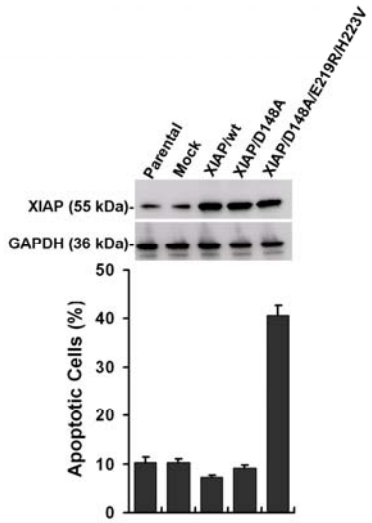
Supplementary Figure 5: Examination of caspases 1-10 expression in HEK293 and MCF-7 cells. Aliquots (50 μg) of cell lysates were subjected to Western blot analysis using each caspase-specific antibody. Antibodies against caspases-1, 2, 6, 8 and 10 were purchased from IMGENEX. Caspases-3, 7 and 9 antibodies were from Cell Signaling. Caspases 4 and 5 antibodies were from GENETEX.



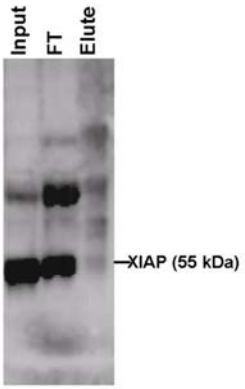
Supplementary Figure 6: (A) Chromosomal DNA fragmentation of MCF-7 cells in response to I-Lys treatment at different time periods. (B) Assessment of cell apoptosis in MCF-7 cells by PI-based Flow-cytometric analysis post-treatment with I-Lys (1 μM) at designated time points. The data from three independent experiments are shown as the mean ± SEM.



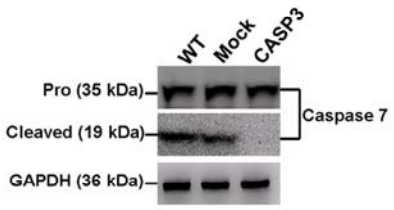
Supplementary Figure 7: Test of caspase 9 proteolysis in MCF-7 cells. Cells were treated with I-Lys or STS (1 μM of each) for indicated time periods. Aliquots (100 μg) of cell lysates were subjected to Western blot analysis using caspase-9 or cleaved-form caspase-9 antibody.



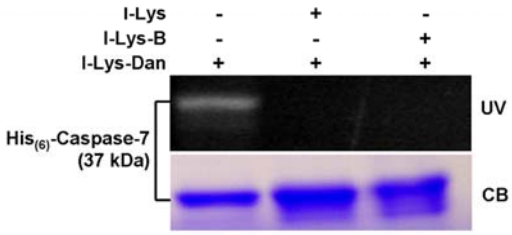
Supplementary Figure 8: Effects of different XIAP mutations on apoptosis of MCF-7 cells. Cells (2 x 10^5/ml) were transfected with pIRES2-EGFP vector without (Mock) or with DNA encoding wild-type (wt), D148A or D148A/E219R/H223V mutant XIAP. (Upper) Protein expression levels were determined by Western blot analysis using XIAP antibody. GAPDH was used as internal control for protein loading. (Lower) After the transfection, parental and transfected MCF-7 cells were treated with STS at 1 μM for 24 hours. Apoptotic cells were estimated by PI-based Flow cytometric analysis. The data from three independent experiments are shown as the mean ± SEM.



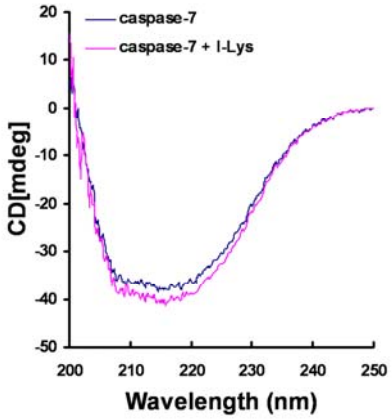
Supplementary Figure 9: Immunoblotting for XIAP in MCF-7 whole cell lysates (input) and in the flow-through (FT) and eluted fractions from streptavidin-affinity column chromatography of I-Lys-Biotin-interacting protein in MCF-7 cell lysates.



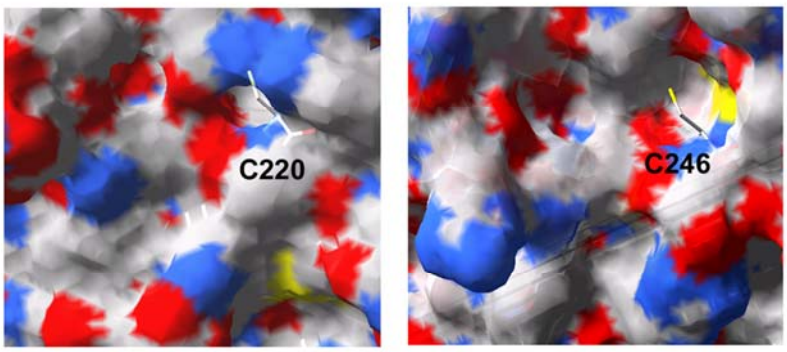
Supplementary Figure 10: Pro and cleaved (p19-casp7)-caspase-7 expression in CASP3-restoring MCF-7 cells. Aliquots (100 μg) of cell lysates were subjected to Western blot analysis using pro-caspase-7, p19-casp7 or GAPDH antibody. GAPDH was used as control for protein loading.



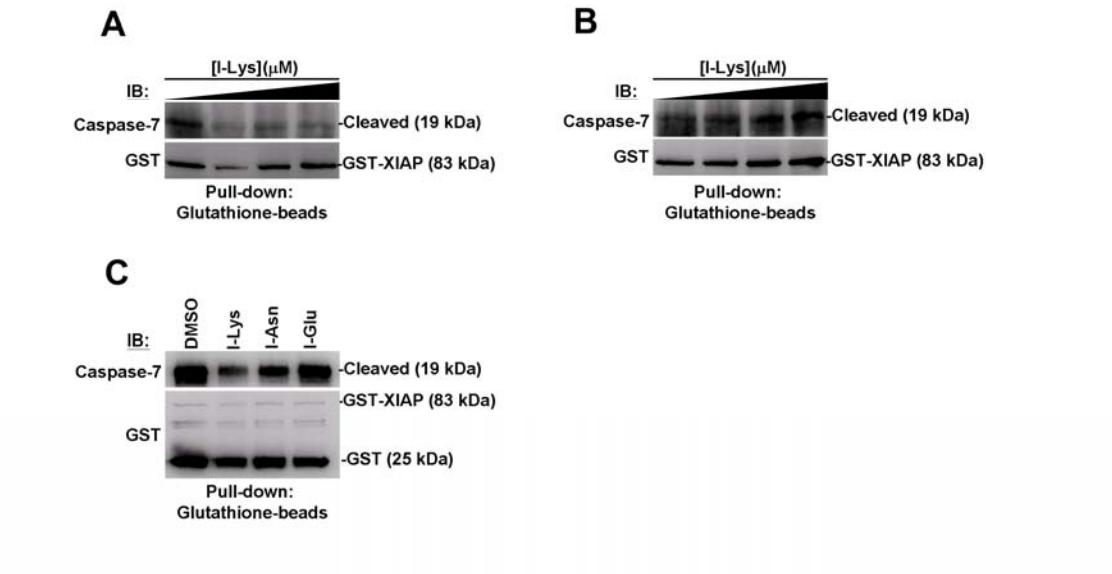
Supplementary Figure 11: Recombinant caspase-7 protein (100 μg) was pre-incubated without or with I-Lys (10 μM) or I-Lys-B (10 μM) for 1 hour prior to additional incubation with I-Lys-Dan (10 μM) for 2 hours. After incubation, 10 μg of protein was analyzed by SDS-PAGE. Fluorescent bands were visualized under a UV lamp prior to Coomassie staining.



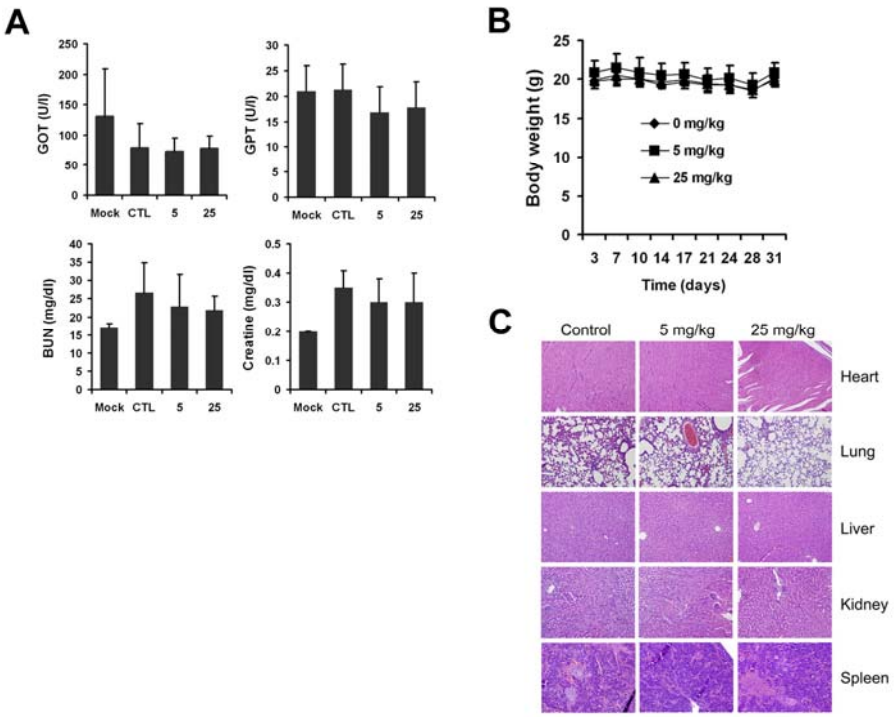
Supplementary Figure 12: Circular dichroism (CD) spectroscopic analysis of recombinant caspase-7 protein labeled with or without I-Lys.



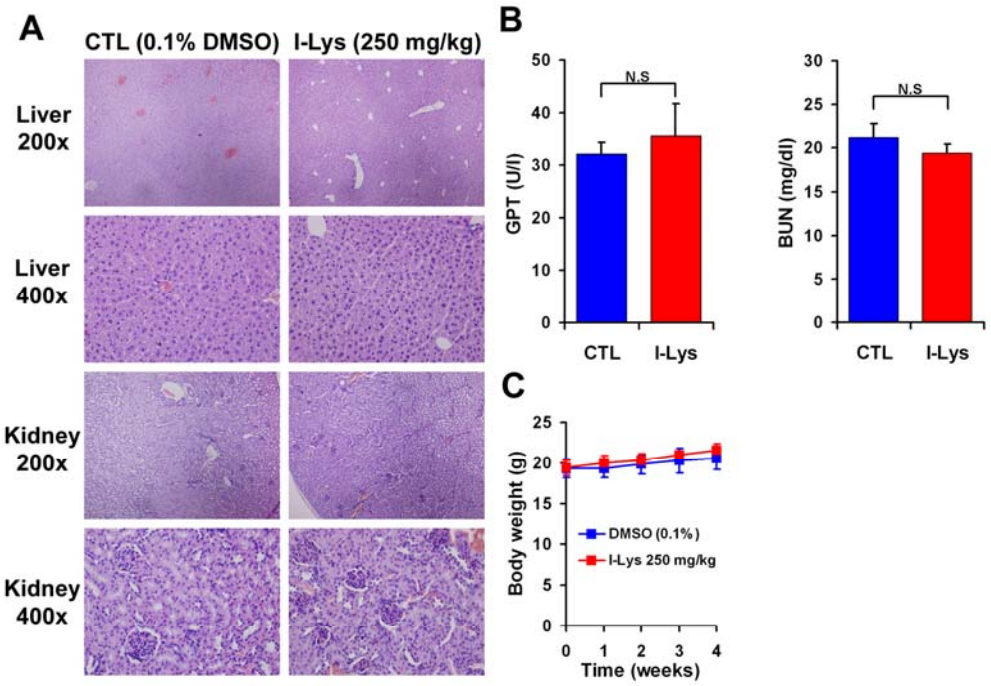
Supplementary Figure 13: Comparison between cleaved-form caspase-3 (PDB: 1I30) and p19/p12-casp7 (PDB: 1I51) protein structures complexed with XIAP at the relative regions. SH-groups of Cys^{220} of caspase-3 and Cys^{246} of capsase-7 are shown as sticks in yellow, and Cys^{220} is embedded in caspase-3. Blue, red and white surfaces represent negative, positive and non-charged fields, respectively.



Supplementary Figure 14: (A, B) Effect of I-Lys on disrupting XIAP complex with wild-type or C246S mutated p19/p12-casp7. Full-length XIAP was co-expressed with wild-type (A) or C246S mutated (B) p19/p12-casp7 as glutathione S-transferase (GST) and hexa-histidine (6xHis)-fused proteins in *E. coli* by using expression vectors pGEX-4T1 and pET-28, respectively, and the protein complexes were purified from bacterial lysates using glutathione and Ni^{2+}-NTA affinity column chromatography, respectively. The complexes were incubated with I-Lys at concentrations (0, 0.1, 0.5 and 1 mM) for 1 hour at 4°C prior to pull-down with glutathione beads. After washes, the pellets were subjected to Western blot analysis using p19-casp7 or GST antibody. (C) Effect of I-Lys, I-Asn or I-Glu on disrupting XIAP:p19/p12-casp7 complex in vitro. The recombinant full-length XIAP:p19/p12-casp7 complex was incubated without or with I-Lys, I-Asn or I-Glu (0.5 mM of each) for 1 hour at 4°C prior to pull-down with glutathione beads. After washes, the pellets were subjected to Western blot analysis using p19-casp7 or GST antibody.



Supplementary Figure 15: (A) Serological examination of mice on end-point of treatment with I-Lys at designated concentrations. Mice treated without (Mock, n = 3) or with PBS containing 0.1 % DMSO (control, CTL; n = 8) or I-Lys at 5 mg/kg or 25 mg/kg (n = 8 of each) were subjected to serological examination of liver (GOT and GPT) and renal (BUN and creatine) functions. (B) Body weights of mice on different time points after treatment with I-Lys at designated concentrations. PBS containing 0.1 % DMSO used as solvent was a control and the data is shown in 0 mg/kg. (C) Hematoxylin and eosin staining of heart, lung, liver, kidney, and spleen tissue samples obtained from mice treated with PBS containing 0.1 % DMSO (control) or I-Lys at 5 mg/kg and 25 mg/kg at the end of treatment.



Supplementary Figure 16: Physiological determinations of mice subjected to maximum-tolerated doses (MTD) analysis against I-Lys. (A) Hematoxylin and eosin staining of liver and kidney tissue samples obtained from mice treated with PBS containing 0.1 % DMSO (control, CTL) or I-Lys at 250 mg/kg at the end of treatment. (B) Serological examination of mice on end-point of treatment with PBS containing 0.1 % DMSO (CTL) or I-Lys at 250 mg/kg. Mice treated with PBS containing 0.1 % DMSO (n = 5) or I-Lys (n = 5) were subjected to serological examination of liver (GPT) and renal (BUN) functions. (C) Body weights of mice on different time points after treatment with PBS containing 0.1 % DMSO or I-Lys at 250 mg/kg.

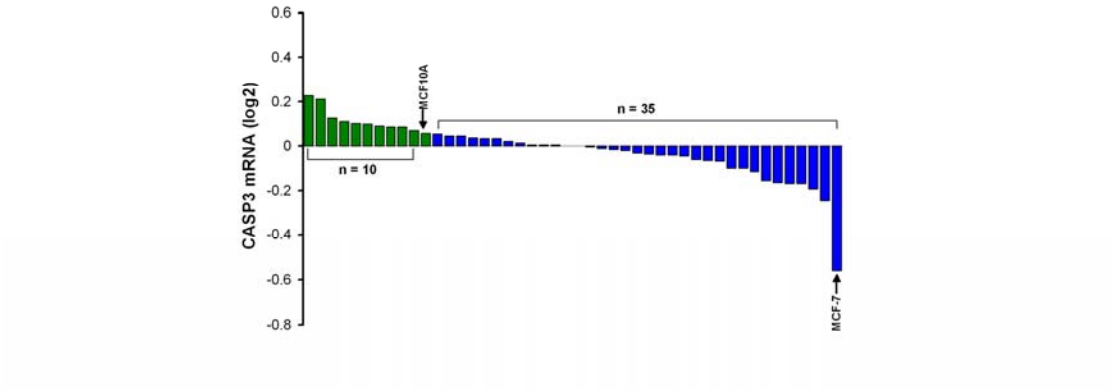
A

Colon Cancers (n = 159)		CASP3(low)/ p19/p12-casp7(high)	Others	Sig.
Metastasis	No	**20** (*29.3*)	**117** (*107.7*)	< 0.001
	Yes	**14** (*4.7*)	**8** (*17.3*)	

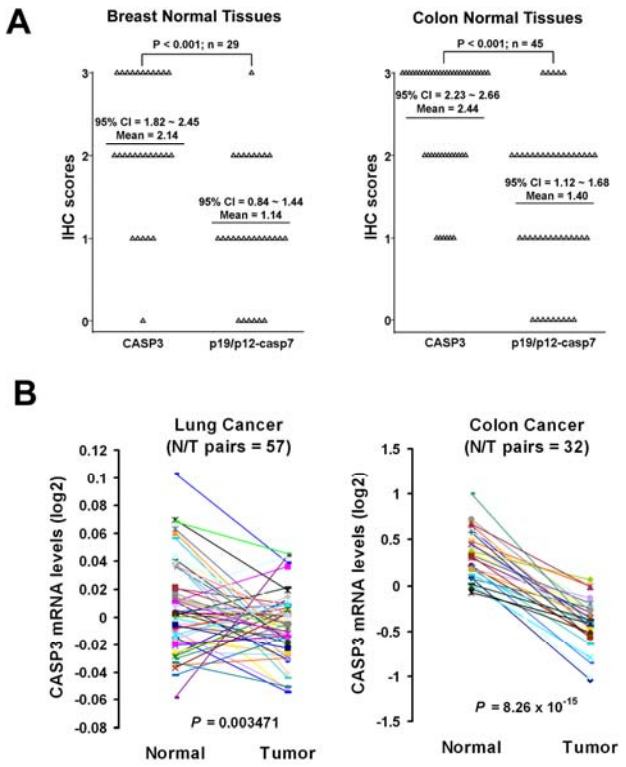
B

Lung Cancers (n = 55)		CASP3(low)/ p19/p12-casp7(high)	Others	Sig.
Metastasis	No	**13** (*16.3*)	**16** (*12.7*)	0.06
	Yes	**18** (*14.7*)	**8** (*11.3*)	

Supplementary Figure 17: (A and B) Relevance between the signature of CASP3(low)/p19/p12-casp7(high) and cancer metastasis in colon (A) and lung (B) cancers. Statistical significances were analyzed by One-way ANOVA using Duncan's Multiple Range Test.



Supplementary Figure 18: Transcriptional profile of CASP3 in MCF10A (indicated by arrow) and 45 breast cancer cell line including MCF-7 (indicated by arrow) (GEO accession no: GSE10890). Caspase-3 mRNA levels were normalized by median and presented as log2 values. Blue columns denote that caspase-3 mRNA levels in those cells are relatively lower than that in MCF10A.



Supplementary Figure 19: (A) Scoring results of IHC staining against CASP3 and p19/p12-casp7 expressions in normal breast (n = 29) (left) and colon (n = 45) (right) tissues obtained from a cohort of healthy subjects. Statistical significances ($P < 0.001$) and 95% confident intervals (CIs) were analyzed by One-way ANOVA using Duncan's Multiple Range Test. (B). Caspase-3 gene expression in paired normal/tumor (N/T) tissues from clinical cohorts with colon (accession number: GSE8671; left) or lung (accession number: GSE32863; right). Statistical differences of caspase-3 expression in normal and tumor tissues were analyzed by paired t-test.

		XIAP		
		Breast Cancer (n = 92)	Lung Cancer (n = 55)	Colon Cancer (n = 159)
CASP3	Pearson's Correlation	-0.265	-0.259	-0.049
	Sig. (2-tailed)	0.011	0.056	N.S
p19/p12-casp7	Pearson's Correlation	0.181	0.453	0.371
	Sig. (2-tailed)	0.084	0.001	< 0.001

Supplementary Figure 20: Pearson's Correlation analysis among XIAP, CASP3 and p19/p12-casp7 expression in IHC experiments in a cohort of subjects with breast, lung or colon cancers. Negative R values indicate that XIAP and CASP3 expression levels are inversely correlated in the detected clinical cancer samples. Conversely, XIAP and p19/p12-casp7 expression levels are correlative. Pearson's test was used to analyze statistical significances of each Correlation test.

DATASET	CANCER TYPE	SUBTYPE	ENDPOINT	COHORT	CONTRIBUTOR	ARRAY TYPE	PROBE ID	N	CUTPOINT	MINIMUM P-VALUE	COX P-VALUE	HR [95% CIlow-Chupp]
GSE13507	Bladder cancer	Transitional cell carcinoma	Disease Specific Survival	CNUH	Kim	Human-6 v2	ILMN_1756787	165	0.81	0.112	0.05	4.01 [0.96 - 16.8]
GSE5287	Bladder cancer		Overall Survival	Aarhus (1995-2004)	Als	HG-U133A	202763_at	30	0.4	0.056	0.064	2.12 [0.96 - 5.03]
GSE13507	Bladder cancer		Overall Survival	CNUH	Kim	Human-6 v2	ILMN_1756787	165	0.81	0.152	0.156	1.36 [0.83 - 3.18]
GSE4475	Blood cancer	B-cell lymphoma	Overall Survival	Berlin (2003-2005)	Hummel	HG-U133A	202763_at	158	0.1	0.004	0.006	2.41 [1.29 - 4.49]
E-TABM-346	Blood cancer	DLBCL	Overall Survival	GELA (1998-2000)	Jais	HG-U133A	202763_at	53	0.53	0.073	0.079	1.95 [0.93 - 4.10]
E-TABM-346	Blood cancer	DLBCL	Event Free Survival	GELA (1998-2000)	Jais	HG-U133A	202763_at	53	0.53	0.081	0.086	1.83 [0.92 - 3.63]
GSE2990	Breast cancer		Distant Metastasis Free Survival	Uppsala, Oxford	Sotiriou	HG-U133A	202763_at	54	0.89	0.206	0.416	24.4 [0.01 - 54017.9]
GSE1378	Breast cancer		Relapse Free Survival	MGH (1987-2000)	Ma	Arcturus 22k	18593	60	0.12	0.027	0.035	2.87 [1.08 - 7.64]
E-TABM-158	Breast cancer		Disease Specific Survival	UCSF, CPMC (1989-1997)	Chin	HG-U133A	202763_at	117	0.22	0.005	0.016	2.54 [1.19- 5.43]
GSE2990	Breast cancer		Relapse Free Survival	Uppsala, Oxford	Sotiriou	HG-U133A	202763_at	62	0.37	0.073	0.082	2.37 [0.90 - 6.27]
E-TABM-158	Breast cancer		Overall Survival	UCSF, CPMC (1989-1997)	Chin	HG-U133A	202763_at	117	0.22	0.013	0.016	2.28 [1.17 - 4.44]
E-TABM-158	Breast cancer		Relapse Free Survival	UCSF, CPMC (1989-1997)	Chin	HG-U133A	202763_at	117	0.22	0.013	0.016	2.28 [1.17 - 4.45]
E-TABM-158	Breast cancer		Distant Metastasis Free Survival	UCSF, CPMC (1989-1997)	Chin	HG-U133A	202763_at	117	0.22	0.118	0.21	1.70 [0.74 - 3.91]
GSE3143	Breast cancer		Overall Survival	Duke	Bild	HG-U95A	36143_at	158	0.62	0.111	0.114	1.65 [0.89 - 3.05]
GSE12945	Colorectal cancer		Disease Free Survival	Berlin	Staub	HG-U133A	202763_at	51	0.12	0.026	0.05	5.97 [1.00 - 35.8]
GSE12945	Colorectal cancer		Overall Survival	Berlin	Staub	HG-U133A	202763_at	62	0.11	0.081	0.097	3.03 [0.82 - 11.2]
GSE14333	Colorectal cancer		Disease Free Survival	Melbourne	Jorissen	HG-U133_Plus_2	202763_at	226	0.12	0.015	0.018	2.31 [115 - 4.62]
GSE17536	Colorectal cancer		Disease Specific Survival	MCC	Smith	HG-U133_Plus_2	202763_at	177	0.54	0.018	0.021	1.97 [1.11 - 3.50]
GSE17536	Colorectal cancer		Disease Free Survival	MCC	Smith	HG-U133_Plus_2	202763_at	145	0.49	0.098	0.021	1.97 [1.11 - 3.50]
GSE11595	Esophagus cancer	Adenocarcinoma	Overall Survival	Sutton	Giddings	CRUKDMF_22K_v 1.0.0	30170	34	0.21	0.020	0.02	2.83 [1.13 - 7.08]
GSE17710	Lung cancer	Squamous cell carcinoma	Overall Survival	UNC	Wilkerson	Agilent-UNC-custom-4X44K	35447	56	0.11	0.011	0.021	4.64 [1.26 - 17.1]
GSE17710	Lung cancer	Squamous cell carcinoma	Overall Survival	UNC	Wilkerson	Agilent-UNC-custom-4X44K	17988	56	0.11	0.011	0.021	4.64 [1.26 - 17.1]
GSE17710	Lung cancer	Squamous cell carcinoma	Relapse Free Survival	UNC	Wilkerson	Agilent-UNC-custom-4X44K	17988	56	0.11	0.029	0.042	3.80 [1.05 - 13.8]
GSE17710	Lung cancer	Squamous cell carcinoma	Overall Survival	UNC	Wilkerson	Agilent-UNC-custom-4X44K	2046	56	0.11	0.051	0.061	2.80 [0.95 - 8.23]
GSE17710	Lung cancer	Squamous cell carcinoma	Relapse Free Survival	UNC	Wilkerson	Agilent-UNC-custom-4X44K	35447	56	0.32	0.008	0.01	2.66 [1.26 - 5.61]
MICHIGAN-LC	Lung cancer	Adenocarcinoma	Overall Survival	Michigan (1994-2000)	Beer	HuGeneFL	U13737_at	86	0.17	0.095	0.103	2.19 [0.85 - 5.62]
GSE14814	Lung cancer	NSCLC	Disease Specific Survival	JRB.10	Zhu	HG-U133A	202763_at	90	0.39	0.052	0.05	2.03 [0.98 - 4.22]
GSE17710	Lung cancer	Squamous cell carcinoma	Relapse Free Survival	UNC	Wilkerson	Agilent-UNC-custom-4X44K	2046	56	0.21	0.083	0.088	1.95 [0.90 - 4.22]
jacob-00182-CANDF	Lung cancer	Adenocarcinoma	Overall Survival	CAN/DF	Shedden	HG-U133A	202763_at	82	0.29	0.059	0.065	1.89 [0.96 - 3.73]
GSE13213	Lung cancer	Adenocarcinoma	Overall Survival	Nagoya (1995-1999, 2002-2004)	Tomida	G4112F	A_23_P92410	117	0.1	0.128	0.134	1.79 [0.84 - 3.83]
GSE4573	Lung cancer	Squamous cell carcinoma	Overall Survival	Michigan (1991-2002)	Raponi	HG-U133A	202763_at	129	0.19	0.036	0.039	1.79 [1.03 - 3.12]
GSE14814	Lung cancer	NSCLC	Overall Survival	JRB.10	Zhu	HG-U133A	202763_at	90	0.39	0.087	0.091	1.73 [0.92 - 3.26]
GSE3141	Lung cancer	NSCLC	Overall Survival	Duke	Bild	HG-U133_Plus_2	202763_at	111	0.61	0.087	0.09	1.62 [0.93 - 2.82]
GSE8894	Lung cancer	NSCLC	Relapse Free Survival	Seoul (1995-2005)	Lee	HG-U133_Plus_2	202763_at	138	0.83	0.199	0.204	1.58 [0.78 - 3.18]
jacob-00182-UM	Lung cancer	Adenocarcinoma	Overall Survival	UM	Shedden	HG-U133A	202763_at	178	0.5	0.128	0.129	1.35 [0.92 - 1.99]
GSE17260	Ovarian cancer		Overall Survival	Nigata (1997-2008)	Yoshihara	G4112A	A_23_P92410	110	0.14	0.043	0.05	2.18 [1.00 - 4.74]
DUKE-OC	Ovarian cancer		Overall Survival	Duke	Bild	HG-U133A	202763_at	133	0.14	0.059	0.064	1.74 [0.97 - 3.13]
GSE26712	Ovarian cancer		Overall Survival	MSKCC (1990-2003)	Bonome	HG-U133_Plus_2	202763_at	185	0.31	0.054	0.05	1.43 [0.99 - 2.06]
GSE9891	Ovarian cancer		Overall Survival	AOCS, RBH, WH, NKI-AVL (1992-2006)	Tothill	HG-U133_Plus_2	202763_at	278	0.34	0.075	0.078	1.41 [0.96 - 2.07]
GSE26712	Ovarian cancer		Disease Free Survival	MSKCC (1990-2003)	Bonome	HG-U133_Plus_2	202763_at	185	0.31	0.049	0.05	1.40 [1.00 - 1.97]
E-DKFZ-1	Renal cell carcinoma		Overall Survival	RZPD	Sueltmann	A-RZPD-20	rzpd.de:huber1:Reporter:IMAGE:49729	59	0.42	0.150	0.253	1.75 [0.67 - 4.54]

Supplementary Table 1: PrognoScan analysis of CASP3/DR towards multiple cancers.

Protein ID	Protein Name	M.W.	Mascot Score	Biochemical Function/ Intracellular Distribution
gi|152032648	Phosphate-binding protein	38.51	145	Apolipoprotein/Cytoplasm
gi|1346343	Keratin	65.98	113	Cytoskeleton/Cytoplasm
gi|4505773	Prohibitin	29.79	77	Tumor suppressor/Cytoplasm
gi|950004	Citrate transporter protein	34.06	59	Citrate carrier/Mitochondria
gi|113459	ADP/ATP translocase 2	32.87	51	ADP/ATP carrier/Mitochondria

Supplementary Table 2: Mass spectrometric identification of I-Lys-biotin-labeled proteins other than caspase-7 in MCF-7 cells.

CASP3(low)/p19/p12-casp7(high) vs. Others	Recurrence Probability	95%CI [low – high]	Sig.	Hazard Ratios	95%CI [low – high]	Sig.
Breast cancer (n = 92)	5.270	1.519 – 18.284	0.009	10.389	2.199 – 49.084	0.003
Lung cancer (n = 58)	3.944	1.763 – 8.819	0.001	3.368	1.677 – 6.764	0.001
Colon cancer (n = 159)	1.412	0.733 – 2.720	0.302	1.776	1.008 – 3.129	0.047

Supplementary Table 3: Multivariate analyses of risk for clinical patients with CASP3/DR lung or colon cancers. Categorical variables, including CASP3(low)/p19/p12-casp7(high), age, grade status and TNM stage, were modeled via a Cox regression test. Statistical significance for each of these variables was analyzed relative to disease-free survival.