Supplementary Data

Supplementary Materials and Methods

Animal Studies

For pIpC injections, baseline labs were drawn when mice reached 6 weeks of age. Treatment with pIpC (1 mg/mL in PBS; Amersham/Pharmacia) was initiated on 7 week-old mice. Mice were treated every other day with a total of 5 intraperitoneal injections (20mg/kg). At 16 weeks post-treatment, mice were sacrificed. Peripheral blood, spleen, thymus, and bone marrow were harvested and analyzed. PCR genotyping of Taspase1 has been described. MMTV-neu and MMTV-wnt mice were treated intravenously with NSC48300 or vehicle (30% propylene glycol and 5% Tween-80 in D5W, pH 4.65). Tumors were measured in two dimensions and volume was calculated as described (1). U251 glioblastoma cells transduced with GPF-luciferase expressing retroviruses were selected for 3 days in puromycin (1.5μg/mL). Cells were then harvested and resuspended in RPMI 1640 in a 2:1 ratio with growth factor reduced Matrigel (BD Biosciences, San Jose, CA). One million cells were engrafted into each flank of male NOD-*scid* *IL2Rγ*$^{-/-}$ mice (Jackson Lab) between 6-8 weeks of age. NSC48300 was administered every other day starting 1 day after implantation.

Compounds

The NCI Diversity Set was kindly provided by the NCI DTP. NSC48300 analogues were provided by the NCI DTP. The Ac-ISQLD-cmk and Ac-ISQLD-aldehyde were generated by the Peptides International (Louisville, KY) and Anaspec (Freemont, CA), respectively. All compounds were dissolved in DMSO. Arsenic acid sodium salt was purchased from MP Biomedicals (Solon, OH).

IVTT-based and FRET-based in vitro cleavage assays

The IVTT-based in vitro cleavage assays were performed as previously described (2). To assess the enzymatic activity of Taspase1, a FRET-based proteolytic reporter (FRPR) was generated (Tufts University Core Facility, Boston, MA). Reactions were conducted in reaction buffer (100mM HEPES, pH 7.9, 10% sucrose, 1mM DTT). To study inhibitors, rTaspase1 was pre-incubated with the indicated compounds for 30 minutes at room temperature. To initiate cleavage reaction, FRPR was added (final concentration at 15μM). Taspase1 activity was monitored by recording the accumulated, emitted fluorescence signal ($\lambda_{excitation}$ = 328nm, slit = 10nm and $\lambda_{emission}$ = 393nm, slit = 10nm) over time. Assays were performed with an LS55 (Perkin Elmer, Inc., Waltham, MA) fluorescence spectrometer in a 96-well plate format. Steady state rates were determined for each reaction. K_M and K_I were determined using Prism software (GraphPad Software, Inc., La Jolla, CA), assuming Michaelis-Menten kinetics.

Toxicity studies

Male C57/Bl6 mice, 6-8 weeks old, were treated with vehicle or NSC48300 at 2.5mg/kg every other day for a total of 5 intravenous doses. Mice were sacrificed for tissue and blood harvest one day after the last treatment. Tissues were subjected histological examination. CBC and serum chemistry were analyzed by Hemavet and Research Animal Diagnostic Lab (Washington University School of Medicine), respectively.

References:

1. Chen DY, Liu H, Takeda S, Tu HC, Sasagawa S, Van Tine BA, et al. Taspase1 functions as a non-oncogene addiction protease that coordinates cancer cell proliferation and apoptosis. Cancer Res. 2010;70:5358-67.
2. Hsieh JJ, Cheng EH, Korsmeyer SJ. Taspase1: a threonine aspartase required for cleavage of MLL and proper HOX gene expression. Cell. 2003;115:293-303.

Supplementary Figures and Tables

Figure S1. The inhibition of Taspase1 by ISQLD peptidomimetics and Bortezomib. A, Ac-KISQLD-aldehyde and Ac-ISQLD-cmk have minimal activity against Taspase1. rTaspase1 was incubated with the indicated concentrations of either Ac-KISQLD-aldehyde (top) or Ac-ISQLD-cmk (bottom) for 30 minutes at room temperature. The IVTT, ^{35}S-methionine labeled, p75MLL cleavage reporter was then added to the enzyme (5ng rTaspase1)/inhibitor mix for 30 minutes at 30°C. The resulting cleavage of p75MLL was resolved by SDS-PAGE and monitored by autoradiography. B, Bortezomib has no activity against Taspase1. rTaspase1 was incubated with the indicated concentrations of Bortezomib for 30 minutes at room temperature. The IVTT, ^{35}S methionine labeled, p75MLL cleavage reporter was then added to the enzyme (5ng rTaspase1)/Bortezomib mix for 30 minutes at 30°C. The resulting cleavage was analyzed as described in A.

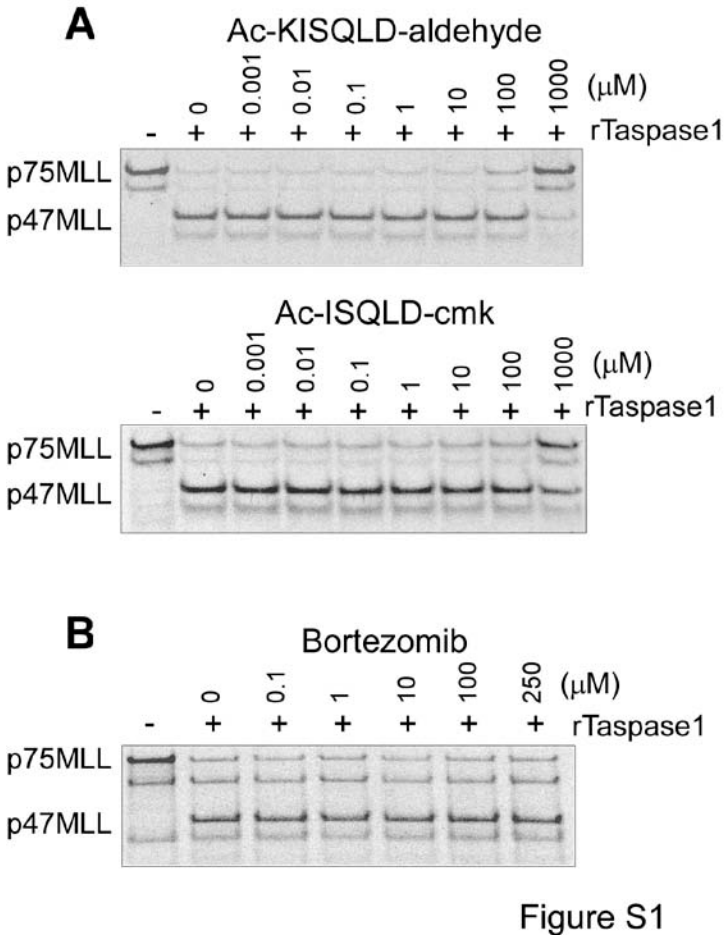

Figure S1

Figure S2. Optimization of rCaspase8-mediated in vitro cleavage of p22Bid. The indicated amounts of rCaspase8 were incubated with IVTT, ^{35}S-methionine labeled, p22Bid cleavage reporter for the indicated periods of time at 30°C. The resulting cleavage of p22Bid was resolved by SDS-PAGE and monitored by autoradiography.

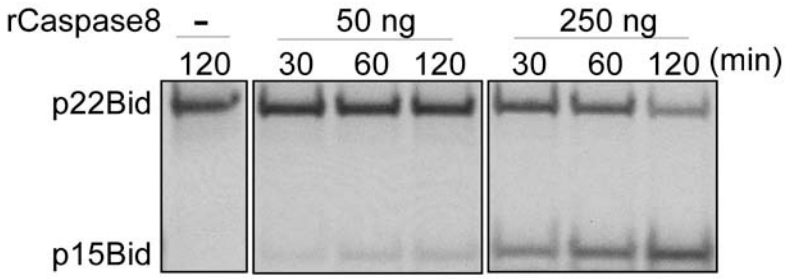

Figure S2

Figure S3. HTI-9 (ISQLAGVDD) is a competitive inhibitor of Taspase1. A, rTaspase1 was incubated with the indicated concentrations of HTI-9 for 30 minutes at room temperature. The IVTT, ^{35}S-methionine labeled, p75MLL cleavage reporter was then added to the enzyme (5ng rTaspase1)/HTI-9 mix for 30 minutes at 30°C. The resulting cleavage of p75MLL was resolved by SDS-PAGE and monitored by autoradiography. B, Increasing concentration of FRPR was incubated with 100 nM of rTaspase1 that was pretreated with the indicated concentrations of HTI-9 for 30 minutes at room temperature. The enzymatic cleavage was performed at 30°C and activity monitored by an LS55 spectrofluorometer. Data presented are mean ± SD of three independent experiments.

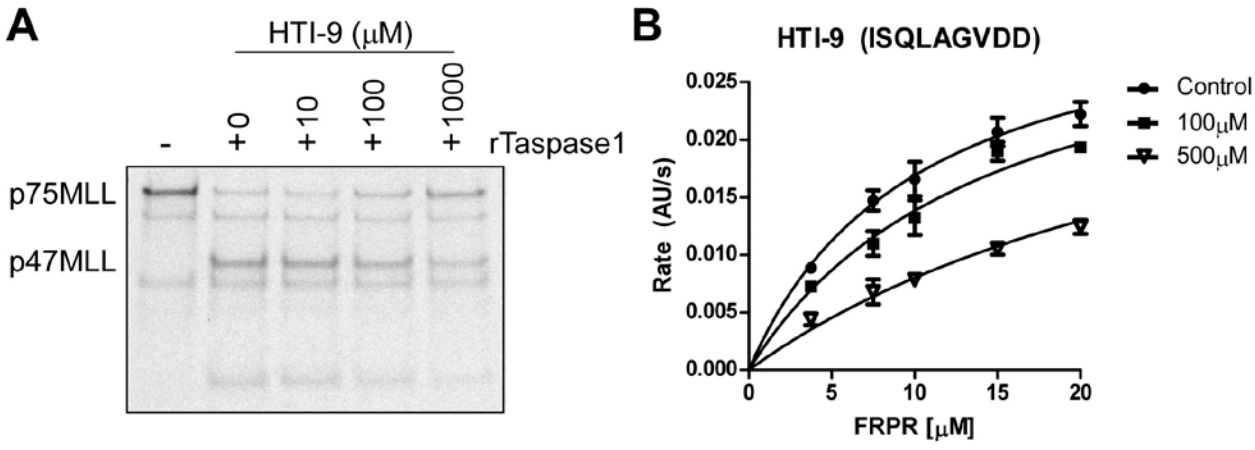

Figure S3

Figure S4. Precursor Taspase1 undergoes intramolecular but not intermolecular autoproteolysis to generate mature enzyme. IVTT, ^{35}S-methionine labeled, precursor Taspase1 (p50T1α-β) was incubated with the indicated amounts of rTaspase1 for 30 minutes at 30°C. The resulting cleavage was resolved by SDS-PAGE and monitored by autoradiography.

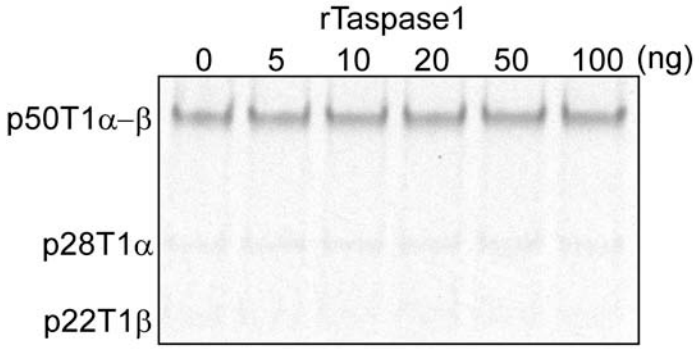

Figure S4

Figure S5. Histological examination of the indicated organs after treatment of NSC48300. Mice were treated with 2.5mg/kg of NSC48300 every other day through intravenous injections for a total of 5 doses. Organs were harvested one day after the last treatment, formalin-fixed, paraffin-embedded, sectioned, and stained by H&E. Scale bar equals 200μm.

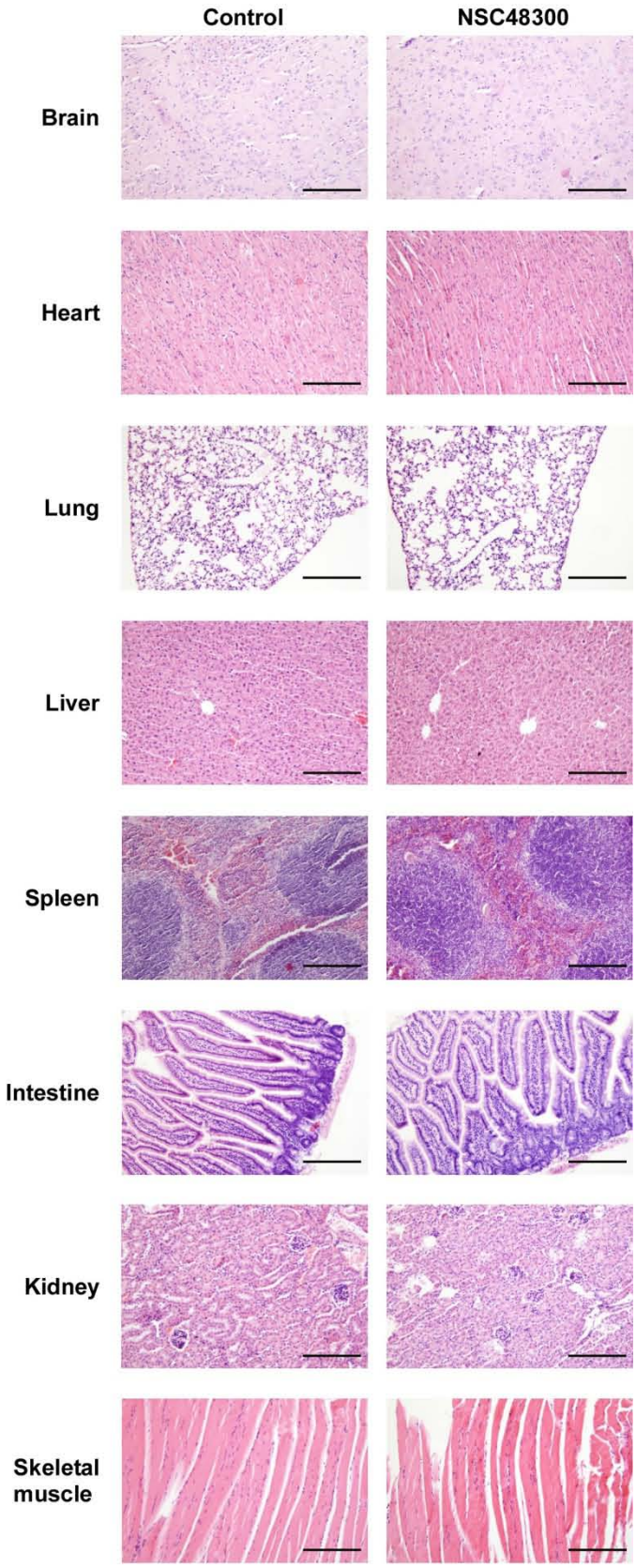
Control
NSC48300
Brain
Heart
Lung
Liver
Spleen
Intestine
Kidney
Skeletal muscle

Figure S5

Figure S6. Hematological profiles after treatment with NSC48300. Mice were subjected to the same treatment as outlined in Figure S5. Blood was collected one day after last treatment and subjected to analysis using HEMAVET. Data are mean ± SD of three animals in each arm. *, $P < 0.05$. NSC denotes NSC48300.

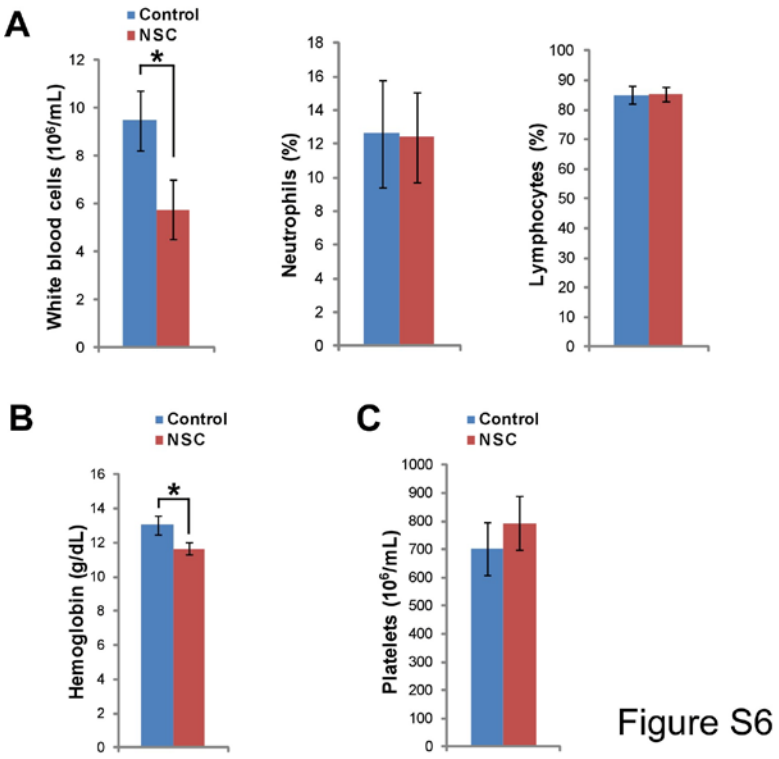

Figure S7. Knockdown of Taspase1 in the indicated breast cancer cell lines. The protein levels were determined by Western blots using the indicated antibodies.

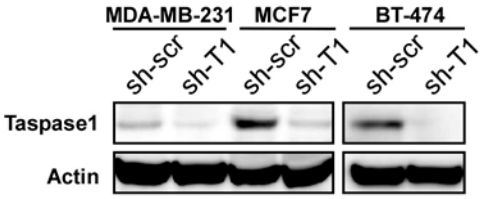

Figure S7

Table S1. Identities of compounds #1-#7 tested in Figure 3.

	PLATEKEY	PLATE	SUFFIX	WELLID	WELLNBR	NSC
#1	3847048	3847	48	G07	79	7215
#2	3855048	3855	48	A08	8	339585
#3	3859048	3859	48	G02	74	163443
#4	3860059	3860	59	A02	2	48300
#5	3863048	3863	48	G11	83	118176
#6	3865048	3865	48	B05	17	172033
#7	3865048	3865	48	H08	92	402959

Table S1

Table S2. Summary of Taspase1 protein expression and the NSC48300 sensitivity based on published and publically available databases.

Cell line	GD2006 T1/Actin	NSC48300 10-09		
		log GI50	log TGI	log LC50
BR:HS578T	1.05	-5.876	-4.908	-4
BR:BT_549	1.35	-5.928	-5.51	-5.092
BR:MDA_MB_231	1.39	-5.996	-5.639	-5.297
BR:T47D	4.58	-6.506	-5.727	-4.625
BR:MCF7	5.72	-6.546	-5.925	-5.443
CNS:SF_295	1.30	-5.556	-5.073	-4.443
CNS:SF_268	1.69	-6.364	-5.732	-5.192
CNS:SNB_75	1.77	-5.874	-5.507	-5.139
CNS:SF_539	2.34	-6.715	-6.266	-5.795
CNS:SNB_19	3.97	-5.939	-5.578	-5.216
CNS:U251	4.38	-6.431	-5.82	-5.407
CO:HCT_15	3.37	-5.932	-5.54	-5.103
CO:KM12	4.13	-5.719	-5.264	-4.619
CO:COLO205	4.34	-6.21	-5.722	-5.359
CO:SW_620	4.38	-6.569	-5.662	-4.169
CO:HT29	4.45	-6.109	-5.623	-5.19
CO:HCT_116	6.41	-6.048	-5.671	-5.335
CO:HCC_2998	7.50	-5.719	-5.434	-5.149
LE:K_562	0.20	-6.576	-5.434	-4
LE:SR	0.91	-6.57	-5.967	-4
LE:CCRF_CEM	1.96	-7.09	-5.978	-4
LE:HL_60	4.34	-5.963	-5.4	-4
LE:RPMI_8226	5.23	-6.546	-6.007	-4
LE:MOLT_4	6.07	-6.34	-5.371	-4
ME:LOXIMVI	3.23	-6.474	-5.85	-5.373
ME:UACC_257	3.61	-6.199	-5.887	-5.431
ME:MDA_MB_435	4.00	-5.892	-5.625	-5.099
ME:MALME_3M	4.46	-5.922	-5.582	-5.242
ME:SK_MEL_5	5.50	-5.878	-5.724	-5.352
ME:M14	6.99	-5.975	-5.609	-5.268
ME:UACC_62	9.37	-6.302	-5.581	-5.28
ME:SK_MEL_28	9.86	-5.871	-5.535	-5.191
ME:SK_MEL_2	15.35	-6.239	-5.542	-5.213
ME:MDA_N		-6.376	-5.783	-5.328
LC:NCI_H226	1.29	-5.422	-5.114	-4.591
LC:HOP_92	1.71	-5.823	-5.535	-5.246
LC:NCI_H23	2.66	-6.113	-5.646	-5.241
LC:NCI_H522	3.46	-6.378	-5.841	-5.346
LC:HOP_62	4.47	-5.883	-5.444	-4.99
LC:EKVX	5.00	-5.946	-5.541	-5.163
LC:NCI_H322M	7.48	-5.531	-5.022	-4.51
LC:A549	8.05	-5.742	-5.419	-5.096
LC:NCI_H460	8.41	-6.041	-5.614	-5.239
OV:IGROV1	1.72	-6.16	-5.711	-5.309
OV:NCI_ADR_RES	2.03	-5.831	-5.168	-4
OV:OVCAR_5	2.41	-5.631	-5.332	-5.02
OV:SK_OV_3	2.42	-5.668	-5.208	-4.789
OV:OVCAR_8	3.49	-6.446	-5.871	-5.397
OV:OVCAR_4	6.49	-5.722	-5.127	-4.358
OV:OVCAR_3	6.70	-6.076	-5.578	-5.107
PR:DU_145	3.80	-6.474	-5.932	-5.396
PR:PC_3	4.04	-6.263	-5.755	-5.376
RE:A498	2.84	-5.674	-5.402	-5.131
RE:786_0	3.25	-6.449	-5.889	-5.442
RE:RXF_393	3.48	-6.384	-5.815	-5.327
RE:SN12C	4.25	-6.194	-5.681	-5.238
RE:UO_31	4.45	-5.907	-5.58	-5.254
RE:CAKI_1	4.72	-6.02	-5.555	-5.065
RE:TK_10	5.28	-6.262	-5.895	-5.463
RE:ACHN	5.90	-5.986	-5.636	-5.318

Table S2

Table S3. Serum chemistry of mice post-NSC48300 treatment. Mice were subjected to the same treatment as outlined in Figure S5. Serum was collected one day after last treatment and subjected to analysis. Data are mean and (SD) of three animals from each arm. NSC denotes NSC48300.

	GLU	BUN	CREAT	LDH	AMYL	ALP	ALT	AST	T. BILI
	mg/dL	mg/dL	mg/dL	U/L	U/L	U/L	U/L	U/L	mg/dL
Control	464.0 (110.9)	24.0 (5.3)	0.23 (0.1)	2470.0 (887.6)	4280.5 (1034.5)	199.3 (70.0)	79.3 (24.3)	213.3 (35.9)	0.9 (0.3)
2.5mg/kg (NSC)	408.7 (58.1)	25.0 (7.2)	0.30 (0)	1145.0 (367.0)	4645.7 (1033.9)	228.7 (37.6)	109.67 (51.4)	157.67 (94.0)	1.0 (0.1)
p-value	0.508	0.885	0.184	0.048	0.816	0.355	0.201	0.522	0.580
	Na+	K+	Cl-						
	mmol/L	mmol/L	mmol/L						
Control	148.7 (8.6)	11.4 (3.4)	117.3 (2.5)						
2.5mg/kg (NSC)	131.3 (5.1)	8.0 (0.8)	103.7 (4.0)						
p-value	0.156	0.187	0.053						

Table S3