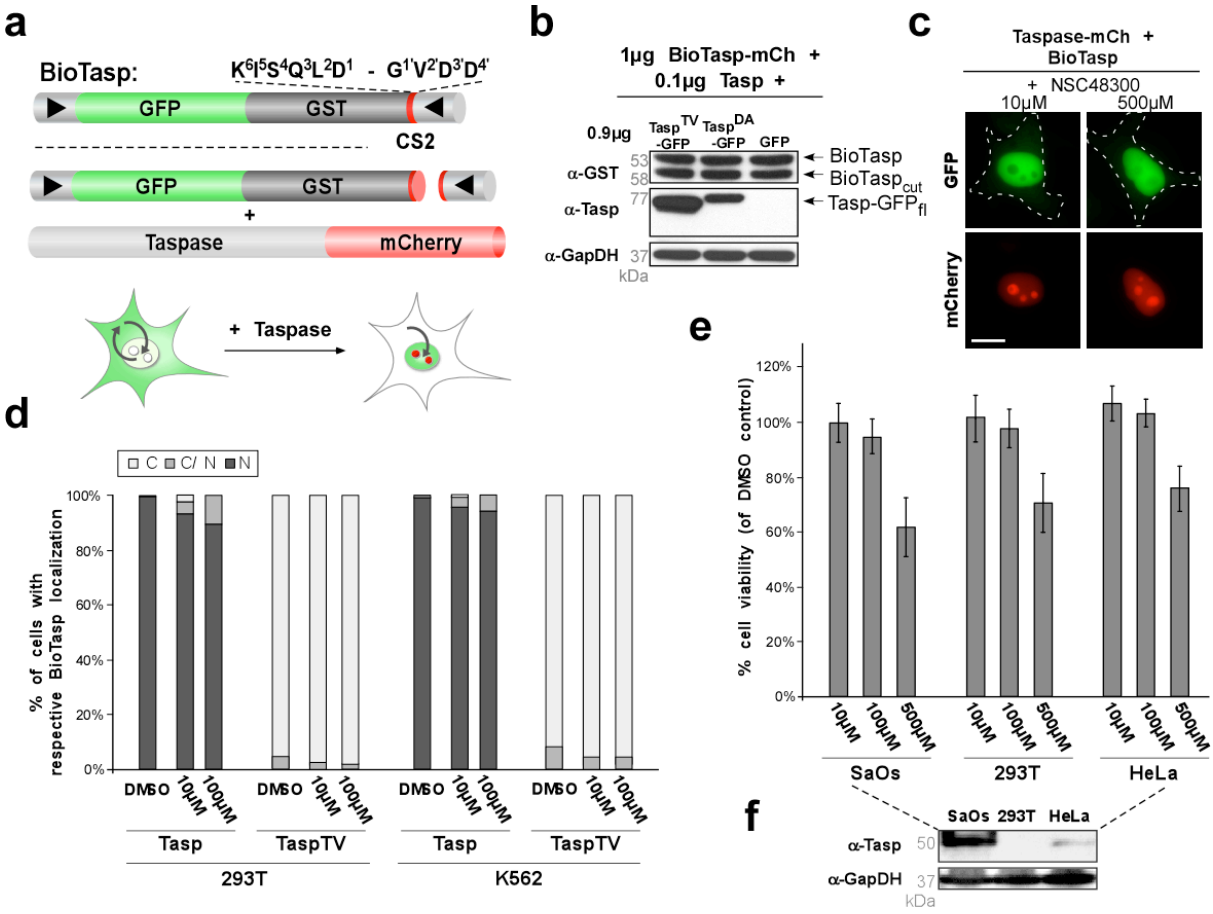
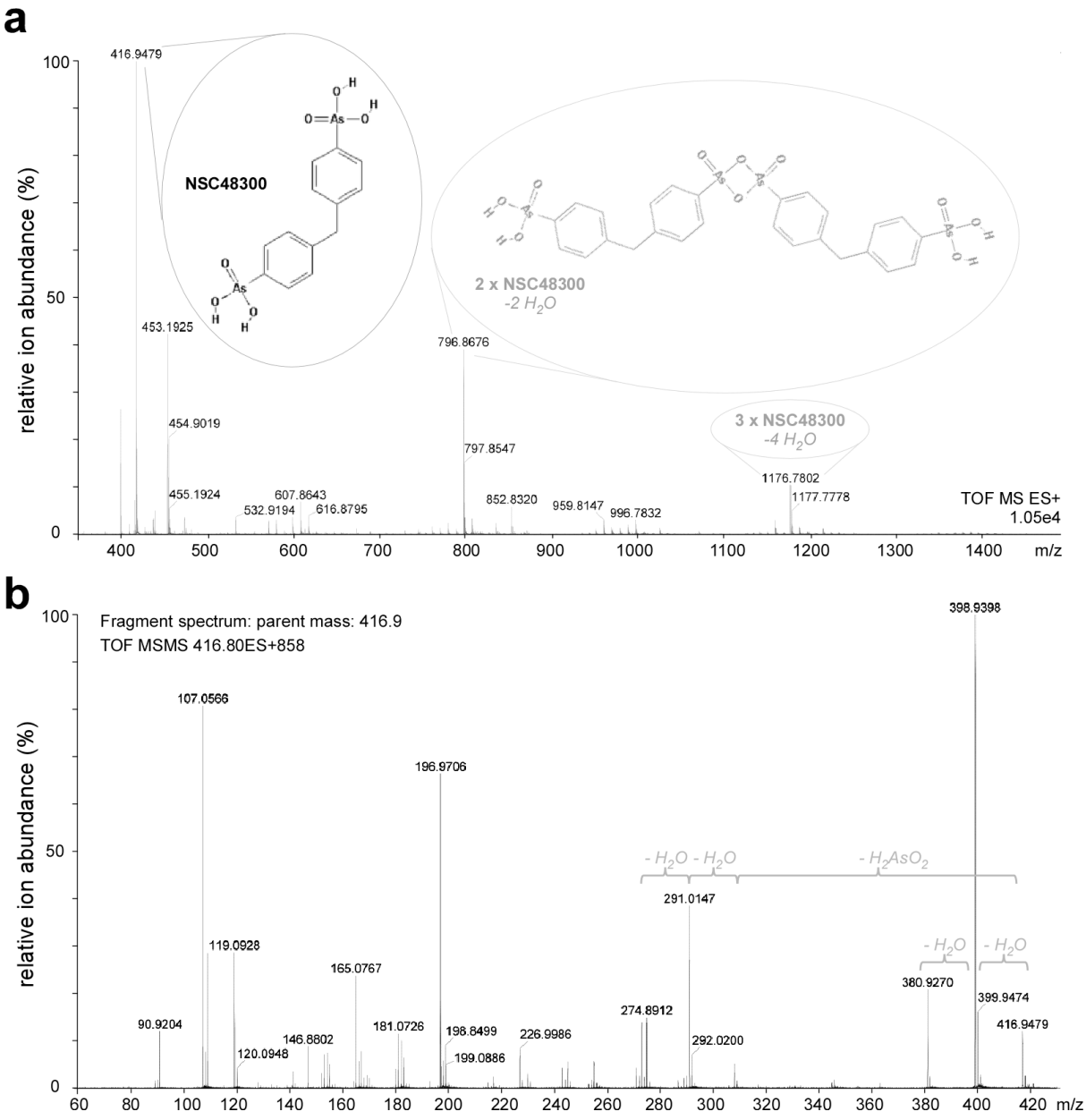
SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURES



SUPPLEMENTARY FIGURE 1. (a) Principle and schematic domain organization of the Taspase1 biosensor assay. BioTasp is composed of GST, GFP, combinations of a nuclear import (NLS) and an export signal (NES), combined with the Taspase1 cleavage site from AF4•MLL. BioTasp localizes predominantly to the cytoplasm but is continuously shuttling between the nucleus and the cytoplasm. Co-expression of Taspase1-mCherry (Tasp-mCherry) results in the removal of the NES by Taspase1-induced cleavage, triggering nuclear accumulation of the green fluorescent indicator protein. **(b)** Immunoblot analysis demonstrates that Tasp^{T234V}- and Tasp^{D233A}-GFP fusions are neither autocatalytically cleaved nor affect wt Taspase1 *trans*-cleavage activity. Proteins were visualized using α-GST and α-Taspase1 Ab. GapDH served as loading control. (**c)** NSC48300 does not inhibit Taspase1's *trans*-cleavage activity. HeLa transfectants coexpressing green fluorescent BioTasp$_G$ and red fluorescent (mCh) Taspase1 were treated with NSC48300, and analyzed 48h later. Representative examples are shown. Scale bars, 10µm. (**d)** Quantitation of BioTasp$_G$ processing. Indicated cell lines coexpressing green fluorescent BioTasp$_G$ and red fluorescent (mCh) wild type or inactive Tasp^{T234V} were treated with DMSO or NSC48300 (10µM or

100μM final concentration), and analyzed 48h later. *Trans*-cleavage induced nuclear translocation of BioTasp$_G$ was not affected by NSC48300 293T or leukemic K562 cells. The number of cells showing cytoplasmic (C), cytoplasmic and nuclear (N/C) or nuclear (N) fluorescence was counted in at least 200 BioTasp$_G$-expressing cells. Results from a representative experiment are shown. Similar results were obtained in three independent experiments. (**e)** Effect of NSC48300 on cell viability does not correlate with Taspase1 expression levels. Indicated cell lines were treated with DMSO or NSC48300, and analyzed 48h later using the Alamar Blue Assay. Columns, mean; bars, ±SD from three independent experiments. DMSO treated cells were set 100%. . (**f)** Endogenous Taspase1 levels were analysed by immunoblot using α-Tasp and -GapDH Abs.



SUPPLEMENTARY FIGURE 2. Mass spectrometry of NSC48300 confirmed compound integrity. The *y* axis measures MS-intensity as a per cent of the most intense peak. *m*/*z*, mass-to-charge ratio. **(a)** Survey spectra of NSC48300 were acquired over a mass range from 300–1,500 Da. The peak (m/z=416.9) corresponding to $(NSC48300+H)^+$ was most abundant, peaks at m/z 796.9 and 1176.8 could be attributed to dimerization/trimerization products accompagnied by loss of $2xH_2O$ (putative structure shown as insert). **(b)** the peak at 416.9 was manually selected for fragmentation in MS/MS mode. Observed fragment ions could be attributed to loss of H_2O and arsinate (H_2AsO_2), (as observed for ESI+ fragment spectra of 3-amino-HPAA by Wershaw et al. (PMID 18969012) further confirming identity of NSC48300. NSC48300 was analysed by ESI-Q-TOF mass spectrometry, representative spectra are shown.

SUPPLEMENTARY TABLES

SUPPLEMENTARY TABLE 1. NSC48300 does not affect nucleo-cytoplasmic transport.

Substrate	t=0		t=20 min		t=40min		t=120min	
Localization	**C**	**N**	**C**	**N**	**C**	**N**	**C**	**N**
GST-TaspNLS-GFP, DMSO	100%	0%	5%	95%	5%	95%	5%	95%
GST-TaspNLS-GFP, NSC48300 (100µM)	100%	0%	4%	96%	4%	96%	4%	96%
GST-SurvNES-GFP, DMSO	0%	100%	54%	56%	95%	5%	98%	2%
GST-SurvNES-GFP, NSC48300 (100µM)	0%	100%	58%	52%	93%	7%	95%	5%

Equal amounts of the indicated recombinant GST-GFP fusion proteins ([2mg/ml]) were microinjected into the cytoplasm of HeLa cells and transport documented in 100 cells by live cell fluorescence microscopy at the indicated time points. Neither nuclear import (GST-TaspNLS-GFP: GST-GFP fusion containing the Taspase1 bipartite nuclear import signal (1)) nor nuclear export (GST-SurvNES-GFP: GST-GFP fusion containing the Survivin nuclear export signal was affected by NSC48300. Cells were treated with NSC48300 (100µM final concentration) 1 h prior to injection and NSC48300 was present throughout the experiment.

SUPPLEMENTARY METHODS

Antibodies (Ab) and NSC48300.

Ab used: α-GapDH (sc-47724; Santa Cruz Biotechnology, Heidelberg, Germany); α-GFP (sc-8334; Santa Cruz Biotechnology, Heidelberg, Germany); α-GST (sc-57753; Santa Cruz Biotechnology, Heidelberg, Germany); α-Taspase1 (directed against the C-terminus, AP1330b BioCat GmbH, Heidelberg, Germany. Appropriate HRP-conjugated secondary antibodies (Santa Cruz Biotechnology, Heidelberg, Germany) were used. Reagents were from Sigma Aldrich (Sigma Aldrich, Munich, Germany) unless stated otherwise. NSC48300 (4-[(4-arsonophenyl)methyl]phenyl] arsonic acid; ChemSpider ID: 210748; $C_{13}H_{14}As_2O_6$; Monoisotopic mass: 415.9 Da) was kindly provided by the NCI *Developmental Therapeutics Program*.

Plasmids.

The red- or green-autofluorescent Taspase1 biosensors ($BioTasp_R$ or $BioTasp_G$) are composed of a NLS, GST, the Taspase1 cleavage site from AF4•MLL, a NES and GFP or mCherry (mCh), respectively, and have been described (1, 2). Expression constructs encoding wild type or inactive Taspase1 variants as untagged or fusions with autofluorescent proteins were described (1, 2).

Cells, transfection, microscopy and measurement of cell viability.

Liquid and adherent cancer cell lines were maintained and transfected as described (1-3). Observation, image analysis and quantitation of living or fixed cells were performed as described in detail (1, 2). Assessment of cell viability was performed using the Alamar Blue Assay (Biozol, Esching, Germany) as described (4).

Protein extraction and immunoblot analysis.

Preparation of whole lysates from cells and immunoblotting were carried out as described (1, 4).

Mass spectrometry analysis.

Identity of NSC48300 was verified by nano-electrospray-ionization quadrupole time-of-flight (nanoESI-QTOF) mass spectrometry as described (5). Briefly, NSC43800 was dissolved in 50%ACN, 0.1%FA at a concentration of 0.1 mg/ml and applied to a gold-coated glass capillary (TypeC nanoflow needle, Waters) and introduced into the nano-ESI source using a capillary voltage of 800-1000V, resulting in a spray of about 50nl min^{-1}. Mass detection was performed on a hybrid orthogonal acceleration QTOF MS/MS (Q-TOF Premier, Waters)

equipped with an ESI interface and operated in a positive electrospray ionization mode. The mass spectrometer was operated in V-mode and tuned to a resolution of 10.000 full-width at half-maximum (FWHM) for singly charged ions. Prominent peaks in the survey mass spectrometry spectrum were manually selected for fragmentation in MS/MS mode using collision energies between 20-40eV. The mass spectrometer was calibrated using a [Glu^1]fibrinopeptide solution (300fmol/µL) according to (5).

In silico molecular docking.

The X-ray structure of the processed Taspase1 dimer (PDB: 2A8J, chain A) (6) served as as a docking target for NSC48300. The mol-formatted file of NSC48300 was translated to PDB format using *Ballview* and charges as well as non-polar hydrogen atoms were added using the *MGLTools 1.5.4*. In the Taspase1 structure, the water molecules were removed prior analysis and docking was performed by *Autodock Vina* with default parameters.

ABBREVIATIONS

α, anti; aa, amino acids; Ab, antibody; AF4•MLL, fusion product of Fused on chromosome4 and Mixed Lineage Leukemia; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; GapDh, Glyceraldehyde-3-phosphate dehydrogenase; DPOLZ, DNA Polymerase Zeta; GFP/BFP/RFP, green/blue/red fluorescent protein; GST, glutathione S-transferase; mCh, mCherry; NES, nuclear export signal; NLS, nuclear import signal; TF2A, Transcription Factor 2A; USF2, upstream stimulatory factor2.

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