Supporting Information

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SI Materials and Methods

Patient Samples. All samples were obtained between June 2010 and May 2013 from the University of California, San Francisco (UCSF) Clinical Laboratories or the San Francisco Veterans Affairs Medical Center Clinical Laboratories. Clinical data, patient diagnoses, treatment regimen, and laboratory values were obtained from the electronic medical record. The sample collection and informed consent protocol was approved by the UCSF Committee on Human Research (Institutional Review Board). For discovery experiments, blood samples were obtained ∼24 h after initiation of treatment in either citrate or EDTA anticoagulant tubes. For targeted quantitation experiments, plasma samples were collected both before chemotherapy initiation (24–96 h before) as well as within ∼96 h of treatment initiation. After blood collection, plasma was separated by centrifugation at 5,000 × *g* for 5 min and then stored at −80 °C.

N-Terminal Labeling and Sample Preparation for MS. Plasma samples were labeled similar to the method described previously (1). Plasma samples were initially thawed rapidly at 37 °C and then placed on ice. For discovery experiments, 1.5 mL of plasma was used; for targeted selected reaction monitoring (SRM) experiments, either 0.25 or 0.5 mL was used. Normal plasma for control experiments was obtained from Innovative Research. Plasma samples were adjusted to a final concentration of 100 mM bicine (pH 8.5), 1 μM protease inhibitor 4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride (Sigma), 1 mM dithiothreitol, 1 mM synthetic peptide ester with biotin tag and aminobutyric acid label (2), and 1 μM engineered enzyme subtiligase (3). For SRM experiments, spike-in internal standards were added: 50 μg each of bovine catalase (Sigma) and yeast alcohol dehydrogenase (Sigma). Labeling occurred for 1 h at room temperature. After labeling, plasma was desalted using NAP-25 columns (GE Healthcare) to remove unbound peptide ester. Guanidine·HCl was added to the protein-containing eluate to a final concentration of 5.3 M. Cysteines were reduced with 5 mM Tris(2-carboxyethyl)phosphine hydrochloride by boiling for 15 min, and then alkylated by 10 mM iodoacetamide in the dark for 1 h at room temperature. Biotinylated peptides were captured on NeutrAvidin resin (Thermo Scientific) overnight. Beads were washed extensively in 5 M guanidine·HCl followed by on-bead trypsin (Promega) digestion overnight at room temperature with agitation. Captured peptides were released from the beads by incubation with tobacco etch virus protease. Peptides were desalted using C_{18} ZipTips (Millipore), evaporated to dryness, and stored at −80 °C.

Dried peptides from discovery samples only were resuspended in 0.1% formic acid and separated into 10 fractions via reverse-phase high-pH fractionation using an XBridge C18 column (1.0 × 100 mm, 3.5 μm; Waters) on a Waters 2796 Bioseparations Module HPLC. We used a 70-min gradient at a flow rate of 50 μL/min, with linear increase from 2 to 38% acetonitrile in water and constant 10% 100 mM ammonium bicarbonate (pH 10.5). Fractions were evaporated to dryness and stored at −80 °C.

Unbiased Discovery Mass Spectrometry and Analysis. Peptide fractions from discovery plasma samples were resuspended in 12 μL 0.1% formic acid. Five microliters of each fraction was injected onto an Eksigent NLC-1DV-500 HPLC with a 75 μm × 15 cm in-house packed C18 column. This column was directly in-line with a QSTAR Elite quadrupole time-of-flight (AB SCIEX) mass spectrometer. Reverse-phase separation was performed in buffer A (0.1% formic acid in water) and buffer B (0.1% formic acid in acetonitrile) at a flow rate of 250 nL/min. The gradient extended from 2 to 30% B over 70 min, followed by a column wash to 60% B over 5 min, and then re-equilibration at 2% B for a total run time of 90 min.

Data-dependent acquisition was performed using Analyst version 2.0 software (AB SCIEX) across an *m/z* range of 310–1,400 with an isolation window of 100 mDa for ions selected for MS/MS with a dynamic exclusion window of 60 s after acquisition. The top two most intense ions in each MS survey scan (MS1) were chosen for fragmentation and sequencing.

Raw data from unbiased MS experiments were processed using Protein Prospector (version 5.9) (University of California, San Francisco). All spectra were searched using the full human SwissProt database (downloaded March 21, 2012 from www.uniprot.org/downloads) with the reverse sequence database for false-discovery rate determination. Search parameters included fixed modification N-terminal aminobutyric acid and cysteine carbamidomethylation; variable modifications methionine-loss (N terminus) and methionine oxidation; up to two missed tryptic cleavages; C-terminal trypsin cleavage; nonspecific cleavage N terminus; parent mass tolerance 150 ppm; and fragment mass tolerance 250 ppm. Expectation value cutoff was adjusted to maintain a <1% false-discovery rate at the peptide level in each sample.

Targeted Discovery Mass Spectrometry and Analysis. For targeted discovery mass spectrometry, we first built a list of 672 peptides of interest for incorporation into a peptide inclusion list as described in the main text. These peptides are listed in Dataset S3. Based on peptide sequence and the Abu residue at the N terminus (as a result of subtiligase labeling), we predicted the monoisotopic *m/z* for each peptide at charge state 2+ and 3+. We included this list of *m/z* values as a parent mass list within Thermo Scientific Xcalibur software controlling a Thermo Scientific LTQ Orbitrap Velos mass spectrometer.

We injected 5 μL of each peptide fraction (remaining material from the same samples as used above for the QSTAR Elite experiments) onto a Waters nanoACQUITY HPLC instrument coupled in-line to the LTQ Orbitrap Velos. Injected samples were first trapped on a Symmetry C18 column (0.18 × 20 mm, 5 μm; Waters) for 5 min at 1% buffer B before starting the gradient. Reverse-phase separation took place on an analytical BEH130 C18 column (0.075 μm × 200 mm, 1.7 μm; Waters) at a flow rate of 600 nL/min. A linear gradient to 30% buffer B was run over 70 min, increased to 50% B over 2 min, and then decreased to 2% B to re-equilibrate the column for a total run time of 90 min. Samples were analyzed at 60,000 resolution in MS1 with collision energy 35 eV over a mass range of 350–1,400 *m/z*. Peptides selected for sequencing were restricted to the top eight most intense ions within 10 ppm of parent masses on the inclusion list. The intensity threshold for MS2 selection was 1,000, the isolation window was 3 Thompson, and the dynamic exclusion after sequencing was 60 s. Peptide fragmentation and sequencing took place in higher-energy collisional dissociation (HCD) mode.

Analysis of raw sequencing data was performed in Protein Prospector as described above, except that parent and fragment mass tolerance were both set to 20 ppm.

Cell-Culture Release of Proteolytic Contents Postchemotherapy. MM1.S and SU-DHL-8 cells were obtained from the American Type Culture Collection, and MOLM-13 was from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Cell lines were cultured in suspension in RPMI-1640 media supplemented with 2 mM L-glutamine, 10% (MM1.S, MOLM-13) or 20% (SU-DHL-8) FBS. Twenty-four hours before treatment,

cells were pelleted and washed twice in RPMI-1640 media without FBS. Cells were divided into culture flasks at ~70% maximal density: 1×10^6 per mL (MM1.S, MOLM-13) or 1.5×10^6 per mL (SU-DHL-8). Forty milliliters of culture was used for separate treatment and control experiments in each cell line. Chemotherapeutics were obtained from Sigma (doxorubicin and cytarabine) and LC Laboratories (bortezomib) and added to the indicated final concentrations to the treatment cell lines. Control cell lines were treated with 0.1% DMSO final concentration. Cell viability was monitored by CellTiter-Glo assay (Promega) and apoptosis induction by Caspase-Glo 3/7 assay (Promega). After either 21-h (MM1.S) or 30-h (SU-DHL-8 and MOLM-13) treatment, cells were pelleted by centrifugation at 4,000 × *g* for 10 min at 4 °C. The supernatant was then syringe-filtered at 0.22 μm followed by high-speed centrifugation at 70,000 × *g* for 1 h at 10 °C to remove any additional cellular fragments or debris. Clarified media were then treated with trichloroacetic acid to a final concentration of 11% (wt/vol) and sodium lauroyl sarcosinate to 0.1% (wt/vol) to precipitate all proteins on ice for 2 h. Precipitated proteins were pelleted by centrifugation at 8,000 × *g* for 15 min at 4 °C. The pelleted proteins were washed twice in ice-cold tetrahydrofuran, air-dried, and frozen at −80 °C.

For subtiligase labeling, precipitated proteins were resuspended in 7.2 M guanidine·HCl, 100 mM bicine (pH 8.5). Cysteines were reduced and alkylated as above, and then the guanidine was removed using PD-10 columns (GE Healthcare). N-terminal labeling was then performed by subtiligase as described above for plasma samples. Unfractionated peptides were analyzed on the QSTAR Elite MS instrument with the same parameters as above for patient samples.

Selected Reaction Monitoring Assay. Crude spot-synthesized peptides were purchased from JPT Peptide Technologies at an estimated scale of ~50 nmol per peptide. Peptides were resuspended in 80% 0.1 M ammonium bicarbonate, 20% acetonitrile to an initial concentration of 1 nmol/μL. Peptides were serially diluted in 0.1% formic acid, mixed, and injected simultaneously at a concentration of ~120 fmol/μL per peptide onto the LTQ Orbitrap Velos instrument. MS analysis parameters were identical to those used above for patient samples.

As described previously (4), we used Skyline software (version 2.1) (5) to generate SRM assays based on the obtained HCD spectra. Briefly, the seven most intense parent–fragment ion pairs (transitions) for each synthetic peptide were exported into a scheduled SRM method (incorporating retention time information) for use on the AB SCIEX QTRAP 5500 triple-quadrupole instrument. The same synthetic peptide mix was injected onto the QTRAP 5500 using identical chromatographic conditions as on the LTQ Orbitrap Velos (identical HPLC instrument, trapping column, analytical column, and gradient conditions). Each transition was monitored with a ±5-min retention time window with a constant duty cycle of 3 s and unit resolution at both the first and third quadrupole (Q1 and Q3). Using this retention time window, a maximum of 258 transitions was monitored concurrently (at 32 min of the gradient). For each synthetic peptide, the four most intense transitions on this initial run were further refined using collision energy optimization. Optimized collision energies and retention times were retained for the final method. For peptides of interest found in plasma that could not be synthesized, we generated SRM transitions as described previously (4), based on data from either N-terminally labeled apoptotic cell lysates or unfractionated plasma from patient AML_001. Using data analysis in Skyline, peptides were selected for further method development based on (*i*) the signal detection (above baseline) of at least five of seven coeluting transitions, (*ii*) a similar rank of order of fragment ion intensities to the HCD spectrum, and (*iii*) a retention time within 5 min of that acquired in the initial HCD spectral library on the LTQ Orbitrap Velos on patient samples. Again, we used collision energy optimization for the four most intense transitions for inclusion in the final SRM assay. Based on these criteria, we were able to develop a final SRM assay for 140 of the 153 peptides of interest from discovery experiments on plasma samples. We also included transitions corresponding to N-terminally labeled peptides from the bovine catalase and yeast alcohol dehydrogenase spike-in standards. All transitions are listed in Dataset S4.

After finalizing this SRM assay, we applied it to unfractionated pre- and postchemotherapy patient samples. Each pre- and posttreatment sample was injected in technical duplicate, except for multiple myeloma samples, where sufficient material was only available for single injections. Column washes consisting of 60% buffer B for 20 min followed by 15 min of re-equilibration at 3% buffer B were performed between pre- and posttherapy samples as well as between patients to avoid bias due to carryover. Data were analyzed in Skyline. Peptides were determined as "identified" if multiple coeluting transitions were detected above baseline noise with retention time and fragment intensity rank order consistent with the library HCD spectrum and SRM data from synthetic peptides and discovery HCD data. We derived an index based on the geometric mean intensity of peptides from the included spike-in proteins and scaled SRM intensity of all peptides in each sample based on the median value of this index. This approach allowed us to normalize for any variable effects of sample preparation or MS conditions. Total peak area of all transitions, normalized by spike-in standards and averaged across technical duplicates, was used to determine fold changes post- vs. prechemotherapy.

All statistical analysis was performed in GraphPad Prism (version 5).

Smac ELISA. Smac sandwich ELISA was purchased from RayBiotech and performed per the manufacturer's protocol. Absolute concentration was determined by the recombinant Smac calibration curve as provided by the manufacturer. Fifty-microliter plasma samples were evaluated in duplicate at 1:2 dilution in assay buffer as recommended by the manufacturer, except for the AML_1 postchemotherapy sample, which required 1:32 dilution for measurement within the standard linear range. A limit of quantification of 1 ng/mL was determined by twofold change in absorbance for twofold increase in recombinant Smac by calibration curve.

Access to Mass Spectrometry Data. Raw spectral data with peptide assignments for unbiased and targeted discovery experiments can be accessed using the MS-Viewer function of Protein Prospector (http://prospector2.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msviewer). The search key corresponding to each dataset is below. It can be entered on the MS-Viewer page under "Search Key" and retrieved by clicking "Get Existing Results." The assigned mass spectrum with all fragment ion matches can be viewed by clicking the hyperlink for each peptide.

Unbiased discovery on QSTAR Elite:
AML_1: ce0rxbywdn
PCL_1: teuii7m5cl
ALL_1: 3knnewq57r
NHL_1: jq2cdpq86t
AML_2: 4qpbf9bnms
Targeted discovery on LTQ Orbitrap Velos:
AML_1: k2l5kudkok
PCL_1: nyhmg1txbp
ALL_1: opfynkybst
NHL_1: u2hymkowb6
AML_2: 25lftt5qcz
Cell-culture samples:
MOLM_13 cytarabine-treated: asmplh9dn7
MOLM_13 control: aqddrpbkeh

SU-DHL-8 doxorubicin-treated: ao55w7zn5q
SU-DHL-8 control: w7bsnxepqq
MM1.S bortezomib-treated: szgejs39z4
MM1.S control: vxbh6cwtrh

For SRM data, we have made publicly available a Skyline (version 2.1) archive. The Skyline document (.sky) along with associated data (.skyd) and spectral library files (.blib) can be accessed at http://wellslab.ucsf.edu/wiita-2014/Full_SRM_method_with_all_patient_data.zip. With all files extracted in a single directory and the .sky file opened in Skyline, this document includes the full SRM method with all finalized transitions, the raw pre- and postchemotherapy data for all 17 patient samples analyzed, a control normal plasma sample, and data for all synthetic peptides used to build the SRM method. Samples are named with the patient identification, "pre-" or "post-" to indicate whether the sample was collected before or after therapy, as well as "1" or "2" to reflect technical duplicate sample injection. Note that in this Skyline document the automatically software-selected peak does not necessarily reflect a peak chosen for quantification; these were manually selected for each sample. Peptides quantified in each sample are listed in Dataset S4. The note attached to each peptide includes the protein name. Peptides noted in red indicate those derived from typically intracellular proteins, those in green are typically plasma proteins, and those in purple are peptides from spike-in standards for intensity normalization.

Instructions on installing and using Skyline can be found at https://skyline.gs.washington.edu/labkey/project/home/software/Skyline/begin.view.

1. Wildes D, Wells JA (2010) Sampling the N-terminal proteome of human blood. *Proc Natl Acad Sci USA* 107(10):4561–4566.
2. Shimbo K, et al. (2012) Quantitative profiling of caspase-cleaved substrates reveals different drug-induced and cell-type patterns in apoptosis. *Proc Natl Acad Sci USA* 109(31):12432–12437.
3. Mahrus S, et al. (2008) Global sequencing of proteolytic cleavage sites in apoptosis by specific labeling of protein N termini. *Cell* 134(5):866–876.
4. Wiita AP, et al. (2013) Global cellular response to chemotherapy-induced apoptosis. *Elife* 2:e01236.
5. MacLean B, et al. (2010) Skyline: An open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics* 26(7):966–968.

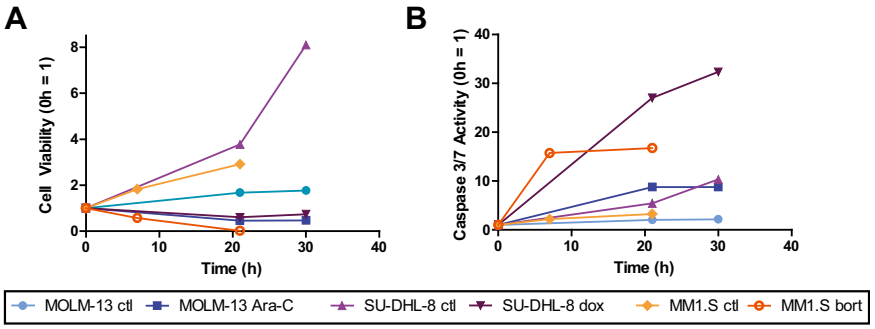

Fig. S1. Cell-culture studies of proteolytic fragment release postchemotherapy. CellTiter-Glo (Promega) assay for cell viability (*A*) and Caspase-Glo 3/7 (Promega) assay to monitor induction of apoptosis (*B*). Cell lines and drugs studied are MOLM-13 (acute myeloma leukemia) treated with 10 μM cytarabine (Ara-C), SU-DHL-8 (diffuse large B-cell lymphoma) treated with 10 μM doxorubicin (dox), and MM1.S (multiple myeloma) treated with 20 nM bortezomib (bort). Results show apoptotic activation under each treatment condition. DMSO (0.1%) was added to control (ctl) conditions. All experiments were in FBS-free media.

Table S1. Additional information on hematologic malignancy patients studied in discovery and targeted MS identification experiments

Patient	AML_1	PCL_1	ALL_1	NHL_1	AML_2
Diagnosis	Acute myeloid leukemia	Plasma cell leukemia	B-acute lymphoblastic leukemia	Diffuse large B-cell lymphoma	Acute myeloid leukemia
Treatment	Clofarabine	Bortezomib, cisplatin, doxorubicin, cyclophosphamide, etoposide	Cyclophosphamide, daunorubicin, vincristine, prednisone	Rituximab, vincristine, cyclophosphamide	Cytarabine, daunorubicin
Malignant circulating cells pretreatment ($\times 10^6$ cells/mL blood)	70.0	16.4	47.5	7.1	29.9
Malignant circulating cells at sample collection ~24 h postinitiation ($\times 10^6$ cells/mL blood)	4.0	7.8	6.2	0.1	10.0
Total no. of unique peptides found by QSTAR Elite discovery	195	124	104	177	110
Total no. of proteins found by QSTAR Elite discovery	100	63	46	78	50
No. of peptides from proteins not found in normal plasma* by QSTAR Elite	60	16	5	35	5
Total no. of unique peptides found by Orbitrap Velos-targeted identification	174	120	115	214	106
Total no. of proteins found by Orbitrap Velos-targeted identification	111	56	55	137	58
No. of peptides from proteins not found in normal plasma* by Orbitrap Velos	75	8	10	94	5

Samples used for labeling were 1.5 mL plasma. "Unique peptides" refers to peptide sequences identified without duplicates or variable modifications.
*Normal plasma proteolytic peptides derived from Wildes and Wells (1).

Table S2. Detailed information on studies of cell-free media from hematologic malignancy cell lines treated with clinically relevant chemotherapeutics

Cell line	MM1.S	MOLM-13	SU-DHL-8
Derived from malignancy	Multiple myeloma	Acute myeloid leukemia	Diffuse large B-cell lymphoma
Treatment	20 nM bortezomib	10 μM cytarabine	10 μM doxorubicin
Duration of treatment, h	21	30	30
No. of peptides identified in cell-free media after treatment	71	126	41
No. of peptides identified in cell-free media without treatment	7	94	14
D-at-P1 peptides in treated sample	28	4	23
D-at-P1 peptides in untreated sample	3	3	1

All experiments were performed after seeding cells in FBS-free media for 24 h. For control samples, the same number of cells (40 mL at 1×10^6 cells per mL density for MM1.S and MOLM-13; 1.5×10^6 cells per mL density for SU-DHL-8) was grown for the same duration in FBS-free media as treated samples but without addition of drug. "Peptides identified" refers to unique peptides without duplicates or variable modifications. "D-at-P1" refers to peptides with an Asp residue inferred at the P1 proteolytic position, immediately N-terminal to the identified cleavage site, suggestive of caspase cleavage.

Table S3. Summary of findings from high-yield postchemotherapy patient plasma

Peptide characteristic	No. of peptides	Percentage
Total potential biomarkers of therapeutic response from discovery and targeted MS*	153	
Caspase-cleaved[†]	47	30.7 (of total)
Derived from typically intracellular proteins[‡]	142	92.8 (of total)
Low-abundance (<10 ppm)[§]	39	27.5 (of intracellular)
Medium-abundance (10–100 ppm)[§]	45	31.6 (of intracellular)
High-abundance (>100 ppm)[§]	58	40.8 (of intracellular)

*Proteolytic peptides derived from proteins identified in postchemotherapy patient plasma, not previously found in an extensive study of proteolytic peptides in normal human plasma and serum (1), plus a caspase-cleaved peptide from gelsolin.

[†]Peptides with an inferred Asp residue at the P1 cleavage position, suggestive of caspase cleavage.

[‡]Peptides derived from typically intracellular proteins with >10-fold increased abundance in cells compared with plasma based on the PaxDb (2).

[§]Abundance of intracellular proteins from which proteolytic peptides are derived, as measured by the PaxDb (2).

1. Wildes D, Wells JA (2010) Sampling the N-terminal proteome of human blood. *Proc Natl Acad Sci USA* 107(10):4561–4566.
2. Wang M, et al. (2012) PaxDb, a database of protein abundance averages across all three domains of life. *Mol Cell Proteomics* 11(8):492–500.

Table S4. Patient characteristics and data from quantitative SRM assay

Patient	Diagnosis	Treatment	Hours after induction posttherapy sample collected	Circulating malignant cells pretherapy (10^6/mL blood)	Circulating malignant cells at posttherapy collection (10^6/mL blood)	Peptides identified by SRM assay posttherapy	Peptides increased >twofold post- vs. pretherapy
AML_1	Acute myeloid leukemia	Clofarabine	24	70.0	4.0	100	77
AML_3	Acute myeloid leukemia	Ara-C	12	6.1	1.5	49	18
NHL_2	Mantle cell lymphoma	Ara-C	96	15.0	0.0	21	1
NHL_3	T-prolymphocytic leukemia	Alemtuzumab	48	46.0	23.0	38	22
ALL_2	B-acute lymphoblastic leukemia	Ritux, Dox, Dex, vincristine	96	0	0	30	1
PCL_2	Plasma cell leukemia	Dex, Bort	96	5.0	3.3	26	1
AML_4	Acute myeloid leukemia	Decitabine	96	47.0	23.9	29	9
AML_5	Acute myeloid leukemia	Ara-C, Dauno	36	17.7	4.6	29	1
AML_6	Acute myeloid leukemia	Ara-C, Dauno, Dex	24	0.2	0.04	30	8
ALL_3	B-acute lymphoblastic leukemia	Ara-C, vincristine, Dauno	12	35.4	21.7	25	1
AML_7	Acute myeloid leukemia	Etoposide, Dauno, Ara-C, Dex	78	30.0	6.8	26	3
MM_1	Multiple myeloma	Dex, Bort, cisplatin, Dox, cpm, etoposide	24	0	0	30	1
MM_2	Multiple myeloma	Carfilzomib	24	0	0	24	2
MM_3	Multiple myeloma	Dex, Bort, cisplatin, Dox, cpm, etoposide	24	0	0	26	0
NHL_4	Diffuse large B-cell lymphoma	Ritux, cpm, Dox, vincristine, prednisone	24	0	0	39	22
AML_8	Acute promyelocytic leukemia	ATRA, arsenic trioxide	24	20.1	8.6	28	1
AML_9	Acute myeloid leukemia	Ara-C, Dauno	24	0.7	0.0	24	4

Paired pre- and posttherapy plasma samples were either 250 or 500 μL for each patient. Ara-C, cytarabine; ATRA, *all-trans* retinoic acid; Bort, bortezomib; cpm, cyclophosphamide; Dauno, daunorubicin; Dex, dexamethasone; Dox, doxorubicin; Ritux, rituximab; No circulating malignant cells indicates either bone marrow- or lymph node-confined disease.

Dataset S1. Analyzed MS data from unbiased discovery experiments (.xslx). Worksheets AML_1, NHL_1, ALL_1, PCL_1, AML_2, and Control show N-terminal peptides identified by Protein Prospector searches of mass spectrometry data from fractionated QSTAR Elite experiments. The first five are discovery patient samples, and Control shows data from 1.5 mL of normal commercial plasma N-terminally labeled and analyzed in the same way. Peptides highlighted in yellow indicate those not identified either in the normal plasma data here nor in the prior study of Wildes and Wells (1). The caspase-cleaved fragment of gelsolin is highlighted in green. Worksheet Patient N Termini Not in Normal lists the 98 unique N-terminal proteolytic peptides across patient samples derived from proteins not previously found in normal plasma

Dataset S1

Dataset S2. Analyzed MS data from cell-culture experiments (.xslx). Worksheets MM1S_bort, MM1S_control, MOLM13_cytarabine, MOLM13_control, SUDHL8_dox, and SUDHL_control show N-terminal peptides identified by Protein Prospector searches of mass spectrometry data from unfractionated QSTAR Elite experiments. Worksheet Peptides Cell cx and pts lists those overlapping peptides that were found both in cell-culture studies of proteolytically released fragments as well as in postchemotherapy patient samples in **Dataset S1**

Dataset S2

Dataset S3. Targeted discovery experiments method development and analyzed MS data (.xslx). Worksheets AML_1, NHL_1, ALL_1, PCL_1, and AML_2 show N-terminal peptides identified by Protein Prospector searches of mass spectrometry data from fractionated LTQ Orbitrap Velos experiments with peptides identified based on the applied parent mass list. All peptides used for *m/z* at 2+ or 3+ charge state on the parent mass list, along with the reason for targeting, are listed in the worksheet N-Term Peptides Inclusion List. Worksheet Patient N Termini Not in Normal lists the unique peptides found in targeted discovery experiments across patient plasma samples derived from proteins not previously found in Wildes and Wells (1)

Dataset S3

Dataset S4. Selected reaction monitoring method development and quantified data (.xslx). Worksheet SRM Method Development lists N-terminal peptides found in unbiased and targeted discovery experiments that were further explored for targeted quantitation. Worksheet SRM Transitions lists the parent and fragment ion *m/z* values used in the QTRAP 5500 method. Worksheet SRM Intensity Data shows intensity-corrected (based on spike-in standards) SRM data for identified peptides in each patient sample

Dataset S4