Supplemental Data

Figure Legends

Figure 1. Supplemental Data. Duplicate of Figure 5A from original manuscript - only at a reduced exposure time.

Figure 2. Supplemental Data. Ex vivo treatments with Velcade, vinblastine, Taxol, and tamoxifen do not induce changes in plasma serpin A1 and A3 S-glutathionylation. Cancer free (all samples designated 1) and cancer (AML, all samples designated 2) were treated with vehicle control, 0.1μmol/L, 1μmol/L, and 5μmol/L of Velcade *(A),* vinblastine *(B)*, Taxol *(C)*, or tamoxifen *(D)* for 1 hour. Immunoblots of S-glutathionylation (PSSG), serpin A1, serpin A3 and albumin levels (loading control) are presented.

Methods

Tandem Mass spectroscopy

Purified serpin A1 and A3 proteins were treated with 100μmol/L PABA/NO and 1mmol/L for 30 minutes at 37°C, digested with Lys-C and analyzed via liquid chromatography (LC)-electrospray ionization (ESI) -tandem mass spectrometry (MS/MS) on a linear ion trap mass spectrometer (LTQ, Thermo Finnigan) coupled to an LC Packings nano LC system. *S*-glutathionylated serpin A3 peptides required additional digestion with trypsin for 6hr prior to analyses. A 35cm 75micron C-18 reversed phase LC column (packed in house) was utilized with a 60 minute gradient from 2% acetonitrile, 0.1% formic acid to 60%

acetonitrile, 0.1% formic acid. A blank was analyzed between samples to limit carry over. Data Dependant Analysis was utilized on the LTQ to perform MS/MS on the 10 most intense ions in each MS spectra with a minimum ion count of 500. Dynamic Exclusion was set to exclude ions from MS/MS selection for 3 minutes after being selected twice in a 30 second window. MS/MS data were searched against the human database utilizing Thermo Finnigan Bioworks 3.3.1 SP1 software. Variable modifications of methionine oxidation, carboxamido-methylation of cysteines, *S*-glutathionylation of cysteines, and phosphorylation of serine, threonine, and tyrosine were considered. Protein identifications must meet the minimum criteria of a Protein Probability of 1.0 E-3 or better and have an Xcorr vs charge state > 1.5, 2.0, 2.5 for +1, +2, and +3 ions, with at least 2 unique peptides matching the protein, and a good match for at least 4 consecutive y or b ion series from the MS/MS spectra.

Cloning and protein purification

Human serpin A1 (NM_00295) and A3 cDNAs (NM_001085) were ordered from Thermo Scientific (Rockford, IL). Fragments were the cloned into the *SalI I* and *Not1* recognition sites of pET-28b for expression in a bacterial system. Clones were sequenced to ensure alignment and integrity of the insert. For expression of recombinant proteins pET-28/A1 and pET-28/A3 plasmids were transformed into BL21 (DE3) competent *Esherichia coli* cells. Cultures were grown at 37°C in Luria-Bertani media supplemented with 50μg/mL kanamycin. At an A_{600nm} of 0.5 bacterial cultures were induced with 1mmol/L isopropyl-L-thio-B-

D-galactopyranoside for an additional 3 hours. Cells were pelleted at 4°C for 15 minutes at 6000rpm and resuspended in bacterial lysis buffer pH 8.0 (20mmol/L sodium phosphate buffer, 500mmol/L NaCl, 1x protease inhibitor cocktail (Roche, Indianapolis, IN) and sonicated on ice using 180 one-second bursts at high intensity with a three-second cooling period. The lysate was then centrifuged at 4°C for 30 minutes at 13,000 rpm and the cleared supernatant loaded onto a Ni^{2+} column pre-equilibrated with lysis buffer. The column was then washed with wash buffer (lysis buffer with 10mmol/L imidazole) and protein was eluted in elution buffer (lysis buffer plus 250mmol/L imidazole). Protein was dialyzed at 4°C for 24 hours in dialysis buffer (25mM HEPES (pH 7.3), 1mmol/L NaCl, and 1mmol/L EDTA). Protein purity was confirmed by SDS-PAGE and immunoblot.

Supplemental plasma treatments

Equal amounts of total plasma protein, as determined by Bradford assay (Bio-Rad Laboratories), were treated with Velcade, vinblastine, Taxol, and tamoxifen at 0.1μmol/L, 1μmol/L, and 5μmol/L for 1 hour at 37°C. Doses chosen reflect steady state drug concentrations in pharmacokinetic studies of patients on typically used dosing regimens. Velcade and vinblastine were prepared and diluted in phosphate buffered saline and Taxol and tamoxifen in DMSO. Proteins were separated on non-reducing SDS-polyacrylamide gels, transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories), and probed with a monoclonal anti-GSSG antibody to detect S-glutathionylation, or polyclonal antibodies for serpins A1 and A3.