# Supporting Information

## Seo and Carroll 10.1073/pnas.0903015106

### SI Materials and Methods

**General Experimental.** Unless otherwise noted, all reactions were performed under an argon atmosphere in oven-dried glassware. All purchased materials were used without further purification. Thin layer chromatography (TLC) was carried out using Analtech Uniplate silica gel plates. TLC plates were visualized using a combination of UV, *p*-anisaldehyde, ceric ammonium molybdate, ninhydrin, and potassium permanganate staining. Flash chromatography was performed following the method of Still et al. (1), using Sorbent Technologies Incorporated silica gel (32–63 $\mu$M, 60-Å pore size).

NMR spectra were obtained on a Varian Inova 400 (400 MHz for $^1$H; 100 MHz for $^{13}$C), or a Varian Mercury 300 (300 MHz for $^1$H; 75 MHz for $^{13}$C NMR) spectrometer. $^1$H and $^{13}$C NMR chemical shifts are reported in parts per million (ppm) relative to TMS, with the residual solvent peak used as an internal reference. Low-resolution electrospray ionization (ESI) mass spectra were obtained on a Water-Micromass LCT spectrometer at the University of Michigan Mass Spectrometry Laboratory. Reverse-phase HPLC purifications were performed on a Beckman Coulter System Gold 126P equipped with System Gold 166P detector ($\lambda$ = 220) using a C18 (21.2 × 150 mm) Beckman Coulter Ultraprep with a gradient of 0.1% TFA in $H_2O$ and $CH_3CN$ as the mobile phase.

**Chemical Synthesis of Hapten.** See *SI Appendix*.

**Cell Culture.** Jurkat cells were cultured in RPMI media supplemented with 10% FBS and 1% penicillin-streptomycin-L-glutamate (PSG). Cells were incubated in a 5% $CO_2$ humidified incubator at 37 °C and were typically seeded at 0.5 × 10$^6$/mL and grown to a density of 2 × 10$^6$/mL.

**Dimedone Labeling of Cell Lysate.** Jurkat cells (6 × 10$^6$) were collected by centrifugation (1,000 × *g* for 5 min) and washed three times with sterile PBS. The media free cell pellet was then suspended in 100 $\mu$L ice-cold lysis buffer (1.0% Nonidet P-40, 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, and protease inhibitors) and incubated on ice for 30 min with frequent mixing. The supernatant was collected by centrifugation (20,000 × *g* for 20 min) at 4 °C. The supernatant was transferred to a new tube and subjected to the same centrifugation protocol. The protein concentration of the lysate was determined by Bradford assay (Bio-Rad). Cell lysate (1 mg/mL, in 30-$\mu$L total reaction volume) was untreated or treated with 1, 2.5, 5, or 10 mM dimedone or DMSO (2.5% wt/vol) for 1 h at room temperature. The reaction was terminated by adding 10% $\beta$-mercaptoethanol in XT sample buffer and analyzed by Western blot using the $\alpha$-hapten antibody.

**Dimedone Labeling of Cells in Culture.** Jurkat cells in RPMI with 2% FBS and 1% PSG were incubated with 10 mM dimedone or DMSO (5% vol/vol) for 0, 1, 2, 4, or 8 h at 37 °C. At the end of each time point, cells were harvested, washed 3 times with PBS and a lysate was generated and analyzed as described above.

**Identification of Sulfenic Acid-Modified Proteins.** Proteins were immunoprecipitated from lysates made from dimedone-treated cells using GAPDH, PrxI, and actin-specific antibodies (Santa Cruz Biotechnology). After capture on protein A-Sepharose, samples were analyzed by Western blot using the $\alpha$-hapten antibody.

**Comparative Labeling of GAPDH-SOH With Sulfenic Acid Probes.** DAz-1, DAz-1-biotin and *p*-biotin were synthesized as previously described (4). GAPDH (4 mg/mL in PBS, pH 7.4) was treated with 1 mM DTT for 20 min. After reduction, DTT was removed by ultrafiltration using an Amicon Ultra-4 10 KDa MWCO centrifugal filter unit (Millipore). Stocks of dimedone, DAz-1, and DAz-1-biotin used in labeling studies were made up in DMSO-0.1 M Bis-Tris·HCl, pH 7.4 (1:1). GAPDH (5 $\mu$M in PBS) was treated with 500 $\mu$M dimedone, 500 $\mu$M DAz-1-biotin, 500 $\mu$M DAz-1, or DMSO (2.5% vol/vol) followed by $H_2O_2$ (0, 2.5, or 5 $\mu$M) and incubated for 30 min at rt. DAz-1 treated GAPDH was further incubated with 250 $\mu$M *p*-biotin for 2 h at 37 °C. Proteins were resolved by SDS/PAGE using Criterion XT 4–12% Bis-Tris gels (Bio-Rad) in XT Mes running buffer, transferred to PVDF membrane, and then blocked with 5% nonfat dried milk in PBST overnight at 4 °C or for 1 h at room temperature. The membrane was then washed in PBST (2 × 10 min) and probed with $\alpha$-hapten antibody (1:200) or HRP-streptavidin (1:100,000) for 1 h at room temperature. The membrane was then washed with PBST (3 × 10 min) and incubated with goat $\alpha$-rabbit-HRP (1:100,000) for 1 h at room temperature, washed with PBST (3 × 10 min), and developed using ECL Plus detection reagent (GE Healthcare). Equal protein loading was verified by probing with $\alpha$-GAPDH.

**Viability Assay of Cultured Cells.** Jurkat cells were cultured as described above. Cells were incubated in RPMI media containing 10 mM dimedone, 2% FBS, and 1% PSG at 37 °C for 0, 1, 2, 4, 6, and 8 h. At the end of each time point, cells (100 $\mu$L) were mixed with trypan blue solution (100 $\mu$L) and quantified by counting with a hemocytometer.

**Dot Blot.** PVDF membrane was treated with methanol for 10 min before spotting proteins. Using narrow-mouth pipet tip, 1.3 $\mu$L GADPH (0.31 mg/mL in PBS, pH 7.4) was spotted on the membrane and dried for 5 min at room temperature. The membrane was then untreated or incubated with 0.1 mM $H_2O_2$ for 5 min at room temperature and washed with PBS (2 × 10 min). Next, the membrane was incubated with 5 mM dimedone in PBS or DMSO (2.5% vol/vol) for 15 min at room temperature. The membrane was then washed with PBS (2 × 10 min), blocked with 2% nonfat milk in TBST for 1 h at room temperature and washed with TBST (3 × 10 min). The membrane was incubated with $\alpha$-hapten antibody in TBST (1:2,000) for 1 h at room temperature, washed with TBST (3 × 10 min), and incubated with goat $\alpha$-rabbit-HRP (1:5,000) in TBST for 1 h at room temperature. After washing with TBST (3 × 10 min), the membrane was developed with ECL plus detection reagent.

**Source and Preparation of Samples for Protein Microarray.** Protein Biotechnologies Inc. provides pharmaceutical, biotechnology, government, and academic institutions with human clinical specimen derivatives and high-throughput protein and tissue microarrays. The SomaPlex™ line of protein microarray research products is prepared from normal and tumor tissue collected from the same patient. Tissues for microarray research products are obtained through a global network of participating medical centers that employ IRB approved protocols and strict ethical guidelines to ensure patient confidentiality and safety. Identical procedures are used to prepare all patient samples. Specimens are flash frozen to −120 °C within 5 min of removal to minimize autolysis, oxidation, and protein degradation. Tissue specimens

are homogenized in modified RIPA buffer (PBS, pH 7.4, 1 mM EDTA, and protease inhibitors) to obtain the soluble proteins, and centrifuged to clarify. Proteins are spotted in triplicate at a protein concentration of 1 mg/mL and each spot contains approximately 5 ng total protein. The proteins have not been subjected to denaturing or reducing conditions. This allows the microarray to be used in a variety of applications; to study protein-protein interaction, ligand binding, and immunological analysis for the detection of specific protein targets and post-translational modifications (5–7).


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2. Khan AT, Ali MA, Goswami P, Choudhury LH (2006) A mild and regioselective method for bromination of $\alpha$-keto esters and 1,3-diketones using bromodimethylsulfonium bromide (BDMS). *J Org Chem* 71:8961–8963.
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7. Meyer R, et al. (2009) Overexpression and mislocalization of the chromosomal segregation protein separase in multiple human cancers. *Clin Cancer Res* 15:2703–2710.
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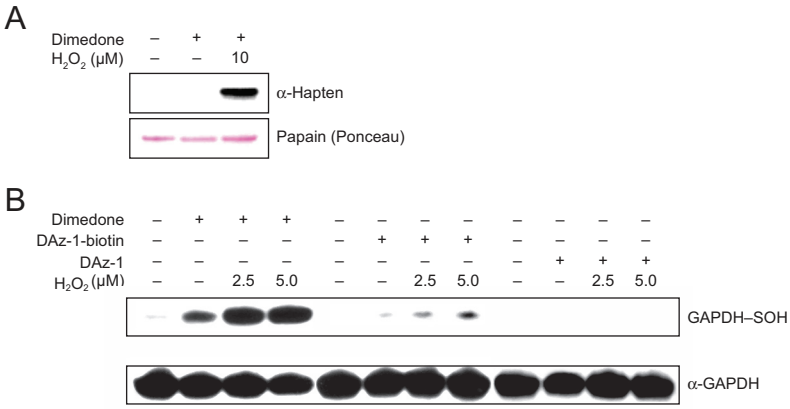


**Fig. S1.** Trapping and tagging sulfenic acid modified proteins. (*A*) Sulfenic acid-modified papain is detected by the α-hapten antibody. Top, papain (10 μM) was treated with dimedone (0 or 5 mM) followed by $H_2O_2$ (0 or 10 μM) for 30 min at room temperature and analyzed by Western blot with the α-hapten antibody. (*B*) Comparative labeling of sulfenic acid-modified proteins using dimedone, DAz-1-biotin and DAz-1. GAPDH (5 μM in PBS, pH 7.4) was treated with 500 μM dimedone, 500 μM DAz-1-biotin, 500 μM DAz-1, or DMSO (2.5% vol/vol) followed by $H_2O_2$ (0, 2.5, or 5 μM) for 30 min at room temperature. DAz-1 treated GAPDH was further incubated with 250 μM *p*-biotin for 2 h at 37 °C. Proteins were resolved by SDS/PAGE using Criterion XT 4−12% Bis-Tris gels (Bio-Rad) in XT Mes running buffer, transferred to PVDF membrane, and then blocked with 5% nonfat dried milk in PBST overnight at 4 °C or for 1 h at room temperature. The membrane was then washed in PBST (2 × 10 min) and probed with α-hapten antibody (1:200) or HRP-streptavidin (1:100,000) for 1 h at room temperature. The membrane was then washed with PBST (3 × 10 min) and incubated with goat α-rabbit-HRP (1:100,000) for 1 h at room temperature, washed with PBST (3 × 10 min), and developed using ECL Plus detection reagent (GE Healthcare). Equal protein loading was verified by probing with α-GAPDH.

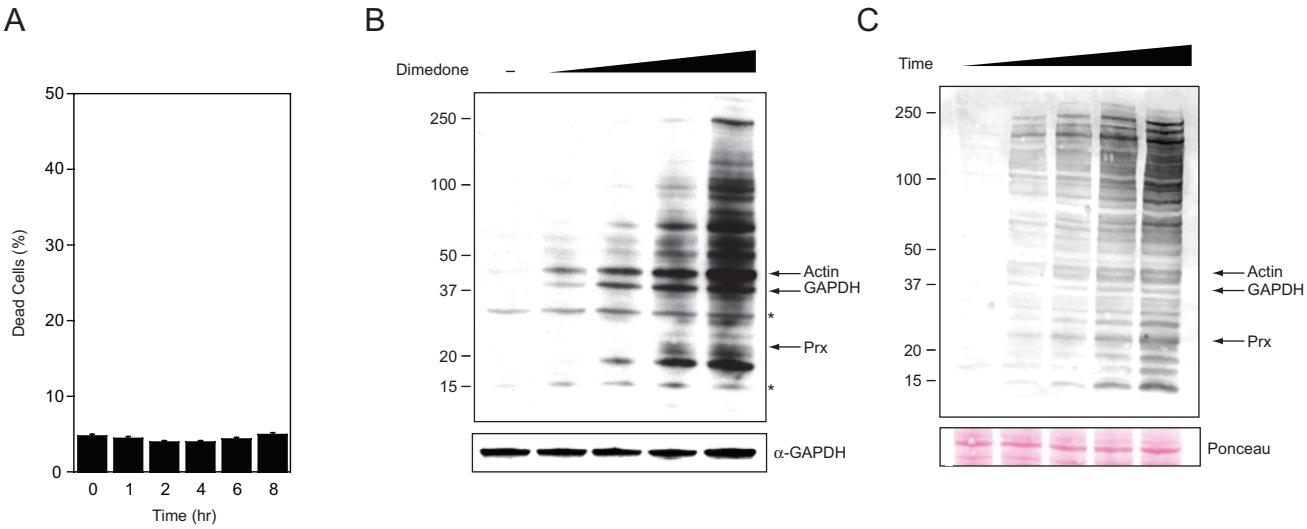


**Fig. S2.** α-Hapten antibody specifically recognizes sulfenic acid-modified proteins in vitro and in vivo. (*A*) Viability assay of Jurkat cells treated with dimedone. Jurkat cells were incubated in media containing 10 mM dimedone for 0, 1, 2, 4, 6, and 8 h. At the end of each time point, cells (100 μL) were mixed with trypan blue solution (100 μL) and dead cells were quantified by counting with a hemocytometer. (*B*) Dose-dependence of dimedone labeling. Jurkat cell lysate (1 mg/mL) was treated with dimedone (0, 1, 2.5, 5, or 10 mM) for 1 h at room temperature and analyzed by Western blot with the α-hapten antibody. Bands corresponding to actin, GAPDH, and PrxI are indicated on the right. (*C*) Time-dependence of dimedone labeling. Jurkat cells were incubated in media containing 5 mM dimedone for 0, 1, 2, 4, and 8 h. Cell lysate was analyzed by Western blot with the α-hapten antibody. Bands corresponding to actin, GAPDH and PrxI are indicated on the right. Asterisks denote a background immunoreactive band.

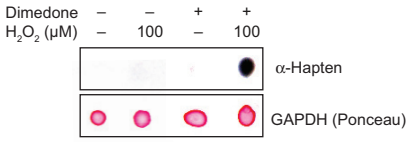


**Fig. S3.** Detecting thiol oxidation in GAPDH in dot blot format. Sulfenic acid-modified GAPDH is detectable with the dot blot format. Top, PVDF membrane as spotted with GAPDH (0.4 $\mu$g) was untreated or incubated with 0.1 mM $H_2O_2$ followed by 5 mM dimedone of DMSO (2.5% vol/vol). The dot blot was then analyzed with the $\alpha$-hapten antibody.

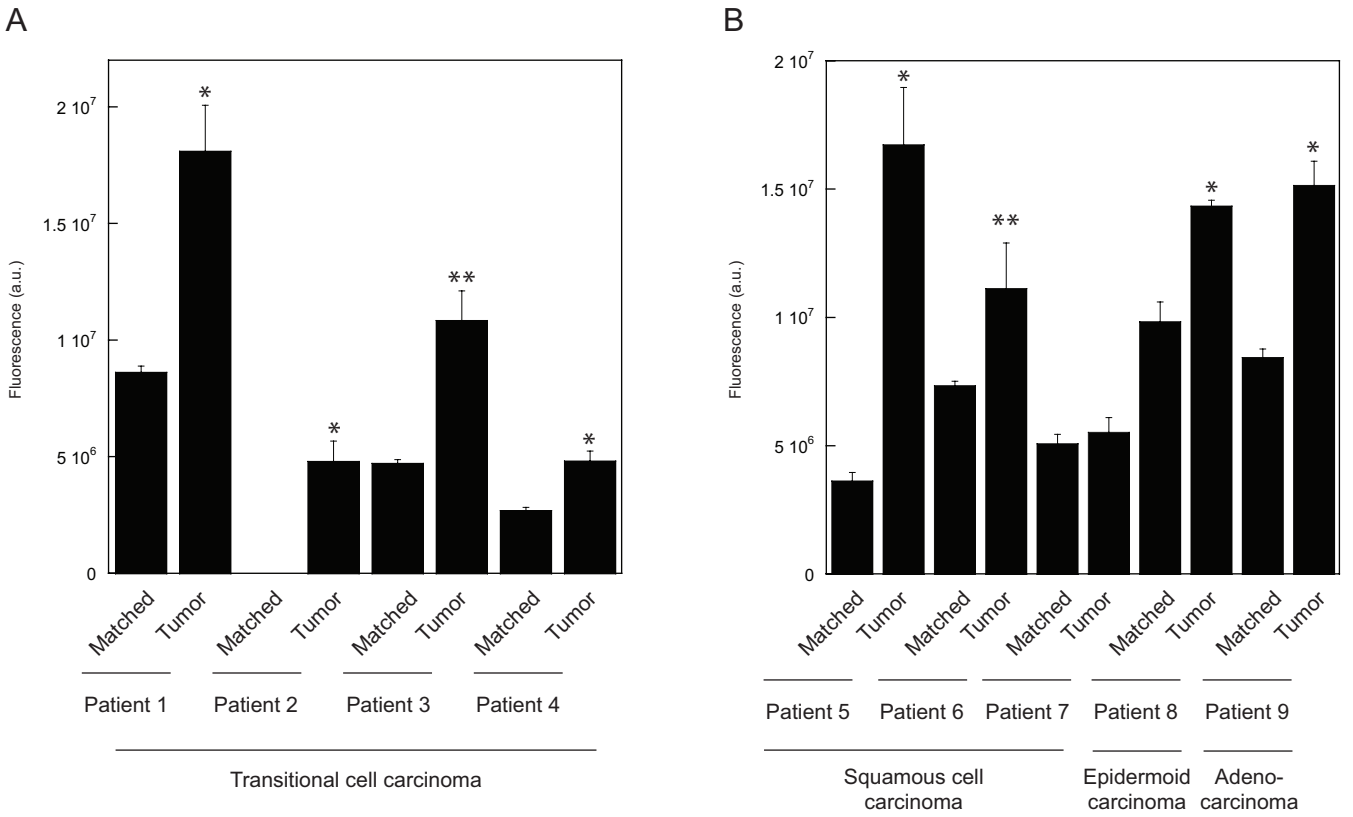


**Fig. S4.** Comparison of sulfenic acid levels in bladder tumors to corresponding matched normal tissue. The protein microarray was incubated in buffer containing 2 mM dimedone for 1 h and probed for sulfenic acids with the $\alpha$-hapten antibody. The fluorescence intensities of microarray spots are indicated with bars. Error bars represent s.d. of three replicate spots. (*A*) Transitional cell carcinoma tissue and matched normal tissue in four patient samples. (*B*) Squamous cell, epidermoid, and adenocarcinoma and matched normal tissue in patient samples. The Student's *t* test was used to determine statistical significance of the observed differences (*, $P < 0.001$; **, $P < 0.005$).

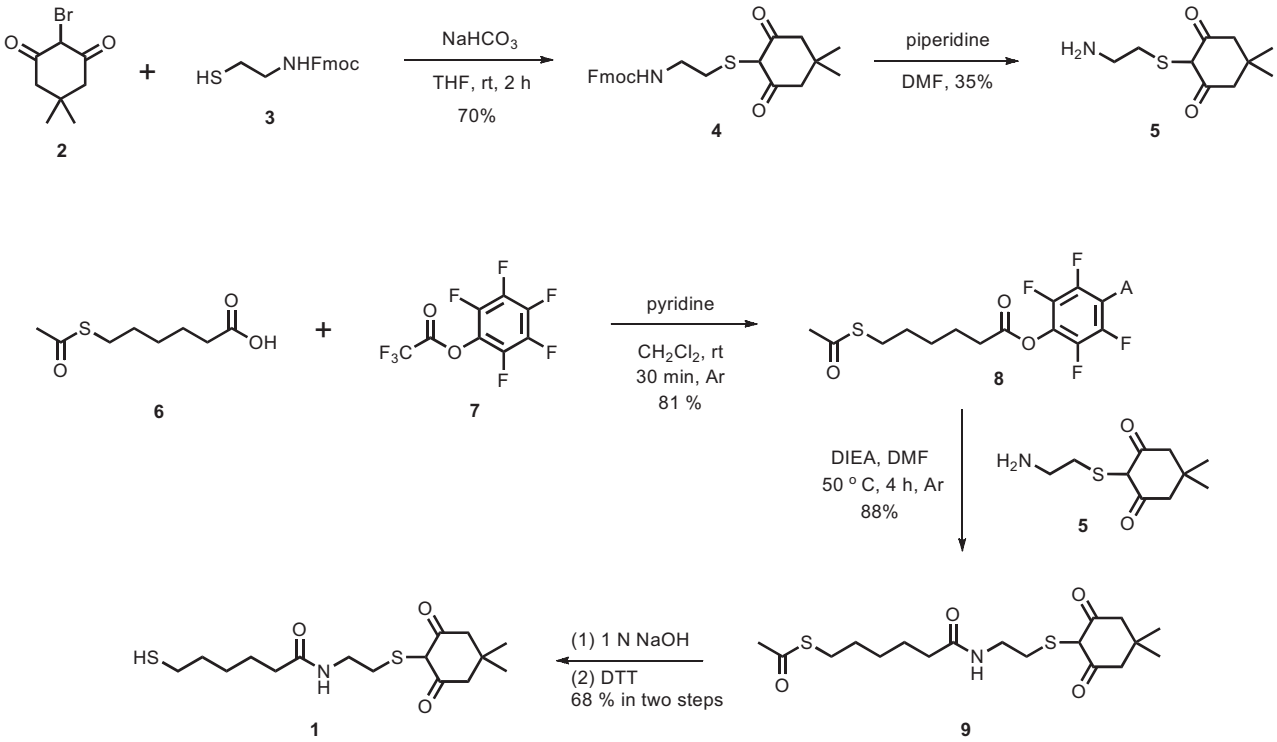


**Fig. S5.** Chemical synthesis of the hapten conjugate used to elicit sulfenic acid-specific antibodies.

**Table S1. Source, clinical, and pathological features of tumors used to derive breast cancer cell lines used in this study, adapted from ref. 8**

| | Cell line | Gene cluster | ER | PR | HER2 | TP53 | Source | Tumor type | Age | Ethnicity | Culture media | Culture condition |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | BT20 | BaA | − | [−] | | $++^{WT}$ | P.Br | IDC | 74 | W | DMEM, 10% FBS | 37 °C, 5% $CO_2$ |
| 2 | BT474 | Lu | + | [+] | + | + | P.Br | IDC | 60 | W | RPMI, 10% FBS | 37 °C, 5% $CO_2$ |
| 3 | HS578T | BaB | − | [−] | | $+^{M}$ | P.Br | IDC | 74 | W | DMEM, 10% FBS | 37 °C, 5% $CO_2$ |
| 4 | MCF7 | Lu | + | [+] | | $+/-^{WT}$ | PE | IDC | 69 | W | DMEM, 10% FBS | 37 °C, 5% $CO_2$ |
| 5 | MDAMB231 | BaB | − | [−] | | $++^{M}$ | PE | AC | 51 | W | L15, 10% FBS | 37 °C, no $CO_2$ |
| 6 | MDAMB468 | BaA | − | [−] | | [+] | PE | AC | 51 | B | L15, 10% FBS | 37 °C, no $CO_2$ |

AC, adenocarcinoma; BaA, Basal A; BaB, Basal B; IDC, invasive ductal carcinoma; Lu, Luminal; P.Br, primary breast; PE, pleural effusion; W, White; B, Black. ER/PR/HER2/TP53 status: ER/PR positivity, HER2 overexpression, and TP53 protein levels and mutational status (M, mutant protein; WT, wild-type protein) are indicated. Square brackets indicate that levels are inferred from mRNA levels alone where protein data is not available. Media conditions: FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium, GIBCO #11965–092; RPMI, RPMI medium 1640, GIBCO #27016–021; L15, Leibovitz's L-15 medium, GIBCO #11415–064.

## Other Supporting Information Files

*SI Appendix*