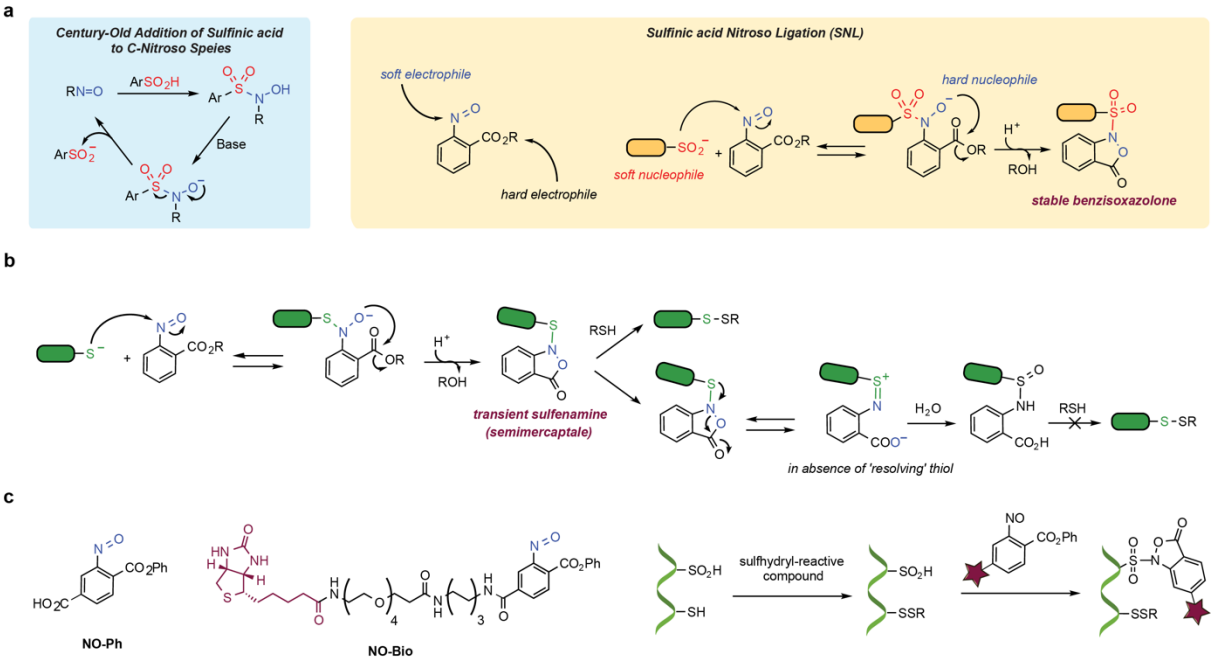
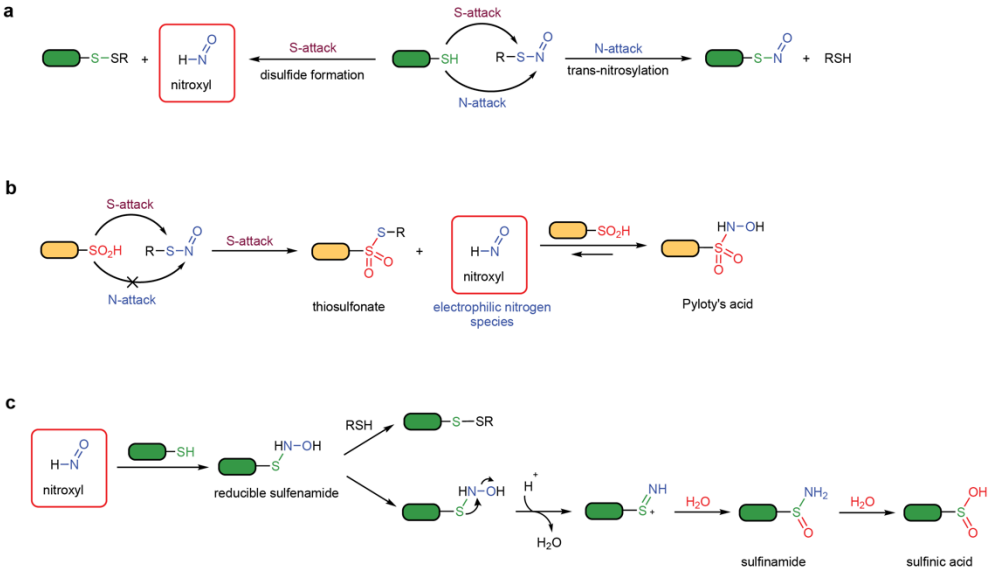
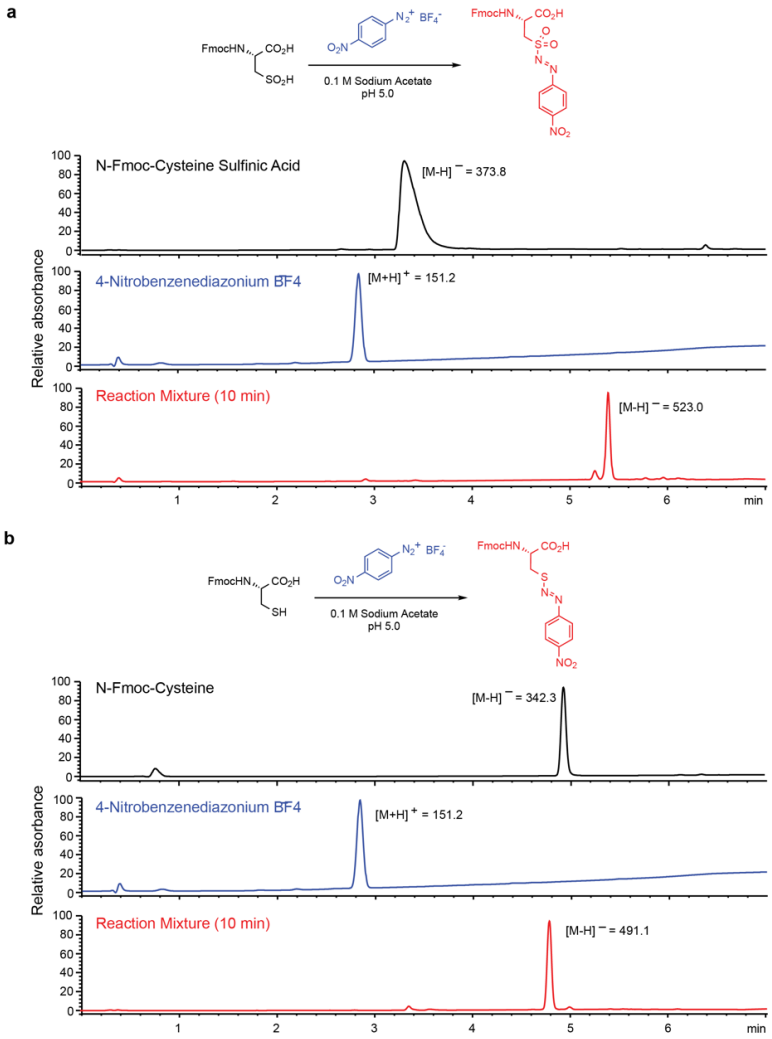


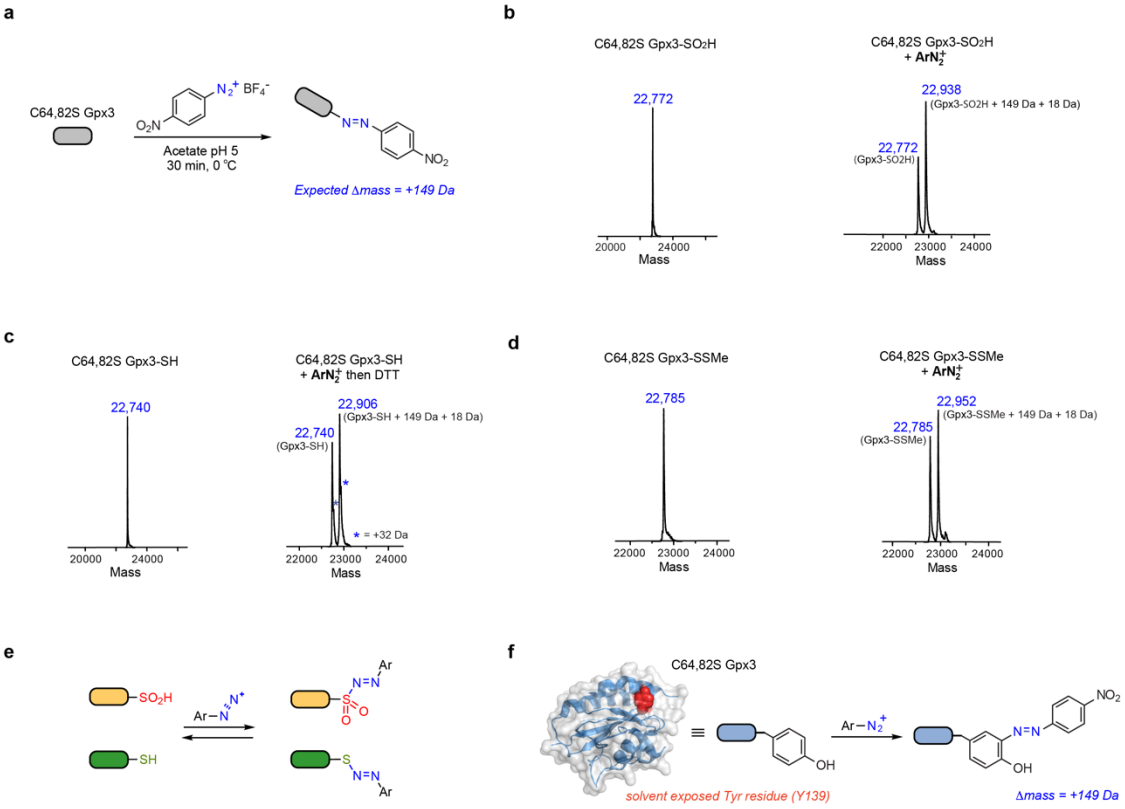
Supplementary Figure 1 | Reactivity of sulfinic acids and thiols toward *C*-nitroso compounds. (**a**) The addition of sulfinic acid to the electron-poor *C*-nitroso compound yields a base-label sulfonyl hydroxylamine. In the SNL, the incorporation of an ester in the ortho position of the *C*-nitroso compound allows, instead, the formation of a stable benzisoxazolone *via* an intramolecular trans-esterification. (**b**) Reactivity of *C*-nitroso compounds with thiols. In the absence of a resolving thiol, the transient sulfenyl rearranges to yield a stable, not reducible, adduct. (**c**) Elaboration of a two-step approach for selective detection of protein sulfinic acid using *C*-nitroso based probes.



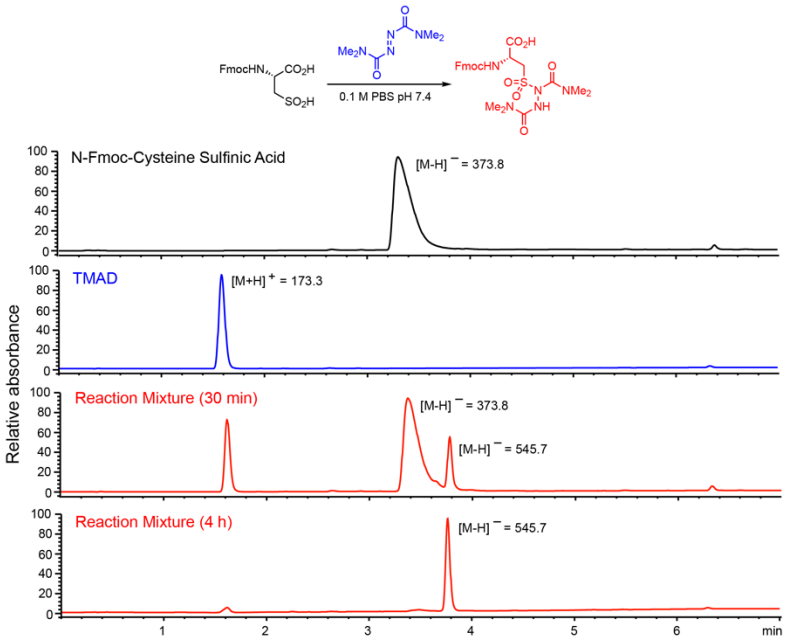
Supplementary Figure 2 | Reactivity of sulfinic acids and thiols toward *S*-nitrosothiols. (**a**) General reactivity of *S*-nitrosothiol toward thiols. (**b**) Sulfinic acid may attack only the electron-deficient sulfur atom of the *S*-nitrosothiol to yield a thiosulfonate ester and concomitant release of nitroxyl, which forms Pyloty's acid with a second molecule of sulfinic acid. (**c**) Reactivity of nitroxyl toward thiols. (**d**) In the absence of nearby thiols, nitroxyl quickly dimerizes to hyponitrous acid, which is dehydrated to nitrous oxide or reacts with molecular oxygen to form peroxynitrite.



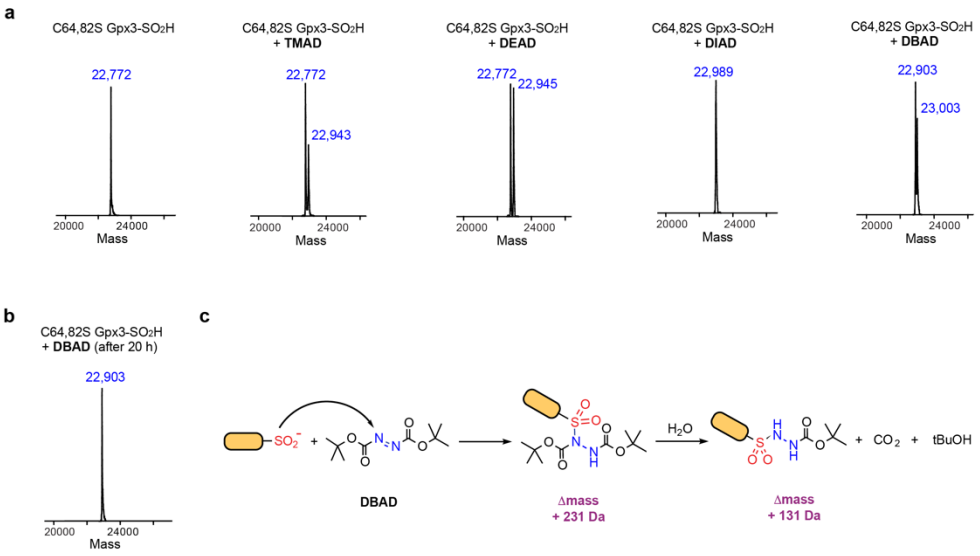
Supplementary Figure 3 | Reactivity of 4-nitrobenzenediazonium tetrafluoroborate (NDF) diazonium salt toward small-molecule sulfinic acids and thiols. (**a**) Extracted ion chromatograms (XIC) showing reactivity of diazonium salt toward *N*-Fmoc cysteine sulfinic acid. A solution of sulfinic acid (100 mM) was prepared in 100 mM sodium acetate buffer, pH 5. A solution of NDF (100 mM) was prepared in ddH_2O. One equiv (10 µL) of the diazonium solution and one equiv (10 µL) of the sulfinic acid solution were added to 80 µL of 100 mM acetate buffer, pH 5. After 10 min, 10 µL of the reaction was removed, diluted with 490 µL of acetonitrile/water 3:1 (0.1% of formic acid) and analyzed by LC-MS. (**b**) XIC showing reactivity of NDF toward *N*-Fmoc cysteine. A solution of cysteine (100 mM) was prepared in 100 mM sodium acetate buffer, pH 5. One equiv (10 µL) of the diazonium solution and one equiv (10 µL) of the cysteine solution were added to 80 µL of 100 mM sodium acetate buffer, pH 5. After 10 min, 10 µL of the reaction was removed and analyzed, as above. Representative data from three independent experiments with one LC-MS run for each are shown.



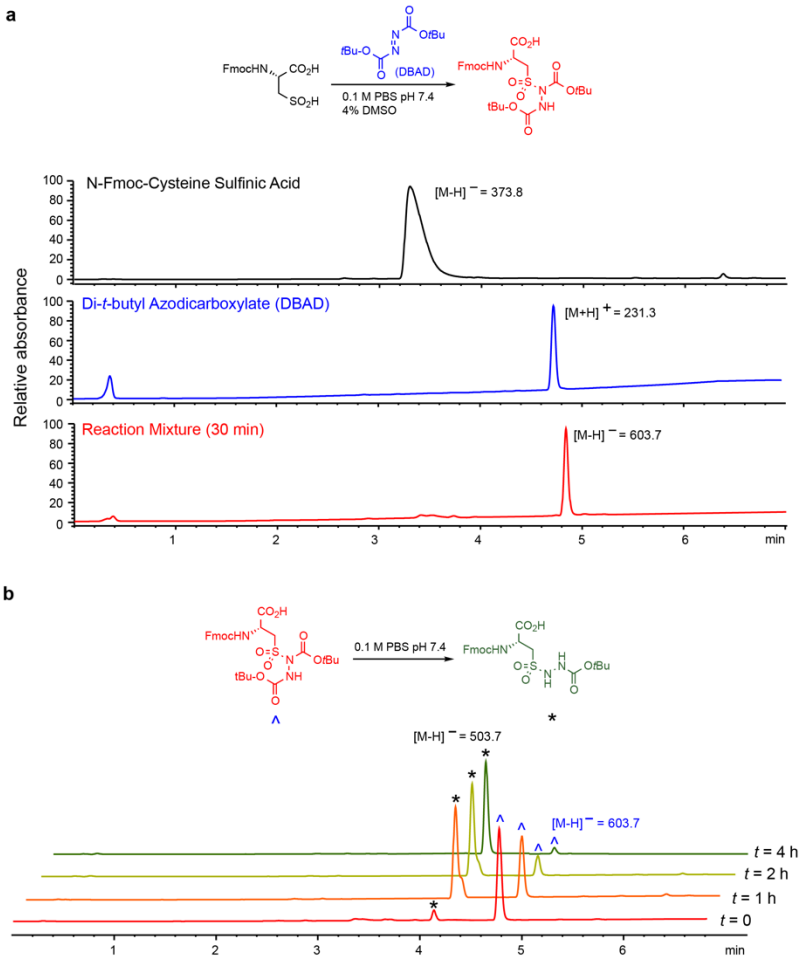
Supplementary Figure 4 | Reactivity of protein sulfinic acids and thiols toward nitrosobenezene diazonium salt. (**a**) C64,82S Gpx3 (25 µM) was incubated with diazonium salt (1 mM) at 0 °C for 30 min. The addition of one molecule of diazonium salt to the protein would yield an expected mass increase of 149 Da. (**b**) Deconvoluted MS spectra of C64,82S Gpx3-SO_2H before and after treatment with diazonium salt. (**c**) Deconvoluted MS spectra of Gpx3-SH before and after treatment with diazonium salt, followed by incubation with DTT (2 mM). (**d**) Deconvoluted MS spectra of Gpx3-SSMe before and after treatment with diazonium salt. C64,82S Gpx3 (100 µM) protein was incubated with MMTS (1 mM) for 1 h to generate Gpx3-SSMe, which was then incubated with diazonium salt. (**e**) The addition of diazonium salt to sulfinic acids and thiols appears to be a reversible reaction. (**f**) Diazonium salt likely reacts with Gpx3 solvent-exposed tyrosine, Y139. Representative data from three independent experiments with one LC-MS run for each are shown.



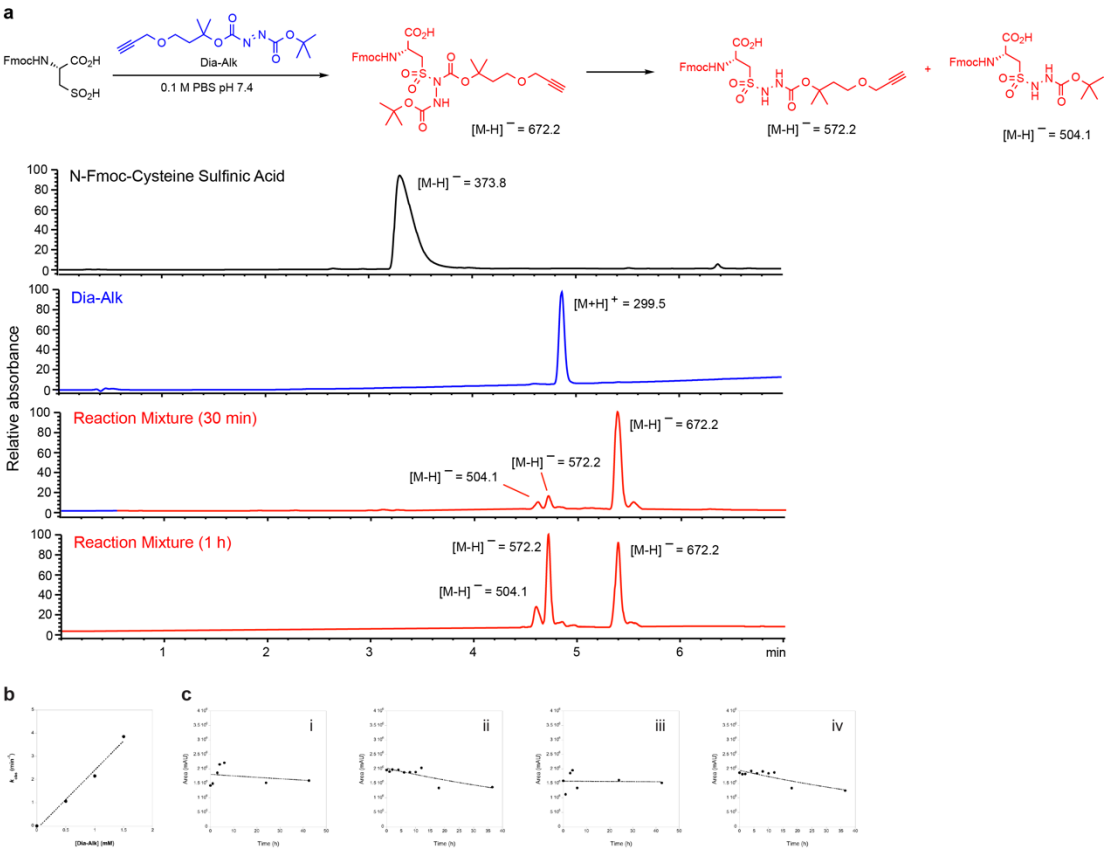
Supplementary Figure 5 | Reactivity of TMAD toward small-molecule sulfinic acids. Extracted ion chromatograms showing the reactivity of TMAD toward *N*-Fmoc cysteine sulfinic acid. A solution of sulfinic acid (100 mM) was prepared in PBS, pH 7.4. A solution of TMAD (100 mM) was prepared in ddH_2O. One equiv of the TMAD (10 µL, 1 mM) and one equiv of the cysteine sulfinic acid solution (10 µL, 1 mM) was added to 80 µL of PBS, pH 7.4. After 30 min or 4 h at rt, 10 µL of the reaction mixture was removed, diluted with 490 µL of acetonitrile/water 3:1 (0.1% of formic acid) and analyzed by LC-MS. Representative data from three independent experiments with one LC-MS run for each are shown.



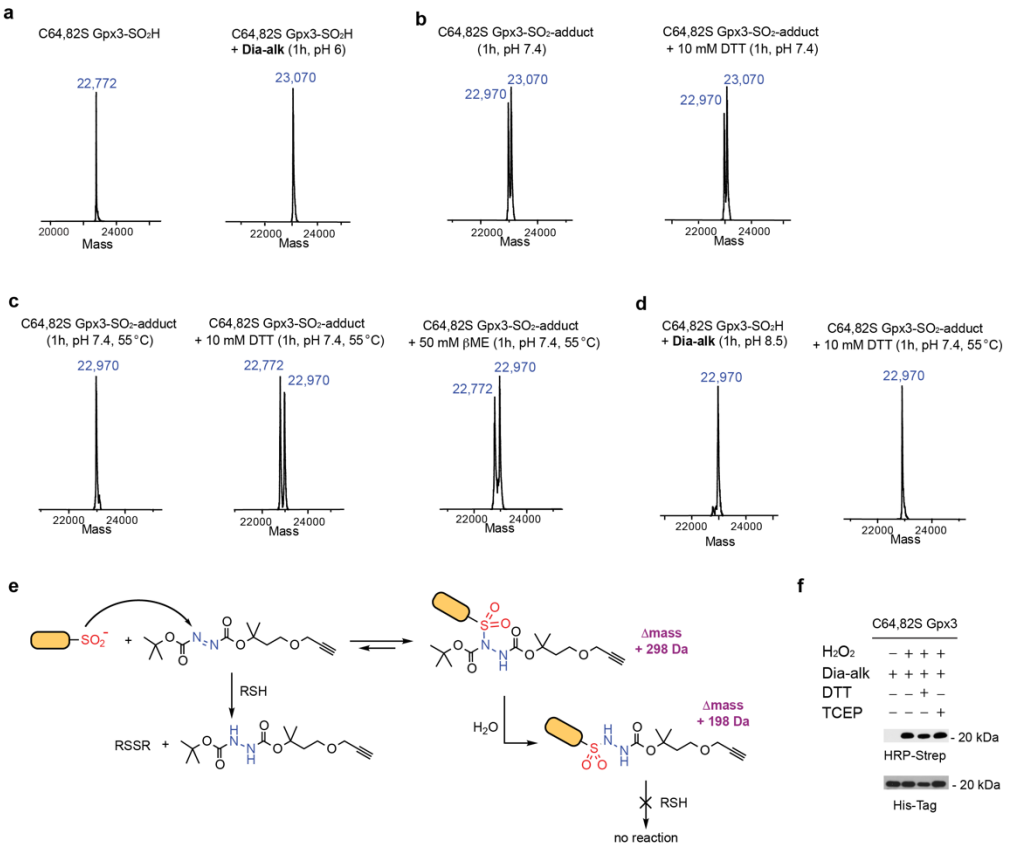
Supplementary Figure 6 | Comparison in labeling efficiency of various electron-deficient diazenes toward Gpx3-SO_2H. (**a**) Deconvoluted MS spectra of C64,82S Gpx3-SO_2H before and after treatment with various electron-deficient diazenes. A stock solution of the diazene (TMAD, DEAD, DIAD, or DBAD, 100 mM) was prepared in DMSO. Oxidized C64,82S Gpx3 was buffer exchanged using one Micro Bio-Spin P-30 column pre-equilibrated with PBS, pH 7.4. The recombinant protein (25 μM) was incubated with diazene compound (1 mM) in the dark at rt. After 1 h, the reaction was quenched by passage through one Micro Bio-Spin P-30 column pre-equilibrated with 50 mM ammonium bicarbonate, pH 8.0 for analysis by LC-MS. (**b**) Deconvoluted MS spectrum of the adduct formed by the addition of Gpx3-SO_2H to DBAD after 20 h. (**c**) Scheme of the reaction between protein sulfinic acid and DBAD. Intact MS analysis revealed that the sulfonamide adduct (23,003 Da) loses a *tert*-butyloxy carboxyl group (BOC), forming a cleaved product (22,903 Da) in a time- and pH-dependent manner. Representative data from three independent experiments with one LC-MS run for each are shown.



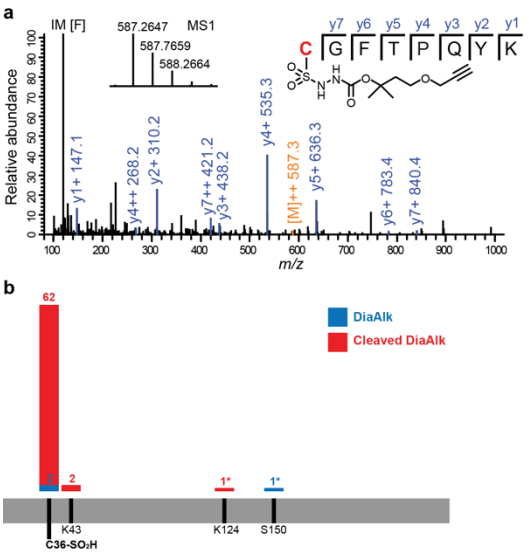
Supplementary Figure 7 | Reactivity of DBAD toward small-molecule sulfinic acids. (**a**) Extracted ion chromatograms showing the reactivity of DBAD toward *N*-Fmoc cysteine sulfinic acid. Conditions as reported in **Supplementary Figure 5**. *N*-Fmoc cysteine sulfinic acid (1 equiv, 1 mM) was incubated with DBAD (1 equiv, 1 mM) and the reaction was monitored over time by LC-MS. (**b**) Cleavage of the *tert*-butyloxy carbonyl group from the adduct over time. Representative data from three independent experiments with one LC-MS run for each are shown.



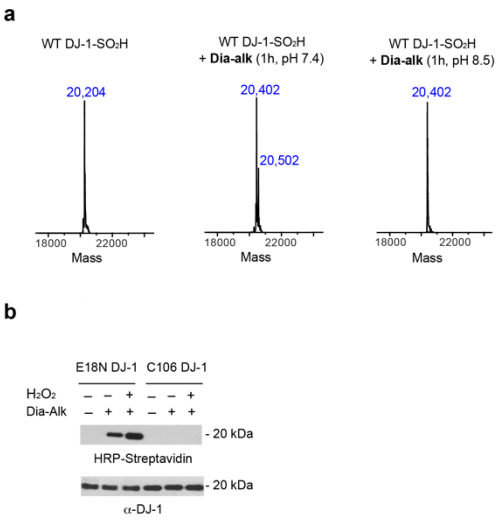
Supplementary Figure 8 | Reactivity DiaAlk toward small-molecule sulfinic acids. (**a**) Extracted ion chromatograms showing the reactivity of DiaAlk toward *N*-Fmoc cysteine sulfinic acid. Conditions as reported in **Supplementary Figure 5**. *N*-Fmoc cysteine sulfinic acid (1 mM) was incubated with DiaAlk (1 mM) and the reaction was monitored over time by LC-MS. Small-molecule sulfinic acid may attack both positions of the N=N, as indicated by the presence of two different cleaved products, although the less hindered position remains the most favorable. (**b**) Plot of k_{obs} versus [DiaAlk] obtained in PBS/ACN (2:1), pH 7.4 at rt under pseudo-first order conditions gives a second-order rate constant for reaction with *N*-Fmoc cysteine sulfinic acid of 2.5 x 10^3 M^{-1} min^{-1} to give the "uncleaved" or di-BOC product ($M\text{-}H^-$ = 672.2). (**c**) Stability of "cleaved" or mono-BOC DiaAlk-*N*-Fmoc cysteine sulfinic acid product ($M\text{-}H^-$ = 572.2, 250 µM) under conditions employed in the chemoproteomic workflow: i) PBS, pH 7.4 at rt ii) 10-fold excess DTT at rt, iii) 50 mM ammonium acetate at 37 °C, and iv) 0.1% formic acid in ddH_2O at rt. In all cases, the $t_{1/2}$ of the "cleaved" product was greater than 48 h. Representative data from three independent experiments with one LC-MS run for each are shown.



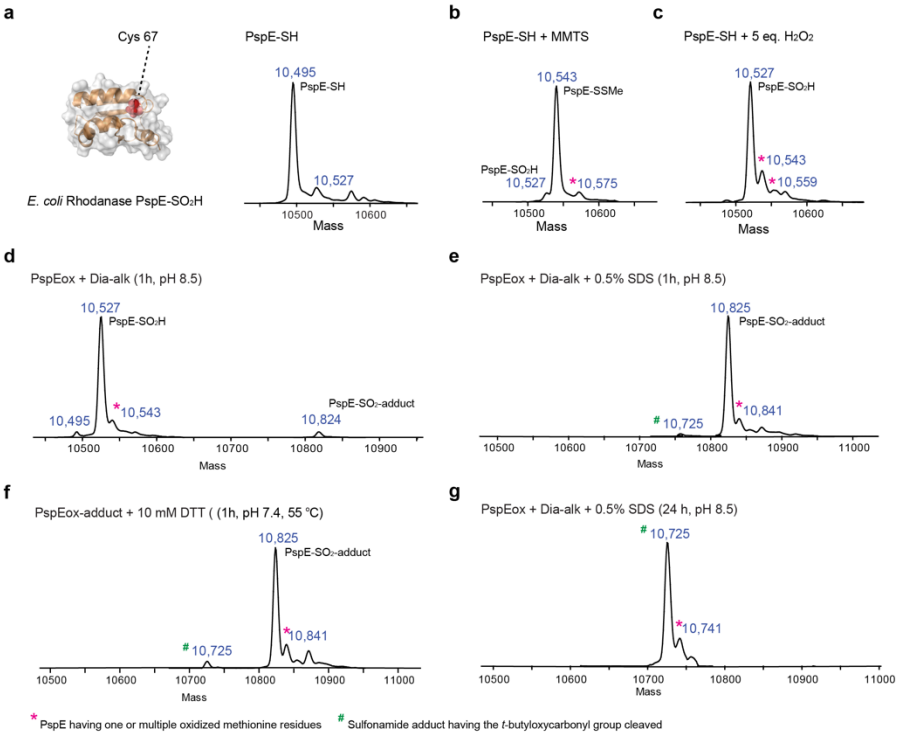
Supplementary Figure 9 | Stability of the sulfonamide adduct formed by the addition of Gpx3-SO_2H to DiaAlk. (**a**) Reaction conditions and sample processing as reported in **Supplementary Figure 6**. Generation of an "un-cleaved" adduct (23,070 Da) obtained by treating Gpx3-SO_2H with DiaAlk at pH 6. (**b**) Stability of "un-cleaved" adduct in the presence of 10 mM of DTT at rt. (**c**) Stability of "un-cleaved" adduct in the presence of 10 mM of reducing agent at 55 °C. (**d**) Stability of "cleaved" adduct in the presence of 10 mM of DTT at 55 °C. (**e**) Scheme to explain the difference in stability of the "un-cleaved" and "cleaved" adducts. (**f**) Immunoblot of the adduct after treatment with various reducing agents. Gpx3-SO_2H (25 μM) was untreated or exposed to H_2O_2 (50 μM) and then incubated in the presence or absence of probe (1 mM) at pH 8.5, followed by 1 h treatment with reducing agent (10 mM) at 55 °C. Representative data from three independent experiments with one LC-MS run or immunoblot for each are shown.



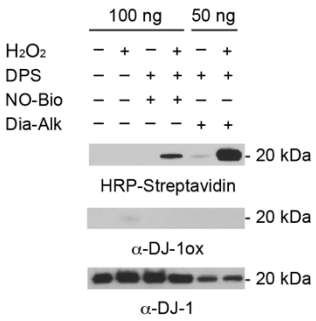
Supplementary Figure 10 | DiaAlk selectively labels *S*-sulfinylated C36 of Gpx3 C64,82S. (**a**) MS/MS spectrum of the partial cleaved adduct of DiaAlk at Gpx3 C36. The experiment was repeated independently twice with similar results. (**b**) The colored bars show the spectral counts identified for each site. Asterisks indicate potential false positive identifications due to the absence of modification-specific fragment ions. RAW files were analyzed by pFind studio. Precursor ion mass and fragmentation tolerance were 10 ppm and 20 ppm, respectively for the database search. A specific-tryptic search was employed with a maximum of three missed cleavages allowed. Modifications of +15.9949 Da (Methionine oxidation, M), +330.1427 (DiaAlk-SO_2, C), +230.0903 (Cleaved DiaAlk-SO_2, C), +314.1478 (DiaAlk-SO, C), +214.0953 (Cleaved DiaAlk-SO, C), +298.1526 Da (DiaAlk, CHKSTY), +198.1004 (Cleaved DiaAlk, CHKSTY) were searched as dynamic modifications. No fixed modifications were searched.



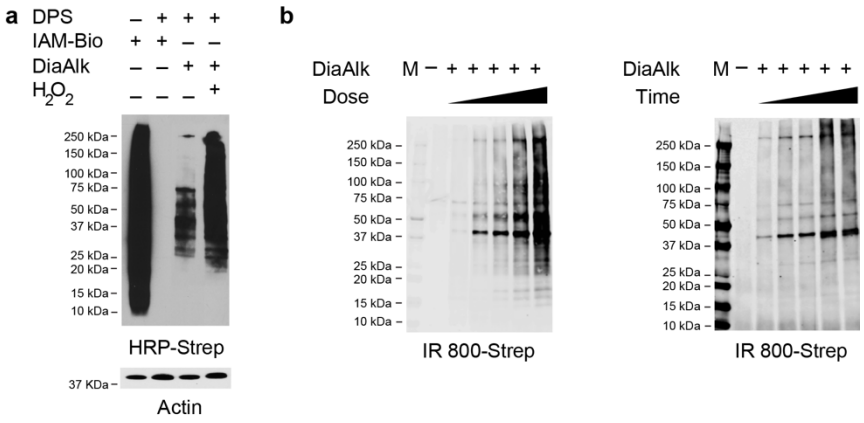
Supplementary Figure 11 | Reactivity of DiaAlk toward wild-type and mutant DJ-1. (**a**) Deconvoluted MS spectra of DJ-1 before and after treatment with Dia-Alk. Reaction conditions and sample processing as reported in **Supplementary Figure 6**. DJ-1 (25 μM) was incubated in the presence or absence of DiaAlk (1 mM) at rt for 1 h, followed by a 30 min treatment with reducing agent (2 mM) at rt. Labeling efficiency was measured by intact mass analysis. (**b**) Immunoblot showing detection of protein sulfinic acid in two different mutants of DJ-1. E18N DJ-1 has a higher propensity to form a stable sulfinic acid compared to wild type, as expected. The site of *S*-sulfinylation is further verified by the absence of signal in the C106S DJ-1 mutant. Representative data from three independent experiments with one LC-MS run or immunoblot for each are shown.



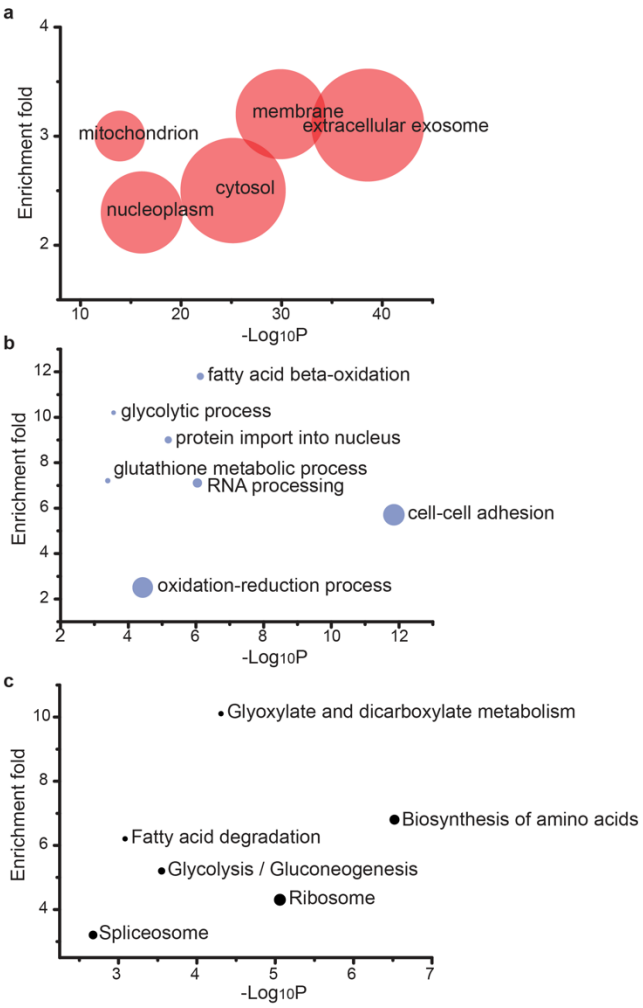
Supplementary Figure 12 | Reactivity of DiaAlk toward PspE. (**a**) Structure and deconvoluted MS spectrum of reduced PspE (PDB: 2JTQ). PspE has a single cysteine residue, which is buried inside its catalytic pocket. (**b**) Treatment of the PspE with MMTS confirmed the presence of a small amount of sulfinic acid in the "as-purified protein" (10,527 Da; see also **Fig. 2h**). (**c**) Deconvoluted MS spectrum of oxidized PspE. PspE-SO_2H was generated by incubating PspE with 5 equiv of H_2O_2. (**d**) DiaAlk, by itself, does not efficiently label the hindered sulfinic acid of PspE. (**e**) Adding 0.2% SDS to the buffer allows efficient labeling of the buried sulfinic acid in PspE. (**f**) The "uncleaved" adduct formed by the addition of PspE-SO_2H to DiaAlk is stable in the presence of a reducing agent even at 55°C, ostensibly owing to steric hinderance. (**g**) The "cleaved" adduct can be readily obtained by labeling the protein in the presence of 0.5% of SDS. *PspE having one or multiple oxidized methionine residues; #Sulfonamide adduct having the *tert*-butyloxycarbonyl group cleaved. Representative data from three independent experiments with one LC-MS run for each are shown.



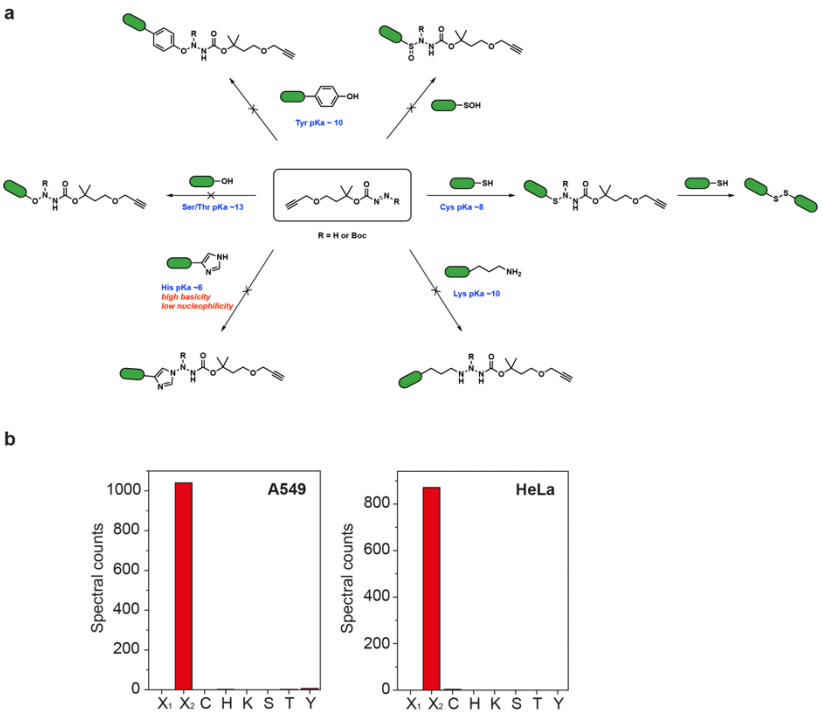
Supplementary Figure 13 | Benchmarking DiaAlk against current art. Comparison among the sensitivities of various probes for sulfinic acids detection (antibody against oxidized DJ-1, NO-Bio and DiaAlk) as described in the **Online Methods**. Total protein (100 or 50 ng) was loaded per lane, as indicated. Representative data from three independent experiments are shown.



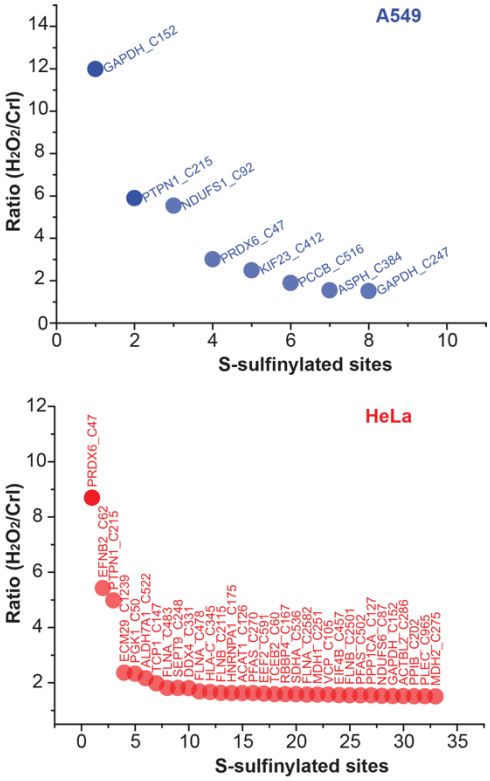
Supplementary Figure 14 | Redox- dose- and time-dependent detection of protein *S*-sulfinylation from cells using DiaAlk. (**a**) Immunoblot showing DiaAlk detection of protein sulfinic acids and total actin in HeLa cells. Cells were treated with or without H_2O_2 for 15 min at rt at the indicated concentration and processed for lysate in the presence of 200 U/mL catalase. Lysates treated with 4-DPS (200 mM) and then labeled with DiaAlk or IAM-Bio (1 mM or 250 µM, respectively). (**b**) Immunoblot showing dose- and time-dependence of sulfinic acid detection in Hela lysates derived from H_2O_2-treated (1 mM, 15 min) cells by DiaAlk at: 0, 0.1, 0.2, 0.5, 1, and 2 mM DiaAlk at rt for 2 h (left panel) or 0, 15, 30, 60, 90, 120 min at rt with DiaAlk (1 mM, right panel). M, molecular weight ladder. Representative data from three independent experiments are shown.



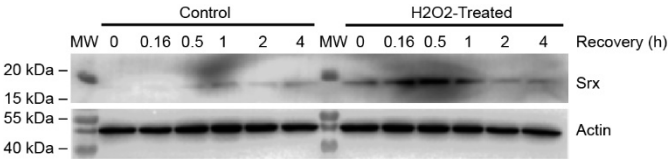
Supplementary Figure 15 | GO classification of the *S*-sulfinylome. (**a**) Cellular compartment. (**b**) Biological processes. (**c**) KEGG pathway.



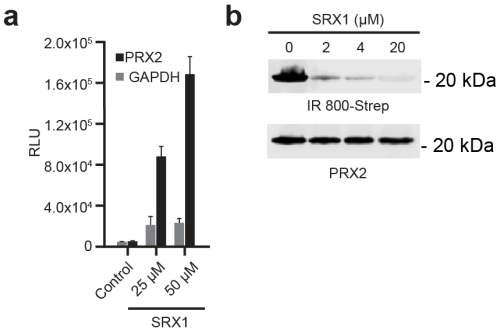
Supplementary Figure 16 | DiaAlk probe selectively labels cysteine *S*-sulfinic acid in complex proteomes. (a) Possible side-reactions between DiaAlk and protein nucleophiles. (**b**) All MS/MS data was computationally examined for potential cross-reactivity of DiaAlk with other nucleophilic amino acids. The search did not identify a single case of sulfoxide adduct (possible by-product of reaction with sulfenic acid) or a meaningful number of DiaAlk-derived adducts with residues containing nucleophilic functional groups. Bars show spectral counts identified for each ostensible adduct. X_1 indicates cysteine sulfenic acid ('sulfoxide'-DiaAlk). X_2 indicates cysteine sulfinic acid ('sulfone'-DiaAlk). The chemoproteomic data obtained from A549 and HeLa cells were searched against Homo sapiens Uniprot canonical database (Dec 2, 2016, 20,130 entries) using pFind studio. Precursor ion mass and fragmentation tolerance were 10 ppm and 20 ppm, respectively for the database search. A specific-tryptic search was employed with a maximum of two missed cleavages allowed. In addition to modifications of +15.9949 Da (methionine oxidation, M) and +57.0214 Da (iodoacetamide alkylation, C), +387.1754 (cleaved DiaAlk adducts on -SO_2H, C, Light), and +393.1955 (cleaved DiaAlk adducts on -SO_2H, C, Heavy), other potential modifications of +371.1805 (cleaved DiaAlk adducts on -SO, C, Light), and +377.2006 (cleaved DiaAlk adducts on -SO, C, Heavy), +355.1856 (cleaved DiaAlk adducts, CHKSTY, Light), +361.2057 (cleaved DiaAlk adducts, CHKSTY, Heavy) were searched as dynamic modifications. No fixed modifications were searched.



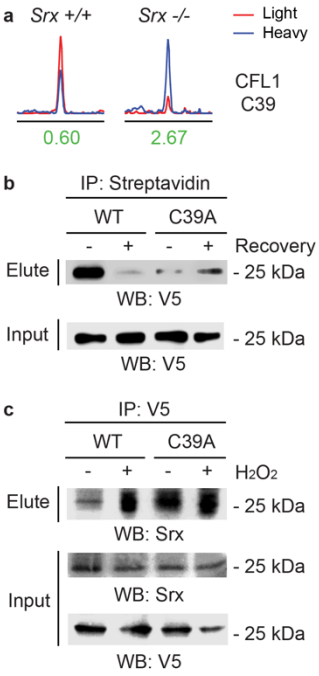
Supplementary Figure 17 | Dynamic protein *S*-sulfinylation events with measured ratios >1.5 in A549 (blue) and HeLa (red) cells in response to H_2O_2. Related to Fig. 4d.



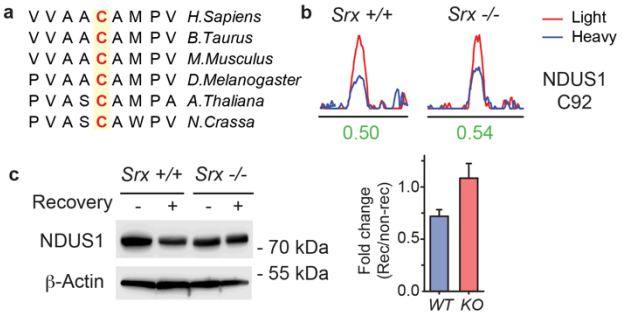
Supplementary Figure 18 | Induction of SRX expression in *Srx (+/+)* MEFs. SRX protein expression is induced by H_2O_2 treatment (1 mM, 15 min) reaching a peak at ~30 min. After 2 h, SRX returns to basal levels, representing “recovery”. Representative data from three independent experiments are shown.



Supplementary Figure 19 | SRX reduces *S*-sulfinylation in PRDX2. (**a**) Biochemical assay of SRX activity was measured as luminescence by incubating *S*-sulfinylated PRDX2 or GAPDH with recombinant SRX at the indicated concentration in buffer containing 100 mM Hepes pH 8, 100 mM NaCl, 2 mM DTT, 1 mM ATP and 2 mM $MgCl_2$, followed by 2 h incubation at 37 ºC. The ADP product generated *via* SRX reduction of protein sulfinic acid was detected using the ADP-Glo™ assay (Promega) following the manufacturer's protocol. The increase in signal in the absence of SRX was subtracted as background; the control reaction lacks ATP. Error bars represent the s.d. and experiments were repeated at least three times with similar results. (**b**) *S*-sulfinylation of recombinant PRDX2 is reduced by recombinant SRX. H_2O_2-treated PRDX2 (20 µM) was incubated with increasing concentrations of SRX in the presence of 2 mM DTT, 1 mM ATP and 2 mM $MgCl_2$ at 37 ºC for 1 h. Residual *S*-sulfinylated PRDX2 was detected by labeling with BioDiaAlk (100 µM) and analysis by HRP-streptavidin blot. Representative data from three independent experiments are shown.

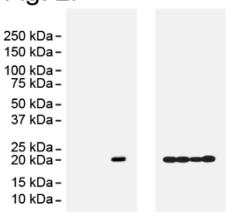


Supplementary Figure 20 | Verification of CF1 C39 as a substrate for SRX reduction in cells. (**a**) Extracted ion chromatograms of DiaAlk-modified peptides from CFL1 (C39) from *Srx* +/+ and *Srx* -/- MEF cells. The determined heavy (recovery) to light (control) ratios calculated from two independent experiments are displayed below the individual chromatograms, respectively. (**b**) *S*-sulfinylation of CFL1 C39 is reversible in cells. HEK293T cells transfected with V5-tagged WT or C39A CFL1 were treated with H_2O_2 (1 mM) for 15 min. Cells were either harvested or placed in H_2O_2-free medium for 2 h of recovery. *S*-sulfinylated proteins were detected in cell lysates by labeling with DiaAlk and conjugation to azido biotin *via* click chemistry. Biotinylated proteins were then enriched on streptavidin beads and detected by immunoblotting using the indicated antibodies. (**c**) H_2O_2 treatment promotes CFL1-SRX binding. HEK293T cells transfected with V5-tagged WT and C39A CFL1 were treated with or without H_2O_2 (1 mM) for 15 min. Immunoprecipitation and immunoblotting analyses were carried out using antibodies against the indicated proteins. Note: While the H_2O_2-dependent increase in SRX association is clear in wild-type CFL1, a stronger basal signal is detected observed for the C39A mutant. However, the mutant signal does not increase significantly with H_2O_2 stress. One possible explanation is that the C39A mutation alters its association with SRX (*e.g.,* slows dissociation). Other explanations, including enhanced antibody recognition of C39A, are also possible.



Supplementary Figure 21 | NDUS1 C92 *S*-sulfinylation is recovered in both *Srx* +/+ and *Srx* -/- MEF cells. (**a**) C92 of NDUS1 is an evolutionarily conserved site. (**b**) Extracted ion chromatograms of DiaAlk-modified peptides from NDUS1 (C92) from *Srx* +/+ and *Srx* -/- MEF cells. The determined heavy (recovery) to light (control) ratios calculated from two biological replicates are displayed below the individual chromatograms, respectively. (**c**) Immunoblots showing only slight or no change of the protein level of NDUS1 in *Srx* +/+ and *Srx* -/- MEF cells during the 2 h recovery period.

Fig. 2f



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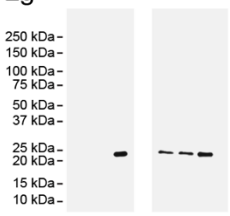


2h

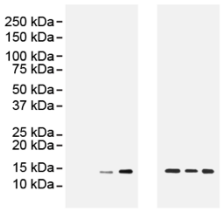


Fig. 4g

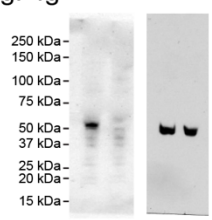


Fig. 5d

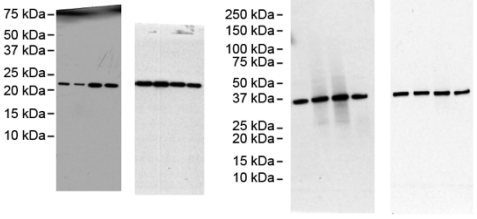
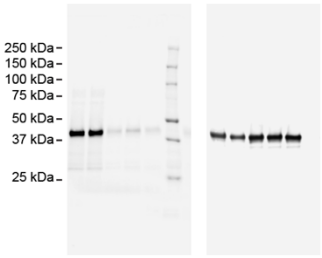
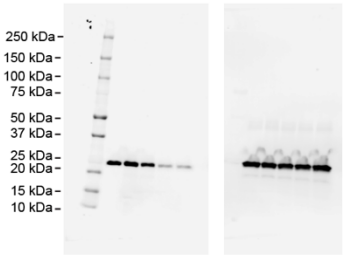


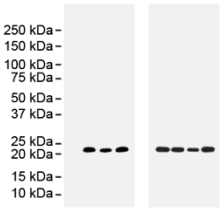
Fig. 5f



5g



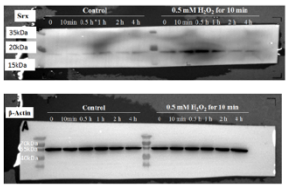
Supplementary Fig. 9f



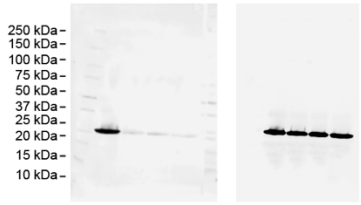
Supplementary Fig.11b

Supplementary Fig. 13

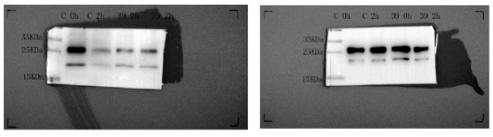
Supplementary Fig. 18



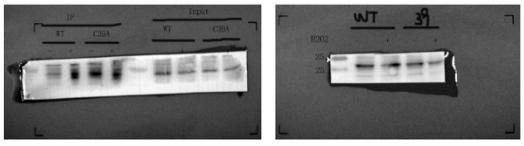
Supplementary Fig. 19b



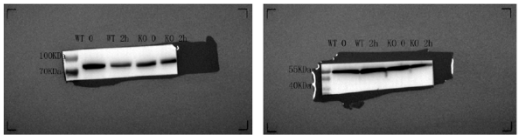
Supplementary Fig. 20b



20c



Supplementary Fig. 21



Supplementary Figure 22 | Raw immunoblots from current study.

Supplementary Dataset 1. *S*-Sulfinylated cysteines identified and quantified in response to exogenous oxidants in A549 and HeLa cells as shown in **Fig. 4**. After serum starvation overnight, A549 or HeLa cells were stimulated with or without 1 mM H_2O_2 for 15 min, lysed and labeled with 5 mM DiaAlk for 2 h. The resulting cell lysates were processed and analyzed as indicated in **Online Methods**. Quantitative measurements were acquired for 242 and 220 *S*-sulfinylated cysteines in A549 and HeLa cells, respectively, with a medium coefficient of variation of 10.3 % (A549) or 6.8 % (HeLa) across three independent experiments with one technical LC-MS/MS run for each. For the purpose of the quality control of sequence alignment and quantification, identification scores and interference scores exported from the pFind Studio are also provided for each site.

Supplementary Dataset 2. *S*-Sulfenylated cysteines identified and quantified in response to exogenous oxidants in A549 and HeLa cells as shown in **Fig. 5**. After serum starvation overnight, A549 or HeLa cells were stimulated with or without 1 mM H_2O_2 for 15 min, lysed and labeled with 5 mM BTD for 2 h. The resulting cell lysates were processed and analyzed as indicated in **Online Methods**. Quantitative measurements were acquired for 1173 and 1098 S-sulfenylated cysteines in A549 and HeLa cells, respectively, with a medium coefficient of variation of 13.9 % (A549) or 6.4% (HeLa) across two independent experiments with two technical LC-MS/MS runs for each. For the purpose of the quality control of sequence alignment and quantification, identification scores and interference scores exported from the pFind Studio are also provided for each site.

Supplementary Dataset 3. *S*-Sulfinylated cysteines identified and quantified in *Srx* +/+ and *Srx* -/- MEF cells as shown in **Fig. 6**. After serum starvation overnight, cells were stimulated with 1 mM H_2O_2. After 15 min treatment, cells were either harvested or subjected to 2 h recovery were probed with DiaAlk, processed as indicated in **Online Methods**. Quantitative measurements were acquired for 680 and 407 S-sulfinylated cysteines in *Srx* +/+ and *Srx* -/- MEF cells, respectively, with a medium coefficient of variation of 8.2 % (*Srx* +/+) or 14.8 % (*Srx* -/-) across two independent experiments with

two technical LC-MS/MS runs for each. For the purpose of the quality control of sequence alignment and quantification, identification scores and interference scores exported from the pFind Studio are also provided for each site.