ONLINE SUPPLEMENT

ONLINE METHODS

Immunoprecipitation of HDAC2: Cell or lung tissue extracts were prepared by using 100 µL of immunoprecipitation buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1.0% Triton X-100, 0.5% CHAPS, 0.5% deoxycholate) containing 1% complete protease inhibitor cocktail (Roche, Morristown, NJ). The lysis mixture was incubated on ice for 15 min and microcentrifuged for 10 min at 4°C. Extracts were pre-cleared with 20 µL protein A/G Sepharose beads (a 50:50 mix) from Thermo Scientific (Rockford, IL) and 2 µg of normal IgG. After microcentrifugation, 20 µL of protein A/G Sepharose conjugated with 5 µg of antibody was used to precipitate HDAC2 (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C with rotation. The immune complexes were pelleted with gentle centrifugation, washed twice with 1 mL of immunoprecipitation buffer, and divided equally after the final wash for the activity assay and immunoblotting. For the HDAC assay, immunoprecipitates were resuspended in the activity assay buffer (50 mM Tris-HCl, pH 8.0, with 10% glycerol, 0.1 mM EDTA, and complete protease inhibitor cocktail). For HDAC2-specific immunoblotting, immune complexes were washed three times with immunoprecipitation buffer and finally resuspended in Laemmli buffer and analyzed by SDS-PAGE. In addition, a metal chelator, 0.1 mM EDTA, was added to the buffers to eliminate free metals that catalyze the loss or gain of *S*NO bonds. All reactions were performed under non-reducing conditions and in the dark at 4°C and pH 7.4, since UV light and pH shifts disrupt SNO bonds.

Saville assay: The assay was performed as published elsewhere (1). In brief, a total of 20 µg of sample in 50 µl buffer was added to 50 µl of solution A (1% [w/v] sulfanilamide in 0.5 M HCl) in the wells of a 96-well plate. In separate wells, 50 µl of each sample was added to 50 µl of solution B (Solution A containing 0.2% (w/v) $HgCl_2$). Samples were incubated for 5 min at room temperature, and then 100 µL of solution C (0.02% [w/v] *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 0.5 M HCl) was added to each sample. After 5 min, the absorbance of the samples was measured spectrophotometrically at 540 nm. The amount of *S*NO was determined in the sample by subtracting the absorbance of the sample in solution A (nitrite) from the absorbance of the sample in solution B (nitrite + *S*NO). Standard curves were generated by repeating the above steps by using two-fold serial dilutions of GSNO from 250 to 15 µM. Control samples containing GSNO in the absence of protein were used to determine the background absorbance generated by residual GSNO in the samples. This assay was performed in the dark. The values are expressed as relative fold change.

Biotin-switch assay: The assay was performed as published elsewhere (2). In brief, cells were lysed in HEN buffer (250 mM HEPES, 1 mM EDTA, and 100 mM neocuproine) and adjusted to contain 0.4% CHAPS. Samples were homogenized, and free cysteines were blocked for 1 h at 50°C in three volumes of blocking buffer (HEN buffer plus 2.5% SDS [HENS]) containing methylmethanethiosulfonate (200 mM). Proteins were acetone-precipitated at -20°C and resuspended in 100 µL of HENS solution. After the addition of fresh ascorbic acid (20 mM) and 1 mM biotin-HPDP (Thermo Scientific, Rockford, IL), proteins were incubated at room temperature for 1 h. Biotinylated proteins were resuspended in 100 µL HENS buffer containing 200 µL of

neutralization buffer (20 mM HEPES, pH 7.8, with 100 mM NaCl, 1 mM EDTA, and 0.5% Triton X-100) and precipitated with 50 µL of streptavidin-agarose beads at room temperature for 1 h. The beads were washed five times at 4°C with neutralization buffer containing 600 nM NaCl. Biotinylated proteins were eluted with 50 µL elution buffer (20 mM HEPES, pH 7.8**,** with 100 mM NaCl, 1 mM EDTA, and 100 mM β-mercaptoethanol) and heated at 95°C for 5 min in reducing SDS–PAGE loading buffer, then immunoblotted with specific antibodies. A no biotin-HPDP treatment was used as a control for specificity. Denitrosylation of HDAC2 by reducing agents, including ascorbate, UV, and $HgCl_2$, was also used as specificity controls. This assay was performed in the dark.

Modified biotin-switch assay for assessing the fraction of HDAC2 *S*-nitrosylation in alveolar macrophages from COPD patients: To assess the relative fraction of *S*-nitrosylated-HDAC2 in alveolar macrophages from patients with COPD, we used a modified biotin-switch assay. In brief, HDAC2 was immunoprecipitated from alveolar macrophages isolated from each patient and divided into two portions: a) one portion was treated with a reducing agent, dithiothreitol (DTT), and then processed according to the biotin-switch method, and b) the second portion was processed normally according to the biotin-switch method, but without DTT. At the step before streptavidin-bead pull-down of the biotin-HPDP-labeled proteins, we ran the samples on a PAGE gel optimized for the LiCoR Odyssey protocol and then transferred them to a nylon membrane. The membrane was then probed with an infrared-labeled anti-streptavidin antibody to detect the biotinylated bands under Odyssey. The gel is shown in gray scale in Figure 1G (upper blot). The input was optimized by immunoblotting with anti-HDAC2

antibody using the ECL-plus chemiluminescence method. Samples from a total of six COPD patients were used for analysis.

Denitrosylation assay (3)**:** The denitrosylation reaction was performed at pH 7.8 in a reaction buffer containing reduced glutathione (L-GSH) at a final concentration of 1 mM and 0.1 mM diethylenetriaminepentaacetate (DPTA, to prevent metal-catalyzed degradation of *S*NO) at 25°C. The nitrosylation reaction was also performed at pH 7.8, but the reaction buffer did not include DTPA. All reactions were performed in the dark, with the final volume maintained at 100 µL.

pH dependence: We carried out the denitrosylation reaction with GSNO-treated recombinant HDAC2 protein at a neutral pH range (pH 7.4, in phosphate buffer, Supplemental Figure 9C, Part I; or at pH 7.8, in Tris buffer, Supplemental Figure 9C, Part II) and at basic pH (pH 9.0, in borate buffer, Supplemental Figure 9C, Part III); acidic pH inhibited this reaction (pH 4.5, acetate buffer, Supplemental Figure 9C, Part IV), as assessed by Saville assay.

Time for reaction completion: The reaction was carried out in Tris buffer (pH 7.8) for different time periods; the optimal time for complete denitrosylation was 30 min to 1 h.

Nuclear extract preparation: We used the NEPER kit from Promega (Madison, WI) and followed the manufacturer's instructions for preparing the nuclear extracts.

Cytokine assays: For human IL-8 and mouse IL-6 and MCP-1 proteins, we used Quantikine ELISA kits from R&D Systems (Minneapolis, MN) according to the manufacturer's instructions.

Immunoblot assays: All immunoblots were performed using protocols as described previously (4). Rabbit anti-HDAC2 antibody (Santa Cruz Biotechnology) was used at

1:500 dilution. Rabbit anti-*S*-nitroso-cysteine (anti-*S*NO) antibody (Alpha Diagnostics, San Antonio, TX) was used at 1:500 dilution. Rabbit anti-nitro-tyrosine antibody (Cell Signaling Technology, San Antonio, TX) was used at 1:250 dilution. Rabbit anti-GR antibody (Santa Cruz Biotechnology) was used at 1:1000 dilution. Rabbit anti-acetyl-lysine antibody (Abcam, Cambridge, MA) was used at 1:750 dilution. Rabbit anti-lamin-B1 (Santa Cruz Biotechnology) was used at 1:1000 dilution as a loading control. The Image J program was used for densitometry analyses.

Quantitative real-time PCR (qRT-PCR) assays: Total RNA was extracted from the cells by using the Qiagen RNeasy kit (Qiagen, Valencia, CA), and qRT-PCR was performed by using Assay-on-Demand primers and probe sets for human IL-8, NQO1, GCLM, and iNOS from Applied Biosystems (Foster City, CA) as described previously (4). We used the ABI 7000 Taqman system (Applied Biosystems) to perform these assays. β-actin was used as the normalization control.

Chromatin immunoprecipitation (ChIP)-PCR assays: Chromatin immunoprecipitation was performed as described previously (5). In brief, cells were fixed in ~1% formaldehyde in cell growth medium for 10 min, followed by glycine fixation. They were then incubated on ice for 30 min in ChIP-lysis buffer containing a protease inhibitor cocktail, then sonicated. After sonication, the samples were centrifuged at maximum speed for 12 min in a cold microcentrifuge. The supernatants were then used as input samples. A portion of the input sample was pre-cleared by incubation with a 50% slurry of protein A/G beads in immunoprecipitation (IP) buffer for 2 h in the cold, and the beads were then centrifuged and the supernatant was used for IP. The pre-cleared samples then were incubated with 2 μg of anti-pan-acetylated histone H4 antibody

(ChIP assay kit; Millipore, Upstate Biotechnology, Billerica, MA) or anti-HDAC2 or anti-DDK antibody (for the HDAC2-Myc-DDK overexpression construct-transfected THP-1 cells) or anti-GR antibody from Santa Cruz Biotechnology (Santa Cruz, CA). As a control, we used anti-rabbit IgG antibody for 1 h in the cold. The protein A/G bead slurry was added to each reaction and was rotated overnight. The next day, the beads were washed five times, and the DNA was reverse-crosslinked, eluted in elution buffer, and stored at -80°C until used for sequencing or PCR validation assays. The eluted DNA was checked for 100- to 300-bp fragment enrichments by using 8% polyacrylamide gel electrophoresis. This immunoprecipitated DNA was used for PCR amplification by using specific gene (IL-8 for humans) promoter PCR assays. The following PCR primers were used for the IL-8 promoter: forward, 5'-TTCCTTCCGGTGGTTTCTTC-3' and reverse, 5'-GGGCCATCAGTTGCAAATC-3'.

DAF-FM DA staining for NO levels: THP-1 cells were incubated in growth medium with 10 mM DAF-FM DA (Invitrogen, Carlsbad, CA) for up to 4 h, and fluorescence readings were taken every 10 min at 37°C.

HDAC2 deletion mutants and site-directed mutants: The HDAC2-Myc-DDK (C-terminal Myc-DDK-tagged) construct was obtained from Origene (Rockville, MD). The HDAC2 deletion constructs were generated by using PCR primers containing *Mlu*I and *Asi*SI restriction sites for cloning into the pCMV vector. The primers for Del 1 were: forward, 5’- GAGGCGATCGCCCAGCTCTCAACTGGCGGTTCAG-3’ and reverse, 5’-GCGACGCGTGGGTTGCTGAGCTGTTCTG-3’. Those for Del 2 were: forward, 5’-GAGGCGATCGCCGTAGAAGTTGTAAAAACTTTTAAC-3’ and reverse, 5’-GCGACGCGTGGGGTTGCTGAGCTGTTCTG-3’. Those for Del 3 were: forward, 5’-

GAGGCGATCGCCATGGCGTACAGTCAAGGAGG-3' and reverse, 5'-GCGACGCGTTCGAGCAACATTACGGATTGTG-3'. Site-directed mutagenesis to synthesize cysteine-to-alanine point mutations was carried out by using the Quick Change II site-directed mutagenesis kit (Stratagene, Agilent, Santa Clara, CA) according to the manufacturer's instructions.

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3. Hu TM, Chou TC. The kinetics of thiol-mediated decomposition of S-nitrosothiols. AAPS J. 2006;8(3):E485-92.

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ONLINE SUPPLEMENTARY DATA

Supplementary Table 1: Characteristics of patients with COPD (peritoneal lung tissue assay).

Supplementary Table 2: Characteristics of patients with COPD (alveolar macrophage analysis).

Supplementary Table 1: Characteristics of patients with COPD (peritoneal lung tissue assay)

Characteristic	Normal Samples	COPD Samples
Sex: male/female	6/0	12/0
Age: median (IQR), years	70.0 (68.6, 79.0)	72.5 (69.1, 84.0)
Pack-years smoked: median (IQR)	49.2 (45.7, 55.9)	57.2 (50.6, 67.3)
FEV_1 % predicted: median (IQR)	93.2 (92.2, 98.1)	49.7 (36.9, 69.2)
FVC % predicted: median (IQR)	87.9 (86.1, 90.2)	58.4 (49.0, 74.3)

IQR: Inter-quartile range (1^{st} and 4^{th} quartile)

FEV_1: Forced expiratory volume at 1 sec

FVC: Function vital capacity

Pack-years: (Packs smoked per day) x (years as a smoker)

Supplementary Table 2: Characteristics of patients with COPD (alveolar macrophage analysis)

Characteristic	Sample
Sex: male/female)	10/12
Age: median (IQR), years	50.0 (46.5, 72.0)
Pack-years smoked: median (IQR)	46.5 (30.0, 84.0)
FEV_1 % predicted: median (IQR)	58.0 (50.0, 74.0)
FVC % predicted: median (IQR)	75.0 (68.0, 102.0)

IQR: Inter-quartile range (1^{st} and 4^{th} quartile)

FEV_1: Forced expiratory volume at 1 sec

FVC: Function vital capacity

Pack-years: (Packs smoked per day) x (years as a smoker)

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: (**A**) Immunoblots for analysis of HDAC2 protein levels in nuclear and cytosolic fractions from COPD alveolar macrophages. (**B**) Evaluation of anti-SNO antibody specificity by immunoblot, using immunoprecipitated HDAC2 from alveolar macrophages from patients with COPD in the presence of NO donor GSNO or a reducing agent (ascorbate, $HgCl_2$, UV, or DTT). (**C-D**) *S*-nitrosoglutathione (GSNO, 0.5 mM, 2 h) treatment of immunoprecipitated HDAC2 (**C**) or r-HDAC2 (**D**) induces HDAC2 *S*-nitrosylation that is reversed by GSH treatment in a cell-free *in vitro* reaction, as assessed by means of biotin-switch and Saville assays, respectively. (**E-F**) GSNO treatment also decreases the *in vitro* activity of immunoprecipitated HDAC2 (**E**) and r-HDAC2 (**F**), as assayed by means of a fluorigenic substrate; this reaction is reversed by GSH treatment. (**G**) Characterization of the pH dependence of the glutathione (GSH, 1 mM)-mediated denitrosylation reaction with the following: I) phosphate buffer (pH 7.4), II) Tris buffer (pH 7.8), III) borate buffer (pH 9.0), and IV) acetate buffer (pH 4.5). (**H**) Characterization of the time dependence of the denitrosylation reaction completion in Tris buffer (pH 7.8). Data are means ± S.D. All assays were repeated three times (n = 4 samples per group).

Supplementary Figure 2: Pre-treatment with the HDAC chemical inhibitor trichostatin A or the NO donor *S*-nitrosoglutathione induces dexamethasone insensitivity. (**A**) Lipopolysaccharide (LPS, 10 μg/mL, 4 h) induces IL-8 promoter histone H4 acetylation (anti-pan-H4 acetyl antibody, anti-H4 acetyl ChIP) and decreases HDAC2 (Myc-DDK-tagged, anti-DDK antibody ChIP) binding to IL-8 promoter region, whereas dexamethasone (DEX, 1 μM) reverses this effect. However, trichostatin A (TSA, 10 nM

in ethanol, 2 h) reverses the effect of DEX on the IL-8 promoter. (**B**) Decline in HDAC2 activity after TSA treatment. (**C-D**) LPS induces IL-8 mRNA expression, as assessed by qRT-PCR (**C**), and IL-8 secreted protein levels, as assessed by an ELISA assay (**D**); DEX pre-treatment (0.5 h) before LPS exposure lowers LPS-induced IL-8 levels. Again, TSA pre-treatment before DEX exposure leads to DEX resistance and a failure to inhibit the IL-8 expression induced by LPS. (**E**) *S*-nitrosoglutathione (GSNO, 0.5 mM, 2 h) induces HDAC2 *S*-nitrosylation, as assessed by the biotin-switch assay. (**F-G**) GSNO enhances LPS-induced HDAC2 *S*-nitrosylation (**F**) and decreases HDAC2 enzymatic activity (**G**), which is not reversed by DEX treatment, as assessed by Saville assay. (**H-I**) In conjunction with HDAC2 *S*-nitrosylation, GSNO treatment enhances the levels of LPS-induced IL-8 mRNA (**H**) and secreted protein (**I**), as assessed by qRT-PCR and ELISA assays, respectively. Data are means ± S.D. All assays were repeated three times (n = 4 samples per group).

Supplementary Figure 3: Treatment of THP-1 cells with a cell-permeable GSH-ester (GSH-e) decreases GSNO-induced HDAC2 *S*-nitrosylation. (**A**) *S*-nitrosoglutathione (GSNO, 0.5 mM, 2 h) induces HDAC2 *S*-nitrosylation, which is reversed by GSH-e (0.5 mM, 2 h) treatment in THP-1 cells, as assessed by biotin-switch assay. (**B**) GSNO pre-treatment enhances LPS-induced IL-8 mRNA expression, as assessed by qRT-PCR, and increases DEX resistance, which can be reversed by GSH-e. Data are means ± S.D. All assays were repeated three times (n = 4 samples per group).

Supplementary Figure 4: Cysteines in the HDAC2 protein are targeted for *S*-nitrosylation. (**A**) Schematic representation of the HDAC2 wild-type (WT) protein and the three deletion constructs (Del 1-3), showing the various cysteines present in each

construct. (**B**) THP-1 cells transfected with site-directed mutant vectors bearing either a single or double cysteine-to-alanine (C→A) mutation (as indicated in the top figure) show a reduction in HDAC2 enzymatic activity in all the double mutants and a complete loss of activity in the triple-cysteine (Cys-152+262+274-Ala) mutant after S-nitrosoglutathione (GSNO 0.5 mM, 2 h) exposure, as assessed by biotin-switch assay. (**C**) Quantitation of the immunoprecipitated HDAC2 (by anti-DDK antibody immunoprecipitation) in the samples in **B,** as assessed by *in vitro* fluorigenic substrate activity assay, shows a loss of activity for cysteine-152 (catalytic core cysteines), the double mutant in which both cysteine-262 and -274 are mutated to alanines, and the triple mutant. Ascorbate, UV, and $HgCl_2$ were used as reducing agents to reverse *S*-nitrosylation and to show the specificity of the biotin-switch assay. Data are means ± S.D. All assays were repeated three times (n = 4 samples per group).

Supplementary Figure 5: Sulforaphane restores corticosteroid sensitivity in CS-exposed mouse alveolar macrophages isolated from CS-exposed mouse lungs in an Nrf2-dependent manner. (**A**) Total reduced glutathione (GSH) levels in CS (5 h/day, 250 TSP, 1 month)-exposed alveolar macrophages isolated from *Nrf2*$^{+/+}$ and *Nrf2*$^{-/-}$ with and without sulforaphane (SUL, 5 μM, 16 h) (n = 6 mice per group). (**B-E**) mRNA expression of Nrf2 target detoxification genes: Nqo1 (**B**); Ho1 (**C**); and glutathione synthesis enzymes, Gclm (**D**); and Gclc (**E**) as assessed by qRT-PCR. Data are means ± S.D. All assays were repeated three times, n = 6 / group. *Significant vs. vehicle samples, as analyzed by ANOVA, followed by Bonferroni post-test; †significant between genotypes; and ‡significant between two compared samples shown with the arrows, using Student's *t*-test. Data are means ± S.D.; $P < 0.01$.

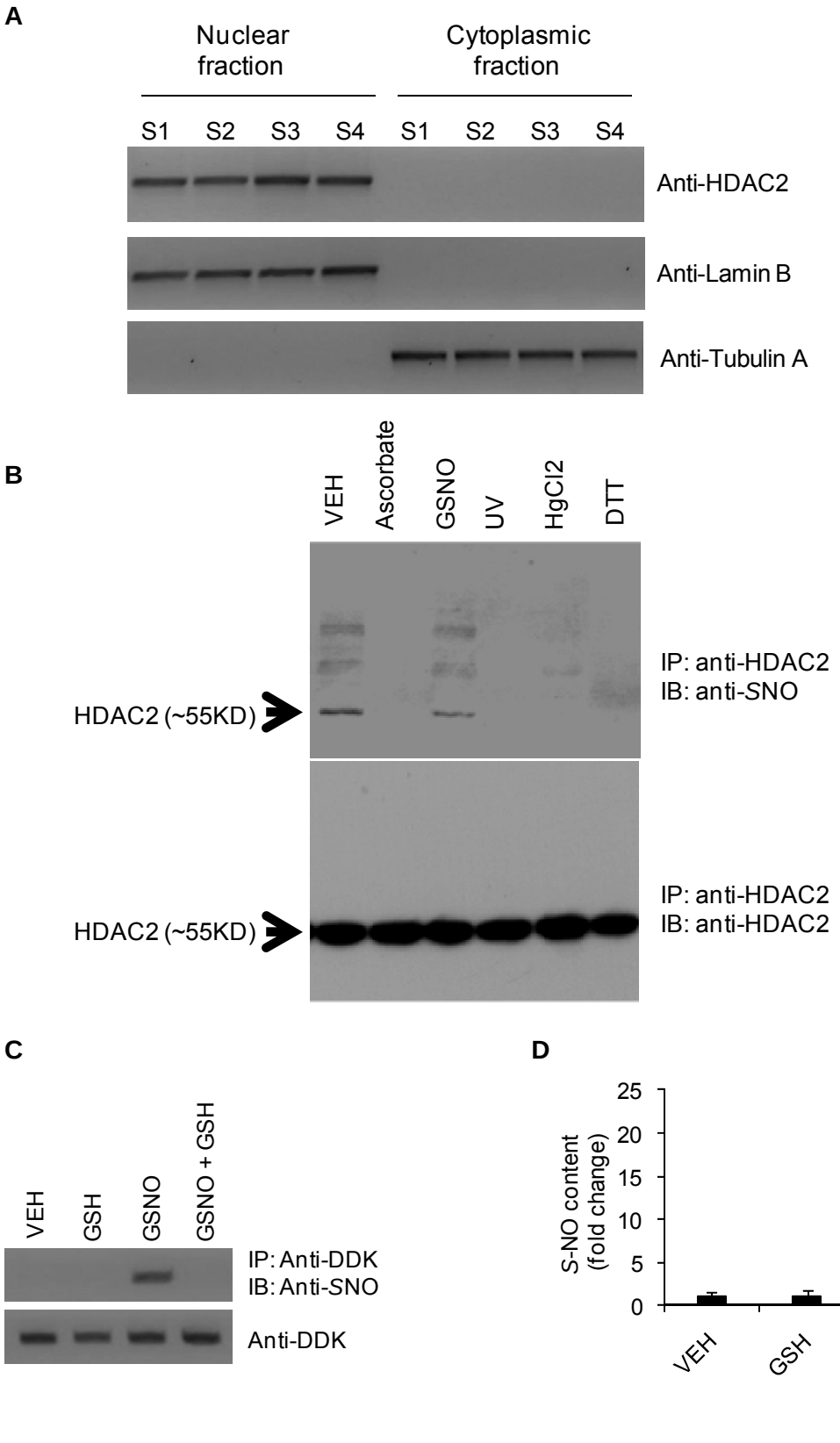
Supplementary Figure 6: Sulforaphane fails to restore corticosteroid sensitivity in alveolar macrophages isolated from patients with COPD in the presence of L-buthionine sulfoximide. (**A-B**) Lipopolysaccharide (LPS, 10 μg/mL, 4 h)-induced histone acetylation in the promoter of the IL-8 gene (**A**); and IL-8 mRNA expression (**B**) in alveolar macrophages from patients with COPD, co-treated with sulforaphane (SUL, 5 μM, 16 h) and/or L-buthionine sulfoximide (BSO, 1 mM, 16 h). (**C**) GSH levels in alveolar macrophages from patients with COPD exposed to SUL and/or BSO after LPS challenge. *Significant vs. vehicle samples, as analyzed by ANOVA, followed by Bonferroni post-test; †significant between SUL- and DEX-treated samples with or without LPS treatment; and ‡significant between two compared samples shown with the arrows, using Student's *t*-test. Data are means ± S.D.; $P < 0.01$. n = 12 per group.

Supplementary Figure 7: Pretreatment of sulforaphane-exposed macrophages with the HDAC inhibitor trichostatin A showed dexamethasone insensitivity. (**A-B**) Lipopolysaccharide (LPS, 10 μg/mL, 4 h) induces IL-8 mRNA expression, as assessed by qRT-PCR (**A**), IL-8 secreted protein levels are assayed by means of ELISA (**B**), and DEX pre-treatment (0.5 h) before LPS exposure shows dexamethasone (DEX, 1 μM) resistance that is reversed by SUL pre-treatment. In contrast, trichostatin A (TSA, 10 mM in ethanol, 2 h) treatment before DEX exposure in sulforaphane (SUL, 5 μM, 16 h)-exposed cells fails to reverse DEX resistance and fails to inhibit IL-8 expression induced by LPS. Data are means ± S.D. All assays were repeated three times (n = 4 samples per group).

Supplementary Figure 8: Sulforaphane treatment restores HDAC2 protein levels, but has no effect on HDAC2 mRNA expression. (**A-B**) HDAC2 nuclear protein levels, as

assessed by immunoblot (**A**) and quantified by densitometry (**B**) in alveolar macrophages from patients with COPD (n = 12) after sulforaphane (SUL, 5 μM, 16 h) treatment. (**C**) The mRNA levels of HDAC2 did not change in alveolar macrophages from patients with COPD (n = 12 per group) after SUL treatment. Data are means ± S.D. The assay was repeated three times.

FIGURE 1


A
Nuclear fraction
Cytoplasmic fraction
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Anti-Lamin B
Anti-Tubulin A
B
VEH
Ascorbate
GSNO
UV
HgCl2
DTT
HDAC2 (~55KD)
IP: anti-HDAC2
IB: anti-SNO
HDAC2 (~55KD)
IP: anti-HDAC2
IB: anti-HDAC2
C
VEH
GSH
GSNO
GSNO + GSH
IP: Anti-DDK
IB: Anti-SNO
Anti-DDK
D
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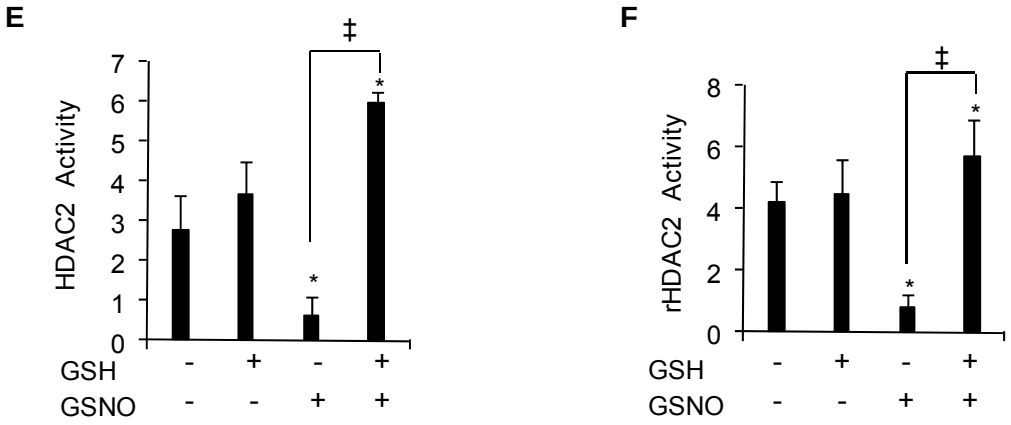
20
15
10
5
0
*
†
VEH
GSH
GSNO
GSNO+GSH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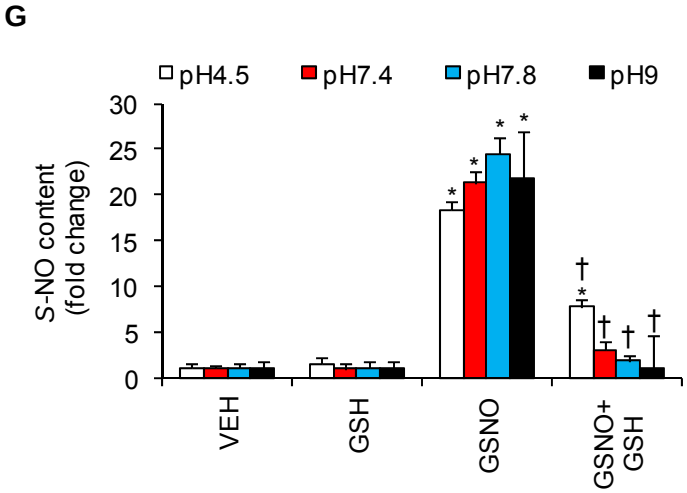
GSH
GSNO
F
rHDAC2 Activity
GSH
GSNO


G
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pH7.4
pH7.8
pH9
S-NO content (fold change)
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GSH
GSNO
GSNO+ GSH

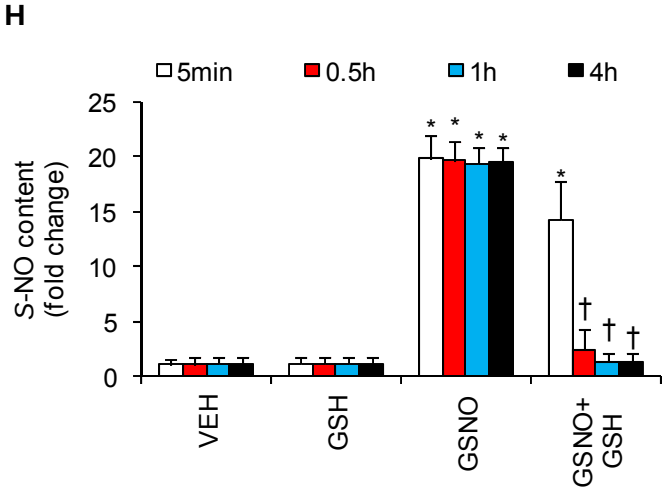
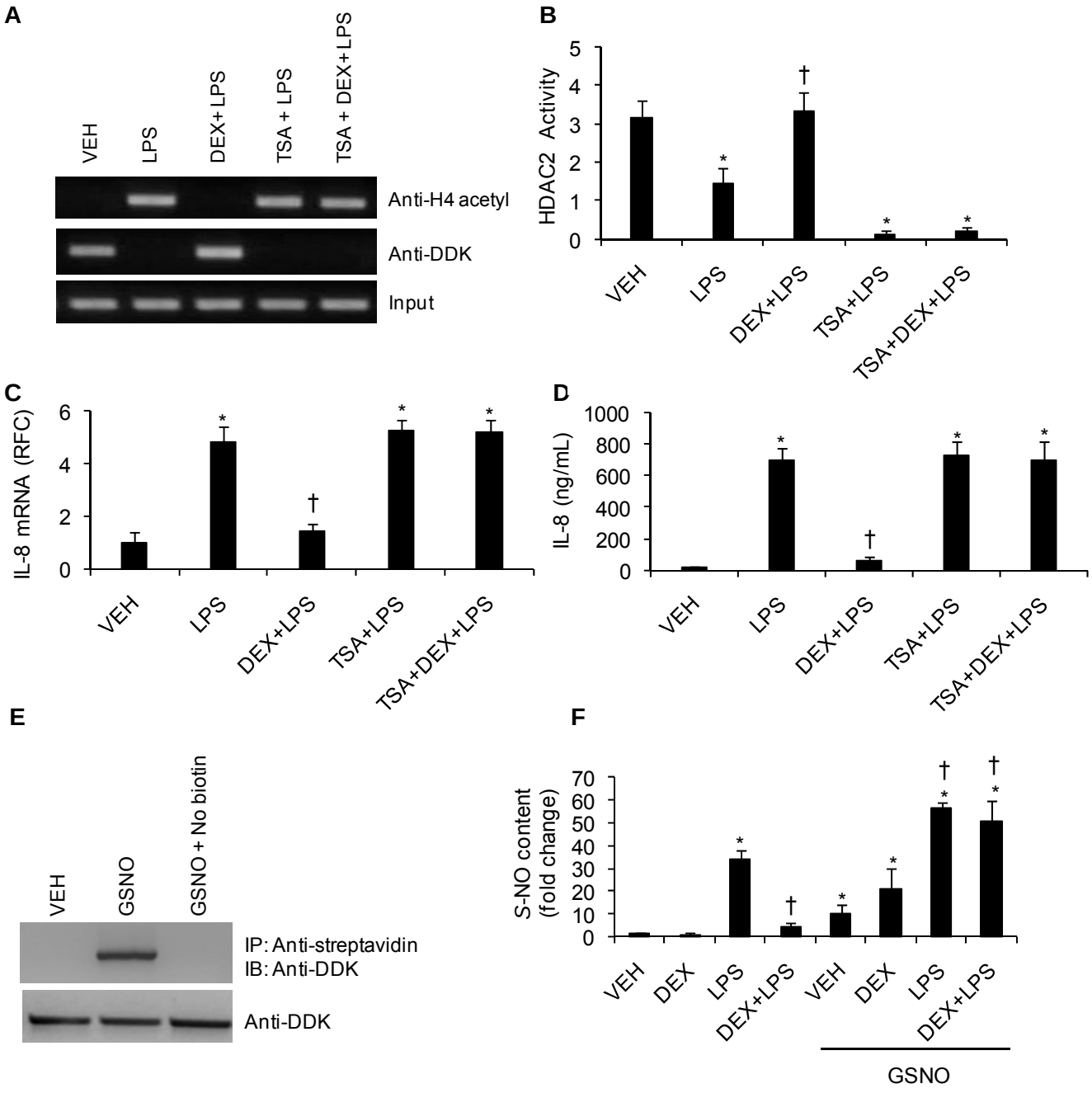

H
5min
0.5h
1h
4h
S-NO content (fold change)
VEH
GSH
GSNO
GSNO+ GSH

FIGURE 2


A
VEH
LPS
DEX+LPS
TSA + LPS
TSA + DEX+LPS
Anti-H4 acetyl
Anti-DDK
Input
B
HDAC2 Activity
VEH
LPS
DEX+LPS
TSA+LPS
TSA+DEX+LPS
C
IL-8 mRNA (RFC)
D
IL-8 (ng/mL)
E
VEH
GSNO
GSNO + No biotin
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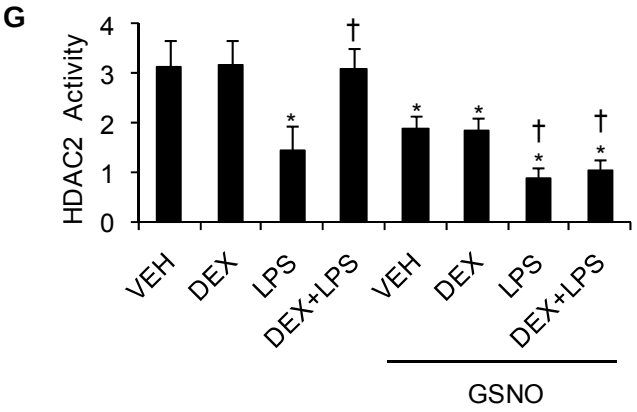
DEX
LPS
DEX+LPS
GSNO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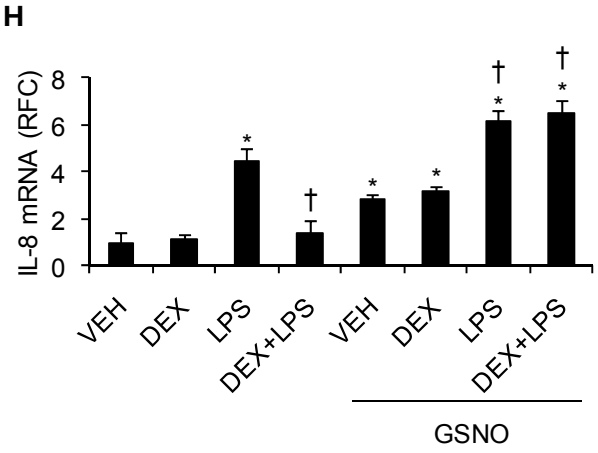
2
3
4
VEH
DEX
LPS
DEX+LPS
VEH
DEX
LPS
DEX+LPS
GSNO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6
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VEH
DEX
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VEH
DEX
LPS
DEX+LPS
GSNO

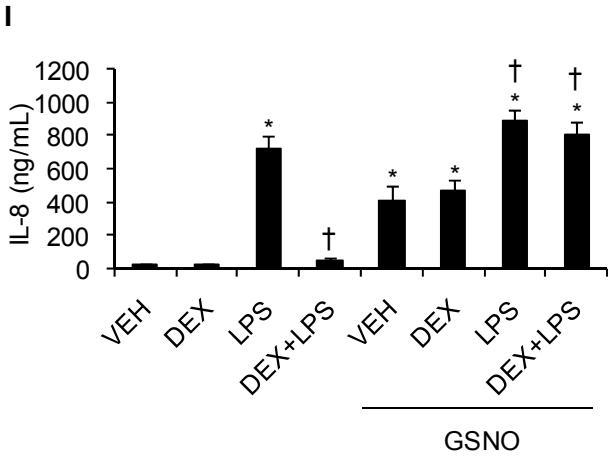
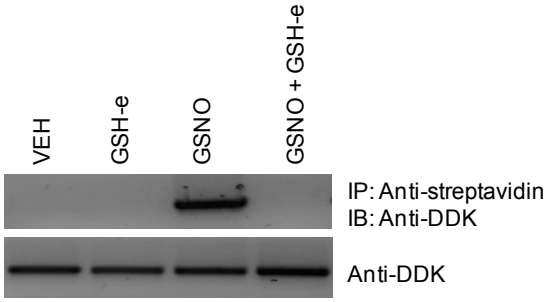

I
IL-8 (ng/mL)
0
200
400
600
800
1000
1200
VEH
DEX
LPS
DEX+LPS
VEH
DEX
LPS
DEX+LPS
GSNO

FIGURE 3

A


VEH
GSH-e
GSNO
GSNO + GSH-e
IP: Anti-streptavidin
IB: Anti-DDK
Anti-DDK

B

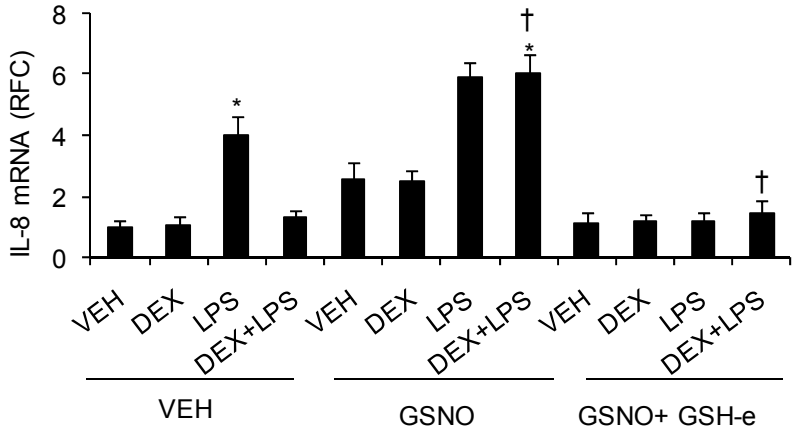

IL-8 mRNA (RFC)
8
6
4
2
0
*
†
*
†
VEH
DEX
LPS
DEX+LPS
VEH
DEX
LPS
DEX+LPS
VEH
DEX
LPS
DEX+LPS
VEH
GSNO
GSNO+ GSH-e

FIGURE 4

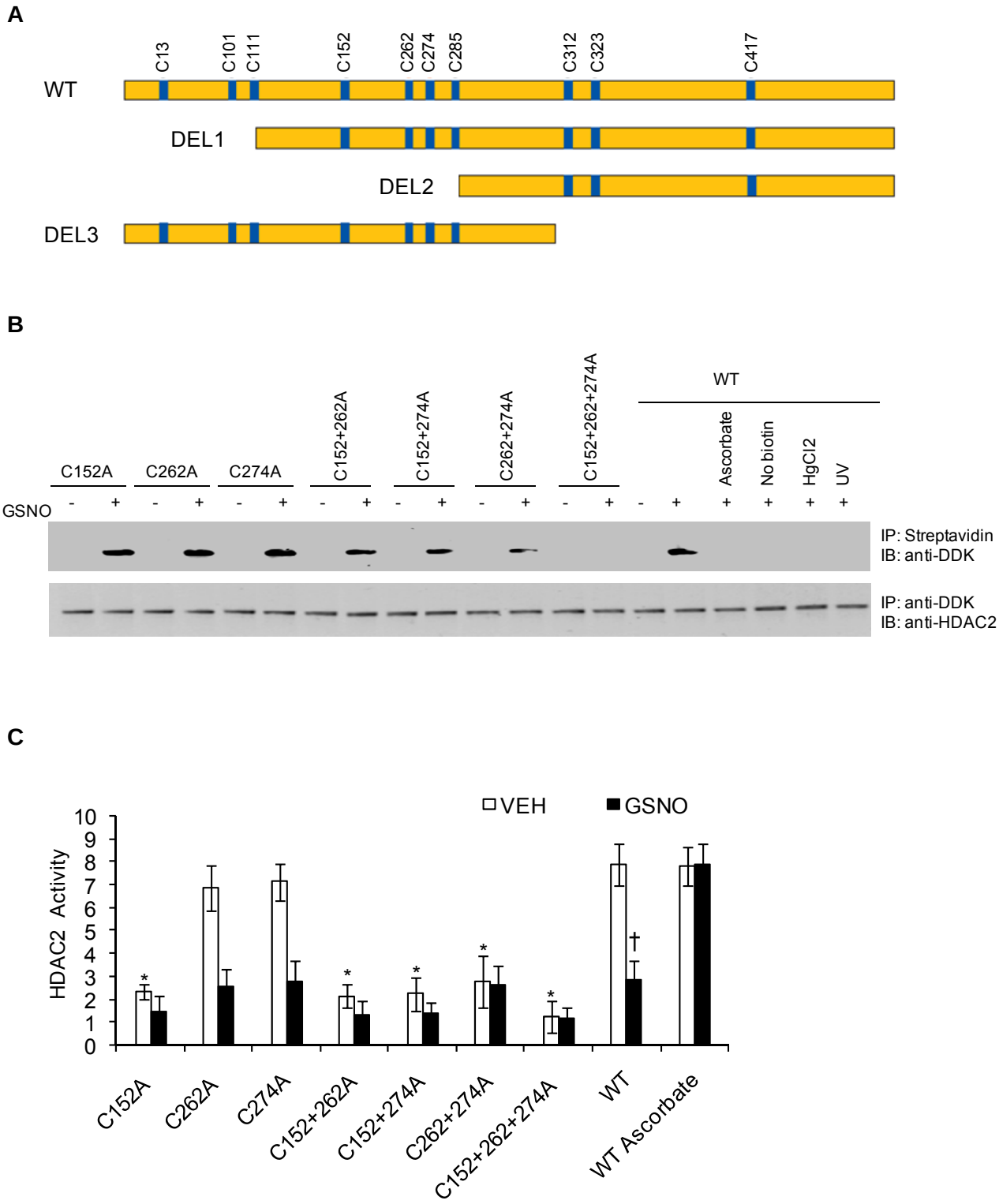

A
C13
C101
C111
C152
C262
C274
C285
C312
C323
C417
WT
DEL1
DEL2
DEL3
B
C152A
C262A
C274A
C152+262A
C152+274A
C262+274A
C152+262+274A
WT
Ascorbate
No biotin
HgCl2
UV
GSNO
IP: Streptavidin
IB: anti-DDK
IP: anti-DDK
IB: anti-HDAC2
C
VEH
GSNO
HDAC2 Activity
C152A
C262A
C274A
C152+262A
C152+274A
C262+274A
C152+262+274A
WT
WT Ascorbate

FIGURE 5

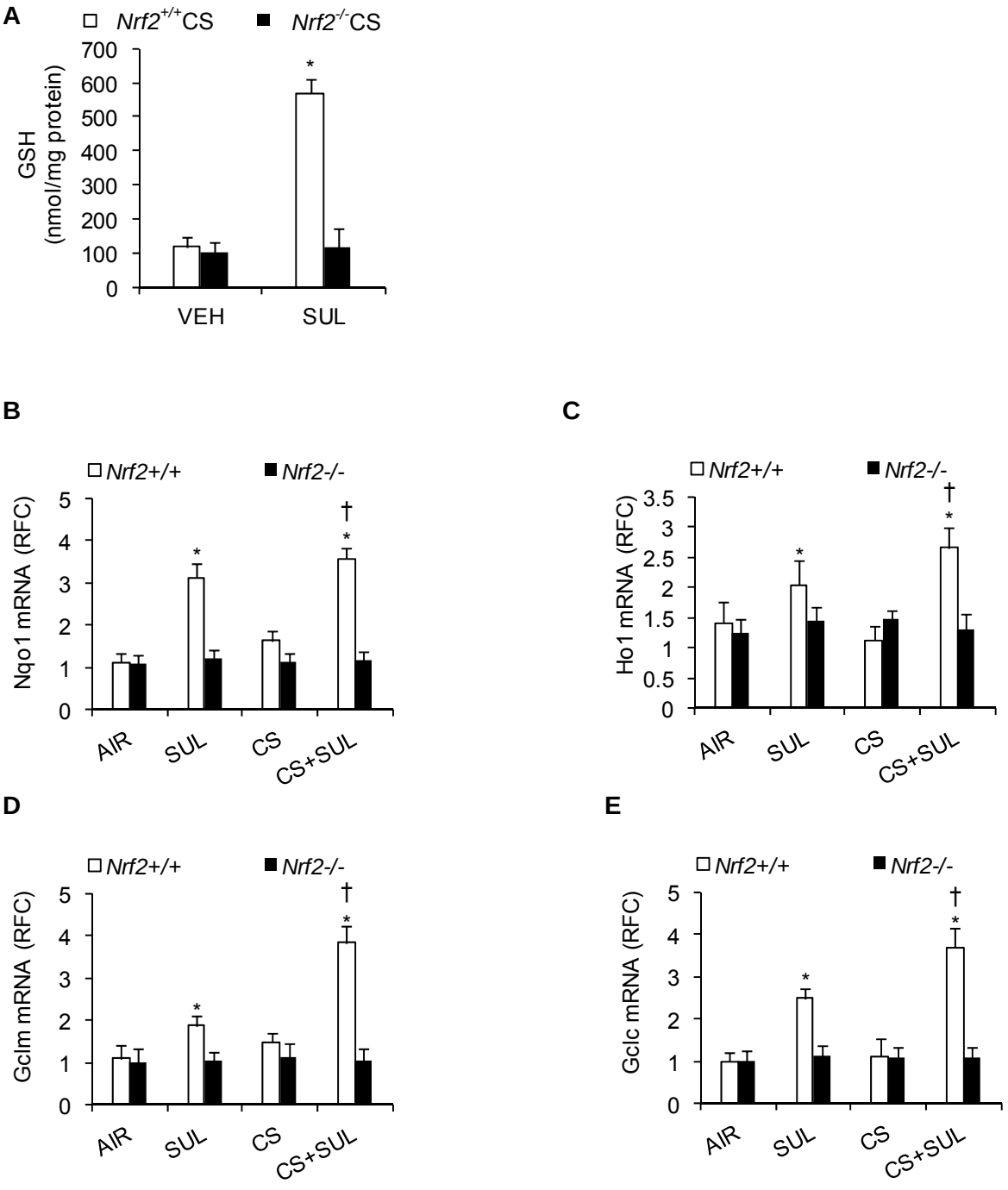

A
Nrf2+/+CS
Nrf2-/-CS
GSH (nmol/mg protein)
700
600
500
400
300
200
100
0
VEH
SUL
B
Nrf2+/+
Nrf2-/-
Nqo1 mRNA (RFC)
AIR
SUL
CS
CS+SUL
C
Ho1 mRNA (RFC)
D
Gclm mRNA (RFC)
E
Gclc mRNA (RFC)

FIGURE 6

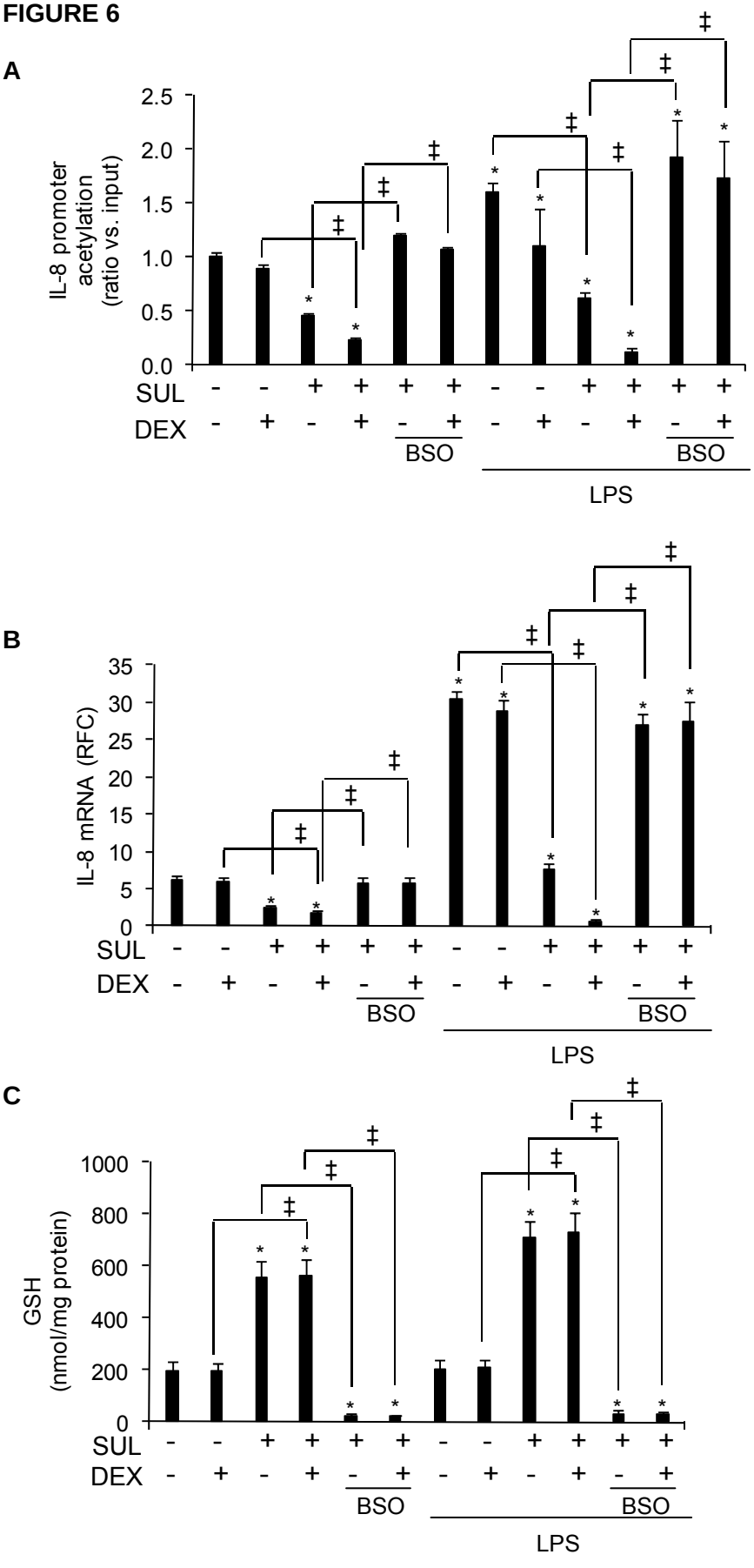
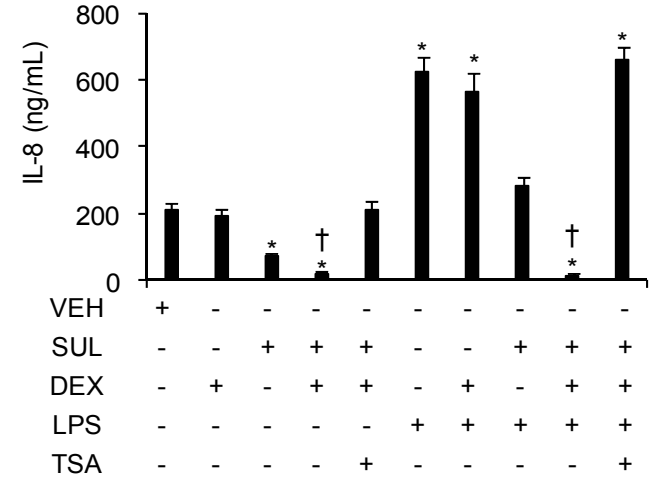

A
IL-8 promoter acetylation (ratio vs. input)
2.5
2.0
1.5
1.0
0.5
0.0
SUL - - + + + + - - + + + +
DEX - + - + - + - + - + - +
BSO
BSO
LPS
B
IL-8 mRNA (RFC)
35
30
25
20
15
10
5
0
SUL - - + + + + - - + + + +
DEX - + - + - + - + - + - +
BSO
BSO
LPS
C
GSH (nmol/mg protein)
1000
800
600
400
200
0
SUL - - + + + + - - + + + +
DEX - + - + - + - + - + - +
BSO
BSO
LPS

FIGURE 7

A


IL-8 (ng/mL)
800
600
400
200
0
VEH + - - - - - - - - -
SUL - - + + + - - + + +
DEX - + - + + - + - + +
LPS - - - - - + + + + +
TSA - - - - + - - - - +

B

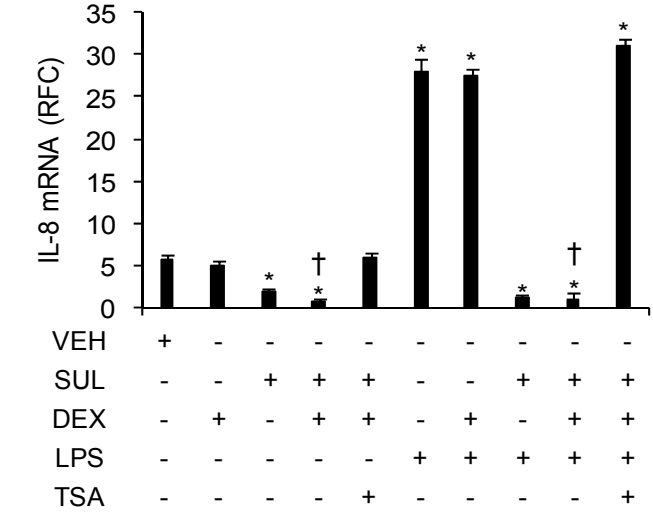
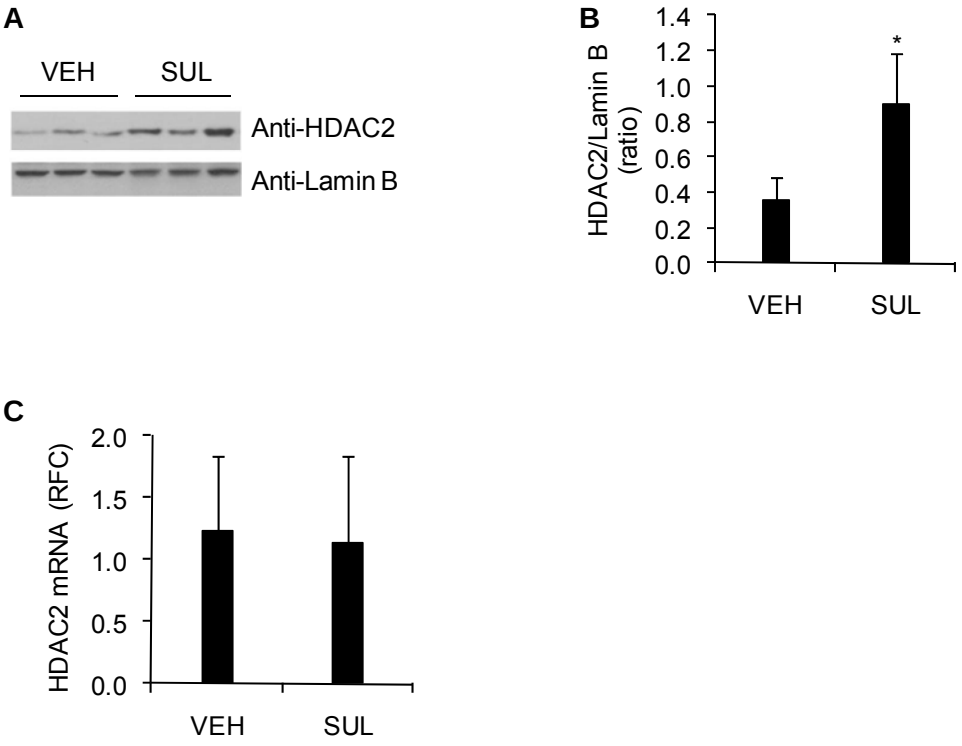

IL-8 mRNA (RFC)
35
30
25
20
15
10
5
0
VEH + - - - - - - - - -
SUL - - + + + - - + + +
DEX - + - + + - + - + +
LPS - - - - - + + + + +
TSA - - - - + - - - - +

FIGURE 8


A
VEH
SUL
Anti-HDAC2
Anti-Lamin B
B
HDAC2/Lamin B (ratio)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
VEH
SUL
C
HDAC2 mRNA (RFC)
2.0
1.5
1.0
0.5
0.0
VEH
SUL