

Supplementary Material

		<i>p</i> -value	statistical analysis
Fig. 2	Ctl vs. P* (1 mM)	0.0391	paired t-test
Fig. 3, (a)	Ctl vs. NaHS (0.5 mM)	0.8594	
	Ctl vs. NaHS (1 mM)	0.8694	
	Ctl vs. P* (0.5 mM)	0.3741	
	Ctl vs. P* (1 mM)	0.6623	
	Ctl vs. P* (0.5 mM)	> 0,9999	
	Ctl vs. P* (1 mM)	0,9961	
	NaHS (1 mM) vs. P* (1 mM)	> 0,9999	
	Ctl. vs. pos. Ctl	< 0,0001	
	NaHS (1 mM) vs. pos. Ctl	< 0,0001	
	P* (1 mM) vs. pos. Ctl	< 0,0001	
Fig. 3, (b)	Ctl vs. NaHS (0.5 mM)	0.8594	
	Ctl vs. NaHS (1 mM)	0.8694	
	Ctl vs. P* (0.5 mM)	0.3741	
	Ctl vs. P* (1 mM)	0.6623	
	NaHS (1 mM) vs. P* (1 mM)	0.9932	
Fig. 4, (a)	Ctl vs. NaHS (0.5 mM)	0,2090	
	Ctl vs. P* (0.5 mM)	0,1095	
	NaHS (0.5 mM) vs. P* (0.5 mM)	0,8702	
	Ctl vs. NaHS (0.5 mM)	0.0907	unpaired t-test
	Ctl vs. P* (0.5 mM)	0.0466	unpaired t-test
Fig. 4, (b) 6 h	Ctl vs. NaHS (0.5 mM)	0.9310	
	Ctl vs. NaHS (1 mM)	0.2405	
	Ctl vs. P* (0.5 mM)	0.7272	
	Ctl vs. P* (1 mM)	0.7913	
	NaHS (1 mM) vs. P* (1 mM)	0.7942	
	Ctl vs. NaHS (1 mM)	0.2159	unpaired t-test
	Ctl vs. P* (1 mM)	0.0354	unpaired t-test
Fig. 4, (b) 12 h	Ctl vs. NaHS (0.5 mM)	0.9990	
	Ctl vs. NaHS (1 mM)	0.9961	
	Ctl vs. P* (0.5 mM)	0.7391	
	Ctl vs. P* (1 mM)	0.0412	
	NaHS (1 mM) vs. P* (1 mM)	0.1161	
Fig. 4, (b) 24 h	Ctl vs. NaHS (0.5 mM)	> 0.9999	
	Ctl vs. NaHS (1 mM)	> 0.9999	
	Ctl vs. P* (0.5 mM)	0.8799	
	Ctl vs. P* (1 mM)	0.0027	
	NaHS (1 mM) vs. P* (1 mM)	0.0035	
Fig. 4, (c)			

	Ctl vs. FR P* (0.5 mM)	0.0083	
	Ctl vs. ON Cys	> 0.9999	
	Ctl vs. ON P* (0.5 mM)	< 0.0001	
	ON Cys vs. ON P* (0.5 mM)	< 0.0001	
	FR P* (0.5 mM) vs. ON P* (0.5 mM)	0.0055	
Fig. 5, (a)			
	neg ctl siRNA: Ctl vs. P* (0.5 mM)	0.0001	
	Nrf2 siRNA: Ctl vs. P* (0.5 mM)	0.9554	
	neg ctl siRNA Ctl vs. Nrf2 siRNA Ctl	< 0.0001	
	neg ctl siRNA P* (0.5 mM) vs. Nrf2 siRNA P* (0.5 mM)	< 0.0001	
Fig. 5, (b)			
	neg ctl siRNA Ctl vs. Nrf2 siRNA Ctl	< 0.0001	unpaired t-test
Fig. 5, (c)			
	p-Akt (T308): Ctl vs. P* (0.5 mM)	0.0450	unpaired t-test
	p-Akt (S473): Ctl vs. P* (0.5 mM)	0.0204	unpaired t-test
Fig. 5, (d)			
	Ctl vs. P* (0.5 mM)	0.0015	unpaired t-test
Fig. 5, (e)			
	Ctl vs. SB	> 0.9999	
	Ctl vs. U	> 0.9999	
	Ctl vs. LY	> 0.9999	
	Ctl vs. P* (0.5 mM)	0.0238	
	Ctl vs. SB + P* (0.5 mM)	0.7949	
	Ctl vs. U + P* (0.5 mM)	0.0179	
	Ctl vs. LY + P* (0.5 mM)	0.9409	
	P* (0.5 mM) vs. SB + P* (0.5 mM)	0.3274	
	P* (0.5 mM) vs. U + P* (0.5 mM)	> 0.9999	
	P* (0.5 mM) vs. LY + P* (0.5 mM)	0.1842	
Fig. 5, (f)			
	Ctl vs. P* (0.5 mM)	0.0017	
	Ctl vs. NAC	0.9993	
	Ctl vs. NAC + P* (0.5 mM)	0.9025	
	P* vs. NAC + P* (0.5 mM)	0.0053	
Fig. 6, (a)			
	Ctl vs. Men	< 0.0001	
	Ctl vs. NaHS (0.25 mM)	0.0400	
	Ctl vs. NaHS (0.5 mM)	0.1003	
	Ctl vs. NaHS (1 mM)	0.0620	
	Ctl vs. P* (0.25 mM)	0.7254	
	Ctl vs. P* (0.5 mM)	0.9454	
	Ctl vs. P* (1 mM)	0.9215	
	Men vs. NaHS (0.25 mM)	< 0.0001	
	Men vs. NaHS (0.5 mM)	< 0.0001	
	Men vs. NaHS (1 mM)	< 0.0001	
	Men vs. P* (0.25 mM)	< 0.0001	
	Men vs. P* (0.5 mM)	< 0.0001	
	Men vs. P* (1 mM)	< 0.0001	
	NaHS (1 mM) vs. P* (1 mM)	0.5007	
Fig. 6, (b)			

	Ctl vs. Men	< 0.0001
	Men vs. Men + NaHS (0.25 mM)	0.0235
	Men vs. Men + NaHS (0.5 mM)	0.0074
	Men vs. Men + NaHS (1 mM)	0.0017
	Men vs. Men + P* (0.25 mM)	0.0024
	Men vs. Men + P* (0.5 mM)	0.0010
	Men vs. Men + P* (1 mM)	0.0015
	Men + NaHS (1 mM) vs. Men + P* (1 mM)	> 0.9999
Fig. 6, (c)		
	neg ctl siRNA: Ctl vs. Men	0,5599
	neg ctl siRNA: Men vs. P* (1 mM)	0,0415
	Nrf2 siRNA: Ctl vs. Men	0,8961
	Nrf2 siRNA: Men vs. P* (1 mM)	0,9616
	neg ctl siRNA P* (1 mM) vs. Nrf2 siRNA P* (1 mM)	0,0028
Fig. 7, (a)		
	Stim vs. NaHS (0.25 mM)	> 0.9999
	Stim vs. NaHS (0.5 mM)	> 0.9999
	Stim vs. NaHS (1 mM)	0.5419
	Stim vs. P* (0.25 mM)	0.9353
	Stim vs. P* (0.5 mM)	0.5179
	Stim vs. P* (1 mM)	0.0018
	NaHS (1 mM) vs. P* (1 mM)	0.0496
Fig. 7, (b)		
	Stim vs. P* (0.25 mM)	0.0266
	Stim vs. P* (0.5 mM)	0.0146
	Stim vs. P* (1 mM)	< 0.0001
Fig. 7, (c)		
	Stim vs. P* (1 mM) pi	0.0004
	Stim vs. P* (1 mM) sim	0.0030
	Stim vs. P* (1 mM) post	0.0007
Fig. 7, (d)		
	Stim vs. NaHS (0.5 mM)	0.7111
	Stim vs. P* (0.5 mM)	< 0.0001
	NaHS (0.5 mM) vs. P* (0.5 mM)	< 0.0001
Fig. 7, (e)		
	Stim vs. P* (0.5 mM) pi	0.0003
	Stim vs. P* (0.5 mM) sim	0.0006
	Stim vs. P* (0.5 mM) post	0.0028
Fig. 7, (f)		
	Stim vs. NaHS (0.5 mM)	0.6639
	Stim vs. P* (0.5 mM)	0.0004
	NaHS (0.5 mM) vs. P* (0.5 mM)	0.0035
Fig. 8, (a)		
	Stim vs. P* (0.25 mM)	0.6812
	Stim vs. P* (0.5 mM)	< 0.0001
	Stim vs. P* (1 mM)	< 0.0001
Fig. 8, (b)		
	neg ctl siRNA: Stim vs. P* (0.25 mM)	0,1181
	neg ctl siRNA: Stim vs. P* (0.5 mM)	<0,0001

neg ctl siRNA: Stim vs. P* (1 mM)	<0,0001
Nrf2 siRNA: Stim vs. P* (0.25 mM)	>0,9999
Nrf2 siRNA: Stim vs. P* (0.5 mM)	0,3428
Nrf2 siRNA: Stim vs. P* (1 mM)	0,0012
neg ctl siRNA P* (1 mM) vs. Nrf2 siRNA P* (1 mM)	0,9055

Fig. 8, (c)

Stim vs. P* (0.25 mM)	0.0324
Stim vs. P* (0.5 mM)	0.0008
Stim vs. P* (1 mM)	< 0.0001
Stim vs. NAC	0.9623
NAC vs. NAC + P* (0.25 mM)	0.0485
NAC vs. NAC + P* (0.5 mM)	0.0011
NAC vs. NAC + P* (1 mM)	0.0002
P* (1 mM) vs. NAC + P* (1 mM)	0.8847

Fig. 8, (d)

Stim vs. FR P* (0.25 mM)	0.5526
Stim vs. FR P* (0.5 mM)	0.0009
Stim vs. FR P* (1 mM)	< 0.0001
Stim vs. Cys	> 0.9999
Cys vs. ON P* (0.25 mM)	0.3104
Cys vs. ON P* (0.5 mM)	0.0919
Cys vs. ON P* (1 mM)	0.0044
FR P* (1 mM) vs. ON P* (1 mM)	0.0030

Fig. 9, (a)

15 min p-NF-κB/NF-κB

Ctl vs. Stim	0.8953
Stim vs. NaHS (1 mM)	0.9146
Stim vs. P* (0.25 mM)	0.9466
Stim vs. P* (0.5 mM)	0.7999
Stim vs. P* (1 mM)	0.9130
NaHS (1 mM) vs. P* (1 mM)	> 0.9999

30 min p-NF-κB/NF-κB

Ctl vs. Stim	0.3319
Stim vs. NaHS (1 mM)	0.9703
Stim vs. P* (0.25 mM)	0.9587
Stim vs. P* (0.5 mM)	0.9074
Stim vs. P* (1 mM)	0.5940
NaHS (1 mM) vs. P* (1 mM)	0.9444

Fig. 9, (b)

15 min p-p38/p38

Ctl vs. Stim	0.0002
Stim vs. NaHS (1 mM)	0.9793
Stim vs. P* (0.25 mM)	0.9996
Stim vs. P* (0.5 mM)	> 0.9999
Stim vs. P* (1 mM)	0.4226
NaHS (1 mM) vs. P* (1 mM)	0.1607

30 min p-p38/p38

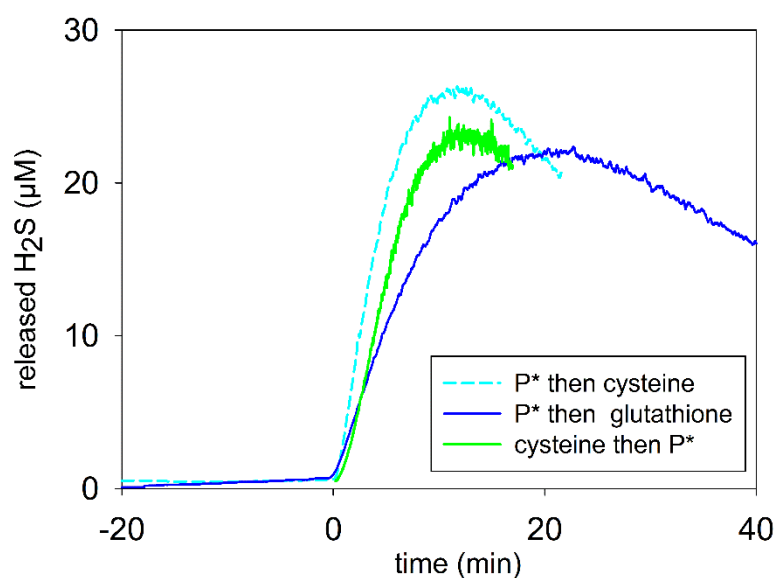
Ctl vs. Stim	0.1749
Stim vs. NaHS (1 mM)	0.3047
Stim vs. P* (0.25 mM)	0.3802
Stim vs. P* (0.5 mM)	0.0238
Stim vs. P* (1 mM)	0.0749

	NaHS (1 mM) vs. P* (1 mM)	0.9469	
15 min p-ERK1/2 / ERK1/2			
	Ctl vs. Stim	0.9084	
	Stim vs. NaHS (1 mM)	0.0098	
	Stim vs. P* (0.25 mM)	> 0.9999	
	Stim vs. P* (0.5 mM)	0.9987	
	Stim vs. P* (1 mM)	> 0.9999	
	NaHS (1 mM) vs. P* (1 mM)	0.0073	
30 min p-ERK1/2 / ERK1/2			
	Ctl vs. Stim	0.9844	
	Stim vs. NaHS (1 mM)	0.0116	
	Stim vs. P* (0.25 mM)	> 0.9999	
	Stim vs. P* (0.5 mM)	0.6600	
	Stim vs. P* (1 mM)	0.9904	
	NaHS (1 mM) vs. P* (1 mM)	0.0311	
Fig. 9, (c)			
	Stim vs. P* (0.25 mM)	0.2914	
	Stim vs. P* (0.5 mM)	0.1803	
	Stim vs. P* (1 mM)	0.0454	
	Stim vs. SB	0.9998	
	SB vs. SB + P* (0.25 mM)	0.8615	
	SB vs. SB + P* (0.5 mM)	0.2507	
	SB vs. SB + P* (1 mM)	0.0207	
	P* (1 mM) vs. SB + P* (1 mM)	0.0112	
Fig. 9, (d)			
	Stim vs. P* (0.25 mM)	0.2914	
	Stim vs. P* (0.5 mM)	0.1803	
	Stim vs. P* (1 mM)	0.0454	
	Stim vs. U	0.0324	
	U vs. U + P* (0.25 mM)	0.9933	
	U vs. U + P* (0.5 mM)	0.3814	
	U vs. U + P* (1 mM)	0.0627	
	P* (1 mM) vs. U + P* (1 mM)	0.9294	
Supplemental figure S4			
	Ctl vs. NaHS (0.5 mM)	0,9981	
	Ctl vs. NaHS (1 mM)	0,9331	
	Ctl vs. P* (0.5 mM)	0,0957	
	Ctl vs. P* (1 mM)	0,2610	
	NaHS (1 mM) vs. P* (1 mM)	0,6358	
	Ctl vs. NaHS (1 mM)	0,0658	unpaired t-test
	Ctl vs. P* (1 mM)	0,1223	unpaired t-test
Supplemental figure S6			
	Ctl vs. P* (0.5 mM)	0,6130	unpaired t-test
Supplemental figure S7			
	Ctl vs. Men	< 0,0001	
	Men vs. Men + hemin (10 µM)	0,0005	
Supplemental figure S10, (a)			
	Stim vs. P* (1 mM)	0,0016	
	Stim vs. penicillamine (1 mM)	0,1041	
	P* (1 mM) vs. penicillamine (1 mM)	0,0175	

Supplemental figure S10, (b)

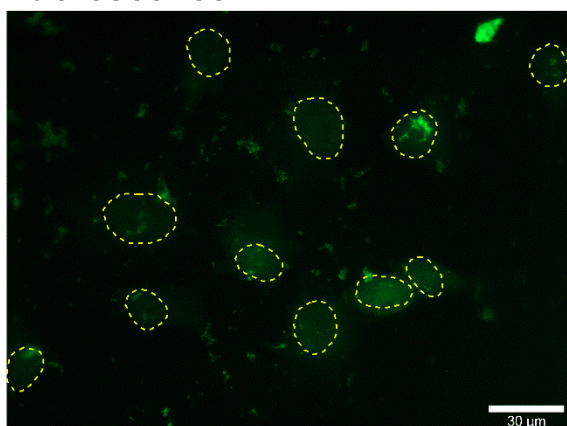
Stim vs. P* (1 mM)	0,0016
Stim vs. penicillamine (1 mM)	0,0555
P* (1 mM) vs. penicillamine (1 mM)	0,0002

Supplemental Table S1. Summary of meaningful *p*-values. If not otherwise stated, one-way ANOVA with Tukey's posthoc test was used for statistical comparison.

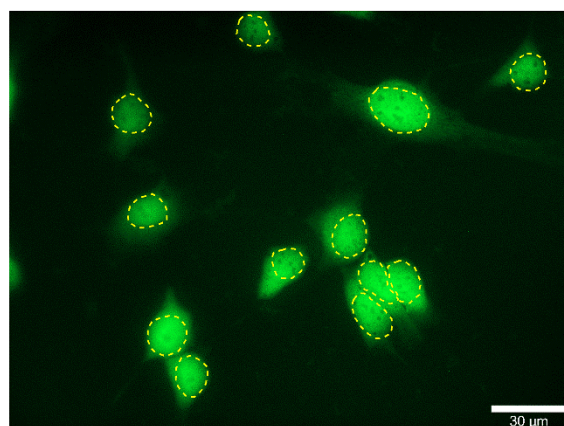


Supplemental figure S1. H₂S generation by P*. Thiol-induced H₂S release was monitored with the ISO-H₂S-2 sensor. A first substrate (P*, blue traces, or cysteine, green trace) was incubated in phosphate buffer (20 mM pH 7.4, containing 500 μM DTPA) for 20 minutes, then the second substrate (P*, cysteine or glutathione) was added (*t* = 0). Final concentrations were 40 μM for P* and 4 mM for L-cysteine or glutathione.

fluorescence

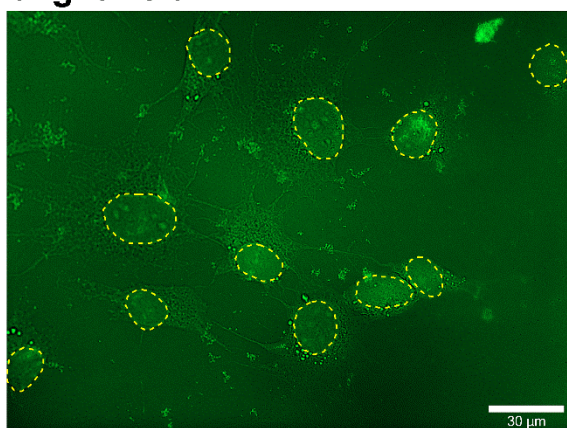


Ctl

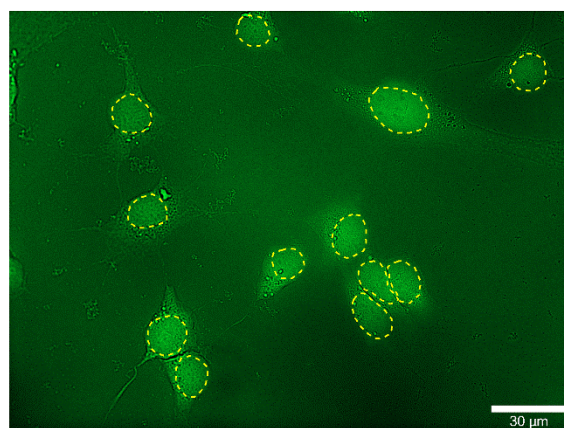


P*

bright-field

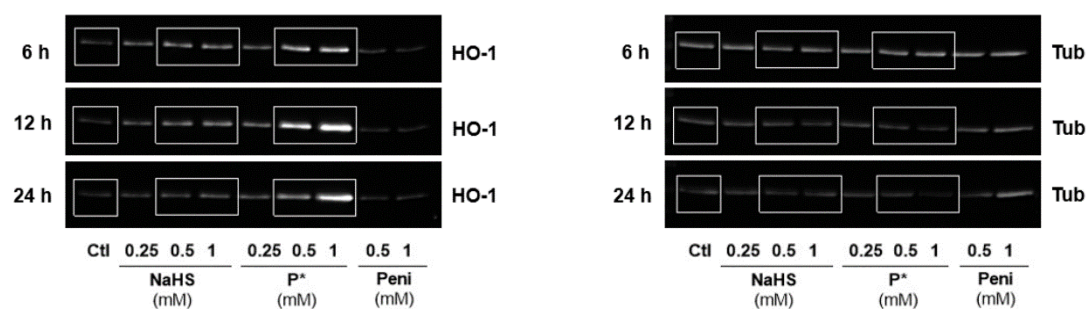


Ctl

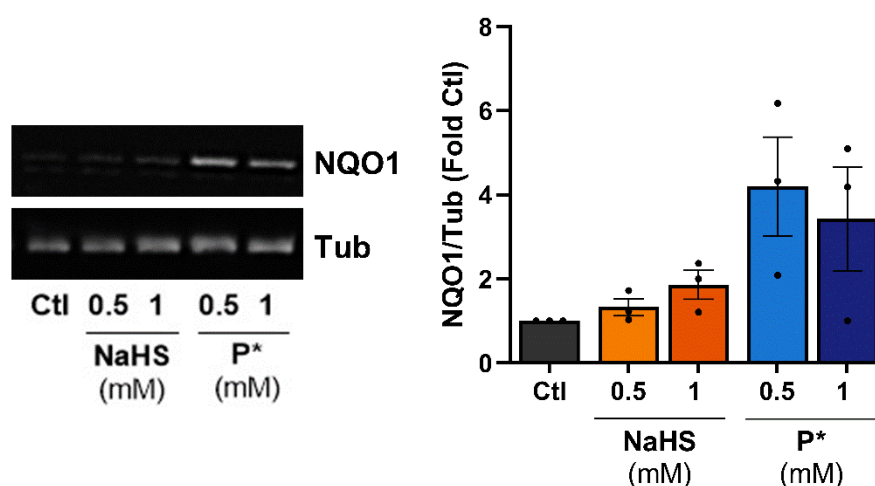


P*

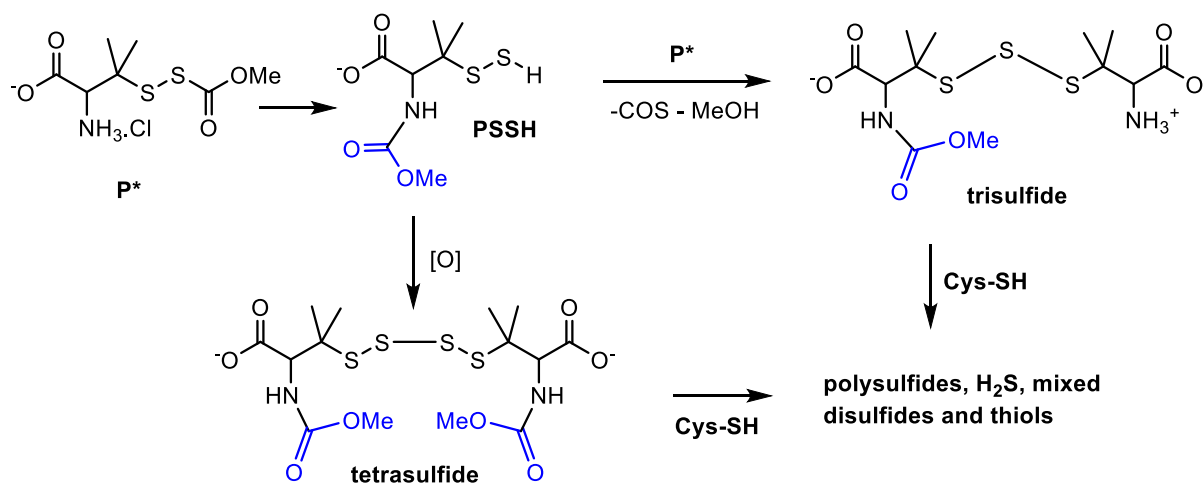
Supplemental figure S2. Fluorescence and bright-field images. ATDC5 cells were incubated for 30 min with the H₂S fluorescent probe WSP-5 followed by incubation with 1 mM P* for 30 min. Approximate nuclear outlines (yellow) were traced from the corresponding bright-field images presented above.



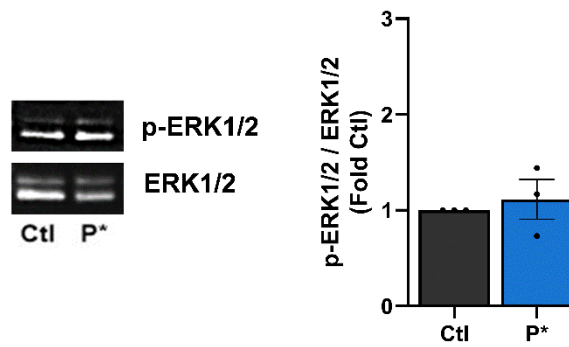
Supplemental figure S3. Original uncropped Western blot images related to Figure 4, P* induces HO-1 expression. ATDC5 cells were incubated with increasing concentrations of NaHS or P* for 6, 12 or 24 h. Representative full Western blots of the expression of HO-1 and α , β -tubulin (Tub).



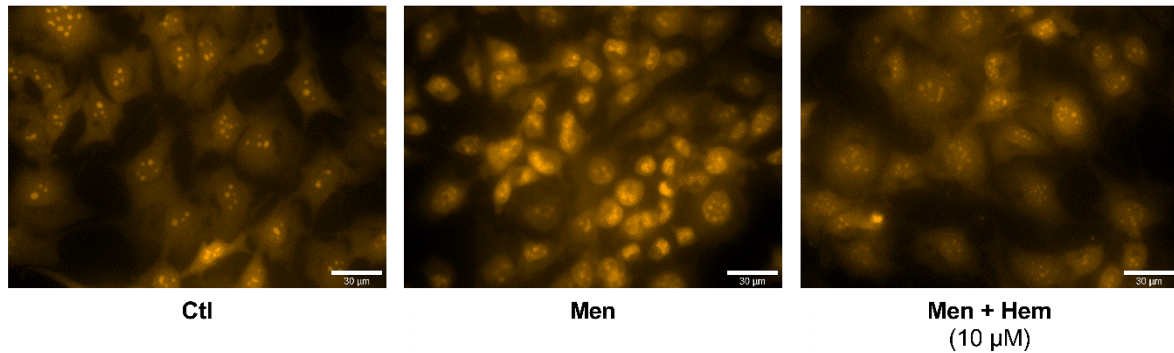
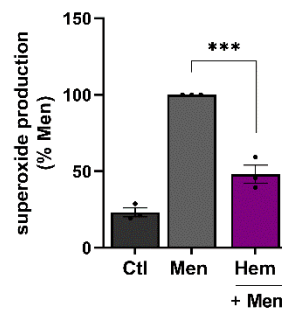
Supplemental figure S4. P* induces NQO1 expression. ATDC5 cells were incubated with increasing concentrations of NaHS or P* for 24 h. Representative Western blots of the expression of NQO1 and α , β -tubulin (Tub). Densitometry analysis values of NQO1 were normalized against Tub and then compared with untreated cells (Ctl) set to 1, n = 3.



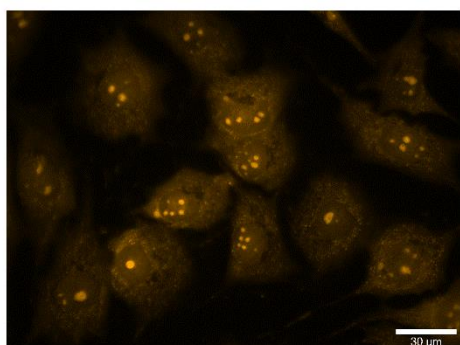
Supplemental figure S5. Formation of tri- and tetrasulfides by P*. P* is converted to its persulfide, PSSH, in a pH-driven S-to-N methoxycarbonyl transfer. P* may react with PSSH to give the trisulfide along with the volatiles COS and methanol, and PSSH can be oxidized to the tetrasulfide by traces of metal from the buffer. All these compounds may react with additional thiols to give complex dynamic mixtures of thiols, disulfides, polysulfides and hydrogen sulfide.



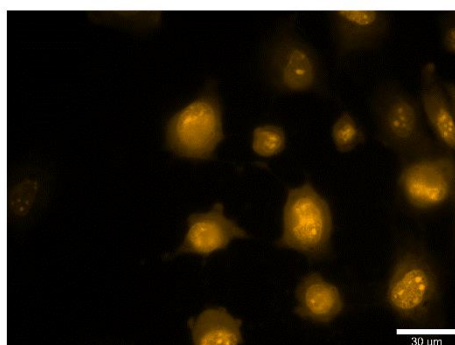
Supplemental figure S6. P* does not affect ERK1/2 phosphorylation. ATDC5 cells were incubated with 0.5 mM P* for 6 h. Representative Western blots of the expression of p-ERK1/2 and ERK1/2. Densitometry analysis values of p-ERK1/2 were normalized against ERK1/2 with respect to untreated cells (Ctl) set to 1, n = 3.



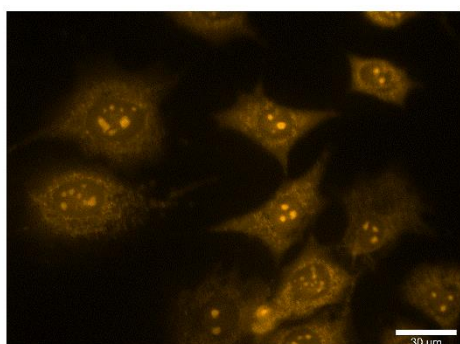
Supplemental figure S7. Hemin reduces menadione-induced superoxide production. ATDC5 cells were incubated with 10 μM hemin (Hem) for 24 h followed by 50 μM menadione (Men) for 4 h. Superoxide production was measured and normalized to Men (100 %), n = 3, *** $p < 0.001$.



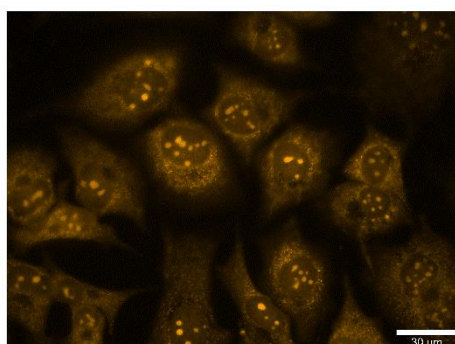
Ctl



Men

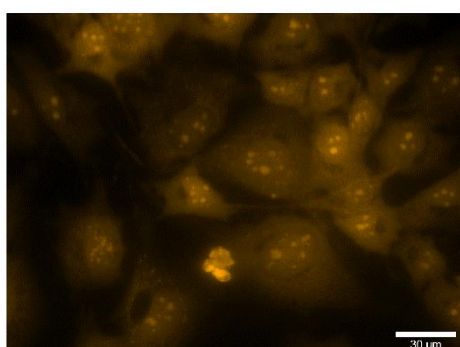


NaHS
(1 mM)

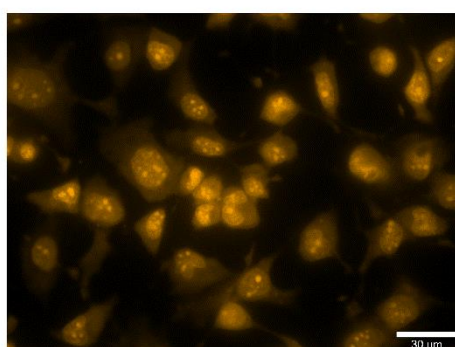


P*
(1 mM)

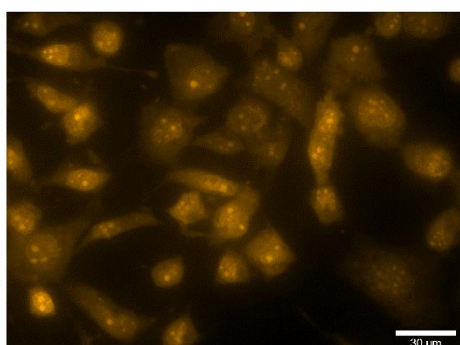
(a)



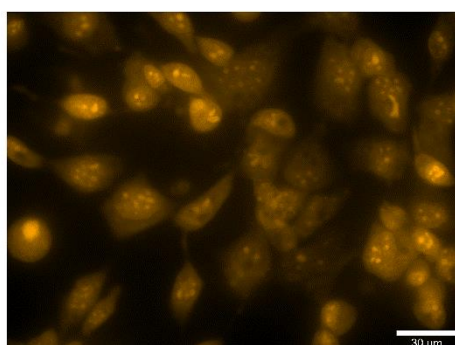
Ctl



Men



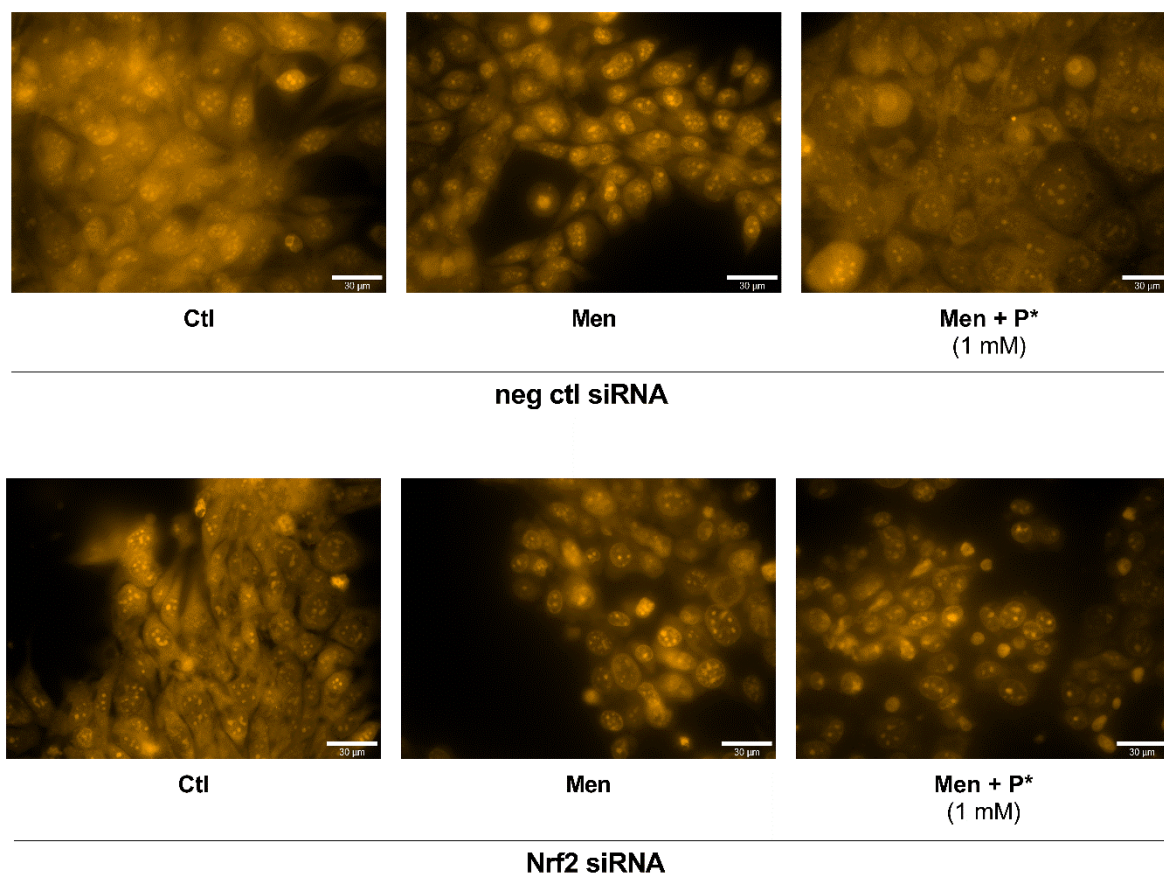
Men + NaHS
(1 mM)



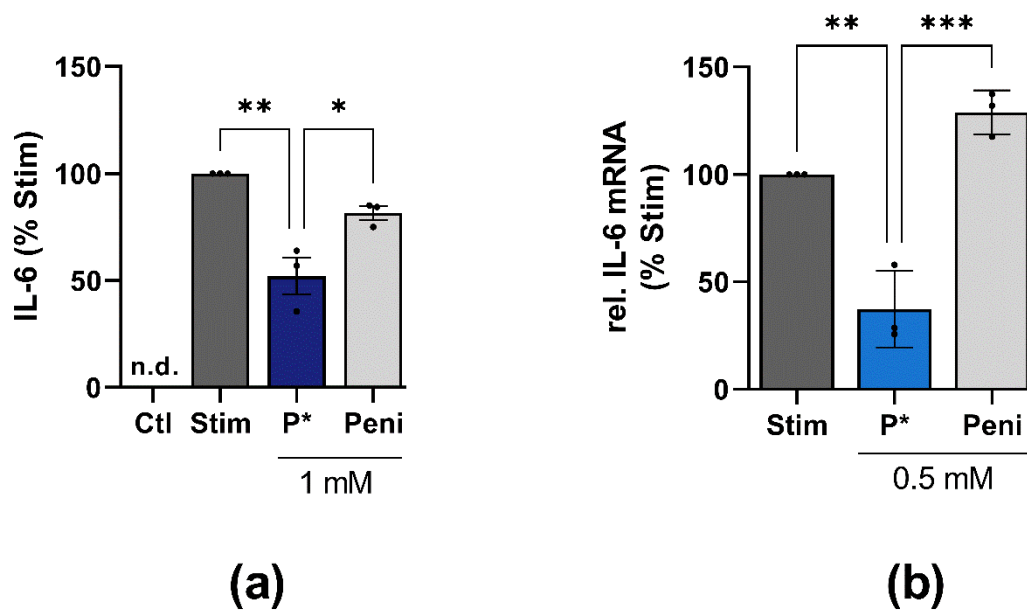
Men + P*
(1 mM)

(b)

Supplemental figure S8. Representative images of superoxide measurement. (a) ATDC5 cells were incubated for 1 h with NaHS or P* followed by 4 h of incubation with 10 μ M hydroethidine with or without 100 μ M menadione (Men) before fluorescence imaging was performed. (b) ATDC5 cells were incubated for 24 h with NaHS or P*, washed and incubated with 10 μ M hydroethidine with or without 100 μ M menadione (Men) for 4 h before fluorescence imaging was performed.



Supplemental figure S9. Representative images of superoxide measurement in transfected ATDC5 cells. ATDC5 cells transfected with Nrf2 (Nrf2 siRNA) or negative control (neg ctrl siRNA) siRNA were incubated for 24 h with 1 mM P*, washed and incubated with 10 μ M hydroethidine with or without 100 μ M menadione (Men) for 4 h before fluorescence imaging was performed.



Supplemental figure S10. Penicillamine does not affect IL-6 levels. (a). ATDC5 cells were incubated with 1 mM P* or penicillamine (Peni) for 1 h followed by stimulation with IL-1 β /IFN- γ for 24 h. IL-6 levels were determined by ELISA and normalized against stimulated cells (Stim) set to 100 %, $n = 3$, * $p < 0.05$, *** $p < 0.001$. (b) Primary human chondrocytes from OA patients were preincubated for 1 h with 0.5 mM P* or Peni and stimulated for 24 h with IL-1 β . IL-6 mRNA levels were determined by qPCR with SDHA as a reference gene and normalized to Stim (100 %), $n = 3$, ** $p < 0.01$, *** $p < 0.001$.