
Supplementary information

Hydropersulfides inhibit lipid peroxidation and ferroptosis by scavenging radicals

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SUPPLEMENTARY INFORMATION

Persulfides inhibit lipid peroxidation and ferroptosis by scavenging free radicals

Uladzimir Barayeu^{1,2}, Danny Schilling^{1,2,+}, Mohammad Eid^{1,2,+}, Tamara Nishida Xavier da Silva³, Lisa Schlicker^{4,5}, Nikolina Mitreska⁶, Christopher Zapp⁷, Frauke Gräter⁷, Aubry K. Miller⁸, Reinhard Kappl⁶, Almut Schulze⁴, José Pedro Friedmann Angeli³, Tobias P. Dick^{1,2,*}

¹Division of Redox Regulation, German Cancer Research Center (DKFZ), Heidelberg, Germany

²Faculty of Biosciences, Heidelberg University, Heidelberg, Germany

³Rudolf-Virchow-Zentrum - Center for Integrative and Translational Bioimaging, University of Würzburg, Würzburg, Germany

⁴Division of Tumor Metabolism and Microenvironment, German Cancer Research Center (DKFZ), Heidelberg, Germany

⁵Proteomics Core Facility, German Cancer Research Center (DKFZ), Heidelberg, Germany

⁶Department of Biophysics, Faculty of Medicine, Center for Integrative Physiology and Molecular Medicine (CIPMM), Saarland University, Homburg, Germany

⁷Molecular Biomechanics, Heidelberg Institute for Theoretical Studies (HITS), Heidelberg, Germany

⁸Research Group Cancer Drug Development, German Cancer Research Center (DKFZ), Heidelberg, Germany

⁺Equal contribution

^{*}Corresponding author

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Supplementary Table 1. Plasmids and siRNAs used in this study

Plasmids:

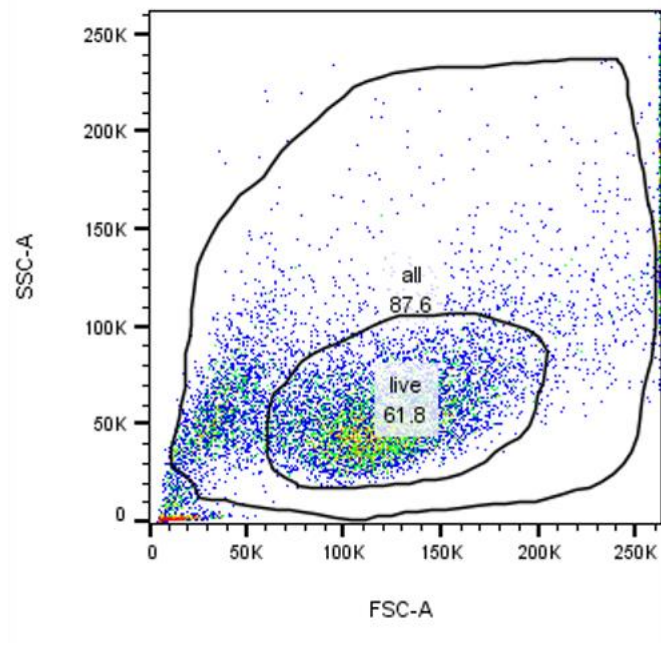
APEX2	72558, pTRC-APEX2, Addgene
CSE	114777419, GPCF, DKFZ
ETHE	194472089, GPCF, DKFZ
pMD2.G	12259, Addgene

siRNAs:

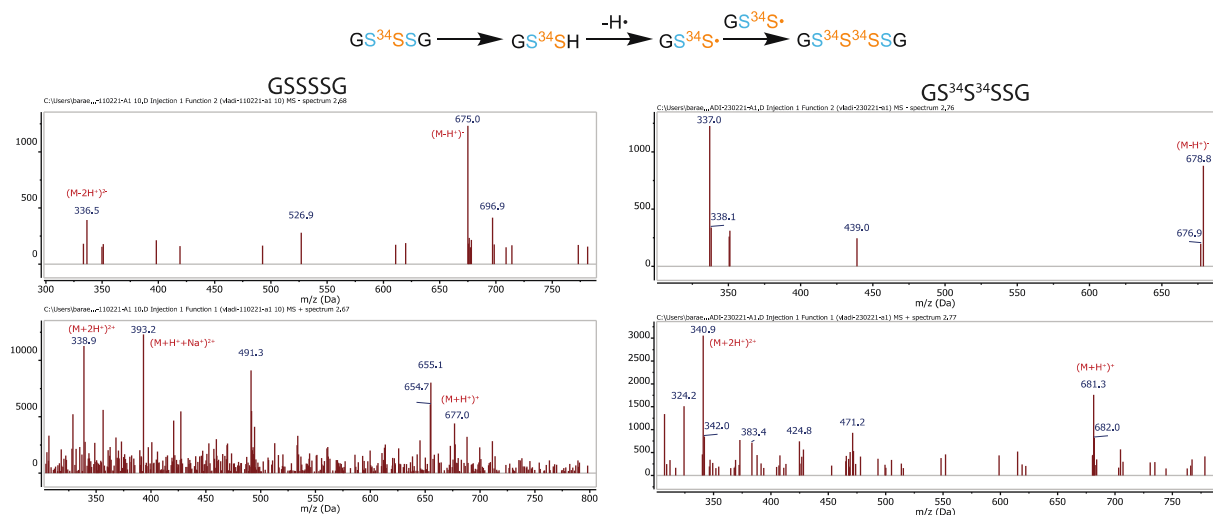
CSE	003481, ON-TARGETplus, SMARTpool, Horizon Discovery
ETHE1	012508, ON-TARGETplus, SMARTpool, Horizon Discovery
SQR	008271, ON-TARGETplus, SMARTpool, Horizon Discovery
MST	010119, ON-TARGETplus, SMARTpool, Horizon Discovery

Supplementary Table 2. MS/MS transition list for per/polysulfides

Metabolite	MBB derivative	Parent ion (M+H ⁺)	Fragment ion (M+H ⁺)
CSSSC	-	273,0032	122,0274
GSSSG	-	645,1313	387,0471
GSSSSG	-	677,1034	372,0139
H ₂ S	Bim-S-Bim	415,1435	193,0971
H ₂ S ₂	Bim-SS-Bim	447,1155	192,0893
GSH	Bim-SG	498,1653	225,0693
GSSH	Bim-SSG	530,1374	192,0893
GSSSH	Bim-SSSG	562,1095	192,0891
Cys	Bim-Cys	312,1013	192,089
CysSH	Bim-SCys	344,0733	192,089



Supplementary Figure 1. FACS gating strategy
Gating of the live cell population is indicated.



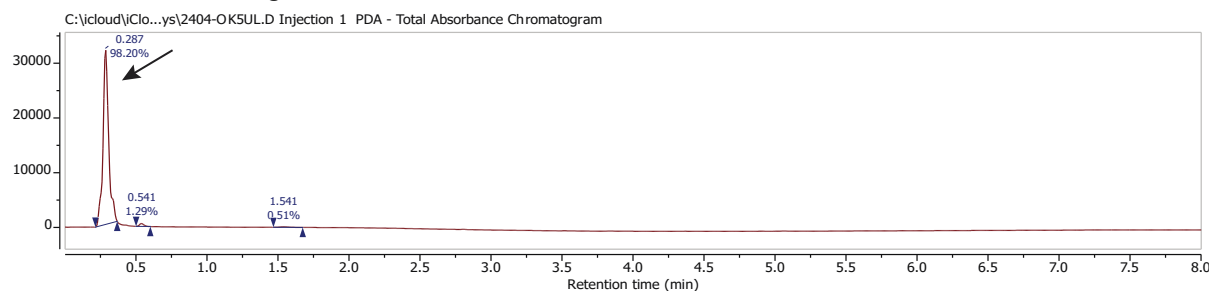
Supplementary Figure 2. MS-based detection of isotopically labeled GSSSG.

The reaction scheme on top explains the generation of doubly-labeled GS³⁴S³⁴SSG from singly-labeled GS³⁴SSG. GS³⁴SSG is reduced to GS³⁴SH, which then reacts with a radical to form GS³⁴S[•]. The perthiyl radical self-recombines to generate GS³⁴S³⁴SSG. Mass spectra of unlabeled GSSSG (left panels) and doubly-labeled GS³⁴S³⁴SSG (right panels) formed in the reaction of ferric cytochrome c with GSSH and GS³⁴SH, respectively. Negative (upper panels) and positive ion mode (lower panels).

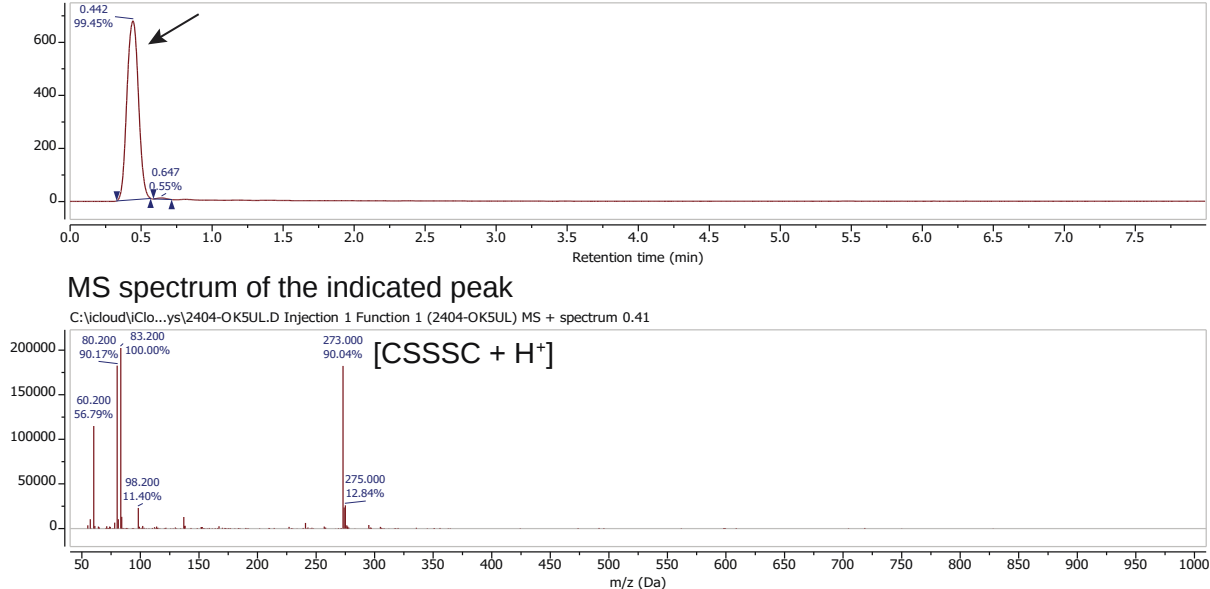
Supplementary Note 1. LC/MS-based characterization of synthesized CSSSC

Cysteine trisulfide (CSSSC)

HPLC chromatogram



MS spectrum of the indicated peak



MS² spectrum

