

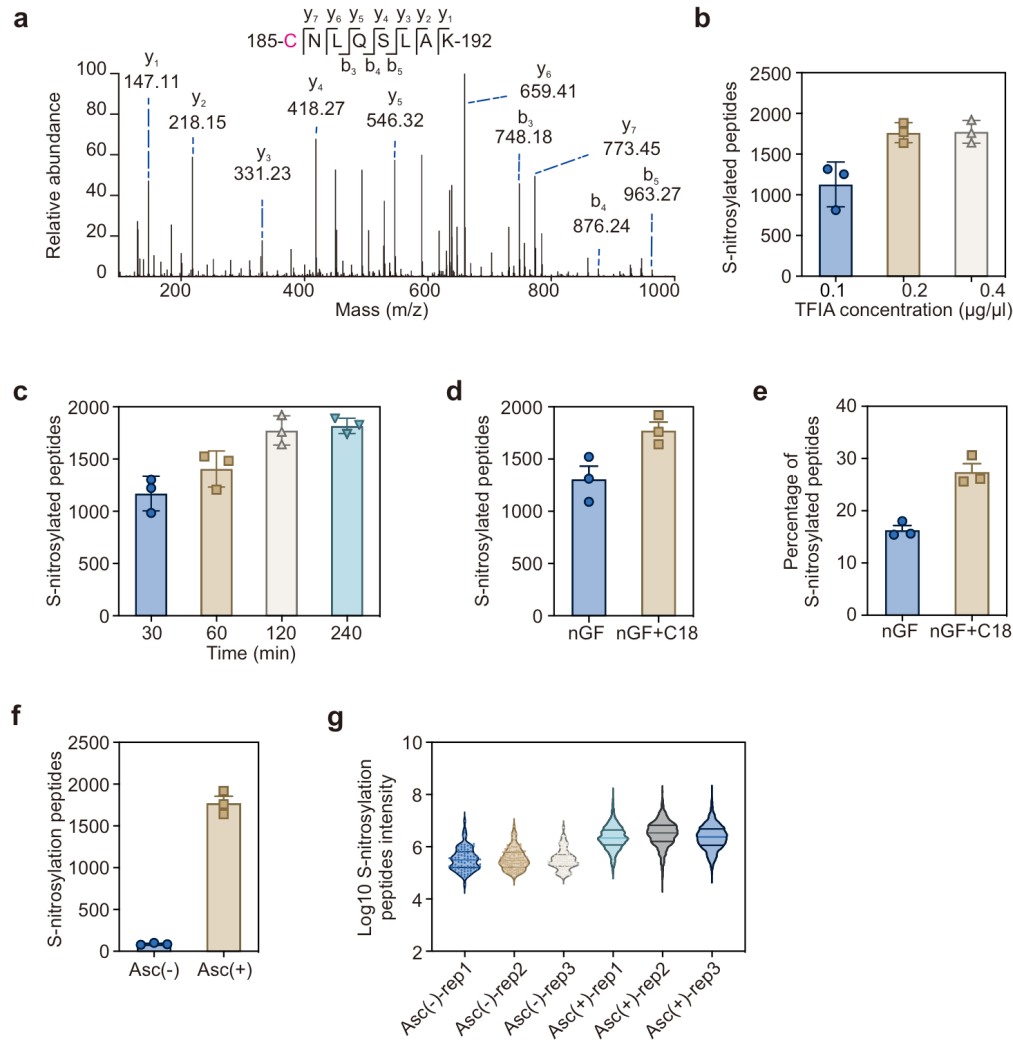
**FAT-switch-based quantitative S-nitrosoproteomics reveal a key role of GSNOR1 in
regulating ER functions**

Qin et al.

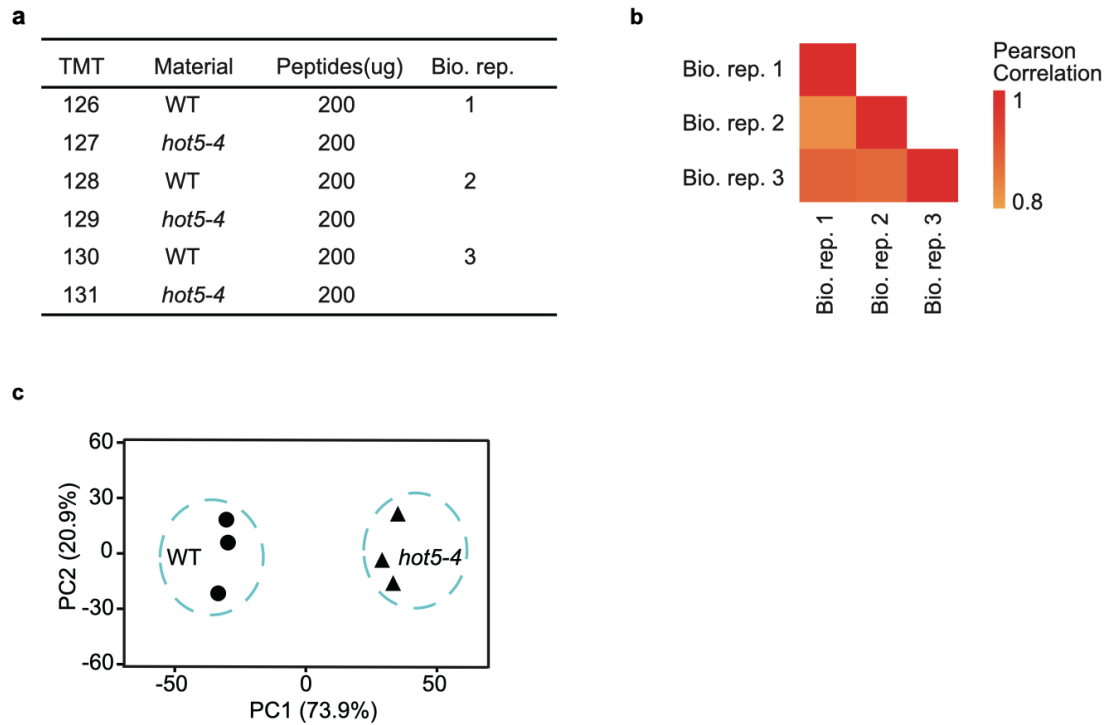
Supplementary Information

This file includes:

Supplementary Figure 1 to Figure 5 with figure legends, Supplementary Table I and II
with references, and list of Supplementary Datasets.

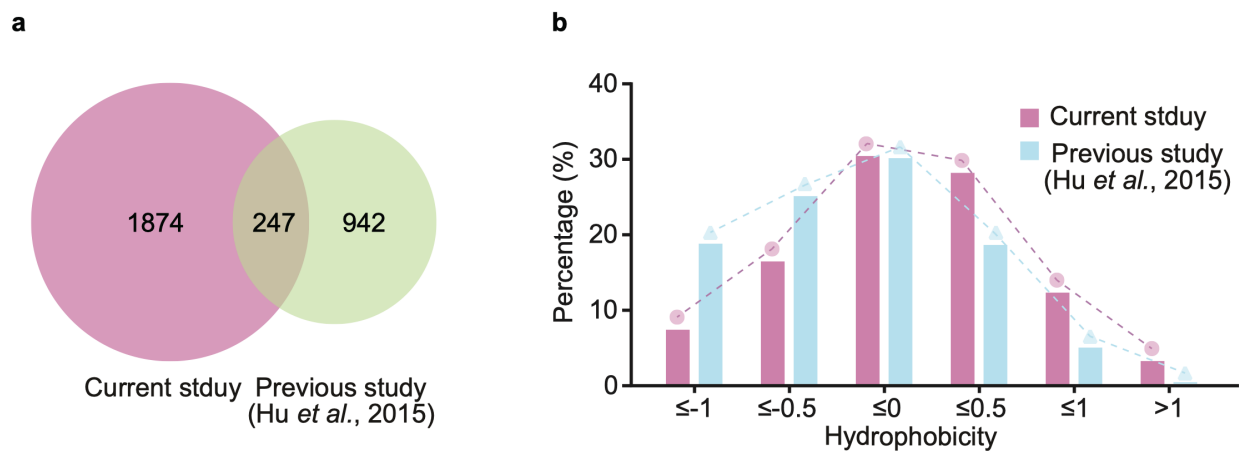


Supplementary Fig. 1 FAT-switch method shows higher sensitivity than biotin-switch method. **a** MS/MS spectrum of C¹⁸⁵NLQSLAK peptide from PYL4 protein. The increase of 417.04 Da on the cysteine residue indicates that the site was labeled by TFIA. **b** The number of S-nitrosylated peptides identified by LC-MS/MS in different TFIA concentration. Error bars indicate SD (n = 3 biologically independent samples). **c** The number of S-nitrosylated peptides identified by LC-MS/MS in different incubation time with TFIA. Error bars indicate SD (n = 3). **d** The number of identified S-nitrosylated peptides with or without C18 enrichment. Error bars indicate SD (n = 3 biologically independent samples). **e** The percentage of identified S-nitrosylated peptides in all peptides with or without C18 enrichment. Error bars indicate SD (n = 3). **f** The number of S-nitrosylation peptides identified by FAT-switch method with or without ascorbate (Asc), three replicates were performed. Error bars indicate SD (n = 3 biologically independent samples). **g** Boxplots of log₁₀ MS/MS intensity of all identified S-nitrosylation peptides in fluoros tag (F-tag) switch approach with or without ascorbate (Asc). The horizontal lines in the boxplots indicate the median; whisker caps and circles indicate the 10th-90th and 1st-99th percentiles, respectively.

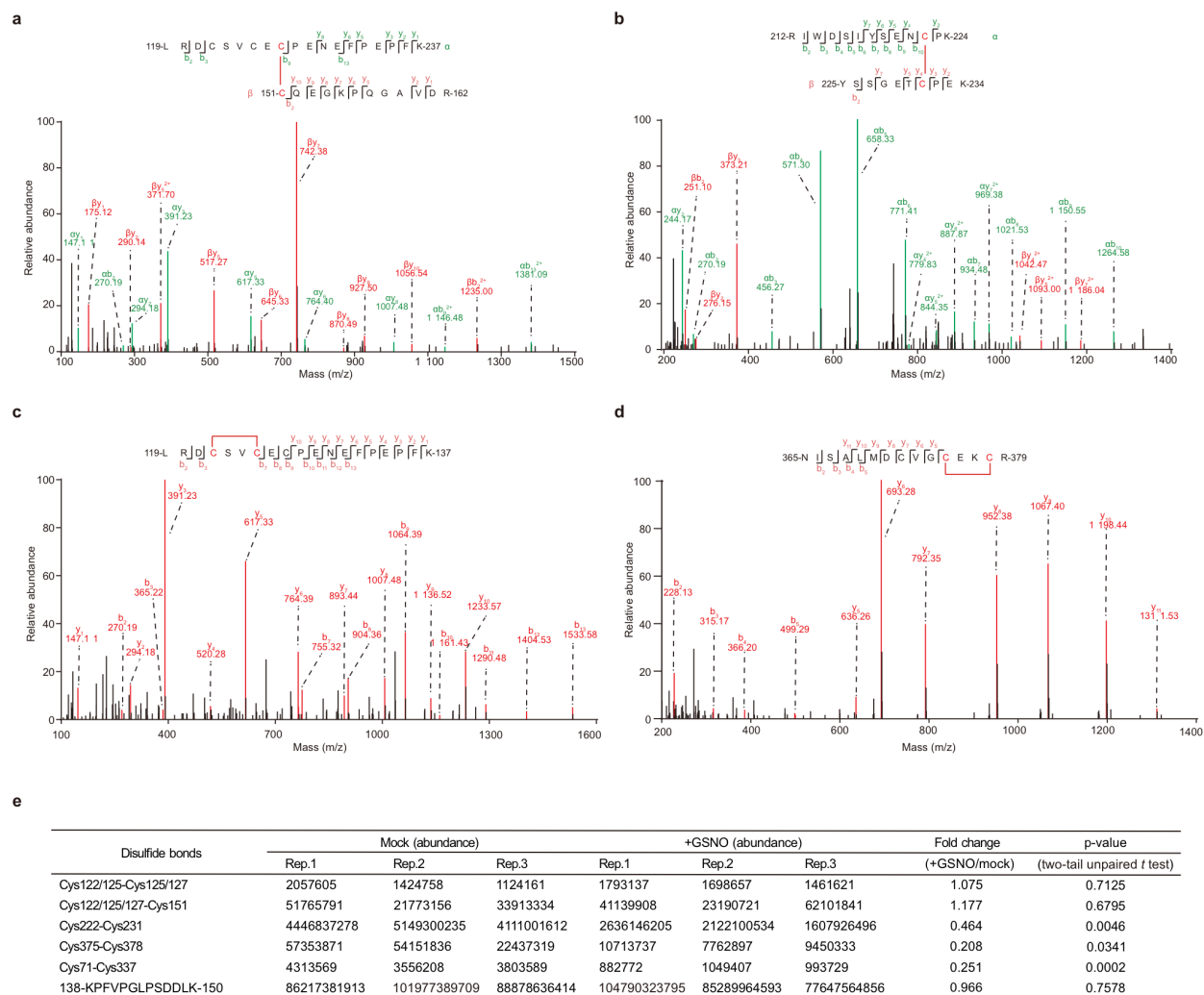


Supplementary Fig. 2 Comparison of the biological replicates in TMT-sixplex labeling.

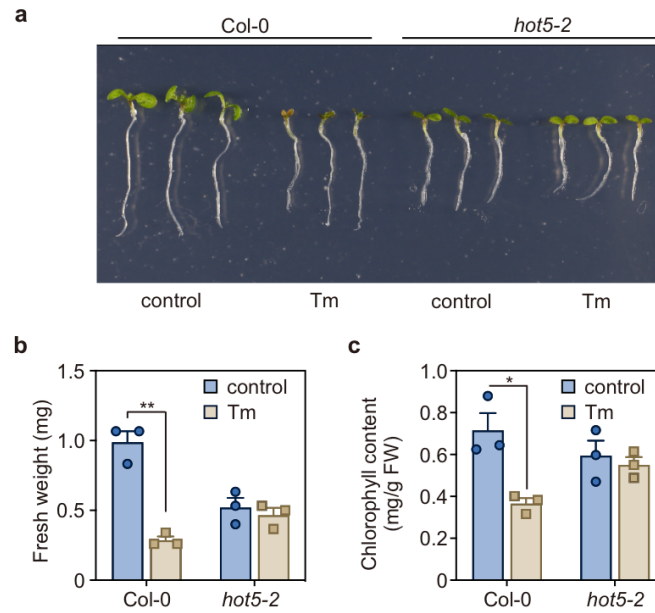
a TMT six-plex labeling of wild type and *hot5-4* seedlings. **b** Pearson's correlation of *hot5-4*/WT S-nitrosylation peptide ratios between biological replicates. **c** Principal component analysis (PCA) of the S-nitrosylation sites across all six samples.



Supplementary Fig. 3 Comparison of S-nitrosylated peptides identified by this study and previous studies. a The number of S-nitrosylated peptides identified in this study and previous study. **b** Hydrophobic distribution of all identified S-nitrosylation peptides in in this study (red) and previous study (blue).



Supplementary Fig. 4 Identified disulfide bonds with in ERO1 by LC-MS/MS. a MS/MS spectrum of the disulfide bonds linked peptides between Cys122/125/127 and Cys151 in ERO1 protein. **b** MS/MS spectrum of the disulfide bonds linked peptides between Cys222 and Cys231 in ERO1 protein. **c** MS/MS spectrum of the disulfide bonds linked peptides between Cys122-Cys125 in ERO1 protein. **d** MS/MS spectrum of the disulfide bonds linked peptides between Cys375 and Cys378 in ERO1 protein. **e** the relative abundance of the disulfide bonds linked peptide in ERO1 protein after incubating with or without GSNO.



Supplementary Fig. 5. *hot5-2* mutant shows the tolerance phenotype of ER stress under Tm treatment. **a** Phenotype of wild type (Col-0) and *hot5-2* with or without Tm treatment. Photograph were taken after growing at 22 °C for 9 days. **b** Fresh weight of Col-0 wild type and *hot5-2* plants with or without Tm treatment. Error bars, SD (each dot represents one biological replicate with 3 seedlings). ** $p < 0.01$, two-tail unpaired t -test. **c** Chlorophyll content of WT and *hot5-4* plants with or without Tm treatment. Error bars, SD (each dot represents one biological replicate with 3 seedlings). * $p < 0.05$, two-tailed unpaired t -test.

Supplementary Table I. Comparison of SNO proteomics studies in plants.

Method	Material	Number of SNO proteins/peptides/sites	Amount of starting material/condition/mutant	Quantification	Year	Ref.
Biotin-switch	Arabidopsis	105 proteins		No	2005	1
Biotin-switch	Arabidopsis	16 proteins	hypersensitive response	No	2008	2
Biotin-switch	<i>Brassica juncea</i>	20 proteins	cold	No	2009	3
Biotin-switch	<i>Citrus aurantium</i>	49 proteins	salinity	No	2009	4
Biotin-switch	Arabidopsis leave mitochondria	11 proteins	harpin	No	2010	5
Biotin-switch	Arabidopsis cells	53 peptides	2 mg proteins	No	2011	6
Biotin-switch	Arabidopsis	58 proteins	<i>Pst</i> DC3000/ <i>gsnor1</i>	No	2011	7
Biotin-switch	Pea peroxisomes	6 proteins		No	2012	8
Biotin-switch	tobacco	11 peptides	<i>Phytophthora cryptogea</i>	No	2012	9
Biotin-switch	Arabidopsis	5 proteins	auxin	No	2013	10
Biotin-switch	pea	29 proteins	salinity	No	2013	11
Biotin-switch	Potato leaves	80 proteins		No	2013	12
Biotin-switch	<i>Brassica juncea</i>	110 spots	cold	No	2013	13
Biotin-switch	Arabidopsis plantlets	62 peptides	cold stress	No	2014	14
Biotin-switch	<i>Brassica juncea</i>	271 proteins	PAs	No	2013	15
Biotin-switch	<i>Brassica juncea</i>	48 proteins	cold stress	No	2014	16
Biotin-switch	Populus	32 proteins	Acute ozone fumigation	No	2014	17
Biotin-switch	Arabidopsis seedlings	926 proteins, 1,195 peptides	<i>gsnor1-3</i>	No	2015	18
Biotin-switch	wheat	44 proteins	dehydration	No	2016	19
Biotin-switch	sunflower	61 proteins	30 µg proteins	No	2018	20
iodoTMT	tomato	334 proteins, 425 sites	1 mg/ <i>GSNOR</i> RNAi line	Yes	2019	21
Biotin-switch	Cucumber	165 proteins	GSNO/cPTIO/ <i>gsnor1</i>	No	2019	22
iodoTMT	Tea leaves	191 proteins, 228 sites	1 mg proteins	Yes	2019	23
Biotin-switch	<i>Lotus japonicus</i>	281 proteins		No	2020	24
iodoTMT/ iTRAQ	Arabidopsis leave peels	35 proteins, 41 peptides	100 peels/Flg22	Yes	2020	25
FAT-switch	Arabidopsis seedlings	1,595 proteins, 2,121 peptides	0.5 g seedlings/ <i>hot5-4</i>	Yes	2022	This study

Supplementary Table II. Primers used in this study.

Primers	Sequence
bip3-L	CACGGTTCCAGCGTATTTCAAT
bip3-R	ATAAGCTATGGCAGCACCCGTT
shd-L	GAAGGAAGCATTCAAGGAGCTA
shd-R	TCTTTGATGATAGGGTGTCTGTG
bip1,2-L	TCACTTGGGAGGTGAGGACTTT
bip1,2-R	CTCACATTCCCTTCGGAGCTTA
actin-L	GGTAACATTGTGCTCAGTGGTGG
actin-R	AACGACCTTAATCTTCATGCTGC
His-MSB-BamHI-ERO1-F	GAAAACCTCTACTTCCAAGGATCCATGAATTCCAATGTCGGTTTC
His-MSB-NotI-ERO1-R	TCGACTTAAGCATTATGCGGCCGCTTACCAGAATGAGACAGC
His-MSB-BamHI-PDI9-F	GAAAACCTCTACTTCCAAGGATCCATGTATAAAATCACCATTA
His-MSB-NotI-PDI9-R	TCGACTTAAGCATTATGCGGCCGCTCACAACATCATCCTTAGA
MSB-ERO1-C337S-F	ACAGCTTCTCCTGTTCCGTTTGATGAA
MSB-ERO1-C337S-R	AACAGGAGAAGCTGTTTGAGTTTCGG
HOT5-2 GABI_315D11-LP	ATGGTTCGACGCATATTTTTC
HOT5-2 GABI_315D12-RP	GGAAAGAGACCTTCAGGATCC
ero1-3-Salk_096805-LP	GATTCTTGGTTTCGACCCATC
ero1-3-Salk_096805-RP	CTTTAGCGACTTTGGTTGTCTG
LBb1.3	ATTTTGCCGATTTCGGAAC
GABI	ATATTGACCATCATACTCATTGC

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List of Supplementary Datasets:

Supplementary Dataset 1: S-nitrosylation peptides identified by biotin-switch method

Supplementary Dataset 2: S-nitrosylation peptides identified by FAT-switch method

Supplementary Dataset 3: Quantitative S-nitroso-proteomics by FAT-switch method

Supplementary Dataset 4: S-nitrosylation peptides identified by previous studies

Supplementary Dataset 5: S-nitrosylation peptides enriched in *hot5-4* mutant

Supplementary Dataset 6: Proteomics analysis of WT and *hot5-4* mutant seedlings

Supplementary Dataset 7: GO and KEGG analyses of *hot5-4* enriched S-nitrosylated proteins