

Figure S1. Synthesis of the S-nitrosoglutathione-vinyl sulfone silica.- (i) reaction of vinyl silica with oxidized glutathione (GSSG) in 100 mM phosphate buffer pH 7.6 at rt; (ii-iii) blockage of the unreacted vinyl sulfone groups (VS) with β -mercaptoethanol (bETSH), generation of glutathione (GSH) and further reduction with DTT; (iv) S-nitrosylation by treatment with $\text{NO}_2\text{Na}/\text{HCl}$ to yield S-nitrosoglutathione-vinyl sulfone silica

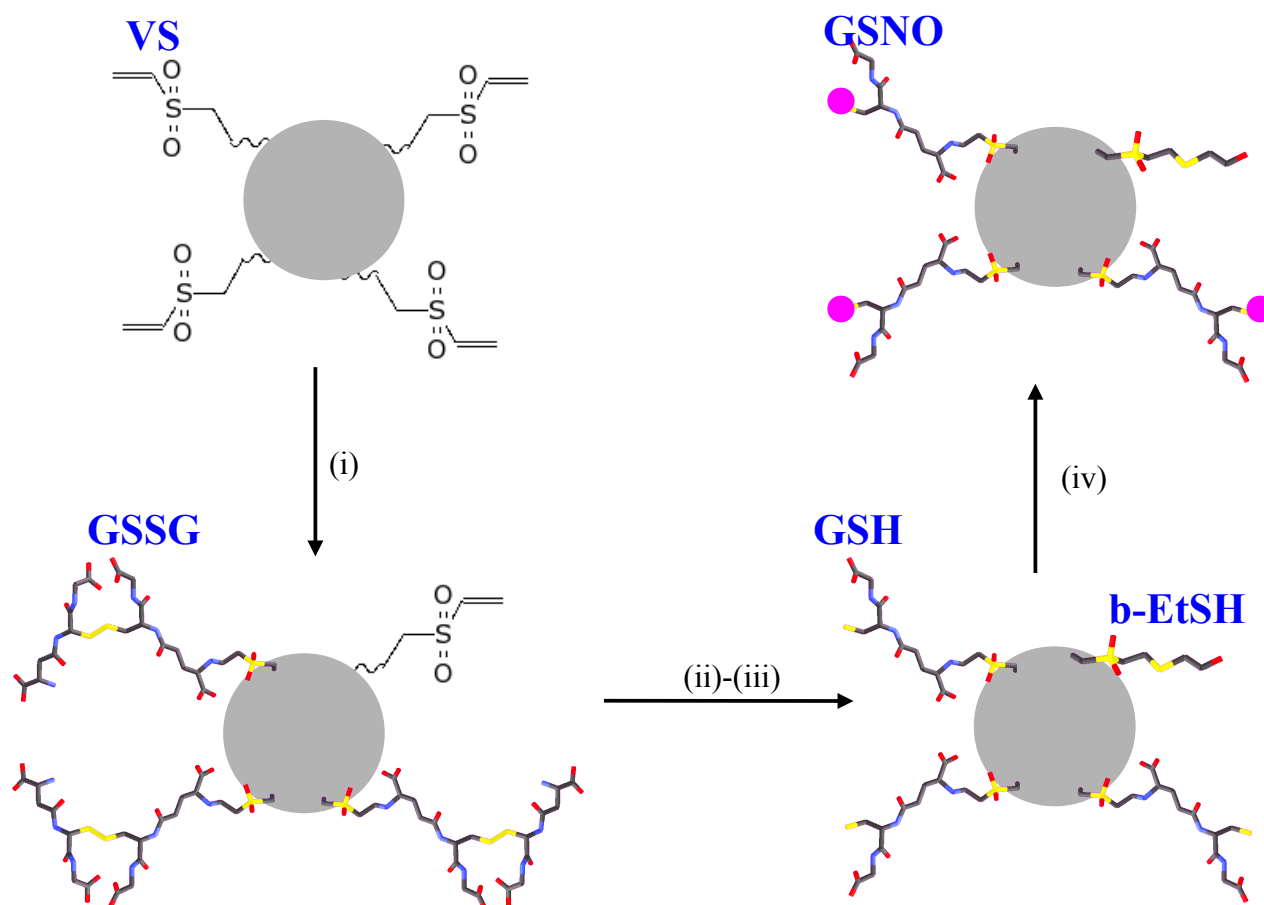


Figure S2. Profile of the affinity chromatography of the pea leaves crude extract monitored at three wavelengths to detect both protein (A280) and S-nitrosothiol (A340 and A550) signal and the types of interactions that are disrupted (insert).- A volume of 150 ml of crude extract of pea leaves in 50 mM HEPES pH 7.6 supplemented with 10 mM EDTA and 50 mM NaCl. After equilibration with 50 mM HEPES pH 7.6 with 50 mM NaCl to remove the non-bounded or weakly bounded proteins, the column was washed with 200 mM NaCl in the same buffer to discard those proteins retained by unspecific interactions and then eluted by increasing the ionic strength of the buffer to 1500 mM NaCl. Finally, the column was cleaned with 2000 mM NaCl and 100 mM DTT to removed those proteins that formed a disulfide bond with the resin.

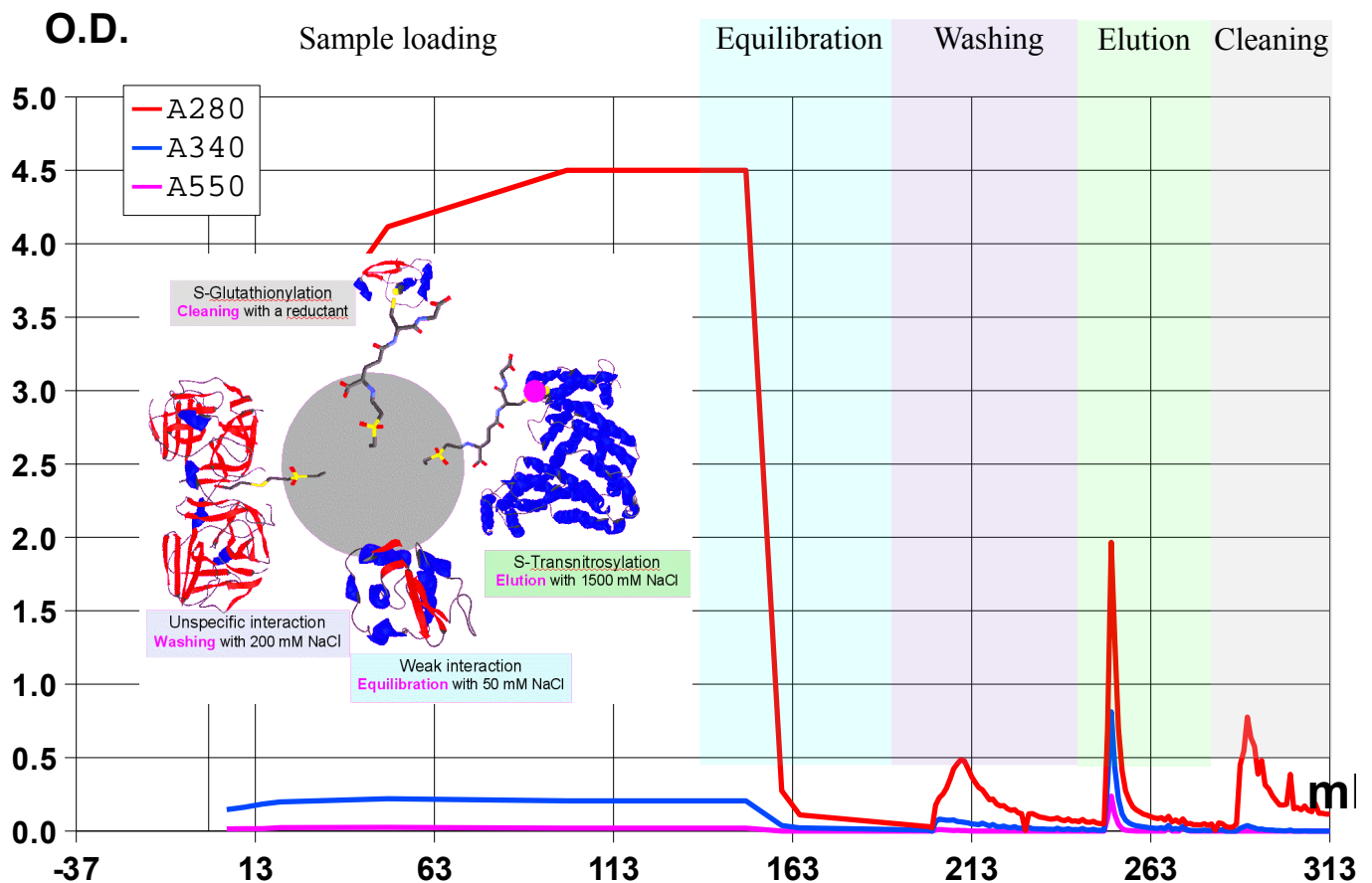


Figure S3. Bidimensional electrophoresis of the peak fraction of pea leaves eluted at 1500 mM NaCl that showed the highest protein and S-nitrosothiol signal. The gel was stained with Spypro Ruby. Molecular-mass standards are indicated on the left in kDa. Spots picked, digested with trypsin and analyzed by mass spectrometry are numbered.

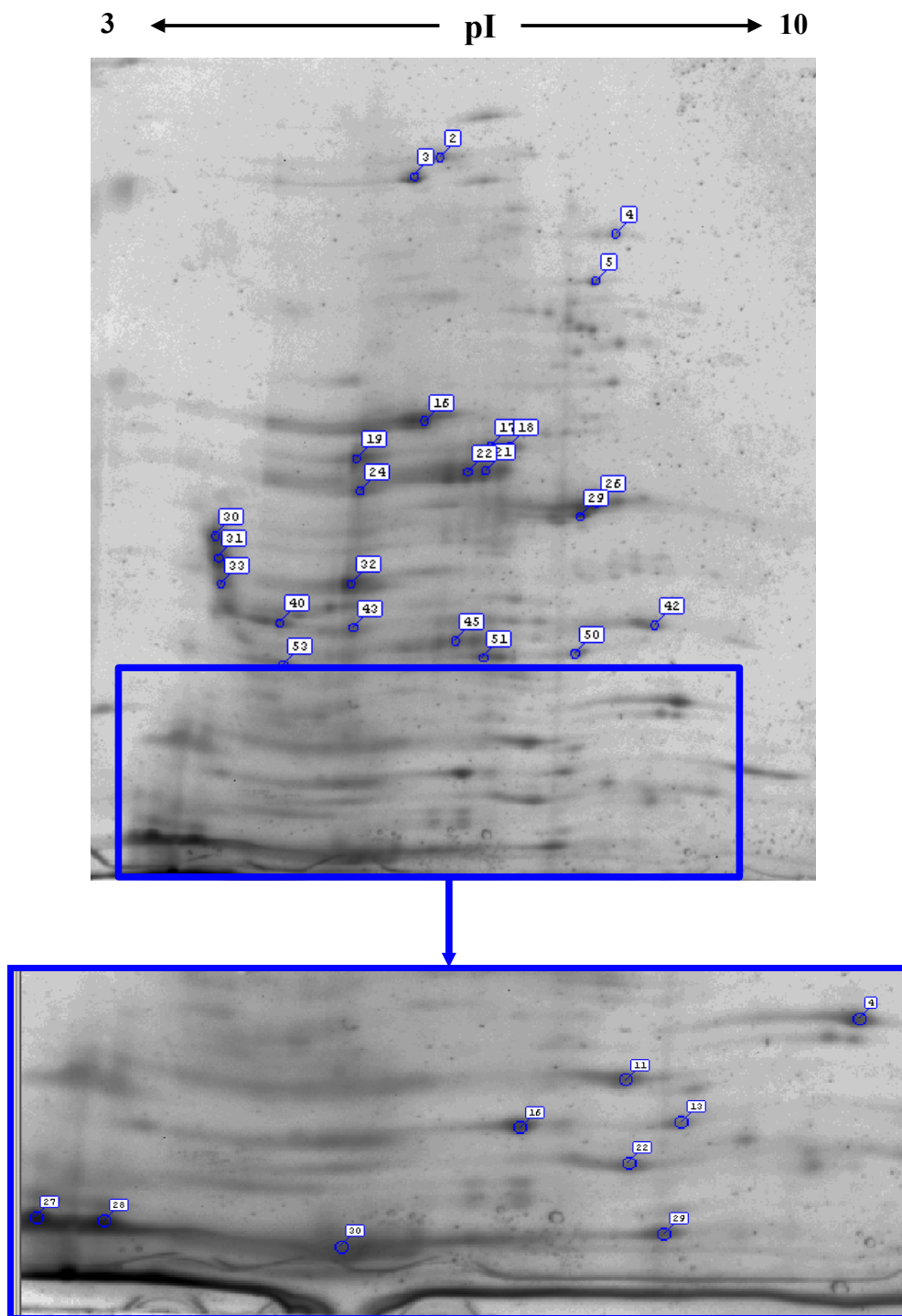


Figure S4. Bidimensional electrophoresis of the peak fraction of sunflower hypocotyls eluted at 1500 mM NaCl. (a) The gel was stained with Spypro Ruby. Molecular-mass standards are indicated on the left in kDa. (b) Spots that were picked, digested with trypsin and analyzed by mass spectrometry.

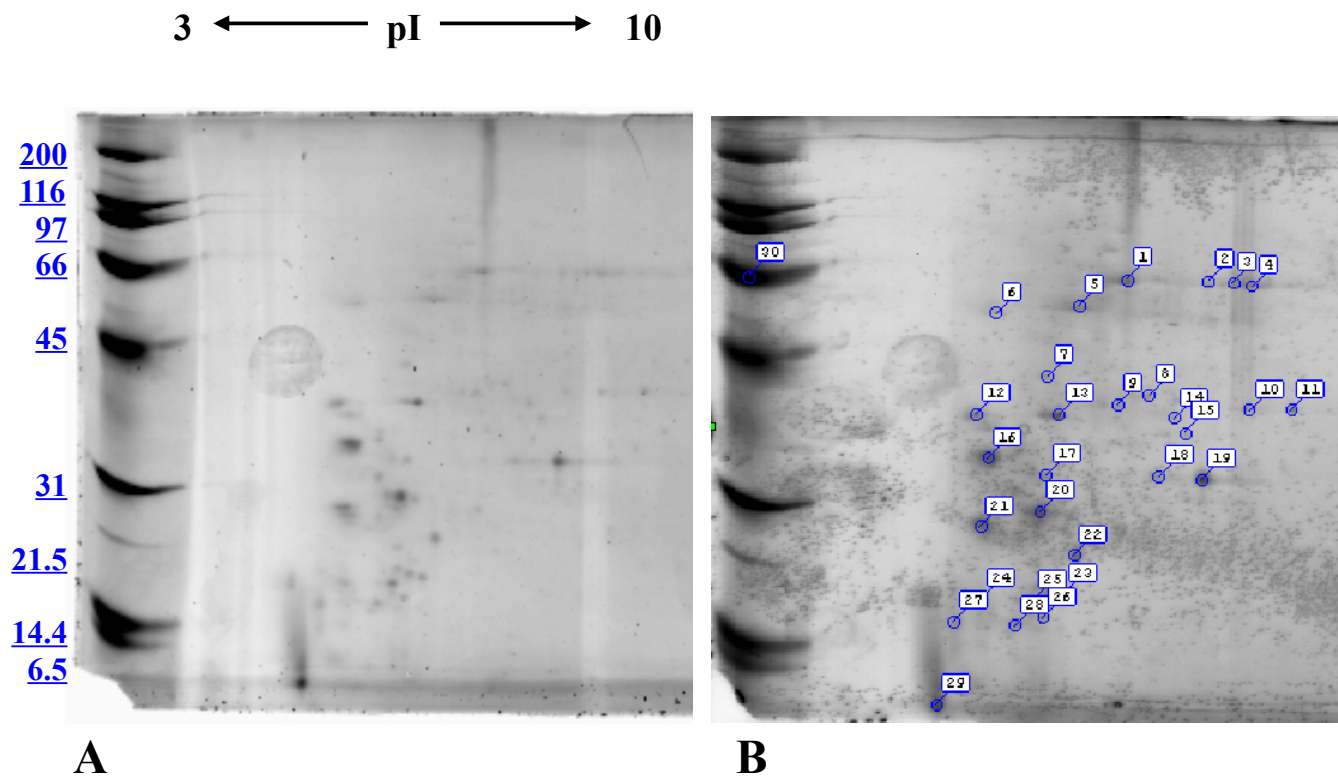


Table S1. Summary of the positive identifications by mass spectrometry of the peak fraction of the pea leaf sample eluted from a GSNO-vinyl sulfone resin that showed the highest protein and S-nitrosylation signals. Criteria to consider an identification as positive were: i) agreement between both molecular weight and isoelectric point inferred from the 2D gel and those reported in literature or predicted from the primary structure and ii) either fragmentation or mass fingerprint yielded a protein score C.I. better than 97% (instead of the less stringent 95% cutoff generally accepted). Peptides labeled as (*) were confirmed by MS/MS

spot	Protein score C.I.%	Peptide count	Protein species	MW (kDa)	pI	ID	identification method matched peptides [(*) Peptides identified by MS/MS]
5	7	12	CRK1 protein. <i>Beta vulgaris subsp vulgaris</i>	66.7	9.26	Q9LDC1	Peptide mass fingerprinting KHPMTR DISSRAR NVVEGNEPK RIITHANAK IITHANAKSK YPPSKEMDAK FDNLEPESVK LCGSPSDEYWK MGCVFGKESLAPEGR VDLPVADVVSQWDTGK GVGQHWGSLVDPASAGGPR DFPPSALSLLIDPAER
16	100	15	Heat shock protein Hsp70 <i>Medicago truncatula</i>	75.8	5.19	Q1SKX2	MS/MS (total ion C.I.:%: 99.998%) IAGLEVL TPVENSLR IPAVQELVK DNKSLGSFR DIDEVILVGGSTR SFAAEEISAQVLR SFAAEEISAQVLRK AVVTVPAYFNDSQR (*) QAVVNPENTFFSVK SLGSFRLDGIPPAPR AKFEELCSDLLDR MSEVDEESKQVSYR QAVVNPENTFFSVKR IINEPTAASLAYGFER LSIKDIDEVILVGGSTR (*)
17	98.8	11	Inducer of CBF expression 1 protein <i>Populus suaveolens.</i>	60.8	6.51	Q1HIU3	Peptide mass fingerprinting IMHLGIK NLMAQWR AVNIHMFCGR [oxidation M6] AVNIHMFCGRK GLPAKNLMAQWR MQLNDRLYTMR LDEISVNPLFSSR LLSPLETYPHGR GDQDSIKDLTFSPR IMDQLCPSSLSPNGQPAR

spot	Protein score C.I.%	Peptide count	Protein species	MW (kDa)	pI	ID	identification method matched peptides [(*) Peptides identified by MS/MS]
							LLSPLETYPIHGRSATLRFQR
18	99.7	10	S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase <i>Arabidopsis thaliana</i>	41.6	6.21	Q9LS20	Peptide mass fingerprinting LVPAFHK MALTFVGR NDFSLFLR MALTFVGRK CFISGVPGSFYSR LFERYTYHLAER RLASDAKPVVVETVK NKPTVQFFVSLTRK SITEPMLGAHFGDAIMDR ASIIRSITEPMLGAHFGDAIMR
19	100	9	Chloroplastic Fructose-bisphosphatase (with transit peptide) <i>Pisum sativum</i>	42,3	5.00	P46275	MS/MS (total ion C.I.%: 99.998%) HVNGSGVR IYSFNEGNYK YIGSLVGFHR TLLYGGIYGYP VLDIQPTEIHQR (*) CVFDAKSVLSSSR KLDVISNEVFSNCLR VPLYIGSTEEVEKVEK YIDDLKEPGPSGKPYSAR (*)
21	100	11	Phosphoribulokinase <i>Pisum sativum</i>	39.2	5.40	P93681	MS/MS (total ion C.I.%: 100) LIQKAGVK GHSLESIK FFYGPETYK (*) DLFEQIVASR FYGEVTQQMLK (*) RLTSVFGGAAEPPK IRDLFEQIVASR KPDFEAYIDPQK ANDFDLMYEQVK (*) ILVIEGLHPMYDSR HADFPGSNNGTGLFQTIVGLK
24	100	9	Chloroplastic Fructose-bisphosphatase (mature protein) <i>Pisum sativum</i>	35.3	5.02	P46275	MS/MS (total ion C.I.%: 100) QIASLVQR ANISNLTEDQK IYSFNEGNYK EPGPSGKPYSAR YIGSLVGFHR (*) TLLYGGIYGYP (*) VLDIQPTEIHQR VPLYIGSTEEVEKVEK YIDDLKEPGPSGKPYSAR (*)
26	100	7	Hypothetical protein (predicted as chloroplastic) <i>Arabidopsis thaliana</i>	42.8	8.19	Q9SA52	MS/MS (total ion C.I.%: 100) ILIMGGTR KILIMGGTR EGHQVTLFTR DQHFFASVEK GALYVSASSEKK

spot	Protein score C.I.%	Peptide count	Protein species	MW (kDa)	pI	ID	identification method matched peptides [(*) Peptides identified by MS/MS]
							AGGFPEPEIVHYNPK SSLSAEGFDVVYDINGR (*)
29	97.1	9	Putative F-box protein <i>Arabidopsis thaliana</i>	38.1	9.80	Q9FVT4	Peptide mass fingerprinting ADNSATRVR WTEFAMSR VKALTLEMK LESGSMLELK MADNSATRVR GVPFPMFKVK NVENMSLDVR MMNFHLKINK SLSHRLDLHDVIPR
42	100	9	Ferredoxin:nadp+ reductase (with transit peptide) <i>Pisum sativum</i>	40.2	8.60	P10933	MS/MS (total ion C.I.%: 100) MYIQTR FKPKPEYVGR GIDDIMVSLAAK LVYTNDAGEVVK (*) RLVYTNDAGEVVK KDNTFVYMCGLK APENFRLDFAVSR LYSIASSAIGDFGDSK DPNATVIMLGTGTGIAPFR
43	100	8	Chloroplatic Fructose-bisphosphatase (nicked form) <i>Pisums sativum,</i>	34.0		P46275	MS/MS (total ion C.I.%: 100) SVLSSRR IYSFNEGNYK EPGPSGKPYSAR YIGSLVGDFHR (*) TLLYGGIYGYP VLDIQPTEIHQR VPLYIGSTEEVEK YIDDLKEPGPSGKPYSAR (*)
45	98.8	8	GTP-binding protein <i>Pisums sativum,</i>	25.0	5.70	Q08147	Peptide mass fingerprinting NIVILVGNK STIGVEFQTR ANSGGYGDANQR FSRNEFSLDSK MANSGGYGDANQR DVPTEDAKEFAEK GAVGAMLVYDITKR SDLENQRDVPTEDAK
50	100	8	ferredoxin: nadp+reductase (mature protein) <i>Pisum sativum</i>	33.0	6.06	P10933	MS/MS (total ion C.I.%: 100) DGIDWLEFK GIDDIMVSLAAK LVYTNDGGEIVK ITGPVGKEMLMPK RLVYTNDGGEIVK LGFSVSREQTNEK QEEGIVVNKFKPK LYSIASSAIGDFGDSK (*)

spot	Protein score C.I.%	Peptide count	Protein species	MW (kDa)	pI	ID	identification method matched peptides [(*) Peptides identified by MS/MS]
51	100	6	26S proteasome alpha-3 chain <i>Spinacia oleracea</i>	27.5	6.11	O24362	MS/MS (total ion C.I.%: 99.999) YFGAAIGK MMLPGSNR VFQIEYAAK (*) MMLPGSNRR CKDGIVLGVEK HSGMAVAGLAADGR
Bottom part of the gel							
4	100	8	Proteasome subunit alpha type-7 <i>Cicer arietinum</i>	27.2	6.86	Q9SXU1	MS/MS (total ion C.I.%: 99.871%) KGNAAVGVR YIAGLQQK (*) NIEVAVMTK ALLEVVESGGK GTDNVVLGVEK GTDNVVLGVEKK NSNSIREFLEK NFKETSGQETVK
11	100	10	Proteasome subunit alpha type-6 <i>Glycine max</i>	27.5	5.83	O48551	MS/MS (total ion C.I.%: 100) TLVQQAR NEAAEFR AAGITSIGVR LFQVEYAFK (*) HITIFSPEGR DQEAINFLEK YLGLLATGMTADAR ATSAGLKDQEAINFLEK (*) VLTTEIDEHLTAISER VLTTEIDEHLTAISERD
16	100	7	Proteasome subunit alpha type-2-A <i>Arabidopsis thaliana</i>	25.7	5.53	O23708	MS/MS (total ion C.I.%: 100) EPIPVTQLVR NIEIGKIGADK ASAMGKNVSNK LPSILVDEASVQK KLPSILVDEASVQK (*) LYKEPIPVTQLVR (*) LVQIEHALTAVGSGQTSGLIK
29	100	6	Putative beta4 proteasome subunit <i>Nicotiana tabacum</i>	14.6	6.40	Q93X32	MS/MS (total ion C.I.%: 97.36) GELATALR IMILDCHK GELATALRK NVALYQFR (*) LMGASGEAGDR NGIPLTTAAAANFTR

Table S2. Summary of the positive identifications by mass spectrometry of the peak fraction of the sunflower hypocotyls sample eluted from a GSNO-vinyl sulfone resin that showed the highest protein and S-nitrosylation signals. Criteria to consider an identification as positive were: i) agreement between both molecular weight and isoelectric point inferred from the 2D gel and those reported in literature or predicted from the primary structure and ii) either fragmentation or mass fingerprint yielded a protein score C.I. better than 97% (instead of the less stringent 95% cutoff generally accepted). Peptides labeled as (*) were confirmed by MS/MS

spot	Protein score C.I.%	Peptide count	Protein species	MW (kDa)	pI	ID	identification method matched peptides [(*) Peptides identified by MS/MS]
3	99.8	12	Ferredoxin-nitrite reductase <i>Spinacia oleracea</i>	67.1	6.51	P05314	Peptide mass fingerprinting DLGFRGNR NNSIRCQK RMPEQVLER IADVYGSSELK HHYGRFMMR LQADEMEELAR ERYSPEPPILMK IDSLLEPLLKER MMWLIDELGMEAFR VTEEVQRLVSVTRPVR LFIEDGISDLATLSMEEVDK AVSPAAETA AVSPSVDAARLEPR
4	97.6	9	Transcriptional adapter ADA2b <i>Arabidopsis thaliana</i>	56.1	6.05	Q9ATB4	Peptide mass fingerprinting KAEQNMK EEYFSPPK ENEEGMNRGK NLLYPNPFK VDRVYDMLVK EEYFSPPKVK ENDTPEEHELK RLFSQDMSHVAGK FHSKEEHDELLR
16	99.9	11	Expressed protein <i>Oryza sativa</i>	43.0	5.72	Q2QWE0	Peptide mass fingerprinting TMLVEEK LPDCTFGKK TMLSEEKVK LKTMLSEEK LKTMLVEEK TMLVEEKDK LLKTIFVEEK NEHYKTMLAK NMLEEEKDNNK AVEVIQTSSEVK AEHHKNMLEEEK
18	99.9	10	At2g18860 <i>Arabidopsis thaliana</i>	34.0	5.87	Q501A1	Peptide mass fingerprinting WQLEEFK ETLVAQIEATPK IWSSPNFNLSR MNVNSFDLWQK

spot	Protein score C.I.%	Peptide count	Protein species	MW (kDa)	pI	ID	identification method matched peptides [(*) Peptides identified by MS/MS]
							WQLEEFKAVR QFVTAIENQIHR MMVVNSFDLWQK ESTNSSLVENVMEVSAK (oxid. M12) SRESTNSSLVENVMEVSAK ESTNSSLVENVMEVSAK
19	99.9	11	Pentatricopeptide repeat-containing protein-like protein <i>Arabidopsis thaliana</i>	42.0	8.81	Q9CAY8	Peptide mass fingerprinting WGLKYR EKGMVEK LWNEYK AEKLMTR YLDRASR LMTRWGLK MSMTLSLQR VDESLREMK LCSKACIGDK FLADWEGTVK NGTLSTNQTLHGR
20	99.6	10	Hypothetical protein At2g46320 <i>Arabidopsis thaliana</i>	39.3	9.37	Q8S8M6	Peptide mass fingerprinting AMTMTR QIEKNTDR MQAFKGTQR SKSIPNENLK GMFSGAGARVGR NTDRAMTMTR MLWTGLGAQLAR MVEEAQLDPKSK ITGSASVCSDNQYK QTLAEIWRDGGMR
21	100	14	Ferredoxin-nitrite reductase precursor (fragment) <i>Spinacia oleracea</i>	67.1	6.51	P05314	Peptide mass fingerprinting SGINPAEK AMLEAFR DGFWVLK LEPRVEER EYLGVHPQK NNSIRCQK RMPEQVLER HHYGRFMMR IDSLLEPLLK LQADEMEELAR ERYSPEPPILMK MPEQVLERASSEELVQK LFIEDGISDLATLSMEEVDK MMWLIDELGMEAFRGEVEK
22	99.9	4	Pectinesterase <i>Solanum tuberosum</i>	24.4	8.84	Q43838	MS/MS (total ion C.I.%: 99.977) ENVEVTSRK CQLVARKPSK YQKNMVTAQGR IDAYQDTLYAHSQR (*)
24	98.7	7	Catalase 1 (fragment)	34.2	5.9	Q93XK3	Peptide mass fingerprinting

spot	Protein score C.I.%	Peptide count	Protein species	MW (kDa)	pI	ID	identification method matched peptides [(*) Peptides identified by MS/MS]
			<i>Pinus pines</i>				FPDMVHALKPNPK HMDGSGVHTFTLVNK DGMKFPDMVHALKPNPK TWPEDIFPLQPVGRMVLNK GFFEVDHSHLTCADFLR EGNFDLVGNNFPVFFVRDGMK ILDFFSHHPESLHMFTFLFDDLGIPLNYR
25	98	4	Thioredoxin peroxidase <i>Nicotinum tabacum</i>	30.1	8.2	Q8RVF8	MS/MS (total ion C.I.%: 99.407) SVDETLR SYNVLIPDQGIALR (*) SGGLGDLNYPLISDVTK EGVIQHSTINNLGIGRSVDETLR
26	99.7	6	Cytochrome P450 (fragment) <i>Teucrium canadense</i>	14.6	6.06	Q5EKR9	Peptide mass fingerprinting GSNVHVNWALAR SNANVKIGGYDIPK AQEEMDRVIGYER TSTTMEWAMTELIK FLEEDVDMKGHDFR IGGYDIPKGSNVHVNWALAR
29	99.8	3	Calmodulin <i>Arabidopsis thaliana</i>	15.6	4.20	P25069	MS/MS (total ion C.I.%: 99.836) LTDEEVDEMIK DTDSEELKEAFR VFDKQNGFISAAELR (*)