Supplementary Data

Title: *In vivo* protein tyrosine nitration in *Arabidopsis thaliana*

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Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Putative Y-nitrated proteins identified from Arabidopsis and the corresponding functional Y-nitrated counterparts in other organisms.

Table S2. Identification of potentially Y nitrated proteins by MALDI-TOF peptide fingerprinting of the most abundant 2D gel-excised spots from anti-3-nitroY-immunoprecipitated Arabidopsis proteins.

Table S3. Identification of potential targets of 3-amino-Y modification by shotgun LC-MS/MS analysis.

Fig. S1. Potential Y nitration targets in glyceraldehyde-3-phopsphate dehydrogenase, serine hydroxymethyltransferase, transketolase, Rubisco large subunit and Rubisco activase are conserved in different plants and other organisms.

Fig. S2. Conservation and structural modeling analysis of plant methionine synthases.

Fig. S3. Alignment of 3D structure models of rat and Arabidopsis glyceraldehyde-3-phosphate dehydrogenases.

Fig. S4. Scheme displaying primary carbon and sulphur metabolism enzymes highlighting those that have been identified in this work as potentially nitrated in Arabidopsis.

Fig. S5. Confirmation of the presence of proteins identified through shotgun proteomic analysis in the immnunopurified nitroproteome. The entire gels for Western blots performed in Fig. 2 are shown to account for specificity of the antibodies.

Fig. S6. ROS and NO detection in roots of wild type plants grown under standard conditions. Nitroblue tetrazolium (NBT) staining of roots at different zones

(A,B). Roots were pre-incubated with 10 U/mL of superoxide dismutase (SOD) prior to NBT staining (C,D). DAF-FM DA staining of roots pre-treated (G,H) or not (E,F) with the NO scavenger cPTIO under UV-illumination (E,G) or bright field (F,H).

Table S1. Putative Y-nitrated proteins identified from Arabidopsis and the corresponding functional Y-nitrated counterparts in other organisms.

Identified Arabidopsis protein (AGI locus)	**Protein (Organism)**	**References**
TCPA_ARATH (At3g20050)	T-complex polypeptide 1 alpha subunit TCP-1(Rat ; Human)	Suzuki et al., 2005 Guesquière et al., 2009
G3PB_ARATH (At1g42970) G3PA_ARATH (At3g26650) G3PC_ARATH (At3g04120)	Glyceraldehyde-3-phosphate dehydrogenases (Human; Rat)	Sultana et al., 2006 Guesquière et al., 2009
ATPA_ARATH (AtCg00120) ATPB_ARATH (AtCg00480) ATPG1_ARATH (At4g04640) VATB_ARATH (At1g76030)	ATP synthases (Human ; Mouse)	Casoni et al., 2005; Sultana et al., 2006; Zhang et al., 2007
CAHC_ARATH (At3g01500) CAH2_ARATH (At5g14740)	Carbonic anhydrases (Human)	Sultana et al., 2006
ENO_ARATH (At2g36530)	Enolase (Mouse; Human)	Casoni et al., 2005; Sultana et al., 2006
CATA3_ARATH (At1g20620)	Catalase (Mouse)	Ghosh et al., 2006
PGKH_ARATH (At1g56190)	3-phosphoglycerate kinase (Yeast ; Human)	Bacharach et al., 1977 Guesquière et al., 2009
ACA9_ARATH (At3g21180)	Ca-ATPase (Rat)	Knyushko et al., 2005
HDA5_ARATH (At5g61060)	Histone deacetylase HDAC2 (mammals cells)	Ito et al., 2004
ACT7_ARATH (At5g09810) ACT2_ARATH (At3g18780)	Actins (mammals cells ; Mouse)	Casoni et al., 2005; Zhang et al., 2007
TBA6_ARATH (At4g14960) TBA3_ARATH (At5g19770) TBB4_ARATH (At5g44340) TBB2_ARATH (At5g62690) TBB1_ARATH (At1g75780)	Tubulins (Pig; Human ; mammals cells; Mouse)	Fiore et al., 2006; Zhang et al., 2007
GLNA2_ARATH (At5g35630)	Glutamine synthetase (Rat; Mouse)	Görg et al., 2006; Zhang et al., 2007
TYW23_ARATH (At4g04670) SYV_ARATH (At1g67220) SYM_ARATH (At4g13780)	t-RNA synthetases (Human)	Guesquière et al., 2009
HSP83_ARATH (At5g56010)	Heat shock protein 90 (mammals cells, Mouse)	Zhang et al., 2007
HSP71_ARATH (At5g02500) HSP73_ARATH (At3g09440)	Heat shock 71-kDa protein (Mouse)	Casoni et al., 2005; Zhang et al., 2007
AAT5_ARATH (At4g31990)	Aspartate aminotransferase (Cow; Mouse)	DiCola et al., 1976; Zhang et al., 2007
MDHP_ARATH (At3g47520) MDHG1_ARATH (At5g09660)	Malate:NADP+ oxidoreductases (Pigeon; Mouse, Human)	Chang et al., 1980; Zhang et al., 2007 Guesquière et al., 2009
RH56_ARATH (At5g11200)	ATP-dependent RNA helicase (Human)	Guesquière et al., 2009
FBX10_ARATH (At1g51290) FBK84_ARATH (At4g19865) FBK19_ARATH (At1g32430)	F-Box proteins (Human)	Guesquière et al., 2009
EF1A_ARTH (At1g07940)	Elongation factor 1-alpha (Human)	Guesquière et al., 2009
PGMP_ARATH (At5g51820)	Phosphoglucomutase (Rabbit)	Layne et al., 1979
2AAA_ARATH (At1g25490)	Ser/Thr-protein phosphatase (Human)	Guesquière et al., 2009
IF5A2_ARATH (At1g26630)	Eukaryotic translation initiation factor (Human)	Guesquière et al., 2009
PGKH_ARATH (At1g56190)	PGK1 Protein (Mouse)	Zhang et al., 2007
WBC16_ARATH (At3g55090)	ABC transporter	Guesquière et al., 2009
GBLP_ARATH (At1g18080)	Guanine nucleotide-binding protein beta (Human)	Guesquière et al., 2009
DRP1D_ARATH (At2g44590)	Dynamin (Mouse)	Zhang et al., 2007
GBLP_ARATH (At1g18080)	Guanine Nucleotide Binding Protein (Mouse)	Zhang et al., 2007
SCP37_ARATH (At3g52010)	Carboxypeptidase (Bovine)	Zisapel 1978

The Y-nitrated proteins identified by LC-MS/MS in immunopurified samples from Arabidopsis seedlings and the corresponding counterparts from different organisms in parenthesis in the second column.

Table S2. Identification of potentially Y nitrated proteins by MALDI-TOF peptide fingerprinting of the most abundant 2D gel-excised spots from anti-3-nitroY-immunoprecipitated Arabidopsis proteins.

Description	**AGI code**	**Mr Theor**	**Mr Exper**	**Mascot Score**	**Peptides (n.r.)**	**Coverage (%)**
Ribulose bisphosphate carboxylase large chain precursor	AtCg00490	52.9	53.4	348	20	41
Ribulose bisphosphate carboxylase/oxygenase activase	At2g39730	52.0	52.3	251	14	50
Serine Hydroxymethyltransferase	At4g13930	51.7	52.1	238	11	25
Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	At3g04120	36.9	37.0	176	11	49
Trigger factor type chaperone family protein	At5g55220	61.7	62.1	120	11	27
Putative microtubule-associated motor	At5g60930	145.2	44.4	101	17	11
Probable 125 kDa kinesin-related protein	At2g36200	119.3	120.4	81	23	26
Carbonic anhydrase, chloroplast precursor	At3g01500	37.4	37.8	78	9	34
Hypothetical 213.7 kDa protein ycf1.2	AtCg01130	213.7	214.6	77	25	12
DNA repair protein RAD50	At2g31970	152.8	153.6	73	22	20
Putative nuclear matrix constituent protein 1-like protein (NMCP1-like)	At5g65770	121.2	121.8	70	21	17
DNA topoisomerase 1	At5g55300	102.8	103.2	69	19	18
Mitochondrial import inner membrane translocase subunit Tim9	At3g46560	10.7	10.9	67	8	78
60S ribosomal protein L3-1 (Protein EMBRYO DEFECTIVE 2207)	At1g43170	44.5	44.7	65	11	23
Glycosyl hydrolase family 1 protein	At5g25980	53.4	54.3	64	7	28
Glycine-rich RNA-binding protein 7	At2g21660	16.8	16.9	64	9	78
Glyceraldehyde-3-phosphate dehydrogenase C-2	At1g13440	36.9	36.9	63	8	22
Transketolase, putative	At3g60750	79.9	80.3	62	12	34
Ras-related protein ARA-4	At2g43130	24.0	24.1	61	10	50
Probable nucleolar GTP-binding protein 1	At1g50920	76.8	77.3	60	17	25
Probable mannitol dehydrogenase	At4g39330	38.9	39.3	59	8	26
Protein AIG1	At1g33960	40.1	40.3	59	13	34
Arginine/serine-rich-splicing factor RSP40	At4g25500	40.1	40.3	59	12	60

Samples containing 3-nitroY immunopurified proteins were separated by 2-DE and identified by MALDI-TOF as described in Materials and Methods. The AGI identifiers for each identified protein are included along with the corresponding Mascot score and the number of peptides (n.r., non redundant) with matched sequences as well as the sequence coverage (%).

Figure S1

Gliceraldehyde-3-phosphate dehydrogenase

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Arabidopsis        DAKAGIALSDKFVKLVSWYDNEWGYSSRVVDLIVHMSKA- 338
Nicotiana          DAKAGIALSKNFVKLVSWYDNEWGYSSRVIDLICHMASVA 326
Oryza              DAKAGIALNDNFVKLVAWYDNEWGYSNRVIDLIRHMAKTQ 337
Rattus             DAGAGIALNDNFVKLISWYDNEYGYSNRVVDLMAYMASKE 333
Mus                DAGAGIALNDNFVKLISWYDNEYGYSNRVVDLMAYMASKE 333
Homo               DAGAGIALNDHFVKLISWYDNEFGYSNRVVDLMAHMASKE 335
Escherichia        DAKAGIALNDNFVKLVSWYDNETGYSNKVLDLIAHISK-- 331
Saccharomyces      DASAGIQLSPKFVKLVSWYDNEYGYSTRVVDLVEHIAKA- 332
                   ** *** *. :****::***** ***.:*:**: :::.
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Serine Hydroxymethyltransferase

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Arabidopsis        TSGGKKISATSIYFESLPYKVNFTTGYIDYDKLEEKALDFRPKLLICGGSAYPRDWDYAR 200
Oryza              TAGGKKISATSIYFESLPYKVSAATGYIDYEKLEEKALDFRPKLIICGGSAYPRDWDYAK 200
Rattus             TDK-KKISATSIFFESMPYKVYPDTGYINYDQLEENASLFHPKLIIAGTSCYSRNLDYAR 410
Mus                TDK-KKISATSIFFESMPYKVYPETGYINYDQLEENASLFHPKLIIAGTSCYSRNLDYAR 207
Homo               TDK-KKISATSIFFESMPYKVNPDTGYINYDQLEENARLFHPKLIIAGTSCYSRNLEYAR 213
Nicotiana          TDT-KKISAVSIFFETMPYRLNESTGYIDYDQLEKSATLFRPKLIVAGASAYARLYDYAR 243
Saccharomyces      TEN-RKISAVSTYFESFPYRVNPETGIIDYDTLEKNAILYRPKVLVAGTSAYCRLIDYKR 204
Escherichia        ----SPVNFSGKLYNIVPYGID-ATGHIDYADLEKQAKEHKPKMIIGGFSAYSGVVDWAK 185
                         :.  .  :: .** :   ** *:*  **:.*  .:**::: * *.*    :: :
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Transketolase

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Arabidopsis        VKNGNTGYDEIRAAIKEAKTVTDKPTLIKVTTTIGYGSPNKANSYSVHGAALGEKEVEAT 352
Nicotiana          VKNGNTGYDEIRAAIKEAKTVTDKPTMIKVTTTIGFGSPNKANSYSVHGSALGAKEVEAT 355
Oryza              VKNGNDGYDEIRAAIKEAKAVTDKPTLIKVTTTIGFGSPNKANSYSVHGSALGTKEVEAT 355
Saccharomyces      VDKGDDDMESISSALEKAKLSKDKPTIIKVTTTIGFGSLQQG-TAGVHGSALKADDVKQL 275
Escherichia        EIDG-HDPQAVKEAILEAQSVKDKPSLIICRTVIGFGSPNKAGKEEAHGAPLGEEEVALA 272
Rattus             VDGH-----SVEELCKAFGQAKHQPTAIIAKTFKGRGITGIEDKEAWHGKPLPKN----- 297
Mus                VDGH-----SVEELCKAFGQAKHQPTAIIAKTFKGRGITGIEDKEAWHGKPLPKN----- 265
Homo               VDGH-----SVEELCKAFGQAKHQPTAIIAKTFKGRGITGVEDKESWHGKPLPKN----- 265
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Rubisco large subunit

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Chlamydomonas      LSAKNYGRAVYECLRGGLDFTKDDENVNSQPFMRWRDRFLFVAEAIYKAQAETGEVKGHY 239
Micromonas         LSAKNYGRAVYECLRGGLDFTKDDENVNSQPFMRWRDRFLFVAEAIYKAQAETGEIKGHY 177
Arabidopsis        LSAKNYGRAVYECLRGGLDFTKDDENVNSQPFMRWRDRFLFCAEAIYKSQAETGEIKGHY 239
Oryza              LSAKNYGRACYECLRGGLDFTKDDENVNSQPFMRWRDRFVFCAEAIYKSQAETGEIKGHY 239
Nicotiana          LSAKNYGRAVYECLRGGLDFTKDDENVNSQPFMRWRDRFLFCAEALYKAQAETGEIKGHY 239
Spinacia           LSAKNYGRAVYECLRGGLDFTKDDENVNSQPFMRWRDRFLFCAEALYKAQAETGEIKGHY 239
Nostoc             LSAKNYGRAVYECLRGGLDFTKDDENINSAPFQRWRDRFLFVADAITKAQAETGEIKGHY 240
                   ********* ****************:** ** ******:* *:*: *:******:****

Chlamydomonas      LNATAGTCEEMMKRAVCAKELGVPIIMHDYLTGGFTANTSLAIYCRDNGLLLHIHRAMHA 299
Micromonas         LNVTAATSEEMIKRTVCAKELGLPIVMHDYITGGFTSNTSLSLYCRDHGLLLHIHRAMHA 237
Arabidopsis        LNATAGTCEEMIKRAVFARELGVPIVMHDYLTGGFTANTSLSHYCRDNGLLLHIHRAMHA 299
Oryza              LNATAGTCEEMIKRAVFARELGVPIVMHDYLTGGFTANTSLAHYCRDNGLLLHIHRAMHA 299
Nicotiana          LNATAGTCEEMIKRAVFARELGVPIVMHDYLTGGFTANTSLAHYCRDNGLLLHIHRAMHA 299
Spinacia           LNATAGTCEDMMKRAVFARELGVPIVMHDYLTGGFTANTTLSHYCRDNGLLLHIHRAMHA 299
Nostoc             LNVTAPTCEEMLKRAEYAKELKQPIIMHDYLTAGFTANNTLARWCRDNGLLLHIHRAMHA 300
                   **.** *.*:*:**:  *:**  **:****:*.***:*.:*: :***:************
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Rubisco activase

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Chlamydomonas      ARVYDDMVRQWITDTGVDKIGQQLVNAR--QKVAMPKVSMDLNVLIKYGKSLVDEQENVK 380
Micromonas         ARVYDDKVREWIRETGIEAMGPLLVNPKRGSKVTFEPPRMSLDILLQYGKALEMEQENVK 384
Arabidopsis        ARVYDDEVRKFVESLGVEKIGKRLVNSRE-GPPVFEQPEMTYEKLMEYGNMLVMEQENVK 408
Spinacia           ARVYDDEVRKWVNSVGVDNVGKKLVNSKD-GPPVFEQPEMTLQKLMEYGNMLVQEQENVK 406
Nicotiana          ARVYDDEVRKWVSGTGIEKIGDKLLNSFD-GPPTFEQPKMTIEKLLEYGNMLVQEQENVK 412
Oryza              ARVYDDEVRKWVSDTGVENIGKRLVNSRE-GPPEFEQPKMTIEKLMEYGYMLVKEQENVK 399
Nostoc             SRIYDIQIRDFIHKVGFERISLRVVNSLE-APPEFKKPDFSLAHLIESGNLVLGEQQRVD 281
                   :*:**  :*.::   *.: :.  ::*.       :    :    *:: *  :  **:.*.
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Figure S2

A

Methionine synthases

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AtMS1           ELESTLSGLN-VLVETYFADIPAEAYKTLTSLKGVTAFGFDLVRGTKTLDLVKAG-FPEG 285
AtMS2           ELESTLSGLN-VLVETYFADIPAEAYKTLTSLKGVTAFGFDLVRGTKTIDLIKSG-FPQG 285
StMS            DLESSLSGLN-VLVETYFADVPAEAFKTLTALKGVTAFGFDLVRGTQTLELIKSS-FPSG 285
GmMS            ELAPALSGLN-VLVETYFADIPAEAYKTLTSLNGVTAYGFDLVRGTNTLDLIKGG-FPSG 285
OsMS            ELESALSGLN-VLIETYFADIPAESYKTLTSLNSVTAYGFDLIRGSKTLDLVKSAGFPSG 286
HvMS            ELESTLSGLN-VLVETYFADVPAESYKTLTSLSSVTAYGFDLERGTKTLELVKSG-FPAG 285
ZmMS            ELESSFSGLN-VLIETYFADIPAESYKTLTSLSGVTAYGFDLIRGAKTLDLIRSS-FPSG 285
MET6            YFGEQ-SNLPKITLATYFGTVVPN-LDAIKGLP-VAALHVDFVRAPEQFDEVVAA-IGNK 292
NcMS            AIAAAGDAVPKVVVATYFGDIVHN-FDVLPAFSGAAGLHVDLVRNPEQLEPVLKQ-LGPN 288
                 :    . :  : : ***. :  :  ..: .:  .:.  .*: * .: :: :    :

AtMS1           KYLFAGVVDGRNIWANDFAASLSTLQALEGIVGKDKLVVSTSCSLLHTAVDLINETKLDD 345
AtMS2           KYLFAGVVDGRNIWANDLAASLITLQSLEGVVGKDKLVVSTSCSLLHTAVDLINETKLDA 345
StMS            KYLFAGVVDGRNIWANDLAASLALLQSLEGVVGKDKLVASTSCSLLHTAVDLINETKLDD 345
GmMS            KYLFAGVVDGRNIWANDLAASLTTLQGLEGIVGKDKLVVSTSSSLLHTAVDLVNETKLDD 345
OsMS            KYLFAGVVDGRNIWADDLAASLTTLESLEAIVGKDKLVVSTSCSLMHTAVDLVNETKLDS 346
HvMS            KYLFAGVVDGRNIWADDLAASLATLQSLEAIVGKDKLVVSTSCSLMHTAVDLVNETKLDD 345
ZmMS            KYLFAGVVDGRNIWADDLAASLSTLHSLEAVAGKDKLVVSTSCSLMHTAVDLVNETKLDD 345
MET6            QTLSVGIVDGRNIWKNDFKKSSAIVNKAIEKLGADRVVVATSSSLLHTPVDLNNETKLDA 352
NcMS            QILSAGVVDGRNIWKNDFAKSLEILQTAVKALGSERVIVATSSSLIHTPHTLASEKKLPS 348
                : * .*:******* :*:  *   :.      * ::::.:**.**:**.  * .*.**
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B

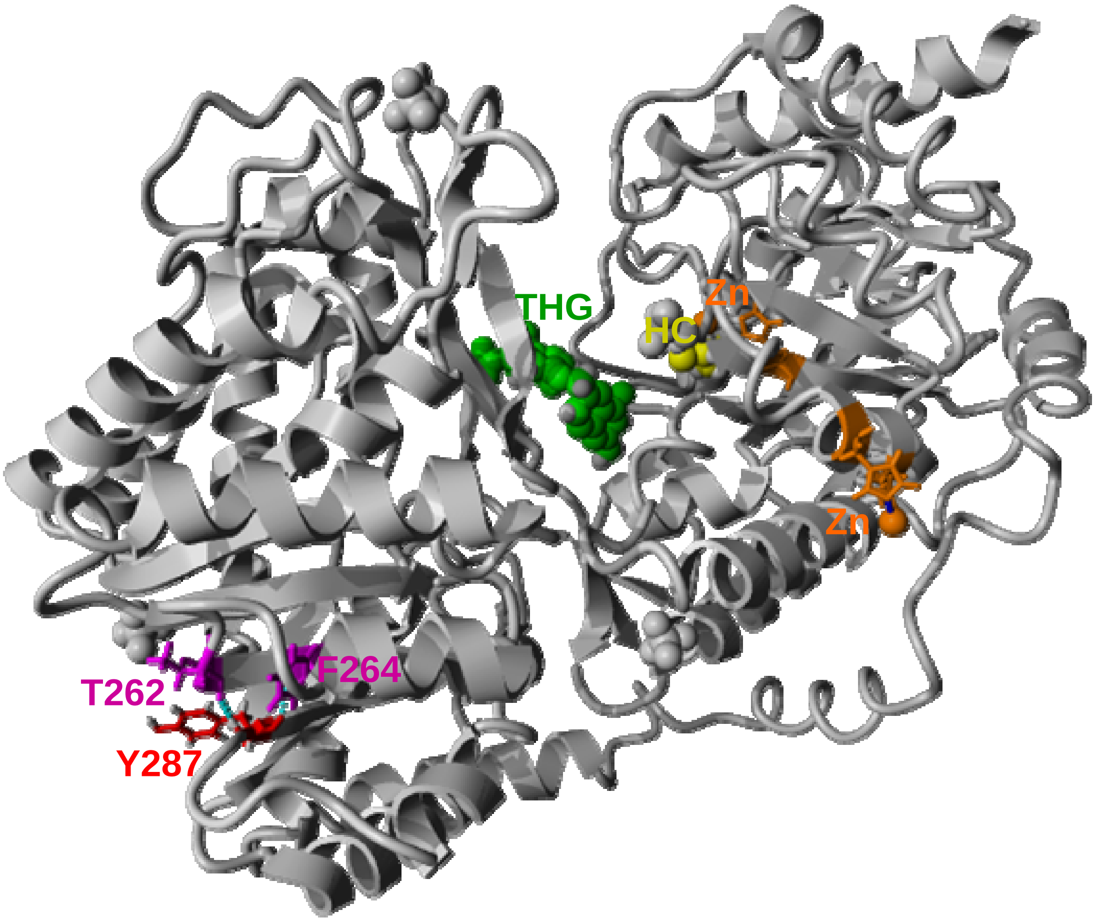


Figure S3

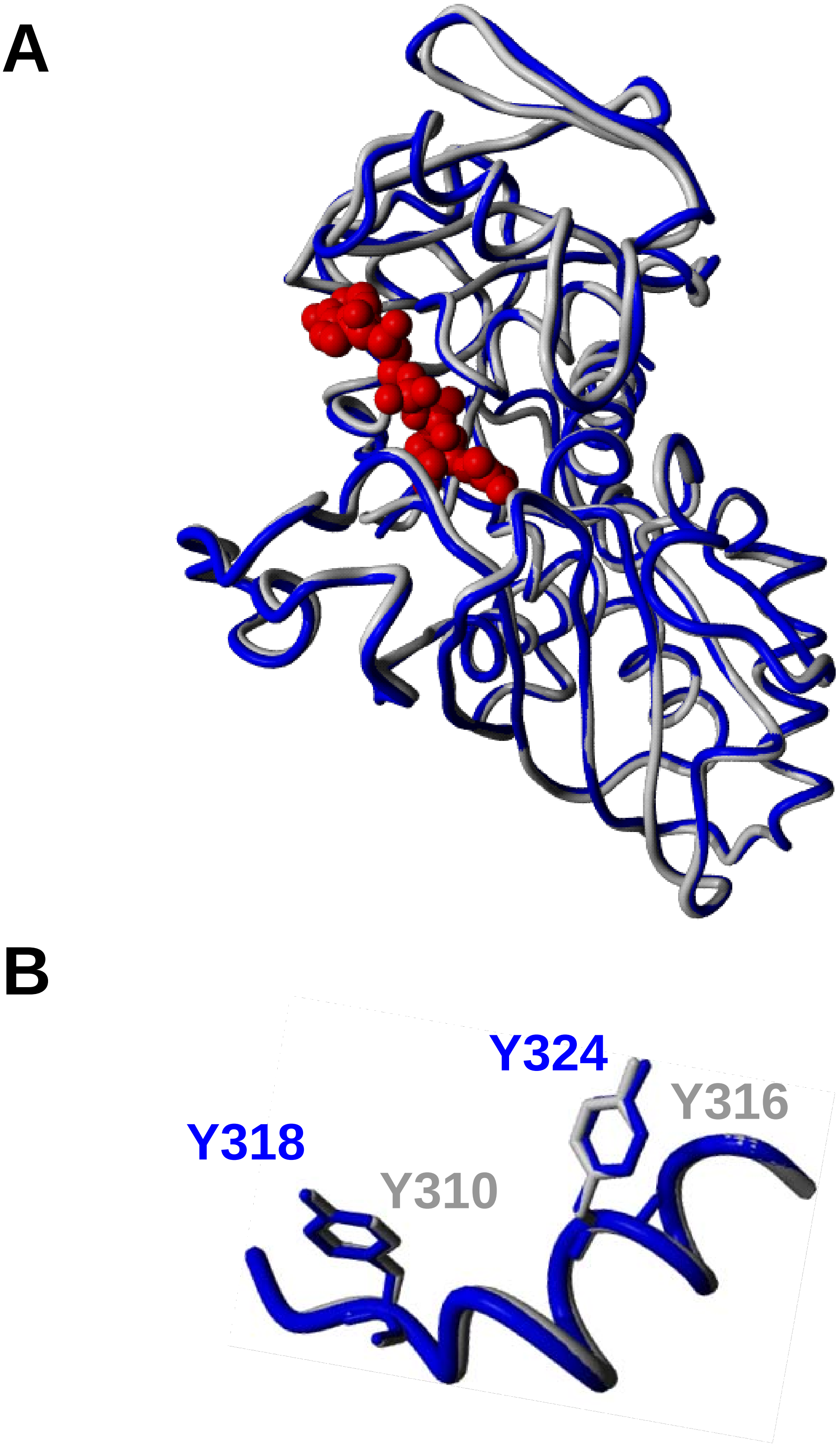


Figure S4

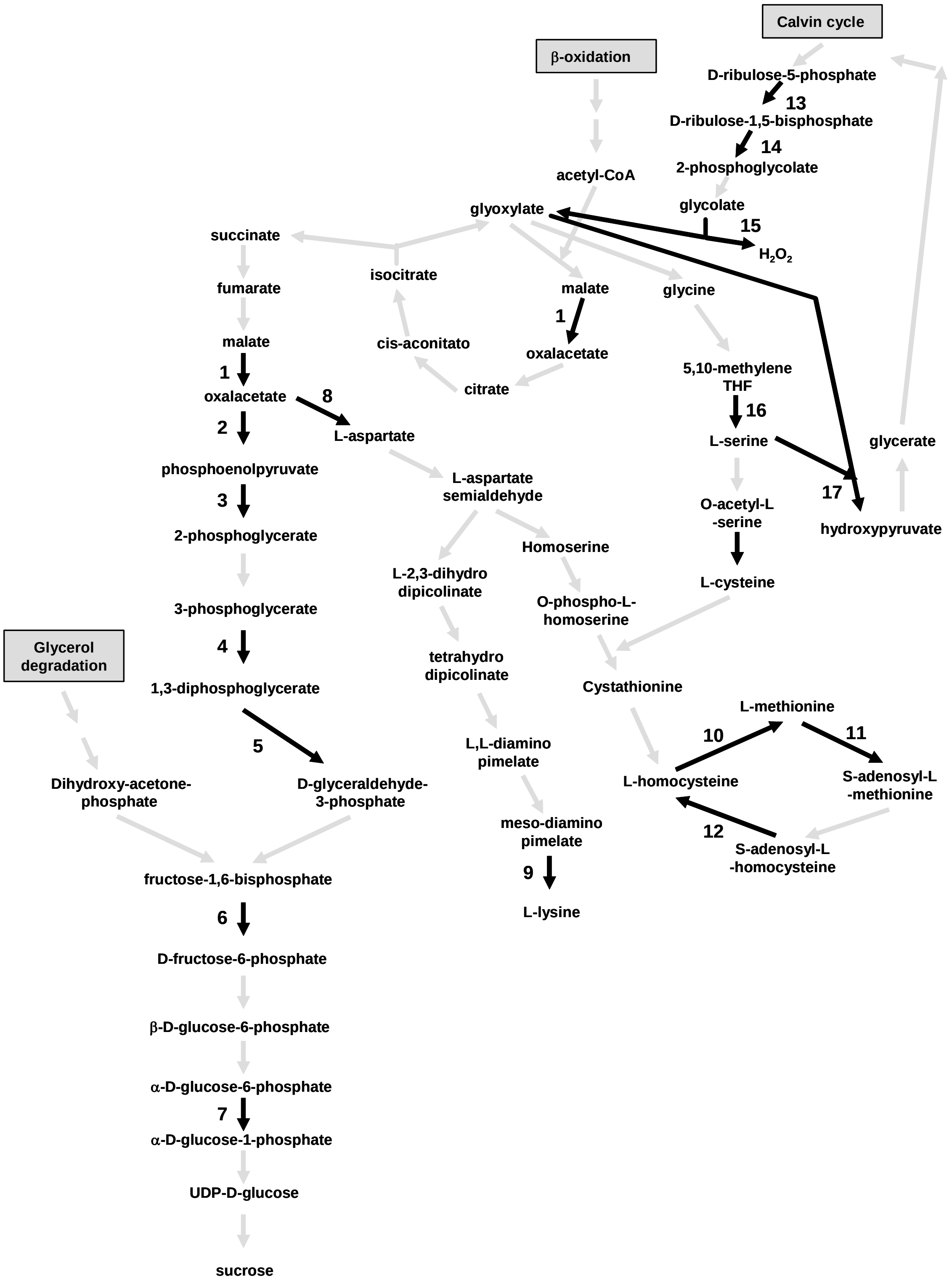
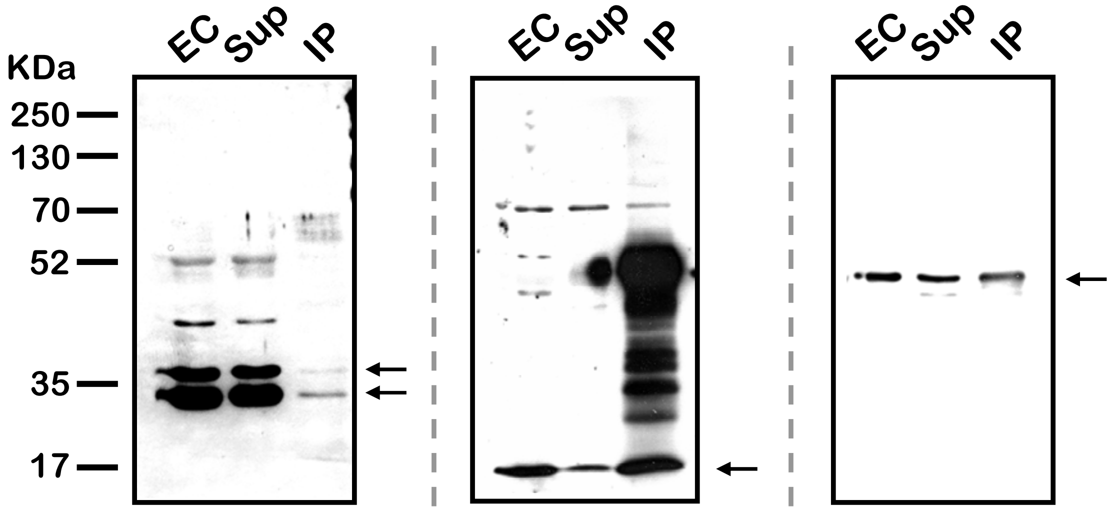

Calvin cycle
β-oxidation
D-ribulose-5-phosphate
13
D-ribulose-1,5-bisphosphate
14
2-phosphoglycolate
acetyl-CoA
glycolate
glyoxylate
15
H_2O_2
succinate
isocitrate
malate
glycine
fumarate
1
malate
cis-aconitato
oxalacetate
5,10-methylene THF
1
oxalacetate
8
citrate
16
2
L-aspartate
L-serine
glycerate
phosphoenolpyruvate
L-aspartate semialdehyde
17
3
O-acetyl-L -serine
hydroxypyruvate
2-phosphoglycerate
Homoserine
L-2,3-dihydro dipicolinate
L-cysteine
O-phospho-L-homoserine
3-phosphoglycerate
Glycerol degradation
4
tetrahydro dipicolinate
Cystathionine
1,3-diphosphoglycerate
L-methionine
10
11
5
L,L-diamino pimelate
Dihydroxy-acetone-phosphate
D-glyceraldehyde-3-phosphate
L-homocysteine
S-adenosyl-L -methionine
meso-diamino pimelate
12
S-adenosyl-L -homocysteine
fructose-1,6-bisphosphate
9
L-lysine
6
D-fructose-6-phosphate
β-D-glucose-6-phosphate
α-D-glucose-6-phosphate
7
α-D-glucose-1-phosphate
UDP-D-glucose
sucrose

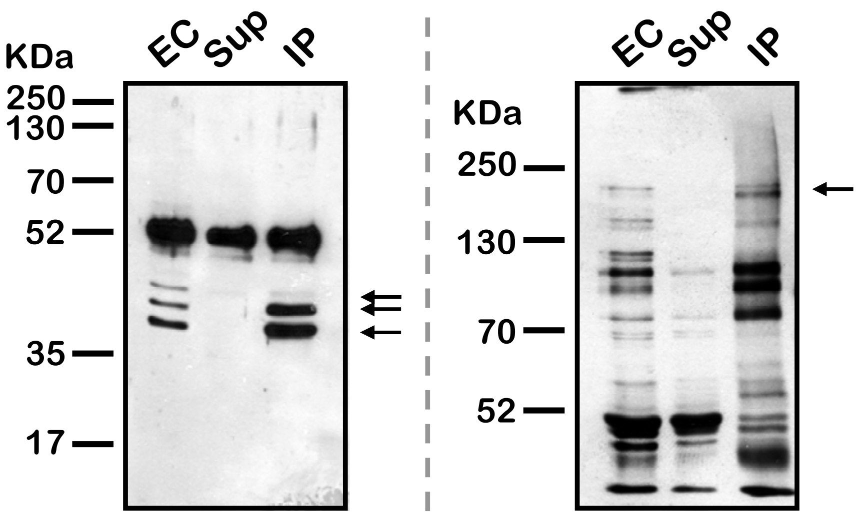
Figure S5



IP: anti-3-NitroY
WB: anti-CA
10% SDS-PAGE

IP: anti-3-NitroY
WB: anti-GRP7
10% SDS-PAGE

IP: anti-3-NitroY
WB: anti-FBPase
10% SDS-PAGE



IP: anti-3-NitroY
WB: anti-GAPDH
12% SDS-PAGE

IP: anti-3-NitroY
WB: anti-PKL
8% SDS-PAGE

Figure S6