

Table S1. Primers for site-directed mutagenesis and DNA sequencing.

Primers name	Sequence (5' to 3')
Site-directed mutagenesis	
AtGOX1-T4Vf	CATATGGCTAGCGAGATCGTTAACGTTACCGAGTATG
AtGOX1-T4Vr	CATACTCGGTAACGTTAACGATCTCGCTAGCCATATG
AtGOX1-T4Df	GCCATATGGCTAGCGAGATCGATAACGTTACCGAGTATGATG
AtGOX1-T4Dr	CATCATACTCGGTAACGTTATCGATCTCGCTAGCCATATGGC
AtGOX2-T4Vf	TCGCGGATCCATGGAGATCGTTAACGTTACCGAGT
AtGOX2-T4Vr	ACTCGGTAACGTTAACGATCTCCATGGATCCGCGA
AtGOX2-T4Df	TCGCGGATCCATGGAGATCGATAACGTTACCGAGT
AtGOX2-T4Dr	ACTCGGTAACGTTATCGATCTCCATGGATCCGCGA
ZmGO1-T5Vf	GCTAGCGGGGAGATCGTCAATGTCATGGAGT
ZmGO1-T5Vr	ACTCCATGACATTGACGATCTCCCCGCTAGC
ZmGO1-T5Df	TGGCTAGCGGGGAGATCGACAATGTCATGGAGTACC
ZmGO1-T5Dr	GGTACTCCATGACATTGTCGATCTCCCCGCTAGCCA
AtGOX-T158Vf	TGCTCTCACTGTAGACGTCCCAAGGCTAGG
AtGOX-T158Vr	CCTAGCCTTGGGACGTCTACAGTGAGAGCA
AtGOX-T158Df	TGCTCTCACTGTAGACGACCCAAGGCTAGG
AtGOX-T158Dr	CCTAGCCTTGGGTCGTCTACAGTGAGAGCA
ZmGO1-T159Vf	GCTCACCGTCGACGTGCCGCGCCTTGGC
ZmGO1-T159Vr	GCCAAGGCGCGGCACGTGACGGTGAGC
ZmGO1-T159Df	CGCTCACCGTCGACGACCCGCGCCTTGGCCG
ZmGO1-T159Dr	CGGCCAAGGCGCGGGTCGTGACGGTGAGCG
AtGOX1-S212Af	ACATCCTTCCAGGCTAAGGTACGGTCAATT
AtGOX1-S212Ar	AATTGACCGTACCTTAGCCTGGAAGGATGT
AtGOX1-S212Df	ACATCCTTCCAGTCTAAGGTACGGTCAATT
AtGOX1-S212Dr	AATTGACCGTACCTTAGACTGGAAGGATGT
AtGOX2-S212Af	GGATATCCTTCCAGGCCAAGGTACGGTCAA
AtGOX2-S212Ar	TTGACCGTACCTTGGCCTGGAAGGATATCC
AtGOX2-S212Df	GGATATCCTTCCAGTCCAAGGTACGGTCAA
AtGOX2-S212Dr	TTGACCGTACCTTGGACTGGAAGGATATCC
ZmGO1-S213Af	TGACGTCTTTCCAGGCCAGGGTGCGGTCGA
ZmGO1-S213Ar	TCGACCGCACCCCTGGCCTGGAAAGACGTCA
ZmGO1-S213Df	TGACGTCTTTCCAGTCCAGGGTGCGGTCGA
ZmGO1-S213Dr	TCGACCGCACCCCTGGACTGGAAAGACGTCA

AtGOX1-T265Af	GGGCCGAGATGGCTGCTGGGACATAGTC
AtGOX1-T265Ar	GACTATGTCCCAGCAGCCATCTCGGCCC
AtGOX1-T265Df	ACTATGTCCCAGCAGACATCTCGGCCCTTG
AtGOX1-T265Dr	CAAGGGCCGAGATGTCTGCTGGGACATAGT
AtGOX2-T265Af	GATTATGTCCCAGCAGCAATCTCAGCCCTTG
AtGOX2-T265Ar	CAAGGGCTGAGATTGCTGCTGGGACATAATC
AtGOX2-T265Df	CTTGATTATGTCCCAGCAGATATCTCAGCCCTTGAA
AtGOX2-T265Dr	TTCAAGGGCTGAGATATCTGCTGGGACATAATCAAG
ZmGO1-T266A-f	CGCGCTGATGGCCGCCGGCACGT
ZmGO1-T266A-r	ACGTGCCGGCGGCCCATCAGCGCG
ZmGO1-T266D-f	CTACGTGCCGGCGGACATCAGCGCGCTG
ZmGO1-T266D-r	CAGCGCGCTGATGTCCGCCGGCACGTAG

DNA sequencing

T7	TAATACGACTCACTATA
T7term	GCTAGTTATTGCTCAGCGG

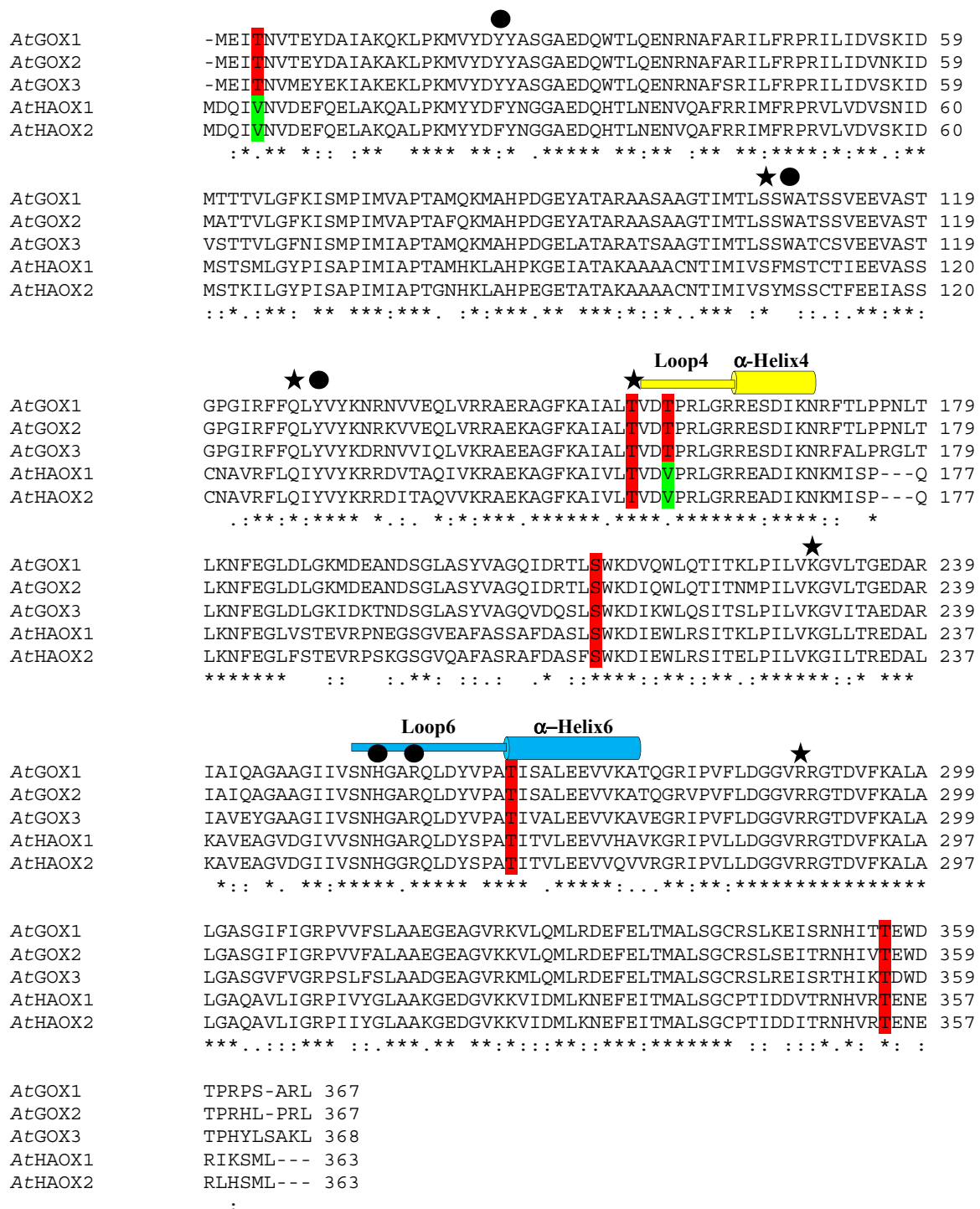


Figure S1. *AtGOX1*, *AtGOX2*, *AtGOX3*, *AtHAOX1* and *AtHAOX2* protein sequence alignments showing the conservation of phosphorylated residues and other important residues and structures

Sequence alignment was carried out using Clustal Omega software (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Phosphorylated amino acids T4, T155, T158, S212, T265 and T355 are highlighted in red. When the phosphorylated amino acid is replaced by a valine in *AtHAOX1* and *AtHAOX2* proteins, they are highlighted in green. Loop4 and α-helix4 are highlighted in yellow and loop6 and α-helix6 are highlighted in blue. Residues

involved in FMN binding are marked by stars [27]. Residues involved in the catalytic site are marked by circles [28,29]. UniProtKB: Q9LRR9 (*AtGOX1*), F4JFV6 (*AtGOX2*), O49506 (*AtGOX3*), Q9LJH5 (*AtHAOX1*) and Q24JJ8 (*AtHAOX2*).

Aerococcus	-----mnnndieynapseikyidvntydleee---askvvpghggnfyaga	44
Lactococcus	mhlssdvnfiknrtrtkmtytktstenkaleivnvkslegkvkqsmearagnkggfyirgg	60
Homo_HAOX2	-----mslvcltdfqah---areqlskstrdfiegg	28
Chlamydomonas	-----madlsflnlleeveee---akkvmpkmafdyystg	31
Nostoc	-----mtaisspinlfeyeql---akthlsqmafdyysg	32
Homo_GOX1	-----mlprlicindyeqh---aksvlpksiydyysrg	30
Arabidopsis_HAOX1	-----mdqivnvdefqel---akqalpkmyydfyngg	29
Arabidopsis_HAOX2	-----mdqivnvdefqel---akqalpkmyydfyngg	29
Arabidopsis_GOX3	-----meitnvmeyeki---akeklpkmydydyasg	28
Zea	-----mgeitnvmeygai---akqklpkmaydydyasg	29
Spinacia	-----meitnvmeygai---akqklpkmaydydyasg	28
Arabidopsis_GOX1	-----meitnvtneydai---akqklpkmydydyasg	28
Arabidopsis_GOX2	-----meitnvtneydai---akaklpkmvydydyasg	28
Brassica	-----meitnvtneygai---akeklpkmydydyasg	28
Vitis	-----meitnvtneygai---akqklpkmydydyasg	28
Nicotiana	-----meevtnvmeygai---akkklpkmvydydyasg	29
Populus	-----meitnvtneygai---akqklpkmafdydyasg	28
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Aerococcus	sgdewtkrandrawkhkllpyrlaqdveapdtsteilghkikapfimapiaahglahttk	104
Lactococcus	sedewtlntentsafnkkqimprvlrgidsadlstslfgiklktpiiqapvaaqglahaeg	120
Homo_HAOX2	addsitrddniaafkrirrlrprylrdvsevdtrttiggeeisapiciaptgfhclvwpdg	88
Chlamydomonas	sdtcytvgenrscfsrylllprmlrnvsrvdtshelfgirssmpvwvapmamhglahpgr	91
Nostoc	agdeitlqenravferiklrprmlvdvsqinltsvlgqplqlplliapmafqlahteg	92
Homo_GOX1	andeetladniaaafsrwklyprmlrnvaetdlstsvlgqrvsmpicvgatamqrmahvdg	90
Arabidopsis_HAOX1	aedqhtlnenvqafrrimfrprvlvdvsnidmstsmlygypisapimiaptamhklahpkg	89
Arabidopsis_HAOX2	aedqhtlnenvqafrrimfrprvlvdvskidmstkilgypisapimiaptgnhklahpeg	89
Arabidopsis_GOX3	aedqwtlqenrnafsrilfrprilidvskidvsttvlgfnismpimiaptamqkmahpdg	88
Zea	aedewtlqenreafrsilfrprilidvskidmtttvlgfkismpimvaptamqkmahpdg	89
Spinacia	aedqwtlaenrnafsrilfrprilidvtnidmtttlilgfkismpimiaptamqkmahpeg	88
Arabidopsis_GOX1	aedqwtlqenrnafarilfrprilidvskidmtttvlgfkismpimvaptamqkmahpdg	88
Arabidopsis_GOX2	aedqwtlqenrnafarilfrprilidvskidmtttvlgfkismpimvaptafqkmahpdg	88
Brassica	aedqwtlqenrnafarilfrprilidvskidmtttvlgfkismpimvaptamqkmahpeg	88
Vitis	aedqwtlyqnrfhafsqilfrprilidvskidmtttvlgfkismpimiaptamqkmahpeg	88
Nicotiana	aedqwtlaenrnafsrilfrprilidvskidmtttvlgfkismpimiaptamqkmahpeg	89
Populus	aedqwtlaenrnafsrilfrprilidvskidiattvlgfkismpimiaptafqkmahpeg	88
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Aerococcus	eagtaravsefgtimsisaysgatfeeisegln---ggprwffqiyamakddqgnrdildea	161
Lactococcus	evatakamaevgsifsistygstsvedaakaap---dapqffqlymskddkdfneflkka	177
Homo_HAOX2	emstaraaqaagicyitstfascslediviaap---eglrfwqlyvhpdqlnlkqliqrv	145
Chlamydomonas	evatcraaaaagvpftfstvatsslqeiqetgh---dnrifqlyvirnrevrrrwtea	147
Nostoc	elatamaaasagtgmvltstlstsleevaevgskfspslqwfqlyihkdrgltralvera	152
Homo_GOX1	elatvraqslgtgmmlsswatssieevaeagp---ealrwlqlylykdrevtkklvrqa	147
Arabidopsis_HAOX1	eiatakaaaacntimivsfmstctieevasscn---avrflqiyvykrrdvtaiqvkra	145
Arabidopsis_HAOX2	etatakaaaacntimivsymssctfeeiascn---avrflqiyvykrrditaqvakra	145
Arabidopsis_GOX3	elataratsaagtimtlsswatssveevastgp---girffqlyvykdrnvviqlvakra	144
Zea	enataraaaaagtimtlsswatssveevastgp---girffqlyvykdrkvveqlvrria	145
Spinacia	eyataraasaagtimtlsswatssveevastgp---girffqlyvykdrnvvaqlvrria	144
Arabidopsis_GOX1	eyataraasaagtimtlsswatssveevastgp---girffqlyvykdrnvvaqlvrria	144
Arabidopsis_GOX2	eyataraasaagtimtlsswatssveevastgp---girffqlyvykdrnvvaqlvrria	144
Brassica	eyataraasaagtimtlsswatssveevastgp---girffqlyvykdrnvvaqlvrria	144
Vitis	eyataraasatgtimtlsswatssveevastgp---girffqlyvykdrhvaqlvrria	144
Nicotiana	eyataraasaagtimtlsswatssveevastgp---girffqlyvykdrnvvaqlvrria	145
Populus	eyataraasaagtimtlsswatssveevastgp---girffqlyvykdrnvvaqlvrria	144
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Aerococcus	ksdgataiiltadstvsognrdrdvknkfvyppfgmpi--vqrylrg-----taegmslenn	213
Lactococcus	vsagvkaaiiltadstlgygredivnhfqqfppmpn--laafses-----dgtgkgise	229
Homo_HAOX2	eslgfkalvitldpvcgnrrhdirnqlrr--nltltdlqs-----pkkgnaiipy	193
Chlamydomonas	esrgfkalmtvdapvlgqrerdrnefvlpplghlanlttisglni--phapgesglft	206
Nostoc	yaagykalcltvdapvlgqrerdrnefvlpplghlanlttisglni--phapgesglft	210
Homo_GOX1	ekmgykaifvtvdpylgnrlldvnrfrklppqlrmknfetstlsfspeenfgddsglaa	207
Arabidopsis_HAOX1	ekagfkaivltvdvprlgrreadiknkmispq---lknfeglvste---vrpnegsgvea	199
Arabidopsis_HAOX2	ekagfkaivltvdvprlgrreadiknkmispq---lknfeglvste---vrpsksgsvqa	199
Arabidopsis_GOX3	eeagfkaialtvdvprlgrresdiknrfalprgltlknfegldlgk---idktndsglas	201
Zea	eragfkaialtvdvprlgrreadiknrfvlpphltnknfegldlgk---mdqaadsglas	202

Spinacia	eragfkaialtvdtprlgrreadiknrfvlppfltlknfegldlgk---mdkandsglss	201
Arabidopsis_GOX1	eragfkaialtvdtprlgrresdiknrftlppnltlknfegldlgk---mdeandsglas	201
Arabidopsis_GOX2	ekagfkaialtvdtprlgrresdiknrftlppnltlknfegldlgk---mdeandsglas	201
Brassica	ekagfkaialtvdtprlgrresdiknrftlppnltlknfegldlgk---mdeandsglas	201
Vitis	eragfkaialtvdtprlgrreadiknrfvlppfltlknfegldlgk---mdkaddsglas	201
Nicotiana	eragfkaialtvdtprlgrreadiknrfvlppfltlknfegldlgk---mdqasdsclas	202
Populus	eragfkaialtvdtprlgrresdiknrftlppfltlknfegldlgk---mdkandsglas	201
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Aerococcus	iyg-askqkispdreeeiaghsglpvfvgkqhpedadmaikrgasgiwvsnhgarqlye	272
Lactococcus	iya-aakqglvlediqkikkitnlpvkvqspidaddainagadgiwvsnhggrqldg	288
Homo_HAOX2	fgmtpistslcnwldswfqsitrlpiilkgiltkedaelavkhnvvgiivsnhggrqldg	253
Chlamydomonas	lftsevdsltwefipwlrgrvgtklpiivkgllspadaelavqygvdgiwvsnhggrqldy	266
Nostoc	yfaqqlnpaltwddlewlqslsplvlkgilrgddaaraveygakaivvsnhggrqldg	270
Homo_GOX1	yvakaidpsiswedikwlrsltpivakgilrgddareavkhngilvsnhgarqldg	267
Arabidopsis_HAOX1	fassafdaslskdielwlrsltpilvkglltredalkaveagvdiwvsnhgarqldy	259
Arabidopsis_HAOX2	fasrafdasfswkdielwlrsltpilvkgiltredalkaveagvdiwvsnhggrqldy	259
Arabidopsis_GOX3	yvagqvdsqslskdikwlsitltpilvkgvitaedariaaveygaagiivsnhgarqldy	261
Zea	yvagqvdrtslwkdvkwltitltpilvkgvltaedtrlavangaagiivsnhgarqldy	262
Spinacia	yvagqidrslskdvawltitltpilvkgvitaedarlavqhaagiivsnhgarqldy	261
Arabidopsis_GOX1	yvagqidrtlskdvqwlqtitltpilvkgvltgedariaiqagaagiivsnhgarqldy	261
Arabidopsis_GOX2	yvagqidrtlskdkiqwlqtitnmpilvkgvltgedariaiqagaagiivsnhgarqldy	261
Brassica	yvagqidrtlskdvqwlqtitnmpilvkgvltgedariaiqagaagiivsnhgarqldy	261
Vitis	yvagqidrtlskdvkwltitltpilvkgvltaedtrlaiqagaagiivsnhgarqldy	261
Nicotiana	yvagqidrtlskdvqwlqtitltpilvkgvltaedarlavqagaagiivsnhgarqldy	262
Populus	yvagqidrtlskdvqwlqtitltpilvkgvltaedarlsiqagaagiivsnhgarqldy	261
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Aerococcus	apgsfdtlpaiaervnk---rvpivfdsgvrrgehvakalasgadvvvalgrpvlfglalg	329
Lactococcus	gpasidvlpiaiksvnh---rvpivfdsgvrrgehvfkalaaggadvavgrpvlyglnlg	345
Homo_HAOX2	vlasidaltevvaavkg---kievylldggvrtgndvfkalalgakciflgrpvlwglack	310
Chlamydomonas	apsglhmlpavvaavrgcgssipvlvdggvrrgtdvfkalalgasgvllgrpvlyglavg	326
Nostoc	aialdalpeivaavng---kaevlldggirrgtdiikalaigaqavligrpvlwglavg	327
Homo_GOX1	vpasidvlpiveaveg---kvevflldggvrrgtdvfkalalgakavfgrpvwglafq	324
Arabidopsis_HAOX1	spatitvleevvhavkg---ripvllldggvrrgtdvfkalalgagavligrpivyglak	316
Arabidopsis_HAOX2	spatitvleevvqvrrg---ripvllldggvrrgtdvfkalalgagavligrpivyglak	316
Arabidopsis_GOX3	vpatisaleevvkaavg---ripvflldggvrrgtdvfkalalgasgvfgrpslflaad	318
Zea	vpatisaleevvkaavg---qlpvfvdggvrrgtdvfkalalgagvfvgrpvvflslaaa	319
Spinacia	vpatisaleevvkaavg---ripvflldggvrrgtdvfkalalgagvfvgrpvvflslaae	318
Arabidopsis_GOX1	vpatisaleevvkatqg---ripvflldggvrrgtdvfkalalgasgfigrpvfvflslaae	318
Arabidopsis_GOX2	vpatisaleevvkatqg---rvpvfldggvrrgtdvfkalalgasgfigrpvfvfalaae	318
Brassica	vpatisaleevvkatqg---rvpvfldggvrrgtdvfkalalgasgfigrpvfvflslaae	318
Vitis	vpatisaleevvkaavg---rvpvfldggvrrgtdvfkalalgasgfigrpvfvflslaae	318
Nicotiana	vpstimalaleevvkaavg---ripvflldggvrrgtdvfkalalgasgfigrpvfvflslaae	319
Populus	vpstimalaleevvkaavg---rvpvfldggvrrgtdvfkalalgasgfigrpvfvflslase	318
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Aerococcus	gwqgaysvldyfqkdltrvmqltgsqnvledlkgldlfdnpygyey-----	374
Lactococcus	gakgvqsvfelnkelsitmqlagtknieeikhtslid-----	383
Homo_HAOX2	gehgvkevlniltnefhtsmaltgcrsvaeinrnlvqfsrl-----	351
Chlamydomonas	ggagvervqlrlrseielsmalagcsvgqigpqlllpapsagpappmpaaqlckl	382
Nostoc	ggagvshvisllqkelnvamaligcsqlqdidtsflhl-----	365
Homo_GOX1	gekqvqdvleilkeefrlamalsgcqnkvkvidktlvkrknplavski-----	370
Arabidopsis_HAOX1	gedgvkkvidmlknefeitmalsgcptiddvtrnhvrteneriksm-----	363
Arabidopsis_HAOX2	gedgvkkvidmlknefeitmalsgcptidditrnhvrtenerlhsm-----	363
Arabidopsis_GOX3	geagvrkmlqmlrdefeltmalsgcrslreisrthiktdwdtphylsakl-----	368
Zea	geagvsnvlrmlrdefeltmalsgctslaeitrkhiitesdklsaisprl-----	369
Spinacia	geagvkkvlqmrrdefeltmalsgcrslkeisrshiaadwdgpssrav--arl---	369
Arabidopsis_GOX1	geagvrkvlqmlrdefeltmalsgcrslkeisrnhitewdtprrsarl-----	367
Arabidopsis_GOX2	geagvkkvlqmlrdefeltmalsgcrslseitrnhivtewdtprrhlprl-----	367
Brassica	geagvrkvlqmlrdefeltmalsgcrslseitrnhitewetprhlprl-----	367
Vitis	geagvrkvlqmlreefeltmalsgcrslkeitrhivtewevphpgsrplprl---	371
Nicotiana	geagikkvlqmlrdefeltmalsgcrslneitrnhivtewdapraa--lpaprl---	371
Populus	geagvrkvlqmlrdefeltmalsgcrslkeitrnhivtewdhpral--lapk-l---	369
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Figure S2. Amino acid sequence alignment of *Lactococcus lactis*, *Aerococcus viridians*, *Nostoc* and *Chlamydomonas reinhardtii* lactate oxidases with glycolate oxidases and hydroxy-acid oxidases of human and plants.

Sequence alignment was carried out using Clustal Omega software (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Phosphorylated amino acids T4, T155, T158, S212, T265 and T355 from *Arabidopsis thaliana* and their equivalents in other proteins are highlighted in red. Amino acids in yellow were presumed to be involved in substrate binding (Hackenberg et al, 2011). UniProtKB: Lactate oxidase: Q44467 (*Aerococcus viridians* IF012219), Q9CG58 (*Lactococcus lactis* Il1403), Q8Z0C8 (*Nostoc* sp. PCC 7120), A8IEL8 (*Chlamydomonas reinhardtii*) ; HAOX : Q9LJH5 (*Arabidopsis thaliana*, HAOX1), Q24JJ8 (*Arabidopsis thaliana*, HAOX2), Q9NYQ3 (Homo sapiens, HAOX2) ; GOX : C0P702 (*Zea mays*), Q9LRR9 (*Arabidopsis thaliana*, AtGOX1), F4JFV6 (*Arabidopsis thaliana*, AtGOX2), O49506 (*Arabidopsis thaliana*, AtGOX3), Q3L1H0 (*Brassica napus*), A5B1R1 (*Vitis vinifera*), E1AXT8 (*Nicotiana benthamiana*), A0A4U5NR44 (*Populus alba*), P05414 (*Spinacia oleracea*), and Q9UJM8 (Homo sapiens, GOX1).

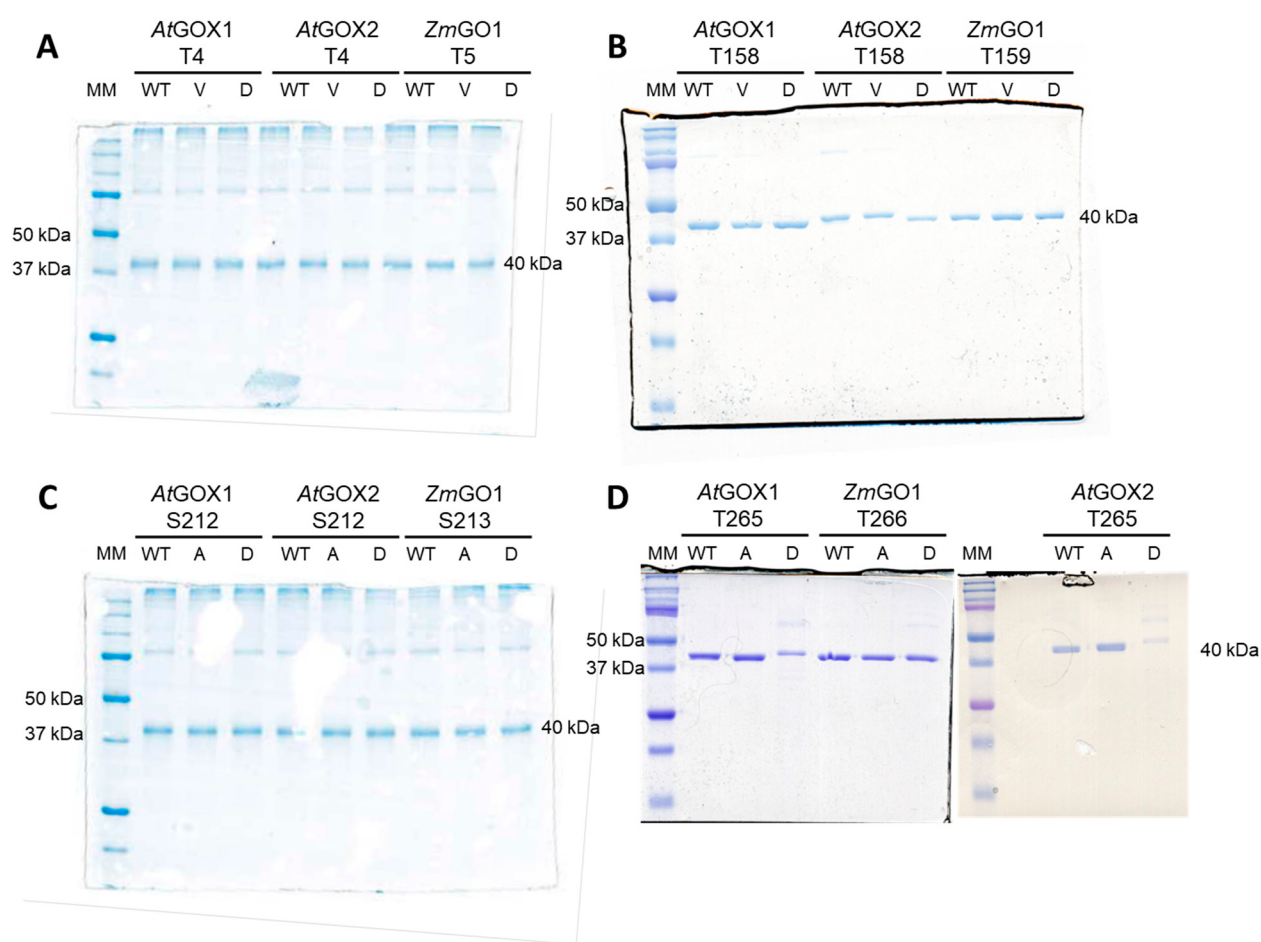


Figure S3. Purified recombinant His-tag proteins of wild-type *AtGOX1*, *AtGOX2* and *ZmGO1* and their phosphosite mutated forms on Coomassie-stained SDS-PAGE gels. Two micrograms of desalted purified GOX proteins were separated by SDS-PAGE with 10% acrylamide and stained with Coomassie Blue R-250. Purified recombinant proteins of wild-type *AtGOX1*, *AtGOX2* and *ZmGO1* and (A) *AtGOX1/2* T4V/D and *ZmGO1* T5V/D, (B) *AtGOX1/2* T158V/D and *ZmGO1* T159V/D, (C) *AtGOX1/2* S212A/D and *ZmGO1* S213A/D, and (D) *AtGOX1/2* T265A/D and *ZmGO1* T266A/D. Molecular mass markers (MM) are shown with the molecular masses of 50 kDa and 37 kDa indicated. Each GOX recombinant protein was estimated to be ~40 kDa.