

GLX1-1

```

GLX1-1_1  MVRIIPMAASSIRPSLACFSDSPRPFISLLSRNLRTLHVPQSQFLGTLTKHLLRRSVNC
GLX1-1_2  -----
GLX1-1_1  LGVAESGKAAQATTQDDLLTWKNDKRRMLHVYRVGDMDRITIKFYTECLGMKLLRKRD
GLX1-1_2  -----MLHVYRVGDMDRITIKFYTECLGMKLLRKRD
*****

GLX1-1_1  PEEKYTNAFLGYGPDSSHFIETLYNYGVDKYDIGAGFGHFGIAVDDVAKTVELVKAKGG
GLX1-1_2  PEEKYTNAFLGYGPDSSHFIETLYNYGVDKYDIGAGFGHFGIAVDDVAKTVELVKAKGG
*****

GLX1-1_1  KVSREPGFVKGGKTVIAFIEDPDGYKFELLERGPTPEPLCQVMLRVGDLRAIKFYEKAF
GLX1-1_2  KVSREPGFVKGGKTVIAFIEDPDGYKFELLERGPTPEPLCQVMLRVGDLRAIKFYEKAF
*****

GLX1-1_1  GMELLRTRDNPEYKTIAMMGYPEDKFPVLELTYNYGVTYDKGNAYAQIAIGTDDVYK
GLX1-1_2  GMELLRTRDNPEYKTIAMMGYPEDKFPVLELTYNYGVTYDKGNAYAQIAIGTDDVYK
*****

GLX1-1_1  TAAAIKLFGGKITREPGLPGISTKITACLDPDGKSVFVDNIDFLKELE
GLX1-1_2  TAAAIKLFGGKITREPGLPGISTKITACLDPDGKSVFVDNIDFLKELE
*****

```

GLX1-2

```

GLX1-2_1  -----MAEASDLEWPKKNRRFLHV
GLX1-2_2  -----MAEASDLEWPKKNRRFLHV
GLX1-2_3  -----MAEASDLEWPKKNRRFLHV
GLX1-2_4  -----MAEASDLEWPKKNRRFLHV
GLX1-2_5  -----MAEASDLEWPKKNRRFLHV
GLX1-2_6  MNEIASASMLRLCQCFCISICNVHFSMRAESSFLSRNMAEASDLEWPKKNRRFLHV
*****

GLX1-2_1  VYRVGDLDRITIEFYTEVFGMKLLRKRDIPEEKYSNAFLGFGPETSNFVVELTYNYGVSSY
GLX1-2_2  VYRVGDLDRITIEFYTEVFGMKLLRKRDIPEEKYSNAFLGFGPETSNFVVELTYNYGVSSY
GLX1-2_3  VYRVGDLDRITIEFYTEVFGMKLLRKRDIPEEKYSNAFLGFGPETSNFVVELTYNYGVSSY
GLX1-2_4  VYRVGDLDRITIEFYTEVFGMKLLRKRDIPEEKYSNAFLGFGPETSNFVVELTYNYGVSSY
GLX1-2_5  VYRVGDLDRITIEFYTEVFGMKLLRKRDIPEEKYSNAFLGFGPETSNFVVELTYNYGVSSY
GLX1-2_6  VYRVGDLDRITIEFYTEVFGMKLLRKRDIPEEKYSNAFLGFGPETSNFVVELTYNYGVSSY
*****

GLX1-2_1  DIGTGFHFAISTQDVSKLVENVRAGGNVTRPGPVKGGGSVIAFVKDPDGYTFELIQR
GLX1-2_2  DIGTGFHFAISTQDVSKLVENVRAGGNVTRPGPVKGGGSVIAFVKDPDGYTFELIQR
GLX1-2_3  DIGTGFHFAISTQDVSKLVENVRAGGNVTRPGPVKGGGSVIAFVKDPDGYTFELIQR
GLX1-2_4  DIGTGFHFAISTQDVSKLVENVRAGGNVTRPGPVKGGGSVIAFVKDPDGYTFELIQR
GLX1-2_5  DIGTGFHFAISTQDVSKLVENVRAGGNVTRPGPVKGGGSVIAFVKDPDGYTFELIQR
GLX1-2_6  DIGTGFHFAISTQDVSKLVENVRAGGNVTRPGPVKGGGSVIAFVKDPDGYTFELIQR
*****

GLX1-2_1  GPTPEPFCQVMLRVGDLRAIKFYEKALGMRLLRKIERPEYKTYIGMMGYAEYESIVLE
GLX1-2_2  GPTPEPFCQVMLRVGDLRAIKFYEKALGMRLLRKIERPEYKTYIGMMGYAEYESIVLE
GLX1-2_3  GPTPEPFCQVMLRVGDLRAIKFYEKALGMRLLRKIERPEYKTYIGMMGYAEYESIVLE
GLX1-2_4  GPTPEPFCQVMLRVGDLRAIKFYEKALGMRLLRKIERPEYKTYIGMMGYAEYESIVLE
GLX1-2_5  GPTPEPFCQVMLRVGDLRAIKFYEKALGMRLLRKIERPEYKTYIGMMGYAEYESIVLE
GLX1-2_6  GPTPEPFCQVMLRVGDLRAIKFYEKALGMRLLRKIERPEYKTYIGMMGYAEYESIVLE
*****

GLX1-2_1  LTYNYDVTEYTKGNAYAQIAIGTDDVYKSSEVIRKIVNQELGGKITREAGPLGLGTRKIVS
GLX1-2_2  LTYNYDVTEYTKGNAYAQIAIGTDDVYKSSEVIRKIVNQELGGKITREAGPLGLGTRKIVS
GLX1-2_3  LTYNYDVTEYTKGNAYAQIAIGTDDVYKSSEVIRKIVNQELGGKITREAGPLGLGTRKIVS
GLX1-2_4  LTYNYDVTEYTKGNAYAQIAIGTDDVYKSSEVIRKIVNQELGGKITREAGPLGLGTRKIVS
GLX1-2_5  LTYNYDVTEYTKGNAYAQAQMMCTKAVKLLR-----
GLX1-2_6  LTYNYDVTEYTKGNAYAQIAIGTDDVYKSSEVIRKIVNQELGGKITREAGPLGLGTRKIVS
*****

GLX1-2_1  FLDPDGWKTIVLDNKKDFLKELE
GLX1-2_2  FLDPDGWKTIVLDNKKDFLKELE
GLX1-2_3  FLDPDGWKTIVLDNKKDFLKELE
GLX1-2_4  FLDPDGWKTIVLDNKKDFLKELE
GLX1-2_5  -----
GLX1-2_6  FLDPDGWKTIVLDNKKDFLKELE

```

GLX1-3

```

GLX1-3_1  -----MASEARESPA
GLX1-3_2  -----MASEARESPA
GLX1-3_3  -----MASEARESPA
GLX1-3_4  MSSYSIASAISRISPLIRFVKPYSTGFSFITACNSTRPKRFDQLCVSMASEARESPA
*****

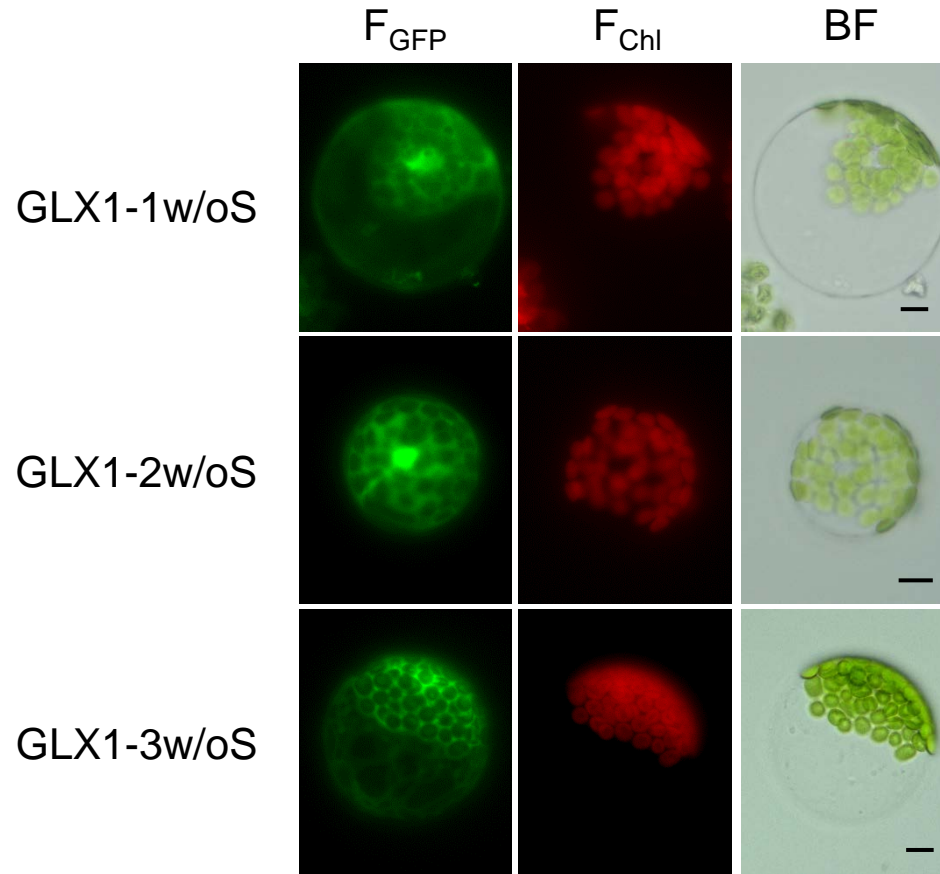
GLX1-3_1  NNPGSLSTNRDEATKGYIMQQTMFRIKDPKASLDFYSRVLGMSLLKRLDFSEMKFSLYFLG
GLX1-3_2  NNPGSLSTNRDEATKGYIMQQTMFRIKDPKASLDFYSRVLGMSLLKRLDFSEMKFSLYFLG
GLX1-3_3  NNPGSLSTNRDEATKGYIMQQTMFRIKDPKASLDFYSRVLGMSLLKRLDFSEMKFSLYFLG
GLX1-3_4  NNPGSLSTNRDEATKGYIMQQTMFRIKDPKASLDFYSRVLGMSLLKRLDFSEMKFSLYFLG
*****

GLX1-3_1  YEDTTAPTDPDPTERTVWTFGQPATIELTHNWGTESDPEFKGYHNGNSEPRGFHIGVTV
GLX1-3_2  YEDTTAPTDPDPTERTVWTFGQPATIELTHNWGTESDPEFKGYHNGNSEPRGFHIGVTV
GLX1-3_3  YEDTTAPTDPDPTERTVWTFGQPATIELTHNWGTESDPEFKGYHNGNSEPRGFHIGVTV
GLX1-3_4  YEDTTAPTDPDPTERTVWTFGQPATIELTHNWGTESDPEFKGYHNGNSEPRGFHIGVTV
*****

GLX1-3_1  DVHKACERFEELGVEFAKKPNDGKMKNIATIKDPDGYWIEIFDLKTI GTTTVNA
GLX1-3_2  DVHKACERFEELGVEFAKKPNDGKMKNIATIKDPDGYWIEIFDLKTI GTTTVNA
GLX1-3_3  DVHKACERFEELGVEFAKKPNDGKMKNIATIKDPDGYWIEIFDLKTI GTTTVNA
GLX1-3_4  DVHKACERFEELGVEFAKKPNDGKMKNIATIKDPDGYWIEIFDLKTI GTTTVNA
*****

```

Supplemental Fig. S1. Amino acid sequences for each AtGLX1 splice variant.



Supplemental Fig. S2. Subcellular localisation of GLX1w/oS–GFP fusion proteins expressed in *A. thaliana* mesophyll protoplasts. Fluorescence images of GFP (F_{GFP}), chlorophyll autofluorescence (F_{Chl}), and the bright field (BF) are shown. Black bars = 10 μm .

S6803GLX1 LLHTMIRVGDLDK-----SLQFYCDILGMNLLRKKDYPSEFTPLAFVGYGK-----ESENAVIELTHNWGTDK-----
 EcGLX1 LLHTMIRVGDLDK-----SIDFYTKVLGMKLLRSTENPEYKYSLAFVGYGP-----ETEEAVIELTYNWGVDK-----
 OsGLX1-1 LLHVYVRVGDIDR-----TIKFYTECLGMKLLRKRDIPEEKYTNAFVLGYGA-----EDNHFVVELTYNYGVDK-----
 ZmGLX1-1 LLHVYVRVGDLDK-----TIKFYTECLGMKLLRKRDIPEEKYSNAFLGYGP-----EESHFVVELTYNYGVDK-----
 AtGLX1-1 MLHVYVRVGDMDR-----TIKFYTECLGMKLLRKRDIPEEKYTNAFVLGYGP-----EDSHFVIELTYNYGVDK-----
 MpGLX1-1 MLHVYVRVGDLDK-----TIKFYTECLGMKLLRKRDFPDEKYTNAFVLGYGP-----EDSHFVVELTYNYGVFS-----
 SmGLX1-1 MLHVYVRVGDLDK-----TIKFYTECLGMKLLRKRDIPEERYTNAFVLGYGP-----EDSHFVVELTYNYGVDK-----
 OsGLX1-3 MLHVYVRVGDLDK-----TIKFYTECLGMKLLRKRDIPEERYTNAFVLGYGP-----EDSHFVVELTYNYGVES-----
 ZmGLX1-4 LLHVYVRVGDLDK-----TIKFYTECLGMKLLRKRDIPEERYTNAFVLGYGP-----EDSHFVVELTYNYGVES-----
 CvGLX1-1 MLHAVYRVGDDMA-----TIKYQDCFCGMKLLRFRDIKEEKYSNAFLGYGP-----EETHFAMELTYNYGVDS-----
 OsGLX1-2 LLHAVYRVGDLDR-----TIKCYTECFGMKLLRKRDPPEEKYTNAFVLGFGE-----EDTNFAVELTYNYGVDK-----
 ZmGLX1-3 MLHAVYRVGDLDR-----TIKYYTECFGMKLLRKRDPPEEKYTNAFVLGFGE-----ENTNFAVELTYNYGVDK-----
 ZmGLX1-2 MLHAVYRVGDLDRTIKYAHLQLRVMLFVHYTECFGMKLLRKRDIPEEKYTNAFVLGFGE-----EDTNFAVELTYNYGVDK-----
 AtGLX1-2 FLHVYVRVGDLDLDR-----TIEFYTEVFGMKLLRKRDIPEEKYSNAFLGFGE-----ETSNFVVELTYNYGVSS-----
 MpGLX1-2 IQQTMIRIKDPKV-----SLDFYTRILGMTLLKRLDFPEMKFSLYFVGYED---PASIPTDPAERMAYTF---VLELTHNWGTESDENFK
 MpGLX1-3 IQQTMIRIKDPKV-----SLDFYTRILGMTLLKRLDFPEMKFSLYFVGYED---PASIPTDPAERMAYTFSSKATLELTHNWGTESDENFK
 SmGLX1-2 VQQTMIRIKDPKA-----SLDFYSRVLMGMTLLKRLDFPDSKFSLYFVGYED---SAAEPKDPPIERVWTFRRKATIELTHNWGTETDPDFK
 OsGLX1-4 MQQTMIRIKDPKV-----SLDFYSRVLMGMTLLKRLDFPEMKFSLYFVGYED---VESAPTDPVKRTVWTFGQRATLELTHNWGTENDPEFK
 ZmGLX1-5 LQQTMIRIKDPKV-----SLDFYSRVLMGMTLLKRLDFPEMKFSLYFVGYED---VTLAPDDHIKRTWTFRQKATLELTHNWGTENDPEFK
 AtGLX1-3 MQQTMIRIKDPKA-----SLDFYSRVLMGMTLLKRLDFSEMKFSLYFVGYED---TTTAPTDPTERTVWTFGQPATIELTHNWGTESDPEFK
 CvGLX1-2 FQQTMIRIKDPQP-----SLDFYTRVLMGMTLLKRLDFADMKFSLYFLAYQS---PEDVPADPVERAKWMFGLPACLELTHNWGTESDPDFK
 PtGLX1 FSQTMIRIKDPKR-----SLAFYKAMGMKLLSEKHFN---DFSLYFLGSSN---VADG-----ADTKTLFQPVLELTHNHGTENDDDFR
 CmGLX1 FAQTMIRIKDPK-----SRQFYEALG-MNFLTFRDFPPELSFSLYFFALEKDPVPAEDAPQPERAKWLFSRQYPTLELTHNWGTEDKPNFK
 ScGLX1 LNHITCLRVKDPAR-----AVKFYTEHFGMKLLSRKDFEEAKFSLYFLSFPK-----DDIPKNKNGEPDVFAGHVLELTHNWGTENKPNFYK

S6803GLX1 →---YDLNGFGHIALGVEDIYSTCDKIRDK--GGKVVRPEPGPMKHGTTVIAFVEDPDGYKIEL
 EcGLX1 ---YELGTAYGHIALSVDNAEAACEKIRQN--GGNVTREAGPVKGGTTVIAFVEDPDGYKIEL
 OsGLX1-1 ---YDIGAGFGHFIADVDDVAKTVELIRAK--GGKVTRPEPGPVKGGKTVIAFVEDPDGYKFEI
 ZmGLX1-1 ---YDIGAGFGHFIADVDDVAKTVELIRAK--AGKVIREAGPVKGGGETVIAFVEDPDGYKFEI
 AtGLX1-1 ---YDIGAGFGHFIADVDDVAKTVELVKAK--GGKVSREPGPVKGGKTVIAFIEDPDGYKFEL
 MpGLX1-1 ---YDIGTGFHFGIADVDDVQKVDLVKAK--GGVVSREPGPVKGGKSIVIAFVDDPDGYKFEL
 SmGLX1-1 ---YDIGTGFHFGIADVDDVYKTVDLVKAK--GGKVSREAGPVKGGSTVIAFVDDPDGYKFEL
 OsGLX1-3 ---YDIGTAFGHFIADVDDVAKTVDLIKAK--GGTVTREPGPVKGGKSIVIAFIEDPDGYKFEL
 ZmGLX1-4 ---YNIPTGFHFGIADVDDVAKTVELIKAK--GGTVTREPGPVKGGKSIVIAFIEDPDGYKFEL
 CvGLX1-1 ---YDLGEGFGHFIATPDAYKMEAVKAK--GGRVTREPGPTKGGKTVIAFVEDPTGYKFEL
 OsGLX1-2 ---YDIGAGFGHFAIATEDVYKLAEEKISSCCCKITREPGPVKGGSTVIAFAQDPDGYMFEL
 ZmGLX1-3 ---YDIGTGFHFGFAIANDDVYKLAENIKS--KGGKITREPGPVKGGSTVIAFAQDPDGYMFEL
 ZmGLX1-2 ---YDIGTGFHFGFAIANEDG-----GKITRDPGPVKGGSSTVIAFAQDPDGYMFEL
 AtGLX1-2 ---YDIGTGFHFGFAISTQDVSKLVENVRAK--GGNVTREPGPVKGGGSIVIAFVKDDPDGYTFEL
 MpGLX1-2 GHHNGNSEPRGYGHIGITVDDTYKACERFEKM--GVKPVKKPDDGK--MKGVAFIQDPDGYWIEI
 MpGLX1-3 GHHNGNSEPRGYGHIGITVDDTYKACERFEKM--GVKPVKKPDDGK--MKGLAFIQDPDGYWIEI
 SmGLX1-2 GYHNGNADPRGYGHIGISVDDTYRACERFEKL--GVEFVKKPDDGS--MKGLAFIKDPDGYWIEI
 OsGLX1-4 GYHNGNSDPRGFGHIGVTVDVHKACERFERL--GVEFVKKPDDGK--MKGIAFIKDPDGYWIEI
 ZmGLX1-5 GYHNGNSDPRGFGHIGVTVDVHKACERFERL--GVEFVKKPDDGK--IKGIAFIKDPDGYWIEI
 AtGLX1-3 GYHNGNSEPRGFGHIGVTVDVHKACERFEEL--GVEFAKKPNDGK--MKNIAFIKDPDGYWIEI
 CvGLX1-2 GYHNGNTQPRGFGHIGLCVPDVEAACARFEEL--GVEFVKKPNDGK--MRNLAFIKDPDGYWIEI
 PtGLX1 YYNGNEDRQGRFGHIGFLVDDVYKACDALRPL--GFGFRKEPDGGS--MKGLAFAYDPDGYWIEI
 CmGLX1 YANGNTPEPKGYGHIGFLVDDLYASCAAVEKA--GYVVSRRKPGPFQ--VGEIAFVRDPDGYWIEL
 ScGLX1 INNGNEEPRHGFHICFSVSDINKTCEELESQ--GVKPKRLSEGR--QKDIAPALDPDGYWIEL

Supplemental Fig. S3. Sequence comparison of the Glyoxalase domain (Pfam, PF00903) in the N-terminus of glyoxalase I (GLX1) among oxygenic phototrophs. Blue shading represents the metal-binding sites; orange shading represents substrate-binding sites. Navy arrows indicate the regions specific to Zn²⁺-dependent GLX1. S6803, cyanobacterium *Synechocystis* sp. PCC 6803; Cv, green alga *Chlorella variabilis*; Mp, liverwort *Marchantia polymorpha*; Sm, fern *Selaginella moellendorffii*; At, C₃ plant *Arabidopsis thaliana*; Os, C₃ plant *Oryza sativa*; Zm, C₄ plant *Zea mays*; Cm, red alga *Cyanidioschyzon merolae*; Pt, diatom *Phaeodactylum tricornutum*; Ec, heterotrophic prokaryote *Escherichia coli*; and Sc, heterotrophic eukaryote *Saccharomyces cerevisiae*.

OsGLX2-2 EYTVKNLKFILTVEPDNEKVKQKLEWAQKQREANQPTIPSTIGEEFETNTFMVRD-----LPEIQAKFGAKSPVEALREVRKTKDNW
 ZmGLX2-2 EYTVKNLKFMLTLEPENKTKQKLEWAQKQREANQPTVPSTIGDEFEINTFMVRD-----LPEIQAKFSVNSPVEAMREVRKTKDNW
 AtGLX2-2 EYTVKNLEFALTVEPNNGKIQQKLAWARQQRQADLPTIPSTLEEELETNPFMRVD-----KPEIQEKLKCKSPIDTMREVRNKKDQW
 SmGLX2-2 EYTAKNLKFAMSVDPHNDALKQKVAWTEEQRNRDKPTVPSTIREELQTNPFMRVN-----VKEFQVHMGESDPVELLASLRAAKDQF
 CmGLX2 EYTVANLAFALTVEPNNESIRNKLKEAERLRAENKATIPSTVGGEQQWNVFLRA-----ADAQWMTELREKKNRF
 AtGLX2-4 EYTLSNSKFALSIEPTNEVLQSYAAYVAELRDKKLPTIPTTMKMEKACNPFLRT---ENTDIRRALGIPETADEAEALGIIRRAKDNF
 AtGLX2-5 EYTLSNSKFALSLEPNNEVLQSYAAHVAELRSKKLPTIPTTVKMEKACNPFLRS---SNTDIRRALRIPEAADEAEALGIIRRAKDDF
 OsGLX2-1 EYTLSNSKFALSIEPGNKDLQEYAANAADLRKRNTPTVPTTIGREKQCNPFLLRT---SSPEIKNTLSIPDHFDDARVLEVVRRAKDNF
 ZmGLX2-1 EYTLNNAKFALSVEPGNKALQEYAANAAELRNKNIPTVPTTIGREKECNPFLLRT---SNPEIKRTLSPVDHDFEDRVLGVVRRAKDNF
 CvGLX2 EYTASNAKFAAHVDEGNEDLQRMKADIEAKRARGEPTVPSQLGDELKCNPFLLRPGTLDSPAIRSKLGVPEGASNDVAFGAIRAAKDTF
 PtGLX2 EYTSSNAKFALAIIEPGNSALVSRAEEIKATREERGEPTVPSNLGVEKQTNPFLLR---CDMSAEIRQNIQVKISDSADVFGRIRKAKDKF
 MpGLX2-1 EYTLNNAKFAMSVEPQNEALSSRYEKVAELRRKGLPTVPTSLGEEKSFNPFLLRP---FSQELRKSVHLNSSASDVETFAAVRLAKDRY
 SmGLX2-1 EYTLNNAKFAMTIEPNPALNSHFEEKVKQLRDSGLATIPSSVGEEKKFNPFLLRP---ASREIRRSNLNLSDDASDSVFAAVRKAKDRA
 MpGLX2-2 EYTASNIRFAVTVEPHNEDLLSQRQLVEQLRQKGQPTIPTTIGQENSFNPFLLRP---FVESVRQSLNKSASENNVEVFTALRLAKDKF
 S6803GLX2 EYTLGNLKFALTVDPSNKLQERFQTVQGDQRQATIPSWLGTEKRTNPFLLRW---DNPAIQARVGMTEP---ARVFGKLRGMKDNF
 EcGLX2 EYTLNNAKFALSILPHDLSINDYYRKVKELRAKNQITLPVILKNERQINVFLRTED--IDLINVINEETLLQQPEERFAWLRSKKDRF
 ScGLX2 EYTSNDNVKFVRKIYPQVGENKALDELEQFCCKHEVTAGRFTLKDEVEFNPFMRLE--DPKVQKAAGDTNNSWDRAQIMDKLRAMKNRM

Supplemental Fig. S4. Sequence comparison of the hydroxyacylglutathione hydrolase domain (Pfam, PF16123) in the C-terminus of glyoxalase II (GLX2) among oxygenic phototrophs. Orange shading indicates substrate-binding sites. S6803, cyanobacterium *Synechocystis* sp. PCC 6803; Cv, green alga *Chlorella variabilis*; Mp, liverwort *Marchantia polymorpha*; Sm, fern *Selaginella moellendorffii*; At, C₃ plant *Arabidopsis thaliana*; Os, C₃ plant *Oryza sativa*; Zm, C₄ plant *Zea mays*; Cm, red alga *Cyanidioschyzon merolae*; Pt, diatom *Phaeodactylum tricornutum*; Ec, heterotrophic prokaryote *Escherichia coli*; and Sc, heterotrophic eukaryote *Saccharomyces cerevisiae*.