

Supplementary Materials:

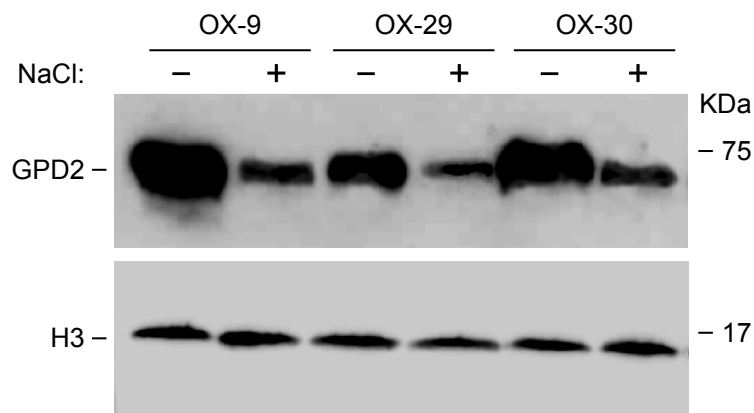


Figure S1. Analysis of GPD2 protein abundance in overexpression strains OX-9, OX-29, and OX-30. The upper panel shows an immunoblot of transgenic GPD2 protein abundance, detected with an anti AcV5 monoclonal antibody, in cells cultured for 6 hours in minimal HS medium or HS medium containing 100 mM NaCl. Immunodetection of histone H3 was used as a control for equivalent loading of the lanes (bottom panel).

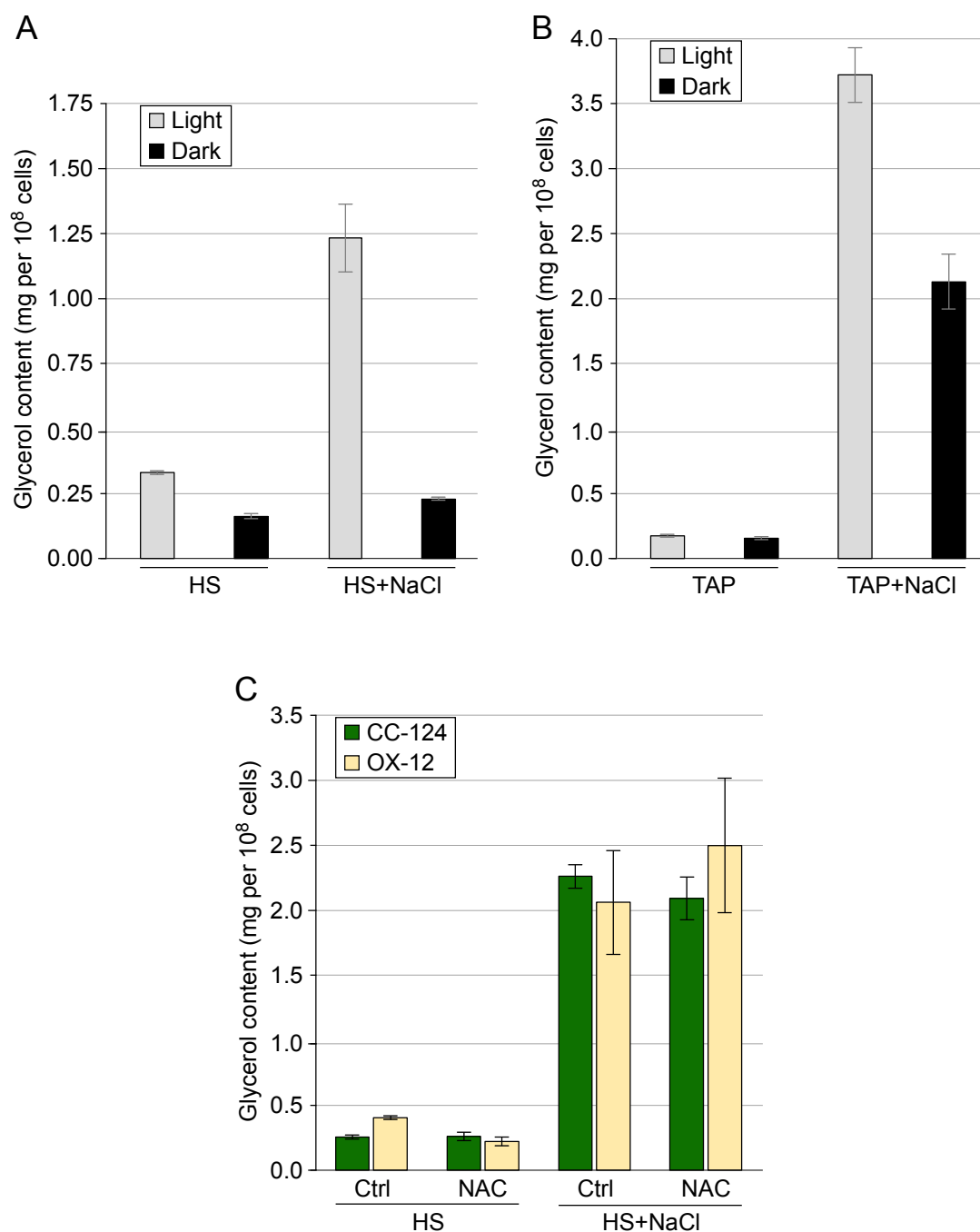


Figure S2. Effect of light and the ROS scavenger *N*-acetyl-L-cysteine (NAC) on glycerol accumulation by the wild type and OX-12 strains. **(A)** Glycerol content in the wild type cultured in minimal HS medium or HS medium containing 100 mM NaCl (HS+NaCl) for 6 hours under continuous light (Light) or under complete darkness (Dark). Cells, in the middle of the logarithmic phase, were grown for three days in minimal HS medium under continuous darkness (to deplete them from stored starch) prior to the experiment. Values shown are the mean \pm SD of three independent experiments ($n=3$). **(B)** Glycerol content in the wild type cultured in TAP medium or TAP medium containing 100 mM NaCl (TAP+NaCl) for 6 hours under continuous light (Light) or under complete darkness (Dark). Values shown are the mean \pm SD of three independent experiments ($n=3$). **(C)** Glycerol content in the wild type and OX-12 strains cultured in HS medium or HS medium containing NaCl, in the absence (Ctrl) or presence of *N*-acetyl-L-cysteine (NAC). Values shown are the mean \pm SD of three independent experiments ($n=3$).

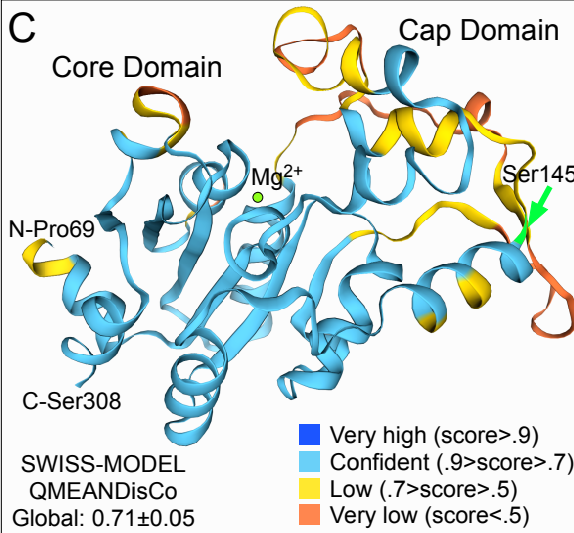
A

Experiment	Sequence	Modifications	Quality PEP	Quality q-value	#PSMs	Theo. MH+ [Da]	Confidence MS Amanda 2.0	Confidence Sequest HT
HS-exp1	LNLINCSPPDIR	1xCarbamidomethyl [C6]	1.00E+00	3.10E-01	1	1429.71039	Low	
HS-exp2	LNLINCSPPDIR	1xCarbamidomethyl [C6]	4.60E-01	2.50E-02	1	1585.81150	Medium	
HS+NaCl-exp1	LNLINCSPPDIR	1xPhospho [S7]	5.90E-01	3.60E-02	2	1608.75637	Medium	Low
HS+NaCl-exp2	LNLINCSPPDIR	1xPhospho [S7]	8.50E-01	3.00E-02	2	1608.75637	Medium	Medium

B

Dsa_6IUy_A	FMGIEDEAQSLEQANRGEINLTAKAFEDRLAKLNFTPTDIDRFLEEHPAHTRLVPGVENL
Dte_AJP36315	FMGIEDEAQSLEQANRGEINLTAKAFEDRLAKLNFTPTDIDRFLEEHPAHTRLVPGVENL
Dvi_ACD84643	FMGLKDEV--LRHEAMDGTMLPDTMAERLAINCSPEDIQQFLLEHPPKERLVPGVEEL
Psp_CAG9463212	FKGVGQAVADITNSAMDGVMDLEESLQARMQIINPSPDDIARYNKQVDPREVRVPGAKEL
CspUWO_KAG1665202	FMGVGAECTKITNQAMDGTLDLSQSLEARLNIINCTPKDVKRFIKANPPQSRLTPIGIVQF
Ede_KAG2496545	FMGCKEEVEELTNKAMDGVMLNTSLEERLNLINCSPSDLRAFLKAHPPASRLAPGVREL
Cin_KAG2424635	FMGVKDEVEALTTKAMDGTMSLTRSLEERLNLINCSPPDIRRFKAHPPQSRLAPGIKEL
Cre_XP_042928911	FMGVKKEVEELTNKAMDGTMSLTRSLEERLNLINCSPPDIRRFKAYPPQSRLAPGIKEL
Csc_KAG2432760	FMGVKDEVEELTNKAMDGTMSLTRSLEERLNLINCSPPDIRRFKAYPPQSRLAPGIKEL
Pst_GLC60827	FMGVKEPVEALTHKAMEGSLSLERALEERLNLINCSPPDIKNFISAHPPSSRLAPGIKEL
Vaf_GIL57042	FMGVKEQVEELTNKAMDGSLSLERALEERLNLINCSPPDIKNFIAHPPSSRLAPGIKEL
Ssp_KAF6254612	FMGCGDLVAAVTNRAMDGSMLNLEQALEERLRLINCTPADIKRFIRAHPPESRLVPGIESL
Cso_PRW58014	WMGVGEQVAALTNAMDGMSLLEDALEKRLEIINCTPADIQAFLEHRAHPPESRLVPGAREL
Cva_XP_005847643	FMGVGEQVAALTNAMDGMSLLEDALEQRLAVINCTPADIQGFLKAHPPESRLTPGAKEL
Mco_PSC74868	FMGVGESVAALTNAMDGMSLLEDALEKRLNVLINCTPADIQAFLEHRAHPPESRLTPGAREL
Consensus	: * : * * : * : : * : * : * : : hN×[S/T]P×D1

C



D

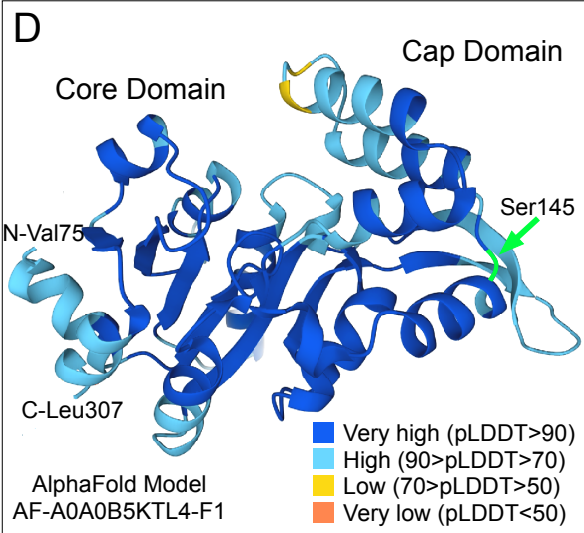


Figure S3. Phosphorylation of the GPP domain of GPD2 in OX-12 cells exposed to salinity stress. **(A)** Phosphorylated GPD2 peptide identified by LC-MS/MS exclusively in NaCl-treated samples. The phosphorylated residue corresponds to Ser145 in the GPP domain. PEP, posterior error probability; PSM, peptide spectral match. **(B)** The phosphorylated serine (S/T, indicated in red) is within a conserved motif in bidomain GPDs from diverse species. Consensus: h, hydrophobic residues; l, aliphatic residues. Species: Cin, *Chlamydomonas incerta*; Cre, *C. reinhardtii*; Csc, *Chlamydomonas schloesseri*; Cso, *Chlorella sorokiniana*; Csp, *Chlamydomonas* sp. UWO 241; Cva, *Chlorella variabilis*; Dsa, *Dunaliella salina*; Dte, *Dunaliella tertiolecta*; Dvi, *Dunaliella viridis*; Ede, *Edaphochlamys debaryana*; Mco, *Micractinium conductrix*; Psp, *Pedinophyceae* sp. YPF-701; Pst, *Pleodorina starrii*; Ssp, *Scenedesmus* sp. NREL 46B-D3; Vaf, *Volvox africanus*. **(C)** Predicted three-dimensional structure of the GPD2 GPP domain generated by Swiss Model [41], based on the crystal structure of *D. salina* GPDH 6iuy.1.a. The model is shown in cartoon representation and colored according to confidence class. The location of Ser145 is shown in light green. The green sphere corresponds to a Mg²⁺ atom. **(D)** AlphaFold [43] predicted model of the GPP domain of *C. reinhardtii* GPD2. The model is shown in cartoon representation and colored according to confidence class.