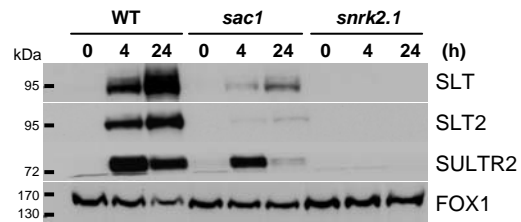


# Supplemental Figure S1

<b>A</b>	<b>CrSULTR1</b>	RRNRPSSAGSAVAQADGSMAPSTSLLLHPDGAGAALPSSSSMMPTDCSVINRHRGAAGASSGGAOMEFHWENTNGNADRSSVHGILQKQWERSKSSYLKMSITYSALDWLAFPLPCVRMLR
	<b>CrSULTR2</b>	MKRNISN-----VDTGVVPAPLNS---TPSTRLIQNS-YGDSKYET--ERMEFPPEDPRYHPRDSVKG---ANEWKEDHHHRVATYNNWVDWLAFFIPCVRMHR
	<b>AtSULTR4;1</b>	MSYASLS-----VKDLDS---LVSRSGTGSSSSLKPPQGRPVKVIPLQHPDTSNEARPPSTIP-----FDIFSGWTAKIKRMRLVDWIDTIFPCSRMLR
	<b>AtSULTR1;2</b>	-----MSSRAHPVDGSPAITDGHVPMKPSPTRHVKVGFEPKQNMFKDMYTFKTE---FFHDPLDRDFKQPKSKQMLQLQSVPVDFWGR
	<b>ShSHST1</b>	MSQRVSD-----QVMADVIAETRSNSSSHRRGGGSGDDTTSLPYMHKVGTFPKQFLFQEIKHSFNED---FFPKPFPGFKFDQSGFRKLELGLQVIFPILLEWGR
	<b>CrSULTR1</b>	TYKIREYLFADIVAGISVGFVWVWPGQMSYANLAGLESVYGLYQAFLEVITVALVGSRRQLANGPVAVVSTLHCSSLQELVPGAETISLNPIQ-LTPDQVIECKYNNLAIQSLVLAHLYT
	<b>CrSULTR2</b>	TYR-RSYLLNDIVAGISVGFVWVWPGQMSYANLAGLESVYGLYQAFLEVITVALVGSRRQLANGPVAVVSTLHLLKLDLPEAAGISNPNIPGSELDPAVOBYRLAIIQAFVLAQVLT
	<b>AtSULTR4;1</b>	TYRWSEYFKLDLIVAGISVGFVWVWPGQMSYANLAGLESVYGLYSSVFVVFVAIFGSSRRDIAIGPVAVVSLLLVSNALGGIA-----DNBELHIELALHILVGLIEC
	<b>AtSULTR1;2</b>	NYTFKKRFG-DLISGHTVAGLCPIDPQISYAKLANLDPFVGLYSSVFVVFVAIFGSSRRDIAIGPVAVVSLLLGLLRAEIDP-----NPSDVEYLRLAFTAFFAFSITEA
	<b>ShSHST1</b>	HYDLKKFRG-DFIAGITIASLCIPQDLAYAKLANLDPVGLYSSVFVVFVAIFGSSRRDIAIGPVAVVSLLLGLLRAEIDP-----NKGSHVYLRLAFTAFFAFSVQTQ
	<b>CrSULTR1</b>	SVGFVRLGFIINFLSHVIGSFTSGAAITIGLSCVCHVWLKYLHGISIP---RERLHDQVSYTIEFIRNLKWOQFIMGSTFVLLVITWRKVKRSRHRFVWLRPLGELISVCHALLAVY
	<b>CrSULTR2</b>	GVSHFRLGFVWFLSHAVIGSFTSGAAITIGLSCV---KYLIGSIP---RDRLDQQAQKYVDNMMKWOQFIMGSTFVLLVITWRKVKRSRHRFVWLRPLGELISVCHALLAVY
	<b>AtSULTR4;1</b>	IMGHRLGLLRFVSHSVISGFTSASAIYGLSCL---KYFLGYSTA---RBSKTVFIVESIITAGADKQWPPFVWMSLITVILVQVMKVKRKKLQELQRAAAPLIGVILGTIAIK
	<b>AtSULTR1;2</b>	ALGFVRLGFIIDFLSHAAVVGFTSGAAITIGLSCV---KGLFGKCK---FTKKTDLISVLSVFKAAHGWNVQITLLGASFTLLETLSKILCKRSKRLWPAIAPLISVYVDFVRY
	<b>ShSHST1</b>	LLSVKRLGFIIDFLSHAAVVGFTSGAAITIGLSCV---KGLLGISNNTTKKTDIISVMSVWTVHVEGWNVQITLLGASFTLLETLSKILCKRSKRLWPAIAPLISVYVDFVRY
	<b>CrSULTR1</b>	TSVDRKGRKIKGAIKKGLETP-TVGVWAPMPDFVLDLPIAHVVMVLDLESTSIARALANKKRYELVANGELVGLGANFASAAHGYSTTGSFSSRAVNNESSGAKTGLAGFVTAWVVG
	<b>CrSULTR2</b>	VGNVQNKGRKIKGAIKAGLEP-TVSMVMPPEISQLEFPAIVVMVLDLESTSIARALANKKRYELVANGELVGLGANFASAAHGYSTTGSFSSRAVNNESSGAKTGLAGFVTAWVVG
	<b>AtSULTR4;1</b>	VFHPPS--TSVWGTPOGLE---TFSFPRSFDAKTIETTSALITGPILESVGLAKALAAKNYELSDSDFGLGVANILSFPSPVATGSPSSRAVNNESSGAKTGLSGLTTRITIG
	<b>AtSULTR1;2</b>	ITRADKQGVVILKIDCGINPSSFLIYFGNDLAKGRIGVAGMVAITPAVALCRITFRAMKVDIIDCKMDFVLEEMANVSSMSCYATGSPSSRAVNNESSGAKTGLAGFVTAWVVG
	<b>ShSHST1</b>	ITRADKQGVVILKIDCGINPSSFLIYFGNGLYGVAGMVAITPAVALCRITFRAMKVDIIDCKMDFVLEEMANVSSMSCYATGSPSSRAVNNESSGAKTGLAGFVTAWVVG
	<b>CrSULTR1</b>	FVLLFLTPVREKLEPYHLSAIVCSVSVIGLHVEYEAIVLWKVVKLDFVMMASLCHLHSHSIEGLSIAHGLAMLIVYESAHPHTAMGRIPGSGVVRNKOYVPSQITPCHTVMRISDF
	<b>CrSULTR2</b>	FVLEFLTPVRAHLPYHLSAIVCSVSVIGLHVEYEAIVLWKVVKLDFVMMASLCHLHSHSIEGLSIAHGLAMLIVYESAHPHTAMGRIPGTRIRNKOYPAQIAPGLVFRIDAP
	<b>AtSULTR4;1</b>	CSLLFLTPFKYVQCALAAIVTSAVSGLVYDEAFLWRVDRKDFSLTITSTTILDFGIEIGLVGCVGSDIARVHESANPHAVLGLRPGTVVRNKOYPAQIAPGLVFRIDAP
	<b>AtSULTR1;2</b>	LTLLFLTPFKYVQCALAAIIVNAVIPDLIQALLKVKDKLDFACIGAFGVIFQVEYETRDQLAISNENKDFHLTARSQFAVLEKWEFFVHDAVYVQVQVQSSNLEDK---
	<b>ShSHST1</b>	LTLLVITLTPFKYVQCALAAIIVRAVNVNIBAMVLWKIDKDFVACVQGFVGFHKSVEIGLLIAVAISPAKILLOVTRPRTAVLGLRPGTVVRNKOYPAQIAPGLVFRIDAP
	<b>CrSULTR1</b>	LYFANVQMIKRLRVYEDRHRDWS---GEGHTKLEFALIDMSPVTHIDATGVHLESWIEHFVAVGTOVLVLSVSVKVIRELDTAIVPDMGKDMFVTVHDAVYVQVQSSNLEDK---
	<b>CrSULTR2</b>	LYFANVQMIKRELREGASAHRWS---QEHGVPLEYVLDSPVTHIDATGHLEIVTETLAGHGTQVVLNANSEQETIADMRRGGLFDMGQVDFVHVTNVAVYVQVQSSNLEDK---
	<b>AtSULTR4;1</b>	LYFANVQMIKRLREYEAADKYTNRELVDRINVELEMSPVTHIDATGSSVALEKELKYQVEYETRDQLAISNENKDFHLTARSQFAVLEKWEFFVHDAVYVQVQVQSSNLEDK---
	<b>AtSULTR1;2</b>	LYFNSNVIYRETRQRLWHEEKEVK-AASLPRIQVLEEMSPVTHIDATGSHALDPLKSLQKRDQLLANEGPLVTKLHLSHFADMGQDNYLYTVADVAQVQVQSSNLEDK---
	<b>ShSHST1</b>	LYFNSNVIYKERILRWLIDEGAQRT-ESELPETQHLIEMSPVTHIDATGSHALDPLKSLQKREVQLLANEGPLVLEKHLASKLLETIGEDKLEHVTADVAVYVQVQSSNLEDK---
	<b>CrSULTR1</b>	-----PLSMQQPSSTSDE-----
	<b>CrSULTR2</b>	NTSSYPHFGSRRTPGALPAPSSQLDSSPSTVPESTSGTPAAGTYVSGIAGVAVAGHTAAGNGGSHSPSAQPGVQLTTTGSQRQO
	<b>AtSULTR4;1</b>	-----HLSPTRRYGSSNNSSSNALLKEPLLVEK-----
	<b>AtSULTR1;2</b>	-----
	<b>ShSHST1</b>	-----
<b>B</b>	<b>CrSLT1</b>	-----
	<b>CrSLT2</b>	-----
	<b>CrSLT3</b>	-----
	<b>PpST</b>	MTRSMPLYRGEQEMWFSHTESIKTTPSATTNAPLSDGIRIPRPHVGRVGGPDMHRNPDLRNVAVLLSCSVQGEVLDLGVVPGAKPALYCNFGFMISSLNLCVMNCLFEFDFVESAENS
	<b>CrSLT1</b>	-----MAALSNQGIIVAVITFALAVVMAADWVGDITFTVLLAFLTAFDQCIIVTAKAAAGYQNTGLLTVVFLYVWABGITOQGGLELIMNYVLRGRSRVHWALVRSMFPVMVLS
	<b>CrSLT2</b>	-----MGRSNQGSYIAFLAVVMAADWVGDITFTVLLAFLTAFDQCIIVTAKAAAGYQNTGLLTVVFLYVWABGITOQGGLELIMNYVLRGRSRVHWALVRSMFPVMVLS
	<b>CrSLT3</b>	-----MAALSNQGIIVAVITFALAVVMAADWVGDITFTVLLSWLTAFTDCKIITVAKAAAGYQNTGLLTVVFLYVWABGITOQGGLELIMNYVLRGRSRVHWALVRSMFPVMVLS
	<b>PpST</b>	GRELRRSDKVMQLGNSYLVATLTIAGVMAADWVGDITFTVFLMVGFLTAICR---VITVKESTETGFSQNGVLTWVFLVWABGITOQGGMKALANLIGKATSPFWALTIRMFIPVAVITS
	<b>CrSLT1</b>	AFILNNTPCVTFMIPILLSWGRGCVPIKLLIPLSYAAVVGCTCTSIGTSTNLVIVGLQDARYRQSKVDQAKFOIFDIAPYGVYALWGFVILLACGFLLPGNSSRYAKDLLLAVRVL
	<b>CrSLT2</b>	AFILNNTPCVTFMIPILLSWGRGCVPIKLLIPLSYAAVVGCTCTSIGTSTNLVIVGLQDARYRQSKVDQAKFOIFDIAPYGVYALWGFVILLACGFLLPGNSSRYAKDLLLAVRVL
	<b>CrSLT3</b>	AFILNNTPCVTFMIPILLSWGRGCVPIKLLIPLSYAAVVGCTCTSIGTSTNLVIVGLQDARYRQSKVDQAKFOIFDIAPYGVYALWGFVILLACGFLLPGNSSRYAKDLLLAVRVL
	<b>PpST</b>	AFILNNTPCVTFMIPILLSWGRGCVPIKLLIPLSYAAVVGCTCTSIGTSTNLVIVGLQDARYRQSKVDQAKFOIFDIAPYGVYALWGFVILLACGFLLPGNSSRYAKDLLLAVRVL
	<b>CrSLT1</b>	PSSSVVKKLKDSDGLQCGFDVAIVRNGOLIKISDESIVLDGGDILVYSGELDVVEFVGEENGLAVNCOBE---IAAERPFSGSEEVFSAANGAAPYHR---LVQAQKSKTSDLIGRT
	<b>CrSLT2</b>	PSSSVVKKLKDSDGLQCGFDVAIVRNGOLIKISDESIVLDGGDILVYSGELDVVEFVGEENGLAVNCOBE---IAAERPFSGSEEVFSAANGAAPYHR---LVQAQKSKTSDLIGRT
	<b>CrSLT3</b>	TT-----IQOS-----SNEPTTRACROVDEDTVLEENDILYAGLEDOVVEFVGEENGLAVNCOBE---IAAERPFSGSEEVFSAANGAAPYHR---LVQAQKSKTSDLIGRT
	<b>PpST</b>	PEVVAANTVREAGLQMERLPLVVRGSGVTHVAGVQYLLEPDDILYFCGLEDOVVEFVGEENGLAVNCOBE---IAAERPFSGSEEVFSAANGAAPYHR---LVQAQKSKTSDLIGRT
	<b>CrSLT1</b>	VREVSWQGRFGLIPVAIQRNGREDGRINDVLAAGDVLDDTTPFYDEDRERHINFNFGKLEAVDKGAKEFVIGVKVKSSEVWNTVSAAGLRGIPGLFVLSVDRADGSSVSDSYL
	<b>CrSLT2</b>	VREVSWQGRFGLIPVAIQRNGREDGRINDVLAAGDVLDDTTPFYDEDRERHINFNFGKLEAVDKGAKEFVIGVKVKSSEVWNTVSAAGLRGIPGLFVLSVDRADGSSVSDSYL
	<b>CrSLT3</b>	VREVSWQGRFGLIPVAIQRNGREDGRINDVLAAGDVLDDTTPFYDEDRERHINFNFGKLEAVDKGAKEFVIGVKVKSSEVWNTVSAAGLRGIPGLFVLSVDRADGSSVSDSYL
	<b>PpST</b>	LDQIDFRKREDVAVLGLRGETHQEGEISEVWNAVDVLLGDNEEVLQKPVKAVEK-IVEKLEALBKEVLTENKVTNFRKGVKGTIVYDAGLRGIPGLFVLSVDRADGSSVSDSYL
	<b>CrSLT1</b>	YKIQPDITWIAADVAAGVLSKFPGLLEVQOQVDKTGTSILYRHLVQAAVSHKGLPVGKTVRDRFRTLYNAAVVAHREGNARHPLKQVDIVLQCGDVLILSCHTNWADEHRHDKSFV
	<b>CrSLT2</b>	YKIQPDITWIAADVAAGVLSKFPGLLEVQOQVDKTGTSILYRHLVQAAVSHKGLPVGKTVRDRFRTLYNAAVVAHREGNARHPLKQVDIVLQCGDVLILSCHTNWADEHRHDKSFV
	<b>CrSLT3</b>	YKIQPDITWIAADVAAGVLSKFPGLLEVQOQVDKTGTSILYRHLVQAAVSHKGLPVGKTVRDRFRTLYNAAVVAHREGNARHPLKQVDIVLQCGDVLILSCHTNWADEHRHDKSFV
	<b>PpST</b>	TVVETCDTLNFGSVQGFHPLFKISGLERSQAVQVSKLRADILYRHLVQAAVSHKGLPVGKTVRDRFRTLYNAAVVAHREGNARHPLKQVDIVLQCGDVLILSCHTNWADEHRHDKSFV
	<b>CrSLT1</b>	LVQVPDSSPKRSRMLIGVLLATGMVLQIIVGG-LKKKEYIHLWPAVLLTAALMLLTCMNADQARKAIMDVLVITAAAFVGSAALEGTGVAAFANALISIGKLAGGTGAALIAIYI
	<b>CrSLT2</b>	LVQVPDSSPKRSRMLIGVLLATGMVLQIIVGG-LKRSEYIHLWPAVLLTAALMLLTCMNADQARKAIMDVLVITAAAFVGSAALEGTGVAAFANALISIGKLAGGTGAALIAIYI
	<b>CrSLT3</b>	LVQVPDSSPKRSRMLIGVLLATGMVLQIIVGG-LKRKEYIHLWPAVLLTAALMLLTCMNADQARKAIMDVLVITAAAFVGSAALEGTGVAAFANALISIGKLAGGTGAALIAIYI
	<b>PpST</b>	LISGVESSPVKRSRMLIGVLLATGMVLQIIVSSITGGTILNLFIAIILSGLMLLTCGLSDAQARNSILRVVITFALAFASFCWCKSKLARALADIRHLSIESIGMGRASVAIYI
	<b>CrSLT1</b>	ATALLSELLTNNAAAGAIMYPAIADGALKIPKDTSVAILMGASAGFINPFSYQTNLMVYAAGNYSVREFAIIVGAPFQVLMIVAGFILLVNRQHWQVIVSWICTAGIVLLPALYFLL
	<b>CrSLT2</b>	ATALLSELLTNNAAAGAIMYPAIADGALKIPKDTSVAILMGASAGFINPFSYQTNLMVYAAGNYSVREFAIIVGAPFQVLMIVAGFILLVNRQHWQVIVSWICTAGIVLLPALYFLL
	<b>CrSLT3</b>	ATAVVSELLTNNAAAGAIMYPAIADGALKIPKDTSVAILMGASAGFINPFSYQTNLMVYAAGNYSVREFAIIVGAPFQVLMIVAGFILLVNRQHWQVIVSWICTAGIVLLPALYFLL
	<b>PpST</b>	ATALLSELLTNNAAAGAIMYPAIADGALKIPKDTSVAILMGASAGFINPFSYQTNLMVYAAGNYSVREFAIIVGAPFQVLMIVAGFILLVNRQHWQVIVSWICTAGIVLLPALYFLL
	<b>CrSLT1</b>	PTRIKIKIDGFFERIAAVLNPKAALERRSRRQ-----VSHRTTDSGSSSSE---LPAKLVIA-
	<b>CrSLT2</b>	PTVQVIRIDAFDRVAQNLNPKAALERRSRRQ-----ASATSGSGSSDSPR-ALCVKIVITA
	<b>CrSLT3</b>	HTVQNRMAFFDRIDAEINPRAALQRRRSRRQCSFGGKAMSVDGTSRDTGSSSTPVALTFTIEMPKQVVR
	<b>PpST</b>	BEVPAIRSRFSFGSKE-----KTEKIEQ-----

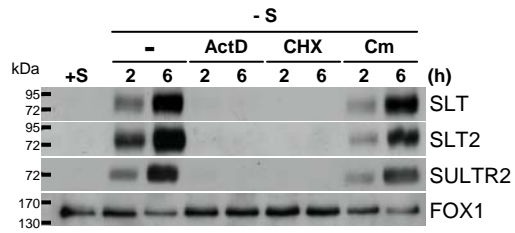
**Supplemental Figure S1.** Amino acid sequence alignments of  $\text{SO}_4^{2-}$  transporter proteins. **A.** Predicted CrSULTR1 and CrSULTR2 amino acid sequences were aligned with each other and with representative *Arabidopsis thaliana* and *Stylosanthes hamata* high-affinity  $\text{SO}_4^{2-}$  transporters using BioEdit version 7.0.9.0 software. **B.** Predicted CrSLT1, CrSLT2, CrSLT3 proteins were aligned with each other and a putative  $\text{SO}_4^{2-}$  permease from *Physcomitrella patens* (PpST). Black and grey shadings indicate identical and similar amino acid residues, respectively. The red bar (**A**) highlights the C-terminal STAS domain and the blue bar (**A**) represents the region of CrSULTR2 used as an antigen for antibody production. The magenta bar (**B**) indicates the peptide sequence in SLT2 that is recognized by the SLT2 antibody; the orange bar indicates the TrkA-C domain, and the green bar (**B**) shows the region recognized by the general SLT antibody.

## Supplemental Figure S2



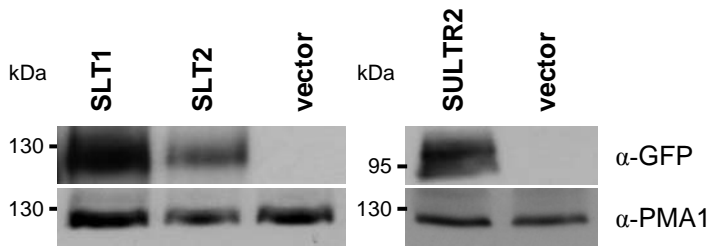
**Supplemental Figure S2.** SULTR2, SLT1, and SLT2 polypeptide abundances in wild-type 21gr (WT), *sac1*, and *snrk2.1* strains. The time courses show accumulation of SLT1, SLT2, and SULTR2 polypeptides following transfer of cells from S-replete to S-deficient medium. Samples were taken prior to, and 4 and 24 h after the cells were transferred. The ferroxidase, FOX1, protein served as a loading control.

## Supplemental Figure S3

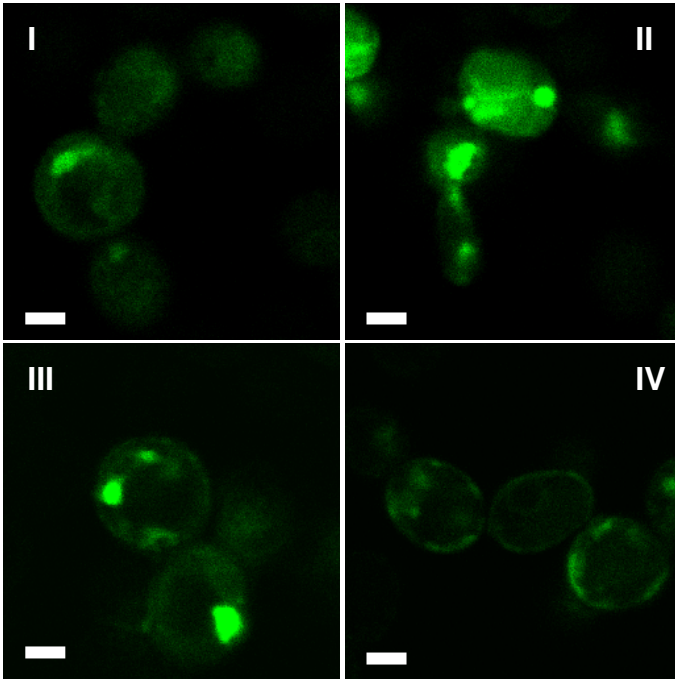


**Supplemental Figure S3.** Cycloheximide inhibition of accumulation of the  $\text{SO}_4^{2-}$  transporter protein during S deprivation. *Chlamydomonas* cells were grown in TAP and then transferred to TAP-S medium. At the time of S removal, a transcriptional inhibitor actinomycin D (ActD), a cytosolic translational inhibitor cycloheximide (CHX), or an organellar translational inhibitor chloramphenicol (Cm), was added to the cultures. Samples were taken prior to starvation as well as 2 and 6 h after the removal of S. The ferroxidase protein, FOX1, served as the loading control (accumulation of FOX1 is S-independent).

A



B



**Supplemental Figure S4.** The expression of  $\text{SO}_4^{2-}$  transporter-GFP fusion proteins in *S. cerevisiae* cells. **A.** CP60-1C cells transformed with an empty pDR196-GW-GFP, plasmids carrying SLT1, SLT2, or SULTR2 were grown to mid-logarithmic phase and the microsomal fraction was isolated, separated by SDS-PAGE and the immunoblot performed to detect the chimeric transporters tagged with GFP and a plasma membrane ATPase, PMA1. **B.** Confocal images of CP60-1C expressing the fusion proteins SULTR2-GFP (I), SLT1-GFP (II), SLT2-GFP (III) or AtSULTR1;2-GFP (IV). The bar on the image represents 3  $\mu$ m.

# Supplemental Figure S5

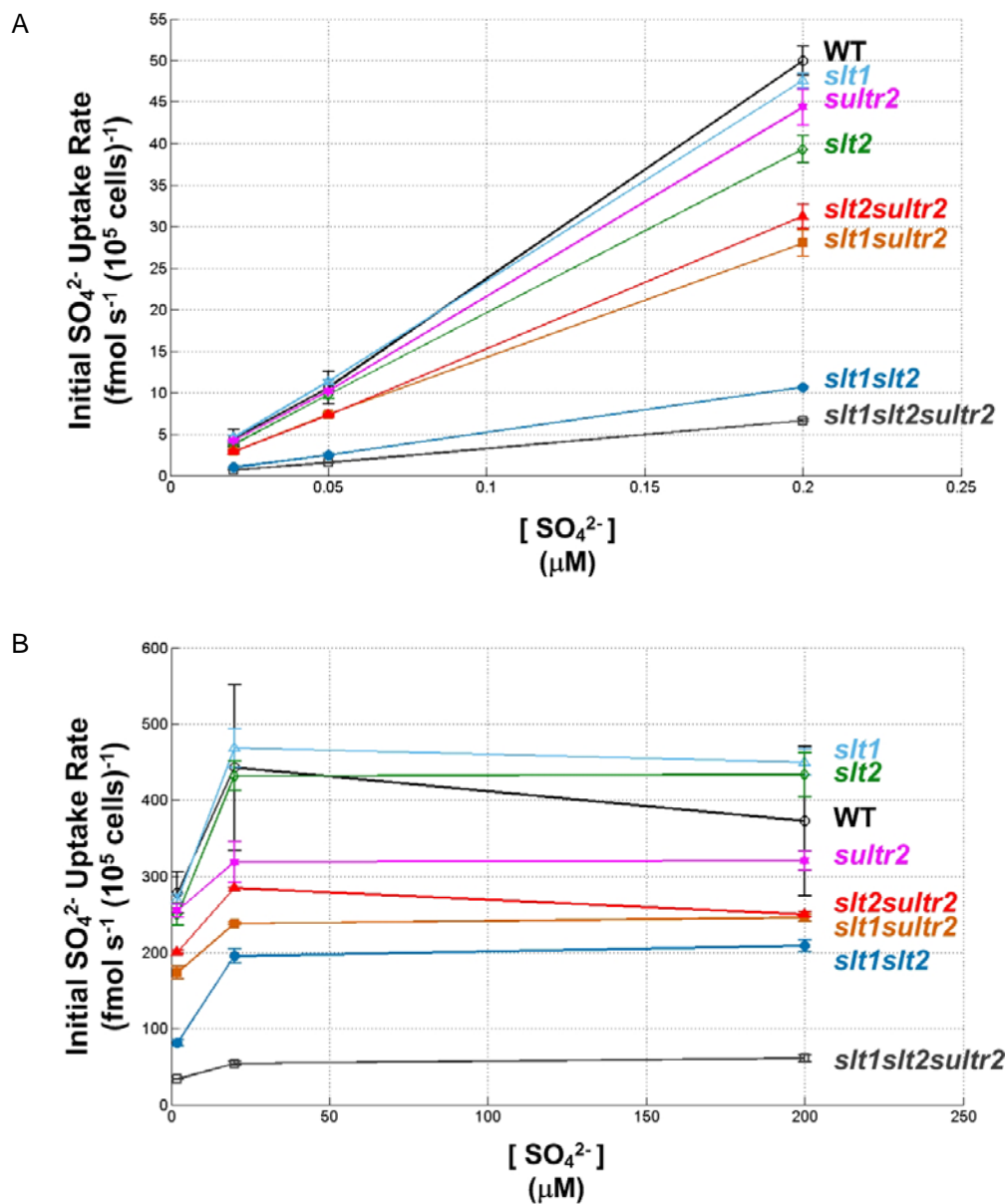
**A**

<b>WT-SLT1</b>	MAALSWQGI VAVTFTALAFV VMAADWV GPDITFTVLLAFLTA FDFGQIVTVAKAAAGYGN TGLLTVVFLYVVAEGITQTGGLELIMNYVLGRSRSVHVALVRSMPFV MVLSAFLNNTPCVT
<b>m-SLT1</b>	MAALSWQGI VAVTFTALAFV VMAADWV GPDITFTVLLAFLTA FDFGQIVTVAKAAAGYGN TGLLTVVFLYVVAEGITQTGGLELIMNYVLGRSRSVHVALVRSMPFV MVLSAFLNNTPCVT
<b>WT-SLT1</b>	FMPILILISWGRRCGVP IKKLLIPLSYAAVLGGTCTSI GTSTNLVIVGLQDARYAKSKQVDQAKFQIFDIAPYGV PYPALWGFVIFLLAQGFLLPGNSSRYAKDLLLAVRVL PSSSVVKKLL
<b>m-SLT1</b>	FMPILILISWGRRCGVP IKKLLIPLSYAAVLGGTCTSI GTSTNLVIVGLQDARYAKSKQVDQAKFQIFDIAPYGV PYPALWGFVIFLLAQGFLLPGNSSRYAKDLLLAVRVL PSSSVVKKLL
<b>WT-SLT1</b>	KDSGLLQNGFVDTAIYRNGQLIKISDPSIVLDGGDILYVSGELDVVEFV GEEYGLALVNQEQELAAERPF GSGEEAVFSANGAAPYHKLVQAKLSKTSDLIGRTVREVSWQGRFGLIPV
<b>m-SLT1</b>	KDSGLLQNGFVDTAIYRNGQLIKISDPSIVLDGGDILYVSGELDVVEFV GEEYGLALVNQEQELAAERPF GSGEEAVFSANGAAPYHKLVQAKLSKTSDLIGRTVREVSWQGRFGLIPV
<b>WT-SLT1</b>	AIQRNGREDGR LSDVLLAAGDVLLLDITTFYDEDREDIKTNFDGK LHAVKDGAKEFVIGVKVKSAE VVGKTVSAAGLRGIPGLFVLSVDHADGTSV DSSDYLYKIQPDDTIWAADV
<b>m-SLT1</b>	AIQRNGREDGR LSDVLLAAGDVLLLDITTFYDEDREDIKTNFDGK LHAVKDGAKEFVIGVKVKSAE VVGKTVSAAGLRGIPGLFVLSVDHADGTSV DSSDYLYKIQPDDTIWAADV
<b>WT-SLT1</b>	AAVGF LSKFPGLELVQEQVDK TGTSTILYRHLVQA AVSHKGLPVGKTVRDRVFRFTLYNAAVVA VHENARIP LKVDIVLQGGDVLLI SCHTNWADEHRHDKS FVLVQVPDSSPKR SR
<b>m-SLT1</b>	<u>LTWPPWASCPSSLAWSWCSRSRWRTRPGPSSSTATWCRPP</u>
<b>WT-SLT1</b>	MIIGVLLATGMVLTQIIGGLKNKEYIHLWPCAVLTAALMLLTGCMNADQTRKAIMD VYLTIAAAFVSAALEGTVAAK FANAIISIGKGAGGTGAALIAIYIATALLSELLTNNAAGA
<b>m-SLT1</b>	MIIGVLLATGMVLTQIIGGLKNKEYIHLWPCAVLTAALMLLTGCMNADQTRKAIMD VYLTIAAAFVSAALEGTVAAK FANAIISIGKGAGGTGAALIAIYIATALLSELLTNNAAGA
<b>WT-SLT1</b>	IMYPAAIAGDALKITPKDTSVAIMLGASAGFVNPFSYQTNLMVYAAGNYSVREFAIVGAPQVWLMIVAGF ILVYRNQWHQVIVSWICTAGIVLLPALYFLLPTRIQIKIDGFFERIA
<b>m-SLT1</b>	IMYPAAIAGDALKITPKDTSVAIMLGASAGFVNPFSYQTNLMVYAAGNYSVREFAIVGAPQVWLMIVAGF ILVYRNQWHQVIVSWICTAGIVLLPALYFLLPTRIQIKIDGFFERIA
<b>WT-SLT1</b>	AVLNPKAALERRRSLRRQVSHTRTDDSGSSGSPLPAPKIVA
<b>m-SLT1</b>	AVLNPKAALERRRSLRRQVSHTRTDDSGSSGSPLPAPKIVA

**B**

<b>WT-SULTR2</b>	MKRNTSNVD TGGVPAPLNSTPSTR LIQNGYGD SKYETERMEFPFPEDPRYHPRDSVKGAW EKVKEDHHRVATYNWVDWLAFFIPCVRWLRTYRRSYLLNDIVAGISVGMVMPVQGLSYA
<b>m-SULTR2</b>	MKRNTSNVD TGGVPAPLNSTPSTR LIQNGYGD SKYETERMEFPFPEDPRYHPRDSVKGAW EKVKEDHHRVATYNWVDWLAFFIPCVRWLRTYRRSYLLNDIVAGISVGMVMPVQGLSYA
<b>WT-SULTR2</b>	NLAGLPSVYGLYGAFLPCIVSVL GSSRQLAVGPVAVT SLLLGTKLKDILPEAAGISNPNIPGSELPDAVQEKYNRLAIQLAFLVACLTYG VGIIFRLGFVTFNFLSHAVIGGFTSGAAITI
<b>m-SULTR2</b>	NLAGLPSVYGLYGAFLPCIVSVL GSSRQLAVGPVAVT SLLLGTKLKDILPEAAGISNPNIPGSELPDAVQEKYNRLAIQLAFLVACLTYG VGIIFRLGFVTFNFLSHAVIGGFTSGAAITI
<b>WT-SULTR2</b>	GLSQVKYILGISIPRQDRLQDQAKTYVDNMHNMKWQEFIMGTTFLLV LFKVEVGRSKRFKWRP IGPFLTVCIIGLCAVYVGNVQNKGIKIIGAIKAGLPAPT VSWFPMPEISQLFPT
<b>m-SULTR2</b>	GLSQVKYILGISIPRQDRLQDQAKTYVDNMHNMKWQEFIMGTTFLLV LFKVEVGRSKRFKWRP IGPFLTVCIIGLCAVYVGNVQNKGIKIIGAIKAGLPAPT VSWFPMPEISQLFPT
<b>WT-SULTR2</b>	AIVVMLVDLLESTSIARALARKNKYELHANQEIVGLGLANFAGAI FNCTYTTGFSFRSAVNNEGAKTGLACFI TAWVVG FVLIIFLTPVFAHLPYCTLGAI IVSSIVGLLEYEQAIYLWK
<b>m-SULTR2</b>	AIVVMLVDLLESTSIARALARKNKYELHANQEIVGLGLANFAGAI FNCTYTTGFSFRSAVNNEGAKTGLACFI TAWVVG FVLIIFLTPVFAHLPYCTLGAI IVSSIVGLLEYEQAIYLWK
<b>WT-SULTR2</b>	VNKL DVLVWMA SFLGVLFI SVEIGL GAIAGLAILIVIYESAF FNTALVGRIPGTTIWRNKIQYPNAQLAPGLLVFRIDAPIYFANI QWIKERLEGFASAH RVWSQEHGVPLE YVILDFSP
<b>m-SULTR2</b>	VNKL DVLVWMA SFLGVLFI SVEIGL GAIAGLAILIVIYESAF FNTALVGRIPGTTIWRNKIQYPNAQLAPGLLVFRIDAPIYFANI QWIKERLEGFASAH RVWSQEHGVPLE YVILDFSP
<b>WT-SULTR2</b>	VTHIDATGLH TLETIVETLAGHGTVQVLANP SQEIIALMR RGLFDMIGR DYVITVNEAVTFC SRQMAERGYAVKEDNTSSYPHFGSRRTPGALPAPSQ LDDSSPPTSVTESTGTPAA
<b>m-SULTR2</b>	VTHIDATGLH TLETIVETLAGHGTVQVLANP SQEIIALMR RGLFDMIGR DYVITVNEAVTFC SRQMAERGYAVKEDNTSSYPHFGSRRTPGALPAPSQ LDDSSPPTSVTESTGTPAA
<b>WT-SULTR2</b>	GTYSSIGGAVPAVAGHTAAGNGGSHSPSAQPGVQLIT TGSQRQQ
<b>m-SULTR2</b>	<u>GTYSSIGGAVPAVAGHTAAGNGGSHSPSAQPGVQLIT TGSQRQQ</u>

**Supplemental Figure S5.** Amino acid sequence alignments of wild-type and the *SLT1* (A) and *SULTR2* (B) mutant gene products. The amino acid sequence of the proteins encoded by the *slt1* and *sultr2* mutant genes was deduced. In both cases, the insertions caused a frame-shift mutation generating a premature stop codon and the production of truncated proteins. The regions of amino acid sequences in the *slt1* and *sultr2* strains deviated from the corresponding wild-type polypeptides are underlined. Predicted proteins from wild-type (WT) and mutant (m) strains were aligned using BioEdit version 7.0.9.0 software.



**Supplemental Figure S6.** Characteristics of SO<sub>4</sub><sup>2-</sup> transport in wild-type cells and single, double and triple mutants (progeny of a cross between a wild-type strain and an *slt1slt2sultr2* triple mutant) deprived of S for 24 h. Transport assays were performed as a function of external SO<sub>4</sub><sup>2-</sup> concentrations: **A.** 0.02-0.2 μM; **B.** 2-200 μM. Initial rates of uptake are expressed as fmol of SO<sub>4</sub><sup>2-</sup> s<sup>-1</sup> (10<sup>5</sup> cells)<sup>-1</sup>. Values are averages of 2-3 technical replicates, and error bars represent one standard deviation.

## Supplemental Table S1

Plasmid	Doubling time (h)
Vector (pDR196-GW-GFP)	5.91 ± 0.33
AtSULTR1;2-GFP	3.52 ± 0.11
SULTR2-GFP	5.49 ± 0.12
SLT1-GFP	5.24 ± 0.25
SLT2-GFP	5.09 ± 0.27

**Supplemental Table S1.** Growth rates of the CP60-1C strain harboring genes encoding Arabidopsis SULTR1;2 or various Chlamydomonas  $\text{SO}_4^{2-}$  transporters. Doubling time (in h) is measured as a change in  $A_{600}$ .



Primer	Sequence
SULTR2-5'UTR-F1	5'- TAACGGGCCTCCGCAAGACA -3'
SULTR2-F8	5'- TTCCCTCCGTGTACGGCCTGTA -3'
SULTR2-F9	5'- GCCGCCTTAGCCGAAGCTTAGT -3'
SULTR2-D	5'- GCTTCCTTCCTGGGAGTGCT -3'
SULTR2-E	5'- AGGATGCGGCTCTACCCAAT -3'
SULTR2-K	5'- CTGTGAGGCGAGGCGATAGATG -3'
SULTR2-G	5'- AGCAGGCAGTGGGATTGAAA -3'
SULTR2-R5	5'- GCCCAGACCGATCTCCACACTGA -3'
SULTR2-I	5'- CGAAGTGCGGATAGGAGGAG -3'
SULTR2-J	5'- TGCAGGGAAGTGCCTGGTA -3'
SLT1-5'UTR-F1	5'-TGCTCACTTACATAGTCAGGCGCG-3'
SLT1-SEQ-F3	5'-AAGTCCAAGCAGGTCGACCA-3'
SLT1-SEQ-F5	5'-GCAAGACCAGTGACCTGATCG-3'
SLT1-SEQ-F7	5'-GCAGGAGCAGGTGGACAAGA-3'
SLT1-SEQ-F10	5'-ACCTCCGTCGCCATCATGCT-3'
SLT1-3'UTR-R1	5'-GCTTCTGTTCACAGGATTACATTCAA-3'
SLT1-SEQ-R3	5'-TGGGTAGCAGGAAGTACAGCGC-3'
SLT1-SEQ-R4	5'-TGTCCTGGACCTTGAGCGGGAT-3'
SLT1-SEQ-R5	5'-TGTC AAGCAGCAGCACATCGCC-3'
SLT1-SEQ-R6	5'-TCCTGCAGACCCACGATGACCA-3'
P-SLT2-F1	5'- CGCTGCTGGAAAAGCATATGCAATTC -3'
SLT2-SEQ-F2	5'- CAACCTGGTCATCGTCGGTC -3'
SLT2-F8	5'- GCGGGTACTGGACAGTTGGACACA -3'
SLT2-F6	5'- TTGCCCTATCACACAGGATGACAC -3'
SLT2-F2	5'- CAACCGGGTTGCAACTTCCTGAT -3'
SLT2-R10	5'- GAAACCCGTTCCCTGCTGCAGT -3'
SLT2-R8	5'- TGGTGTCCAGGATGAGCACGTC -3'
SLT2-R13	5'- CTGTGTGATAGGGGCAACGACAAT -3'
SLT2-R12	5'- TTGAATTGCGGCAGATGGTGTAAC -3'
SLT2-3'UTR-R2	5'- TCGGTCCGCGCAACTTCTTTGT -3'

**Supplemental Table S2.** List of *SULTR2*-, *SLT1*- and *SLT2*-specific primers used for PCR screening of the insertion library.

<b>Strain</b>	<b>K<sub>1/2</sub> (μM)</b>
Wild-type	4.12 ± 2.25
<i>slt1</i>	4.75 ± 1.42
<i>slt2</i>	6.85 ± 0.49
<i>sultr2</i>	4.42 ± 1.04
<i>slt1slt2</i>	3.11 ± 1.15
<i>slt1sultr2</i>	4.69 ± 0.94
<i>slt2sultr2</i>	4.80 ± 0.32
<i>slt1slt2sultr2</i>	5.92 ± 1.59

**Supplemental Table S3.** Characteristics of SO<sub>4</sub><sup>2-</sup> transport in wild-type cells, single, double and triple SO<sub>4</sub><sup>2-</sup> transporter mutants after 24 h of S deprivation. K<sub>1/2</sub> (in μM) is calculated from the initial rates using a Michaelis-Menten equation. Values are averages of 2-4 biological replicates with each experiment performed in duplicate. Error bars represent one standard deviation.

## SUPPLEMENTAL MATERIALS AND METHODS

**Yeast strain, media and growth conditions:** The strain of *Saccharomyces cerevisiae* used in this study was CP60-1C (*MATa his3 leu2 ura3 trp1 sul1-1 sul2-1*), which harbors mutations in both high-affinity  $\text{SO}_4^{2-}$  transporters (*SUL1* and *SUL2*) (Cherest et al., 1997). CP60-1C transformants were grown at 30°C in synthetic defined (SD) –Met –Ura liquid medium supplemented with 100  $\mu\text{M}$   $\text{MgSO}_4$ . Cell growth was evaluated by measuring optical density of the cultures at 600 nm ( $A_{600}$ ) in a DU640 spectrophotometer (Beckman Coulter, CA).

**Construction of GFP-tagged  $\text{SO}_4^{2-}$  transporters, yeast transformation and microscopy:** Full-length cDNAs encoding  $\text{SO}_4^{2-}$  transporters were cloned into pDR196-GW (no tag) or pDR196-GW-GFP (GFP was fused in-frame to the transporters at the carboxyl terminus). The pDR196-GW and pDR196-GW-GFP plasmids were kind gifts from Dr. Dominique Loque. The transporters were expressed in yeast under the control of the constitutive *PMA1* promoter. The constructs carrying  $\text{SO}_4^{2-}$  transporters were transformed into the yeast mutant CP60-1C, using the lithium-acetate procedure (Rose et al., 1990). Transformants were selected on SD medium lacking uracil and then grown in liquid medium to mid-logarithmic phase. For microscopy, a drop of cell suspension was mounted onto slides and GFP fluorescence was detected as previously described (using a Nikon TMD200 inverted fluorescence microscope equipped with a Nikon 60X 1.2-numerical aperture water immersion objective and a Biorad MRC 1024 confocal head) (Shibagaki and Grossman, 2004).

**Yeast protein isolation and immunoblot analysis:** Cells in mid-logarithmic phase ( $A_{600} \sim 0.2$ ) were harvested by centrifugation (3000 X *g* for 5 min), washed once with ice-cold STE10 buffer (10% w/v sucrose, 5 mM Tris pH 7.4, 10 mM EDTA) and resuspended in the same buffer containing protease inhibitor cocktail (Calbiochem, Gibbstown, NJ). Cells were disrupted by agitation with glass beads (425 – 600  $\mu\text{M}$ ) and the cell debris was removed by a brief centrifugation (3000 X *g* for 5 min). The supernatant was centrifuged at 100,000 X *g* for 50 min to obtain a microsomal pellet, which was then resuspended in the STE10 buffer. An equal vol of loading buffer (6.25 mM Tris-HCl, pH

6.8, 5 % SDS, 6 M urea, 500 mM dithiothreitol, 10 % glycerol and 0.002 % bromophenol blue) was added to the samples prior to an incubation at 42°C for 15 min. Solubilized polypeptides were resolved by SDS-PAGE and the immunoblot performed as described in the **MATERIALS AND METHODS**. Dilutions of primary antibodies used were: 1:1000 anti-PMA1 (ABCam, Cambridge, MA) and 1:1000 anti-GFP (Roche, Nutley, NJ). A 1:10,000 dilution of horseradish peroxidase-conjugated anti-rabbit IgG (Promega, Madison, WI) or 1:10,000 dilution of horseradish peroxidase-conjugated anti-mouse IgG (Sigma, St. Louis, MO) was used as a secondary antibody. The peroxidase activity was detected by an enhanced chemiluminescence assay (Amersham Biosciences, Sweden).

### **SUPPLEMENTAL REFERENCES**

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