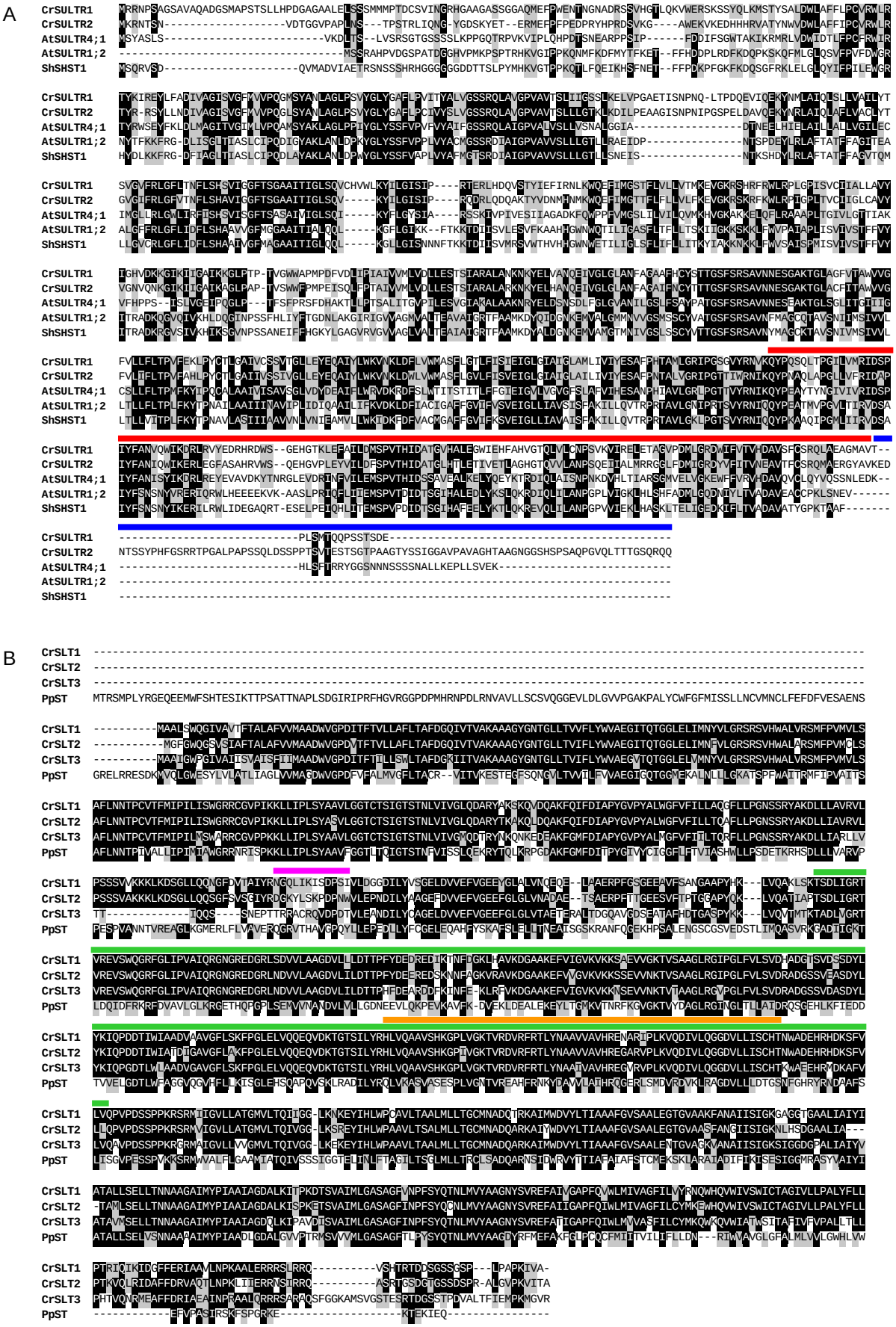
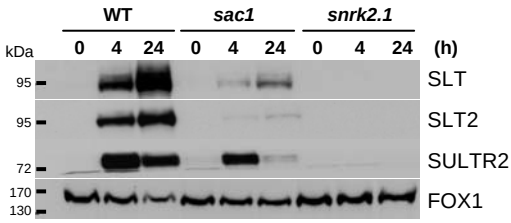
Supplemental Figure S1



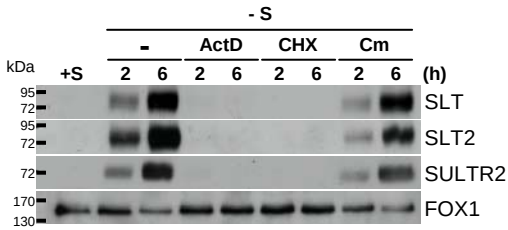
Supplemental Figure S1. Amino acid sequence alignments of SO_4^{2-} transporter proteins. **A.** Predicted CrSULTR1 and CrSULTR2 amino acid sequences were aligned with each other and with representative *Arabidopsis thaliana* and *Stylosanthes hamata* high-affinity SO_4^{2-} transporters using BioEdit version 7.0.9.0 software. **B.** Predicted CrSLT1, CrSLT2, CrSLT3 proteins were aligned with each other and a putative 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



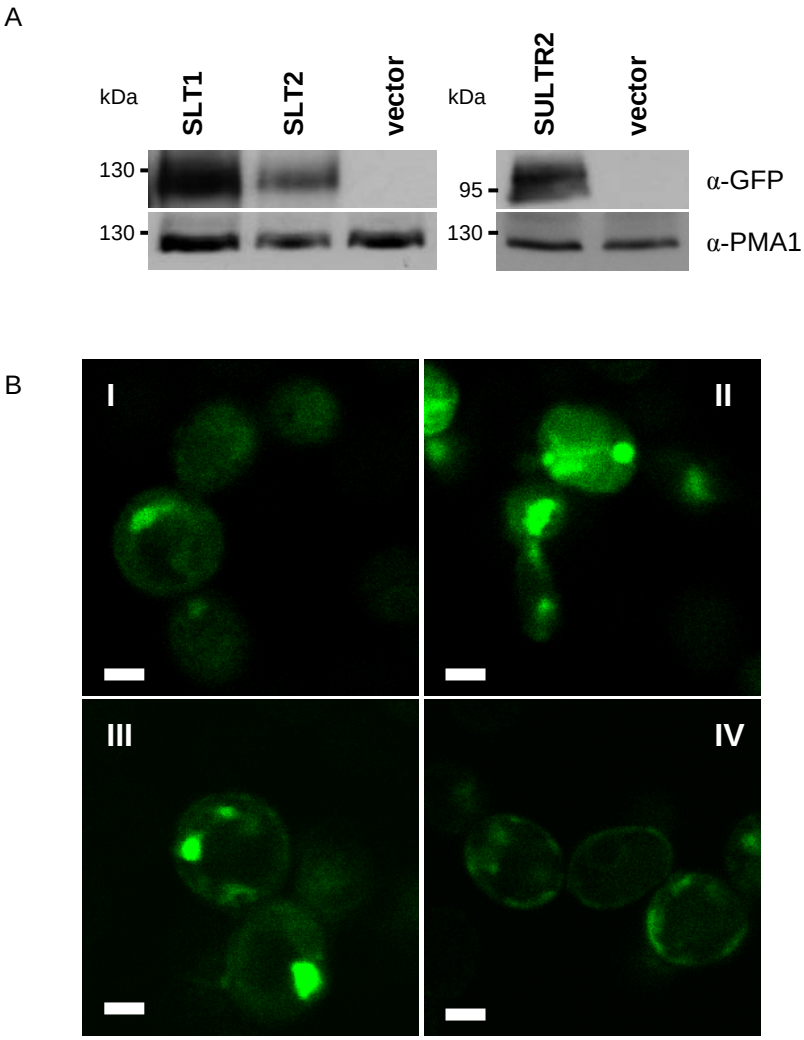
Supplemental Figure S2. SULTR2, SLT1, and SLT2 polypeptide abundances in wild-type 21gr (WT), *sac1*, and *snrk2.1* strains. The time courses show accumulation of SLT1, SLT2, and SULTR2 polypeptides following transfer of cells from S-replete to S-deficient medium. Samples were taken prior to, and 4 and 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 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).

Supplemental Figure S4



Supplemental Figure S4. The expression of 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 µm.

Supplemental Figure S5

A

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WT-SLT1  MAALSWQGIVAVTFTALAFVVMAADWVGPDITFTVLLAFLTAFDGQIVTVAKAAAGYGNTGLLTVVFLYWVAEGITQTGGLELIMNYVLGRSRSVHWALVRSMFPVMVLSAFLNNTPCVT
m-SLT1   MAALSWQGIVAVTFTALAFVVMAADWVGPDITFTVLLAFLTAFDGQIVTVAKAAAGYGNTGLLTVVFLYWVAEGITQTGGLELIMNYVLGRSRSVHWALVRSMFPVMVLSAFLNNTPCVT

WT-SLT1  FMIPILISWGRRCGVPIKKLLIPLSYAAVLGGTCTSIGTSTNLVIVGLQDARYAKSKQVDQAKFQIFDIAPYGVPYALWGFVFILLAQGFLLPGNSSRYAKDLLLAVRVLPSSSVVKKKL
m-SLT1   FMIPILISWGRRCGVPIKKLLIPLSYAAVLGGTCTSIGTSTNLVIVGLQDARYAKSKQVDQAKFQIFDIAPYGVPYALWGFVFILLAQGFLLPGNSSRYAKDLLLAVRVLPSSSVVKKKL

WT-SLT1  KDSGLLQQNGFDVTAIYRNGQLIKISDPSIVLDGGDILYVSGELDVVEFVGEEYGLALVNQEQELAAERPFGSGEEAVFSANGAAPYHKLVQAKLSKTSDLIGRTVREVSWQGRFGLIPV
m-SLT1   KDSGLLQQNGFDVTAIYRNGQLIKISDPSIVLDGGDILYVSGELDVVEFVGEEYGLALVNQEQELAAERPFGSGEEAVFSANGAAPYHKLVQAKLSKTSDLIGRTVREVSWQGRFGLIPV

WT-SLT1  AIQRGNGREDGRLSDVVLAAGDVLLLDTTPFYDEDREDIKTNFDGKLHAVKDGAAKEFVIGVKVKKSAEVVGKTVSAAGLRGIPGLFVLSVDHADGTSVDSSDYLYKIQPDDTIWIAADV
m-SLT1   AIQRGNGREDGRLSDVVLAAGDVLLLDTTPFYDEDREDIKTNFDGKLHAVKDGAAKEFVIGVKVKKSAEVVGKTVSAAGLRGIPGLFVLSVDHADGTSVDSSDGKTTCTRSSPMTPSGSP

WT-SLT1  AAVGFLSKFPGLELVQQEQVDKTGTSILYRHLVQAAVSHKGPLVGKTVRDVRFRTLYNAAVVAVHRENARIPLKVQDIVLQGGDVLLISCHTNWADEHRHDKSFVLVQPVPDSSPPKRSR
m-SLT1   LTWPPWASCPSSLAWSWCSRSRWTRPGPPSSTATWCRRP

WT-SLT1  MIIGVLLATGMVLTQIIGGLKNKEYIHLWPCAVLTAALMLLTGCMNADQTRKAIMWDVYLTIAAAFGVSAALEGTGVAAKFANAIISIGKGAGGTGAALIAIYIATALLSELLTNNAAGA
m-SLT1

WT-SLT1  IMYPIAAIAGDALKITPKDTSVAIMLGASAGFVNPFSYQTNLMVYAAGNYSVREFAIVGAPFQVWLMIVAGFILVYRNQWHQVWIVSWICTAGIVLLPALYFLLPTRIQIKIDGFFERIA
m-SLT1

WT-SLT1  AVLNPKAALERRRSLRRQVSHTRTDDSGSSGSPLPAPKIVA
m-SLT1
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B

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WT-SULTR2  MKRNTSNVDTGGVPAPLNSTPSTRLIQNGYGDSKYETERMEFPFPEDPRYHPRDSVKGAWEKVKEDHHHRVATYNWVDWLAFFIPCVRWLRTYRRSYLLNDIVAGISVGFMVVPQGLSYA
m-SULTR2   MKRNTSNVDTGGVPAPLNSTPSTRLIQNGYGDSKYETERMEFPFPEDPRYHPRDSVKGAWEKVKEDHHHRVATYNWVDWLAFFIPCVRWLRTYRRSYLLNDIVAGISVGFMVVPQGLSYA

WT-SULTR2  NLAGLPSVYGLYGAFLPCIVYSLVGSSRQLAVGPVAVTSLLLGTKLKDILPEAAGISNPNIPGSPELDAVQEKYNRLAIQLAFLVACLYTGVGIFRLGFVTNFLSHAVIGGFTSGAAITI
m-SULTR2   NLAGLPSVYGLYGAFLPCIVYSLVGSSRQLAVGPVAVTSLLLGTKLKDILPEAAGISNPNIPGSPELDAVQEKYNRLAIQLAFLVACLYTGVGIFRLGFVTNFLSHAVIGGFTSGAAITI

WT-SULTR2  GLSQVKYILGISIPRQDRLQDQAKTYVDNMHNMKWQEFIMGTTFLFLLVLFKEVGKRSKRFKWLRPIGPLTVCIIGLCAVYVGNVQNKGIKIIGAIKAGLPAPTVSWWFPMPEISQLFPT
m-SULTR2   GLSQVKYILGISIPRQDRLQDQAKTYVDNMHNMKWQEFIMGTTFLFLLVLFKEVGKRSKRFKWLRPIGPLTVCIIGLCAVYVGNVQNKGIKIIGAIKAGLPAPTVSWWFPMPEISQLFPT

WT-SULTR2  AIVVMLVDLLESTSIARALARKNKYELHANQEIVGLGLANFAGAIFNCYTTTGSFSRSAVNNESGAKTGLACFITAWVVGFVLIFLTPVFAHLPYCTLGAIIVSSIVGLLEYEQAIYLWK
m-SULTR2   AIVVMLVDLLESTSIARALARKNKYELHANQEIVGLGLANFAGAIFNCYTTTGSFSRSAVNNESGAKTGLACFITAWVVGFVLIFLTPVFAHLPYCTLGAIIVSSIVGLLEYEQAIYLWK

WT-SULTR2  VNKLDWLVWMASFLGVLFISVEIGLGIAIGLAILIVIYESAFPNTALVGRIPGTTIWRNIKQYPNAQLAPGLLVFRIDAPIYFANIQWIKERLEGFASAHRVWSQEHGVPLEYVILDFSP
m-SULTR2   VNKLDWLVWMASFLGVLFISVEIGLGIAIGLAILIVIYESAFPNTALVGRIPGTTIWRNIKQYPNAQLAPGLLVFRIDAPIYFANIQWIKERLEGFASAHRVWSQEHGVPLEYVILDFSP

WT-SULTR2  VTHIDATGLHTLETIVETLAGHGTQVVLANPSQEIIALMRRGGLFDMIGRDYVFITVNEAVTFCSRQMAERGYAVKEDNTSSYPHFGSRRTPGALPAPSSQLDSSPPTSVTESTSGTPAA
m-SULTR2   VTHIDATGLHTLETIVETLAGHGTQVVLANPSQEIIALMRRGGLFDMIGPAQIGPATSPPPLVGDVPT

WT-SULTR2  GTYSSIGGAVPAVAGHTAAGNGGSHSPSAQPGVQLTTTGSQRQQ
m-SULTR2
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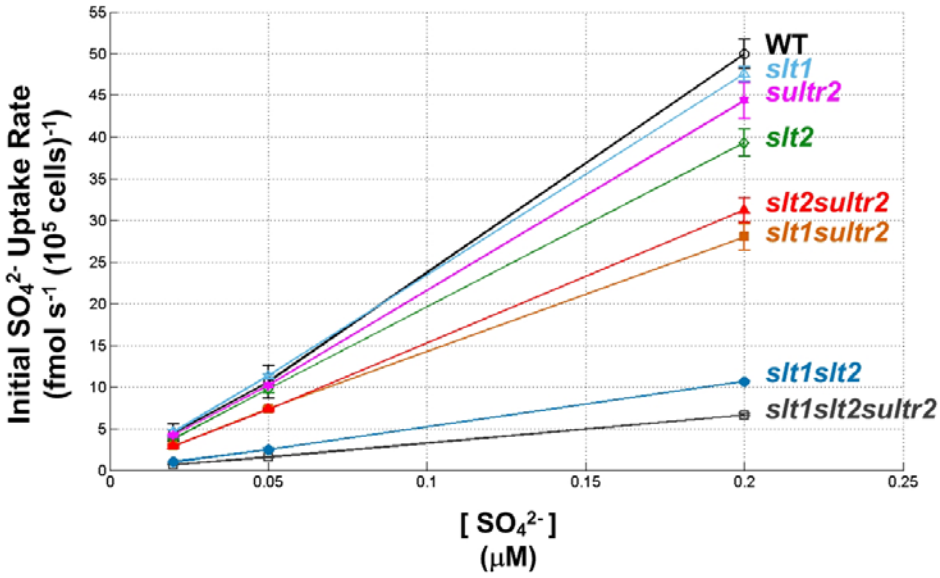
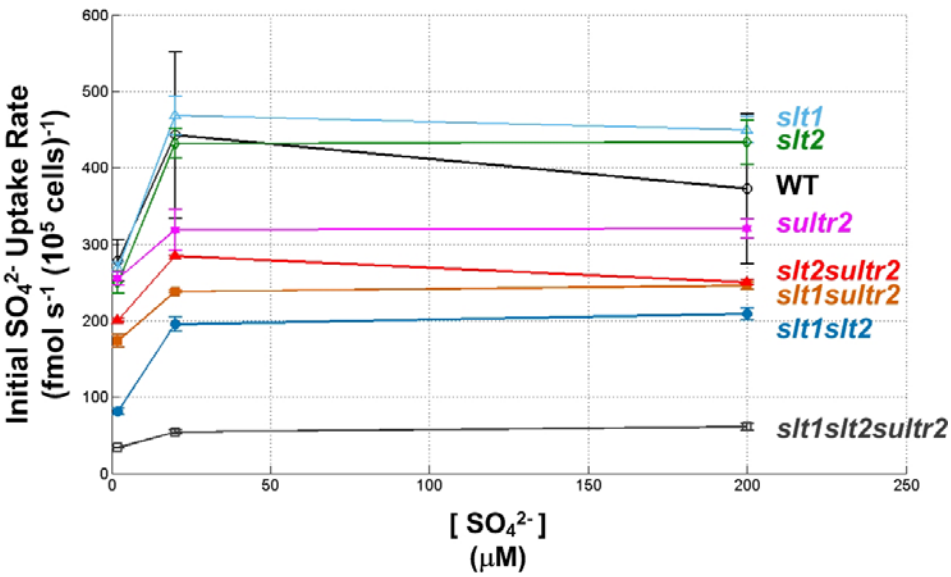
Supplemental Figure S5. Amino acid sequence alignments of wild-type and the *SLT1* (**A**) and *SULTR2* (**B**) mutant gene products. The amino acid sequence of the proteins encoded by the *slt1* and *sultr2* mutant genes was deduced. In both cases, the insertions caused a frame-shift mutation generating 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

A



B



Supplemental Figure S6. Characteristics of SO_4^{2-} 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_4^{2-} concentrations: **A**. 0.02-0.2 μM; **B**. 2-200 μM. Initial rates of uptake are expressed as fmol of SO_4^{2-} s^{-1} (10^5 cells)$^{-1}$. 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 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'- TGCAGGGAACTGCACTGGTA -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'-TGTCAAGCAGCAGCACATCGCC-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'- TTGCCCCTATCACACAGGATGACAC -3'
SLT2-F2	5'- CAACCGGGTTGCAACTTCCTGAT -3'
SLT2-R10	5'- GAAACCCGTTCCCCTGCTGCAGT -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.

Strain	$K_{1/2}$ (μM)
Wild-type	4.12 ± 2.25
slt1	4.75 ± 1.42
slt2	6.85 ± 0.49
sultr2	4.42 ± 1.04
slt1slt2	3.11 ± 1.15
slt1sultr2	4.69 ± 0.94
slt2sultr2	4.80 ± 0.32
slt1slt2sultr2	5.92 ± 1.59

Supplemental Table S3. Characteristics of SO_4^{2-} transport in wild-type cells, single, double and triple SO_4^{2-} transporter mutants after 24 h of S deprivation. $K_{1/2}$ (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 (*MAT***a** *his3 leu2 ura3 trp1 sul1-1 sul2-1*), which harbors mutations in both high-affinity SO_4^{2-} transporters (*SUL1* and *SUL2*) (Cherest et al., 1997). CP60-1C transformants were grown at 30ºC in synthetic defined (SD) –Met –Ura liquid medium supplemented with 100 µM $MgSO_4$. 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 SO_4^{2-} transporters, yeast transformation and microscopy: Full-length cDNAs encoding 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 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} ~ 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 µM) 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 42ºC for 15 min. Solubilized polypeptides were resolved by SDS-PAGE and the immublot 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

Cherest, H., Davidian, J.-C., Thomas, D., Benes, V., Ansorge, W., and Surdin-ker Jan, Y. (1997). Molecular characterization of two high affinity sulfate transporters in *Saccharomyces cerevisiae*. Genetics 145: 627-637.

Rose, M.D., Winston, F., and Heiter, P. (1990). Methods in Yeast Genetics. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Shibagaki, N., and Grossman, A.R. (2004). Probing the function of STAS domains of the Arabidopsis sulfate transporters. J Biol Chem 279: 30791-30799.