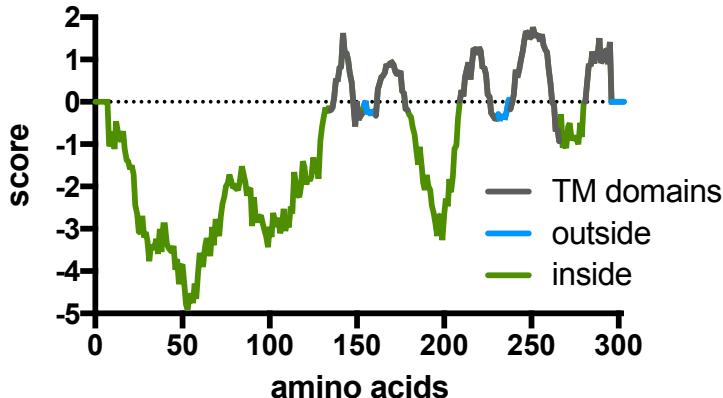


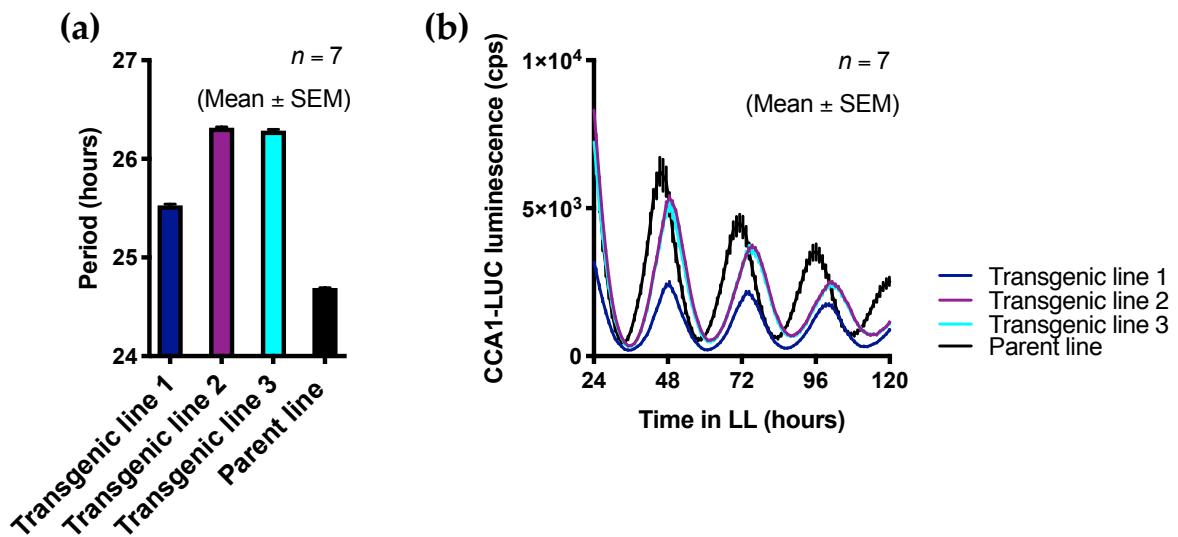
(a)

| | | | |
|-----------|---|-------------------------|------|
| OtMgtE | -TMLVGAGGNAGNQSAIHVIRGLATGEMENTNE----- | CMRKTLCVEQFQVGLM---LGVV | 51 |
| MgtE | VPVLLGTGGNTGNQSATLIIRALATRDLDL----- | dWRRVFLKEMGVGLL---LGLT | 50 |
| SLC41A3*1 | VPPLVGLKGNLLEMTLASRLSTAANTGQIDDPQE----- | QHRVISSNLALIQVQATVVGLL | 55 |
| SLC41A2*1 | VPALLGLKGNLLEMTLASRLSTAANIGKMDSPIE----- | KWNLIIGNLALKQVQATVVGFL | 55 |
| SLC41A1*1 | VPALLGLKGNLLEMTLASRLSTAANIGHMDTPKE----- | LWRMITGNMALIQVQATVVGFL | 55 |
| SLC41A3*2 | TPVICGVGGNLVAIQTSRISTYLHMSAPGVLPQLQMKKFWPNPCSTFCTSEIN----- | 53 | |
| SLC41A2*2 | TPVINGIGGNLVAIQASRISTYLHLHSIPGELPD-EPKGCYYPFRFFFPGPVN----- | 52 | |
| SLC41A1*2 | TPVINGVGNNLVAVQASRISTFLHMNGMPGENSEQAPRRCPSPTFFFSPDVN----- | 53 | |
| | : * ** : : | : | |
| OtMgtE | LSSAGFVRVLLTSPQGTDDLIG-----PFAIATALFAIVTT----- | STCVGTALP | 96 |
| MgtE | LSFLLVGKVYWD---GHPLLLP-----VVGVSVLVLIVFFAN-----LV--GAMLP | 90 | |
| SLC41A3*1 | AAVAALLL-GVV-SREEVDVAKVELLCA--SSVLTAFLAFAFALG-----VLMVCIV | 102 | |
| SLC41A2*1 | AAVAAILL-GWI-PEGKYLDHSILLCS--SSVATAFIASLILQG-----IIMVGVI | 102 | |
| SLC41A1*1 | ASIAAVVF-GWI-PDGHSIPHAFLLCA--SSVATAFIASLVLG-----MIMIGVI | 102 | |
| SLC41A3*2 | SMSARVLLLVLV-PGHLIFFYIIYV-EGQSVINSQTFFVLYLLLAGLIQVTILLYLAEVMV | 111 | |
| SLC41A2*2 | NKSAQVLLLVI-PGHLIFLYTIHLMSGHTSLTIIIFIVVYLFGAVLQVFTLLWIADWMV | 111 | |
| SLC41A1*2 | SRSARVLFLLVV-PGHLVFLYTISCMQGGHTTLTLIFIIFYMTAALLQVLILLYIADWMV | 112 | |
| | . | : | |
| OtMgtE | FLLMRLNQD PANAGTSVQVVM-DVSGVIIT ----- | 125 | |
| MgtE | FLLRLLGVD PALVSNPLVATLSDVTGLLIYL ----- | 121 | |
| SLC41A3*1 | IGARKLGVNP DNIATPIAASLGDLITLISILA ----- | 133 | |
| SLC41A2*1 | VGSKKTG INPDNVATPIAASFGDLITLAILA ----- | 133 | |
| SLC41A1*1 | IGSRKIG INPDNVATPIAASLGDLITLALL ----- | 132 | |
| SLC41A3*2 | RLTWHQALD PDNHCIPIYLTGLGDLLGSS ----- | 139 | |
| SLC41A2*2 | HHFWRKGKD PDSFSIPIYLTALGDLLGTALLA ----- | 142 | |
| SLC41A1*2 | HWMWGRGLD PDNFSIPIYLTALGDLLGTGLLAXSLCA ----- | 148 | |
| | : * | . | : *: |

(b)



Supplementary Figure 1: (a) The *OtMgtE* MgtE domain shares homology with the bacterial MgtE domain (here from *Thermus thermophiles*) and all three human SLC41 proteins as shown with this multiple sequence alignment. The two conserved motifs outlined by Wabakken *et al.* (2003) are shown in green. * denotes a fully conserved residue, : denotes conservation between groups of strongly similar properties and . denotes conservation between groups of weakly similar properties. **(b)** The hydrophobicity plot predicting transmembrane domains indicates that this protein is a transmembrane protein.



Supplementary Figure 2: Luminescent traces and period analysis of *OtMgtE* overexpression lines. (a) Period analysis of 3 transgenic lines and parent line. (b) Luminescent traces: *OtMgtE* overexpression induces a long period phenotype compared to the parent line in free running conditions (constant light). Figure shows CCA1 bioluminescent traces over 4 days.

Supplementary Table 1: Protein accession numbers for proteins used in figure 4

| <i>Species</i> (NCBI taxonomic ID) | NCBI/Uniprot accession number, (homolog number where >1) |
|---|--|
| <i>Homo sapiens</i> (9606) | Q8IVJ1 (1) (SLC41A2) Q96JW4 (SLC41A3) Q96GZ6 |
| <i>Drosophila melanogaster</i> (7227) | M9PHE6 |
| <i>Crassostrea gigas</i> (29159) | K1QPNO |
| <i>Amphimedon queenslandica</i> (400682) | XP_019864491.1 |
| <i>Monosiga brevicollis</i> MX1 (431895) | A9VBR3 |
| <i>Salpingoeca rosetta</i> (946362) | F2UAH4 |
| <i>Chlamydomonas reinhardtii</i> (3055) | A8J2E0 |
| <i>Ostreococcus tauri</i> (70448) | A0A096P9B9 |
| <i>Emiliania huxleyi</i> (2903) | (1) R1EK90 (2) R1DRZ5 (3) R1FDF9 (4) R1CTW0 (5) R1CBM8 (6) R1EBL0 (7) R1F8I7 |
| <i>Thalassiosira pseudonana</i> (35128) | B8CF51 |
| <i>Cyanidioschyzon merolae</i> (45157) | M1V9W9 |
| <i>Naegleria gruberi</i> (5762) | D2VNX4 |
| <i>Chlamydia trachomatis</i> (813) | A0A0E9C5A4 |
| <i>Pseudomonas aeruginosa</i> (208964) | Q9I544 |
| <i>Bacillus subtilis</i> (224308) | O34442 |
| <i>Providencia stuartii</i> (471874) | B2PYL8 |
| <i>Tolypothrix campylonemoides</i> (1136105) | WP_071838811.1 |
| <i>Treponema azotonutricium</i> (545695) | F5YDZ2 |
| <i>Pelolinea submarina</i> (913107) | WP_116224794.1 |
| <i>Thermus thermophilic</i> (274) | Q5SMG8 |
| <i>Acidobacteriaceae bacterium</i> KBS 96 (1267535) | WP_020720448.1 |
| <i>Oceanotoga teriensis</i> (515440) | WP_109604606.1 |
| <i>Natronomonas pharaonic</i> (348780) | A0A1U7EW96 |
| <i>Pyrococcus abyssi</i> (272844) | Q9UZ17 |
| <i>Hyperthermus butylicus</i> (54248) | WP_048061491 |
| <i>Methanosaarcina mazei</i> (2209) | A0A0F8MPI8 |

Ulva mutabilis proteome accessed from ORCAE, gene ID: UM021_0210.1