Reduced expression of a gene encoding a Golgi localized monosaccharide transporter (*OsGMST1*) confers hypersensitivity to salt in rice (*Oryza sativa*). *Hong Cao, Siyi Guo, Yunyuan Xu, Kun Jiang, Alan M. Jones, Kang Chong*

SUPPLEMENTARY DATA

Figure S1. Alignment of OsGMST1 with other members in rice pGlcT (plastid glucose transporter) subfamily.

Os09g23110 Os01g04190 OsGMST1		-MQPPPPPPHPAALLPLPLPLPFCLPAAPRVRGRGGTAVAALGAL-WEPPRLVAVESRPP- MAGTGASVKMIRCVVKCGGQQQHVVVAARGDRKSPAIAAPPPATVRMPGRVLCCGMRSRG VPSRDAAMDLDVE-	:	57 60 25
Os09g23110 Os01g04190 OsGMST1	:::::::::::::::::::::::::::::::::::::::	PPSSPASASAPPPLPESAAAGLDAGIGGGGGGGGGGGGGGADLGWLRVF ADLAGVEMAAGPQPQGGVSGLFRGPRSSPRYSRVRATATVDPEDIPLEKVQAKSSGSVLP TPAKMADGGAPSWRMSLP	:	104 120 43
Os09g23110 Os01g04190 OsGMST1	: :	HVLTASMANFLEGYHIGVMNGPIBDIARELGFQGNPFLQGLVVSIFIVGAFFGSLGSSAL YVGVACLGAILFGYHLGVVNGALBYLAKDLGISENAVLQGMVVSTTLAGATAGSFTGGAL HVCVATLTSFLFGYHSGVVNEPLBSISTDLGFAGNTLAEGLVVSICLGGAFVGCLFSGSI	::	164 180 103
Os09g23110 Os01g04190 OsGMST1	: :	VDNFCCKRTLQIDSIPLILGALLSAQADSLDEMLLGRFLVGIGIGINTVLVPLYVSBVAB ADKFGRTRTFILDAIPLAVGAFLSATAHDVRTMIIGRLLAGIGIGISSALVPLYISBISB ADGIGRRRAFQLSALEMIIGAAVSALTNSLEGMLLGRFLVGTCMGLGPPVASLYITBVSP	::	224 240 163
Os09g23110 Os01g04190 OsGMST1		TKYRGSLGTLCQIGTCLGTIAAFSLGIESESDPHWWRTMLYAACVEGVLIVAGMQFAVES TEIRGALGSVNOLFICIGILAALVAGLELAGNPAWWRTMFGISIVESILLALGMAVSPES PSVRGTYGSFVQIATCLGIVVSLLIGTEVKDIDRWWRVCFWVAAVEATLQALGMEFCAES	::	284 300 223
Os09g23110 Os01g04190 OsGMST1	: : :	BRWLAKVGRIDDARNVVEHVWGPSEVEKSWEEIQSVVANDDSQ-ASWSELLEEPHNRVAL BRWLFQQGKLSQAETAIKKLYGREKVAEVWYDLKAASQGSSEPDAGWLDLFSKRYWKVVS BQWLYKCGRTTEAEIQFEKLLGPLHVKSAWAELSRSERGDDGENVKYSELFYGRNFNVVF	: :	343 360 283
Os09g23110 Os01g04190 OsGMST1		IEGSLEFLOOFACINGVLYFSSLTERDVCITSGILASLYVCITNFACAIVASILMDKOCR VGAAMELFOOLACINAVVYYSTSVERSACIASDVAASALVCAANVFCTMIASSLMDKOCR ICTTLEALOOLSCINSVFYFSSTVERSVCVPP-NLANICMCIANLSCSIVAMLLMDKLCR	::	403 420 342
Os09g23110 Os01g04190 OsGMST1		KKLUTGSYLGMALAMFLIVYAISFPLDEGVSHGLSITGTLLYIFTBAIGAGPVTGIIIPE KSLLITSFSGMAASMLLLSLSFTWKALAPYSGPLAVAGTVLYVLSPALGAGPVPALLLPE KVLLSGSFL <u>GMA</u> FAMGLQAVGANRHHLGSASVYLSVGGMLLFVLTBSLGAGPVPGLLLPE		463 480 402
Os09g23110 Os01g04190 OsGMST1		LSGARTRSKVMGFSFTVHWICNELVGOYELELAKKLGVGAVMAGBGGVSLLSALFAYNFI IFASRIRAKAVALSLGMHWVSNEFIGUYELSVVNKFGISTVMLGBASVCALAVVYIAGNV IFPNKIRAKAMALCMSVHWVVNEFVSTLELRLLEQLGPQVLMTMESSACVVAAIFVRHV		523 540 462
Os09g23110 Os01g04190 OsGMST1		VETKCRSLEEIEMSLSPAAPGKRE : 547 VETKCRSLEEIERALSSAS : 559 VETKCKTLQEIEVSLLQTQ : 481		

Figure S2. Growth of hexose transporter deficient yeast cells transformed with sense and antisenes *OsGMST1* on plates supplemented with sugars. S1, S2 are two dependent transformant lines of EBY VW4000 expressing *OsGMST1* in sense orientation, AS1 is one transformant line expressing *OsGMST1* in antisense orientation, and Vector is one line transformed with the empty vector pEX-Tag. Transformant lines (S1, S2, AS1, Vector) were grown on maltose for 2 days, and on other monosaccharides (glucose, fructose, mannose, galactose, ribose, xylose) as the sole carbon source for 3 days.



Figure S3 Distribution of stress related cis-elements in the *OsGMST1* promoter region.



Figure S4. Molecular identification and phenotype observation of OsGMST1 knockdown transgenic rice plants. A) Diagram of OsGMST1 antisense construct for rice transformation. LB, left border; RB, right border; Gus, β -glucoronidase; Ubi, ubiquitin promoter; NOS, NOS terminator; *Hpt*, hygromicin phosphotransferase. B) Southern blot analysis of antisense independent transgenic rice lines. WT indicates the wild type, AS-L4, L12, L18 indicate the antisense transgenic lines. Rice genome DNA in the left 4 lanes are digested by EcoRI and the right 4 lanes are digested by HindIII. C) Real time PCR analysis of the OsGMST1 relative expression in knockdown lines. WT indicates the wild type rice, and AS4, 5, 12, 18 indicate the antisense transgenic rice lines. Data are means \pm SD (n = 3). ** indicates P < 0.01 by student's T-test. D) Quantitative PCR analysis of the Os01g04190 and Os09g23110 relative expression in OsGMST1 knockdown lines. WT indicates the wild type rice, and AS12, 18 indicate the knockdown transgenic rice lines. Data are means ± SD. E) Phenotype observation of the OsGMST1 antisense transgenic plants under normal growth conditions WT and AS-L12 indicate wild type and the OsGMST1 knockdown rice respectively. a, Seeds. b, Seeds germinated for 2 days. c, 4-week-old seedlings. d, Rice at grain filling stage. a, b, Bars = 0.5 cm. c, Bar = 5 cm. d, Bar = 20 cm.



Figure S5. Molecular identification and phenotype observation of OsGMST1 overexpressed transgenic rice plants. A) Southern blot analysis of independent transgenic rice lines. WT indicates the wild type, OE3, OE8, OE24 indicate the transgenic lines. Rice genome DNA in the left 4 lanes are digested by EcoRI and the right 4 lanes are digested by *HindIII. B*) Real time PCR analysis of the OsGMST1 relative expression in overexpressor lines. WT indicates the wild type rice, and OE3, OE6, OE8, OE24 indicate the transgenic rice lines. Data are means \pm SD (n = 3). C) Growth of WT and OsGMST1 overexpressed transgenic seedlings before (left) and after (right) NaCl treatment. 2-week-old seedlings were treated by 200 mM NaCl for 11 days, and then recovered for 7 days. D) Survival rate of WT and OsGMST1 transgenic seedlings after treated with 150 mM and 200 mM NaCl. 24 seedlings of 3 weeks old were used in each repeat. Error bars are SE of three replicates. E) Phenotype observation of OsGMST1 overexpressed rice plants under normal growth conditions. WT and OE3 indicate wild type and OsGMST1 overexpressed rice respectively. a, Seeds. b, Seeds germinated for 2 days. c, 4-week-old seedlings. d, Rice at grain filling stage. a, b, Bars = 0.5 cm. c, Bar = 5 cm. d, Bar = 20 cm.

