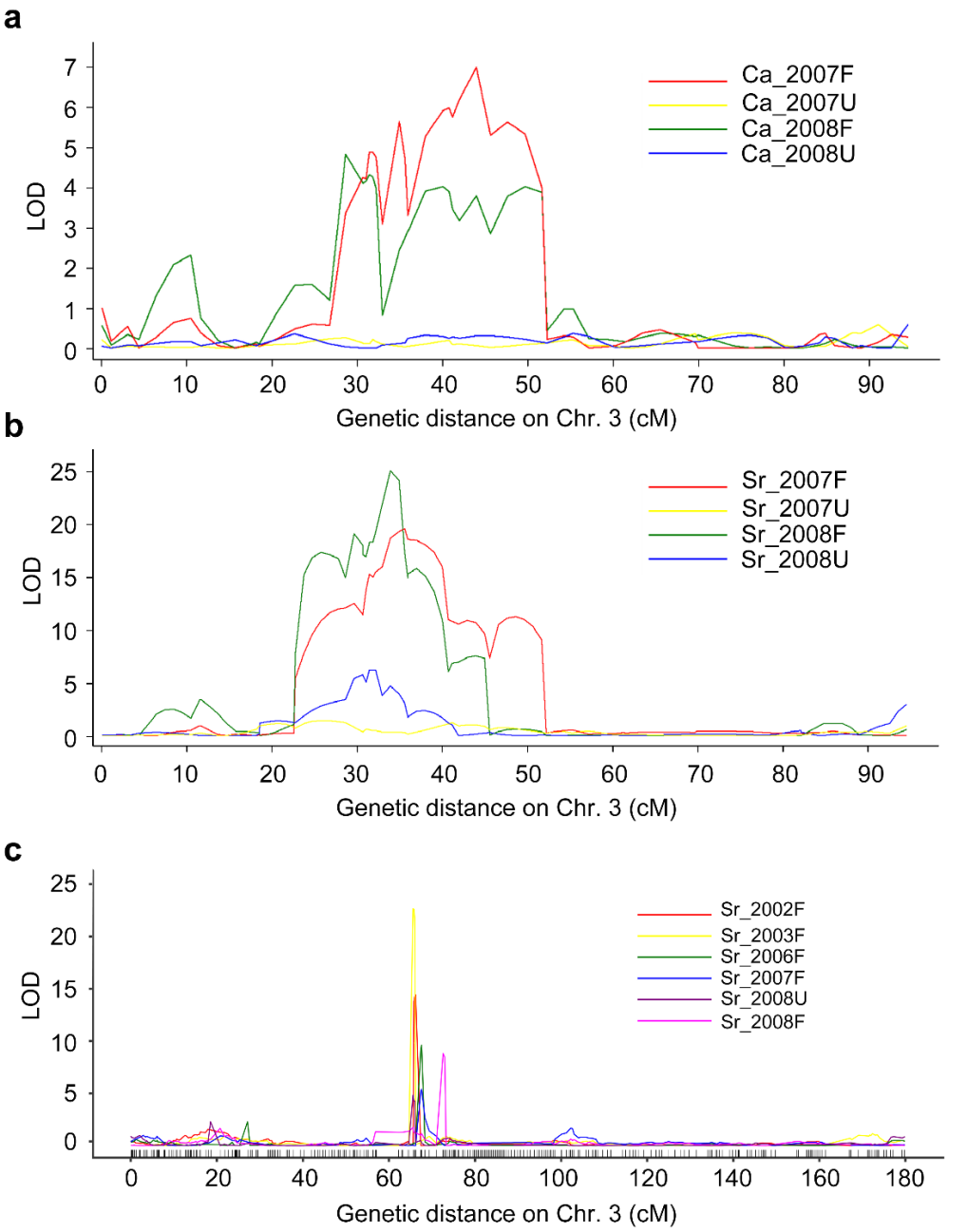
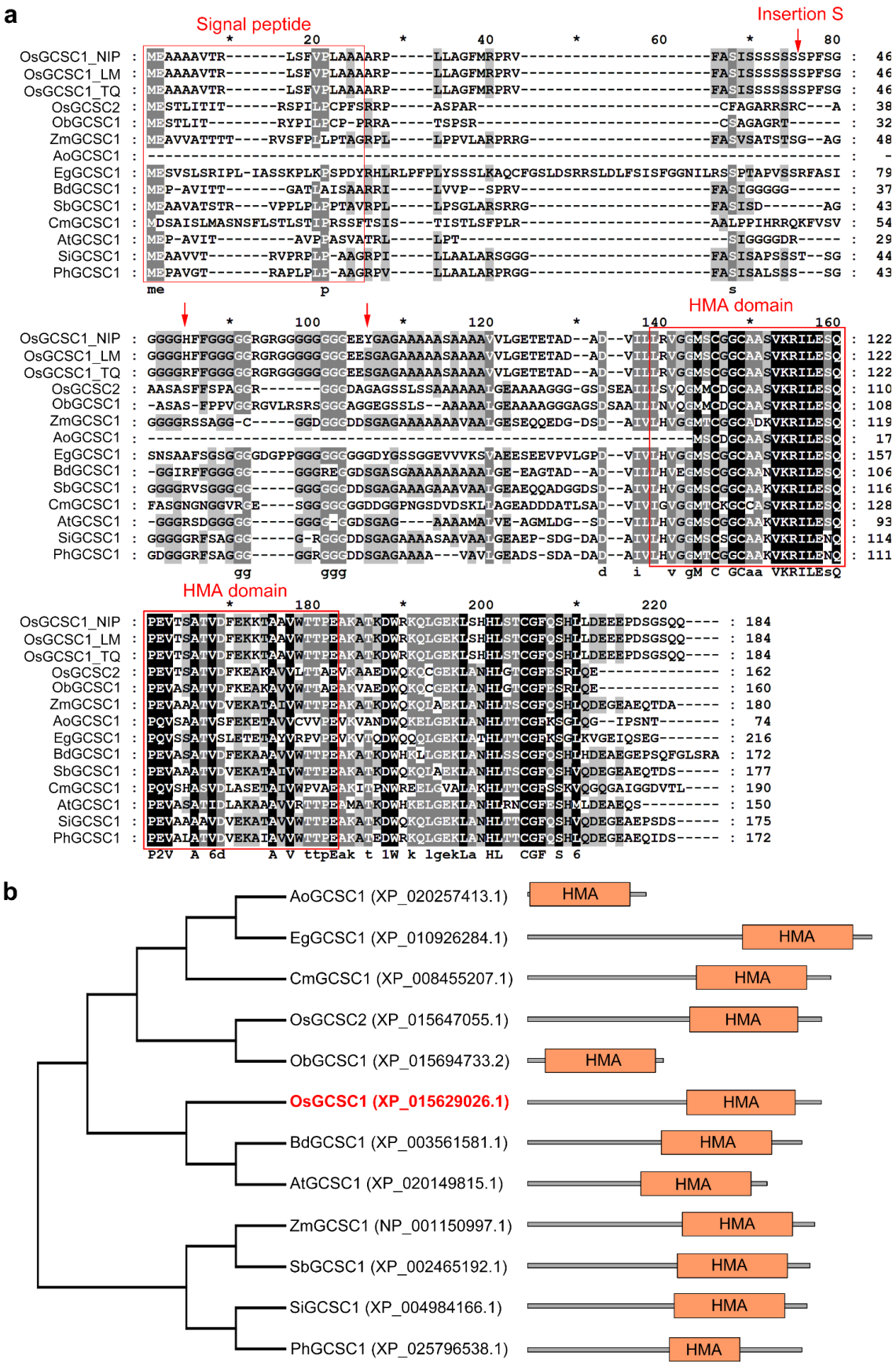
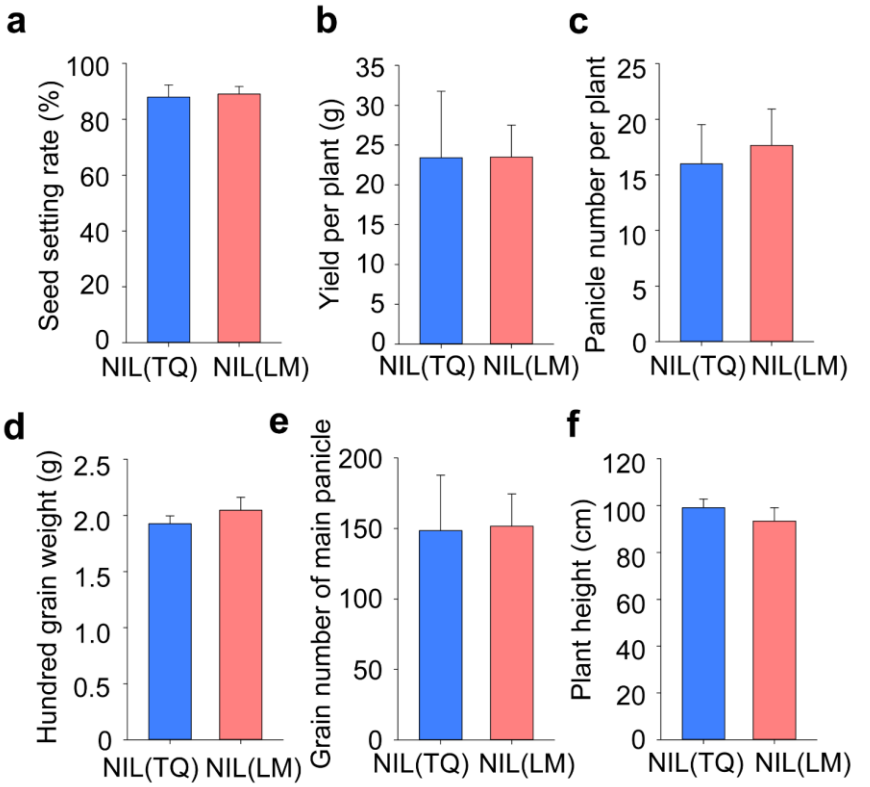


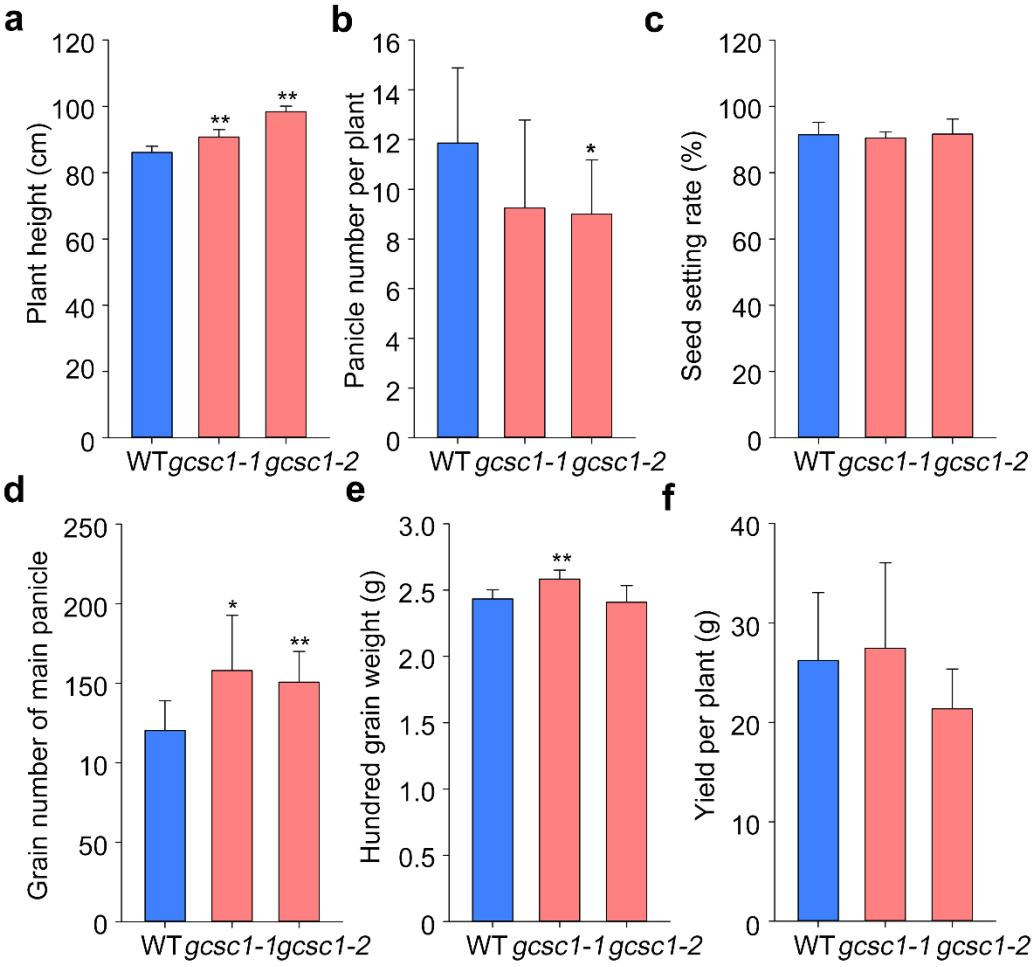
Extended Data Fig. 1 QTL analysis of *qGCa3* in TIL population and LT-RIL population. **a,b,** The LOD profiling of *qGCa3* on chromosome 3 in the TIL population grown in multiple years under different conditions. QTL analyses were performed on the grain concentrations of Ca **(a)** or Sr **(b)** by using SSR markers. **c,** QTL analysis on grain Sr concentration of LT-RIL population grown in multiple years under different conditions by using SNPs derived from whole genome resequencing. F, flooded; U, unflooded.



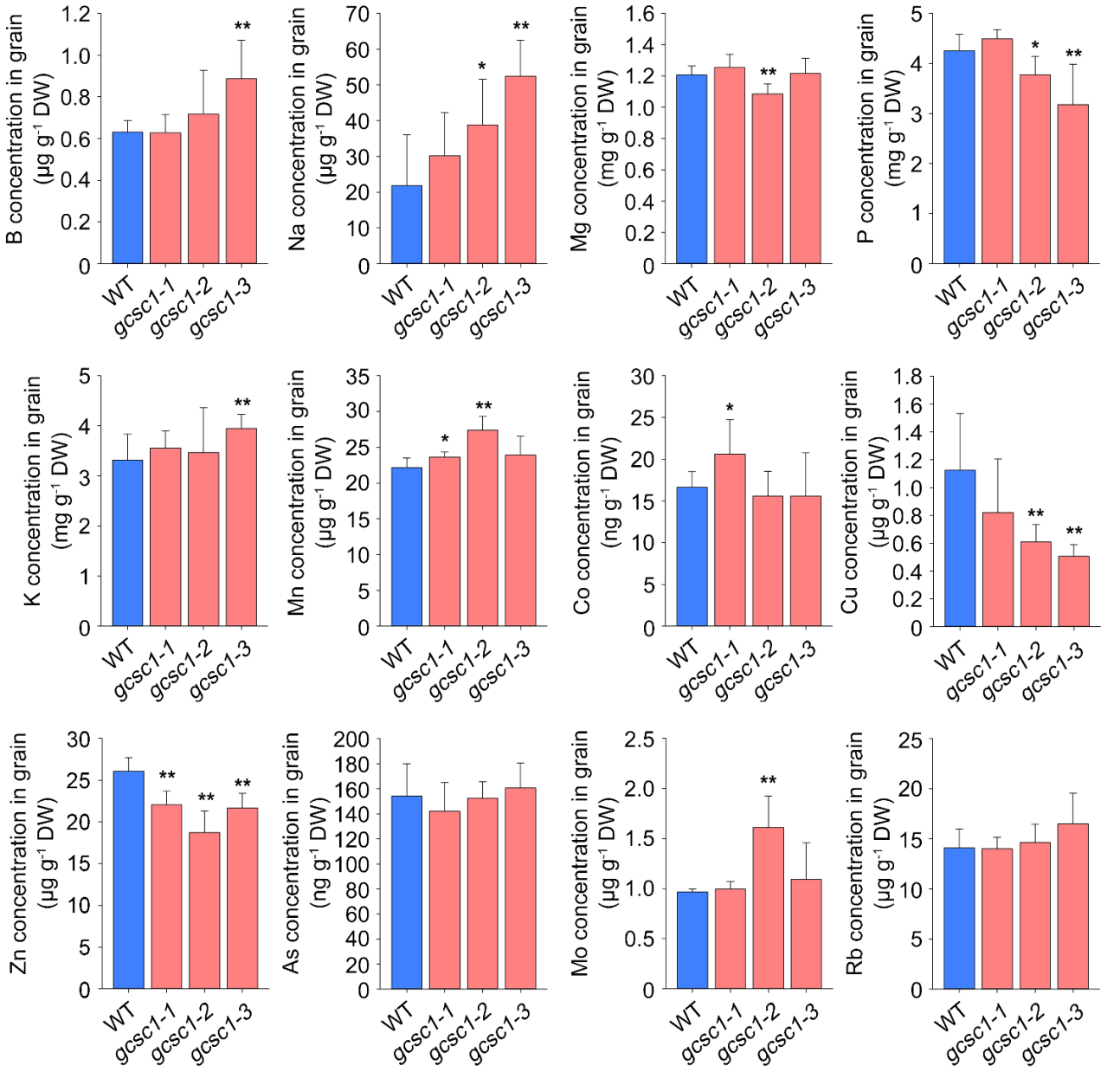
Extended Data Fig. 2 Sequence alignment and phylogenetic analysis of GCSC1 proteins. **a,** Sequence alignment of GCSC1 proteins from different plant species. Sequence alignment was performed in MEGA-X by using Clustal W. Identical and similar residues are displayed in black or grey background. Signal peptide and conserved HMA domain were marked with red boxes. The red arrows indicated the variable amino acids in GCSC1 protein among rice germplasms. Accession numbers: AoGCSC1: XP_020257413.1 [*Asparagus officinalis*]; EgGCSC1: XP_010926284.1 [*Elaeis guineensis*]; BdGCSC1: XP_003561581.1 [*Brachypodium distachyon*]; SbGCSC1:XP_002465192.1 [*Sorghum bicolor*]; CmGCSC1: XP_008455207.1 [*Cucumis melo*]; OsGCSC1: XP_015629026.1 [*Oryza sativa Japonica Group*]; AtGCSC1: XP_020149815.1 [*Aegilops tauschii subsp. tauschii*]; ObGCSC1: XP_015694733.2 [*Oryza brachyantha*]; ZmGCSC1: NP_001150997.1 [*Zea mays*]; SiGCSC1: XP_004984166.1 [*Setaria italica*]; PhGCSC1: XP_025796538.1 [*Panicum hallii*]; OsGCSC2: XP_015647055.1 [*Oryza sativa*]. **b,** Phylogenetic tree of GCSC1. Neighbor-joining tree was constructed by using MEGA-X. The HMA domain of GCSC1 was showed as orange block.



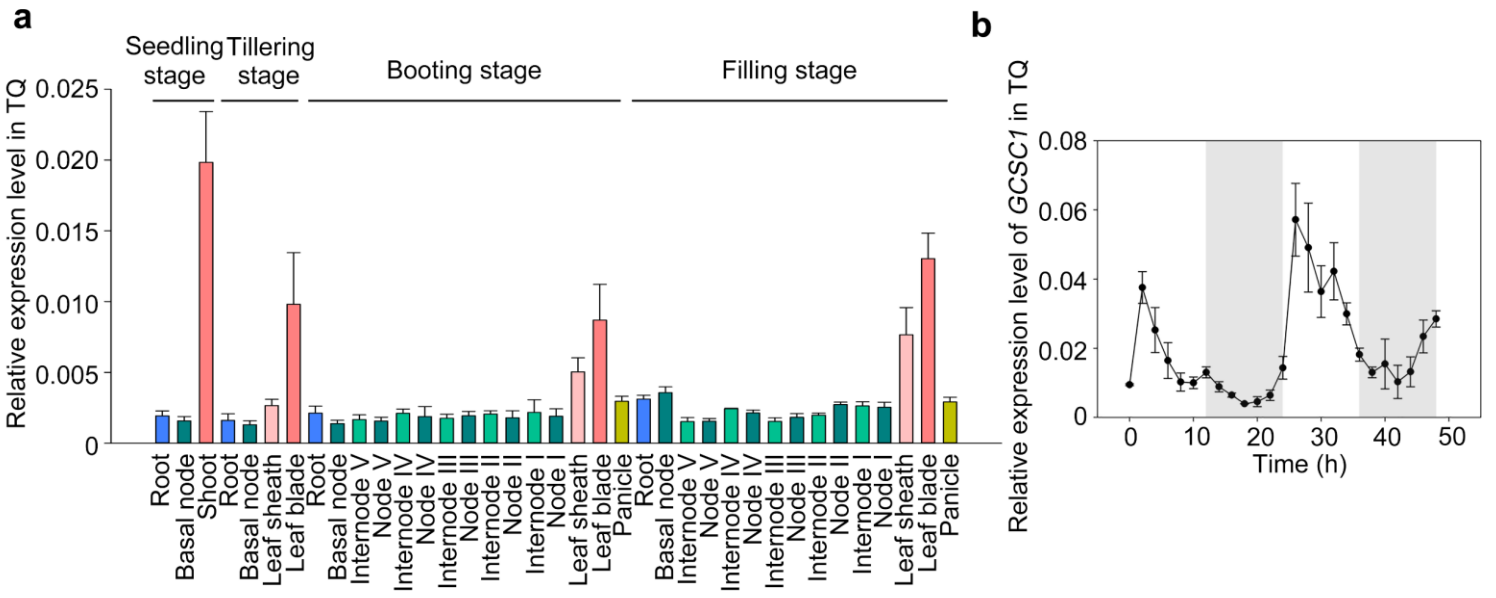
Extended Data Fig. 3 Agronomic traits of NIL(TQ) and NIL(LM). The NILs were grown in a paddy field and the major agronomic traits were determined at harvesting stage. **a,** Seed setting rate of the main panicle. **b,** Total weight of seeds per plant. **c,** Panicle number per plant. **d,** 100-grain weight of main panicle. **e,** Grain number of the main panicle. **f,** Plant height at harvesting stage. Data are presented as mean± SD with n = 12. NIL(TQ) and NIL(LM) are NILs with genomic fragment at *qGCa3* from TQ or LM, respectively.



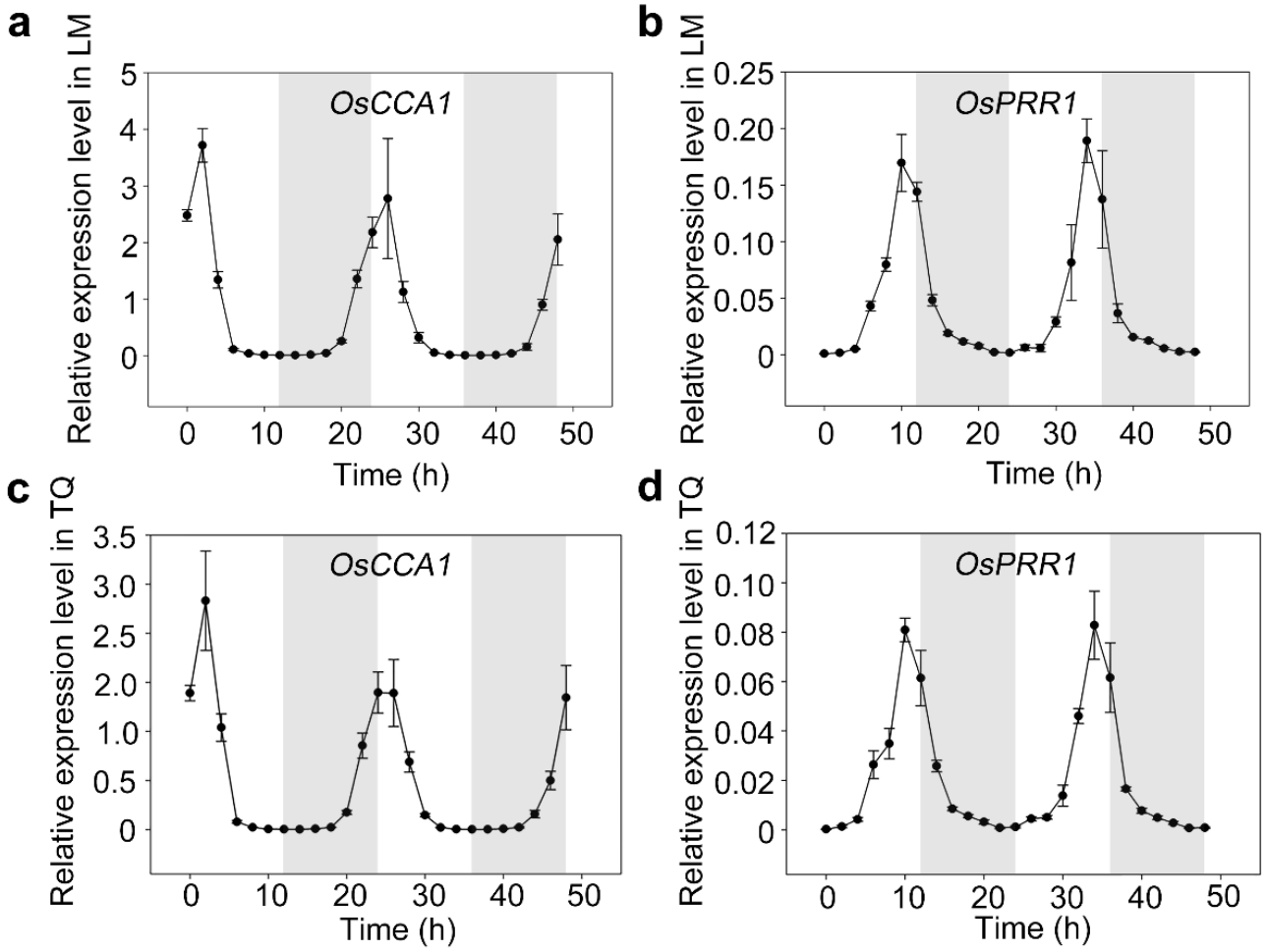
Extended Data Fig. 4 Agronomic traits of WT and *gcsc1*. Plants were grown in a paddy field and the major agronomic traits were determined at harvesting stage. **a,** Plant height at harvesting stage. **b,** Panicle number per plant. **c,** Seed setting rate of the main panicle. **d,** Gain number of the main panicle. **e,** 100-grain weight of the main panicle. **f,** Total weight of seeds per plant. Data are presented as mean ± SD with $n = 12$. * and ** indicate significant difference between WT and *gcsc1* at $p \leq 0.05$ and $p \leq 0.01$, respectively (Student's *t*-test).



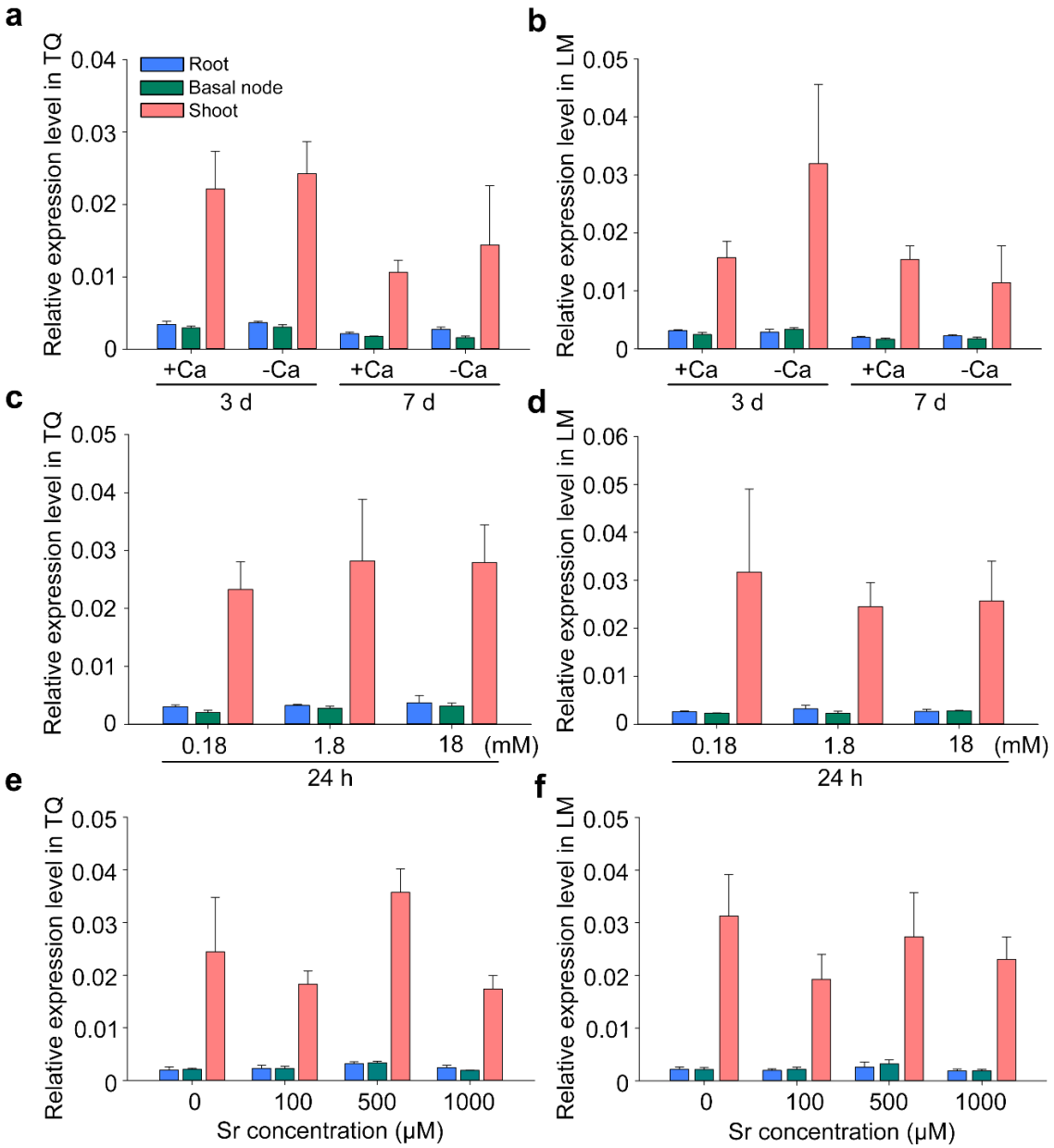
Extended Data Fig. 5 Concentrations of 12 elements in the grains of WT and *gcsc1*. Plants were grown in a paddy field and elemental concentrations in grains were determined by ICP-MS. Data in are presented as mean ± SD with n = 4-9. Statistical significance was determined by Student's t-test. *, $p \leq 0.05$; **, $p \leq 0.01$. DW, dry weight.



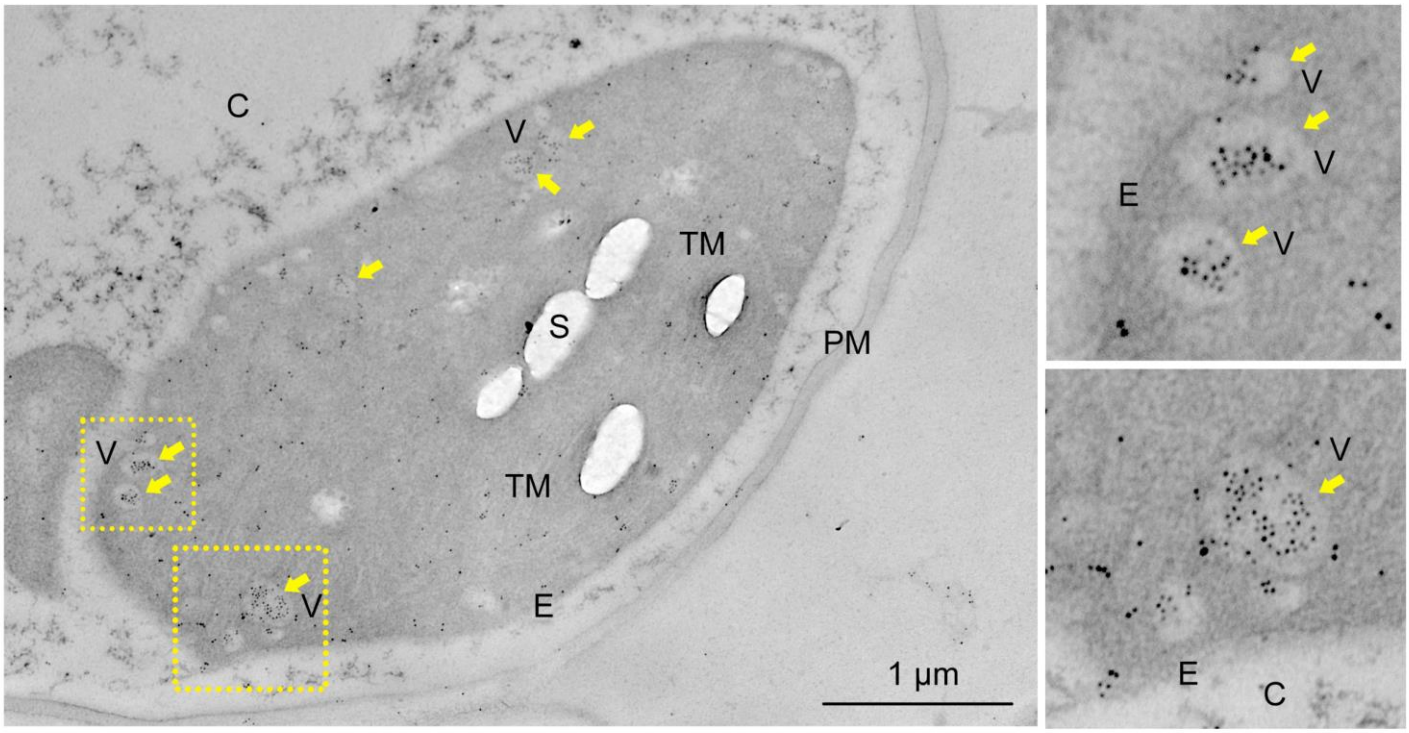
Extended Data Fig. 6 Tissue-specific expression pattern and diurnal rhythmic expression of *GCSC1* in TQ. a, Expression level of *GCSC1* in different organs at different growth stages. Samples were taken from TQ grown in a paddy field. **b,** Diurnal rhythmic expression of *GCSC1* in the leaf blade of TQ. The leaf blades of TQ seedlings were sampled every 2 h for 48 h. The white and grey background represent light and dark conditions, respectively (0 = dawn). The relative expression level of *GCSC1* was normalized to the rice *OsACTIN* gene and presented as mean ± SD with three biological replicates.



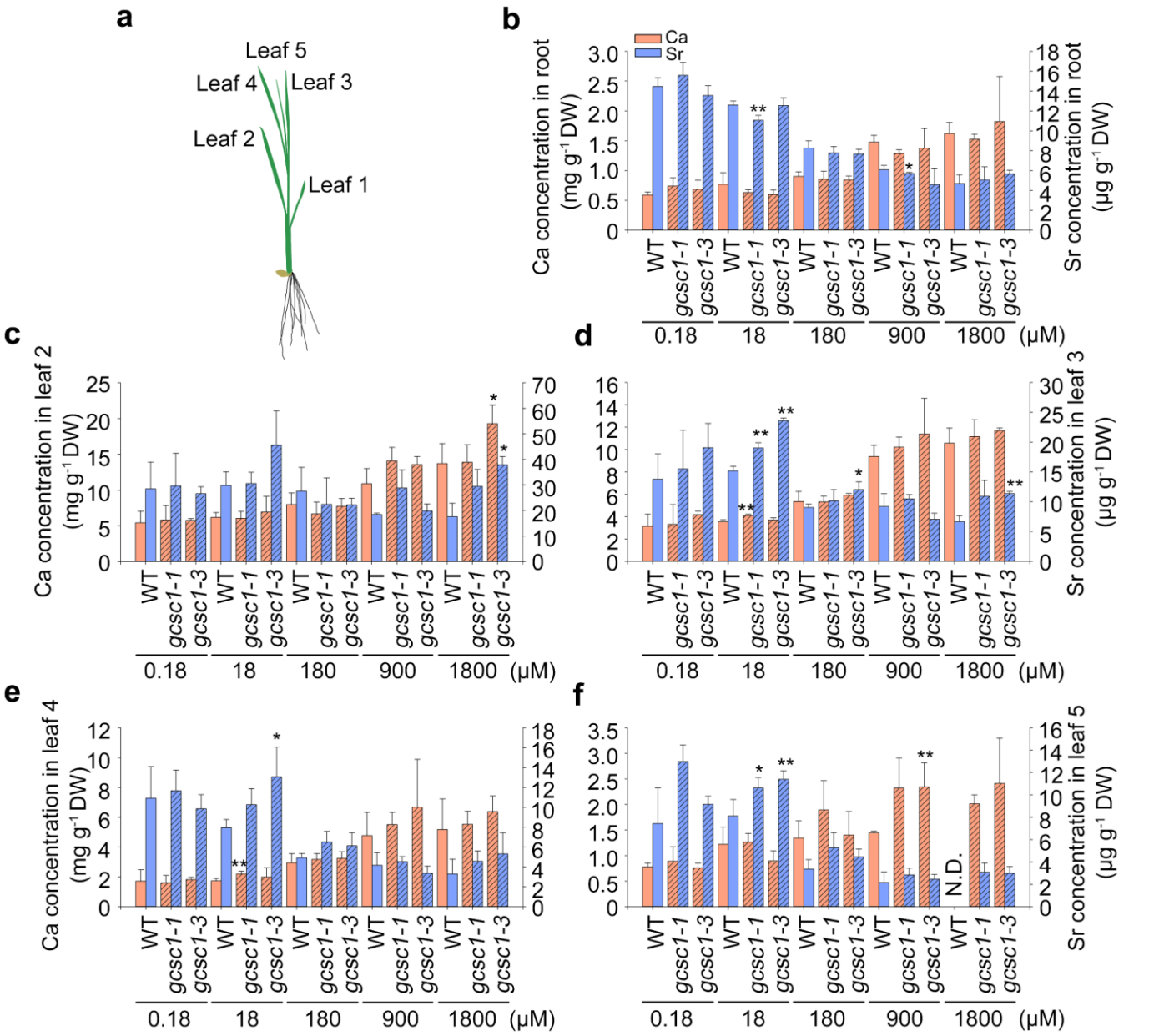
Extended Data Fig. 7 Diurnal rhythmic expression of *OsCCA1* and *OsPRR1* in the leaf blade of LM and TQ. The leaf blades of the LM (**a**, **b**) or TQ (**c**, **d**) seedlings were sampled every 2 h for 48 h. The white and grey background represent light and dark conditions, respectively (0 = dawn). The relative expression level of *OsCCA1* (**a**, **c**) or *OsPRR1* (**b**, **d**) was normalized to the rice *OsACTIN* gene and presented as mean ± SD with three biological replicates.



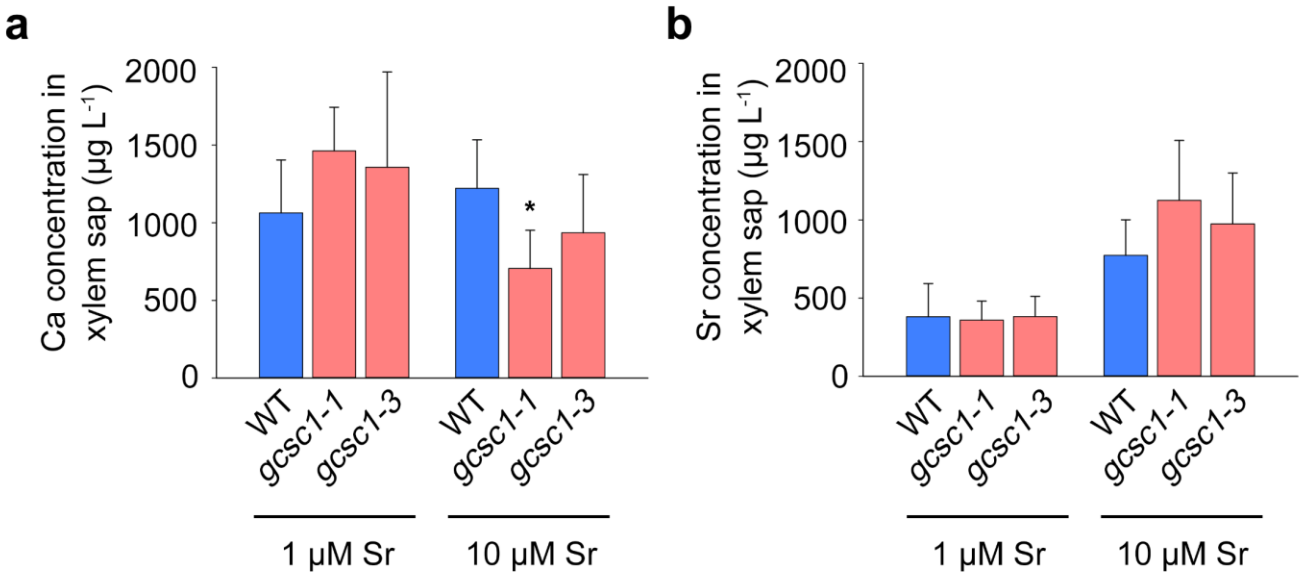
Extended Data Fig. 8 Expression of *GCSC1* in response to different levels of Ca or Sr. a,b, Expression of *GCSC1* in root, basal node and shoot of TQ (**a**) and LM (**b**) under Ca deficient condition. Four-week-old TQ or LM plants were treated in the nutrient solution with (+ Ca) or without Ca added (- Ca) for 3 or 7 d. **c,d,** Expression of *GCSC1* in response to excess levels of Ca. Four-week-old TQ (**c**) or LM (**d**) plants grown in the nutrient solution containing 0.18 mM Ca were treated in the nutrient solution containing 0.18, 1.8, or 18 mM Ca for 24 hours. Expression of *GCSC1* in root, basal node and shoot of TQ and LM were determined by qRT-PCR. **e,f,** Expression of *GCSC1* in root, basal node, and shoot of TQ **(e)** or LM **(f)** grown in nutrient solution with different concentrations of Sr. Four-week-old TQ and LM plants were treated with 0, 100, 500, 1000 μM Sr for 24 hour. The relative expression level of *GCSC1* was normalized to the rice *OsACTIN* gene and presented as mean ± SD with three biological replicates.



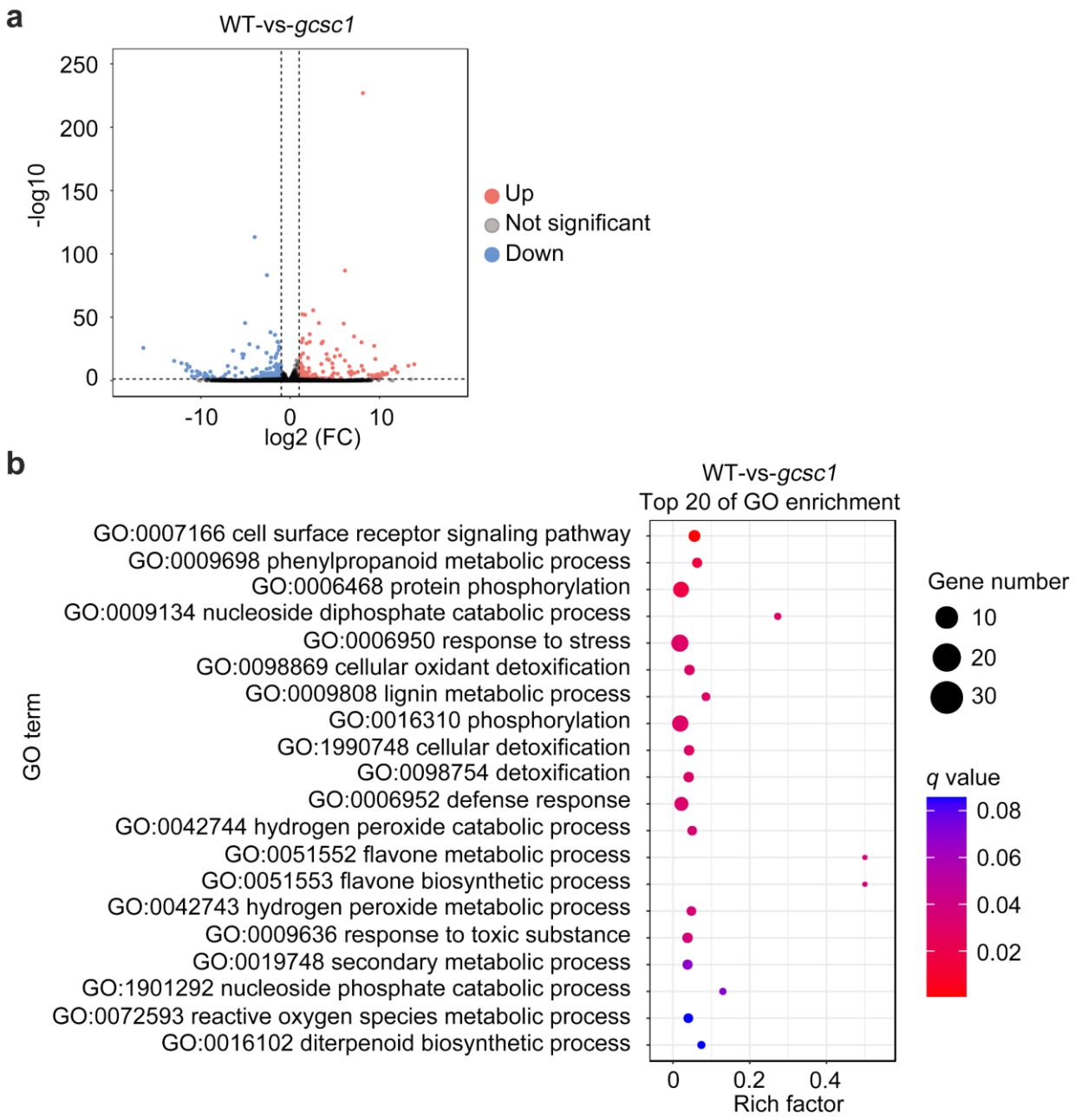
Extended Data Fig. 9 Localization of GCSC1 to the chloroplast vesicles as revealed by immunogold electron microscopy analysis. Ultrathin sections were prepared from the leaf of the *UBIpro::GCSC1-GFP* transgenic line followed by immunogold labeling using GFP antibodies. The yellow rectangles indicate the enlarged parts shown on the right panel. Yellow arrows denote the chloroplast vesicles. E, envelope membrane; V, vesicle; TM, thylakoid membranes; S, starch; C, cytosol; PM, plasma membrane.



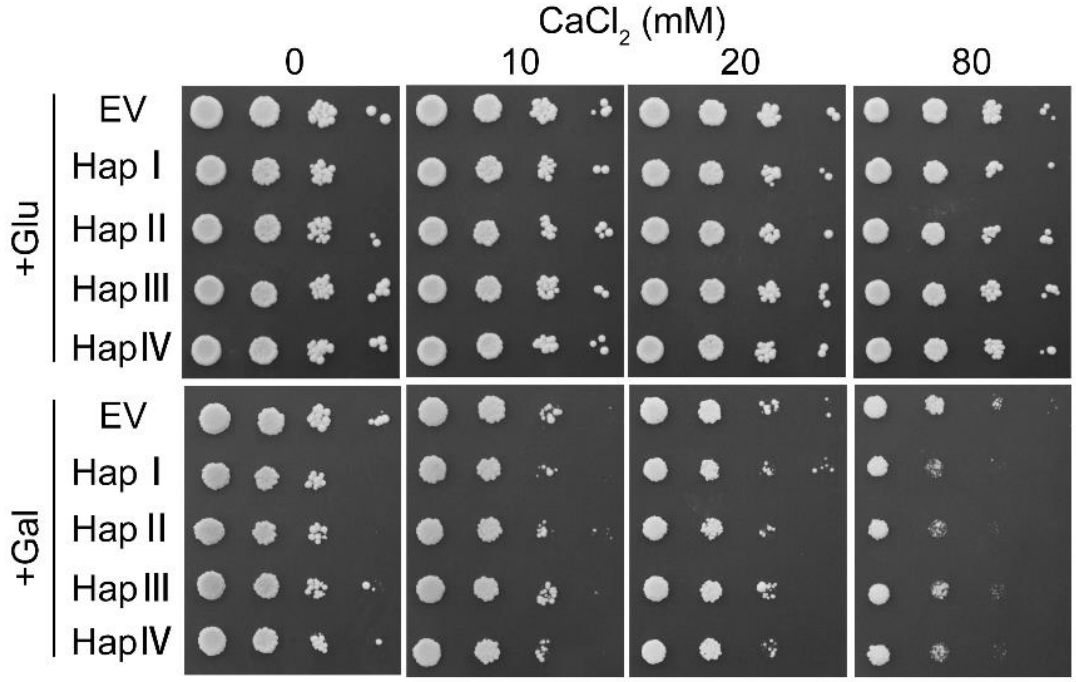
Extended Data Fig. 10 The concentrations of Ca and Sr in roots and different leaves of WT and *gcsc1*. a, Diagram of the seedlings at sampling stage showing the different leaves harvested for determining the concentrations of Ca and Sr. **b-f,** The concentrations of Ca and Sr in the roots (**b**) and different leaves (**c-f**) of WT and two independent *gcsc1* knockout mutants. Plants were grown hydroponically in nutrient solution containing with 0.18, 18, 180, 900, or 1800 μM of Ca^{2+} for 4 w. Before sampling, the nutrient solution was supplemented with 0.5 μM Sr and seedlings were grown for another 3 d. The fifth leaf of WT did not emerge so the concentrations of Ca and Sr were not detectable (N.D.). Data are presented as mean ± SD with $n = 3$. Significant differences of Ca or Sr concentration between WT and *gcsc1* are indicated (Student's *t*-test): *, $p \leq 0.05$ and **, $p \leq 0.01$. DW, dry weight.



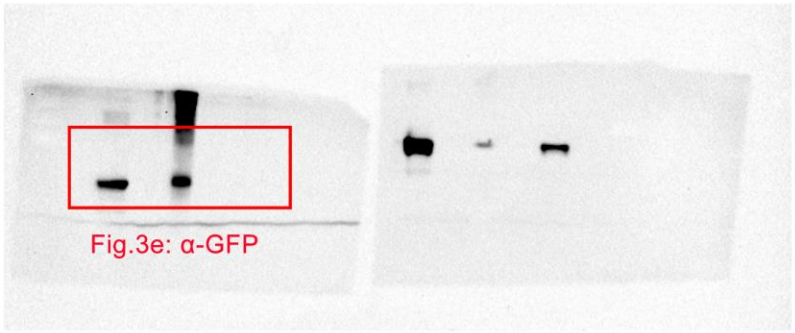
Extended Data Fig. 11 The concentrations of Ca and Sr in the xylem sap of WT and *gcsc1*. Plants were grown hydroponically in nutrient solution containing with 180 μM for 4 w. Xylem sap was collected after 1 or 10 μM Sr treatment for 3 d and the concentrations of Ca (**a**) and Sr (**b**) were determined. Data are presented as mean ± SD with n = 4-6. Significant differences of Ca or Sr concentrations between WT and *gcsc1* are determined by Student's t-test. *, $p \leq 0.05$. DW, dry weight.

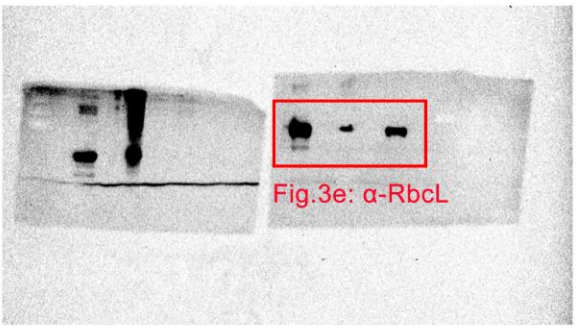


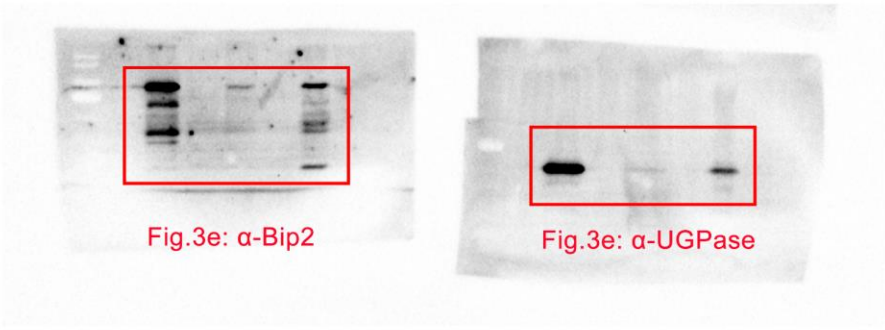
Extended Data Fig. 12 Transcriptomic analysis in the flag leaves of WT and *gcsc1* grown in the paddy field at grain filling stage. **a,** Differentially expressed genes in *gcsc1* compared with WT. **b,** GO analysis of differentially expressed genes between WT and *gcsc1*. RNA-seq analysis was performed in the flag leaves of plants grown in the paddy field at grain filling stage. Criteria for differential expression were set as FDR ≤ 0.05 and |log2(fc)|≥1.

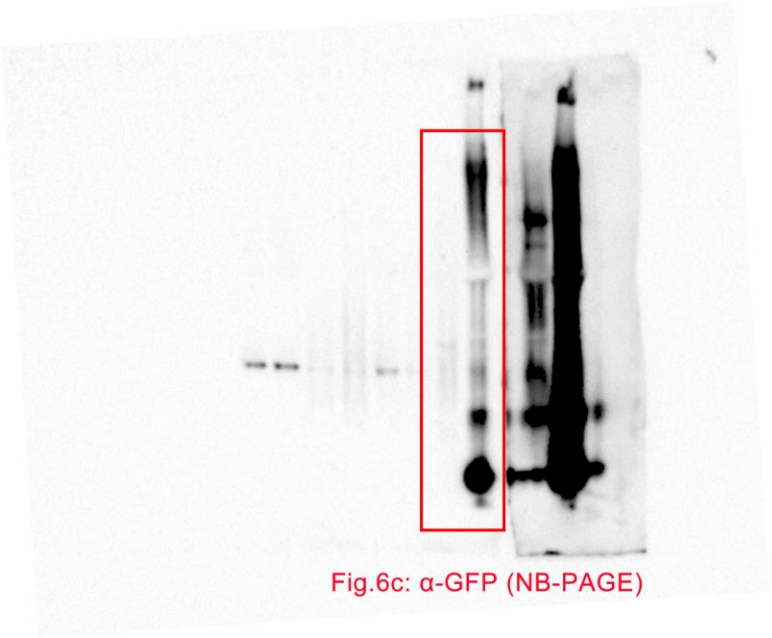


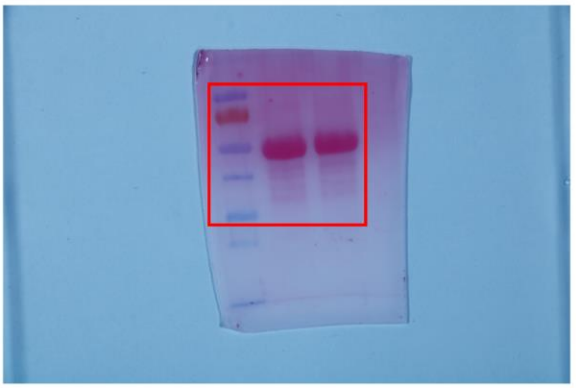
Extended Data Fig. 13 Ca^{2+} transport activities of four different GCSC1 haplotypes in yeast. Overnight yeast mutant *k667* cells transformed with empty vector (EV) or different haplotypes of *GCSC1* were serially diluted (1:10) and spotted on the media supplied with indicated concentration of $CaCl_2$. Expression of *GCSC1* was induced on the media supplemented with galactose (Gal) but not glucose (Glu). Pictures were taken after incubation at 30 °C for 3 d.











Extended Data Fig. 14 Source data of full uncropped versions of western blot images in Fig 3e and Fig 6c. The red rectangles indicate the cropped area of the images shown in Fig 3e or Fig 6c.