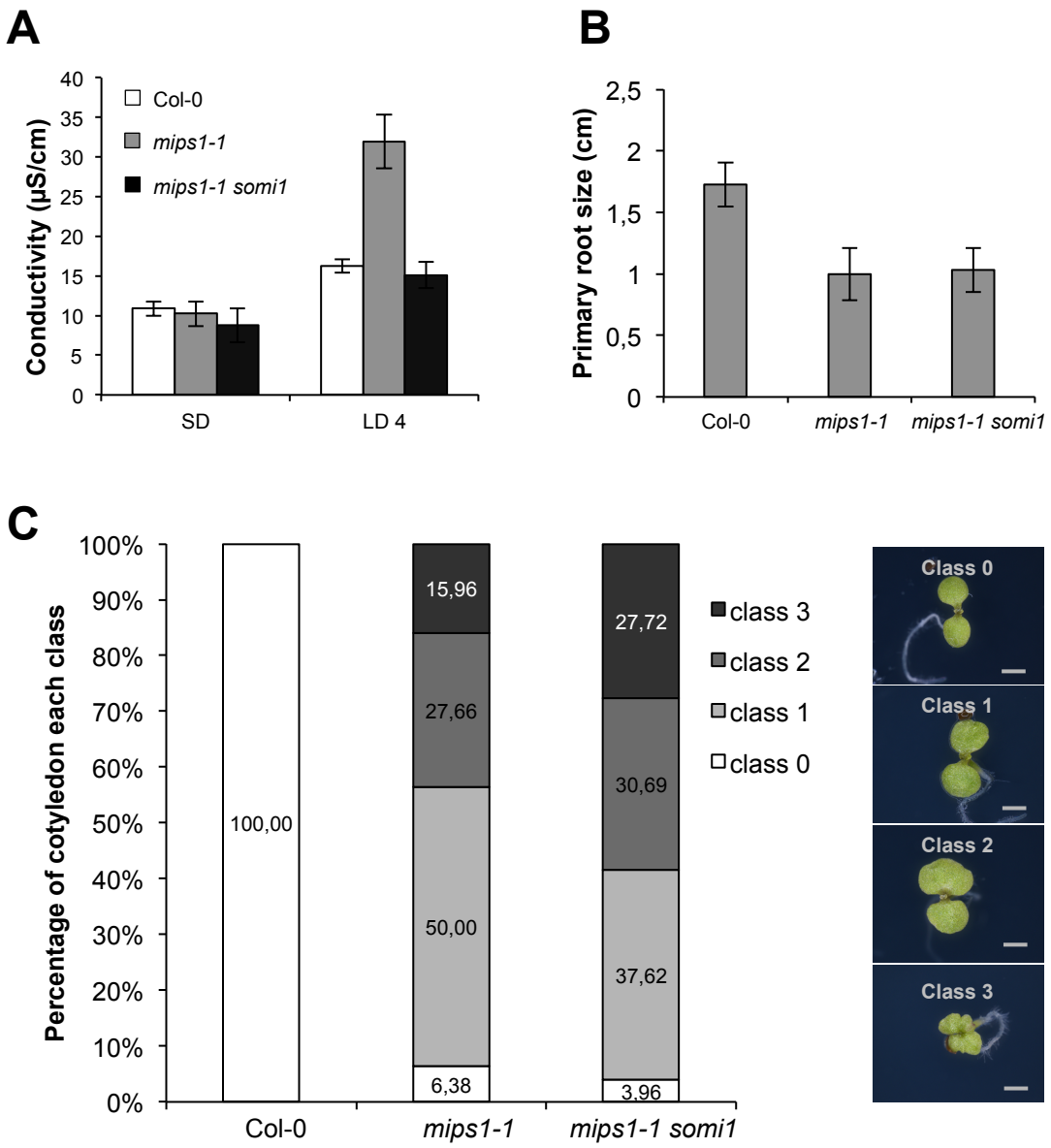

Figure S1. Phenotypic analyses of the *mips1-1 hxk1-1* suppressor mutant. A, Ion leakage assays, with means and standard deviations calculated from six leaf discs per treatment with three biological replicates grown in SD conditions and transferred to LD conditions for 4 days (LD 4). B, Primary root lengths of the indicated genotypes. C , Relative proportion of each cotyledon class in the indicated genotypes. Bar = 1 cm.

A

```
A. thaliana      FPVKQTSLSSGSLIKWTKGFSIEEAVGQDVVGALNKALERVG-LDMRIAALVNDTVGTLAGGRY-YNPDVVAAVILGTGTNAAYVERATAIPKWH-GL--
N. benthamiana   FPVMQTSINSGTIMRWTKGFSIDDAVGQDVVGELTKAMKRKG-VDMRVSALVNDTVGTLAGGKY-THNDVAVAVILGTGTNAAYVERVQAIPKWH-GP--
V. vinifera      FPVRQSSIASGSLIKWTKGFSIEDAVGQDVVGELTKAMERIG-LDMRVSALVNDTIGTLAGGRY-YDQDVVAAVILGTGTNAAYVERAQSIPKWH-GL--
P. patens        FPVRQTSVKSGIVIHWTKGFKVDDAVGKDIVKQFQDAISRSN-HQIMISALVNDTVGTLAGGRFNFDEETMIGCIIGTGTNACYVERADAVHKWD-EP--
O. satyva        FPVHQTSIASGTLIRWTKAFAVDDAIGEDVVAALQAAMSERG-LDMRVSALINDTVGTLAAGSY-YDEDVVAAVILGTGTNAAYVEDATAIAKLHPSQ--
S. cerevisiae    YPASQNKINEGILQRWTKGFDIPNVEGHDVVPLLQNEISKRE-LPIEIVALINDTVGTLIASYY-TDPETKMGVIFGTGVNGAFYDVVSDIEKLEGKLAD
C. reinhardtii   FAVEQSGLAAGKLLDWTKGFKCSGVIGNDPVKLLTAALERAG-CPCRVLALLNDTVGVLAAQRY-LDHHTDVGVIIGTGTNACYVEDAAKLTKWRPPAG-
M. musculus      FPCRQSKIDEAVLITWTKRFKASGVEGADVVKLLNKAIKKRGDYDANIVAYVNDTVGTMMTCGY-DDQQCEVGLIIGTGTNACYMEELRHIDLVE-----
H. sapiens       FPCQQSKIDEAILITWTKRFKASGVEGADVVKLLNKAIKKRGDYDANIVAYVNDTVGTMMTCGY-DDQHCEVGLIIGTGTNACYMEELRHIDLVE-----
                 %pv.Q.si.sg.li.WTKgF....a.G.DvV..l..a..r.g..d.r!.AlvNDT!Gt$a.g.%..d.....gvI.GTGtNaa%v#.a..i.kw......
```

B

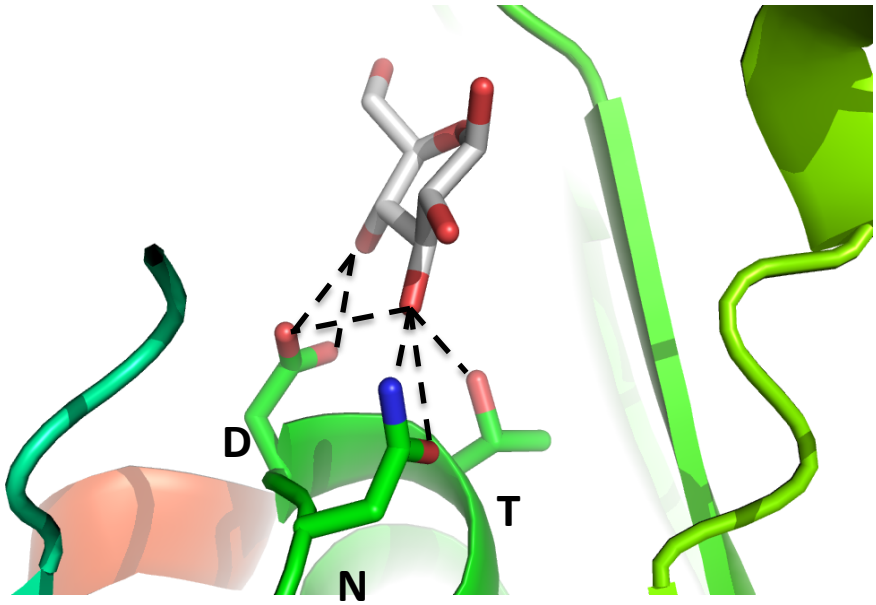

Figure S2. Position of threonine 231 in HXK1 primary protein sequences

A - Sequence analysis. Protein sequences of the indicated species were collected from public resources (www.ncbi.nlm.nih.gov), and multi alignment was performed with the online software Multalin (multalin.toulouse.inra.fr/multalin). The arrow indicates threonine 231.

B – Structure analysis. Close view of the active site of the glucose-bound form of the yeast hexokinase from *Kluyveromyces lactis* (Kuettner et al., 2010). The figure was prepared using the PyMOL Molecular Graphics System and the PDB entry 3O5B. The protein is shown as a cartoon colored by spectrum. The side chains of the three conserved residues N, D and T are highlighted in sticks colored by atom type, as the bound glucose. H-bonds between the three residues and glucose are shown as black dashed lines.

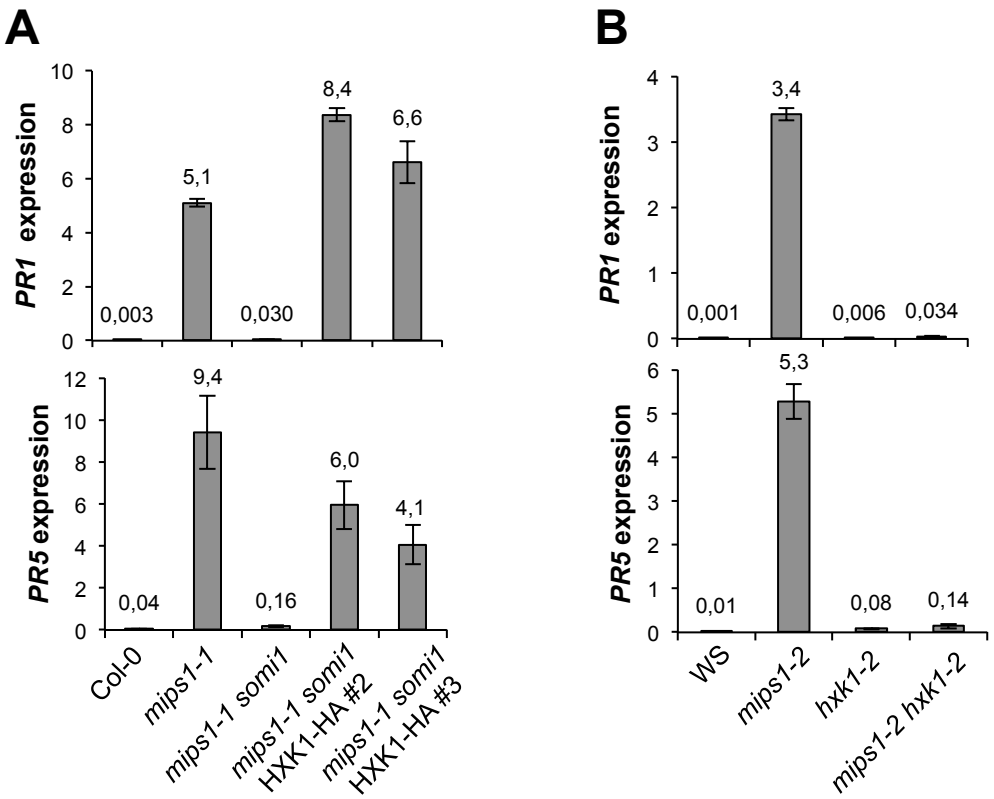

Figure S3. Relative expression of *PR1* and *PR5*

A and B, RT-qPCR analysis of *PR1* and *PR5* expression in the rosette leaves of plants of the indicated genotypes, 4 days after transfer to LD conditions. Transcript abundance is expressed relative to *UBQ10* transcript abundance. Means and standard deviations were calculated from two biological replicates.

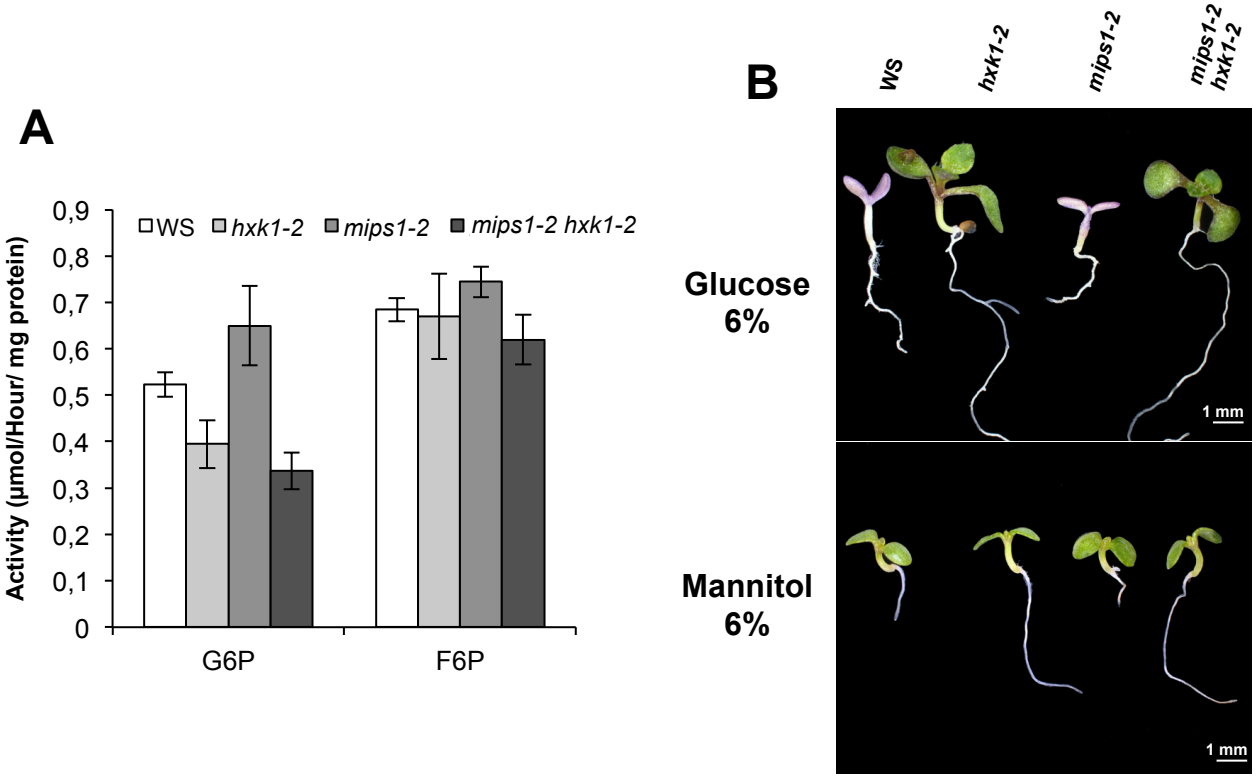

Figure S4. HXK1 metabolic and signalling functions in the mutants *hxk1-2 and mips1-2 hxk1-2*

A, Glucokinase and fructokinase activity measurement in the indicated lines. G6P, glucose-6-phosphate; F6P, fructose-6-phosphate. Samples were harvested two days after transfer to LD conditions, and means and standard error were calculated from four biological replicates. B, Glucose sensitivity assay. Plants were grown on a 6% glucose (Glc) or mannitol (Man) MS medium, for 10 days.

.

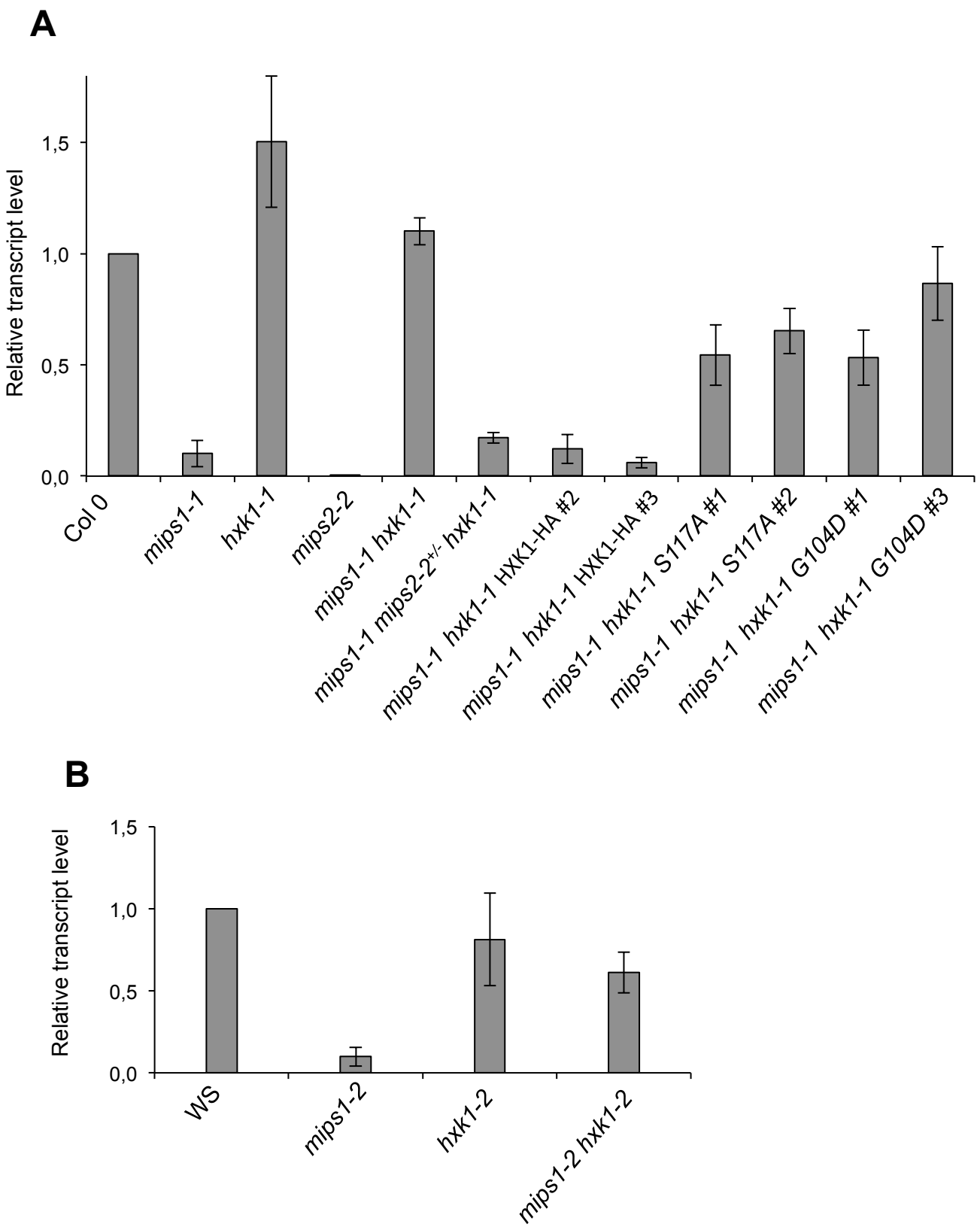

Figure S5. Effect of *mips1*, *mips2*, *hxk1* mutations and of complementation on *MIPS2* expression

RT-qPCR analysis of *MIPS2* expression in rosette leaves from indicated genotypes in Col-0 (A) and WS (B) backgrounds, 4 days after transfer in LD conditions. Transcript abundance is expressed relative to *UBQ10* transcripts abundance. Means and standard deviations were calculated from two biological replicates.