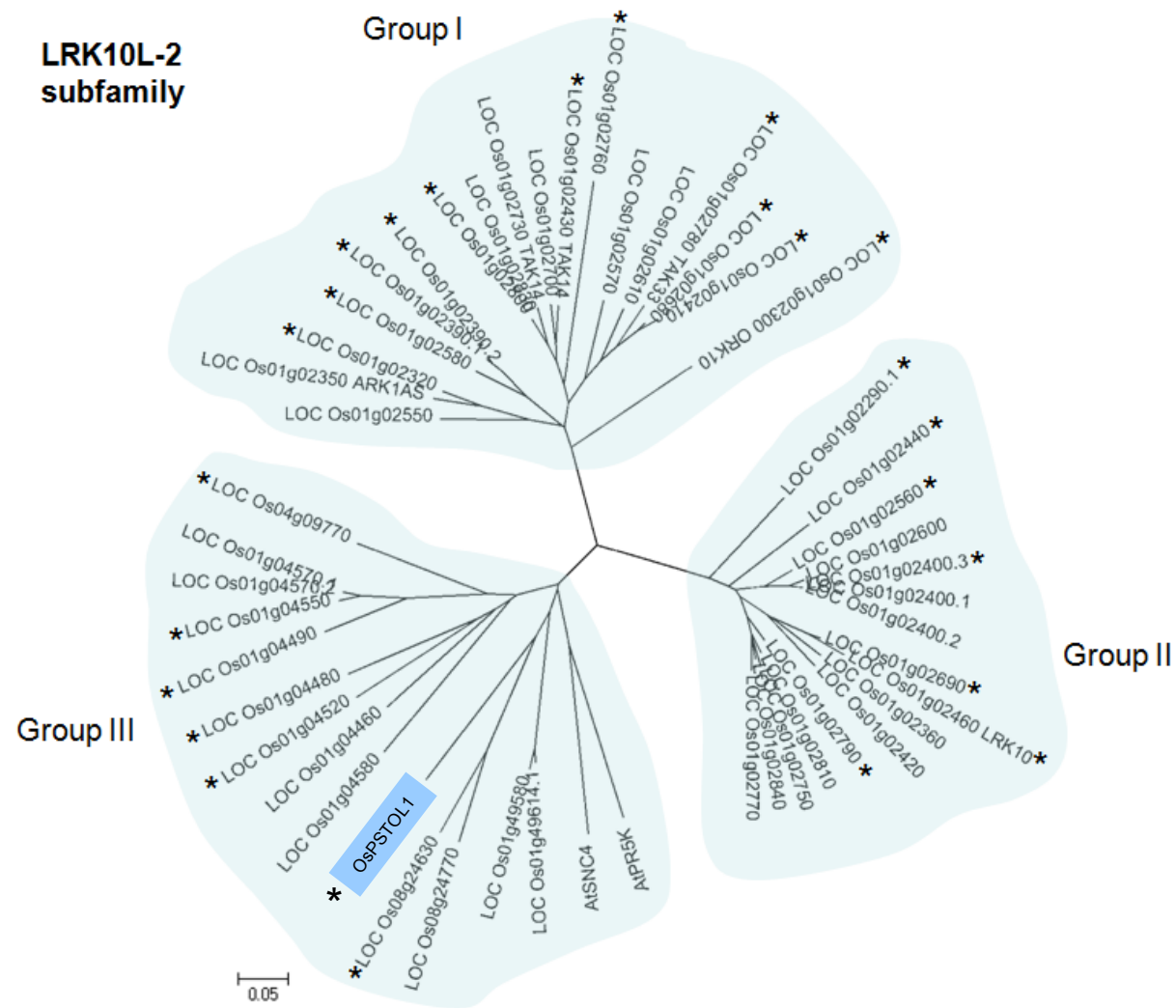




Supplemental Figure 1. OsPSTOL1 is a receptor-like cytoplasmic kinase.
Based on sequence comparisons of the OsPSTOL1 conserved kinase domain with publicly available protein kinase amino acid sequences, OsPSTOL1 was identified as a member of the LRK10L-2 subfamily. Three distinct groups can be differentiated within this subfamily. OsPSTOL1 belongs to group III. The two most similar *Arabidopsis* proteins of group III were included for comparison. Proteins marked with an asterisk lack an N-terminal domain and are therefore classified as receptor-like cytoplasmic kinases (RLCKs).

OsPSTOL1 1
 LOC_Os01g04570.2 1
 LOC_Os01g04570.1 1
 LOC_Os04g09770 1
 LOC_Os01g04460 1
 LOC_Os01g04480 1
 LOC_Os01g04520 1
 LOC_Os01g49614.1 1
 LOC_Os01g49580 1
 LOC_Os08g24770 1
 LOC_Os08g24630 1

OsPSTOL1 1
 LOC_Os01g04570.2 1
 LOC_Os01g04570.1 104
 LOC_Os04g09770 1
 LOC_Os01g04460 111
 LOC_Os01g04480 111
 LOC_Os01g04520 111
 LOC_Os01g49614.1 117
 LOC_Os01g49580 70
 LOC_Os08g24770 121
 LOC_Os08g24630 1

OsPSTOL1 1
 LOC_Os01g04570.2 1
 LOC_Os01g04570.1 215
 LOC_Os04g09770 1
 LOC_Os01g04460 210
 LOC_Os01g04480 222
 LOC_Os01g04520 231
 LOC_Os01g49614.1 232
 LOC_Os01g49580 184
 LOC_Os08g24770 241
 LOC_Os08g24630 1

OsPSTOL1 1
 LOC_Os01g04570.2 22
 LOC_Os01g04570.1 277
 LOC_Os04g09770 9
 LOC_Os01g04460 265
 LOC_Os01g04480 279
 LOC_Os01g04520 296
 LOC_Os01g49614.1 302
 LOC_Os01g49580 304
 LOC_Os08g24770 341
 LOC_Os08g24630 1

OsPSTOL1 1
 LOC_Os01g04570.2 22
 LOC_Os01g04570.1 277
 LOC_Os04g09770 9
 LOC_Os01g04460 265
 LOC_Os01g04480 279
 LOC_Os01g04520 296
 LOC_Os01g49614.1 302
 LOC_Os01g49580 424
 LOC_Os08g24770 341
 LOC_Os08g24630 1

OsPSTOL1 1
 LOC_Os01g04570.2 22
 LOC_Os01g04570.1 277
 LOC_Os04g09770 9
 LOC_Os01g04460 265
 LOC_Os01g04480 279
 LOC_Os01g04520 296
 LOC_Os01g49614.1 302
 LOC_Os01g49580 424
 LOC_Os08g24770 341
 LOC_Os08g24630 1

OsPSTOL1 78
 LOC_Os01g04570.2 119
 LOC_Os01g04570.1 374
 LOC_Os04g09770 105
 LOC_Os01g04460 362
 LOC_Os01g04480 376
 LOC_Os01g04520 404
 LOC_Os01g49614.1 417
 LOC_Os01g49580 664
 LOC_Os08g24770 455
 LOC_Os08g24630 42

OsPSTOL1 196
 LOC_Os01g04570.2 234
 LOC_Os01g04570.1 489
 LOC_Os04g09770 220
 LOC_Os01g04460 477
 LOC_Os01g04480 491
 LOC_Os01g04520 519
 LOC_Os01g49614.1 534
 LOC_Os01g49580 781
 LOC_Os08g24770 575
 LOC_Os08g24630 161

```

OsPSTOL1      310 LESNGRDLPLPPKGL-----
LOC_Os01g04570.2 350 FERSLDELEMPKQNFSELEHHPAQEINTESTSSTINTKAAQALSEVLRVEETSLVNSKDPTVTTNYAKR
LOC_Os01g04570.1 605 FERSLDELEMPKQNFSELEHHPAQEINTESTSSTINTKAAQALSEVLRVEETSLVNSKDPTVTTNYAKR
LOC_Os04g09770   336 FERSADELEIPPKHCFYSAIQ-----
LOC_Os01g04460   591 LERSISELEMPKPFLLCSPIHSTNVSSYKSVNFTMSASS-----
LOC_Os01g04480   606 LEKNINEMEVPKPFLLS-----
LOC_Os01g04520   -----
LOC_Os01g49614.1 649 LEGSTSGLELPPKVLLSW-----
LOC_Os01g49580   896 LEGSTSGLELPPKVLLSW-----
LOC_Os08g24770   692 FDRSLTELQLPPRISCCGNYNESFG-----
LOC_Os08g24630   279 FDRSMHELQLPPRMSCCGIDNPSIV-----

```

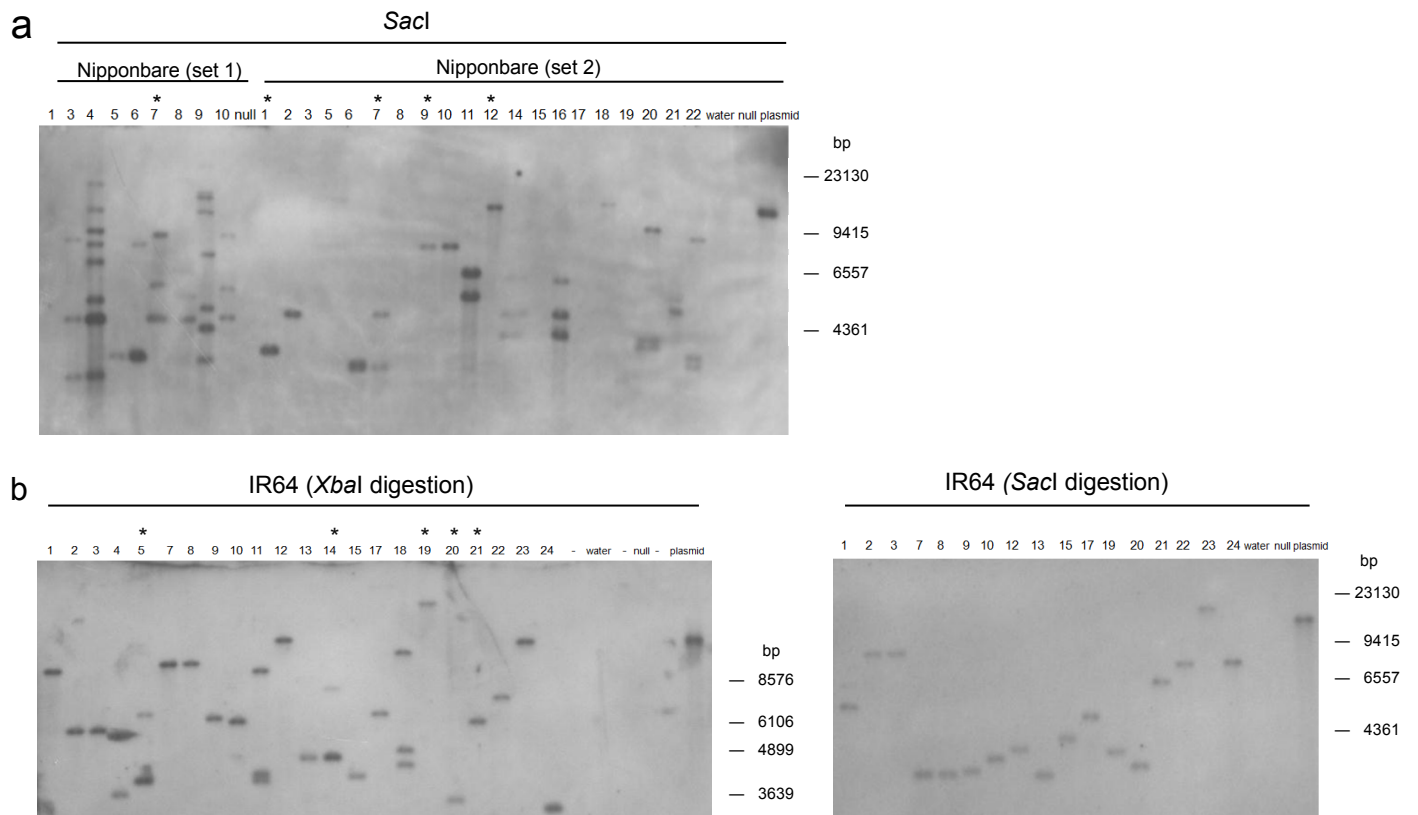
Supplemental Figure 2. OsPSTOL1 protein sequence alignment with most similar rice protein kinases.

Rice protein kinases most similar to OsPSTOL1 were identified by BLASTn and BLASTp searches in public databases and the ten most similar protein sequences were used for a multiple sequence alignment using MAFFT (<http://mafft.cbrc.jp/alignment/server/index.html>). The conserved protein kinase catalytic domain (underlined) is composed of 281 amino acids in OsPSTOL1. Black shading: Identical AA; gray shading: similar AA.

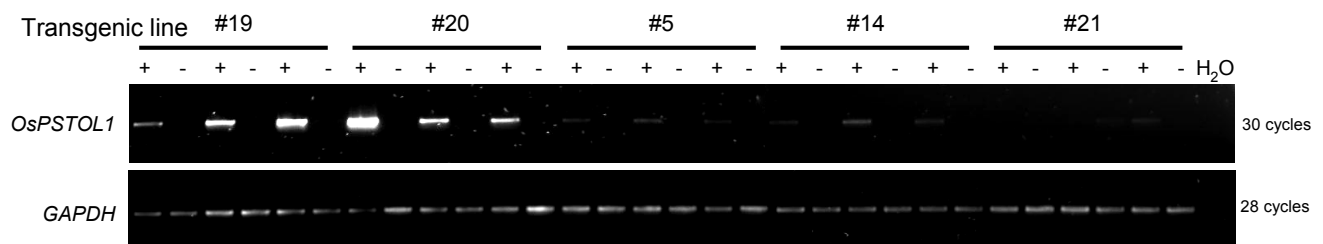
OsPSTOL1	1	-----KCDYTWPGFIMTTTAVSLPTNGF-----
AtPR5K	1	-----MVEGFSLSLMLLVSHFFVSGVMSTNFIEN-----
AtSNC4	1	MNSQQSTRKQMLQGSSTHLLCGVLLQLFAAQVDAQRSTSPWQLSGDAPLVIARGGFSGLFPDSSLAAYQFAMVVSADVVWCDVQLTKDGHGICFPDLNLANASNEEVYPNRQKS
LOC_Os01g49580	1	-----MVLPPRLLLFFLLLIQVHASVSHGSPSLPPTYDPSICSN-----LDGSKQTPFIQLNGENYITILEIYYDSRTIV
LOC_Os01g49614.1	1	-----MRLLCLAAMAHLPDLLLSFLVLIGVHASVSHGSPPLPSTYDPSICSSSECGGVNISYFFYLSNATDYTRFSCGYTDLKISCSRDRDGRNETPTILLGGDNYTVLDIYYDSHTIV
LOC_Os08g24630	1	-----
LOC_Os08g24770	1	MRDRDQDGTTFKFLSIVPLVNYRTTRTGWCNSICHKFFFPQDAGNSSHDNSSKAQKQANATAARLRSAMAMAGNHRSSQLHLLLLFCCTTTLRAAALSFDYDFSDAADAKNLVMFGDAAH
OsPSTOL1	1	-----
AtPR5K	56	SLKKGESRVINVPSPWSGRLWGRSFCSTSSSTGNFSCATGDCGSGKIECSDSGARPPTTLIDFTLDATDGDGFYDVSVDGYNLPIVVVPQRIGSGRSTCSNVGCVVNLNKTCPSELKVMGS
AtSNC4	121	YPVNGVTTKGWFPIDFSLELQKVLFSRLIGILSRSGKFDENGYSISTVQNVATQMKPALFWLNVQHDEFYEQHNLMSMSSFLSTSRVSIIDFISSEPEVNFRRKIAGGFGNGGSPFVQF
LOC_Os01g49580	70	LADTDALRG-SCPRVR-----HNVTFGQAYPWLQYTGSRDNLTFFFGCKNLNPPIDPGLVSLADKHQINCKDFSNWPDSDGDSFVFTSGEALPEAPVESELARCCSQVIVVPVN-----GSV
LOC_Os01g49614.1	117	LADTDALRGSGCPRVR-----HNITFAQADEWLQYTGPRDNLTFFFGCKNLNPPIDPGLVSLADKHQINCKDFSNWPDSDGDSFVFTSALEAPVESELARCCSQVIVVPVN-----GDI
LOC_Os08g24630	1	-----
LOC_Os08g24770	121	AGDRINLTNLGVWRAG-----RVAHRQLVRLWDDDVGGGRTTTTSFTTAFSFAIGRNSTNQPADGMAFFVGLPRDNLPPHSDGAFFGLLSNNYFGPYGSPRTVGVEFDTSFNPMDPEGT
OsPSTOL1	1	-----
AtPR5K	176	SNKEHPIACMNACQKFLPEFCYGEYGPAPKQPTLYSTNFKNECPLAYSYAYDNEENNTFRCSNSPNYVITFCPNDISSMSQPSK-----
AtSNC4	241	MKGEDFEPTTNRTYSGILSNLSFVKTFASGILVPKSYILPLDDQYLLPHTSLVQDAHKAGLKLKLYASGFANDVDIAYNYSWDPVSEYLSFVNGNFSVDGMLSDPPLTASASVDCFSHIG
LOC_Os01g49580	179	LNSNQSALPSGGYGQVLKMG--FDLAWNSKDEQCYKCEQSKGHCSYSQNRFLGCLCSDGKVSSTDCRNGASNSALTMLLSLCLRLPLLLVLVAASHGDASGDTYDTSMLCQKPT
LOC_Os01g49614.1	227	LNSNQSALPSGGYGQVLNKG--FDLAWNSRKDEQCYCEQSQGHCSYSQNRVFLDCLCSDGKGVNQDCRNSGASNS-----
LOC_Os08g24630	1	-----
LOC_Os08g24770	236	VDHVGIDVNTVTSKNTTAMPT--LSLLAGVMRAEVSYDAAARMVAVTLRTLDGMSYSVEAADLRAAGLPQDAAVGFSAAATGDLVESHQLLSWSFNSSTDGSVSSTG-----
OsPSTOL1	1	-----
AtPR5K	262	-----
AtSNC4	361	RNATKQVD FLVISKNGASGEYPGCTKLAYEKAIKDGSVDVDCPVOMSSDGI PPC SSSIDLVNSTTVGOTHLNRSSI VP ISSVAGIFITFSLTWHETOSLTPAISNPFRENGMGRNPNR
LOC_Os01g49580	297	TCGNVISYYPFYFATKTKDINGSSNSYCGYPLAIDCDDGKPLQLNGTEKYKVNYINYSITNVSADLEVVDDSSGCPRVNDHNTVPIQISWFLFSGISVDYLVFRLCSFTTFAPKFA
LOC_Os01g49614.1	302	-----
LOC_Os08g24630	1	-----
LOC_Os08g24770	341	-----
OsPSTOL1	1	-----
AtPR5K	262	-----
AtSNC4	481	NGGNLISLIEFLNLAKNSTLSGLISLENVYVLRKKGLDVVKVVLNRLTETGYIVGTGKVMIOSTRLVLVDFKNOSTYKTVYKIKETIGNITDSDAIEDIKKFAVANVINKASVEPNS
LOC_Os01g49580	417	NFNPIACGSFINLTRSFVFPDELVPNGWSQLCEETFEVPLVKYQLMEMDSNGNAWNNSGYAQLVRQGFLSVNESRRPNCSCQESQGRCGYSQAGEFTGCLCPNGVRVSRLCDPDS
LOC_Os01g49614.1	302	-----
LOC_Os08g24630	1	-----
LOC_Os08g24770	341	-----
OsPSTOL1	1	-----
AtPR5K	262	-----
AtSNC4	601	DSFTTGOTTNVLRLQKFLPVYVELFQNEFVSPDPFPADETVEINAYIFGAGNGITTEFFPYTAARYKRNRLGREGVEPPYMLPVNPGGVLTLISTSSLPAQDPNPFI THDDVTEPP
LOC_Os01g49580	537	LAGEVMDKTRYSLMPFVSPNPLHANIIAGTSS-----VLLCLLSFACLFGLKKRYRR-----
LOC_Os01g49614.1	302	-----STLRYSSLKGRMKLYIIAGTSS-----VLLCLLSFPCLFSLKKYRHR-----
LOC_Os08g24630	1	-----
LOC_Os08g24770	341	-----SPLVSESKKRKITYIIAGTSS-----LLGISVVLAVFLVYKHKHCLL-----
OsPSTOL1	7	-----
AtPR5K	304	-----ASKNAPRIESFLQKQETSNPKRYTLSEVKRMT TSFAHKLGRGGGTIVYKGSLED GRE TAVKMLKDK
AtSNC4	721	LPPVIAKSPSTLTGTPSTIAKPLRNFLKIVIRIVSWSVAGVVLFLVLLTLVFCFHRKRETRLRQQLKALIPLEHYTYAQVKRMT TSBAEVLGCGGTIVYKGRLEDS GRV VAVKMLKDK
LOC_Os01g49580	593	-----ISKGTPRIESFLQNGTTLHPKRYTYTEVKRMT TSBAEKLGGGTGAVYRGNLSD CRQ VAVKMLKDK
LOC_Os01g49614.1	346	-----ISKGTPRIESFLQNGTTLHPKRYTYTEVKRMT TSBAEKLGGGTGAVYRGNLSD CRQ VAVKMLKDK
LOC_Os08g24630	1	-----TSBAHTLGGGTGAVYRGNLSD-----CSE LAVKMLKDK
LOC_Os08g24770	385	-----PWQRSTAPRLHSLRS-QLKSYTYSEVRKMT TSSTHTLGGGTIVYKGSLED GST TAVKMLKDK
OsPSTOL1	74	GDCERFINEVASHGSHNNVTLGLFSLQGSKRALLIYEYIPNGSLDRYSFG--DSSVQGDNTLSMDRLNNTIVGIARGLEYLHCHNIRIVHFDIKPONTLLAQDFCPKISDFGLAKLC
AtPR5K	367	GNCEEFINEVASHGSHNNVTLGLFCEYKKNRAIYIEYIPNGSLDKYISAN-----MSTKMBERLMDVAGISRGLEYLHNRQVTRIVHFDIKPONTLLMDENLCPKISDFGLAKLC
AtSNC4	840	GNCEEFINEVATMSHNNVTLGLFCSEGSKRALLIYEYIPNGSLDKFILG-----KTSVNMMDTALRLALGWAHGLEYLHLSKTRIVHFDIKPONTLLDSDFCPKISDFGLAKLC
LOC_Os01g49580	660	GDCERFINEVASHGSHNNVTLGLFCLHGSKRALLIYEYIPNGSLERYAFR--NNSE-GEHSTHEKLMDVAGISRGLEYLHNRQVTRIVHFDIKPONTLLDQDFCPKISDFGLAKLC
LOC_Os01g49614.1	413	GDCERFINEVASHGSHNNVTLGLFCLHRSKRALLIYEYIPNGSLERYAFR--NNSK-GEHSTHEKLMDVAGISRGLEYLHNRQVTRIVHFDIKPONTLLDQDFCPKISDFGLAKLC
LOC_Os08g24630	38	DAEDFINEVASHGSHNNVTLGLFCLHRSKRALLIYEYIPNGSLDKYAVGVVDTVGE-KSLWEKLEHLLVGIAGGLDYLRWNNRNVHFDIKPONTLLDQDFCPKISDFGLAKLC
LOC_Os08g24770	451	NDCEFINEVSSHGSHNNVTLGLFCLQHGSKRALLIYEYIPNGSLDKFAVGNDTMOQKEFLISWEKLEHLLVGIAGGLDYLRWNNRNVHFDIKPONTLLDQDFCPKISDFGLAKLC
OsPSTOL1	192	HIKESRIS SLNGIRGTGTYAPETVFSROY SAS SKSDVYSYGMVLL DMACAKN---INVSTGSSSRVFPQW WYNLDQFCCTPGE ---ISSQTDLV KKVVVGLWCICLV TD RFPM
AtPR5K	481	KNKESIS SLHMRGTGTYAPETVFSKNF AVS SKSDVYSYGMVLL DMACANIEK-VEYESNNGSVYFPEWYKDFEKGTEITRIFG-NSITEEEEKFAK VLVGLWCICLV TD RFPM
AtSNC4	954	EKKESIS SLDLTRGTGTYAPETVFSRVY ENV SKSDVYSYGMVLL DMACANEKANOACASNTSSVYFPEWYRDLES-CKSGRHIEDGINSDEDELA KKTLVGLWCICLV TD RFPM
LOC_Os01g49580	777	SNKESIS SLAGARTGTYAPETVYSKQF SAIS SKSDVYSYGMVLL DMVGAERN---IDANESSESHYFPCW WYEHLDYECISSE ---IDGETTELVR KVVVGLWCICLV TD RFPM
LOC_Os01g49614.1	530	ANKESIS SLAGARTGTYAPETVYSKQF SAIS SKSDVYSYGMVLL DMVGAERN---IEANESSESHYFPCW WYEHLDYECISSE ---IDGETTELVR KVVVGLWCICLV TD RFPM
LOC_Os08g24630	157	KKESKIS SLGGARTGTYAPETVWRH CAV TSKSDVYSYGMVLL DMVGAERN---TNASMQTVSRVFPW WYNLDNQFCGAATEGIDSRNTCISEVAKV TVT IGWCICLV TD RFPM
LOC_Os08g24770	571	KKESKIS SLGGARTGTYAPETVWRH CAV TSKSDVYSYGMVLL DMVGAERN---INATESGSRVFPW WYNLDNQFCGVPSSGIDG-SNSTSEVAKV TVT IGWCICLV TD RFPM
OsPSTOL1	304	REVLMEL SGNRD LP PFKGL-----
AtPR5K	599	IKVTEML EGNLEA QVPPNPLFSPEETVPD TLEDSDDTSTFFNP SHFERGTLLASEDVLQHGSRSS
AtSNC4	1073	NRVTEML EGSLEA QVPPRPVLQQIPISNLHESSILSEDVSVYTEG-----
LOC_Os01g49580	890	TRVTEML EGSTSG ELPPKVL LSW -----
LOC_Os01g49614.1	643	TRVTEML EGSTSG ELPPKVL LSW -----
LOC_Os08g24630	273	SEVIDM DRSMHE QLPPRMSCCGIDNPSIV-----
LOC_Os08g24770	686	SEVIDM DRSLTE QLPPRISCCGNYNESFG-----

Supplemental Figure 3. Alignment of OsPSTOL1 with most similar rice and *Arabidopsis* protein kinases.

The predicted OsPSTOL1 amino acid (AA) sequence was aligned with the most similar rice and *Arabidopsis* proteins. The conserved kinase domain is underlined (thick black line). The predicted ATP-binding site is indicated by a dotted line and the predicted Serine/Threonine protein kinase active site is indicated by a broken line. OsPSTOL1 and the two most similar rice protein kinases (see above) show highest sequence similarity with the *Arabidopsis* proteins AtPR5K and AtSNC4. A transmembrane domain absent from OsPSTOL1 is underlined (thin line). Other domains absent from OsPSTOL1 but present in the aligned proteins are additionally indicated: Thaumatin domain in AtPR5K (thin double underline), glycerophosphodiester phosphodiesterase domain repeat 1 in AtSNC4 (thin underline, italics), glycerophosphodiester phosphodiesterase domain repeat 2 of AtSNC4 (thin double underline, italics). Black shading: Identical AA; gray shading: similar AA.

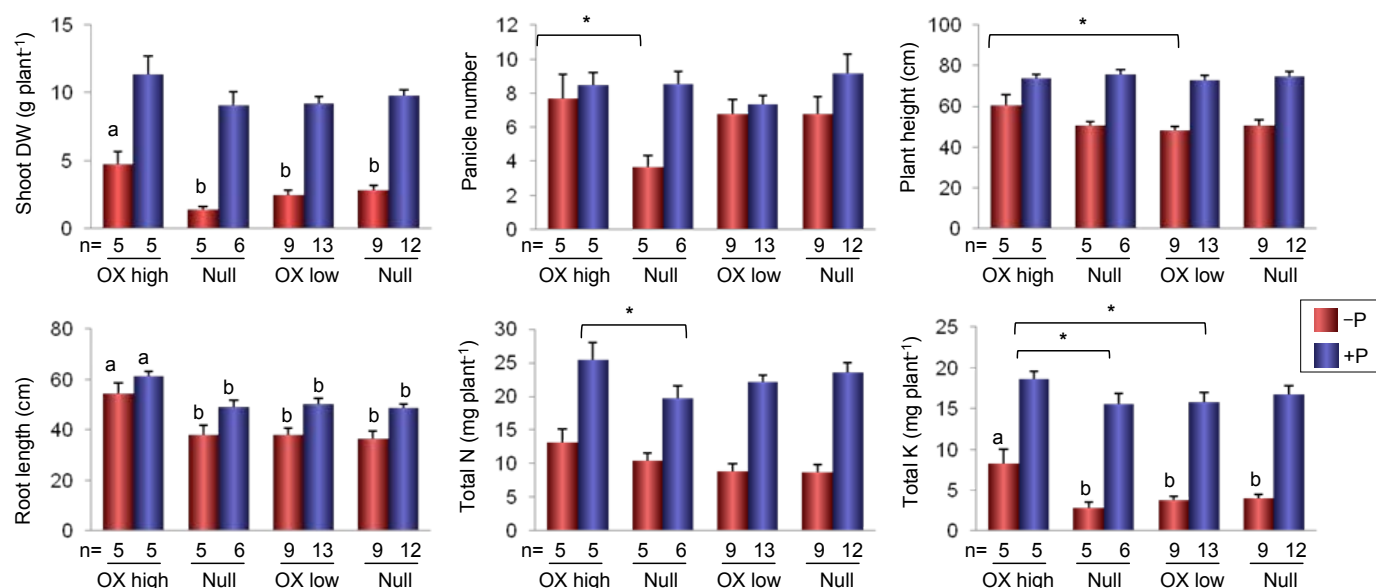


Supplemental Figure 4 Southern blot analyses of 35S::OsPSTOL1 transformants. **a**, Genomic DNA of Nipponbare 35S::OsPSTOL1 primary transformants (T0) was digested with *Sac*I. **b**, Genomic DNA of IR64 35S::OsPSTOL1 T0 plants was digested with *Xba*I (left) and *Sac*I (right). DNA was blotted on membranes and hybridized with a DIG-labeled hygromycin phosphotransferase gene probe. Independent transgenic lines selected for further evaluations are marked with an asterisk.



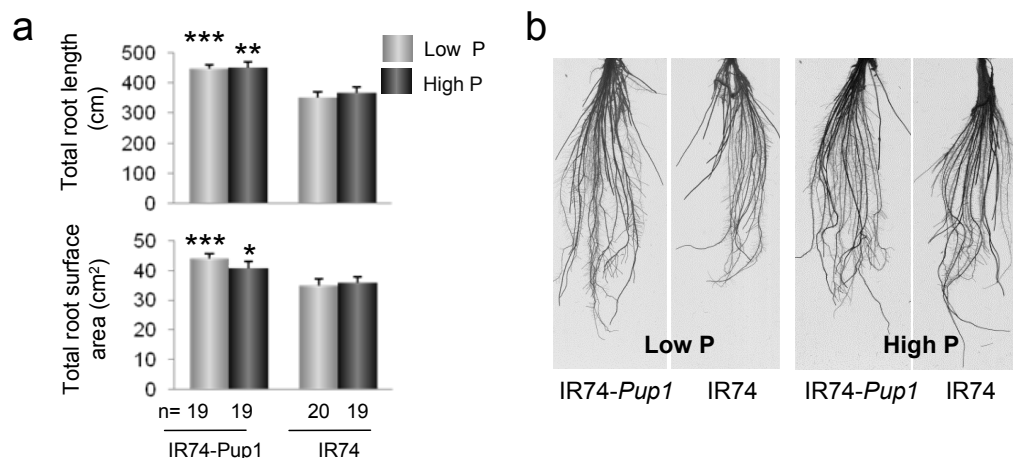
Supplemental Figure 5. Transgene expression in IR64 35S::OsPSTOL1 plants.

Expression of the *OsPSTOL1* transgene was analyzed by semi-quantitative RT-PCR using leaf RNA samples from individual transgenic plants (+) and Null segregants (-) of the five indicated independent lines. Plants were grown in P-deficient soil. The PCR cycle number is indicated. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was included as a control. H₂O, water control.



Supplemental Figure 6. Phenotypic data and nutrient content in IR64 35S::OsPSTOL1.

Additional phenotypic data of 35S::OsPSTOL1 and Nulls with high (OX high) and low (OX low) transgene expression grown in P-deficient soil under stress (-P/dry down) and control (+P/well-watered,) conditions. See main text for details. Significance is indicated by different letters (ANOVA and Tukey's HSD test) and asterisks (paired t test p < 0.05).



Supplemental Figure 7. Root phenotype of IR74-Pup1 NILs in hydroponics.

a, Total root length and surface area of IR74 near-isogenic lines with (IR74-Pup1) and sister lines without (IR74) the Kasalath *Pup1* locus grown for 21 days in hydroponics under high-P (100 μM) and low-P (10 μM) conditions. Roots were scanned using WinRhizo. **b**, Representative root scans of analyzed plants. Error bars indicate standard error. The data were analyzed by paired t test (95%) and significance levels are indicated as * 0.05 > p ≥ 0.01, ** 0.01 > p ≥ 0.001, *** 0.001 > p; no asterisk: not significant.

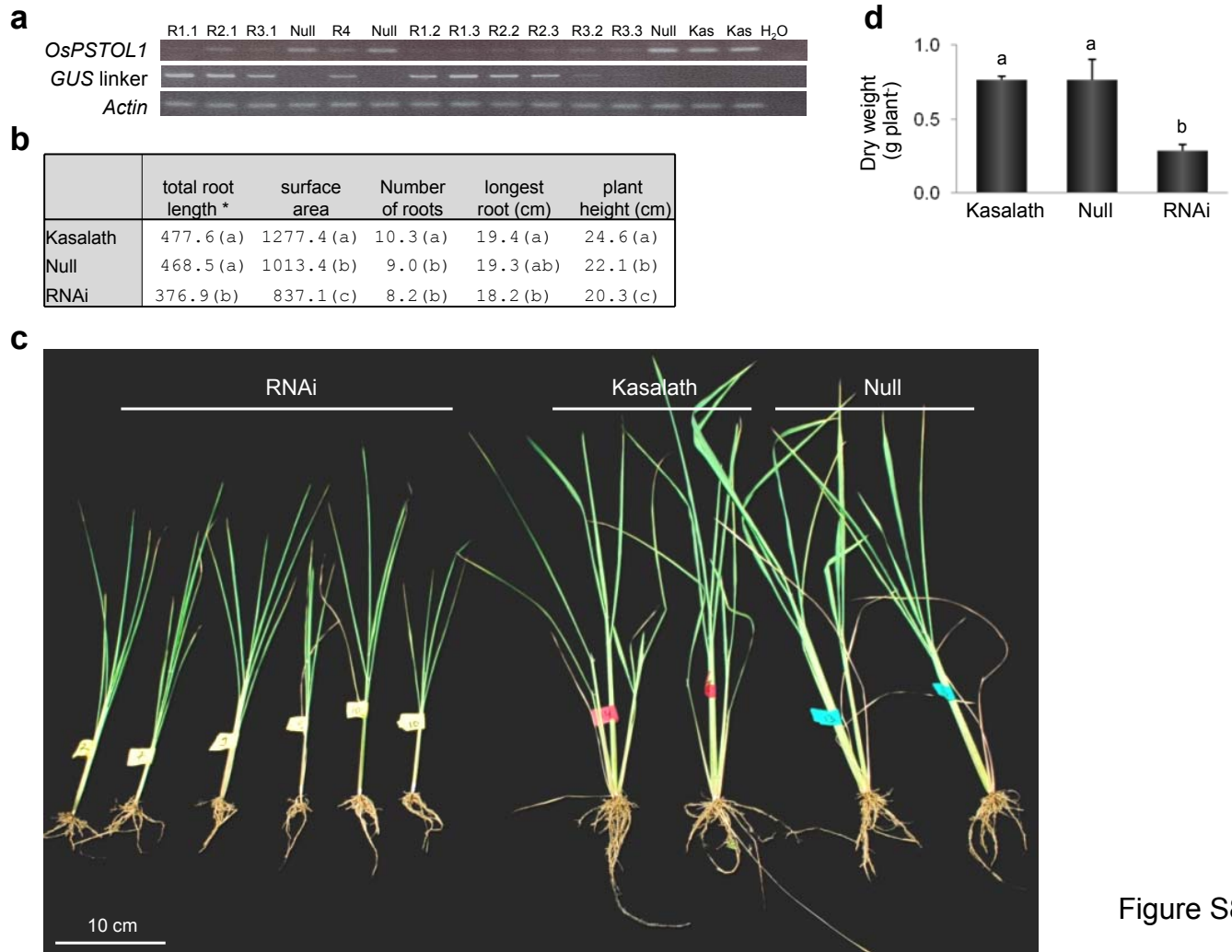


Figure S8

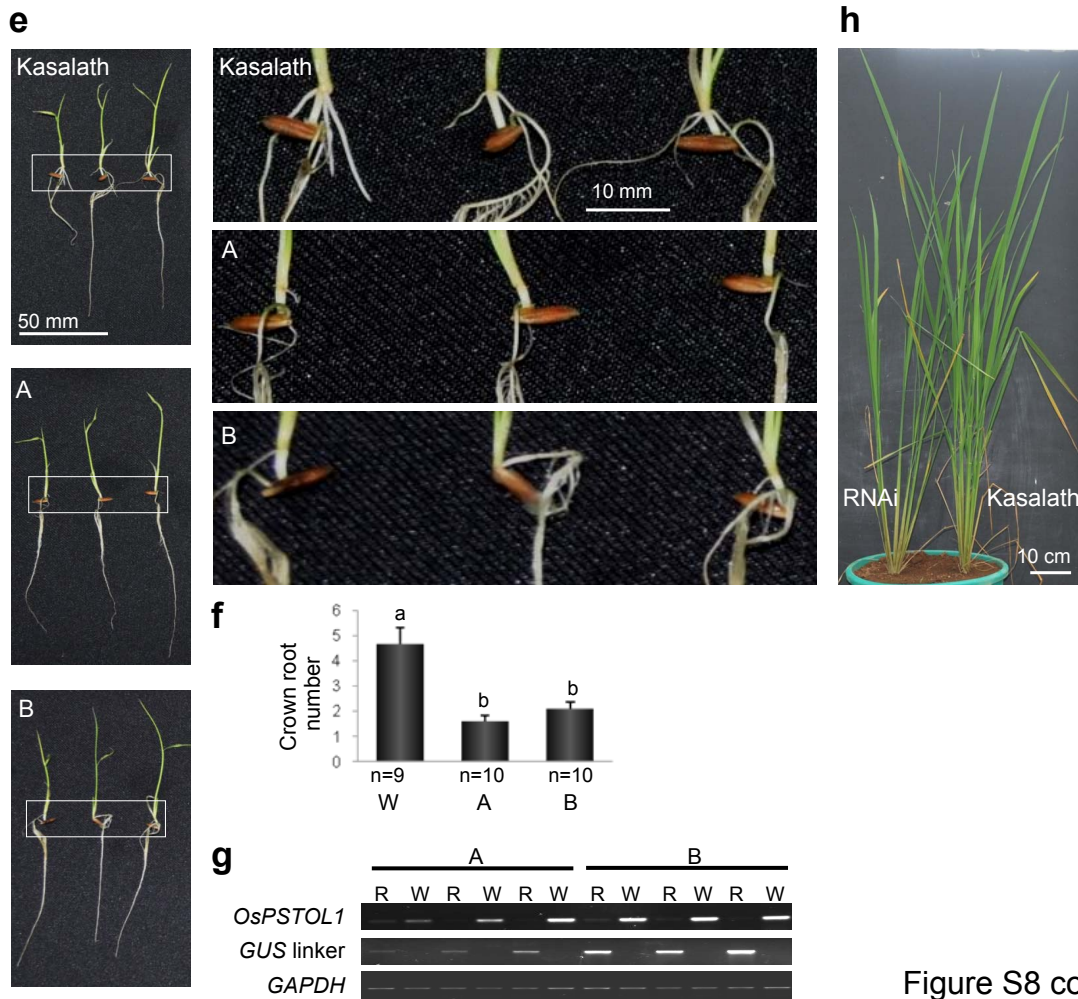
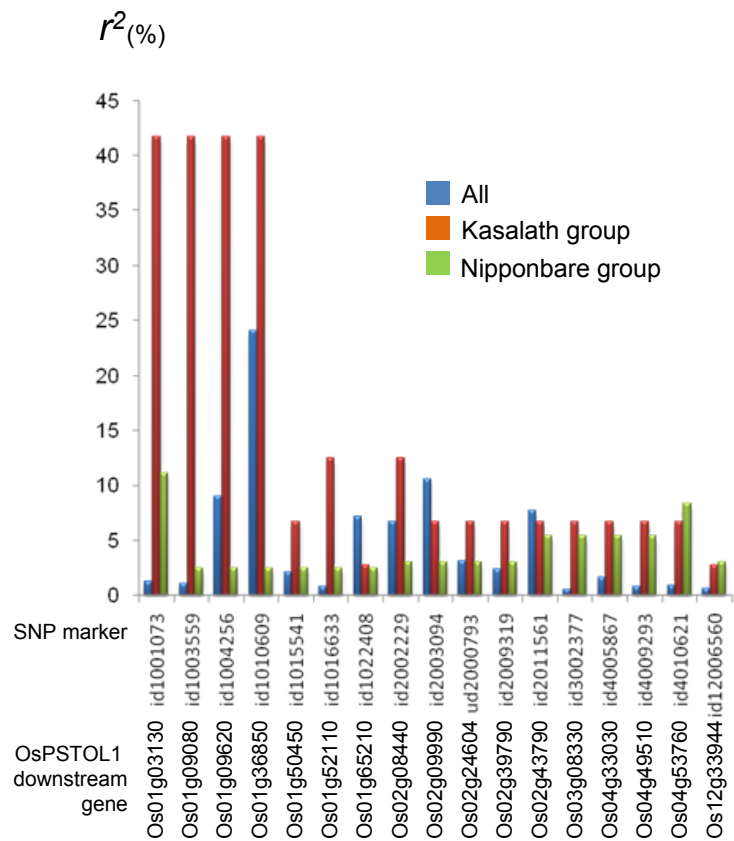


Figure S8 continued

Supplemental Figure 8. *OsPSTOL1* RNAi transformation experiment.

a, The rice variety Kasalath was transformed with an RNAi construct and knock-down of *OsPSTOL1* was analyzed by semi-quantitative RT-PCR of root RNA samples from representative plants of four transgenic lines (R1-R4; T3 generation) in comparison to Null segregants and Kasalath wild type controls. **b**, Plants were grown in -P hydroponics for 12 days and roots were scanned to obtain total root length and surface area. The other data were obtained manually. *Different letters indicate significant difference (Tukey HSD All-Pairwise Comparisons; $\alpha = 0.05$). *roots with diameter $2.000 < L \leq 5.000$. **c**, Representative RNAi plants, Kasalath wild type and Null segregants grown in P-deficient soil for 60 days. **d**, Dry weight of Kasalath, Null segregants, and RNAi plants at 60 days after sowing. Significance is indicated by different letters (ANOVA and Tukey's HSD test). **e**, Crown root emergence in representative RNAi plants from two independent lines (A, B) and Kasalath grown in hydroponics culture solution at 8 days after germination (DAG). **f**, Crown root number at 8 DAG. Significance is indicated by different letters (ANOVA and Tukey's HSD test). **g**, *OsPSTOL1* expression was determined by semi-quantitative RT-PCR of representative RNAi plants (R) and Kasalath wild type controls (W). **h**, Representative RNAi plant (R) and Kasalath (W) at 84 DAG grown in P-deficient soil.



Supplemental Figure 9. Allelic association between *OsPSTOL1* and putative downstream genes.

Eight rice accessions with the *OsPSTOL1* gene (Kasalath group) and fourteen accessions without *OsPSTOL1* (Nipponbare group) were used to analyze allelic association between *OsPSTOL1* and SNP markers located within 1 Mb distance to the putative *OsPSTOL1* downstream genes (see Supplemental Table 2 and Figure 4 for details on the genes; for some genes SNP markers were not available).