



Figure S1. DN-PRK2a overexpression reduced the germination rate of pollen grain in Arabidopsis.

(A) Schematic diagram of the truncated AtPRK2a defined as DN-PRK2a. The signal peptide (SP), the transmembrane domain (TM), and the cytoplasm kinase domain (KD) were shown. (B) *In vitro* germination assay showed the defected pollen germination in DN-PRK2a OX lines. 8-3, 23-12, and 34-4 are three independent lines overexpressed DN-PRK2a.

Figure S2. Alignment showing the variable C-terminal region of AtRopGEFs.

The alignment was produced by MUSCLE3.6 using the full amino acid sequences of AtRopGEFs and presented by GeneDoc. The red arrows indicated the S460 and S480 sites of RopGEF1, and the blue arrow showed the S510 site in RopGEF9.

Chang_Fig. S3

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      *           20           *           40           *           60           *           80           *           100           *           120
ROPGEF9 : ADSLARKQCTGEETSDGGKI---ATETDSASAGSSNYSGEEIEKLES---QNSSKTTLLDFI---GWSDNS-----SKGQSEKPPKSPRMTPK--KLSYL-EKLENLNGFRSPKDRH : 100
ROPGEF8 : TDTLALKQTLLEAETS DGGRTT-----ETDSESAGSSNGEEAEKHDPHKTLLDFM---GWNDNS-----SKGGDKPTKSPNLTPKK--LSYL-EKLENLNGFRSPKDRH : 95
ROPGEF12 : ADRAGN-----KRNTPLEAEEET---LVGSMTLSDFM---GWDFDQ---AANALESK-----DLPDDPLIKEKLSVVTTKKTSYL---ETLGGVRSPTARH : 81
Araly1.874 : TDSLAL---KQTLLEAETS DGGRTTETDSE--SAGSSNGEETEKLDPH-YSKT---LLDFM---GWNDNS-----SKGGDKPTKSPNITPKK--LSYLEKLENL-NGFRSPKDRH : 96
Araly1.883 : ADSLARKQCTGEETSDGGKGL---ATETDSARSSNYSGEETEKLESQ---NSSKTTLLDFI---GWSDNS-----SKGQSEKPPKSPKMTPK--KFSYL-EKLENLNGFRSPKDRH : 98
Araly1.477 : ADRAGN-----KRNTPMESEDET---LVGSMTLSDFM---GWDFDQ---AGNADLDSK-----DLSDDPLVKEKLSVVTTKKTSYL---ETLGGVRSPTARH : 81
Bra013249 : TDSLALKQALLAEETPDGGR-----TET--DSESPGSSNSGEETEKLDRQHSKT--LDFM---GWS-----DKPTTTPSVTPPK--LSYLEKLENF-NGFRSPKDRNL : 89
Bra015068 : TDSLAL---KETLLAEETS DGGR-TETDSE--SAGSSNGEETEKLDPH-YSKT---LLDFM---GWSDNS---SKSSD-KPTKSP-----GLTPK-KFSYLENL---NGFRSPKARH : 92
Bra036671 : TDSLALQ--KQALLAEADAGR---TSETDSE--SAGSSHSGEETEKLDPH-YSKT---LLDFM---GWSDNS-----SKGGDKPTKSPSLTPKK--LSYLEKLENF-NGFRSPKDRH : 94
Bra003536 : ADRAGN-----KRSTPMEPEEETLVGSMT--LDFM---GWDFDQ---GNEDL-DSKKD-----MSDDDLVKEKLNVTATKTSYL---ETLGGVRSPTARH : 81
Thhalv1001 : ADRAGN-----KRSTPRETEDET---LVGSMTLSDFM---GWDLDQ---SKK-----DTSDDLVLVKEKLSVVTTKKTSYL---ETLGGVRSPTARH : 74
POPTR_0001 : AVYVTRNPSHAGHKRNTLKEAPQV-----PASPREGMEKNS EDTNASMT--LDFM---GWGSEQ---NESAA-KKDPFGSDELLKDDRYKQKLTN---ISTNR-KPSYL---DNNGALRSPTARH : 106
POPTR_0003 : ADHVARNPSHAGHTSNTLKE---APQVLVSPKEGMEKNS EDTNASMT--LDFM---GWSQEQNDTAKKDPFGSDEL-----LKDDDKCTQKLANLSTNRKPSYL---DNNGASRSPTARH : 106
POPTR_0018 : ADSLAQ-----SPQLESN-----QKPEEELDNL TSAETPTSKTLSDFM---GWKVDQGEANMNKTSTDNM-----ENCQDKIKDKTDTTPKK--FSYL-EKLENL-SGLRSPTARQF : 96
Cucsa.0620 : AVYVTQNPQSASGKKNPSRE---TPVSSGT---DKFPPGEETQNA AETPTSMT---LDFM---NMGPI---ADSEA-KKESPPANS DNLPTGEVVKHVHKLQNI VTNKLVSYL---ENNGCLRSPARH : 113
Cucsa.3592 : ADGLTQ---NPSQIATRRK-----PTSE--PPMEKLEELNNGPETPASMT---LDFM---GWGDQ---NETEM-KKESFGNSDDLNL-DSDLKQGNKAGNI VTNK--KVSYL---ENNSAVRSPTARH : 104
Vv.1782614 : ADSLAQ---NPSLAQTGR---KPSAVCLPLTGSTREETEKNPTET---QTPTSMTLSDFM---GWHLMG-----DTEPGKLNK---VAIHKKSNSYL-DKLENL-GCLRSPTSRH : 93
Vv.1782737 : SSL-----DPEPLNF---PNPKEEIEKLST---GTPQSMTLSDFM---GWNLEK---EDGEKKKSGGNSEEL---SKNDEKPMTKIANIITNKKVSYL-EKLENL-GCLRSPARH : 95
      d                                     L DF6          gW                                     SY6          g SP Rh

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ROPGEF9 : - : -
ROPGEF8 : - : -
ROPGEF12 : - : -
Araly1.874 : - : -
Araly1.883 : - : -
Araly1.477 : - : -
Bra013249 : S : 90
Bra015068 : - : -
Bra036671 : - : -
Bra003536 : - : -
Thhalv1001 : - : -
POPTR_0001 : - : -
POPTR_0003 : - : -
POPTR_0018 : L : 97
Cucsa.0620 : - : -
Cucsa.3592 : - : -
Vv.1782614 : - : -
Vv.1782737 : - : -

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Figure S3. Alignment showed the C-terminal regions of the RopGEF9 Orthologous Genes.

The alignment was produced using full amino acid sequences. The read arrow indicated the conserved S510 site in RopGEF9.

Chang_Fig. S4

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LePRK1 : -----MSVAYRYSNHNRHHHHHLLLFVLLQVI-----VPIKSDNNEAE-I-----LRFKSKLQKNDAT---AN-----NTKVSFCDKKTDRFPNNDVI-GE---NGF---VFG : 88
LePRK2 : -----MSSQKNYKKNHVLFLVMIMCCLAFV-----TEANLSEPE-V-----LVEFKELLNTSLL-----DSS-----KGTNPNDDNNK---MFEVQ-CD---NNNNNVLQAL : 82
LePRK3 : -----MAAHVLIIVLVFFSITSC-----VSIGDDQ-V-----LVEFKELLNTSLL-----DSS-----KGTNPNDDNNK---MFEVQ-CD---NNNNNVLQAL : 73
AT5G35390 : -----MPPMQARTLSVYVNMVPLVCLLFF-----STPHGLSDSE-A-----LTKFKNSLVVQEN---ALAS---NAKSPBCT---MSEVLCN---GGS---VWR : 78
AT2G07040 : -----MESKCLMFVSIIVSVMFVNVG-----VSETE-T-----LTKFKNSLVVGRAN---ALEA---NRRNPPCK---MTEVLCN---RGF---VWL : 68
AT3G42880 : -----MTAVLFLFCFLIC-----FSFTPSLQNVSESE-P-----LVRFKRSVNIKGD---LNS---RTGTDENCKG---MFEIY-CQ---KQOT---VSG : 71
AT3G20190 : -----MLTWETPVMLASNTASTKKLAFITTFLLIIVLCP-----VTMVMVQPQADVLPLPSADDC-----LRFKMDTVNASFI---SS---DPSISECKRNSEN---MFEVLCV---TGN---VWL : 99
AT1G50610 : MRNWEDPFTLACNTALKKNLPSCIFLIFISVLCPVAMS-----QVVVPSDSDAC-----LRFKMDTVANGSEF---RS---DPLSSECGQNTAN---MFEVLCV---NY---VWL : 93
AT5G20690 : -----MAAVLNPGFFLLILLFSFI-----SPSLQVYSESE-P-----LVRFKNSVITKGD---LNS---REGTDESCKG---MFEIY-CQ---KGLT---VSG : 76
AT4G31250 : -----MTRDDKFPVYVLLLVLLFV-----SPIYGDGAD-A-----LTKFKSSLVNASSL---CG---DSGPEKSGDKGSDKVKVMS---NGS---VFA : 79
AT1G10850 : -----MASSSSSSSSTVSVVFAFTVFC-----LVTPARSSDVEAE-----LTLKSSLDPSNSI---S---RGTDLN---MFEVRCM---NGR---VSK : 75
AT1G60630 : -----MISSSSCMFFLVFAFFLISPVR-----SDVE-A-----LTLKSSLDPSNSI---S---RGTDLN---MFEVRCM---KGR---VSK : 66
AT1G72460 : -----MAVAWLWPTVLSLTALSA-----NSITSESE-S-----LTKFKNSNTKSL---DS---TPESECGASQR---MTEVLCN---KNS---VFG : 70
AT3G50230 : -----MTLFFFYSLLFLLLLR-----STASLPATNYFDSFLPSDAVA-----LTSFKSADLKNL---LYSL---TEPYDYQ---MREGVD-CQ---QDR---VWR : 79
AT5G43020 : -----MLLRNCLPISGLFLFCFLFTIVASSSSSSNHTKRVFHSRDV-----LTFKRSKADLNKI---NTSHFYQ---MREGVD-CQ---GNR---VWR : 80
AT5G67200 : -----MTLNFDYLPFFFFIFILRV-----SAGAEPNYFNSLPSDAVA-----LTSFKSTADLNKI---LYSL---TERYDYQ---MREGVD-CQ---QDR---VWR : 77
LOC Os02g0 : -----MARRPPGLLVAIAAAALVAVCLCGPASAAAAAGGAGGGAGPEGD-V-----LAPRPTRCGPDGAGPPGPRAT---GTPAVYCRKASQ---MFEVSCV---GNS---VQL : 98
LOC Os02g5 : -----MPTPPSPCLFLLFLFFMSSHSH-----LGAASDAD-A-----LALAKSADRSDR---P---RRDTARLCS---MFEVRCQ---QPPRRR---VTK : 79
LOC Os04g5 : -----MRHRLLLLLVLAAYAAAGDVASSYSQPTLPAVPPQVAVRPPPAVAVGGGSHSQPTFFPRAPVRAVPPATLVSTATPGAL---LALAKADPSAHL---R---PLAVSECS---HPAVS-CV---ADGQ---VTR : 120
LOC Os06g0 : -----MAQPLLLLAIAAAAVAVV-----VAQVQNMADAE-A-----LQKRSQNTNMSL---SS---LITNTDGDKSECGPSGHE---MFEVSCV---RKG---VTL : 82
LOC Os06g4 : -----MAGVAARVLLQLAAALALAAA-----AAAGPEAA-T-----LAPRAGRPHGAPPEPSQ---ATTTPECGAGAGTGVSLYVTV-CV---QRTQ---VRL : 88
LOC Os08g4 : -----MAGAPCLRVLAVSSCLLADLA-----ARLLAAAPAEEDA-----LTKLAGVDGGGA---LDT---AAGTSGDGTSA---MFEVSCV---KGS---VLG : 82
LOC Os09g2 : -----MEKSKVVFAGRVLAIVALLAC-----CCMAAAAQGGGARVRE---S-----LIGELTEAGGDKL-RARGIG---DASVPEGDNRVT---MFEVCGNAPAGDGR---VTA : 94
LOC Os11g4 : -----MVTLLAFRLSTFLLLAAGATAVDPDGAAPDTAAAPDEAAA-----LRLKASLDPTNA---LEA---SPSSPSCDETHR---MFEVSCV---NGV---VIG : 90

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LePRK1 : QLENKGLSCTI-DVDAKDPNFTLSVMNMFECPIEN-I--SKAGLATAVFNKFKSCQDNSFFEGHWLKKHIGNNQISGKTFVSVF-GQLEPKTEIRENKKEGCOIPDF--NQERIDMIFANNSSCGEFTHGLA--SLKPSAEGN--NL--DGPF : 240
LePRK2 : NLENKGLSCTI-DLDIKENLRTISVFKKFCGPHI-L-NKPTLKSAMFNKFKSCGPHIDONIEFGNSKKHIANNEFTGELPEIF-----GDMENL-----RELNQNKKFEFTIPESYS--HLYLPAIDGN-DGLG-EPP : 213
LePRK3 : LLGGKLSGSL-DVDVTVLQGLRVNLSNMFSGSIPF-F-FRIGALKSLFDGNOFSGDIPDFDFSKASWKTWFSNFKSGLPESL-ASLKYLEHHEHNEETGCTIPSL-SQPNATINLSNKKQGLIPQSIS--KFGSNPQCN-PDLG-EQI : 226
AT5G35390 : QMENELSGSI-DIEALSGTSLRTSFMNKKFCGPHI-F-FKALASLYLSNNOGCDIPGDAFEGGWKKKHIAQNKFTGQIPSSV-AKPKLELRDGDGQCTGIEEF--RHQHLLNLNSNAITGIPESLS--MTDPKVEGN-KGY-EKPL : 230
AT2G07040 : RLENELSGSI-DIEALMGNSLRSEFINKFKGPEPE-F-KKVALASLYLSNNOGCDLEPKDAFDGWWKKKHIEGNNFIEIETSI-VKSKLELRDGDGQCTGIEEF--RRHFNMLNLSNAIACQIENSFS--TMDPKLEGN-KGLG-EKPL : 220
AT3G42880 : HVFRGLSGTI-NIEDKDPNLTIRLDNLLSGPHI-F-FKPLGLSLISNNSFSGEADDFKTEPQKRVLDNNRLEKTFEASL-MQLAGEEHVGQGGCTGIEEIPPLTDGNKVKSLDLSNNDIEGIEFITISDRKNLEMKEGN-QRDG-EPP : 228
AT3G20190 : QLEGVGLCKL-DLEPAALKNLRTSFMNKKFNGSIPF-V--KNFALSLSLYSNNRFTCEPADAFDGHHLKLLANNAFRGTFPSSL-AYLPMLELRINGCQHGIEIYF--KQDKKLASFEKNDIEGIPESLS--NMDPVSSEGN-KNLG-EPP : 252
AT1G50610 : QLEGVGLCKL-NLDPLVPMKLRISFMNKNFNGMPQ-V--KRFALSLSLYSNNRFTCEPADAFDGLKLLANNAFRGTFPSSL-ASLPMLELRINGCQHGQIQPF--QQDKKLASFEKNDIEGIPESLS--NMDPVSSEGN-KGLG-DAP : 246
AT5G20690 : HVFRGLSGTI-TVDDKDPNLTIRLDNLLSGPHI-F-FKPLGLSLISNNSFSGEADDFKTEPQKRVLDNNRLEKTFEASL-MQLAGEEHVGQGGCTGIEEIPPLTDGNKVKSLDLSNNDIEGIEFITISDRKNLEMKEGN-QRDG-EPP : 228
AT4G31250 : RLENELSGSL-DVQALGSRGLKLSFMRHFKGKPRGI--DGVSIAHLHYAHNOFTCEIDGDLFSGKALKLVHUEGNRFSGETPESL-GKLPKTEHNEEDMTEGTPAF--KQKNLVTVNVAHQEGRIPLTLG--LMNITFSEGN-KGLG-EAP : 233
AT1G10850 : VLEYNLKCSL-NEKSNQDQLRVLSFKANSLSGSEFN-L-SGVNLSLYLNDNNSFSGEPESLTS-IHRKTHFVSRNRFSGTFPSSL-LRLSRYTYNVEDLITGSIPL--NQTSRYFNVSNNKSGOIBLTRALKQFDESSTGN-VALG-DQI : 229
AT1G60630 : VLENELSGSL-NGKSNQDQLRVLSFKANSLSGSEFN-L-SGVNLSLYLNDNNSFSGEPESLTS-IHRKTHFVSRNRFSGTFPSSL-LRLSRYTYNVEDLITGSIPL--NQATRFVFNVSNNKSGOIBLTRALKRNFESSTGN-VALG-DQI : 220
AT1G72460 : QLEQVGLSKV-DVAPKDPNLTIRLDNLLSGPHI-F-NRVALASLYLSNNSFSGEADDFKTEPQKRVLDNNRLEKTFEASL-MQLAGEEHVGQGGCTGIEEIPPLTDGNKVKSLDLSNNDIEGIEFITISDRKNLEMKEGN-QRDG-EPP : 228
AT3G50230 : ILDQVLRGTF-SPEVSRDQLRVLSLENNISGSEPD-L-SGVNLSLTLKNSFSGTSSSILS-IHRTTELDNSFNSGELPESG-NALSRSSNNEFRNLNGLPPL--NLSSISFNVSNNKTEIPLVPLTKLLRNFASSSGLG-EI : 233
AT5G43020 : VEDHYLGRRI-IPDSANKDQLRVLSLENTSLGPHI-F-SGVNLSLTLKNSFSGTSSSILS-IHRTTELDNSFNSGELPESG-VLSDRILYRDSNRNCGVPEL--NQSTHTFNVSNNTCAVRYVPLTKLLRNFASSSGLG-EI : 234
AT5G67200 : VLSGVLRGYF-SSATLSRQDLRVLSLENSLFGPHI-F-SHVNLSLFLSRNRFSGAFPSSILS-IHRMLISLSHNNSFSGTFPSSL-NAIDRYSNNDNFRNGLPPL--NQSTHTFNVSNNTCAVRYVPTLSRFDASSRSGN-PGLG-EI : 231
LOC Os02g0 : QLEKGLSCAAPDLGLAAEPGLRVLSLANALGAFENF--SAJAMLALMYLNRNRFSGFPDGTHTVRLKRLHSSNLSGTFPSSI--TSFRVYDVSNNSGTFEGLIS--RFNASMSEGN-EYLG-EKPL : 251
LOC Os02g5 : VLENELKCVI-TATLAPSELRVLSLANALGAFENF--ALPAAVNLKALMYLNRNRFSGFPDGTHTVRLKRLHSSNLSGTFPSSI--TTLPRTSLDLNRLNGLPPL--PQPTDRLLNVSANRSGEISVSLVA-TKFNASSLAN-ADLG-EPP : 234
LOC Os04g5 : VLESSENGTF-APATLSRVELRVLSLANALGAFENF--SPENLALMYLNRNRFSGFPDGTHTVRLKRLHSSNLSGTFPSSI--TTLPRTSLDLNRLNGLPPL--PQPTDRLLNVSANRSGEISVSLVA-TKFNASSLAN-ADLG-EPP : 234
LOC Os06g0 : RLVNRLGCVI-DVGAUVGHFNRSVAFAGNSGPHI-F--DRITKSMFSDNOFTCVDDDFFSKSHLKKLWHDHNSLGPAST--AQATSLVHHEAHNAHSGELPPL--PPPALVFDVSNNSGTFEGLIS--RFNASMSEGN-EYLYVPTS : 276
LOC Os06g4 : RLEYGLGCPADMAPAAVRLRALSANLNLGPHI-F--SMPLALMYLNRNRFSGFPDGTHTVRLKRLHSSNLSGTFPSSI--TTLPRTSLDLNRLNGLPPL--PQPTDRLLNVSANRSGEISVSLVA-TKFNASSLAN-ADLG-EPP : 241
LOC Os08g4 : QLEKGLSGEL-DLAPKSLRTRTFMDFACAVED-V-KGGLLHLYLNRNRFSGEPADAFAGGWKKVSRNRFSGTFEASL-AAVPRLDVQNDNKTGKIPDF--PKDKLVVDVSNNSGTFEASL--SIDPQMEGN-KKLG-EAP : 235
LOC Os09g2 : VLRRKGLDCTINAASLCAAPALVSLGELALRCDPAAT--SGCARLHLYLNRNRFSGEPADAFAGGWKKVSRNRFSGTFEASL-SKL-GVRFVNDNRCALPPL--ELSRFEHFS--ANNHNTGPDAG--DFGRDSSNSDGLG-EAP : 248
LOC Os11g4 : RLRNRLSGTF-DFAALSRPGLHSNLRNRFSGEPASL--AARSLHLYLNRNRFSGEPADAFAGGWKKVSRNRFSGTFEASL-AAVPRLDVQNDNKTGKIPDF--PKDKLVVDVSNNSGTFEASL--SIDPQMEGN-KKLG-EAP : 246

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LePRK1 : ---SKT-----*-----340-----*-----360-----*-----380-----*-----400-----*-----420-----*-----440-----*-----460-----*-----480-----*
LePRK2 : A--KSN-----KEDEKKEKESSSSSSSGKALIVLWVVVVVLCIIVFVL-----INRRNNKHQEVVLGGSSLT-----SSSPTSQDKLIPQSHDLHNKMEQGSSAASTPDRACNDGGKRAE-----: 321
LePRK3 : G--REK-----AVIYEKESSESGTKWIVLWVLLLVAVI-----LFSKRRKDDQPEFKLEKENLD-----EAVVHLLNKRSMSTRSMSSRRKGRSRG-----: 310
AT5G35390 : E--TED-----SPYIEHPQSEARPKSSSRGPIITAVAAALTIITLGVIFLL-----NRSYANKKPRLAVETGFPSSL-----QKKTGIREADQSRDRKKADHRKSGTTRKMGAAAG-----: 334
AT2G07040 : D--TKS-----SPYHNSPEKSKTKTSSKFLYLAAVAALAASTIITLGVIFL-----TRRRKKQPLLSAEPGFPSSL-----QMRQAQIESERGGQGSYHQNRAAKM-----: 315
AT3G42880 : N--IED-----EKPSSSTGSGNEKNNTAKAFMVLLFLITIFVVAIT-----TRWKKRQPEFRMLGKDHLSDDQESVEVVRVPSIKKPIDSSKKRSNAEGSSKGGSSHNGKAGGGGPG-----: 335
AT3G20190 : ---SPS-----SDSG-----SSPDLSPSTTEKNKNSFFLAIIVIVIGIITMIISLVCI-----LHTRRRKLSLAYSAPAGQDR-----EKYNDQSTDKDKAADSVTYSYTRRGAV-----: 349
AT1G50610 : ---SPS-----SSSPGVVPPVPS-----PVDPKSTSPPTGKAGSFYTAIITIVIGIITVIIALVFCF-----VQSRRRNFLSAYPSSAGKER-----IESYNYHQSTNNKNKPAESVNHTRRGSMP-----: 352
AT5G20690 : D--VGE-----NIELNDPQEGQPPSKSPSSVPETSNAKANAVISISLLLFFIIVGI-----KRRNKKNPDFRMLANNREN-----DVVEVRISESSSTAKRSDTSRRKGGHSDDGSTKGVSNIGKGGNGGGGALG-----: 361
AT4G31250 : ---LPR-----YTRPPFTVFLIALTILAVVLLTVFELSVCIL-----SRROGQDQIQNHGVGFFA-----GOVYQPEQQQHSKSSQDSKVYRKLANETVQRDSTKTSGAISVGLLSPDEDKR-----: 343
AT1G10850 : G--SPG-----ISPAPSAKPTPIPKSKSKAKLIGTAGSVAGGVLVILLLTLIV-----CWRKRNRNAPREDRKGKGI-----AEAEGATTAETERDIERKDRGFSWERGEE-----: 329
AT1G60630 : F--NSN-----SAKPAIPVAKTRSRKRLIGLISGSGCGGLLILLLTFLICL-----LWRRRKRKSRREERRSKRVA-----ESKEAKTAEETEGSDQMKRFRSWEKESSE-----: 325
AT1G72460 : S--TPP-----QPKN-----STASITIEGTMKDANKSKYFAFSTLGVLLIVLWLSLAFR-----KKKKRRRKKARTSEQDMS-----DDQIQVTVEGSNSSRQSRSSRSGELNKGV-----: 323
AT3G50230 : N--RSGLHSSSPFFSGPKNTSSTSSSSSEAPVIQSEQ-----NGEAMIVPPVVKVKNKWLGLFTIGLALSLVGLCLVVFSLFKRNEEDYDDVIIITQPKREE-----ENKEIKIQFTTAPSKKRI-----: 356
AT5G43020 : H--KENPRAKFFTPVTAAPSFKMVLGQIA-----QIGGARLRSPQNKHSRFFVLGFTAGLALSLVGLCLVVFVACVIGA-----VKRRSKTEKQKKESTAVV-----TFDAAETAEVAARAEQESEIEEKVKLQA-----: 352
AT5G67200 : N--RAAA-----SRSPFFGSTNKTTSSEAPLQQAQQAQGGAVVIPPVTKKKGKESGLVGLFTAGLALSLVGLCLVVFV-----SLVIKRRNDGIVPEGPGDPT-----ASLSQQQSQSQNTPRTRAVVPLNSDTESKRKEVQFQTEQRI-----: 368
LOC_Os02g0 : D--TPD-----KIASPSNMSTFMTIAVILVIVGVILAAAGATVIGIRRRRRRRRRRPPGEPGEGDQTP-----SNPKLHTAPAVNINRGSATAAATAAAGTSAAGTASAGGGGAAAKRGRRR-----: 361
LOC_Os02g5 : R--IOGA-----APTAPAAAA-----FTPLPPRSNRSRRAKNAGIAGATVAGVIVLGIILVAAVVM-----ASRRGNKRVAGVDVKGAMP-----EEEEEQQQQPQAQPREINASAASASAVSERRRGREFSWER-----: 353
LOC_Os04g5 : R--REER-----GSHLLFFHGPNGNSAAPVQSAATGDDGQRDDISLPSDSTPRSKLRRRAALVAATAAAVALLLCAVIAAM-----KRCRKRNRSSAAAYPSFKS-----AAMSEVSRDMDTLGVCEVPADEETAAMMPPEKARRL-----: 412
LOC_Os06g0 : D--RPP-----RVQAAAASSRSKPMAFVILVSVVIVLVLVCLCC-----NRSSVHDTFAHRRGGDGLD-----ERPVMYVGFSTTKGRSAVHLGKRTGSSLRGHRRAASAADKDELG-----: 342
LOC_Os06g4 : G--APP-----EVPILASP-----SPSPLSSSSWPKLKLMLIALVWVVALAFAGATAM-----LARRSATTEQGGGGVGHFA-----ANAAA-ARMAKTPNPAVTSAGWGGGGGGEVAVPAKRRGRRR-----: 361
LOC_Os08g4 : D--AKE-----APSPAATTSPPAATSGKGTGSPSPATAAETTTGTVAEEGQCATKPKGTSEFVGAALFALIGFAVVE-----LQRRREYNTQNFPAASTKPT-----TLPSPAPATKPTHAATAAATAATGGGARVSATVPAKRRGGKA-----: 383
LOC_Os09g2 : F--PPPP-----PPPSGENDGRRRLRIGLVMCGVLLIGAVIAAVFLYVM-----MCSRKGVSVLGGKTAATIT-----TSSSVTPKATKPTGVAALPMSERMAATAAAAARAVATPASLVVLQSG-----: 359
LOC_Os11g4 : SGAGAA-----AAAPGAHT-----AMPMSAADYFAVQEETSVMCGIIMLVVLLVAGAMVLM-----RQDEGTSTASSGYEHPAIGA-----PSGNLSVPHAAGAAAQAQLVMEQGGSGAAGAGGCVGVGGAR-----: 366

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LePRK1 : AVQOKLLEFK-----DDIE--KEDPDLKASAEVLGS-----VFSSTYKAAISRC--RVMVVKRFQMN-----VCKED--EHEHRRLCRUSKKNLPLVVAAYYRKEELLVSEVYVNNVSLAVHHE--N-KSRGQPSLQWPTRLKIIVKGVAKGL : 480
LePRK2 : VAGQKLEFK-----DDIE--KEDPDLKASAEVLGS-----VFSSTYKAAISRC--PVMVVKRFHMN-----VCKED--EHEHRRLCRUSKKNLPLVIAFYRKEELLVSEVYVNNVSLAVYHE--NKSRRGNQSLQWPTRLKIIVKGVSKGI : 457
LePRK3 : SDMGDLVWVN-----DEKE--IIGMPDLKAAAEVLGN-----GLSAYKALVLSG--VLSVVKRLETKN-----FNKEC--DAEELRRARIRKKNLLOPLAAYHYGKEELLVSEYLPKGSLLYLFHC--D-RGTAHAQLNWCIRVKIILGVANCM : 446
AT5G35390 : VENTKLSFTR-----EDRE--KEDPDLKASAEVLGS-----CFASAYKALVLSG--QMMVVKRFQMN-----AGRDE--EHEHRRLCRUSKKNLPLVVAAYYRKEELLVSEVYVNNVSLAVHHE--N-OSLGKPSLQWPTRLKIIVKGVAKGL : 469
AT2G07040 : IHTTKLSFTR-----DDKG--KEEDPDLKASAEVLGS-----CFASAYKILVLSG--SVMVVKRFQMN-----AGRDE--EHEHRRLCRUSKKNLPLVVAAYYRKEELLVSEVYVNNVSLAVHHE--H-KSLGQPSLQWPTRFNIIVKGVGRGL : 450
AT3G42880 : SGMGDLVWVN-----SEKG--SFGPDLKAAAEVLGN-----SLSAYKVMANQ--LVMVVKRLEDMN-----IAREA--DTEQRFKCRKRNPLVPLAAYHYRKEELLVSEVYVNNVSLAVHHE--D-RGVYHSELQWPTRLKIIVKGVAKGL : 484
AT3G20190 : PDQNKLELQ-----DDIQ--REDPDLKASAEVLGS-----SFSSTYKATVLSG--QMLVVKRFQMN-----VGRDE--EHEHRRLCRUSKKNLPLVVAAYYRKEELLVSEVYVNNVSLAVHHE--N-HSVDPQLQWPTRLKIIVKGVAKGL : 474
AT1G50610 : DPGGRLEFVR-----DDIQ--REDPDLKASAEVLGS-----TFASAYKALVLSG--QTLVVKRLEDMN-----VGRDE--EHEHRRLCRUSKKNLPLVVAAYYRKEELLVSEVYVNNVSLAVHHE--N-NNSAGLQWPTRLKIIVKGVAKGL : 484
AT5G20690 : GGMGDLVWVN-----TDKG--SFGPDLKAAAEVLGN-----SLSAYKVMANQ--LVMVVKRLEDMN-----IAREP--DKEVRRFRCRKNPLVPLAAYHYRKEELLVSEVYVNNVSLAVHHE--D-RGIYHSELQWPTRLKIIVKGVAKGL : 496
AT4G31250 : GDQKLEFVR-----NDQE--RTPDMLKAAAEVLGS-----GFSSAYKALVLSG--RAVMVVKRFQMN-----IAREE--DYDHKKTCRUSKKNLPLVIAFYRKEELLVSEVYVNNVSLAVHHE--N-RTPGQVLLQWPTRLKIIVKGVAKGL : 478
AT1G10850 : GAVGTLEFVG-----TSDSGETVTVRTEEDLLKASAEVLGR-----TLSSAYKAVESG--FIVTVKRLNARY-----PRMEE--KRRHEILGRUSKKNLPLVPLAAYHYRKEELLVSEVYVNNVSLAVHHE--TRAGSGKPLQWPTRLKIIVKGVAKGL : 470
AT1G60630 : GSVGTLVFLG-----RDIIVTVRTEEDLLKASAEVLGR-----TLSSAYKAVESG--FIVTVKRLDAGF-----PRMDE--KRRHEILGRUSKKNLPLVPLAAYHYRKEELLVSEVYVNNVSLAVHHE--KTRAGSGKPLQWPTRLKIIVKGVAKGL : 463
AT1G72460 : AGTSDLVWVN-----KEKG--VGRSDDLKAAAEVLGN-----GGVSAKAVLSG--VTVVVKRFTVMNQ-----MSVDV--DKEELRKLGRUSKKNLPLVPLAAYHYRKEELLVSEVYVNNVSLAVHHE--D-HEEQQLQWPTRLKIIVKGVAKGL : 467
AT3G50230 : FRNGDLIFCG-----EGGGGGEA--MVTIDOLMRASAEVLGR-----SVCTHYKAVLNQ--MIVTVKRFAPSKT-----AITSLE--BENQEVYCGKRNPLVPLAAYHYRKEELLVSEVYVNNVSLAVHHE--S-RTSKAKPLQWPTRLKIIVKGVAKGL : 497
AT5G43020 : TKSGLVFCG-----GEAH--VYTVDOIMRASAEVLGR-----TVCTHYKALDSR--LIVTVKRLDARL--AGVGRDK--BENHESVCAHCHENPLVPLAAYHYRKEELLVSEVYVNNVSLAVHHE--T-KSSRAKPLQWPTRLKIIVKGVAKGL : 489
AT5G67200 : PNSGNLVECC-----ESRSQGMVTEGDMRASAEVLGR-----SVCTHYKAVLNQ--LIVTVKRLDAAKT--AVTSEEA--BENHESVCAHCHENPLVPLAAYHYRKEELLVSEVYVNNVSLAVHHE--S-RSSRAKPLQWPTRLKIIVKGVAKGL : 507
LOC_Os02g0 : DEHGRLEFVG-----ESRK--REEDPDLKASAEVLGS-----MFSSTYKALQER--FIVTVKRFQMN-----VCREDE--EHEHRRLCRUSKKNLPLVVAAYYRKEELLVSEVYVNNVSLAVHHE--N-RRGSELQWPTRLKIIVKGVAKGL : 493
LOC_Os02g5 : EGIKLEFVG-----GVAE--MSEEDLLKASAEVLGR-----EVCTHYKAVTEG--FIVTVKRFQMN-----AGAAE--LGRRAEELCRUSKKNLPLVVAAYYRKEELLVSEVYVNNVSLAVHHE--SSSRTSKGKPLQWPTRLKIIVKGVAKGL : 490
LOC_Os04g5 : ERSGCITFCA-----GEGA--SYSEOLMRASAEVLGR-----SVCTHYKAVLDGR--LVIVTVKRLDAAKIGAAAEAEA--EENQDAVGRUSKKNLPLVPLAAYHYRKEELLVSEVYVNNVSLAVHHE--S-RSSRAKPLQWPTRLKIIVKGVAKGL : 551
LOC_Os06g0 : GGAGDLVWVN-----NCKG--VFGPDLKAAAEVLGS-----GFASAYKAVMANQ--VAMVVKRLEDMN-----ATKDA--EAEELRKLGRUSKKNLPLVPLAAYHYRKEELLVSEVYVNNVSLAVHHE--D-RGMDYAGLQWPTRLKIIVKGVAKGL : 474
LOC_Os06g4 : DHDHGRLEFVG-----EGRE--REEDPDLKASAEVLGS-----SFSAYKAVLEVE--QSMVVKRFQMN-----VGRQD--EHEHRRLCRUSKKNLPLVVAAYYRKEELLVSEVYVNNVSLAVHHE--GSSMAYVPPRRKLEIKGVTRGI : 499
LOC_Os08g4 : GEGGRLEFVR-----DDDRGRFEEDPDLKASAEVLGA-----ANLVCYRATLVEG--QSMVVKRFQMN-----VGRQD--EHEHRRLCRUSKKNLPLVVAAYYRKEELLVSEVYVNNVSLAVHHE--E-GRRVKLELQWPTRLKIIVKGVAKGL : 520
LOC_Os09g2 : TAASTVMTNTAAAAAAEAAR--KLRFEEDLLKASAEVLGR-----RFSAYKAVLVVPG--AALAVKRVDAAG-----AEEDDE--RRRREYVCKARSPVPLAAYHYRKEELLVSEVYVNNVSLAVHHE--S-TESSQVLLQWPTRLKIIVKGVAKGL : 502
LOC_Os11g4 : KQVAEFLVLS-----NAAG--EFGPDLKASAEVLGN-----TLSSAYKAVMANQ--VTVVVKRLEDMN-----VGRAE--EHEHRRLCRUSKKNLPLVVAAYYRKEELLVSEVYVNNVSLAVHHE--D-QSPERVLLQWPTRLKIIVKGVAKGL : 501

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66 a Ae 6G g G Y4a 6 g 6 VKR f g H n66 a5 E L 6 5 sL Hg 6 W 6 g

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LePRK1 : 660 * 680 * 700 * 720 * 740 * 760 * 780 * 800 * 820
LePRK1 : LLYNELPSLT-----A-----PHGHLKSSNVLLNESVEPLLTDYADL-----PVVNLEHAQEH---MIAVYKSPFF---K-----HNG-R-IRNDVWTLGLIIEMLTGKFPENLQQKGSQSDT---DLATNVRSVVNEPMT : 593
LePRK2 : LLYNELPSLT-----S-----PHGHLKSSNVLLTENFEAVLTDYADL-----PVVNAEHAHEH---MISYKAPFL---K-----QSG-K-VNRRKTDVMTLGMILIEILTGKFPENLKGKQDSD---DLATNVTTLGGESS : 570
LePRK3 : KPLHSEFGSYD-----V-----PHGHLKSSNVLLSANNPELLTDYAFY-----PLVNSQAVQS---LFAVYKSPFA---I-----LNQ-Q-VNRRKTDVMTLGMILIEILTGKFPENLQKQKFTGT---DVAQVQSAIENNRV : 559
AT5G35390. : FYLHQDLPSLM-----A-----PHGHLKSSNVLLTKTFEPLLTDYGTI-----PLINQEKQAMH---MAAVYKSPFY---L-----QHR-R-LNRRKTDVMTLGMILIEILTGKFPENLQKQKFTGT---DVAQVQSAIENNRV : 579
AT2G07040. : LYLHKNLPSLM-----A-----PHGHLKSSNVLLSEKPEPLLMDYGTI-----PMINEESAQEL---MVAVYKSPFY---V-----KQS-R-VNRRKTDVMTLGMILIEILTGKLELESFQVQKESSE---DLASVNRSSFKGWT : 563
AT3G42880. : DEHEEFASYD-----I-----PHGHLKSSNVLLSETVEPLLISDYAFI-----PLVQPNASQA---LFAVYKSPFF---V-----QNQ-Q-VNRRKTDVMTLGMILIEVMTGKFPENLQKQKFTGT---DVAQVQSAIENNRV : 582
AT3G20190. : GYFDELPTLT-----I-----PHGHLKSSNVLLDESPEPLLTDYADR-----PVMNSEQSHNL---MISYKSPFY---S-----LKG-Q-VNRRKTDVMTLGMILIEILTGKFPENYLQSGYDANM---SLVTNVSMMVKEKKT : 597
AT1G50610. : SYFDELPTLT-----I-----PHGHLKSSNVLLDDSEPLLTDYADR-----PMSSEHANNF---MTAVYKSPFY---R-----PSKQIILNRRKTDVMTLGMILIEVLTGKFPENYLQSGYDANM---SLVTNVSMMVKEKKT : 599
AT5G20690. : KPEHEEFASYD-----L-----PHGHLKSSNVLLSETVEPLLISDYAFI-----PLVQPNASQA---LFAVYKSPFF---A-----QTQ-Q-VNRRKTDVMTLGMILIEILTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 608
AT4G31250. : AYLVRVFPDLN-----L-----PHGHLKSSNVLLDPNEPELLTDYAVV-----PVMNRDQSQF---MVAVYKAPFF---T-----QQD-R-TNRRKTDVMTLGMILIEILTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 591
AT1G10850. : LYLHQNPG-----L-----PHGHLKSSNVLLGPDPEESCLTDYGS-----TLHDDPSVEETSAVSLFYKAPFC---R-----DPR-KASQPADVMSFGVLLIEILTGKFPENLQKQKFTGT---DVAQVQSAIENNRV : 582
AT1G60630. : VYLHQNPG-----L-----PHGHLKSSNVLLGPDPEESCLTDYGS-----TLHDDPSYIEDTSAASLIFYKAPFC---R-----DLR-KASQPADVMSFGVLLIEILTGKFPENLQKQKFTGT---DVAQVQSAIENNRV : 575
AT1G72460. : WYLHRELGLFN-----L-----PHGHLKSSNVLLFAEDGPELLSDFGQ-----KLNINPDAQSQS---LVAVYKSPFA---D-----RDG-T-VNRRKTDVMTLGMILIEILTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 580
AT3G50230. : HYLHQSAKF-----L-----PHGHLKSSNVLLGHDFEAGVTDYCS-----VITDSSVPPND---PDISSYKAPFI---R-----KSTDVMSFGVLLIEILTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 608
AT5G43020. : SYLHQAWQ-----L-----VHGNLKSSNVLLGQDFEAGVTDYCS-----VITDSSVPPND---PDISSYKAPFI---R-----KSTDVMSFGVLLIEILTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 605
AT5G67200. : YYLHQSSALV-----L-----VHGNLKSSNVLLGQDFEAGVTDYCS-----VITDSSVPPND---PDISSYKAPFI---R-----KSTDVMSFGVLLIEILTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 619
LOC_Os02g0. : GHYDELPLMT-----V-----PHGHLKSSNVLLDGDMEAVLTDYAVV-----PVMNPSAAAQV---MVAVYKAPCVAAA---AAG-K-PKSDVMSFGVLLIEVLTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 609
LOC_Os02g5. : VHLHQSPFAGI-----V-----PHGHLKSSNVLLGPDPEESCLTDYGVV-----PTLLPSSHADLASSTVLRYKAPFI---R-----RTA-HRTPASDMSFGVLLIEILTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 603
LOC_Os04g5. : AYLHQASR-----V-----VHGNLKSSNVLLGSDPEAGLTDYCAA-----PMSSEVKNDF---MTAVYKAPFN---M-----KSN-RHRRKTDVMTLGMILIEVLTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 657
LOC_Os06g0. : AYLHGEIAGHE-----V-----PHGHLKSSNVLLIAPDEPELLVDYGS-----GHVNHMQSPNS---MIAVYKAPFC---A-----AGH-P-VGADVMSFGVLLIEVLTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 589
LOC_Os06g4. : AYLHDELPLMT-----V-----PHGHLKSSNVLLDAPEPELLSDYAVV-----PVMTPRHAQV---MVAVYKSPFC---G-----ETG-R-PKSDVMSFGVLLIEVLTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 606
LOC_Os08g4. : QYLHDELPLMT-----V-----PHGHLKSSNVLLNDRPEPELLTDYGSV-----PVMNQSASHAQL---MVAVYKSPFR---R-----QFG-R-SKSDVMSFGVLLIEILTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 636
LOC_Os09g2. : AYLHGAALRGDGGDGANANLSFSSSYEEDEAGGAIAPHGNLKSSNVLLTATAPPELLSDFGVT-----APPPSSAPAA-----ALADVMSFGVLLIEILTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 617
LOC_Os11g4. : SYLHEKLGIPA-----L-----MRLVSMTGADPDAAPPPPHGHLKSSNVLLDAHEPELLVDYGF-----PLVNTSQAHPA---MFAVYKSPFA---ASAAAAGAGAAAQRA-A-LASDMSFGVLLIEVLTGKFPENYLQKQKFTGT---DVAQVQSAIENNRV : 641
6 6 HG 6Ks N6 1 E 6 d pe D6 G6 61E663G4 p 6

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LePRK1 : 840 * 860 * 880 * 900 * 920 *
LePRK1 : EVDVFEKEM---RGTTN---SEGEVKKLLKALGCCD---LDMKKRFDLKEAERIEEVKRD-----GDDDFYSTRGLSDDYTQVSMNI----- : 669
LePRK2 : EKEVFDKEM---KGTKD---CESEMKKLLKGLSCCE---ADVEKCDLKEAERIEEDVKK---GKGFDFSSNVASNEVDNMHTSR----- : 642
LePRK3 : S-ELIDPEI---ETEKD---SLEMVEKFIYGAACE---SDHHRFDYKEARRRIEEHDLM----- : 612
AT5G35390. : F-SLFDKGM---GKTSH---CEGQVKKLLTGLNCC---PDVEKRLDQGAERIEELKREG-----DDDDFYSTVYVSETDGRSSKGESCESISFA----- : 662
AT2G07040. : Q-ELFDQEM---GKTSN---CEAHLNLMRLGLSCCE---VDVEKRLDREARERMEDLMKEREQ-----GDDDFYSTVYASEADGRSSRGLSSEGINLS----- : 647
AT3G42880. : E-ELIDPEI---ASNTD---SIKQVVELLRGAACIA---SNPNBRQNKETIRRIERVTL----- : 633
AT3G20190. : G-DVFDKEM---TGKKN---CKAEMNLLKGLSCCE---EDEERMEMRDAERIERKEGE-----FDNDFASTTHNVFASRLIDDDDFGFAMNR----- : 679
AT1G50610. : G-DVFDKEM---KGKKN---CKAEMNLLKGLRCC---EEEEERMDREVEMVMDREGESEDDF-----GSMDRHRTHNNVYSMLLDDDDDFGFSMNR----- : 686
AT5G20690. : E-ELIDPEI---VNNTS---SMRQVVELLRGAACIA---SNPDBELDREARERIEQVKT----- : 659
AT4G31250. : A-DVFDKEM---KAGKE---HEAQMLKLLKGLRCCD---WDIEKRIEHEANDRIEEDRDAGG-----GQESVRSYVTASDGDHRSRAMTEEFSLM----- : 676
AT1G10850. : SGEEPTS---SGNEA---SEEKQALLSHATVCVT---IQPDNRPVREVMKWRDARAEAPFSS---NSSEHSPGRWSDTVQSLPRDDQVSI----- : 663
AT1G60630. : VSEELNA-----SEEKQALLSHATAVA---VKPENRPAVREVMKWRDARAEAAALFSF---NSSDHSPPGRWSDTVQSLPREDHMSI----- : 652
AT1G72460. : M-DLHPMVTAAAEK---IMEEENLVRGVRCR---EDFDQPNTEV---VDELTIED-----SNDDFITIET----- : 644
AT3G50230. : S-----K-----EENGEMTQACLQV---TSPEQPTMKEVKMQLQKGSVV-MTE-----ENEKFL----- : 660
AT5G43020. : K-----KGNWRE---DRDKFGMTETAVASL---ASPEQPTMVOVKMLQELKEAVM-----ECELVMDSANSESS----- : 669
AT5G67200. : T-----EDNRKGMVTETACLQV---TSPEQPTMROVKMQLQKESVMAE-----ENDPFR----- : 669
LOC_Os02g0. : G-EVFDKEM---AAAGAG---AEDDMKLLHVLGCCD---ADVDRWEIKTANARIEERIVPDPPTP-----AAAAAEEPSPTTTTTNSGETRS----- : 692
LOC_Os02g5. : SGESASAS-----GG---TEEKGALLISAAACVV---ADPARPTPEVIRVREARAEAM-SSS-----NSSDRSPARWSDAVQVQMGMGVPRDQGELGLLT----- : 690
LOC_Os04g5. : DVERSLMDVDSACVR---SSPESPTAVQVVKMLQELKEADTAGD-----NDSDLTNS----- : 710
LOC_Os06g0. : R-DLFDKAI---TSAWKF---ALPDARLMRNVADQVE---TDADKSPDKVAARVEEVAAAMATVRRERHQAGGESRSSSHQYVVRDGMORITVSGERSSRRGSNDYSS----- : 693
LOC_Os06g4. : G-EVFDQEM---RGARG---GEGEMVKKLLKGLGCC---SDVDRWDVDAARIEERDRADAGAGA-----DDSSAASSVAGGGGGEASRSHSS----- : 688
LOC_Os08g4. : E-KVVDAMIRKWEDEE---SKGEMVKKLLKGMACE---AAVDSEWIKTAVESIEEIKGKKEEDAN-----DEHSFYSSIDGDEFASVAIN----- : 717
LOC_Os09g2. : A-EVFDRAMLSSAGCGDVTASEQRVRLQVAMRCDDASSPSPPTVREVAGVWNAHREE-----DDMSLSSEA----- : 687
LOC_Os11g4. : Q-EVVDPMV-----AAG---AGPAAVRLRIGVRCI---PEPESPEPSADVARMVQVAGGGGGGAS----- : 697
C I 6

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**Figure S4. Alignment data used to produce the PRKs
phylogenetic tree in Figure 1A.**

The alignment was produced by MUSCLE 3.6 and presented by
GeneDoc without adjustment.

Chang_Fig. S5

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360          *          380          *          400
RepGEF1 : NIDYNKDVQSILEYSRVMSIAFNATARDVLYVDAME : 357
RepGEF2 : CIDYNKDVQALLESYRVLEGAFNANARDVLYVKTRM : 362
RepGEF3 : CIDYNKDVQALLESYRVLEGAFNANARDVLYVKTRM : 362
RepGEF4 : CIDYNKDVQALLESYRVLEGAFNANARDVLYVKTRV : 361
RepGEF5 : CIDYNKDVQALLESYRVLEGAFNANARDVLYVDLTR : 362
RepGEF6 : SKIDYNKDVKSLLESYRVLESIAFGVSEEDVLYVMDISK : 353
RepGEF7 : SKIDYNKDICKSLESYRVLESIAFNANARDVLYVDLTR : 364
RepGEF8 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 350
RepGEF9 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVSLAR : 350
RepGEF10 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVO : 352
RepGEF11 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLQTO : 347
RepGEF12 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLRAGN : 349
RepGEF13 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLQTO : 352
RepGEF14 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLSIN : 359
Aralyl.314 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLQTO : 347
Aralyl.477 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLRAGN : 349
Aralyl.479 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLQTO : 352
Aralyl.483 : CIDYNKDVQALLESYRVLEGAFNANARDVLYVVRTVR : 349
Aralyl.485 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVMDISK : 353
Aralyl.486 : SKIDYNKDICKSLESYRVLESIAFNANARDVLYVDLTR : 365
Aralyl.488 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVO : 352
Aralyl.490 : CIDYNKDVQALLESYRVLEGAFNANARDVLYVKTRM : 362
Aralyl.490 : NIDYNKDVQSILEYSRVMSIAFNATARDVLYVDAME : 357
Aralyl.497 : CIDYNKDVQALLESYRVLEGAFNANARDVLYVKTRV : 361
Aralyl.861 : SKIDYNKDICKSLESYRVLESIAFNANARDVLYVDLTK : 363
Aralyl.874 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 350
Aralyl.883 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVSLAR : 350
Bra00971 : SKIDYNKDVQALLESYRVLEGAFNANARDVLYVKTRM : 362
Bra002246 : SKIDYNKDICKSLESYRVLESIAFNANARDVLYVRLVO : 352
Bra003536 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLRAGN : 349
Bra004945 : SKIDYNKDVQALLESYRVLEGAFNANARDVLYVVRTVR : 361
Bra006510 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 347
Bra007183 : SKIDYNKDVQALLESYRVLEGAFNANARDVLYVMDISK : 353
Bra009152 : SKIDYNKDICKSLESYRVLESIAFNANARDVLYVDLTK : 362
Bra009621 : SKIDYNKDICKSLESYRVLESIAFNANARDVLYVDLTR : 364
Bra013249 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 350
Bra015010 : NIDYNKDVQALLESYRVLESIAFNANARDVLYVDLAL : 368
Bra015068 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 350
Bra018956 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLQTO : 347
Bra020048 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVO : 352
Bra021162 : SKIDYNKDICKSLESYRVLESIAFNANARDVLYVRLQTO : 335
Bra027189 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLQTO : 351
Bra030396 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVQ : 347
Bra036671 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 346
Bra037342 : CIDYNKDVQALLESYRVLEGAFNANARDVLYVKTRM : 362
Cucsa.0620 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVVRTVR : 351
Cucsa.0666 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDLTK : 358
Cucsa.1164 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 347
Cucsa.1193 : SKIDYNKDVQALLESYRVMSIAFNANARDVLYVDAIK : 360
Cucsa.1355 : SKIDYNKDVQALLESYRVLEGAFNANARDVLYVRSIR : 360
Cucsa.1363 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 350
Cucsa.1758 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 347
Cucsa.3592 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLQTO : 349
mgv1a00322 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 350
mgv1a00378 : SKIDYNKDVQALLESYRVMSIAFNANARDVLYVDAIK : 361
mgv1a00399 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDAIK : 363
mgv1a00461 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVMDISK : 353
mgv1a00530 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVQ : 338
mgv1a00594 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 363
mgv1a00778 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 297
mgv1a02184 : SKIDYNKDVQALLESYRVLEGAFNANARDVLYVDTLAL : 361
mgv1a02262 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 363
mgv1a02280 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVQ : 346
mgv1a02642 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 349
POPTR_0001 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVVRTVR : 349
POPTR_0002 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDAIK : 371
POPTR_0002 : SKIDYNKDVQALLESYRVLEGAFNANARDVLYVVRTVR : 359
POPTR_0003 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 349
POPTR_0004 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDAIK : 364
POPTR_0005 : NIDYNKDVQSILEYSRVMSIAFNATARDVLYVDAIK : 363
POPTR_0006 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 366
POPTR_0008 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 358
POPTR_0009 : SKIDYNKDVQALLESYRVMSIAFNANARDVLYVDAIK : 364
POPTR_0010 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 358
POPTR_0014 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVQ : 357
POPTR_0016 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 364
POPTR_0018 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVQ : 349
Thhalv1000 : SKIDYNKDVQALLESYRVLEGAFNANARDVLYVVRTVR : 361
Thhalv1000 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 350
Thhalv1000 : CIDYNKDVQALLESYRVLEGAFNANARDVLYVKTRM : 362
Thhalv1001 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVMDISK : 353
Thhalv1001 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLQTO : 347
Thhalv1001 : SKIDYNKDICKSLESYRVLESIAFNANARDVLYVDLTK : 364
Thhalv1001 : SKIDYNKDICKSLESYRVLESIAFNANARDVLYVDLTR : 364
Thhalv1001 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVQ : 351
Thhalv1001 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLRAGN : 352
Thhalv1001 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLRAGN : 352
Thhalv1002 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLQTO : 352
Thhalv1002 : NIDYNKDVQSILEYSRVMSIAFNATARDVLYVDAME : 359
Thhalv1002 : SKIDYNKDVQALLESYRVMSIAFNANARDVLYVDAME : 301
Thhalv1002 : SKIDYNKDVQALLESYRVMSIAFNANARDVLYVDAME : 301
Thhalv1002 : CIDYNKDVQALLESYRVLEGAFNANARDVLYVKTRM : 358
Vv.1782614 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVQ : 346
Vv.1782686 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 366
Vv.1782737 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 342
Vv.1783220 : SKIDYNKDVQALLESYRVMSIAFNANARDVLYVDAIK : 364
Vv.1783447 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 358
Vv.1783965 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 358
LOC_080194 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 347
LOC_080195 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 349
LOC_080196 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 349
LOC_080291 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 344
LOC_080494 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 361
LOC_080593 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 362
LOC_080594 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 350
LOC_080792 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 352
LOC_080993 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDAIK : 371
LOC_081094 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVVRTVR : 366
GRMZM2G059 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 347
GRMZM2G065 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 365
GRMZM2G071 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRSIR : 368
GRMZM2G087 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDAIK : 355
GRMZM2G105 : NIDYNKDVQSILEYSRVMSIAFNATARDVLYVDAIK : 364
GRMZM2G131 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 353
GRMZM2G132 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 349
GRMZM2G144 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 357
GRMZM2G158 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 374
GRMZM2G173 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 343
GRMZM2G359 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 350
GRMZM2G377 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 349
GRMZM2G442 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 343
Selmol.431 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 357
Selmol.623 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 357
Selmol.627 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVRLVH : 362
Selmol.629 : SKIDYNKDVQALLESYRVLESIAFNANARDVLYVDTLAL : 362
Riq_dg_syr_e_a_d
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Figure S5. Alignment data used to produce the RopGEFs phylogenetic tree in Figure 7A.

The alignment was produced by MUSCLE 3.6 and presented by GeneDoc without adjustment.

Table S1: The Segregation Ratios of the *LAT52:DNPRK2* T2 plants

Mutant line ID	Bar ^R	Bar ^S	Bar ^R :Bar ^S
LDN-1	72	15	4.8 :1
LDN-2	68	2	34 :1
LDN-3	69	35	2 :1
LDN-4	152	22	7 :1
LDN-5	172	48	3.6 :1
LDN-6	98	24	4 :1
LDN-7	117	57	2 :1
LDN-8	98	56	1.8 :1
LDN-9	100	0	∞
LDN-10	53	20	2.7 :1
LDN-11	112	44	2.5 :1
LDN-12	48	11	4.4 :1
LDN-13	64	1	64 :1
LDN-14	51	3	17 :1
LDN-15	46	23	2 :1
LDN-16	109	31	3.5 :1
LDN-17	42	39	1.1 :1
LDN-18	53	5	10.6 :1
LDN-19	120	50	2.4 :1
LDN-20	98	50	2 :1
LDN-21	53	29	1.8 :1
LDN-22	47	1	47 :1
LDN-23	92	57	1.6 :1
LDN-24	165	62	2.7 ;1
LDN-25	79	33	2.4 :1
LDN-26	65	0	∞
LDN-27	52	23	2.3 :1
LDN-28	67	32	2.1 :1
LDN-29	53	6	8.8 :1
LDN-30	73	31	2.4 ;1
LDN-31	61	23	2.7 :1
LDN-32	53	23	2.3 :1
LDN-33	NA	NA	NA
LDN-34	42	21	2 :1
LDN-35	66	21	3.1 :1
LDN-36	103	7	14.7 :1
LDN-37	85	30	2.8 :1
LDN-38	67	27	2.5 :1
LDN-39	70	28	2.5 ;1
LDN-40	41	11	3.7 :1

Table S2. Information of the ropgef and prk T-DNA Insertion Mutants

Genes	AGI number	Alleles	Accession No.	Background	Marker	Insert. posi.	mRNA levels (by RT PCR)
RopGEF1	At4g38430	<i>ropgef1-1 (586B11)</i>	GABI_586B11	Col	Sulfadiazine	3rd Exon	not detectable
RopGEF9	At4g13240	<i>ropgef9-1 (717A10)</i>	GABI_717A10	Col	Sulfadiazine	5th Exon	not detectable
RopGEF12	At1g79860	<i>ropgef12-1 (103614)</i>	SALK_103614	Col	Kan (2:1)	4th Exon	not detectable
RopGEF14	At1g31650	<i>ropgef14-1 (064617)</i>	SALK_064617	Col	Kan (3:1)	1st Intron	suppression
		<i>ropgef14-2 (046067)</i>	SALK_046067	Col	Kan (lost)	1st Intron	not detectable
AtPRK1	At5g35390	<i>prk1-1</i>	SALK_112241	Col	Kan(3:1)	1st Exon	not detectable
		<i>prk1-2</i>	SALK_054149	Col	Kan(3:1)	1st Exon	not detectable
AtPRK2	At2g07040	<i>prk2-1</i>	SALK_110661	Col	Kan(lost)	1st Exon	suppression
AtPRK5	At1g50610	<i>prk5-1</i>	SALK_016815	Col	Kan(lost)	3rd Exon	suppression

Table S3. Phosphorylation in C-terminal regions is critical for RopGEF1 activity

	Length^a	Width^a	Width/Length
GFP	469 ± 11	9.1 ± 0.8	0.02
GFP-GEF1	21 ± 7	29.4 ± 4	1.41
GFP-S458A	39 ± 11	39.9 ± 8	1.03
GFP-S460A	422 ± 89	13.8 ± 2.3	0.03
GFP-S480A	174 ± 40	5.7 ± 0.5	0.03
GFP-S484A	63 ± 15	11.3 ± 0.7	0.58
GFP-S488A	70 ± 12	35.6 ± 0.7	0.51
GFP-S501A	24 ± 5	22.7 ± 3.8	0.94

The length and the maximum tip width of tobacco pollen tubes were measured 6 hours after bombardment. ^aData are the mean ± SD.