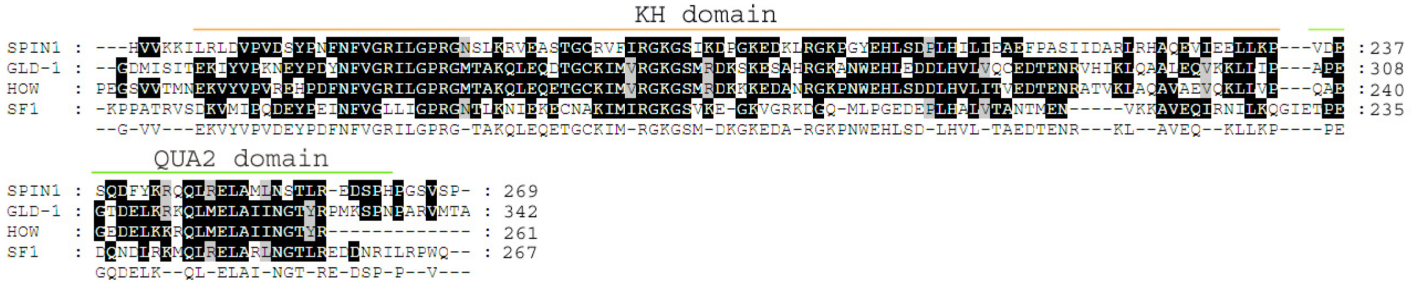
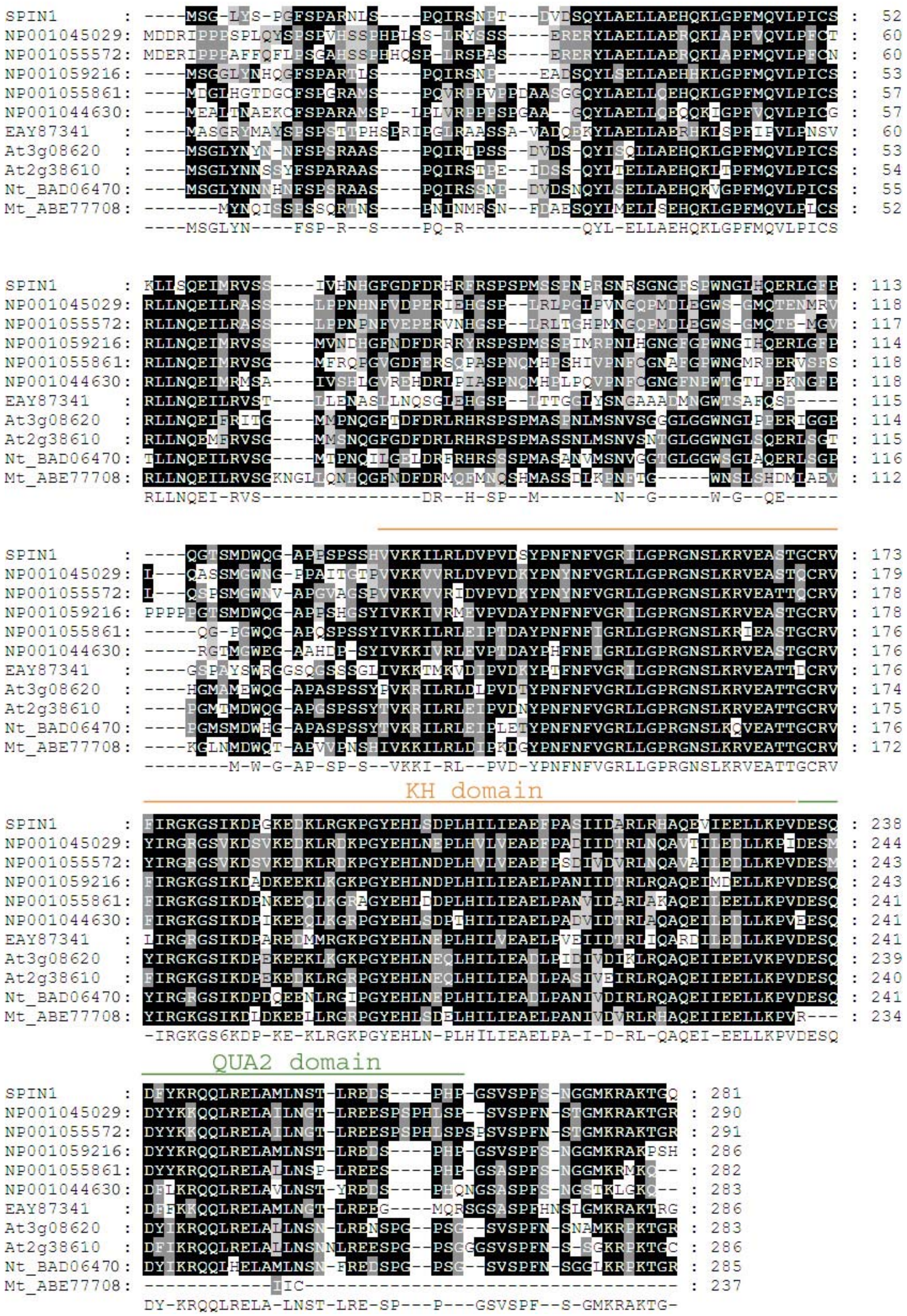
Supplemental data Vega-Sanchez et al (2008). SPIN1, a K homology domain protein negatively regulated and ubiquitinated by the E3 ubiquitin ligase SPL11, is involved in flowering time control in rice



Supplemental figure 1. Alignment of the STAR domain of SPIN1 with mammalian protein members of the family. Gld-1, from *C. elegans*; Sf1 from human; How, from *Drosophila melanogaster*. Multiple sequence alignment was done using ClustalX and conserved residue shading performed using GeneDoc. Amino acid similarity between SPIN1 and GLD-1, HOW and SF-1 is 69%, 69% and 59%, respectively.



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SPIN1      : ----MSG-LYS-PGFSPARNLS----PQIRSNPT---DVDSQYLAELLAEHQKLGPFMQVLPICS :  52
NP001045029: MDDRIPPPSPLQYSPSPVHSSPHPLSS-LRYSSS----ERERYLAELLAERQKLAPFVQVLPFCT :  60
NP001055572: MDERIPPPAFFQFLPSGAHSSPHHQSP-LRSPAS----ERERYLAELLAERQKLAPFMQVLPFCN :  60
NP001059216: ----MSGGLYNHQGFSPARTLS----PQIRSNP----EADSQYLSELLAEHHKLGPFMQVLPICS :  53
NP001055861: ----MDGLHGTDGCFSPGRAMS----PQVRPPVPPDAASGGQYLAELLQEHQKLGPFMQVLPICS :  57
NP001044630: ----MEALTNAEKCFSPARAMSP--LPLVRPPPSPGAA--GQYLAELLQEQQKIGPFVQVLPICG :  57
EAY87341   : ----MASGRYMAYSPSPSTTPHSPRIPGLRAASSA-VADQEKYLAELLAERHKLSPFIPVLPNSV :  60
At3g08620  : ----MSGLYNYN-NFSPSRAAS----PQIRTPSS--DVDS-QYISQLLAEHQKLGPFMQVLPICS :  53
At2g38610  : ----MSGLYNNSSYFSPARAAS----PQIRSTPE--IDSS-QYLTELLAEHQKLTPFMQVLPICS :  54
Nt_BAD06470: ----MSGLYNNNHNFSPSRAAS----PQIRSSNP--DVDSNQYLSELLAEHQKVGPFMQVLPICS :  55
Mt_ABE77708: -------MYNQISSPSSQRTNS----PNINMRSN--FDAESQYLMELLSEHQKLGPFMQVLPICS :  52
             ----MSGLYN----FSP-R--S----PQ-R-----------QYL-ELLAEHQKLGPFMQVLPICS

SPIN1      : KLLSQEIMRVSS----IVHNHGFGDFDRHRFRSPSPMSSPNPRSNRSGNGFSPWNGLHQERLGFP : 113
NP001045029: RLLNQEILRASS----LPPNHNFVDPERIEHGSP--LRLPGLPVNGQPMDLEGWS-GMQTENMRV : 118
NP001055572: RLLNQEILRASS----LPPNPNFVEPERVNHGSP--LRLTGHPMNGQPMDLEGWS-GMQTE-MGV : 117
NP001059216: RLLNQEIMRVSS----MVNDHGFNDFDRRRYRSPSPMSSPIMRPNLHGNGFGPWNGIHQERLGFP : 114
NP001055861: RLLNQEIMRVSG----MFRQPGVGDFERSQPASPNQMHPSHIVPNFCGNAFGPWNGMRPERVSFS : 118
NP001044630: RLLNQEIMRMSA----IVSHLGVREHDRLPIASPNQMHPLPQVPNFCGNGFNPWTGTLPEKNGFP : 118
EAY87341   : RLLNQEILRVST----ILENASLLNQSGLEHGSP--LTTGGLYSNGAAADMNGWTSAFQSE---- : 115
At3g08620  : RLLNQEIFRITG----MMPNQGFTDFDRLRHRSPSPMASPNLMSNVSGGGLGGWNGLPPERIGGP : 114
At2g38610  : RLLNQEMFRVSG----MMSNQGFGDFDRLRHRSPSPMASSNLMSNVSNTGLGGWNGLSQERLSGT : 115
Nt_BAD06470: TLLNQEILRVSG----MTPNQILGELDRFRHRSSSPMASANVMSNVGGTGLGGWSGLAQERLSGP : 116
Mt_ABE77708: RLLNQEILRVSGKNGLLQNHQGFNDFDRMQEMNQSHMASSDLKPNFTG-----WNSLSHDMLAEV : 112
             RLLNQEI-RVS---------------DR--H-SP--M-------N--G-----W-G--QE-----

SPIN1      : ----QGTSMDWQG-APPSPSSHVVKKILRLDVPVDSYPNFNFVGRILGPRGNSLKRVEASTGCRV : 173
NP001045029: I---QASSMGWNG-PPAITGTPVVKKVVRLDVPVDKYPNYNFVGRLLGPRGNSLKRVEASTQCRV : 179
NP001055572: I---QSPSMGWNV-APGVAGSPVVKKVVRIDVPVDKYPNYNFVGRLLGPRGNSLKRVEATTQCRV : 178
NP001059216: PPPPPGTSMDWQG-APPSHGSYIVKKIVRMEVPVDAYPNFNFVGRILGPRGNSLKRVEASTGCRV : 178
NP001055861: -----QG-PGWQG-APQSPSSYIVKKILRLEIPTDAYPNFNFIGRLLGPRGNSLKRIEASTGCRV : 176
NP001044630: -----RGTMGWEG-AAHDP-SYIVKKIVRLEVPTDAYPHFNFIGRLLGPRGNSLKRVEASTGCRV : 176
EAY87341   : ----GSPAYSWRGGSQGSSSGLIVKKTMKVDIPVDKYPTFNFVGRILGPRGNSLKRVEATTDCRV : 176
At3g08620  : ----HGMAMEWQG-APASPSSYPVKRILRLDLPVDTYPNFNFVGRLLGPRGNSLKRVEATTGCRV : 174
At2g38610  : ----PGMTMDWQG-APGSPSSYTVKRILRLEIPVDNYPNFNFVGRLLGPRGNSLKRVEATTGCRV : 175
Nt_BAD06470: ----PGMSMDWHG-APASPSSYTVKRILRLEIPLETYPNFNFVGRLLGPRGNSLKQVEATTGCRV : 176
Mt_ABE77708: ----KGLNMDWQT-APVVPNSHIVKKILRLDIPKDGYPNFNFVGRLLGPRGNSLKRVEATTGCRV : 172
             ---------M-W-G-AP-SP-S--VKKI-RL--PVD-YPNFNFVGRLLGPRGNSLKRVEATTGCRV

SPIN1      : FIRGKGSIKDPGKEDKLRGKPGYEHLSDPLHILIEAEFPASIIDARLRHAQEVIEELLKPVDESQ : 238
NP001045029: YIRGRGSVKDSVKEDKLRDKPGYEHLNEPLHVLVEAEFPADIIDTRLNQAVTILEDLLKPIDESM : 244
NP001055572: YIRGRGSVKDSVKEDKLRDKPGYEHLNDPLHVLVEAEFPSDIVDVRLNQAVAILEDLLKPVDESM : 243
NP001059216: FIRGKGSIKDADKEEKLKGKPGYEHLNDPLHILIEAELPANIIDTRLRQAQEIMDELLKPVDESQ : 243
NP001055861: FIRGKGSIKDPNKEEQLKGRAGYEHLDDPLHILIEAELPANVIDARLAKAQEILEELLKPVDESQ : 241
NP001044630: FIRGKGSIKDPIKEEQLKGRPGYEHLSDPTHILIEAELPADVIDTRLAQAQEILEDLLKPVEESQ : 241
EAY87341   : LIRGRGSIKDPAREDMMRGKPGYEHLNEPLHILVEAELPVEIIDTRLIQARDILEDLLKPVDESQ : 241
At3g08620  : YIRGKGSIKDPEKEEKLKGKPGYEHLNEQLHILIEADLPIDIVDIKLRQAQEIIEELVKPVDESQ : 239
At2g38610  : FIRGKGSIKDPEKEDKLRGRPGYEHLNEQLHILIEADLPASIVEIRLRQAQEIIEELLKPVDESQ : 240
Nt_BAD06470: YIRGRGSIKDPDQEENLRGIPGYEHLNEPLHILIEADLPANIVDIRLRQAQEIIEELLKPVDESQ : 241
Mt_ABE77708: YIRGKGSIKDLDKEELLRGRPGYEHLSDELHILIEAELPANIVDVRLRHAQEIIEELLKPVR--- : 234
             -IRGKGS6KDP-KE-KLRGKPGYEHLN-PLHILIEAELPA-I-D-RL-QAQEI-EELLKPVDESQ

SPIN1      : DFYKRQQLRELAMLNST-LREDS----PHP-GSVSPFS-NGGMKRAKTGQ : 281
NP001045029: DYYKKQQLRELAILNGT-LREESPSPHLSP--SVSPFN-STGMKRAKTGR : 290
NP001055572: DYYKKQQLRELAILNGT-LREESPSPHLSPSPSVSPFN-STGMKRAKTGR : 291
NP001059216: DYYKRQQLRELAMLNST-LREDS----PHP-GSVSPFS-NGGMKRAKPSH : 286
NP001055861: DYYKRQQLRELALLNSP-LREES----PHP-GSASPFS-NGGMKRMKQ-- : 282
NP001044630: DFLKRQQLRELAVLNST-YREDS----PHQNGSASPFS-NGSTKLGKQ-- : 283
EAY87341   : DFFKKQQLRELAMLNGT-LREEG----MQRSGSASPFHNSLGMKRAKTRG : 286
At3g08620  : DYIKRQQLRELALLNSN-LRENSPG--PSG--SVSPFN-SNAMKRPKTGR : 283
At2g38610  : DFIKRQQLRELALLNSNNLREESPG--PSGGGSVSPFN-S-SGKRPKTGC : 286
Nt_BAD06470: DYIKRQQLHELAMLNSN-FREDSPG--PSG--SVSPFN-SGGLKRPKTGR : 285
Mt_ABE77708: ------------IIC----------------------------------- : 237
             DY-KRQQLRELA-LNST-LRE-SP---P---GSVSPF--S-GMKRAKTG-
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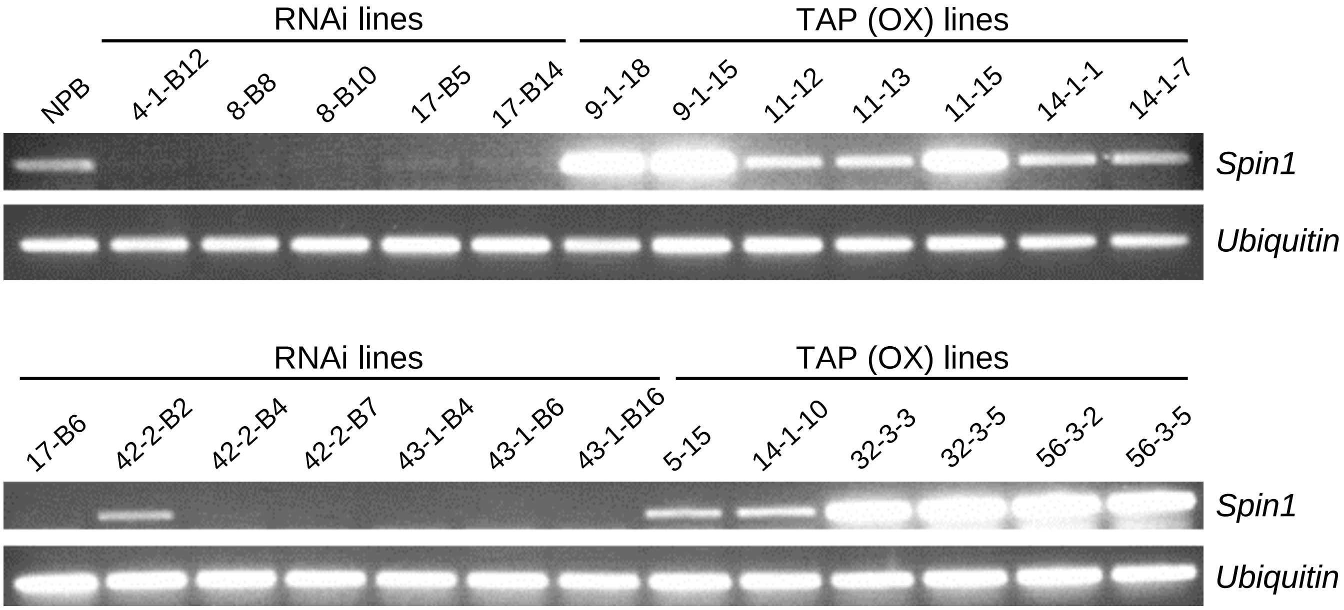
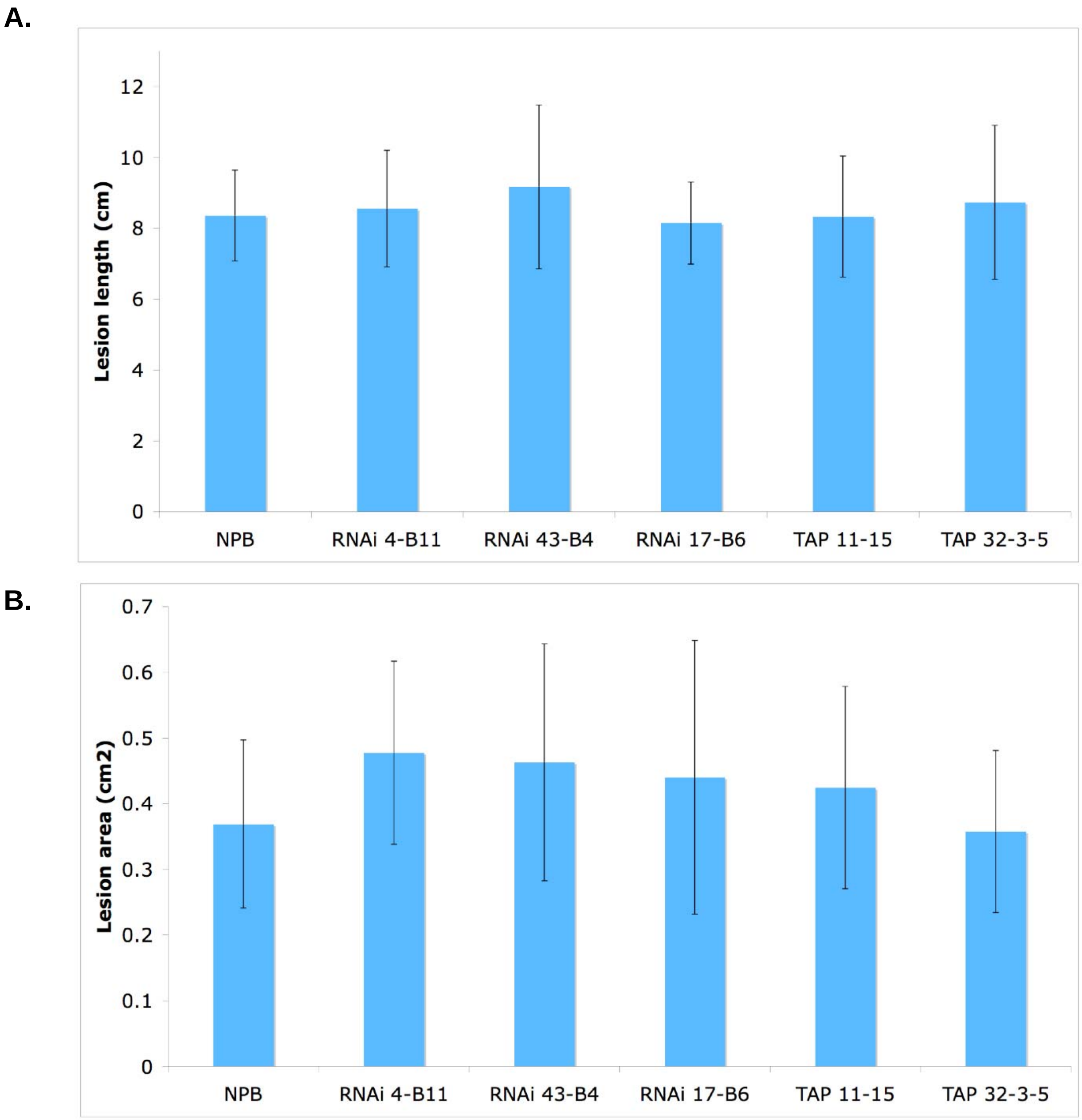
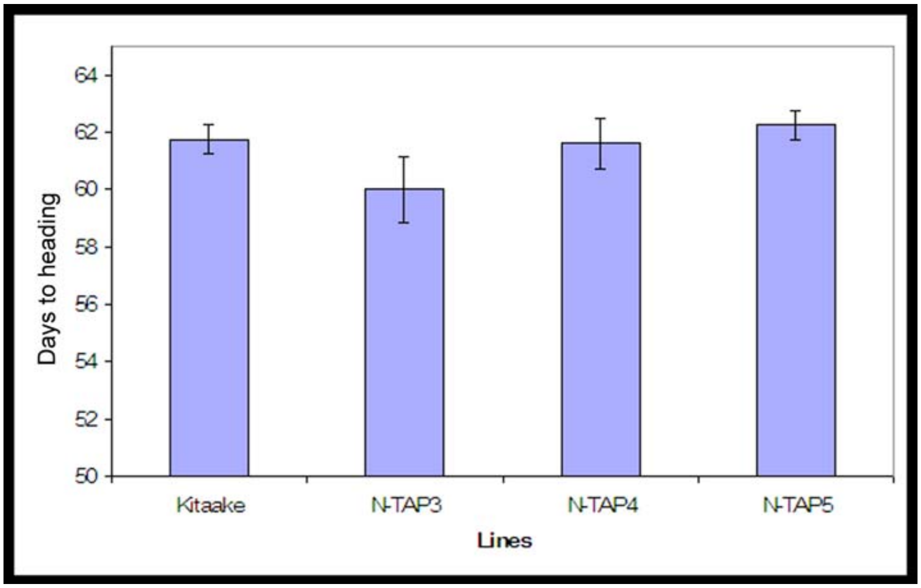
Supplemental figure 2. Alignment of plant SPIN1-like proteins. Alignment was done as described for supplemental figure 1.



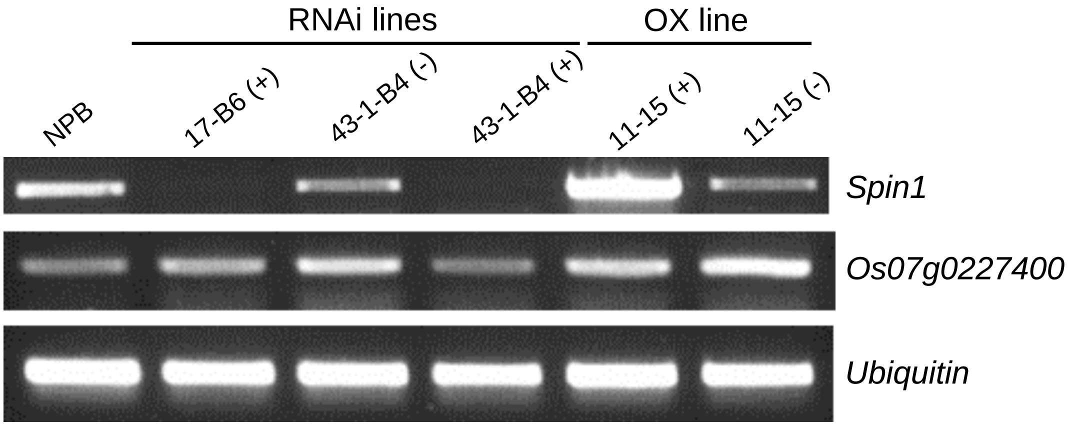
Supplemental Figure 3. RT-PCR analysis of *Spin1* expression in T2 transgenic RNAi and TAP (overexpression) lines. NPB: Nipponbare (recipient cultivar).



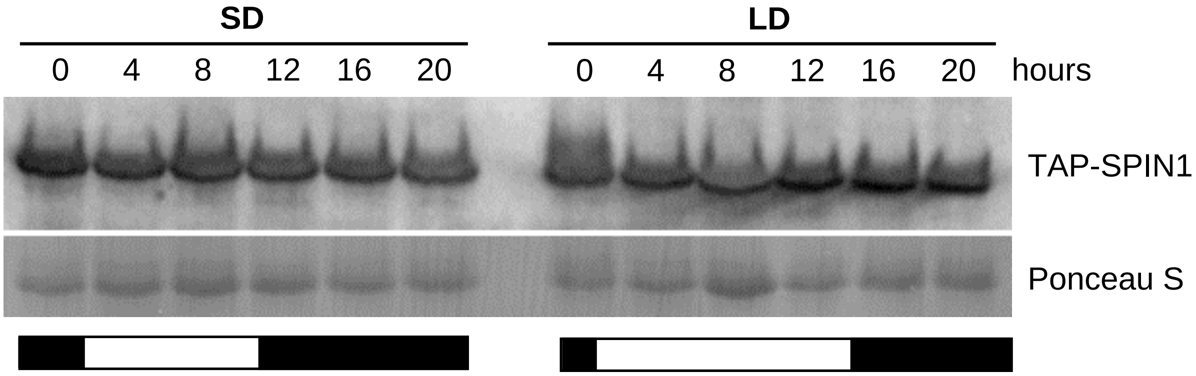
Supplemental figure 4. Rice blast and bacterial blight inoculation response of *Spin1*-RNAi and *Spin1*-OX lines. **A.** Lesion length of *Spin1* transgenic plants inoculated with *X. oryzae* pv. *oryzae* race J22. TAP lines are lines overexpressing *Spin1*. **B.** Lesion area of *Spin1* transgenic plants inoculated with *M. oryzae* isolate Che86. Error bars: +/- standard deviation. A minimum Of 10 leaves per genotype were inoculated.



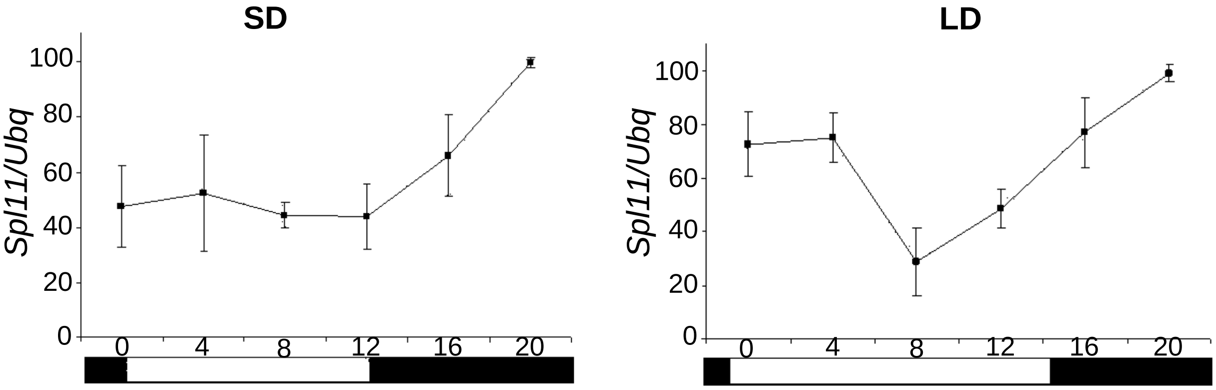
Supplemental Figure 5. Days to heading in NTAP transgenic lines transformed with the empty TAP tag vector used for generation of *Spin1*-TAP lines. Recipient cultivar for transformation is Kitaake.



Supplemental Figure 6. Expression analysis of *Spin1* and its closest paralog *Os07g0227400* by RT-PCR in T5 *Spin1*-RNAi and *Spin1*-TAP lines. +/- indicates sibling containing or not the transgene, respectively.



Supplemental Figure 7. Accumulation of TAP-SPIN1 protein in SD and LD over a 24h period. Ponceau S staining of the blot after transfer is shown as loading control. Western blot performed with PAP antibody.



Supplemental figure 8. RT-PCR analysis of *Spl11* expression in Spin1-TAP (OX) line #11-15 in SD and LD over a 24h period. White and black boxes indicate light and dark periods. Error bars are +/- standard deviation from an average of three experiments.

Supplemental Table 1. Summary of yeast-two hybrid screen using SPL11-ARM as bait

Gene name	Independent positive clones	Interactive strength	Homolog/putative function
Spin1	11	Strong	KH domain/Pre-mRNA processing
Spin2	7	Intermediate	Putative myosin heavy chain
Spin3	4	Between strong and intermediate	MOM-like protein
Spin4	2	Between strong and intermediate	Pre-mRNA cleavage complex II protein Pcf11, S-locus protein 4-like protein
Spin5	2	Between strong and intermediate	Acetylglutamate kinase-like protein
Spin6	1	Weak	Rho-GTPase activating protein
Spin7	1	Between strong and intermediate	No hits
Spin8	1	Intermediate	No hits

Supplemental Table 2. List of primers and constructs used in this study

Primer	Sequence	Purpose
SPIN1-NF	5’CGCGTCGACCATGTCGGGGCTGTA CAGC3’	pPC86SPIN1-N construct
SPIN1-NR	5’CCCGCGGCCGCTACAACATGAGAA CTAG3’	
SPIN1-KHF	5’CGCGTCGACCAAGATTCTGCGTTTGGATG3’	pPC86SPIN1-KH construct
SPIN1-KHR	5’CCCGCGGCCGCCACTGGTTTTAGCAGTT3’	
SPIN1-QR	5’CCCGCGGCCGCAGGATGGGGGCTGTCCTC3’	pPC86SPIN1-KHQ
SPIN1-CR	5’CCCGCGGCCGCCTACTGGCCCGTTTTCG3’	pPC86SPIN1-C
Spin1-XhoI F	5’GCGCTCGAGCTATGTCGGGGCTGTACAG3’	To clone into pGD series
Spin1-BamHI R	5’GCGGGATCCCTACTGGCCCGTTTTCGC3’	
SPIN1HAR	5’GCGGGATCCCTAAGCGTAATCTGGAACATCGTATGGGTACTGGCCCGTTTTCGCGC3’	C terminal HA (underlined)
spin1rnai-f	5’CACCAGCCAAGAAATTATGCGG3’	RNAi construct
spin1rnai-r	5’ACATGAGAACTAGGGCTTGG3’	
Spin1tap F	5’CACCATGTCGGGGCTGTACAGCCC3’	Overexpression construct
Spin1tap R	5’CTACTGGCCCGTTTTCGCGCGTTTC3’	
spin1gexF	5’GCGGGATCCATGTCGGGGCTGTACAGCC3’	pGEXSPIN1 construct
spin1gexR	5’GCGCTCGAGCTACTGGCCCGTTTTCGCGC3’	
spin1NgexR	5’GCGCTCGAGCTAAACATGAGAACTAGGGC3’	pGEXSPIN1-N construct
SPIN1-BKHF	5’CCCGGATCCAAGATTCTGCGTTTGGATG3’	pGEXSPIN1-KH construct
SPIN1-XKHR	5’CCCCTCGAGCTACACTGGTTTTAGCAGTT3’	
SPIN1-XCR	5’CCCCTCGAGCTACTGGCCCGTTTTCG3’	pGEXSPIN1-C construct
11Y2Harm-F	5’ATAAGCTAGCCCAGACACTGAGGAGCAGAG3’	Spl11-ARM in pDBLeu
11Y2Harm-R	5’TCTACCATGGCTCTTGTTGCTGGACTAGGAA3’	
Y2HPPC86F	5’TATAACGCGTTTGGAATCACT3’	Screening Y2H positive clones
Y2HPPC86R	5’GTAAATTTCTGGCAAGGTAGAC3’	
Spin1cdF	5’TTAGGAGCAACCCGACGGATG3’	Spin1 RT-PCR
Spin1cdR	5’GGGTCACTCAGATGTTCGTAGC3’	Spin1 RT-PCR
UbqF	5’AAGAAGCTGAAGCATCCAGC3’	Ubiquitin RT-

		PCR
UbqR	5’CCAGGACAAGATGATCTGCC3’	
Spin1NB-F	5’GCGGTCGACATGTCGGGCCTGTACAGC3’	BiFC SPIN1 N terminus
Spin1NB-R	5’GCGGGATCCAACATGAGAACTAGGGC3’	
Spin1CB-F	5’GCGGTCGACATGAAGATTCTGCGTTTG3’	BiFC SPIN1 C terminus
Spin1CBR	5’GCGGGATCCCTGGCCCGTTTTCGCGC3’	