# Supplemental Figure S1

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AtSnRK1.1       MFKRVDEFNLVSSTIDHRIFKSRMDGSG-TGSRSGVESILPNYKLGRTLGIGSFGRVKIA 59
SlSnRK1         ----------------------MDGTA-VQGTSSVDSFLRNYKLGKTLGIGSFGKVKIA 36
AtSnRK1.2       ----------------------MDHSSNRFGNNGVESILPNYKLGKTLGIGSFGKVKIA 37
AtSnRK1.3       ----------------------MDGSS-EKTTNKLVSILPNYRIGKTLGHGSFAKVKLA 36
AtSnRK2.1       ---------------------------------------MDKYDVVKDLGAGNFGVARLL 21
AtSnRK2.2       --------------------MDPATNSPIMPIDLPIMHDSDRYDFVKDIGSGNFGVARLM 40
AtSnRK2.3       --------------------MDRAP-VTTGPLDMPIMHDSDRYDFVKDIGSGNFGVARLM 39
AtSnRK3.1       ----------------------MEK---------KGSVLMLRYEVGKFLGQGTFAKVYHA 29
AtSnRK3.2       ----------------------MEN---------KPSVLTERYEVGRLLGQGTFAKVYFG 29
AtSnRK3.3       ----------------------MESPYPKSPEKITGTVLLGKYELGRRLGSGSFAKVHVA 38
                                                             .* . : :* *.*. .

AtSnRK1.1       EHALTGHKVAIKILNRRKIKNMEMEEKVRREIKILR-LFMHPHIIRLYEVIETPTDIYLV 118
SlSnRK1         EHTLTGHKVAVKILNRRKIRNMDMEEKVRREIKILR-LFMHPHIIRLYEVIETPSDIYVV 95
AtSnRK1.2       EHVVTGHKVAIKILNRRKIKNMEMEEKVRREIKILR-LFMHPHIIRQYEVIETTSDIYVV 96
AtSnRK1.3       LHVATGHKVAIKILNRSKIKNMGIEIKVQREIKILR-FLMHPHIIRQYEVIETPNDIYVV 95
AtSnRK2.1       RHKDTKELVAMKYIER----GRKIDENVAREIINHR-SLKHPNIIRFKEVILTPTHLAIV 76
AtSnRK2.2       TDRVTKELVAVKYIER----GEKIDENVQREIINHR-SLRHPNIVRFKEVILTPSHLAIV 95
AtSnRK2.3       RDKLTKELVAVKYIER----GDKIDENVQREIINHR-SLRHPNIVRFKEVILTPTHLAII 94
AtSnRK3.1       RHLKTGDSVAIKVIDKERILKVGMTEQIKREISAMR-LLRHPNIVELHEVMATKSKIYFV 88
AtSnRK3.2       RSNHTNESVAIKMIDKDKVMRVGLSQQIKREISVMR-IAKHPNVVELYEVMATKSRIYFV 88
AtSnRK3.3       RSISTGELVAIKIIDKQKTIDSGMEPRIIREIEAMRRLHNHPNVLKIHEVMATKSKIYLV 98
                    * . **:* :::       :  .: ***   *    **::::.  **: * . : .:

AtSnRK1.1       MEYVNSGELFDYIVEKGRLQEDEARNFFQQIISGVEYCHRNMVVHRDLKPENLLLDSKCN 178
SlSnRK1         MEYVKSGELFDYIVEKGRLQEDEARNFFQQIISGVEYCHRNMVVHRDLKPENLLLDSKWN 155
AtSnRK1.2       MEYVKSGELFDYIVEKGRLQEDEARNFFQQIISGVEYCHRNMVVHRDLKPENLLLDSRCN 156
AtSnRK1.3       MEYVKSGELFDYIVEKGKLQEDEARHLFQQIISGVEYCHRNMIVHRDLKPENVLLDSQCN 155
AtSnRK2.1       MEYASGGELFDRICTAGRFSEAEARYFFQQLICGVDYCHSLQICHRDLKLENTLLDGSPA 136
AtSnRK2.2       MEYAAGGELYERICNAGRFSEDEARFFFQQLISGVSYCHAMQICHRDLKLENTLLDGSPA 155
AtSnRK2.3       MEYASGGELYERICNAGRFSEDEARFFFQQLLSGVSYCHSMQICHRDLKLENTLLDGSPA 154
AtSnRK3.1       MEHVKGGELFNKVST-GKLREDVARKYFQQLVRAVDFCHSRGVCHRDLKPENLLLDEHGN 147
AtSnRK3.2       IEYCKGGELFNKVAK-GKLKEDVAWKYFYQLISAVDFCHSRGVYHRDIKPENLLLDDNDN 147
AtSnRK3.3       VEYAAGGELFTKLIRFGRLNESAARRYFQQLASALSFCHRDGIAHRDVKPQNLLLDKQGN 158
                :*:  .***:  :   *:: *  *   * *:  .:.:**   : ***:* :* ***

AtSnRK1.1       --VKIADFGLSNIM---RDGHFLKTSCGSPNYAAPEVISGKLYAGPEVDVWSCGVILYAL 233
SlSnRK1         --VKIADFGLSNIM---RDGHFLKTSCGSPNYAAPEVISGKLYAGPEVDVWSCGVILYAL 210
AtSnRK1.2       --IKIADFGLSNVM---RDGHFLKTSCGSPNYAAPEVISGKLYAGPEVDVWSCGVILYAL 211
AtSnRK1.3       --IKIVDFGLSNVM---HDGHFLKTSCGSPNYAAPEVISGKPYG-PDVDIWSCGVILYAL 209
AtSnRK2.1       PLLKICDFGYSKSS---ILHSRPKSTVGTPAYIAPEVLSRREYDGKHADVWSCGVTLYVM 193
AtSnRK2.2       PRLKICDFGYSKSS---VLHSQPKSTVGTPAYIAPEILLRQEYDGKLADVWSCGVTLYVM 212
AtSnRK2.3       PRLKICDFGYSKSS---VLHSQPKSTVGTPAYIAPEVLLRQEYDGKIADVWSCGVTLYVM 211
AtSnRK3.1       --LKISDFGLSALSDSRRQDGLLHTTCGTPAYVAPEVISRNGYDGFKADVWSCGVILFVL 205
AtSnRK3.2       --LKVSDFGLSALADCKRQDGLLHTTCGTPAYVAPEVINRKGYEGTKADIWSCGVVLFVL 205
AtSnRK3.3       --LKVSDFGLSALPEHRSNNGLLHTACGTPAYTAPEVIAQRGYDGAKADAWSCGVFLFVL 216
                  :*: *** *           ::: *:* * ***::  . *    .* ***** *:.:

AtSnRK1.1       LCGTLPFDDENIPNLFKKIKGGIYTLPS------HLSPGARDLIPRMLVVDPMKRVTIPE 287
SlSnRK1         LCGTLPFDDENIPNLFKKIKGGIYTLPS------HLSAGARDLIPRMLIVDPMKRMTIPE 264
AtSnRK1.2       LCGTLPFDDENIPNLFKKIKGGIYTLPS------HLSSEARDLIPRMLIVDPVKRITIPE 265
AtSnRK1.3       LCGTLPFDDENIPNVFEKIKRGMYTLPN------HLSHFARDLIPRMLMVDPTMRISITE 263
AtSnRK2.1       LVGAYPFEDPNDPKNFRKTIQRIMAVQYKIPDYVHISQECKHLLSRIFVTNSAKRITLKE 253
AtSnRK2.2       LVGAYPFEDPQEPRDYRKTIQRILSVTYSIPEDLHLSPECRHLISRIFVADPATRITIPE 272
AtSnRK2.3       LVGAYPFEDPEEPRDYRKTIQRILSVKYSIPDDIRISPECCHLISRIFVADPATRISIPE 271
AtSnRK3.1       LAGYLPFRDSNLMELYKKIGKAEVKFPN------WLAPGAKRLLKRILDPNPNTRVSTEK 259
AtSnRK3.2       LAGYLPFHDTNLMEMYRKIGKADFKCPS------WFAPEVKRLLCKMLDPNHETRITIAK 259
AtSnRK3.3       LAGYVPFDDANIVAMYRKIHKRDYRFPS------WISKPARSIIYKLLDPNPETRMSIEA 270
                * *  ** * :    :.*                 ::    :: :::  :   *::

AtSnRK1.1       IRQHPWFQAHLPRYLAVPPPDTVQQAKKI-------------DEEILQEVINMGFDRNHL 334
SlSnRK1         IRLHPWFQAHLPRYLAVPPPDTTQQAKKI-------------DEEILQEVVKMGFDRNNL 311
AtSnRK1.2       IRQHRWFQTHLPRYLAVSPPDTVEQAKKI-------------NEEIVQEVVNMGFDRNQV 312
AtSnRK1.3       IRQHPWFNNHLPLYLSIPPLDTIDQAKKI-------------EEEIIQNVVNIGFDRNHV 310
AtSnRK2.1       IKNHPWYLKNLPKELLESA-QAAYYKRDT-------------SFSLQSVEDIMKIVGEAR 299
AtSnRK2.2       ITSDKWFLKNLPGDLMDEN-RMGSQFQEP-------------EQPMQSLDTIMQIISEAT 318
AtSnRK2.3       IKTHSWFLKNLPADLMNES-NTGSQFQEP-------------EQPMQSLDTIMQIISEAT 317
AtSnRK3.1       IMKSSWFRKGLQEEVK--ESVEEETEVDA----EAEGNASAEK----EKKRCINLNAFEI 309
AtSnRK3.2       IKESSWFRKGLHLKQKKMEKMEKQQVREATNPMEAGGSGQNENGENHEPPRLATLNAFDI 319
AtSnRK3.3       VMGTVWFQKSLEISEFQSSVFELDRFLEK-----------------EAKSSNAITAFDL 312
                :    *:   *                .                   .     :
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# Supplemental Figure S1 contd.

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AtSnRK1.1       IESLRNRTQNDGTVTYYLILDNRFR--ASSGYLGAEFQETMEG-TPRMHPAESVASPVSH 391
SlSnRK1         TESLRNRVQNEGTVAYYLLLDNRHR--VSTGYLGAEFQESMEYGYNRINSNETAASPVGQ 369
AtSnRK1.2       LESLRNRTQNDATVTYYLLLDNRFR--VPSGYLESEFQETTWF----------------- 353
AtSnRK1.3       VDSLANRIQNEATVAYHLILDNRNQNSVPNDPFQSKFKEISDGIFNSTLPVQNITSHVGH 370
AtSnRK2.1       NPAPSTSAVKSSGSGADEEEEEDVEAEVEEEEDDEDEYEKHVKEAQSCQESDKA------ 353
AtSnRK2.2       IPTVRNRCLDDFMADN-LDLDDDMDDFDSESEIDVDSSGEIVYAL--------------- 362
AtSnRK2.3       IPAVRNRCLDDFMTDN-LDLDDDMDDFDSESEIDIDSSGEIVYAL--------------- 361
AtSnRK3.1       ISLSTGFDLSGLFEKGEEKEEMRFTSNREASEITEKLVEIGKDLKMKVRKK-EHEWRVKM 368
AtSnRK3.2       IALSTGFGLAGLFGDVYDKRESRFASQKPASEIISKLVEVAKCLKLKIRKQGAGLFKLER 379
AtSnRK3.3       ISLSSGLDLSGLFER-RKRKEKRFTARVSAERVVEKAGMIGEKLGFRVEKK--EETKVVG 369
                                        :               .

AtSnRK1.1       RLPGLMEYQGVGLRSQYPVERKWALGLQSRAHPREIMTEVLKALQDLNVCWKKIGHYNMK 451
SlSnRK1         RFPGIMDYQQAGAR-QFPIERKWALGLQSRAHPREIMTEVLKALQELNVCWKKIGQYNMK 428
AtSnRK1.2       ---------------------------QSYAHT--------------------------- 359
AtSnRK1.3       SFSALYGLKSNVKD-----DKTWTLGLQSQGSPYDIMTEIFKALQNLKICWKKIGLYNIK 425
AtSnRK2.1       ------------------------------------------------------------
AtSnRK2.2       ------------------------------------------------------------
AtSnRK2.3       ------------------------------------------------------------
AtSnRK3.1       SAEAT----VVEAEVFEIAPSYHMVVLKKSGGDTAEYKRVMK--ESIRPALIDFVLAWH- 421
AtSnRK3.2       VKEGKNGILTMDAEIFQVTPTFHLVEVKKCNGDTMEYQKLVE--EDLRPALADIVWVWQG 437
AtSnRK3.3       LGKGR---TAVVVEVVEFAEGLVVADVKVVVEGEEEEEEVESHWSELIVELEEIVLSWHN 426

AtSnRK1.1       CRWVPNSS--ADGMLSNSMHDNNYFGDESSIIENEAAVKSPNVVKFEIQLYKTRDDKYLL 509
SlSnRK1         CRWVPSLPGHHEGMGVNSMHGNQFFGDDSSIIENDGATKLTNVVKFEVQLYKTREEKYLL 488
AtSnRK1.2       ------------------------------------------------------------
AtSnRK1.3       CRWVRSFAYYKN----------------HTIEDECAIILPTVIKFEIQLYKVREGKYLL 468
AtSnRK2.1       ------------------------------------------------------------
AtSnRK2.2       ------------------------------------------------------------
AtSnRK2.3       ------------------------------------------------------------
AtSnRK3.1       ------------------------------------------------------------
AtSnRK3.2       EKEKEEQLLQDEQGEQEPS---------------------------------------- 456
AtSnRK3.3       ------------------------------------------------------------


AtSnRK1.1       DLQRVQGPQFLFLDLCAAFLAQLRVL 535
SlSnRK1         DLQRLQGPQFLFLDLCAAFLAQLRVL 514
AtSnRK1.2       -------------------------
AtSnRK1.3       DILRIDGPQFIFFDLCVAFLRELGVL 494
AtSnRK2.1       -------------------------
AtSnRK2.2       -------------------------
AtSnRK2.3       -------------------------
AtSnRK3.1       -------------------------
AtSnRK3.2       -------------------------
AtSnRK3.3       -------------------------
```

**Supplemental Figure S1**. Alignment of SnRK proteins from tomato and *Arabidopsis*. The following SnRK sequences were aligned using clustalW: SlSnRK1 (shown in bold; AF143743), AtSnRK1.1 (NP850488), AtSnRK1.2 (NP974375), AtSnRK1.3 (NP198760), AtSnRK2.1 (P43292), AtSnRK2.2 (Q39192), AtSnRK2.3 (Q39193), AtSnRK3.1 (P92937), AtSnRK3.2 (Q9LYQ8), AtSnRK3.3 (Q9SUL7). The invariant lysine responsible for ATP binding is shown in yellow outlined in black; K48 in *Sl*SnRK1. The activation phosphorylation site, T175 in SlSnRK1, is shown in red outlined in black.

# Supplemental Figure S2 contd.

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AtAKINβ1          MGNANGKDEDAAAGSGGADVTSSSARSNGGDPSARSRH----RRPSSDSMSSSPPGSPAR 56
SlGal83           MGNANAR-EDGAAGDGDGDGQVSGRRSNVESGIVEDHHALNSRVPSADLMVNSPPQSPHR 59
SlSip1            MGNVNGREENEGNIPSGVEGVDG--IDSGGVQDIMAVHQ-----VDGEFMGQSPPSSPRA 53
SlTau2            MGNVNGREE-----------ID---QSSVGIQETMDAR-------DGEFMGQSPPSSPRA 39
AtAKINβ2          MGNVNAREEANSNNASAVEDED----AEICSREAMSAASDGNHVAPPELMGQSPPHSPRA 56
SlTau1            MGNVSGKKKEGESAESSGIKNQ-----EHGEEEYMEYG------LFPDSMVQSPPHSPKA 49
HsAMPKβ1          MGNTSSERAALERHGGHKTPRR----------------------DSSGGTKDGDRPK 35
HsAMPKβ2          MGNTTSDRVSGERHG-AKAARS----------------------EGAGGHAPGKEHK 34
                  ***...                                      :       .

AtAKINβ1          -SPSPFLFAPQVPVAPLQRANAPPPNNIQW-NQS-QRVFDNPP-EQGIPTIITWNQGGND 112
SlGal83           -SASPLLFGPQVPVVPLQGGDGNPVSNQMWGNEC-EDASDHSL-EGGIPTLITWSYGGNN 116
SlSip1            -SRSPLMFRPEMPVVPLQRPDEGHGPSISWSQT--TSGYEEPCDEQGVPTLISWTLDGKE 110
SlTau2            -SHSPLMFRPQMPVVPLQRPEELHISNPSWMQN--TSGYEDLNEEKGVPTLISWTYEGKD 96
AtAKINβ2          -TQSPLMFAPQVPVLPLQRPDEIHIPNPSWMQSP-SSLYEEASNEQGIPTMITWCHGGKE 114
SlTau1            YHHSPLDFTPQVPIFPLQRPDEILMQNQSGNIVQKTMEYGDMPCENGIPTMITWSHGGHE 109
HsAMPKβ1          -----ILMDSPEDADLFHSEEIKAPEKEEFLAWQHDLEVNDKAPAQARPTVFRWTGGGKE 90
HsAMPKβ2          -----IMVGSTDDPSVFSLPDSKLPGDKEFVSWQQDLEDSVKPTQQARPTVIRWSEGGKE 89
                     : . .      :   :     .                    . **:: *   *::

AtAKINβ1          VAVEGSWDNWRSRKKLQKSGKDHSILFVLPSGIYHYKVIVDGESKYIPDLPFVADEVGNV 172
SlGal83           VAIQGSWDNWTSRKILQRSGKDYTVLLVLPSGIYHYKFIVDGEVRYIPELPCVADETGVV 176
SlSip1            VAVEGSWDNWKSRMPLQKSGKDFTILKVLPSGVYQYRFIVDGQWRCSPDLPCVQDEAGNT 170
SlTau2            IAVEGSWDNWKSRNILQRSGKDFTILKVLPSGVYQYRFIVDGQWRCSPDLPCVQDEAGNT 156
AtAKINβ2          IAVEGSWDNWKTRSRLQRSGKDFTIMKVLPSGVYEYRFIVDGQWRHAPELPLARDDAGNT 174
SlTau1            VAIEGSWDGWKTKDFLQRTDKDFTVMKVFPSGVYHYRFIVDGQWRYAPDYPYERDDTGNV 169
HsAMPKβ1          VYLSGSFNNWS-KLPLTRSHNNFVAILDLPEGEHQYKFFVDGQWTHDPSEPIVTSQLGTV 149
HsAMPKβ2          VFISGSFNNWSTKIPLIKSHNDFVAILDLPEGEHQYKFFVDGQWVHDPSEPVVTSQLGTI 149
                  : :.**::.*  :  * :: ::.  :  :*.* :.*:.:***:    *. *   .: *

AtAKINβ1          CNILDVHNFVPENPESIVEFEA----------PPSPDHSYGQTLPAA--EDYAKEPLAVP 220
SlGal83           FNLLDVNDNVPENLESVAEFEA----------PPSPDSSYAQALMGE--EDFEKEPVAVP 224
SlSip1            YNLLDMKDYVPEDIESISGFEP----------PQSPDSSYNNLHLVS--EDYAKEPPVVP 218
SlTau2            YNILDVKDYVPEDIESISGFEP----------PLSPDSSYSNLELGA--EDYAKEPPLVP 204
AtAKINβ2          FNILDLQDYVPEDIQSISGFEP----------PQSPENSYSNLLLGA--EDYSKEPPVVP 222
SlTau1            FNVLDLQDIIPEVLNNTNWSDA----------PPSPESSYSNAPFSS--EDFSEKLPDLP 217
HsAMPKβ1          NNIIQVKKTDFEVFDALMVDSQKCSDVS--ELSSSPPGPYHQEPYVCKPEERFRAPPILP 207
HsAMPKβ2          NNLIHVKKSDFEVFDALKLDSMESSETSCRDLSSSPPGPYGQEMYAFRSEERFKSPPILP 209
                  *::.::.   *  :     .           . **  .* :       *:  .    :*

AtAKINβ1          PQLHLTLLG--TTEETA---IATKPQHVVLNHVFIEQGWTPQSIVALGLTHRFESKYITV 275
SlGal83           PQLHLTVLGSENSEEAP---SSPKPQHVVLNHLFIEKGWASQSIVALGLTHRFQSKYVTV 281
SlSip1            PHLQMTLLNVSPSHMEI-PPPLSRPQHVVLNHLYMQKDRSTPSVVALGSTNRFLSKYVTV 277
SlTau2            PHLQMTLLNVPSSPMEILPPPLSRPQHVVLNHLYMQKGKSNPSLVALSSTNRFLFKYVTV 264
AtAKINβ2          PHLQMTLLNLPAANPDI-PSPLPRPQHVILNHLYMQKGKSGPSVVALGSTHRFLAKYVTV 281
SlTau1            PLLQQTPLDQPSSSAGS-VETFRKPLPAVLNHLYIQKTRSSQSMVVLSSTHRFRTKYVTA 276
HsAMPKβ1          PHLLQVILNKDTGISCD-PALLPEPNHVMLNHLYALS--IKDGVMVLSATHRYKKKYVTT 264
HsAMPKβ2          PHLLQVILNKDTNISCD-PALLPEPNHVMLNHLYALS--IKDSVMVLSATHRYKKKYVTT 266
                  * *  . *.             .*  .:***::  .     .::.*. *:*:  **:*.

AtAKINβ1          VLYKPLTR 283
SlGal83           VLYKPLKR 289
SlSip1            VLYKSIQS 285
SlTau2            VLYKSIQR 272
AtAKINβ2          VLYKSLQR 289
SlTau1            VLFKSLKK 284
HsAMPKβ1          LLYKPI-- 270
HsAMPKβ2          LLYKPI-- 272
                  :*:*.:
```
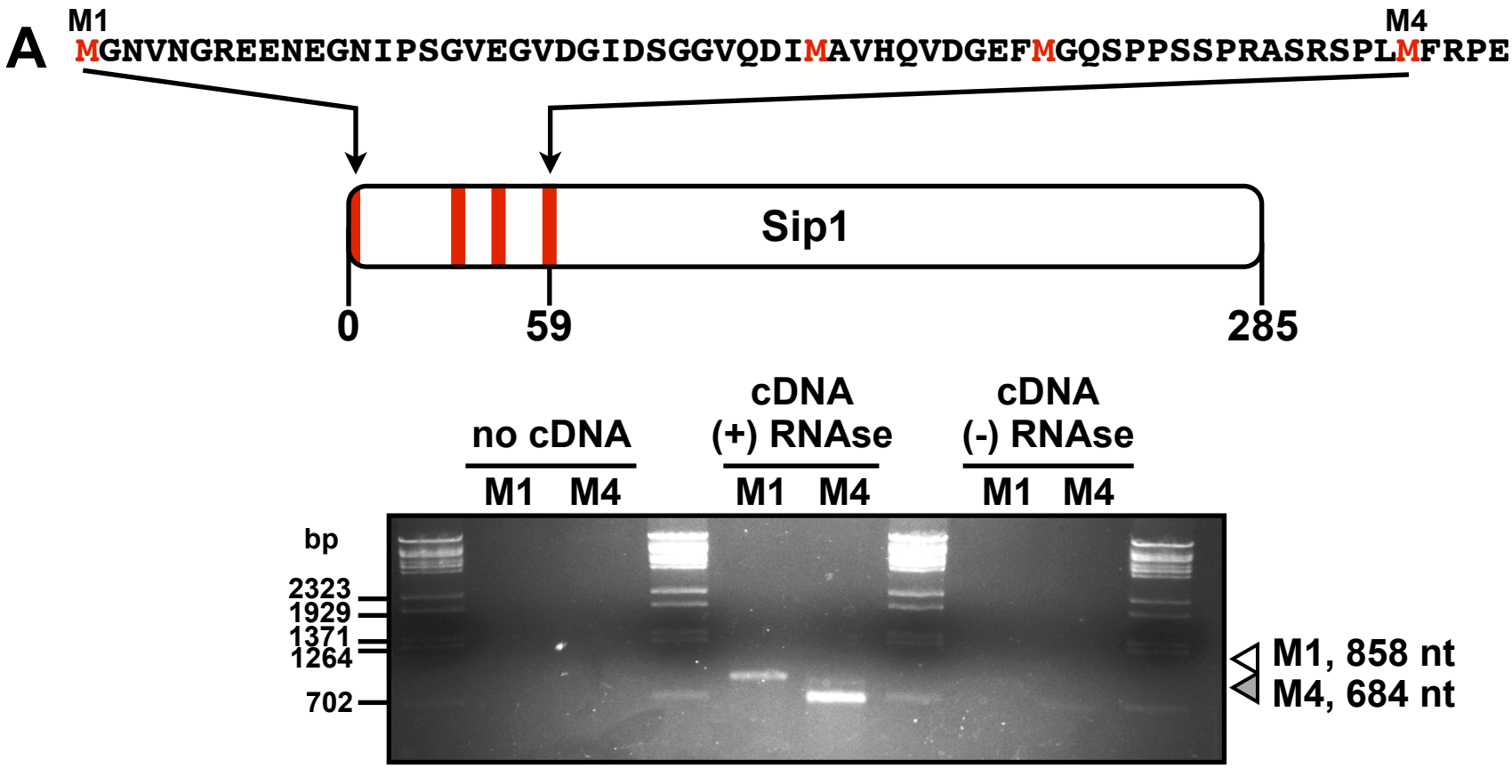
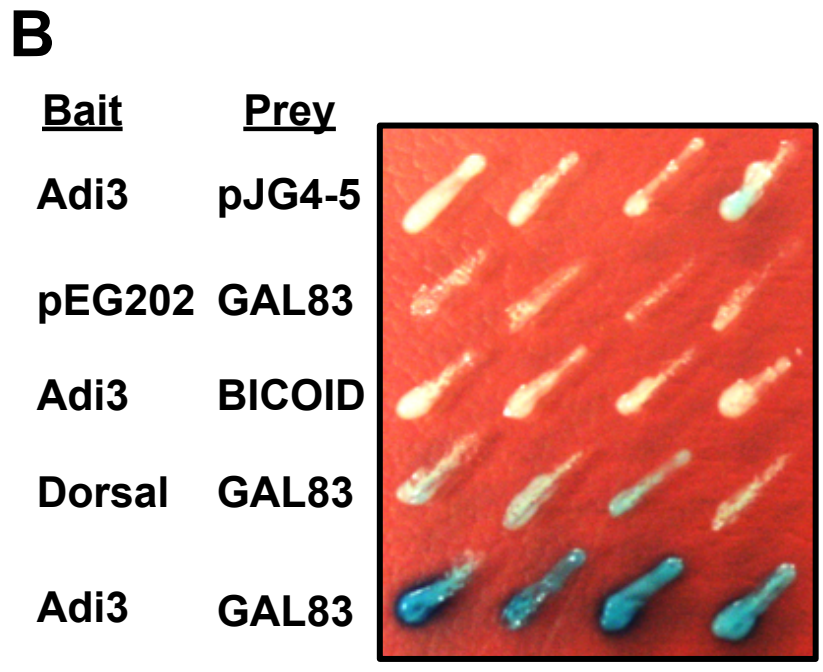

**Supplemental Figure S2**. Alignment of SnRK complex β-subunits. The following β-subunit protein sequences were aligned using clustalW: SlGal83 (JF895513), SlSip1 (JF8955212), SlTau1 (XXXX), SlTau2 (XXXX), *At*AKINβ1 (AAM6584), *At*AKINβ2 (CAB64719), *Hs*AMPKβ1 (NP_006244), and *Hs*AMPKβ2 (NP_005390). The N-terminal end of the original SlGal83 (D18) is shown in purple and outlined in black. The Adi3 phosphorylation site on SlGal83 (S26) and the MS identified phosphorylation site S30 are shown in red outlined in black. The originally identified SlSip1 start site (M33) is shown in orange outlined in black. The HsAMPKβ1 phosphorylation sites are shown in pink outlined in black.

# Supplemental Figure S3

Avila et al.




**Supplemental Figure S3**. RT-PCR amplification of SlSip1 and Adi3/SlGal83 yeast two-hybrid interaction. A, Schematic of SlSip1 showing originally identified start site (M4) and newly identified start site (M1). Also shown below is the RT-PCR gel showing amplification of the larger SlSip1 from tomato leaf total RNA. B, Yeast two-hybrid interaction between Adi3 and Gal83. The indicated bait and prey constructs were tested in a standard yeast two-hybrid assay for expression of the *lacZ* gene on X-Gal plates.

# Supplemental Figure S4

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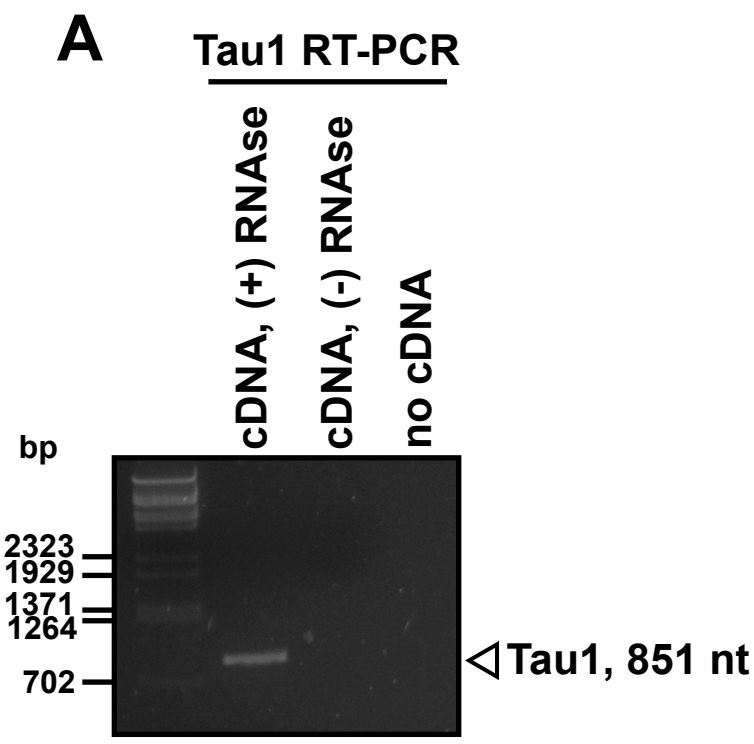
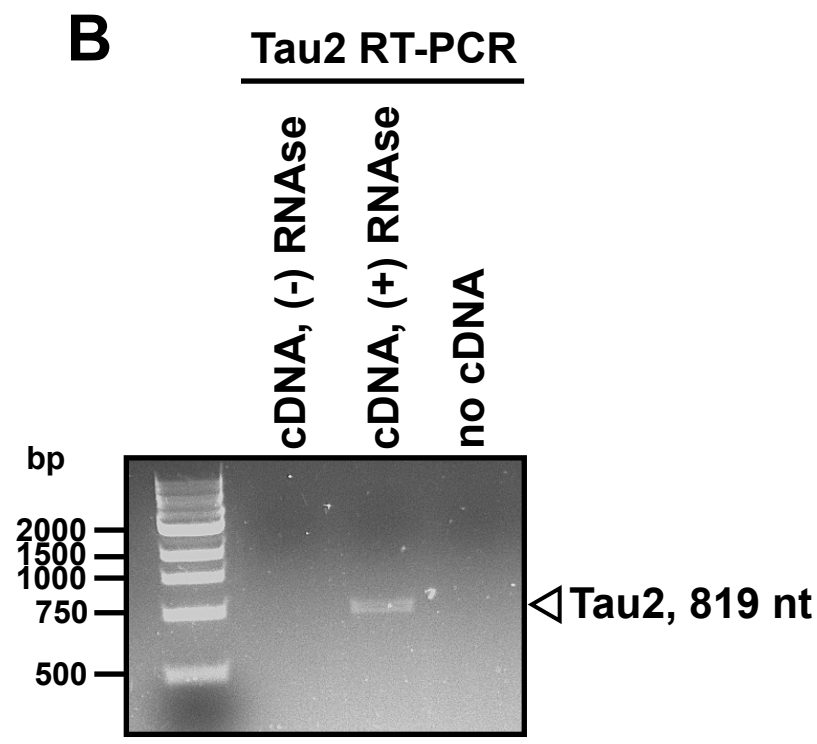
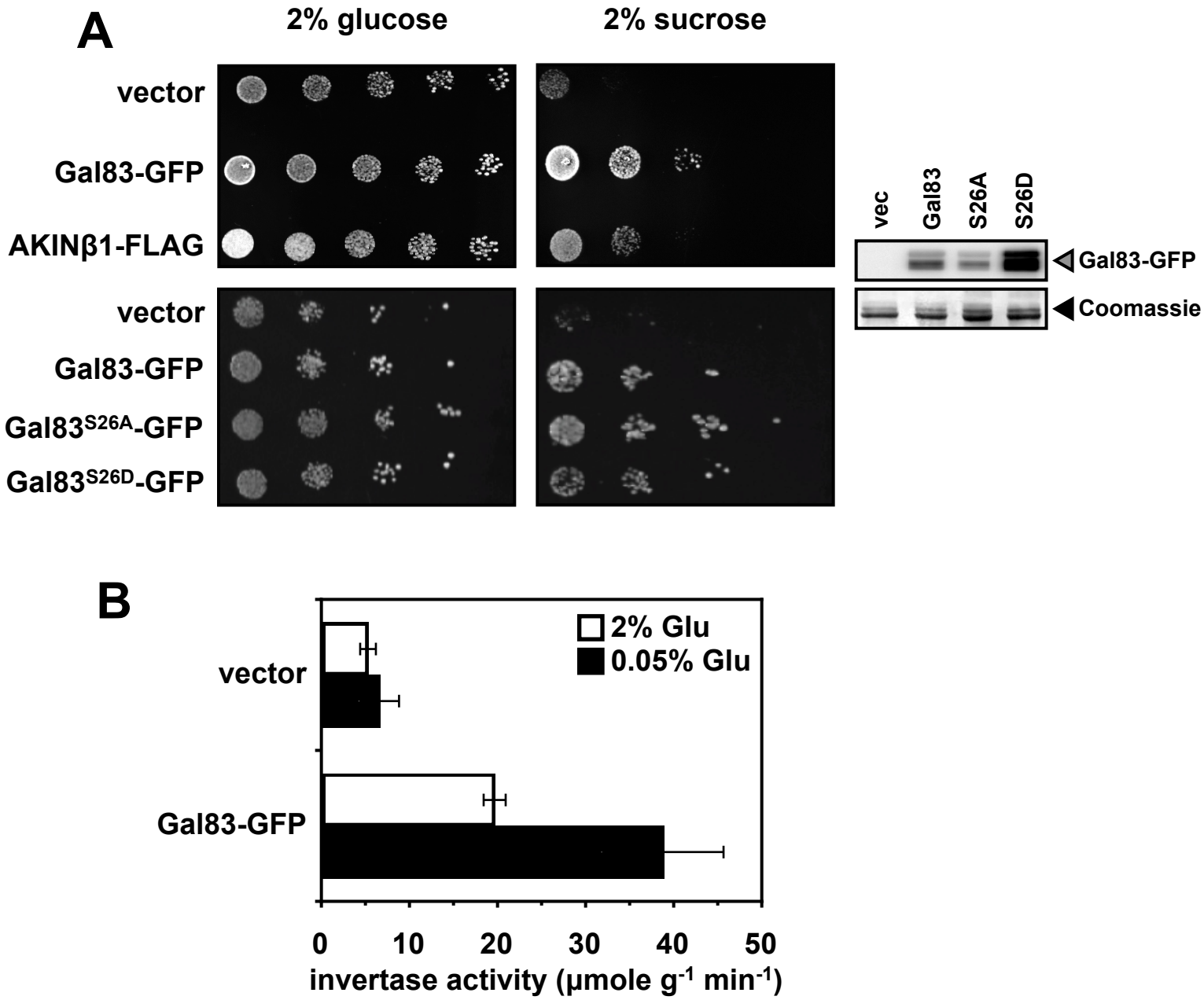



**Supplemental Figure S4**. RT-PCR amplification of SlTau1 and SlTau2. A, RT-PCR gel showing amplification of the SlTau1 cDNA from tomato leaf total RNA. B, RT-PCR gel showing amplification of the SlTau2 cDNA from tomato leaf total RNA.

# Supplemental Figure S5

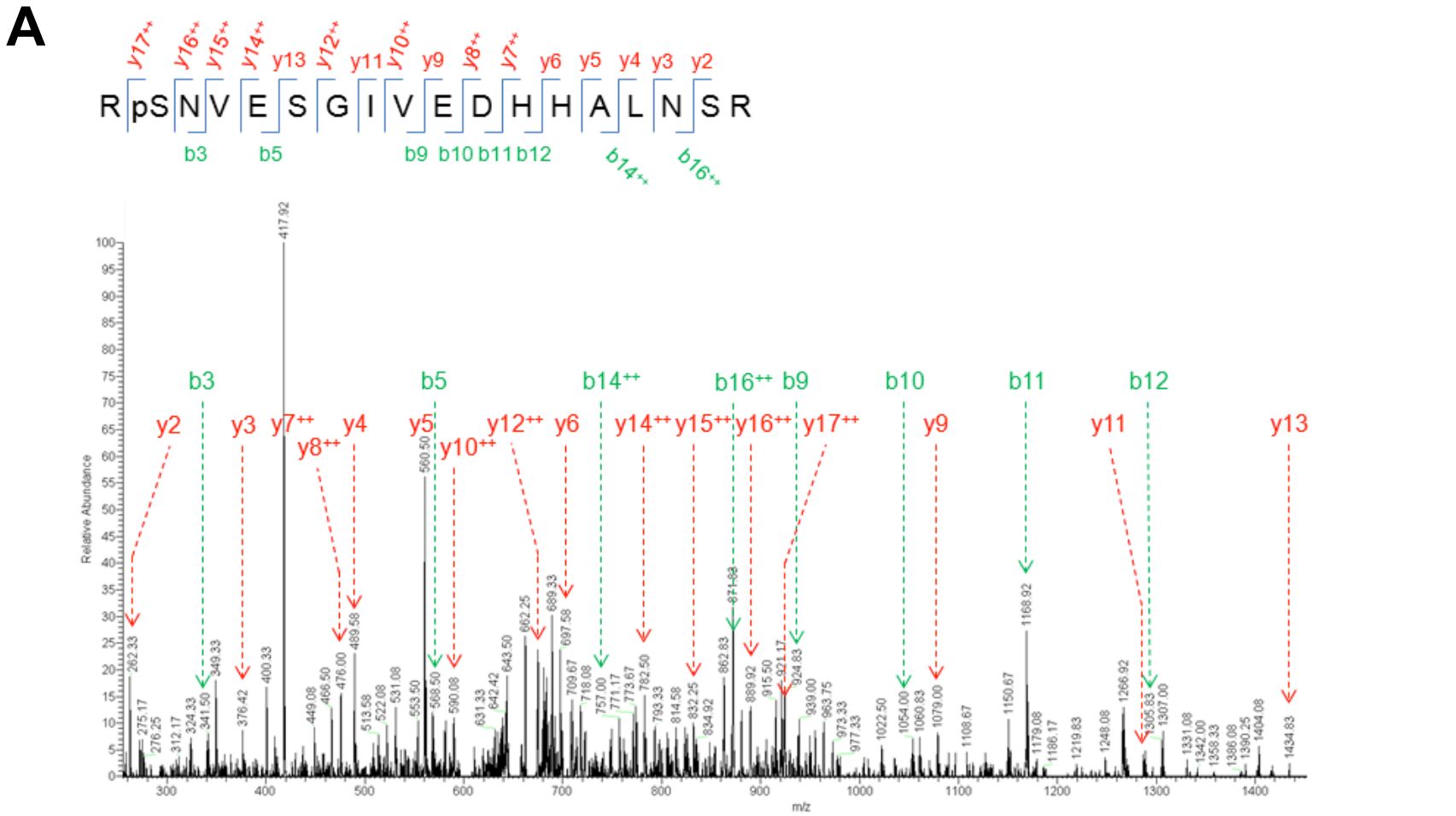
Avila et al.




**Supplemental Figure S5**. SlGal83 complementation of *sip1Δsip2Δgal83Δ* yeast. A, Tomato Gal83 can complement the yeast β-subunit triple knockout. *sip1Δsip2Δgal83Δ* yeast cells were transformed with empty vector, the indicated SlGal83 constructs, or AKINβ1 and plated on 2% glucose or sucrose CM plates at 5-fold dilutions. SlGal83-GFP protein expression detected by α-GFP western blot is shown on the right. B, SlGal83 complementation of yeast invertase activity. The indicated constructs were transformed into *sip1Δsip2Δgal83Δ* yeast and extracts from the yeast tested for invertase activity in the presence of high (2%) and low (0.05%) glucose. Values are averages of three independent experiments and error bars are standard error.

# Supplemental Figure S6

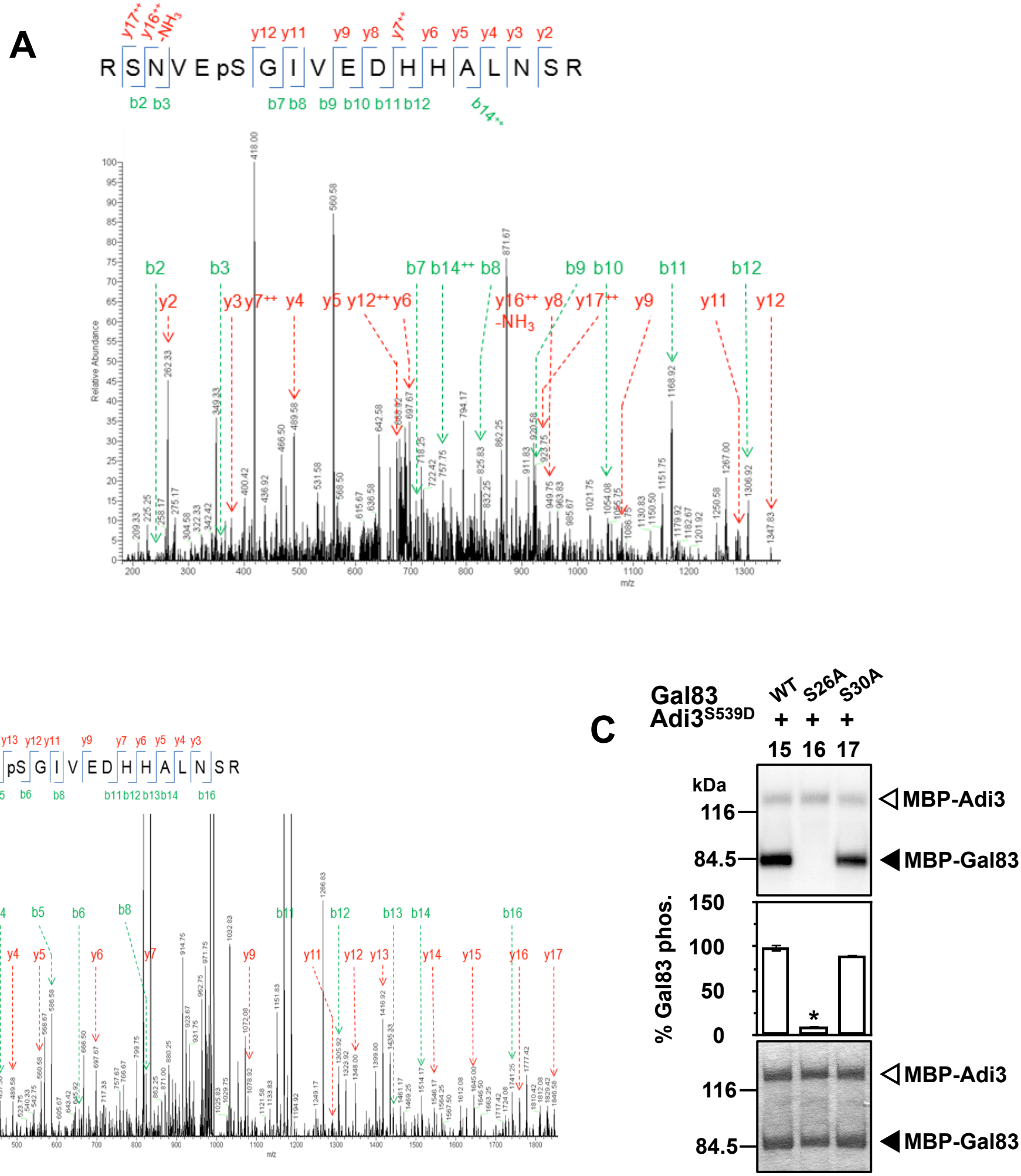
Avila et al.




**Supplemental Figure S6**. MS identification of SlGal83 S26 phosphorylation and α-GFP immunoprecipitation of SlGal83-GFP. A, Identification of SlGal83 S26 phosphorylation *in vitro* in peptide RpSNVESGIVEDHHALNSR. B, SlGal83-GFP can be pulled down with an α-GFP antibody. Protoplasts expressing SlGal83-GFP for 16 hrs were lysed, immunoprecipitated with α-GFP antibody, and analyzed by α-GFP western blot. C, Immunoprecipitated SlGal83-GFP used for MS analysis. SlGal83-GFP was expressed and immunoprecipitated as in (A) and the sample separated by SDS-PAGE. The band corresponding to SlGal83-GFP was cut from the gel, trypsin digested and analyzed by MS.

# Supplemental Figure S7

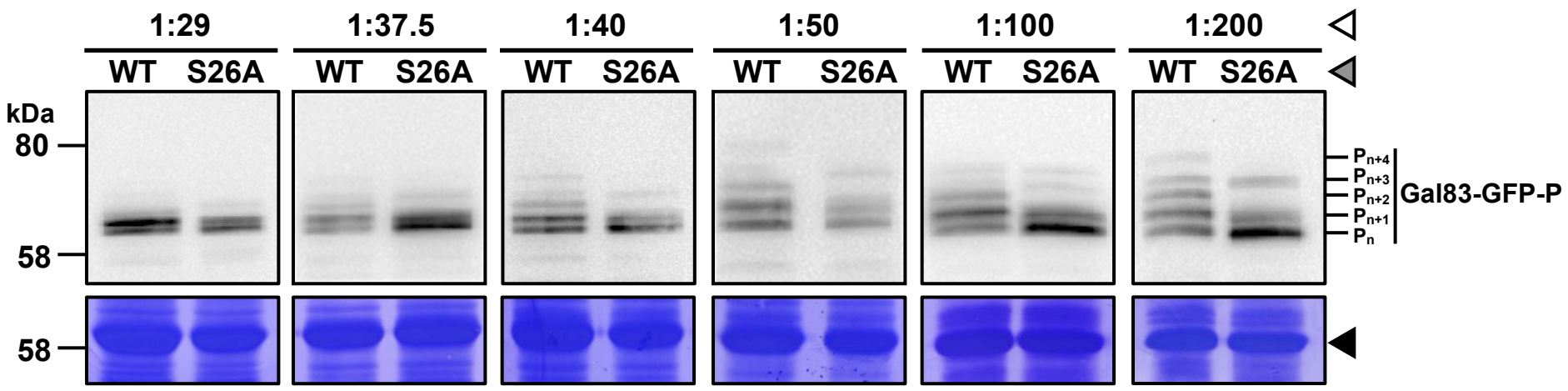
Avila et al.




**Supplemental Figure S7**. MS identification of SlGal83 S30 phosphorylation. A, Identification of SlGal83 S30 phosphorylation *in vitro* in peptide RSNVEpSGIVEDHHALNSR. B, Identification of SlGal83 S30 phosphorylation *in vivo* in peptide RSNVEpSGIVEDHHALNSR. C, Adi3 does not phosphorylate SlGal83 S30 *in vitro*. One asterisk indicates significant decrease in phosphorylation of SlGal83 Ser to Ala mutants compared to wild-type SlGal83 phosphorylation (Student's *t* test, $p < 0.05$).

# Supplemental Figure S8

Avila et al.




**Supplemental Figure S8**. Separation of SlGal83-GFP phosphoproteins by SDS-PAGE with varying bis-acrylamide:acrylamide ratios. SlGal83-GFP was stably transformed into *Arabidopsis*, protein extracts made from leaf tissue, and the extracts analyzed by SDS-PAGE. Open triangle, bis-acrylamide:acrylamide ratio; grey triangle, SlGal83-GFP protein; black triangle, RuBisCo

# Supplemental Table S1

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| Gene | Primer Name | Puropose | Direction | Restriction site | Sequence |
|---|---|---|---|---|---|
| Adi3 | Adi3 BamHI-F | Cloning into pGEX | Forward | BamHI | CACGGATCC**ATG**GAAAGGATACCTGAAGTT |
| Adi3 | Adi3 EcoRI-R | Cloning into pGEX | Reverse | EcoRI | CACGAATTC**CTA**AAAGAACTCAAAGTCAAG |
| SnRK1 | SnRK EcoRI-F | ORF Amplification Cloning into pEG202/pJG4-5 | Forward | EcoRI | CACGAATTC**ATG**GACGGAACAGCAGTG |
| SnRK1 | SnRK BamHI-R | ORF Amplification Cloning into pEG202 | Reverse | BamHI | CACGGATCC**TTA**AAGTACTCGAAGCTG |
| SnRK1 | SnRK PstIR | Cloning into pMAL | Reverse | PstI | CACCTGCAG**TTA**AAGTACTCGAAGCTG |
| SnRK1 | SnRK T175D-F | Mutagenesis | Forward | | GGTCATTTTCTGAAG**GAT**AGTTGCGGAAGCCCA |
| SnRK1 | SnRK T175D-R | Mutagenesis | Reverse | | TGGGCTTCCGCAACT**ATC**CTTCAGAAAATGACC |
| SnRK1 | SnRK K48Q-F | Mutagenesis | Forward | | CACAAAGTTGCTGTC**CAG**ATTCTTAATCGTCGA |
| SnRK1 | SnRK K48Q-R | Mutagenesis | Reverse | | TCGACGATTAAGAAT**CTG**GACAGCAACTTTGTG |
| Snf4 | Snf4 EcoRI | ORF Amplification Cloning into | Forward | EcoRI | CACGAATTC**ATG**CAGGCAACAGCGGAG |
| Snf4 | Snf4 SalI | ORF Amplification Cloning into | Reverse | SalI | CACGTCGAC**TCA**CTGCAAAAACTCAG |
| Sip1 | Sip1 EcoRI | ORF Amplification Cloning into pMAL | Forward | EcoRI | CACGAATTC**ATG**TTTAGACCTGAGATG |
| Sip1 | Sip1 BamHI | ORF Amplification Cloning into pMAL | Reverse | BamHI | CACCTCGAG**TCA**CCTCTGTATTGACTTG |
| Gal83 | Gal83 EcoRI | ORF Amplification Cloning into pMAL, | Forward | EcoRI | CACGAATTC**ATG**GGGAATGCGAACGCC |
| Gal83 | Gal83 SalIR | ORF Amplification Cloning into | Reverse | SalI | CACGTCGAC**TCA**CCTCTTCAGTGGCTTG |
| Gal83 | Gal83 BamHI | Cloning into MBB263, pTEX cGFP | Forward | BamHI | CACGGATCC**ATG**GGGAATGCGAACGCC |
| GFP | GFP EcoRI | Cloning of Gal83-GFP into MBB263 | Reverse | EcoRI | CACGAATTC**TTA**TTTGTATAGTTCATCC |
| Gal83 | Gal83 SalIR NS | Cloning Gal83 without stop codon into pTEX cGFP | Reverse | SalI | CACGTCGAC**CCT**CTTCAGTGGCTTGGTAG |
| Gal83 | S22A | Mutagenesis | Forward | | GGCGACGGT CAGGTA**GCG** GGAAGAAGA TCTAATG |
| Gal83 | S22A | Mutagenesis | Reverse | | CATTAGATCTTCTTCC**CGC**TACCTGACCGTCGCC |
| Gal83 | S26A | Mutagenesis | Forward | | CGGGAAGAAGA**GCT**AATGTTGAATCTGG |
| Gal83 | S26A | Mutagenesis | Reverse | | CCAGATTCAACATT**AGC**TCTTCTTCCG |
| Gal83 | S45A | Mutagenesis | Forward | | CGCGAGTGCCT**GCG**GCTGACTTGATGG |
| Gal83 | S45A | Mutagenesis | Reverse | | CCATCAAGTCAGC**CGC**AGGCACTCGCG |
| Gal83 | S60/62A | Mutagenesis | Forward | | GCAGAGTCCACATCGT**GCA**GCT**GCA**CCTCTCTTGTTCGG |
| Gal83 | S60/62A | Mutagenesis | Reverse | | CCGAACAAGAGAGG**TGC**AGC**TGC**ACAGTGTGGACTCTGC |
| Gal83 | S122A | Mutagenesis | Forward | | GTTGCTATCCAAGGA**GCT**TGGGACAACTGGAC |
| Gal83 | S122A | Mutagenesis | Reverse | | GTCCAGTTGTCCCA**AGC**TCCTTGGATAGCAAC |
| Gal83 | S135A F | Mutagenesis | Forward | | GGAAAATTCTCCAAAGA**GCA**GGCAAGGACTATACCG |
| Gal83 | S135A R | Mutagenesis | Reverse | | CGGTATAGTCCTTGCC**AGC**TCTTTGGAGAATTTTCC |
| Gal83 | S147A | Mutagenesis | Forward | | CTTTTGGTCCTTCCA**GCG**GGTATATATCATTAC |
| Gal83 | S147A | Mutagenesis | Reverse | | GTAATGATATATACC**CGC**TGGAAGGACCAAAAG |
| Gal83 | S192A | Mutagenesis | Forward | | CCAGAGAACCTCGAA**GCT**GTTGCAGAGTTTGAG |
| Gal83 | S192A | Mutagenesis | Reverse | | CTCAAACTCTGCAAC**AGC**TTCGAGGTTCTCTGG |
| Gal83 | S204/205A | Mutagenesis | Forward | | CCACCATCACCT**GACGCT**GCCTATGCGCAAGCTTTG |
| Gal83 | S204/205A | Mutagenesis | Reverse | | CAAAGCTTGCGCATAGG**CAGCGTC**AGGTGATGGTGG |
| Gal83 | S234A | Mutagenesis | Forward | | CTAACTGTTCTTGGT**GCT**GAAAACTCAGAAGAAGC |
| Gal83 | S234A | Mutagenesis | Reverse | | GCTTCTTCTGAGTTTTG**AGC**ACCAAGAACAGTTAG |
| Gal83 | S237A | Mutagenesis | Forward | | GGTTCTGAAAAC**GCA**GAAGAA GCACCTTC |
| Gal83 | S237A | Mutagenesis | Reverse | | GAAGGTGCTTC TTC**TGC**GTTTTC AGAACC |
| Gal83 | S242/243A | Mutagenesis | Forward | | CAGAAGAAGCACCT**GCTGCT** CCAAAACCCCAGCACG |
| Gal83 | S242/243A | Mutagenesis | Reverse | | GTGCTGGGGTTTTGG**AGCAGC**AGGTGCTTCTTCTG |
| Gal83 | S262A/S264R | Mutagenesis | Forward | | GAGAAAGGATGGGCT**GCT**CAA**GCC**ATTGTTGCTCTTGG |
| Gal83 | S262A/S264R | Mutagenesis | Reverse | | CCAAGAGCAACAAT**CCG**TTG**AGC**AGCCCATCCTTTCTC |
| Tau1 | Tau1-F | ORF amplification | Forward | | **ATG**GGGAATGTGAGTGGG |
| Tau1 | Tau1-R | ORF amplification | Reverse | | **TCA**CTTTTTCAAGGACTTAAAAAG |
| Tau1 | Tau1 EcoRI | Cloning into pMAL | Forward | EcoRI | CACGAATTC**ATG**GGGAATGTGAGTGGG |
| Tau1 | Tau1 SalI | Cloning into pMAL | Reverse | SalI | CACGTCGAC**TCA**CTTTTTCAAGGACTTAAAAAG |
| Tau2 | Tau2-F | ORF amplification | Forward | | **ATG**GGGAATGTTAATGGAAGAG |
| Tau2 | Tau2-R | ORF amplification | Reverse | | **TCA**CCTCTGTATGGACTTGTAAAG |
| Tau2 | Tau2 EcoRI | Cloning into pMAL | Forward | EcoRI | CACGAATTC**ATG**GGGAATGTTAATGGA |
| Tau2 | Tau2 SalI | Cloning into pMAL | Reverse | SalI | CACGTCGAC**TCA**CCTCTGTATGGACTTGTA |

**Supplemental Table S1**. Primers used in this study are listed by gene name and purpose. In the primer sequences restriction sites are underlined, start and stop codons are in bold, and mutation sites are in bold and underlined.