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Supplemental Information

Reciprocal Regulation of the TOR Kinase and ABA Receptor Balances Plant Growth and Stress Response

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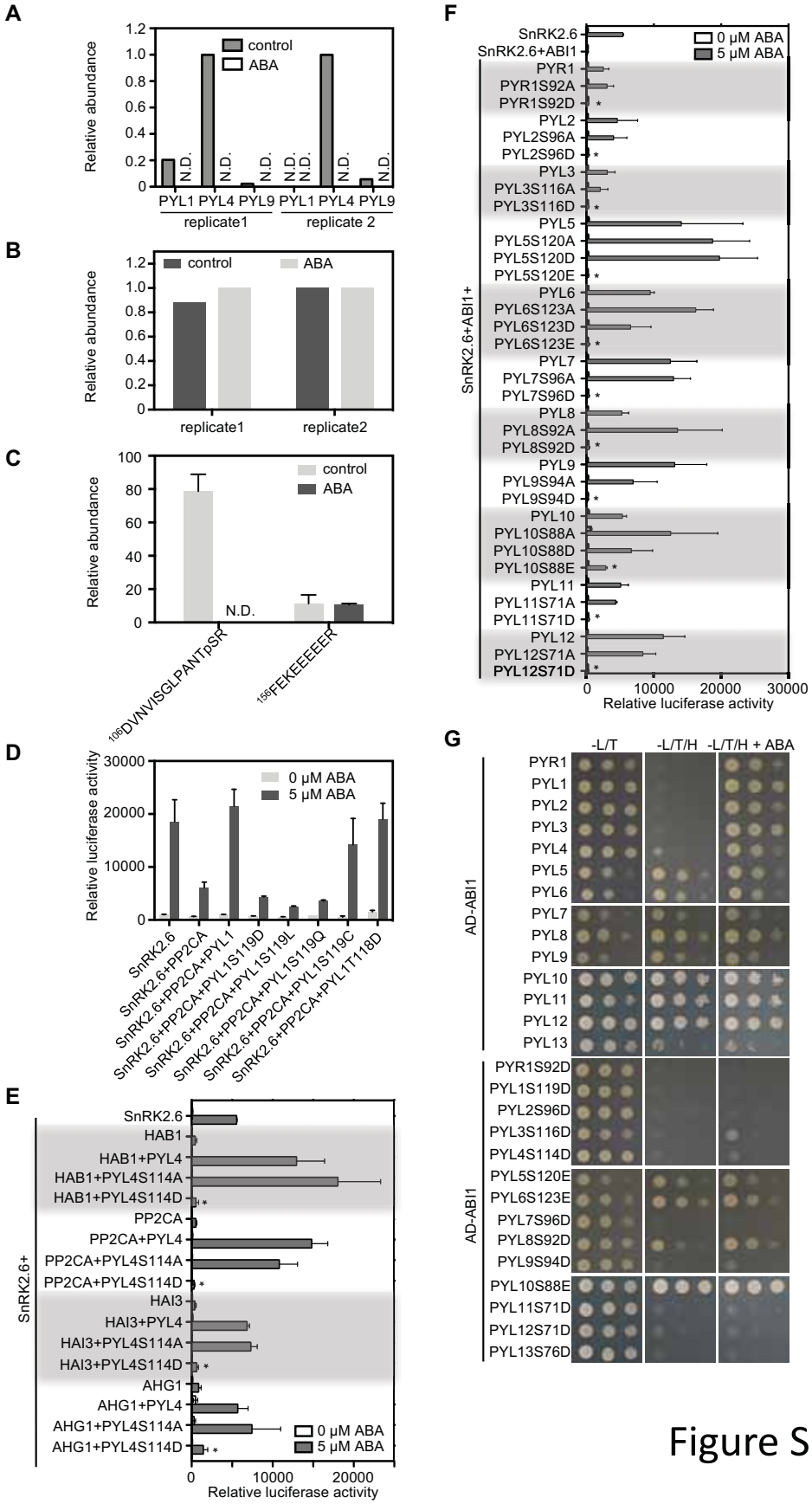

A
Relative abundance
control
ABA
N.D.
PYL1 PYL4 PYL9 PYL1 PYL4 PYL9
replicate1
replicate 2
B
Relative abundance
control
ABA
replicate1
replicate2
C
Relative abundance
control
ABA
N.D.
105DVNVISGLPANTpSR
156FEKEEEEER
D
Relative luciferase activity
0 μM ABA
5 μM ABA
SnRK2.6
SnRK2.6+PP2CA
SnRK2.6+PP2CA+PYL1
SnRK2.6+PP2CA+PYL1S119D
SnRK2.6+PP2CA+PYL1S119L
SnRK2.6+PP2CA+PYL1S119Q
SnRK2.6+PP2CA+PYL1S119C
SnRK2.6+PP2CA+PYL1T118D
E
SnRK2.6+
SnRK2.6
HAB1
HAB1+PYL4
HAB1+PYL4S114A
HAB1+PYL4S114D
PP2CA
PP2CA+PYL4
PP2CA+PYL4S114A
PP2CA+PYL4S114D
HAI3
HAI3+PYL4
HAI3+PYL4S114A
HAI3+PYL4S114D
AHG1
AHG1+PYL4
AHG1+PYL4S114A
AHG1+PYL4S114D
0 μM ABA
5 μM ABA
Relative luciferase activity
F
SnRK2.6+ABI1+
SnRK2.6
SnRK2.6+ABI1
PYR1
PYR1S92A
PYR1S92D
PYL2
PYL2S96A
PYL2S96D
PYL3
PYL3S116A
PYL3S116D
PYL5
PYL5S120A
PYL5S120D
PYL5S120E
PYL6
PYL6S123A
PYL6S123D
PYL6S123E
PYL7
PYL7S96A
PYL7S96D
PYL8
PYL8S92A
PYL8S92D
PYL9
PYL9S94A
PYL9S94D
PYL10
PYL10S88A
PYL10S88D
PYL10S88E
PYL11
PYL11S71A
PYL11S71D
PYL12
PYL12S71A
PYL12S71D
0 μM ABA
5 μM ABA
Relative luciferase activity
G
-L/T
-L/T/H
-L/T/H + ABA
AD-ABI1
PYR1
PYL1
PYL2
PYL3
PYL4
PYL5
PYL6
PYL7
PYL8
PYL9
PYL10
PYL11
PYL12
PYL13
AD-ABI1
PYR1S92D
PYL1S119D
PYL2S96D
PYL3S116D
PYL4S114D
PYL5S120E
PYL6S123E
PYL7S96D
PYL8S92D
PYL9S94D
PYL10S88E
PYL11S71D
PYL12S71D
PYL13S76D

Figure S1

Figure S1. Phosphomimic Mutations in PYLs abolish their activity in protoplasts and yeast, Related to Figure 1.

(A) Quantitative analysis of phosphopeptides containing Ser119 in PYL1, Ser114 in PYL4 and Ser94 in PYL9 in seedlings with or without ABA treatment. The relative abundance of individual phosphopeptides was normalized to the amount of PYL4 phosphopeptide in the control samples. N.D., not identified.

(B) Quantitative analysis of phosphopeptide containing Ser9 in PYL1 in seedlings with or without ABA treatment. The relative abundance of individual phosphopeptides was normalized by the amount of PYL1 Ser9 phosphopeptide in the ABA treatment samples.

(C) Quantitative analysis of phosphopeptides containing Ser119 in PYL1 from the PYL1 proteins immunoprecipitated from *pyr1pyl24-PYL1-Myc* seedlings, with or without ABA treatment. A non-phosphorylated, negative charged peptide FEKEEEEER was used to indicate the equal amount of PYL1 in each sample. N.D., not identified. Error bars indicate s.e.m. (n = 3).

(D) Substitution of PYL1 Ser119 with D(Asp), L(Leu) or Q(Gln), but not the substitution of Ser119 with C(Cys) or Thr118 with D(Asp) abolished its ability to inhibit PP2CA in transient transcription assays in protoplasts. Error bars indicate s.e.m. (n = 3).

(E) Phosphomimic mutation of PYL4 abolished its ability to inhibit different PP2Cs in transient transcription assays in protoplasts. Error bars indicate s.e.m. (n = 3).

(F) Phosphomimic mutation of PYR1 and PYLs abolished their activity in transient transcription assays in protoplasts. For (C to E), *RD29B::LUC* and *ZmUBQ::GUS* were used as the ABA-responsive reporter and internal control, respectively. After transfection, protoplasts were incubated for 5 hours under light and in the absence of ABA (open bars) or in the presence of 5 μM ABA (filled bars). Error bars indicate s.e.m. (n = 3).

(G) Interaction between ABI1 and phosphomimic mutation of PYLs in yeast two-hybrid assay.

A


β3
β4
β5
AT4G17870.1 (PYR1)
80
90
100
110
120

B

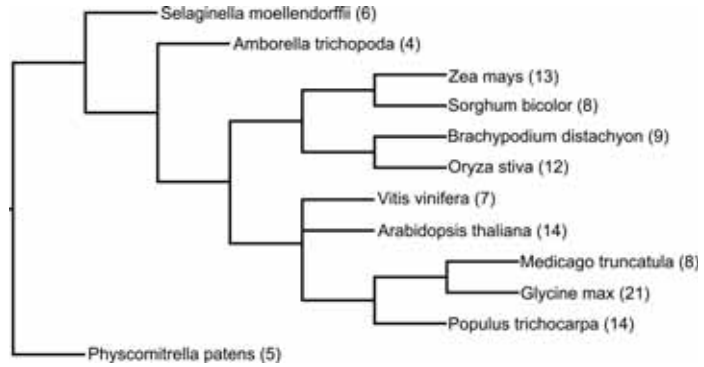

Selaginella moellendorffii (6)
Amborella trichopoda (4)
Zea mays (13)
Sorghum bicolor (8)
Brachypodium distachyon (9)
Oryza stiva (12)
Vitis vinifera (7)
Arabidopsis thaliana (14)
Medicago truncatula (8)
Glycine max (21)
Populus trichocarpa (14)
Physcomitrella patens (5)

Figure S2

Figure S2. The Serine corresponding to Ser119 in PYL1 is evolutionarily conserved, Related to Figure 2.

(A) Serine corresponding to Ser119 in PYL1 is evolutionarily conserved in 121 PYLs in 12 different species.

(B) Phylogenetic tree of the 12 species shown in (A).

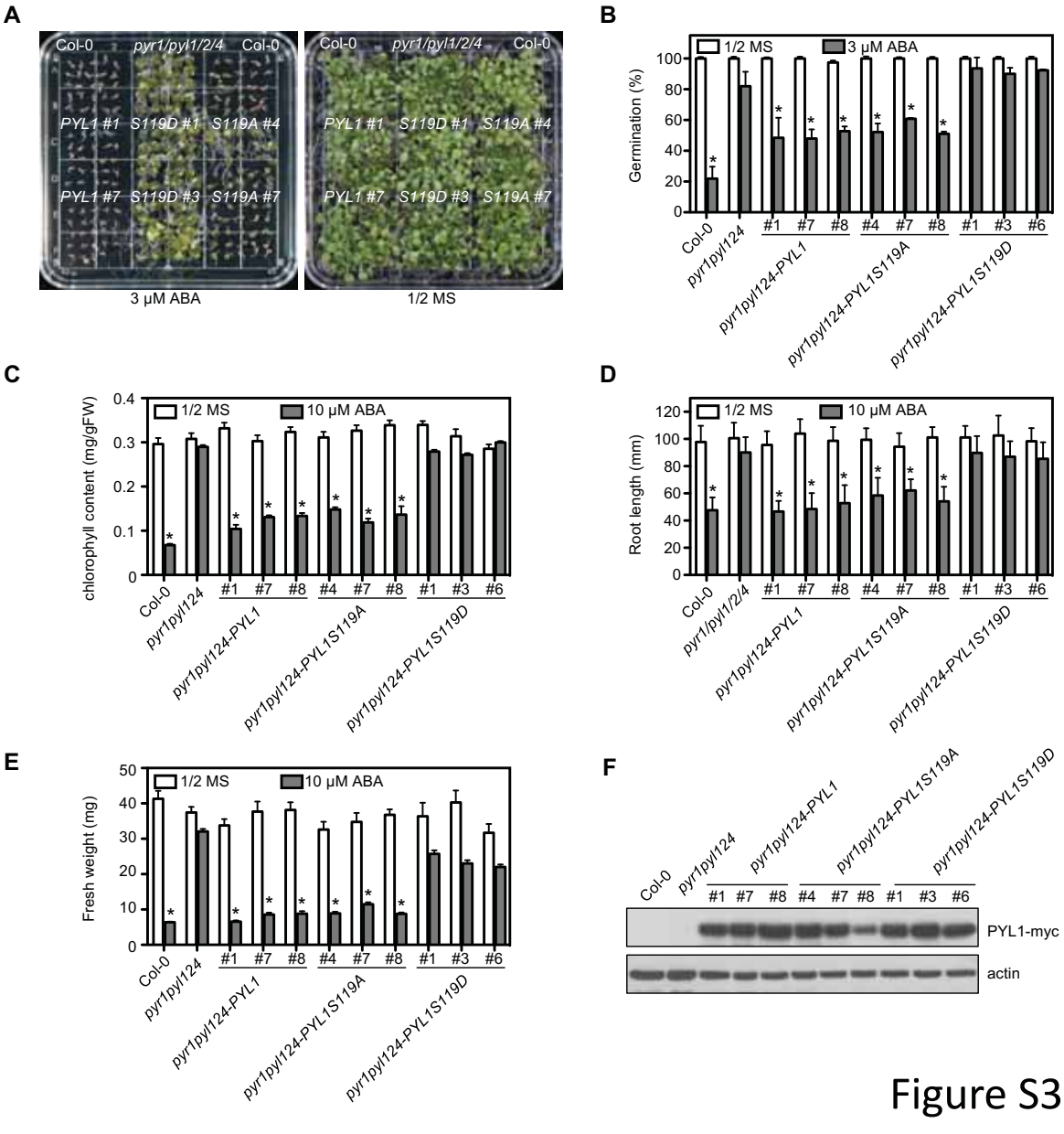

A
Col-0
pyr1/pyl1/2/4
Col-0
PYL1 #1
S119D #1
S119A #4
PYL1 #7
S119D #3
S119A #7
3 μM ABA
1/2 MS
B
1/2 MS
3 μM ABA
Germination (%)
Col-0
pyr1pyl124
pyr1pyl124-PYL1
pyr1pyl124-PYL1S119A
pyr1pyl124-PYL1S119D
C
1/2 MS
10 μM ABA
chlorophyll content (mg/gFW)
D
1/2 MS
10 μM ABA
Root length (mm)
pyr1pyl1/2/4
E
1/2 MS
10 μM ABA
Fresh weight (mg)
F
PYL1-myc
actin

Figure S3

Figure S3. Response of Transgenic Plants Carrying Wild Type and Phosphomimic Mutated PYL1 to ABA Treatment, Related to Figure 3.

(A) 10-day-old seedlings grown on 1/2 Murashige-Skoog (MS) medium containing 3 μM ABA (left panel) or without ABA (right panel).

(B) Germination rate of seeds treated as in (A). Radicle emergence rate was assessed 3 days post plating. Error bars indicate s.e.m. $p < 0.05$ (n = 3), Student's *t*-test, compared with *pyr1pyl124*.

(C) Total chlorophyll content of the seedlings 11 days after transfer to the medium containing 10 μM ABA. Error bars indicate s.e.m. (n = 3). * $p < 0.05$, Student's *t*-test, compared with *pyr1pyl124*.

(D) Root growth of seedlings 11 days after transfer to the medium containing 10 μM ABA. Error bars indicate s.e.m. ($n = 3$). * $p < 0.05$, Student's *t*-test, compared with *pyr1pyl124*.

(E) Fresh weight of the seedlings 11 days after transfer to the medium containing 10 μM ABA. Error bars indicate s.e.m. (n = 3). * $p < 0.05$, Student's *t*-test, compared with *pyr1pyl124*.

(F) Immunoblot shows protein abundance of transgenic lines carrying c-Myc tagged wild type PYL1, PYL1S119A and PYL1S119D used in this study.

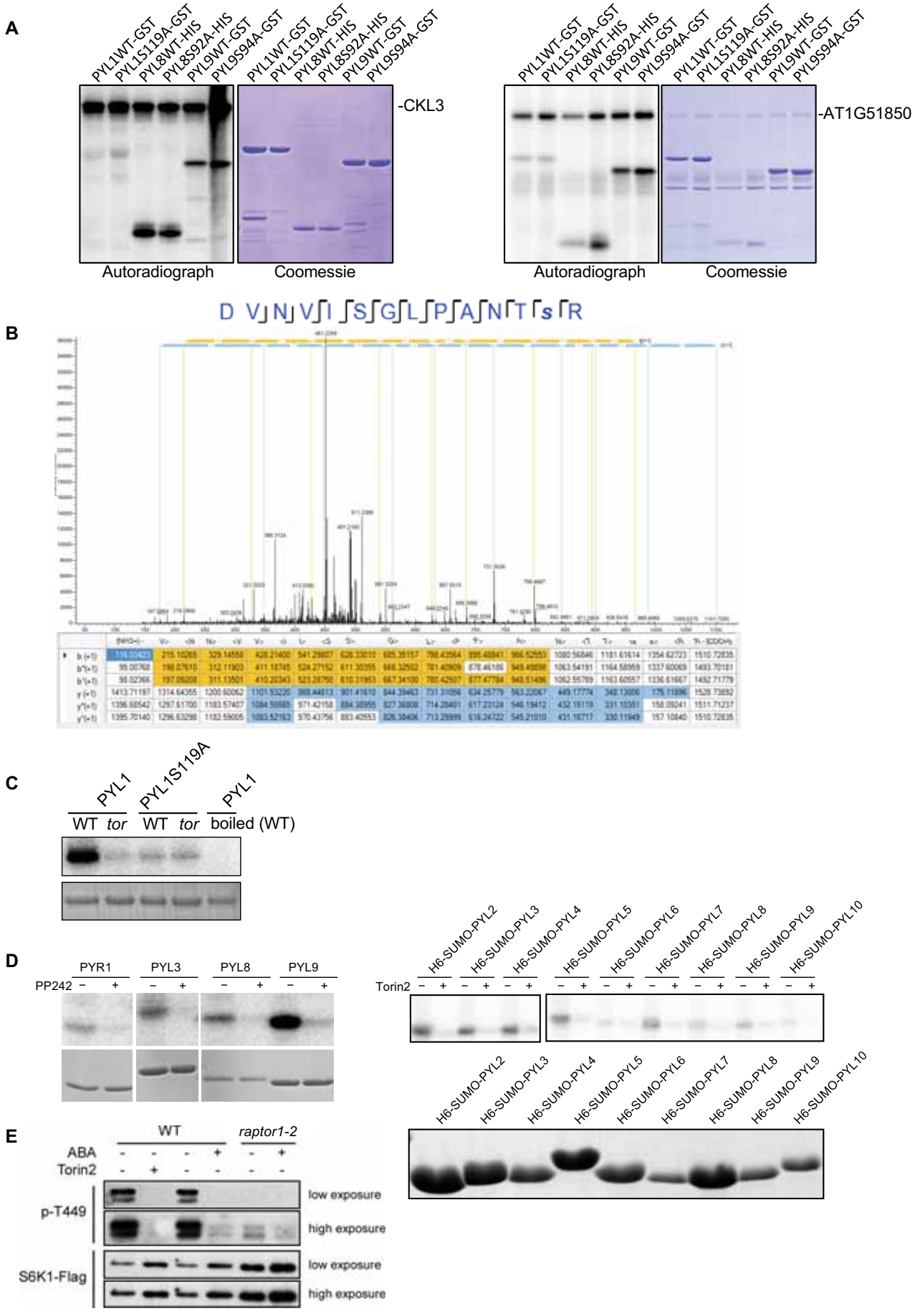

A
PYL1WT-GST
PYL1S119A-GST
PYL8WT-HIS
PYL8S92A-HIS
PYL9WT-GST
PYL9S94A-GST
-CKL3
-AT1G51850
Autoradiograph
Coomessie
B
D V J N J V ∫ I ∫ S ∫ G ∫ L ∫ P ∫ A ∫ N ∫ T ∫ s ∫ R
C
PYL1
PYL1S119A
PYL1
WT tor WT tor boiled (WT)
D
PYR1
PYL3
PYL8
PYL9
PP242
H6-SUMO-PYL2
H6-SUMO-PYL3
H6-SUMO-PYL4
H6-SUMO-PYL5
H6-SUMO-PYL6
H6-SUMO-PYL7
H6-SUMO-PYL8
H6-SUMO-PYL9
H6-SUMO-PYL10
Torin2
E
WT
raptor1-2
ABA
Torin2
p-T449
S6K1-Flag
low exposure
high exposure

Figure S4

Figure S4. The Phosphorylation of PYLs by TOR Kinase, Related to Figure 4.

(A) Phosphorylation of recombinant wild type and mutated PYL proteins by recombinant AT1G51850 and CKL3. Autoradiograph and Coomassie staining show phosphorylation and loading of purified protein kinase and PYL proteins.

(B) MS/MS spectrum showing that the ^{18}O-phosphopeptide DVNVISGLPANTSR containing the phosphoserine at S119 in PYL1 after incubation with immunoprecipitated TOR kinase.

(C) The phosphorylation of PYL1 and PYLS119A by anti-TOR immunoprecipitates from wild type and *es-tor* seedlings. Boiled immunoprecipitate was used as a control.

(D) Phosphorylation of recombinant PYLs by immunoprecipitated TOR kinase. The recombinant PYL proteins are shown in the lower panel.

(E) Phosphorylation of S6K1 at T449 site in wild type and *raptor1-2* seedlings. S6K1-HA was overexpressed in the seedlings to facilitate S6K1 detection using anti-HA immunoblot.

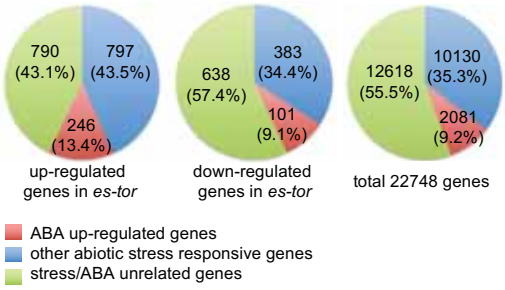

790
(43.1%)
797
(43.5%)
246
(13.4%)
up-regulated
genes in es-tor
638
(57.4%)
383
(34.4%)
101
(9.1%)
down-regulated
genes in es-tor
12618
(55.5%)
10130
(35.3%)
2081
(9.2%)
total 22748 genes
ABA up-regulated genes
other abiotic stress responsive genes
stress/ABA unrelated genes

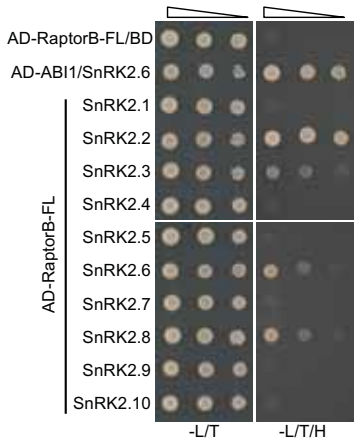
Figure S5

Figure S5. Stress/ABA Responsive Genes are Up-Regulated in the *es-tor* Line, Related to Figure 5.

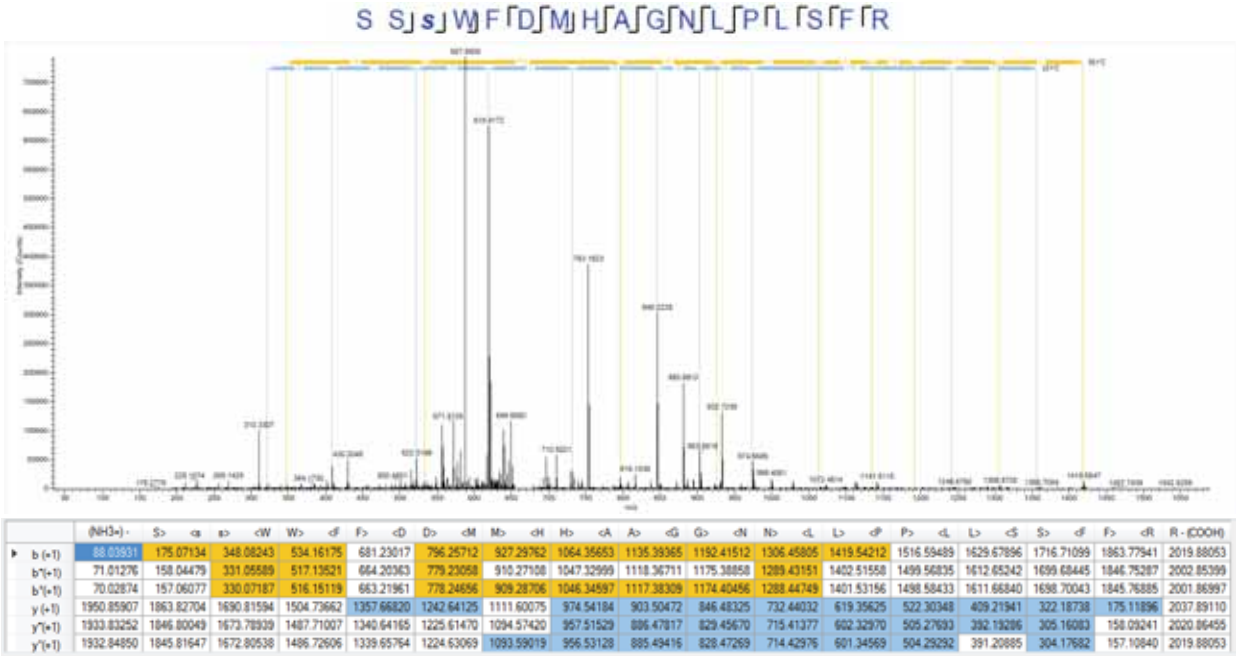
Percentage of ABA up-regulated genes, other abiotic stress responsive genes and stress/ABA unrelated genes in the up-regulated (left panel) and down-regulated genes (middle panel) in *es-tor*. The percentage of these genes out of the total 22748 genes on the microarray is shown in the right panel.

See also Table S1 to S4.

A



B



	(NH3+) -	S>	a	a>	W	W>	F	F>	D	D>	M	M>	H	H>	A	A>	G	G>	N	N>	L	L>	P	P>	L	L>	S	S>	F	F>	R	R - (COOH)
b (+1)	88.03931	175.07134	348.08243	534.16175	681.23017	796.25712	927.29762	1064.35653	1135.39365	1192.41512	1306.45805	1419.54212	1516.59489	1629.67896	1716.71099	1863.77941	2019.88053
b*(+1)	71.01276	158.04479	331.06589	517.13521	664.20363	779.23058	910.27108	1047.32999	1118.36711	1175.38858	1289.43151	1402.51558	1499.56835	1612.65242	1699.68445	1846.75287	2002.85399
b°(+1)	70.02874	157.06077	330.07187	516.15119	663.21961	778.24656	909.28706	1046.34597	1117.38309	1174.40456	1288.44749	1401.53156	1498.58433	1611.66840	1698.70043	1845.76885	2001.86997
y (+1)	1950.85907	1863.82704	1690.81594	1504.73662	1357.66820	1242.64125	1111.60075	974.54184	903.50472	846.48325	732.44032	619.35625	522.30348	409.21941	322.18738	175.11896	2037.89110
y*(+1)	1933.83252	1846.80049	1673.78939	1487.71007	1340.64165	1225.61470	1094.57420	957.51529	886.47817	829.45670	715.41377	602.32970	505.27693	392.19286	305.16083	158.09241	2020.86455
y°(+1)	1932.84850	1845.81647	1672.80538	1486.72606	1339.65764	1224.63069	1093.59019	956.53128	885.49416	828.47269	714.42976	601.34569	504.29292	391.20885	304.17682	157.10840	2019.88053

C

Identified phosphopeptides	Modifications
[K].LNNNPIANWDTRFETGTK.[T]	1x18O-Phospho [T11]
[K].PQSSSLLTSLPSIAK.[F]	1x18O-Phospho [S4]
[K].SVVEDEFDDDEK.[I]	1x18O-Phospho [S1]
[K].PLLGGADASQEIAAK.[R]	1x18O-Phospho [S9]
[R].SSSWFDMHAGNLPLSFR.[T]	1x18O-Phospho [S3]
[RK].FETGTKTALLHPFSPIVVAADENER.[I]	1x18O-Phospho [T]
[K].LAAASYWKPQSSSLLTSLPSIAK.[F]	1x18O-Phospho [S/T]
[R].ISSSPLGSSGLMQGSPLSDDSSLHSDSGMMHDSVSNGAVHQPR.[L]	1x18O-Phospho [S8]

D

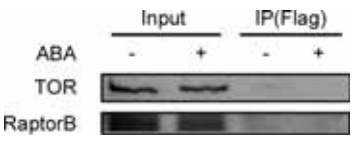


Figure S6

Figure S6. SnRK2s interact with and phosphorylate RaptorB, Related to Figure 6.

(A) Interaction between RaptorB and SnRK2s in yeast two-hybrid assay.

(B) MS/MS spectrum showing that the ^{18}O-phosphopeptide SSSWFDMHAGNLPLSFR containing the phosphoserine at S897 in RaptorB fragment after incubation with recombinant SnRK2.6.

(C) Identified ^{18}O-phosphopeptides in RaptorB fragment after incubation with recombinant SnRK2.6.

(D) Immunoprecipitation with anti-FLAG antibody was used as a negative control of Figure 6J.