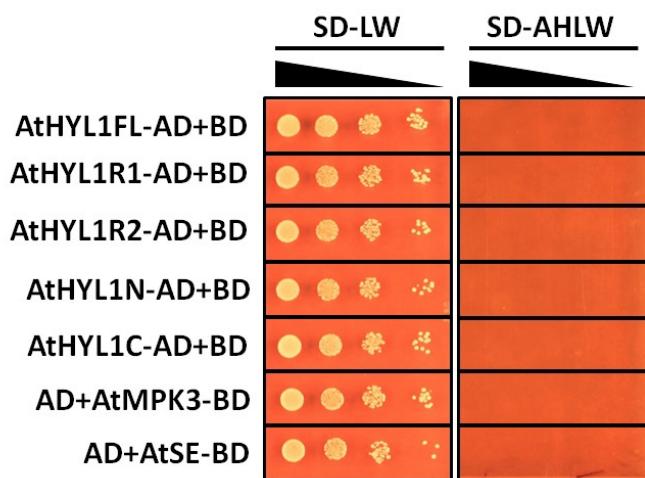


## Supplementary Information

A



B



**Figure S1. Controls of Y2H assay** (Related to Figure 1.) (A) Protein-protein interaction using empty vectors with AtHYL1 and its variants along with AtMPK3 and AtSE in SD-LW and SD-AHLW plates. (B) Distribution of putative phosphorylation sites in AtHYL1 as detected by NetPhos. Blue and red arrow head represents the potential serine and threonine phosphorylation residues.

**A**

1-NESTWSSGCVNCVYKTEQVQYKLYTPEVLTNIDGEGHIIPOSTVILDEVEVNSLPGPNSKAA  
2-QRSAYAVALRELAKSSELSQCVSPYVECTGKLLQSGVQGNAAPLPIQCGKELRALPENIPLV  
3-GFASVQEAGTCAATLSEKAGAQLQCLDNLYNPQTIVLICKK-AIQAIPIKETVTLWAK  
4-QPKKKACKG-CG-NLEPESCHCGNGNEAAPSVEEKEEST

NLEPESCHCGNGNEAAPSVEEKEEST

5-HLEPTFCGGGNRGRANAGYGFPLVPPVLTIPPL2PFPS2CAGLKLGAAGIHPSEYKREIE-TNLNNESSCNSLKE  
6-BAPGSVTPK-7-TTNUJLIUFGNGLKTRATATGGVITFENLTNTNLS88CMCGH172AATCGVNT-TASTIA - 419

**B**

AtHYL1	1	<b>MTS1VS</b> GCVNCVYFKSLRLQEYQAKLIPVYELKEGHSHKSLFSTVILLGVRYS	60
AlHYL1	1	MTA <i>N</i> ASSCVNCVYFKSLRLQEYQAKLIPVYELKEGHSHKSLFSTVILLGVRYS	60
AaHYL1	1	M <b>TNTPS</b> PGVSCVYFKSLRLQEYQAKLIPVYELKEGHSHKSLFSTVILLGVRYS	60
AtHYL1	61	LPGFNRKAQSAAEVALKELAKSSLSQPVHEGLCKNLLQEYQAKNVAIPLY	120
AlHYL1	61	LPGFNRKAQSAAEVALKELAKSSLSQPVHEGLCKNLLQEYQAKNVAIPLY	120
AaHYL1	61	<b>LPGFNRKAQSAAEVALKELAKSSLSQPVHEGLCKNLLQEYQAKNVAIPLY</b>	120
AtHYL1	121	QCGKWK5LGRVQPTCIVLIGG1KVYGAATTKVHDAPISAGRTALTAQSDTENILAYN	180
AlHYL1	121	QCGKWK5LGRVQPTCIVLIGG1KVYGAATTKVHDAPISAGRTALTAQSDTENILAYN	180
AaHYL1	121	<b>QCGKWK5LGRVQPTCIVLIGG1KVYGAATTKVHDAPISAGRTALTAQSDTENILAYN</b>	180
AtHYL1	181	QIQTVPDCEK-TIQAIPKETVILKAKA-QPKKAK-QGSKTVAMPEBIIPIPQPTIH	240
AlHYL1	181	QIQTVPDCEK-TIQAIPKETVILKAKA-QPKKAK-QGSKTVAMPEBIIPIPQPTIH	240
AaHYL1	181	<b>QIQTVPDCEK-TIQAIPKETVILKAKA-QPKKAK-QGSKTVAMPEBIIPIPQPTIH</b>	240
AtHYL1	241	CQN-QSEK-ET-TIN-LEPS3CNYGLKEAAAFGSVEI-CINTPNEPPS2CNGKEAAGFS	299
AlHYL1	241	CQN-QSEK-ET-TIN-LEPS3CNYGLKEAAAFGSVEI-CINTPNEPPS2CNGKEAAGFS	299
AaHYL1	241	<b>CQN-QSEK-ET-TIN-LEPS3CNYGLKEAAAFGSVEI-CINTPNEPPS2CNGKEAAGFS</b>	297
AtHYL1	300	VEKSEKET-TINLEQIISCKG-KEAFAQS-WEIETKDTNNLDESSCNGKEAAGFSVII	358
AlHYL1	300	CGQGVLVCKSNTYI-----VIEV1FPR01TMD-IMYLNSSSLRKLVLW-L8L29	351
AaHYL1	298	METR-----APPNPESQDONG KEAFAQSME---IATP-----PSGQGNGKEAAGFSVII	350
AtHYL1	358	RKNSK-TRQ-NMLPT10-----FLRK-----NRCPTF-----	376
AlHYL1	352	RKNSK-TRQ-NMLPT10-----FLRK-----NRCPTF-----	376
AaHYL1	351	<b>RKNSK-TRQ-NMLPT10-----FLRK-----NRCPTF-----</b>	355
AtHYL1	419	:	:
AlHYL1	-	:	:
AaHYL1	-	:	:

**C**

Arabidopsis\_thaliana 1 MTS1VSQGVNCVYFKSLRLQEYQAKLIPVYELKEGHSHKSLFSTVILLGVRYS 60  
Brassica\_napus 1 MTT1VWVGGSVCNVCFVKSLRLQEYQAKLIPVYELKEGHSHKSLFSTVILLGVRYS 60  
Brassica\_oleracea 1 MTIANLVSQGSVCNVCFVKSLRLQEYQAKLIPVYELKEGHSHKSLFSTVILLGVRYS 60  
Brassica\_rapa 1 MTIANLVSQGSVCNVCFVKSLRLQEYQAKLIPVYELKEGHSHKSLFSTVILLGVRYS 60

\*\*\*\*\*; \*\*\*\*\*;

Arabidopsis\_thaliana 61 LPGFNRKAQSAAEVALKELAKSSLSQPVHEGLCKNLLQEYQAKNVAIPLY 120

Brassica\_napus 61 LPGFNRKAQSAAEVALKELAKSSLSQPVHEGLCKNLLQEYQAKNVAIPLY 120

Brassica\_oleracea 61 LPGFNRKAQSAAEVALKELAKSSLSQPVHEGLCKNLLQEYQAKNVAIPLY 120

Brassica\_rapa 61 LPGFNRKAQSAAEVALKELAKSSLSQPVHEGLCKNLLQEYQAKNVAIPLY 120

\*\*\*\*\*; \*\*\*\*\*;

Arabidopsis\_thaliana 120 **QCQVETLQRVQ**PTCTVELGQGLGAATTKTEKAAISAGRTALATQSQQKNNLH-Y 1/9

Brassica\_napus 120 QCQSETTQGAPICPTCTVELGQGLGAATTKTEKAAISAGRTALATQSQQKNNLH-Y 1/9

Brassica\_oleracea 121 QCQSETTQGAPICPTCTVELGQGLGAATTKTEKAAISAGRTALATQSQQKNNLH-Y 1/9

Brassica\_rapa 121 QCQSETTQGAPICPTCTVELGQGLGAATTKTEKAAISAGRTALATQSQQKNNLH-Y 1/9

\*\*\*\*\*; \*\*\*\*\*;

Arabidopsis\_thaliana 180 ITOLTVLPCEKTKLQAPL-KETVKEIKLAKA-QAFKKA-CUGKRIVANPPEL1 IIPPOTU 239

Brassica\_napus 181 ITOLTVLPCEKTKV2A-APF-KETK1-KTEKAF-QAFKKA-CUGKRIVANPPEL1 IIPPOTU 236

Brassica\_oleracea 181 ITOLTVLPCEKTKV2A-APF-KETK1-KTEKAF-QAFKKA-CUGKRIVANPPEL1 IIPPOTU 236

Brassica\_rapa 181 ITOLTVLPCEKTKV2A-APF-KETK1-KTEKAF-QAFKKA-CUGKRIVANPPEL1 IIPPOTU 236

\*\*\*\*\*; \*\*\*\*\*;

Arabidopsis\_thaliana 240 HCQNQDQEKEKTFNLEPSCONINGKEA-EESVETED-FPTFTHLPSCONINGKEA 297

Brassica\_napus 237 HCQNQDQEKEKTFNLEPSCONINGKEA-EESVETED-FPTFTHLPSCONINGKEA 297

Brassica\_oleracea 237 HCQNQDQEKEKTFNLEPSCONINGKEA-EESVETED-FPTFTHLPSCONINGKEA 297

Brassica\_rapa 237 HCQNQDQEKEKTFNLEPSCONINGKEA-EESVETED-FPTFTHLPSCONINGKEA 278

\*\*\*\*\*; \*\*\*\*\*;

Arabidopsis\_thaliana 358 GSUEFEKITEIPFUSLPSCONINGKEA-EEETKETT2NIESPSCMNCIKSAFPGSV 356

Brassica\_napus

Brassica\_oleracea

Brassica\_rapa

\*\*\*\*\*; \*\*\*\*\*;

Arabidopsis\_thaliana 357 EKKEKTFNLEPSCONINGKEA-EEETKETT2NIESPSCMNCIKSAFPGSV 416

Brassica\_napus

Brassica\_oleracea

Brassica\_rapa

\*\*\*\*\*; \*\*\*\*\*;

Arabidopsis\_thaliana 417 EKKEKTFNLEPSCONINGKEA-EEETKETT2NIESPSCMNCIKSAFPGSV 419

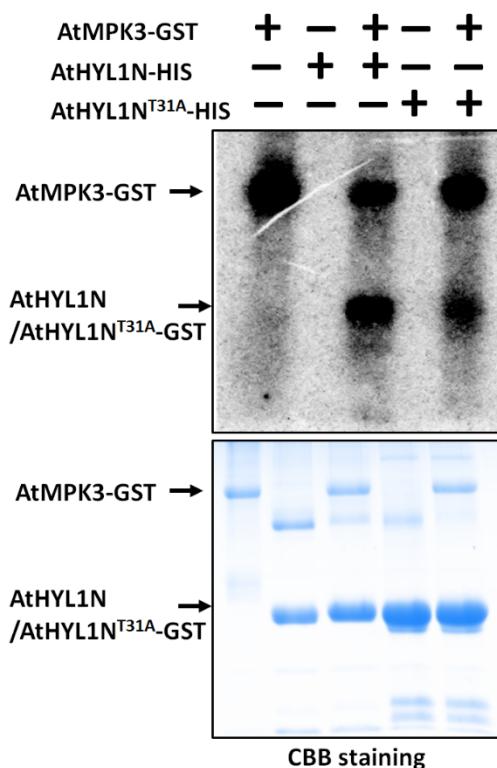
Brassica\_napus

Brassica\_oleracea

Brassica\_rapa

\*\*\*\*\*; \*\*\*\*\*;

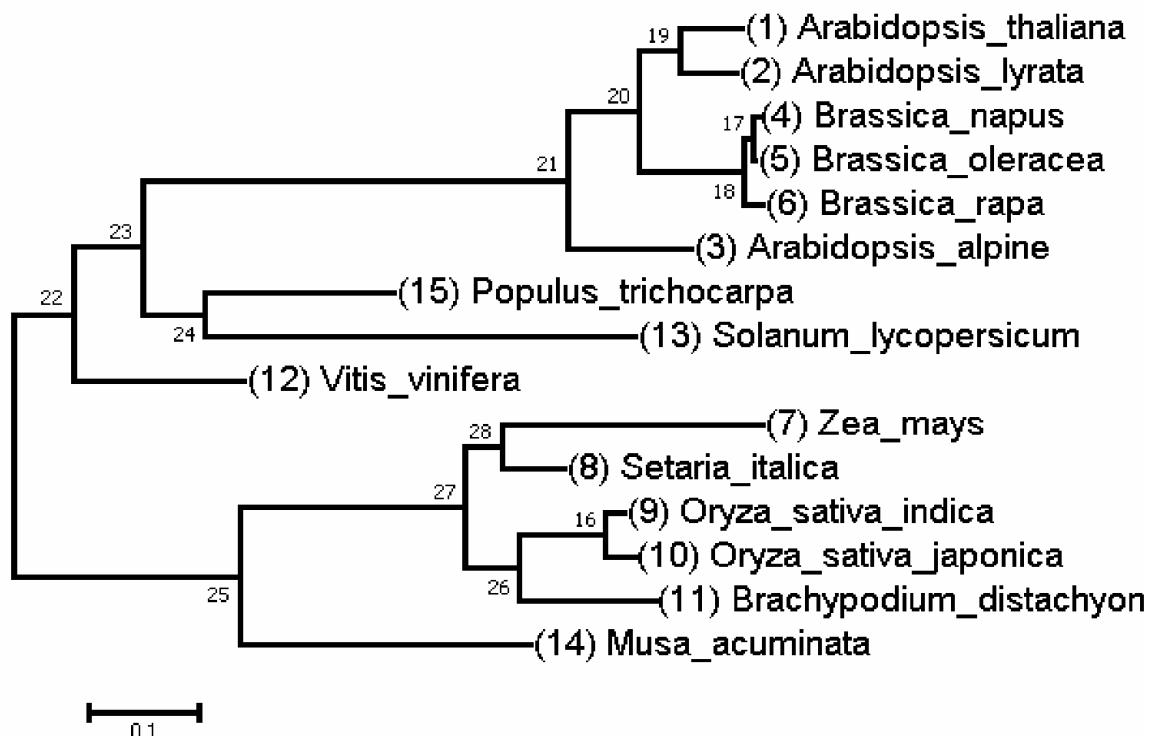
**Figure S2.** (A) Protein sequence of AtHYL1. Two dsRBDs are highlighted by light and dark grey colour. Bi-partite NLS is highlighted in green colour. The 28 amino acid repeat at C-terminal is in red box. The canonical MAP kinase phosphorylation site, i.e. threonine is in red colour. (B) Multiple protein sequence alignment of AtHYL1 and its close members *Arabidopsis lyrata* (Al) and *Arabidopsis alpine* (Aa). Blue arrow head indicates the evolutionarily conserved canonical MAP kinase phosphorylation site and red arrow heads represent other putative MAP kinase target sites. A non-canonical MAP kinase site, serine-42 is indicated in red box. (C) Multiple protein sequence alignment of AtHYL1 and its close members of Brassica.



**Figure S3.** *In-vitro* phosphorylation assay using a bacterially purified AtHYL1N wild type and AtHYL1N<sup>T31A</sup> by AtMPK3. The canonical evolutionarily conserved threonine-31 was mutated to alanine, a non-phosphorylatable amino acid.



**Figure S4.** Multiple protein sequence alignment showing the evolutionarily conservation of serine-42 of AtHYL1 in other plant species indicated by blue arrow head within red box.



**Figure S5.** Phylogenetic tree showing the relation of AtHYL1 with its homologue proteins in other plants.

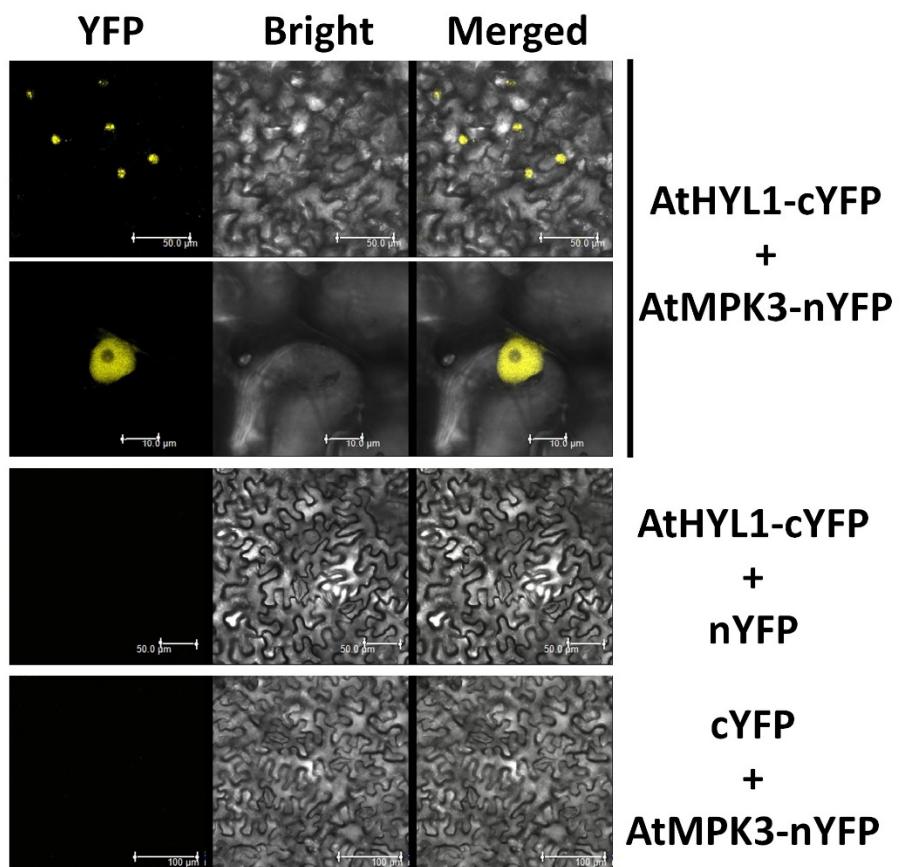
**A**

1- MTSTDVSSGVSNCYVFKSRLQEYAQKYKLPTPVYEIVKEGSPHKSLFQSTVILDGV  
 RYNSLPGFFNRKAAEQSAAEVALRELAKSSELSCQCVSQPVHETGLCKNLLQEYAQK  
 MNYAIPLYQCQKVETLGRVTQFTCTVEIGGIKYTGAATRTKKDAEI SAGR TALLAI  
 QSDTKNNLANYNTQLTVLPCEKKTIQAAIPLKETVKTLKARKA QFKKKAQKGK RTV  
**AKNPEDIIIPPPQPTDHQNDQSEKIEETTPNLEPSSCMNGLK**  
**EAAAFGSVETEKIEETTPNLEPPSCMNGLK**  
**EAAAFGSVETEKIEETTPNLEPPSCMNGLK**  
**EAAAFGSVETEKIEETTPNLESSSCMSGLK**  
**EAAAFGSVETEASHA-419**

**B**

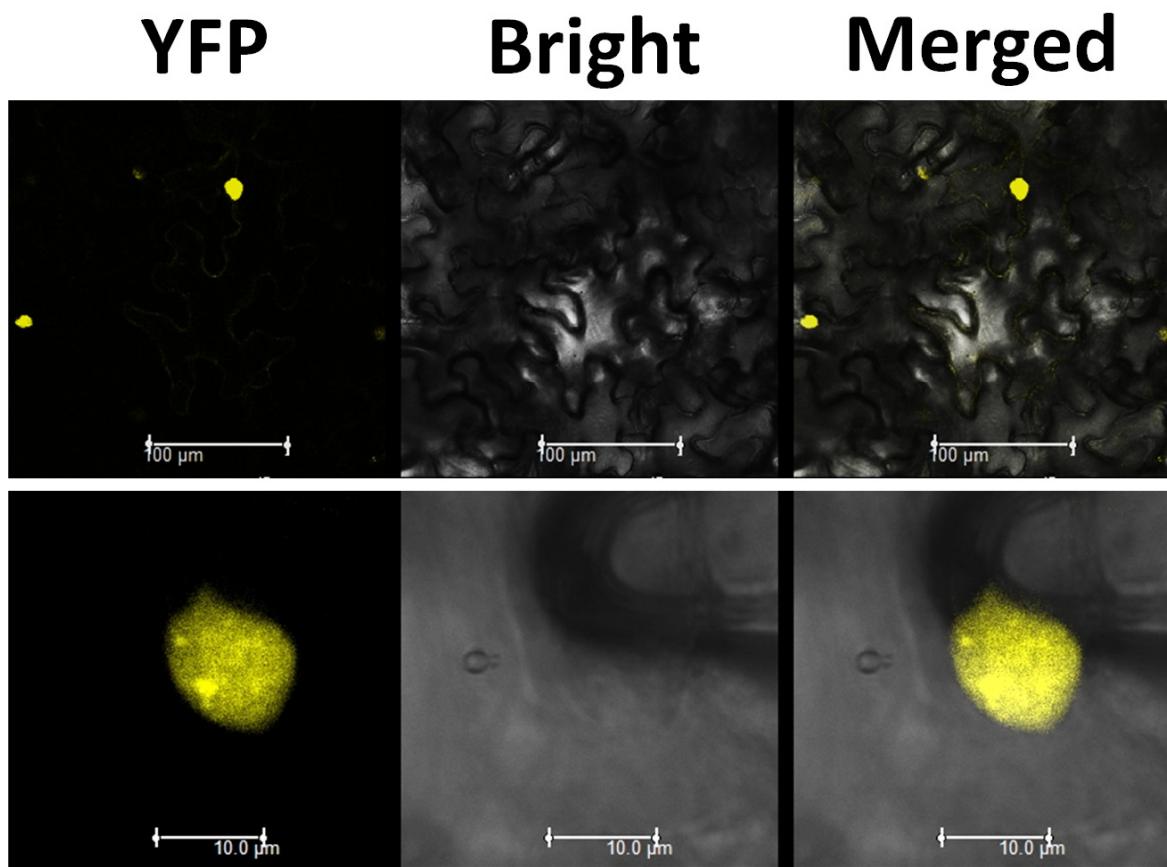
Cleavage site	N-terminal Native (kDa)	C-terminal Native (kDa)
R-222	24.6	20.9
K-226	25	20.5
K-248	27.5	18.4
K-265	29.3	16.2
K-276	30.4	15
K-293	32.3	13.2
K-304	33.4	12.1
K-321	35.2	10.2
K-332	36.4	9.1
K-349	38	7.3
K-360	39.4	6.1
K-377	41.2	4.3
K-388	42.3	3.1
K-405	44.1	1.4

**Figure S6.** (A) AtHYL1 protein sequence showing the presence of putative protease site for trypsin like protease. The multiple lysine (K) residues are highlighted by green and multiple putative MAP kinase sites threonine by red colour. A single arginine (R) residue is present within the NLS indicated with red colour. (B) The putative molecular weight of cleaved peptide at indicated target sites are presented in the tabular form.



**Figure S7.** BiFC assay showing the interaction between AtHYL1-cYFP and AtMPK3-nYFP in *N. benthamiana* leaves under confocal microscope.

# AtHYL1-cYFP + AtHYL1-nYFP



**Figure S8.** BiFC assay showing the interaction between AtHYL1-cYFP and AtHYL1-nYFP in *N. benthamiana* leaves under confocal microscope.

**Table S1: Putative phosphorylation sites on AtHYL1.** Phosphorylation -sites of AtHYL1 protein were predicted by the program of NetPhos 2.0 server (<http://www.cbs.dtu.dk/services/NetPhos-2.0/>). All possible threonine and serine sites of phosphorylation are given. Yellow highlighted are the putative MAP kinase phosphorylation sites.

No	Position	Group	score
<b>Threonine</b>			
1	2	---MTSTDV	0.631
2	31	YKLPTPVYE	0.313
3	152	AATRTKKDA	0.936
4	205	ETVKTLKAR	0.818
5	252	KIETTPNLE	0.845
6	274	GSVETEKIE	0.851
7	280	KIETTPNLE	0.845
8	302	GSVETEKIE	0.851
9	308	KIETTPNLE	0.845
10	330	GSVETEKIE	0.851
11	336	KIETTPNLE	0.845
12	358	GSVETEKIE	0.851
13	364	KIETTPNLE	0.845
14	386	GSVETEKIE	0.851
15	392	KIETTPNLE	0.845
<b>Serine</b>			
1	7	STDVSSGVS	0.629
2	8	TDVSSGVSN	0.808
3	42	KEGPSHKSL	0.987
4	60	VRYNSLPGF	0.729
5	85	ELAKSSELS	0.973
6	89	SSEL\$QCVS	0.815
7	159	DAEISAGRT	0.897

**Table S2: List of primers used in the present study**

SN	Gene	Sequence	Use
1	AtMPK3	For: ATCCGGAATTCATGAACACCGGCCGGTGG Rev: ATCGCGGATCCCCTAACCGTATGTTGGATTGAGTGC	Y2H
2	AtHYL1/DRB1	For: CGGGAATTCATGACCTCCACTGATGTTTC Rev: CCGCTCGAGTGCCTGGCTTGCTTC	Protein Expression for in-vitro phosphorylation assay
	AtHYL1-N	For: CGGGATCCGCATGACCTCCACTGATGTTTC Rev: ATCCGCTCGAGTGAATGGATCGCTAAAAGAG	
	AtHYL1-C	For: CGGGATCCGCACACTAAAAACAACCTTG Rev: GTCCGGAATTCTTATGCGTGGCTTGCTCTGTCTCC	
	AtHYL1-N	For: GAATTCATGACCTCCACTGATGTTTC Rev: CTCGAGAGGAAGTACAGTAAGCTGAGTG	
	AtHYL1-RDM-I	For: CTGCATATGACCTCCACTGATGTTTC CGGGAAATTCATGACCTCCACTGATGTTTC Rev: CGGGAAATTCTTGTGAAACACATTGGCTTAG	
	AtHYL1-RDM-II	For: CTGCATATGTTCACGAAACGGGATTATGC Rev: CGGGAAATTCAAGGAAGTACAGTAAGCTGAGTG	
	AtHYL1-C	For: CTGCATATG TGTGAGAAGAAGACAATACAG Rev: CGGGAAATTGACACTGTTATGCGTGGCTTG	
3	AtSE	For: ATCCGGAATTCATGGCCGATGTTAACCTCCTCC Rev: ATCGCGGATCCCCTACAAGCTCCTGTAATCAATAACGG	Y2H
4	OsMPK3	For: GGATCCATGGGGATGGACGGGGCGCCGGTG Rev: CGGAATTCCGCTAGTACCGGATGTTGGGTTCATCTCGAT	Protein expression
5	OsDRB1-1	For: ATCGCGGATCCCATGAAGAAAAAAAGTGCCTCC Rev: GTCCGGAATTCTCAGGCTACCTCAGGTGTTG	Protein expression
	OsDRB1-1-N	For: CGGGATCCGCATGAAGAAAAAAAGTGCTC Rev: ATCCGCTCGAGACCTTGGATTGCCAGAAGAG	
	OsDRB1-1-C	For: CGGGATCCCCAATCAGAGGGTTCTGCAAATG Rev: GTCCGGAATTCTCAGGCTACCTCAGGTGTTG	
6	OsDRB1-2	For: CGGGATCCGCATGGACATGCCGCCAC Rev: CCGCTCGAGTTCTCGCTCATATTAGT	
	OsDRB1-2-N	For: CGGGATCCGCATGGACATGCCGCCAC Rev: ATCCGCTCGAGACCTTGGATTGCCAGAAGAG	
	OsDRB1-2-C	For: CGGGATCCCCAATCAGAGGGTTCTGCAAATG Rev: CCGCTCGAGTTCTCGCTCATATTAGT	
7	OsDRB1-4	For: CGGGATCCGCATGGCGGCCGCCACCGCC Rev: CCGCTCGAGCTGTGCAACTCTTCTTC	Protein expression
	OsDRB1-4-N	For: CGGGATCCGCATGGCGGCCGCCACCGCC Rev: ATCCGCTCGAGAGCTAAAGTGAATTGACCG	
	OsDRB1-4-C	For: CGGGATCCGCACAAATTACACTTCCATG Rev: CCGCTCGAGCTGTGCAACTCTTCTTC	
8	AtHYL1	For: CACCATGACCTCCACTGATGTTTC	Gateway

		Rev: TGC GTGGCTTGCTTCTG	Cloning in pENTR
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