a
MAX2

## Lj3g3v2851190（EDR1－like）


$\Psi$ MAX2－like
Lj0g3v0059919（EDR1－like）
1 kb

b

|  |  | $\stackrel{20}{1}$ |  | ${ }_{1}^{40}$ |  | ${ }_{1}^{60}$ |  | ${ }_{1}^{80}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| MAX2 | mSNAAETTVR | hlpeeillkv | FSGVSDTRTR | NSLSLVCRSF | YCFERRTRSS | LTLRGIARDL | YLIPTCFAHV | ThldLsLlsp 80 |
| ¢MAX2－like | Magvneigms | dLPE－ILANV | ESADTDTETE | ESLCLMCES | LKLEKKTET | LTUENVFE | HS | THensiose 80 |
| AX2－like $\Delta^{\text {T } 453}$ | Magvnitigms |  | － |  | LKL | Nv | HSIETIERE | 80 |
|  |  | ${ }_{10}^{10}$ |  | ${ }_{1}^{120}$ |  | ${ }_{1}^{140}$ |  | 160 1 1 |
| MAX2 | wghallcss | TAD－－－－－P | HHLAQRLRDA | vr | ， | RH |  | GSDFA |
| ЧMAX2－like | Whelfrtd | ESQLPTDSK | QLIALEIRA | EHEASLTM |  | cus－mplar | －KLIEMEEA | EADLFRIRIR 159 |
| ЧMAX2－like 土T453 $^{\text {a }}$ | WCYEFRTD | ESQLPTDSK | QLIELELERI | A T－ | － | － |  | EATSSESED 159 |
|  |  | ${ }_{180}^{180}$ |  | ${ }^{200}$ |  | ${ }_{1}^{220}$ |  | $\stackrel{240}{1}$ |
| MAX2 | tlfsrcrslt | SLDLSAFYHW | PE－DLPPVLT | ANPAAAASLR | RLnLlktsft | EGFKSHEIES | ITASCPNLE | FLVACTFDPR 23 |
| ¢MAX2－like | RPLRALPVAH | LAGPLQLLRL | EHLEPPFGAE | IKFGHHRVDA | AAESPYGLAQ | GGYavg．－．－ |  | 216 |
| чMAX2－like $\triangle$ T453 | G［1FEHCESEII |  | STWNLISAIK | SNEVTTASME | ELIEILTASLK |  | ITragenter | DEK 239 |
|  |  | ${ }_{2}^{260}$ |  | ${ }_{1}^{280}$ |  | ${ }^{300}$ |  | $\begin{array}{r}320 \\ 1 \\ \hline\end{array}$ |
| max2 | YIGFVGDETL | SAIPSNCPKL | SLLHLADTSS | FSNRSDDDGN | GGEDARISHE | TLVALFSGLP | LLEELVLDV | KNVRESSFAM 313 |
|  |  |  |  |  |  |  |  |  |
|  | HSTY（GEETI | LAIIASNEEEL | THEHEGITA | LRG【ped | TCVEAEISA | AMVEFFCELE | － | DSCFLL 319 |
|  |  | ${ }_{1}^{340}$ |  | ${ }_{3}^{360}$ |  | ${ }_{3}^{380}$ |  | 400 |
| max2 | EVLSKKCPNL | RVLRLGQFQG | iclaigskld | GIALCQGLRS | LSihgcadid | Mglieiarg | csrlvafela | KLVtekgl 393 |
| $\Psi$ MAX2－ike |  |  |  |  |  |  |  |  |
| ЧMAX2－ilike DT453 $^{\text {a }}$ | EVIGTK | ILQRandw | MGLEIGPQLH | ［Q | ISNGEILT | GGIVIIAEG | K | WGIMEEGI |
|  |  | ${ }_{1}^{420}$ |  | $440$ |  | ${ }^{460}$ |  | 480 |
| max2 | gtmacllek | dvgrvscev | dtaAalRa | PIRDRIER | HVDCVWNGL | SDDNmGGG | LLNF－DLndp | NGGAEIMDCF 472 |
| ¢MAX2－like |  |  |  |  |  |  |  | －－ 216 |
| $\Psi$ MAX2－like $\Delta T 453$ | KALTCLIERI | 【MEV｜ |  | ELIEDEVE | HVECVISEL | ETNVDLNNL | EECHSEINID | －．－－EflVG【S 47 |
|  |  | ${ }^{500}$ |  |  |  | ${ }_{540}^{10}$ |  | ${ }_{5}^{56}$ |
| max2 | GDEECEDPSK | RKRQRCEYGL | EgDDSLVQSN | GNGYY－GKNW | DRLRYLSLWI | Vgdllnllp | Vagledcpa | EIRVKVEGD 55 |
| \％MAX2－like |  | ．－ | －．－－．－．－． |  |  |  |  | 216 |
| $\Psi$ MAX2－like $\Delta T 453$ | ©SVDLEGTS |  | SSSSSEME－ | －NGYQCSRS | ERLEMSIM | G\CIVITPLE | Magiederid | EEIPIRUEGD |
|  |  | ${ }_{580}^{18}$ |  |  |  | ${ }_{1}^{620}$ |  | ${ }_{6}^{60}$ |
| max2 | CRGQPKPAES | efglsilacy | PQLSKMQLDC | gdtrgyvlta | PSGQMDLSLW | ERFFLNGISS | LSLNELHYWP | PQDEDVNLRS 631 |
| ЧMAX2－like $\triangle$ T453 | SE | G |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
| max2 | vslpaaglla | Ecytlrklil | hgtahehfmn | FFLKIPNLRD | VQLREDYYPA | PASDMSTEIR | vgscsrfeda | LNRRHICD＊ 710 |
| $\Psi$ MAX2－like |  |  |  |  |  | －．．．．－． | －－．－．．．．．－ | －．．．．．．－＊ 216 |
| MAX2－1ike 土T453 $^{\text {d }}$ | 1 $\mathrm{S}_{\text {K }}$ | F |  |  | － | TES－－－．－ | －scastevef | 1（18E－VRE＊ 699 |

## Supplementary Figure 1 ｜MAX2－like underwent pseudogenization．

（a）Schematic representation of the synthenic regions containing the MAX2 and MAX2－like loci in L．japonicus．Coloured arrows and black lines show exons and introns respectively．（b）Protein alignment of LjMAX2，LjMAX2－like and an artificial LjMAX2－like with a deletion of the thymine at the position 453 in the coding sequence（LjMAX2－like $\Delta T 453$ ）．Position of the nucleotide deletion is indicated in the translated sequence by a red triangle．Amino－acid conservation between MAX2 and MAX2－like is indicated by a dark background．


## Supplementary Figure 2 | Organ-specific accumulation of D14, KAI2a, KAI2b and MAX2 transcripts.

(a-c) Transcript accumulation in wild-type of $D 14, K A I 2 a, K A I 2 b$ and $M A X 2$, in (a) leaf, stem, flower and root of plants grown in pots, and in (b) hypocotyl and roots of 1 wpg plants grown on Petri dishes in 8 h light / 16h dark cycles, and in (c) roots of 2 wpg plants grown on Petri dishes in 16h light / 8h dark cycles $(n=3)$.


Supplementary Figure 3 | Subcellular localisation of LjD14, LjKAI2a, LjKAI2b and LjMAX2 in Nicotiana benthamiana leaves.
(a) Subcellular localization of LjD14, LjKAI2a, LjKAI2b and LjMAX2 in $N$. benthamiana leaf epidermal cells. LjD14, LjKAI2a and LjKAI2b are N-terminally fused with mOrange. LjMAX2 is N-terminally fused with T-Sapphire. Scale bars: $25 \mu \mathrm{~m}$. (b) Western blot of protein extracts from N. benthamiana, showing that the mOrange tag fused with LjD14, LjKAl2a and LjKAI2b was not cleaved at detectable amounts.


## Supplementary Figure 4 | SDS-PAGE of purified SUMO fusion proteins.

200 pmol (approx. $8 \mu \mathrm{~g}$ ) of purified proteins were separated by $12 \%$ SDS-PAGE containing 2,2,2 trichlorethanol as a vizualization agent. Below each lane is the calculated protein size in kiloDaltons. S, protein size standards (Precision Plus Dual Color Standards, Bio-Rad \#1610394) with corresponding sized in kDa shown on the left. Optimal exposures of recombinant proteins and size standards were taken separately under UV transillumination and red epi-illumination, respectively. The two images were merged in post-processing, and the junction between them is indicated by a vertical line.

AtKAl2 MGVVEEAHNVKVIGSG-EATIVLGHGFGTDQSVWKHLVPHLV-DDYRVVLYDNMGAGTTNPDYFDFDRYSNLEGYSFDLI 78 OsD14L MGIVEEAHNLRVVGEGKRGVIVLAHGFGTDQSVWKHLVPHLVAD- YRVVLFDTMGAGPTNPDYFDFSRYATLEGYALDLL 79 LjKAI2a MGIVEEAHNVKVLGSGTR-AIVLAHGFGTDQSLWKHFVPYLT-NDFRVILYDNMGAGTTNPEYFDFERHSSLEGYAYDLL 78

Ps_contig23049 MGIVEEAHNVKVLGTGNR-FIVLAHGFGTDQSVWKHFVPHLV-DGFRVVLYDNMGAGTTNPEYFDSEHHSSLEGYAYDLL 78 | $M t-5 g 016150$ | MGIVEEAHNVKVLGTGNR-YIVLAHGFGTDQSVWKHFVPYLV-DDFRVVLYDNMGAGTTNPEYFDSERHSSLEGYAYDLL 78 |
| ---: | :--- |
| $O C 100780802 ~ M G I A A E A H N V K I ~ L G S G T E-Y I V L A H G F G T D Q S V W K H F V P Y L V-D N F R V I L Y D N M G A G T T N P D Y F D F E R H S I L E G Y A S D L L ~$ |  |
| 78 |  |

 LjKAI2b MG IVEEAHNVKVLGSGSR-FIVLAHGFGTDQSVWKHLVPHLLNDDFRVLLYDNMGAGTTNPDYFDFDRYSTLQGYAYDLL 79
Ps_contig22784 MGIVEEAHNVKVLGTGSR-FIVLAHGFGTDQSVWKHLVPHLQ-EDFRVILYDNMGAGTTNPDYFDFERYSTLEGYAYDLL 78 Mt_4g095310 MGIVEEAHNVKVLGSGSR-FIVLAHGFGTDQSVWKHLVPHLL-DEFRVILYDNMGAGTTNPDYFDFERYSTLEGYAYDLL 78



## $\nabla \nabla^{100}$

ATKAI2 AILEDLKIESCIFVGHSVSAMIGVLASLNRPDLFSKIVMISASPRYVNDVDYQGGFEQEDLNQLFEAIRSNYKAWCLGFA 158 OsD14L AI LQELRVASC I YVGHSVSAVIGAIASI SRPDLFSKLVLLSASPRYLNDVDYYGGFEQEDLDELFEAMGSNYKAWCSGFA 159 LjKAI2a SILEELRVDSCIFVGHSVSAMIGAVASI SRPDLFSKI IMVGASPRYLNDVNYYGGFEQEDLNQLFDAMAANYKAWCLGFA 158
Ps_contig23049 SI LEELQVESCIFVGHSVSAMIGAIASI SRPDLFLKLIMVSGSPRYLNDVNYFGGFEQEDLNQLFTAMSENYKAWCYGFA 158 Mt_5g016150 AILEELQIDSCIFVGHSVSAMIGAIASITRPDLFLKLIMVSSSPRYLNDVNYFGGFEQEDLNQLFNAMAENYKAWCYGFA 158

 LjKAI2b AILEELQVRSCIFVGHSVSGMIGTIASISRPDLFSKLIMVSASPRYLNDVDYFGGFEQEDLDQLFDAMAANYKAWCSGWA 159
Ps_contig22784 AI LQELGVDSCIFVGHSVSAMIGTVASI SRPDLFAKI IM I SASPRYLNDSNYFGGFEQEDLDQLFNAMASNYKAWCSGFA 158 Mt_4g095310 A I LEELRVDSCIFVGHSVSAMIGTVASI SRPDLFNKI I L I SASPRYLNDRDYFGGFEQEDLDQLFDAMASNYKSWCSGFA 158


$\nabla \stackrel{\nabla}{\nabla} \stackrel{180}{\nabla} \overbrace{i}^{200}$ AtKAI2 PLAVGGDMDSIAVQEFSRTLFNMRPDIALSVGQTIFQSDMRQILPFVTVPCHILQSVKDLAVPVVVSEYLHANLGCESVV 238 OsD14L PLCVGGDMESVAVQEFSRTLFNIRPDIALSVAQTIFQSDVRSLLPLVTVPCHIVQSTKDLAVPVVVSEYLHKHLGGDSIV 239 LjKAI2a PLAVGGDMESVAVQEFSRTLFNMRPDIALTVSRTIFQSDMRGILSLVTVPCHIIQAQKDMAVPVVVSEFLHQHLGGVSIV 238
Ps_contig23049 PLAVGGDMDSVAVQEFSRTLFNMRPDIALIVSRTIFQSDMRQILKLVTVPCHIIQAEKDMAVPVMVSEYLHQQLGGQSIV 238 Mt 5g016150 PLAVGGDMD SVAVQEFSRTLFNMRPDIALIVSRTIFQSDMRQI LNLVTVPCHIIQAEKDMAVPVMVSEYLHQHLGGQSIV 238

 LjKAI2b PMA I GGDMESVAVQEFSRTLFNMRPD I ALSVLQT I FQSDMRQVLSLVTVPCHI IQSKKDLAVPVVVAEYLHQHVGGESIV 239
Ps_contig22784 PMAIGGDMESVAVQEFSRTLFNMRPDIALSVLQT IFKSDMRQILCLVSVPCHIIQSMKDLAVPVVVAEYLHQHVGTESIV 238 Mt_4g095310 PMAVGGDMESVAVQEFSRTLFNMRPDIALSVLQTIFKSDMRQILCMVTVPCHIIQSMKDLAVPVVVAEYLHQHVGSESIV 238

 $260 \quad 280 \quad 300$


## Supplementary Figure 5 | Amino acid differences between the legume KAl2a and KAl2b clades.

Protein sequence alignment of KAI2a and KAI2b homologs from the legumes Lotus japonicus, Pisum sativum, Medicago truncatula and Glycine max, in comparison with Arabidopsis KAI2 and rice D14L. Residues conserved within the KAI2a and KAI2b clades but different between these clades are coloured in green and blue. Residues of the catalytic triad are coloured in red. A non-conserved tryptophan in LjKAI2b located in the protein cavity is coloured in violet. Yellow and orange triangles indicate amino acid residues located in the ligand-binding cavity of the proteins. Orange triangles indicate the three amino acids responsible for differences in


Supplementary Figure 6 | Transcript accumulation in the L. japonicus KAR and SL receptor mutants.
(a) qRT-PCR based transcript accumulation of LjKAI2a and LjKAI2b, in roots of wild type and kai2a-1, kai2b-1, kai2b-3, kai2a1 kai2b-1 and max2-4 as well as LjMAX2 and LjD14 in max2-4 and d14-1, respectively (n=4). (b) LjKAl2b transcript accumulation in wild-type, kai2b-1 (stop codon) and kai2b-3 (LORE1 insertion) mutants by semi-quantitative RT-PCR using primer pairs located 5' and $3^{\prime}$ of the mutations, as well as flanking (ML) the mutations. Transcript accumulation of the housekeeping gene Ubiquitin is also shown.
a

b

c

d


Supplementary Figure 7 | Characterisation of the kai2a-1 allele.
(a) Schematic representation of mis-splicing caused by the LORE1 insertion in the kai2a-1 mutant. (b) cDNA alignment showing the absence of nucleotides 369 to 383 in the kai2a-1 transcript, causing a deletion of amino acids 124 to 128 (orange). (c) Protein model of LjKAI2a based on the AtKAI2-KAR complex $4 J Y M{ }^{5}$ showing $K A R_{1}$ in green, residues of the catalytic triad in red and the amino acids missing in a hypothetical LjKAI2a-1 protein in orange. (d) Hypocotyl elongation at 6 dpg in Arabidopsis kai2-2 mutants transgenically complemented with genomic and the cDNA of wild-type LjKAI2a and Ljkai2a-1 driven by the AtKAI2 promoter ( $\mathrm{n}=75-106$ ). Plants were grown in 8 h light / 16h dark cycles. Letters indicate different statistical groups (ANOVA, post-hoc Tukey test).
a

b


## Supplementary Figure 8 | Suppression of $L$. japonicus hypocotyl growth by KAR treatment depends on MAX2.

(a) Hypocotyl length of wild-type and max2-4 seedlings one-week post germination after treatment with solvent (Mock), $1 \mu \mathrm{M}$ $\mathrm{KAR}_{1}, 1 \mu$ KAR $_{2}(\mathrm{n}=66-96)$. Asterisks indicate significant differences of the compounds versus mock treatment (ANOVA, posthoc Dunnett test, N.S.>0.05, *<0.05, ** $\leq 0.01,{ }^{* * * \leq 0.001) . ~(b) ~ C o m p a r i s o n ~ o f ~ D L K 2 ~ t r a n s c r i p t ~ a c c u m u l a t i o n ~ i n ~ h y p o c o t y l s ~ o f ~ m o c k ~}$ treated wild-type, kai2a-1, kai2b-3, kai2a-1 kai2b-1 and max2-4 displayed in Fig 7C ( $\mathrm{n}=3$ ). Letters indicate different statistical groups (ANOVA, post-hoc Tukey test).


## Supplementary Figure 9 | Refreshing rac-GR24 in the medium does not influence root architecture.

PER density of wild-type plants at 2 wpg and treated with solvent (Mock) $1 \mu \mathrm{M} \mathrm{KAR} 1$, $1 \mu \mathrm{M} \mathrm{KAR}$, or $1 \mu \mathrm{M}$ rac-GR24 ( $\mathrm{n}=43-51$ ). Plants were transferred onto fresh hormone-containing medium after 5 days. Asterisks indicate significant differences (ANOVA, Dunnett test, N.S.>0.05, * $\leq 0.05$ ).


Supplementary Figure 10 | Small overlap between transcriptional responses of Lotus japonicus roots to KAR and rac-GR24.

Number of differentially expressed genes (DEGs, adjusted p-value < 0.01) as assessed by microarray analysis. Left panel: DEGs responding to $1 \mu \mathrm{M} \mathrm{KAR}$, after $1 \mathrm{~h}, 2 \mathrm{~h}$ and 6 h incubation. Middle panel: DE genes responding to $1 \mu \mathrm{M}$ rac-GR24 $1 \mathrm{~h}, 2$, $6 h$ incubation. Right panel: comparison of DE genes responding to $2 h$ treatment with $K_{A R}$ and rac-GR24.


Supplementary Figure 11 | KAR perception mutants are less responsive to KAR ${ }_{1}$ treatment.
(a-c) Post-embryonic-root (PER) density of L. japonicus plants, 2 wpg after treatment with solvent (Mock) or $1 \mu M K A R_{1}$, of wildtype, (a) kai2a-1, kai2b-1 and kai2a-1 kai2b-1 (n=32-50); (b) max2-4 ( $\mathrm{n}=34-43$ ); (c) kai2a-1, kai2b-3 and kai2a-1 kai2b-1 ( $\mathrm{n}=$ 37-72). (a-c) Asterisks indicate significant differences versus mock treatment (Welch t.test, * $\leq 0.05,{ }^{* *} \leq 0.01,{ }^{* * *} \leq 0.001$ ).


Supplementary Figure $12 \mid K A R_{1}$ response in roots requires LjKAI2a or LjKAI2b and LjMAX2.
Primary-root length (PRL) and post-embryonic-root (PER) number of L. japonicus plants, 2 wpg after treatment with solvent (Mock) or $3 \mu \mathrm{MAR}_{1}(\mathrm{n}=34-72$ ) displayed in Fig 9A. Asterisks indicate significant differences versus mock treatment (Welch t.test, ${ }^{* \leq 0.05, ~ * * \leq 0.01, ~ * * * \leq 0.001) . ~}$

Supplemental Table 1| L. japonicus mutants used in this study and information on seed production.

| allele | type | reference | position from ATG | comments |
| :--- | :--- | :--- | :--- | :--- |
| Ljd14-1 | EMS | SL4580 | C685T (Q > stop) | hardly produce flowers |
| Ljkai2a-1 | LORE1 insertion | 30008990 | 387 | - |
| Ljkai2b-1 | EMS | SL1281 | C640T (Q > stop) | - |
| Ljkai2b-2 | EMS | SL2723 | G462A (W > stop) | produced no seeds |
| Ljkai2b-3 | LORE1 insertion | 30034333 | 535 | - |
| Ljmax2-1 | LORE1 insertion | 30031159 | 83 | hardly produce flowers |
| Ljmax2-2 | LORE1 insertion | P0860_3 | 504 | hardly produce flowers |
| Ljmax2-3 | LORE1 insertion | 30019601 | 1132 | produce few flowers |
| Ljmax2-4 | LORE1 insertion | 30049531 | 1230 | produce few flowers |

## Supplemental Table 3 | Primers.

Primers used for LORE1 insertion mutant genotyping. Forward primer was used to amplify specifically LORE1 insertion with a specific P2 primer (CCATGGCGGTTCCGTGAATCTTAGG).

| mutant | Forward |  | Reverse |  |
| :--- | :--- | :--- | :--- | :--- |
| Ljkai2a-1 | Sc403 | TATGGTCTCTCACGCTGTTTCCGCC <br> ATGATCG | Sc283 | TCCACAATAGACACGCCACC |
| Ljkai2b-3 | Sc285 | CCTCCGTTGACATGACCTCC | Sc17 | TTGAAGACTACCCCTTAAACA <br> AGGGGTTTGAG |
| Ljmax2-1 | Sc130 | ATGAAGACTTTACGGGTCTCACACC <br> ATGAGTAACGCTGCTGAAAC | CG416 | CAGTAGAAGCTCCGGCAAAC |
| Ljmax2-2 | CG383 | TTGGGGAGGGGTTTAATAGG | CG424 | CGATTTCGTGAGACTTGAAGC |
| Ljmax2-3 | Sc163 | TCACCTCGCTGGATCTCTC | Sc131 | TTGAAGACTACCACCTCCCAT <br> GTTGTCATC |
| Ljmax2-4 | Sc131 | TTGAAGACTACCACCTCCCATGTTG <br> TCATC | Sc163 | TCACCTCGCTGGATCTCTC |

Primers used for EMS mutant genotyping. dCAPS strategy was used to genotype EMS mutants.

| mutant | Forward |  | Reverse | Site |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Ljd14-1 | Sc429 | GCCGGCGGCGGCCGCGAGGT <br> ACCTG | Sc242 | TTTCGTCTCACCTTGTGTGCCC <br> CCGCCAGTGC | Pstl <br> (Cut WT) |
| Ljkai2b-1 | Sc431 | GGTAACTGTGCCATGTCACAG <br> TATA | Sc285 | CCTCCGTTGACATGACCTCC | Accl <br> (Cut WT) |

Primers used for cloning.

| Use | Primers |  |
| :---: | :---: | :---: |
| cloning promoter AtD14 in LI | Sc224 | TTTCGTCTCAGCGGGTCTACACATTCATCAATCTCGC |
|  | Sc225 | TTTCGTCTCACAGATTTTTTATGTGTTTGGGTTTGAG |
| cloning promoter AtKAI2 fragment 1 in LI | Sc232 | TTTCGTCTCAGCGGGGCGATTCAGTGCCATGATT |
|  | Sc233 | TTTCGTCTCACGATTCGTTCAGATTCTCGCT |
| cloning promoter AtKAI2 fragment 2 in LI | Sc234 | TTTCGTCTCAATCGACTCGAATTTGATGGATCTTTC |
|  | Sc235 | TTTCGTCTCACAGACTCTCTAAAGAAGATTCTTC |
| cloning genomic AtD14 in LI | Sc236 | TTTCGTCTCACACCATGAGTCAACACAACATCTTAGAAG |
|  | Sc237 | TTTCGTCTCACCTTTCACCGAGGAAGAGCTCGCC |
| cloning genomic AtKAl2 in LI | Sc238 | TTTCGTCTCACACCATGGGTGTGGTAGAAGAAG |
|  | Sc239 | TTTCGTCTCACCTTTCACATAGCAATGTCATTACGAATG |
| cloning genomic LjD14 in LI | Sc240 | TTTCGTCTCACACCATGGCCACTTCAATCCTCGACG |
|  | Sc241 | TTTCGTCTCACCTTTCAGTGTGCCCCCGCCAGTG |
| cloning genomic LjKAl2a and cDNA Ljkai2a-1 in LI | Sc243 | TTTCGTCTCACACCATGGGGATAGTGGAGGAAGCTCAC |
|  | Sc244 | TTTCGTCTCACCTTTTACACCCCACTAAATTTTACATCAC |


| cloning genomic LjKAl2b in LI | Sc246 | TTTCGTCTCACACCATGGGGATAGTGGAAGAAGCTC |
| :---: | :---: | :---: |
|  | Sc247 | TTTCGTCTCACCTTTCAAGCTGCAATATCATGGCAAATG |
| cloning genomic Ljkai2a-1 in LI | Sc243 | TTTCGTCTCACACCATGGGGATAGTGGAGGAAGCTCAC |
|  | ST97 | CAAATCCTTCCATAGTAATTTGCGGAAGAAAATCATC |
|  | ST96 | TTGAAGACTATCTTCAGATATCTCATATAC |
|  | Sc244 | TTTCGTCTCACCTTTTACACCCCACTAAATTTTACATCAC |
| cloning cDNA LjKAI2a (3b) fragment 1 in LO | Sc505 | ATGAAGACTTCCATCGGAGCCCACCCTAAAC |
|  | ST161 | ATGAAGACTTTACGTCGTCTCACACCATGGG |
| cloning cDNA LjKAI2a (3b) fragment 2 in Lo | ST163 | ATGAAGACTTATGGCGGTGGGTGGAGACATG |
|  | ST164 | ATGAAGACTTCGCAAAACGGTTAGAGCAATATC |
| cloning cDNA LjKAl2a (3b) fragment 3 in Lo | ST165 | ATGAAGACTTTGCGGACCATTTTTTCAGAGC |
|  | Sc498 | ATGAAGACTACAGACGTCTCACCTTTTACACCCCACTAA ATTTTAC |
| cloning cDNA LjKAI2b (3a) fragment 1 in Lo | Sc506 | ATGAAGACTTCCAGCGGGGCAAAGCCTGAAC |
|  | ST169 | ATGAAGACTTTACGTCGTCTCACACCATGGG |
| cloning cDNA LjKAI2b (3a) fragment 2 in LO | ST171 | ATGAAGACTTCTGGCTATCGGAGGAGACATG |
|  | ST172 | ATGAAGACTTTGCGATACGCTTAAGGCTATG |
| cloning cDNA LjKAI2b (3a) fragment 3 in Lo | ST173 | ATGAAGACTTCGCAGACAATTTTTCAAAGTG |
|  | Sc503 | ATGAAGACTACAGACGTCTCACCTTTCAAGCTGCAATATC |
| cloning pSUMO LjKAl2a (2b) | Sc604 | CGTGGTGTTTAGGGTTTGCTCCGATGGCGGTG |
|  | Sc605 | CACCGCCATCGGAGCAAACCCTAAACACCACG |
| cloning pSUMO LjKAI2b (2a) | Sc606 | CATGGTGTTCAGGCTGGGCCCCGCTGGCTATC |
|  | Sc607 | GATAGCCAGCGGGGCCCAGCCTGAACACCATG |

Primers used for gene amplification by RT-qPCR.

| Use |  |  |
| :--- | :--- | :--- |
| qPCR Ubiquitin | Ubi F | ATGCAGATCTTCGTCAAGACCTTG |
|  | Ubi R | ACCTCCCCTCAGACGAAG |
| qPCR LjMAX2 | Sc302 | GAATGTTACACCCTGAGGAAGC |
|  | Sc303 | TCAGGTTTGGGATCTTGAGG |
| qPCR LjKAl2a | Sc282 | CGGTGCAGGAGTTTAGCAGA |
|  | Sc283 | TCCACAATAGACACGCCACC |
| qPCR LjKAI2b | Sc284 | AAGAAAGACCTGGCGGTTCC |
|  | Sc285 | CCTCCGTTGACATGACCTCC |
| qPCR LjDLK2 | MG027 | CTCCTTGGTGCTTCTCCCAG |
|  | MG028 | AAAGCCGAAGCCAGTTTTCA |
| qPCR LjD14 | D14_qPCR_F | ACAGCGTCCGAGAAAACTC |
|  | D14_qPCR_R | AGCAATGGAGGCCAACTAC |

## Supplemental Table 4 | Plasmids.

| Name | Description |
| :---: | :---: |
| Golden Gate Level 0 |  |
| LO cLjKAl2a ${ }^{\text {M160, } L 190, W 157}$ A | PCR amplification of $L$. japonicus Gifu cDNA with primers Sc505 +ST161. Assembly by Stul cut ligation into LO-Amp (BB01) |
| L0 cLjKAl2a ${ }^{\text {M160, } L 190, \mathrm{~W} 157}$ B | PCR amplification of $L$. japonicus Gifu cDNA with primers ST163 +ST164. Assembly by Stul cut ligation into LO-Amp (BB01) |
| LO cLjKAl2a ${ }^{\text {M160, } L 190, W 157} \mathrm{C}$ | PCR amplification of $L$. japonicus Gifu cDNA with primers ST165 +Sc498. Assembly by Stul cut ligation into LO-Amp (BB01) |
| LO cLjKAI2b ${ }^{\text {L161,S199,FF58 }} \mathrm{A}$ | PCR amplification of $L$. japonicus Gifu cDNA with primers Sc506 +ST169. Assembly by Stul cut ligation into LO-Amp (BB01) |
| LO cLjKAl2b ${ }^{\text {L161,S19, FFis8 }}$ B | PCR amplification of $L$. japonicus Gifu cDNA with primers ST171 +ST172. Assembly by Stul cut ligation into LO-Amp (BB01) |
| LO cLjKAl2b ${ }^{\text {L66,S199,FF58 }} \mathrm{C}$ | PCR amplification of $L$. japonicus Gifu CDNA with primers ST173 +Sc503. Assembly by Stul cut ligation into LO-Amp (BB01) |
| Golden Gate Level I |  |
| LI Esp31 pAtKAl2 A | PCR amplification of L. japonicus Gifu genomic DNA with primers Sc232 + Sc233. Assembly by Stul cut ligation into LI-pUC57 (BB02) |
| LI Esp31 pAtKAl2 B | PCR amplification of L. japonicus Gifu genomic DNA with primers Sc234 + Sc235. Assembly by Stul cut ligation into LI-pUC57 (BB02) |
| LI Esp31 pAtD14 | PCR amplification of L. japonicus Gifu genomic DNA with primers Sc224 + Sc225. Assembly by Stul cut ligation into LI-pUC57 (BB02) |
| LI Esp3I gAtKAl2 | PCR amplification of L. japonicus Gifu genomic DNA with primers Sc238 + Sc239. Assembly by Stul cut ligation into LI-pUC57 (BB02) |
| LI Esp3I gAtD14 | PCR amplification of L. japonicus Gifu genomic DNA with primers Sc237 + Sc238. Assembly by Stul cut ligation into LI-pUC57 (BB02) |
| LI Esp3I gLjKAI2a | PCR amplification of L. japonicus Gifu genomic DNA with primers Sc243 + Sc244. Assembly by Stul cut ligation into LI-pUC57 (BB02) |
| LI Esp3I gLjKAI2b | PCR amplification of L. japonicus Gifu genomic DNA with primers Sc246 + Sc247. Assembly by Stul cut ligation into LI-pUC57 (BB02) |
| LI Esp3I gLjD14 | PCR amplification of L. japonicus Gifu genomic DNA with primers Sc240 + Sc241. Assembly by Stul cut ligation into LI-pUC57 (BB02) |
| LI Esp3I gLjkai2a-1 | PCR amplification of L. japonicus kai2a-1 genomic DNA with primers Sc243 + ST97 and ST96 +Sc244. Assembly by Bpil and Stul cut ligation into LI-pUC57 (BB02) |
| LI Esp3I cLjkai2a-1 | PCR amplification of L. japonicus kai2a-1 coding DNA with primers Sc243 + Sc244. Assembly by Stul cut ligation into LI-pUC57 (BB02) |
| LI Esp3I CLjKAI2a | PCR amplification of $L$. japonicus Gifu coding DNA with primers Sc243 + Sc244. Assembly by Stul cut ligation into LI-pUC57 (BB02) |
| LI Esp3I cLjKAI2b | PCR amplification of $L$. japonicus Gifu cDNA with primers Sc246 + Sc248. Assembly by Stul cut ligation into LI-pUC57 (BB02) |
| $\begin{aligned} & \text { LI Esp3I } \\ & \text { cLjKAI2a }{ }^{\text {M160, L190,W157 }} \end{aligned}$ | Assembled by Bpil cut ligation from: L0 cLjKAl2a ${ }^{\text {M160, L190,W157 }} \mathrm{A}+\mathrm{LO}$ cLjKAI2a ${ }^{\text {M160, L190,W157 }} \mathrm{B}$ + LO CLjKAI2a ${ }^{\text {M160, L190, W157 }} \mathrm{C}+\mathrm{LI}$-Bpil (BB03) |
| LI Esp3I cLjKAl2b ${ }^{\text {L161,S191,F158 }}$ | Assembled by Bpil cut ligation from: LO cLjKAI2b ${ }^{L 161, S 191, F 158} \mathrm{~A}+\mathrm{LO} \mathrm{c} L j K A 12 b^{L 161, S 191, F 158} \mathrm{~B}+$ LO cLjKAI2b ${ }^{L 161, S 191, F 158} \mathrm{C}+\mathrm{LI}$-Bpil (BB03) |
| Golden Gate Level II |  |


| Lllc F 1-2 POI:GOI:HygroR | Assembled by Bsal cut ligation from: LI A-B POI (G082) + LI B-C dy (BB06) + LI C-D GOI + LI D-E dy (BB08) + LI E-F nos-T (G006) + LI F-G HygroR (G095) + Lllc F 1-2 (BB30) |
| :---: | :---: |
| Lllc R 3-4 p35S:mCherry | $\begin{aligned} & \text { Assembled by Bsal cut ligation from: LI A-B p35S (G005) + LI B-C dy (BB06) + LI C-D } \\ & \text { mCherry (G023) + LI D-E dy (BB08) + LI E-F 35S-T (G059) + LI F-G dy (BB09) + LIIc R 3-4 } \\ & \text { (BB34) } \end{aligned}$ |
| Golden Gate Level III |  |
| LIIIß POI:GOI:HygroR | Assembled by Bpil cut ligation from: LIlc F 1-2 POI:GOI:HygroR + LII 2-3 ins (BB43) + LIIc R 3-4 p35S:mCherry + LII 4-6 dy (BB41) + LIIIß F A-B (BB53) |
| LIIIß pAtKAl2:gAtKAI2 | Assembled by Esp3I cut ligation from: LIII F A-B POI:GOI:HygroR + LI Esp3I pAtKAI2 A + LI Esp3I pAtKAI2 A + LI Esp3I gAtKAI2 |
| LIIIß pAtKAI2:gAtD14 | Assembled by Esp3I cut ligation from: LIII F A-B POI:GOI:HygroR + LI Esp3I pAtKAI2 A + LI Esp3I pAtKAI2 A + LI Esp3I gAtD14 |
| LIIIß pAtKAl2:gLjKAl2a | Assembled by Esp3I cut ligation from: LIII $\beta$ F A-B POI:GOI:HygroR + LI Esp3I pAtKAI2 A + LI Esp3I pAtKAl2 A + LI Esp3I gLjKAI2a |
| LIIIß pAtKAl2:gLjKAI2b | Assembled by Esp3I cut ligation from: LIII F A-B POI:GOI:HygroR + LI Esp3I pAtKAI2 A + LI Esp3I pAtKAI2 A + LI Esp3I gLjKAI2b |
| LIIIß pAtKAI2: gLjkai2a-1 | Assembled by Esp3I cut ligation from: LIIIß F A-B POI:GOI:HygroR + LI Esp3I pAtKAI2 A + LI Esp3I pAtKAI2 A + LI Esp3I gLjkai2a-1 |
| LIIIß pAtKAI2: cLjkai2a-1 | Assembled by Esp3I cut ligation from: LIII F A-B POI:GOI:HygroR + LI Esp3I pAtKAI2 A + LI Esp3I pAtKAI2 A + LI Esp3I cLjkai2a-1 |
| LIIIß pAtKAI2:gLjD14 | Assembled by Esp3I cut ligation from: LIIIß F A-B POI:GOI:HygroR + LI Esp3I pAtKAI2 A + LI Esp3I pAtKAI2 A + LI Esp3I gLjD14 |
| LIIIß pAtD14:gAtD14 | Assembled by Esp3I cut ligation from: LIII F A-B POI:GOI:HygroR + LI Esp3I pAtKD14 + LI Esp3I gAtD14 |
| LIIIß pAtD14:gAtKAl2 | Assembled by Esp3I cut ligation from: LIII F A-B POI:GOI:HygroR + LI Esp3I pAtKD14 + LI Esp3I gAtKAl2 |
| LIIIß pAtD14:gLjD14 | Assembled by Esp3I cut ligation from: LIII F A-B POI:GOI:HygroR + LI Esp3I pAtKD14 + LI Esp3I gLjD14 |
| LIIIß pAtD14:gLjKAI2a | Assembled by Esp3I cut ligation from: LIIIß F A-B POI:GOI:HygroR + LI Esp3I pAtKD14 + LI Esp3I gLjKAI2a |
| LIIIß pAtD14:gLjKAI2b | Assembled by Esp3I cut ligation from: LIIIß F A-B POI:GOI:HygroR + LI Esp3I pAtKD14 + LI Esp3I gLjKAI2b |
| Protein induction |  |
| pSUMO cLjKAI2a | PCR amplification from LI Esp3I cLjKAI2a with primers MW1002 + MW1003. Assembly by Gibson cloning |
| pSUMO cLjKAI2b | PCR amplification from LI Esp3I cLjKAI2b with primers MW1002 + MW1004. Assembly by Gibson cloning |
| pSUMO <br> cLjKAI2a ${ }^{\text {M160, } L 190, \text { W157 }}$ | PCR amplification from LI Esp3I cLjKAI2a (3b) with primers MW1002 + MW1003. Assembly by Gibson cloning |
| pSUMO <br> cLjKAI2b ${ }^{L 161, S 191, F 158}$ | PCR amplification from LI Esp3I cLjKAl2b (3a) with primers MW1002 + MW1004. Assembly by Gibson cloning |
| pSUMO cLjKAI2a ${ }^{\text {M160, L190 }}$ | Rolling circle PCR amplification from pSUMO LjKAl2a (3b) with primers Sc604 + Sc605. |
| pSUMO <br> cLjKAI2b ${ }^{L 161, S 191, F 158}$ | Rolling circle PCR amplification from pSUMO LjKAI2b (3a) with primers Sc606 + Sc607. |

## Supplemental Table 5 | Statistical results of ANOVA for multiple

## comparisons.

| Figure | genotype/treatment/gene | post hoc test | p-value | F-value |
| :---: | :---: | :---: | :---: | :---: |
| Fig. 2a |  | Tukey | $\leq 0.001$ | $\begin{gathered} \mathrm{F}_{14 / 4338}= \\ 125.3 \end{gathered}$ |
| Fig. 2c | - | Tukey | $\leq 0.001$ | $\mathrm{F}_{11 / 132}=45.6$ |
| Fig. 3a | WT (Ler) | Tukey | $\leq 0.001$ | $\mathrm{F}_{2 / 311}=244$ |
|  | kai2-2 |  | $=0.18$ | $\mathrm{F}_{2 / 300}=1.71$ |
|  | AtKAI2 \#1 |  | $\leq 0.001$ | $\mathrm{F}_{2 / 122}=31.9$ |
|  | AtKAI2 \#3 |  | $\leq 0.001$ | $\mathrm{F}_{2 / 303}=116.4$ |
|  | LjKAl2a \#10b |  | $\leq 0.001$ | $\mathrm{F}_{2 / 316}=65.7$ |
|  | LjKAl2a \#11b |  | $\leq 0.001$ | $\mathrm{F}_{2 / 313}=42$ |
|  | LjKAl2b \#1b |  | $\leq 0.001$ | $\mathrm{F}_{2 / 296}=33.4$ |
|  | LjKAI2b \#5b |  | $\leq 0.001$ | $\mathrm{F}_{2 / 288}=87.4$ |
| Fig. 3b | WT (Col) | Tukey | $\leq 0.001$ | $\mathrm{F}_{2 / 311}=158.3$ |
|  | K02821 |  | $\leq 0.001$ | $\mathrm{F}_{2 / 353}=100.3$ |
|  | WT (Ler) |  | $\leq 0.001$ | $\mathrm{F}_{2 / 384}=499.6$ |
|  | htl-2 |  | $\leq 0.05$ | $\mathrm{F}_{2 / 391}=3.2$ |
|  | \#18 |  | $\leq 0.001$ | $\mathrm{F}_{2 / 383}=104.8$ |
|  | \#23 |  | $\leq 0.001$ | $\mathrm{F}_{2 / 253}=127$ |
| Fig. 3c | WT (Col) | Tukey | $\leq 0.001$ | $\mathrm{F}_{2 / 415}=1008$ |
|  | d14-1 kai2-2 |  | $=0.22$ | $\mathrm{F}_{2 / 353}=1.54$ |
|  | LjKAl2a \#32 |  | $\leq 0.001$ | $\mathrm{F}_{2 / 287}=50$ |
|  | LjKAl2a \#46 |  | $\leq 0.001$ | $\mathrm{F}_{2 / 184}=85$ |
|  | LjKAI2b \#29 |  | $\leq 0.001$ | $\mathrm{F}_{2 / 883}=9.4$ |
|  | LjKAI2b \#31 |  | $\leq 0.05$ | $\mathrm{F}_{2 / 244}=3.9$ |
| Fig. 4b | LjKAl2a | Dunnett | $\leq 0.0001$ | $\mathrm{F}_{5 / 12}=96.1$ |
|  | LjKAI2a ${ }^{\text {M160,L190 }}$ |  | $\leq 0.001$ | $\mathrm{F}_{5 / 12}=9.5$ |
|  | LjKAl2a ${ }^{\text {M160,L190, W157 }}$ |  | $=0.227$ | $\mathrm{F}_{5 / 12}=1.63$ |
|  | LjKAI2b |  | $=0.632$ | $\mathrm{F}_{5 / 12}=0.70$ |
|  | LjKAl2b ${ }^{\text {L161,M191 }}$ |  | $=0.001$ | $\mathrm{F}_{5 / 12}=8.9$ |
|  | LjKAl2b ${ }^{\text {L161,M191,F158 }}$ |  | $\leq 0.0001$ | $\mathrm{F}_{5 / 12}=56.9$ |
| Fig. 6c | - | Tukey | $\leq 0.001$ | $\mathrm{F}_{6 / 103}=35$ |
| Fig. 6d | - | Tukey | $\leq 0.001$ | $\mathrm{F}_{6 / 605}=26.5$ |
| Fig. 7a | KAR1 | Tukey | $\leq 0.001$ | $\mathrm{F}_{3 / 396}=33.1$ |
|  | KAR2 |  | $\leq 0.001$ | $\mathrm{F}_{3 / 390}=16.5$ |
|  | rac-Gr24 |  | $\leq 0.001$ | $\mathrm{F}_{3 / 392}=35$ |
| Fig. 7b | WT | Dunnett | $\leq 0.001$ | $\mathrm{F}_{2 / 313}=30$ |
|  | kai2a-1 |  | $=0.08$ | $\mathrm{F}_{2 / 234}=2.51$ |
|  | kai2b-1 |  | $\leq 0.001$ | $\mathrm{F}_{2 / 302}=29.3$ |
|  | kai2b-3 |  | $\leq 0.001$ | $F_{2 / 308}=14.2$ |
|  | kai2a-1 kai2b-1 |  | $=0.99$ | $\mathrm{F}_{2 / 272}=0.01$ |
| Fig. 7c | WT | Dunnett | $\leq 0.001$ | $\mathrm{F}_{3 / 8}=28.4$ |


|  | kai2a-1 |  | $\leq 0.001$ | $\mathrm{F}_{3 / 8}=53$ |
| :---: | :---: | :---: | :---: | :---: |
|  | kai2b-3 |  | $\leq 0.001$ | $\mathrm{F}_{3 / 8}=26$ |
|  | kai2a-1 kaizb-1 |  | $\leq 0.001$ | $\mathrm{F}_{3 / 8}=105.8$ |
|  | max2-4 |  | $=0.99$ | $\mathrm{F}_{3 / 8}=0.04$ |
| Fig. 8a | KAR1 PRL | Tukey | $\leq 0.001$ | $\mathrm{F}_{3 / 209}=7.40$ |
|  | KAR1 PER |  | $\leq 0.001$ | $\mathrm{F}_{3 / 209}=11.1$ |
|  | KAR1 PER density |  | $\leq 0.01$ | $\mathrm{F}_{3 / 209}=5.51$ |
|  | KAR2 PRL |  | $=0.51$ | $\mathrm{F}_{3 / 217}=0.77$ |
|  | KAR2 PER |  | $=0.18$ | $\mathrm{F}_{3 / 217}=1.64$ |
|  | KAR2 PER density |  | $=0.72$ | $\mathrm{F}_{3 / 217}=0.44$ |
|  | rac-GR24 PRL |  | $=0.74$ | $\mathrm{F}_{3 / 203}=0.42$ |
|  | rac-GR24 PER |  | $=0.07$ | $\mathrm{F}_{3 / 203}=2.45$ |
|  | rac-GR24 PER density |  | $=0.43$ | $\mathrm{F}_{3 / 203}=0.92$ |
| Fig. 8b | WT | Tukey | $\leq 0.001$ | $\mathrm{F}_{2 / 9}=30.7$ |
|  | max2-4 |  | $=0.20$ | $\mathrm{F}_{2 / 9}=1.97$ |
| Supp. Fig. 6a | KAl2a | Tukey | $\leq 0.001$ | $\mathrm{F}_{5 / 18}=39.5$ |
|  | KAl2b |  | $\leq 0.001$ | $\mathrm{F}_{5 / 18}=33.7$ |
| Supp. Fig. 7d | - | Tukey | $\leq 0.001$ | $\mathrm{F}_{9 / 714}=178.8$ |
| Supp. Fig. 8a | WT | Dunnett | $\leq 0.001$ | $\mathrm{F}_{2 / 246}=51$ |
|  | d14-1 |  | $\leq 0.001$ | $\mathrm{F}_{2 / 260}=74.3$ |
|  | max2-4 |  | $=0.25$ | $\mathrm{F}_{2 / 204}=1.38$ |
| Supp. Fig. 8b | - | Tukey | $\leq 0.001$ | $\mathrm{F}_{4 / 10}=148$ |
| Supp. Fig. 9 | - | Dunnett | $\leq 0.01$ | $\mathrm{F}_{3 / 188}=4.1$ |

