

SUPPLEMENTARY ONLINE DATA

Inter- and intra-molecular interactions of *Arabidopsis thaliana* DELLA protein RGL1

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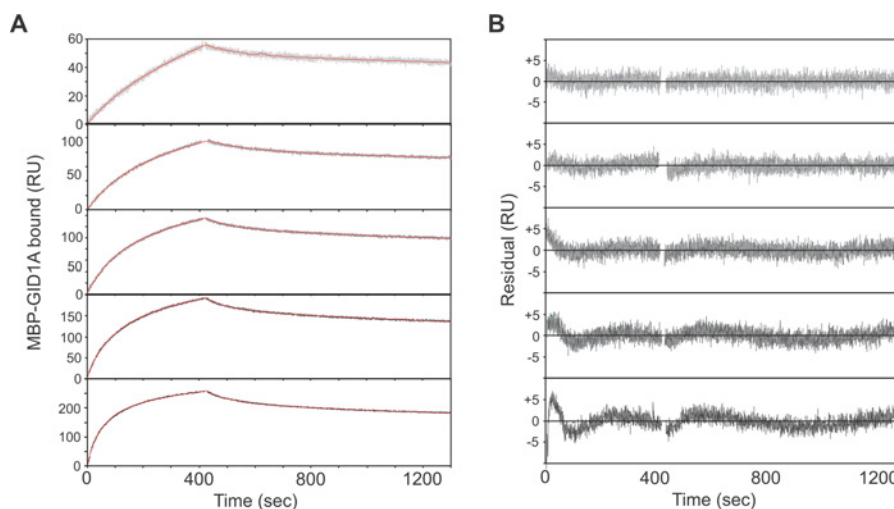


Figure S1 Conformational change kinetic modelling of the gibberellin-dependent GID1A–RGL1N interaction

(A) Gibberellin-dependent association and dissociation data for the interaction between GID1A and immobilized RGL1^N, detected by SPR. Interactions were performed for 100, 200, 400, 800 and 1600 nM solutions of GID1A (top to bottom). A calculated two-state kinetic model was fitted to individual curves, indicated in red, using BiaEvaluation software version 3.1. (B) Residual plot for variance in response units (RU), of the kinetic data from the calculated model for each GID1A concentration.

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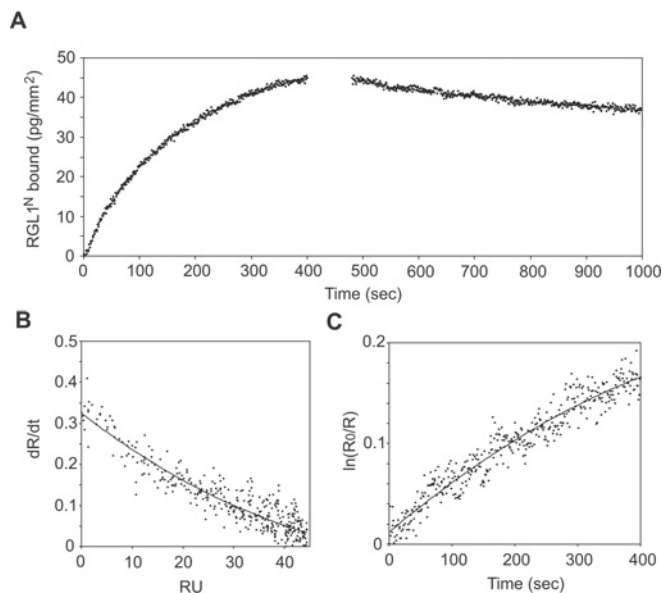


Figure S2 Gibberellin-dependent binding of RGL1^N to immobilized GID1A

(A) Binding of purified recombinant RGL1^N (residues 1–137) from a 1 μ M solution to immobilized MBP–GID1A in the presence of gibberellin in GA₄. Association, 0–420 s; dissociation, 420–1000 s. RGL1^{1–137} was prepared by rTEV protease cleavage from the MBP–fusion protein and subsequent anion–exchange chromatography as described previously [1]. Dissociation of immobilized MBP–GID1A subtracted (baseline drift, approximately -15 pg/mm² at 400 s and -28 pg/mm² at 1000 s). Gibberellin (5 μ M) was present in all solutions and running buffers. **(B)** dR/dt against *R* linearization (Scatchard plot) of the association phase. **(C)** ln(R₀/R) against time linearization of the dissociation phase.

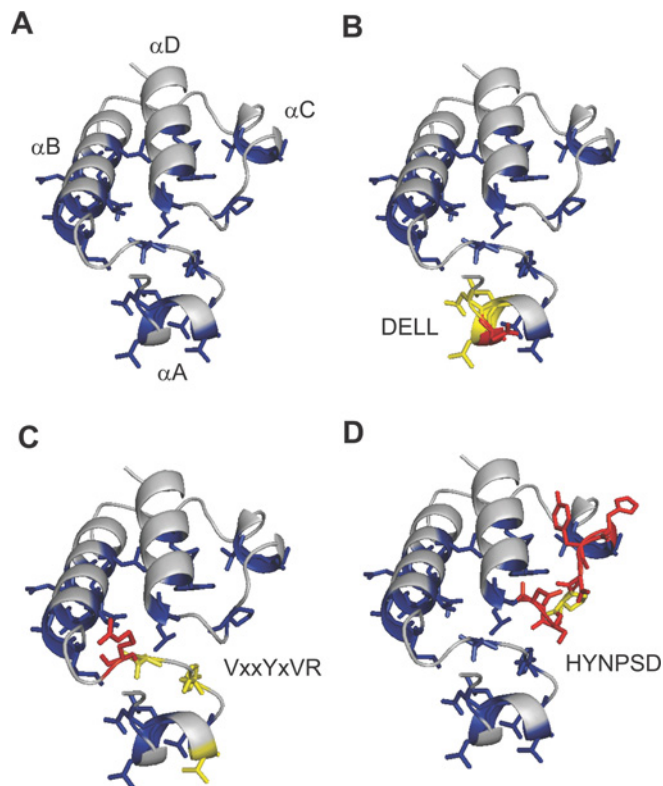


Figure S3 Structural prediction of the RGL1 N-terminal DELLA domain when in complex with GID1A

(A) Predicted RGL1^N (residues 1–137) tertiary structure, modelled from the GID1A/GA4:GA1^{11–113} crystal structure using SwissModel (PDB code 2ZSI) [2,3]. Conserved residues that form direct interactions between GAI and GID1A [2] are shown in blue. **(B–D)** RGL1^N model, indicating monoclonal antibody epitopes: 6C8 **(B)**, BC9 **(C)** and AD7 **(D)**. Antibody epitopes are highlighted in red and yellow. Yellow indicates a residue that also forms a direct GAI–GID1A interaction, whereas red residues do not.

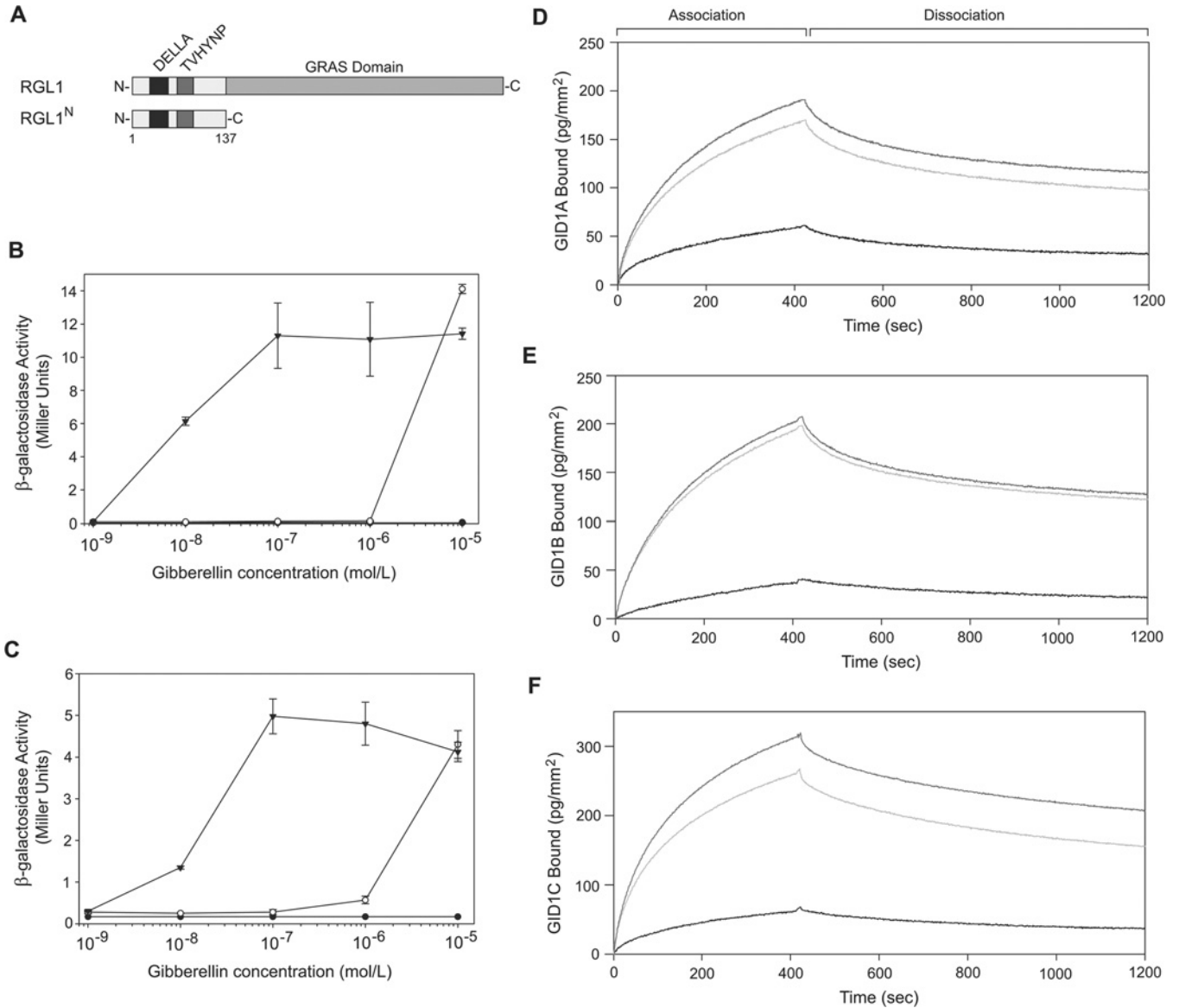


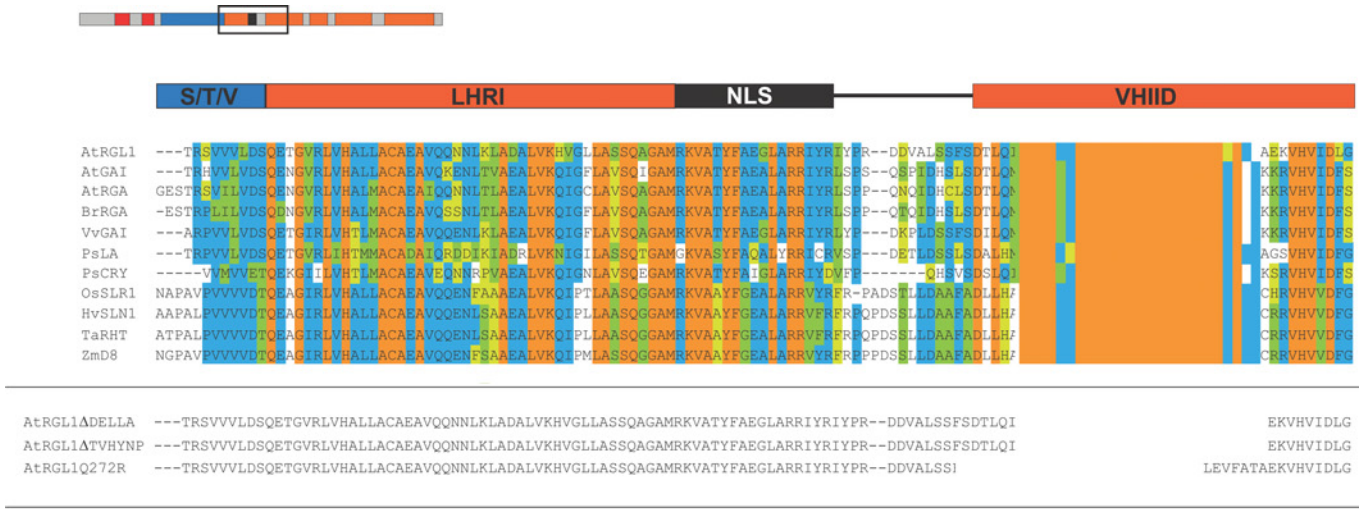
Figure S4 Comparison of the GA₃- and GA₄-dependent GID1A-RGL1^N interaction *in vivo* and *in vitro*

(A) Schematic representation of RGL1, and the N-terminal 137 residues of RGL1, RGL1^N, used in *in vitro* experiments. (B and C) Dose-response curves of yeast two- and three-hybrid assays. (B) Two-hybrid assay of the interaction between the Gal4 DNA-binding domain fusion of GID1A and the Gal4 activation-domain fusion of RGL1. (C) Three-hybrid assay of the interaction between the Gal4 DNA-binding domain fusion of SLY1 and the Gal4 activation domain fusion of RGL1 in the presence of GID1A. LacZ (β -galactosidase) reporter gene activity, from *Saccharomyces cerevisiae* grown in absence of gibberellins ((EPS)) and the presence of GA₃ ((EPS)) or GA₄ ((EPS)). The experiment was performed in duplicate (from two independent transformants); β -galactosidase assays were performed in triplicate for each transformant. Error bars show ± 1 S.D. (D-F) *In vitro* association and dissociation of gibberellin-saturated GID1A-C and RGL1^N, monitored using SPR. Interaction of RGL1^N with: (D) GID1A, (E) GID1B or (F) GID1C; in the absence (black), or presence of 100 M GA₃ (light grey) or 100 M GA₄ (dark grey). Gibberellins were mixed with GID1A-C 30 min prior to the binding assay and excluded from running buffer during the dissociation phase. Association, 0–420 s; dissociation, 420–1200 s. The amount of bound GID1A-C is shown as pg/mm² of surface area.

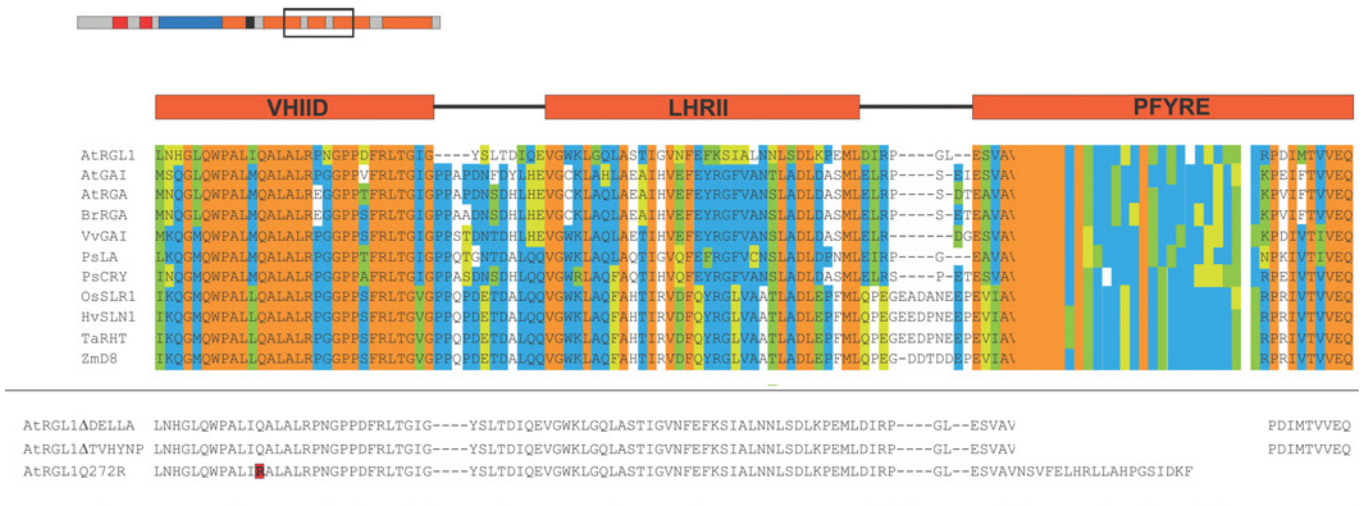


Figure S5 DELLA protein alignment

The full-length sequences for DELLA proteins from a range of plant species were aligned using AlignX (Vector NTI software, Invitrogen). Absolutely conserved residues are highlighted in orange; highly conserved residues are highlighted in blue; highly similar residues are highlighted in green; and similar residues are highlighted in yellow. The RGL1 gain-of-function mutants used in the present paper are displayed. The sequences of several DELLA gain-of function mutations are also displayed, indicating in-frame deletions or amino acid replacements. The *A. thaliana* (*At*) *gai-1*, *rga*^{Δ17}; grape (*Vv*, *Vitis vinifera*) *gai-1*; (*Zm*, *Zea mays*) rice (*Os*, *Oryza sativa*) *slr1w*^{ΔDELLA}, *slr1*^{ΔSPACE}, *slr1*^{ΔTVHYNP}, *slr1*^{ΔS/T/V}; barley (*Hv*, *Hordeum vulgare*) *sln1-d*; wheat (*Ta*, *Triticum aestivum*) *rht*, *rht-B1b*, *rht-D1b*; and maize field mustard (*Br*, *Brassica rapa*) *rga1-d*; *d8-MP* have been previously described as semi-dominant gibberellin-insensitive mutations [4–11]. *Ps*, pea (*Pisum sativum*).

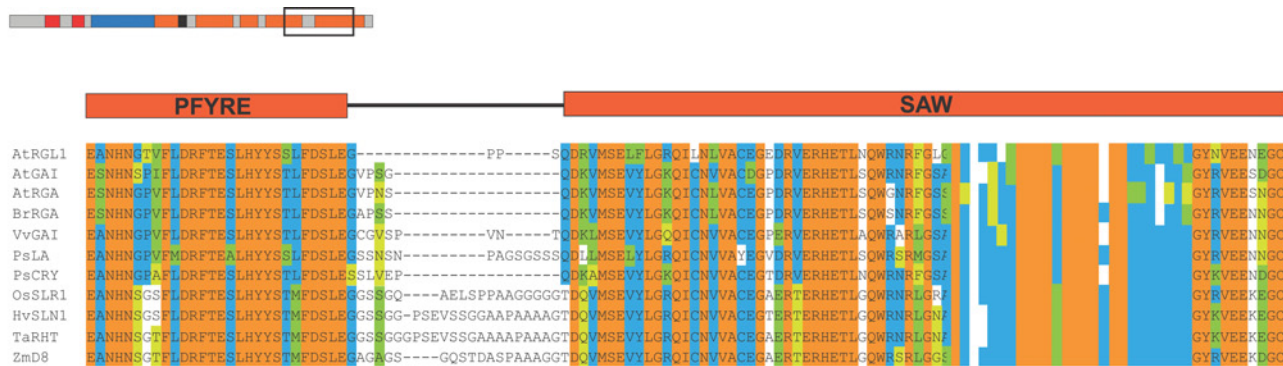


AtRGL1ΔDELLA	---TRSVVVLDSQETGVRLVHALLACAEAVQNNLKLADALVKHVGLLASSQAGAMRKVATYFAEGLARRIYRIYPR--DDVALSSFSDTLQI	EKVHVIDLG
AtRGL1ΔTVHYNP	---TRSVVVLDSQETGVRLVHALLACAEAVQNNLKLADALVKHVGLLASSQAGAMRKVATYFAEGLARRIYRIYPR--DDVALSSFSDTLQI	EKVHVIDLG
AtRGL1Q272R	---TRSVVVLDSQETGVRLVHALLACAEAVQNNLKLADALVKHVGLLASSQAGAMRKVATYFAEGLARRIYRIYPR--DDVALSS	LEVFATAEKVHVIDLG
AtGAI-1	---TRHVVLVDSQENGVRLVHALLACAEAVQENLTVAEALVKQIGFLAVSQIGAMRKVATYFAEALARRIYRLSPS--QSPIDHSLSDTLQ	QKRRVHVIDFS
AtRGA17	GESTRSVILVDSQENGVRLVHALLACAEAVQNNLKLAEALVKQIGFLAVSQAGAMRKVATYFAEALARRIYRLSP--NQIDHCLSDTLQMHFYETCI	VHVIDFS
VvGAIXX	---ARPVVLVDSQETGIRLVHTLMACAEAVQENLKLAEALVKQIGFLAVSQAGAMRKVATYFAEGLARRIYRLYP--DKPLDSSFSIDLQ	FEGKRRVHVIDFS
BrRGA-d	-ESTRPLILVDSQDNQVRLVHALLACAEAVQSSNLTAEALVKQIGFLAVSQAGAMRKVATYFAEALARRIYRLSP--QTQIDHSLSDTLQ	EGKRRVHVIDFS
OsSLR1ΔDELLA	NAPAVVVVVDTQEAGIRLVHALLACAEAVQENFAAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRF-PADSTLLDAAFADLLHAHFYESC	RHVHVDG
OsSLR1ASPACE	NAPAVVVVVDTQEAGIRLVHALLACAEAVQENFAAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRF-PADSTLLDAAFADLLHAHFYESC	RHVHVDG
OsSLR1ΔTVHYNP	NAPAVVVVVDTQEAGIRLVHALLACAEAVQENFAAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRF-PADSTLLDAAFADLLHA	HRVHVDG
OsSLR1AS/T/V	NAPAVVVVVDTQEAGIRLVHALLACAEAVQENFAAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRF-PADSTLLDAAFADLLHAHFYESC	RHVHVDG
Osslr1-d4	NAPAVVVVVDTQEAGIRLVHALLACAEAVQENFAAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRF-PADSTLLDAAFADLLHA	AFAGCHRHVHVDG
HvSLN1-d	AAPALPVVVVDTEAGIRLVHALLACAEAVQENLSAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVFRFRFPDSSLDAAFADLLHA	AGCRHVHVDG
TaRht	ATPALPVVVVDTEAGIRLVHALLACAEAVQENLSAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVFRFRFPDSSLDAAFADLLHA	CRHVHVDG
TaRHTXXX	ATPALPVVVVDTEAGIRLVHALLACAEAVQENLSAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVFRFRFPDSSLDAAFADLLHA	FAGCRHVHVDG
TaRHTXXX	ATPALPVVVVDTEAGIRLVHALLACAEAVQENLSAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVFRFRFPDSSLDAAFADLLHA	FAGCRHVHVDG
ZmD8-MP	NGPAVPPVVVDTEAGIRLVHALLACAEAVQENFSAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVFRFRFPDSSLDAAFADLLHA	AGCRHVHVDG



AtRGL1ΔDELLA	LNHGLQWPALIQALALRPNPDPFRLTGIG---YSLTDIQEVGWKLGQLASTIGVNFEFKSIALNNLSDLKPEMLDIRP---GL--ESVAV	PDIMTVVEQ
AtRGL1ΔTVHYNP	LNHGLQWPALIQALALRPNPDPFRLTGIG---YSLTDIQEVGWKLGQLASTIGVNFEFKSIALNNLSDLKPEMLDIRP---GL--ESVAV	PDIMTVVEQ
AtRGL1Q272R	LNHGLQWPALIQALALRPNPDPFRLTGIG---YSLTDIQEVGWKLGQLASTIGVNFEFKSIALNNLSDLKPEMLDIRP---GL--ESVAVSVFELHRLLAHPGSDIKF	
AtGAI-1	MSQGLQWPALMQALALRPGGPPVFRLTGIGPPAPDNFDYLVHEVGCKLAHLAEAIHVEFYRGEFVANTLADLDASMLELRP---S-EIESVAV	QIKPEIFTVVEQ
AtRGA17	MNQGLOWPALMQALALRREGGPPFRLTGIGPPAPDNDHLEHVGVCKLAQLAEAIHVEFYRGEFVANSIADLDASMLELRP---S-DTEAVAVSVFELI	IFTVVEQ
VvGAIXX	MKQGMQWPALMQALALRPGGPPSFRLTGIGPPSPDNDTHLHEVGWKLQAQFAETIHVEFYRGEFVANSIADLDASMLELRP-----DGESVAV	KDMKPDIVTVEQ
BrRGA-d	MNQGLOWPALMQALALRREGGPPSFRLTGIGPPAPDNDHLEHVGVCKLAQLAEAIHVEFYRGEFVANSIADLDASMLELRP---S-ETEAVAVSVFELHKLGRGGIEKV	
OsSLR1ΔDELLA	IKQGMQWPALLQALALRPGGPPSFRLTGVGPPQDETDALQQVGVKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEADANEEPEVI	RIVTVEQ
OsSLR1ASPACE	IKQGMQWPALLQALALRPGGPPSFRLTGVGPPQDETDALQQVGVKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEADANEEPEVI	RIVTVEQ
OsSLR1ΔTVHYNP	IKQGMQWPALLQALALRPGGPPSFRLTGVGPPQDETDALQQVGVKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEADANEEPEVI	PRIVTVEQ
OsSLR1AS/T/V	IKQGMQWPALLQALALRPGGPPSFRLTGVGPPQDETDALQQVGVKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEADANEEPEVI	RIVTVEQ
Osslr1-d4	IKQGMQWPALLQALALRPGGPPSFRLTGVGPPQDETDALQQVGVKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEADANEEPEVI	HAVRPRIVTVEQ
HvSLN1-d	IKQGMQWPALLQALALRPGGPPSFRLTGVGPPQDETDALQQVGVKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEEDPNEEPEVI	AVRPRIVTVEQ
TaRht	IKQGMQWPALLQALALRPGGPPSFRLTGVGPPQDETDALQQVGVKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEEDPNEEPEVI	RPRIVTVEQ
TaRHTXXX	IKQGMQWPALLQALALRPGGPPSFRLTGVGPPQDETDALQQVGVKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEEDPNEEPEVI	RAVRPRIVTVEQ
TaRHTXXX	IKQGMQWPALLQALALRPGGPPSFRLTGVGPPQDETDALQQVGVKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEEDPNEEPEVI	RAVRPRIVTVEQ
ZmD8-MP	IKQGMQWPALLQALALRPGGPPSFRLTGVGPPQDETDALQQVGVKLAQFAHTIRVDFQYRGLVAATLADLEPFMLQPEG-DDTDDEPEVI	AVRPRIVTVEQ

Figure S5 Continued



AtRGL1ADELLA	EANHNGTVFLDRFTESLHYYSFLDSLEG-----PP-----SQRVMSEFLGRQILNLVACEGEDRVERHETLNQWRNRFLGL	YNVEENEGC
AtRGL1ATVHYNP	EANHNGTVFLDRFTESLHYYSFLDSLEG-----PP-----SQRVMSEFLGRQILNLVACEGEDRVERHETLNQWRNRFLGL	YNVEENEGC
AtRGL1Q272R	EANHNGTVFLDRFTESLHYYSFLDSLEG-----PP-----SQRVMSEFLGRQILNLVACEGEDRVERHETLNQW	LYAGADGYNVEENEGC

AtGAI-1	ESNHNSPIFLDRFTESLHYYSFLDSLEGVPSG-----QDKVMSEVYLGKQICNVVACDGPDRVERHETLSQWRNRFGSA	GEGYRVEESDGC
AtRGA17	ESNHNGPVFLDRFTESLHYYSFLDSLEGVPSN-----QDKVMSEVYLGKQICNLVACEGPDPRVERHETLSQWRNRFGSSGLAPAH	VEESNGC
VvGAIXXX	EANHNGTVFLDRFTESLHYYSFLDSLEGCVSP-----VN-----TQDKLMSEVYLGKQICNVVACEGPERVERHETLAQWRARLGS	GGDGYRVEENNGC
BrRGA-d	ESNHNGPVFLDRFTESLHYYSFLDSLEGAPSS-----QDKVMSEVYLGKQICNLVACEGPDPRVERHETLSQWRNRFGSS	GEGYRVEENNGC
OsSLR1ADELLA	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRAGFEPVH	RVEEKEGC
OsSLR1ASPACE	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRAGFEPVH	RVEEKEGC
OsSLR1ATVHYNP	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRA	YRVEEKEGC
OsSLR1AS/T/V	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRAGFEPVH	RVEEKEGC
Osslr1-d4	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRA	GGDGYRVEEKEGC
HvSLN1-d	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ--PSEVSSGGAAPAAAAGTDQVMSEVYLGKQICNVVACEGTERTERHETLGQWRNRLGNA	GDGYKVEEKEGC
TaRht	EANHNSGTVFLDRFTESLHYYSFLDSLEGSSGQGGPSEVSSGAAAAAAAAGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGNA	GYKVEEKEGC
TaRHTXXX	EANHNSGTVFLDRFTESLHYYSFLDSLEGSSGQGGPSEVSSGAAAAAAAAGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGNA	GGDGYKVEEKEGC
TaRHTXXX	EANHNSGTVFLDRFTESLHYYSFLDSLEGSSGQGGPSEVSSGAAAAAAAAGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGNA	GGDGYKVEEKEGC
ZmD8-MP	EANHNSGTVFLDRFTESLHYYSFLDSLEGAGAGS---GQSTDASPAAGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGGE	GGDGYRVEEKDGC



SAW

AtRGL1	LLLGWQTRPLIATSAWRINRVE-----
AtGAI	LLLGWHTRPLIATSAWKLSTN-----
AtRGA	LLLGWHTRPLIATSAWKLSTAA-----
BrRGA	LLLSWHTRPLIATSAWKLSAVH-----
VvGAI	LLLGWHTRPLIATSAWQLANKPALPSSTPASN
PsLA	LLLGWHTRPLIATSAWKLPSESK-----
PcCRY	LLLGWHTRPLIATSAWKLAANSMVVSH-----
OsSLR1	LTLGWHTRPLIATSAWRVAAA-----
HvSLN1	LTLGWHTRPLIATSAWRLAAP-----
TaRHT	LTLGWHTRPLIATSAWRLAGP-----
ZmD8	LTLGWHTRPLIATSAWRVAAAAAP-----

AtRGL1ADELLA	LLLGWQTRPLIATSAWRINRVE-----
AtRGL1ATVHYNP	LLLGWQTRPLIATSAWRINRVE-----
AtRGL1Q272R	LLLGWQTRPLIATSAWRINRVE-----

AtGAI-1	LLLGWHTRPLIATSAWKLSTN-----
AtRGA17	LLLGWHTRPLIATSAWKLSTAA-----
VvGAIXXX	LLLGWHTRPLIATSAWQLANKPALPSSTPASN
BrRGA-d	LLLSWHTRPLIATSAWKLSAVH-----
OsSLR1ADELLA	LTLGWHTRPLIATSAWRVAAA-----
OsSLR1ASPACE	LTLGWHTRPLIATSAWRVAAA-----
OsSLR1ATVHYNP	LTLGWHTRPLIATSAWRVAAA-----
OsSLR1AS/T/V	LTLGWHTRPLIATSAWRVAAA-----
Osslr1-d4	LTLGWHTRPLIATSAWRVAAA-----
HvSLN1-d	LTLGWHTRPLIATSAWRLAAP-----
TaRht	LTLGWHTRPLIATSAWRLAGP-----
TaRHTXXX	LTLGWHTRPLIATSAWRLAGP-----
TaRHTXXX	LTLGWHTRPLIATSAWRLAGP-----
ZmD8-MP	LTLGWHTRPLIATSAWRVAAAAAP-----

Figure S5 Continued

Table S1 Oligonucleotide sequences for amplification and sequencing

Description	Sequence
RGL1 forward primer for pACT2 (XmaI)	5'-TCCCCCGGGTATGAAGAGAGAGACAACCACC-3'
RGL1 reverse primer for pACT2 (SacI)	5'-CGAGCTCGTTATCCACACGATTGATTCGCC-3'
RGL1 internal nt93 reverse primer	5'-GACGTGGCACACAAGCTTG-3'
RGL1 internal nt145 forward primer	5'-CACTCCGGCAGCTTCTTC-3'
RGL1 internal nt213 reverse primer	5'-AGCATGCTCTCGGATCTTGAC-3'
RGL1 internal nt268 forward primer	5'-ATTCGAGATCCATACCAAGAAC-3'
RGL1 internal mutagenic primer reverse	5'-CTTTGATTGCGACTCTTGCTTTAC-3'
RGL1 internal nt830 forward primer	5'-CCGGCCATTGTAACCATGG-3'
RGL1 internal nt411 reverse primer	5'-GTTCTTCTCCTTACTCATTCCGCCACCCGTAGAGGATAACTCCGAT-3'
RGL1 internal nt412 forward primer	5'-GCATGGATGAACATACAAGGAGGGGTCGCTCTGTGGTGGTTTTGGATTC-3'
RGL1 forward for pMalc2x (BamHI)	5'-CGGGATCCATGAAGAGAGACACAACCACC-3'
RGL1 reverse N-term internal for pMalc2x (Sall)	5'-GACGCGTCGACTACGTAGAGGATAACTCCGATCAA-3'
GID1A forward primer for pGBKT7 (EcoRI)	5'-GGAATTCATGGCTGCGAGCGATGAAG-3'
GID1A reverse primer for pGBKT7 (BamHI)	5'-CGGGATCCGTTAACATCCGCGTTTACAAC-3'
GID1A forward primer for pBridge MCSII (NotI)	5'-ATAAGAATCGCGCCGCTATGGCTGCGAGCGATGAAG-3'
GID1A reverse primer for pBridge MCSII (NotI)	5'-ATAAGAATCGCGCCGCTATTAACATCCGCGTTTACAAC-3'
GID1B forward primer for pGBKT7 (EcoRI)	5'-GGAATTCATGGCTGCTGGTAAACGAAGT-3'
GID1B reverse primer for pGBKT7 (BamHI)	5'-CGGGATCCGCTAAGGAGTAAGAAGCACAGG-3'
GID1C forward primer for pGBKT7 (EcoRI)	5'-GGAATTCATGGCTGGAAGTGAAGAAGTT-3'
GID1C reverse primer for pGBKT7 (BamHI)	5'-CGGGATCCGTTCAATGGCATTCTGCGTTTAC-3'
SLY1 forward primer (EcoRI)	5'-CGGAATTCATGAAGCGCAGTACTACCGAC-3'
SLY1 reverse primer (BamHI)	5'-CGCGGATCCGTTATTTGGATTCTGGAAGAGGTC-3'
SLY1 mutagenic primer reverse primer (BamHI)	5'-CGCGGATCCGTTATTTGGATTCTGGAAGAGGTCCTCTTAGTAAACTCATCTTCTGTAG-3'
GFP forward primer	5'-ATCGAGGATATCCTCTACGGGTGGCGAATGAGTAAAGGAGAAGAAC-3'
GFP reverse primer	5'-GAATCCAAAACACCACAGAGCGACCCCTCTTTGTATGTTATCCACAGC-3'
pACT2 forward sequencing primer	5'-CTATCTATTGATGATGAAGATAC-3'
pACT2 reverse sequencing primer	5'-AGTTGAAGTGAACCTTGGGGTT-3'
pGBKT7 forward sequencing primer (T7)	5'-TAATACGACTCACTATAGGG-3'
pGBKT7 / pBridge MCSI reverse sequencing primer	5'-TAAGAGTCACTTAAAAATTTGTAT-3'
pBridge MCSII forward sequencing primer	5'-TTGGGAACGTGTTGGTTG-3'
pBridge MCSII reverse sequencing primer	5'-CCGTATTACCGCCTTTGAGT-3'
pGADT7 forward sequencing primer	5'-TAATACGACTCACTATAGGG-3'
pGADT7 reverse sequencing primer	5'-GTGAACCTTGGGGTTTTTCAGTATCTACGATT-3'
pMALc2x forward sequencing primer	5'-GGTCGTGAGACTGTCGATGAAGCC-3'
pMALc2x reverse sequencing primer	5'-CGCCAGGGTTTTCCAGTCCACGAC-3'

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