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SUPPLEMENTARY ONLINE DATA

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

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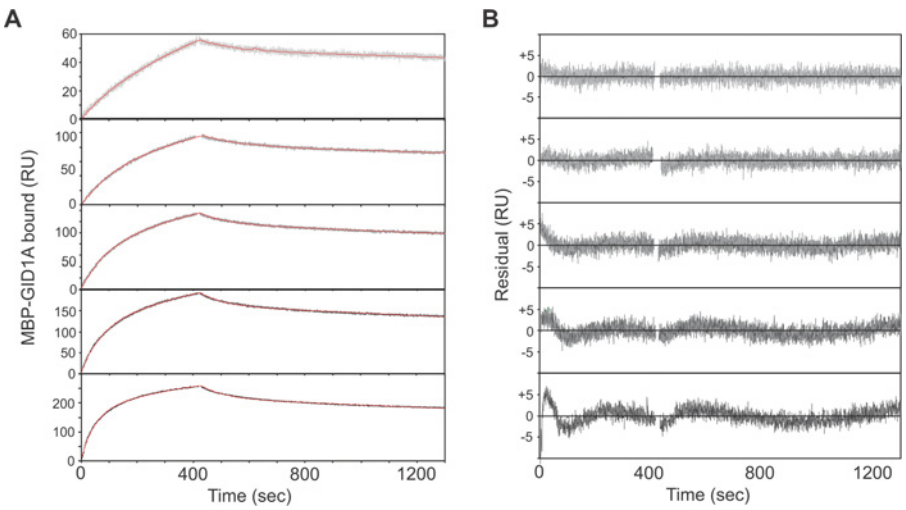

Figure S1 Conformational change kinetic modelling of the gibberellin-dependent GID1A–RGL1N interaction

(**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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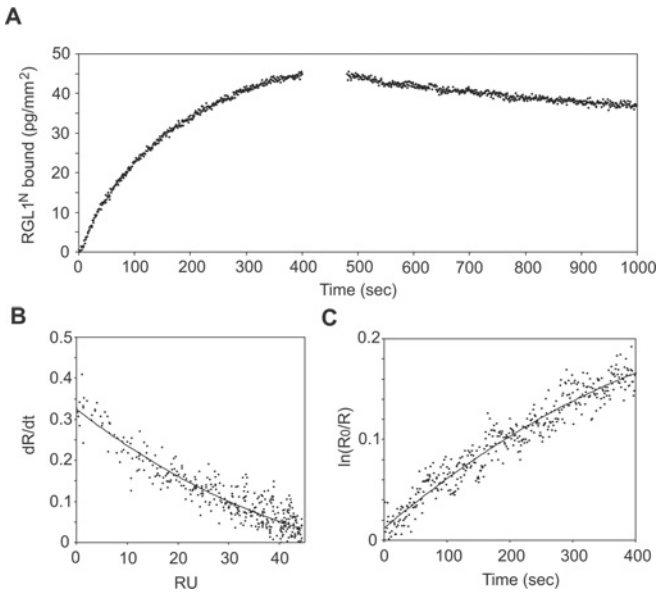

Figure S2 Gibberellin-dependent binding of RGL1^N to immobilized GID1A

(**A**) Binding of purified recombinant RGL1^N (residues 1–137) from a 1 μM solution to immobilized MBP–GID1A in the presence of gibberellin GA_4. 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/mm^2 at 400 s and $-$ 28 pg/mm^2 at 1000 s). Gibberellin (5 μM) was present in all solutions and running buffers. (**B**) dR/dt against R linearization (Scatchard plot) of the association phase. (**C**) $\ln(R_0/R)$ against time linearization of the dissociation phase.

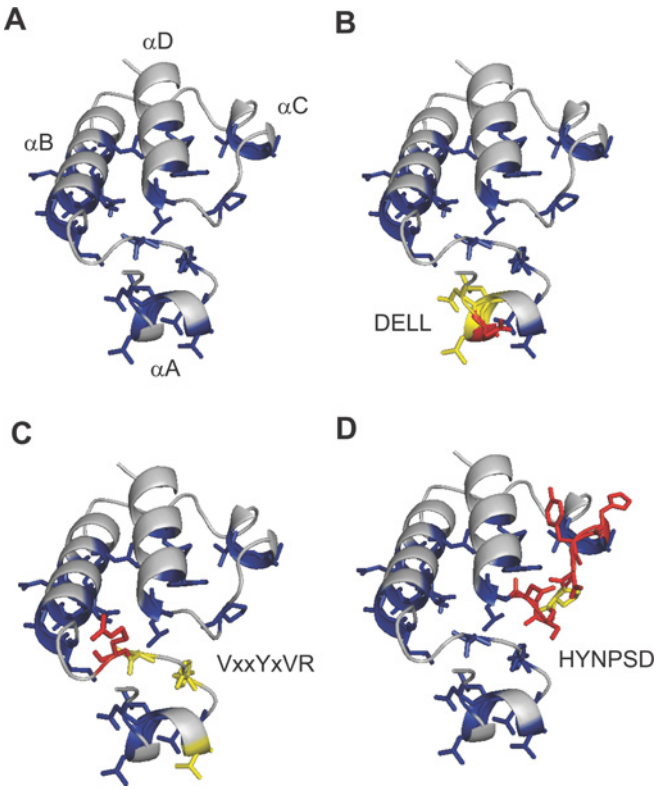

Figure S3 Structural prediction of the RGL1 N-terminal DELLA domain when in complex with GID1A

(**A**) Predicted RGL1^N (residues 1–137) tertiary structure, modelled from the GID1A/GA4:GAI$^{11–113}$ 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.

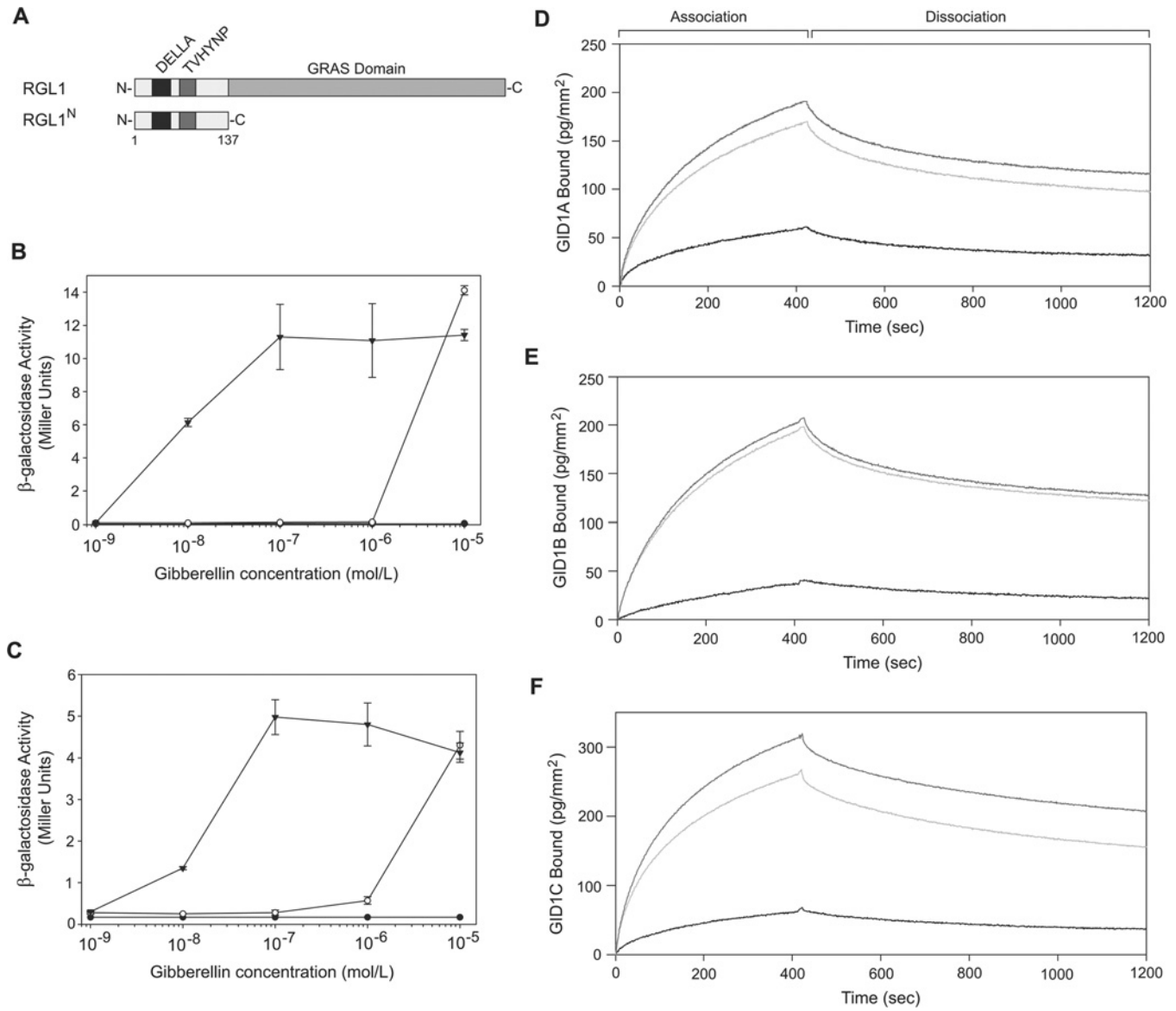

Figure S4 Comparison of the GA_3- and GA_4-dependent GID1A–RGL1^c interaction *in vivo* and *in vitro*

(**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 GA_3 ([EPS]) or GA_4 ([EPS]). The experiment was performed in duplicate (from two independent transformants); β-galactosidase assays were performed in triplicate for each transformant. Error bars show $\pm$ 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 GA_3 (light grey) or 100 M GA_4 (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/mm^2 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*$^{\Delta 17}$; grape (Vv, *Vitis vinifera*) *gai-1*; (Zm, *Zea mays*) rice (Os, *Oryza sativa*) *slr1*w$^{\Delta DELLA}$, *slr1*$^{\Delta SPACE}$, *slr1*$^{\Delta TVHYNP}$, *slr1*$^{\Delta S/T/V}$; 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*).

Figure S5 Continued

Figure S5 Continued

Table S1 Oligonucleotide sequences for amplification and sequencing

Description	Sequence
RGL1 forward primer for pACT2 (XmaI)	5′-TCCCCCGGGTATGAAGAGAGAGCACAACCACC-3′
RGL1 reverse primer for pACT2 (SacI)	5′-CGAGCTCGTTATTCCACACGATTGATTCGCC-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′-ATTCGAGATTCCATCACCAAGAAC-3′
RGL1 internal mutagenic primer reverse	5′-CTTTGATTCGAGCTCTTGCTTTAC-3′
RGL1 internal nt830 forward primer	5′-CCGGCCATTGTAAACCATGG-3′
RGL1 internal nt411 reverse primer	5′-GTTCTTCTCCTTTACTCATTCCGCCACCCGTAGAGGATAACTCCGAT-3′
RGL1 internal nt412 forward primer	5′-GCATGGATGAACTATACAAAGGAGGGGTCGCTCTGTGGTGGTTTTGGATTC-3′
RGL1 forward for pMalc2x (BamHI)	5′-CGGGATCCATGAAGAGAGAGCACAACCACC-3′
RGL1 reverse N-term internal for pMalc2x (SalI)	5′-GACGCGTCGACTTACGTAGAGGATAACTCCGATTCAA-3′
GID1A forward primer for pGBKT7 (EcoRI)	5′-GGAATTCATGGCTGCGAGCGATGAAG-3′
GID1A reverse primer for pGBKT7 (BamHI)	5′-CGGGATCCGTTAACATTCCGCGTTTACAAAC-3′
GID1A forward primer for pBridge MCSII (NotI)	5′-ATAAGAATGCGGCCGCTATGGCTGCGAGCGATGAAG-3′
GID1A reverse primer for pBridge MCSII (NotI)	5′-ATAAGAATGCGGCCGCTATTAACATTCCGCGTTTACAAAC-3′
GID1B forward primer for pGBKT7 (EcoRI)	5′-GGAATTCATGGCTGGTGGTAACGAAGT-3′
GID1B reverse primer for pGBKT7 (BamHI)	5′-CGGGATCCGTCTAAGGAGTAAGAAGCACAGG-3′
GID1C forward primer for pGBKT7 (EcoRI)	5′-GGAATTCATGGCTGGAAGTGAAGAAGTT-3′
GID1C reverse primer for pGBKT7 (BamHI)	5′-CGGGATCCGTTCATTGGCATTCTGCGTTTAC-3′
SLY1 forward primer (EcoRI)	5′-CGGAATTCATGAAGCGCAGTACTACCGAC-3′
SLY1 reverse primer (BamHI)	5′-CGCGGATCCGTTATTTGGATTCTGGAAGAGGTC-3′
SLY1 mutagenic primer reverse primer (BamHI)	5′-CGCGGATCCGTTATTTGGATTCTGGAAGAGGTCTCTTAGTGAAACTCATCTTCTTGTAG-3′
GFP forward primer	5′-ATCGGAGTTATCCTCTACGGGTGGCGAATGAGTAAAGGAGAAGAAC-3′
GFP reverse primer	5′-GAATCCAAAACCACCACAGAGCGACCCCCTCCTTTGTATAGTTCATCCAGC-3′
pACT2 forward sequencing primer	5′-CTATCTATTCGATGATGAAGATAC-3′
pACT2 reverse sequencing primer	5′-AGTTGAAGTGAACTTGCGGGGTT-3′
pGBKT7 forward sequencing primer (T7)	5′-TAATACGACTCACTATAGGG-3′
pGBKT7 / pBridge MCSI raeverse sequencing primer	5′-TAAGAGTCACTTTAAAATTTGTAT-3′
pBridge MCSII forward sequencing primer	5′-TTGGGGAACTGTGGTGGTTG-3′
pBridge MCSII reverse sequencing primer	5′-CCGTATTACCGCCTTTGAGT-3′
pGADT7 forward sequencing primer	5′-TAATACGACTCACTATAGGG-3′
pGADT7 reverse sequencing primer	5′-GTGAACTTGCGGGGTTTTTCAGTATCTACGATT-3′
pMALc2x forward sequencing primer	5′-GGTCGTCAGACTGTCGATGAAGCC-3′
pMALc2x reverse sequencing primer	5′-CGCCAGGGTTTTCCCAGTCCACGAC-3′

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