Title: Proteolysis-independent down-regulation of DELLA repression in Arabidopsis by the gibberellin receptor GIBBERELLIN INSENSITIVE DWARF1.

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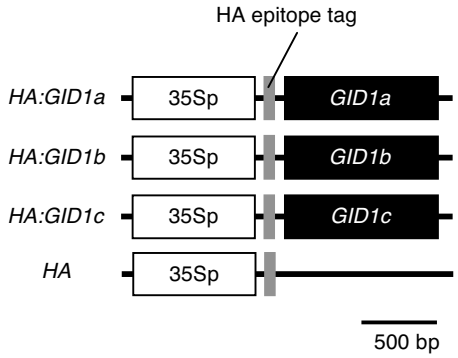
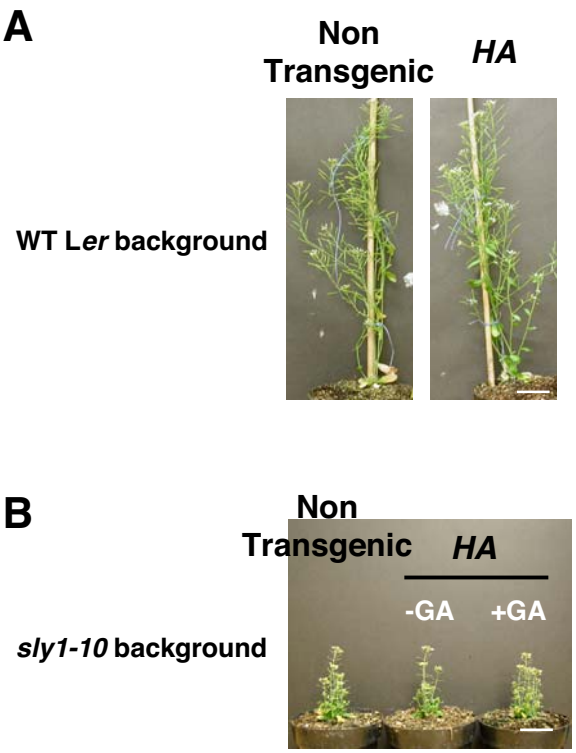


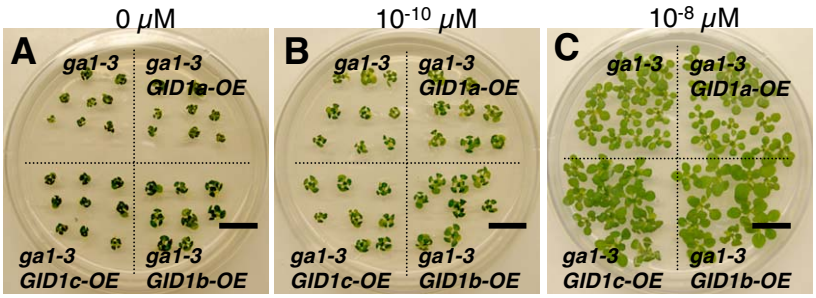
Supplemental Figure 1.

Schematic diagram of the chimeric HA:GID1 constructs. The Arabidopsis *GID1a*, *GID1b* and *GID1c* genes were overexpressed under control of the constitutive 35S CaMV promoter. The HA epitope tag was fused in-frame with the *GID1* coding region at the N terminal position to allow determination of GID1 protein expression levels. A construct in which only the HA epitope tag is overexpressed was created as a control. These constructs are named as *HA:GID1a*, *HA:GID1b*, *HA:GID1c* and *HA*.



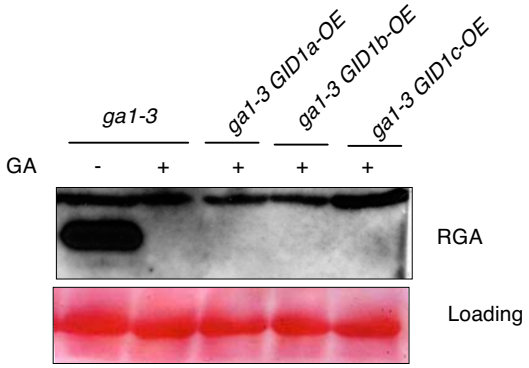
Supplemental Figure 2.

The *HA* control construct had no effect on growth and development. A construct overexpressing only the HA epitope tag was introduced into WT (**A**) and the *sly1-10* (**B**) mutant backgrounds. No effect on growth or fertility was observed after 36-d of growth. Transgenic plants were treated with and without 10 μM GA_4 every 3 d. Bar = 5 cm.



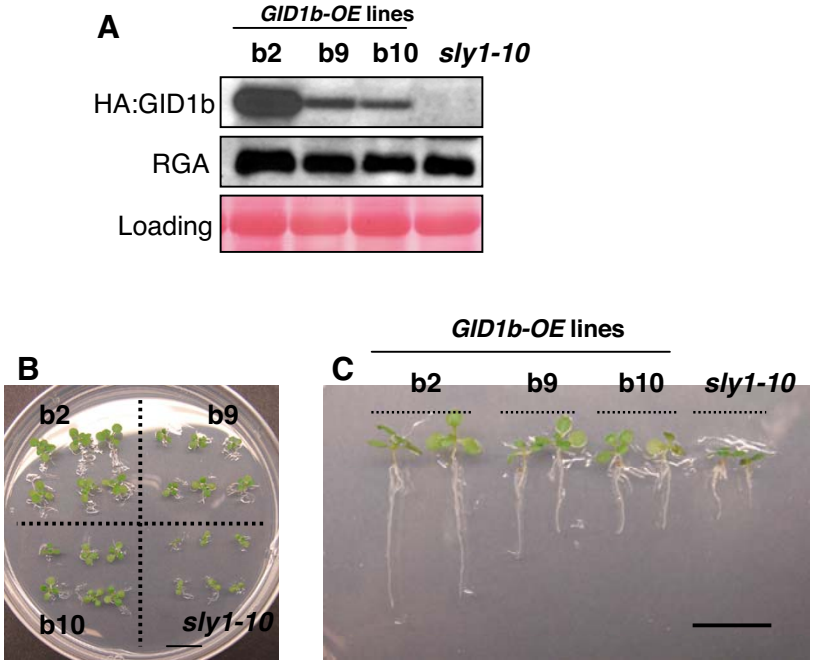
Supplemental Figure 3.

ga1-3 plants used for measurements shown in Figure 2 show enhanced GA sensitivity when transformed with *GID1-OE* constructs. The *ga1-3* mutant and the transgenic *ga1-3* mutant plants in which each *GID1* gene was overexpressed (*GID1a-OE*, *GID1b-OE* and *GID1c-OE* lines) were grown on MS-agar containing different concentrations of GA (0, 10^{-10}, and 10^{-8} M) at 22°C for 10 d. Seeds were incubated in an aqueous 10^{-5} M GA_4 solution at 4°C for 3 d to stimulate seed germination (See Methods). (**A**) 0 μM, (**B**) 10^{-10} μM, (**C**) 10^{-8} μM. Bar = 1 cm.



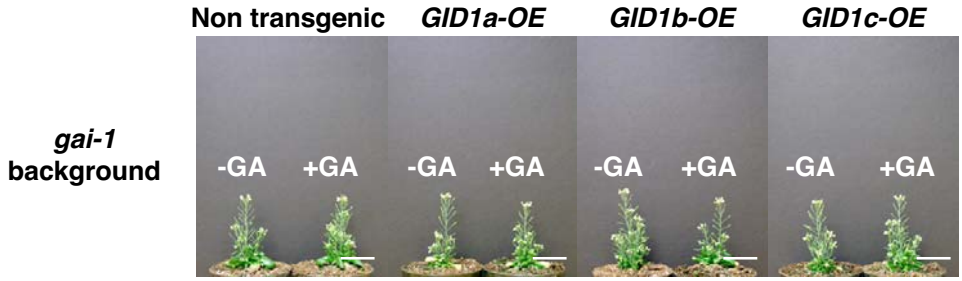
Supplemental Figure 4.

RGA protein accumulation after GA treatment in *ga1-3 GID1-OE* plants. After 10-d-old *ga1-3* and *ga1-3 GID1-OE* plants were treated with 100 μM GA_3 for 3 h, total protein was extracted and 40 μg was analyzed by immunoblot. Protein from the 10-d-old *ga1-3* seedling without GA treatment was also loaded. Equal loading was confirmed by ponceau staining. Bar = 1 cm.



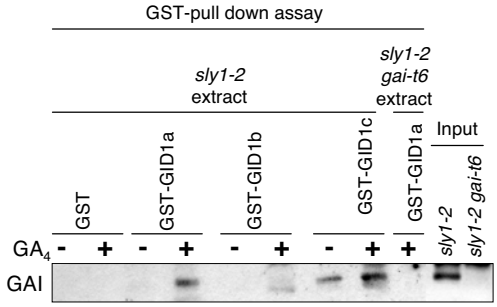
Supplemental Figure 5.

HA:GID1b levels correlate with *sly1-10* seedling growth. (**A**) Protein blot analysis to detect HA:GID1b and RGA protein accumulation from 14-d-old seedling of *sly1-10* and independent *sly1-10 GID1b-OE* lines (b2, b9 and b10). 40 µg of total protein was loaded. 14-d-old seedling appearance (**B**) and root development (**C**) in *sly1-10* and independent *sly1-10 GID1b-OE* lines. Bar = 1 cm.



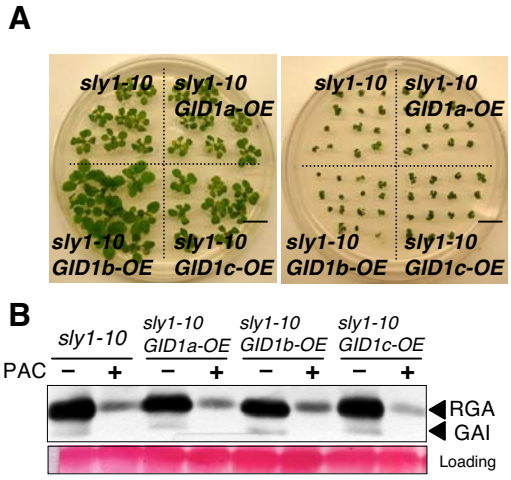
Supplemental Figure 6.

HA:GID1 constructs did not suppress growth defects of the *gai-1* mutant. *HA:GID1a*, *HA:GID1b* and *HA:GID1c* constructs were introduced into *gai-1* mutant background. Growth of 36-d-old plants were compared with and without GA treatment. Bar = 5 cm.



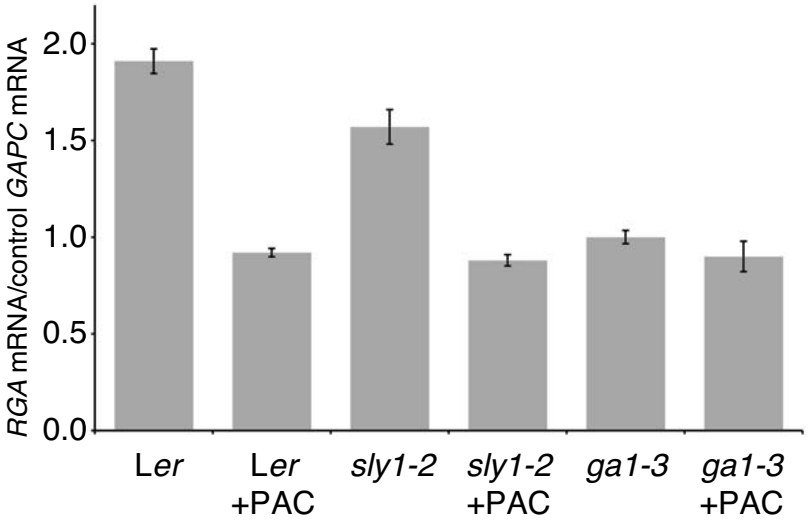
Supplemental Figure 7

GST-GID1 pull down assay shows interaction with DELLA GAI protein. GST pull-down assays of GAI protein using GST-GID1a, GST-GID1b, and GST-GID1c were performed according to Griffiths et al., (2006) with the following modifications. Five gram of 11d-old seedlings of *sly1-2* and *sly1-2 gai-t6* were harvested, ground in liquid nitrogen, and suspended in 3 ml buffer B. The extracted protein was centrifuged, and the supernatant was transferred to new tubes. The 2 mg of total protein extract from the *sly1-2* and/or *sly1-2 gai-t6* was mixed with 80 µl of beads from recombinant GST-GID1 proteins, and with 25 µl beads from control GST protein. The mixture was agitated for 2 h at 4°C in the absence (-) and presence (+) of 100 µM GA_4. The beads were washed five times with buffer A, and resuspended with 10 µl of 6x SDS sample buffer. 15 µl of beads for each sample were loaded for protein blot analysis. GAI protein was detected using affinity purified SLR1 antibody (1:10,000; Itoh et al., 2002). Incubation with secondary antibody and detection of signals were performed as described in Materials and Methods.



Supplemental Figure 8.

The GA biosynthesis inhibitor PAC blocks rescue of *sly1-10* by *GID1* overexpression. **(A)** 10 d-old *sly1-10* and the *sly1-10 GID1-OE* plants were transferred to MS-agar with and without 1 μM PAC treatment and incubated for 12 d at 22°C. Bar = 1 cm. **(B)** The effect of PAC treatment on the RGA and GAI protein accumulation was determined by protein blot analysis using RGA antibody. 40 μg of total protein from (**A**) was loaded, and equal loading confirmed by ponceau staining.



Supplemental Figure 9

RGA mRNA accumulation in WT L*er*, *sly1-2* and *ga1-3* mutants in the absence and presence of PAC treatment. 10d-old seedlings of L*er*, *sly1-2* and *ga1-3* plants were moved to the fresh MS media with and without PAC application. After 12-d incubation at 22^{o}C, total mRNA was extracted, cDNA was synthesized and qRT-PCR analysis was performed as described in Materials and Methods. Mean values for at least three independent experiments are shown. Error bars show standard deviation.

Supplemental Table 1

Table S1a Final plant height (cm) in the *sly1-10* background.

NT		*HA*		*GID1a-OE* (a6)		*GID1a-OE* (a7)		*GID1b-OE* (b2)		*GID1b-OE* (b3)		*GID1c-OE* (c3)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
17.7 ± 0.4	17.6 ± 0.9	18.1 ± 0.6	17.6 ± 0.9	20.2 ± 0.6[a]	20.0 ± 0.8[a]	21.5 ± 0.8[b]	21.4 ± 0.7[b]	31.8 ± 1.1[b]	31.7 ± 1.4[b]	30.4 ± 1.2[b]	30.8 ± 1.2[b]	22.0 ± 0.3[b]	21.7 ± 0.7

Table S1b Final plant height (cm), in the *sly1-2* mutant background.

NT		*GID1a-OE* (a3)		*GID1b-OE* (b6)		*GID1b-OE* (b8)		*GID1c-OE* (c4)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
11.3 ± 0.5	10.9 ± 0.6	20.5 ± 0.5[b]	20.2 ± 1.0[b]	31.1 ± 0.5[b]	31.7 ± 0.9[b]	30.7 ± 1.5[b]	30.3 ± 0.6[b]	22.4 ± 0.7[b]	22.2 ± 0.5[b]

Table S1c Final plant height (cm), in the WT L*er*.

NT		*HA*		*GID1a-OE* (a6)		*GID1b-OE* (b1)		*GID1c-OE* (c7)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
28.4 ± 0.7	31.5 ± 0.6	28.2 ± 0.8	31.0 ± 0.4	32.2 ± 0.3[b]	33.1 ± 0.4[a]	38.3 ± 0.5[b]	41.6 ± 0.6[b]	34.5 ± 0.4[b]	35.9 ± 0.2[b]

Table S1d Final height (cm), in the *ga1-3* background.

NT		*GID1a-OE* (a7)		*GID1abOE* (b2)		*GID1c-OE* (c7)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
N.D.	17.7 ± 0.9	N.D.	20.2 ± 0.4[a]	N.D.	26.0 ± 0.5[b]	N.D.	23.0 ± 0.4[b]

Supplemental Table 1

Supplemental Table 1

Table S1e Final plant height (cm), in the *rga*-Δ*17* background.

NT		*GID1a-OE* (a6)		*GID1b-OE* (b2)		*GID1c-OE* (c7)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
10.5 ± 0.3	10.4 ± 0.4	10.1 ± 0.3	10.2 ± 0.4	10.6 ± 0.6	10.8 ± 0.7	10.6 ± 0.6	10.5 ± 0.9

Table S1f Final plant height (cm), in the *gai1-1* background.

NT		*GID1a-OE* (a1)		*GID1b-OE* (b1)		*GID1c-OE* (c1)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
13.0 ± 0.5	13.6 ± 0.2	13.5 ± 0.6	12.7 ± 0.7	13.7 ± 0.6	13.0 ± 0.4	13.3 ± 0.6	13.0 ± 0.4

NT, not transformanted
HA, transformants with construct *HA* (Supplemental Figure 1).
GID1a-OE, transformants with construct *HA:GID1a*.
GID1b-OE, transformants with construct *HA:GID1b*.
GID1c-OE, transformants with construct *HA:GID1c*.
Independently transformed lines indicated in ().
A significant difference from untransformed plants is indicated as a, $P < 0.05$ or b, $P < 0.01$ as determine by *t* test.

Supplemental Table 2

Table S2a Fertility, number of seeds/silique in the *sly1-10* background.

N T		*HA*		*GID1a-OE* (a7)		*GID1b-OE* (b3)		*GID1c-OE* (c3)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
18.0 ± 2.8	18.8 ± 2.1	17.4 ± 2.1	18.2 ± 2.4	45.2 ± 3.8[b]	45.1 ± 2.6[b]	41.6 ± 2.1[b]	38.7 ± 4.4[b]	61.5 ± 3.5[b]	61.6 ± 7.6[b]

Table S2b Fertility, number of seeds/silique in the *sly1-2* background.

NT		*GID1a-OE* (a3)		*GID1b-OE* (b8)		*GID1c-OE* (c4)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
12.1 ± 2.0	10.6 ± 1.9	48.7 ± 3.3[b]	47.6 ± 3.2[b]	40.6 ± 3.1[b]	42.9 ± 1.9[b]	63.5 ± 3.5[b]	64.8 ± 1.6[b]

Table S2c Fertility, number of seeds/silique in WT Ler.

NT		HA		*GID1a-OE* (a6)		*GID1b-OE* (b1)		*GID1c-OE* (c7)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
71.3 ± 2.2	71.7 ± 2.8	71.0 ± 3.0	72.0 ± 2.9	71.2 ± 2.0	72.2 ± 1.6	72.7 ± 2.8	70.3 ± 2.1	74.3 ± 6.3	73.7 ± 2.9

Table S2d Fertility, number of seeds/silique in the *ga1-3* background.

NT		*GID1a-OE* (a7)		*GID1b-OE* (b2)		*GID1c-OE* (c7)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
N.D.	25.8 ± 2.8	N.D.	26.4 ± 3.0	N.D.	27.4 ± 3.0	N.D.	27.6 ± 4.8

Table S2e Fertility, seeds/silique in the *rga*-Δ*17* background.

NT		*GID1a-OE* (a6)		*GID1b-OE* (b2)		*GID1c-OE* (c7)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
64.3 ± 1.9	66.0 ± 2.5	62.0 ± 3.9	61.2 ± 4.6	60.4 ± 6.0	69.3 ± 5.4	64.0 ± 2.6	66.0 ± 2.5

Table S2f Fertility, seeds/silique in the *gai1-1* background.

NT		*GID1a-OE* (a1)		*GID1b-OE* (b1)		*GID1c-OE* (c1)	
-GA	+GA	-GA	+GA	-GA	+GA	-GA	+GA
50.0 ± 4.6	48.4 ± 3.9	49.9 ± 3.4	52.8 ± 7.4	47.8 ± 5.9	54.1 ± 5.1	53.1 ± 5.0	51.8 ± 3.7

NT, Not transformed plants
HA, transformants with *HA* vector (Supplemental Figure 1).
GID1a-OE, transformed with construct 35S:*HA::G1D1a*.
GID1b-OE, transformed with construct 35S:*HA::G1D1b*.
GID1c-OE, transformed with construct 35S:*HA::GID1c*.
Independently transformed lines indicated in ().
A significant difference from untransformed plants is indicated as a, $P < 0.05$ or b, $P < 0.01$ as determine by *t* test.

Supplementary Table 3. Primer sequences used for this study.

Amplified PCR fragment	Primer-F	Primer-R	Purpose
GID1a	ATGGCTGCGAGCGATGAAGT	TTAACATTCCGCGTTTACAAACGCCGAA	Construction of overexpression vector
GID1b	ATGGCTGGTGGTAACGAAGT	CTAAGGAGTAAGAAGCACAG	Construction of overexpression vector
GID1c	ATGGCTGGAAGTGAAGAAGT	TCATTGGCATTCTGCGTTTACAAAT	Construction of overexpression vector
Hemagglutinin (HA)	ATGGCAGGTTACCCATACGAC	CATATGGTGGACGCCTCTAGA	Construction of overexpression vector
RGA	AGAAGCAATCCAGCAGA	GTGTACTCTCTTCTTACCTTC	Quantitative RT-PCR
GAPC	AGCTGCTACCTACGATG	CACACGGGAACTGTAAC	Quantitative RT-PCR