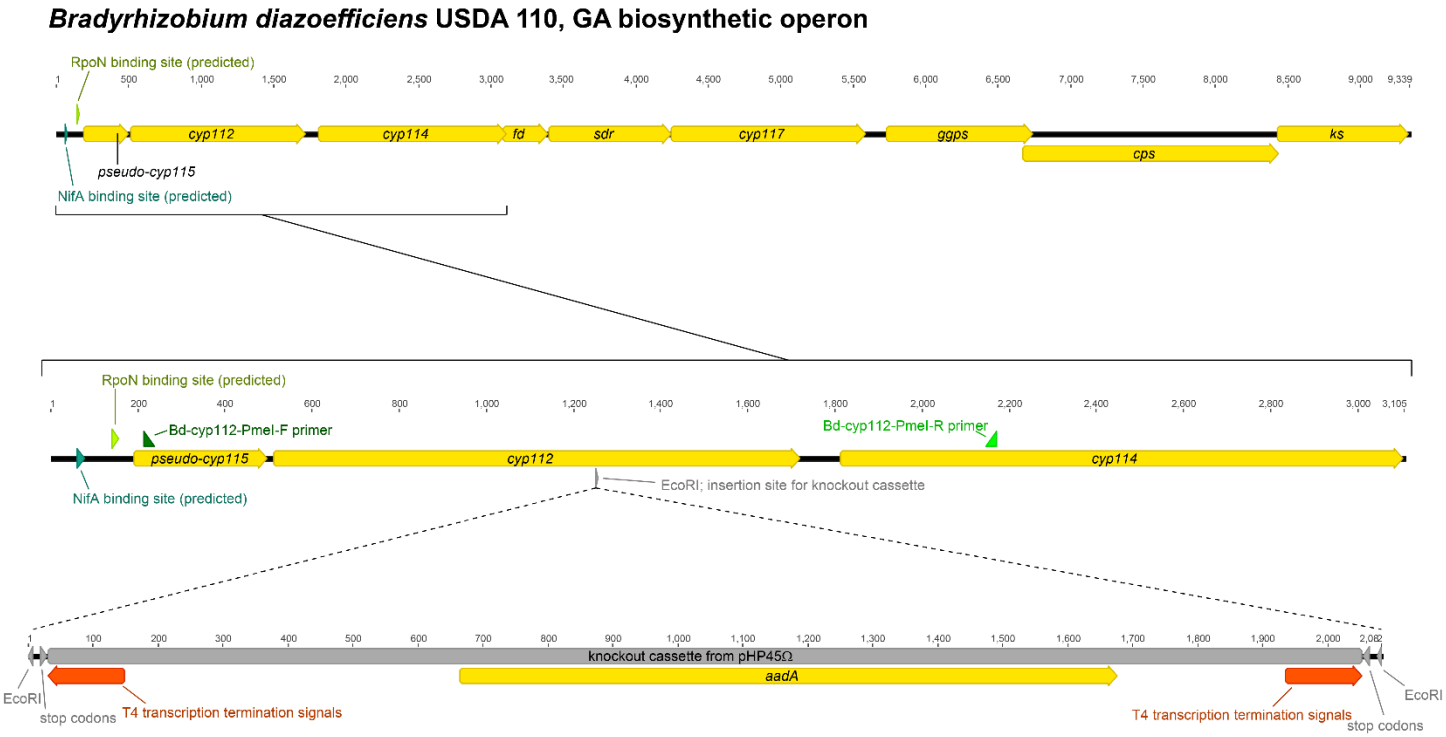
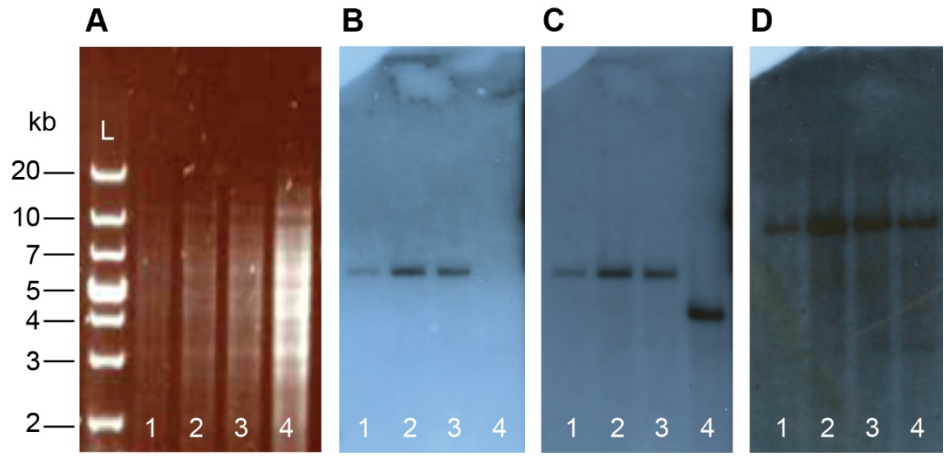
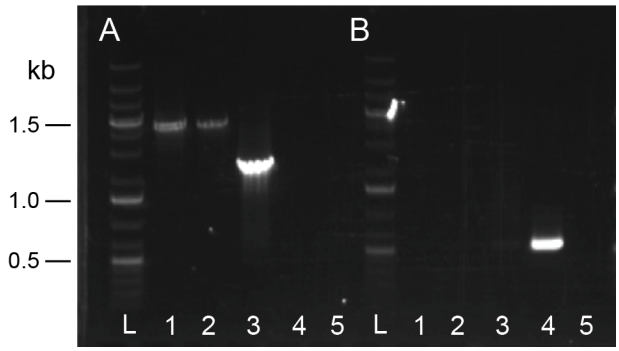
SUPPLEMENTARY INFORMATION



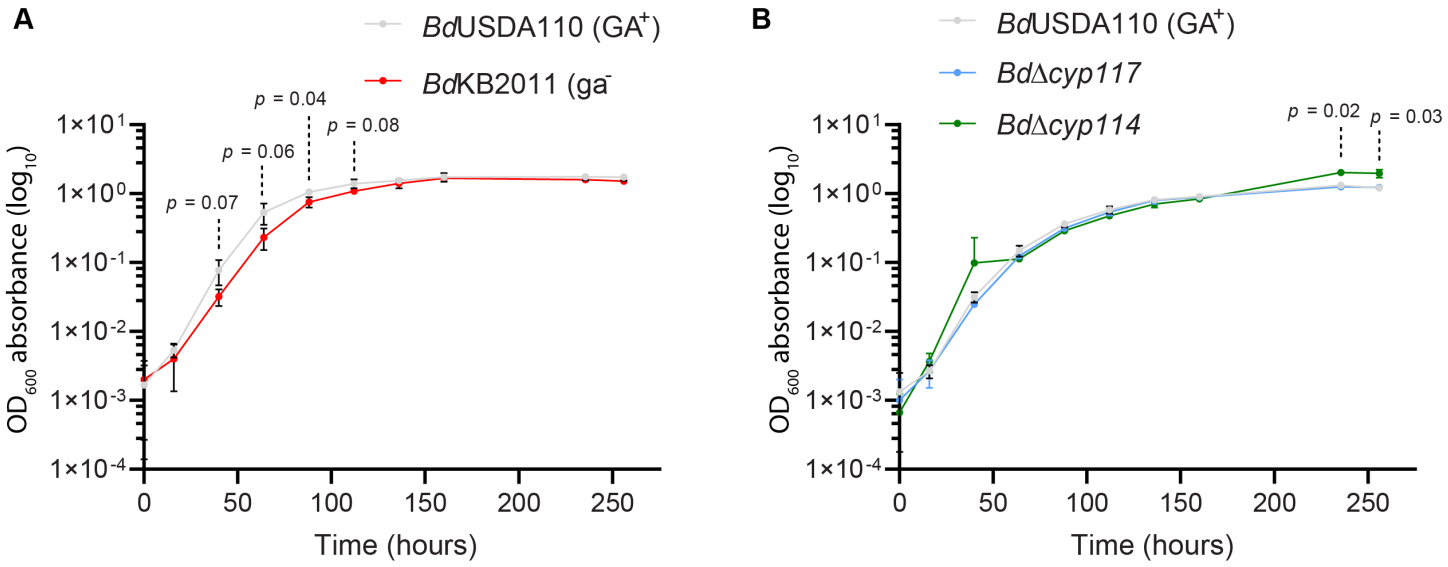
Supplementary Figure 1. Schematic of GA operon knockout strategy. Polar knockouts of the GA operon in *Bradyrhizobium diazoefficiens* USDA 110 were constructed by inserting a knockout cassette containing *aadA* and flanking terminators (derived from pHP45Ω [1]) into *cyp112*.



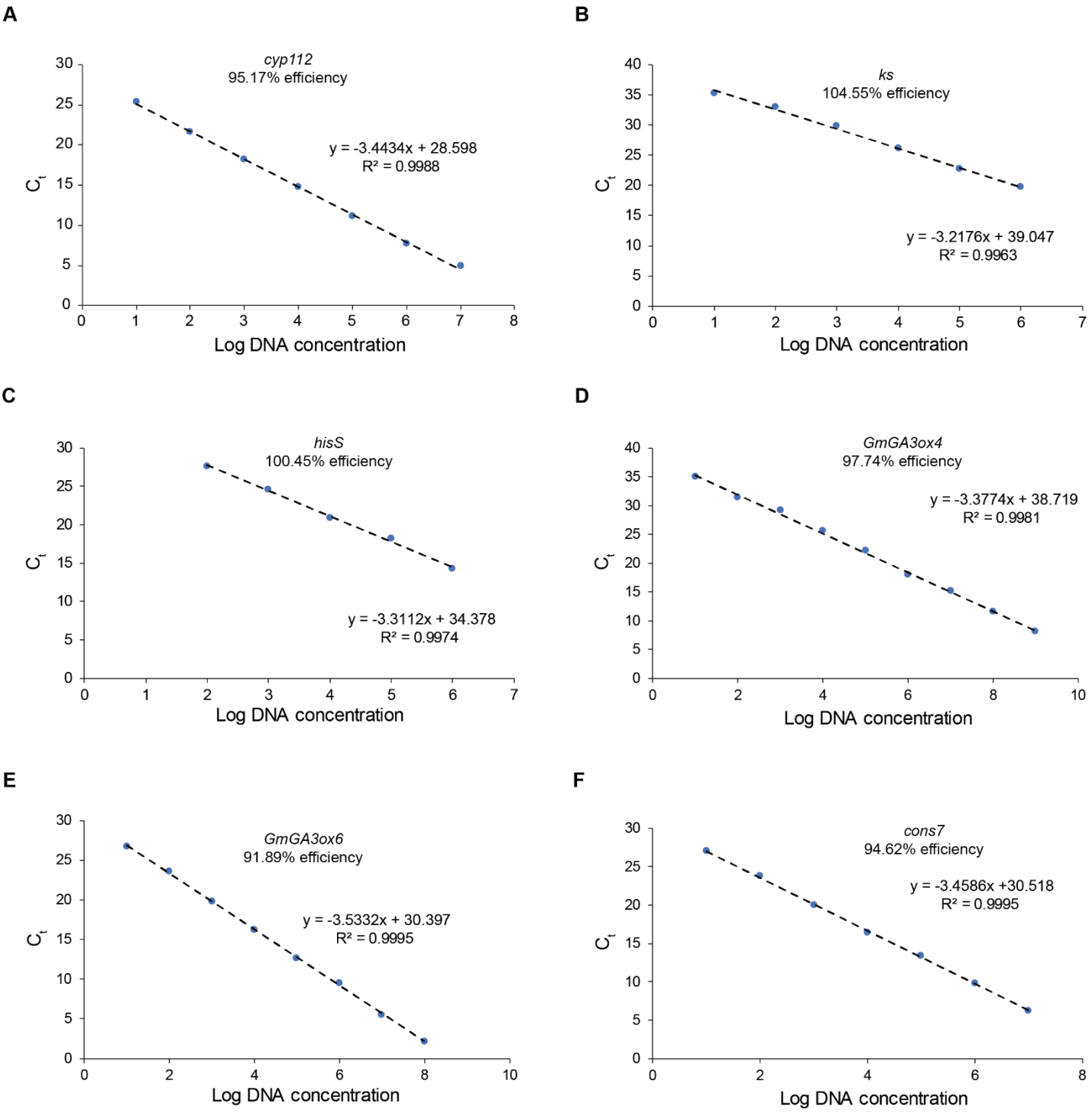
Supplementary Figure 2. Southern blot analysis of *B. diazoefficiens* USDA 110 GA operon knockout strains KB0903, KB0904, and KB2011. **A**) Agarose gel of XhoI gDNA digests. The gel was loaded as follows: L = DNA ladder, 1 = KB0903, 2 = KB0904, 3 = KB2011, and 4 = *B. diazoefficiens* USDA 110 (wild type). Panels **B-D** are blots of the gel in panel **A** probed with the following: **B**) *aadA*, **C**) *cyp112*, **D**) *cyp114.* kb = kilobase



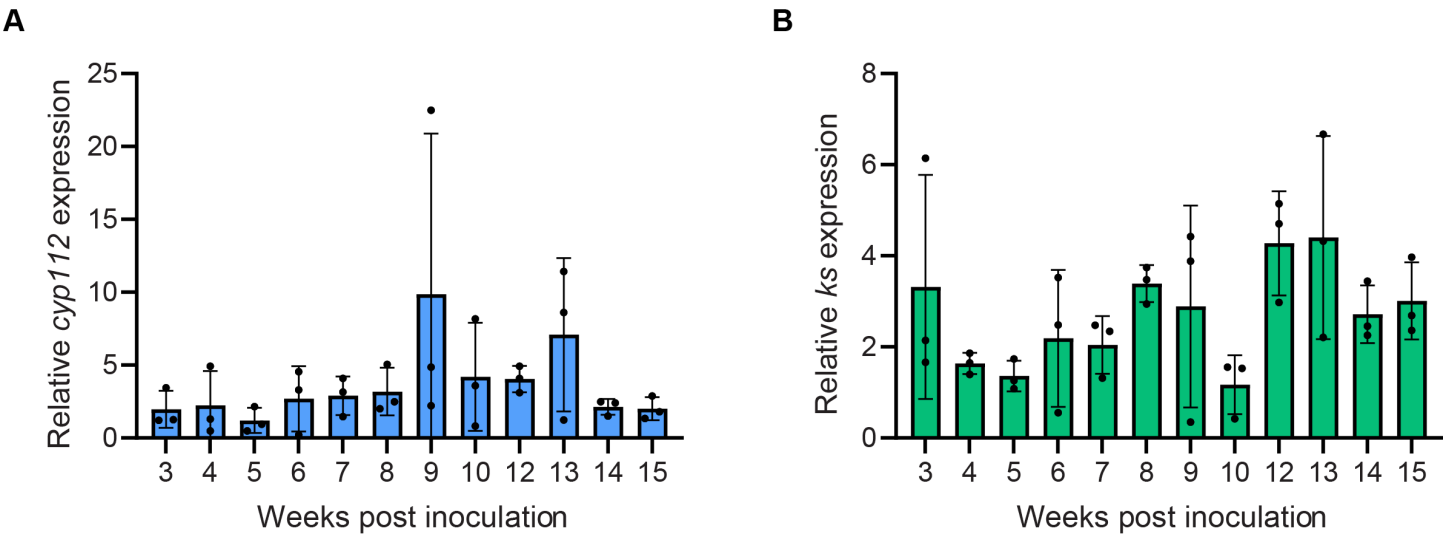
Supplementary Figure 3. **PCR analysis of *B. diazoefficiens* USDA 110 GA operon knockout strains KB0904 and KB2011. a**) PCR with primer set targeting the chromosomal region outside of the insertion of the *aadA* interruption cassette (primers bind to the *pseudo cyp115* and *cyp114* genes). **b**) PCR with primer set targeting the *sacB* gene of the pLOBJ3 suicide vector used for introducing the interruption cassette. Lanes 1-5 correspond to the following template DNA: 1 = KB2011, 2 = KB0904, 3 = *B. diazoefficiens* USDA 110 (wild type), 4 = pLOBJ3 mating vector, and 5 = no DNA; L = DNA ladder. kb = kilobase



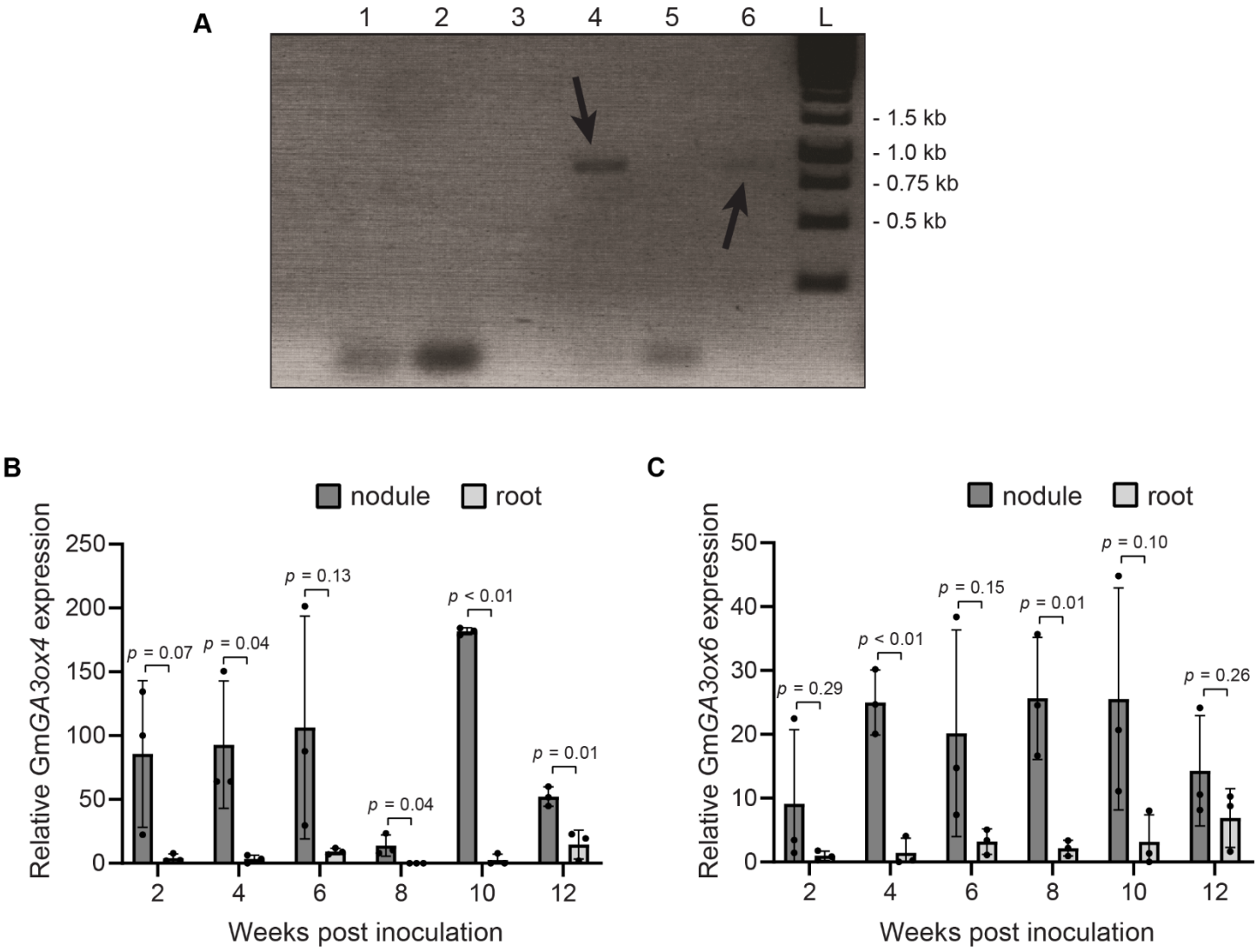
Supplementary Figure 4. Aerobic growth curves of *B. diazoefficiens* USDA110 (GA$^+$) and GA operon mutants. A) GA$^+$ (*Bd*USDA110) growth compared to that of ga$^-$ (*Bd*KB2011), as measured by the optical density of the cultures measured at a wavelength of 600 nm (OD_{600}). Note that the y-axis is on a $\log_{10}$ scale. Statistical significance for each time point was determined with an unpaired t-test. *P*-values less than 0.1 are shown above the relevant time points. All other time points had a *P*-value > 0.1. n = 3 cultures for each strain with the mean ± SD shown. **B)** GA$^+$ growth compared to that of *cyp117* and *cyp114* deletion mutants (*BdΔcyp117* and *BdΔcyp114*, respectively). Note that the y-axis is on a $\log_{10}$ scale. n = 3 cultures for each strain with the mean ± SD shown. After 40 hours, one of the GA$^+$ cultures was contaminated, so only two replicates were measured for subsequent time points. Statistical significance for each time point was determined with separate unpaired t-tests between GA$^+$/*BdΔcyp117* and GA$^+$/*BdΔcyp114*. All *p* values were > 0.05 except for those indicated in the figure for *BdΔcyp114*.



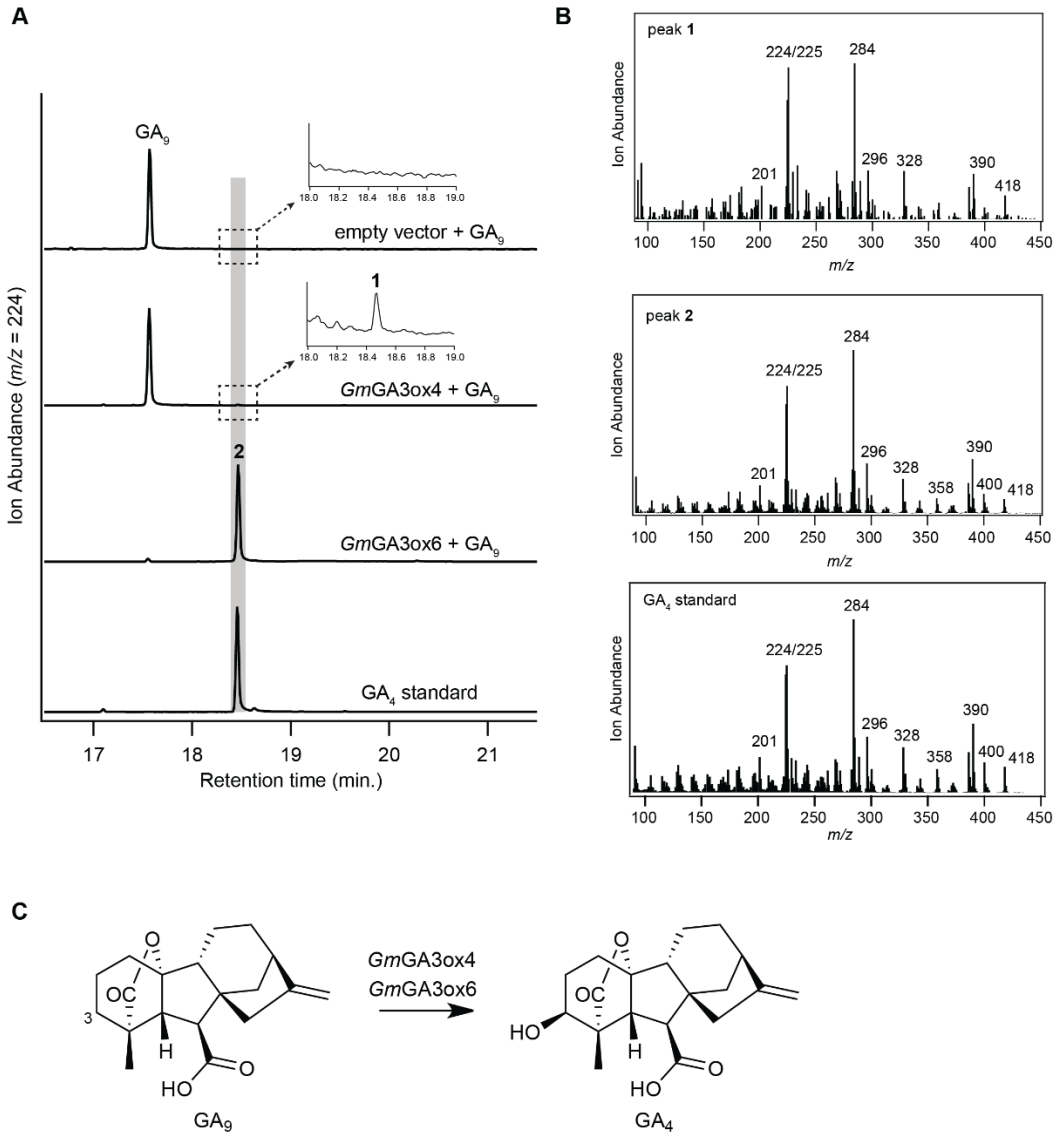
Supplementary Figure 5. qPCR primer efficiencies. Shown are the calculated cycle threshold (C_t) values plotted against the corresponding DNA template concentration (log scale) for the qPCR primer sets used to quantify expression for **A**) *cyp112*, **B**) *ks*, and **C**) *hisS* (reference gene) from *B. diazoefficiens* USDA 110, along with **D**) *GA3ox4*, **E**) *GA3ox6*, and **F**) *cons7* (reference gene) from *G. max* cv. Williams ’82.



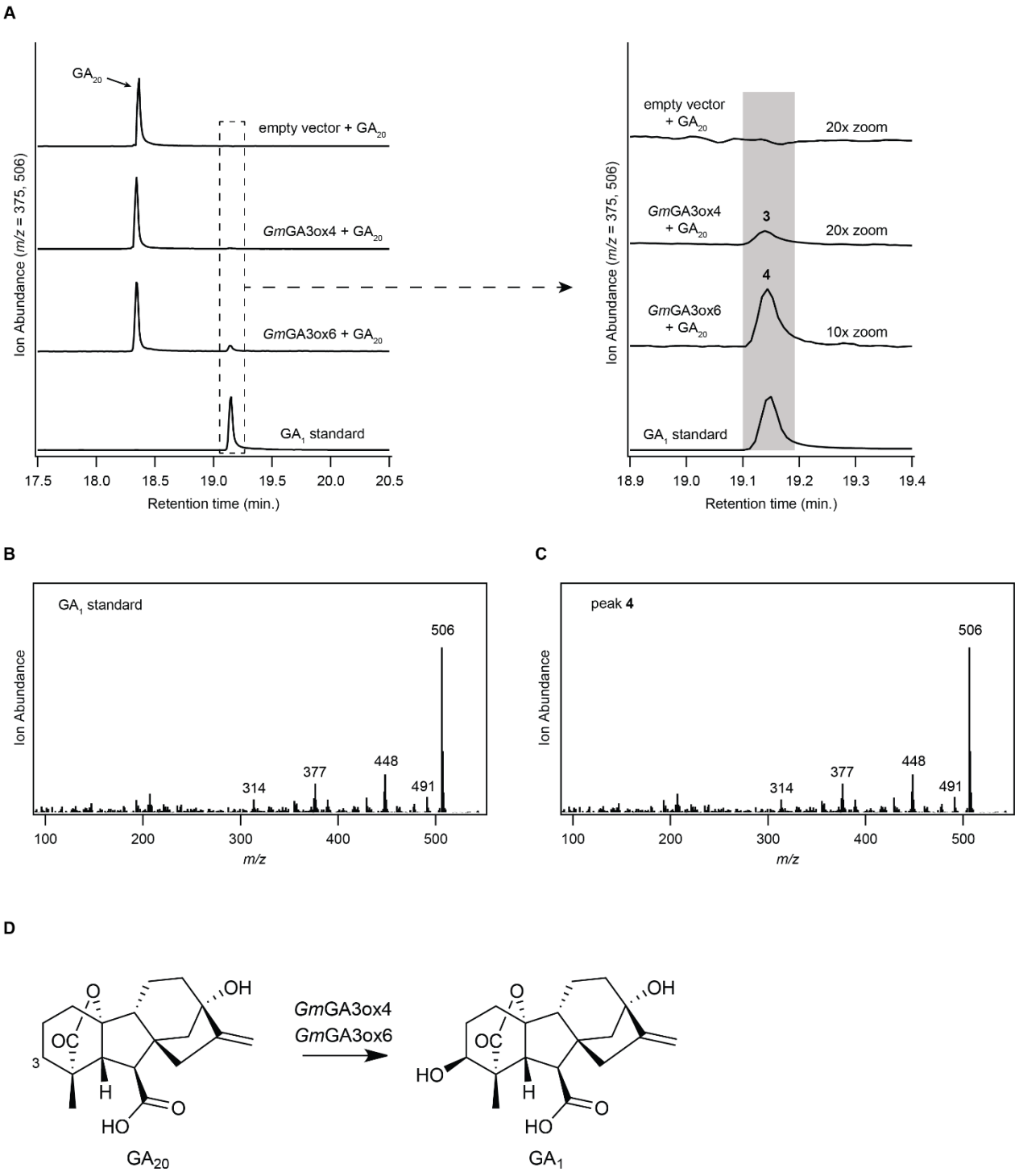
Supplementary Figure 6. Expression of GA operon genes during the *Bradyrhizobium diazoefficiens*-soybean symbiosis. Bacteroid expression of **A**) *cyp112* and **B**) *ks* over the course of *B. diazoefficiens* USDA 110 symbiosis with soybean. Target gene expression was normalized using *B. diazoefficiens* USDA 110 *hisS* as a reference gene, and relative expression was then calculated by dividing all values by the lowest mean expression value of the data set. n = 3 biological replicates (nodules derived from three different plants) for each time point with ± standard deviation (SD) shown.



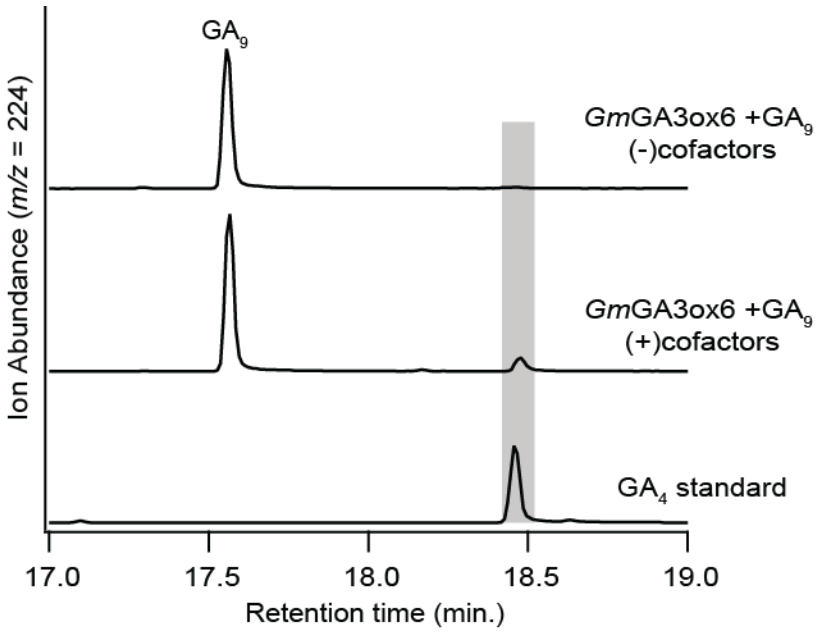
Supplementary Figure 7. Expression of soybean GA 3-oxidase isoforms in nodule tissue. A) PCR with cDNA isolated from mature nodules (during flowering stage) demonstrates that two isoforms are noticeably expressed in this tissue, as shown via agarose gel analysis. Primers for this PCR were design to produce amplicons of roughly 800-1000 base pairs. Arrows indicate detected amplicon bands. 1: Gm*GA3ox1*, 2: Gm*GA3ox2*, 3: Gm*GA3ox3*, 4: Gm*GA3ox4*, 5: Gm*GA3ox5*, 6: Gm*GA3ox6*, L: DNA ladder. kb = kilobase. Expression of **B)** Gm*GA3ox4* and **C)** Gm*GA3ox6* was confirmed via qPCR analysis of cDNA prepared from soybean nodule and root tissue over 12 weeks of development. Target gene expression was normalized using *G. max* cv. Williams '82 *cons7* as a reference gene, and relative expression was then calculated by dividing values by the mean expression value of the lowest mean within the data set. n = 3 for each tissue at each time point. Each replicate represents cDNA samples prepared from tissue derived from different plants. Shown for each condition is the mean ± SD. Statistical comparisons for each time point were made using two-tailed t-tests.



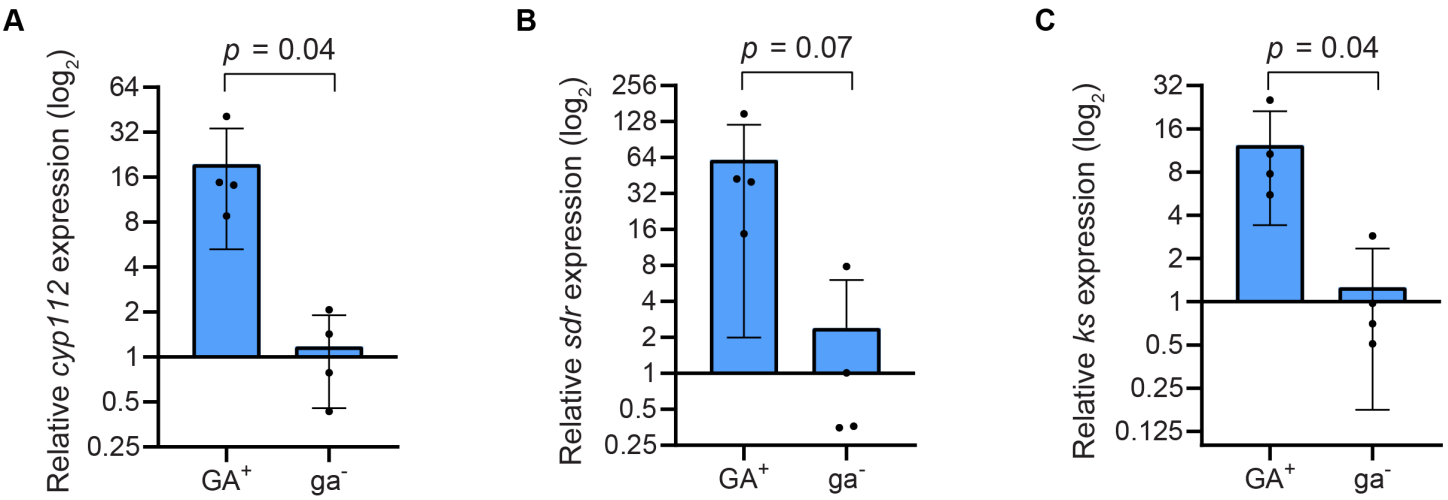
Supplementary Figure 8. Confirmation of GA 3-oxidase activity by *Gm*GA3ox4 and *Gm*GA3ox6. A) Incubating GA_9 in cells expressing synthetic clones of either *Gm*GA3ox4 or *Gm*GA3ox6 results in consumption of GA_9 and production of GA_4, as shown here through extracted ion chromatograms from GC-MS analysis. **B**) Mass spectra of the putative GA_4 products and an authentic GA_4 standard. The chromatograms and spectra shown are representative of the methyl ester (GA_9) or methyl ester, trimethylsilyl ether (GA_4) of the corresponding compounds. **C)** Reaction proposed to be catalyzed by *Gm*GA3ox4 and *Gm*GA3ox6 - 3β-hydroxylation of GA_9 to produce bioactive GA_4.



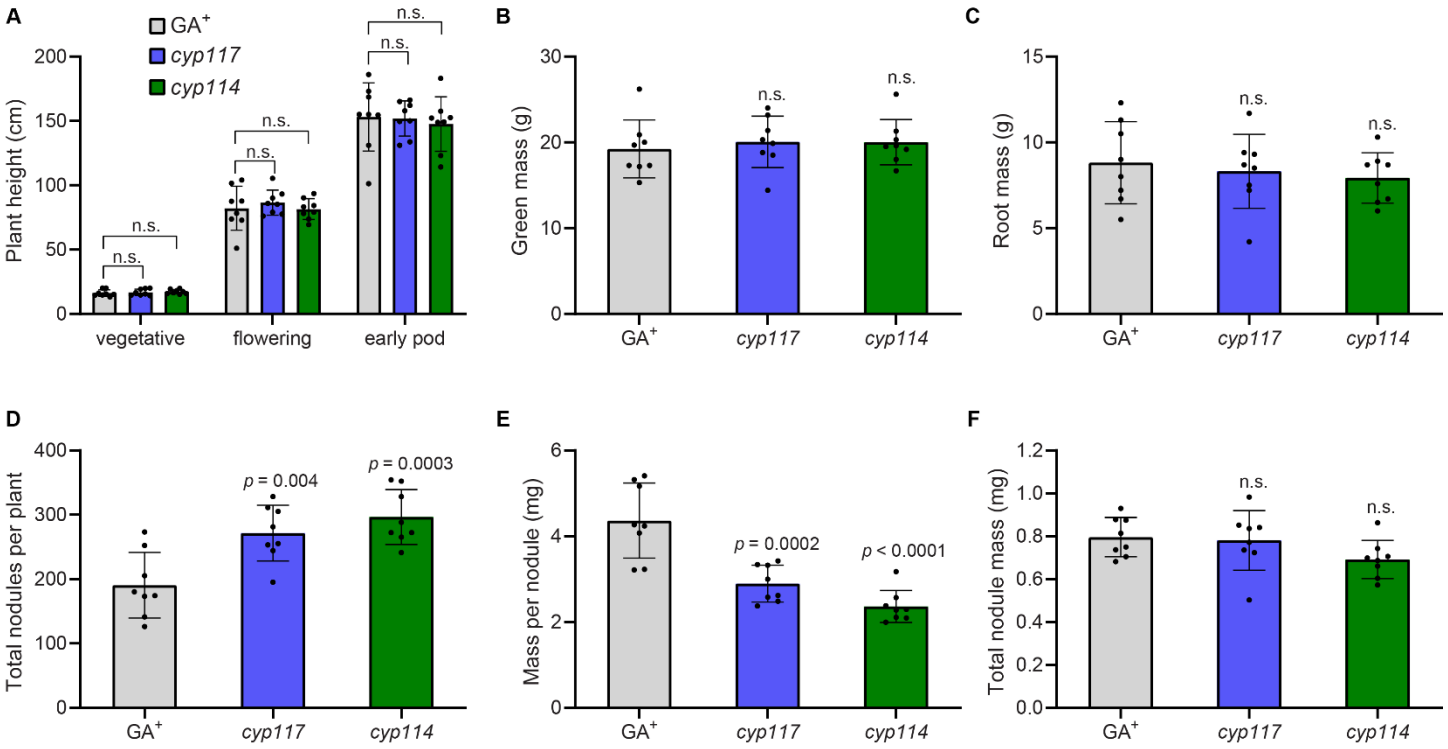
Supplementary Figure 9. *Gm*GA3ox4 and *Gm*GA3ox6 exhibit GA 3-oxidase activity with GA_{20}. A) Incubation of GA_{20} in *E. coli* cells expressing either *Gm*GA3ox4 or *Gm*GA3ox6 results in formation of GA_1, as shown here via extracted ion chromatograms from GC-MS analysis. **B**) Mass spectrum of an authentic GA_1 standard. **C**) Mass spectrum of peak **4**, a putative GA_1 peak produced from incubation of GA_{20} with *Gm*GA3ox6-expressing cells. The abundance of peak **3** was too low for a high-quality mass spectrum to be derived. The chromatograms and spectra shown are representative of the methyl ester, trimethylsilyl ether of the corresponding compounds. **D)** Proposed 3β-hydroxylation of GA_{20} by *Gm*GA3ox4 and *Gs*GA3ox6 to produce bioactive GA_1.



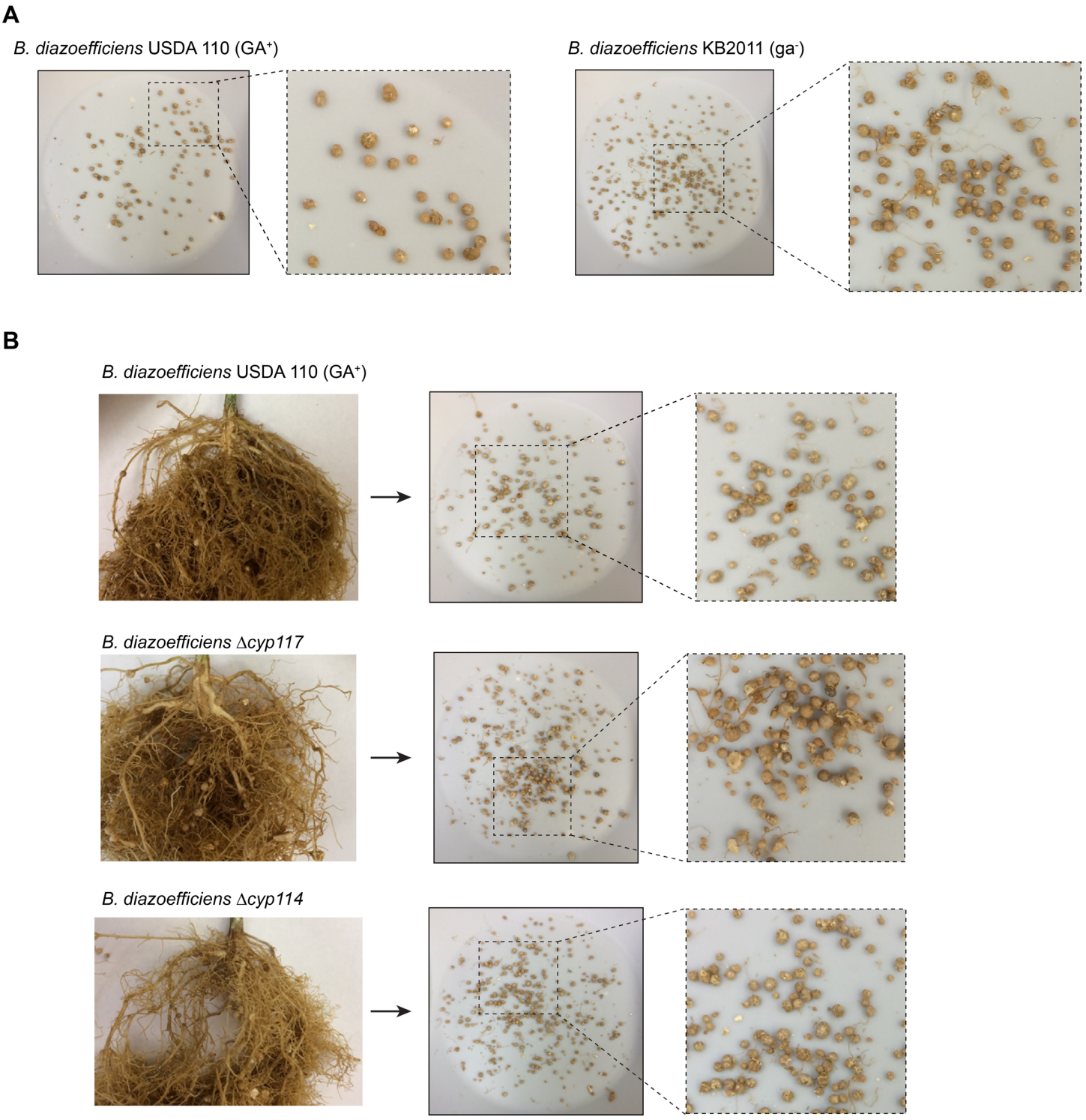
Supplementary Figure 10. Confirmation of *Gm*GA3ox6 as a 2ODD enzyme. Lysates from cells expressing *Gm*GA3ox6 were incubated with GA_9 in either the presence or absence of Fe(II)/2-oxoglutarate dependent dioxygenase (2ODD) cofactors (2-oxoglutarate, ascorbate, and iron). Production of GA_4 was detected with GC-MS, as shown here with representative extracted ion chromatograms. The chromatograms shown correspond to the methyl ester (GA_9) or methyl ester, trimethylsilyl ether (GA_4) of the corresponding compounds. Due to the high homology and similar functionality demonstrated between *Gm*GA3ox4 and *Gm*GA3ox6, the status of *Gm*GA3ox4 as a 2ODD enzyme is inferred.



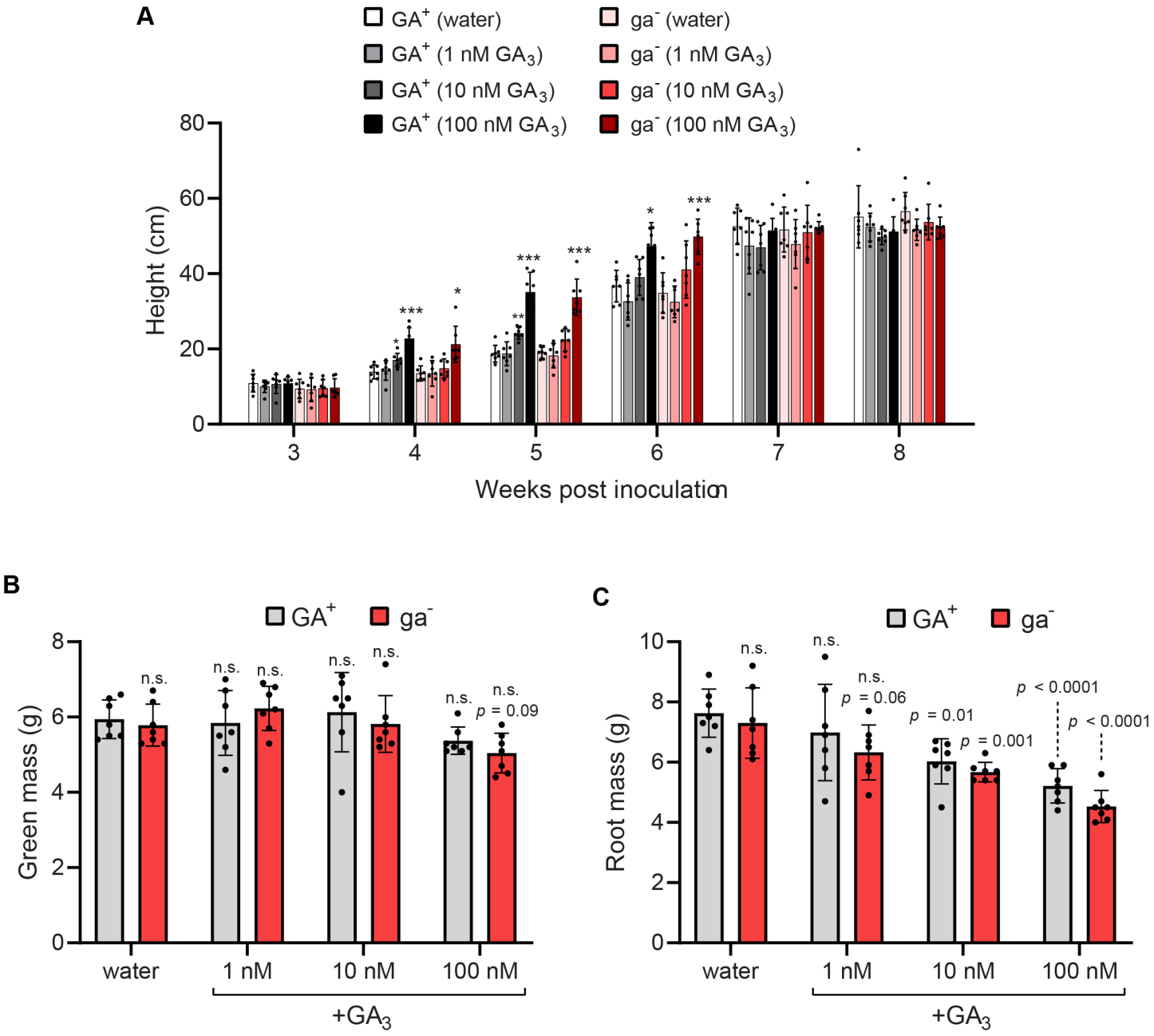
Supplementary Figure 11. Expression of GA operon genes in GA$^+$ and ga$^-$ *B. diazoefficiens* bacteroids. Relative expression of **A**) *cyp112*, **B**) *sdr*, and **C**) *ks* as determined via qPCR with cDNA generated from post-flowering stage GA$^+$ or ga$^-$ bacteroids. Target gene expression was normalized using *B. diazoefficiens* USDA 110 *hisS* as a reference gene, and relative expression was then calculated by dividing values by the mean expression value of the corresponding ga$^-$ expression data. n = 4 biological replicates with ± SD shown. Data is shown on a $\log_2$ scale, with statistics performed on the non-log-transformed data. Statistical significance was assessed using a one-tailed Welch's t-test. Trace amounts of GA operon gene amplification in the ga$^-$ strain are presumably due to low levels of contaminating bacterial gDNA.



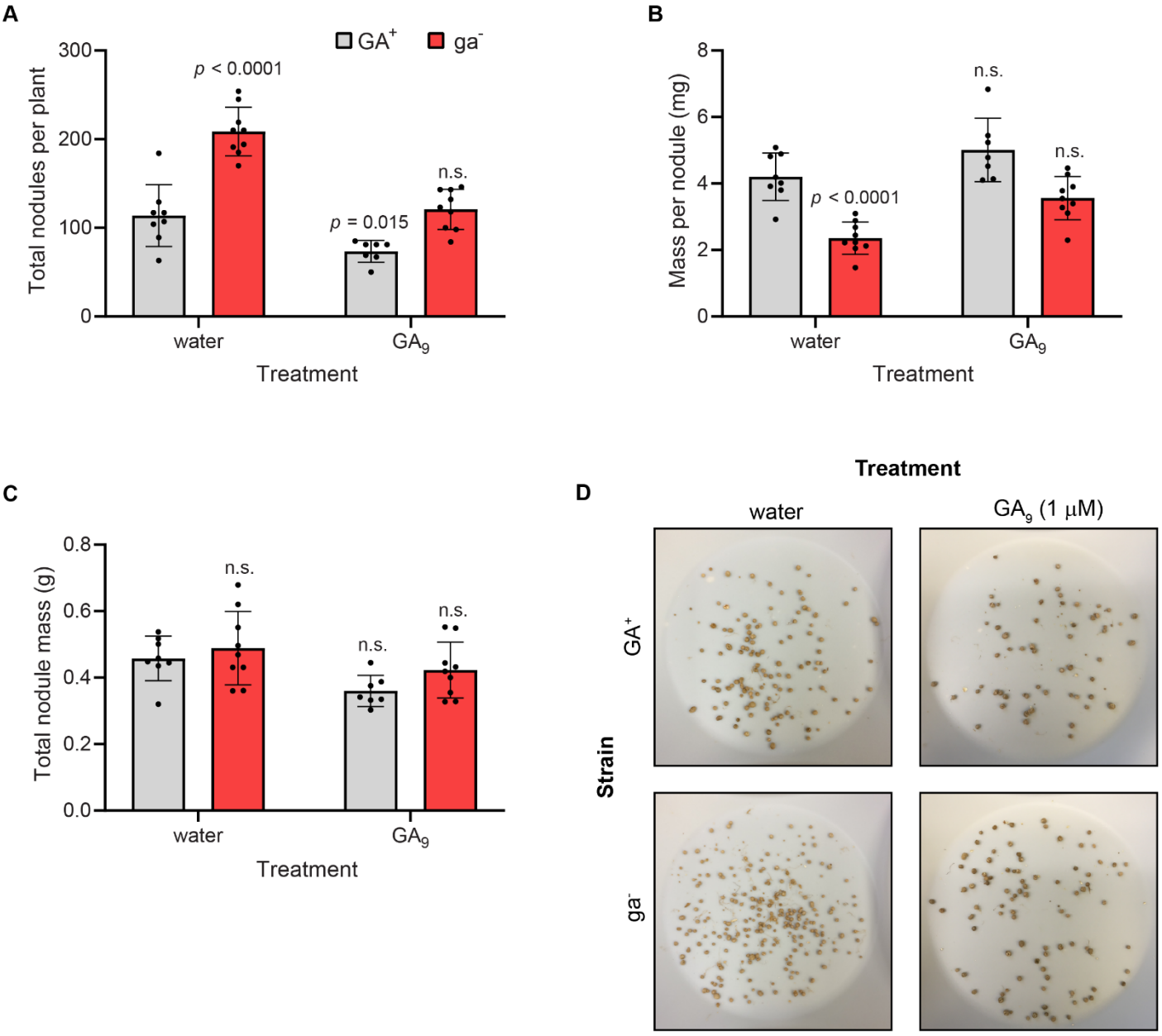
Supplementary Figure 12. Soybean phenotypes associated with knockout of *cyp117* and *cyp114* in *B. diazoefficiens*. Soybean plants were inoculated with wild-type (GA^+) *B. diazoefficiens*, or with strains containing a knockout of *cyp117* (*BdΔcyp117* or *cyp114* (*BdΔcyp114*). **A**) Plant height was measured for three different time points. At the early pod stage of the plants, the following phenotypic parameters were assessed: **B**) green mass, **C**) root mass, **D**) nodule number per plant, **E**) average mass per nodule, and **F**) total nodule mass per plant. n = 8 for each treatment with means ± SD shown. Statistical significance was assessed by using a two-way ANOVA and Dunnett's multiple comparison test with GA^+ as the control group. n.s. = not significant ($p > 0.05$). For height measurements, statistical significance was determined between treatments within a single time point.



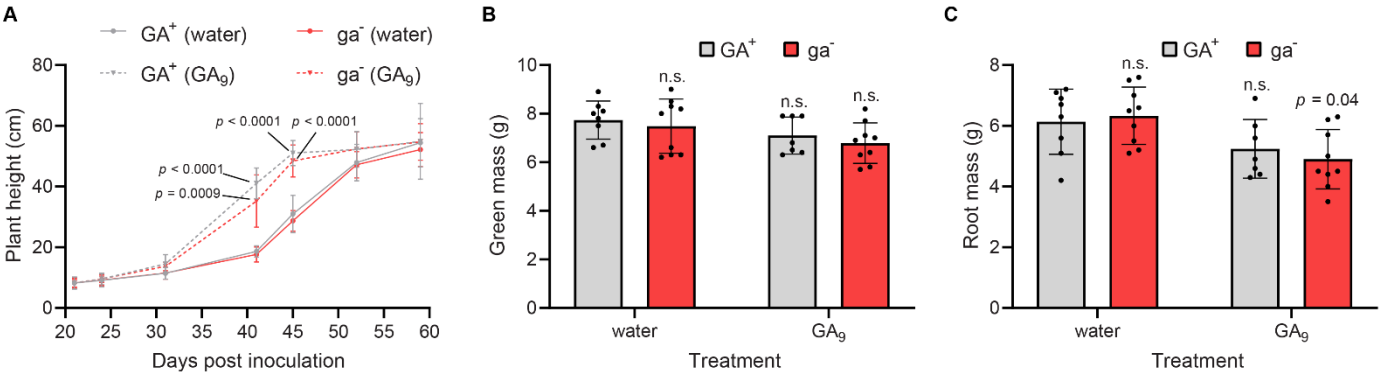
Supplementary Figure 13. Representative images of GA$^+$ and ga$^-$ nodules. A) All of the nodules from single representative *B. diazoefficiens* USDA 110 (GA+) and *B. diazoefficiens* KB2011 (ga-) plants are shown, as well as zoomed in images to better demonstrate nodule morphology and appearance. Each of these plants were from the same experiment. **B)** Roots, total nodules, and zoomed in nodule images from representative GA+, *Bd*Δ*cyp117*, and *Bd*Δ*cyp114* plants. These plants were all from the same experiment.



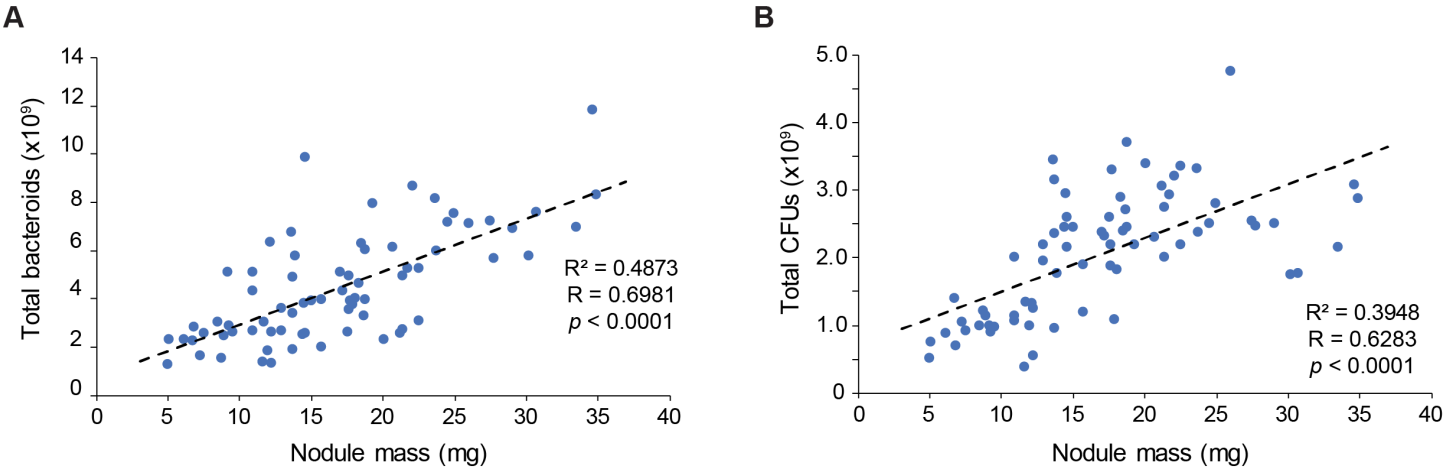
Supplementary Figure 14. Effects of a range of GA_3 concentrations on plant height and mass. The soil substrate of soybean plants in symbiosis with either GA^+ or ga^- *B. diazoefficiens* were treated with several concentrations of GA_3 (1, 10, or 100 nM) or water. **A**) Plant height was measured over time. The following phenotypic measurements were assessed at the early pod stage: **B**) green mass and **C**) root mass. n = 7 for each treatment with means ± SD shown. Statistical significance was assessed using a two-way ANOVA and Dunnett's multiple comparison test, with the GA^+ water treatment used as the control. n.s. = not significant ($p > 0.05$). For height measurements, statistical significance was only assessed between experimental treatments within a single time point. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. An absence of asterisks in panel "a" infers $p > 0.05$. Individual p values are shown if ≤ 0.1.



Supplementary Figure 15. Chemical complementation with GA_9 rescues the ga⁻ nodule phenotypes. Nodule phenotypic characteristics were measured at the early pod stage following weekly application of GA_9 (1 µM) to the soil substrate of soybean plants nodulated with GA^+ or ga⁻ *B. diazoefficiens*. **A**) Average number of nodules per plant. **B**) Average mass per nodule. **C**) Total nodule pass per plant. **D)** Representative root nodules from each experimental group. Each image shows nodules isolated from one plant. n = 7-9 plants per treatment. Shown for each experimental group is the mean ± SD. Statistical significance was assessed using a two-way ANOVA and Dunnett's multiple comparison test with the GA^+ water treatment as the control group. n.s. indicates $p > 0.05$. Individual p values are shown when ≤ 0.1.



Supplementary Figure 16. Effect of exogenous GA_9 on soybean growth phenotypes. Following nodulation of soybean plants with GA^+ and ga^- *B. diazoefficiens*, 1 µM GA_9 was applied twice per week to the soil. **A**) Plant height for these plants was measured over time. The following phenotypic characteristics were measured at the early pod stage of the plant: **B**) green mass and **C**) root mass. n = 7-9 per treatment with means ± SD shown. Statistical significance was determined using a two-way ANOVA and Dunnett's multiple comparison test, with the GA^+ water treatment used as the control. For height measurements in panel **A**, statistical significance was only assessed between treatments within a single time point. n.s. = not significnt ($p > 0.05$). In panel "a", if no p value is given, then the experimental group has a p value > 0.05.



Supplementary Figure 17. Correlations between nodule size and bacteroid numbers. A) Total bacteroid cells extracted from nodules of varying sizes, as counted with flow cytometry, in relation to the mass of the nodule from which they were isolated. **B**) The total number of viable cells in the same samples was determined by plating for colony-forming units (CFUs). Data sets were analyzed in JMP Pro 13 to obtain statistical parameters of linear regressions.

Supplementary Table 1. Bacterial strains used in this study.

Strain	Description	Source
One Shot® TOP10 Chemically Competent *E. coli*	F- *mcrA, Δ(mrr-hsdRMS-mcrBC), Φ80lacZΔM15, Δ lacX74, recA1, araD139, Δ(araleu)7697, galU, galK, rpsL(StrR), endA1, nupG;* for plasmid propagation and cloning	Thermo Fisher Scientific
One Shot® BL21 Star™ (DE3) Chemically Competent *E. coli*	F- *ompT hsdSB (rB-, mB-) galdcmrne131* (DE3); for pET101 expression of soybean GA 3-oxidases	Thermo Fisher Scientific
E. coli S17-1 λ *pir*	F-, *RP4-2(Km::Tn7,Tc::Mu-1)*, *pro-82*, *LAMpir*, *recA1*, *endA1*, *thiE1*, *hsdR17*, *creC510*; for creating the *B. diazoefficiens* insertional mutant strain *Bd*KB2011	[2]
Bradyrhizobium diazoefficiens USDA 110 (*Bd*USDA110, formerly *B. japonicum* USDA 110,)	wild-type rhizobial symbiont of *Glycine max;* Cm^R, Tm^R	USDA; Beltsville, MD
B. diazoefficiens KB2011 (*Bd*KB2011)	*B. diazoefficiens* USDA 110 derived strain containing an insertion in *cyp112* for disruption of the entire GA operon	This study
BdΔcyp117	*cyp117* deletion strain derived from *B. diazoefficiens* USDA 110 parent strain; Cm^R	[3]
BdΔcyp114	*cyp114* deletion strain derived from *B. diazoefficiens* USDA 110 parent strain; Cm^R	[3]

Supplementary Table 2. Primers used in this study**.** Restriction sites are indicated with underlined nucleotide sequence. bp = base pairs, kb = kilobase.

Primer	Sequence (5' to 3')	Description
For cloning synthetic soybean GA 3-oxidases		
GmGA3ox4 F	CACCATGGTTACCACAC TGAGCGAAG	Forward primer for amplifying synthetic GmGA3ox4. Contains a 5' CACC for cloning into pET101/D-TOPO.
GmGA3ox4 R	TTAGTTATTATTCAGCA TGCTAATCAGGC	Reverse primer for amplifying synthetic GmGA3ox4. Contains the native stop codon to prevent the addition of a His tag in pET101/D-TOPO.
GmGA3ox6 F	CACCATGGCAACCAC ACTGAGCGAAG	Forward primer for amplifying synthetic GmGA3ox6. Contains a 5' CACC for cloning into pET101/D-TOPO.
GmGA3ox6 R	TTAGTTTTTCAGCAT GCTAATCAGGCTC	Reverse primer for amplifying synthetic GmGA3ox6. Contains the native stop codon to prevent the addition of a His tag in pET101/D-TOPO.
For amplifying a fragment containing cyp112 and flanking sequences for creation of the B. diazoefficiens KB2011 insertional mutant		
Bd-cyp112-PmeI-F	CCACGTTTAAACTCG AACCTCCTTCACCAAT CCGTA	Forward primer upstream of *cyp112*. Contains PmeI restriction site at the 5' end.
Bd-cyp112-PmeI-R	CTCCGTTTAAACTGTC GATCTGGCCCATGGT GAAAT	Reverse primer downstream of *cyp112*. Contains PmeI restriction site at the 5' end.
For confirming insertion within cyp112 in B. diazoefficiens KB2011		
Bd-CYP112-kb F	TGGTGGGTGACAGGCT ATGACGAGG	Forward primer 5' to the ~2 kb cassette insertion site in *cyp112*.

		Wild-type amplicon = 762 bp; insertion mutant = 2.8 kb
Bd-CYP112-kb R	TCACGTCGGTCCTCGGA TAGCGCATG	Reverse primer 3' to the ~2 kb cassette insertion site in *cyp112*. Wild-type amplicon = 762 bp; insertion mutant = 2.8 kb
For confirming clean deletion strains of B. diazoefficiens USDA 110		
Bd-CYP117ko-check F	ATCGTCAACATGTCGTC GTGCCAGG	Forward primer ~400 bp upstream of *cyp117* to check for presence or deletion of this gene; wild-type fragment = 2.1 kb, knockout = 0.8 kb
Bd-CYP117ko-check R	TGCGCCGGCAGCCAAA CAGAGC AAG	Reverse primer ~400 bp downstream of cyp117 to check for presence or deletion of this gene; wild-type fragment = 2.1 kb, knockout = 0.8 kb
Bd-CYP114ko-check F	ATTCCCGCGGAGAGCA AGGTGC	Forward primer ~400 bp upstream of *cyp114* to check for presence or deletion of this gene; wild-type fragment = 2.2 kb, knockout = 0.8 kb
Bd-CYP114ko-check R	ATAGCCGCCGAGCCATC AATGT CGGC	Reverse primer ~400 bp downstream of *cyp114* to check for presence or deletion of this gene; wild-type fragment = 2.2 kb, knockout = 0.8 kb
To amplify fragments for use as templates in determining qPCR primer efficiency		
BdCYP112 frag-F	TGGTGGGTGACAGGCT ATGACGAGG	Forward primer; amplicon = ~ 800 bp
BdCYP112 frag-R	TCACGTCGGTCCTCGGA TAGCGCATG	Reverse primer; amplicon = ~ 800 bp
BdKS frag-F	ATGATCCAGACTGAAC GCGCGGTG	Forward primer; amplicon = 831 bp
BdKS frag-R	TGGTCGAGGTCCGGTAG TACTGC	Reverse primer; amplicon = 831 bp
BdhisS frag-F	ACCCCAGAAACTGAAG GCGCGCCTG	Forward primer; amplicon = 1.5 kb

BdhisS frag-R	CTAGCCCCAGCTCACGTCATGGC	Reverse primer; amplicon = 1.5 kb
GmGA3ox4 frag-F	ATGCATGGCCTCAATCTGAAGATGG	Forward primer; amplicon = 863 bp
GmGA3ox4 frag-R	TCAAGGAACGAAACCGAGGCAAGG	Reverse primer; amplicon = 863 bp
GmGA3ox6 frag-F	TTCACTTAGGACCTTACCTGATTCG	Forward primer; amplicon = 877 bp
GmGA3ox6 frag-R	AATCAAGAACCAAAGGAGAAACCAC	Reverse primer; amplicon = 877 bp
cons7 frag-F	AGTCTCCTGGTAACATTGAGCAAAG	Forward primer; amplicon = 540 bp
cons7 frag-R	ATGAGAGTGCCCAATATTACAGGTG	Reverse primer; amplicon = 540 bp

To check for expression of the 6 GA3ox isoforms in soybean

GmGA3ox1 check-F	ACTGTCAACCCAATGATGATGCATC	Forward primer; amplicon = 796 bp after splicing of an intron from the gene
GmGA3ox1 check-R	CATCGGTGGAGAATAGAAATAAGCC	Reverse primer; amplicon = 796 bp after splicing of an intron from the gene
GmGA3ox2 check-F	ATGGTCTCACTCTCAACCCAACGATG	Forward primer; amplicon = 851 bp after splicing of an intron from the gene
GmGA3ox2 check-R	ACAGAGTCAACTAAAGGAGAAACC	Reverse primer; amplicon = 851 bp after splicing of an intron from the gene
GmGA3ox3 check-F	ACACAAGCACCCTGACTTAAACTCC	Forward primer; amplicon = 952 bp after splicing of an intron from the gene
GmGA3ox3 check-R	TGCCAAGGTACTCATTCCAAGTCAC	Reverse primer; amplicon = 952 bp after splicing of an intron from the gene

GmGA3ox4 check-F	ATGCATGGCCTCAATCT GAAGATGG	Forward primer; amplicon = 863 bp after splicing of an intron from the gene
GmGA3ox4 check-R	TCAAGGAACGAAACCG AGGCAAGG	Reverse primer; amplicon = 863 bp after splicing of an intron from the gene
GmGA3ox5 check-F	AGTCTTACACTTGGACA CACCATGG	Forward primer; amplicon = 909 bp after splicing of an intron from the gene
GmGA3ox5 check-R	AGGTACTCATTCCAAGT CACTGCC	Reverse primer; amplicon = 909 bp after splicing of an intron from the gene
GmGA3ox6 check-F	TTCACTTAGGACCTTAC CTGATTCG	Forward primer; amplicon = 877 bp after splicing of an intron from the gene
GmGA3ox6 check-R	AATCAAGAACCAAAGG AGAAACCAC	Reverse primer; amplicon = 877 bp after splicing of an intron from the gene

Supplementary Table 3. Plasmids and expression constructs used in this study.

Plasmid/construct	Description	Source
Plasmids		
pCR™-Blunt II-TOPO®	For cloning and propagation of blunt-end PCR constructs; Km^R	Thermo Fisher Scientific
pET101/D-TOPO®	For directional cloning and expression; contains C-terminal His-tag; Cb^R	Thermo Fisher Scientific
pJET	For cloning of the DNA fragments from *B. diazoefficiens* used to create the insertional mutant *Bd*KB2011; Cb^R	Thermo Fisher Scientific
pLO1	Mobilizable, mating/SacB counterselection vector; Km^R	[4]
pLOBJ3	pLO1 containing the GA operon knockout cassette used to created the insertional knockout strain *Bd*KB2011; Km^R	This study
Expression constructs		
pET101-sGmGA3ox4	For expression of synthetic soybean GA3ox4 in *E. coli*; Cb^R	This study
pET101-sGmGA3ox6	For expression of synthetic soybean GA3ox6 in *E. coli*; Cb^R	This study

Supplementary Table 4. Primers used for qPCR for selected *Bradyrhizobium diazoefficiens* USDA 110 and soybean (*Glycine max* cv. Williams ’82) gene transcripts.

target	forward primer (5’-3’)	reverse primer (5’-3’)	amplicon size (bp)
B. diazoefficiens genes			
cyp112	ATCGAATTCGGCCTGCTA	AGGATTTCCTCTACCGCCTT	97
sdr	CATGCTGTCGTCCTCACTTG	GAGGAGTCGCTCGGTCAT	94
ks	CTACGCGAACGTGTTCTGTT	CAAGGTCGCCATATCCAGC	64
hisS	GATGGAATACACCGACGCGCT	AACGCAAGCTAATCCACTGCTCG	106
G. max genes			
GmGA3ox4	ATCTGGCATAATGACTGTGC	GTTGGTTGAACCAATCCACC	150
GmGA3ox6	TCCATGTGATGATGCCAAAAAG	TTTTGCTCCTCAGAAATGCCC	150
cons7	ATGAATGACGGTTCCCATGTA	AGCATTAAGGCAGCTCACTCT	114

SUPPLEMENTARY REFERENCES

1. Prentki P, Krisch HM. In vitro insertional mutagenesis with a selectable DNA fragment. *Gene* 1984; **29**: 303–313.
2. de Lorenzo V, Eltis L, Kessler B, Timmis KN. Analysis of *Pseudomonas* gene products using *lacI*^{q}*/Ptrp-lac* plasmids and transposons that confer conditional phenotypes. *Gene* 1993; **123**: 17–24.
3. Nett RS, Montanares M, Marcassa A, Lu X, Nagel R, Charles TC, et al. Elucidation of gibberellin biosynthesis in bacteria reveals convergent evolution. *Nat Chem Biol* 2017; **13**: 69–74.
4. Lenz O, Schwartz E, Dernedde J. The *Alcaligenes eutrophus* H16 *hoxX* gene participates in hydrogenase regulation. *J Bacteriol* 1994; **176**: 4385–4393.