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Supplemental Information

A GH81-type β -glucan-binding protein enhances colonization by mutualistic fungi in barley

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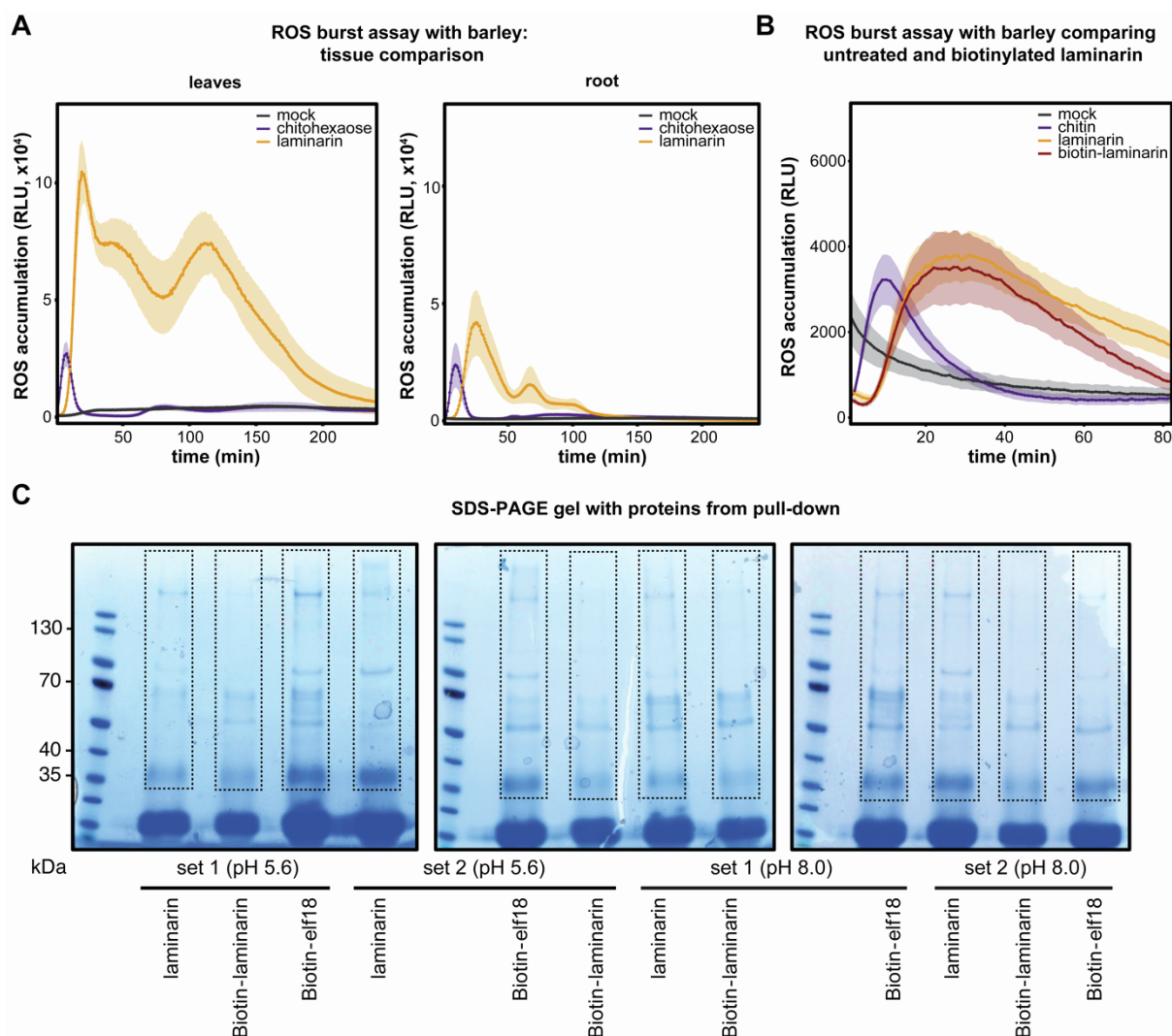


Figure S1. Protein pull-down with biotinylated laminarin in barley leaves. Related to Figure 1. (A) Barley leaf and root tissues respond similarly to laminarin treatment. Apoplastic ROS accumulation after treatment of seven-day-old barley root pieces and leaf discs with chitohexaose ($10 \mu\text{M}$) and laminarin ($4 \text{ mg}\cdot\text{mL}^{-1}$). Treatment with Milli-Q water was used as mock control. Values represent mean \pm SEM from 16 wells. The experiment was repeated at least three times with similar results. (B) Apoplastic ROS accumulation after treatment of two-week-old barley leaf discs with untreated and biotinylated laminarin (each $6 \text{ mg}\cdot\text{mL}^{-1}$). Treatment with Milli-Q water was used as mock control. Values represent mean \pm SEM from 8 wells. (C) Separation of pull-down samples on SDS-PAGE gel prior to submission for mass spectroscopy. Pull-down was performed with untreated laminarin, biotinylated laminarin and biotinylated elf18 at two different pH values. Areas indicated by dotted lines were excised from the gel and further processed for mass spectrometric analyses. Elf18, peptide from bacterial elongation factor Tu; ROS, reactive oxygen species; RLU, relative luminescence units.

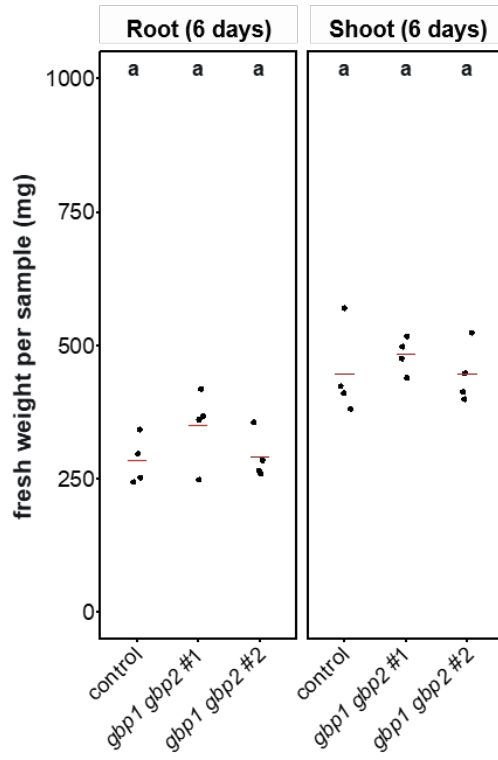
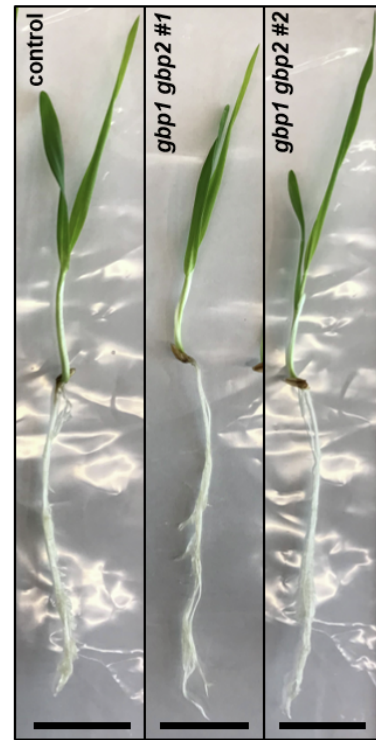
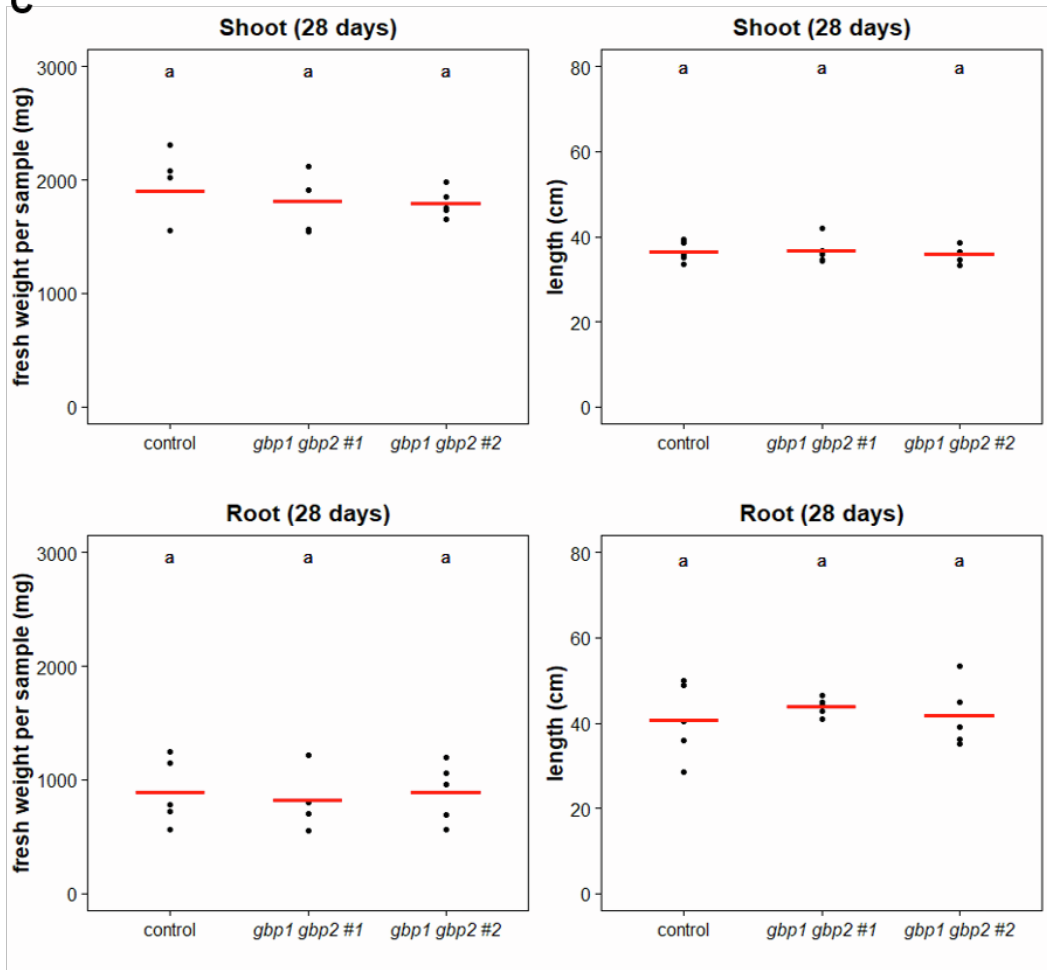
A**B****C**

Figure S2. Quantification of root and shoot tissue of the barley control and *gbp1 gbp2* mutant lines. Related to Figure 1. (A) The control line and *gbp1 gbp2* mutant lines were germinated on wet filter paper for 4 days and then grown on 1/10 PNM medium for 6 days under sterile conditions. Root and shoot fresh weight of the 10-day-old barley plants were measured. Black dots represent biological replicates, and the red bar indicates the average fresh weight ($n = 4$, each replicate consists of 4 barley plants). Different letters represent statistically significant differences based on one-way ANOVA (significance threshold: $p \leq 0.05$). (B) Representative images of barley control and *gbp* mutant lines grown on 1/10 PNM under sterile conditions for 7 days. In total, 12 seedlings were grown for each genotype (scale bar = 3 cm). (C) The control line and *gbp1 gbp2* lines were germinated on wet filter paper for 4 days and then grown in pre-sterilized soil in pots for 28 days. Root and shoot fresh weight as well as length of the 32-day-old barley plants were measured. Black dots represent biological replicates, and the red bar indicates the average fresh weight (left graphs) or length (right graphs) ($n = 5$). Different letters represent statistically significant differences based on one-way ANOVA (significance threshold: $p \leq 0.05$).

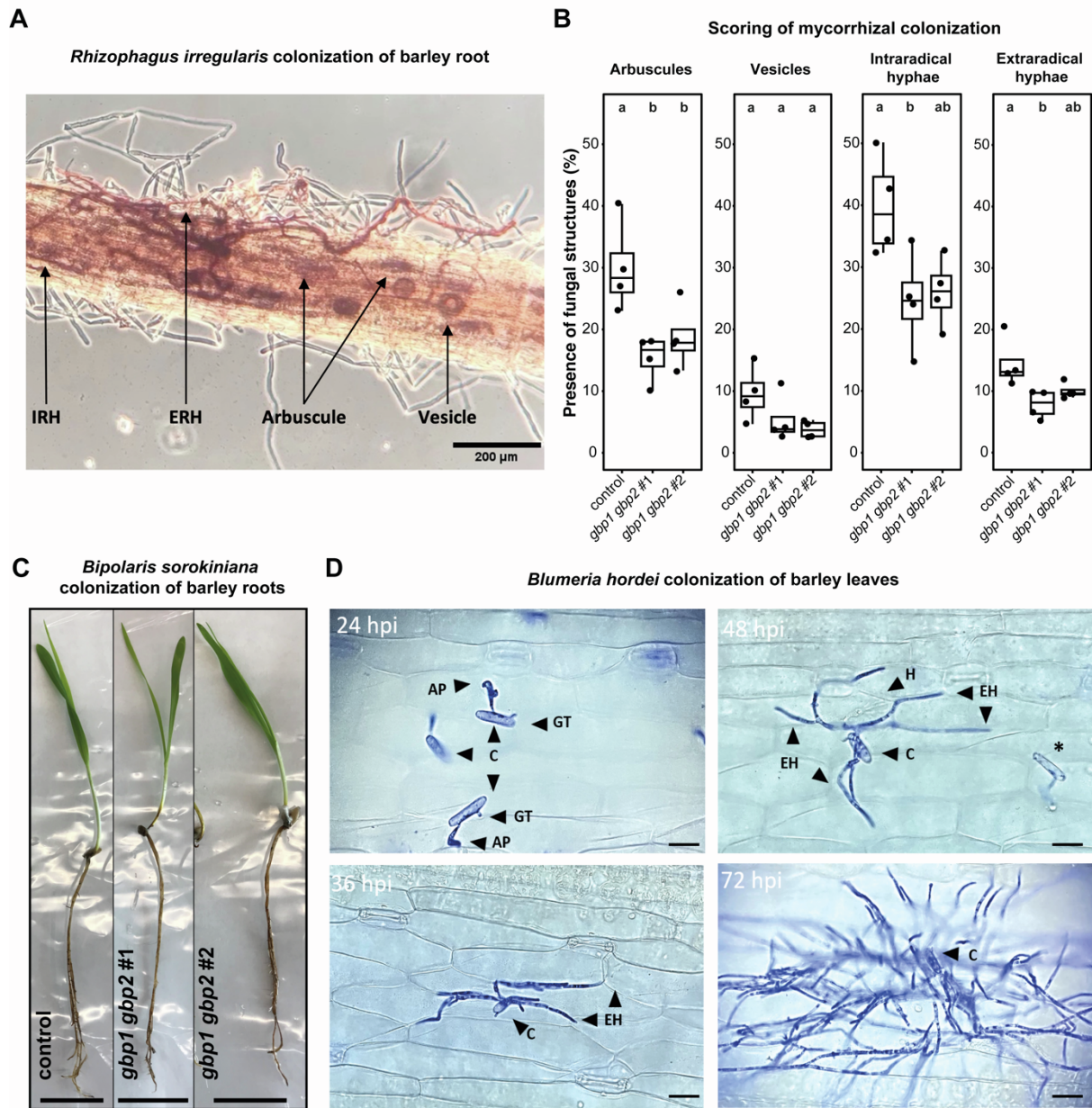
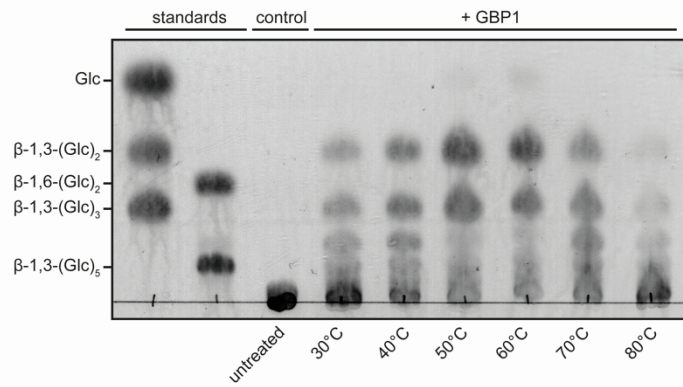


Figure S3. Barley *gbp1 gbp2* mutant lines exhibit decreased colonization by mutualistic and pathogenic fungi. Related to Figure 1 and 2. (A) Example of *R. irregularis* structures in barley roots at 28 dpi. *R. irregularis* structures were stained with 5% ink (Pelikan) and fungal structures were observed using a light microscope (AxioStar, Carl Zeiss, Jena, Germany) at 10X magnification. (B) The control line and *gbp1 gbp2* mutants were inoculated with *R. irregularis* spores and roots were harvested at 28 dpi. Barley roots were analyzed at 300 randomly chosen sections (covering 30 cm of root length) for the presence of *R. irregularis* structures including arbuscules, vesicles, intraradical hyphae (IRH), and extraradical hyphae (ERH). All data points are plotted on graphs as circles ($n = 4$). Boxplot elements in this figure: center line, median; box limits, upper and lower quartiles; whiskers, $1.5 \times$ interquartile

range. Different letters represent statistically significant differences based on one-way ANOVA and Tukey's post hoc test (significance threshold: $p \leq 0.05$). (C) Images of barley control and *gbp* mutant line seedlings during *B. sorokiniana* colonization. Barley control and *gbp* mutant lines were inoculated with *B. sorokiniana* and grown in jars under axenic conditions for 7 days. Images were taken at 7 dpi to assess any shoot or root phenotypes of the *gbp* mutants (scale bar = 3 cm). Mock-inoculated images can be found in Figure S2B. (D) Representative images of *B. hordei* infection structures on barley leaves. Barley leaves colonized by *B. hordei* were analyzed for penetration success using bright field microscopy (scale bar = 20 μm). Fungal structures were stained using Coomassie brilliant blue, and secondary hyphae formation was assessed from 50 germinated conidia spores in both the tip and middle area of each leaf.

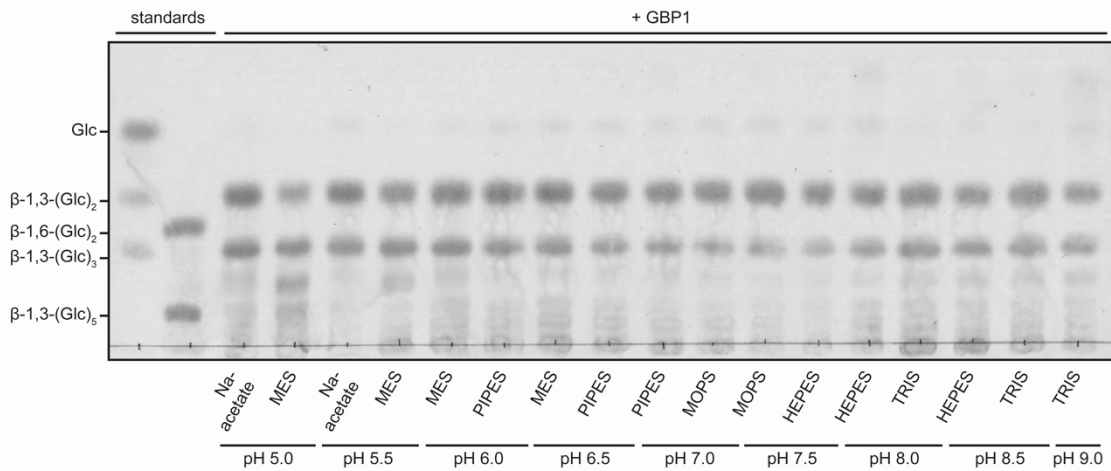
A

Thin layer chromatography of temperature-dependent digestion of laminarin



B

Thin layer chromatography of buffer-dependent digestion of laminarin



C

MALDI-TOF digestion profiles of cell surface glycan substrates derived from *Serendipita indica*

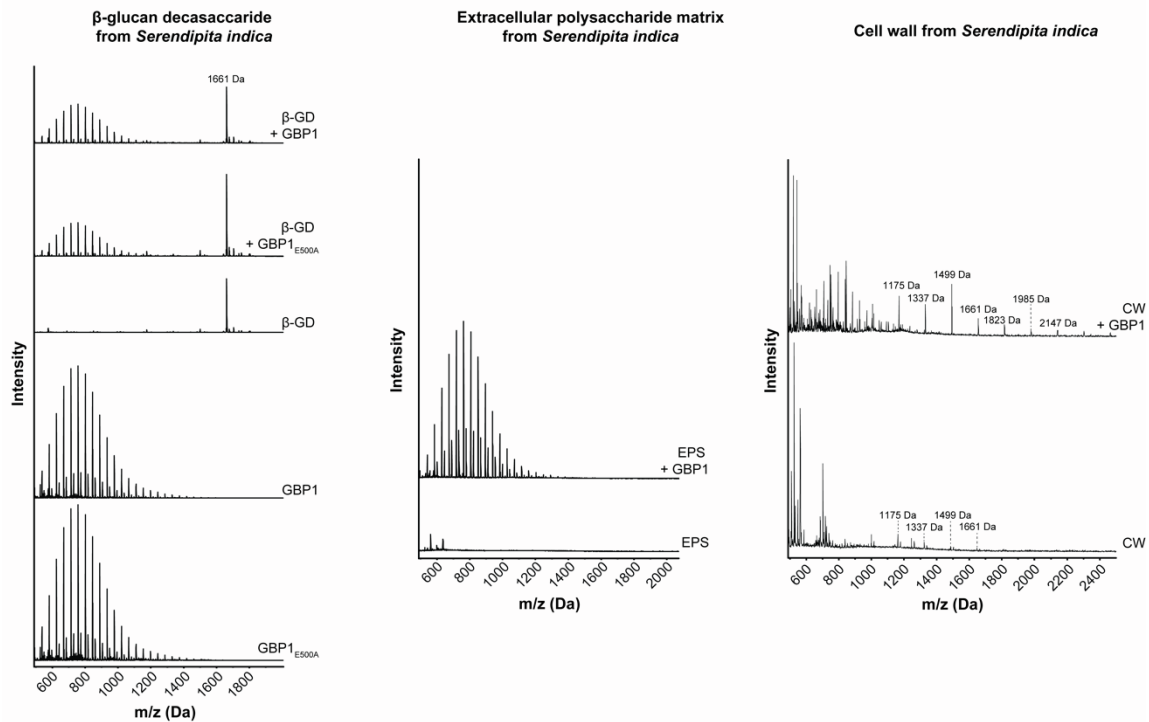


Figure S4. Digestion of commercial and native glucan substrates by GBP1. Related to Figure 3 and 4. Temperature- and buffer-dependency of GBP1 activity on laminarin. (A) Laminarin ($4 \text{ mg} \cdot \text{mL}^{-1}$) was digested with GBP1 (70 nM) for 10 min at different temperatures. Sample without enzymes (UT) was mock-digested at 80 °C. (B) Laminarin ($4 \text{ mg} \cdot \text{mL}^{-1}$) was digested with purified GBP1 (70 nM) in different buffers (10 mM, pH 5-9). Digestions were performed at 60 °C for 10 min. Products from GBP1-catalyzed laminarin digestion assays in (A) and (B) were separated by thin layer chromatography. Quantification of thin layer chromatographs in Figure 4C. (C) Analysis of GBP1-digested β -glucan decasaccharide, extracellular polysaccharides, or cell wall fractions from *Serendipita indica* by MALDI-TOF mass spectrometry. While GBP1 does not act on the EPS matrix and the derived β -GD from *S. indica*, it releases minor oligosaccharide fractions [(m/z 1175 (Hexose₇) – m/z 1247 (Hexose₁₃))] from the CW. Oligosaccharide peaks of interest were labeled with m/z (M+Na)⁺ masses. The digestion assays using the β -GD were performed two times with similar results and the digestion assays using the EPS and CW were performed once. β -GD, β -glucan decasaccharide; CW, cell wall; EPS, extracellular polysaccharides.

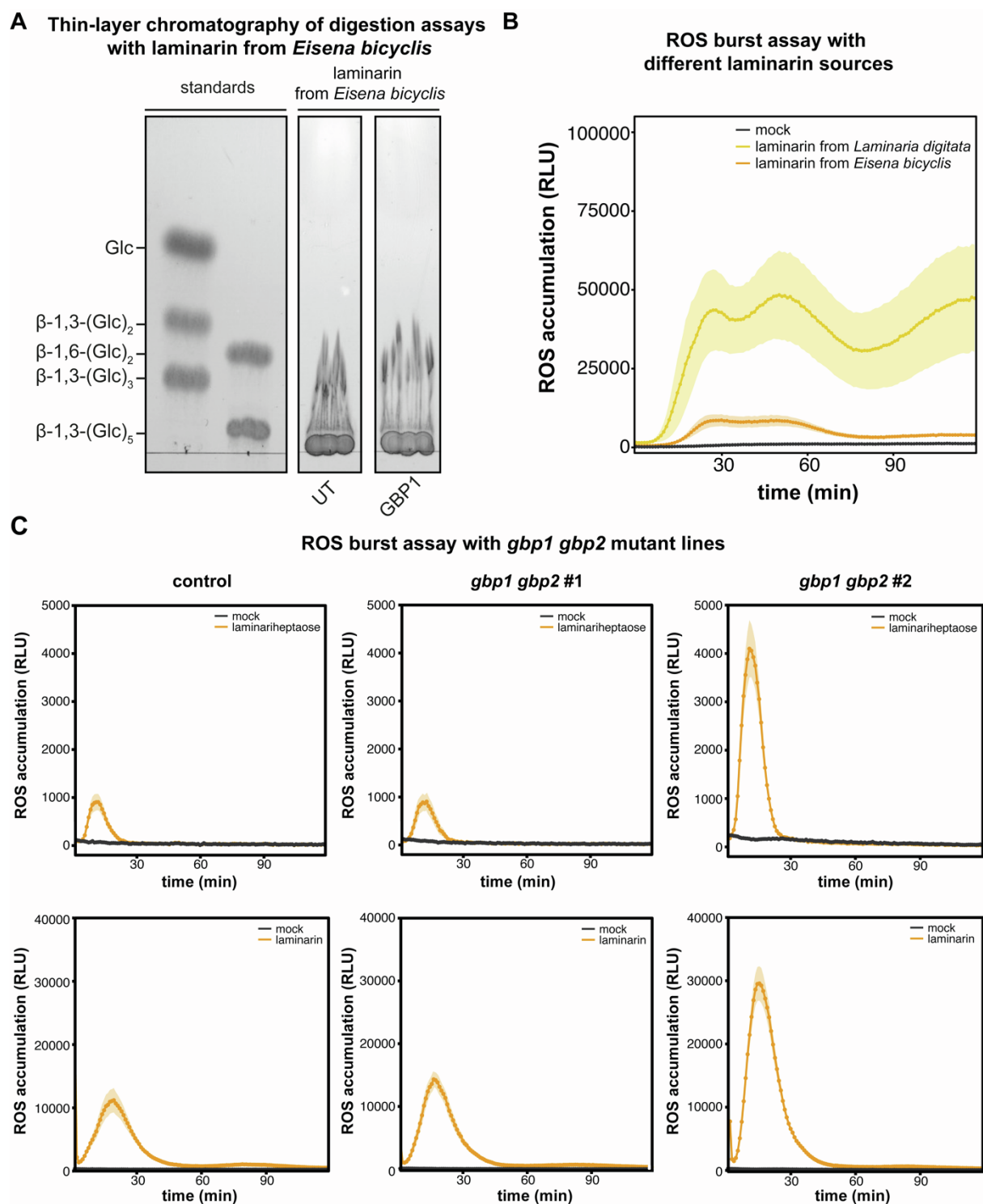


Figure S5. Effects of highly branched laminarin and GBPs on ROS production in barley roots. Related to Figure 4. Highly branched laminarin from *Eisena bicyclis* is inert to GBP1 hydrolysis and triggers only low production of ROS in barley roots. (A) Laminarin from *E. bicyclis* (2.4 mM) was digested with GBP1 (72 nM) for 1 h at 60 °C. The digestion products were analyzed by thin layer chromatography. (B) Apoplastic ROS accumulation after treatment of barley root pieces with laminarin from either *Laminaria digitata* (low frequency of β -1,6 linked branches) or *E. bicyclis* (high

frequency of β -1,6 linked branches) was monitored by ROS burst assay. Treatment with Milli-Q water (mock) was used as control. Values represent mean \pm SEM from eight wells. (C) ROS burst assays in the barley control and *gbp1 gbp2* mutant lines. Apoplastic ROS accumulation after treatment of barley roots with laminariheptaose (250 μ M) or laminarin (4 mg \cdot mL $^{-1}$) was quantified in the control line and *gbp1 gbp2* mutant lines. Treatment with Milli-Q water (mock) was used as control. Values represent mean \pm SEM from 16 wells. The experiment was performed twice with similar results. ROS, reactive oxygen species; RLU, relative luminescence units; UT, untreated.

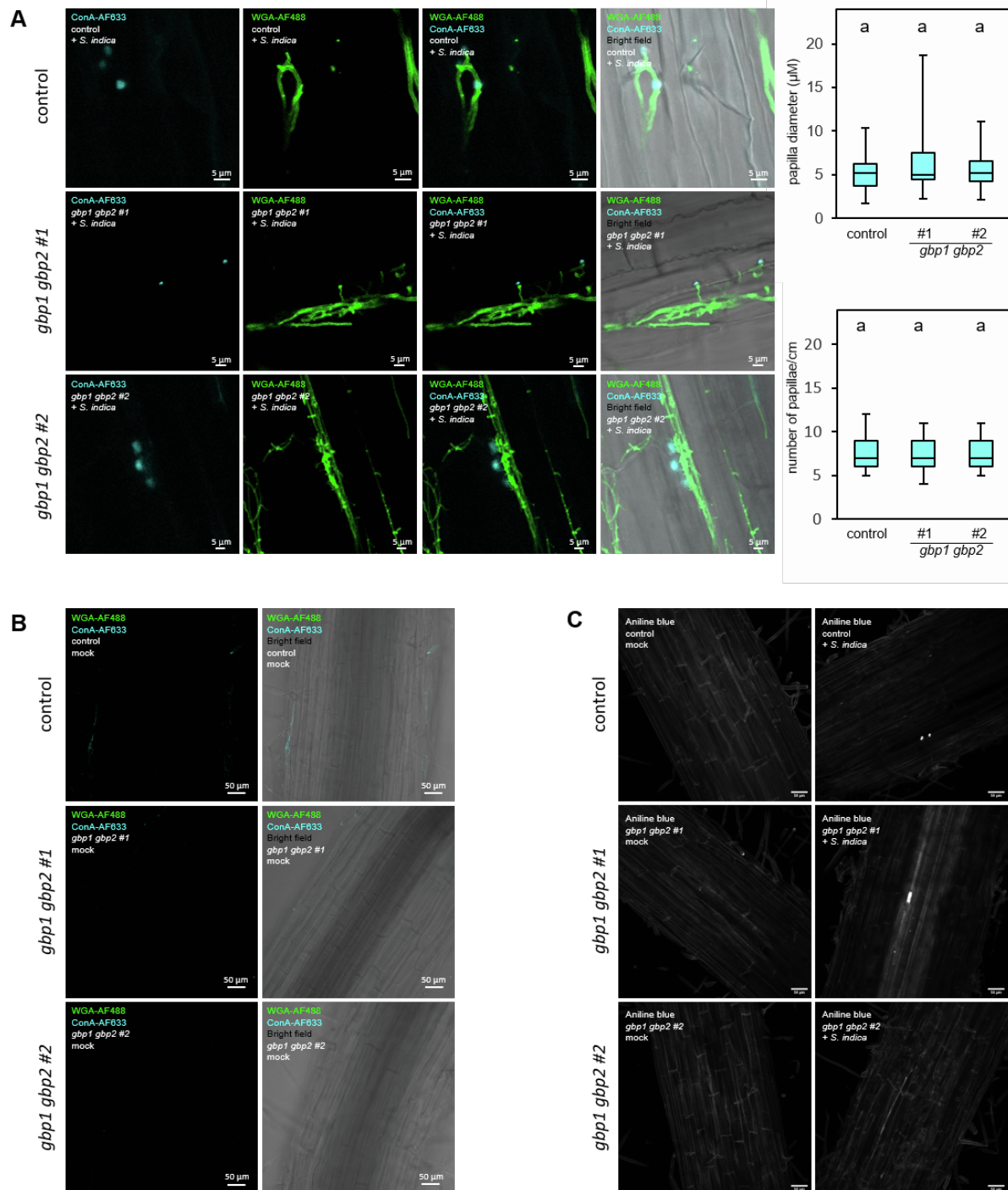


Figure S6. Formation of cell wall responses in control and *gbp1 gbp2* mutant barley lines during *S. indica* colonization. Related to Figure 6. (A) The number and size of papillae upon *S. indica* colonization does not differ between the control and *gbp1 gbp2* mutant lines. *S. indica*-colonized roots of the barley control line and *gbp1 gbp2* mutant lines were stained with concanavalin A (ConA-AF633, cyan) and wheat germ agglutinin (WGA-AF488, green) for visualization of papillae and fungal structures, respectively. Images were acquired using a confocal microscope. The diameter of papillae found in the control and *gbp1 gbp2* mutant lines colonized by *S. indica* ($n = 61$, upper boxplot). The number of papillae were quantified in the control

line and *gbp1 gbp2* mutant lines colonized by *S. indica* ($n = 25$, lower boxplot). Different letters represent statistically significant differences based on one-way ANOVA (significance threshold: $p \leq 0.05$). (B) The barley control and *gbp1 gbp2* mutant lines do not form spontaneous papillae or CW responses in absence of fungal colonization. Mock-treated roots of the control and *gbp1 gbp2* mutant lines were stained with concanavalin A (ConA-AF633, cyan) and wheat germ agglutinin (WGA-AF488, green) for visualization of CWAs and fungal structures, respectively. Images were acquired using a confocal microscope. (C) Aniline blue stained callose deposition differs in control and *gbp1 gbp2* mutant lines during *S. indica* colonization. Barley control and *gbp1 gbp2* mutant lines were inoculated with sterile water (mock) or *S. indica* and grown under axenic conditions. Root samples were harvested at 6 dpi and stained with aniline blue to visualize callose deposition. Images were acquired with a confocal microscope. ConA, concanavalin A; CW(A), cell wall (appositions); WGA, wheat germ agglutinin.

Oligo Name	Sequence	Purpose
Clal_GBP1_F	5'-GACGGTATCGATAAAATGCCGCCACATGGTAGACG-3'	Cloning
GBP1_noSTOP_Xmal_R	5'-ATAACTCCCGGGATGGCCATATTGACGATACCAACAGC-3'	Cloning
GBP1_E500A_F	5'-CAGGCATCAACATCAGAAGCAGTG-3'	Site-directed mutagenesis
GBP1_E500A_R	5'-GTTCTACCATCTCCAACTCAGTC-3'	Site-directed mutagenesis
GBP1_gRNA1	5'-CCCGGCACGCTTCTTCGCGCCGG-3'	CRISPR/Cas9 gRNA
GBP1_gRNA2	5'-TGGCGCCTTCGGATGAACAGCGG-3'	CRISPR/Cas9 gRNA
GBP2_gRNA1	5'-TAAGATCCGTCTGAGGCAGTATGG-3'	CRISPR/Cas9 gRNA
GBP2_gRNA2	5'-GTACAGCCGTTGCTACCCGACGG-3'	CRISPR/Cas9 gRNA
SiTEF_F	5'-GCAAGTTCTCCGAGCTCATC-3'	qPCR primer for <i>Serendipita indica</i> colonization
SiTEF_R	5'-CCAAGTGGTGGGTACTCGTT-3'	qPCR primer for <i>Serendipita indica</i> colonization
SvTEF_F	5'-ATCCCAAGCAAGCCAATGTG-3'	qPCR primer for <i>Serendipita vermifera</i> colonization
SvTEF_R	5'-TGCCGTCAGTCTTCTCAACA-3'	qPCR primer for <i>Serendipita vermifera</i> colonization
BsTEF_F	5'-CGCCGTACCGGAAAGTCTG-3'	qPCR primer for <i>Bipolaris sorokiniana</i> colonization
BsTEF_R	5'-GGCGAAACGACCAAGAGGA-3'	qPCR primer for <i>Bipolaris sorokiniana</i> colonization
HvUBI_F	5'-ACCCTCGCCGACTACAACAT-3'	qPCR primer for barley colonization
HvUBI_R	5'-CAGTAGTGGCGGTCTGAAGTG-3'	qPCR primer for barley colonization
HvPR10_F	5'-GGAGGGCGACAAGGTAAGTG-3'	qPCR primer for defense gene expression analysis
HvPR10_R	5'-CGTCCAGCCTCTCGTACTCT-3'	qPCR primer for defense gene expression analysis

Table S2. Oligonucleotides used in this study. Related to Figure 1, 2 and 6.