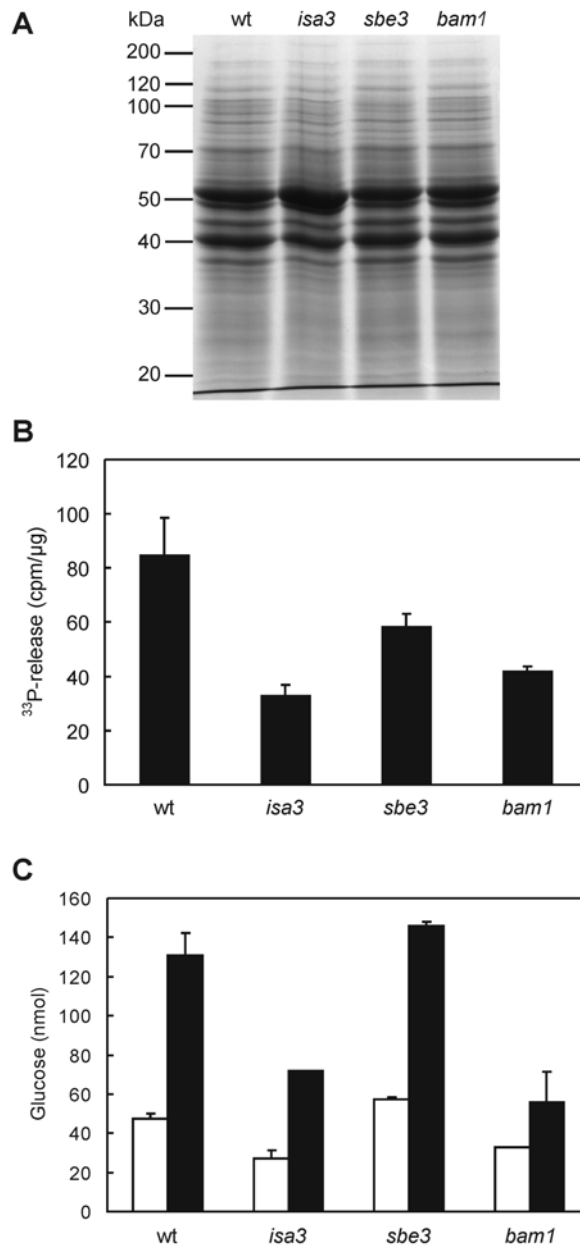
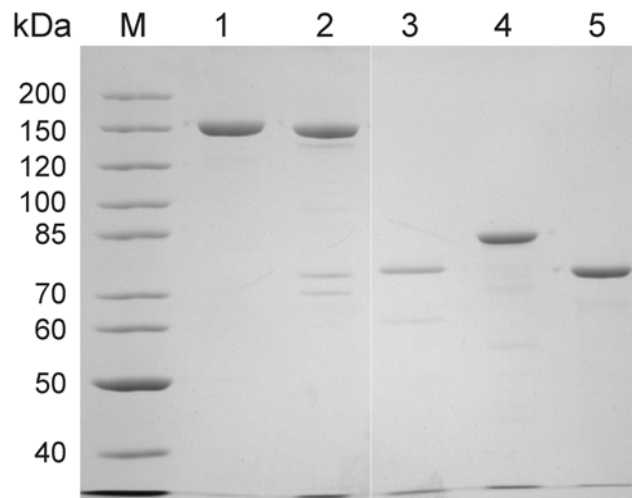


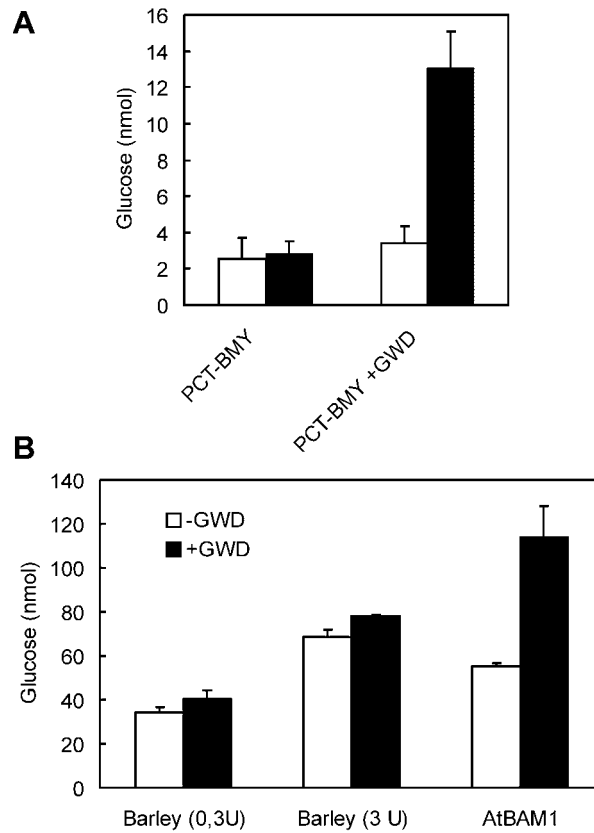
SUPPLEMENTAL FIGURES



Supplemental figure 1. Analysis of protein fractions purified from wild-type or mutants defective in BAM1 (*bam1*), ISA3 (*isa3*) or SBE3 (*sbe3*). Leaf extracts were precipitated with ammonium sulfate (45% saturation). Precipitated proteins were dissolved in buffer A, desalted and subjected to anion-exchange chromatography (Q-Sepharose FF, 1 ml column). Proteins were successively eluted with 112.5 mM, 300 mM and 750 mM NaCl in buffer A. Proteins eluted with 300 mM NaCl were further analyzed. **A**, Equal amounts of protein were separated by SDS-PAGE and stained with Coomassie Blue. **B**, Release of ³³P from pre-labeled starch (radioactive test). **C**, Non-radioactive test: Each sample contained 1.5 μg recombinant StGWD and 45 μg of protein prepared from the different mutants. The release of glucosyl-equivalents from *sex1-3* starch granules in the presence (black bars) or absence (white bars) of ATP was determined (t = 90 min).



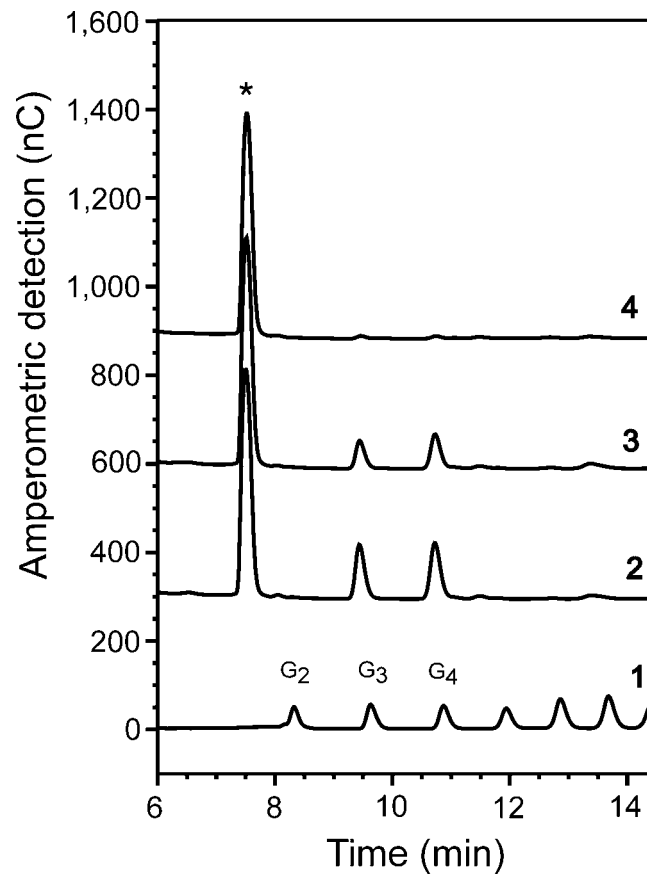
Supplemental figure 2. SDS-PAGE analysis of purified recombinant proteins. 0.5 μg of the following recombinant proteins were applied per lane: StGWD (1), AtGWD (2), StISA3 (3), GST-BAM1 (4), GST-BAM3 (5).



Supplemental figure 3. Breakdown of *sex1-3* granules by recombinant potato PCT-BMY or commercial β -amylase from barley flour (Megazyme) with and without simultaneous glucan phosphorylation.

A, N-terminally His-tagged PCT-BMY (Scheidig et al., 2002) was expressed in *E.coli* and purified. Compared to BAM1 and BAM3 from Arabidopsis the specific activity of recombinant PCT-BMY was very low. *Sex1-3* starch was incubated with 10 μ g PCT-BMY with or without 4.5 μ g StGWD in the presence (black bars) or absence (white bars) of 0.25 mM ATP for 90 min.

B, Breakdown of *sex1-3* starch granules by β -amylase from barley flour (Megazyme) in an ATP-containing buffer with (black bars) or without (white bars) 2 μ g StGWD. For comparison barley-BAM was replaced by BAM1 of Arabidopsis (GST fusion protein, 4 μ g) in a control sample.



Supplemental figure 4. Release of maltotriose and maltotetraose from starch by StISA3 following pretreatment of the granules with BAM3 +/- StGWD. *Sex1-3* starch granules (3 mg) were incubated in an ATP-containing buffer with 4 μ g GST-BAM3 +/- StGWD (5 μ g) for 45 min at 25°C. The supernatant was then removed and granules were washed twice in 2% (w/v) SDS and three times in water. Subsequently granules were incubated with 0.75 μ g StISA3 for 45 min at 25°C. Equal volumes of supernatant were analyzed by HPAEC-PAD. Maltodextrin standard (1), supernatant of StISA3 digested starch that had been pretreated with GST-BAM3 and StGWD (2), supernatant of StISA3 digested starch that had been pretreated with GST-BAM3 (3), supernatant of StISA3 digested starch that had not been pretreated (4). The peak labeled with an asterisk (*) comprises HEPES.