Supporting Information

Baroja-Fernández et al. 10.1073/pnas.1117099109

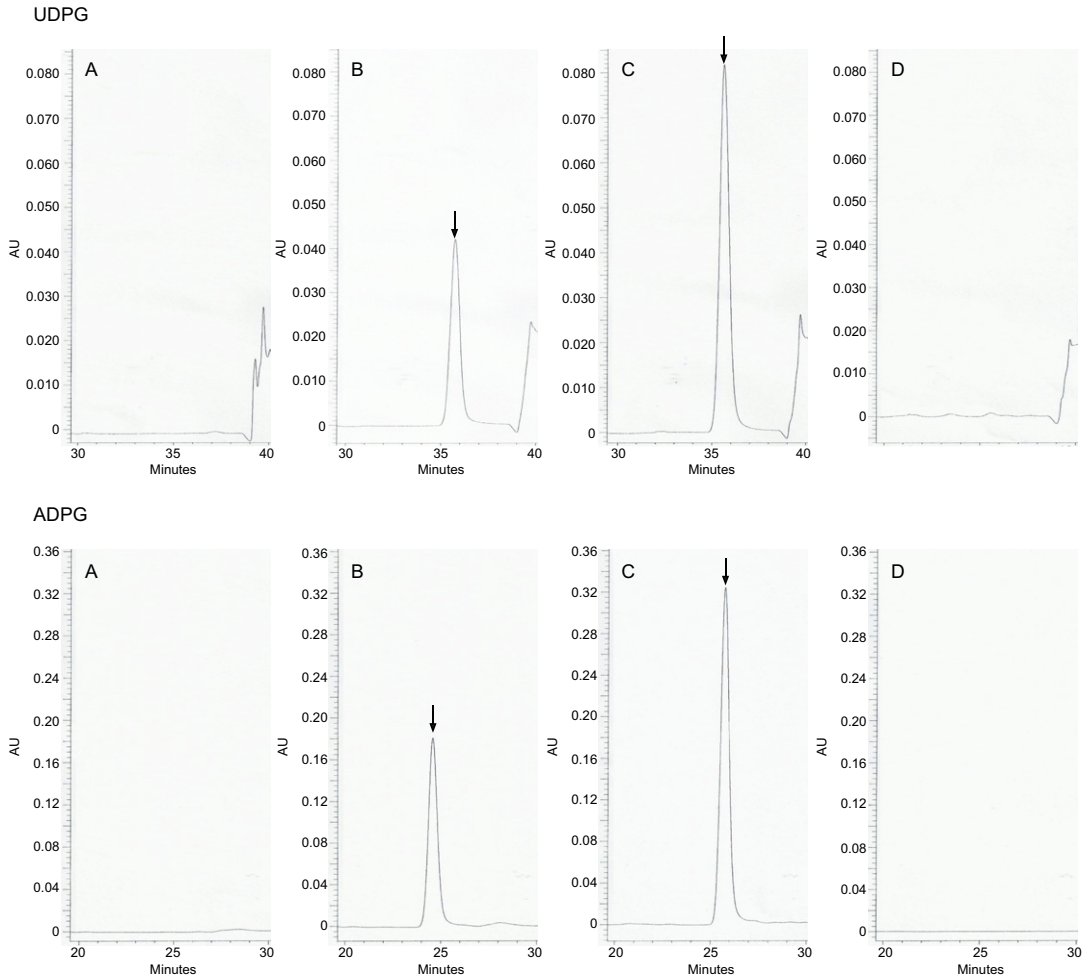

Fig. S1. UDPG (*Upper*) and ADPG (*Lower*) detection in 30 μL of SUS assay reaction mixture including recombinant SUS1. The SUS assay mixture contained 50 mM Hepes (pH 7.0), 200 mM sucrose, 2 mM of UDP or ADP, and recombinant SUS1 (10 ng). After 3 min at 37 °C, reactions were stopped by boiling the assay mixture for 1 min. UDPG and ADPG were then measured by HPLC as described in *Materials and Methods*. In *A*, recombinant SUS1 heated at 100 °C for 1 min was included in the assay mixture. In *B*, the assay mixture included nonheated, active recombinant SUS1. In *C* and *D*, once the SUS reaction was stopped, 5 nmol of commercially available UDPG/ADPG and 1 U of USPP/ASPP were added to the assay mixture, respectively. UDPG and ADPG elution peaks are indicated by arrows. UDP and ADP elution times were 40.3 min and 40.5 min, respectively.

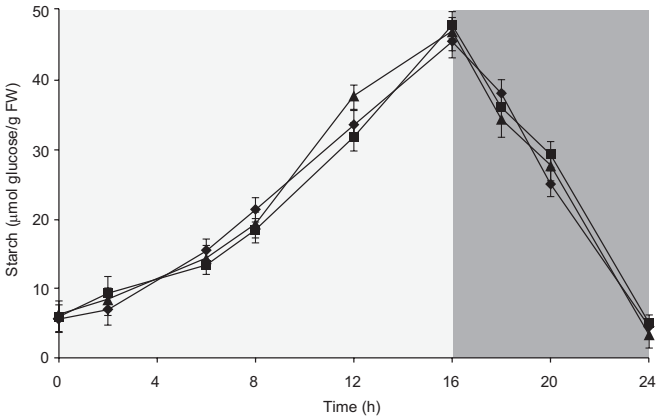

Fig. S2. Time course of starch accumulation in leaves of WT (squares), *sus1/sus2/sus3/sus4* (diamonds) and *sus5/sus6* (triangles) plants cultured under a 16-h light (100 μmol photons sec^{-1} m^{-2})/8-h dark photoperiod. The results are the mean ± SE of three independent experiments.

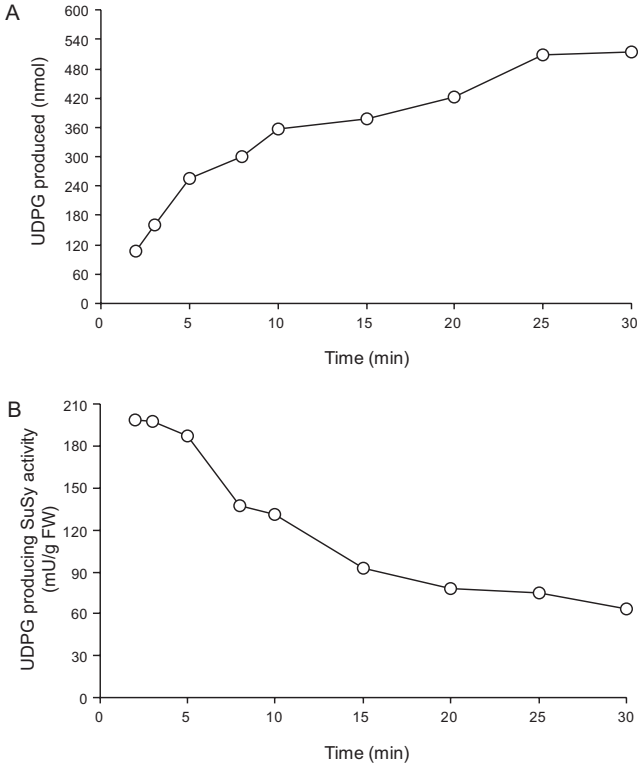

Fig. S3. Time course of (*A*) UDPG production by SUS and (*B*) apparent velocity of SUS in crude extracts of WT leaves. The reaction took place at 37 °C in 50 μL of assay mixture containing 50 mM Hepes (pH 7.0), 200 mM sucrose, 2 mM UDP, and 30 μg of protein extract. At the indicated incubation periods, the reactions were stopped by boiling the assay mixture for 1 min. UDPG was then measured by HPLC as described in *Materials and Methods*. Note that initial velocity conditions occur during the first 5 min of reaction.

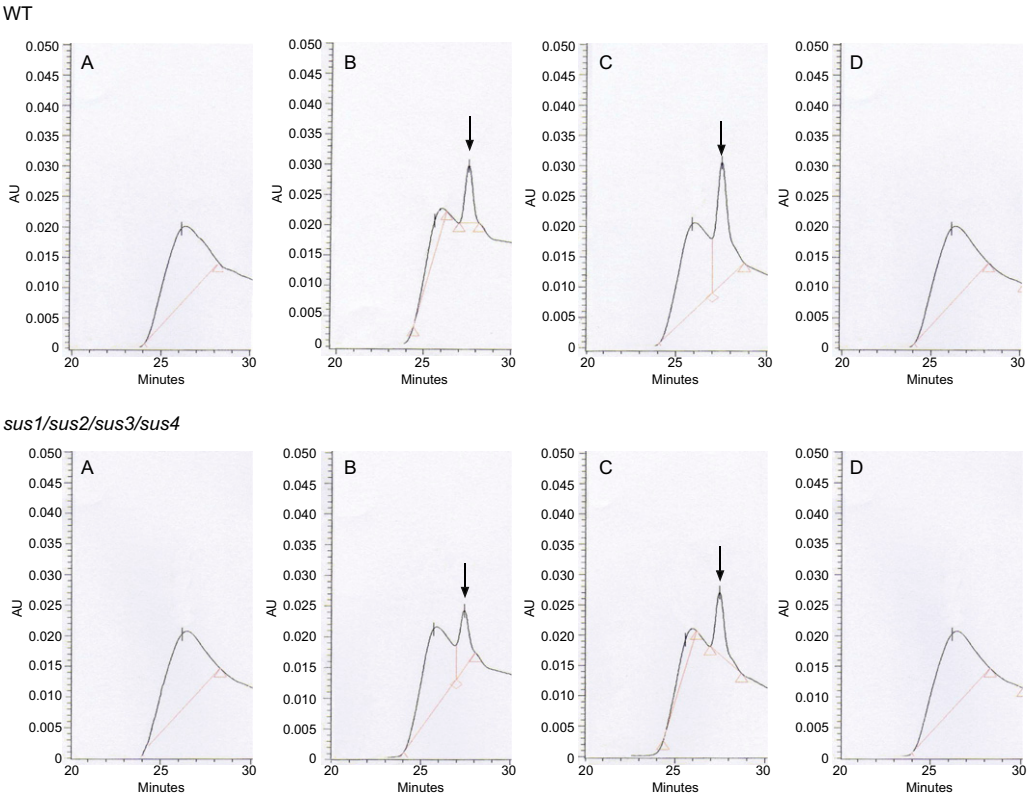

Fig. S4. UDPG detection in 30 μL of SUS assay reaction mixture including crude extracts from WT and *sus1/sus2/sus3/sus4* leaves. The SUS assay mixture contained 50 mM Hepes (pH 7.0), 200 mM sucrose, 2 mM UDP, and crude extract. After 3 min at 37 °C (still under initial velocity conditions, see Fig. S3) reactions were stopped by boiling the assay mixture for 1 min. UDPG was then measured by HPLC as described in *Materials and Methods*. In *A*, crude extract heated at 100 °C for 1 min was included in the assay mixture. In *B*, the assay mixture included fresh crude extract. In *C* and *D*, once the SUS reaction was stopped, 5 nmol of commercially available UDPG and 1 U of USPP were added to the assay mixture, respectively. UDPG elution time was 27.5 min. UDPG elution peaks are indicated by arrows.

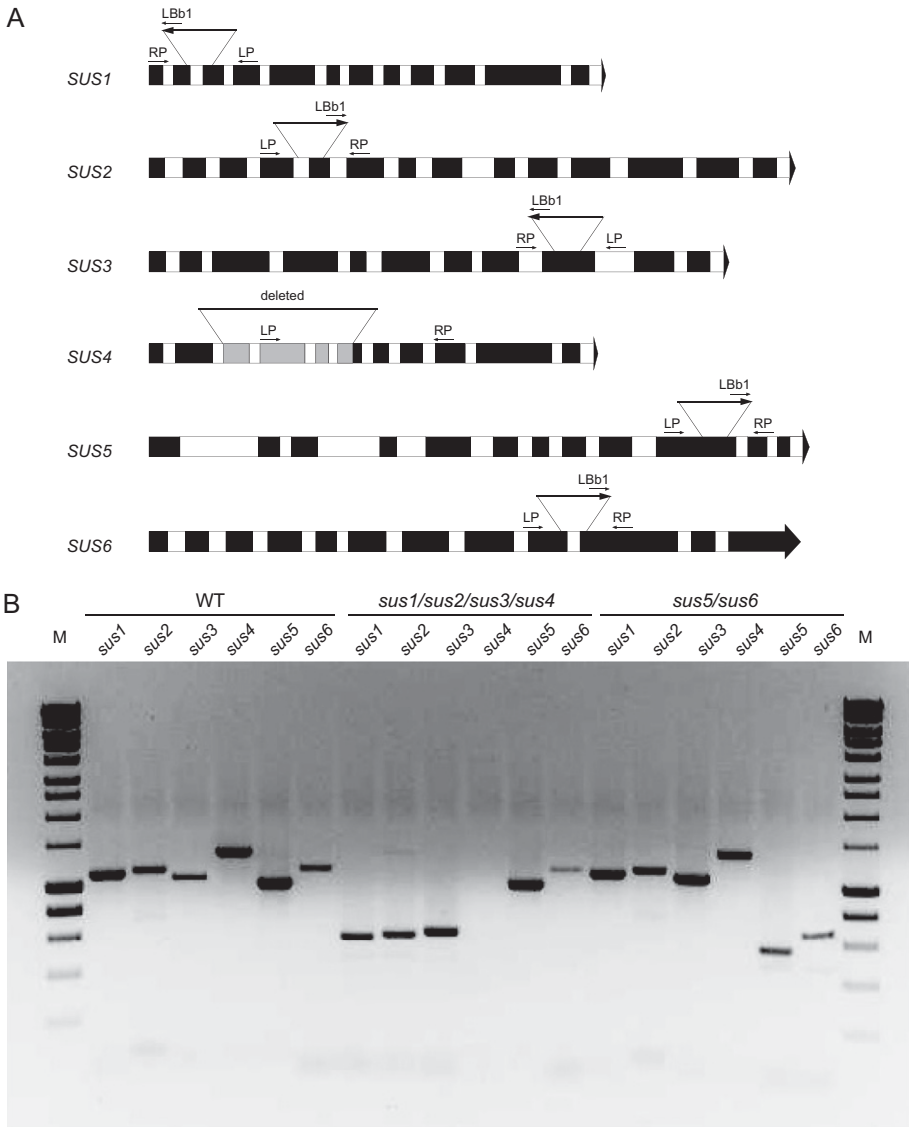

Fig. S5. (*A*) Schematic representation of the six *SUS* genes occurring in the *Arabidopsis* genome, and sites of T-DNA insertions and mutations in the different knockouts lines used in this study [for further details see Bieniawska (1) and Bieniawska et al. (2)]. Black and white boxes correspond to exons and introns, respectively. (*B*) PCR analysis of genomic DNA from WT, and homozygous *sus1/sus2/sus3/sus4* and *sus5/sus6* plants, using gene-specific "left" and "right" primers (LP and RP, respectively) (Table S1), and the left border T-DNA oligonucleotide LBb1 (5′-GCGTGGACCGCTTGCTGCAACT-3′). PCR of *SUS4* was performed using LP and RP only. "M" corresponds to DNA Hyperladder (Bioline) molecular marker.

1. Bieniawska Z (2006) Functional analysis of the sucrose synthase gene family in *Arabidopsis thaliana*. Thesis dissertation. University of Potsdam. March 2006.
2. Bieniawska Z, et al. (2007) Analysis of the sucrose synthase gene family in Arabidopsis. *Plant J* 49:810–828.

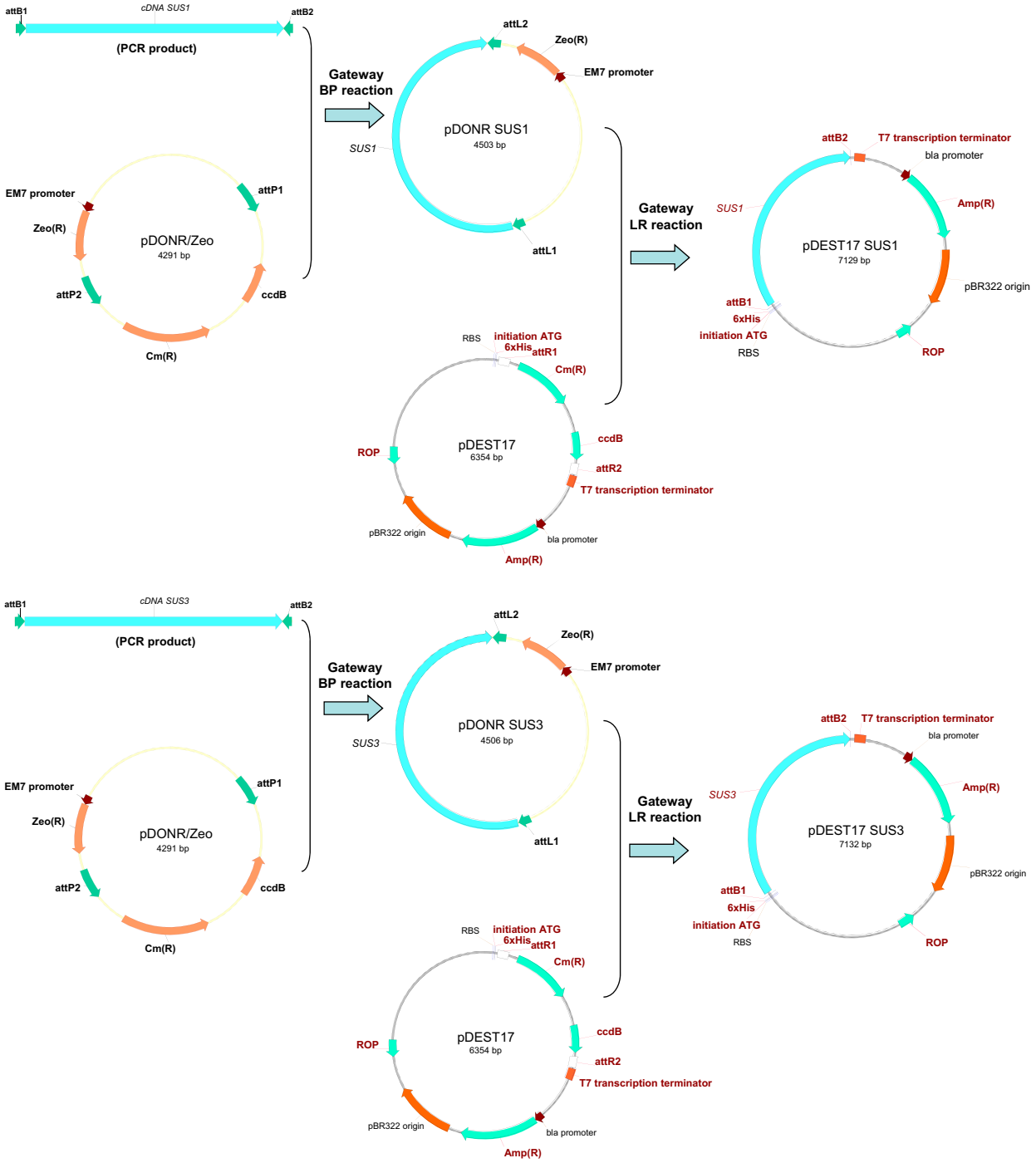

Fig. S6. Stages to produce the constructs used in this work. Primers used for the synthesis and cloning of full-length SUS-encoding cDNAs into pDEST17 expression vector are listed in Table S2.

Table S1. Primers used to confirm homozygous *sus1/sus2/sus3/sus4* and *sus5/sus6* mutants (Fig. S5)

Gene	Orientation	Sequence
SUS1	LP	5′-CTCAAGAGTGCAAGGATCAGG-3′
	RP	5′-ACGCTGAACGTATGATAACGC-3′
SUS2	LP	5′-CATGGGTAATGGTTTTGGTTG-3′
	RP	5′-ATGCGGAGACAAAATCACAAC-3′
SUS3	LP	5′-TTGGAGACCAGCGTCTGATAC-3′
	RP	5′-ATCGATGTGTTTGATCCGAAG-3′
SUS4	LP	5′-CAATAGGATCCAGAACCTAAAC-3′
	RP	5′-CCGTGAACTACACGGTACAAC-3′
SUS5	LP	5′-ATTTCCCTTTTACCGCACAAG-3′
	RP	5′-TTGTTTGGCCAGTTTCTGATC-3′
SUS6	LP	5′-TGACACGGTTAATACCGGAAG-3′
	RP	5′-ATCCATCTGAATTTCCCCTTG-3′

LP, left primer; RP, right primer.

Table S2. Primers used for the synthesis and cloning of full-length SUS encoding cDNAs into pDEST17 expression vector

Primer	Orientation	Sequence
SUS1-attB1	Forward	5′-**ggggacaagtttgtacaaaaaagcaggctta**atggcaaacgctgaacgtatg-3′
SUS1-attB2	Reverse	5′-**ggggaccactttgtacaagaaagctgggta**tcaatcatcttgtgcaagagg-3′
SUS3-attB1	Forward	5′-**ggggacaagtttgtacaaaaaagcaggctta**atggcaaaccctaagctcac-3′
SUS3-attB2	Reverse	5′-**ggggaccactttgtacaagaaagctgggta**tcagtcatcggcggttgaagg-3′

Primer sequences for attB sites (Fig. S6) are indicated in bold.

Table S3. Primers used in real time PCR analyses of *SUS* expression

Gene	Orientation	Sequence
18S RNA (At3g41768)	Forward	5′-GGGCATTCGTATTTCATAGTCAGAG-3′
	Reverse	5′-CGGTTCTTGATTAATGAAAACATCCT-3′
SUS1 (At5g20830)	Forward	5′-AGTTCACTGCGGATATTTTCGC-3′
	Reverse	5′-CCCAACAGTTTCTTTGCTTCCA-3′
SUS2 (At5g49190)	Forward	5′-TGCCATGAATAATGCCGATTTC-3′
	Reverse	5′-TTGCCCAACATTGTTCTTGCTT-3′
SUS3 (At4g02280)	Forward	5′-GACCAGACTGATGAGCATGTCG-3′
	Reverse	5′-TCTTCACTTTGTCGAGCCTCG-3′
SUS4 (At3g43190)	Forward	5′-AAGGAATCGTTCGCAAATGG-3′
	Reverse	5′-TTTCAGCGGCAACATCCTC-3′
SUS5 (At5g37180)	Forward	5′-GAACGTAGCGTTCGCAGTAA-3′
	Reverse	5′-TGAGATATTGCGTGCTCGAT-3′
SUS6 (At1g73370)	Forward	5′-CGGAGGCCAGGTTGTTTACAT-3′
	Reverse	5′-AGGCTTGAATCCGAGACCTTGT-3′