LETTER

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Expression of barley SUSIBA2 transcription factor yields high-starch low-methane rice

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Atmospheric methane is the second most important greenhouse gas after carbon dioxide, and is responsible for about 20% of the global warming effect since pre-industrial times[1,2]. Rice paddies are the largest anthropogenic methane source and produce 7–17% of atmospheric methane[2,3]. Warm waterlogged soil and exuded nutrients from rice roots provide ideal conditions for methanogenesis in paddies with annual methane emissions of 25–100-million tonnes[3,4]. This scenario will be exacerbated by an expansion in rice cultivation needed to meet the escalating demand for food in the coming decades[4]. There is an urgent need to establish sustainable technologies for increasing rice production while reducing methane fluxes from rice paddies. However, ongoing efforts for methane mitigation in rice paddies are mainly based on farming practices and measures that are difficult to implement[5]. Despite proposed strategies to increase rice productivity and reduce methane emissions[4,6], no high-starch low-methane-emission rice has been developed. Here we show that the addition of a single transcription factor gene, barley *SUSIBA2* (refs 7, 8), conferred a shift of carbon flux to *SUSIBA2* rice, favouring the allocation of photosynthates to aboveground biomass over allocation to roots. The altered allocation resulted in an increased biomass and starch content in the seeds and stems, and suppressed methanogenesis, possibly through a reduction in root exudates. Three-year field trials in China demonstrated that the cultivation of *SUSIBA2* rice was associated with a significant reduction in methane emissions and a decrease in rhizospheric methanogen levels. *SUSIBA2* rice offers a sustainable means of providing increased starch content for food production while reducing greenhouse gas emissions from rice cultivation. Approaches to increase rice productivity and reduce methane emissions as seen in *SUSIBA2* rice may be particularly beneficial in a future climate with rising temperatures resulting in increased methane emissions from paddies[9,10].

High-starch content and low-methane emissions are two important traits in rice breeding that are difficult to achieve simultaneously. In 2002, high-yielding rice cultivars with improved productivity were proposed as a strategy for reducing methane emissions[4,6]. However, no such high-starch low-methane-emission rice has as yet been reported. Here we report the first example, to our knowledge, of such a rice, *SUSIBA2* rice, generated via transcription factor technology.

Sugar signalling in barley 2 (SUSIBA2) is a plant-specific transcription factor[7,8,11] that regulates sugar-inducible gene expression, thereby mediating source–sink communication[7,8]. High expression of *SUSIBA2* is associated with an increase in sink strength and starch biosynthesis[7,8]. We hypothesized that the overexpression of *SUSIBA2* in the seeds and stems of rice would increase sink strength in aboveground tissues and generate a high-starch low-methane-emission rice variety.

Two stable rice lines (numbers 77 and 80) of homozygote transformants were selected in this study and defined as *SUSIBA2* rice (*SUSIBA2-77* and *SUSIBA2-80*, respectively). *SUSIBA2-77* and its control, Nipponbare (Nipp), were cultivated in Fuzhou, China during the summers of 2012 and 2013. Results showed that cultivation of *SUSIBA2-77* cut methane emissions to around 10% of control levels before flowering, and almost to zero (0.3% of the control level) at 28 days after flowering (Fig. 1a). Genomic sequencing demonstrated that the observed emission trait was related to the introduced *HvSUSIBA2* activity rather than its physical insertion site in the rice genome (Extended Data Fig. 1). Subsequent phytotron experiments corroborated the significant reduction of methane emissions in *SUSIBA2* rice and showed that the measured methane emissions occurred in a linear fashion over time (Extended Data Fig. 2).

To illustrate the role of *SUSIBA2* rice in reducing methane emissions in different ecological environments and climates, we cultivated *SUSIBA2-77* and *SUSIBA2-80* in the autumn of 2014 in Fuzhou, Guangzhou and Nanning, China at locations >500 km apart, and measured diurnal and seasonal methane emissions (Extended Data Fig. 3). Both rice lines displayed similar traits in methane emissions, that is, less reduction in the morning, but significant reduction during the day (Extended Data Fig. 3). Interestingly, the observed pattern of emission reduction matched the predicted pattern of SUSIBA2-controlled sugar metabolism, which increases during the day and in the summer.

To elucidate the mechanism underpinning the reduced methane emissions from *SUSIBA2* rice, we took a three-pronged approach: (1) cultivating *SUSIBA2* and control rice in phytotrons/fields and quantifying rhizospheric methanogen communities; (2) measuring phenotypic traits of *SUSIBA2* rice; and (3) characterizing genotypic traits.

Strong blue-green autofluorescence when excited with light at 420 nm wavelength is a characteristic of all methanogens due to the presence of the cofactor F420 (ref. 12). Fluorescence microscopy revealed that the major methanogenic autofluorescence was associated with the root tip and proximal regions, and much less signal was apparent in *SUSIBA2-77* compared with Nipp (Fig. 1b). Quantification of the methanogenic communities indicated that the gene copy numbers of total archaea and methanogens and the orders Methanobacteriales, Methanomicrobiales and Methanocellales and two families Methanosaetaceae and Methanosarcinaceae of the order Methanosarcinales were significantly lower in *SUSIBA2* rice compared with Nipp in both phytotron (Fig. 1c and Extended Data Fig. 4) and field conditions (Extended Data Fig. 5). We propose that the low-methane emissions from *SUSIBA2* rice were due to a decrease in the rhizospheric abundance of methanogens. Methanocellales are the dominant methanogens in rice paddies[13,14], although recent reports indicate that other methanogenic groups can also be abundant in certain paddies[15]. One plausible reason for the broad representation of methanogens detected in this study could be that plant residues were included as fertilisers in field and phytotron soils, possibly favouring richness in the methanogenic population[16,17]. When we looked into the

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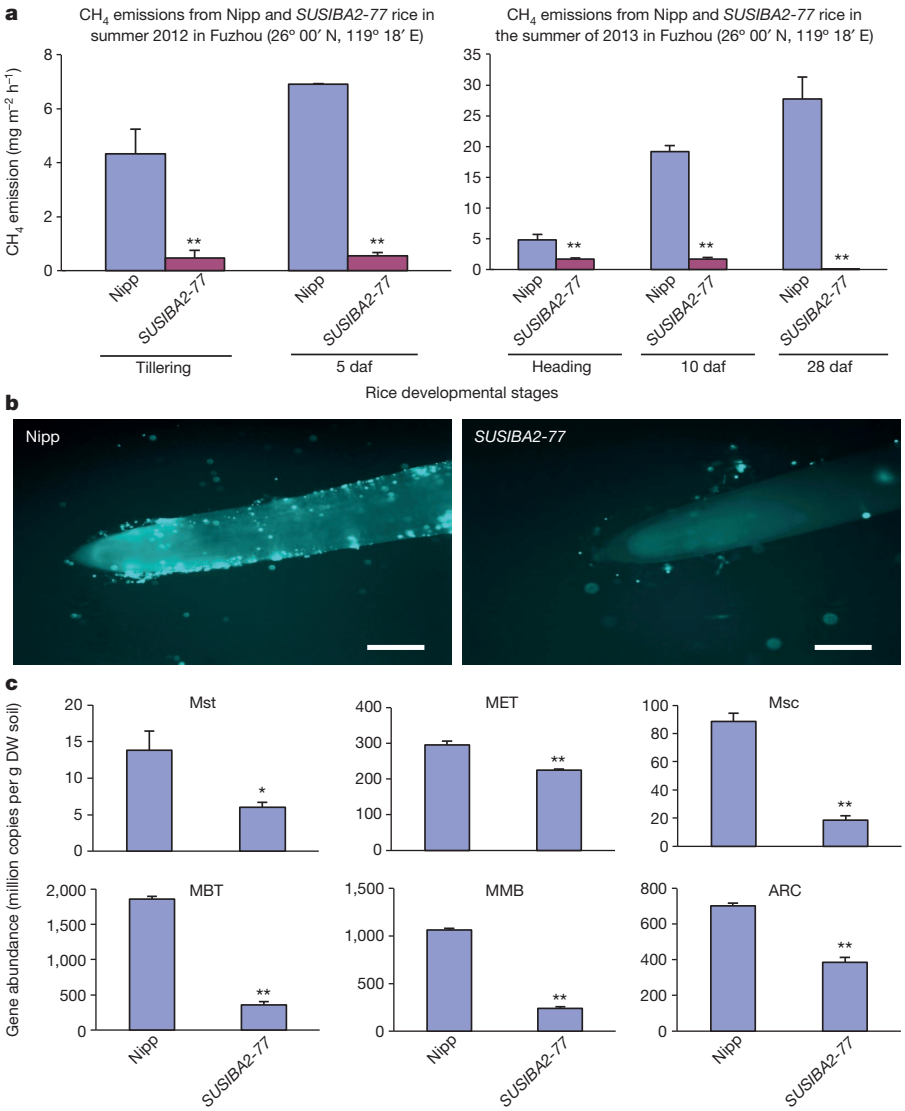

Figure 1 | *SUSIBA2* rice reduces methane emissions from rice paddies. **a**, Methane emissions from four rice plants ($n = 4$) for 2013 and three ($n = 3$) for 2012. **b**, Fluorescent microscopy of Nipp and *SUSIBA2-77* roots. Scale bar, 1 mm. **c**, Quantification of methanogens in soil and root samples from three rhizospheric positions ($n = 3$) of three plants ($n = 3$). Technical triplicates per position were applied. Quantification was performed for total archaea (ARC) and methanogens (MET), and the orders Methanobacteriales (MBT), Methanomicrobiales (MMB) and Methanocellales and two families, Methanosaetaceae (Mst) and Methanosarcinaceae (Msc), of the order Methanosarcinales, respectively. Typical results from soil sample 1 of plant 1 are shown. Differences between Nipp and *SUSIBA2-77* were statistically significant (one-way ANOVA, $*P \leq 0.05$ or $**P \leq 0.01$, error bars show s.d.). daf, days after flowering; DW, dry weight.

specific group of Methanocella[14] in the order of Methanocellales, we found that it was also significantly reduced in *SUSIBA2* rice (Extended Data Fig. 5c).

To investigate if photosynthate partitioning in *SUSIBA2* rice was altered, we examined phenotypic traits in *SUSIBA2-77* and Nipp. Compared with Nipp, *SUSIBA2-77* has larger panicles with a higher proportion of filled grains, resulting in more drooping panicles (Fig. 2a, left and middle panels). In contrast, *SUSIBA2-77* has a smaller root system than Nipp (Fig. 2a, right panel). Consistent with these observations, measurements of total dry biomass in aboveground and belowground tissues (Fig. 2b) showed that significantly more aboveground biomass and less root biomass were found with *SUSIBA2-77*. The increased aboveground biomass was associated with grain dry biomass and the numbers of filled grains (Fig. 2b). Plant height, thousand-grain weight (TGW), and numbers of panicles and tillers per plant were similar in *SUSIBA2-77* and Nipp (Fig. 2b).

Starch content in filled grains of *SUSIBA2-77* increased to 86.9% dry weight compared with 76.7% in Nipp (Fig. 2c). Electron microscopy examination showed that the starch granule sizes in mature seeds of *SUSIBA2-77* were reduced (Fig. 2d). As the grain size was unaffected in *SUSIBA2-77*, we suggest that one possible reason for the increased starch content in mature *SUSIBA2-77* grains is a more dense packing of smaller granules. A closer examination of starch content during seed development showed an elevation from 14 days after flowering (daf) (Fig. 2c). Notably, at the same developmental stage (14 daf), the starch content in stems was also significantly increased, but not in leaves and roots (Fig. 2c).

To link the phenotypic traits with genotypic profiles, we examined the integration of *HvSUSIBA2* in the *SUSIBA2* rice genome, along with the gene and protein expression of *HvSUSIBA2* and other representative genes. Southern blot analysis revealed two copies of *HvSBEIIb* p:*HvSUSIBA2* in the *SUSIBA2-77* and *SUSIBA2-80* genome (Fig. 3a), in agreement with the sequencing results from *SUSIBA2-77* (Extended Data Fig. 1b). The presence of sugar-responsive elements (SUREs) in the promoters of genes targeted by SUSIBA2 is critical for executing the sugar-signalling cascade that controls starch biosynthesis[7,8]. Using

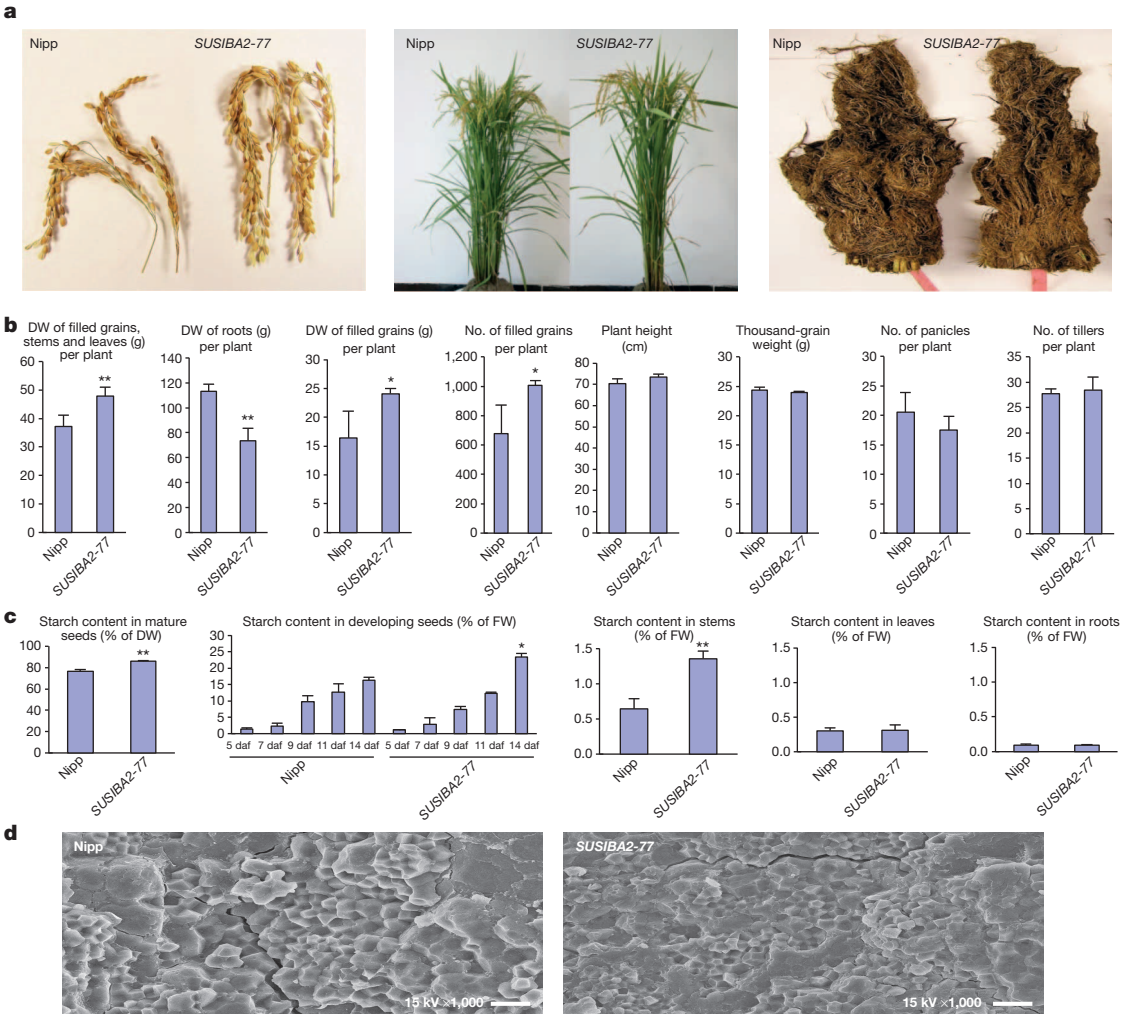

Figure 2 | Phenotypic profiling of *SUSIBA2* rice and Nipponbare (Nipp). **a**, Panicles (left), aboveground plants (middle) and roots (right). **b**, For *SUSIBA2-77* we observed significantly increased dry weight of aboveground biomass and filled grains, increased number of filled grains, and significantly decreased root dry weight. Plant height, thousand-grain weight, number of panicles and tillers were not significantly altered in *SUSIBA2-77*. Four plants were used ($n = 4$). **c**, For *SUSIBA2-77* starch content in mature seeds, developing seeds and stems at 14 daf changed significantly, but did not change in leaves and roots. Three plants ($n = 3$) were used. **d**, Starch granules from mature seeds. Scale bar, 10 μm. One-way ANOVA was used for statistical analysis (*$P \leq 0.05$ or **$P \leq 0.01$, error bars show s.d.). DW, dry weight; FW, fresh weight.

the protocol described in ref. 7, we demonstrated the SURE-binding activity in rice by HvSUSIBA2 (Extended Data Fig. 6). To investigate gene expression, we selected 24 genes[18] associated with sugar metabolism, including *HvSUSIBA2* and *OsSUSIBA2-like*. Gene expression analyses showed that *HvSUSIBA2* was highly expressed in early developing seeds and stems, and at very low levels in leaves, roots and late developing seeds (Fig. 3b, upper two panels). The expression pattern correlated with the activity of the *HvSBEIIb* promoter in rice (Extended Data Fig. 7). The expression level of selected genes, apart from the control gene *TIP41-like*, followed the *HvSUSIBA2* expression pattern with significant differences between *SUSIBA2* rice and Nipp in seeds (Fig. 3b and Extended Data Figs 8 and 9) and, at least for some genes, in stems but not in leaves or roots. (Fig. 3b, Extended Data Fig. 8 and Supplementary Table 2). Following transcriptomic analysis, the same tissues of *SUSIBA2-77* were subjected to protein expression analysis. Protein analysis indicated that all five selected proteins could be found at higher levels in stems, but not in leaves and roots in *SUSIBA2-77* compared with Nipp (Fig. 3c, upper panel). In developing seeds, the membrane protein (SUT5) and starch granule-bound protein (GBSSI) were expressed more in *SUSIBA2-77* than in Nipp (Fig. 3c, lower panel). The same trend was not obvious for the soluble proteins. *In vitro* degradation experiments suggested that the unchanged levels of soluble proteins in *SUSIBA2-77* compared with Nipp might represent a higher turnover of soluble proteins in *SUSIBA2-77* (Fig. 3d), supported by a zymogram activity assay of a soluble phase of starch branching enzyme I (Fig. 3e).

We have proposed a model for how *SUSIBA2* rice works (Fig. 4). The *HvSBEIIb* promoter activity is sugar-inducible via the activation of *HvSUSIBA2* (refs 7, 8). When photosynthates (sugars) are available, *HvSBEIIb* p:*HvSUSIBA2* enhances sugar-inducible activities of targeted genes, including the *HvSBEIIb* p construct. The augmented gene expression increases sink strength in tissues where *HvSUSIBA2* is expressed. The increased sink strength draws more sugars from source tissues to yield more biomass and more filled grains associated with higher starch content. The increased allocation of photosynthates further activates *HvSBEIIb* p:*HvSUSIBA2*, causing a 'snowball effect' for increasing sink strength. The snowball effect was illustrated by the exogenous sugar treatment of leaves and field trial experiments. Expression levels of the sugar-inducible genes were significantly higher in *SUSIBA2* rice than in Nipp when sucrose was available (Extended Data Fig. 10). Methane emission reduction was more effective during the summer than during the autumn, and at noon rather than in the morning or later afternoon (Fig. 1a and Extended Data Fig. 3). The data indicate that the higher temperature and enhanced sugar metabolism during the summer and at noon may favour the SUSIBA2-derived snowball effect and carbon allocation to aboveground biomass. As suggested in the model, less sugar

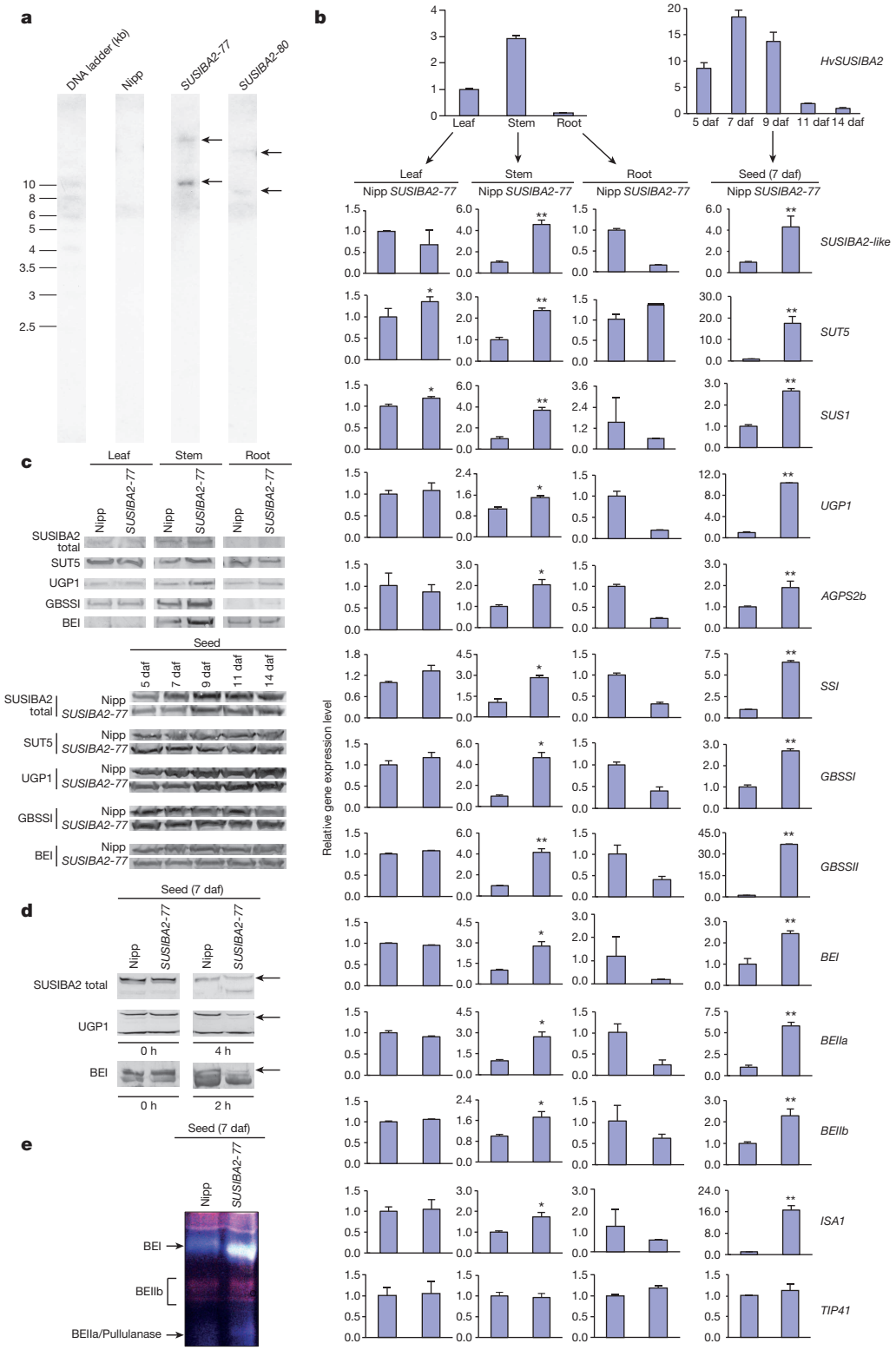

Figure 3 | Genotypic profiling of *SUSIBA2* rice and Nipponbare (Nipp). **a**, Two copies of *HvSUSIBA2* were detected in *SUSIBA2-77* and *SUSIBA2-80*. **b**, Downstream effects of *HvSUSIBA2* expression in *SUSIBA2-77*. High expression of *HvSUSIBA2* enhanced expression of 12 genes in stems and early developing seeds, with a corresponding reduction of gene expression in roots. Three plants ($n = 3$) and technical triplicates per plant were used. **c**, Western blot analysis. SUSIBA2 total represents levels of HvSUSIBA2 and OsSUSIBA2-like. Protein levels increased in stems of *SUSIBA2-77*, but not in leaves and roots (upper panel). Protein levels also increased in seeds for membrane proteins (SUT5) and starch granule-bound proteins (GBSSI), but not for soluble proteins (lower panel). **d**, *In vitro* study of degradation efficiency of soluble proteins in *SUSIBA2-77* seeds. A higher degradation efficiency was detected in *SUSIBA2* rice. **e**, Zymogram of starch branching enzyme activity. Soluble branching enzyme I (BEI) activity increased in *SUSIBA2-77*. One-way ANOVA was used for statistical analysis (*$P \leq 0.05$ or **$P \leq 0.01$, error bars show s.d.).

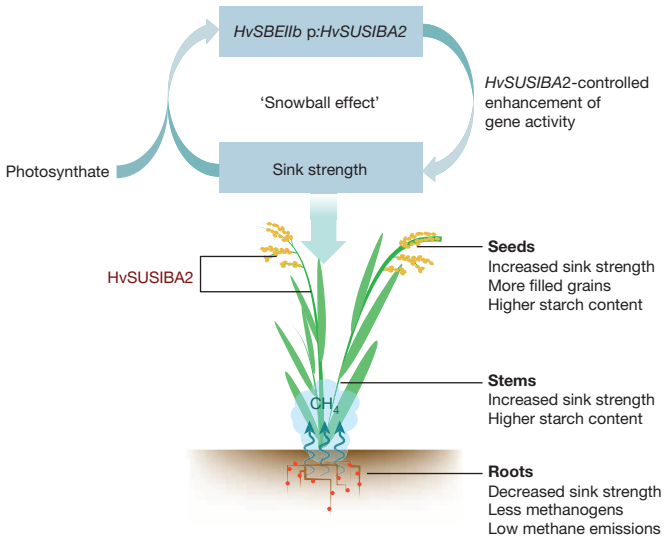

Figure 4 | Model depicting high-starch low-methane-emission *SUSIBA2* rice. Sugar-inducible *HvSUSIBA2* expression generates a snowball effect that ultimately leads to a rice plant with enhanced starch accumulation in seeds and stems and decreased carbon allocation to roots, which reduces methanogenic growth and methane emissions. Red dots represent methanogens.

availability in *SUSIBA2* rice roots results in a rhizosphere with less organic biomass and hence less root exudates as nutrients for inhabiting methanogenic consortia. As a consequence, methane emissions decreased. Due to the biomass differences between *SUSIBA2* rice and Nipp, we do not exclude the possibility that the emission reduction is due in part to impaired methane transport in *SUSIBA2* rice.

Finally, we suggest that the use of *SUSIBA2* rice in cutting methane emissions from paddies may become more relevant with global warming. Increased temperatures accelerate methane emissions in all ecosystems including paddies[9,10], but they also favour SUSIBA2-derived carbon allocation to seeds and aboveground biomass, thus counteracting the temperature-driven acceleration of rice paddy methane emissions.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions J.S., Z.C., Q.G., Y.W. and D.Z. performed measurements of methane emissions from paddies; J.S. also performed western blot and zymogram analyses, methanogen quantification and starch determination. C.H. was responsible for plasmid constructions, rice transformation, Southern blot analysis and phenotypic trait characterization. X.Y. carried out gene expression analysis, starch determination, sugar induction experiments and phenotypic trait characterization. Y.J. performed plasmid validation, insertion site identification, methanogen quantification, measurements of methane emissions in phytotrons and sugar induction experiments, electrophoretic mobility shift assay (EMSA), qPCR and light microscopy. C.J. was involved in the initiation, layout and discussions concerning the work and manuscript revision. F.W. was involved in the planning of rice transformation and field trial settings. A.S. revised the manuscript and helped with methane and methanogen determinations. C.S. initiated and coordinated the work, designed the experiments, performed some experiments, and drafted and revised the manuscript.

Author Information The sequence of construct containing *HvSBEIIb p:HvSUSIBA2* has been deposited in GenBank under accession number KR935231. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.S. (Chuanxin.Sun@slu.se) or F.W. (wf@fjage.org).

METHODS

Plant materials and growth conditions. Rice plants of variety Nipponbare (*Oryza sativa* L. ssp. *Japonica*) and transformed homozygote lines *SUSIBA2-77* and *SUSIBA2-80* were grown in open fields or in a phytotron. Open field cultivation was performed in a similar way to that described previously[18], but with rice straw in soil under natural conditions in Fuzhou, Guangzhou and Nanning, southern China, respectively. Phytotron conditions were applied to mimic field conditions, but with limited high temperatures. In the phytotron, rice plants were grown in cylinder-type pots (30 cm high with an upper diameter of 29 cm and bottom diameter of 19 cm) with organic soil containing plant residues. Phytotron growth management was similar to that described previously[19] with a modified setting for rice, 14 h light/10 h dark at 30 °C/21 °C, a constant relative humidity of 80% and light intensity of 400 μmol photons m^{-2} s^{-1}.

Plasmid construction and rice transformation. Plasmid construction and general molecular cloning procedures were performed according to previously developed protocols[7,8,20]. Nucleotides 247–2067 of GenBank accession number AY323206, encoding barley *SUSIBA2*, were fused to nucleotides 1–1010 of barley *SBEIIb* promoter (*HvSBEIIb* p; GenBank accession number AF064563). The fused DNA fragment was cloned in the pCAMBIA 1301 binary vector (Extended Data Fig. 1a) and sequenced at Macrogen Europe (Amsterdam, the Netherlands). The sequence of the construct is in the Supplementary Information and deposited under GenBank accession number KR935231. The plasmid construct was used for *Agrobacterium*-mediated transformation of rice following the protocol in ref. 21. Screening of post-transformants was based on hygromycin resistance and PCR determination of T-DNA insertion. Out of 14 positive lines, five homozygous lines were selected for characterization. Eventually, two homozygote lines *SUSIBA2-77* (Extended Data Fig. 1b) and *SUSIBA2-80* were used for detailed studies. A binary vector containing *HvSBEIIb* p:*GUS* was also constructed and transformed to Nipponbare (Extended Data Fig. 7). The final construct was verified by DNA sequencing, and transformed into *Agrobacterium tumefaciens* strain EHA105.

Gene expression analysis by quantitative PCR (qPCR). RNA isolation, cDNA synthesis and qPCR analysis were performed in accordance with previous reports[8,18]. In brief, plant materials from different tissues were ground into fine powders in liquid nitrogen and total RNA was isolated by the Spectrum Plant Total RNA Kit (Sigma-Aldrich) according to the manufacturer's protocol using approximately 30 mg of plant materials. All samples were treated with DNase I (Sigma-Aldrich) to remove trace amounts of DNA contamination. Total RNA of 1 μg was used as a template for the cDNA synthesis with the Quanta qScript cDNA synthesis kit (Quanta Biosciences). The synthesized cDNA was adjusted to a concentration of 5 ng μl^{-1} and 15 ng used for qPCR analysis. qPCR reactions with at least 90% amplification efficiency were performed in a volume of 20 μl containing 5 μM specific primers and a SYBR Green PCR master mix (Applied Biosystems, Life Technologies Europe BV). The PCR programme consisted of an initial temperature of 95 °C for 4 min, and then 40 cycles of 30 s at 95 °C and 30 s at 60 °C. The melt curve was performed by increasing the temperature from 60 °C to 95 °C at a speed of 0.05 °C per second. qPCR-specific amplification was verified by a single band product in gel analysis. Data were calculated with the comparative C_t method[18] and one-way ANOVA[18] was used for statistical analysis. The gene expression level by qPCR was normalized using housekeeping genes *ACT11* (ref. 22) in developing seeds and *Ubiquitin10* (ref. 22) in vegetative tissues, and using *TIP41-like*[23] as a control gene.

Electrophoretic mobility shift assay (EMSA). Overexpressed HvSUSIBA2 protein from *E. coli* was purified and used for EMSA. EMSA was performed essentially as described in ref. 7. The SURE sequences in the rice *ISA1* promoter were found by manual search in the promoter sequence[18]. The binding assay of HvSUSIBA2 protein to labelled SURE oligonucleotides was performed by incubating the protein with a DNA probe at room temperature for 30 min and then visualizing a protein–DNA complex on a native 5% polyacrylamide gel. The gel electrophoresis was conducted at 200 V for 2 h. A DNA fragment of 'A-rich stretch' was used as a negative control. The gel was dried and autoradiography was carried out on an X-ray film overnight.

Exogenous sucrose induction. Leaf blades were excised from rice plants after 10 h in the dark during the early tillering stage and incubated with 100 mM sucrose solutions in a falcon tube (1 blade per tube) containing 2 ml sucrose solution for 24 h at 22 °C in the dark. Immediately after collection, the leaf blades were frozen in liquid nitrogen and stored at −70 °C until further analysis.

Validation of T-DNA insertion sites. The genomic DNA of *SUSIBA2-77* was isolated using a DNA isolation kit (Qiagen GmbH, Hilden, Germany). The genomic DNA was fragmented by HindIII and ligated to a DNA adaptor also generated by HindIII. The HindIII-digested adaptor was purified before ligation using a PCR product purification kit (Qiagen). PCR cloning was performed using the ligated template to obtain a specific PCR product with the primers against T-DNA board sequences and the adaptor sequences. The resulting PCR products were then sub-cloned into a PCR cloning vector by the TOPO10 TA cloning kit (Life Technologies Europe BV) for sequencing.

qPCR quantification of methanogenic communities. Soil or root samples from three independent positions in the underground vicinity around the root tip and proximal regions (5 cm from the rice plant and 5 cm depths, and 5 cm between positions) of three independent plants for Nipp, *SUSIBA2-77* and *SUSIBA2-80* were collected at 3.00 p.m. from rice paddies or phytotrons, and the DNA was subsequently isolated using a DNA isolation kit for soil organisms (FastDNA SPIN Kit for Soil; MP Biomedicals, LLC). The DNA was then diluted into four different series concentrations. qPCR quantification was performed for all four diluted DNA samples using a standard of a previously cloned 16S rRNA gene fragment[24–26] for the individual groups of targeted methanogens and a newly PCR cloned 16S rRNA gene fragment using described primers[14] from the strain *Methanocella conradii* DSM 24694 (German Collection of Microorganisms and Cell Cultures GmbH, Germany) for the genera of *Methanocella*. Only DNA copy numbers that were in agreement from two series of diluted samples were used for copy number determination. All methanogenic groups were analysed by using group-specific primers (Supplementary Table 1) with at least 90% amplification efficiency. Existing PCR products in all qPCR reactions were verified by gel analysis, showing a single band of the expected size. The abundance of each group was calculated and translated to DNA copy numbers for each gram of dry root and/or soil. The abbreviations for each group are Mst (Methanosaetaceae), Msc (Methanosarcinaceae), MBT (Methanobacteriales), MMB (Methanomicrobiales), ARC (archaea) and MET (methanogens) according to ref. 26, and Met for *Methanocella-specific* (Supplementary Table 1). The qPCR programme for Mst was as follows: 95 °C for 7 min, then 54 cycles of 40 s at 95 °C, 1 min at 61 °C and 40 s at 72 °C; for MBT: 95 °C for 7 min, followed by 54 cycles of 40 s at 95 °C, 1 min at 58 °C and 40 s at 72 °C; for MMB: 95 °C for 7 min, followed by 54 cycles of 40 s at 95 °C, 1 min at 66 °C and 40 s at 72 °C; for Msc, ARC and MET: 95 °C for 7 min, followed by 54 cycles of 40 s at 95 °C, 1 min at 60 °C and 40 s at 72 °C; for *Methanocella*-specific (Met) analysis: 94 °C for 4 min, followed by 40 cycles of 30 s at 94 °C, 1 min at 60 °C. All melting curves are from 55 °C to 95 °C with an increase of 0.05 °C per second.

Autofluorescence analysis of methanogens. Rice root tip and proximal regions at/after 28 days after flowering were picked at 3.00 p.m. and rinsed with pure water and then examined under a microscope at an excitation light wavelength of 420 nm. Multiple observations on different batches of rice plants were performed.

Southern blot analysis. Southern blot analysis was performed according to a previous protocol[20]. Briefly, rice genomic DNA was isolated from leaves using a CTAB method and 10 μg DNA was used for restriction digestion at 37 °C for 2 h with BamHI. Digested DNA was applied to agarose gel separation immediately with an electrophoresis condition of 50 V for about 7 h. The separated DNA was denatured by an alkaline solution and transferred to the Hybond-N membrane overnight through capillary blotting with 10× SSC solution, and then the DNA was cross-linked to the membrane by UV-light for 5 min. The hybridization was performed overnight with a probe labelled with α-^{32}P dCTP using a random prime labelling kit (rediprime II, GE Healthcare) in a volume of 20 ml hybridization solution at 42 °C. After hybridization, the membrane was washed under a moderate stringent condition, 1× SSC at 50 °C. Autoradiography was conducted with an X-ray film on the membrane.

Western blot analysis. Western blot analysis was performed as described previously[7]. Five proteins were selected for analysis: a membrane protein (sucrose transporter 5, SUT5), a starch granule-bound protein (granule-bound starch synthase I, GBSSI), and three soluble/soluble-phase proteins (SUSIBA2 total = barley HvSUSIBA2 and rice OsSUSIBA2-like; UDP-glucose pyrophosphorylase 1, UGP1; branching enzyme I, BEI), respectively. Peptide antibodies against the five proteins were obtained from the Beijing Genomics Institute. The antibodies were raised against peptide positions of 300–330, 510–540, 440–470, 260–290 and 480–510 of GenBank accession numbers NM_001066651, DQ072593, DQ395328.1, X62134 and D11082, for OsSUSIBA2-like, SUT5, UGP1, GBSSI and BEI, respectively. Rice tissue samples were collected at 3.00 p.m. and ground into fine powder. Total proteins were extracted from approximately 200 mg samples using the plant total protein extraction kit (Sigma-Aldrich, St. Louis, MO, US). The proteins were separated in a 4–12% gradient PAGE gel under a condition of 150 V for 1.5–2 h, and then transferred to PVDF membrane by electro-blotting. The PVDF membrane was blocked in 5% milk in a TBS buffer for 1 h, and then incubated with the first antibody in the blocking solution overnight at room temperature. Incubation with the second antibody conjugated with a phosphatase was performed for approximately 2 h after the membrane was washed by TBST and TBS solutions. Immuno-reacted bands were visualized in a BCIP/NBT (substrates for the phosphatase) solution for colour development.

Methane collection in rice paddies and phytotrons. Methane sampling and determination were based on published protocols[27–29]. Individual rice plants were covered with a sealed plastic cylinder (diameter: 15 cm, height: 45 cm for early stages and 95 cm for late stages) and 3 × 25 ml gas samples were taken from six independent plants by a syringe from the headspace after 10, 20 and 30 min and pooled in a sealed airbag. Sampling time in rice paddies was 8.00 a.m. for the summer (June–August) of 2012 and 2013, and 8.00 a.m., 12.00 p.m. and 4.00 p.m. for the autumn (September–November) of 2014, respectively. Temperatures were recorded. The samples in the phytotrons were taken from six independent plants at 3.00 p.m. after 15 min coverage for each plant. Four technical repeats, that is, four vials, were used for each plant methane collection. All gas samples were analysed by gas chromatography with appropriate methane standards. Methane in samples was quantified by calculating the sample peak area in comparison with the standard peak area of known amounts. An air methane concentration of 1.8 p.p.m. was used as the background for calculations. Methane flux calculation was according to the formula described by ref. 29.

Degradation efficiency analysis. Soluble proteins were extracted from the seeds at 7 daf without the addition of protease inhibitors according to a protocol described previously[7,8]. The protein extracts were adjusted to the same concentration at time = 0 and incubated at 28° C for 2 or 4 h. Western blot analysis was used to check the remaining protein levels.

Total starch analysis. Total starch was extracted and analysed using a total starch assay kit (Megazyme, Bray, Co. Wicklow, Ireland). Rice tissues were collected at 3.00 p.m. and ground into fine powder in liquid nitrogen and 100 mg samples of tissues were used for total starch analysis after removing the soluble sugars with 80% ethanol. Total starch quantification and calculation followed the manufacturer's protocol exactly.

Zymogram analysis of starch branching enzyme (SBE) activity. Zymogram analysis was performed according to a protocol described previously[8]. Equal amounts of total soluble proteins from Nipp and *SUSIBA2-77* developing seeds at 7 daf were separated in a 10% (w/v) polyacrylamide gel containing 1% (w/v) starch. After electrophoresis, the gel containing starch branching enzymes was washed with water and incubated at 30 °C in a buffer containing 100 mM sodium citrate (pH 7.0) for 4–8 h. The gel was then transferred to an I_2/Kl solution to visualize reddish-purple bands against the dark blue starch gel for SBE activity.

Scanning electron microscopy of starch granules. Starch granules of Nipponbare and *SUSIBA2-77* were isolated as described[8]. Coating of the granules was carried out in a high-resolution sputter coater, with platinum/palladium as the target. Scanning electron microscopy was performed on a JSM-6320F (JEOL Ltd, Akishima, Tokyo, Japan).

Molecular cloning of *Methanocella*-specific 16S rRNA genes. Genomic DNA of *Methanocella conradii* DSM 24694 were isolated by the DNA isolation kit for soil organisms (FastDNA SPIN Kit for Soil; MP Biomedicals, LLC) and used as PCR templates for PCR-based cloning of 16S rRNA. PCR-amplified products were subcloned into a PCR cloning vector by the TOPO10 TA cloning kit (Life Technologies Europe BV). The cloned genes were verified by DNA sequencing.

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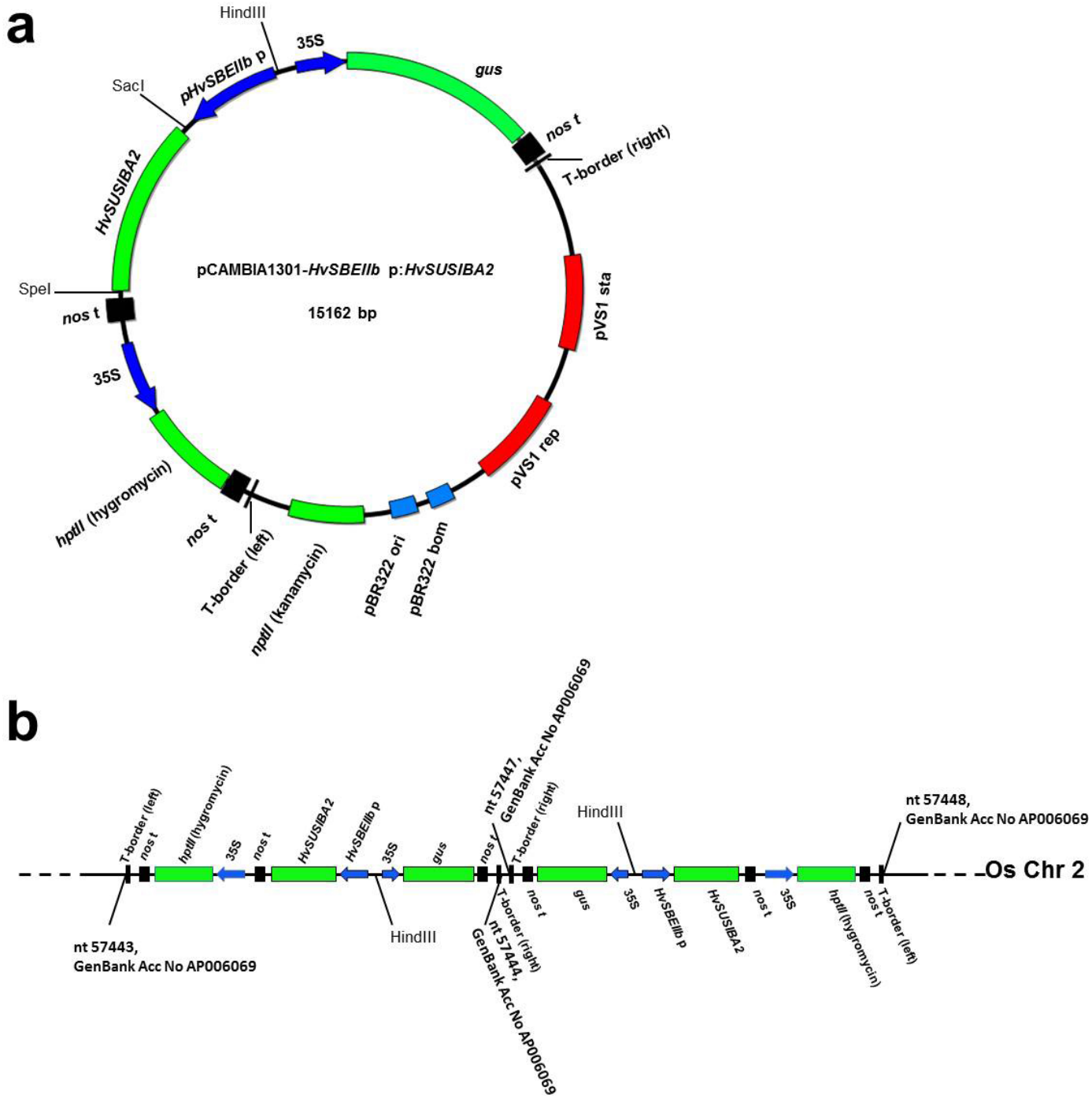
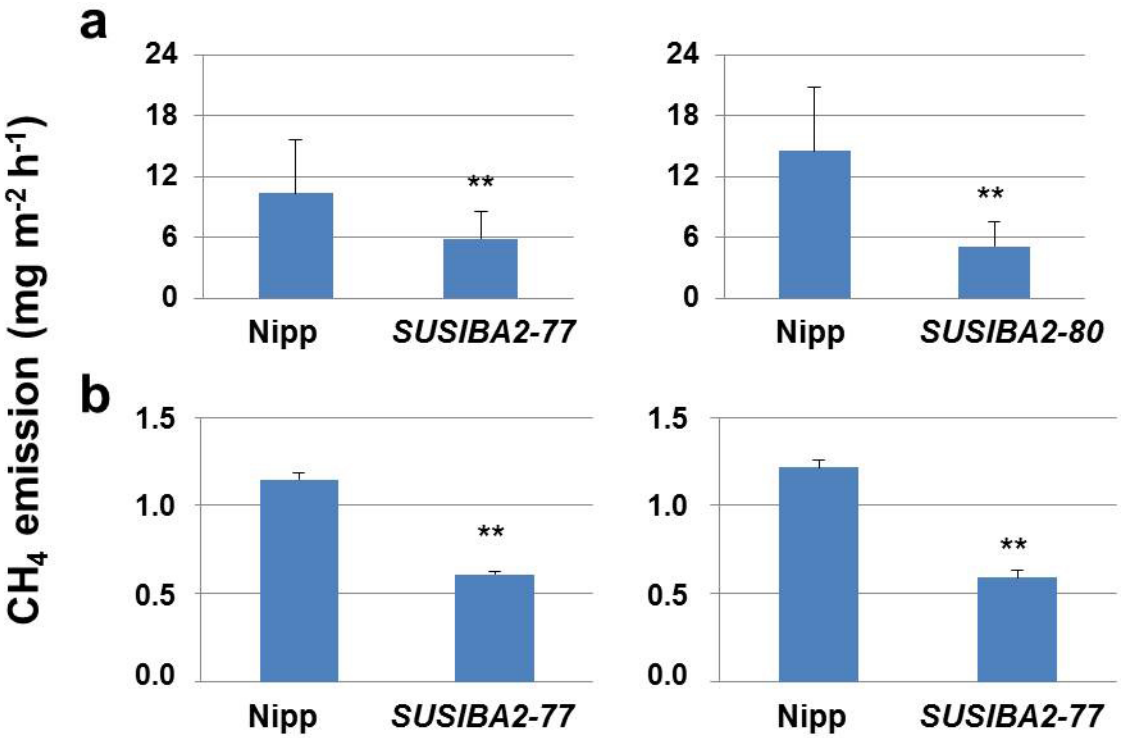
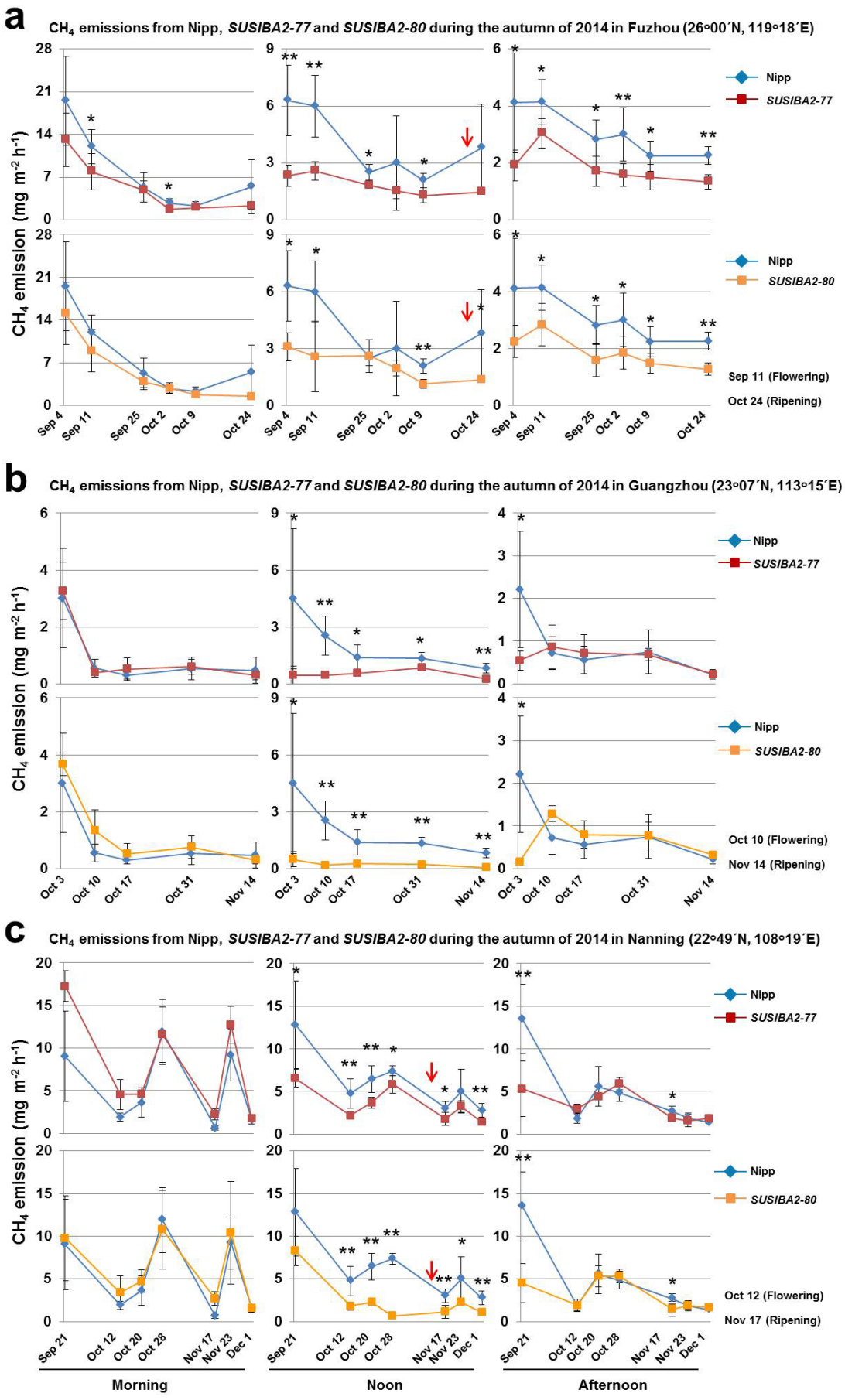


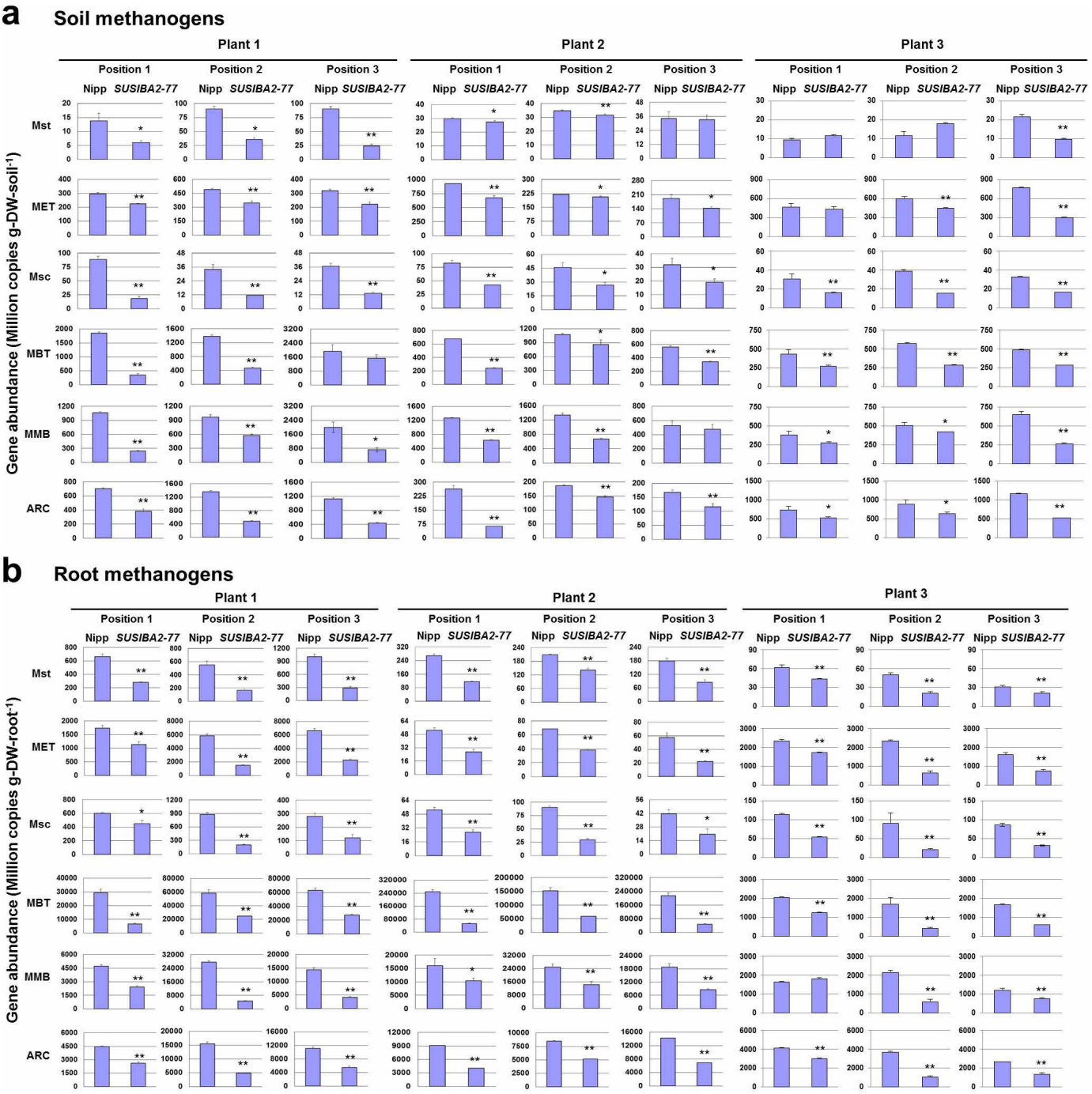
Extended Data Figure 1 | Validation of an expression cassette containing barley *SBEIIb* promoter and barley *SUSIBA2* (*HvSBEIIb* p:*HvSUSIBA2*) in a binary vector and rice genome. **a**, Construction of an expression cassette containing *HvSBEIIb* p:*HvSUSIBA2* in a binary vector was performed as described in the Methods. **b**, Validation of the construct in the rice genome was performed by PCR-based cloning using primers and HindIII-adaptor ligation, followed by sequencing. Two insertion sites were identified in rice chromosome 2, from nucleotides 57443 to 57444 and 57447 to 57448 (GenBank accession number AP006069), respectively.



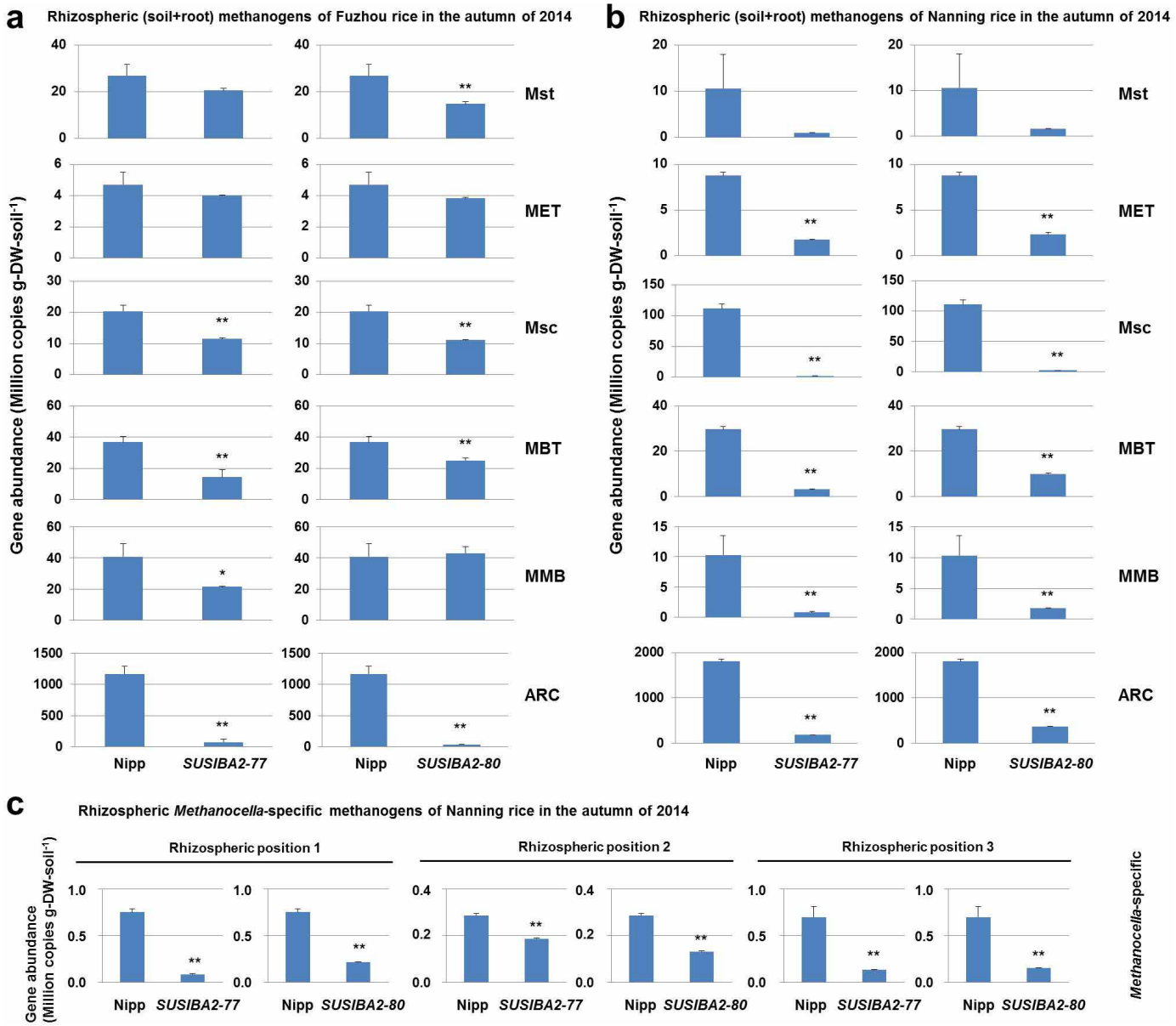
Extended Data Figure 2 | Methane emissions of *SUSIBA2* rice compared with Nipponbare (Nipp) in phytotrons. **a**, Methane emission of Nipp, *SUSIBA2-77* and *SUSIBA2-80* rice (15 daf). Six independent plants ($n = 6$) from each rice line were used for measurements. **b**, Methane emission of Nipp and *SUSIBA2-77* (28 daf) at 30 min (left panel) or 60 min (right panel) after plants were covered. A linear relationship over time for the measured methane concentrations at 30 and 60 min was found, as presented by similar methane fluxes determined from the different time points. Three plants ($n = 3$) from each rice line were used for time point measurements. A statistically significant reduction of methane emission in *SUSIBA2* rice is indicated (one-way ANOVA, $^{**}P \leq 0.01$ or $^{*}P \leq 0.05$, error bars show s.d.).



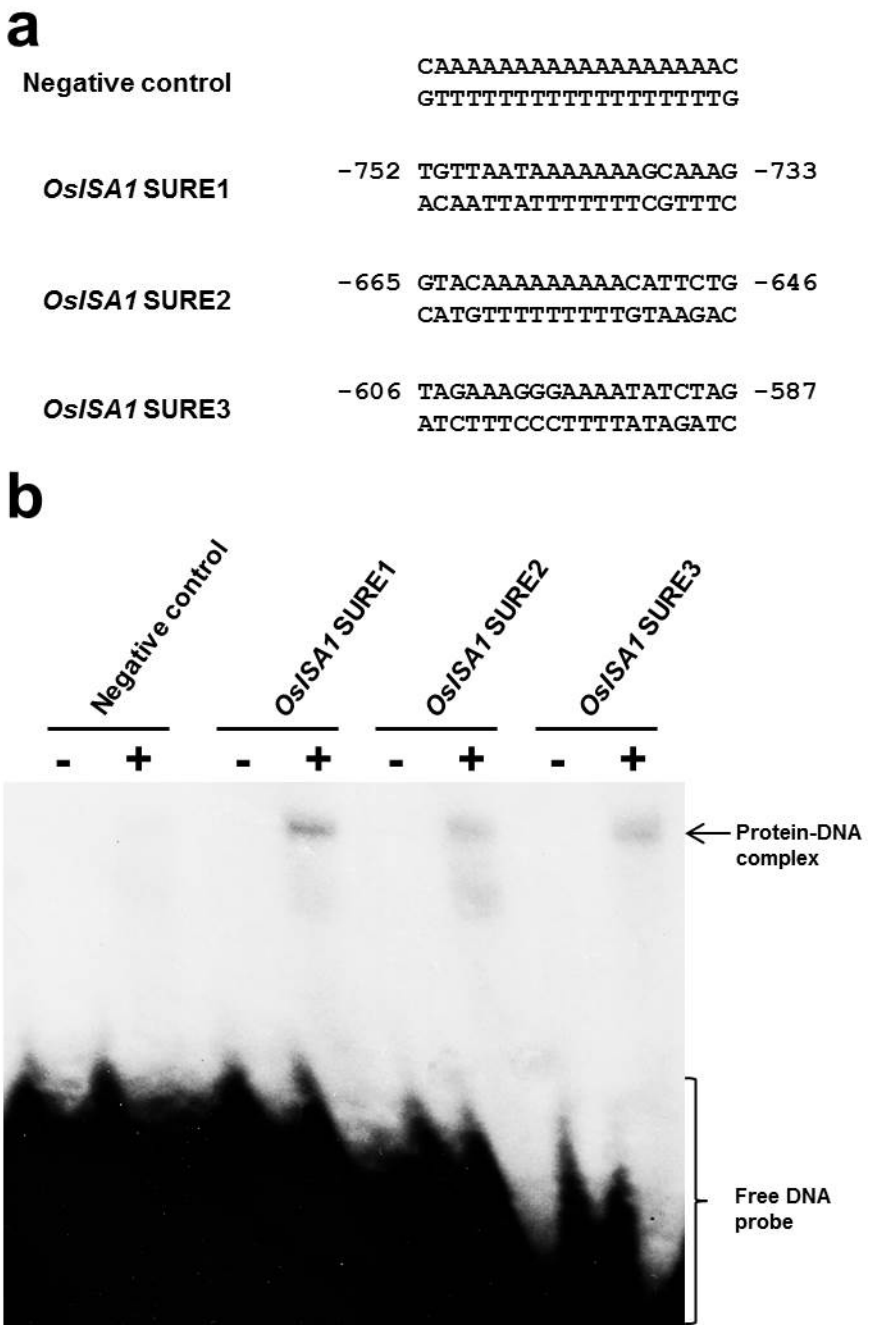
Extended Data Figure 3 | Diurnal and seasonal methane emissions from *SUSIBA2-77* and *SUSIBA-80* rice compared with Nipponbare (Nipp) in autumn 2014 at three sites in China. Methane emissions of six independent plants ($n = 6$) from three time points during the day (morning 8.00 a.m., noon 12.00 p.m. and afternoon 4.00 p.m.) on different dates are presented. Key rice development stages for the corresponding dates are indicated. Time points for sampling soil and roots for methanogen analysis are indicated by red arrows. **a**, Methane emission from Fuzhou. **b**, Methane emission from Guangzhou. **c**, Methane emission from Nanning. Reported statistically significant reduction of methane emission in *SUSIBA2* rice is indicated (one-way ANOVA, $^{**}P \leq 0.01$ or $^{*}P \leq 0.05$, error bars show s.d.).



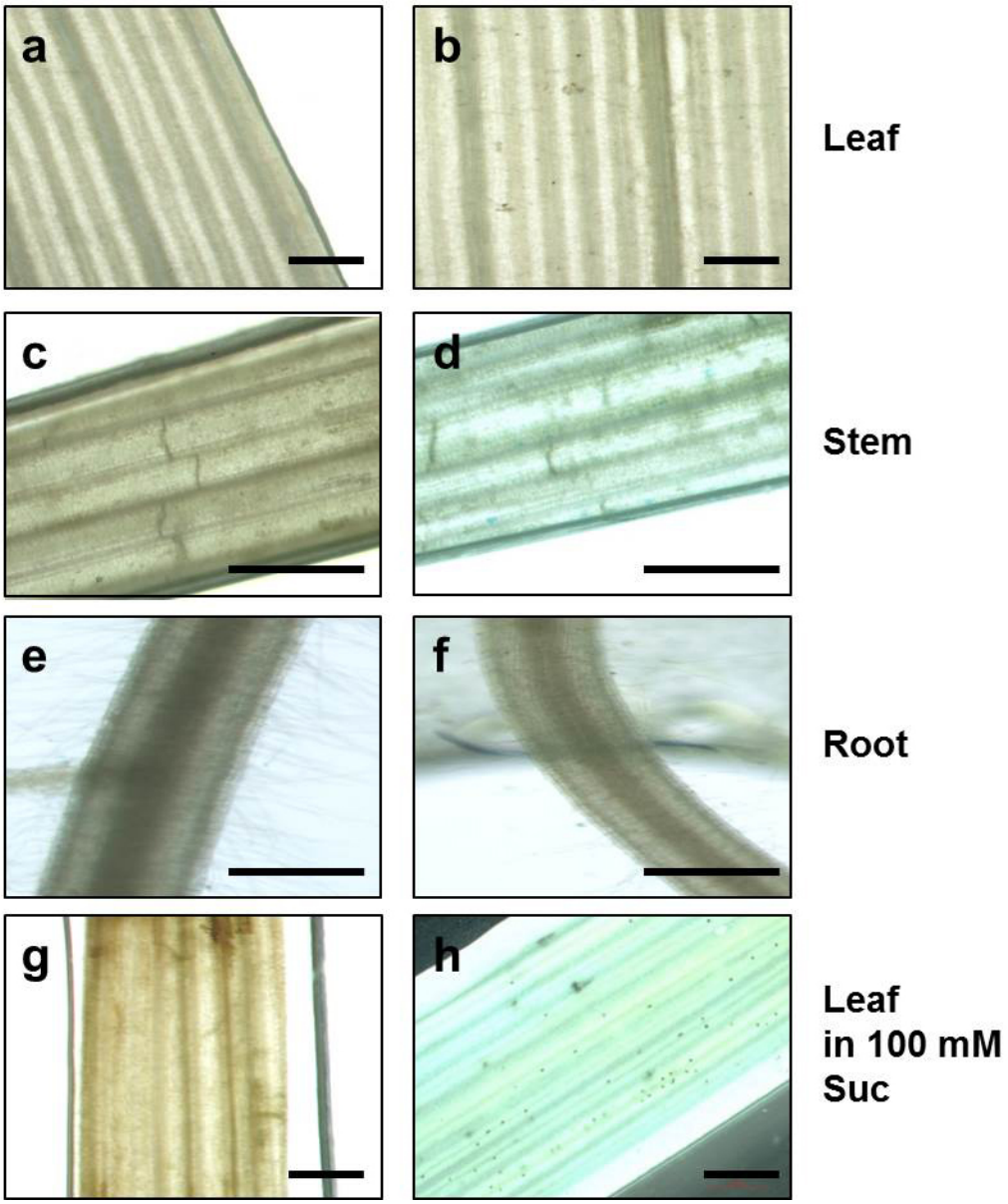
Extended Data Figure 4 | qPCR quantification of rhizospheric methanogenic communities associated with *SUSIBA2-77* rice and Nipp in phytotrons. Soil and root samples from three independent positions, positions 1–3 ($n = 3$), in the underground vicinity close to the root tip and proximal regions of three independent plants ($n = 3$) for Nipp and *SUSIBA2-77* rice were collected and analysed. Technical triplicates per position were applied. Six pairs of primers (Supplementary Table 1) were used to quantify total archaea (ARC) and methanogens (MET), and the orders Methanobacteriales (MBT), Methanomicrobiales (MMB) and Methanocellales and two families Methanosaetaceae (Mst) and Methanosarcinaceae (Msc) of the order Methanosarcinales, respectively. Results from soil and root samples are shown in **a** and **b**, respectively. Existing numbers of all methanogenic groups and total archaea were significantly reduced in the *SUSIBA2* rhizosphere compared to that of Nipp (one-way ANOVA, $^{**}P \leq 0.01$ or $^{*}P \leq 0.05$, error bars show s.d.).



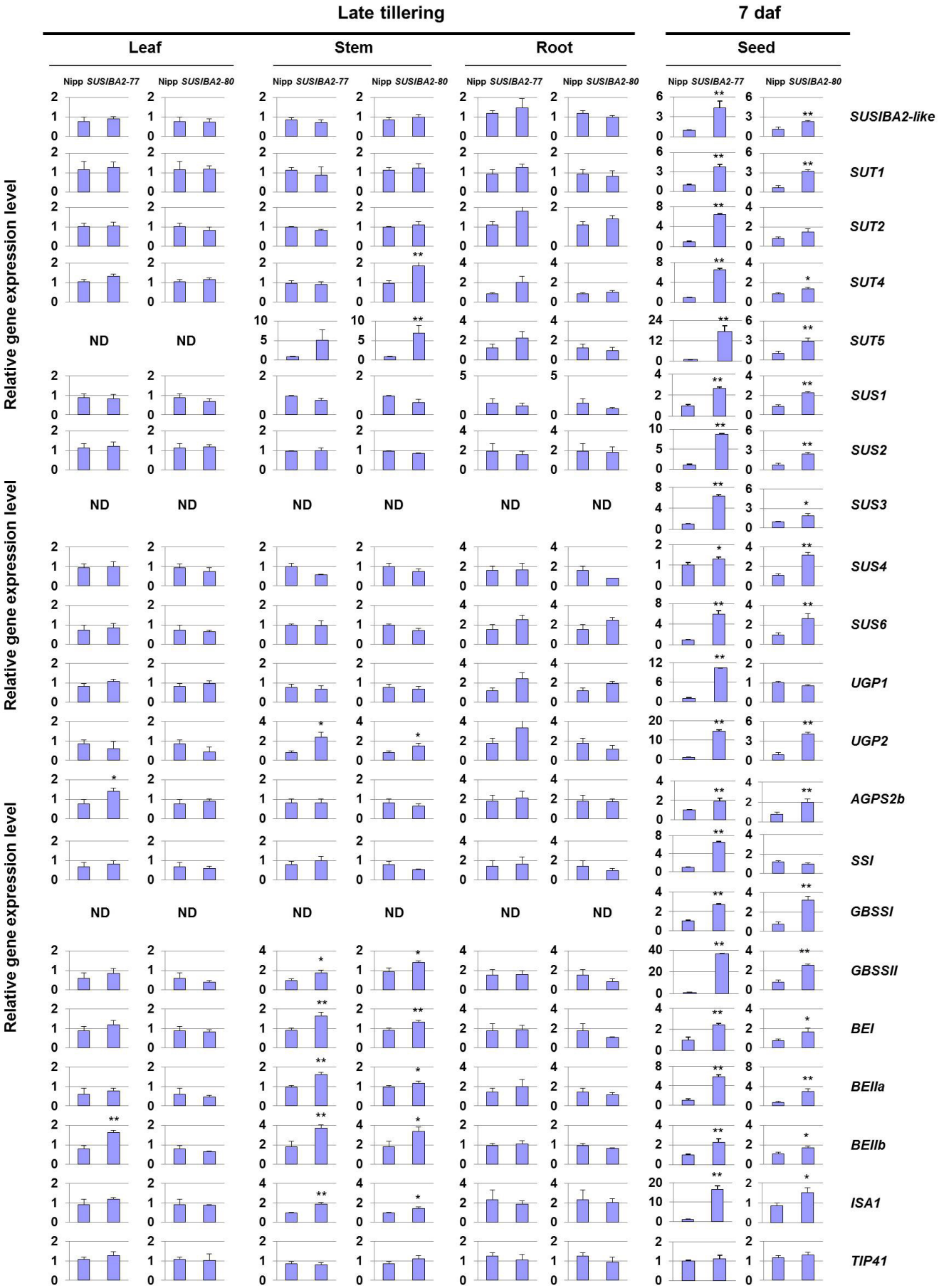
Extended Data Figure 5 | qPCR quantification of rhizospheric methanogens associated with *SUSIBA2* rice and Nipponbare (Nipp) from rice paddies. Soil and root samples (a mixture) from three positions ($n = 3$) close to the root tip and proximal regions of three independent plants ($n = 3$) for Nipp, *SUSIBA2-77* and *SUSIBA2-80* rice, respectively, were collected. The sampling time and sites are indicated in Extended Data Fig. 3. Technical triplicates per position were applied. Six pairs of primers (Supplementary Table 1) were used to quantify total archaea (ARC) and methanogens (MET), and the orders Methanobacteriales (MBT), Methanomicrobiales (MMB) and Methanocellales and two families Methanosaetaceae (Mst) and Methanosarcinaceae (Msc) of the order Methanosarcinales, respectively. Primers (Supplementary Table 1) specific to Methanocella were also used for quantification. **a**, Methanogenic communities in samples from Fuzhou. **b**, Methanogenic communities in samples from Nanning. **c**, *Methanocella* in samples from Nanning. All methanogenic groups and total archaea were significantly reduced in the *SUSIBA2* rice rhizosphere compared with Nipp (one-way ANOVA, $^{**}P \leq 0.01$ or $^{*}P \leq 0.05$, error bars show s.d.).



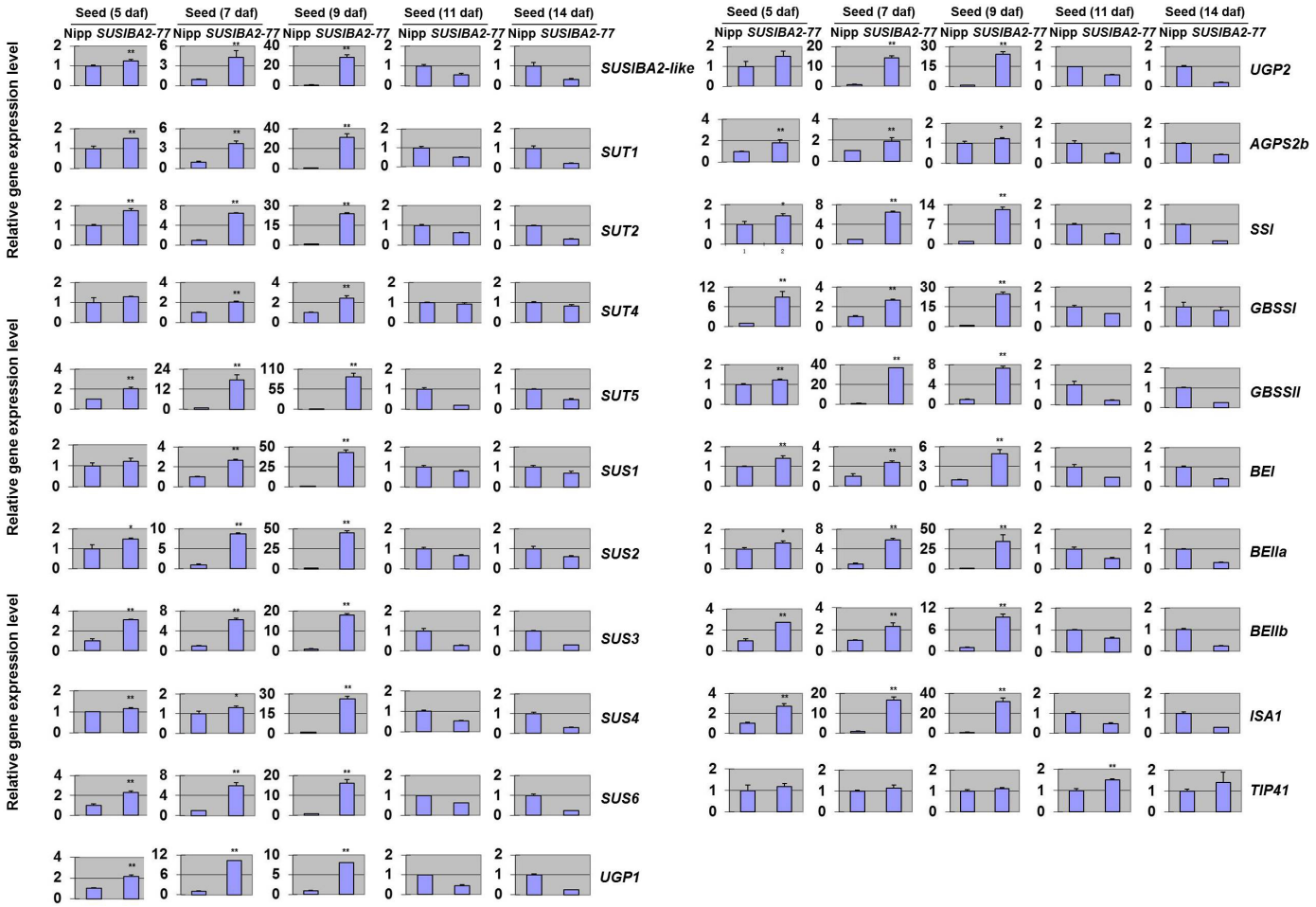
Extended Data Figure 6 | Binding activity of HvSUSIBA2 to SURE sequences in the rice *ISA1* promoter. **a**, Three SURE sequences in the rice *ISA1* promoter (GenBank accession number AB093426) were used and a negative ('A stretch') control was included. **b**, Barley SUSIBA2 protein (HvSUSIBA2) was overexpressed from *E. coli* and used for electrophoretic mobility shift assay (EMSA).



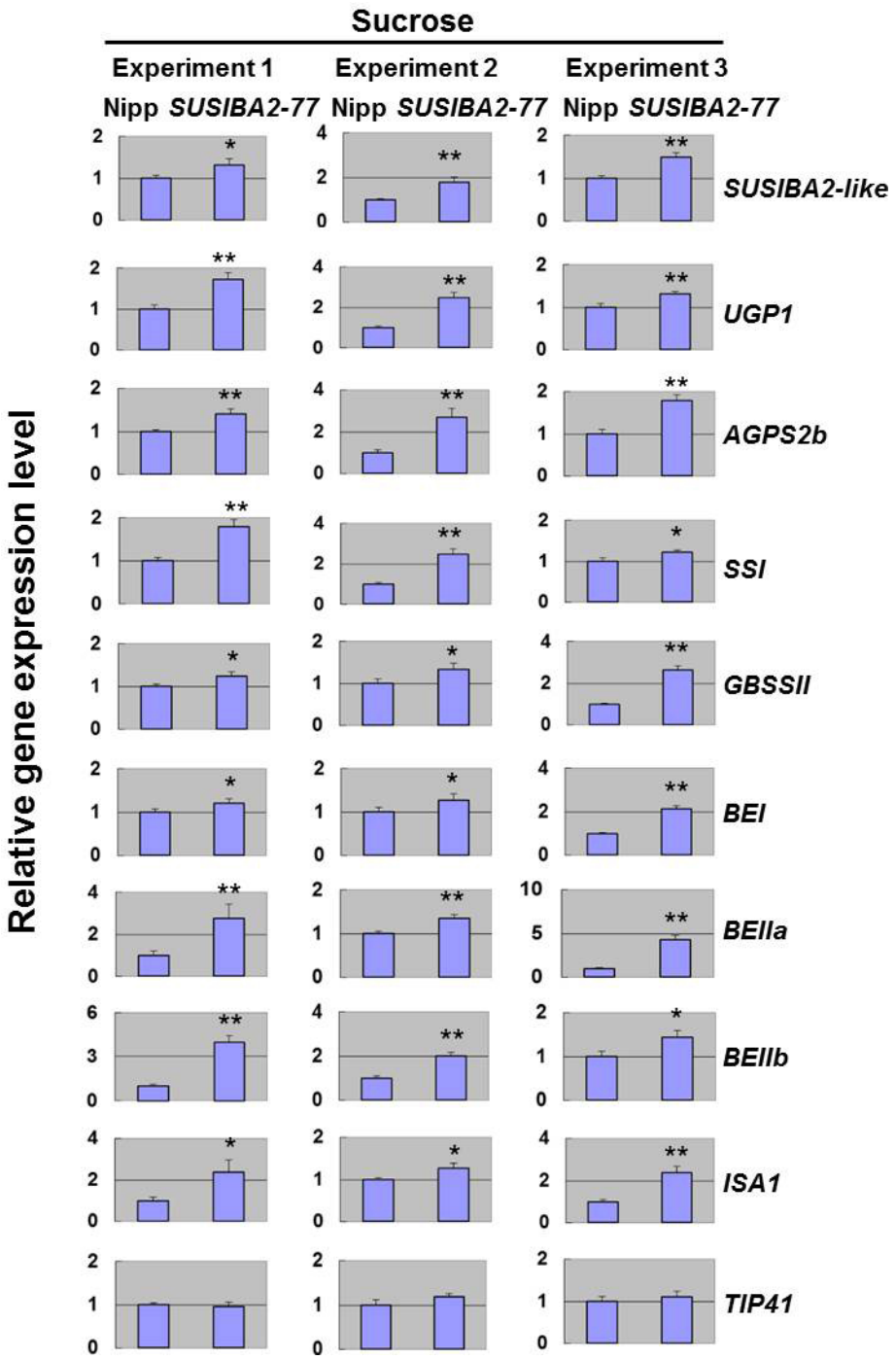
Extended Data Figure 7 | *HvSBEIIb* promoter activity analysis in rice seedlings. **a**, *HvSBEIIb* p:*GUS* was introduced in Nipponbare (Nipp). GUS activity was stained in different tissues of transformant and Nipp lines. The GUS activity was found in transformant stems and induced by sucrose (Suc) in leaves. **a**, Nipp leaf. **b**, Transformant leaf. **c**, Nipp stem. **d**, Transformant stem. **e**, Nipp root. **f**, Transformant root. **g**, Nipp leaf induced by 100 mM sucrose. **h**, Transformant leaf induced by 100 mM sucrose. Scale bars, 2 mm.



Extended Data Figure 8 | Transcriptomic analysis of genes related to sugar metabolism in late tillering plants of *SUSIBA2* rice and Nipponbare (Nipp). Relative expression levels of 23 genes together with a housekeeping gene, *TIP41-like,* were analysed by qPCR and compared between Nipp and *SUSIBA2-77* and *SUSIBA2-80* rice in leaves, stems and roots at late tillering stage and in seeds at 7 daf. Three plants were used ($n = 3$) and technical triplicates were performed for each rice line. *SUT3* and *SUS5/7* transcripts were not detected in all samples (not shown) and *HvSUSIBA2* expression was similar in *SUSIBA2-77* and *SUSIBA2-80* rice, as presented in Fig. 3b. One-way ANOVA was used for statistical analysis (*$P \leq 0.05$ or **$P \leq 0.01$, error bars show s.d.). ND, not detected.



Extended Data Figure 9 | Transcriptomic analysis of 23 genes related to sugar metabolism in developing rice seeds of *SUSIBA2* rice and Nipponbare (Nipp). Relative expression levels of 23 genes together with a housekeeping gene, *TIP41-like*, were analysed by qPCR and compared between *SUSIBA2-77* rice and Nipp at 5, 7, 9, 11 and 14 daf. Seeds from three independent plants were used ($n = 3$) and technical triplicates were performed. *SUT3* and *SUS5/7* transcripts were not detected in all samples (not shown) and *HvSUSIBA2* expression is presented in Fig. 3b. One-way ANOVA was used for statistical analysis ($^{*}P \leq 0.05$ or $^{**}P \leq 0.01$, error bars show s.d.).



Extended Data Figure 10 | Sugar-induction in Nipponbare (Nipp) and *SUSIBA2* rice. Rice leaves of Nipp and *SUSIBA2-77* were depleted for sucrose in the dark before induction with 100 mM sucrose. Sugar induction was carried out in the dark for 24 h. An enhanced expression of HvSUSIBA2-regulated genes (9 of the 12 genes except *SUT5*, *SUS1* and *GBSSI* that were not detected in either rice cultivar under dark conditions) in *SUSIBA2-77* was observed compared with Nipp. One-way ANOVA was used for statistical analysis (*$P \leq 0.05$ or **$P \leq 0.01$, error bars show s.d.). The experiment was repeated at least three times ($n = 3$).