LETTERS

A silicon transporter in rice

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Silicon is beneficial to plant growth and helps plants to overcome abiotic and biotic stresses by preventing lodging (falling over) and increasing resistance to pests and diseases, as well as other stresses[1–3]. Silicon is essential for high and sustainable production of rice[4], but the molecular mechanism responsible for the uptake of silicon is unknown. Here we describe the Low silicon rice 1 (*Lsi1*) gene, which controls silicon accumulation in rice, a typical silicon-accumulating plant. This gene belongs to the aquaporin family[5] and is constitutively expressed in the roots. Lsi1 is localized on the plasma membrane of the distal side of both exodermis and endodermis cells, where casparian strips are located. Suppression of *Lsi1* expression resulted in reduced silicon uptake. Furthermore, expression of *Lsi1* in *Xenopus* oocytes showed transport activity for silicon only. The identification of a silicon transporter provides both an insight into the silicon uptake system in plants, and a new strategy for producing crops with high resistance to multiple stresses by genetic modification of the root's silicon uptake capacity.

Silicon is the second most abundant element in the Earth's crust and soil and is contained in significant amounts in all plants. However, plant species differ greatly in silicon accumulation, ranging from 0.1% to 10% in top dry weight[1,6]; this difference is attributed to the difference in the ability of roots to take up silicon[7]. Rice can accumulate silicon to the level of up to 10% of shoot dry weight, which is often several times higher than that of essential macronutrients such as nitrogen, phosphate and potassium[4]. Silicon is taken up by roots in the form of silicic acid, an undissociated molecule[8,9]. Physiological studies have shown that silicon uptake by rice roots is mediated by a type of transporter[10]. After it is taken up, silicon is translocated to the shoot in the form of monomeric silicic acid[11,12] and is finally deposited on cell wall material as a polymer of hydrated, amorphous silica, forming silica–cuticle double layers and silica–cellulose double layers on the surface of leaves, stem and hulls[13]. Silicon enhances resistance of plants to diseases, pests and lodging through deposition in the apoplast and induced resistance, improves the light-interception ability by plants in a community, and minimizes transpiration losses[1,6,14]. Therefore, plants need to accumulate large amounts of silicon.

lsi1 (low silicon rice 1) is a rice mutant defective in silicon uptake[15]. This mutant accumulates less silicon in the shoot throughout its growth period compared with the wild type (Fig. 1a) and is susceptible to pests and diseases (Fig. 1b, c). The mutant has a grain yield one-tenth of that of wild-type rice (Fig. 1d). We roughly mapped the gene (*Lsi1*) controlling silicon uptake to chromosome 2 (ref. 16). For fine mapping of *Lsi1*, we used about 1,000 homozygotes with low silicon uptake, which were selected from F_2 plants derived from a cross between *lsi1* and an indica cultivar Kasalath. We developed new markers and mapped the candidate region of *Lsi1* to 88 kilobases (kb) between the markers lsi1-4 and E60168 and to 13.9 kb between the markers lsi1-a and lsi1-6 (Fig. 2a, b). Using gene prediction software we predicted a gene in the candidate region (Fig. 2b), sequenced it and made comparisons between the wild type and *lsi1* mutant. We found a mutation in the DNA sequence of the candidate gene (G in the wild type; A in the mutant) that results in an amino acid change from alanine in the wild type to threonine in the mutant at position 132 (Fig. 2d). Thus, we considered this candidate gene to be *Lsi1*. The gene consists of five exons and four introns (Fig. 2c). The complementary DNA of this gene is 1,409-base-pairs (bp) long and the deduced protein comprises 298 amino acids (Fig. 2d).

The gene is predicted to encode a membrane protein similar to water channel proteins (aquaporins)[5]. The predicted amino acid sequence has six transmembrane domains and two Asn-Pro-Ala (NPA) motifs, which is well conserved in typical aquaporins (Fig. 2d). BLAST search and ClustalW analysis revealed that Lsi1 belongs to a Nod26-like major intrinsic protein (NIP) subfamily (Fig. 2f). We found three close homologues in maize (ZmNIP2-1,

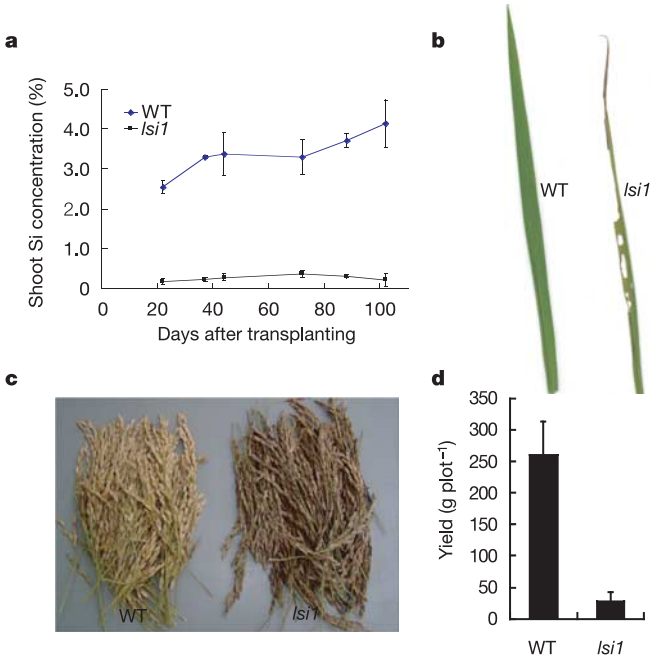

Figure 1 | Phenotype of the *lsi1* mutant. **a**, Silicon concentration of shoots at each growth stage in wild-type rice (WT; cv. Oochikara) and an *lsi1* mutant grown in a field. **b**, A mature leaf showing pest damage in the mutant due to low silicon. **c**, Panicles at harvest showing that panicles of the *lsi1* mutant are infected by diseases, resulting in discoloration. **d**, Rice grain yield per plot (70 cm × 70 cm). Data are means ± s.d. ($n = 3$).

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ZmNIP2-2 and ZmNIP2-3) sharing 77–83% sequence identity[17], and one homologue in rice (Os06g12310, named Lsi6) with 77% identity (Fig. 2f). However, the transport selectivity and physiological roles of ZmNIP2—as with all members of the subgroup—are unknown. We show here that one of the NIPs, Lsi1, is a transporter for silicic acid in rice roots (Supplementary Fig. 1). Maize is also able to accumulate silicon, suggesting that ZmNIP2-1, ZmNIP2-2 and ZmNIP2-3 might be involved in silicon uptake. Alanine at position 132 seems to be a critical residue, because substitution of this amino acid in the mutant significantly alters the conformation according to the modelling of the native and mutant proteins. Thus, substitution of Ala for Thr at position 132 (Ala132Thr) provoked severe steric interactions with Val 55 and Val 59 in helix 1 (H1), facilitating a movement of H1. This unfavourable interaction would affect the conformation of Asn 108, the pore-forming residue in the P-loop (Fig. 2e).

We found that *Lsi1* was mainly expressed in the roots (Fig. 3a). This expression was constitutive, but regulated by silicon level; the expression was decreased by one-quarter by continuous silicon supply for 3 days (Fig. 3b). Results of *in situ* hybridization showed that *Lsi1* messenger RNA was localized at the exodermis and endodermis (Supplementary Fig. 2). We investigated the subcellular localization of Lsi1 by delivering a translational fusion between Lsi1 and green fluorescent protein (GFP) into onion epidermal cells by particle bombardment. Cells expressing the Lsi1–GFP fusion showed a GFP signal only at the plasma membrane (Supplementary Fig. 3), whereas the signal for cells expressing GFP alone was found in the nucleus and cytosol.

To examine further the localization of Lsi1 in rice roots, we generated transgenic rice plants carrying the open reading frame for *Lsi1* fused with GFP under the control of the *Lsi1* promoter region (2 kb). The GFP fluorescence signal was observed in the main and lateral roots, but not in root hairs (Fig. 3c, d). This is consistent with

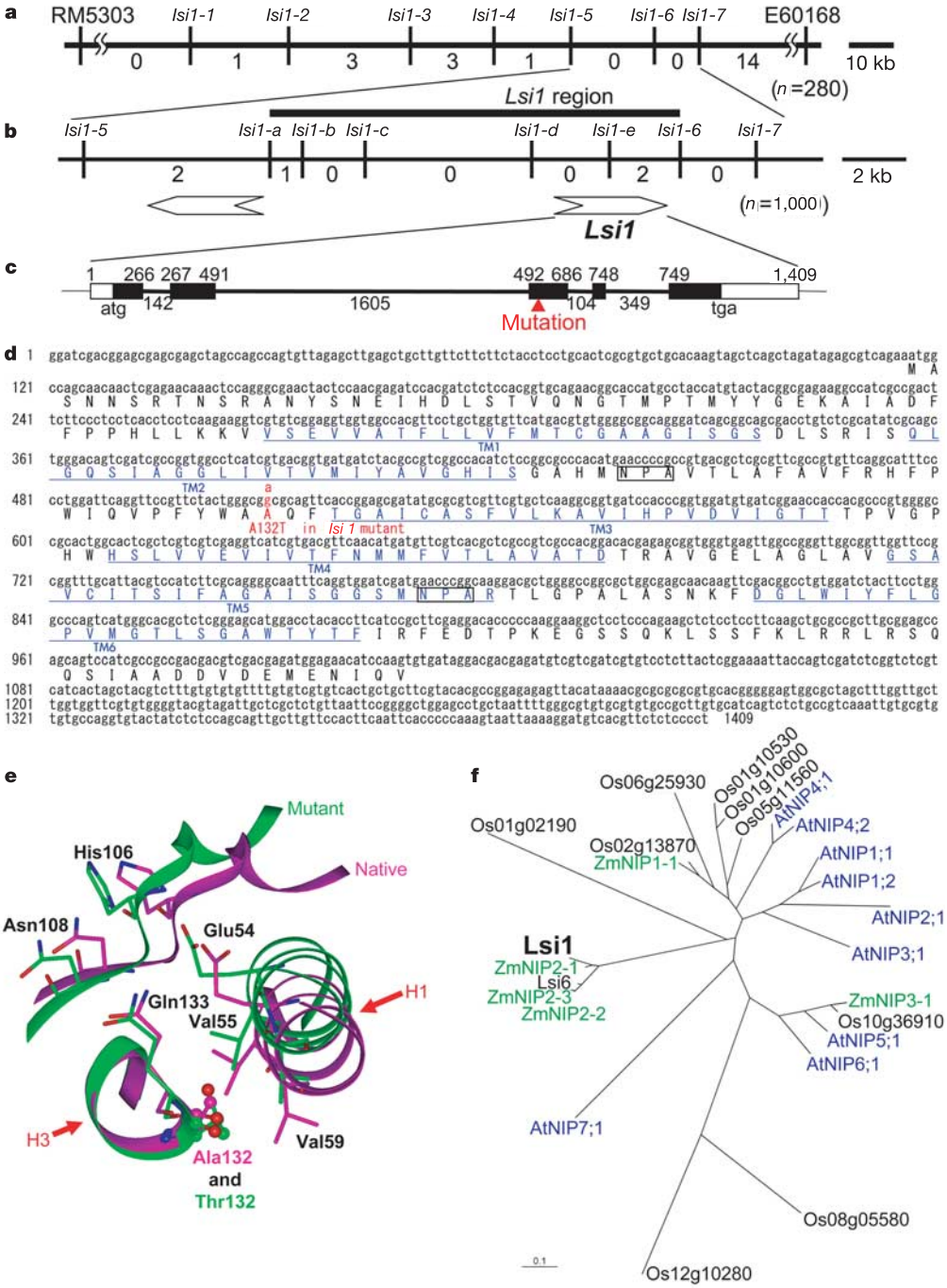

Figure 2 | Mapping of *Lsi1* and gene structure. **a**, **b**, The *lsi1* mutation was mapped on the long arm of chromosome 2 between markers RM5303 and E60168. The number of recombinants between the molecular markers is indicated below the high-resolution map. **c**, *Lsi1* gene structure at genomic sequence. Five exons are boxed; a black box shows the open reading frame. **d**, *Lsi1* cDNA and predicted amino acid sequence based on full-length cDNA clone (accession number AK069842). Red letters show position of *lsi1* mutation. Predicted transmembrane domains (TM) are underlined and the conserved NPA motif is boxed. **e**, Model of the Lsi1 protein from the wild-type and mutant rice. **f**, Phylogenetic relationship of Lsi1 proteins in rice (black), *Arabidopsis* (blue) and maize (green).

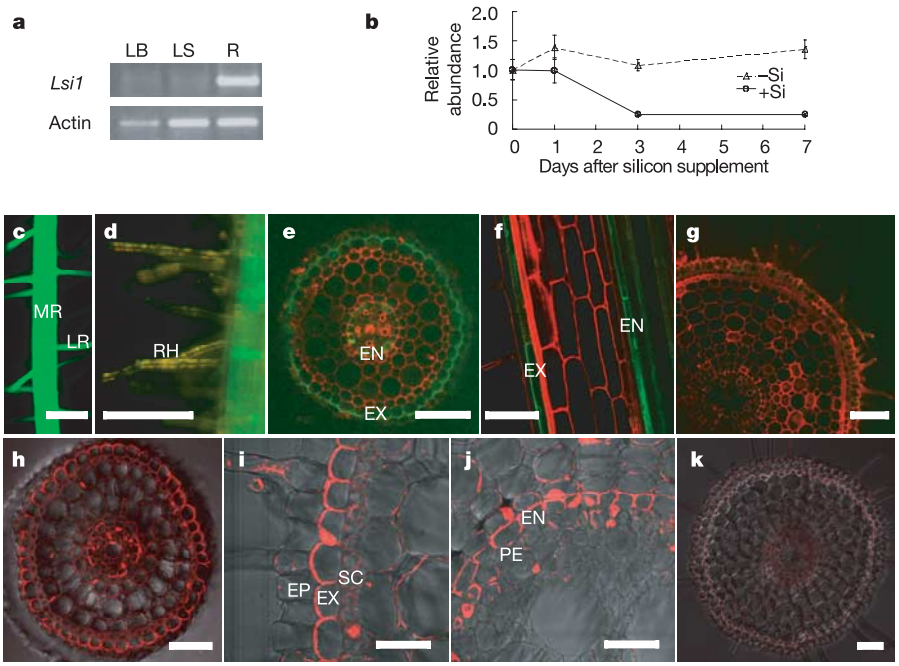

Figure 3 | Expression and localization of Lsi1. **a**, Expression analysis of *Lsi1* and Actin (internal standard) in the leaf blade (LB), leaf sheath (LS) and root (R). **b**, Time-dependent expression of *Lsi1*. Data are means ± s.d. ($n = 3$). **c–f**, Fluorescence of the Lsi1–GFP fusion protein in transgenic plants. The main root (MR), lateral root (LR), root hair (RH), endodermis (EN) and exodermis (EX) are shown. **c**, **d**, Stereoscopic microscope images. Scale bars, 500 μm. **e**, **f**, Cross-section (**e**) and longitudinal section (**f**) observed by laser scanning confocal microscopy, counterstained with propidium iodide. **g**, Control (wild-type root). Scale bar, 50 μm. **h–j**, Lsi1 immunolocalization stained with anti-Lsi1 polyclonal antibody. Epidermis (EP), exodermis, sclerenchyma (SC), endodermis and pericycle (PE) are shown. **k**, Control (non-immunized serum). Scale bars, 50 μm (**h**, **k**) and 20 μm (**i**, **j**).

the results of a previous physiological study that root hairs do not have any demonstrable role in silicon uptake, but that lateral roots contribute significantly to silicon uptake[18]. Within a root, we found that the GFP fluorescence signal was observed on the plasma membrane of both exodermis and endodermis (Fig. 3e, f), where casparian strips exist (Supplementary Fig. 4). To confirm this subcellular localization of Lsi1, we stained the roots with an anti-Lsi1 polyclonal antibody. Lsi1 was localized on the plasma membrane of the distal side of both exodermis and endodermis cells, which is similar to the localization seen for the transgenic plants carrying the GFP fusion (Fig. 3h–j). Because solutes are unable to pass casparian strips freely[19], transporters are needed to reach the stele for translocation from the roots to the shoot. Localization of Lsi1 at the plasma membrane of the distal side of both exodermis and endodermis cells indicates that Lsi1 is a transporter responsible for silicon uptake, and therefore for silicon accumulation in rice.

Because plants were not generated from calluses of the cultivar (Oochikara) used, probably due to the specificity of this cultivar, we could not produce transgenic plants by introducing *Lsi1* into the silicon-uptake-defective (*lsi1*) mutant. Therefore, we used RNA interference (RNAi) to suppress the expression of *Lsi1* in the Nipponbare cultivar, which has the same gene and same silicon uptake capacity as the Oochikara cultivar. In RNAi transgenic lines, silicon uptake was significantly reduced compared with vector control plants; expression of *Lsi1* in RNAi transgenic lines was significantly suppressed (Fig. 4a, b). The RNAi transgenic lines also showed higher resistance to Ge toxicity (Supplementary Fig. 5); however, there was no difference in water uptake between RNAi lines and vector control lines (data not shown).

We also investigated the silicon transport activity of Lsi1 by injecting cRNA encoding Lsi1 or injecting water into *Xenopus laevis* oocytes. We measured the silicon or glycerol inside the oocytes after incubation in a solution containing silicic acid or glycerol for 30 min. Oocytes expressing Lsi1 had a silicon uptake rate 2.4 times greater than the control (water-injected) oocytes (Fig. 4c). However, the difference in the transport activity for glycerol was very small between control oocytes and those injected with cRNA encoding Lsi1. Furthermore, the transport activity of silicon was unaffected by the presence of equimolar amounts of glycerol (data not shown), suggesting that Lsi1 is specific for silicon transport. Kinetic analysis showed that silicon transport activity increased with increasing silicon concentrations in external solution (Fig. 4d).

Our results support the concept that Lsi1 is a transporter for silicon in rice roots. Silicon is ubiquitous in the environment, and all living organisms take up silicon. However, the genes responsible for silicon uptake have not been identified so far in higher plants. A gene family encoding silicon transporters has been identified in the marine diatom *Cylindrotheca fusiformis*, which requires silicon as an essential element[20,21]; however, this gene has no similarity to *Lsi1*. Furthermore, silicon uptake was not increased by introducing one of the diatom silicon transporter genes into tobacco[22], indicating that the silicon uptake system in higher plants is different compared with that in diatoms. To our knowledge, this is the first report of a silicon transporter in higher plants, and it provides a molecular basis for their silicon transport system. Many plants, especially dicotyledonous species, are unable to accumulate silicon in sufficient amounts to be beneficial. Identification of *Lsi1* may provide a new strategy for producing plants with resistance to multiple stresses by genetic

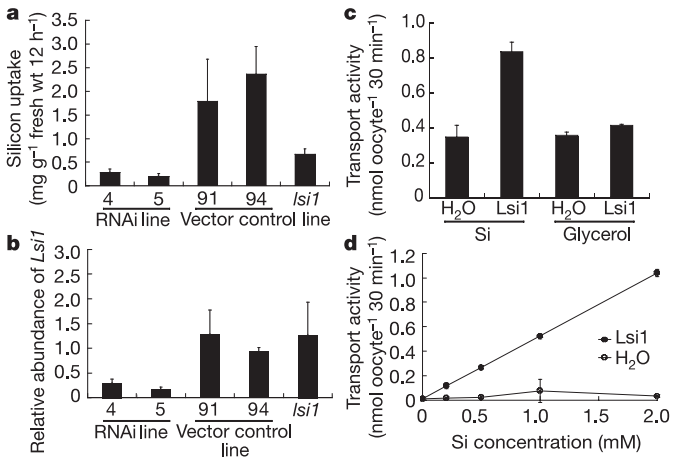

Figure 4 | Transport activity of Lsi1. **a**, Effect of *Lsi1* suppression by RNAi silencing on silicon uptake. **b**, Relative root transcript level of *Lsi1*. Actin was used as an internal control and relative value to vector control is shown. **c**, Uptake of silicon and glycerol by *Xenopus* oocytes that were injected with water or *Lsi1* cRNA. **d**, Kinetics of silicon uptake by *Xenopus* oocytes injected with water or *Lsi1* cRNA. Oocytes were exposed to a solution containing different silicon concentrations labelled with ^{68}Ge for 30 min. Data are means ± s.d. ($n = 3$).

modification of the root's silicon uptake capacity. As silicon has been implicated in optimal bone and connective tissue development in the human body[23], enhanced silicon uptake in plants may also result in increased silicon accumulation in food, thereby improving silicon nutrition in humans.

METHODS

Plant growth. We grew wild-type (cv. Oochikara) and mutant (*lsi1*) rice in a field from May to September in 2003. We obtained shoot samples at various growth stages and determined the silicon accumulation as described previously[24]. Grain yield per plot (70 cm × 70 cm) was investigated at harvest.

Map-based cloning of *Lsi1*. We evaluated about 4,000 F_2 plants derived from a cross between *lsi1* and the Kasalath cultivar for silicon uptake, and selected about 1,000 homozygote plants showing low silicon uptake for high-resolution mapping. Linkage analysis using both SSR and PCR markers revealed that *Lsi1* was restricted to a 13.9-kb genomic region on the bacterial artificial chromosome (BAC) clone OJ1118_G04. Only one gene was predicted in this region by rice GAAS, and the sequence of this gene was determined for both the wild type and the mutant.

Immunohistological fluorescence staining. Rice roots were fixed in 4% (w/v) paraformaldehyde and 60 mM sucrose and then embedded in 5% agar. Sections sliced to 50-μm thickness were incubated in PBS containing 0.1% (w/v) pectolyase Y-23 and then in PBS containing 0.3% (v/v) Triton X-100. The nonspecific reaction was blocked with 5% (w/v) BSA in PBS. Then we incubated the slides with purified rabbit anti-Lsi1 polyclonal antibodies and subsequently with secondary antibodies (Alexa Fluor 555 goat anti-rabbit IgG; Molecular Probes). We observed the sections with a laser scanning confocal microscope (LSM510; Zeiss).

Generation of transgenic rice. To investigate the cellular and subcellular localization of Lsi1, we introduced a construct consisting of the promoter (2 kb) and *Lsi1* cDNA fused with *GFP* to calluses (cv. Nipponbare) using an *Agrobacterium*-mediated transformation system[25]. We selected transformed calluses by hygromycin resistance, and from them regenerated plants. We examined fluorescence in the transgenic rice roots by laser-scanning confocal microscopy after counterstaining with propidium iodide.

To generate the hairpin RNAi construct, we cloned two copies of a 299-bp fragment (15–313 bases from transcriptional start) of *Lsi1* cDNA at inverted repeats into the pHELLSGATE vector under control of a 35S promoter[26] and subsequently introduced it to calluses (cv. Nipponbare) as described above. We measured the silicon uptake in two independent RNAi transgenic lines as well as in two independent vector control lines and the mutant *lsi1* as described[15], and examined the expression level of *Lsi1* with real-time PCR as described below.

Real-time PCR. We extracted total RNA from the leaf blade, leaf sheath and root supplied with or without silicon for 1, 3 and 7 days or from transgenic rice roots and then converted it to cDNA. The *Lsi1* and Actin (internal control) cDNAs were amplified using SYBR green I real-time PCR with pairs of primers: *Lsi1*, 5′-CGGTGGATGTGATCGGAACCA-3′ (forward) and 5′-CGTCGAACTT GTTGCTCGCCA-3′ (reverse); Actin, 5′-GACTCTGGTGATGGTGTCAGC-3′ (forward) and 5′-GGCTGGAAGAGGACCTCAGG-3′ (reverse).

Transport activity in oocytes. We performed *in vitro* transcription from pXßG-ev1 poly(A)$^+$ vector carrying *Lsi1* cDNA. We injected the capped cRNA (50 nl, 1 ng nl^{-1}) or distilled water into *Xenopus* oocytes selected according to size and developmental stage. After a 1-day incubation, the oocytes were exposed to a solution containing 2 mM silicon as silicic acid or 2 mM glycerol labelled with ^{14}C (40 MBq mmol^{-1}) for 30 min. The oocytes were then washed with a buffer without silicon and homogenized with 0.1 N HNO_3. We determined the concentration of silicon in the lysates as described previously[24] and that of glycerol by a liquid scintillation counter. The kinetics of silicon uptake was performed by exposing the oocytes to a solution containing different silicon concentrations ranging from 0.2 to 2.0 mM labelled with ^{68}Ge (10 MBq mmol^{-1}) following the method reported[27]. After 30 min, we washed the oocytes with the solution without silicon five times and then measured the radioactivity by a liquid scintillation counter 24 h later.

Modelling of the Lsi1 structure. We used the crystal structure of the monomeric structure of aquaporin (Protein Data Bank 1J4N) to build the model of the monomer of Lsi1. Sequence alignment and the initial homology modelling were performed with the homology module installed in Insight II (version 2000, Molecular Simulations Inc.). $Si(OH)_4$ was roughly docked into the substrate-filtering site. We minimized the initial complex model and optimized the whole structure of the complex model by the molecular dynamics/minimization procedure without any structural constraints. We selected the lowest-energy structure as an energy-refined complex model. We generated the Ala132Thr mutant by replacing Ala with Thr and optimized the mutant structure.

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Author Information The nucleotide sequence data reported in this paper has been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases under accession number AB222272. Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to J.F.M. (maj@rib.okayama-u.ac.jp).