Supporting Information

Sugimoto et al. 10.1073/pnas.0911965107

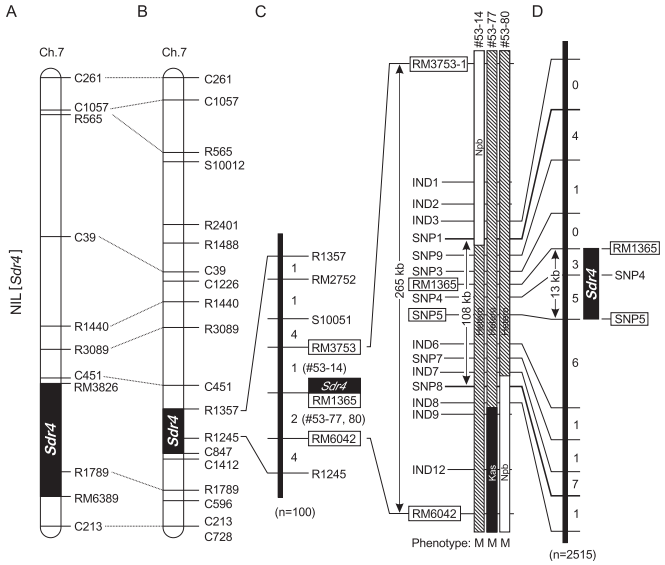

Fig. S1. Coarse- and high-resolution mapping of *Sdr4*. (*A*) Graphical genotype of chromosome 7 in NIL[*Sdr4*]. The introgressed segment from Kasalath is indicated by a black bar. The positions of RFLP markers and SSR markers are based on physical distance. (*B*) Chromosomal locations of *Sdr4*, based on genetic distance. (*C*) Coarse mapping of *Sdr4* using a population of $n = 100$. Numbers of recombinants between each set of markers are indicated. Phenotype "M" indicates mildly resistant to preharvest sprouting. By using this population, the *Sdr4* candidate region was delimited to 108 kbp. (*D*) High-resolution mapping using a population of $n = 2,515$. Numbers of recombinations between pairs of markers are shown. Black regions are chromosomal segments homozygous for the Kasalath allele; white regions are homozygous for the Nipponbare allele; hatched regions are heterozygous. Primer sequences of newly designed markers are listed in Table S2*A*.

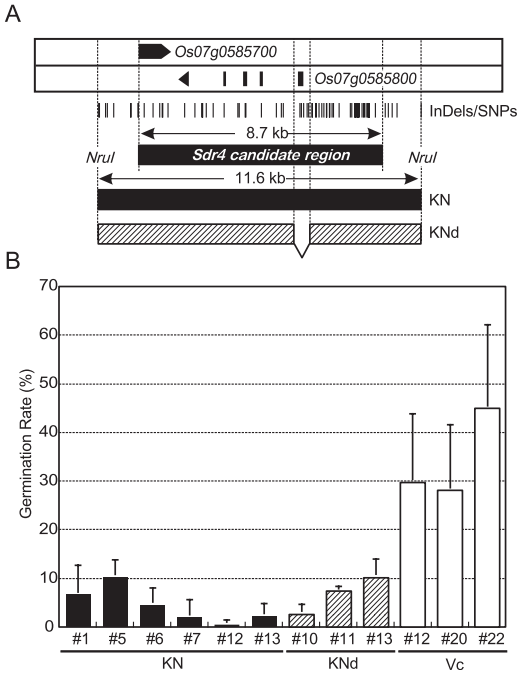

Fig. S2. Genetic complementation of *Sdr4* using the genomic fragment encompassing the entire candidate genomic region and its derivative. (*A*) Candidate genomic region of *Sdr4* and predicted genes. Genes to the right of the black arrow are those predicted by RAP-DB in the Nipponbare genomic sequence around the *Sdr4* candidate region. The 8.7-kb *Sdr4* candidate region was defined on the basis of high-resolution mapping. Vertical bars indicate InDels and SNPs located in the 11.6-kb region from *NruI* to *NruI*. The 11.6-kb fragment (KN) and 11.2-kb fragment with a 0.4-kb deletion around the first exon of the *Os07g0585800* (KNd) were used for complementation analysis. (*B*) Germination rates of T_3 seeds harboring the KN fragment, the KNd fragment, and empty vector (Vc). The presence of the KN fragment reduced germination rates, indicating that *Sdr4* was present in this fragment. The KNd fragment complemented the function of *Sdr4*, demonstrating that *Os07g0585800* was not the *Sdr4*.

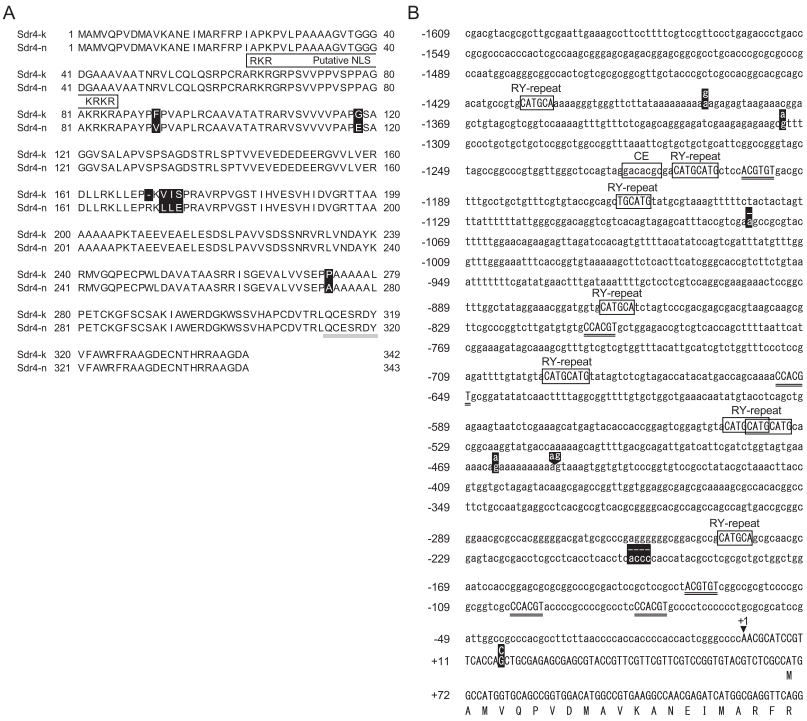

Fig. S3. Deduced amino acid and promoter sequence comparison between *Sdr4-n* and *Sdr4-k*. (*A*) Amino acid sequences of predicted ORFs in *Sdr4-k* and *Sdr4-n* were compared. Putative NLS and deletion in *sdr4* mutant (gray line) are shown. (*B*) Promoter sequences of *Sdr4-k* from −1,609 to +132. SNPs and InDels are indicated by black boxes, and RY repeats and ABRE-CEs are indicated by clear boxes. ABREs are indicated by double underlines.

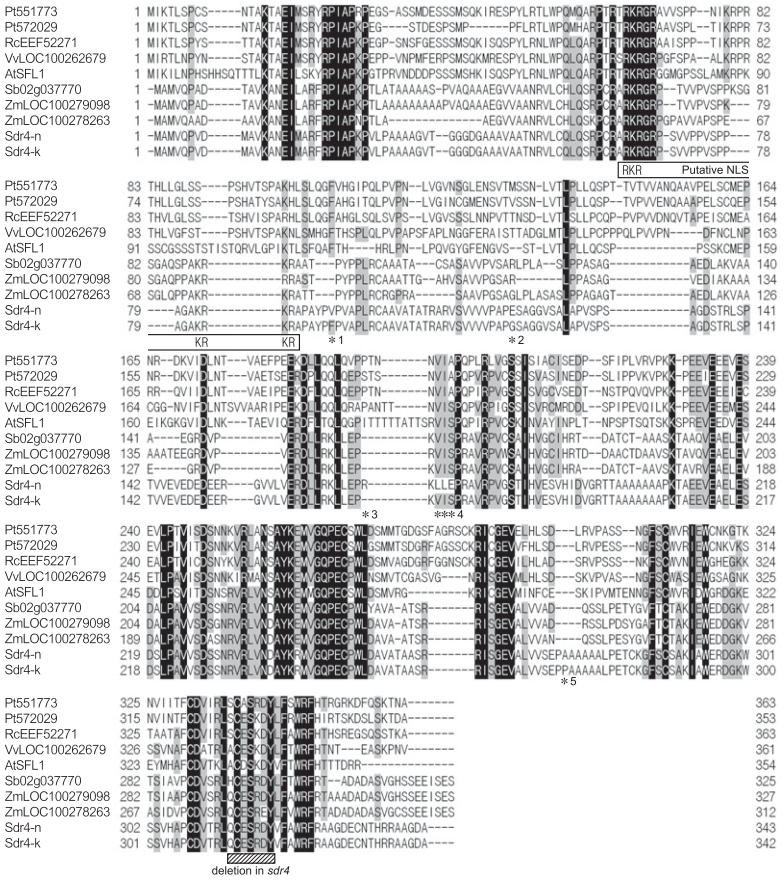

Fig. S4. Deduced amino acid sequence comparison between *Sdr4* and homologs. Amino acid sequences of predicted ORFs were compared between Sdr4 and homologs. Amino acid substitutions between Sdr4-n and Sdr4-k are marked with as asterisk followed by a number. Putative NLS and deletion in *sdr4* mutants are indicated by clear and hatched boxes, respectively.

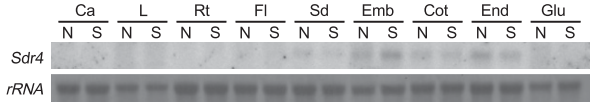

Fig. S5. Tissue specificity of *Sdr4* expression. *Sdr4* mRNA levels in various tissues of plants or seeds 14 days after heading of Nipponbare (N) or NIL[*Sdr4*] (S) were detected by RNA gel blot analysis. Ca, callus; L, leaf; Rt, root; Fl, flower; Sd, whole seed; Emb, embryo; Cot, seed coat; End, endosperm; Glu, outer glume.

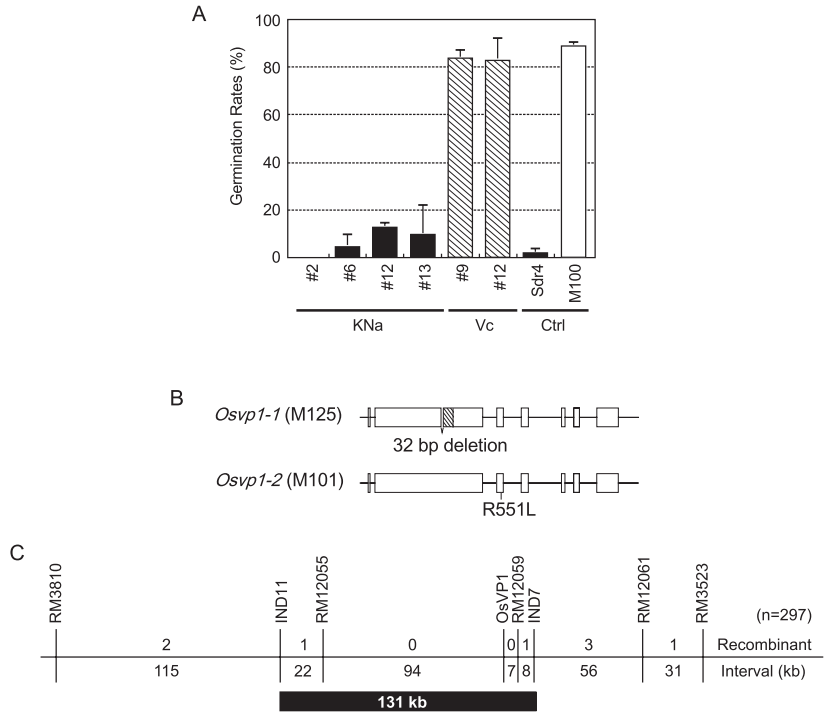

Fig. S6. Complementation of *sdr4* mutation, mutations in *Osvp1* mutants, and linkage analysis of M101 viviparous phenotype and *Osvp1-2* mutation. (*A*) Complementation of *sdr4* mutant (M100) was performed with a 3.3-kb *Sdr4-k* genomic fragment (Fig. 1 *C* and *D*). The germination rates of transgenic lines with *Sdr4-k* (KNa) were lower than those of the vector control lines (Vc), indicating that the viviparous phenotype of the M100 line was due to the mutation in *Sdr4*. Germination rates of NIL[*Sdr4*] (Sdr4) and the *sdr4* mutant (M100) are shown together. (*B*) Mutations in the *OsVP1* gene are summarized. (*C*) Tight linkage of *Osvp1-2* mutation and viviparous phenotype shown by genetic mapping using a mapping population derived from F_2 seeds selected by preharvest sprouting at 4 weeks after heading from M101 × Kasalath cross F_1 plants. This result shows that the *Osvp1-2* mutation and the causal mutation of the viviparous phenotype lay within the indicated 131-kb region. The sequences of the PCR primers for markers were 5′-CGT CAG TGC ACC GAA GTT T-3′ and 5′-CTG GCT TTT GGA GAA GAT CG-3′ for IND11, and 5′-GAT TAA GCA CGG CTC ACC AC-3′ and 5′-GGT TAA CGG GTC TCC ACA CA-3′ for IND7.

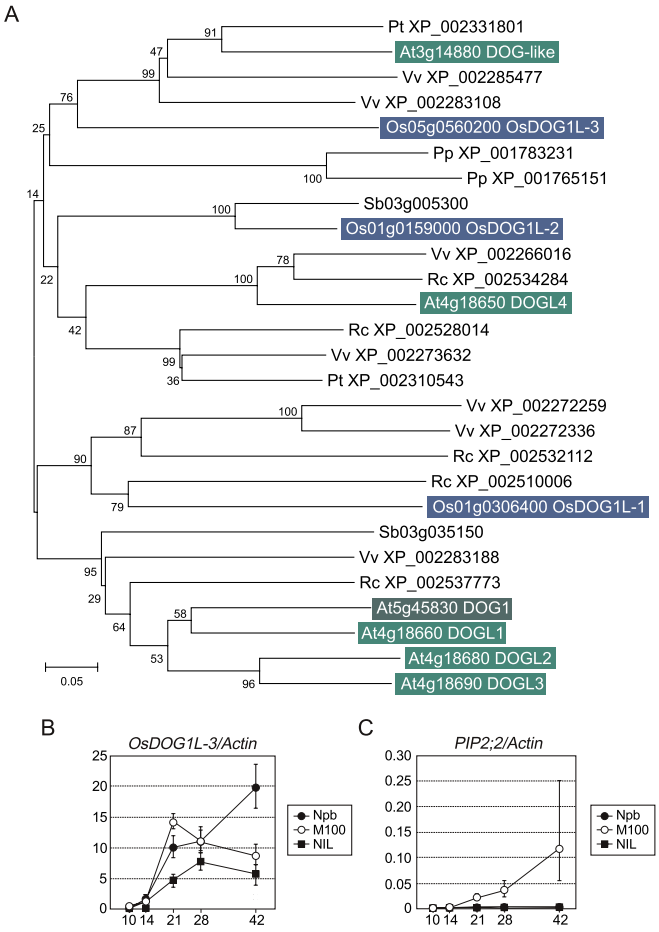

Fig. S7. Phylogenetic analysis of *DOG1 homologs* and expression analysis of *DOG1 like-3* and *PIP2;2*. (*A*) DOG1 homologs were searched using the BLASTP program of NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the reference protein sequence database. Those with *E* values $<e^{-10}$ were selected for phylogenetic analysis. Sequences were aligned using Genetyx version 9 software (Genetyx). Evolutionary history was inferred using the neighbor-joining method (1). The optimum tree with a sum of branch lengths of 6.23632813 is shown. The percentages of replicate trees in which the 27 associated amino acid sequences clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the distances used to infer the phylogenetic tree. All positions containing gaps and missing data were eliminated from the dataset. The final dataset contained a total of 88 positions. Phylogenetic analyses were conducted using MEGA4 software (2). (*B*) Temporal changes (DAF) in mRNA levels in embryos of the *OsDOG1 like-3* gene was monitored by real-time PCR in Nipponbare (Npb), the *sdr4* mutant (M100), and NIL[*Sdr4*] (NIL), with three biological repeats. Expression levels are normalized to *Actin-1* expression. (*C*) Temporal changes (DAF) in mRNA levels in embryos of the aquaporin gene (*PIP2;2*) monitored by real-time PCR.

1. Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425.
2. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599.

	SNP name	SNP1	SNP2	Sdr4	SNP3	SNP4	SNP5	
	Distance (Mb)	-1.6	-0.1	0	+0.1	+0.3	+1.2	
Code	Chr. position (Mb)	22.9	24.4	24.5	24.6	24.8	25.7	Sub Group
	Nipponbare	C	T	n	T	A	T	
	Kasalath	T	C	k	G	T	C	
WRC01	Nipponbare	C	T	n	T	A	T	Jp
WRC43	Dianyu 1	C	T	n	T	A	T	Jp
WRC47	Jaguary	C	T	n	T	A	T	Jp
WRC53	Tima	C	T	n	T	A	T	Jp
WRC51	Urasan 1	C	T	n	T	A	T	Jp
WRC46	Khao Nok	T	T	n	T	A	T	Jp
WRC48	Khau Mac Kho	T	T	n	T	A	T	Jp
WRC49	Padi Perak	T	T	n	T	A	T	Jp
WRC68	Khao Nam Jen	T	T	n	T	A	T	Jp
WRC52	Khau Tan Chiem	T	T	n	T	A	T	Jp
WRC45	Ma sho	T	T	n	T	A	T	Jp
WRC67	Phulba	T	T	n	T	A	T	Jp
WRC50	Rexmont	T	T	n	T	A	T	Jp
WRC13	Asu	T	T	n	T	A	C	In-2
WRC42	Local Basmati	T	T	n	T	A	C	In-1
WRC29	Kalo Dhan	T	T	n	T	A	C	In-1
WRC28	Jarjan	T	T	n	T	A	C	In-1
WRC21	Shwe Nang Gyi	T	T	n	T	A	C	In-2
WRC66	Bingala	T	T	n	T	A	C	In-2
WRC27	Nepal 8	T	T	n	T	A	C	In-1
WRC04	Jena 035	T	T	n	T	A	C	In-1
WRC26	Jhona 2	T	T	n	T	A	C	In-1
WRC34	ARC 7291	T	T	n	T	A	C	In-1
WRC25	Muha	T	T	n	T	A	C	In-1
WRC44	Basilanon	T	T	n	T	T	C	In-1
WRC09	Ryousuisannkoumai	T	T	n	T	T	C	In-2
WRC55	Tupa729	T	T	n	G	A	C	Jp
WRC03	Bei Khe	T	T	n	G	A	C	In-2
WRC07	Davao 1	T	C	n	T	A	C	In-2
WRC38	ARC 11094	T	C	n	T	A	C	In-1
WRC62	Kemasin	C	C	n	G	A	C	In-2
WRC64	Padi Kuning	C	C	n	G	T	C	In-2
WRC06	Puluik Arang	C	C	n	G	T	C	In-2
WRC39	Badari Dhan	T	T	n	G	T	C	In-1
WRC36	Ratul	T	T	n	G	T	C	In-1
WRC11	Kinkagin	T	C	n	G	T	C	In-2
WRC18	Seiyu	T	C	n	G	T	C	In-2
WRC19	Touchusai	T	C	n	G	T	C	In-2
WRC20	Tadukan	T	C	n	G	T	C	In-2
WRC57	Mitusyou 23	T	C	k	G	T	C	In-2
WRC05	Naba	T	C	k	G	T	C	In-2
WRC10	QuiZhaoZong	T	C	k	G	T	C	In-2
WRC37	ARC 7047	T	C	k	T	T	C	In-1
WRC40	Nepal 555	T	C	k	G	T	C	In-1
WRC33	Surjamukhi	T	T	k	G	T	C	In-1
WRC35	ARC 5955	T	C	k	G	T	C	In-1
WRC30	Anjana Dhan	T	C	k	G	T	C	In-1
WRC31	Shoni	T	C	k	G	T	C	In-1
WRC02	Kasalath	T	C	k	G	T	C	In-1
WRC32	Tupa 121-3	T	C	k	G	T	C	In-1
WRC58	Neang Menh	T	C	k	G	T	C	In-2
WRC59	Neang Phtong	T	C	k	G	T	C	In-2
WRC60	Hakphaynhay	T	C	k	G	T	C	In-2
WRC61	Radin Goi Sesat	T	C	k	G	T	C	In-2
WRC63	Bleiyo	T	C	k	G	T	C	In-2
WRC65	Rambhog	T	C	k	G	A	C	In-2

Fig. S8. SNPs in the peripheral region of *Sdr4*. Typing of world rice core collection around the *Sdr4* locus was performed as follows. Chromosomal regions located −1.6, −0.3, +0.4, +1.0, and +1.2 Mb from *Sdr4* in the world rice core collection (positions given in Table S2C) were amplified by PCR. They included introns and intergenic regions. PCR fragments were treated with ExoSap nuclease (GE Healthcare UK) and used as templates for sequencing reactions. Primers for this experiment are listed in Table S2C. SNPs were then summarized. Chromosomal positions of SNPs are based on the RAP-DB Build4 sequence. The *Sdr4* alleles *Sdr4-k* and *Sdr4-n* are represented by k and n, respectively. The *Indica*(In-1 or In-2)/*japonica* subgroups of each cultivar are shown as In-1, In-2, or Jp (*japonica*).

Other Supporting Information Files

Table S1. SNPs and InDels in the *Sdr4* gene in *O. sativa* cultivars and 46 accessions of *O. rufipogon*

Table S1 (DOC)

Amino acid substitutions relative to *Sdr4-n* are shown in the first line. Positions from the first ATG of each substitution are shown in the next line. SNPs and InDels of *Sdr4-n*, *Sdr4-k*, and *Sdr4-k′* are been added as Nipponbare, Kasalath, and Co13, respectively. Sequences of large insertions and deletions are given in the bottom line.

Table S2. Primer sequences of newly designed DNA markers used in the linkage mapping (*A*), primer sequences used to monitor mRNA levels by semi-quantitative RT-PCR or real-time PCR (*B*), and primer sequences used for SNP typing by sequencing (*C*)

Table S2 (DOCX)

In A, for InDel markers, amplified fragment sizes of Nipponbare and Kasalath are shown. For CAPS markers, target SNPs, restriction enzymes used to detect polymorphisms, and amplified sizes of larger fragments are shown. For SNP markers, primers for preamplification, acylCo primers, target SNPs, and amplified fragment sizes are shown. RAP-ID, RAP-DB accession number.