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Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes

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Heterosis, or hybrid vigor, is exploited by breeders to produce elite high-yielding crop lines, but beneficial phenotypes are lost in subsequent generations owing to genetic segregation. Clonal propagation through seeds would enable self-propagation of F_1 hybrids. Here we report a strategy to enable clonal reproduction of F_1 rice hybrids through seeds. We fixed the heterozygosity of F_1 hybrid rice by multiplex CRISPR–Cas9 genome editing of the *REC8*, *PAIR1* and *OSD1* meiotic genes to produce clonal diploid gametes and tetraploid seeds. Next, we demonstrated that editing the *MATRILINEAL* (*MTL*) gene (involved in fertilization) could induce formation of haploid seeds in hybrid rice. Finally, we combined fixation of heterozygosity and haploid induction by simultaneous editing of all four genes (*REC8*, *PAIR1*, *OSD1* and *MTL*) in hybrid rice and obtained plants that could propagate clonally through seeds. Application of our method may enable self-propagation of a broad range of elite F_1 hybrid crops.

Hybrid offspring of genetically distant individuals have increased vigor relative to their homozygous parents owing to heterosis (also known as hybrid vigor). Heterosis has been widely applied in agriculture to improve the productivity and adaptability of crops[1,2]. However, hybrid seed production is prohibitively expensive for many crops. Synthetic apomixes has been proposed as a way to fix the heterosis of F_1 hybrid crop varieties[3]. Apomixis is an asexual reproductive strategy in which offspring are generated through seeds without meiosis or fertilization. Although it has been described in many flowering plant taxa[4], apomixis has not been reported in crops. Combined mutations of three genes that mediate crucial meiotic processes create a genotype named *MiMe* (*Mitosis instead of Meiosis*) in which meiosis is replaced by a mitosis-like division, resulting in the production of male and female clonal diploid gametes in *Arabidopsis* and rice (*Oryza sativa*)[5,6]. However, the self-fertilization of *MiMe* plants doubles the ploidy at each generation. Crossing *Arabidopsis MiMe* with a CENH3-mediated chromosome-elimination line produced clonal diploid offspring[7]. However, this system still relies on crossing different plants, and the CENH3-mediated chromosome elimination seems unlikely to transfer to other species[8]. Therefore, we set out to devise a method for broadly applicable heterosis fixation in self-pollinated F_1 hybrids.

First, to test the feasibility of *MiMe* technology in hybrid rice varieties, we chose 'Chunyou84' (CY84), an elite inter-subspecific hybrid rice from a cross between the maternal Chunjiang 16 A (16 A), a *japonica* male-sterile line, and the paternal C84, an *indica-japonica* intermediate-type line (Supplementary Fig. 1). To ensure rapid generation of *MiMe* in the hybrid CY84 background, we simultaneously edited the *REC8*, *PAIR1* and *OSD1* genes using our previously developed multiplex CRISPR–Cas9 system[9] (Fig. 1a and Supplementary Table 1). Seven of 32 primary transformed plants were identified as frameshift triple mutants, and three of these were analyzed in detail (Supplementary Fig. 2a,b). The triple mutant (*MiMe*) could not be distinguished from the wild-type CY84 on the basis of its growth or morphology (Supplementary Fig. 3). To test whether meiosis had been changed into a mitosis-like division, we investigated male meiotic chromosome behavior in both wild-type and *MiMe* plants. In the wild-type CY84 (Supplementary Fig. 4a–f), 12 bivalents were scattered at diakinesis and aligned along the equatorial plate at metaphase I. The 12 pairs of homologous chromosomes separated at anaphase I and produced tetrads after the second meiotic division. In *MiMe* (Supplementary Fig. 4g–i), 24 univalents were found at diakinesis and aligned at metaphase I. In anaphase I, 24 pairs of chromatids segregated into two groups and produced dyads, suggesting that the meiosis had been turned into a mitosis-like division. We next examined the ploidy of spores of *MiMe* by performing fluorescence in situ hybridization analyses using a 5 S rDNA-specific probe, which identifies chromosome 11 of rice. Only one signal was observed in CY84 spores ($n = 30$), whereas two signals were consistently observed in *MiMe* spores ($n = 40$, Fig. 1b), showing that diploid gametes were generated in *MiMe*. We also investigated the fertility of the *MiMe* mutant and found a seed-setting rate in *MiMe* of 81.2% ($n = 4{,}043$), which was comparable with that of the wild type (79.1%, $n = 3{,}876$) (Fig. 1c and Table 1), suggesting that simultaneous editing of these three genes did not obviously affect fertility in this hybrid variety. The ploidy of the progeny of the *MiMe* plant was investigated by flow cytometry, and all ($n = 123$) were found to be tetraploid plants (Fig. 1d and Table 1). Furthermore, these progeny plants ($n = 123$) completely retained the heterozygosity of the CY84 parent for ten tested insertion-deletion (indel) markers (Fig. 1e and Supplementary Table 2). The self-fertilized progeny of *MiMe* displayed reduced fertility, increased grain size and elongated awn length compared with the wild type, all of which are characteristics of tetraploid rice (Fig. 1f). These results show that the *MiMe* phenotype can be rapidly introduced into hybrid rice varieties using the CRISPR–Cas9 genome-editing technique.

MiMe clonal gametes participate in normal self-fertilization, giving rise to progeny with the doubled ploidy. This ploidy doubling

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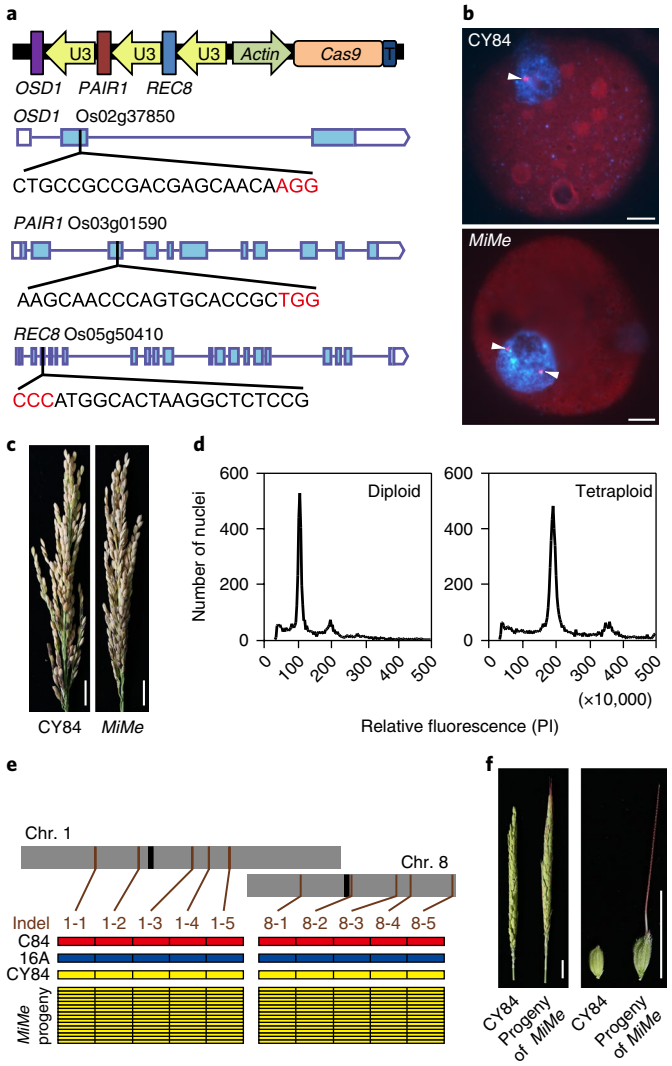

Fig. 1 | Turning meiosis into mitosis in hybrid rice variety CY84. a, The structure of CRISPR–Cas9 vector targeting *OSD1*, *PAIR1* and *REC8*. **b**, The chromosomes of CY84 and *MiMe* were probed by digoxigenin-16-dUTP-labeled 5 S rDNA (red signal, indicated with a white arrow) in spores, showing one signal in wild-type CY84 and two signals in *MiMe*. The DNA is stained with 4′,6-diamidino-2-phenylindole (DAPI, blue signal). Scale bars, 5 μm. **c**, Panicles of wild-type CY84 and *MiMe*. The fertility of *MiMe* was as high as that of wild-type CY84. Scale bars, 2 cm. **d**, Ploidy analysis of CY84 (left) and the progeny of *MiMe* (right) by flow cytometry, which were found to be diploid and tetraploid, respectively (Table 1); PI, propidium iodide. **e**, Genotype analysis of the paternal C84, maternal Chunjiang 16 A (16 A), hybrid variety CY84 and the progeny siblings of *MiMe*. Ten indel markers distributed on chromosomes 1 and 8 were used to identify the genotype of the offspring of *MiMe*. Positions of markers (brown) and centromeres (black) are indicated along the chromosomes. For each marker, plants carrying the C84 allele are in red, plants carrying the 16 A allele are in blue, and plants with both C84 and 16 A alleles appear in yellow. Each row represents one plant, and each column indicates a locus. **f**, Panicles and grain shape of CY84 and the progeny of *MiMe*. The progeny of *MiMe* displayed reduced fertility, increased glume size and elongated awn length. Scale bars, 2 cm.

must be prevented to achieve apomixis. Recently, it was reported that mutation of the *MATRILINEAL* (*MTL*) gene (also known as *NOT LIKE DAD* and *PHOSPHOLIPASE A1*), which encodes a sperm-specific phospholipase, triggers haploid induction in maize[10–13]. To test whether the homologous gene in rice could be manipulated to induce haploidy in self-fertilized hybrid rice, we edited the *MTL* gene in CY84 (Fig. 2a and Supplementary Table 1). Eleven of 32 transformed plants were identified as having frameshift mutants, and three of them were analyzed (Supplementary Fig. 5a,b). The *mtl* mutants showed normal vegetative growth (Supplementary Fig. 3), but the seed-setting rate was reduced to 11.5% ($n = 5{,}180$; Fig. 2b and Table 1). Twelve indel markers (one per chromosome; Supplementary Table 2) that were polymorphic between the two parents were used to determine the genotype of the progeny of the *mtl* plants. In the progeny of wild-type CY84, no plants homozygous at all markers were found ($n = 220$; Table 1). In contrast, 11 plants from 248 *mtl* progeny appeared to be homozygous for all markers (Fig. 2c and Table 1). Flow cytometry showed that nine of these plants were indeed haploid, whereas two were diploid, presumably resulting from spontaneous doubling of haploid embryos (Fig. 2d and Table 1). To further classify the genotypes of these identified plants, the whole genomes of two haploids, two doubled haploids of *mtl* progeny and two offspring plants of wild-type CY84 were resequenced with 30-fold coverage. A total of 78,909 single nucleotide polymorphisms (SNPs) that differed between two parents were screened out for detailed genotype analysis. Whole-genome sequencing revealed that the haploids and doubled haploids were homozygous at all chromosomes (Fig. 2e) and recombinant compared with the parental genome, suggesting that they were each derived from a single gamete. The haploid plants showed reduced plant height, decreased glume size and loss of fertility, whereas the doubled haploid plant displayed normal vegetative and panicle growth (Fig. 2f). The results demonstrate that recombinational haploid plants can be generated by self-fertilization of hybrid varieties.

Since turning meiosis into mitosis and eliminating the paternal genome are both possible in self-fertilized F_1 hybrid rice, we next tested the possibility of inducing heterozygosity fixation without additional crossing in hybrid rice by simultaneously editing four genes—namely, *OSD1*, *PAIR1*, *REC8* and *MTL*—in CY84 (Fig. 3a,b). Out of 22 transgenic plants, 3 were identified by DNA sequencing as *osd1 pair1 rec8 mtl* quadruple mutants (named *Fix*, for *Fixation of hybrids*) and were used for further analysis (Supplementary Fig. 6a,b). The *Fix* mutants grew normally during the vegetative stage (Fig. 3c). During the reproductive stage, the male meiotic chromosome behavior was investigated and found to be indistinguishable from that of *MiMe* (Supplementary Fig. 4j–l). The seed-setting rate was 4.5% ($n = 5{,}850$) (Fig. 3c and Table 1), which was slightly lower than that of the *mtl* mutant. In the progeny seedlings, we investigated the ploidy using flow cytometry. Among 145 progeny plants of *Fix* mutants, 136 were identified as tetraploid and 9 as diploid (Fig. 3d and Table 1). To investigate whether the heterozygosity was fixed in these diploid offspring, the genomes of two diploid and two tetraploid offspring plants of *Fix* were resequenced with an average of 30-fold coverage. Bioinformatic analysis using the 78,909 SNPs revealed that both the diploid and tetraploid progeny plants were genetically identical to the hybrid rice CY84 (Fig. 3e). Finally, we investigated the phenotype of the potential clonal plants of *Fix*. All these potential clones displayed similar plant morphology to the hybrid rice CY84, with normal vegetative growth, normal glume size and normal awn length (Fig. 3f), whereas the seed-setting rate of these plants (6.2%, $n = 5{,}889$) was greatly reduced compared with that of the wild-type controls (81.9%, $n = 4{,}103$), as was the case also for the parent *Fix* plants (Fig. 3c). Taking these results together, the diploid progeny of *Fix* plants displayed the same ploidy, the same heterozygous genotype, and a phenotype similar to that of the parent *Fix* plants, implying that *Fix* is able to produce clonal seeds and fix the heterozygosity of F_1 hybrid rice.

Our findings revealed that hybrids can be self-pollinated to produce true-breeding progeny through seeds by targeted editing of four endogenous genes in a rice F_1 hybrid variety. Simultaneous editing of *REC8*, *PAIR1* and *OSD1* genes did not have obvious adverse

Table 1 | Ploidy analysis of the progeny of CY84, *MiMe*, *mtl* and *Fix*

Genotype	Plant No.	Seed-setting rate (filled grains/florets)	Progeny tested	Tetraploid	Haploid + DH (haploid + DH/ progeny tested)	Diploid (clonal seeds/progeny tested)	Estimated frequency of clonal seeds
CY84	1	77.2% (1,151/1,490)	65	0	0	65	
	2	81.3% (951/1,170)	73	0	0	73	
	3	79.1% (962/1,216)	82	0	0	82	
MiMe	7	81.9% (1,178/1,439)	35	35	0	0	
	8	79.2% (877/1,108)	43	43	0	0	
	21	82.1% (1,228/1,496)	45	45	0	0	
mtl	1	9.1% (101/1,103)	77	0	6 + 0 (6/77 = 7.8%)	71	
	2	13.6% (217/1,601)	90	0	2 + 1 (3/90 = 3.3%)	87	
	3	11.3% (280/2,476)	81	0	1 + 1 (2/81 = 2.5%)	79	
Fix	6	3.7% (63/1,725)	39	37	0	2 (2/39 = 5.1%)	5.1% × 3.7% = 0.19%
	10	5.2% (124/2,373)	64	61	0	3 (3/64 = 4.7%)	4.7% × 5.2% = 0.24%
	22	4.3% (76/1,752)	42	38	0	4 (4/42 = 9.5%)	9.5% × 4.3% = 0.41%

DH, doubled haploids.

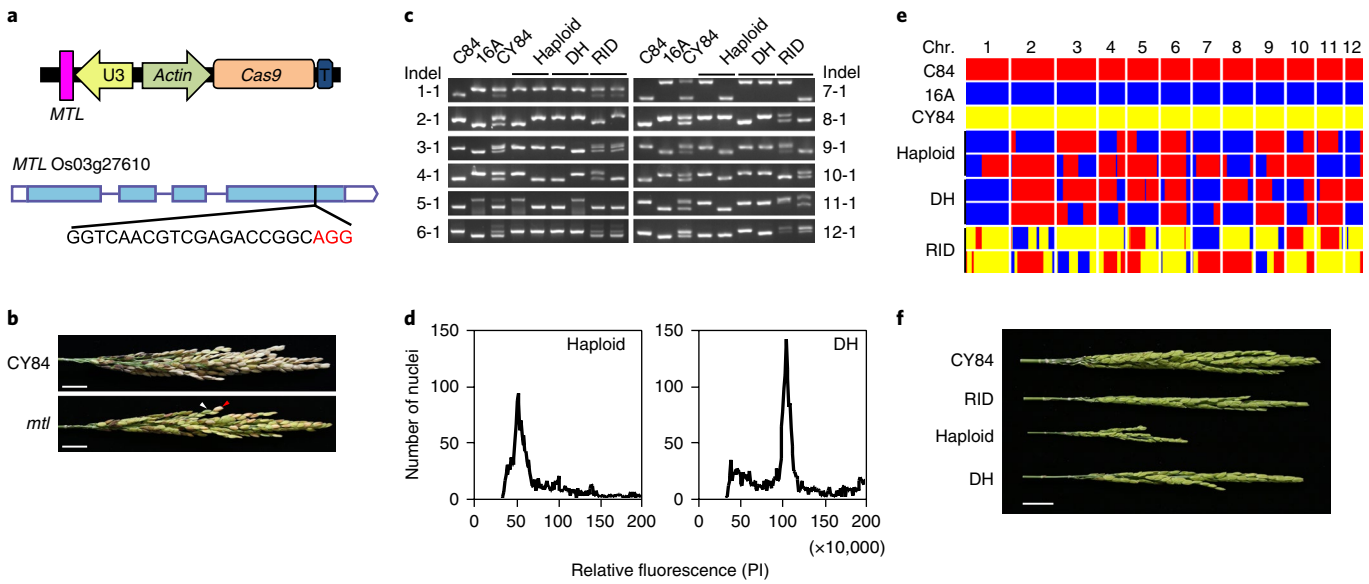

Fig. 2 | Generation of a haploid inducer line by editing the *MTL* gene in hybrid rice variety CY84. a, Schematic diagram of the structure of the CRISPR-Cas9 vector targeting *MTL*. **b**, Panicles of the WT and *mtl* in the CY84 background. The fertility was decreased in *mtl*; a white arrow indicates an aborted seed and a red arrow shows a fertile seed. Scale bars, 2 cm. **c**, Cropped gels indicate the genotype of haploids, doubled haploids (DH) and recombinant inbred diploids (RID) using 12 indel markers (1 per chromosome). Plants homozygous at all markers in the progeny siblings of *mtl* were identified as haploid or DH. **d**, Ploidy analysis of the haploid and DH by flow cytometry (Table 1); PI, propidium iodide. **e**, Whole-genome sequencing of the haploid, DH and RID plants. Twelve blocks represent 12 chromosomes. The SNPs of C84 allele are in red, the SNPs of 16 A allele are in blue, and the coexistence of both alleles is in yellow. **f**, Panicles of wild-type CY84 and *mtl* progeny, including RID, haploid and DH plants. Scale bars, 2 cm.

effects on the growth and reproduction of the hybrid. By contrast, the *MTL* gene used to induce paternal genome elimination had negative effects on hybrid fertility and was not fully penetrant, effects that are consistent with the observations obtained from inbred varities[14]. Consequently, when the four genes were simultaneously mutated in hybrid rice by genome editing, the *Fix* plants displayed a similar reduced fertility. Although the fertility was reduced mainly because of the *MTL* mutation, the *Fix* plant was able to produce clonal seeds with the same ploidy and heterozygous genotype.

Improvements in fertility, such as by modifying the *MTL* gene or looking for different haploid-inducing genes, will be required to enable our technology to be commercialized for rice. However, our Fix strategy could be immediately applied to crop varieties in which fertility and seed production are less important, such as pasture and forage sorghum. Genome-editing technology is now available for a wide range of crops, and the genes that we targeted are conserved, so we anticipate that our method can readily be applied to crops in which generation of F_1 hybrids is not currently commercially viable.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41587-018-0003-0.

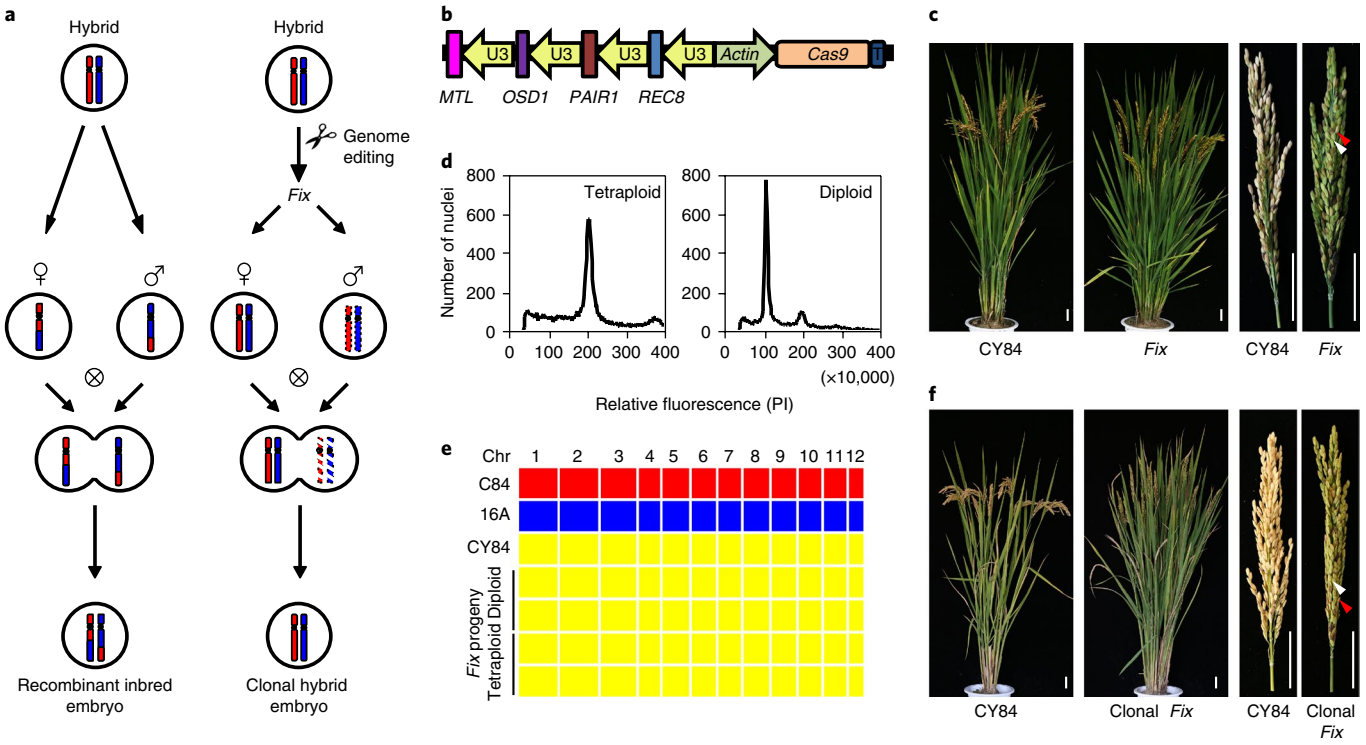

Fig. 3 | Fixation of rice heterozygosity by multiplex gene editing in hybrid rice variety CY84. a, A model for fixation of heterozygosity of the hybrid. In normal sexual reproduction (left), recombinant inbred embryos are generated by fusion of recombined haploid gametes. The clonal reproduction strategy (right) is based on two components: meiosis is turned into mitosis to produce clonal diploid gametes (*MiMe*), and the genome of the male gamete is eliminated by knocking out the *MTL* gene. The progeny of self-pollinated *Fix* are genetically identical to the hybrid parent. **b**, The CRISPR–Cas9 vector simultaneously targeting *OSD1*, *PAIR1*, *REC8* and *MTL*. **c**, Comparison of the plant morphology and panicles of CY84 and *Fix* (*osd1 pair1 rec8 mtl*) grown in paddy fields. The *Fix* plant exhibited a low seed-setting rate (Table 1). An aborted seed is indicated with a white arrow and a normally developed seed with a red arrow. Scale bars, 5 cm. **d**, Ploidy analysis of the progeny of *Fix* by flow cytometry, including tetraploid (left) and diploid (right) examples; PI, propidium iodide. **e**, Whole-genome sequencing of the diploid and tetraploid progeny of *Fix* and the diploid progeny of clonal *Fix*. The SNPs of the C84 allele are in red, the SNPs of the 16 A allele are in blue, and the coexistence of both alleles is in yellow. Twelve blocks represent 12 chromosomes. The diploid and tetraploid progeny of *Fix* and the diploid progeny of clonal *Fix* are heterozygous and identical to CY84. **f**, Comparison of the plant morphology and panicles of wild-type CY84 and the diploid progeny of *Fix* (clonal *Fix*) grown in paddy fields. The clonal *Fix* displayed a low seed-setting rate that was similar to that of parent *Fix* plant. An aborted seed is indicated with a white arrow and a normally developed seed with a red arrow. Scale bars, 5 cm.

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Author contributions

C.W. and K.W. conceived and designed the study. C.W., Y.S., Y.H. and Z.C. performed the lab experiments. Q.L. and T.S. conducted the computational analyses. Y.H. and J.W. carried out the field experiments. J.L. and M.W. provided the rice varieties and helped with the field management. C.W., R.M. and K.W. wrote the manuscript.

Competing interests

The authors have submitted a patent application (application no. 201810325528.4 and 201811205889.1) based on the results reported in this paper.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41587-018-0003-0.

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Methods

Plasmid construction. The plasmids expressing the CRISPR–Cas9 system were constructed via the isocaudamer ligation method, as previously described[9]. The modified single-guide RNAs (sgRNAs) scaffold and *ACTIN1* promoter-driven Cas9 were used to increase the mutation rate in this study[15]. Briefly, the double-stranded overhangs of target oligonucleotides (Supplementary Table 1) were ligated into the SK-sgRNA vectors digested with AarI. Then the sgRNAs for *OSD1* (digested with KpnI and SalI), *PAIR1* (digested with XhoI and BglII) and *REC8* (digested with BamHI and NheI) were assembled into one pC1300-ACT:Cas9 binary vector (digested with KpnI and XbaI), using T4 ligase, to obtain the vector pC1300-ACT:Cas9-sgRNA^{OSD1}-sgRNA^{PAIR1}-sgRNA^{REC8} for generation of *MiMe*. The sgRNA for *MTL* (digested with KpnI and NheI) was assembled into the pC1300-ACT:Cas9 binary vector (digested with KpnI and XbaI) to obtain the vector pC1300-ACT:Cas9-sgRNA^{MTL} for generation of *mtl*. The sgRNA of *MTL* (digested with KpnI and NheI) was assembled into the pC1300-ACT:Cas9-sgRNA^{OSD1}-sgRNA^{PAIR1}-sgRNA^{REC8} vector (digested with KpnI and XbaI) to obtain the vector pC1300-ACT:Cas9-sgRNA^{OSD1}-sgRNA^{PAIR1}-sgRNA^{REC8}-sgRNA^{MTL} for generation of *Fix*.

Rice transformation and growth conditions. The hybrid rice 'Chunyou 84' (CY84) was used as the host variety in this study. The generation of transgenic rice, by *Agrobacterium*-mediated transformation with the strain EHA105, was performed by Hangzhou Biogle Co., Ltd (Hangzhou, China). In summer, the plants were grown in the transgenic paddy fields of the China National Rice Research Institute in Hangzhou, China. In winter, the plants were grown in a greenhouse maintained at average day and night temperatures of 34 and 25 °C, respectively, 12-h light/12-h dark cycles, and 75% relative humidity.

Detection of genome modifications. Genomic DNA was extracted from approximately 100 mg rice leaf tissue via the cetyltrimethylammonium bromide (CTAB) method. PCR was conducted with KOD FX DNA Polymerase (Toyobo, Osaka, Japan) to amplify the genomic regions surrounding the target sites. The primers are listed in Supplementary Table 2. The fragments were sequenced by the Sanger method and decoded by the degenerate sequence decoding method[16]. The Sanger sequences of the analyzed mutants are listed in Supplementary Figs. 2b, 5b and 6b, respectively.

Cytological analyses. Young rice panicles at meiosis stage were harvested and fixed in Carnoy's solution (ethanol:glacial acetic acid, 3:1). Microsporocytes undergoing meiosis were squashed in an acetocarmine solution. Slides were frozen in liquid nitrogen, and the coverslips were removed rapidly with a blade. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) in an antifade solution (Vector Laboratories, Burlingame, CA, USA). Microscopy was conducted using an Olympus BX61 fluorescence microscope fitted with a micro charge-coupled device camera.

Fluorescence in situ hybridization analysis was conducted as described previously[17]. The plasmid pTa794 was used as the probe to quantify the 5 S rDNA.

Genotyping with indel markers. Insertion-deletion (indel) markers to distinguish between heterozygous and homozygous genotypes were designed on the basis of the whole-genome sequences of C84 and 16 A. The primers are listed in Supplementary Table 2. The genotyping was performed by a standard PCR program using 2 × Taq Master Mix (Novoprotein Scientific, China), and the PCR products were detected by electrophoresis on 5% agarose gels.

Flow cytometry determination of DNA content in leaf cell nuclei. The ploidy of leaf cells was determined by estimating nuclear DNA content using flow cytometry. All procedures were done at 4 °C or on ice. Approximately 2 cm^2 leaf tissue was chopped using a new razor blade for 2 to 3 min in 1 mL LB01 buffer (15 mM Tris, 2 mM disodium EDTA, 0.5 mM spermine tetrahydrochloride, 80 mM KCl, 20 mM NaCl, 0.1% (v/v) Triton X-100, 15 mM β-mercaptoethanol, pH 7.5, filtered through a 0.22-μm filter). The homogenate was filtered through a 40-μm nylon filter followed by centrifugation (135*g*, 5 min) to collect the nuclei. The supernatant was discarded, and the pellet was resuspended in 450 μL fresh LB01 buffer. Then 25 μL 1 mg/mL propidium iodide (PI, Sigma P4170) and 25 μL 1 mg/mL DNase-free RNase A (Sigma V900498) were added to stain the DNA. The stained samples were incubated on ice in darkness for 10 min before analysis. The samples were analyzed using a BD Accuri C6 flow cytometer with laser illumination at 552 nm and a 610/20 nm filter. The gating strategy is provided in the Supplementary Data 1. Samples with the same result as CY84 were deemed to be diploids, with the first peak of relative fluorescence at ~100 (×10,000). Samples with the first peak of relative fluorescence at ~50 (×10,000) were deemed to be haploids, whereas samples with the first peak of relative fluorescence at ~200 (×10,000) were deemed to be tetraploids.

Whole-genome resequencing and genotype calling. The 150-bp paired-end reads were generated by Illumina Hiseq2500 at an average depth of approximately 30-fold coverage for each sample. The raw paired-end reads were first filtered to create clean data using NGSQCtoolkit v2.3.3[18]. The cutoff value for PHRED quality score was set to 20. Clean reads of each accession were aligned against the rice reference genome (IRGSP 1.0) using the software SOAPaligner (soap version 2.21)[19] with parameters "-m 200 -x 1000 -l 35 -s 42 -v 5" and "--p 8". To obtain high-quality SNPs, reads that could be mapped to different genomic positions were excluded by SOAPsnp[20]. Uniquely mapped single-end and paired-end results were used in SNP calling. Genotype calling was carried out on the whole genome using these SNPs, which were heterozygous in the parent. The window size (the number of consecutive SNPs in a window) was 0.1 K, and a recombination map was constructed for each chromosome.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data supporting the findings of this study are available in the article and its supplementary figures and tables or are available from the corresponding author on request. For sequence data, rice LOC_Os identifiers (http://rice.plantbiology.msu.edu/): LOC_Os02g37850 (*OSD1*), LOC_Os03g01590 (*PAIR1*), LOC_Os05g50410 (*REC8*) and LOC_Os03g27610 (*MTL*). Whole-genome sequencing data are deposited in the NCBI Sequence Read Archive with accession codes SRP149641 and SRP149677.

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☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	A Perl script (v5.16.3) was used to analysis the SNP data.
Data analysis	NGSQCtookit v2.3.3, SOAPaligner (soap version 2.21), SOAPsnp were used to analyze the whole genome sequencing data.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Whole genome sequencing data are deposited in the NCBI Sequence Read Archive (SRP149641 and SRP149677).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Three individual genome-edited rice plants were chosen in each experiment. Between four and six panicles of each plant were chosen to analyse the seed-setting rate. No statistical tests were used to determine the sample size.
Data exclusions	No data was excluded.
Replication	All attempts at replications were successful.
Randomization	Progenies tested for each plant were chosen at random.
Blinding	Not applicable. This is not relevant to biochemical/cell biology studies and samples were not blinded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	All procedures were done at 4 °C or on ice. Approximately 2 cm2 leaf tissue was chopped using a new razor blade for 2 to 3 min in 1 mL LB01 buffer. The homogenate was filtered through a 40 µm nylon filter followed by centrifugation (1200 × rpm, 5 min) to collect the nuclei. The supernatant was discarded, and the pellet was resuspended in 450 µL fresh LB01 buffer, then 25 µL 1 mg/mL propidium iodide and 25 µL 1 mg/mL DNase-free RNase A were added to stain the DNA. The stained samples were incubated on ice in darkness for 10 min prior to analysis.
Instrument	BD Accuri C6 flow cytometer, with the laser illumination at 552 nm and a 610/20 nm filter.
Software	BD Accuri C6 software
Cell population abundance	The rice leaf nuclei were used to determine the ploidy level. In this ploidy analysis experiment, cell sorting and purification steps were not involved. After filtration, cells in the suspension were used to perform the experimental procedures.
Gating strategy	See the examples provided for gates used in the Supplementary Information. A negative control was used to establish gate P1 to eliminate irrelevant debris. CY84 was used as the diploid template. The single nuclei were enclosed by polygonal region P2.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.