Supplementary information, Data S1 MATERIALS AND METHODS

Growth of *Arabidopsis* and rice plants

Arabidopsis thaliana ecotype Columbia (Col-0) was used in all experiments. Seeds were sown on MS plates and stratified for 3 days at 4 ℃, then grown under long-day conditions (16 h light/8 h dark) at 22 ℃ for 5 days before being transplanted in soil. Rice plants were grown under standard greenhouse condition (16h light at 30 ℃ /8h night at 22 ℃).

Vector construction

The coding sequence of hSpCas9 was cloned from vector pX260[1] using primers Cas9-F and Cas9-R (Table S2) and subcloned into pA7-GFP with XhoI and BamHI to replace the GFP gene, which provided a 2x 35S promoter and a Nos terminator. Then the Cas9 expression cassette was subcloned into the pBluescript SK+ vector (Stratagene Inc., San Diego, CA) and designated 35S-Cas9-SK.

The AtU6-26 promoter was cloned from *Arabidopsis* wild type Col-0 genomic DNA by PCR with primers AtU6-26F and AtU6-26R (Table S2) adding KpnI and XhoI on the two ends, respectively, and put into the pEasy Blunt vector (Transgen Biotech, China). They were then subcloned into the pBluescript SK+ vector (Stratagene Inc., San Diego, CA) using KpnI and XhoI sites. The 85bp chimeric guide RNA region containing two BbsI digest sites was amplified from the vector pX330[1] by PCR using AtU6-26-85F and AtU6-26-85R (Table S2) and fused to the AtU6-26 promoter, which resulted in AtU6-26SK. After the designed oligos (20bp targeting sequences) were cloned into the BbsI sites, these chimeric RNA expression cassettes between KpnI and SalI were either cloned into the 35S-Cas9-SK for transient assay, or into the KpnI and EcoRI region of pCambia1300 vector (Cambia, Canberra, Australia) together with the SalI and EcoRI fragment of the Cas9 expression cassette for stable transformation of *Arabidopsis*.

The OsU6-2 promoter was cloned from rice wild type Nipponbare genomic DNA by PCR using OsU6-F and OsU6-R (Table S2) and put into the pEasy Blunt vector (Transgen Biotech, China). Then transfer PCR was conducted using TPCR-OsU6F and TPCR-OsU6R (Table S2) to replace the AtU6-26 promoter in the AtU6-26SK vector, which produced the OsU6SK vector with the 85nt guide RNA region. After target oligos were successfully inserted into the BbsI sites of the OsU6SK vector, the chimeric RNA expression cassettes between KpnI and HindIII were similarly cloned into the pCambia1300 vector (Cambia, Canberra, Australia) between the KpnI and EcoRI sites together with the HindIII and EcoRI Cas9 expression cassette for stable transformation of rice.

Transient YFP-HR reporter assay

A HR-based transient YFP reporter was constructed based on the pA7-YFP vector. The 1-510 bp and 229-720 bp coding sequences of YFP were cloned by PCR and fused together with an

18 bp linker (GGATCC ACTAGT GTCGAC), creating a split YFP with 282 bp overlapping. The isolation and PEG transformation of *Arabidopsis* mesophyll protoplasts were as described[2]. The transformed protoplasts were examined using a flow cytometer (BECKMAN COULTER MoFlo™ XDP, USA) after 16-24 hours of incubation in the dark according to the manufacturer's instructions.

Generation of *Arabidopsis* and rice stable transgenic plants

The pCambia1300 vectors containing the hSpCas9 expression cassette and the guide RNA expression cassettes were transformed into *Agrobacterium* strain GV3101 and EHA105 by the freeze-thaw method for transformation of *Arabidopsis* and rice, respectively. Healthy *Arabidopsis* Col-0 wild type plants at the flowering stage were used for transformation by the floral dipping method[3]. The collected seeds were screened on MS plates with 20 μg/L hygromycin. *Agrobacterium*-mediated transformation of the callus of rice cultivar Kasalath was conducted as described[4].

RFLP analysis of genome modification

Genomic DNA was extracted from stable transgenic plants from hygromycin selection and wild type control plants. PCR was performed using specific primers for each target (Table S2). After purification, about 400 nanograms of PCR product was digested overnight with the corresponding restriction enzymes designed for each target site. Digested DNA was separated on an ethidium bromide-stained agarose gel (1.5%). The digest-resistant bands were recovered and cloned into the pZeroBack Blunt vector (Tiangen Biotech, China), and mutations were identified by Sanger sequencing of individual clones.

SUPPLEMENTARY NOTE

Sequence of the sgRNA and Cas9 expression cassettes

>AtU6-26 sgRNA

GGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTGCCCTTAA
GCTTCGTTGAACAACGGAAACTCGACTTGCCTTCCGCACAATACATCATTTCTTCTT
AGCTTTTTTTCTTCTTCTTCGTTCATACAGTTTTTTTTTGTTTATCAGCTTACATTTTC
TTGAACCGTAGCTTTCGTTTTCTTCTTTTTAACTTTCCATTCGGAGTTTTTGTATCTT
GTTTCATAGTTTGTCCCAGGATTAGAATGATTAGGCATCGAACCTTCAAGAATTTGA
TTGAATAAAACATCTTCATTCTTAAGATATGAAGATAATCTTCAAAAGGCCCCTGGG
AATCTGAAAGAAGAGAAGCAGGCCCATTTATATGGGAAAGAACAATAGTATTTCTT
ATATAGGCCCATTTAAGTTGAAAACAATCTTCAAAAGTCCCACATCGCTTAGATAA
GAAAACGAAGCTGAGTTTATATACAGCTAGAGTCGAAGTAGTGATTGGGTCTTCGA
GAAGACCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA
ACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGTCCCTTCGAAGGGCCTTTCT
CAGATATCCATCACACTGGCGGCCGCTCGAGGTCGACGGTATCGATAAGCTT

The AtU6-26 sequence and sgRNA are highlighted in magenta and yellow, respectively.

>OsU6-2 sgRNA

GGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTGCCCTTG
GATCATGAACCAACGGCCTGGCTGTATTTGGTGGTTGTGTAGGGAGATGGGGAGA
AGAAAAGCCCGATTCTCTTCGCTGTGATGGGCTGGATGCATGCGGGGGAGCGGGA
GGCCCAAGTACGTGCACGGTGAGCGGCCCACAGGGCGAGTGTGAGCGCGAGAGG
CGGGAGGAACAGTTTAGTACCACATTGCCCAGCTAACTCGAACGCGACCAACTTAT
AAACCCGCGCGCTGTCGCTTGTGTGGGTCTTCGAGAAGACCTGTTTTAGAGCTAG
AAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGA
GTCGGTGCTTTTTTTGTCCCTTCGAAGGGCAATTCTGCAGATATCCATCACACTGGC
GGCCGCTCGAGGTCGACGGTATCGATAAGCTT

The OsU6-2 sequence and sgRNA are highlighted in green and yellow, respectively.

>2×35S-Cas9-Nos

AAGCTTGCATGCCTGCAGGTCAACATGGTGGAGCACGACACACTTGTCTACTCCA
AAAATATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACA
AAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTAT
TGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAA
GGAAAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCC
CACCCACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGC
AAGTGGATTGATGTGATAACATGGTGGAGCACGACACACTTGTCTACTCCAAAAAT
ATCAAAGATACAGTCTCAGAAGACCAAAGGGCAATTGAGACTTTTCAACAAAGGG
TGATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTATTGTGA
AGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAA

GGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCC
ACGAGGAGCATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTG
GATTGATGTGATATCTCCACTGACGTAAGGGATGACGCACAATCCCACTATCCTTCG
CAAGACCCTTCCTCTATATAAGGAAGTTCATTTCATTTGGAGAGGACCTCGACCTC
AACACAACATATACAAAACAAACGAATCTCAAGCAATCAAGCATTCTACTTCTATT
GCAGCAATTTAAATCATTTCTTTTAAAGCAAAAGCAATTTTCTGAAAATTTTCACCA
TTTACGAACGATACTCGAGATGGACTATAAGGACCACGACGGAGACTACAAGGAT
CATGATATTGATTACAAAGACGATGACGATAAGATGGCCCCAAAGAAGAAGCGGA
AGGTCGGTATCCACGGAGTCCCAGCAGCCGACAAGAAGTACAGCATCGGCCTGGA
CATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCC
AGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAAC
CTGATCGGAGCCCTGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTG
AAGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTATCTG
CAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGAC
TGGAAGAGTCCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATCTT
CGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCAC
CTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTATC
TGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCT
GAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTAC
AACCAGCTGTTCGAGGAAAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCC
ATCCTGTCTGCCAGACTGAGCAAGAGCAGACGGCTGGAAAATCTGATCGCCCAGC
TGCCCGGCGAGAAGAAGAATGGCCTGTTCGGAAACCTGATTGCCCTGAGCCTGGG
CCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAG
CTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGC
GACCAGTACGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGC
TGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCTC
TATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTCTC
GTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGA
ACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTT
CATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTG
AACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCC
CACCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTT
ACCCATTCCTGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCAT
CCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATTCGCCTGGATGACC
AGAAAGAGCGAGGAAACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAG
GGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGC
CCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTA
TAACGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTC
CTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGG
AAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTC
GACTCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACAT
ACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAA
CGAGGACATTCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGAG
ATGATCGAGGAACGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGTGATGA

AGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAGCCGGAAGCTGA
TCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTC
CGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACC
TTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACG
AGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGAC
AGTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGAGAA
CATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAA
CAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCA
GATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTA
CCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGAACTGGACATCA
ACCGGCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGAC
GACTCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCGGGGCAAGAGC
GACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAG
CTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTGACCAAGGCCG
AGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGG
TGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAA
CACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTG
AAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCGCG
AGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTCGTGGGAAC
CGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTAC
AAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAG
GCTACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGAT
TACCCTGGCCAACGGCGAGATCCGGAAGCGGCCTCTGATCGAGACAAACGGCGA
AACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCACCGTGCGGAAAGTG
CTGAGCATGCCCCAAGTGAATATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCT
TCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTGATCGCCAGAAA
GAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTAT
TCTGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGT
GTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATC
CCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCAT
CAAGCTGCCTAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTG
GCCTCTGCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATG
TGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCCGAGGA
TAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATC
ATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGG
ACAAAGTGCTGTCCGCCTACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGC
CGAGAATATCATCCACCTGTTTACCCTGACCAATCTGGGAGCCCCTGCCGCCTTCA
AGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAAGAGGTGCT
GGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATCGAC
CTGTCTCAGCTGGGAGGCGACAAAAGGCCGGCGGCCACGAAAAAGGCCGGCCAG
GCAAAAAAGAAAAAGTAAGGATCCTGATTGATCGATAGAGCTCGAATTTCCCCGAT
CGTTCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCG
ATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATG
CATGACGTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTTAA

TACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCGCGCGCGGT GTCATCTATGTTACTAGATCGGGAATTC

The 2x35S, 3xFLAG, NLS, hSpCas9 and Nos terminator sequences are highlighted, respectively.

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