

Supplementary information

Elevating RNA m⁵C methylation provides a promising strategy for crop productivity

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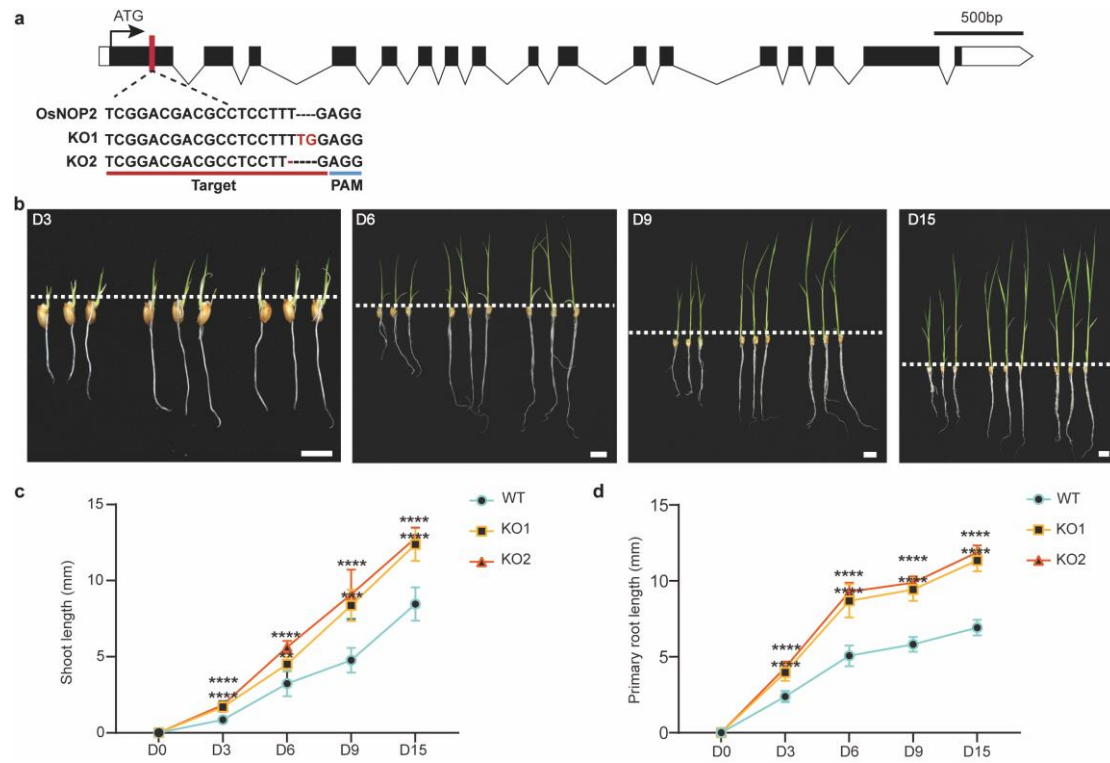
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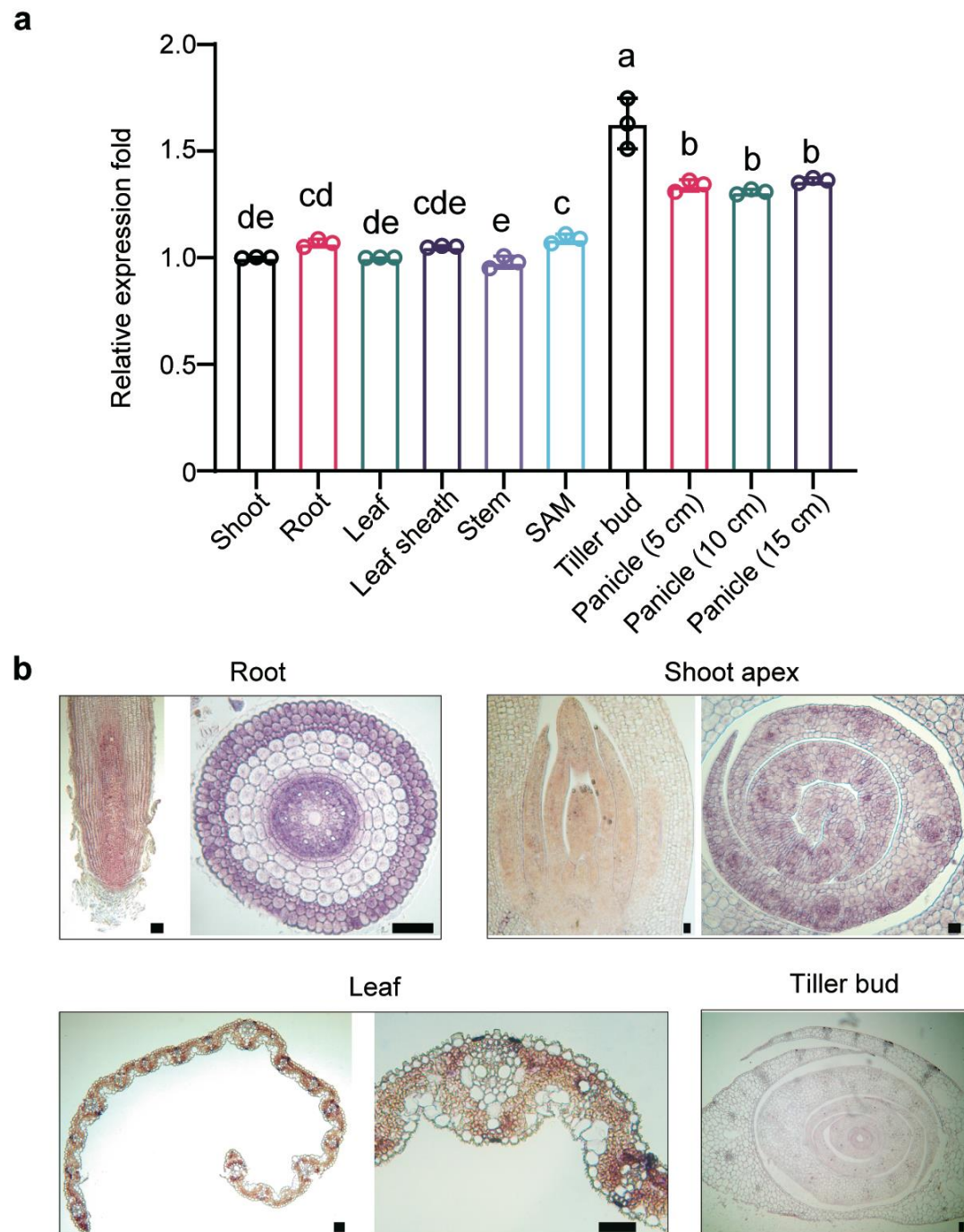
Contains Supplementary Figs. 1-19 and Supplementary Tables 1-20.

Supplementary Figs. 1-19



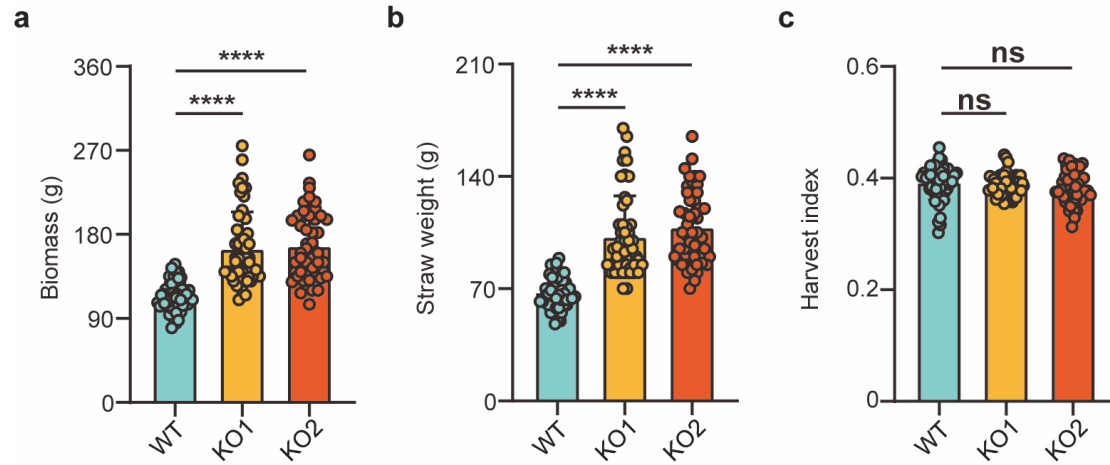
Supplementary Fig. 1| OsNOP2 is a negative regulator for seedling development.

a, The gene structure and mutation sites of KO1 and KO2. PAM, protospacer adjacent motif. **b**, Phenotype of seedlings at different developmental stages (Day 3, Day 6, Day 9, and Day 15). **c-d**, Statistical analysis of the shoot length (**c**) and primary root length (**d**) at different developmental stages in WT, KO1, and KO2 seedlings. Data are mean $\pm$ S.D. ($n > 10$ seedlings). $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed).



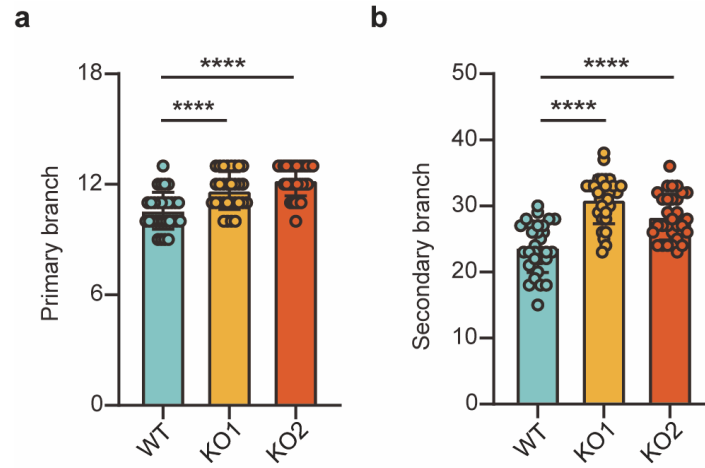
Supplementary Fig. 2| The expression patterns of *OsNOP2* in different tissues. a, RT-qPCR shows the expression levels of the *OsNOP2* in different tissues (shoot, root, leaf, leaf sheath, stem, SAM, tiller bud, and panicles of different length). b, *OsNOP2* expression was validated by *in situ* hybridization assay in different tissues (root, shoot apex, leaf, and tiller bud). Scale bar, 50 μ m. Data are mean $\pm$ S.D. (a, n = 3 biological

replicates). Letters indicate statistical significance among tissues by two-tailed Student's t-tests.

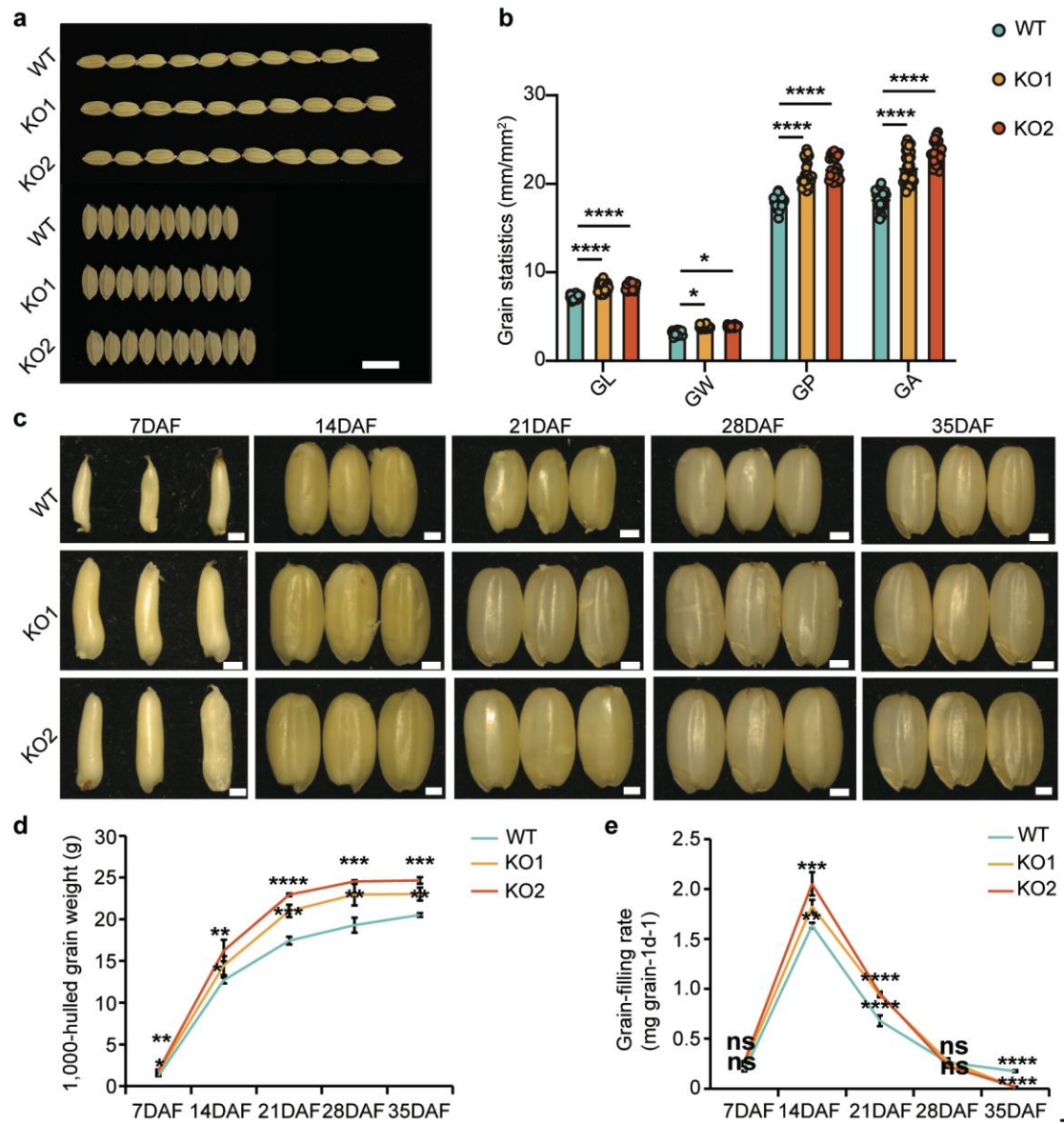


Supplementary Fig. 3| *OsNOP2* knockout alters relevant parameters of rice

biomass. a-c, Statistical analysis of biomass (a), straw weight (b), and harvest index (c) from WT, KO1, and KO2 plants at the mature stage. Data are mean ± S.D. (n = 30 plants). ns, no significance, **** $P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed).

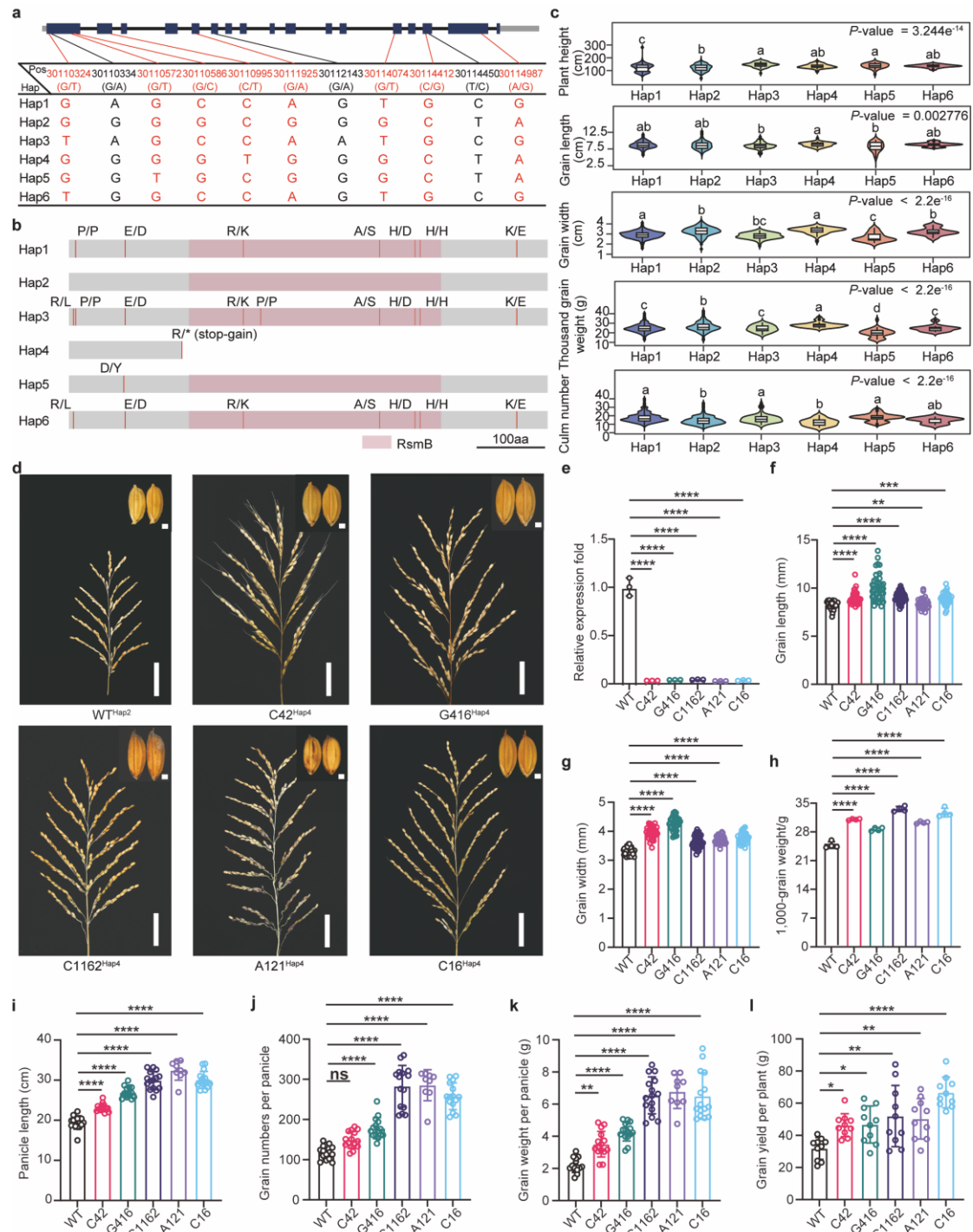


Supplementary Fig. 4| *OsNOP2* knockout increases relevant parameters of panicle traits. a-b, Statistical analysis of primary branch (**a**) and secondary branch (**b**) from WT, KO1, and KO2 plants at the mature stage. Data are mean $\pm$ S.D. (**a-b**, $n = 30$ panicles). **** $P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed).



Supplementary Fig. 5| *OsNOP2* knockout promotes grain size and rice grain filling rate. **a**, Grain morphologies of WT, KO1, and KO2 plants. Scale bar, 1 cm. **b**, Statistical analysis of grain length (GL), grain width (GW), grain perimeter (GP), and grain area size (GA) from WT, KO1, and KO2 plants. **c**, Morphological changes in brown rice grains of WT, KO1, and KO2 plants at five stages. Scale bar, 2 mm. **d**, Statistical analysis of brown rice weight and grain-filling rate changes of WT, KO1, and KO2 plants at five stages. Data are mean $\pm$ S.D. (**b**, n = 50 grains; **d-e**, n = 3

biological replicates). ns, no significance, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$,
 $****P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed).



Supplementary Fig. 6| Genetic diversity of *OsNOP2* in the 3K RG dataset. a,

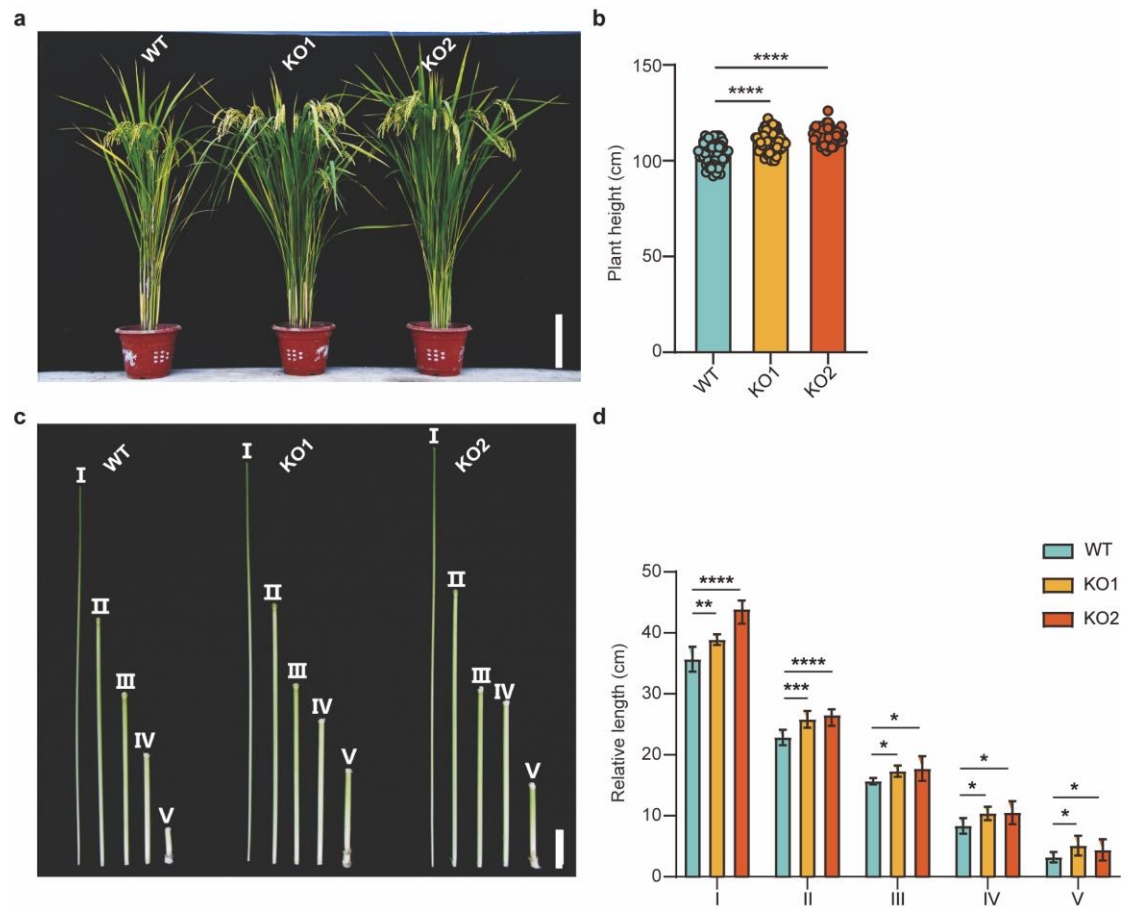
Haplotypes of *OsNOP2* in 2,753 accessions of 3K RG (rare haplotypes of < 100

accessions are not shown) using 11 SNPs in the CDS region. **b,** The protein structures

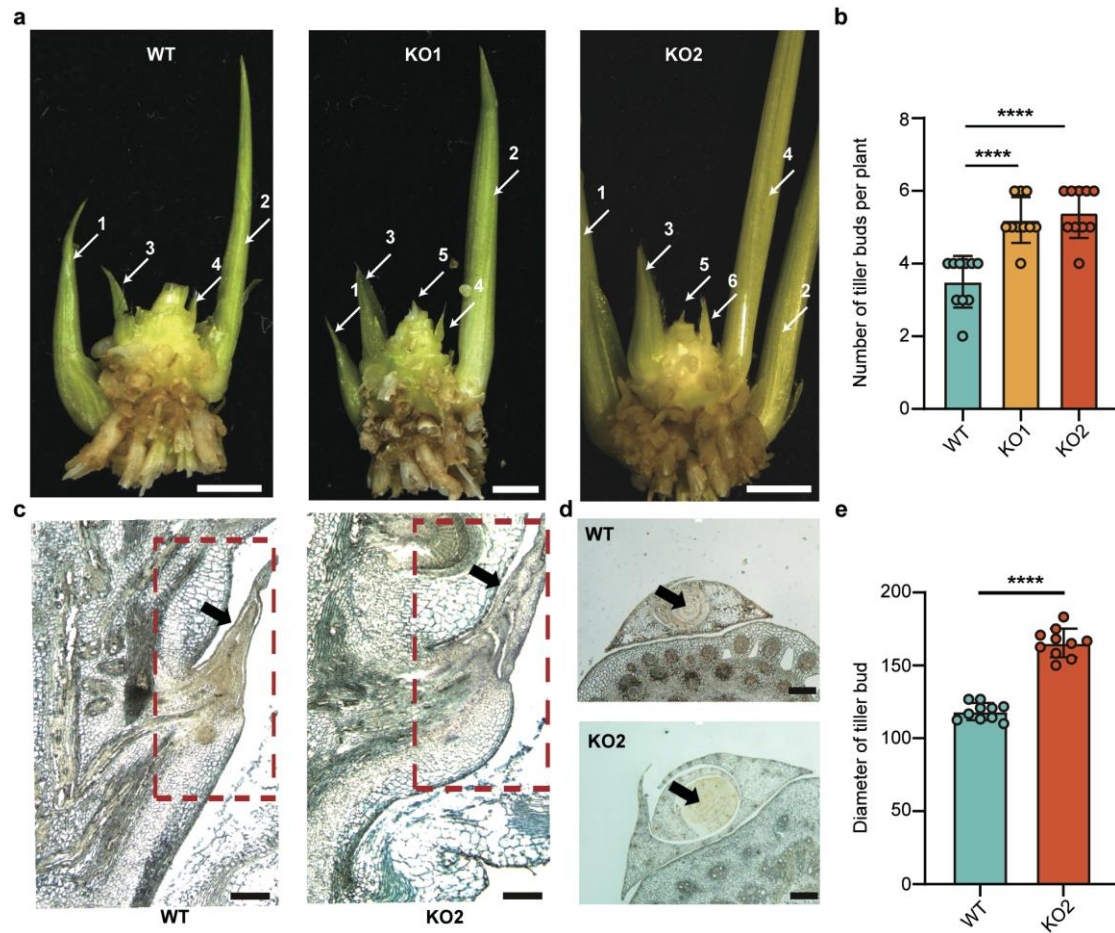
of six haplotypes. The reference and the alternative amino acid are represented by the

abbreviation before and after slash, respectively. Stop-gain indicates the early

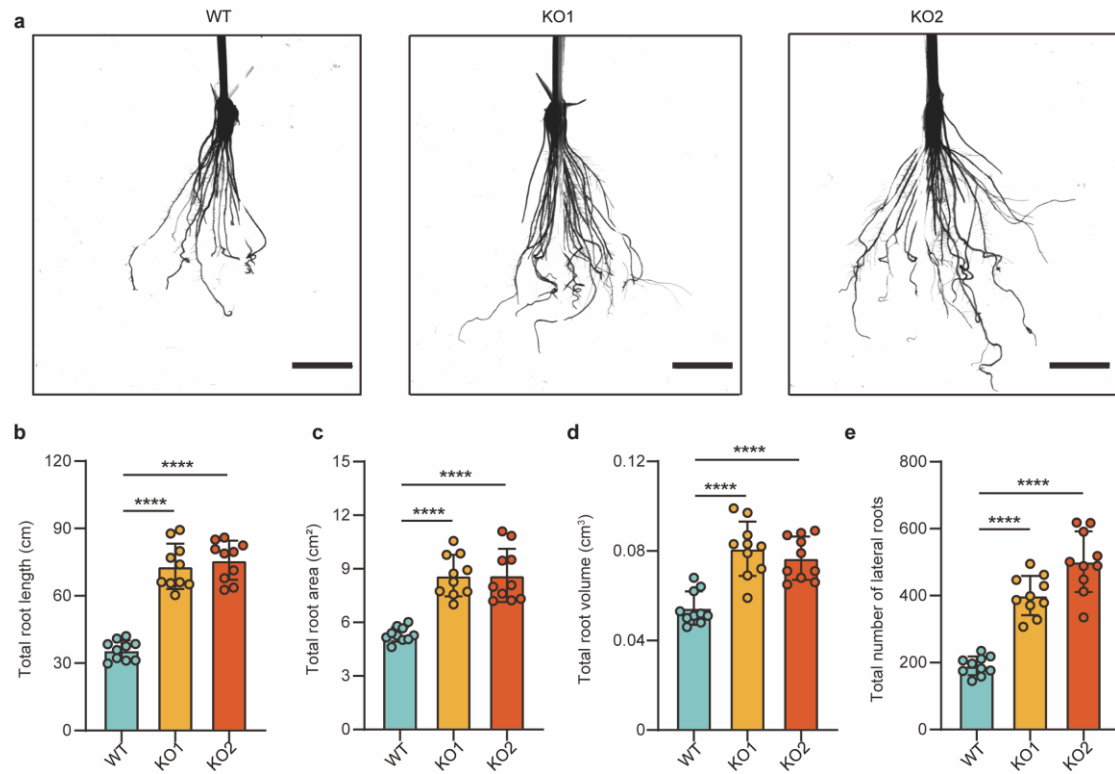
termination of translation. **c**, Performance distribution of different haplotypes of *OsNOP2* on plant height, grain length, grain width, thousand grain weight and culm number in 3K RG. Different letters on the boxplots indicate statistically significant differences ($P < 0.01$, Duncan's new multiple range test). **d**, Panicle and grain morphology of some accessions from the Hap2 (WT) and Hap4 (C42, G416, C1162, A121, and C16). Scale bar, 3 cm. **e**, Relative expression levels of *OsNOP2* in WT^{Hap2}, C42^{Hap4}, G416^{Hap4}, C1162^{Hap4}, A121^{Hap4}, and C16^{Hap4} plants. **f-l**, Statistical analysis of grain length (**f**), grain width (**g**), 1,000-grain weight (**h**), panicle length (**i**), grain numbers per panicle (**j**), grain weight per panicle (**k**), and grain yield per plant (**l**) of the accessions from the Hap2 and Hap4. Data are mean $\pm$ S.D. (**e**, $n = 3$ biological replicates; **f-g**, $n \geq 20$ grains; **h**, $n = 4$ biological replicates; **i-k**, $n = 15$ panicles; **l**, $n = 10$ plants). ns, no significance, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed).



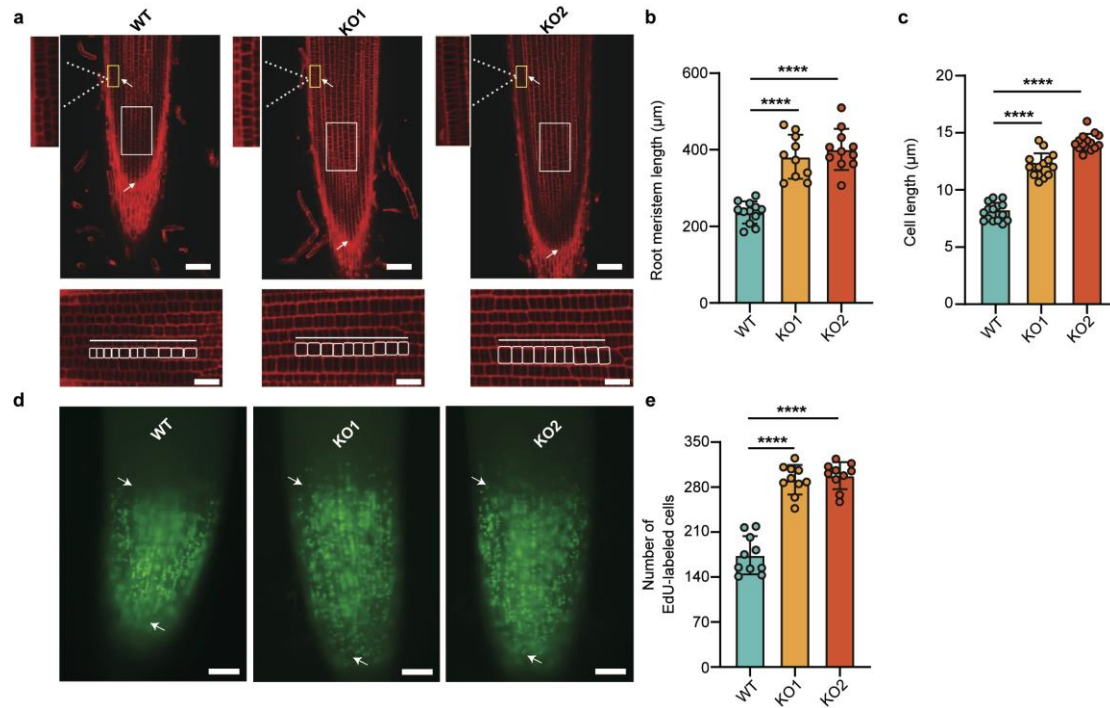
Supplementary Fig. 7| *OsNOP2* knockout alters rice morphology. a, c, Phenotype of single-plant (**a**) and internode (**c**) of WT, KO1, and KO2 plants at the grain-filling stage. Scale bars, 15 cm (**a**) and 3 cm (**c**). **b, d**, Statistical analysis of the plant height (**b**) and relative length of internodes (**d**) of WT, KO1, and KO2 plants at the mature stage. Data are mean $\pm$ S.D. (**b**, n = 30 plants; **d**, n = 10 plants). * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001; P values are from one-way ANOVA (and nonparametric or mixed).



Supplementary Fig. 8| *OsNOP2* knockout shows simultaneously increased number and size of tiller buds. **a**, Phenotypes of tiller buds in 2-month-old seedlings from WT, KO1, and KO2 plants. Scale bar, 1 mm. **b**, The number of tiller buds of WT, KO1, and KO2 plants were counted. **c-d**, Microphenotypes of vertical section (**c**) and cross section (**d**) of tiller buds from WT and KO2 plants by paraffin section. Scale bar, 50 μ m. **e**, The diameter of tiller buds were measured. Data are mean $\pm$ S.D. (**b**, $n = 10$ plants; **e**, $n = 10$ plants). **** $P < 0.0001$; In **b**, P values are from one-way ANOVA (and nonparametric or mixed). In **e**, P values are from two-tailed Student's t -tests.

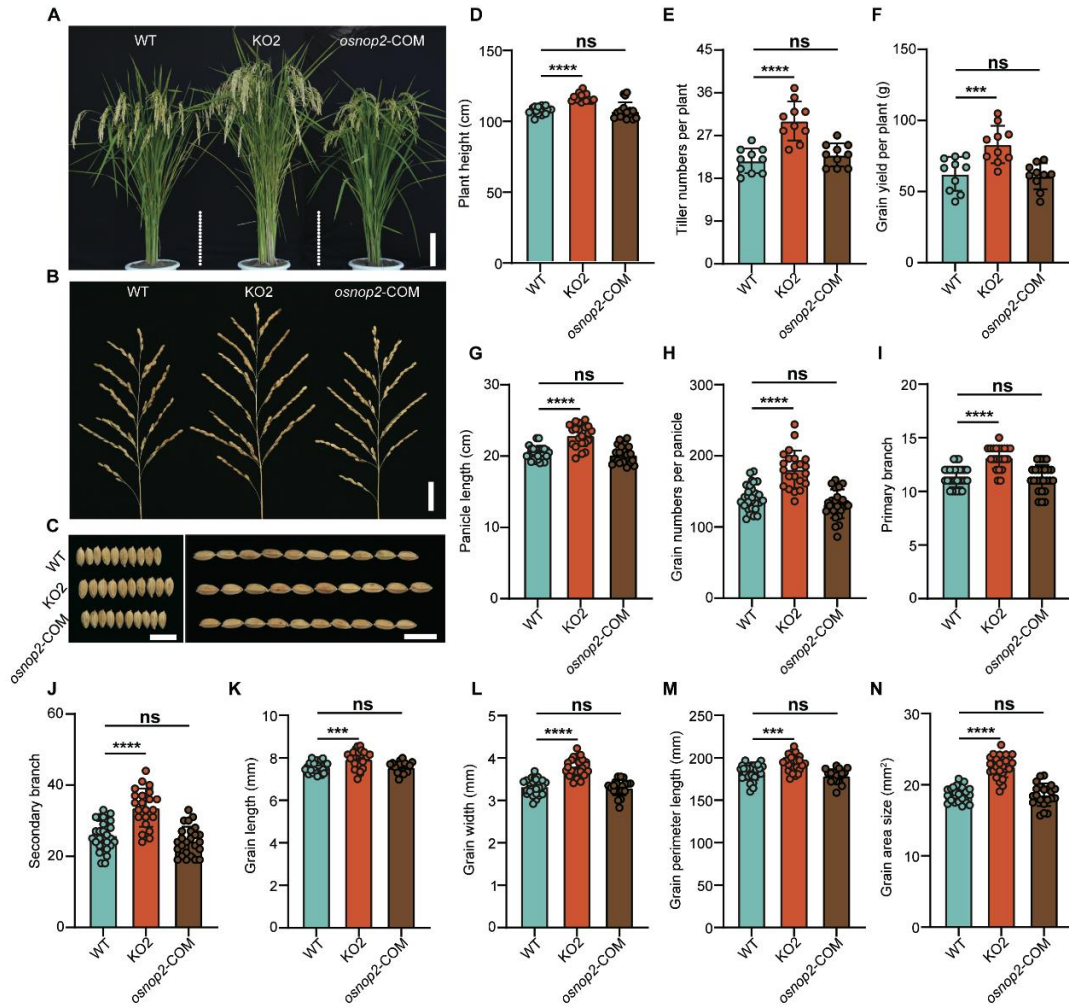


Supplementary Fig. 9| *OsNOP2* knockout improves the phenotype and relevant parameters of root morphological. **a**, The scan images of WT, KO1, and KO2 roots at seedling stage hydroponically grown in the greenhouse by using EPSON scanner (Epson Expression 1600pro). Scale Bar, 2 cm. **b-e**, Statistical analysis of total root length (**b**), total root area (**c**), total root volume (**d**), and total number of lateral roots (**e**) of WT, KO1, and KO2 seedlings by using WinRHIZO software (Regent Instruments). Data are mean $\pm$ S.D. (**b-e**, $n = 10$ plants). **** $P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed).



Supplementary Fig. 10| *OsNOP2* knockout positively regulates root meristem cell

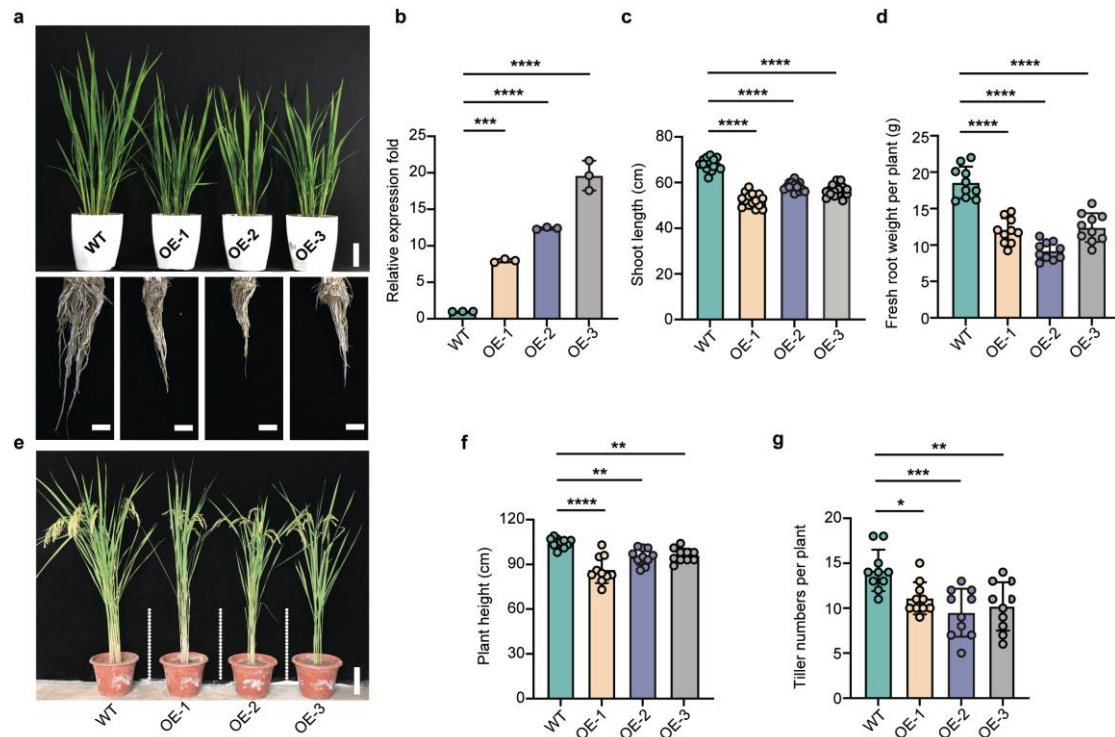
proliferation. **a**, PI staining of root tips in 3-day-old WT, KO1, and KO2 rice seedlings. **b-c**, Quantitative analysis of root meristem length (**b**) and cell length (**c**) within the same length of the root meristem (as indicated in white boxes and magnified images at the bottom). Arrowheads indicate range of roots meristems. The cell length was calculated from the same length of the root meristem as indicated by the white lines. Scale bar, 50 μ m. **d**, Longitudinal view of EdU-labeled cells in root meristems of 3-day-old WT, KO1, and KO2 rice seedlings. Scale bar, 50 μ m. **e**, Statistical analysis of numbers of EdU-labeled cells in root tips were counted. Arrowheads indicate range of roots meristems. Data are mean $\pm$ S.D. (**b**, $n = 10$ plants; **c**, $n = 15$ cells; **e**, $n = 10$ plants). **** $P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed).



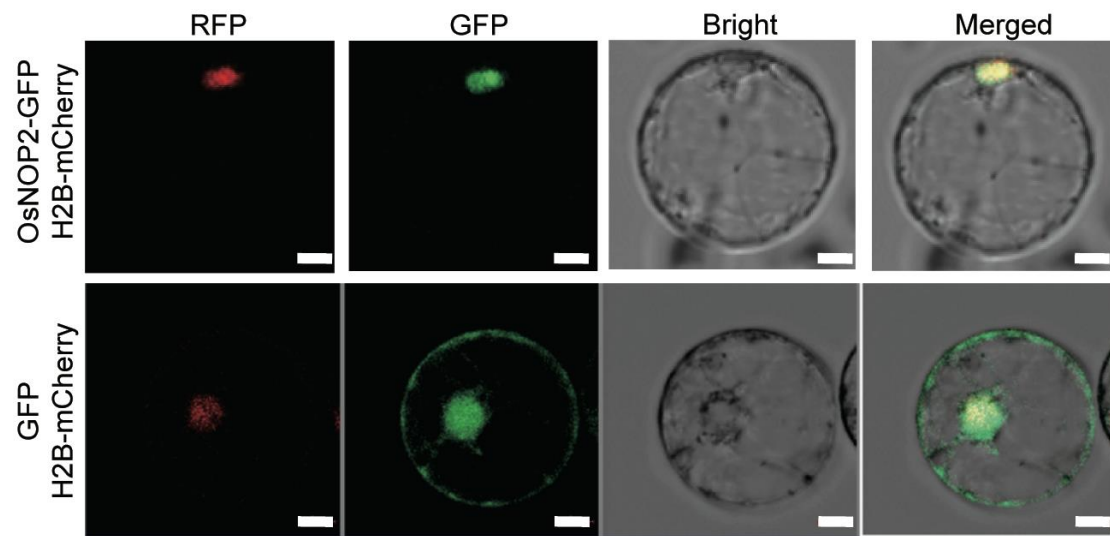
Supplementary Fig. 11| *OsNOP2* complementation plants rescue the *OsNOP2*

knockout phenotype. a-c, Morphology (**a**), panicle morphology (**b**), and grain morphology (**c**) of WT, KO2, and *osnop2*-COM plants. Scale bars, 10 cm (**a**), 3 cm (**b**), and 1 cm (**c**). **d-n**, Statistical analysis of plant height (**d**), tiller numbers per plant (**e**), grain yield per plant (**f**), panicle length (**g**), grain numbers per panicle (**h**), primary branch (**i**), secondary branch (**j**), grain length (**k**), grain width (**l**), grain perimeter length (**m**), and grain area size (**n**) in WT, KO2, and *osnop2*-COM plants. Data are mean $\pm$ S.D. (**d-f**, $n = 10$ plants; **g-j**, $n \geq 20$ panicles; **k-n**, $n \geq 20$ grains). ns, no significance, *** $P < 0.001$, **** $P < 0.0001$; P values are from one-way ANOVA (and

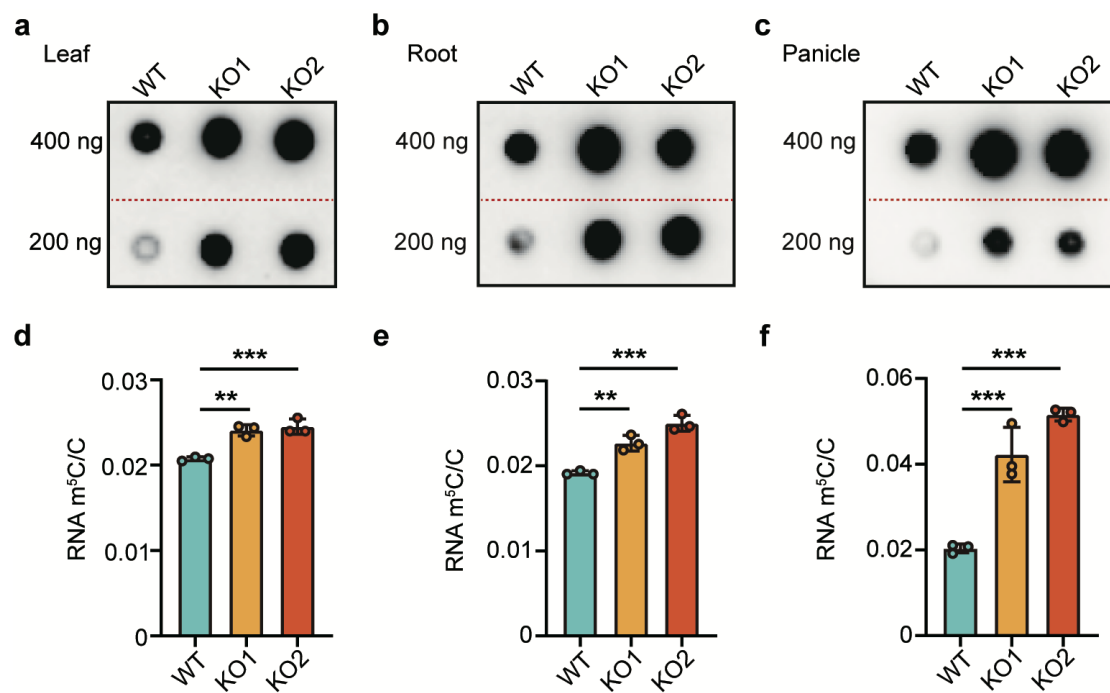
nonparametric or mixed).



Supplementary Fig. 12| *OsNOP2* overexpression decreases the shoot length and the fresh root weight at tillering stage, and decreases the plant height and the tiller numbers at grain-filling stage. **a**, Phenotypes of shoot and root in tillering-stage seedlings of WT, OE1, OE2, and OE3 plants. Scale bar, 5 cm. **b**, Relative expression levels of *OsNOP2* in the WT and *OsNOP2*-overexpressing plants (OE-1, OE-2, and OE-3). **c-d**, Statistical analysis of shoot length (**c**) and fresh root weight per plant (**d**) of WT, OE1, OE2, and OE3 plants were measured. **e**, Morphology of WT, OE1, OE2, and OE3 plants at the grain-filling stage. Scale bar, 10 cm. **f-g**, Statistical analysis of plant height (**f**) and tiller numbers per plant (**g**) from WT, OE1, OE2, and OE3 plants. Data are mean $\pm$ S.D. (**b**, $n = 3$ biological replicates; **c-d**, and **f-g**, $n = 10$ plants). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed).



Supplementary Fig. 13| Subcellular localization of OsNOP2. Nuclear localization of OsNOP2 in rice protoplasts. The empty vector pAN580 (GFP) was used as negative control. The nuclear protein H₂B fused with mCherry (RFP) was used as a nuclear marker. Scale bar, 20 μ m.

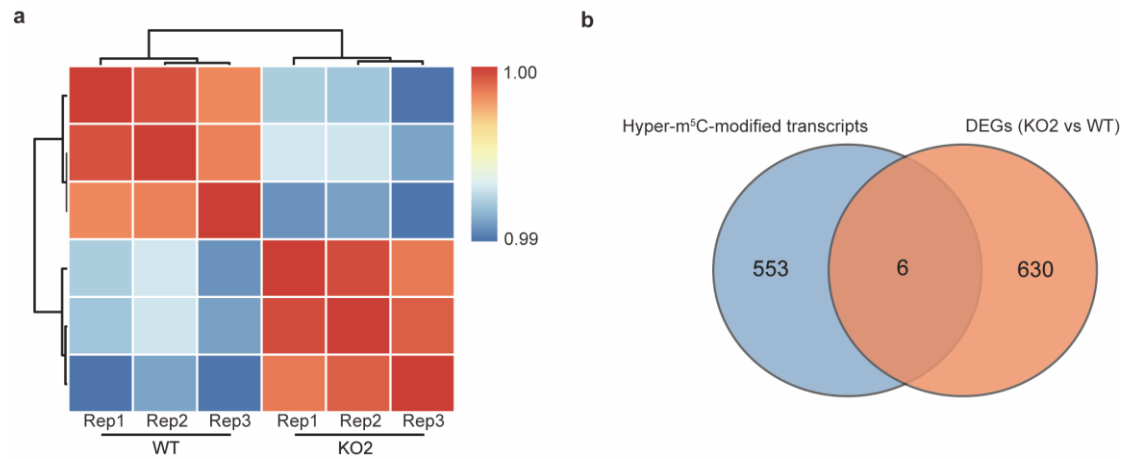


Supplementary Fig. 14| Detection of total RNA m⁵C level in different tissues. a-c, Detection of total RNA m⁵C level of leaf (**a**), root (**b**), and panicle (**c**) in WT, KO1, and KO2 plants by dot blot. **d-f**, Detection of total RNA m⁵C level of leaf (**d**), root (**e**), and panicle (**f**) in WT, KO1, and KO2 plants by LC-MS/MS. Data are mean $\pm$ S.D. (**a-f**, $n = 3$ biological replicates). ** $P < 0.01$, *** $P < 0.001$; P values are from one-way ANOVA (and nonparametric or mixed).

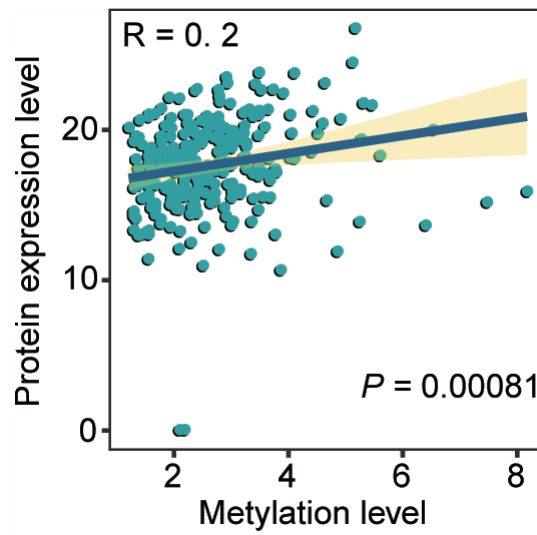
A

NOP2	MGRKLDPTKEKRGPRGRKARKQKGAETELVRFLPAVSDENSKRLSSARKRAAKRRLLGSVEAPKTNKSPFAKPLPGKLPKG	80
OsNO2	MAKKGAPRRQPPPPPPRC.....LAAGKKGKASPKAAKRAAPKKQRLLES..SSDDSELEGGGGLQEV	62
	m k p p s a kraa e e g l	
NOP2	ISAGAVQTAGKKGPGSLFNAPRGKKRPAPGSEEEEEEDSE.EDGNVNHGDLVGSSEDDADTVDDYGADSNSEDEEEGEAL	159
OsNO2	ESGSDLDVPSDSGAEEELSDSDASFEGGDSGDEEEEDDEDGDDPLADDFLAGSDDESOGGDSGVDSDESDD.....	136
	s g l deeeee d d l gs d d dd g ds d	
NOP2	LPI ERAARKQKAREAAAGI CWSSEETEDEEEEKEVTPESGPPKVEEADGGICINVDEEPFVLPAPAGEECDAQAPDLICR	238
OsNO2	LEAKSRAIDE.....EKEKAEAEAEELK.....LINRSEDEFRLPTKEELKEEALRPPNLPN	190
	k r e e e e e l i f l p e e a p l	
NOP2	VHKRIQDI VGI LRDFGACREGRSRSEYLNRLKKDLAIYYSYGDFLLGKLMDLFPLSELVEFLEANEVPRPVTLRNTLTK	318
OsNO2	LKRRISEI VRVLSNESKLRCKQVPRKDYVNCLEKTDIMSYGYNDFLVEAFIEMFPAVELVELLESFEKRPPCELRNTLTK	270
	r i i v l f r r y n l k d y y y d f l f p e l v e l e e p l r t n t l k	
NOP2	TRRRDLAALINRGVNLDPLGKWSKTGLVVDSSVPIIGATPEYLAGHYMLQGASSMLPVMALAPQEHRI LDMCCAPGGK	398
OsNO2	TRRRDLAALIPRGFNLDPLGKWSKVGLVVDSTISAGATVEYMAGHYMLQGASSFLPVMALAPQEKERI VDMAAPGGK	350
	t r r r d l a a l i r g n l d p g k w s k g l v v y d s g a t e y a g h y m q g a s s l p v m a l a p q e e r i d m a p g g k	
NOP2	TSYWAQLMKNTGVI LANDANAERLKSIVGNLHRLGVTNTIISHYDGRQFPKVVG...FDRVLLDAPCSGTGVI SKDPAVK	476
OsNO2	TTYI GALMKNTGI IYANEFNEKRLHGLLGNHRLGVTNTI VCNVDCKELPKVLGMNSVDRVLLDAPCTGTGTI VKDQPIK	430
	t y l n k n t g i a n n r l g n h r g v t n t i y d g p k v g d r v l l d a p c g t g i k d p k	
NOP2	TNKDEKDI LRCAHLCKELLLSAIDSVATSKTGGYLVYCTCSI TVEENEVVDYALKKRNVRVLPITGLDFGQEGFTRFRE	556
OsNO2	TSKGI EDI RDCAFVCKCLLLAAILDVCANSKTGGYVYSTCSLMI PENEAVVDYALKKRNVLKLPVCGLDGFRPGFI RFRE	510
	t k d i c a q k l l l a i d v a s k t g g y v y t c s e n e v v d y a l k k r n v l p g l d f g g f r f r e	
NOP2	RRFHPSLRSTRRFYPHTNMDGFFIAKPKKFSNSIPQSQTGNSETATPTNVDLPQVI PKSENSSQPAKAKGAAKTKQCL	636
OsNO2	HRFHTSLDKTTRRFYPHVNMDGFFIAKPKKFSNTIP.....VASESSNVPEEAI EKADPSSDDPCKCP....QSKK	578
	r f h s l t r r f y p h n n d g f f a k k k s n i p a i k s s k	
NOP2	CKQCHPKKASFQKLNGISKGADSELSTVPSVTIKTCASSFQDSSCPAGKAEI REPKVTEKLRQSPKLGSSKKVAFLRQ	716
OsNO2	HKDVKTNEETSILDGVTK.....DKRQTHETLKNHKK GKKRNGPESTKI KGDQKE.....TH	631
	k l g k k q k g k g k	
NOP2	NAPPKGTDTQTPAVLSPSKTQATLKPQDHQPLGRAKGEKQCLPEGPFKAFAFKCNDTPKGPQPPTVSPIRSSRPPPA	796
OsNO2	NEEPTSEKKCPVSAKIKKSVPKRISGNKGKLLDTGKGEKRRNWNVRDWEAYKKSRSKCV	693
	n p k l k g a k	
NOP2	KRKKSQSRGNSQLLS	812

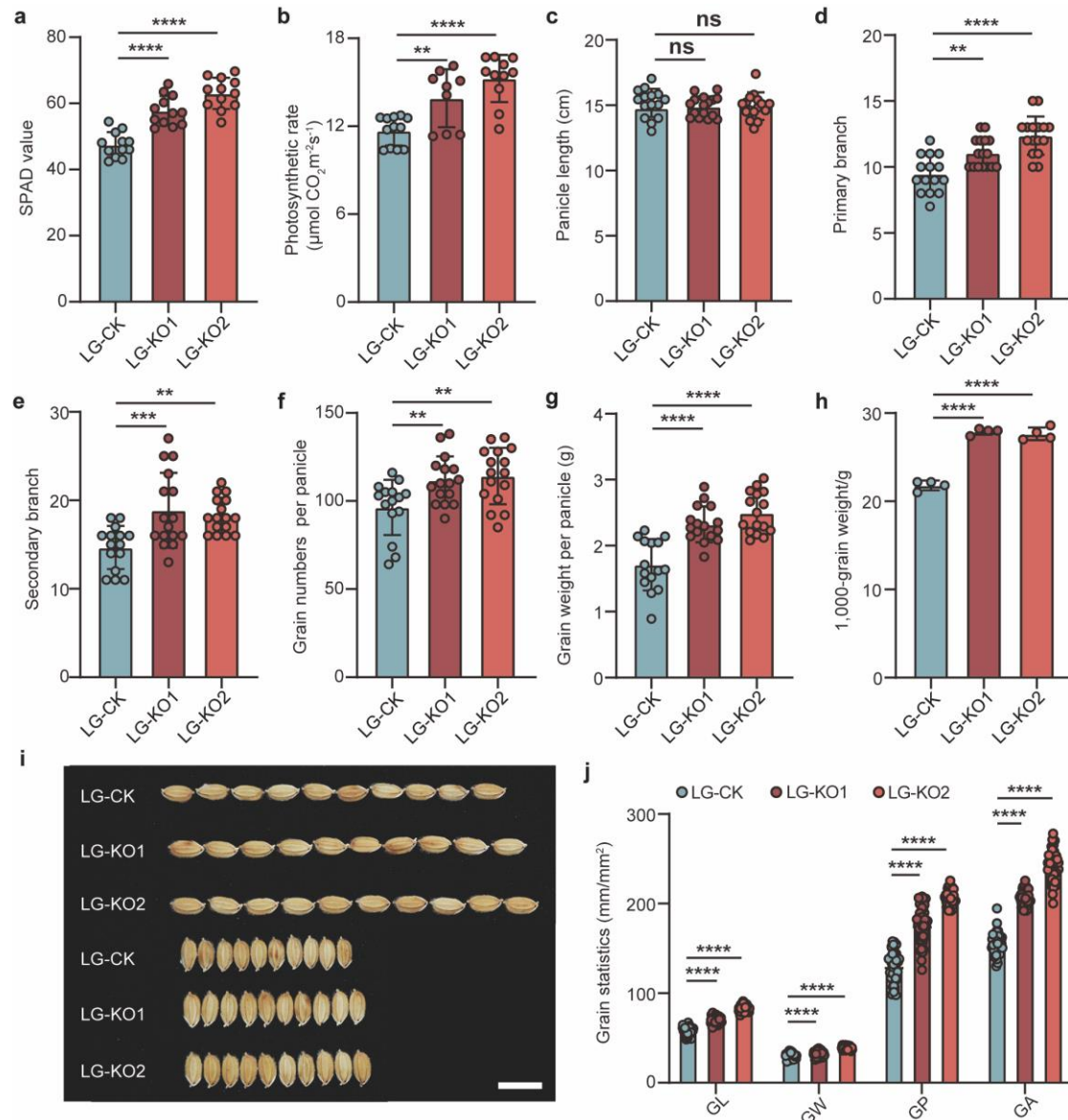
Supplementary Fig. 15| Comparison of the protein sequence of OsNOP2 and NOP2.



Supplementary Fig. 16| The OsNOP2-dependent RNA m⁵C modification nearly affects the transcription level of target transcripts. **a, The heatmap shows the Pearson correlation coefficient between the biological replicates. **b**, Overlap between hyper-m⁵C-modified transcripts with differentially expressed genes (DEGs) in KO2 versus WT plants.**

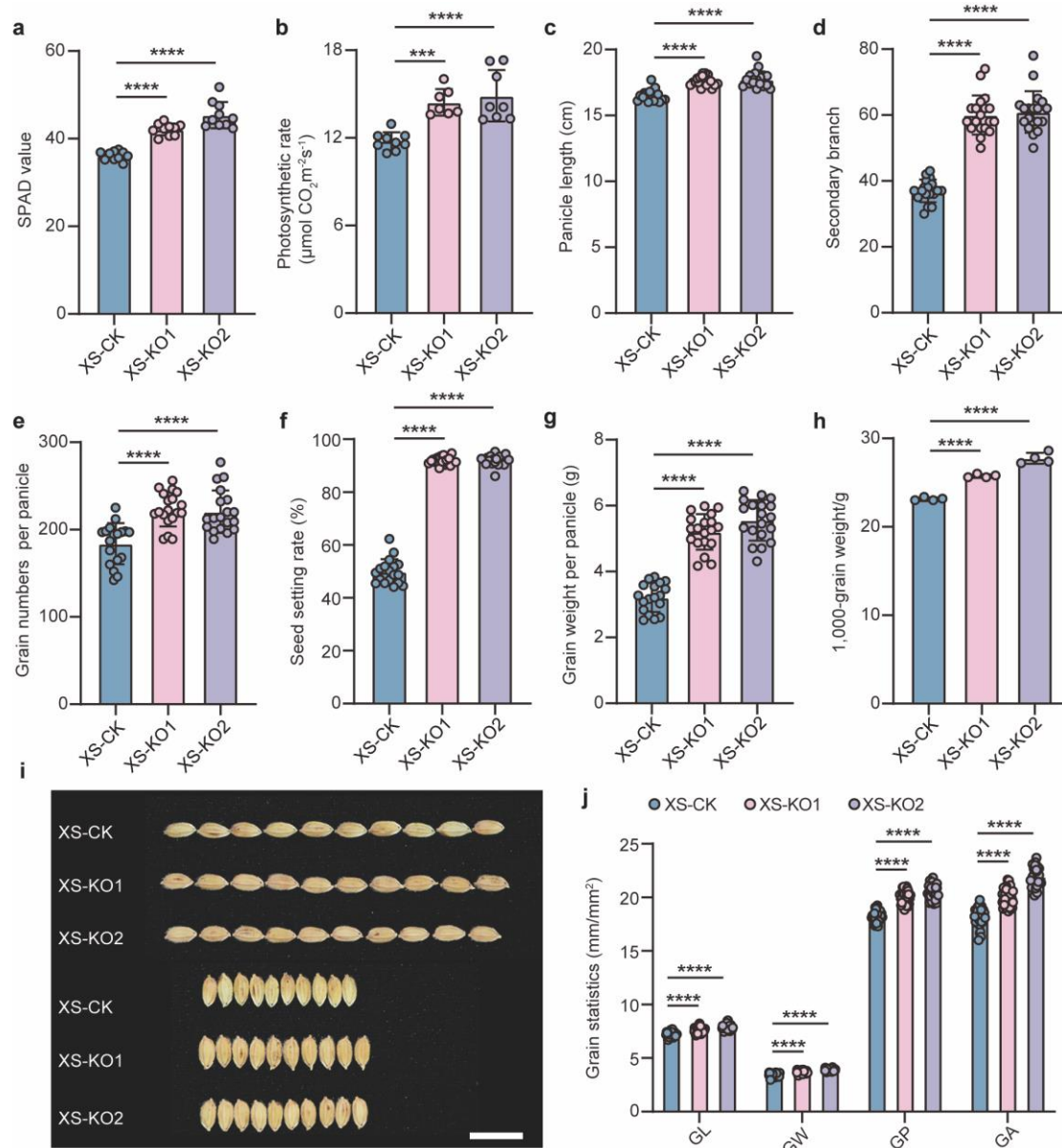


Supplementary Fig. 17| Pearson correlation coefficient of the proteomics between the biological replicates in WT and KO2 plants.



Supplementary Fig. 18| *OsNOP2* knockout improves the parameters of agronomic traits in variety Longgeng31. **a-b**, Statistical analysis of the SPAD value (**a**) and photosynthetic rate (**b**) from Longgeng31 (LG-CK), LG-KO1, and LG-KO2 plants grown in the field in Langfang. **c-h**, Statistical analysis of the panicle length (**c**), primary branch (**d**), secondary branch (**e**), grain numbers per panicle (**f**), grain weight per panicle (**g**), and 1,000-grain weight (**h**) from Longgeng31 (LG-CK), LG-KO1, and LG-KO2 plants grown in the field in Langfang. **i**, Grain morphologies of LG-CK, LG-KO1, and LG-KO2 grains. Scale bar, 1 cm. **j**, Statistical analysis of grain length (GL), grain width (GW), grain perimeter length (GP), and grain area size (GA)

of LG-CK, LG-KO1, and LG-KO2 plants. Data are mean $\pm$ S.D. (**a-b**, $n \geq 10$ plants; **c-g**, $n = 15$ panicles; **h**, $n = 4$ biological replicates; **j**, $n > 30$ grains). ns, no significance, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$; In **a-h**, P values are from one-way ANOVA (and nonparametric or mixed). In **j**, P values are from two-way ANOVA (and nonparametric or mixed).



Supplementary Fig. 19| *OsNOP2* knockout enhances the parameters of

agronomic traits in variety Xiushui134. a-b, Statistical analysis of the SPAD value

(a) and photosynthetic rate **(b)** from Xiushui134 (XS-CK), XS-KO1, and XS-KO2

plants grown in the field in Langfang. **c-h**, Statistical analysis of the panicle length

(c), secondary branch **(d)**, grain numbers per panicle **(e)**, seed setting rate **(f)**, grain

weight per panicle **(g)**, and 1,000-grain weight **(h)** from Xiushui134 (XS-CK), XS-

KO1, and XS-KO2 plants grown in the field in Langfang. **i**, Grain morphologies of

XS-CK, XS-KO1, and XS-KO2 grains. Scale bar, 1 cm. **j**, Statistical analysis of grain

length (GL), grain width (GW), grain perimeter length (GP), and grain area size (GA) of XS-CK, XS-KO1, and XS-KO2 plants. Data are mean $\pm$ S.D. (**a-b**, $n \geq 7$ plants; **c-g**, $n = 20$ panicles; **h**, $n = 4$ biological replicates; **j**, $n > 30$ grains). *** $P < 0.001$, **** $P < 0.0001$; In **a-h**, P values are from one-way ANOVA (and nonparametric or mixed). In **j**, P values are from two-way ANOVA (and nonparametric or mixed).

Supplementary Tables 1-20

Supplementary Table 1| Agronomic traits of rice plants grown in the field in Langfang in 2020.

	WT	KO1	KO2	n ^a
Plant height (cm)	106.2±2.81	110.5±3.89 ****	114.9±2.99 ****	15
Tiller number per plant	23.4±1.22	26.43±1.34 ****	26.93±1.44 ****	15
Panicle length (cm)	20.43±1.42	21.79±0.59 **	21.93±0.89 ***	15
Grain number per panicle	141.4±18.15	159.08±20.01 *	163.07±14.69 **	15
Primary branch	11.4±0.74	12.5±0.8 ***	12.87±0.64 ****	15
Secondary branch	26.47±3.56	30.42±3.26 **	32.07±2.79 ****	15
1,000-grain weight (g)	22.1±0.56	24.03±0.65 ***	25.61±0.5 ****	1,000 ⁴
Grain length (mm)	7.56±0.26	8.01±0.38 ****	7.91±0.27 ****	> 30
Grain width (mm)	3.28±0.15	3.6±0.13 ****	3.54±0.11 ****	> 30
Grain perimeter length (mm)	18.44±0.76	19.88±0.81 ****	19.73±0.84 ****	> 30
Grain area size (mm ²)	18.37±0.99	20.74±1.12 ****	20.52±0.94 ****	> 30
Plant height (cm)	106.2±2.81	110.5±3.89 ****	114.9±2.99 ****	15

a: Number of biological replicates.

Agronomic traits of WT, KO1, and KO2 rice were obtained from field-grown plants in Langfang (grown from May to October, 2020). Data are presented as means ± S.D. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed).

Compared with WT.

Supplementary Table 2| Agronomic traits of rice plants grown in the field in Langfang in 2021.

	WT	KO1	KO2	n ^a
Plant height (cm)	112.7±2.81	120.2±2.2 ****	124±1.89 ****	30
Tiller number per plant	20.7±3.74	28.6±3.75 ***	30.1±4.12 ****	30
Panicle length (cm)	20.43±0.87	21.49±1.03 **	22.9±1.55 ****	60
Grain number per panicle	146.43±23.97	162.28±22.06 **	181.18±26.22 ****	60
Primary branch	11.4±0.97	12.14±1.19 *	13.23±1.15 ****	60
Secondary branch	27.07±5.19	31.83±4.6 ***	33.68±5.35 ****	60
1,000-grain weight (g)	23.1±0.74	26.55±0.81 ***	27.75±0.3 ****	1,000 ⁴
Grain length (mm)	7.26±0.3	8.08±0.24 ****	8.63±0.26 ****	> 30
Grain width (mm)	3.14±0.17	3.7±0.12 ****	3.89±0.17 ****	> 30
Grain perimeter length (mm)	17.02±0.78	20.5±0.61 ****	21.63±0.7 ****	> 30
Grain area size (mm ²)	18.42±1.19	21.75±0.79 ****	24.39±1.38 ****	> 30
Seed setting rate (%)	77.73±7.36	75.51±7.46 ^{ns}	87.42±3.33 **	60
Biomass (g)	149.73±17.57	185.8±18.94 *	191.74±37.04 **	30
Grain yield per plant (g)	59.49±10.2	74.93±10.41 *	78.14±17.54 **	30
Harvest index	0.4±0.03	0.4±0.04 ^{ns}	0.41±0.02 ^{ns}	30

a: Number of biological replicates.

Agronomic traits of WT, KO1, and KO2 rice were obtained from field-grown plants in Langfang (grown from May to October, 2021). Data are presented as means ± S.D. ns, no significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed). Compared with WT.

Supplementary Table 3| Agronomic traits of rice plants grown in the field in Hainan in 2021.

	WT	KO1	KO2	n ^a
Plant height (cm)	72.7±4.81	76.2±3.28 **	78.3±2.98 **	15
Tiller number per plant	16.8±3.34	25.86±4.62 ****	23.2± 3.89 ***	15
1,000-grain weight (g)	24.1±0.48	27.15±0.34 ****	27.85±0.25 ****	1,000 ⁴
Grain length (mm)	8.04±0.19	8.51±0.29 ****	8.57±0.3 ****	> 30
Grain width (mm)	3.44±0.13	3.7±0.09 ****	3.84±0.12 ****	> 30
Grain perimeter length (mm)	19.86±0.97	21.29±1.06 ****	21.67±0.69 ****	> 30
Grain area size (mm ²)	20.53±0.89	22.34±1.2 ****	24.53±1.08 ****	> 30

a: Number of biological replicates.

Agronomic traits of WT, KO1, and KO2 rice were obtained from field-grown plants in Hainan (grown from December 2021 to April 2022). Data are presented as means ± S.D. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed). Compared with WT.

Supplementary Table 4| Agronomic traits of rice plants grown in the field in Langfang in 2022.

	WT	KO1	KO2	n ^a
Plant height (cm)	103.8±5.9	110.4±5.2 ****	113.2±3.9 ****	50
Tiller number per plant	13.24±2.04	16.14±2.25 ****	15.96±1.89 ****	50
Panicle length (cm)	20.36±1.18	22.06±1.03 ****	22.03±1.12 ****	30
Grain number per panicle	133.7±15.6	162±16.22 ****	158.87±13.66 ****	30
Primary branch	10.6±0.89	11.27±1.05 ****	12.23±0.86 ****	30
Secondary branch	23.7±3.77	30.93±3.6 ****	28.33±3.49 ****	30
Grain weight per panicle (g)	3.19±0.41	3.56±0.35 ****	3.61±0.3 ****	30
1,000-grain weight (g)	24.13±0.85	25.5±0.05 ****	26.58±0.3 ****	1,000 ⁴
Grain length (mm)	7.82±0.28	8.02±0.23 ****	8.58±0.34 ****	> 30
Grain width (mm)	3.46±0.14	3.61±0.09 *	3.89±0.097 *	> 30
Grain perimeter length (mm)	19.34±0.71	20.18±0.57 ****	21.64±0.704 ****	> 30
Grain area size (mm ²)	19.44±0.96	20.96±0.74 ****	23.9±0.75 ****	> 30
Seed setting rate (%)	90.47±7.63	87.59±3.2 ^{ns}	86.47±4.7 ^{ns}	30
Biomass (g)	113.04±15.63	164.52±39.67 ****	167.68±36.41 ****	50
Grain yield per plant (g)	45.24±7.16	62.16±14.82 ****	59.42±14.38 ****	50
Grain yield per plot (kg)	4.31±0.5	5.5±0.43 *	5.54±0.53 *	150 ³

a: Number of biological replicates.

Agronomic traits of WT, KO1, and KO2 rice were obtained from field-grown plants in Langfang (grown from May to October, 2022). Data are presented as means ± S.D. ns, no significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed). Compared with WT.

Supplementary Table 5| Agronomic traits of rice plants grown in the field in Hainan in 2022.

	WT	KO1	KO2	n ^a
Plant height (cm)	73.36±2.78	77.93±2.57 ****	79.9±2.68 ****	20
Tiller number per plant	23.21±4.27	25±4.53 ****	27.9±3.21 ****	20
Panicle length (cm)	16.71±1.56	18.37±1.72 **	18.31±0.89 **	20
Grain number per panicle	67±13.54	101.3±30.12 ****	95.8±9.26 ***	20
Primary branch	5.4±0.97	7±1.15 ****	7.3±0.95 ****	20
Secondary branch	13.3±2.75	18.4±4.35 ****	18.3±2.95 ****	20
Grain weight per panicle (g)	1.35±0.19	1.62±0.18 ***	1.57±0.32 **	20
1,000-grain weight (g)	25.06±0.18	27.28±0.13 ****	28.49±0.15 ****	1,000 ⁴
Grain length (mm)	8.04±0.3	8.49±0.18 ****	8.74±0.36 ****	> 30
Grain width (mm)	3.61±0.16	3.81±0.09 ****	3.89±0.15 ****	> 30
Grain perimeter length (mm)	20.16±1.05	21.57±1.1 ****	22.22±0.86 ****	> 30
Grain area size (mm ²)	20.73±0.91	22.71±1.02 **	25.18±1.27 ****	> 30
Seed setting rate (%)	72.53±5.9	71.86±8.9 ^{ns}	72.02±2.58 ^{ns}	20
Grain yield per plant (g)	20.25±2.51	28.22±4.48 ****	30.14±4.52 ****	20

a: Number of biological replicates.

Agronomic traits of WT, KO1, and KO2 rice were obtained from field-grown plants in Hainan (grown from December 2022 to April 2023). Data are presented as means ± S.D. ns, no significance, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed). Compared with WT.

Supplementary Table 6| Agronomic traits of rice plants grown in the field in Langfang in 2023.

	WT	KO1	KO2	n ^a
Plant height (cm)	107.8±3.51	119.03±2.91 ****	123.13±3.66 ****	60
Tiller number per plant	15.8±2.93	21.6±4.72 ****	21.3±3.46 ****	60
Panicle length (cm)	19.63±1.03	21.26±0.9 ****	22.16±1.02 ****	30
Grain number per panicle	120.4±9.1	147.81±26.65 ****	150.29±14.15 ****	30
Primary branch	10.87±0.92	12.25±1.18 ****	12.71±1.05 ****	30
Secondary branch	22.47±3.8	26±3.39 ****	26.82±4.45 ****	30
Grain weight per panicle (g)	2.88±0.32	3.43±0.34 ****	3.52±0.25 ****	30
1,000-grain weight (g)	25±0.43	26.45±0.25 ****	26.8±0.16 ****	1,000 ⁴
Grain length (mm)	7.88±0.37	8.41±0.25 ****	8.32±0.31 ****	> 30
Grain width (mm)	3.54±0.15	3.88±0.17 ****	3.84±0.18 ****	> 30
Grain perimeter length (mm)	19.67±0.8	21.42±0.7 ****	21.13±0.77 ****	> 30
Grain area size (mm ²)	20.27±1.19	24.09±1.2 ****	23.8±1.1 ****	> 30
Seed setting rate (%)	93.51±2.19	94.63±2.4 ^{ns}	94.5±2.7 ^{ns}	30
Biomass (g)	101.06±13.27	154.32±15.56 ****	162.03±16.23 ****	50
Grain yield per plant (g)	32±5.92	44.43±8.19 ****	46.43±6.15 ****	50
Grain yield per plot (kg)	8.69±0.23	10.46±1.46 *	10.07±0.12 *	232 ³

a: Number of biological replicates.

Agronomic traits of WT, KO1, and KO2 rice were obtained from field-grown plants in Langfang (grown from May to October, 2023). Data are presented as means ± S.D. ns, no significance, * $P < 0.05$, **** $P < 0.0001$; P values are from one-way ANOVA (and nonparametric or mixed). Compared with WT.

Supplementary Table 7| Primers used for construction of transformation vectors and characterization of transgenic plants.

Name	Primer (5'-3')	Usage
OsNOP2-KO-F	GCGGGGAAGAAGGGGAAG	Identification of <i>OsNOP2</i> KO mutants (<i>Nipponbare</i> , <i>LG31</i> , <i>XS134</i>)
OsNOP2-KO-R	TAGCTCGCGTGAATGCTACA	
OsNOP2-OE-SmaI-F	GTAGAAGAGGTACCCGGGATGGC GAAGAAGGGCGCGCC	<i>OsNOP2</i> overexpression in <i>Nipponbare</i>
OsNOP2-OE-XbaI-R	GCAGGTCGACTCTAGATCATACTT GTTTACTCCTCGA	
OsNOP2-COM-Kpn1-F1	ACGAATTCGAGCTCGGTACCACT AGCTGATAATTCCACATTC	Construction of transformation vector for creating complementary materials
OsNOP2-COM-R1	GTCGTCGGAGGACGACTCGAGCA GCCTCTGCTTCTTG	
OsNOP2-COM-F2	CTCGAGTCGTCTCCGACGACTC CGAGCTGGAGCAGC	
OsNOP2-COM-Kpn1-R2	TCTAGAGGATCCCCGGGTACCTC ATACTTGTTTACTCCTCGA	
Ta-F	CTCGGACGCCGGTAGCAGCGAGG	Identification of <i>OsNOP2</i> KO mutants in Wheat (KN199)
Ta-R	CCCATCTGATTCCTCTCCAGAGT	
Sl-F	GTGATAGTGAAGGAACGGATGGG	Identification of <i>OsNOP2</i> KO mutants in Tomato (AC)
Sl-R	GTCCGTAGAGAAATTGGTCTAGG	

Supplementary Table 8| Primers used for vector construction.

Name	Primer (5'-3')	Usage
pAN580-OsNOP2-F	TCCGGAGCTAGCTCTAGAATGGC	Subcellular localization
	GAAGAAGGGCGCGCC	
pAN580-OsNOP2-R	CACCATGGATCCCCCGGGTCATAC	
	TTGTTTACTCCTCGA	OsNOP2 expression
OsNOP2-GST-F	GGTTCCGCGTGGATCCATGGCGA	
	AGAAGGGCGCGCC	
OsNOP2-GST-R	GTCGACCCGGGAATTCTACTTGTT	OsNOP2 ^{mut} expression
	TACTCCTCGACTTC	
OsNOP2 ^{mut} -GST-F1	GGTTCCGCGTGGATCCATGGCGA	
	AGAAGGGCGCGCC	OsNOP2 ^{mut} expression
OsNOP2 ^{mut} -GST-R1	CGCTGTCTCCTCCCTCAAGGAGG	
	CGTCGTCCGAGTC	
OsNOP2 ^{mut} -GST-F2	GACTCGGACGACGCCTCCTTGAG	Verify the enzyme activity of OsNOP2 designed by prediction
	GGAGGAGACAGCG	
OsNOP2 ^{mut} -GST-R2	GTCGACCCGGGAATTCAGTCATC	
	AGACTCATCTGAG	Verify the enzyme activity of OsNOP2 designed by prediction
OsNOP2 ^{T422V} -GST-F	GGTTCCGCGTGGATCCATGGCGA	
	AGAAGGGCGCGCC	
OsNOP2 ^{T422V} -GST-R	GGATCCTTCCAAATGACCCCTGT	Verify the enzyme activity of OsNOP2 designed by prediction
	GCCTGTGCAGGGT	
OsNOP2 ^{H375K} -GST-F	GGTTCCGCGTGGATCCATGGCGA	
	AGAAGGGCGCGCC	Verify the enzyme activity of OsNOP2 designed by prediction
OsNOP2 ^{H375K} -GST-R	GCGATGTATGTTGCCCAAAAGTC	
	CCTTCAGCCTTTTC	
OsNOP2 ^{A2G} -GST-F	GGTTCCGCGTGGATCCATGGGGA	Verify the enzyme activity of OsNOP2 designed by prediction
	AGAAGGGCGCGCC	
OsNOP2 ^{A2G} -GST-R	CTCATTGAACTCATTTGCATAAAT	
	TATTCCAGTATTC	NOP2 expression
NOP2-GST-F	GGTTCCGCGTGGATCCATGGGGC	
	GCAAGTTGGACCCTACG	
NOP2-GST-R	GTCGACCCGGGAATTCAGATAGC	NOP2 expression
	AGCAGCTGGCTGTTGCCCTG	

Supplementary Table 9| Fragments used for demethylase enzyme activity and EMSA.

Name	Primer (5'-3')	Usage
Bio-m ⁵ C-RNA	/5Biosg/rGrGrCrGrArUrGrCrGrG/i5M	Enzyme activity <i>in vitro</i> and EMSA
Bio-C-RNA	-rC-TOM/rGrGrCrGrGrArGrGrCrCrG /5Biosg/rGrGrCrGrArUrGrCrGrGrCrG rGrCrGrGrArGrGrCrCrG	

Supplementary Table 10| Primers used for identification of the stable transgenic lines with site-directed mutagenesis of amino acids.

Name	Primer (5'-3')	Usage
OsNOP2 ^{T422V} -F	TTGAATTGGGTGCAGCATTT	Identification of the stable transgenic lines
OsNOP2 ^{T422V} -R	GCACAGTCTCTGATGTCCTCAAT	
OsNOP2 ^{H375K} -F	TGTCATGCACAGGAATAATTTATGC	
OsNOP2 ^{H375K} -R	GATTAAAATGGACCAAAAATTATTG	

Supplementary Table 11| Primers used for qRT-PCR and RNA m⁵C-IP-qPCR.

Name	Primer (5'-3')
qOsUBQ-F	GCTCCGTGGCGGTATCAT
qOsUBQ-R	CGGCAGTTGACAGCCCTAG
qOsNOP2-F	GAGGAAGACGATGAGGATGG
qOsNOP2-R	CATCTGAGTCCACACCAGAG
RCA-P1-F1	CGATCGTCATCTTGGGCTGCTCG
RCA-P1-R1	CACGGGTGTGGAGAACATTGGC
RCA-P2-F2	CGAAGACGAGCTCACACTGG
RCA-P2-R2	GGCTTCTACATCGCCCCTGC
RCA-control-F	CCACAGGCCACACGCTGAAG
RCA-control-R	CACAGACTCCGATCCAGGTCC
PsbA-P1-F1	CCATTATTCTACTTCTGCGGCG
PsbA-P1-R1	CAATTAGCTCATAAGGACCACC
PsbA-P2-F2	CGGATTCAATTTCAACCAATCTG
PsbA-P2-R2	GTTGTGAGCATTACGTTCTGTGC
PsbA-control-F	GCCGAGTTGGTACGAATTCC
PsbA-control-R	CATATACCTATTTACATAAC
OsACS6-P1-F1	GGCGGAGCGGCAACGGCGGC
OsACS6-P1-R1	GCCCCCGCGTCCCTGCACC
OsACS6-P2-F2	CAGGTCTTACAGCGAGAAAGG
OsACS6-P2-R2	GTTGCTCAATGTCGTGAAGC
OsACS6-control-F	AACAGATATTGATTGATACAAAG
OsACS6-control-R	GCCACCAAACGATTCATACCAACA
PDIL1-P1-F1	GGCTGGGGCCGACTCTGCCTTCTC
PDIL1-P1-R1	CTGCGAACGATGTGCCAAGCGAG
PDIL1-P2-F2	CGTAGAACTCGACGACCATGAAC
PDIL1-P2-R2	GGCTTGGATCTCGCTGCTGCTC
PDIL1-control-F	CAGATGTTTGCGAAAATCAAC
PDIL1-control-R	GGTGGAGAGGAATCTCCGAGC
OsLIR1-peak-F	CGCGCAACGCAACACGGCGAGGAG
OsLIR1-peak-R	CTAGCAGTGTCGTTGGATTGTCGG
OsCHLP-peak-F	CGGGTCGTACGTGGTGCCTACAAC
OsCHLP-peak-R	CTCCTGGAACGCGATGGCGTACTCG
OsPORA-peak-F	GCATCGCGACGACGGGCTTGTTC
OsPORA-peak-R	CGCCCACCACCTGCGCCAGCCGC
OsCAO1-peak-F	GGTGGAGATGCGTCGAACATTGC
OsCAO1-peak-R	CAAGGATACTGGGATCCATACCCG
Fd-GOGAT1-peak-F	CTGCGCGGGCTTCTCCGCGGCCTC
Fd-GOGAT1-peak-R	GCCACGCTCCCACGTGCGGCGG
OsNIA1-peak-F	GCGGCAGTTTCGGCCACCTCGAGC
OsNIA1-peak-R	CGCGGCGGTGGCGAGACGAGCGG
Sl-RCA-peak-F	GAAGACAGAATTGGTGTTTGC
Sl-RCA-peak-R	CAATTCCAGTACTTCCAATC

Sl-PsbA-peak-F	CCCAATCTGGGAAGCGGCATC
Sl-PsbA-peak-R	GATTCCTAGAGGCATACCATC
Sl-ACS6-peak-F	ATGACAAGGTCACGGAACCG
Sl-ACS6-peak-R	CGTTTTAGGTAGAGTTGCC
Sl-PDIL1-peak-F	CAGCGGAAATTTTAAGCCAG
Sl-PDIL1-peak-R	GCATCCTTGGATGATTTAATTTC
Ta-RCA-peak-F	ATGGCTGCTGCCTTCTCCTC
Ta-RCA-peak-R	GCAAGACCCTTCCACTTGTC
Ta-PsbA-peak-F	GTAGATATTGATGGTATTCG
Ta-PsbA-peak-R	TAAGAAGTGTAGAACAATTAG
Ta-ACS6-peak-F	CTCTTCTACTTCCTCCAGCTC
Ta-ACS6-peak-R	GGGTGGTAGGGGTCGTCGGCG
Ta-PDIL1-peak-F	CCTCATTCAAGACAGTGACTC
Ta-PDIL1-peak-R	CTAGCTTCTTGCAGTGTCCGCAC

Supplementary Table 12| Primers used for RIP-qPCR.

Name	Primer (5'-3')
RCA-RIP-F	GGCTTGACGCTTGCTATTAATT
RCA-RIP-R	AACAAAAACTTGTCATGCCAG
PsbA-RIP-F	TTCCAGGCAGAGCATAACATC
PsbA-RIP-R	GATTCGTTTTTCAGTGGTTTCCC
OsACS6-RIP-F	ATAATGAAAGCATCGTGACAGC
OsACS6-RIP-R	TCTTGCGCAATCTCTCTCTATT
PDIL1-RIP-F	CGCTGATAACGTCCATGATTTT
PDIL1-RIP-R	CTTAGCGATGACAACATCCTTG

Supplementary Table 13| Primers used for relative luciferase activity.

Name	Primer (5'-3')
RCA-LUC-HindIII-F	GAGGTCGACGGTATCGATAAGCTTATGAACATCGCCGACA ACC
RCA-LUC-BamHI-R	GCCGCTCTAGAACTAGTGGATCCAAAGGTGTAAAGGCAGC TGCCG
PsbA-LUC-HindIII-F	GAGGTCGACGGTATCGATAAGCTTATGATCCCTACCTTATT GAC
PsbA-LUC-BamHI-R	GCCGCTCTAGAACTAGTGGATCCAGCAGCCAAGAAGAAG TGT
OsACS6-LUC-HindIII-F	GAGGTCGACGGTATCGATAAGCTTGCAGAGGTTGTTGATG ATCTAG
OsACS6-LUC-BamHI-R	GCCGCTCTAGAACTAGTGGATCCATTGACCTTTGCCTCCTC CAAT
PDIL1-LUC-HindIII-F	GAGGTCGACGGTATCGATAAGCTTAGTCCAAGAAATTTTT GAAGG
PDIL1-LUC-BamHI-R	GCCGCTCTAGAACTAGTGGATCCGACCAGCGGTTTCCTTG TTCTTC

Supplementary Table 14| MeRIP-seq of the WT and *OsNOP2*-KO2

transcriptomes in 3-week-old seedlings (Excel file).

Supplementary Table 15| List of amino acid sequences from multiple species aligned with OsNOP2 (Excel file).**Supplementary Table 16| List of designed sites for mutant proteins based on diff values (Excel file).****Supplementary Table 17| List of hyper-m⁵C-modified transcripts in *OsNOP2*-KO2 plants compared to WT (Excel file).****Supplementary Table 18| Go and KEGG enrichment analysis of the hyper-m⁵C-modified transcripts (Excel file).****Supplementary Table 19| List of differentially expressed genes between WT and KO2 seedlings (Excel file).****Supplementary Table 20| Proteomics FPKM expression data between WT and KO2 seedlings (Excel file).**