New Phytologist **Supporting Information**

Intragenic Heterochromatin-mediated Alternative Polyadenylation Modulates MiRNA and Pollen Development in Rice

Li-Yuan You, Juncheng Lin, Hua-Wei Xu, Chun-Xiang Chen, Jun-Yu Chen, Jinshan Zhang, Jian Zhang, Ying-Xin Li, Congting Ye, Hui Zhang, Jing Jiang, Jian-Kang Zhu, Qingshun Q. Li, Cheng-Guo Duan

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Fig. S13. The relative expression of representative *MIRNAs* and stem-loops

Fig. S14. The relative accumulation of miRNAs in inflorescence tissues.

Fig. S15. The flowering time phenotype of different *XRNL* transgenic plants

Fig. S16. Sequence alignment between *Arabidopsis* and rice AIPP1 proteins

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Fig. S19. The poly(A) site usage and expression analysis of flowering time- and pollen development-related genes in different tissues.

Fig. S1. Phylogenetic analysis of OsASI1 protein. Phylogenetic tree was generated using SALAD SEARCH (https://salad.dna.affrc.go.jp/CGViewer/en/index.html).

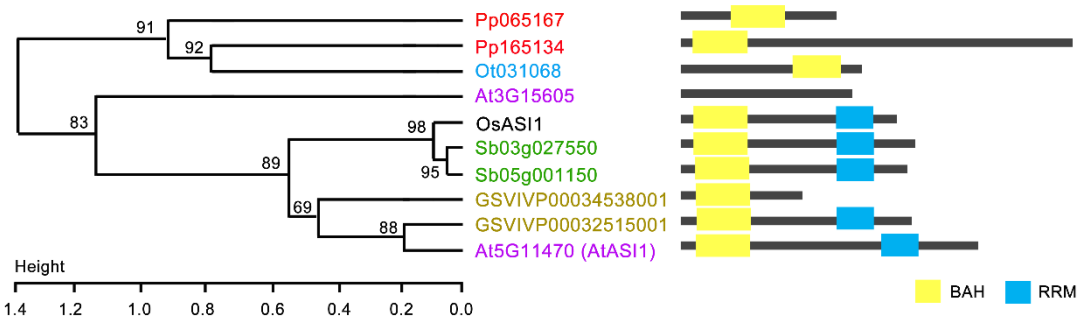


Fig. S2. Mutation information of *OsASI1*, *OsEDM2* and *OsAIPP1a*. A-C. For each gene, two mutant alleles were generated by CRISPR/Cas9-mediated editing. Red boxes indicate the target regions of guide RNAs. Black and gray boxes represent coding exons and untranslated regions, respectively. (A) Upper panel: the mutation information of *osasi1-1* and *osasi1-2 mutants*. Lower panel: the premature termination and alternative ORF information in *osasi1-1* and *osasi1-2* mutants. (B) the mutation information of *osaipp1a*. (C) the mutation information of *osedm2*.

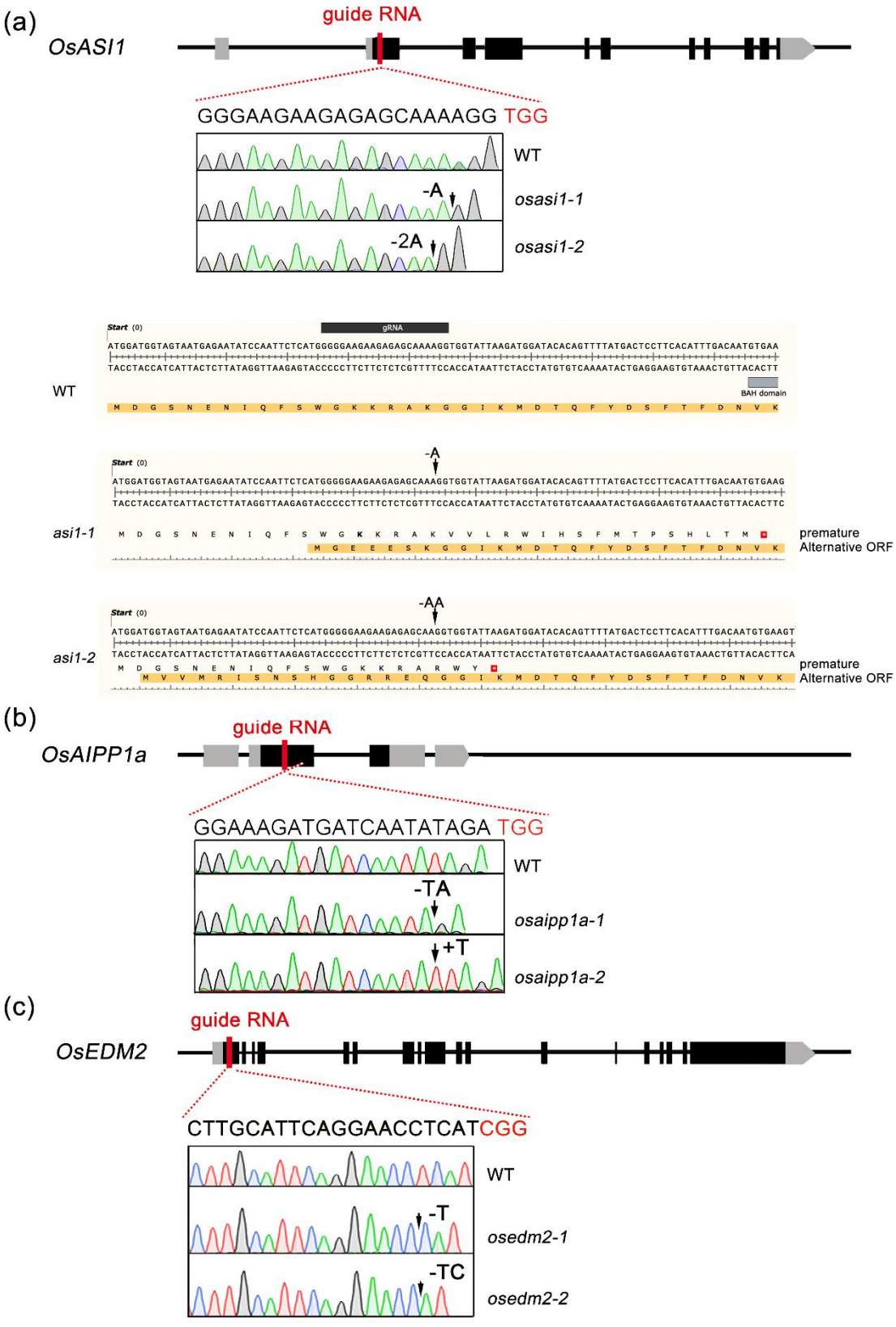

(a)
OsASI1
guide RNA
GGGAAGAAGAGAGCAAAAGG TGG
WT
-A
osasi1-1
-2A
osasi1-2
Start (0)
gRNA
ATGGATGGTAGTAATGAGAATATCCAATTCTCATGGGGGAAGAAGAGAGCAAAAGGTGGTATTAAGATGGATACACAGTTTTATGACTCCTTCACATTTGACAATGTGAA
TACCTACCATCATTACTCTTATAGGTTAAGAGTACCCCCTTCTTCTCTCGTTTCCACCATAATTCTACCTATGTGTCAAAATACTGAGGAAGTGTAAACTGTTACACTT
WT
BAH domain
M D G S N E N I Q F S W G K K R A K G G I K M D T Q F Y D S F T F D N V K
-A
ATGGATGGTAGTAATGAGAATATCCAATTCTCATGGGGGAAGAAGAGAGCAAAGGTGGTATTAAGATGGATACACAGTTTTATGACTCCTTCACATTTGACAATGTGAAG
TACCTACCATCATTACTCTTATAGGTTAAGAGTACCCCCTTCTTCTCTCGTTTCCACCATAATTCTACCTATGTGTCAAAATACTGAGGAAGTGTAAACTGTTACACTTC
asi1-1
M D G S N E N I Q F S W G K K R A K V V L R W I H S F M T P S H L T M
M G E E E S K G G I K M D T Q F Y D S F T F D N V K
premature
Alternative ORF
-AA
ATGGATGGTAGTAATGAGAATATCCAATTCTCATGGGGGAAGAAGAGAGCAAGGTGGTATTAAGATGGATACACAGTTTTATGACTCCTTCACATTTGACAATGTGAAGT
TACCTACCATCATTACTCTTATAGGTTAAGAGTACCCCCTTCTTCTCTCGTTCCACCATAATTCTACCTATGTGTCAAAATACTGAGGAAGTGTAAACTGTTACACTTCA
asi1-2
M D G S N E N I Q F S W G K K R A R W Y
M V V M R I S N S H G G R R E Q G G I K M D T Q F Y D S F T F D N V K
premature
Alternative ORF
(b)
OsAIPP1a
guide RNA
GGAAAGATGATCAATATAGA TGG
WT
-TA
osaipp1a-1
+T
osaipp1a-2
(c)
OsEDM2
guide RNA
CTTGCATTCAGGAACCTCATCGG
WT
-T
osedm2-1
-TC
osedm2-2

Fig. S3. The morphological phenotypes of pistil and stamen of *osasi1* and *osxrnl* mutants. Scale, 0.5 mm.

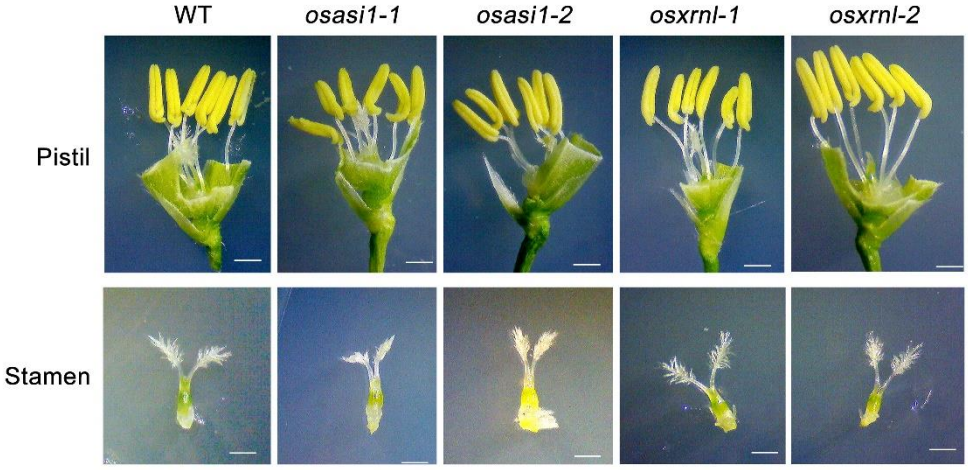


Fig.S4. The expression level of OsASI1 protein in *OsASI1ox* plants. Western blotting result showing the expression levels of OsASI1 proteins in different transgene lines. Ponceaus staining serves as protein loading control.

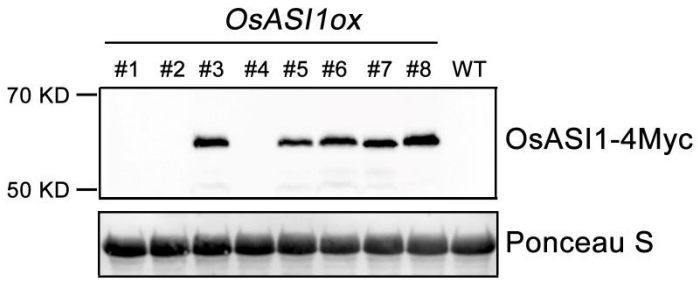


Fig. S5. The effect of *OsASI1* dysfunction on global gene expression. (a). Volcano diagram showing the differentially expressed genes in the *osasi1-1* mutant compared to WT plants. **(b-cz).** Heatmap diagrams showing the expression patterns of selected development (B) and stress response-related (C) DEGs in *osasi1-1* mutant and WT plants. The names of representative annotated genes are labeled. **(d).** RT-qPCR results showing the relative expression of two flowering-related genes *OsHd3a* and *OsRFT1* in *osasi1* mutants and WT plants. Error bars represent the ±SD of three biological replicates. Unpaired two-tailed Student's t test was performed and *, p-value < 0.01.

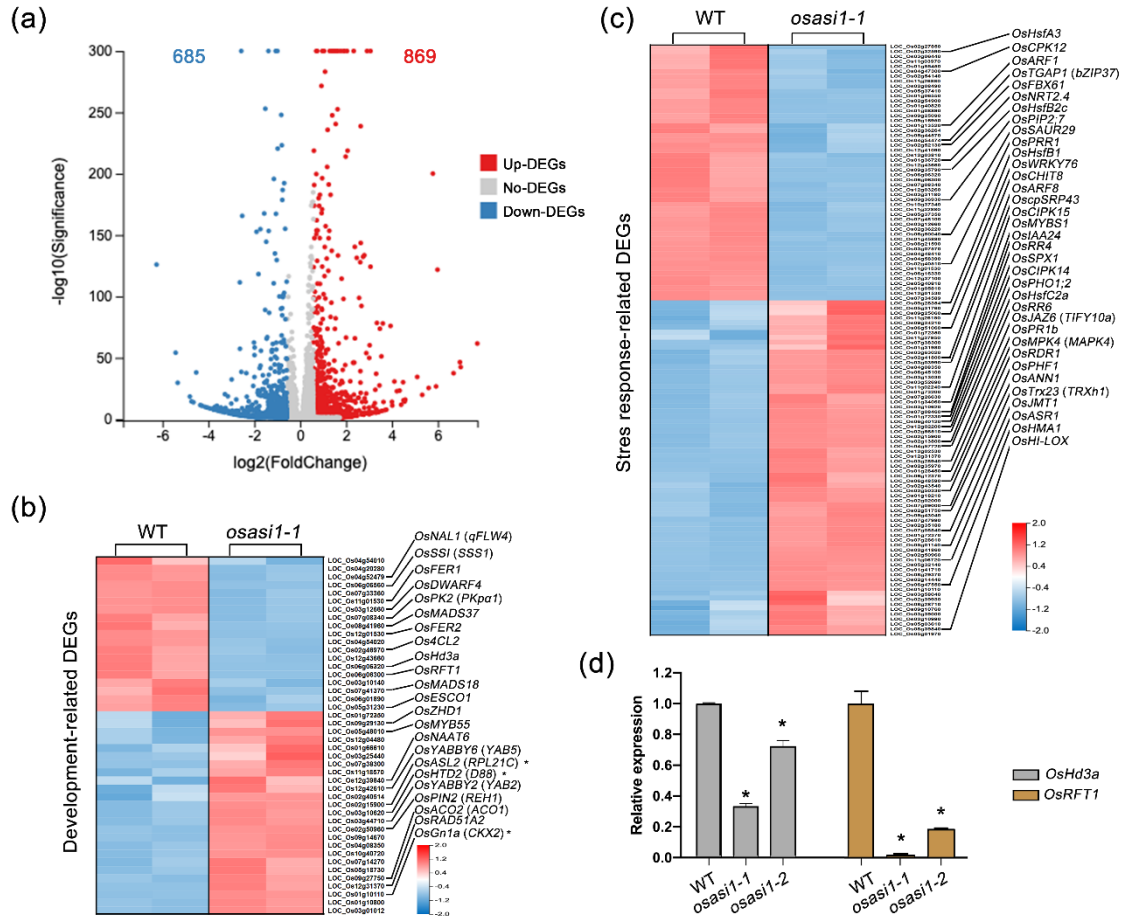
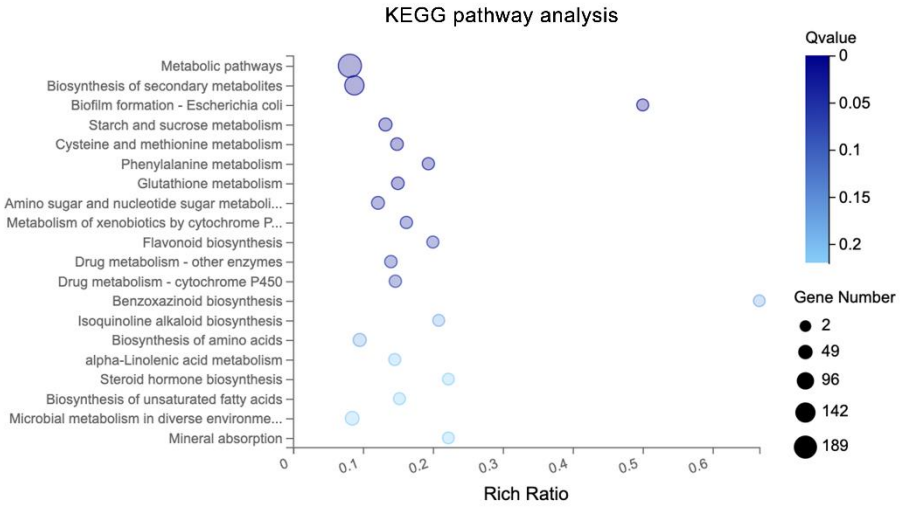


Fig. S6. The enrichment analysis of *osasil1-1* DEGs. (a). The statistics of Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment. **(b).** The statistics of Gene Ontology (GO) enrichment

(a)



(b)

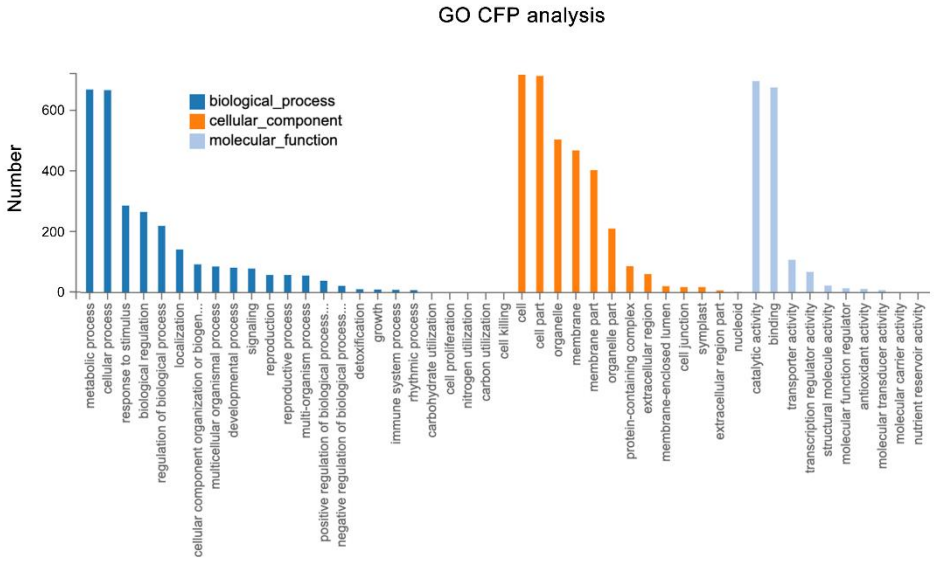


Fig. S7. Subcellular localization of *OsASI1*, *OsEDM2*, *OsAIPP1a* and *OsAIPP1b* proteins. GFP-fused proteins were transiently expressed in rice protoplast cells. NLS-RFP (RFP) served as a nuclear marker.

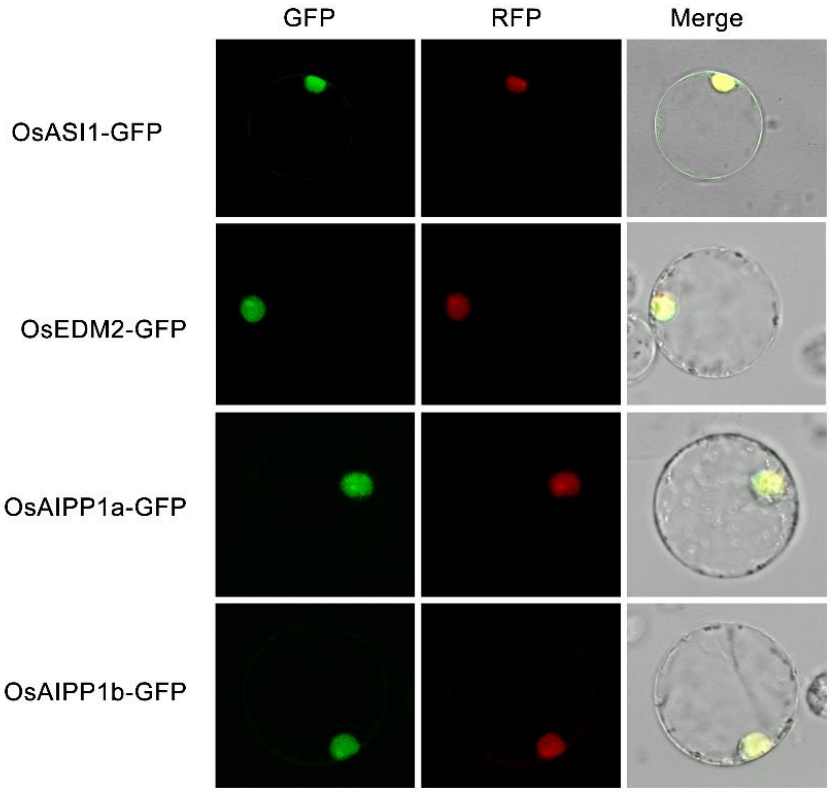


Fig. S8. The effect of OsASI1 dysfunction on the alternative polyadenylation of 3'UTR. (a). Variation of 3' UTR length in *osasi1-1* compared to WT. 'Long' indicates lengthening, 'Short' indicates shortening. The *padj* <0.05 was used a threshold to identify significant 3' UTR length variation events. **(b).** IGV screenshots of 3' UTR lengthening events. **(c).** Overlapping of 3' UTR lengthening genes, 3' UTR shortening genes, TE-excluding genes and TE-containing genes.

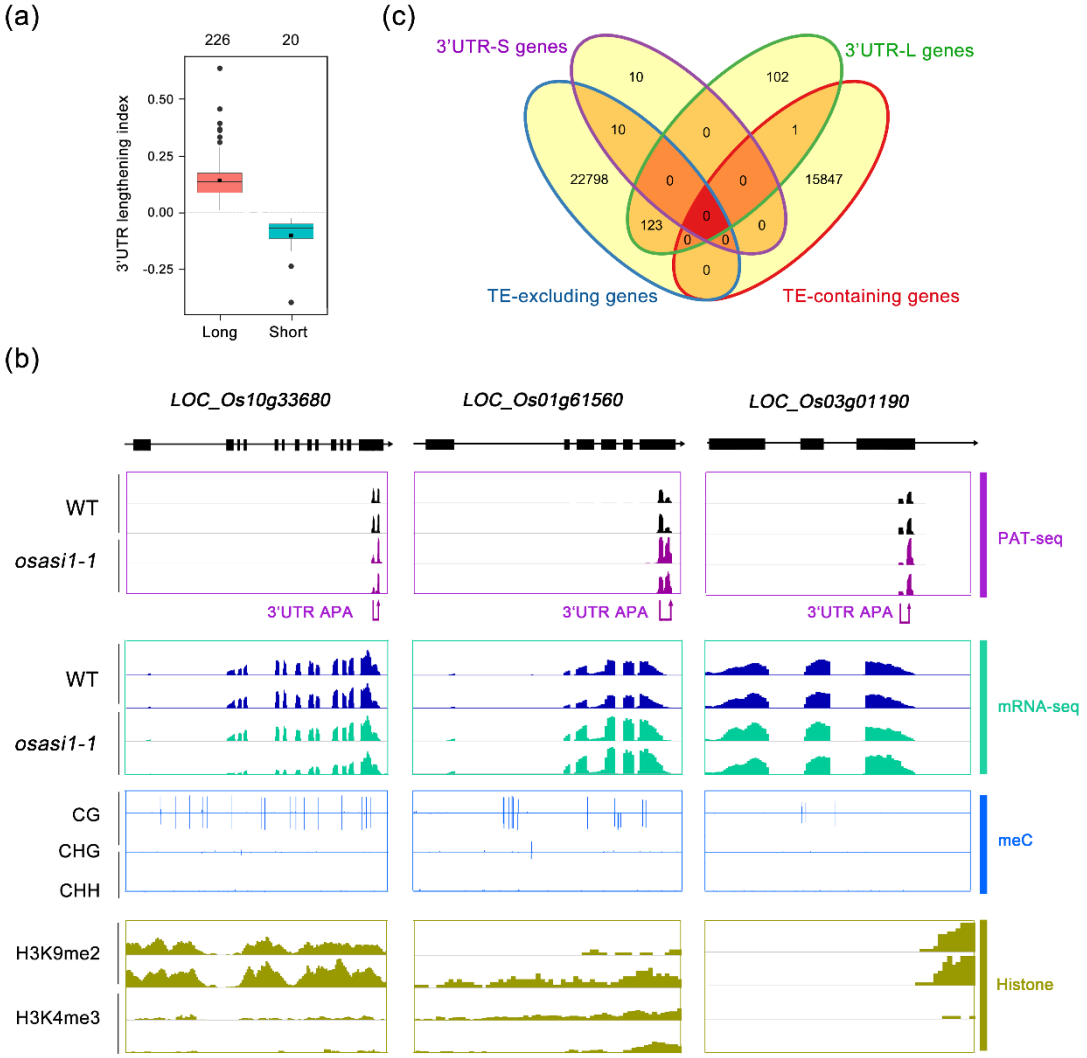


Fig. S9. Gene Ontology analysis of deAPA genes in *osasi1-1*. Red dots indicate Molecular Function category, cyan dots indicate Biological Processes category. FDR: false discovery rate.

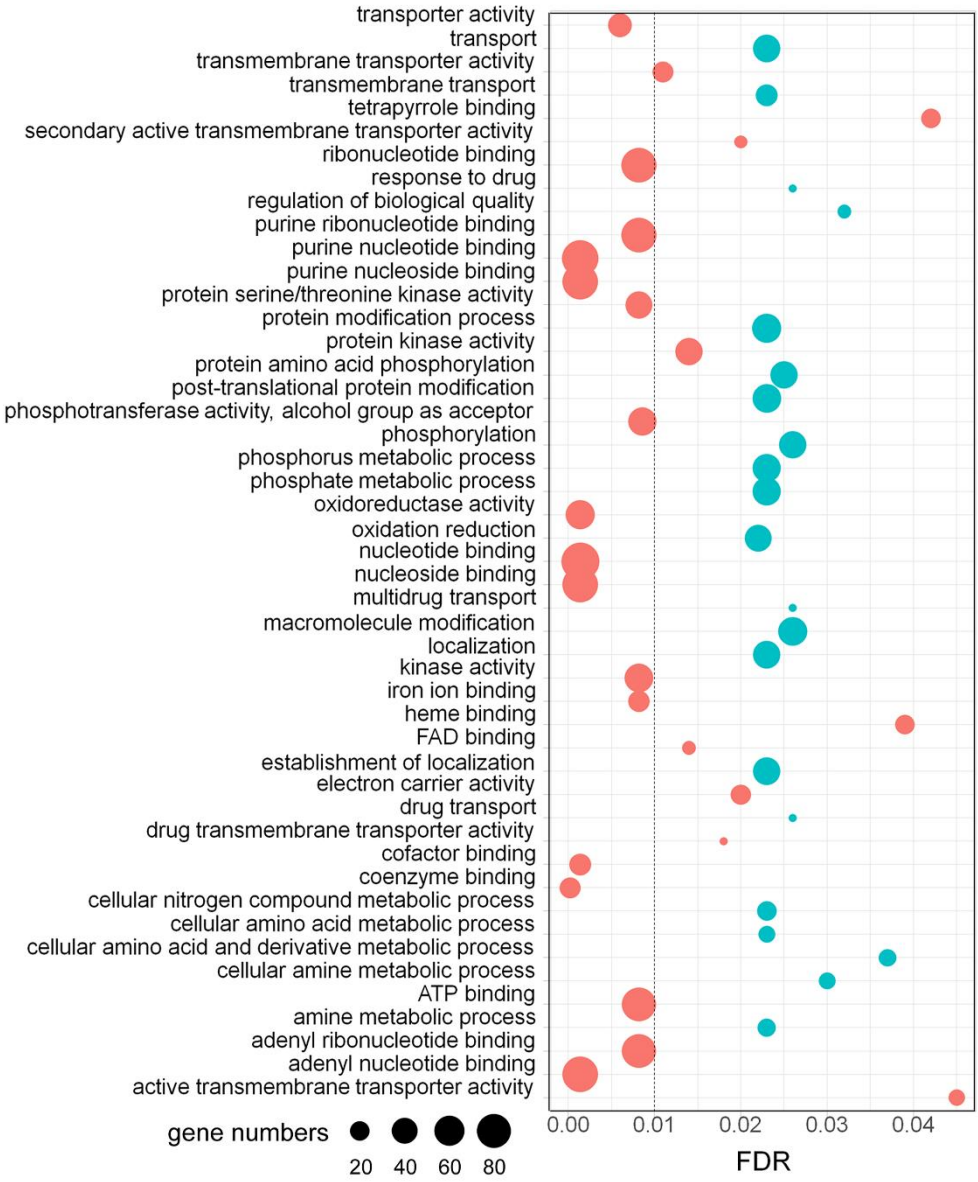


Fig. S10. ChIP-qPCR analysis of H3K9me2 density at selected target genes of OsASI1. (a-b). H3K9me2 enrichment was normalized to input. Error bars represent ±SD of three biological replicates. Unpaired two-tailed Student's t test was performed and *, p-value < 0.01. The positions of the test regions were labeled in x-axis as shown in Fig. 4B. (A) the H3K9me2 enrichment in 14-day-old seedlings. **(b)** the enrichment of H3K9me2 in the inflorescence tissues of wild-type plants.

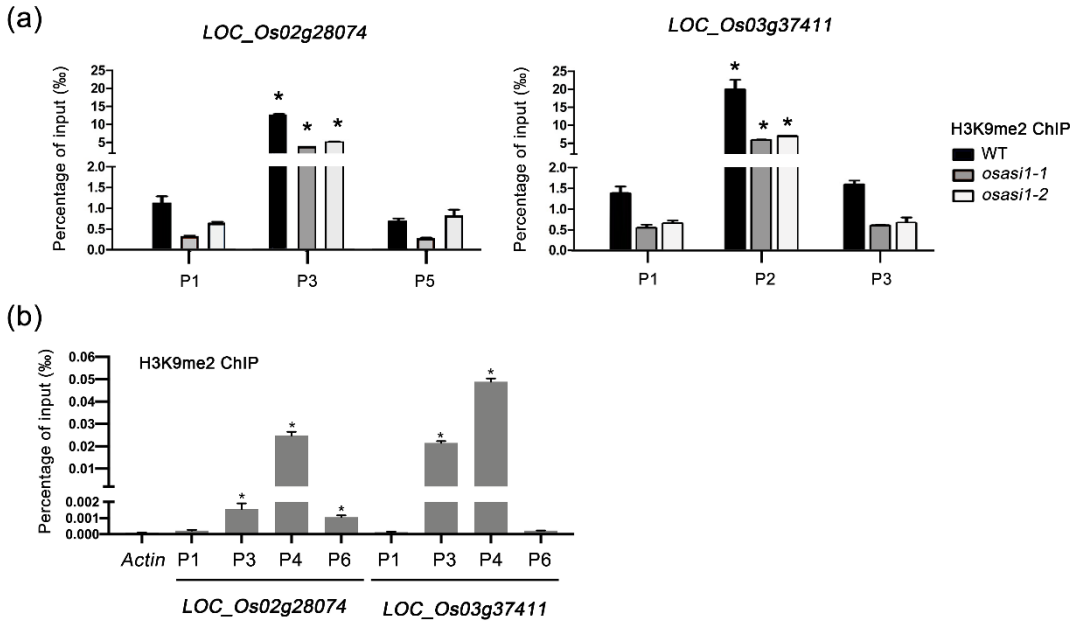


Fig. S11. The subcellar localization of OsXRNL protein and mutation information of *OsXRNL* gene. (a). Subcellular analysis showing a nuclear localization pattern of the OsXRNL protein. The localization assay was performed in rice protoplast cells. An NLS-RFP fusion (RFP) was co-expressed to indicate the nuclei. **(b).** Two mutant alleles were generated by CRISPR/Cas9-mediated editing. Red boxes indicate the target regions of guide RNAs. Black and gray boxes represent coding exons and untranslated regions, respectively.

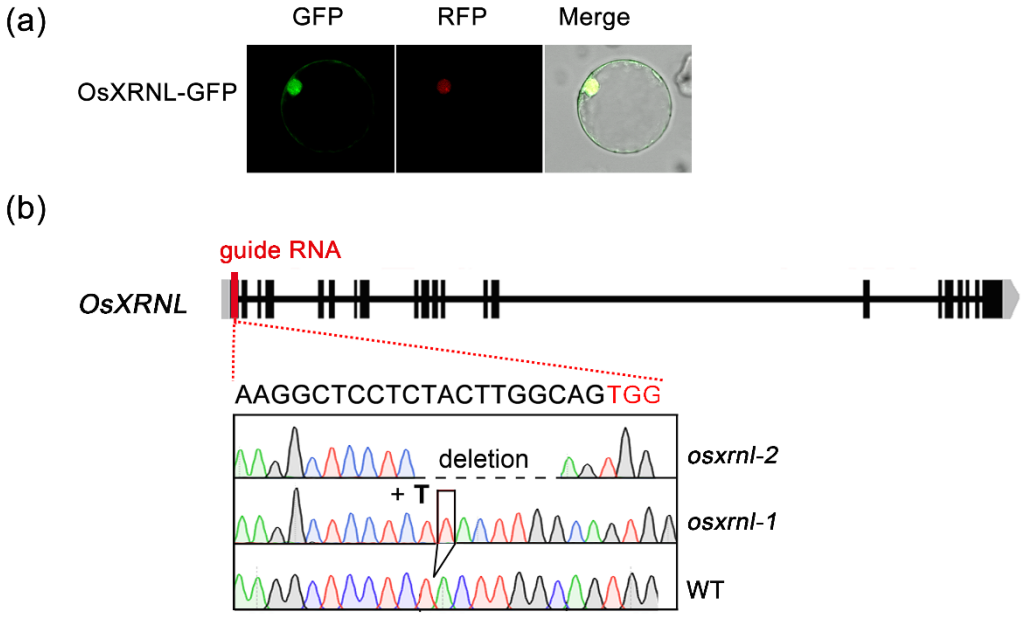


Fig. S12. Length distribution of mapped small RNAs from WT, *osasi1-1* and *osxrnl-1*. The Y axis indicates the percentage of abundance of different lengths among the total abundance of small RNAs.

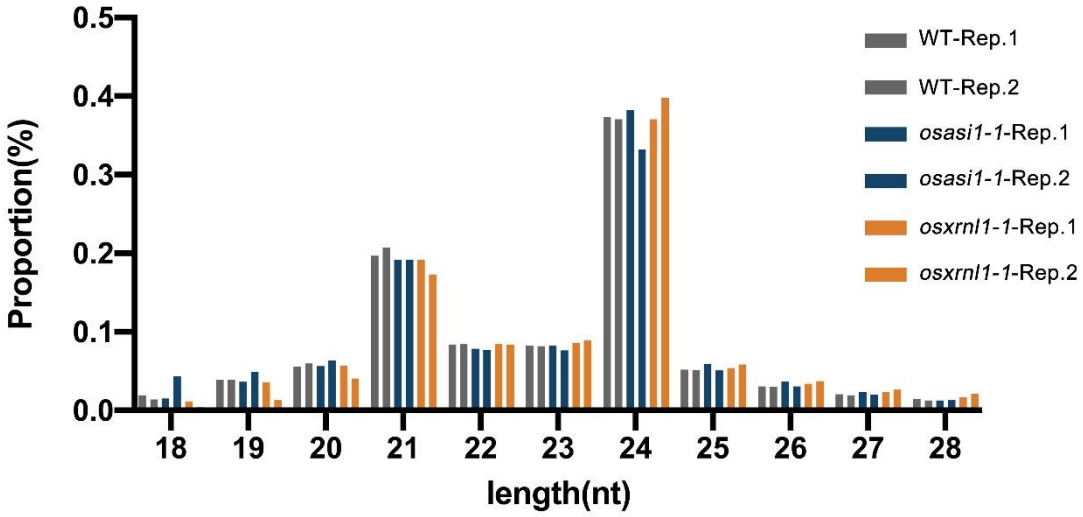


Fig. S13. The relative expression of representative *MIRNAs* and stem-loops. RT-qPCR results showing the ectopic expression of the pri-miRNAs and stem-loops of selected miR1882e and miR398b in WT plants and *osasi1* and *osxrnl* mutants. Error bars represent the ±SD of three biological replicates. Unpaired two-tailed Student's t test was performed and *, p-value < 0.0, ns no significance.

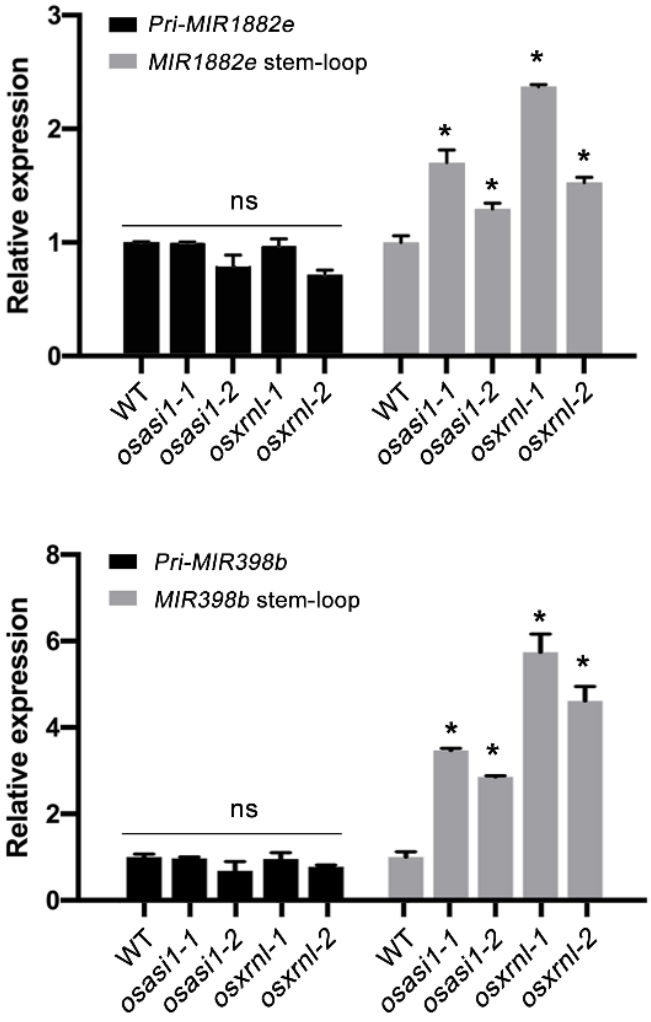


Fig. S14. The relative accumulation of miRNAs in inflorescence tissues. qPCR results showing the relative expression of selected miRNAs in inflorescence tissues of WT, *osasi1* and *osxrnl* mutants. Error bars represent the ±SD of three biological replicates. Unpaired two-tailed Student's t test was performed and *, p-value < 0.05.

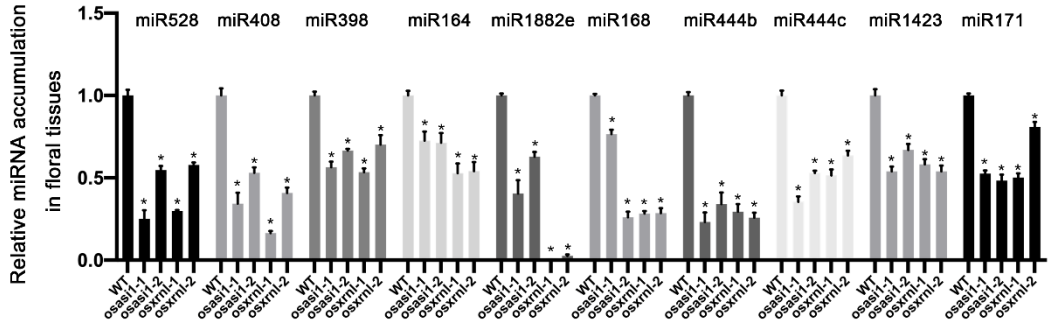


Fig. S15. The flowering time phenotype of different *XRNL* transgenic plants.
The photographs were taken at the heading time of wild-type plants. Scale, 5 cm.

Fig. S16. Sequence alignment between *Arabidopsis* and rice AIPP1 proteins.
Blue lines indicate the region of RRM motif.

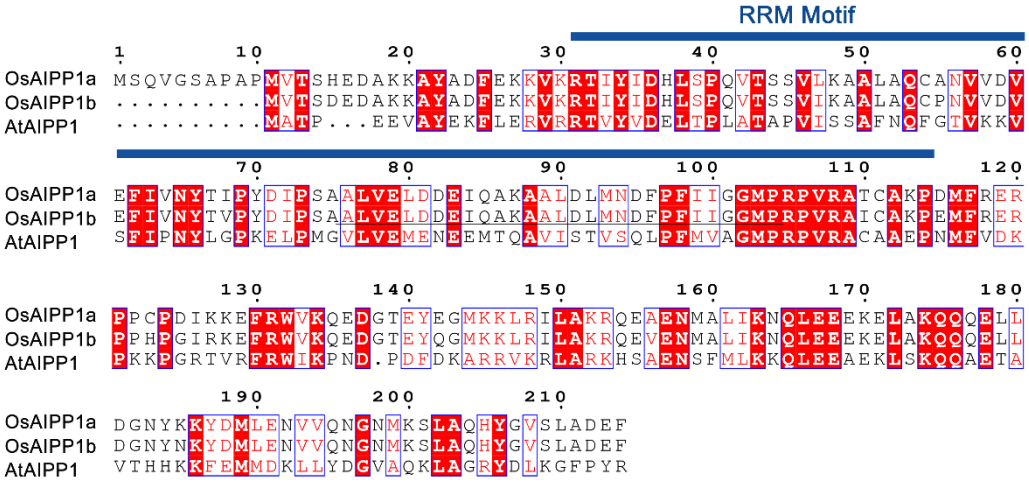


Fig. S17. The relative expression of *OsASI1*, *OsAIPP1a*, *OsAIPP1b* and *OsEDM2* in different tissues. RT-qPCR results showing the relative expression of *OsASI1*, *OsAIPP1a*, *OsAIPP1b* and *OsEDM2* in different tissues of WT plants. Error bars represent the ±SD of three biological replicates.

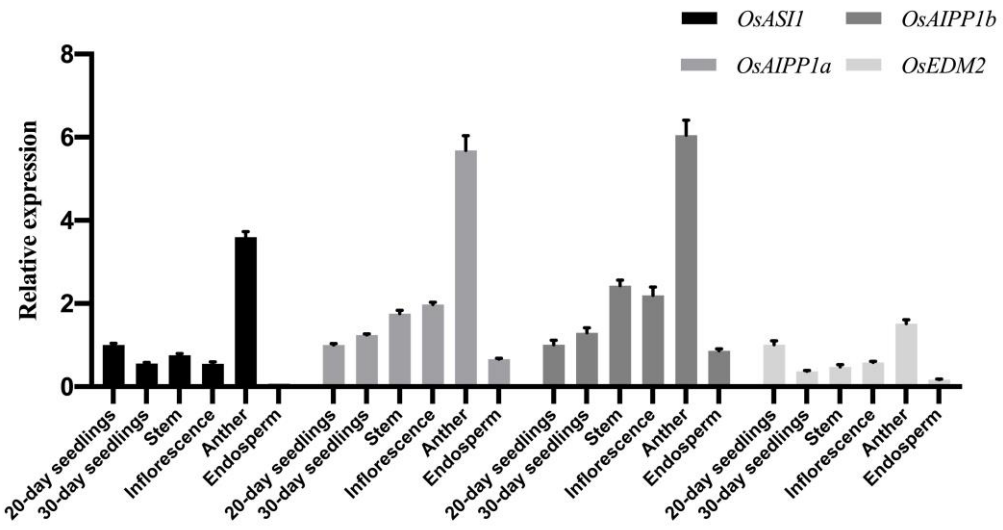


Fig. S18. Statistics analysis of flowering time, pollen defective rate and seed-setting rate of *osedm2* and *osaipp1a* mutants. The column diagrams showing the days to heading (a), pollen defective rate (b) and seed setting rate (c) of the selected mutants. The data points are shown as dots. Error bars represent the ±SD (n=15 to 40). Unpaired two-tailed Student's t test was performed. *, p-value < 0.01.

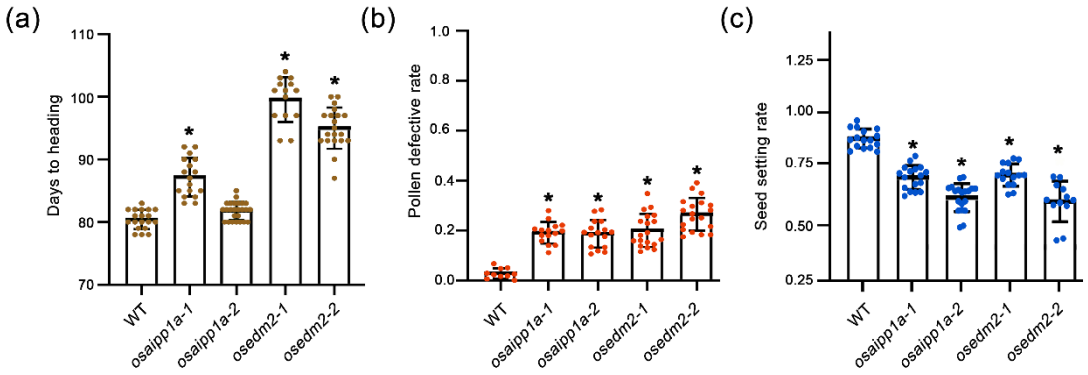


Fig. S19. The poly(A) site usage and expression analysis of flowering time- and pollen development-related genes in different tissues. (a). IGV snapshots of PAT-seq and mRNA-seq data showing the poly(A) usasge patterns and gene expression of flowering time- and pollen development-related genes in WT and mutants. One representative replicate was shown for each sequencing. **(b).** RT-qPCR results showing the relative expression of *Hd3a* and *RFT1* in 60-day-old plants and inflorescence tissues. Error bars represent the ±SD of three biological replicates. Unpaired two-tailed Student's t test was performed and *, p-value < 0.05.