Supplemental Data

miR2105 and kinase OsSAPK10 co-regulate OsbZIP86 to mediate drought–induced ABA biosynthesis in rice

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Supplementary Data include:

Supplemental Tables 1-2

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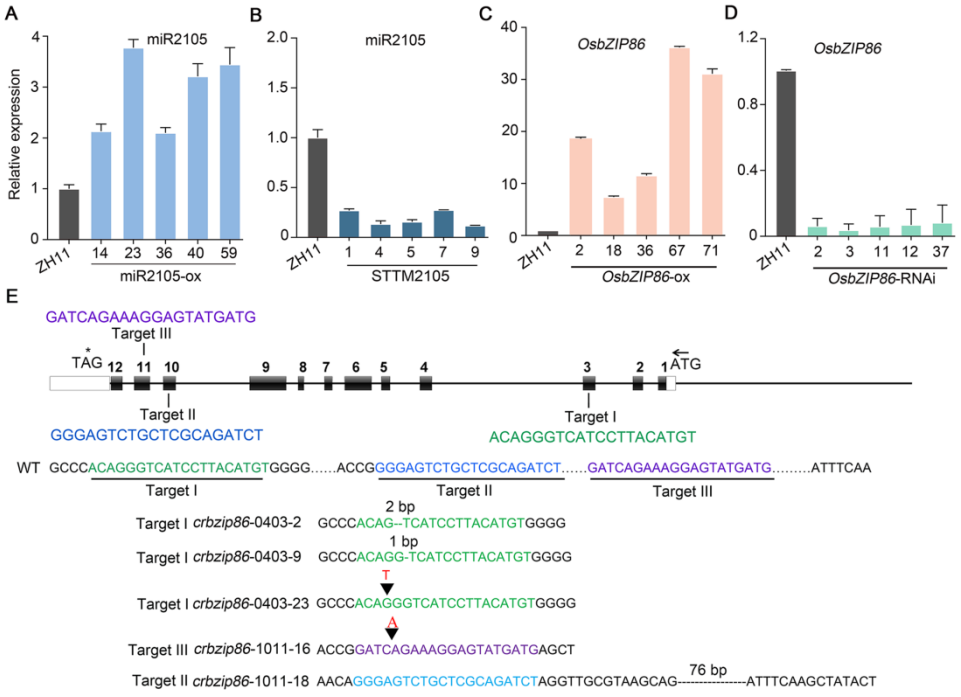
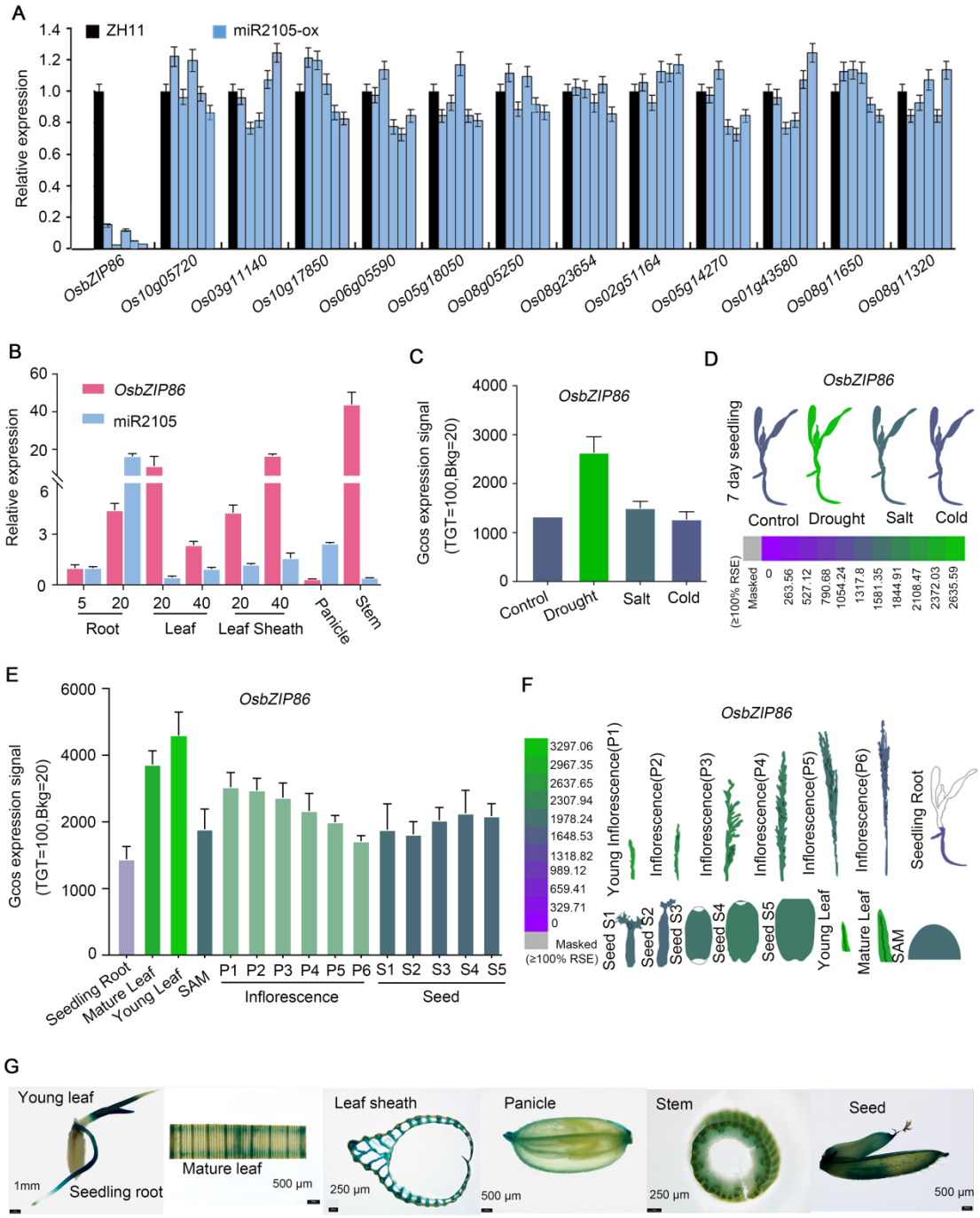


Supplemental Figure S1. Identification of miR2105 and *OsbZIP86* transgenic rice.

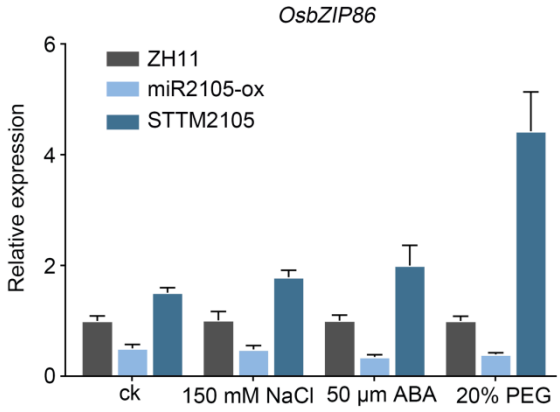
(A,B) Expression levels of miR2105 in seedlings of transgenic rice overexpressing miR2150 (miR2105-ox) or with downregulation of miR2105 (STTM2105). *U6* was used as a miRNA reference gene. (C,D) Expression levels of *OsbZIP86* in seedlings of transgenic rice overexpressing *OsbZIP86* (*OsbZIP86*-ox) and *OsbZIP86*-RNAi. *e-EF-1a* was used as an mRNA reference gene. Means ± SD (n = 3) are shown in (A–D). All qRT-PCR analyses for gene expression were performed using three biological replicates with similar results. (E) Generation of target-site mutations of *OsbZIP86* in representative knockout lines (*crbzip86*) using the CRISPR/Cas9 system. The sequences located in the third, tenth, and 11th exons of *OsbZIP86* were selected as target sites of the sgRNA. Filled black bars indicate exons and lines represent introns of *OsbZIP86*. The white boxes represent the 3′ and 5′ untranslated regions. The black arrow shows the start codon, and the black asterisk indicates the stop codon. The “T” and “A” insertions are marked with red; deletions are marked with green or black dashes.



Supplemental Figure S2. *OsbZIP86* is a target gene of miR2105 and the expression pattern analyses of *OsbZIP86*.

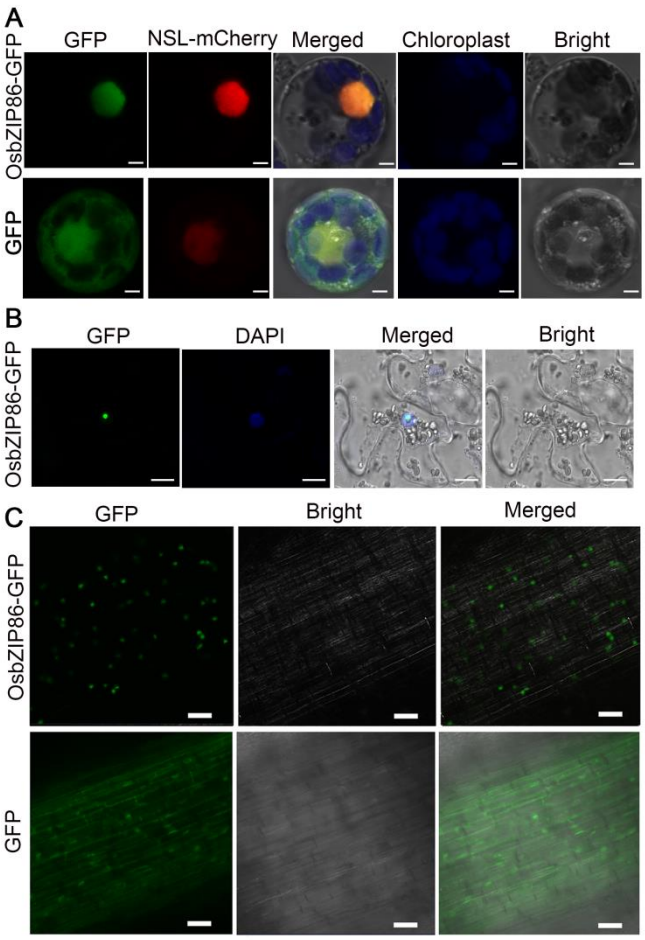
(A) Expression levels of the predicted 13 target genes of miR2105 in leaves of the wild-type ZH11 and miR2105-ox lines. Means ± SD (n = 3) are shown. All qRT-PCR analyses for genes expression were performed with three biological replicates with similar results. (B) Expression patterns of miR2105 and *OsbZIP86* in various organs of ZH11 under normal growth conditions by qRT-PCR. Expression of roots of 5-day-old seedlings were normalized as 1. Means ± SD (n = 3) are shown. All qRT-PCR

analyses for gene expression were performed with three biological repeats with similar results. (C–F) Expression patterns of *OsbZIP86* under drought, salt, and cold stresses (C,D) and in various organs of rice (E,F). Datas are from the Rice eFP Browser (http://bar.utoronto.ca/efprice/cgi-bin/efpWeb.cgi). The GeneChip operating software (GCOS) was used for calculation of the expression values with a target intensity (TGT) value of 100. Most tissues were sampled in triplicate. The image was generated with Plant eFP (http://bar.utoronto.ca/eplant). Values are means ± SD of three biological replicates. (G) Activity of *OsbZIP86* promoter in different tissues in transgenic rice by GUS staining of *OsbZIP86pro:GUS* transgenic rice under normal conditions.



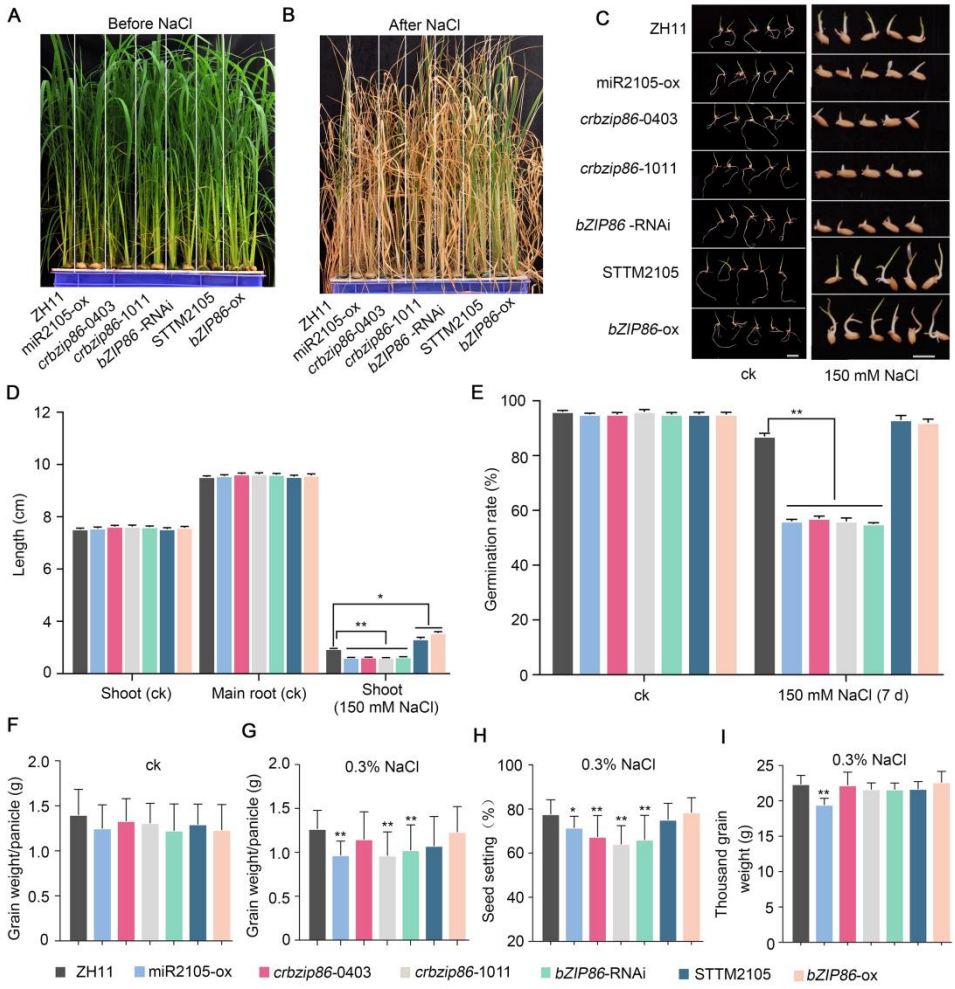
Supplemental Figure S3. Expression changes of *OsbZIP86* in ZH11, miR2105-ox and STTM2105 under normal conditions and treatment of salt, ABA and PEG.

Total RNA was isolated from 2-week-old rice seedlings grown in Yoshida solution. For salt, ABA and PEG treatments, 2-week-old seedlings grown in Yoshida solution were treated with 150 mM NaCl, 50 μM ABA or 20 % PEG for 2 h. *e-EF-1a* was used as mRNA reference gene, and the *OsbZIP86* expression of ZH11 under different condition was normalized as 1, respectively. Means ± SD (n = 3) are shown.



Supplemental Figure S4. Subcellular localization of OsbZIP86.

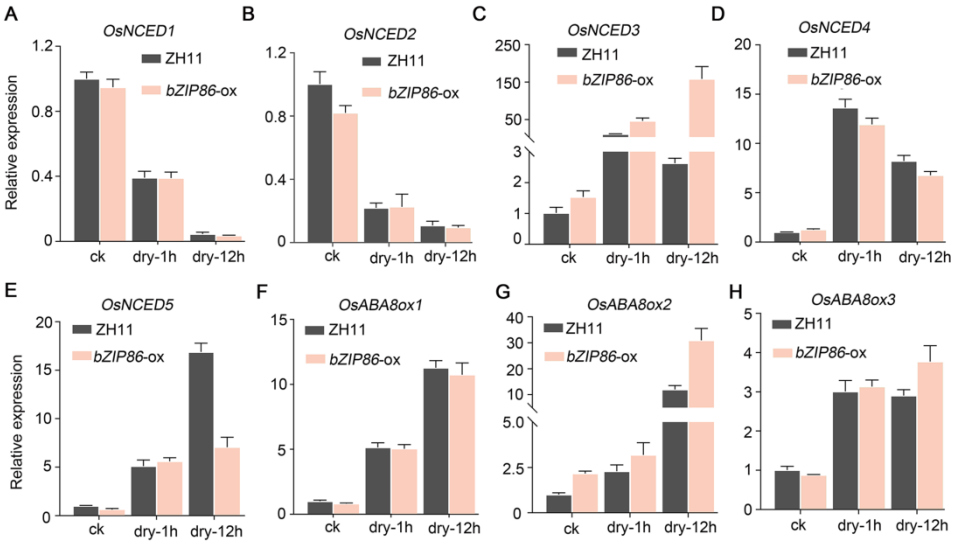
(A) Subcellular localization of OsbZIP86 in rice protoplasts. Vector construct *35S: OsbZIP86-GFP* and *35S: GFP* were co-transiently expressed with a nuclear marker (NSL-mCherry) in rice protoplasts, respectively. (B) DAPI staining assays of OsbZIP86 in tobacco leaves. (C) Subcellular localization of OsbZIP86 in roots of stable transgenic rice expressing *Ubi: OsbZIP86-GFP* or *Ubi: GFP*. Scale bars, 3 μm (A), 250 μm (B) and 10 μm (C).



Supplemental Figure S5. miR2105 and *OsbZIP86* mediate salt resistance and grain yield of rice under salt treatment.

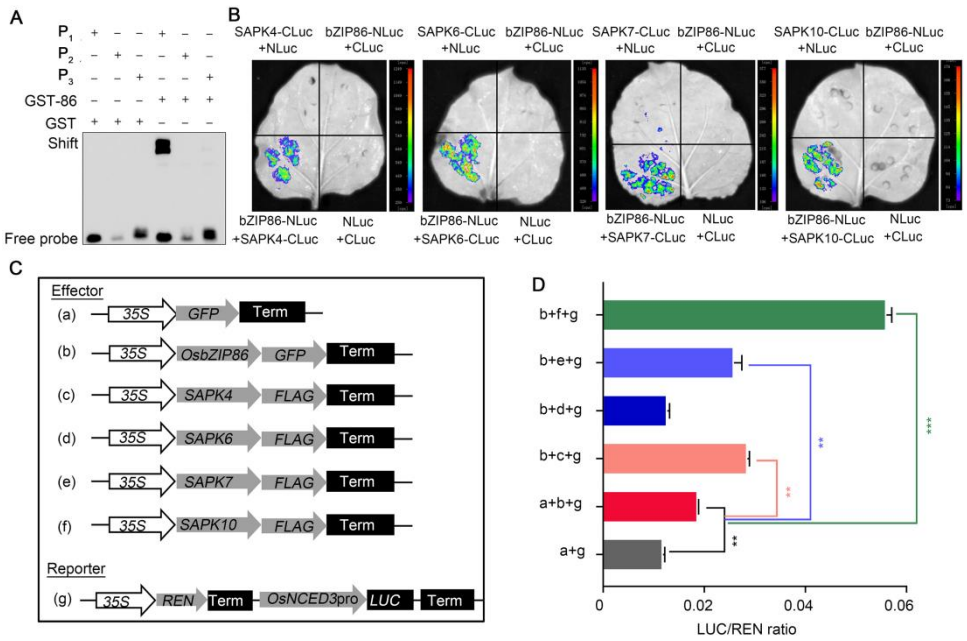
(A,B) Phenotypes of miR2105 and *OsbZIP86* transgenic rice seedlings under 150 mM NaCl treatment. 4-week-old seedlings were grown in Yoshida solution and then transferred into Yoshida solution with 150 mM NaCl for 10 d, then recovered with Yoshida solution for one week. Experiments were performed with three biological replicates with similar results. (C–D) Photographs (C), lengths of shoots and main roots (D), and rates of seed germination (E) of miR2105 and *OsbZIP86* transgenic rice seedlings under salt stress. Seeds were placed on double sheets of filter paper in a 9-cm Petri dish moistened with 150 mM NaCl for 7 d. Scale bar, 1 cm. Experiments used three biological replicates with similar results, and two independent lines were used for each transgenic construction. Each repeat was measured in 30 independent seeds. Values are means ± SD of 30 independent plants. (F–I) Statistics of main agronomic traits of miR2105 and *OsbZIP86* transgenic rice under normal and NaCl conditions. Rice plants were grown in boxes filled with field soil. For salt treatment, the plants were grown under normal conditions until flowering, then treated with 0.3% NaCl from flowering to mature

grain. Each repeat was measured in at least 20 independent plants. Values are means ± SD; *$p < 0.05$, **$p < 0.01$ according to student's *t*-test (D-I).



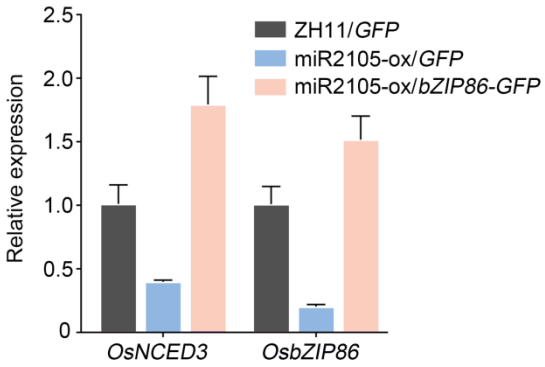
Supplemental Figure S6. Expression changes of ABA biosynthetic and metabolic genes in *OsbZIP86* overexpression transgenic rice.

Five ABA biosynthetic genes (*OsNCED1* (A), *OsNCED2* (B), *OsNCED3* (C), *OsNCED4* (D) and *OsNCED5* (E)) and three ABA metabolic genes (*OsABA8ox1* (F), *OsABA8ox2* (G) and *OsABA8ox3* (H)) were checked in seedlings dried for 1-12 h. Means ± SD (n = 3) are shown. All qRT-PCR analyses for gene expression were performed using three biological replicates with similar results.

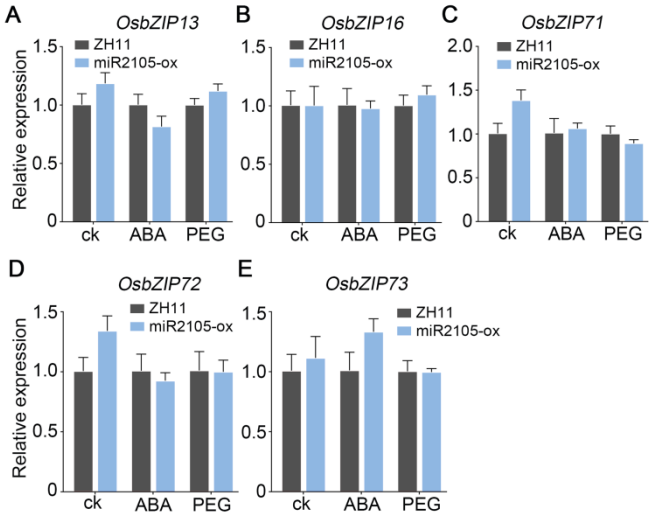


Supplemental Figure S7. OsbZIP86 binds to promoter fragments of *OsNCED3*, and OsbZIP86 interacts with OsSAPKs to regulate *OsNCED3* expression.

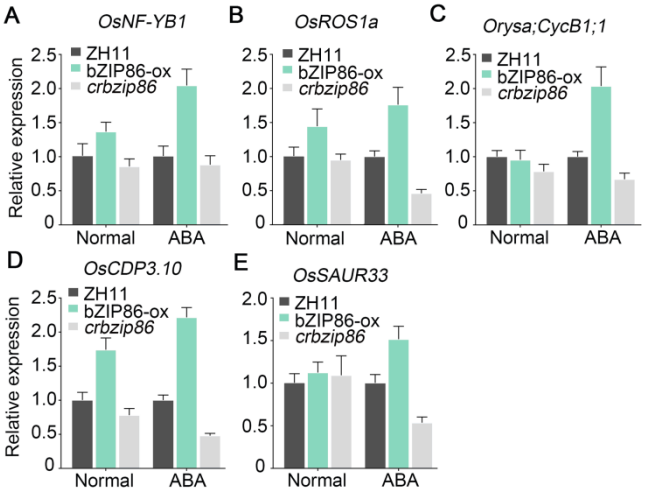
(A) *In vitro* electrophoretic mobility shift assay (EMSA) using G-box sequences from promoter of *OsNCED3* as probes. The P_1-P_3 (as shown in Figure 5B) probes were three biotin-labelled fragments of the *OsNCED3* promoter containing the G-box motif. GST-tagged OsbZIP86 was purified, and 2 μg protein was used. GST-86, fusion protein GST-OsbZIP86; GST, negative control. (B) Luciferase complementation imaging (LCI) assays of the interaction between OsbZIP86 and rice SAPK family members in tobacco leaves. OsbZIP86 and OsSAPKs (OsSAPK1-OsSAPK10) were fused with NLuc and CLuc, respectively, then co-transformed into tobacco leaves by *Agrobacterium* GV3101. Results of four OsSAPKs interacted with OsbZIP86 are showed. (C, D) *In vivo* luciferase assay. Schematic diagram of various constructs used in the luciferase activity assay (C). Relative activity of the *OsNCED3* promoter in tobacco leaves. *35S*: *REN-OsNCED3pro: LUC* was constructed as the reporter. *35S: bZIP86-GFP*, *35S: SAPK4-FLAG*, *35S: SAPK6-FLAG*, *35S: SAPK7-FLAG* and *35S: SAPK10-FLAG* were constructed as effectors. Free GFP was used as a negative control (D). Error bars indicate SD with biological triplicates (n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$according to student's *t*-test.



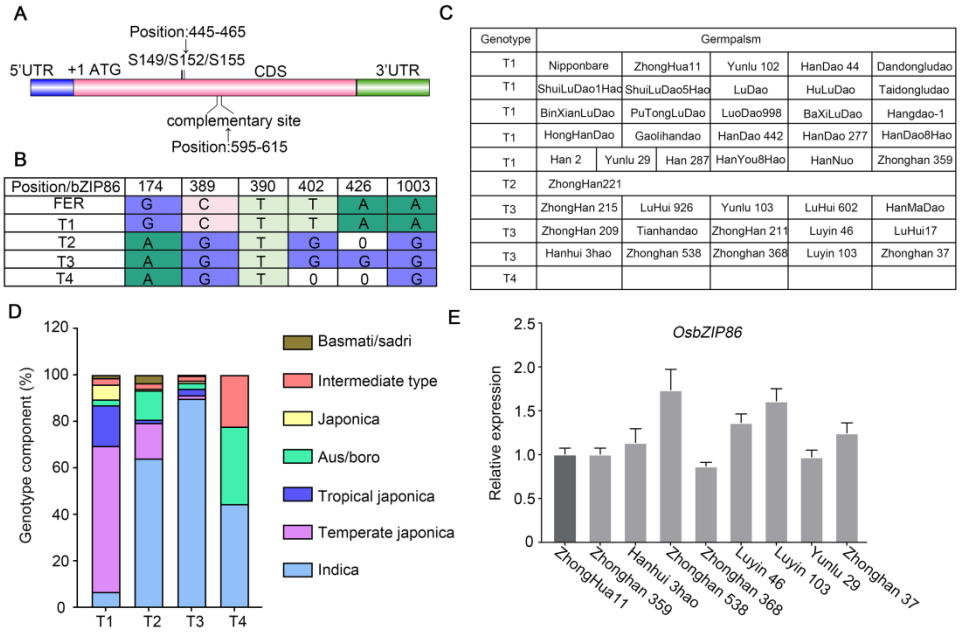
Supplemental Figure S8. Expression changes of *OsNCED3* and *OsbZIP86* in the rice protoplasts of ZH11/GFP, miR2105-ox/*GFP* and miR2105-ox/*bZIP86-GFP*. *ZH11/GFP*: The *pCAMBIA1300-GFP* plasmid was introduced into rice protoplasts of ZH11; miR2105-ox/*GFP*: The *pCAMBIA1300-GFP* plasmid was introduced into rice protoplasts of miR2105-ox; miR2105-ox/*bZIP86-GFP*: The *pCAMBIA1300-bZIP86-GFP* plasmid was introduced into rice protoplasts of miR2105-ox. *GFP* was used as reference genes, Means ± SD (n = 3) are shown.



Supplemental Figure S9. The expression changes of several bZIPs in ZH11 and miR2105-ox. miR2105 displayed no effect on the expression of other members of bZIPs, including *OsbZIP13* (A), *OsbZIP16* (B), *OsbZIP71* (C), *OsbZIP72* (D) and *OsbZIP73* (E). RNA was isolated from 2-week-old rice seedlings grown in Yoshida solution (ck). For ABA and PEG treatments, 2-week-old seedlings grown in Yoshida solution were treated with 50 μM ABA or 20 % PEG for 2 h. The expression of bZIPs under different condition was normalized as 1, respectively. *e-EF-1a* was used as mRNA reference gene, Means ± SD (n = 3) are shown.



Supplemental Figure S10. Expression changes of early endosperm development genes (A–C) and seed vigor-related genes (D–E) in ZH11 and *OsbZIP86* transgenic rice. *e-EF-1a* was used as mRNA reference genes, Means ± SD (n = 3) are shown.



Supplemental Figure S11. The genotype of OsbZIP86 CDS in various rice germpalsm.

(A) The schematic structure of the cleavage site targeted by miR2105 in the *OsbZIP86* mRNA and the predicted phosphorylation sites of OsbZIP86. (B) The genotype analysis of OsbZIP86 CDS in various rice germpalsm using online tool MBKbase (http://www.mbkbase.org/). FER: The genotype of Nipponbare. (C) Genotype distribution of different upland rice. (D) The genotype component in various upland rice. (E) Expression levels of *OsbZIP86* in seedlings of ZH11 and several upland rice.

RNA was isolated from 2-week-old ZH11 and upland rice seedlings grown in Yoshida solution (ck). *e-EF-1a* was used as mRNA reference gene , Means ± SD (n = 3) are shown.

Supplemental Table S1. The description of the predicted 13 target genes of miR2105.

The sequence of pre-miR2105	**5'-uugugaugugaaugauucau -3'**	
The predicted 13 target genes	**Gene ID in MSU**	**Target Description**
	LOC_Os10g05720	cDNA|LTPL37 - Protease inhibitor/seed storage/LTP family protein precursor, expressed
	LOC_Os03g11140	cDNA|pleckstrin homology domain-containing protein-related taxo, putative, expressed
	LOC_Os12g13170（OsbZIP86）	cDNA|transcription factor, putative, expressed
	LOC_Os10g17850	cDNA|retrotransposon protein, putative, Ty3-gypsy subclass
	LOC_Os06g05590	cDNA|OsFBX186 - F-box domain containing protein, expressed
	LOC_Os05g18050	cDNA|retrotransposon protein, putative, unclassified
	LOC_Os08g05250	cDNA|retrotransposon protein, putative, Ty3-gypsy subclass, expressed
	LOC_Os08g23654	cDNA|retrotransposon protein, putative, Ty3-gypsy subclass, expressed
	LOC_Os02g51164	cDNA|expressed protein
	LOC_Os05g14270	cDNA|expressed protein
	LOC_Os01g43580	cDNA|kinesin motor domain containing protein, putative, expressed
	LOC_Os08g11650	cDNA|transposon protein, putative, CACTA, En/Spm sub-class, expressed
	LOC_Os08g11320	cDNA|transposon protein, putative, CACTA, En/Spm sub-class, expressed

Supplemental Table S2. Primers used in this study.

List of the primers used in this study			
Purpose	**Primer name**	**Sequence (5′-3′)**	**Vector**
qRT-PCR primers	miR2105-RF	GACTGTTGTGATGTGAATGAT	
	miR2105-RR	GTGCAGGGTCCGAGGTATTC	
	U6-R-F	CGATAAAATTGGAACGATACAGA	
	U6-R-R	ATTTGGACCATTTCTCGATTTGT	
	eEF-1a -q-F	GCACGCTCTTCTTGCTTTC	
	eEF-1a -q-R	AGGGAATCTTGTCAGGGTTG	
	OsbZIP86 -q-F	TGGTAAAACCTCTGGGGCATC	
	OsbZIP86 -q-R	AACTTTGCCTGGGATGGTGAA	
	OsNCED1 -q-F	CTACATCCCTCCTGCTGCTT	
	OsNCED1 -q-R	CTACCAACTGCTCGTCCTCT	
	OsNCED2 -q-F	TTCCAAGGTACGCCAAGGAT	
	OsNCED2 -q-R	TCTCCTCTCCATCAGCCTCT	
	OsNCED3 -q-F	TTCGCCATCACCGAGAACTA	
	OsNCED3 -q-R	GAGCATCTCCTGGAGCTTGA	
	OsNCED4 -q-F	GTCCAAGCCGTACCTCAAGT	
	OsNCED4 -q-R	TCCTGGAGCTTGAACACGAT	
	OsNCED5 -q-F	AGGTGTGGCAAGAAGAAGGA	
	OsNCED5 -q-R	GCACATTCGTGATGAACCCT	
	OsABA8ox1 -q-F	GTCATCGGCGTCATCTTCGC	
	OsABA8ox1 -q-R	TGACCCGGCTCGTCATCTTC	
	OsABA8ox2 -q-F	CTGCTCACCGACGACCAGAT	
	OsABA8ox2 -q-R	CCCTCGTTGGCCACGTAGAT	
	OsABA8ox3 -q-F	TGGCCCTAACCCACAAGGTG	
	OsABA8ox3 -q-R	CGGCATCACCTTCCATCCCT	
	OsbZIP72 -q-F	TGTATTCGCTGACGTTCGAC	
	OsbZIP72 -q-R	GAGCAGCTCGTCCATGTTC	
	OsbZIP71 -q-F	TGTGTGCCCTAACTGACATCCTGA	
	OsbZIP71 -q-R	AAGTCTATGGGTGGCTGGTTCCAT	
	OsbZIP16 -q-F	TCCAACATTGCATACCATCCTAG	
	OsbZIP16 -q-R	GCATTTGCTTCATCTTCAGTCAC	
	OsbZIP73 -q-F	TCAGTTCCCACTACCACAGCAACA	
	OsbZIP73 -q-R	TACCACAGGGAGCAATTCCTGGAT	
	OsbZIP13 -q-F	TGGTTCTTCGCCTGTT	
	OsbZIP13 -q-R	TCCCGATTAGACTGCTTCCT	
	OsNF-YB1 -q-F	CAGGGAACAAAAAGCGTGGTGGC	
	OsNF-YB1 -q-R	ATCGGCAGCTCGGCGTTGGT	
	OsROS1a-q-F	CCTAAGGACAGAGCACCAAG	
	OsROS1a-q-R	GCACATAGTTCACCATTCTC	
	OsCycB1;1-q-F	AATCTCACCGTTCCTACAGC	
	OsCycB1;1-q-R	AGTAGAGTGCGAGGTAACAAG	
	OsCDP3.10 -q-F	TCGTTCTTCAGCGCGTTTAGCG	
	OsCDP3.10 -q-R	TTCTCCAATTCTTCCCGGCGAGTG	
	OsSAUR33 -q-F	CGGGTAATATCTCCGTCCTTTGCC	
	OsSAUR33 -q-R	TGGCATATCAGCACCGAAACAGG	
Primers for ChIP-qPCR	*OsNCED3* -P_3-q-F	CGCCAGCCACTCTGGCGCACT	
	OsNCED3 -P_3-q-R	GTGGCTGGCGCCGTCGTTCAA	
	OsNCED3 -P_2-q-F	AATCCAGTTGGCACCACAGGACTC	
	OsNCED3 -P_2-q-R	TACTACACTTAAAAACTTTGTCTC	
	OsNCED3 -P_1-q-F	TCGCGTCGCGCGGTCGCCGTCCCC	
	OsNCED3 -P_1-q-R	ACGGGGGCGTGGGCGGTTTGAACG	
	Actin -F	GCCACATCACCACAGATGTT	
	Actin -R	ACTTGGTTGAAGGGTGGTC	
Reverse transcript primers	miR2105-RT	GTCGTATCCAGTGCAGGGTCCGAG GTATTCGCACTGGATACGACATGAATCA	
	U6-RT	ATTTGGACCATTTCTCGATTTGT	
Primers for 5' RACE	Adaptor-5RACE	GCUACACucGGUUUGCUGGCUUUGAUGAAA	
	5' RACE-out/F	GCTACACTCGGTTTGCTGGCTT	
	5' RACE-in/F	CGGTTTGCTGGCTTTGATG	
	OsbZIP86 -5' RACE-out/R	GCTCAGGAGCATTGCACGATAG	
	OsbZIP86 -5' RACE-in/R	CTCCTGCCTCATCAGTTTTGTG	

Supplemental Table S2. Primers used in this study.

Purpose	Primer name	Sequence (5′-3′)	Vector
Primers for vector construction			
OE-construct	G-4368	GAAGATCTCTGCAAGGCGATTAAGTTGGGTAAC	*pCAMBIA1301*
	G-4369	CCCCTTAAGGCGGATAACAATTTCACACAGGAAACAG	
	I miR2105-s	agTTGTGATGTGAATGATTCATcaggagattcagtttga	
	I miR2105-a	tgATGAATCATTCACATCACAActgctgctgctacagcc	
	III miR2105*s	ctATGAAACATACACATCACAAttcctgctgctaggctg	
	IV miR2105*a	aaTTGTGATGTGTATGTTTCATagagaggcaaaagtgaa	
	OsbZIP86 -CDS-6HA-GFP-F	CCCAAGCTTATGGGTAGCAGTGGCGCAGA	*pXU1301-6HA-GFP*
	OsbZIP86 -CDS-6HA-GFP-R	CGGGGTACCGTTTCCTTTTTTCTGGC	
Mutant construct	STTM2105-F	GAAGCTTatgaatcattCTAcacatcacaaGTTGTTGTTGTTATGGTCTAG	*pXU1301*
	STTM2105-R	TGGATCCttgtgatgtgTAGaatgattcat ATTCTTCTTCTTTAGACCATATTCTTCT	
	OsbZIP86 -RNAi-F	CGGGGTACCACTAGTCGTAAGCAGGCTGAATGTGA	*pTCK303*
	OsbZIP86 -RNAi-R	GCGGGATCCGAGCTCCTGCCTCATCAGTTTTGTGTT	
Crispr-cas9 vector	*OsbZIP86* -I-CRISPR-F	CATGTAAGGATGACCCTGTgttttagagctagaaat	*pYLCRISPR/Cas9Pubi-H*
	OsbZIP86 -I-CRISPR-R	ACAGGGTCATCCTTACATGTgccacggatcatctgc	
	OsbZIP86 -II-CRISPR-F	GGAGTCTGCTCGCAGATCTgttttagagctagaaat	
	OsbZIP86 -II-CRISPR-R	AGATCTGCGAGCAGACTCCCgccacggatcatctgc	
	OsbZIP86 -III-CRISPR-F	CATCATACTCCTTTCTGATCgttttagagctagaaat	
	OsbZIP86 -III-CRISPR-R	GATCAGAAAGGAGTATGATGTgccacggatcatctgc	
Subcellular location	*OsbZIP86* -5'GFP-F	GGACTAGTATGGGTAGCAGTGGCGCA	*pUC18*
	OsbZIP86 -5'GFP-R	GGGGTACCGTTTCCTTTTTTCTGGC	
GUS histochemical analysis	Pro-*OsbZIP86* -F	GAATTCGAGCTCGGTACCAAATACCCACACCCACAC	*pCAMBIA1301*
	Pro-*OsbZIP86* -R	AAATTTACCCTCAGATCTCAGATACACAGAGCAGTGAT	
LCI	*OsbZIP86* -Nluc-F	GGGACGAGCTCGGTACCATGGGTAGCAGTGGCG	*pCAMBIA1300-NLuc*
	OsbZIP86 -Nluc-R	TACGAGATCTGGTCGACGTTTCCTTTTTTCTGGC	
	OsSAPK1 -CLuc-F	CGTCCCGGGGCGGTACCATGGAGCGGTACGAGGTGATG	*pCAMBIA1300-CLuc*
	OsSAPK1 -CLuc-R	AAGCTCTGCAGGTCGACTCACAAGGCGCACACGAAG	
	OsSAPK2 -CLuc-F	CGTCCCGGGGCGGTACCATGGAGAGGTACGAGGTGATCAA	
	OsSAPK2 -CLuc-R	AAGCTCTGCAGGTCGACTCACAATGCGCACACGAAGT	
	OsSAPK3 -CLuc-F	CGTCCCGGGGCGGTACCATGGAGGAGAGGTACGAGGCG	
	OsSAPK3 -CLuc-R	AAGCTCTGCAGGTCGACTCAGTAGGTGTCATACTCATCGGC	
	OsSAPK4 -CLuc-F	CGTCCCGGGGCGGTACCATGGAGAAGTACGAGGCGGTG	
	OsSAPK4 -CLuc-R	AAGCTCTGCAGGTCGACTCATATGCGCAGTGAGCTCATAC	
	OsSAPK5 -CLuc-F	CGTCCCGGGGCGGTACCATGGAGAAATACGAGCCAGTTCG	
	OsSAPK5 -CLuc-R	AAGCTCTGCAGGTCGACTCAGGAGATTTGGAGGTTTGACA	
	OsSAPK6 -CLuc-F	CGTCCCGGGGCGGTACCATGGAGAAGTACGAGCTGCTCAA	
	OsSAPK6 -CLuc-R	AAGCTCTGCAGGTCGACTCAGCTCTTCTGCAAGTCACAGC	
	OsSAPK7 -CLuc-F	CGTCCCGGGGCGGTACCATGGAGAGGTACGAGCTGCTCA	
	OsSAPK7 -CLuc-R	AAGCTCTGCAGGTCGACTCAGCTGAGCTGAAACTCACCA	
	OsSAPK8 -CLuc-F	CGTCCCGGGGCGGTACCATGGCAGCGGCGGGGGCC	
	OsSAPK8 -CLuc-R	AAGCTCTGCAGGTCGACTTACATCGCATAGACGATCTCGC	
	OsSAPK9 -CLuc-F	CGTCCCGGGGCGGTACCATGGAGAGGGCGGCGGCG	
	OsSAPK9 -CLuc-R	AAGCTCTGCAGGTCGACTTACATGGCATATACGATCTCTCCG	
	OsSAPK10 -CLuc-F	CGTCCCGGGGCGGTACCATGGACCGGGCGGCGCTG	
	OsSAPK10 -CLuc-R	AAGCTCTGCAGGTCGACTCACATAGCGTATACTATCTCCCCA	
BiFC	cYFP-*OsbZIP86* -F	TCGAGCTCAAGCTTCGAATTCTATGGGTAGCAGTGGCG	*pSATN-cYFP-C1*
	cYFP-*OsbZIP86* -R	GTACCGTCGACTGCAGAATTCTTTAGTTTCCTTTTTTCTGGC	
	nYFP-*OsSAPK10* -F	GGTACCGCGGGCCCGGGATCCATGGACCGGGCGGCGCTG	*pSATN-nYFP-C1*
	nYFP-*OsSAPK10* -R	GACTCTAGATCAGGTGGATCCTCACATAGCGTATACTATCTCCCCA	
Pull down	GST-*OsbZIP86* -F	GATCTGGTTCCGCGTGGATCCATGGGTAGCAGTGGCG	*pGEX4T-1*
	GST-*OsbZIP86* -R	GATGCGGCCGCTCGAGTCGACTTAGTTTCCTTTTTTCTGGC	
	MBP-*OsSAPK10* -F	TTCAGAATTCGGATCCATGGACCGGGCGGCGCTG	*pMAL-c2x*
	MBP-*OsSAPK10* -R	CTTGCCTGCAGGTCGACTCACATAGCGTATACTATCTCCCCA	
Co-IP	*OsbZIP86* -FLAG-F	TCTCTCTCAAGCTTGGATCCATGGGTAGCAGTGGCG	*pHB-3×FLAG*
	OsbZIP86 -FLAG-R	ATACCGTCACTAGTGGATCCGTTTCCTTTTTTCTGGC	
	OsSAPK10 -GFP-F	ACTAGTGGATCCGGTACCATGGACCGGGCGGCGCTG	*pCAMBIA1300-GFP*
	OsSAPK10 -GFP-R	CTTGCTCACCATGGTACCCATAGCGTATACTATCTCCCCA	
EMSA	Biotin-*OsNCED3* -EMSA-P_1F	CCTCCCCCCCGGAGCGCACCACGTGGGCACCCAACCCCCTC	
	Biotin-*OsNCED3* -EMSA-P_1R	GAGGGGGTTGGGTGCCCACGTGGTGCGCTCCGGGGGGAGG	
	Biotin-*OsNCED3* -EMSA-P_2F	GCTGCTACTTGGCCGATCACGTTTTACTGCCAACAGAATTT	
	Biotin-*OsNCED3* -EMSA-P_2R	AAATTCTGTTGGCAGTAAAACGTGATCGGCCAAGTAGCAGC	
	Biotin-*OsNCED3* -EMSA-P_3F	CCACCTCAGCATCACCCCGCCACGTCGGCAGGGGGACGG	
	Biotin-*OsNCED3* -EMSA-P_3R	CCGTCCCCCTGCCGACGTGGCGGGGTGATGCTGAGGTGG	
Nonlabelled probe	*OsNCED3* -EMSA-P_1F	CCTCCCCCCCGGAGCGCACCACGTGGGCACCCAACCCCCTC	
	OsNCED3 -EMSA-P_1R	GAGGGGGTTGGGTGCCCACGTGGTGCGCTCCGGGGGGAGG	
Dual-Luciferase assay	Pro-*OsNCED3* -pGreenII-Luc-F	GACGGTATCGATAAGCTTGGCGTAACTTTCTCATACGG	*pGreenII0800-LUC*
	Pro-*OsNCED3* -pGreenII-Luc-R	TCTAGAACTAGTGGATCCATCGCGTCGATCGCACAAC	
	OsbZIP86 -GFP-F	ACTAGTGGATCCGGTACCATGGGTAGCAGTGGCGCAGA	*pCAMBIA1300-GFP*
	OsbZIP86 -GFP-R	CTTGCTCACCATGGTACCGTTTCCTTTTTTCTGGC	
	OsSAPK4 -FLAG-F	TCTCTCTCAAGCTTGGATCCATGGAGAAGTACGAGGCGGTG	*pHB-3×FLAG*
	OsSAPK4 -FLAG-R	ATACCGTCACTAGTGGATCCTATGCGCAGTGAGCTCATAC	
	OsSAPK6 -FLAG-F	TCTCTCTCAAGCTTGGATCCATGGAGAAGTACGAGCTGCTCAA	
	OsSAPK6 -FLAG-R	ATACCGTCACTAGTGGATCCGCTCTTCTGCAAGTCACAGC	
	OsSAPK7 -FLAG-F	TCTCTCTCAAGCTTGGATCCATGGAGAGGTACGAGCTGCTCA	
	OsSAPK7 -FLAG-R	ATACCGTCACTAGTGGATCCGCTGAGCTGAAACTCACCA	
	OsSAPK10 -FLAG-F	TCTCTCTCAAGCTTGGATCCATGGACCGGGCGGCGCTG	
	OsSAPK10 -FLAG-R	ATACCGTCACTAGTGGATCCCATAGCGTATACTATCTCCCCA	
	OsbZIP86 $^{S149A/S152A/S155A}$-GFP-R	CGCACCTAAAGCTCCTTTGGCTCTTTTAATGGGACTT	*pCAMBIA1300-GFP*
	OsbZIP86 $^{S149A/S152A/S155A}$-GFP-F	GCCAAAGGAGCTTTAGGTGCGTTGAATATGATTACA	
	OsSAPK10 ^{S177A}-FLAG-F	GGCTTTTGGTTGCGAATGAAGAACAGA	*pHB-3×FLAG*
	OsSAPK10 ^{S177A}-FLAG-R	ACCAAAAGCCACTGTTGGAACTCCGGC	

Note: Red marks indicate restriction sites.