

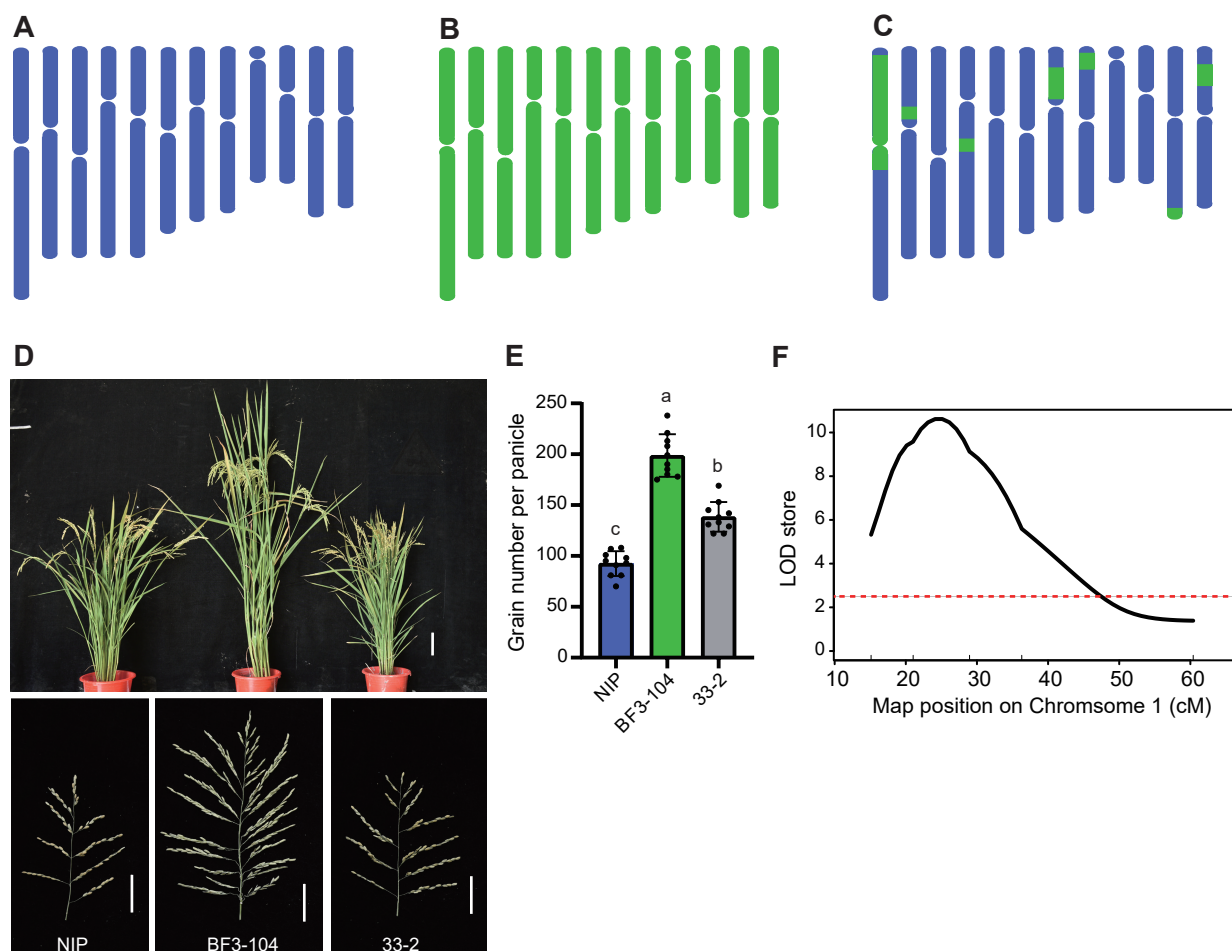


Figure S1. Phenotype of genetic materials.

(A-C) Chromosome diagram of NIP (A), BF3-104 (B) and 33-2 (C).

(D) Mature plant architecture (upper panel) and main panicle morphology (lower panel) of NIP, BF3-104, and 33-2 plants. Scale bar: 10 cm for plant architecture, 5 cm for main panicle.

(E) Comparison of grain number per panicle between NIP, BF3-104, and 33-2 (n=10) rice lines. The data are shown as means $\pm$ SD. Different alphabetical letters indicate significant differences determined by one-way ANOVA with post-hoc Tukey's multiple comparison tests.

(F) LOD (Logarithm of the Odds) score map in the initial mapping of *GNI.1* locus, with the threshold value of the LOD score set to 2.5.

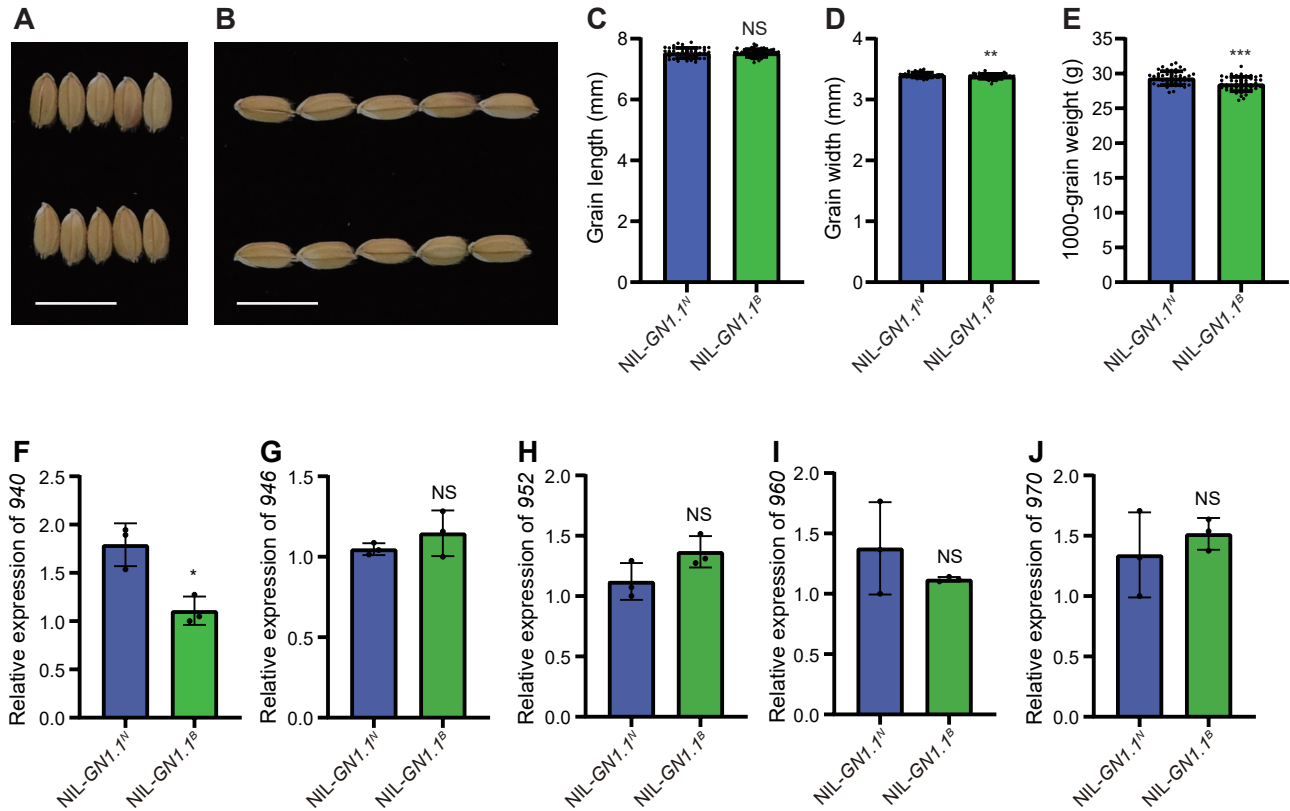


Figure S2. Grain size between NIL-*GN1.1^N* and NIL-*GN1.1^B* rice lines and the relative expression of five candidate genes.

(A-B) Phenotype of grain width (A) and grain length (B) between NIL-*GN1.1^N* (upper panel) and NIL-*GN1.1^B* (lower panel) rice lines. Scale bar, 1 cm.

(C-E) Comparisons of grain length (C), grain width (D), and 1000-grain weight between NIL-*GN1.1^N* and NIL-*GN1.1^B* rice lines (n=49). The data are shown as means $\pm$ SD. ****P* < 0.001; ***P* < 0.01; NS, not significant as determined by Student's *t*-test.

(F-J) Relative gene expression of 940 (*LOC_Os01g11940*) (F), 946 (*LOC_Os01g11946*) (G), 952 (*LOC_Os01g11952*) (H), 960 (*LOC_Os01g11960*) (I), 970 (*LOC_Os01g11970*) (J) in 5 mm lengths of young panicle between NIL-*GN1.1^N* and NIL-*GN1.1^B* plants (n=3). The data are shown as means $\pm$ SD. **P* < 0.05; NS, not significant as determined by Student's *t*-test.

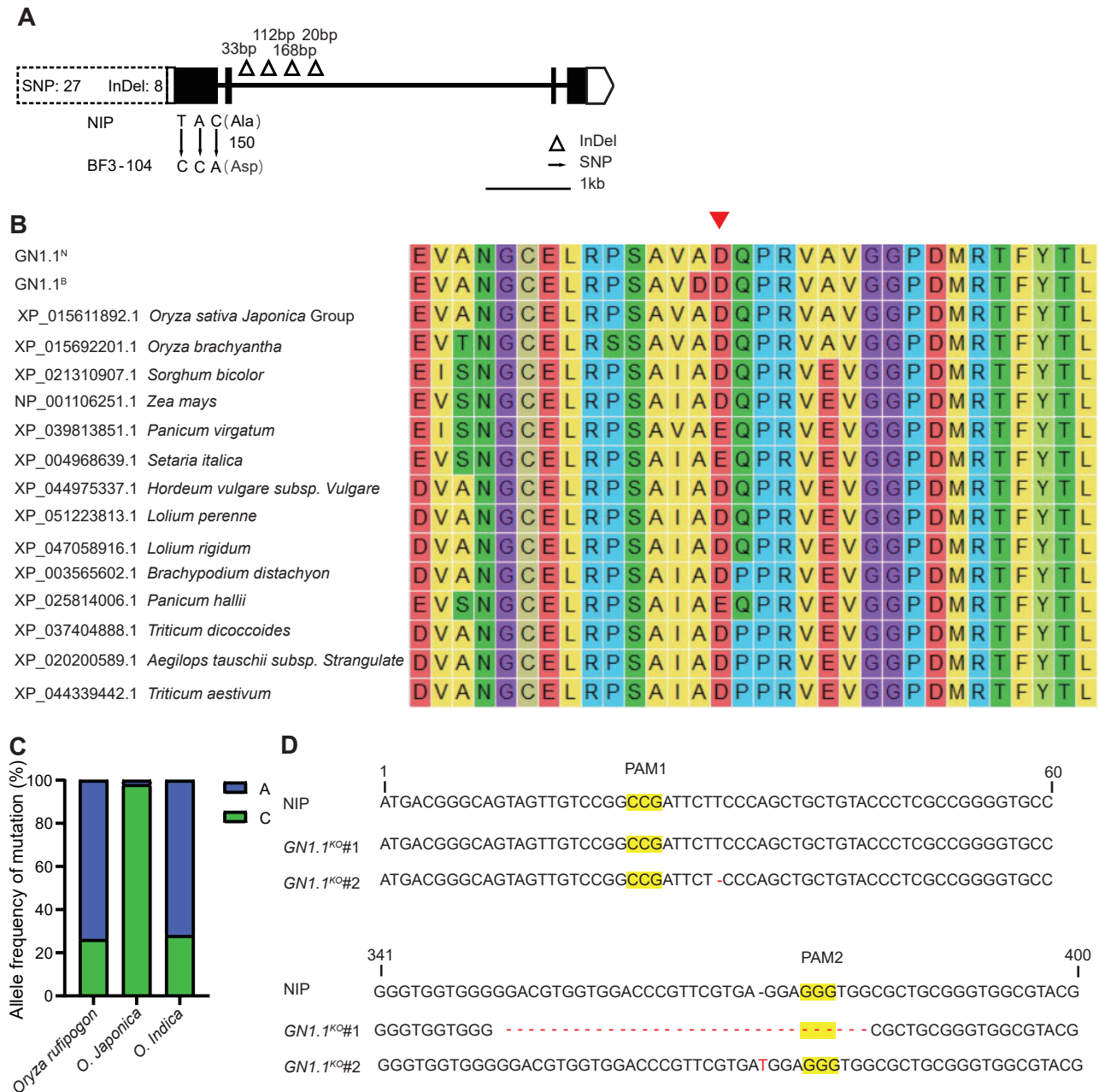


Figure S3. Analysis of *FT-L1* sequences, amino acid conservation at mutation sites, and the sequences of *GN1.1* knockout targets.

(A) Diagram of *FT-L1* sequence comparisons between NIP and BF3-104 rice lines.

(B) Analysis of amino acid conservation at mutation sites, which varied between NIL-*GN1.1*^N and NIL-*GN1.1*^B. The red inverted triangle denotes the specific locus of mutation.

(C) The allele frequency of the C449A site mutation in *GN1.1* in *Oryza rufipogon*, *O. japonica*, and *O. indica*.

(D) Diagram of *GN1.1* PAM1 and PAM2 knockout targets, and the mutation sequences of *GN1.1*^{KO}#1 and *GN1.1*^{KO}#2 knockout lines compared with NIP.

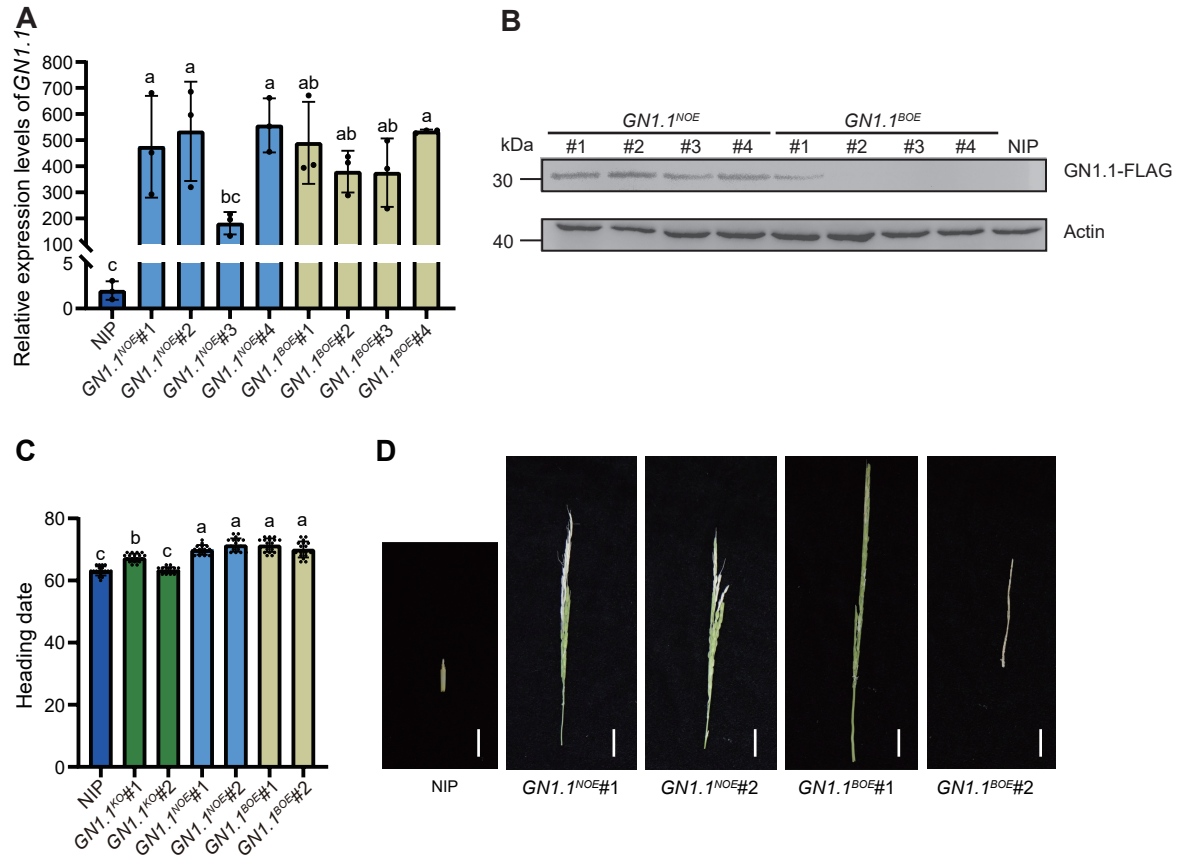


Figure S4. Expression levels of *GN1.1* and accumulation of GN1.1 proteins in *GN1.1^{OE}* plants, and heading date in *GN1.1* transgenic rice plants.

(A) Relative expression levels of *GN1.1* in the leaves of NIP, *GN1.1^{NOE}*, and *GN1.1^{BOE}* plants at the reproductive stage (n=3).

(B) Total accumulation levels of GN1.1^N-FLAG and GN1.1^B-FLAG proteins in transgenic rice seedlings.

(C) Comparisons of heading date between NIP, *GN1.1^{NOE}#1*, *GN1.1^{NOE}#2*, *GN1.1^{BOE}#1*, *GN1.1^{BOE}#2*, *GN1.1^{BOE}#1*, and *GN1.1^{BOE}#2* plants (n=18, 18, 18, 15, 18, 18 and 17 respectively). The data are shown as means $\pm$ SD. Statistical differences determined by one-way ANOVA with post-hoc Holm-Sidak multiple comparison tests, and are indicated by different alphabetical letters.

(D) Young panicle phenotypes of NIP, *GN1.1^{NOE}*, and *GN1.1^{BOE}* plants were observed simultaneously during the initiation of young panicle development in NIP plants. Scale bar, 1 cm.

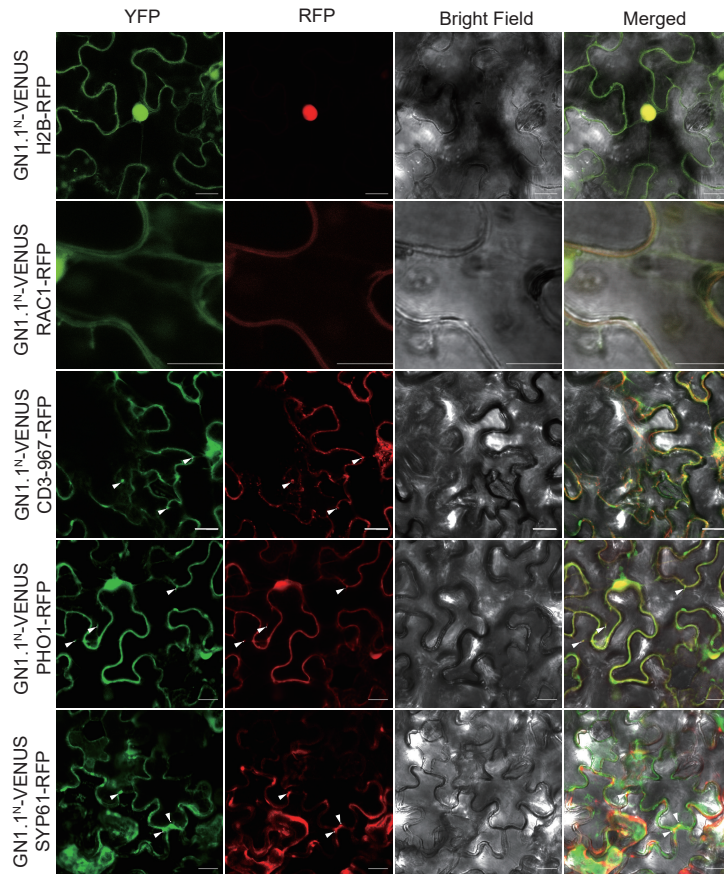


Figure S5. Subcellular localization of GN1.1-VENUS.

Subcellular localization of GN1.1-VENUS with H2B-RFP, RAC1-RFP, CD3-967-RFP, PHO1-RFP, SYP61-YFP in leaf epidermal cells of *N. benthamiana* plants. Scale bar, 20 μ m. White arrows indicate punctate signals.

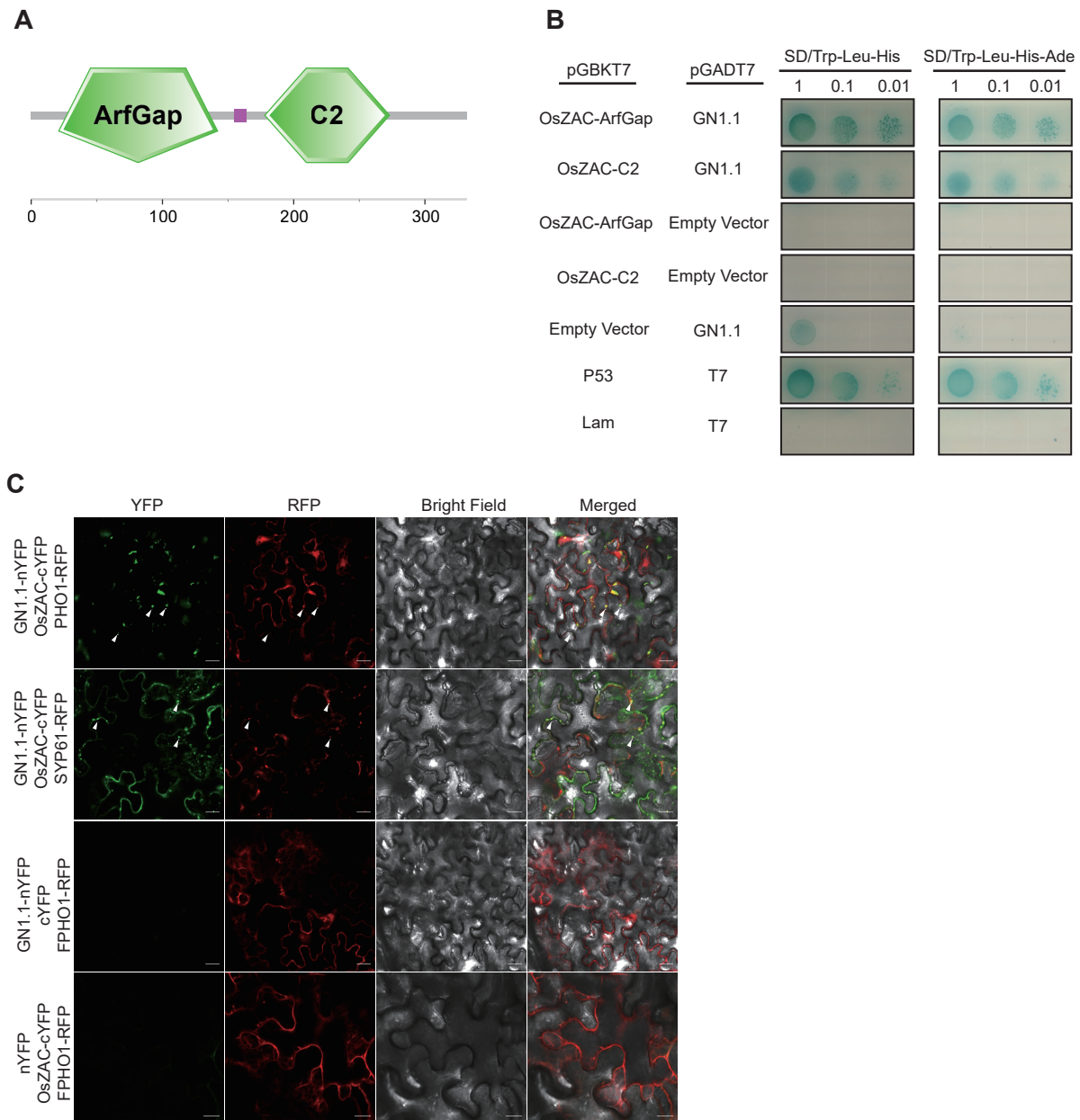


Figure S6. Yeast two hybrid analysis of OsZAC ArfGap, C2 domain, and GN1.1 interactions, and BiFC analysis of interactions between GN1.1 and OsZAC.

(A) Diagram of OsZAC protein domains.

(B) Y2H assays demonstrating the interaction between GN1.1 with OsZAC ArfGap and the C2 domain. The yeast cells were cultured on synthetic dropout medium lacking Trp, Leu and His (SD/-Trp-Leu-His) and drop out medium lacking Trp, Leu, His and Ade (SD/-Trp-Leu-His-Ade).

(C) GN1.1 was fused with the N-terminus of YFP (nYFP) while OsZAC was fused with the C-terminus of YFP (cYFP). Different combinations were co-expressed with PHO1-RFP and SYP61-RFP in leaf epidermal cells of *N. benthamiana* plants. Scale bar, 20 μ m. White arrows indicate punctate signals.

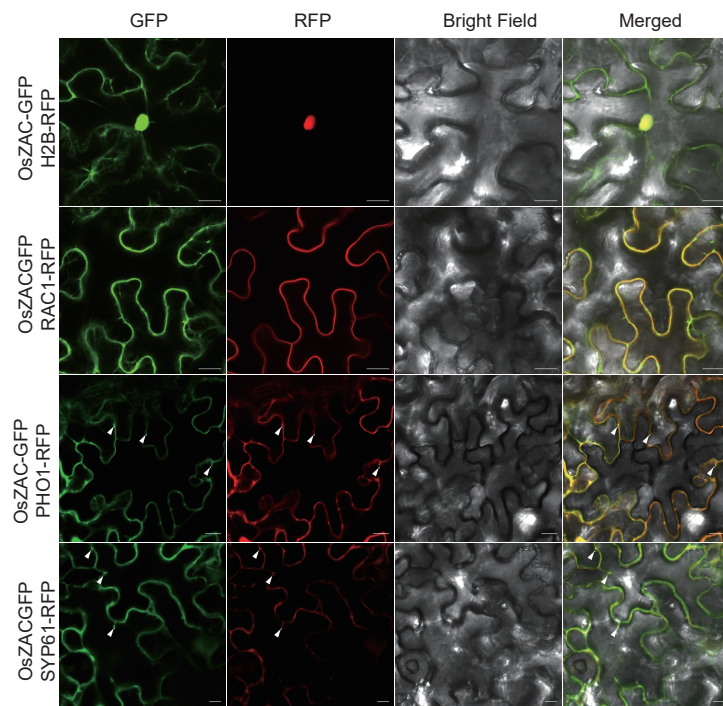


Figure S7. Subcellular localization of OsZAC-GFP.

Subcellular localization of OsZAC-GFP with H2B-RFP, RAC1-RFP, PHO1-RFP, and SYP61-YFP in leaf epidermal cells of *N. benthamiana* plants. Scale bar, 20 μ m. White arrows indicate punctate signals.

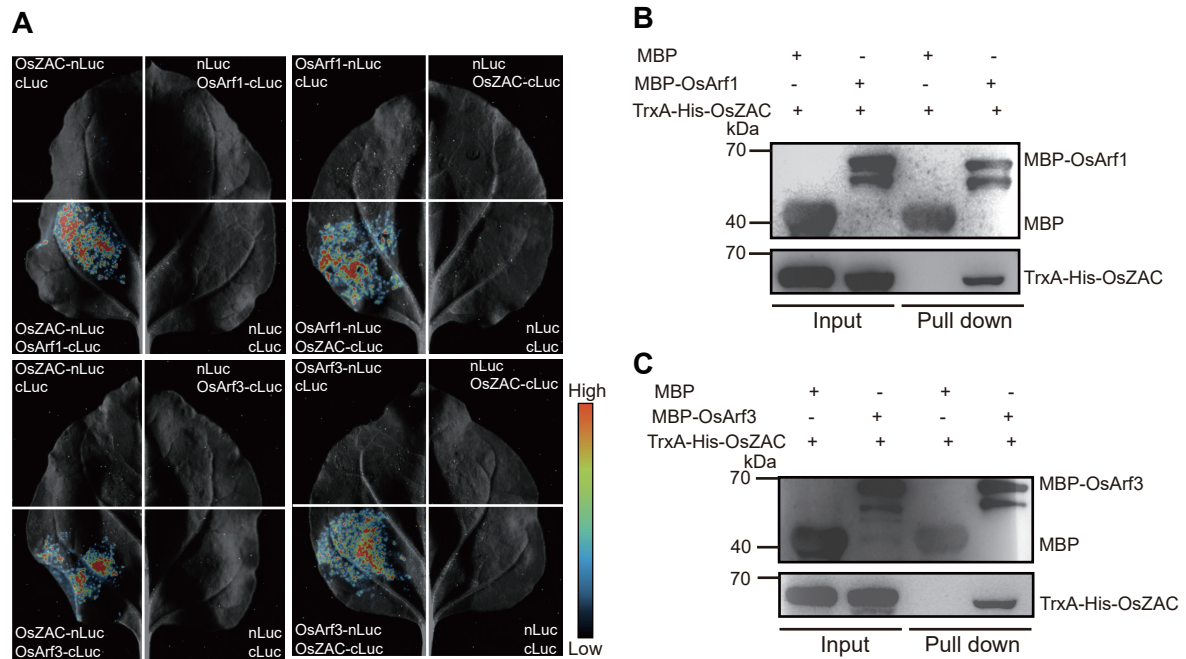


Figure S8. OsZAC interacts with OsArf1 and OsArf3.

(A) LCI assays demonstrating interactions between OsZAC with OsArf1 and with OsArf3 in *N. benthamiana* leaves. The bar graph on the right indicates fluorescence intensity.

(B-C) Pull-down assays showing direct interaction of OsZAC with OsArf1 (B), and OsZAC with OsArf3 (C) *in vitro*.

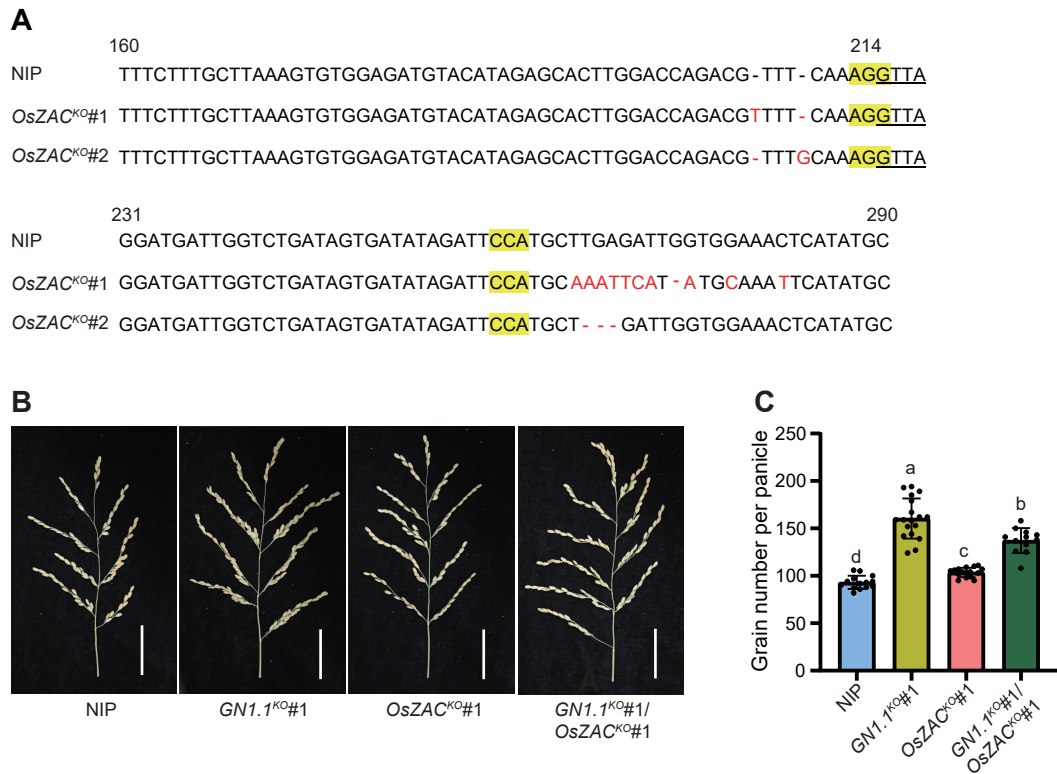


Figure S9. Sequences of *OsZAC* knockout targets and *GN1.1^{KO}#1/OsZAC^{KO}#1* panicle phenotypes.

(A) Diagram of *OsZAC* PAM1 and PAM2 knockout targets, and the mutated sequences of *OsZAC^{KO}#1* and *OsZAC^{KO}#2* knockout lines, compared with NIP.

(B) Main panicle morphology of NIP, *GN1.1^{KO}#1*, *OsZAC^{KO}#1* and *GN1.1^{KO}#1/OsZAC^{KO}#1* plants. Scale bar, 5 cm.

(C) Comparisons of grain number of the main panicle between NIP, *GN1.1^{KO}#1*, *OsZAC^{KO}#1* and *GN1.1^{KO}#1/OsZAC^{KO}#1* plants (n=14, 18, 16 and 12, respectively). The data are shown as means ± SD. Statistical differences determined by one-way ANOVA with post-hoc Holm-Sidak multiple comparison tests, and are indicated by different alphabetical letters.

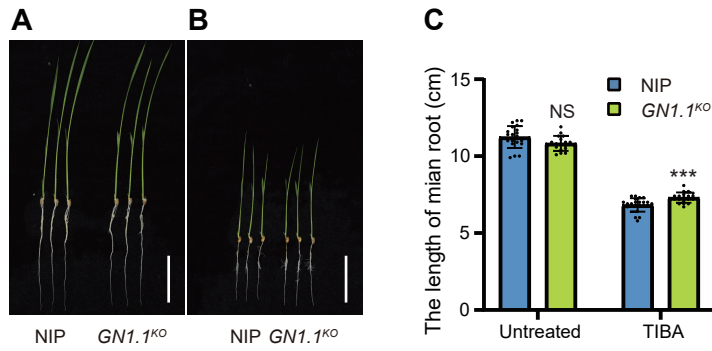


Figure S10. TIBA treatment of *GN1.1*^{KO} plants.

(A-B) NIL-*GN1.1*^N and NIL-*GN1.1*^B rice seedlings were treated with water **(A)** or TIBA **(B)**.

(C) Comparisons of the lengths of primary roots between NIP and *GN1.1*^{KO} plants treated with water or TIBA (n=21 for NIP, and 17 for *GN1.1*^{KO} untreated, 22 for NIP, and 19 for *GN1.1*^{KO} with TIBA treatment, respectively). The data are shown as means ± SD. ****P* < 0.001; NS, not significant as determined by Student's *t*-test.

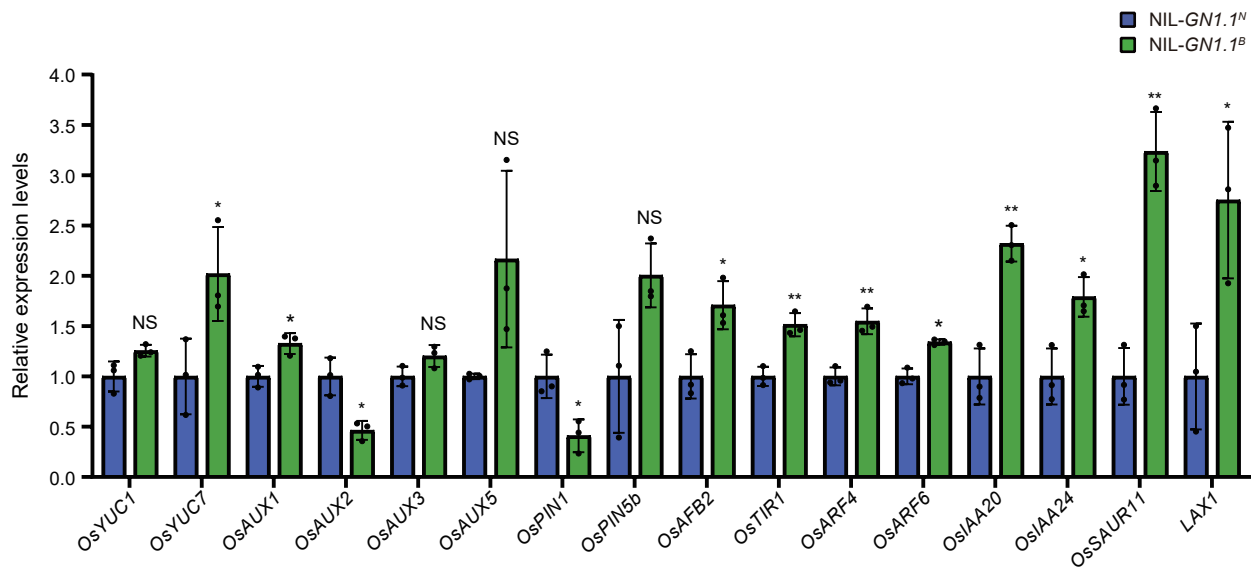


Figure S11. The relative expression of auxin-relevant genes in NIL-GN1.1^N and NIL-GN1.1^B plants.

The relative expression of auxin-relevant genes including *OsYUC1*, *OsYUC7*, *OsAUX1*, *OsAUX2*, *OsAUX3*, *OsAUX5*, *OsPIN1*, *OsPIN5b*, *OsAFB2*, *OsTIR1*, *OsARF4*, *OsARF6*, *OsIAA20*, *OsIAA24*, *OsSAUR11*, and *LAX1* in the young panicles of NIL-GN1.1^N and NIL-GN1.1^B plants (n=3). The data are shown as means $\pm$ SD. ** $P < 0.01$; * $P < 0.05$; NS, not significant as determined by Student's *t*-test.

Table S1. *GN1.1* is a QTL contributing to grain number.

LOD	%VAR	Additive effect	Molecular marker
10.698	28.40%	-4.8	1-21.03~RM1220

Table S2. The primer sequences used in this paper

Primer name	Forward sequence (5'-3')	Reverse sequence (5'-3')	Note
W01-15119	ATTATTCCAAACTCAGTAGGTGC	AATGTATCCACGAGTCATGGT	Mapping
RM1220	GGGACTACTCGAGCAAGCTAATGC	GTCCAATCTAATCGACCTCCAAGAGC	Mapping
RM7075	GCGTTGCAGCGGAATTTGTAGG	CCCTGCTTCTCTCGTGCAGTCG	Mapping
W01-88719	TTTAAACGGTTTGATTATCCTT	ACGTGGATTGTAAATAGTGAGT	Mapping
1-21.03	TTGACCCAATCGACACAC	GCACTTGTTTGAGTCAATGG	Mapping
1-22.831	CGAGAGAGCAGAGAAGAC	CTGGGTGCATTGAATTGAAT	Mapping
1-24.883	CTAAGGTAGTGTCATGTGG	TGTGACAGATGCCAGAGA	Mapping
1-26.527	TATCTTGGTTGAGGGGTTC	ACTAGTAGTGGGCCATAAC	Mapping
1-36.299	CTCTCAAAACCCTAACCCC	GAGAGCACAGACTAGTAAGC	Mapping
1-25.3476	CAGGAACGGTTGGTAGGCTAT	GCATCTCTTAAACACAATCTCACG	Mapping
1-25.8704	TTGTTGAGCAGAAACGAGAGG	CGACGATTAGCTGGCATCC	Mapping
1-26.102	CATCGAGGTCGTCTTCTCCA	CGGGTGAGGGCGTCCATCGT	Mapping
1-26.5627	GATCACACCATGGTGGCGAA	AAACCTGTGACGAGAGAG	Mapping
1-26.7733	GTCTTCCTCTTAGCTTGGATTGA	AGCCTGCACCTGCAAGAA	Mapping
1-27.883	AGCTACACTTTCGCTCTC	CAAACATGCCATTACGCC	Mapping
940-qPCR	CGGCTACCACAGGAGTCTCT	GTTGTAGAGCTCGGCGAAGT	RT-qPCR
946-qPCR	AGTATGCTACAAACCAACTCGA	ACAGTTTCAGATATTCGTCGT	RT-qPCR
952-qPCR	ACTTTTAGAGAACGACTTCGGT	TCTGCAACACTACGTCTAACTT	RT-qPCR
960-qPCR	GCGAGAGGAAAGAGGATTTCTA	CTGAGGCCGCTATTAGAGTATC	RT-qPCR
970-qPCR	GTACACTTGAGTTGATTCGCTC	AGGCAGATAGTCTCAAAACACA	RT-qPCR
ZAC-qPCR	CTCAAACCACTGTTAAACCGAG	CAGAGAACGTATCATGGTCGTA	RT-qPCR
OsYUCCA1-qPCR	TCATCGGACGCCCTCAACGTCGC	GGCAGAGCAAGATTATCAGTC	RT-qPCR
OsYUCCA7-qPCR	CACTGCTGTGTCCTACAATATCAC	GGAGGTGCATCTCCGTCATCTTC	RT-qPCR
OsAUX1-qPCR	GGTAGAAGAAGAAGAGGGC	CCAAACAAACACAAGGACA	RT-qPCR
OsAUX2-qPCR	GTTTACAGCAGCGTCAATACTG	GCACCAAAATCACACGTAATGT	RT-qPCR
OsAUX3-qPCR	CTGCTGCTGCAGGTGTTCTACG	GTATATGTTACTTGACAGGCGAT	RT-qPCR
OsAUX5-qPCR	TACCTCATCAGCGTGCTCTA	ACCACTGGATGACATGGTTG	RT-qPCR
OsPIN1-qPCR	GTCTGCTTCAGGTGGAACCT	GAACAGACCGAGACTGAACAT	RT-qPCR
OsPIN5b-qPCR	GTCTGCACCTCGCCATCAT	GCGTAATATGCAATCAGAATCGG	RT-qPCR
OsAFB2-qPCR	CGTTTGTCAATATCTGGTC	GACATCCAAAGGGATCGCA	RT-qPCR
OsTIR1-qPCR	GATGCTTTCAATCGCCTTCG	ACAAGCACGACGACATCCAAAG	RT-qPCR
OsARF4-qPCR	GAACCTCCGTGTTGGTGTAGAC	CTGGTGCCTCTTCTCCTTCAA	RT-qPCR
OsARF6-qPCR	CCAGCAAGCAGCCAACAA	GTCTCCAGCAACAGTCTCT	RT-qPCR
OsIAA20-qPCR	TTGTACGTGAACGGGATTATTTTG	CATGCTTATGAAATTGCTGAAACA	RT-qPCR
OsIAA24-qPCR	GGCTTGTGCTCTTCGTTGCT	CCTCTTGGATTGAGAAACACTGAA	RT-qPCR
SAUR11-qPCR	CTCGATCGAGTAGGACTCAAAC	CACAGAAAGGGGAGGAACAATA	RT-qPCR

LAX1-qPCR	TTCCCCATCCACCCGCAGCCG	GCTCTGCGGGTCGGTGG	RT-qPCR
UBQ5--qPCR	ACCACTTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT	RT-qPCR
GN1.1-KO-T1	gccGTACAGCAGCTGGGAAGAAT	aaacATTCTTCCCAGCTGCTGTA	CRISPR/Cas9
GN1.1-KO-T2	ggcaGGTGGACCCGTTCTGTAGGA	aaacTCCTCACGAACGGGTCCACC	CRISPR/Cas9
OsZAC-KO-T1	gccgACTTGGAACAGACGTTTCAA	aaacTTGAAACGTCTGGTCCAAGT	CRISPR/Cas9
OsZAC-KO-T2	ggcaGTTTCCACCAATCTCAAGCA	aaacTGCTTGAGATTGGTGGAAAC	Overexpression
pUN1301-GN1.1	aggtcgactctagaggatccATGACGGGCAGTAGTTGTCCGGC	ccaagggcgaattgggtaccCATTCTTCTTCCTCCGGTTC	Complement
pCAMBIA1300-proGN1.1-EGFP-F	gctcgggtaccggggatccCTTCCCGTCTTTGGCAAGGGATGGCGG		Complement
proGN1.1-R		CCGGCTGCCGGCCGACGACGACTAAACC	Complement
proGN1.1-CDS-F	GTGCGCCGGCAGCCGGATGACGGGCAGTAGTTGTCC		Complement
GN1.1-CDS-R		CATTCTTCTTCCTCCGGTTCCAG	Complement
GN1.1-CDS-R-EGFP-F	CATGGTACTAGTGTGACCAATTCTTCTTCCTCCGGTTCCAG		Complement
pCAMBIA1300-proGN1.1-EGFP-R		agtgccaaagcttgatgcctgcagTTACTTGTACAGCTCGTCCATGCC	Complement
proGN1.1N:GUS	agcttgcatgcctgcagggtgcacCATGGACCTCTCGGCGGGATGCCAAAC	ccctcagatctaccatgggtaccCCGGCTGCCGGCCGACGACGAC	GUS staining
pCAMBIA1300-GN1.1-EGFP	aggcaggggtaccggggatccATGACGGGCAGTAGTTGTCCGG	gctcaccatgggtactgtgcacCATTCTTCTTCCTCCGGTTCC	Subcelluar Localization
pCAMBIA1300-GN1.1-VEUNS	tttcatttggagagaacacggagctcATGACGGGCAGTAGTTGTCCGG	tcctcctcctcctcctccggatccCATTCTTCTTCCTCCGGTTCC	Subcelluar Localization
pCAMBIA1300-OsZAC-GFP	ggacaggggtaccggggatccATGAGTACCACTAATCACTATC	caccatgggtactagtgtgcacGGGTATTGGAACCCATTCC	Subcelluar Localization/Co-IP
BK-GN1.1	catatggccatggaggccgaattcATGACGGGCAGTAGTTGTCCGGC	ggtatgcggccgtgcaggtgcacggatTCACATTCTTCTTCCTCCGGTTC	Y2H
AD-GN1.1	catatggccatggaggccagtgatcATGACGGGCAGTAGTTGTCC	catctgcagctcgagctcgatggatccTCACGTCTCCCTCCTCCTCCTCT	Y2H
BK-OsZAC	catatggccatggaggccgaattcATGAGTACCACTAATCACTATCAAC	ggtatgcggccgtgcaggtgcacggatCTAGGGTATTGGAACCCATTCCAG	Y2H
AD-OsZAC	catatggccatggaggccagtgatcATGAGTACCACTAATCACTATCAAC	catctgcagctcgagctcgatggatccCTAGGGTATTGGAACCCATTCCAG	Y2H
BK-ZAC-Arf	catatggccatggaggccgaattcAGAAAATTGAAGGATCTAATGT	tgcgccgctgcaggtgcacggatTCATGTAATGCGCAAACCTTGCTTCAA	Y2H
AD-ZAC-Arf	tatggccatggaggccagtgatcAGAAAATTGAAGGATCTAATGT	catctgcagctcgagctcgatggatTCATGTAATGCGCAAACCTTGCTTCAA	Y2H
BK-ZAC-C2	catatggccatggaggccgaattcGAGCTGAACATTACAGTGGTAAAGAG	tgcgccgctgcaggtgcacggatTCAATCTCCAAAGGCCATGACGGCTGT	Y2H
AD-ZAC-C2	tatggccatggaggccagtgatcGAGCTGAACATTACAGTGGTAAAGAG	catctgcagctcgagctcgatggatTCAATCTCCAAAGGCCATGACGGCTGT	Y2H
pMAL-C5Xs-GN1.1	gccgcgatatcgtcgacggatccACGGGCAGTAGTTGTCCGGCCGAT	tatttaattacctgcagggaattcTCACATTCTTCTTCCTCCGGTTCC	Pull-down/Cell-free degradation
GN1.1-R		CATTCTTCTTCCTCCGGTTCCAGACT	Cell-free degradation
GN1.1-FLAG-F	GAACCGGAGGAAGAAGAATGggatcagaatttgccaccGACTACAAGGACG		Cell-free degradation
pMAL-C5Xs-GN1.1-FLAG-R	ACGAT	tatttaattacctgcagggaattcTCATTGTGTCATCATCGTCTTTGTAGTCAC	Cell-free degradation
pET32a-OsZAC	ggccatggctgatatcggtatccATGAGTACCACTAATCACTATC	ggtgctcgagtcggccccaageCTAGGGTATTGGAACCCATTCC	Pull-down/Enzyme activity assay
pMAL-C5Xs-Arf1	gccgcgatatcgtcgacggatccATGGGGCTCACGTTACGAAGCTGT	cttatttaattacctgcagggaattcAGCCTTGTTGGCAATGTTGTTGGA	Pull-down/Enzyme activity assay
pMAL-C5Xs-Arf3	gccgcgatatcgtcgacggatccGGCATCGTCTTCACGGGCTCTTCT	cttatttaattacctgcagggaattcTTAGCTGCTTTTGGAATTTAGTGCAT	Pull-down/Enzyme activity assay
pCAMBIA1305-35S-GN1.1-FLAG	agctcgggtaccggggatccATGACGGGCAGTAGTTGTCC	gtcaagcttgcatgcctgcagCATTCTTCTTCCTCCGGTTC	Co-IP
GN1.1-nLuc	ggagagaacacgggggacgagctcATGACGGGCAGTAGTTGTCC	gggacgcgtacgagatctggtgcacCATTCTTCTTCCTCCGGTTC	LCI
GN1.1-eLuc	ctctacgcgtcccggggcgtaccATGACGGGCAGTAGTTGTCC	gatgatacgaacgaaagctctgcagTCACATTCTTCTTCCTCCGG	LCI
OsZAC-nLuc	ggagagaacacgggggacgagctcATGAGTACCACTAATCACTATCAAC	gggacgcgtacgagatctggtgcacGGGTATTGGAACCCATTCCAGTTCT	LCI
OsZAC-cLuc	ctctacgcgtcccggggcgtaccATGAGTACCACTAATCACTA	gatgatacgaacgaaagctctgcagCTAGGGTATTGGAACCCATT	LCI
OsArf1-nLuc	ggagagaacacgggggacgagctcATGGGGCTCACGTTACGAAGCTGT	gggacgcgtacgagatctggtgcacAGCCTTGTTGGCAATGTTGTTGGA	LCI

OsArf1-cLuc	ctcgtacgcgtcccggggcggtaccATGGGGCTCACGTTACGAAGCTGT	gatgatacgaacgaaagctctgcagTCAAGCCTTGTTGGCAATGTTGTTG	LCI
OsArf3-nLuc	ggagagaacacgggggacgagctcATGGGCATCGTCTTCACGCGGCT	gggacgcgtacgagatctggcgacGCTGCTTTTGGATTTCAGTGCATT	LCI
OsArf3-cLuc	ctcgtacgcgtcccggggcggtaccATGGGCATCGTCTTCACGCGGCT	gatgatacgaacgaaagctctgcagTTAGCTGCTTTTGGATTTCAGTGC	LCI
