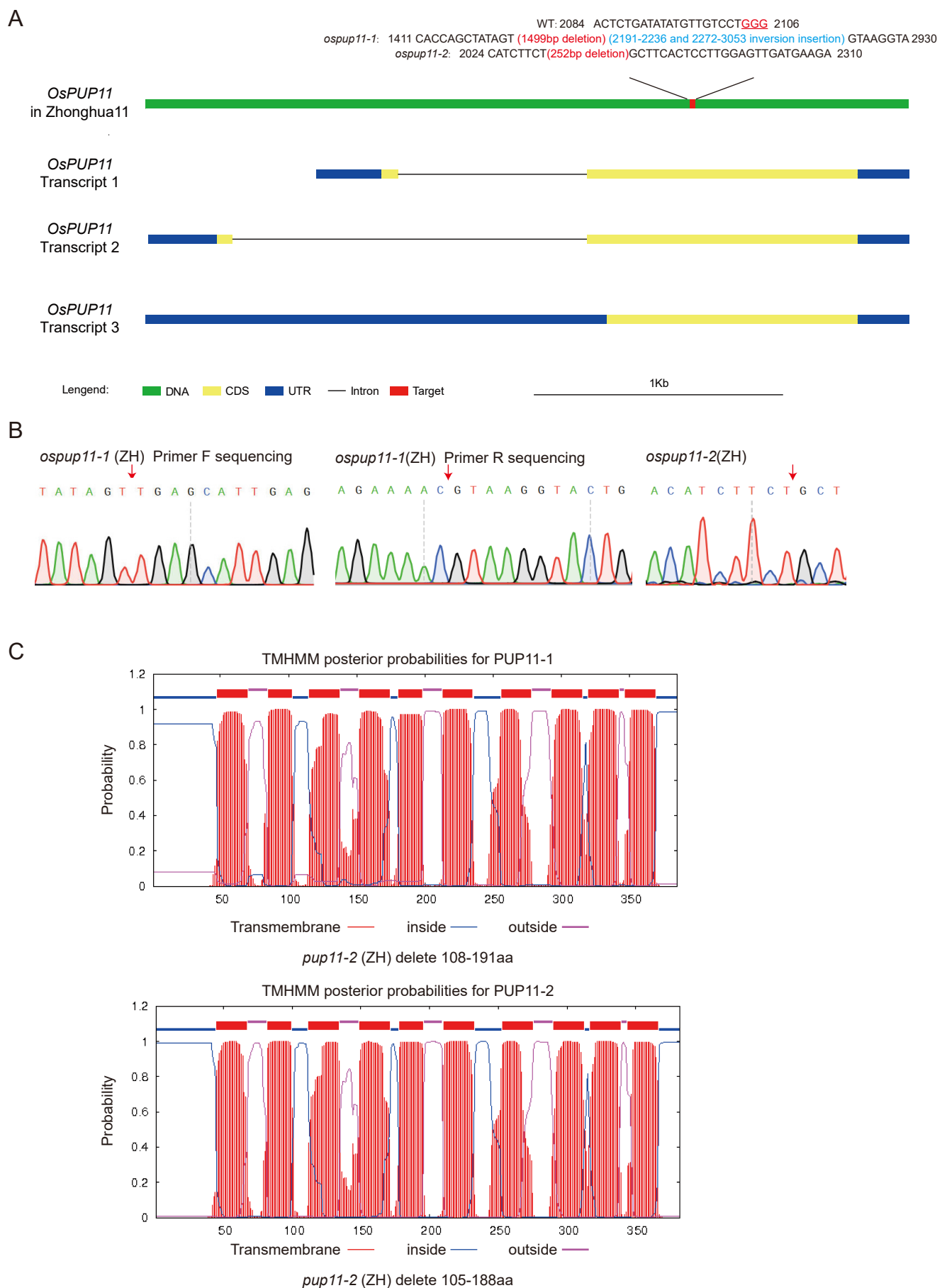
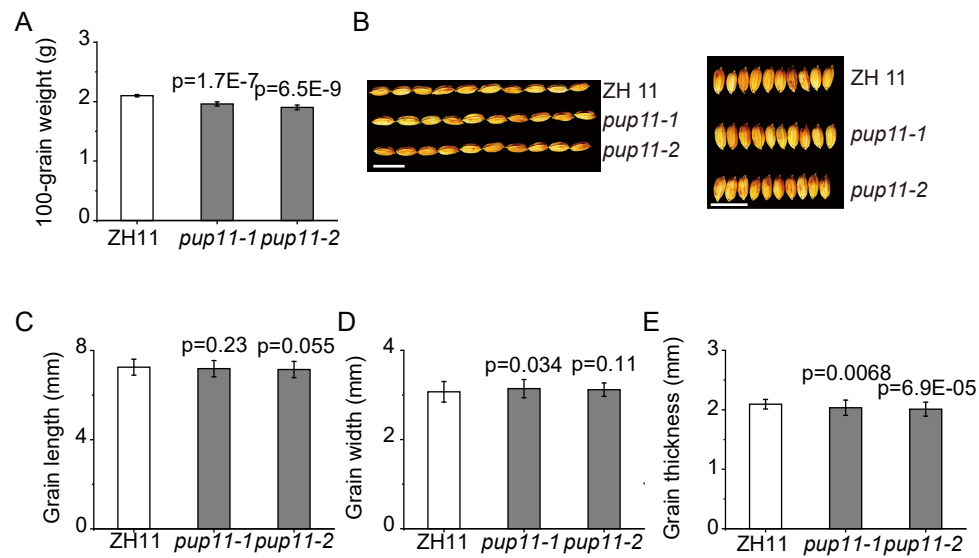
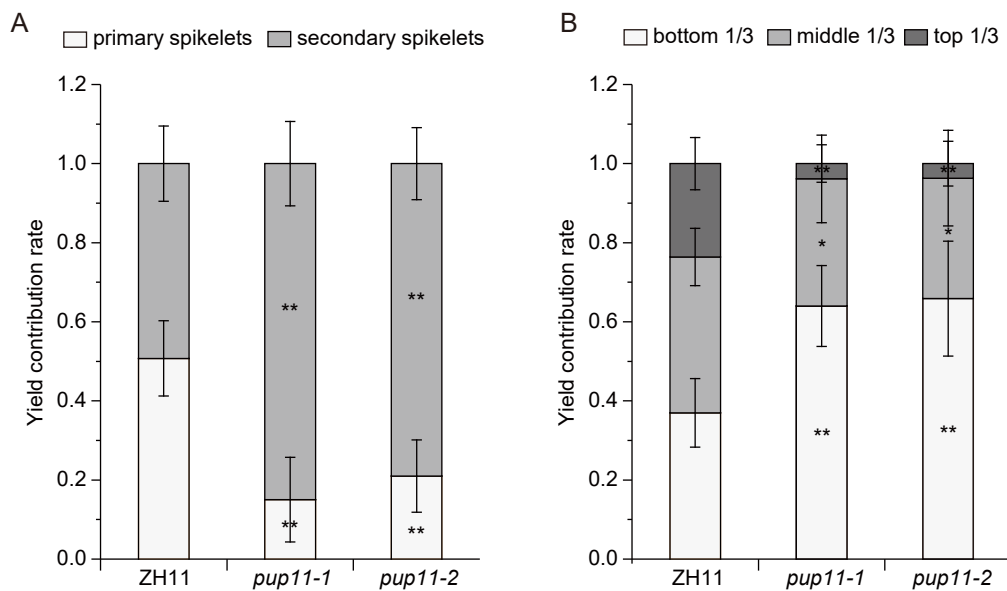




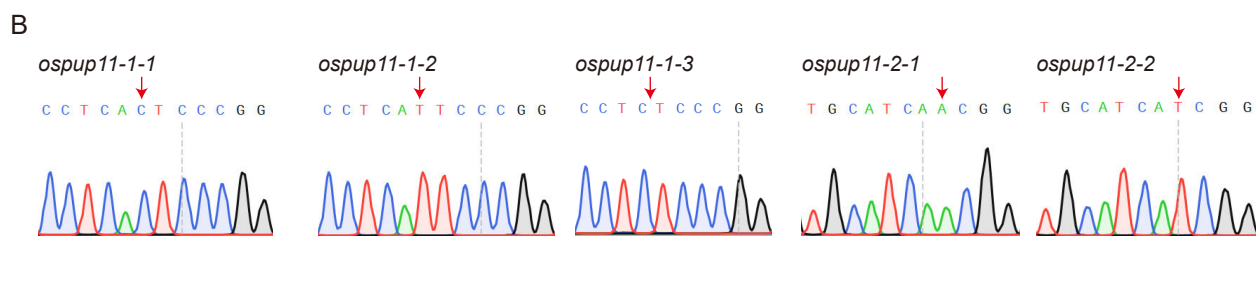
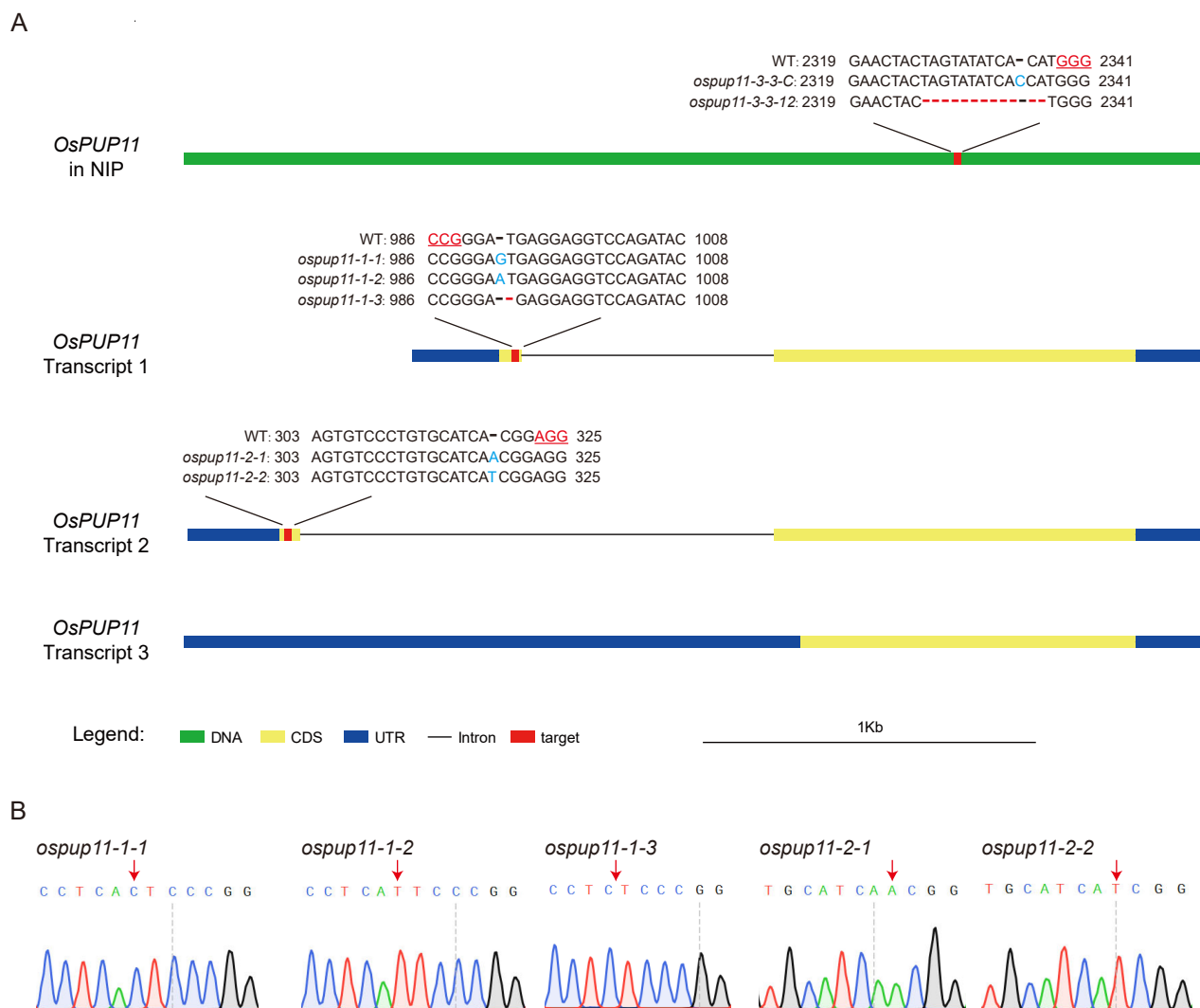
Supplemental Figure S1. Gene structures and mutation details of *PUP11* in ZH11 background. (A) Gene structures of three transcripts and the mutation details of the CRISPR edited lines targeting *PUP11*. Solid green box represents the whole DNA of *PUP11*, solid yellow boxes represent exons, solid lines represent introns, solid blue boxes represent untranslated regions, red boxes represent target sequences, and protospacer adjacent motif (PAM) sequences are shown in red with a solid red line underneath. (B) Chromatograms of the *pup11* mutant lines. The sequencing results of each mutation line. Red arrows point to mutant sites. (C) The transmembrane helix regions prediction of two *PUP11* transcript by TMHMM, and showed the protein sequence deletion in *pup11-2* mutant.



Supplemental Figure S2. Grain phenotypic characterization of Zhonghua11 (ZH11) and *pup11* mutant plants in 2020. (A) Measurement of the 100-grain weight in ZH11 and *pup11* mutants (n=15). (B) grain length and width phenotypic features of ZH11 and *pup11* mutants, images shown were digitally extracted and scaled for comparison; scale bar = 1 cm. (C-E) measurement of grain length (C), grain width (D), and grain thickness (E) of ZH11 and *pup11* mutants, n > 50 for each index. P values indicate the level of statistical significance between ZH11 and *pup11* mutants determined using the Student's *t*-test.



Supplemental Figure S3. Yield contribution rate of Zhonghua11 (ZH11) and *pup11* mutant plants in 2022. (A) Yield contribution rate of primary spikelets and secondary spikelets in ZH11 and *pup11* mutants. (B) Yield contribution rate of grains from bottom to top in ZH11 and *pup11* mutants.



Supplemental Figure S4. Gene structures and mutation details of *PUP11* in NIP background. (A) Gene structures of three transcripts and the mutation details of the CRISPR edited lines targeting *PUP11*. Solid green box represents the whole DNA of *PUP11*, solid yellow boxes represent exons, solid lines represent introns, solid blue boxes represent untranslated regions, red boxes represent target sequences, and protospacer adjacent motif (PAM) sequences are shown in red with a solid red line underneath. (B) Chromatograms of all the *pup11* mutant lines. The sequencing results of each mutation line. Red arrows point to mutant sites.

Supplemental Table S1 Phenotypes of vegetative organs in ZH11 and *pup11* mutants in 2022

	plant height (cm)	Flag leaf length (cm)	Flag leaf width (cm)	Top second leaf length (cm)	Top second leaf width (cm)	Top third leaf length (cm)	Top third leaf width (cm)	tiller number
ZH11	106.8±4.3	31.7±3.0	1.46±0.07	48.4±5.0	1.07±0.09	43.4±5.5	0.85±0.07	13.4±2.6
<i>pup11-1</i>	103.2±4.1 *	35.7±3.7 **	1.66±0.05 **	46.8±4.9	1.23±0.07 **	43.8±4.6	0.97±0.09 **	15.1±4.0
<i>pup11-2</i>	99.7±3.0 **	37.5±4.4 **	1.57±0.06 **	50.0±4.4	1.18±0.07 **	46.2±4.2	0.92±0.07 **	12.1±3.3

* for significantly different from ZH11 at P < 0.05, ** for significantly different from ZH11 at P < 0.01 (Student's *t*-test), n > 20.

Supplemental Table S2 Phenotypes of panicle structure in ZH11 and *pup11* mutants from 2020-2022

	primary branches	secondary branches	primary spikelets	secondary spikelets	spikelets per panicle	panicles per plant
ZH11	14.9±1.5	39.3±5.7	83.8±6.7	119.0±20.7	202.8±21.9	9.1±2.0
2020 <i>pup11-1</i>	14.8±0.7	48.7±5.3 **	79.1±5.7 *	158.4±20.2 **	237.5±21.4 **	10.4±1.6
<i>pup11-2</i>	14.1±1.1	44.5±5.6 *	79.3±8.0	143.5±20.4 **	222.7±23.3 *	9.2±1.6
ZH11	14.6±2.0	37.6±5.7	91.0±12.4	112.0±23.2	203.0±18.6	11.4±2.6
2021 <i>pup11-1</i>	15.5±1.6	43.9±8.6 *	92.9±10.7	136.6±31.8 *	229.5±39.7 *	12.5±3.8
<i>pup11-2</i>	15.1±1.4	44.9±4.8 **	95.4±9.1	139.0±17.0 **	234.4±20.8 **	12.1±3.2
ZH11	14.4±1.8	33.7±5.9	90.1±11.0	93.1±19.1	183.3±22.3	11.8±3.4
<i>pup11-1</i>	15.3±1.7	37.7±5.1 *	93.7±9.7	108.2±18.6 *	201.9±20.7 *	12.6±3.1
2022 <i>pup11-2</i>	16.0±1.7 *	39.5±7.4 *	99.7±9.3 **	105.4±24.6	205.1±25.1 *	11.4±3.0

* for significantly different from ZH11 at P < 0.05, ** for significantly different from ZH11 at P < 0.01 (Student's *t*-test), n > 20.

Supplemental Table S3 Phenotypes of vegetative organs in NIP and *pup11* mutants in 2022

	plant height (cm)	Flag leaf length (cm)	Flag leaf width (cm)	tiller number
NIP	90.9±1.9	39.4±2.6	1.55±0.05	19.2±3.7
<i>pup11-1-1</i>	88.3±3.1 **	40.2±3.4	1.52±0.07	18.6±4.5
<i>pup11-1-2</i>	83.3±4.2 **	34.8±2.9 **	1.46±0.05 **	18.3±3.4
<i>pup11-1-3</i>	88.7±3.3 *	35.5±2.7 **	1.48±0.06 **	19.8±3.3
<i>pup11-2-1</i>	86.5±2.7 **	38.8±4.3	1.42±0.05 **	22.2±4.6 **
<i>pup11-2-2</i>	89.0±2.4 **	38.0±2.8	1.48±0.07 **	20.1±4.4

* for significantly different from ZH11 at $P < 0.05$, ** for significantly different from ZH11 at $P < 0.01$ (Student's *t*-test) , $n > 20$.

Supplemental Table S4 Phenotypes of panicle structure in NIP and *pup11* mutants in 2021 and 2022

		primary branches	secondary branches	primary spikelets	secondary spikelets	spikelets per panicle	panicles per plant
2021	NIP	10.1±1.1	19.1±3.5	59.9±7.4	52.2±11.0	112.2±14.2	
	<i>pup11-1-1</i>	9.9±0.8	18.0±3.8	58.1±4.7	49.6±11.5	107.6±12.8	
	<i>pup11-1-2</i>	9.4±0.8	15.1±3.9 **	56.9±4.6	39.9±12.4 **	96.8±12.8 **	
	<i>pup11-1-3</i>	10.6±1.2	13.0±5.1 **	62.1±7.4	34.5±14.5 **	96.6±19.0 **	
	<i>pup11-2-1</i>	9.8±1.2	11.1±3.5 **	55.5±7.8	27.4±10.4 **	82.9±12.8 **	
	<i>pup11-2-2</i>	10.2±0.9	12.1±3.5 **	59.7±5.6	30.4±10.6 **	90.1±13.2 **	
	<i>pup11-3-3-C</i>	10.3±1.1	11.8±3.3 **	60.9±8.0	30.9±10.4 **	91.9±14.3 **	
	<i>pup11-3-3-12</i>	10.0±1.0	14.6±4.1 **	60.9±5.6	39.9±12.2 **	100.8±11.3 *	
	<i>pup11-3-3-C/12</i>	10.4±1.2	17.1±3.3	63.6±8.3	46.6±9.5	110.1±11.7	
2022	NIP	10.1±1.1	17.0±2.6	58.3±6.3	45.5±8.1	103.9±9.9	17.7±4.6
	<i>pup11-1-1</i>	9.6±0.8	13.2±3.9 **	55.3±5.0	34.2±10.8 **	89.5±13.7 **	18.3±3.8
	<i>pup11-1-2</i>	10.5±1.0	9.0±3.3 **	60.1±6.5	21.1±9.3 **	81.2±12.6 **	18.6±2.9
	<i>pup11-1-3</i>	10.7±1.2	10.5±2.6 **	61.0±5.9	26.3±8.4 **	87.4±9.8 **	18.8±2.4
	<i>pup11-2-1</i>	10.3±0.9	9.6±3.3 **	58.8±5.4	23.9±9.3 **	82.7±11.4 **	19.8±3.7
	<i>pup11-2-2</i>	11.4±1.6 **	11.5±2.3 **	66.8±7.1 **	28.3±6.5 **	95.1±9.1 **	18.7±4.6
	<i>pup11-3-3</i>	10.4±1.5	8.6±2.5 **	56.6±8.1	21.2±6.4 **	77.8±13.3 **	

* for significantly different from ZH11 at P < 0.05, ** for significantly different from ZH11 at P < 0.01 (Student's *t*-test), n > 20.

Supplemental Table S5 Details of the primers used in the study

Primer name	Primer sequence	Usage
PUP11-1/2-NIP-mF	ACTCCCTCCATCCTGCTTC	Genotyping
PUP11-1/2-NIP-mR	ATAAGCCCGAGAGAACGAAC	
PUP11-3-NIP-mF	GATGTCCACATTCGTCCAAA	
PUP11-3-NIP-mR	ATGATGGGTAGAGCTAGGGT	
PUP11-1-ZHmF	AACGCAGATGAAATGAACTC	
PUP11-1-ZHmR	TTCTCTTGCTTGATACATCC	
PUP11-2-ZHmF	AACGCAGATGAAATGAACTC	
PUP11-2-ZHmR	TCTACCCATCATTCCTGTTT	
PUP11-ZH-sgF	GGCAACTCTGATATATGTTGTCCT	Construct mutants
PUP11-ZH-sgR	AAACAGGACAACATATATCAGAGT	
PUP11-1-NIP-sgF	GGCAGTATCTGGACCTCCTCATCC	
PUP11-1-NIP-sgR	AAACGGATGAGGAGGTCCAGATAC	
PUP11-2-NIP-sgF	GGCAAGTGTCCCTGTGCATCACGG	
PUP11-2-NIP-sgR	AAACCCGTGATGCACAGGGACACT	
PUP11-3-NIP-sgF	GGCAGAACTACTAGTATATCACAT	
PUP11-3-NIP-sgR	AAACATGTGATATACTAGTAGTTC	
PUP11-proXbaF1	TCTAGAGCAGCCCGAGTATTTTTGTCAT	Construct GUS vectors
PUP11-1-proBamHR2	GGATCCGCCCCGTCGTTGGGGGCGCTGCTCC	
PUP11-2-proBamHR2	GGATCCCGAGAGGCCGACAGCGACCAGTG	
pup11-terSacF	GAGCTCTCATCACAATGTTGCCTAGAATGC	
pup11-terEcoRR	GAATTCTCTGGTTTTGTGGTTAAGGGATC	
PUP11-1cdsHindF	AAGCTTATGGCCGGTGACAGCGGCAACAG	Construct GFP/mCherry vectors
PUP11-2cdsHindF	AAGCTTATGAGCAACCCATGCCGAGTGT	
PUP11-1/2cdsKpnR	GGTACCGGAAGTTTCCTCTACACTGACT	
PUP11-1rtF	TATCAACTCCCTCTCTCTCC	q-RT PCR
PUP11-2rtF	ATGCTCACTGGTCGCTGTC	
PUP11-1/2rtR	CAAGAAGTGTAGATGCGGTC	
RR1rtF	CAAGGAAGAAGGCAAGGAGCAG	
RR1rtR	TCCTCTGGGGCTCGTTTTCC	
RR2rtF	CGGAGATGACAGGATACGAT	
RR2rtR	CGCTGGACATCGTTCATCTT	
RR3rtF	CGAGTTACGACGGTGGATAG	
RR3rtR	CCGCCGACTCCTTGACCCTCT	
RR4rtF	TCTTCTGAGAATGTGCCTGCAA	
RR4rtR	TGTGTGGCGGCTTATCTGGTG	
RR6rtF	GTCCCCAACGTCAACATGATC	
RR6rtR	TGAGACGATTCTTGACGCG	
RR9rtF	TGCTTTCTCCTTGAGTCTCTTCTG	
RR9rtR	CGGAATCAACAGTGGTAAC	
SUT1rtF	GCTTTCAACCAGGGTGTGTCAG	
SUT1rtR	ACTTCCGGCACATTGGTTC	

SUT2rtF	CACCCTAGCACAGAACAGGA
SUT2rtR	ATCCAATCCATGTGAGGGCA
SUT4rtF	TTTAGCTAAGCCGTCCCAAAG
SUT4rtR	CACGGCATGATGATGATATTG
SWEET11rtF	GGGATTTCTGGCTAGTTTCT
SWEET11rtR	CGAGGTAGAGGACGATGTAG
SWEET15rtF	CTTCACCTTTGGCATCTTAG
SWEET15rtR	AACGCGTAGTACATCCACA
SSSIrtF	GGGCCTTCATGGATCAACC
SSSIrtR	CCGCTTCAAGCATCCTCATC
SSSII-3rtF	GGCCAAGTACCAATGGTGAA
SSSII-3rtR	GCATGATGCATCTGAAACAAAGC
SSSIII-2rtF	CTCAAACAGGTGAAGCCACA
SSSIII-2rtR	CCCATGACAGGCACTTCTTT
