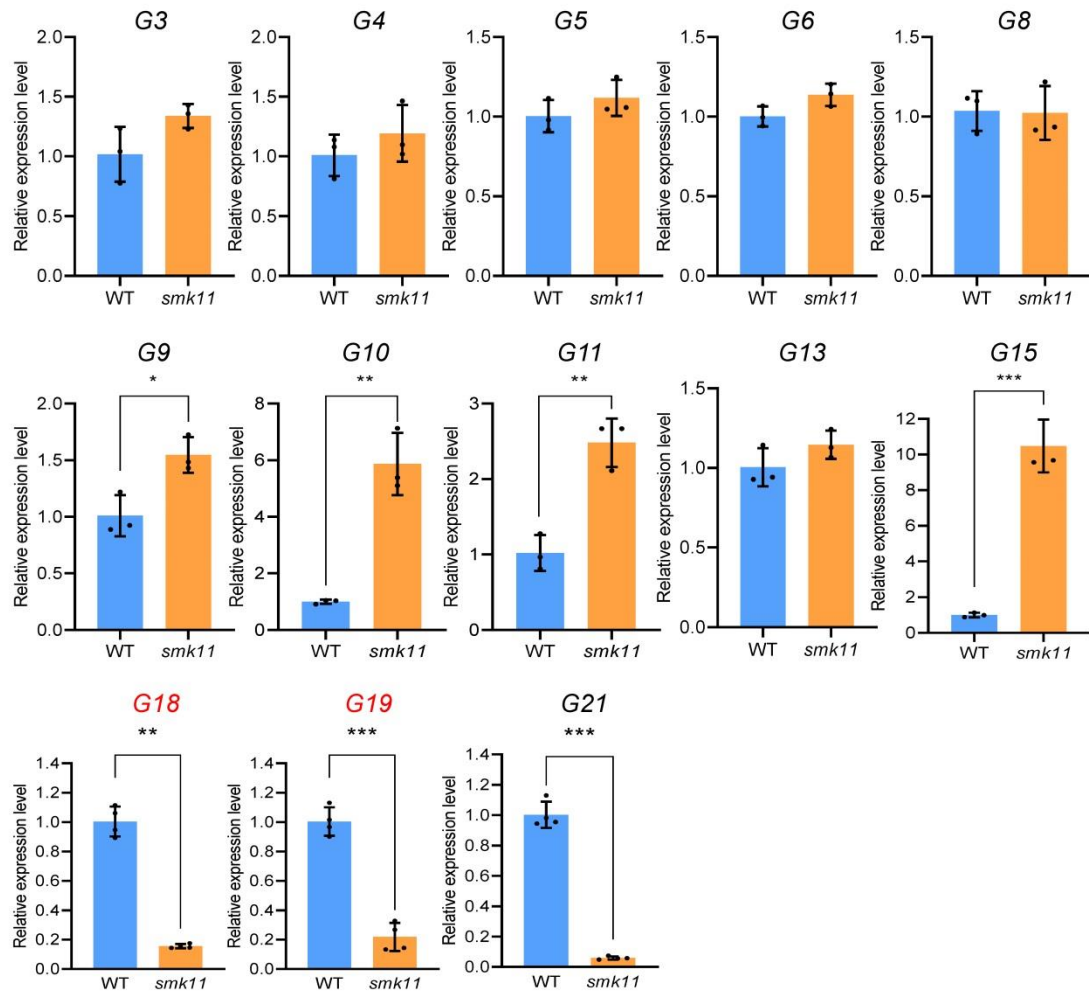


Supplemental Figure S1 The homozygous *smk11* plants could produce small and inviable progeny.

(a) WT and *smk11* mutant (orange arrows) plants at 70 days after planting. (b) Randomly selected mature WT kernels from the segregated F₂ population. (c) Randomly selected mature *smk11* kernels from the segregated F₂ population. (d) The selfed progenies of homozygous F₂ mutant kernels at maturity. (e-g) Comparison of grain length (n=30 seeds) (e), grain width (n=30 seeds) (f), and starch content (n=3 ears) (g) between WT and *smk11* kernels. Values are the average ± S.E. (***) $p < 0.001$, Student's *t*-test). Scale bars=10 cm (a) ; 2 cm (b-d).



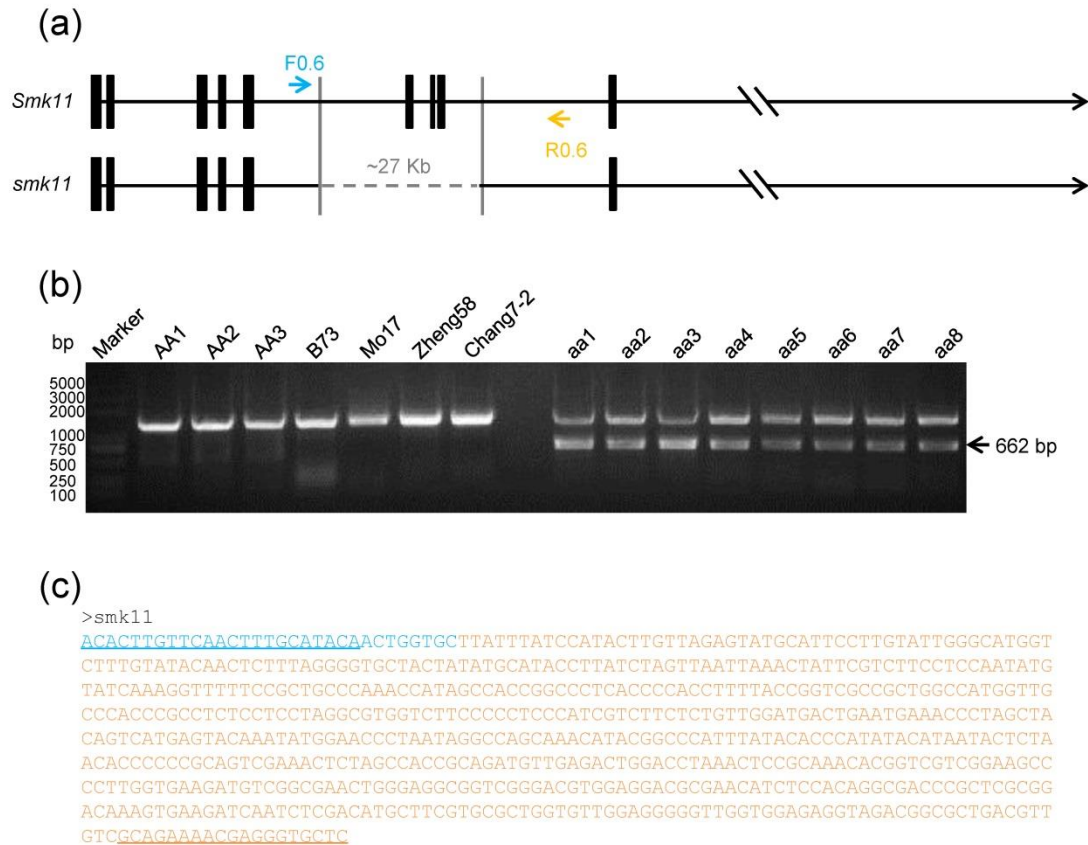
Supplemental Figure S2 RT-qPCR analysis of 13 expressed genes in candidate interval.

ZmActin was used as an internal control. Data are the average \pm S.E. of three biological replicates. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Student's *t*-test).

GCATGTGAAAGCTGAACCACATATTTGGAAGCAATTGAGATCCTGGAGGAATCATTGGACCTGATGATATACGG
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ACAGTTCAGCAATGATTCAATCAGGATTACAGAAGGACAATTAATTCCTTCAAGAAAAGATCAGAATAAAATTAAC
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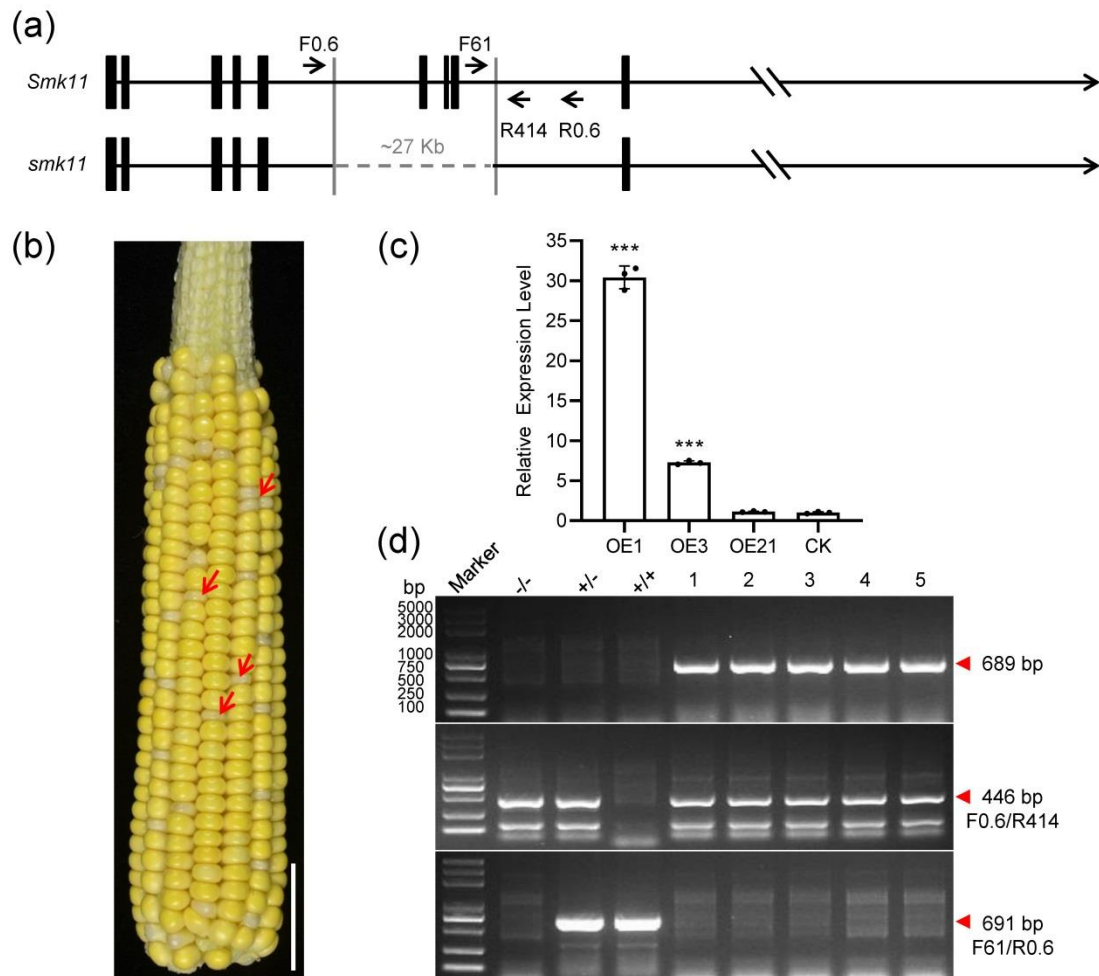
Supplemental Figure S3 The cDNA sequence of *LOC100192003* in inbred line B73 obtained using 3'RACE.

The sequence highlighted in orange represents the location of inner primer, the letters highlighted in blue represent the stop codon, and the sequence underlined stands for 3' UTR.



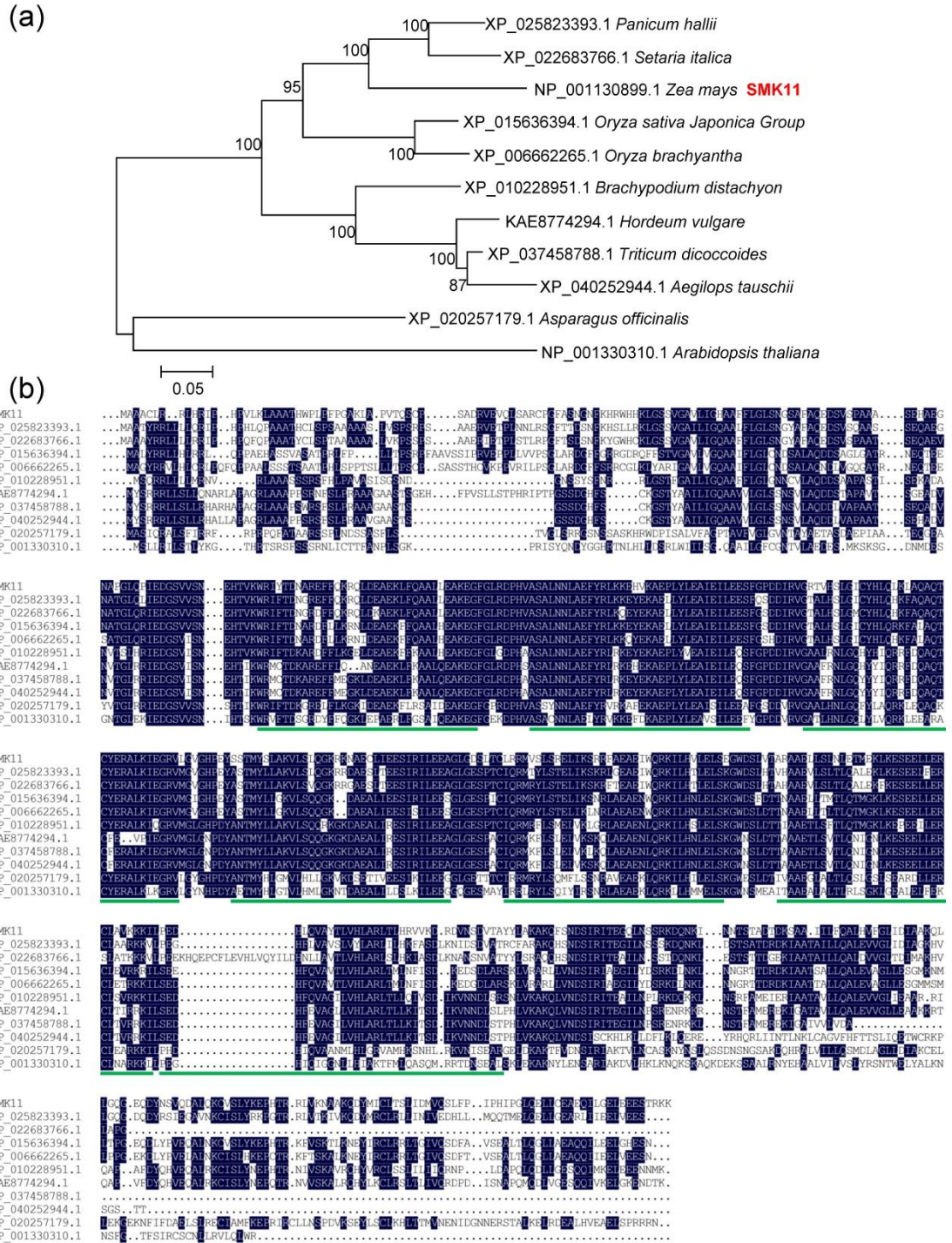
Supplemental Figure S4 The sequencing conducted on the Pacific Bioscience Sequel II platform was confirmed by PCR amplification.

(a) Diagram showing the gene structure of *Smk11* and *smk11*. The lines represent introns, the black boxes represent the first nine exons. The gray dashed line stands for the deletion fragment in *smk11*. The expected amplification product using specific primer pairs are indicated. (b) The validation of genotype in *Smk11* (wild type) and *smk11* mutant. Genomic DNA from homozygous WT kernels (AA1 to AA3 from three ears), B73, Mo17, Zheng58, Chang7-2, and eight homozygous mutants kernels (aa1 to aa8 from three ears) were extracted and amplified using primer pairs F0.6/R0.6. (c) The sequencing of 662 bp in Fig.S4b. The sequences underlined stand for the location of primer pairs. The letters highlighted in blue represent the 5' flanking sequence of the deletion, the letters highlighted in orange represent the 3' flanking sequence of the deletion.



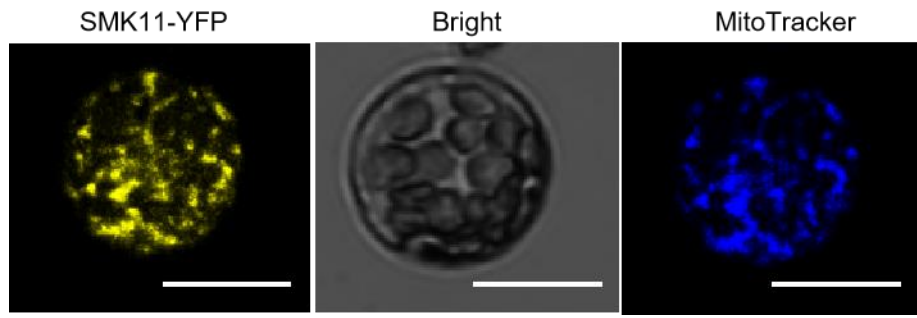
Supplemental Figure S5 Functional complementation test of *smk11*.

(a) Diagram showing the gene structure of *Smk11* and *smk11*. The lines represent introns, the black boxes represent the first nine exons. The gray dashed line stands for the deletion fragment in *smk11*. The expected amplification products using different primer pairs are indicated. **(b)** T_2 ear of self-pollinated plants with heterozygous transgene and homozygous *smk11* mutant. Red arrows indicate the small kernels. Scale bar = 3 cm. **(c)** qRT-PCR analysis of *Smk11* gene in three independent transformants (OE1, OE3 and OE21). CK stands for non-transgenic maize used as a negative control. *ZmActin* was used as an internal control. Data are the average \pm S.E. of three biological replicates. (***) $p < 0.001$, Student's *t*-test). **(d)** The transgenic lines (T_0) were crossed to the homozygous mutant (*smk11/smk11*) and then self-pollinated to obtain F_2 ears. Using the molecular markers linked to the *smk11* locus (F0.6/R414 and F61/R0.6) and the primers cross *Smk11* and *Nos* (Smk11-CX-F/Nos-R2), five containing homozygous *smk11* alleles and transgenic positive kernels were sorted out, a homozygous mutant (-/-) kernel, a heterozygous WT (+/-), and a homozygous WT (+/+) kernel were used as controls.



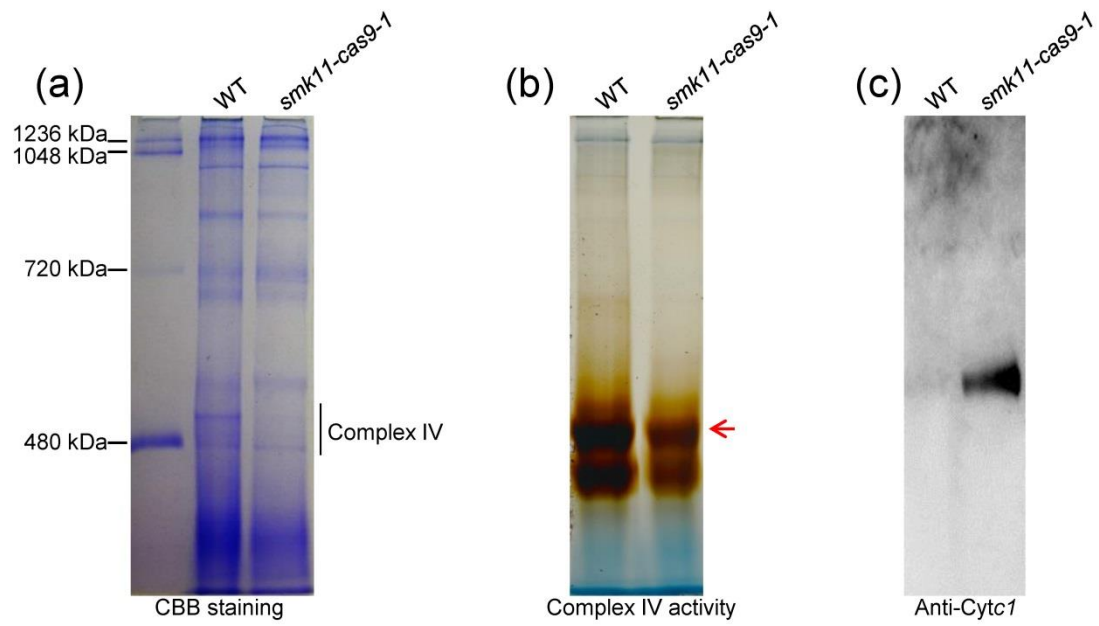
Supplemental Figure S6 Phylogenetic relationships and amino acid alignment of SMK11 and its homologs.

(a) The SMK11 protein and its homologs from NCBI were aligned by ClustalW, and the phylogenetic tree was constructed using MEGA 5.0 software. Distances were estimated with a neighbor-joining algorithm, and bootstrap support is indicated to the left of branches. Scale bar indicates the average number of amino acid substitutions per site. (b) The amino acid sequences of SMK11 homologs were downloaded from Phytzome v.12.1 (<https://phytozome.jgi.doe.gov>) and were aligned with DNAMAN. The TPR domains were highlighted with green lines.



Supplemental Figure S7 Localization of SMK11 in maize protoplasts.

MitoTracker Red was used to label mitochondria. Scale bar = 10 μm .

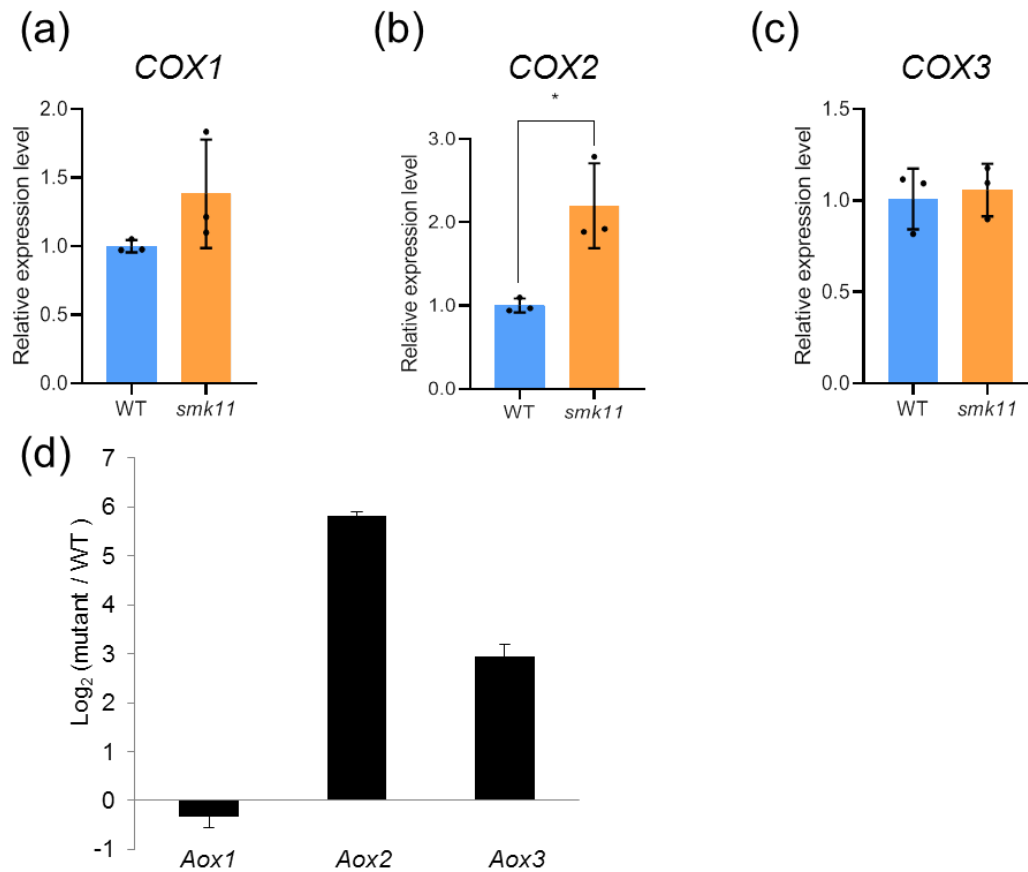


Supplemental Figure S8 Impact on mitochondrial complexes in *smk11-cas9-1* kernels at 15 DAP.

(a) BN (blue native) gel was stained with Coomassie Brilliant Blue (CBB). The position of mitochondrial complex IV is indicated. About 100 μ g of mitochondrial protein was loaded in each lane.

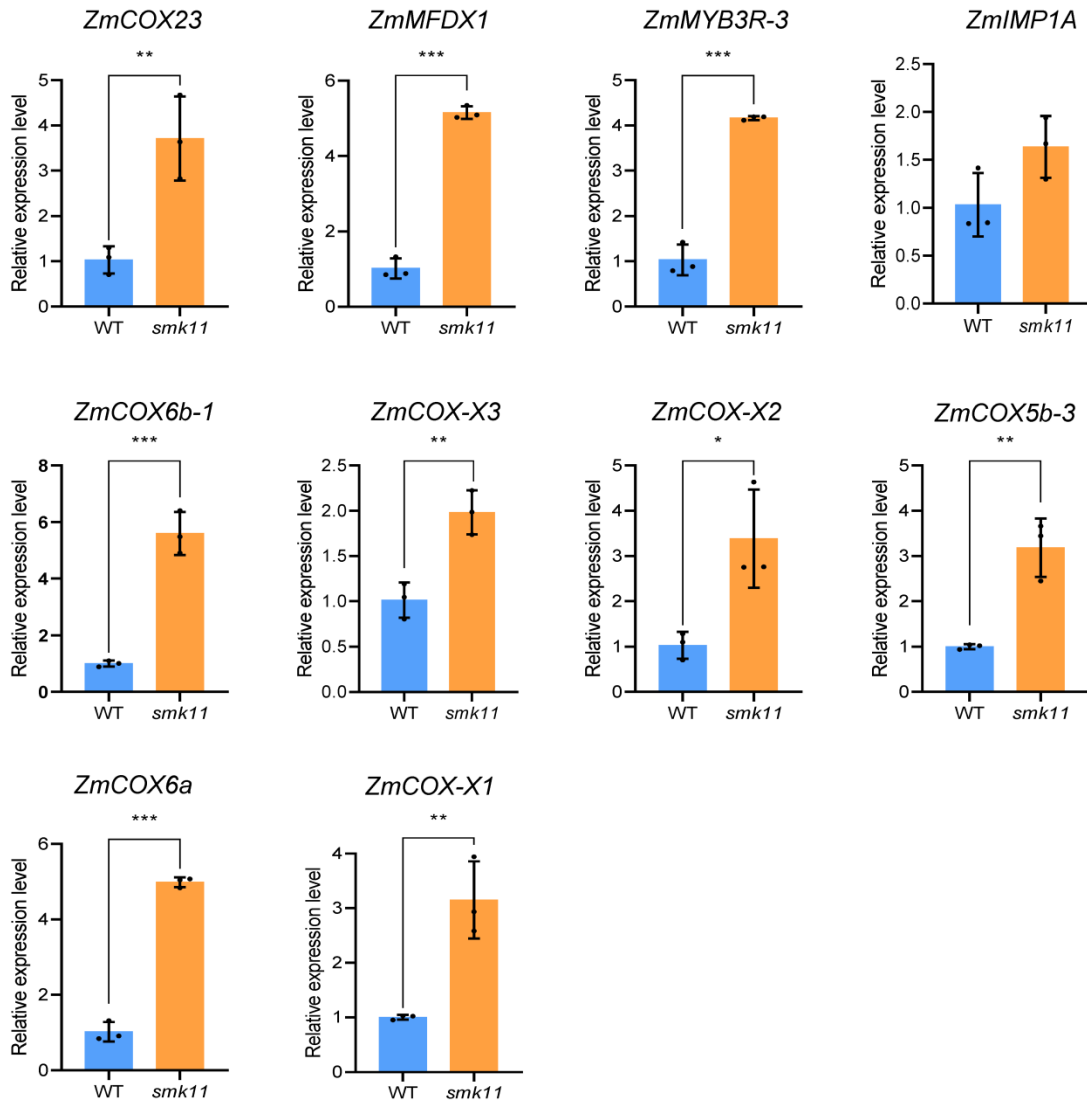
(b) BN gels were used for activity staining of complex IV. The position of mitochondrial complex IV is indicated in red arrow.

(c) BN gels transferring to PVDF membranes were probed with anti-Cytc₁ (a subunit of complex III).



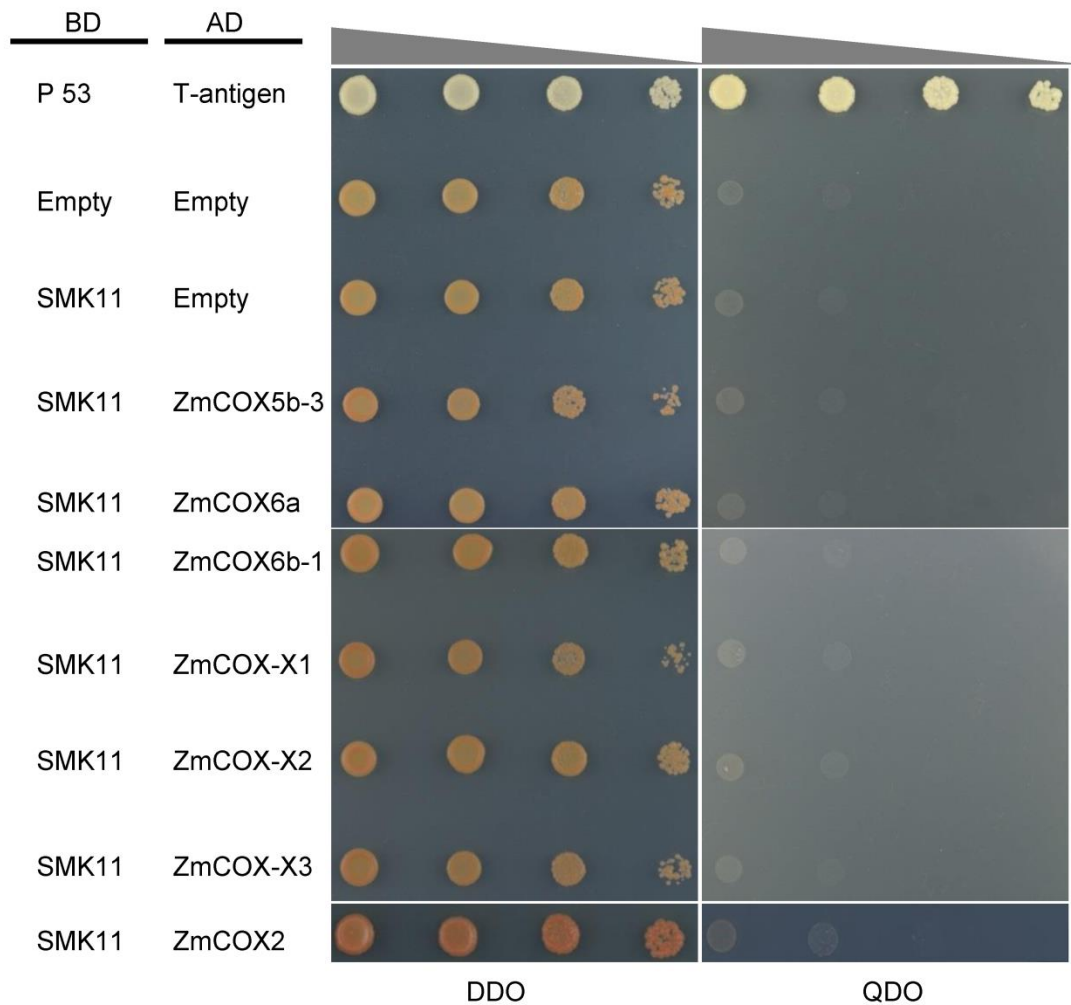
Supplemental Figure S9 RT-qPCR analysis of *Cox1*, *Cox2*, *Cox3*, as well as *Aox1*, *Aox2*, and *Aox3* expression in *smk11* kernels at 15 DAP.

(a) RT-qPCR analysis of *COX1* expression in *smk11* mutant. (b) RT-qPCR analysis of *COX2* expression in *smk11* mutant. (c) RT-qPCR analysis of *COX3* expression in *smk11* mutant. (d) RT-qPCR analysis of *Aox1*, *Aox2*, and *Aox3* expression in *smk11* mutant. *ZmActin* was used as an internal control. Data is the average \pm S.E. of three biological replicates. (* $P < 0.05$, Student's *t*-test).



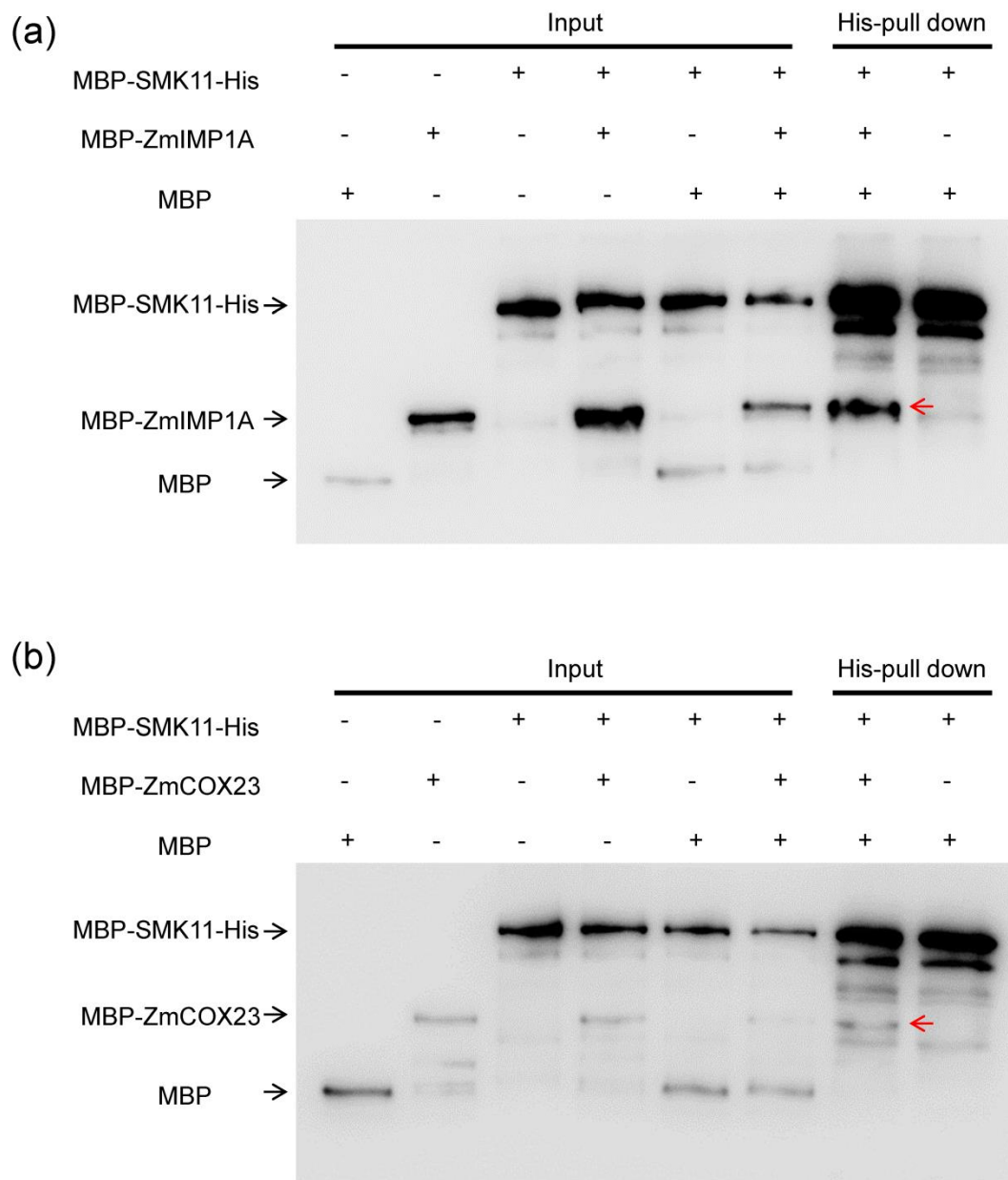
Supplemental Figure S10 RT-qPCR analysis of the expression of four assembly factors and six subunits of complex IV.

ZmActin was used as an internal control. Data are the average \pm S.E. of three biological replicates. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Student's *t*-test).



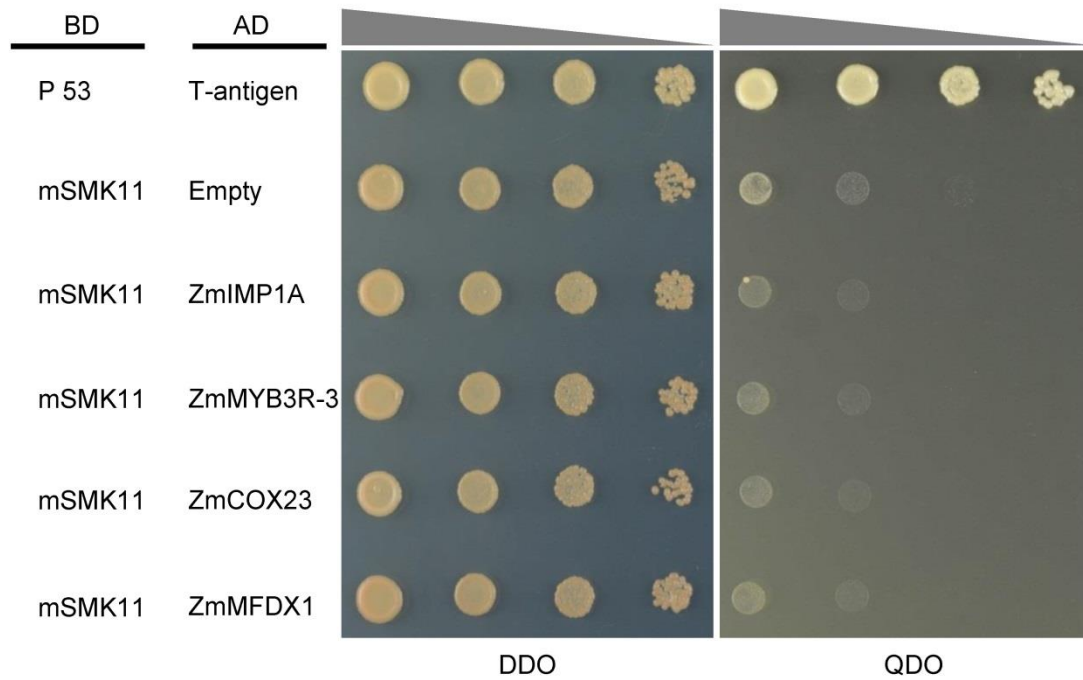
Supplemental Figure S11 The SMK11 does not interact with the seven subunits of complex IV.

Yeast two-hybrid (Y2H) analysis between SMK11 and the seven subunits (ZmCOX5b-3, ZmCOX6a, ZmCOX6b-1, ZmCOX-X1, ZmCOX-X2, ZmCOX-X3, and ZmCOX2) of complex IV. The Y2H Gold strain harboring the indicated bait and prey constructs were spotted on SD-Trp-Leu (DDO) and SD-Trp-Leu-His-Ade (QDO). The interaction between Human P53 and T-antigen was used as a positive control. AD, activating domain; BD, binding domain.



Supplemental Figure S12 Recombinant protein MBP-SMK11-His interacts with MBP-ZmIMP1A and MBP-ZmCOX23, as determined using in vitro His pull-down assays.

(a) Recombinant protein MBP-SMK11-His interacts with MBP-ZmIMP1A in vitro by His pull-down assays. **(b)** Recombinant protein MBP-SMK11-His interacts with MBP-ZmCOX23 in vitro by His pull-down assays. '+' and '-' indicate the presence and absence of the corresponding proteins in the reactions, respectively. Red arrows indicate the positions of interacting proteins.



Supplemental Figure S13 Mutation of SMK11 (mSMK11) could not interact with the four assembly factors of complex IV.

Yeast two-hybrid (Y2H) analysis between mSMK11 and the four assembly factors (ZmIMP1A, ZmMYB3R-3, ZmCOX23, and ZmMFDX1) of complex IV. The Y2H Gold strain harboring the indicated bait and prey constructs were spotted on SD-Trp-Leu (DDO) and SD-Trp-Leu-His-Ade (QDO). The interaction between Human P53 and T-antigen was used as a positive control. AD, activating domain; BD, binding domain.

>Cox1-WT
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>Cox1-smk11
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>Cox2-WT
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>Cox2-smk11
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ATGTAACC

>Cox3-WT
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>Cox3-smk11
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Supplemental Figure S14 Sequence analysis of *Cox1*, *Cox2* and *Cox3* transcripts in WT and *smk11*.

Supplemental Table S1. Primers used in this study.

Primer name	Primer sequence 5'-->3'	Use for
umc2578-F	CGGAAGGGAGAACTTGTTACGTT	
umc2578-R	GATGCCGTCGTCCTTTTCTG	
umc2035-F	ACCACA ACTACTACGGCCGAAAC	
umc2035-R	ACTGTTGCTGGTACGAAGAGTTCC	
umc1048-F	TGCGCTGTTTCCTACTCAGACTAA	
umc1048-R	AAGACAAGTCCAGTGACGAAGAGC	
umc2294-F	ATTGGAGTGGCTCCATTGCTT	
umc2294-R	CCCACCATTCTATATATTGTTGCCA	
ID24.160-F	GAAAGAAGGTAGATGTGAGGCA	
ID24.160-R	ACCGTTCGACCAAGCTAACT	
ID26.019-F	TGTCGTGTAACCTAAGATGCAC	Map-based cloning of
ID26.019-R	CCCAATCAATACAGCAGCGT	<i>Smk11</i>
ID27.495-F	AAGTCCCCTTGTTGATGCAA	
ID27.495-R	ACCATAGTTTAGAGTTCCAGCAA	
ID27.884-F	CAGCAAGCAACTCTCGCAAT	
ID27.884-R	CAGCAGGCAGGAAAACGATG	
SNP074239-F	AGATAAGGCTGAACAGAACCT	
SNP074239-R	ACCAGATTATGATTCGAGACCA	
SNP305415-F	AGGAGCATTGTTGGGAGGGTC	
SNP305415-R	CCCGAAGTGCTAGTGGATCT	
SNP035779-F	GCATCTCTTTCTTACTTAGTCAGCA	
SNP035779-R	GGTTGCCAGGATGACGTG	
3RACE-Outer-F	CGAACACACGGTCAAATGGA	Amplification of full-length
3RACE-Inner-F	GCATGTGAAAGCTGAACCAC	<i>Smk11</i> transcripts

SMK11-MT1-BsF	ATATATGGTCTCTGGCGGCTGCGGCGAGCTTAAGGACGTT	Construct pBUE411-2gRNA-Smk11 vector
SMK11-MT1-F0	CGGCTGCGGCGAGCTTAAGGACGTTTTAGAGCTAGAAATAGC	
SMK11-MT2-R0	AACGTGCCAAGTTGGCTCCGGTCCGCTTCTTGGTGCC	
SMK11-MT2-BsR	ATTATTGGTCTCTAAACGTGCCAAGTTGGCTCCGGTCC	
35S-Smk604-inF	GGACTCTTGACCATGGCCGCCGCCTG	Construct p3301-35S-Smk11 vector RT-PCR analysis of <i>Smk11</i> gene expression
35S-Smk604-inR	ATTCGAGCTGGTCACCTCATTGCTTCTTCCTAGTACTCTCT	
Smk604-F	CACCCTCAGTCCCCACATG	
Smk604-R	ATGGGTGTTGGTTCCTACGT	
ZmAox1-qF	CCTATTGGACCGTCAAATTACTGC	qRT-PCR analysis of <i>Aox</i> gene expression
ZmAox1-qR	CACTGTTTCCAGCATCATAGCAC	
ZmAox2-qF	CCAAGACGCTGATGGATAAGGT	
ZmAox2-qR	CCACGGTTTCCAGCATCAT	
ZmAox3-qF	CGGCACCGAGAAGCATGA	
ZmAox3-qR	CTGGTCCACTTCCACTCCGT	
G3-qF3	TTACTGTTTCCTGGCTCCCA	qRT-PCR analysis of 13 candidate genes expression
G3-qR3	AACGCACCAATGTCAAAGCT	
G4-qF2	AAGCTCTACGAGAAGTCCGG	
G4-qR2	ATCTTGAGGTCCGGCATGAA	
G5-qF2	TGCCTCCAAATAGCTGTCCT	
G5-qR2	TCCTACCAAGTCCACACACC	
G6-qF2	TGCCCGTGTTTCTGTCAATC	
G6-qR2	AATGGCTCCTCGAACCTTCA	
G8-qF3	GTGCCAAGCCAAGACAGAAA	
G8-qR3	CGTGCCATGTCCTCAAGAAA	
G9-qF3	AGTCCTCAACCTTCTCACGG	
G9-qR3	AACCATCGTCTGCTTCCTCA	

G10-qF1	TGTTGATCAAATGCCGCAA	
G10-qR1	CAACCAGGCCCTCACATTTT	
G11-qF2	TCACCCAGGAGCATGTCATT	
G11-qR2	GGAGAACCCTTTGCACCGAAG	
G13-qF1	CCCATACCGTTCCCTACTCC	
G13-qR1	ACCTTCTTGCTGTCGTCGTA	
G15-qF3	AGGGACAATACGCATGGCTA	
G15-qR3	TCCAAGCTTCTCTGGAGTGG	
G18-qF3	CTGATAATGCGCGGGAGTTT	
G18-qR3	GGTCCAAATGATTCTCCAGG	
G19-qF2	GGCAAGGGGAGCAAGACTAT	
G19-qR2	CCGGTATGTGTGGAATGGGA	
G21-qF2	ATGACGTTCGGCCTCATGTA	
G21-qR2	CCCAGCCAGTACACCCAG	
ZmActin-qF	ATGGTCAAGGCCGGTTTTCG	qRT-PCR internal control
ZmActin-qR	TCAGGATGCCTCTCTTGGCC	
pGWC-Smk11-F	AGCAGGCTTTGACTTTATGGCCGCCGCCTG	Construct pGWC-Smk11
pGWC-Smk11-R	TGGGTCTAGAGACTTTCCTTGCTTCTTCCTAGTACTCTCT	
pGBKT7-Smk11-F	CATGGAGGCCGAATTCATGGCCGCCGCCTG	Construct pGBKT7-Smk11
pGBKT7-Smk11-R	GCAGGTGCACGGATCCTCATTGCTTCTTCCTAGTACTCTCT	
nLUC-Smk11-F	CACGGGGGACGAGCTCGGTACCATGGCCGCCGCCTG	Construct nLUC-Smk11
nLUC-Smk11-R	ACGCGTACGAGATCTGGTCGACTTGCTTCTTCCTAGTACTCTCT	
pGWC-3660-F	AGCAGGCTTTGACTTTATGCTGCCGTCCCTGAA	Construct pGWC vectors
pGWC-3660-R	TGGGTCTAGAGACTTTTTAAGACTGCTTCGTTGCGTC	
pGWC-2938-F	AGCAGGCTTTGACTTTATGTATCCTACGTTTACGGCC	
pGWC-2938-R	TGGGTCTAGAGACTTTTTAAATCGCACACAGCATAACC	

pGWC-1501-F	AGCAGGCTTTGACTTTTATGCCTCGCCGCAGCT	
pGWC-1501-R	TGGGTCTAGAGACTTTTTAGACAGACACACCACCACG	
pGWC-7104-F	AGCAGGCTTTGACTTTTATGTCCATGTTAGAAGCTGCTCA	
pGWC-7104-R	TGGGTCTAGAGACTTTTCAGTGTGGTTTCGGTACATAGC	
pGADT7-3660-F	GGAGGCCAGTGAATTCATGCTGCCGTCCCTGAA	
pGADT7-3660-R	CGAGCTCGATGGATCCTTAAGACTGCTTCGTTGCGTC	
pGADT7-2938-F	GGAGGCCAGTGAATTCATGTATCCTACGTTTACGGCC	
pGADT7-2938-R	CGAGCTCGATGGATCCTTAAATCGCACACAGCATAACC	
pGADT7-1501-F	GGAGGCCAGTGAATTCATGCCTCGCCGCAGCT	
pGADT7-1501-R	CGAGCTCGATGGATCCTTAGACAGACACACCACCACG	
pGADT7-7104-F	GGAGGCCAGTGAATTCATGTCCATGTTAGAAGCTGCTCA	
pGADT7-7104-R	CGAGCTCGATGGATCCTCAGTGTGGTTTCGGTACATAGC	
pGADT7-5396-F	GGAGGCCAGTGAATTCATGACGGCTGGCTACATTGTT	
pGADT7-5396-R	CGAGCTCGATGGATCCCTACTCAGTGGACTTGACGATGAAG	Construct pGADT7 vectors
pGADT7-2925-F	GGAGGCCAGTGAATTCATGGCCGCGGAAGGC	
pGADT7-2925-R	CGAGCTCGATGGATCCTTACAGAGGTCCAGGGAAGGT	
pGADT7-9691-F	GGAGGCCAGTGAATTCATGACGGAGGCCCCGAG	
pGADT7-9691-R	CGAGCTCGATGGATCCTTAATCTTTATGCGAATGGGC	
pGADT7-7512-F	GGAGGCCAGTGAATTCATGATAATGGCAAATTGACAATG	
pGADT7-7512-R	CGAGCTCGATGGATCCTCAGCCAGAGGCAGTCTTCTT	
pGADT7-4218-F	GGAGGCCAGTGAATTCATGGCTTCGGCGGCG	
pGADT7-4218-R	CGAGCTCGATGGATCCTCAGTGGTGGTCCTTCGTCTC	
pGADT7-4738-F	GGAGGCCAGTGAATTCATGTGGCGCCGCCTC	
pGADT7-4738-R	CGAGCTCGATGGATCCTTAGTGATGTCCATCATCTTCATC	
cLUC-3660-F	TACGCGTCCCGGGGCGGTACCATGCTGCCGTCCCTGAA	
cLUC-3660-R	TCCTTGTAGTCCATTTGTTGTTAAGACTGCTTCGTTGCGTC	Construct cLUC vectors

cLUC-2938-F	TACGCGTCCCGGGGCGGTACCATGTATCCTACGTTTACGGCC	
cLUC-2938-R	TCCTTGTAGTCCATTTGTTGTTAAATCGCACACAGCATACC	
cLUC-1501-F	TACGCGTCCCGGGGCGGTACCATGCCTCGCCGCAGCT	
cLUC-1501-R	TCCTTGTAGTCCATTTGTTGTTAGACAGACACACCACCAG	
cLUC-7104-F	TACGCGTCCCGGGGCGGTACCATGTCCATGTTAGAAGCTGCTCA	
cLUC-7104-R	TCCTTGTAGTCCATTTGTTGTCAGTGTGGTTTCGGTACATAGC	
pGBKT7-3660-F	CATGGAGGCCGAATTCATGCTGCCGTCCCTGAAC	
pGBKT7-3660-R	GCAGGTGCACGGATCCTTAAGACTGCTTCGTTGCGTC	
pGBKT7-2938-F	CATGGAGGCCGAATTCATGTATCCTACGTTTACGGCC	
pGBKT7-2938-R	GCAGGTGCACGGATCCTTAAATCGCACACAGCATACC	Construct pGBKT7 vectors
pGBKT7-1501-F	CATGGAGGCCGAATTCATGCCTCGCCGCAGCT	
pGBKT7-1501-R	GCAGGTGCACGGATCCTTAGACAGACACACCACCAG	
pGBKT7-7104-F	CATGGAGGCCGAATTCATGTCCATGTTAGAAGCTGCTCA	
pGBKT7-7104-R	GCAGGTGCACGGATCCTCAGTGTGGTTTCGGTACATAGC	
Smk11-CX-F	CCTCGCAAAGCAAACAGTT	
Nos-R2	GTAACATAGATGACACCGCGC	Genotype analysis of transgenic complementation kernels
F0.6	ACACTTGTTCAACTTTGCATACA	
R414	CAGTCTCAACATCTGCGGTG	
F61	AGTATTTGCGGCTACCCTGT	
R0.6	GAGCACCCCTCGTTTTCTGC	
cox1-F1	GGCCCCTCTCTGATAAGGTT	
cox1-R1	GTTAAGGCAAAGCCCAAACA	
cox2-F1	GTCCTACTTCTGGTGCTGCC	
cox2-R1	GAGAATTGCATTTCCGCTTC	
cox3-F1	TCAATCCACTTATTCGTTCCC	
cox3-R1	GTTTACATACAACCGGGGCA	

5396-qF2	GGTCGGATCCTTTGCCATTG
5396-qR2	GCCACCACTCCTTCTCAGAA
4738-qF2	ATAGTCGGTTGTCCTGGTGG
4738-qR2	TATTGCGAGCAGACTGGACA
2925-qF1	AAGGCATTGTTTCACACGCT
2925-qR1	TACAGAGGTCCAGGGAAGGT
9691-qF1	CGAGCCGTAGGTTGCCTCT
9691-qR2	GGCTTGTGTACTTGTCCACC
7512-qF2	GTGAAGAGAATGGGAGGGCA
7512-qR2	ACGGAGAGTGTACCTTCAA
4218-qF1	TGGGAGAAGATCACCTACGC
4218-qR1	CGGATGTGCAGATAGGGGTA
3660-qF2	AAAGCGACTCCTCCAGAACC
3660-qR2	CCAAACCCGGCAAAAAGATCT
2938-qF2	ATTGGGCTTCCTGGTGAGAA
2938-qR2	ACCGAATGCCCTAGAATCCC
1501-qF1	TGCTCAAGGTATGGCGTTTG
1501-qR1	AGCTTCTGTGGCAAGAGTCT
7104-qF1	GTCCATGTTAGAAGCTGCTCA
7104-qR1	GTCTCTGTGAGCCCAAATGC

pMAL-SMK11-F	TTCAGAATTCGGATCCATGGCCGCCGCCTG
pMAL-SMK11-HisR	C GACTCTAGAGGATCCttaATGATGATGATGATGATGTTGCTTCTTCCTAGTACTCTCT
pMAL- ZmIMP1A-F0.3	TTCAGAATTCGGATCCATGCTGCCGTCCCTGAAC
pMAL- ZmIMP1A-HisR0.3	C GACTCTAGAGGATCCttaATGATGATGATGATGATGAGACTGCTTCGTTGCGTC
pMAL- ZmIMP1A-R0.3	C GACTCTAGAGGATCCttaTTAAGACTGCTTCGTTGCGTC
pMAL- ZmMYB3R-3-F0.3	TTCAGAATTCGGATCCATGTATCCTACGTTTACGGCC

Construct pMAL vectors

pMAL- ZmMYB3R-3-HisR0.3	CGACTCTAGAGGATCCttaATGATGATGATGATGATGAATCGCACACAGCATAACC
pMAL- ZmMYB3R-3-R0.3	CGACTCTAGAGGATCCttaTTAAATCGCACACAGCATAACC
pMAL- ZmMFDX1-F0.3	TTCAGAATTCGGATCCATGTCCATGTTAGAAGCTGCTCA
pMAL- ZmMFDX1-HisR0.3	CGACTCTAGAGGATCCttaATGATGATGATGATGATGGTGTGGTTTCGGTACATAGC
pMAL- ZmMFDX1-R0.3	CGACTCTAGAGGATCCttaTCAGTGTGGTTTCGGTACATAGC
pMAL- ZmCOX23-F0.4	TTCAGAATTCGGATCCATGCCTCGCCGCAGCT
pMAL- ZmCOX23-HisR0.4	CGACTCTAGAGGATCCttaATGATGATGATGATGATGGACAGACACACCACCACG
pMAL- ZmCOX23-R0.4	CGACTCTAGAGGATCCttaTTAGACAGACACACCACCACG
pMAL- ZmCOX6a-F0.3	TTCAGAATTCGGATCCATGGCTTCGGCGGCG
pMAL- ZmCOX6a-R0.3	CGACTCTAGAGGATCCttaTCAGTGGTGGTCCTTCGTCTC
pMAL- ZmCOX-X1-F0.3	TTCAGAATTCGGATCCATGACGGAGGCCCGAG
pMAL- ZmCOX-X1-R0.3	CGACTCTAGAGGATCCttaTTAATCTTTATGCGAATGGGC
pMAL-ZmCOX-X3-F0.4	TTCAGAATTCGGATCCATGATAATGGCAAATTGACAATG
pMAL-ZmCOX-X3-R0.4	CGACTCTAGAGGATCCttaTCAGCCAGAGGCAGTCTTCTT

Supplemental Table S2. The expression patterns of 21 candidate genes in a 1 Mb Interval*

Gene Name	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21
Gene ID	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ	GRMZ
	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G	M2G
	074239	392823	032648	032910	033926	034276	338853	073351	374385	073017	073034	073109	073114	404920	105987	432697	039370	142266	005199	305093	305446
Em10	0	0	3.38	19.75	7.47	23.68	0	3.94	50.89	2.5	1.12	0	0.95	0	0.26	0	0	15.98	5.25	0	59.66
Em12	0	0	2.16	37	6.71	23	0	1.46	37.29	2.8	2.2	0	2.92	0	0.76	0	0	10.14	4.93	0	84.85
Em14	0	0	1.14	26.22	11.75	26.97	0	0.2	33.84	2.33	2.31	0	1.12	0	0	0	0	9.71	7.07	0	64.48
Em16	0	0	2.08	26.55	7.16	23.22	0	0.75	36.49	1.63	1.35	0	2.8	0	0.17	0	0	10.53	5.67	0	117.31
Em18	0	0	1.25	35.96	11.99	26.2	0	0.28	47.08	2.29	1.96	0	1.44	0	0.19	0	0	10.9	4.66	0	131.35
Em20	0	0	0.42	26.82	13.12	20.34	0	0.3	42.01	2.6	2	0	2.21	0	0	0	0	10.62	3.53	0	65.96
Em22	0	0	0.98	27.61	14.89	17.75	0	1.31	33.43	2.19	1.6	0	1.7	0	0.42	0	0	7.83	6.27	0	369.53
Em24	0	0	0.42	37.94	16.96	23.71	0	1.51	27.33	2.28	0.94	0	1.94	0	0.03	0	0	5.64	6.02	0	321.41
Em26	0	0	0.42	41.76	14.98	14.28	0	4.96	19.27	1.47	1.96	0	1.05	0	0.26	0	0	4.46	4.96	0	910.81
Em28	0	0	0.6	42.79	16.69	14.84	0	2.91	28.56	1.57	0.27	0	0.58	0	0.79	0	0	5.03	4.24	0	579.22
Em30	0	0	0.14	31.63	17.8	16.12	0	1.96	33.68	2	1.7	0	1.62	0	0.21	0	0	7.53	5.06	0	276.1
Em32	0	0	0.1	21.37	25.42	18.17	0	2.58	39.66	2.14	2.24	0	0.78	0	0.04	0	0	7.4	4.34	0	251.21
Em34	0	0	0.3	20.79	17.81	17.97	0	9.78	27.48	1.81	1.46	0	2.41	0	0.27	0	0	7.41	4.21	0	854.19
Em36	0	0	0.09	20.88	23.6	18.58	0	12.1	29.35	1.72	1.92	0	12.48	0	0.31	0	0	5.07	4.06	0	638.68
Em38	0	0	0.19	14.1	22.07	19.49	0	16.59	28.58	1.85	1.57	0	4.16	0	0.23	0	0	4.93	4.32	0	777.58
En6	0	0	0.51	11.18	6.5	17.45	0	0.01	62.6	1.82	0.23	0	0	0	0.43	0	0	15.21	4.33	0	0.7
En8	0	0	0.16	8.21	6.35	22.27	0	0.1	64.82	1.66	0.84	0	0	0	0.3	0	0	14.95	5.57	0	0.36
En10	0	0	0.09	0.92	7.49	16.69	0	0	40.9	1.4	0.63	0	0	0	0.1	0	0	11.82	2.87	0	2.26
En12	0	0	0	0.28	6.34	10.86	0	0.02	15.88	0.93	0.29	0	0	0	0.1	0	0	4.8	2.48	0	1.66
En14	0	0	0.01	0.32	4.87	11.06	0	0	11.37	0.56	0.59	0.21	0	0	0	0	0	4.89	2.06	0	17.19

En16	0	0	0	0.37	4.54	15.23	0	0	12.88	0.84	0.57	0	0	0	0	0	0	4.86	1.69	0	14.54
En18	0	0	0.06	0.57	5.71	17.28	0	0	12.39	0.81	1.44	0	0	0	0.03	0	0	4.88	2.72	0	50.77
En20	0	0	0.09	0.49	4.17	15.23	0	0.12	14.12	0.94	0.6	0	0	0	0.08	0	0	4.78	3.01	0	34.43
En22	0	0	0.04	1.05	4.38	9.83	0	0.02	11.8	0.6	0.73	0	0.51	0	0.14	0	0	5.23	2.51	0	28.05
En24	0	0	0	1.48	4.89	10.77	0	0.02	10.2	0.75	1.17	0	0.03	0	0.07	0	0	5.28	3.41	0	53.37
En26	0	0	0	1.64	5.67	12.13	0	0.34	7.51	0.35	0.54	0	0.35	0	0	0	0	4.62	2.72	0	164.23
En28	0	0	0	3.48	5.76	9.69	0	1.19	6.02	0.4	3.07	0	0.23	0	0.59	0	0	1.42	1.54	0	638.25
En30	0	0	0	1.55	4.9	13.85	0	0.71	10.49	0.36	1	0	0	0	0.26	0	0	4.9	2.71	0.13	247.33
En32	0	0	0.08	7.74	10.77	17.1	0	4.16	12.4	1.08	2.39	0	0	0	0.03	0	0	4.35	2.84	0	770.18
En34	0	0	0	10.21	13.44	22.86	0	5.48	15.2	1.28	2.08	0	0.34	0	0.4	0	0	4.97	3.18	0	634.11
En36	0	0	0	16.54	10.16	12.81	0	11.12	10.31	0.58	0.77	0	0	0	0.09	0	0	2.52	1.58	0	1696.8 4
En38	0	0	0	24.5	18.49	16.27	0	16	12.36	1.4	0.73	0	0.09	0	0	0	0	4.19	1.97	0	1944.7 5
S0	0	0	1.71	34.36	23.72	40.12	0	2.3	25.43	3.36	6.21	0	1.06	0	3.36	0	0.13	10.62	8.49	0	0
S2	0	0	0.79	33.96	21.69	27.92	0	2.47	28.52	1.96	7.12	0	1.18	0	4.17	0.22	0	8.48	7.74	0	1.15
S3	0	0	1.29	47.73	12.69	31.26	0	1.33	24.9	2.03	4.48	0	0.4	0	4.03	0.42	0.13	8.14	4.71	0	0.13
S4	0	0	1.29	35.61	12.06	30.06	0	1.06	23.85	3.86	3.39	0	0.13	0	3.34	0.7	0	10.05	5.83	0	0.53
S6	0	0	0.76	18.41	10.12	30.49	0	0.7	24	2.57	4.46	0	0	0	2.04	0	0	7.12	6.41	0	0.38
S8	0	0	0.54	8.28	12.33	27.1	0	0.2	38.49	1.6	1.95	0	0	0	1.65	0	0	11.65	7.39	0	0.37
S10	0	0	0.31	3.95	6.86	21.3	0	0.05	32.53	1.33	1.69	0	0	0	1.1	0	0	10.62	4.41	0	1.97
S12	0	0	0.55	5.27	6.47	15.35	0	0.12	14.52	0.52	0.93	0	0.16	0.05	0.65	0	0.08	4.93	4	0	5.56
S14	0	0	0.07	5.3	5.07	12.2	0	0.09	13.43	0.84	1.17	0	0.16	0	0.21	0	0	4.26	3.37	0	15.55
S16	0	0	0.29	6.59	7.74	19.79	0	0.11	21.61	1.85	0.91	0.07	0.26	0	0.15	0	0	6.45	4.7	0	17.28
S18	0	0	0.19	7.08	6.4	18.45	0	0.01	19.22	1.27	0.71	0	0.1	0	0.79	0	0	6.02	3.87	0	40.26

S20	0	0	0.14	8.27	6.46	18.09	0	0.17	19.99	1.18	1.64	0	0.7	0	0.41	0.09	0	6.24	4.03	0	59.44
S22	0	0	0.16	8.67	13.79	17.99	0	0.27	17.03	1.3	0.94	0	0	0	0.57	0	0	4.94	6.37	0	59.22
S24	0	0	0.3	7.28	5.24	13.75	0	0.18	13.84	0.81	1.82	0	0.19	0.05	0.6	0	0	3.64	3.52	0	79.63
S26	0	0	0.09	9.58	7.67	17.13	0	0.26	16.31	1.06	3.39	0	0	0	1.48	0	0	4.74	4.92	0	89.89
S28	0	0	0.04	10.34	9.74	15.89	0	1.62	17.89	1.46	3.16	0	0.55	0	0.72	0	0	4.51	3.73	0	196.07
S30	0	0	0.36	22.81	12.31	14.95	0	13.48	19.77	1.11	2.48	0.64	13.55	0	1.59	0	0	5.18	4.51	0	1049.78
S32	0	0	0.12	16.34	17.6	25.37	0	4.58	28.67	2.99	1.03	0	7.91	0	2.59	0	0	5.71	4.24	0	350.28
S34	0	0	0.13	20.61	15.46	19.05	0	10.68	21.06	2.54	1.1	0	7.23	0	2	0	0	5.58	4.73	0	524.02
S36	0	0	0.12	14.31	16.97	22.56	0	6.22	25.29	1.44	0.83	0	6.32	0	0.38	0	0	7.01	3.74	0	198.88
S38	0	0	0.22	19.83	16.09	18.99	0	21.19	19.1	1.56	1.44	0	6.06	0	0.97	0	0	5.4	4.08	0	744.77
Shoots	0.15	0	0.96	5.31	6.89	8.97	0	44.17	8.62	0.75	0.33	0	2	0	1.05	0	0	1.13	3.26	0.08	0.29
Roots	0.32	0	0.79	6.67	6.7	5	0	0.19	8.35	0.69	0.28	0	3.27	0	20.93	0	0.06	1.44	5.57	0.08	0.16
Leaf 1	0	0	2.67	12.74	5	4.31	0	100.49	8.7	0.84	0.31	0	0.2	0	0.13	0.08	0	3.6	2.5	0	1.24
Leaf 2	0.12	0	0.89	13.75	9.09	8.25	0	242.2	5.09	0.42	0.75	0	0.08	0	0.2	0	0	0.89	2.61	0	0.05
Leaf 3	0	0	3.54	1.24	4.32	4.85	0	170.59	1.68	0.26	0.37	0	0	0	0.06	0	0	0.35	2.56	0	0
Leaf 4	0	0	3.92	1.17	1.89	4.74	0	147.98	3.7	0.18	0.28	0	0	0	0.21	0	0	0.62	2.08	0	0
Leaf 5	0	0	3.01	5.13	2.8	7.3	0	85.24	3.42	0.15	0.7	0	0.06	0	0	0.08	0	0.41	2.41	0	0
Leaf 6	0	0	1.97	7.98	8.4	16.99	0	71.06	26.43	2.04	1.46	0	0	0	0.16	0	0	8.58	3.09	0	0.06
Leaf 7	0	0	2.33	7.5	7.75	16.78	0	79.21	25.24	2.18	0.91	0	0.09	0	0.34	0	0	8.96	3.27	0	0
SAM 1	0	0	3.94	35.73	5.43	14.25	0	1.91	37.54	3.51	1.25	0	0	0	0.3	0.06	0	15.64	3.39	0	0.2
SAM 2	0	0	3.02	32.82	5.31	19.02	0	2.06	45.25	3.34	1.2	0	0.29	0	0.23	0	0	16.33	2.86	0	0.12
SAM 3	0	0	1.41	0.33	1.6	6.82	0	0.18	10.44	0.97	0	0	0	0	0	0	0	2.92	0.76	0	0
Ear 1	0	0	5.81	27.87	3.81	17.56	0	2.32	41.68	2.69	1.22	0	5.41	0	1.18	0	0	16.6	2.93	0	0.07
Ear 2	0	0	5.49	20.79	2.76	10.25	0	1.89	25.26	2.82	0.82	0	4.77	0	1.23	0	0	6.33	1.6	0	0.2

Tassel 1	0	0	5.36	25.61	6.86	17.96	0	6.31	41.31	1.78	0.8	0	8.03	0	1.45	0	0	11.93	3.42	0	0
Tassel 2	0	0	5.87	26.41	6.86	17.81	0	6.05	41.78	2.26	0.96	0	7.02	0.06	1.12	0	0	10.37	3.7	0	0
Tassel 3	0	0	5.37	21.19	5.92	12.9	0	5.27	29.58	2.6	0.95	0.14	9.04	0	1.17	0	0	5.69	2.47	0	0
Tassel 4	0	0	1.46	20.74	5.53	9.83	0	24.22	7.61	0.97	0.83	0.18	23.09	0	0.63	0	0	1.63	7.24	0.14	0
Tassel 5	0	0	1.46	24.17	5.88	8.08	0	20.49	5.28	1.04	0.83	0	25.7	0	0.8	0	0	1.68	5.29	0.23	0
Cob 1	0	0	7.8	50.67	3.53	17.44	0	9.53	16.33	2.65	0.9	0	5.98	0	1.89	0.24	0	5.29	3.79	0	0.17
Cob 2	0	0	7.45	55.34	3.41	14.62	0	20.56	15.24	2.26	0.72	0.58	35.34	0	2.85	0.4	0	6.43	3.71	0	0.26
Silk	0	0	5.22	42.6	7.2	14.53	0	32.73	6.43	1.4	2.16	0	3.36	0	0.66	0	0.06	1.2	5.87	0	1.85
Anthers	0	0	1.42	1.84	14.85	2.35	0	1.58	0.55	0.1	0.2	0	0.04	0	1.37	0	0	0.13	1.64	0	0
Ovule	0	0	6.53	81.75	4.69	11.61	0	21.27	10.26	2.09	1.03	0	28.42	0	1.28	0.49	0	3.2	3.47	0.09	0.27
Pollen	0	0	0.88	0.08	8.31	0.18	0	0.02	0.06	0.03	0	0	0.04	0	0	0	0	0	0.78	0	0

Supplemental Table S3. Segregating ratio of heterozygous *smk11-cas9*+ ears.

Ear No.	No. (NS)	No. (MS)	Ratio		
			No.(NS)/No.(MS)	χ^2 value	χ^2 (0.05)
smk11-cas9-1	216	66	3.27	0.3	3.841
smk11-cas9-2	231	96	2.41	3.08	
smk11-cas9-3	247	64	3.86	3.01	
smk11-cas9-4	175	51	3.43	0.59	
smk11-cas9-5	237	71	3.34	0.52	
smk11-cas9-6	279	88	3.17	0.15	
smk11-cas9-7	269	78	3.45	1.05	
smk11-cas9-8	178	63	2.83	0.11	
smk11-cas9-9	276	72	3.83	3.22	
smk11-cas9-10	299	80	3.74	2.86	

NS, normal seeds, MS, mutant seeds.

Supplemental Table S4. Complementation statistics (15:1) of *smk11* mutation with *Smk11* transgene.

Ear No.	No.(NS)	No.(MS)	Ratio No.(NS) / No.(MS)	χ^2 value	χ^2 (0.05)
536-1	286	21	13.62	0.183	3.841
536-4	348	24	14.50	0.026	
536-12	220	17	12.94	0.345	
536-15	257	21	12.24	0.807	
536-16	331	19	17.42	0.403	
537-5	454	22	20.64	2.154	
537-10	437	29	15.07	0.001	
537-11	391	26	15.04	0.000	
537-12	396	27	14.67	0.013	
537-13	239	13	18.38	0.512	

536-1, 536-4, 536-12, 536-15 and 536-16 lines were from the cross between homozygous mutant *smk11/smk11* (maternal parent) and the transgenic T0 lines (male parent) ; 537-5, 537-10, 536-11, 536-12 and 536-13 lines were from the cross between the transgenic T0 lines (maternal parent) and homozygous mutant *smk11/smk11* (male parent). NS, normal seeds, MS, mutant seeds.

Supplemental Table S5. List of SMK11-interacting proteins identified by Y2H, LUC and BIFC assays.

Number	Gene ID	Gene Name	Annotation
1	GRMZM5G833660	ZmIMP1A	Mitochondrial ATP-independent protease
2	GRMZM2G142938	ZmMYB3R-3	Peptidase S24/S26A/S26B/S26C family protein
3	GRMZM2G031501	ZmCOX23	Twin CX9C domain protein.
4	Zm00001d037104	ZmMFDX1	Mitochondrial ferredoxin 1