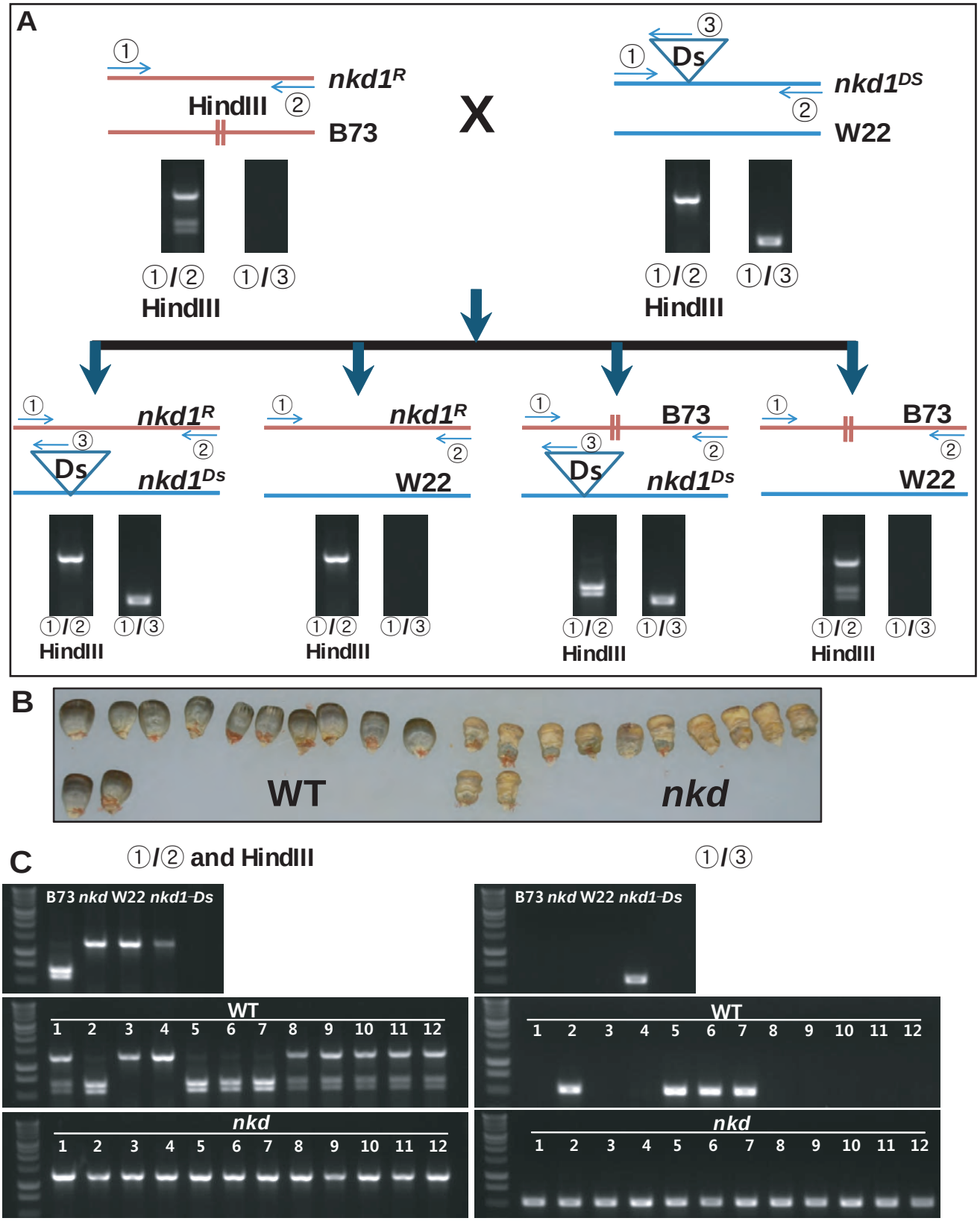
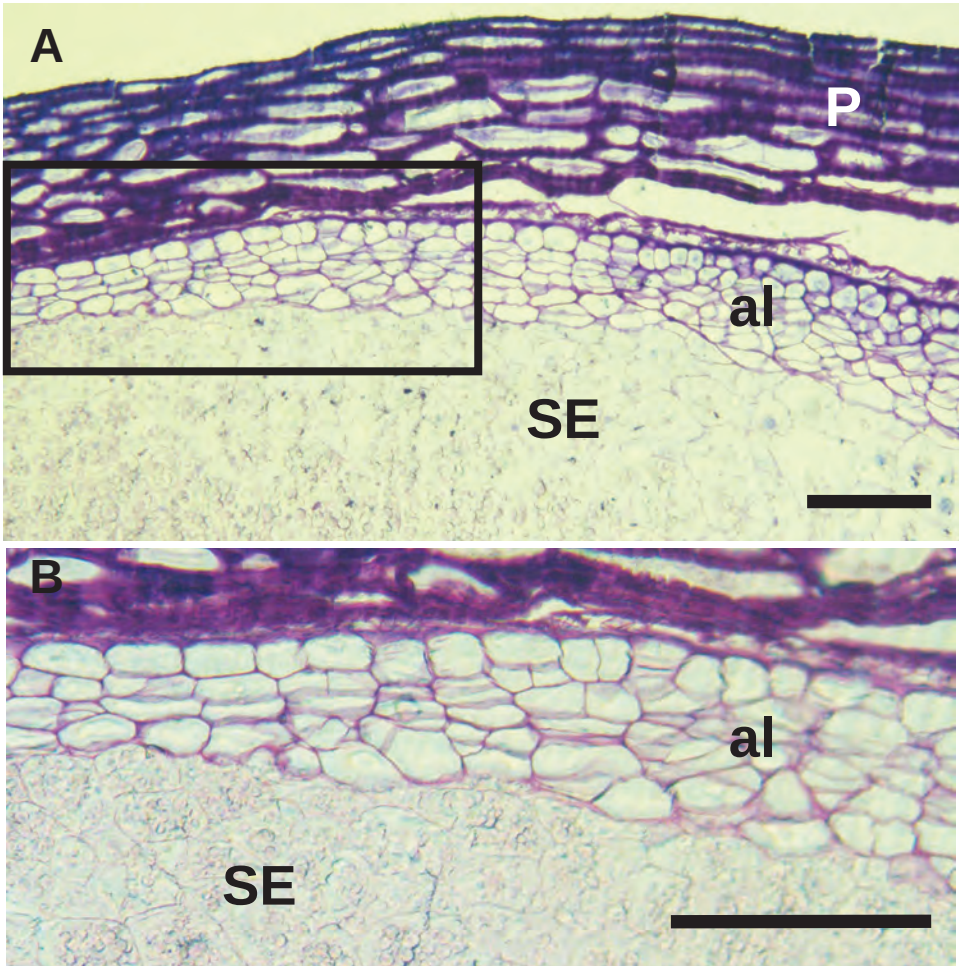
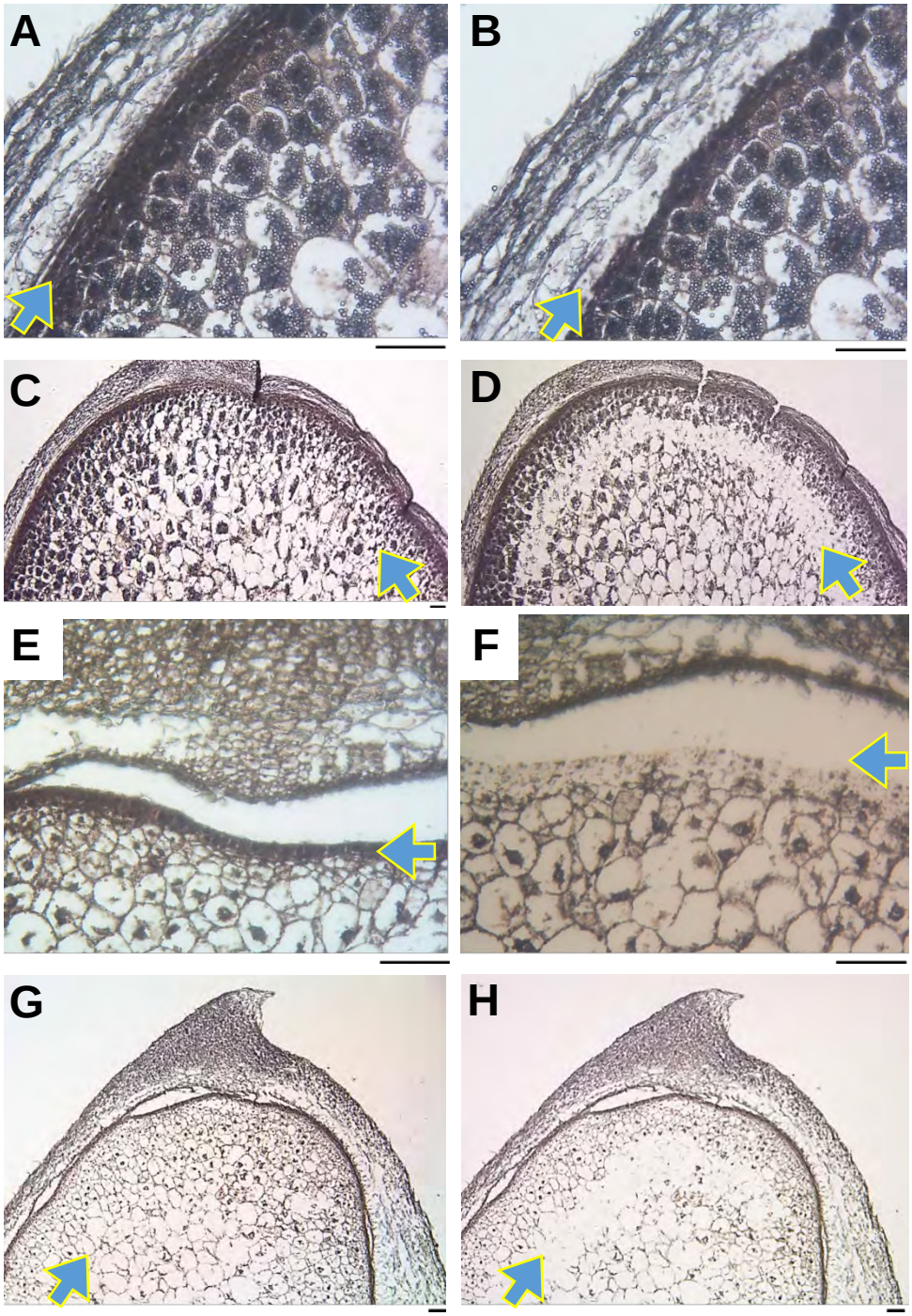


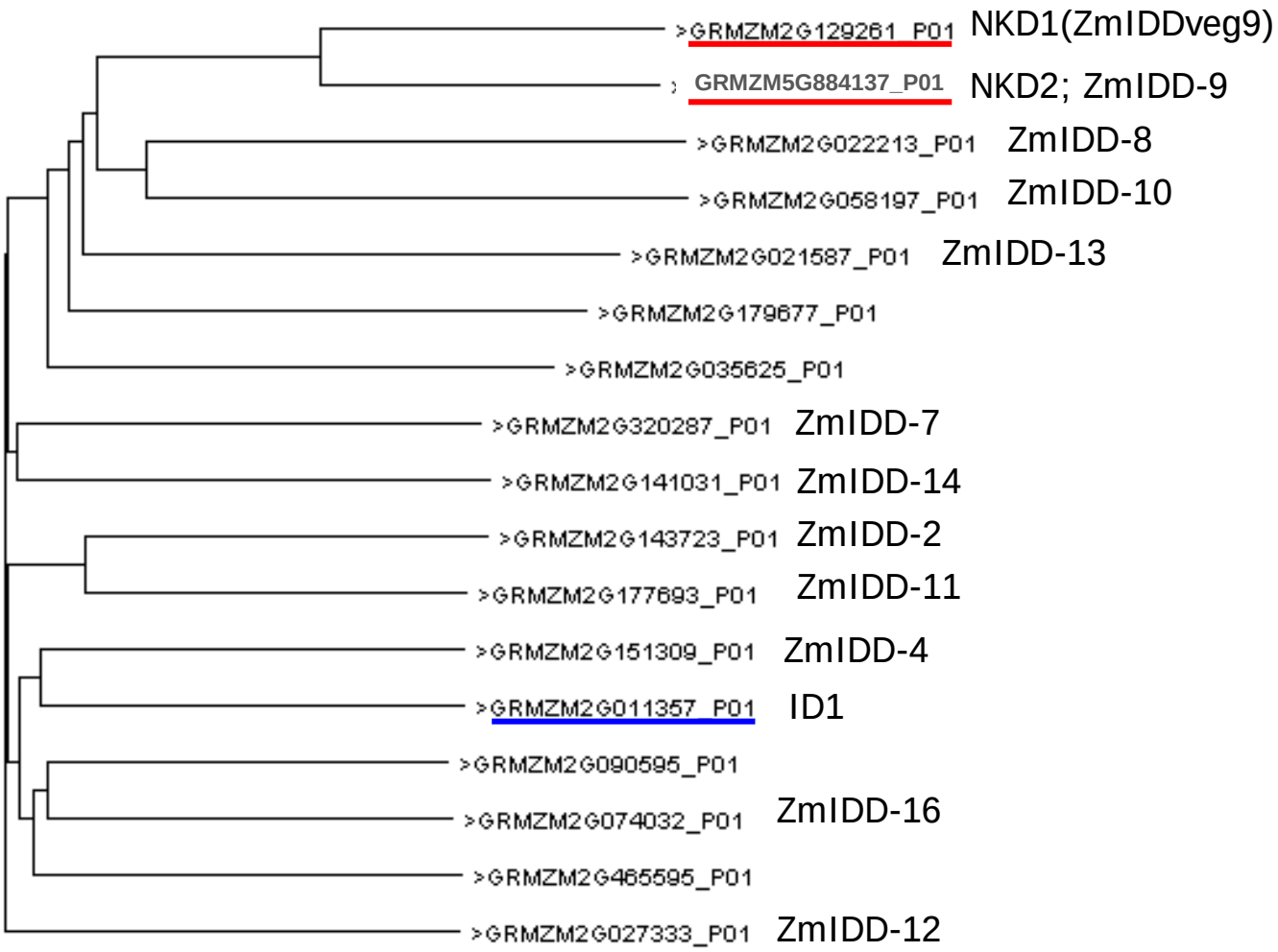
Supplemental Figure 1. Complementation test with *nkd1-Ds* allele. **(A)** Cross performed for the complementation test. Note that all the progeny are *nkd2-R/+* heterozygous from the cross of maternal nkd2-R/nkd2-R and paternal +/+. Four different progeny genotypes were identified by marker analysis. WT *Nkd1-B73* can be distinguished from *nkd1-R* and *Nkd1-W22* by susceptibility to *Hind*III digestion of the PCR product produced by primer pair 1/2. The *Ds* insertion allele was detected by PCR with *Ds*-specific primer 3 and gene specific primer 1. **(B)** Ears derived from the designated cross segregated 3:1 for WT:mut. 12WT kernels and 12 mutant kernels were genotyped. **(C)** Genotyping for 12 WT and 12 *nkd* kernels. All the mutant kernels inherited *nkd1-R*, as indicated by lack of *Hind*III digestion of the maternal allele, and *nkd1-Ds* as indicated by amplification with the *Ds*-specific primer 3. All the WT kernels inherited either the *Nkd1-B73* maternal allele, the *Nkd1-W22* paternal allele or both.



Supplemental Figure 2. Endosperm phenotype of a *nkd1-Ds* and *nkd2-Ds0297* double mutant. **(A)** Histological section of a nearly mature kernel showing a phenotype similar to the original *nkd1-R; nkd2-R* mutant. The multiple layers of peripheral cells are highly vacuolated rather than showing the dense cytoplasm characteristic of normal aleurone cells.
(B) Increased magnification of inset in (A). SE, starchy endosperm; P, pericarp; “al”, abnormal aleurone cells. Size bars = 100 μm.



Supplemental Figure 3. Laser capture microdissection (LCM) of aleurone (AL), and starchy endosperm (SE) cells.
B73 WT and *nkd* mutant kernels (in B73 background) were collected at 15DAP, fixed and paraffin embedded before sectioning and LCM. **A-D** depict sections of B73 WT kernels before (**A, C**) and after (**B, D**) capture of aleurone **(A,B)** and starchy endosperm cells **(C,D).** **E, F, G** and **H** show *nkd* mutant sections before (**E, G**) and after (**F, H**) capture. Captured cells are denoted by arrows. Scale bars represent 100μm.



Supplemental Figure 4. The IDD protein family in maize.
Gene sequences were identified by searching the reference maize genome B73 v.2 with BLASTP using the IDD region of ID1 as a query. Protein sequences were aligned with ClustalW and the phylogeny was produced with MEGA4 using default parameters of the UPGMA method.

Table S1. Marker data for *nkd1* positional cloning on chromosome 2.

Marker	Forward primer; Reverse primer	Position on IBM7 (cM)	Mapping Population	Maker Type	No. Recombinant
IDP7746[a]	CTCGTACCGAGCTATCACCC; CAAGTTAGGAGCCGTCAAGC	52.8	H99xnkd F2	dominant	15/120~30/120 [b]
491I18-7h99	CAGCTAAAAGTGGGAGCGCGC; TGCTAGTGCTAGTGCTACCTAT	nd[c]	H99xnkd F2	dominant	11/218~22/218
190B15-6,7	ACGTGCCTCTGACATGTGGGTCAC; CCGATCTCTTGCGATGCATCGAG	nd	H99xnkd F2	dominant	16/338~32/338
190B15-3,2	CACTTGCACCTATCCAAAATTCTCT; TGTCTCCGGTGCTCTTAATGATG	nd	H99xnkd F2	dominant	2/118~4/118
266H09-9,10	CGTGAGCTGGACATTTTACAG; GTGCATACGGGTCTTCATAGTAC	nd	H99xnkd F2	codominant	5/328
nkd1 position					
166I20-1,5	AGAACGAGAAGGGGACCTCATAA; TCCCTCCATTCCATTGATTAAGATG	nd	H99xnkd F2	codominant	2/332
IDP1612	AATTGCACATAACAGAGGCG; CTCTTCCAATCGGGTTTGC	65.9	H99xnkd F2	codominant	2/120
IDP668	GCTCCGAACTTTGAAAGAGG; CGCCGTAATTTACAACCAGC	67.3	H99xnkd F2	codominant	2/124
IDP7439	ATGCTTCTCATCAGGTTGGG; TGATCTCACTCGACACAGGC	73.6	H99xnkd F2	codominant	3/122
IDP2485	GAGCTGTGCATTCAAACACG; GAGCAGCATCTCCGAGTACC	75.4	H99xnkd F2	codominant	7/124

[a] Information on IDP makers comes from Fu et al. 2006.

[b] Because of the dominant nature of the marker, heterzygotes could not be distinguished from homozygotes. Therefore a range of possible recombinant frequencies is presented.

[c] nd: not determined

Table S2. Marker data for *nkd1* positional cloning on chromosome 10.

Maker	Forward primer; Reverse primer	Position on IBM7 (cM)	Mapping Population	Maker Type	No. Recombinant
IDP3853[a]	AATCTGCGCAATCCATAACC; CTCTCGCTTGTTTCTGTAGCC	65.5	H99xnkd F2	codominant	38/92
IDP8518	CTTGGTGAAGGTCTCGAAGC; ACCTGTACCTGACCGTGGG	65.5	Mo17xnkd F2	codominant	8/72
IDP1978	ACGACCGAATGTTGCTAAGG; TGGCAGAGTGATCTTTGTCG	71.4	H99xnkd F2 (Mo17xnkd F2)[b]	codominant	40/122 (8/78)
IDP8526	GAAGGCCTACGACATGAACC; TGGTAGTCCTGGAAGTTGGC	74.4	H99xnkd F2	codominant	11/330
nkd2 position					
IDP8334	CTCAACCAAGAGAGCGTGC; GAGATGATTCTCCAGGCTGC	76.4	H99xnkd F2 (Mo17xnkd F2)	codominant	6/328 (6/76)
IDP250	TGGGAACAATTCTCCAAACC; AAGAGGCACTCCAACATTCC	92.1	H99xnkd F2	codominant	9/90
IDP7345	CGGTGACTGCCTACATTGG; AGCTCTTCACAGGCAGAAGC	99.8	Mo17xnkd F2	codominant	20/70

[a] Information on IDP makers come from Fu et al. 2006

[b] In several cases, multiple populations were used. The secondary population and corresponding recombination data are shown parenthetically.

Table S3. PCR primers used in this study

Primer	Sequence	Use
491I18-7h99f	CAGCTAAAAGTGGGAGCGCGC	Mapping
491I18-7h99r	TGCTAGTGCTAGTGCTACCTAT	Mapping
190B15-6	ACGTGCCTCTGACATGTGGGTCAC	Mapping
190B15-7	CCGATCTCTTGCGATGCATCGAG	Mapping
266H09-9	CGTGAGCTGGACATTTTACAG	Mapping
266H09-10	GTGCATACGGGTCTTCATAGTAC	Mapping
166I20-1	AGAACGAGAAGGGGACCTCATAA	Mapping
166I20-5	TCCCTCCATTCCATTGATTAAGATG	Mapping
2ZNF-Xba1*	acgt*tctaga*ATGGCATCGAATTCATCGGCG	GFP fusion
2ZNF-BamH1*	tgac*ggatcc*TGGCATCCTGCCTCCGTTGAAG	GFP fusion
10ZNF-Xba1*	acgt*tctaga*ATGGCGTCGAATTCACCGGCG	GFP fusion
10ZNF-BamH1*	tgac*ggatcc*TGGCATCCTGCCTCCATTGAAG	GFP fusion
10ZNF3EF-Avr2*	agg*cctagg*GAGGGACAGCTTCATCACG	RNAi
10ZNF3ER-Asc1*	tca*ggcgcgcc*TCATGGCATCCTGCCTCCA	RNAi
10ZNF3EF-Xma1*	tca*cccggg*GAGGGACAGCTTCATCACG	RNAi
10ZNF3ER-Spe1*	agg*actagt*TCATGGCATCCTGCCTCCA	RNAi
291F22-5	CATATATGAGTCTGCGGGTG	RT PCR
291F22-7	TGCTGCCGCAGATGTCGGCGACA	RT PCR
291F22-8	TCGGTCATGGCATCCTGCCTCCG	RT PCR, genotyping
2ZNF-5	GATTCCGGTCGTGCATGCACACTGC	RT PCR
2ZNF-8	CTGTGTGACATGGAGCACGAGGT	RT PCR
10ZNF-12	TACCGCTGCGACTGCGGCACGC	RT PCR
10ZNF-13	AGGCTTGTTGGCCTGCAGCTGAA	RT PCR
10ZNF-17	CGACATGGCGGAGCGCGAGGG	RT PCR
10ZNF-2	TCTGTCATGGCATCCTGCCTCCA	RT PCR
Nkd2F209	CTCTGACTAATGGAGCAGTAAGCTG	RT PCR
Nkd2R317	CAACAAGAACGAGACCAGCAGAAT	RT PCR

VP1-RT3	CTTCAGATAAGCGGCAGGG	RT PCR
VP1-RT4	CCAAAACCTGTACCGCATG	RT PCR
Waxy GK-1	CCAGTTCAAATTCTTTTAGGCTCACC	RT PCR
2ZNF-4	TATCTTATCCGTCGATGCGTTG	Genotyping
JS-R01	GTTCGAAATCGATCGGGATA	Genotyping

* Additional sequences were written in lower case and restriction enzyme sites are *italic*.

SUPPLEMENTAL METHODS

Kernel Genotyping

For the genotyping shown in Supplemental Figure 1, 12 WT kernels and 12 mutant kernels from a cross between *nkd1-R/+; nkd2-R/nkd2-R* in B73 background and *nkd1-Ds/+; +/+* in W22 background were genotyped. Four different progeny genotypes of *Nkd1* locus were identified by two different PCR reactions and following *HindIII* digestion for one of them. WT *Nkd1-B73* was distinguished from *nkd1-R* and *Nkd1-W22* by susceptibility to *Hin*dIII digestion of the PCR product of a primer pair 2ZNF-4 and 291F22-8. The nkd1-*Ds* allele was detected by PCR with *Ds* specific primer JS-R01 (Ahern et al., 2009) and gene specific primer 2ZNF-4.