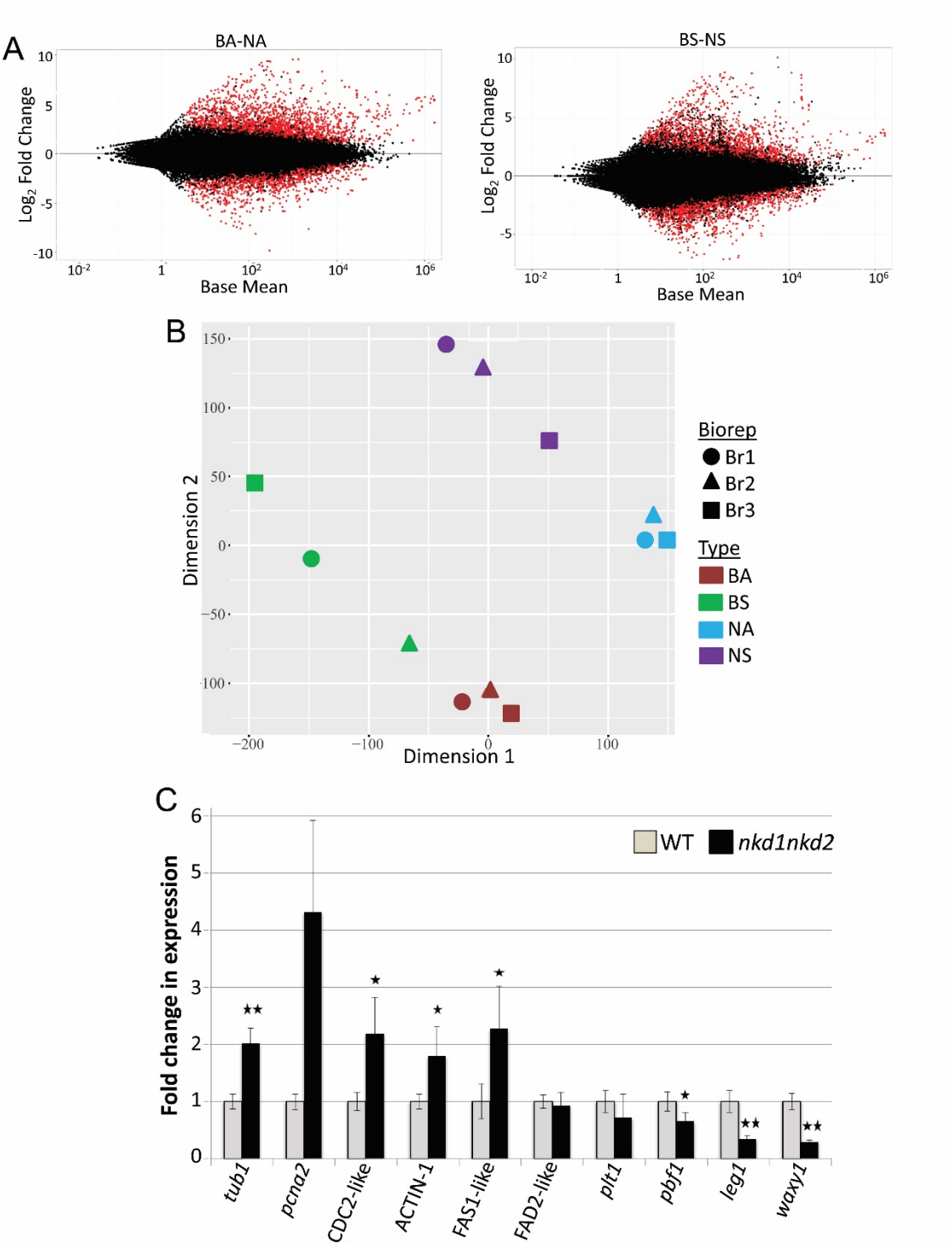
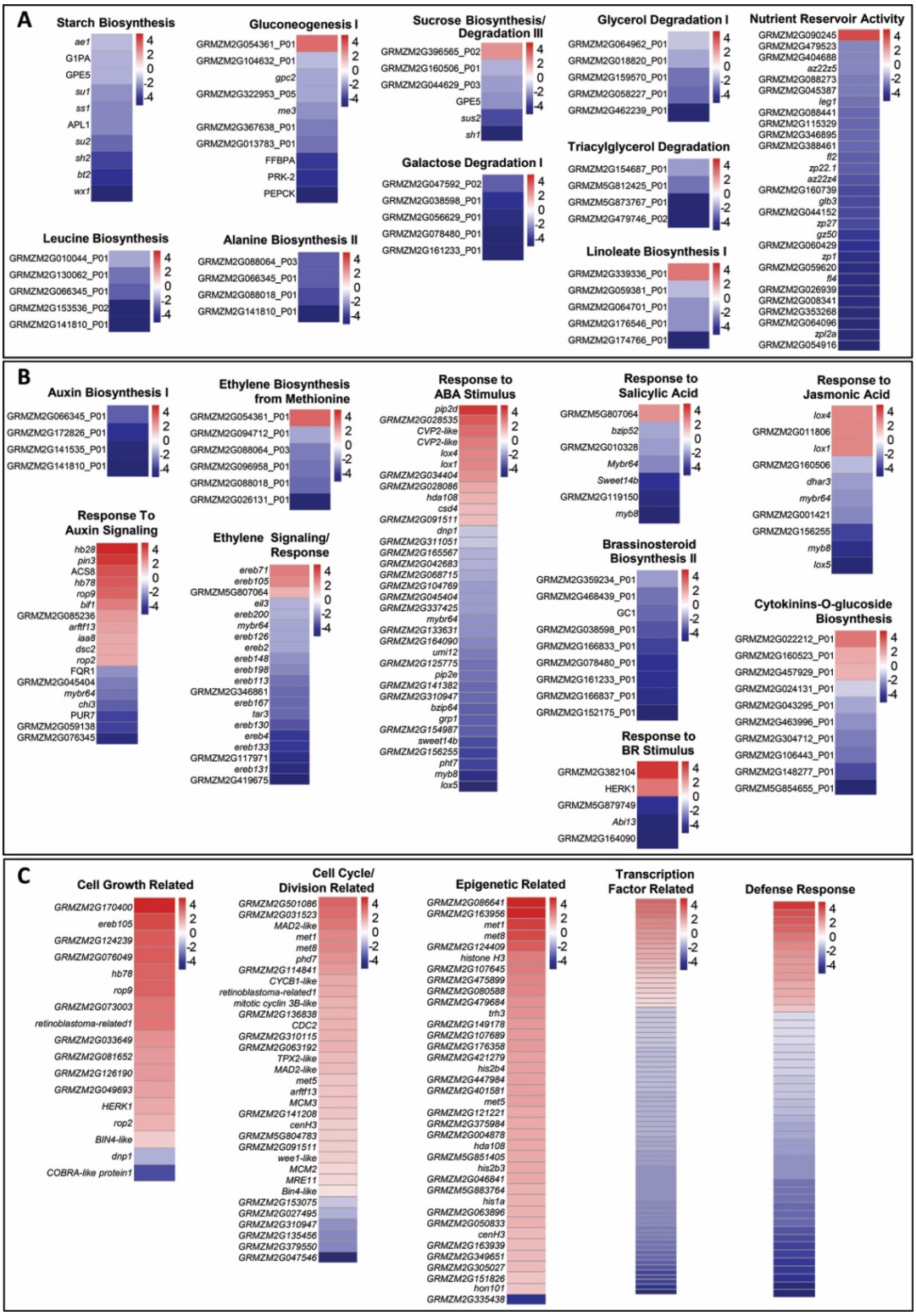


Supplemental Figure 1. Maize V3 LCM-RNAseq Transcriptome Assembly and DE Gene Confirmation

LCM-RNAseq data reported in Yi et al., 2015 (GSE61057 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE61057) was realigned to the Maize V3 reference genome using Tophat2 version 2.1.0 and BEDTools package.

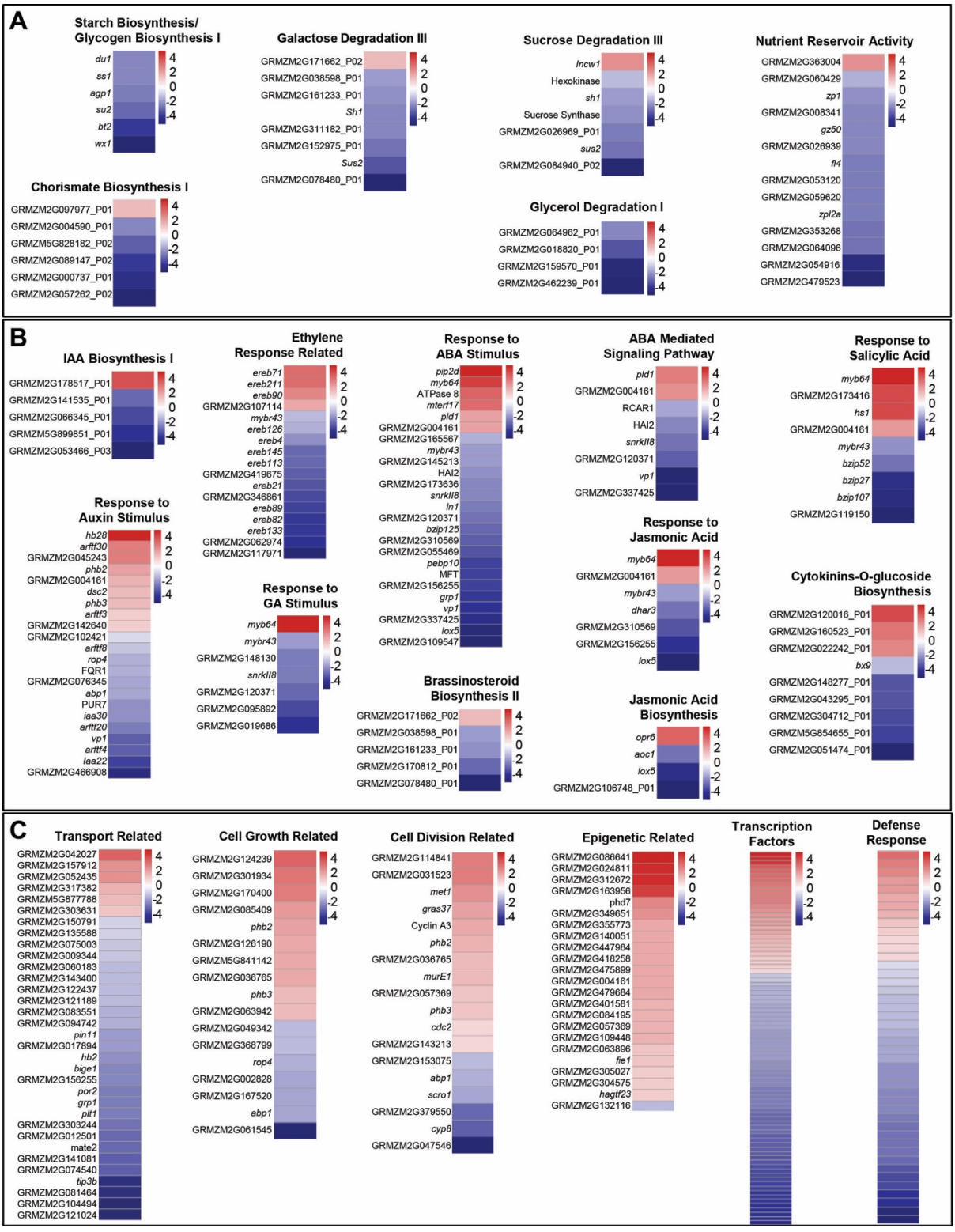
(A) MA plots of $\log_2$ fold change in transcript abundance of *nkd1 nkd2* mutant relative to WT plotted against mean normalized read counts in mutant and WT aleurone (BA-NA) and starchy endosperm (BS-NS). Black dots represent genes detected with ≥1 read count and red dots represent DE genes (adjusted p-value⩽ 0.01).
(B) Two dimensional scatter plot generated by MDS (Multidimensional scaling) analysis of DE genes in the three biological replicates from WT and *nkd1 nkd2* mutant endosperm analysis.
(C) Bar diagram showing subset of tested genes using qRT-PCR from Figure 1. Error bars represent standard error of the mean of three biological replications. The statistically significant DE genes are indicated by asterisks. *denotes P<0.05, ** for P<0.01.



Supplemental Figure 2. Disrupted Pathways in *nkd1 nkd2* Mutant Aleurone

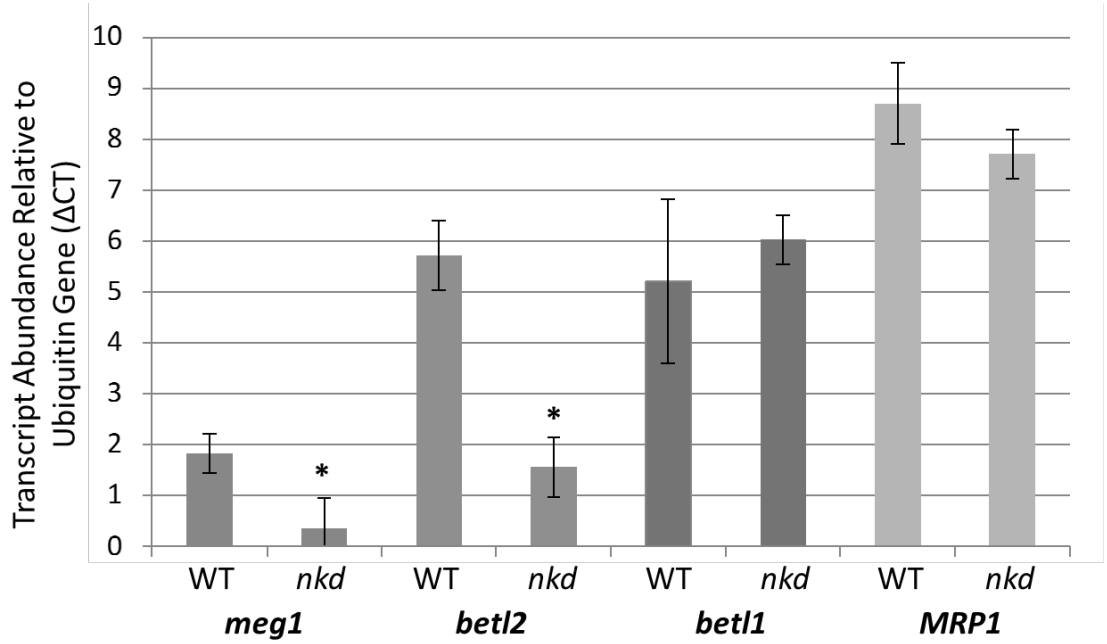
Log_2 fold change heat maps of DE genes functioning in **(A)** Resource reserve metabolism, **(B)** Hormone biosynthesis, signaling and response, and **(C)** Cell division/

growth regulation, defense response, epigenetic and transcriptional regulation of gene expression. See Supplemental Dataset 1 for further details.



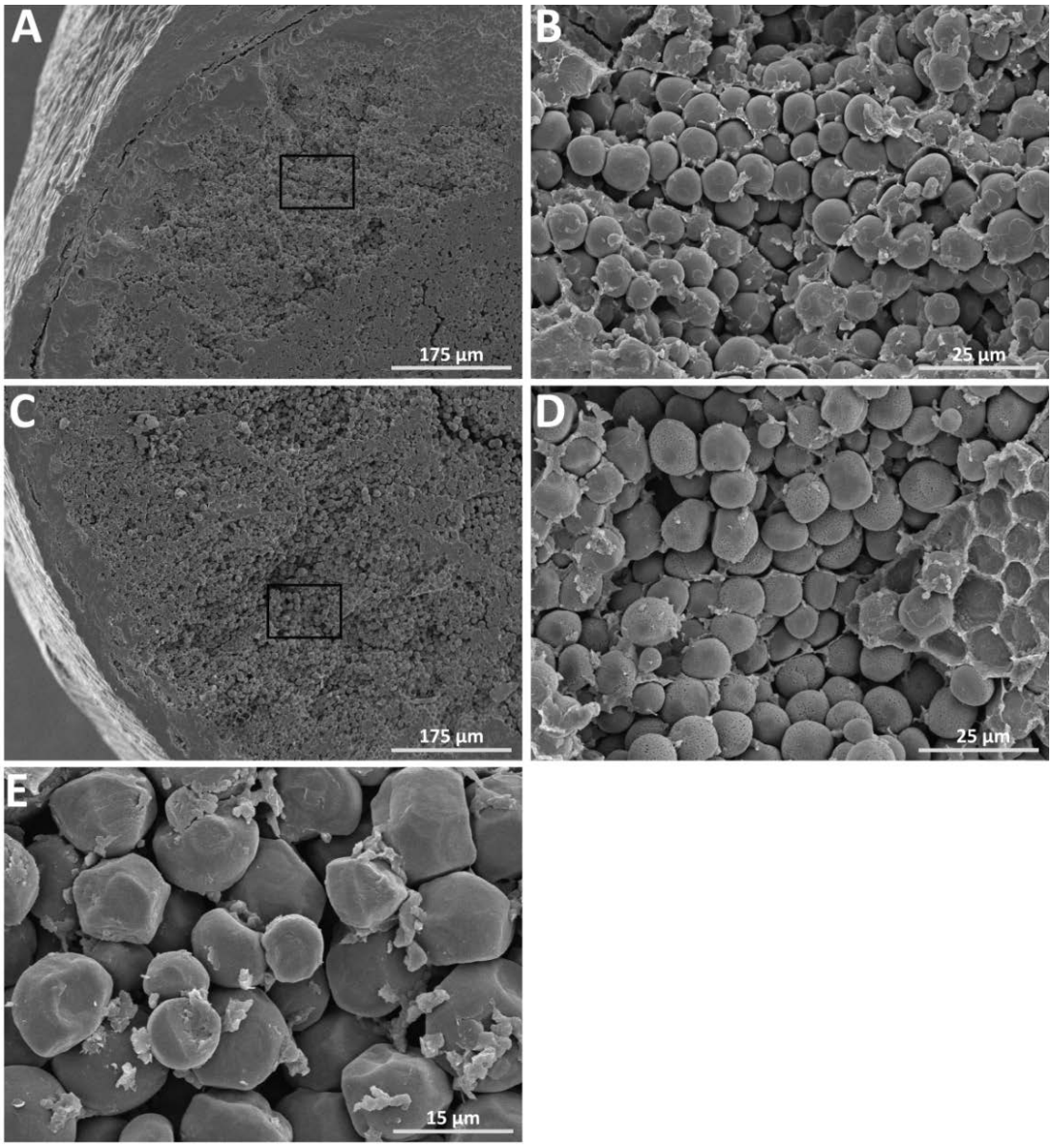
Supplemental Figure 3. Disrupted Pathways in *nkd1 nkd2* Mutant Starchy Endosperm

Log_2 fold change heat maps of DE genes functioning in **(A)** Resource reserve metabolism, **(B)** Hormone biosynthesis, signaling and response, and **(C)** Cell division/ growth regulation, defense response, nutrient transport, epigenetic and transcriptional regulation of gene expression. See Supplemental Dataset 2 for further details.



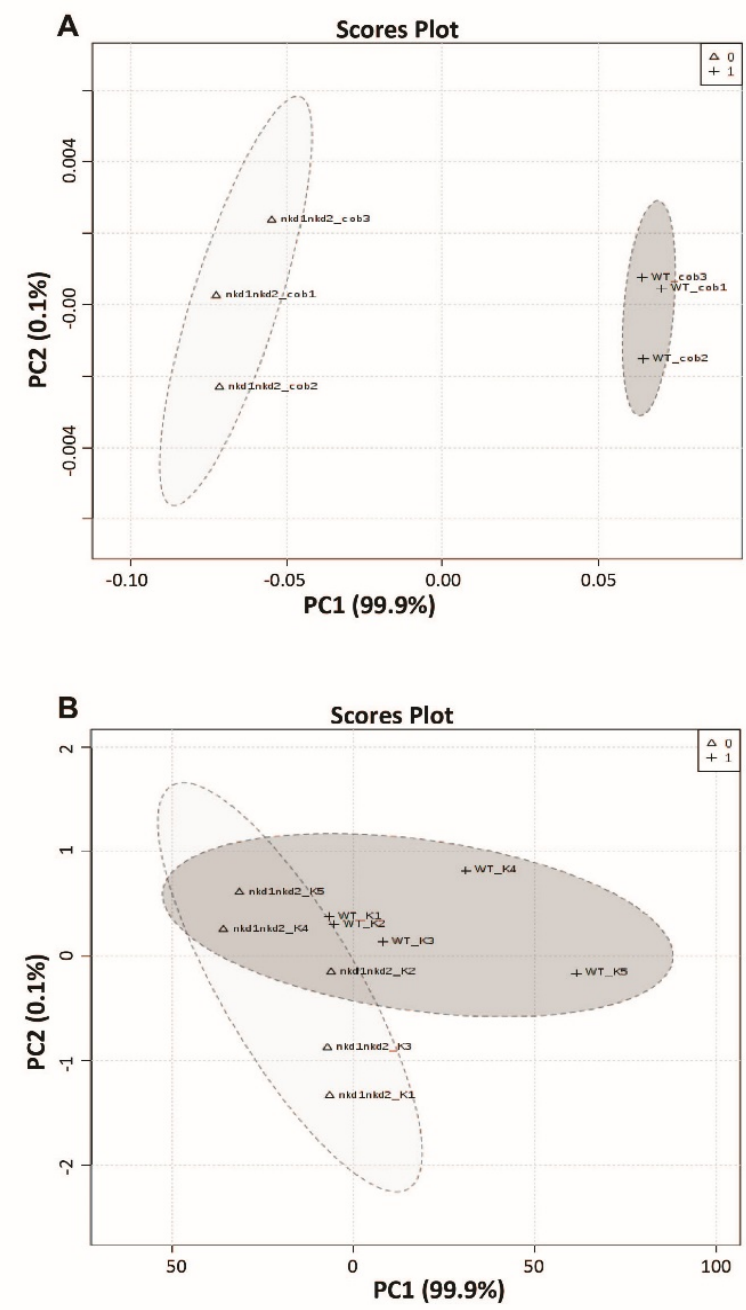
Supplemental Figure 4. BETL qRT-PCR

qRT-PCR on independent RNA samples from the lower quarter of segregating wildtype (WT) and *nkd1 nkd2* mutant (*nkd*) endosperms at 12DAP. Differential expression was determined by comparing WT to *nkd1 nkd2* mutant BETL marker gene expression using standard delta CT methodology relative to *ubiquitin* gene. * denotes significant differential expression. Error bars represent the standard deviation from the mean of three biological replicates each with three technical replicates.



Supplemental Figure 5. *nkd1 nkd2* Mutant Starch Granule SEM

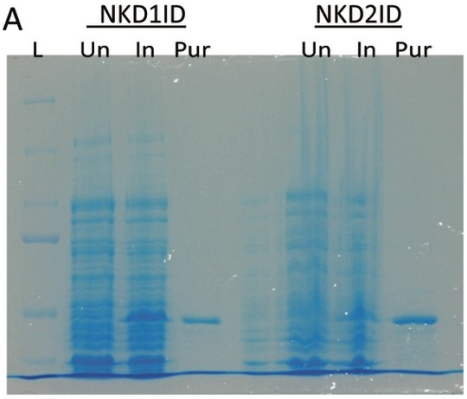
(A and B) Mature WT kernels from a segregating F2 ear described in Figure 3 C to F.
(C to E) Mutant *nkd1-Ds;nkd2-Ds0297* starch granules.
(A and C) Black boxes show position of images in panels **(B)** and **(D),** respectively.
(E) *nkd1 nkd2* mutant starch granules were sometimes faceted.



Supplemental Figure 6. Principal Component Analysis (PCA) of Wildtype and *nkd1-Ds nkd2-Ds0297* Mutant Resource Reserve Datasets

(A) PCA of WT and *nkd1 nkd2* mutant kernel weights and total protein datasets from independent segregating cobs (cob1-3).
(B) PCA of starch branch chain length and total starch content datasets from WT and *nkd1 nkd2* mutant kernels (k1-5).
(A and B) The first principal component is graphed on the x-axis and the second principal component is graphed on the y-axis. A third principal component was not generated for either analysis.

A



B

Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Peptide
1007.56	1006.56	1006.55	11.0	1	30	39	R.RGHNLPWK.L
1115.48	1114.47	1114.45	13.7	0	30	15	R.CDCGTLFSR.R
1193.66	1192.65	1192.64	9.3	1	38	5	R.EQNLQLHRR.G
1338.63	1337.63	1337.60	17.1	0	42	1.8	R.AFCDALAQESARL.L

Matched peptides shown in red

1 MASNSSAAAV AAMFGIRDAD HQDQMKPLLA QQHQHQHQHQ MPPAPLLNAA
51 SSSAGPGQAA GATPPAVKKK RNLPDPDAEV IALSPKTLLA TNRFVCEVCN
101 KGFQREQNLQ LHRRGHNLPW KLKQKDPSQA QRRRVYLCPE PTCAHHDPSR
151 ALGDLTGIKK HFCRKHGEKK WKCDKCSKRY AVQSDWKAHS KVCGTREYRC
201 DCGTLFSRRD SFITHRAFCD ALAQESARLP PP

C

Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Peptide
1007.52	1006.51	1006.55	-31.34	1	34	17	R.RGHNLPWK.L
1338.57	1337.57	1337.60	-28.01	0	48	0.38	R.AFCDALAQESAR.L

Matched peptides shown in red

1 MASNSSAAFF GVRDGDQQDQ IKPLISPQQQ LAAALPGVAG GVPAASGQGA
51 PPAQPPPKKK RTMPDPDAEV IALSPKTLMA TNRFVCEVCN KGFQREQNLQ
101 LHRRGHNLPW KLKQKNPLQA QRRRVYLCPE PTCVHHDPAR ALGDLTGIKK
151 HFCRKHGEKK WKCDKCSKRY AVQSDWKAHS KICGTREYRC DCGTLFSRRD
201 SFITHRAFCD ALAQESARLP PT

Supplemental Figure 7. Verification of NKD-ID GST Fusion Proteins

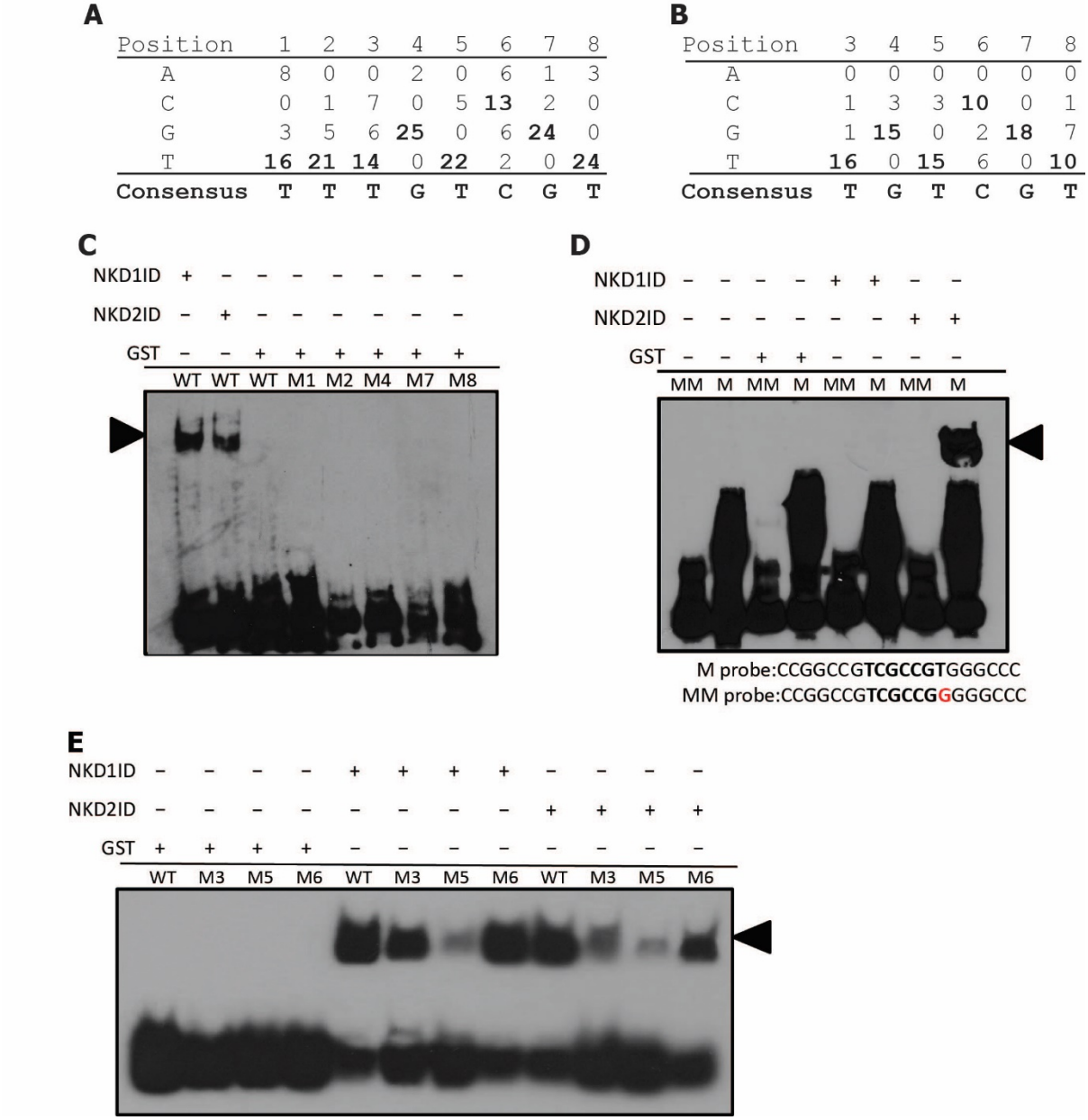
(A) SDS-PAGE gel stained with Coomassie stain of IPTG un-induced, induced and purified NKD1ID and NKD2ID GST fusion proteins. Protein concentrations were determined via Bradford Protein Assay (Bio-Rad) and equivalent protein concentrations were loaded for each sample. “L” represents protein size ladder (Precision Plus Protein™ Dual Color Standards #1610374), “Un” represents total soluble un-induced protein, “In” represents IPTG induced total soluble protein, and “Pur” represents purified GST fusion proteins purified with Glutathione Sepharose 4B (GE Healthcare). Purified protein bands were cut out of the gel and were verified by trypsin digest QSTAR MS/MS. QSTAR MS/MS data and aligned fragments for NKD1-ID GST fusion protein **(B)** and NKD2-ID GST fusion protein **(C)** indicated by bold red lettering.

A

Position	1	2	3	4	5	6	7	8
A	8	0	0	2	0	6	1	3
C	0	1	7	0	5	**13**	2	0
G	3	5	6	**25**	0	6	**24**	0
T	**16**	**21**	**14**	0	**22**	2	0	**24**
Consensus	**T**	**T**	**T**	**G**	**T**	**C**	**G**	**T**

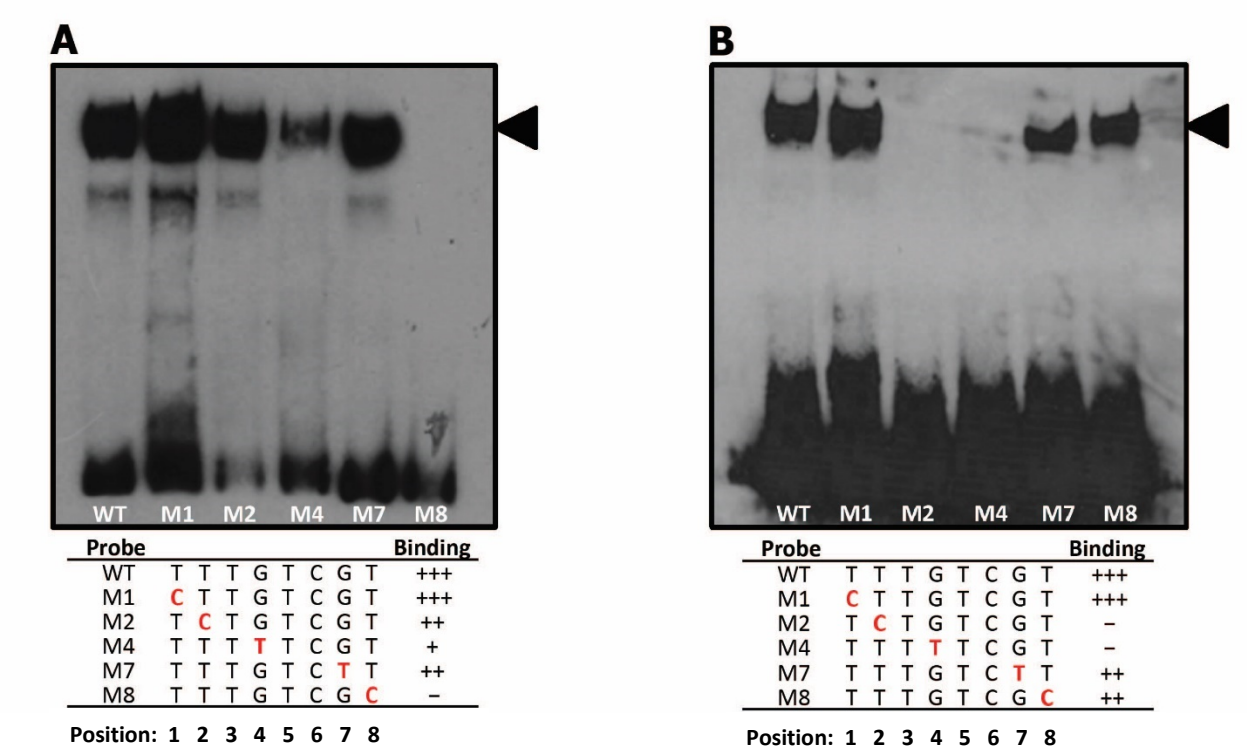
B

Position	3	4	5	6	7	8
A	0	0	0	0	0	0
C	1	3	3	**10**	0	1
G	1	**15**	0	2	**18**	7
T	**16**	0	**15**	6	0	**10**
Consensus	**T**	**G**	**T**	**C**	**G**	**T**



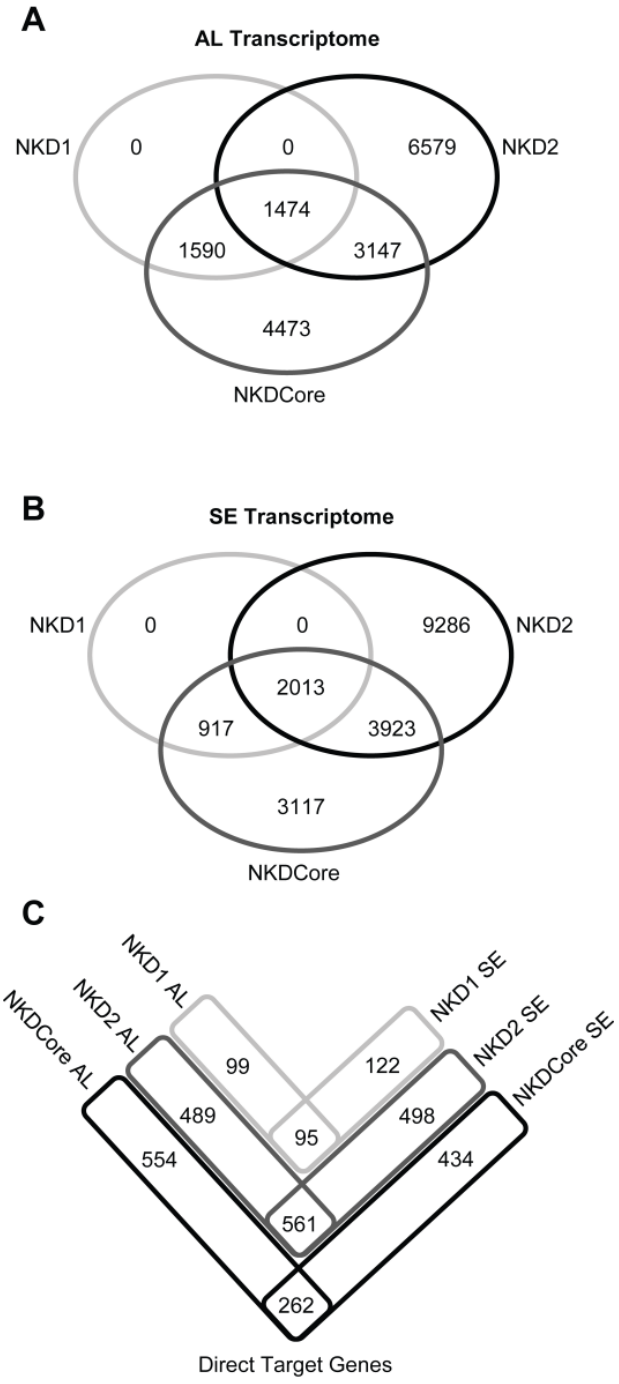
Supplemental Figure 8. EMSA Controls and Additional Tested Mutant Probes

(A and B) Positional base Frequency of MEME selected SAAB sequences for NKD1ID **(A)** and NKD2ID **(B)**.
(C and D) EMSA controls show the GST tag does not bind probes.
(D and E) Binding affinity of NKD1ID and NKD2ID GST fusion proteins for additional point mutated variants of the BCS probe. Sequences of probes used in **(D)** are shown, while for **(C and E**), probes are as indicated in Figure 5C. The NKD/DNA complex is indicated by an arrowhead. + indicates protein used and '–' indicating protein not used for each lane.



Supplemental Figure 9. Scanning Mutagenesis EMSA Overexposure

Over exposure of EMSAs described in Figure 5 for **(A)** NKD1ID and **(B)** NKD2ID GST fusion proteins. Note that no shift was detected for NKD1ID GST with point mutant probe variant M8 nor for NKD2ID GST with M2 and M4 point mutant probe variants.



Supplemental Figure 10. Overlap in NKD Motifs and Predicted Direct Target Genes

(A and B) RNAseq detected genes with NKD1, NKD2, and/or NKDCore binding motif in proximal promoter region in **(A)** aleurone and **(B)** starchy endosperm.
(C) Shared and unique NKD1, NKD2 and NKDCore direct target genes in aleurone and starchy endosperm.

A

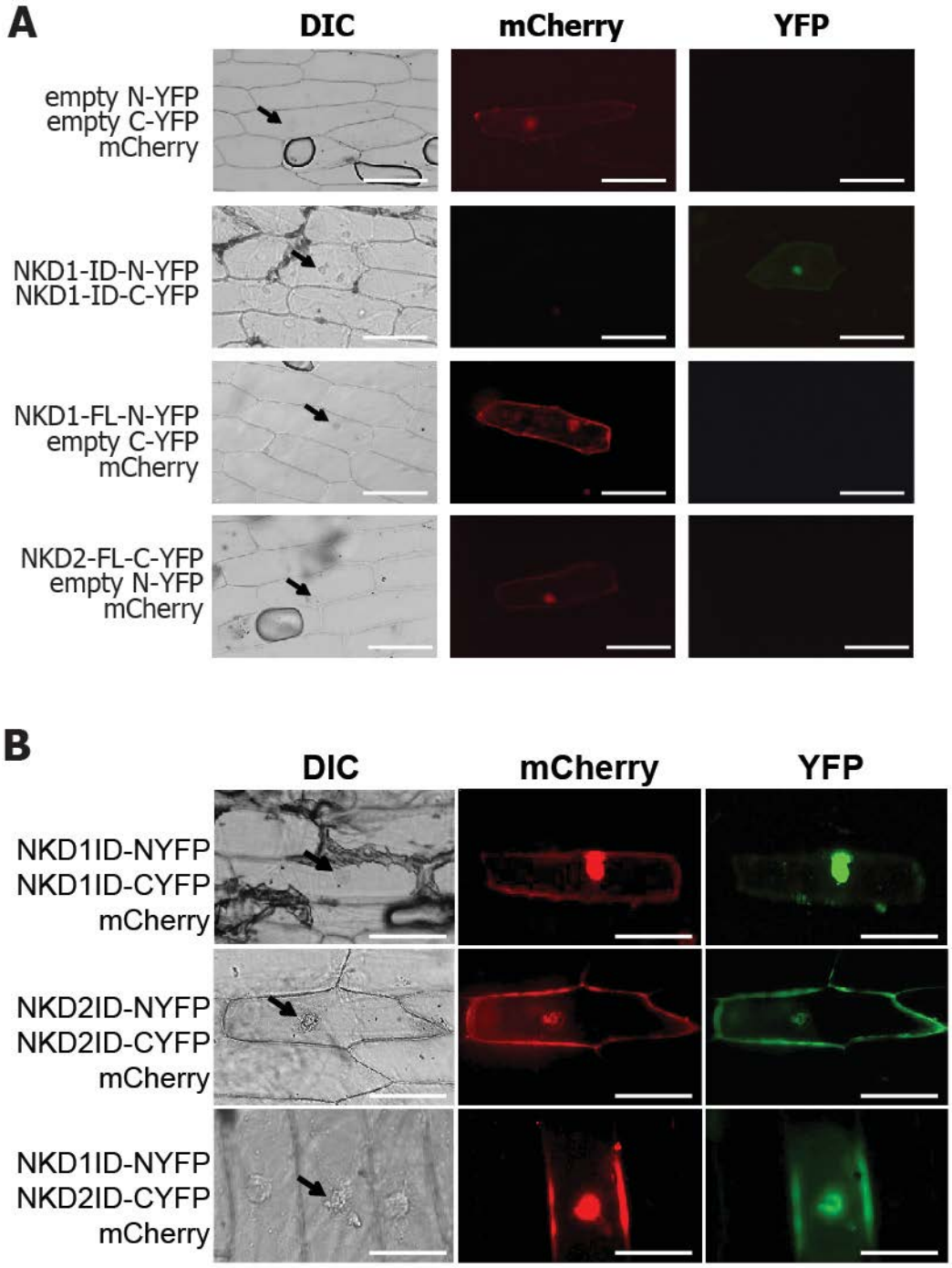
Motif	DE Al Genes ≥ 1 Motif in P.P.	Al Transcriptome ≥ 1 Motif in P.P.	Enrichment
NKD1 Control1 	241	3614	(241 /2135) vs (3614/32621) p-value=0.778
NKD1 Control2 	168	2903	(168/2135) vs (2903/32621) p-value=0.145
NKD1 Control3 	277	3972	(277/2135) vs (3972/32621) p-value=0.345
NKD2 Control1 	1364	20742	(1364 /2135) vs (20742/32621) p-value=0.900
NKD2 Control2 	1102	16538	(1102/2135) vs (16538/32621) p-value=0.645
NKD2 Control3 	1040	16320	(1040/2135) vs (16320/32621) p-value=0.509

B

Motif	DE S.E. Genes ≥ 1 Motif in P.P.	S.E. Transcriptome ≥ 1 Motif in P.P.	Enrichment
NKD1 Control1 	243	3473	(243 /2192) vs (3473/31389) p-value=0.972
NKD1 Control2 	192	2799	(192/2192) vs (2799/31389) p-value=0.847
NKD1 Control3 	260	3848	(260/2192) vs (3848/31389) p-value=0.663
NKD2 Control1 	1366	19932	(1366 /2192) vs (19932/31389) p-value=0.606
NKD2 Control2 	1060	15889	(1060/2192) vs (15889/31389) p-value=0.242
NKD2 Control3 	1044	15647	(1044/2192) vs (15647/31389) p-value=0.247

Supplemental Figure 11. NKD Motif Enrichment Shuffled Controls

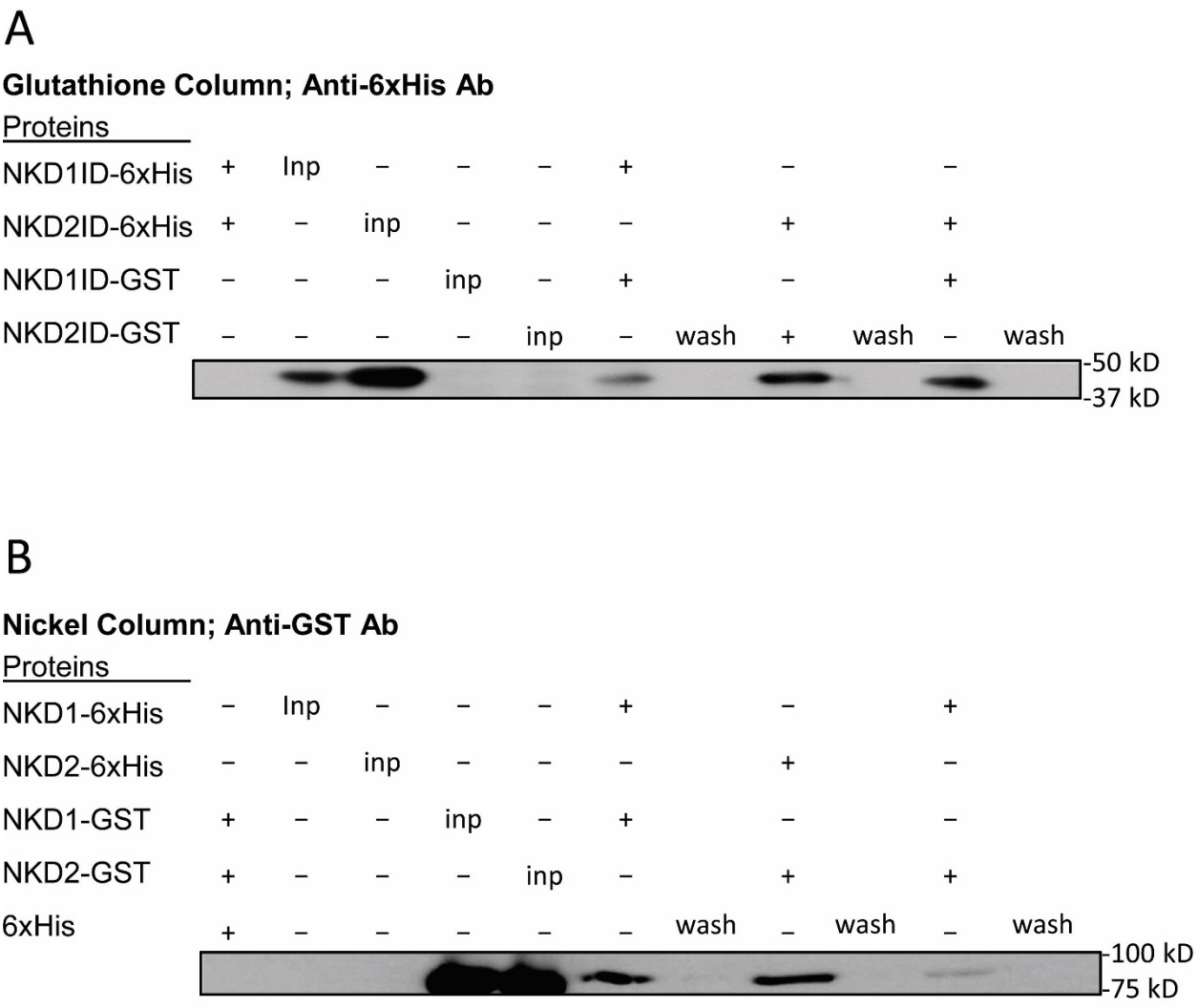
(A) Enrichment analysis for NKD1ID and NKD2ID shuffle control BCS occurrence in aleurone differentially expressed (DE) genes.
(B) BCS shuffle controls for starchy endosperm DE genes. Enrichment was determined relative to all genes detected in each RNAseq transcriptome.



Supplemental Figure 12. BiFC Controls and NKD ID domain interactions.

(A) Control bombardments consisting of combinations of empty vector CYFP + empty vector NYFP, NKD1 and NKD2 NYFP + empty vector CYFP or NKD1 and NKD2 CYFP + empty vector NYFP did not produce YFP fluorescence. Bombardments of NKD1ID-CYFP + NKD1ID-NYFP without mCherry was visualized with the mCherry filter to verify

specificity of each florescent filter set for the respective fluorophore. Constructs used in each control assay are listed at the left of each row.
(B) BiFC NKD1ID and NKD2ID proteins. Vectors used for each BiFC assay are listed to the left of each row. Arrows designate nuclei viewed under differential interference contrast (DIC). mCherry florescence marks transient cells transformation and YFP florescence indicates a positive protein-protein interaction.
(A and B) size bars = 100µm.



Supplemental Figure 13. Reciprocal Tag Co-Pull Downs

(A) Reciprocal Co-pull downs described in Figure 6 for NKD1ID and NKD2ID 6xHis and GST tagged proteins.
(B) Reciprocal Co-pull downs with NKD1 and NKD2 full length fusion proteins.

Supplemental Tables:

Sample	No. Reads (x 10^6)	% Uniquely mapped	% paired & mapped
WT AL	281	87.44	72.26
nkd1 nkd2 AL	310	75.65	59.09
WT SE	292	56.87	40.71
nkd1 nkd2 SE	256	72.07	54.07

Supplemental Table 1: Summary of aleurone (AL) and starchy endosperm (SE) RNA sequencing reads from Yi et al., 2015 re-aligned and mapped to the maize reference genome (B73 RefGen-V3, GSE61057).

Cell type	Expressed genes	Common genes	DE genes	Common DE genes	Higher in *nkd1 nkd2*	Lower in *nkd1 nkd2*
AL	34014	31792	2188	935	799	1389
SE	32629	31792	2193	935	915	1278

Supplemental Table 2: Summary of gene expression analysis in endosperm cell types

	NKD1 SAAB Selected Sequences
1	**TTTGTCGT** tttgtgtgaacc
2	accgtgtatt **TTTGTCGT** a
3	tattcggtgt **TTTGTCGT** a
4	ccgt **TTTGTGGT** ttatgtgc
5	tttttaagtt **TGTGTCGT**
6	acact **ATCGTCGT** atcacca
7	tcgcagttac **TTGGTGGT**
8	cgc **ATGGTCGT** aacgtaagt
9	gatc **ATTGTAGT** acatactc
10	tgcagcatg **TTCGCCGT** caa
11	a **TTGGCCGT** atttgtcgaa
12	attggccgta **TTTGTCGA** ac
13	ggtttacgca **GTGGTCGT**
14	c **TTCGCAGT** tacttggtgg
15	tgcatgaac **ATTGCAGT** aca
16	ac **GTCGTAGT** gctgtatca
17	gcgcgcga **TTTGTACT** cgca
18	g **ATTATCGT** gatcatcctg
19	attggc **AGCGTGGT** acgtga
20	tacttat **TTGGTGGA** tcctt
21	gtacc **GTTGTTGT** gattgac
22	ttagtcccta **TTCATGGT** gc
23	tgga **TTGGCTGT** attacatg
24	gtacgaac **AGTGTCGA** tgct
25	ctggtctttc **ACTGTAGT** a
26	tcta **TGTGTGCT** atcttcga
27	**TGCGTCAT** caaacaggcg

Supplemental Table 3: NKD1 SAAB Selected Sequences

	NKD2 SAAB Selected Sequences
1	gaacccattttt **TGTCGT** ta
2	**TGTCGG** cctaaccgttaga
3	cccgtctctggcg **TGTCGG** c
4	gccgt **TGTCGG** tatcaaccc
5	gtct **TGCCGT** ttggtgcgtg
6	cgttcaac **TGTTGT** ctcc
7	caagt **TGCCGT** ctatgtgcc
8	acagtggtacgc **TGTTGT** ca
9	**TGTGGT** agtaaagttactga
10	ttgtta **TGTGGT** tacatata
11	tctgcta **TGTTGG** agttagt
12	tatagat **TCTCGG** ctcggtg
13	tatggttcagcc **TCTCGG** cc
14	cagtactattt **TGTCGC** ta
15	g **TGCTGT** tcaattgaataaa
16	atg **CGTTGT** tgcggtgaact
17	cac **GGTTGT** gtattagctgg
18	agagtttctcaa **TCTCGG**

Supplemental Table 4: NKD2 SAAB Selected Sequences

Gene ID	Gene Annotation	*nkd1 nkd2* aleurone log2FC	*o2* logFC	*o2* p-value
GRMZM2G012806	subtilisin-chymotrypsin inhibitor CI-1B	-6.41	-6.49	6.69E-301
GRMZM2G353268	19 kD zein Z1A	-5.83	-2.03	1.71E-49
GRMZM2G026939	19 kD zein Z1A	-5.67	-1.2	1.20E-02
GRMZM2G059620	19 kD zein Z1A	-5.5	-1.58	2.36E-92
GRMZM2G138689	50 kD γ-zein	-4.94	-1.15	3.30E-06
GRMZM2G348914	Unknown	-4.69	-2.02	1.18E-04
GRMZM2G100018	18 kDδ-zein	-4.43	-194.82	4.10E-24
GRMZM2G138727	27 kD γ-zein	-4.38	-1.32	2.22E-10
GRMZM2G044152	22 kD α-zein	-4.32	-40.31	6.84E-70
GRMZM2G160739	22 kD α-zein	-4.22	-37.46	1.25E-231
GRMZM2G086294	14 kD β-zein	-4.08	-6.06	0.00E+00
GRMZM2G346897	22 kD α-zein	-4.01	-14.41	1.02E-93
GRMZM2G117956	proline oxidase	-3.97	-3.93	3.71E-03
GRMZM2G044625	22 kD α-zein	-3.89	-12.21	0.00E+00
GRMZM2G397687	22 kD α-zein	-3.83	-24.63	0.00E+00
GRMZM2G388461	22 kD α-zein	-3.72	-79.37	6.60E-62
GRMZM2G346895	22 kD α-zein	-3.61	-75.76	2.30E-91
GRMZM2G088441	22 kD α-zein	-3.56	-154.55	1.70E-23
GRMZM2G312877	Lactoylglutathione lyase	-3.5	-2.01	3.55E-05
GRMZM2G045387	22 kD α-zein	-3.22	-107.44	1.87E-194
GRMZM2G088273	22 kD α-zein	-3.07	-1.68E+06	7.60E-05
GRMZM2G181362	LKR/SDH	-2.91	-4.57	2.48E-17
GRMZM2G088365	22 kD α-zein	-2.88	-1.39E+07	9.78E-41
GRMZM2G147424	DNA-binding protein S1FA2	-2.17	-1.65	6.84E-05
GRMZM2G069651	Heat shock protein Hsp90	2.75	1.32	1.48E-02
GRMZM2G458208	Chaperonin Cpn60/TCP-1	3.85	1.88	2.03E-03
GRMZM2G156632	Bowman-Birk type wound-induced proteinase inhibitor WIP1 Precursor	-6.46	1.39	1.67E-02
GRMZM2G304548	Trypsin/factor XIIA inhibitor Precursor	-4.91	2.38	0.00E+00
GRMZM2G005633	Endochitinase B Precursor	-4.67	3.14	9.19E-42
GRMZM2G410134	plant lipid transfer protein/seed storage	-4.25	1.34	1.61E-12
GRMZM2G474575	Proteinase inhibitor I3, Kunitz legume	-3.52	2.37	3.51E-02
GRMZM2G174883	11-S seed storage protein	-3.48	2.12	2.77E-58
GRMZM2G030717	cysteine proteinase inhibitor B	-3.46	5.14	1.45E-13
GRMZM2G058358	subtilisin-chymotrypsin inhibitor CI-1C	-3.27	2.18	7.36E-11
GRMZM2G146283	Dof zinc finger protein PBF	-3.22	2.38	4.87E-08
GRMZM2G001500	Heat shock protein 70	-2.16	1.58	1.07E-02
GRMZM2G130062	2-isopropylmalate synthase B	-2.09	1.21	4.03E-02
GRMZM2G076239	hydroxyacid oxidase 1	-2.07	1.57	5.49E-03
GRMZM2G091481	Protein disulfide-isomerase (PDI)	-1.82	1.3	9.41E-05
GRMZM2G019325	40S ribosomal protein S11	-1.82	1.58	3.13E-04
GRMZM2G448151	30S ribosomal protein S3, chloroplastic	3.05	-1.22	4.69E-02
GRMZM5G811749	30S ribosomal protein S16, chloroplastic	3.2	-1.29	5.34E-05

Supplemental Table 5: NKD1, NKD2 and O2 Aleurone Co-Regulated Genes

Gene ID	Gene Annotation	*nkd1 nkd2* starchy endosperm log2FC	*o2* logFC	*o2* p-value
GRMZM2G012806	subtilisin-chymotrypsin inhibitor CI-1B	-4.69	-6.49	6.69E-301
GRMZM2G353268	19 kD zein Z1A	-3.96	-2.03	1.71E-49
GRMZM2G059620	19 kD zein Z1A	-3.71	-1.58	2.36E-92
GRMZM2G026939	19 kD zein Z1A	-3.39	-1.2	1.20E-02
GRMZM2G138689	50 kD γ-zein	-3.37	-1.15	3.30E-06
GRMZM2G348914	Unknown	-2.35	-2.02	1.18E-04
GRMZM2G100018	18 kDδ-zein	-2.26	-194.82	4.10E-24
GRMZM2G168330	60S ribosomal protein L14	0.87	1.82	6.55E-11
GRMZM2G091383	40S ribosomal protein S24	0.91	1.69	4.98E-03
GRMZM2G100467	60S ribosomal protein L39	1.01	1.4	3.22E-02
GRMZM2G377797	40S ribosomal protein S16	1.19	1.35	2.86E-02
GRMZM2G142640	60S ribosomal protein L24	1.19	2.07	2.20E-05
GRMZM2G178968	60S ribosomal protein L7	1.4	1.51	3.34E-03
GRMZM2G116292	60S ribosomal protein L40	1.72	2.47	2.91E-12
GRMZM2G069651	Heat shock protein Hsp90	2.58	1.32	1.48E-02
GRMZM2G156632	Bowman-Birk type wound-induced proteinase inhibitor WIP1 Precursor	-5.81	1.39	1.67E-02
GRMZM2G474575	Proteinase inhibitor I3, Kunitz legume	-3.18	2.37	3.51E-02
GRMZM2G005633	Endochitinase B Precursor	-2.59	3.14	9.19E-42
GRMZM2G304548	Trypsin/factor XIIA inhibitor Precursor	-1.84	2.38	0.00E+00
GRMZM2G146283	Dof zinc finger protein PBF	-1.54	2.38	4.87E-08
GRMZM2G448151	30S ribosomal protein S3, chloroplastic	3.17	-1.22	4.69E-02

Supplemental Table 6: NKD1, NKD2 and O2 Starchy Endosperm Co-Regulated Genes

GO term	Ontology	Description	Number in co-DE	Number in Suggested Background	p-value	FDR
GO:0045735	F	nutrient reservoir activity	18	100	1.90E-32	4.20E-31
GO:0004866	F	endopeptidase inhibitor activity	6	82	4.50E-09	3.30E-08
GO:0030414	F	peptidase inhibitor activity	6	82	4.50E-09	3.30E-08
GO:0004857	F	enzyme inhibitor activity	6	180	4.00E-07	2.20E-06
GO:0030234	F	enzyme regulator activity	6	368	2.20E-05	9.60E-05

Supplemental Table 7: Enriched Gene Ontologies in NKD1, NKD2 and O2 Aleurone Co-Regulated Genes

GO term	Ontology	Description	Number in co-DE	Number in Suggested Background	p-value	FDR
GO:0006412	P	translation	6	980	5.30E-05	0.0024
GO:0003735	F	structural constituent of ribosome	6	649	5.30E-06	2.90E-05
GO:0005198	F	structural molecule activity	6	767	1.40E-05	3.70E-05
GO:0033279	C	ribosomal subunit	7	301	1.40E-09	1.40E-07
GO:0005840	C	ribosome	8	772	3.70E-08	1.40E-06
GO:0044445	C	cytosolic part	6	280	4.20E-08	1.40E-06
GO:0022626	C	cytosolic ribosome	6	323	9.50E-08	2.30E-06
GO:0030529	C	ribonucleoprotein complex	8	894	1.10E-07	2.30E-06
GO:0043228	C	non-membrane-bounded organelle	8	1579	8.10E-06	0.00012
GO:0043232	C	intracellular non-membrane-bounded organelle	8	1579	8.10E-06	0.00012
GO:0032991	C	macromolecular complex	8	2550	0.00025	0.003
GO:0005829	C	cytosol	6	1316	0.00027	0.003

Supplemental Table 8: Enriched Gene Ontologies in NKD1, NKD2 and O2 Starchy Endosperm Co-Regulated Genes

Gene ID	Forward primer	Reverse primer	Amplicon size (bp)
Tubulin 1 (*tub1*), GRMZM2G164696	CAATACCCTGTTGCGCTTTG	CAGTTCCAGAAACATGAGCAAATA	99
Proliferating cell Nuclear Antigen2 (*pcna2*), GRMZM2G108712	CACATCTGGAGAAATTGGGAGTG	CCGGCTCCTGCATCTCTATAA	98
Cell Division Cycle2-like (CDC2-like), GRMZM2G068193	GACGAAGCTGTCTGCTCTTG	CATCTCCCTTCCTCACATCTTTG	146
Granule Bound Starch Synthase (*waxy1*), GRMZM2G024993	GCGTACGAGGAGATGGTGAG	CAGCACGTTCTCCCAGTTCT	81
Endo Betachitinase 2, GRMZM2G005633	GTGTAGGCGATCACTGTTTCA	CACAGAATGGGTAGAAGACCAC	112
Floury1, GRMZM2G094532	CAGAAGCAGATGTATGACCGG	ACAAGGCTCGATCAGTTATGG	106
Actin-1, GRMZM2G047055	AAGGCTGAGTACGACGAGT	CTCTCGGCTTTGCATCTCTT	86
Nac Transcription factor ASN1/Nac36, GRMZM2G154182	CTAGCTAGTACATGCACCGTATC	CCAAACTCGAATCTGCTTCTTAC	114
Fatty acid Desaturase 2, GRMZM2G064701	TCGGCGAGTACTACCAGTT	TGCTGTTGTACCAGAAGACG	123
Phospholipid Transfer Protein homolog1 (*plt1*), GRMZM2G101958	GCTCCAGGGTGAACTGAA	GTCGTGATCATGCGTAGGTAG	136
Opaque2, GRMZM2G015534	CCAGAAGTACAACGACGCTAAC	CAGGGAGTCCTCTCCCATC	94
Faciata1-like, GRMZM2G010105	GAAGAAATTAACCAGCCCAATCC	CACCACCTTATGGATACCATCTC	126
Prolamin-box Binding Factor1 (*pbf1*), GRMZM2G146283	CAACGCTAGCTCTAGCAATATGA	TGGGAGCACATTAGGGAATAAG	106
Legumin1 (*leg1*), GRMZM2G174883	GACGAGAACAACAACATCAACC	GAAGATGTTCTCGGCGAAGT	102
Ubiquitin Conjugating Enzyme (AL qRT), GRMZM2G132759	GCCATTGTTCCCTTGGATTTG	TGGTAGGCCGACGATATACA	101
betl1 ,GRMZM2G082785	CTGTTGCCATTCTGTCCTCACTG	CACCGTCCTTGAAACCCTTGGA	186
betl2, GRMZM2G152655	TGCACGCACAACAAGTGGGCAC	AGCATGGCCCGTCGTCATTACG	116
meg1, GRMZM2G354335	CTTTGCTGCTCATGCGCATGG	GCATGCATGACTACACTGAGCC	202
mrp1, GRMZM2G111306	CCGTGGACAACATGGACATGATG	CTTCCATTTACCACGGCCAAACAC	191
Ubiquitin Conjugating Enzyme (BETL qRT), GRMZM2G027378	AGGAAGTTTGGTTTGCTAGCG	CTGTTGGATCCCATGACGG	168

Supplemental Table 9: Primer sequences used for qRT-PCR

Primers	Forward	Reverse
GST-NKD1-ID	n1ID-BamH1: GGATCCGCATCGAATTCATCGGCGGCGTTCTTTG	n1ID-Sal1: aaaGTCGACCGTCGGCGGCAGGCGCGCGCTTTC
GST-NKD2-ID	n2ID-BamH1: GGATCCGCGTCGAATTCACCGGCGGCGGCG	n2ID-Sal1: aaaGTCGACGGTCGGCGGCAGCCGCGCGCTC
GST-NKD1-FL	nkd1BamH1: cttGGATCCGCATCGAATTCATCGGCGGCGTTCTTTG	nkd1Sal1: catGTCGACTGGCATCCTGCCTCCGTTGAAGGACGAGG
GST-NKD2-FL	nkd2BamH1: cttGGATCCGCGTCGAATTCACCGGCGGCGGCG	nkd2Sal1: aaGTCGACTGGCATCCTGCCTCCATTGAAGGACG
NKD1-ID-His	n1ID-BamH1: GGATCCGCATCGAATTCATCGGCGGCGTTCTTTG	n1ID-HINDIII: tttAAGCTTCGTCGGCGGCAGGCGCGCGCTTTC
NKD2-ID-His	n2ID-BamH1: GGATCCGCGTCGAATTCACCGGCGGCGGCG	n2ID-HINDIII: tatAAGCTTGGTCGGCGGCAGCCGCGCGCTC
NKD1-FL-His	nkd1BamH1: cttGGATCCGCATCGAATTCATCGGCGGCGTTCTTTG	nkd1BamH1 Rev: catGGATCCTGGCATCCTGCCTCCGTTGAAGGACGAGG
NKD2-FL-His	nkd2BamH1: cttGGATCCGCGTCGAATTCACCGGCGGCGGCG	nkd2BamH1 Rev: aaGGATCCTGGCATCCTGCCTCCATTGAAGGACG

Supplemental Table 10: Primers used to generate NKD GST and 6x His tag expression constructs

Primers	Forward	Reverse
WT	CCGGCCTTTGTCGTGGGCCC, 5'Biotinylated (no Biotin for unlabeled)	GGGCCCACGACAAAGGCCGG
M1	CCGGCCCTTGTCGTGGGCCC, 5'Biotinylated	GGGCCCACGACAAGGGCCGG
M2	CCGGCCTCTGTCGTGGGCCC, 5'Biotinylated (no Biotin for unlabeled)	GGGCCCACGACAGAGGCCGG
M3	CCGGCCTTCGTCGTGGGCCC, 5'Biotinylated	GGGCCCACGACGAAGGCCGG
M4	CCGGCCTTTTTCGTGGGCCC, 5'Biotinylated	GGGCCCACGAAAAAGGCCGG
M7	CCGGCCTTTGTCTTGGGCCC, 5'Biotinylated	GGGCCCAAGACAAAGGCCGG
M8	CCGGCCTTTGTCGCGGGCCC, 5'Biotinylated (no Biotin for unlabeled)	GGGCCCGCGACAAAGGCCGG
MM3	CCGGCCTTCGTCGCGGGCCC, 5'Biotinylated	GGGCCCGCGACGAAGGCCGG
M	CCGGCCGTCGCCGTGGGCCC, 5'Biotinylated	GGGCCCACGGCGACGGCCGG
MM	CCGGCCGTCGCCGGGGGCCC, 5'Biotinylated	GGGCCCCCGGCGACGGCCGG

Supplemental Table 11. Oligonucleotides used in electrophoretic mobility shift assays (EMSA)

Primers	Forward	Reverse
NKD1-ID C and N-terminal YFP halves	Sal1-nkd1: ttGTCGACATGGCATCGAATTCATCGGCGGCG	n1ID-BamH1: tttGGATCCCCGTCGGCGGCAGGCGCGCGCTTTC
NKD2-ID C and N-terminal YFP halves	Sal1-nkd2: ttGTCGACATGATGGCGTCGAATTCACCGGCG	n2ID-BamH1: tatGGATCCCGGTCGGCGGCAGCCGCGCGCTC
NKD1-FL C and N-terminal YFP halves	Sal1-nkd1: ttGTCGACATGGCATCGAATTCATCGGCGGCG	BamH1-nkd1: aaGGATCCGTGGCATCCTGCCTCCGTTGAAGG
NKD2-FL C and N-terminal YFP halves	Sal1-nkd2: ttGTCGACATGATGGCGTCGAATTCACCGGCG	BamH1-nkd2: aaGGATCCGTGGCATCCTGCCTCCATTGAAGGAC
35s-mCherry	Mch-kpn1: tttGGTACCATGGTGAGCAAGGGCGAGGAG	Mch-BamH1: tatGGATCCTTACTTGTACAGCTCGTCCATGCC

Supplemental Table 12. Primers and restriction sites used for cloning of constructs used in BiFC

Primers	Forward	Reverse
35S$_{pro}$:NKD1	Sal1-nkd1: ttGTCGACATGGCATCGAATTCATCGGCGGCG	BamH1-nkd2: aaGGATCCGTGGCATCCTGCCTCCGTTGAAGG
35S$_{pro}$:NKD2	Sal1-nkd2: ttGTCGACATGATGGCGTCGAATTCACCGGCG	BamH1-nkd1: aaGGATCCGTGGCATCCTGCCTCCATTGAAGGAC
35S$_{pro}$:RLUC	Rluc-NCO1: aataCCATGGATGACTTCGAAAGTTTATGATCCAGAAC	Rluc-BamH1: atttGGATCCTTATTGTTCATTTTTGAGAACTCGCTCA
X1P-1$_{pro}$:LUC	XIP1-Kpn1: aaGGTACCGACCGACCTAGACTCCTCTAATC	XIP1-Nhe1: ttGCTAGCGTCTCGTTGGTTAATTGCTGCTAC
o2$_{pro}$:LUC	O2-Sac1: aaGAGCTCGCTCCAAAATATAGCAAGTCACAG	O2-Nhe1: ttGCTAGCCACATTTTGAAGGCCTCGAG
zp22.1$_{pro}$:LUC	Azein22.3-Sac1: aaGAGCTCACTTGAGGTTCGCACCAATTGC	Azein22.3-Nhe1: ttGCTAGCGTTGTTAGGTTGTTGCTAATGTGC
nkd1$_{pro}$:LUC	Nkd1-MlU1: aataACGCGTGAAGCTCTATATTGATGGTTTGTAAGCC	Nkd1-BGlIIR: atttAGATCTGACTTCCTCCCGATTTATCCTGG
vp1$_{pro}$:LUC	Vp1-MlU1: aataACGCGTGCTAGTGTTCTTTGAGCTCTTGTGCTTG	Vp1-Xho1: atttCTCGAGCAGAGAGACACGACGACAAAGGG
vp1-mut$_{pro}$:LUC	Vp1-MlU1: aataACGCGTGCTAGTGTTCTTTGAGCTCTTGTGCTTG	Vp1-Xho1: atttCTCGAGCAGAGAGACACGGCGACAAAGGG
JIP$_{pro}$:LUC	JIP-Sac1: aaGAGCTCACTCAGCAAAGAACCGAATTCCAG	JIP-Nhe1: ttGCTAGCATTACCTGCAACGATCGAGCAAC
MTF$_{pro}$:LUC	MFT-like-Kpn1: aaGGTACCGGTTCGGAAACTGATCCTTAAGCGG	MFT-like-Nhe1: ttGCTAGCGATCAACGCGACCTTGGGTAGAG
WRKY29$_{pro}$:LUC	Wrky29-Mlu1: aaACGCGTGGCCTTTACTCCTGTCTCCTAG	Wrky29-Xho1: ttCTCGAGAGAGAGAGAGAGAGAAATTAAGCG

Supplemental Table 13. Primers and restriction sites used for cloning of transcription assay constructs.

Construct	Description	Experiments
NKD1-ID-N-YFP	C-terminal YFP (N-terminus)	BiFC
NKD1-ID-C-YFP	C-terminal YFP (C-terminus)	BiFC
NKD2-ID-N-YFP	C-terminal YFP (N-terminus)	BiFC
NKD2-ID-C-YFP	C-terminal YFP (C-terminus)	BiFC
NKD1-FL-N-YFP	C-terminal YFP (N-terminus)	BiFC
NKD1-FL-C-YFP	C-terminal YFP (C-terminus)	BiFC
NKD2-FL-N-YFP	C-terminal YFP (N-terminus)	BiFC
NKD2-FL-C-YFP	C-terminal YFP (C-terminus)	BiFC
35s-mCherry	Full length mCherry CDS	BiFC
GST-NKD1-ID	NKD1 ID CDS, N-terminal GST tag	Co-Pull Down, EMSA, SAAB
GST-NKD2-ID	NKD2 ID CDS, N-terminal GST tag	Co-Pull Down, EMSA, SAAB
GST-NKD1-FL	NKD1 CDS, N-terminal GST tag	Co-Pull Down, EMSA
GST-NKD2-FL	NKD2 CDS, N-terminal GST tag	Co-Pull Down, EMSA
NKD1-ID-His	NKD1 ID CDS, C-terminal 6x His tag	Co-Pull Down
NKD2-ID-His	NKD2 ID CDS, C-terminal 6x His tag	Co-Pull Down
NKD1-FL-His	NKD1 CDS, C-terminal 6x His tag	Co-Pull Down
NKD2-FL-His	NKD2 CDS, C-terminal 6x His tag	Co-Pull Down
X1P-1$_{pro}$:LUC	X1P-1 proximal promoter region (-596bp from TSS to -4bp from ATG), Firefly Luciferase	Transcription Assays
*o2*pro:LUC	*opaque2*proximal promoter region (-570bp from TSS to +432 from ATG), Firefly Luciferase	Transcription Assays
zp22.1$_{pro}$:LUC	*zp22.1*proximal promoter region (-510bp from TSS to -1bp from ATG), Firefly Luciferase	Transcription Assays
nkd1$_{pro}$:LUC	*nkd1*proximal promoter region (-966bp from TSS to -1bp from ATG), Firefly Luciferase	Transcription Assays
vp1$_{pro}$:LUC	*vp1* proximal promoter region (-1592bp from TSS to +256bp from TSS), Firefly Luciferase	Transcription Assays
VP1-mut$_{pro}$:LUC	*vp1* proximal promoter region (-1592bp from TSS to +256bp from TSS) with point mutation in second NKD BCS TTGTCGT to TTGTCGC, Firefly Luciferase	Transcription Assays
JIP$_{pro}$:LUC	JIP proximal promoter region (-600bp from TSS to ATG), Firefly Luciferase	Transcription Assays
MTF$_{pro}$:LUC	MTF proximal promoter region (-343bp from TSS to -8bp from ATG), Firefly Luciferase	Transcription Assays
WRKY29$_{pro}$:LUC	*WRKY29* proximal promoter region (-600bp from TSS to -19bp from ATG), Firefly Luciferase	Transcription Assays
35S$_{pro}$:NKD1	2x 35S promoter-NKD1 CDS	Transcription Assays
35S$_{pro}$:NKD2	2x 35S promoter-NKD2 CDS	Transcription Assays
35S$_{pro}$:null	2x 35S promoter-MCS-TGA	Transcription Assays
35S$_{pro}$:RLuc	2x 35S promoter-Renilla Luciferase CDS	Transcription Assays

Supplemental Table 14. Constructs cloned in this study.