

Figure S1. The phenotype of *dek504* in different hybridization backgrounds and phenotypes of *dek504* alleles. (a–c) A selfed ear segregates 3:1 for the wild-type (WT) and *dek504* mutant kernels (arrows). (a–c) belong to B73, Mo17 and Z58 backgrounds respectively. (d) The phenotype in the reproductive stages of *dek504* and WT ('B73') plants in the field respectively. (e) A homozygous ear of *dek504*. (a–e) Scale bar = 1 cm. (d) Scale bar = 0.5 m.

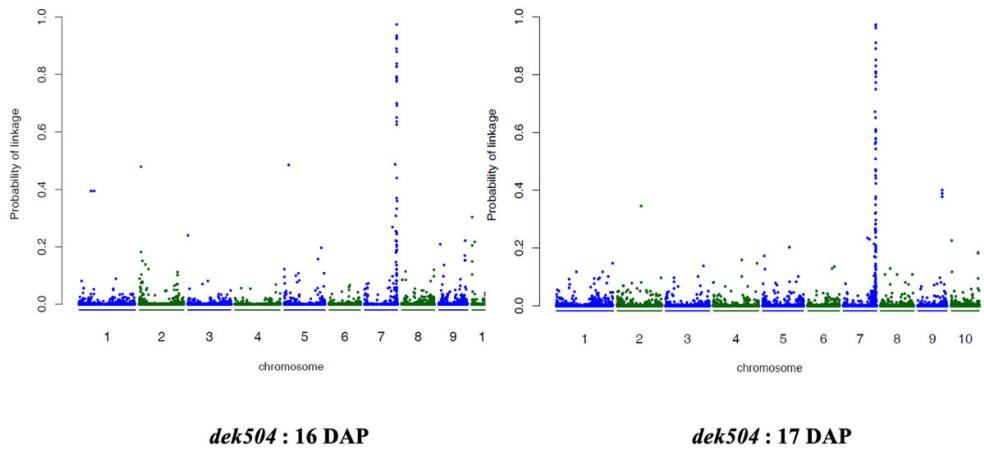


Figure S2. BSR-seq mapping of *dek504*. Mapping of *dek504* gene was performed using RNA sequencing of plant pools of wild type (WT) and *dek504-ref* kernels (pericycle removed) collected from the same ear. The probability of linkage of each SNP (Single Nucleotide Polymorphism) with the causal gene was determined by a Bayesian approach and plotted versus genomic coordinate of the SNP.

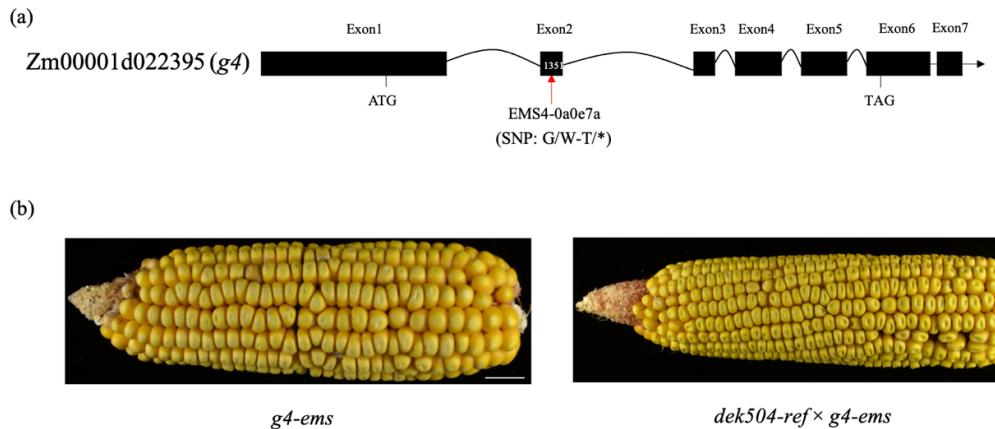


Figure S3. Allelic tests between *dek504-ref* heterozygotes and *g4-ems* homozygous mutants.

(a) Structure and mutation site of the *Zm00001d022395* gene. The red arrow denotes the mutation site of *Zm00001d022395*. Lines represent introns and black boxes represent exons. (b) Crossed ear between *dek504-ref* heterozygotes and *g4-ems* homozygous display WT phenotype. Bar, 1cm.

WT:

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ATGCATTCCGCCGCCCTTACCAATACCACCGCCTTGGCCGCCGCCGCTCTACGGCT  
CTGTGGCGCTGCCCTTCCTGCCCTCCGCAAGCAGCTCATGCCCAATCTCGCAC  
GTTAACAGCCATTCTCCATACATTCCTCTCCAAACTCTCTCCCTCTCCGCCCC  
CGGCCCTCTGCCCTGCCGCCAACATTCCTCTCTCTCTCTCTCCACCCGGGCC  
ACCCGCTTCACTAACATGCTAACATCGCTTATCGCTCATGCCGCCGACACCTC  
CTTCGCCCTCTCTCGCATGCTGCCGCCAGCTGCCGCTCGACCCGACGATAC  
ACCCCTCCCTCTCTCTCGCCGCCGCCCTGCCGCCACCGGCCCTGCCGCC  
CGGCCATGCTCTCTCGAGAAGATGCTCGGTGACCCAGAACACCGTCACTC  
CTCATCACCATGTACTCTGCTTGTAGATCACCTGGCCGGAGGGTGTGAGC  
GAATTCACCCGGATGTCGTTCTTGGAACTGATGTTGAAGGCATAAGGGCG  
AGGGATGGTGGCGAGGGTGAAGGATGTCGCGTGATGGTACCGAGGGCGAAGT  
GGCACCCATGGGAGGGTGGGGAGGGTGGGGTGGAGCTGCGGATGGTGGAC  
TGGTGCCTGGAGGGTGGGGAGGGTGGGGTGGAGCTGCGGATGGTGGAC  
TGGCTATGGGTCAGCCTGGGGATGTTGAAGGATGAGGTGGAGAGATGGCAGGG  
CACGGCGTGTGTTGAGCATGACAAGGATGCTGCGATGGAATGCCATG  
CACCGGTTATGCCAAAGGATGCTGAAATOAAGGATAGCTTGTGTTACAGCATG  
AGGGAAAGCTGGGCTGTCAGGAGATAACCTTGGTGGTGTCTCTCATCTGGG  
CAGCAGTTGTCAGTGAAGCTGGAGTGAATTGGATACCTGCGATGCCAGGG  
CTCTACACAACTGCTAGAACGCGCTAGTGGACATGTTGAGCTGGAG  
ATCTTGAGAAAAGCCGATGTTTGGAGATGCCATCAAATAGGCTCATG  
GAATGCAATTATCTGTTGACTCTGCTTCAACGGTGGAGCTGATGCCATTCAG  
TGAACAAATGAGAGATGAGAAAGGACTCAACCGAGATATCACATTCTAGGGT  
GCTTGGCTGTTGATGTCATGCTGGCTACTCGAGTGGCCCGAGTGTGCAATTCT  
TGACACCTGTTTAAGATCATCTCTAGAATTGAGACTCTCTCATGTTGATTG  
TGGCACGGTGTGTCATTAGAAGAACGATGGGATTITATAGGAAATGCCGG  
GGTAGATGCCGTCATGTTAGGGCTTGTGCTGCTGCTGCCGAAATGCAAGAAC  
GAGGTTGGAGGGTGTGTCATAAAGGATTATGAGGCTGAGCTGAGCATCTGGA  
ACTACGGTGTGTCATCCAAGATTATGCAACCTCGAGATAAAATGGATGAAATCTGGA  
ATGATAGGGCAATGAGGGAGGGGGTGTCAACAAAGACTCAGGGTGCAGCTGGGTT  
GAGGTTAATGAAAAGTTCTGAACTCTGCAACGACTGAACAGCATGGTGGGG  
AAGATATGATCAACTCATGGGGTACTCTGTTAAATGAGATGAGACTAAAGGGATATG  
CCAAACCTTGATCTGGTGTAG*
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dek504-ref:

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ATGCATTCCGCCGCCCTTACCAATACCACCGCCTTGGCCGCCGCCGCTCTACGGCT  
CTACGGCTCTGTGGCGCTGCCGTTCCCTGCCCTCCGCAAGCAGCTCATG  
CCACATCTGCGACGCTCAACAGCCCATTCCTACATCTCTCTCTCT  
CAAACCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT  
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT  
CAATCCGCTTATTCGCTCATGCCGCCGAGACCTCCCTGCGCTCTCT  
CAGCATGCTGCCGCCAGGGTGGCTGCCGACCCGACGCTATACACCTCCC  
TTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT  
CGCCCAATGCTCTCTGAGAAGATCGTCTGGTACCCAGACACACCGT  
TCGGCACCCGCTTCCCGCATCAACCCCTCCCGCTCGGCACCCGTTCC  
AAGACGGGCTGTTGGTCAAGCCGGCAACGTTCAAATTGAGAGAAAAATG  
CTGCAGCCGCCGACTACAACAGGATGGTACGCCCTCACAAAAG  
AAGGATAG*
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Figure S4. The ORF of *Zm00001d022394* in WT and *dek504-ref*. The ORF of *Zm00001d022394* produced in WT and *dek504-ref*. The red brackets represent the 6.9-kb deletion in *dek504-ref*. The predicted stop codon of *Zm00001d022394* is indicated in red in WT and *dek504-ref*.

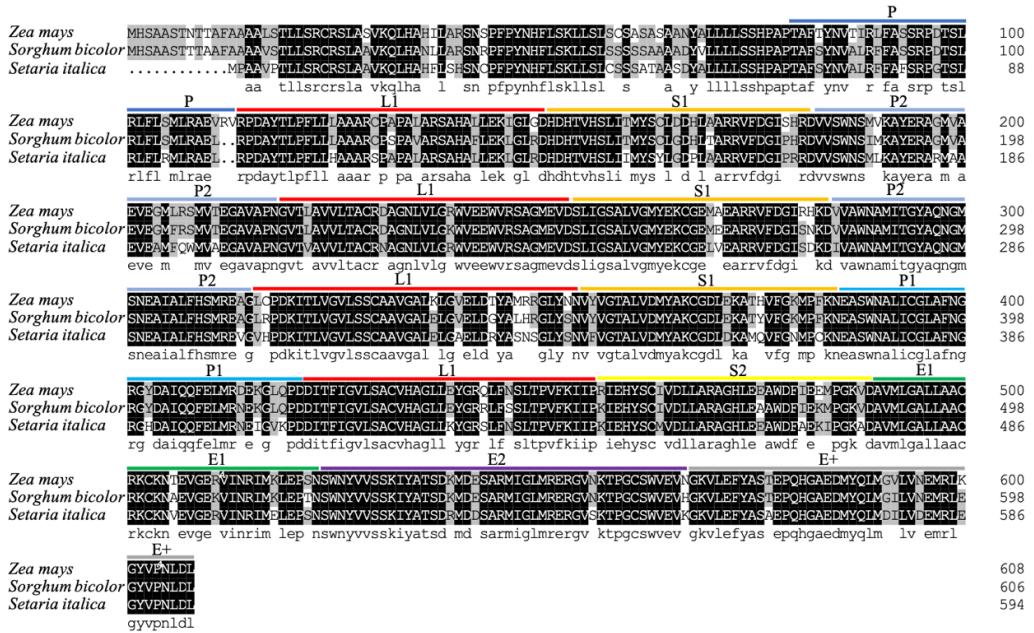


Figure S5. Sequence alignment of DEK504 and its homologues from *Sorghum bicolor* and *Setaria italica*. The amino acid sequence of DEK504 homologs, including the *Sorghum bicolor* (XP 021308964.1) and *Setaria italica* (XP 014661047.1), was downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>) and was aligned with DNAMAN.

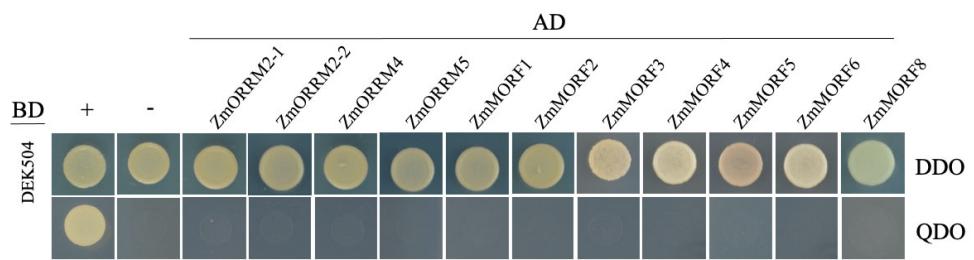


Figure S6. A yeast two hybrid (Y2H) assay between DEK504 with organelle RRM proteins (ORRMs) and multiple organelle RNA editing factors/RNA-editing factor interacting proteins (MORFs/RIPs). The colony pictures were taken after three days incubation at 30°C in SD/- Trp-Leu dropout (DDO) plates, as well as three days incubation at 30°C in SD/-Trp-Leu-His-Ade dropout (QDO) plates.

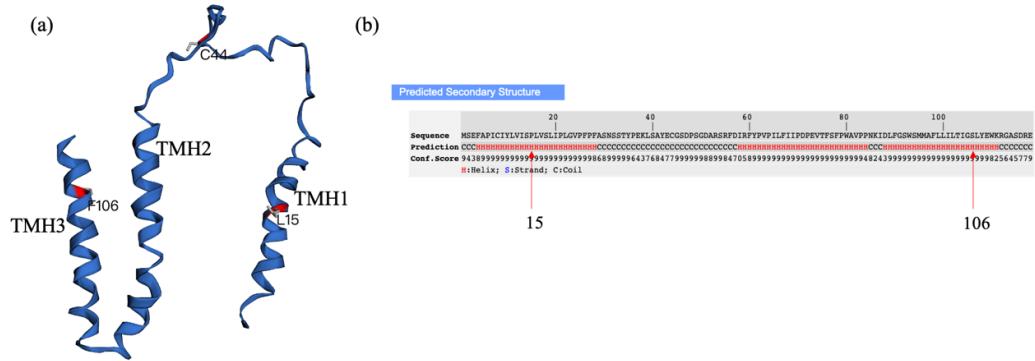


Figure S7. Predicted protein structure of NAD3 in the WT. **(a)** The 3D structure was analyzed according to its homology with c6humC_. The L15 and F106 has already been edited, C44 is the important amino acid to access for chemical modification in only the D-form. **(b)** the predicted secondary structure was analyzed by I-TASSER. The amino acids need to be edited is numbered.