

Article title: **Pentatricopeptide repeat protein DEK40 is required for mitochondrial function and kernel development in maize**

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Supplementary Fig. S1-S5

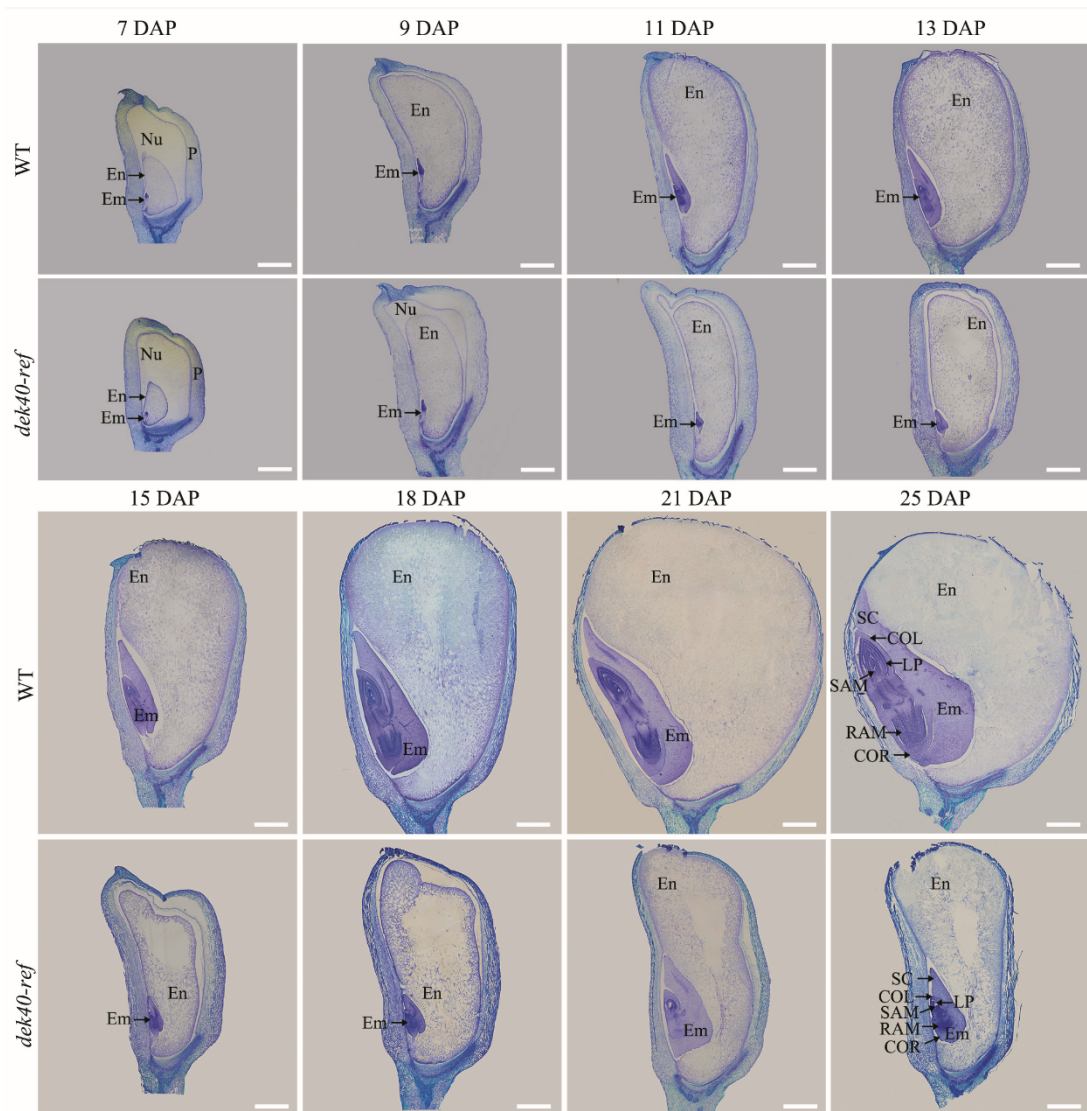


Fig. S1 Paraffin sections analysis of WT and *dek40-ref* kernels at different developmental stages

En, endosperm; Em, embryo; Nu, nucellus; P, pericarp; LP, leaf primordia; RAM, root apical meristem; SAM, shoot apical meristem; SC, scutellum; COL, coleoptile; COR, coleorhiza. Scale bars, 1 mm.

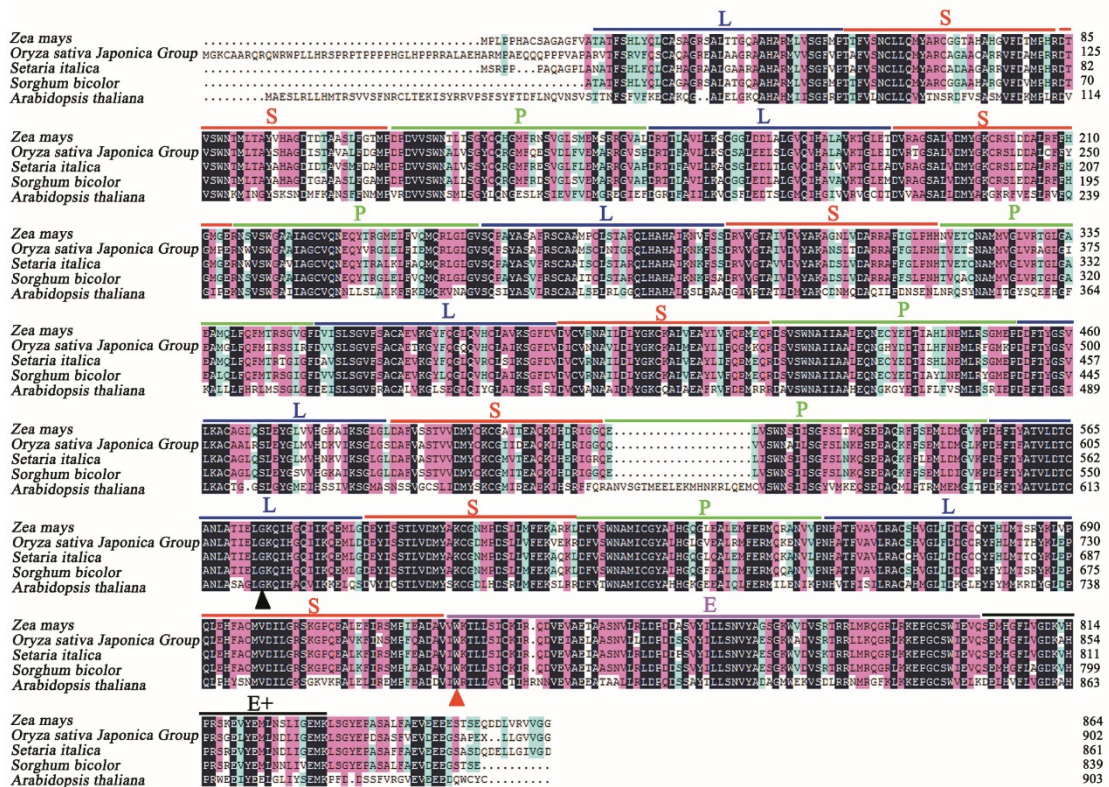


Fig. S2 Amino acids alignment of maize DEK40 with homologous PPR proteins in other plant species

PPR elements are indicated. S type is indicated by red line; L type is indicated by blue line; P type is indicated by green line; E domain is indicated by purple line; E+ domain is indicated by black line. The mutant sites in *dek40-ref* and *dek40-1* are indicated by black arrowhead and red arrowhead, respectively.

Arabidopsis thaliana, sequence ID NP_186882.2; *Setaria italica*, sequence ID XP_012698197.1; *Oryza sativa Japonica Group*, sequence ID XP_015612764.1; *Sorghum bicolor*, sequence ID XP_002441797.1; *Zea mays*, sequence ID XP_008662428.1. All data obtained from the NCBI (<https://www.ncbi.nlm.nih.gov/>).

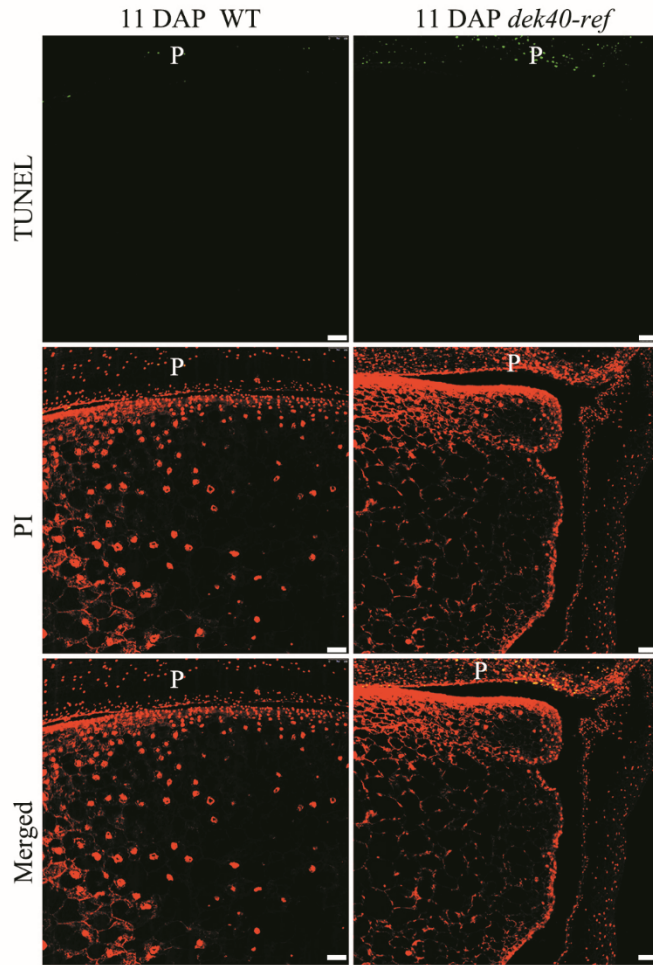


Fig. S3 Nuclear DNA fragmentation was detected in WT and *dek40-ref* endosperm at 11 DAP by TUNEL assay

The green is the TUNEL signals. The red is PI indicated nuclei. The yellow is merged of TUNEL detected nuclei and PI detected nuclei. P, pericarp. Scale bars, 100 μ m.

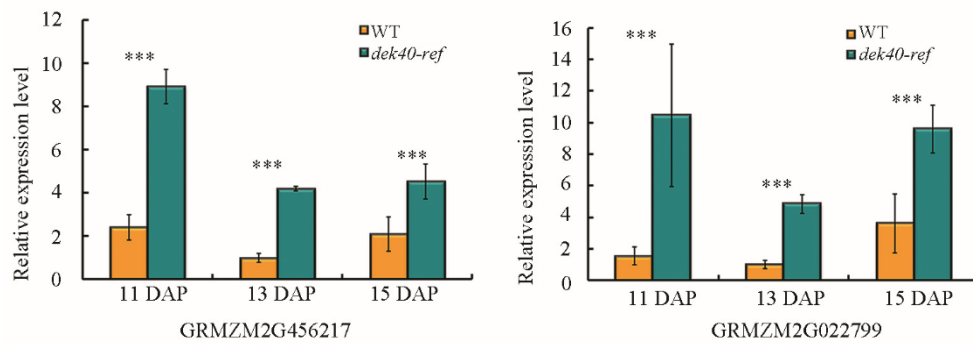


Fig. S4 The expression levels of PCD associated genes

The expression levels of PCD associated genes (Cysteine proteinase gene, GRMZM2G456217; Metacaspase gene, GRMZM2G022799) in WT and *dek40-ref* at 11, 13 and 15 DAP. Values and bars represent the mean and standard deviation of three biological replicates, respectively. The RNA levels were normalized to that of maize *ZmActin* gene (GRMZM2G126010). Significant differences are indicated. $**P < 0.01$, $***P < 0.001$ (Student's t-test).

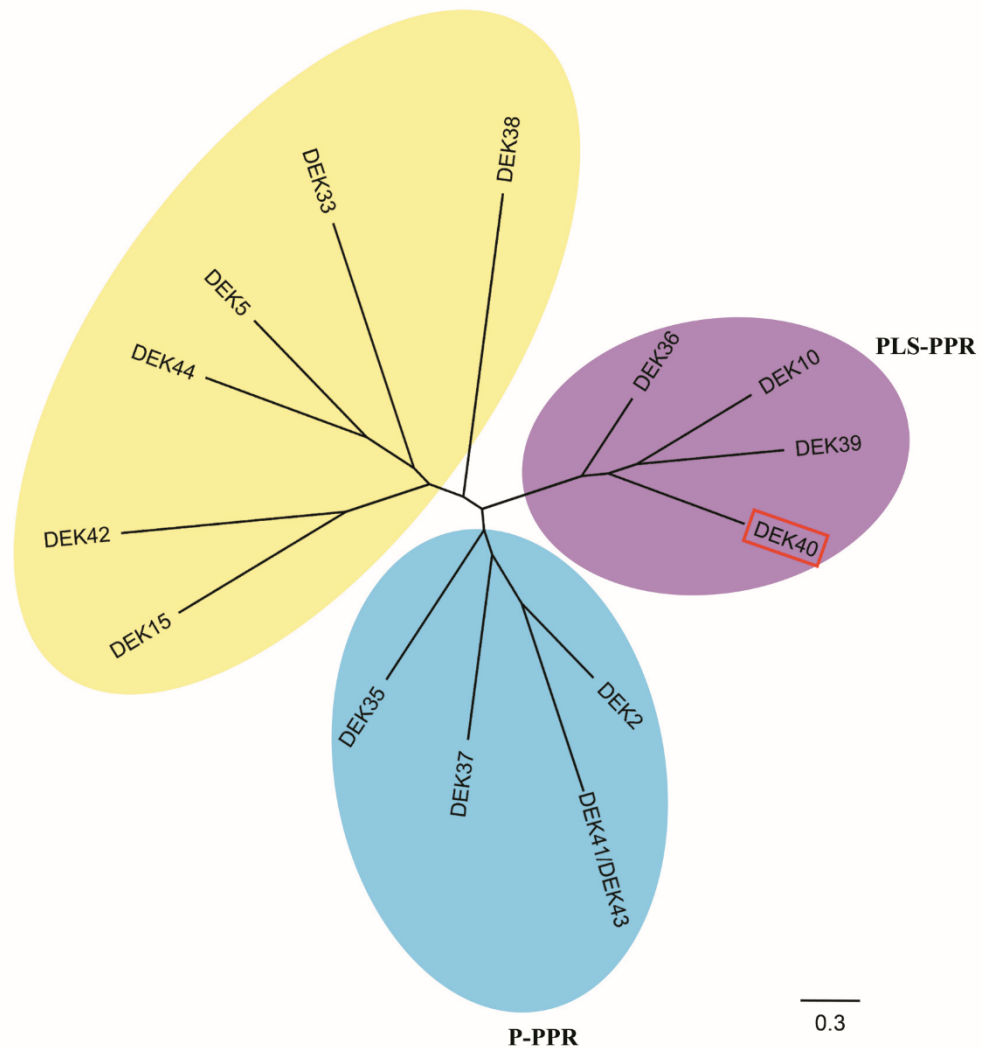


Fig. S5 Phylogenetic relationships between the DEK40 and other maize DEK proteins.

Several *Dek* genes involved in maize kernel development had been reported in previous studies. *Dek2* encodes a P-type PPR protein (Qi *et al.*, 2017b). *Dek5* encodes a protein homologous to rice SUBSTANDARD STARCH GRAIN4 (SSG4) and *E.coli* tamB (Zhang *et al.*, 2019). *Dek10* encodes an E subgroup PPR protein (Qi *et al.*, 2017a). *Dek15* encodes a homolog of SISTER CHROMATID COHESION PROTEIN 4 (SCC4) (He *et al.*, 2019). *Dek33* encodes the pyrimidine reductase in riboflavin biosynthesis (Dai *et al.*, 2019). *Dek35* encodes a P-type PPR protein (Chen *et al.*, 2017). *Dek36* encodes an E+ subgroup PPR protein (Wang *et al.*, 2017). *Dek37* encodes a P-type PPR Protein (Dai *et al.*, 2018). *Dek38* encodes a TTI2

(Tel2-interacting protein 2) molecular cochaperone (Garcia *et al.*, 2017). *Dek39* encodes an E subclass PPR protein (Li *et al.*, 2018). *Dek41/Dek43* encodes a P-type PPR protein (Zhu *et al.*, 2019). *Dek42* encodes an RRM_RBM48 type RNA-binding protein (Zuo *et al.*, 2019). *Dek44* encodes a putative 50S ribosomal protein L9 (Qi *et al.*, 2019). The purple part is PLS-type PPR proteins (PLS-PPR), the light blue part is P-type PPR proteins (P-PPR), the yellow part is other types proteins. DEK40 is indicated in red box. Bar, 0.3.

References

- Chen X, Feng F, Qi W, Xu L, Yao D, Wang Q, Song R.** 2017. *Dek35* encodes a PPR protein that affects *cis*-splicing of mitochondrial *nad4* intron 1 and seed development in maize. *Molecular Plant* **10**, 427-441.
- Dai D, Luan S, Chen X, Wang Q, Feng Y, Zhu C, Qi W, Song R.** 2018. Maize *Dek37* encodes a P-type PPR protein that affects *cis*-splicing of mitochondrial *nad2* intron 1 and seed development. *Genetics* **208**, 1069-1082.
- Dai D, Tong H, Cheng L, Peng F, Zhang T, Qi W, Song R.** 2019. Maize *Dek33* encodes a pyrimidine reductase in riboflavin biosynthesis essential for oilbody formation and ABA biosynthesis during seed development. *Journal of Experimental Botany* doi: 10.1093/jxb/erz268
- Garcia N, Li Y, Dooner HK, Messing J.** 2017. Maize *defective kernel* mutant generated by insertion of a *Ds* element in a gene encoding a highly conserved TTI2 cochaperone. *Proceedings of the National Academy of Sciences of the United States of America* **114**, 5165-5170.
- He Y, Wang J, Qi W, Song R.** 2019. Maize *Dek15* encodes the cohesin-loading complex subunit SCC4 and is essential for chromosome segregation and kernel development. *The Plant Cell* **31**, 465–485.
- Li X, Gu W, Sun S, Chen Z, Chen J, Song W, Zhao H, Lai J.** 2018. *Defective Kernel 39* encodes a PPR protein required for seed development in maize. *Journal of Integrative Plant Biology* **60**, 45-64.
- Qi W, Lu L, Huang S, Song R.** 2019. Maize *Dek44* encodes mitochondrial ribosomal

protein L9 and is required for seed development. *Plant Physiology* doi: 10.1104/pp.19.00546

Qi W, Tian Z, Lu L, Chen X, Chen X, Zhang W, Song R. 2017a. Editing of mitochondrial transcripts *nad3* and *cox2* by Dek10 is essential for mitochondrial function and maize plant development. *Genetics* **205**, 1489-1501.

Qi W, Yang Y, Feng X, Zhang M, Song R. 2017b. Mitochondrial function and maize kernel development requires Dek2, a pentatricopeptide repeat protein involved in *nad1* mRNA splicing. *Genetics* **205**, 239-249.

Ren RC, Wang LL, Zhang L, Zhao YJ, Wu JW, Wei YM, Zhang XS, Zhao XY. 2019. DEK43 is a P-type PPR protein responsible for the *cis*-splicing of *nad4* in maize mitochondria. *Journal of Integrative Plant Biology* doi: 10.1111/jipb.12843

Wang G, Zhong M, Shuai B, Song J, Zhang J, Han L, Ling H, Tang Y, Wang G, Song R. 2017. E+ subgroup PPR protein *defective kernel 36* is required for multiple mitochondrial transcripts editing and seed development in maize and Arabidopsis. *New Phytologist* **214**, 1563-1578.

Zhang J, Wu S, Boehlein SK, McCarty DR, Song G, Walley JW. 2019. *Maize defective kernel5* is a bacterial TamB homologue required for chloroplast envelope biogenesis. *Journal of Cell Biology* doi: 10.1083/jcb.201807166

Zhu C, Jin G, Fang P, Zhang Y, Feng X, Tang Y, Qi W, Song R. 2019. Maize pentatricopeptide repeat protein DEK41 affects *cis*-splicing of mitochondrial *nad4* intron 3 and seed development. *Journal of Experimental Botany* doi: 10.1093/jxb/erz193

Zuo Y, Feng F, Qi W, Song R. 2019. *Dek42* encodes an RNA binding protein that affects alternative pre-mRNA splicing and maize kernel development. *Journal of Integrative Plant Biology* **61**, 728-748.