

Fig.S1. The *Mu* insertion in *Emp12* links with the empty pericarp phenotype in the *emp12-673* allele.

Individual seedlings of *Emp12* heterozygotes and WT were differentiated by using *Emp12* specific primers of EMP12-R and *Mu*-TIR primer TIR8. PCR products at 653 bp are indicative of a *Mu* insertion at 673 bp (*emp12-673*) downstream of the ATG of *Emp12*. The *emp12* phenotype was observed in the cob after selfing. “N”: non-segregating WT plants; “S”: segregating heterozygous *Emp12/emp12* plants.

EMP12	1	:	MLELVRRHR-RLALPFSS--LHA-PAPA----PAPTL
Sb06g030430	1	:	MLELVRRHR-RLALPFST--LPA-PAPASNPTPTETP
LOC_Os04g55090	1	:	MISLFR--R-RHGLPFST--LHT-PAGD----VAATP
AT1G80550	1	:	MLLLRRLNRVRIASPYSVRLLSVKPIISNVDDAKFRSQ
EMP12	69	:	PTAATLARAVDILGKHFEFPLATSL---LLSHHDPG-
Sb06g030430	73	:	PTAATLARAVDILGKHFEFPLATSL---LLSHHDEE-
LOC_Os04g55090	67	:	PTPATVARAVDVLGKHFEFPLATSL---LVSHHDPGR
AT1G80550	79	:	HTTETFNRVIDILGKYFEFEISWALINRMIGNTESV-
EMP12	144	:	SFHLLVDALCDHRRVDEADHLCFGKD--PPPF--PGT
Sb06g030430	148	:	SFHLLVDALCDHRRVDEADHLCFGKD--PPPF--PGT
LOC_Os04g55090	144	:	SFHLLVDALCDHRRVDEAHHLFCFGKD--PPPF--EVT
AT1G80550	153	:	SEYNLVDALCEHKKHVEAEELFCFGKNVIGNGEFSVSN
EMP12	221	:	ALAKSGKPWKAFFKVFKEKQKRGIRIDTVAYNTAIHAV
Sb06g030430	225	:	ALAKSGKPWKTFFKVFKEKQKGVRIIDTVAYNTAIHAV
LOC_Os04g55090	221	:	ALAKSGKPWKAFFKVFKEKQKGMALDVMAYNTAIHVS
AT1G80550	233	:	IMCKSGKPWKAFFKLYKEMKSRRMKLDVMAYNTVIRAI
EMP12	301	:	GYAFVQQMRKTGCKPDVLTYHCFQYLSRPQEVVLGLF
Sb06g030430	305	:	GYAFVQQMHTGCKPDVLTYHCFQYLSRPQEVVLGLF
LOC_Os04g55090	301	:	GYAFVQQMHKFGTEPNVLTYHCFQYLSRPQEVVLGLF
AT1G80550	313	:	AYRMLDEMPKRGCCQPSITYMCLFSRLEKPKSEILSLF
EMP12	381	:	SPDAFAYNALIDALLQKGMVDLARKYDEKMLAKGLSP
Sb06g030430	385	:	SPDAFAYNALIDALLQKGMVDLARKYDEEMLAKGLSP
LOC_Os04g55090	381	:	SPDAFAYNSLIDALLQKGMVDLARKYDEEMLSKGLSP
AT1G80550	393	:	TPDSAAYNAVIDALIQKGMVDMAREYEEMIERGLSP

Fig.S2. The amino acid alignment of EMP12 homologs.

The amino acid sequences of EMP12 homologs were retracted from the phytozyme database (<https://phytozome.jgi.doe.gov/pz/portal.html>). The maize EMP12 was compared with the homologous sequences of the monocotyledonous sorghum (Sb06g030430), rice (LOC_Os04g55090) and the dicotyledonous Arabidopsis (AT1G80550).

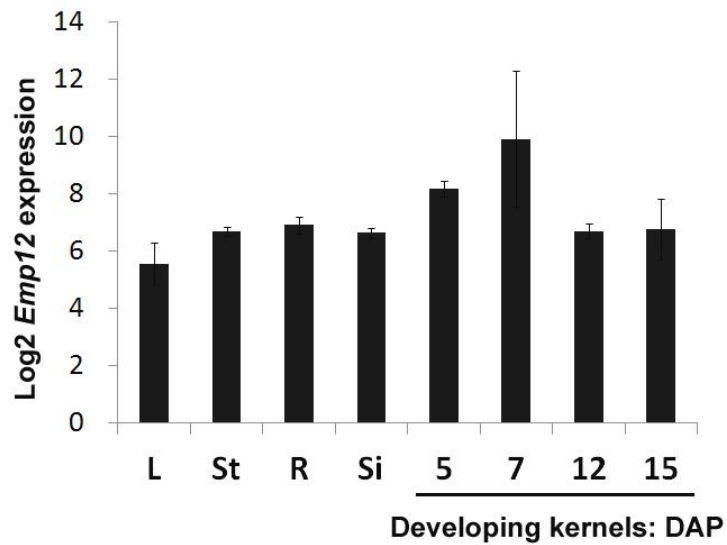


Fig.S3. Quantitative RT-PCR analysis of *Emp12* expression in different tissues and kernels at different developing stages.

The tissues of maize, including leaf (L), stem (St), root (R), silk (Si) were collected from a maize plant pending for pollination. Total RNA was extracted from at least three replicates and transcribed for gene expression analysis. *Ubiquitin* was used as an internal control. DAP, day after pollination.

A

PPR motif	P	P	P	P	P	P	P	P	P	P	Position
	A	A	R	H	N	S	N	N	V	N	6
	L	F	D	G	D	D	N	D	D	K	1'
<i>nad2</i> intron 1	g	c	a	g	c	u	c	u	u	u	
<i>nad2</i> intron 2	g	u	a	u	c	g	c	u	u	u	
<i>nad2</i> intron 4	g	c	a	g	u	g	c	u	u	u	

B

PPR motif	P	P	P	P	P	P	P	P	Position
EMP16	N	N	N	T	S	S	G	N	6
	T	D	T	N	N	D	H	D	1'
<i>nad2</i> intron 4	c	u	c	g	a	u	g	u	

Fig. S4. Predicted binding sites of EMP12 and EMP16 in *nad2* introns.

The amino acids at positions 6 and 1' in PPR motifs of EMP12 (A) and EMP16 (B) are predicted according to Cheng *et al.*, 2016. The binding sequence of *nad2* intron 1, intron 2, and intron 4 is determined by the frequencies of co-occurrence of amino acids at positions 6, 1' and nucleotides of introns (Barkan *et al.*, 2012; Takenaka *et al.*, 2013b). The stronger binding of nucleotide combinations is shown by yellow color, the moderate binding is shown by green color, whereas the weak binding is shown in gray green color. "P" stands for classical PPR motifs.

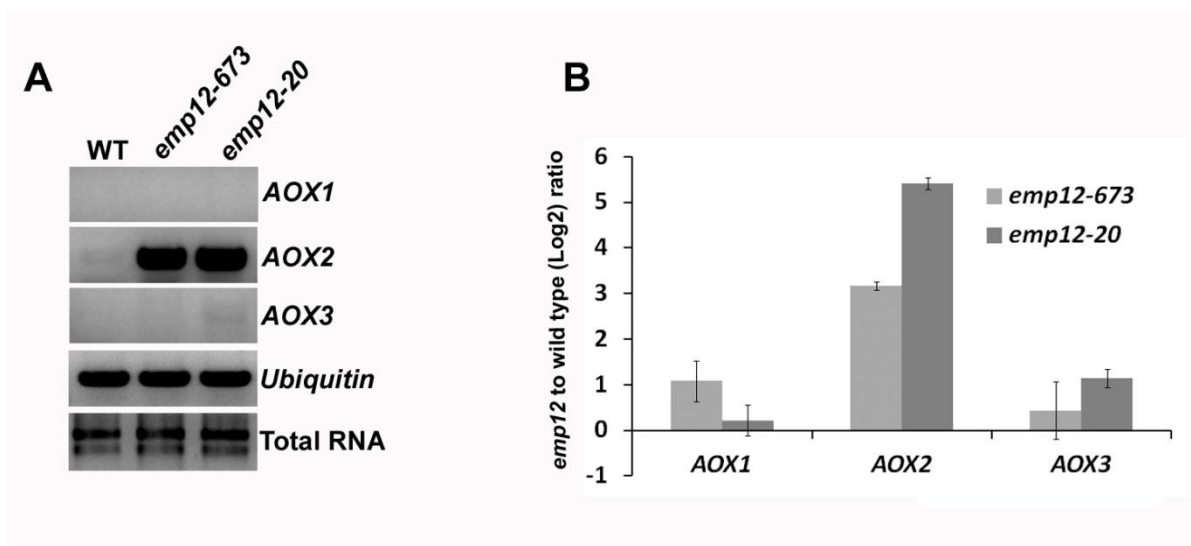


Fig. S5. AOX2 expression is increased in *emp12* mutants.

(A) RT-PCR analysis of the *Alternative Oxidase* (AOX) gene expression of *emp12* mutant kernels at 12 DAP.

(B) Quantitative qRT-PCR analysis of AOX expression of in *emp12* mutant kernels at 12 DAP.

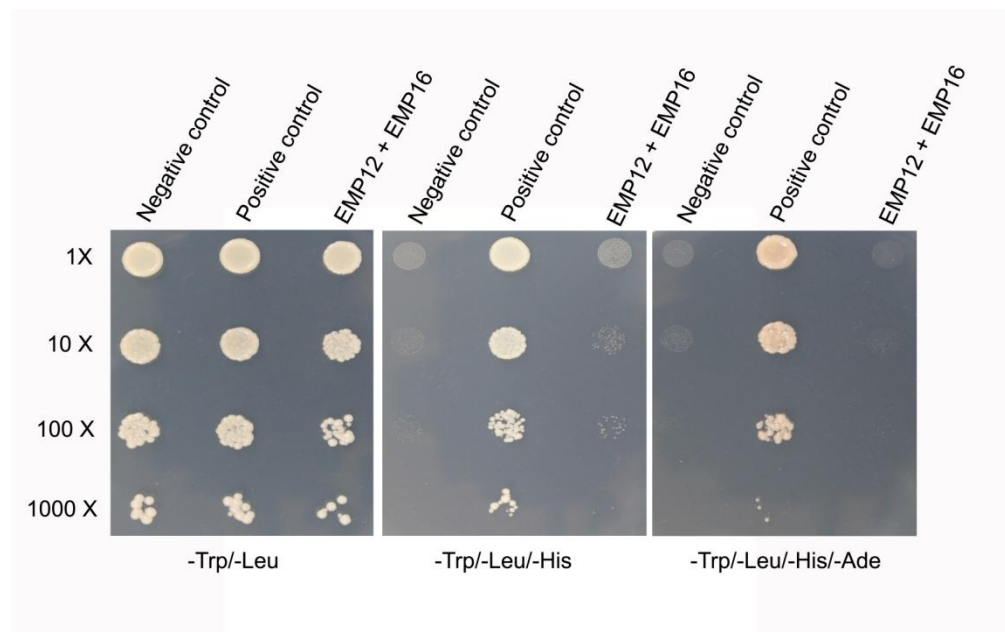


Fig.S6. EMP12 did not interact with EMP16 as demonstrated by yeast two-hybrid assay.

Mature EMP12 and EMP16 without signal peptide were co-transformed and sprayed on dropout medium of DDO (SD/-Leu/-Trp), TDO (SD/-Leu/-Trp/-His) and QDO (SD/-Leu/-Trp/-His/-Ade) . Blank vectors of BD (pGBKT7) and AD (pGADT7) and positive controls were co-transformed according to the manual of Clontech.