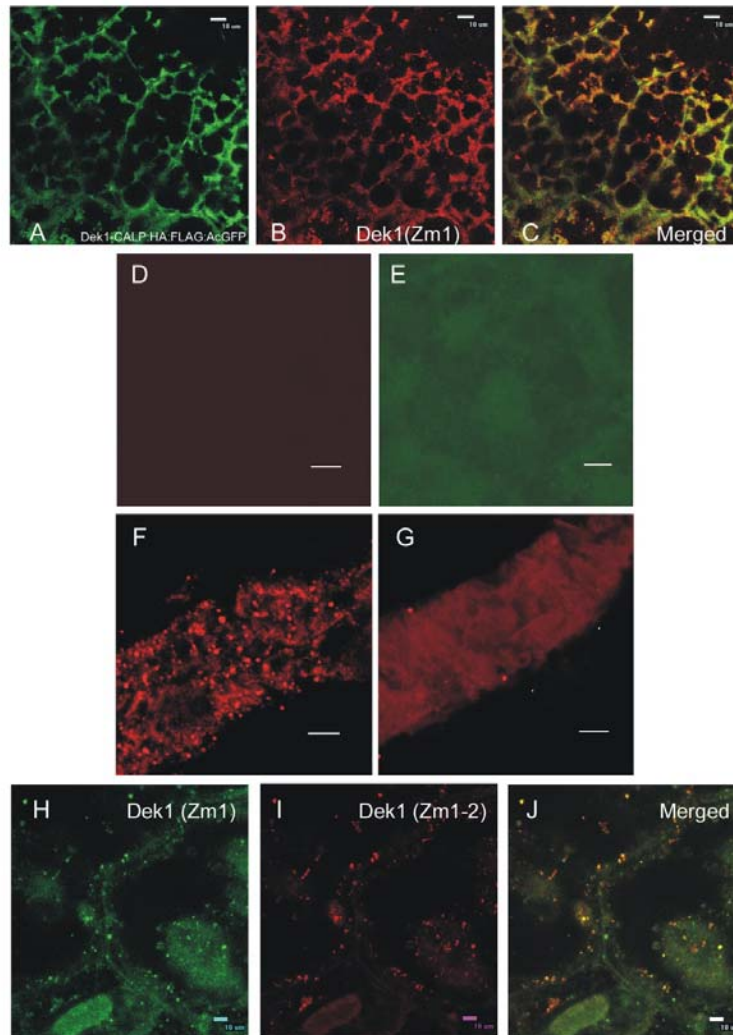


Supplemental Data. Tian et al. 2007. Subcellular localization and functional domain studies of DEFECTIVE KERNEL 1 in maize and *Arabidopsis thaliana* suggest a model for aleurone cell fate specification involving CRINKLY 4 and SUPERNUMERARY ALEURONE LAYER 1



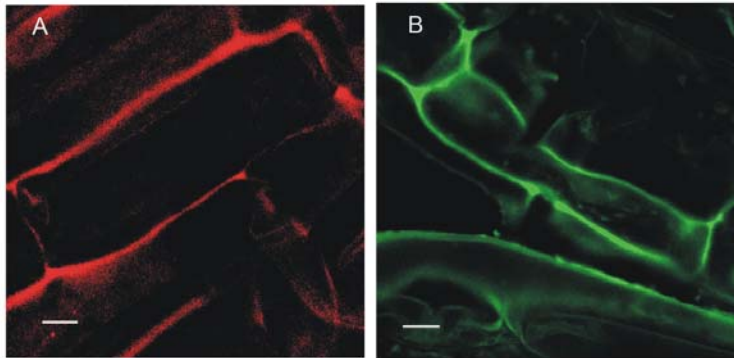
Supplemental Figure 1. Immunostaining of DEK1.

(A)-(C) Immuno-staining of ZmDEK1-CALP:HA:FLAG:AcGFP fusion protein. **(A)** GFP signal in endosperms at 20 DAP is mainly located in the cytosol. **(B)** Immunostaining of the same endosperm section as in (A) with DEK1 Zm1 antibody (red). **(C)** Merged picture of (A) and (B); yellow color shows overlapping staining. Scale=10 μ m

(D)-(E) Negative controls for DEK1 Zm1 antibody staining. **(D)** Staining performed as shown in (B), but omitting the primary antibody and labeled with TRITC-conjugated secondary antibody. **(E)** Staining performed as shown in (D), but with FITC- conjugated secondary antibody. Scale=10 μ m

(F)-(G) Immunolabeling of DEK1 using Zm8 antibodies in the presence of negative control and Zm8 peptides. **(F)** Section of maize in vitro endosperm labeled with Zm8 primary antibody incubated with a negative control peptide (SPNDLK YDFKMDGKL). **(G)** Similar section labeled with the same primary antibody as in (F) but incubated with the Zm8 peptide (DKGLDPNFSYMLKDK). Secondary antibody used in these experiments was donkey anti-rabbit Rhodamine Red. Scale=10 μ m

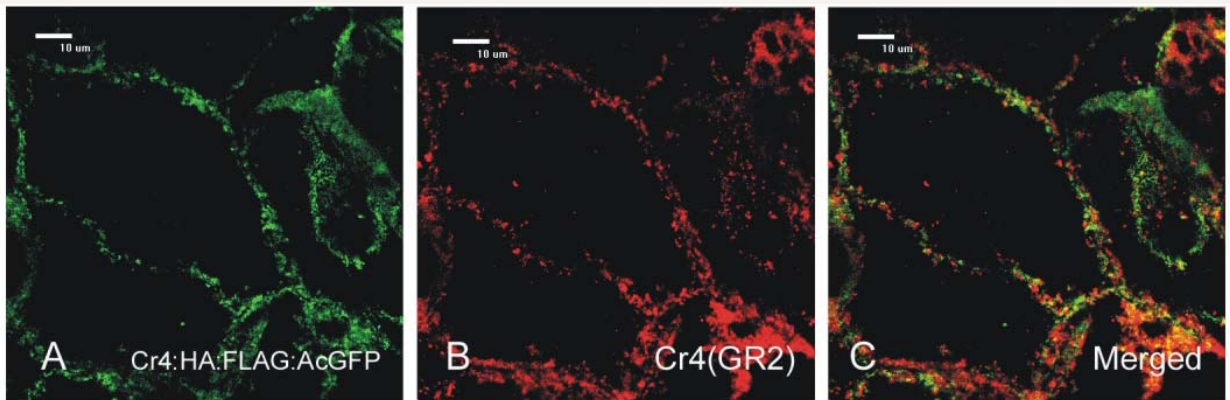
(H)-(J) DEK1 Zm1 peptide antibody raised in rabbits and rats recognize the same subcellular structures. In vitro endosperm section immunostained with DEK1 rabbit antibody Zm1**(H)** and rat antibody Zm1-2 **(I)**. **(J)** Overlapping staining is indicated by yellow structures in the merged picture of **(A)** and **(B)**. Scale=10 μ m



Supplemental Figure 2. Detection of AtDEK1-MEM and AtDEK1-MEM-DEL in *Arabidopsis*.

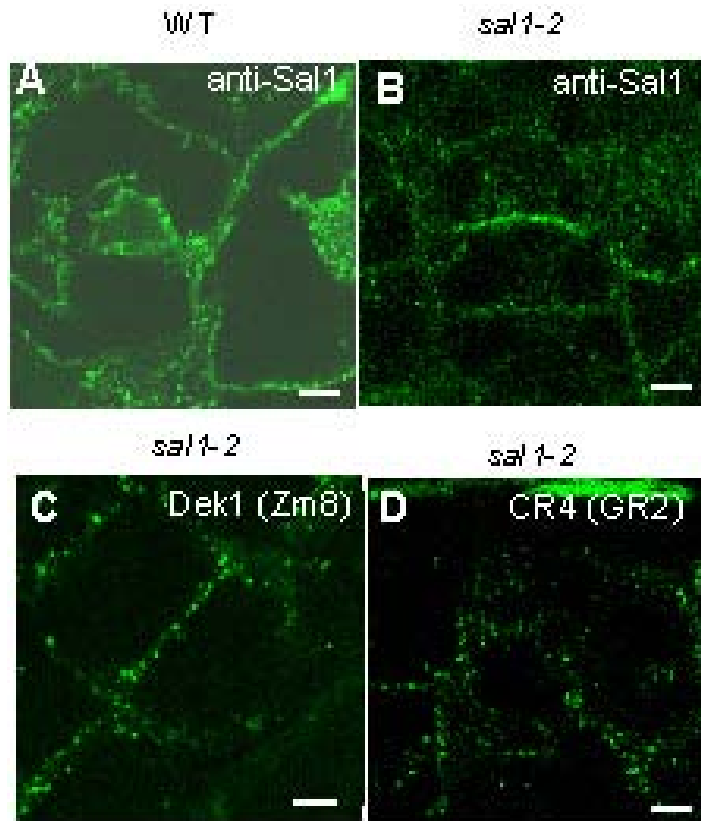
(A) Root cells of a AtDEK1-MEM-GFP expressing plant immunolabeled with anti-GFP conjugated with Alexa594 and excited by a 543 nm HeNe laser. The Alexa594 was used to overcome the strong background fluorescence using the 488 nm Ar laser. Ten transgenic plants were tested with the similar result. Scale=10µm

(B) AtDEK1-MEM-DEL is targeted to the plasma membrane. Root cells of an AtDEK1-MEM-DEL-FLAG expressing plant labeled with anti-FLAG-M2 conjugated with FITC and excited by an Ar-Ion 488 nm laser. Fifteen transgenic plants were tested with similar result. Scale=10µm

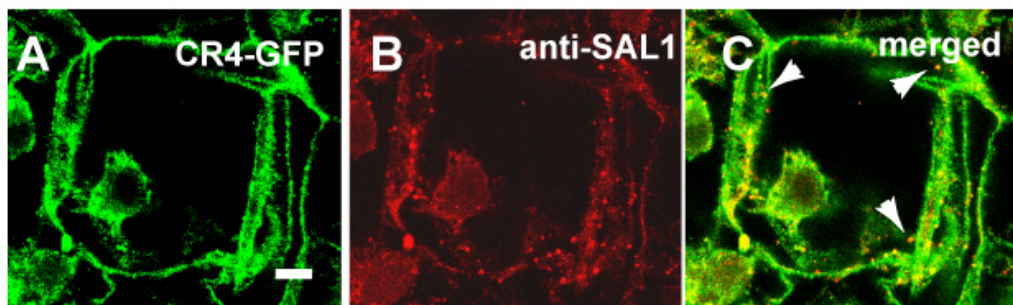


Supplemental Figure 3. CR4 peptide antibody recognizes the CR4:HA:FLAG:AcGFP fusion protein in sections of in vitro endosperm.

(A) GFP signal from 9 DAP CR4:HA:FLAG:AcGFP endosperms on plasma membrane and punctuate cytoplasmic structures. **(B)** Immunostaining of CR4 using GR2 antibody in the same section as in (A). **(C)** Merged picture of (D) and (E). Yellow indicate overlapping staining patterns. Scale= 10 µm.



Supplemental Figure 4. SAL1, DEK1 and CR4 localization in *sal1-2* mutant endosperm. Immunostaining of SAL1 (**A-B**), DEK1 (**C**), and CR4 (**D**) in 9 DAP wild type (WT) (**A**) or *sal1-2* mutant (**B-D**) in vitro endosperms using antibodies as indicated in the figure and FITC-conjugated secondary antibody. Scales=10 µm.



Supplemental Figure 5. Co-localization of CR4 and SAL1 proteins.

(**A-C**) Confocal images of CR4:HA:FLAG:AcGFP transgenic endosperm immunolabeled with anti-SAL1 antibodies. (**A**) GFP fluorescence of CR4:HA:FLAG:AcGFP. (**B**) Same section stained with SAL1 antibodies. (**C**) Merged picture of (**A**) and (**B**) showing SAL1 and CR4 co-localization (arrows).