

Figure S1. Embryo rescue experiment and phenotypic features of developing wild-type and *sh4* kernels. (A) Immature embryo (18 DAP) cultures rescue attempts of WT and *sh4* on Murashige and Skoog (MS) medium. This was the phenotype after 6 days of cultivation. (B) Heterozygote *sh4* ears from 9 to 21 DAP. Arrows show the *sh4* kernels. (C) Comparison of developing wild-type and *sh4* kernels at different stages. Bar = 1 cm.

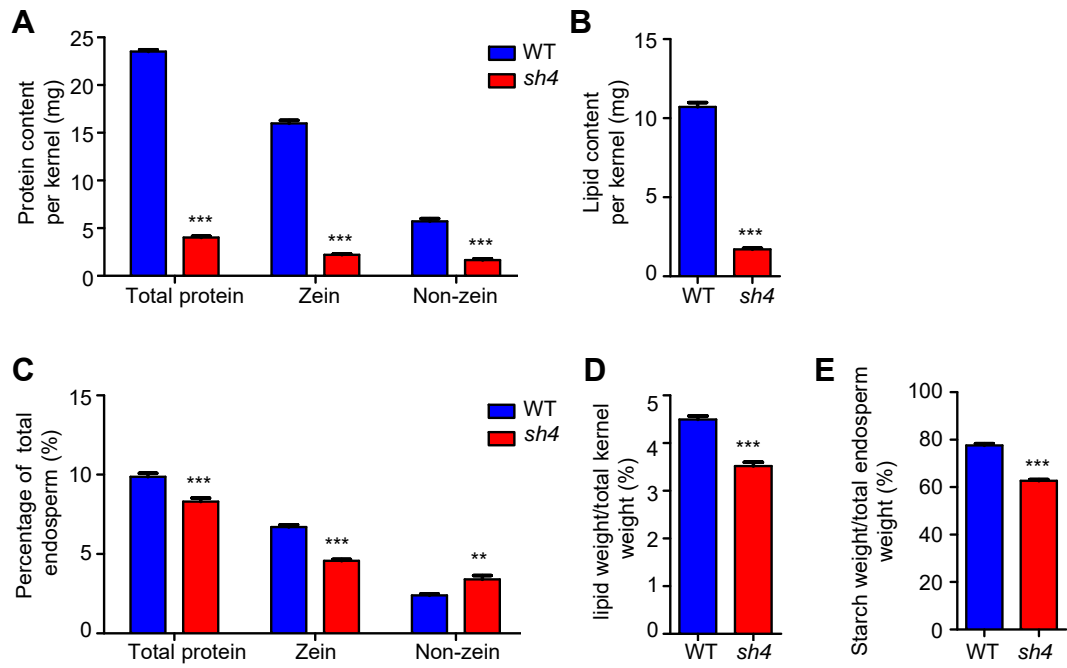


Figure S2. Comparison of protein (total, zein, and nonzein protein) (A), and lipid (B) contents of mature WT and *sh4* endosperm of individual kernels. Comparison of protein (total, zein, and nonzein protein) (C), and lipid (D), and starch (E) contents of mature WT and *sh4* endosperm according to the endosperm weight. Values are means \pm SE; n = 3 (***, P < 0.001; Student's t-test).

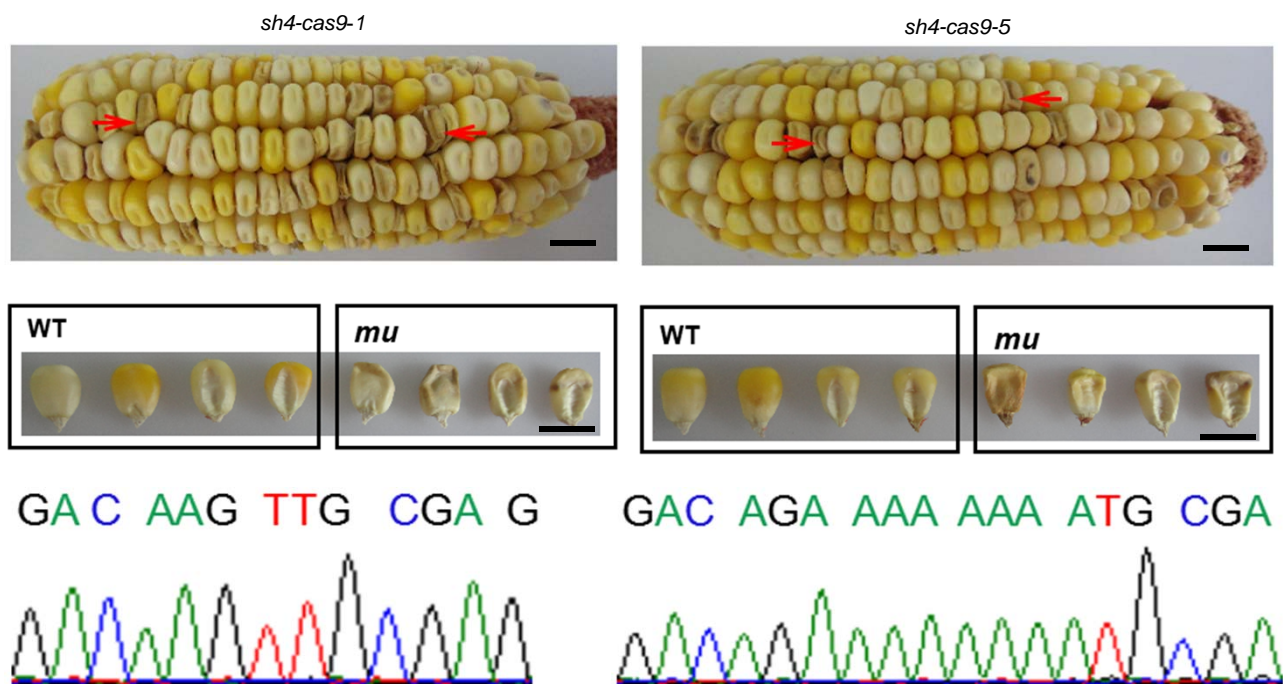


Figure S3. Mature F_2 ear of *sh4-cas9* × W22 line. Arrows show the mutant kernels. Bar = 1 cm.

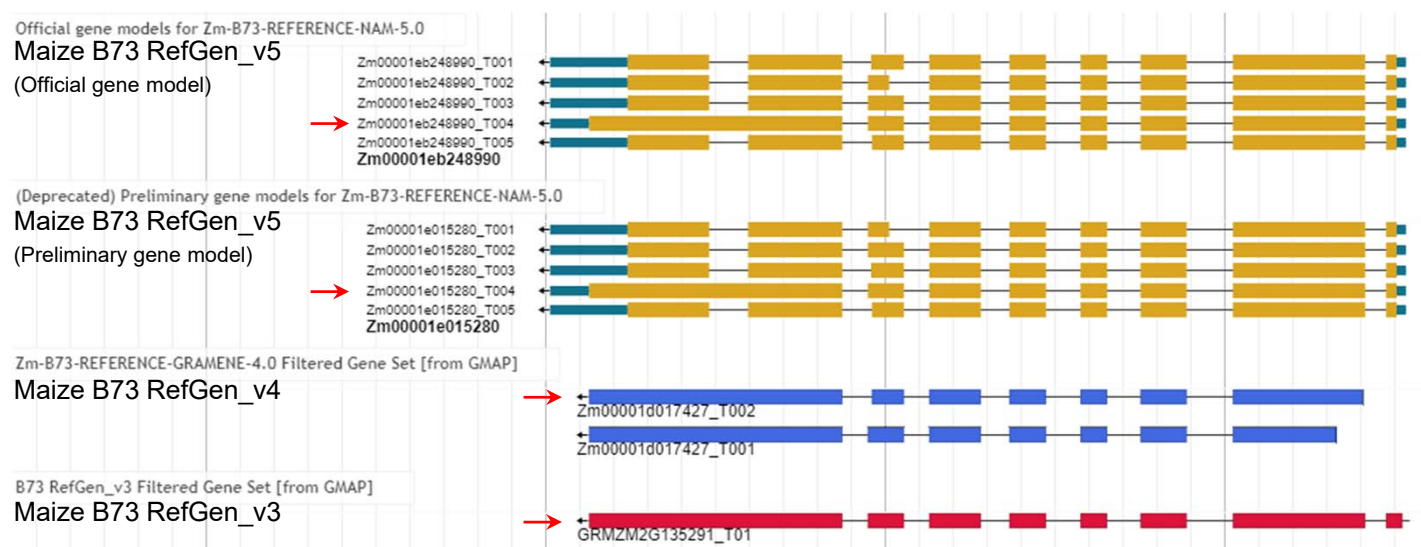


Figure S4. The schematic structure of the *Sh4/ZmYSL2* gene in the maize genome (<https://www.maizegdb.org/>). Arrows indicate canonical transcripts.

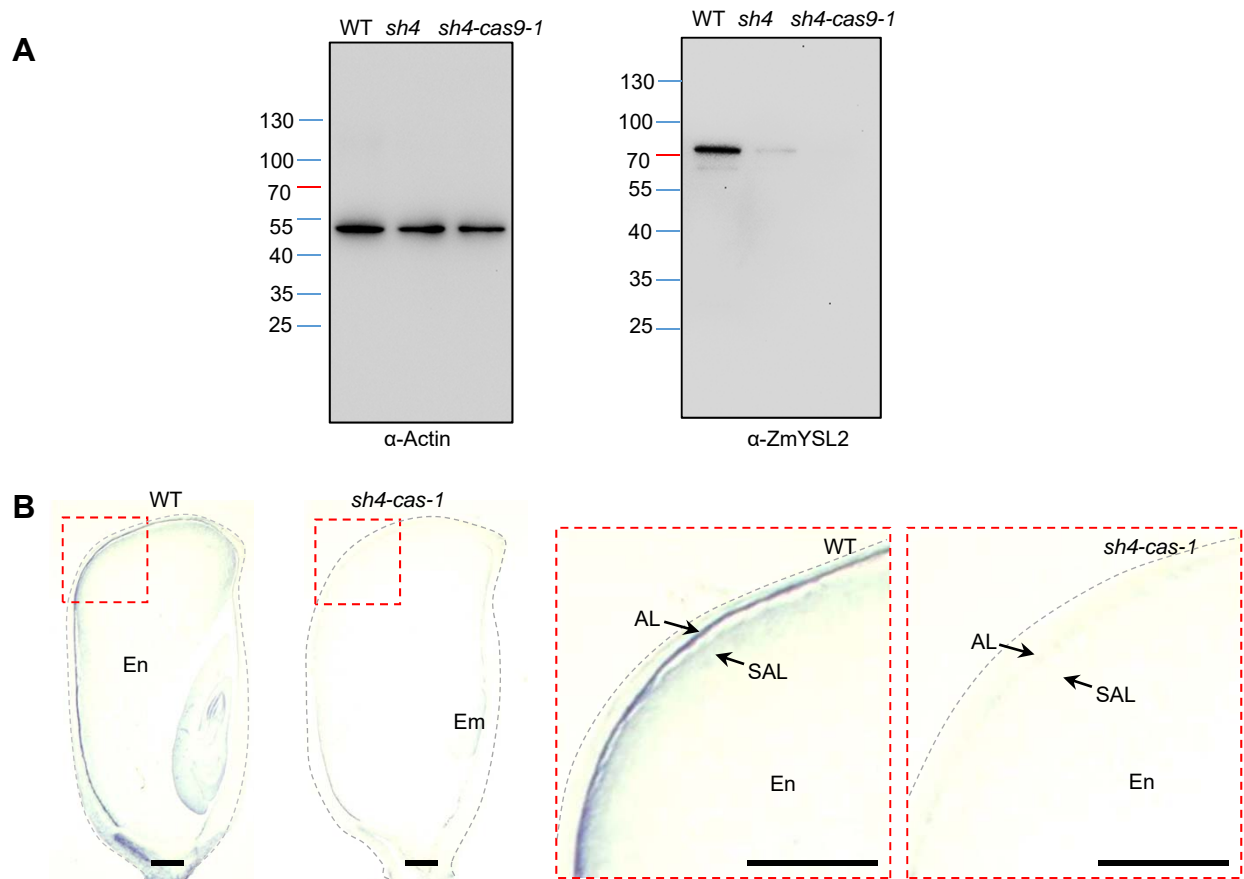


Figure S5. Antibody specificity was detected in the developing kernel. (A) Full images of the immunoblots in Figure 6E. (B) Immunostaining with anti-ZmYSL2 antibody was performed in longitudinal sections of 15 DAP developing WT and *sh4-cas9-1* kernel. Bar = 1 mm. The insets show the enlarged image of boxed regions. Bar = 1 mm.

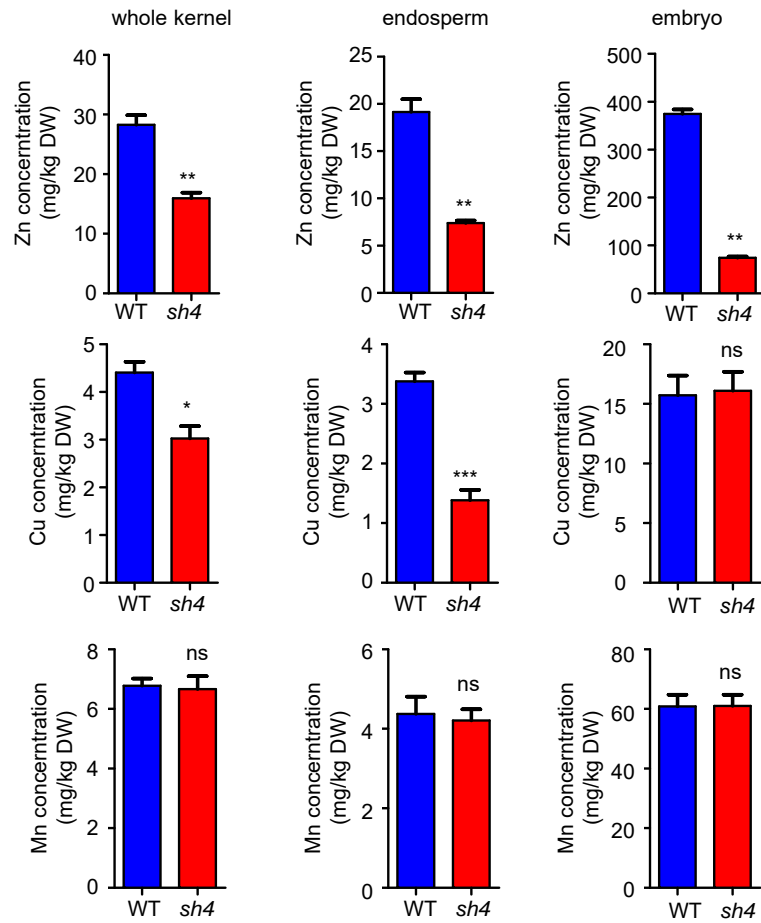


Figure S6. Comparison of the Zn, Cu, and Mn concentration in the whole kernel, endosperm, and embryo of mature WT and *sh4* kernels. Values are means with \pm SE; $n = 3$ (ns, not significant, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; Student's t-test).

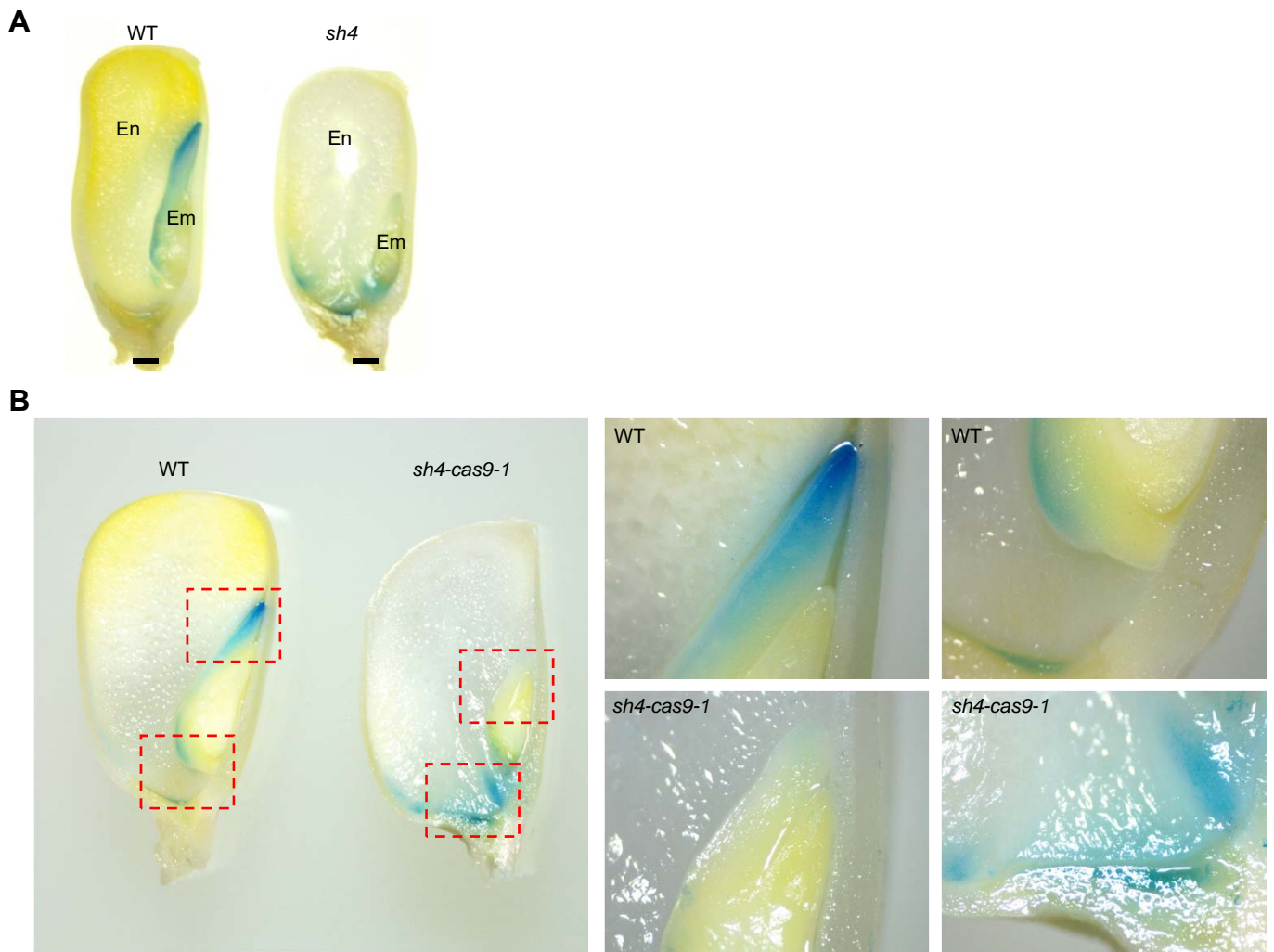


Figure S7. Fe localization in longitudinal sections of WT, *sh4* (A), and *sh4-cas9-1* (B) developing (15 DAP) kernels by Perl's staining. En, endosperm; Em, embryo. Bars = 1 mm.

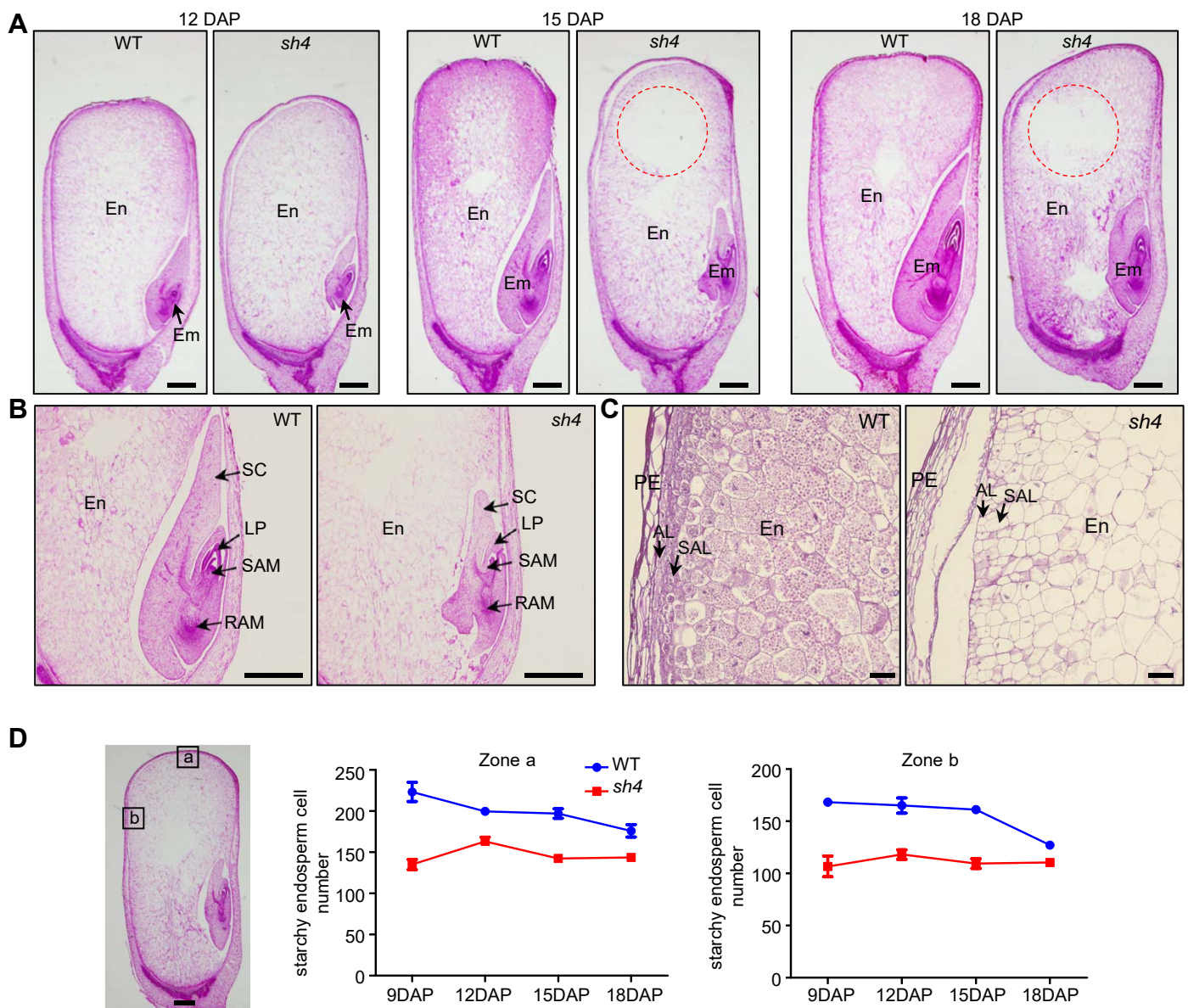


Figure S8. Longitudinal sections of developing WT and *sh4* kernels. (A) Longitudinal sections of developing WT and *sh4* kernels at 12, 15, and 18 DAP. The red circle shows a central cavity in the *sh4* endosperm. En, endosperm; Em, embryo. Bars = 1 mm. (B-C) The magnified images of the embryo (B) and endosperm (C) at 15 DAP in (A). PE, pericarp. Bars = 1 mm in (B); Bars = 100 μ m in (C). (D) Starchy endosperm cell number were counted in two zones (indicated a and b) of the developing wild type and *sh4* kernels. Values are means with \pm SE; n = 3. Bar = 1 mm.

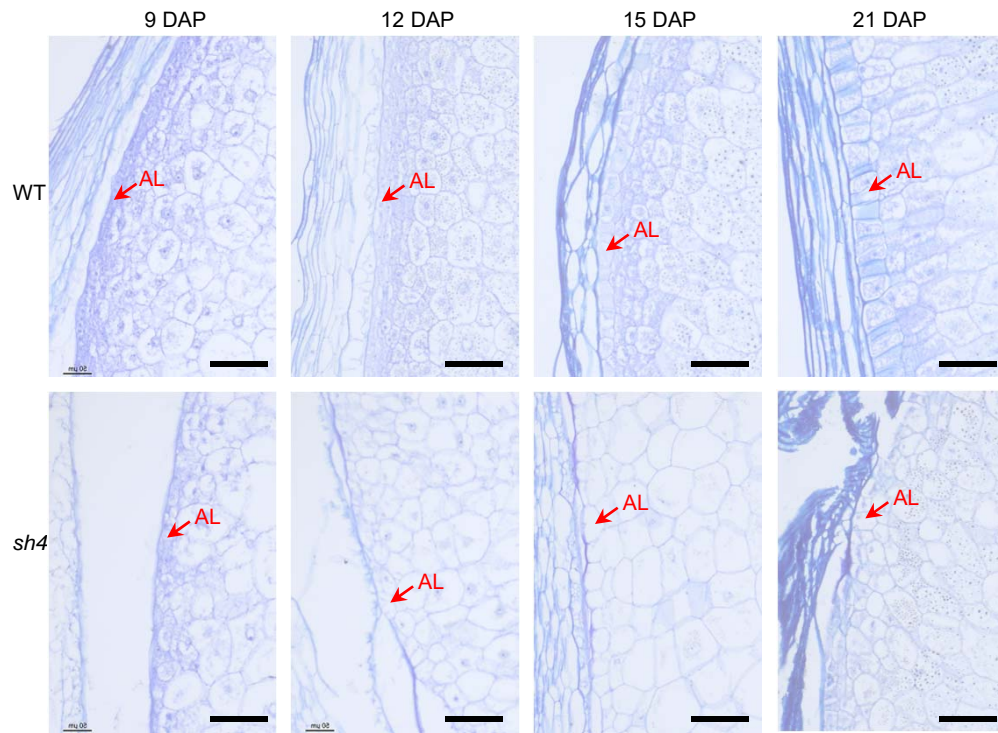


Figure S9. Cell morphology of wild type and *sh4* endosperm aleurone layers at different developmental stages. Bars = 100 μm.