

Figure S1. Double heterozygous mutant siliques contain abnormal, easy-recognized seeds. Seeds from *ca1ca1ca2CA2* or WT siliques were isolated and photographed under binocular microscope (Left panels) and under Scanning Electronic Microscope (SEM, right panels). Double mutant seeds are collapsed.

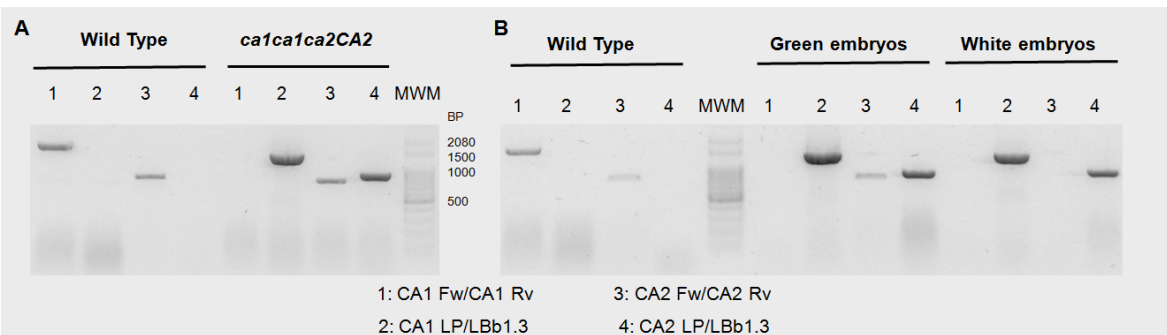


Figure S2. Genotyping of mutant plants and embryos. Genomic DNA was isolated from: **A**, WT or 40 plants of a F2 progeny from crosses between *ca1ca1* and *ca2ca2* plants. In a representative gel, it is shown a sample corresponding to a knockout for the *CA1* gene and heterozygous for the *CA2* gene or **B**, immature embryos (green and white) observed in *ca1ca1ca2CA2* siliques. In a representative gel, it is shown that green embryos correspond to WT or heterozygous for *CA2* gene and that white embryos correspond to double *ca1ca2* knockout mutants. The same results are obtained when genotyping seedlings. Primers pairs were combined in order to detect non-disrupted alleles (1 and 3) and disrupted alleles (2 and 4) as indicated in M & M section.

HAP

48

72

240

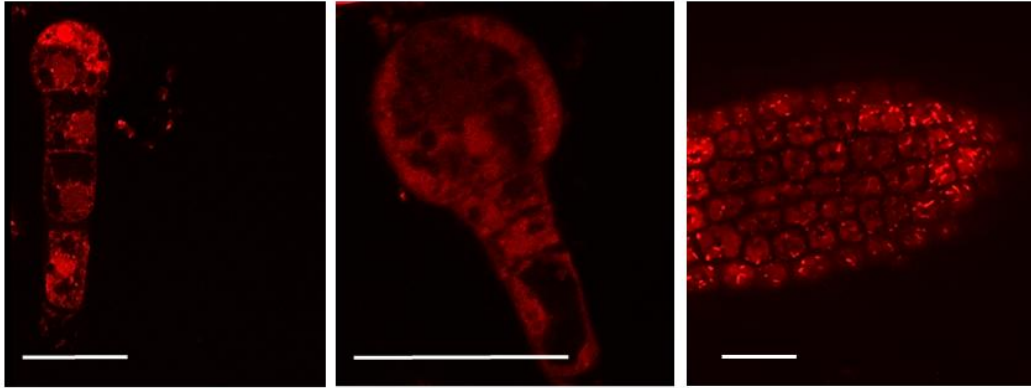


Figure S3. Mitochondrial membrane potential in *ca1ca1CA2CA2* embryos is similar to WT embryos. Flowers were emasculated and manually pollinated. Then, at the indicated times, embryos were extracted from seeds, mounted on slides with TMRM and incubated during 15 minutes. Images were taken on fluorescence confocal microscope, with 40X oil objective. Bars: 25 μ m. HAP: Hours After Pollination.

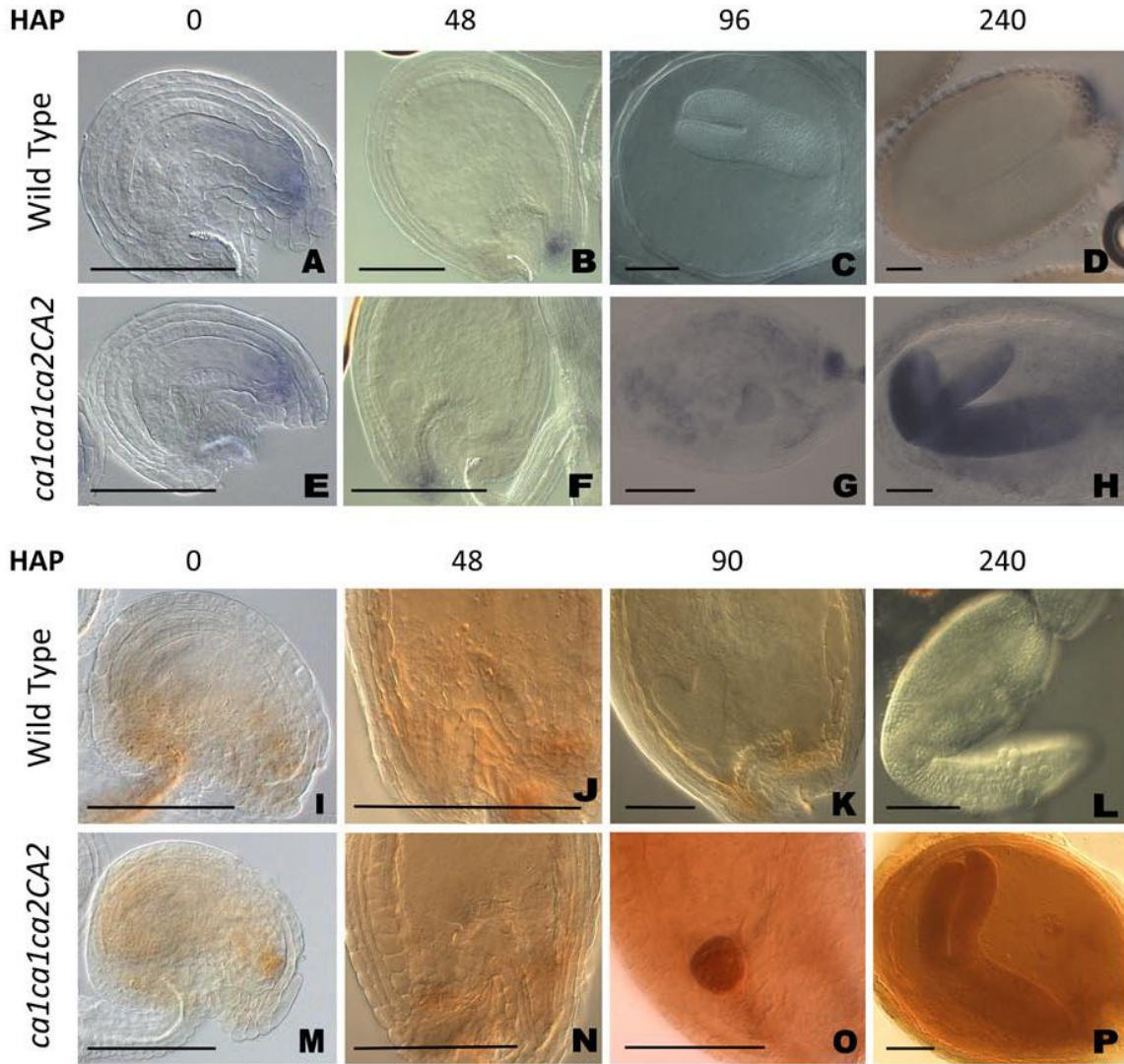


Figure S4. Reactive oxygen species (ROS, peroxide and superoxide) are accumulated in *ca1ca1ca2ca2* delayed embryos. WT and *ca1ca1ca2CA2* flowers were emasculated and manually pollinated. At indicated time, siliques were cutted longitudinally and incubated in Nitroblue tetrazolium (NBT superoxide stain, pictures A to H) or in Diaminobenzidine (DAB peroxide stain, pictures I to P). Images were taken in optical microscope by Nomarsky (DIC) optics. Bars: 50 μ m.

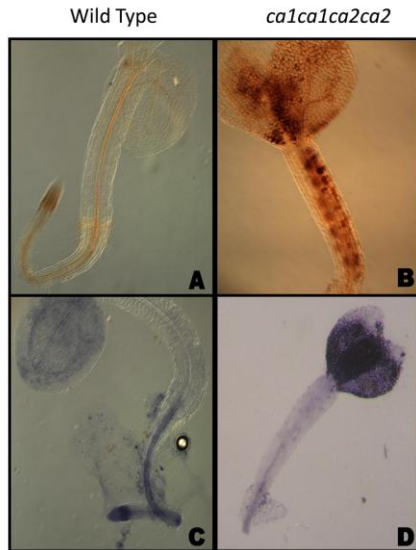


Figure S5. *ca1ca1ca2ca2* late germinating seedlings accumulate high amounts of ROS. WT and *ca1ca1ca2ca2* (wrinkled) seeds were sown as described in Figure S4. Three days (for WT and *ca1ca1ca2CA2*) and 14 days (for *ca1ca1ca2ca2*) after sowing, the seedlings were stained with DAB (to detect hydrogen peroxide, pictures A and B) or NBT (to detect superoxide, pictures C and D).

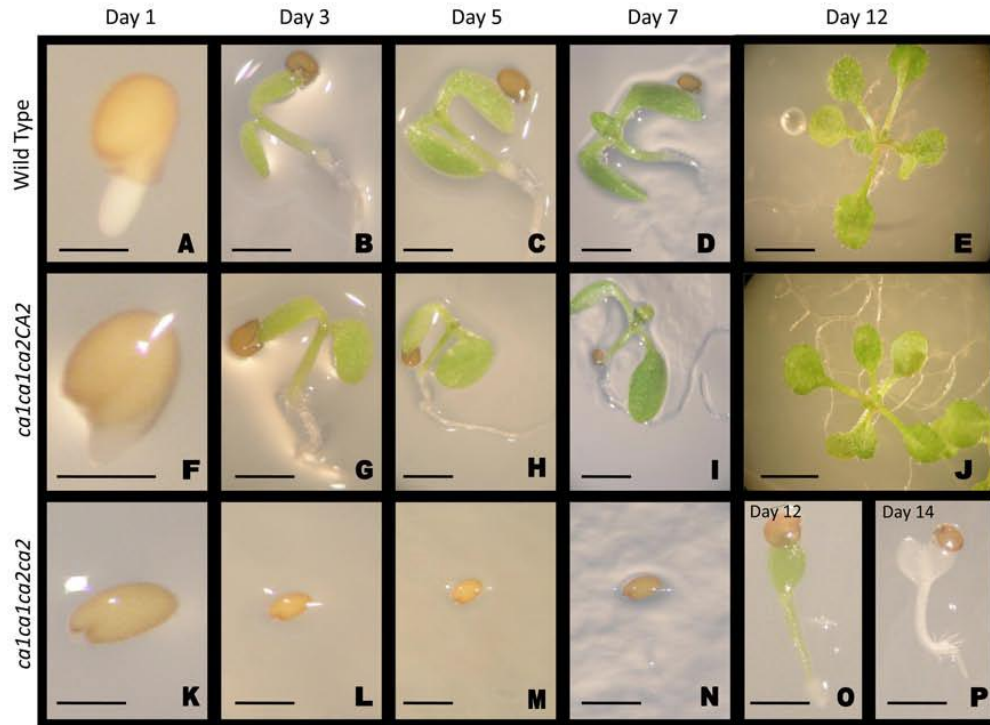


Figure S6. Double knockout *ca1ca2* seeds germinate twelve days later than normal. Wild Type, *ca1ca1ca2CA2* and *ca1ca1ca2ca2* (shriveled) seeds were sown on MS containing Gamborg's vitamins medium, stratified 48 hours at 4°C on darkness and then grown under standard conditions (22°C, 16/8 L/D photoperiod). Images were taken daily until day 14. Bars: A, F and K: 0.5mm. B to E, G to J and L to P: 1mm.

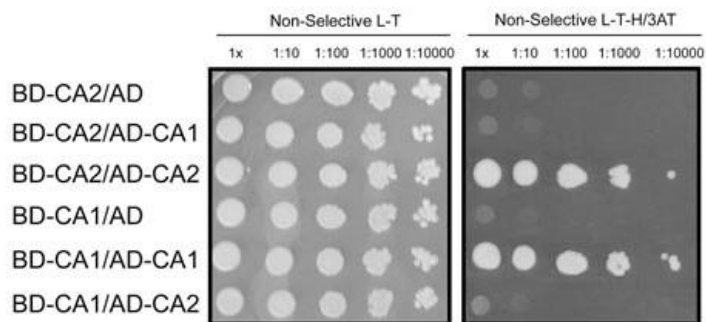


Figure S7. CA1 and CA2 proteins do not interact. The coding sequence of CA1 and CA2 were introduced into the pGBKT7 (containing the binding domain-BD) and pGADT7 (containing the activation domain-AD). These constructs and controls were used to transform the Yeast strain Y190 and let grow at 30 °C for 48 hours in non selective medium until an OD=2.0. After that, 10 ml and serial dilutions were plated on non-selective medium and in selective medium without histidine and containing 3-AT. Plates were incubated for additional time at 30 °C.

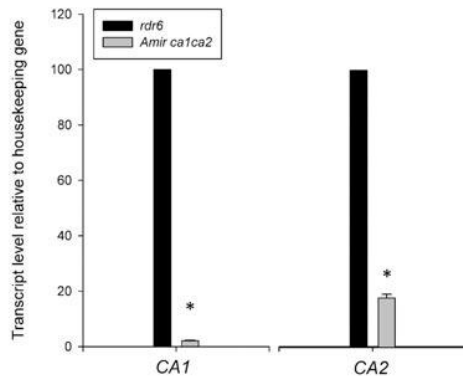
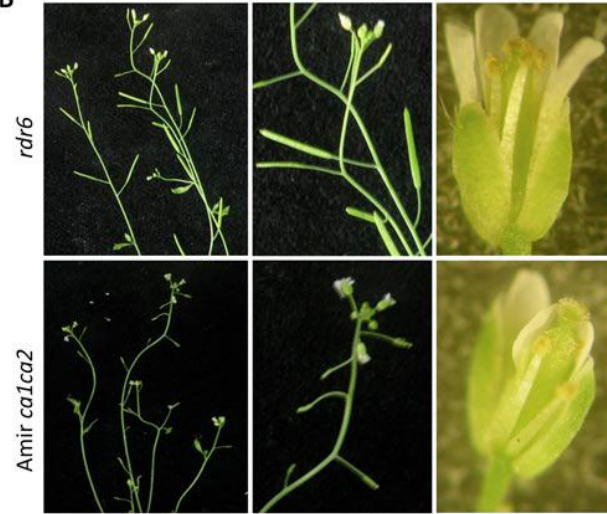
A**B**

Figure S8. *amiRca1ca2* plants show reduced fertility. The *rdr6* background was transformed with two independent artificial microRNAs targeting *CA1* and *CA2* genes. A, Total RNA was then extracted and quantitative PCR assays using specific primers for each gene were performed. Values are normalized using *UBQ5* and *ACT2* as housekeeping genes. Two technical and three biological replicates were used. Asterisks indicate statistically significant differences compared with the WT ($*P \leq 0.001$). B, bolts of transgenic plants showing small siliques. Right panels, flowers at anthesis of transgenic plants showing short stamens in comparison to those of *rdr6* background.