

Additional file 1: Figure S1

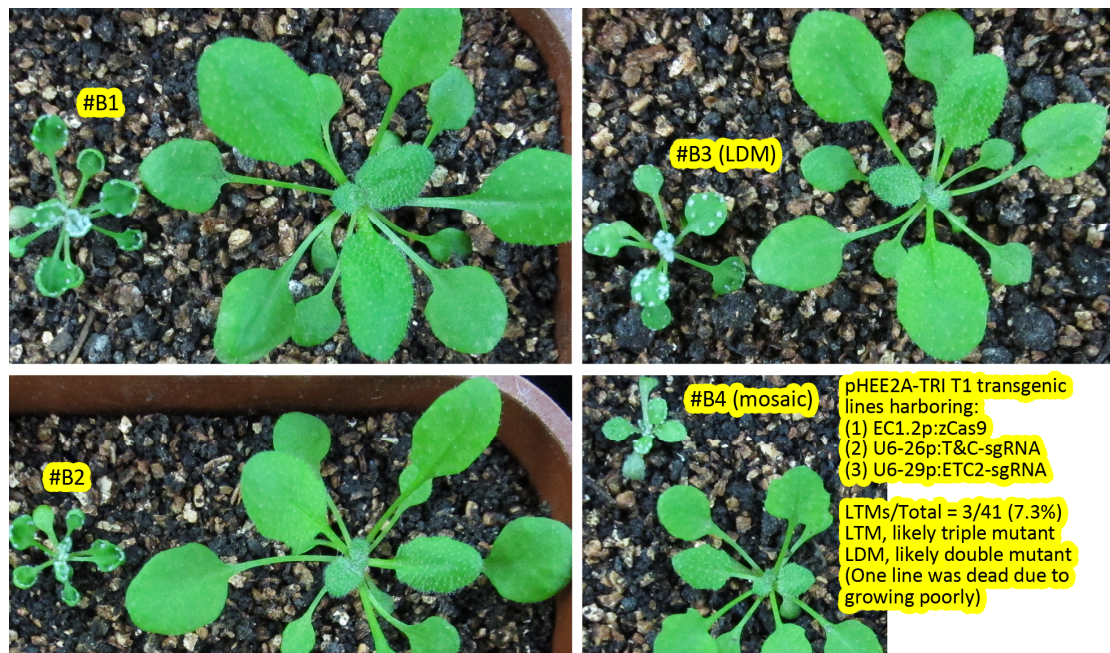


Figure S1. *Arabidopsis* T1 likely triple mutants obtained from the second round of transformation. Seeds from T0 plants were sown on MS medium containing 25 mg/L hygromycin, vernalized at 4°C for 3 days, and grown under long-day conditions (16 h light/8 h dark) at 22°C for 11 days. Hygromycin-resistant seedlings (T1) were transplanted to soil and allowed to grow for 26 days before photographing.

Additional file 2: Figure S2

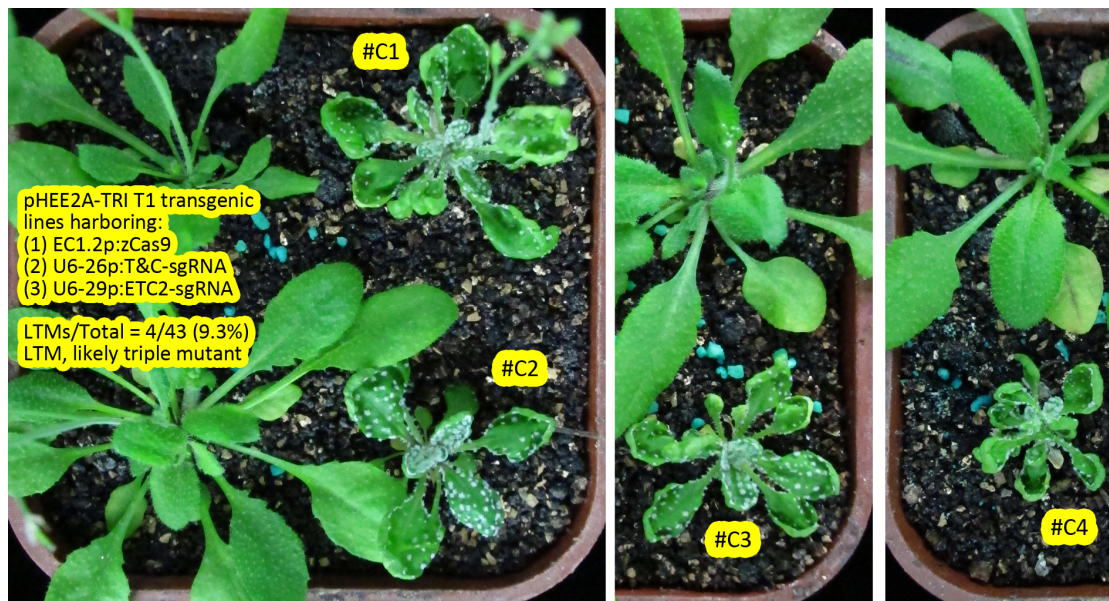


Figure S2. *Arabidopsis* T1 likely triple mutants obtained from the third round of transformation. Seeds from T0 plants were sown on MS medium containing 25 mg/L hygromycin, vernalized at 4°C for 3 days, and grown under long-day conditions (16 h light/8 h dark) at 22°C for 8 days. Hygromycin-resistant seedlings (T1) were transplanted to soil and allowed to grow for 32 days before photographing.

Additional file 2: Figure S3

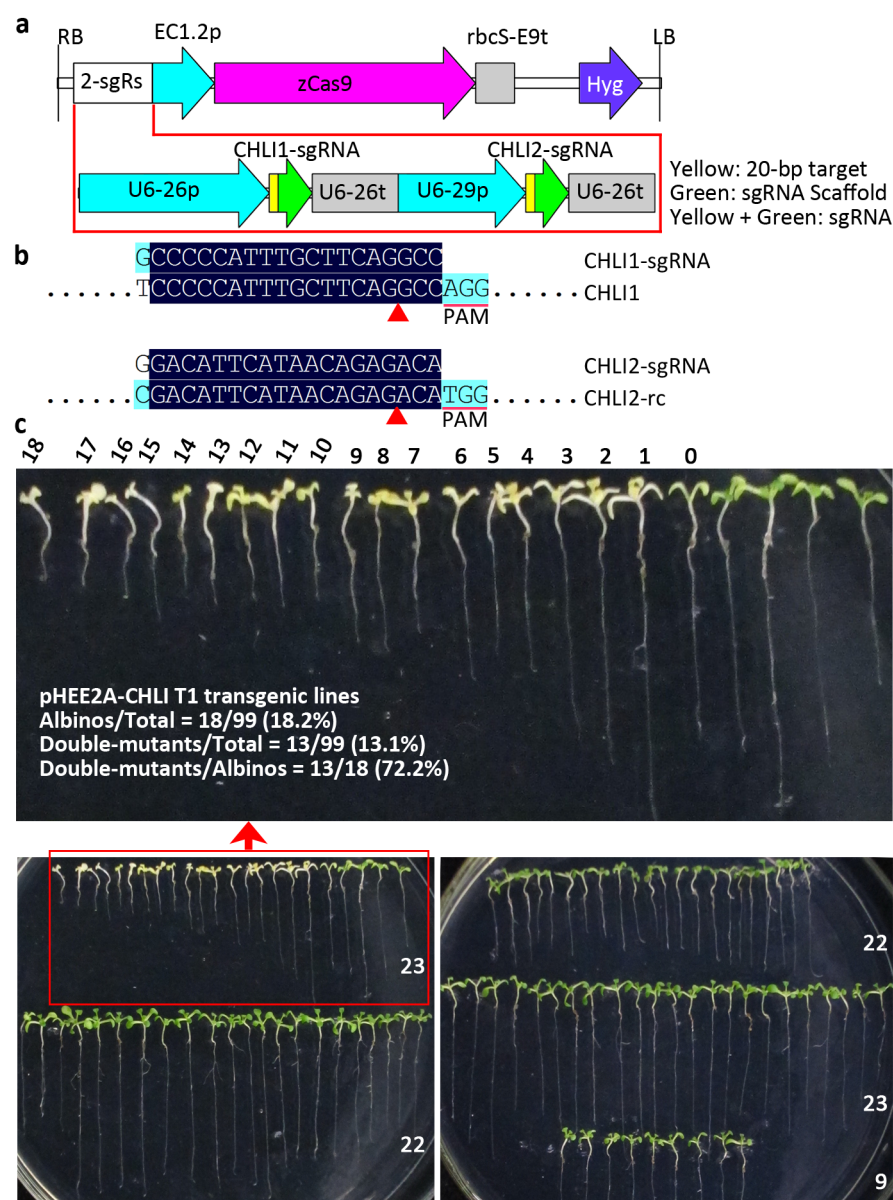


Figure S3. *Arabidopsis* T1 homozygous double mutants obtained via EPC CRISPR/Cas9.

(a) Physical maps of the T-DNA of CRISPR/Cas9 binary vector carrying *Cas9* driven by the egg-cell specific *EC1.2* gene promoter and two sgRNA genes driven by Pol-III promoters U6-26p and U6-29p, respectively. See Figure 1 for RB, LB, EC1.2p, rbcS-E9t, 2-sgRs, zCas9, U6-26p, U6-29p, U6-26t, and *Hyg*. **(b)** The alignment of the sgRNA with its target genes and potential off-targets. Only aligned regions of interest are displayed. -rc, reverse complement. **(c)** Phenotypes of all transgenic seedlings from a single screening. Seeds from T0 plants were sown on MS medium containing 25 mg/L hygromycin, vernalized at 4°C for 3 days, and grown under long-day conditions (16 h light/8 h dark) at 22°C for 7 days.

Hygromycin-resistant seedlings (T1) were transferred to two fresh MS plates and allowed to recover for 3 days before photographing. The number of seedlings in each row of a plate is indicated. Photographs of albino seedlings are enlarged and numbered.