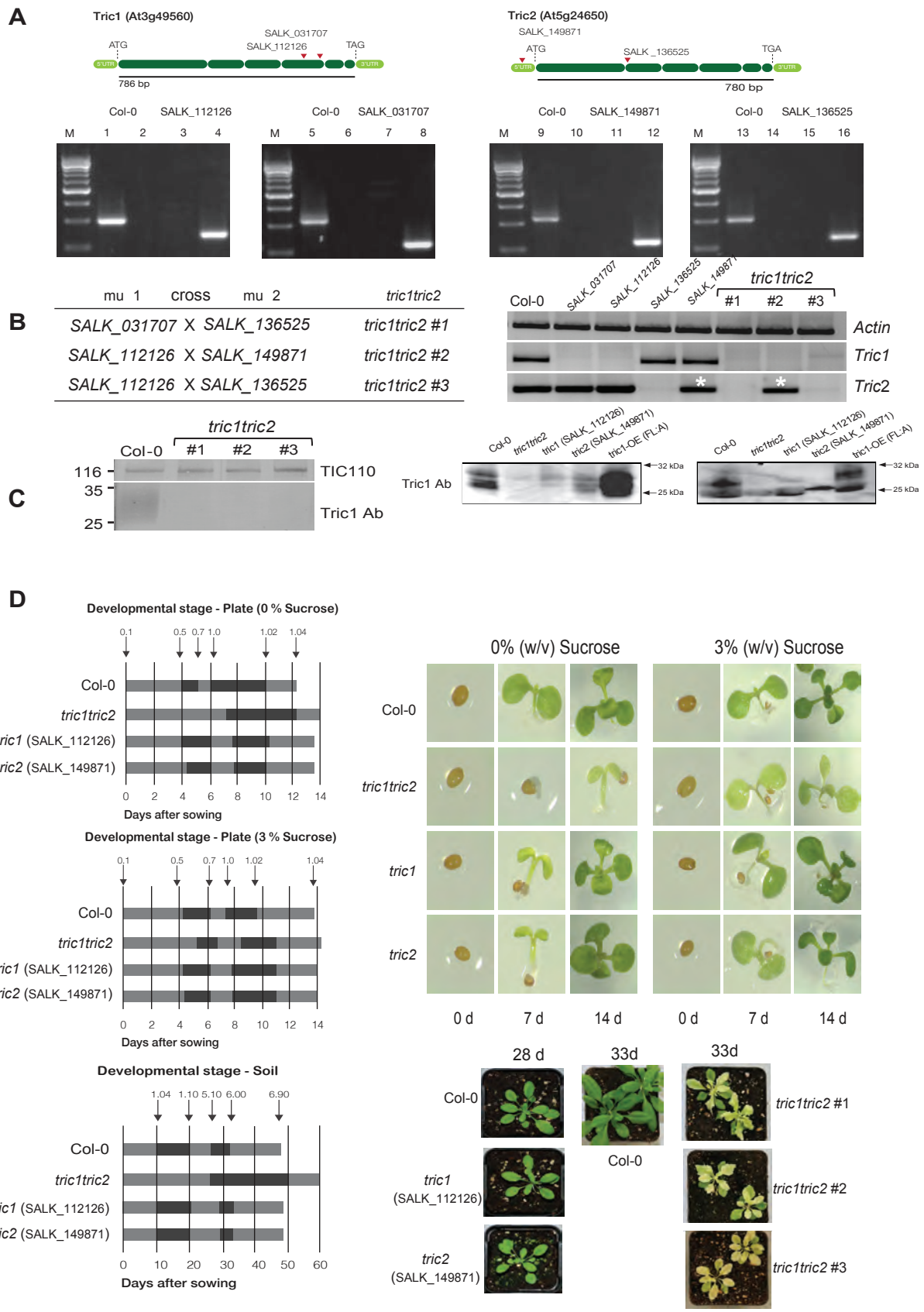

Figure S4. Phenotyping of single and double T-DNA insertion knock-outs for Tric1 and Tric2. A , Screening of T-DNA insertion lines for AtTric1 and AtTric2. Two T-DNA insertion lines where obtained for Tric1 (SALK_112126 and SALK_ 031707) and Tric2 (SALK_136525 and SALK_149871) and plants were screened for homozygosity using primers as listed in ST1. **B**, In total, three independent double knock-out lines (*tric1tric2* #1, #2, #3) were generated by crossing the different SALK lines as indicated and rescreened as above. RT-PCR analysis of *Tric1* and *Tric2* transcript content in Col-0, SALK_031707, SALK_112126, SALK_136525, SALK_149871 single mutant lines as well as *tric1tric2* #1, #2, #3. RNA was prepared from 14-day-old seedlings and reverse transcribed into cDNA. PCR reactions were conducted with primers listed in ST1. As positive control, constitutively expressed actin was used. Residual *Tric2* mRNA amplified on the single mutant SALK_149871 and on the double mutant *tric1tric2* #2 is indicated by asterisks. Because in SALK_149871 the T-DNA insertion is located in the 5'-UTR of *Tric2* (compare A), it is possible that residual transcripts can be amplified by the C-terminal primers used for RT-PCR. However, these transcripts were non-functional for translation into Tric2 proteins, as demonstrated by the lack of protein signals in immunoblots as well as by the chlorotic phenotype of *tric1tric2* #2 (see C). **C**, (Left) Immunoblot of Tric1 on equally loaded 10 µg proteins isolated from leaf material of 33-day-old Col-0 and *tric1tric2* double mutant lines #1, #2, #3. Antiserum against the inner envelope protein TIC110 was used as loading control. (Right) To confirm absence of Tric protein in organelles, mitochondria and chloroplasts were isolated from single (*tric1* and *tric2* and *tric1tric2* #2 lines and immunodetected with antibodies raised against AtTric2. Numbers indicate the molecular mass of proteins in kDa. **D**, Plate- and soil-based growth progression analysis of Col-0, and respective Tric mutant lines. Arrows define the time (days after sowing) that Col-0 plants have reached the growth stages as defined by Boyes et al. (2001) (Boyes et al., 2001). Boxes define the time between the growth stages, and shading indicates the occurrence of each growth stage. Stage 0.1, imbibition; stage 0.5, radical emergence; stage 0.7, hypocotyl emerge from seed coat; stage 1.0, cotyledons fully opened; stage 1.02, two rosette leaves >1 mm in length; stage 1.04, four rosette leaves > 1mm in length. Data are given as averages for 10 plants. Days are relative to the days after sowing after a 3-d stratification at 4°C. Stage 1.10, 10 rosette leaves >1 mm; stage 5.10, first flower buds visible; stage 6.00, first flower opens; stage 6.90, flowering complete. Please note that for all following experiments, we used the mutant lines *tric1tric2* #1 (for microscopy) and #2 for all molecular characterisation.