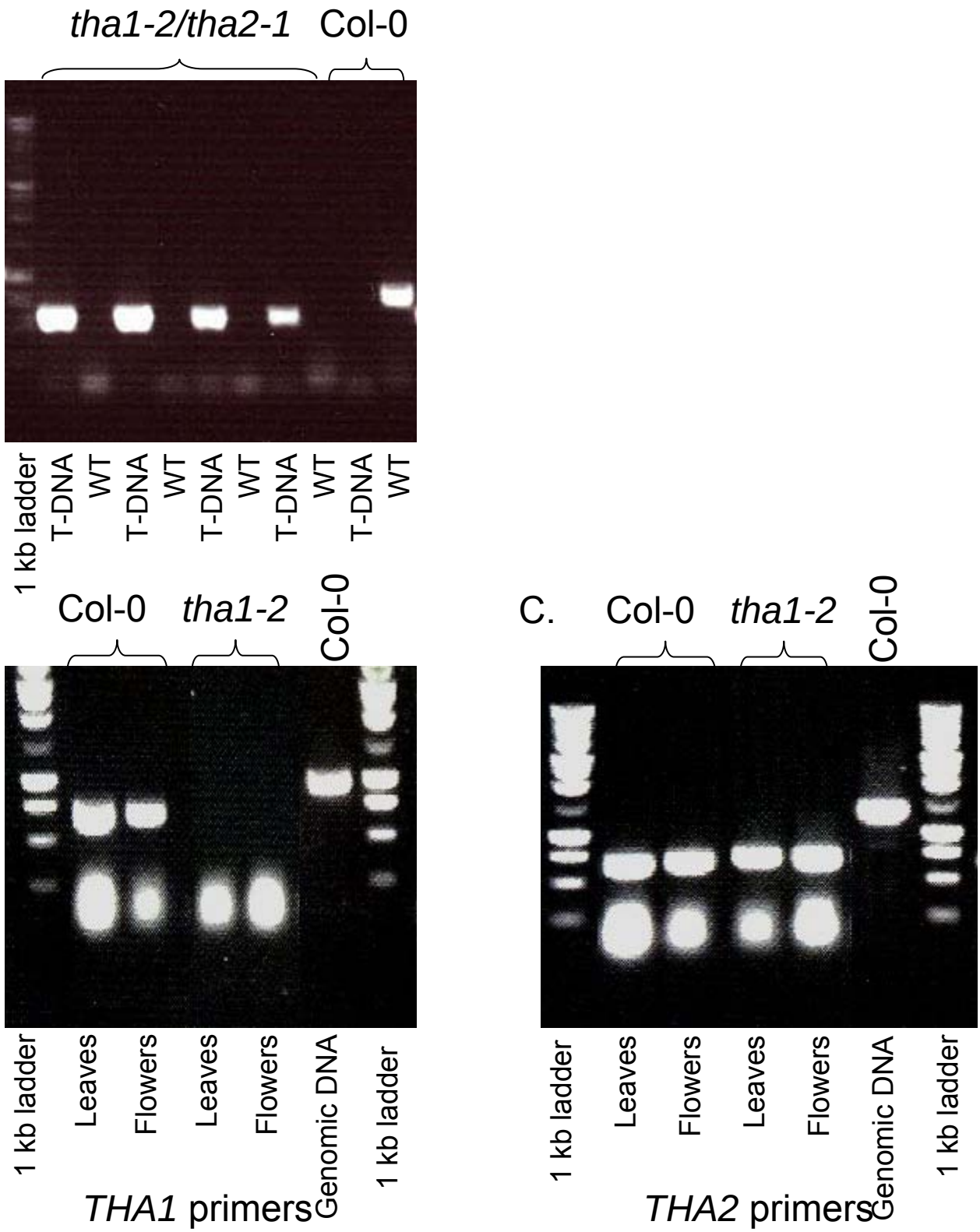
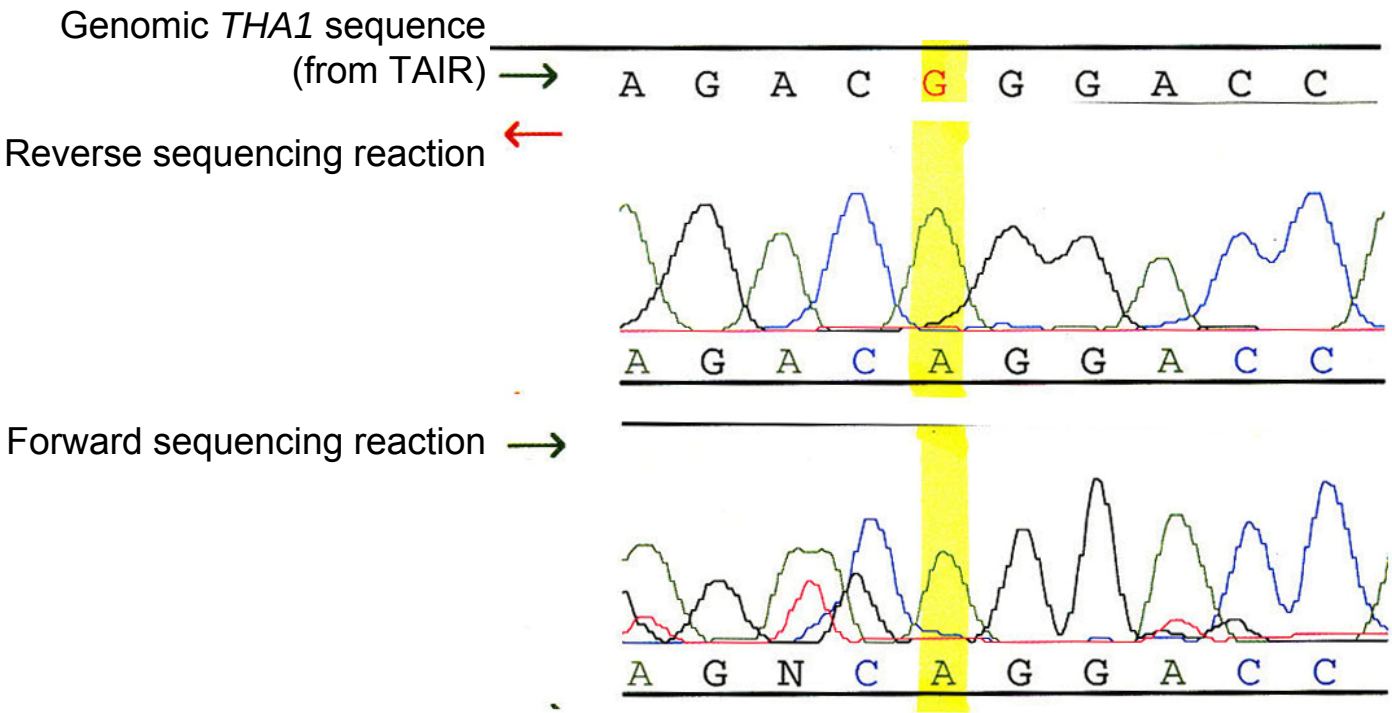
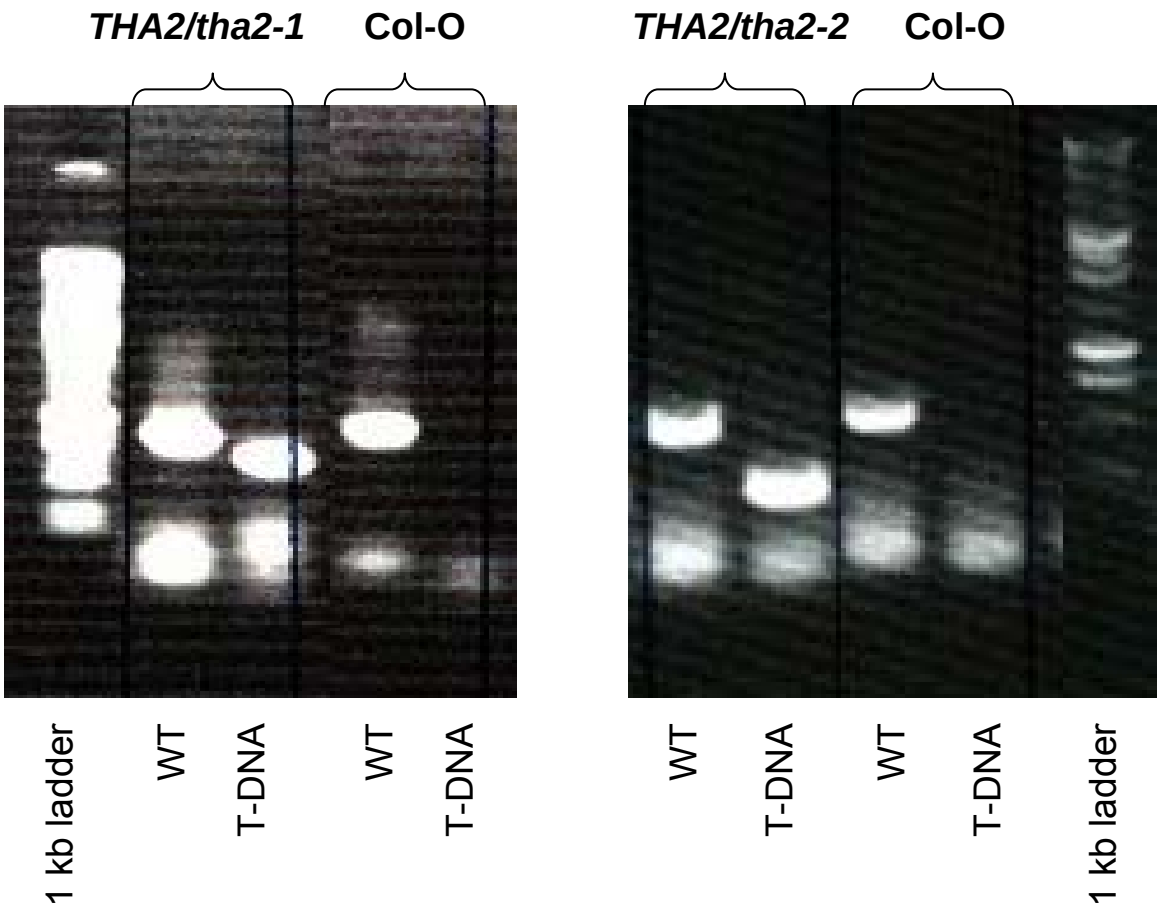


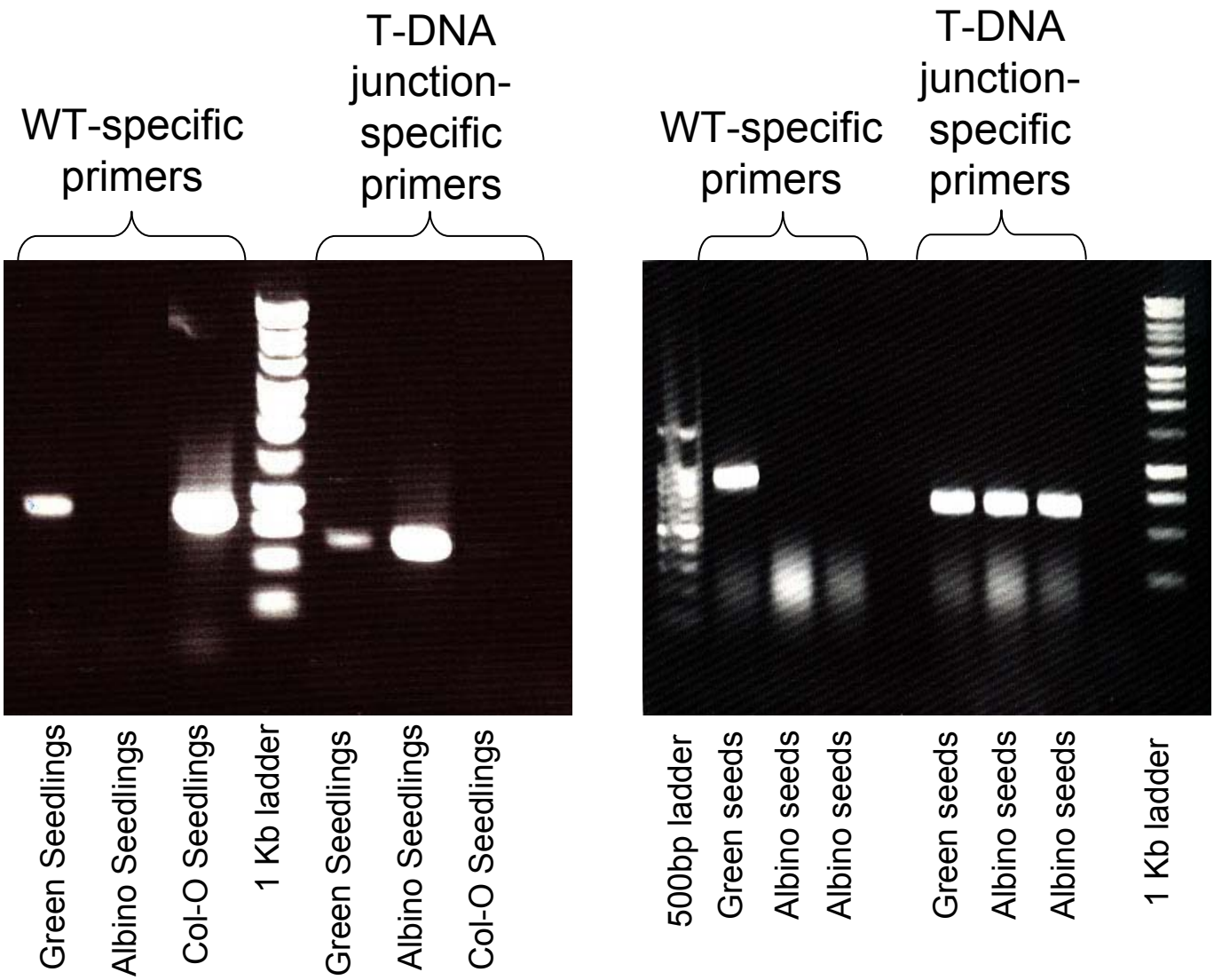
Supplemental Figure 3A. Top gel: genotyping with T-DNA junction (primer GAB-SEQ, Supplemental Table 2) and wildtype-specific primers (*tha1-2* P1 and *tha1-2* P2) shows that *tha1-2* mutants are homozygous. Bottom gels: RT-PCR using gene-specific primers (TA5F and TA6R, Supplemental Table 2) shows that the *tha1-2* mutant is a complete transcriptional knockout. (C) Transcription of *THA2* is unaffected by the *tha1-2* insertional mutation (using primers RNAt2-1 P1 and RNAt2-1 P2, Supplemental Table 2).



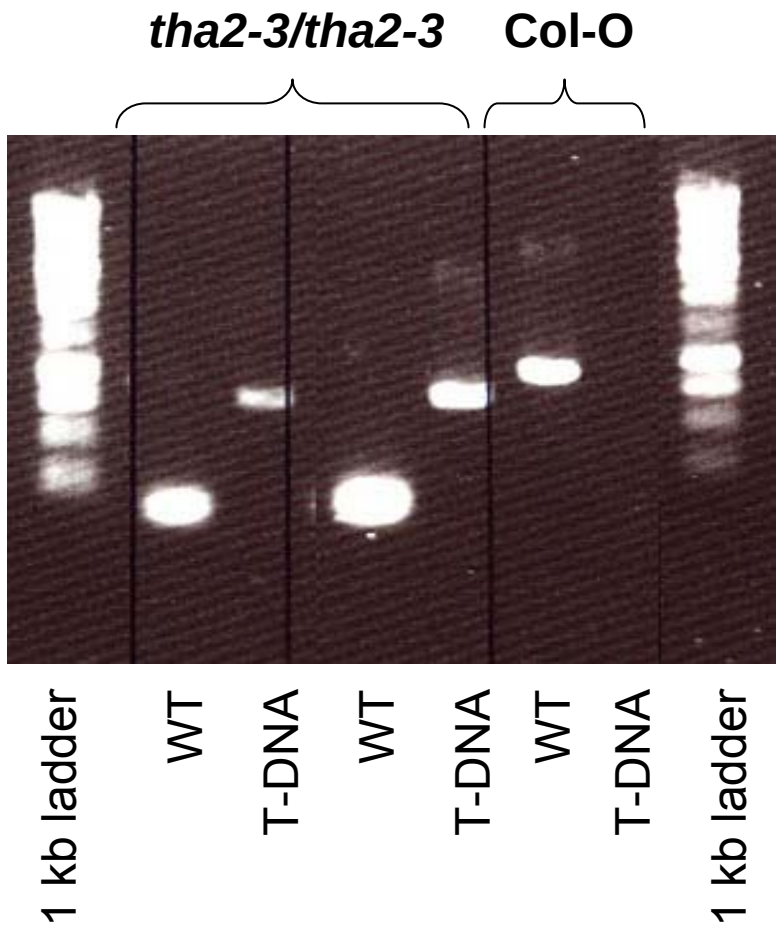
Supplemental Figure 3B. DNA sequencing shows that the *tha1-1* line used in these experiments has a homozygous G to A change (Primers used TA6F and TA6R, Supplemental Table 2).



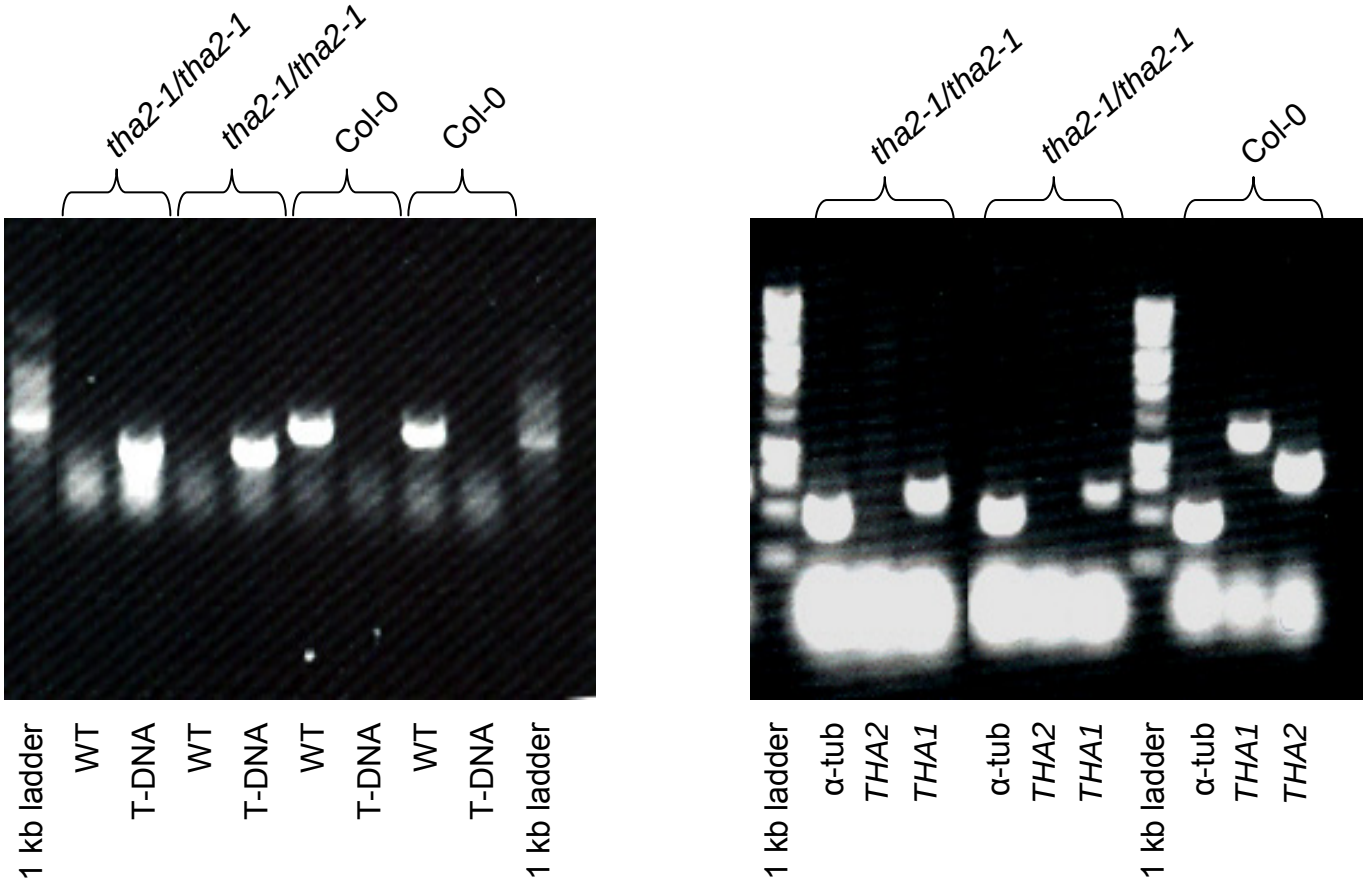
Supplemental Figure 3C. PCR amplification with T-DNA junction (LB3 and LBa1 for tha2-1 and tha2-2 respectively, Supplemental Table 2) and wildtype specific primers (for *tha2-1*; *tha2*-P1and *tha2-1*P2; while for *tha2-2*; *tha2-2* P1 and *tha2-2*P2 respectively, Supplemental Table 2) shows that the *THA2/tha2-1* and *THA2/tha2-2* lines are heterozygous.



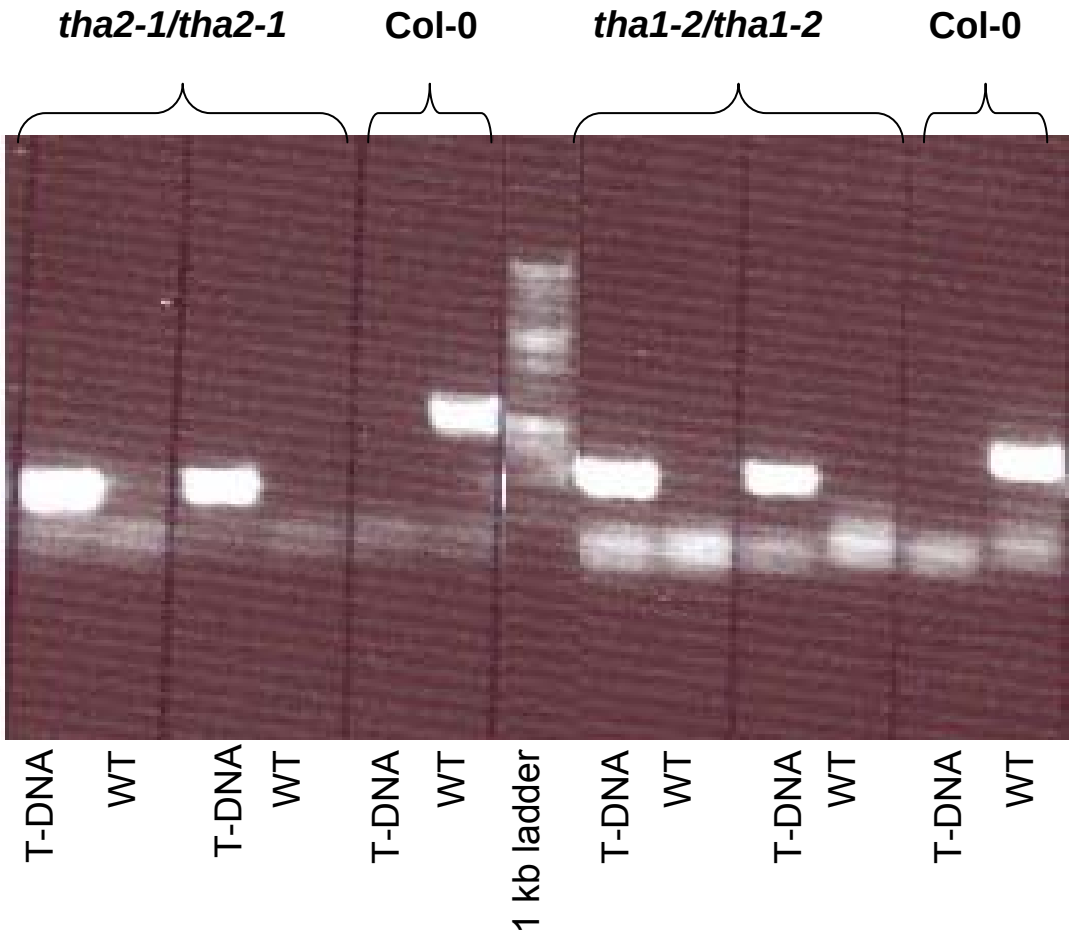
Supplemental Figure 3D. PCR-based genotyping (using T-DNA specific primer LB3 and gene specific primers *tha2*-P1and *tha2-1*P2, Supplemental Table 2) shows that albino seedlings (left gel) and seedlings (right gel) are homozygous *tha2*, whereas pooled tissue of their green siblings is heterozygous.



Supplemental Figure 3E. PCR-based genotyping with T-DNA junction (primer LBa1, Supplemental Table 2) and wildtype-specific primer pairs (*tha2-3* P1and*tha2-3* P2, Supplemental Table 2) shows that the *tha2-3* line is homozygous mutant.



Supplemental Figure 3F. Confirmation of homozygous *tha2-1* knockout mutations in the presence of feedback-insensitive Thr deaminase (*omr1-5*)
Left gel: PCR amplification to identify homozygous *tha2-1/tha2-1* isolates. DNA from two isolates each of Col-0 and homozygous *tha2-1/tha2-1* P35S*omr1-5* were subjected to PCR amplification with primers specific to the wild type *THA2* gene (*tha2-1*_P1 and *tha2-1*_P2, Supplemental Table 2) and the T-DNA insertion allele (Lb3 and *tha2-1*_P2, Supplemental Table 2). Lanes are labeled WT and T-DNA, respectively. Whereas Col-0 DNA only shows amplification with primers specific to *THA2,* the homozygous *tha2-1/tha2-1* mutant DNA shows only amplification with the primers specific to the T-DNA insertion. This shows that the mutants are homozygous *tha2-1.*
Right gel: RT-PCR to study *THA2* in Col-0 wild type and *tha2-1/tha2-1* homozygous mutant plants*.* Primers specific to α-tubulin (At-tub-f and At-tub-r, Supplemental Table 2), , THA2 (RNAt2-1 P1 and RNAt2-1 P2, Supplemental Table 2), and THA1 (TA5F and TA6R, Supplemental Table 2), were used to amplify cDNA from Col-0 wild type and *tha2-1/tha2-1* P35S*omr1-5* (two isolates) plants. Whereas Col-0 showed amplification with all three primer pairs, there was no amplification of *tha2-1/tha2-1* P35S*omr1-5* with the *THA2*-specific primer pair. This indicates an undetectable level of *THA2* expression in the mutant lines.



Supplemental Figure 3G. PCR-based genotyping with T-DNA junction (GAB-SEQ and Lb3 for *THA1* and *THA2* respectively, Supplemental Table 2) and wild type specific primers (TA8F and TA8R for *THA1* and*tha2-1* P2 and TNAt2-1 P2 *for THA2,* Supplemental Table 2*)* shows that the *tha2-1/tha2-1 tha2-1/tha2-1 P35S-omr1-5* triple mutant is homozygous for T-DNA insertions in both Thr aldolase genes.