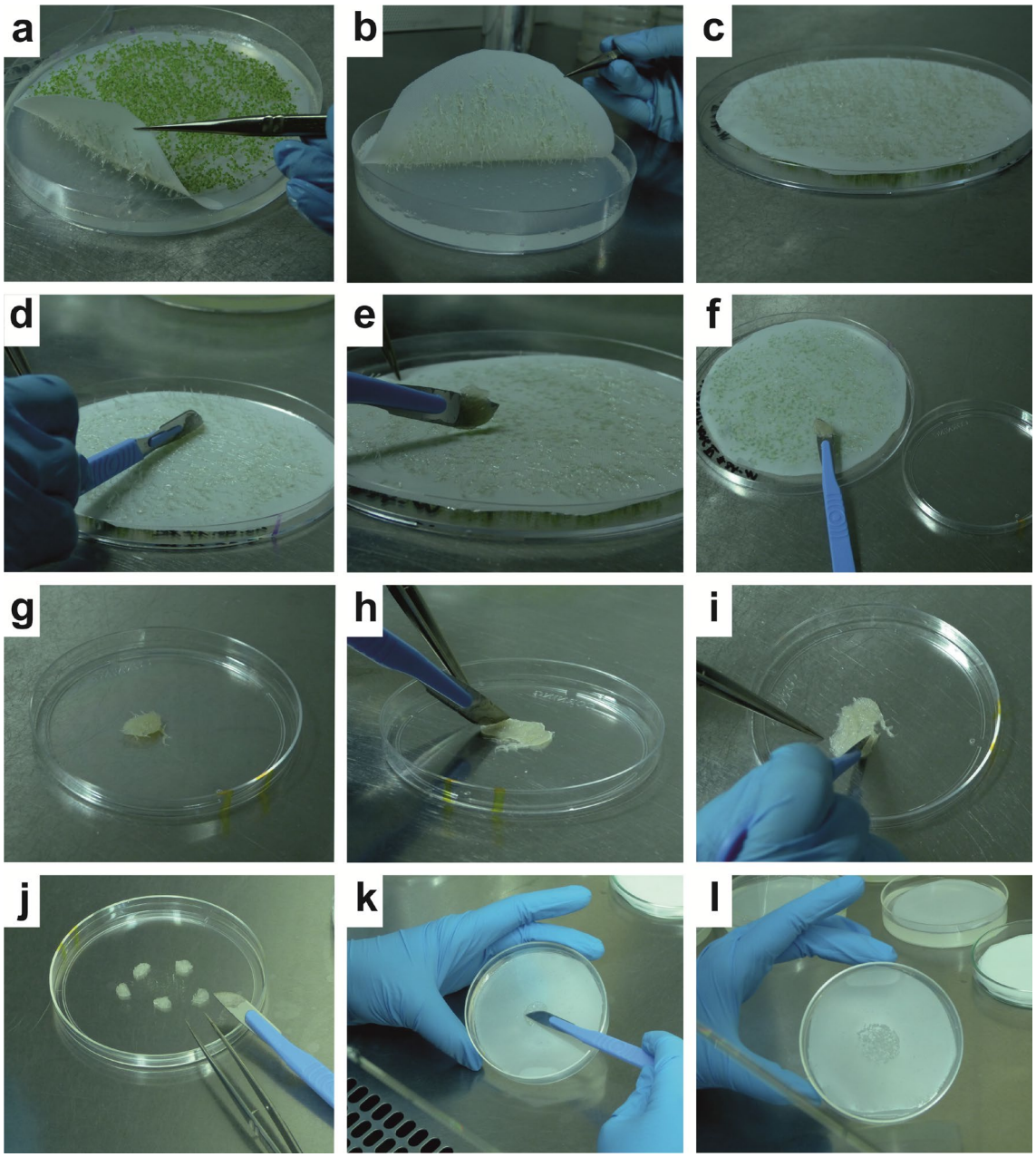
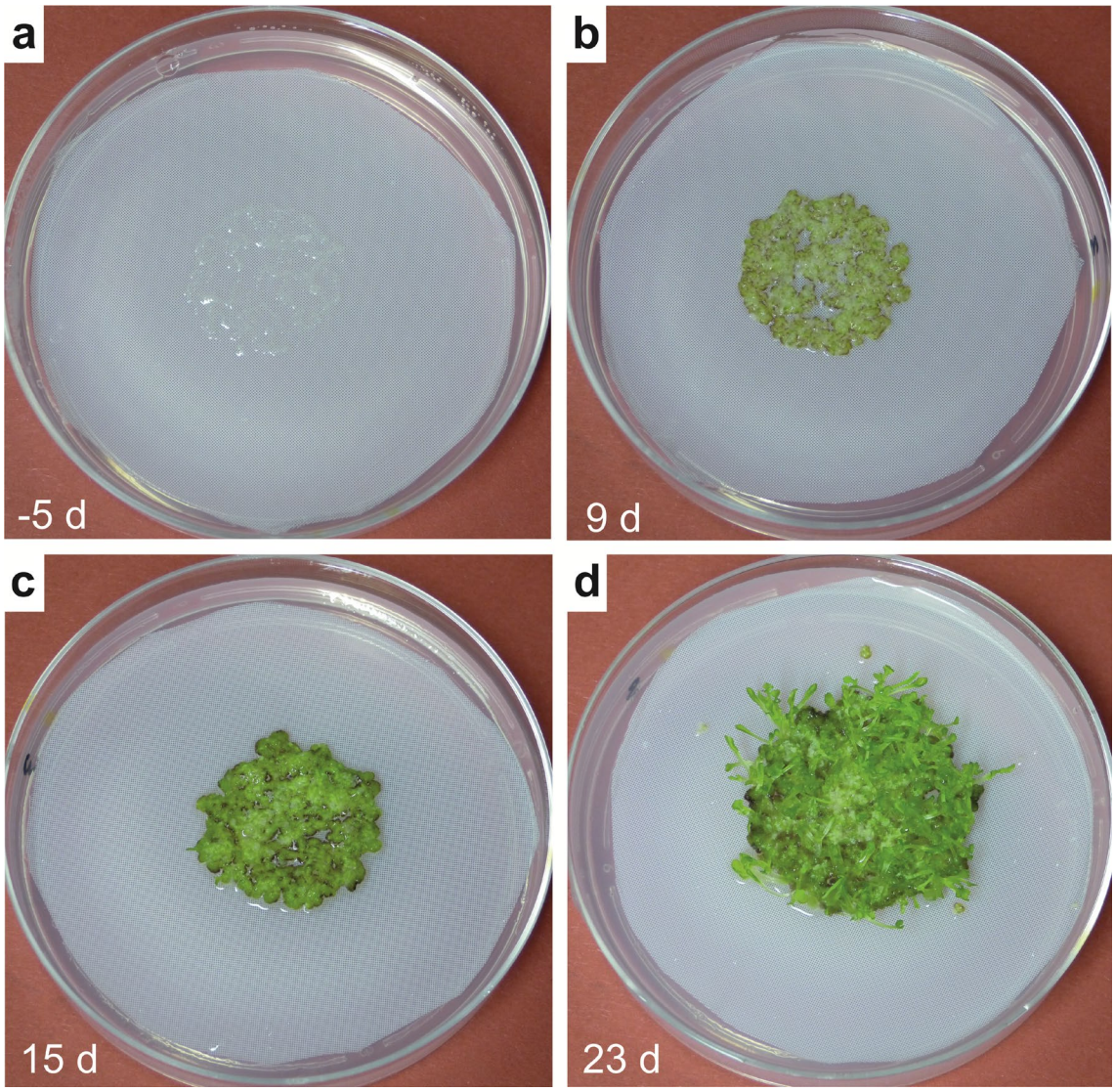
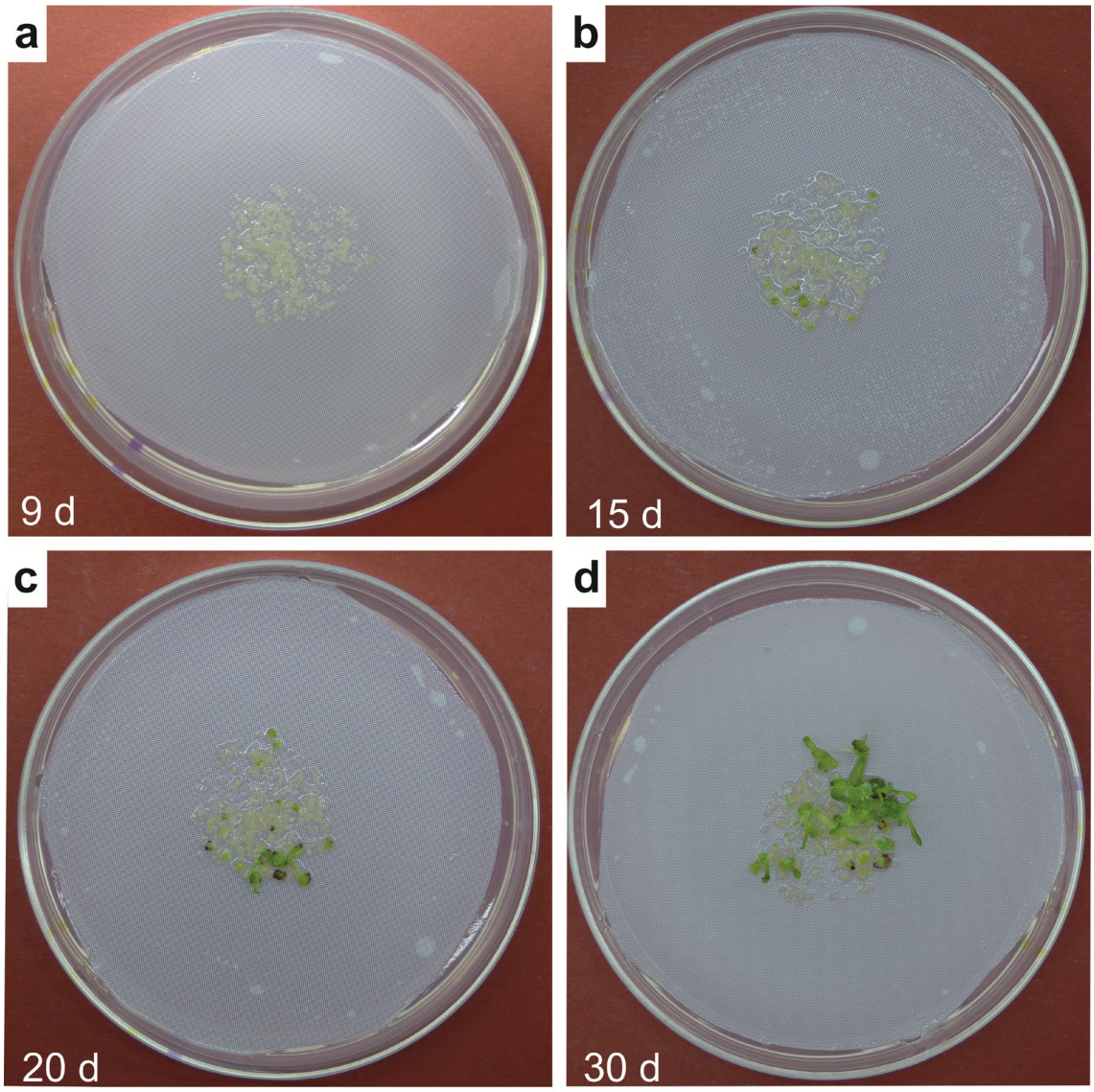


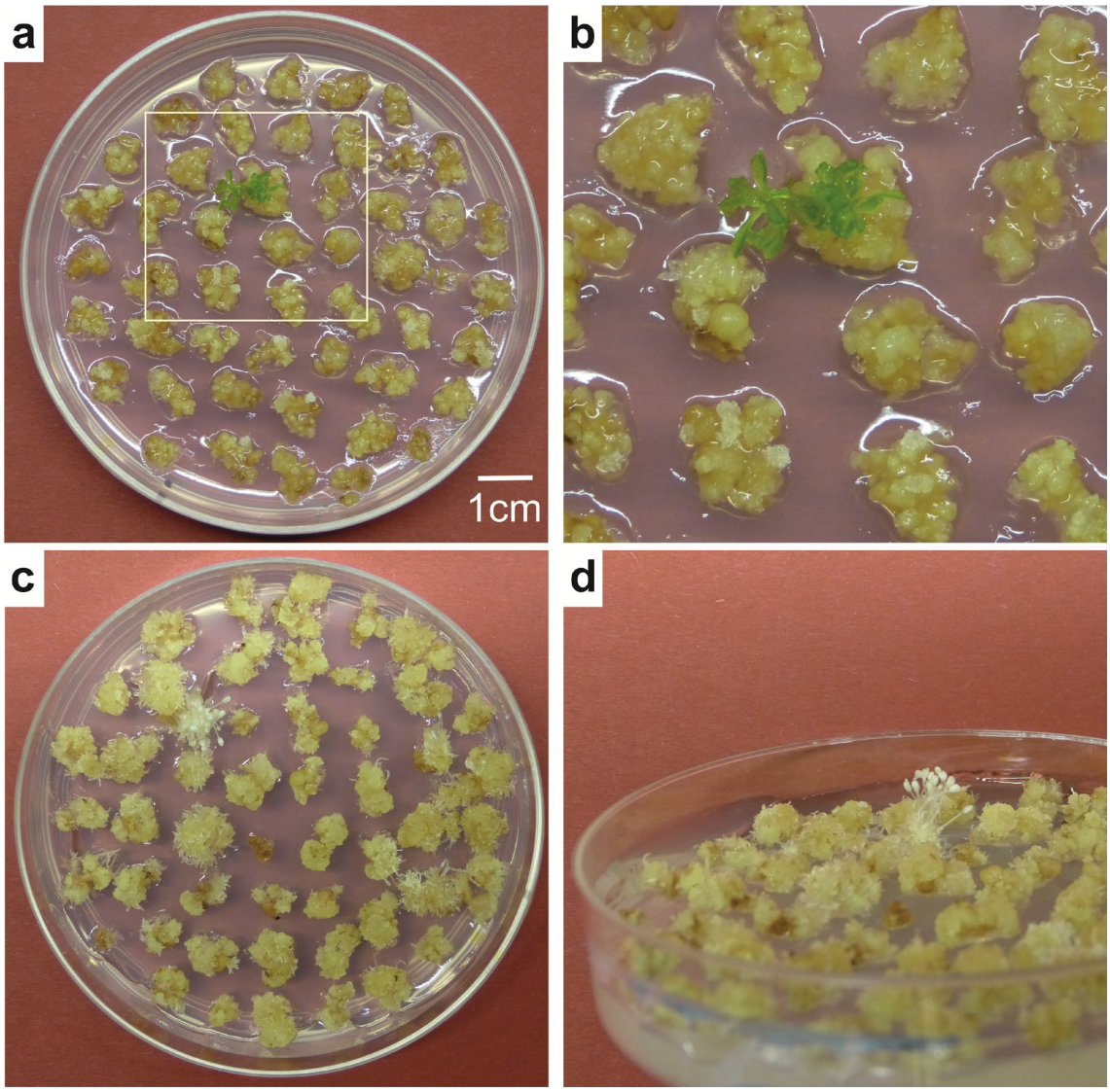
Supplementary Fig. 1. Preparation of plant material for biolistic transformation experiments in *Arabidopsis thaliana*. (**a**) *In vitro* germinated seedlings grown on netting to facilitate harvest of root tissue. (**b**) Removal of the netting for root harvest. (**c**) Netting placed upside-down in the lid of the Petri dish. (**d**) Removal of the adhering agar with the blunt edge of the scalpel. (**e,f**) Root harvest by scraping off the roots with the sharp edge of the scalpel. (**g**) Transfer of the harvested root material to the lid of a Petri dish. (**h,i**) Cutting of the roots into small pieces. (**j**) Aliquoting of the cut root material for transformation. (**k**) Spreading of the root material onto the surface of netting on synthetic medium. (**l**) Arrangement of the root material in a circular area (diameter ~3.5 cm) for biolistic bombardment.



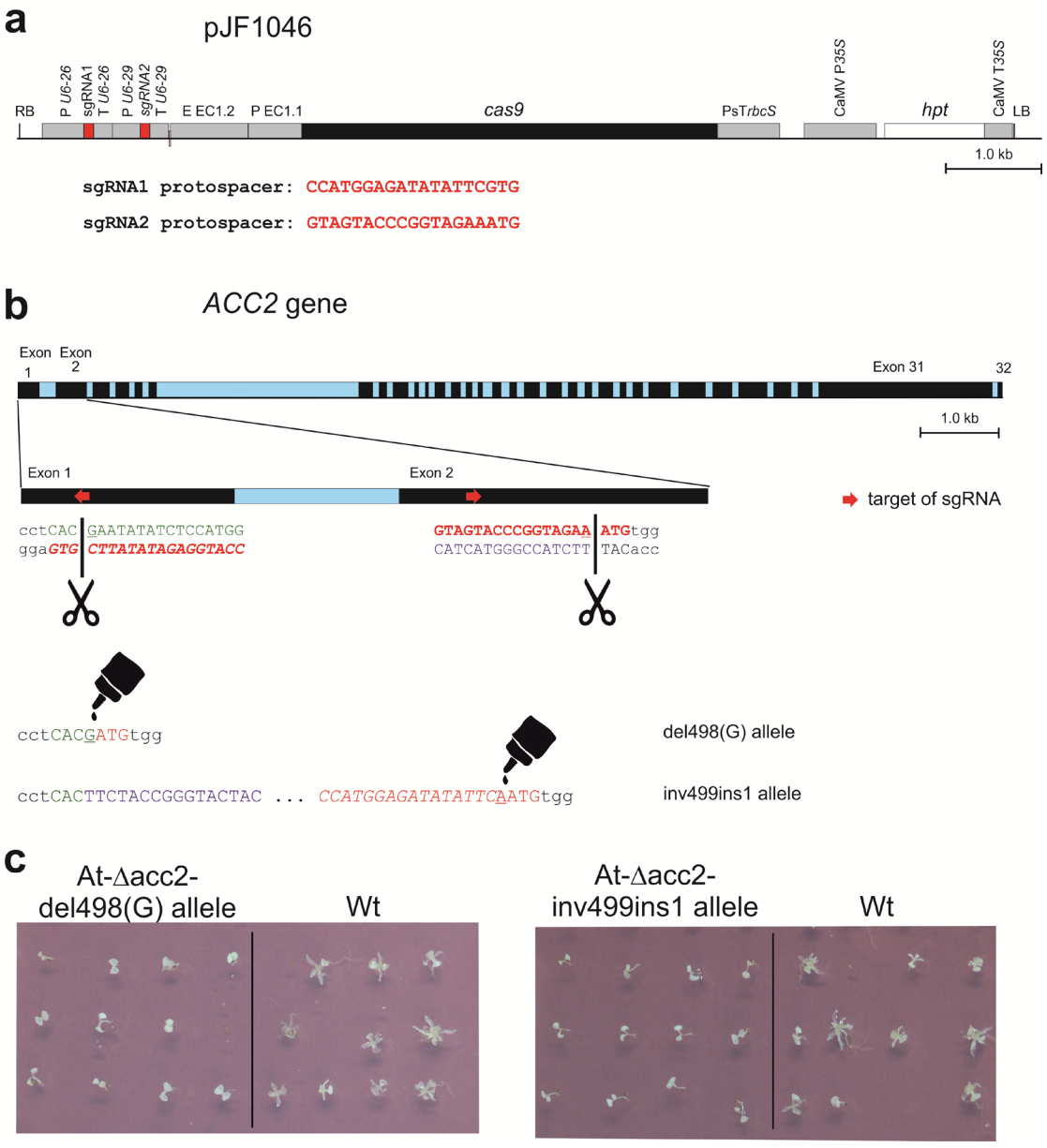
Supplementary Fig. 2. Regeneration of *Arabidopsis* plantlets from root tissue in the absence of selection. The numbers in the left bottom corner of each photograph give the time point relative to the day of bombardment (with -5 d indicating that, typically, root harvest would occur 5 days prior to bombardment). (**a**) Root tissue immediately after harvest. The tissue is arranged in a circular area for biolistic bombardment. (**b**) Callus growth and incipient greening 9 days after bombardment and transfer to AtSIM3 medium. (**c**) Incipient shoot regeneration 15 days after transformation. (**d**) Massive shoot regeneration 23 days after bombardment. These experiments were repeated independently more than 75 times with similar results.



Supplementary Fig. 3. Selection of nuclear transformants after biolistic bombardment of *Arabidopsis* root-derived microcallus tissue. Selection was performed on AtSIM3 regeneration medium supplemented with 50 µg/mL kanamycin. See Methods for details. The numbers in the left bottom corner of each photograph give the days that have elapsed after bombardment. (**a**) Bombarded tissue 9 days after transformation. (**b**) Selection plate 15 days after bombardment. (**c**) Selection plate 20 days after bombardment, when green resistant tissue begins to emerge. (**d**) Selection plate 30 days after bombardment. Regeneration of multiple kanamycin-resistant shoots is clearly visible. These transformation experiments were repeated independently more than 150 times with similar results.

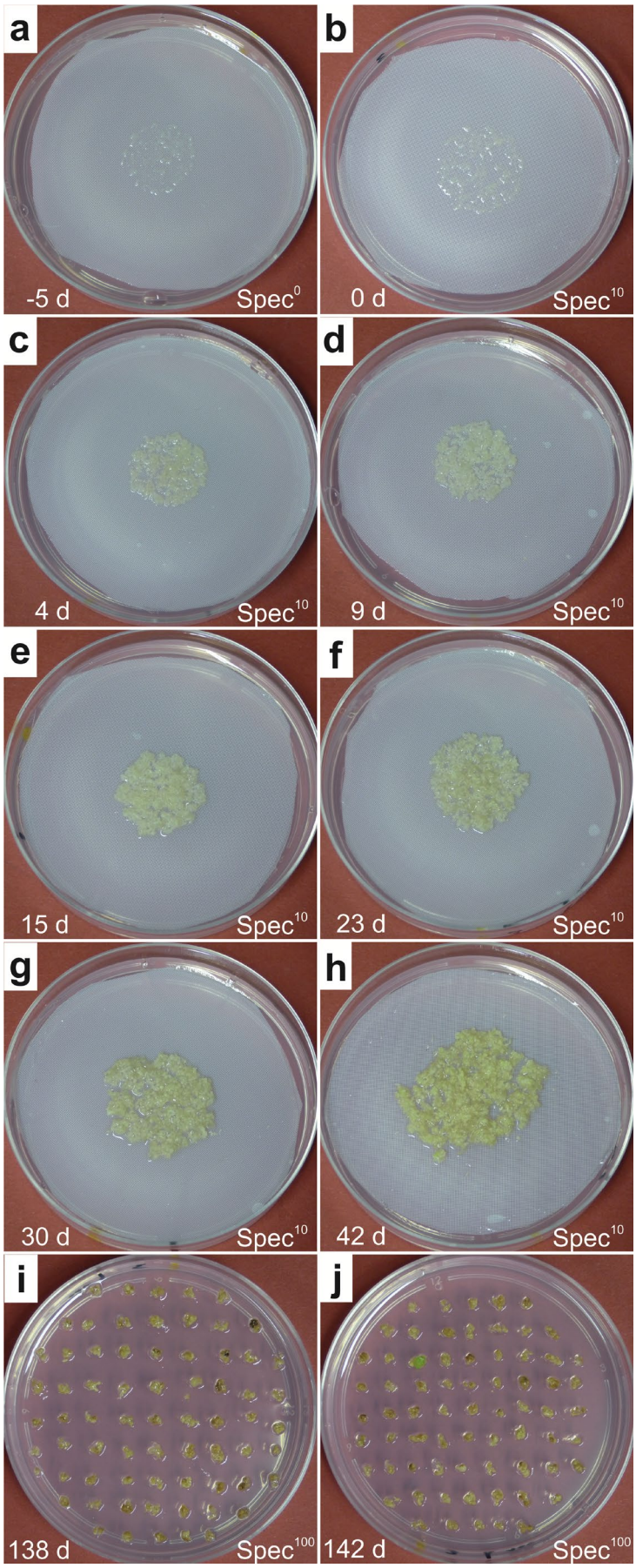


Supplementary Fig. 4. Selection of the transplastomic line shown in Fig. 2b. The event was obtained from biolistic bombardment of root-derived tissue of wild-type *Arabidopsis thaliana* C24 with vector pCH8. Selection was performed on regeneration medium with 10 µg/mL spectinomycin. (**a**) Primary transplastomic line obtained by bombardment with vector pCH8 after 16 weeks of selection on spectinomycin-containing medium (cf. Fig. 2b; Table 1). Medium change took place every two weeks. (**b**) Close-up of the region boxed in (a). (**c**) Inefficiency of spectinomycin selection in the wild type. Substantial callus growth and background regeneration of untransformed (white) tissue is clearly visible. (**d**) Side view and close-up of the selection plate in (c) showing a flowering escape regenerant. Transformation experiments with the wild-type *Arabidopsis* strain C24 and vector pCH8 were repeated independently 507 times (cf. Table 1), and resulted in similar background growth of the bombarded calli.

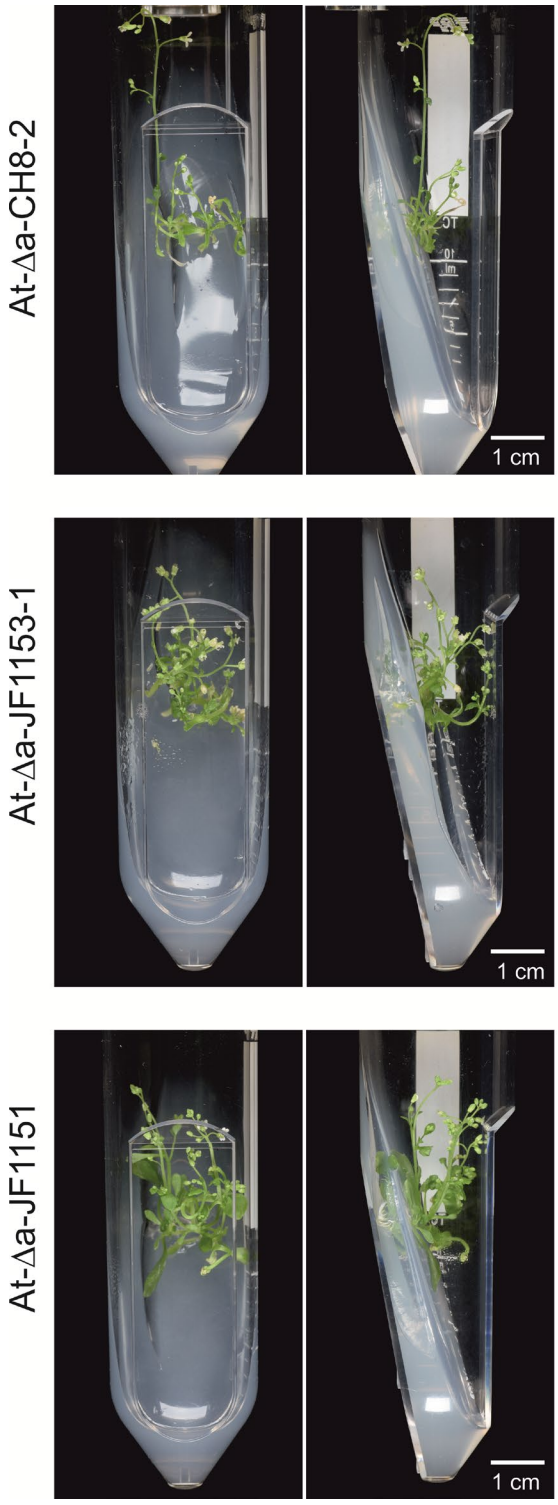


Supplementary Fig. 5. Generation of *ACC2* knock-out lines using the CRISPR/Cas9 system for genome editing. (**a**) Physical map of the T-DNA region in the nuclear transformation vector pJF1046 for CRISPR/Cas9-mediated knock-out of *ACC2* in *Arabidopsis thaliana* C24. The vector harbors two sgRNAs whose protospacer sequences are shown below the map. The hygromycin resistance gene *hpt* serves as selectable marker gene for plant transformation. (**b**) Map of the *ACC2* gene in the *Arabidopsis thaliana* nuclear genome. Exons are shown as black boxes, introns as light blue boxes. The expected sites of Cas9 cleavage are indicated by scissors. See Methods for details. The sequences of the resealed cleavage sites in the two knock-out mutants analyzed in detail are shown below the map. The del498(G) allele carries the deletion intended to be induced with the two sgRNAs, but has an unexpected G at the religation

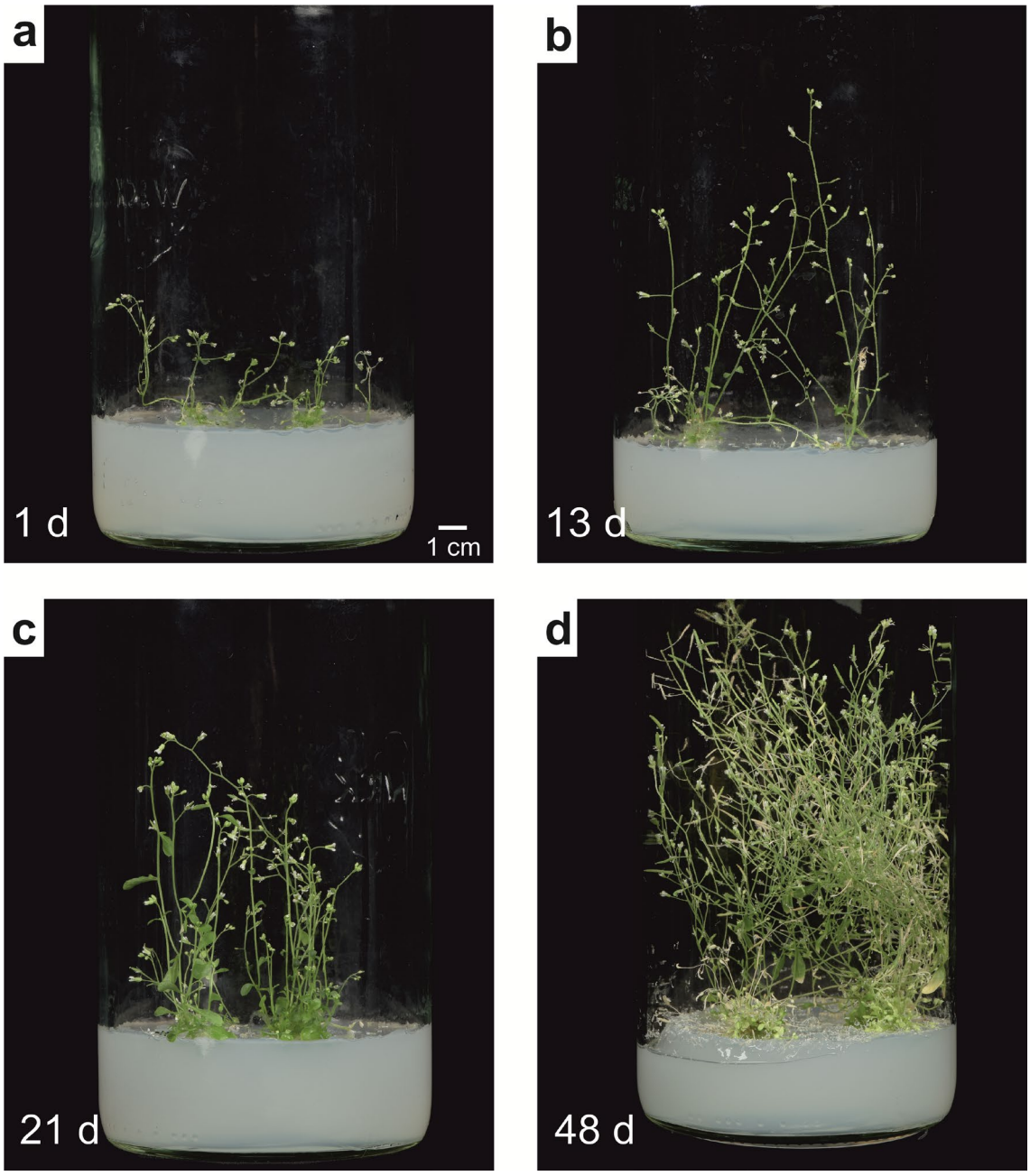
site that could come from either imprecision of Cas9 cleavage or post-cleavage insertion. The inv499ins1 allele has the excised fragment inverted and an additional A inserted (resulting in a premature stop codon). (**c**) Confirmation of *ACC2* inactivation by growth assays with germinating seedlings on synthetic medium with 100 mg/L spectinomycin. Note arrested growth at the cotyledon stage in the two knock-out mutants and outgrowth of true leaves in the wild type. These assays were repeated independently four times with similar results.


a
-5 d
Spec0
b
0 d
Spec10
c
4 d
Spec10
d
9 d
Spec10
e
15 d
Spec10
f
23 d
Spec10
g
30 d
Spec10
h
42 d
Spec10
i
138 d
Spec100
j
142 d
Spec100

Supplementary Fig. 6. Generation of transplastomic *Arabidopsis* lines using the At-Δacc2 recipient line and vector pCH8. (**a**) Harvested root material spread on AtCIM1 medium without antibiotic. (**b**) Root material immediately after biolistic bombardment and transfer to AtSIM3 medium with 10 mg/L spectinomycin. (**c**) Bombarded sample after 4 days on AtSIM3 medium with 10 mg/L spectinomycin. (**d**) Bombarded sample after 9 days on AtSIM3 medium with 10 mg/L spectinomycin. (**e**) Bombarded sample after 15 days on AtSIM3 medium with 10 mg/L spectinomycin. (**f**) Bombarded sample after 23 days on AtSIM3 medium with 10 mg/L spectinomycin. (**g**) Bombarded sample after 30 days on AtSIM3 medium with 10 mg/L spectinomycin. (**h**) Bombarded sample after 42 days on AtSIM3 medium with 10 mg/L spectinomycin, one day before transfer of the material to fresh plates and separation of the calli. (**i**) A bombarded sample after 138 days on AtSIM3 medium with 100 mg/L spectinomycin. No transformation event resulted from this selection plate. (**j**) A bombarded sample after 142 days on AtSIM3 medium with 100 mg/L spectinomycin. The transplastomic event is identical with the one shown in Fig. 2c. The medium was exchanged every 2 weeks, the shift from 10 mg/L to 100 mg/L spectinomycin was done after 8 weeks. The transformation experiments with the At-Δacc2 recipient line and vector pCH8 were repeated independently 98 times with similar results.



Supplementary Fig. 7. Growth of transplastomic shoots. When 0.5 to 1 cm tall, plantlets are transferred from Petri dishes to agar slants and grown on AtRIM1 medium for approximately two weeks to stimulate root induction and flowering. The front view (left) and the side view (right) are shown for one transplastomic line per transformation construct. These experiments were repeated independently for 25 transplastomic lines with similar results.



Supplementary Fig. 8. Seed production from transplastomic *Arabidopsis* plants. For unhindered growth and efficient seed production, plantlets are transferred from agar slants (Supplementary Fig. 7) to larger containers (glass jars) and grown to maturity. (**a**) Plantlets 1 day after transfer. (**b**) Plants 13 days after transfer. (**c**) Plants 21 days after transfer. (**d**) Plants 48 days after transfer. Note abundant flowers and siliques with seeds (cf. Figs. 1b and 3). These experiments were repeated independently for 22 transplastomic lines with similar results.

Supplementary Table 1. List of oligonucleotides used in this study.

Oligonucleotide	**Sequence (5‘ → 3‘)**
oJF212	TGCCAAGCTTCGACTTGCCT
oJF213	TTGTGGCGCGCCTGTTGGTCTCTCAATCACTACTTCGACTCTAGC
oJF214	AACAGGCGCGCCACAAGGTCTCAGTTTTAGAGCTAGAAATAGCAAG
oJF215	ATTCACTAGTATAACCATGGTATTGG
oJF217	ACCAGGTCTCAATTGCCATGGAGATATATTCGTGGTTTTAGAGCTAGAAATAGCAAG
oJF218	TGGTGGTCTCTAAACCATTTCTACCGGGTACTACCAATCTCTTAGTCGACTCTACC
oJF219	ATTGTTCTCAGCTTCACTGTCGA
oJF324	AACAGGATCCCTTGAGACTATCTGGCTTGG
oJF325	TGTTGGTACCTCGTAAGCCCATGTTCTGAC
oSR2	CGGGAAATATTGTAATTTTTTC
oSR1	ATCGGGACGAGGTGGTAC