Supplemental File S1: MATERIALS AND METHODS

TALEN design and synthesis

TALEN target sites within the tomato *PRO* gene (Solyc11g011260.1) were identified using TAL Effector Nucleotide Targeter (TALE-NT) 2.0 program (Doyle et al., 2012). Two TALEN target sites were selected, and TAL effector arrays were constructed using the Golden Gate method (Cermak et al. 2011; Zhang et al. 2013). All information about TAL effector sequences and binding sites are listed in Table S1. Fully assembled TALEN monomers were cloned into the Gateway compatible entry clone pZHY013 using *Xba*I and *Bam*HI or *Nhe*I and *Bgl*II sites. pZHY013 contains two heterodimeric FokI nuclease domains separated by a T2A translational skipping sequence to allow the left and right TALEN to be expressed from a single transcript (Halpin et al., 1999). A Gateway LR reaction (Invitrogen) was performed to recombine the entry clone with the pFZ19 expression vector to generate the TALEN expression vectors pTAL423/4_FZ19 and pTAL425/6_FZ19. These vectors place TALEN expression *in planta* under the control of the XVE promoter (Zuo et al., 2000).

Agrobacterium-mediated tomato transformation

pTAL423/4_FZ19 and pTAL425/6_FZ19 expression vectors were transformed into *Agrobacterium tumefaciens* strain AGL1. Stable transgenic tomato (cv. M82) plants were generated using a modification of the Agrobacterium-mediated tomato transformation method of McCormick (1991). Briefly, tomato seeds were surface sterilized in 10% sodium hypochlorite and germinated on 1X Murashige and Skoog (MS) medium. The medium had macronutrients, micronutrients and vitamins according to Murashige and Skoog (1962); the pH was adjusted to 5.6 and solidified with 0.5% Phytoagar (referred hereafter as solid 1X MS). Plates with seeds were placed in a growth chamber under 16 hr light/8 hr dark at 24°C**.** Cotyledons were excised (cutting away the blade tip and petiole) from one-week-old seedlings and pre-cultured for two days on co-cultivation medium (pH 5.6) containing 3% sucrose, 1X MS, 1X Nitsch and Nitsch, 2mg/L zeatin-riboside, 2mg/L indol-butyric acid and 0.5% Phytoagar in a 15X100mm petri plate. Agrobacterium was grown in 50mL Luria-Bertani (LB)

medium with 50µg/L of kanamycin for two days at 28°C and then diluted with sterile LB to 0.6 OD_{600nm}. The cells were recovered from 50mL of the diluted culture by centrifugation in a Beckman JA-20 rotor at 4000 rpm and re-suspended in 50mL of sterile 1X MS solution pH 5.6 with 50µM acetosyringone. Explants were co-cultivated for 30 min. with slow agitation and then placed back onto co-cultivation media. After two days, the explants were transferred to shooting media (co-cultivation media with 400mg/L cefotaxime and 25mg/L hygromycin-B) in a 25X100mm petri plate. Explants were transferred to fresh shooting medium every three weeks or as needed until shoots formed. The shoots were excised and transferred to magenta boxes containing solid 1X MS. Once roots were visible, the plants were transferred to BM2 soil (Berger) and were designated as primary transformants (pTAL423/4 T_0 or pTAL425/6 T_0; Figure S3A). Primary transformants were grown in a growth chamber under 16 hr light/8 hr dark at 24°C and transferred to a greenhouse after three weeks. Seeds were extracted from ripe fruits, washed with 2.0% HCl for 45 min and air-dried overnight at room temperature.

PCR screening for TALEN activity in primary transgenic plants

PCR/restriction enzyme digestions assays were performed to test if TALENS were active in T_0 plants (Zhang et al. 2010). Five T_0 plants were sprayed with 100µM of β-estradiol, and leaf DNA was extracted 7 days later using the DNeasy Plant Mini Kit (Qiagen). The region encompassing the TALEN target site on the *PRO* gene was amplified by PCR using the forward primer (5' CCAATCTTGAACCCCATCTC 3') and reverse primer (5' GCGAATTTGAGATAAGGGCAAG 3'). Each PCR reaction used 50ng of genomic DNA template in a 50µL volume using Takara ExTaq polymerase (Clonetech). Thermocycler conditions were set according to manufacturer recommendations with the annealing temperature at 55°C and elongation time set for 1 min. *PRO* PCR amplicons were digested with *Sm1*I, which cuts the spacer region between the TALEN binding sites, and 10µL was run on a 0.8% agarose gel. A second round of PCR using 10µL of the digested *PRO* amplicons as template was performed to enrich for *Sm1*I-resistant *PRO* amplicons. Then

the amplicons were digested with *Sm1*I and separated on a 0.8% agarose gel. Presence of *Sm1*I-resistant *PRO* amplicons indicates that the TALENs are active *in planta* and have induced DSBs at the target site. *Sm1*I-resistant *PRO* amplicons were gel extracted, TA cloned into pCR2.8 TOPO vector (Invitrogen), and the presence of TALEN-induced mutations was confirmed by DNA sequencing.

Production of heritable TALEN-induced mutations

Seeds from the primary TALEN transformant were surface sterilized and germinated on solid 1X MS plates. Fifty cotyledons were excised from 7-10 day old seedlings for a second round of tissue culture with hygromycin-B as the selective agent. Because the seedlings are a segregating population, hygromycin-B is needed to select for explants with the TALEN transgene. Following tissue excision, explants were immersed in 100µM β-estradiol/1X MS solution with slow agitation for 30 min. and placed on shooting media. This process was repeated every seven days for a total of three weeks. Plants were regenerated from the explants as described above and the regenerated plants were designated βE-pTAL425/6 M_0 (Figure S3B). βE-pTAL425/6 M_0 plants were screened by the PCR/restriction enzyme assay as described above to identify lines with TALEN-induced mutations in the *PRO* gene. Primers (forward: 5' GCTTTCAGCTTCGATGTAGG 3' and reverse: 5' GGCGAGTACTTCTACACAGC 3') were designed to amplify the hygromycin-B resistance gene to test if the transgene segregated away from the *pro* mutation.

Statistical Analysis

Phenotypes were recorded for 293 segregating *pro*_2 M_1 plants. A Pearson's Chi square test was conducted in R using chisq.test to test if the mutant allele is inherited in a Mendelian manner.

Literature Citations:

Halpin C, Cooke SE, Barakate a, El Amrani a, Ryan MD (1999) Self-processing 2A-polyproteins--a system for co-ordinate expression of multiple proteins in transgenic plants. Plant J **17**: 453–9

McCormick S (1991) Transformation of tomato with Agrobacterium tumefaciens. Plant Tissue Cult Man 1–9

Murashige T, Skoog F (1962) A Revised Medium for Rapid Growth and Bio Agsays with Tohaoco Tissue Cultures. Physiol Plant **15**: 473–497

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

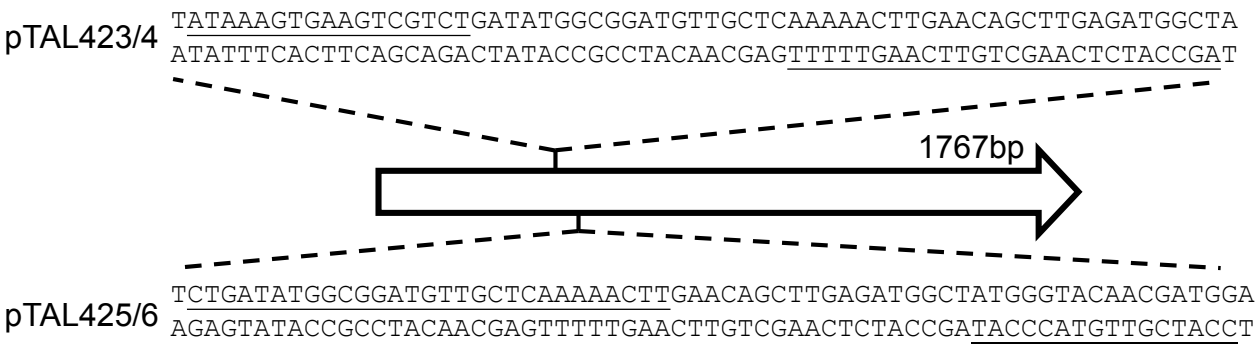

Figure S1. Location of TALEN target sites. Arrow depicts *PROCERA* (Solyc11g011260.1) gene model. Underlined sequences are the binding sites recognized by the TALEN pairs.

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TCTGATATGGCGGATGTTGCTCAAAAACTTGAACAGCTTGAGATGGCTATGGGTACAACGATGGAAGATGGT  WT
TCTGATATGGCGGATGTTGCTCAAAAACTTGAACAGCTTGA--TGGCTATGGGTACAACGATGGAAGATGGT -2
TCTGATATGGCGGATGTTGCTCAAAAACTTGAACAG------ATGGCTATGGGTACAACGATGGAAGATGGT -6
TCTGATATGGCGGATGTTGCTCAAAAACTTGAACAGCT------GGCTATGGGTACAACGATGGAAGATGGT -6
TCTGATATGGCGGATGTTGCTCAAAAACTTGAACA-------ATGGCTATGGGTACAACGATGGAAGATGGT -7
------------------------------------------------------------------------ -168
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Figure S2. Sequences of TALEN-induced mutations generated in somatic cells. Left and right TALEN pair pTAL425/6 binding sites are underlined in wild-type sequence. Deletion sizes (-) are indicated to the right of sequences.

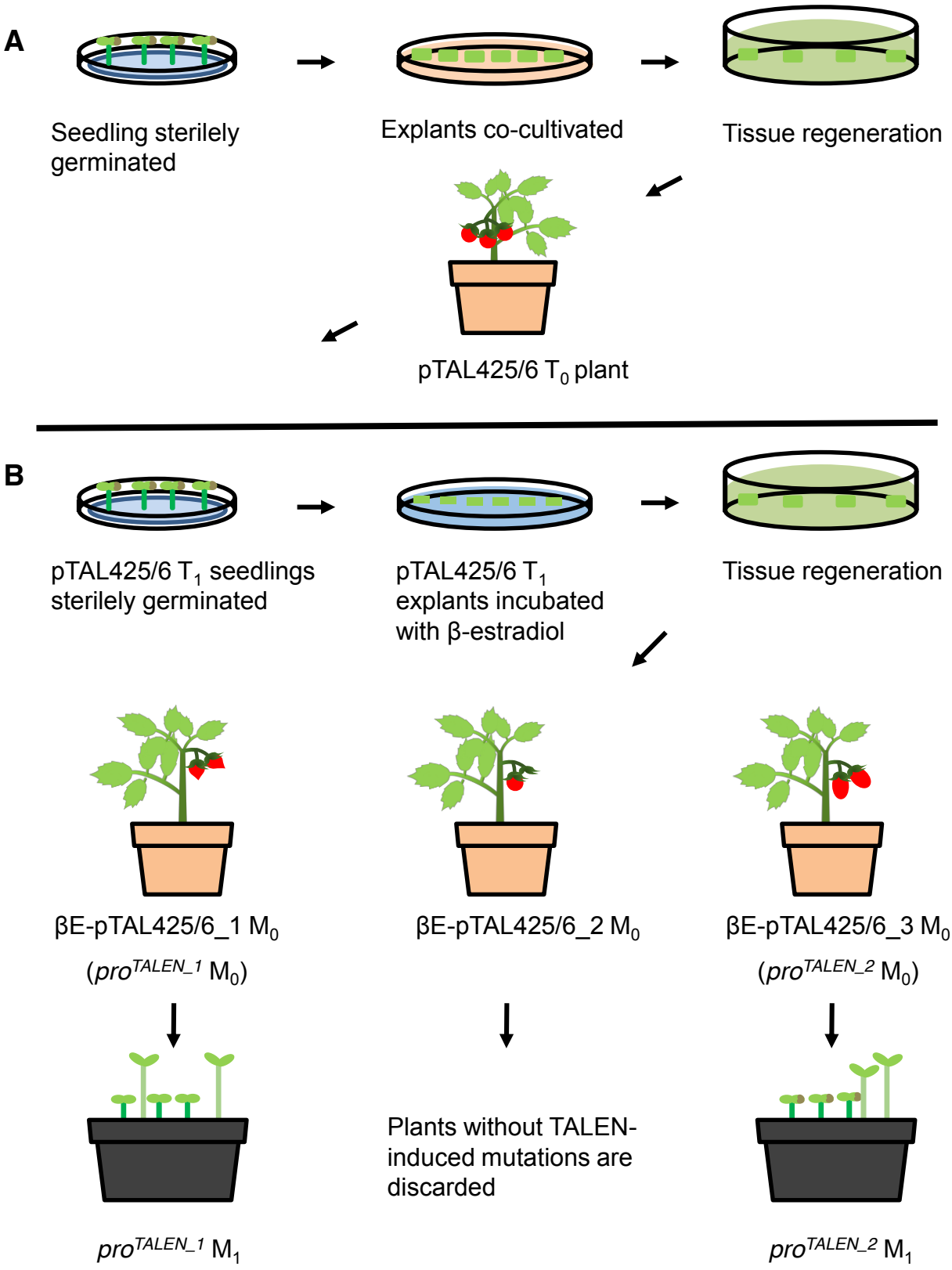
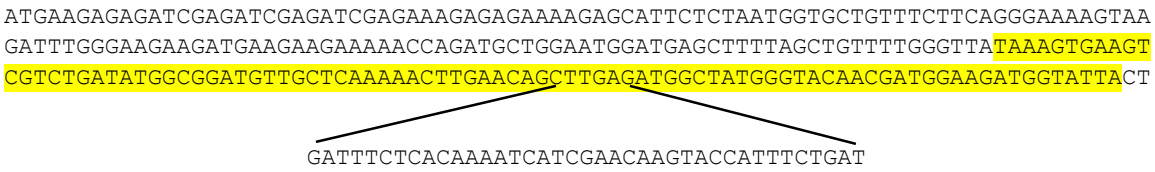


Figure S3. Strategy for regenerating pTAL425/6 TALEN-induced mutants. A, Transgenic plants expressing the pTAL425/6 TALEN are produced in the first round of tissue culture in the absence of β-estradiol to avoid TALEN cytotoxicity. B, pTAL425/6 T_1 seeds are collected from primary transformants in A and sterilely germinated for a second round of tissue culture. Cotyledons are excised and incubated with β-estradiol to induce TALEN expression, and plants, designated as βE-pTAL425/6 M_0, are regenerated and screened by PCR to identify lines with TALEN-induced mutations. βE-pTAL425/6 M_0 plants with TALEN-induced mutations are grown to maturity and *pro*^{TALEN} M_1 seeds are harvested from them.



CATCTTTCTACTGATACCGTTCATAAAAACCCATCTGATATGGCTGGTTGGGTACAAAGTATGTTATCTTCGATTTCGAC
AAACTTTGATATGTGTAATCAGGAAAACGATGTGCTTGTATCTGGTTGTGGTTCTTCTTCTTCTATAATCGATTTCTCAC
AAAATCATCGAACAAGTACCATTTCTGATGATGATTTAAGAGCTATACCTGGTGGTGCTGTTTTCAATTCGGATAGTAAT
AAAAGACACAGATCAACAACTTCTAGTTTTTCAACTACATCCTCATCTATGGTGACAGATTCATCAGCAACGAGACCTGT
TGTACTAGTTGATTCACAAGAAACTGGGGTTCGTCTTGTTCATACTTTAATGGCGTGTGCTGAAGCTGTACAACAAGAAA
ATTTAACTTTAGCGGATCAACTTGTTAGACATATTGGTATTCTTGCGGTTTCACAATCTGGTGCTATGAGAAAAGTTGCT
ACTTACTTTGCTGAAGCATTAGCAAGAAGAATCTACAAAATTTATCCACAAGATTCAATGGAATCATCATATACAGATGT
TTTACAAATGCATTTCTATGAAACTTGCCCTTATCTCAAATTCGCTCATTTTACTGCTAATCAAGCCATTCTTGAAGCGT
TTACAGGTTGTAACAAAGTTCATGTAATTGATTTCAGCTTAAAACAGGGTATGCAATGGCCTGCACTTATGCAAGCTTTA
GCTTTACGCCCCGGTGGACCTCCGGCATTTAGACTCACCGGAATCGGACCTCCACAGCCGGATAACACAGATGCCTTGCA
ACAAGTTGGATGGAAGTTGGCTCAGTTAGCGGAAACTATTGGGGTTGAATTTGAATTCAGGGGATTTGTTGCTAATTCGT
TAGCAGATCTTGATGCGACTATACTTGATATAAGGCCAAGTGAAACTGAAGCAGTAGCTATAAACTCTGTTTTTGAGCTT
CATCGATTGTTATCCCGGCCGGGAGCAATTGAAAAAGTGTTGAACTCTATTAAACAGATTAACCCGAAGATTGTTACTCT
TGTTGAGCAAGAAGCGAATCATAACGCAGGGGTTTTTATTGATAGATTTAACGAAGCTTTGCATTATTACTCAACCATGT
TTGATTCGTTAGAAAGCTCTGGGTCTTCGTCTTCAGCTTCACCAACTGGGATTCTTCCTCAACCTCCGGTGAACAATCAA
GATTTGGTGATGTCGGAGGTTTATTTAGGGAGACAGATTTGTAACGTGGTGGCTTGTGAAGGTTCAGATCGAGTTGAACG
ACATGAAACACTGAATCAATGGAGGGTTAGGATGAACTCATCTGGGTTCGATCCGGTTCATCTGGGTTCAAATGCGTTCA
AACAAGCTTCCATGCTTTTAGCTCTGTTCGCCGGCGGCGATGGTTACAGGGTGGAAGAAAACGATGGGTGTCTTATGTTG
GGGTGGCATACACGGCCACTTATAGCTACCTCCGCCTGGAAGCTATTGCCGGACTCCGGCACCGGCGCCGGAGAAGTCGA
GTTGTAA

Figure S4. The coding region of *PRO* showing the sequences of the *pro*$^{TALEN\_1}$ and one of the *pro*_7 alleles. The 88bp that are deleted in one of the *pro*_7 alleles (Figure 1B) are highlighted. The insertion sequence of *pro*$^{TALEN\_1}$ allele is shown.

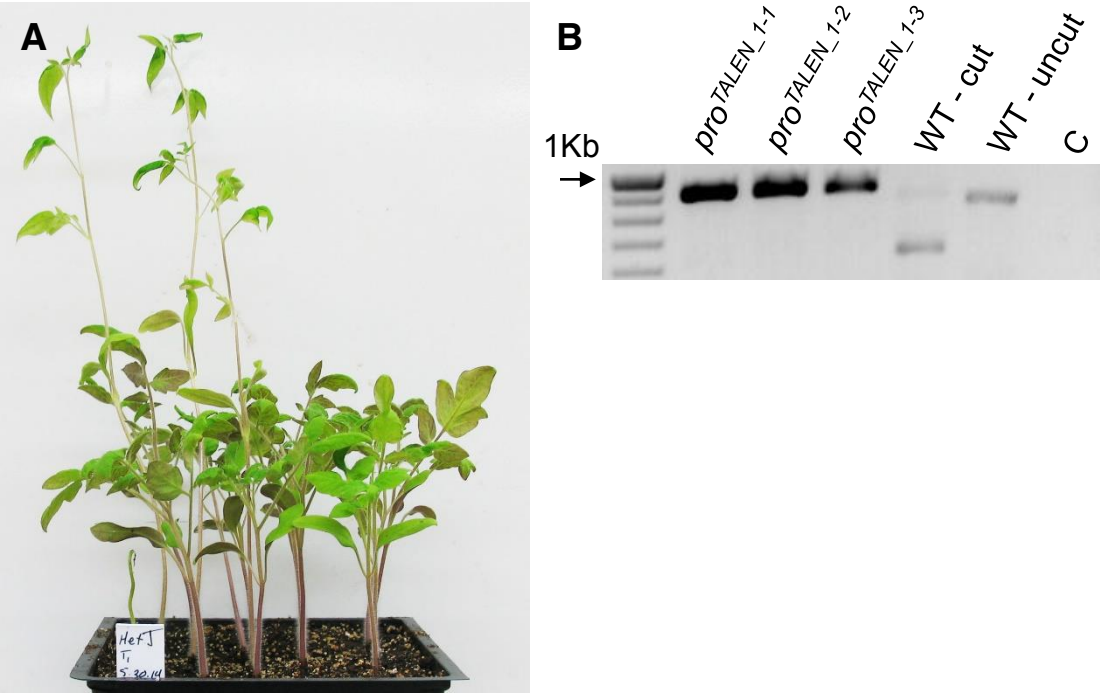


Figure S5. A, Six-week-old seedling segregating for the *pro*$^{TALEN\_1}$ allele. B, PCR–based genotyping confirmed the tall seedlings in A are homozygous for *pro*$^{TALEN\_1}$. The no template control (C) is the negative control.

Table S1. TALEN RVD sequences and TALEN target sequences

TALEN ID	Repeat Number	Spacer Length	Repeat Variable Diresidues (RVDs)	Target Sequence (5'➔ 3')
pTAL423 left	17		NI NG NI NI NI NN NG NN NI NI NN NG HD NN NG HD NG	ATAAAGTGAAGTCGTCT
		19		GATATGGCGGATGTTGCTC
pTAL424 right	26		NI NN HD HD NI NG HD NG HD NI NI NN HD NG NN NG NG HD NI NI NN NG NG NG NG NG	AAAAACTTGAACAGCTTGAG ATGGCT
pTAL425 left	29		HD NG NN NI NG NI NG NN NN HD NN NN NI NG NN NG NG NN HD NG HD NI NI NI NI NI HD NG NG	CTGATATGGCGGATGTTGC TCAAAAACTT
		18		GAACAGCTTGAGATGGCT
pTAL426 right	16		HD HD NI NG HD NN NG NG NN NG NI HD HD HD NI NG	ATGGGTACAACGATGG