

## SUPPLEMENTARY INFORMATION

### **Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion**

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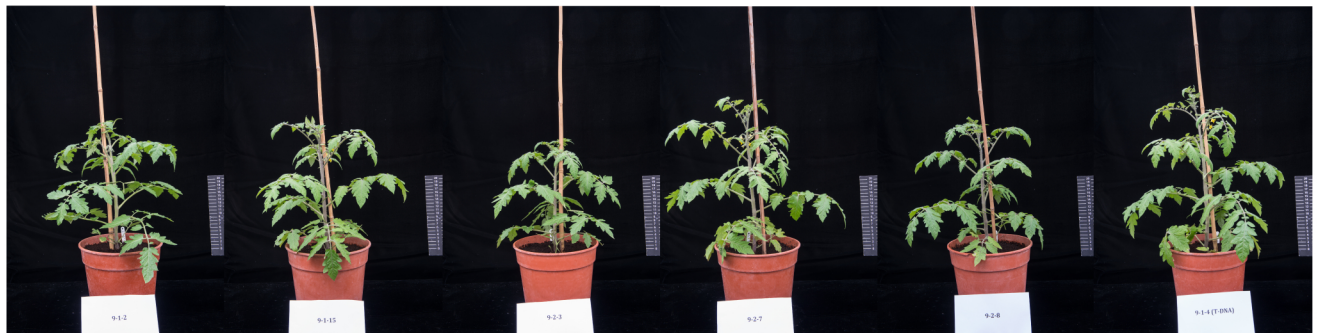
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**WT**

**T-DNA:**    -                      -                      -                      -                      -                      -



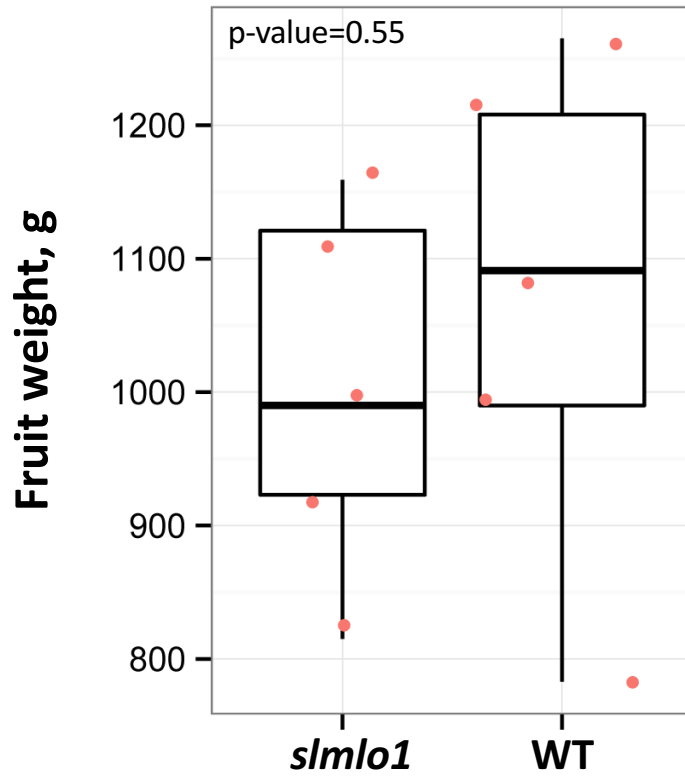
***slm1o1***

**T1 plant:**    **8-1**                      **8-2**                      **8-3**                      **8-4**                      **8-5**                      **8-6**

**T-DNA:**    -                      -                      -                      -                      -                      +

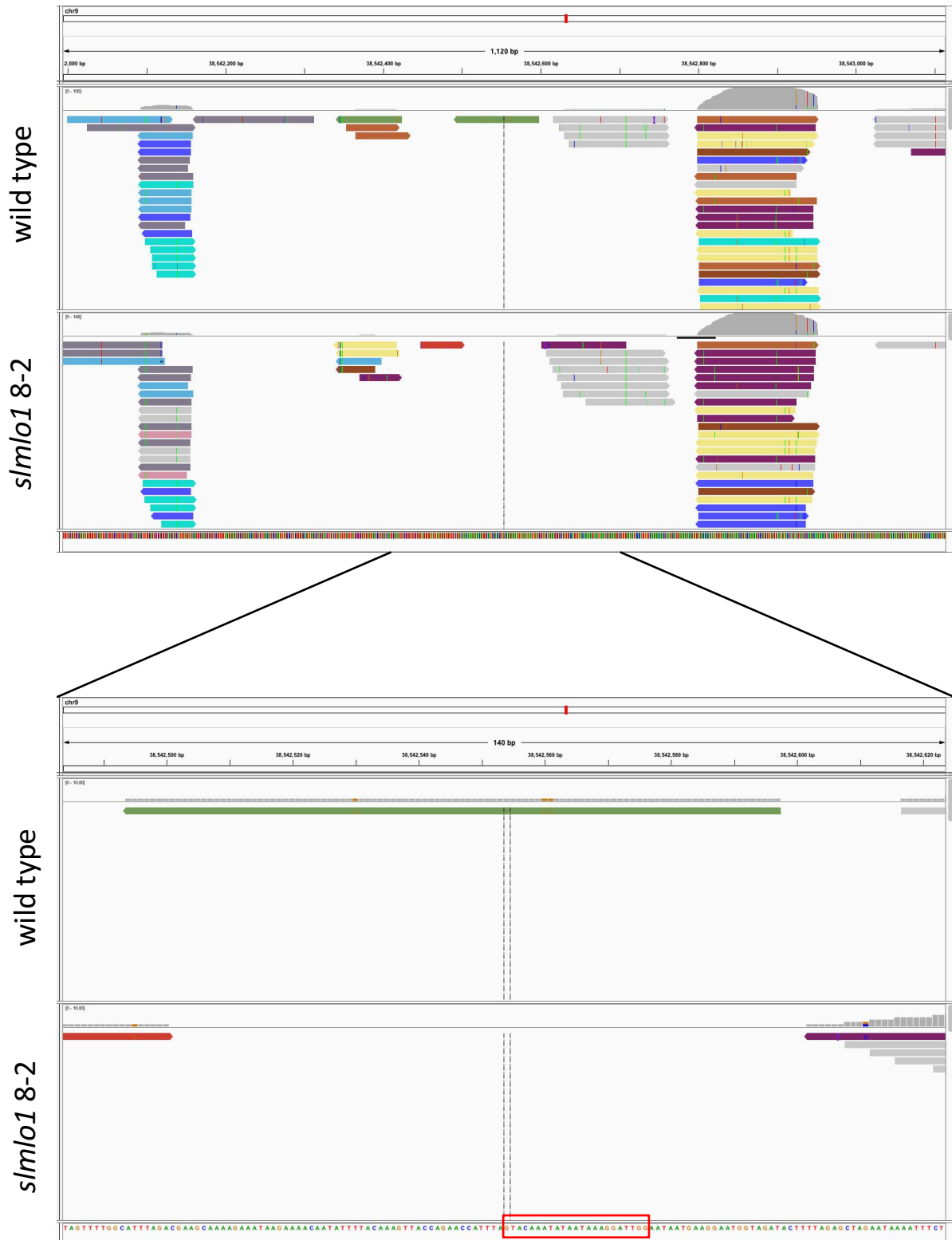
**Supplementary Figure 1.**

Wild type and *slm1o1* tomato plants at the 8 week stage.



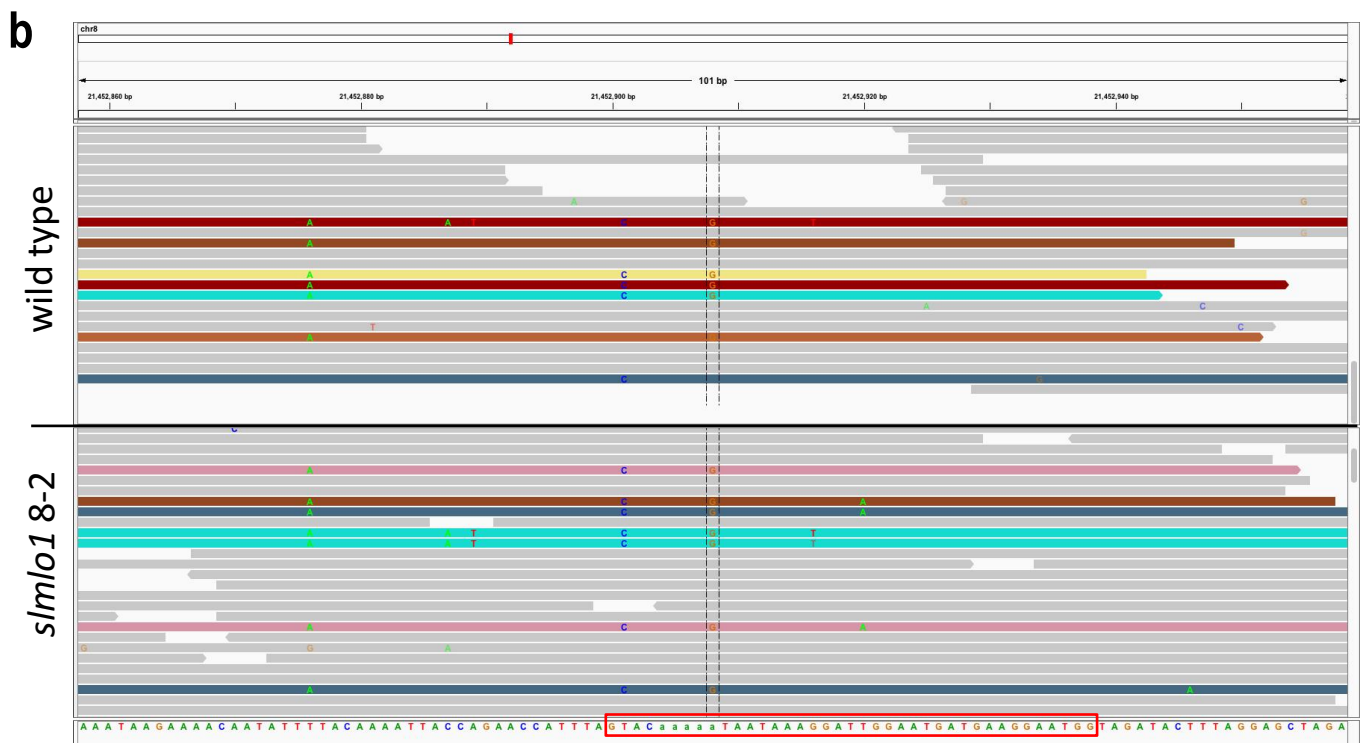
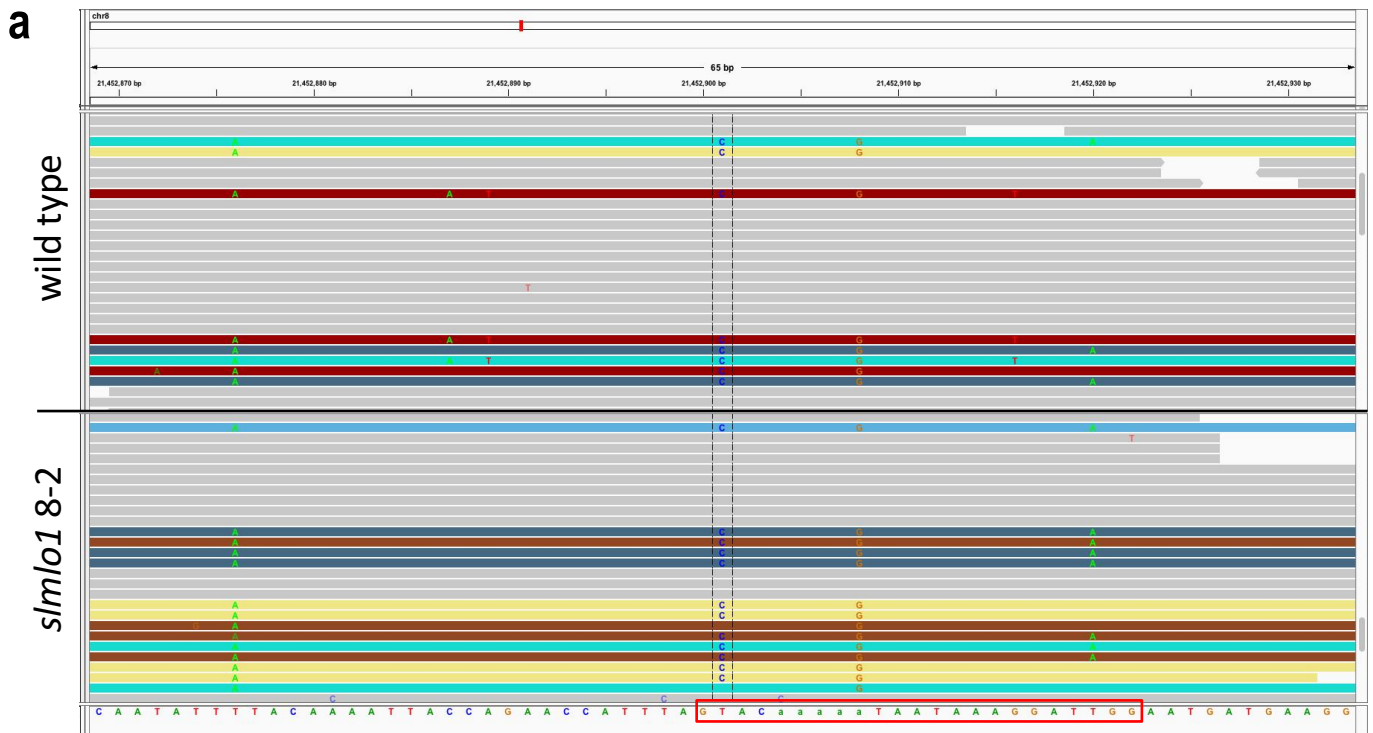
**Supplementary Figure 2.**

Fruit harvest from wild type (WT) and *slmlo1* tomato plants. The graph represents average fruit harvest from five wild type and five *slmlo1* plants.



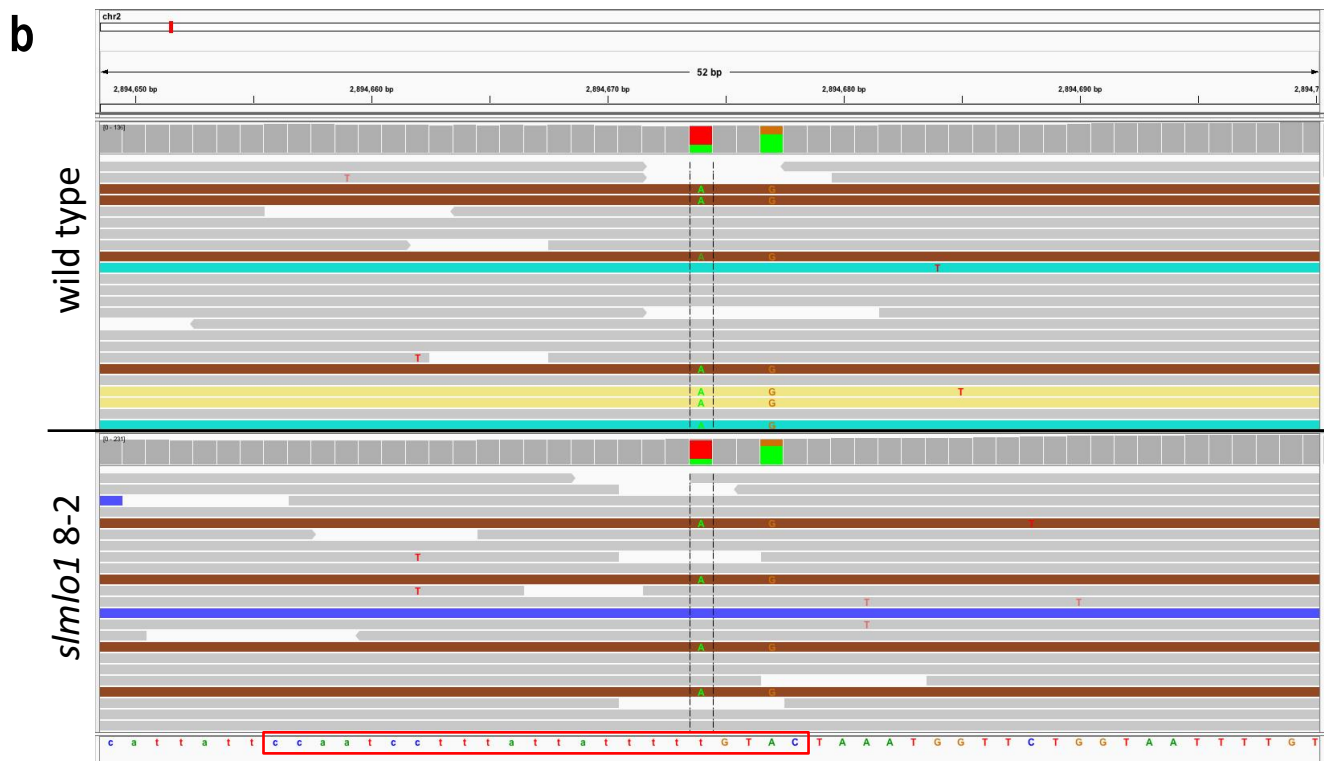
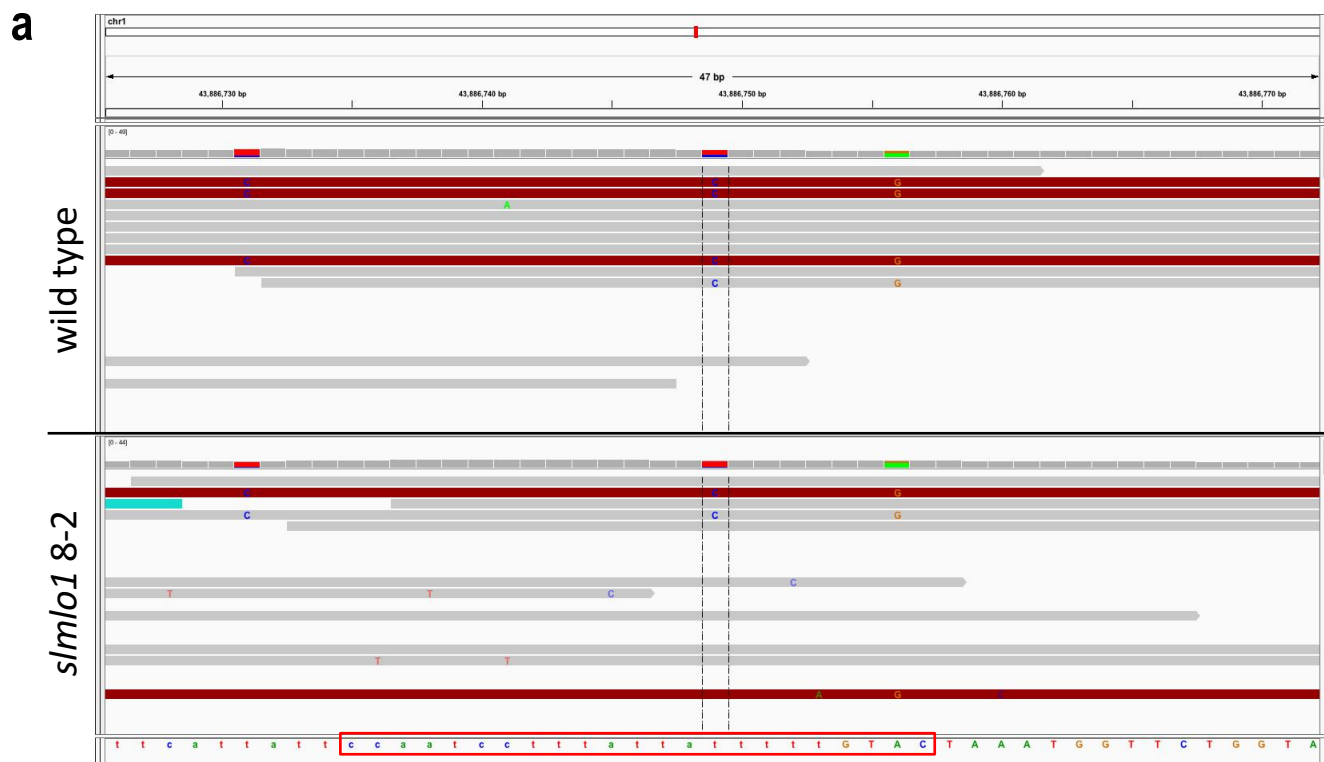
**Supplementary Figure 3.**

Illumina coverage of the off-target on chromosome 9, position 38,542,554.



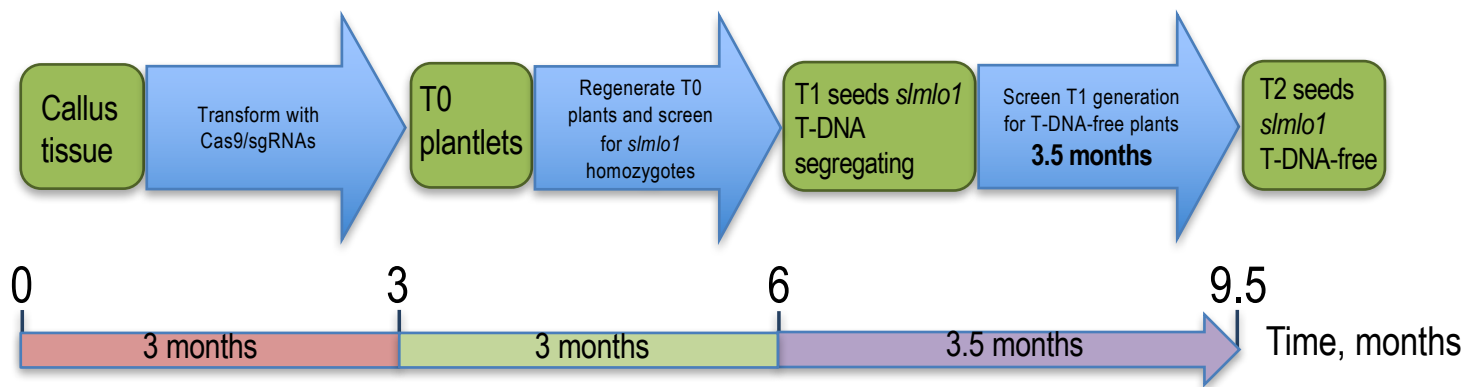
**Supplementary Figure 4.**

Illumina data showing heterozygous SNPs on chromosome 8 at positions 21,452,901 (a) and 21,452,908 (b) in both wild type and *slm1 8-2*.



**Supplementary Figure 5**

Illumina data showing heterozygous SNPs on chromosome 1 at position 43,886,749 (a) and chromosome 2 at position 2,894,674 (b) in both wild type and *slmlo1 8-2*.



**Supplementary Figure 6.**

The timescale of generating nontransgenic *slm1* knockout tomato lines.

**Supplementary Table 1.** Summary of the Illumina sequencing data.

|  | wild type   | <i>slm1o1</i> |             |            |            |            |            |
|--|-------------|---------------|-------------|------------|------------|------------|------------|
|  |             | 8-1           | 8-2         | 8-3        | 8-4        | 8-5        | 8-6        |
| <b>Total Sequences (paired reads)</b>    | 335,281,574 | 102,736,318   | 447,224,142 | 84,559,994 | 89,901,369 | 95,910,142 | 86,911,928 |
| <b>Sequences flagged as poor quality</b> | 0           | 0             | 0           | 0          | 0          | 0          | 0          |
| <b>Sequence length</b>                   | 35-151      | 35-151        | 35-151      | 35-151     | 35-151     | 35-151     | 35-151     |
| <b>%GC</b>                               | 35          | 35            | 35          | 35         | 35         | 35         | 34         |
| <b>Genome coverage</b>                   | 112X        | 34X           | 149X        | 28X        | 30X        | 32X        | 29X        |



**Supplementary Table 8.** Primers used in this study.

| <b>Primer name</b> | <b>Primer sequence</b>                                       |
|--------------------|--|
| Mlo1_target1_f     | TGTGGTCTCAATTGGTGTACCTGTGGTGGAGACGTTTTAGAGCTA<br>GAAATAGCAAG |
| Mlo1_target2_f     | TGTGGTCTCAATTGTACAAAGTTAATCAAGAATGTTTTAGAGCTAG<br>AAATAGCAAG |
| sgRNAr             | TGTGGTCTCAAGCGTAATGCCAACTTTGTAC                              |
| Cas9_2f            | GAGGAAGACAAGACTATCACTCCCTGGAAC TTCGAG                        |
| Cas9_2r            | GAGGAAGACAAATCTTCTTTGAGCTGTTTCACGGTAACT                      |
| SIMloseq3f         | AAGTATGTTTCATGATTGACTAAAG                                    |
| SIMloseq1r         | CCAAGTTAGGAGTTTGTACCG  |
| M13F               | GTTGTAAAACGACGGCCAGT   |
| M13R               | CAGGAAACAGCTATGACC   |