



Virus-induced transgene- and tissue culture-free heritable genome editing in tomato

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Genome editing has emerged as a powerful tool for genome manipulation and trait improvement in crops. However, most commonly used approaches rely on tissue culture and transgenic materials, which are time-consuming, labor-intensive, and often strongly genotype-dependent. Here, we developed a Tobacco rattle virus (TRV)-based system to deliver the compact ISYmu1 TnpB endonuclease, coupled with in planta shoot regeneration, to achieve somatic and heritable genome editing across different tomato cultivars without tissue culture. By targeting *SIPDS*, we successfully generated virus-free homozygous mutant progeny in a single generation. Furthermore, we extended this system to the functional analysis of the previously uncharacterized *SIDA1* locus, revealing its involvement in organ size regulation, and recovered transgene-free *SIDA1* mutants displaying enlarged fruits. Given the wide host range of TRV, our system should be broadly applicable for rapid, nontransgenic and less genotype-dependent heritable genome editing, thereby advancing both functional genomics and crop improvement.

transgene- and tissue culture-free | heritable genome editing | compact TnpB endonuclease | virus-induced gene editing | enlarged tomato fruit

The emergence of genome-editing technology offers tremendous potential for accelerating crop improvement (1, 2). However, despite the rapid adoption of genome editing in many major crops, two significant bottlenecks remain: efficient delivery of genome-editing reagents and the efficient generation of transgene-free plants. Conventional plant genome editing often relies on the use of tissue culture and plant transformation, which typically involves introducing the editing construct into plants through *Agrobacterium tumefaciens*-mediated transformation, selecting transgenic plants that carry the desired mutation, and subsequently removing the transgenic components by crossing (3, 4). This method is time- and labor-intensive and is limited to specific crop species and genotypes, thereby hindering the broader application of genome editing in crop plants.

To overcome these barriers, several plant viruses have been engineered to deliver single-guide RNAs (gRNAs) directly to transgenic plants expressing SpCas9 for virus-induced genome editing (VIGE) in both dicotyledonous and monocotyledonous crop species (5–9). For example, some RNA viral vectors, such as tobacco rattle virus (TRV) and barley stripe mosaic virus (BSMV) have been shown to deliver gRNA to meristematic and germline cells to achieve heritable editing, in part with the help of RNA mobility sequences (10–13). However, most plant viruses are excluded from meristematic tissues, limiting access to the germline and thus reducing the potential to generate gene-edited progeny (14, 15). Moreover, due to the restricted cargo capacity of most viruses, the application of these methods still requires the additional steps of using tissue culture and transformation to obtain the Cas9-overexpressing line as well as crossing to segregate away the transgenes after edits are obtained (16).

A recent strategy to circumvent these limitations was to engineer TRV to deliver the compact ISYmu1 TnpB nuclease and the gRNA, to enable transgene-free germline editing in *Arabidopsis* (17). It remains unknown, however, whether this approach can be applied to crop species. Alternatively, large-capacity vectors such as barley yellow striate mosaic virus (BYSMV) have been used to deliver Cas9 and gRNAs for heritable genome editing in wheat (18, 19). However, this approach faces limitations due to its complexity and dependence on insect transmission, making it difficult to adapt to other plant species. Thus, the development of simple, rapid, and broadly applicable methods for producing transgene-free, heritable genome-edited crops without the need for tissue culture remains an urgent priority.

In this study, we established a strategy for TRV-mediated delivery of the compact ISYmu1 TnpB endonuclease and gRNAs in tomato. Using this system, we first targeted the *SIPDS* gene to successfully generate somatically edited shoots, and also observed

Significance

Efficient genome editing without the need for transgenesis or tissue culture remains a major challenge in crop breeding. Here, we establish a simple single-step system for transgene- and tissue culture-free genome editing in tomato based on Tobacco rattle virus-mediated delivery of the compact RNA-guided TnpB enzyme ISYmu1 and its guide RNA. This strategy enabled somatic editing of de novo shoots and heritable transmission of targeted mutations to the next generation. Notably, editing an agronomically relevant gene produced tomato plants with larger fruits, highlighting the potential of this system for crop improvement.

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transmission of edited alleles to the next generation. We further applied this system to the previously uncharacterized *SIDA1* locus and generated mutants with enlarged fruits. Together, these results indicate that our platform enables efficient somatic editing and stable transmission of targeted mutations in a crop, thereby providing a streamlined, one-step strategy for heritable, transgene-free genome editing in tomato that bypasses tissue culture.

Results

Viral Delivery of ISYmu1 for Somatic Genome Editing in Tomato.

Given their ultracompact size, TnpBs represent attractive nucleases for virus-induced genome editing, as viral vectors can deliver and express the entire genome-editing reagents (17), thereby enabling editing without the need for Cas9-overexpressing plants and eliminating the requirement for transgenesis and tissue culture in crops such as tomato. To this end, we engineered a TRV vector to express both ISYmu1 TnpB endonuclease and its guide RNA, fused to a truncated mutant FT sequence of tomato (mSIFT, with a mutation in the start codon) (Fig. 1A), which has been shown to enhance mobility of the gRNA between cells (20). We then generated a pTRV2-TnpB-gRNA^{SIPDS}-mSIFT vector that expresses a gRNA targeting the tomato phytoene desaturase gene (*SIPDS*), which was delivered to a Red cherry-type cultivar via an *A. tumefaciens* cotyledon infiltration method (Fig. 1B and SI Appendix, Fig. S1A). Three weeks postinfiltration, DNA was isolated from young leaves and used as a template for PCR, followed by NGS analysis of PCR amplicons flanking the *SIPDS* target site. Of the 20 plants infiltrated, we observed three (15%) plants with somatic editing, with indel frequencies ranging from 0.8 to 6.93% indels in systemically infected leaves (SI Appendix, Fig. S1B). We collected seeds from the #17 plant showing 6.93% somatic editing to screen for heritability of edited alleles, however, these somatic mutations failed to be transmitted to the progeny seedlings, which may be attributed to a combination of low efficiency of *Agrobacterium* delivery, viral spread, and TnpB activity, and/or instability of the engineered TRV RNA2. Collectively, these data indicate that TRV can deliver ISYmu1 and gRNA to induce somatic genome editing in tomato.

Induction of Genome Edited De Novo Shoots via Viral Delivery of ISYmu1 and gRNA^{SIPDS}.

Emerging in planta regeneration approaches offer the potential for more efficient induction of de novo shoots (21, 22). This inspired us to explore a heritable editing strategy in which genome editing was first induced in somatic cells, followed by the regeneration of de novo shoots from the edited cells. To achieve this, we removed the shoot apical meristem (SAM) and all axillary shoots from Red cherry-type tomato plants, and then injected *Agrobacterium* encoding TRV-TnpB-gRNA^{SIPDS}-mSIFT into all wounding sites of the main stem and axillary regions (Fig. 1C and SI Appendix, Fig. S2A–C). Five weeks after injection, we observed new shoots initiating from axillary regions, or the formation of callus-like tissues, from which additional shoots subsequently emerged, suggesting that de novo shoots arose from either direct or indirect regeneration, or both (SI Appendix, Fig. S2D and E). A total of 49 plants were injected, from which 72 de novo shoots were initiated (SI Appendix, Table S1). One of the de novo shoots (#3) showed full or partial white sectors on the plant, similar to the known *SIPDS* mutant phenotype (Fig. 1D–G), suggesting biallelic mutations in the *SIPDS* gene. To confirm this, we first performed Sanger sequencing on three randomly selected green leaves of this shoot, which all revealed a 5-bp deletion as indicated by the presence of two sets of peaks in

the sequencing traces at the target site (Fig. 1I). Further amplicon-sequencing (amp-seq) analysis showed that this plant exhibited 56.66% somatic editing efficiency in green leaves (Fig. 1J), consisting of 48.51% of the 5-bp deletion and another 8.15% with a 4-bp deletion. Interestingly, as the plant grew, the petals of all the flowers were white (Fig. 1H), but the fruits did not display a photobleaching phenotype. In addition, we isolated genomic DNA from different organs to measure the mutation frequency. Amp-seq analysis showed that different tissues displayed distinct mutation patterns and editing efficiencies (SI Appendix, Fig. S3A and B), indicating that TRV-TnpB-gRNA^{SIPDS}-mSIFT#3 shoot was chimeric and genome editing continued to occur late in the development of this shoot.

For other newly developed shoots without a photobleaching phenotype (Fig. 1K–M), we first performed Sanger sequencing as a preliminary screening. The results displayed mixed peaks in TRV-TnpB-gRNA^{SIPDS}-mSIFT#7, #17, #43, and #70 lines out of 72 shoots (SI Appendix, Fig. S4A–F), suggesting genome editing had occurred. To quantify this editing, tissue samples from these lines were collected for amp-seq analysis, which revealed somatic mutation frequencies ranging from 25.21 to 49.69% (Fig. 1N–P and SI Appendix, Fig. S5). Notably, #7 and #17 shoots appeared to carry a single predominant deletion with a frequency of roughly 50% (Fig. 1N and O), suggesting these lines were heterozygous for a mutant allele that likely arose during early development of the de novo shoot. These data demonstrate that TRV-TnpB-gRNA^{SIPDS}-mSIFT can induce a high level of somatic edits in de novo shoots.

Heritable Genome Editing via Viral Delivery of ISYmu1 and gRNA^{SIPDS}.

We next investigated whether targeted mutations could be transmitted to the next generation. A total of 96 seeds from four lines (#3, #7, #17, and #43) were sown on 1/2 MS plates. After 1 wk, albino seedlings were observed in the progeny of shoots #3 and #17, suggesting biallelic mutations in the *SIPDS* gene (Fig. 2A, C, E, and G). To characterize the genotypes of the seedlings, we performed Sanger sequencing. Analysis of seedlings from shoot #3 revealed 27 homozygous mutants (28%) with a 5-bp frameshift deletion, 39 heterozygous mutants (41%), and 30 wild type (31%), consistent with a roughly 3:1 segregation ratio and confirming that the parent plant was likely heterozygous for the 5-bp deletion (Fig. 2I). Similarly, the seedlings of shoot #7 showed 23 homozygotes for a 9-bp deletion (24%), 54 heterozygotes (56%), and 19 wild type (20%) (Fig. 2I), and shoot #17 produced 27% homozygotes (8-bp deletion) and 51% heterozygotes. The progeny of shoot #43, however, showed a lower transmission of mutations, with only 2% homozygotes and 26% heterozygotes for a 3-bp deletion. It is not surprising that no albino phenotypes were observed in the progeny of #7 and #43 plants (Fig. 2B, D, F, and H), since these lines carried 9-bp or 3-bp deletions, respectively, which result in in-frame deletions. Together, these data indicate that TRV-mediated delivery of ISYmu1 and gRNA fused to mSIFT is capable of inducing heritable editing in tomato.

Previous studies have shown that TRV is not transmitted to the next generation in plants (17, 23). To verify the absence of TRV in the progeny of TRV-infected plants, reverse transcription PCR was performed on five albino progeny seedlings of both #3 and #17 parent shoots. As expected, TRV and TnpB mRNA signals were not present in any of the albino offspring seedlings (Fig. 2J), and PCR analyses further indicated the absence of viral T-DNA integration (SI Appendix, Fig. S8), demonstrating that the TRV-TnpB-gRNA-mSIFT system enables the generation of edited virus- and transgene-free progeny.

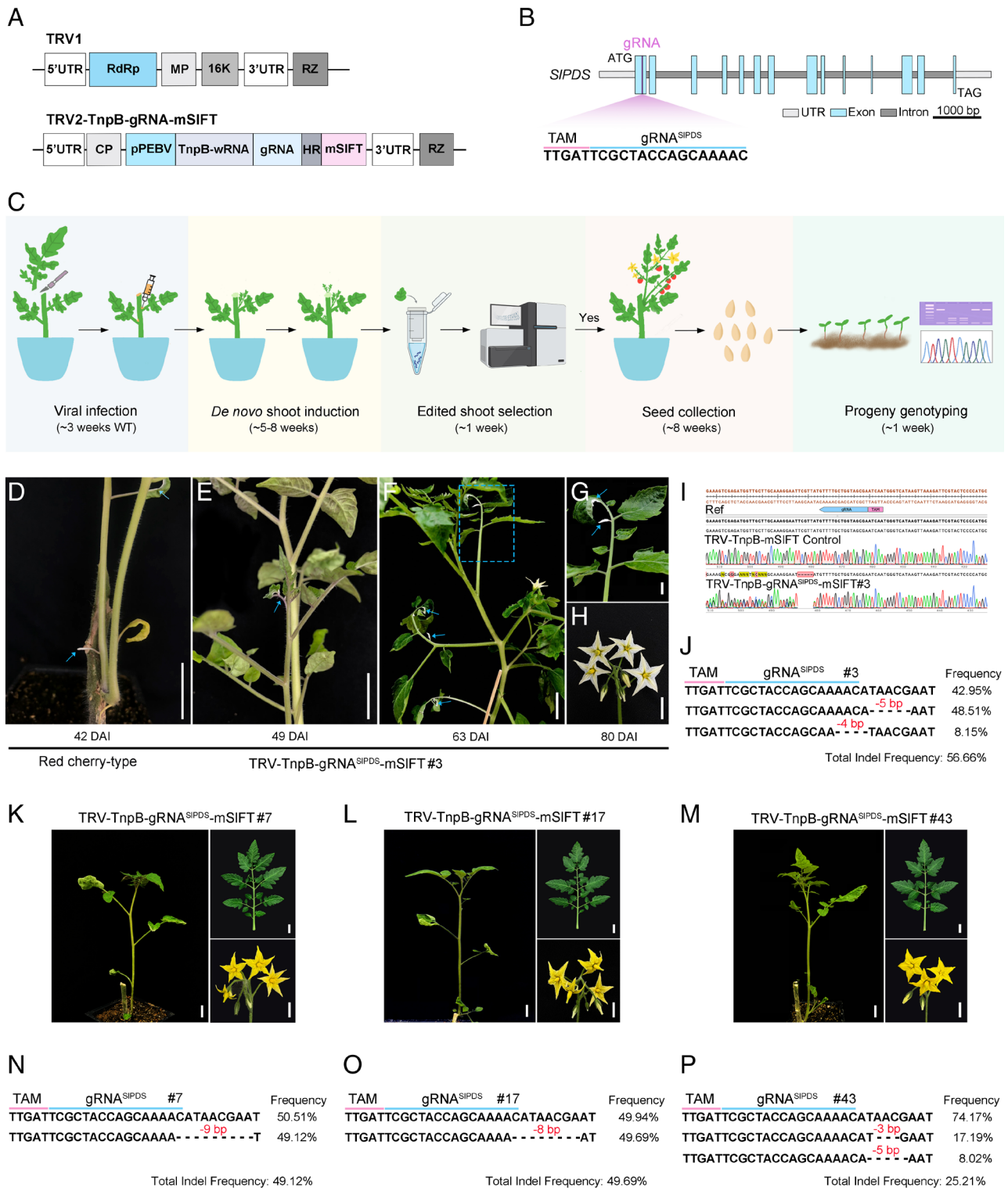


Fig. 1. Virus-induced genome editing in de novo shoots of tomato using TRV-TnpB-gRNA^{SIPDS}-mSIFT. (A) Schematic diagram of the TRV1 and TRV2 plasmids. The tomato mutated *FT* mobility element (mSIFT) was fused to the 3' end of the gRNA in TRV2. RdRp: RNA-dependent RNA polymerase; MP: Movement Protein; CP: Coat Protein; pPEBV: *Pea Early Browning Virus* subgenomic promoter; HR: HDV Ribozyme; mSIFT: mutated tomato *FT* mobility element. (B) Schematic representation of *SIPDS* genomic structure. The light blue box represents the UTR sequence, and dark gray represents introns. The TAM sequence and target sequence are highlighted by the pink and blue lines, respectively. (C) The procedure for TRV-TnpB-gRNA^{SIPDS}-mSIFT-mediated genome editing system in tomato plants. Edited de novo shoots were screened and validated by Sanger and NGS amplicon sequencing. (D-H) Different growth stages of TRV-TnpB-gRNA^{SIPDS}-mSIFT#3 plant displaying albino sectors in leaves and flowers. White leaflets in leaves and the flowers exhibited white petals (H). (G) is a close-up view of (F); DAI: Days After Injection. (Scale bar, 1 cm.) (I) Sanger sequencing of the *SIPDS* gene target site in TRV-TnpB-mSIFT Control (Top) and TRV-TnpB-gRNA^{SIPDS}-mSIFT#3 (Bottom) plants. (J) Indel frequency analysis of the *SIPDS* gene in the TRV-TnpB-gRNA^{SIPDS}-mSIFT#3 shoot. Genomic DNA was extracted from green leaf tissue and subjected to amplicon sequencing using NGS. The TAM sequence and target sequence are highlighted by the pink and blue lines, respectively. Deleted nucleotides are marked by black dotted lines. The different indel types are shown on the Left. The percentage of reads corresponding to each indel type is shown on the Right. The total indel efficiency was calculated by the total number of indel reads divided by total number of reads. (K-M) Phenotype of the TRV-TnpB-gRNA^{SIPDS}-mSIFT#7 (K), #17 (L) and #43 (M) shoots, respectively, showing no obvious albino sectors. Images include the whole plant (Left), a leaf (Top Right), and flowers (Bottom Right). (Scale bar, 1 cm.) (N-P) Indel frequency analysis of the *SIPDS* gene from the TRV-TnpB-gRNA^{SIPDS}#7 (N), #17 (O), and #43 (P) shoots. The reads of sequences with frequencies >5% are shown as well as the total indel frequency.

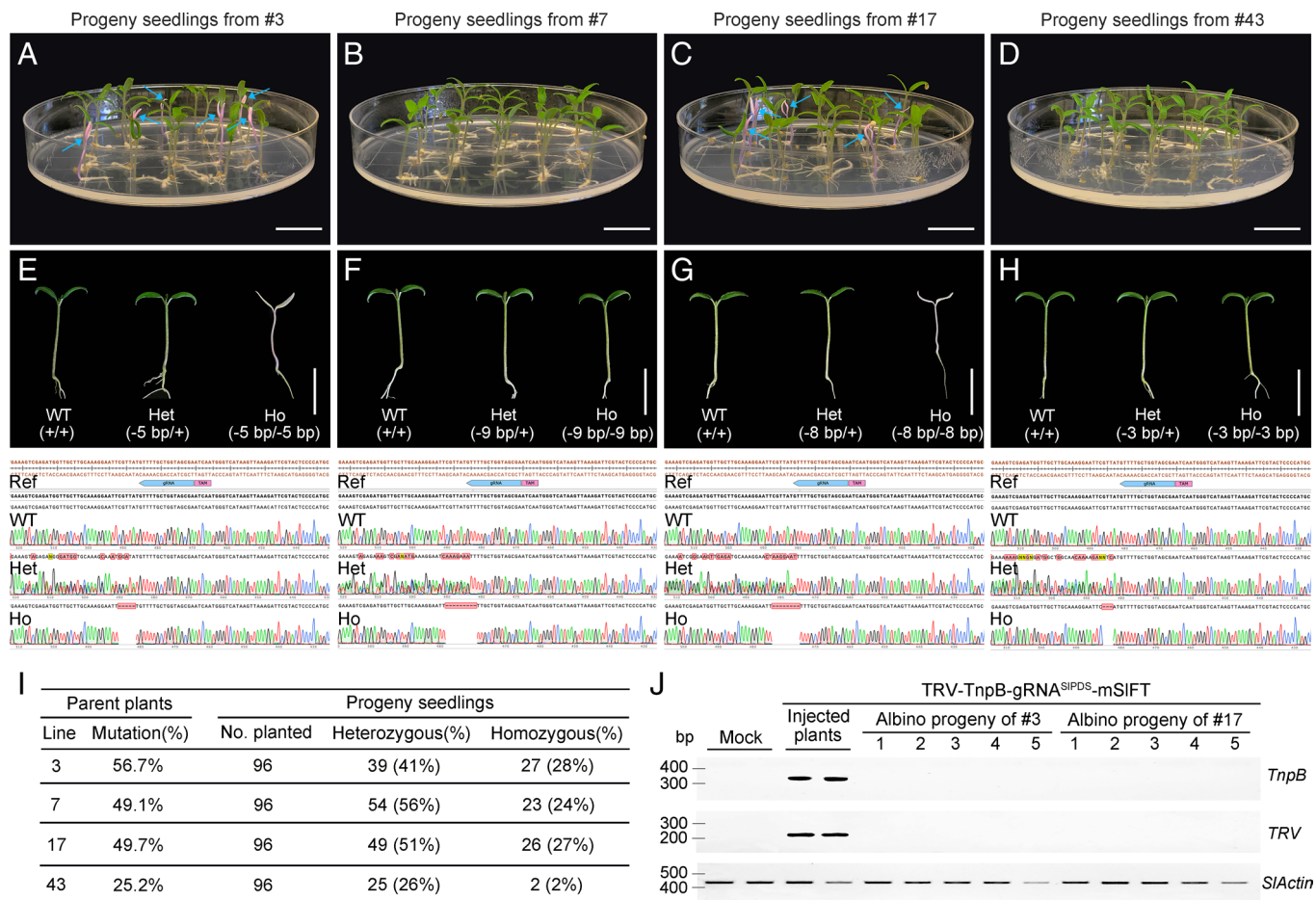


Fig. 2. Transgene-free and heritable genome editing in TRV-TnpB-gRNA^{SID1}-mSIFT infected tomato plants. (A–D) Phenotype of 1-wk-old progeny seedlings from TRV-TnpB-gRNA^{SID1}-mSIFT#3 (A), #7 (B), #17 (C), and #43 (D) parent plants. The albino seedlings are marked with blue arrows. (Scale bar, 2 cm.) (E–H) Phenotyping and genotyping analysis of progeny seedlings from TRV-TnpB-gRNA^{SID1}-mSIFT#3 (E), #7 (F), #17 (G), and #43 (H) parent plants. Representative image of 1-wk-old progeny seedlings (Top). Sanger sequencing trace file of PCR product from progeny seedlings (Bottom). Ref: Reference sequence; WT: wild type; Het: Heterozygous; Ho: Homozygous. (Scale bar: 1 cm.) (I) Table summarizing the percentage of progeny seedlings carrying heterozygous and homozygous mutations from the four parent plants shown in panels A–H. (J) RT-PCR performed using total RNA from two control plants, two TRV injected plants (tissue shortly after injection), and 10 albino progeny seedlings to detect *TnpB* (Upper) or TRV mRNA (Middle) expression. The *SIActin* gene served as an endogenous control (Bottom). The experiment was repeated three times with similar results.

Efficient Somatic and Heritable Editing of *SIDA1* in De Novo Shoots Using TRV to Express ISYmu1 and gRNA in Different Tomato Cultivars. Many prior demonstrations of novel genome-editing approaches have relied on targeting genes with readily observable phenotypic readout, such as *PDS* (24–26). To evaluate whether our system can support the editing of other genes, particularly agronomically relevant loci, we targeted the *SIDA1* gene of tomato, whose homologous genes play important roles in the regulation of seed and organ size in other plant species (27–30), but remains uncharacterized in tomato. To this end, we first conducted a BLAST search using the *Arabidopsis* DA1 protein sequence as a query against the tomato genome database and combined with phylogenetic analysis, identified *Solyc04g079840* as the likely tomato DA1 ortholog (Fig. 3A), hereafter referred to as *SIDA1*. We then generated a TRV-TnpB-gRNA^{SIDA1}-mSIFT construct and utilized the same method (SI Appendix, Fig. S2) to infect multiple tomato cultivars, including M82, Ailsa Craig and Sweet 100, to test whether our system functions effectively in different tomato cultivars (Fig. 3B). Approximately 5 wk after injection, 41 de novo shoots were initiated from 27 injected M82 plants (SI Appendix, Table S1). Three random leaves from each of the 41 de novo shoots were collected for genomic DNA extraction, and the target region was subsequently amplified by PCR and subjected to Sanger sequencing. We observed mixed sequencing peaks near

the *SIDA1* target site in four of the TRV-TnpB-gRNA^{SIDA1}-mSIFT infected shoots #3, #23, #32, and #38 (SI Appendix, Fig. S6A), suggesting the presence of mutations. Further amp-seq analysis of these four shoots detected indel frequencies from 64.36 to 99.68% in the *SIDA1* gene. Notably, shoots #3 and #23 contained more than 99% edited reads, consisting of approximately 50% of one indel type and 50% of another, suggesting that these shoots are biallelic mutants with two different mutations that arose on the two homologous chromosomes very early during shoot development. Similar editing outcomes were also observed in the Ailsa Craig and Sweet 100. In Ailsa Craig, four edited shoots were identified among 47 de novo shoots with indel frequencies ranging from 35.76 to 89.62% (Fig. 3C and SI Appendix, Figs. S6B and S7B). Similarly, in Sweet 100, two edited shoots were recovered out of 24 shoots with editing efficiencies of 84.03% and 99.42%, respectively (Fig. 3C and SI Appendix, Figs. S6C and S7C).

To assess whether edited alleles are heritable, we first screened progeny derived from TRV-TnpB-gRNA^{SIDA1}-mSIFT #3, #23, #32, and #38 plants in the M82 background. For each line, 48 seeds were germinated on plates and analyzed. As no visible phenotypes were observed in the progeny seedlings, Sanger sequencing was performed to examine the presence of targeted mutations. Notably, all seedlings from #3 and #23 plants harbored biallelic or homozygous mutations. In contrast, progeny from line #32

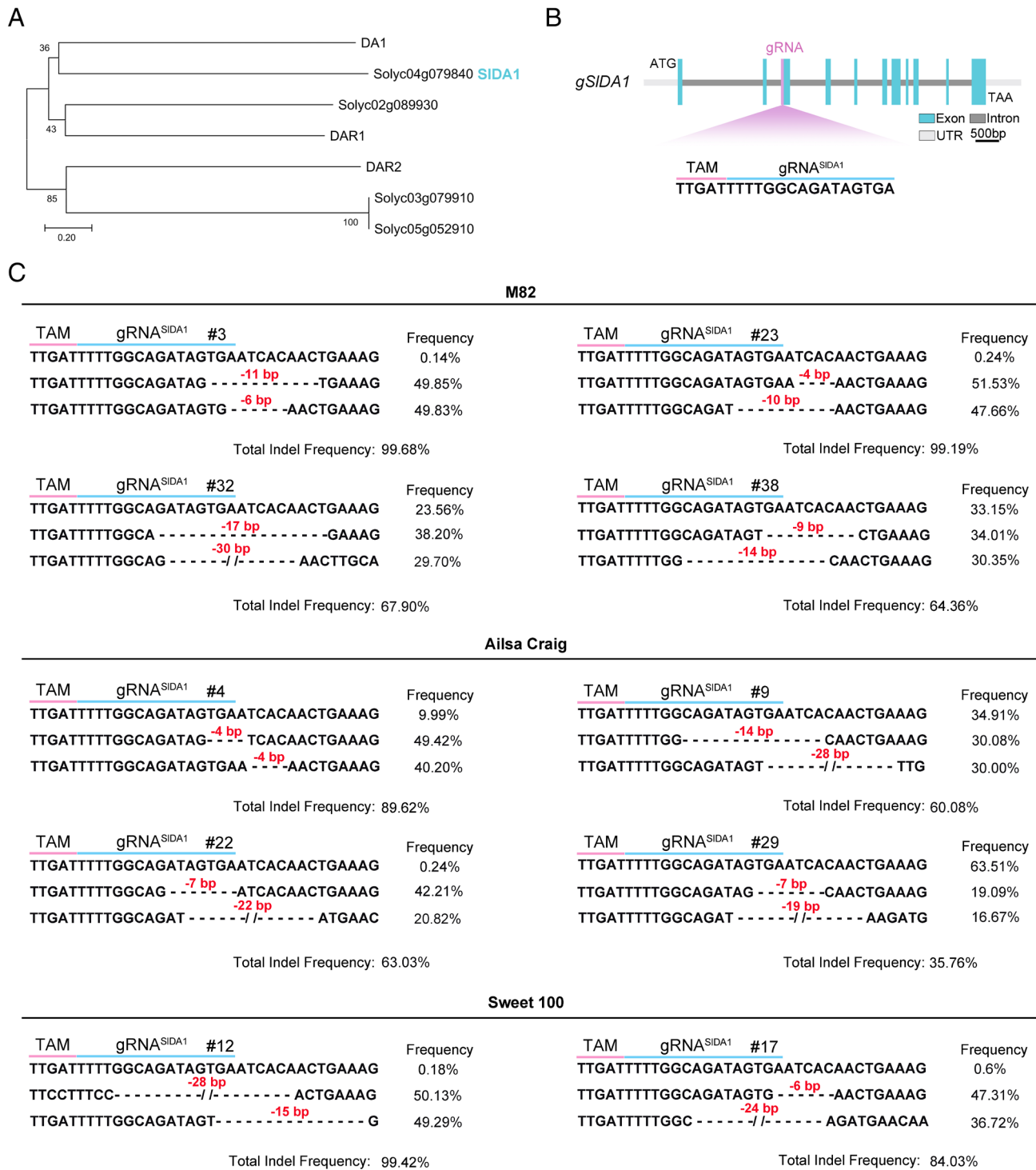


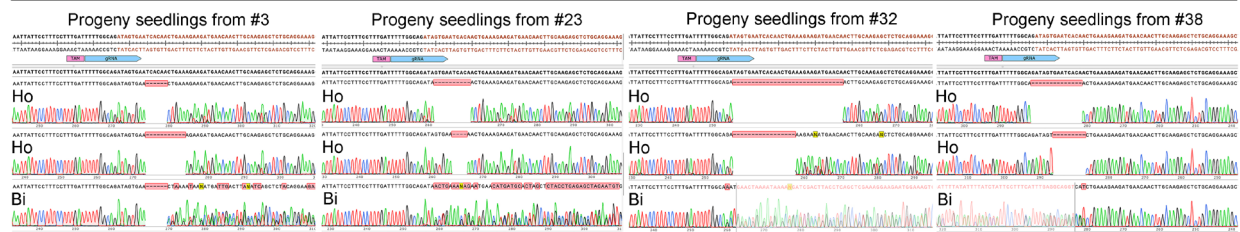
Fig. 3. TRV-mediated delivery of TnpB and guide RNA enables somatic editing of *SIDA1* in different tomato cultivars. (A) Phylogenetic analysis of tomato and *Arabidopsis* DA1 homologs. *SIDA1* is marked in cyan color. (B) Schematic diagram of genomic structure. The cyan box represents exons, light gray represents the UTR sequence, dark gray represents introns, the purple box represents the gRNA site. The TAM and target sequences are highlighted by the pink and blue lines, respectively. (C) Indel frequency analysis of edited shootings in different tomato cultivars infected with TRV-TnpB-gRNA^{SIDA1}-mSIFT. Genomic DNA was extracted from green leaf tissue and subjected to amplicon sequencing using NGS. The TAM and target sequences are highlighted by pink and blue lines, respectively. Deleted nucleotides are marked by black dotted lines. The different indel types are shown on the *Left*. The percentage of reads corresponding to each indel type is shown on the *Right*. The total indel efficiency was calculated by the total number of indel reads divided by the total number of reads. Mutant sequences with frequencies >5% are shown as well as the total indel frequency.

plant exhibited 38% homozygous and 44% biallelic mutations, whereas those from line #38 plant showed 42% homozygous and 38% biallelic mutations (Fig. 4A and D). Similarly, progeny from Ailsa Craig and Sweet 100 edited plants also carried heritable *SIDA1* mutations at substantial frequencies (Fig. 4B–D). Moreover, RT-PCR analysis indicated that neither TRV2 coat protein RNA

nor TnpB transcripts were detected in any of the progeny seedlings (Fig. 4E), suggesting that the progeny were virus-free. PCR analysis further confirmed the absence of viral vector sequences (SI Appendix, Fig. S8), indicating that the progeny did not contain T-DNA insertions. Together, these results demonstrate that TRV-TnpB-gRNA-mSIFT-mediated transgene-free heritable editing can be

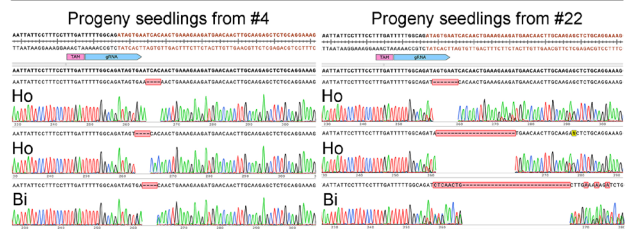
A

M82



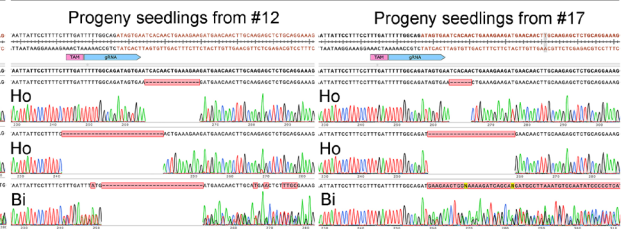
B

Ailsa Craig



C

Sweet 100



D

Cultivar	Parent plants		Progeny seedlings		
	Line	Mutation(%)	No. planted	Homozygous(%)	Biallelic(%)
M82	3	99.7%	48	22 (46%)	26 (54%)
	23	99.2%	48	25 (52%)	23 (48%)
	32	67.9%	48	18 (38%)	21 (44%)
	38	64.4%	48	20 (42%)	18 (38%)
Ailsa Craig	4	89.6%	48	35 (73%)	13 (27%)
	22	63.0%	48	26 (54%)	22 (46%)
Sweet 100	12	99.4%	48	21 (44%)	27 (56%)
	17	84.0%	48	26 (54%)	22 (46%)

E

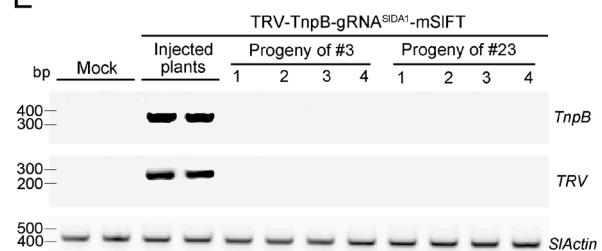


Fig. 4. Transgene-free and heritable genome editing in TRV-TnpB-gRNA^{SIDA1}-mSIFT infected tomato plants. (A) Sanger sequencing trace file of PCR product from progeny seedlings of edited M82 plants. (B) Sanger sequencing trace file of PCR product from progeny seedlings of edited Ailsa Craig plants. (C) Sanger sequencing trace file of PCR product from progeny seedlings of edited Sweet 100 plants. Ho: Homozygous; Bi: Biallelic. (D) Frequencies of biallelic or homozygous mutations in progeny seedlings. (E) RT-PCR performed using total RNA from two control plants, two TRV injected plants (tissue shortly after injection), and four progeny seedlings to detect *TnpB* (Upper) or TRV mRNA (Middle) expression. The *SActin* gene served as an endogenous control (Bottom). The experiment was repeated three times with similar results.

applied to a gene of agronomic interest and in a variety of different tomato cultivars.

Loss of *SIDA1* Function Leads to Enlarged Fruit Phenotypes. Fruit size is a major target trait in modern tomato breeding, as it is closely associated with both yield and commercial value (31, 32). To investigate the phenotypic effects of *SIDA1* mutations, three homozygous mutant lines carrying distinct frameshift mutations were selected for analysis. All three independent *SIDA1* mutant lines exhibited visibly enlarged fruits compared to wild-type plants (Fig. 5A), and quantitative measurements further confirmed these observations. Both fruit height and fruit diameter were significantly increased in the mutants (Fig. 5B and C). Compared with wild-type plants, fruit fresh weight in the *SIDA1* mutants was increased by 22 to 30% (Fig. 5D). The flowers also appeared visibly larger (Fig. 5A). Together, these results indicate that disruption of *SIDA1* promotes organ growth in tomato, leading to increased fruit size, consistent with the conserved role of DA1 homologs in regulating organ size of plants (27–30).

Discussion

To advance modern crop genome editing, it is important to develop rapid and convenient strategies to eliminate the requirement for stable transgene integration and reduce regulatory barriers to generate transgene-free and heritable genome edited crops. VIGE has emerged as a promising approach to achieve these goals (33). In a

number of recent studies, tissue culture-free heritable gene editing was achieved by delivering the gRNA to Cas9-expressing tomato plants using TRV (20, 34, 35). However, this approach still requires the generation of Cas9-expressing plants via tissue culture and plant transformation, as well as self- or backcrossing to remove the transgenic components after the edits are obtained. In this study, TRV-mediated delivery of TnpB and its gRNA efficiently generated targeted mutations at the *SIPDS* and *SIDA1* genes in de novo shoots, with stable transmission to the progeny. Thus, our method simultaneously overcomes two major obstacles for tomato genome editing: dependence on tissue culture and the requirement of transgenic components. We targeted the *SIPDS* gene, a commonly used marker with an easily scorable phenotype, and the previously uncharacterized *SIDA1* gene, demonstrating that its disruption increased fruit size and validated the robustness of the approach across four tomato cultivars. This suggests that our system will be useful for gene function analysis and crop improvement, and potentially be effective in a wide range of tomato cultivars. Additionally, we obtained plants with enlarged fruits in tomato by knocking out *SIDA1*, which also provides a valuable genetic resource for tomato improvement to increase yield.

FT-based mobile RNA elements have been proposed to enhance long-distance transport of genome-editing reagents to the SAM (13). However, when tomato seedlings were inoculated with the TRV-TnpB-gRNA^{SIPDS}-mSIFT vector via cotyledon injection, low levels of somatic editing were observed and no edited progenies were recovered. These results are consistent with previous studies

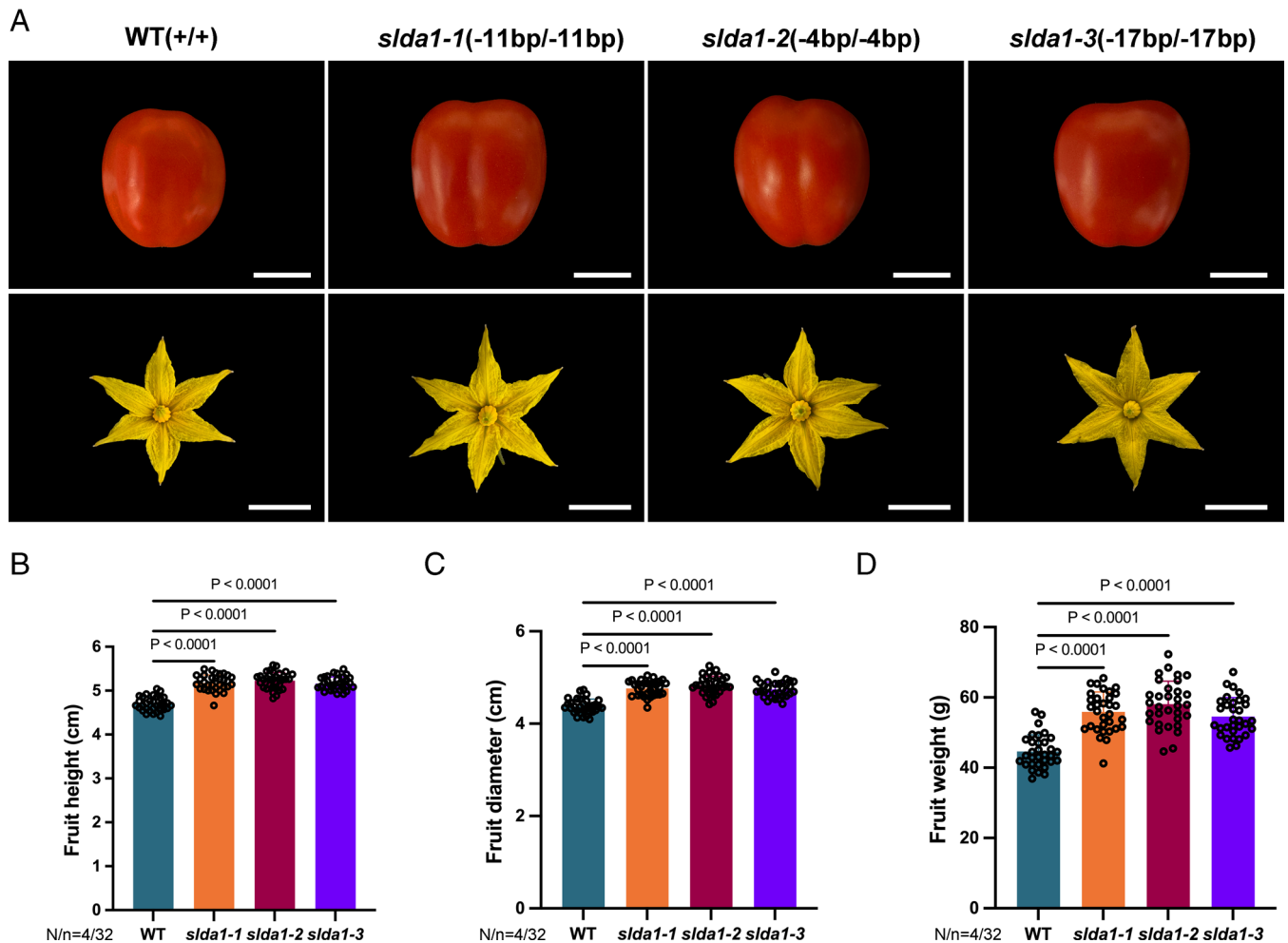


Fig. 5. *SIDA1* mutants exhibit increased fruit size in M82 tomato plants. (A) Phenotypes of fruits (*Top*) and flowers (*Bottom*) of WT and different *SIDA1* mutant lines. Scale bar: 2 cm in *Top* panel and 1 cm in *Bottom* panel. (B–D) Measurement of fruit height (B), diameter (C), and weight (D) in WT and *SIDA1* mutant plants. Fruits at the red-ripe stage were collected from four independent plants (N = 4) per genotype, with eight fruits sampled per plant. Each dot represents an individual fruit, and bars represent the mean \pm SE (N = 32). Statistical significance compared with WT was determined by one-way ANOVA followed by Dunnett's test.

in both tomato and pepper plants, in which TRV-mediated delivery of gRNA^{PDS}-FT into Cas9-expressing plants resulted in partial photobleaching phenotypes that were absent in later-developing leaves at normal growth temperature and failed to yield heritable mutations (34, 36). This phenomenon has been attributed, at least in part, to activation of plant antiviral immune responses that restrict viral replication and systemic movement, thereby limiting viral accumulation and persistence in apical tissues. For example, a very recent study showed that TRV-mediated delivery of CRISPR/AsCas12f achieved heritable, tissue culture- and transgene-free genome editing in tomato only under conditions of RdRPs knock-down or low-temperature growth (37), highlighting the constraints imposed by host antiviral defenses. Moreover, even if low levels of virus reach the shoot apex, the relatively modest editing efficiency of the compact nuclease TnpB may further constrain successful mutagenesis of germline cells. Finally, as TRV replicates and spreads, it can utilize template switching to recombine out the TnpB and/or wRNA cargo sequences, resulting in smaller, more efficiently replicating viruses that are incapable of genome editing (19, 38).

To overcome these limitations, we adopted a de novo shoot-based editing strategy (34), in which genome editing occurs shortly before the initiation and formation of new shoot meristems to circumvent the need for long-distance viral movement, thereby facilitating stable inheritance of targeted mutations. In contrast to flooding-based inoculation approaches previously

employed in *Arabidopsis*, which benefit from the small size and tractability of that model species (10, 17), our strategy is less dependent on whole-plant long distance viral movement and may therefore be readily adaptable to larger plants and diverse crop species. Moreover, the TRV vector in this study possesses a broad host range of more than 400 plant species (39), suggesting that the approach described here should be applicable to many additional crops. In addition, ISYmu1 is compact in size and can likely be incorporated into other viral vectors with cargo capacities comparable to TRV, such as potato virus X (PVX) and pea early browning virus (PEBV) (7, 40), further expanding the range of crops that could benefit from this approach. Given that in planta viral infection strategies have already been demonstrated with many different viruses and plant species, the potential number of systems that could utilize ISYmu1 is large. Our approach should be especially useful in recalcitrant species that are difficult to genetically transform. In the future, further improving editing efficiency and the target sequence range of ISYmu1 and related TnpB enzymes will be essential to fully realize the potential of this strategy. For example, improved TRV VIGE systems have recently been reported using engineered high-activity TnpBs, and multiplexed editing has also been achieved with this system (41–44).

In summary, by integrating the advantages of a compact RNA-guided nuclease, viral infection, and de novo shoot regeneration, this

work demonstrates a faster, simpler, and more cost-effective heritable genome-editing method that could advance functional genomics and precision breeding in tomato and other crops.

Methods and Materials

Plant Material and Growth Conditions. In this study, four tomato genotypes were used for viral infection, including a Red cherry-type tomato (Amazon.com), M82 (USDA), Ailsa Craig (USDA), and Sweet 100 (Zachary Lippman lab). Seeds were soaked in water at 55 °C for 20 min, then planted in germination trays and placed in a long-day growth chamber (16-h light and 8-h dark; day 25 °C/night 21 °C; light intensity: 150 $\mu\text{mol}/\text{m}^2/\text{s}$; 50% humidity) for about 3 wk.

A different procedure was followed for growing plants used for seed harvesting. Two weeks after germination, small seedlings were transferred from germination trays into large pots. They were grown in a greenhouse with long-day conditions (16-h light and 8-h dark; day 24 °C/night 21 °C; light intensity: 150 $\mu\text{mol}/\text{m}^2/\text{s}$; 50% humidity) for about 3 mo.

Plasmid Construction. To generate the TRV2-TnpB-wRNA-ccdb-mSIFT (pYL001) plasmid, the TnpB-wRNA-ccdb-HR-mSIFT sequence with 20 bp overlapping as a gene block was synthesized from Integrated DNA Technologies (IDT). The pMK435 vector was digested by SacI (NEB, R3156S) and PmlI (NEB, R0532S) for 2 h and the largest fragment was purified using the QIAquick Gel Cleanup kit (Qiagen, 28704). Next, the gene block and digested purified pMK435 plasmid fragment were used to make the pYL001 plasmid using NEBuilder HiFi DNA Assembly (NEB, E2621) according to the manufacturer's protocol. Finally, the reaction was transformed into the ccdB Survival2 T1R Competent Cells (Invitrogen, A10460).

The pYL001 plasmid was used as a base vector to construct the TRV2 vector with *SIPDS* and *SIDA1* gRNA using the Anneal and Golden Gate strategy. First, two target site oligos were synthesized from Integrated DNA Technologies (IDT) and diluted to 100 μM , and then phosphorylated and annealed. Next, the annealed double-stranded DNA and the pYL001 plasmid were used in a Golden Gate reaction using PaqCI^{*} (NEB, R0745) and T4 DNA Ligase (NEB, M202), followed by the Golden Gate Assembly Protocol. Finally, the reaction was transformed into the 10-beta competent *Escherichia coli* (NEB, C3019). All the plasmids were confirmed using Primordium whole-plasmid sequencing (Plasmidsaurus). The target site primers are listed in *SI Appendix, Table S2*.

Viral Infection and De Novo Shoot Induction. TRV1 and TRV2 related plasmids were transformed into *Agrobacterium* strain GV3101 electrocompetent cells (Goldbio, CC-207) and grown on LB plates with Kan (50 $\mu\text{g}/\text{mL}$) and RIF (30 $\mu\text{g}/\text{mL}$) for 48 h at 28 °C. A single colony was selected and moved to 2 mL LB liquid media with antibiotics and shaken overnight at 28 °C. After confirmation by bacterial PCR, 1 mL of *Agrobacterium* liquid was transferred into 100 mL of LB liquid media containing KAN (50 $\mu\text{g}/\text{mL}$), RIF (30 $\mu\text{g}/\text{mL}$), 10 mM MES, and 25 μM acetosyringone and grown overnight in a 28 °C incubator. The next day, the *Agrobacterium* culture was centrifuged for 15 min at 4,000 rpm. The supernatant was discarded and the pellet was resuspended in infection buffer containing 10 mM MgCl₂, 10 mM MES and 250 μM acetosyringone to an OD₆₀₀ of 0.6. The *Agrobacterium* cells were then incubated at room temperature for 3 h in the dark. After approximately 3 h, the TRV1 and TRV2 *Agrobacterium* were mixed in a 1:1 ratio and prepared for injection into the tomato plants.

Three-week-old tomato seedlings with about four true leaves were pruned, removing the main shoot and all the axillary shoots, only leaving two true leaves and two cotyledons. TRV1 and TRV2 *Agrobacterium* mix was injected near the axillary site using 1 mL syringes. Successful injection was confirmed by observing the *Agrobacterium* buffer seeping from the axillary site. Plants were then placed in a dark and 50% humidity growth chamber at 25 °C for 2 d, and then grown at 20 °C for 2 d. Next, plants were grown in a greenhouse at a 16/8-h day/night cycle at 24/21 °C. We removed any new shoots that appeared at the axillary sites in the first month to promote the de novo shoot initiation.

Edited Shoots Screening and Next-Generation Amplicon Sequencing. Approximately 35 DPI, 3 random green leaves from each shoot were harvested for genomic DNA extraction by using the Qiagen DNeasy Plant Mini Kit (Qiagen, 69106) according to manufacturer instructions. Target sites were amplified using Q5 DNA polymerase (NEB, M0491s) and PCR products were directly sent

for Sanger sequencing. If editing was observed, plants were transferred to larger pots. Otherwise, the unedited shoots were cut at their base to promote new shoot initiation.

Deep next-generation amplicon sequencing was performed for analysis of the mutation frequency of edited plants. DNA libraries were prepared by using a 2-step PCR amplification method. For the first round of PCR, target regions were amplified with primers close to the target site using Phusion™ High-Fidelity DNA Polymerases (Thermo Fisher, F530S) and the reaction was performed under the following cycling conditions: 98 °C for 30 s, 98 °C for 30 s, 55 °C for 20 s, 72 °C for 30 s, 25 cycles (Step 2); 72 °C for 5 min. Then, the PCR products were purified by using 1.0 \times Ampure XP bead purification (Beckman Coulter, A63881). Purified PCR products were then used as the template for the second round PCR by using Illumina indexing primers under the following cycling conditions: 98 °C for 30 s, 98 °C for 30 s, 60 °C for 20 s, 72 °C for 30 s, 12 cycles (Step 2); 72 °C for 5 min. Subsequently, the second-round PCR products were purified and quantified. Finally, equal amounts of the second-round PCR products were mixed and single-end next-generation sequencing (NGS) on the Illumina NovaSeqX platform was performed. The primers are listed in *SI Appendix, Table S2*.

Mutation Frequency Analysis. Amplicon sequencing analysis was conducted as previously described (45). Single-end reads were processed by adapter trimming with Trim Galore using default parameters, and the resulting reads were aligned to the target genomic region with BWA (v0.7.17) employing the BWA-MEM algorithm. The resulting BAM files were sorted, indexed, and subsequently analyzed with the CrispRvariants R package (v1.14.0). For each sample, distinct mutation patterns and their associated read counts were extracted using CrispRvariants. Based on the assessment of control samples, a stringent classification criterion was applied; only reads containing indels of ≥ 1 bp (insertions or deletions of identical size starting at the same position) with a minimum of 10 supporting reads per sample were considered edited. Single-nucleotide variants were excluded from the analysis.

Progeny Genotyping. Seeds were harvested from the edited parent plants and dried in a 37 °C incubator for 3 d. Seeds were sterilized using a 55 °C water incubator for 10 min, 75% ethanol for 1 min, and washed three times with sterile water, 3% NaClO for 7 min. Seeds were then put in 1/2 MS plates and incubated in darkness at 25 °C for 3 d, followed by growth with long-day conditions (16-h light and 8-h dark; day 25 °C/night 20 °C; light intensity: 150 $\mu\text{mol}/\text{m}^2/\text{s}$; 50% humidity) for about 5 d. Once the cotyledons were fully expanded, half of a cotyledon was collected for DNA extraction using the Invitrogen Platinum Direct PCR Universal Master Mix (A44647500) following the manufacturer's protocol. The extracted DNA was subsequently used as a template for PCR, and the PCR products were subjected to Sanger sequencing. Quantification of indel frequencies was performed with the ICE CRISPR Analysis Tool.

Genotypes were classified according to indel frequencies as follows: Shoots or seedlings with a total indel frequency of 0 to 10% were considered wild type (WT); samples with a single unique sequence modification at 35 to 65%, the nonmodified sequence frequency between 35% and 65%, and the total frequency of single unique sequence modification and nonmodified sequence between 85% and 100% was designated as heterozygous (Het); samples with a single unique sequence modification at 85 to 100% were classified as homozygous (Ho); and samples with two distinct sequence modifications each at 35 to 65% and the total modification frequency at 85 to 100% were designated as biallelic (Bi) for two different alleles.

RT-PCR. Total RNA from progeny seedlings was extracted using the Zymo Research Direct-zol RNA MiniPrep Kit (R2052). Total RNA was reverse transcribed to synthesize first-strand cDNA using the Invitrogen SuperScript IV VILO Master Mix (11766050). The synthesized cDNA was diluted and then used for PCR with specific primers to detect the TRV virus and TnpB expression by using New England Biolabs Q5 High-Fidelity 2 \times Master Mix (M0492L) according to manufacturer instructions. The *SIActin* gene was used as the internal control. PCR products were fractionated by 2% agarose gel electrophoresis. The primers are listed in *SI Appendix, Table S2*.

Fruit Phenotypic Measurement and Statistical Analysis. All wild-type and mutant plants were grown on the same bench in a greenhouse under identical conditions (16-h light and 8-h dark; day 25 °C/night 21 °C; light intensity:

150 $\mu\text{mol}/\text{m}^2/\text{s}$; 50% humidity). To standardize fruit load, the first inflorescence was removed after flowering, and only the four central flowers were retained on each of the second, third, and fourth inflorescences. Fruits were harvested at the red-ripe stage when they had fully developed red coloration. Fruits used for phenotypic measurements were collected from the second to fourth inflorescences. For wild type and each mutant line, four independent plants were analyzed, and eight fruits per plant were selected for measurements of fruit length, diameter, and fresh weight.

Quantitative data are presented as mean \pm SD. Statistical significance among different genotypes was evaluated using one-way ANOVA. Graphs and statistical analyses were performed using GraphPad Prism (V 10.0).

1. A. Tuncel, C. Pan, J. S. Clem, D. Liu, Y. Qi, CRISPR-Cas applications in agriculture and plant research. *Nat. Rev. Mol. Cell. Biol.* **26**, 419–441 (2025).
2. B. Li, C. Sun, J. Li, C. Gao, Targeted genome-modification tools and their advanced applications in crop breeding. *Nat. Rev. Genet.* **25**, 603–622 (2024).
3. F. Altpeter *et al.*, Advancing crop transformation in the era of genome editing. *Plant Cell* **28**, 1510–1520 (2016).
4. R. A. Nasti, D. F. Voytas, Attaining the promise of plant gene editing at scale. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2004846117 (2021).
5. Z. Ali *et al.*, Efficient virus-mediated genome editing in plants using the CRISPR/Cas9 system. *Mol. Plant* **8**, 1288–1291 (2015).
6. Q. Liu, C. Zhao, K. Sun, Y. Deng, Z. Li, Engineered biocontainable RNA virus vectors for non-transgenic genome editing across crop species and genotypes. *Mol. Plant* **16**, 616–631 (2023).
7. M. Uranga *et al.*, Efficient Cas9 multiplex editing using unspaced sgRNA arrays engineering in a Potato virus X vector. *Plant J.* **106**, 555–565 (2021).
8. S. Y. Lee *et al.*, Development of virus-induced genome editing methods in Solanaceous crops. *Hortic. Res.* **11**, uhad233 (2024).
9. B. Ghoshal *et al.*, A viral guide RNA delivery system for CRISPR-based transcriptional activation and heritable targeted DNA demethylation in Arabidopsis thaliana. *PLoS Genet.* **16**, e1008983 (2020).
10. U. Nagalakshmi, N. Meier, J. Y. Liu, D. F. Voytas, S. P. Dinesh-Kumar, High-efficiency multiplex biallelic heritable editing in Arabidopsis using an RNA virus. *Plant Physiol.* **189**, 1241–1245 (2022).
11. T. Li *et al.*, Highly efficient heritable genome editing in wheat using an RNA virus and bypassing tissue culture. *Mol. Plant* **14**, 1787–1798 (2021).
12. S. Tamilselvan-Nattar-Amutha *et al.*, Barley stripe mosaic virus-mediated somatic and heritable gene editing in barley (*Hordeum vulgare* L.). *Front. Plant Sci.* **14**, 1201446 (2023).
13. E. E. Ellison *et al.*, Multiplexed heritable gene editing using RNA viruses and mobile single guide RNAs. *Nat. Plants* **6**, 620–624 (2020).
14. G. Bradamante, O. Mittelsten Scheid, M. Incarbone, Under siege: Virus control in plant meristems and progeny. *Plant Cell* **33**, 2523–2537 (2021).
15. H. Wu *et al.*, WUSCHEL triggers innate antiviral immunity in plant stem cells. *Science* **370**, 227–231 (2020).
16. A. R. Steinberger, D. F. Voytas, Virus-induced gene editing free from tissue culture. *Nat. Plants* **11**, 1241–1251 (2025).
17. T. Weiss *et al.*, Viral delivery of an RNA-guided genome editor for transgene-free germline editing in Arabidopsis. *Nat. Plants* **11**, 967–976 (2025).
18. J.-H. Qiao *et al.*, Transgene- and tissue culture-free heritable genome editing using RNA virus-based delivery in wheat. *Nat. Plants* **11**, 1252–1259 (2025).
19. T. Yoshida, M. Ishikawa, S. Toki, K. Ishibashi, Heritable tissue-culture-free gene editing in Nicotiana benthamiana through viral delivery of SpCas9 and sgRNA. *Plant Cell Physiol.* **65**, 1743–1750 (2024).
20. H. Lee, J. E. Baik, K.-N. Kim, Development of an efficient and heritable virus-induced genome editing system in *Solanum lycopersicum*. *Hortic. Res.* **12**, uhae364 (2024).
21. A. Sebiani-Calvo, A. Hernández-Soto, G. Hensel, A. Gatica-Arias, Crop genome editing through tissue-culture-independent transformation methods. *Front. Genome Ed.* **6**, 1490295 (2024).
22. J. G. Bélanger, T. R. Copley, V. Hoyos-Villegas, J.-B. Charron, L. O'Donoghue, A comprehensive review of in planta stable transformation strategies. *Plant Methods* **20**, 79 (2024).
23. G. H. Kang, Y. Ko, J. M. Lee, Enhancing virus-mediated genome editing for cultivated tomato through low temperature. *Plant Cell Rep.* **44**, 22 (2025).
24. Q. Shan *et al.*, Targeted genome modification of crop plants using a CRISPR-Cas system. *Nat. Biotechnol.* **31**, 686–688 (2013).
25. B.-C. Kang *et al.*, Precision genome engineering through adenine base editing in plants. *Nat. Plants* **4**, 427–431 (2018).
26. Z. Zhong *et al.*, Efficient plant genome engineering using a probiotic sourced CRISPR-Cas9 system. *Nat. Commun.* **14**, 6102 (2023).
27. Y. Li, L. Zheng, F. Corke, C. Smith, M. W. Bevan, Control of final seed and organ size by the DA1 gene family in *Arabidopsis thaliana*. *Genes Dev.* **22**, 1331–1336 (2008).
28. H. Liu *et al.*, TaDA1, a conserved negative regulator of kernel size, has an additive effect with TaGW2 in common wheat (*Triticum aestivum* L.). *Plant Biotechnol. J.* **18**, 1330–1342 (2020).
29. J.-L. Wang *et al.*, Down-regulation of BnDA1, whose gene locus is associated with the seeds weight, improves the seeds weight and organ size in *Brassica napus*. *Plant Biotechnol. J.* **15**, 1024–1033 (2017).
30. C. Li *et al.*, OsDA1 positively regulates grain width in rice. *Crop. J.* **12**, 92–101 (2024).
31. L. Aguirre, A. Hendelman, S. F. Hutton, D. M. McCandlish, Z. B. Lippman, Idiosyncratic and dose-dependent epistasis drives variation in tomato fruit size. *Science* **382**, 315–320 (2023).
32. M. Alonge *et al.*, Major impacts of widespread structural variation on gene expression and crop improvement in tomato. *Cell* **182**, 145–161.e123 (2020).
33. J.-H. Qiao, Q. Gao, X.-B. Wang, Virus-induced genome editing: Toward crop breeding applications. *Trends Plant Sci.*, 10.1016/j.tplants.2026.01.007 (2026).
34. D. Liu *et al.*, Heritable gene editing in tomato through viral delivery of isopentenyl transferase and single-guide RNAs to latent axillary meristematic cells. *Proc. Natl. Acad. Sci. U.S.A.* **121**, e2406486121 (2024).
35. Y. Oh *et al.*, Heritable virus-induced germline editing in tomato. *Plant J.* **122**, e70115 (2025).
36. B. Kang *et al.*, Virus-induced systemic and heritable gene editing in pepper (*Capsicum annuum* L.). *Plant J.* **122**, e70257 (2025).
37. M. Hu, L. Zhang, L. Herrera-Estrella, D. Liu, Heritable, tissue culture-independent and transgene-free genome editing in plants via viral delivery of CRISPR/AsCas12f. *Plant Biotechnol. J.* **24**, 84–86 (2026).
38. A. Khakhar, D. F. Voytas, RNA viral vectors for accelerating plant synthetic biology. *Front. Plant Sci.* **12**, 668580 (2021).
39. S. A. Macfarlane, Tobravirus-Plant pathogens and tools for biotechnology. *Mol. Plant Pathol.* **11**, 577–583 (2010).
40. Z. Ali, A. Eid, S. Ali, M. M. Mahfouz, Pea early-browning virus-mediated genome editing via the CRISPR/Cas9 system in *Nicotiana benthamiana* and Arabidopsis. *Virus Res.* **244**, 333–337 (2018).
41. T. Weiss *et al.*, Efficient transgene-free multiplexed germline editing via viral delivery of an engineered TnpB. *Plant Biotechnol.*, 10.1111/pbi.70644 (2026).
42. B. W. Thornton *et al.*, Engineered TnpB genome editors for plants and human cells identified by ribonucleoprotein mutational scanning. *Nat. Biotechnol.*, 10.1038/s41587-026-03059-7 (2026).
43. U. Nagalakshmi *et al.*, High-efficiency, transgene-free plant genome editing by viral delivery of an engineered TnpB. *Nat. Plants* **12**, 503–511 (2026), 10.1038/s41477-026-02237-4.
44. Z. Zhou *et al.*, Stepwise DNA unwinding gates TnpB genome-editing activity. *bioRxiv* [Preprint] (2026). <https://doi.org/10.64898/2026.01.09.698545> (Accessed 10 January 2026).
45. Z. Li *et al.*, Genome editing in plants using the compact editor CasΦ. *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2216822120 (2023).
46. Y. Liu, Data from, "TRV-TnpB-gRNA-mSIFT induced transgene- and tissue culture-free heritable genome editing in tomato." NCBI Bioproject. <https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1453053>. Deposited 19 May 2026.

Data, Materials, and Software Availability. The amp-seq data generated in this study are accessible at NCBI Sequence Read Archive under BioProject PRJNA1453053 (46). All other data are included in the manuscript and/or SI Appendix.

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