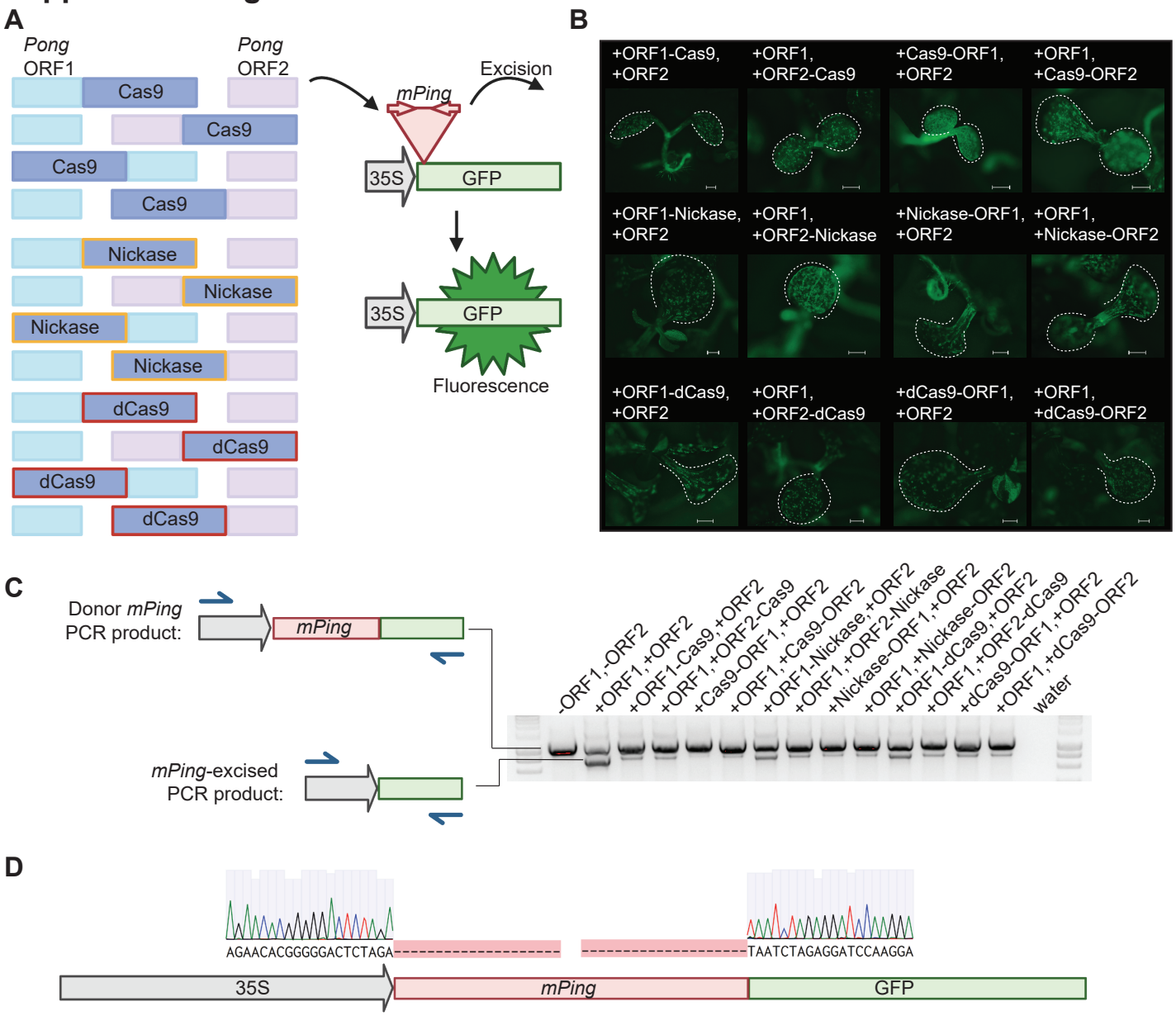
Supplemental Figure 1



Supplemental Figure 1 - Transposable element excision generated by synthetic CAST proteins

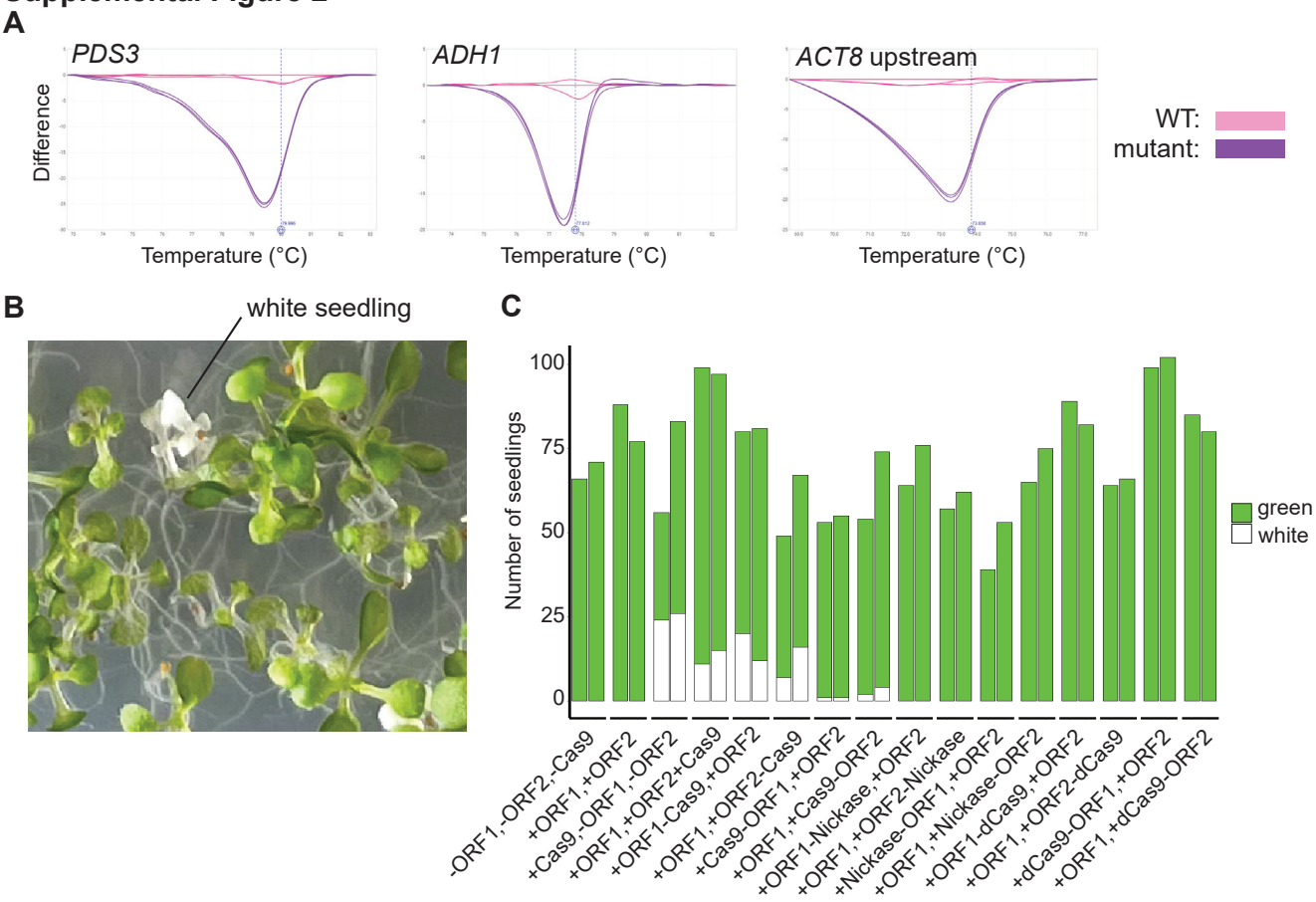
A. Diagram of fusion proteins tested. Twelve different transgenes were created and transformed into Arabidopsis. Cas9 and derivative proteins were fused either to the *Pong* transposase ORF1 or ORF2 protein coding regions. Both N- and C-terminal translational fusions were created using the G4S flexible linker. Three different versions of Cas9 were used: double-strand cleavage Cas9, the single stranded nickase, and the catalytically dead dCas9. When a functional transposase protein is generated by expression of ORF1 and ORF2, it excises *mPing* out of the 35S-GFP donor location in the Arabidopsis genome, producing fluorescence.

B. Excision of the *mPing* TE from GFP restores the plant's ability to generate fluorescence. Images of representative Arabidopsis seedlings showing GFP fluorescence for all 12 fusion proteins. The cotyledons are outlined with a white dashed line. Size bars represent 500 µm.

C. Excision of m*Ping* assayed by PCR in pooled seedlings. The top band represents *mPing* within GFP (donor position), and the smaller band is generated upon *mPing* excision. The blue arrows indicate the pair of primers used for PCR.

D. Sanger sequencing of the PCR product upon *mPing* excision. Gray bars behind the sequencing peaks represent quality scores in that base call.

Supplemental Figure 2



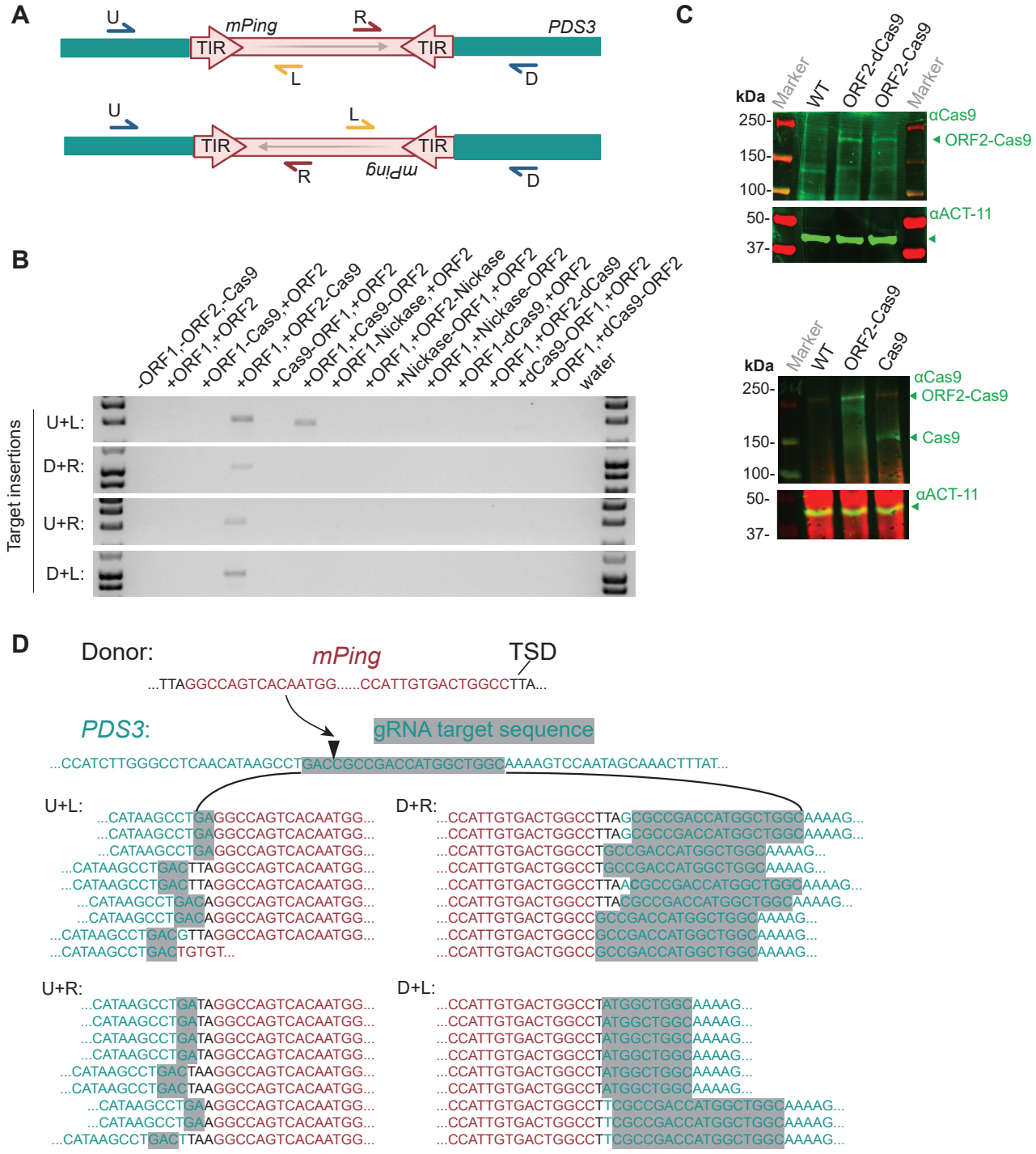
Supplemental Figure 2 - Cas9-induced cleavage ability of synthetic CAST proteins

A. High Resolution Melt (HRM) analysis to test gRNA efficiency. Mutations created by Cas9 were detected for genomic loci *PDS3*, *ADH1*, or the region upstream of *ACT8*. PCR product melting dynamics differed between the WT plants (pink) and Cas9 positive control lines with the indicated gRNA (purple). The melting temperature difference is caused by the generation of short indels and SNPs upon Cas9 cleavage repair by NHEJ, verifying that all three gRNAs are functional in Arabidopsis plants.

B. Representative *pds3* homozygous mutant white seedling in plants with the catalytically-active Cas9 fusion protein.

C. The ratios of green and white *pds3* T2 seedlings for all synthetic CAST proteins tested.

Supplemental Figure 3



Supplemental Figure 3 - Targeted insertions of mPing at the *PDS3* gRNA target site

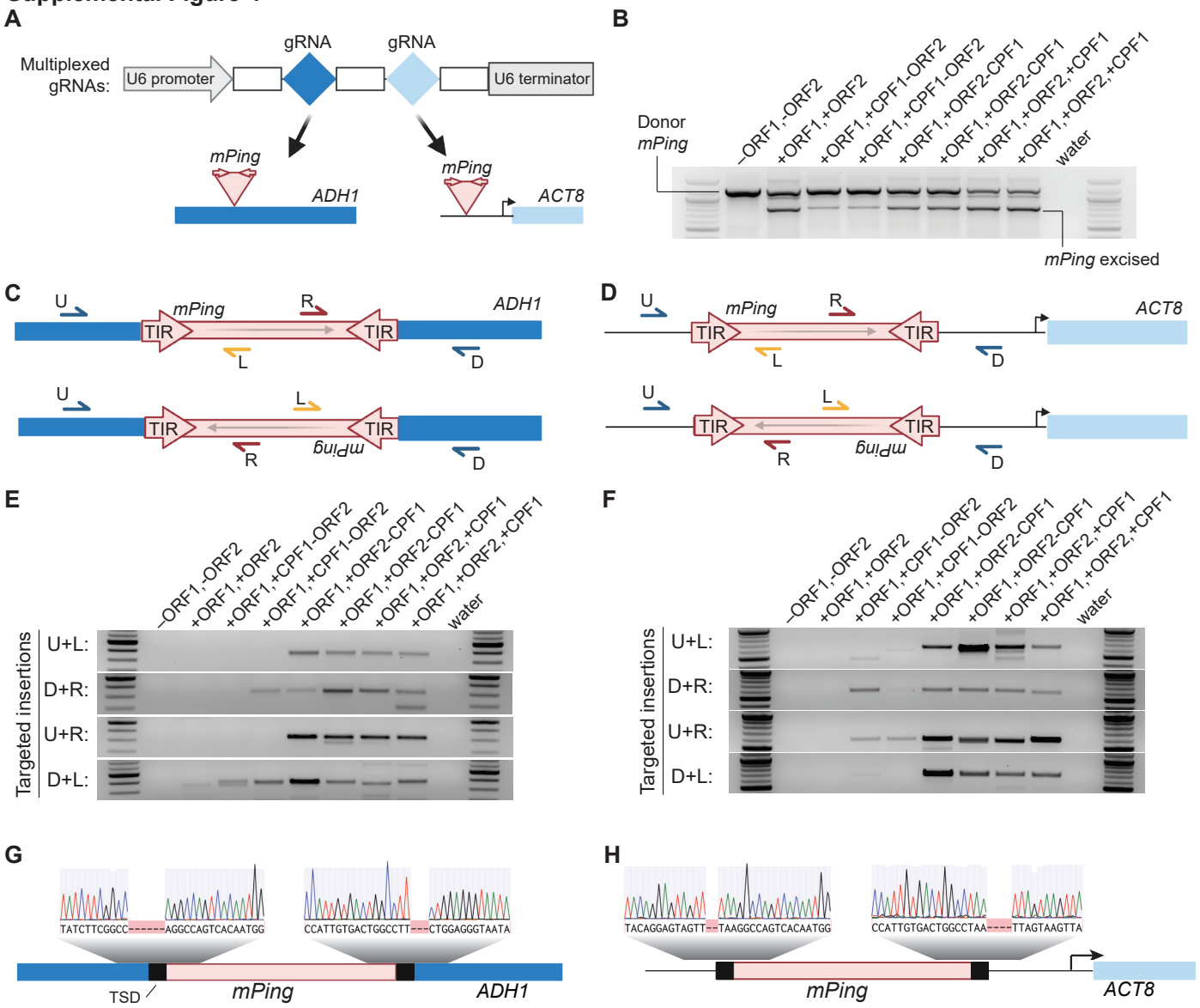
A. PCR primer placement and strategy to detect targeted insertions into the *PDS3* gene. *mPing* can insert in the forward or reverse orientation relative to *PDS3*, and therefore 2 orientations multiplied by two sides of *mPing* to assay, equals four PCR reactions to test for targeted insertion: U+L, D+R, U+R, D+L.

B. PCR assay as described in panel A for the 12 fusion proteins generated in **Supplemental Figure 1A** and controls. PCR negative controls include a line lacking the Cas9, ORF1 and ORF2 proteins (-ORF1,-ORF2,-Cas9), a line with ORF1 and ORF2 but no Cas9 (+ORF1,+ORF2), and a no-template DNA PCR reaction (water). Among the 12 fusion proteins, only ORF2-Cas9 displays the correct size band for targeted insertions. Insertions were verified by Sanger sequencing of these PCR products.

D. Western blots using the Cas9 or Actin11 antibodies, showing that the ORF2-Cas9 and ORF2-dCas9 proteins are expressed in transgenic plants as full-length fusion proteins. Upper panel shows that both ORF2-Cas9 and ORF2-dCas9 have the expected size of ~216 kDa (Cas9 is 150 kDa and ORF2 is 66 kDa). Lower panel compares the size of the unfused Cas9 with the ORF2-Cas9 fusion protein.

E. Sanger sequencing of the junctions of targeted integration events into the *PDS3* gene. PCR products from panel B were cloned into the pCR4_TOPO TA vector and 9 individual colonies were sequenced per PCR reaction. The triangle represents where Cas9 cuts in the gRNA target sequence. The TSD is present at some junctions and absent in others. The *PDS3* sequence is shown in green, the gRNA target site is highlighted in gray, *mPing* is shown as red text, and the TSD sequences are shown in black text.

Supplemental Figure 4



Supplemental Figure 4 - CPF1-mediated targeted insertions

A. Diagram of the multiplexed vector cassette that generates two distinct CPF1 gRNAs, one that targets *ADH1* and one that targets upstream of *ACT8*.

B. PCR assay to detect excision of *mPing* generated by functional ORF1 and ORF2 proteins. Fusing these proteins to CFP1 does not stop excision activity.

C. Diagram of the four PCR-based assay to detect targeted insertions into *ADH1*. Arrows indicate primers used to detect targeted insertions: U+L, D+R, U+R, D+L.

D. Diagram of the four PCR-based assay to detect targeted insertions into the region upstream of *ACT8*.

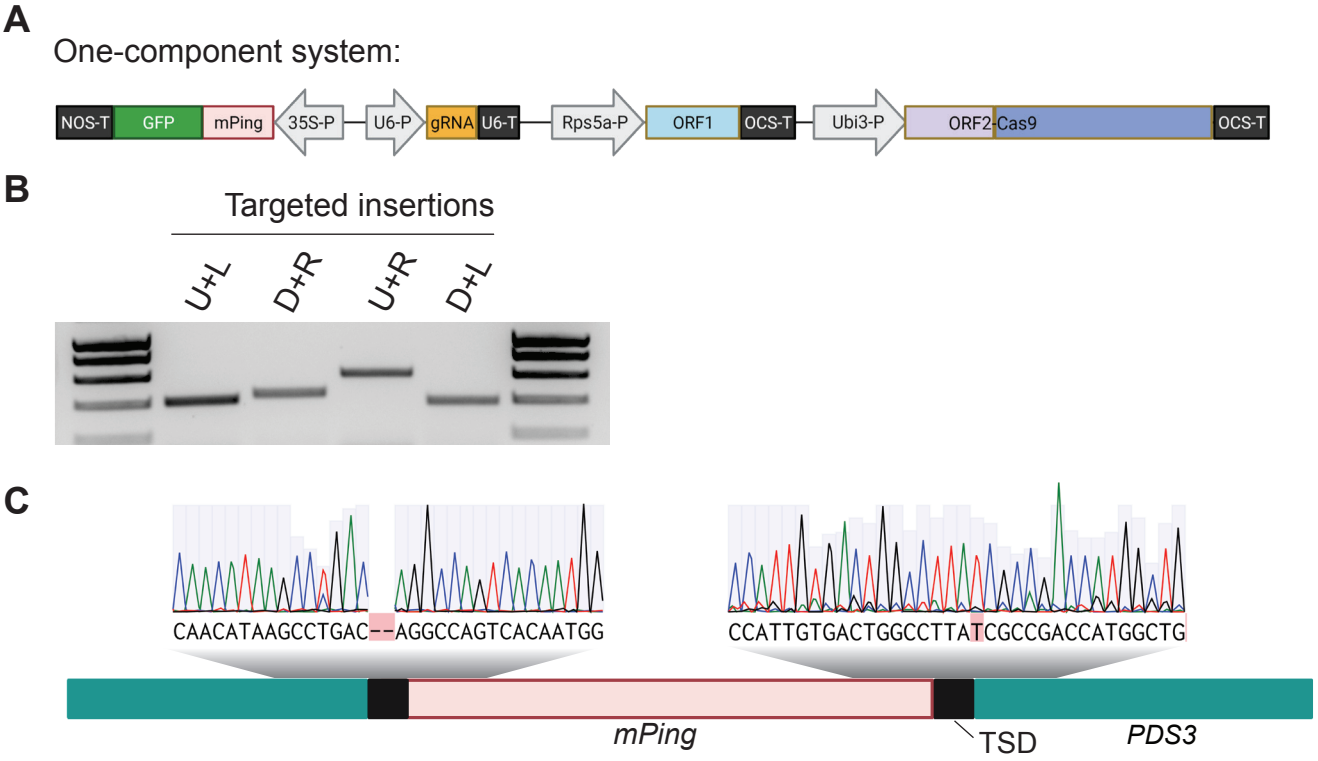
PCR assay to detect targeted insertion of *mPing* into *ADH1*. Targeted insertions are detected for both protein fusions to CPF1 as well as in the unfused configuration.

E. PCR assay to detect targeted insertion of *mPing* into the region upstream of *ACT8*. Targeted insertions are detected for both protein fusions to CPF1 as well as in the unfused configuration.

F. Sanger sequencing of a *mPing* targeted insertion into *ADH1* mediated by CPF1 cleavage.

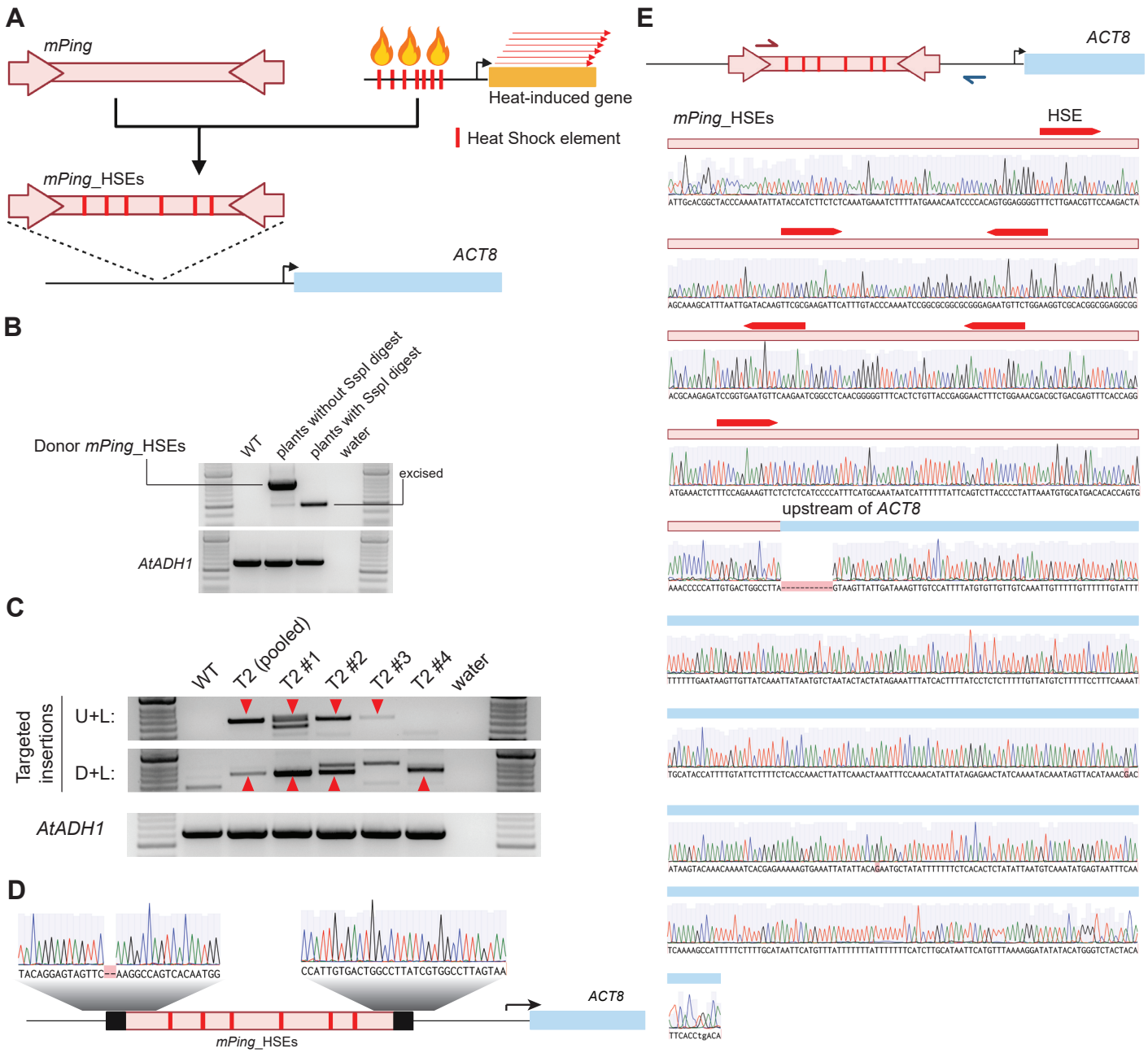
G. Sanger sequencing of a *mPing* targeted insertion into the region upstream of *ACT8* mediated by CPF1 cleavage.

Supplemental Figure 5



Supplemental Figure 5 - Targested insertions directed by a one-component transgene system
A. Map of a single vector containing the *mPing* donor element, the gRNA and protein machinery required to obtain mPing targeted insertions (+ORF1,+ORF2-Cas9).
B. PCR based targeted insertion assay (as in **Supplemental Figure 3A**) in pooled seedlings using the one-component transgene system. Targeted insertions are detected in each reaction.
C. Sanger sequencing of the junctions of a targeted insertion event in the Arabidopsis *PDS3* gene generated from the single vector one-component transgene system shown in panel A.

Supplemental Figure 6



Supplemental Figure 6 - Insertion of heat shock elements (HSEs) as mPing cargo

A. Experimental design and generation of the synthetic 444 bp '*mPing*_HSEs' element.

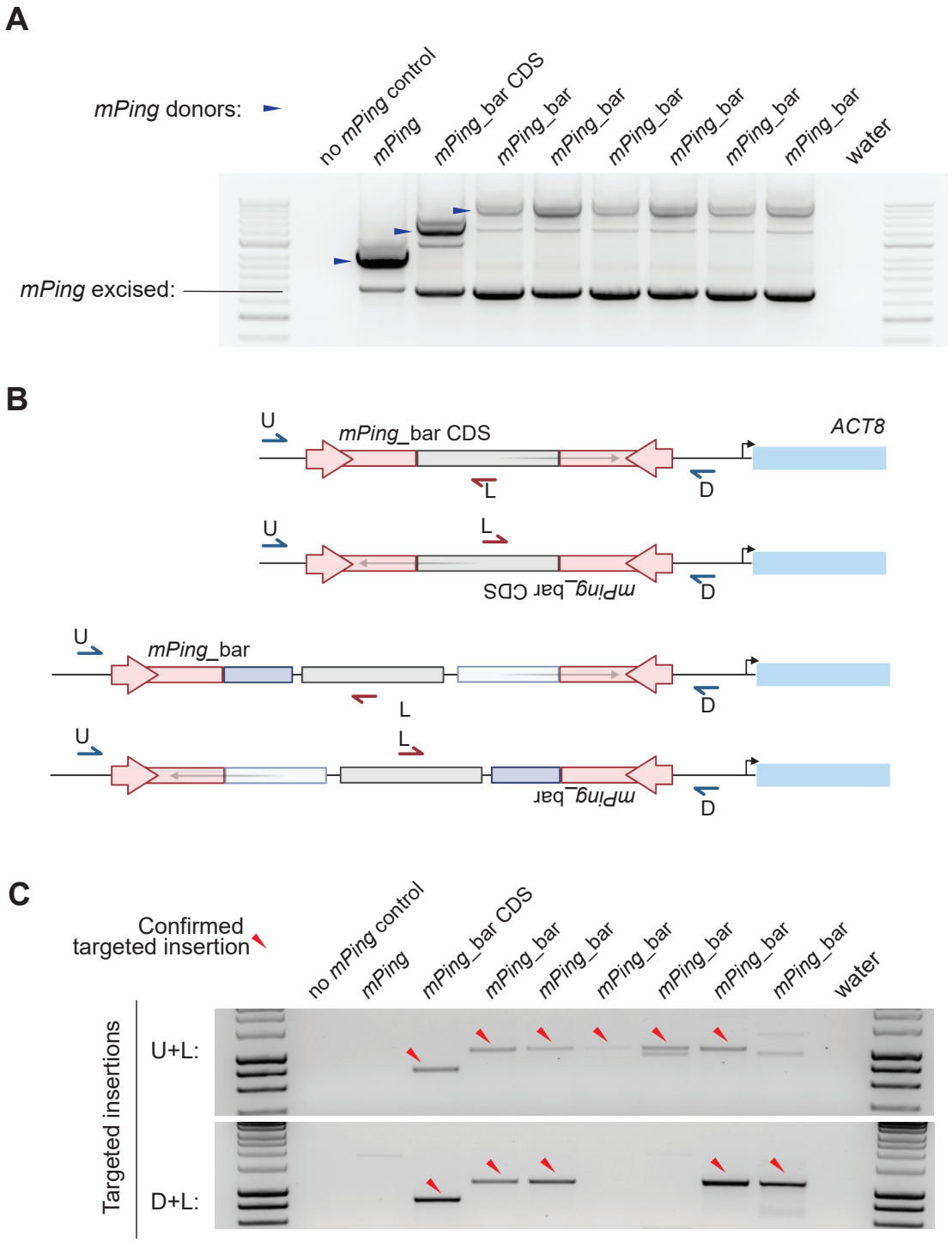
B. Excision assay by PCR (as in **Supplemental Figure 1C**) in pooled seedlings shows the *mPing*_HSEs element is capable of excision. The excised product is easier to detect if the genomic DNA is digested with the *Ssp*I restriction enzyme before PCR (*Ssp*I site is in *mPing*_HSEs).

C. PCR assay detecting targeted insertions (as in **Supplemental Figure 4D**) of *mPing*_HSEs into the region upstream of the *ACT8* gene. The 'T2 (pooled)' sample represents a pool of T2 seedlings, while 'T2 #1', 'T2 #2', etc… are individual T2 plants. Red arrowheads denote PCR products that were verified as targeted insertions by Sanger sequencing.

D. Sanger sequencing of the junctions of a *mPing*_HSEs targeted insertion into the region upstream of *ACT8*.

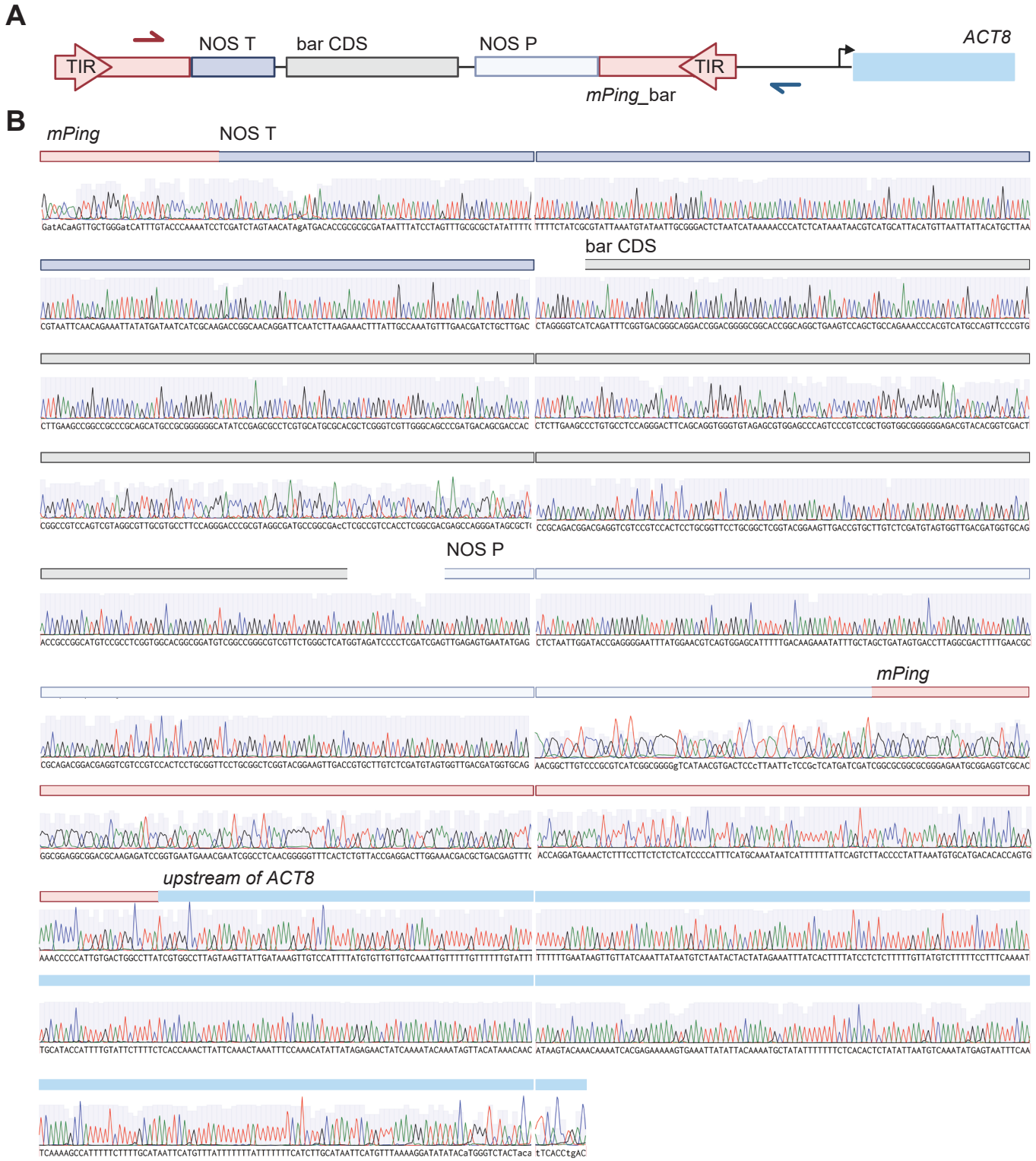
E. Sanger sequencing across the majority of the *mPing*_HSEs element and into the region upstream of *ACT8* demonstrates that all six HSEs were successfully delivered to this region. The red and blue arrows on the top cartoon indicate the pair of primers used for PCR. The Sanger sequencing represents the contig of several sequencing reactions from a single TOPO TA plasmid clone of a PCR product. The sequence is annotated above, including the six HSEs as red pointed boxes.

Supplemental Figure 7



Supplemental Figure 7 - Targeted insertion of a gene and CDS as *mPing* cargos
A. Excision assay by PCR (as in **Supplemental Figure 1C**) in pooled seedlings shows the *mPing*_bar CDS and *mPing*_bar versions are capable of excision. Blue arrowheads indicate the expected size of the amplicon with different sized *mPing* versions before excision.
B. PCR strategy and primer placement to detect targeted insertions of *mPing*_bar CDS and *mPing*_bar into the region upstream of *ACT8*. Arrows indicate primers used to detect targeted insertions: U+L, D+L. The "L" primer is the same for *mPing*_bar CDS and *mPing*_bar versions.
C. PCR detecting targeted insertions of *mPing*_bar CDS and *mPing*_bar into the region upstream of the *ACT8* gene. Red arrows indicate correct size PCR products that were verified as targeted insertions by Sanger sequencing. There is no PCR product in the '*mPing*' sample because this "L" PCR primer site is in the bar CDS region (see panel B).

Supplemental Figure 8

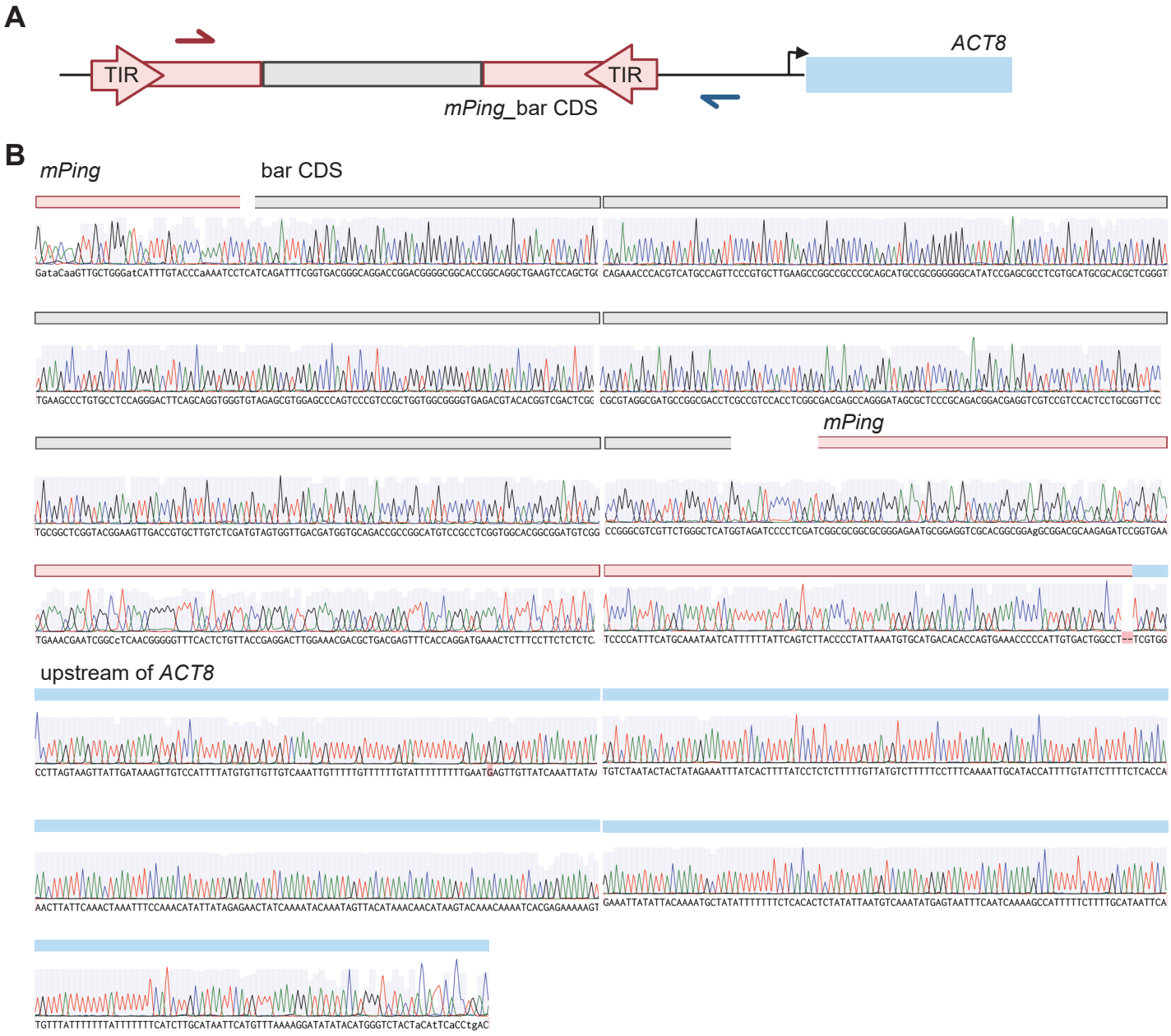


Supplemental Figure 8 - Targeted insertion of the intact bar gene cassette

A. PCR strategy and primer placement to detect targeted insertions of the *mPing*_bar element into the region upstream of the *ACT8* gene. The red and blue arrows indicate the pair of primers used for PCR.

B. Sanger sequencing across the majority of the *mPing*_bar element and into the region upstream of *ACT8* demonstrates the successful delivery of the complete bar gene cassette (including promoter and terminator) into this region. The Sanger sequencing represents the contig of several sequencing reactions from a single TOPO TA plasmid clone of a PCR product.

Supplemental Figure 9

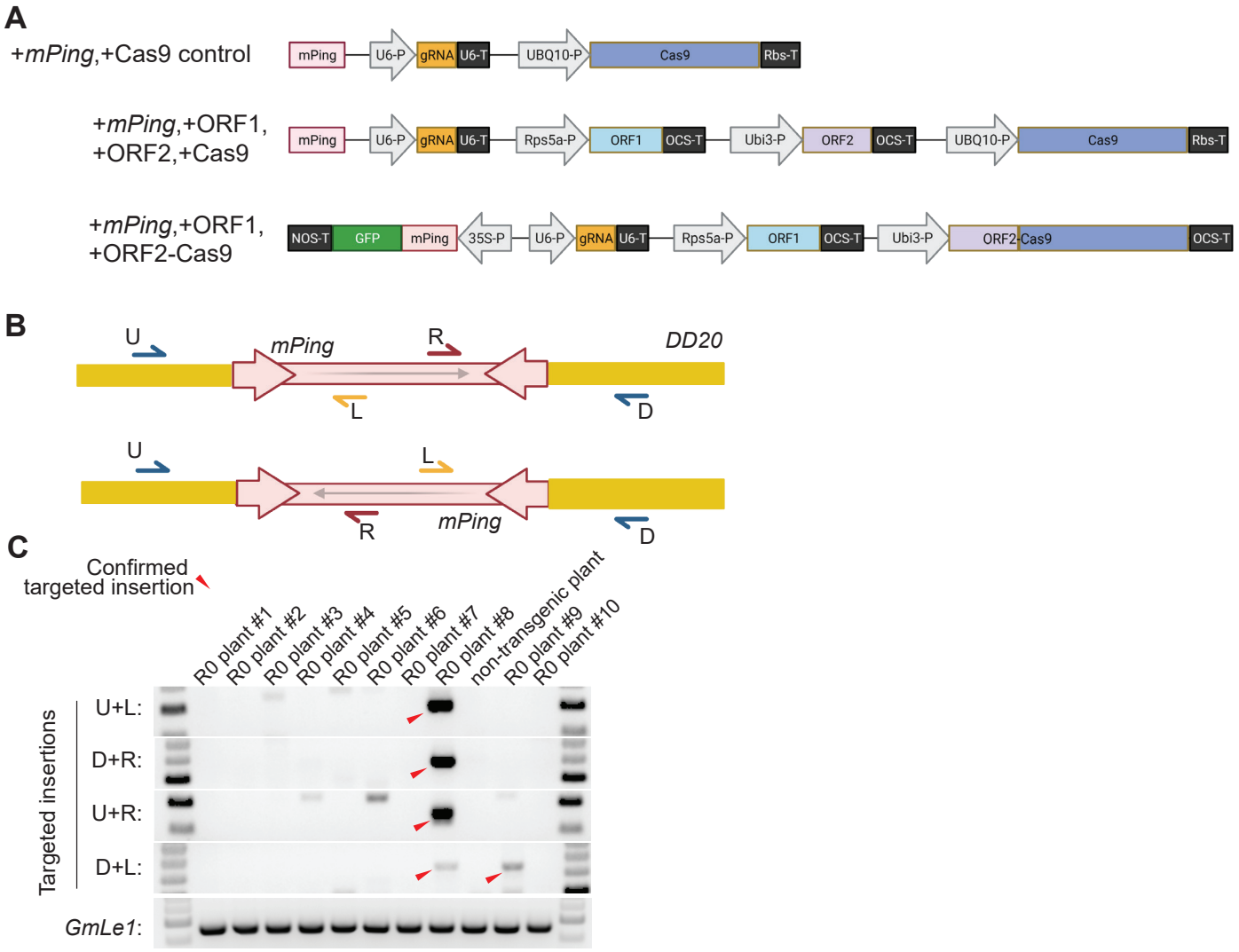


Supplemental Figure 9 - Targeted insertion of the intact bar CDS

A. PCR strategy and primer placement to detect targeted insertions of the *mPing*_bar CDS element into the region upstream of the *ACT8* gene. The red and blue arrows indicate the pair of primers used for PCR.

B. Sanger sequencing across the majority of the *mPing*_bar CDS element and into the region upstream of *ACT8* demonstrates the successful delivery of the complete bar CDS into this region. The Sanger sequencing represents the contig of several sequencing reactions from a single TOPO TA plasmid clone of a PCR product.

Supplemental Figure 10



Supplemental Figure 10 - Targeted insertions of mPing in the soybean genome

A. Vector maps of three transgenes transformed into soybean plants. The '+*mPing*,+Cas9' transgene has a gRNA and is used as a positive control of Cas9 cleavage of the *DD20* target site. The '+*mPing*,+ORF1,+ORF2,+Cas9' transgene is the same as the unfused Cas9 configuration tested in Arabidopsis. The '+*mPing*,+ORF1,+ORF2-Cas9' transgene has the ORF2-Cas9 fusion protein as in **Supplemental Figure 1A**. The *mPing*_HSEs transgene transformed into soybean is identical to the '+*mPing*,+ORF1,+ORF2,+Cas9' transgene, with *mPing*_HSEs substituting for *mPing*.

B. PCR strategy and primer placement to detect targeted insertions of *mPing* and *mPing*_HSEs into the *DD20* loci in the soybean genome. Arrows indicate primers used to detect targeted insertions: U+L, D+R, U+R, D+L.

C. PCR assay to detect targeted insertions of *mPing* into the *DD20* loci in soybean. Each sample is a different regenerated transgenic R0 soybean plant, and a representative round of 10 plants post-transformation and regeneration are shown here. Of these ten, two show targeted insertion of mPing. A summary of all soybean samples is shown in **Figure 4A**. Red triangles indicate targeted insertions confirmed by Sanger sequencing.