Supplemental Materials for Renyu Li, Charles Vavrik and Cristian H. Danna (2020)

"Proxies of CRISPR/Cas9 activity to aid in the identification of mutagenized Arabidopsis plants"

GGTCTCAGGTCAGAGCTTGTTCAGGACTCGAGcatcttcattcttaagatatg
aagataatcttcaaaaggcccctgggaatctgaaagaagagaagcaggcccatttatatgggaaagaaca
atagtatttcttatataggcccatttaagttgaaaacaatcttcaaaagtcccacatcgcttagataagaaaacg
aagctgagtttatatacagctagagtcgaagtagtgattg**TTTGTAAGTAAATGGCGGA**
TGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTT
ATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTGAGCTCcatc
ttcattcttaagatatgaagataatcttcaaaaggcccctgggaatctgaaagaagagaagcaggcccattta
tatgggaaagaacaatagtatttcttatataggcccatttaagttgaaaacaatcttcaaaagtcccacatcgc
ttagataagaaaacgaagctgagtttatatacagctagagtcgaagtagtgattg**TTCTGACGAT**
GCGGTTCCATGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTT
TGAGCTCcatcttcattcttaagatatgaagataatcttcaaaaggcccctgggaatctgaaagaagag
aagcaggcccatttatatgggaaagaacaatagtatttcttatataggcccatttaagttgaaaacaatcttcaa
aagtcccacatcgcttagataagaaaacgaagctgagtttatatacagctagagtcgaagtagtgattg**GC**
TCGCATAAGCGTTGTGACGTTTTAGAGCTAGAAATAGCAAGTTA
AAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGT
CGGTGCTTTTGGATCCCTGATGAGACC

Figure S1. *In silico* designed and *in vitro* synthesized sgRNA expression cassettes. Each AtU6 promoter sequence is depicted in lowercase. Each sgRNAs is depicted in uppercase brown (*JAR1*), blue (*GL1*) and green (*EIN2*). Each crRNA sequence is depicted in bold font. The RNA polymerase III transcription start site "g" is depicted in lower case red font and the stop site (poly T tail) in uppercase red font. The 32 and 17 extra nucleotides at 5' and 3' of the synthetic DNA fragment are shown in back uppercase font.

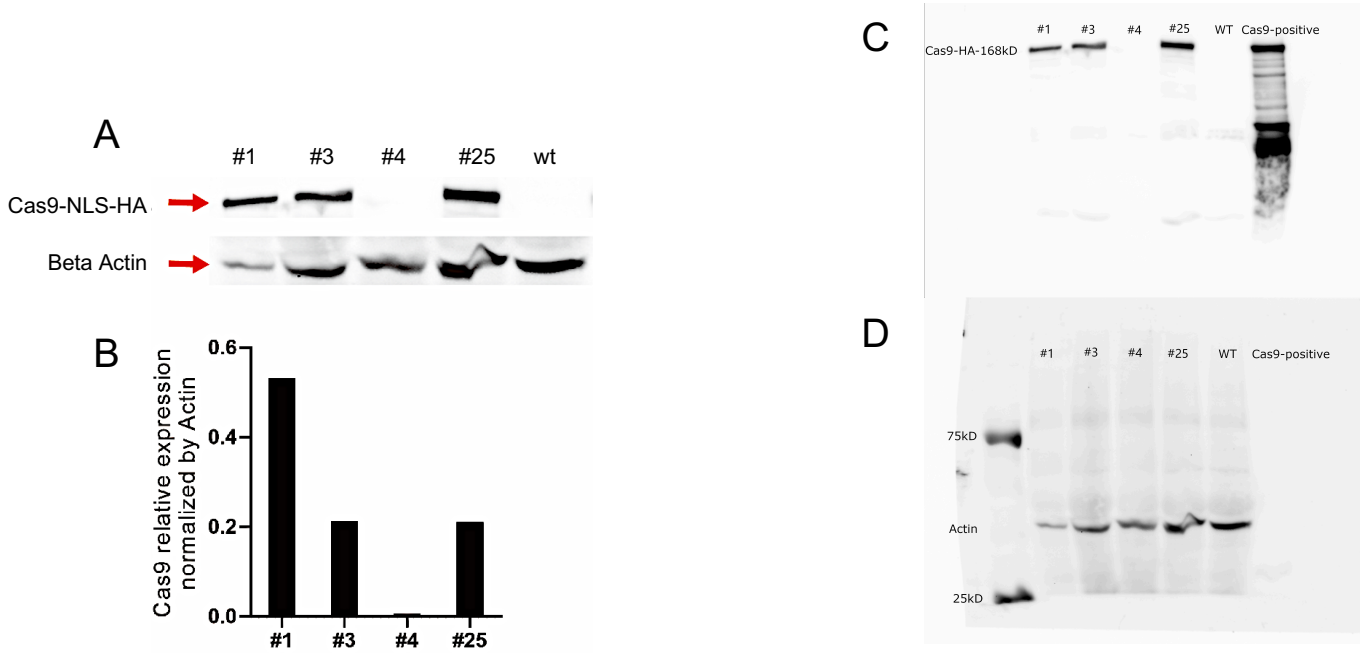

Figure S2 Cas9 expression in somatic tissue of T1 plants. Cas9 expression in 4 independent T1 transgenic plants (#1, #3, #4, #25) and non-transgenic wild type plants (wt) were tested via western blot (A). Quantification of Cas9 expression normalize by Beta-Actin (B). Western blot raw images showing Cas9 (C) and beta-actin (D) expression. Protein extracts of T1 transgenic lines #1, #3, #4 and #25 or non-transgenic wild type plants (wt) leaf tissue were run in SDS-PAGE gel, transferred to PVDF filters and incubated with rabbit anti-HA primary antibody (top panel) or monoclonal mouse anti-β-Actin primary antibody (lower panel). HRP conjugated anti-rabbit IgG secondary antibody or IR dye-conjugated anti-mouse secondary antibody where use to detect HA and beta-actin, respectively. Images were taken with a Bio-Rad® ChemiDoc MP® system. This experiment was repeated three times with similar results

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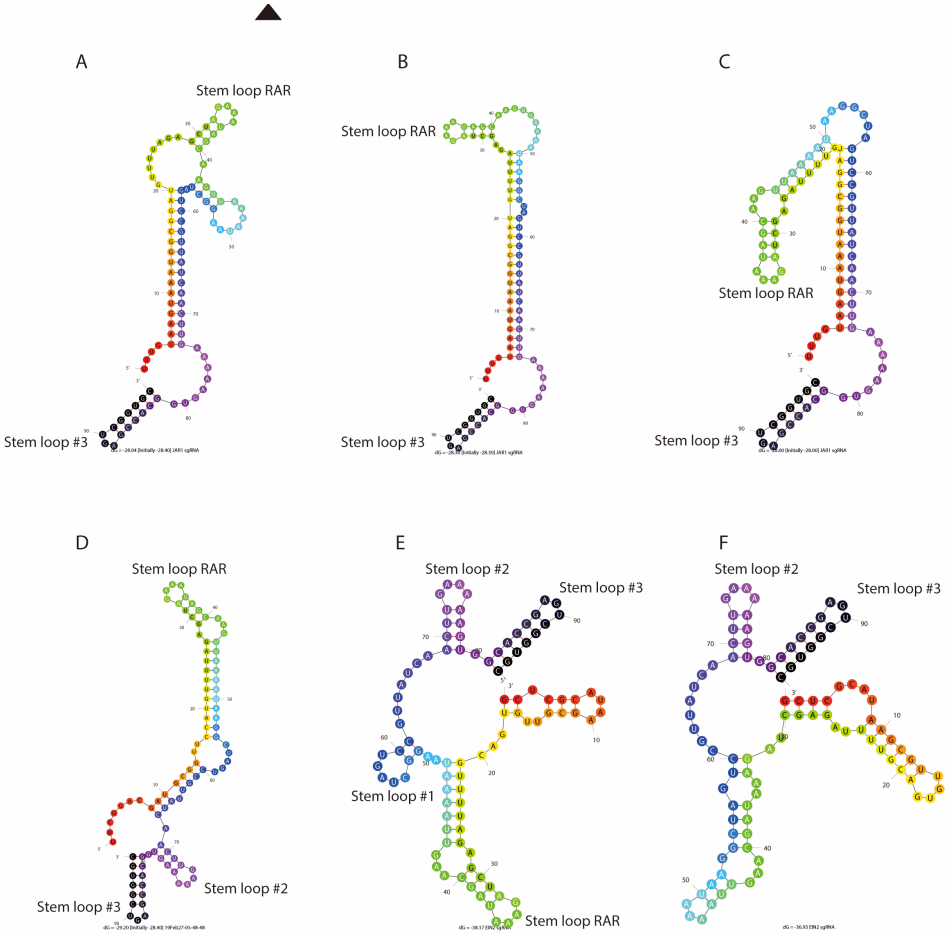

Figure S3 sgRNA secondary structure. *In-silico* predictions of *JAR1* (**A-C**), *GL1* (**D**) and *EIN2* (**E-F**) sgRNA secondary structure obtained with Mfold http://unafold.rna.albany.edu/?q=mfold (Zuker et al. 2003). Nucleotides were color coded as red and black for the 5' and 3' ends, respectively. Stem loops #1, #2, #3 as well as the predicted repeat and anti-repeat region (stem loop RAR) are labeled for ease of identification.

Table S1. Gene-specific 20mer crRNA sequence composition and predicted efficiency

AGI [a]	Target sequence (5' to 3')	DNA strand	# Off-target sites	Specificity Score	PAM sequence	GC content	# CBP [b]	# IBP [c]	S3 Figure predicted structure
AT3G27920 (*GL1*)	TTCTGACGATGCGGTTCCAT	antisense (exon1)	5	98	TGG	50	11	0	D
AT2G46370 (*JAR1*)	TTTGTAAGTAAATGGCGGAT	antisense (exon2)	9	98	TGG	35	16	0	A
							16	0	B
							16	0	C
AT5G03290 (*EIN2*)	GCTCGCATAAGCGTTGTGAC	sense (exon1)	3	99	TGG	55	0	5	E
							8	2	F

[a] Arabidopsis Gene Identifier for *GL1*, *JAR1* and *EIN2.*
[b] Number of consecutive base pairs
[c] Number of internal base pairs

Figure S4. Representative Sanger sequencing chromatograms of PCR products obtained from leaves of CRISPR mutagenized plants. Wild type (WT) or heterozygous (HET) alleles are shown. Uppercase letters indicate gene specific target site. The yellow box indicates the PAM site in each gene. The red arrow indicates the end of the wild type and the beginning of mutated DNA sequence downstream of the NHEJ-mediated DNA repair.