The unequal functional redundancy of the Arabidopsis *INCURVATA11* and *CUPULIFORMIS2* genes is not dependent on genetic background

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Supplementary Figures and Tables

Supplementary Material not included in this file:

Supplementary Table S2

A Features of sgRNA and its target

sgRNA name: ICU11_sgRNA1

sgRNA sequence: GCGAGGCAAGATTGAAGCTTCGG

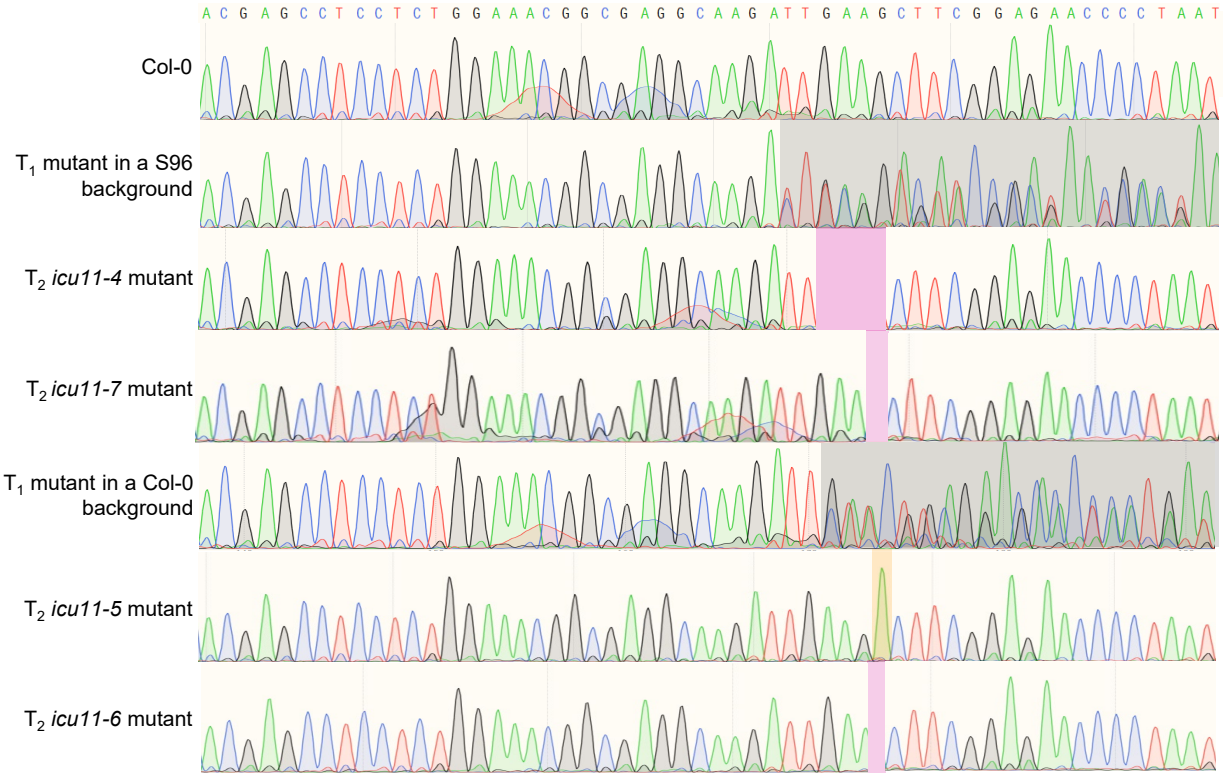
Target location: Chr1:8127046. AT1G22950, first exon

Target strand: complementary

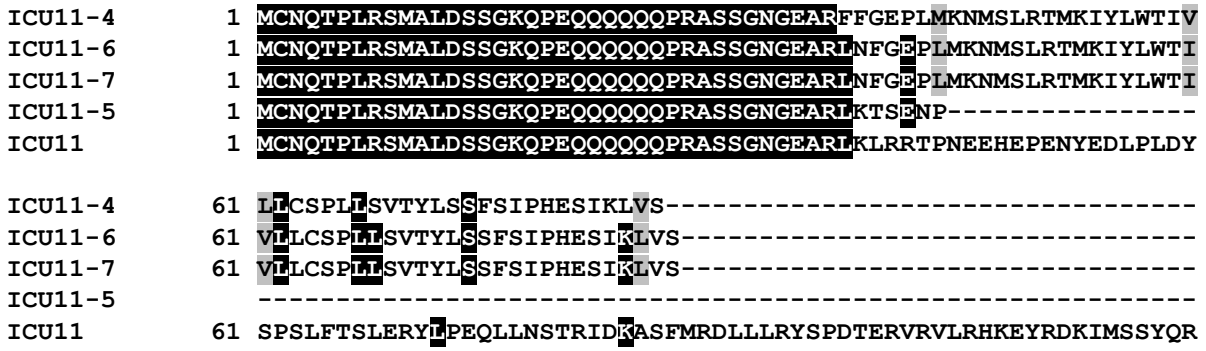
sgRNA efficiency: 49.72%

sgRNA predicted off-targets: MM(0):0, MM(1):0, MM(2):0, MM(3):0, MM(4):2

B



C

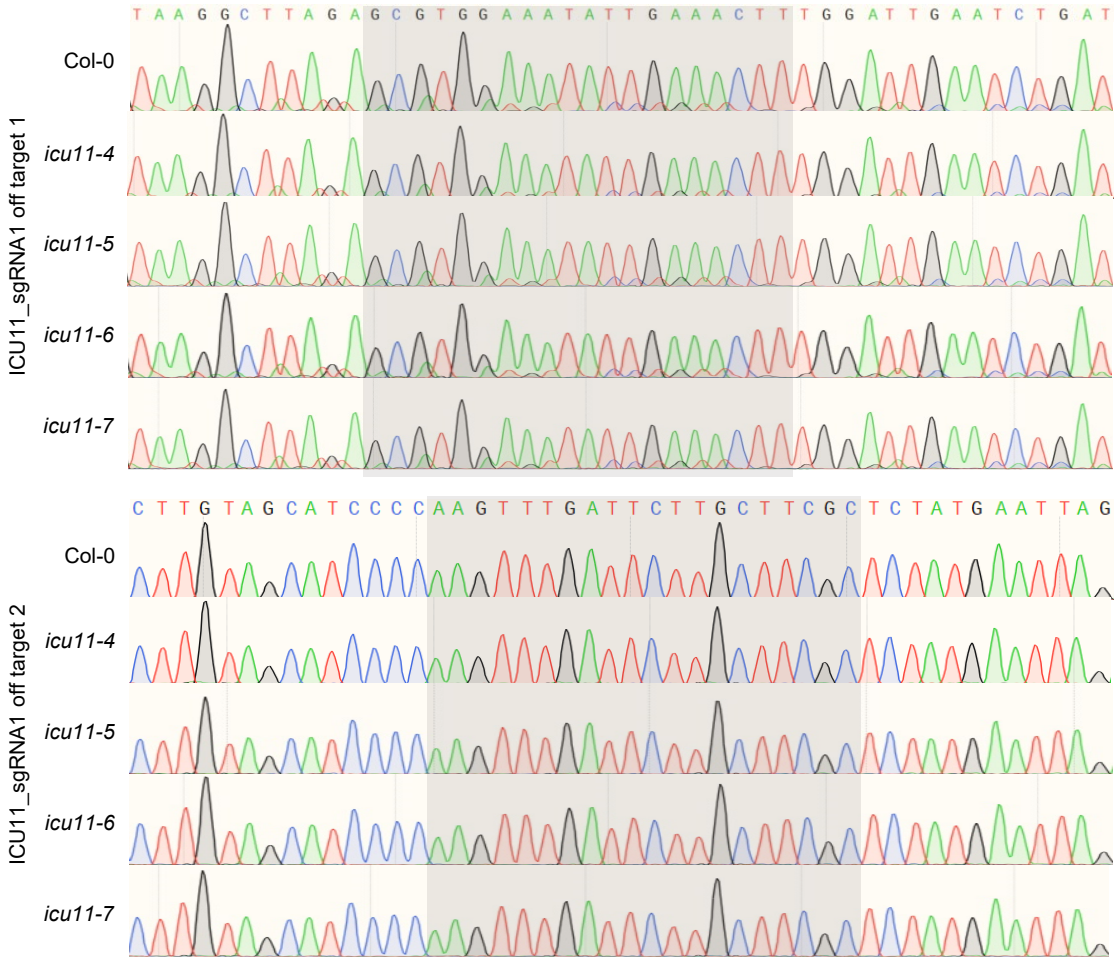


Supplementary Figure S1. Design and effects of the CRISPR/Cas9 mutagenesis of *ICU11*. **(A)** Details of the *ICU11* sgRNA1 target. The PAM sequence is shown in red. On-target mutation efficiency was calculated using the "Rule Set 2" scoring model, which provides values ranging from 0 to 100 (Doench et al., 2016). Possible off-target events are represented according to the number of mismatches [MM (number)]. **(B)** Electropherograms of the *ICU11* sgRNA1 target site in wild-type plants, and T_1 and T_2 transgenic plants. The gray, magenta, and orange shaded areas indicate chimeric, deletion, and insertion mutations, respectively. **(C)** Multiple amino acid sequence alignment of the predicted proteins translated from wild-type and CRISPR/Cas9 alleles showing that all the latter produce truncated proteins. Identical and similar residues are shaded in black and gray, respectively. Numbers indicate residue positions.

A Details of two putative off-targets of ICU11_sgRNA1

	ICU11_sgRNA1 off-target 1	ICU11_sgRNA1 off-target 2
Chromosome	1	3
Position	19132845	2280675
Strand	Forward	Complementary
Gene	Intergenic, between AT1G51590 (*MNS1*) and AT1G51600 (*GATA28*)	First exon of AT3G07170 (*IRP1*)
Mismatches	ICU11-T1: **GCGAGGCAAGATTGAAGCTTCGG** Col-0: **GCGTGGAAATATTGAAACTTTGG**	ICU11-T1: **GCGAGGCAAGATTGAAGCTTCGG** Col-0: **GCGAAGCAAGAATCAAACTTGGG**

B



Supplementary Figure S2. Testing of two putative CRISPR/Cas9 off-targets in the new *icu11* mutant lines. **(A)** Off-targets were found using Cas-OFFinder, a bioinformatic tool developed by Bae et al. (2014). Mismatches are shown in red, with the first nucleotide of the PAM sequence (NGG) is in blue. **(B)** Sanger sequencing electropherograms of putative off-targets in T_3 mutant and wild-type plants. The gray shaded area corresponds to the putative off-target sequence.

Supplementary Table S1. Primer sets used in this work

Purpose	Oligonucleotide name(s)	Oligonucleotide sequences (5′ → 3′)	
		Forward primer (L or F)	Reverse primer (R)
Genotyping	ICU11-Off-target1_F/R	TGGTGGGTTTGGTTTGTCTC	CTCGGTCATTGGAGCAACTT
	ICU11-Off-target2_F/R	TGAGTCTGGAAGCAGGAAGG	AATGGGCAAATCAGAGAGTCC
	At1g22950_1F/R	ACCCTAACCTCTCAAACAAACCA	AGACTTTGTTAACCCAATCCGAC
	At1g22950_4F/R	CCTCTCAAACAAACCATCATCA	CGCTCAGTATCAGGGGAATATC
	SAIL_1215_B02_L/R	GAGCGATAACAGTGAGCTTGG	GACATTTTCAAACCATTCATGC
	SAIL_658_E12_L/R	AGAGGCAAGAGACGAAAAAGC	CCTTTGAGCCTGTAGCATCAG
	SAIL_621_G08_L/R	TGAGAGCGAAAGCTTTCATTC	AACAAATGACTGGAGCAGAGC
	gis-5_F/R	GAAGCAAGAACAGGTTTCTATG	AGCTAGTTACACTCGAGGATA
	icu2-1_F/R	TGTTGAAGGAGGTCAGTTATTCT	CACAAGTGTTTTGGATGACTGAA
	clf-2_F/R	ATGGCGTCAGAAGCTTCGCC	CTGGACCTCTCTCCTCCGC
	tfl2-2_F/R	TATCAGCGGTGATCGGTGTG	CGCCGTAATTCTCCCGGTAA
	ebs-1_F/R	TGAAGGTGTGAACAATGCAT	GAAAACTCGACCTGGTGTCG
	fas1-1_F/R	TGAGCTGTTCTTCTGCATCATG	ACTATGGTAGCTGTGAAGAGTG
	AT5G51230_1F/R	TGTAATGGTTCAGAGATCAATAGAA	GTCCGTGCAATCTTGAGAATG
	SALK_131712_L/R	CAGAAGAAGATCGTCCGAGTG	TGAACTTCCCCACTCTTCATG
	SALK_056440_L/R	TGGTCAGATGGGCTAGAATTG	AACGCGTTGCTGTAGAAACTC
	SAIL_826_A06_L/R	AGCAGCAGAAGAAGAAGCATG	TTTGGCCTACAAAGACACCAG
	SALK_021316_L/R	GAGCCGTCTCATCAAACTGAC	TTGCAGGAGCAAATATGGAAC
	SALK_150863_L/R	AGATCGCTTCCAGAGTTAGCC	TTGTCGCAAAAAGCAAAAGAG
	SAIL_223_F05_L/R	GGATCAGCCAAAAGGTTAAGG	TCATTCACTTTGCATCACTCG
	SAIL_809_E03_L/R	GCGTGTACCAGTTTCAAGGAG	TAAAGAGCCCAGTTGTGAAGC
	SALK_045303_L/R	CCAGTTAAGGACAGAACACCG	TCGTCTTTCGATCAAATCCAC
	SALK_022363_L/R	ATCAATGTGGCATCTAGTGGC	ACCCGCCTCTTCTTCATCTAC

Supplementary Table S1 (continued). Primer sets used in this work

Purpose	Oligonucleotide name(s)	Oligonucleotide sequences (5′ → 3′)	
		Forward primer (L or F)	Reverse primer (R)
Genotyping	SAIL_97_E06_L/R	CTTTCCCAGTTTTTACTGCCC	AATCACTCGCTTCTTCCACTG
	SALK_149002_L/R	AATGAAAGCATGCGGATACAC	TCCGTGTTGACTGGAAAGATC
	SALK_130607_L/R	TTTCTCTTGTCCGGTGAAATG	CCTGCAACAATCAGTGTGATG
	SAIL_240_H01_L/R	TTGAGATGAATCTGGAGACCG	AAACGACGACGTATTGGAGTG
	SALK_149692_L/R	TCTTGTGACAGGTGCAACTTG	AAACAAAGCTAGGCACAAGGC
	SALK_080380_L/R	AGGGAACATGTCATCCATGAG	AGGGAGAATCTGAGAACCTGC
	SALK_027726_L/R	ATGGTGTGCGAATCTATGACC	ACGGAGAGGAAAGCTCAAGAC
	LB1[1]	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC	
	LbB1.3[2]	ATTTTGCCGATTTCGGAAC	
	Cas9_F/R	GCTTCATCAAGAGACAGCTGG	GGACTTGCCCTTTTCCACTTT
Cloning	ICU11_sgRNA1_F/R	ATTGCGAGGCAAGATTGAAGCTT	AAACAAGCTTCAATCTTGCCTCGC

[1,2]These primers were used for genotyping [1]SAIL and [2]SALK lines, and their sequences were taken from [1]Sessions et al. (2002) and [2]T-DNA Primer Design (http://signal.salk.edu/tdnaprimers.2.html).

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