

a Plant_gRNA512-13-4 (T_2 generation)

G1

62

G3

G1

G2

G3

b Plant_gRNA512-13-5 (τ , generation)

c Plant_gRNA512-13-6 (T₂ generation)

G1

G2

G3

d Plant_gRNA512-13-7(T_2 generation)

	20	40	60	80	
CRUC G1	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
CRUC G2	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
CRUC G3	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACGTTCGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 1	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 2	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 3	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 4	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 5	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 6	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 7	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 8	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 9	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 10	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 11	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 12	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 13	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 14	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 15	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCT-AACGG
colony sequence 16	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCT-AACGG
colony sequence 17	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCT-AACGG
colony sequence 18	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCT-AACGG
colony sequence 19	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCT-AACGG
colony sequence 20	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCT-AACGG
colony sequence 21	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCT-AACGG
colony sequence 22	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCT-AACGG
colony sequence 23	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTCGT CCT-AACGG
colony sequence 24	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACGTTCGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 25	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACGTTCGGGG	TTCTCCCTCGT CCTT-AACGG
colony sequence 26	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACGTTCGGGG	TTCTCCCTCGT CCTT-AACGG

G1

G2

G3

e Plant_gRNA512-13-10 (T₂ generation)

f Plant_gRNA512-1-46 (T₂ generation)

g Plant_gRNA512-1-47 (T₀, generation)

h Plant_gRNA512-1-69 (T₂ generation)

i Plant_gRNA512-1-93 (T₂ generation)

	20	40	60	80	
CRUC G1	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
CRUC G2	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
CRUC G3	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCG	ACGTTGGGG	TTCTCCCTGT
colony sequence 1	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 2	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 4	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 5	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 6	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 7	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 8	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 9	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 10	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 11	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 12	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 13	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 14	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 15	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 16	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 17	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 18	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 19	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 20	ACGGGTAAGC	TCAGCAATCT	CCTCGTTGCA	ACATTGGGG	TTCTCCCTGT
colony sequence 21	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCG	ACGTTGGGG	TTCTCCCTGT
colony sequence 22	ATGGGTAAGC	TCGGCAATCT	CCTCGTTGCG	ACGTTGGGG	TTCTCCCTGT
colony sequence 23	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCG	ACGTTGGGG	TTCTCCCTGT
colony sequence 24	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCG	ACGTTGGGG	TTCTCCCTGT
colony sequence 25	ATGGGTAAGC	TCAGCAATCT	CCTCGTTGCG	ACGTTGGGG	TTCTCCCTGT
colony sequence 26	ACGGGTAAGC	TCAGCAATCT	CCTCGTTGCG	ACGTTGGGG	TTCTCCCTGT

j Plant_gRNA512-1-69-8 (T₃ generation)

k Plant_gRNA512-1-69-8-7-11 (T₅ generation)

Figure S3. CRISPR-derived mutations identified in *CsCRUC*. **a-k** Alignment of *CsCRUC* sequences PCR amplified and cloned from plants listed in Table 1. Sequences are from independent colony clones of the *CsCRUC* amplicon generated using plant genomic DNA as template. Cas9 cut site is indicated by an arrow. Homoeologues are indicated and assigned based on SNPs. Grouped sequences designated G1, G2 and G3 correspond to the *CsCRUC* genes from the three sub-genomes (*CsCRUC-G1*, *CsCRUC-G2*, and *CsCRUC-G3*). * indicates sequence with mutation at the Cas9 cut site, and likely represents a heritable mutation. ‡ indicates a unique mutated sequence.