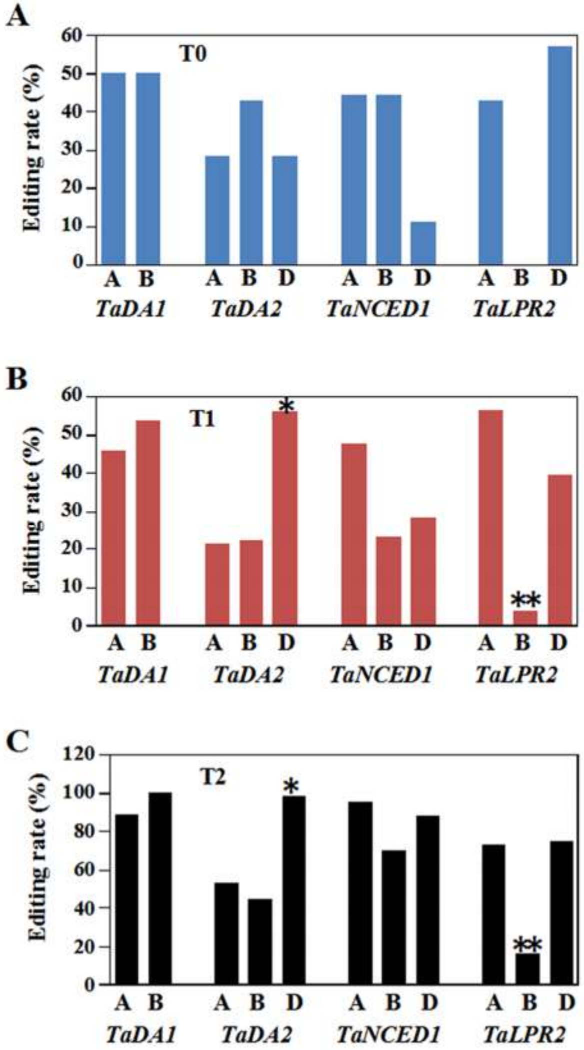
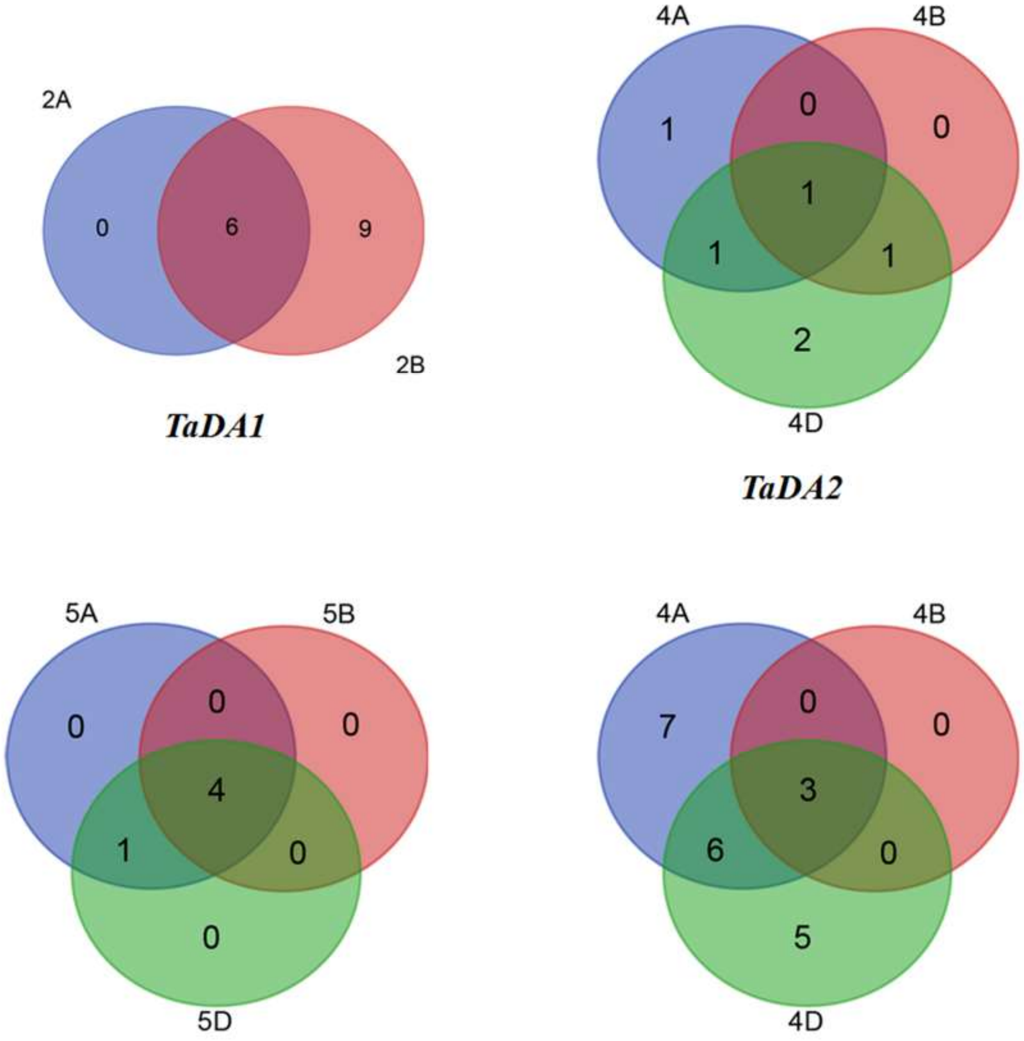


Figure S1. Editing rates of the target genes in each subgenome across the T0 (**A**), T1 (**B**) and T2 (**C**) generations. Asterisks indicate a significant difference from the other subgenomes of each target gene at the *, *P*-value < 0.05 level of significance or **, the *P*-value < 0.01 level of significance by Fisher's exact test.



TaDA1

TaDA2

TaNCED1

TaLPR2

Figure S2. Overlap of editing types in each subgenome among the target genes.

Table S1. sgRNA selection.

Target Gene	sgRNA Sequence (5′-3′)	GC Content (%)	Targeted Genome
TaPinb	CTTAGCTTCGGCCGCTCCTG	65%	5D
TaDA1	ACTCATGAGATGATGCATGCA	43%	2A, 2B
TaDA2	GTCTTACTTGTGGCTCGAGT	50%	4A, 4B, 4D
TaNCED1	GCTGAGCGCGAAGAGCTCGC	70%	5A, 5B, 5D
TaLPR2	CGGAGCCGACGACGTGGAAG	70%	4A, 4B, 4D

Table S2. Primers for mutation detection in transgenic wheat plants.

Target Gene	Oligo Sequence (5′-3′)
pinb-F	ggagtgagtacggtgtgcTGTAGCGAGCACAACCTT
pinb-R	gagttggatgctggatggGTGGTGCTATCTGGCTCA
DA1-F	ggagtgagtacggtgtgcGACTTTGCGAGTCTCTAAT
DA1-F	gagttggatgctggatggAGAGCGAAGACGTGGTATT
DA2-F	ggagtgagtacggtgtgcTTGTTCGATTAATGGCACCTTCAG
DA2-R	gagttggatgctggatggGTGTCATTGGCAATCTGATGCATG
NCED1-F	ggagtgagtacggtgtgcCGGCCTCGTCTACTTCAAC
NCED1-R	gagttggatgctggatggTACTTGAGGTACGGCTTCT
LPR2-F	ggagtgagtacggtgtgcCTCAGTGGCAGCCTGAGTACTTCG
LPR2-R	gagttggatgctggatggTGACGACGTCAAACGACTCGGACAC

Table S3. Heredity of genotypes from the T0 to T1 generations.

Target Gene	T0 Line	Genotype of T0 Lines	Bar Positive of T1 Plants	Genotypes of T1 Plants
TaPinb	1-22	-	132	9HetD:1ChiD
TaDA1	6	HetAHetB	3	5HomAChiB:1HetAHetB:2HetB:2HetAChiB:1HomAHetB:1HetAChiB
	14	HetAHetB	12	8HomAChiB:1HomAHetB:2BiaAChiB:1HetAChiB:2BiaAHetB:1HetAHetB:1HomB:1HomAHetB:2BiaB:1HetB
	15	ChiAHetB	7	1HomAHomB:8BiaAChiB:14HomAChiB:1BiaABiaB:1ChiB
	17	HetAChiB	20	1BiaAHetB:9HomAChiB:2HomAHetB:3HetAChiB:3HetAHetB:1HomAHomB:2ChiB:1ChiAHetB:1HomB:2ChiB:1ChiAChiB
TaDA2	3	HetD	14	14HetD: 1HetAHetD
	5	HetD	12	1HomAHomBChiD:3HetD:4HetBHetD:
	58	BiaAHomBHomD	19	1HomAHomBHomD:5HomAHomBBiaD:2HomAHomBChiD:1HomABiaBChiD:4BiaBBiaD:1HomABiaD:2HomBBiaD:1BiaABiaD:1HomAChiD
	60	BiaABiaB	19	1HetAHetBHetD:3HomAChiD:2HetAChiD:1HomABiaD:1HetBHetD:1BiaD:5HetD
TaNCED1	53	HetAHetBHetD	9	3HetD: 2HetAHetD: 2HetAHetBHetD
	84	HetAHetB	18	8HetA: 6HetAHetBHetD
	98	HetAHetB	9	6HetA: 10HetAHetBHetD
	107	HetAHetB	5	7HetA: 4HetAHetBHetD
TaLPR2	1	ChiABiaD	16	1BiaAHomBBiaD:5ChiAHomD:5BiaABiaD:1BiaAChiD:6HomAChiD:2HomABiaD:2BiaAHomD
	54	HomD	18	9HomA:3BiaA:1HetAHomD:1BiaABiaD:4BiaAHomD:4HomAHomD:1HetAHetD
	57	HomABiaD	17	4HomAHomD:5HomABiaD:4HetA:1HomA
	59	ChiAChiD	21	1BiaAHomBHomD:3HetA:2HomB:2HomAHomD:1HetAHomD:1HetABiaD:1ChiD:1BiaA:1HetAHetD:1HetAHomB

Hom, homozygous; Het, heterozygous; chi, chimeras.

Table S4. Heredity of genotypes from the T1 to T2 generations.

Target Gene	T1 Line	Genotype of T1 Lines	Bar Detection(+/-) of T1 Plants	Genotype of T2 Plants
TaPinb	47	HetD	+	1HetD
	30	HetD	+	7HomD:9HetD
	34	HetD	+	1HomD:1HetD
	44	HetD	+	1HomD:1HetD
	48	HetD	+	1HetD
TaDA1	6-3	HetAHetB	+	9HomAChiB:3HetAHetB:3HetAChiB: 2HetB
	14-1	HomB	+	11HomAChiB:1HomAHomB:1HomAHetB:1HomABiaB:3ChiB:2BiaB
	14-15	HomAChiB	+	18HomAChiB:1BiaAChiB
	14-6	HomAChiB	-	16HomAChiB:2HomAHetB
	15-7	BiaABiaB	+	5HomAChiB:10BiaAChiB
	15-8	HomAChiB	-	11HomAChiB:3HomAHomB:1HomB
	17-4	HetB	+	1HomAHetB:5HomAChiB:1HomABiaB:1BiaABiaB:4BiaAChiB:3ChiB:1HetB
TaDA2	3-13	HetAHetD	+	12HetD
	3-12	HetD	-	13HetD
	5-13	HomAHomBChiD	+	16HetBHetD
	58-6	HomAHomBChiD	+	6BiaABiaD:12BiaAChiD:2BiaAHetD:1BiaA
	58-10	HomAHomBChiD	+	9HomAHomBChiD:4BiaAHomBBiaD:3HomAChiD
	58-18	HomAHomBHomD	+	1HomAHomBHomD:1HomAHomBChiD:1BiaAHomBHomD:1HomAHomBBiaD:1HomAHomBHetD:2HomAHomBHetD:2HomAHetBChiD:2HomAHetBHetD:1BiaAHetBHetD
	60-3	HetD	-	1HetD
TaNCED1	53-1	HetD	+	2ChiAChiBChiD:1ChiAHetBHetD:3HetAHetBHetD:2HetBHetD:3HetAHetD: 5HetD
	53-6	HetAHetBChiD	+	8ChiAChiBChiD:12HetAHetBHetD
	53-10	HetAHetBHetD	+	15HetAHetBHetD
	84-6	HetA	+	4HetAHetBHetD:6HetA
	84-10	HetAHetBHetD	+	14HetAHetBHetD
	98-3	HetAHetBHetD	-	8HetAHetBHetD
	98-9	HetAHetBHetD	+	8HetAHetBHetD:1ChiAHetBChiD:7HetAHetD

	98-12	HetA	+	5HetAHetBHetD:2HetAHetD:7HetA
	98-13	ChiAHetD	+	4ChiAHetBHetD:3HetAHetBHetD:3ChiAHetD:1HetAHetD
	107-2	HetA	+	1HetAHetBHetD: 2HetA
TaLPR2	1-13	BiaAHomBBiaD	+	2BiaAHomBHomD:1BiaAHomBBiaD:3BiaABiaD:5BiaAHomD:3HomABiaD:5HomAHomD:1ChiAHetD
	54-23	HetAHetD	-	13HomD:7HetD
	57-1	HetA	+	1HomA:1HetAHetD:1HomAHetD:8HetA:2ChiA:
	59-4	BiaA	+	1HomAHomBHomD:4HomAHomD:1ChiAChiD:1BiaAHomD:1BiaA:4ChiAHetD:2HomA:2HomD
	59-11	HomB	+	2HetAHomBHomD:3HetAHomBHetD:5HomBHomD:7HomBHetD:1HomABiaD:1HomAChiD:1HomB
	59-14	BiaAHomBHomD	+	2HomAHomBHomD:7HomAHomB:2HomAHomD:1HomABiaD:1HomB:2HomD
	59-16	HetAHomD	+	12HomA:4HomAHomD:1BiaA:1HetA

Hom, homozygous; Het, heterozygous; chi, chimeras.

Table S5. Mutations in the putative CRISPR/Cas9 off-target sites.

Target	Genome Location	Sequence	No.of Mismatching Bases	Primers	No. Of Plants Detected	No. Of Plants with Mutations
Pinb-Off target	chr3B_591364632..591365116	CCGCATGGAAGCGGCCGAAGCTGAG	3	F:5′-GTCGTGCCATATCGTTGGCAA-3′ R:5′-GGCTCTTGACTTCCTCTTG-3′	24	0
DA1-Off target	chr3B_404628830..404629312	GAAATGAGATGATGCATGCATGG	3	F:5′-GCTATTGTCCGTATCATTGGC-3′ R:5′-GTTCCTTATTCATACGATCCAC-3′	24	0
DA2-Off target	chr7B_126807007..126807487	GTCTTACTTG-GACTCGAG-CGG	2	F:5′-GCACCAGCAGTTAAGTAGA-3′ R:5′-AACGAGGAAGAGTAGAACAC-3′	24	0
NCED1-Off Target1	chr1A_7311594..7312076	CCGGCGACCTCTCCGCGCTCTGC	3	F:5′-GAGACGAAGCTCATCGAGGA-3′ R:5′-TGCCGAAGGCGTAGGCGTTG-3′	24	0
NCED1-Off Target2	chr2A_376194240..376194722	CCGGATAGCTCTTCGCGTTCAGC	3	F:5′-TCGGAGATGGCATCAATAC-3′ R:5′-TCAGGCAAGGCTAAGAAC-3′	24	0
LPR2-Off target	chr2A_387111290..387111770	GTGTT-CTTGT-GCTCGAGTGGG	3	F:5′-CCACCTTGCGGTGTAAAACCAT-3′ R:5′-CCTAGCCACCACCATAGAGA-3′	24	0