Supporting information

Claeys et al., “Coordinated gene upregulation in maize through CRISPR/Cas-mediated enhancer insertion”

Methods

Identification of PE

A 16-bp consensus enhancer described in literature[2,8] and known to be bound by native plant transcription factors[9,10] was used as the query in a standard BLAST search against the reference genomes of maize (B73v4[11]), soybean (Wm82.a4[12]), rice (Os-Nipponbare-Reference-IRGSP-1.0[13]) and barley (MorexV3[14]). In all cases, 5-7 copies of a shorter but similar sequence (`GTAAGCGCTTAC`) were found in these genomes; we termed this sequence PE.

gRNA design

Gene models were manually corrected using publicly available CAGE data[15], and gRNAs were designed in a window of 40-200 bp upstream of the corrected TSS using Geneious Prime (Biomatters Ltd), ensuring a minimum of 3 mismatches with the most similar off-target sequence in the B104 genome for specificity.

Biolistics transformation of Cas9-expressing embryos

Immature embryos were harvested at 13 days after pollination from plants carrying a homozygous T-DNA containing ZmUbi::Cas9 in the B104 background crossed to wild-type B104; the resulting embryos are hemizygous for the Cas9 transgene, making it easier to segregate away this T-DNA afterwards. Embryos were incubated in the dark at 25°C for 2 days on BOM1 media (for media recipes, see Table S5), and 3-5 hours prior to bombardment moved to BOM2 media. 800 ng synthetic sgRNA (IDT), 800 ng double-stranded 3xPE dsODNs (modified with 5’ phosphorylation and two phosphorothioate linkages at both ends; see Table S6 for sequences; IDT) and 2 µg plasmid DNA (containing pZmUbi::mScarlet::tZmUbi and p35S::pat::tNOS cassettes) were coated onto 400 µg 0.6 µm gold particles (Bio-Rad) using isopropanol/ammonium acetate[16] and resuspended in 100 µl ethanol. Twelve µl gold solution was loaded onto a macrocarrier used for bombardment of 25 immature embryos with the Bio-Rad PDS-1000 instrument using 650 psi rupture disks. After bombardment, embryos were incubated for 1 day in the dark at 25°C. Then, embryos were incubated at 28°C in continuous light (50 µmol/m^2/s), and transferred through a series of media: ML4 for one week, ML5 for two weeks, and ML6 for two weeks. Then, callus was moved to 16 hours dark/8 hours light (150 µmol/m^2/s) at 28°C, and grown on ML7 for two to three weeks and ML8 for one week to regenerate, before finally transferring regenerated seedlings to soil.

Amplicon sequencing for edit detection

gDNA was prepared using the DNeasy Plant Mini kit (Qiagen) or using CleanNGS magnetic beads (CleanNA); for this method, frozen ground tissue was mixed with extraction buffer (50 mM Tris-HCl pH 8, 300 mM NaCl, 10% sucrose) and incubated at 60°C for 20 minutes followed by centrifugation, after which gDNA was extracted from the supernatant by adding magnetic beads and washing twice with 80% ethanol before elution. Prescreening for insertions was performed by PCR using GoTaq G2 Green (Promega) with primers flanking the insertion site (Table S6), and resolving reaction products on a 2% agarose gel to look for longer amplicons. Amplicons were generated with Phusion Flash (ThermoScientific) or Q5 High Fidelity DNA Polymerase (NEB) using primers flanking the insertion site (Table S6) and carrying standard adapters, followed by amplicon clean-up using Kapa Pure beads (Roche). Amplicons were sequenced on the Illumina MiSeq platform (150 bp paired-end). Fastqc (0.11.9) and trimmomatic (v0.38) were used with standard parameters for quality control and cleaning of the raw fastq reads. Cleaned reads were mapped using BWA (v0.7.17_cv1) to the amplicon reference sequence, and the BAM file was then passed to CrispRVariants[17] along with positions of where the guide/PAM falls along the amplicon. Edits were then detected in a 100 bp window with the guide RNA positioned in the middle. Enhancer insertions were further characterized by analyzing amplicon sequences using an alternative pipeline. Briefly, paired end reads were merged into single fragments using FLASH2 (v2.2.00). Next, PCR duplicates were collapsed via UMIs, and identical merged reads were counted. All forward and reverse complement reads were combined into a fasta with their occurrence being shown as the header, the reference sequence was added, and MAFFT (v7.407) was used (with the parameters --inputorder --adjustdirection --anysymbol --clustalout --auto) to generate an alignment file. The alignment file was then manually inspected.

Gene expression analysis

To sample roots, plants were grown as described[18], and after 6 days, the primary root was cut 4 cm above the tip and frozen in liquid nitrogen. To sample leaves, plants were grown in soil at 25°C (16 hours light/8 hours dark), and after 6 days, the tip of the first leaf was harvested and frozen in liquid nitrogen. After tissue grinding, RNA was prepared using the Direct-Zol RNA Miniprep kit (Zymo Research), and 500 ng total RNA was used to generate cDNA using the SuperScript VILO cDNA synthesis kit (Invitrogen). qRT-PCR was performed using SsoAdvanced Universal SYBR Green (Bio-Rad) on a Bio-Rad CFX-96 qPCR machine with primers specific to the target gene (Table S6) in two technical replicates. Actin1 (Zm00001d010159) was used for

normalization. Ct values were determined using the Bio-Rad CFX Maestro software, and converted to relative expression values using the ΔΔCt method.

Soy protoplast isolation, transfection and microscopy

Protoplasts were isolated from etiolated soy epicotyls grown 11 days on ½ MS growth media (2.215 g/L Murashige and Skoog basal salts w/ vitamins, 30 g/L sucrose, 100 mg/L myo-inositol, 3 g/L Phytagel) by making thin lateral slices using a razor blade and submerging these tissues in 10 mL soy protoplast enzyme solution (0.45 M mannitol, 20 mM MES pH 5.7, 2% Cellulase Onozuka R-10 [Yakult Pharma Co], 0.5% Macerozyme R-10 [Yakult Pharma Co]) in a deep-well petri-dish. The submerged tissues underwent a 10 min vacuum infiltration and then were incubated 15h at 26°C rotating at 25 RPM. The digested solution was filtered through a 40 µm nylon filter and washed twice with W5 solution (154 mM NaCl, 125 mM $CaCl_2$, 5 mM KCl, 2 mM MES pH 5.7). Viability of isolated protoplasts was measured using Evan's Blue staining and cell concentration was measured via hemocytometer.

Isolated cells were concentrated to a density of 10^6 cells/mL in MMG solution (0.6 M mannitol, 15 mM $MgCl_2$, 4 mM MES pH 5.7) for transfection. Each transfection sample received 200 µL cells and 20 µg plasmid DNA and then tapped to mix. 220 µL of 40% PEG (0.25 M mannitol, 0.2 M $CaCl_2$, 40% PEG 4000) was next added to sample tubes followed by incubation at room temperature for 15 min. Samples were washed with W5 and resuspended in 1 mL W5 for final incubation at 28°C in a 6-well plate. Sample wells were imaged at 16, 24, 40, and 48 hours post-transfection for GFP fluorescence using the Biotek Cytation 1 multi-mode imager. Additionally 8 end-point readings spanning the entire well were taken and averaged for each well at the same time points to measure sample fluorescence intensity using the same instrument.

Whole-genome sequencing and off-target insertion detection

Prior to sequencing and subsequent data analysis, an in-silico read depth estimation was performed by simulating fastq reads from an *in-silico* genome with heterozygous insertion of 200 3xPE elements using the randomreads.sh from bbmap (v38.20) with *paired interleaved length=250 mininsert=200 maxinsert=400 gaussian* parameters. Reads were processed as mentioned below. Simulations showed that using 2 x 250bp short reads at 25X coverage resulted in a recovery rate of 90%, while increasing sequencing depth only marginally improved recovery rate. gDNA was prepared from single T1 individuals of 5 different lines that were selected to carry all expected on-target insertions based on genotyping the T0 parent. Sequencing was performed

on the Illumina NovaSeq platform (250 bp paired end; Novogene) to 25X coverage. Fastqc (0.11.9) and trimmomatic (v0.38) were used with standard parameters for quality control and cleaning of the raw fastq reads. Cleaned reads were mapped using BWA (v0.7.17_cv1) to the B104 genome supplemented with the full plasmid used for bombardment and the T-DNA present in the parental Cas-expressing line with indel sensitive *-A2 -E1* parameters. Detailed genome-wide coverage analysis with mosdepth (v0.3.2) showed very high coverage, with 87% of the genome covered at a depth of 25X and about 97% of the genome covered at a depth of 10X.

In order to detect off-target insertions, genome-wide SNP and INDEL calling was performed using bcftools mpileup (v1.9-1-deb_cv1) followed by bcftools call and *-mv -Ob* parameters. After genome-wide INDEL calling, all insertions were extracted using the bcftools filter (v1.9-1-deb_cv1) and the *--include 'strlen(REF)<strlen(ALT)'* filter. No additional filters were applied on the insertions. For every insertion a fuzzy match partial ratio score was calculated against the 12bp 1xPE element using the partial ratio function of the fuzzywuzzy python package (0.18.0). Only insertions larger than 10 bp and with a partial ratio score larger than 91 were kept (practically allowing for one bp mismatch with a single PE element). Integration of plasmid fragments into the genome was assessed by extracting reads that might originate from the borders of that insertion and therefore map to both plasmid and a chromosome. This was accomplished by extracting all reads that map to the plasmid in any way using samtools (1.9). Custom python code was created to extract reads that map to both the plasmid and a chromosome location (split reads) and read pairs where one of the reads maps to the plasmid (split pairs). Low complexity and repeat reads were filtered out and no multi-mappers were allowed. Split reads were extracted by filtering on the SA (supplementary alignments) tag in the bam file and had to match with at least 40bp to both plasmid and a chromosome, the total matching percentage of a read was set at higher than 90%. Split pairs had to have at least one perfectly matching read. All remaining reads were reformatted and visualized as a circular genome using the interacCircos R package. As this methodology might be insensitive to small insertions (< 40bp), we also mapped all insertions detected by genome-wide INDEL calling (see above) to the plasmid using the Geneious 6.0.3 Read Mapper, and no significant hits were detected.

Phenotyping

For NUE experiments, seeds from segregating populations were sown in vermiculite in 9x9x10 cm square pots placed in 18-pot trays, and placed in growth chambers with 16 hours light/8 h dark and a constant temperature of 25°C. Vermiculite was kept humid by watering with tap water

until two weeks after sowing, and from this point onwards pots were watered twice a week with 50 ml Hoagland's solution without nitrogen (Caisson Labs) supplemented with either 5 mM KNO_3 or 20 mM KNO_3 (Sigma). Trays were regularly randomized. 35 days after sowing, SPAD was measured on a position halfway along the leaf blade of leaf 3 using a MultiSpeq V2 device (PhotoSynq Inc.), senescence was scored visually, and plants were harvested for destructive measurement of shoot biomass. Data analysis and statistical testing were performed in R (version 4.0.5).

Biolistics transformation of B104 embryos with RNPs

B104 immature embryos were harvested at 13 days after pollination. Embryos were incubated in the dark at 25°C for 2 days on BOM1 media (for media recipes, see Table S5), and 3-5 hours prior to bombardment moved to BOM2 media. 40 µg Cas protein was complexed with 5.7 µg gRNA (IDT) by incubating for 10 min at room temperature, and these RNPs were then coated onto 400 µg 0.6 µm gold particles (Bio-Rad) together with 800 ng double-stranded 3xPE dsODNs (IDT) and 2 µg plasmid DNA using TransIT-2020 (Mirus Bio)[19] and resuspended in 80 µl water. Ten µl gold solution was loaded onto a macrocarrier used for bombardment of 25 immature embryos with the Bio-Rad PDS-1000 instrument using 650 psi rupture disks. Callus induction and regeneration were performed as described above.

References

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Table S1: Targets and gRNAs used

Gene name	Identifier (B73V4)	gRNA sequence	Distance from TSS
Gln1-3	Zm00001d017958	TACACGTACGATTACAACCA	91
NLP5	Zm00001d015201	CTTGGGTTTAACTAATCCGT	167
ENOD93	Zm00001d045390	AGCTAATGGGAGAGTAGCCA	235
NAC60	Zm00001d013003	AAGGATATATAGCGGCGGGC	52
AlaATb	Zm00001d014258	AAGATGGAGGATTGCAAGTT	154
Dof1	Zm00001d031278	GACGCGAGTGGGGGCCCACG	93
		CCCGGTTCCAGGACGCGAGT	82
GOGAT1	Zm00001d043845	GGGGCGAAAATATAATCAGT	141
AspAT	Zm00001d043382	GACGTGGACGGCTGTTTTTG	79
NAC49	Zm00001d034601	TTCCTCGGGGATAGGGGATG	86
		TTGGCACGTGTGGTTGGGCG	150
AlaATa	Zm00001d030557	GTGCTTATAAAAGGCGGCCA	50
GS2	Zm00001d026501	TGGCTTTTGCTGAGGGGATA	85

Table S2: Insertion efficiency across all targets examined

For NAC49 and Dof1 two gRNAs were tried. Note that efficiencies mentioned here are based on size difference in flanking PCR, and not all have been validated by sequencing.

Gene	Lines generated	Lines with insertion	% insertion efficiency
Dof1 (gRNA 2)	85	5	6%
GOGAT1	61	7	11%
NAC49 (gRNA 2)	42	5	12%
ENOD93	57	8	14%
NLP5	62	10	16%
NAC60	58	11	19%
AlaATa	30	8	27%
Dof1 (gRNA 1)	9	3	33%
AlaATb	11	4	36%
Gln1-3	36	14	39%
AspAT	51	23	45%
GS2	25	13	52%
NAC49 (gRNA 1)	38	23	61%

Table S3: Summary of enhancer insertions recovered in whole-genome sequencing of 5 lines with enhancer insertions.

Expected (on-target) insertions are in black, the off-target insertion is in red. A single PE unit is underlined in the first sequence.

line	chr	insertion sequence	closest gene	distance to gene (bp)	insertion size
1	1	GTAAGCGCTTACGTAAGCGCTTACGTAAGCGCTTA	Dof1	93	35
	5	GTAAGCGCTTACGTAAA	NAC60	52	17
2	1	GTAAGCGCTTACGTAAGCGCTTACGTAAGCGCTTAC	NAC49	86	36
	2	GTAAGCGCTTACGTAAGCGCTTACGTAATCGCTTACGTAAGCGCTTACGTAAGCGCTTAC		>20 kb	60
	5	TGTAAGCGCTTACTAAGCGCTTACGTAAGCGCTTACGTAAGCGCTTAC	NAC60	52	48
3	10	GTAAGCGCTTACGTAAGCGCTTACGTAAGCGCTTACGTAAGCGCTTAC	GS2	85	48
4	1	GTAAGCGCTTACGTAAGCGCTTCCGTAAGCGCGCTTTCGTAAGCGCTTACGTAAGCGCTTAC	AlaATa	50	62
	3	ACCAAGCGCTTACTAAGCGCTTACGTAAGCGCGCTTACGAAAGCGCTTACGTAAGCGCTTAC	AspAT	79	62
	5	GTAAGCGGTTACGTAAGCGCTTACGTCAGCGCTTAC	AlaATb	154	36
5	1	GTAAGCGCTGAGTAAGCGCTTACGTAAGCGCTGACGTAAGCGCTTAC	AlaATa	50	47
	3	GTAAGCGCTTAC	AspAT	79	12
	5	GTAAGCTCTTACGTATGCGCTTTCGTAAGCGCTTACGTAAGCGCTTACGTAAGCGCTTACGTAAGCGCTTAC	AlaATb	154	72

Table S4: Summary of editing at KO target in selected T0 individuals
Alleles shown in bold are present at either 50% or 100% and likely to be heritable.

Plant	% editing	Alleles found
ZM205-3A	33.3%	21% +1, 3% -1, 2% -4, 1% -5, 1% -6, 1% +1 + additional at <1%
ZM212-1A	**98.5%**	**52% +1(T), 46% +1(C)**
ZM212-1B	3.3%	1% +1, 1% -3, 1% -6 + additional at <1%
ZM212-1C	4.4%	4% +1
ZM212-1D	1.7%	No alleles at >1%
ZM212-7A	**99.5%**	**99.5% +1(A)**
ZM212-7B	15.0%	9% +1, 2% -1, 1% -3, 1% -4 + additional at <1%
ZM212-7C	14.1%	6% +1, 3% -1, 1% -5, 1% -7 + additional at <1%

Table S5: Media used in transformation

		unit	*BOM1*	*BOM2*	*ML3*	*ML4*	*ML5*	*ML6*	*ML7*	*ML8*
MS Salts	Duchefa	g/L	4.33	4.33	4.33	4.33	4.33	4.33	4.33	4.33
Sucrose	Duchefa	g/L	30	30	30	30	30	45	60	30
myo-Inositol	Duchefa	g/L	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Casein Hydrolysate	Duchefa	g/L	0.1	0.1	0.1	0.1	0.1	0.05		
L-Proline	Duchefa	g/L	2.76	0.69	0.7	0.7	0.7	0.35		
MES	Duchefa	g/L			0.5	0.5	0.5	0.5	0.5	0.5
Sorbitol	Duchefa	g/L		36.4						
Mannitol	Duchefa	g/L		36.4						
Gelrite/Gelzan	Duchefa	g/L	3	3	2.3	2.3	2.3	2.5	2.5	3
pH			5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
autoclaving										
Silver Nitrate (100mM)	Sigma	mL/L	0.9	0.9	0.88	0.88	0.88	0.059		
Dicamba-NaOH (6,63g/L)	Duchefa	mL/L	0.5	0.5	0.5	0.5	0.5			
NAA-NaOH (1mg/mL)	Duchefa	mL/L						0.5		
BAP (1mg/mL)	Duchefa	mL/L						1		
Abscisic acid-NaOH (1mg/mL)	Duchefa	mL/L						2.5		
CuSO4 (1mg/ml)	Sigma	mL/L						5		
PPT (20mg/mL)	Duchefa	mL/L				0.075	0.25	0.25	0.3	
Carbenicillin (100mg/mL)	Duchefa	mL/L			2.5	2.5	2.5	2.5	1.25	
Mc Cown Woody Vitamins (1000x)	Duchefa	mL/L			1	1	1	1	1	1
MS Vitamins (1000x)	Duchefa	mL/L	1	1						

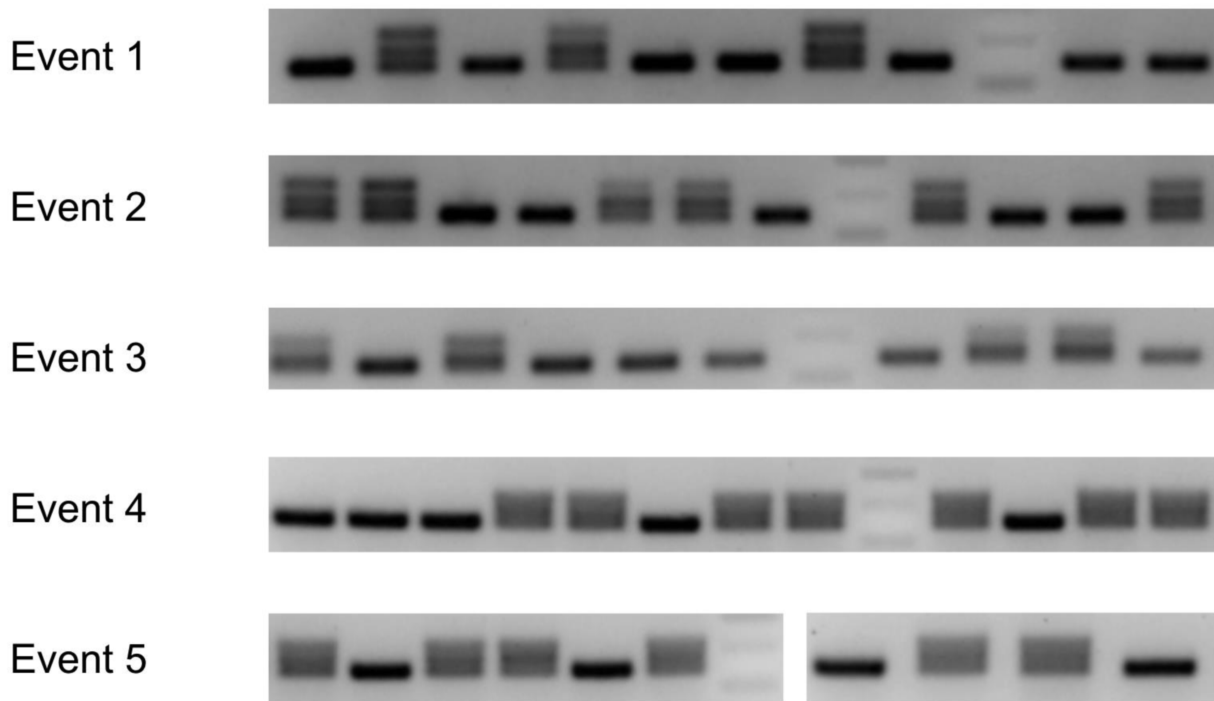
Table S6: Primers used

3xPE	/5Phos/G*T*AAGCGCTTACGTAAGCGCTTACGTAAGCGCTT*A*C
Detection of oligo insertion (gel shift)	
Gln1-3-F	GCCGAATATATTATGCGCGGAAC
Gln1-3-R	AGGGATACGAAACAAGTTGGCG
NLP5-F	ATCATAGATTCGTACTCATAAACAG
NLP5-R	TGATGCGTTGGTGGATGTG
ENOD93-F	GCACTTTTTAAGCCTGCTTGTTC
ENOD93-R	CTCAAGATTTGGGAGGGGGC
NAC60-F	CTTTCCCTCCCGAACCCAC
NAC60-R	GCGCTGTCATTGAAGCTGAG
AlaATb-F	AGCATTACATGTTGACCCCG
AlaATb-R	GCTGAAGATGGACTTGCTTG
Dof1-F	CACCACGTCCCTCCCTGC
Dof1-R	GGCTGGCTTTGCCTTCTCTC
GOGAT1-F	GCTGAGATAGCCTCACCTAAC
GOGAT1-R	CTCTCCTCGTCTCGCGTG
AspAT-q-F	AACAAGGGACACACGCGCTC
AspAT-q-R	GTCGCGTATAAAACAGCGGG
NAC49-q-F	CAACCACACGTGCCAATTCC
NAC49-q-R	GTTGTCGAAGCTGAGAACCG
AlaATa-F	GTGCGCCTCTTGTTTGTCTG
AlaATa-R	CTCTCTCACAGTGCGGCTTC
GS2-F	TTGCCTGCCTCTGATGCTC

GS2-R	AAAGTACTTGGCATCCAGTGG
Amplicon sequencing	
Gln1-3-F	CGAGGGATACGAAACAAGTTGGCG
Gln1-3-R	CGAGTCCACATGTAACTGACAGGTGG
NLP5-F	GTGATATCCAGTAATCATAGATTCG
NLP5-R	TCAGCAACTACTGAGACCAC
ENOD93-F	TGCCTACCGAAAACTGGGAG
ENOD93-R	CTCAAGATTTGGGAGGGGGC
NAC60-F	CTTTCCCTCCCGAACCCAC
NAC60-R	CCGAGAACTAGAAGCGGTGG
AlaATb-F	AGCATTACATGTTGACCCCG
AlaATb-R	GAAAGCGAGTGGAAGGAGACC
Dof1-F	TGTACGGGGGGCTGGGTTGGC
Dof1-R	CGAGCGAGCGAGCGAGTTGAAAG
GOGAT1-F	AGGGGTACAACGGAATCCTG
GOGAT1-R	TATAGAGGGGAGGAGCGAGG
AspAT-q-F	CCAGTCCACATCTCCACGG
AspAT-q-R	GGGAAACGAGAGCGGGGG
NAC49-q-F	CGTCCAATCCAATCCCTGGG
NAC49-q-R	GGGAGGGATATATAGCGGCG
AlaATa-F	GTGCGCCTCTTGTTTGTCTG
AlaATa-R	AACACGAGGGATCAGCTTCG
GS2-F	TTTGCCTGCCTCTGATGCTC
GS2-R	CTCACCTCACCTGTGACGAG
qRT-PCR	
Act1-F	GGATCTACCATGTTCCCTGG

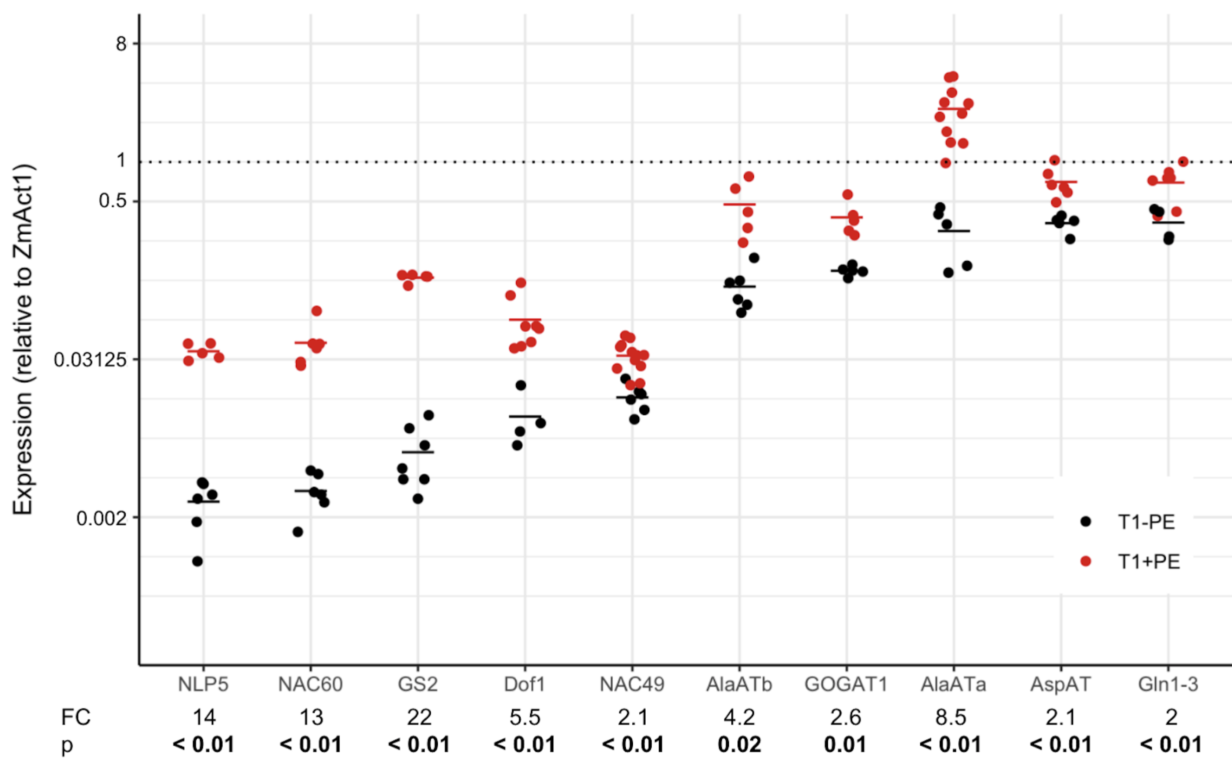
Act1-R	CTTGGAGATCCACATCTGCTG
Gln1-3-q-F	AGGAGCCGTGGTATGGTATTGAG
Gln1-3-q-R	TTCCACAGTAGTAAGGACCCTGAG
Gln1-4-q-F	TCATGTGCGATTGCTACACC
Gln1-4-q-R	ACTCCTGCTCGATACCATACCAG
NLP5-q-F	AGTCTCGGTGTTTGTCCGAC
NLP5-q-R	CTTGATCTTGTCCAGGGAGC
ENOD93-q-F	CTGGGATGGCCTACTTCAT
ENOD93-q-R	GCTCTGGACGAAGGCCAC
NAC60-q-F	CTCCCACTCGCAGATCAC
NAC60-q-R	TTGGGCACCATCATCGCC
AlaATb-q-F	GGATTTGGTCAAGTTCCTGG
AlaATb-q-R	ACACACAGCGTAACAGTTTAG
Dof1-q-F	GACGAGGACTCGTTCGTGTG
Dof1-q-R	GCAGAAGAATCAAAGCGCTCC
GOGAT1-q-F	GTCCTGAAGCGACAATAGCTG
GOGAT1-q-R	GGCAAACACACCATCGACAC
AspAT-q-F	TGACATCTGATGGGAGGATC
AspAT-q-R	GAAAGCTGCGATACTATCCTC
NAC49-q-F	GGTCGCTTAGGTTGGATGAC
NAC49-q-R	AGTGTGATTGGGAGTGGGAG
AlaATa-q-F	GGATTTGGCCAAGTTCCTG
AlaATa-q-R	CTCAGCCACTGTAACAGTTC
GS2-q-F	GGAACCTTCTCATGGGGTG
GS2-q-R	GGTCTTCCAGGTAACCTTTCC

Figure S1



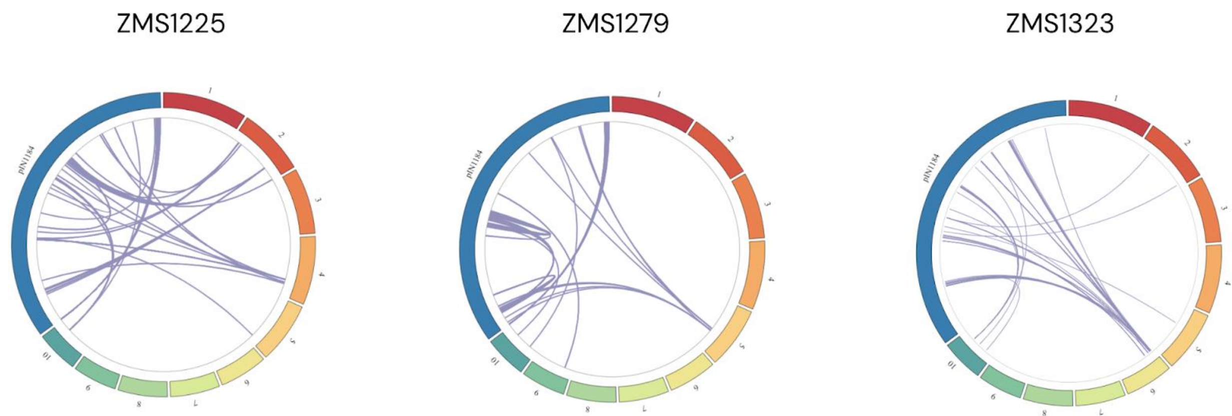
Genotyping of T1 individuals resulting from a backcross of a T0 with a heterozygous 3xPE insertion with B104. Each lane represents one individual, and PCR was performed with primers flanking the insertion site; in plants with an insertion, a second band that is higher can be seen, in addition to the band coming from the unedited allele. In all cases, segregation is 1:1 as expected.

Figure S2



Expression of 10 target genes in primary roots of lines with 3xPE insertion in the corresponding gene. Dots represent data points from individual plants, horizontal line segments show the average per group. FC, fold change in individuals with 3xPE versus individuals without 3xPE. p-values calculated by Student’s t-test.

Figure S3



Maps showing junctions between plasmid sequence (blue) and each of the ten maize chromosomes (rainbow, dark red to turquoise). Each line represents a junction. For 2 out of 5 events, no plasmid reads were found.

Figure S4

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Promoter enhancer target                                                KO target

REF TTATCTCTG-----------------------------------AGGAGGAGG   CGCCCTCCTCGC-CGAGGTAGTATTG

ln1 TTATCTC--GTAAGCGCTTACGTAAGCGCTTACGTAAGCGCTTACAGGAGGAGG   CGCCCTCCTCGCTCGAGGTAGTATTG
    TTATCTC--GTAAGCGCTTAC-------------------------AGGAGGAGG   CGCCCTCCTCGCCCGAGGTAGTATTG

ln2 TTATCTCTG-----------------------------------AGGAGGAGG   CGCCCTCCTCGCACGAGGTAGTATTG
    TTATCTCTGGTAAGCGCTTAC-------------------------AGGAGGAGG   CGCCCTCCTCGCACGAGGTAGTATTG
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Alleles recovered in two lines from transformation to introduce 3xPE in the promoter of an enhancer target (left) and small indels in the coding sequence of a KO target (right). Line 1 (ln1) shows biallelic edits at both targets, while line 2 (ln2) is heterozygous for a 1xPE insertion in the promoter and homozygous for a 1 bp insertion in the KO target. The PAM (NGG) is underlined.