

Figure S1. Maize *ARGOS8* gene expression. The transcript abundance of *ARGOS8* in various tissues of maize inbred PH184C was measured by RNA sequencing. Samples were taken from the plants at the developmental stage of V10, VT/R1 and R4. TPM, transcript per ten million.

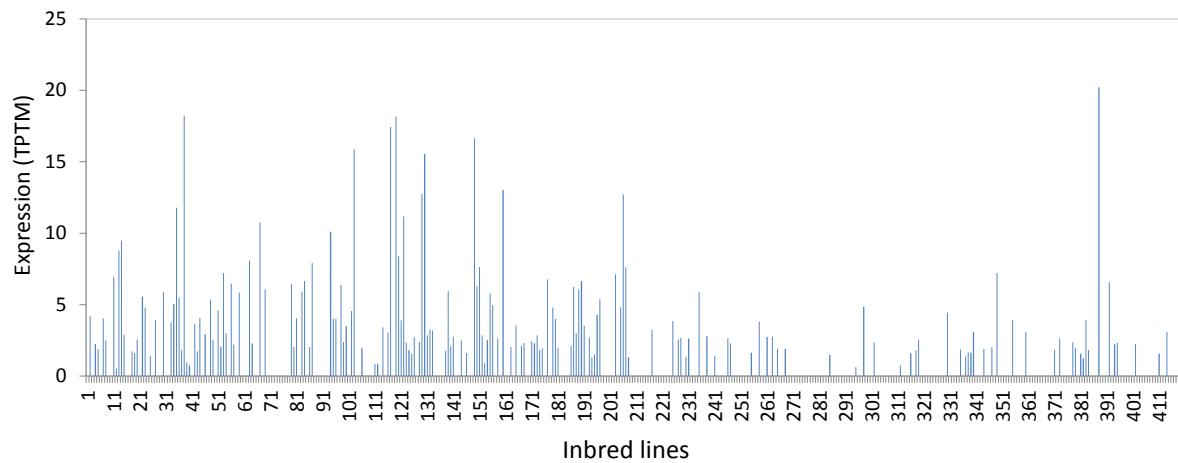


Figure S2. Comparison of *ARGOS8* gene expression among 419 maize inbred lines. The *ARGOS8* transcript levels in leaves of 3-week-old seedlings were measured by RNA sequencing. TPM, transcript per ten million.

(a)



(b)

CR1

GC GTGCATCG ATCCATCGC G TTTTAGAGCT AGAAATAGCA AGTAAAATA AGGCTAGTCC GTTATCACT TGAAAAAGTG GCACCGAGTC GGTGCT

CR2

GCATCATATC TATCCGTAGC CGTTTAGAG CTAGAAATAG CAAGTTAAA TAAGGCTAGT CCGTTATCAA CTTGAAAAAG TGGCACCGAG TCGGTGCT

CR3

GTTACTTCTC TAAGCACGGC GTTTAGAGC TAGAAATAGC AAGTTAAAAT AAGGCTAGTC CGTTATCAC CTTGAAAAAGT GGACCCGAGT CGGTGCT

Figure S3. Single guide RNA genes used in editing the genomic sequence of *ARGOS8*. (a) Scheme illustrating the structure of a single guide RNA (sgRNA) gene. (b) DNA sequences of the transcription region in the sgRNA genes *CRISPR RNA1 (CR1)*, *CR2* and *CR3*. U6 PRO, maize U6 polymerase III promoter; crRNA, CRISPR RNA (in red font) functioning as a guide; tracrRNA, trans-activating CRISPR RNA, the sgRNA scaffold.

>ID189_HR1 junction PCR product (P1/P2), ARGOS8-v1

GCGCCATTCCCTAAAGGTAACTACTAAACAGAACACAATTCTAGGCCATCTCAGCAGGCTGTGAAACAGCCGTGCAAATGTTTTGCA
CTGTTGTACAGTGAAGTTAAATAAAGAGTAAGATAGGAAATGAGATAAAGAGTATTCTGGAGATAGTCTTACATCACCGTAGCAACGCATT
AATATATACCTAGTTAATATTATGATTAAATTCTAAAATACTTGACGCCATCTAGTGAAGTTAGTTATAATACATATTATCTCTCAA
TACATGAACATCCTTTAGAATACGTATAGGACGATCACCGATACGGAGGATCTAGGCAAACAACATAAGAACATCGAGCGAGTGTAGGAGCATC
TCTAAAAGACTAATAAAATAACTCGTAAGTTAAATTAGATACTCAAGAGATAAATAACTATTAAACAGACTCGTTTTGACTCATCAATATACC
CTTCAAAATTGACTCTCTACATCTAAACTGGCTAGCCACTATGACTAAACTAGATAGATACTTTGGTGGAGTAAGATGCTATATAAAGTG
TAATATTATAAAATGATAAAATAAAAGCTAAAATAAGAGTTACTCTCTAAGCAGGCTGATTAGGCCCTAGTCTACTAGTACTAGCT
ACACGACGTGCACGCATCATCACAGCAACTAGACACGCACAGCAGCTGCACGGCCGGGGAAACCAACTGATTCCCCCTCCCGCGC
GGTTGATTCTCTCTGGTACGGATCCATCTGAGGGCTTGGCGTTTCCAACACATGTATTGGATGGGATTGAAAAAAATGAGAA
GAAGTTGACTGTTGGATTAAACCCATCCAATCCACTCAATCACATGGATTGAGAGCTAACCGAACAGCCCTCATAGTACATACCTGGTA
CGGATCCATATCATAGTACATAGCTCAGTAGAATAGAAGGTGATCCGACCGCCGGCTTGCCTGTTCCCCTGGCCATTGAACCTGCCAACCC
TCCTAACACAGGCACGCAAACCGGGCTCCGGCACCCGCCACCGCCACCTGGCCACCTCTAACCCCCAATCCAGGGGGGGGG
GGGGGCACCATGCGTCATCGATCCATGCTGTCCAAGCGTCACTTACGATTAGC

>ID189_HR2 junction PCR product (P5/P4), ARGOS8-v1

ACCGTCCTTATCTGCATCATCTATCTGCTAGTTCTGCGTCTCAAATATTCCGGCTGTGACTGATGACTGATAATATGATTGGCA
GCTTCTGCTATAAGAACACAAATCAAAGCTGATCAGCTCGGTCTCACAAAACCTCAACACCAAGTTCCATGAGCGCTGGCGCGGGTCCG
GCGGGCGGTCTGTGAGGGCAAATTATATAGGCTAGTGGTACCGGCTACGGATAGATATGATGCTGCACTGCACATTGGTATATCTGAGG
CTCCTCGCCGCCCTGGCAGGTCTGTCATCGGGCGATGCCGAGGAAGAGGAAGCCGGTGGCGACGACGACCATGGCGGGGGCAA
GGTGGCGGGCGCTGCTGGCACGGCGCGCTGCTGCTGCTCCCCTGCGCTGCCGCTGCCGCCACCGCAGCTGTTGTTCG
TCCCCGTGGCTTGTCTGCTCCCTGTCGGCTCCCTCGCCTGCCCCCGCGCACCCCTCGCCGATGCTGATGCCGCCACACGGGTCGTT
CGGGACCACTGGATCACCGCACCTATGTTGACGCTGACGATGAGAGATCTGTCGATGACGGTGGCTTGAATGGCGCTGGAATCAAGCAG
TTCGACACAC

>ID664_HR1 junction PCR product (P1/P2), ARGOS8-v2

GCGCCATTCCCTAAAGGTAACTACTAAACAGAACACAATTCTAGGCCATCTCAGCAGGCTGTGAAACAGCCGTGCAAATGTTTTGCA
CTGTTGTACAGTGAAGTTAAATAAAGAGTAAGATAGGAAATGAGATAAAGAGTATTCTGGAGATAGTCTTACATCACCGTAGCAACGCATT
AATATATACCTAGTTAATATTATGATTAAATTCTAAAATACTTGACGCTATGAGTTAGTTATAATACATATTATCTCTCAA
TACATGAACATCCTTTAGAATACGTATAGGACGATCACCGATACGGAGGATCTAGGCAAACAACATAAGAACATCGAGCGAGTGTAGGAGCATC
TCTAAAAGACTAATAAAATAACTCGTAAGTTAAATTAGATACTCAAGAGATAAATAACTATTAAACAGACTCGTTTTGACTCATCAATATACC
CTTCAAATTGACTCTCTACATCTAAACTGGCTAGCCACTATGACTAAACTAGATAGATACTTTGGTGGAGTAAGATGCTATATAAAGTG
TAATATTATAAAATGATAAAATAAAAGCTAAAATAAGAGTTACTCTCTAAGCAGGCTGATCACCGTCACTTACGATTAGC

>ID664_HR2 junction PCR product (P5/P4), ARGOS8-v2

ACCGTCCTTATCTGCATCATCTATCTGCTAGTTCTGCGTCTCAAATATTCCGGCTGTGACTGATGACTGATAATATGATTGGCA
GCTTCTGCTATAAGAACACAAATCAAAGCTGATCAGCTCGGTCTCACAAAACCTCAACACCAAGTTCCATGGTACGGATAGATATGCT
GCACTGCACATTGGCTATATCTGAGGCTCTGCGCGCCTTGGCAGGTGTCATGCGGGCGATGCCGAGGAAGAGGAAGCCGGTGG
CGACGACGACCATGGCGGGGGCAAGGTGGCGCGCTGCTGGCACGGCGCGCTGCTGCTGCTCCCCTGCGCTGCCGCCACCCCTGCC
GCCGCCACCGCAGCTGTTGTCCTCGTGGCTCTGCTGCTGCCGCTGCCGCCACCGCAGCTGCTGCTGCTGCCGCC
ATGCATGCCGCCACCGGCTGTCGGACACTGGATCACCGCACCTATGTTGACGCTGACGATGAGAGATCTGTCGATGACGGTGGCT
TGCAATGGCGCTCGGAATCAAGCAGTTGCACAC

>ID336_PCR product (P1/P4), ARGOS8-v3

GCGCCATTCCCTAAAGGTAACTACTAAACAGAACACAATTCTAGGCCATCTCAGCAGGCTGTGAAACAGCCGTGCAAATGTTTTGCA
CTGTTGTACAGTGAAGTTAAATAAAGAGTAAGATAGGAAATGAGATAAAGAGTATTCTGGAGATAGTCTTACATCACCGTAGCAACGCATT
AATATATACCTAGTTAATATTATGATTAAATTCTAAAATACTTGACGCTATGAGTTAGTTATAATACATATTATCTCTCAA
TACATGAACATCCTTTAGAATACGTATAGGACGATCACCGATACGGAGGATCTAGGCAAACAACATAAGAACATCGAGCGAGTGTAGGAGCATC
TCTAAAAGACTAATAAAATAACTCGTAAGTTAAATTAGATACTCAAGAGATAAATAACTATTAAACAGACTCGTTTTGACTCATCAATATACC
CTTCAAATTGACTCTCTACATCTAAACTGGCTAGCCACTATGACTAAACTAGATAGATACTTTGGTGGAGTAAGATGCTATATAAAGTG
TAATATTATAAAATGATAAAATAAAAGCTAAAATAAGAGTTACTCTCTAAGCAGCTGCACTGACATTGGCTATCTGAGGCTCTGCGCGCCTTGGC
GCAAATTATAGGTCTAGTGGTACCGGCTACGGATAGATATGCTGCACTGACATTGGCTATCTGAGGCTCTGCGCGCCTTGGC
CAGGTGTCATGCCGAGTGCCGAGGAAGAGGAAGCCGGTGGCGACGACGACCATGGCGGGGGCAAGGTGGCGCTGCTGGC
CACGGCGGGCGCGCTGCTGCTGCCGCTGGCGTCCGCCGCGCCACCGCAGCTGTTGTCCTGGCTGGTCTGCT
CCTCGTGGCTCCCTCGCCTGCCCCCGCGCACCCCTCGCCGCGATGCTGATGCCGCCACCGGCTGTTGGGACCACGGGACTGGATCACC
GCACCTATGTTGACGCTGACGATGAGAGATCTGTCGATGACGGTGGCTTGAATGGCGCTCGGAATCAAGCAGTTGCACA

Figure S4. DNA sequences of the junction PCR products amplified from *ARGOS8* variants

Table S1. Frequency of the GOS2 promoter insertion with one target site and the promoter swap with two target sites in T0 maize plants

	Insertion	Swap
Target sites	CTS1	CTS2 and CTS3
No. of embryos bombarded	1050	1150
No. of shoots regenerated	194	334
No. of shoots with CTS modified	190	172 *
No. of shoots positive with junction PCR	7	23
HR1 junction-PCR positive	2	9
HR2 junction-PCR positive	3	11
Both junction-PCR positive	2	3
No. of T0 plants regenerated	3	8
HR1 junction-PCR positive	1	3
HR2 junction-PCR positive	1	3
Both junction-PCR positive	1	2

* Both CRISPR-RNA target site 2 (CTS2) and CTS3 were modified.

Table S2. Primers and probes used for generation and characterization of *ARGOS8* genome editing variants

Target	PCR type	Primer name	Primer type	Primer sequence (5'-3')
CTS1	qPCR	P11	Forward	CCTCTCCAACCCCAAATCCA
		P12	Reverse	CCACTAGACCTATATAAATTGCCCTCA
			Probe	CGCCAGCGATGGAT
CTS2	qPCR	P13	Forward	TGTGAGGGCAAATTATAGGTCTAGTG
		P14	Reverse	AGCCTCAGATATAGCCAATGTGCA
			Probe	CCGGCTACGGATAGA
CTS3	qPCR	P15	Forward	AGATAGATATTTGGTGGAGTAAGATGCT
		P16	Reverse	GCTAGTACTAGTAGAGGACTAGAGGCCTG
			Probe	AATCCAGCCGTGCTT
HR1 junction	PCR	P1	Forward	GCGCCATTCCCTAAAGGTAAC
		P2	Reverse	GCTAATCGTAAGTGACGCTTGGAA
HR2 junction	PCR	P5	Forward	ACCGTCCTTATCTCTGCATCATCT
		P4	Reverse	GTGTGCGAACTGCTTGATTCCG
HR2 junction	PCR	P3	Forward	GCTCGTCCAAGCGTCACTACGATTAGCT
		P4	Reverse	GTGTGCGAACTGCTTGATTCCG
<i>ARGOS8</i> locus	Long PCR	P1	Forward	GCGCCATTCCCTAAAGGTAAC
		P4	Reverse	GTGTGCGAACTGCTTGATTCCG
Cas9	PCR	P21	Forward	AACTGGTGGACAGCACAGACAA
		P22	Reverse	GAAGTGGCCGCGGAAC
			Probe	CTCGCGCATATGAT
sgRNA	PCR	P23	Forward	CTAACACAAGAGTGGAGCGTACCTT
		P24	Reverse	AGCCTTATTTAACCTGCTATTCTAGCTCT
			Probe	CCGAGCCGCAAGCA
PMI	PCR	P25	Forward	CGCCGGAGATATCGTTCA
		P26	Reverse	CCTCTCCGAGCAGAGTCGATT
			Probe	CTCAATCACATCACGCA
ODP2	PCR	P27	Forward	TTGGTGTACTTCTGCAGGTGAA
		P28	Reverse	AGAGGGAGAAAGCGAGCCAGT
			Probe	ATCCATGGCCACTGTGAA
WUS	PCR	P29	Forward	CTCCCTGCACCTGCCATG
		P30	Reverse	GCTGCCGCTGCCCTC
			Probe	CGCTGAGATAGAGGCATG

Table S3. Plant height (PLHT), ear height (EARHT) and grain moisture (MST) of *ARGOS8* genome edited variants and wild type under flowering stress, grain-filling stress and optimal (well-watered) conditions.

	PLHT (cm)	EARHT (cm)	MST %
<i>Flowering Stress (FS)</i>			
<i>ARGOS8-v1</i>	213.1	94.2	20.1
<i>ARGOS8-v2</i>	213.1	95.0	20.4
WT	213.5	92.8	20.3
<i>Optimal (OPT)</i>			
<i>ARGOS8-v1</i>	273.3	143.0	21.4
<i>ARGOS8-v2</i>	275.6*	144.5*	21.6
WT	273.0	141.3	21.5
<i>Grain-filling Stress (GFS)</i>			
<i>ARGOS8-v1</i>	nd	nd	18.2*
<i>ARGOS8-v2</i>	nd	nd	18.7
WT	nd	nd	19.0

Asterisks indicate predicted difference significant at $P < 0.1$. nd, not determined.