

## Supporting Information

### File S1

#### Material and Methods

**Nucleotide Variation in *tga1*.** We augmented the sample of teosinte individuals used by Wang *et al.* (2005) for nucleotide diversity analysis by sequencing an additional 11 teosinte plants (Table S1) for the 1042 bp control region defined by Wang *et al.* (2005) which includes the first 18 bp of the ORF and 1024 bp upstream of the ORF. The primer sequences, PCR programs, and sequencing protocols are the same as those used by Wang *et al.* (2005). For heterozygous DNA samples, PCR fragments were cloned into TOPO<sup>®</sup>-TA vectors (Life Technologies) and multiple clones were sequenced to acquire the individual allele sequences without potential Taq polymerase incorporated errors. To determine the putative causative site underlying the glume architecture difference between maize and teosinte, we aligned the complete set of 16 maize and 20 teosinte sequences and visually inspected the alignment for fixed differences between maize and teosinte.

**Screening for *tga1* Mutants and Identification of *neighbor of tga1* (*not1*).** Efforts to obtain a loss-of-function allele of *tga1* were made by screening several maize mutant collections, including maize targeted mutagenesis (May *et al.* 2003), maize TILLING project (Till *et al.* 2004) and Pioneer Hi-Bred International's Trait Utility System for Corn (TUSC). We also performed informatics searches of the databases for the RescueMu (Lunde *et al.* 2003) and UniformMu (Settles *et al.* 2007) projects. No *tga1* mutants were found in any of these collections, however, five *Mu* insertion events were identified in *neighbor of tga1* (*not1*), a gene closely related to *tga1* (Preston *et al.* 2012), from TUSC using a *Mu* terminal repeat primer (5'-agagaagccaacgccawcgcctcyatttcgtc-3') with a primer designed from *tga1* (5'agaaagcgtctggacgggcacaatc3').

**Quantitative PCR.** RNA extractions were performed, as previously described (Wang *et al.* 2005), on immature top ears that were 25mm in length. All gene expression comparisons were based on plants from the same nursery location and year. cDNA was produced as previously reported (Wang *et al.* 2005) and then used for quantitative PCR (RT-qPCR). RT-qPCR was performed on ABI Prism 7000 sequence detection system (Applied Biosystems) with Power SYBR<sup>®</sup> Green PCR Master Mix (Applied Biosystems) according to the manufacturer's instructions. Specific forward primers were used for *tga1* (5'-cagtgacgacaggttccatc-3') and *not1* (5'-cgcatcactcaccaaatcca-3') along with a common reverse primer (5'gaagcttatctgcctctc3'). We sequenced PCR products from 20 independent clones for each primer set to confirm that they were specific to either *tga1* or *not1*. A maize actin gene (AY104722, GRMZM2G126010) was selected as a control, and amplified using the primers 5'-ccaaggccaacagagagaaa

-3' and 5'- ccaaacggagaatagcatgag -3' (Bomblies 2003). Gene expression quantification was performed using 10 biological replicates for each genetic stock.

**Protoplast Transient Assays.** The TGA1 N-terminal coding sequences (amino acids 1-103) from the maize, teosinte and ems alleles were fused to a GAL4-DNA Binding Domain (DBD) followed by the *Nopaline synthase (Nos) terminator* (maize-GD, teosinte-GD and ems-GD respectively). These fusion gene effectors were expressed using the rice *Actin1* promoter. An effector with the GAL4-DBD alone (GD) served as the negative control. The effector GD-IAA17(I), encodes a GAL4-DBD fused to the IAA17 protein domain I, which is an known repressor (Tiwari et al. 2004). This effector served as a positive control for trans-repression. The effector LD-VP16 encodes an *E. coli* LexA-DBD fused to the *Herpes simplex virus* VP16 activation domain (LaMarco and McKnight 1989). This effector serves as a positive control for activation. Both the GD-IAA17(I) and LD-VP16 effectors are under the control of the cauliflower mosaic virus 35S minimal promoter (-46bp). These two effectors were generous gifts from Dr. Tom J. Guilfoyle at the University of Missouri-Columbia.

Two reporters were constructed for the transient assays. The LG-Fluc reporter contains a firefly luciferase gene under the control of the cauliflower mosaic virus 35S minimal promoter (-46bp) with two LexA and two GAL4 binding sites upstream. The LexA and GAL4 binding sites upstream of the minimal promoter allowed the fusion gene effectors containing Gal4-DBD or LexA-DBD to bind and drive the expression of the firefly luciferase gene. The Actin-Rluc reporter contains a *Renilla* luciferase gene under rice *Actin1* promoter. This reporter was co-transformed into the protoplasts with the LG-luc reporter and served as an internal control. Using both firefly and *Renilla* luciferase enabled us to take advantage of using the dual-luciferase reporter assay system (Promega Corp., Madison, WI) to take two readings from the same samples.

Transient expression assays using maize mesophyll protoplasts were performed following a detailed protocol from the Sheen's lab ([http://molbio.mgh.harvard.edu/sheenweb/protocols\\_reg.html](http://molbio.mgh.harvard.edu/sheenweb/protocols_reg.html)). Briefly, 2-3 x 10<sup>5</sup> freshly isolated protoplasts in 400  $\mu$ l electroporation buffer were mixed with 50  $\mu$ l of plasmids which include different combination of effectors and reporters. All the plasmids were prepared with EndoFree Plasmid Mega Kit (Qiagen) and each effector : reporter LG-Fluc : reporter Actin-Rluc mixture contained 25  $\mu$ g : 15  $\mu$ g : 5  $\mu$ g, respectively. The protoplast-plasmid mixes were transferred in 0.5 ml cuvettes and electroporated with a Gene Pulser II Electroporation System (Bio-rad) set to 250 Volts for 1.5 msec. Three pulses spaced 20 sec apart were used for each sample. After electroporation, protoplasts were incubated for 18 hrs at 25 °C before harvesting for quantification. The harvested protoplasts were then lysed with CCLR (Cell Culture Lysis Reagent, Promega) and subjected to dual-luciferase assay using a Dual-Luciferase Reporter Assay System (Promega) following the manufacturer's instruction. Six biological replicates were measured per effector/reporter mixture.

**Heterologous Protein Expression and Western Blot Assays.** Tagged TGA1 proteins were generated by cloning various full-length *tga1* cDNA alleles, and a *tga1* maize allele with an N-terminal coding region deletion, into the pET151/D-TOPO<sup>®</sup> vector (Invitrogen) and transforming into BL21 codonPlus (DE3)-RIL cells (Stratagene). All of the proteins cloned into the pET151/D-TOPO<sup>®</sup> vector are expressed with a 6xHis-V5-tag derived from the vector. Overexpressed proteins were extracted using BugBuster<sup>®</sup> Master Mix (Navagen) and then solubilized from inclusion bodies and purified under denaturing condition (with 6M urea) using a His-bind purification Kit (Navagen). The purified proteins were then re-natured by dialyzing through serial refolding solution with decreasing urea concentration (4 M, 2 M, 1 M to 0 M). The refolding solution consists of 0.1 M Tris, pH 8.0, 0.4M L-Arginine, 0.1 mM ZnCl<sub>2</sub>, 5 mM DTT, 0,2 mM PMSF and plant protease inhibitor cocktail (Sigma). Dialysis was performed in 2 liter volume at 4 °C with at least 2 hours in between solution exchanges. The purity and integrity of the proteins were assessed by SDS-PAGE and western analysis.

A soluble tag-free maize-TGA1 was generated, but could only be retained at low concentrations ( $\leq 5$  ng/ $\mu$ l). The maize full-length *tga1* cDNA was cloned into a pVP-GW vector (Singh et al, 2005), which can produce proteins with S-His-tag and enhanced solubility. Proteins were overexpressed in *E. coli*, and then extracted and purified using Ni-NTA Superflow Columns (Qiagen) following the manufacturer's instructions. After purification, the S-His-tag was cleaved off using AcTEV protease (Invitrogen). All proteins were diluted to low concentration (< 40 ng/ $\mu$ l) prior to the TEV protease reaction to prevent precipitation of TGA1 upon cleavage of the tag. Final purification of the tag-free TGA1 proteins was performed by passing them through Ni-NTA Superflow Columns.

Western blot assays were performed on immature ear tissue 25mm in length using the anti-TGA1 antibody as previously described (Wang et al. 2005). The mini gel for the western blot in Figure 1B was run at 150 Volts for 80 min. The blot was probed with anti-TGA1 first, and then stripped and re-probed with anti-ACTIN to confirm equal loading. A large gel that was run at 150 Volts for 18 hours, was used for the western blot in Figure 1C to resolve TGA1 and NOT1. Immature *not1-Mu2* ears were used for the western blot in Figure 5B.

**PCR-Assisted Binding Site Selection.** PCR-assisted binding site selections were performed via Electrophoretic Mobility Shift Assays (EMSAs) as described previously (Tang and Perry 2003). A 76-mer oligo (5'-actcgaggaattcggcaccgccgggt(n)<sub>26</sub>tggatccggagagctccaacgcgt -3') containing a core of 26 randomized nucleotides was synthesized and then converted to double-stranded DNA by PCR using two primers that corresponding to the non-degenerate ends of the

76-mer (5'-actcgaggaattcgctacccccgggt -3' and 5'- acgcgttgggagctctccggatcca -3'). The double-stranded 76-mer population was labeled with [ $\alpha$ -<sup>32</sup>P]dATP and purified on a 8% polyacrylamide gel for use as an EMSA probe.

EMSA were performed as reported previously (Wang et al. 2004) with slight modifications. Approximately 100 ng of maize-TGA1 or teosinte-TGA1 was pre-incubated in 1× binding buffer (50 mm Tris-HCl, pH 7.5, 50 mm NaCl, 1 mM ZnCl<sub>2</sub>, 1 mM DTT, 10% glycerol, 0.1 μg μl<sup>-1</sup> poly(dI-dC), 0.5 μg μl<sup>-1</sup> BSA) at room temperature for 10 min before adding 10<sup>4</sup>-10<sup>5</sup> cpm of the radiolabeled DNA fragments. Then, the entire reaction mix (20 μl) was incubated for an additional 10 min and analyzed on a 5% PAGE gel containing 0.5 x TBE and 2.5% glycerol. The gel was dried and exposed to a PhosphorImager screen (Amersham Biosciences). The regions corresponding to shifted bands were excised and electroeluted. Then, an aliquot from each elutant was used for PCR amplification and radiolabeling to generate new probes. The new probes were used for the next round of binding and EMSA and this procedure was repeated for 5 rounds. Finally, the enriched TGA1 bound oligonucleotides were cloned into pCR2.1-TOPO<sup>®</sup> cloning vector (Invitrogen) and at least 40 clones from each shifted band were sequenced and aligned to obtain the consensus sequence.

**EMSA Testing *in vitro* Interaction Between maize-TGA1 and *not1* Promoter.** An 825 bp of promoter segment of *not1-maize* as well as 480 bp of 5' end sequence of *not1-teosinte* were isolated using the GenomeWalker Universal Kit (Clontech, Mountain View, CA) following the manufacture's instructions. A DNA fragment corresponding to the *not1* regulatory region (from -389 to -343) and containing a GTAC motif (-372 to -361) was generated using two partially complementary oligonucleotides that were extend by PCR. The two oligonucleotides were as follows: oligonucleotide 1, 5'- ttgctacagtcgcaactgtctgtctgcaaaGTACgactgct -3', and oligonucleotide 2, 5'- ccggaactgggggtggagtgggagcagtcGACTttgagacaca -3'. A mutated version of the DNA fragment was created using oligonucleotide 3 (same as oligonucleotide 1 except that the GTAC was changed to CTAC) and oligonucleotide 4 (same as oligonucleotide 2 except that except that the GTAC was changed to CTAC). The wild-type and mutant DNA fragments were labeled with [ $\alpha$ -<sup>32</sup>P]dATP and EMSA performed as described above.

**Chromatin Immunoprecipitation (ChIP) Assays.** ChIP assays were performed to verify *in vivo* binding of TGA1 to DNA fragments containing the GTAC motif from the *not1* regulatory region. Immature ear primordia (1-5 cm in length) from *not1-Mu2* plants were isolated and sliced to pieces, with at least one dimension less than 5 mm, and then fixed in 1% formaldehyde. The experimental procedure was done as described previously (Gendrel et al. 2002; Wang et al. 2002). An anti-TGA1 polyclonal antibody was used for chromatin immunoprecipitation. DNA populations recovered from ChIP were then used as templates for

qPCR with a maize actin gene as the control. Primers specific to *not1* promoter region are 5'-acaggtgcacagcacaacat-3' and 5'-agcagcagccaacaagatt-3'. qPCR was performed as described above using 7 biological replicates.

***tga1-RNAi* Plants Generation and Phenotyping.** An RNAi vector that targets *tga1* was constructed by generating a hairpin loop with an inverted exon3 sequence (amplified using the primers 5'-GAGAGTCCATATCACATCACTCACC-3' and 5'-CACTAAAGCCAGTATCTCCCTACCAGATCGTC-3') link to intron2-exon3 of *tga1* (5'-AGTCTGTAAGTTCGAATACATCTAC-3' and 5'-CACTAAAGCCAGTATCTCCCT-3'). The hairpin structure was driven with a maize ubiquitin promoter (Christensen and Quail 1996) and transformed into Hi-II maize using the Agrobacterium-mediated method. Maize transformation was done at the Plant Transformation Facility at Iowa State University (<http://agron-www.agron.iastate.edu/ptf/index.aspx>).

Different transgenic events of *tga1-RNAi* were recovered and crossed to W22. Then, 80 progeny from each cross were grown out and subjected to a BASTA painting assay. Briefly, a 1% solution of glufosinate (BASTA) solution with 0.01% Tween was painted on the tip of an expanded leaf when plants were at 8-10 leaf stage. Four days after the BASTA application, the plants were scored for resistance or susceptibility to the herbicide. The correlation between RNAi transgene presence and BASTA resistance in plants were further validated by western blot (Figure S4). Western blots using anti-TGA1 were performed with four BASTA resistant and four susceptible plants for each event using young ear tissue. Consistent with expectation, TGA1 was not detected, or had a weak signal, in all ear samples from BASTA resistant plants, while samples from BASTA susceptible plants produced a strong signal with western blot analysis.

Thirty BASTA resistant and 30 susceptible plants, from segregating F<sub>2</sub> families derived from a cross between BASTA resistant T<sub>0</sub> plants with W22, were phenotyped for plant and ear architecture traits. These include lateral branch number, length of the uppermost primary lateral branch, blade length of the first husk leaf of the top ear, number of nodes with prop roots, glume length, the weight of 50 kernels, ear diameter, and ear length. To measure glume length, the kernels were removed from the middle of the ears and then the cobs were broken to expose the glumes for measurement. Eight glumes were measured from each ear and the average value calculated. T-tests were used to evaluate whether the trait values were significantly different between plants with/without the *tga1-RNAi* transgene. Segregating families from four independent transgenic events were assayed.

## Literature Cited

- Bomblies, K. 2003 Duplicate FLORICAULA/LEAFY homologs *zfl1* and *zfl2* control inflorescence architecture and flower patterning in maize. *Development* 130: 2385–2395.
- Christensen, A. H., and P. H. Quail. 1996 Ubiquitin promoter-based vectors for high-level expression of selectable and/or screenable marker genes in monocotyledonous plants. *Transgenic Res.* 5: 213–218.
- Gendrel A., Z. Lippman, and C. Yordan, 2002 Dependence of heterochromatic histone H3 methylation patterns on the *Arabidopsis* gene DDM1. *Science.* 297: 1871–1873.
- LaMarco, K. L., and S. L. McKnight. 1989 Purification of a set of cellular polypeptides that bind to the purine-rich cis-regulatory element of herpes simplex virus immediate early genes. *Gene. Dev.* 3: 1372–1383.
- Lunde, C. F., D. J. Morrow, L. M. Roy, and V. Walbot. 2003 Progress in maize gene discovery: a project update. *Func. Integr. Genomics* 3: 25–32.
- May, B. P., H. Liu, E. Vollbrecht, L. Senior, P. D. Rabinowicz, et al. 2003 Maize-targeted mutagenesis: A knockout resource for maize. *Proc. Natl. Acad. Sci. USA* 100: 11541–11546.
- Preston, J. C., H. Wang, L. Kursel, J. Doebley, and E. A. Kellogg. 2012 The role of teosinte glume architecture (*tga1*) in coordinated regulation and evolution of grass glumes and inflorescence axes. *New Phytol.* 193: 204–215.
- Settles, M., D. R. Holding, B. C. Tan, S. P. Latshaw, J. Liu, et al. 2007 Sequence-indexed mutations in maize using the UniformMu transposon-tagging population. *BMC Genomics* 8: 116.
- Singh, S., C.C. Cornilescu, R. C. Tyler, G. Cornilescu, M. Tonelli, et al. 2005 Solution structure of a late embryogenesis abundant protein (LEA14) from *Arabidopsis thaliana*, a cellular stress-related protein. *Protein Sci.* 14: 2601-2609.

Tang, W., and S. E. Perry. 2003 Binding site selection for the plant MADS domain protein AGL15: an in vitro and in vivo study. *J. Biol. Chem.* 278: 28154–28159.

Till, B. J., S. H. Reynolds, C. Weil, N. Springer, C. Burtner, et al. 2004 Discovery of induced point mutations in maize genes by TILLING. *BMC Plant Biol.* 4: 12.

Tiwari, S. B., G. Hagen, and T. J. Guilfoyle. 2004 Aux/IAA Proteins Contain a Potent Transcriptional Repression Domain. *Plant Cell.* 16: 533–543.

Wang H., Tang W., Zhu C., Perry S., 2002 A chromatin immunoprecipitation (ChIP) approach to isolate genes regulated by AGL15, a MADS domain protein that preferentially accumulates in embryos. *Plant J.* 32: 831–843.

Wang, H., L. V. Caruso, A. B. Downie, and S. E. Perry. 2004 The Embryo MADS Domain Protein AGAMOUS-Like 15 Directly Regulates Expression of a Gene Encoding an Enzyme Involved in Gibberellin Metabolism. *Plant Cell* 16: 1206–1219.

Wang, H., T. Nussbaum-Wagler, B. Li, Q. Zhao, Y. Vigouroux, et al. 2005 The origin of the naked grains of maize. *Nature* 436: 714–719.





```

NOT1-MAIZE MDWDLKAVGAWDLAELEQDHAAATAAAGPSEGHATDTAAAGTGTGTGTERPP---GAGA
NOT1-TEOSINTE MDWDLKAAGAWDLAELEQDHAAATAAAGPSEGHAAANTAAAGTG--MGTERPP---GAGA
TGA1-MAIZE MDWDLNAAGAWDLAELEQDHAAAPSSG---GHAANAAGTGT----ESRPPAPGAAGA
TGA1-TEOSINTE MDWDLKAAGAWDLAELEQDHAAAPSSG---GHAANAAGTGT----ESRPPAPGAAGA

NOT1-MAIZE PAECSDVLDKLGGMGECELGAGAATACREREEAAGATKRPRPAGQQQ---QQCPSCAVDGC
NOT1-TEOSINTE PAECSDVLDKLGGMGECELGAGAATARREREEAAGATKRPRPAGPGG---QQCPSCAVDGC
TGA1-MAIZE PAECSDVLDKLGGMGECEPGA----ARREREEAAGAAKRPRPAGPGGQQQQCPSCAVDGC
TGA1-TEOSINTE PAECSDVLDKLGGMGECEPGA----ARREREEAAGAAKRPRPAGPGGQQQQCPSCAVDGC

NOT1-MAIZE RADLSKCRDYHRRHKVCEAHSKTPVVVVAGREMRFCQQCSRFHLLAEFDADKRSCRKRLD
NOT1-TEOSINTE RADLSKCRDYHRRHKVCEAHSKTPVVVVAGREMRFCQQCSRFHLLAEFDADKRSCRKRLD
TGA1-MAIZE RADLGGKCRDYHRRHKVCEAHSKTPVVVVAGREMRFCQQCSRFHLLAEFDADKRSCRKRLD
TGA1-TEOSINTE RADLGGKCRDYHRRHKVCEAHSKTPVVVVAGREMRFCQQCSRFHLLAEFDADKRSCRKRLD

NOT1-MAIZE GHNRRRRKQPDTMASASISITSQOQTRFSPFAPRLEASW-PGVMKTEESPYRITHQIHL
NOT1-TEOSINTE GHNRRRRKQPDTMASASFITSQOQTRFSPFAPRLEASW-PGVMKTEESPYRITHQIHL
TGA1-MAIZE GHNRRRRKQPDTMASASFIASQOQTRFSPFAHPRLEASWPPGVMKTEESPYRITHQIPL
TGA1-TEOSINTE GHNRRRRKQPDTMASASFIASQOQTRFSPFAHPRLEASWPPGVMKNEESPYRITHQIPL

NOT1-MAIZE GSSSS-SRQQHFV--GAATSAYAKEGPRFPFLQEGEISFATGVVLEPPAAAPACQPLLKS
NOT1-TEOSINTE G-SSS-SRQQHFV--GAATSAYAKEGQRFPFLQEGEISFATGVVLKSGA-----
TGA1-MAIZE GSSSS-SRQQHFVALGAATPAYAKEGRRFPFLQEGEISFATGVVLEPPAAAPACQPLLRT
TGA1-TEOSINTE GSSSSGNRQQHFVALGAATPAYAKEGRRFPFLQEGEISFATGVVLEPPASAPACQPLLRT

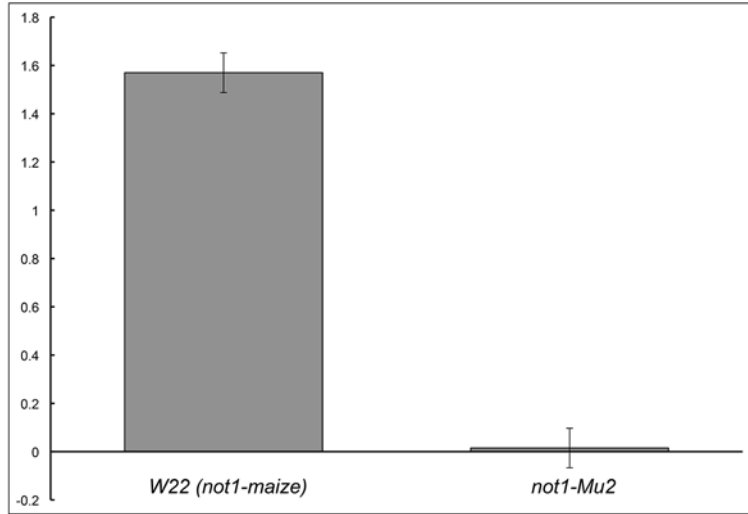
NOT1-MAIZE GAPPPESSSAGGGKMFSDQGLTRVLDSDCALSLLSAPANYSGIDVSRMVRPTEHVMAQQ
NOT1-TEOSINTE --PPPESSSAGGGKMFSDQGLTRVLDSDCALSLLSSPANYSGIDVSRMVRPTEHVMAQQ
TGA1-MAIZE GA-PSESSGAGGSKMFSDQGLARVLDSDCALSLLSAPANSSGIDVSRMVRPTEHVMAQQ
TGA1-TEOSINTE GA-PSESSGAGGSKMFSDQGLARVLDSDCALSLLSAPANSSGIDVSRMVRPTEHVMAQQ

NOT1-MAIZE LVVSGLQFGSASWFRPQASTGGSFVSSCPAVQVEGEQQLNAVLGPNDSEVSMNYGGMFH
NOT1-TEOSINTE LVVSGLQFGSASWFRPQASTGGSFVSSCPAVQVEGEQQLNAVLGPNDNEVSMNYAGMFH
TGA1-MAIZE PVVPGLQFGSASWFRPQASTGGSFVVPFCPAA-VEGEQQLNAVLGPNDSEVSMNYGGMFH
TGA1-TEOSINTE PVVPGLQFGSASWFRPQASTGGSFVVPFCPAA-VEGEQQLNAVLGPNDSEVSMNYGGMFH

NOT1-MAIZE VGGGSGGGEGSSDGGTSSSMFFSWQ
NOT1-TEOSINTE VGGGGE-----
TGA1-MAIZE VGGGSGGGEGSSDGGTSSSMFFSWQ
TGA1-TEOSINTE VGGGSGGGEGSSDGGTSSSMFFSWQ

```

**Figure S2** Amino acid alignment of TGA1 and NOT1 from the maize inbred W22 and the teosinte parent of the mapping population used by Wang *et al.* (2005).



**Figure S3** Column graph showing results of RT-qPCR using *not1* specific primers. Expression of *not1* was normalized to a maize actin gene. *not1-Mu* expression ( $0.015 \pm 0.082$ ) was 100-fold less than *not1-maize* ( $1.570 \pm 0.082$ ) in W22 and not statistically different from 0 (no expression).

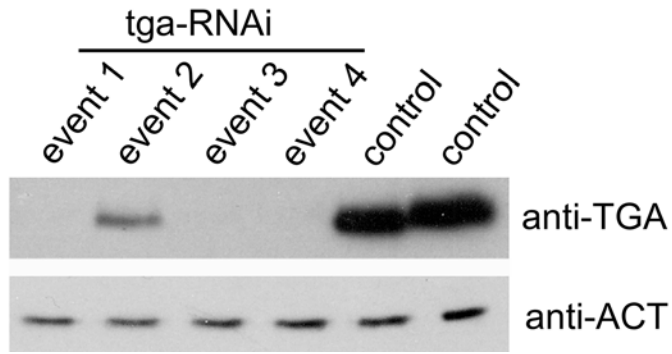
>*not1-maize* promotor (from W22)

ATCATTTCCACTTCTTGAGTTACCGTTTCCAATTTTCATTTTTTTAAAAAATATA  
AAAATAAAAATACTTTTAATATTTATCACTGTTCCGTCCTGCCTCCCTTGCTA  
CAGTCGCAACTGTCTGTGTCTGCAAAGTACGTGACTGCTCCCACTCCACCCCA  
GTTCCGGGCCTCTTGGGCATCGCTTCTGTTGCTGCAAATCTTTGGGCCTGTTG  
GTTCAGCTTTTTTCTGACCAGCTTTTCTGAGAATCTGGCTGTAGGAAGAATCT  
GGCTGTGTAGAGAATCTGTGTATCATTAGGATTACGTGCGGAGGAAGATAAA  
GGTGTCCATAGGACTCAGGATATAGAAAGTGACGGATTCTACTATTGCAAC  
GACTCAACCGATTATGTGTTTATGTTGATTTTGAATGATTTTTACTCAAACGA  
ATTTTATAGAAGCTGACTGAAAAGCTGAGCGTTTGGCAGTCCACAACAGCTT  
TTGGTGGCGAGAAGCTCAAAAAGCTGAAACAAACAGTGGCTTTGTTGCCTG  
CTGCTGCCTTGCTATCTATCTATACACCGCCCGCACCTTCCATTCTCTCCTC  
CGAAGCAGCAGGCAGCAGCTGCATCGCACCTCACACCTCTCGTGTCCATCGA  
TCCAGCCGCCCGCCGAGCTGCAGCTCTCACTGTTGCTGTGCCACCTCCTCGT  
CGCCTGTAGTGTGACAGAGAAACGCCCGCTGAATGAGAGGGAAGGAACGG  
AAGCTGCAGCGGGCGCGCGAGTGCAAGGACTAGGACTAGCGGTTGCAACGT  
CGGCGCGCGCGCGCGTGCAGGTACGTCCGGGCATG

>*not1-teosinte* promotor (from W22:*tga1*)

AAATACGTATATATTACTAATTTTTTTGTGGATGTACAACCTTGTGTGAATT  
TTTTAAGTCAATTGAGGGTGGATGTTATTTTACCAATTGTATTTAGATTC  
CATACGTTTATTTTAGGGAAATTTGGATCCATACCATTAAAAGATCACCCT  
TTGGATCCATACCATTACTATCTCACTTACATGTGGGTCCACATGAGTCAATG  
ACATGTGGGGTCCATGGTATATATCTAAAGTTTGGATCTTTTAATGGTATAGA  
TCCAATTGTTCTTATTTTTATACCAAAGTTTGTTTTTCATGTACACGAATTA  
CTGATATCATTTTCACTTCTGGAGTTACCGTTTCTAATTTCAATTCCTGAAAAA  
TGTAATAATAAAAATACTTTTAATATTTATCGCTGTTTCCGTCCTGCCTCCCT  
GCTACAGTCGCAGCTGTCTGTGTCTGCAAAGTACGTGACTGCTCCCACTCCACT  
CCACCCCAAGTTCCGGGCCACTCGGGCATCGTTTCTGTTGCTGCAAATCTTTG  
TTGGCTGCTGCTGCCTTGCTATCTATCTATACACCGCCCGCACCTTCCATTCT  
CCTCCTCCGAAGCAGCAGGCAGCAGCTGCATCGCACCTCACACCTCTCGTGT  
CCATCGATCCAGCCGCCGCCGAGCTCTCACTTCACTGTTGCTGTGCCACCTC  
CTCGTCGCCTGTAGTGTGACAGAGAAACGCCCGCGGAATGAGAGGGAAGG  
AACGGAAGCTGCAGCGGGCGCGCGAGTGCAAGGACTAGGACTAGCGGTTGC  
AACGTCCGGGCATG

**Figure S4** Nucleotide sequences of the maize and teosinte *not1* promoter sequences. The GTAC motif bound by *tga1* is highlighted in yellow. The start codon (ATG) for the ORFs are shown in bold.



**Figure S5** Western blot showing the TGA1 and NOT1 protein accumulation were knocked down or out in 4 different *tga1-RNAi* events. Blot was probed with anti-TGA1 first (top panel), then it was stripped and probed with anti-ACTIN show equal loading. Controls is from plants with out transgene.

**Table S1** List of additional teosinte accessions for diversity analysis

Sample	Genbank ID	Accession	Source	Taxon	Country	State/Province	Collector	Collection	Altitude	Latitude	Longitude
S1663	KR261108	G-120	HHI	<i>Z. mays</i> ssp. <i>huehuetenangensis</i>	Guatemala	Huehuetenango	H. Iltis	G-120	1350	N15:39	W91:46
S0149	KR261105	11369	CIMMYT	<i>Z. mays</i> ssp. <i>mexicana</i>	Mexico	Guanajuato	T. A. Kato	K-69-7	1925	N20:10	W101:5
S0190	KR261106	11387	CIMMYT	<i>Z. mays</i> ssp. <i>mexicana</i>	Mexico	Chihuahua	Wilkes, Sanchez, Taba	WST-85-2	1850	N26:14	W106:58
S0897	KR261107	11372	CIMMYT	<i>Z. mays</i> ssp. <i>mexicana</i>	Mexico	Guanajuato	T. A. Kato	K-69-10	2100	N20:5	W101:13
TA175	KR261102	8765	CIMMYT	<i>Z. mays</i> ssp.	Mexico	Michoacan	T. A. Kato	K-67-22	950	N19:5	W100:28

TA176	KR261103	8765	CIMMYT	parviglumis Z. mays ssp. parviglumis	Mexico	Michoacan	T. A. Kato	K-67-22	950	N19:5	W100:28
TA074	KR261099	69	INIFAP	Z. mays ssp. parviglumis	Mexico	Mexico	Sanchez & Ordaz	JSG Y LOS - 172	1620	N19:10	W100:12
TA075	KR261100	69	INIFAP	Z. mays ssp. parviglumis	Mexico	Mexico	Sanchez & Ordaz	JSG Y LOS - 172	1620	N19:10	W100:12
TA076	KR261101	69	INIFAP	Z. mays ssp. parviglumis	Mexico	Mexico	Sanchez & Ordaz	JSG Y LOS - 172	1620	N19:10	W100:12
TA012	KR261098	18	INIFAP	Z. mays ssp. parviglumis	Mexico	Jalisco	Sanchez & Ordaz	JSG Y LOS - 3	1520	N20:25	W102:21
TI28	KR261104	G-347-3	Kermicle	Z. mays ssp. mexicana	Mexico	Chihuahua	Kermicle	G-347-3	1850	N26:14	W106:58