Figure S1  (a, b) BTx642 tissues on EMM with IMZ selection following transformation with pPHP81814. (c) Plantlets growing on rooting media with IMZ.
Figure S2 Plants from (a) RTx430 and (b) BTx642 transformed with pPHP81814. In both, plants null for Zm-Bbm and Wus2 (right) and plants retaining introduced Zm-Bbm and Wus2 (left) have shorter stature, twisted leaves and poorer seed set, compared to wild-type.
Figure S3  Schematic representation of the molecular components of constructs. (a) pPHP83911, used for generating pGL193 and pGL198, is a pENTR vector. (b) pPHP85425 is a destination vector for morphogene-assisted transformation (MAT). Molecular components between left and right borders are shown. (c) pGL193 is a pENTR vector for MAT-mediated editing with gRNAs. The remaining backbone part is from pPHP83911. (d) pGL198 is a pENTR vector for MAT-mediated editing with the maize ubiquitin promoter and maize codon-optimized SpCas9 for carrying gRNAs. The remaining backbone part is also from pPHP83911.
Figure S4 Two gRNAs targeting *pds* in pGL196; yellow rectangles indicate exons; light gray lines indicate intron regions. gRNA1.1 is located in the third exon of *pds* genes. gRNA1.3 is located in the fifth exon of *pds* genes.
Figure S5 Molecular cloning strategy for constructing sorghum CRISPR/Cas9 vectors. Cloning vector for gRNAs containing BsaI sites for sequential insertion of two or three gRNAs with tRNAs that forms polycistronic cassettes (gRNA-tRNA), driven by rice U3 promoter (Os-U3pro). The gRNA-tRNA and Cas9 expression cassettes were mobilized into pPHP85425 through Gateway recombination, generating pGL196 and pGL199 for Agrobacterium-mediated sorghum transformation.
**Figure S6** Determination of transgene independent integration (TII) with adapter ligation-mediated PCR. Genomic DNA with a T-DNA insert (i) was digested with EcoRI and HindIII (ii). Adapters are ligated to digested sites creating adapter-flanked templates (iii). Only the longer arm of the adapter contains a sequence exactly matching the adapter primers. Adapter-to-adapter amplification does not occur due to lack of primer match sites on the shorter arm of adapters. If T-DNA is present in the template (black line, Fig. 4a.i), T-DNA primers (yellow arrow, left border; dark blue arrow, right border, Fig. 4a.iii) will bind to their corresponding sites and initiate synthesis of a complementary strand (iv). PCR products will contain adapter primer-binding sites derived from the complement of the longer arm of the adapter.
### Table S1. Primers for genotyping putative transformed plants

| Gene symbol | Protein name                  | Construct name | Primers (F/R) (5'-3')                           | Amplicon length (bp) | Tm (°C) |
|-------------|-------------------------------|----------------|-------------------------------------------------|----------------------|---------|
| ALS2        | Acetolactate synthase         | pPHP81814      | CTTTGCCCATGGAACGA ATCTCTTTTATCGCTGCGC           | 576                  | 58.2    |
| ZSG         | ZsGreen                       | pPHP81814      | CTCCTGCGGAGAAGATCATC ACCCTATCGAGATCTGAGTC       | 277                  | 55      |
| RFP         | Red fluorescence protein      | pANIC10A       | GGCCATTATACGTGCGACTT GACATGTGCAATTTCGTTG        | 155                  | 60      |
| Bbm         | Baby boom                     |                | GCCGGAGCAACCACTACAT TCCGCCACCATTGTCTC           | 132                  | 61      |
| hph         | Hygromycin phosphotransferase | pANIC10A       | GAAAGAATCTCGTGCTTTCAGCTTCG CAAGCTCTGTAGATGGTGAAGACC | 741                  | 64      |
| Bbm         | Baby boom                     | pGL190/ pGL196/ pGL199 | GGTCGTCAAGTCTATTAGTGTGGCT AAGTAAGATCTCTTCTTTCCCTGCAACT | 299                  | 63      |
| gRNA unit   | gRNA-tRNA polycistronic cassette | pGL196/ pGL199 | CCTAGAAGGCCACCCCAGGGATAAAAATTTTCA CTGCAGGCATGCACGCCTAATTAAAC | 1,157                | 60      |
| PDS-06g-1   | Phytoene desaturase           | pGL196         | TGTAAGTTGGGAAATTTCGAGGGAACCACCTA GCAGATTAAGCTCAACGGGTAATGTGTAGTG | 1,127                | 60      |
| PDS-06g-2   | Phytoene desaturase           | pGL199         | TAATTTAATCCATCATTGTCTCAGC GGTGTTATAATGTGTAGAAACCACATCA | 1,061                | 60      |
| Accession Name | Purpose                  | Primer Name | Sequences 5 -> 3                  |
|---------------|--------------------------|-------------|-----------------------------------|
| pPL01         | pGL190 construction      | attL4 R     | caacctttctatacaaaagtggcattataaaaaagcatggcttatcaat |
| pPL02         | “                        | attL4_pUBI F| ggcaactttgtatagaaagttgctgcccttaagggccaatttcaagattcattcaaca |
| pPL03         | “                        | attL3_tUBI R| ggtgaccgggccgcgaagcctaccaaaagccaagcgttgtcga |
| pPL04         | “                        | attL3 F     | ggccgcgcgggtcaccgggt              |
| pPL05         | “                        | ZS Green 2 R| accctatcgagatctgagtc              |
| pPL06         | pGL193 construction      | Cas9 F      | ctttaacttagcttgaagcttgagcagctgactacaagagataaaggtgcttatcaat |
| pPL07         | “                        | Cas9 R      | actgcccatttgatcccttttttttttgcccttgagagctagaa |
| pPL08         | “                        | pENTR F     | ggtaccaatggccagttacacagtccagtct |
| pPL09         | “                        | pENTR R     | ggtaccaatggccagttacacagtccagtct |
| pPL10         | “                        | gRNA unit F | cctagaagccacccagggataacattttcacacaagagataaaggtgcttatcaat |
| pPL11         | “                        | gRNA unit R | aacctttgtataataaagttgccccttttgacagcagagagtgcag |
| pPL12         | gRNAs for sorghum PDS    | PDS-gR1.1 F| taGGTCTCCAGATGTGGGgttttagagctgaga |
| pPL13         | “                        | PDS-gR1.1 F| cgGGTCTCAATCTCTTGCTCtgaccagcggg |
| pPL14         | “                        | PDS-gR1.2 F| taGGTCTCCGGAGAATTCAACGgtaatttagtagtaaaaggtgcttatcaat |
| pPL15         | “                        | PDS-gR1.2 F| cgGGTCTCAGTCCCTGGGTGTgacaccagcgg |
| pPL16         | “                        | PDS-gR1.3 F| taGGTCTCCGGACGGACAGACGGgttttagagctgaga |
| pPL17         | “                        | PDS-gR1.3 R| cgGGTCTCATCGCGAGCTGCTgaccagcgg |
| pPL18         | Determining independent  | LSA 1       | gtaatacgacttacatagggaccgcggttgcagcggccgggggtcgc |
| pPL19         | “                        | SSAH3       | phosphate-AGCTGCAGCCCG-amino C7 ‡ |
| pPL20         | “                        | SSAE1       | phosphate-AATTGCAGCCCG-amino C7 ‡ |
| pPL21 | “”   | LBa1 | tcacaatccacacacatacgagcc |
|-------|-------|------|--------------------------|
| pPL22 | “”   | RBa1 | gccagctggcgaatagcgaaga   |
| pPL23 | “”   | AP1  | gtaatagcactactatagggcacgcg |
| pPL24 | “”   | LBB1 | attaattgctgctgctactgccc   |
| pPL25 | “”   | RBB1 | ccccaacagtgccagcctgaatgccaat |
| pPL26 | “”   | AP2  | tggtcagggccggtgctgc    |
| pPL27 | pGL198 construction | ZM-UBI F | gccaacctttgtataaaaaagtgcagtgccagcagtgaccg |
| pPL28 | “”   | ZM-opt Cas9 R | actggcctatggtaccttcttcgctgccgctgccg |
| pPL29 | pGL199 construction | PDS-gR2.1 F | taGGTCTCCAACGGTTTACTGgttttagagctagaa |
| pPL30 | “”   | PDS-gR2.1 R | cgGGTCTCAGTGGTAGTCCgaccagcgggg |
| pPL31 | “”   | PDS-gR2.2 F | taGGTCTCCTGAGGAGACgttttagagctagaa |
| pPL32 | “”   | PDS-gR2.2 R | cgGGTCTCAGCCACCATCAATGGGCgaccagcgggg |
| pPL33 | Transgene independent insertion (TII) determination for altruistic MAT§ | LBa1_81814 | ccgattaatcggtgctttgct |
| pPL34 | “”   | LBB1_81814 | cggccctctgctccagagtagctatgt |

† Capitalized letters indicate gRNA sequences.
‡ Capitalized letters indicate oligonucleotides.
§ MAT
**Table S3. Medium composition for sorghum transformation**

| Medium name                        | Medium components                                                                 |
|------------------------------------|------------------------------------------------------------------------------------|
| PHI-I (infection medium)           | MS salts plus vitamins, 4.43 g/L, thiamine-HCl 1mg/l, 2,4-D 1.5 mg/l, sucrose 68.5 g/l, glucose 36 g/l, acetosyringone 39.24 mg/l, pH 5.2. |
| Co-cultivation                     | MS salts plus vitamins, 4.43 g/L, thiamine-HCl 1mg/l, 2,4-D 2 mg/l, sucrose 20 g/l, glucose 10 g/l, L-proline 0.7 g/l, MES buffer 0.5 g/l, acetosyringone 39.24 mg/l, ascorbic acid 10 mg/l, thymidine 100mg/l, pH 5.8. |
| Resting                            | MS salts plus vitamins, 4.43 g/L, thiamine-HCl 1mg/l, 2,4-D 2 mg/l, sucrose 20 g/l, glucose 10 g/l, L-proline 0.7 g/l, MES buffer 0.5 g/l, acetosyringone 39.24 mg/l, ascorbic acid 10 mg/l, carbenicillin 25 mg/l, pH 5.8. |
| ‡ Embryo-maturation medium (EMM)   | MS salts plus vitamins, 4.43 g/L, zeatin 0.05 mg/l, copper sulfate 1.25mg/l, L-proline 0.7 g/l, sucrose 60 g/l, IAA 1 mg/l, ABA 0.026mg/l, thidiazuron 0.1 mg/l, BAP 1 mg/l, carbenicillin 250mg/l, pH 5.6. |
| ‡ Rooting (RM)                      | MS salts 4.43 g/l, sucrose 40 g/l, pH 5.6                                          |

† Added after medium is autoclaved and cooled to ~55°C
‡ For EMM and RM, selection agent (hyg or IMZ) differs depending on construct
**Table S4.** Constructs used for sorghum transformation

| Construct     | Construct Size | Purpose                          | Plant selectable marker gene (selection agent)                  | Bacterial selectable marker gene (selection agent) |
|---------------|----------------|----------------------------------|-----------------------------------------------------------------|-----------------------------------------------------|
| pPHP81814     | 27.8 kb        | MAT †                            | Acetolactate synthase, ALS (Imazapyr)                           | Adenylyltransferase, aadA (spectinomycin)            |
| pANIC10A      | 17.4 kb        | Altruistic MAT                   | hygromycin phosphotransferase, hph (hygromycin)                | Aminoglycoside phosphotransferase, nptII, (kanamycin) |
| pGL190        | 31.4 kb        | Altruistic MAT                   | Acetolactate synthase, ALS (Imazapyr)                           | Adenylyltransferase, aadA (spectinomycin)            |
| pGL193        | 10.3 kb        | Entry vector for gRNAs targeting pds | NA                                                             | Aminoglycoside phosphotransferase, nptII, (kanamycin) |
| pGL196        | 36.1 kb        | MAT-mediated CRISPR editing target for Pds | Acetolactate synthase, ALS                                    | Adenylyltransferase, aadA (spectinomycin)            |
| pGL198        | 10.4 kb        | Entry vector                     | NA                                                             | Aminoglycoside phosphotransferase, nptII, (kanamycin) |
| pGL199        | 36.1 kb        | MAT-mediated CRISPR editing target for Pds | Acetolactate synthase, ALS                                    | Adenylyltransferase, aadA (spectinomycin)            |

† MAT- morphogene-assisted transformation
| Gene Symbol | Gene Name                    | Construct name | Initial Denature | Denature | Annealing | Extension | Extension | Hold       | Polymerase                  |
|-------------|------------------------------|----------------|------------------|----------|-----------|-----------|-----------|------------|-----------------------------|
| ALS2        | Acetolactate synthase        | pPHP81814      | 95°C-3 min       | 95°C-30  sec | 54°C-30 sec | 72°C-60 sec | 72°C-5 min | 12°C-∞     | EconoTaq ® PLUS GREEN 2X Master Mix |
| ZSG-1       | ZsGreen                      | pPHP81814      | 95°C-4 min       | 95°C-30  sec | 55°C-30 sec | 72°C-50 sec | 72°C-7 min | 12°C-∞     | PrimeSTAR GXL                |
| RFP         | Red fluorescence protein     | pANIC10A       | 95°C-3 min       | 95°C-30  sec | 53°C-30 sec | 72°C-30 sec | 72°C-5 min | 4°C-∞      | PrimeSTAR GXL                |
| Bbm         | Baby Boom                    | pGL190         | 95°C-3 min       | 95°C-30  sec | 53°C-30 sec | 72°C-30 sec | 72°C-5 min | 4°C-∞      | PrimeSTAR GXL                |
| hph + Bbm   | Hygromycin phospho-transferase + Baby Boom | pGL190 + pANIC10A | 95°C-3 min | 95°C-30  sec | 57°C-30 sec | 72°C-60 sec | 72°C-5 min | 4°C-∞      | PrimeSTAR GXL                |
| gRNA unit   | gRNA-tRNA                    | pGL196         | 98°C-3 min       | 98°C-10  sec | 60°C-15 sec | 68°C-75 sec | 68°C-5 min | 4°C-∞      | PrimeSTAR GXL                |
| Pds         | Phytoene desaturase          | pGL199         | 98°C-3 min       | 98°C-10  sec | 60°C-15 sec | 68°C-5 sec  | 68°C-5 min | 4°C-∞      | PrimeSTAR GXL                |
### Table S6. Primers and probes for digital droplet PCR

| Gene symbol | Protein name                      | Primers (F/R) (5’-3’)              | Probe sequence (5’-3’)            | Amplicon length (bp) | Tm (°C) |
|-------------|-----------------------------------|------------------------------------|-----------------------------------|----------------------|---------|
| ALS2†       | Acetolactate synthase             | CTTTGCTCATGGAACGA                  | CATATTGTGGCTGGATCTCCTCA TTAGAT    | 154                  | 59      |
| RFP†        | Red fluorescence protein          | CGATGGCGACTCTTTTCATCT              | TGCCACCCACCACACTCATACT            | 184                  | 60      |
| PP2A‡       | Serine/threonine protein phosphatase | CCGATCTGTGATATGGGACG              | TGGTTGGTTTTGTGCCTGG CCGG          | 209                  | 60      |

†ALS and RFP probes: labeled with FAM™, double-quenched with ZEN™ and Iowa Black Hole Quencher®
‡PP2A: labeled with HEX™, double-quenched with ZEN™ and Iowa Black Hole Quencher®
| Symbol    | Name                        | Reference                                                                 |
|-----------|-----------------------------|---------------------------------------------------------------------------|
| Wus2      | Zea mays Wuschel2           | (Lowe et al. 2007)                                                        |
| Zm-Bbm    | Zea mays Baby Boom          | (Gordon-Kamm, W.J., Helentjaris, T.G., Lowe, K.S., Shen, B., Tarczynski, M.C., Zheng, P., n.d.) et al. 2005 |
| Zs-GREEN1 | Green fluorescence protein, Zoanthus sp. | (Matz et al. 1999)                                                       |
| moCRE     | Zea mays-optimized CRE recombinase | (Odell et al. 1990)                                                       |
| loxP      | Recombinase target site for CRE recombinase | (Odell et al. 1990)                                                       |
| Sb-ALSpro | Sorghum bicolor ALS promoter | SB-ALS promoter and 5'UTR, DOE-JGI Sbi v3.1, SBChr04, bases 49239164-49240031. |
| Zm-Axig1pro | Zea mays auxin-inducible promoter 1 | (Garnaat, Lowe, and Roth 2005), NCBI accession AR883375.1 bases 1-1240 |
| Zm-PLTPpro | Zea mays phospholipid transferase promoter | GenBank sequence MN380778                                                   |
| Sb-Ubi1pro | Sorghum bicolor ubiquitin promoter | Unpublished Corteva Agriscience sequence                                   |
| Zm-Glb1pro | Zea mays globulin 1 promoter | (Liu et al. 1998)                                                         |
| Zm-Hsp26pro | Zea mays heat shock promoter | Unpublished Corteva Agriscience sequence                                   |
| Zm-Ubi1pro | Zea mays ubiquitin promoter | (Xing et al. 2014)                                                        |