SUPPLEMENTARY FIGURES

STRUCTURAL VARIATION AT THE MAIZE *WUSCHEL1* LOCUS ALTERS STEM CELL ORGANIZATION IN INFLORESCENCES

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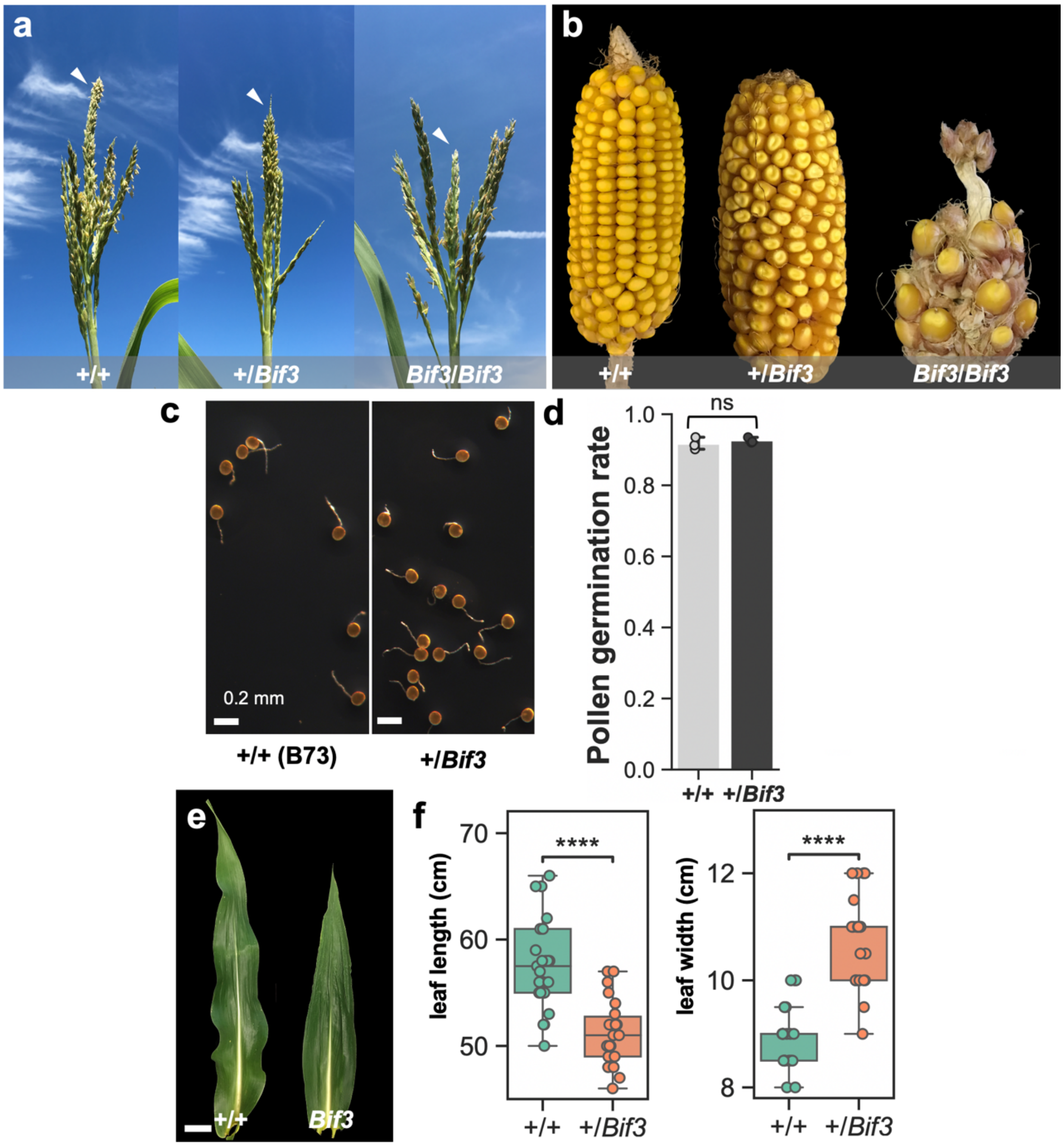
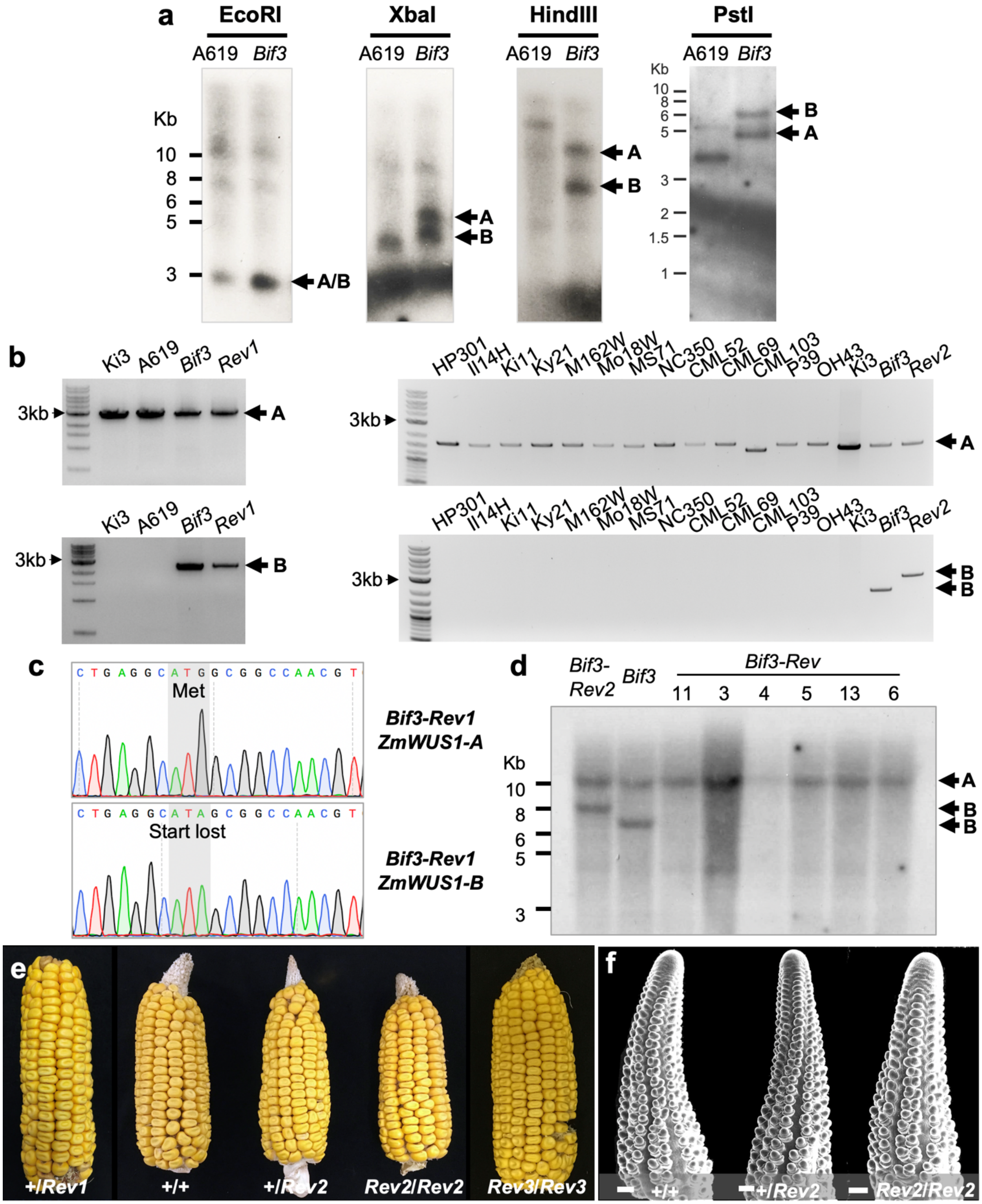
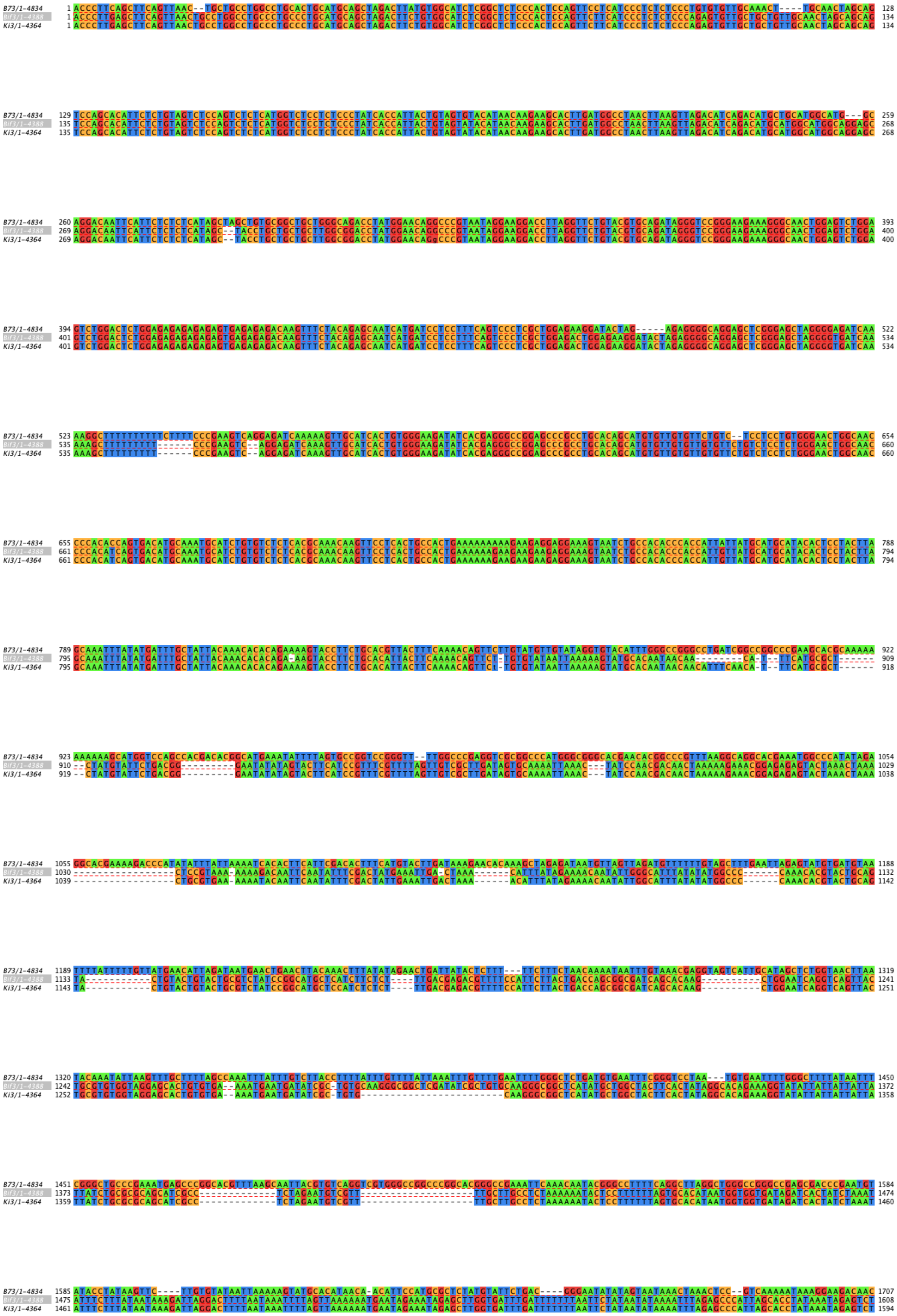


Supplementary Figure 1. *Bif3* mutant phenotype. (**a**) Mature tassel phenotype in B73. Normal tassels produce spikelets and flowers on branches and central spikes. Central spikes are reduced in *Bif3* homozygous mutants. White arrowheads point to the central spikes. (**b**) Mature ear phenotype in B73. Ears are reduced in size in *Bif3/Bif3* mutants and display disorganized rows of kernels in +/*Bif3* mutants. (**c**,**d**) Pollen germination in wild type and *+/Bif3* mutant in B73. Quantification of germination rate: 91.6% (3 plants, 10 anthers/plant, n=1400 pollen grains) in B73 and 92.6% (3 plants, 10 anthers/plant, n=4952 pollen grains) in *+/Bif3* (**d**); plotted data represent the mean, +/- standard deviation. (**e**,**f**) Leaves (the top third ones) from wild type and heterozygous *Bif3* plants in

A619. The *Bif3* leaves are broader in width and shorter in length (n_{WT} = 25, n_{Bif3} = 22, two-tailed Student’s *t*-test, **** $p<0.0001$). Box plot center line, median; box limits, upper and lower quartiles; whiskers, maximum and minimum values. Single datapoints outside of the whiskers represent outliers. Scale bars: 1 cm in **b**, 200 μm in **c**; 5 cm in **e**.



Supplementary Figure 2. Duplication of *ZmWUS1* in *Bif3*. (**a**) Southern blot analysis of *ZmWUS1* copies in wild type (A619) and *Bif3* mutants. A, *ZmWUS1-A*; B, *ZmWUS1-B*. (**b**) PCR amplification in different inbred lines of *ZmWUS1-A* and *ZmWUS1-B* by copyspecific primers (*ZmWUS1-A,* 1428WUS-FWD/WUSseqR3; *ZmWUS1-B*, 6968F7/WUSseqR3 in left panels; *ZmWUS1-A,* 1428WUS-FWD/WUS-R; *ZmWUS1-B*, 6968F6/WUS-R in right panels). (**c**) Chromatograph snapshot of *ZmWUS1-A* and *ZmWUS1-B* start codons in *Bif3-Rev1*. (**d**) Southern blot analysis of *ZmWUS1*. (**e**) A normal ear phenotype is observed in *Rev1*, *Rev2* and *Rev3* plants. (**f**) SEM analysis of *Rev2* ear primordia, showing a regular arrangements of AMs at the IM flanks. Scale bars, 100μm.


B73/1-4834
Bif3/1-4388
Ki3/1-4364
1 128
1 134
1 134
129 259
135 268
135 268
260 393
269 400
269 400
394 522
401 534
401 534
523 654
535 660
535 660
655 788
661 794
661 794
789 922
795 909
795 918
923 1054
910 1029
919 1038
1055 1188
1030 1132
1039 1142
1189 1319
1133 1241
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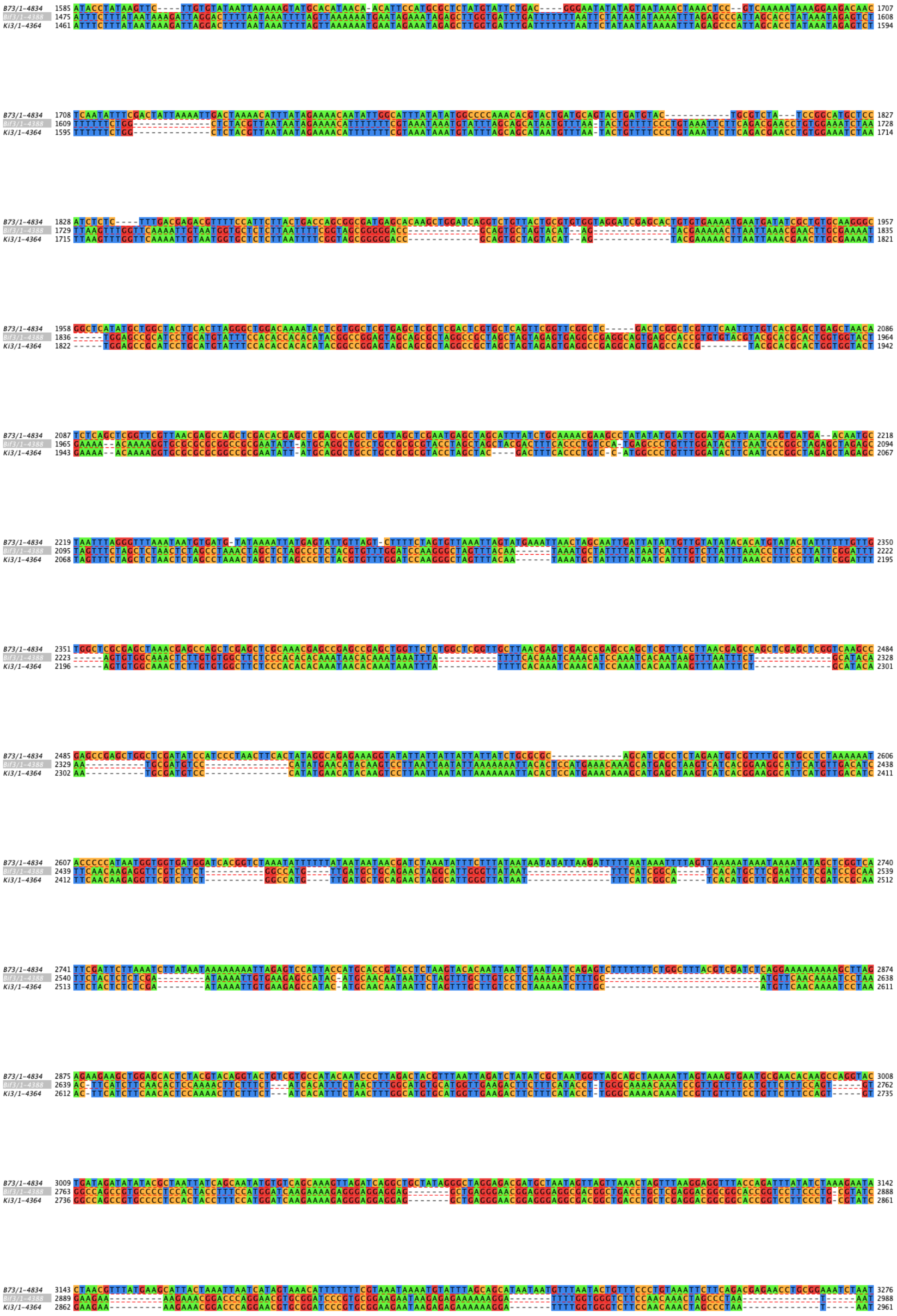
1585 1707
1475 1608
1461 1594


B73/1-4834 1585 1707
Bif3/1-4388 1475 1608
Ki3/1-4364 1461 1594
B73/1-4834 1708 1827
Bif3/1-4388 1609 1728
Ki3/1-4364 1595 1714
B73/1-4834 1828 1957
Bif3/1-4388 1729 1835
Ki3/1-4364 1715 1821
B73/1-4834 1958 2086
Bif3/1-4388 1836 1964
Ki3/1-4364 1822 1942
B73/1-4834 2087 2218
Bif3/1-4388 1965 2094
Ki3/1-4364 1943 2067
B73/1-4834 2219 2350
Bif3/1-4388 2095 2222
Ki3/1-4364 2068 2195
B73/1-4834 2351 2484
Bif3/1-4388 2223 2328
Ki3/1-4364 2196 2301
B73/1-4834 2485 2606
Bif3/1-4388 2329 2438
Ki3/1-4364 2302 2411
B73/1-4834 2607 2740
Bif3/1-4388 2439 2539
Ki3/1-4364 2412 2512
B73/1-4834 2741 2874
Bif3/1-4388 2540 2638
Ki3/1-4364 2513 2611
B73/1-4834 2875 3008
Bif3/1-4388 2639 2762
Ki3/1-4364 2612 2735
B73/1-4834 3009 3142
Bif3/1-4388 2763 2888
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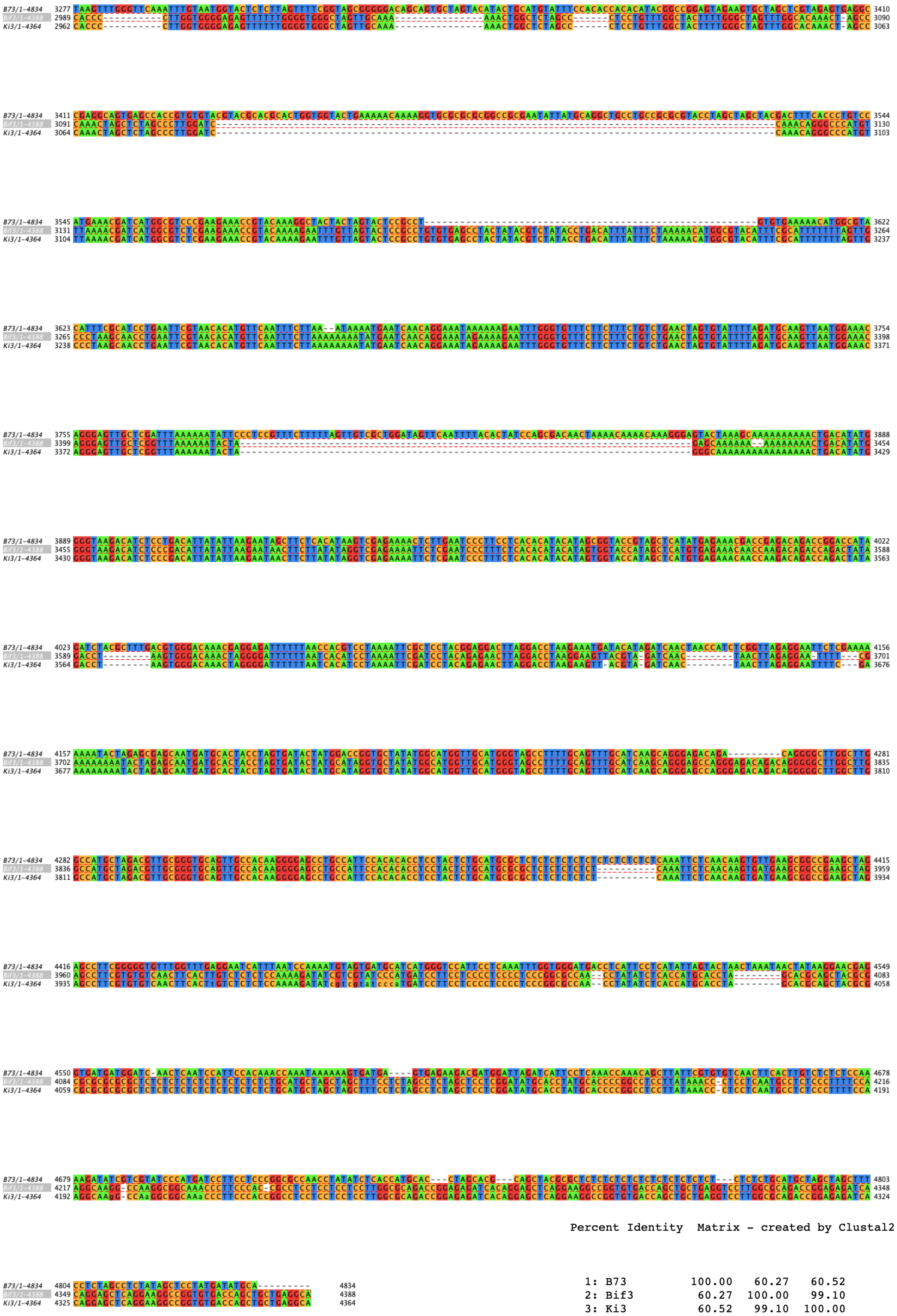
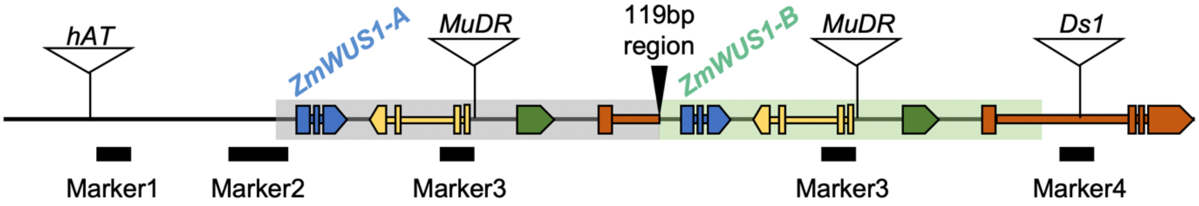
Ki3/1-4364 2862 2961



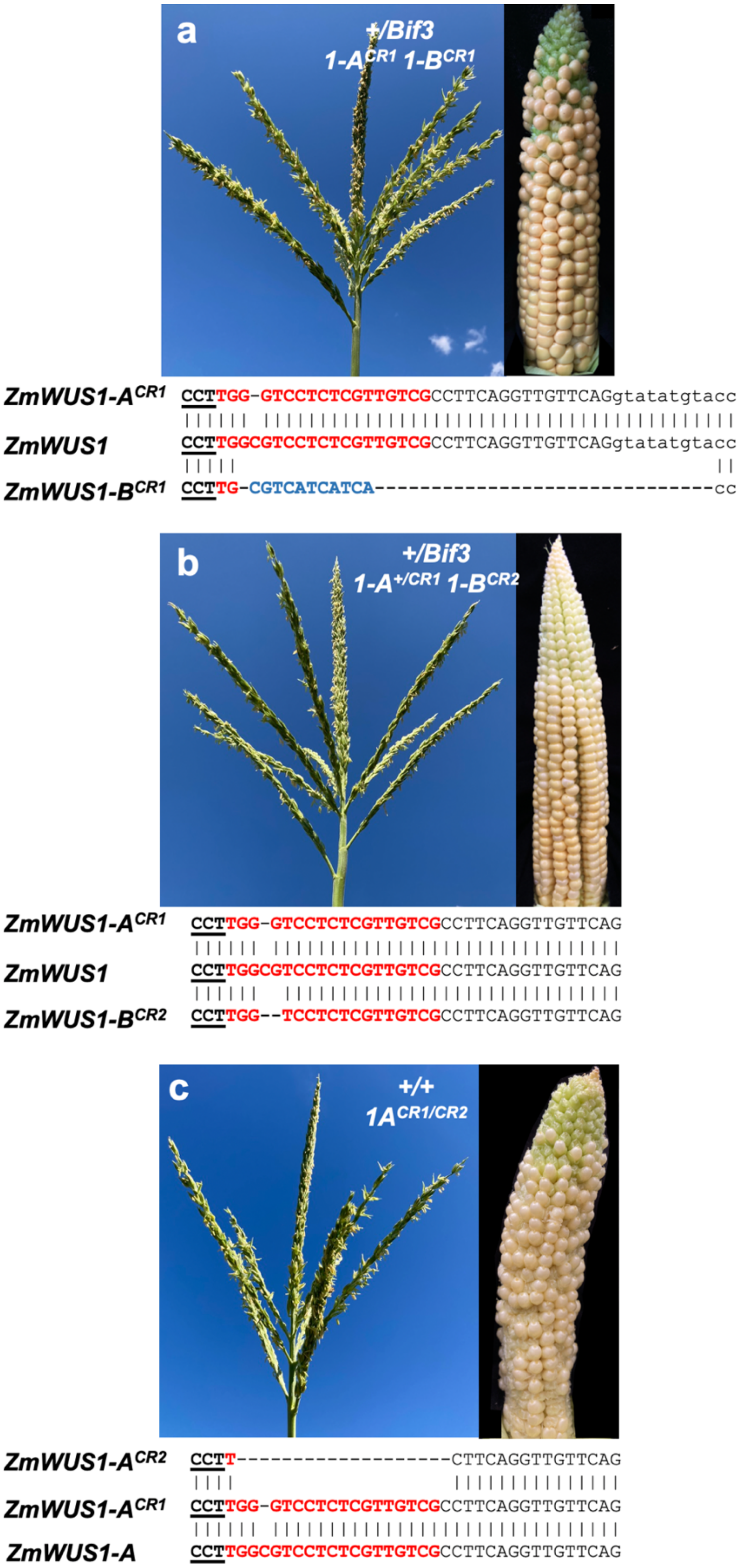
```
Percent Identity  Matrix - created by Clustal2.1

     1: B73         100.00   60.27   60.52
     2: Bif3         60.27  100.00   99.10
     3: Ki3          60.52   99.10  100.00
```

Supplementary Figure 3. Clustal Omega multiple sequence alignment of the *ZmWUS1* promoter in *Bif3*, Ki3 and B73. Partial section (B73 region coordinates -4,978 to -144 from *ZmWUS1* start site). Alignment visualized in Jalview (version 2.11.1.3) [1].



Revertant	Marker1	Marker2	*ZmWUS1-A*	Marker3	MuDR	*ZmWUS1-B*	Marker4	phenotype
Rev1	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	+ (G -> A)	*Bif3/Bif3*	WT
Rev2	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	+ (longer promoter)	*Bif3/Bif3*	WT
Rev3	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev4	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev5	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev6	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev7	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev8	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev9	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev10	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev11	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev12	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev13	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev14	*Bif3/Bif3*	*+/Bif3*	+	*+/Bif3*	+	-	*+/Bif3*	WT
Rev15	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev16	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev17	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev18	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev19	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT
Rev20	*Bif3/Bif3*	*Bif3/Bif3*	+	*Bif3/Bif3*	+	-	*Bif3/Bif3*	WT

Supplementary Figure 4. Genotyping of the *Bif3* locus in different revertant individuals. Each original M1 revertant was self-crossed and homozygous *Bif3/Bif3* plants were selected based on Marker 1 for genotyping with additional markers spanning the entire region. Details on the markers used are provided in Supplementary Data 2.

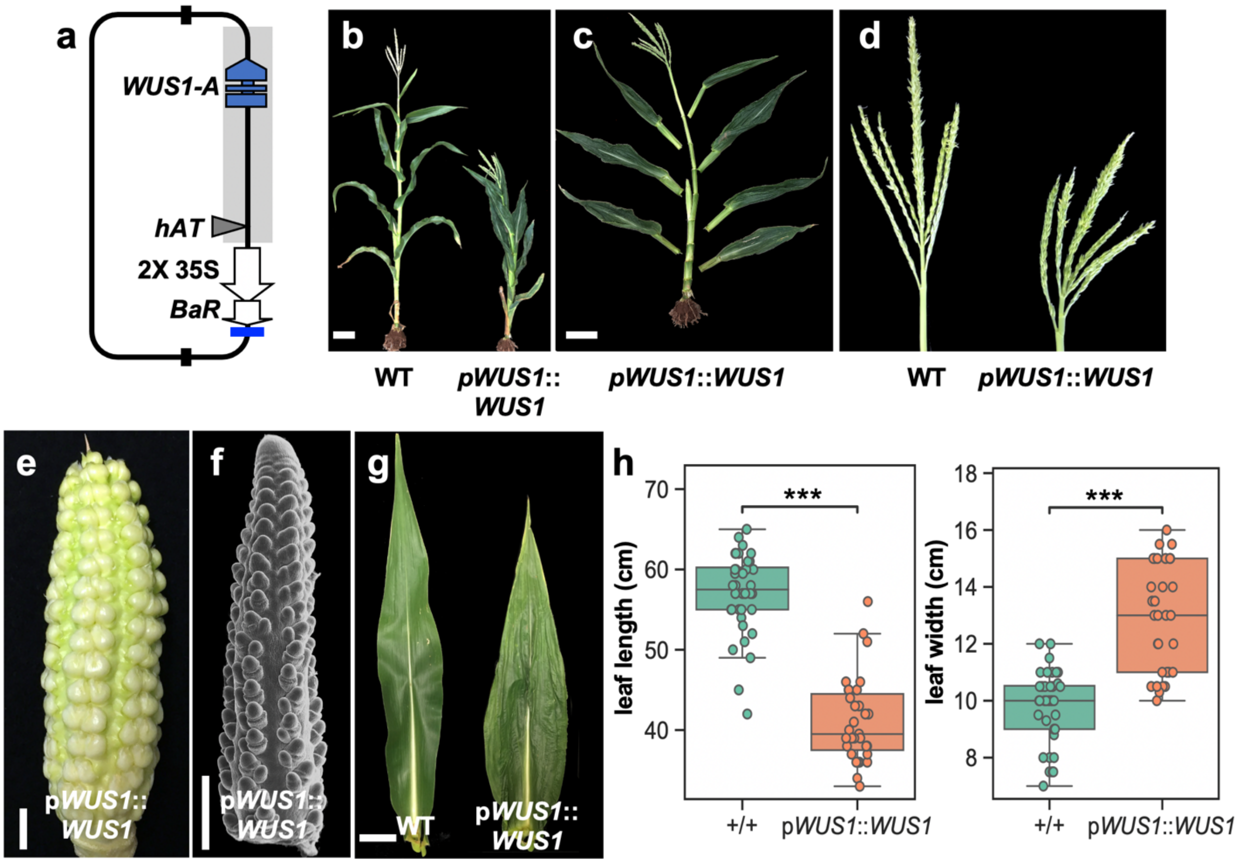


```
ZmWUS1-A^CR1  CCTTGG-GTCCTCTCGTTGTCGCCTTCAGGTTGTTCAGgtatatgtacc
              |||||| ||||||||||||||||||||||||||||||||||||||||||
ZmWUS1        CCTTGGCGTCCTCTCGTTGTCGCCTTCAGGTTGTTCAGgtatatgtacc
              |||||                                          ||
ZmWUS1-B^CR1  CCTTG-CGTCATCATCA------------------------------cc
```

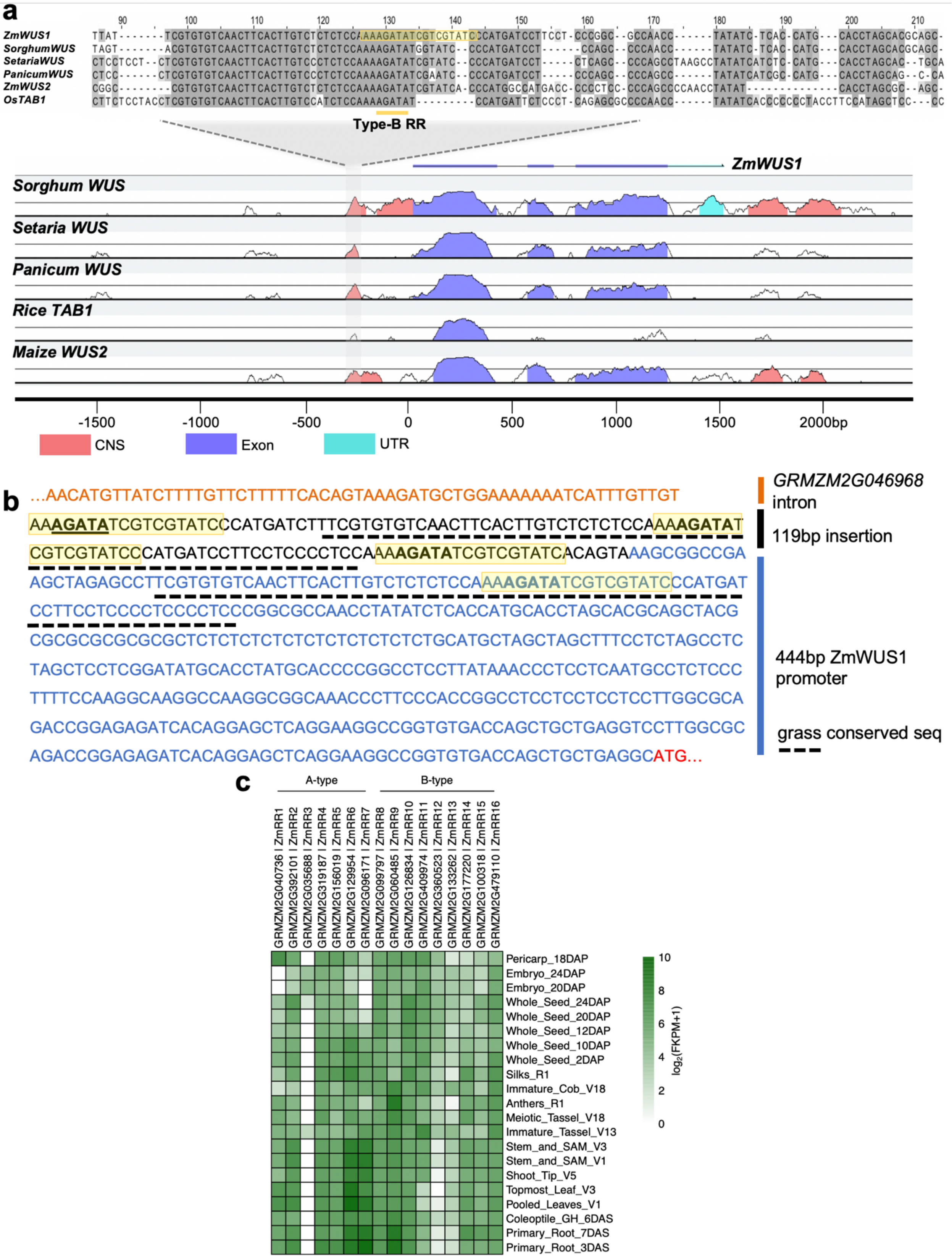
```
ZmWUS1-A^CR1  CCTTGG-GTCCTCTCGTTGTCGCCTTCAGGTTGTTCAG
              |||||| |||||||||||||||||||||||||||||||
ZmWUS1        CCTTGGCGTCCTCTCGTTGTCGCCTTCAGGTTGTTCAG
              ||||||  ||||||||||||||||||||||||||||||
ZmWUS1-B^CR2  CCTTGG--TCCTCTCGTTGTCGCCTTCAGGTTGTTCAG
```

```
ZmWUS1-A^CR2  CCTT-------------------CTTCAGGTTGTTCAG
              ||||                   |||||||||||||||
ZmWUS1-A^CR1  CCTTGG-GTCCTCTCGTTGTCGCCTTCAGGTTGTTCAG
              |||||| |||||||||||||||||||||||||||||||
ZmWUS1-A      CCTTGGCGTCCTCTCGTTGTCGCCTTCAGGTTGTTCAG
```

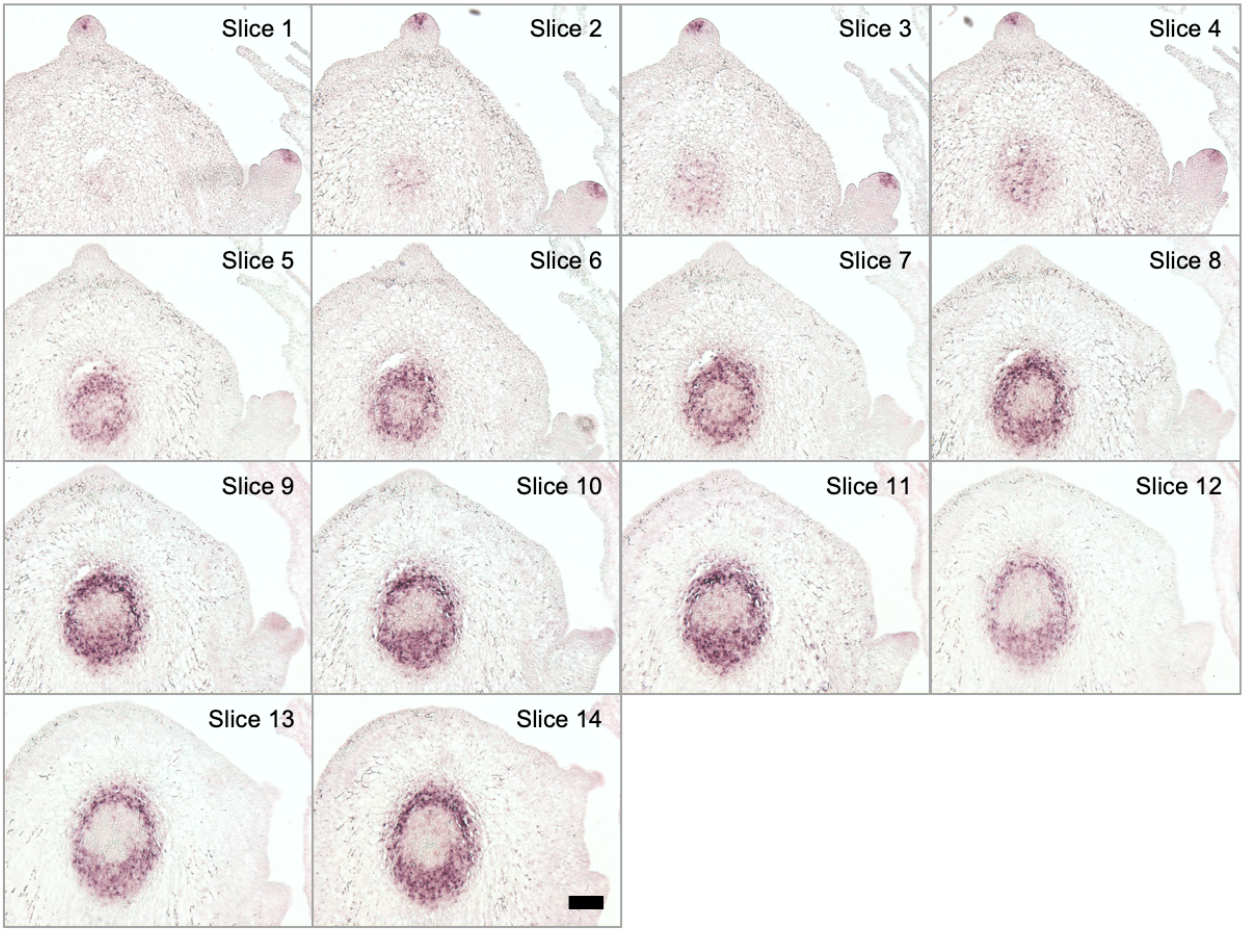
Supplementary Figure 5. CRISPR-Cas9 loss-of-function *ZmWUS1-A* and *ZmWUS1-B* alleles. Additional knockout events in both genes in *Bif3* mutant (**a**,**b**) and wild type (**c**) plants show a wild type phenotype in both tassels and ears. Events are homozygous, unless otherwise noted.



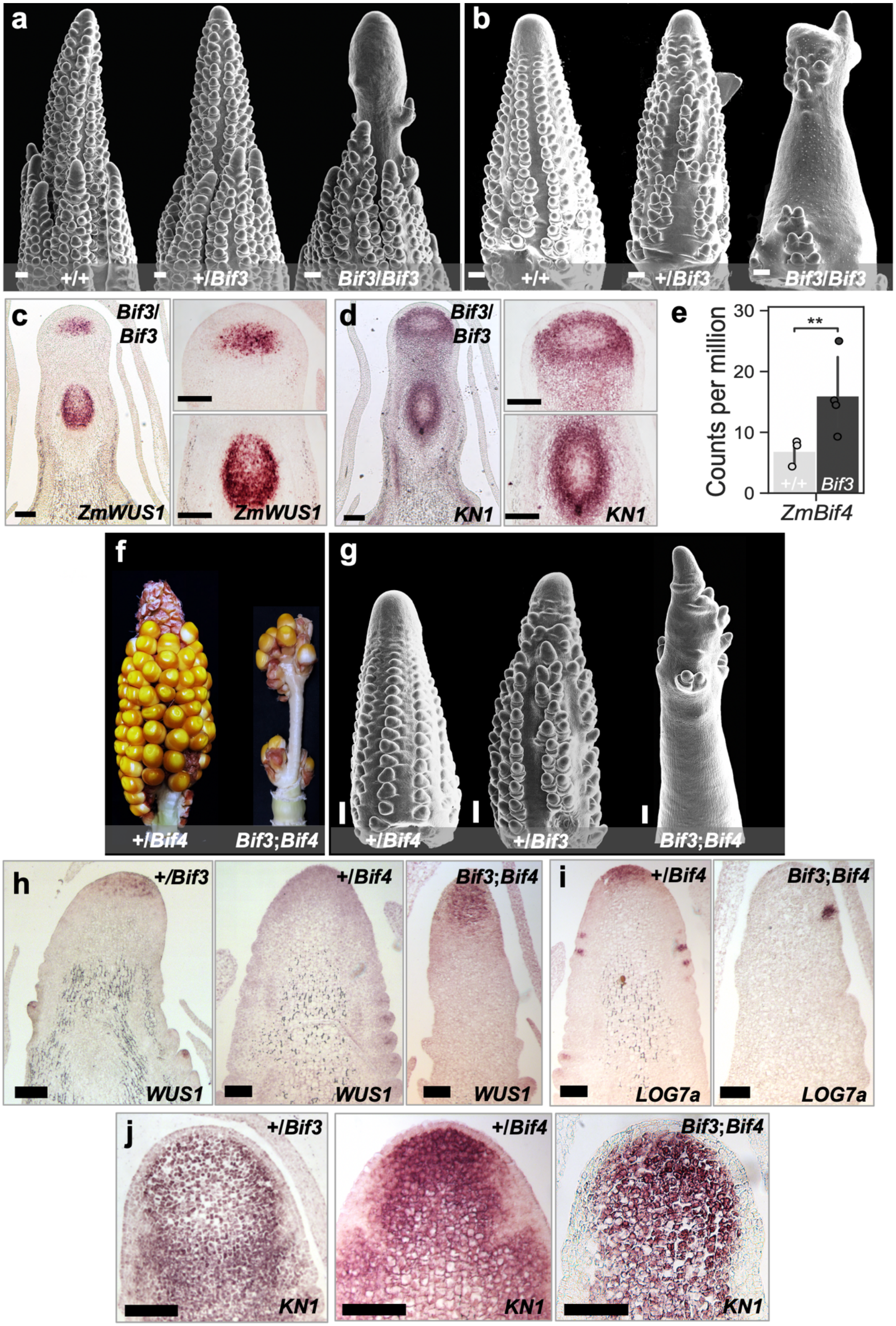
Supplementary Figure 6. Analysis of *pWUS1-A::ZmWUS1-A* transgenic plants. (**a**) Binary *pZmWUS1-A*::*ZmWUS1-A* construct used for transformation. (**b**,**c**) *pWUS1-A* transgenic plants are shorter and more compact. (**d**) Normal tassels of *pWUS1-A* transgenic plants. (**e**) Normal ear appearance of *pWUS1-A* transgenic plants. (**f**) SEM image of *pWUS1-A* transgenic ear primordia. (**g**,**h**) *pWUS1-A* transgenic plants have wider and shorter leaves ($n_{WT} = 36$, $n_{pWUS1\text{-}A} = 31$, two-tailed Student's *t*-test, ***p<0.001). Box plot center line, median; box limits, upper and lower quartiles; whiskers, maximum and minimum values. Single datapoints outside of the whiskers represent outliers. Bars: 10cm in **b** and **c**, 1cm in **e**, 500µm in **f**, 5cm in **g**.


a
90
100
110
120
130
140
150
160
170
180
190
200
210
ZmWUS1 TTAT-------TCGTGTGTCAACTTCACTTGTCTCTCTCCAAAAGATATCGTCGTATCCCATGATCCTTCCT-CCCGGC--GCCAACC------TATATC-TCAC-CATG---CACCTAGCACGCAGC-
SorghumWUS TAGT-------ACGTGTGTCAACTTCACTTGTCTCTCTCCAAAAGATATGGTATC--CCCATGATCCT------CCAGC--CCCAACC------TATATC-TCAC-CATG---CACCTAGCACGCAGC-
SetariaWUS CTCCTCCT--CTCGTGTGTCAACTTCACTTGTCCCTCTCCAAAAGATATCGTATC--CCCATGATCCT-----CTCAGC--CCCAGCCTAAGCCTATATCATCTC-CATG---CACCTAGCAC--TGCA
PanicumWUS CTCC------CTCGTGTGTCAACTTCACTTGTCCCTCTCCAAAAGATATCGAATC--CCCATGATCCT-----CCCAGC--CCCAGCC------TATATCATCGC-CATG---CACCTAGCAG--C-CA
ZmWUS2 CGGC--------CGTGTGTCAACTTCACTTGTCTCTCTCCAAAAGATATCGTATCA-CCCATGGCCATGACC-CCCCTCC-CCCAGCCCCAACCTATAT--------------CACCTAGCGC--AGC-
OsTAB1 CTTCTCCTACCTCGTGTGTCAACTTCACTTGTCCATCTCCAAAAGATAT---------CCATGATTCTCCCT-CAGAGCGCCCCAACC------TATATCACCCCCCTACCTTCCATAGCTCC---CC
Type-B RR
ZmWUS1
Sorghum WUS
Setaria WUS
Panicum WUS
Rice TAB1
Maize WUS2
-1500
-1000
-500
0
500
1000
1500
2000bp
CNS
Exon
UTR
b
...AACATGTTATCTTTTGTTCTTTTTCACAGTAAAGATGCTGGAAAAAAATCATTTGTTGT
AAAGATATCGTCGTATCCCATGATCTTTCGTGTGTCAACTTCACTTGTCTCTCTCCAAAAGATAT
CGTCGTATCCCATGATCCTTCCTCCCCTCCAAAAGATATCGTCGTATCACAGTAAAGCGGCCGA
AGCTAGAGCCTTCGTGTGTCAACTTCACTTGTCTCTCTCCAAAAGATATCGTCGTATCCCATGAT
CCTTCCTCCCCTCCCCTCCCGGCGCCAACCTATATCTCACCATGCACCTAGCACGCAGCTACG
CGCGCGCGCGCTCTCTCTCTCTCTCTCTCTCTCTGCATGCTAGCTAGCTTTCCTCTAGCCTC
TAGCTCCTCGGATATGCACCTATGCACCCCGGCCTCCTTATAAACCCTCCTCAATGCCTCTCCC
TTTTCCAAGGCAAGGCCAAGGCGGCAAACCCTTCCCACCGGCCTCCTCCTCCTCCTTGGCGCA
GACCGGAGAGATCACAGGAGCTCAGGAAGGCCGGTGTGACCAGCTGCTGAGGTCCTTGGCGC
AGACCGGAGAGATCACAGGAGCTCAGGAAGGCCGGTGTGACCAGCTGCTGAGGCATG...
GRMZM2G046968 intron
119bp insertion
444bp ZmWUS1 promoter
grass conserved seq
c
A-type
B-type
GRMZM2G040736 | ZmRR1
GRMZM2G392101 | ZmRR2
GRMZM2G035688 | ZmRR3
GRMZM2G319187 | ZmRR4
GRMZM2G156019 | ZmRR5
GRMZM2G129954 | ZmRR6
GRMZM2G096171 | ZmRR7
GRMZM2G099797 | ZmRR8
GRMZM2G060485 | ZmRR9
GRMZM2G126834 | ZmRR10
GRMZM2G409974 | ZmRR11
GRMZM2G360523 | ZmRR12
GRMZM2G133262 | ZmRR13
GRMZM2G177220 | ZmRR14
GRMZM2G100318 | ZmRR15
GRMZM2G479110 | ZmRR16
Pericarp_18DAP
Embryo_24DAP
Embryo_20DAP
Whole_Seed_24DAP
Whole_Seed_20DAP
Whole_Seed_12DAP
Whole_Seed_10DAP
Whole_Seed_2DAP
Silks_R1
Immature_Cob_V18
Anthers_R1
Meiotic_Tassel_V18
Immature_Tassel_V13
Stem_and_SAM_V3
Stem_and_SAM_V1
Shoot_Tip_V5
Topmost_Leaf_V3
Pooled_Leaves_V1
Coleoptile_GH_6DAS
Primary_Root_7DAS
Primary_Root_3DAS
10
8
6
4
2
0
log2(FKPM+1)

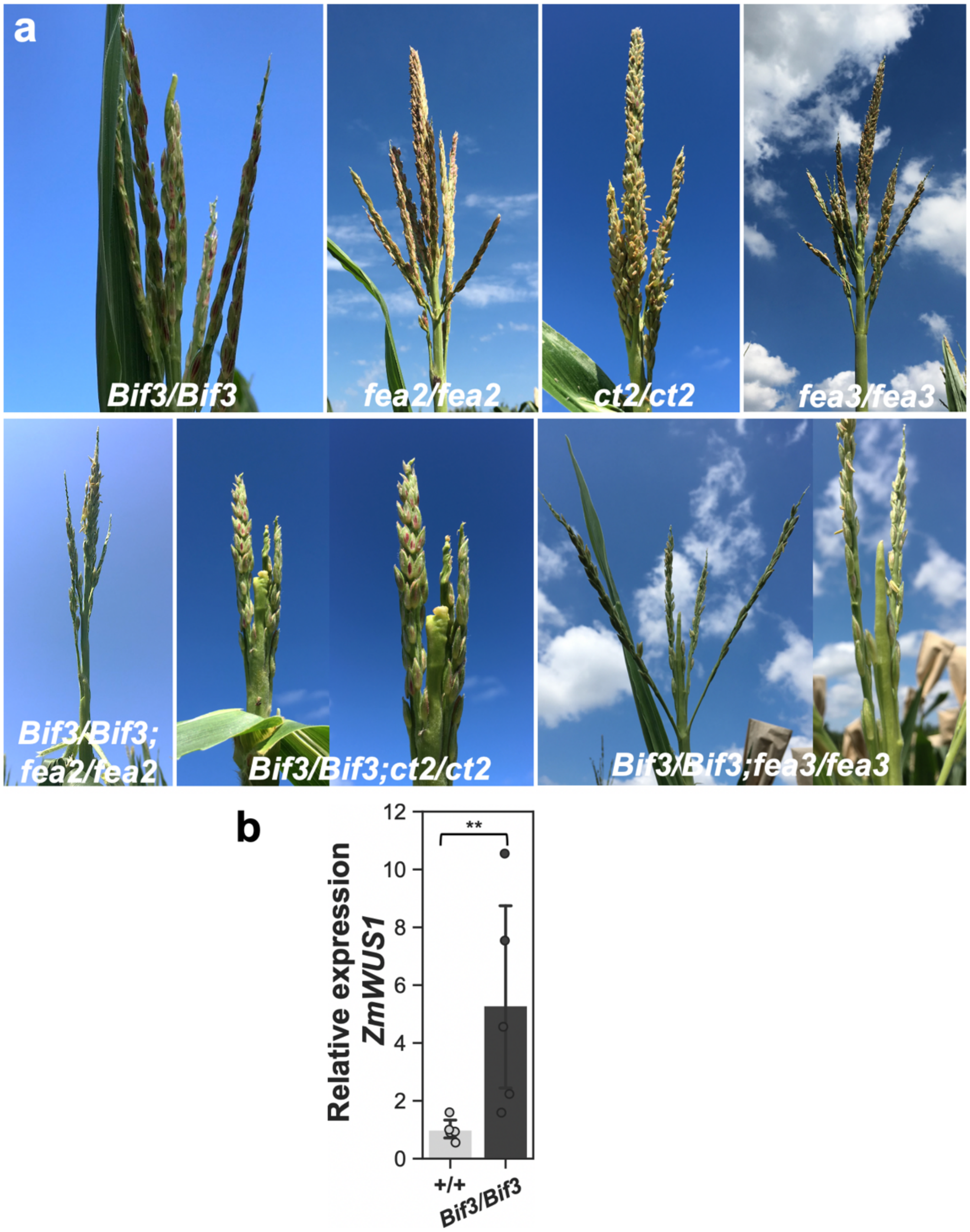
Supplementary Figure 7. Conservation of type-B RR binding sites in the promoter of *WUS* in different species. (**a**) Alignment of the conserved region in the proximal promoter region of the *WUS1* gene in different grass species. In yellow is the 17bp element repeated in the unique 119bp region of the *Bif3* mutant. (**b**) Proximal promoter sequence of the *ZmWUS1-B* gene in *Bif3* mutants. (**c**) RNA-seq expression levels of *ZmRR* genes in different tissues, from Stelpflug et al. 2016 [2].



Supplementary Figure 8. RNA *in situ* hybridizations. *ZmWUS1* antisense probe used on consecutive longitudinal sections of ear primordia show a hollow spherical expression domain in *Bif3* inflorescence meristem (A619 background). Bar: 100 µm.


a
+/+
+/Bif3
Bif3/Bif3
b
+/+
+/Bif3
Bif3/Bif3
c
Bif3/ Bif3
ZmWUS1
ZmWUS1
d
Bif3/ Bif3
KN1
KN1
e
Counts per million
30
20
10
0
**
+/+
Bif3
ZmBif4
f
+/Bif4
Bif3;Bif4
g
+/Bif4
+/Bif3
Bif3;Bif4
h
+/Bif3
WUS1
+/Bif4
WUS1
Bif3;Bif4
WUS1
i
+/Bif4
LOG7a
Bif3;Bif4
LOG7a
j
+/Bif3
KN1
+/Bif4
KN1
Bif3;Bif4
KN1

Supplementary Figure 9. *Bif3* mutant phenotype in the B73 background and genetic interaction between *Bif3* and *Bif4* mutants. (**a**,**b**) SEMs of wild-type and *Bif3* tassel (**a**) and ear (**b**) primordia. *Bif3* inflorescences shows reduced number of SPMs and single SMs. (**c**,**d**) mRNA *in situ* hybridizations of *ZmWUS1* (**c**) and *KN1* (**d**) in *Bif3/Bif3* inflorescence meristems show two OC domains. (**e**) The *BIF4* gene is upregulated about 1.7-fold in *Bif3* ear primordia; two-tailed Student's *t*-test **p<0.01; plotted data represent the mean, +/- standard deviation; $n_{+/+}$=3, $n_{Bif3/Bif3}$=4 (*n* pools of three ear samples). (**f**,**g**) Enhanced *Bif3* ear phenotype in heterozygous *Bif3*;*Bif4* double mutants. (**h**-**j**) Expression pattern of *ZmWUS1*, *ZmLOG7a* and *KN1* in *Bif3* heterozygous and *Bif3*;*Bif4* double mutants. Scale bars: 200 µm in **a**, **b**, **g;** 100 µm in **c**, **d**, **h**, **i**.



Supplementary Figure 10. Double mutant analysis. Mature tassel phenotype in single and double mutant combinations of *Bif3* and different fasciated mutants. The central spike is particularly reduced in length in *Bif3/Bif3;ct2/ct2* and *Bif3/Bif3;fea3/fea3* mutants (n≥8) (**b**) qRT-PCR showing *ZmWUS1* overexpression in developing embryos 12 days after pollination. Two-tailed Student's t-test $^{**}p<0.01$; plotted data represent the mean, +/- standard deviation; $n_{+/+}=5$, $n_{Bif3/Bif3}=5$ (*n* pools of three ear samples).

REFERENCES

1. Waterhouse, A.M. *et al.* Jalview Version 2--a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**, 1189-91 (2009).
2. Stelpflug, S.C. *et al.* An Expanded Maize Gene Expression Atlas based on RNA Sequencing and its Use to Explore Root Development. *Plant Genome* **9**(2016).