

## Supplementary Materials for **Control of sexuality by the *sk1*-encoded UDP-glycosyltransferase of maize**

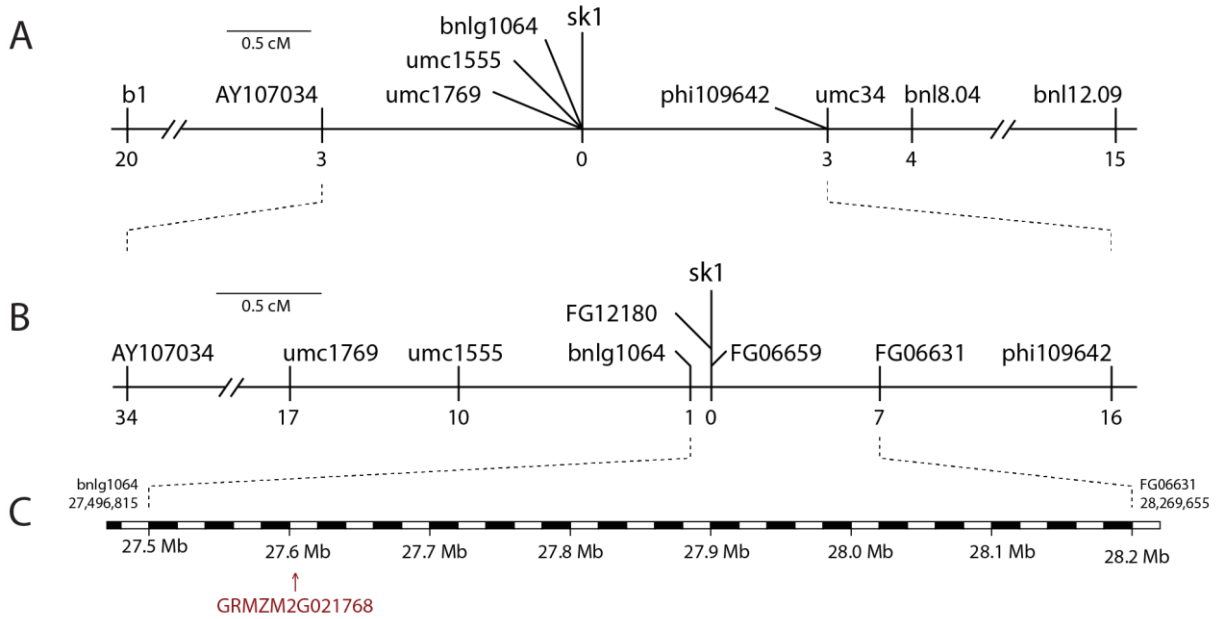
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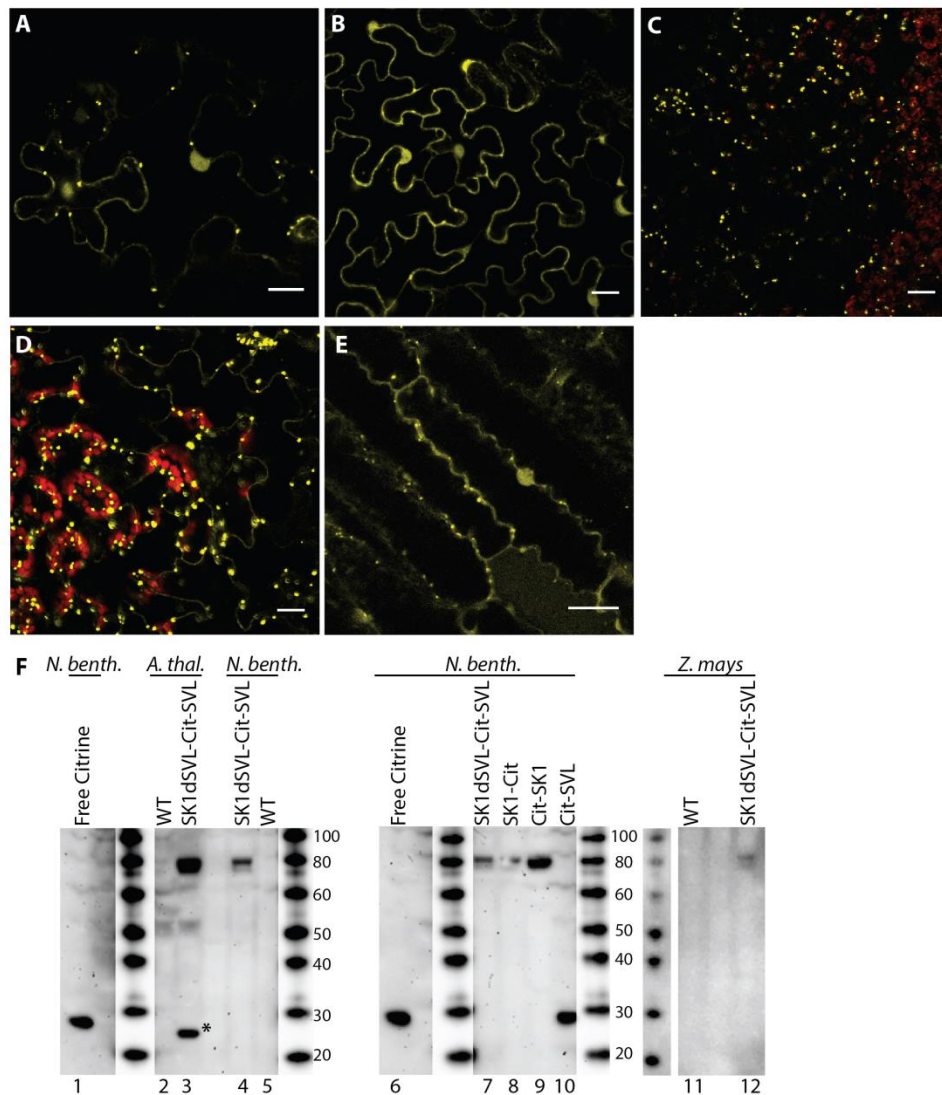
**Supplemental Materials:**



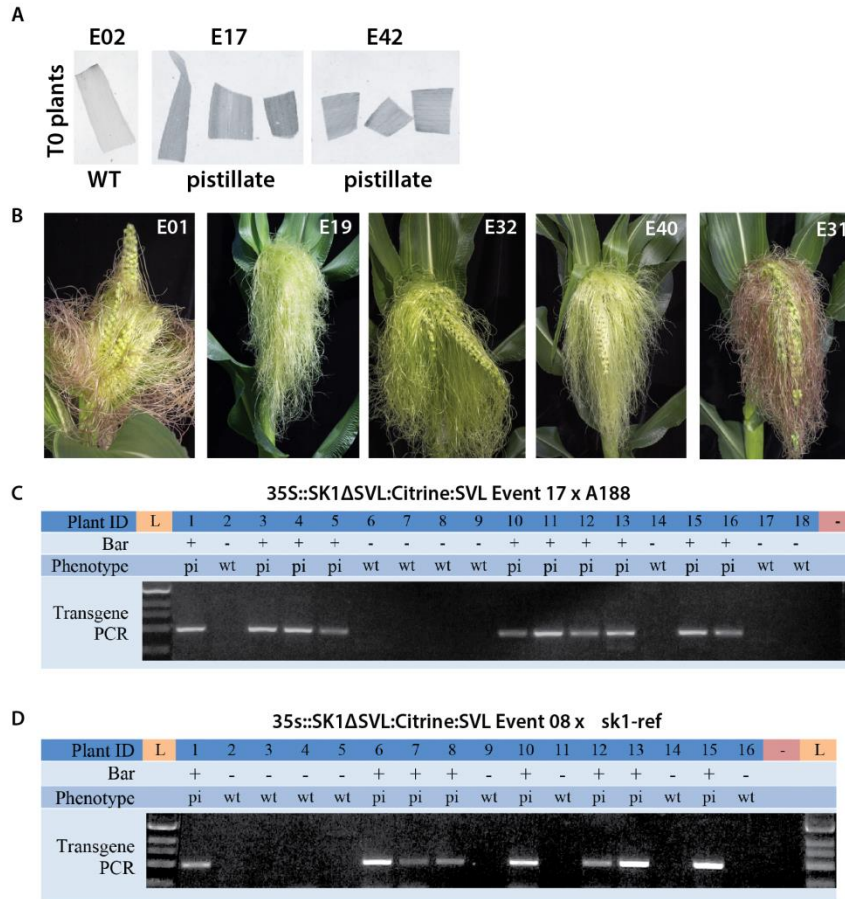
**fig. S1. Identification of the *skI* genetic and genomic interval.** (A) Genetic map interval of *skI*. The number of recombination breakpoints is shown below each marker. (B) Refined map interval of *skI*. (C) Genomic region of the *skI* on Chromosome 2 showing positions of flanking markers used to define the *skI* genetic interval. The approximate location of GRMZM2G021768 at Chr2:27,602,064...27,606,189 is also shown. All positions are based on B73 RefGen\_v3 (<http://www.maizegdb.org>).



**fig. S2. *sk1* encodes a Group N UGT.** (A) Bayesian unrooted tree of the five most highly related SK1 proteins from *B. dystachion*, *O. sativa*, *S. italica*, *S. bicolor*, and *Z. mays*. Genes clustering with SK1 (GRMZM2G021786), shaded in red, were retained for further analysis. (B) Bayesian rooted tree containing putative SK1 homologs from *B. dystachion*, *O. sativa*, *S. italica*, *S. bicolor*, and *Z. mays*. The Arabidopsis nearest hit to SK1, AT3G22250.1 (UGT82A1), was used as the outgroup. Bayesian posterior probabilities are indicated at each node. (C) Clustal Omega (v1.2.1 at <http://www.ebi.ac.uk/Tools/msa/clustalo/>) amino acid alignment of Arabidopsis AT3G22250.1 (UGT82A1) and maize SK1 (GRMZM2G021786). Position of conserved amino acids indicated as fully conserved (\*), strongly similar amino acids (:), with Gonnet PM250 matrix score >0.5, and weakly similar (.) with score ≤0.5. Details for amino acid alignments and tree construction can be found in the Materials and Methods.

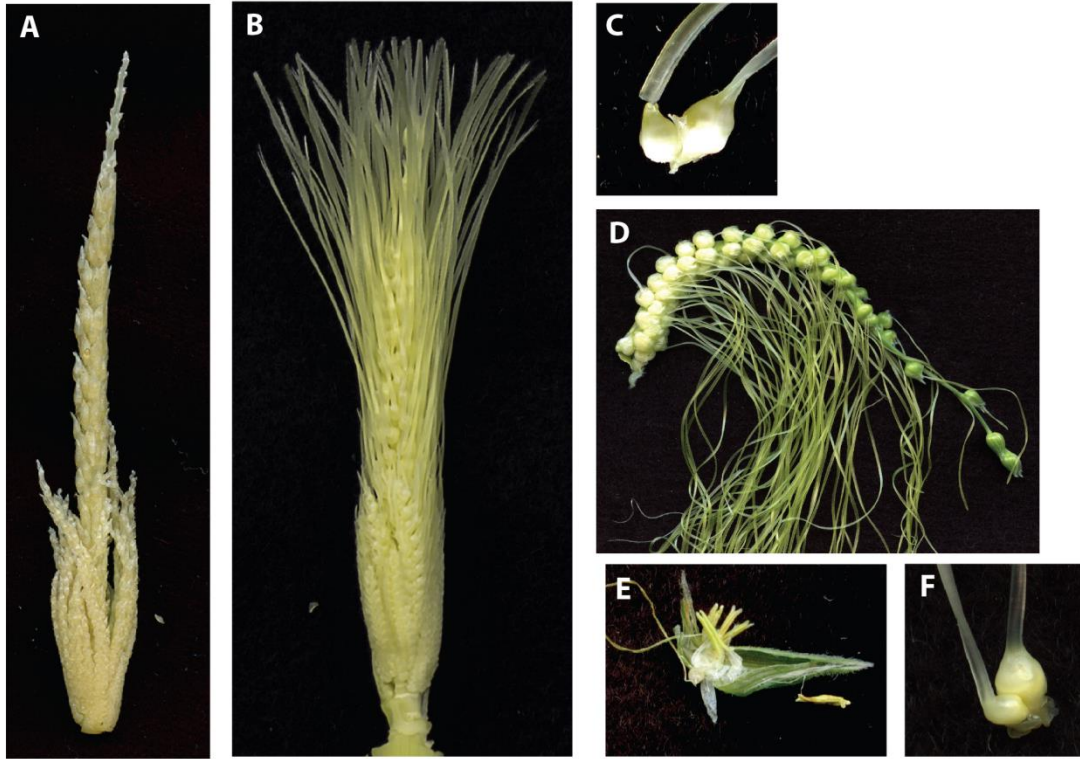


**fig. S3. *sk1* localizes to peroxisomes.** (A) Citrine:SVL localizes to punctate bodies when transiently expressed in *N. benthamiana*. Scale bar is 20  $\mu$ m. (B) SK1:Citrine shows diffuse cytoplasmic localization and not punctate localization when transiently expressed in *N. benthamiana*. Scale bar is 20  $\mu$ m. (C) SK1 $\Delta$ SVL:Citrine:SVL localizes to punctate bodies in stable transgenic Arabidopsis leaf tissue. Scale bar is 20  $\mu$ m. (D) SK1 $\Delta$ SVL:Citrine:SVL localizes to punctate bodies in stable transgenic *N. benthamiana* leaf tissue. Scale bar is 20  $\mu$ m. (E) SK1 $\Delta$ SVL:Citrine:SVL localizes to punctate bodies in stable transgenic maize leaf tissue. Scale bar is 20  $\mu$ m. (F) Western blots confirm expression of the fluorescent proteins described here and in Fig. 2. Asterisk indicates position of Citrine cleavage product.



**fig. S4. Pistillate phenotype cosegregates with the SK1-GoF transgene in maize.** (A) Representative examples of the SK1ΔSVL:Citrine:SVL T<sub>0</sub> plant screening process. Leaf tissue was screened for Citrine fluorescence using a Typhoon imager. A single plant from event E02 that was negative for Citrine fluorescence, shown here, was maintained and displayed a wild type staminate tassel phenotype. Plants positive for Citrine fluorescence ( $n = 72$ ), such as those from event E17 and event E42, were scored at flowering and all Citrine-positive plants developed a complete pistillate phenotype. (B) Pistillate phenotype of SK1ΔSVL:Citrine:SVL T<sub>0</sub> maize representing five independent transformation events. (C, D) The pistillate terminal inflorescence phenotype cosegregated perfectly with SK1ΔSVL:Citrine:SVL transgene. T<sub>0</sub> SK1ΔSVL:Citrine:SVL plants were crossed to either A188 (C) or *sk1-ref* (D). Individual T<sub>1</sub> progeny were scored for phosphinothricin herbicide resistance encoded by the physically linked selectable marker *bar* used in the transformation vector to determine the presence or absence of the SK1ΔSVL:Citrine:SVL transgene cassette. Plants were also scored for the presence of the SK1ΔSVL:Citrine:SVL transgene by PCR. The pistillate (pi) phenotype cosegregated perfectly with the presence of the SK1ΔSVL:Citrine:SVL transgene and the *bar* selectable marker.





**fig. S5. Characterization of SK1-GoF transgenic maize plants.** (A) Staminate wild type tassel at 8 cm. (B) Pistillate SK1 $\Delta$ SVL:Citrine:SVL tassel at 8 cm. (C) Spikelet from an SK1 $\Delta$ SVL:Citrine:SVL tassel showing both upper and lower pistillate florets. (D) A branch of an SK1 $\Delta$ SVL:Citrine:SVL tassel displaying nearly complete penetrance of the pistillate phenotype. Only the most terminal florets display a cosexual phenotype. (E) Example of a rare cosexual terminal spikelet from an SK1 $\Delta$ SVL:Citrine:SVL tassel. (F) Spikelet from an SK1 $\Delta$ SVL:Citrine:SVL ear showing both the upper and lower floret are pistillate.

**table S1. *sk1* alleles used in this study.**

<i>Allele</i>	<i>Mutation</i>	<i>Target site duplication</i>	<i>Position*</i>	<i>Reference (source)</i>
<i>sk1-ref</i>	>4 kb <i>Helitron</i> insertion	none	1562 (intron 1)	Jones et al. 1925 (Maize Coop)
<i>sk1-rMu</i>	1379 bp <i>Mu1</i> insertion	GCTGGCGCT	2537 (exon 2)	Rescue Mu lines (Maize Coop)
<i>sk1-Allie1</i>	3549 bp uncharacterized insertion	GTACA	2544 (intron 1)	This study

\* Based on B73 RefGen\_v3 genomic DNA sequence, [http://maizgdb.org/gene\\_center/gene?id=GRMZM2G021786](http://maizgdb.org/gene_center/gene?id=GRMZM2G021786)



**table S2. Selected primers used in this study.**

Primer ID	Primer sequence (listed 5' to 3')	Purpose
1811	AAAGTGTCCCTGGCTTGCAGATACC	Amplification of SSR marker AY107034 for mapping of the <i>sk1</i> locus
1825	AAGCATTCTAGGGCACACATTGAT	Amplification of SSR marker AY107034 for mapping of the <i>sk1</i> locus
1655	AAGGCTCGTGGCATACTGTAGT	Amplification of SSR marker b1 (umc1776) for mapping of the <i>sk1</i> locus*
1656	GCTGTACGTACGGGTGCAATG	Amplification of SSR marker b1 (umc1776) for mapping of the <i>sk1</i> locus*
782	GTCATCACTCATCAATCCCAGC	Amplification of indel marker bn18.04 for mapping of the <i>sk1</i> locus
783	TCAACCCCCACCTCTCTATTATA	Amplification of indel marker bn18.04 for mapping of the <i>sk1</i> locus
773	CCTACCCGCTACAACCTGGACATAA	Amplification of CAPS (HaeIII) marker bn12.09 for mapping of the <i>sk1</i> locus
781	CAGTACTCGTTTGTGCAGTTTGCT	Amplification of CAPS (HaeIII) marker bn12.09 for mapping of the <i>sk1</i> locus
1573	CTGGTCCGAGATGATGGC	Amplification of SSR marker bnlg1064 for mapping of the <i>sk1</i> locus*
1574	TCCATTCTGCATCTGCAAC	Amplification of SSR marker bnlg1064 for mapping of the <i>sk1</i> locus*
1571	CTCTCTTTCTTCCGACTTTCC	Amplification of SSR marker phi109642 for mapping of the <i>sk1</i> locus*
1572	GAGCGAGCGAGAGAGATCG	Amplification of SSR marker phi109642 for mapping of the <i>sk1</i> locus*
1621	ATAAAACGAACGACTCTCTACCG	Amplification of SSR marker umc1555 for mapping of the <i>sk1</i> locus*
1622	ATATGCTGACGAGCTTCGACACC	Amplification of SSR marker umc1555 for mapping of the <i>sk1</i> locus*
1727	GACGCGACTTATTACGACCCAC	Amplification of indel marker umc1769 for mapping of the <i>sk1</i> locus
1733	ATTGTTTACAGCGCTGCCGTTA	Amplification of indel marker umc1769 for mapping of the <i>sk1</i> locus
661	CAACTTCGAGGCAGTTCGTTTAT	Amplification of indel marker umc34 for mapping of the <i>sk1</i> locus
662	AGCTCTGTGTGCAGGAAGTAGGAC	Amplification of indel marker umc34 for mapping of the <i>sk1</i> locus
2159	GCGTTGTTTGGTAGATCGTTAGCC	Amplification of CAPS (Mwo1) marker FG12180
2160	CATATGCATCAGGTCAAGCAAGGA	Amplification of CAPS (Mwo1) marker FG12180
2180	ACTGCATCTCACTTGTACCCGTCT	Amplification of CAPS (SacII) marker FG06631
2187	TGCAGCTTAAATTTATGACGCTG	Amplification of CAPS (SacII) marker FG06631
2205	GCCGAGGATTTCTGCTGAAG	Amplification of CAPS (BsiHKAI) marker FG06659
2206	GCTCATGTTGCTTCACAACCTCTC	Amplification of CAPS (BsiHKAI) marker FG06659
TA_BC1F	ACACTCTTTCCCTACACGACGCTCTT CCGATCTAGCTT	Forward adapter used to create the <i>sk1</i> Taq <sup>q</sup> I library for plant P19-33
TA_BC1R	[Phos]AGCTAGATCGGAAGAGCGTCG TGTAGGGAAAGAGTG	Reverse adapter used to create the <i>sk1</i> Taq <sup>q</sup> I library for plant P19-33
TA_BC2F	ACACTCTTTCCCTACACGACGCTCTT CCGATCTGCTAT	Forward adapter used to create the <i>sk1</i> Taq <sup>q</sup> I library for plant P22-24
TA_BC2R	[Phos]TAGCAGATCGGAAGAGCGTCG TGTAGGGAAAGAGTG	Reverse adapter used to create the <i>sk1</i> Taq <sup>q</sup> I library for plant P22-24
TA_BC3F	ACACTCTTTCCCTACACGACGCTCTT CCGATCTCTAGT	Forward adapter used to create the <i>sk1</i> Taq <sup>q</sup> I library for plant P4-48
TA_BC3R	[Phos]CTAGAGATCGGAAGAGCGTCG TGTAGGGAAAGAGTG	Reverse adapter used to create the <i>sk1</i> Taq <sup>q</sup> I library for plant P4-48
TA_BC4F	ACACTCTTTCCCTACACGACGCTCTT CCGATCTGATGT	Forward adapter used to create the <i>sk1</i> Taq <sup>q</sup> I library for plant P13-27
TA_BC4R	[Phos]CATCAGATCGGAAGAGCGTCG TGTAGGGAAAGAGTG	Reverse adapter used to create the <i>sk1</i> Taq <sup>q</sup> I library for plant P13-27
CommonF	CTCGCATTCTGCTGAACCGCTCTT CCGATCT	Forward adapter used to create the <i>sk1</i> Taq <sup>q</sup> I library for all four <i>sk1</i> plants
CommonR	[Phos]GATCGGAAGAGCGGTTACGCA GGAATGCCGAG	Reverse adapter used to create the <i>sk1</i> Taq <sup>q</sup> I library for all four <i>sk1</i> plants
Buc PCR1	AATGATACGGCGACACCGAGATCT ACACTCTTTCCCTACACGACGCTCTT CCGATCT	Primer used for Illumina library amplification
Buc PCR2	CAAGCAGAAGACGGCATAACGAGATC GGTCTCGGCATTCTGCTGAACCGCT CTTCCGATCT	Primer used for Illumina library amplification

\* Previously reported SSRs; for original source, see [www.maizegdb.org](http://www.maizegdb.org)