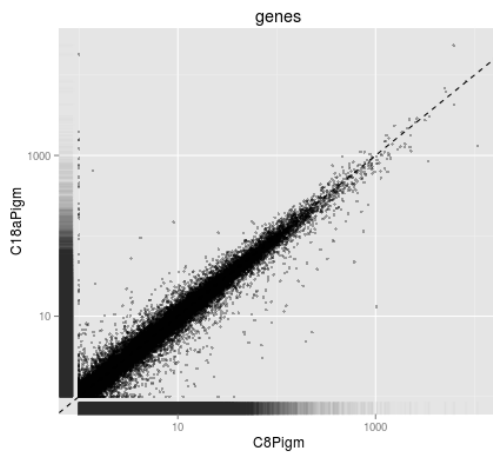


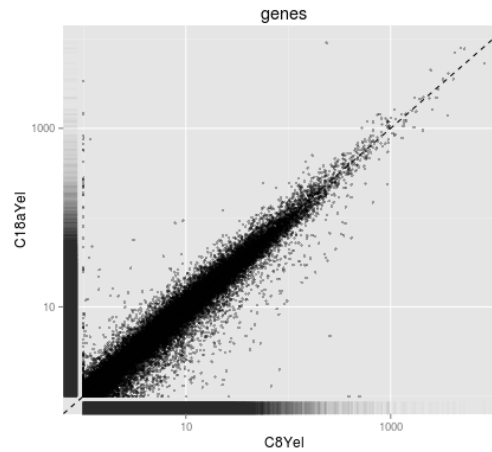
Supplemental Figure 1. The Pedigree of Clark Isolines that Show the Saddle Pattern Phenotype.

The saddle variety having the recessive *k1* allele, Clark 18a, is a mutant of Clark which has the dominant *K1* allele with *i*. Another variety showing the saddle pattern phenotype, Clark 8, is an isoline resulting from six generations of backcrossing the *i* allele into the pigmented Clark UC9 isoline which carries the recessive *i* allele. The seed insets represent the phenotypes. The C or UC numbers are internal lab numbers. The PI numbers are the official USDA germplasm numbers for these lines created by Richard L. Bernard, USDA, in the 1960-70's and are searchable at the national GRIN (Germplasm Resources Information Network) database. The Clark UC9 *i* mutation (PI547474) is listed as received in 1965 from Portageville, Missouri and is a deletion of *CHS* genes in the *I* locus region (Todd and Vodkin, 1996). The Clark 18a *k1* mutation (PI547439) used in this study is listed as found in Ames, Iowa in 1956.

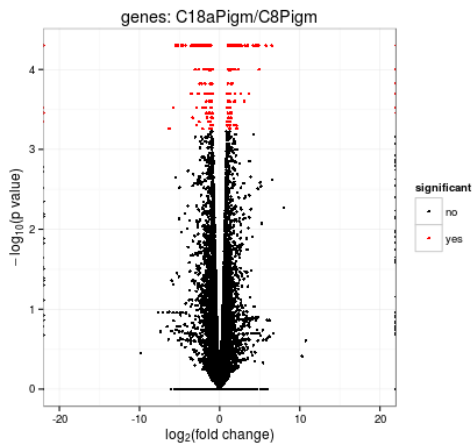
A



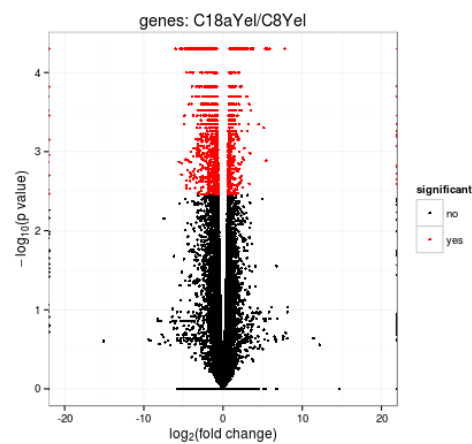
B



C



D



Supplemental Figure 2. Cufflinks Plots Comparing RNA-Seq Data From Clark (*k1*) and Clark 18a (*k1*).

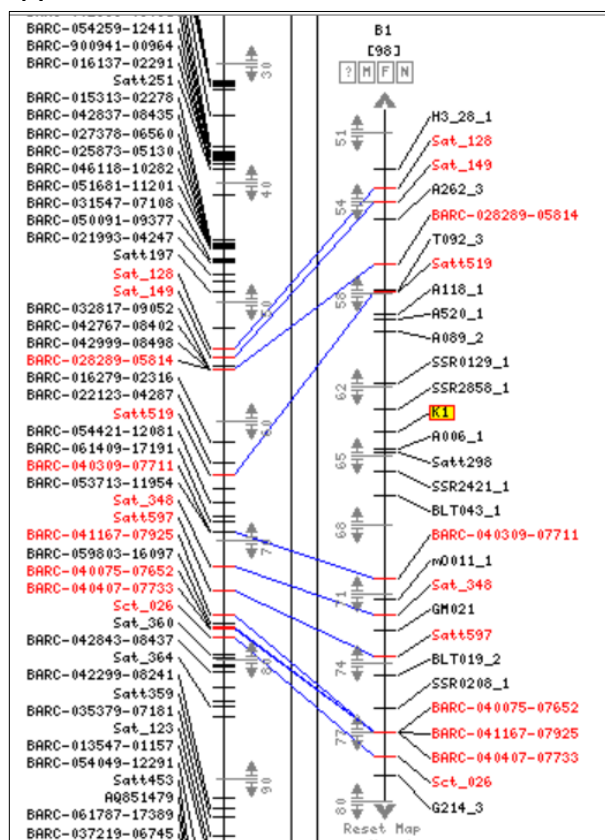
These plots assess quality of RNA-Seq using the Cufflinks package (Trapnell et al., 2012).

C8: Clark 8; C18a: Clark 18a; Pigm: pigmented region; Yel: non-pigmented region

(A) and (B). Scatter plots highlight general similarities in comparison of gene expression between Clark 8 and Clark 18a (*k1*) pigmented region (A) or Clark 8 and Clark 18a non-pigmented region (B). Each dot represents an individual gene.

(C) and (D). Volcano plots show the observed differences in gene expression relating to the significance under the statistical model of the Cufflinks package. Genes with an adjusted p value <0.05 are indicated by red dots. There are fewer differentially expressed genes in the comparison of the black saddle regions from Clark 8 (*K1*) to Clark 18a (*k1*) (C) than in the comparison of the yellow sectors (D).

A



B

Object Name	LG	Start cM	Stop cM	Object Type
Sat_128	B1	53.41	53.41	SSR
Sat_149	B1	54	54	SSR
BARC-028289-05814	B1	56.7	56.7	SNP
Satt519	B1	57.91	57.91	SSR
SSR0129_1	B1	61.9	61.9	SSR
SSR2858_1	B1	63	63	SSR
K1	B1	64	64	Gene
Satt298	B1	64.91	64.91	SSR
SSR2421_1	B1	65.7	65.7	SSR
BARC-040309-07711	B1	70.34	70.34	SNP
Sat_348	B1	71.97	71.97	SSR
Satt597	B1	73.76	73.76	SSR
SSR0208_1	B1	76	76	SSR
BARC-040075-07652	B1	77.01	77.01	SNP
BARC-040407-07733	B1	77.01	77.01	SNP
BARC-041167-07925	B1	77.01	77.01	SNP
Sct_026	B1	78.12	78.12	SSR
Sat_360	B1	80.19	80.19	SSR

C

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TGACTCTAAAGCAGAACAAGCAAAC[A/T]TCTACCTTAAAT
CCCCAATCCCCAACTTTAAGCCAGCATTGCTCAATAGAGATC
TCCTTCCTTGIGAGTTTCAATTACACAGTCTGCAGTTGGATAG
GAGACACAAGTAAGCACAAAGCCTTCCTTCAGTTGGTTGTTCAT
CGAGGAATGAGCCATCAGACTGGTCCACAGAACAGCAACAAT
CTCCCAGCACAGGTAGAGCATGCTCCGGCTCGGCAAGAGTAA
    
```

Supplemental Figure 3. Mapping Information Resources at Soybase for the *K1* Locus.

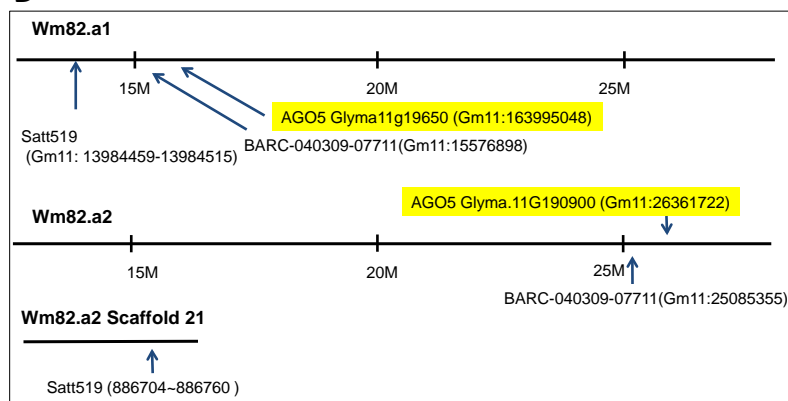
(A) Screenshot of markers surrounding the position of the *K1* locus (yellow) on Chromosome 11 (historical linkage group B1) as shown at Soybase (Grant et al., 2010) <http://soybase.org/> for the soybean composite marker map.

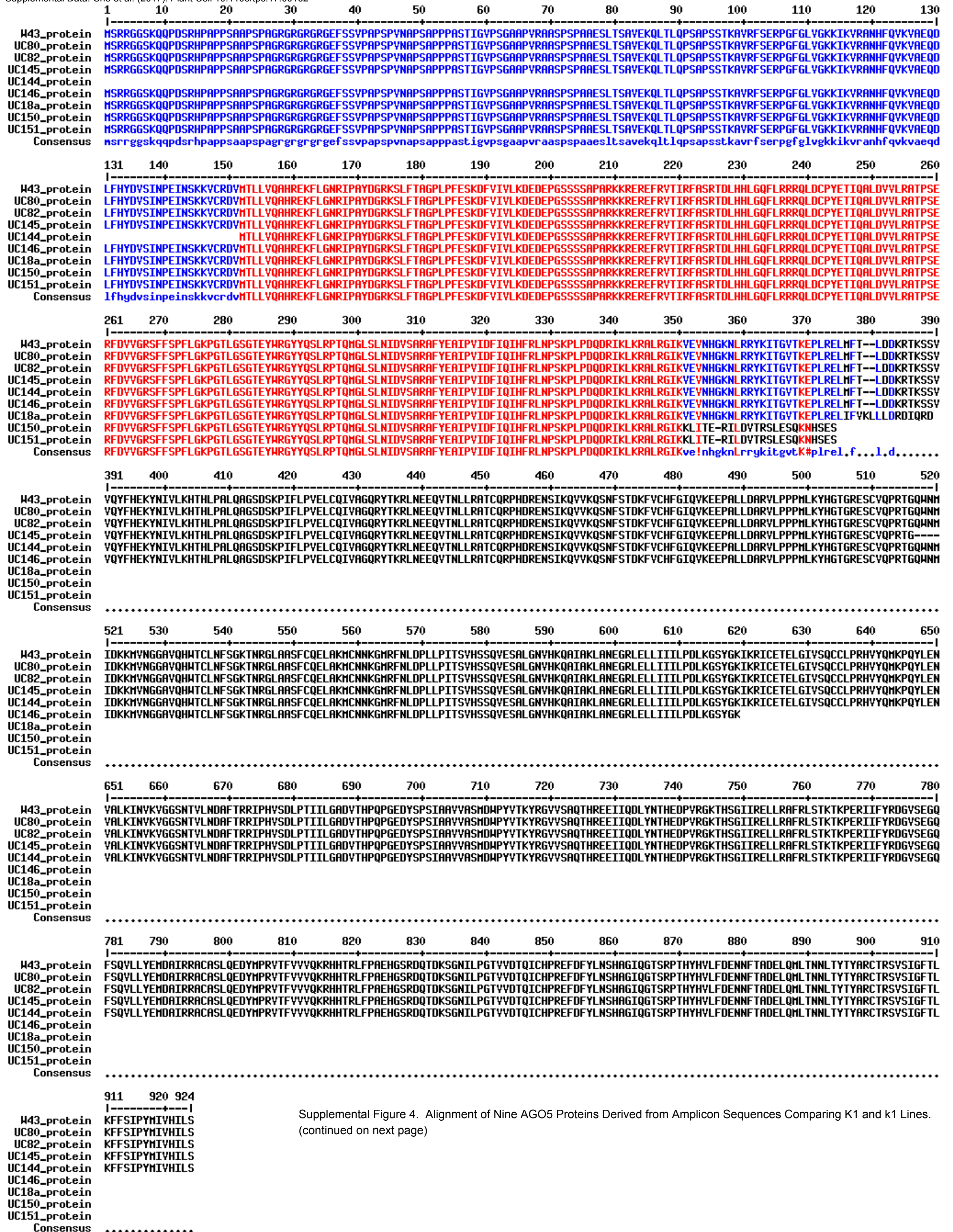
(B) Download of all SSR and SNP markers surrounding the *K1* gene locus (yellow) between 50 and 80 centimorgans showing two putative flanking markers. Not all markers have available sequences.

(C) Sequence surrounding BARC-040309-07711 used to find its position in the reference genome.

(D) Our diagram of the position of the markers on the two versions of the soybean reference genome (Wm82.a1 and Wm82.a2) showing the relative position of the Glyma model representing the AGO5 gene (yellow) which we identify in this report to be the *K1* locus. In the a2 assembly of the genome, only one of the markers (BARC-040309-07711) was located on chromosome 11 while the other marker (Satt519) was found on scaffold 21, possibly due to a discrepancy in the genome assembly.

D





Supplemental Figure 4. Alignment of Nine AGO5 Proteins Derived from Amplicon Sequences Comparing *K1* and *k1* Lines. (continued from previous page)

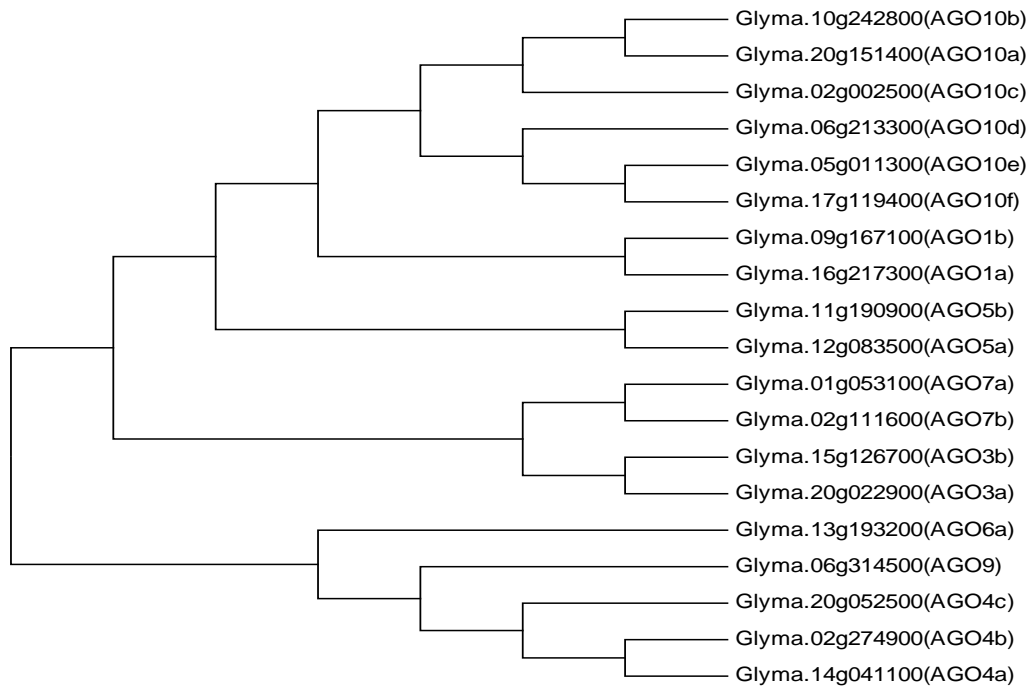
The cultivar or mutant genotypes of the following nine predicted proteins derived from amplicon sequencing were aligned with Multalin <http://multalin.toulouse.inra.fr/multalin/>

W43	PI548631	Williams	Reference	<i>K1</i>
UC80	PI548362	Lincoln	Parent line	<i>K1</i>
UC82	PI548527	Calland	Parent line	<i>K1</i>
UC145	PI634895	L88-5422	saddle pattern mutant from Lincoln 1945	<i>k1</i>
UC144	PI634873	L88-5344	saddle pattern mutant from Calland 1970	<i>k1</i>
UC146	PI634896	L88-5424	saddle pattern mutant from Lincoln 1954	<i>k1</i>
UC18a	PI547439	L67-3479	saddle pattern mutant from Clark 1956	<i>k1</i>
UC150	PI506948	Kurakake	saddle pattern variety from Japan, 1987	<i>k1</i>
UC151	PI506949	Kurakake Daizu	saddle pattern variety from Japan, 1986	<i>k1</i>



Supplemental Figure 5. Alignment of Two Closely Related Soybean AGO5 Coding Sequence Transcripts.

The coding sequences of Glyma.11G190900.1 (2673 bp) and Glyma.12G083500.1 (2886 bp) were aligned using Multalin at <http://multalin.toulouse.inra.fr/multalin/>.



Supplemental Figure 6. Phylogenetic Tree for Soybean Argonaute Genes

(Top) Phylogenetic analysis for 20 soybean AGO genes was performed with MUSCLE and MEGA v6.0 programs by the maximum-likelihood method with 500 bootstrap replicates as Liu et al. (2014) described.

(Right) The gene model number of AGO genes in both soybean reference genomes. The list of Wm82.a1.v1.1 was reported in Liu et al., 2014 and the a, b, c, etc. subclassification follows that nomenclature to distinguish homologs.

Annotation	Wm81.a1.v1.1	Wm82.a2.v1
AGO1a	Glyma16g34300	Glyma.16G217300
AGO1b	Glyma09g29720	Glyma.09G167100
AGO3a	Glyma20g02820	Glyma.20G022900
AGO3b	Glyma15g13260	Glyma.15G126700
AGO4a	Glyma14g04510	Glyma.14G041100
AGO4b	Glyma02g44260	Glyma.02G274900
AGO4c	Glyma20g12070	Glyma.20G052500
AGO5a	Glyma12g08860	Glyma.12G083500
AGO5b	Glyma11g19650	Glyma.11G190900
AGO6a	Glyma13g26240	Glyma.13G193200
AGO6b	Glyma15g37170	no correspondence
AGO7a	Glyma01g06370	Glyma.01G053100
AGO7b	Glyma02g12430	Glyma.02G111600
AGO9	Glyma06g47230	Glyma.06G314500
AGO10a	Glyma20g28970	Glyma.20G151400
AGO10b	Glyma10g38770	Glyma.10G242800
AGO10c	Glyma02g00510	Glyma.02G002500
AGO10d	Glyma06g23920	Glyma.06G213300
AGO10e	Glyma05g08170	Glyma.05G011300
AGO10f	Glyma17g12850	Glyma.17G119400
AGO10g	Glyma04g21450	Glyma.04G150900