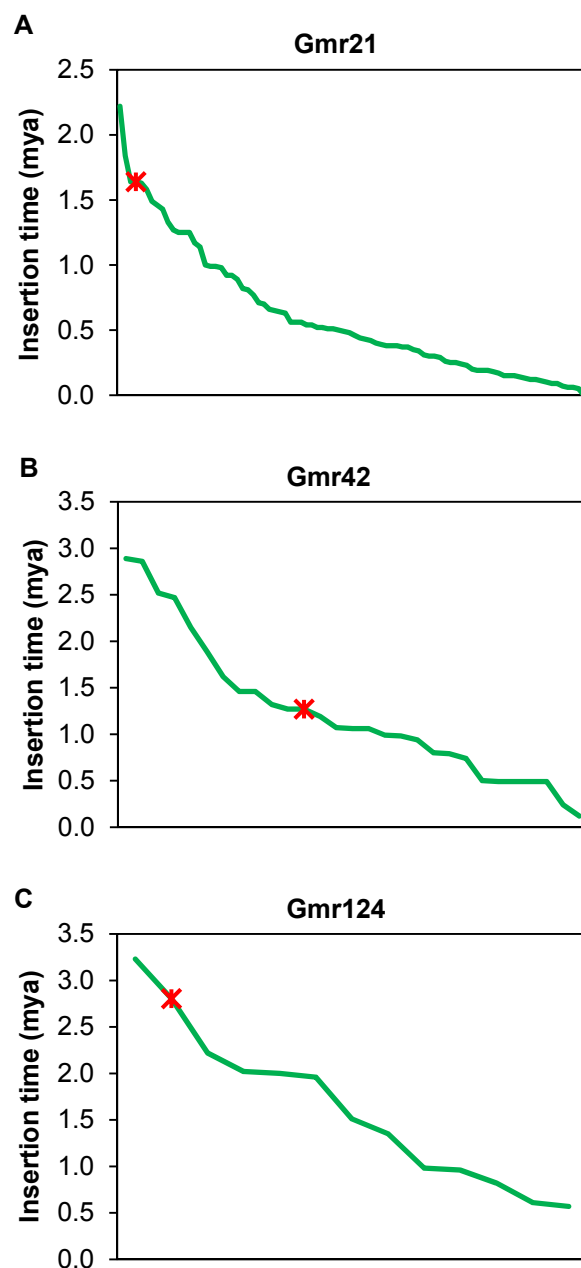


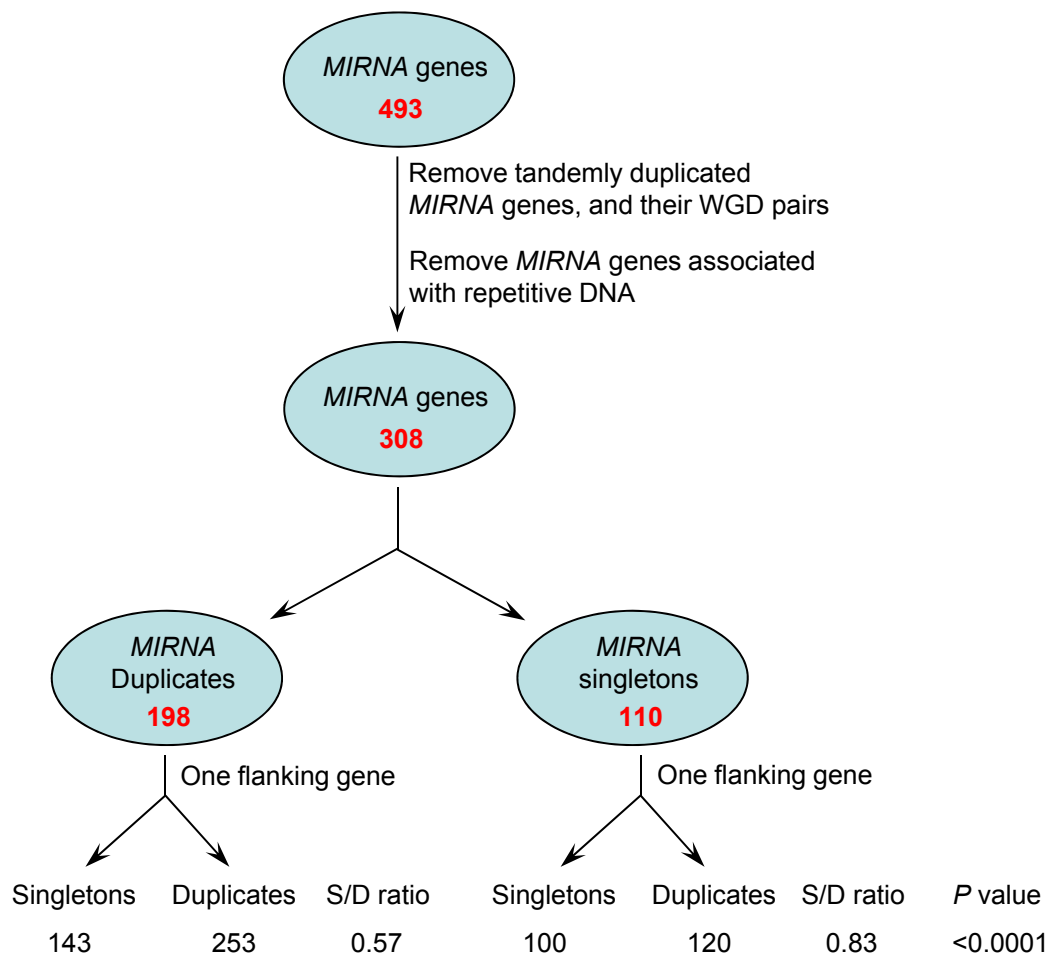
Supplemental Figure 1. Schematic Description of *MIRNA* Genes Co-located with Intact Transposable Elements.

- (A) An example in a mutator element.
- (B) An example in a Helitron.
- (C) An example in a CACTA element.
- (D) Examples in LTR retrotransposons.



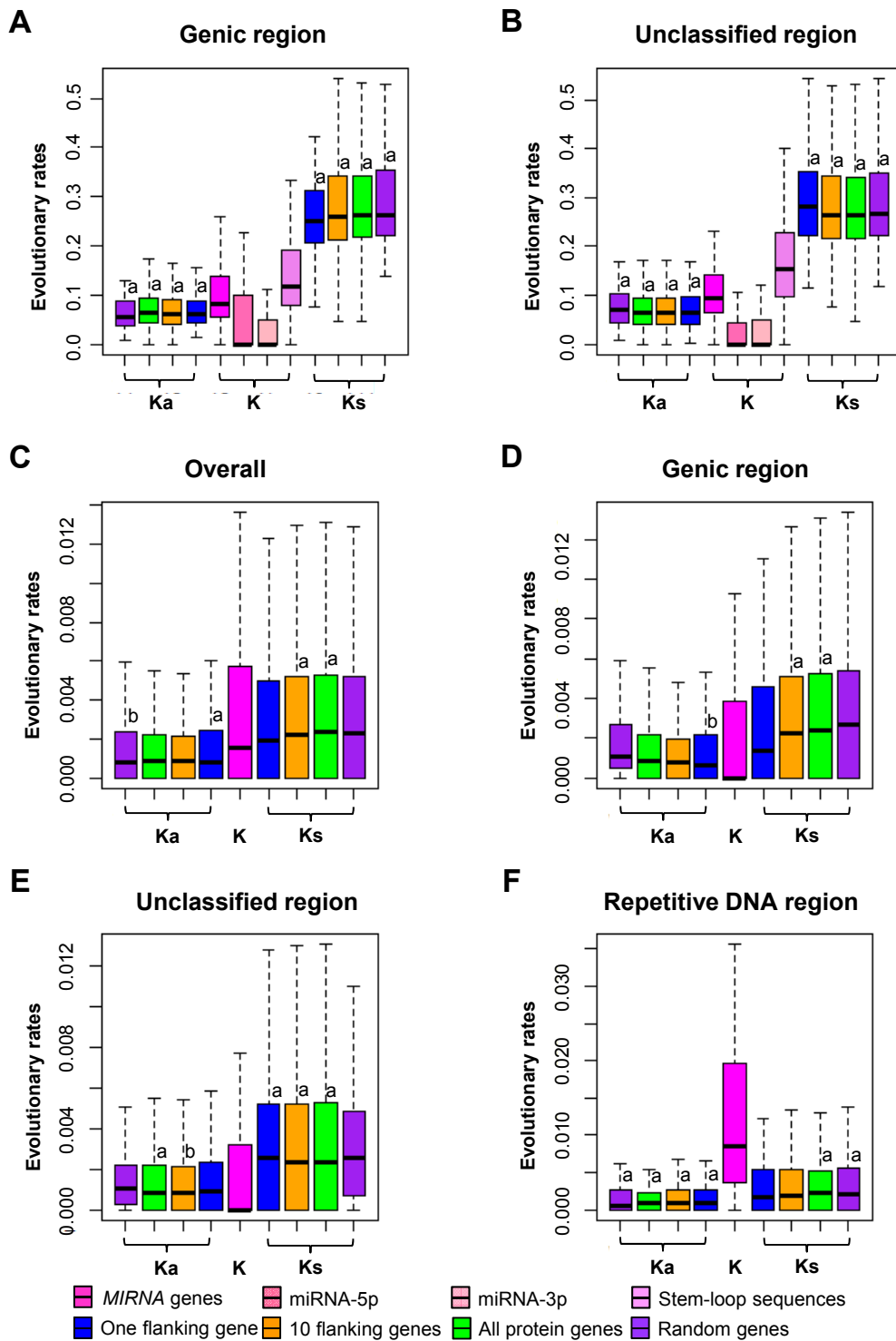
Supplemental Figure 2. Age Distribution of the Elements of Three LTR-RT Families *Gmr21*, *Gmr42* and *Gmr124*.

The x-axis represents all the intact LTR-RT elements in the family, and the y-axis represents the ages of the corresponding elements in the x-axis. The red star indicates that the element of the family contains *MIRNA* genes.



Supplemental Figure 3. Comparison of the Duplicate Status of One Flanking Genes of *MIRNA* Duplicates and *MIRNA* Singletons.

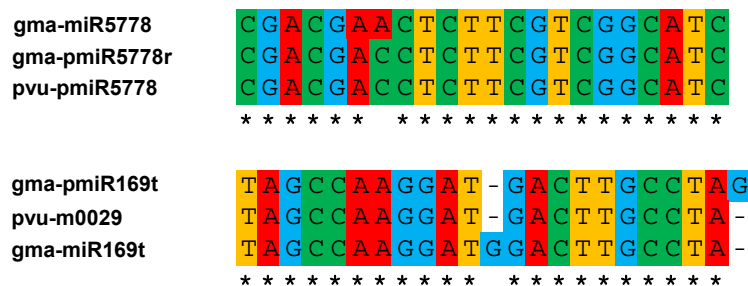
The statistical analysis was conducted using a χ^2 goodness-of-fit test.



Supplemental Figure 4. Comparison of Evolutionary Rates between Different Categories of *MIRNA* Genes and PEGs in Soybean.

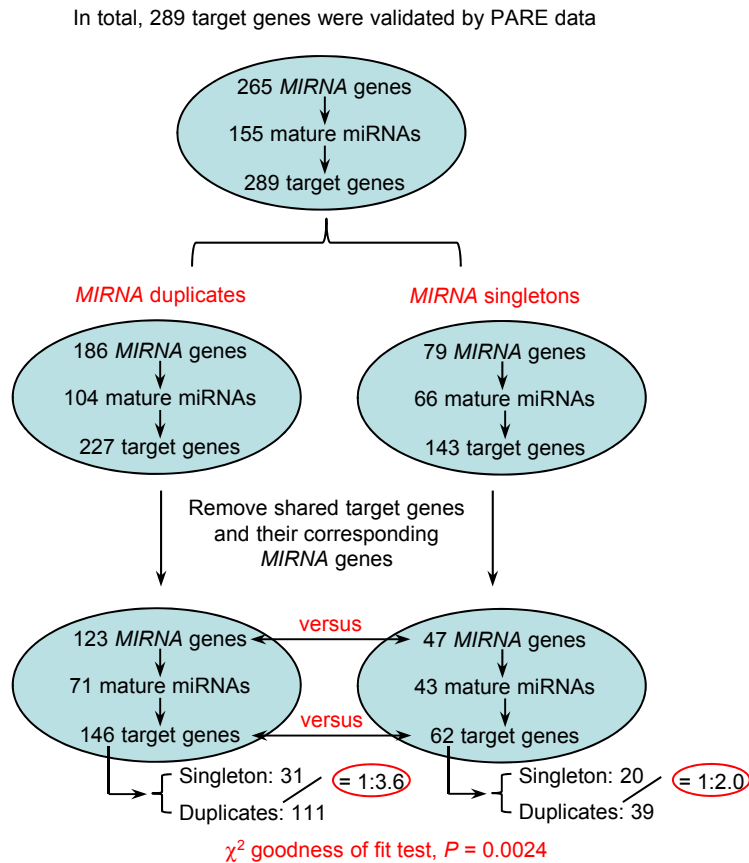
(A) and (B), Ka, Ks and K were calculated by pairwise comparisons between soybean and common bean.

(C), (D), (E) and (F), Ka, Ks and K were calculated by pairwise comparison among seven pan genomes of *Glycine soja*. The statistical analysis was conducted between each set of *MIRNA* genes and protein-encoding genes by Student's *t* test. The little "a" above each column indicates $P < 0.01$, and "b" indicates $P < 0.05$.



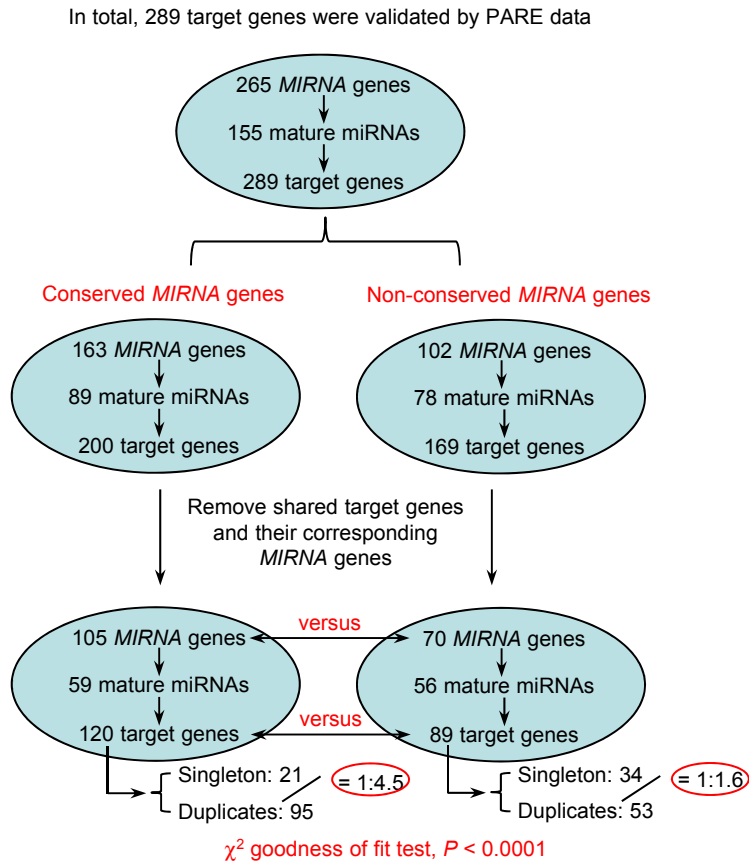
Supplemental Figure 5. Two Representative miRNAs May Have Gained Function via Mutation or Indels.

The abbreviation “pvu” indicates *Phaseolus vulgaris*. Colors indicate different nucleotides to facilitate comparisons across sequences.



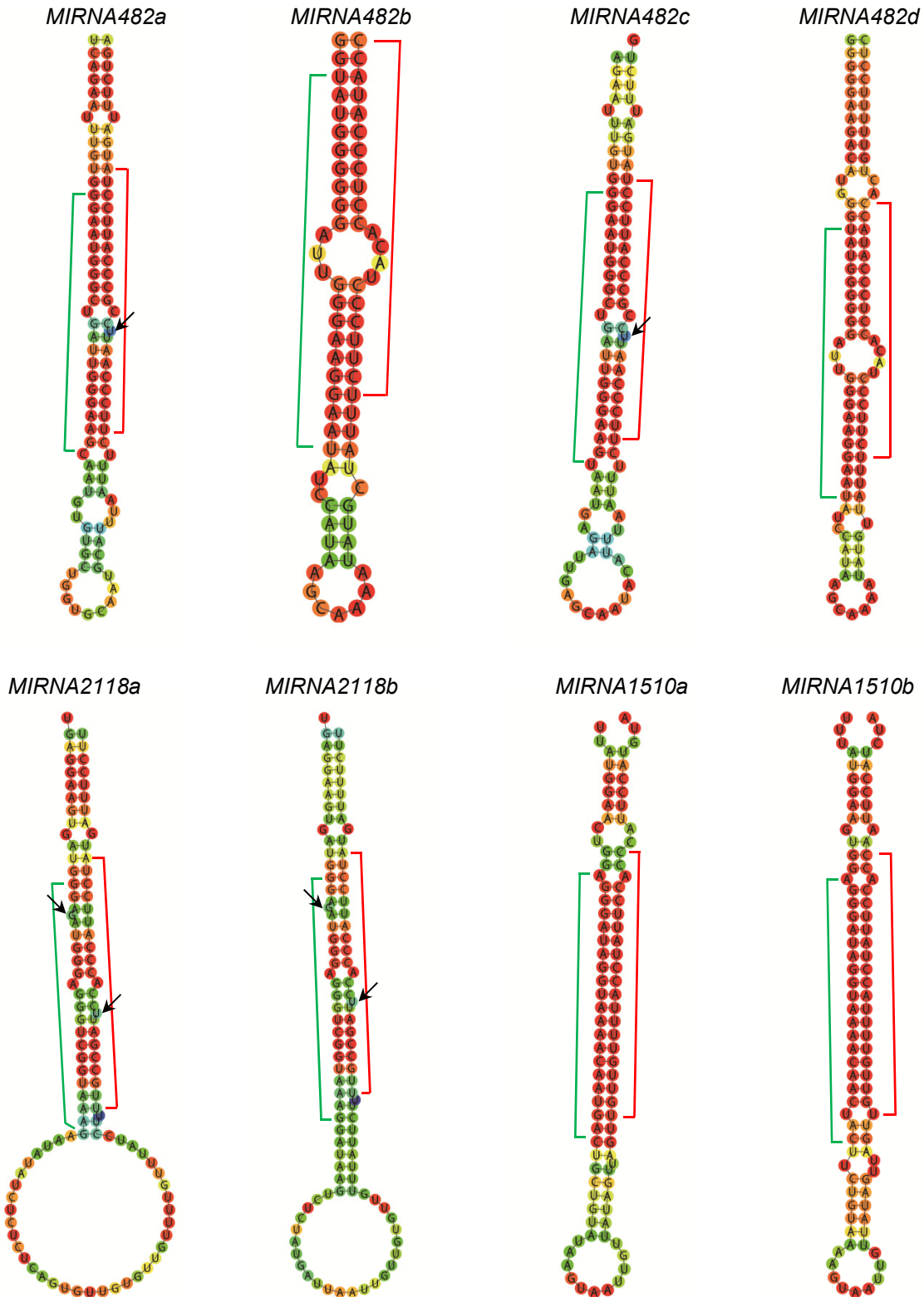
Supplemental Figure 6. Comparisons among *MIRNA* Duplicates and *MIRNA* Singletons, and among Their Target Genes.

In total, 289 miRNA targets that have been validated by using three parallel analysis of RNA ends (PARE) libraries (Song et al., 2011; Shamimuzzaman et al., 2012; Hu et al., 2013) were selected. These targets correspond to 265 *MIRNA* genes and 155 mature miRNAs. The 265 *MIRNA* genes were separated into two sets “*MIRNA* duplicates” and “*MIRNA* singletons” according to their duplication status. Shared targets and their corresponding *MIRNA* genes were further removed. The statistical analysis was conducted on the ratios of singletons to duplicates of the target genes of *MIRNA* duplicates and *MIRNA* singletons by χ^2 goodness of fit test.



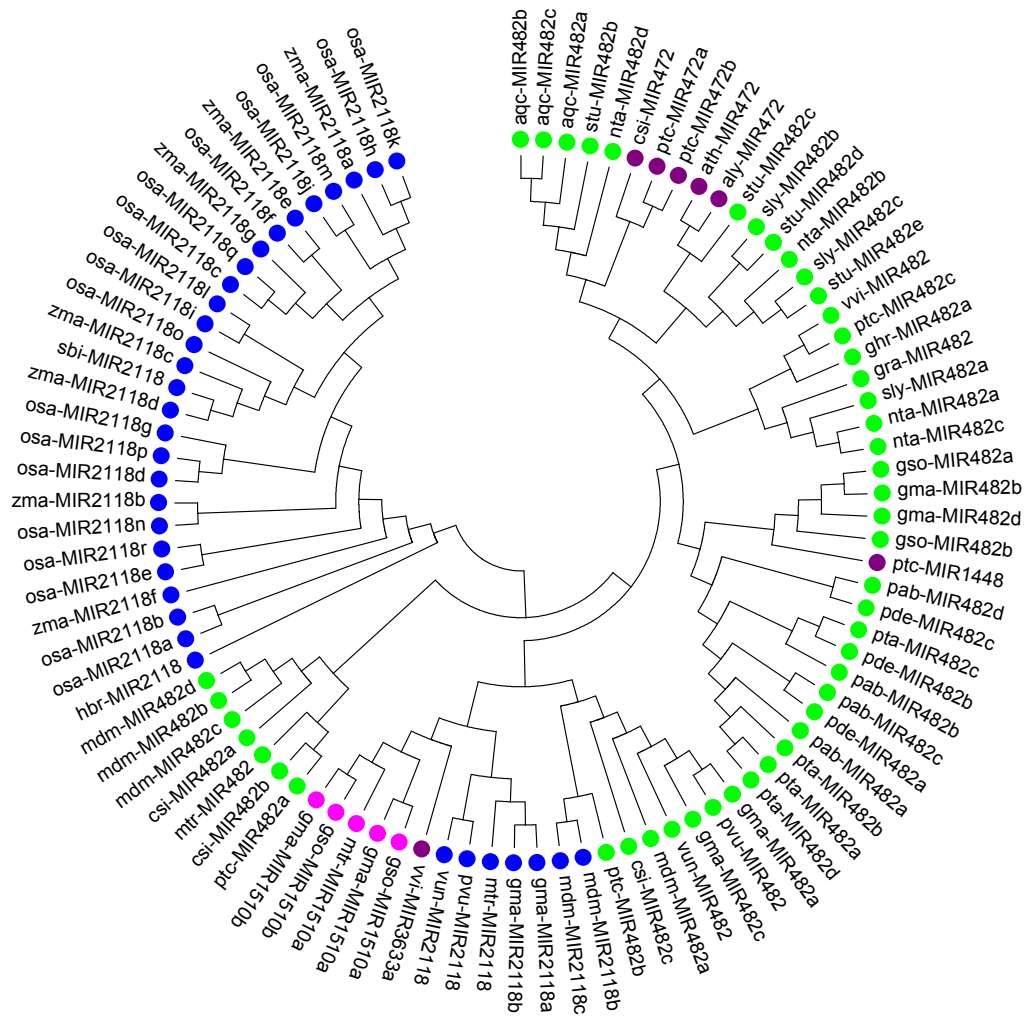
Supplemental Figure 7. Comparisons among Conserved and Non-conserved *MIRNA* Genes, and among Their Target Genes.

In total, 289 miRNA targets that have been validated by using three parallel analysis of RNA ends (PARE) libraries (Song et al., 2011; Shamimuzzaman et al., 2012; Hu et al., 2013) were selected. These targets correspond to 265 *MIRNA* genes and 155 mature miRNAs. The 265 *MIRNA* genes were separated into two sets “conserved *MIRNA* genes” and “non-conserved *MIRNA* genes”. Shared targets and their corresponding *MIRNA* genes were further removed. The statistical analysis was conducted on the ratios of singletons to duplicates of the target genes of conserved *MIRNA* genes and non-conserved *MIRNA* genes by χ^2 goodness of fit test.



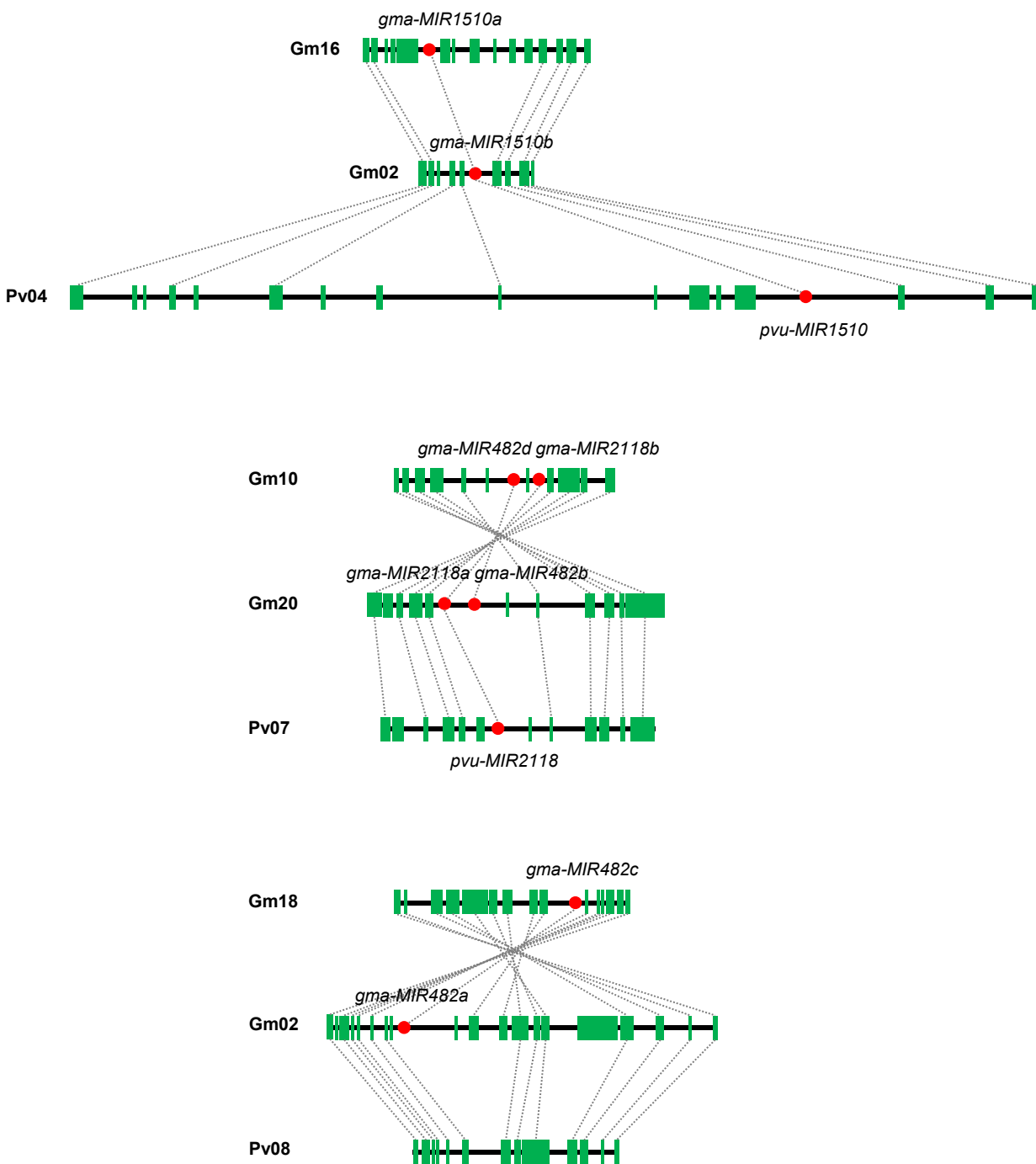
Supplemental Figure 8. Predicted Stem-loop Structures of Transcripts from *MIR482*, *MIR2118* and *MIR1510* in Soybean.

The secondary structures were predicted by CentroidFold, and the green and red lines indicate miRNA-5p and miRNA-3p. The bulges in miRNA-5p/miRNA-3p duplex regions are indicated by black arrows.



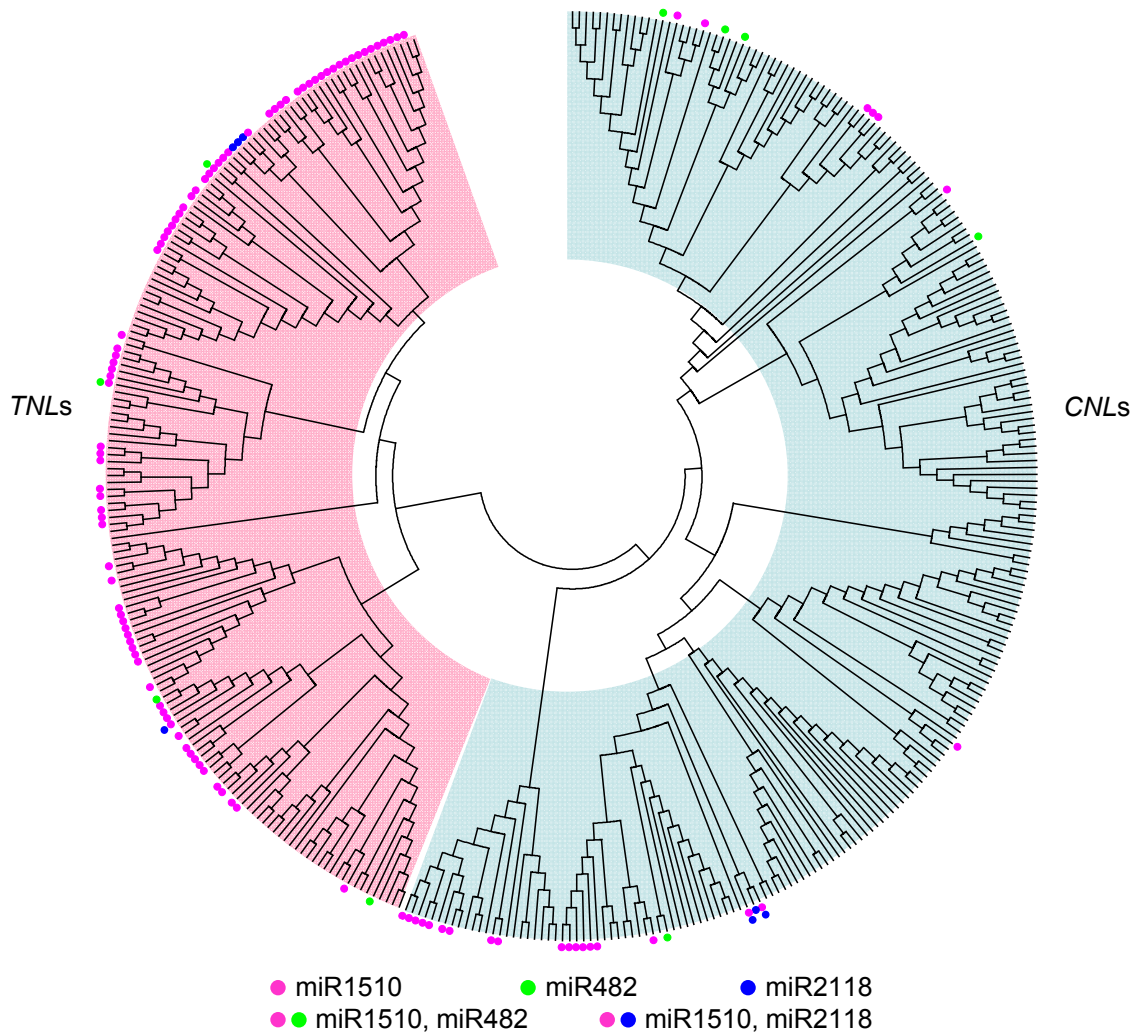
Supplemental Figure 9. Phylogenetic Relationship of *MIRNA* Genes Related to *MIR482*, *MIR2118* and *MIR1510*.

The miRNA-5p, miRNA-3p and their flanking upstream or downstream sequences were combined and used to construct the phylogenetic trees. For *MIR1510*, the combined sequence contains eight bp upstream sequences of miRNA-5p, miRNA-5p, miRNA-3p and eight bp downstream sequences of miRNA-3p. For *MIRNA482* and *MIR2118*, the combined sequence contains miRNA-5p, eight bp downstream sequences of miRNA-5p, eight bp upstream sequences of miRNA-3p and miRNA-3p. *MIR482*, *MIR2118*, *MIR1510* and other miRNA genes are indicated by green, blue, pink and purple dots, respectively.



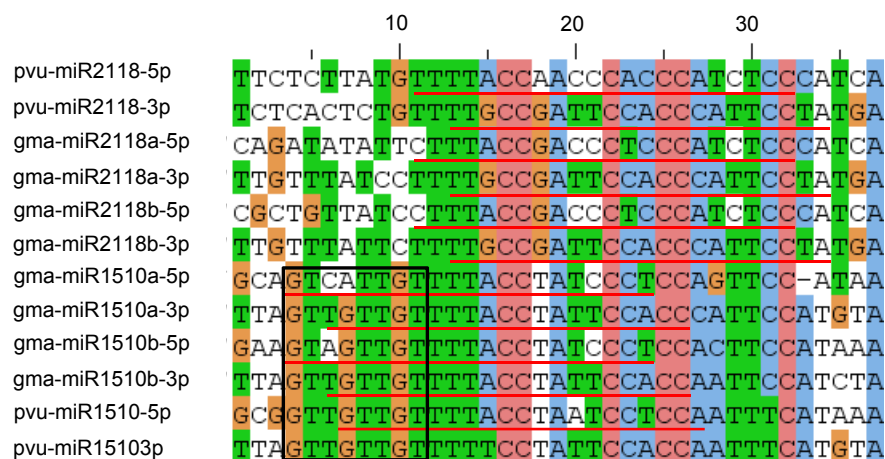
Supplemental Figure 10. Orthologous Relationships of *MIR1510*, *MIR2118* and *MIR482* between Common Bean and Soybean.

The green boxes indicate protein-encoding genes, and the red dots represent *MIRNA* genes.



Supplemental Figure 11. Phylogenetic Analysis of *NB-LRR* Genes in Soybean.

The nucleotide sequences of the conserved P-loop domains described in Meyers et al., 2003 were used to construct the phylogenetic tree. The P-loop motifs of miR1510, miR482, and miR2118 predicted targets are indicated by pink, green and blue dots, respectively. Two dots mean the gene is both the predicted targets of two miRNAs. All others are the remaining *NB-LRR* genes in the soybean reference genome. The targets of miR1510, miR482 and miR2118 were predicted at or under the penalty score (≤ 5). *TNLs*, *TIR-NB-LRRs*. *CNLs*, *CC-NB-LRRs*.



Supplemental Figure 12. Comparative Analysis of the miRNA-5p and miRNA-3p Regions of *MIR1510* and *MIR2118*.

The miRNA-5p and its flanking upstream and downstream sequences were extracted, reversed and complemented, and compared with miRNA-3p and its flanking upstream or downstream sequences. The red lines beneath each sequence indicate the miRNA-5p and miRNA-3p regions. The black box represents the region conserved in *MIR1510*, but diverged in *MIR2118*.

Supplemental Data. Zhao et al. (2015). Plant Cell 10.1105/tpc.115.00048

Supplemental Table 1. Orientations of *MIRNA* Genes Co-located with Protein-encoding Genes

	Same strand	Opposite strand	Total
Intron	39	9	48
Exon	16	8	24
5' UTR	28	1	29
3' UTR	20	4	24
Junction	34	3	37
Total (%)	137 (84.6%)	25 (15.4%)	162

Supplemental Data. Zhao et al. (2015). Plant Cell 10.1105/tpc.115.00048

Supplemental Table 2. Description of *MIRNA* Genes with RNA-seq Reads Matched in the Soybean 28 Tissues

	Total	No. of <i>MIRNA</i> genes matched by the RNA-seq reads
Intron	48	26
Exon	24	ND
5' UTR	29	ND
3' UTR	24	ND
Multiple categories	37	1
Intergenic_5' UTR	4	ND
5' UTR_exon	13	ND
5' UTR_exon_3' UTR	1	ND
5' UTR_exon_intron	1	ND
Exon_intron/Intron_exon	6	ND
Exon_intron_exon	1	1
Intron_5' UTR	1	ND
Intron_exon_3' UTR	1	ND
Exon_3' UTR	8	ND
3' UTR_intergenic	1	ND

ND, Not determined

Supplemental Data. Zhao et al. (2015). Plant Cell 10.1105/tpc.115.00048

Supplemental Table 3. Description of Soybean *MIRNA* Genes with Orthologs and Homologs in Common Bean

Features	Total ^a	Duplicates			Singletons		
		<i>MIRNA</i> genes	Orthologs ^b	Homologs ^c	<i>MIRNA</i> genes	Orthologs ^b	Homologs ^c
Genic							
Intron	48	11	4	1	37	1	6
Exon	24	19	13	1	5	0	2
5' UTR	29	21	15	1	9	5	0
3' UTR	24	16	13	1	7	1	0
Junctions	37	24	22	1	13	4	2
Subtotal	162	91	67	5	71	11	10
Unclassified							
Subtotal	213	157	106	16	56	14	10
Repetitive DNA							
Subtotal	79	11	2	0	68	3	2
Total (%)	454	259	175	21	195	28	22

^aTotal number of expressable *MIRNA* genes in soybean.

^bNo. of orthologous *MIRNA* genes of soybean shared by common bean.

^cNo. of homologous *MIRNA* genes of soybean shared by common bean.

Supplemental Data. Zhao et al. (2015). Plant Cell 10.1105/tpc.115.00048

Supplemental Table 4. Statistics Analysis of Differential Ratios of Singletons to Duplicates of *MIRNA*s and Protein-Encoding Genes in Soybean

	Singletons	Duplicates	S/D ratio ^a	<i>P</i> value ^b
<i>MIRNA</i> s	110	198	0.56:1	–
First flanking genes	243	373	0.65:1	0.0528
Second flanking genes	216	400	0.54:1	0.7383
All protein genes	14964	31524	0.47:1	<0.0001

^aThe ratio of Singletons to Duplicates.

^bThe statistic analysis was conducted on the ratios of singletons to duplicates between *MIRNA*s and each set of protein-encoding genes by χ^2 goodness of fit test. *MIRNA*s and their WGD pairs associated with tandem duplication and those located in repetitive DNAs were not included. Tandem duplicated protein-encoding genes were also excluded in this analysis.

Supplemental Data. Zhao et al. (2015). Plant Cell 10.1105/tpc.115.00048

Supplemental Table 5. Comparison of Evolutionary Rates among Different Regions of *MIRNA* Hairpins

Comparison ^a	Nucleotide divergence		<i>P</i> ^c
	Region1	Region2	
miRNA-5p vs. miRNA-3p	0.0317 ± 0.0571	0.0383 ± 0.0735	0.2937
miRNA-5p vs. LS ^b	0.0317 ± 0.0571	0.1300 ± 0.0768	<0.0001
miRNA-3p vs. LS ^b	0.0383 ± 0.0735	0.1300 ± 0.0768	<0.0001

^aThe nucleotide divergence were calculated by pairwise comparison between soybean *MIRNA* hairpins and their orthologs in the common bean genome.

^bloop and other stem sequences.

^cStudent's paired *t* test.

Supplemental Data. Zhao et al. (2015). Plant Cell 10.1105/tpc.115.00048

Supplemental Table 6. Comparison of Evolutionary Rates of *MIRNA*s Involved and Not Involved in Tandem Duplication

Do not remove *MIRNA* genes in repetitive DNA region

Comparison	Duplicates	Singletons	<i>P</i> ^a	Comparison	Tandem	Non-tandem	<i>P</i> ^a
Tandem	0.0016 ± 0.0025	0.0026 ± 0.0036	0.2569	Duplicates	0.0016 ± 0.0025	0.0025 ± 0.0049	0.0507
Non-tandem	0.0025 ± 0.0049	0.0109 ± 0.0149	<0.0001	Singletons	0.0026 ± 0.0036	0.0109 ± 0.0149	<0.0001

Remove *MIRNA* genes in repetitive DNA region

Comparison	Duplicates	Singletons	<i>P</i> ^a	Comparison	Tandem	Non-tandem	<i>P</i> ^a
Tandem	0.0016 ± 0.0025	0.0023 ± 0.0029	0.2656	Duplicates	0.0016 ± 0.0025	0.0018 ± 0.0034	0.5655
Non-tandem	0.0018 ± 0.0034	0.0077 ± 0.0112	<0.0001	Singletons	0.0023 ± 0.0029	0.0077 ± 0.0112	<0.0001

^aStudent's *t* test.

Supplemental Data. Zhao et al. (2015). Plant Cell 10.1105/tpc.115.00048

Supplemental Table 7. Comparisons of the miRNA Expression Levels of *MIRNA* Genes Located in Different Regions

Comparison	Genic region	Unclassified region	<i>P</i> ^a
Overall	9402 ± 54636	4077 ± 22210	0.3898
Duplicates	19437 ± 78735	4889 ± 26484	0.2507
Singletons	412 ± 1311	2882 ± 13956	0.2838

Comparison	Genic region	Repetitive DNA region	<i>P</i> ^a
Overall	9402 ± 54636	286 ± 813	0.1151
Duplicates	19437 ± 78735	263 ± 507	0.1178
Singletons	412 ± 1311	290 ± 857	0.6046

Comparison	Unclassified region	Repetitive DNA region	<i>P</i> ^a
Overall	4077 ± 22210	286 ± 813	0.1017
Duplicates	4889 ± 26484	263 ± 507	0.1973
Singletons	2882 ± 13956	290 ± 857	0.2604

^aStudent's *t* test.

Supplemental Data. Zhao et al. (2015). Plant Cell 10.1105/tpc.115.00048

Supplemental Table 8. Comparison of the Expression Values of the 11 Duplicated miRNAs with Mutations

miRNA	Genomic location	TPM	miRNA pair	Genomic location	TPM	Fold change
gma-miR395h	Arm	0	gma-miR395m	Arm	0	-
gma-miR1510a	Arm	14715.26	gma-miR1510b	Arm	36885.09	2.51
gma-miR482c	Arm	2203.61	gma-miR482a	Arm	167177.08	75.87
gma-miR1512a	Arm	3.05	gma-miR1512b	Arm	98.37	32.25
gma-miR169j	Arm	3.53	gma-miR169l	Arm	4.65	1.32
gma-miR1509b	Peri	211.52	gma-miR1509a	Arm	1123.34	5.31
gma-miR5770b	Arm	0.35	gma-miR5770a	Peri	139.45	398.43
gma-miR1514b	Arm	11.45	gma-miR1514a	Arm	565.16	49.36
gma-miR172k	Arm	0	gma-miR172b	Arm	8.12	-
gma-miR319n	Arm	50.56	gma-miR319l	Arm	1996.35	39.48
gma-miR4415b	Peri	0	gma-miR4415a	Arm	3.92	-