Supplementary Information

Table S1. Statistics of miRNA amounts detected from small RNA deep sequencing.

Sample	Amount of miRNAs	Total Numbers		Shared Numbers	
WRRh	780 (152)	891 (170)	921 (188)	680(147)	579 (102)
WRRl	791 (165)				
XHh	718 (133)	797 (165)		671 (109)	
XHl	750 (141)				

The value in brackets is the number of novel miRNAs. WRRh, WRRl, XHh, and XHl indicated the group of Recessive White Rock with high body weight, Recessive White Rock with low body weight, Xinhua Chickens with high body weight, and Xinhua Chickens with low body weight, respectively.

Table S2. Novel miRNAs have read counts more than 1000 reads in the libraries.

miRNA	Read Counts	Chromosome	Strand	Star	End
15_2275	854,769	15	−	473,792	473,870
1_6441	27,093	1	−	130,059,859	130,059,906
2_9878	6819	2	−	131,256,102	131,256,146
3_11306	5729	3	−	95,198,063	95,198,113
Z_18074	4786	Z	−	71,507,867	71,507,915
Z_17969	4779	Z	−	42,745,528	42,745,607
4_12521	1528	4	−	91,911,592	91,911,653
4_11967	1186	4	+	92,169,305	92,169,366
3_11169	1062	3	−	68,697,828	68,697,899
5_12580	1032	5	+	5,381,982	5,382,053

Table S3. Details of shared differentially expressed miRNAs among four contrasts.

Conjoint Contrast	Differentially Expressed miRNAs	Amount
WRRh *vs.* WRRl＆ XHh *vs.* XHl	1_4060, 1_4903, 1_5489, 10_133, 14_1786, 17_2567, 18_3584, 2_9448, 20_7112, 24_7725, 24_7726, 28_8412, 3_11092, 4_11437, 4_11488, 4_12339, 4_12341, 6_13832, 6_14144, Z_17439, Z_17672, Z_18026, miR-1416-3p, miR-146b-5p, miR-1736-5p, miR-1744-3p, miR-1751-5p, miR-1756a, miR-1791-3p, miR-205b, miR-460b-3p, miR-460b-5p, miR-6544-5p, miR-9-5p	54
WRRh *vs.* XHh＆WRRl *vs.* XHl	1_4836, 1_5155, 1_5163, 1_5364, 1_5442, 1_5571, 1_5572, 1_6316, 1_6441, 1_6464, 1_6603, 1_6696, 1_6743, 10_168, 10_237, 11_641, 13_1554, 14_1780, 14_1987, 15_2097, 15_2173, 19_3786, 19_3829, 2_8666, 2_8901, 2_8915, 2_8923, 2_8924, 2_9621, 2_9815, 20_6874, 20_6890, 20_6954, 20_6992, 20_7045, 21_7211, 21_7222, 21_72124, 24_7682, 28_8325, 28_8457, 3_10168, 3_10670, 4_11479, 4_11670, 4_11987, 4_12193, 4_12255, 4_12363, 4_12411, 5_13090, 5_13621, 5_13622, 8_16601, Z_17715, miR-1329-5p, miR-142-5p, miR-146b-3p, miR-155, miR-1603, miR-1635, miR-1648-5p, miR-1674, 1684b-3p, miR-1716, miR-1747, miR-184, miR-194, miR-200a, miR-200b, miR-21, miR-222b-3p, miR-222b-5p, miR-223, miR-34b, miR-34c, miR-3524a, miR-3529, miR-458a-3p, miR-6557-3p, miR-6570-5p, miR-6586-5p, miR-6677-5p, miR-6681-5p	84

Table S3. *Cont.*

Conjoint Contrast	Differentially Expressed miRNAs	Amount
All Four Contrasts	1_6510, 15_2093, 19_3787, 23_7539, 24_7684, 26_7910, 26_8039, 26_8040, 4_11833, 4_12372, 5_12997, 5_13077, 7_15435, Z_17414，Z_18085, Z_18086, miR-122, miR-1329-3p, miR-1587, miR-1736-3p, miR-1769-3p, miR-1769-5p, miR-1773-5p, miR-205a, miR-31, miR-375	26

WRRh *vs*. WRRl indicated the comparison between the two-tail samples of Recessive White Rock; XHh *vs*. XHl indicated the comparison between the two-tail samples of Xinhua Chickens; WRRh *vs*. XHh indicated the comparison between the groups of Recessive White Rock and Xinhua Chickens with high body weight; WRRl *vs*. XHl indicated the comparison between the groups of Recessive White Rock and Xinhua Chickens with low body weight.

Table S4. Top 29 GO terms of gene counts over 1,000 for target genes of all differentially expressed miRNAs.

No.	GO Terms	GO Accession	Gene Numbers	*p* Value
1	protein binding	GO:0005515	2530	1.30×10^{-30}
2	cytoplasm	GO:0005737	1669	1.90×10^{-25}
3	cytoplasmic part	GO:0044444	1058	1.30×10^{-15}
4	cellular process	GO:0009987	3395	8.40×10^{-11}
5	membrane-bounded organelle	GO:0043227	1924	3.10×10^{-8}
6	intracellular membrane-bounded organelle	GO:0043231	1921	3.60×10^{-8}
7	binding	GO:0005488	5116	4.60×10^{-8}
8	localization	GO:0051179	1106	9.40×10^{-8}
9	cellular metabolic process	GO:0044237	2107	1.10×10^{-7}
10	intracellular part	GO:0044424	2720	1.50×10^{-7}
11	cellular macromolecule metabolic process	GO:0044260	1558	1.50×10^{-6}
12	biological regulation	GO:0065007	2107	9.10×10^{-6}
13	membrane part	GO:0044425	1635	1.20×10^{-5}
14	macromolecule metabolic process	GO:0043170	1804	1.70×10^{-5}
15	regulation of biological process	GO:0005789	2006	2.60×10^{-5}
16	intracellular	GO:0005622	3173	2.90×10^{-5}
17	nucleotide binding	GO:0000166	1171	4.00×10^{-5}
18	regulation of cellular process	GO:0050794	1922	6.20×10^{-5}
19	intrinsic to membrane	GO:0031224	1357	6.90×10^{-5}
20	metabolic process	GO:0008152	2802	1.20×10^{-4}
21	protein metabolic process	GO:0019538	1172	1.20×10^{-4}
22	integral to membrane	GO:0016021	1308	1.70×10^{-4}
23	primary metabolic process	GO:0044238	2302	1.90×10^{-4}
24	organelle	GO:0043226	2251	6.00×10^{-4}
25	intracellular organelle	GO:0043229	2247	7.00×10^{-4}
26	catalytic activity	GO:0003824	2625	1.70×10^{-3}
27	cell	GO:0005623	4723	2.00×10^{-3}
28	cell part	GO:0044464	4723	2.00×10^{-3}
29	nucleus	GO:0005634	1249	6.40×10^{-3}

Table S5. Enriched KEGG pathways for target gene of differently expressed miRNA identified in the four contrasts.

Class	ID	Pathways	*p* Value
WRRh *vs*. WRRl	gga04142	Lysosome	0.0023
	gga01100	Metabolic pathways	0.0061
	gga00052	Galactose metabolism	0.0078
	gga00280	Valine, leucine and isoleucine degradation	0.0078
	gga00511	Other glycan degradation	0.0093
	gga00600	Sphingolipid metabolism	0.0123
	gga00270	Cysteine and methionine metabolism	0.0137
	gga00510	N-Glycan biosynthesis	0.0151
	gga04620	Toll-like receptor signaling pathway	0.0205
	gga00230	Purine metabolism	0.0244
	gga04141	Protein processing in endoplasmic reticulum	0.0256
	gga03040	Spliceosome	0.0292
	gga04145	Phagosome	0.0327
WRRh *vs*. XHh	gga00052	Galactose metabolism	0.0044
	gga00280	Valine, leucine and isoleucine degradation	0.0044
	gga04744	Phototransduction	0.0053
	gga00511	Other glycan degradation	0.0053
	gga00600	Sphingolipid metabolism	0.0070
	gga01100	Metabolic pathways	0.0116
	gga04142	Lysosome	0.0127
	gga00230	Purine metabolism	0.0143
WRRl *vs*. XHl	gga04142	Lysosome	0.0003
	gga01100	Metabolic pathways	0.0045
	gga00052	Galactose metabolism	0.0095
	gga00280	Valine, leucine and isoleucine degradation	0.0095
	gga00511	Other glycan degradation	0.0113
	gga00600	Sphingolipid metabolism	0.0148
	gga00270	Cysteine and methionine metabolism	0.0165
	gga00510	N-Glycan biosynthesis	0.0182
	gga04620	Toll-like receptor signaling pathway	0.0245
	gga04110	Cell cycle	0.0260
	gga00230	Purine metabolism	0.0290
	gga04141	Protein processing in endoplasmic reticulum	0.0304
	gga03040	Spliceosome	0.0345
	gga04145	Phagosome	0.0384
	gga04144	Endocytosis	0.0498

Table S5. *Cont.*

Class	ID	Pathways	*p* Value
XHh *vs.* XHl	gga00280	Valine, leucine and isoleucine degradation	0.0002
	gga04142	Lysosome	0.0023
	gga01100	Metabolic pathways	0.0061
	gga00052	Galactose metabolism	0.0078
	gga04744	Phototransduction	0.0093
	gga00511	Other glycan degradation	0.0093
	gga00600	Sphingolipid metabolism	0.0123
	gga00270	Cysteine and methionine metabolism	0.0137
	gga04270	Vascular smooth muscle contraction	0.0218
	gga04110	Cell cycle	0.0218
	gga04145	Phagosome	0.0327
	gga04530	Tight junction	0.0449

WRRh *vs*. WRRl indicated the comparison between the two-tail samples of Recessive White Rock; XHh *vs*. XHl indicated the comparison between the two-tail samples of Xinhua Chickens; WRRh *vs*. XHh indicated the comparison between the groups of Recessive White Rock and Xinhua Chickens with high body weight; WRRl *vs*. XHl indicated the comparison between the groups of Recessive White Rock and Xinhua Chickens with low body weight.

Table S6. Detail of miRNA quantitative real-time PCR primer.

Gene	Primer Sequence (5′ to 3′)	Annealing Temp (°C)
gga-miR-223	F: GTGTCAGTTTGTCAAATACCCCAA	56
gga-miR-142-5p	F: GCCCATAAAGTAGAAAGCACTACAA	56
gga-miR-205a	F: CCTTCATTCCACCGGAGTCTG	56
gga-miR-222b-5p	F: GCTCAGTAGTCAGTGTAGGATCTG	56
U6	F: GCAGGGCCATGCTAATCTTCTCTGTATCG	56
GHR	F: TTCAACACATCCTACACCTCG R: TGGTGGTGGATCCCATCGTA	60
GAPDH	F: TATGATTCTACACACGGAC R: ATGGTGGTGAAGACACCAGTG	60

F means forward primer; R means reverse primer.

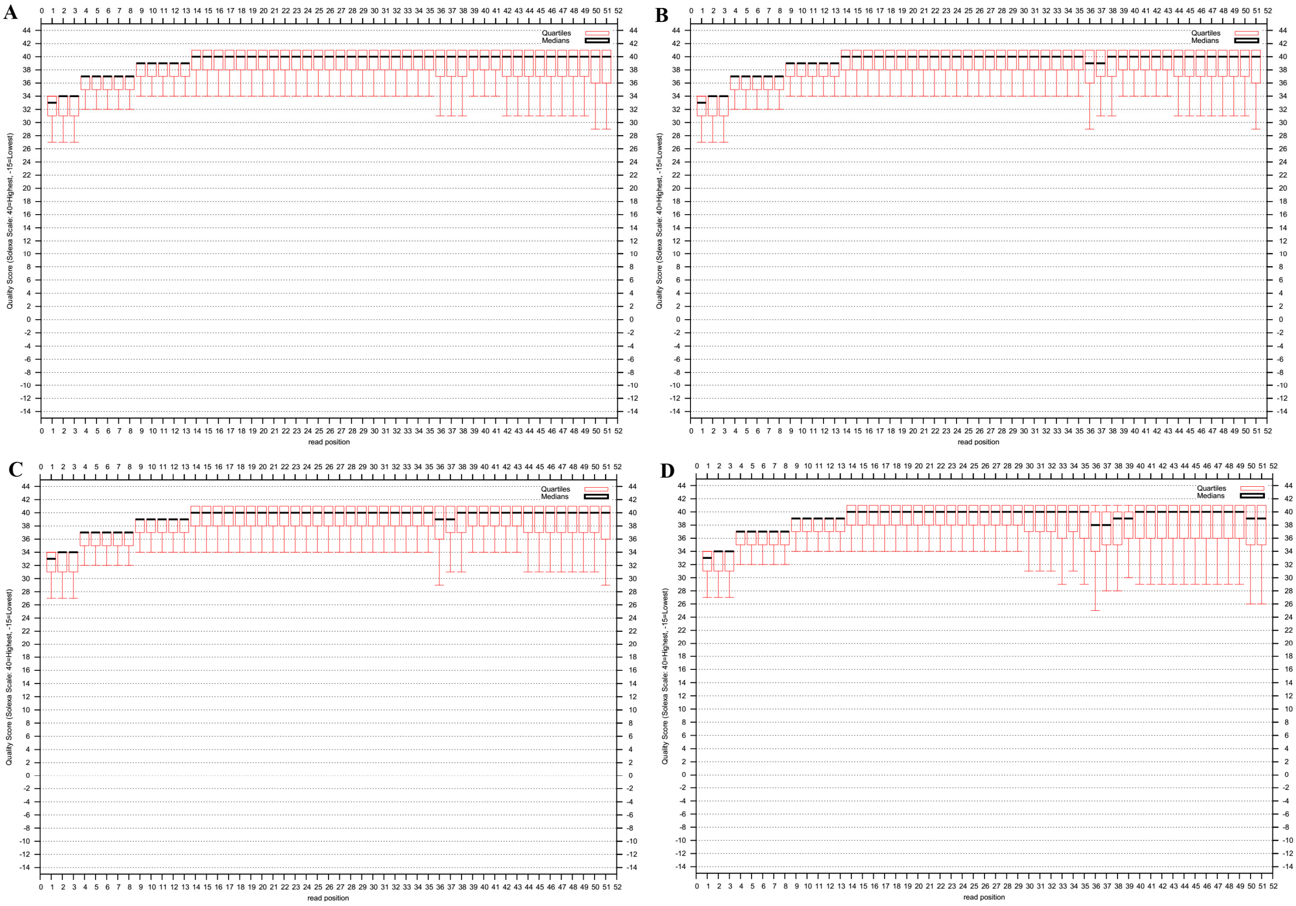
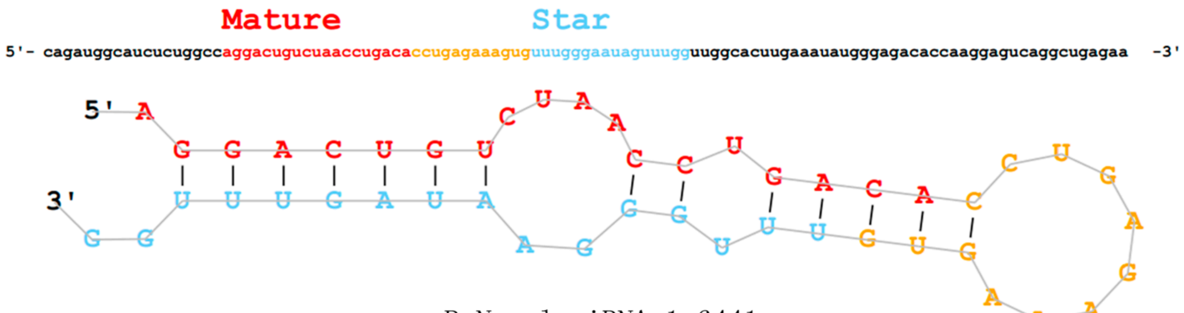


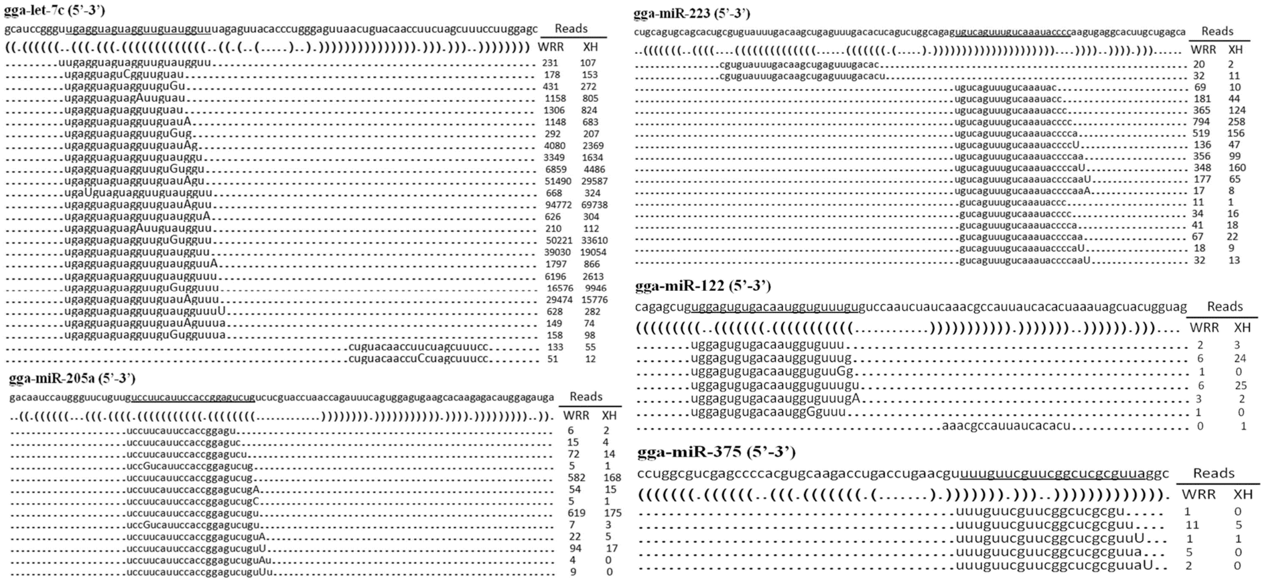
Figure S1. The quality data for RNA samples. (**A**) WRRh (Recessive White Rock with high body weight); (**B**) WRRl (Recessive White Rock with low body weight); (**C**) XHh (Xinhua Chickens with high body weight); (**D**) XHl (Xinhua Chickens with low body weight). Horizontal axis means base of reads 1–51 from 5′ to 3′; vertical axis means quality value of base. Red boxes means quartiles of reads quality, and black bold lines means medians of reads quality. The higher the medians, the lower the error rate of sequencing.

A Novel miRNA 15_2275



B Novel miRNA 1_6441

Figure S2. Predicted secondary structures of two most abundant novel miRNAs. The red color indicated the mature sequence, the yellow color indicated the loop sequence, and the blue color indicated the predicted star sequence.



gga-let-7c (5'-3')

```
gcauccggguugagguaguagguuguaugguuuagaguuacacccugggaguuaacuguacaaccuucuagcuuuccuuggagc   Reads
((.((((((..(((.(((.(((((((((((((..((.(..(.....)..).))))))))))))))).))).)))..))))))))   WRR    XH
.........uugagguaguagguuguaugguu....................................................   231    107
..........ugagguaguCgguuguau........................................................   178    153
..........ugagguaguagguuguGu........................................................   431    272
..........ugagguaguagAuuguau........................................................   1158   805
..........ugagguaguagguuguau........................................................   1306   824
..........ugagguaguagguuguauA.......................................................   1148   683
..........ugagguaguagguuguGug.......................................................   292    207
..........ugagguaguagguuguauAg......................................................   4080   2369
..........ugagguaguagguuguauggu.....................................................   3349   1634
..........ugagguaguagguuguGuggu.....................................................   6859   4486
..........ugagguaguagguuguauAgu.....................................................   51490  29587
..........ugaUguaguagguuguaugguu....................................................   668    324
..........ugagguaguagguuguauAguu....................................................   94772  69738
..........ugagguaguagguuguauagguA...................................................   626    304
..........ugagguaguagAuuguaugguu....................................................   210    112
..........ugagguaguagguuguGugguu....................................................   50221  33610
..........ugagguaguagguuguaugguu....................................................   39030  19054
..........ugagguaguagguuguaugguuA...................................................   1797   866
..........ugagguaguagguuguaugguuu...................................................   6196   2613
..........ugagguaguagguuguGugguuu...................................................   16576  9946
..........ugagguaguagguuguauAguuu...................................................   29474  15776
..........ugagguaguagguuguaugguuuU..................................................   628    282
..........ugagguaguagguuguauAguuua..................................................   149    74
..........ugagguaguagguuguGugguuua..................................................   158    98
.......................................................cuguacaaccuucuagcuuucc.......   133    55
.......................................................cuguacaaccuCcuagcuuucc.......   51     12
```

gga-miR-205a (5'-3')

```
gacaauccaugguucuguuguccuucauuccaccggagucugucucguaccuaaccagauuucaguggagugaagcacaagagacauggagauga   Reads
..((.((((((...(((.((((.(((((((((((.((((((((............)))))))).))))))))))).)))).)))))))))..)).   WRR  XH
....................uccuucauuccaccggagu........................................................   6    2
....................uccuucauuccaccggaguc.......................................................   15   4
....................uccuucauuccaccggagucu......................................................   72   14
....................uccGucauuccaccggagucug.....................................................   5    1
....................uccuucauuccaccggagucug.....................................................   582  168
....................uccuucauuccaccggagucugA....................................................   54   15
....................uccuucauuccaccggagucugC....................................................   5    1
....................uccuucauuccaccggagucugu....................................................   619  175
....................uccGucauuccaccggagucugu....................................................   7    3
....................uccuucauuccaccggagucuguA...................................................   22   5
....................uccuucauuccaccggagucuguU...................................................   94   17
....................uccuucauuccaccggagucuguAu..................................................   4    0
....................uccuucauuccaccggagucuguUu..................................................   9    0
```

gga-miR-223 (5'-3')

```
cugcagugcagcacugcguguauuugacaagcugaguuugacacucagucuggcagagugucaguuugucaaauaccccaagugaggcacuugcugagca   Reads
..(((((((..((((....((((((((((((((......(((((((.(.....).))))))))))))))))))))))....))))..)))).))).....   WRR  XH
................cguguauuugacaagcugaguuugacac........................................................   20   2
................cguguauuugacaagcugaguuugacacu.......................................................   32   11
..........................................................ugucaguuugucaaauac........................   69   10
..........................................................ugucaguuugucaaauacc.......................   181  44
..........................................................ugucaguuugucaaauaccc......................   365  124
..........................................................ugucaguuugucaaauacccc.....................   794  258
..........................................................ugucaguuugucaaauacccca....................   519  156
..........................................................ugucaguuugucaaauaccccU....................   136  47
..........................................................ugucaguuugucaaauaccccaa...................   356  99
..........................................................ugucaguuugucaaauaccccaU...................   348  160
..........................................................ugucaguuugucaaauaccccaaU..................   177  65
..........................................................ugucaguuugucaaauaccccaaA..................   17   8
...........................................................gucaguuugucaaauaccc......................   11   1
...........................................................gucaguuugucaaauacccc.....................   34   16
...........................................................gucaguuugucaaauacccca....................   41   18
...........................................................gucaguuugucaaauaccccaa...................   67   22
...........................................................gucaguuugucaaauaccccaU...................   18   9
...........................................................gucaguuugucaaauaccccaaU..................   32   13
```

gga-miR-122 (5'-3')

```
cagagcuguggagugugacaaugguguuuguguccaaucuaucaaacgccauuaucacacuaaauagcuacugguag   Reads
((((((((..(((((((.((((((((((............))))))))))).)))))))..)))))).)))....   WRR  XH
........uggagugugacaaugguguuu................................................   2    3
........uggagugugacaaugguguuug...............................................   6    24
........uggagugugacaauggguguuGg..............................................   1    0
........uggagugugacaaugguguuugu..............................................   6    25
........uggagugugacaaugguguuugA..............................................   3    2
........uggagugugacaauggGguuu................................................   1    0
...........................................aaacgccauuaucacacu................   0    1
```

gga-miR-375 (5'-3')

```
ccuggcgucgagccccacgugcaagaccugaccugaacguuuuguucguucggcucgcguuaggc   Reads
(((((((.((((((..(((.((((((((.(.......).)))))))).)))..))))))))))))).   WRR  XH
.........................................uuguucguucggcucgcgu.....   1    0
.........................................uuguucguucggcucgcguu....   11   5
.........................................uuguucguucggcucgcguuU...   1    1
.........................................uuguucguucggcucgcguua...   5    0
.........................................uuguucguucggcucgcguuaU..   2    0
```

Figure S3. Various isomiRs of five gga-miRs. Reads alignments of the various isoforms of five gga-miRs are presented. The sequence of the gga-miR hairpin is presented in the top line, and underline letter means mature sequence; the brackets below denote the secondary structure. Reads that aligned with the mature gga-miR sequence are denoted by a series of dots and the mutations are denoted by uppercase. The number of reads for each sequence is presented on the right.