Supplementary Data

1. Supplemental Method and Discussion
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Supplemental Method/Functional group assembly and GO analysis

Genes were annotated by gene ontology functional classification (1). Each gene-to-GO term association was mapped to a GO Slim association. To examine which functional categories are highly targeted by multiple miRNAs, for a functional group, we defined the target percentage as the number of target genes within this group dividing that of the total annotated target genes on a certain seed number. Only seed numbers including at least 5 target genes were considered. Then we calculated the Pearson correlation coefficient between target percentages and seed numbers. We defined the highly targeted category as a functional group which was found with significant positive correlation and the rarely targeted functional category as a functional group which was found with significant negative correlation. The set of functional groups was restricted to those including 100~2100 genes.

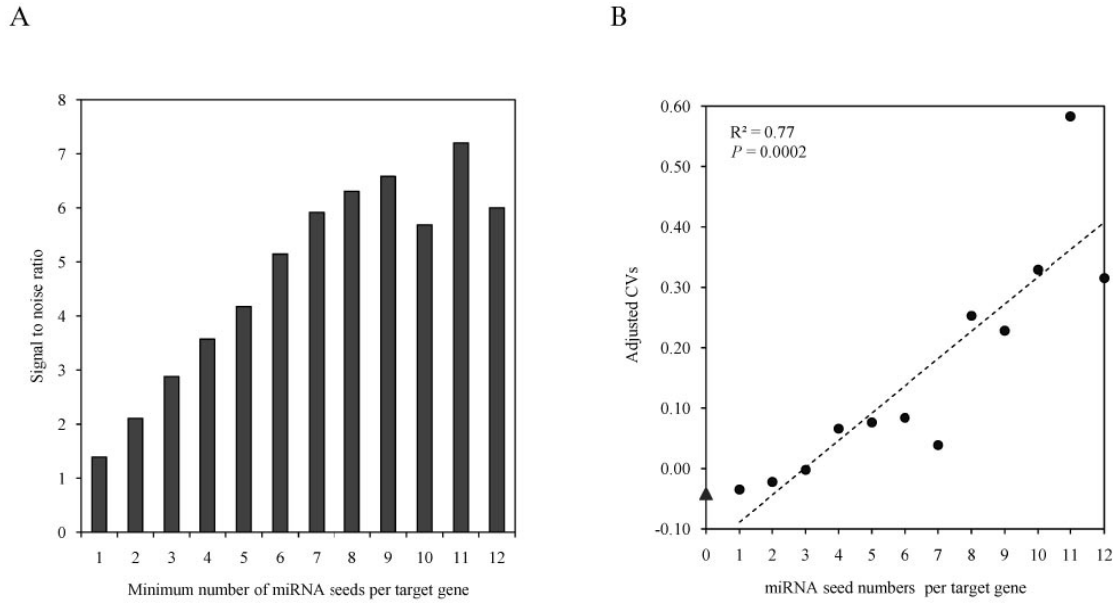
Supplemental Discussion/Gene ontology (GO) analysis

To examine whether the increased variability of target genes can be explained by gene functional categories, we assembled genes into functional groups, and compared the average CVs of target genes and non-miRNA-target genes within each functional group. In most groups (30/38), the average CVs of the target genes are higher than that of the non-miRNA-target genes (Supplemental Fig. 5A). For example, target genes encoding proteins localized to nucleus and target genes that function in structural molecule activity have high expression variability compared with non-miRNA-target genes within the same functional groups. This result indicates that the increased expression variability of target genes is not related to certain functional

groups, but directly to miRNAs.

Although the CV differences between target genes and non-miRNA-target genes are not related with functional groups, there are functional groups showing over-representation of target genes recognized by multiple miRNA seeds. For example, the functional categories in binding, metabolism and regulation of biological process are highly abundant for genes recognized by multiple miRNAs (Supplemental Fig. 5B&C). In contrast, the categories in diversified enzyme activities (such as oxidoreductase, kinase, hydrolase, transferase and ligase) do not show such pattern. Surprisingly, the categories in binding and metabolism take 93% and 80% of the highly regulated target genes (recognized by more than 7 seeds). This observation suggests that the cooperation of miRNAs tends to be enriched in certain functional categories, which may contribute to the different variability between functional categories.

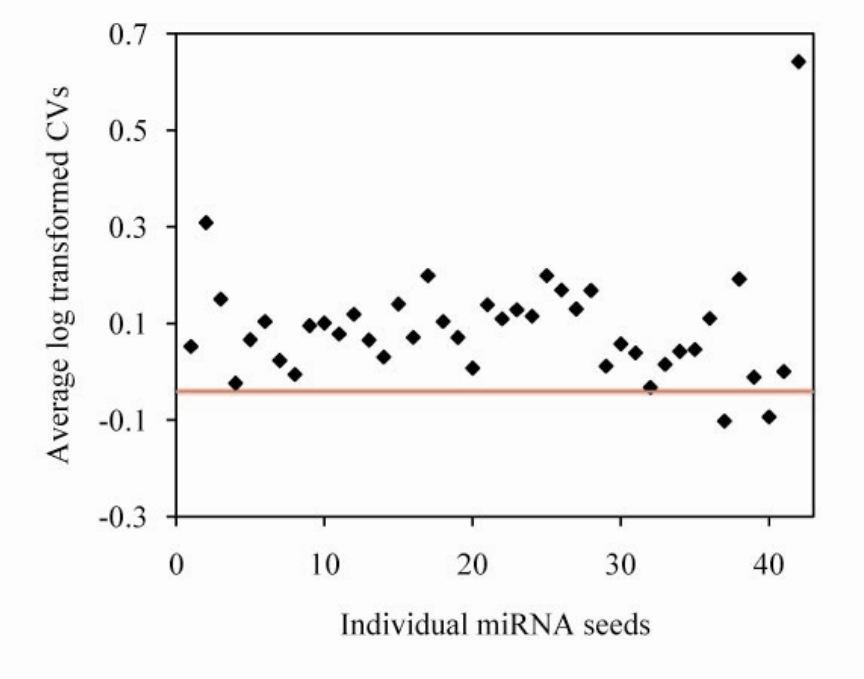
Supplemental Figures and Tables



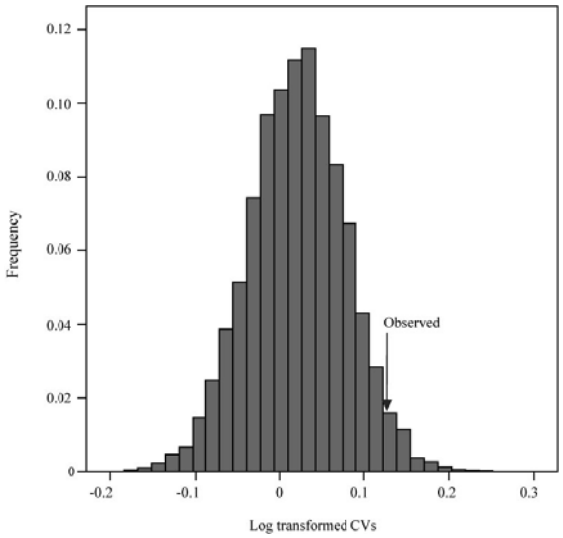
Supplemental Figure 1. Adjusting CVs by signal-to-noise ratio does not affect the positive correlation between miRNA seed numbers and CVs. (A) The ratios of the number of transcripts with at least *n* seeds for real versus random seeds. (B) The correlation between miRNA numbers per target gene and the adjusted CVs. Adjusted average CVs were calculated using the following formula:

$$C_{non} + (R_i - 1)X_i = R_i C_i$$

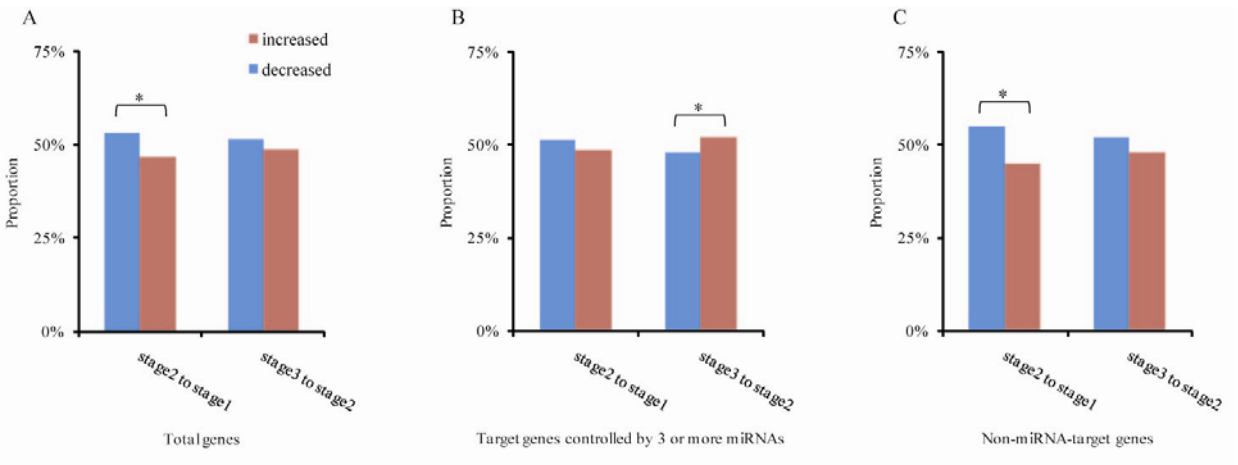
C_{non} is the average CV of the non-miRNA-target genes. R_i is the signal-to-noise ratio of target genes recognized by minimum number of seeds *i*. X_i is the adjusted average CV of group *i*. C_i is the original CV of group *i* .The average CV of the non-miRNA-target genes is indicated in Y axis by triangle. The dash line indicates the linear least square regression line. The R square and *P* value are indicated.



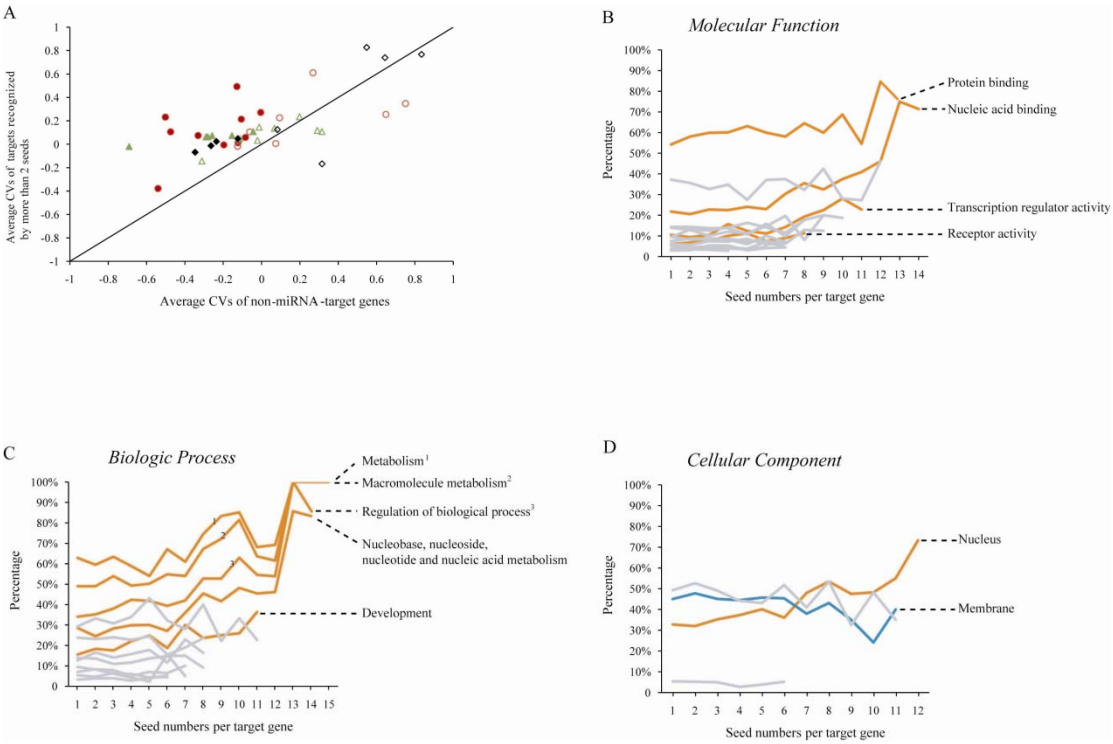
Supplemental Figure 2. Average log transformed CVs of target genes (recognized by three or more seeds) for each miRNA seed. The red line represents the average log transformed CV of non-miRNA-target genes.



Supplemental Figure 3. The average CV of SNP-residing targets is 0.116, slightly larger than that of non-SNP target genes (P = 0.047, probability of the observed CV compared with the normal distribution of the randomly sampled dataset. (see Methods).



Supplemental Figure 4. The proportion of genes with increased or decreased CVs between different age stages. stage1, the young old (65-74); stage2, the middle old (75-84); stage3, the oldest old (those aged 85 and above).* $P < 0.05$ with Two-Tailed Wilcoxon Signed Ranks Test.



Supplemental Figure 5. Gene ontology analysis. (A) The average CVs of target genes recognized by more than 2 seeds versus non-miRNA-target genes within each functional group. The diagonal line indicates equal CVs of the two categories. The red/green/black dots refer to molecular function, biologic process and cellular component respectively. The set of functional groups was restricted to those including at least twenty target genes and at least twenty non-miRNA-target genes. Functional groups with a significantly increased variability of targets (false discovery rate < 0.05 in a Two-Sample T Test) are indicated by solid symbols, and the rest are indicated by open symbols. (B-D) Plot of the percentage of target genes recognized by n seeds within each functional categories. The orange line represents functional categories

highly targeted by multiple miRNAs and the blue line represents functional categories rarely targeted by multiple miRNAs.

Supplemental Table 1. The list of conserved miRNA seeds.

Mature miRNA names	Seed region (position 1~8)
hsa-let-7a/hsa-let-7b/ hsa-let-7c/hsa-let-7e/hsa-let-7i	TGAGGTAG
hsa-let-7d	AGAGGTAG
hsa-miR-100/hsa-miR-99a	AACCCGTA
hsa-miR-103/hsa-miR-107	AGCAGCAT
hsa-miR-124	TAAGGCAC
hsa-miR-125a-5p/hsa-miR-125b	TCCCTGAG
hsa-miR-126	TCGTACCG
hsa-miR-127-3p	TCGGATCC
hsa-miR-128a/hsa-miR-128b	TCACAGTG
hsa-miR-129-5p	CTTTTTGC
hsa-miR-139-5p	TCTACAGT
hsa-miR-140-5p	CAGTGGTT
hsa-miR-143	TGAGATGA
hsa-miR-145	GTCCAGTT
hsa-miR-149	TCTGGCTC
hsa-miR-15a/hsa-miR-16	TAGCAGCA
hsa-miR-181a/hsa-miR-181b	AACATTCA
hsa-miR-185	TGGAGAGA
hsa-miR-191	CAACGGAA
hsa-miR-196a	TAGGTAGT
hsa-miR-204	TTCCCTTT
hsa-miR-20a	TAAAGTGC
hsa-miR-21	TAGCTTAT
hsa-miR-218	TTGTGCTT
hsa-miR-221/hsa-miR-222	AGCTACAT
hsa-miR-23a/hsa-miR-23b	ATCACATT
hsa-miR-24	TGGCTCAG
hsa-miR-25	CATTGCAC
hsa-miR-26a	TTCAAGTA
hsa-miR-27a/hsa-miR-27b	TTCACAGT
hsa-miR-28-5p	AAGGAGCT
hsa-miR-29a/hsa-miR-29b	TAGCACCA
hsa-miR-30a*	CTTTCAGT
hsa-miR-30a/hsa-miR-30b/ hsa-miR-30c/hsa-miR-30d	TGTAAACA
hsa-miR-31	AGGCAAGA
hsa-miR-34a	TGGCAGTG
hsa-miR-451	AAACCGTT
hsa-miR-9	TCTTTGGT
hsa-miR-92a	TATTGCAC
hsa-miR-93	CAAAGTGC
hsa-miR-96	TTTGGCAC
hsa-miR-99b	CACCCGTA

Supplemental Table 2. The relationship between 3'UTR length and CVs. For target genes targeted by the same miRNA seed number, we calculated the Spearman's rho between 3'UTR length and CVs of each gene.

miRNA seed numbers	ρ	P
1	0.094	0.00032**
2	0.1	0.0020**
3	0.007	0.87
4	-0.01	0.81
5	0.11	0.078
6	-0.02	0.76
7	-0.072	0.42
8	0.14	0.27
9	-0.12	0.4
10	0.31	0.067
11	-0.044	0.84
12	0.12	0.66

** $P < 0.01$

Supplemental Table 3. The relationship between 3'UTR length and CVs. For target genes with the same miRNA binding site number, we calculated the Spearman's rho between UTR length and CVs of each gene.

miRNA binding site number	ρ	P
1	0.092	0.00048**
2	0.099	0.0021**
3	0.0066	0.87
4	-0.036	0.46
5	0.122	0.039*
6	-0.076	0.28
7	0.055	0.5
8	0.026	0.81
9	-0.15	0.25
10	0.061	0.69
11	0.022	0.9
12	0.51	0.019*
13	-0.21	0.34
14	-0.032	0.91
15	0.28	0.4

** $P < 0.01$

* $P < 0.05$

Supplemental Table 4. Comparison of gene expression variability between miRNA target genes and non-miRNA-target genes using two additional predicting methods.

	TargetScan method		PITA method	
	Tar1-2 / Non	Tar>2 / Non	Tar1-2 / Non	Tar>2 / Non
Median	0.076 / 0.073	0.08 / 0.073	0.077/0.070	0.082/0.070
P value[#]	0.36	0.02*	0.004*	0.000011*

Tar1-2, target genes recognized by 1-2 miRNA seeds; Tar>2, target genes recognized by 3 or more miRNA seeds; Non, non-miRNA-target genes; [#] Based on Two-Tailed Mann-Whitney Test. For PITA method, the target genes recognized by 1-2 miRNA seeds also have an increased CVs compared with the non-miRNA-target genes. This might be explained by the more accurate prediction of this method compared with other existing algorithms. *$P < 0.05$

Reference:

1. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T. *et al.* (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet*, 25, 25-29.