MicroRNAs tend to synergistically control genes encoding extensively-expressed proteins in humans

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Theoretically, single microRNA (miRNA) targeting plenty of genes makes miRNA-mediated regulation high-throughput but insufficient due to target gene dilution effect. Considering complicated miRNA biogenesis and action mechanisms, it was thought so high energy-consuming for a cell to afford simultaneous overexpression of many miRNAs. Thus it prompts that an alternative miRNA regulation pattern on protein-encoding genes must exist, which has characteristics of energy-saving and precise protein output. In this study, expression tendency of proteins encoded by miRNAs' target genes was evaluated at human organ scale, followed by quantitative assessment of miRNA synergism. Expression tendency analysis suggests that universally expressed proteins (UEPs) tend to physically interact in clusters and participate in fundamental biological activities whereas disorderly expressed proteins (DEPs) would like to relatively independently execute organ-specific functions. Consistent with this, miRNAs that mainly target UEP-encoding genes, such as miR-21, tend to collaboratively or even synergistically act with other miRNAs in fine-tuning protein output. Synergistic gene regulation may maximize miRNAs' efficiency with less dependence on miRNAs' abundance and overcome the deficiency of single miRNA-mediated gene regulation mentioned above. Furthermore, our *in vitro* experiment verified that merely 25 nM transfection of miR-21 be enough to influence the overall state of various human cells. Thus miR-21 was identified as a hub in synergistic miRNA-miRNA interaction network. Our findings suggest that synergistic miRNA-miRNA interaction is an important endogenous miRNA regulation mode, which ensures adequate potency of miRNAs at low abundance, especially those implicated in fundamental biological regulation.

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ABSTRACT

Theoretically, single microRNA (miRNA) targeting plenty of genes makes miRNA-mediated regulation high-throughput but insufficient due to target gene dilution effect. Considering complicated miRNA biogenesis and action mechanisms, it was thought so high energy-consuming for a cell to afford simultaneous overexpression of many miRNAs. Thus it prompts that an alternative miRNA regulation pattern on protein-encoding genes must exist, which has characteristics of energy-saving and precise protein output. In this study, expression tendency of proteins encoded by miRNAs' target genes was evaluated at human organ scale, followed by quantitative assessment of miRNA synergism. Expression tendency analysis suggests that universally expressed proteins (UEPs) tend to physically interact in clusters and participate in fundamental biological activities whereas disorderly expressed proteins (DEPs) would like to relatively independently execute organ-specific functions. Consistent with this, miRNAs that mainly target UEP-encoding genes, such as miR-21, tend to collaboratively or even synergistically act with other miRNAs in fine-tuning protein output. Synergistic gene regulation may maximize miRNAs' efficiency with less dependence on miRNAs' abundance and overcome the deficiency of single miRNA-mediated gene regulation mentioned above. Furthermore, our *in vitro* experiment verified that merely 25 nM transfection of miR-21 be enough to influence the overall state of various human cells. Thus miR-21 was identified as a hub in synergistic miRNA-miRNA interaction network. Our findings suggest that synergistic miRNA-miRNA interaction is an important endogenous miRNA regulation mode, which ensures adequate potency of miRNAs at low abundance, especially those implicated in fundamental biological regulation.

INTRODUCTION

MicroRNAs (miRNAs) belong to a super-family of ~22 nucleotides single-stranded non-coding RNA molecules, which are extensively implicated in pathophysiological activities (Bartel, 2009; Shukla et al., 2011). After a long biogenesis process, functionalities of miRNAs reply on their interaction with argonaute proteins and co-assembling into RNA-induced silencing complex (RISC). Despite energy-consuming, such a unique gene regulation pattern may be of great significance to human cells owing to their macro roles in biological regulation (Adlakha & Seth, 2017; Park et al., 2016; Srinivasan & Das, 2015).

One miRNA usually physically interacts with hundreds of target genes (Zhou and Yang, 2012), suggesting the advantage of high-throughput and integrative gene regulation (Liu et al., 2016). However, it was also suggested that inherent deficiency should be not ignored regarding the operation of a 'one to many' system like this. Targeting hundreds of genes undoubtedly dissipates the efficacy of the miRNA-RISC machine due to the abundance dilution effect of target genes (Arvey et al., 2010). Undoubtedly, a comprehensive and effective regulation covering all target genes needs high miRNA output with sufficient RISC machines in which mature miRNA is embedded. Such a strategy may be uneconomical for widespread adoption due to excessive occupancy of cellular energy and material. Actually, the completed human miRNA expression profiles have revealed that only a very small portion of miRNAs can afford this way, such as miR-1 that is highly expressed in heart and miR-122a that is highly expressed in liver (Ritchie et al., 2010). Just recently, it has been revealed that ~10-15% of human miRNAs are tissue-specific, replying limited cell load capacity for miRNAs (Ludwig et al. 2016).

Indirect and non-physical interactions exist among miRNAs, which constitutes the basis of the overall effect of miRNA-mediated gene regulation. Several possible patterns of miRNA-miRNA interactions

(MMIs) were revealed by far. As one miRNA can physically interact with hundreds of target genes, this causes with a great probability that different miRNAs may competitively bind the 3'-untranslated region of the same gene. Competition for binding is the main and fundamental pattern of miRNA-miRNA interactions (MMIs) in the miRNA world (Jens and Rajewsky, 2015). Expect the competition pattern, different miRNAs may show cooperativity due to being co-regulated by the same transcription factor and targeting genes with functional interconnections (Na and Kim, 2013; Shi et al., 2013; Guo et al., 2014). Restricted MMI is another pattern that two miRNAs have completely or partially complementary structures and constitute an endogenous sense and antisense miRNA pair (Guo et al., 2014). Great expression difference of the two miRNAs in a pair was a major feature of this pattern.

Based on our previous knowledge of miRNA regulation (Yuan et al., 2015; Zhu et al., 2011; Zhu et al., 2013), we proposed that miRNAs might adopt an alternative pattern of MMI that promotes a manner of more economical and efficient gene regulation in response to real-time adjustment of cellular signals. It less depends on miRNA abundance and more rely on synergistic miRNA-miRNA collaboration for fine-tuning protein output (Skommer et al., 2014). On the whole, synergistic gene regulation may optimize the regulatory efficacy of miRNAs (Xu et al., 2011). Importantly, it is less expensive in cellular energy consumption compared to strengthened miRNA expression. In order to justify this hypothesis, we performed a quantitative assessment of miRNA synergism by calculating miRNA synergy score (Zhu et al., 2013). Benefited from the increasingly clear human protein expression atlas (Uhlén et al., 2015), this method allows us explore potential principles in miRNA regulation at the dimension of human organs. Additionally, *in vitro* experiment was performed to show the potential advantage of miRNA synergy by using miR-21 and miR-133a as comparison example. Such efforts aim to shed novel insights into the

biological significance of miRNA-miRNA collaboration and provide implications in better understanding the existence of miRNAs in humans.

MATERIALS AND METHODS

MiRNA-target interactions (MTIs) and protein expression

Three datasets miRecords (Xiao et al., 2009), miRSel (Naeem et al., 2010) and ExprTargetDB (Gamazon et al., 2010) were used for obtaining reliable MTIs in humans. MiRSel is a collection of literature evidence of MTIs. MiRecords and ExprTargetDB belong to secondary tools for MTI prediction, in which integration of different algorithms led to reliable MTI identification. Finally, all MTI data were merged for further analysis. The organ protein expression data was retrieved from the human protein atlas (HPA) database (Uhlén et al., 2015). Twelve human organs were included: breast and female reproductive system (BFS), blood and immune system (BIS), central nervous system (CNS), cardiovascular system (CVS), digestive tract (DT), endocrine glands (EG), liver and pancreas (LP), male reproductive system (MS), placenta (P), respiratory system (RS), skin and soft tissues (SS) and urinary tract (UT). A resilient fraction threshold was adopted for expression verification. Specifically, the fraction threshold was set at 70% for BFS, BIS, CNS, LP and SS; 90% for DT; 100% for CVS, EG, MS, P, RS and UT. The information of the level of annotated protein expression was downloaded and imported into Cytoscape v2.8.3 (Smoot et al., 2011). Notably, the validated marks ‘none’, ‘low’, ‘medium’ and ‘high’ representing proteins expression levels were in advance converted into the digitals 0, 1, 2 and 3, respectively. Afterwards, coefficient of variation (CV) was used to evaluate the cellular expression dispersion degree of each miRNA target gene-encoded protein.

Synergy score and skewness

92 Potential degree of synergistic collaboration between miRNAs was quantitatively assessed by calculating
93 miRNA synergy score as described before (Zhu et al., 2013). For each miRNA, the statistics parameter
94 skewness was used to evaluate the distribution inclination of the log_2CV values of protein expression and
95 determine the regulatory tendency of the miRNA (Mardia, 1970). We used log_2CV instead of CV for
96 skewness calculation, as the distribution of CVs did not pass D'Agostino & Pearson omnibus normality test
97 (D'Agostino and Pearson, 1973). A smaller skewness value means a tendency of regulating genes encoding
98 uniformly expressed proteins (UEPs); however a higher skewness value suggests that a miRNA tend to
99 fine-tune disorderly expressed proteins (DEPs). File S1 was a four-step protocol to reveal miRNAs that
100 tend to regulate UEP-encoding genes. In this study, UEPs and DEPs refer to proteins with CVs of less than
101 40% and CVs of more than 120%, respectively.

102 **Gene ontology (GO) and network topology**

103 A comparison between UEPs and DEPs was investigated from the GO aspect by applying the online
104 DAVID functional annotation tool (Huang et al., 2009). Briefly, the gene official symbols were submitted
105 as gene list and 'Homo Sapiens' chosen as background. Over-represented GO biological processes, cellular
106 components and molecular functions were considered to be significant only if false discovery rate (FDR) <
107 0.05. We also investigated the network topology characteristics of UEPs and DEPs, respectively. The
108 Cytoscape plugins BisoGenet [18] was used for retrieving, trimming, and analyzing experimentally
109 validated protein-protein interactions (PPIs).

110 **Cell culture and miRNA transfection**

111 Human umbilical vein endothelial cell line (HUVEC), breast cancer cell line (MCF7) and hepatic carcinoma
112 cell line (HepG2) were all obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy

of Science (Shanghai, China). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. After that, cells were incubated at 37 °C with 5% CO_2 and 95% air. 24 hours before transfection, cells were transferred to 96-well plates and cultured in fresh medium without antibiotics, According to the manufacturer's instructions, X-treme GENE siRNA transfection reagent (Roche, Switzerland) was used for mono-transfection of negative control or miRNA mimic at 25 nM. Table S1 listed the sequences of the negative control and miRNA mimics used in this study.

Cell viability assay

Cell viability was assessed by measuring mitochondrial dehydrogenase activity, using the colorimetric MTT assay, based on the fact that viable cells (but not dead cells) can reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). After miRNA transfection 24 hours, with or without hypoxia treatment, cells were incubated with MTT of 5 mg/ml at 37 °C for four hours. Hypoxia was induced by exposing cells to hypoxic condition (1% O_2, 94% N_2, 5% CO_2) for 24 hours using a modular incubator. The purple formazan crystal was dissolved with 150 μ L of dimethyl sulfoxide (DMSO) and added to the cells. The absorbance was measured at 490 nm.

Data statistics

All data are expressed as mean ± SEM (Standard Error of Mean). Statistical analysis was performed with Mann–Whitney *U* test or one-way ANOVA (analysis of variance) followed by Tukey's test for multiple comparisons. Differences were only considered to be significant at $p < 0.05$.

RESULTS

Data collecting and screening

Initial data integration identified a total of 11162 target genes and 69618 MTIs for 472 miRNAs from the three databases including miRecords, miRSel, and ExprTargetDB. Only 166 of were retained for further analysis, each of which targeted at least 50 mRNA genes in each of the 12 human organs (Table S2). Totally, they were functionally associated with more than 6328 proteins via 36211 MTIs.

Comparison of UEPs and DEPs in organ expression, network topology and GO terms

After retrieving the protein expression data from the HPA database, we calculated the CV value for each of the 6328 proteins regarding its HPA-defined abundance ranks in human cells. The majority of the proteins were located with the CV range from 40% to 120% (Figure 1A). This distribution characteristic was more clearly observed after log2 transformation of CVs. Totally, we identified 1340 UEPs and 1115 DEPs. Unlike DEPs, UEPs tend to be more uniformly expressed in human organs, have more interacting neighbors, and compose of closer associations with each other in clusters (Figures 1B-D). Comparably, DEPs are not ubiquitously expressed, and more marginalized in the human PPI network and execute functions in isolation. More than these, GO analysis revealed that UEPs tend to be intensively involved in gene transcription and related biological processes, be located in the nucleus or nearby, and play roles in the housekeeping functions such as being transcription regulators (Table S3). This result indicates the important role of UEPs in the maintenance of basic cell activities. In contrast, DEPs are more implicated in organ-specific biological processes and perform molecular functions at plasma membrane and cytoplasmic organelles.

MiRNAs' regulation tendency on genes encoding UEPs or DEPs is independent of miRNA expression

155 We investigated the difference of UEPs and DEPs from the miRNA regulation aspect. UEP-encoding genes
156 instead of those encoding DEPs are averagely under more dense control of miRNAs in the whole genome
157 context (Figure 2A). Even when MTIs are restricted into one organ such as CVS, the preference of miRNAs
158 for UEP-encoding genes is also obvious to observe (Figure 2B). The highest skewness value highlighted
159 miR-133a of its definite trend in regulating DEP-encoding genes in CVS (Figure 2C). Both miR-1 and 133a
160 are cardiac-specific miRNAs. However, we found that they showed different tendencies on gene regulation
161 (Figure 2D). This result suggests that regulating organ-specific genes may be not a necessary condition for
162 the organ-specific functions of miRNAs, such as miR-1 (Yang et al., 2007).

163 **MiRNAs regulate UEP-encoding genes in a synergistic pattern**

164 MiRNAs may act synergistically with each other (Xu et al., 2011). With the evolution of the complexity of
165 biological system, synergism is undoubtedly more advantageous than isolated regulation in terms of
166 management strategy (Corning, 1995; Stelling et al., 2004). Altered expression of only a few numbers of
167 miRNAs caused systemic changes via disrupting synergistic associations between miRNAs (Figures 3A-
168 C). If all possible miRNA pairs are supposed to be synergistic 75.5% of synergistic miRNA interactions
169 will be affected only when half of all the miRNAs undergo altered expression (Figure 3D). In particular, to
170 those the hubs in the miRNA-miRNA synergistic network, adaptation of this management strategy may be
171 more energy-saving due to plenty of synergistic partners (Figure 3E). Further result indicates that miRNAs
172 with small skewness values constitute most of the synergistic miRNA-miRNA associations in human
173 organs (Figure 4). This finding implies that the molecular behaviors of UEPs, rather than those of DEPs,
174 are under the surveillance of a dense synergistic miRNA regulation network. In this invisible network, miR-
175 21 should be paid more attention owing to its more powerful and extensive collaboration with other

176 miRNAs (Figure 5). Compared with other miRNAs, miR-21 that both tends to regulate UEP-encoding
177 genes and act synergistically with other miRNAs really had significant impact on the whole cell state of the
178 three cell lines at a mono-transfection concentration of 25 nM (Figure 6). Furthermore, miR-133a tends to
179 regulate DEP-encoding genes implying its relatively isolated action, our *in vitro* experiment verified its
180 failing to influence the whole cell state of the three cell lines.
181

182 DISCUSSION

183 The new discovery of miRNAs and other kinds of noncoding RNAs greatly consolidates the dominant
184 position of genes in regulating cellular activities (Cech and Steitz, 2014**)**. Genes are not only carriers and
185 messengers of genetic information, but also direct supervisors on whether such information is accurately
186 translated into phenotypes. Despite lot of efforts still it is not clear about the regulation layer that is
187 constituted by thousands of miRNAs by now (Boettger and Braun, 2012; Stroynowska-Czerwinska et al.,
188 2014). In this study, a system-level insight was attempted to shed into the overall effect of miRNAs from
189 the perspective of miRNA-miRNA collaboration.
190 It was confirmed that abundant expression of target genes produces the abundance dilution effect for
191 miRNA-mediated regulation (Arvey et al., 2010). Denzler and his colleagues further proved that the overall
192 abundance of target genes is generally higher than that of the corresponding miRNA (Denzler et al., 2014).
193 This causes insensitive influence of competitive endogenous RNA (ceRNA)-mediated derepression on
194 miRNA activity (Denzler et al., 2016). The above findings imply that miRNAs may do not perform
195 functions in isolation and collaboration of different miRNAs is a means of effective regulation of gene
196 expression.

Functional studies have confirmed that miRNAs might act synergistically, strongly suggesting collaborative gene regulation (Huang et al., 2016; Raut et al., 2016; Xue et al., 2016). The above evidence is well consistent with the conclusion inferred by the systems biology analyses performed before (Xu et al., 2011; Zhu et al., 2013). Extensive miRNA-miRNA synergism may exist in the miRNA layer. Precisely because of this, slight adjustment of miRNAs' abundance can cause apparent variation in the state of cells. Synergistic miRNAs action allows cells to adjust their live-or-death status more flexibly and rigorously to adapt to internal and external alternation. Due to acting synergistically with other miRNAs, low concentration transfection of miR-21 showed obvious impact on the viability of all of the studied cell lines. This finding also implies that altered miRNA expression may not only independently affect the translation of its own target gene products but also produce a radiative impact on other miRNAs that have synergistic associations with it. Put briefly, miRNA synergy amplifies the effects of miRNA expression alteration.

Functional execution of miRNAs requires high cellular energy consumption as a prerequisite (Shukla et al., 2011). Besides of miRNA regulatory crosstalk (Jens and Rajewsky, 2015), miRNA synergy may represent an alternative pattern of energetically optimal miRNA-mediated regulation of post-transcriptional gene expression. However, we supposed that this obvious advantage brought by miRNA synergy might rely heavily on the complexity of biological system. As miRNA synergy score calculation revealed, only dense PPIs between target gene products could lead to meaningful miRNA synergy (Zhu et al., 2011). This explains well that one can expect strong synergistic potential of the cardic-specific miRNA miR-1 instead of miR-133a. Our findings indicate that miR-1 tends to regulate UEP-encoding genes whereas miR-133a tends to regulate DEP-encoding genes. A deeper insight is that only complex life forms such as human can afford the coexistence of hundreds of miRNAs in a cell owing to intricate PPI network and intricate PPI

network is an important condition for miRNA synergy to occur.

Besides, our results further point out that miRNA synergism may be associated with the organ expression of target gene products. UEPs that have broad and balanced expression in human organs are subject to over-supervision by synergistic miRNA regulation. Broad and balanced expression of UEPs is a reasonable reflection of their important biological roles in cellular activities such as significant participation in gene transcription. Uniformed expression of large number of proteins throughout human organs implies that different cells share a similar basic state, which ensures that the communication between cells is relatively equal in physiological environment. Strengthened synergistic miRNA regulation on UEP-encoding genes suggests that miRNA synergism should contribute to the maintaining of their uniformed expression throughout human organs. Due to allowing cells more finely tune the protein abundance of UEPs, miRNA synergism leads to more economical adaptation of cells to the intracellular and extracellular environments.

The complexity of biological system is described by the following characteristics such as robustness, redundancy, and crosstalk (Jia et al., 2009). Biological robustness lies in that altered expression of single gene may be not enough to effectively affect the overall system (Kitano, 2004). Although this ensures the relative stability of biological system, it makes cells difficult to adapt to the external environment changes in a timely manner. Due to the existence of functional linkages between system components, cells can not merely change one component but keep others unchanged when faced with altered conditions. For instance, as cells encounter excessive oxidative stress, adjustment of the overall cellular signals could be observed (Chandra et al., 2000; Kiffin et al., 2006; Reuter et al., 2010). Robustness and adaptability are mutually contradictory. Biological system requires an integrated gene/protein management strategy for coordinating different biological signals. By targeting hundreds of mRNA messengers, single miRNA is able to

accomplish integrated gene regulation (Backes et al., 2017). Compared to mRNA-mediated protein expression control, indirect regulation by miRNAs is high-throughput (Baek et al., 2008). This may be the biological significance of miRNAs' presence in human biology. More importantly, the unique synergistic action mechanism of miRNAs provides richer control skills for cells and better overcomes the huge energy consumption in biosynthesis of miRNAs and assembly of RISC protein complex.

Undoubtedly, more in-depth exploration about constituent factors for effective miRNA target site (Agarwal et al., 2015) would greatly promote our understanding of synergistic interactions between miRNAs. Differential 3'-untranslated region isoforms may lead to inconsistent target gene profiles of a miRNA in different types of cells (Nam et al., 2014). Taken together with the fact that ~10-15% of human miRNAs are tissue-specific (Ludwig et al., 2016), this finding implies that synergistic collaboration between miRNAs may be cell-type-specific or tissue-specific. However, more experimental researches are definitely to explore this.

In conclusion, no longer depending on self-expression lets miRNA synergism maximizes the effectiveness of fine-tuning protein output. To complex biological systems, miRNA synergism seems to be a very energy-economical solution by which cells better deal with the contradictory relationship between system robustness and system adaptability. Our findings provide a new understanding for the biological significance of miRNAs at the organ scale.

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FIGURE LEGENDS

Figure 1. Overview of organ expression of proteins encoded by miRNA targets. A. Frequency distribution of coefficient of variation (CV) of protein expression abundance in 12 human organs. The right upper showed the frequency distribution of $\log_2$CV. The CV interval from 40% to 120% was highlighted with gray box. B. The box and whiskers plot of organ expression percentage of UEPs and DEPs. C. Comparison of average number of neighbors of UEPs and DEPs. *** $p < 0.001$, UEPs versus DEPs; D. Comparison of cluster coefficient of UEPs and DEPs. *** $p < 0.001$, UEPs versus DEPs. UEPs: uniformly expressed proteins; DEPs: disorderly expressed proteins.

Figure 2. Regulation density and tendency of miRNAs on UEP-encoding genes and DEP-encoding genes. A. Comparison of miRNA density on UEP-encoding genes and DEP-encoding genes. The calculated miRNA density refers to miRNA target sites within only 3' untranslated regions of genes. B. Distribution of the number of miRNAs per target genes that encode proteins expressed in cardiovascular system. ** $p < 0.01$, *** $p < 0.001$, UEP-encoding genes versus DEP-encoding genes. C. Skewness assessment of miRNAs in cardiovascular system (CVS). D. Distribution of expression $\log_2$CVs of proteins encoded by miR-133a and miR-1 target genes. The red dotted line at $\log_2$CV of -1.32 corresponds to the CV value of 40%. CV: coefficient of variation. UEPs: uniformly expressed proteins; DEPs: disorderly expressed proteins.

Figure 3. Influence of altered expression and synergy on the overall miRNA regulation. A-C. Percentage of the affected miRNAs and synergistic miRNA interactions at different number of differently expressed miRNAs. n=1, 3, or 5 means that one, three, or five miRNAs are simultaneously dysregulated. D. Percentage of the affected miRNAs and synergistic miRNA interactions by altered miRNA expression. E. Synergistic miRNA-miRNA interactions in cardiovascular system. The red, blue and gray lines represent interactions with synergy scores of > 2.0, > 1.5 and > 1.0, respectively. Two hubs in the synergistic miRNA

interaction network miR-1 and miR-21 were highlighted as big nodes.

Figure 4. Constitution share of low and high skewness miRNAs for top 500 (A), top 100 (B) and top 50 (C) synergistic miRNA interactions in 12 human organs. *** $p < 0.001$, HH versus LL; LL, LH and HH represent synergies between low skewness miRNAs, between low skewness miRNAs and high skewness miRNAs, and between high skewness miRNAs, respectively.

Figure 5. Heatmap of mean synergy scores of miRNAs with skewness values of < 0.3 in 12 human organs. BFS: Breast and female reproductive system (female tissue); BIS: Blood and immune system (hematopoietic); CNS: Central nervous system (brain); CVS: Cardiovascular system (heart and blood vessels); DT: Digestive tract (GI-tract); EG: Endocrine glands; LP: Liver and pancreas; MS: Male reproductive system (male tissues); P: Placenta; RS: Respiratory system (lung); SS: Skin and soft tissues; UT: Urinary tract (kidney and bladder).

Figure 6. Cell viability assay of miRNA transfection on cell lines of HUVEC (A), MCF7 (B), and HepG2 (C). ** $p < 0.01$, versus NC+Hypoxia (HUVEC); * $p < 0.05$, versus NC (MCF7 and HepG2); NC: negative control; n = 6.

SUPPLEMENTAL INFORMATION

Table S1. Sequences of the negative control and miRNA mimics used in this study.

Table S2. Distribution of miRNA target genes in the 12 human organs and within the whole genome.

Table S3. Result of gene ontology (GO) analysis by using DAVID.

File S1. A four-step protocol to reveal miRNAs that tend to regulate UEP-encoding genes.

Figure 1

Figure 1. Overview of organ expression of proteins encoded by miRNA target.

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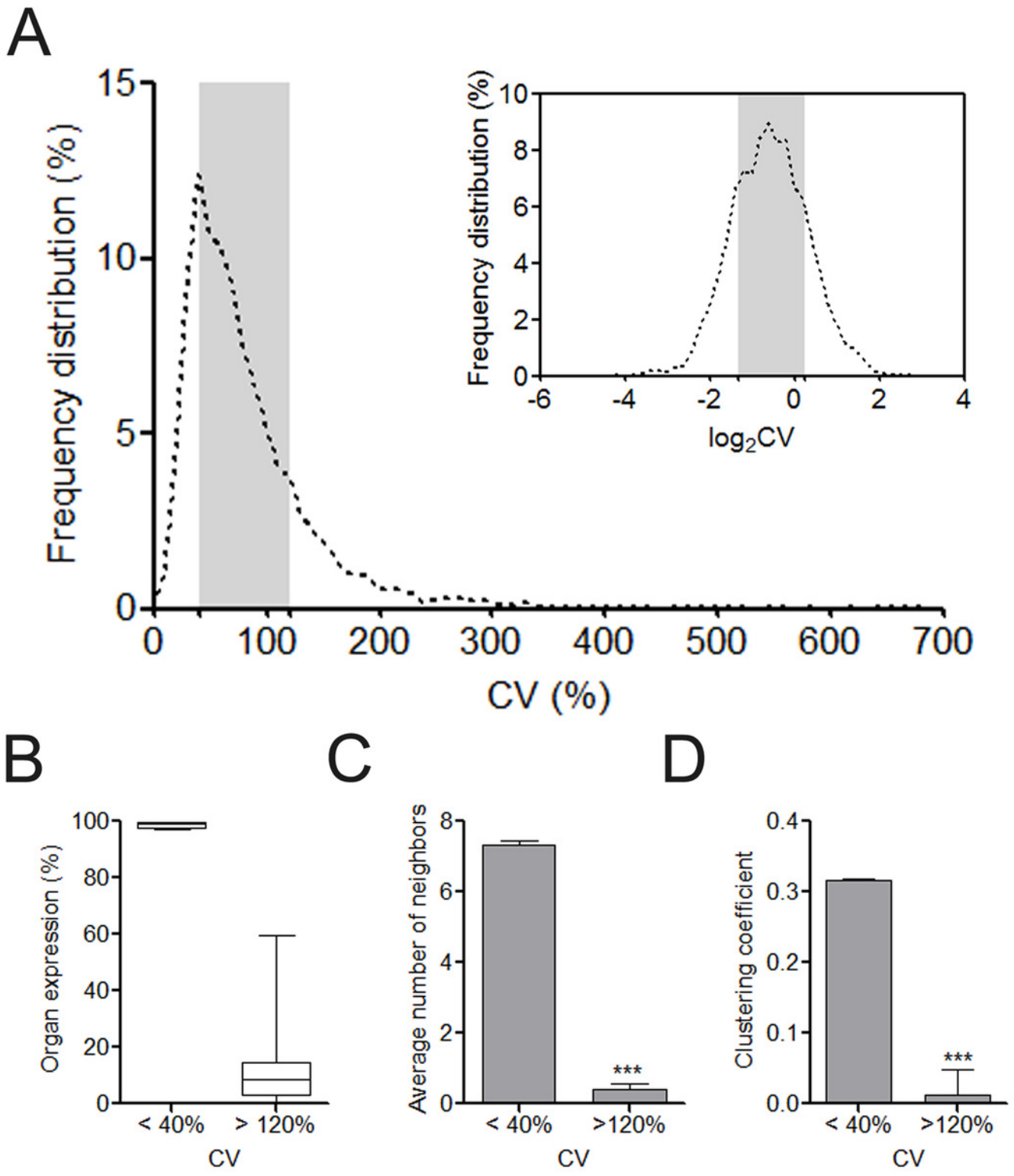

A
Frequency distribution (%)
CV (%)
Frequency distribution (%)
log2CV
B
Organ expression (%)
< 40%
> 120%
CV
C
Average number of neighbors
***
< 40%
>120%
CV
D
Clustering coefficient
***
< 40%
>120%
CV

Figure 2

Figure 2. Regulation density and tendency of miRNAs on UEP-encoding genes and DEP-encoding genes.

A. Comparison of miRNA density on UEP-encoding genes and DEP-encoding genes. The calculated miRNA density refers to miRNA target sites within only 3’ untranslated regions of genes. B. Distribution of the number of miRNAs per target genes that encode proteins expressed in cardiovascular system. ** $p < 0.01$, *** $p < 0.001$, UEP-encoding genes versus DEP-encoding genes. C. Skewness assessment of miRNAs in cardiovascular system (CVS). D. Distribution of expression $\log_2$CVs of proteins encoded by miR-133a and miR-1 target genes. The red dotted line at $\log_2$CV of -1.32 corresponds to the CV value of 40%. CV: coefficient of variation. UEPs: uniformly expressed proteins; DEPs: disorderly expressed proteins.

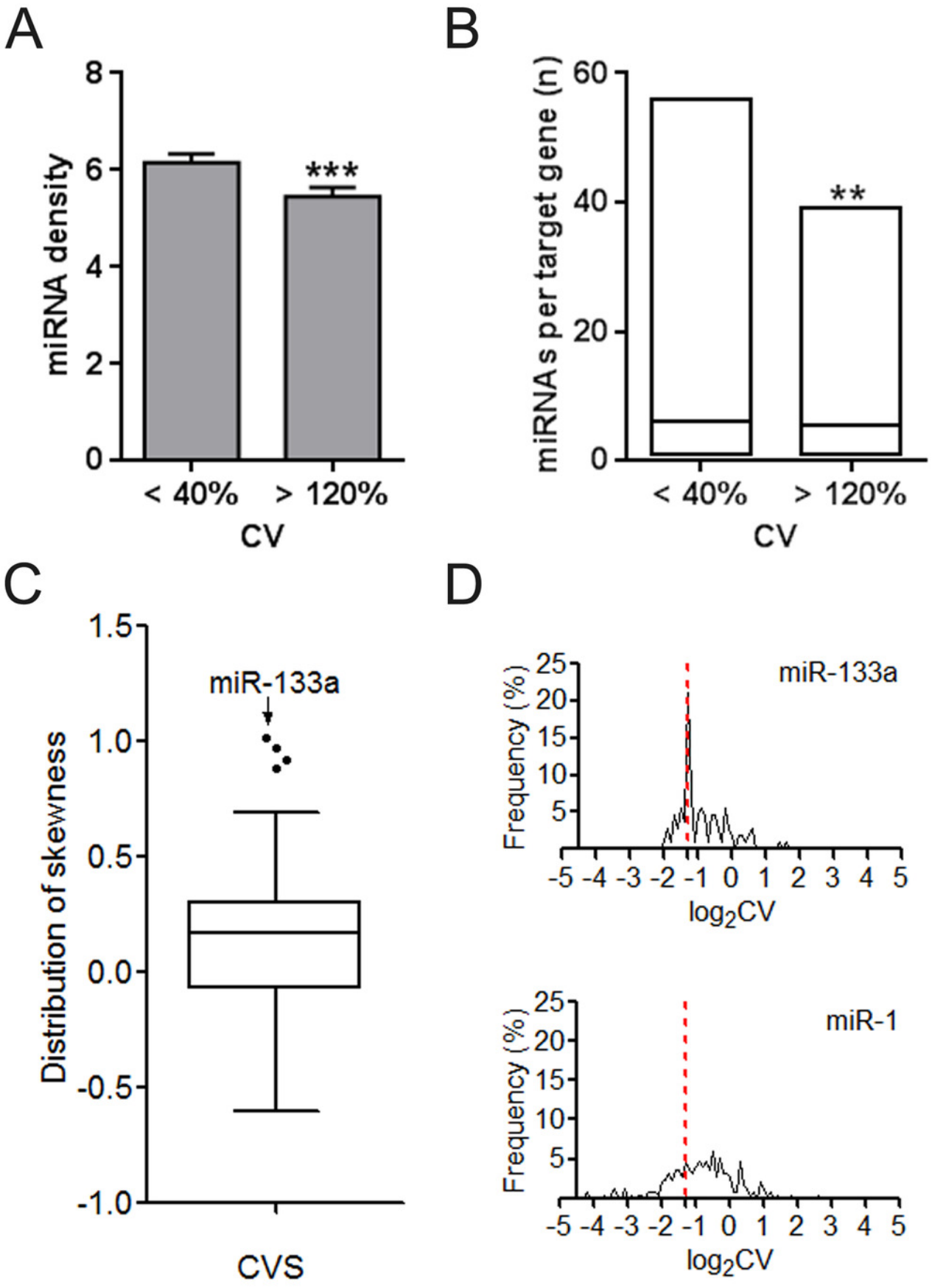
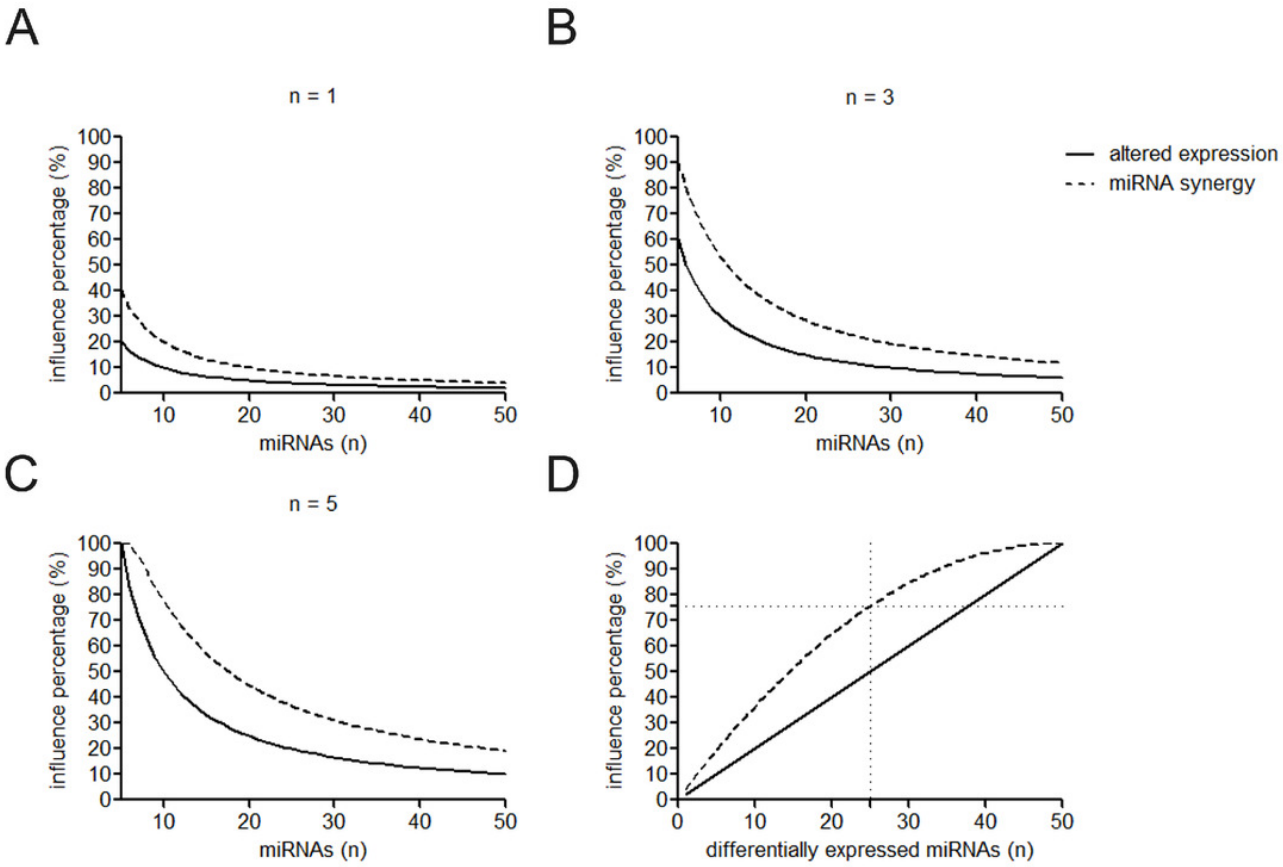

A
miRNA density
8
6
4
2
0
***
< 40%
> 120%
CV
B
miRNAs per target gene (n)
60
40
20
0
**
< 40%
> 120%
CV
C
Distribution of skewness
1.5
1.0
0.5
0.0
-0.5
-1.0
miR-133a
CVS
D
Frequency (%)
25
20
15
10
5
miR-133a
-5 -4 -3 -2 -1 0 1 2 3 4 5
log2CV
Frequency (%)
25
20
15
10
5
miR-1
-5 -4 -3 -2 -1 0 1 2 3 4 5
log2CV

Figure 3

Figure 3. Influence of altered expression and synergy on the overall miRNA regulation.

A-C. Percentage of the affected miRNAs and synergistic miRNA interactions at different number of differently expressed miRNAs. n=1, 3, or 5 means that one, three, or five miRNAs are simultaneously dysregulated. D. Percentage of the affected miRNAs and synergistic miRNA interactions by altered miRNA expression. E. Synergistic miRNA-miRNA interactions in cardiovascular system. The red, blue and gray lines represent interactions with synergy scores of > 2.0, > 1.5 and > 1.0, respectively. Two hubs in the synergistic miRNA interaction network miR-1 and miR-21 were highlighted as big nodes.


A
n = 1
influence percentage (%)
miRNAs (n)
B
n = 3
influence percentage (%)
miRNAs (n)
altered expression
miRNA synergy
C
n = 5
influence percentage (%)
miRNAs (n)
D
influence percentage (%)
differentially expressed miRNAs (n)

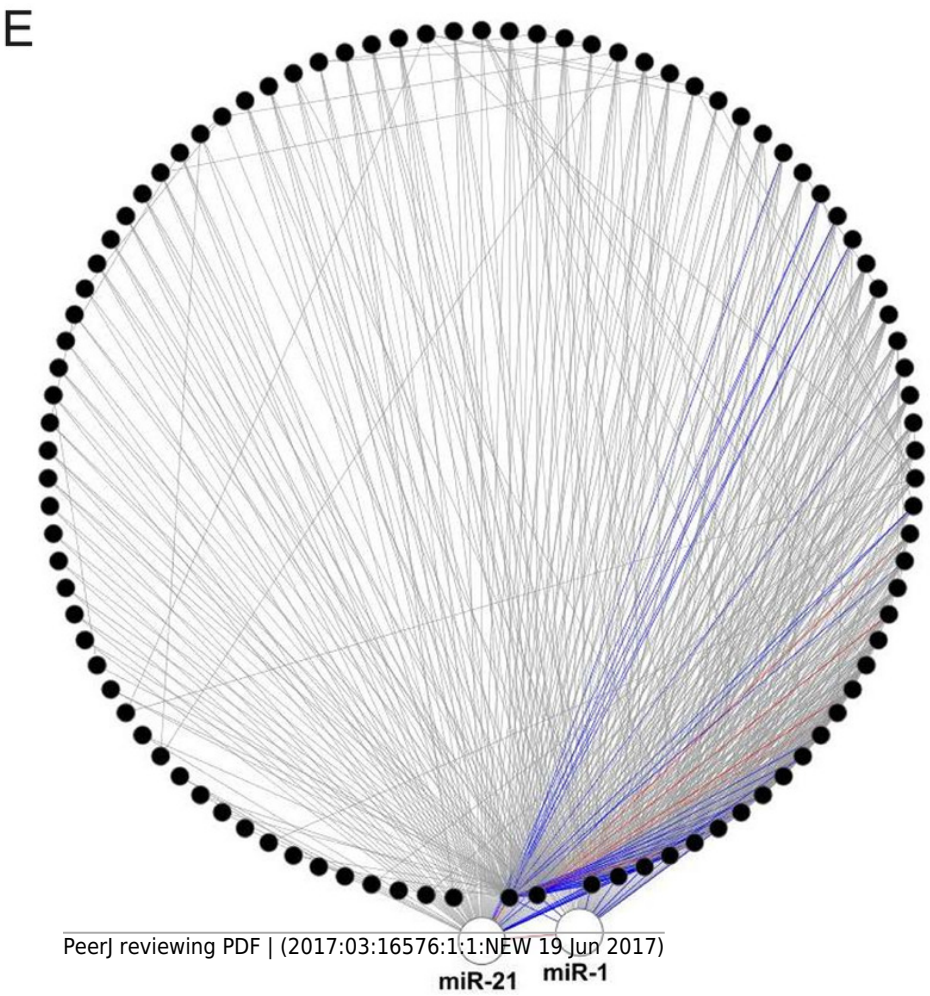

E
miR-21
miR-1

Figure 4

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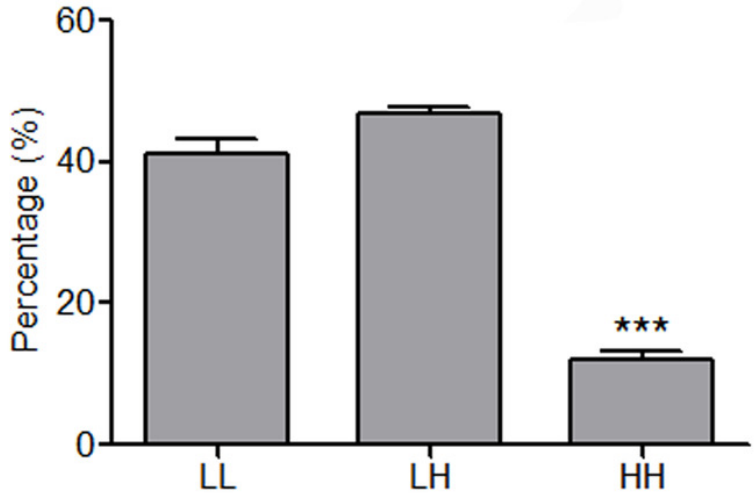
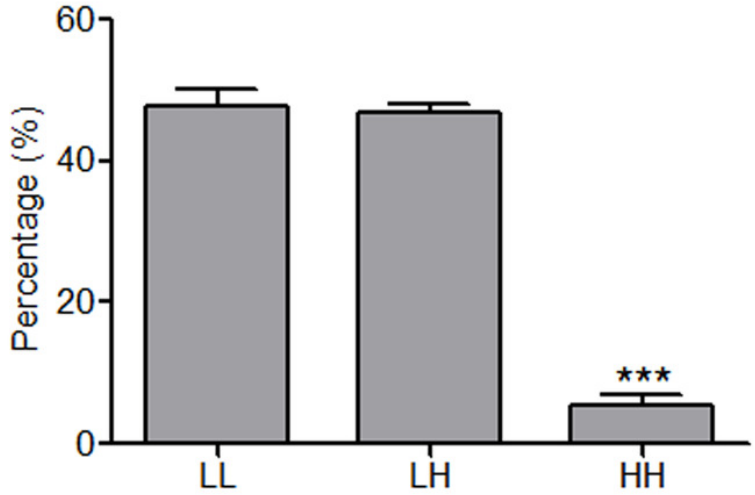
A


60
40
20
0
Percentage (%)
***
LL
LH
HH

B


60
40
20
0
Percentage (%)
***
LL
LH
HH

C

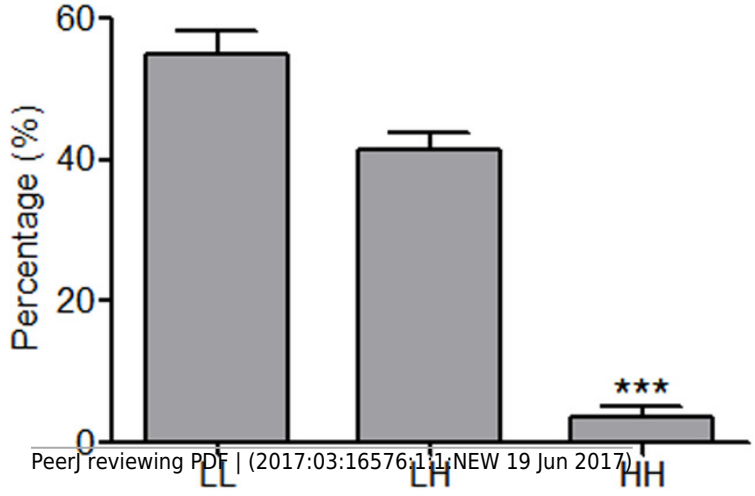

60
40
20
0
Percentage (%)
***
LL
LH
HH

Figure 5

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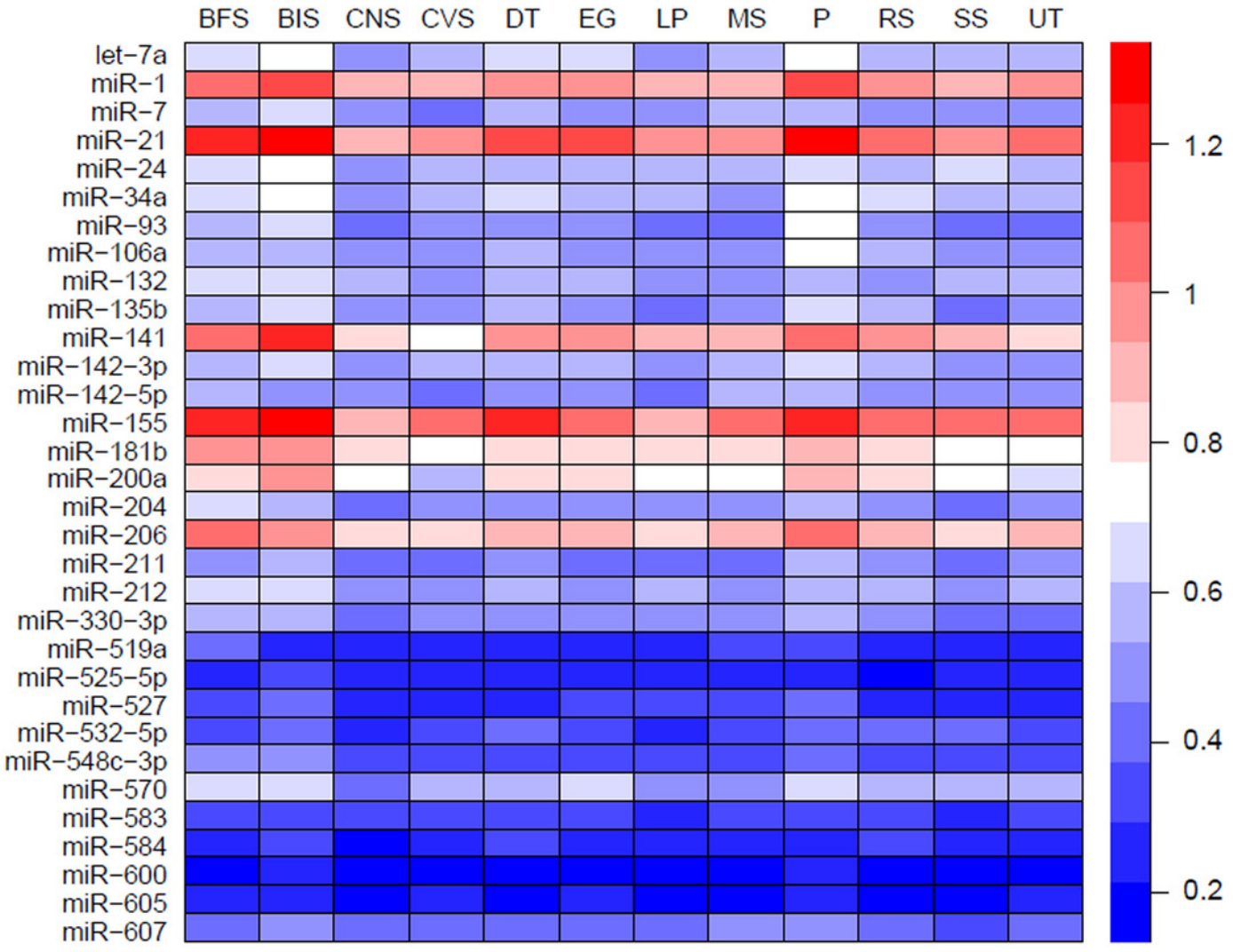

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