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

Xue Chen [1] , **Wei Zhao** [1] , **Ye Yuan** [1] , **Yan Bai** [1] , **Yong Sun** [1] , **Wenliang Zhu** [1] , **Zhimin Du** [Corresp. 1]

[1] Department of Pharmacy, the Second Affiliated Hospital of Harbin Medical University (Institute of Clinical Pharmacy, the Heilongjiang Key Laboratory of Drug Research, Harbin Medical University), Harbin, China

Corresponding Author: Zhimin Du
Email address: dzm1956@126.com

Considering complicated microRNA (miRNA) biogenesis and action mechanisms, it was thought so high energy-consuming for a cell to afford simultaneous over-expression 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 in 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) are inclined to relatively independently execute organ-specific functions. Consistent with this, miRNAs that mainly target UEP-encoding mRNAs, 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 that targeting plenty of genes by single miRNA makes miRNA-mediated regulation high-throughput but insufficient due to target gene dilution effect. Furthermore, our *in vitro* experiment verified that merely 25 nM transfection of miR-21 be sufficient 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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3   Xue Chen, Wei Zhao, Ye Yuan, Yan Bai, Yong Sun, Wenliang Zhu, Zhimin Du*

4   Department of Pharmacy, the Second Affiliated Hospital of Harbin Medical University (Institute of Clinical

5   Pharmacy, the Heilongjiang Key Laboratory of Drug Research, Harbin Medical University), Harbin, China

6   *To whom correspondence should be addressed. Zhimin Du: Address: 246 Xuefu Road, Harbin 150086,

7   Heilongjiang Province, China; Tel/Fax: 86-451-86605353; E-mail: dzm1956@126.com.

ABSTRACT

Considering complicated microRNA (miRNA) biogenesis and action mechanisms, it was thought so high energy-consuming for a cell to afford simultaneous over-expression 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 in 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) are inclined to relatively independently execute organ-specific functions. Consistent with this, miRNAs that mainly target UEP-encoding mRNAs, 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 that targeting plenty of genes by single miRNA makes miRNA-mediated regulation high-throughput but insufficient due to target gene dilution effect. Furthermore, our *in vitro* experiment verified that merely 25 nM transfection of miR-21 be sufficient 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 rely on their interaction with argonaute proteins and co-assembling into the RNA-induced silencing complex (RISC). Despite energy-consuming, such a unique gene regulation pattern may be significant 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 proposed that an 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, 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 shown that only a very small portion of miRNAs can afford this way, such as miR-1 that is strongly 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 forms the basis of the overall effect of miRNA-mediated gene regulation. Several possible patterns of miRNA-miRNA interactions (MMIs)

50   were uncovered by far. As one miRNA can physically interact with hundreds of target genes, this causes

51   with a great probability that different miRNAs may competitively bind the 3'-untranslated region of the

52   same gene. Competition for binding is the main and fundamental pattern of miRNA-miRNA interactions

53   (MMIs) in the miRNA world (Jens and Rajewsky, 2015). Expect the competition pattern, different miRNAs

54   may show cooperativity due to being co-regulated by the same transcription factor and targeting genes with

55   functional interconnections (Na and Kim, 2013; Shi et al., 2013; Guo et al., 2014). Restricted MMI is

56   another pattern that two miRNAs have completely or partially complementary structures and constitute an

57   endogenous sense and antisense miRNA pair (Guo et al., 2014). Great expression difference of the two

58   miRNAs in a pair was a major feature of this pattern.

59   Based on our previous knowledge of miRNA regulation (Yuan et al., 2015; Zhu et al., 2011; Zhu et al.,

60   2013), we proposed that miRNAs might adopt an alternative pattern of MMI that promotes a manner of

61   more economical and efficient gene regulation in response to real-time adjustment of cellular signals. It less

62   depends on miRNA abundance and more rely on synergistic miRNA-miRNA collaboration for fine-tuning

63   protein output (Skommer et al., 2014). On the whole, synergistic gene regulation may optimize the

64   regulatory efficacy of miRNAs (Xu et al., 2011). Importantly, it is less expensive in cellular energy

65   consumption compared to extraordinarily high expression of miRNAs. In order to validate this hypothesis,

66   we performed a quantitative assessment of miRNA synergism by calculating miRNA synergy score (Zhu

67   et al., 2013). Benefited from the increasingly clear human protein expression atlas (Uhlén et al., 2015), this

68   method enables us to explore potential principles in miRNA regulation at the dimension of human organs.

69   Additionally, *in vitro* experiment was performed to show the potential benefit of miRNA synergy. Such

70   efforts are intended to shed novel insights into the biological significance of miRNA-miRNA collaboration

71 and provide implications for better understanding the existence of miRNAs in humans.

72

MATERIALS AND METHODS

MiRNA-target interactions (MTIs) and protein expression

75 Three datasets miRecords (Xiao et al., 2009), miRSel (Naeem et al., 2010) and ExprTargetDB (Gamazon

76 et al., 2010) were used to obtain reliable MTIs in humans. MiRSel is a collection of literature evidence of

77 MTIs. MiRecords and ExprTargetDB belong to secondary tools for MTI prediction, in which integration

78 of different algorithms led to reliable MTI identification. Finally, all MTI data were merged for further

79 analysis. Organ protein expression data were obtained from the human protein atlas (HPA) database (Uhlén

80 et al., 2015). Twelve human organs were included: breast and female reproductive system (BFS), blood and

81 immune system (BIS), central nervous system (CNS), cardiovascular system (CVS), digestive tract (DT),

82 endocrine glands (EG), liver and pancreas (LP), male reproductive system (MS), placenta (P), respiratory

83 system (RS), skin and soft tissues (SS) and urinary tract (UT). A resilient fraction threshold was adopted

84 for expression verification. Specifically, the fraction threshold was set at 70% for BFS, BIS, CNS, LP and

85 SS; 90% for DT; 100% for CVS, EG, MS, P, RS and UT. The information of the level of annotated protein

86 expression was downloaded and imported into Cytoscape v2.8.3 (Smoot et al., 2011). Notably, validated

87 marks 'none', 'low', 'medium' and 'high' representing proteins expression levels were in advance

88 converted into the digitals 0, 1, 2 and 3, respectively. Afterwards, coefficient of variation (CV) was used to

89 assess the cellular expression dispersion degree of each miRNA target gene-encoded protein.

Synergy score and skewness

91 Potential degree of synergistic collaboration between miRNAs was quantitatively assessed by calculating

92 miRNA synergy score as described before (Zhu et al., 2013). For each miRNA, the statistics parameter

93 skewness was used to assess the distribution inclination of the $\log_2$CV values of protein expression and

94 determine the regulatory tendency of the miRNA (Mardia, 1970). We used $\log_2$CV instead of CV for

95 skewness calculation, as the distribution of CVs did not pass D'Agostino & Pearson omnibus normality test

96 (D'Agostino and Pearson, 1973). A smaller skewness value means a tendency of regulating mRNAs

97 encoding uniformly expressed proteins (UEPs); however a higher skewness value suggests that a miRNA

98 tends to fine-tune disorderly expressed proteins (DEPs). File S1 was a four-step protocol to reveal miRNAs

99 that tend to regulate UEP-encoding mRNAs. In this study, UEPs and DEPs refer to proteins with CVs of

100 less than 40% and CVs of more than 120%, respectively.

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

102 A comparison between UEPs and DEPs was investigated from the GO aspect by applying the online

103 DAVID functional annotation tool (Huang et al., 2009). Briefly, gene official symbols were submitted as

104 gene list and 'Homo Sapiens' was selected as the background. Over-represented GO biological processes,

105 cellular components and molecular functions were considered to be significant only if false discovery rate

106 (FDR) was less than 0.05. We also investigated network topology characteristics of UEPs and DEPs,

107 respectively. The Cytoscape plugins BisoGenet [18] was used to retrieve, trim, and analyze experimentally

108 validated protein-protein interactions (PPIs).

109 **Cell culture and miRNA transfection**

110 Human umbilical vein endothelial cell line (HUVEC), breast cancer cell line (MCF7) and hepatic carcinoma

111 cell line (HepG2) were all obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy

112 of Science (Shanghai, China). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM)

113   supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. After that,

114   cells were incubated at 37 °C with 5% CO_2 and 95% air. 24 hours before transfection, cells were transferred

115   to 96-well plates and cultured in fresh medium without antibiotics, According to the manufacturer's

116   instructions, X-treme GENE siRNA transfection reagent (Roche, Switzerland) was used for mono-

117   transfection of negative control or miRNA mimic at 25 nM. Table S1 listed the sequences of the negative

118   control and miRNA mimics used in this study.

119   **Cell viability assay**

120   Cell viability was assessed by measuring mitochondrial dehydrogenase activity, using the colorimetric

121   MTT assay, based on the fact that viable cells (but not dead cells) can reduce 3-(4,5-dimethylthiazol-2-yl)-

122   2,5-diphenyl tetrazolium bromide (MTT). After miRNA transfection 24 hours, with or without hypoxia

123   treatment, cells were incubated with MTT of 5 mg/ml at 37 °C for four hours. Hypoxia was induced by

124   exposing cells to hypoxia condition (1% O_2, 94% N_2, 5% CO_2) for 24 hours using a modular incubator. The

125   purple formazan crystal was dissolved with 150 μL of dimethyl sulfoxide (DMSO) and added to the cells.

126   The absorbance was measured at 490 nm.

127   **Data statistics**

128   All data are expressed as mean ± SEM (Standard Error of Mean). Statistical analysis was performed with

129   Mann–Whitney *U* test or one-way ANOVA (analysis of variance) followed by Tukey's test for multiple

130   comparisons. Differences were only considered to be significant at $p < 0.05$.

131

132   **RESULTS**

133   **Data collecting and screening**

134   Data integration identified a total of 11162 target genes and 69618 MTIs for 472 miRNAs from the three

135   databases including miRecords, miRSel, and ExprTargetDB. Only 166 of were retained for further analysis,

136   each of which targeted at least 50 mRNA genes in each of the 12 human organs (Table S2). Totally, they

137   were functionally associated with more than 6328 proteins via 36211 MTIs.

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

139   After retrieving the protein expression data from the HPA database, we calculated the CV value for each

140   of the 6328 proteins regarding its HPA-defined abundance ranks in human cells. The majority of the

141   proteins were located with the CV range from 40% to 120% (Figure 1A). This distribution characteristic

142   was more clearly observed after log2 transformation of CVs. Totally, we identified 1340 UEPs and 1115

143   DEPs. Unlike DEPs, UEPs tend to be more uniformly expressed in human organs, have more interacting

144   neighbors, and constitute closer associations with each other in clusters (Figures 1B-D). Comparably, DEPs

145   are not ubiquitously expressed, and more marginalized in the human PPI network and execute functions in

146   isolation. More than these, GO analysis disclosed that UEPs tend to be intensively involved in gene

147   transcription and related biological processes, be located in the nucleus or nearby, and play roles in the

148   housekeeping functions such as being transcription regulators (Table S3). This result indicates the crucial

149   role of UEPs in the maintenance of basic cell activities. In contrast, DEPs are more implicated in organ-

150   specific biological processes and perform molecular functions at the plasma membrane and cytoplasmic

151   organelles.

152   **MiRNAs' regulation tendency on mRNAs encoding UEPs or DEPs is independent of miRNA**

153   **expression**

154   We investigated the difference of UEPs and DEPs from the miRNA regulation aspect. UEP-encoding

155   mRNAs instead of those encoding DEPs are averagely under more dense control of miRNAs in the whole

156   genome context (Figure 2A). Even when MTIs are restricted into one organ such as CVS, preference of

157   miRNAs for UEP-encoding mRNAs is also obvious to observe (Figure 2B). The highest skewness value

158   highlighted miR-133a of its definite trend in regulating DEP-encoding mRNAs in CVS (Figure 2C). Both

159   miR-1 and 133a are cardiac-specific miRNAs. However, we found that they showed different tendencies

160   on gene regulation (Figure 2D). This result suggests that regulating organ-specific genes may be not just a

161   necessary condition for the organ-specific functions of miRNAs, such as miR-1 (Yang et al., 2007).

162   **MiRNAs regulate UEP-encoding mRNAs in a synergistic pattern**

163   MiRNAs may act synergistically with each other (Xu et al., 2011). With the evolution of the complexity of

164   a biological system, synergism is undoubtedly more advantageous than isolated regulation in terms of

165   management strategy (Corning, 1995; Stelling et al., 2004). Altered expression of only a few numbers of

166   miRNAs caused systemic changes via disrupting synergistic associations between miRNAs (Figures 3A-

167   C). If all conceivable miRNA pairs are supposed to be synergistic 75.5% of synergistic miRNA interactions

168   will be affected only when half of all the miRNAs undergo altered expression (Figure 3D). In particular, to

169   those the hubs in the miRNA-miRNA synergistic network, adaptation of this management strategy may be

170   more energy-saving due to plenty of synergistic partners (Figure 3E). Further result indicates that miRNAs

171   with small skewness values comprise most of the synergistic miRNA-miRNA associations in human organs

172   (Figure 4). This finding implies that the molecular behaviors of UEPs, rather than those of DEPs, are under

173   the surveillance of a dense synergistic miRNA regulation network. In this invisible network, several

174   miRNAs should be paid more attention owing to their more powerful and extensive collaboration with other

175   miRNAs (Figure 5). They are miR-1, miR-21, miR-141, miR-155, miR-181b, miR-200a, and miR-206.

176   Compared with other miRNAs, miR-21 had a significant impact on the whole cell state of the three cell

177   lines at a mono-transfection concentration of 25 nM (Figure 6). MiR-133a tends to regulate DEP-encoding

178   mRNAs implying its relatively isolated action, our *in vitro* experiment verified its failing to influence the

179   whole cell state of the three cell lines.

180

DISCUSSION

181

182   The new discovery of miRNAs and other kinds of non-coding RNAs greatly consolidates the dominant

183   position of genes in regulating cellular activities (Cech and Steitz, 2014). Genes are not only carriers and

184   messengers of genetic information, but also direct supervisors on whether such information is accurately

185   translated into phenotypes. Despite lots of efforts it is not still clear about the regulation layer that is

186   constituted by thousands of miRNAs by now (Boettger and Braun, 2012; Stroynowska-Czerwinska et al.,

187   2014). In this study, system-level insight was attempted to shed into the overall effect of miRNAs from the

188   perspective of miRNA-miRNA collaboration.

189   It was confirmed that abundant expression of target genes produces the abundance dilution effect for

190   miRNA-mediated regulation (Arvey et al., 2010). Denzler and his colleagues further proved that the overall

191   abundance of target genes is generally higher than that of the corresponding miRNA (Denzler et al., 2014).

192   This causes insensitive influence of competitive endogenous RNA (ceRNA)-mediated derepression on

193   miRNA activity (Denzler et al., 2016). The above findings imply that miRNAs may do not perform

194   functions in isolation and collaboration of different miRNAs is a means of effective regulation of gene

195   expression.

196   Functional studies have confirmed that miRNAs might act synergistically, strongly suggesting

197   collaborative gene regulation (Huang et al., 2016; Raut et al., 2016; Xue et al., 2016). The above evidence

198   is properly consistent with the conclusion inferred by the systems biology analyses performed before (Xu

199   et al., 2011; Zhu et al., 2013). Extensive miRNA-miRNA synergism may exist in the miRNA layer.

200   Precisely because of this, slight adjustment of miRNAs' abundance can cause an apparent variation in the

201   state of cells. Synergistic miRNAs action allows cells to adjust their live-or-death status more flexibly and

202   rigorously to adapt to internal and external alternation. Due to acting synergistically with other miRNAs,

203   low concentration transfection of miR-21 showed an obvious impact on the viability of all of the studied

204   cell lines. Our result is in agreement with the established knowledge that miR-21 is a well-known miRNA

205   that targets tumor suppressor genes and was shown experimentally in many studies to control apoptosis

206   (Buscaglia and Li, 2011). This finding also implies that altered miRNA expression may not only

207   independently affect the translation of its own target gene products but also produces a radiative impact on

208   other miRNAs that have synergistic associations with it. Put briefly, miRNA synergy amplifies the effects

209   of miRNA expression alteration. As cell viability assay is only one way of measuring effects of miRNAs

210   on the cell state and it cannot be excluded that the other examined miRNAs with high potential to regulate

211   UEP-encoding mRNAs can affect the cell state in another way. However, further studies are definitely

212   needed to investigate the miRNAs through other experimental methods.

213   Functional execution of miRNAs requires high cellular energy consumption as a prerequisite (Shukla et

214   al., 2011). Besides of miRNA regulatory crosstalk (Jens and Rajewsky, 2015), miRNA synergy may

215   represent an alternative pattern of energetically optimal miRNA-mediated regulation of post-transcriptional

216   gene expression. However, we supposed that this obvious advantage brought by miRNA synergy might

217   rely heavily on the complexity of the biological system. As miRNA synergy score calculation disclosed,

218   only dense PPIs between target gene products could lead to meaningful miRNA synergy (Zhu et al., 2011).

219   This explains well that one can expect strong synergistic potential of the cardic-specific miRNA miR-1

220   instead of miR-133a. Our findings indicate that miR-1 has a tendency to regulate UEP-encoding mRNAs

221   whereas miR-133a tends to regulate DEP-encoding mRNAs. A deeper insight is that only complex life

222   forms such as human can afford the coexistence of hundreds of miRNAs in a cell owing to the intricate PPI

223   network that is an essential condition for miRNA synergy to occur.

224   Besides, our results further point out that miRNA synergism may be associated with the organ expression

225   of target gene products. UEPs that have broad and balanced expression in human organs are subject to over-

226   supervision by synergistic miRNA regulation. Broad and balanced expression of UEPs is a reasonable

227   reflection of their important biological roles in cellular activities such as significant participation in gene

228   transcription. Uniformed expression of large number of proteins throughout human organs implies that

229   different cells share a similar basic state, which ensures that the communication between cells is relatively

230   equal in the physiological environment. Strengthened synergistic miRNA regulation on UEP-encoding

231   mRNAs suggests that miRNA synergism should contribute to the maintaining of their uniformed expression

232   throughout human organs. Due to allowing cells more finely tune the protein abundance of UEPs, miRNA

233   synergism leads to more economical adaptation of cells to the intracellular and extracellular environments.

234   The complexity of the biological system is described by the following characteristics such as robustness,

235   redundancy, and crosstalk (Jia et al., 2009). Biological robustness lies in that altered expression of a single

236   gene may be insufficient to effectively affect the overall system (Kitano, 2004). Although this guarantees

237   the relative stability of a biological system, it makes cells difficult to adapt to the external environment

238   changes in a timely manner. Due to the existence of functional linkages between system components, cells

239   cannot merely change one component but keep others unchanged when faced with altered conditions. For

240   instance, as cells encounter excessive oxidative stress, adjustment of the overall cellular signals could be

241   observed (Chandra et al., 2000; Kiffin et al., 2006; Reuter et al., 2010). Robustness and adaptability are

242   mutually contradictory. Biological system requires an integrated gene/protein management strategy for

243   coordinating diverse biological signals. By targeting hundreds of mRNA messengers, single miRNA is in

244   a position to accomplish integrated gene regulation (Backes et al., 2017). Compared to mRNA-mediated

245   protein expression control, indirect regulation by miRNAs is high-throughput (Baek et al., 2008). This may

246   be the biological significance of miRNAs' presence in human biology. More importantly, the unique

247   synergistic action mechanism of miRNAs provides richer control skills for cells and better overcomes the

248   huge energy consumption in biosynthesis of miRNAs and assembly of RISC protein complex.

249   Undoubtedly, more in-depth exploration of constituent factors for effective miRNA target site (Agarwal

250   et al., 2015) would greatly promote our understanding of synergistic interactions between miRNAs.

251   Differential 3'-untranslated region isoforms may lead to inconsistent target gene profiles of a miRNA in

252   different types of cells (Nam et al., 2014). Combined with the fact that ~10-15% of human miRNAs are

253   tissue-specific (Ludwig et al., 2016), this finding implies that synergistic collaboration between miRNAs

254   may be cell-type-specific or tissue-specific. However, more experimental researches are definitely to

255   explore this.

256   In conclusion, no longer depending on self-expression lets miRNA synergism maximize the effectiveness

257   of fine-tuning protein output. For complex biological systems, miRNA synergism seems to be a very

258   energy-economical solution by which cells can better deal with the contradictory relationship between

259   system robustness and system adaptability. Our findings provide a new understanding of the biological

260   significance of miRNAs in the organ scale.

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391

FIGURE LEGENDS

393 **Figure 1. Overview of organ expression of proteins encoded by miRNA targets.** A. Frequency

394 distribution of coefficient of variation (CV) of protein expression abundance in 12 human organs. The right

395 upper showed the frequency distribution of $\log_2$CV. The CV interval from 40% to 120% was highlighted

396 with a gray box. B. The box and whiskers plot of organ expression percentage of UEPs and DEPs. C.

397 Comparison of the average number of neighbors of UEPs and DEPs. *** $p < 0.001$, UEPs versus DEPs; D.

398 Comparison of cluster coefficient of UEPs and DEPs. *** $p < 0.001$, UEPs versus DEPs. UEPs: uniformly

399 expressed proteins; DEPs: disorderly expressed proteins.

400 **Figure 2. Regulation density and tendency of miRNAs on UEP-encoding mRNAs and DEP-encoding**

401 **mRNAs.** A. Comparison of miRNA density on UEP-encoding mRNAs and DEP-encoding mRNAs. The

402 calculated miRNA density refers to miRNA target sites within only 3' untranslated regions of mRNAs. B.

403 Distribution of the number of miRNAs per target genes that encode proteins expressed in the cardiovascular

404 system. ** $p < 0.01$, *** $p < 0.001$, UEP-encoding mRNAs versus DEP-encoding mRNAs. C. Skewness

405 assessment of miRNAs in the cardiovascular system (CVS). D. Distribution of expression $\log_2$CVs of

406 proteins encoded by miR-133a and miR-1 target genes. The red dotted line at $\log_2$CV of -1.32 corresponds

407   to the CV value of 40%. CV: coefficient of variation. UEPs: uniformly expressed proteins; DEPs: disorderly

408   expressed proteins.

409   **Figure 3. Influence of altered expression and synergy on the overall miRNA regulation.** A-C.

410   Percentage of the affected miRNAs and synergistic miRNA interactions at different number of differently

411   expressed miRNAs. n=1 means that one miRNA is dysregulated; n=3 or 5 means that three or five miRNAs

412   are simultaneously dysregulated. D. Percentage of the affected miRNAs and synergistic miRNA

413   interactions by altering miRNA expression. E. Synergistic miRNA-miRNA interactions in cardiovascular

414   system. The red, blue and gray lines represent interactions with synergy scores of > 2.0, > 1.5 and > 1.0,

415   respectively. Two hubs in the synergistic miRNA interaction network miR-1 and miR-21 were highlighted

416   as big nodes.

417   **Figure 4. Constitution share of low and high skewness miRNAs for top 500 (A), top 100 (B) and top**

418   **50 (C) synergistic miRNA interactions in 12 human organs.** *** $p < 0.001$, HH versus LL; LL, LH and

419   HH represent synergies between low skewness miRNAs, between low skewness miRNAs and high

420   skewness miRNAs, and between high skewness miRNAs, respectively.

421   **Figure 5. Heatmap of mean synergy scores of miRNAs with skewness values of < 0.3 in 12 human**

422   **organs.** BFS: Breast and female reproductive system (female tissue); BIS: Blood and immune system

423   (hematopoietic); CNS: Central nervous system (brain); CVS: Cardiovascular system (heart and blood

424   vessels); DT: Digestive tract (GI-tract); EG: Endocrine glands; LP: Liver and pancreas; MS: Male

425   reproductive system (male tissues); P: Placenta; RS: Respiratory system (lung); SS: Skin and soft tissues;

426   UT: Urinary tract (kidney and bladder).

427   **Figure 6. Cell viability assay of miRNA transfection on cell lines of HUVEC (A), MCF7 (B), and**

428   **HepG2 (C).** ** $p < 0.01$, versus NC+Hypoxia (HUVEC); * $p < 0.05$, versus NC (MCF7 and HepG2); NC:

429   negative control; n = 6.

430

SUPPLEMENTAL INFORMATION

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

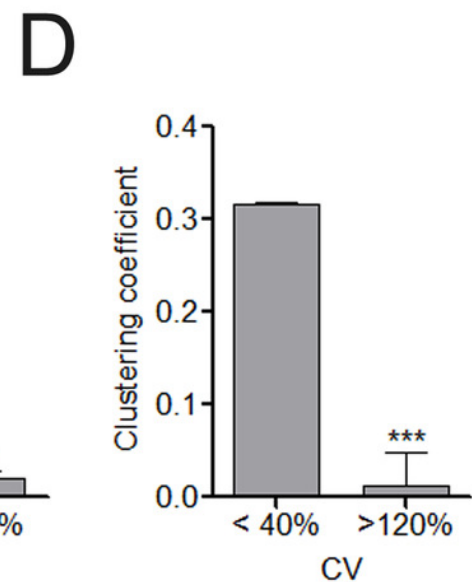
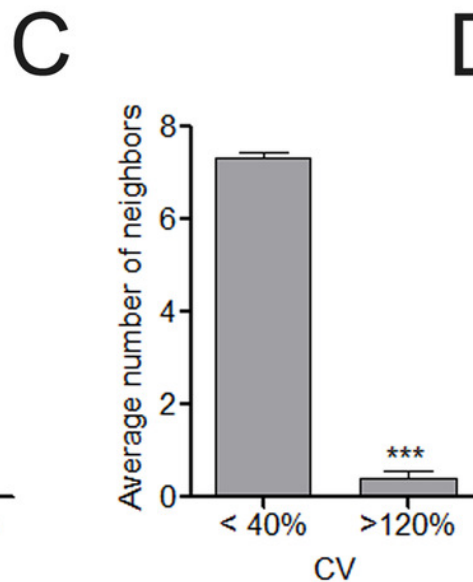
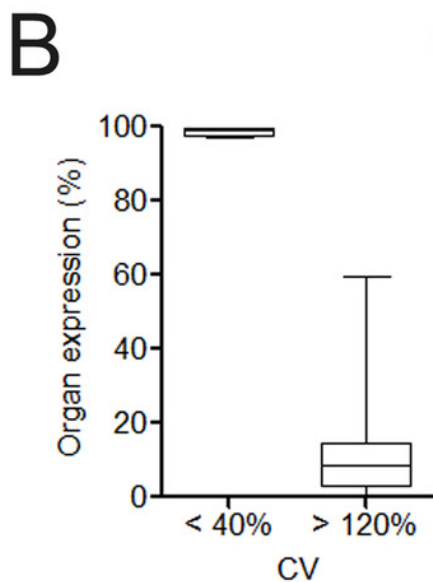
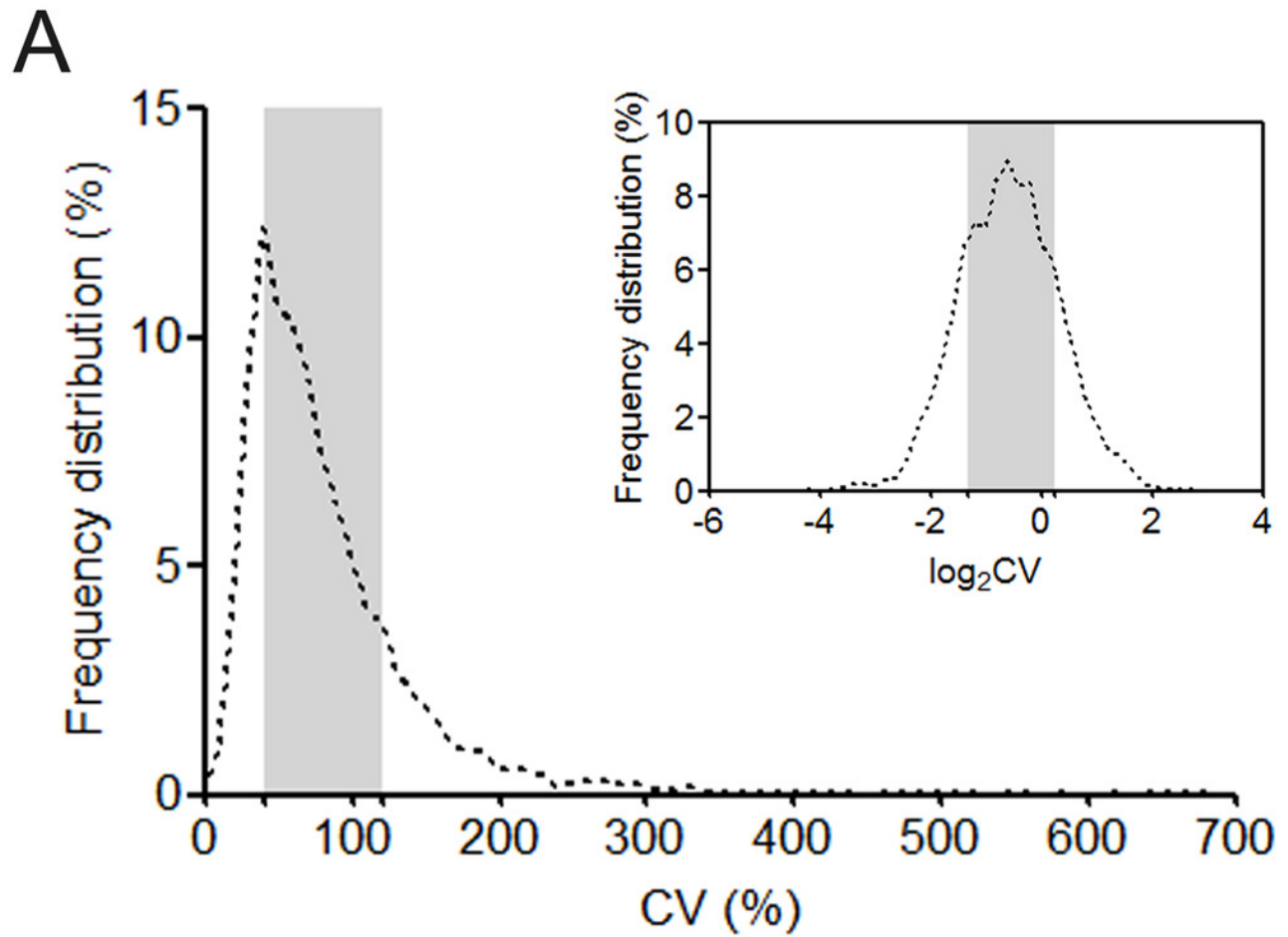
433   **Table S2.** Distribution of miRNA target genes in the 12 human organs and within the whole genome.

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

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

Figure 1

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

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.

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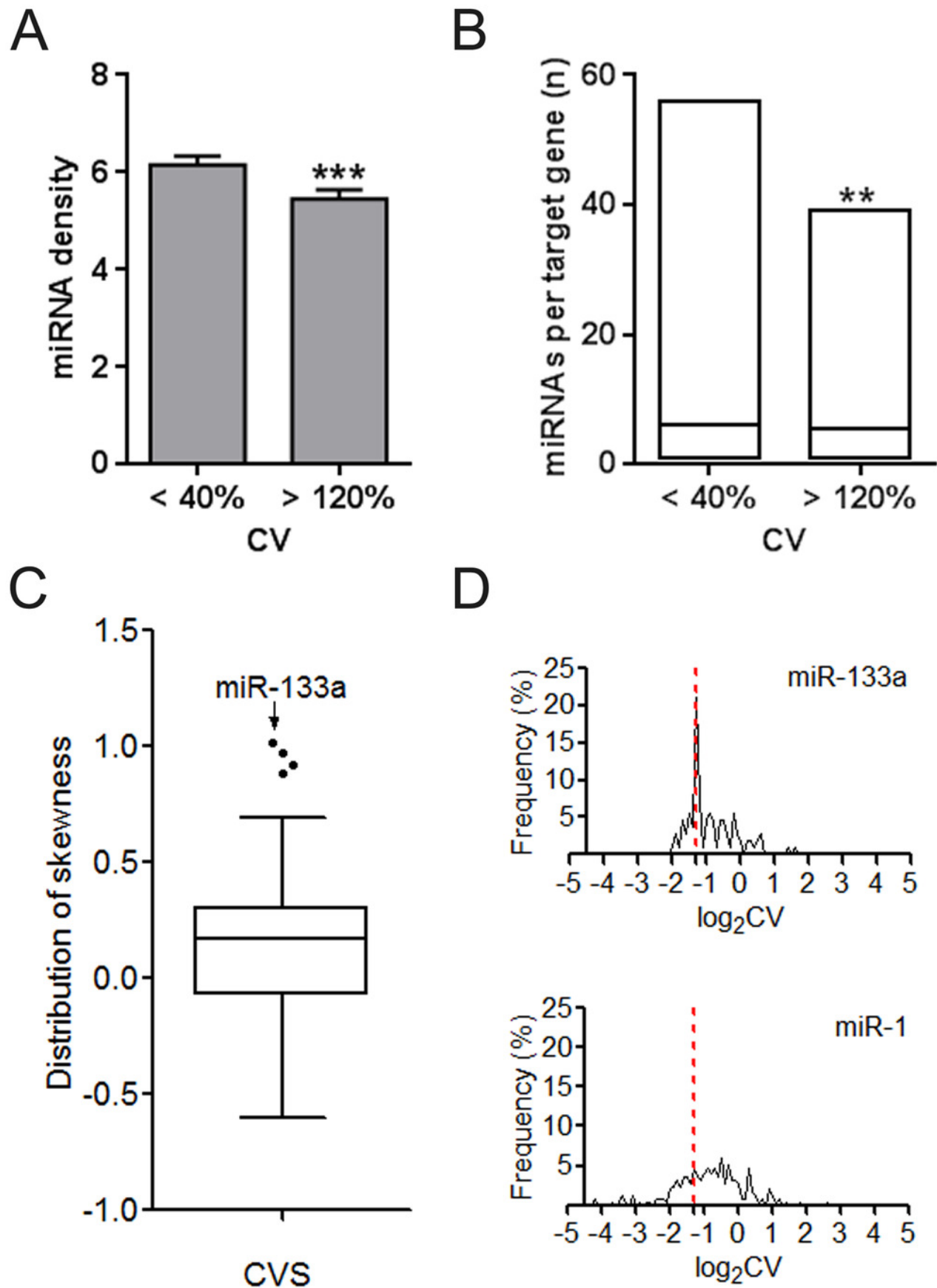
A. Comparison of miRNA density on UEP-encoding genes and DEP-encoding genes. The calculated miRNA density refers to miRNA target sites within only 3' untranslated regions of genes. B. Distribution of the number of miRNAs per target genes that encode proteins expressed in cardiovascular system. ** $p < 0.01$, *** $p < 0.001$, 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.

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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

B

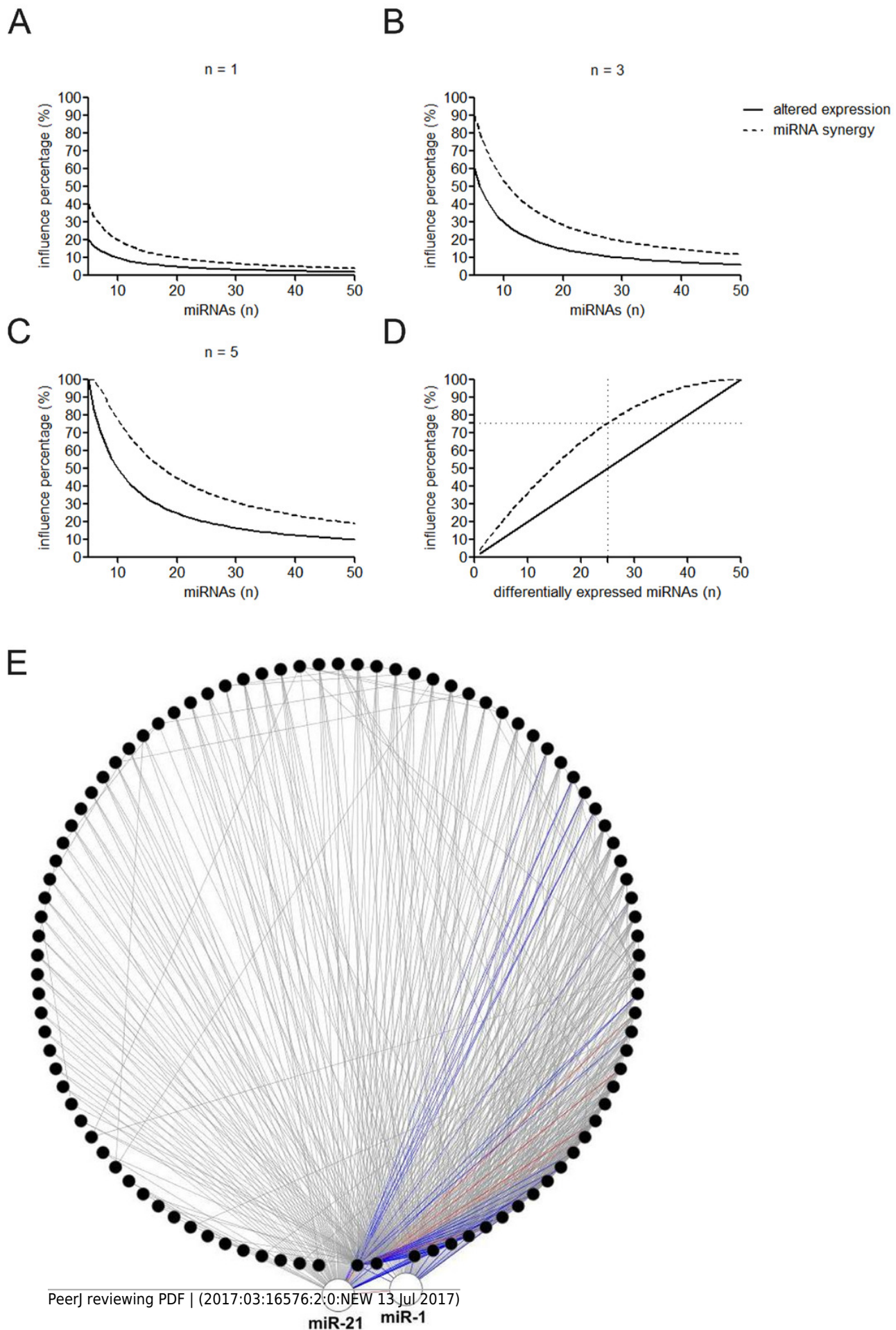
n = 3

— altered expression
- - - miRNA synergy

C

n = 5

D

E

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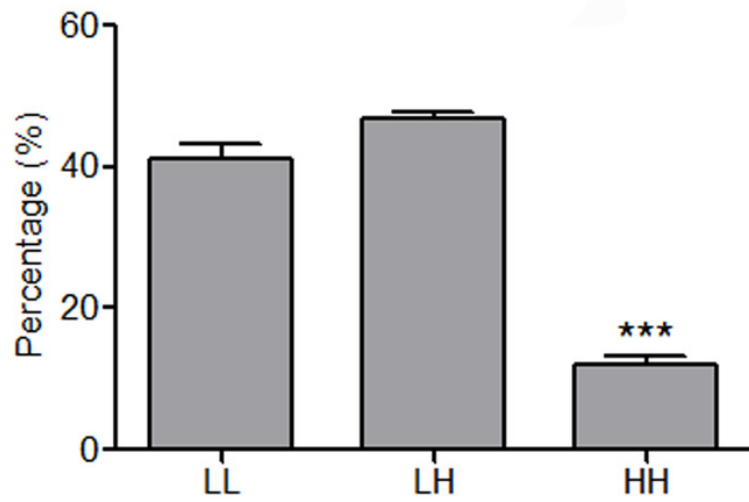
A

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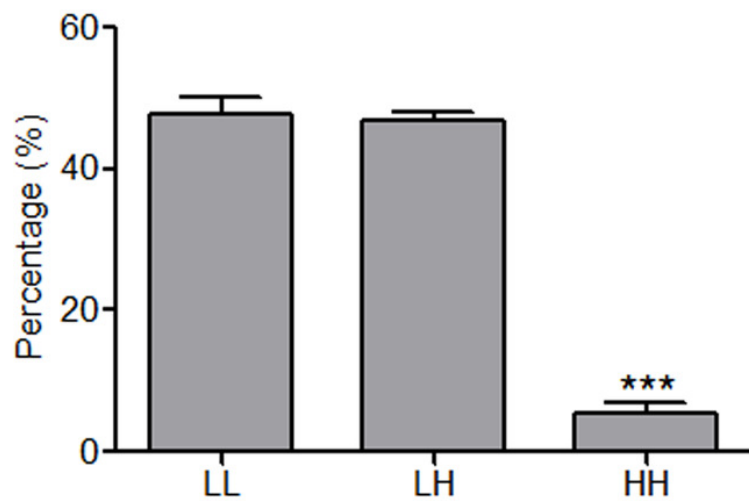
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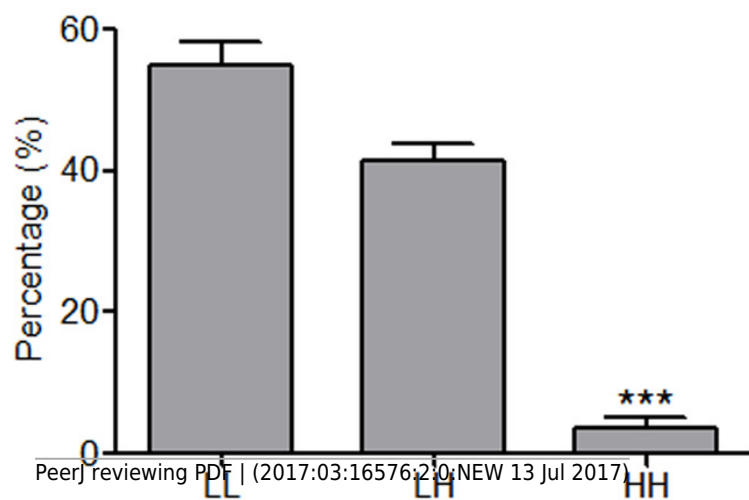
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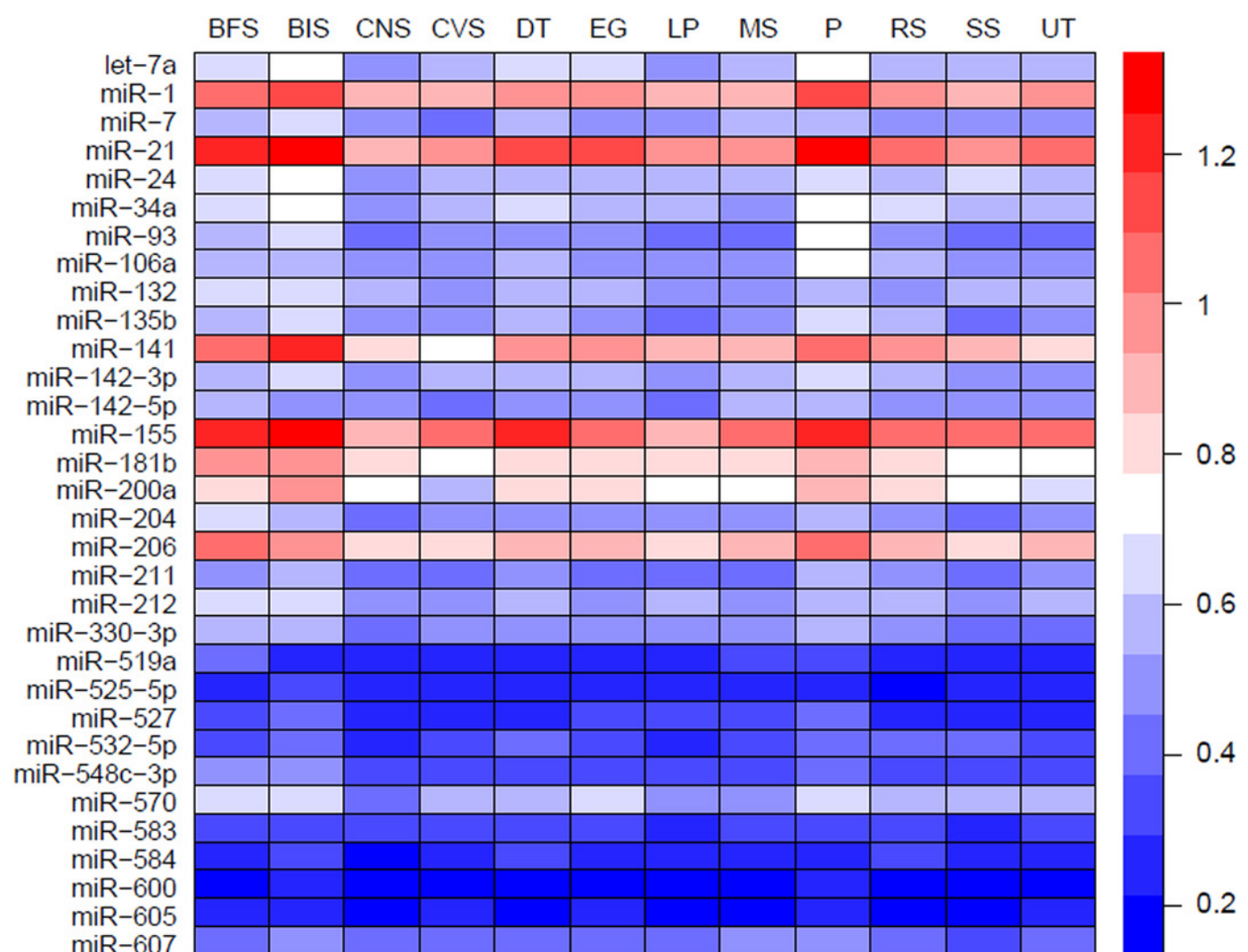
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