Pan-cancer characterization of ncRNA synergistic competition uncovers potential carcinogenic biomarkers

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Abstract

Non-coding RNAs (ncRNAs) act as important modulators of gene expression and they have been confirmed to play critical roles in the physiology and development of malignant tumors. Understanding the synergism of multiple ncRNAs in competing endogenous RNA (ceRNA) regulation can provide important insights into the mechanisms of malignant tumors caused by ncRNA regulation. In this work, we present a framework, SCOM, for identifying ncRNA synergistic competition. We systematically construct the landscape of ncRNA synergistic competition across 31 malignant tumors, and reveal that malignant tumors tend to share hub ncRNAs rather than the ncRNA interactions involved in the synergistic competition. In addition, the synergistic competition ncRNAs (i.e. ncRNAs involved in the synergistic competition) are likely to be involved in drug resistance, contribute to distinguishing molecular subtypes of malignant tumors, and participate in immune regulation. Furthermore,

SCOM can help to infer ncRNA synergistic competition across malignant tumors and uncover potential diagnostic and prognostic biomarkers of malignant tumors. Altogether, the SCOM framework and the resulting web-based database SCOMdb (www.comblab.cn/SCOMdb/) serve as a useful resource for exploring ncRNA regulation and to accelerate the identification of carcinogenic biomarkers.

Author summary

Abundant evidence reveals that ncRNAs are important modulators of gene expression, and it is important for us to understand the regulation of ncRNAs in malignant tumors. In this work, we hypothesize that ncRNAs acting as ceRNAs can crosstalk with each other in the form of synergistic competition. We name the hypothesis as the *ncRNA synergistic competition hypothesis*. To uncover potential carcinogenic biomarkers, we present a framework, SCOM, for identifying ncRNA synergistic competition in malignant tumors. From the perspective of synergistic competition, we have shown that the synergistic competition ncRNAs are likely to involve in drug resistance, contribute to distinguishing molecular subtypes of malignant tumors, and participate in immune regulation. In addition, the synergistic competition ncRNAs are potential diagnostic and prognostic biomarkers of malignant tumors. We believe that the proposed framework SCOM and the web-based database SCOMdb can contribute to the design of reliable diagnosis and treatment biomarkers for malignant tumors.

38 ## Introduction

39 Malignant tumors are a type of complex human diseases characterized by uncontrolled
40 growth and spread of malignant cells and tissue infiltration [1]. According to the latest global
41 cancer statistics from International Agency for Research on Cancer (IARC), malignant
42 tumors are the second leading cause of death worldwide, and severely threaten human life
43 and health [2]. Depending on the type of malignant tumor cells, malignant tumors are mainly
44 divided into two categories: cancer and sarcoma [3]. Compared with healthy cells, the
45 structure and function of genes in tumor cells have changed. It is found that polygenic
46 synergy is one of the key characteristics of malignant tumors [4], suggesting that malignant
47 tumors are usually polygenic disorders and their pathogenesis is caused by multi-gene
48 synergy.

49 Non-coding RNAs (ncRNAs) are a class of various RNA transcripts that usually cannot be
50 translated into proteins. Ordinarily, ncRNAs are categorized into two types: regulatory
51 ncRNAs and structural ncRNAs [5]. For example, microRNAs (miRNAs), long non-coding
52 RNAs (lncRNAs), circular RNAs (circRNAs) and pseudogenes belong to the type of
53 regulatory ncRNAs, while ribosome RNAs (rRNAs) and transfer RNAs (tRNAs) are
54 considered as structural ncRNAs. Existing studies [6,7] have demonstrated that ncRNAs are
55 closely associated with the occurrence and development of malignant tumors, which opens a
56 promising avenue for the diagnosis and preventive treatment of malignant tumors.

57 As regulators, ncRNAs can modulate the expression of thousands of genes in the manner
58 of trans-acting and cis-acting [8], thereby driving cell biological responses and fates [7]. In
59 addition to the role of regulators, by soaking up miRNAs, ncRNAs can act as competing
60 endogenous RNAs (ceRNAs) [9] which compete with the target messenger RNAs (mRNAs)
61 of the soaked miRNAs. In the ceRNA context, miRNAs serve as mediators to connect non-
62 coding and coding RNAs, resulting in the ceRNA network (consisting of ceRNAs and
63 mRNAs), a more comprehensive regulatory network than the miRNA regulatory network
64 [10]. So far, three types of ncRNAs, including lncRNAs, circRNAs and pseudogenes have
65 been widely known to function as ceRNAs [11], and are regarded as potential carcinogenic
66 biomarkers in clinical applications [12,13]. For example, lncRNA *H19* serves as a ceRNA for
67 *LIN28* to promote breast cancer stem cell maintenance [14], circRNA *circTP63* functions as a
68 ceRNA for *FOXM1* to accelerate lung squamous cell carcinoma progression [15], and
69 pseudogene *PTENP1* acts as a ceRNA for *PTEN* in prostate cancer [16].

70 The ceRNA regulation of malignant tumors involves the interactions between multiple
71 non-coding and coding genes. In the ceRNA network in which ncRNAs are involved, the
72 competitive relationships between ncRNAs and target mRNAs are not one-to-one, but many-
73 to-many. For example, previous studies [17–25] have demonstrated that the three ncRNAs,
74 *H19*, *MALAT1* and *PVT1* synergistically compete with the four target genes, *KLF4*, *VEGFA*,
75 *ZEB1* and *ZEB2*. In this work, we call such collective competition by a group of ncRNAs

76 against a group of mRNAs as "ncRNA synergistic competition". It is observed that such
77 complicated gene regulation carries out important tasks in physiological and pathological
78 processes, and is considered to be a key component of cellular regulatory networks [7].
79 Furthermore, the entire cell system presents the characteristics of functional modularity, and
80 each gene functional module (i.e. a group of genes interacting or crosstalking with each other
81 frequently and not by chance) is responsible for specific biological functions in complex
82 human diseases, including malignant tumors [26]. These findings indicate that ncRNAs,
83 acting as ceRNAs, may synergistically rather than just independently play important roles in
84 malignant tumors.

85 In this work, to uncover potential carcinogenic biomarkers, we present the SCOM
86 (Synergistic COMpetition) framework to characterize ncRNA synergistic competition in Pan-
87 cancer. SCOM first systematically predicts ncRNA-related ceRNA networks across 31 tumor
88 types (containing 8001 tumor samples). Based on the proposed *ncRNA synergistic*
89 *competition hypothesis*, SCOM applies three criteria, (i) significant synergistic competition
90 for mRNAs, (ii) significant positive correlation, and (iii) significant sensitive correlation
91 conditioning on the mRNAs synergistically competed against by the ncRNAs, to predict the
92 ncRNA synergistic competition network (consisting of ncRNAs acting as ceRNAs) from the
93 gene expression data of ncRNAs and target mRNAs and the predicted ncRNA-related ceRNA
94 networks. By using SCOM, we provide a landscape of ncRNA synergistic competition in

95 Pan-cancer, contributing to the discovery of potential carcinogenic biomarkers. Ultimately,
96 SCOM provides a framework for discovering the relationships between the synergistic
97 competition ncRNAs (i.e. ncRNAs involved in the synergistic competition) and malignant
98 tumors, and can help to elucidate ncRNA synergistic competition mechanisms in malignant
99 tumors.

100 Materials and methods

101 Synergistic COMpetition (SCOM)

102 The proposed SCOM framework is based on the *ncRNA synergistic competition hypothesis*
103 (**Fig 1a**). For soaking up miRNAs, a pool of ncRNA transcripts (acting as ceRNAs) competes
104 with mRNAs through miRNA response elements (MREs). As a result, the competitive
105 interactions between ncRNAs and mRNAs form a ceRNA network (consisting of ncRNAs
106 acting as ceRNAs and target mRNAs). In the ceRNA network, we have observed that the
107 competitive relationships between ncRNAs and mRNAs are not one-to-one but many-to-
108 many, revealing synergistic competition of ncRNAs. Based on this observation, we
109 hypothesize that ncRNAs acting as ceRNAs can crosstalk with each other in the form of
110 synergistic competition (i.e. ncRNAs synergistically compete with mRNAs). We name the
111 hypothesis as the *ncRNA synergistic competition hypothesis*.

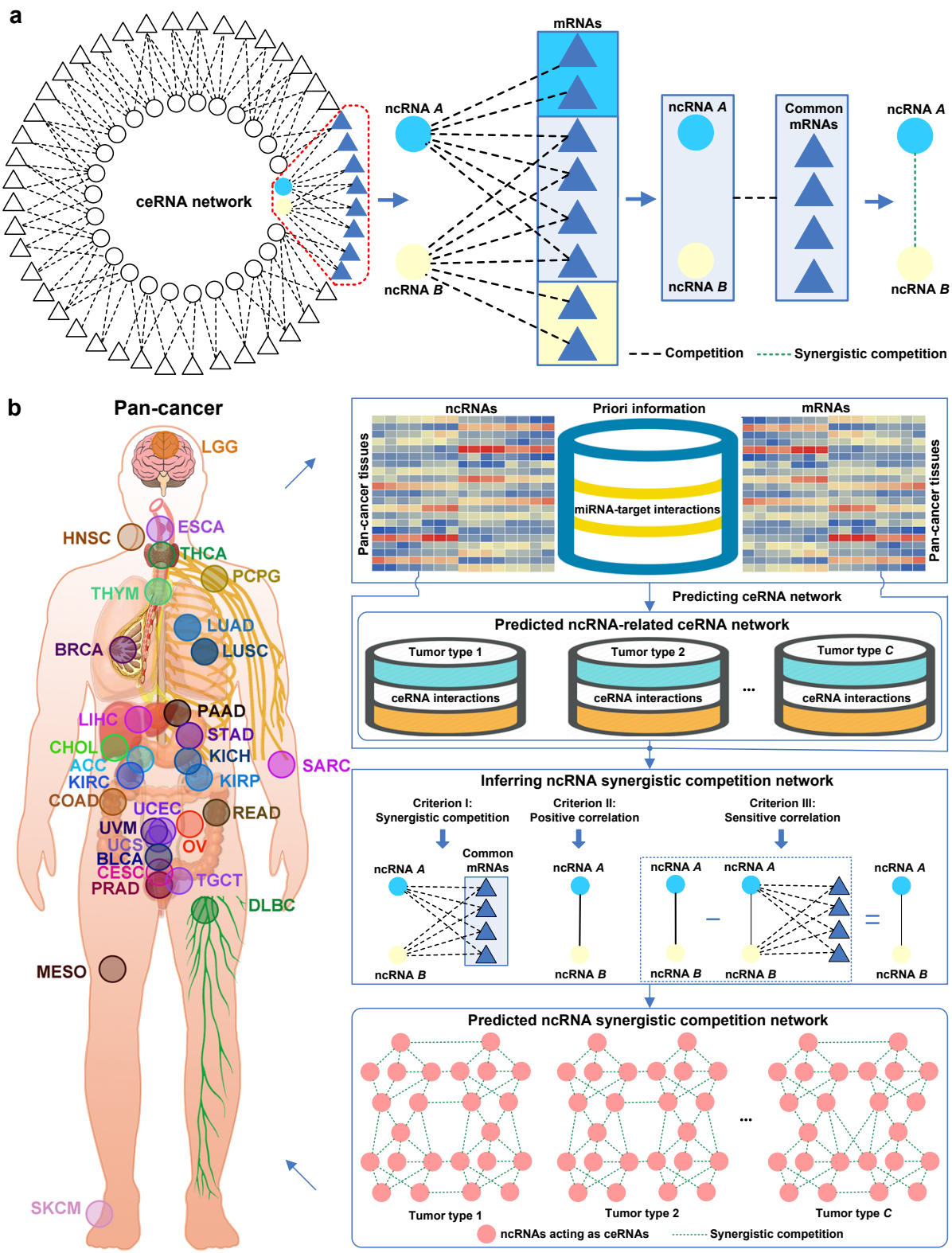


Fig 1. Schematic illustration of SCOM. (a) An illustration of the ncRNA synergistic competition hypothesis. ncRNA *A* and ncRNA *B* acting as ceRNAs collectively compete with mRNAs for soaking up miRNAs. In the context of ceRNA competition, the crosstalk between ncRNA *A* and ncRNA *B* is in the form of synergistic competition. (b) For each tumor type in Pan-cancer, SCOM firstly extends the SPONGE [33] method to predict ncRNA-related (including lncRNA- and pseudogene-related) ceRNA

network by incorporating gene (including miRNAs, lncRNAs, pseudogenes, and mRNAs) expression data in matched tumor tissues and priori information of miRNA-target (including miRNA-lncRNA, miRNA-pseudogene and miRNA-mRNA) interactions. By integrating gene (including lncRNAs and pseudogenes acting as ceRNAs, and target mRNAs) expression data in matched tumor tissues and predicted ncRNA-related ceRNA networks, SCOM utilise three criteria to infer ncRNA synergistic competition network for each tumor type in Pan-cancer. Based on the predicted ncRNA synergistic competition networks across 31 malignant tumors, SCOM further uncovers potential carcinogenic biomarkers.

The first step of SCOM (**Fig 1b**) is to predict ncRNA-related ceRNA network of each malignant tumor type. The matched ncRNA (including miRNA, lncRNA and pseudogene) and mRNA expression data in Pan-cancer (consisting of 8001 matched tumor samples) are obtained from The Cancer Genome Atlas (TCGA) [27] project. The information of miRNA targets is obtained by integrating five well-known databases, including miRTarBase v9.0 [28], TarBase v8.0 [29], LncBase v2.0 [30], NPInter 4.0 [31] and ENCORI [32]. By integrating gene (including ncRNAs and mRNAs) expression data and putative miRNA-target interactions, SCOM extends SPONGE [33] (a three-criterion evaluation method) to predict ncRNA-related ceRNA networks across 31 malignant tumors. The three-criterion evaluation method predicts ceRNA interactions by integrating sample-matched ncRNA (miRNA, lncRNA and pseudogene) and mRNA expression data, and putative miRNA-target interactions. We reason that if a candidate ncRNA-mRNA pair meets the three criteria (significant sharing of miRNAs, significantly positive correlation, and significantly sensitive correlation conditioning on shared miRNAs) with p-value < 0.05, the candidate ncRNA-mRNA pair is regarded as a ceRNA interaction. All of the predicted ceRNA interactions form

140 a large-scale ncRNA-related ceRNA network, with genes (including lncRNAs, pseudogenes
141 and mRNAs) denoted as nodes and the ncRNA-mRNA competition relationships as
142 undirected edges.

143 The second step of SCOM (**Fig 1b**) is to infer ncRNA synergistic competition network of
144 each malignant tumor type. For a malignant tumor type, to predict the ncRNA synergistic
145 competition network, we use the expression data of ncRNAs acting as ceRNAs and target
146 mRNAs and the predicted ncRNA-related ceRNA networks. Following the *ncRNA*
147 *synergistic competition hypothesis*, we consider a ncRNA-ncRNA pair having a synergistic
148 competition relationship if the pair have (i) significant synergistic competition with mRNAs,
149 (ii) significant positive correlation, and (iii) significant sensitive correlation conditioning on
150 their competing mRNAs. The synergistic competition network of a malignant tumor type is
151 then formed by integrating all of ncRNA synergistic competition relationships.

152 **Pan-cancer transcriptomics data**

153 After removing non-matching tumor samples between ncRNAs (including miRNAs,
154 lncRNAs and pseudogenes) and mRNA expression data, the Pan-cancer transcriptomics data
155 includes the expression data of 17,212 ncRNAs (including 894 miRNAs, 4366 lncRNAs, and
156 11,952 pseudogenes) and 19,068 mRNAs in 8001 matched tumor samples covering 31 tumor
157 types (**S1 Data**), and the clinical information of 8001 matched tumor samples (**S2 Data**).

158 **Putative miRNA-target interactions**

159 Three types of putative miRNA-target interactions, including miRNA-mRNA, miRNA-
160 lncRNA, and miRNA-pseudogene are used for predicting ncRNA-related ceRNA networks.
161 The putative miRNA-mRNA interactions are acquired from miRTarBase v9.0 [28] and
162 TarBase v8.0 [29], the putative miRNA-lncRNA interactions are obtained from LncBase v2.0
163 [30] and NPInter 4.0 [31], and the putative miRNA-pseudogene interactions are from
164 ENCORI [32] (the previous version is starBase). In total, we have 762,540 miRNA-mRNA
165 interactions, 225,063 miRNA-lncRNA interactions, and 91,619 miRNA-pseudogene
166 interactions as priori information of miRNA-target interactions.

167 **Predicting ceRNA networks in Pan-cancer**

168 Given the gene expression data of miRNAs, lncRNAs, pseudogenes, and mRNAs, and
169 miRNA-target interactions, three criteria, (i) significant sharing of miRNAs, (ii) significant
170 positive correlation, and (iii) significant sensitive correlation conditioning on shared miRNAs,
171 are used to predict ceRNA network (see **Supplementary Methods** in **S1 File**).

172 In this work, a candidate ncRNA-mRNA pair meeting the above three criteria with *p*-value
173 < 0.05 is regarded as a ceRNA interaction. For each of the 31 tumor types, we integrate all of
174 ceRNA interactions to form the ceRNA network of the tumor type.

175 **Constructing ncRNA synergistic competition networks in Pan-cancer**

176 Given the gene expression data of lncRNAs and pseudogenes acting as ceRNAs, and miRNA
177 targets mRNAs and the predicted ncRNA-related ceRNA networks, three criteria, (i)
178 significant synergistic competition for mRNAs, (ii) significant positive correlation, and (iii)
179 significant sensitive correlation conditioning on synergistically competed mRNAs are used to
180 construct ncRNA synergistic competition network.

181 The first criterion uses a hypergeometric test to assess the significance of synergistic
182 competition of ncRNA *i* and ncRNA *j* for mRNAs. The significance *p*-value of the test is
183 computed as:

184 $$p_{ij} = 1 - \sum_{x_{ij}=0}^{L_{ij}-1} \frac{\binom{M_{ij}}{x_{ij}}\binom{N_{ij}-M_{ij}}{K_{ij}-x_{ij}}}{\binom{N_{ij}}{K_{ij}}} \quad (1)$$

185 where N_{ij} is the number of all mRNAs in a Pan-cancer dataset, M_{ij} and K_{ij} represent the total
186 numbers of mRNAs competing with ncRNA *i* and ncRNA *j* respectively, and L_{ij} is the
187 number of mRNAs synergistically competed by ncRNA *i* and ncRNA *j*.

188 The second criterion requires that ncRNA *i* and ncRNA *j* are positively correlated. The
189 Pearson [34] method is also utilized to evaluate whether the expression level of ncRNA *i* and
190 the expression level of ncRNA *j* are positively correlated at a significant level (e.g. 0.05). The
191 correlation cor_{ij} between ncRNA *i* and ncRNA *j* is computed as:

192 $$cor_{ij}=cor(p,q)=\frac{\sum_{k=1}^{s}(p_k-\overline{p})(q_k-\overline{q})}{\sqrt{\sum_{k=1}^{s}(p_k-\overline{p})^2}\sqrt{\sum_{k=1}^{s}(q_k-\overline{q})^2}} \quad (2)$$

193 where $p=(p_1,p_2,...,p_s)$ and $q=(q_1,q_2,...,q_s)$ denote the expression level of ncRNA *i* and

194 ncRNA *j* respectively, $\overline{p}$ and $\overline{q}$ represent the average expression level of ncRNA *i* and ncRNA

195 *j* respectively, and *s* is the number of samples for each tumor type. Accordingly, the

196 significance *p*-value pc_{ij} is calculated as:

197 $$pc_{ij}=2pt\left(cor_{ij}\sqrt{\frac{s-2}{1-(cor_{ij})^2}}\right) \quad (3)$$

198 where the *pt* function is used to calculate the probability of $cor_{ij}\sqrt{\frac{s-2}{1-(cor_{ij})^2}}$.

199 The third criterion is used to evaluate the influence of synergistically competed mRNAs

200 on ncRNA *i* and ncRNA *j*. The sensitive correlation [35] is also employed to calculate the

201 influence of the mRNAs synergistically competed by ncRNA *i* and ncRNA *j*, and the null

202 model [33] is also applied to evaluate the significance of this influence. The sensitive

203 correlation sc_{ij} is calculated as:

204 $$sc_{ij}=cor_{ij}-pcor_{ij} \quad (4)$$

205 where $pcor_{ij}$ is the partial correlation between ncRNA *i* and ncRNA *j*, i.e. the correlation

206 conditioning on the mRNAs synergistically competed by the ncRNAs, which is the

207 correlation between ncRNA *i* and ncRNA *j* when the influence of the synergistically

208 competed mRNAs is eliminated. $pcor_{ij}$ is calculated as:

$$pcor_{ij} = cor(p,q \mid M) = cor(p,q \mid (M_1, M_2, ..., M_n))$$

209 $$= \frac{cor(p,q \mid (M_1, M_2, ..., M_{n\text{-}1})) - cor(p, M_n \mid (M_1, M_2, ..., M_{n-1}))cor(q, M_n \mid (M_1, M_2, ..., M_{n-1}))}{\sqrt{1 - cor(p, M_n \mid (M_1, M_2, ..., M_{n-1}))^2}\sqrt{1 - cor(q, M_n \mid (M_1, M_2, ..., M_{n-1}))^2}} \quad (5)$$

210 where *p*, *q* and *M* denote the expression level of ncRNA *i*, ncRNA *j*, and the synergistically

211 competed *n* mRNAs respectively, $cor(p,q \mid (M_1, M_2, ..., M_n))$ denotes the partial correlation

212 between *p* and *q* conditioning on $(M_1, M_2, ..., M_n)$, $cor(p,q \mid (M_1, M_2, ..., M_{n-1}))$ represents the

213 partial correlation between *p* and *q* conditioning on $(M_1, M_2, ..., M_{n-1})$,

214 $cor(p, M_n \mid (M_1, M_2, ..., M_{n-1}))$ is the partial correlation between *p* and M_n conditioning on

215 $(M_1, M_2, ..., M_{n-1})$, $cor(q, M_n \mid (M_1, M_2, ..., M_{n-1}))$ is the partial correlation between *q* and M_n

216 conditioning on $(M_1, M_2, ..., M_{n-1})$.

217 To evaluate the significance of sc_{ij}, the null model hypothesizes that the synergistically

218 competed mRNAs do not affect the correlation between ncRNA *i* and ncRNA *j*, i.e. the

219 sensitive correlation (the difference between cor_{ij} and $pcor_{ij}$) between ncRNA *i* and ncRNA *j*

220 is 0. The number of datasets sampled is set to 1E+03 for the null model, and the pre-

221 computed covariance matrices in the R package SPONGE [33] are used to build the null

222 model. Based on the constructed null model, the significance *p*-value of sc_{ij} is calculated.

223 In this study, a candidate ncRNA-ncRNA pair meeting the three criteria for synergistic

224 competition with *p*-value < 0.05 is considered as a ncRNA synergistic competition

225 relationship. For each tumor type, we combine all of the ncRNA synergistic competition

226 relationships to create the ncRNA synergistic competition network for the tumor type.

227 **Network topological analysis**

228 Network topological features yield useful insights into biological networks, e.g. degree, path
229 and density. If the node degree of a biological network obeys a power law distribution, the
230 biological network is regarded as a scale-free network. The path and density features are used
231 to measure the tightness of a biological network. Compared with the random networks, a
232 shorter characteristic path length or higher density of a biological network means that the
233 biological network is more likely to be a small-world network [36,37].

234 The topological features (degree, path and density) of ncRNA-related ceRNA networks
235 and ncRNA synergistic competition networks are analyzed using the R package igraph [38],
236 a useful tool for network analysis and visualization. The ncRNA-related ceRNA networks
237 and ncRNA synergistic competition networks are undirected networks, and the degree of a
238 node is the number of edges connected with the node. The Kolmogorov-Smirnov (KS) test
239 [39] is used to evaluate whether the node degree of a network follows a power law
240 distribution. If the *p*-value of the KS test is smaller than a cutoff (e.g. 0.05), the node degree
241 of a ncRNA-related ceRNA network or a ncRNA synergistic competition network does not
242 follow a power law distribution, then these indicate that the ncRNA-related ceRNA network
243 or the ncRNA synergistic competition network is not a scale-free network. To determine
244 whether the ncRNA-related ceRNA networks and the ncRNA synergistic competition
245 networks are small-world networks, we generate 100 random networks (the same number of

246 nodes and edges with real network) for each ncRNA-related ceRNA network or ncRNA
247 synergistic competition network. The Student's *t*-test [40] is applied to evaluate whether the
248 characteristic path length (or density) of a ncRNA-related ceRNA network or a ncRNA
249 synergistic competition network is smaller (or higher) than that of its corresponding random
250 networks at a significance level. For a ncRNA-related ceRNA network or a ncRNA
251 synergistic competition network, if the *p*-value of the Student's *t*-test is smaller than a cutoff
252 (e.g. 0.05), the characteristic path length (or density) of a ncRNA-related ceRNA network or
253 a ncRNA synergistic competition network is significantly smaller (or higher) than that of its
254 corresponding random networks, then the network is a small-world network.

255 **Network and hub similarity calculation**

256 In terms of ncRNA synergistic competition networks or hub ncRNAs, the network or hub
257 similarity between tumor *c* and tumor *d* is computed as follows.

258 $$sim_{cd} = \frac{|SCR_c \cap SCR_d|}{min(|SCR_c|, |SCR_d|)} \quad (6)$$

259 where SCR_c and SCR_d denote the identified ncRNA synergistic competition regulation
260 (networks or hub ncRNAs) in tumor *c* and tumor *d*, respectively, $|SCR_c \cap SCR_d|$ is the
261 number of common ncRNA synergistic competition relationships or hub ncRNAs between
262 SCR_c and SCR_d, and $min(|SCR_c|, |SCR_d|)$ denotes the smaller number of ncRNA
263 synergistic competition relationships or hub ncRNAs between SCR_c and SCR_d.

264 **Hub ncRNA identification**

265 Hub ncRNAs are defined as highly connected nodes in a ncRNA synergistic competition
266 network. Here, we use the cumulative probability of Poisson distribution to decide whether a
267 ncRNA is a hub ncRNA:

268 $$p(x \geq k) = 1 - \sum_{i=0}^{k-1} \frac{\lambda^i e^{-\lambda}}{i!} \quad (7)$$

269 where $\lambda = np$, $p = \frac{m}{A_n^2}$, n is the number of ncRNAs, m is the number of ncRNA synergistic
270 competition relationships in a ncRNA synergistic competition network, and A_n^2 is the number
271 of all possible ncRNA synergistic competition pairs. Smaller *p*-value of a ncRNA indicates
272 that the ncRNA is more likely to be a hub ncRNA. In this work, the cutoff of the *p*-value is
273 set to 0.05.

274 **Uncovering conserved and rewired interactions and hubs**

275 The ncRNA synergistic competition interaction or hub ncRNA existing in at least two
276 malignant tumors is defined as a conserved ncRNA synergistic competition interaction or hub
277 ncRNA, whereas the ncRNA synergistic competition interaction or hub ncRNA existing in
278 only one malignant tumor is defined as a rewired ncRNA synergistic competition interaction
279 or hub ncRNA. In the case of the identified ncRNA synergistic competition networks and hub
280 ncRNAs across 31 malignant tumors, we can uncover conserved and rewired ncRNA
281 synergistic competition interactions and hub ncRNAs.

282 **Multi-class classification analysis**

283 To understand the classification performance of the discovered conserved ncRNA synergistic
284 competition interactions and hub ncRNAs, we train a classification model to evaluate the
285 discriminating ability of the features in classifying tumor types, and also train a model to
286 classify BRCA subtypes (LumA, LumB, Her2, Basal and Normal) to understand the ability of
287 the features in discriminating cancer subtypes. We carry out multi-class classification
288 analysis by using the Binary Relevance(BR) [41] strategy in the R package utiml [42] to
289 perform multi-class classification analysis. In the BR strategy, the Support Vector Machine
290 (SVM) [43] implemented in the R package e1071 [44] is selected as the base classifier to
291 create the multi-class model. We use 22 metrics (accuracy, average-precision, clp, coverage,
292 F1, hamming-loss, macro-AUC, macro-F1, macro-precision, macro-recall, margin-loss,
293 micro-AUC, micro-F1, micro-precision, micro-recall, mlp, one-error, precision, ranking-loss,
294 recall, subset-accuracy and wlp as described in **S1 File**) and conduct 10-fold cross-validation
295 to evaluate the performance of ncRNAs in conserved ncRNA synergistic competitions and
296 hub ncRNAs. Also, we apply a commonly used baseline method [45] for comparison in the
297 evaluation of the classification performance.

298 **Survival analysis**

299 To compute the risk score of each malignant tumor sample, the multivariate Cox model [46]
300 in the R package survival [47] is fitted by using ncRNAs in conserved ncRNA synergistic

301 competition and hub ncRNAs as features. According to the risk scores of each malignant
302 tumor sample, the top one-third of the malignant tumor samples are put in the high risk group
303 and the remaining two thirds are put in the low risk group. In terms of the gene expression
304 levels of the ncRNAs, the Log-rank test is applied to assess the distinction of the feature
305 ncRNAs between the high and the low risk groups or between different molecular subtypes.
306 In addition, the proportional hazard ratio (HR) between the high and the low risk groups or
307 between different molecular subtypes is also computed.

308 **Enrichment analysis**

309 To understand the biological significance of hub ncRNAs across the 31 malignant tumors, we
310 conduct enrichment analysis in three categories, disease, cell marker and cancer hallmark
311 using LncSEA [48]. The hub ncRNAs are enriched in significant disease, cell marker and
312 cancer hallmark terms with an adjusted *p*-value (adjusted by Benjamini-Hochberg method)
313 cutoff of 0.05. Moreover, LncSEA is also used to uncover whether the synergistic
314 competition ncRNAs are involved in drug resistance or not. The synergistic competition
315 ncRNAs are significantly enriched in drug terms with an adjusted *p*-value (adjusted by
316 Benjamini-Hochberg method) less than 0.05.

317 **Molecular subtyping of malignant tumors**

318 Within the same type of malignant tumor, malignant tumors have great phenotypic
319 differences and genetic heterogeneity. Because of this, molecular subtyping is usually applied

320 to divide malignant tumors into subtypes with distinct molecular and clinical characteristics.
321 To assess the performance of the synergistic competition ncRNAs in molecular subtyping, we
322 use an ensemble method implemented in the CancerSubtypes [49] R package (combining the
323 Similarity Network Fusion (SNF) [50] and Consensus Clustering (CC) [51] methods) to
324 generate malignant tumor subtypes. For each malignant tumor, the number of molecular
325 subtypes is empirically set according to the previous molecular subtyping studies (**S3 Data**).

326 **Immune regulation analysis**

327 Numerous ncRNAs have been shown to play important roles in immune regulation. For the
328 immune regulation analysis, we obtain the significant immune-related ncRNA-pathway and
329 ncRNA-cell pairs from ImmReg [52]. Based on the significance of the enrichment of
330 ncRNAs with immune pathways and the significance of the correlation between ncRNAs and
331 immune cells, we further determine which synergistic competition ncRNAs are significantly
332 enriched in immune pathways and correlated with immune cells in each malignant tumor.

333 Results

334 **The ceRNA regulation landscape in Pan-cancer**

335 Using the transcriptomics data in TCGA, we apply SCOM for detecting two types of ceRNA
336 regulation landscape across the 31 malignant tumors. In the ceRNA regulation landscape, the
337 two types of ceRNA networks (lncRNA- and pseudogene-related ceRNA networks) have
338 different numbers of ceRNA interactions across the 31 malignant tumors. We have found that

339 the number of ceRNA interactions of the pseudogene-related ceRNA networks is
340 significantly larger than that of the lncRNA-related ceRNA networks (**Fig 2a**, *p*-value =
341 1.84E-13 with paired Student's *t*-test). Network topological analysis reveals that 5 out of the
342 31 (16.13%) lncRNA-related ceRNA networks and 16 out of the 31 (51.61%) pseudogene-
343 related ceRNA networks follow a power law distribution (**Fig 2b**). After merging the lncRNA
344 and pseudogene related ceRNA networks of each tumor, 14 out of the 31 (45.16%) merged
345 ncRNA-related ceRNA networks follow a power law distribution (**Fig S1** in **S1 File**).
346 Moreover, 7 out of the 31 (22.58%) lncRNA-related ceRNA networks and none of the
347 pseudogene-related ceRNA networks have shorter characteristic path lengths than their
348 corresponding random networks, and 30 out of the 31 (96.77%) lncRNA (and pseudogene
349 related) ceRNA networks have higher densities than their corresponding random networks
350 (**Fig 2b**). In the merged ncRNA-related ceRNA networks of each tumor, we have found that
351 none of the ncRNA-related ceRNA networks have shorter characteristic path lengths than
352 their corresponding random networks, and all of the ncRNA-related ceRNA networks have
353 higher densities than their corresponding random networks (**Fig S1** in **S1 File**). These
354 observations indicate that less than or nearly half of the ceRNA networks across the 31
355 malignant tumors tend to be scale-free networks, and most or all of the ceRNA networks
356 across the 31 malignant tumors are likely to be small-world networks with higher densities
357 than their corresponding random networks.

358 With the lncRNA and pseudogene related ceRNA networks, the similarity intervals
359 between the 31 malignant tumors are [0.13 0.52] and [0.10 0.44], respectively (**Fig 2c**).
360 Moreover, in terms of the ncRNA (including both lncRNA and pseudogene) related ceRNA
361 networks, the similarity interval between the 31 malignant tumors is [0.12 0.47] (**Fig S2** in **S1**
362 **File**). This result shows that the malignant tumors may have common ncRNA-related ceRNA
363 regulation. We have also discovered that each tumor ceRNA network varies in similarity to
364 other tumor ceRNA networks, revealing known and hidden relationships among these
365 malignant tumors. In addition, we have observed that ~16.45% lncRNA-related ceRNA
366 regulations and ~28.58% pseudogene-related ceRNA regulations only exist in one malignant
367 tumor (**Fig 2d**). Overall, ~24.80% ncRNA-related ceRNA regulations are occurred in one
368 malignant tumor (**Fig S3** in **S1 File**). For malignant tumors, the percentages of the rewired
369 ncRNA-related ceRNA regulations are different, demonstrating different degrees of
370 heterogeneity across malignant tumors (**Fig S4** in **S1 File**). Particularly, 106 (less than 0.01%)
371 ncRNA-related ceRNA regulations are conserved in the 31 malignant tumors (**Fig 2e**),
372 indicating a low conservation of ceRNA regulation in Pan-cancer. By conducting the multi-
373 class classification and survival analysis, we have shown that the conserved ncRNA-related
374 ceRNA regulations in the 31 malignant tumors have better performance than the baseline
375 method in classifying 31 malignant tumors, and can distinguish the high and the low risk
376 groups of each malignant tumor at a significant level (Log-rank p-value < 0.05 and HR > 2)

(**Fig S5** in **S1 File**). This result reveals that the conserved ncRNA-related ceRNA regulations may act as a common core of ceRNA regulation across malignant tumors.

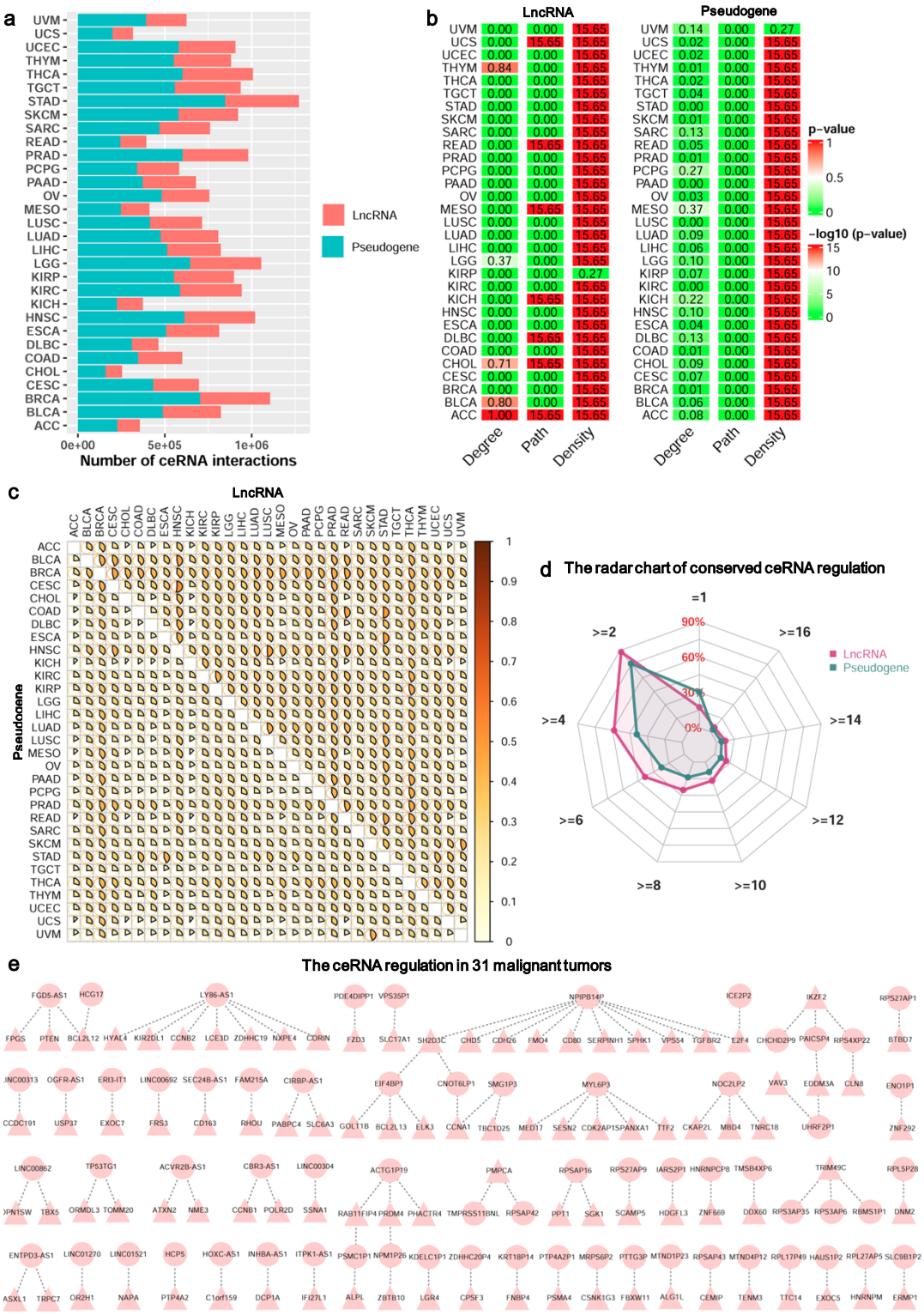


Fig 2. The ceRNA regulation landscape across malignant tumors. (a) The number of ncRNA-related ceRNA interactions in each malignant tumor. (b) The topological characteristics of ncRNA-related ceRNA

382 networks for the 31 malignant tumors. The Degree column is the *p*-value of fitting power-law distribution.
383 The *p*-values equal to or more than 0.05 indicate that the degree of ncRNA-related ceRNA networks obeys
384 the power-law distribution. The Path and Density columns represent the –log10(*p*-value) of Student's *t*-test,
385 and the *p*-values less than 0.05 display that the characteristic path lengths (or densities) of ncRNA-related
386 ceRNA networks are significantly shorter (or higher) than those of their corresponding random networks.
387 (c) The similarity matrix shows the similarity between each pair of lncRNA (upper triangle) and
388 pseudogene (lower triangle) related ceRNA networks across 31 malignant tumors. (d) The radar chart
389 shows the percentage of ceRNA regulations predicted in different numbers of malignant tumors. (e) The
390 conserved ceRNA regulation predicted in 31 malignant tumors.

391 **The ncRNA synergistic competition landscape in Pan-cancer**

392 Taking advantage of the transcriptomics data in TCGA and the predicted ncRNA-related
393 ceRNA interactions, we utilise SCOM for uncovering ncRNA (including lncRNAs and
394 pseudogenes) synergistic competition landscape across 31 malignant tumors. Similar to the
395 number of ceRNA interactions, the number of ncRNA synergistic competition interactions
396 (including lncRNA-lncRNA, lncRNA-pseudogene and pseudogene-pseudogene synergistic
397 competition pairs) also varies across the 31 malignant tumors. The largest and least numbers
398 of ncRNA synergistic competition interactions are inferred in Stomach adenocarcinoma
399 (STAD) and Cholangiocarcinoma (CHOL), respectively (**Fig 3a**). This result may be
400 explained in part by the different number of ceRNA interactions predicted in the 31
401 malignant tumors. Network topological analysis shows that 27 out of the 31 (87.10%)
402 ncRNA synergistic competition networks follow a power law distribution (**Fig 3b**).
403 Furthermore, 19 out of the 31 (61.29%) ncRNA synergistic competition networks have
404 shorter characteristic path lengths than their corresponding random networks, and all of the

405 ncRNA synergistic competition networks have higher densities than their corresponding
406 random networks (**Fig 3b**). These findings indicate that most of the ncRNA synergistic
407 competition networks across the 31 malignant tumors are scale-free networks, and small-
408 world networks with shorter characteristic path lengths and higher densities than their
409 corresponding random networks.

410 With the ncRNA synergistic competition networks, the similarity among the 31 malignant
411 tumors is less than 6% (**Fig 3c**). This low similarity indicates that the malignant tumors may
412 share a small portion of ncRNA synergistic competition with each other. In addition, ~94.06%
413 ncRNA synergistic competition interactions only occur in one malignant tumor, and none of
414 the ncRNA synergistic competition interactions exist in more than six malignant tumors (**Fig**
415 **3d**). Specifically, we have observed that the percentage of the conserved ncRNA synergistic
416 competition interactions in at least two malignant tumors is ~5.94%. This result reveals that
417 the ncRNA synergistic competition interactions of different malignant tumors tend to be
418 rewired in their respective tumor microenvironments. To understand whether the ncRNAs of
419 the conserved synergistic competition in at least two malignant tumors are biologically
420 meaningful, we further perform enrichment analysis in three categories including diseases,
421 cell markers and cancer hallmarks. The functional enrichment analysis has revealed that the
422 ncRNAs of the conserved synergistic competition in at least two malignant tumors are
423 significantly enriched in diseases (e.g. Cancer), cell markers (e.g. Exhausted CD8+ T cell),

and cancer hallmarks (e.g. Epithelial-mesenchymal transition) common to multiple malignant tumors (**S4 Data**). Moreover, the percentage of the rewired ncRNA synergistic competition for each malignant tumor is all more than 80%, demonstrating a great heterogeneity across malignant tumors in terms of ncRNA synergistic competition (**Fig 3e**).

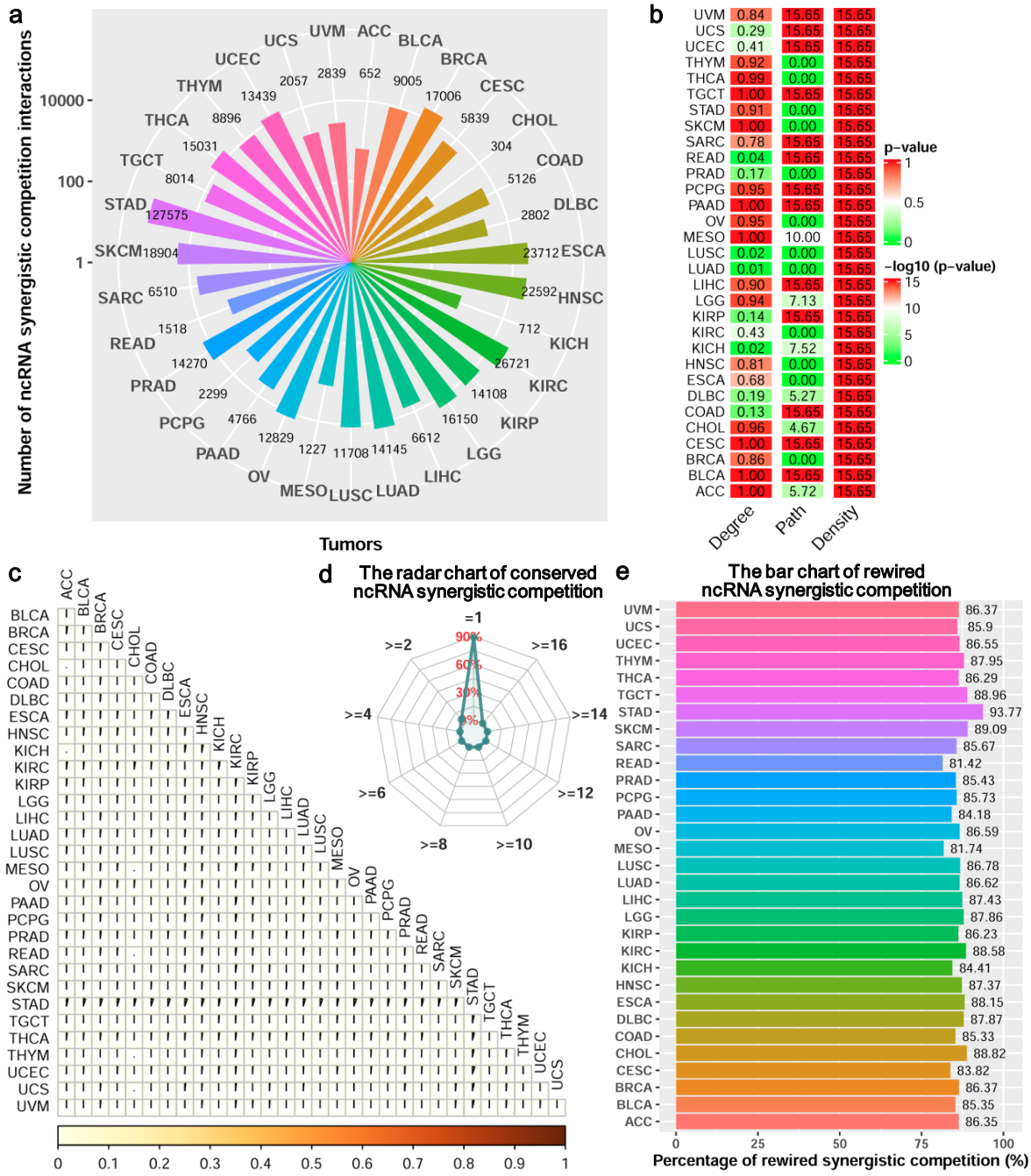


Fig 3. Network analysis of ncRNA synergistic competition across malignant tumors. (a) The number of ncRNA synergistic competition interactions in each malignant tumor. (b) The topological characteristics

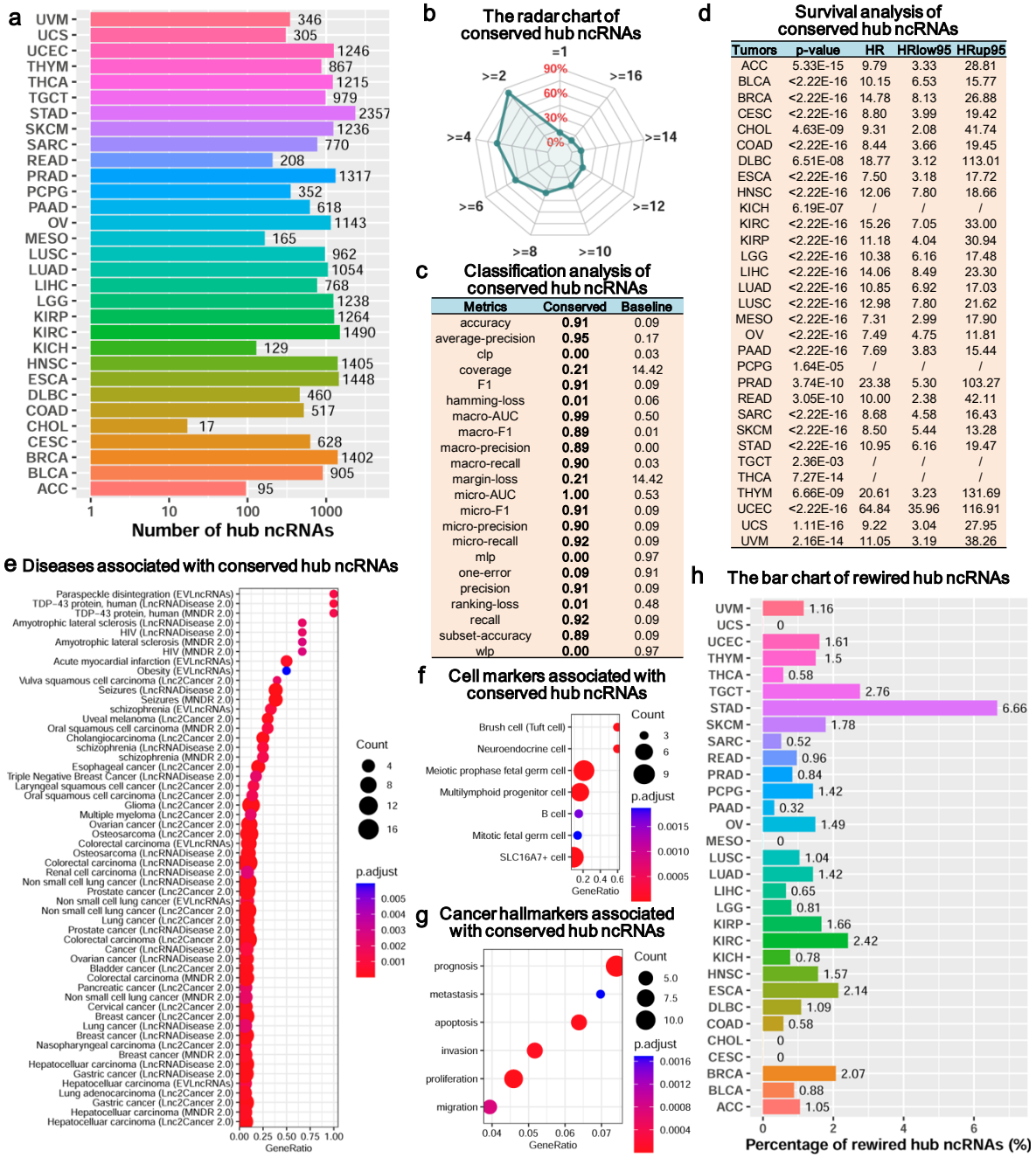
431 of ncRNA synergistic competition networks for the 31 malignant tumors. The Degree column is the *p*-
432 value of fitting power-law distribution. The *p*-values equal to or more than 0.05 indicate that the degree of
433 the ncRNA synergistic competition networks obeys the power-law distribution. The Path and Density
434 columns represent the –log10(*p*-value) of Student's *t*-test, and the *p*-values less than 0.05 suggest that the
435 characteristic path lengths (or densities) of ncRNA synergistic competition networks are significantly
436 shorter (or higher) than those of their corresponding random networks. (c) The similarity matrix showing
437 the similarity between each pair of ncRNA synergistic competition networks across 31 malignant tumors.
438 (d) The radar chart showing the percentages of ncRNA synergistic competition interactions predicted in
439 different number of malignant tumors. (e) The bar chart showing the percentages of ncRNA synergistic
440 competition interactions predicted in one malignant tumor.

441 Based on the topological features of the ncRNA synergistic competition networks, we
442 further identify hub ncRNAs associated with malignant tumors. We have discovered that the
443 number of hub ncRNAs vary across different malignant tumors (**Fig 4a**). In terms of the hub
444 ncRNAs, the similarity between the 31 malignant tumors is [0.07 0.79] (**Fig S6** in **S1 File**).
445 This result shows that the malignant tumors may have common hub ncRNAs. In addition, we
446 have observed that the hub ncRNAs can significantly distinguish the high risk and the low
447 risk groups of each malignant tumor (**Fig S7a** in **S1 File**), and the hub ncRNAs covering
448 96.77% malignant tumors are significantly enriched at least one of Disease, Cell markers and
449 Cancer hallmarks terms (**Fig S7b** in **S1 File**, **S5 Data**). Different from the ncRNA synergistic
450 competition networks, less than 13% hub ncRNAs are only in one malignant tumor but nearly
451 90% hub ncRNAs exist in at least two malignant tumors (**Fig 4b**). In particular, ~7.63% hub
452 ncRNAs are even conserved in at least 16 malignant tumors. This result reveals that the hub
453 ncRNAs of different malignant tumors tend to be conserved in their respective tumor
454 microenvironments.

455 To understand whether there is a common core of hub ncRNAs across malignant tumors,
456 we investigate the conserved hub ncRNAs occurred in at least 16 malignant tumors.
457 Functional analysis indicates that the conserved hub ncRNAs have better performance than
458 the baseline method in classifying the 31 malignant tumors (**Fig 4c**), and can significantly
459 distinguish the high risk and the low risk groups of each malignant tumor (**Fig 4d**). This
460 result shows that the conserved hub ncRNAs may act as potential diagnostic and prognostic
461 biomarkers across malignant tumors. Moreover, the conserved hub ncRNAs are significantly
462 enriched in diseases (**Fig 4e**), cell markers (**Fig 4f**) and cancer hallmarks (**Fig 4g**) common to
463 several malignant tumors. This result suggests that the malignant tumors may have common
464 cell markers and cancer hallmarks.

465 In addition to the conserved hub ncRNAs, the rewired hub ncRNAs switching from tumor
466 to tumor are important to understand the heterogeneity of malignant tumors. We have found
467 that the percentage of the rewired hub ncRNAs for each malignant tumor is less than 7%,
468 revealing a small heterogeneity across malignant tumors in terms of hub ncRNAs (**Fig 4h**).
469 Multi-class classification analysis shows that the rewired hub ncRNAs have better
470 performance than the baseline method in classifying the 31 malignant tumors (**Fig S7c** in **S1**
471 **File**). Moreover, survival analysis displays that the rewired hub ncRNAs can significantly
472 distinguish the high risk and the low risk groups of each malignant tumor (**Fig S7d** in **S1**

File). This result suggests that the rewired hub ncRNAs may also act as potential diagnostic and prognostic biomarkers across malignant tumors.



Metrics	Conserved	Baseline
accuracy	**0.91**	0.09
average-precision	**0.95**	0.17
clp	**0.00**	0.03
coverage	**0.21**	14.42
F1	**0.91**	0.09
hamming-loss	**0.01**	0.06
macro-AUC	**0.99**	0.50
macro-F1	**0.89**	0.01
macro-precision	**0.89**	0.00
macro-recall	**0.90**	0.03
margin-loss	**0.21**	14.42
micro-AUC	**1.00**	0.53
micro-F1	**0.91**	0.09
micro-precision	**0.90**	0.09
micro-recall	**0.92**	0.09
mlp	**0.00**	0.97
one-error	**0.09**	0.91
precision	**0.91**	0.09
ranking-loss	**0.01**	0.48
recall	**0.92**	0.09
subset-accuracy	**0.89**	0.09
wlp	**0.00**	0.97

Tumors	p-value	HR	HRlow95	HRup95
ACC	5.33E-15	9.79	3.33	28.81
BLCA	<2.22E-16	10.15	6.53	15.77
BRCA	<2.22E-16	14.78	8.13	26.88
CESC	<2.22E-16	8.80	3.99	19.42
CHOL	4.63E-09	9.31	2.08	41.74
COAD	<2.22E-16	8.44	3.66	19.45
DLBC	6.51E-08	18.77	3.12	113.01
ESCA	<2.22E-16	7.50	3.18	17.72
HNSC	<2.22E-16	12.06	7.80	18.66
KICH	6.19E-07	/	/	/
KIRC	<2.22E-16	15.26	7.05	33.00
KIRP	<2.22E-16	11.18	4.04	30.94
LGG	<2.22E-16	10.38	6.16	17.48
LIHC	<2.22E-16	14.06	8.49	23.30
LUAD	<2.22E-16	10.85	6.92	17.03
LUSC	<2.22E-16	12.98	7.80	21.62
MESO	<2.22E-16	7.31	2.99	17.90
OV	<2.22E-16	7.49	4.75	11.81
PAAD	<2.22E-16	7.69	3.83	15.44
PCPG	1.64E-05	/	/	/
PRAD	3.74E-10	23.38	5.30	103.27
READ	3.05E-10	10.00	2.38	42.11
SARC	<2.22E-16	8.68	4.58	16.43
SKCM	<2.22E-16	8.50	5.44	13.28
STAD	<2.22E-16	10.95	6.16	19.47
TGCT	2.36E-03	/	/	/
THCA	7.27E-14	/	/	/
THYM	6.66E-09	20.61	3.23	131.69
UCEC	<2.22E-16	64.84	35.96	116.91
UCS	1.11E-16	9.22	3.04	27.95
UVM	2.16E-14	11.05	3.19	38.26

Fig 4. Hub analysis of ncRNA synergistic competition across malignant tumors. (a) The number of hub ncRNAs in each malignant tumor. (b) The radar chart displaying the percentage of hub ncRNAs predicted in different number of malignant tumors. (c) Multi-class classification analysis of conserved hub ncRNAs in at least 16 malignant tumors. (d) Survival analysis of conserved hub ncRNAs in at least 16 malignant tumors. The symbol "/" stands for an infinite value. (e-g) Enrichment analysis (three categories

481 including disease, cell marker and cancer hallmark) of conserved hub ncRNAs in at least 16 malignant
482 tumors. (h) The bar chart indicating the percentage of hub ncRNAs predicted in one malignant tumor.

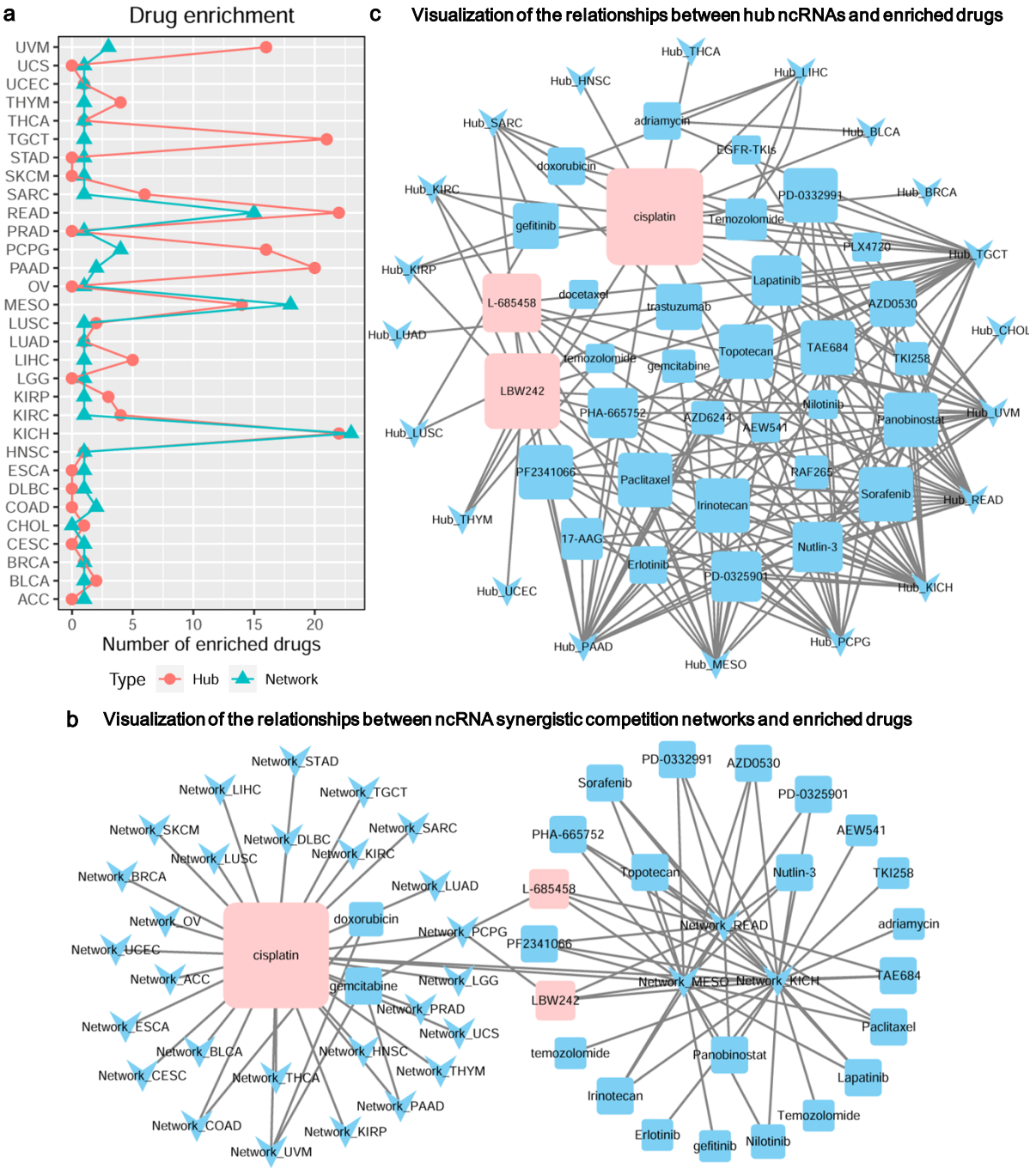
483 **Synergistic competition ncRNAs are involved in drug resistance**

484 It is reported that ncRNAs play important roles in drug resistance, and the dysregulation of
485 them can induce resistance to anti-cancer therapy and cause a failure of malignant tumor
486 clinical treatment [53,54]. Drug enrichment analysis shows that the ncRNA synergistic
487 competition networks of ~96.77% of the 31 malignant tumors and the hub ncRNAs of ~64.52%
488 of the 31 malignant tumors are significantly enriched in at least one drug resistance term (**Fig**
489 **5a**, **S6 Data and S7 Data**). We have found that the ncRNA synergistic competition networks
490 of 29 malignant tumors are involved in the drug *cisplatin* resistance (**Fig 5b**). The drug
491 *cisplatin* is a well-known chemotherapy medication, and can be used to treat a number of
492 cancers including bladder cancer, brain tumors, cervical cancer, esophageal cancer, head and
493 neck cancer, lung cancer, mesothelioma, ovarian cancer, etc [55]. Moreover, the resistance of
494 two drugs *L-685458* and *LBW242* is closely associated with the ncRNA synergistic
495 competition networks of 4 malignant tumors (**Fig 5b**). The drug *L-685458* is a potent
496 transition state analog (TSA) γ-secretase inhibitor (GSI) and is an attractive therapeutic
497 candidate for Alzheimer Disease (AD) and cancers [56], and the drug *LBW242* is a potent and
498 orally active proapoptotic IAP inhibitor and potentiates treat multiple myeloma and ovarian
499 cancer [57,58]. Similar to the ncRNA synergistic competition networks, the top three
500 enriched drugs of hub ncRNAs across malignant tumors are also *cisplatin*, *L-685458* and

501 *LBW242* (**Fig 5c**). This result suggests that the synergistic competition ncRNAs (i.e. ncRNAs

502 involved in the synergistic competition) across malignant tumors are involved in drug

503 resistance, and may act as targets for anti-cancer therapy.



504

505 **Fig 5. Drug resistance analysis of ncRNA synergistic competition networks and hub ncRNAs across**

506 **malignant tumors.** (a) The number of significantly enriched drugs for each malignant tumor. (b)

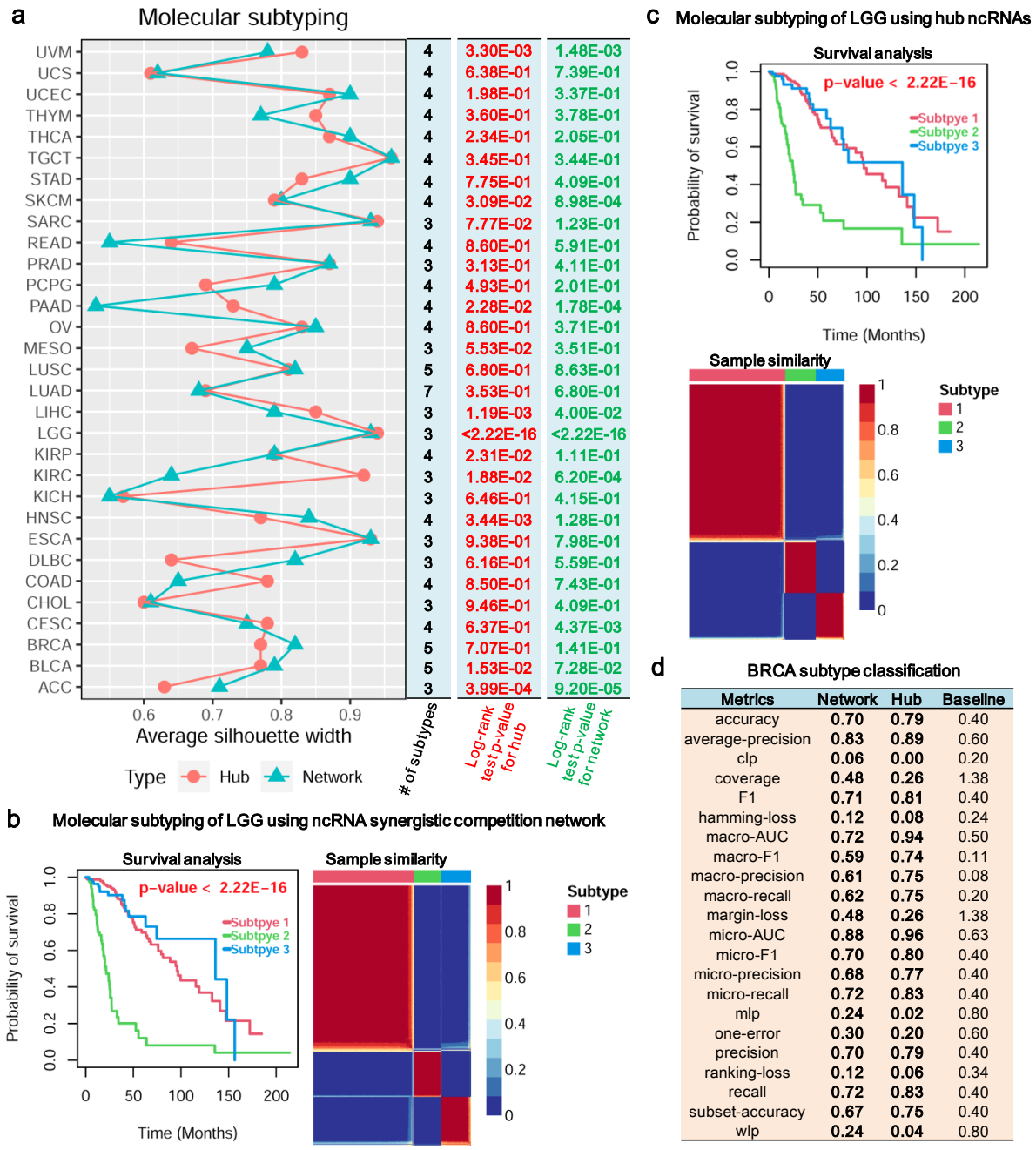
507 Relationships between ncRNA synergistic competition networks and enriched drugs. For example, the

508 node "Network_ACC" denotes the ncRNA synergistic competition network in ACC. Larger drug nodes
509 denote more links with ncRNA synergistic competition networks in malignant cancers. (c) Relationships
510 between hub ncRNAs and enriched drugs. For example, the node "Hub_ACC" denotes the hub ncRNAs in
511 ACC. Larger drug nodes denote more links with hub ncRNAs in malignant cancers.

512 **Synergistic competition ncRNAs contribute to distinguishing molecular subtypes of**
513 **malignant tumors**

514 Malignant tumors are a collection of heterogeneous diseases with both phenotypic and
515 genetic heterogeneity, and a malignant tumor usually possess multiple distinct molecular
516 subtypes [59]. Molecular subtyping analysis of the synergistic competition ncRNAs (i.e.
517 ncRNAs involved in the synergistic competition) shows that the average silhouette width of
518 the molecular subtyping of ~90.32% of the 31 malignant tumors is equal to or greater than
519 0.60 (**Fig 6a**), indicating that the synergistic competition ncRNAs can well assign malignant
520 tumor samples into distinct molecular subtypes. In the case of using hub ncRNAs, the
521 molecular subtypes of ~32.26% of the 31 malignant tumors can be significantly distinguished
522 (Log-rank test p-value < 0.05, **Fig 6a**). Moreover, in the case of using ncRNA synergistic
523 competition networks, the molecular subtypes of ~25.81% of the 31 malignant tumors can be
524 significantly distinguished (Log-rank test p-value < 0.05, **Fig 6a**). For seven malignant tumor
525 types, including Adrenocortical carcinoma (ACC), Kidney renal clear cell carcinoma (KIRC),
526 Brain Lower Grade Glioma (LGG), Liver hepatocellular carcinoma (LIHC), Pancreatic
527 adenocarcinoma (PAAD), Skin Cutaneous Melanoma (SKCM) and Uveal Melanoma (UVM),
528 the hub ncRNAs and ncRNA synergistic competition networks all have contributions to

529 distinguish molecular subtypes of them. For example, in the case of using hub ncRNAs and
530 ncRNA synergistic competition networks, LGG can be divided into three distinct molecular
531 subtypes (**Fig 6b** and **Fig 6c**). This result reveals that the synergistic competition ncRNAs
532 contribute to distinguishing molecular subtypes of malignant tumors, and may act as
533 prognostic biomarkers in several malignant tumors. Since the molecular subtypes (including
534 Luminal A, Luminal B, HER-2, Basal, Normal-like) of Breast invasive carcinoma (BRCA)
535 are mostly accepted by researchers, we focus on studying the BRCA subtype classification
536 performance by using hub ncRNAs and ncRNA synergistic competition networks in BRCA.
537 Multi-class classification analysis shows that the synergistic competition ncRNAs have better
538 performance than the baseline method in classifying BRCA subtypes (**Fig 6d**), suggesting
539 that the synergistic competition ncRNAs may act as potential diagnostic biomarkers of
540 BRCA.



d

BRCA subtype classification

Metrics	Network	Hub	Baseline
accuracy	**0.70**	**0.79**	0.40
average-precision	**0.83**	**0.89**	0.60
clp	**0.06**	**0.00**	0.20
coverage	**0.48**	**0.26**	1.38
F1	**0.71**	**0.81**	0.40
hamming-loss	**0.12**	**0.08**	0.24
macro-AUC	**0.72**	**0.94**	0.50
macro-F1	**0.59**	**0.74**	0.11
macro-precision	**0.61**	**0.75**	0.08
macro-recall	**0.62**	**0.75**	0.20
margin-loss	**0.48**	**0.26**	1.38
micro-AUC	**0.88**	**0.96**	0.63
micro-F1	**0.70**	**0.80**	0.40
micro-precision	**0.68**	**0.77**	0.40
micro-recall	**0.72**	**0.83**	0.40
mlp	**0.24**	**0.02**	0.80
one-error	**0.30**	**0.20**	0.60
precision	**0.70**	**0.79**	0.40
ranking-loss	**0.12**	**0.06**	0.34
recall	**0.72**	**0.83**	0.40
subset-accuracy	**0.67**	**0.75**	0.40
wlp	**0.24**	**0.04**	0.80

Fig 6. Molecular subtype analysis of malignant tumors. (a) Molecular subtyping of the 31 malignant tumors using ncRNA synergistic competition networks and hub ncRNAs. Higher average silhouette width indicates that a malignant tumor sample is better matched with the identified molecular subtypes. (b) Molecular subtyping of LGG using ncRNA synergistic competition network. (c) Molecular subtyping of LGG using hub ncRNAs. (d) Classification analysis of BRCA subtypes.

547 **Synergistic competition ncRNAs participate in immune regulation**

548 As regulatory elements in immune system, ncRNAs can influence immune development and
549 function, leading to immune diseases including autoimmunity and haematological cancers
550 [60,61]. Immune regulation analysis reveals that the ncRNA synergistic competition
551 networks of all the 31 malignant tumors and the hub ncRNAs of 30 malignant tumors are
552 significantly involved in at least one immune-related pathway (**Fig 7a**, **S8 Data and S9**
553 **Data**). For instance, the ncRNA synergistic competition networks of the 31 malignant tumors
554 are closely associated with six immune-related pathways (*Antigen Processing and*
555 *Presentation*, *Cytokine Receptors*, *Antimicrobials*, *Cytokines*, *Natural Killer Cell Cytotoxicity*,
556 *TCR signaling Pathway*) (**Fig 7c**). In addition, the hub ncRNAs of 29 malignant tumors are
557 closely related to two immune-related pathways (*Antigen Processing and Presentation*,
558 *Cytokine Receptors*) (**Fig 7d**). Moreover, the ncRNA synergistic competition networks and
559 the hub ncRNAs of 31 malignant tumors are significantly associated with at least 40 immune
560 cells (**Fig 7b**, **S10 Data and S11 Data**). This result suggests that the synergistic competition
561 ncRNAs (i.e. ncRNAs involved in the synergistic competition) take part in immune
562 regulation, and the dysregulation of them may influence the occurrence and development of
563 immune diseases including malignant tumors.

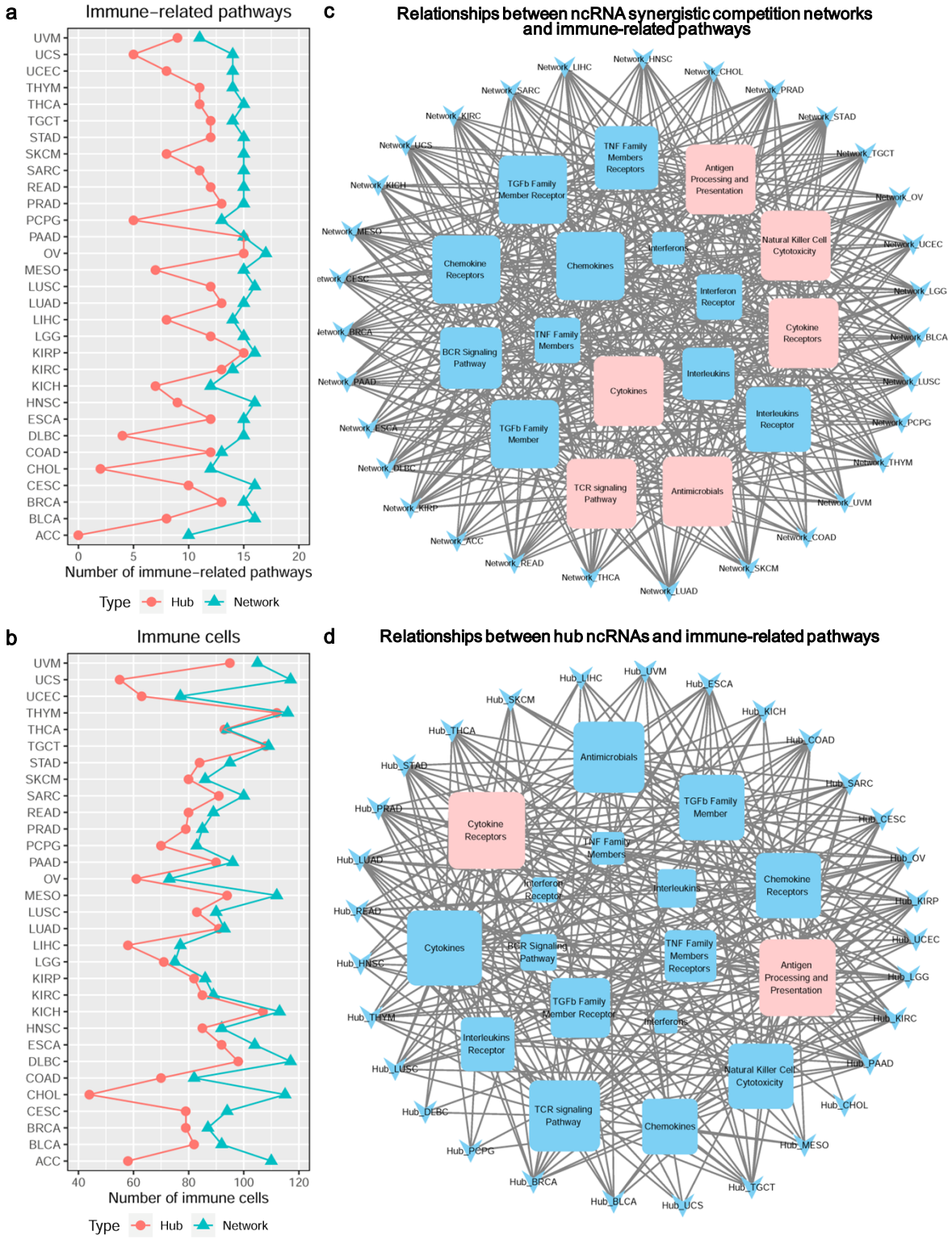


Fig 7. Immune regulation analysis of synergistic competition ncRNAs across malignant tumors. (a) The number of significant immune-related pathways associated with each malignant tumor. (b) The number of significant immune cells associated with each malignant tumor. (c) Relationships between ncRNA synergistic competition networks and immune-related pathways. For example, the node "Network_ACC" denotes the ncRNA synergistic competition network in ACC. Larger immune-related pathway nodes denote more links with ncRNA synergistic competition networks in malignant cancers. (d)

571 Relationships between hub ncRNAs and immune-related pathways. For example, the node "Hub_ACC"
572 denotes the hub ncRNAs in ACC. Larger immune-related pathway nodes denote more links with hub
573 ncRNAs in malignant cancers.

574 **SCOMdb: a web-based resource for ncRNA synergistic competition in Pan-cancer**

575 To provide a convenient and web-accessible resource of ncRNA synergistic competition
576 landscape across malignant tumors for biologists, we have developed a comprehensive and
577 interactive database, SCOMdb (www.comblab.cn/SCOMdb/). By using this web-based
578 resource, users can browse the ceRNA interactions in specific malignant tumor of interest
579 (**Fig 8a**). It also provides the ncRNA synergistic competition interactions in malignant tumors
580 (**Fig 8b**). In addition, users can query the hub ncRNAs of interest across malignant tumors
581 (**Fig 8c**). All the data in this work can be downloaded for downstream analysis (**Fig 8d**). To
582 help users apply SCOM to new datasets, we have also provided the SCOM R package for
583 inferring ncRNA synergistic competition by integrating sample-matched gene (ncRNA and
584 mRNA) expression data and putative miRNA-target interactions (**Fig 8e**). The provided
585 resources will be continuously updated, and should serve as a valuable resource for biologists
586 interested in investigating ncRNA synergistic competition across malignant tumors.

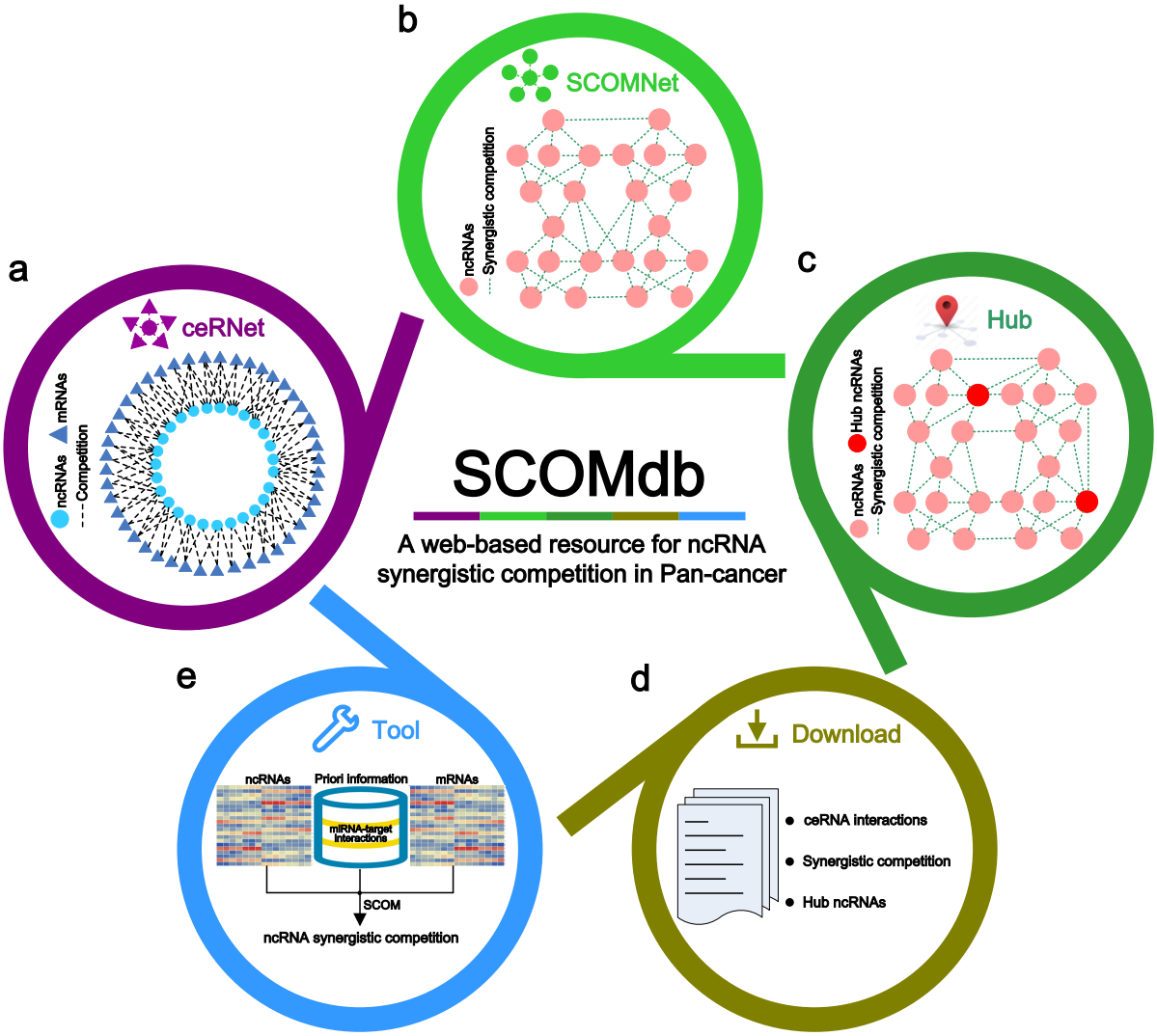


Fig 8. Diagram of SCOMdb database. (a) The search of ceRNA regulation in Pan-cancer. (b) The search of ncRNA synergistic competition in Pan-cancer. (c) The search of hub ncRNAs in Pan-cancer. (d) All resources are provided to be downloaded for further analysis. (e) The SCOM method is available for users to study ncRNA synergistic competition by using their own datasets.

Conclusions and discussion

Abundant evidence reveals that ncRNAs can serve as important regulators across malignant tumors. However, from the perspective of synergistic competition, ncRNA regulation is still unearthed. In this work, we present the *ncRNA synergistic competition hypothesis* and the SCOM framework, and further elaborate the application of the SCOM framework to

597 comprehensively infer the synergistic competition ncRNAs that potentially act as
598 carcinogenic biomarkers. From the perspective of synergistic competition, we have shown
599 that the synergistic competition ncRNAs are potential diagnostic and prognostic biomarkers
600 of malignant tumors. Moreover, the synergistic competition ncRNAs are likely to involve in
601 drug resistance, contribute to distinguishing molecular subtypes of malignant tumors, and
602 participate in immune regulation. The web-based database SCOMdb
603 (www.comblab.cn/SCOMdb/) provides a valuable resource for exploring ncRNA regulation
604 across malignant tumors.

605 As important regulators of gene expression, ncRNAs play important roles in the
606 physiology and development of malignant tumors. Yet, the experimentally validated
607 carcinogenic biomarkers are far from complete. Thus, it is challenging to confirm the
608 accuracy of the findings by the SCOM framework. To show the effectiveness of SCOM, we
609 conduct a series of functional analyses including enrichment analysis, molecular subtyping
610 analysis, multi-class classification analysis, survival analysis, and immune regulation analysis
611 for the synergistic competition ncRNAs. We have found that the conserved ncRNA
612 synergistic competition ncRNAs and hub ncRNAs are significantly enriched in diseases, cell
613 markers and cancer hallmarks. In addition, the conserved and rewired hub ncRNAs may act
614 as potential diagnostic and prognostic biomarkers across malignant tumors. Moreover, the
615 synergistic competition ncRNAs are likely to be involved in drug resistance, contribute to

616 distinguishing molecular subtypes of malignant tumors, and participate in immune regulation.
617 These results indicate that the SCOM framework can effectively infer the synergistic
618 competition ncRNAs with biological significance. Although experimentally validated
619 carcinogenic biomarkers are limited, we have discovered that a significant number of
620 synergistic competition ncRNAs are confirmed as carcinogenic biomarkers by the literature
621 (**Fig S8** in **S1 File**, **S12 Data**).

622 The findings in this paper have provided several biological insights although the identified
623 synergistic competition ncRNAs need to be further confirmed by subsequent biological
624 experiments. Firstly, not all the ncRNA synergistic competition networks follow a power law
625 distribution. For example, the ncRNA synergistic competition networks in Kidney
626 Chromophobe (KICH), Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma
627 (LUSC) and Rectum adenocarcinoma (READ) do not obey the power-law distribution. This
628 result indicates that the synergistic competition ncRNAs in these four malignant tumors
629 might equally interact with each other. Secondly, a majority of the ncRNA interactions
630 involved in the synergistic competition are rewired, but a minority of hub ncRNAs is rewired.
631 This result suggests that malignant tumors are likely to share hub ncRNAs rather than the
632 ncRNA interactions involved in the synergistic competition. Thirdly, the similarity of hub
633 ncRNAs between the 31 malignant tumors is significantly larger than that of ncRNA
634 synergistic competition networks (p-value < 2.20E-16 with paired Student's t-test).

635 Moreover, the percentage of rewired ncRNA synergistic competition interactions is
636 significantly larger than that of rewired hub ncRNAs (p-value < 2.20E-16 with paired
637 Student's t-test). This result reveals that although malignant tumors have great differences in
638 ncRNA synergistic competition networks, the hub ncRNAs between malignant tumors tend to
639 be similar. Fourthly, the synergistic competition ncRNAs can distinguish five BRCA
640 subtypes with accuracy equal to or more than 70%. This result shows that the synergistic
641 competition ncRNAs might be good diagnostic biomarkers in BRCA. Fifthly, we have used
642 in this study the transcriptomics data in TCGA [27], which is one of the most well-known
643 cancer genomics programs. However, the SCOM framework can also be applied to the
644 transcriptomics data in other sources (e.g. CCLE [62] and MET500 [63]) to investigate
645 ncRNA synergistic competition. Finally, SCOM is not limited to the exploration of lncRNA
646 and pseudogene synergistic competition. It can also be used to infer the synergistic
647 competition of other ncRNAs (e.g. circRNAs) that are important modulators to malignant
648 tumors.

649 Prediction of ncRNA-related ceRNA network is a critical step to infer ncRNA synergistic
650 competition. Yet, there is still a lack of study on the prediction of ncRNA-related ceRNA
651 networks. In the current work, we apply three criteria (significant sharing of miRNAs,
652 significant positive correlation, and significant sensitive correlation conditioning on shared
653 miRNAs) to predict ncRNA-related ceRNA networks in 31 malignant tumors. Since the three

654 criteria have been successfully used to investigate ceRNA regulation [33,35,64–66], the
655 ncRNA-related ceRNA network predictions are promising. However, evaluating whether a
656 ncRNA can act as a ceRNA to compete with mRNAs is still challenging. For example, the
657 stoichiometry of miRNAs and ncRNAs is considered as a vital factor to predict ceRNA
658 interactions of a ncRNA of interest [67]. With deeper understanding of ceRNA mechanisms,
659 we can more accurately predict ncRNA-related ceRNA networks to improve the inference of
660 ncRNA synergistic competition and accelerate the discovery of carcinogenic biomarkers.

661 Given gene expression data and miRNA-target interactions, SCOM constructs the
662 landscape of ncRNA synergistic competition across malignant tumors at the bulk level.
663 Recently, increasingly advanced single-cell and spatial RNA sequencing technology has
664 opened a way for exploring ncRNA synergistic competition at the single-cell level. With the
665 advance of single-cell and spatial RNA sequencing technology, SCOM will contribute to
666 uncover more carcinogenic biomarkers.

667 ncRNAs are emerging as potential carcinogenic biomarkers. It is important for us to
668 understand the regulation of ncRNAs in malignant tumors. In-depth study of synergistic
669 competition ncRNAs inferred in this study would contribute to the design of reliable
670 diagnosis and treatment biomarkers for human malignant tumors.

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859 ## Supporting information

860 **S1 Data. The matched tumor types in Pan-cancer dataset.**

861 **S2 Data. Clinical information of the matched 8001 tumor samples.**

862 **S3 Data. The molecular subtypes of malignant tumors in Pan-cancer dataset.**

863 **S4 Data. Enrichment analysis of the conserved ncRNA synergistic competition in at**
864 **least two malignant tumors.**

865 **S5 Data. Enrichment analysis of hub ncRNAs across malignant tumors.**

866 **S6 Data. Drug enrichment of ncRNA synergistic competition networks.**

867 **S7 Data. Drug enrichment of hub ncRNAs.**

868 **S8 Data. Relationships between ncRNA synergistic competition networks and immune-**
869 **related pathways across malignant tumors.**

870 **S9 Data. Relationships between hub ncRNAs and immune-related pathways across**
871 **malignant tumors.**

872 **S10 Data. Relationships between ncRNA synergistic competition networks and immune**
873 **cells across malignant tumors.**

874 **S11 Data. Relationships between hub ncRNAs and immune cells across malignant**
875 **tumors.**

876 **S12 Data. Experimentally validated synergistic competition ncRNAs as biomarkers**
877 **across malignant tumors.**

878 **S1 File. Supporting file.**

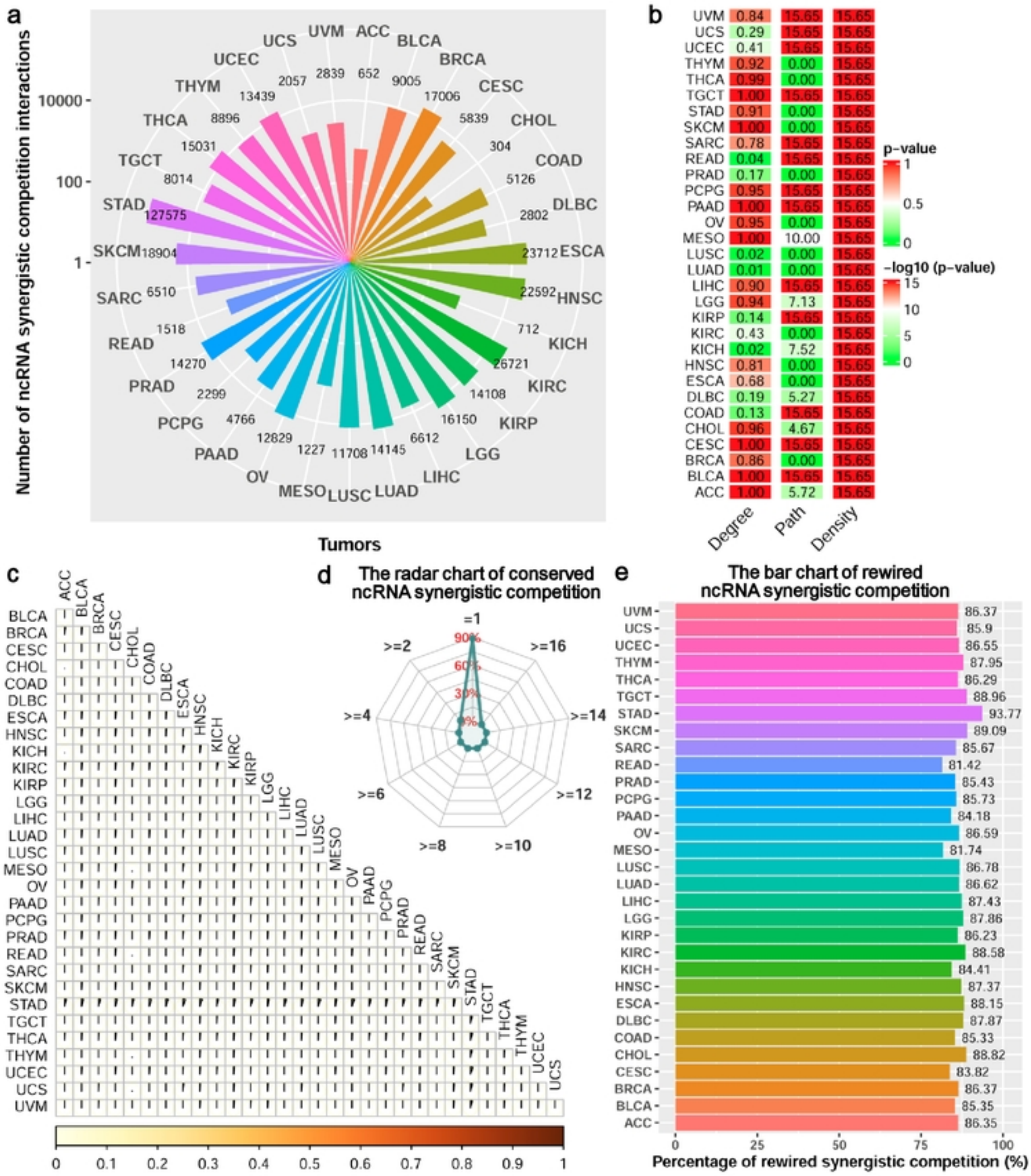
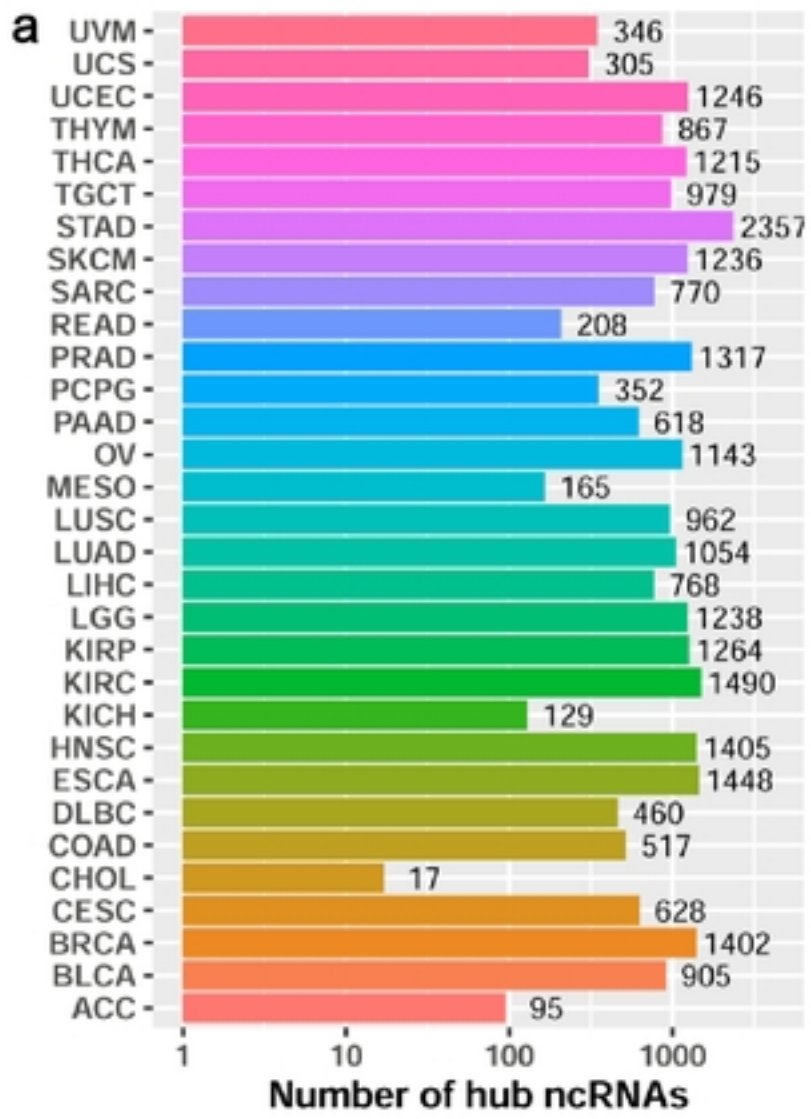
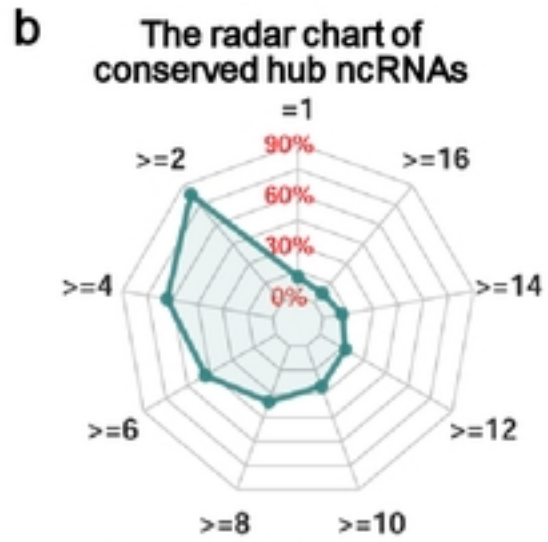

a
Number of ncRNA synergistic competition interactions
10000
100
1
UVM 2839
ACC 652
BLCA 9005
BRCA 17006
CESC 5839
CHOL 304
COAD 5126
DLBC 2802
ESCA 23712
HNSC 22592
KICH 712
KIRC 26721
KIRP 14108
LGG 16150
LIHC 6612
LUAD 14145
LUSC 11708
MESO 1227
OV 12829
PAAD 4766
PCPG 2299
PRAD 14270
READ 1518
SARC 6510
SKCM 18904
STAD 127575
TGCT 8014
THCA 15031
THYM 8896
UCEC 13439
UCS 2057
Tumors
b
UVM 0.84 15.65 15.65
UCS 0.29 15.65 15.65
UCEC 0.41 15.65 15.65
THYM 0.92 0.00 15.65
THCA 0.99 0.00 15.65
TGCT 1.00 15.65 15.65
STAD 0.91 0.00 15.65
SKCM 1.00 0.00 15.65
SARC 0.78 15.65 15.65
READ 0.04 15.65 15.65
PRAD 0.17 0.00 15.65
PCPG 0.95 15.65 15.65
PAAD 1.00 15.65 15.65
OV 0.95 0.00 15.65
MESO 1.00 10.00 15.65
LUSC 0.02 0.00 15.65
LUAD 0.01 0.00 15.65
LIHC 0.90 15.65 15.65
LGG 0.94 7.13 15.65
KIRP 0.14 15.65 15.65
KIRC 0.43 0.00 15.65
KICH 0.02 7.52 15.65
HNSC 0.81 0.00 15.65
ESCA 0.68 0.00 15.65
DLBC 0.19 5.27 15.65
COAD 0.13 15.65 15.65
CHOL 0.96 4.67 15.65
CESC 1.00 15.65 15.65
BRCA 0.86 0.00 15.65
BLCA 1.00 15.65 15.65
ACC 1.00 5.72 15.65
Degree
Path
Density
p-value
1
0.5
0
-log10 (p-value)
15
10
5
0
c
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
d
The radar chart of conserved ncRNA synergistic competition
=1
90%
60%
30%
0%
>=2
>=4
>=6
>=8
>=10
>=12
>=14
>=16
e
The bar chart of rewired ncRNA synergistic competition
UVM 86.37
UCS 85.9
UCEC 86.55
THYM 87.95
THCA 86.29
TGCT 88.96
STAD 93.77
SKCM 89.09
SARC 85.67
READ 81.42
PRAD 85.43
PCPG 85.73
PAAD 84.18
OV 86.59
MESO 81.74
LUSC 86.78
LUAD 86.62
LIHC 87.43
LGG 87.86
KIRP 86.23
KIRC 88.58
KICH 84.41
HNSC 87.37
ESCA 88.15
DLBC 87.87
COAD 85.33
CHOL 88.82
CESC 83.82
BRCA 86.37
BLCA 85.35
ACC 86.35
0
25
50
75
100
Percentage of rewired synergistic competition (%)

Fig 3



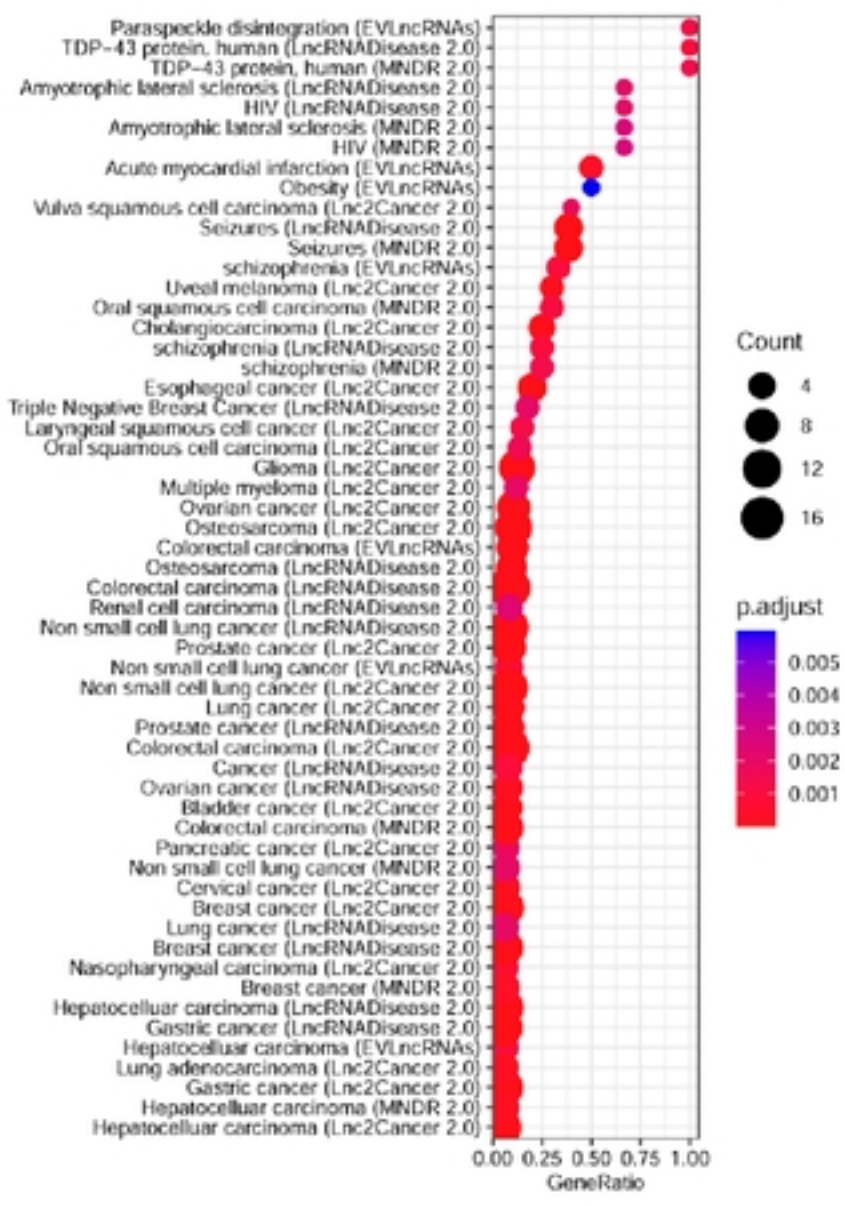


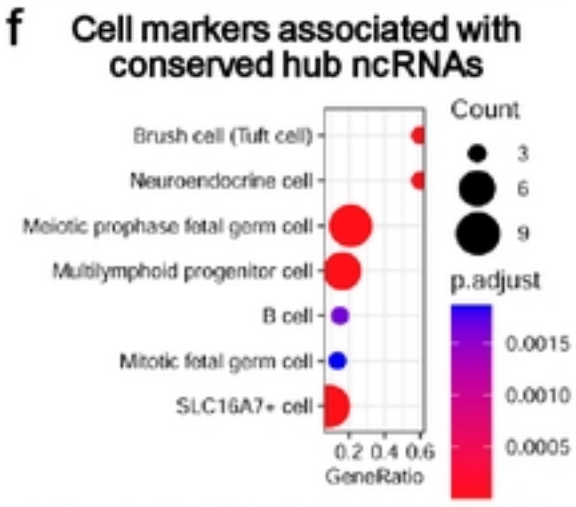
c Classification analysis of conserved hub ncRNAs

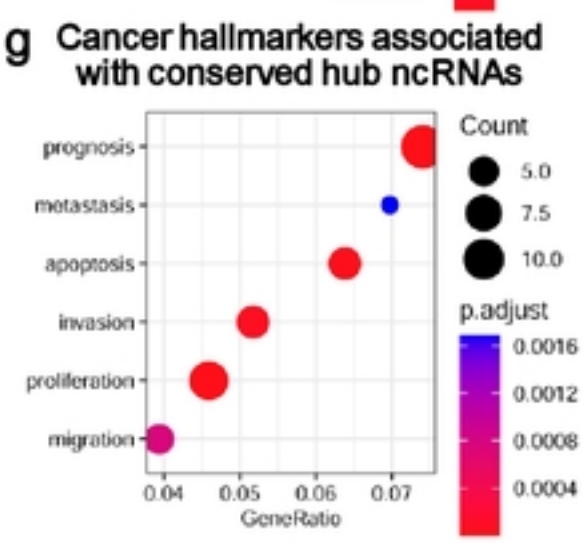
Metrics	Conserved	Baseline
accuracy	**0.91**	0.09
average-precision	**0.95**	0.17
clp	**0.00**	0.03
coverage	**0.21**	14.42
F1	**0.91**	0.09
hamming-loss	**0.01**	0.06
macro-AUC	**0.99**	0.50
macro-F1	**0.89**	0.01
macro-precision	**0.89**	0.00
macro-recall	**0.90**	0.03
margin-loss	**0.21**	14.42
micro-AUC	**1.00**	0.53
micro-F1	**0.91**	0.09
micro-precision	**0.90**	0.09
micro-recall	**0.92**	0.09
mlp	**0.00**	0.97
one-error	**0.09**	0.91
precision	**0.91**	0.09
ranking-loss	**0.01**	0.48
recall	**0.92**	0.09
subset-accuracy	**0.89**	0.09
wlp	**0.00**	0.97

d Survival analysis of conserved hub ncRNAs

Tumors	p-value	HR	HRlow95	HRup95
ACC	5.33E-15	9.79	3.33	28.81
BLCA	<2.22E-16	10.15	6.53	15.77
BRCA	<2.22E-16	14.78	8.13	26.88
CESC	<2.22E-16	8.80	3.99	19.42
CHOL	4.63E-09	9.31	2.08	41.74
COAD	<2.22E-16	8.44	3.66	19.45
DLBC	6.51E-08	18.77	3.12	113.01
ESCA	<2.22E-16	7.50	3.18	17.72
HNSC	<2.22E-16	12.06	7.80	18.66
KICH	6.19E-07	/	/	/
KIRC	<2.22E-16	15.26	7.05	33.00
KIRP	<2.22E-16	11.18	4.04	30.94
LGG	<2.22E-16	10.38	6.16	17.48
LIHC	<2.22E-16	14.06	8.49	23.30
LUAD	<2.22E-16	10.85	6.92	17.03
LUSC	<2.22E-16	12.98	7.80	21.62
MESO	<2.22E-16	7.31	2.99	17.90
OV	<2.22E-16	7.49	4.75	11.81
PAAD	<2.22E-16	7.69	3.83	15.44
PCPG	1.64E-05	/	/	/
PRAD	3.74E-10	23.38	5.30	103.27
READ	3.05E-10	10.00	2.38	42.11
SARC	<2.22E-16	8.68	4.58	16.43
SKCM	<2.22E-16	8.50	5.44	13.28
STAD	<2.22E-16	10.95	6.16	19.47
TGCT	2.36E-03	/	/	/
THCA	7.27E-14	/	/	/
THYM	6.66E-09	20.61	3.23	131.69
UCEC	<2.22E-16	64.84	35.96	116.91
UCS	1.11E-16	9.22	3.04	27.95
UVM	2.16E-14	11.05	3.19	38.26







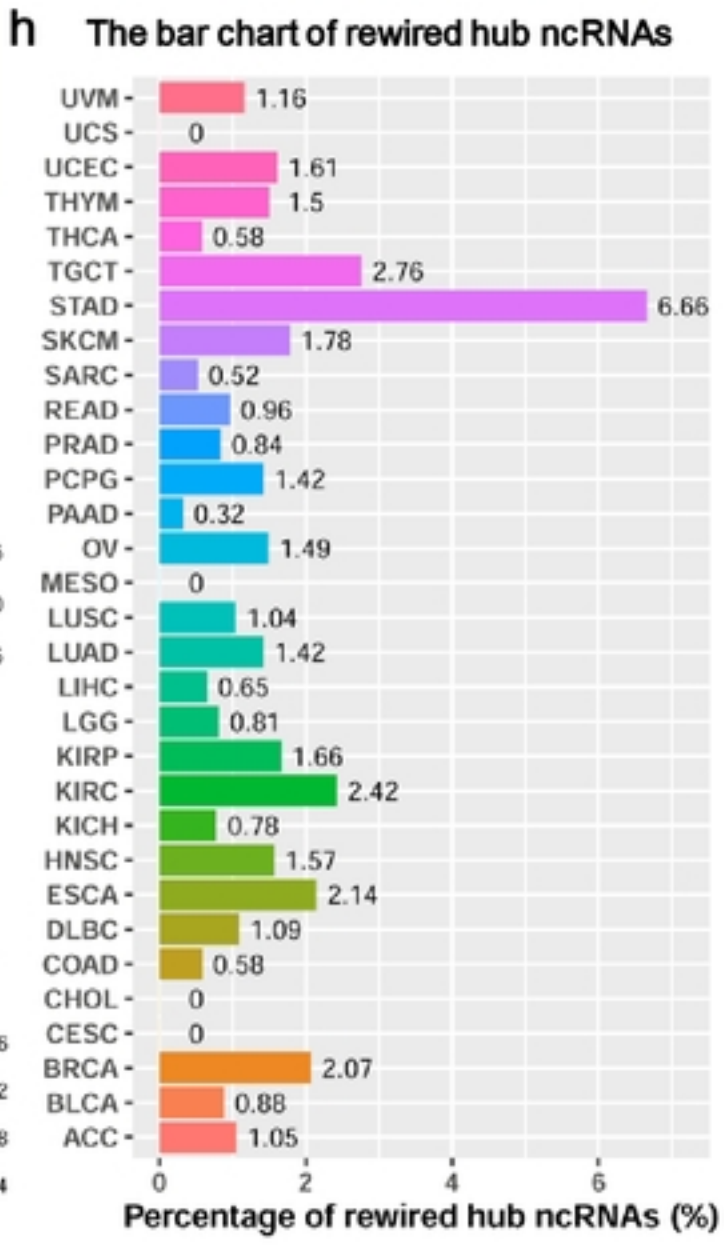


Fig 4

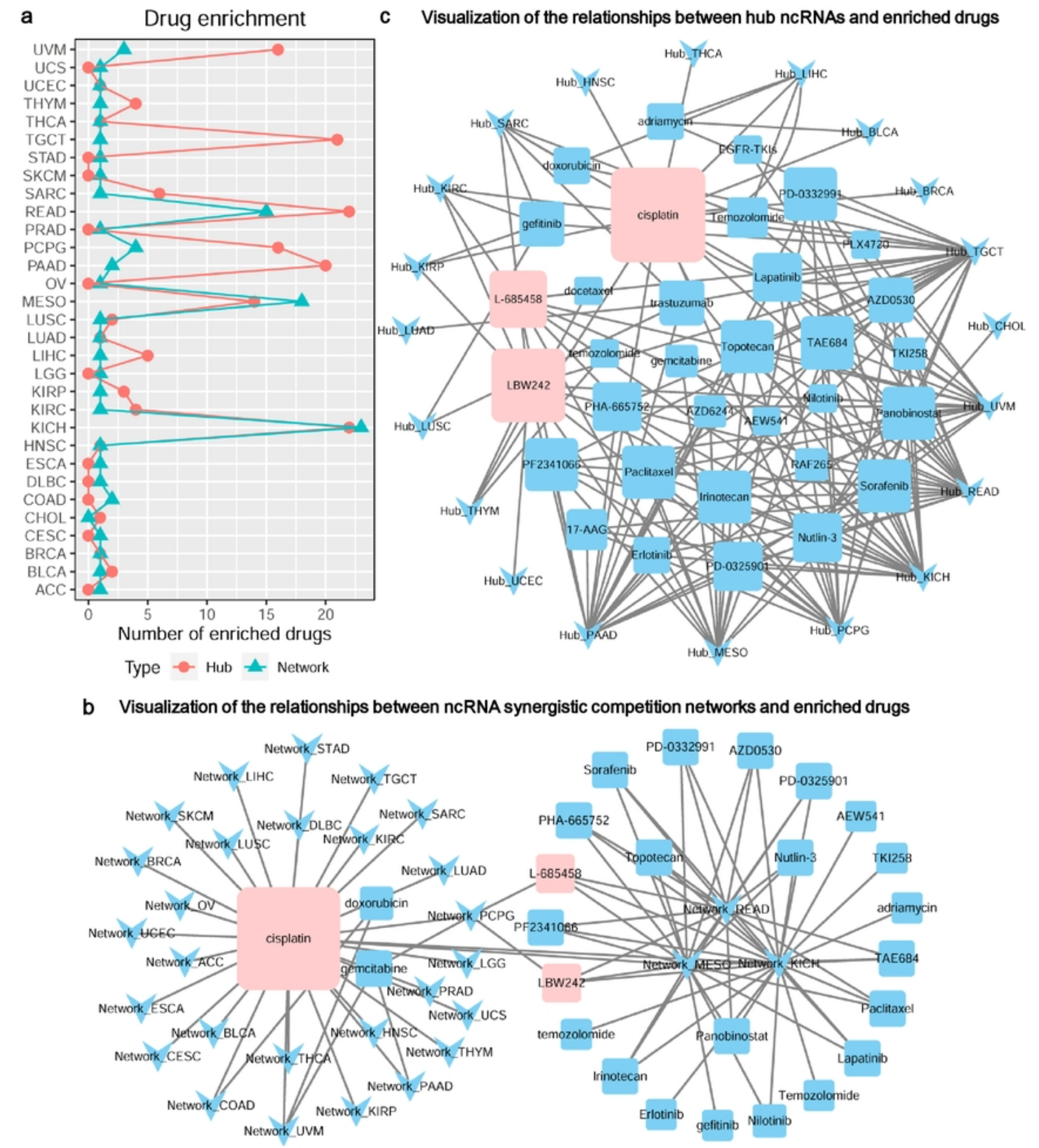
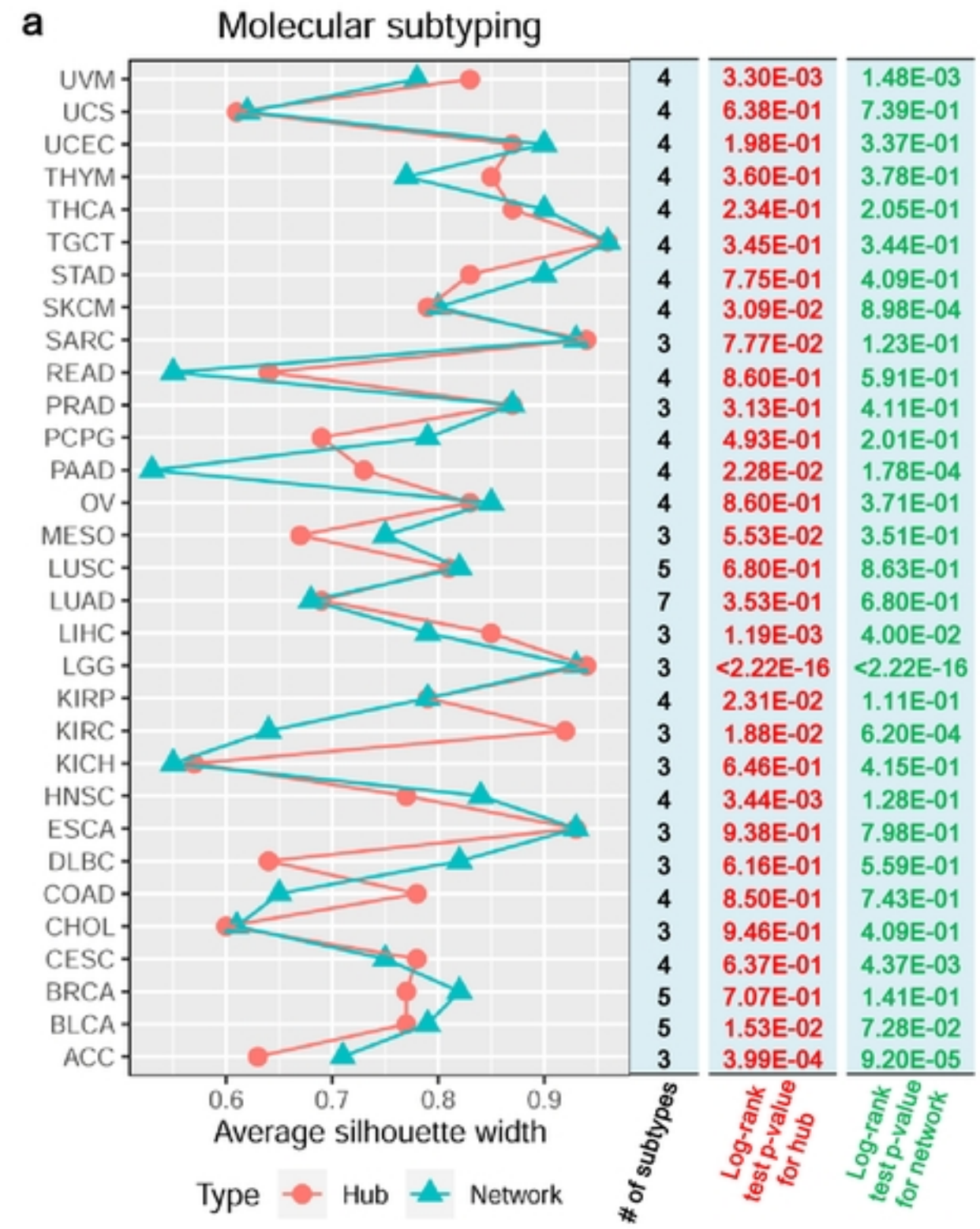
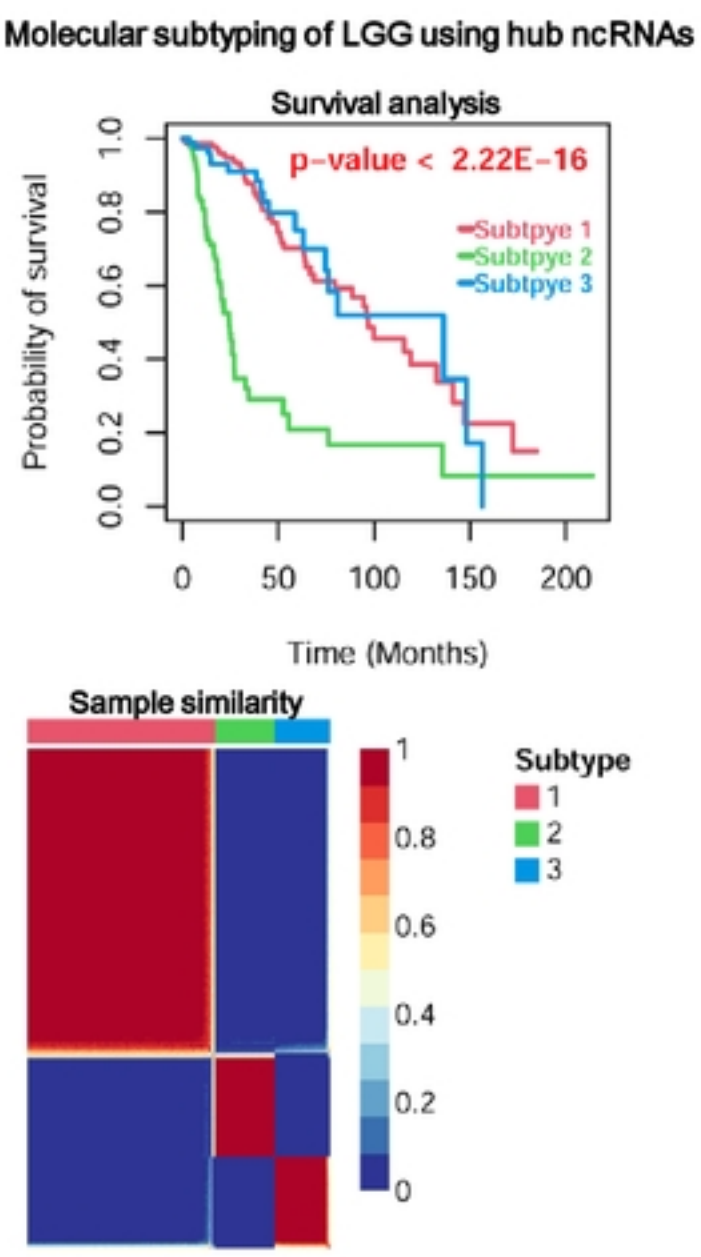
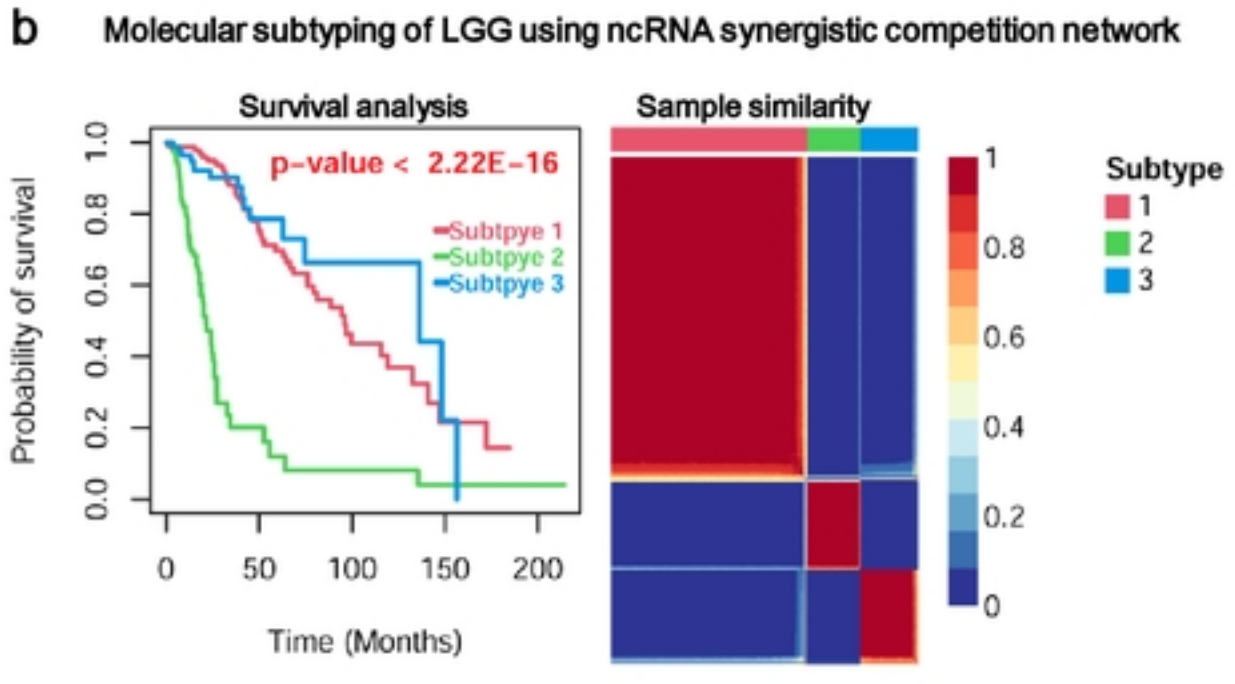

a
Drug enrichment
UVM
UCS
UCEC
THYM
THCA
TGCT
STAD
SKCM
SARC
READ
PRAD
PCPG
PAAD
OV
MESO
LUSC
LUAD
LIHC
LGG
KIRP
KIRC
KICH
HNSC
ESCA
DLBC
COAD
CHOL
CESC
BRCA
BLCA
ACC
0
5
10
15
20
Number of enriched drugs
Type
Hub
Network
c
Visualization of the relationships between hub ncRNAs and enriched drugs
Hub_THCA
Hub_HNSC
Hub_LIHC
Hub_SARC
Hub_BLCA
Hub_KIRC
Hub_BRCA
Hub_TGCT
Hub_KIRP
Hub_LUAD
Hub_CHOL
Hub_UVM
Hub_LUSC
Hub_READ
Hub_THYM
Hub_UCEC
Hub_KICH
Hub_PAAD
Hub_PCPG
Hub_MESO
adriamycin
EGFR-TKIs
doxorubicin
cisplatin
PD-0332991
gefitinib
Temozolomide
PLX4720
Lapatinib
L-685458
docetaxel
trastuzumab
AZD0530
Topotecan
TAE684
temozolomide
gemcitabine
TKI258
LBW242
Nilotinib
PHA-665752
AZD6244
AEW541
Panobinostat
PF2341066
Paclitaxel
RAF265
Sorafenib
Irinotecan
17-AAG
Nutlin-3
Erlotinib
PD-0325901
b
Visualization of the relationships between ncRNA synergistic competition networks and enriched drugs
Network_STAD
Network_LIHC
Network_TGCT
Network_SKCM
Network_DLBC
Network_SARC
Network_LUSC
Network_KIRC
Network_BRCA
Network_LUAD
Network_OV
doxorubicin
Network_PCPG
Network_UCEC
cisplatin
Network_ACC
gemcitabine
Network_LGG
Network_PRAD
Network_ESCA
Network_UCS
Network_BLCA
Network_HNSC
Network_CESC
Network_THCA
Network_THYM
Network_PAAD
Network_COAD
Network_KIRP
Network_UVM
PD-0332991
AZD0530
Sorafenib
PD-0325901
PHA-665752
AEW541
Topotecan
Nutlin-3
TKI258
L-685458
Network_READ
adriamycin
PF2341066
Network_MESO
Network_KICH
TAE684
LBW242
Paclitaxel
temozolomide
Panobinostat
Lapatinib
Irinotecan
Temozolomide
Erlotinib
gefitinib
Nilotinib

Fig 5







d

BRCA subtype classification

Metrics	Network	Hub	Baseline
accuracy	**0.70**	**0.79**	0.40
average-precision	**0.83**	**0.89**	0.60
clp	**0.06**	**0.00**	0.20
coverage	**0.48**	**0.26**	1.38
F1	**0.71**	**0.81**	0.40
hamming-loss	**0.12**	**0.08**	0.24
macro-AUC	**0.72**	**0.94**	0.50
macro-F1	**0.59**	**0.74**	0.11
macro-precision	**0.61**	**0.75**	0.08
macro-recall	**0.62**	**0.75**	0.20
margin-loss	**0.48**	**0.26**	1.38
micro-AUC	**0.88**	**0.96**	0.63
micro-F1	**0.70**	**0.80**	0.40
micro-precision	**0.68**	**0.77**	0.40
micro-recall	**0.72**	**0.83**	0.40
mlp	**0.24**	**0.02**	0.80
one-error	**0.30**	**0.20**	0.60
precision	**0.70**	**0.79**	0.40
ranking-loss	**0.12**	**0.06**	0.34
recall	**0.72**	**0.83**	0.40
subset-accuracy	**0.67**	**0.75**	0.40
wlp	**0.24**	**0.04**	0.80

Fig 6

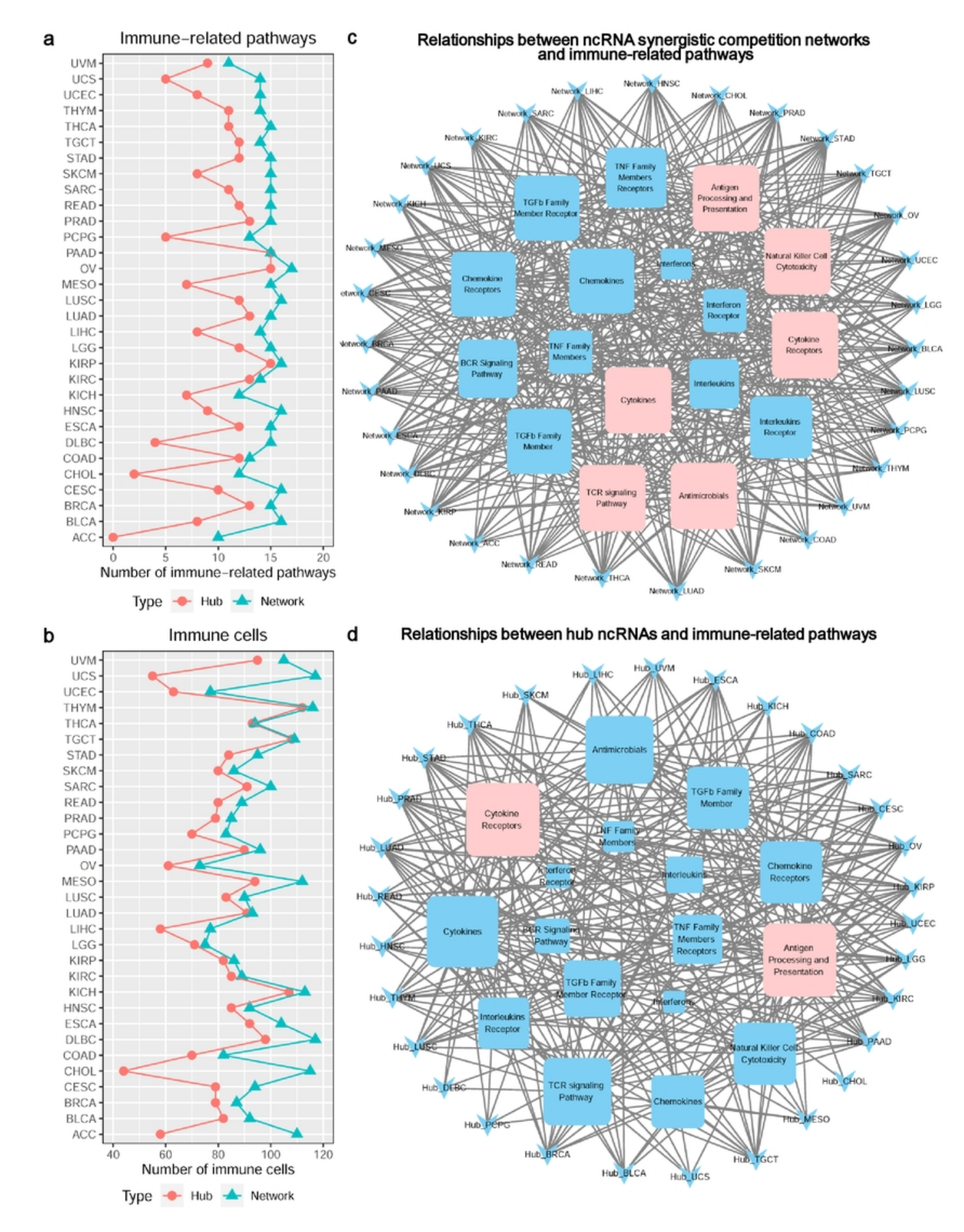

a
Immune-related pathways
UVM
UCS
UCEC
THYM
THCA
TGCT
STAD
SKCM
SARC
READ
PRAD
PCPG
PAAD
OV
MESO
LUSC
LUAD
LIHC
LGG
KIRP
KIRC
KICH
HNSC
ESCA
DLBC
COAD
CHOL
CESC
BRCA
BLCA
ACC
0
5
10
15
20
Number of immune-related pathways
Type
Hub
Network
b
Immune cells
40
60
80
100
120
Number of immune cells
Type
Hub
Network
c
Relationships between ncRNA synergistic competition networks and immune-related pathways
TNF Family Members Receptors
Antigen Processing and Presentation
TGFb Family Member Receptor
Natural Killer Cell Cytotoxicity
Chemokine Receptors
Chemokines
Interferons
Interferon Receptor
Cytokine Receptors
BCR Signaling Pathway
TNF Family Members
Interleukins
Cytokines
Interleukins Receptor
TGFb Family Member
TCR signaling Pathway
Antimicrobials
d
Relationships between hub ncRNAs and immune-related pathways
Antimicrobials
Cytokine Receptors
TGFb Family Member
TNF Family Members
Chemokine Receptors
Interferon Receptor
Interleukins
Cytokines
BCR Signaling Pathway
TNF Family Members Receptors
Antigen Processing and Presentation
TGFb Family Member Receptor
Interferons
Interleukins Receptor
Natural Killer Cell Cytotoxicity
TCR signaling Pathway
Chemokines

Fig 7

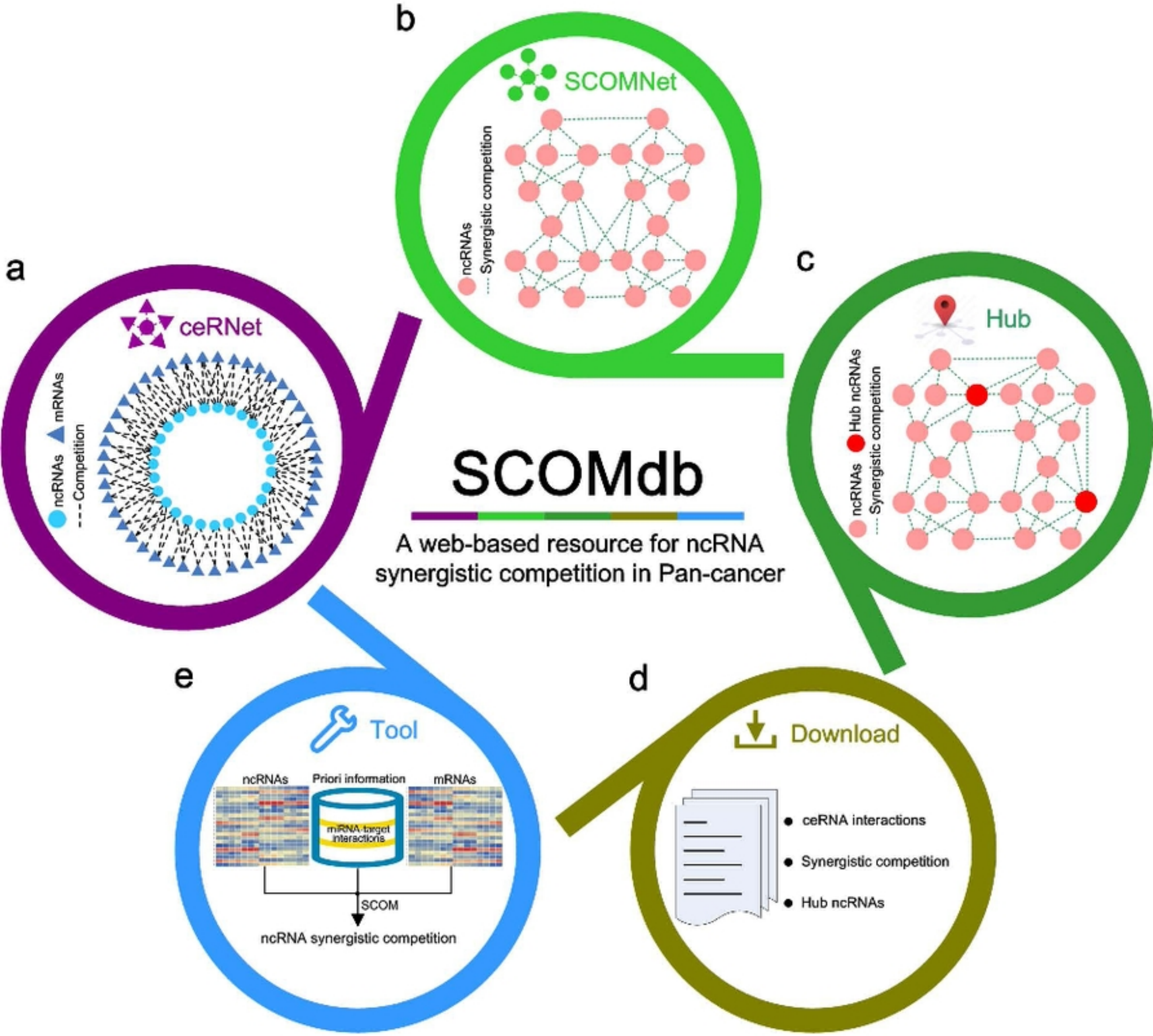

a
ceRNet
ncRNAs
mRNAs
Competition
b
SCOMNet
ncRNAs
Synergistic competition
c
Hub
Hub ncRNAs
ncRNAs
Synergistic competition
SCOMdb
A web-based resource for ncRNA synergistic competition in Pan-cancer
e
Tool
ncRNAs
Priori information
mRNAs
miRNA-target interactions
SCOM
ncRNA synergistic competition
d
Download
ceRNA interactions
Synergistic competition
Hub ncRNAs

Fig 8

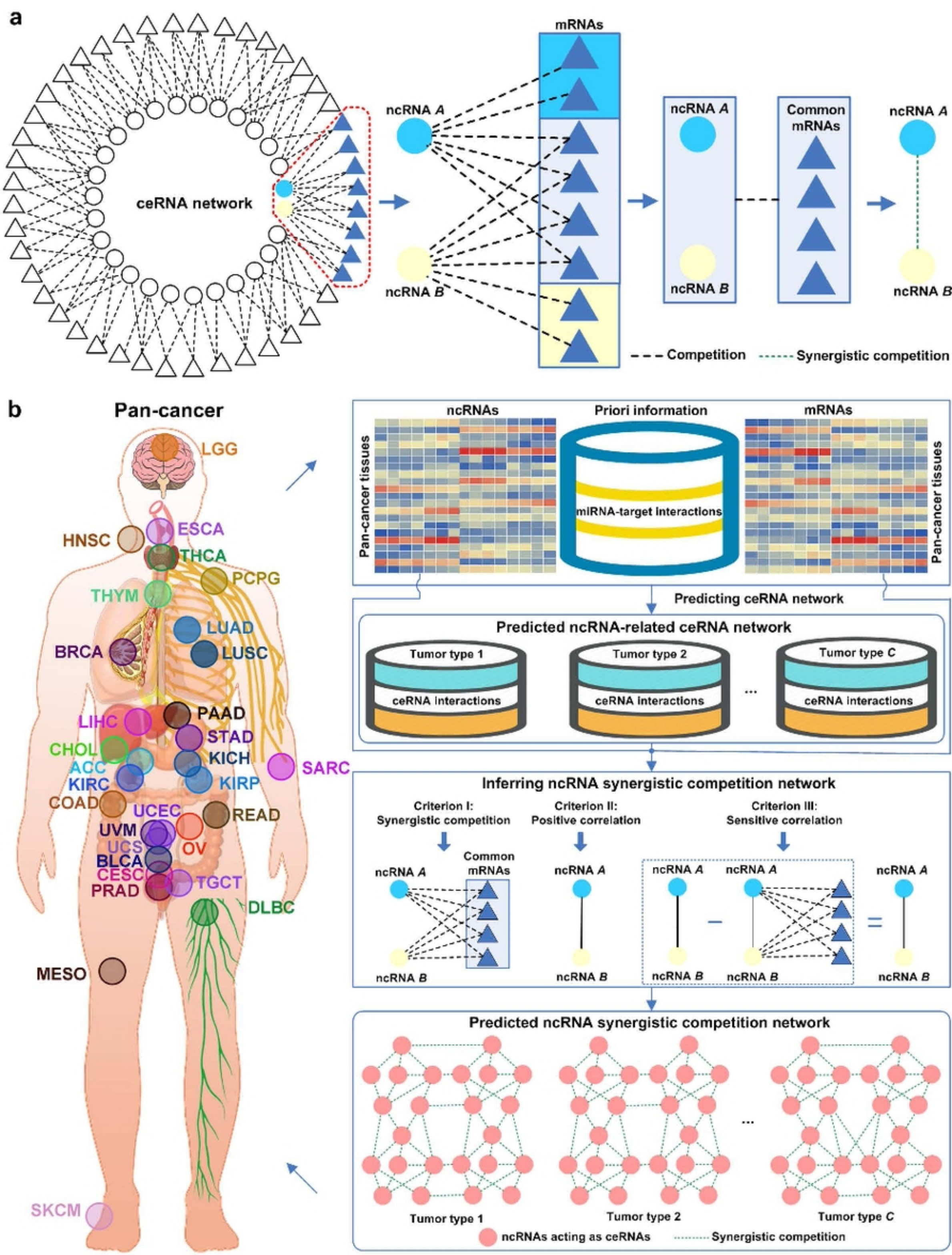

a
ceRNA network
mRNAs
ncRNA A
ncRNA B
Common mRNAs
Competition
Synergistic competition
b
Pan-cancer
LGG
HNSC
ESCA
THCA
PCPG
THYM
LUAD
BRCA
LUSC
LIHC
PAAD
STAD
CHOL
KICH
ACC
SARC
KIRC
KIRP
COAD
UCEC
READ
UVM
UCS
OV
BLCA
CESC
TGCT
PRAD
DLBC
MESO
SKCM
ncRNAs
Priori information
mRNAs
Pan-cancer tissues
miRNA-target interactions
Predicting ceRNA network
Predicted ncRNA-related ceRNA network
Tumor type 1
Tumor type 2
Tumor type C
ceRNA interactions
Inferring ncRNA synergistic competition network
Criterion I: Synergistic competition
Criterion II: Positive correlation
Criterion III: Sensitive correlation
Predicted ncRNA synergistic competition network
ncRNAs acting as ceRNAs
Synergistic competition

Fig 1

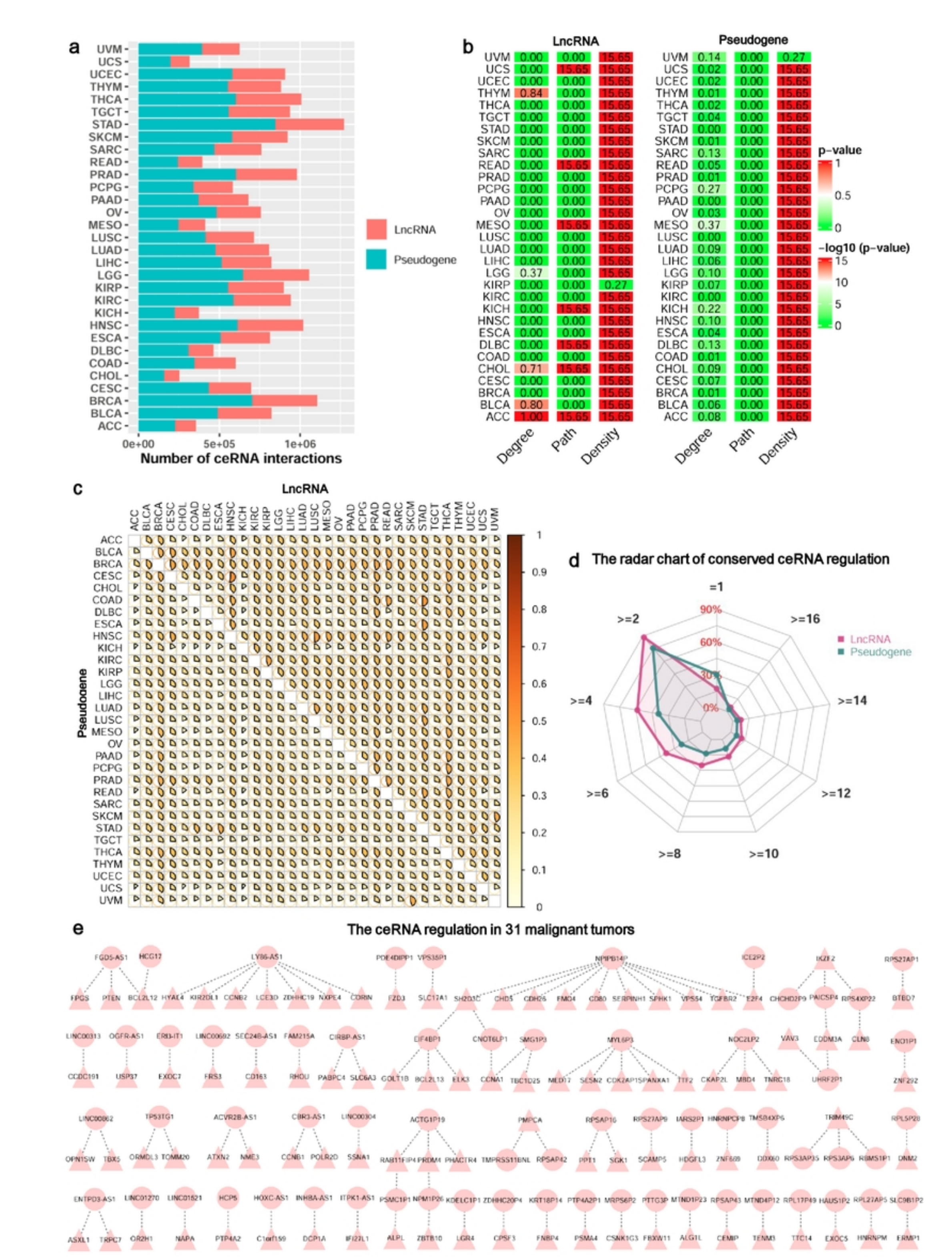

a
UVM
UCS
UCEC
THYM
THCA
TGCT
STAD
SKCM
SARC
READ
PRAD
PCPG
PAAD
OV
MESO
LUSC
LUAD
LIHC
LGG
KIRP
KIRC
KICH
HNSC
ESCA
DLBC
COAD
CHOL
CESC
BRCA
BLCA
ACC
0e+00
5e+05
1e+06
Number of ceRNA interactions
LncRNA
Pseudogene
b
LncRNA
Pseudogene
Degree
Path
Density
p-value
1
0.5
0
-log10 (p-value)
15
10
5
0
c
LncRNA
Pseudogene
d
The radar chart of conserved ceRNA regulation
=1
>=2
>=4
>=6
>=8
>=10
>=12
>=14
>=16
90%
60%
30%
0%
LncRNA
Pseudogene
e
The ceRNA regulation in 31 malignant tumors

Fig 2