

1 **CD4 T-cell transcriptome expression reversal of the lncRNA-mRNA**
2 **co-expression network in elite controller vs. normal-process HIV**
3 **patients**

4

5 Short title: LncRNA-mRNA co-expression network in patients with HIV

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22

23 **Abstract**

24 Elite controller refers to a patient with human immunodeficiency virus infection with
25 an undetected viral load without anti-viral treatment. Studies on gene expression and
26 regulation in these individuals are limited but significant. We enrolled 196 patients
27 and collected CD4 T-cell samples from two elite controllers, two normal-process
28 infected patients, and two healthy controls to perform second-generation
29 transcriptome sequencing. Using the Cuffdiff model, we identified differentially
30 expressed mRNAs and long non-coding RNAs with corrected P value < 0.05 , and
31 constructed a protein-protein interaction network as well a long non-coding
32 RNA-mRNA co-expression network based on the Pearson correlation coefficient.
33 Interestingly, some interactions within the networks were identified as associated with
34 viral infections and immune responses. This was the first study to examine gene
35 transcription in elite controllers and to study their functional relationships. Our results
36 provide a reference for subsequent functional verification at the molecular or cellular
37 level.

38

39 **Author Summary**

40 Some individuals can spontaneously inhibit HIV replication after infection with HIV,
41 and thus lack any symptoms. Studies on these patients, termed elite controllers (ECs)
42 will help researchers and clinicians to understand the interrelationship between HIV
43 and the host. In the present study, we focused on the interactions and functional
44 relationships between significantly differentially expressed long non-coding RNAs

45 (lncRNAs) and mRNAs in ECs vs. normal-process patients (NPs). RNA-sequencing
46 was performed for six representative samples of CD4 T-cells. Using the Pearson
47 correlation test, an lncRNA-mRNA co-expression network was constructed. Several
48 new regulatory relationships between transcripts were revealed that might be closely
49 related to the ability of ECs to maintain a low viral load for long periods without
50 anti-viral treatment. For example, lncRNA *C3orf35* was upregulated in ECs vs. NPs
51 and was positively related to downregulation of *GNG2* mRNA (encoding G protein
52 subunit gamma 2), which functions in chemokine signaling pathways and HIV-1
53 infection. Overall, we identified certain interesting genetic interactions that will
54 provide information about the mechanism of host suppression of viral replication.

55

56 **Introduction**

57 Human immunodeficiency virus (HIV) has been studied for more than 30 years, and
58 research has found that HIV invades host CD4+ cells, integrates its own DNA into the
59 host genome, and establishes a reservoir in the early stage, followed by massive
60 replication, which destroys the normal immune system function of the host, and
61 triggers various concurrent symptoms [1-2]. Ultimately, HIV infection can cause the
62 host to die. Although anti retroviral therapy (ART) treatment can partially reconstitute
63 the immune function of the host, it cannot eradicate the latent HIV pool in the host,
64 which always maintains a low level of virus replication [3]. However, among
65 HIV-infected individuals, a small number of patients are found to be inherently
66 resistant to viral replication, and spontaneously induce suppression of the latent pools

67 without any anti-viral therapy, resulting in an undetectable viral load in plasma for a
68 long period [4-5]. These patients, termed elite controllers (ECs), have attracted
69 significant research interest. Studying the characteristics of the autoimmune factors of
70 ECs is anticipated to identify important factors that control virus replication. Such
71 valuable information could lead to novel methods to treat and alleviate HIV infection.
72 Long-noncoding RNAs (lncRNAs) are transcribed RNA molecules greater than 200
73 nt in length that regulate gene expression by diverse, but as yet not completely
74 understood, mechanisms [6]. Although the function of most lncRNAs is unknown,
75 several have been shown to regulate gene expression at multiple levels from DNA to
76 phenotype [7]. Through a variety of means, including *cis* (near the site of lncRNA
77 production) or *trans* (co-expressed with their target gene) mechanisms, lncRNAs play
78 a vital role in many biological processes [8]. Studies on lncRNAs have become a
79 hotspot in current non-coding RNA research.

80 Recent studies focused on the role of lncRNAs in HIV pathogenesis, especially the
81 relationship between lncRNA regulation of gene expression and viral infection,
82 replication, and latency [9-12]. A few lncRNAs have been characterized and proven
83 to be closely associated HIV, for example, the knockdown of host lncRNA *NEATI*
84 enhanced virus production by increasing nucleus-to-cytoplasm export of
85 Rev-dependent instability element (INS)-containing HIV-1 mRNAs [13]. The
86 knockdown of lncRNA *NRON* enhanced HIV-1 replication through increased activity
87 of nuclear factor of activated T-cells (NFAT) and the viral long terminal repeat (LTR)
88 [14]. Similarly, lncRNA *MALATI* releases epigenetic silencing of HIV-1 replication

89 by displacing the polycomb repressive complex 2 from binding to the LTR promoter
90 [15]. Furthermore, an HIV-encoded antisense lncRNA, *ASP-L*, was proven to promote
91 latency HIV [16-17]. Although, the mechanism of lncRNA function is sometimes
92 elusory and unpredicted, many current studies, which are limited to the screening and
93 functional prediction stage based on chip or sequencing results, also provide us with
94 valuable information and a basis for mechanistic research.

95 In the present study, we conducted a transcriptomics investigation for HIV elite
96 controllers, to identify differences in the transcriptional expression profiles between
97 elite controllers and normal-process HIV patients as compared with healthy controls.
98 The obtained lncRNA-mRNA co-expression network revealed the possible role of the
99 functional relationships between lncRNAs and mRNAs in the ability of elite
100 controllers to inhibit viral replication.

101

102 **Results**

103 **Subjects**

104 A total of 196 individuals infected with HIV were enrolled in our cohort, including
105 patients under treatment (179, 91.3%), untreated normal-process patients (15,7.7%)
106 and elite controllers (2, 1.0%). In addition, we recruited two healthy individuals from
107 the out-patient department of the hospital. Most patients were infected with the virus
108 through heterosexual contact, followed by intravenous infection, and homosexual
109 transmission. Based on the basic principle of intra-group identity, we determined three
110 men and three women who were assigned to each of the three study groups as study

111 subjects. Their ages ranged from 39 to 54 and were free of other diseases, such as
112 tuberculosis, diabetes, or hepatitis. Specific details of the clinical characteristics of the
113 participants are shown in S1 Table. Descriptive statistics are reported as counts
114 (percentage) for dichotomous and categorical variables, and the median (the 25th
115 percentile and 75th percentile) for continuous variables. The experimental design and
116 analysis process of this research are shown in a flowchart (Fig 1).

117 **Identification of differentially expressed (DE) transcripts between health**
118 **controls (HCs) and normal-process infected patients (NPs)**

119 First, we scanned the expression profiles of all transcripts in CD4 T-cells from two
120 HCs, two ECs and two NPs. In all, more than 82000 mRNA transcripts and more than
121 20000 corresponding genes were identified. Likewise nearly 15000 lncRNAs and
122 1000 corresponding gene IDs were identified. Regular grouping between HCs and
123 normal-process patients was first considered. Altogether, 3602 mRNA transcripts and
124 383 lncRNA transcripts were differently expressed according to the threshold of fold
125 change > 2 and p value < 0.05 , among which 2313 mRNAs and 207 lncRNAs were
126 upregulated and 1289 mRNAs and 176 lncRNAs were downregulated. We similarly
127 compared gene expression differences between HCs and ECs though more attention
128 was paid to ECs vs. NPs. The comparison with HCs helped to provide a basic
129 reference background. The lists of fragments per kilobase of transcript per million
130 mapped reads (FPKM) values and regulatory situation of all DE mRNAs and
131 lncRNAs in HCs vs. NPs and HCs vs. ECs are shown in S2 Table and S3 Table
132 respectively.

133 **Identification of DE mRNAs between ECs and NPs**

134 Among all transcripts, 2936 transcripts were differentially expressed according to the
135 threshold of fold change > 2 and p value < 0.05 . There were 1327 mRNAs that were
136 upregulated and 1609 that downregulated in ECs vs. NPs. Gene ontology (GO) and
137 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis was carried
138 out to annotate the function of these DE mRNAs. Certain terms and pathways that
139 might be associated with infection and immunity were significantly enriched (S1 Fig).
140 These terms and pathways included negative regulation of interferon-beta production
141 (GO:0032688), cellular component disassembly involved in execution phase of
142 apoptosis (GO:0006921), regulation of defense response to virus by virus
143 (GO:0050690), negative regulation of type I interferon production (GO:0032480),
144 RIG-I-like receptor signaling pathway (hsa04622), and antigen processing and
145 presentation (hsa04612).

146 **Identification of DE lncRNAs between ECs and NPs**

147 As same, among all transcripts, 3543 official gene symbols that matched in the
148 NONCODE database had corresponding lncRNA transcripts. Three main categories
149 of lncRNAs accounted for the majority, including antisense lncRNAs (2537/14853,
150 17.1%), intronic lncRNAs (8152/14853, 54.9%), and long intergenic noncoding
151 RNAs (lincRNAs; 3344/14853, 22.5%). There were 151 upregulated lncRNAs and
152 160 downregulated lncRNAs in ECs vs. NPs according to the threshold of fold change
153 > 2 and p < 0.05 . In both the mRNA and lncRNA clusters (Fig 2), the samples showed
154 marked intra-group correlations and inter-group differences, which further confirmed

155 the specificity of the samples. In terms of protein-coding mRNAs encoding proteins
156 with specific biological functions, the HC and EC groups showed closer clustering
157 and were relatively distant from the NP group. The list of fragments per kilobase of
158 transcript per million mapped reads (FPKM) values and regulatory situation of all DE
159 mRNAs and lncRNAs are shown in S4 Table.

160 **PPI network of DE mRNAs**

161 Fig 3 shows the top 50 hub genes playing an important role in interaction of DE
162 mRNAs, in which many genes functioned centrally and could be frequently regulated
163 by other transcripts. Considering the differences in the outputs of different algorithms,
164 we identified those genes that were commonly detected by the algorithms and focused
165 on those genes that were regulated by lncRNAs and their functional enrichment
166 analysis. S5 Table shows the intersection list of the top 50 hub-mRNAs calculated
167 using 12 algorithms.

168 **LncRNA-mRNA co-expression network analysis**

169 Almost 0.9 million co-expression relationships were discovered, and the exact form
170 was expressed as a linear orientation whereby one lncRNA targets one mRNA. In all
171 orientations, 1109 DE lncRNAs targeting DE mRNAs were identified. Fig 4a shows
172 the network constructed using these relationships, in which 142 DE lncRNAs were
173 co-expressed with 127 DE mRNAs. Moreover, we found 16 of 24 intersecting hub
174 mRNAs in the diagram, which helped us to quickly locate the target protein coding
175 genes and easily access the enriched functions of these genes. Next, we selected 15
176 annotated lncRNAs and their associated mRNAs from Fig 4a and constructed Fig 4b.

177 We then identified the top 10 GO terms and pathways most closely related to
178 infection and immunity by combining the function of the hub genes. Many significant
179 functions are co-enriched, such as immune response-regulating signaling pathway
180 (GO:0002764) and apoptosis (hsa04215) (Fig 5).

181 **Immune regulation and infection associated lncRNA-mRNA networks**

182 We further investigated the specific functions of the co-expressed mRNAs shown in
183 Fig 4b and found several interesting regulation relationships. Certain genes were
184 enriched to GO terms and pathways that are closely related to immune regulation and
185 infection. These genes and corresponding pathways included *GNG2* (Human
186 cytomegalovirus infection, hsa05163; Chemokine signaling pathway, hsa04062;
187 Kaposi sarcoma-associated herpesvirus infection, hsa05167; Human
188 immunodeficiency virus 1 infection, hsa05170), *RFX5* (Primary immunodeficiency,
189 hsa05340; Tuberculosis, hsa05152; Antigen processing and presentation, hsa04612),
190 *HDAC6* (Viral carcinogenesis, hsa05203), *MAPK8* (Apoptosis - multiple species,
191 hsa04215; NOD-like receptor signaling pathway, hsa04621; Fc epsilon RI signaling
192 pathway, hsa04664; RIG-I-like receptor signaling pathway, hsa04622; Inflammatory
193 mediator regulation of TRP channels, hsa04750; Toll-like receptor signaling pathway,
194 hsa04620), *MAPK4* (IL-17 signaling pathway, hsa04657), and *RARA* (Th17 cell
195 differentiation, hsa04659). Corresponding lncRNAs including *C3orf35*,
196 *TMEM254-AS1*, *ARRDC3-AS1*, *LINC00202-1*, *TRAF3IP2-AS1*, *PSMD5-AS1*,
197 *AC093375.1*, and *SVIL-AS1* were co-expressed with these mRNAs, which suggested
198 several new regulatory relationships that might play an important role in controlling

199 virus replication.

200

201 **Dicussion**

202 In the present study, we systematically analyzed the expression profiles of lncRNAs
203 and mRNAs from CD4⁺ T cells from ECs, NPs, and HCs, and constructed a
204 co-expression network based on the relationships among DE genes and database
205 annotations. The functions of most lncRNAs are not well characterized; therefore, the
206 gene functions in this research mainly referred to those of mRNAs and their
207 expression products. The potential functions of candidate lncRNAs and the
208 lncRNAs-associated biological processes in ECs were predicted using the
209 lncRNA-mRNA network and functional enrichment analysis. We identified certain
210 genes that play a significant role in the ability of ECs to inhibit the replication of
211 latent virus. Most of these genes were downregulated in the HCs and ECs and
212 upregulated in the NPs, which was consistent with their clinical characteristics, in that
213 ECs maintain a similar gene expression pattern to the HCs, which distinguishes them
214 from the NPs. Among these rigorously selected genes, some hub genes were closely
215 related to microbial infections and host immune processes, which are reported to
216 greatly affect the progress of HIV infection [18-19].

217 To the best of our knowledge, the present study was the first to predict
218 lncRNA-mRNA interactions for HIV ECs, in which many of the reported genes had
219 not been previously reported to function in HIV-associated biological process. The
220 *MAPK* family has been associated with the reactivation and replication of the virus in

221 many studies [20-21], and the HIV-1 virus inhibition activity of MAPK pathway
222 inhibitors was the result of the negative regulation of HIV-1 LTR promoter activity
223 [22]. In addition, HDAC6 gene expression product was reported to mediate HIV-1
224 tat-induced proinflammatory responses by regulating the MAPK-NF-kappaB/AP-1
225 pathways in astrocytes [23]. In the present study, we also found that HDAC6 interacts
226 with MAPK8, MAPK4, and MAPK9 (Fig 3). This suggested their related lncRNAs
227 including *C3orf35*, *TMEM254-AS1*, *ARRDC3-AS1*, *LINC00202-1*, *TRAF3IP2-AS1*,
228 *PSMD5-AS1*, and *AC093375.1* may control HIV replication by regulating the
229 expression of these genes. In addition, we identified genes that had not been studied
230 before, whose functions were enriched into the following processes: Virus infection,
231 immunodeficiency, apoptosis, and cytokines. Previous studies had shown that these
232 functions are closely related to viral replication [24-25]. For instance, *GNG2*
233 (encoding G protein subunit gamma 2) showed a strong correlation with HIV in the
234 KEGG pathway analysis. In Kaposi sarcoma-associated herpesvirus infection, *GNG2*
235 activates Ras pathways and PI3K-Akt pathways to affect the production of cellular
236 cytokines such as IL-6, IL-8, VEGF, and COX2, and other inflammatory and cellular
237 life cycle-related biological processes. HIV infection and its concurrent Kaposi
238 sarcoma are also closely related to the occurrence of other cancers [26-27].
239 ART treatment cannot eradicate the latent virus in the host; therefore, the genes
240 identified in the present study may play a non-negligible regulatory role in the
241 relationship between the latent virus and the host. Latent virus is the biggest obstacle
242 to the elimination of the disease; thus, understanding the processes that contribute to

243 its persistence, such as inflammation and immune activation, are crucial for the
244 remission and cure of HIV [28-29].

245 There are also some limitations to this study. First, because of the limited amount of
246 RNA in the samples, the sample size of this study was relatively small. Although we
247 selected eight genes for verification using qRT-PCR in 12 samples and found that the
248 expression trends of four of them were consistent with the RNA sequencing results,
249 these results are not statistically significant because of the small sample size. Second,
250 we only assessed gene expression of CD4+ T cells, which, although they are the main
251 targets of HIV, are only one of the many cell types that are infected by HIV. Further
252 research should be performed to validate the function and mechanism of the genes in
253 a larger sample and in more cell types.

254 In summary, this study identified several important differentially expressed genes
255 associated with the EC phenomenon, which could form the basis for subsequent
256 cellular and molecular studies, and could provide new targets for gene-targeted
257 therapy in the future.

258

259 **Methods**

260 **Ethics statement**

261 All participating medical institutions provided local institutional review board
262 approval, and the participants provided informed consent for this study.

263 **Cohort and patients**

264 In total, 196 patients were recruited into our cohort, from different provinces of

265 China, who were diagnosed in Qingchun Hospital of Zhejiang Province from January
266 2000 to January 2018, and received or voluntarily rejected highly active antiretroviral
267 therapy (HAART) treatment. The viral loads of the patients were detected using the
268 Cobas Taqman system (Roche). The HCs were randomly selected from hospital
269 clinics and were not suffering from any diseases. We chose two ECs, two NPs, and
270 two HCs of similar ages, gender, and traditional risk factors to perform second
271 generation transcriptome sequencing. ECs were defined as HIV-infected patients with
272 and undetectable viral load without any anti-viral treatment for nearly 10 years.

273 **Sample collection and preparation**

274 Peripheral blood mononuclear cells (PBMCs) were isolated from 3–5 mL EDTA-K2+
275 anticoagulant venous blood using density gradient centrifugation. Primary CD4+ T
276 cells were purified through negative selection using a CD4+ T cell isolation Kit
277 (Miltenyi). According to flow cytometry analysis, the purity of the separated CD4+ T
278 cells was more than 90%. Total RNA was extracted using the TRIzol reagent (Life
279 Technologies). RNA purity was checked using a NanoPhotometer®
280 spectrophotometer (IMPLEN). The RNA concentration was measured using a Qubit®
281 RNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies) and its integrity was
282 assessed using an RNA Nano 6000 Assay Kit for the Bioanalyzer 2100 system
283 (Agilent Technologies).

284 **RNA library construction and RNA sequencing**

285 Ribosomal RNA was removed using a Epicentre Ribo-zero™ rRNA Removal Kit
286 (Epicentre), and the rRNA free residue was cleaned up using ethanol precipitation.

287 Subsequently, sequencing libraries were generated using the rRNA depleted RNA by
288 NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina® (NEB)
289 following manufacturer's recommendations. After cDNA synthesis and purification,
290 clustering of the index-coded samples was performed on a cBot Cluster Generation
291 System using a TruSeq PE Cluster Kit v3-cBot-HS (Illumina) according to the
292 manufacturer's instructions. The libraries were then sequenced on an Illumina Hiseq
293 4000 platform and raw data were generated.

294 **RNA sequencing analysis**

295 Clean data were obtained by removing reads containing adapters, reads only
296 containing poly-N, and low quality reads from the raw data. After quality control,
297 paired-end clean reads were aligned to the reference genome downloaded from
298 website using HISAT2 (v2.0.4). Cuffdiff (v2.1.1) was used to calculate the FPKM
299 values of both lncRNAs and mRNAs in each sample [30]. Gene FPKM values were
300 computed by summing the FPKM values of transcripts in each gene group. The
301 Cuffdiff model provides statistical routines to determine differential expression in the
302 digital transcript or gene expression data using a model based on the negative
303 binomial distribution. Transcripts with a corrected P value < 0.05 were assigned as
304 differentially expressed. We plotted a heatmap to observe the clustering between the
305 samples and the genes using Multi Experiment Viewer (v4.9.0).

306 **LncRNA-mRNA co-expression network analysis**

307 *Trans* role is lncRNA binding to target DNA as a RNA:DNA heteroduplex, as
308 RNA:DNA:DNA triplex, or RNA recognition of specific chromatin-like complex

309 surfaces[31]. We calculated the expression correlation between lncRNAs and mRNAs
310 using the Pearson correlation test for target gene prediction and the results were
311 expressed as Pearson correlation coefficients. To narrow the scope of investigation
312 and the number of genes, we first assessed the interaction among all DE mRNAs
313 using the STRING database (v11.0) and constructed a PPI network using the Hubba
314 package in Cytoscape (v3.7.1). After considering 12 synthetic algorithms, the
315 intersection of the top 50 genes yielded 24 hub genes, which occupy a central position
316 in the network of all DE mRNAs. After identifying and retaining the DE mRNAs and
317 lncRNAs in all lncRNA-mRNA relations, we screened 1109 pairs of exact
318 relationships between the two groups and constructed a co-expression network based
319 on the Pearson relation coefficient (all > 0.95). Then, we identified the annotated
320 lncRNAs and associated mRNAs for further functional enrichment analysis. PPI and
321 lncRNA-mRNA network diagrams were also drawn using the Cytoscape software.

322 **Gene ontology and KEGG pathways analysis**

323 By jointly using the David database (v6.8) and KOBAS database (v3.0), gene GO
324 enrichment and KEGG pathways analysis of all DE mRNAs and DE lncRNA
325 co-expressed DE mRNAs were implemented to interpret the biological meaning of the
326 transcripts. GO terms and KEGG pathways with corrected P values less than 0.05
327 were considered significantly enriched by the differentially expressed RNAs.

328

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333

334 **Author Contributions**

335 Chaoyu Chen and Nanping Wu were involved in the conception and design of the
336 study. Chaoyu Chen and Xiangyun Lu were involved in sample preparation, data
337 acquisition, and organization. Chaoyu Chen conducted the data analysis, and all
338 authors were responsible for data interpretation. Chaoyu Chen was the major
339 contributor to the writing of the manuscript. All authors read and approved the final
340 manuscript.

341 **Competing interests**

342 The authors declare that they have no competing interests.

343

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352

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430 **Supporting information**

431 **S1 Figure. Gene ontology (GO) terms and Kyoto Encyclopedia of Genes and**
432 **Genomes (KEGG) pathways enrichment of all differentially expressed (DE)**
433 **mRNAs.**

434 **S1 Table. Clinical characteristics of the participants.**

435 **S2 Table. Lists of differentially expressed (DE) mRNAs and long non-coding**
436 **RNAs (lncRNAs) in health controls (HCs) vs. normal-process infected patients**
437 **(NPs).**

438 **S3 Table. Lists of DE mRNAs and lncRNAs in HCs vs. elite controllers (ECs).**

439 **S4 Table. Lists of DE mRNAs and lncRNAs in ECs vs. NPs.**

440 **S5 Table. The intersection list of the top 50 hub-mRNAs calculated using 12**
441 **algorithms.**

442

443 **Fig 1. Flowchart of the analysis procedure.** lncRNA, long non-coding RNA; EC,
444 elite controller; NP, normal-process infected patient; DE, differentially expressed;
445 GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

446

447 **Fig 2. Heatmaps of differentially expressed (DE) mRNAs and long non-coding**
448 **RNAs (lncRNAs).**

449 Hierarchical clustering analysis of sequencing detected mRNAs and lncRNAs with
450 their expression abundance. Patient groups are on the horizontal axis, and mRNA and
451 lncRNA genes are grouped along the vertical axis. Pinkish-purple bars indicate
452 increased abundance of the corresponding genes, and blue indicates decreased
453 abundance. White bars indicate that the corresponding genes were not detected.

454

455 **Fig 3. Protein-protein interaction (PPI) network of differentially expressed (DE)**
456 **mRNAs.**

457 Top 50 hub mRNAs were included in using EcCentricity algorithm. The intensity of
458 the color indicates the density of the correlation. The closer to red, the more central
459 the gene is. The size of the circle represents the significance of a gene.

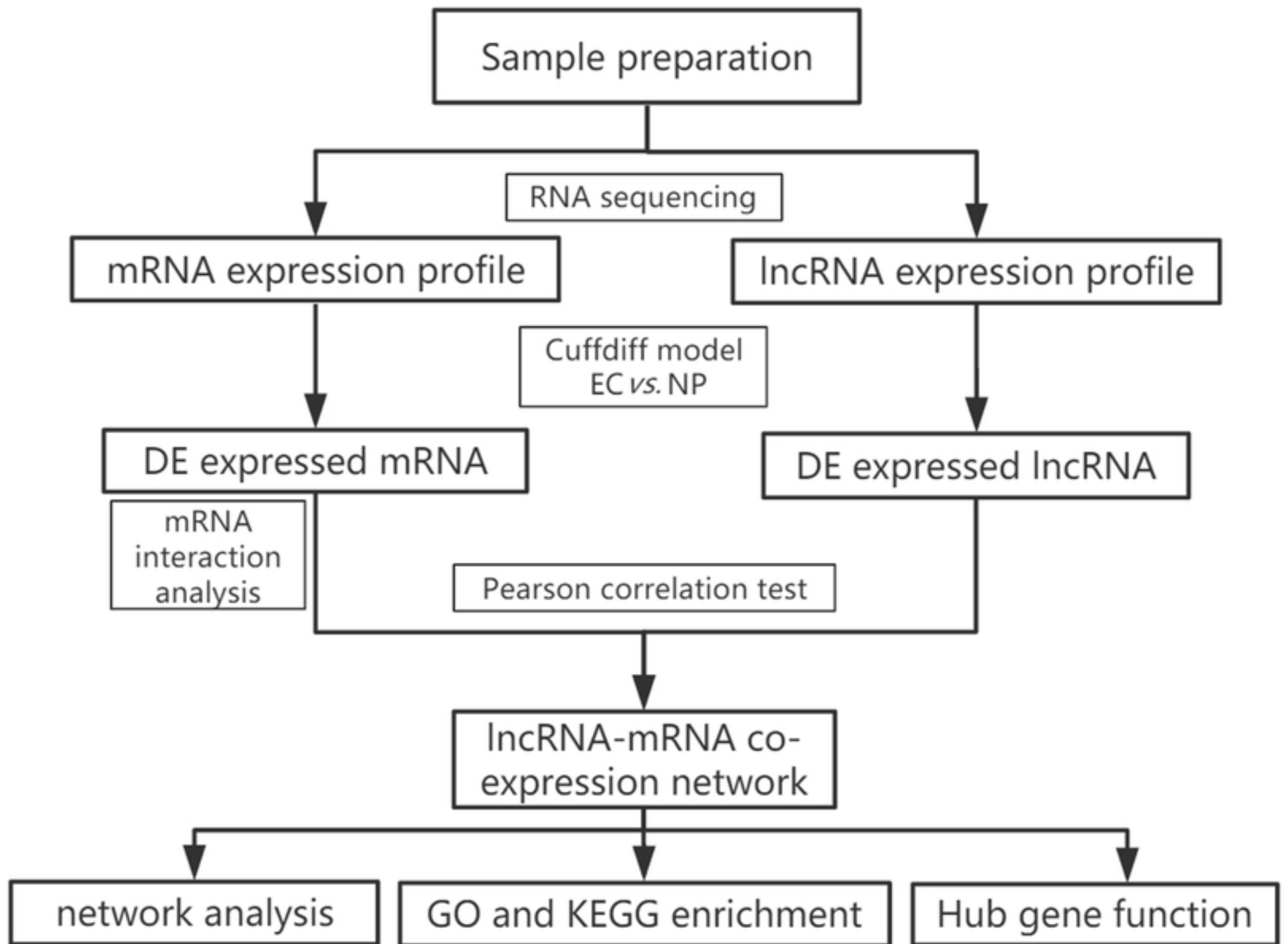
460

461 **Fig 4. Long non-coding RNA (lncRNA)-mRNA co-expression network in elite**
462 **controllers (ECs) and normal-process infected patients (NPs).** The network was
463 based on the Pearson relation coefficient of an lncRNA targeting an mRNA. Round
464 shapes correspond to mRNAs, and squares to lncRNAs. The size of the shape is
465 positively related to the P value. Red means upregulation and green means
466 downregulation. A solid line indicates a positive correlation and a dotted line indicates
467 a negative correlation. All hub-mRNAs and annotated lncRNAs are tagged with large
468 labels. (a) All lncRNAs and co-expressed mRNAs. (b) Annotated lncRNAs and
469 co-expressed mRNAs.

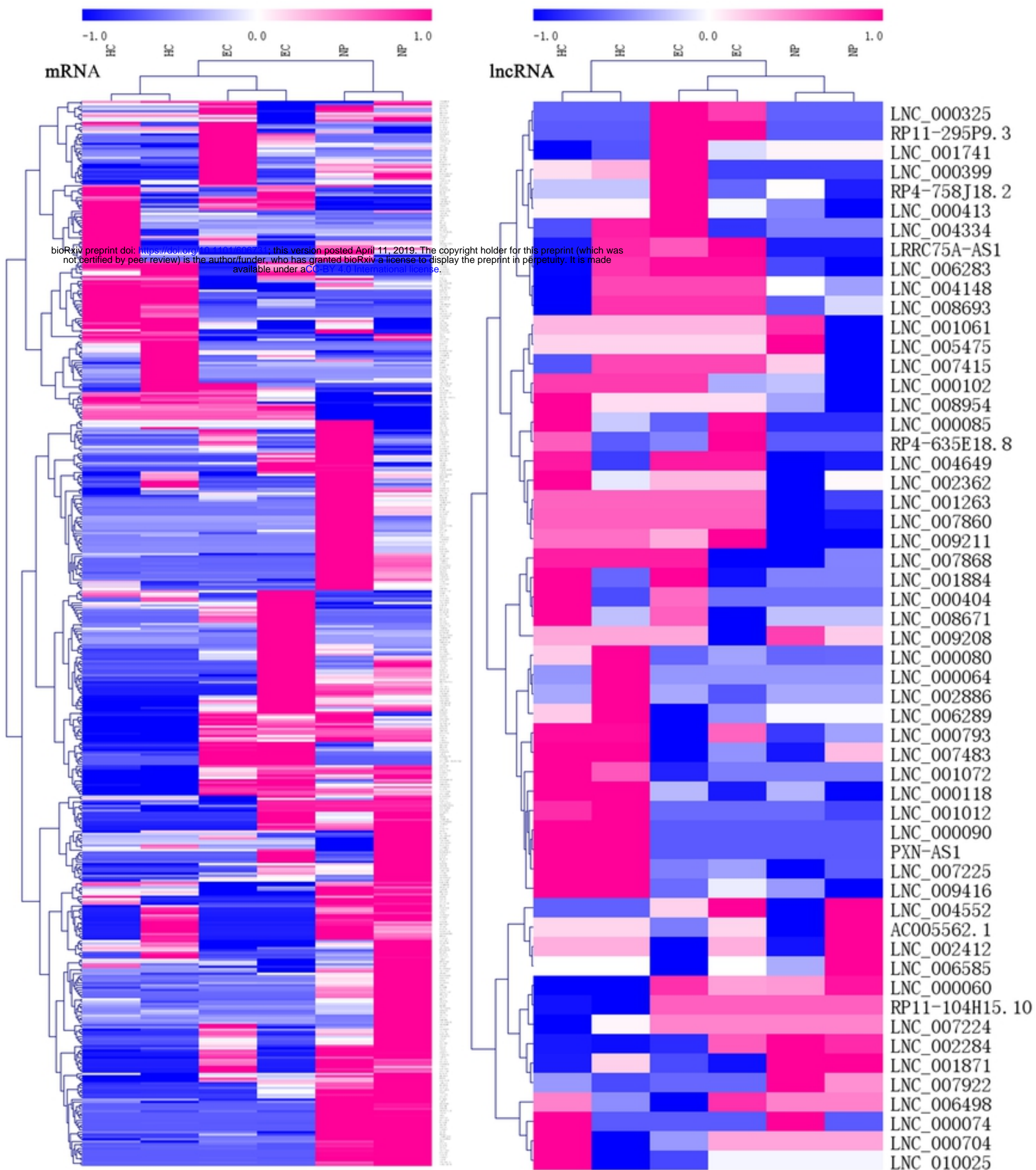
470

471 **Fig 5. Gene ontology (GO) terms and Kyoto Encyclopedia of Genes and**
472 **Genomes (KEGG) enrichment classification of the predicted targeting mRNAs of**
473 **the long non-coding RNA (lncRNA)-mRNA network.** The 10 most significantly
474 enriched KEGG pathways and the 10 most significantly enriched GO terms in the
475 biological process, cellular components, and molecular function categories.

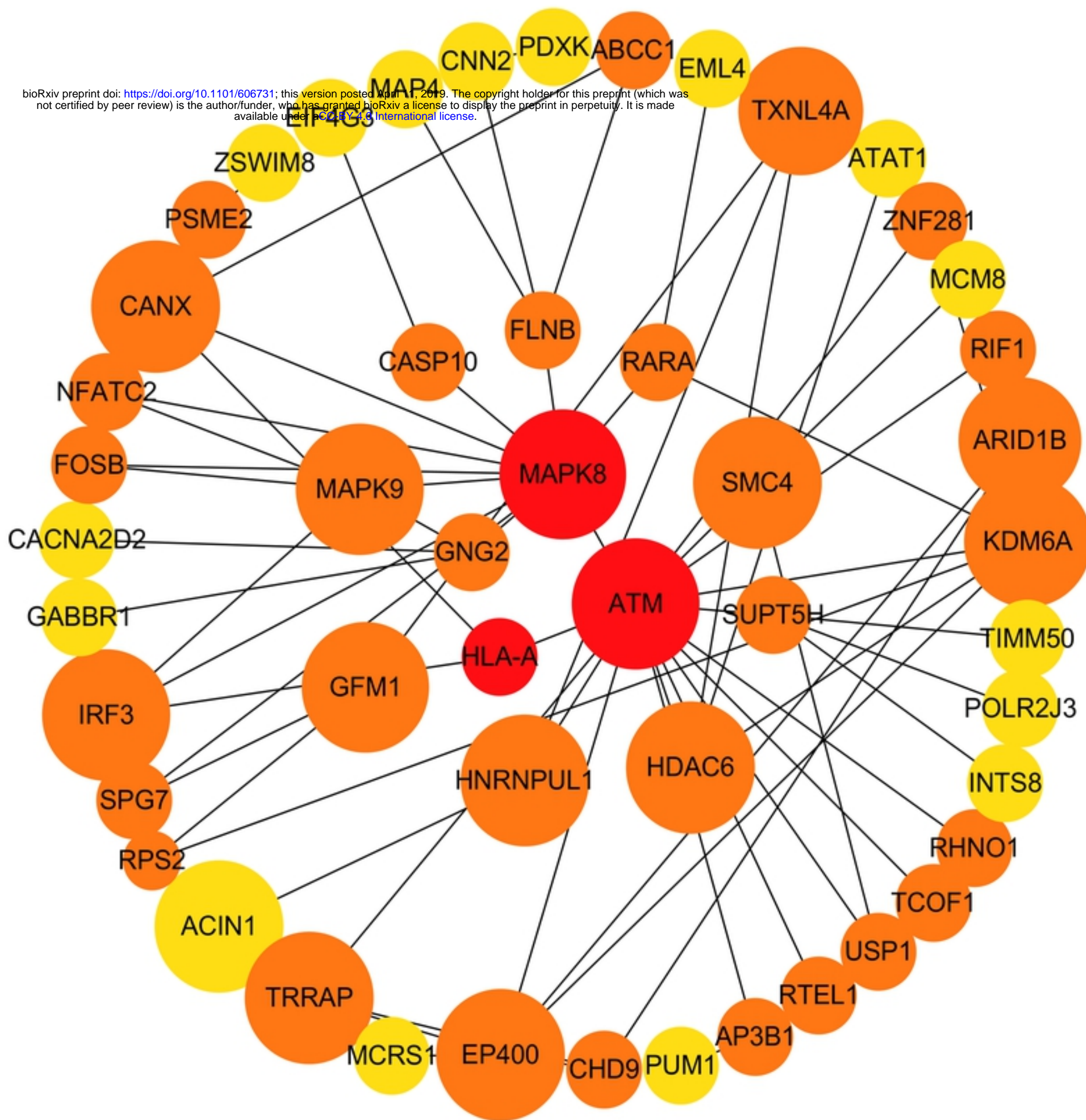
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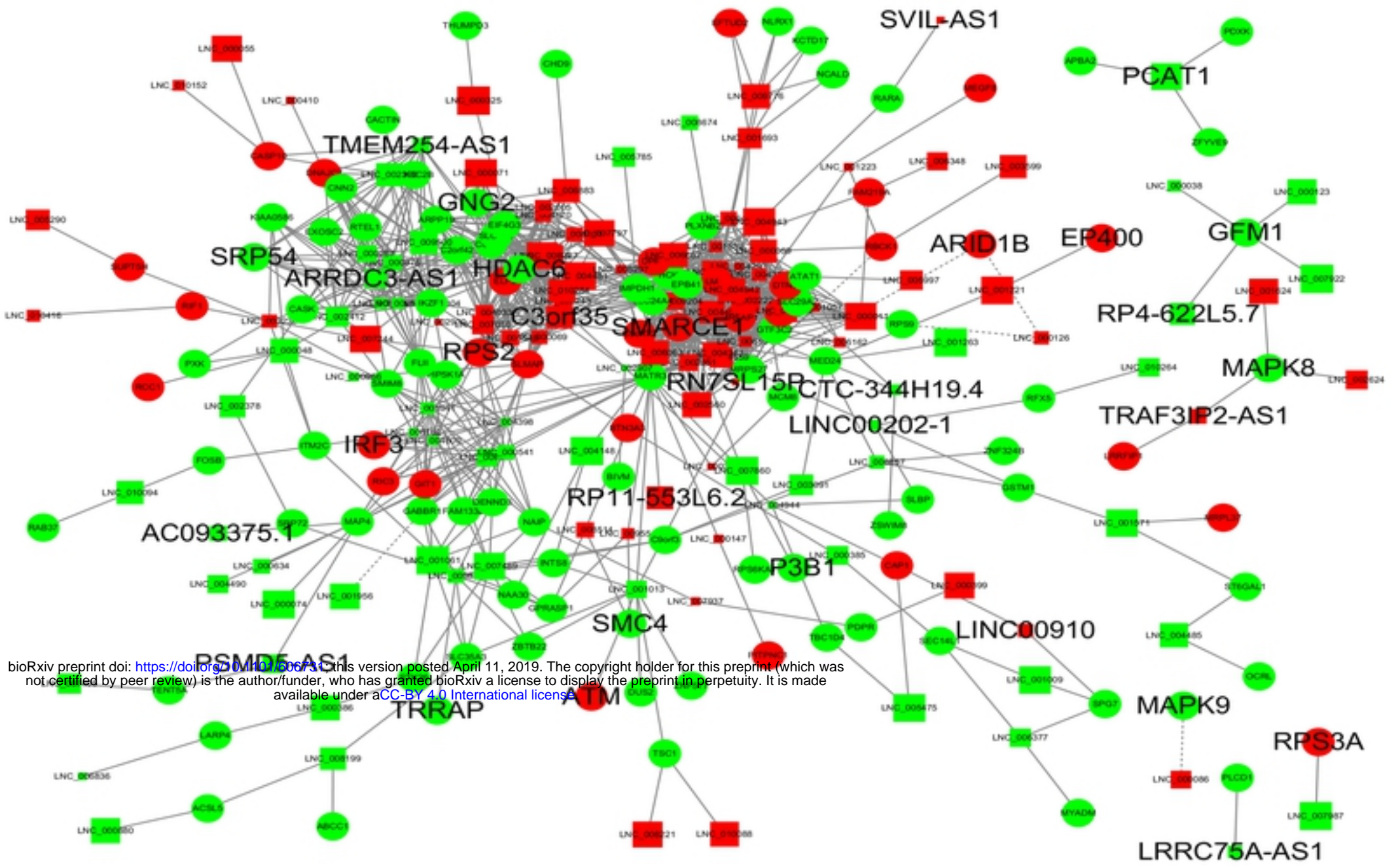
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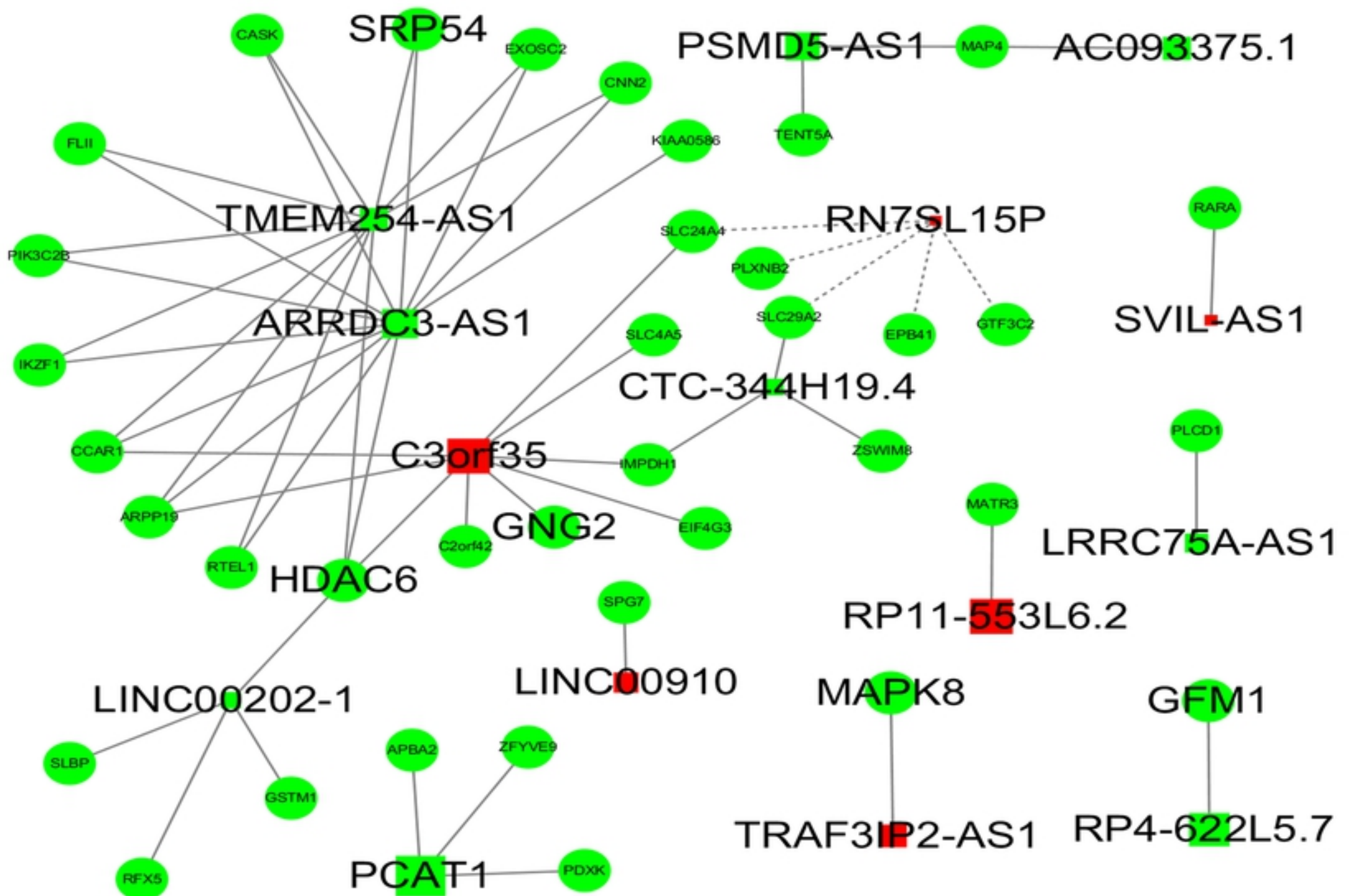
Figure



Figure

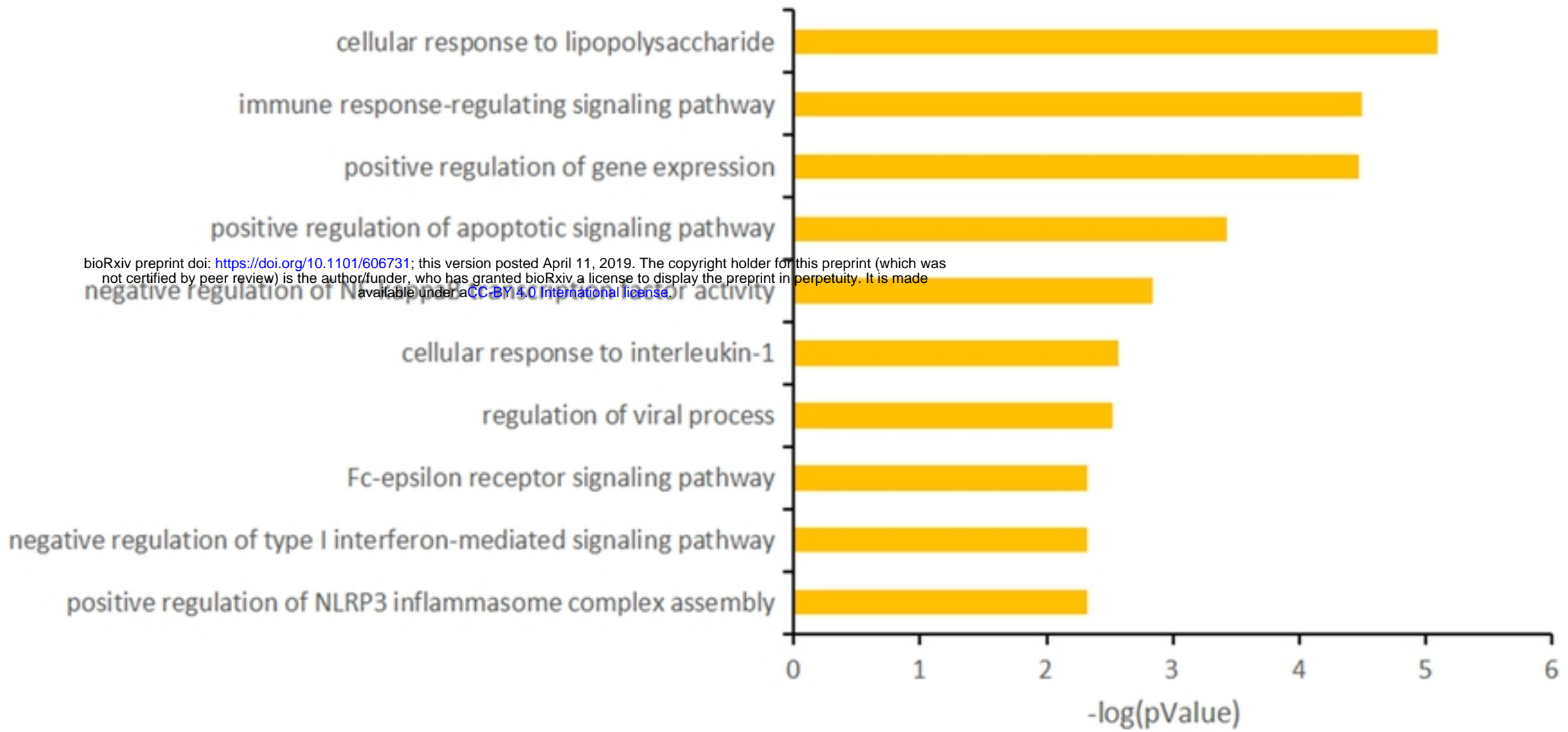


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Figure

GO Terms



KEGG Pathways

