

1 **(5R)-5-Hydroxytriptolide (LLDT-8) induces substantial epigenetic mediated immune response**
2 **network changes in fibroblast-like synoviocytes from rheumatoid arthritis patients**

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27 **Keywords:** lncRNA, LLDT-8, Fibroblast-like Synoviocytes, Rheumatoid Arthritis, Cytokine
28 Regulation, Traditional Chinese medicine (TCM)

29

30 **Abbreviations**

31 RA: Rheumatoid Arthritis; LLDT-8: (5R)-5-Hydroxytriptolide; FLS: Fibroblast-like Synoviocytes

32 lncRNA: Long non-coding RNA; TF: Transcription Factors;

33

34 **Run title:** Transcriptional Regulatory Network Response to LLDT-8

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36 **Figures:** 4

37 **Tables:** 3

38 **Supplementary Tables:** 6

39 **Abstract**

40 *Tripterygium* is a traditional Chinese medicine that has widely been used in the treatment of
41 rheumatic disease. (5R)-5-hydroxytriptolide (LLDT-8) is an extracted compound from *Tripterygium*,
42 which has been shown to have lower cytotoxicity and relatively higher immunosuppressive activity
43 when compared to *Tripterygium*. However, our understanding of LLDT-8-induced epigenomic
44 impact and overall regulatory changes in key cell types remains limited. Doing so will provide
45 critically important mechanistic information about how LLDT-8 wields its immunosuppressive
46 activity. The purpose of this study was to assess the effects of LLDT-8 on transcriptome including
47 mRNAs and long non-coding RNA (lncRNAs) in rheumatoid arthritis (RA) fibroblast-like
48 synoviocytes (FLS) by a custom genome-wide microarray assay. Significant differential expressed
49 genes were validated by QPCR. Our work shows that 394 genes (281 down- and 113 up-regulated)
50 were significantly differentially expressed in FLS responding to the treatment of LLDT-8. KEGG
51 pathway analysis showed 20 pathways were significantly enriched and the most significantly
52 enriched pathways were relevant to Immune reaction, including cytokine-cytokine receptor
53 interaction ($P=4.61\times10^{-13}$), chemokine signaling pathway ($P=1.01\times10^{-5}$) and TNF signaling pathway
54 ($P=2.79\times10^{-4}$). Furthermore, we identified 618 highly negatively correlated lncRNA-mRNA pairs
55 from the selected significantly differential lncRNA and mRNA including 27 cis-regulated and 591
56 trans-regulated lncRNA-mRNAs modules. KEGG and GO based function analysis to differential
57 lncRNA also shown the enrichment of immune response. Finally, lncRNA-transcription factor (TF)
58 and lncRNA-TF-mRNA co-expression network were constructed with high specific network
59 characteristics, indicating LLDT-8 would influence the expression network within the whole FLS
60 cells. The results indicated that the LLDT-8 would mainly influence the FLS cells systemically and
61 specially in the process of immune related pathways

62

63 **Introduction**

64 Rheumatoid arthritis (RA) is the most common chronic inflammatory disease with complex etiology
65 ¹. The inflammation makes the synovium thicken and causes swelling around the joints. Thus, it
66 induces the damage of cartilage. Recent findings indicate that remission of symptoms is more likely
67 when treatment begins early with medications ². Although several widely-used anti-rheumatic drugs,
68 such as methotrexate, steroids, anti-IL-6R and anti-TNF α monoclonal antibodies, have demonstrated
69 some degree of efficacy in the treatment of RA, a substantial proportion of patients exhibit poor
70 response and the incidence of adverse reactions is substantial. The use of plant- and microbial-based
71 compounds to treat a variety of pathologies has an extensive history and includes digitalis, L-Dopa,
72 taxol, quinine, ephedrine, codeine, and penicillin ^{3,4}. In our clinical practice, we found a traditional
73 Chinese medicine *Tripterygium* could be applied to decrease the severity of RA. *Tripterygium* is a
74 genus of plants from the Euonymus Corey Gong. This Traditional Chinese medicine has been used in
75 the treatment of autoimmune disease ^{5,6}, rheumatic disease ^{7,8}, and systemic lupus erythematosus
76 (SLE) ^{9,10}. Triptolide is an extracted compound from *Tripterygium*, which has strong
77 immunosuppressive activity. Triptolide can inhibit interleukin-6 (IL-6) and reduce osteoclastogenesis
78 by inhibiting NF- κ B signaling. An optimized structured analog of Triptolide, (5R)-5-
79 hydroxytriptolide (LLDT-8), has been shown to have both low toxicity and high immunosuppressive
80 ability ¹¹. Previous studies also demonstrated that LLDT-8 can suppress the immune responses on
81 peripheral blood mononuclear cells (PBMC) and T cells ¹¹. Importantly, LLDT-8 also inhibited the
82 differentiation of Th1 and Th17 cells and has effect on the immune responses of RA patients ¹².
83 Increasing evidence indicates that activated synovial fibroblasts, together with macrophage and
84 lymphocyte secreted factors, as part of a complex cellular network, play an important role in the
85 pathogenesis of rheumatoid arthritis ¹³. However, the pharmacological effect of LLDT-8 on synovial
86 fibroblasts has not been investigated. Further, elucidating the underlying molecular mechanisms of

87 how LLDT-8 generates immunosuppression without a high degree of toxicity may provide a
88 platform for the development of novel anti-rheumatologic therapeutics.

89 Long non-coding RNAs (lncRNAs) are a recently discovered class of non-coding functional RNA
90 and some important roles of lncRNAs as regulators in a wide spectrum of biological processes were
91 identified in the past several years¹⁴⁻¹⁶. There are over 16,000 lncRNAs in humans and the functions
92 of majority of them are unknown. Recent evidence also suggests the role of lncRNAs in the
93 pathophysiology of disease processes, especially in cancer and immune disease¹⁷⁻²¹. In the previous
94 studies, lncRNA have been reported to be related to the pathogenesis of RA due to its association of
95 major pathways linked to Inflammation, such as NFkB and TLR signaling²⁰. However, whether
96 lncRNA is involved in the mechanism of therapy of LLDT-8 to rheumatoid arthritis is still unknown.

97 The purpose of our study was to assess the effects of LLDT-8 on the regulation of gene expression
98 on mRNAs and long non-coding RNAs in FLS isolated from RA patients. Agilent Human lncRNA
99 (4 × 180 K, Design ID: 062918) was applied in present study to provide genome-wide ncRNA and
100 mRNA expression profile. With this study, we aimed to demonstrate how LLDT-8 treatment
101 significantly impacts the process of immune regulation.

102 **Material and Methods**

103 **Patients, cell culture and LLDT-8 treatment**

104 FLS cells were derived from the synovial tissues of patients with RA who had undergone total joint
105 replacement surgery during June to August 2015. All the relevant samples were systematically stored
106 in Guanghua Rheumatoid Arthritis Biobank (GHRAB). All patients fulfilled the American College
107 of Rheumatology classification criteria (2010) for RA. This study was conducted in compliance with
108 all relevant ethical regulations, and all research participants provided informed consent under a study
109 protocol approved by the Institutional Review Board (IRB) of the Guanghua Hospital (Shanghai,
110 China). Clinical data were collected at the time of sample collection and detailed clinical information
111 is shown in Table 1.

112 Tissues were minced into 1 x 1 x 1 mm pieces and treated for 2 hours (h) with 2-4 mg/ml of
113 collagenase (Serva, GERMANY) in DMEM at 37°C in 5% CO₂. Dissociated cells were then
114 centrifuged at 500 × g, re-suspended in DMEM supplemented with 10% FCS (Gibco, USA), and
115 plated in 75 cm² flasks. The cultures were kept at 37°C in 5% CO₂ and the culture medium was
116 replaced every 2-3 days. When cells approached confluence, they were passed after diluting 1:3 with
117 fresh medium until used. The purity of the cells was verified by flow cytometric analysis. The FLS,
118 from passages 4, were seeded in 6-well plates.

119 LLDT-8 (Shanghai Pharma, Shanghai) was dissolved with 2% DMSO and diluted with DMEM to
120 100nm/ml and the FLS cells in treatment group were cultured with previous DMEM while the FLS
121 in control group were cultured with DMSO/DMEM for 24 hours. Then all the FLS cells were
122 cultured in DMEM including 10ng/ml TNF-α and 10ng/ml IL-17 (PeproTech, USA) for 12 h. In the
123 process to validate the microarray data, TNF- α treatment is applied to simulate inflammatory
124 reaction and then LLDT8 treatment is applied to check the effect of LLDT-8 to inflammatory
125 reaction. Western Blot and Immunofluorescence were applied to detect the protein expression
126 change and nuclear localization to show the effect of LLDT-8 treatment to RF cells in our validation
127 stage and the procedures are same as the previous study [2].

128 **RNA extraction and microarray hybridization**

129 The total RNA was extracted by TRIzol reagent and quantified by the NanoDrop ND-2000 (Thermo
130 Scientific, Waltham, MA, USA). The integrity of RNA was assessed using the Agilent Bioanalyzer
131 2100 (Agilent Technologies Inc, Santa Clara, CA, USA).

132 Customized Agilent Human lncRNA microarray (4x180K, Design ID: 062918) was used in this

133 study. 30,656 probes located in mRNA transcripts from Entrez Gene and 78,243 probes in lncRNA.
134 the catalog of the lncRNA were collected from the integration of Broad Institute,
135 Human Body Map lncRNA, TUCP catalog, UCSC lncRNA Transcripts, GENCODE 18, NONCODE
136 V4.0, Ensembl, RefSeq, Ultra-conserved region encoding LncRNA (UCR), lncRNADB and ncRNA
137 database. The total RNA was transcribed to double-stranded cDNA, synthesized into cRNA (Low
138 Input Quick-Amp Labeling Kit, one-color, Agilent), and labeled with Cyanine-3-CTP. The labeled
139 cRNAs were then hybridized onto the microarray. After washing, the arrays were scanned by the
140 Agilent Scanner G2505C (Agilent Technologies Inc., Santa Clara, CA, USA). Other parameters in
141 ncRNA labeling, microarray hybridization, and washing procedure were performed according to the
142 manufacturer's standard protocols. Real-time PCR was applied to validate parts of significant
143 differential genes within same samples with > 3 times technical repeats.

144 **Image scanning and data analysis**

145 The array images were analyzed using Feature Extraction software (version 10.7.1.1, Agilent
146 Technologies Inc., Santa Clara, CA, USA), and the raw data were obtained and basically analyzed
147 with Genespring. The raw data were initially normalized with the quantile algorithm. The probes that
148 at least in one out of two conditions had flags in "P" were chosen for further data analysis.
149 Differentially expressed genes or lncRNAs were identified through a combination of fold change and
150 p-values calculated from a t-test (details in the Statistical analysis section). The threshold set for
151 upregulated and downregulated genes was a fold change ≥ 2.0 and a $P \leq 0.05$.

152 **Identification of cis-regulated mRNAs of the differential lncRNAs**

153 Based on these 5 biological replications with or without LLDT-8 treatment, differential expressed
154 lncRNA, cis-regulatory genes/mRNAs were identified. The mRNAs were identified as "cis-regulated
155 mRNAs" when (1) the mRNA loci are within 100 k windows up- and down-stream of the given
156 lncRNA, and (2) the Pearson correlation of lncRNA-mRNA expression is significant ($P \leq 0.01$). GO
157 analysis and KEGG analysis were applied to determine the roles of these differentially expressed
158 mRNAs. Finally, Hierarchical Clustering was performed to display the distinguishable genes'
159 expression pattern among samples.

160 **Functional prediction of selected differential lncRNAs**

161 The overall gene function distribution (co-expression, ontology and pathway) of the differential
162 lncRNAs obtained in the experiment was identified as follows. For each differential lncRNA, the
163 Pearson correlation of its expression value with the expression value of each mRNA was calculated,
164 and a $P < 0.05$ was selected. The enrichment of functional terms in annotation of differential
165 expressing gene or co-expressed mRNAs was statistically evaluated using the hypergeometric
166 cumulative distribution function^{22,23}.

167 **Identification of transcription factors (TF) associated to differential lncRNAs**

168 The transcription factor/chromatin regulation complexes that may possibly play a co-regulatory role
169 with lncRNAs were identified^{24,25}. In brief, the set between the lncRNA co-expression coding genes
170 and the target genes of transcription factors/chromatin regulation complex was collected respectively.
171 The enrichment level of the set was determined using hypergeometric distribution, thus the
172 transcription factors significantly associated with differential lncRNAs were finally screened. Finally,
173 the co-expression networks among lncRNA, TF, and target genes were built with Cytoscape 3.4.0²⁶.

174 **Statistical analysis**

175 One sample t-test was applied to compare the mean difference of the expressed level before and after
176 the treatment of LLDT-8. All the statistical analysis was made using R version 3.2.2. We selected the
177 top signals using the threshold on both the fold change > 2 and the P-value < 0.05 from mRNA and
178 lncRNA. In addition, we calculated the Pearson correlation for the expression values on every

179 different combination of lncRNA and mRNA. The pair of lncRNA-mRNA will be selected if their
180 correlation test reaches the threshold (P -value < 0.05). Also, in order to ensure the selected pairs are
181 meaningful, we only selected the correlated pairs in which lncRNA plays as the inhibition of mRNA
182 expression (correlation coefficient, $r < -0.9$). False discover rate (FDR) correction was performed by
183 Benjamini-Hochberg procedure with default R function.

184 Results

185 Genome-wide mRNA profile of before and after LLDT-8 treatment

186 By analyzing genome-wide lncRNA microarray of RA FLS responding to LLDT-8, we found 394
187 differentially expressed genes ($p < 0.05$, fold change > 2). 71% (281) of them were down-regulated and
188 28.7% (113) were up-regulated (Supp. Table 1). Volcano plot for the differential express genes was
189 shown in Figure 1A. Supervised cluster analysis (Heatmap) showed differentially expressed genes
190 could distinguish the samples before and after LLDT-8 treatment (Figure 1B). In order to check the
191 quality of the microarray data, we validated several canonical immune responses relate genes, such
192 as *NFKB1* ($P = 1.80 \times 10^{-10}$), *MYD88* ($P = 1.26 \times 10^{-25}$), *JUN* ($P = 1.09 \times 10^{-14}$) and *FOS* ($P = 4.41 \times 10^{-22}$)
193 with qPCR and we found the result were highly consistent with microarray results (Figure 1C).
194 These down-regulated T Cell receptor signaling pathway (*NFKB1*, *JUN*, *FOS*) and Toll-like receptor
195 signaling pathway (*MYD88*) genes indicate LLDT-8 could provide significantly immunosuppressive
196 activity with multiple molecular networking approaches. KEGG pathway analysis (Figure 1D) of the
197 differentially expressed genes showed 20 pathways were significantly enriched ($P < 0.05$, FDR < 0.01).
198 Interesting, the top 4 enriched pathways were significantly relevant to Immune reaction, including
199 cytokine-cytokine receptor interaction ($P = 4.61 \times 10^{-13}$), rheumatoid arthritis ($P = 1.90 \times 10^{-6}$),
200 chemokine signaling pathway ($P = 1.01 \times 10^{-5}$) and TNF signaling pathway ($P = 2.79 \times 10^{-4}$). These
201 findings indicated that the LLDT-8 would greatly influence the RA FLS in the process of immune
202 regulation. By analyzing interaction networks based on KEGG, STRING, and BioGRID, we found
203 that these differentially expressed genes were significantly interacting with each other and
204 constructed specific and explicit networks (Figure 2, Supp. Figure 1).

205 Genome-wide lncRNA profile of RA FLS before and after LLDT-8 treatment

206 The lncRNAs with both fold change ≥ 2.0 and P -value ≤ 0.05 from the *t*-test were identified as
207 differentially expressed lncRNAs. Our results showed that 360 lncRNAs of RA FLS were
208 significantly changed with LLDT-8 treatment. Those P -values ranged from 4.88×10^{-2} to 2.92×10^{-7} ,
209 and the fold change spanned from 2.00 to 18.44. Among these lncRNAs, 56% (203) were
210 downregulated and 44% (157) were upregulated (Supp. Table 2). Our result indicates not only
211 mRNAs but also lncRNA transcripts would be widely changed during the treatment of LLDT-8. In
212 addition, we found the target genes of the differential lncRNAs that could also be a strong indication
213 of the change caused by LLDT-8 treatment. In addition, as Figure 1E shows, cluster analysis based
214 on the 30 co-expression genes of differential lncRNA (ENST00000584923) could separate the
215 samples quite accurately with or without LLDT-8 treatment, indicating that the lncRNA network and
216 the mRNA network in the cells were highly interacted and coordinately changed by LLDT-8
217 treatment.

218 Co-expression network analysis between mRNA and lncRNA

219 The lncRNAs co-expressed function was identified through the correlation of lncRNA-mRNA. For
220 each of the lncRNA, we calculate the Pearson correlation of its expression value with the expression
221 value from every different mRNA. The pair of lncRNA and mRNA was selected if their correlation
222 test reached the threshold (P -value < 0.05). Also, in order to ensure the selected pairs is meaningful,
223 we only kept those correlated pairs which show strong negative correlation (correlation coefficient $<$
224 -0.9). Our result indicated that there were 9,666 pairs of lncRNA-mRNA with strong negative
225 correlation. Moreover, among those 9,666 strong negatively correlated lncRNA-mRNA pairs, 618

were also identified as the significantly differential mRNA and lncRNA from the t-test. Also, among those 618 lncRNA-mRNA pairs, 70 pairs of them were located in the same chromosome. Furthermore, 13 out of those 70 pairs were located in chromosome-6, and 10 out of those 70 pairs were located in chromosome-17. Such kind of chromosome-distribution disequilibrium suggests the influence of the LLDT-8 is not a random effect but with specific target and influence to FLS. 27 cis-regulated and 591 trans-regulated lncRNA-mRNAs modules were identified ([Supp. Table 3 and 4](#)).

Functional prediction of selected differential lncRNAs analysis were conducted both with GO and KEGG strategies. Gene Ontology analysis showed that the term of innate immune responses was significantly enriched and 17 differential lncRNAs were identified to be involved ([Supp. Table 5](#)). KEGG analysis identified term of rheumatoid arthritis was significant enriched and 4 differential lncRNAs (NONHSAG028996, NONHSAT142637, ENST00000584934 and NR_028330.1) were identified to be involved ([Supp. Table 6](#)).

We also identified large number of co-expressed lncRNA and TFs. The co-expression network between TF and lncRNA was shown in Figure 3A. We discovered one large sub-network and large number isolated interaction between TFs and lncRNA. This network indicated lncRNA and TF would regulate the gene expression together in FLS cells. When we build lncRNA, TF, target gene network together ([Figure 3B](#)), the result is quite similar as our expectation, large numbers of genes were identified regulated by lncRNA and TF simultaneously.

Cellular regulation effect of LLDT-8 treatment to FLS involved in NF-κB pathway

In order to validate our discoveries that LLDT-8 could provide pharmacological effect to RA therapy via the interaction with rheumatology related pathway ([Figure 1D](#)), we validate the effect of LLDT-8 to one of most important rheumatology related pathway, NF-kappa B signaling pathway. Western-blot shown that the protein level of p-p65 and p-IκB α are strongly inhibited with increasing concentration of LLDT-8 treatment to FLS cells stimulated by TNF- α and IL-17 ([Figure 4A](#)). In addition, we also demonstrate that LLDT-8 would inhibit the nuclear translocation of the p65 ([Figure 4B](#)) which is the significant effect of TNF and IL-17, indicating LLDT-8 has significant inhibition to TNF and IL-17 effects and therefore have potential pharmacological effect to RA therapy.

Discussion

Tripterygium is a traditional Chinese medicine, which has widely been used in the treatment of rheumatic disease. (5R)-5-hydroxytriptolide (LLDT-8) is an extracted compound from *Tripterygium* and has been showed lower cytotoxicity and relatively higher immunosuppressive activity. However, how LLDT-8 influences RA FLS cells is still unknown, especially in the level of lncRNAs. In this study of genome-wide microarray assay, we identified large number of lncRNA and mRNAs responding to the LLDT-8 treatment. In addition, the first LLDT-8 related cis-regulated and trans-regulated lncRNA-mRNA modules were identified. We also constructed the first lncRNA-TF-mRNA co-expression network and demonstrated that LLDT-8 influenced the expression network within the whole FLS cells and therefore provided potential molecular and cellular mechanisms that LLDT-8 could be considered as potential rheumatoid arthritis drugs.

As we know, this is the first study to elaborate the genome-wide lncRNA and mRNA changes with or without LLDT-8 treatment. Our study provided an important landscape to design further studies to investigate the medical applications for lncRNAs in RA therapy. Although, genome-wide association studies (GWAS) have identified hundreds of variants associated with RA, our understanding of the disease mechanisms is still limited. Notably, more than 90% of the risk variants lie in non-coding regions, and almost 10% are corresponding to lncRNA regions ^{17,27,28}. Considering that our result showed the significant change of the lncRNA during LLDT-8 treatment, lncRNA would be play important role in the pathogenesis and therapy of RA.

Our study also provided a probability to compare the lncRNA profiles with RA, primary Sjögren's

273 syndrome²⁹, gastric carcinogenesis³⁰, endometrial carcinoma³¹, bladder cancer³², esophageal
274 squamous cell carcinoma³³, non-small-cell lung cancer³⁴ and cervical cancer³⁵, since our data has
275 been deposited to GEO database. These diseases might share some common aberrant lncRNAs
276 relevant with inflammation and immune response and therefore provide some special insight to the
277 therapy of RA.

278 In summary, our results provided a good prospect in further clinical tests of LLDT-8 for its
279 therapeutic potential in the treatment of RA. We also suggest that more studies need to be done in
280 order to further investigate the biological functions of lncRNAs in more patient samples. However,
281 those initial results suggest that lncRNA would be a good biomarker and drug target in the future
282 drug discovery, especially for LLDT-8.

283 Conclusion

284 Genome-wide lncRNA and transcriptome analysis of response to treatment with (5R)-5-
285 Hydroxytriptolide (LLDT-8) in cells shown LLDT-8 would mainly influence the RA cells
286 systemically and especially in the process of immune network regulation.

287

288 Declarations

289 Acknowledgements

290 We thank all participating subjects for their kind cooperation in this study.

291 Authors' contributions

292 DH and SG contributed to the conception, design and final approval of the submitted version. SG, JL
293 and TJ contributed to the integrated analysis of multiple microarray datasets, batch effect elimination
294 and statistical analysis. TJ, YL, RW, YS, XZ, YW, FB, QD, JZ, GW and XZ collected samples and
295 helped to data cleaning, statistic and draft the manuscript. The final manuscript was completed by
296 SG, SS and DH. All authors read and approved the final manuscript.

297 Competing interests

298 No potential conflicts of interest was disclosed for all the authors

299 Funding

300 This work was funded by the National Natural Science Funds of China (81774114), Shanghai
301 clinical base construction of traditional Chinese medicine (ZY3-LCPT-1-1009, ZY-LCPT-1),
302 Shanghai intensive entity construction of integrated traditional and western medicine rheumatoid
303 arthritis (ZXBZ2012-05), Shanghai clinical intensive subject construction of traditional Chinese
304 medicine-traditional Chinese rheumatology (ZYXK2012012), Shanghai Municipal Planning
305 Commission of science and Research Fund (201640192).

306 Availability of data and material

307 The microarray data was deposited in the Gene Expression Omnibus (GEO accession: GSE71841).

308

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376

377 **Figure and Table Legends**

378 **Figure 1.** Genome-wide differential analysis for the treatment of LLDT-8 in FLS cells from RA
379 patients

380 A. Volcano plot for the differential express genes. B. Heatmap plot for differential expressed
381 genes to show the effect of LLDT-8 to FLS cells. C. qPCR validation to significant
382 differential expressed genes from microarray. D. Gene ontology analysis to differential
383 expressed genes (N=3 technical repeats in control, TNF- α and LLDT8 treatment group).
384 TNF- α treatment is applied to simulate inflammatory reaction and then LLDT8 treatment is
385 applied to check the effect of LLDT8 to inflammatory reaction. E. Heatmap plot to the co-
386 expressed mRNA of differential lncRNA have the power to separate samples with or without
387 LLDT-8 treatment.

388 **Figure 2.** Interaction network analysis to differential expression genes based on STRING

389 A. Evidence view with STRING 9.0, B. BioGRID based Network, C. KEGG based network.

390 **Figure 3.** Co-expression network of lncRNA-TF and lncRNA-TF-targetGene

391 A. Co-expression network of lncRNA-TF, B. Co-expression network of lncRNA-TF-Target Genes.

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393 **Table 1.** Clinical and demographical Characteristics of 5 RA patients

394 **Table 2.** Most significant trans-lncRNA-mRNA regulation pairs for the significant differential
395 lncRNA.

396 **Table 3.** Most significant cis-lncRNA-mRNA regulation pairs for the significant differential lncRNA

Table 1. Clinical and demographical Characteristics of 5 RA patients

ID	Race	City	Disease	COD(Year)	SJC	TJC	ESR	CRP	PGA	DAS28-CRP
RA-001	Han Chinese	Shanghai	RA	5	4	4	19	29.8	40	4.3
RA-002	Han Chinese	Zhejiang	RA	20	10	10	27	25.97	70	5.66
RA-003	Han Chinese	Shanghai	RA	6	1	1	25	6.1	60	3.58
RA-004	Han Chinese	Suzhou	RA	8	1	2	11	1.3	60	3.05
RA-005	Han Chinese	Suzhou	RA	20	18	16	21	18	65	6.19

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398 COD: course of a disease; SJC: swollen joint count; TJC: tender joint count; ESR: erythrocyte sedimentation rate; PGA: patient global
399 assessment; DAS28-CRP: disease activity score in 28 Joints

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415 Table 2. Most significant cis-lncRNA-mRNA regulation pairs for the significant differential lncRNA

LncRNA	Coordination	P	FC	Regulation	Gene Symbol	P-value	FC	Regulation	P	R
<i>FR264384</i>	chr9:2653919-2654254	1.03×10^{-2}	2.67	down	<i>NINJ1</i>	2.30×10^{-5}	2.4	up	2.40×10^{-4}	-0.9
<i>FR344093</i>	chr10:60502900-60507641	9.35×10^{-3}	2.00	down	<i>IFIT2</i>	1.42×10^{-2}	3	up	3.83×10^{-4}	-0.9
<i>LINC00473</i>	chr6:166352947-166401527	1.86×10^{-2}	2.48	down	<i>HSPA1B</i>	7.52×10^{-4}	3	up	2.16×10^{-4}	-0.9
<i>LINC00473-002</i>	chr6:166322290-166401410	1.32×10^{-2}	2.29	down	<i>HSPA1A</i>	3.44×10^{-4}	3	up	3.68×10^{-4}	-0.9
<i>LINC00473-002</i>	chr6:166322290-166401410	1.32×10^{-2}	2.29	down	<i>HSPA1B</i>	7.52×10^{-4}	3	up	2.84×10^{-4}	-0.9
<i>LINC00968-003</i>	chr8:57432874-57472243	1.12×10^{-4}	2.68	down	<i>TNFRSF10B</i>	1.10×10^{-3}	2	up	3.68×10^{-4}	-0.9
<i>linc-C20orf111-BP</i>	chr20:42839626-42855166	4.99×10^{-4}	2.02	up	<i>SPTLC3</i>	6.30×10^{-4}	2	down	1.08×10^{-4}	-0.9
<i>linc-DHX37-4</i>	chr12:127215097-127221919	6.24×10^{-4}	2.73	up	<i>TENC1</i>	4.70×10^{-4}	2.1	down	1.30×10^{-4}	-0.9
<i>linc-DHX37-4</i>	chr12:127215097-127221919	6.24×10^{-4}	2.73	up	<i>TENC1</i>	4.70×10^{-4}	2.1	down	1.30×10^{-4}	-0.9
<i>linc-SCYL1-2</i>	chr11:65222664-65234212	2.97×10^{-3}	2.56	up	<i>FTH1</i>	2.82×10^{-4}	1.9	down	1.51×10^{-4}	-0.9
<i>linc-SLC35F5-2</i>	chr2:114579399-114647368	3.80×10^{-3}	2.17	up	<i>EHD3</i>	1.13×10^{-3}	2.7	down	2.85×10^{-4}	-0.9
<i>linc-SLC35F5-2</i>	chr2:114579399-114647368	3.80×10^{-3}	2.17	up	<i>EHD3</i>	1.13×10^{-3}	2.7	down	2.85×10^{-4}	-0.9
<i>linc-TAGAP-1</i>	chr6:159990580-160016234	5.19×10^{-4}	3.43	up	<i>GJA1</i>	1.72×10^{-3}	2.5	down	3.24×10^{-4}	-0.9
<i>linc-TAGAP-1</i>	chr6:160007988-160015671	2.43×10^{-6}	5.10	up	<i>GJA1</i>	1.72×10^{-3}	2.5	down	2.78×10^{-4}	-0.9
<i>linc-ZNF366-5</i>	chr5:71870012-71876804	6.65×10^{-3}	5.11	up	<i>AQPEP</i>	1.38×10^{-2}	1.9	down	3.62×10^{-4}	-0.9
<i>LOC100130476</i>	chr6:138144806-138189370	1.59×10^{-4}	5.32	up	<i>GJA1</i>	1.72×10^{-3}	2.5	down	3.12×10^{-4}	-0.9
<i>PRLR</i>	chr5:35048860-35118224	1.50×10^{-3}	3.46	up	<i>SPINK13</i>	1.75×10^{-3}	2.6	down	2.38×10^{-4}	-0.9
<i>RP11-212I21.4-001</i>	chr16:55572112-55575939	6.79×10^{-4}	2.12	up	<i>MT2A</i>	2.06×10^{-4}	2.2	down	3.44×10^{-4}	-0.9
<i>RP11-356I2.4-006</i>	chr6:138175999-138179185	1.66×10^{-4}	6.64	up	<i>PRDM1</i>	1.77×10^{-3}	1.9	down	1.06×10^{-4}	-0.9
<i>RP11-809O17.1-002</i>	chr8:142136390-142140060	2.84×10^{-3}	2.09	up	<i>PNMA2</i>	1.46×10^{-3}	2.4	down	2.51×10^{-4}	-0.9
<i>SLC16A7</i>	chr12:60083117-60183635	9.31×10^{-4}	2.34	down	<i>VAMP1</i>	2.01×10^{-5}	2.3	up	1.53×10^{-4}	-0.9
<i>SNORD3A-001</i>	chr17:19091329-19092027	4.55×10^{-5}	11.0	down	<i>CCR10</i>	5.00×10^{-5}	2.8	up	2.17×10^{-4}	-0.9
<i>SNORD3A-001</i>	chr17:19091329-19092027	4.55×10^{-5}	11.0	down	<i>HEXIM2</i>	3.21×10^{-3}	2.1	up	2.88×10^{-4}	-0.9

<i>SPECC1L-ADORA2A</i>	chr22:24666784-24838328	4.18×10^{-5}	2.80	up	<i>TIMP3</i>	9.58×10^{-4}	2.2	down	1.09×10^{-4}	-0.9
<i>TNFRSF10B</i>	chr8:22877647-22926700	5.39×10^{-4}	2.14	up	<i>PLEKHA2</i>	2.59×10^{-3}	3.3	down	1.90×10^{-4}	-0.9
<i>TNFRSF10B</i>	chr8:22877647-22926700	5.39×10^{-4}	2.14	up	<i>SNTB1</i>	9.75×10^{-6}	3.4	down	1.12×10^{-4}	-0.9
<i>WISPI</i>	chr8:134203281-134243932	1.26×10^{-4}	5.31	down	<i>SLCO5A1</i>	3.70×10^{-4}	3.6	up	1.72×10^{-4}	-0.9

416 Footnote: Coordination is based on GRCh37. P-value^A indicate the significance of the differential expression while P-value^B indicate the
 417 significance of the correlation between lncRNA and gene expression. Abbreviation: FC, Fold change; R, Correlation coefficient.

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436 Table 3. Most significant trans-lncRNA-mRNA regulation pairs for the significant differential lncRNA.

lncRNA	Coordination	P-value ^A	FC	R	Gene Symbol	P-value	FC	Regulation	P-value ^B	R
<i>FR407458</i>	chr7:100770384-100771815	1.82×10^{-2}	2.1	down	<i>TIAM2</i>	6.42×10^{-4}	2.15	up	1.01×10^{-4}	-0.9
<i>AC005682.8-001</i>	chr7:22928990-22980809	2.71×10^{-3}	4.1	up	<i>KIAA0226L</i>	2.17×10^{-3}	3.25	down	1.01×10^{-4}	-0.9
<i>PLCB1-IT1-001</i>	chr20:8229372-8237564	7.08×10^{-3}	2.1	down	<i>HSPA1A</i>	3.44×10^{-4}	3.02	up	1.01×10^{-4}	-0.9
<i>CXCLI</i>	chr4:74735108-74737019	4.92×10^{-5}	2.6	down	<i>TNFRSF18</i>	1.47×10^{-3}	4.59	up	1.01×10^{-4}	-0.9
<i>RP11-356I2.4-006</i>	chr6:138175999-138179185	1.66×10^{-4}	6.6	up	<i>CYP24A1</i>	7.06×10^{-5}	9.65	down	1.03×10^{-4}	-0.9
<i>NKX3-1</i>	chr8:23536205-23540434	5.49×10^{-4}	3.9	up	<i>GOS2</i>	3.05×10^{-4}	2.84	down	1.03×10^{-4}	-0.9
<i>FR018579</i>	chr10:63664416-63664655	1.13×10^{-2}	2.3	down	<i>NEK10</i>	1.45×10^{-3}	2.21	up	1.03×10^{-4}	-0.9
<i>HNRNPD1</i>	chr4:83343716-83351378	7.53×10^{-3}	2.1	down	<i>N4BP2LI</i>	6.45×10^{-4}	2.17	up	1.03×10^{-4}	-0.9
<i>FR066129</i>	chr4:74608854-74609068	1.11×10^{-4}	4.3	down	<i>PHLDA2</i>	2.28×10^{-4}	3.3	up	1.04×10^{-4}	-0.9
<i>RP11-356I2.4-006</i>	chr6:138175999-138179185	1.66×10^{-4}	6.6	up	<i>CCL8</i>	2.48×10^{-4}	17.2	down	1.04×10^{-4}	-0.9
<i>TRIB2</i>	chr2:12856997-12882858	5.35×10^{-3}	2	down	<i>LOC727916</i>	2.36×10^{-3}	3.41	up	1.04×10^{-4}	-0.9
<i>FR264384</i>	chr9:2653919-2654254	1.03×10^{-2}	2.7	down	<i>FAMI29A</i>	6.45×10^{-3}	2.08	up	1.04×10^{-4}	-0.9
<i>linc-TRIM29-3</i>	chr11:121899038-121908990	9.03×10^{-4}	2	up	<i>GOS2</i>	3.05×10^{-4}	2.84	down	1.05×10^{-4}	-0.9
<i>linc-DHX37-4</i>	chr12:127215097-127221919	6.67×10^{-4}	3.2	up	<i>KIAA0226L</i>	2.17×10^{-3}	3.25	down	1.05×10^{-4}	-0.9
<i>RP11-356I2.4-006</i>	chr6:138175999-138179185	1.67×10^{-4}	6.6	up	<i>LXN</i>	2.68×10^{-4}	2.83	down	1.05×10^{-4}	-0.9

437 Footnote: Coordination is based on GRCh37. P-value^A indicate the significance of the differential expression while P-value^B indicate the
438 significance of the correlation between lncRNA and gene expression. Abbreviation: FC, Fold change; R, Correlation coefficient.

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Figure 1

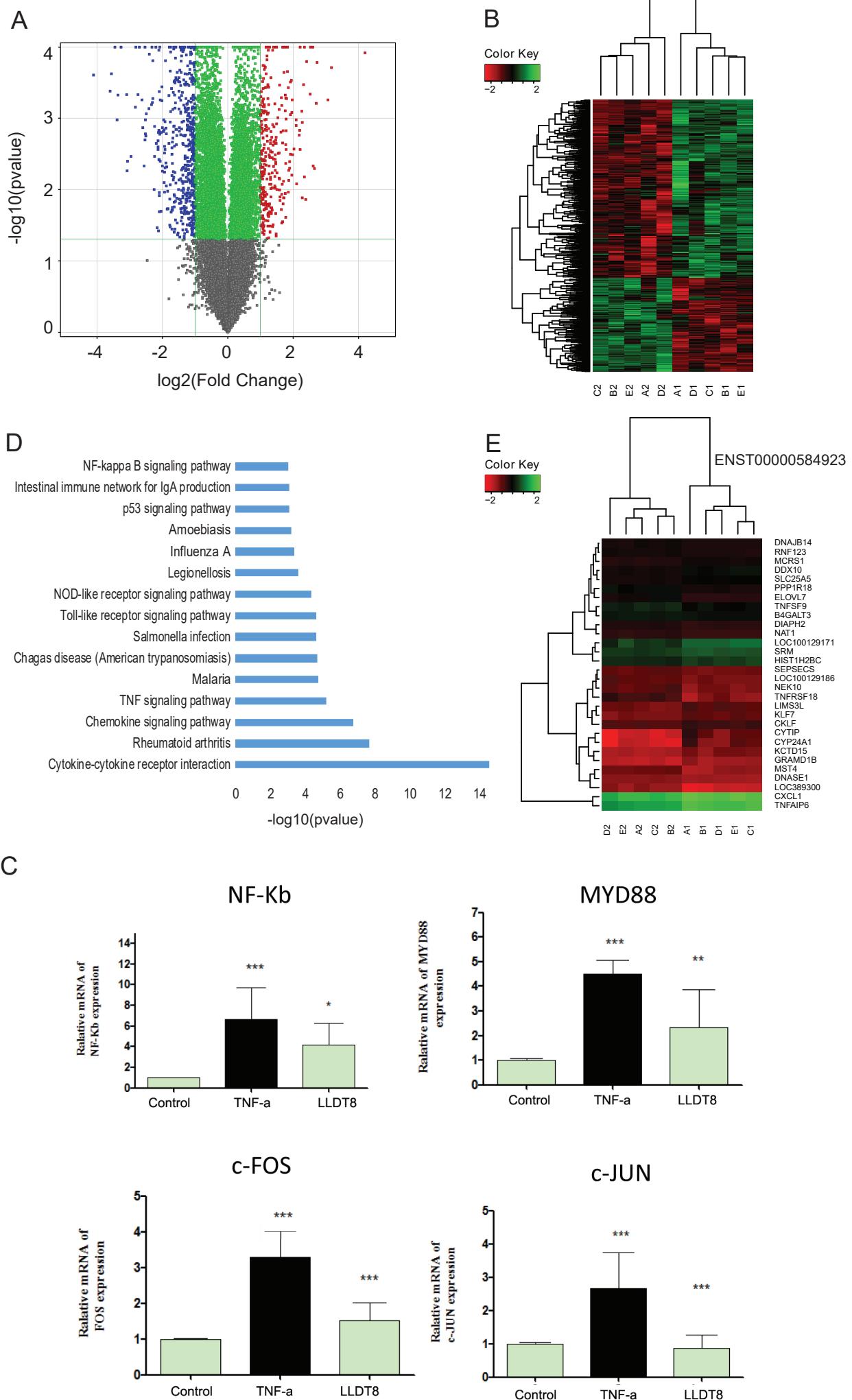


Figure 2

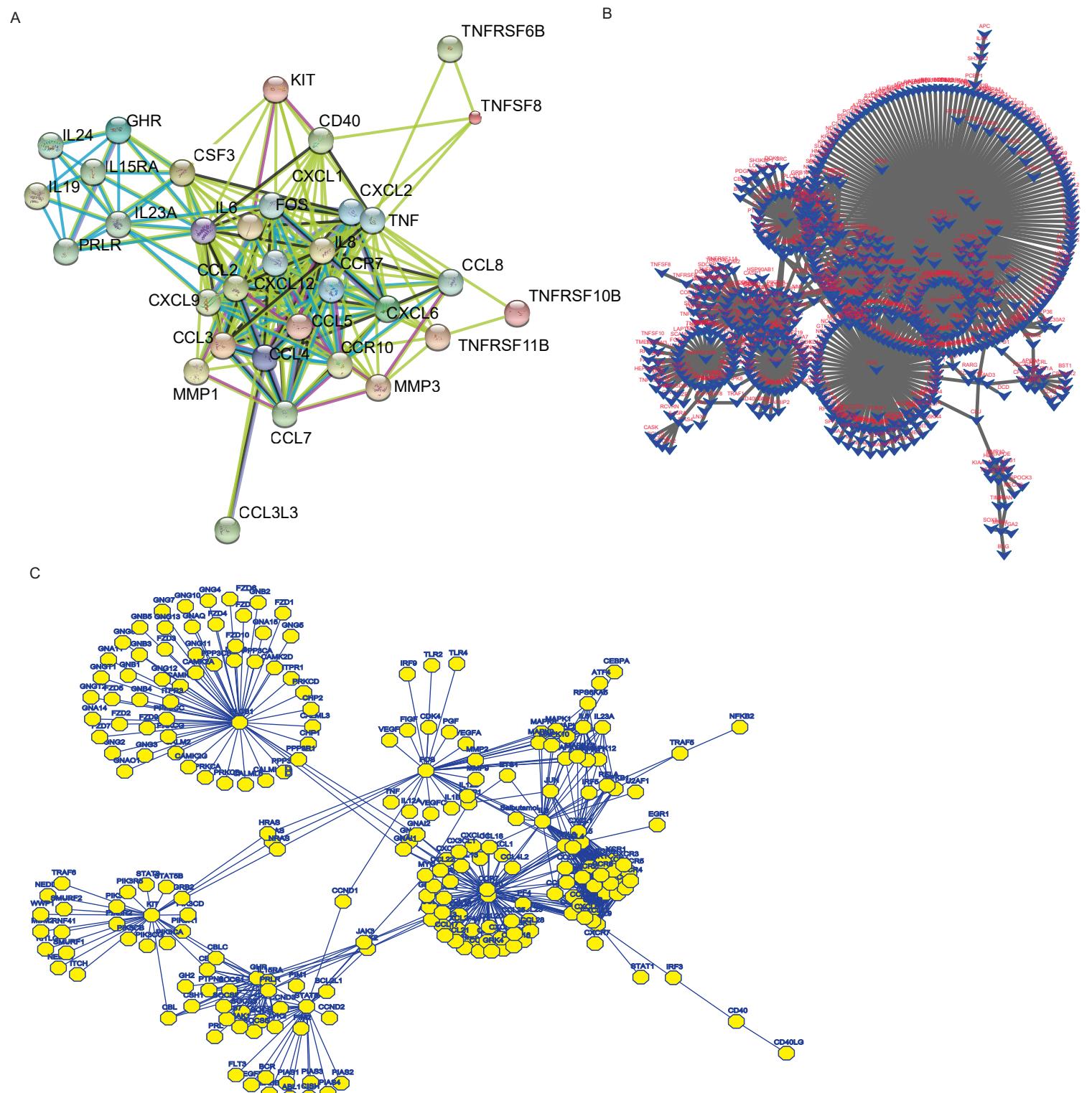
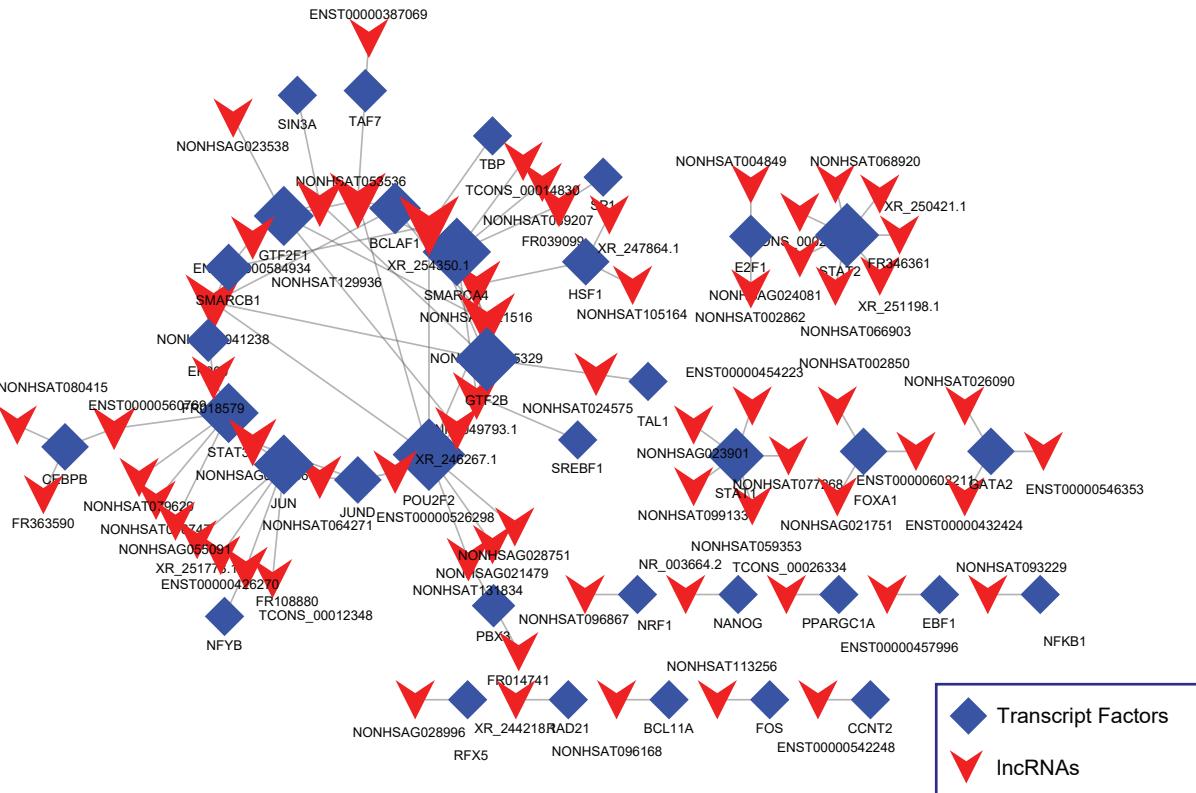


Figure 3

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