Figure S1. PCA of expression data of areca nut chewing samples and non-areca chewing samples. Areca nut chewing and non-areca nut chewing groups are represented by red and turquoise dots, respectively. PCA1 and PCA2 represent two uncorrelated variables following dimension reduction. The components of the two groups demonstrate obvious differences in clustering, indicating that patients who chew areca nut have different characteristic expression profiles than those who do not chew areca nut. PCA, principal component analysis.

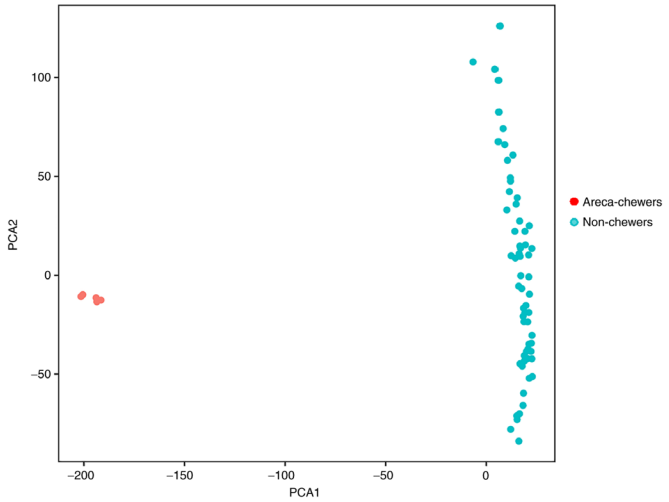
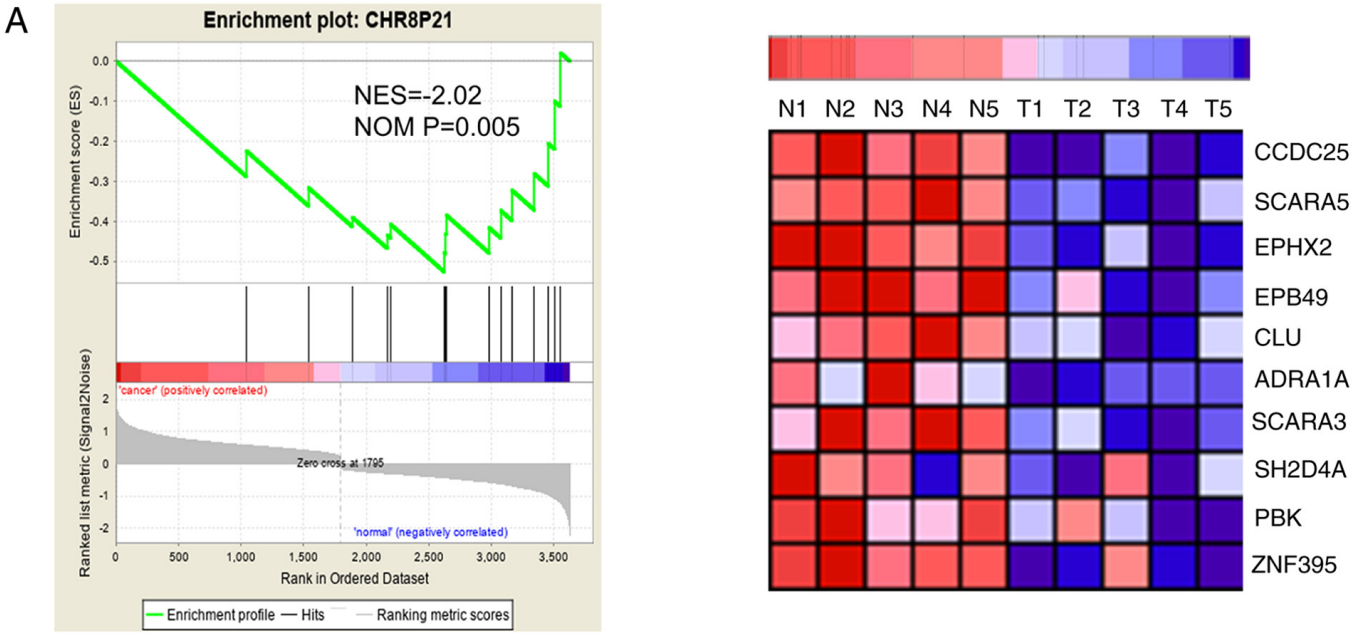


Figure S2. GSEA revealed that differentially expressed genes in TSCC and adjacent normal tissues are enriched in chromosome 8p21. (A) GSEA gene sets demonstrated that several genes located in the 8p21 segment were downregulated in TSCC. (B) Heat map of differentially expressed long non-coding RNAs (asterisks) and mRNAs from the 8p21 segment. GSEA, gene set-enrichment analysis; TSCC, tongue squamous cell carcinoma.



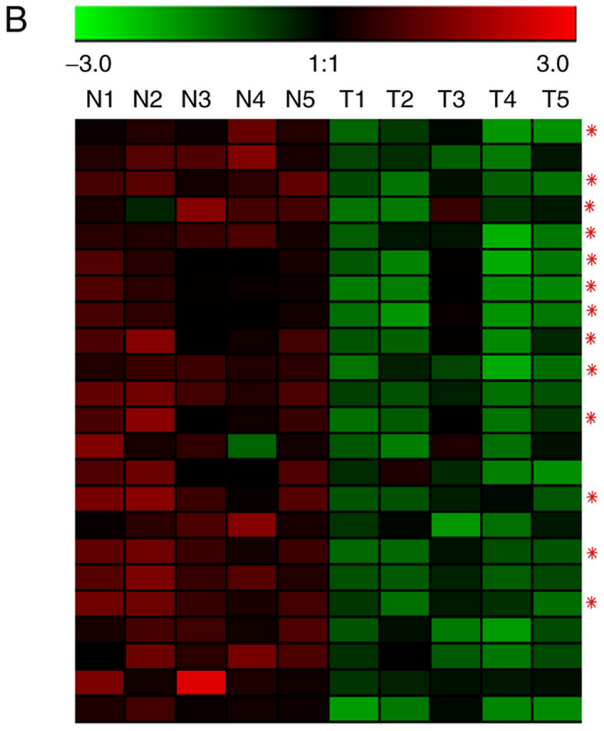


Figure S3. GSEA revealed that differentially expressed genes in TSCC and adjacent normal tissues are enriched in chromosome 3p21. (A) GSEA gene sets demonstrated that several genes located in the 3p21 segment were downregulated in TSCC. (B) Heat map of differentially expressed long non-coding RNAs (asterisks) and mRNAs from the 3p21 segment. GSEA, gene set-enrichment analysis; TSCC, tongue squamous cell carcinoma.

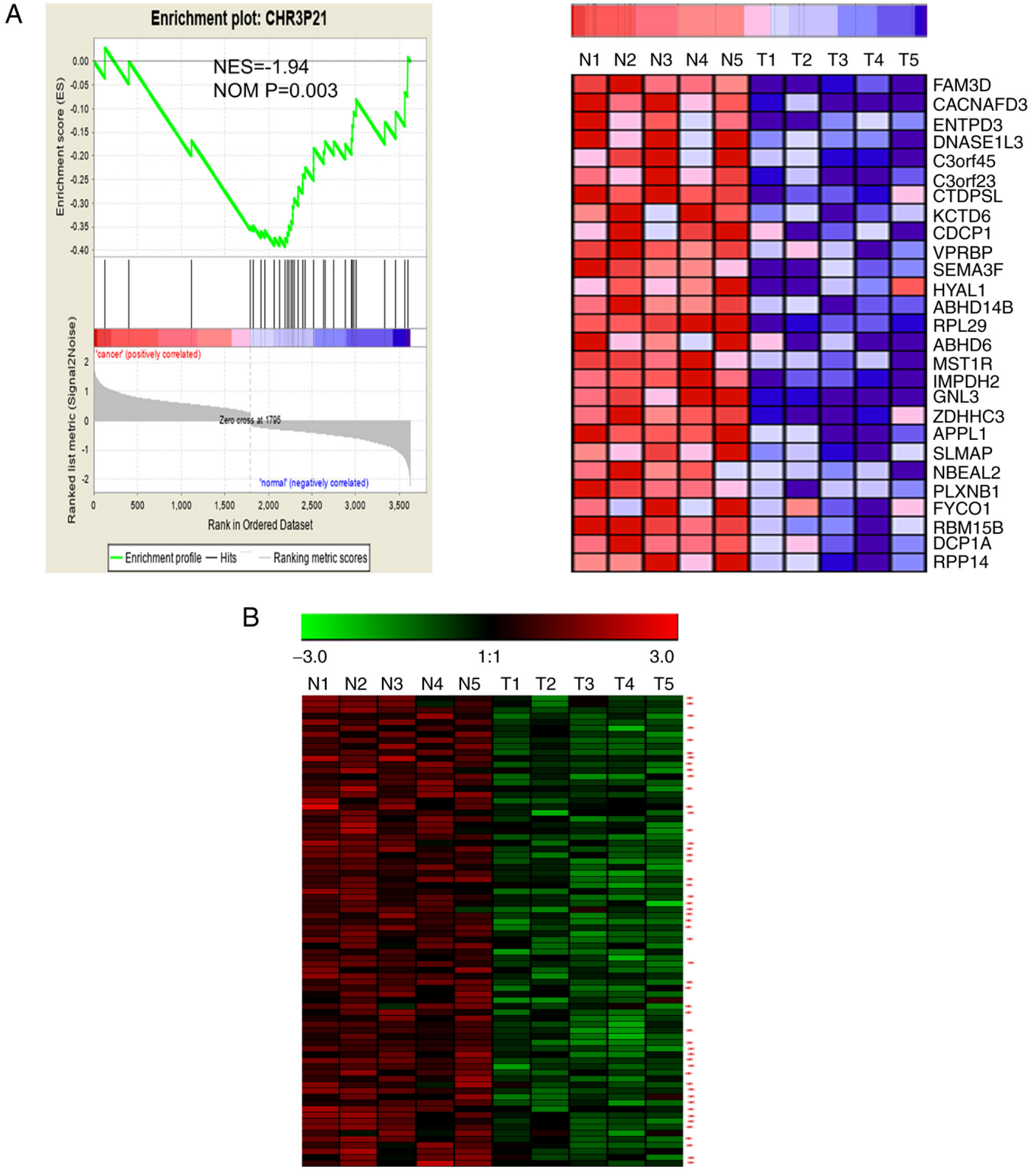


Figure S4. Soft threshold for mRNA and long non-coding RNA co-expression network. Left: Relationship between scale-free topology model fit (R^2) and soft-thresholds (powers). Right: Relationship between the mean connectivity and various soft-thresholds (powers). When the scale-free topology model fit (R^2) over 0.85, and the network constructed accords with scale-free distribution. As in this study, when soft-thresholds (powers)=24, R^2>0.85, so soft threshold is chosen 24.

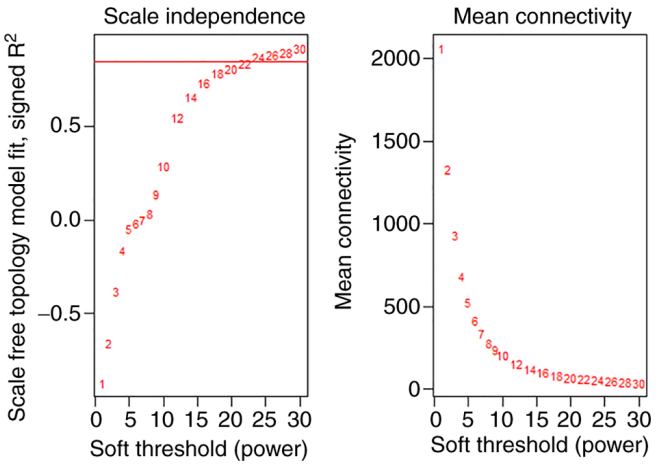


Figure S5. Canonical pathway analysis of differentially expressed genes via IPA. (A) The pathways of differentially expressed mRNAs were predicted via IPA. The top five pathways were Th cell activation pathway, antigen presentation pathway, role of *NFAT* in regulation of the immune response, *GP6* signaling pathway and T cell exhaustion signaling pathway. (B) The co-expression heat map of mRNAs and lncRNAs involved in the Th cell pathway. In the heatmap, the mRNAs were derived from the Th cell pathway gene set in the IPA database, and the lncRNAs were significantly associated with the Th cell activation pathway. IPA, ingenuity pathway analysis; lncRNA, long non-coding RNA.

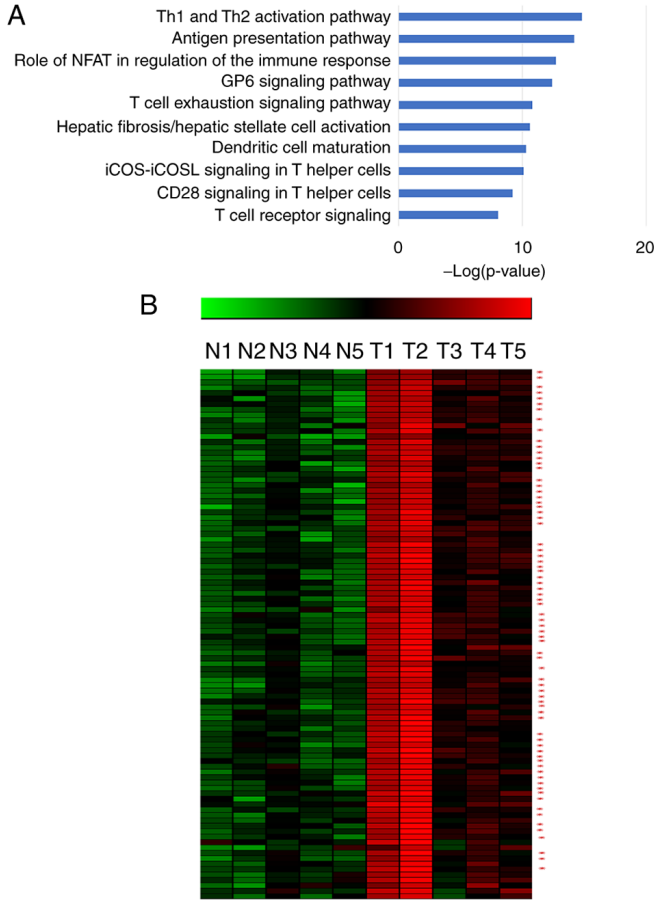


Figure S6. Th cell activation pathway from ingenuity pathway analysis.

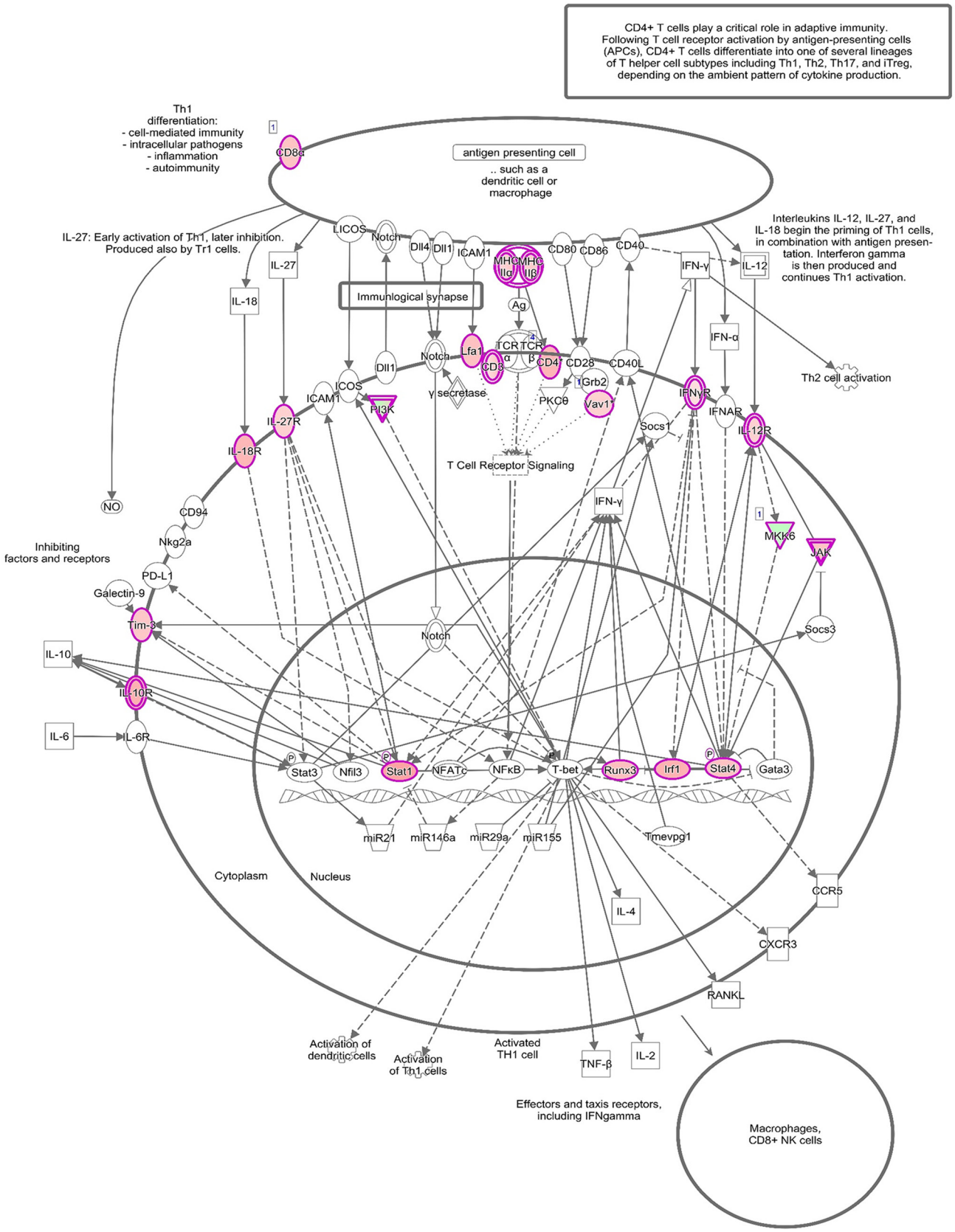


Table SI. Summary of patient characteristics.

Patient number	Sex	Age, years	Diagnosis	Histological grade	TNM stage
1	M	49	TSCC	Well/Moderate	T2N0M0
2	M	57	TSCC	Moderate	T1N2M0
3	M	35	TSCC	Well	T2N0M0
4	M	36	TSCC	Well/Moderate	T2N1M0
5	M	46	TSCC	Well/Moderate	T1N1M0

M, male; TSCC, tongue squamous cell carcinoma; TNM, tumor-node-metastasis.

Table SII. Long non-coding RNAs regulated by interferon regulatory factors.

Number	Probe ID	Source ID
1	CUST_10833_PI429545410	NONHSAT085144
2	CUST_10873_PI429545410	NONHSAT085150
3	CUST_16424_PI429545395	NONHSAT097576
4	CUST_19736_PI429545388	NONHSAT008643
5	CUST_19747_PI429545388	NONHSAG003874
6	CUST_19757_PI429545388	NONHSAT008645
7	CUST_19777_PI429545388	NONHSAT008651
8	CUST_26513_PI429545402	NONHSAT026190
9	CUST_28221_PI429545410	XR_244081.1
10	CUST_31833_PI429545410	XR_254435.1
11	CUST_44437_PI429545399	NONHSAT134375
12	CUST_47066_PI429545395	NONHSAT105044
13	CUST_60364_PI429545410	NR_052021.1
14	CUST_63192_PI429545395	NONHSAT108907
15	CUST_64665_PI429545406	NONHSAG024998
16	CUST_6562_PI429545402	NONHSAT021574
17	CUST_6572_PI429545402	NONHSAT021575
18	CUST_68884_PI429545406	NONHSAG025571
19	CUST_7367_PI429545388	NONHSAG002391
20	CUST_77808_PI429545406	NONHSAT067905
21	CUST_77818_PI429545406	NONHSAT067907
22	CUST_7952_PI429545402	NONHSAT021952
23	CUST_80551_PI429545399	NONHSAT015480
24	CUST_81561_PI429545399	NONHSAT015822

Table SIII. Long non-coding RNAs regulated nuclear factor kappa B.

Number	Probe ID	Source ID
1	CUST_10873_PI429545410	NONHSAT085150
2	CUST_11058_PI429545406	NONHSAT141715
3	CUST_11188_PI429545406	NONHSAT141756
4	CUST_11198_PI429545406	NONHSAT141759
5	CUST_19736_PI429545388	NONHSAT008643
6	CUST_19757_PI429545388	NONHSAT008645
7	CUST_19777_PI429545388	NONHSAT008651
8	CUST_41171_PI429545406	NONHSAT055214
9	CUST_44437_PI429545399	NONHSAT134375
10	CUST_47066_PI429545395	NONHSAT105044
11	CUST_47785_PI429545410	XR_243676.1
12	CUST_63192_PI429545395	NONHSAT108907
13	CUST_6562_PI429545402	NONHSAT021574
14	CUST_6572_PI429545402	NONHSAT021575
15	CUST_7367_PI429545388	NONHSAG002391
16	CUST_81293_PI429545395	NONHSAT123780

Table SIV. Long non-coding RNAs significantly associated with the Th cell activation pathway.

Number	Probe ID	Source ID	Number	Probe ID	Source ID
1	CUST_10833_PI429545410	NONHSAT085144	38	CUST_64665_PI429545406	NONHSAG024998
2	CUST_11058_PI429545406	NONHSAT141715	39	CUST_6492_PI429545402	NONHSAT021561
3	CUST_11188_PI429545406	NONHSAT141756	40	CUST_6562_PI429545402	NONHSAT021574
4	CUST_11198_PI429545406	NONHSAT141759	41	CUST_6572_PI429545402	NONHSAT021575
5	CUST_13293_PI429545410	NONHSAT087145	42	CUST_66040_PI429545388	NONHSAT077459
6	CUST_16418_PI429545388	NONHSAG003418	43	CUST_6682_PI429545402	NONHSAG008449
7	CUST_16424_PI429545395	NONHSAT097576	44	CUST_67024_PI429545410	NR_034176.1
8	CUST_1660_PI429545406	NONHSAT140535	45	CUST_6870_PI429545399	NONHSAT123831
9	CUST_17724_PI429545402	NONHSAT024462	46	CUST_68884_PI429545406	NONHSAG025571
10	CUST_18829_PI429545406	NONHSAT143966	47	CUST_70521_PI429545406	NONHSAT066225
11	CUST_19736_PI429545388	NONHSAT008643	48	CUST_7347_PI429545406	NONHSAT140533
12	CUST_19747_PI429545388	NONHSAG003874	49	CUST_7367_PI429545388	NONHSAG002391
13	CUST_19757_PI429545388	NONHSAT008645	50	CUST_62937_PI429545399	NONHSAT139118
14	CUST_19777_PI429545388	NONHSAT008651	51	CUST_77759_PI429545406	NONHSAT067902
15	CUST_25989_PI429545406	NONHSAT145370	52	CUST_77778_PI429545406	NONHSAG026516
16	CUST_28221_PI429545410	XR_244081.1	53	CUST_77798_PI429545406	NONHSAT067904
17	CUST_31711_PI429545388	NONHSAG028065	54	CUST_77808_PI429545406	NONHSAT067905
18	CUST_31833_PI429545410	XR_254435.1	55	CUST_77818_PI429545406	NONHSAT067907
19	CUST_32151_PI429545395	NONHSAG040238	56	CUST_64658_PI429545406	NONHSAT061494
20	CUST_32957_PI429545388	NONHSAT074730	57	CUST_7952_PI429545402	NONHSAT021952
21	CUST_33216_PI429545402	NONHSAT028253	58	CUST_80551_PI429545399	NONHSAT015480
22	CUST_41171_PI429545406	NONHSAT055214	59	CUST_81361_PI429545406	NONHSAG031109
23	CUST_44437_PI429545399	NONHSAT134375	60	CUST_81561_PI429545399	NONHSAT015822
24	CUST_47066_PI429545395	NONHSAT105044	61	CUST_81566_PI429545399	NONHSAG006607
25	CUST_47076_PI429545395	NONHSAT105045	62	CUST_83852_PI429545402	NONHSAG016585
26	CUST_47785_PI429545410	XR_243676.1	63	CUST_86183_PI429545388	NONHSAT091504
27	CUST_49838_PI429545388	NONHSAT072682	64	CUST_87957_PI429545410	NR_026691.1
28	CUST_53520_PI429545402	NONHSAT033629	65	CUST_18193_PI429545410	XR_251246.1
29	CUST_58795_PI429545388	NONHSAT075575	66	CUST_90590_PI429545384	NONHSAT001802
30	CUST_58938_PI429545402	NONHSAT035089	67	CUST_77264_PI429545380	ENST00000417969
31	CUST_60222_PI429545399	NONHSAG055192	68	CUST_69725_PI429545384	ENST00000602721
32	CUST_60364_PI429545410	NR_052021.1	69	CUST_61100_PI429545384	ENST00000572151
33	CUST_60712_PI429545399	NONHSAG055263	70	CUST_17928_PI429545380	FR010252
34	CUST_61112_PI429545402	NONHSAT035717	71	CUST_68990_PI429545380	FR233826
35	CUST_78365_PI429545384	TEA ncRNAs	72	CUST_75067_PI429545399	NONHSAT013050
36	CUST_88802_PI429545395	NONHSAT120551	73	CUST_22533_PI429545410	XR_242063.1
37	CUST_61634_PI429545406	NONHSAT060794	74	CUST_84643_PI429545388	NONHSAT091147