

# **Profiling of milk miRNAs associated with the innate immune system and pathway analysis in dairy cows with *S. aureus*-infected subclinical mastitis**

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## **SUPPLEMENTARY FILE**

### **Supplementary Materials and methods**

#### **Detailed in-silico analysis descriptions**

##### ***Gene Ontology and Pathway Analysis***

The gene sets that play a role in the “*innate immune response*” of cattle were determined from the Gene Ontology (GO) databases, whose accession number is GO:0045087. Also, the genes involved in the “*defense response of gram-positive bacteria*” for *Bos taurus* have been obtained from GO databases, whose accession number is GO:0050830. The overlapping genes were determined by jvenn (Bardou et al. 2014). To confirm the determined gene sets, the KOBAS online analysis database (Xie et al. 2011) was used to perform the KEGG and Reactome pathways and GO analysis on the overlapping genes.

##### ***PPI Network and Identification of Hub Genes***

The Cytoscape (Su et al. 2014) and STRING (Szklarczyk et al. 2021) tools were used to obtain the protein-protein interaction (PPI) network. Thereafter, the Cytoscape platform utilized applications to identify the hub genes MCODE (Molecular Complex Detection) and cytoHubba

(Chin et al. 2014). The utilization of applications was performed as described previously (Duman et al. 2021).

### ***Prediction of microRNAs of the identified hub genes***

Target microRNAs of identified hub genes, which are functional in *S. aureus* mastitis and the innate immune system, were determined by the miRNet database. The mirTarbase (Hsu et al. 2011) and DIANA TarBase V.8 (Karagkouni et al. 2018) tools only predict experimentally validated microRNAs. Consequently, the miRNet database was used to identify experimentally validated mammary tissue-specific microRNAs in the mirTarbase and Tarbase v.8 tools. The overlapping microRNAs were determined by jvenn. Co-predicted microRNAs identified by both systems were chosen for further research. The target gene 3'UTR sequence was retrieved from the ENSEMBL website. The miRNA-mRNA targets were validated using RNA22v2 (Miranda et al. 2006) and RNAhybrid (Rehmsmeier et al. 2004) tools.

Due to the insufficient database of the bovine miRNA target prediction tools, the human homology of the seed region of identified miRNAs (bta-miR-15a, bta-miR-16b, bta-miR-23a, bta-miR-27a-3p, bta-miR-103, bta-miR-146b, and bta-miR-374b) was examined. In addition, the homology analysis of the seed region in the BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) database on the NCBI website was performed to verify that the miRNAs were conserved between species. An examination of homology revealed that the seed areas of the identified miRNAs are conserved between human and bovine species (Supplementary Table S2).

Furthermore, the DIANA-miRPath online software (<http://snf-515788.vm.okeanos.grnet.gr/>) was used to examine the miRNA pathway analyses. As a result, toll-like receptor pathways, immune system processes, and innate immune response were identified as the most important pathways for chosen miRNAs (bta-miR-15a, bta-miR-16b, bta-miR-23a, bta-miR-27a-3p, bta-miR-103, bta-miR-146b, and bta-mir-374b) by pathway analysis (Supplementary Figure S1).

## Supplementary Tables

**Supplementary Table S1.** Description of the symptoms and parameters used for the group of subclinical animals.

<b>CMT Results</b>	<b>Clinical Signs</b>	<b>Description</b>
0	Negative	No signs of mastitis
(+)	Weak Positive	Mild subclinical mastitis signs
(++)	Positive	Noticeable subclinical mastitis signs
(+++)	Strong Positive	Severe subclinical mastitis signs

**Supplementary Table S2.** The milk composition, CMT score, SCC results, and lactation number information on cows. Cow1-6: The subclinical mastitis cows with *S. aureus*-infected; Cow7-16: healthy cows.

<b>Animal No.</b>	<b>Lactation No.</b>	<b>CMT Score</b>	<b>SCC</b>	<b>Fat, %</b>	<b>Protein, %</b>	<b>Lactose, %</b>
Cow1	1	1: (+)	200,00	1,20	3,20	5,00
Cow2	1	2: (++)	1144,30	5,90	3,80	5,80
Cow3	3	2: (++)	1493,40	1,70	3,10	4,80
Cow4	3	2: (++)	887,40	0,90	3,30	5,00
Cow5	4	2: (++)	212,50	2,20	2,90	4,50
Cow6	4	2: (++)	1391,20	9,60	3,00	4,50
Cow7	1	(-)	<90,000	2,10	3,10	4,80
Cow8	1	(-)	<90,000	2,00	3,30	5,00
Cow9	1	(-)	<90,000	1,50	3,40	5,30
Cow10	1	(-)	<90,000	3,20	3,20	5,00
Cow11	1	(-)	<90,000	1,50	3,20	5,00
Cow12	1	(-)	<90,000	1,30	3,20	4,90
Cow13	1	(-)	<90,000	1,00	3,40	5,30
Cow14	1	(-)	<90,000	2,10	3,40	5,30
Cow15	1	(-)	<90,000	1,50	3,40	5,30
Cow16	1	(-)	<90,000	3,00	3,10	4,80

**Supplementary Table S3** Primer sequence homology and miRBase Access numbers.

<b>miRNAs</b>	<b>Sequence</b>	<b>miRBase Accession</b>
hsa-mir-15a-5p	UAGCAGCACAUAAUGGUUUGUG	<a href="#">MIMAT0000068</a>
bta-miR-15a	UAGCAGCACAUAAUGGUUUGU	<a href="#">MIMAT0004334</a>
hsa-miR-16-5p	UAGCAGCACGUAAAUAUUGGCG	<a href="#">MIMAT0000069</a>
bta-miR-16b	UAGCAGCACGUAAAUAUUGGC	<a href="#">MIMAT0003525</a>
hsa-miR-23a-3p	AUCACAUUGCCAGGGAUUCC	<a href="#">MIMAT0000078</a>
bta-miR-23a	AUCACAUUGCCAGGGAUUCCA	<a href="#">MIMAT0003827</a>
hsa-miR-27a-3p	UUCACAGUGGCUAAGUCCGC	<a href="#">MIMAT0000084</a>
bta-miR-27a-3p	UUCACAGUGGCUAAGUCCG	<a href="#">MIMAT0003532</a>
hsa-miR-103a-3p	AGCAGCAUUGUACAGGGCUAUGA	<a href="#">MIMAT0000101</a>
bta-miR-103	AGCAGCAUUGUACAGGGCUAUGA	<a href="#">MIMAT0003521</a>
hsa-mir-146a-5p	UGAGAACUGAAUCCAUGGGUU	<a href="#">MIMAT0000449</a>
bta-miR-146b	UGAGAACUGAAUCCAUAGGCUGU	<a href="#">MIMAT0009235</a>
hsa-mir-374a-5p	UUAUAAUACAACCUAUAAGUG	<a href="#">MIMAT0000727</a>
bta-mir-374b	AUAUAAUACAACCUAUAAGUG	<a href="#">MIMAT0009302</a>
bta-miR-148	UCAGUGCACUACAGAACUUUGU	<a href="#">MIMAT0003522</a>

**Supplementary Table S4.** Gene information

<b>GO:0050830</b>	<b>GO:0045087</b>	<b>GO:0050830 GO:0045087</b>
P80189	Q8SQB1	Q8SPP7
P61823	Q8SQA6	F1MGC2
W0UV04	Q8SPU5	G8JKZ9
W0UVF2	Q8SPF8	G3X842
E1BPM3	P82943	Q599T9
Q3SZV8	Q1LZA9	Q9TU73
A5D7S4	P80343	F1N720
F1MYW1	Q5E9H3	Q5E9C1
F1MLN4	Q1RMQ0	O02659
E1BJ31	F1MK62	Q3SZJ2
A0A3Q1NDZ8	F1MTR5	F1MQF6
F1MGQ7	F1MVS9	C1ITJ8
A0A140T838	G3X7X6	P22226
A0A3Q1MPE3	G3X823	A0A3Q1LKU2
A0A3Q1M1U5	A6QL48	F1MI53
A0A452DJ61	P48616	G3X698
E1B867	P01030	A0A452DHW6
A7YY75	Q1LZD9	E1BKP4
F1MVZ6	Q2T9X1	A0A3Q1LNK5
A0A3Q1MXH2	Q0IHK5	A0A3Q1NL72
A0A3Q1MD51	Q0IIF7	Q687Y7
A0A3Q1MJW9	Q0X0C4	P00593
Q673L2	Q6WCD5	E1B7V7
Q56JZ2	Q8HXK9	F1MTW5
P46159	G3N0V2	P05059
A0A3Q1MT81	Q2T9U8	Q2KJC3
Q06600	F1MRL0	Q6E804
A7YWM6	F1N3B8	A5PKG9
P39873	Q3SZP0	Q29RS5
Q6B411	E1BP08	P40673
Q6B410	Q3SZM3	A0A3Q1M5C9
A0A3Q1M4Y5	E1BIR9	A0A3Q1M914
A0A3Q1M4R4	Q2HJC9	A0A3Q1LWB5
Q27996	Q6IED8	A6QPV3
A0A3Q1LJT5	C4T7Z1	A0A3Q1MJB3
G5E502	F1MJ63	A0A3Q1MT20
P15467	Q32L31	A0A3Q1NEQ8

G1K230	A0A3Q1NAH0	A0A3Q1MZX7
W0UTH6	A0A3Q1LI40	A0A3Q1NOV6
P80929	F6PXR5	A0A3Q1LIW8
Q70IB2	G3MYA8	P10152
F1MEC3	Q2LGB7	P19660
A6H6X2	Q2LGB5	A0A452DIN2
P04421	Q2LGB3	F1MSQ0
P00669	Q28141	F1N1I1
G3MZ45	Q32LD7	F1MNN7
Q06284	E1BLT8	F1MDI9
Q06283	F1MLZ4	A0A3Q1MZC7
E1BDR1	Q6VE48	Q32P86
E1BH94	Q9TTK4	P54228
E1BBW5	Q3MHL9	P54229
E1BG16	Q5E9E3	P56425
	Q0VD19	E1BAL7
	Q2TBL4	G3MZA1
	Q5TJ59	G3N2G9
	A5D7D9	P33046
	Q5E947	P33046
	F1MZJ0	P08904
	A0A3Q1MEG8	E1B824
	P28783	Q0V7P3
	P28782	E1BEE4
	Q7YRV4	E1BMK7
	A6QQL0	E1BL84
	Q3SZL0	
	Q3SYW2	
	G3N300	
	A2VDM0	
	Q3SYR8	
	Q3SZ20	
	E1BNN6	
	E1BMU6	
	E1BN35	
	Q08DF7	
	E1BMN4	
	A0A3Q1LYZ8	
	E1BMK2	
	A0A3Q1LWM4	
	A0A3Q1M8D9	

	A0A3Q1M8C4	
	F1MLX5	
	F1MM71	
	Q2KI99	
	E1BJC6	
	Q08DN6	
	E1BIU3	
	Q08DQ6	
	F1MKU1	
	A0A3S5ZPK8	
	E1BIF8	
	A0A3S5ZPD6	
	A0A3Q1LUH5	
	E1BA84	
	A0A3Q1LMF9	
	Q5EA36	
	A0A452DI74	
	A0A452DI25	
	A0A452DI18	
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	A0A3Q1LM32	
	A0A3Q1LLL8	
	Q0P5N1	
	F6RSW1	
	Q17QJ6	
	Q17QH6	
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	P79135	
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	A0A140T878	
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	F1MY85	
	A4IF62	
	Q29RU4	
	F1MZ14	
	F1MYR5	



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	A0A3Q1MPX4	
	Q5I2M5	
	Q2HJA4	
	Q4JF29	
	A0A3Q1N0H9	
	P02676	
	Q5EAB4	
	A0A3Q1MNI8	
	Q5EA90	
	Q5EA11	
	A0A3Q1MMD9	
	Q05B79	
	A0JN61	
	A0A3Q1M591	
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	Q9BDI7	
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	Q0VCX1	
	Q0VCP5	
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	Q0VCG9	
	Q3ZBI5	
	Q6QUN5	
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	Q32L49	
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	Q3ZBB1	
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	P07353	
	Q49BZ4	
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	Q95122	
	Q863H3	
	Q2TBI0	
	M0QVZ9	
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	F6PSX0	
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	F1N1P2	
	F1MQ36	
	Q5E9N5	

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	Q04790	
	A0A3Q1MXL6	
	A0A3Q1N9G9	
	F1N3Q7	
	A7MBJ2	
	A0A3Q1N939	
	A7MB69	
	A0A3Q1ME65	
	A0A3Q1MDN3	
	G3MX29	
	Q2UVX4	
	F1MXX7	
	A7LMA0	
	A7LM98	
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	F1MX87	
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	F1MWM8	
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	Q3T0G8	
	A0A3Q1LVU4	
	A0A3Q1MJ95	
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	G5E631	
	Q3SZ90	
	G5E5S6	
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	Q29RQ1	
	Q92176	
	F1MHT9	

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	F1MG80	
	E1BDG5	
	Q3T0A3	
	E1BD59	
	F1MCT8	
	F1MCX4	
	F1MEX6	
	F1MF42	
	Q1RMU2	
	Q0VCA5	
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	F1MX26	
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	Q3ZCM0	
	Q3ZBV6	
	Q3ZBN3	
	G3X6Q8	

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	Q29RL3	
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	A0A3Q1LRY1	
	A0A3Q1M3U5	
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	A0A3Q1LZU2	
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	A0A3Q1MCE9	
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	A0A3Q1MA43	
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	A0A3Q1LJS3	
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	G5E5L8	
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	G5E513	
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	A0A3Q1ML26	
	A0A3Q1N508	
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	A0A3Q1MQM2	
	A0A3Q1MPL7	
	A0A3Q1NB11	
	E1BQ22	
	A5PKF1	
	A0A3Q1M218	
	A0A3Q1M2K4	
	A0A452DJQ7	
	Q2KIJ4	
	F1N5J5	
	F1N713	
	F1MXT2	
	F1N434	
	F1MYM5	
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	F1N541	
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	F1MS12	
	F1MVU7	
	F1MVG5	
	F1N6D0	
	F1MRF8	
	F1MRB0	
	F1N261	
	F1N1Q0	

	F1MNH9	
	F1MMG4	
	F1MLW2	
	F1MYF8	
	F1MJH6	
	F1MJB8	
	F1MIF0	
	F1MI56	
	F1MBS7	
	F1MBR4	
	F1MBB0	
	C4T8B4	
	A6QLU9	
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	Q9TTS6	
	Q32PC2	
	Q32L60	
	A0A3Q1MLJ0	
	A6QPT8	
	A0A3Q1MQY0	
	A0A3Q1N4V0	
	P55918	
	A1A4Q6	
	F1MPI5	
	G3MZJ0	
	E1BAZ1	
	E1BAU5	
	E1BAG6	
	E1BJZ0	
	E1BK51	
	E1BJG5	
	E1BJ49	
	E1BIM8	
	Q09141	
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	G3MXG6	
	G3MX02	
	G3MWK8	
	G3N3Q3	
	G3N342	
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	G3N1J6	
	G3N1H5	
	G3N188	
	G3N0V0	
	G3N148	
	A0JNB0	
	A0JN74	
	Q3T004	
	Q3T166	
	Q58DK8	
	VGNC	
	Q17R13	
	Q17QA1	
	E1BLX4	
	E1B7S4	
	E1B7Z4	
	E1BES5	
	A7MB57	
	E1BGB8	
	E1B7D3	
	Q9BDR7	
	Q9BDJ2	
	P30367	
	P62811	
	E1BD38	
	E1BHM0	
	E1BGW9	
	E1BH62	
	E1BGL7	
	E1B9L3	
	E1B9I3	
	E1B8Z4	
	E1BPI3	
	E1BNL9	
	E1BNE0	
	E1BNA8	
	E1BMP9	
	E1BMC1	
	E1BM69	
	E1BKE6	
	E1B7M2	

	E1B7I6	
	E1B719	
	Q2KIR3	

**Supplementary Table S5.** KOBAS pathway and gene ontology results.

#Term	Database	ID	Input number	Background number	P-Value	Corrected P-Value	Input
NOD-like receptor signaling pathway	KEGG PATHWAY	bta04621	15	162	4,31E-10	4,23E-08	E1BKP4 Q599T9 P19660 P54229 Q3SZJ2 G3X698 Q5E9C1 E1BAL7 Q9TU73 P22226 Q6E804 P33046 P54228 A6QPV3 P56425
Tuberculosis	KEGG PATHWAY	bta05152	10	171	3,24E-01	1,81E+01	Q599T9 P19660 P54229 P54228 Q6E804 Q9TU73 P22226 P33046 Q3SZJ2 P56425
Staphylococcus aureus infection	KEGG PATHWAY	bta05150	7	62	1,14E+01	4,47E+02	O02659 P19660 P54229 P54228 P22226 P33046 P56425
Innate Immune System	Reactome	R-BTA-168249	11	475	3,23E+02	1,15E+03	Q599T9 G3MZA1 Q32P86 A5PKG9 Q5E9C1 Q6E804 Q8SPP7 E1BKP4 P33046 P08904 Q3SZJ2
Defense response to Gram-positive bacterium	Gene Ontology	GO:0050830	24	68	5,21E-37	2,04E-34	O02659 Q599T9 Q32P86 E1BKP4 Q3SZJ2 P40673 A6QPV3 Q8SPP7 F1N1I1 P56425 Q0V7P3 Q29RS5 P00593 P19660 P54229 A5PKG9 P05059 P22226 P54228 Q2KJC3 G3MZA1 C1ITJ8 P33046 P08904
Innate immune response	Gene Ontology	GO:0045087	20	211	7,25E-18	1,42E-15	Q599T9 P40673 Q29RS5 Q2KJC3 G3MZA1 P19660 P54229 A5PKG9 Q5E9C1 C1ITJ8 Q6E804 P05059 Q8SPP7 P22226 E1BKP4 P33046 P08904 P54228 Q3SZJ2 P56425

**Supplementary Table S6.** Target miRNAs of identified hub genes

mir_id	mir_acc	symbol	embl	entrez	experiment	pmid	mirtarbase	tarbase	tissue
hsa-mir-26b-5p	MIMAT0000083	CASP4	ENSG00000196954	837	Microarray	19088304	1	1	Mammary gland
hsa-let-7d-5p	MIMAT0000065	CASP4	ENSG00000196954	837	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-122-5p	MIMAT0000421	CASP4	ENSG00000196954	837	Biotin-Microarrays, Microarrays	tarbase	0	1	Mammary gland
hsa-mir-143-3p	MIMAT0000435	CASP4	ENSG00000196954	837	PAR-CLIP	tarbase	0	1	Mammary gland
hsa-mir-153-3p	MIMAT0000439	CASP4	ENSG00000196954	837	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-30a-5p	MIMAT0000087	CASP4	ENSG00000196954	837	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-30b-5p	MIMAT0000420	CASP4	ENSG00000196954	837	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-30c-5p	MIMAT0000244	CASP4	ENSG00000196954	837	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-30d-5p	MIMAT0000245	CASP4	ENSG00000196954	837	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-30e-5p	MIMAT0000692	CASP4	ENSG00000196954	837	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-671-5p	MIMAT0003880	CASP4	ENSG00000196954	837	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-877-3p	MIMAT0004950	CASP4	ENSG00000196954	837	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-103a-3p	MIMAT0000101	CASP4	ENSG00000196954	837	RIP-Seq	tarbase	0	1	Mammary gland
hsa-mir-23b-3p	MIMAT0000418	CASP4	ENSG00000196954	837	RNA-Seq	tarbase	0	1	Mammary gland
hsa-mir-210-3p	MIMAT0000267	CASP4	ENSG00000196954	837	Microarrays	tarbase	0	1	Mammary gland
hsa-mir-452-5p	MIMAT0001635	CASP4	ENSG00000196954	837	Microarrays	tarbase	0	1	Mammary gland
hsa-mir-374a-5p	MIMAT0000727	CASP4	ENSG00000196954	837	Microarrays	tarbase	0	1	Mammary gland
hsa-mir-148b-3p	MIMAT0000759	CASP4	ENSG00000196954	837	Microarrays	tarbase	0	1	Mammary gland

hsa-mir-133a-3p	MIMAT0000427	CASP4	ENSG00000196954	837	Microarrays	tarbase	0	1	Mammary gland
hsa-mir-21-5p	MIMAT0000076	MYD88	ENSG00000172936	4615	Chip//ELISA//Flow cytometry//Luciferase reporter assay//QRT-PCR//Western blot	23633945	1	0	Mammary gland
hsa-mir-203a-3p	MIMAT0000264	MYD88	ENSG00000172936	4615	Luciferase reporter assay//qRT-PCR//Western blot	23522925	1	1	Mammary gland
hsa-mir-149-5p	MIMAT0000450	MYD88	ENSG00000172936	4615	ELISA//qRT-PCR//Western blot	24375488	1	0	Mammary gland
hsa-mir-155-5p	MIMAT0000646	MYD88	ENSG00000172936	4615	Western blot	21030878	1	1	Mammary gland
hsa-mir-103a-3p	MIMAT0000101	MYD88	ENSG00000172936	4615	PAR-CLIP	tarbase	0	1	Mammary gland
hsa-mir-107	MIMAT0000104	MYD88	ENSG00000172936	4615	PAR-CLIP	tarbase	0	1	Mammary gland
hsa-mir-15a-5p	MIMAT0000068	MYD88	ENSG00000172936	4615	PAR-CLIP	tarbase	0	1	Mammary gland
hsa-mir-15b-5p	MIMAT0000417	MYD88	ENSG00000172936	4615	PAR-CLIP	tarbase	0	1	Mammary gland
hsa-mir-16-5p	MIMAT0000069	MYD88	ENSG00000172936	4615	PAR-CLIP	tarbase	0	1	Mammary gland
hsa-mir-17-3p	MIMAT0000071	MYD88	ENSG00000172936	4615	PAR-CLIP	tarbase	0	1	Mammary gland
hsa-mir-218-5p	MIMAT0000275	MYD88	ENSG00000172936	4615	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-23a-3p	MIMAT0000078	MYD88	ENSG00000172936	4615	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-23b-3p	MIMAT0000418	MYD88	ENSG00000172936	4615	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-424-5p	MIMAT0001341	MYD88	ENSG00000172936	4615	PAR-CLIP	tarbase	0	1	Mammary gland
hsa-mir-522-5p	MIMAT0005451	MYD88	ENSG00000172936	4615	IMPACT-Seq	tarbase	0	1	Mammary gland

hsa-mir-9-5p	MIMAT0000441	MYD88	ENSG00000172936	4615	PAR-CLIP	tarbase	0	1	Mammary gland
hsa-mir-124-3p	MIMAT0000422	MYD88	ENSG00000172936	4615	Microarrays	tarbase	0	1	Mammary gland
hsa-mir-21-3p	MIMAT0004494	MYD88	ENSG00000172936	4615	Microarrays	tarbase	0	1	Mammary gland
hsa-mir-210-3p	MIMAT0000267	MYD88	ENSG00000172936	4615	Microarrays	tarbase	0	1	Mammary gland
hsa-mir-27a-5p	MIMAT0004501	MYD88	ENSG00000172936	4615	Microarrays	tarbase	0	1	Mammary gland
hsa-mir-29c-3p	MIMAT0000681	MYD88	ENSG00000172936	4615	Microarrays	tarbase	0	1	Mammary gland
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hsa-mir-192-5p	MIMAT0000222	NOD2	ENSG00000167207	64127	Microarray	19074876	1	1	Mammary gland
hsa-mir-10a-5p	MIMAT0000253	NOD2	ENSG00000167207	64127	Luciferase reporter assay//qRT-PCR	25281418	1	0	Mammary gland
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hsa-mir-320a	MIMAT0000510	NOD2	ENSG00000167207	64127	Luciferase reporter assay//qRT-PCR//Western blot	26752466	1	0	Mammary gland
hsa-mir-320b	MIMAT0005792	NOD2	ENSG00000167207	64127	Luciferase reporter assay//qRT-	26752466	1	0	Mammary gland

					PCR//Western blot				
hsa-mir-320c	MIMAT0005793	NOD2	ENSG00000167207	64127	Luciferase reporter assay//qRT - PCR//Western blot	26752466	1	0	Mammary gland
hsa-mir-10b-5p	MIMAT0000254	NOD2	ENSG00000167207	64127	Microarrays	tarbase	0	1	Mammary gland
hsa-mir-27a-3p	MIMAT0000084	NOD2	ENSG00000167207	64127	Microarrays	tarbase	0	1	Mammary gland
hsa-mir-203a-3p	MIMAT0000264	TBK1	ENSG00000183735	29110	Luciferase reporter assay//qRT - PCR//Western blot	26584294	1	0	Mammary gland
hsa-mir-221-3p	MIMAT0000278	TBK1	ENSG00000183735	29110	Microarray//Reporter assay//Western blot	20018759	1	1	Mammary gland
hsa-mir-200b-3p	MIMAT0000318	TBK1	ENSG00000183735	29110	PAR-CLIP	24398324	1	1	Mammary gland
hsa-mir-186-5p	MIMAT0000456	TBK1	ENSG00000183735	29110	PAR-CLIP	23446348  20371350	1	0	Mammary gland
hsa-mir-200c-3p	MIMAT0000617	TBK1	ENSG00000183735	29110	Luciferase reporter assay//PAR - CLIP//Western blot	22991189  24398324	1	1	Mammary gland
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hsa-mir-452-5p	MIMAT0001635	TBK1	ENSG00000183735	29110	Microarray	23807165	1	0	Mammary gland
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hsa-let-7b-5p	MIMAT0000063	TBK1	ENSG00000183735	29110	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-let-7c-5p	MIMAT0000064	TBK1	ENSG00000183735	29110	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-let-7e-5p	MIMAT0000066	TBK1	ENSG00000183735	29110	HITS-CLIP	tarbase	0	1	Mammary gland
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hsa-mir-21-5p	MIMAT0000076	TBK1	ENSG00000183735	29110	HITS-CLIP	tarbase	0	1	Mammary gland
hsa-mir-29a-5p	MIMAT0004503	TBK1	ENSG00000183735	29110	PAR-CLIP	tarbase	0	1	Mammary gland
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hsa-mir-338-5p	MIMAT0004701	TBK1	ENSG00000183735	29110	HITS-CLIP	tarbase	0	1	Mammary gland
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hsa-mir-378a-3p	MIMAT0000732	TBK1	ENSG00000183735	29110	HITS-CLIP, PAR-CLIP	tarbase	0	1	Mammary gland
hsa-mir-378c	MIMAT0016847	TBK1	ENSG00000183735	29110	PAR-CLIP	tarbase	0	1	Mammary gland
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hsa-mir-98-5p	MIMAT0000096	TBK1	ENSG00000183735	29110	HITS-CLIP	tarbase	0	1	Mammary gland
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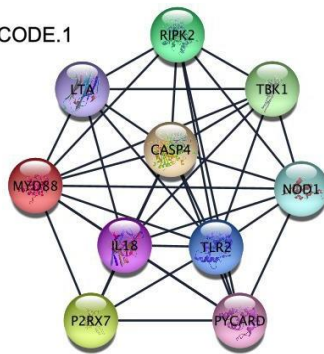
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hsa-mir-155-5p	MIMAT0000646	TBK1	ENSG00000183735	29110	Chimeric fragments	tarbase	0	1	Mammary gland
hsa-mir-221	MIMAT0000278	TBK1	ENSG00000183735	29110	Western blot	20018759	0	0	Mammary gland
hsa-mir-19a-3p	MIMAT0000073	TLR2	ENSG00000137462	7097	Luciferase reporter assay//qRT - PCR//Western blot	22105995  2807802	1	0	Mammary gland
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hsa-mir-146a-5p	MIMAT0000449	TLR2	ENSG00000137462	7097	flow//Luciferase reporter assay//qRT - PCR//Western blot	20375304	1	1	Mammary gland
hsa-mir-106b-5p	MIMAT0000680	TLR2	ENSG00000137462	7097	Microarray	17242205	1	1	Mammary gland

hsa-mir-205-5p	MIMAT0000266	TLR2	ENSG00000137462	7097	Microarrays	tarbase	0	1	Mammary gland
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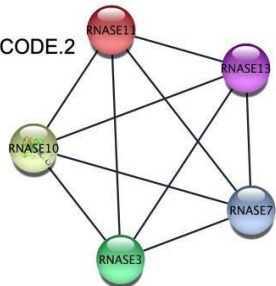


MCODE.1



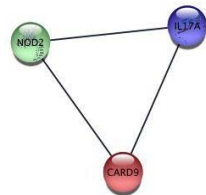
GO	Category	Description	Count	%	Log10(P)	Log10(q)
GO:0050830	GO Biological Processes	defense response to Gram-positive bacterium	9	100.00	-21.80	-17.33
hsa04621	KEGG Pathway	NOD-like receptor signaling pathway	7	77.78	-14.00	-10.13
GO:0001819	GO Biological Processes	positive regulation of cytokine production	8	88.89	-13.42	-9.64
GO:0031349	GO Biological Processes	positive regulation of defense response	7	77.78	-12.43	-8.80

MCODE.2



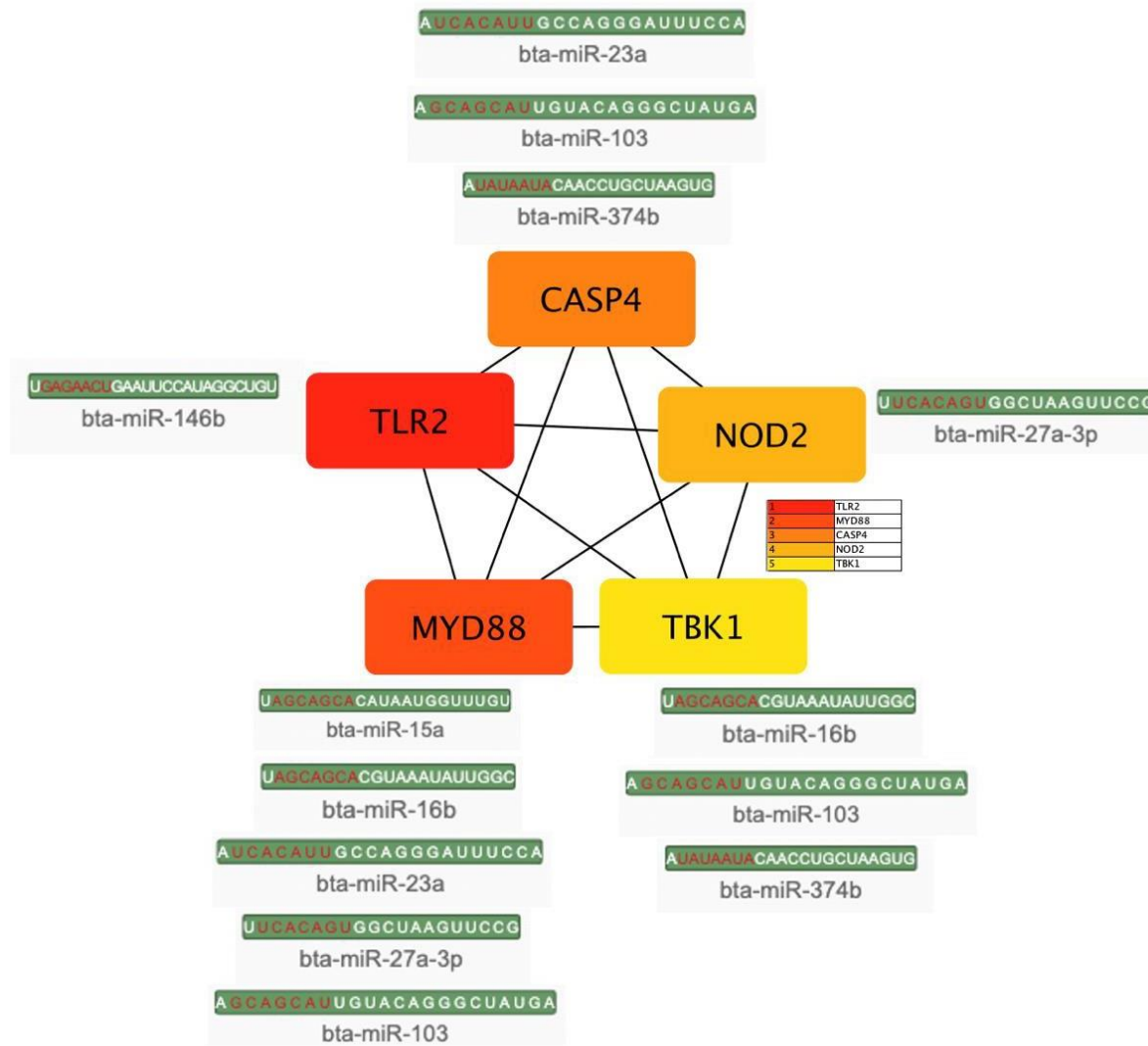
GO	Category	Description	Count	%	Log10(P)	Log10(q)
GO:0050830	GO Biological Processes	defense response to Gram-positive bacterium	5	100.00	-12.08	-7.60
GO:0004519	GO Molecular Functions	endonuclease activity	3	60.00	-6.16	-2.29

MCODE.3

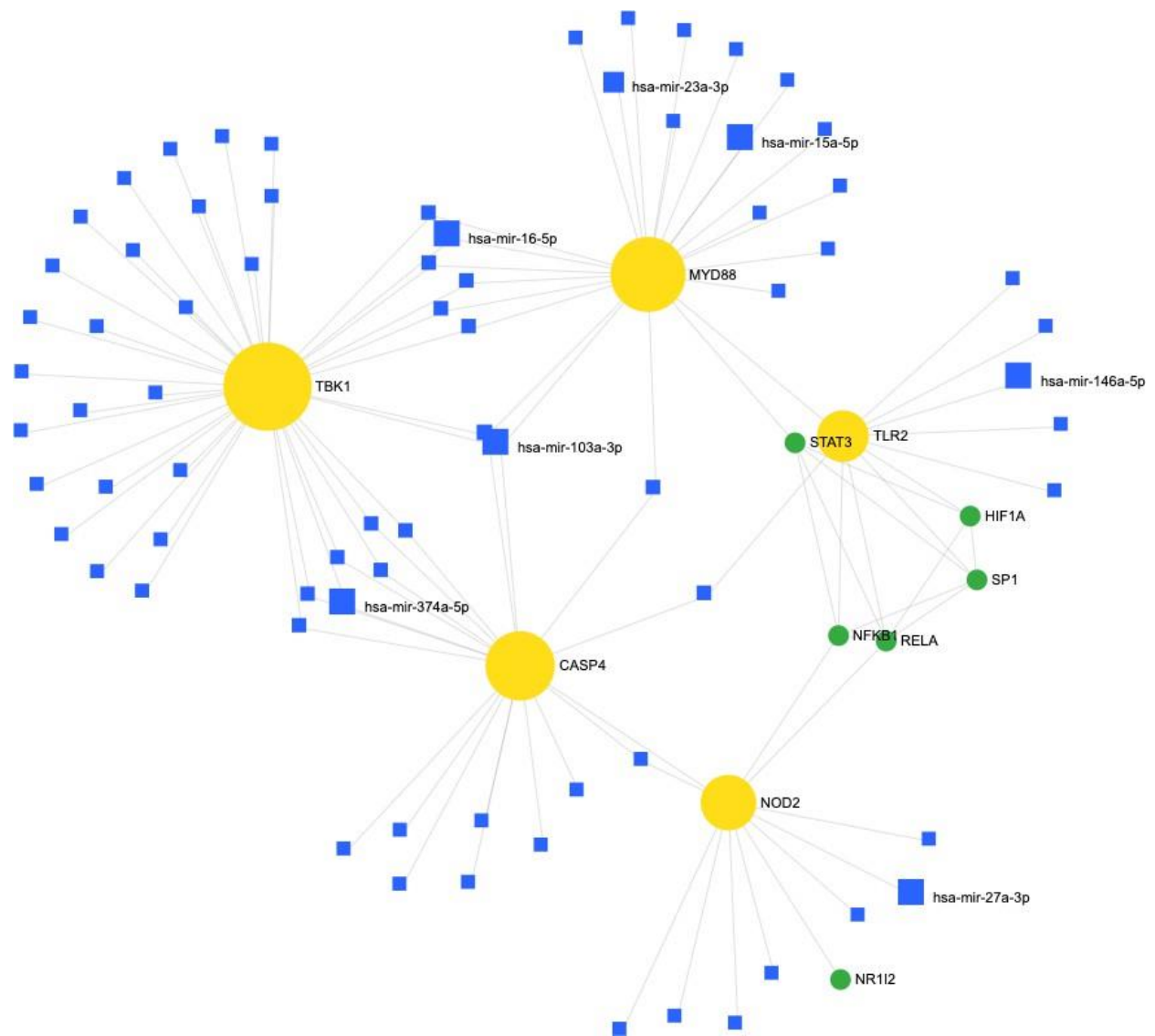


GO	Category	Description	Count	%	Log10(P)	Log10(q)
GO:1900017	GO Biological Processes	positive regulation of cytokine production involved in inflammatory response	3	100.00	-9.15	-4.67

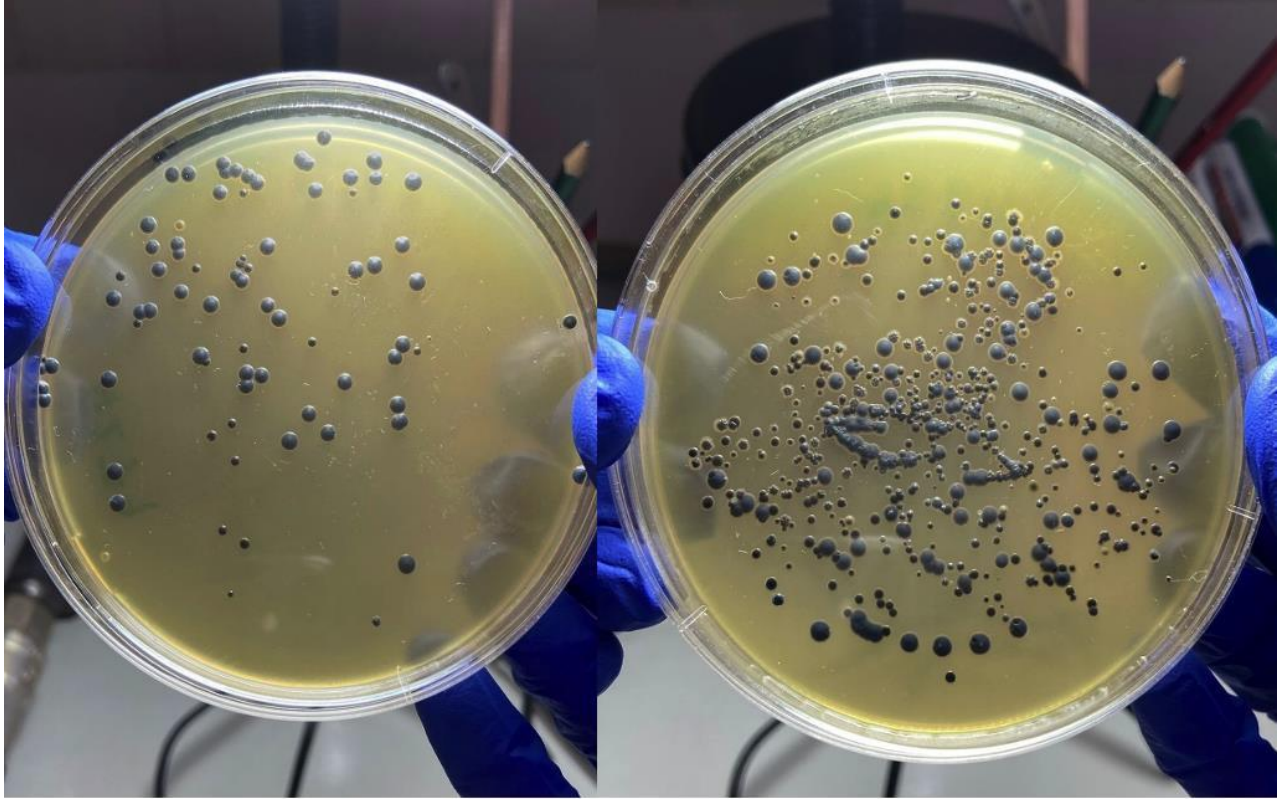
**Supplementary Figure S3.** The three significant modules identified from the PPI network and top-ranked clusters of enrichment terms of the target genes MCODE1-3; MOCODE.1: Module 1; MOCODE.2: Module 2; MOCODE.3: Module 3.



**Supplementary Figure S4.** The networks of the top 5 hub genes and their target miRNAs sequence.

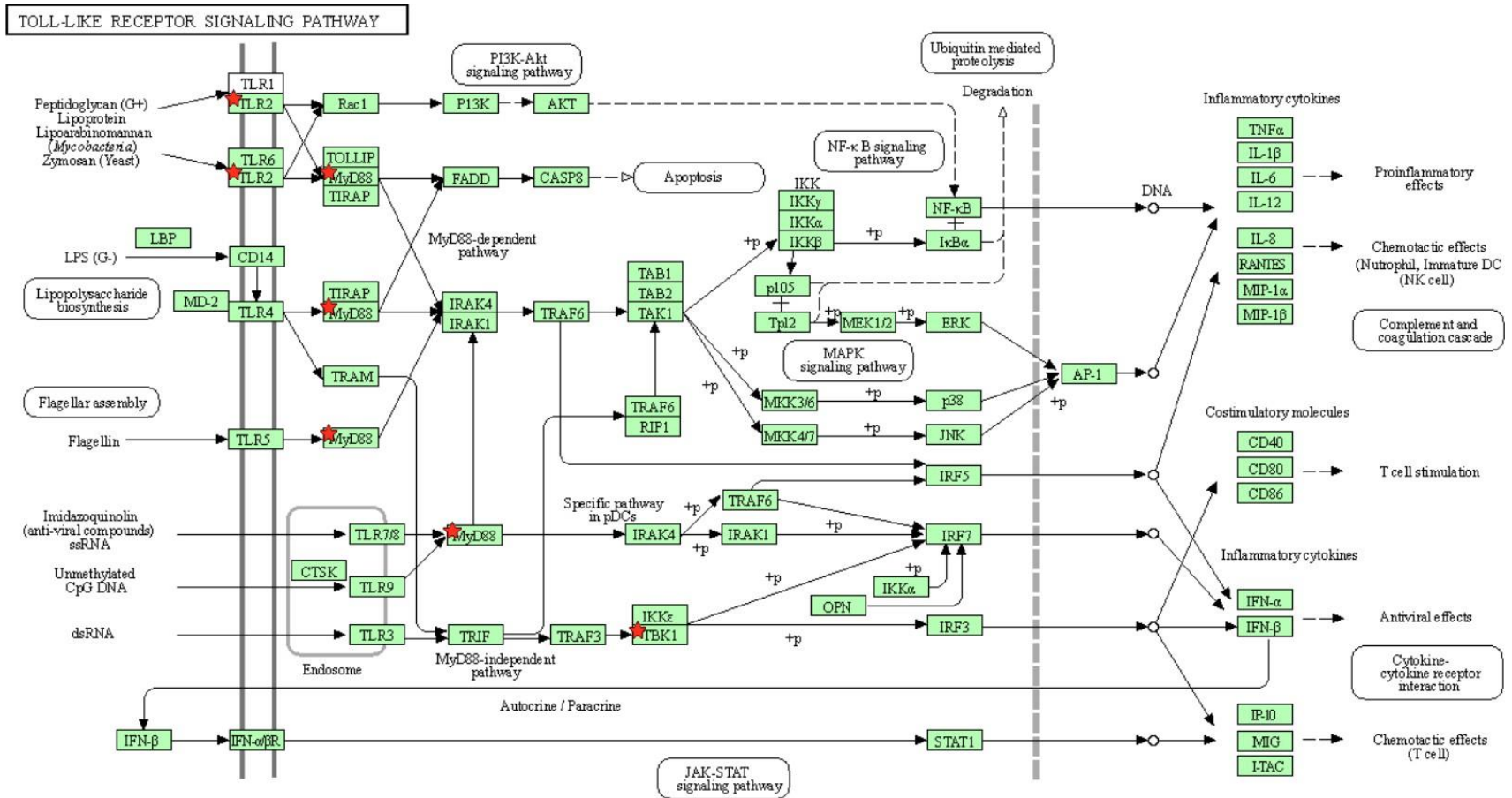


**Supplementary Figure S5** Determined hub gene-miRNA interaction network. Yellow colors represent target genes; Blue colors represent microRNAs; Green color represents Transcription Factors (TFs).



**Supplementary Figure S6.** Culture results of Baird-Parker agar-specific to *S. aureus* colonies.





**Supplementary Figure S7.** Enriched pathway result for determined five hub genes. Genes marked with a red asterisk represent *TLR2*, *MYD88*, and *TBK1*, which are functional in the Toll-like receptors (TLRs) signaling pathway.

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