

**Table S1. Statistics of miRNA sequencing.**

Type	Control Group		<i>S. aureus</i> Group	
	Count	Percent (%)	Count	Percent (%)
Total reads	21,293,853		18,588,177	
high_quality	21,253,265	100	18,565,803	100
3' adapter_null	5959	0.03	3756	0.02
insert_null	8499	0.04	3612	0.02
5' adapter_contaminants	330,968	1.56	22,295	0.12
smaller_than_18nt	60,374	0.28	31,238	0.17
polyA	465	0.00	127	0.00
clean_reads	20,847,000	98.09	18,504,775	99.67

**Table S2. The quality control of mRNAs.**

Sample	A260/A280	Volume (μL)	28S/18S	RIN
A1	2.10	95	1.3	7.9
B1	2.11	95	1.6	8.7
C1	2.06	95	1.5	7.5
A2	2.10	95	2.1	8.5
B2	2.11	95	1.5	8.9
C2	2.12	95	1.9	8.5

**Table S3. The variation coefficient of samples used for microarray test.**

Sample	Chip number	Fluorescent mark	CV (%)	Result
A1	10691-1	cy3	3.38921	Success
B1	10692-1	cy3	4.26303	Success
C1	10693-1	cy3	3.66861	Success
A2	10691-2	cy3	4.82096	Success
B2	10692-3	cy3	4.03989	Success
C2	10693-4	cy3	3.78828	Success

**Table S4. Comparison of the expression levels of seven miRNAs detected by transcriptome sequencing and qRT-PCR.**

miRNAs	miRNA-seq		qRT-PCR
	Log <sub>2</sub> (fold change)	Log <sub>2</sub> (fold change)	Log <sub>2</sub> (fold change)
bta-miR-196a	-1.14		-1.53
bta-miR-205	-1.36		-1.07
bta-miR-200b	-1.05		-1.35
bta-miR-223	+4.88		+5.68
bta-miR-184	+4.82		+4.19
bta-miR-1246	+8.41		+7.56

**Table S5. Comparison of the expression levels of eight mRNAs detected by microarray and qRT-PCR.**

Genes	Microarray	qRT-PCR
	Log <sub>2</sub> (fold change)	Log <sub>2</sub> (fold change)
<i>DGAT2</i>	+3.63	+3.85
<i>FADS2</i>	+2.07	+2.21
<i>ALDH3A2</i>	-1.55	-2.04
<i>EHHADH</i>	-2.68	-3.01
<i>FASN</i>	-2.19	-2.12
<i>LPL</i>	-3.42	-2.99
<i>SCD</i>	-1.59	-1.35
<i>SLC27A6</i>	-5.02	-5.26

**Table S6. Functional annotations of key DEGs and their potential target miRNAs.**

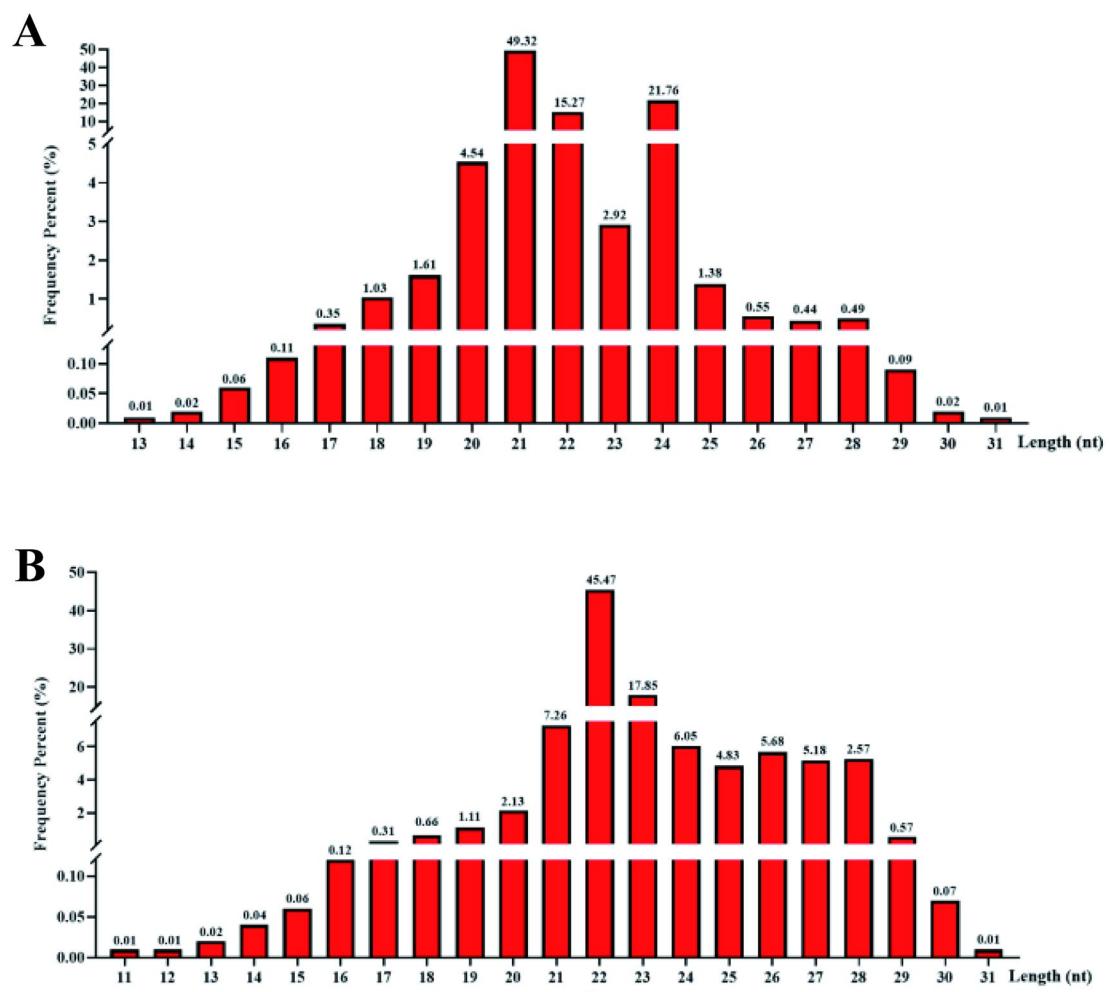
Genes	Log <sub>2</sub> (fold change)	Target miRNAs	Go terms	Pathways
<i>CD14</i>	1.25	bta-miR-664b	cellular response to organic substance cellular response to oxygen-containing compound cellular response to biotic stimulus cellular response to lipopolysaccharide response to lipopolysaccharide cellular response to molecule of bacterial origin	none
<i>GNG2</i>	1.84	bta-miR-23b-3p bta-miR-378b bta-miR-664b bta-miR-874	cellular response to organic substance cellular response to oxygen-containing compound cellular response to acid chemical	PI3K-Akt signaling pathway Chemokine signaling pathway Kaposi sarcoma-associated herpesvirus infection Ras signaling pathway
<i>COL4A1</i>	1.20	bta-miR-23b-3p bta-miR-24 bta-miR-380-3p	cellular response to organic substance cellular response to oxygen-containing compound cellular response to acid chemical cellular response to amino acid stimulus	PI3K-Akt signaling pathway Amoebiasis Human papillomavirus infection
<i>MAPRE2</i>	2.50	bta-miR-135a bta-miR-19b bta-miR-204 bta-miR-23b-3p bta-miR-380-3p bta-miR-410 bta-miR-664b bta-miR-1 bta-miR-139 bta-miR-19b bta-miR-200b bta-miR-23b-3p bta-miR-2431-3p bta-miR-26a bta-miR-32 bta-miR-380-3p	positive regulation of hydrolase activity regulation of cell migration	none
<i>RAP1B</i>	1.35	bta-miR-331-5p bta-miR-664b	cellular response to organic substance cellular response to oxygen-containing compound	Rap1 signaling pathway Leukocyte transendothelial migration Chemokine signaling pathway Ras signaling pathway
<i>IL17A</i>	4.22	bta-miR-331-5p bta-miR-664b	cellular response to organic substance leukocyte migration inflammatory response	IL-17 signaling pathway Rheumatoid arthritis
<i>LDOC1</i>	1.12	bta-miR-19b bta-miR-331-5p	cellular response to organic substance cellular response to oxygen-containing compound cellular response to biotic stimulus cellular response to lipopolysaccharide response to lipopolysaccharide cellular response to molecule of bacterial origin response to molecule of bacterial origin	none
<i>LDLR</i>	1.67	bta-miR-19b bta-miR-20a bta-miR-380-3p bta-miR-410 bta-miR-450b	cellular response to organic substance cellular response to oxygen-containing compound cellular response to acid chemical inflammatory response	Toxoplasmosis
<i>S100A9</i>	4.07	bta-miR-2431-3p	positive regulation of hydrolase activity leukocyte migration neutrophil chemotaxis inflammatory response	IL-17 signaling pathway

**Table S7. The primers used for qRT-PCR to validate the small RNA sequencing.**

<b>miRNAs</b>	<b>Forward Primers (5'-3')</b>
bta-S18(F)	CACCGAGGATGAGGTGGA
bta-S18(R)	TATTGGCGTAGGATTCTGC
bta-miR-196a	GCTGCGACCGTAGGTAGTTCAT
bta-miR-205	TCCTTCATTCCACC GGAGTCTG
bta-miR-200b	GCTGACGGTGCTAATACTGCCT
bta-miR-223	CCTGTCAGTTGTCAAATACCCCA
bta-miR-184	TGGACGGAGAACTGATAAGGGTAAA
bta-miR-1246	GAATGGATTGGAGCAGGAA

**Table S8. The primers used for qRT-PCR to validate the microarray test.**

<b>Gene</b>	<b>Forward Primers (5'-3')</b>	<b>Reverse Primers (5'-3')</b>	<b>Length (bp)</b>	<b>GenBank ID</b>
<i>β-actin</i>	CATCCTGACCCCTCAAGTA	CTCGTTGAGAAGGGTGTG	91	NM_173979.3
<i>DGAT2</i>	GTGTACTCTGCTTCTCTGA	ATGCTAACGTGACCCCTAACAT	117	NM_205793
<i>FADS2</i>	GGTACCCAATGGGACAAAGA	TAGTGGGTTAACCTCCAGATGC	131	NM_001083444
<i>ACOX3</i>	GCTGACTACTTGTAAATTGGGA	AAAGAACGGACTGGCAT	113	NM_001103236
<i>ALDH3A2</i>	TGTTCACTGCTGTTGTAT	GCAGGAAACTTGAAGATTG	105	NM_001101984
<i>EHHADH</i>	ATGAGATTGCTTCTTCCAGT	CGGCTGGGAAATAACCTCTAA	100	NM_001075780
<i>FASN</i>	TAAGGTTCAAATTGCTCGT	GAGCGAAGGAGAGATTAAATCA	134	NM_001012669
<i>LPL</i>	TGTGACTTGTGTTGGCA	AGAGTCCCAGGGCCATA	126	NM_001075120
<i>SCD</i>	TGCAGAAGTGGCTGGTATAAA	CTTCTACCCCTCCATCAAATGTG	142	NM_173959
<i>SLC27A6</i>	TTAGGTGCCACTTGTGTGTTA	GCAAAGTAGTGACAAAGTTC	117	NM_001101169
<i>SREBF1</i>	CAGGTGACTCAACTGTTCC	GCATCTGAGAACTCCTTGT	110	NM_001113302



**Figure S1.** The length distribution of small RNAs in (A) control group and (B) *S. aureus*-inoculated group.

# **Integrative analysis of miRNA and mRNA expression profiles in mammary glands of Holstein cows artificially infected with *Staphylococcus aureus***

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