Going further post-RNA-seq: *In silico* functional analyses revealing candidate genes and regulatory elements related to mastitis in dairy cattle

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SUPPLEMENTARY FILE

Table S1 List of top 5% up and down regulated genes in alveolar mammary tissue 3 h post-inoculation with *Streptococcus agalactiae* from inoculated quarters compared to not inoculated quarters.

Up-regulated			Down-regulated		
Gene Symbol	**Fold Change**	**P-value**	**Gene Symbol**	**Fold Change**	**P-value**
LOC515333	8.48	2.45E-04	*LOC526630*	-8.91	5.09E-07
FOS	8.09	8.17E-05	*SCD*	-7.45	8.67E-06
STAT3	8.07	4.93E-05	*LOC515887*	-7.29	1.24E-04
NFKBIA	7.80	2.00E-04	*LPIN1*	-7.23	3.88E-04
CD14	7.75	4.50E-04	*FABP4*	-7.19	2.09E-04
CCL5	7.66	6.27E-04	*APOC2*	-7.05	1.61E-07
SAA3	7.63	3.91E-05	*MPP6*	-6.87	4.72E-04
IL8	7.34	5.00E-05	*FABP3*	-6.39	1.56E-04
DMXL2	7.26	3.04E-05	*DRAM1*	-6.39	1.19E-04
SFRP2	6.90	1.44E-05	*HSPB8*	-6.37	2.20E-04
CDCA2	6.66	1.52E-06	*LCMT2*	-6.33	5.88E-04
HYKK	6.39	2.16E-10	*CD46*	-6.19	7.20E-06
KDM5A	6.33	1.28E-06	*LOC617415*	-6.19	2.15E-04
FBN1	6.22	6.61E-05	*ARSG*	-6.18	4.12E-05
C20orf196	6.20	1.84E-08	*CLNK*	-6.15	4.55E-05
TLR2	5.78	5.00E-05	*RPS26*	-6.13	9.22E-06
KLK8	5.62	2.56E-05	*LOC100335608*	-6.11	2.50E-05
WDR63	5.61	3.36E-05	*NOP16*	-6.10	1.49E-04
PPP4R1	5.59	1.02E-04	*KRT19*	-6.05	4.83E-05
PDE3B	5.53	2.25E-04	*RAB4A*	-6.04	5.03E-05
MMP7	5.48	3.63E-05			
CHTF18	5.39	4.18E-05			
TTC3	5.28	2.74E-05			
LOC100139881	5.25	6.19E-08			
ABCA5	5.23	1.84E-06			
AIPL1	5.19	2.62E-07			
ATP1B3	5.15	4.58E-07			
C11orf54	5.04	8.82E-06			
SUPT6H	4.99	6.16E-05			

Source: Sbardella *et al*. (2019); Weller *et al*. (2019)

Real-time PCR and data analyses

For real-time PCR, first strand cDNA was synthesized using the Super Script III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA) and the cDNA was then stored at -20°C until use. Real-time PCR was performed using SYBR Green PCR Master Mix kit during amplification on ABI Prism 7300 Sequence Detection Systems (Applied Biosystems, Foster City, CA, USA), according to manufacturer's recommendation. Reactions were performed in duplicates contained cDNA, a pair of primers (a concentration was optimized for each target). The primers were designed using the Primer Express program (Applied Biosystems) from the sequences obtained in the GenBank (http://www.ncbi.nlm.nih.gov) (Supplemental file 2). The genes *Beta-actin*, *RPLP0* (ribosomal protein large P0) and *HPRT* (hypoxanthine-guanine phosphoribosyltransferase) were tested for endogenous control. Then they were analyzed with the geNorm program and the two endogenous controls considered the most stable were *Beta-actin* and *HPRT*. Relative mRNA abundance (fold change) was estimated using the $2-\Delta Ct$ method of Livak and Shmittgen (2001).

Table S2 Sequences of primers employed in the quantitative real-time PCR

Gene	Primer sequence (5'-3')
NFKBIA	F: CGTTCCTGCACTTAGCCATCA
	R: TCACTTGGCGGACCACTTC
SAA3	F: GCACGGCCACAGGATGA
	R: CCCAGGATCAGGAAGCAGAA
CD14	F: ACAGTCCAGCCGACAACCA
	R: TAGGGCACGCACACCATAGTC
STAT3	F: TCTATCTCTACCCCGACATTCCA
	R: TCTCCGGCCGACAGTACTTT
SCD	F: GGGAAACAAGGCAGGAAGCT
	R: AGAGTCAGGAGAGAAAGGGAGCATA
RPLP0	F: CAACCCTGAAGTGCTTGACAT
	R: AGGCAGATGGATCAGCCA
HPRT	F: GCCGACCTGTTGGATTACAT
	R: ACACTTCGAGGGGTCCTTTT
BETA-ACTIN	F: AGCAAGCAGGAGTACGATGAGT
	R: ATCCAACCGACTGCTGTCA

Table S3 Candidate transcription factors of up and down regulated gene groups.

TF gene *up-regulated*	TF gene *down-regulated*
RELA	*INSM1*
Stat3	*Pax2*
REL	*Pax5*

EBF1	*Stat3*
NFE2L1::MafG	*HIF1A::ARNT*
NFYA	*Myf*
NFKB1	*Mycn*
NFATC2	*YY1*
NR1H2::RXRA	*PPARG*
SPI1	*EBF1*
Gata1	*EWSR1-FLI1*
STAT1	*STAT1*
NF-kappaB	*REST*
EWSR1-FLI1	*Gfi*
Nr2e3	*Egr1*
FOXO3	*AP1*
NFIL3	*GABPA*
Spz1	*NFKB1*
Tal1::Gata1	*ESR1*
NR3C1	*NKX3-1*
	RXR::RAR_DR5
	NFE2L1::MafG

Table S4 List of up and down-regulated miRNAs in alveolar mammary tissue 3 h post-inoculation with *Streptococcus agalactiae* from inoculated quarters compared to not inoculated quarters.

miRNA	Fold change	P-value	miRNA	Fold change	P-value
bta-mir-375	3.43	1.34E-06	bta-mir-2285a	-1.21	6.00E-04
bta-mir-2324	2.29	3.23E-06	bta-mir-2444	-1.45	2.16E-04
bta-mir-363	1.87	1.59E-04	bta-mir-2401	-1.46	1.93E-04
bta-mir-2284T	1.78	2.59E-04	bta-mir-2353	-1.70	5.58E-04
bta-mir-2380	1.49	3.14E-06	bta-mir-129-1	-1.75	2.01E-04
bta-mir-95	1.45	2.57E-04	bta-mir-2285d	-1.85	4.45E-04
bta-mir-2344	1.30	3.60E-04	bta-mir-2284h	-2.62	1.66E-04
bta-mir-2363-1	1.28	3.54E-04	bta-mir-193a	-2.87	4.23E-04
bta-mir-148b	1.26	2.83E-05	bta-mir-205	-3.17	2.01E-05
bta-mir-128-2	1.25	4.79E-04	bta-mir-2446	-3.31	3.49E-04
bta-mir-2460	1.19	3.64E-04	bta-mir-2363-2	-3.37	1.57E-04
bta-mir-2284J	1.11	1.47E-04	bta-let-7e	-4.09	1.19E-04
bta-mir-2429	1.09	6.33E-04	bta-mir-2339	-4.19	3.14E-04
bta-mir-2310	1.01	2.64E-04	bta-mir-2475	-4.36	3.16E-06

Source: Sbardella *et al*. (2019); Weller *et al*. (2019)