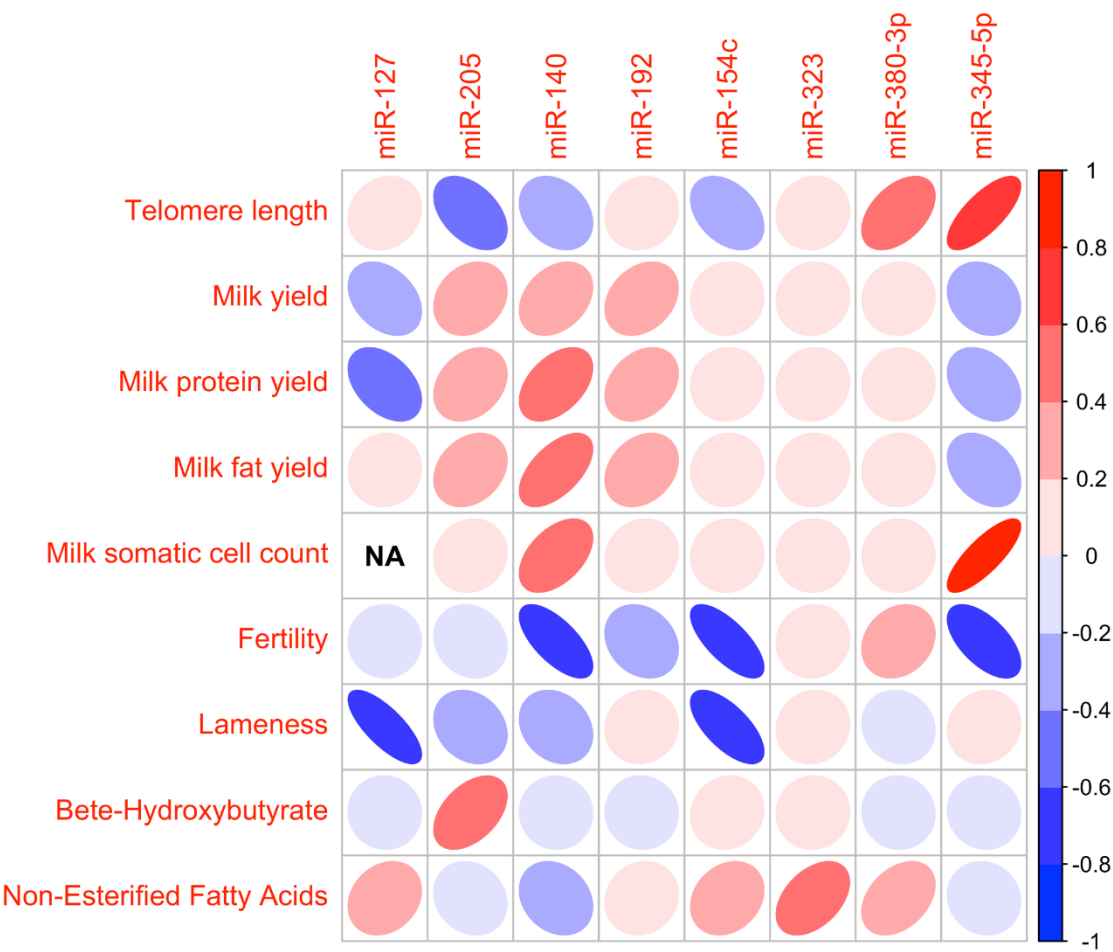


Supplementary Information

Association of plasma microRNA expression with age, genetic background and functional traits in dairy cattle

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Supplementary Figure S1



Supplementary Figure S1. Correlations between traits and individual microRNAs. Only microRNAs that were significant for a trait have been considered. Correlation between miR-127 and milk somatic cell count (NA) could not be estimated. Strength of correlation is indicated both by colour intensity and shape.

Supplementary Tables

Supplementary Table S1. MiRNA differences between calves and mature cows. MiRNAs with differential expression in plasma between select calves and mature cows, using PCR arrays.

Benjamini-Hochberg corrected p-values are shown under 'P-value'.

MiRNA	Calf - normalised expression	Mature cow - normalised expression	Fold-change (Cow / Calf)	P-value
miR-31	0.04	0.25	5.62	0.026
miR-205	0.05	0.29	5.35	0.025
miR-215	1.05	3.68	3.50	0.034
miR-29a	3.70	12.52	3.38	0.025
miR-27a-3p	7.27	22.92	3.15	0.026
miR-362-3p	0.02	0.07	3.06	0.042
miR-143	0.63	1.85	2.94	0.019
miR-29c	2.99	8.11	2.71	0.026
miR-378b	0.38	0.96	2.52	0.034
miR-23b-3p	0.74	1.72	2.31	0.026
miR-29d-3p	0.60	1.34	2.25	0.026
miR-132	0.13	0.28	2.16	0.014
miR-23a	11.82	24.90	2.11	0.025
miR-660	0.40	0.82	2.05	0.026
miR-126-5p	1.95	3.98	2.05	0.029
miR-142-5p	5.21	8.87	1.70	0.014
miR-30c	1.09	1.83	1.67	0.026
miR-378c	0.02	0.03	1.58	0.029
miR-423-3p	8.33	6.32	0.76	0.042
miR-484	3.21	2.00	0.62	0.044
miR-1307	1.33	0.67	0.51	0.014
miR-6123	0.60	0.27	0.45	0.026
miR-483	1.86	0.27	0.15	0.043
miR-380-3p	1.07	0.08	0.08	0.048
miR-154c	0.37	0.03	0.07	0.042
miR-127	1.82	0.09	0.05	0.042

Supplementary Table S2. MiRNA differences between genetic lines. MiRNAs with significantly different expression levels between control and select mature milking cows using PCR arrays. Benjamini-Hochberg corrected p-values are shown under 'P-value'.

MiRNA	Control -normalised expression	Select - normalised expression	Fold-change (Select / Control)	P-value
miR-382	0.84	5.42	6.48	0.038
miR-323	0.11	0.67	5.99	0.038
miR-345-5p	0.06	0.27	4.78	0.038
miR-224	0.17	0.56	3.36	0.038
miR-328	0.37	0.85	2.31	0.039

Supplementary Table S3. MiRNA stability in bovine plasma. The ten miRNAs with the most stable expression across all samples in the PCR array dataset, sorted by ascending stability value generated using the NormFinder algorithm.

MiRNA	NormFinder stability value	Cq-value
bta-miR-101	0.199	24.5
bta-miR-148a	0.201	22.9
bta-miR-532	0.219	27.0
bta-miR-19a	0.232	19.7
bta-miR-19b	0.241	20.2
bta-miR-15a	0.247	22.0
bta-miR-16a	0.250	17.9
bta-miR-20a	0.251	19.9
bta-miR-30e-5p	0.252	20.1
bta-miR-16b	0.255	17.9

Supplementary Table S4. Details of experimental design.

Age group, genetic line	No. of animals	Age (days, median)	Standard Deviation
Calves, control line	9	7	4.2
Calves, select line	12	8	6.8
Heifers, control line	7	384	31.7
Heifers, select line	6	370	15.7
1st lactation milking cows, control line	11	771	36.9
1st lactation milking cows, select line	8	773	52.7
Mature milking cows* control line	9	1,462	418.3
Mature milking cows*, select line	11	1,472	282.0

*Included cows at 2nd (n=8), 3rd (n=10), 4th (n=1) and 5th (n=1) lactations

Both genes targeted by siRNAs, which were differentially expressed between calves and mature cows ($P < 0.05$)

[illegible]

Pathways targeted by miRNAs which were differentially expressed between control and subset genetic lines (P < 0.05)									
KEGG pathway	Group	P-value	No. genes affected	No. of miRNAs targeting miRNAs	MiRNAs targeting pathway				
Adhesion junction	Cell-cell adhesion, cell movement	0.000	49	16	miR-199a-5p, miR-138-5p, miR-224-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Angion stenosis pathway	DNA damage repair	0.000	49	16	miR-497-5p, miR-224-5p, miR-138-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Gene expression	Metabolism	0.000	109	34	miR-199a-5p, miR-138-5p, miR-224-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Protein synthesis in endoplasmic reticulum	Gene expression	0.000	37	19	miR-224-5p, miR-138-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
ECM remodeling pathway	Cell cycle	0.000	37	19	miR-138-5p, miR-1224-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
RNA splicing pathway	Gene expression	0.000	17	16	miR-434-3p, miR-206-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Endothelium	Cell cycle	0.000	98	18	miR-1224-5p, miR-323a-5p, miR-224-5p, miR-230-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
ECM signaling pathway	Gene expression	0.000	33	18	miR-330-5p, miR-434-3p, miR-206-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Immunity	Cell cycle	0.000	97	19	miR-224-5p, miR-230-5p, miR-497-5p, miR-138-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Stress response	Gene expression	0.001	61	15	miR-224-5p, miR-486-5p, miR-230-5p, miR-497-5p, miR-138-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p				
ECM signaling pathway	ECM signaling pathway	0.001	33	16	miR-434-3p, miR-206-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Neurotransmission signaling pathway	Gene expression	0.002	62	16	miR-224-5p, miR-497-5p, miR-138-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Cell cycle	Cell cycle	0.002	59	17	miR-497-5p, miR-230-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
RNA transport	Gene expression	0.003	14	16	miR-138-5p, miR-230-5p, miR-224-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Stress response	Gene expression	0.003	61	16	miR-434-3p, miR-206-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
DNA degradation	Gene expression	0.006	40	16	miR-138-5p, miR-138-5p, miR-497-5p, miR-230-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
ECM signaling pathway	Gene expression	0.009	37	16	miR-224-5p, miR-138-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Oocyte meiosis	Gene expression	0.009	40	17	miR-224-5p, miR-138-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Stress response	Gene expression	0.014	55	17	miR-323a-5p, miR-434-3p, miR-206-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
ECM signaling pathway	DNA damage repair	0.015	49	16	miR-4				
ECM signaling pathway	Gene expression	0.018	47	16	miR-373a-5p, miR-34a-5p, miR-196a-5p, miR-497-5p, miR-138-5p, miR-224-5p, miR-383-5p, miR-118-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Metabolism	Gene expression	0.023	65	16	miR-224-5p, miR-138-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Ubiquitin mediated proteolysis	Gene expression	0.023	60	16	miR-196a-5p, miR-497-5p, miR-230-5p, miR-146a-5p, miR-98b-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				
Down-regulation of actin cytoskeleton	Cell cycle	0.042	15	12	miR-138-5p, miR-497-5p, miR-196a-5p, miR-224-5p, miR-146a-5p, miR-154-5p, miR-196a-5p, miR-323a-5p, miR-230-5p, miR-188-5p, miR-149-5p, miR-385-5p				
Down-regulation of actin cytoskeleton	Gene expression	0.057	16	12	miR-230-5p, miR-98b-5p, miR-146a-5p, miR-187-5p, miR-196a-5p, miR-149-5p, miR-323a-5p, miR-1224-5p, miR-385-5p, miR-486-5p				