

Supplementary Table S1. Associations between *MSTN* variant presence/absence and gross milk production traits

Milk trait	Variant	Mean \pm SE ¹				<i>P</i> ²
		Absent	n	Present	n	
Daily milk yield (liters/day)	<i>A</i>	23.7 \pm 0.58	53	23.0 \pm 0.46	377	0.151
	<i>B</i>	22.9 \pm 0.46	302	23.6 \pm 0.49	128	<i>0.077</i>
	<i>C</i>	23.1 \pm 0.46	357	23.1 \pm 0.56	73	0.984
	<i>D</i>	23.1 \pm 0.45	337	23.1 \pm 0.54	93	0.816
Fat (%)	<i>A</i>	4.8 \pm 0.09	53	5.1 \pm 0.07	377	<i>0.064</i>
	<i>B</i>	4.9 \pm 0.07	302	4.9 \pm 0.08	128	0.892
	<i>C</i>	5.0 \pm 0.07	357	4.9 \pm 0.09	73	<i>0.068</i>
	<i>D</i>	5.0 \pm 0.07	337	4.9 \pm 0.08	93	0.368
Protein (%)	<i>A</i>	4.0 \pm 0.05	53	4.0 \pm 0.04	377	<i>0.079</i>
	<i>B</i>	4.0 \pm 0.04	302	4.0 \pm 0.04	128	0.154
	<i>C</i>	4.0 \pm 0.04	357	4.0 \pm 0.05	73	<i>0.084</i>
	<i>D</i>	4.0 \pm 0.04	337	4.0 \pm 0.04	93	0.244

¹The predicted means and standard error of those means were derived from the GLMMs. Cow age (age of the cow in years), herd and days in milk (DIM; being the integer number of days from the commencement of lactation for each individual cow), were fitted to the models as fixed effects.

²*P* < 0.05 in bold; 0.05 \leq *P* < 0.10 in italics

Supplementary Table S2. Associations between *MSTN* variant presence/absence and individual milk fatty acid component levels

Fatty acid (FA)	Variant	Mean FA level \pm SE ¹ (g/100 g milk FA)				<i>P</i> ²
		Absent	n	Present	n	
C4:0	A	1.28 \pm 0.022	53	1.28 \pm 0.017	377	0.898
	B	1.28 \pm 0.017	302	1.27 \pm 0.018	128	0.475
	C	1.28 \pm 0.017	357	1.28 \pm 0.021	73	0.999
	D	1.28 \pm 0.017	337	1.29 \pm 0.020	93	0.441
C6:0	A	1.55 \pm 0.019	53	1.57 \pm 0.014	377	0.479
	B	1.57 \pm 0.015	302	1.56 \pm 0.015	128	0.396
	C	1.56 \pm 0.014	357	1.56 \pm 0.018	73	0.722
	D	1.57 \pm 0.014	337	1.55 \pm 0.017	93	0.294
C8:0	A	1.18 \pm 0.016	53	1.19 \pm 0.013	377	0.343
	B	1.12 \pm 0.013	302	1.18 \pm 0.014	128	0.164
	C	1.19 \pm 0.013	357	1.18 \pm 0.015	73	0.917
	D	1.19 \pm 0.012	337	1.17 \pm 0.015	93	0.021
C10:0	A	3.23 \pm 0.064	53	3.25 \pm 0.050	377	0.605
	B	3.26 \pm 0.051	302	3.22 \pm 0.054	128	0.320
	C	3.24 \pm 0.050	357	3.26 \pm 0.061	73	0.807
	D	3.28 \pm 0.049	337	3.16 \pm 0.058	93	0.009
C10:1	A	0.27 \pm 0.008	53	0.29 \pm 0.006	377	0.081
	B	0.29 \pm 0.006	302	0.28 \pm 0.006	128	0.066
	C	0.28 \pm 0.006	357	0.28 \pm 0.007	73	0.439
	D	0.29 \pm 0.006	337	0.28 \pm 0.007	93	0.187
C11:0	A	0.06 \pm 0.003	53	0.06 \pm 0.002	377	0.258
	B	0.06 \pm 0.002	302	0.06 \pm 0.003	128	0.129
	C	0.06 \pm 0.002	357	0.05 \pm 0.003	73	0.082
	D	0.06 \pm 0.002	337	0.06 \pm 0.003	93	0.211
C12:0	A	3.90 \pm 0.085	53	3.95 \pm 0.067	377	0.479
	B	3.96 \pm 0.068	302	3.90 \pm 0.073	128	0.233
	C	3.94 \pm 0.067	357	3.95 \pm 0.082	73	0.879
	D	3.98 \pm 0.066	337	3.82 \pm 0.078	93	0.006
C12:1	A	0.09 \pm 0.003	53	0.09 \pm 0.002	377	0.184
	B	0.09 \pm 0.003	302	0.09 \pm 0.003	128	0.050
	C	0.09 \pm 0.002	357	0.09 \pm 0.003	73	0.845
	D	0.09 \pm 0.002	337	0.09 \pm 0.003	93	0.022
C13:0	A	0.11 \pm 0.005	53	0.19 \pm 0.004	377	0.245
	B	0.12 \pm 0.004	302	0.11 \pm 0.004	128	0.172
	C	0.12 \pm 0.004	357	0.11 \pm 0.004	73	0.188
	D	0.12 \pm 0.004	337	0.11 \pm 0.004	93	0.198

C13:0 <i>iso</i>	A	0.08 ±0.003	53	0.08 ±0.002	377	0.104
	B	0.08 ±0.002	302	0.08 ±0.002	128	0.097
	C	0.08 ±0.002	357	0.08 ±0.003	73	0.404
	D	0.08 ±0.002	337	0.08 ±0.003	93	0.090
C13:0 <i>anteiso</i>	A	0.04 ±0.001	53	0.04 ±0.001	377	0.763
	B	0.04 ±0.001	302	0.04 ±0.001	128	0.201
	C	0.04 ±0.001	357	0.04 ±0.001	73	0.714
	D	0.04 ±0.001	337	0.04 ±0.001	93	0.623
C14:0	A	12.52 ±0.148	53	12.48 ±0.117	377	0.795
	B	12.51 ±0.118	302	12.46 ±0.126	128	0.652
	C	12.45 ±0.116	357	12.61 ±0.141	73	0.170
	D	12.53 ±0.115	337	12.37 ±0.136	93	0.123
C14:1 <i>cis</i> -9	A	0.94 ±0.037	53	0.98 ±0.029	377	0.263
	B	0.98 ±0.030	302	0.95 ±0.032	128	0.227
	C	0.97 ±0.029	357	0.96 ±0.035	73	0.590
	D	0.97 ±0.029	337	0.95 ±0.034	93	0.502
C15:0	A	1.45 ±0.029	53	1.47 ±0.023	377	0.275
	B	1.47 ±0.023	302	1.46 ±0.024	128	0.438
	C	1.47 ±0.022	357	1.47 ±0.028	73	0.882
	D	1.47 ±0.022	337	1.45 ±0.027	93	0.303
C15:1	A	0.28 ±0.005	53	0.28 ±0.004	377	0.346
	B	0.28 ±0.004	302	0.28 ±0.005	128	0.282
	C	0.28 ±0.004	357	0.28 ±0.005	73	0.300
	D	0.28 ±0.004	337	0.28 ±0.005	93	0.650
C15:0 <i>iso</i>	A	0.29 ±0.005	53	0.29 ±0.004	377	0.333
	B	0.29 ±0.004	302	0.29 ±0.004	128	0.031
	C	0.29 ±0.004	357	0.29 ±0.004	73	0.148
	D	0.29 ±0.004	337	0.29 ±0.004	93	0.087
C15:0 <i>anteiso</i>	A	0.62 ±0.016	53	0.63 ±0.013	377	0.537
	B	0.63 ±0.013	302	0.62 ±0.014	128	0.273
	C	0.62 ±0.013	357	0.63 ±0.015	73	0.361
	D	0.63 ±0.013	337	0.62 ±0.015	93	0.374
C16:0	A	36.94 ±0.518	53	37.18 ±0.408	377	0.593
	B	37.01 ±0.412	302	37.29 ±0.441	128	0.388
	C	37.26 ±0.505	357	36.69 ±0.493	73	0.254
	D	37.04 ±0.404	337	37.37 ±0.476	93	0.350
C16:1 <i>cis</i> -9	A	1.36 ±0.043	53	1.35 ±0.034	377	0.894
	B	1.36 ±0.034	302	1.34 ±0.037	128	0.506
	C	1.36 ±0.034	357	1.35 ±0.041	73	0.853
	D	1.33 ±0.034	337	1.40 ±0.040	93	0.031
C17:0	A	0.70 ±0.012	53	0.70 ±0.683	377	0.920

	<i>B</i>	0.70 ±0.009	302	0.71 ±0.010	128	0.264
	<i>C</i>	0.70 ±0.009	357	0.70 ±0.011	73	0.736
	<i>D</i>	0.70 ±0.009	337	0.70 ±0.011	93	0.339
C17:1	<i>A</i>	0.21 ±0.004	53	0.21 ±0.003	377	0.327
	<i>B</i>	0.21 ±0.003	302	0.21 ±0.004	128	0.674
	<i>C</i>	0.21 ±0.003	357	0.21 ±0.004	73	0.281
	<i>D</i>	0.21 ±0.003	337	0.21 ±0.004	93	0.300
C17:0 <i>iso</i>	<i>A</i>	0.56 ±0.012	53	0.56 ±0.009	377	0.719
	<i>B</i>	0.56 ±0.009	302	0.56 ±0.010	128	0.788
	<i>C</i>	0.56 ±0.009	357	0.57 ±0.011	73	0.253
	<i>D</i>	0.56 ±0.009	337	0.56 ±0.011	93	0.555
C18:0	<i>A</i>	8.82 ±0.220	53	8.58 ±0.173	377	0.298
	<i>B</i>	8.60 ±0.175	302	8.71 ±0.187	128	0.429
	<i>C</i>	8.61 ±0.173	357	8.73 ±0.210	73	0.486
	<i>D</i>	8.64 ±0.172	337	8.65 ±0.203	93	0.911
C18:1 <i>cis</i> -9	<i>A</i>	13.76 ±0.263	53	13.45 ±0.207	377	0.167
	<i>B</i>	13.51 ±0.210	302	13.54 ±0.224	128	0.850
	<i>C</i>	13.48 ±0.206	357	13.64 ±0.251	73	0.445
	<i>D</i>	13.45 ±0.205	337	13.73 ±0.242	93	0.124
C18:1 <i>trans</i> -11	<i>A</i>	2.52 ±0.125	53	2.64 ±0.099	377	0.277
	<i>B</i>	2.62 ±0.100	302	2.59 ±0.107	128	0.695
	<i>C</i>	2.61 ±0.098	357	2.60 ±0.120	73	0.905
	<i>D</i>	2.63 ±0.098	337	2.54 ±0.115	93	0.302
C18:2 <i>cis</i> -9,12	<i>A</i>	0.71 ±0.014	53	0.69 ±0.011	377	0.199
	<i>B</i>	0.70 ±0.011	302	0.69 ±0.012	128	0.098
	<i>C</i>	0.69 ±0.011	357	0.70 ±0.014	73	0.315
	<i>D</i>	0.69 ±0.011	337	0.70 ±0.013	93	0.186
C18:2 <i>trans</i> -9,12	<i>A</i>	0.35 ±0.010	53	0.35 ±0.008	377	0.330
	<i>B</i>	0.36 ±0.008	302	0.34 ±0.009	128	0.056
	<i>C</i>	0.35 ±0.008	357	0.36 ±0.010	73	0.090
	<i>D</i>	0.35 ±0.008	337	0.35 ±0.009	93	0.791
C18:2 <i>cis</i> -9, <i>trans</i> -12	<i>A</i>	0.07 ±0.003	53	0.07 ±0.002	377	0.786
	<i>B</i>	0.07 ±0.002	302	0.07 ±0.003	128	0.373
	<i>C</i>	0.07 ±0.002	357	0.07 ±0.003	73	0.973
	<i>D</i>	0.07 ±0.002	337	0.07 ±0.003	93	0.312
C18:2 <i>trans</i> -9, <i>cis</i> -12	<i>A</i>	0.44 ±0.021	53	0.45 ±0.016	377	0.408
	<i>B</i>	0.45 ±0.016	302	0.45 ±0.018	128	0.882
	<i>C</i>	0.45 ±0.016	357	0.46 ±0.020	73	0.540
	<i>D</i>	0.45 ±0.016	337	0.44 ±0.019	93	0.203
C18:2 <i>cis</i> -9, <i>trans</i> -13	<i>A</i>	0.29 ±0.006	53	0.29 ±0.005	377	0.306
	<i>B</i>	0.29 ±0.005	302	0.28 ±0.005	128	0.062

	<i>C</i>	0.28 ± 0.005	357	0.29 ± 0.006	73	0.064
	<i>D</i>	0.29 ± 0.005	337	0.29 ± 0.006	93	0.167
C18:3 <i>cis</i> -6,9,12	<i>A</i>	0.08 ± 0.002	53	0.07 ± 0.002	377	0.331
	<i>B</i>	0.07 ± 0.002	302	0.07 ± 0.002	128	0.462
	<i>C</i>	0.07 ± 0.002	357	0.08 ± 0.002	73	0.514
	<i>D</i>	0.07 ± 0.002	337	0.07 ± 0.002	93	0.635
C18:3 <i>cis</i> -9,12,15	<i>A</i>	0.80 ± 0.020	53	0.78 ± 0.015	377	0.203
	<i>B</i>	0.79 ± 0.016	302	0.77 ± 0.017	128	0.061
	<i>C</i>	0.78 ± 0.015	357	0.80 ± 0.019	73	0.141
	<i>D</i>	0.78 ± 0.015	337	0.79 ± 0.018	93	0.425
C20:0	<i>A</i>	0.13 ± 0.003	53	0.13 ± 0.002	377	0.968
	<i>B</i>	0.13 ± 0.002	302	0.12 ± 0.003	128	0.851
	<i>C</i>	0.12 ± 0.002	357	0.13 ± 0.003	73	0.275
	<i>D</i>	0.13 ± 0.002	337	0.12 ± 0.003	93	0.626
C20:1 <i>cis</i> -5	<i>A</i>	0.06 ± 0.003	53	0.06 ± 0.002	377	0.597
	<i>B</i>	0.06 ± 0.002	302	0.06 ± 0.002	128	0.739
	<i>C</i>	0.06 ± 0.002	357	0.06 ± 0.002	73	0.608
	<i>D</i>	0.06 ± 0.002	337	0.06 ± 0.002	93	0.647
C20:1 <i>cis</i> -9	<i>A</i>	0.15 ± 0.004	53	0.15 ± 0.003	377	0.151
	<i>B</i>	0.15 ± 0.003	302	0.15 ± 0.004	128	0.798
	<i>C</i>	0.15 ± 0.003	357	0.15 ± 0.004	73	0.145
	<i>D</i>	0.15 ± 0.003	337	0.15 ± 0.004	93	0.575
C20:1 <i>cis</i> -11	<i>A</i>	0.08 ± 0.002	53	0.08 ± 0.002	377	0.687
	<i>B</i>	0.08 ± 0.002	302	0.08 ± 0.002	128	0.354
	<i>C</i>	0.08 ± 0.002	357	0.08 ± 0.002	73	0.823
	<i>D</i>	0.08 ± 0.002	337	0.08 ± 0.002	93	0.504
C20:3 <i>cis</i> -8,11,14	<i>A</i>	0.03 ± 0.001	53	0.03 ± 0.001	377	0.412
	<i>B</i>	0.03 ± 0.001	302	0.03 ± 0.001	128	0.242
	<i>C</i>	0.03 ± 0.001	357	0.03 ± 0.001	73	0.017
	<i>D</i>	0.03 ± 0.001	337	0.03 ± 0.001	93	0.478
C20:4 <i>cis</i> -5,8,11,14	<i>A</i>	0.04 ± 0.001	53	0.03 ± 0.001	377	0.275
	<i>B</i>	0.03 ± 0.001	302	0.03 ± 0.001	128	0.334
	<i>C</i>	0.03 ± 0.001	357	0.03 ± 0.001	73	0.749
	<i>D</i>	0.03 ± 0.001	337	0.03 ± 0.001	93	0.638
C22:1 <i>trans</i> -13	<i>A</i>	0.07 ± 0.002	53	0.07 ± 0.002	377	0.706
	<i>B</i>	0.07 ± 0.002	302	0.07 ± 0.002	128	0.393
	<i>C</i>	0.07 ± 0.002	357	0.07 ± 0.002	73	0.004
	<i>D</i>	0.07 ± 0.002	337	0.07 ± 0.002	93	0.450
C20:5 <i>cis</i> -5,8,11,14,17	<i>A</i>	0.09 ± 0.002	53	0.09 ± 0.002	377	0.166
	<i>B</i>	0.09 ± 0.002	302	0.09 ± 0.002	128	0.270
	<i>C</i>	0.09 ± 0.002	357	0.09 ± 0.002	73	0.131

	<i>D</i>	0.09 ±0.002	337	0.09 ±0.002	93	0.687
C22:5 <i>cis</i> -7,10,13,16,19	<i>A</i>	0.13 ±0.004	53	0.12 ±0.003	377	0.436
	<i>B</i>	0.12 ±0.003	302	0.12 ±0.004	128	0.712
	<i>C</i>	0.12 ±0.003	357	0.12 ±0.004	73	0.853
	<i>D</i>	0.12 ±0.003	337	0.13 ±0.004	93	0.296
C24:0	<i>A</i>	0.05 ±0.002	53	0.04 ±0.001	377	0.240
	<i>B</i>	<i>0.04 ±0.001</i>	<i>302</i>	<i>0.05 ±0.001</i>	<i>128</i>	<i>0.060</i>
	<i>C</i>	0.04 ±0.001	357	0.05 ±0.002	73	0.507
	<i>D</i>	0.04 ±0.001	337	0.05 ±0.001	93	0.331
CLA <i>cis</i> -9, <i>trans</i> -11	<i>A</i>	0.94 ±0.051	53	0.99 ±0.040	377	0.265
	<i>B</i>	0.99 ±0.041	302	0.95 ±0.043	128	0.312
	<i>C</i>	0.97 ±0.040	357	0.98 ±0.049	73	0.747
	<i>D</i>	0.98 ±0.040	337	0.96 ±0.047	93	0.549

¹The predicted means and standard error of those means were derived from the GLMMs. Cow age (age of the cow in years), herd and days in milk (DIM; being the integer number of days from the commencement of lactation for each individual cow), were fitted to the models as fixed effects.

² $P < 0.05$ in bold; $0.05 \leq P < 0.10$ in italics.

Supplementary Table S3. Associations between *MSTN* variant presence/absence and various grouped milk fatty acid (FA) component levels

Fatty acid (FA) ¹	Variant	Mean FA level \pm SE ² (g/100 g milk FA)				<i>P</i> ³
		Absent	n	Present	n	
SCFA	A	4.00 \pm 0.048	53	4.02 \pm 0.037	377	0.531
	B	4.03 \pm 0.037	302	4.00 \pm 0.040	128	0.252
	C	4.02 \pm 0.036	357	4.01 \pm 0.045	73	0.871
	D	4.02 \pm 0.036	337	4.00 \pm 0.043	93	0.400
MCFA	A	19.64 \pm 0.274	53	19.69 \pm 0.216	377	0.841
	B	19.73 \pm 0.218	302	19.59 \pm 0.233	128	0.397
	C	19.63 \pm 0.215	357	19.81 \pm 0.261	73	0.397
	D	19.78 \pm0.212	337	19.35 \pm0.251	93	0.021
LCFA	A	48.26 \pm 0.458	53	48.28 \pm 0.361	377	0.962
	B	48.13 \pm 0.364	302	48.51 \pm 0.389	128	0.182
	C	48.39 \pm 0.359	357	47.94 \pm 0.436	73	0.207
	D	48.20 \pm 0.357	337	48.52 \pm 0.421	93	0.308
Omega 3	A	1.01 \pm 0.020	53	0.99 \pm 0.016	377	0.125
	B	1.00 \pm 0.016	302	0.98 \pm 0.017	128	0.107
	C	0.99 \pm 0.016	357	1.01 \pm 0.019	73	0.124
	D	0.99 \pm 0.016	337	1.00 \pm 0.019	93	0.345
Omega 6	A	0.847 \pm 0.015	53	0.83 \pm 0.012	377	0.141
	B	0.838 \pm 0.012	302	0.82 \pm 0.013	128	0.140
	C	0.829 \pm 0.012	357	0.84 \pm 0.015	73	0.245
	D	0.829 \pm 0.012	337	0.84 \pm 0.014	93	0.247
MUFA	A	20.24 \pm 0.317	53	20.05 \pm 0.243	377	0.499
	B	20.13 \pm 0.248	302	20.04 \pm 0.264	128	0.636
	C	20.06 \pm 0.243	357	20.20 \pm 0.301	73	0.562
	D	20.04 \pm 0.242	337	20.24 \pm 0.287	93	0.379
PUFA	A	3.94 \pm 0.082	53	3.96 \pm 0.065	377	0.776
	B	3.99 \pm 0.065	302	3.90 \pm 0.070	128	0.094
	C	3.94 \pm 0.064	357	4.02 \pm 0.078	73	0.193
	D	3.96 \pm 0.064	337	3.95 \pm 0.076	93	0.891
branched FA	A	1.58 \pm 0.026	53	1.59 \pm 0.020	377	0.558
	B	1.60 \pm 0.021	302	1.58 \pm 0.022	128	0.155
	C	1.58 \pm 0.020	357	1.61 \pm 0.025	73	0.209
	D	1.60 \pm 0.020	337	1.57 \pm 0.024	93	0.196
C12:1 index	A	2.23 \pm 0.062	53	2.29 \pm 0.049	377	0.276
	B	2.30 \pm 0.049	302	2.24 \pm 0.053	128	0.104
	C	2.28 \pm 0.049	357	2.26 \pm 0.059	73	0.686
	D	2.28 \pm 0.048	337	2.26 \pm 0.057	93	0.573

C16:1 index	<i>A</i>	<i>3.52 ± 0.097</i>	<i>53</i>	<i>3.50 ± 0.077</i>	<i>377</i>	<i>0.831</i>
	<i>B</i>	<i>3.53 ± 0.078</i>	<i>302</i>	<i>3.46 ± 0.083</i>	<i>128</i>	<i>0.243</i>
	<i>C</i>	<i>3.50 ± 0.076</i>	<i>357</i>	<i>3.52 ± 0.093</i>	<i>73</i>	<i>0.709</i>
	<i>D</i>	<i>3.47 ± 0.076</i>	<i>337</i>	<i>3.59 ± 0.089</i>	<i>93</i>	<i>0.083</i>
C18:1 index	<i>A</i>	<i>65.77 ± 0.603</i>	<i>53</i>	<i>66.01 ± 0.463</i>	<i>377</i>	<i>0.643</i>
	<i>B</i>	<i>66.08 ± 0.472</i>	<i>302</i>	<i>65.78 ± 0.502</i>	<i>128</i>	<i>0.438</i>
	<i>C</i>	<i>65.97 ± 0.461</i>	<i>357</i>	<i>65.93 ± 0.572</i>	<i>73</i>	<i>0.942</i>
	<i>D</i>	<i>65.90 ± 0.460</i>	<i>337</i>	<i>66.13 ± 0.546</i>	<i>93</i>	<i>0.589</i>

¹SCFA = Short Chain Fatty Acid, MCFA = Medium Chain Fatty Acid, LCFA = Long Chain Fatty Acid, PUFA = Polyunsaturated Fatty Acid, MUFA = Monounsaturated Fatty Acid.

²The predicted means and standard error of those means were derived from the GLMMs. Cow age (age of the cow in years), herd and days in milk (DIM; being the integer number of days from the commencement of lactation for each individual cow) were fitted to the models as fixed effects.

³ $P < 0.05$ in bold; $0.05 \leq P < 0.10$ in italics.

