

Fig. S1. Immunopathology-associated miRNA profile in the gastrocnemius muscle from FSHD-like mouse models. (A, B) Heat maps depicting the miRNA profile as determined using the immunopathology panel in (A) the moderate DUX4 induced FSHD-like mouse model and (B) the DUX4 chronic FSHD-like mouse models (n=8 animals for *ACTA1-MCM;FLEXD/+* untreated, n=8 animals for *ACTA1-MCM;FLEXD/+* MD3, n=8 animals for *ACTA1-MCM;FLEXD/+* MD9, n=7 animals for *ACTA1-MCM/+* 6 months-old, n=9 animals for chronic MCM/FLEXD-mature, n=6 animals for *ACTA1-MCM/+* 14-18 months-old and n=8 animals for chronic MCM/FLEXD-aged experimental groups).

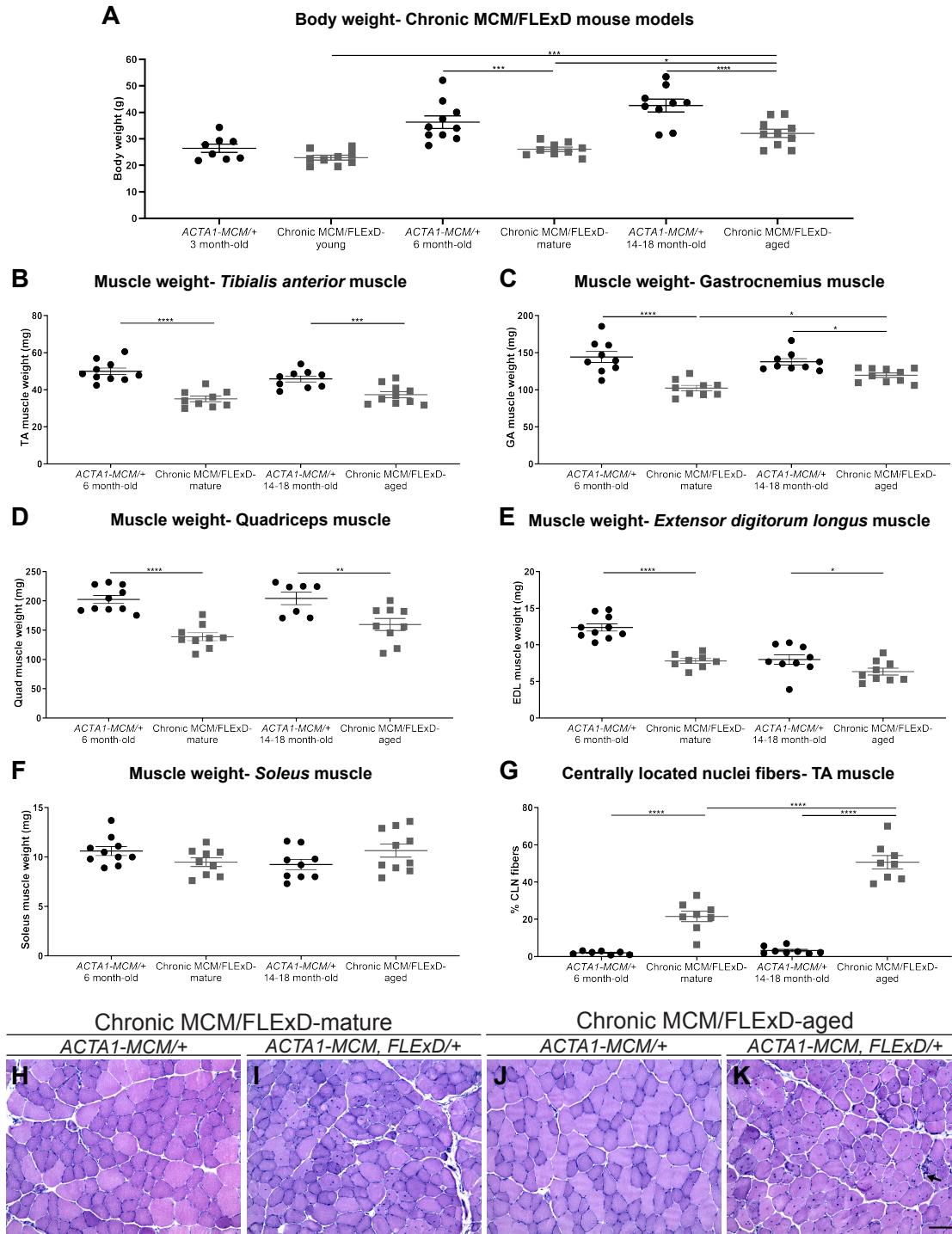


Fig. S2. Body and muscle weight of skeletal muscles and histopathology analysis of *tibialis anterior* muscle. (A) Body weight of chronic MCM/FLExD mouse models. Statistical analysis was performed with two tailed one-way ANOVA (uncorrected Fisher's LSD) with an n=8 animals for *ACTAI-MCM/+* 1 month-old, n=9 animals for *ACTAI-MCM;FLExD/+* 1 month-old, n=10 animals for *ACTAI-MCM/+* 6 months-old, n=9 animals for chronic MCM/FLExD-mature, n=9 animals for *ACTAI-MCM/+* 14-18 months-old and n=10 animals for chronic MCM/FLExD-aged experimental groups. (B-F) Muscle weight of *tibialis anterior* (n=10 animals for *ACTAI-MCM/+* 6 months-old, n=9 animals for chronic MCM/FLExD-mature, n=9 animals for *ACTAI-MCM/+* 14-18 months-old and n=10 animals for chronic MCM/FLExD-aged experimental groups) (B), gastrocnemius (n=9 animals for *ACTAI-MCM/+* 6 months-old, n=9 animals for chronic MCM/FLExD-mature, n=9 animals for *ACTAI-MCM/+* 14-18 months-old and n=10 animals for chronic MCM/FLExD-aged experimental groups) (C), quadriceps (n=9 animals for *ACTAI-MCM/+* 6 months-old, n=9 animals for chronic MCM/FLExD-mature, n=7 animals for *ACTAI-MCM/+* 14-18 months-old and n=9 animals for chronic MCM/FLExD-aged experimental groups) (D), *extensor digitorum longus* (n=10 animals for *ACTAI-MCM/+* 6 months-old, n=8 animals for chronic MCM/FLExD-mature, n=9 animals for *ACTAI-MCM/+* 14-18 months-old and n=9 animals for chronic MCM/FLExD-aged experimental groups) (E) and *soleus* (n=10 animals for *ACTAI-MCM/+* 6 months-old, n=9 animals for chronic MCM/FLExD-mature, n=9 animals for *ACTAI-MCM/+* 14-18 months-old and n=10 animals for chronic MCM/FLExD-aged experimental groups) (F) muscles. Statistical analysis was performed with two tailed one-way ANOVA (uncorrected Fisher's LSD). (G) Centrally located nuclei fibers quantification in *tibialis anterior* muscle. Statistical analysis was performed with two tailed one-way ANOVA (uncorrected Fisher's LSD) with an n=7 animals for *ACTAI-MCM/+* 6 months-old, n=8 animals for chronic MCM/FLExD-mature, n=8 animals for *ACTAI-MCM/+* 14-18 months-old and n=8 animals for chronic MCM/FLExD-aged experimental groups. Data is presented as mean \pm s.e.m.; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (H-K) Hematoxylin and Eosin staining of *tibialis anterior* (TA) muscles from (H, I) chronic MCM/FLExD-mature mice and age matched 6 month-old *ACTAI-MCM/+* controls and (J, K) chronic MCM/FLExD-mature mice and age matched 14-18 months-old *ACTAI-MCM/+* controls. Black arrow (K) depicts a damaged fiber with immune infiltrate. Representative images from n=3 animals for each experimental group Bar = 50 μ m.

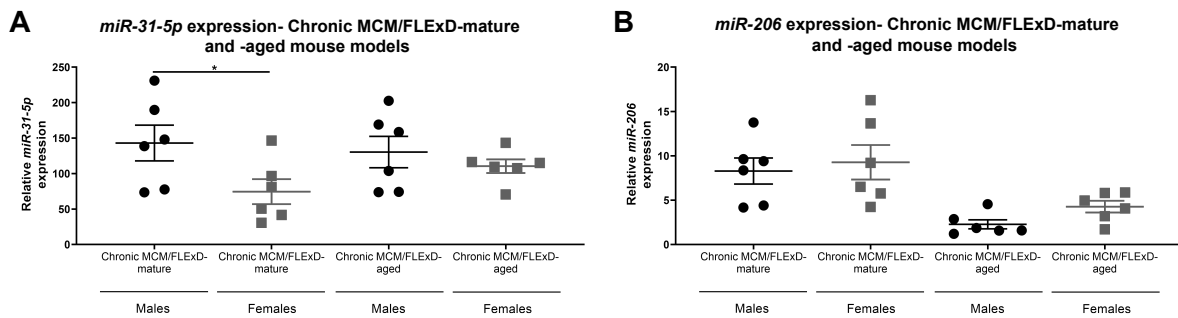


Fig. S3. miRNA expression in the chronic DUX4 mouse models. (A) *miR-31-5p* expression in the gastrocnemius muscle from male and female chronic MCM/FLEXD-mature and -aged mouse models. (B) *miR-206* expression in the gastrocnemius muscle from male and female chronic MCM/FLEXD-mature and -aged mouse models. Statistical analysis was performed with two-tailed one-way ANOVA (uncorrected Fisher's LSD) with an n=6 animals for chronic MCM/FLEXD-mature and aged experimental groups. Data is presented as mean ± s.e.m.; * p<0.05.

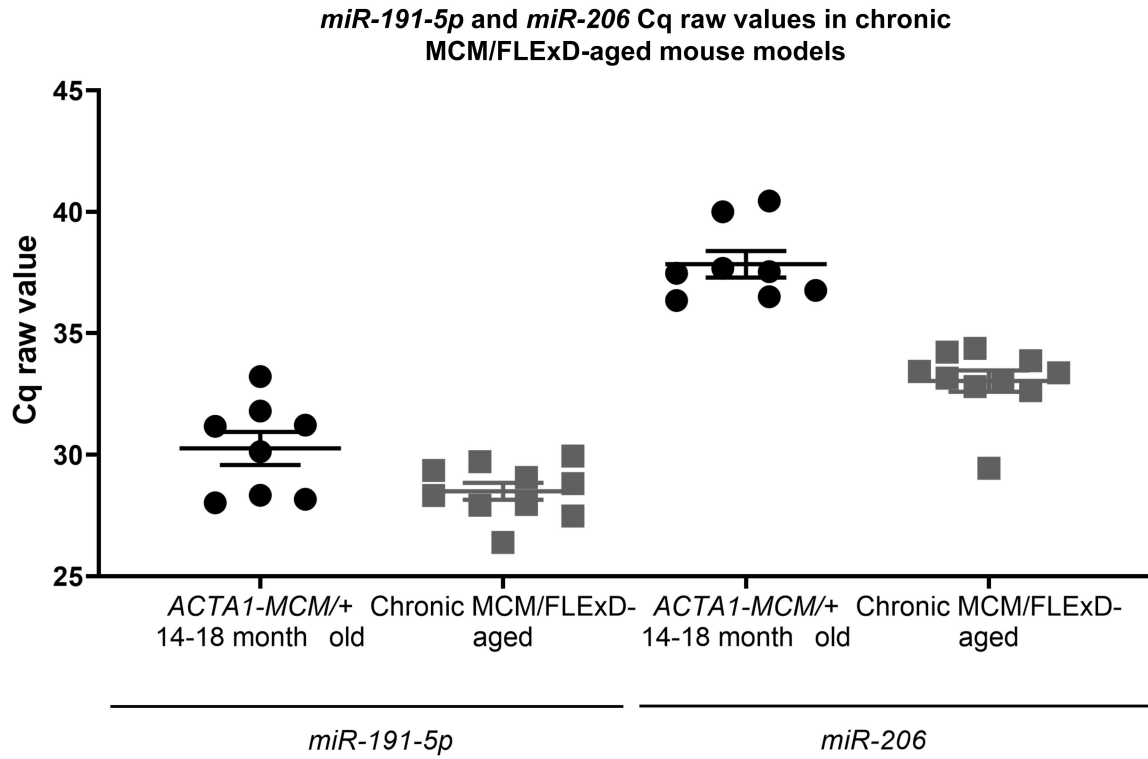


Fig. S4. Serum analysis of *miR-191-5p* and *miR-206* expression in the chronic MCM/FLExD-aged mouse model. Cq raw values for *miR-191-5p* and *miR-206* expression in the serum from *ACTA1-MCM/+* 14-18-month-old and chronic MCM/FLExD-aged mice.

Table S1. Fold change relative expression raw data for miScript analysis. Raw fold change values for relative expression in the miRNA profile panel. Red boxes: Statistically significant upregulation, fold change above a 2-fold threshold. Blue boxes: Statistically significant downregulation, fold change below 0.5-fold change. Gray boxes: Statistically significant fold changes above or below the defined analysis threshold.

miRNA ID	MD3	MD9	1 month old	6 months old	14-18 months old
<i>mmu-let-7a-5p</i>	0.73	0.76	0.81	0.85	1.01
<i>mmu-let-7c-5p</i>	0.96	1.12	1.52	1.02	1.04
<i>mmu-let-7d-5p</i>	0.67	0.60	0.66	0.55	0.58
<i>mmu-let-7e-5p</i>	0.89	0.73	0.90	1.10	0.90
<i>mmu-let-7g-5p</i>	0.85	0.75	0.74	0.84	0.72
<i>mmu-miR-103-3p</i>	0.65	0.82	0.77	0.92	0.93
<i>mmu-miR-105</i>	0.67	0.81	0.85	0.84	0.83
<i>mmu-miR-106a-5p</i>	0.56	0.85	0.61	0.55	0.50
<i>mmu-miR-125a-5p</i>	0.93	1.27	1.00	0.93	0.96
<i>mmu-miR-125b-5p</i>	0.87	1.02	0.98	1.19	0.97
<i>mmu-miR-126a-3p</i>	0.79	0.95	0.78	0.76	0.62
<i>mmu-miR-129-5p</i>	0.61	0.93	1.05	0.94	1.78
<i>mmu-miR-130a-3p</i>	0.71	0.94	0.69	0.99	0.78
<i>mmu-miR-132-3p</i>	0.99	1.32	1.18	1.51	1.15
<i>mmu-miR-134-5p</i>	0.74	1.17	0.72	2.52	1.00
<i>mmu-miR-135a-5p</i>	0.96	1.99	0.84	1.15	0.75
<i>mmu-miR-135b-5p</i>	1.02	1.67	0.98	0.96	0.40
<i>mmu-miR-138-5p</i>	0.93	0.98	1.18	1.35	0.67
<i>mmu-miR-140-5p</i>	0.85	0.86	0.76	0.90	0.76
<i>mmu-miR-142a-3p</i>	1.40	2.80	1.21	2.02	2.84
<i>mmu-miR-142a-5p</i>	1.53	4.50	1.69	0.87	3.46
<i>mmu-miR-145a-5p</i>	0.45	0.59	0.56	0.58	0.42
<i>mmu-miR-146a-5p</i>	2.74	2.19	1.85	2.75	3.31
<i>mmu-miR-146b-5p</i>	1.01	1.03	0.91	1.47	1.54
<i>mmu-miR-147-3p</i>	0.85	1.26	1.10	0.95	1.37
<i>mmu-miR-148a-3p</i>	0.72	0.95	0.67	1.17	2.14
<i>mmu-miR-149-5p</i>	0.54	0.49	0.60	0.84	0.74
<i>mmu-miR-150-5p</i>	0.71	0.56	0.51	0.74	0.97
<i>mmu-miR-152-3p</i>	0.97	1.19	0.96	0.83	0.59
<i>mmu-miR-155-5p</i>	1.17	1.07	0.82	1.17	1.70
<i>mmu-miR-15a-5p</i>	0.41	0.82	0.48	0.92	0.70
<i>mmu-miR-15b-5p</i>	1.15	1.32	0.91	1.35	2.01
<i>mmu-miR-16-5p</i>	0.44	0.89	0.65	1.05	0.83
<i>mmu-miR-181a-5p</i>	0.53	0.53	0.71	0.47	0.71
<i>mmu-miR-182-5p</i>	3.68	7.36	3.42	2.03	3.82
<i>mmu-miR-183-5p</i>	4.98	4.97	4.19	3.87	11.10
<i>mmu-miR-184-3p</i>	0.78	0.96	0.75	1.00	0.98
<i>mmu-miR-185-5p</i>	0.46	0.48	0.43	0.35	0.31
<i>mmu-miR-186-5p</i>	0.50	0.67	0.65	0.59	0.72
<i>mmu-miR-187-3p</i>	0.76	1.31	1.00	1.03	0.65
<i>mmu-miR-18a-5p</i>	1.00	2.18	1.28	1.36	1.42
<i>mmu-miR-18b-5p</i>	0.67	0.77	0.64	0.84	0.86
<i>mmu-miR-191-5p</i>	0.72	0.70	0.65	0.73	0.91
<i>mmu-miR-194-5p</i>	0.74	0.74	0.67	0.80	1.04
<i>mmu-miR-195a-5p</i>	0.80	0.86	0.67	0.95	0.89
<i>mmu-miR-196a-5p</i>	0.63	0.61	0.55	0.67	0.55
<i>mmu-miR-19a-3p</i>	0.36	0.71	0.48	0.72	0.70
<i>mmu-miR-19b-3p</i>	0.37	0.76	0.46	0.49	0.42
<i>mmu-miR-200a-3p</i>	0.76	0.90	0.68	0.84	1.00
<i>mmu-miR-203-3p</i>	1.27	1.47	0.97	1.34	2.31
<i>mmu-miR-205-5p</i>	0.61	1.05	0.92	0.66	1.62
<i>mmu-miR-207</i>	0.67	1.10	1.49	0.58	0.44
<i>mmu-miR-20a-5p</i>	0.46	0.76	0.55	1.00	0.73
<i>mmu-miR-20b-5p</i>	0.63	0.87	0.56	0.80	0.68
<i>mmu-miR-21a-5p</i>	1.17	1.61	0.96	1.15	1.42
<i>mmu-miR-210-3p</i>	0.57	0.96	0.75	0.80	0.83
<i>mmu-miR-214-3p</i>	1.00	1.48	1.25	1.88	1.05
<i>mmu-miR-223-3p</i>	1.36	1.99	1.44	2.41	2.92
<i>mmu-miR-23b-3p</i>	0.86	0.80	0.91	0.97	0.88
<i>mmu-miR-26a-5p</i>	0.53	0.57	0.53	0.74	0.60
<i>mmu-miR-26b-5p</i>	0.60	0.57	0.51	0.61	0.60
<i>mmu-miR-27a-3p</i>	0.81	1.18	0.99	1.09	1.14
<i>mmu-miR-27b-3p</i>	0.78	1.03	0.90	1.05	1.10
<i>mmu-miR-28a-5p</i> <i>mmu-miR-28c</i>	0.58	0.69	0.57	0.98	0.53
<i>mmu-miR-298-5p</i>	1.72	3.04	3.03	2.08	1.57
<i>mmu-miR-299a-3p</i>	0.48	0.85	0.70	0.90	1.07
<i>mmu-miR-29b-3p</i>	0.39	0.32	0.36	0.28	0.35
<i>mmu-miR-29c-3p</i>	0.52	0.47	0.49	0.57	0.67
<i>mmu-miR-30b-5p</i>	0.46	0.53	0.42	0.64	0.60
<i>mmu-miR-30c-5p</i>	0.47	0.52	0.50	0.66	0.59
<i>mmu-miR-30e-5p</i>	0.33	0.55	0.54	0.60	0.52
<i>mmu-miR-31-5p</i>	5.51	7.56	8.06	13.78	95.20
<i>mmu-miR-320-3p</i>	0.48	0.70	0.68	0.87	0.63
<i>mmu-miR-325-3p</i>	0.78	0.86	0.61	0.85	0.79
<i>mmu-miR-335-5p</i>	1.13	1.84	0.79	1.61	2.42
<i>mmu-miR-34a-5p</i>	1.54	1.62	1.44	1.23	0.94
<i>mmu-miR-34c-5p</i>	8.31	8.97	8.86	1.98	9.73
<i>mmu-miR-383-5p</i>	1.05	0.84	2.69	1.19	0.58
<i>mmu-miR-409-3p</i>	1.01	1.55	1.30	1.12	0.69
<i>mmu-miR-451a</i>	0.41	0.67	0.47	0.72	0.35
<i>mmu-miR-493-3p</i>	1.08	1.38	2.21	0.75	0.73
<i>mmu-miR-574-3p</i>	0.99	1.29	0.92	1.14	0.92
<i>mmu-miR-9-5p</i>	0.95	0.95	0.75	1.22	1.05
<i>mmu-miR-98-5p</i>	0.81	0.82	0.68	0.53	0.70

Table S2. Participant information. DRA = D4Z4 reduced allele. NA = Not applicable. Shortest chromosome 4qA D4Z4 EcoRI/BlnI fragment is >41kb and therefore not FSHD1.

Subject ID	Gender	Age	Status	DRA (EcoRI/BlnI)
1	Male	78	Clinically affected FSHD1	19kb
2	Female	31	Clinically affected FSHD1	18kb
3	Male	60	Clinically affected FSHD1	22kb
4	Male	53	Clinically affected FSHD1	26kb
5	Male	79	Clinically affected FSHD1	34kb
6	Male	40	Clinically affected FSHD1	22kb
7	Male	48	Clinically affected FSHD1	22kb
8	Female	51	Clinically affected FSHD1	16kb
9	Male	17	Clinically affected FSHD1	16kb
10	Male	32	Clinically affected FSHD1	24kb
11	Male	56	Clinically affected FSHD1	26kb
12	Female	72	Clinically affected FSHD1	27kb
13	Female	72	Clinically affected FSHD1	27kb
14	Male	30	Clinically affected FSHD1	27kb
15	Male	59	Clinically affected FSHD2	42kb
16	Male	51	Healthy	NA
17	Male	37	Healthy	NA
18	Female	68	Healthy	NA
19	Female	52	Healthy	NA
20	Female	57	Healthy	NA
21	Male	43	Healthy	NA
22	Male	66	Healthy	NA
23	Male	81	Healthy	NA
24	Male	47	Healthy	NA
25	Male	52	Healthy	NA
26	Female	36	Healthy	NA
27	Female	32	Healthy	NA
28	Female	24	Healthy	NA
29	Male	77	Healthy	NA
30	Male	73	Healthy	NA