Supporting Information for
Sarcopenia phenotype and impaired muscle function in male mice with fast-twitch muscle-specific knockout of the androgen receptor

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Other supporting materials for this manuscript include the following:

Dataset S1

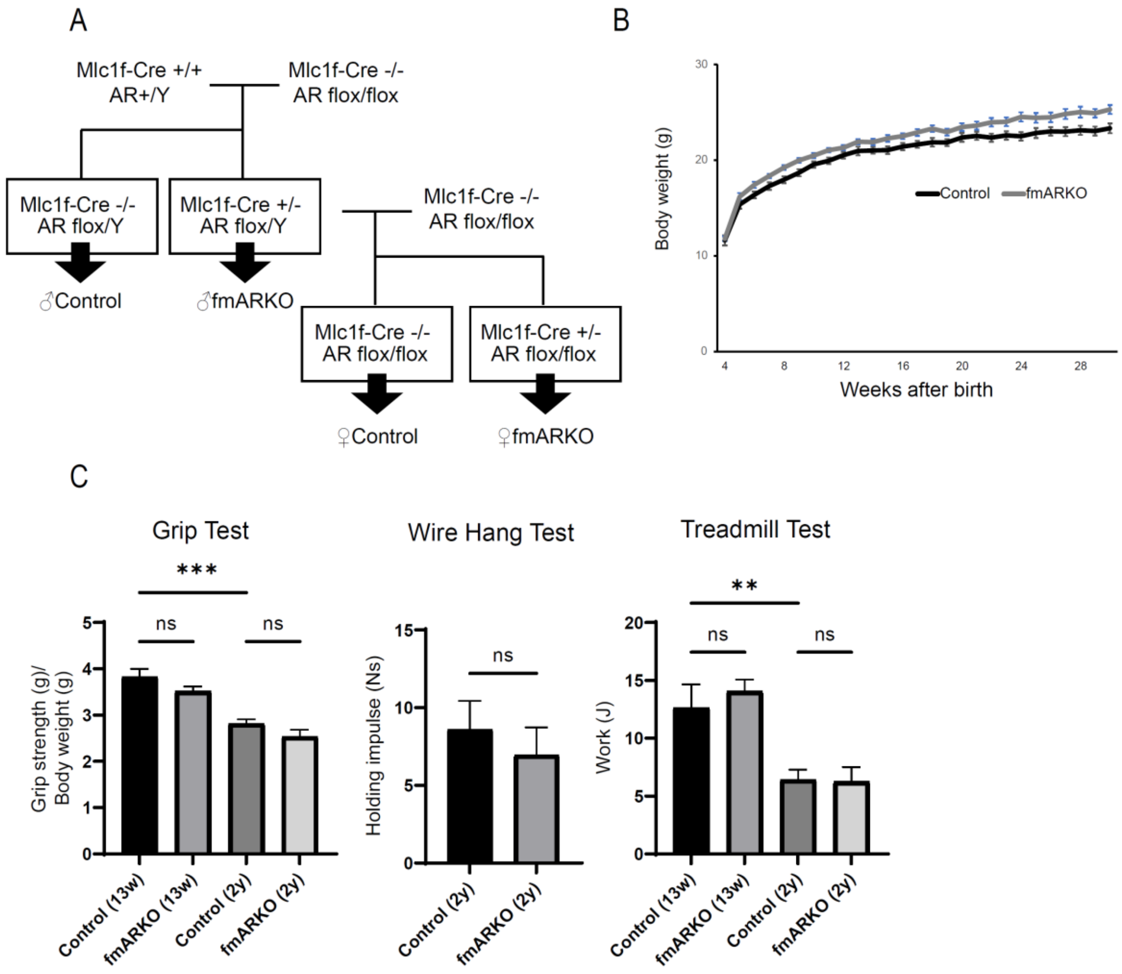

Fig. S1. Phenotypes observed in female fmARKO mice.

(A) The strategy used to generate Mlc1f-Cre+/-/ARflox/flox mice (referred to as female fmARKO mice).

(B) Growth curves of control and fmARKO females (n=13-14/group).

(C) The results of grip test, wire hang test and treadmill test were compared between control and fmARKO females at 13-wk-old or 2-yr-old. (n=5-13/group).

Error bars indicate SEM; $^{**}p<0.01$, $^{***}p< 0.001$

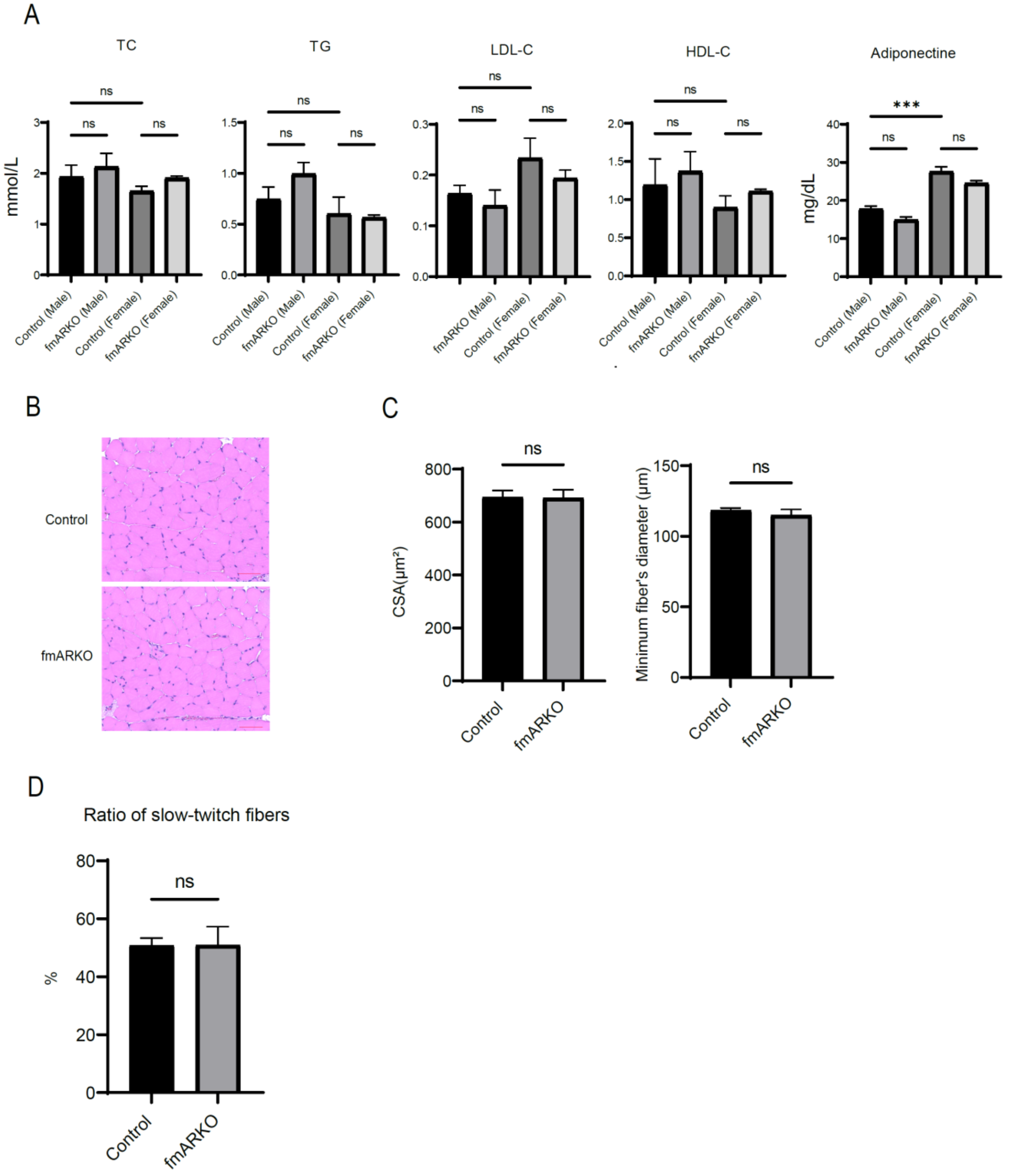

Fig. S2. Histological analysis of skeletal muscles in female fmARKO mice.

(A) Serum lipid levels; total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and adiponectin showed no difference between control and fmARKO mice. (n=3/group)

(B, C) Histological analysis of H&E-stained soleus muscles of 13-week-old control and fmARKO female mice (scale bar, 50 μm). Mean fiber cross-sectional area and minimum fiber's diameter are shown (n=3/group).

(D) The mean ratios of slow-twitch fibers in control and fmARKO females (n=3/group).

Error bars indicate SEM; ***$p < 0.001$

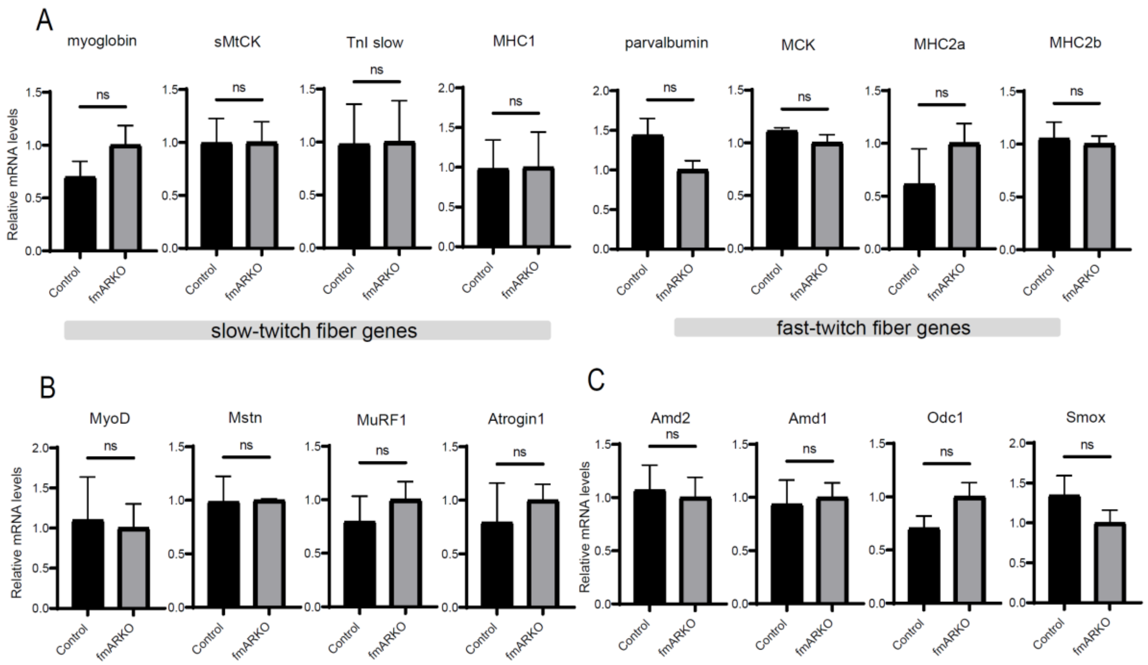

Fig. S3. The gene expression differences observed in male fmARKO mice were not observed in female fmARKO mice.

(A-C) Real-time PCR was used to measure gene expression of (A) fiber type-specific transcripts, (B) muscle mass-related transcripts, and (C) polyamine biosynthesis-related transcripts (n=3/group).

Error bars indicate SEM

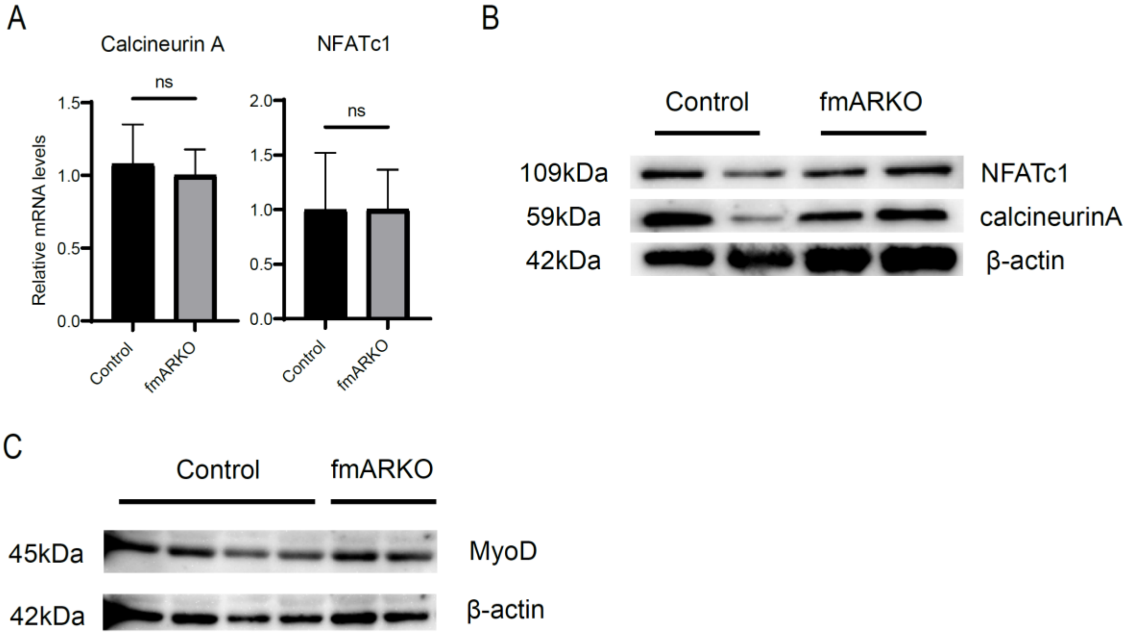

Fig. S4. The shift in muscle fiber type observed in fmARKO mice was independent of the Calcineurin A/nuclear factor of activated T cells cytoplasmic 1 pathway in the translational level.

(A) Real-time PCR showed no change in *CnA* and *NFATc1* gene expression in the gastrocnemius muscles of mice after androgen receptor knockout (n=6/group).

(B) The protein levels of *CnA* and *NFATc1* in control and fmARKO mice were measured (n=2/group).

(C) The protein levels of *MyoD* in control and fmARKO mice were measured (n=2-4/group).

Error bars indicate SEM

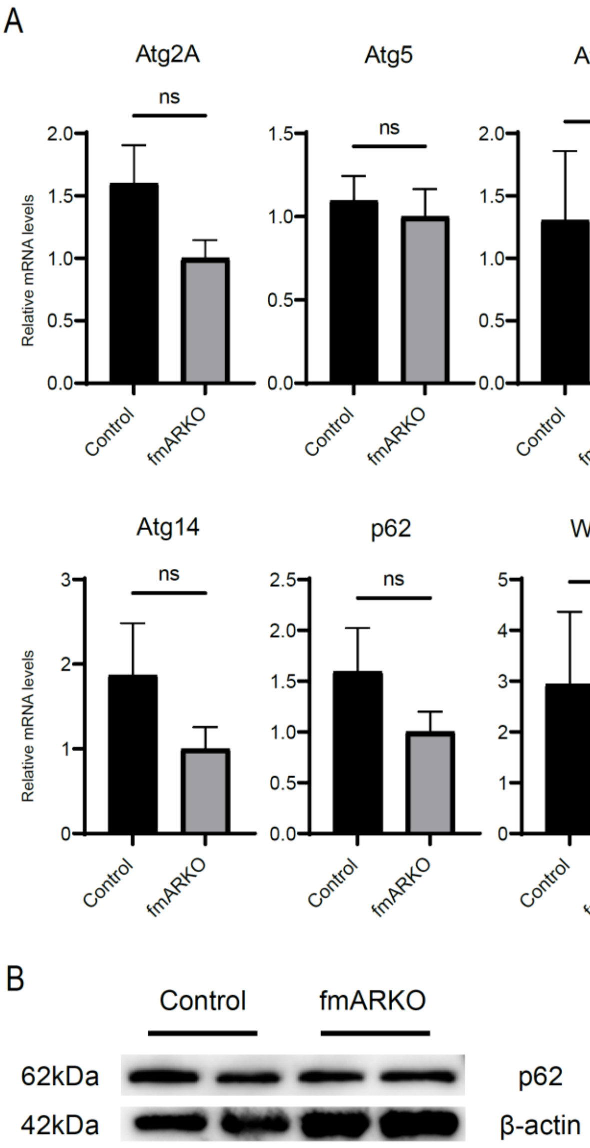

Fig. S5. Autophagy in fmARKO mice.

(A) Real-time PCR was used to measure the gene expression levels of autophagy-related genes (n=6/group).

(B) The protein levels of *p62* in control and fmARKO mice (n=2/group).

Error bars indicate SEM

Table S1. Total body weight and hind limb muscle, and heart mass (mg) normalized by body weight (g) in female mice

	13wk		2yr	
	Control (n=5)	fmARKO (n=5)	Control (n=7)	fmARKO (n=9)
Total body weight (g)	21.2 ± 1.0	21.7 ± 0.6	27.7 ± 1.5	29.8 ± 1.0
Gastrocnemius (mg/g)	5.5 ± 0.04	5.4 ± 0.1	4.1 ± 0.2	3.9 ± 0.2
Soleus (mg/g)	0.35 ± 0.02	0.37 ± 0.02	0.24 ± 0.01	0.24 ± 0.01
EDL (mg/g)	0.43 ± 0.01	0.45 ± 0.02	0.30 ± 0.01	0.30 ± 0.01
Tibialis (mg/g)	2.9 ± 0.2	2.9 ± 0.2	1.6 ± 0.04	1.6 ± 0.1
Heart (mg/g)	5.4 ± 0.2	5.5 ± 0.2	5.7 ± 0.3	5.6 ± 0.3

Data are expressed ± SEM

Table S2. List of primers used in this study

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
36B4	GTTCAGCATGTTCAGCAGTGTG	AATCTCCAGAGGCACCATTGA
AR	GGACAGTACCAGGGACCATG	TCCGTAGTGACAGCCAGAAG
myoglobin	GCACAAGATCCCGGTCAAGTACCTGGAG	CTGACGAAGGCCACTTTGCACCTCTG
TnI slow	TGCCGGAAGTTGAGAGGAAATCCAAGAT	CCAGCACCTTCAGCTTCAGGTCCTTGAT
sMtCK	GATGGCCAGTGCCTTCTCAAAGTTGCTA	TGTCCACTCCAGTTTGGATGCACTGGTC
MHC1	GCCAACTATGCTGGAGCTGATGCCC	GGTGCGTGGAGCGCAAGTTTGTCATAAG
parvalbumin	GCAGGATGTCGATGACAGACGTGCTCAG	CCACTTACGTTTCAGCCACCAGAGTGGA
MCK	GATGTCATCCAGACTGGGGTGGACAACC	TGAACTCGCCCGTCAGGCTGTTGAG
MHC2a	TGACAACTCGTCTCGCTTTG	AAGTGACCCGGGACTTCTCT
MHC2b	CGAAGGCGGAGCTACGGTCA	CGGCAGCCACTTGTAGGGGT
MyoD	AGCACTACAGTGGCGACTCAG	AGGCGGTGTCGTAGCCATTC
Myostatin	CTGTAACCTTCCCAGGACCA	TCTTTTGGGTGCGATAATCC
MuRF1	TAACTGCATCTCCATGCTGGTG	TGGCGTAGAGGGTGTCAAACTT
Atrogin1	GTCGCAGCCAAGAAGAGAAAGA	TGCTATCAGCTCCAACAGCCTT
Amd1	GGATCTGGGGATCTTCGTACC	CTGCTTGTCAGTCTTTGTCACAC
Amd2	TCACGTTCAACTTTCGCTCACG	AGCGAACAACCAACAACCACAG
Odc1	GCAGTCAACATCATTGCCAA	TGTTCATTTGACTCATCTTCATCGT
Smox	CACAGAGAGCTCCAAGACAGC	GGGCACTTGGATGGTAAAAG
CnA (PPP3CA)	CAACACTCGCTACCTCTTC	CCATACAGGCGTCATAAA
NFATc1	CCTTCGGAAGGGTGCCTTTT	AGGCGTGGGGCCTCAGCAGG
Atg2A	CTATCTGTTCCCAGGTGAACGGAGTGG	CTGGATGCAGCTGTGTCACGATGG
Atg5	GACAAAGATGTGCTTCGAGATGTG	GTAGCTCAGATGCTCGCTCAG
Atg7	CCTGCACAACACCAACACAC	CACCTGACTTTATGGCTTCCC
Atg9A	CGGAATTCATGGCACAGTTTGACACTGA	GGGAAGCTTCTATACCTTGTGCACTTG
Atg12	AATCCGGAAAGCAGCTGTGTTG	TTGCAGTGCAGTCTAGTTGTGG
Atg14	GAGGGCCTTTACGTGGCTG	AATAGACGAAATCACCGCTCTG
p62	CCCAGTGTCTTGGCATTCTT	AGGGAAAGCAGAGGAAGCTC
WIPI2	CCAGGATAACACGTCCCTAGCTGTTGG	CTCTCCACAATGCAGACATCTTCAGTGTCAG
ULK1	AAGTTCGAGTTCTCTCGCAAG	ACCTCCAGGTCGTGCTTCT
FIP200	GGAATCTCTGGTCAGGAAGTGC	GTCCAAGGCATACAGCCGATCT

Dataset S1 (separate file).

(Tab1) GO Term extracted (down in fmARKO mice)

(Tab2) List of down-regulated genes in fmARKO mice

(Tab3) GO Term extracted (up in fmARKO mice)

(Tab4) List of up-regulated genes in fmARKO mice