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The exerkin apelin reverses age-associated sarcopenia

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Supplementary Table 1

Table 1: Profile of sarcopenic subjects in MAPT cohort

	No Sarcopenia	Sarcopenia	<i>P</i> value
N	27	34	
Male/Female	8/19	10/24	
Age (years)	75.0 (73.0-78.0)	76.5 (73.7-80.2)	NS
Newman's index	2.200 (1.680-2.910)	-1.885 (-2.300-1.335)	<i>p</i> <0.0001
ASM/height ²	7.721 (7.364-8.583)	5.983 (5.541-6.441)	<i>p</i> <0.0001
BMI (kg/cm ²)	27.51 (24.30-29.41)	23.16 (21.51-26.17)	<i>p</i> =0.002
Total lean mass (kg)	46.35 (42.62-51.78)	35.28 (32.64-39.46)	<i>p</i> <0.0001
Glucose (g/L)	0.98 (0.88-1.06)	1.02 (0.92-1.19)	NS
Insulin (pg/ml)	188.6 (113.1-372.7)	192.1 (55.23-437.1)	NS
Apelin (pg/ml)	216.8 (201.1-246.4)	189.3 (170.5-199.9)	<i>p</i> <0.0001
Leptin (pg/ml)	1397 (636-2246)	1397 (559-2777)	NS
NGF (pg/ml)	2.202 (1.818-3.819)	2.796 (1.818-3.404)	NS
Interleukin-8 (pg/ml)	1.465 (0.866-1.628)	1.155 (0.967-1.972)	NS
Interleukin-6 (pg/ml)	0.66 (0.28-1.02)	0.60 (0.18-1.33)	NS
TNF α (pg/ml)	0.58 (0.47-0.87)	0.69 (0.51-1.25)	NS
MCP-1 (pg/ml)	106.7 (82.4-124.0)	92.3 (68.7-110.4)	NS
HGF (pg/ml)	196.7 (128.7-283.3)	189.3 (140.6-252.3)	NS

Newman index is calculated by dividing appendicular lean mass (aLM) by height and total fat mass. ASM: appendicular skeletal muscle mass. BMI: body mass index. NGF: nerve growth factor. TNF α : tumor necrosis factor. MCP-1: monocyte chemoattractant protein. HGF: hepatocyte growth factor. *P* value is calculated by two-tailed unpaired Student's t-test. NS corresponds to *P*>0.05

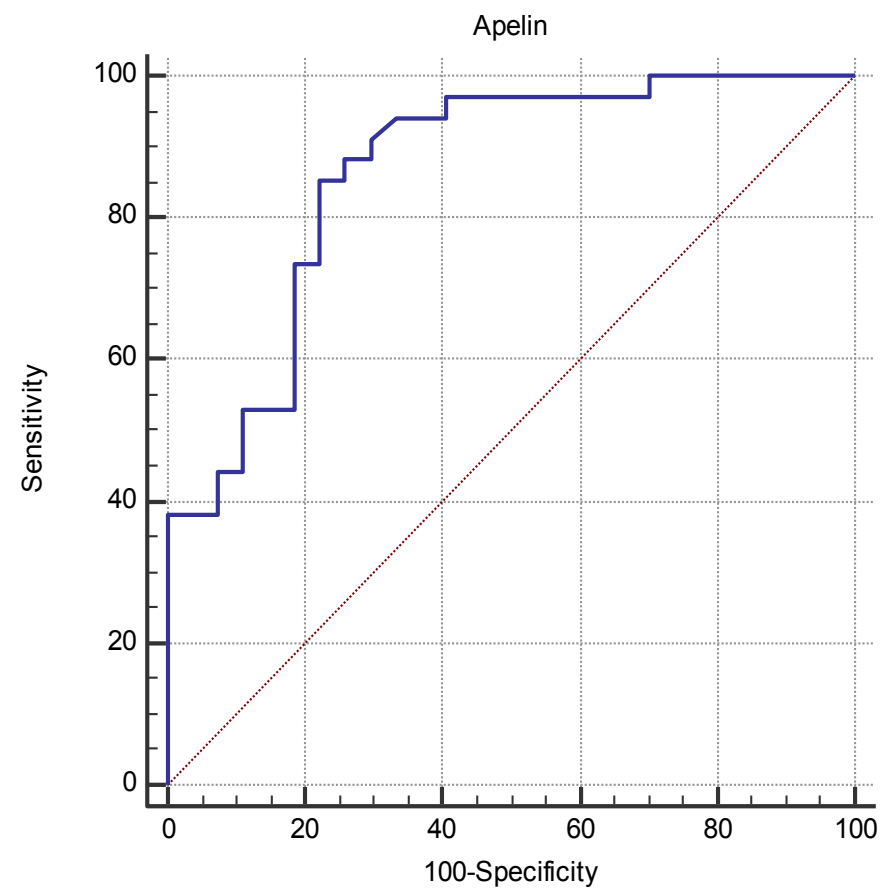
Supplementary Table 2

Table 2: Multivariate analysis between sarcopenic and non sarcopenic subjects

Parameter	P value	Odd ratio	95% CI	
			Inferior	Superior
Age	0.10	1.15	0.97	1.35
BMI	0.06	0.82	0.67	1.01
Sex	0.86	1.16	0.23	5.79
Pl. apelin	0.0008	0.93	0.89	0.97

Abbreviations: BMI: body mass index, CI: confident interval, Pl.: Plasma
Goodness of fit was 0.85 with the Hosmer-Lemeshow test. Area under the ROC curve (AUC) =0.86

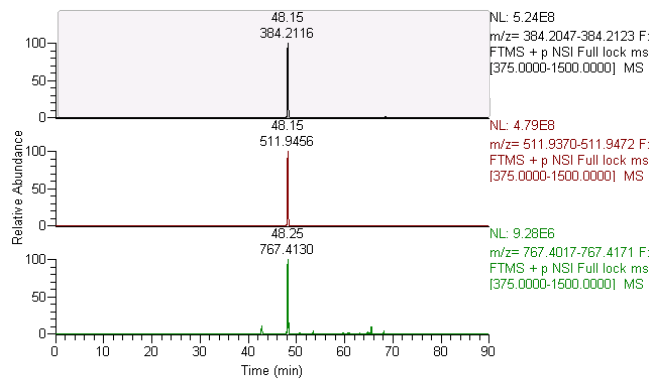
Supplementary Fig. 1



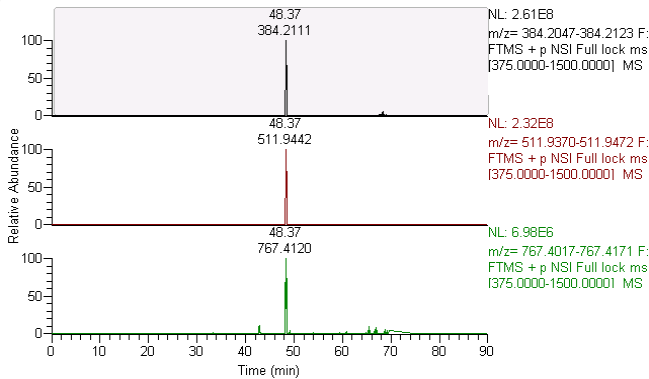
Criterion	Sensitivity	Specificity	+LR	-LR	+PV	-PV
≤200,426	85,29	77,78	3,84	0,19	82,9	80,8

Supplementary Fig. 2

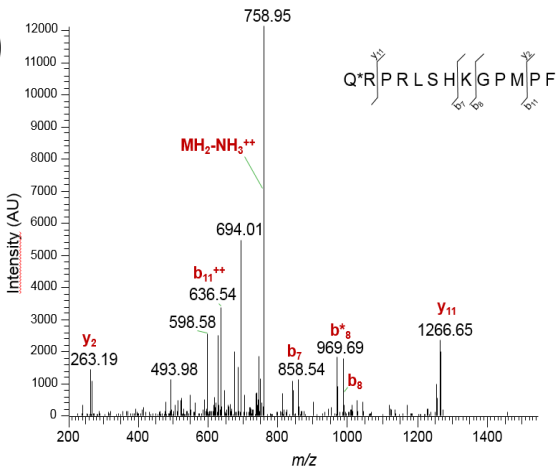
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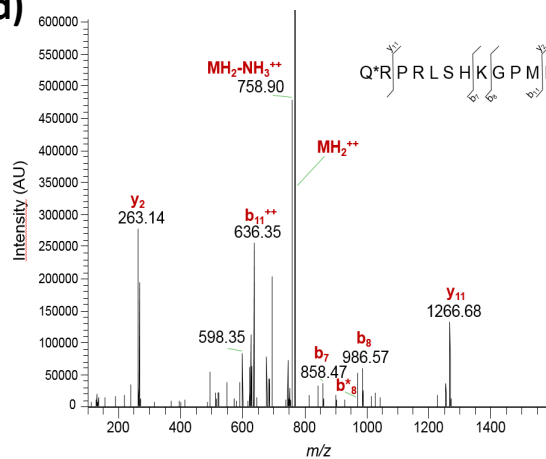
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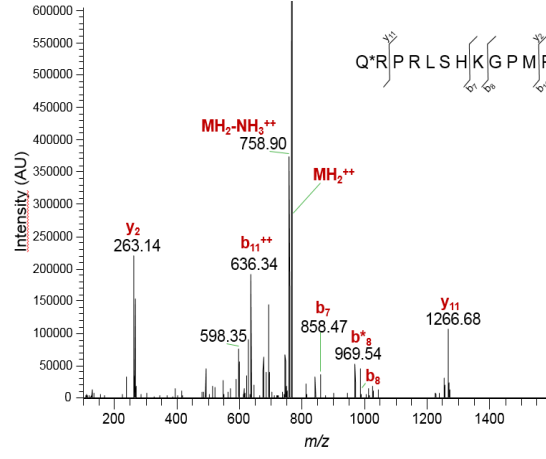
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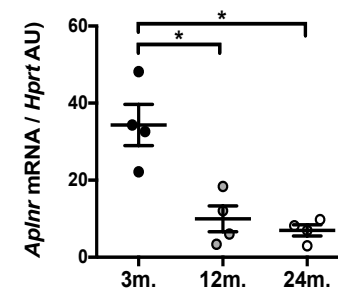
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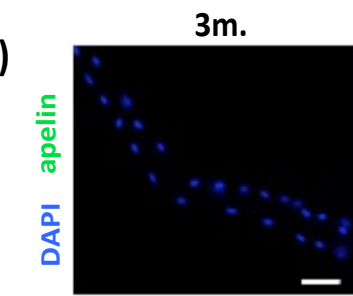
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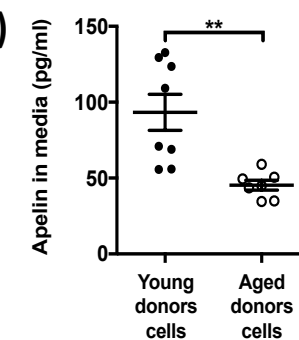
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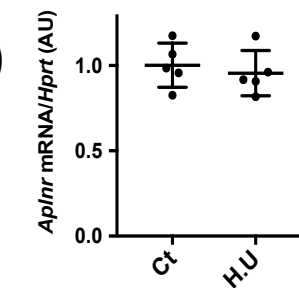
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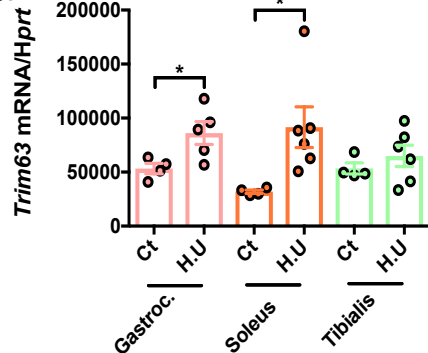
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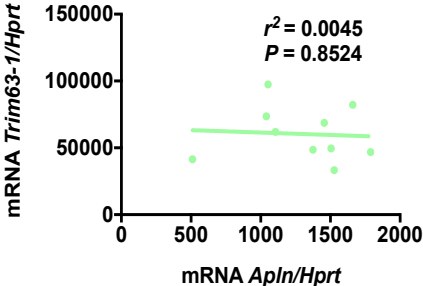
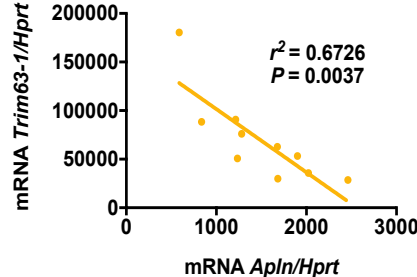
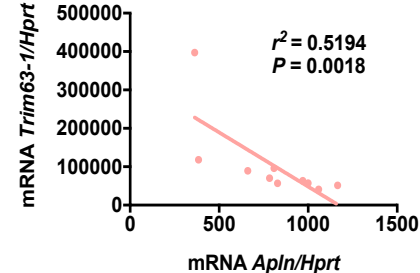
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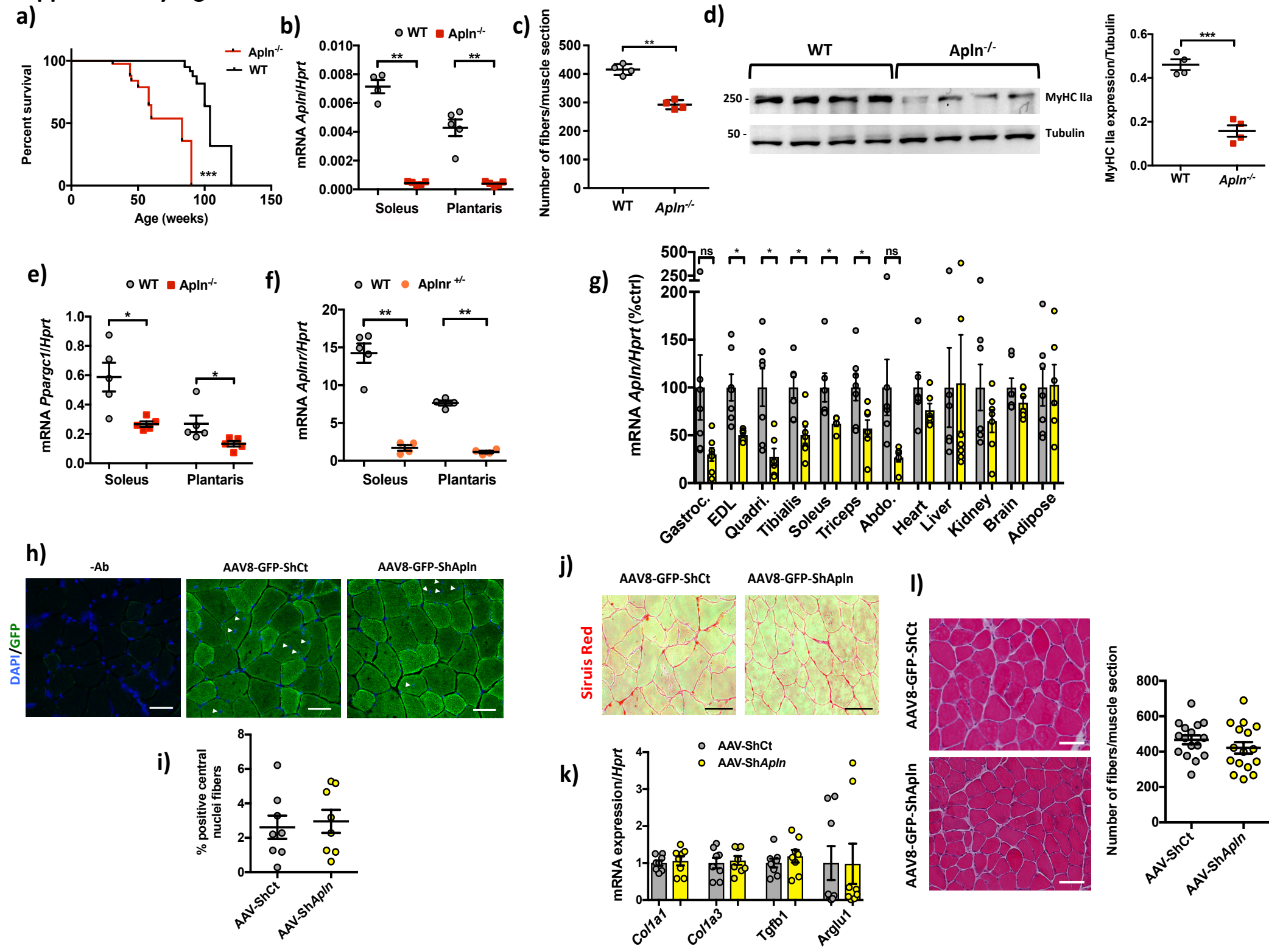
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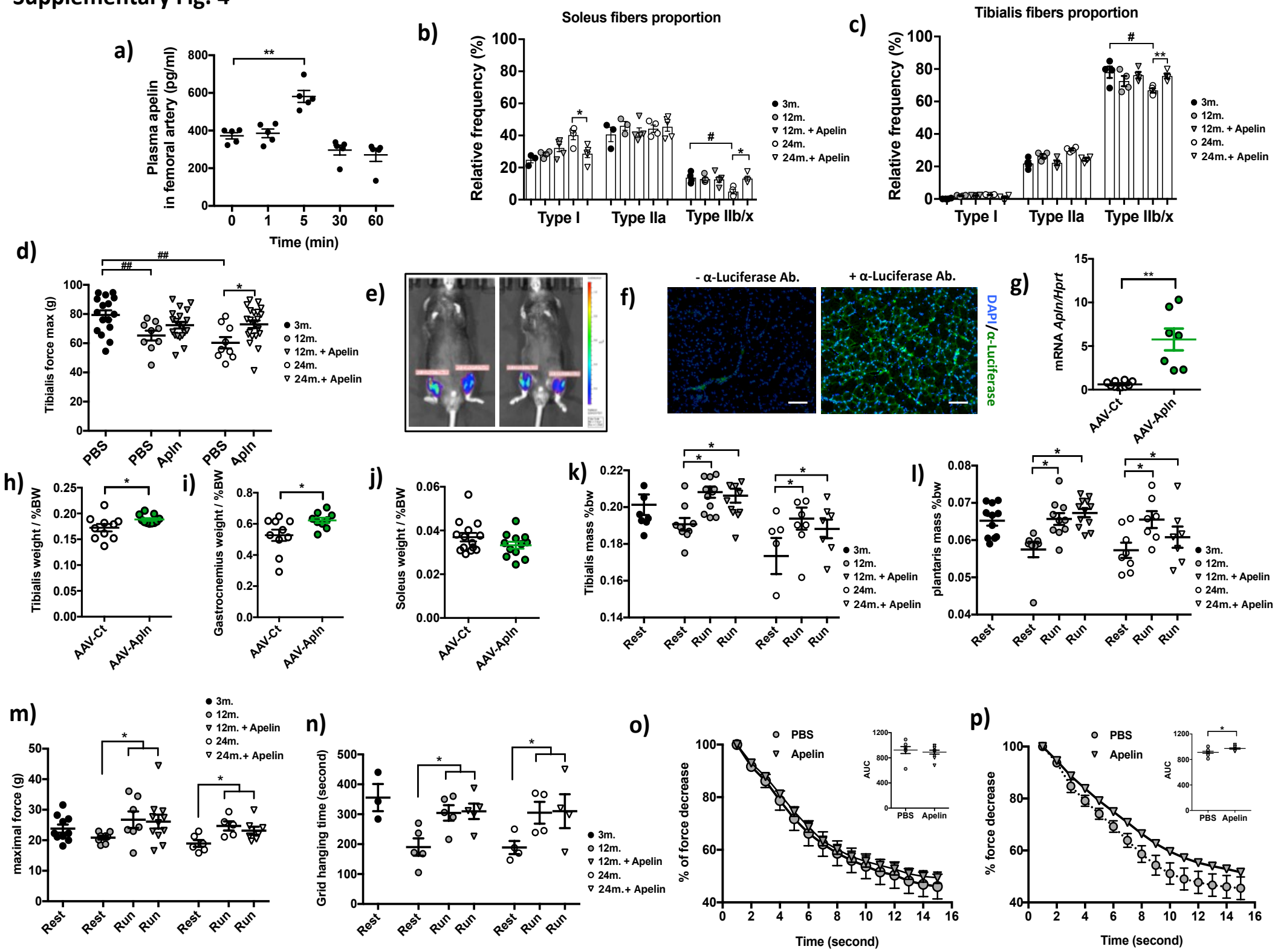
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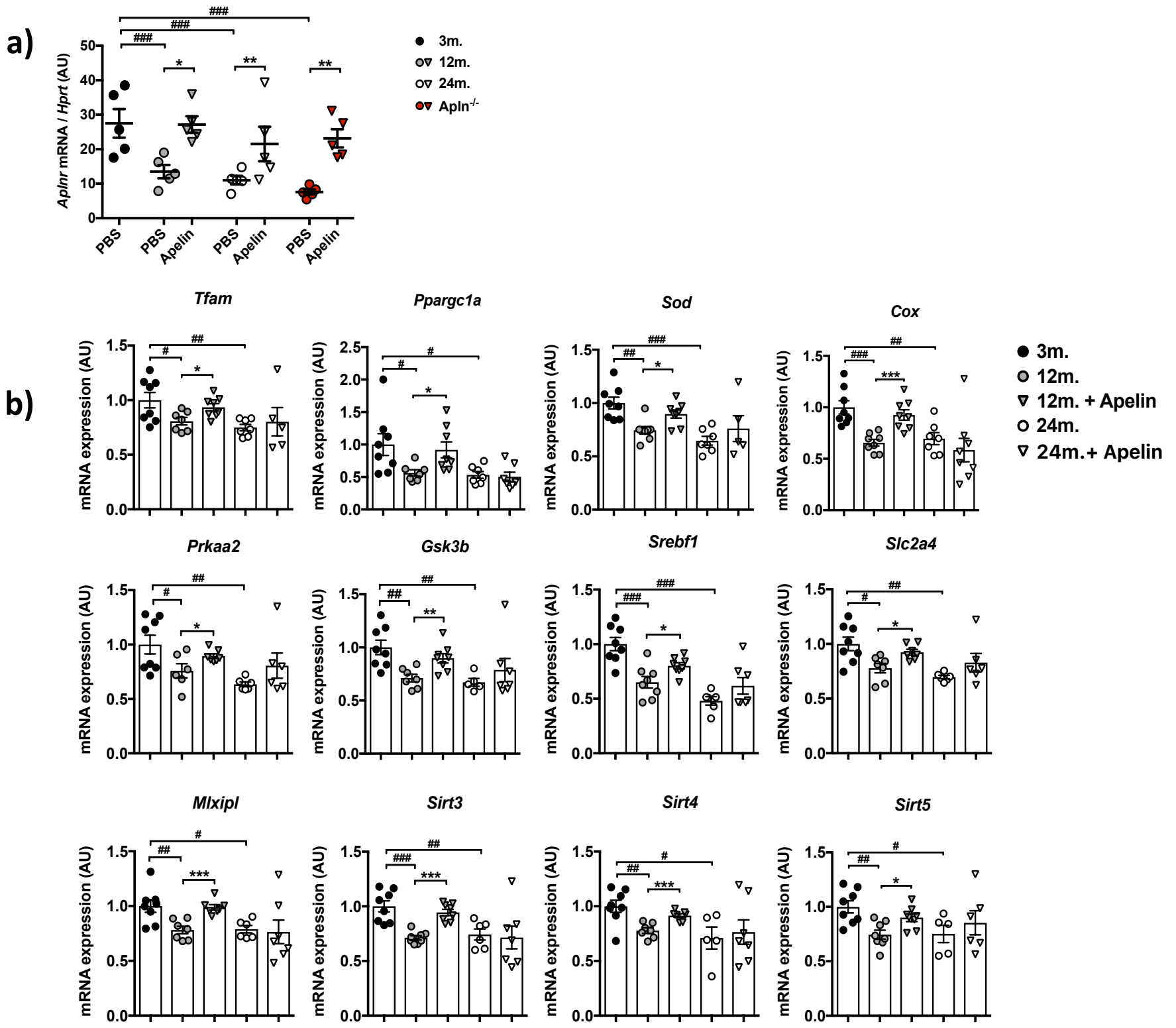
Supplementary Fig. 3



Supplementary Fig. 4

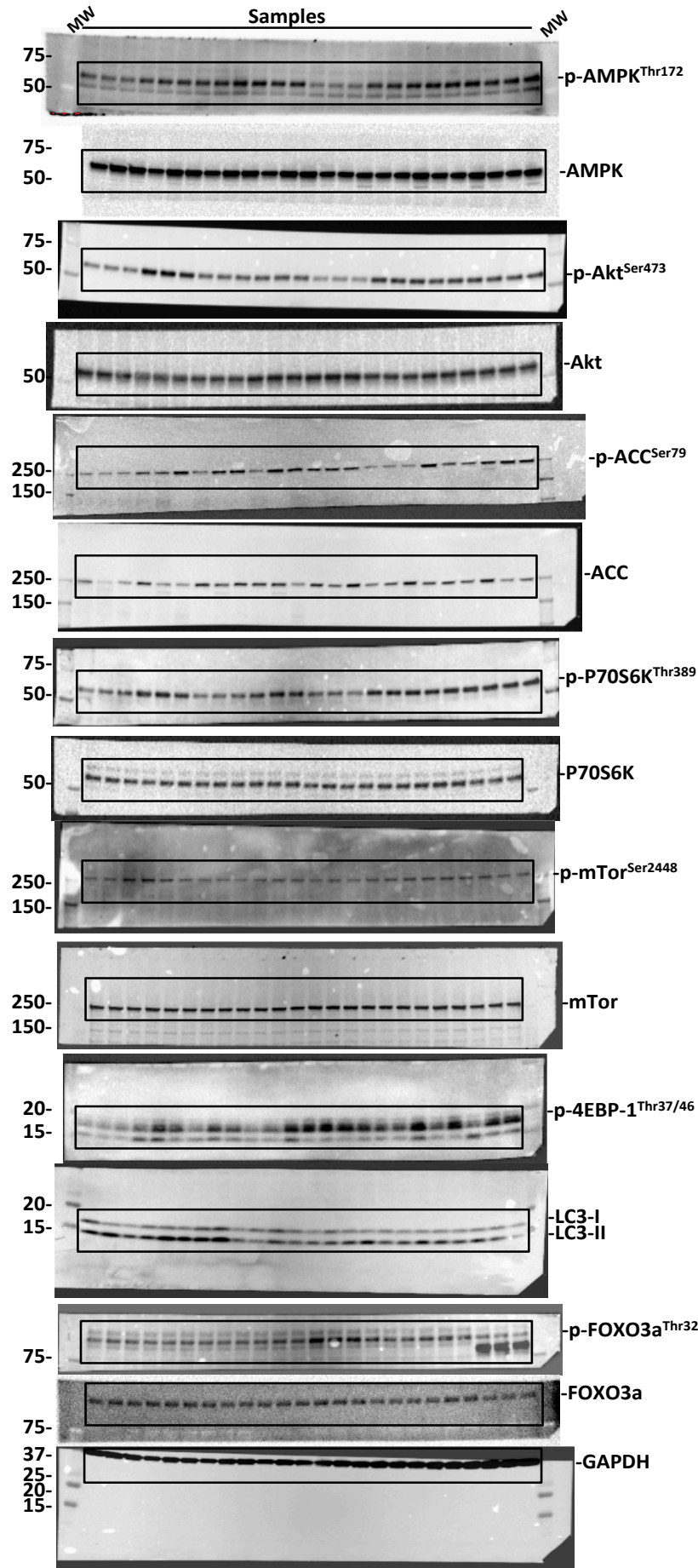


Supplementary Fig. 5

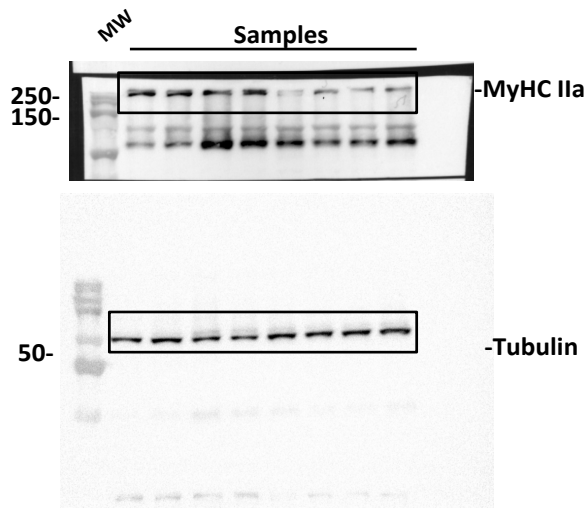


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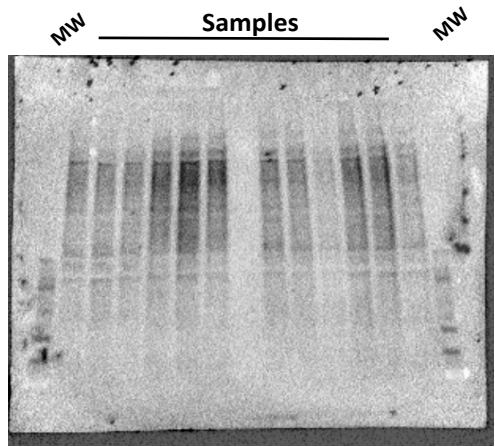
Uncropped gels of figure 4a



Uncropped gels of supplementary figure 3e



Uncropped gel of figure 4d



Supplementary Fig. 1 Receiver operating characteristic (ROC) curve for multivariate relationship between sarcopenia and plasma apelin immunoreactivity level measured in MAPT cohort (see **Supplementary Table 1**). The area under the ROC curve (AUC) $\pm$ standard error was 0.86. Goodness of fit was 0.85 with the Hosmer-Lemeshow test.

Supplementary Fig. 2 Apelin reduction is associated with aging and muscle atrophy. **(a-e)** [Pyr-1]Apelin 13 was analyzed by high-resolution nanoLC-MS/MS using an automated data dependent acquisition (DDA) mode. Extracted ion chromatograms (XIC) are displayed for 3 different charge states of [Pyr-1]apelin-13 (m/z at 384.2085 (4+), m/z at 511.9421 (3+), and m/z at 767.4094 (2+)) in serum samples collected from young **(a)** and aged **(b)** mice. The ratio “young/aged” of [Pyr-1]apelin-13 is calculated from the XIC by dividing the sum of the areas for the 3 different charge states in “young” and “aged” samples, respectively. It is equal to 1.9. **(c,d)** MS/MS spectra of the doubly charged ion (MH_2^{++}) of [Pyr-1]apelin-13 in serum samples collected from young **(c)** and aged **(d)** mice. **(e)** Corresponding MS/MS spectrum of standard [Pyr-1]apelin 13. [Pyr-1]Apelin 13 was identified with a Mascot score of 37, 38, and 35 in young, aged, and standard samples, respectively. The [Pyr-1]Apelin 13 amino acid sequence and the main MS/MS fragmentations observed are indicated. Q*: pyro-Glutamic acid residue. AU: Arbitrary Unit. **(f)** Relative *Aplnr* mRNA expression of plantaris muscle ($n = 4$ animals/group). **(g)** Representative picture of negative control of apelin immunofluorescence obtained in plantaris muscle of 3 month-old mice. To test the specificity of the anti-apelin antibody, overnight co-incubation of the antibody and the peptide (1mM) has been done prior to realize the immunostaining. Scale bar, 30 μ m. ($n = 3$ animals, 1 picture/animal). **(h)** Apelin concentration accumulated in media (2h) of young- and aged-donor myocytes (3 different donors per group) after 4 days of differentiation. ($n = 3$ independent experiments). **(i)** *Aplnr* expression in soleus muscles of control and immobilized

mice in soleus muscle ($n = 4$ animals/group). **(j)** *Trim-63* expression in hindlimb muscles (gastrocnemius, soleus and tibialis) of control and immobilized mice ($n = 6$ animals/group). **(k-m)** Correlations between *Apln* and *Trim-63* mRNA expression in gastrocnemius, soleus and tibialis muscles respectively ($n = 10$ animals/group). Line indicates linear regression, and the Spearman correlation coefficient is shown. *Hprt* was used as a standard housekeeping gene **(f,i,j,k,l and m)**. * $P < 0.05$; 1-way ANOVA with post-hoc Tukey's multiple comparison test **(f)**, * $P < 0.05$; two-tailed Student's *t*-test **(h,i,j)**.

Supplementary Fig. 3 Consequences of apelin deficiency on muscle physiology. **(a)** Kaplan–Meier curve obtained by following 10 WT (black) and 10 *Apln*^{-/-} (red) mice. Two-tailed Student *t*-test comparison between the latter two strains showed statistically significant difference ($p < 0.0001$). **(b)** *Apln* mRNA expression in muscles collected from wild type (grey circles) and *Apln*^{-/-} (red squares) mice ($n = 5$ animals/group). **(c)** Tibialis fibers number quantification per whole muscle section in WT and *Apln*^{-/-} mice ($n = 4$ animals/group). **(d)** Western blot (left panel) and quantification (right panel) of Myosin Heavy Chain IIa in tibialis muscles of wild type and *Apln*^{-/-} mice ($n = 4$ animals/group). Tubulin is used as loading control. **(e)** Relative *Ppargc1a* mRNA expression in tibialis muscles of wild type and *Apln*^{-/-} mice ($n = 5$ animals/group). **(f)** *Aplnr* mRNA expression in muscles collected from wild type (grey circles), *Aplnr*^{+/-} (orange circles) mice ($n = 5$ animals/group). **(g)** mRNA expression of *apln* in tissues collected from AAV8-tMCK-*Apln*-ShRNA-treated mice (yellow circles) and control littermates (grey circles) ($n = 8$ animals/group). **(h)** Determination of AAV8-MCK-*Apln*-ShRNA-eGFP viral infection efficiency by α -GFP immunofluorescence in plantaris muscle of AAV8-tMCK-Ct-ShRNA- (middle panel) and AAV8-tMCK-*Apln*-ShRNA-treated (right panel) mice. Left panel represents negative control without α -GFP primary antibody. White arrowheads represent the presence of central nuclei. Scale bars, 30 μ m. ($n = 5$ animals,

1 picture/animal). **(i)** Quantification of central nuclei in quadriceps muscle of AAV8-tMCK-Ct-ShRNA- (grey circles) and AAV8-tMCK-*Apln*-ShRNA-treated mice (yellow circles) ($n = 8$ animals/group, 3 pictures/animal). **(j)** Representative picture of sirius red staining in quadriceps muscle of AAV8-tMCK-Ct-ShRNA- and AAV8-tMCK-*Apln*-ShRNA-treated mice. Scale bars, 80 μm . ($n = 3$ animals/group, 1 picture/animal). **(k)** mRNA expression of *Colla1*, *Col3a1*, *Tgfb1*, and *Argl1* in quadriceps from AAV8-tMCK-*Apln*-ShRNA-treated mice (yellow circles) and control littermates (grey circles) ($n = 8$ animals/group). **(l)** Representative picture of hematoxylin/eosin staining (left panel) and associated quantification of number of fibers (right panel) in gastrocnemius from AAV8-tMCK-*Apln*-ShRNA-treated mice (yellow circles) and control littermates (grey circles) ($n = 8$ animals/group, 2 pictures/animal). *Hprt* was used as a standard housekeeping gene (b,e,f,k) P value, two-tailed unpaired *t*-test. *** $P < 0.001$; Gehan-Breslow-Wilcoxon test (a), « ns » $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; two-tailed unpaired Student's *t*-test (b,c,d,e,f,g,i).

Supplementary Fig. 4 Validation of apelin supplementation strategies. **(a)** Plasma apelin immunoreactive level in blood collected from femoral artery before (0) or 1, 5, 30 and 60 minutes after *i.p* injection of [Pyr]apelin-13 (0.5 pmol/kg) ($n = 5$ animals). **(b,c)** Tibialis and soleus fibers type proportion relative to total fibers number in 3, 12 and 24 mo mice treated or not by apelin ($n = 4$ animals/group). **(d)** Tibialis maximal strength evaluated *in situ* by electrical sciatic nerve stimulation. ($n = 9$ animals for PBS treated groups and $n = 18$ animals for *Apln* treated groups and 3 mo mice). **(e)** Representative pictures of bioluminescence associated to luciferase activity of 24-month old aged mice intramuscularly injected with AAV1-Luc-Ct (left panel) or AAV1-Luc-*Apln* (right panel) 7 days after virus injection. (3

animals/group, 1 picture/animal). (f) Viral infection efficiency evaluated by immunofluorescent labeling of luciferase in AAV1-Luc-apln injected tibialis. Scale bars, 100 μ m. (n = 5 animals/group, 3 pictures/animal). (g) mRNA *Apln* expression in tibialis collected from 24 mo mice treated by AAV1-Luc-Ct (white) or AAV-Luc-apln (green). Muscles were harvested 35 days post injection (n = 7 animals/group). (h,i,j). Tibialis, gastrocnemius and soleus muscles masses relative to body weight in AAV1-Luc-Ct- (white) and AAV1-Luc-apln- (green) injected 24 mo mice at 35 days post injection (n = 10 animals/group). (k,l) Tibialis and plantaris masses expressed as percentage of body weight in mice submitted (Run) or not (Rest) to treadmill-induced chronic exercise and treated (triangles) or no (circles) with apelin (n = 10 for 3 and 12 mo mice and 7 for 24 mo mice). (m) Plantaris maximal specific force in mice submitted (Run) or not (Rest) to treadmill-induced chronic exercise and treated (triangles) or not (circles) with apelin (n = 10 for 3 and 12 mo mice, n = 7 for 24 mo mice). (n) Grid hanging capacities evaluated in mice submitted (Run) or not (Rest) to treadmill-induced chronic exercise treated (triangles) or no (circles) with apelin (n = 5 for 3 and 12 mo, n = 4 mice for 24 mo mice). (o) Tibialis and (p) plantaris *in situ* concentric torque force of middle-aged (12 mo) PBS-(triangles) and apelin-(squares) treated mice submitted to treadmill-induced chronic physical exercise. Insert represents area under curve (AUC). (n = 6 animal/group). ** $P < 0.01$; two-tailed paired Student's *t*-test (a). *or # $P < 0.05$; **or ## $P < 0.001$; ***or #### $P < 0.001$; two-tailed unpaired Student's *t*-test.

Supplementary Fig. 5 Muscle transcriptomic consequence of apelin supplementation. (a) *Aplnr* mRNA expression in mice treated (triangles) or not (circles) with daily *i.p* injection of apelin during 28 days (n = 5 animals/group). (b) Genes expressions measured by microarray in tibialis muscle of 3 (black), 12 (grey) and 24 (white) months-old mice treated (triangles) or

not (circles) by apelin ($n = 8$ animal/group). *or $\#P < 0.05$; **or $\##P < 0.001$; ***or $\###P < 0.001$; two-tailed unpaired Student's t -test.