Supplementary materials and methods

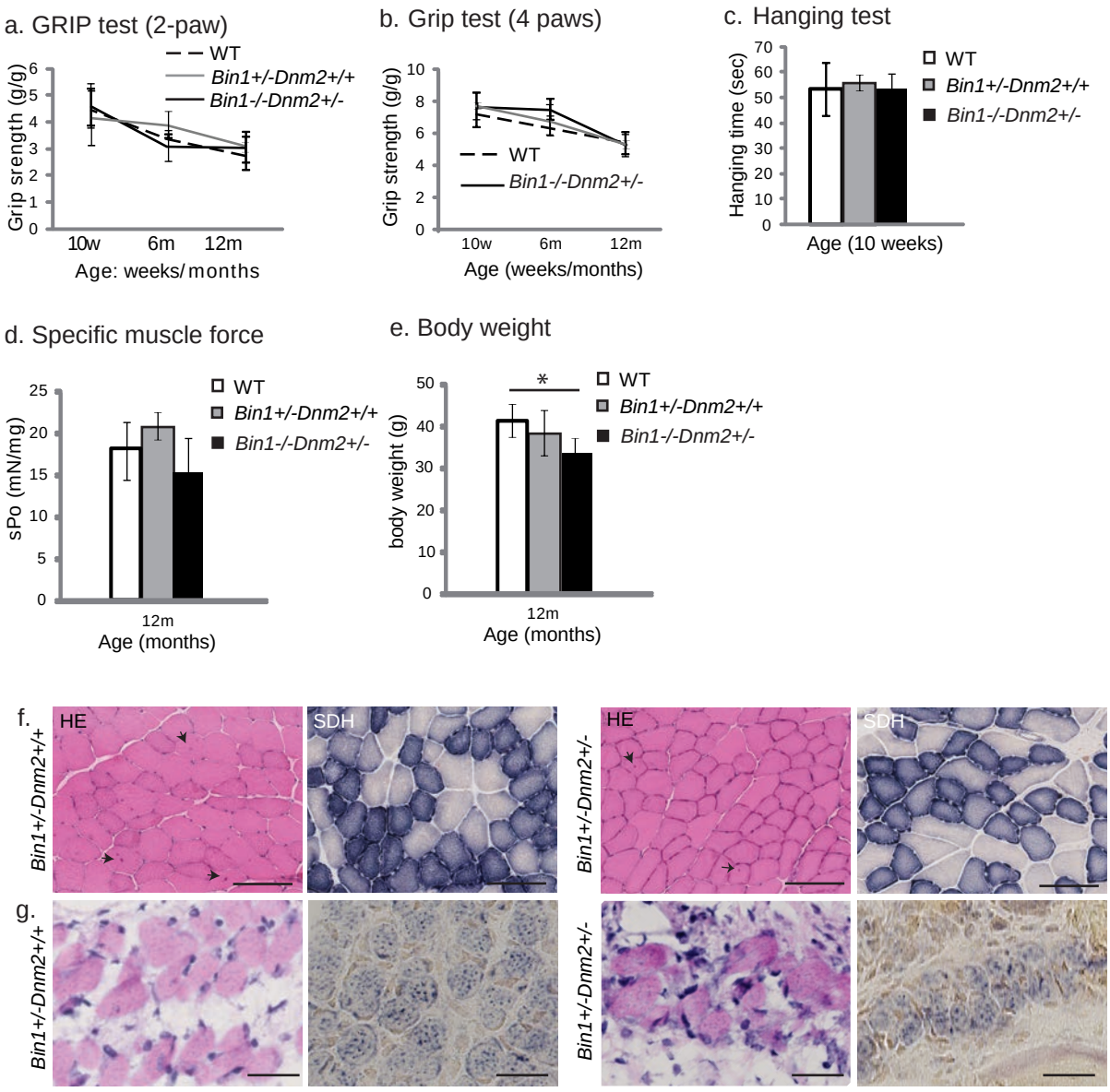
Materials. Primary antibodies used were: mouse anti-RYR1 (clone 34C; Sigma-Aldrich), anti-Serca (MA3–911; ABR), and anti-Dysferlin (abcam) antibodies.

Histological analysis of brain, liver and heart. Brain, liver and heart were dissected and fixed overnight in formalin and then transferred to 70% ethanol until embedding in paraffin. Sections (10μm) were stained with haematoxylin and eosin (HE) or Masson's trichrome stain in case of the heart.

Videos.
Supplementary video 1 : 4 month old *Bin*1+/+*Dnm*2+/+ (wild type) and *Bin*1-/-*Dnm*2-/+ mice
Supplementary video 2 : 12 month old *Bin*1+/+*Dnm*2+/+ (wild type) mouse
Supplementary video 3 : 12 month old *Bin*1-/-*Dnm*2-/+ mouse

Day 2	*Bin1-/-*CMV	*Bin1-/-*HSA
Expected :	25%	25%
Outcome :	0%	0%

Supplementary table 1. Expected and obtained percentage of mice identified at postnatal day two with *Bin1-/-* genotype after excision with Cre recombinase under the control of the CMV (all tissues) or HSA (skeletal muscle) promoters.



Supplementary Figure 1. *Bin1+/-Dnm2+/-* mice exhibit normal motor performance in clinical analysis. Grip test at 10 weeks (10w), 6 months (6m), and 12 months, performed using 2 (a) or 4 (b) paws. (c) Hanging test, the ability of mice to hang from the lid of a cage for 60 seconds was performed with 10 week old mice. (d) Specific muscle force (sPo), that is the maximum force relative to muscle mass, was measured in 12 month old mice. (e) Whole body weight was also measured. (f) Transverse TA sections from 12 month old mice were stained with HE or SDH. Arrows indicate mislocalized nuclei. Scale bar 100μm (g) Transverse hindlimb muscle sections from embryonic 18.5 day old muscles stained for HE and SDH. All graphs depict mean ± s.e.m. Statistical analysis was performed using an unpaired 2-tailed student's t-test for all graphs (*p<0.05) (n=minimum 3 mice per group) (w=weeks, m=months).

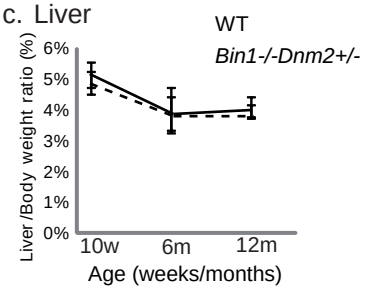
a. General bloodwork

		Total proteins	Glucose	Insulin	Calcium	Creatinine	Urea
		g/l	mmol/l	µg/l	mmol/l	µmol/l	mmol/l
WT	Average	50.25	11.39	5.33	2.61	11.27	8.68
	s.e.m	4.06	0.47	3.01	0.16	2.45	0.82
Bin1-/-Dnm2+/-	Average	53.00	10.72	1.63	2.53	10.81	9.65
	s.e.m	2.00	2.18	0.36	0.09	0.33	1.95
	t-test	0.50	0.75	0.25	0.68	0.77	0.42

b. Cholesterol metabolism

		Total Chol.	TG	FFA	True Triglycerides	Glycerol
		mmol/l	mmol/l	mEq/l	mmol/l	µmol/l
WT	Average	3.11	1,31	0.59	1.03	220.93
	s.e.m	0.42	0.25	0.11	0.27	16.11
Bin1-/-Dnm2+/-	Average	3.09	0.96	0.62	0.54	313.30
	s.e.m	0.38	0.33	0.03	0.29	129.30
	t-test	0.62	0.58	0.56	0.33	0.20

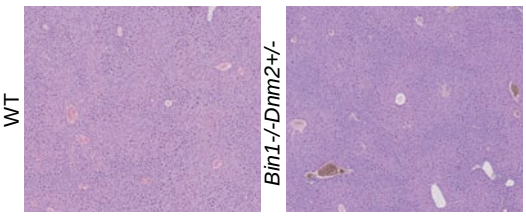
c. Liver



d. Liver function

		ASAT	ALAT
		U/l	U/l
WT	Average	81.60	23.80
	s.e.m	23.38	5.21
	Average	109.33	24.33
	s.e.m	44.36	3.56
	t-test	0.26	0.60

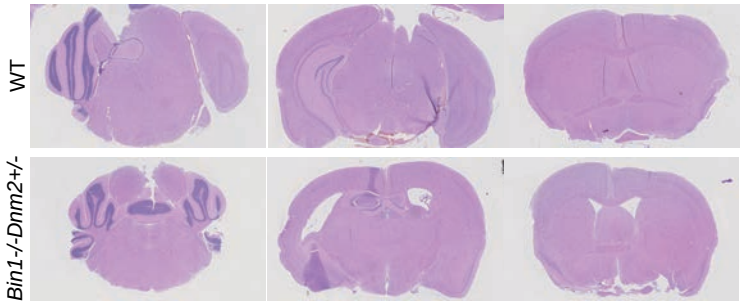
e. Liver histology



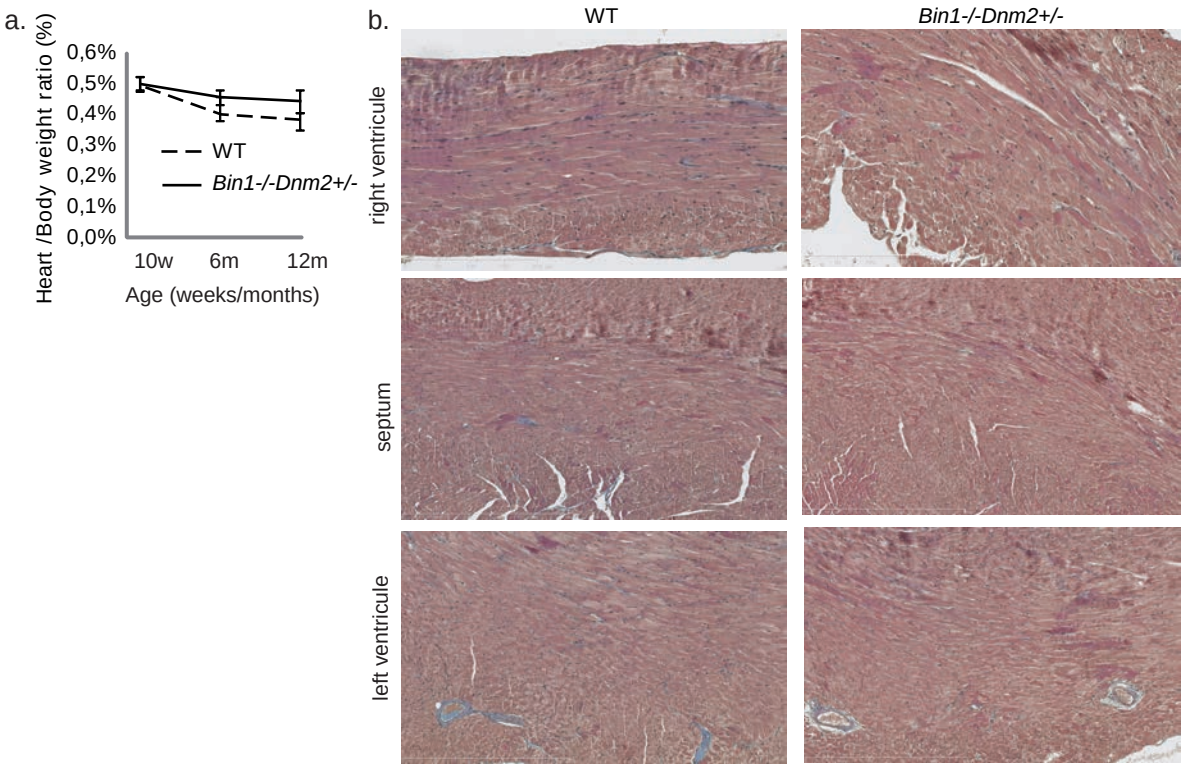
f. Muscle function

		CK	IGF-1
		U/l	ng/ml
WT	Average	814.2	324.7
	s.e.m	281,46	140.7
Bin1-/-Dnm2+/-	Average	642,33	250.1
	s.e.m	430.7	13.7
	t-test	0.77	0.64

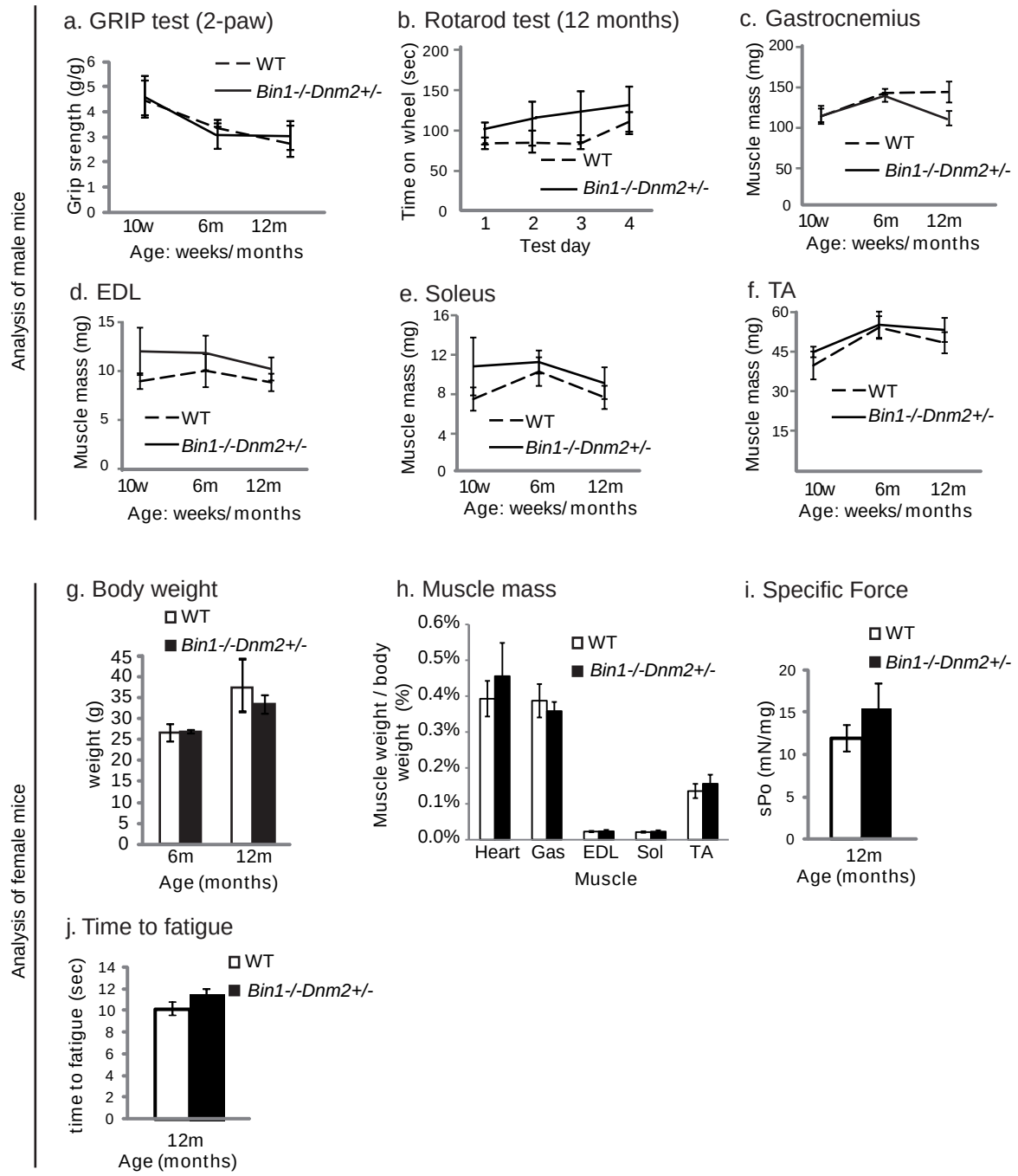
g. Brain histology



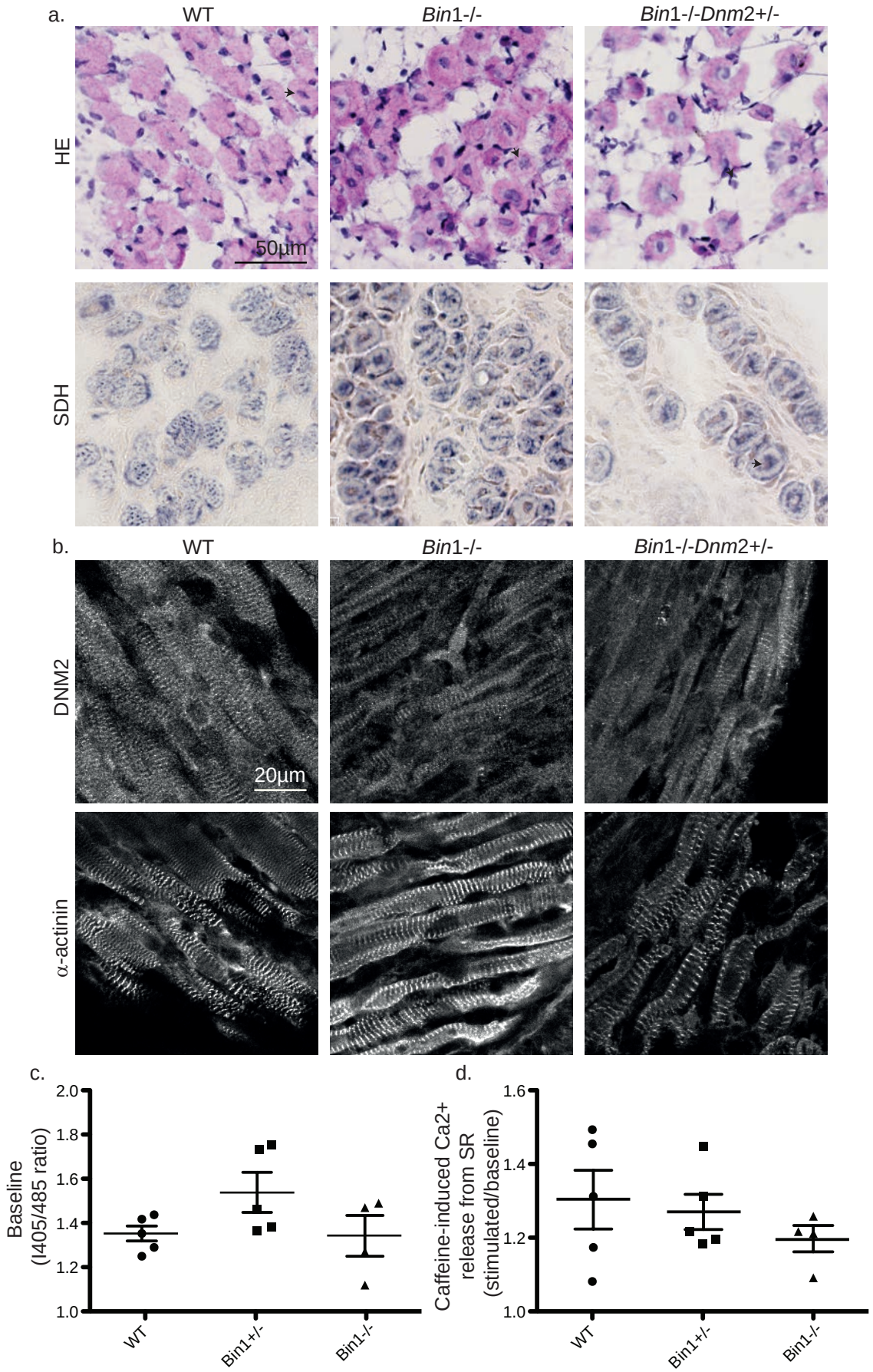
Supplementary Figure 2. Analysis of several organs and whole body metabolism in *Bin*1-/-*Dnm*2+/- mice. (a) Plasma levels of total proteins (g/l), glucose (mmol/l), insulin (µg/l), calcium (mmol/l), creatinine (µmol/l), and urea (mmol/l). (b) Cholesterol metabolism measured from blood plasma. Liver organ/body weight (c), liver enzymes from plasma (d) and 10µm HE histological sections (e); scale bar 250µm. (f) Creatine kinase and IGF-1 total levels measured in blood plasma. (g) HE staining of 10µm brain sections; scale bar 1mm. Statistical analysis was performed using an unpaired 2-tailed student's t-test for graph and tables. n=minimum 4 mice per group.



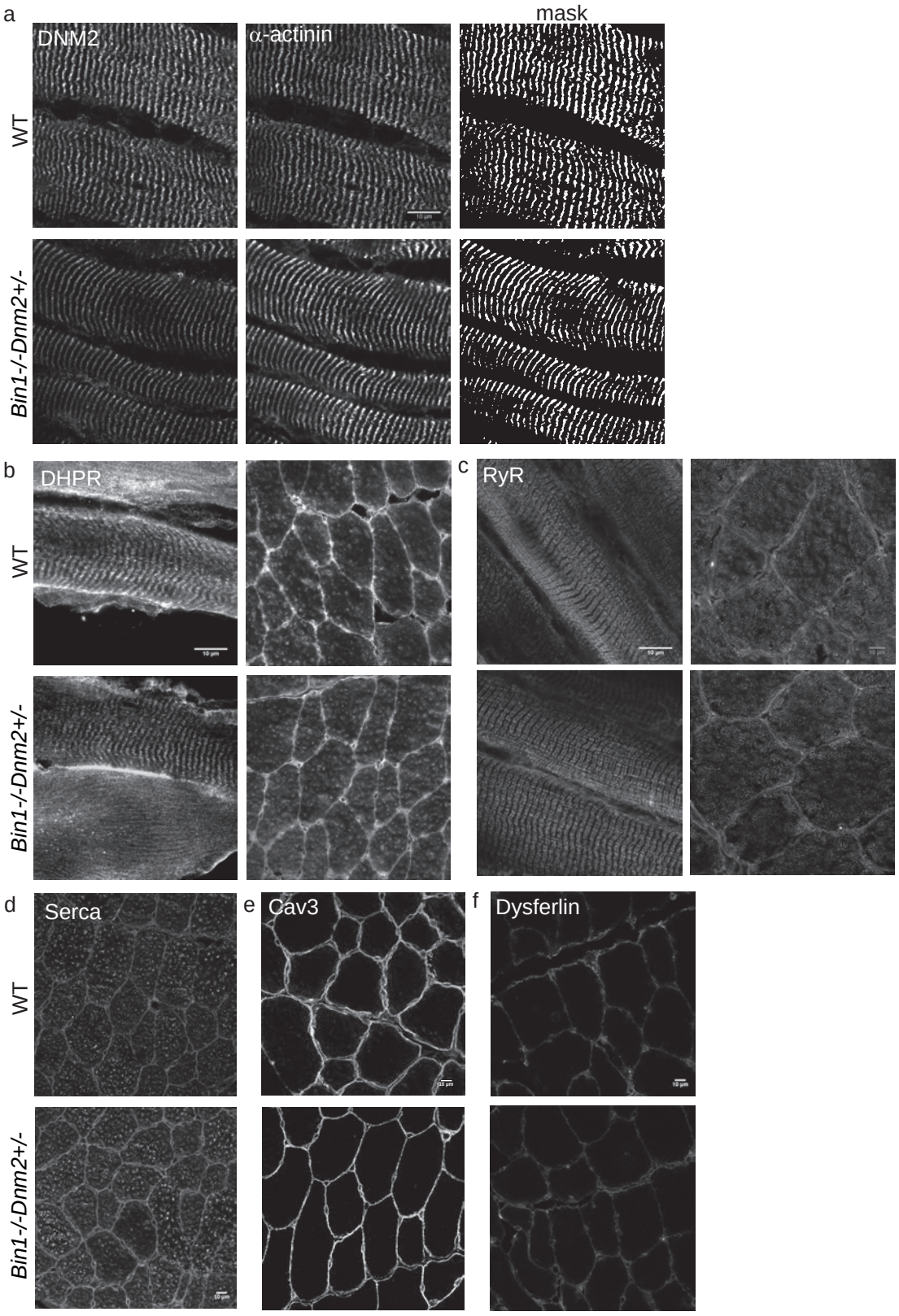
Supplementary Figure 3. Normal heart weight and histology in *Bin*1-/-*Dnm*2+/- mice. (a) Heart/body weight ratios (%) for male mice. Graph depicts mean ± s.e.m. Statistical analysis was performed using an unpaired 2-tailed student's t-test for graph and tables. n=minimum 4 mice per group.(w=weeks, m=months). (b) 10μm HE histological sections of the right ventricle (upper panel), septum (middle panel), and left ventricle (lower panel), from 12 month old male mice. Scale bar 200μm.



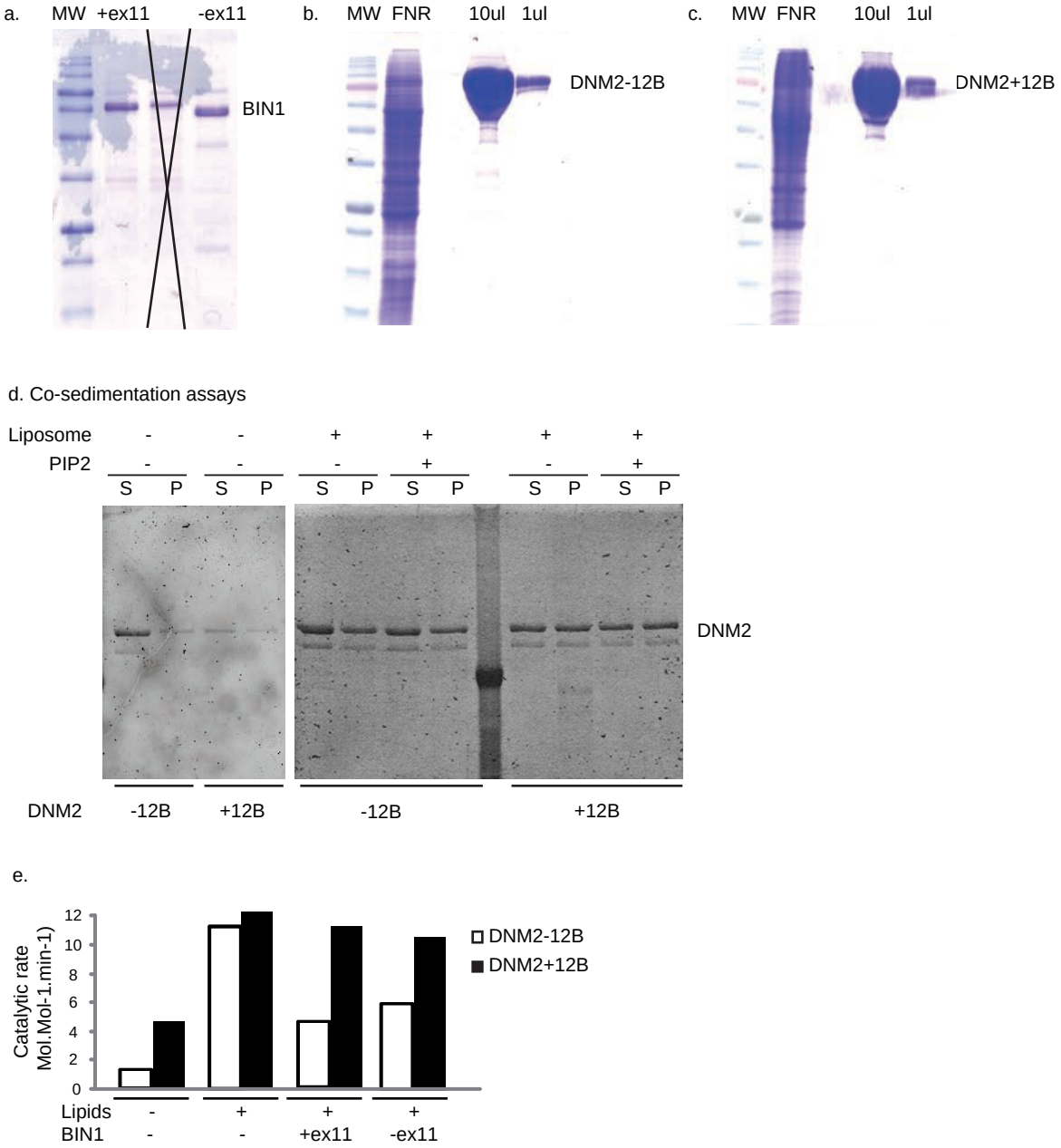
Supplementary Figure 4. *Bin*1-/-*Dnm*2+/- mice exhibit normal motor performance in clinical analysis. Characterization of male mice (a)-(f). (a) 2 paw grip test at 10 weeks (10w), 6 months (6m), and 12 months (12m). (b) Rotarod test performed in 12 month old mice under acceleration mode (40rpm in 5 minutes). n=3 trials/mouse/day. Muscle mass of gastrocnemius (c), extensor digitorum longus (EDL) (d), soleus (e), tibialis anterior (TA)(f). Characterization of female mice (g)-(j). (g) Body weight of 6 and 12 months old female mice (grams, g). (h) Muscle/body weight ratio (%) of heart, gastrocnemius (Gas), extensor digitorum longus (EDL), soleus (Sol), and tibialis anterior (TA) muscles. (i) Specific muscle force (sPo, mg/mN) measured in 12 month old mice. (j) Time to fatigue in 12 month old mice, measured as the time taken to reach 50% of the maximum muscle force (seconds). All graphs depict mean ± s.e.m. Statistical analysis was performed using an unpaired 2-tailed student's t-test for all graphs with the exception of (b) for which a one-way Anova test was performed as described in the methods section (*$p < 0.05$) . n=minimum 3 mice per group (w=weeks, m=months).



Supplementary Figure 5. Structure and function of embryonic day 18.5 skeletal muscles from *Bin1-/-* mice. (a) Transverse hind limb sections from embryonic 18.5 day old mice were stained with HE or SDH. Scale bar 50µm. (b) Immunofluorescence staining of 8µm longitudinal sections with DNM2 (upper panel), and α-actinin (lower panel). Images representative of DNM2 in transverse (mature) localization. Longitudinal DNM2 localization (immature) can be observed in figure 4d. Scale bar 20µm. Primary myoblasts were harvested from hindlimbs of e18.5 day old WT, *Bin*1+/- and *Bin*1-/- mice (n=4-5 mice per genotype). (c) The basal level of calcium in the cytoplasm was measured using the Indo 1 probe (I405/485 ratio). (d) Following basal measurements, calcium release from the sarcoplasmic reticulum (SR) was measured upon caffeine stimulation in calcium (Ca2+) free medium using the Indo 1 probe (I405/485 ratio). The ratio of caffeine-stimulated relative baseline measurements is shown. Graphs represent mean+/-s.e.m. Statistical test performed (one-way ANOVA, Dunn's Multiple Comparison test) showed no signifcant difference between groups.

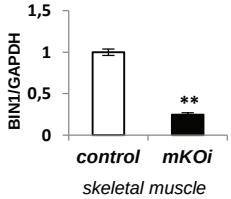


Supplementary Figure 6. Skeletal muscle triad markers are unaltered in *Bin1-/-Dnm2+/-* mice. (a) Immunofluorescence staining of 8μm longitudinal sections with DNM2 (left panel), and α-actinin (middle panel). Mask image shows areas of co-localization (right panel). (b) Longitudinal and transverse skeletal muscle sections stained with DHPR antibody, or for RyR (c). Transverse sections stained for Serca (d), Caveolin 3 (Cav3) (e) and Dysferlin (f). Sections taken from mice at 10 weeks of age. Scale bar 10μm.



Supplementary figure 7. BIN1 inhibits DNM2 in an isoform-dependent manner. SDS-page analysis of purified recombinant protein expression for BIN1 + or -exon 11 (a), DNM2-12B (b) and DNM2+12B (c). FNR=Fraction Not Retained in the column (flow through). (d) SDS-page analysis of co-sedimentation assays. Gel represents 1 of 4 experiments performed. P=pellet, S=supernatant, PIP2=PI4,5P2. (e) Malachite green assay with DNM2 (+ or -12B) and BIN1 (+ex11 (Iso8) and –ex11 (Iso9)) isoforms, with DNM2:BIN1 at a ratio of 1:1. Results are shown as catalytic rate (Mol.Mol-1.min-1) relative to DNM2-12B alone.

a.



b.

	Control	mKOi
Mice injected (n)	5	5
Survival 15w PI. (%)	100%	100%

Supplementary figure 8. *Bin1* exon 20 deletion in adult muscle is not lethal. Tamoxifen induced deletion was performed in mice at 7 weeks of age. (a) 15 weeks after tamoxifen induced excision of exon 20, mice were sacrificed and immunoblot of BIN1 and GAPDH loading control was performed on TA muscle lysates. Control (L2 HSA Cre ERT2-), mKOi (L2 HSA Cre ERT2+). Quantification of BIN1 protein level, relative to GAPDH loading control is shown. Graphs depicts mean ± s.e.m. Statistical analysis was performed using an unpaired 2-tailed student's t-test (**$p < 0.01$)(n=5 mice per group). (b) Percentage of mice that survived after knockdown of BIN1 expression was induced in adult mice.