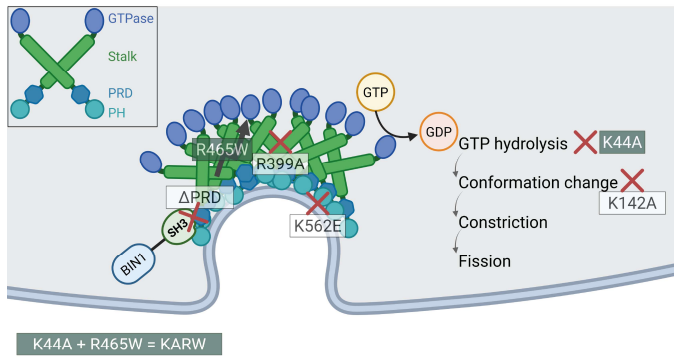


Supplementary figures

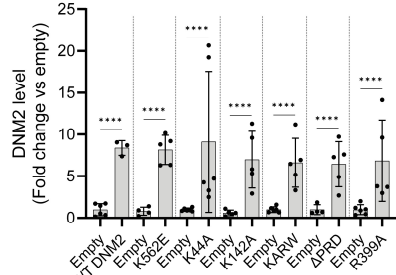
DNM2 lipid binding drives centronuclear myopathy and represents a potential therapeutic target

Raquel Gomez-Oca, Xènia Massana- Muñoz, David Reiss, Juliana De Carvalho Neves, Nadege Diedhiou, Roberto Silva-Rojas, Belinda S. Cowling, Marie Goret, Jocelyn Laporte

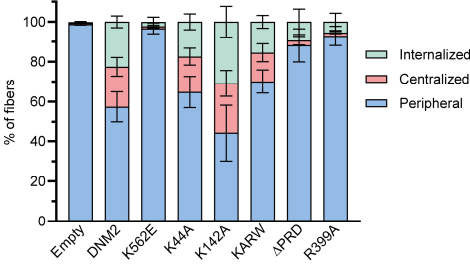
A Effects of DNM2 mutants on protein function



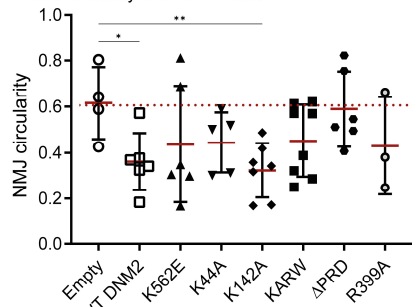
B Western blot DNM2 in TA of WT mice



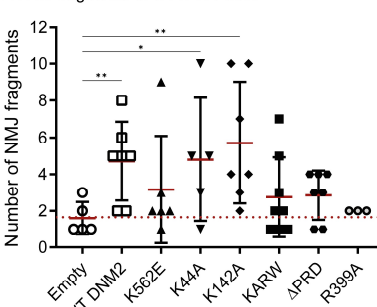
C Nuclei localization in TA of WT mice



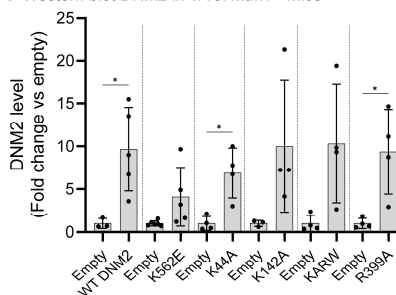
D NMJ circularity in TA of WT mice



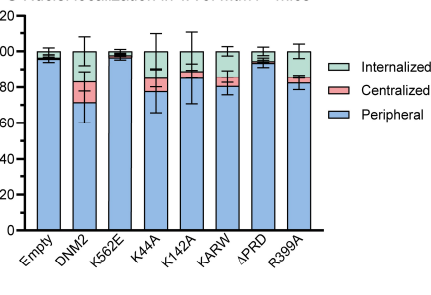
E NMJ fragments in TA of WT mice



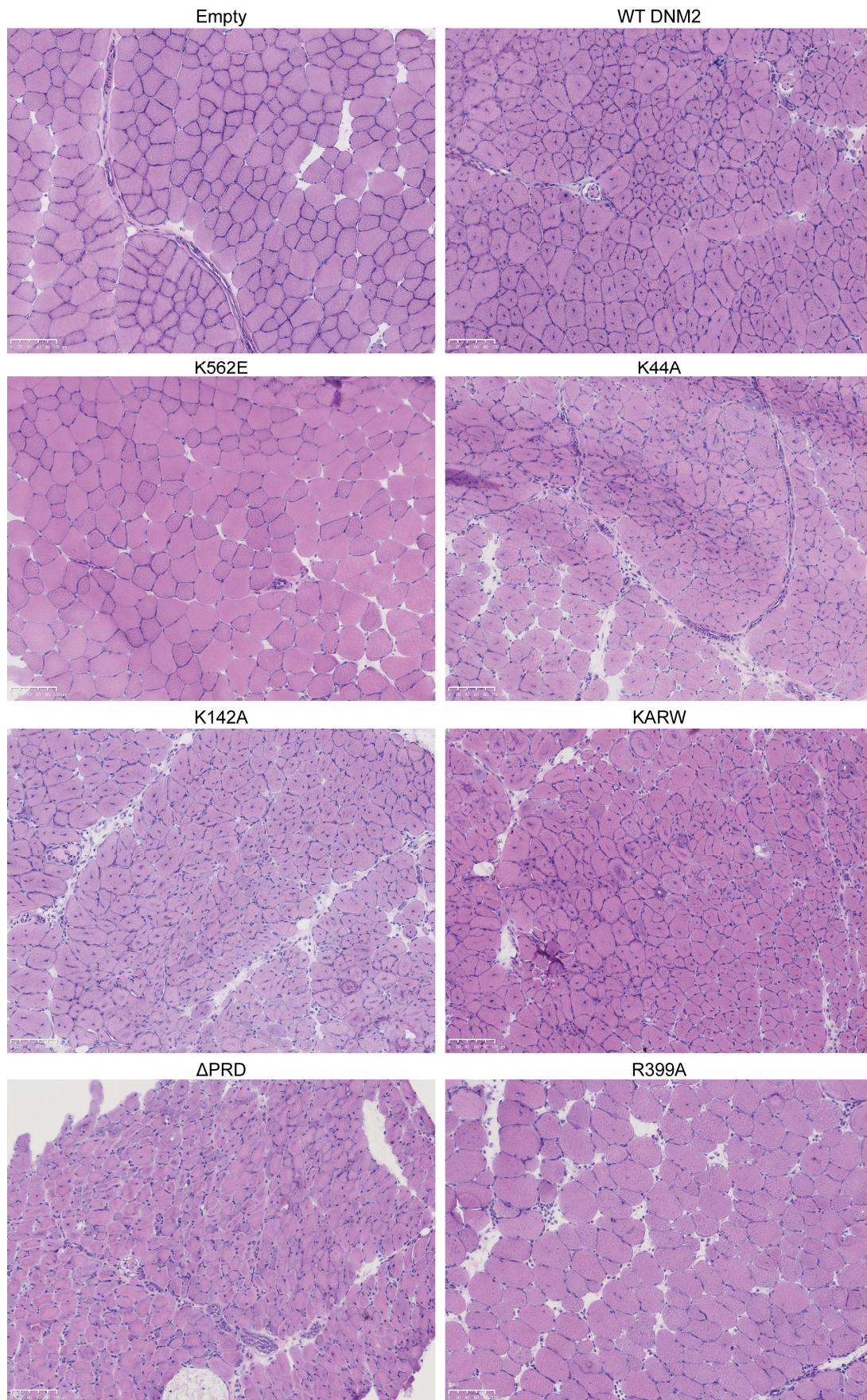
F Western blot DNM2 in TA of *Mtm1*^{-/-} mice



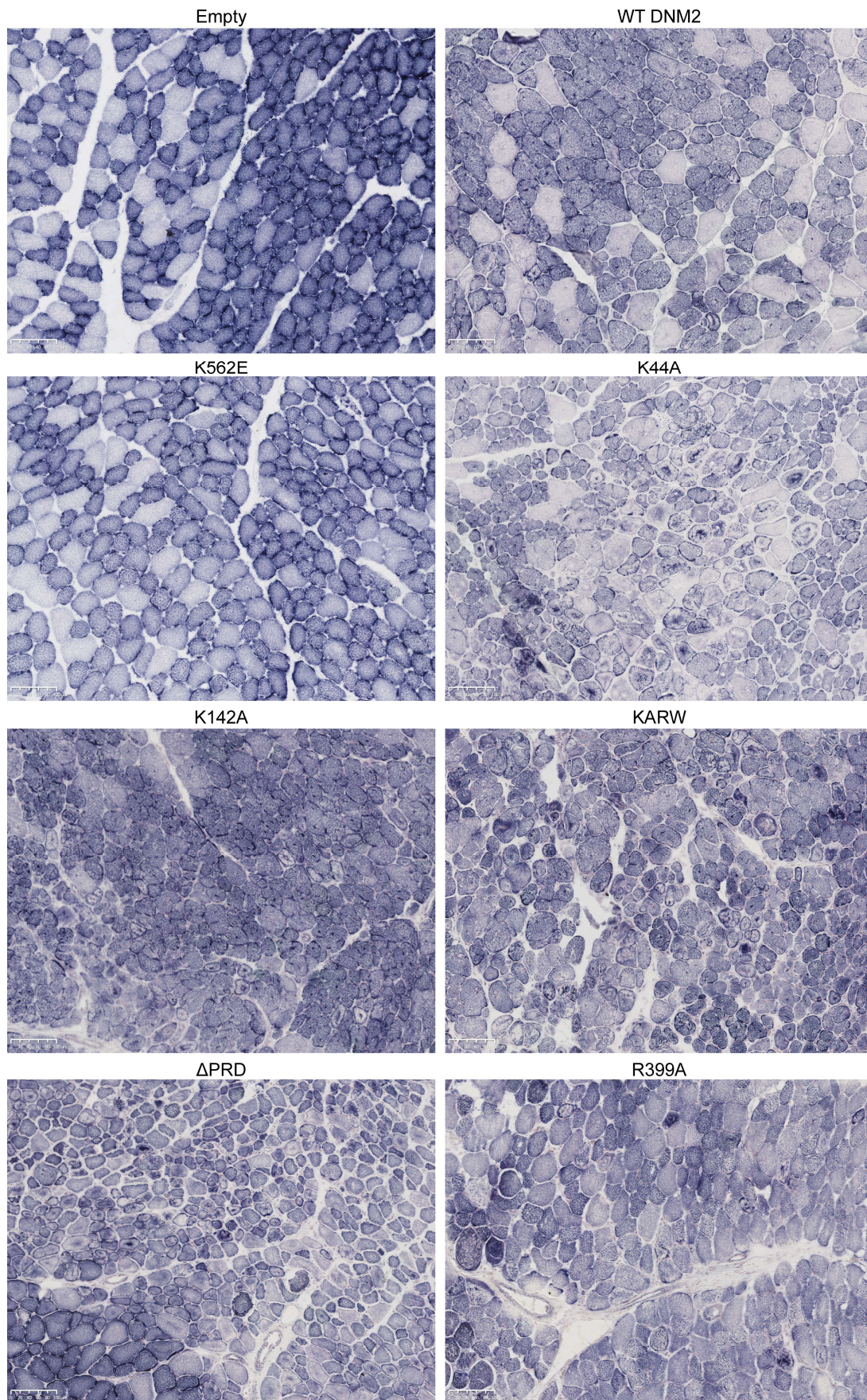
G Nuclei localization in TA of *Mtm1*^{-/-} mice



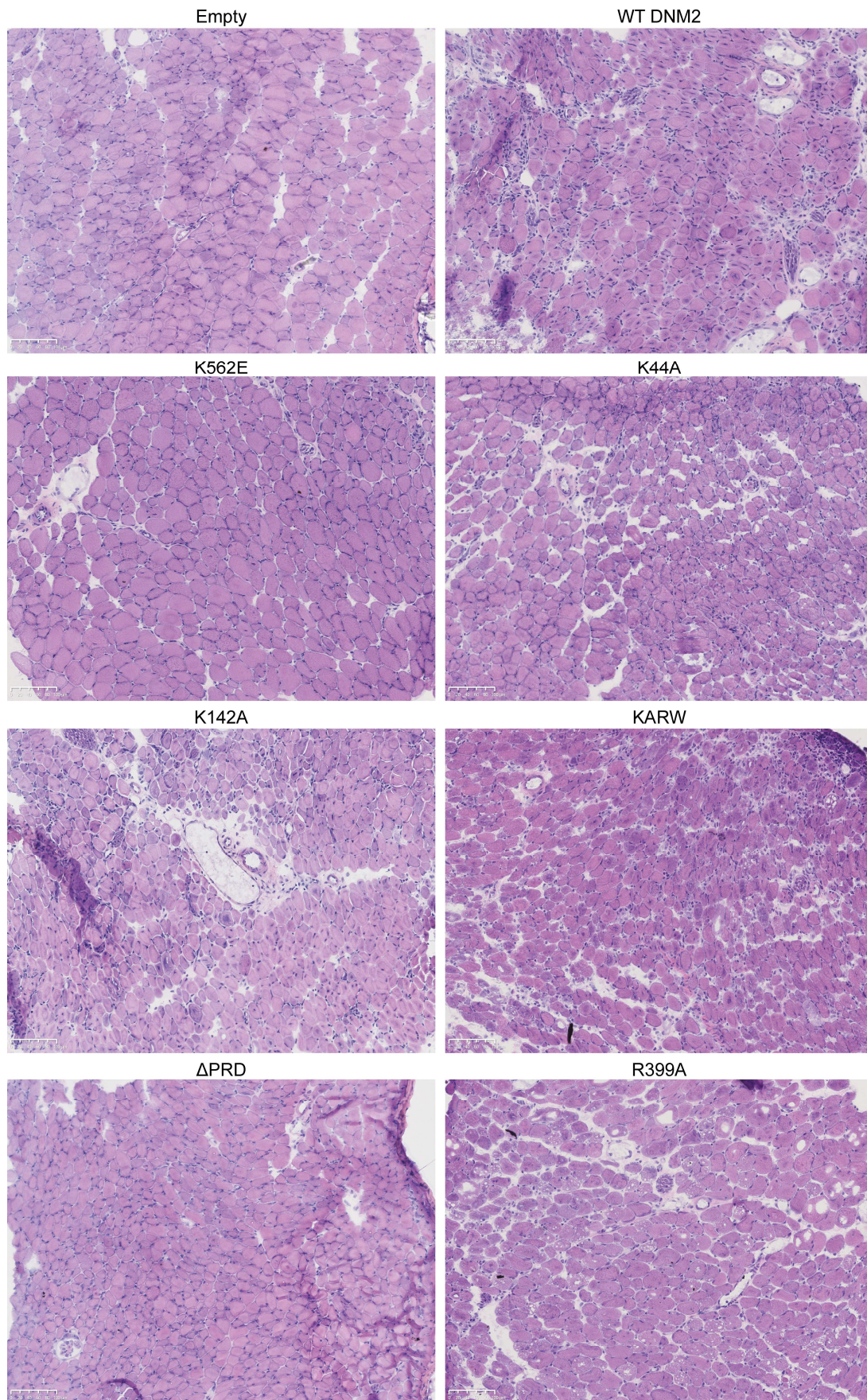
Supplementary Fig. 1. Additional data on injected DNM2 mutants. (A) Theoretical model of DNM2 function in membrane fission, placing the mutated isoforms analyzed in this study. DNM2 domains are detailed in the dimer representation (upper-left corner). Created in BioRender. Goret, M. (2026) <https://BioRender.com/ezxeirw>. **(B)** DNM2 protein quantification in TA after AAV injection, normalized to β -actin depicted in supplementary material (3 $\leq$ n $\leq$ 6). **(C)** Percentage of myofibers with central, internal or peripheral nuclei in injected WT TA (4 $\leq$ n $\leq$ 5). **(D)** NMJ circularity (0 corresponding to a line and 1 to a circle) in longitudinal TA sections (n = 3-8 NMJs from 1 mouse). **(E)** Number of fragments per NMJ in longitudinal TA sections (n = 3-8 NMJs from 1 mouse). **(F)** DNM2 protein quantification in *Mtm1*^{-/-} TA after AAV injection, normalized to β -actin depicted in supplementary material (3 $\leq$ n $\leq$ 6). **(G)** Percentage of myofibers with central, internal or peripheral nuclei in injected *Mtm1*^{-/-} TA (3 $\leq$ n $\leq$ 5). (B, F) Each dot represents one mouse. (D, E) Each dot represents one NMJ. Values are shown as mean $\pm$ SD, *p<0.05, **p<0.01, ****p<0.0001. (B, D, F) ANOVA. (E) Kruskal-Wallis.



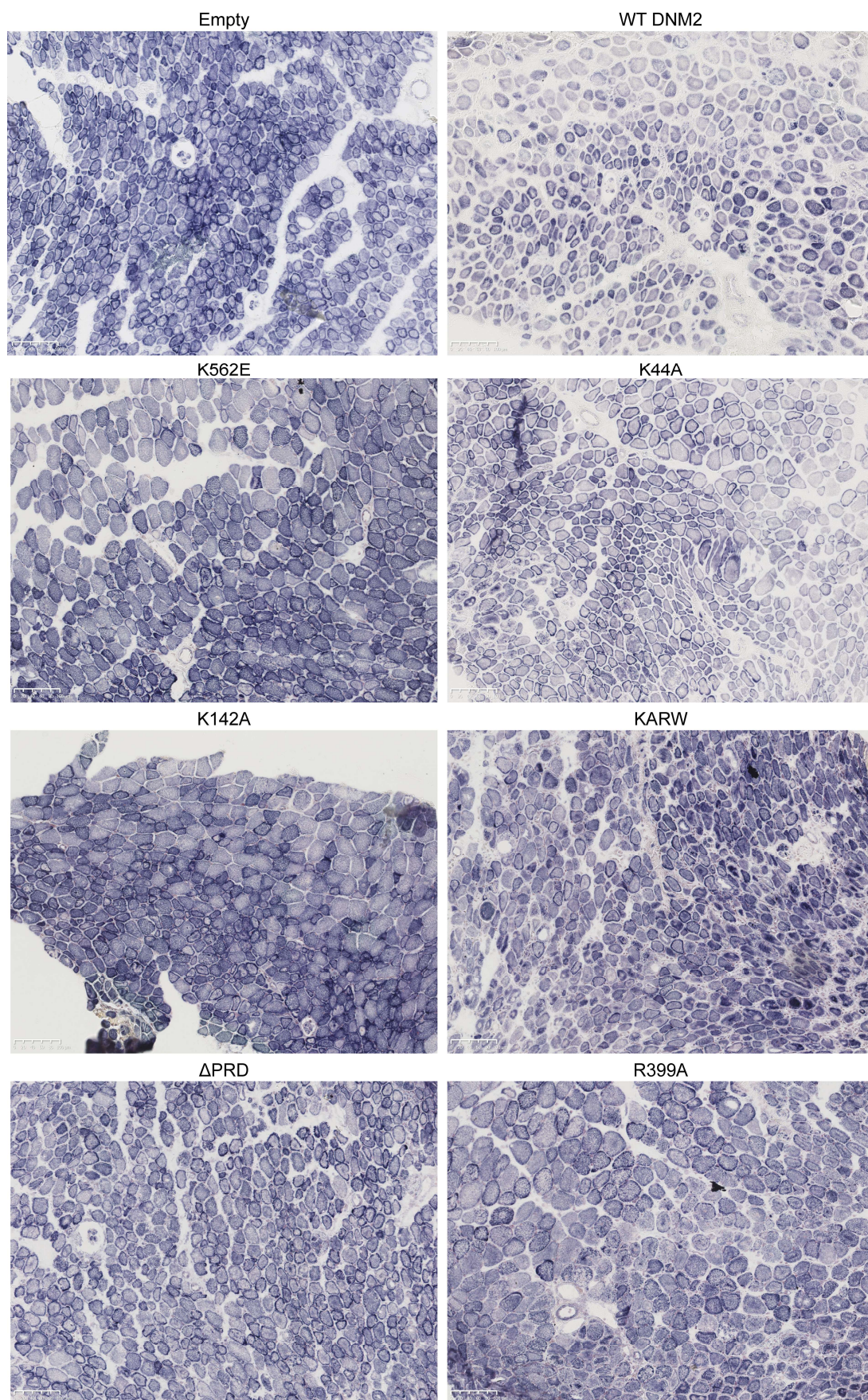
Supplementary Fig. 2. Transversal sections of TA muscles from WT mice injected with DNM2 variants, stained with hematoxylin and eosin. Scale bar = 100 μ m.



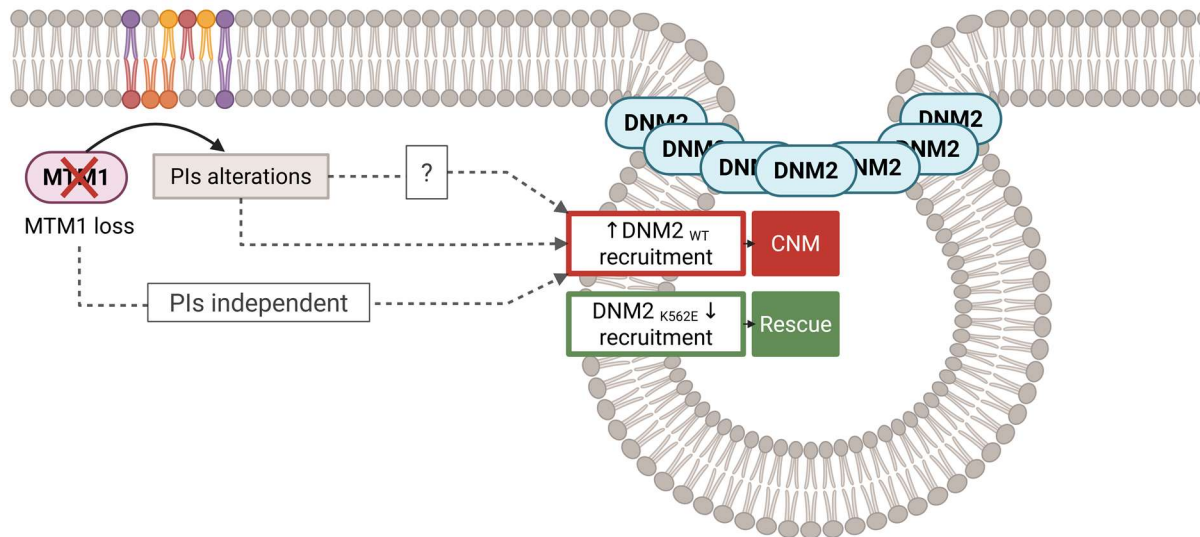
Supplementary Fig. 3. Transversal sections of TA muscles from WT mice injected with DNM2 variants, stained for succinate dehydrogenase activity. Scale bar = 100 μ m.



Supplementary Fig. 4. Transversal sections of TA muscles from *Mtm1*^{-/-} mice injected with DNM2 variants, stained with hematoxylin and eosin. Scale bar = 100 μ m.



Supplementary Fig. 5. Transversal sections of TA muscles from *Mtm1*^{-/-} mice injected with DNM2 variants, stained for succinate dehydrogenase activity. Scale bar = 100 μm.



Supplementary Fig. 6. Hypothetical model linking MTM1, phosphoinositides, DNM2, and CNM pathology and the proposed rescue by the K562E variant. Schematic representation of a conceptual model illustrating how loss of MTM1 function may alter phosphoinositides (PIs) homeostasis and consequently affect DNM2 membrane occupancy, contributing to the development of centronuclear myopathy (CNM) pathology. This model is intended to provide a conceptual framework for the relationship between MTM1, PIs, and DNM2 in CNM and does not represent mechanistic conclusions derived from new experimental data. Created in BioRender. Laporte, J. (2026) <https://BioRender.com/atzx61i>.

Test	<i>Mtm1</i> ^{y/-}	<i>Dnm2</i> ^{K562E/+}
Survival		
Body mass		
Hanging time		
Locomotor activity		
Rear activity		
Paw angle		
Body stretch		
Muscle force		
TA mass		
Fiber size		
Nuclei localization		
SDH localization		
Desmin localization		
β1-integrin localization		
Ultrastructure		
DNM2 level		

Legend

No initial phenotype

Crossing provided:

Phenotype worsening

No rescue

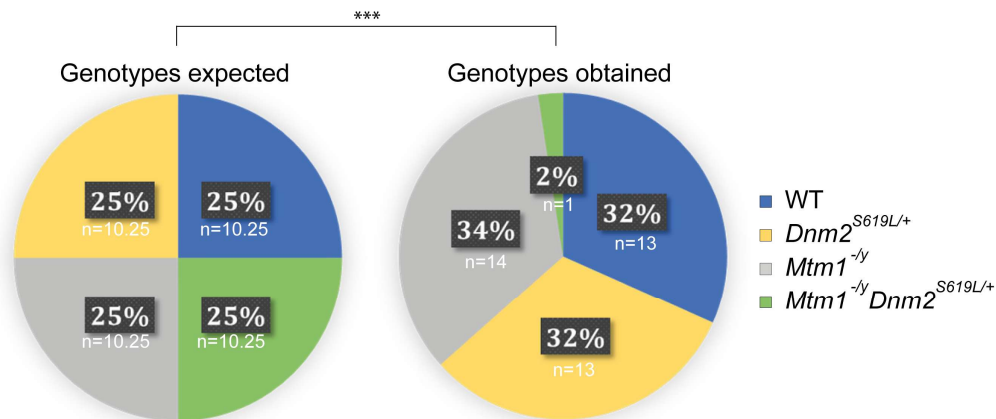
Tendency to rescue

Partial rescue

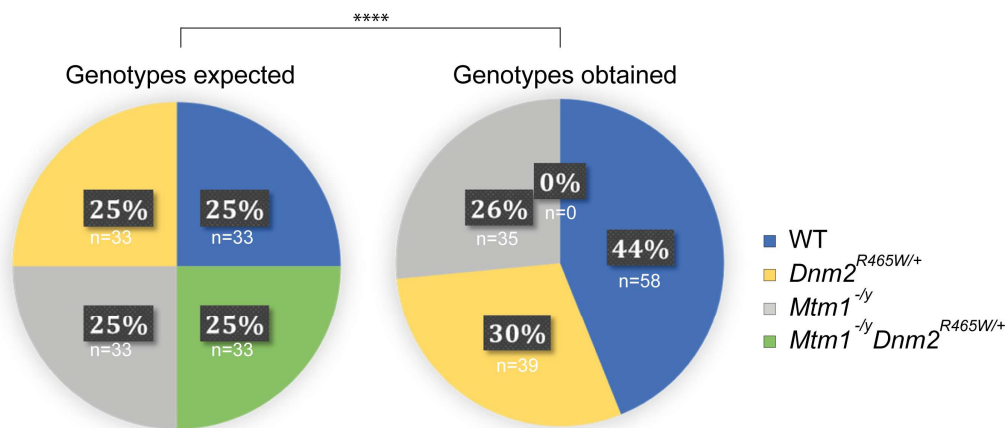
Total rescue

Supplementary Fig. 7. Overview of the improvements observed when combining the two mutants *Mtm1*^{y/-} and *Dnm2*^{K562E/+} over each individual mouse model. Summary of whole-body motor performance and muscle phenotypes at 8 weeks, comparing the double mutant to each single mutant. Tendency to rescue= double mutant not statistically different from both single mutant and WT mice. Partial rescue= double mutant differs from both single mutant and WT mice. Rescue= double mutant differs from single mutant but is similar to WT. TA = Tibialis Anterior.

A Birth ratios $Mtm1^{-/-}$ x $Dnm2^{S619L/+}$ (males)



B Birth ratios $Mtm1^{-/-}$ x $Dnm2^{R465W/+}$ (males)



Supplementary Fig. 8. Perinatal lethality of $Mtm1^{-/-}Dnm2^{S619L/+}$ and $Mtm1^{-/-}Dnm2^{R465W/+}$ mice. (A) Expected versus obtained birth ratios for male offspring from $Mtm1^{+/-}$ x $Dnm2^{S619L/+}$ mice breeding, in percentage and n number (n=41). Only $Mtm1^{+/-}$ males will develop the myopathy. **(B)** Expected versus obtained birth ratios for male offspring from $Mtm1^{+/-}$ and $Dnm2^{R465W/+}$ crosses, in percentage and n number (n=132). ***p<0.001. ****p<0.0001. (A-B) Chi-squared test.