

YMTHE, Volume 26

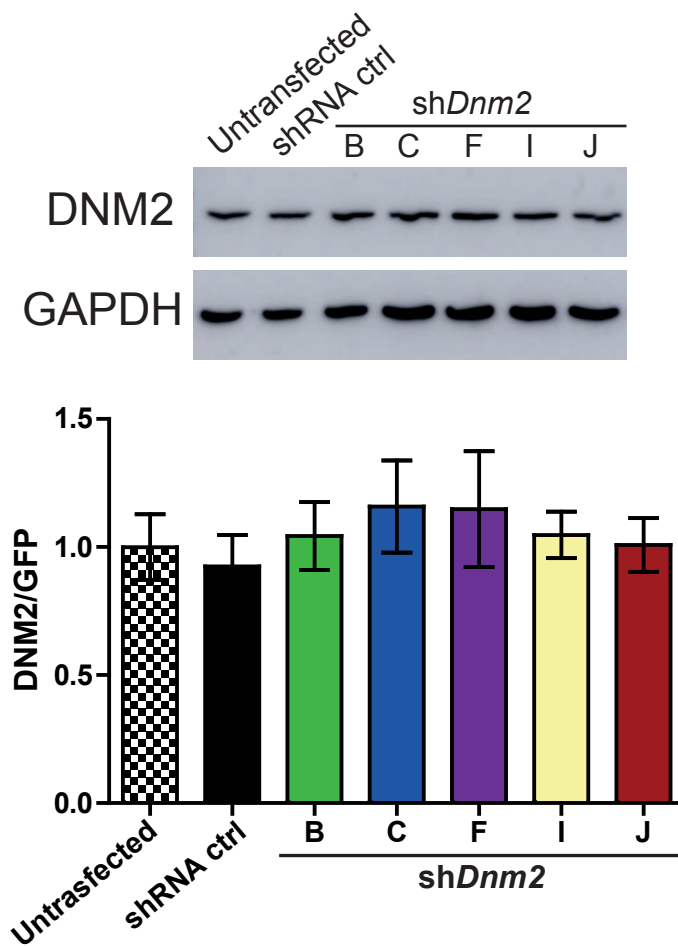
Supplemental Information

Single Intramuscular Injection of AAV-shRNA

Reduces DNM2 and Prevents Myotubular

Myopathy in Mice

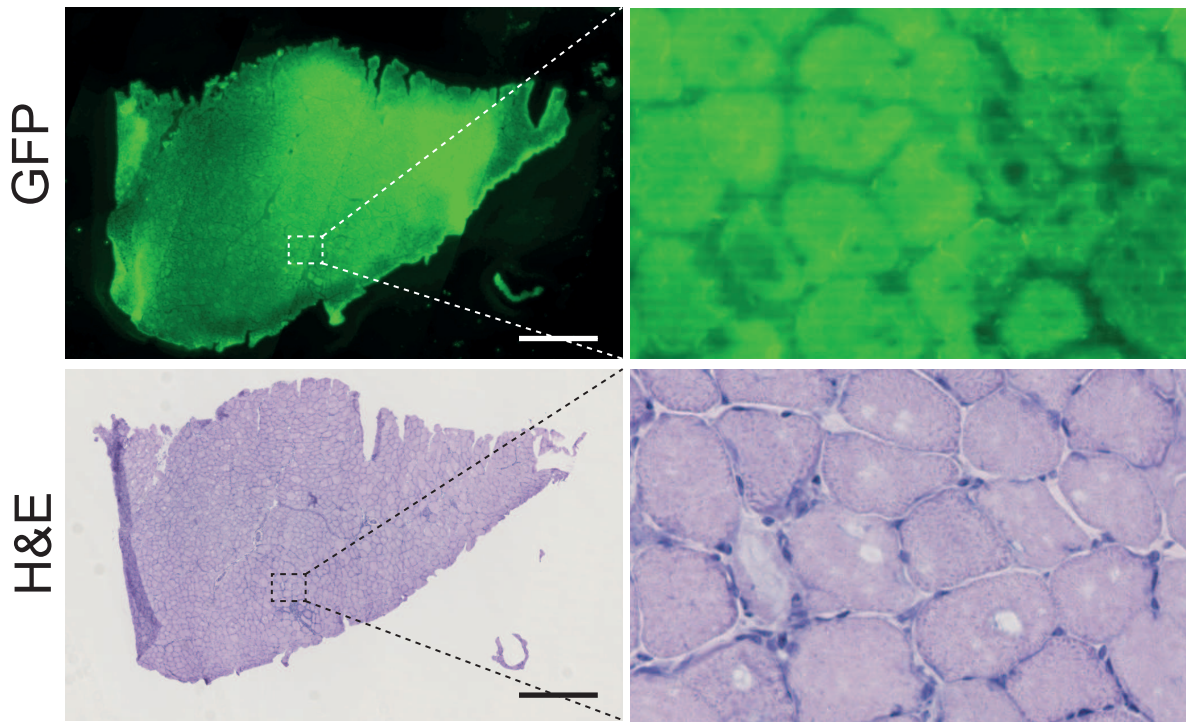
Hichem Tasmaout, Valentina M. Lionello, Christine Kretz, Pascale Koebel, Nadia Messaddeq, Deborah Bitz, Jocelyn Laporte, and Belinda S. Cowling



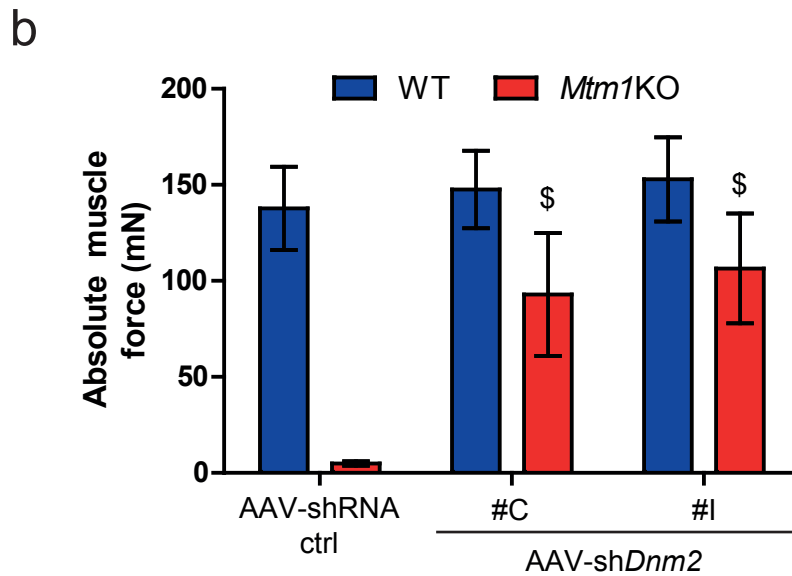
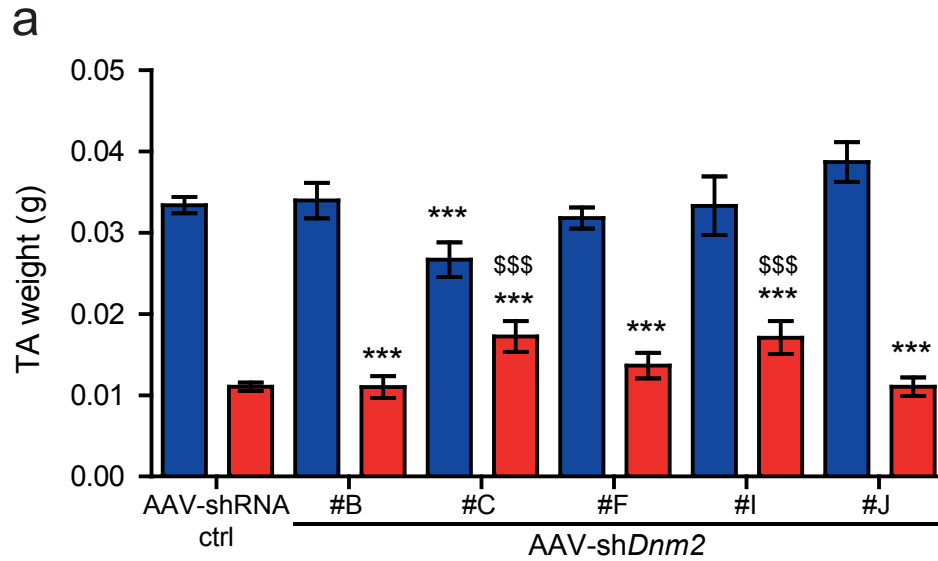
Supplemental figure S1: *In vitro* validation of shRNA targeting *Dnm2*.

Representative western blot from shRNA-treated C2C12 mouse myoblast. Cells were electroporated with shRNA ctrl, or sh*Dnm2* (B, C, F, I or J). DNM2 protein levels was determined by densitometry and standardized to GAPDH. *n*=3 per each group. Data represent an average of three independent experiments \pm SEM.

*Mtm1*KO muscle injected with sh*Dnm2* #C

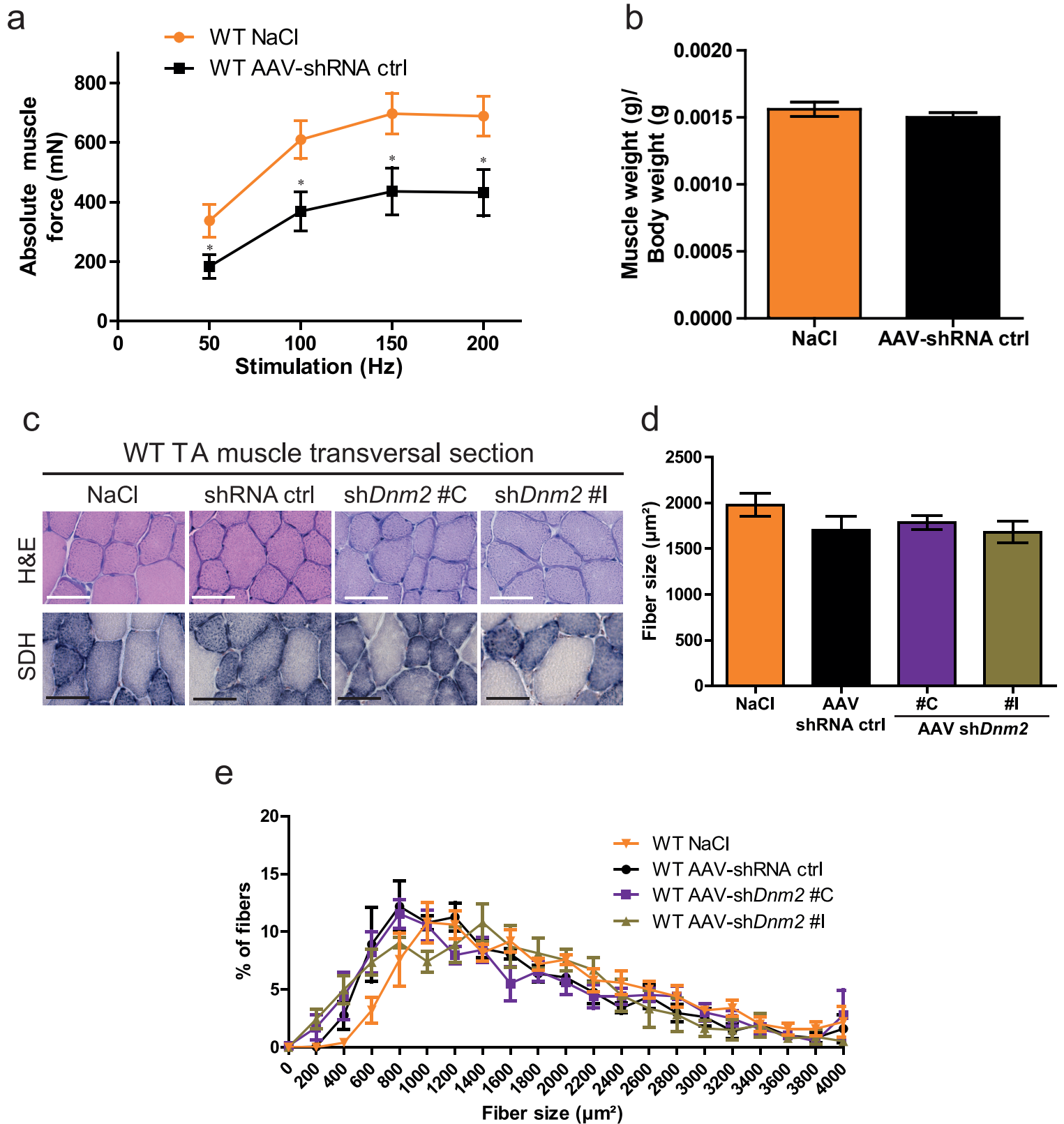


Supplemental figure S2: GFP expression and H&E staining of serial sections at 4 weeks after AAV-sh*Dnm2* injection. Heterogeneous GFP expression was visualized on 8 μ m TA cross section of 7-weeks old *Mtm1*KO.



Supplemental Figure S3: Positive effects of AAV shRNA-mediated *Dnm2* knockdown on *Mtm1KO* muscle mass and absolute force.

4 weeks after AAV-sh*Dnm2* #C or #I injections, improvements in absolute muscle mass (a) and muscle maximal force (measured at 150 Hz) (b) were noted in *Mtm1KO* muscles. n=5-7 mice/group, ***p<0.001 vs WT TA treated with shRNA ctrl. \$p<0.05, \$\$\$p<0.01 *Mtm1KO* treated with AAV-sh*Dnm2* vs *Mtm1KO* treated with AAV-shRNA ctrl (ANOVA test).



Supplemental figure S4: Characterization of AAV-shRNA ctrl and AAV-shDnm2 impact on muscle mass, force and histology. (a) TA muscle of WT mice treated with NaCl or AAV-shRNA ctrl were weighed 4 weeks after a single injection. (b) Absolute muscle forces of TA muscle were recorded after sciatic nerve stimulation with 50, 100, 150 and 200Hz. $n=5-7$ mice/group. (c) TA muscle sections of WT mice treated with AAV-shRNA ctrl, AAV-shDnm2 (#C or #I) or NaCl were stained with H&E and SDH to visualize fiber size, shape, nuclei positioning and mitochondrial distribution (scale bars: 50 μ m). (d) Myofiber area was calculated on 300-400 fibers per sample. (e) Fiber size distribution of WT TA muscles according to size groups. $n=5$ mice/group. * $p<0.05$ vs WT TA treated with NaCl (t -test).