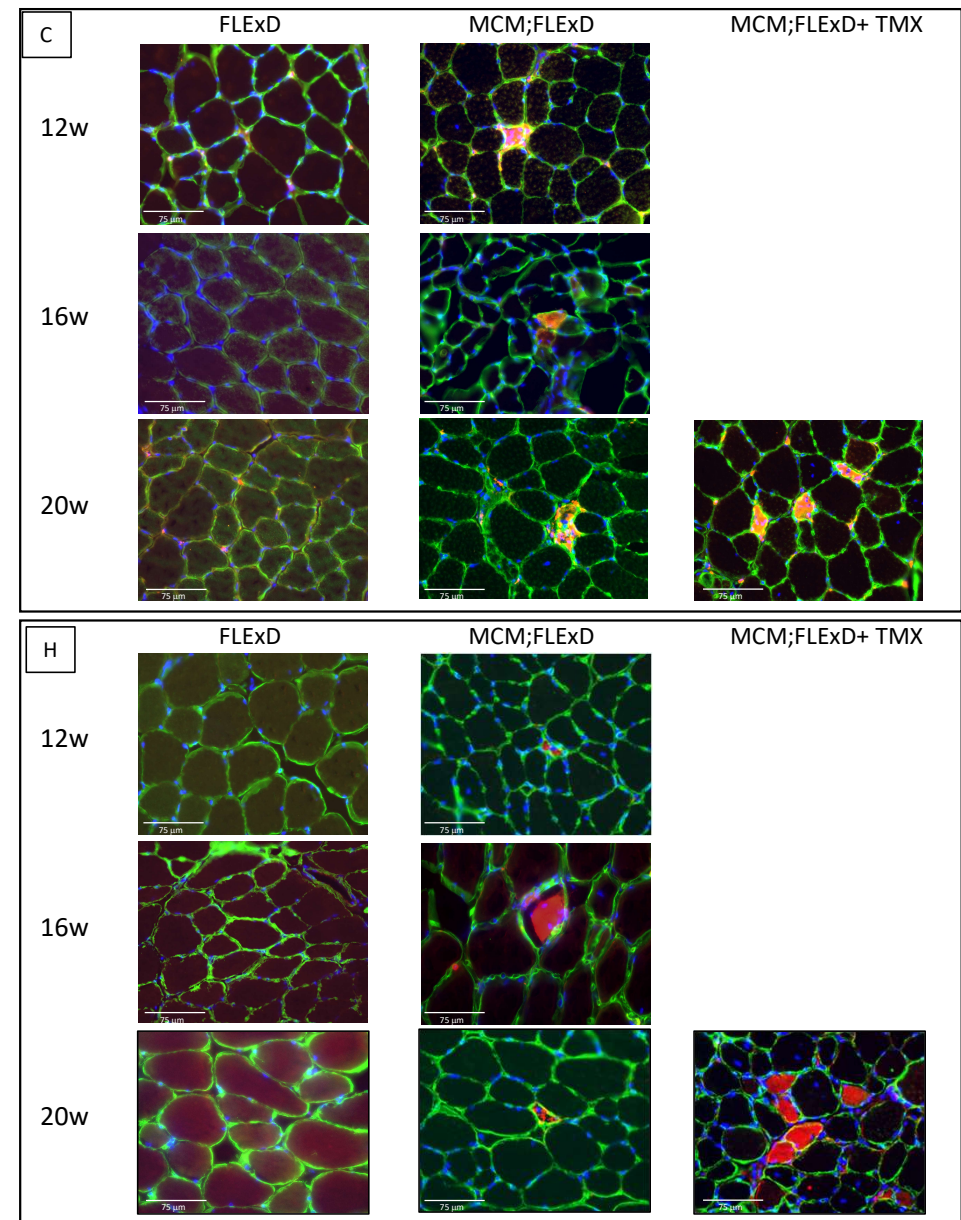
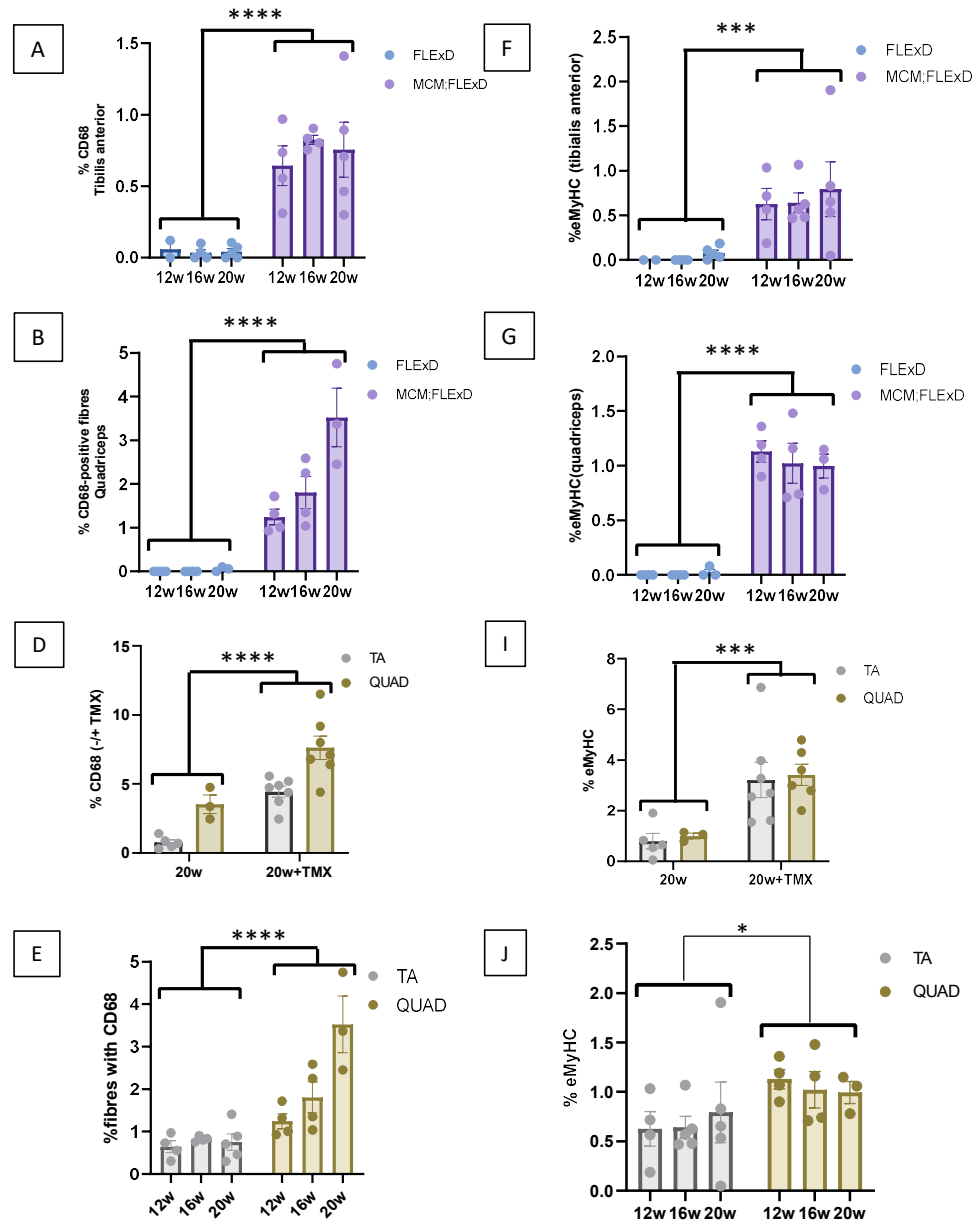


Supplementary Figure S1: Acta1-MCM;FLEXDUX4/+ females present a slow dystrophic process.

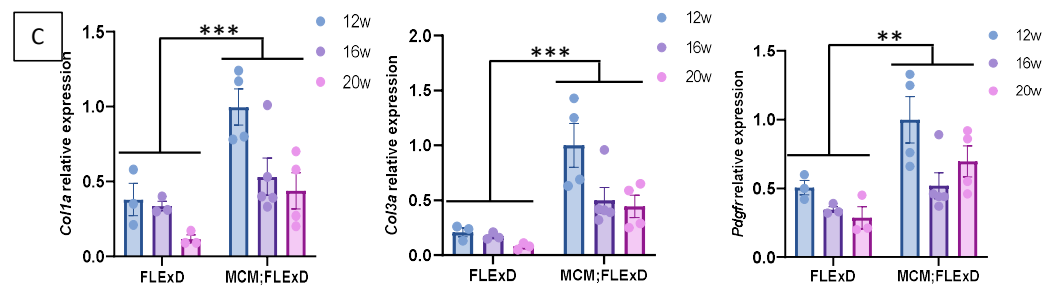
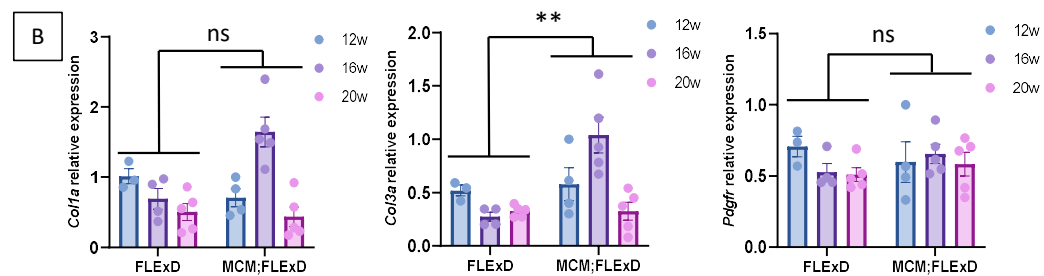
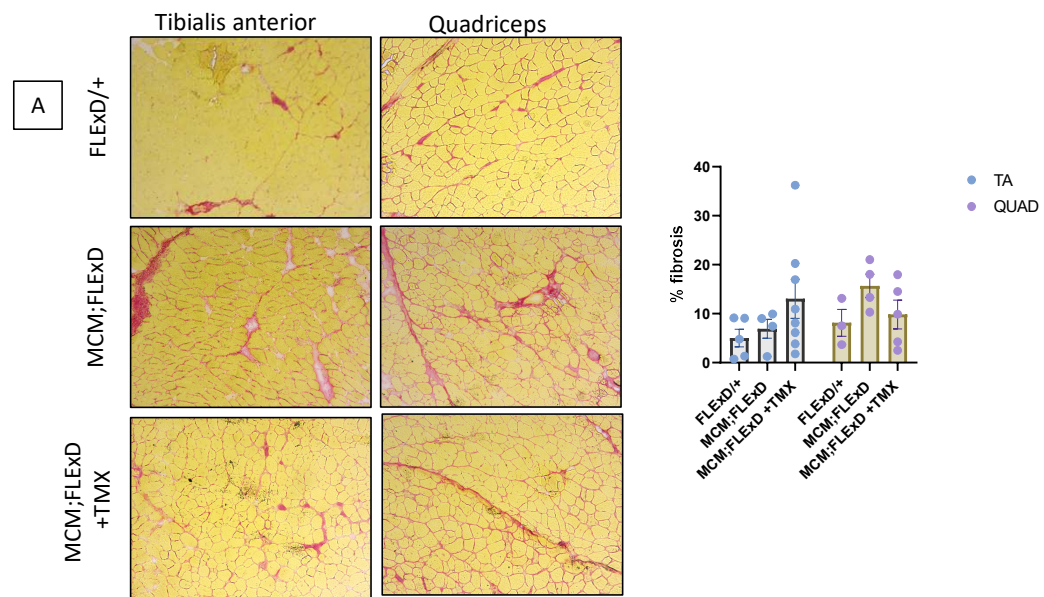
The total body (A), tibialis anterior weight (B), and quadriceps weight (C), and the percentage of centrally located nuclei in the tibialis anterior (D and F) and the quadriceps (E and F) were measured at 12, 16 and 20 weeks in females. In some animals, TMX (2.5 mg/kg weekly for 4 weeks) was injected (H).

Error bars indicate the mean of SEM of 3-5 biological replicates. p-values were calculated using GraphPad Prism9, 2-way ANOVA followed by a Fisher's LSD post hoc test. *p < 0.05; **p<0.01; ***p<0.001; ****p<0.0001.



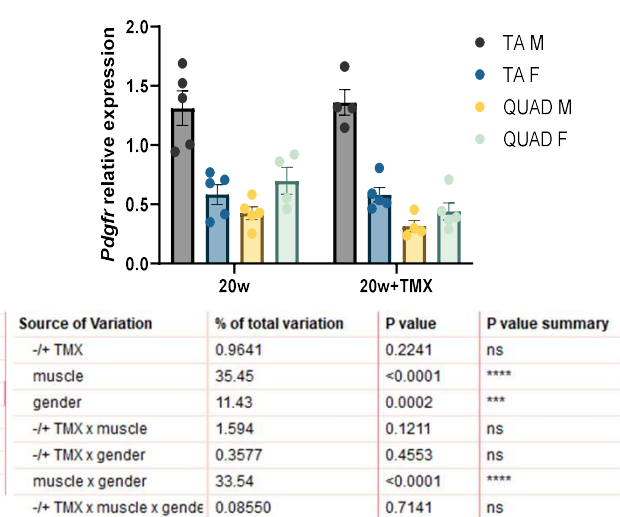
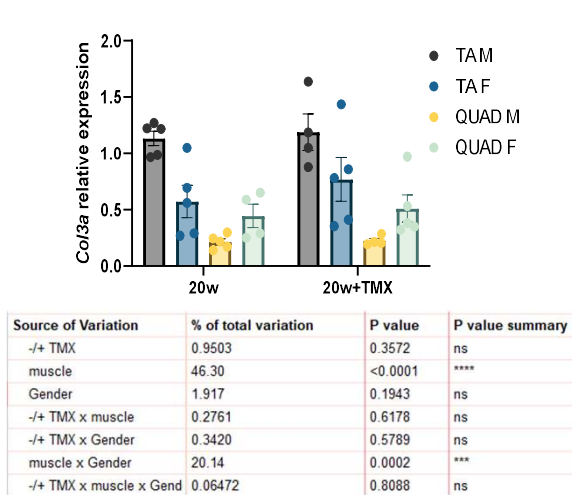
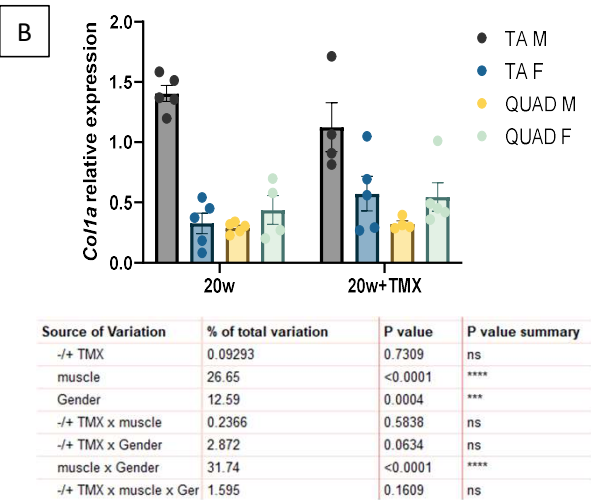
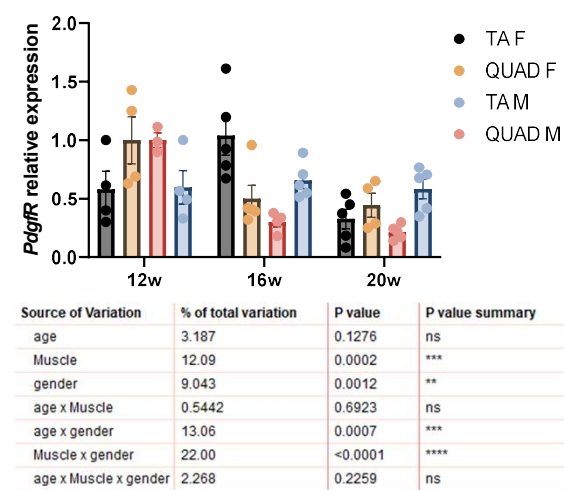
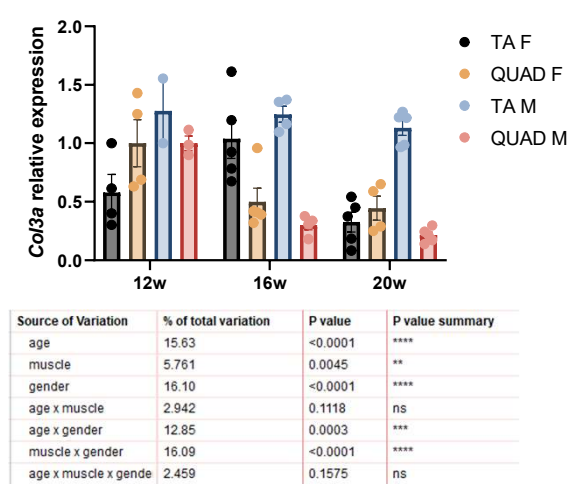
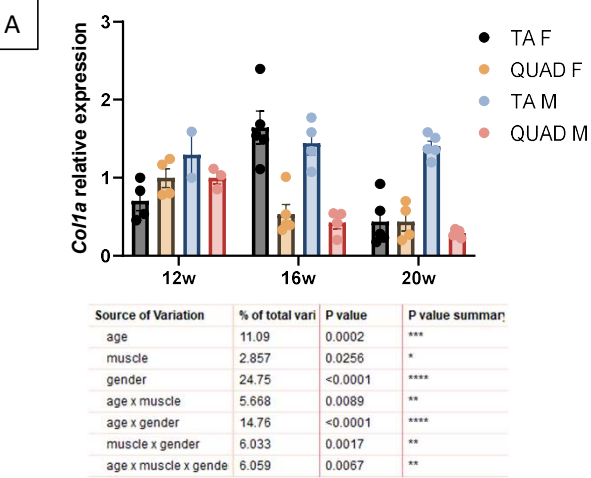
Supplementary Figure S2: Acta1-MCM;FLEXDUX4/+ animals present inflammation and regenerative fibres.

The percentage of fibres positive for CD68 staining (A-E) and the percentage of eMyHC positive fibres (F-J) were measured in the tibialis anterior (A, F) and the quadriceps (B, G) at 12, 16, and 20 weeks in FLEXDUX4/+ and ACTA1-MCM/+;FLEXDUX4/+ (MCM;FLEXD) males. In some animals, TMX (2.5 mg/kg weekly for 4 weeks) was injected (C,D, H, I). The number of fibres positive for CD68 staining or eMyHC was compared in the TA and QUAD muscles (E&J, respectively). Error bars indicate the mean of SEM of 3-5 biological replicates. p values were calculated using GraphPad Prism9, 2-way ANOVA followed by a Fisher's LSD post hoc test. *p < 0.05; **p<0.01; ***p<0.001; ****p<0.0001.; TMX: tamoxifen.



Supplementary Figure S3: Acta1-MCM;FLExDUX4/+ present fibrotic infiltration.

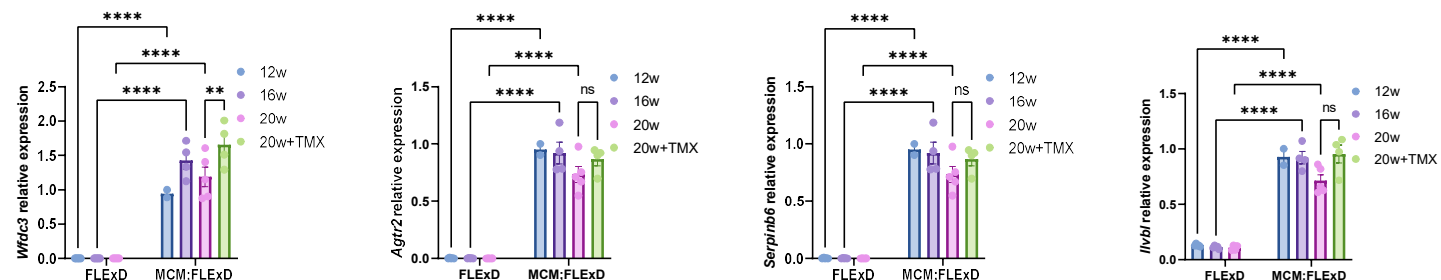
Acta1-MCM;FLExDUX4/+ or FLExDUX4/+ females were treated with or without tamoxifen. Picrosirius red was used to stain collagen on the tibialis anterior (upper panel) and quadriceps (lower panel) sections (A) in animals killed at the age of 20 weeks. The expression of several genes involved in fibrosis was investigated in the tibialis anterior (B) and quadriceps (C) in the females at the age of 12, 16 or 20 weeks. Error bars indicate the mean of SEM of 3-5 biological replicates. p values were calculated using GraphPad Prism9, 2-way ANOVA followed by a Fisher's LSD post hoc test. *p < 0.05; **p<0.01; ***p<0.001; ****p<0.0001.; TMX: tamoxifen.



Supplementary Figure S4: The expression of Col1A, Col3A and Pdgfr were compared in the TA or QUA muscles isolated from MCM;FLEXDUX4/+ males or females at the age of 12, 16 or 20 weeks (A). Some animals were injected with tamoxifen (TMX) at the age of 16 weeks and muscles were harvested at the age of 20 weeks and compared to age-matched mice (B). Error bars indicate the mean of SEM of 3-5 biological replicates. p values were calculated using GraphPad Prism9, 3-way ANOVA followed by a Fisher's LSD post hoc test.

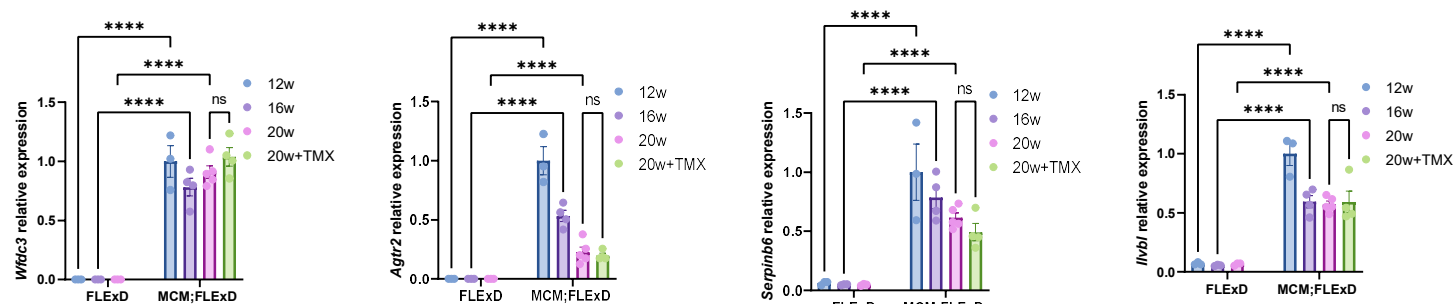
A

TA male



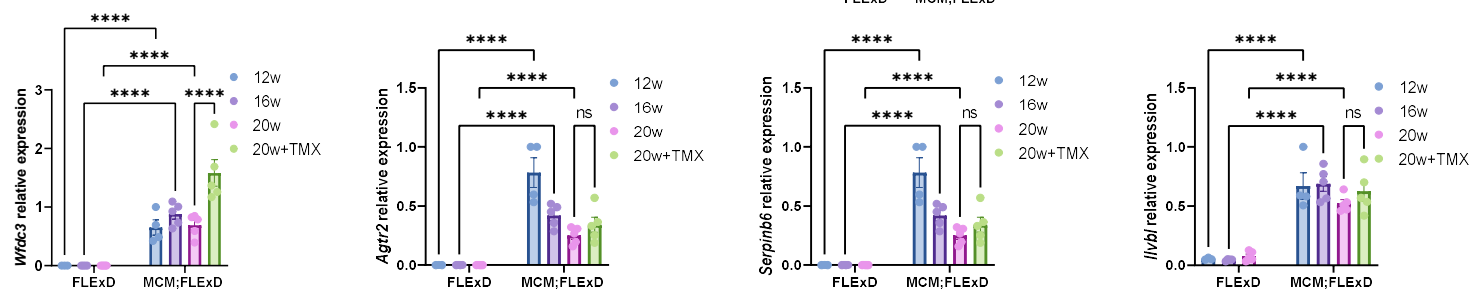
B

QUAD male



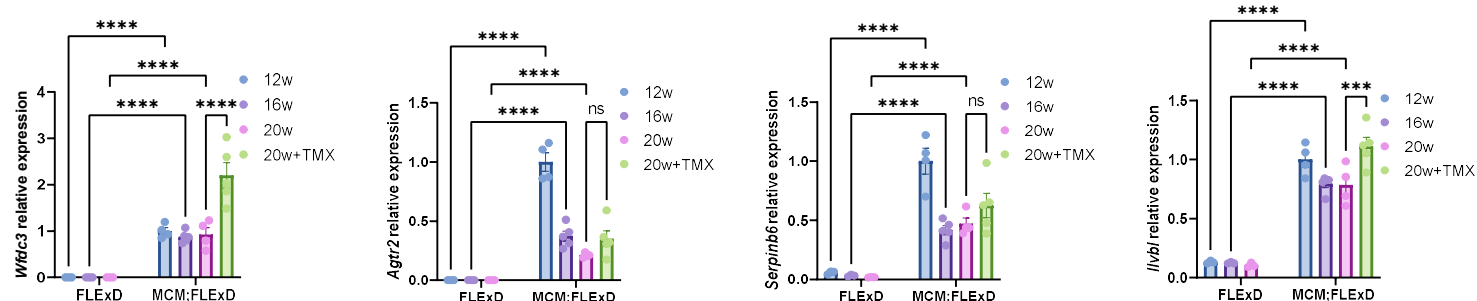
C

TA female



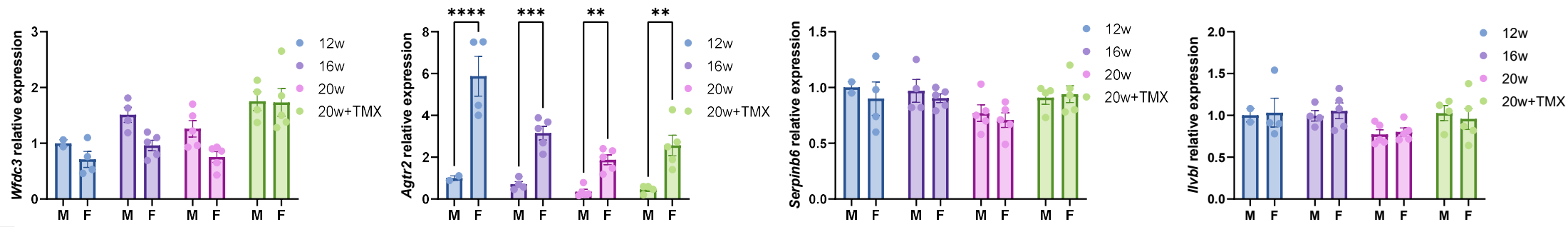
D

QUAD female

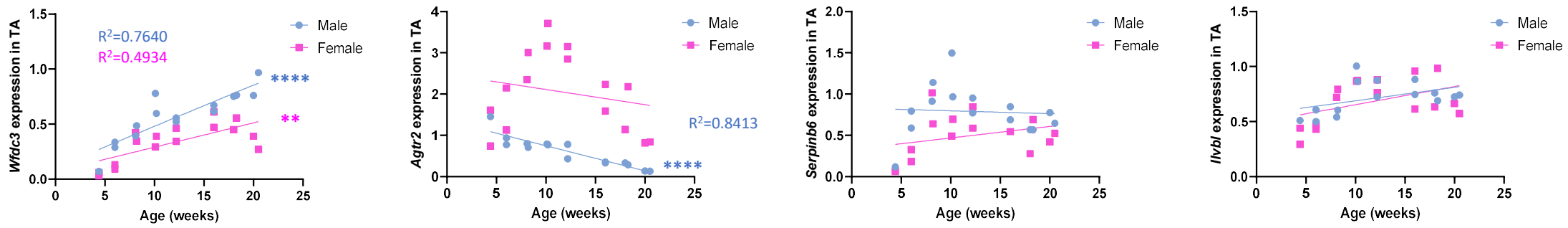


Supplementary Figure S5: expression of genes downstream of DUX4 in males (A&B) and females (C&D). RT-PCR for Wfdc3, Agtr2, Serpinb6 and Ilvbl in the tibialis anterior (A&C) and the quadriceps (B&D) muscles of FLExDUX4/+ and MCM;FLExDUX4/+ animals at the age of 12, 16 or 20 weeks. Some animals were injected with tamoxifen at the age of 16 weeks and muscles were harvested at the age of 20 weeks and compared to age-matched mice. Error bars indicate the mean of SEM of 3-5 biological replicates. p values were calculated using GraphPad Prism9, 2-way ANOVA followed by a Fisher's post hoc test. *p < 0.05; **p<0.01; ***p<0.001; ****p<0.0001. TMX: tamoxifen.

A

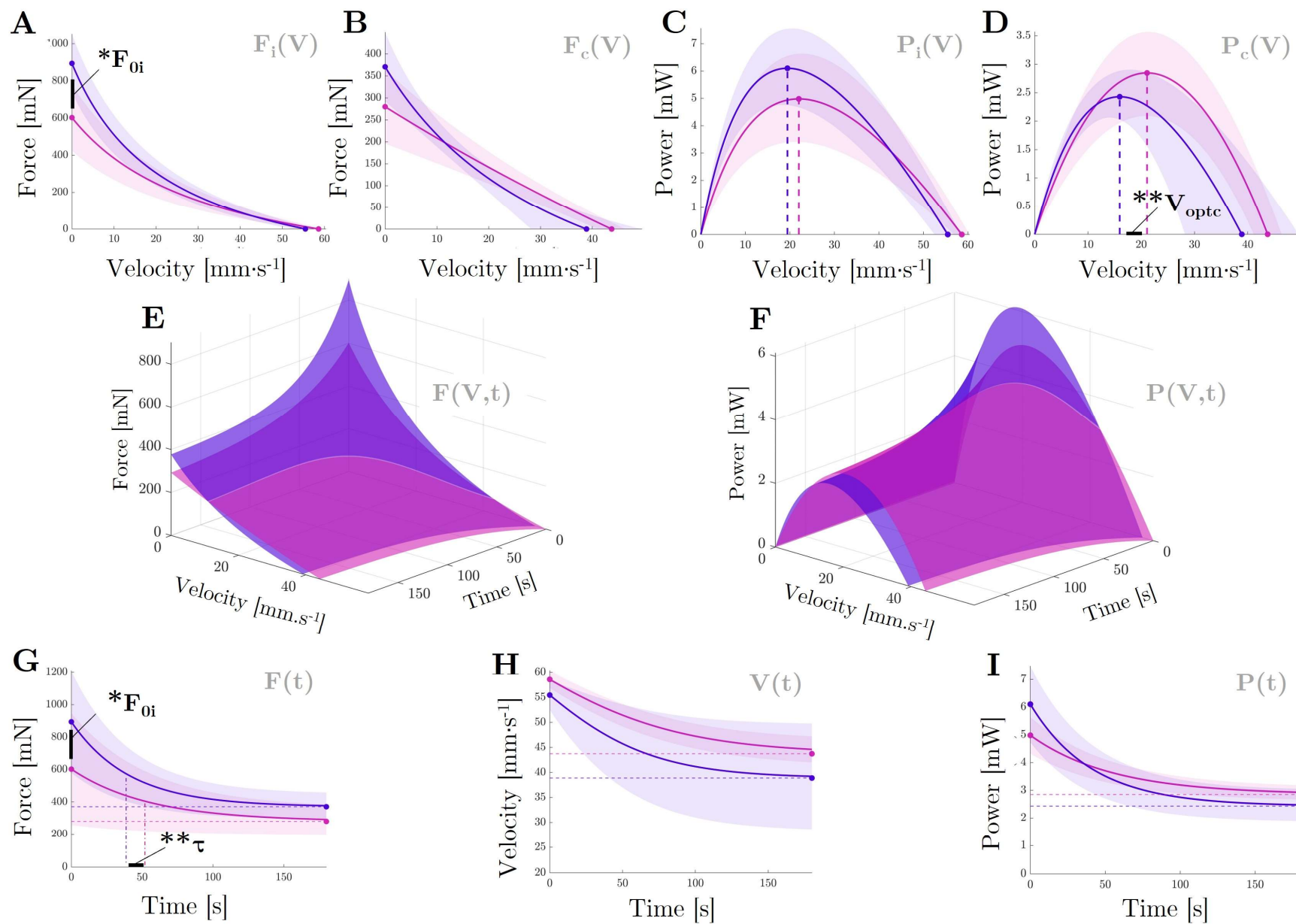


B



Supplementary Figure S6: Expression of the genes downstream of DUX4 in the MCM;FLEXDUX4/+ tibialis anterior muscles

The expression *Wfdc3*, *Agtr2*, *Serpinb6* and *Ilvbl* was compared in the tibialis anterior of MCM;FLEXDUX4/+, at the age of 12, 16 or 20 weeks, and in the absence or presence of tamoxifen (A). Error bars indicate mean of SEM of 3-5 biological replicates. P-values were calculated using Graphpad Prism9, 2-way ANOVA followed by a Fisher's LSD post hoc test. In B and C, the levels of *Wfdc3*, *Agtr2*, *Serpinb6* and *Ilvbl* were followed from 4 to 20 weeks of MCM;FLEXDUX4/+ males or female. P-values were calculated using Graphpad Prism9 after a simple linear regression analysis. A low P-value (< 0.05) means that the slope is likely not to equal zero. R² are indicated on each graph p<0.05. TMX: tamoxifen ; M : male ; F : female. p < 0.05; **p<0.01; ***p<0.001; ****p<0.0001.



Supplementary Figure S7: Absolute 3D Force (E) and Power (F) -velocity-time relationships in ACTA1-MCM/+ (purple) and Acta1-MCM;FLEXDUX4/+ mice (pink). 2D projections are presented : (A) initial force-velocity; (B) critical force-velocity; (C) initial power-velocity; (D) critical power-velocity; (G) maximal force-time; (H) maximal velocity-time; (I) maximal power-time. p values were calculated using GraphPad Prism9: 2-way ANOVA followed by a Fisher's LSD post hoc was performed. When significant, indices are represented graphically on 2D projections: F_{0i} : initial muscle maximal force in isometric condition (i.e. static, null velocity); V_{optc} : critical optimal contraction velocity (i.e. maximizing power production); τ : characteristic decay time (at τ seconds $\approx 63\%$ of the final decay is reached); * $p < 0.05$; ** $p < 0.01$.