Overexpression of eIF3f protein protects mouse skeletal muscle from disuse atrophy during hindlimb immobilization

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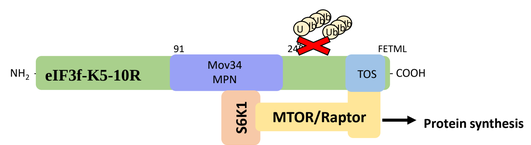
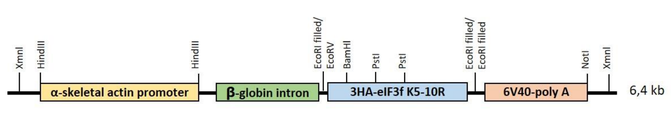
INTRODUCTION

Muscle mass homeostasis is controlled by protein synthesis and degradation. The eukaryotic initiation factor 3f, a subunit of eIF3 complex, plays an important role in the atrophy/hypertrophy antagonism. eIF3f is a fundamental element of MTORC1 pathway, allowing physical interaction between S6K1 and MTOR. Phosphorylation of downstream effectors of MTORC1 pathway like S6 leads to enhanced translational capacity and myotubes hypertrophy. eIF3f is also an ubiquitination target of MAFbx ligase, which is activated when proteolysis is stimulated. Once ubiquitinated, eIF3f is degraded by proteasome while MTORC1 pathway and consecutively, protein synthesis are inhibited in myotubes. Ubiquitination sites were suppressed to create a mutant form of eIF3f which is resistant to proteolysis. When transfected in myotubes, eIF3f K5-10R induces hypertrophy. Generation of transgenic mice carrying *eIF3f-K5-10R* gene allows to further define eIF3f roles in the atrophy/hypertrophy antagonism *in vivo*.

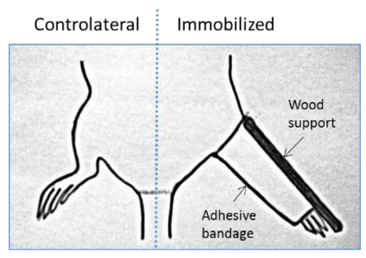
MATERIAL & METHODS

Mouse *eIF3f* gene was modified on six lysine residues of C-terminal domain to generate a eIF3f-K5-10R mutant form. Three HA tags were inserted and 3HA-eIF3f-K5-10R transgene was placed under HSA promoter control. Transgenic mice were produced by ADN microinjection into fertilized oocytes on a C57BL/6 genetic background. Simple transgenic mice were intercrossed in our laboratory to generate double transgenic individuals (eIF3f $^{Tg/Tg}$).





6/9-months-old wild-type (WT) and eIF3f $^{Tg/Tg}$ males mice were submitted to unilateral hindlimb immobilization. Mice were anesthetized by isoflurane inhalation to gently fix adhesive bandage. A puromycin injection was performed 15 minutes before cervical dislocation. Analysis of mass, cross-sectional areas (CSA), synthesis flows, MTORC1 pathway activity and eIF3f expression were all conducted on *gastrocnemius* muscles. Opposed hindlimb was used as an internal control.

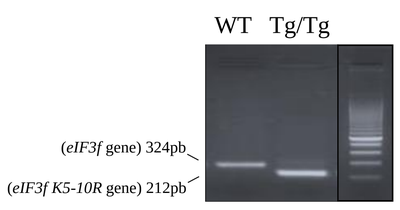


MODEL CHARACTERIZATION

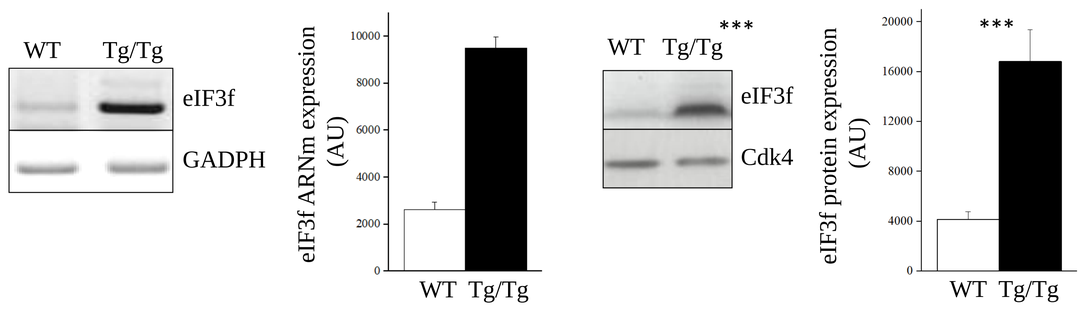
Values are mean ± SEM

*Significantly different at * : p <0.05, ** : p <0.01 or *** : p <0.001.*

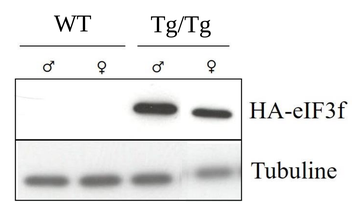
Genotyping of eIF3f $^{Tg/Tg}$ mice



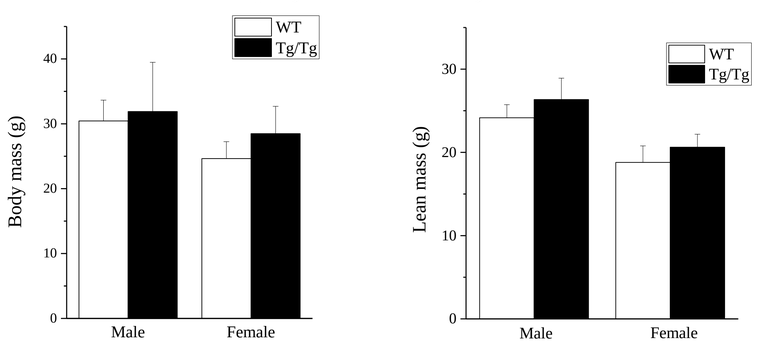
eIF3f ARNm and protein expression levels are higher in eIF3f $^{Tg/Tg}$ skeletal muscle



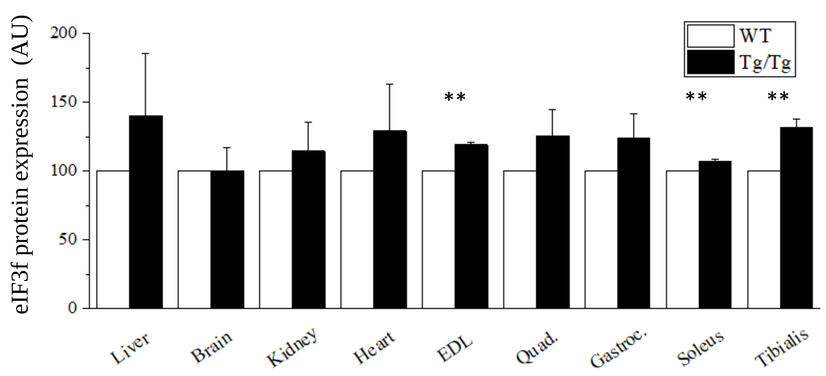
eIF3f $^{Tg/Tg}$ mice express HA-eIF3f in skeletal muscle



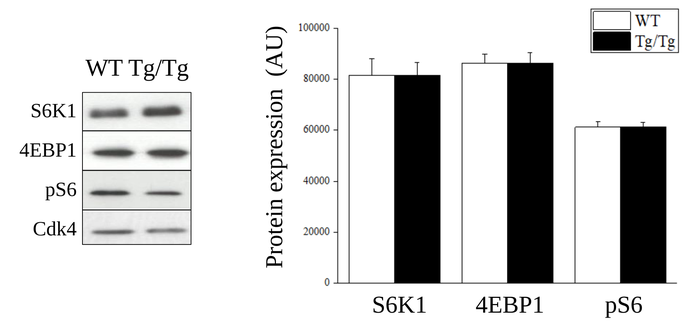
eIF3f $^{Tg/Tg}$ mice display a normal body mass and composition



Skeletal muscles mass is slightly increased in eIF3f $^{Tg/Tg}$ mice

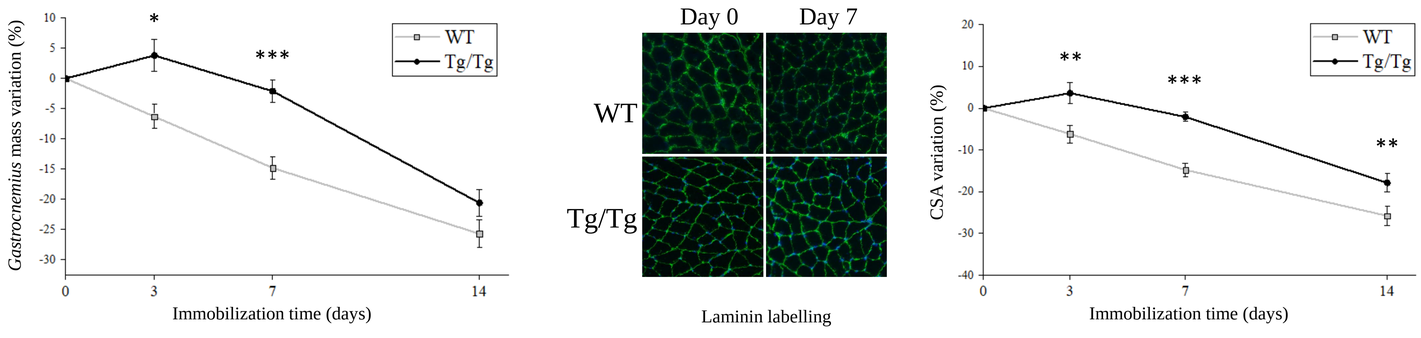


Mutation does not impact MTORC1 pathway activity

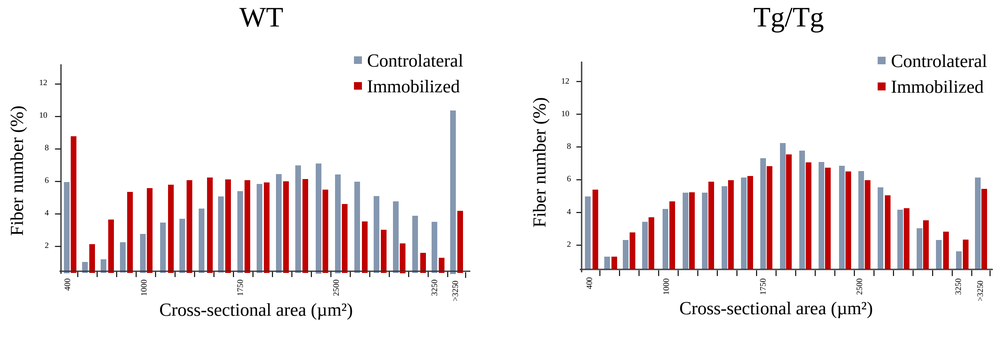


HINDLIMB IMMOBILIZATION STUDY

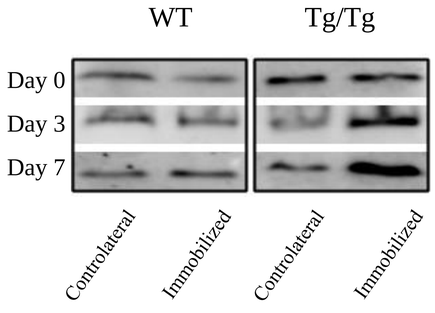
Muscle mass loss and CSA reduction of *gastrocnemius* are slowed down in eIF3f $^{Tg/Tg}$ mice



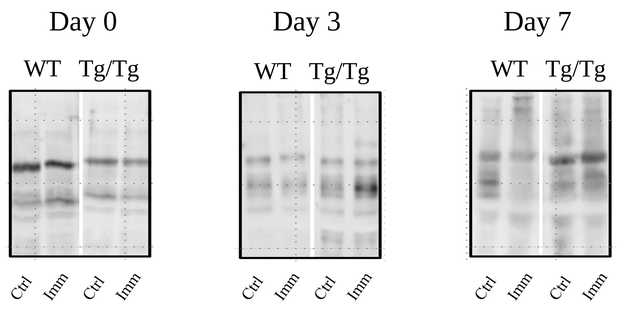
Fiber size distribution of *gastrocnemius* is conserved in eIF3f $^{Tg/Tg}$ mice after 7 days



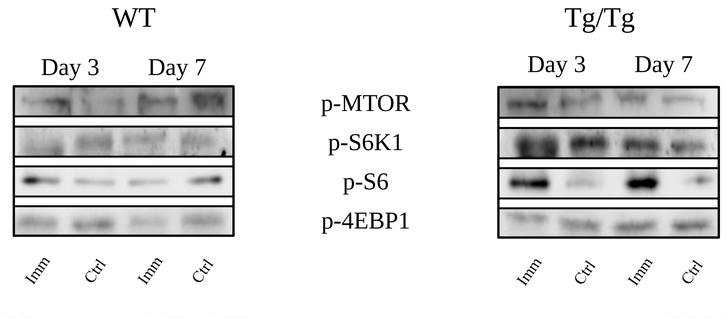
eIF3f expression is induced

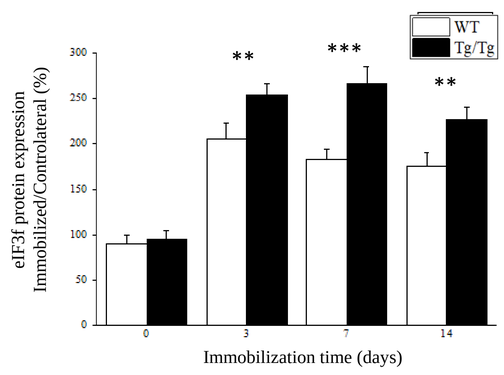


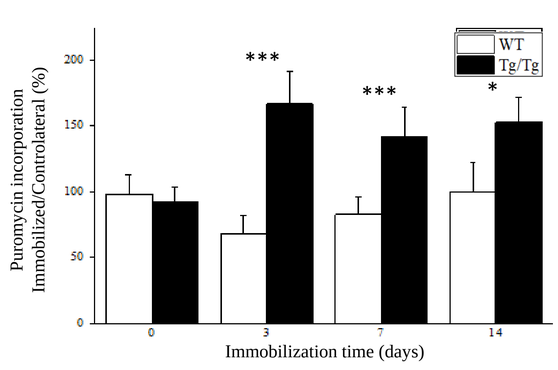
Protein synthesis is stimulated in eIF3f $^{Tg/Tg}$ mice

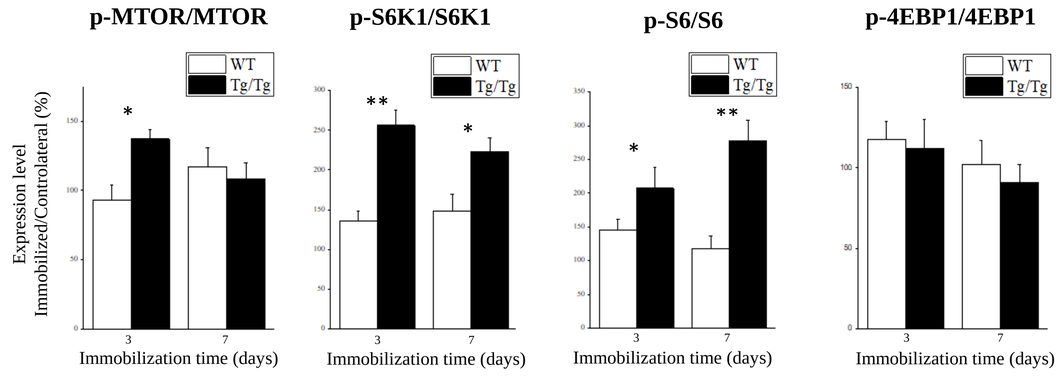


MTORC1 pathway activity is higher in eIF3f $^{Tg/Tg}$ mice

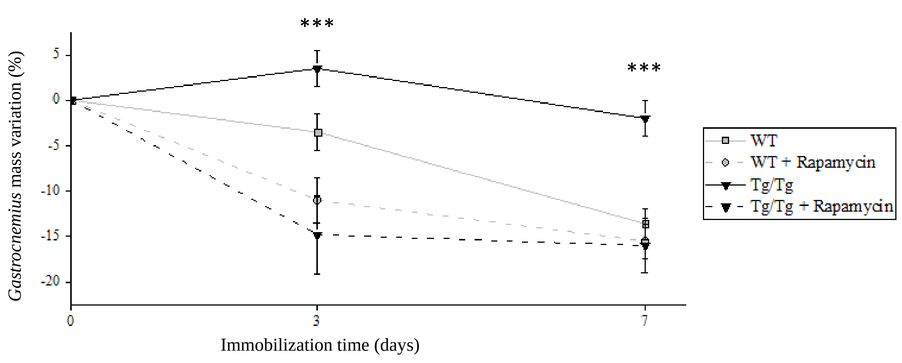




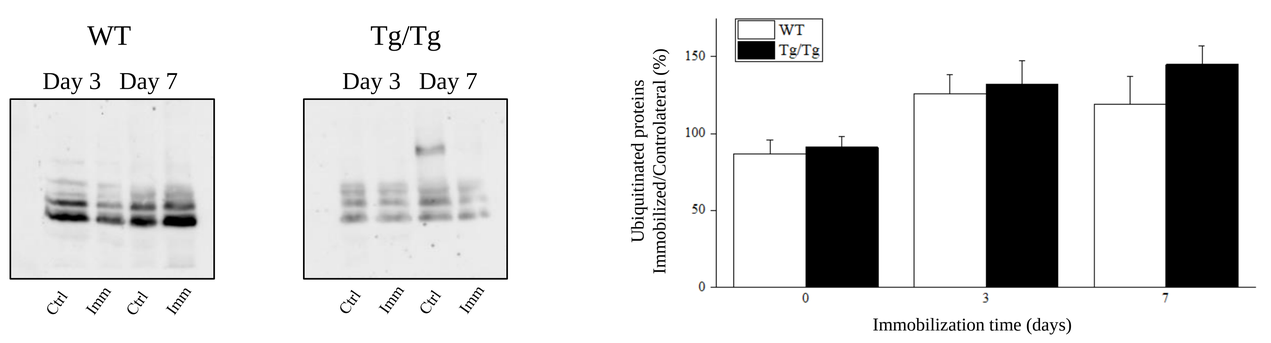




Resistance to immobilization-induced atrophy in eIF3f $^{Tg/Tg}$ mice is MTOR-dependent



Proteins ubiquitination does not differ between WT and eIF3f $^{Tg/Tg}$ mice



CONCLUSION

Double transgenic mice carrying *eIF3f K5-10R* gene display elevated eIF3f ARNm and protein expression levels in skeletal muscles. eIF3f $^{Tg/Tg}$ mice present a normal phenotype with a slight increase in skeletal muscles mass. At basal state, higher eIF3f protein expression does not impact protein synthesis rate and MTORC1 pathway activity. During immobilization, decreases of *gastrocnemius* muscle mass and CSA are slower eIF3f $^{Tg/Tg}$ mice. Maintain of fiber size distribution after one week of immobilization confirms transgenic mice resistance to induced-atrophy. eIF3f overexpression during disuse is associated with protein synthesis stimulation and higher MTORC1 activity whereas proteolysis does not seem to be affected.

These results highlight that eIF3f overexpression in skeletal muscle promotes MTOR-dependent protein synthesis, and thus, protects muscle from disuse atrophy.