eIF3f depletion impedes mouse embryonic development, reduces adult skeletal muscle mass and amplifies muscle loss during disuse

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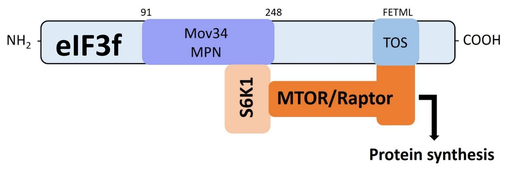
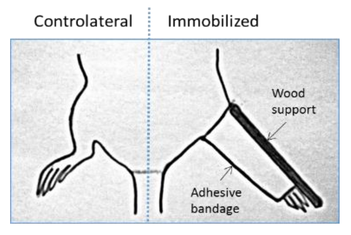
INTRODUCTION

Muscle mass homeostasis is controlled by protein synthesis and degradation. Eukaryotic initiation factor 3f, a subunit of eIF3 complex, 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. Despite its important role in protein synthesis regulation shown *in vitro*, its physiological role in skeletal muscle is poorly described. Generation of mice carrying a mutation in the *eIF3f* gene allows to further define *in vivo* eIF3f roles in muscular homeostasis.

MATERIAL & METHODS



Entire *eIF3f* allele coding sequence was substituted by *lacZ* reporter gene to generate heterozygous mice (eIF3f $^{+/-}$). eIF3f $^{+/-}$ mice intercrosses were done to generate homozygous eIF3f knockout. Genotyping using eIF3f and lacZ primers allowed to identify newborn mutants. eIF3f mRNA and protein expression were analyzed in skeletal muscles of eIF3f $^{+/-}$ and wild-type (WT) mice. Then, 6/9-months-old C57BL/6 WT and eIF3f $^{+/-}$ males mice were submitted to unilateral immobilization for 3, 7 or 14 days. 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 and MTORC1 pathway activity were all conducted on quadriceps muscle. Opposed hindlimb was used as an internal control.



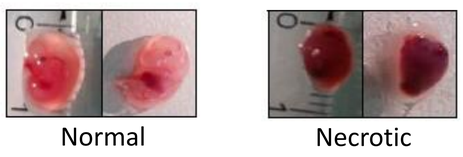
MODEL CHARACTERIZATION

Values are mean ± SEM

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

Embryonic death occurs at early stage of development

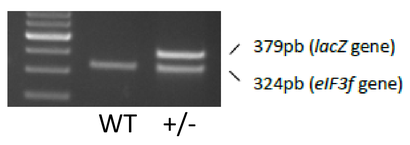
Embryo phenotype	Normal	Necrotic
Percentage (n=116)	76,7	23,3



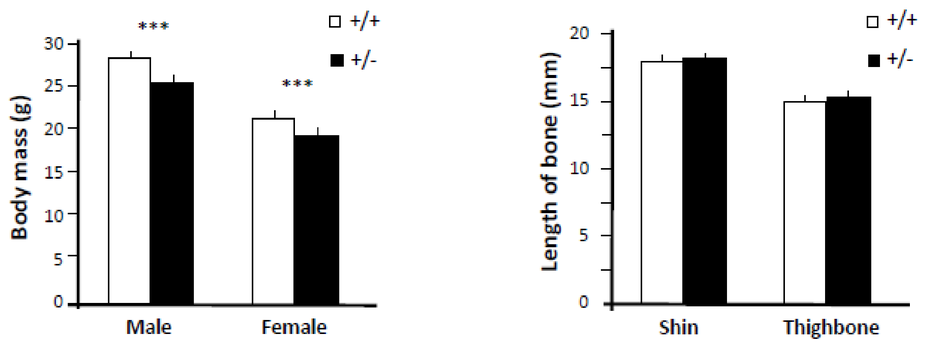
Any homozygous eIF3f $^{-/-}$ embryo was recovered

Embryo genotype	+/+	+/-	??
Percentage (n=116)	25,8	50,2	24,0

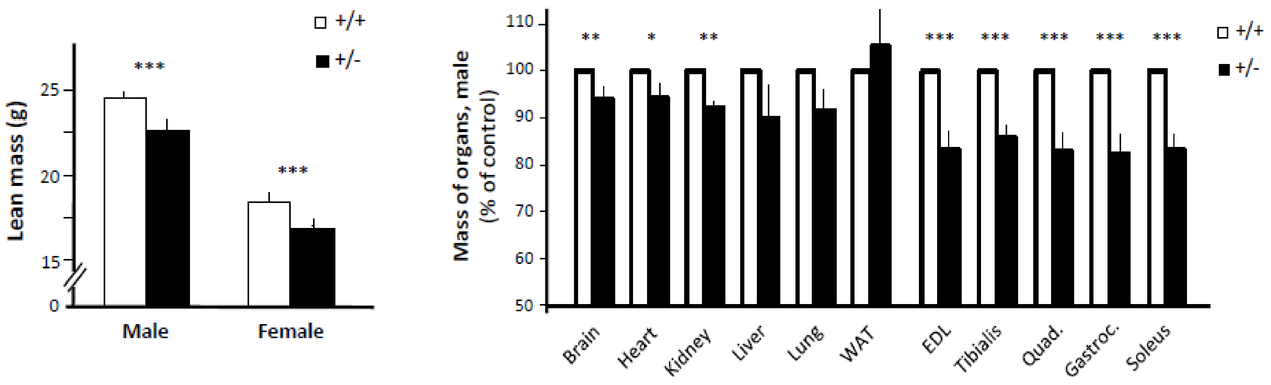
lacZ gene allows to identify eIF3f $^{+/-}$ mice



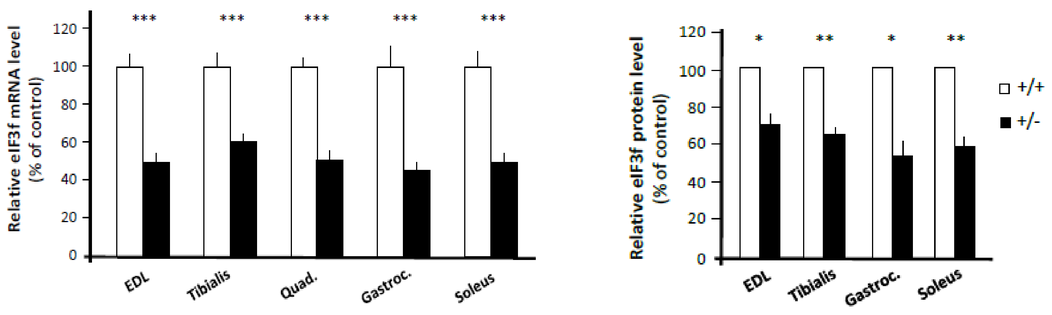
eIF3f $^{+/-}$ are viable and fertile and display diminushed body mass



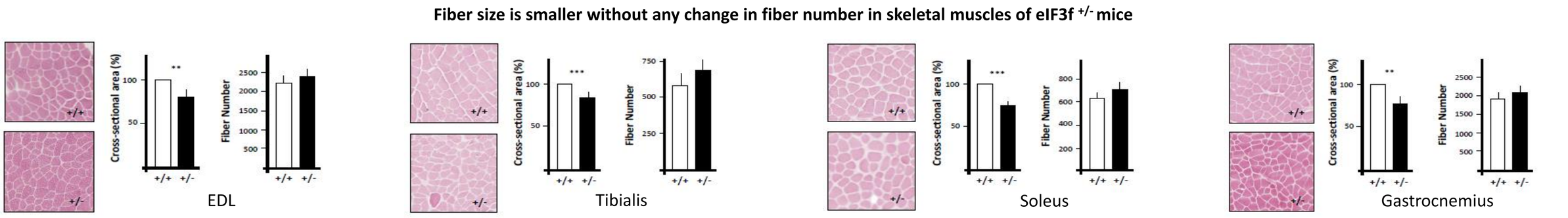
Reduced eIF3f expression mainly impacts skeletal muscles mass



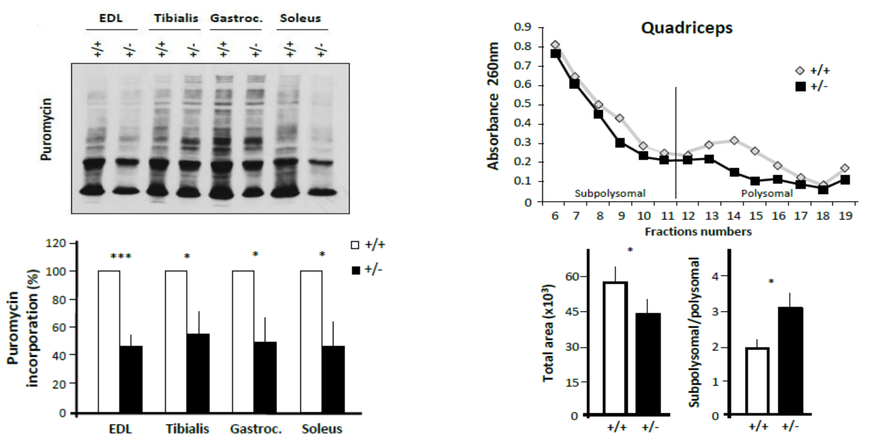
eIF3f mRNA and protein expression levels are lower in eIF3f $^{+/-}$ skeletal muscles



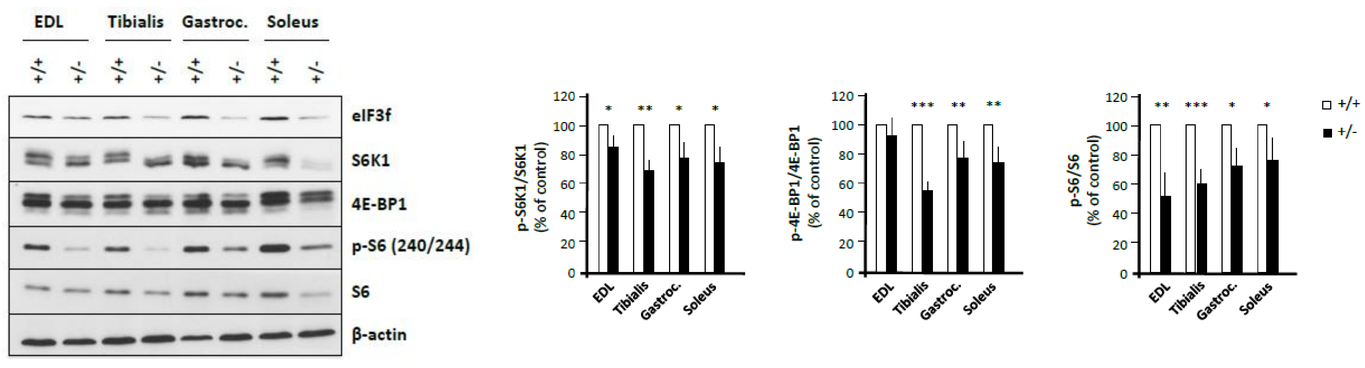
Fiber size is smaller without any change in fiber number in skeletal muscles of eIF3f $^{+/-}$ mice



Protein synthesis activity and efficiency are altered in eIF3f $^{+/-}$ mice

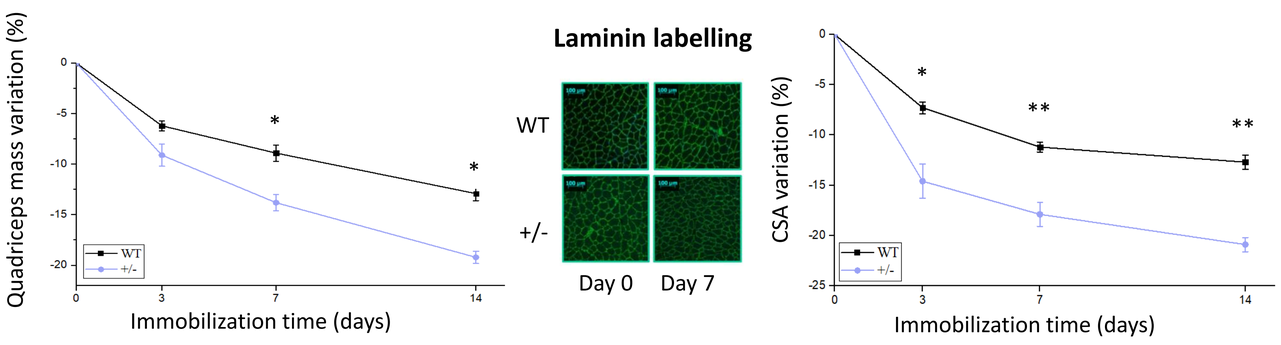


MTORC1 dowstream effectors activity is decreased in skeletal muscles of eIF3f $^{+/-}$ mice

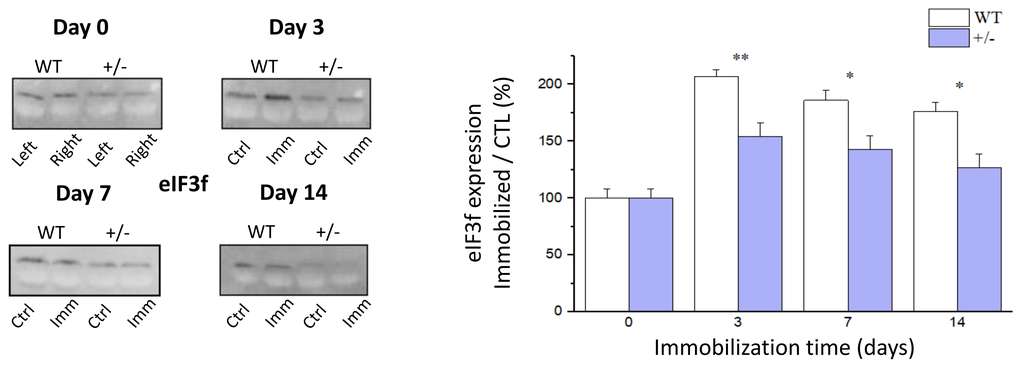


HINDLIMB IMMOBILIZATION STUDY

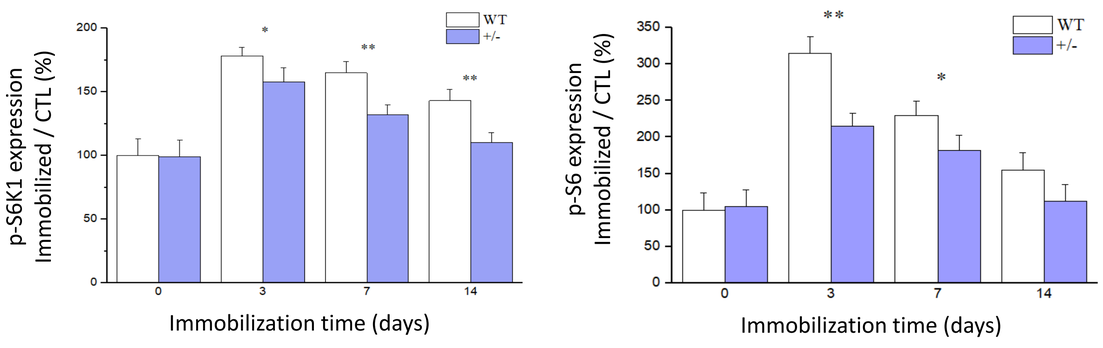
Decrease of muscle mass and CSA is larger in eIF3f $^{+/-}$ mice



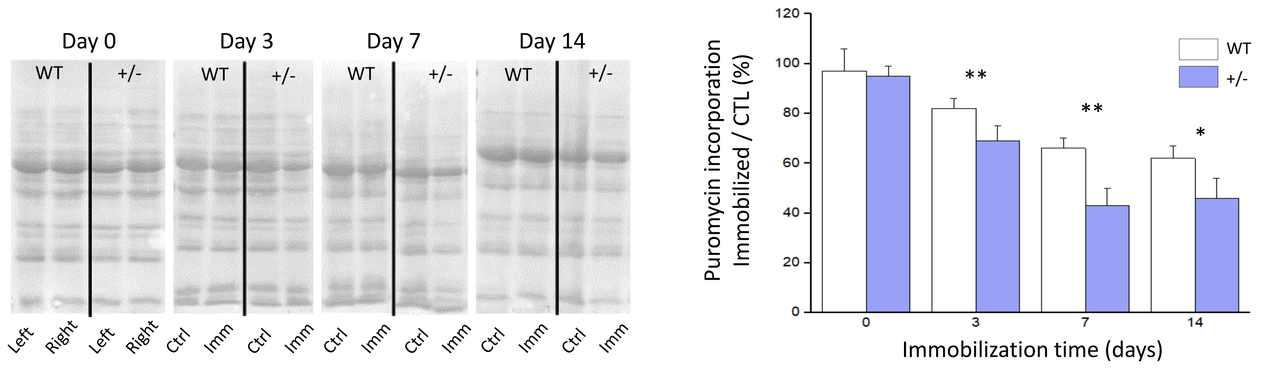
Induction of eIF3f expression is decreased in eIF3f $^{+/-}$ mice



Activities of S6K1 and S6 are reduced in eIF3f $^{+/-}$ mice



Reduced eIF3f expression level exacerbates protein synthesis decrease



CONCLUSION

Embryonic death suggests an essential role of eukaryotic Initiation Factor 3f for mice development. Whole body partial depletion allows normal growth despite a body mass reduction without change in body size. Mass lowering concerns only lean mass, skeletal muscles of heterozygous mice displaying a smaller fiber size. eIF3f mRNA and protein expressions are reduced in muscles of eIF3f $^{+/-}$ mice, as well as protein synthesis and MTORC1 pathway activity. During immobilization, reduction of eIF3f induction results in a stronger atrophy, with a larger decrease in muscle mass and CSA in eIF3f $^{+/-}$ mice. Protein synthesis is also reduced and associated with a lower MTORC1 pathway activity in eIF3f $^{+/-}$ mice.

These results confirm *in vivo* the essential role of eIF3f in development, muscular growth and muscle mass homeostasis.