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## **FoxO3a overexpression prevents both glycogen overload and autophagic buildup in skeletal muscle of Pompe disease**

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## Background and Objectives

Pompe disease (glycogen storage disease type II) is a lysosomal storage disorder caused by the mutation of acid  $\alpha$ -glucosidase (Gaa), the unique enzyme degrading glycogen in glucose into lysosomes. A **massive glycogen overload** is described in Pompe patients, mainly in skeletal and cardiac muscles. Furthermore, severe impairment of autophagic flux has been described, highlighted by **autophagic buildup**. Contrary to cardiac muscle, **no treatment currently allows to cure efficiently and durably the skeletal muscle**. We have identified the transcription factor **Forkhead box O3 (FoxO3a)** as a potential target to alleviate skeletal muscle impairments through its **key role on regulation of both glycogen homeostasis and autophagy**.

The objectives of the study were:

1/ to explore the preventive effect of FoxO3a overexpression on :

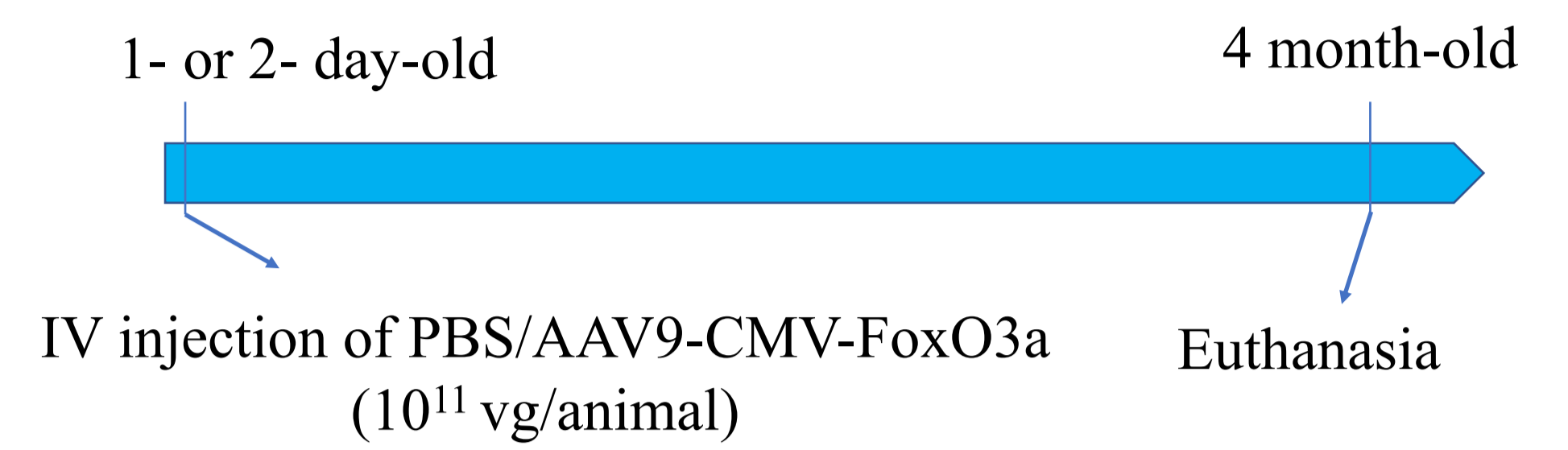
- glycogen overload
- autophagic flux impairments

2/ to investigate the impact of FoxO3a overexpression on skeletal muscle remodeling

## Material and Methods

### Experimental design

Gaa<sup>+/+</sup>: n = 5 male  
Gaa<sup>-/-</sup>: n = 5 male  
Gaa<sup>-/-</sup>-FoxO3a: n = 5 male

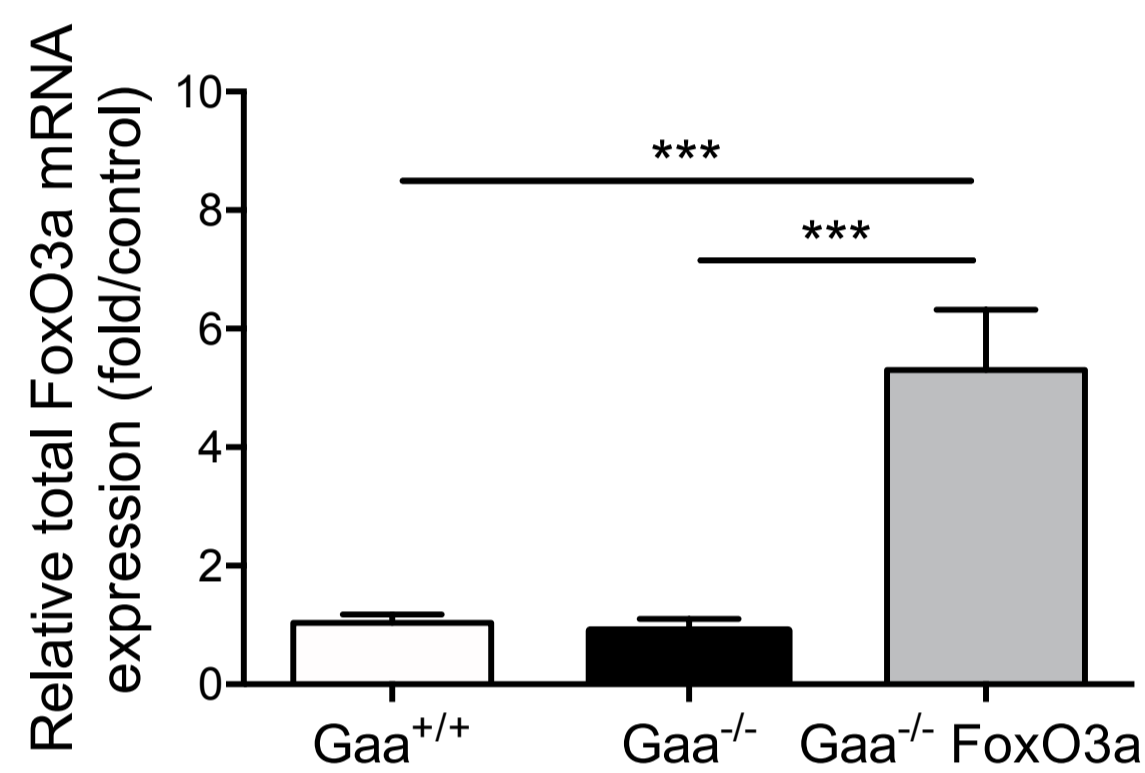


### Analyses performed

- **Characterization of the glycogen overload:** Periodic Acid Schiff (PAS) staining and glycogen assay
- **Exploration of the autophagic buildup:** Immunolabelling of LC3 (autophagosomal marker) and WGA (a lysosomal content marker); western blot against p62 (autophagic flux impairment marker)
- **Investigation of the morphological damages:** Anisocytosis and centronucleation quantification

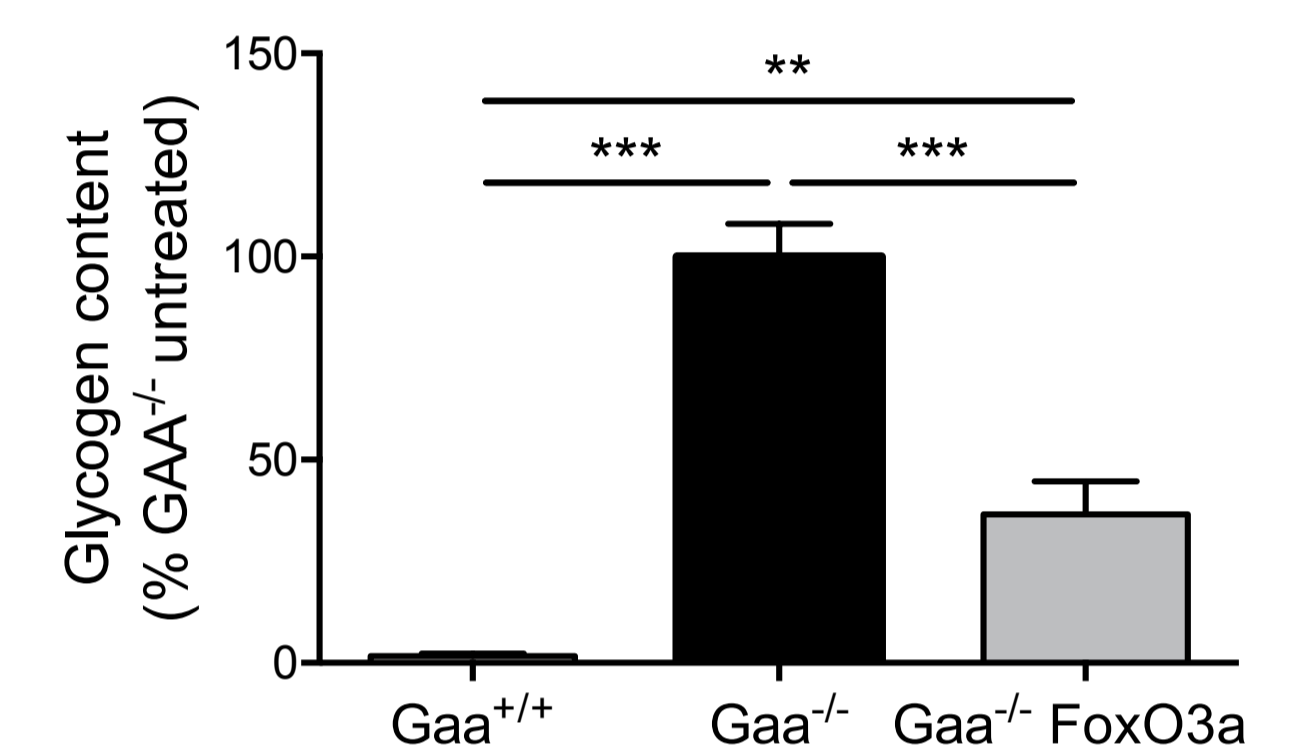
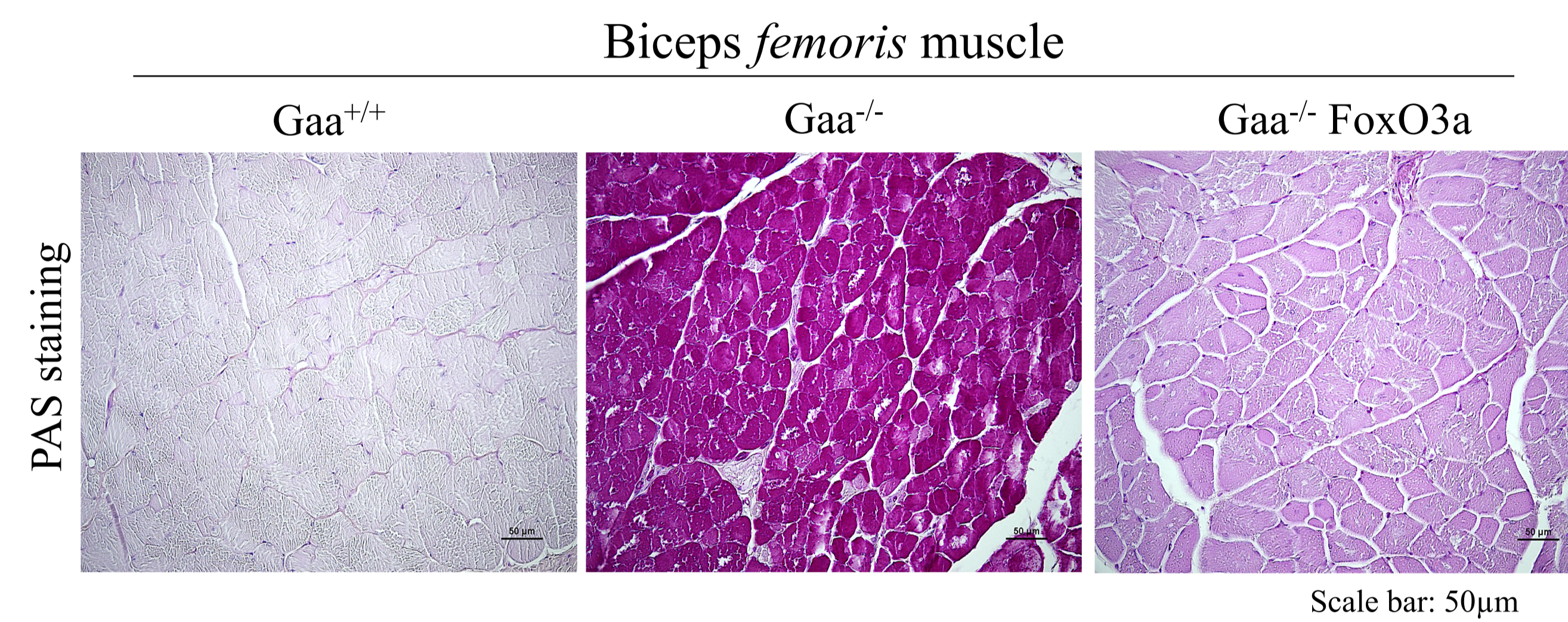
## Results

### 1/ Validation of FoxO3a overexpression



Quantification of total FoxO3a mRNA expression in *Biceps femoris* muscle by qPCR. Statistical analysis : One-way ANOVA, Newman-Keuls post hoc test. \*\*\*p<0,001. n=5 mice/group.

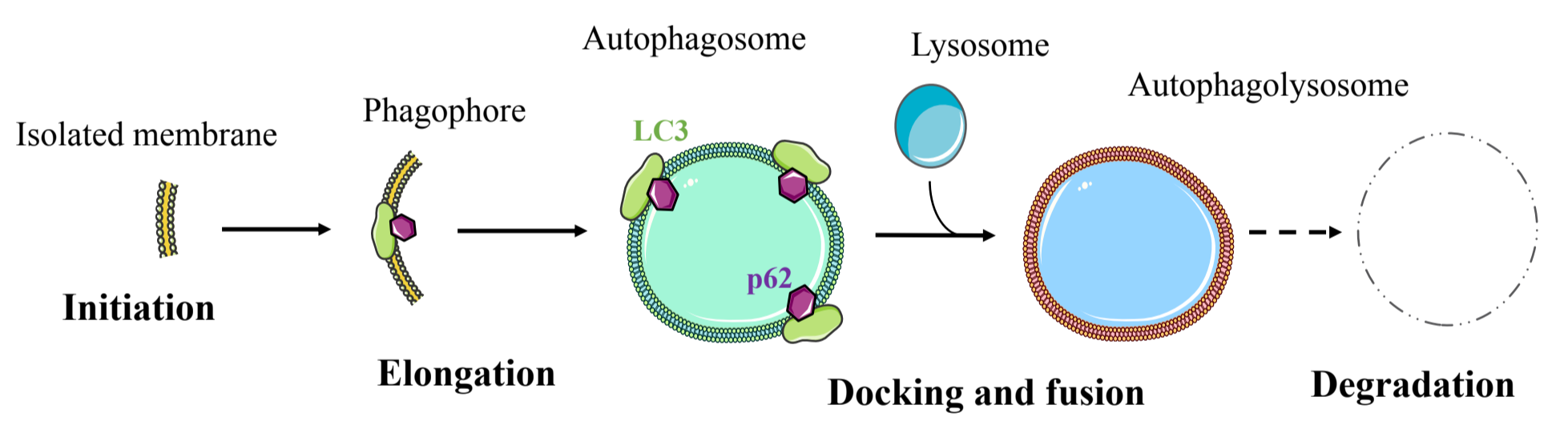
### 2/ FoxO3a overexpression prevents the glycogen overload in skeletal muscle



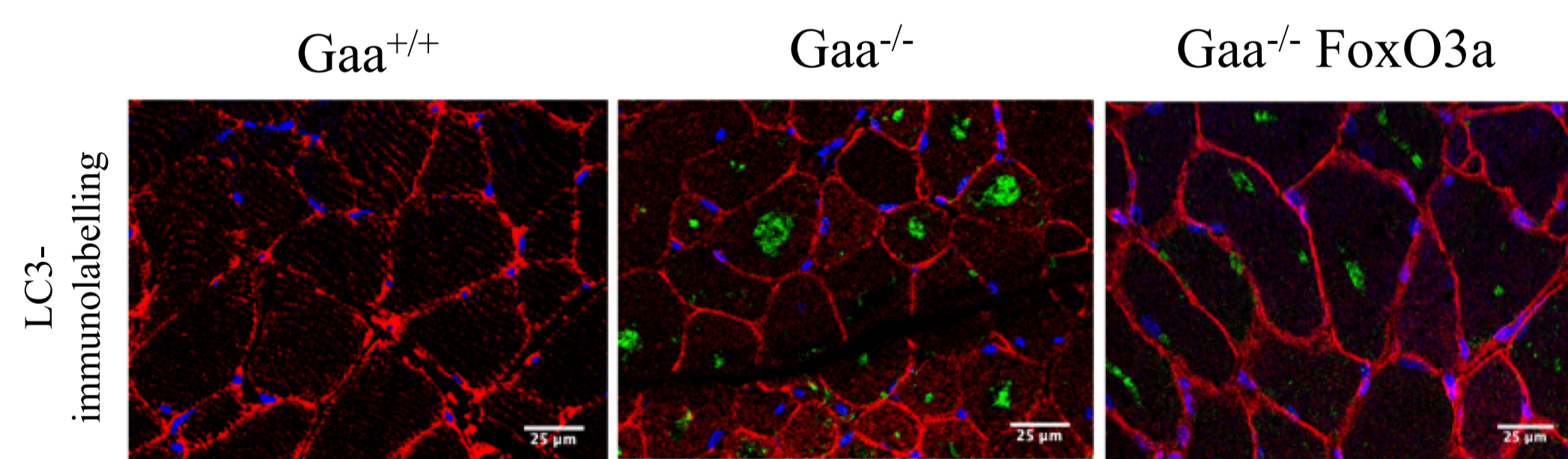
Glycogen assay of *Biceps femoris* muscle extract. Statistical analysis : One-way ANOVA, Newman-Keuls post hoc test. \*\*p<0,01, \*\*\*p<0,001. n=5 mice/group.

### 3/ FoxO3a overexpression prevents the autophagic buildup accumulation in skeletal muscle

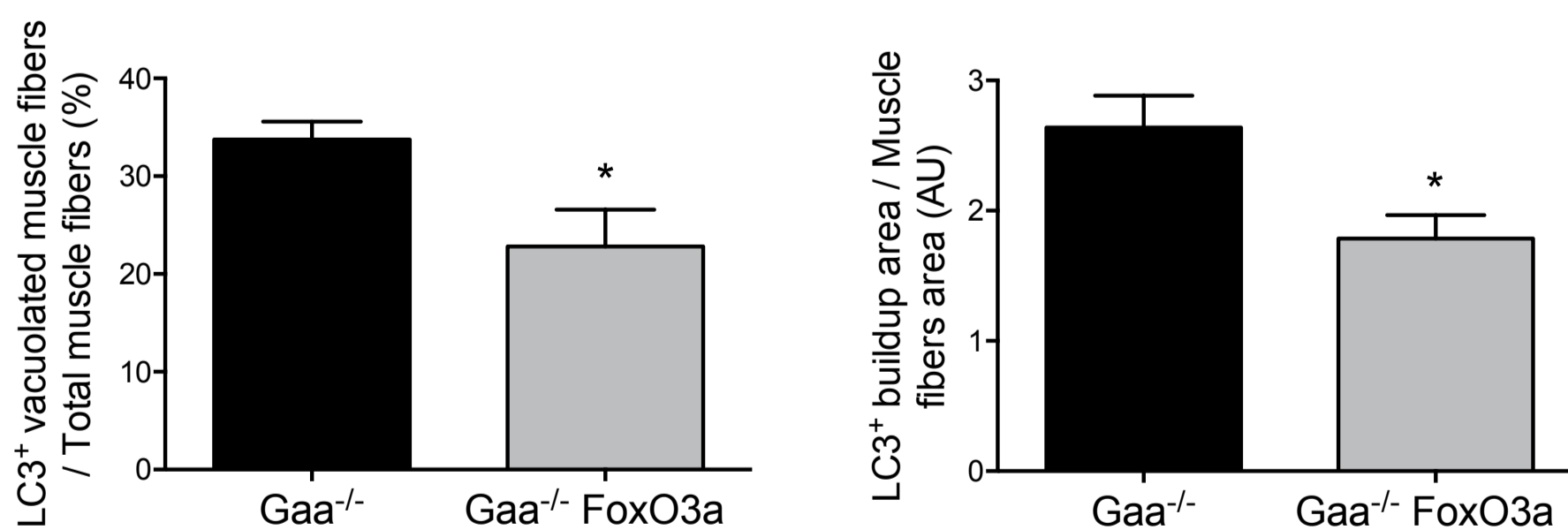
#### Autophagic flux markers



#### Prevention of the autophagic buildup

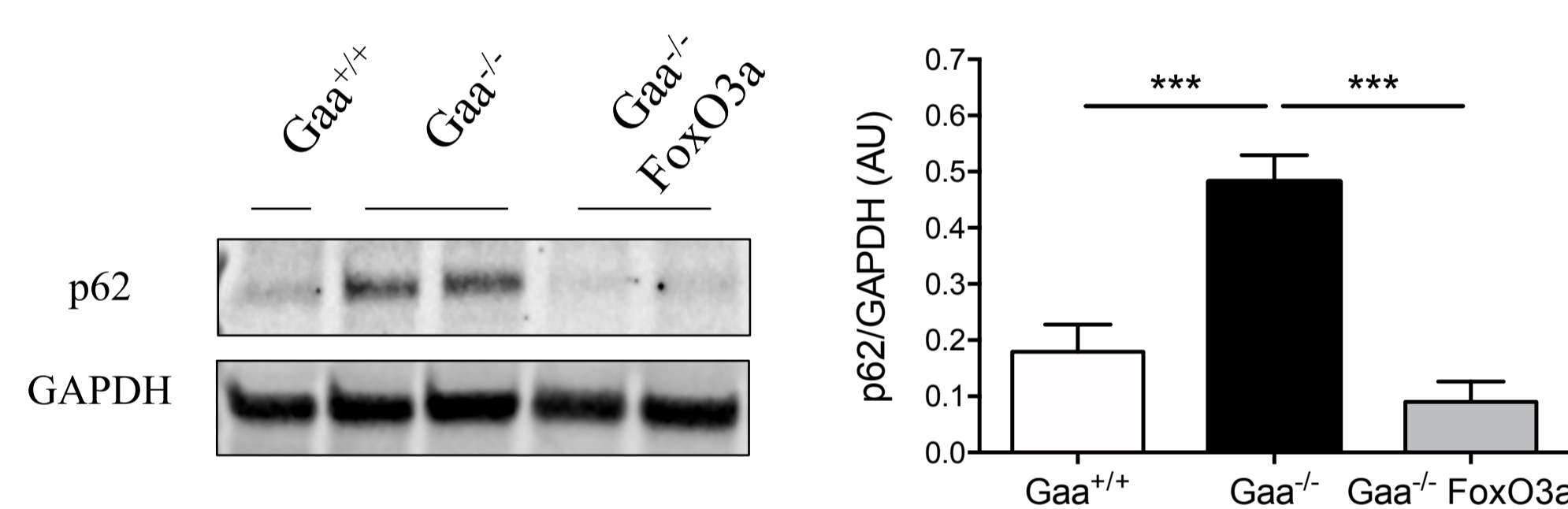


Representative LC3 (green) and dystrophin (red) immunolabelling of *Biceps femoris* muscle. Nuclei were counterstained using Hoechst. Scale bar: 25µm



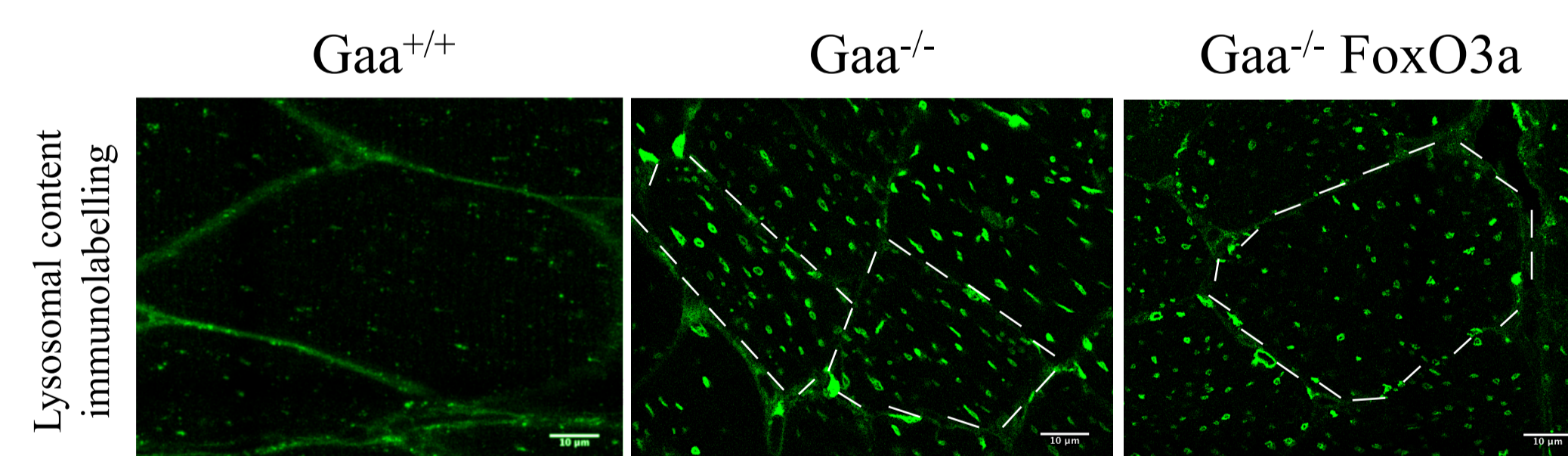
Quantification of LC3<sup>+</sup>-vacuolated fibers (left) and of the LC3<sup>+</sup>-buildup size (right) in *Biceps femoris* muscle. Statistical analysis : Mann-Whitney test, \*p<0,05. n=5 mice/group.

#### Prevention of the autophagic flux impairments

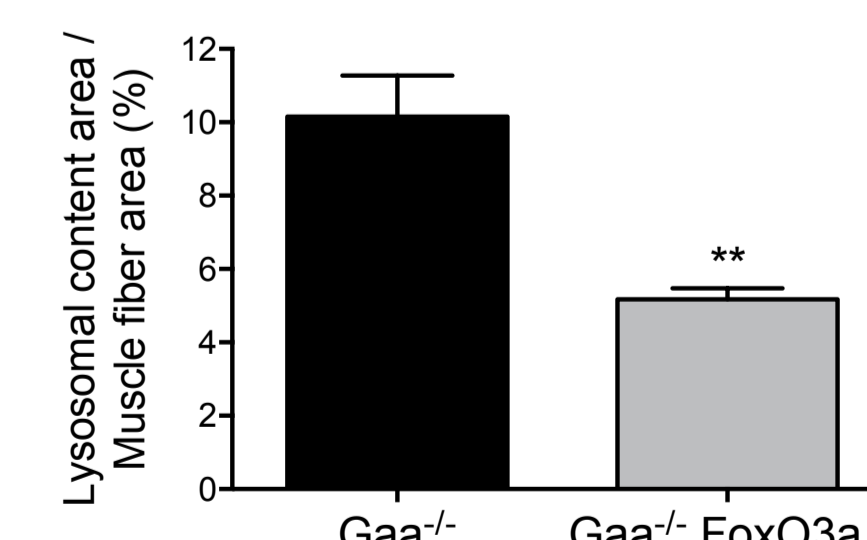


Representative western blot analysis of *Biceps femoris* muscle lysates using anti-p62 antibody. An anti-GAPDH antibody was used as a loading control. Statistical analysis : One-way ANOVA, Newman-Keuls post hoc test. \*\*\*p<0,001. n=5 mice/group.

#### Prevention of the lysosome overload



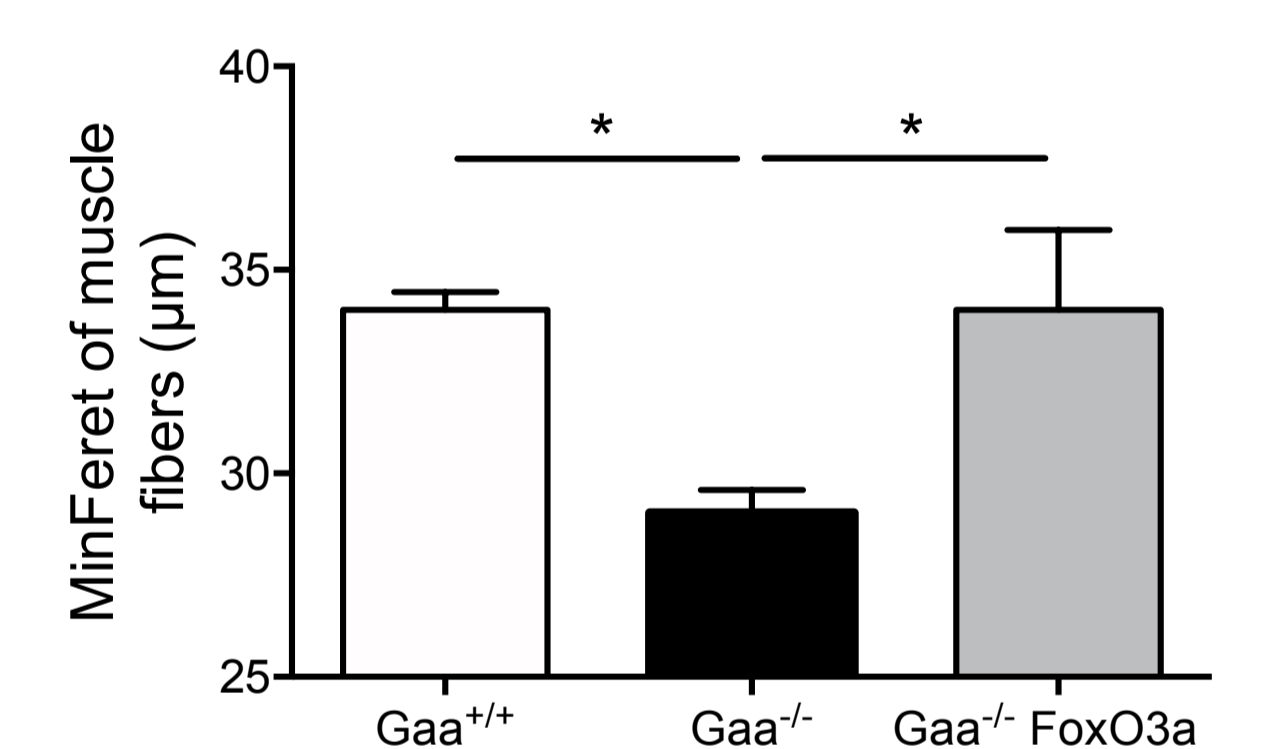
Representative lysosomal content immunolabelling using WGA (green) of *Biceps femoris* muscle. Edges of muscle fibers were marked using white line dots for Gaa<sup>-/-</sup> and Gaa<sup>-/-</sup>-FoxO3a. Scale bar: 10µm.



Quantification of lysosomal content (vesicles WGA<sup>+</sup>) size in *Biceps femoris* muscle. Statistical analysis : Mann-Whitney test, \*\*p<0,01. n=5 mice/group.

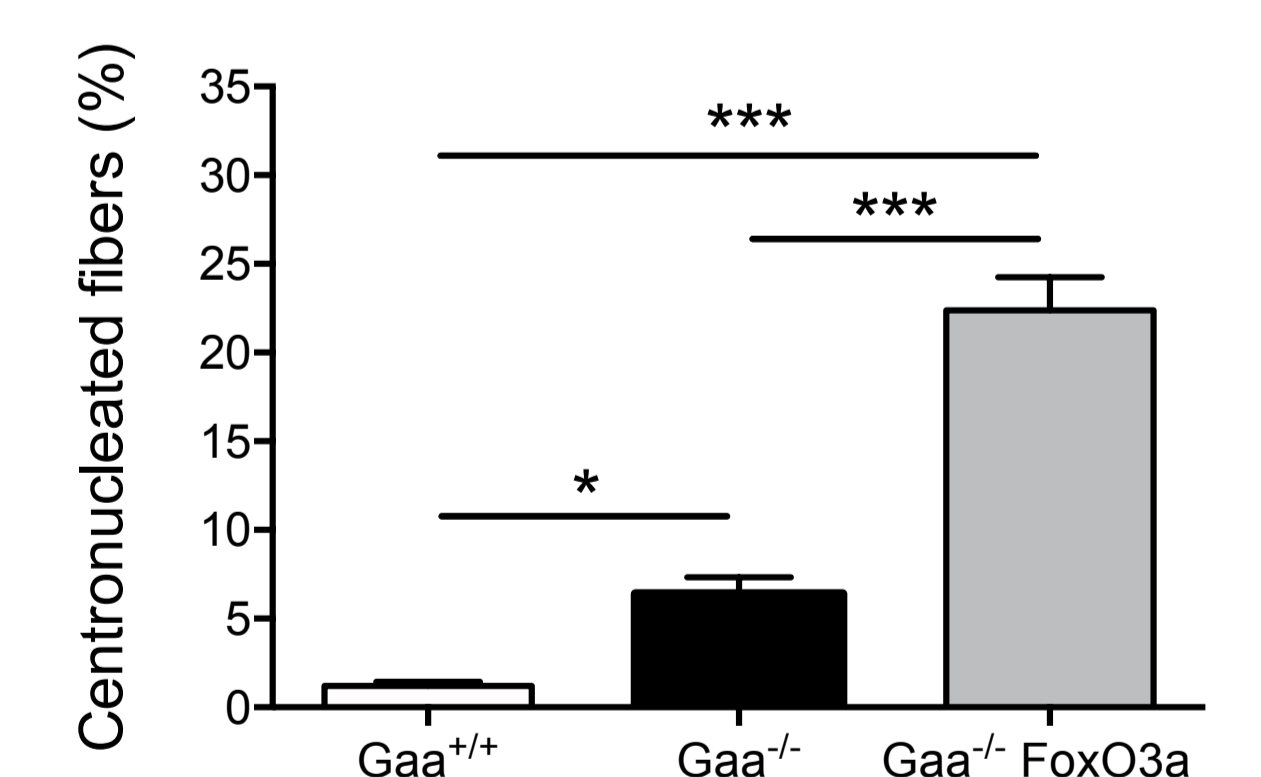
### 4/ FoxO3a overexpression prevents skeletal muscle remodeling

#### Prevention of atrophy



Analysis of the minimum Feret diameter (MinFeret; shortest distance between two parallel tangents of the muscle fiber edges) of *Biceps femoris* muscle. Statistical analysis: Two-way ANOVA, Newman-Keuls post hoc test. \*p<0,05. n=5 mice/group.

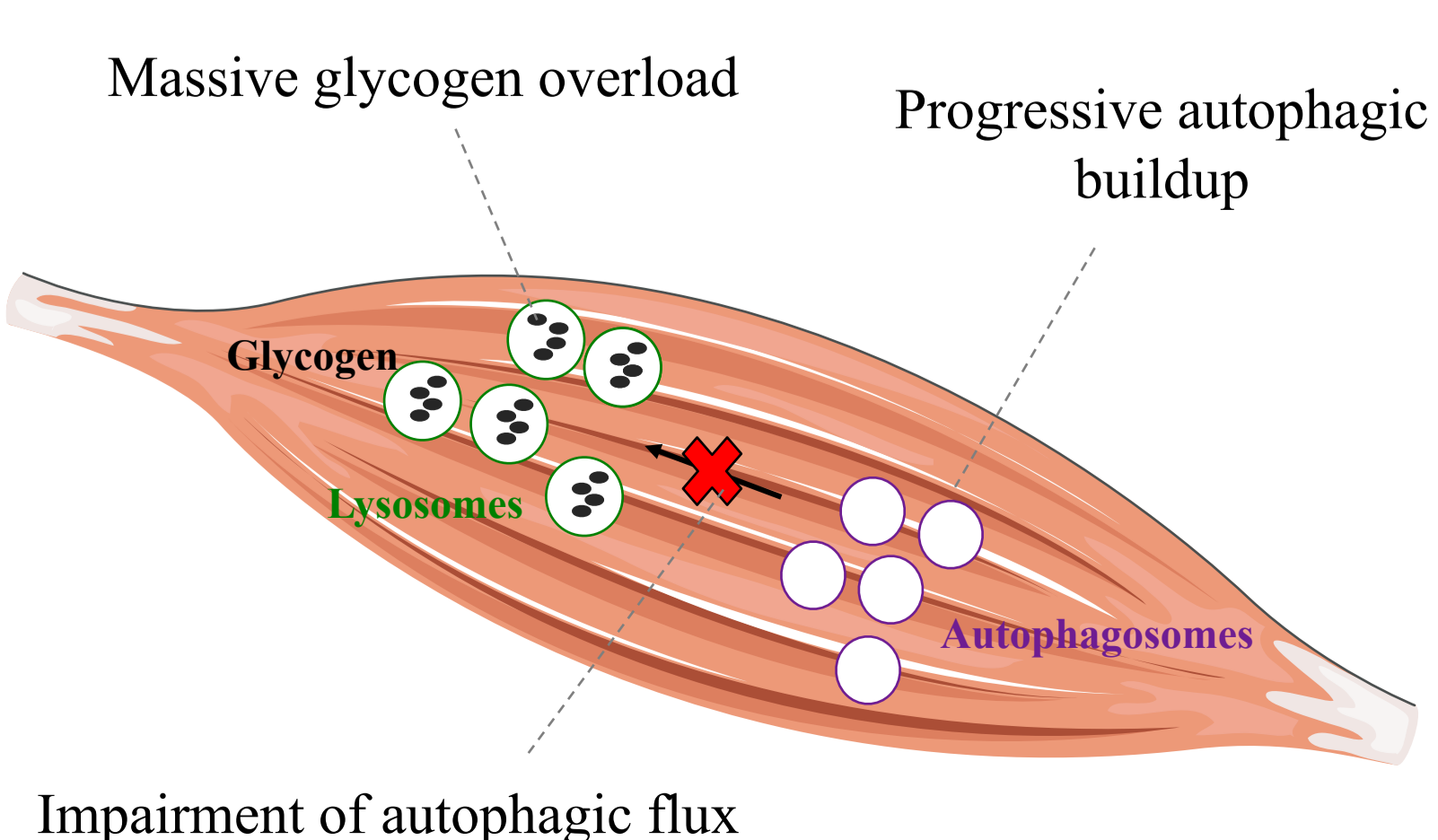
#### Increase of regenerative fibers



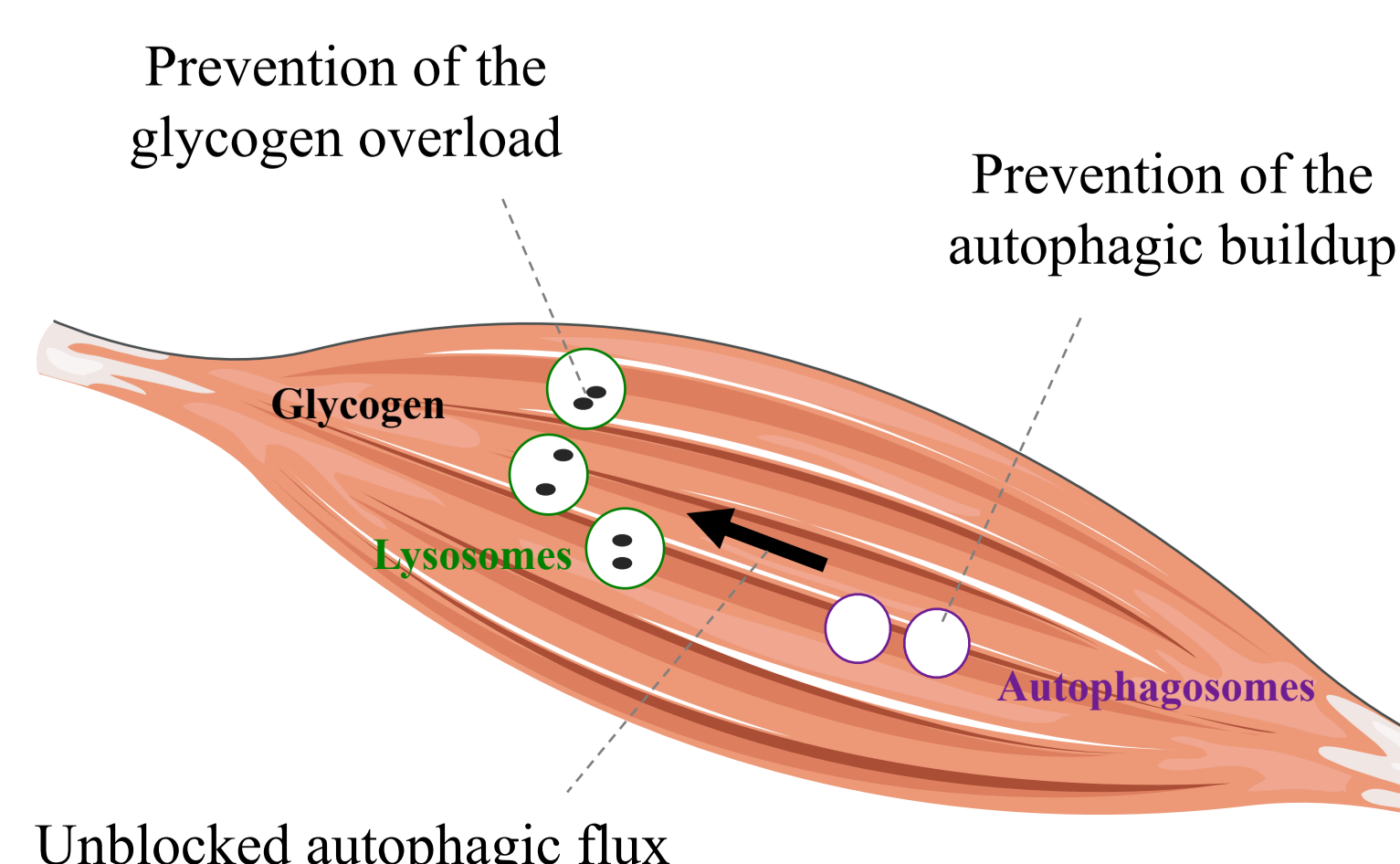
Quantification of centronucleated muscle fibers in *Biceps femoris* muscle. Statistical analysis: One-way ANOVA, Newman-Keuls post hoc test. \*p<0,05, \*\*\*p<0,001. n=5 mice/group.

## Conclusion

### Gaa<sup>-/-</sup> skeletal muscle



### Gaa<sup>-/-</sup> FoxO3a skeletal muscle



## Take home messages

- FoxO3a overexpression prevents both the glycogen overload and the autophagic flux impairment in skeletal muscle of Pompe disease.
- FoxO3a overexpression prevents the skeletal muscle remodeling occurring in Pompe disease.
- FoxO3a overexpression could exhibit a therapeutic relevance to efficiently correct skeletal muscle in Pompe disease, in combination with gene therapy inducing overexpression of GAA or enzyme replacement therapy (ERT).