The role of microRNA and oxidised microRNA-133 in muscle wasting during ageing and cachexia

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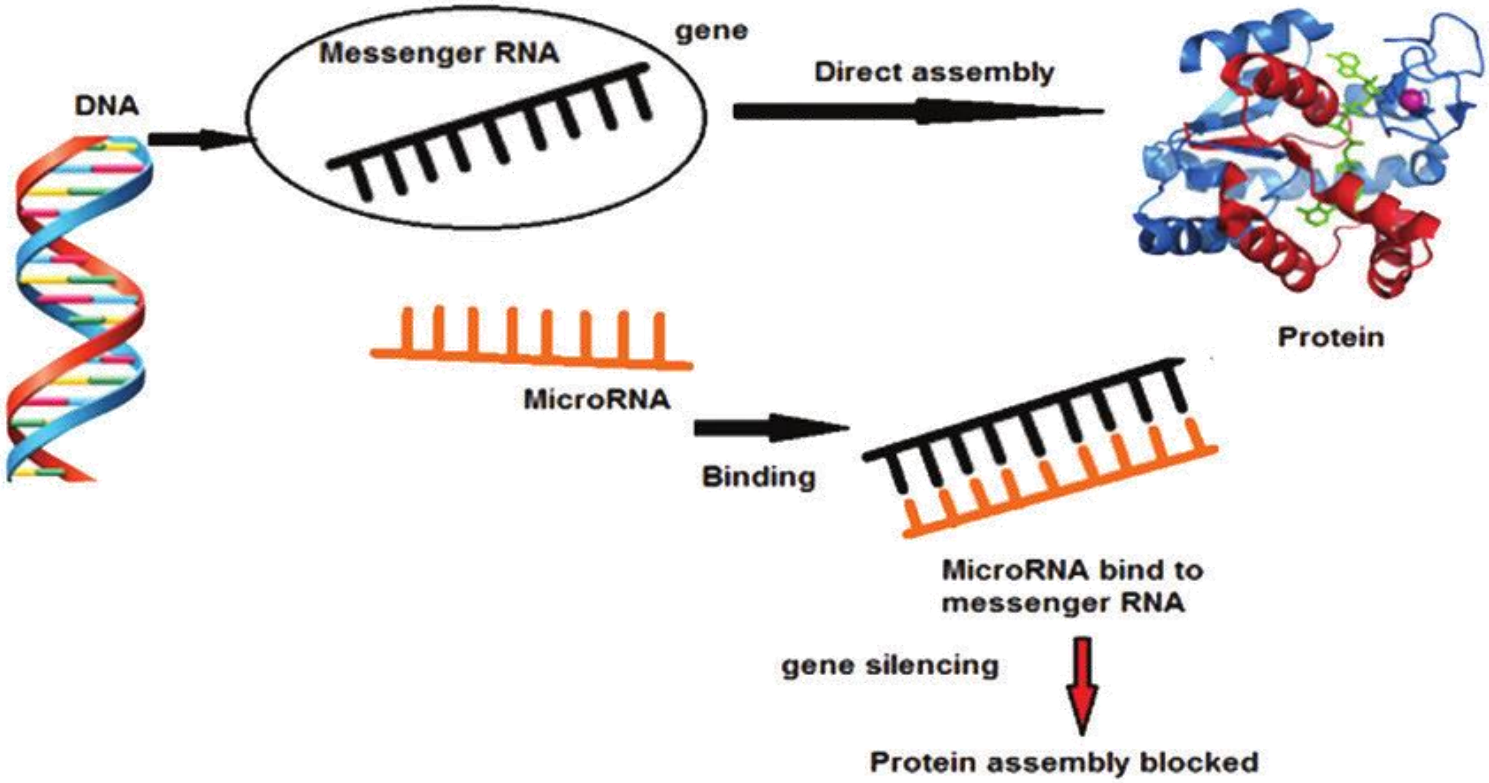
miRNAs and myomiRNAs



miRNAs, are small non-coding RNAs that play a central role in post-transcriptional gene expression regulation, coordinating and fine-tuning many cellular and physiological processes.

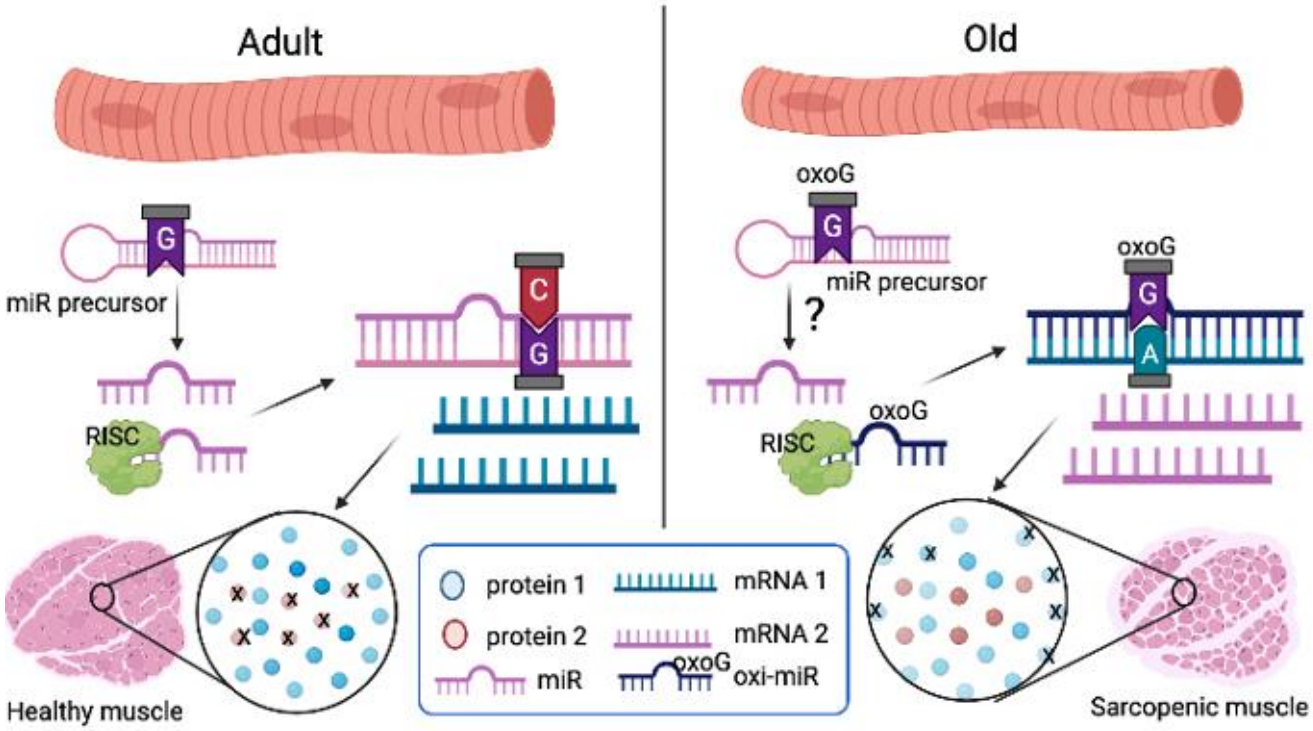
microRNAs control muscle development and homeostasis (***Goljanek-Whysall K, et al. Clin Sci, 2012***).

Several miRNAs, such as miR-1, -206, -208, -486, -499 and miR-133, are classified as **myomiRNAs,** are expressed at high levels in skeletal muscle and have been implicated in maintaining muscle homeostasis in health and disease (***Yu H, et al. Curr drug target, 2014***). miR-1, -133a, -133b and -206 are associated with function and size of skeletal muscles during ageing in mice (***Mytidou C, et al. Frontiers in Physiol, 2020***).

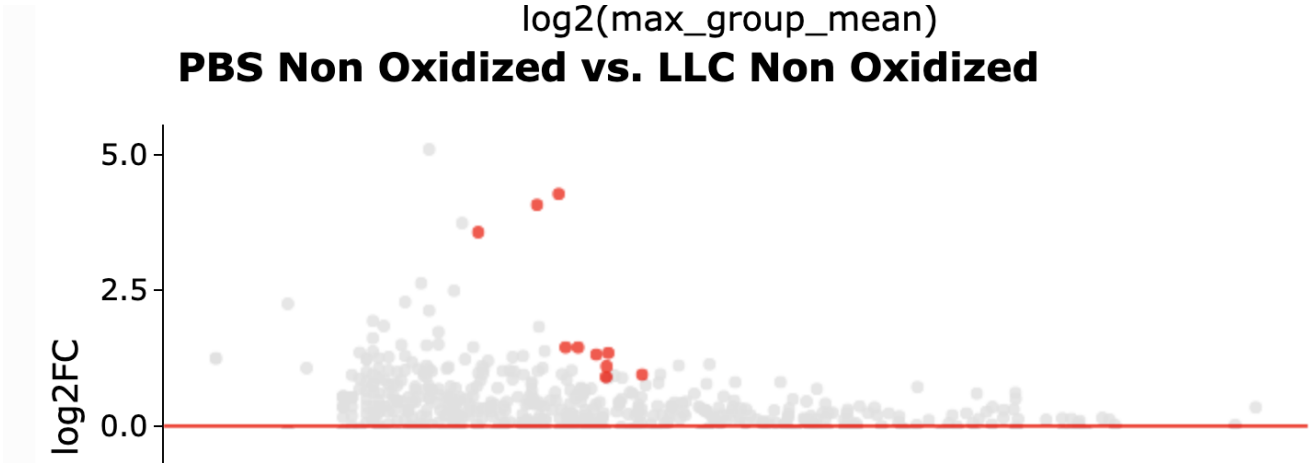
miRNAs, ageing and cancer cachexia

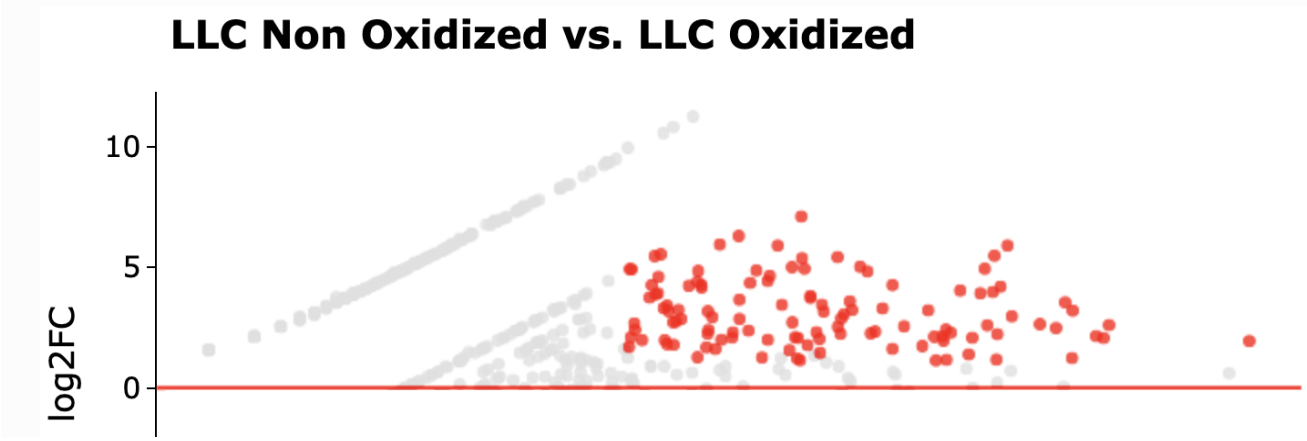
A common mechanisms underlying loss of muscle mass and function during ageing (sarcopenia) and disease, such as cancer cachexia, is disrupted redox homeostasis.

We hypothesise, that as redox homoeostasis is disrupted in muscle during ageing or cachexia, and considering the rather long half-life of a great number of miRNAs (including miR-133 family), oxidative modification of miRNAs could affect interactions with their target genes and be one of the mechanisms associated with loss of muscle mass and function.



Ageing and cancer cachexia induces changes in microRNA oxidation





Mice were subcutaneously injected with LCCs. 3 weeks later GAS muscle was dissected and miR-Seq and oxo-miR-Seq were performed.
Similar observations were made for muscle from old mice compared to adult mice.
Among oxidised miRs upregulated in muscle during ageing and cachexia is oxidised miR-133.

Gonzalez & Goljanek-Whysall et al., unpublished data

miR-133 *in vivo ageing* model

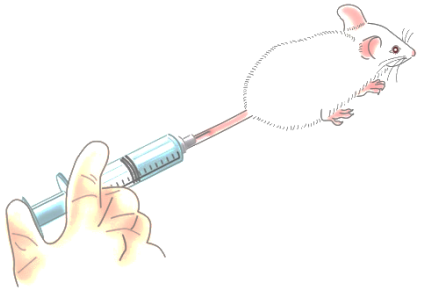
C57JL/6J male mice
11 (adult) and 111 old weeks (old mice)

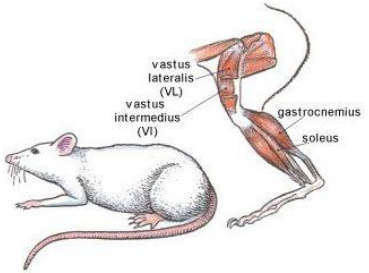
T= 0 — T=1 week — T=3 weeks

Injection of miR-133 and AM oxo-miR-133

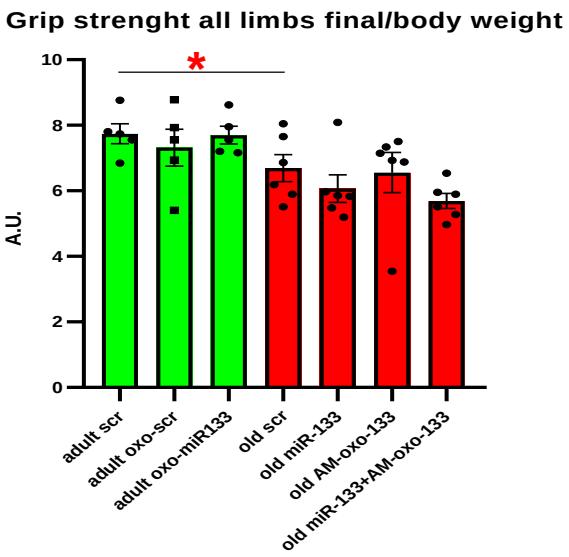
Injection of miR133 and AM oxo-miR-133

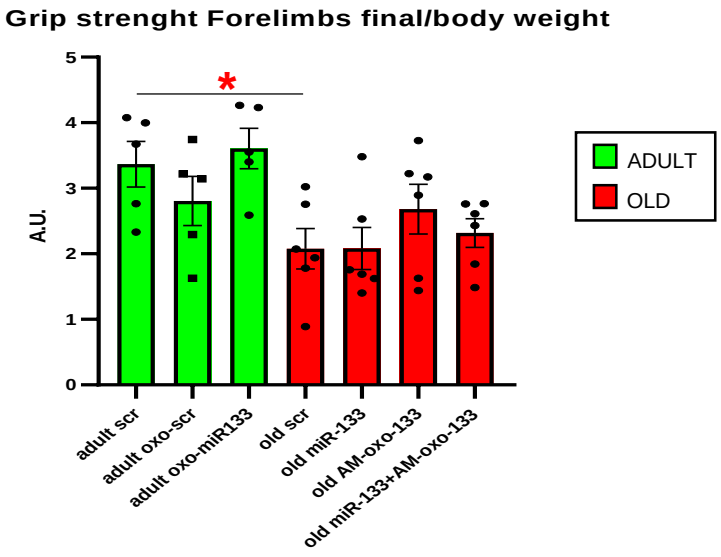
Sacrifice by cervical dislocation and muscle dissections



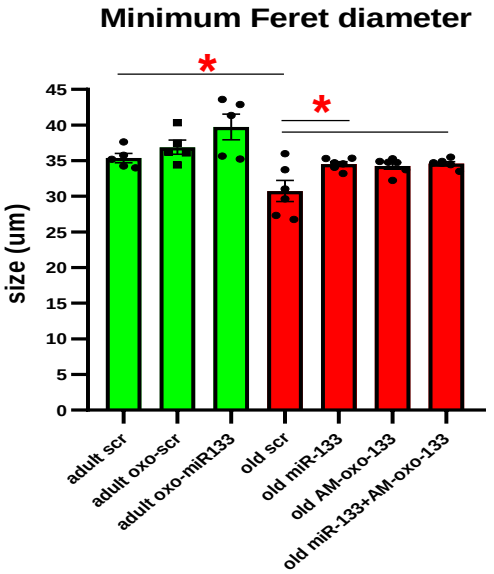


GRIP STRENGHT

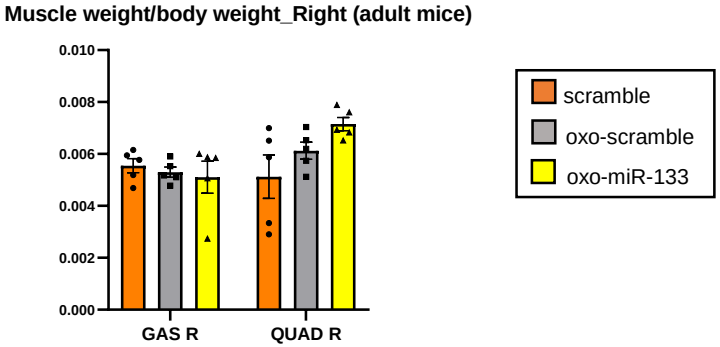


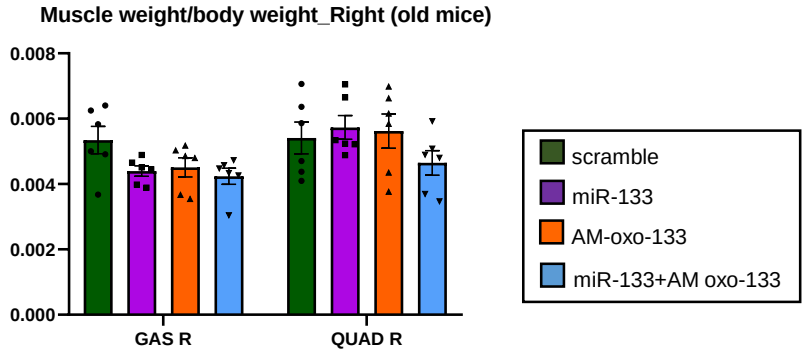


MUSCLE FIBRE SIZE



MUSCLE WEIGHT





Loss of fiber size during ageing can be restored through miR-133 overexpression or combined overexpression of miR-133 with inhibition of oxidised miR-133.

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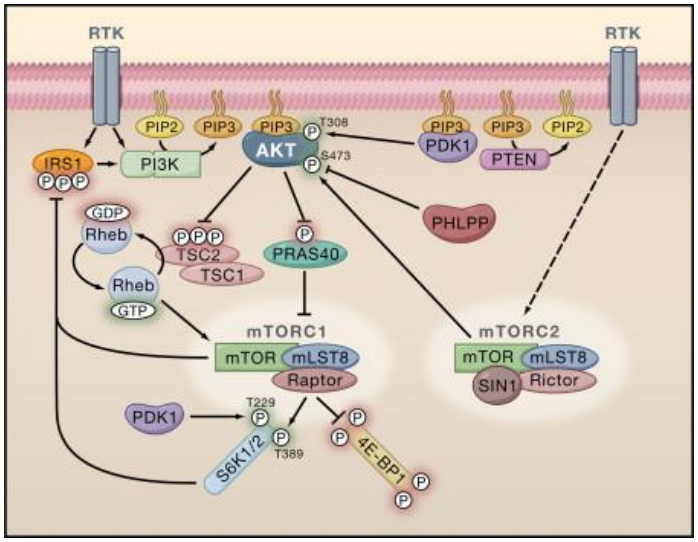
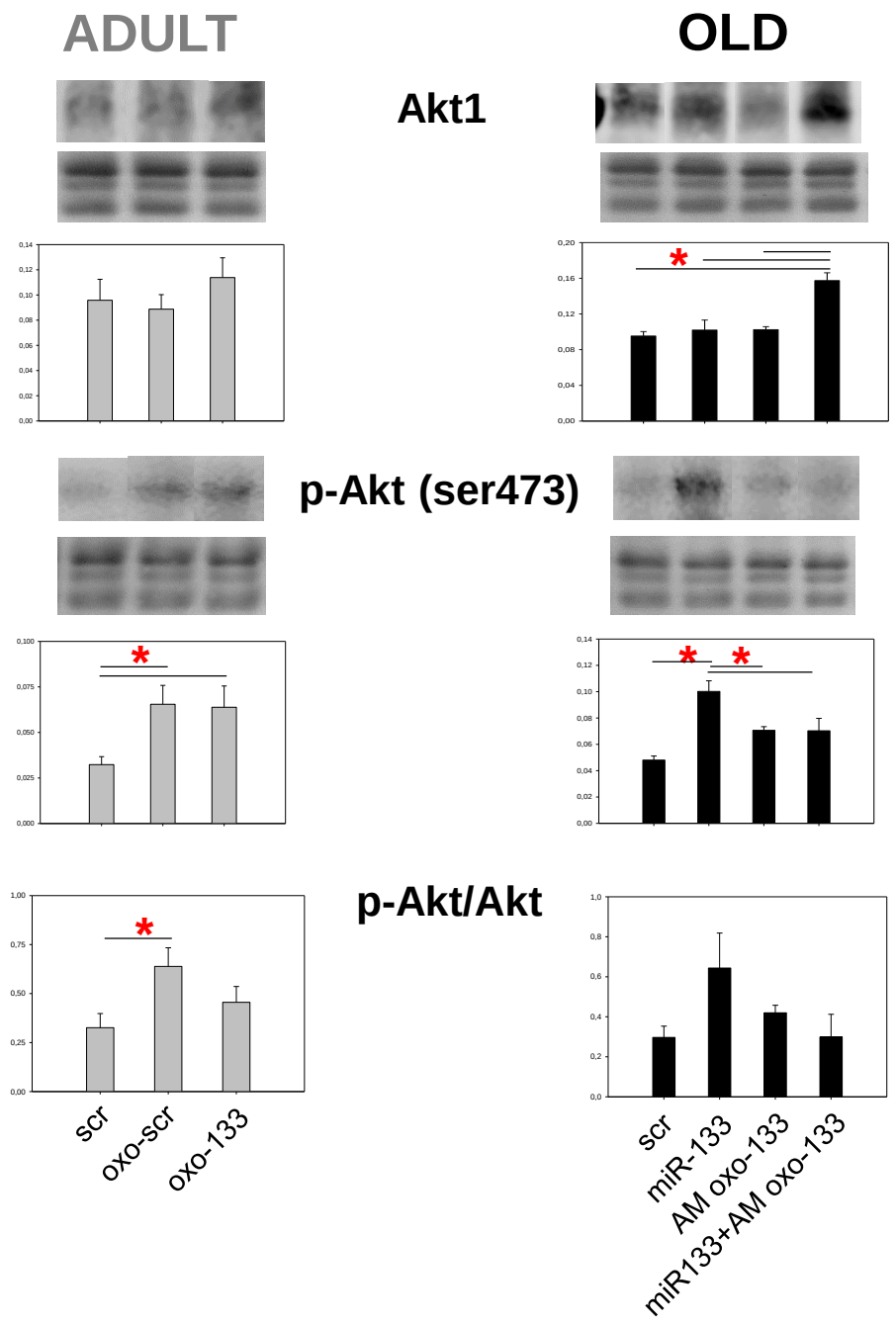
*$p \leq 0.05$: Statistical significance, ANOVA test

Gonzalez & Goljanek-Whysall et al., unpublished data

miR-133 predicted targets: AKT signaling pathway



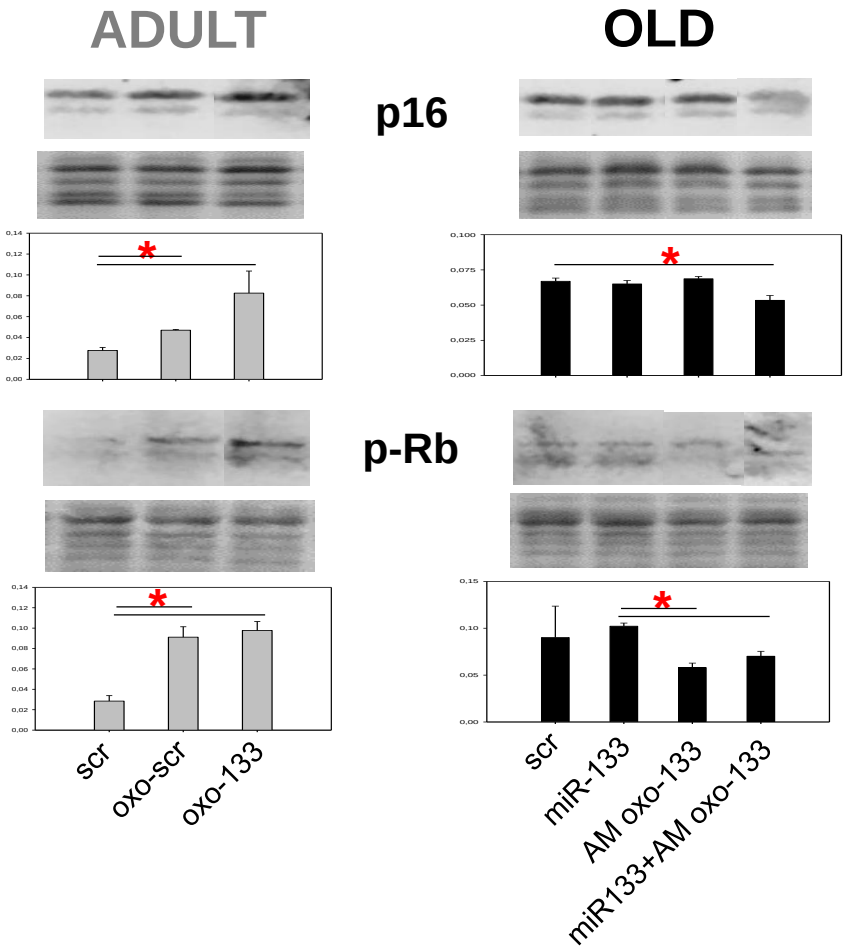
AKT pathway activation can be associated with muscle hypertrophy.
miR-133 has been previously shown to hyper-activate AKT pathway (*Lu XC, et al. Cel Physiol and Biochem, 2015*), however oxidised miR-133 does not activate this pathway.



*$p \leq 0.05$: Statistical significance, ANOVA test

Gonzalez & Goljanek-Whysall et al., unpublished data

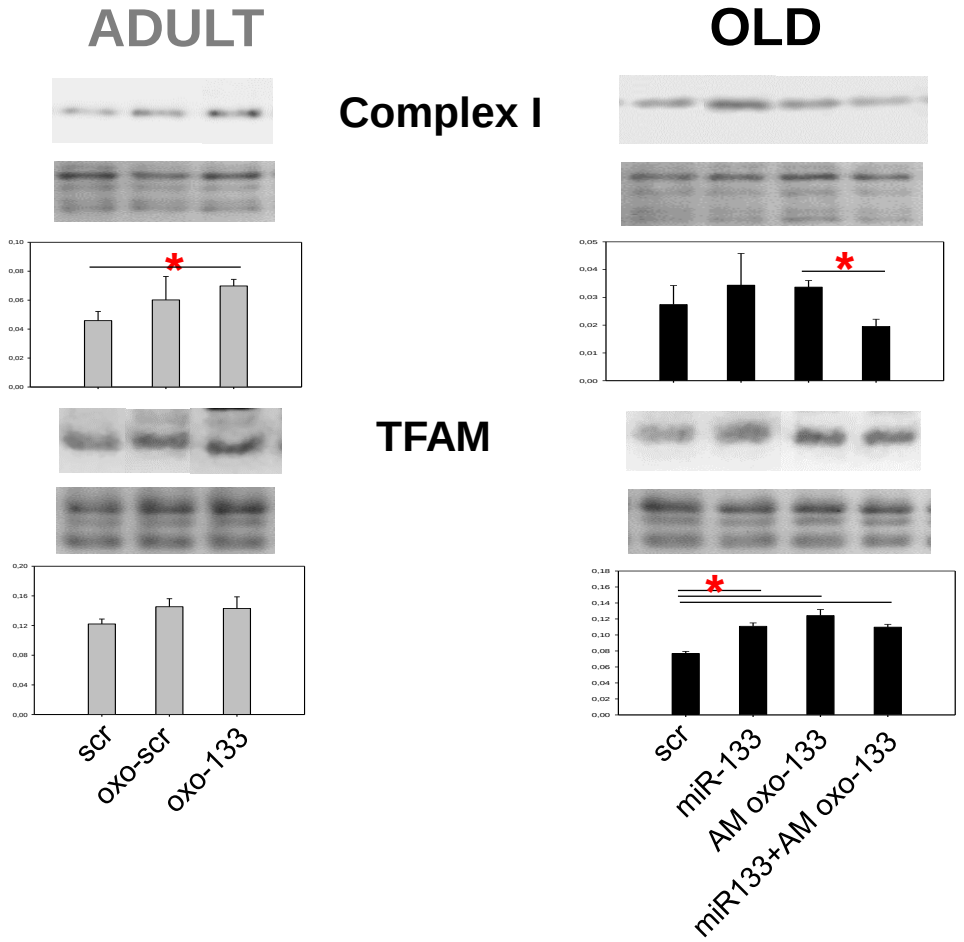
miR-133 predicted targets: Cellular senescence



The treatment of adult mice with oxidised microRNA (Scr or miR-133) leads to increased expression of senescence-associated genes. The expression of senescence-associated genes is downregulated in muscle of old mice following AM-oxi-miR-133 or miR-133 with AM-oxi-miR-133 injections. These data strongly suggest the regulation of senescence pathways by oxidised microRNAs.

miR-133 predicted targets: Mitochondria

(Stefanatos R, et al. *Cell Cycle*, 2011) (Nuo S, et al. *Mol Cel*, 2016)



Oxo-miR-133 treatment leads to increased complex I abundance in adult mice. And combination of miR-133 and antagomir to oxidised miR-133 treatment depletes mito-complex I in aged mice, and leads to increased expression of TFAM (like to mitochondrial biogenesis).

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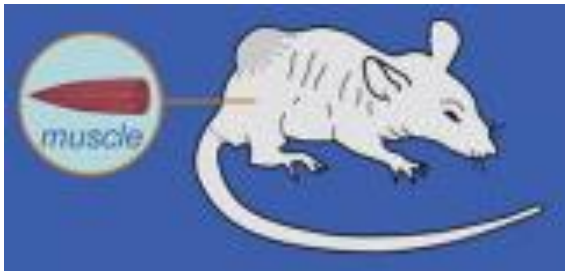
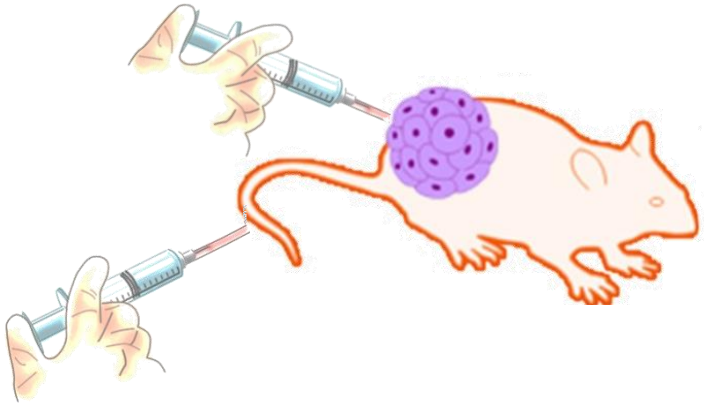
miR-133 *in vivo cancer cachexia* model

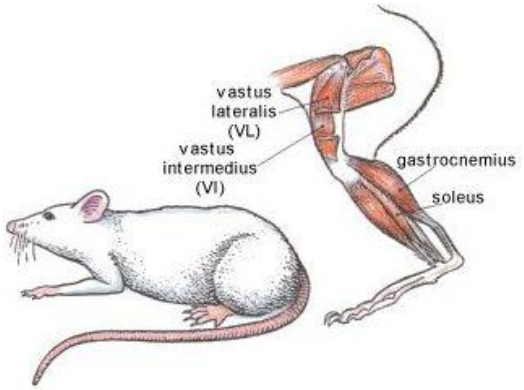


C57JL/6J adult mice (11 old weeks)
14 males and 12 females

T= 1 day — T=10 days — T=21 days

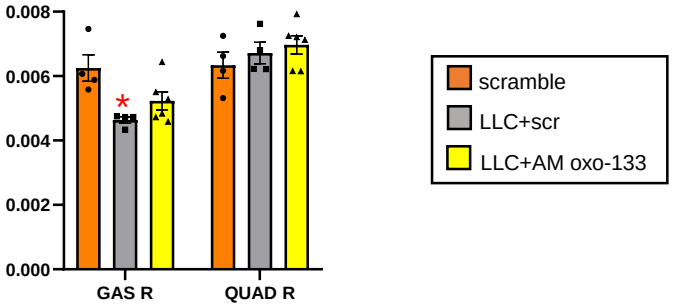
Inoculation LLC cells and AM oxo-133 — **AM oxo-133 inoculation** — **Sacrifice and muscle dissections**



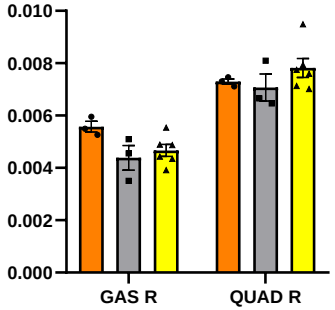


MUSCLE WEIGHT

Muscle weight/body weight_Right (MALES)

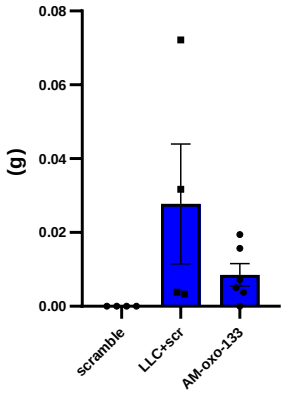


Muscle weight/body weight_Right (FEMALES)

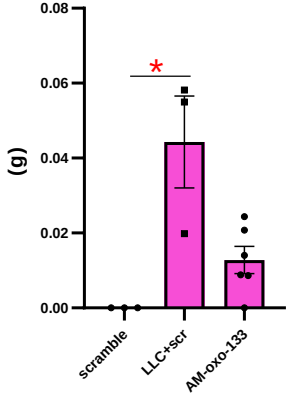


TUMOR SIZE

Tumor size/body weight (MALE)

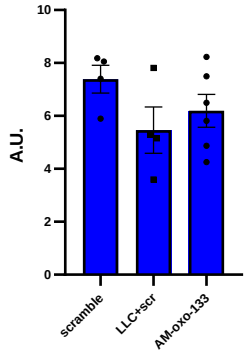


Tumor size/body weight (FEMALE)

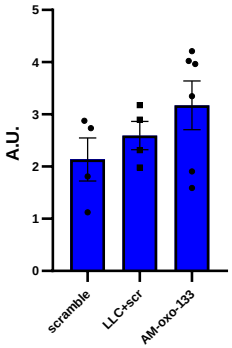


GRIP STRENGHT

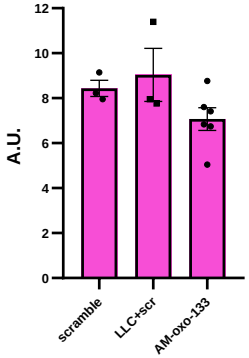
All limbs final/body weight (MALE)



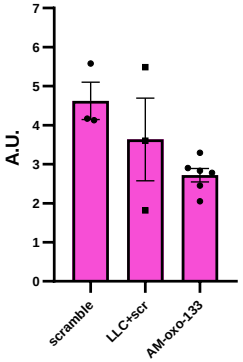
Forelimbs final/body weight (MALE)



All limbs final/body weight (FEMALE)

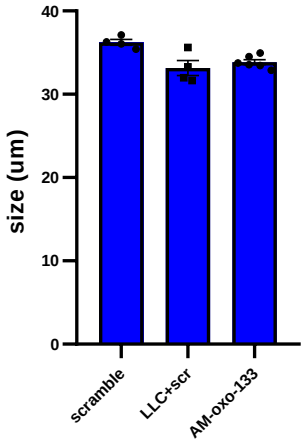


Forelimbs final/body weight (FEMALE)

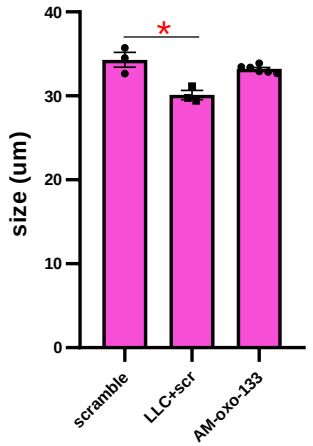


MUSCLE FIBRE SIZE

Minimum Feret diameter (MALE)



Minimum Feret diameter (FEMALE)



Antagomir to oxidised miR-133 ameliorates muscle loss in mice injected with LLC cells, however does not have an effect on grip strength. Antagomir to oxidised miR-133 treatment leads to a decrease in tumour size in male and female cachexic mice.

*$p \leq 0.05$: Statistical significance, ANOVA test

Gonzalez & Goljanek-Whysall et al., unpublished data

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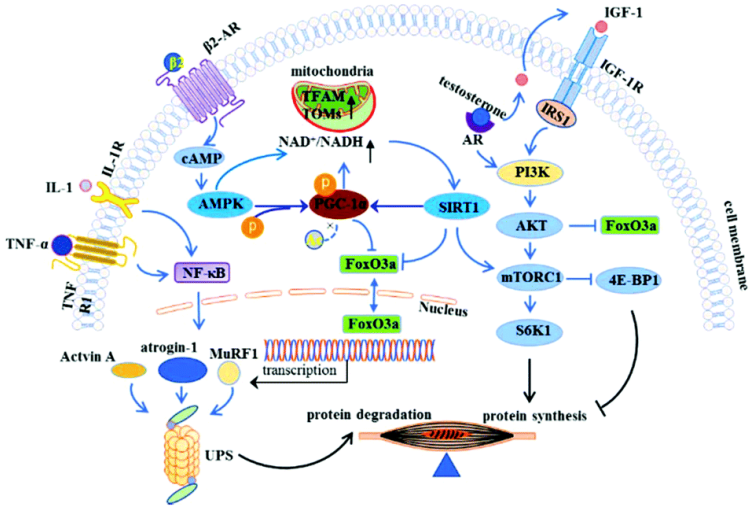
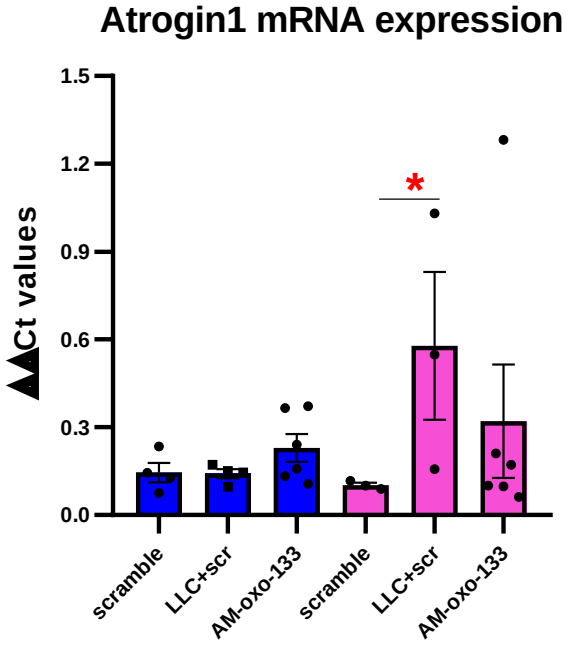
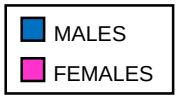
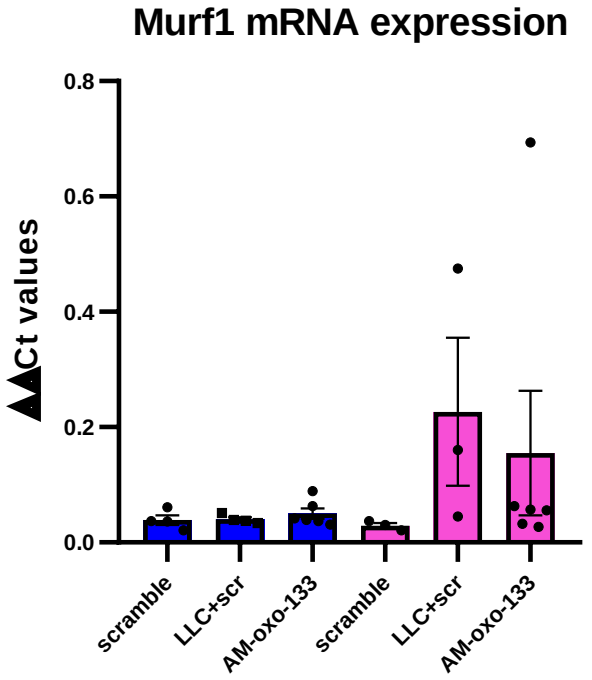
miR-133 predicted targets: Muscle atrophy



Muscle atrophy markers elevated in mice injected with LLC cancer cells in female mice were downregulated in mice injected with LLCs and treated with antagomiR to oxidised miR-133.







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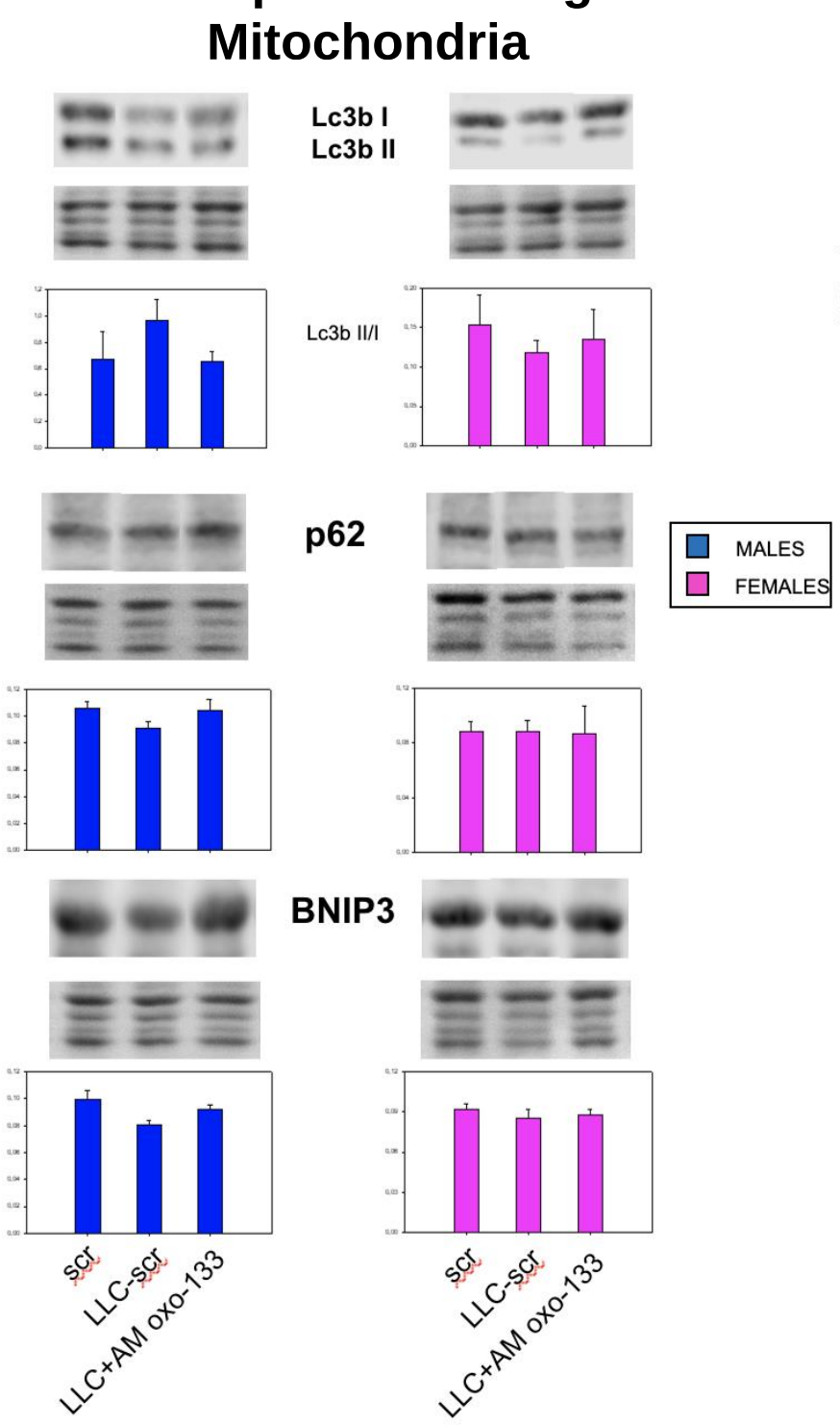
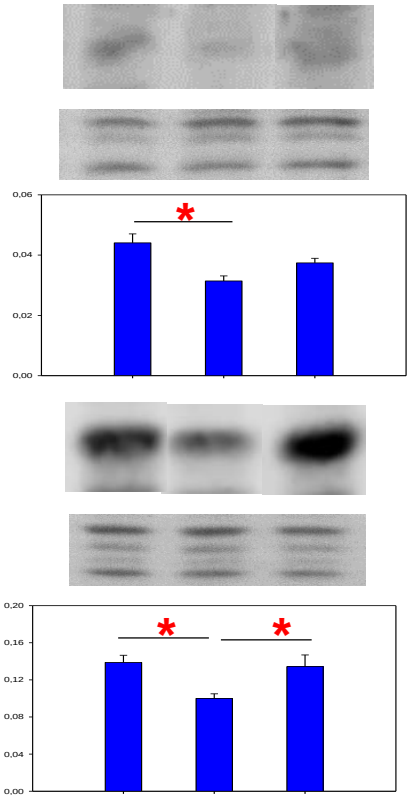
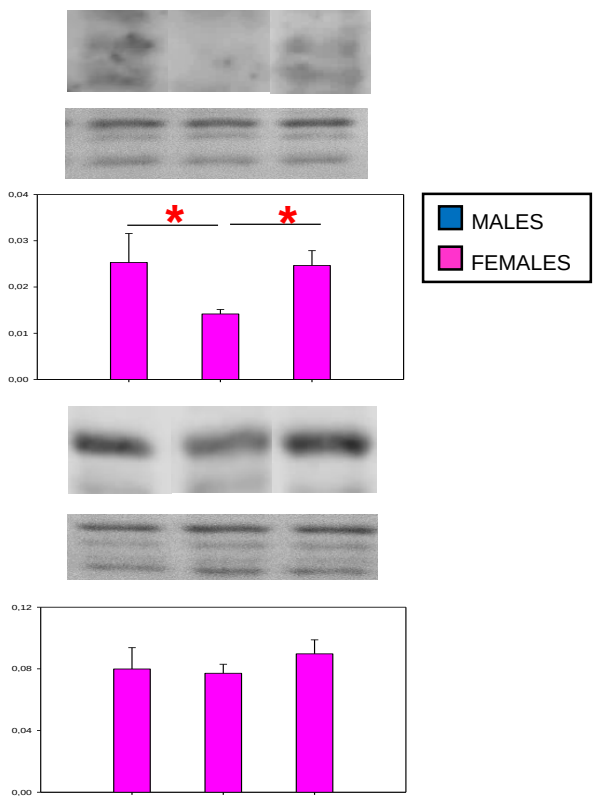
miR-133 predicted targets: Mitochondria







AntagomiR to oxidised miR-133 miR-133 recovers scramble value of autophagy/mitophagy markers. And prevents loss of abundance of mitochondrial complex I, and VDAC protein levels in cachexic mice. Potentially by regulation of mitophagy.

*$p \leq 0.05$: Statistical significance, ANOVA test

Gonzalez & Goljanek-Whysall et al., unpublished data

CONCLUSIONS

- In **old mice**, both miR-133 and inhibition of oxidised miR-133 lead to increase in myofiber size; this is likely related to regulation of AKT signaling, senescence and mitochondrial dynamics.

- In early-stage **CACHEXIC** mice, the changes in muscle fiber size and the tumor size following LLCs injections, were restored by antagomir to oxidised 133 treatment. This was associated with regulation of mitochondrial markers.

- In both studies, molecular and physiological changes were sex-dependent – further works are exploring the mechanistic insights into these changes.

- Oxidised microRNAs likely contribute in the regulation of loss of muscle mass and function during ageing and cancer cachexia.

Gonzalez & Goljanek-Whysall et al., unpublished data

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