Role of Mrf4 gene in homeostasis of adult skeletal muscle

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The process which promotes skeletal muscle growth during embryonic development is known as myogenesis. Disorders in this process constitute one of the underlying causes of different types of muscle dystrophies. That is the reason why the role of the transcription factors (TFs) that are implicated in the determination, differentiation and specification of this tissue has been extensively studied. They are known as MRFs (Myogenic Regulatory Factors) (1). A link between MRFs function and muscle atrophy/hypertrophy has been established (2). Specifically, *Mrf4* is the only MRF highly expressed in all adult skeletal muscles, but a complete understanding of its function is lacking as previous KOs models affect the expression of another MRF, Myf5, in *cis* (3). Thus, by using CRISPR/Cas9 technology, in our laboratory we have recently generated new KO models named Mrf4 $^{L1/L1}$ and Mrf4 $^{L2/L2}$. Preliminary data show that both alleles develop mild muscle hypertrophy and have alterations in muscle metabolism, without affecting *Myf5* expression in *cis*. This project is focused on elucidating how this TF is involved in muscle growth, function, and homeostasis.

Additionally, satellite cells, which are the muscle stem cells, are mitotically quiescent. They are activated by muscle damage, when they proliferate, differentiate and fuse with each other regenerating myofibers. Besides muscle repair, they have also been reported to be responsible for skeletal muscle hypertrophy (4). Furthermore, as adult satellite cells emerge from embryonic founder cells in which *Mrf4* expression was activated, we are also studying satellite cell biology and function in the absence of *Mrf4*.

METHODS

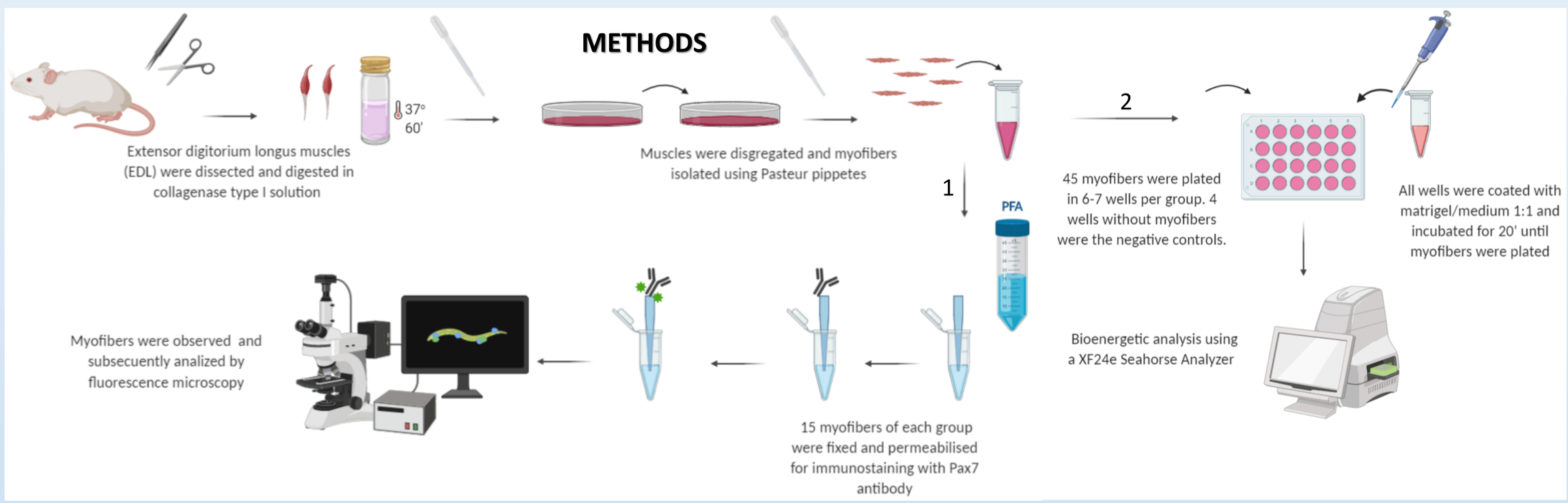


FIG. 1.

RESULTS

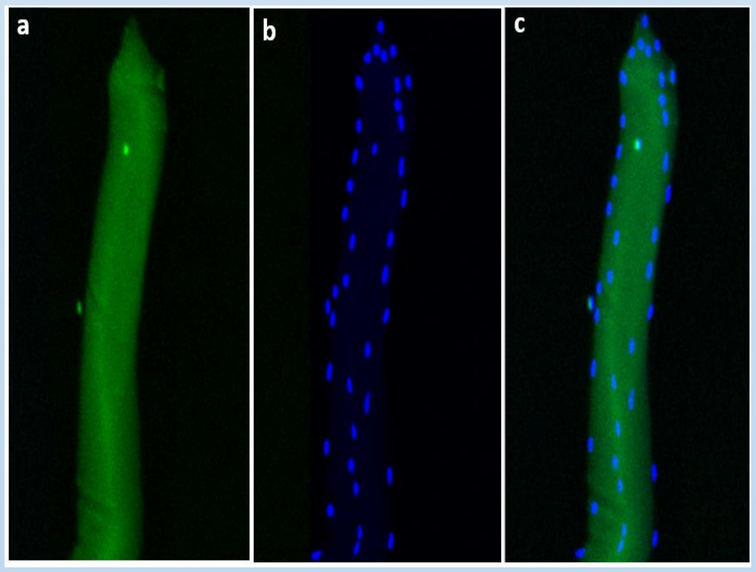


FIG. 1. Muscle stem cells express *Pax7* when they are quiescent. Freshly isolated myofibers from WT, *Mrf4* $^{L1/L1}$ and *Mrf4* $^{L2/L2}$ were fixed and immunostained with anti-*Pax7* antibody to quantify the number of satellite cells per myofiber after isolation (a). DAPI staining identifies all myonuclei (b). Proportion of total cells expressing *Pax* 7 (green) per nuclei (blue) (c). Distribution of satellite cells in myofibers of EDL muscles from young WT and mutant mice. Both mutants have a higher content in satellite cells than WT animals, although this difference is only significant in the *Mrf4* $^{L1/L1}$ line (d). However, the ratio between the number of satellite cells and total myonuclei is significantly higher in *Mrf4* $^{L1/L1}$ mice with respect to *Mrf4* $^{L2/L2}$ counterparts. On the contrary, WT and *Mrf4* $^{L2/L2}$ animals show similar values which suggest not only that number of Satellite Cells in WT is less than in mutants phenotypes, also that number of nuclei in proportion to them (d). Additionally, Myofiber Surface Area (MSA) (2 x pi x fiber radius x fiber length, expressed in mm2) is higher for WTs than for the mutants (f). Importantly, the number of myonuclei per MSA is significantly higher in both mutants, suggesting that, during development, more satellite cells fused to form mutant fibers (g).

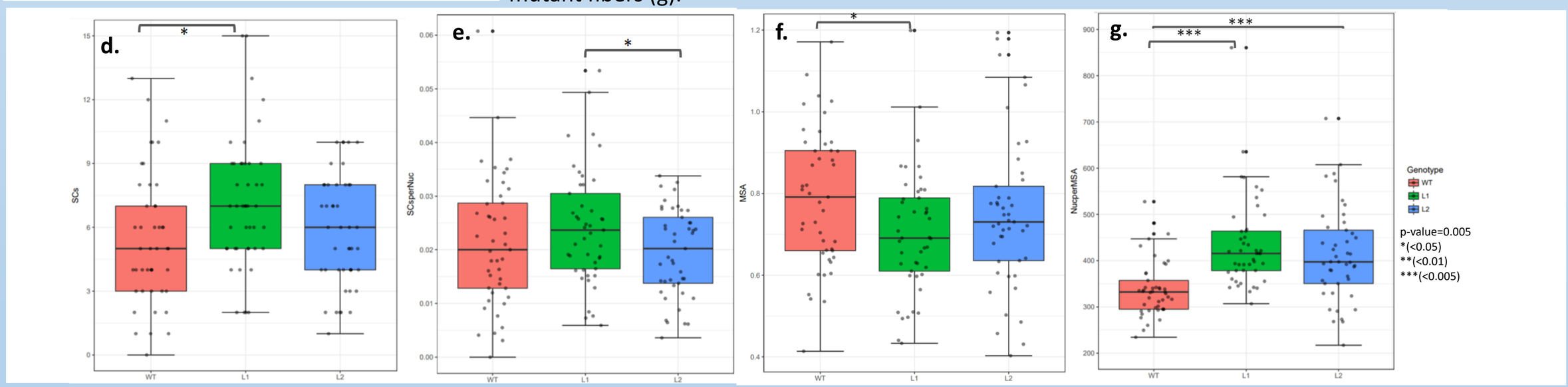
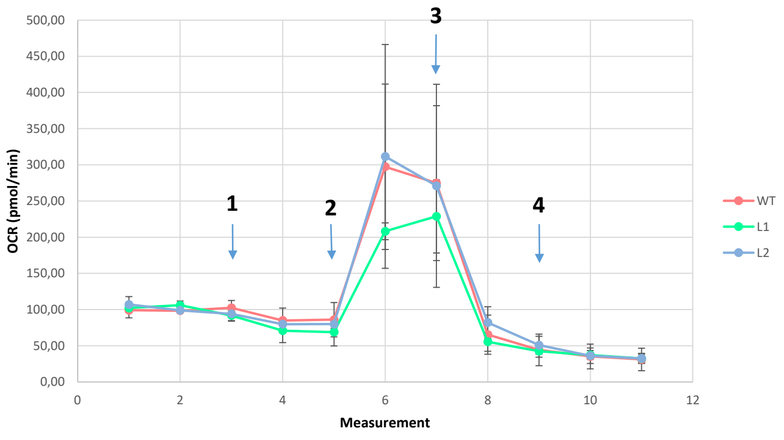


FIG. 2. The Oxigen Consumption Rate (OCR) of isolated muscle myofibers from WT, *Mrf4* $^{L1/L1}$ and *Mrf4* $^{L2/L2}$ mice was measured as an indicator of mitochondrial respiration in response to bioenergetic modulators. This value has been used as a proxy for metabolic function. Basal OCR was measured followed by four injections of inhibitors: oligomycin (1), FCCP (2), rotenone (3) and antimycin (4). FCCP stimulates respiration in mitochondria by uncoupling ATP synthesis from electron transport, while oligomycin, antimycin and rotenone inhibit respiration by inhibiting ATP synthase and complexes III and I, respectively. The experiment was performed 24 hours after isolation. Preliminary results indicate there are no significant differences between groups a priori; more experiments are needed. (p-value=0.05)

FIG. 2. SEAHORSE ANALYSIS



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