SUPPLEMENTAL MATERIAL

Methods

Tissue processing and Immunofluorescence staining for green fluorescence expressing tissue

Animal experiments were performed in accordance with institutional guidelines and national regulations.
Rats expressing GFP[1] and wild type rats were sacrificed by a lethal dose of pentobarbital (150 mg/kg i.p.) at postnatal day 21 for the isolation of muscle tissues from the hind limb. Tissues were immersion-fixed in 4% paraformaldehyde / 0.1M PBS (pH 7.4) for 48 hours and cryoprotected by immersion in 15% sucrose in 0.1M PBS. After freezing in isopentane at -80°C, the muscle tissues were sectioned at 30 µm on a freezing microtome (Leica CM3050). The sections were mounted onto Superfrost Plus coated microscope slides.

Slides were then heated for 45 min at 75°C in citrate buffer pH 6.9 and after cooling down the tissue sections were washed in 0.1 M PBS and protein blocked with 10% horse serum (HS) in 0.4% Triton X-100 PBS for 60 min. Following 3 x 5 min. in PBS sections were incubated overnight at 4°C with rabbit monoclonal anti-GFP antibodies (Cell Signaling) diluted 1:250 in PBS containing 0.1% Triton X-100 and 2.5% HS. After three washes in PBS the sections were incubated for 2 hours with Alexa® Fluor donkey anti-rabbit 594nm (1:250, Molecular Probes) diluted in PBS containing 0.1% Triton-X-100 and 2.5% HS. Thereafter, the sections were washed for 4 x 10 min. in PBS, mounted and then covered with a solution containing 50% PBS and 50% glycerol. Fluorescence pictures were recorded using an Olympus epifluorescence microscope (BX51) equipped with a digital camera (Olympus DP72).

1. Inoue H, Ohsawa I, Murakami T, Kimura A, Hakamata Y, Sato Y, et al. Development of new inbred transgenic strains of rats with lacz or gfp. *Biochem Biophys Res Commun*. 2005;329:288-295