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Supplementary information

Immunocytochemistry methods and materials

PHMs were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% foetal bovine serum (FBS) (Biowest, Nuaillé, France) and 2% penicillin-streptomycin (P/S) until confluent. The cells were induced using DMEM with 2% FBS and 2% P/S over a period of seven days. The images contained in this review were specifically selected for the positive expression of PAX 3/7, MyoD, MyoG and Desmin. The micrographs were included for visual purposes only and did not form part of any specific experiments or subjected to other conditions than those stated above. PHMs were fixed in 4% paraformaldehyde (pH 6.9 – 7.2; Sigma-Aldrich Chemie, Darmstadt, Germany) for 15 minutes (min), permeabilized for 5 min in 0.2% Triton-X-100 (Sigma-Aldrich Chemie, Darmstadt, Germany) and blocked in a solution of phosphate buffered solution with 5% FBS and 0.3 M glycine (Sigma-Aldrich Chemie, Darmstadt, Germany) for 1 hour (h). PHMs were stained with the primary antibody solution over night at 4^{o}C and thereafter with the corresponding secondary antibody solution for 1 h. Coverslips were mounted onto glass slides using Fluoromount^{TM} (Sigma-Aldrich Chemie, Darmstadt, Germany) and imaged using a Zeiss LSM 800 confocal microscope (Carl Zeiss Werke, Göttingen, Germany). Desmin was visualised using rabbit anti-human desmin (1:500; Cell Signaling, Danvers, Massachusetts (MA), United States of America (USA)) and anti-mouse immunoglobulin G (IgG) conjugated to Alexa Fluor (AF) 488 (1:400; Abcam, Cambridge, United Kingdom (UK)). MyoD was visualised using mouse anti-human MyoD (1:200; Thermo Fisher Scientific, Carlsbad, CA, USA) and anti-mouse IgG conjugated to Alexa Fluor (AF) 555 (1:400; Abcam, Cambridge, UK). MyoG was visualised using rabbit anti-human myogenin (1:400; Abcam, Cambridge, UK) and anti-rabbit IgG conjugated to AF 488 (1:400; Abcam, Cambridge, UK). PAX 3/7 was visualised using mouse paired box protein (Pax) 3/7 (1:50; Santa Cruz Biotechnologies, Dallas, TX, USA) and anti-mouse IgG conjugated to Alexa Fluor (AF) 555 (1:400; Abcam, Cambridge, UK). Actin was stained using tetramethylrhodamine (TRITC)-phalloidin (1:400; Sigma-Aldrich Chemie, Darmstadt, Germany). Nuclei were visualised with 4’,6-diamidino-2-phenylindole (1:1000; DAPI; Thermo Fisher, Waltham, MA, USA).