SUPPLEMENTARY MATERIAL

Human satellite cells as a manageable tool for gene therapy

Andreas Marg, Helena Escobar, Markus Kufeld, Sina Gloy, Joseph Zacher, Andreas Spuler, Carmen Birchmeier, Zsuzsanna Izsvák, Simone Spuler

Supplemental Figure 1



Single human muscle fiber fragment after manual dissection. Bar 200 μm

Supplementary Table 1. Summary of experiments

Number of muscle	Age and gender	Procedure	Number of
biopsy specimens	of donors		experiments
69	20-80 years	HMFF ¹ characterization	580 HMFF
	34 male, 35 female	Characterization of	304 HMFF
		colonies that grew onto	
		culture dish	
		Characterization of	272 HMFF
		colonies after hypothermic	
		treatment (4°C)	
6	44-64 years	Transplantation	33 transplantations
	3 male, 3 female		in TA muscle

¹ Human muscle fiber fragment

Supplementary Figure 2



Surface marker profile of human satellite cells. Upper panel: Skeletal muscle cryosections from a three months old infant. MET positive cells (red) are frequent in the satellite cell niche. In muscle section from adult probands MET^+ cells could not be detected. Second and third panel: CXCR4 (red) is expressed in endothelial cells of skeletal muscle tissue; Ulex European Agglutinin (UEA), green. Some CXCR4 positive cells are located in the interstitial space outside of the basal lamina (β 2 laminin, green). Lower panel: Endothelial cells of skeletal muscle tissue express high levels of CD34 (CD34: red, UEA: green). No cells in the satellite cell niche are positive for CD34. Bars: 50/100/50/50 µm. Supplementary Video 1 Viable satellite cells in single human fiber fragment.

Satellite cells migrating in the satellite cell niche of human a single fiber. The fiber has been cultured for 13 days prior to recording for 17 h. Images were taken every 15 min. Note the sliding cell inside of the surrounding membrane. Bar: $75 \mu m$.

Supplementary Figure 3



Colony formation of cells outgrowing the HMFF could be observed 10 days after initiating the HMFF irrespective whether the fiber was placed on Matrigel® (left) or on plastic (right). Bar: 200 µm.

Supplemental Table 2: Antibodies used for immunohistochemistry

Antibody	Species	Working	Catalog number, Company
		concentration	
Anti-BrdU	rat	10 µg/ml	ab 6326, Abcam, Cambridge, UK
Anti-CD34	rabbit	2 µg/ml	sc-9095, Santa Cruz Biotechnology,
			Santa Cruz, CA, USA
Anti-CXCR4	rabbit	2.5 µg/ml	ab 2074, Abcam
Anti-c-met	rabbit	2 µg/ml	sc-161, Santa Cruz Biotechnology
Anti-desmin	mouse	1:500 (cells)	M 0760, Dako, Glostrup, Denmark
		1:50 (histology)	
Anti-desmin	rabbit	4 µg/ml	ab 8592, Abcam
Anti-hu Lamin A/C	rabbit	1:2000	ab 108595, Epitomics, Cambridge, UK
Anti-β2 laminin	mouse	0.1 µg/ml	Novus Biologicals, Littleton, CO, USA
Anti-Myf5	rabbit	0.2 µg/ml	sc-302, Santa Cruz Biotechnology
Anti-MyoD	mouse	1 µg/ml	sc-32758, Santa Cruz Biotechnology
Anti-NCAM (CD56)	mouse	2 µg/ml	130-090-955, Miltenyi Biotech, Bergisch
			Gladbach, Germany
Anti-Pax7	mouse	Supernatant,	DSHB, Iowa City, Iowa, USA
		undiluted	

Supplemental Figure 4



Effect of irradiation (18 Gy) on mouse tibialis anterior (TA) muscle. Cardiotoxin (CTX) was injected into TA muscle without irradiation (left) or 24 h after irradiation (middle). Irradiation completely abolished muscle regeneration, but had no effect on muscle morphology in the absence of CTX (right). Histology was obtained 9 days after irradiation Bar, 100 µm.