

SUPPLEMENTARY MATERIAL

Lenzi et al.

Differentiation of control and ALS mutant human iPSCs into functional skeletal muscle cells, a tool for the study of neuromuscular diseases

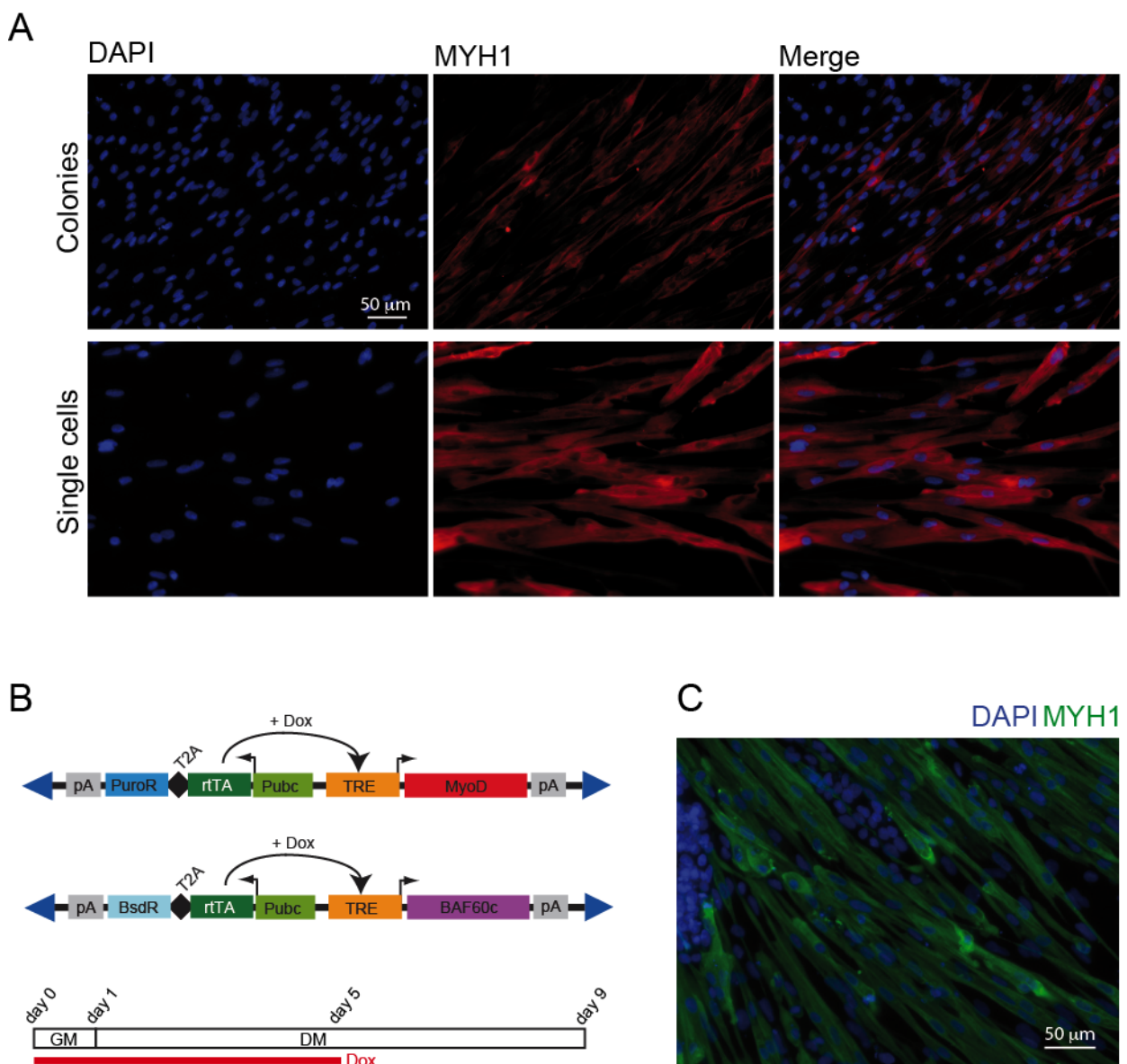


Figure S1 - Myosin Heavy Chain staining and muscle differentiation in presence of BAF60c

(A) Single channels of the Myosin Heavy Chain (MYH1) immunofluorescence shown in Figure 1C. Nuclear exclusion of the signal indicates specificity of the staining. (B) Co-expression of MyoD and

BAF60c induces myogenic conversion in the absence of preliminary multilineage differentiation. Top: schematic representation of the epB-Puro-TT-mMyoD and epB-Bsd-TT-BAF60c constructs. pA: polyadenylation signal; PuroR: puromycin resistance gene; BsdR: blasticidin resistance gene; T2A: self-cleavage peptide; rtTA: TET transactivator protein gene; Pubc: human Ubiquitin C constitutive promoter; TRE: TET responsive element; Dox: doxycycline. Blue triangles represent terminal repeats of the transposon. These constructs were co-transfected in WT I iPSCs together with the transposase plasmid, generating double transgenic iPSCs that were selected with puromycin and blasticidin. Bottom: diagram of the differentiation protocol. GM: myoblast growth medium; DM: myoblast differentiation medium. Red line: time in doxycycline (Dox). Relevant time points are indicated above. (C) Immunostaining for the muscle marker MYH1 at the end of differentiation of double transgenic iPSCs. Note that iPSCs transduced with MyoD alone fail to undergo muscle differentiation under the same conditions (see main text), suggesting that the epigenetic factor BAF60c is necessary in pluripotent stem cells for this process to occur, as previously shown by others (Albini et al., 2013).

Table S1

Gene	Oligo	Cat. N.
MYH1	Hs_MYH1_2_SG QuantiTect Primer Assay	QT01671005
Myogenin	Hs_MYOG1_SG QuantiTect Primer Assay	QT00001722
Dystrophin	Hs_DMD_1_SG QuantiTect Primer Assay	QT00085778
MYOD	Hs_MYOD1_1_SG QuantiTect Primer Assay	QT00209713
MEF2C	Hs_MEF2C_1_SG QuantiTect Primer Assay	QT00053368

Gene	Oligo Forward	Oligo Reverse
ATP50	ACTCGGGTTTGACCTACAGC	GGTACTGAAGCATCGCACCT
GAPDH	CACCATCTTCCAGGAGCGAG	CCTTCTCCATGGTGGTGAAGAC
Brachyury	CACCTGCAAATCCTCATCCTCAG	TGTCATGGGATTGCAGCATGGA

Primers for real-time qPCR and RT-PCR. QuantiTect Primer Assay primer sets were purchased from Qiagen.