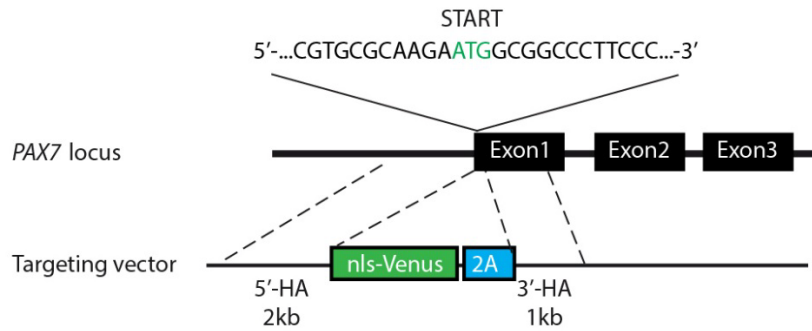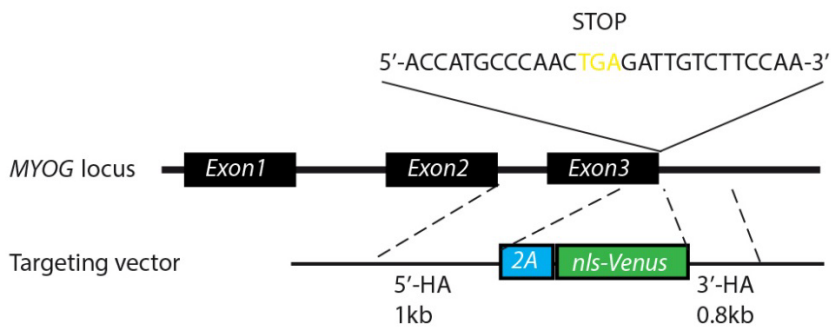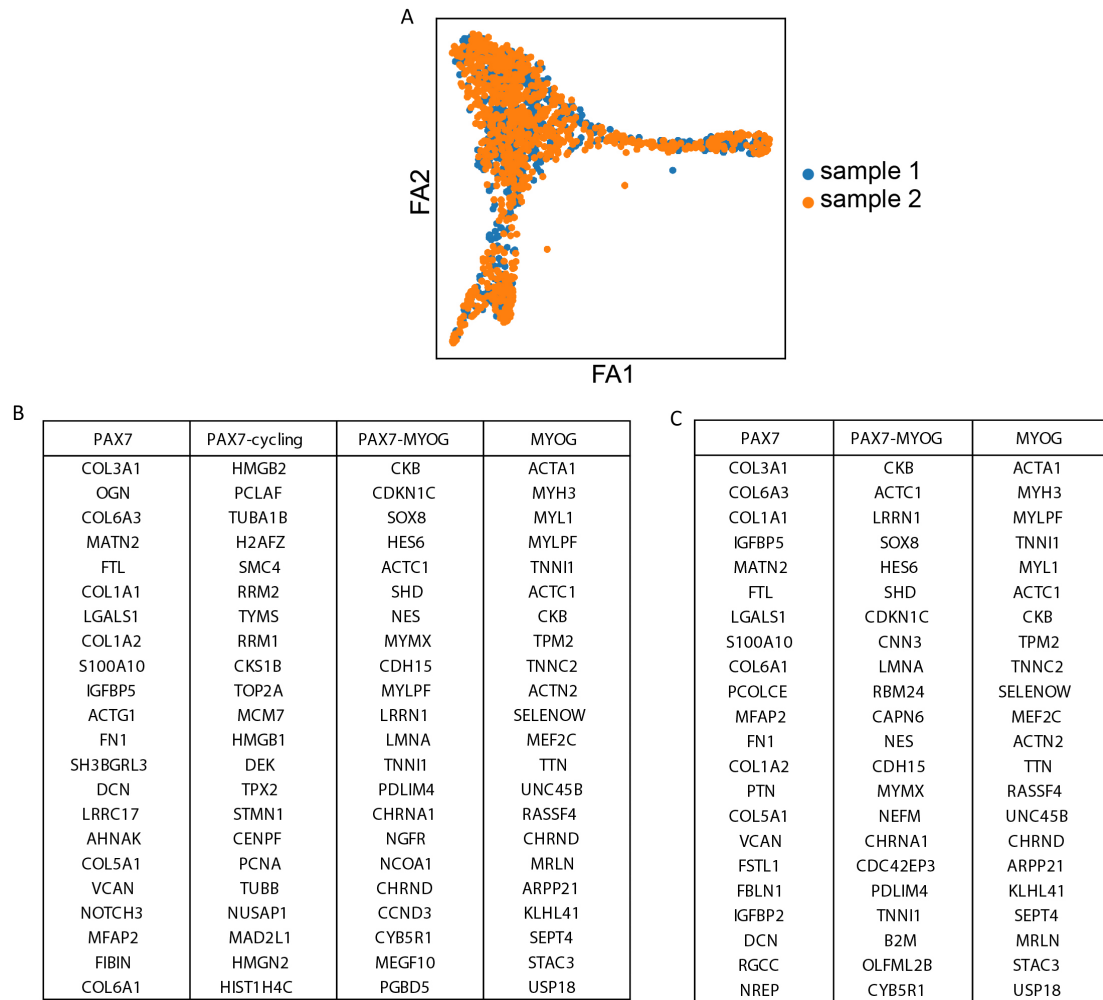## SUPPLEMENTARY FIGURES

A

B

Figure S1: Targeting strategy for iPS reporters

Targeting strategies for hPAX7^{Venus} (A), and hMYOG^{Venus} (B) with CRISPR/CAS9. HA, homology arm; nls, nuclear localization signal; HA, homology arm; H2B for nuclear localization.

A

B

PAX7	PAX7-cycling	PAX7-MYOG	MYOG
COL3A1	HMGB2	CKB	ACTA1
OGN	PCLAF	CDKN1C	MYH3
COL6A3	TUBA1B	SOX8	MYL1
MATN2	H2AFZ	HES6	MYLPF
FTL	SMC4	ACTC1	TNNI1
COL1A1	RRM2	SHD	ACTC1
LGALS1	TYMS	NES	CKB
COL1A2	RRM1	MYMX	TPM2
S100A10	CKS1B	CDH15	TNNC2
IGFBP5	TOP2A	MYLPF	ACTN2
ACTG1	MCM7	LRRN1	SELENOW
FN1	HMGB1	LMNA	MEF2C
SH3BGRL3	DEK	TNNI1	TTN
DCN	TPX2	PDLIM4	UNC45B
LRRC17	STMN1	CHRNA1	RASSF4
AHNAK	CENPF	NGFR	CHRND
COL5A1	PCNA	NCOA1	MRLN
VCAN	TUBB	CHRND	ARPP21
NOTCH3	NUSAP1	CCND3	KLHL41
MFAP2	MAD2L1	CYB5R1	SEPT4
FIBIN	HMGN2	MEGF10	STAC3
COL6A1	HIST1H4C	PGBD5	USP18

C

PAX7	PAX7-MYOG	MYOG
COL3A1	CKB	ACTA1
COL6A3	ACTC1	MYH3
COL1A1	LRRN1	MYLPF
IGFBP5	SOX8	TNNI1
MATN2	HES6	MYL1
FTL	SHD	ACTC1
LGALS1	CDKN1C	CKB
S100A10	CNN3	TPM2
COL6A1	LMNA	TNNC2
PCOLCE	RBM24	SELENOW
MFAP2	CAPN6	MEF2C
FN1	NES	ACTN2
COL1A2	CDH15	TTN
PTN	MYMX	RASSF4
COL5A1	NEFM	UNC45B
VCAN	CHRNA1	CHRND
FSTL1	CDC42EP3	ARPP21
FBLN1	PDLIM4	KLHL41
IGFBP2	TNNI1	SEPT4
DCN	B2M	MRLN
RGCC	OLFML2B	STAC3
NREP	CYB5R1	USP18

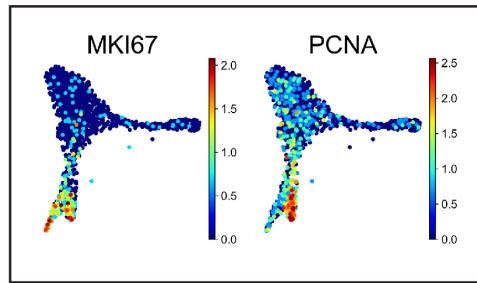## Figure S2: Single cell analysis of PAX7-Venus cells

(A) ForceAtlas2 directed layout showing overlap between PAX7-Venus positive cells analyzed from two myogenic cultures.

(B) Top 20 enriched transcripts for the four Louvain clusters relative to all other clusters detected by Wilcoxon rank-sum test. Transcripts are ranked by FDR-corrected p-values (Benjamini-Hochberg). See also Table S4.
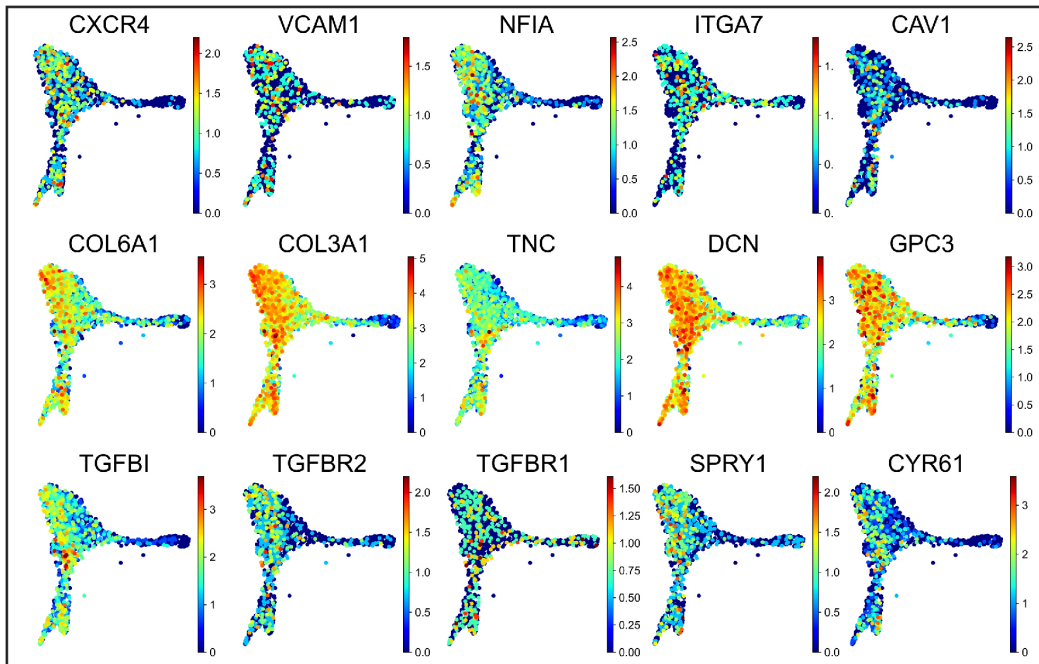
(C) Top 20 enriched transcripts for the three Louvain clusters identified after regression of cell cycle genes relative to all other clusters detected by Wilcoxon rank-sum test. Transcripts are ranked by FDR-corrected p-values (Benjamini-Hochberg). See also Table S4.
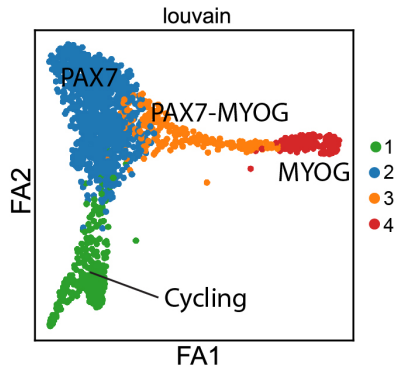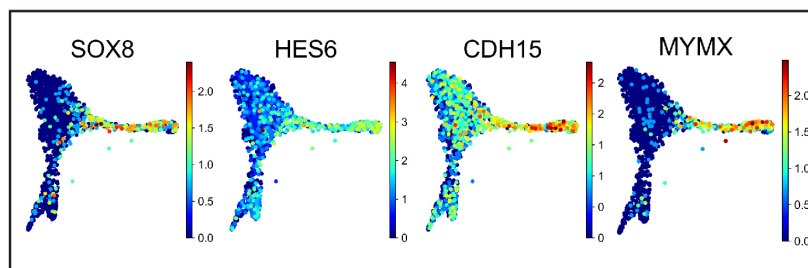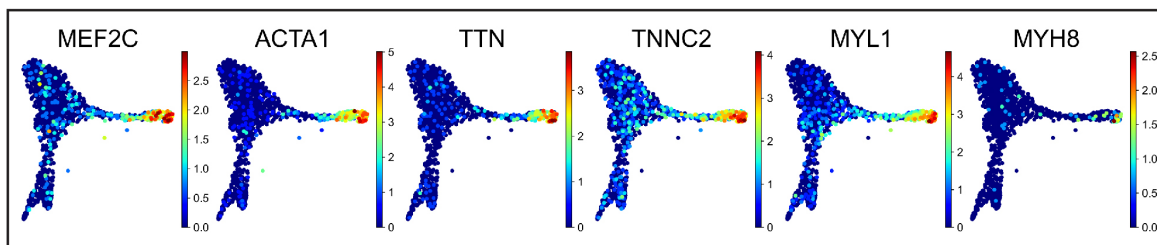
louvain

Cluster 1: cycling PAX7

Cluster 2: PAX7

Cluster 3: PAX7-MYOG

Cluster 4: MYOG

Figure S3: Single cell analysis of PAX7-Venus cells

ForceAtlas2 directed layout showing Louvain clusters and representative transcripts for each cluster.

Scale represents log-normalized transcript counts of indicated genes.

Table S1. List of genes upregulated in human PAX7Venus+ cells compared with undifferentiated hiPSCs

Click here to Download Table S1

Table S2. List of genes upregulated in mouse Pax7Gfp+ cells compared with undifferentiated ES

Click here to Download Table S2

Table S3. List of genes upregulated in human MYOGVenus+ cells compared with undifferentiated hiPSCs

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Table S4. Top 500 enriched transcripts for the four Louvain clusters shown in Fig. 2A relative to all the other clusters detected by the Wilcoxon rank-sum test. Transcripts are ranked by FDR-corrected P-values (Benjamini-Hochberg)

Click here to Download Table S4
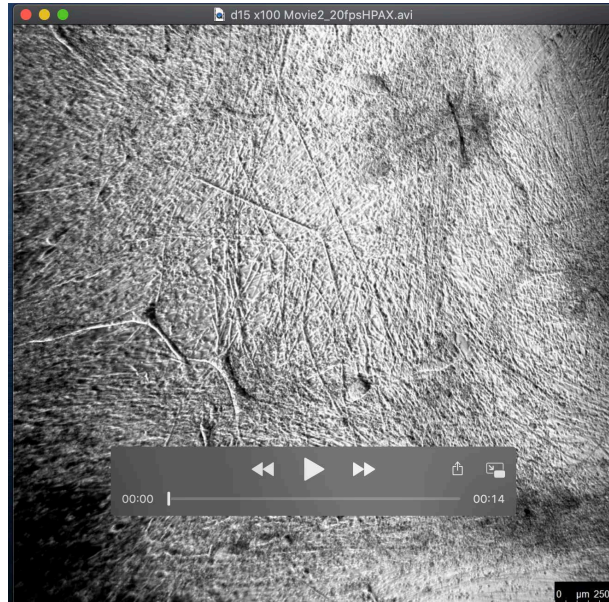
Movie 1. Twitching of human muscle fibers differentiated *in vitro* from PAX7$^{\text{Venus+}}$ cells FACS-sorted from 3-week-old cultures and replated for 2 weeks.