Supplemental information

Intravital imaging reveals cell cycle-dependent myogenic cell migration during muscle regeneration.

Yumi Konagaya[1, 5], Kanako Takakura[2], Maina Sogabe[3], Anjali Bisaria[1], Chad Liu[1], Tobias Meyer[1], Atsuko Sehara-Fujisawa[3], Michiyuki Matsuda[4, 5], Kenta Terai[5, 6, *]

[1]*Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA, 94305, USA.*

[2]*Imaging Platform for Spatio-Temporal Regulation, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan.*

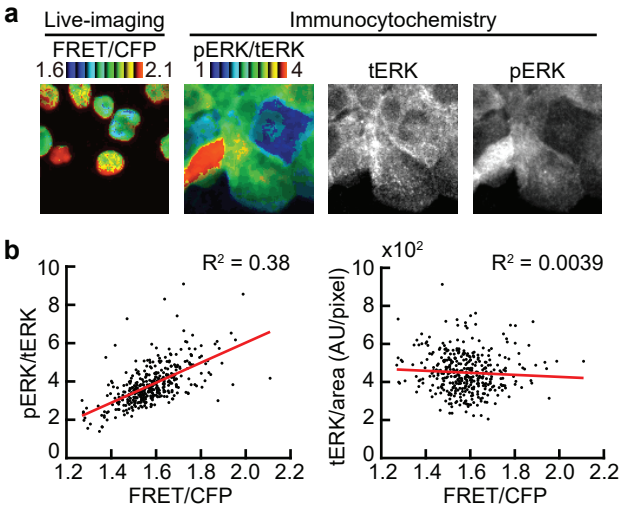
[3]*Department of Regeneration Science and Engineering, Institute of Frontier Life and Medical Sciences, Kyoto University, Kyoto 606-8507, Japan*

[4]*Department of Pathology and Biology of Diseases, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan*

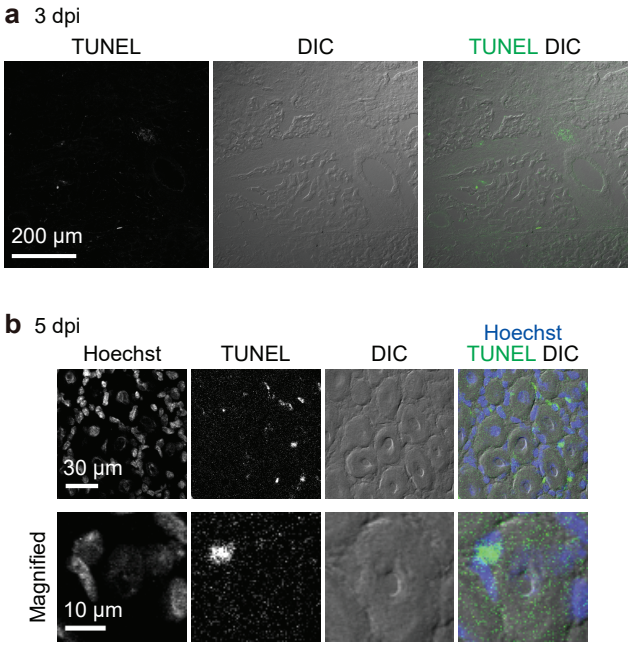
[5]*Laboratory of Bioimaging and Cell Signaling, Graduate School of Biostudies, Kyoto University, Kyoto 606-8501, Japan*

[6]*Lead Contact*

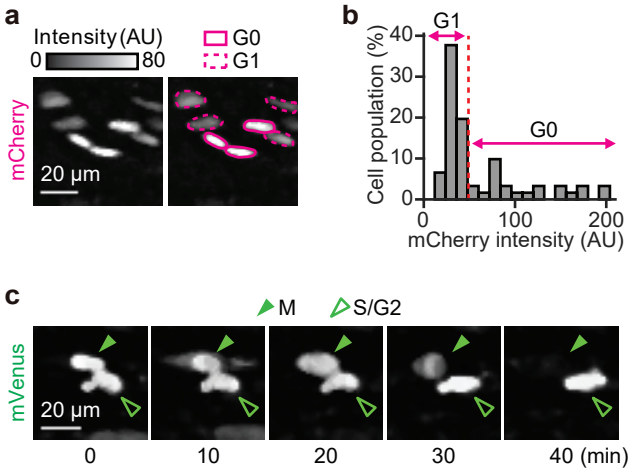
*Correspondence: terai.kenta.5m@kyoto-u.ac.jp



Supplemental Figure 1. Correlation between ERK FRET ratio by imaging and ERK phosphorylation by immunocytochemistry. (a) Representative images of the MCF10A cells expressing ERK FRET biosensor in the nucleus. The cells in same view field of FRET/CFP and phospho-ERK/total-ERK are shown in pseudo-color (IMD mode). Total-ERK and phospho-ERK detected by immunohistochemistry are shown in grayscale. (b) Scatter plot of phospho-ERK/total-ERK against FRET/CFP in MCF10A cells. Mean values of phospho-ERK/total-ERK in the nuclei are measured (left). Scatter plot of total-ERK against FRET/CFP in MCF10A cells. Mean values of total-ERK in the cytoplasmic region (5 pixels around the nuclear region) are measured (right).



Supplemental Figure 2. Apoptosis during muscle regeneration detected by TUNEL assays. (a) Representative images of regenerating muscles at 3 dpi. Snap frozen muscle sections were assayed for TUNEL, and observed together with differential interference contrast (DIC). (b) Representative images of regenerating muscles at 5 dpi. Snap frozen muscle sections were assayed for Hoechst and TUNEL, and observed together with DIC.



Supplemental Figure 3. Classification of G0, G1, S/G2, and M phase cells. (a) Representative images of myogenic cells expressing mCherry-hCdt1 at 3 dpi. Solid and dashed circles indicate G0 and G1 cells, respectively. (b) Histogram of the mCherry-hCdt1 intensity in myogenic cells at 3 dpi (1 of N = 3 mice). A red dashed line indicates a threshold to discriminate cells in G0 and G1 phase. The threshold was defined as an intersection of two Gaussian distributions fitted to the data. (c) Representative time-lapse images of myogenic cells expressing mVenus-hGeminin at 3 dpi. Solid and open arrows indicate M and S/G2 cells, respectively.

Supplemental Table 1. Percentage of proliferative myogenic cells at 5 dpi in different studies and their methods.

References	Assay	Proliferative cells (%)	Thymidine nucleoside analog	Mouse genotype	Tamoxifen	Drug for muscle damage	Mouse strain	Mouse age
This study	Live-imaging	2.2% mVenus-hGeminin(+) (Figure 4B)	NA	Pax7CreERT2 (Lepper et al., 2009)	0 to 14 days before injury	10 µL of 1 mg/mL cardiotoxin	C57BL6	2–6 months
Evano et al., Cell Rep. 2020	Immuno-histochemistry	23.1% EdU(+) (n = 40/173 H3.1-SNAP cells; Figure 2G, right)	4 days post-injury (twice, 0.3 ug/g, 23 h prior to sacrifice, 2 hr apart)	Pax7CreERT2 (Mathew et al., 2011)	4 days post-injury (14 h prior to sacrifice)	15 ml of 10 mM notexin	F1:C57BL6/DBA2	6–8 weeks
Joe et al., Nat Cell Biol. 2010	Flow cytometry (CD31(-)/CD45(-)/Sca1(-)/α7integrin(+))	> 10% BrdU(+) (Figure 5c)	4 days post-injury (twice, 100 mg/kg, 24 h prior to sacrifice, 12 hr apart)	WT	NA	0.15 µg of notexin	C57BL6	> 8 weeks
Rocheteau et al., Cell. 2012	Flow cytometry (Pax7-GFP(+))	> 95% EdU(+)/BrdU(+) (Figures S1D and S1E)	3 days post-injury with EdU (five times, 200 mg/injection 8 hr apart) followed by BrdU (twice 8 hr apart, 8 hr after EdU)	Tg:Pax7-nGFP (Sambasivan et al., 2009)	NA	Notexin	?	6–10 weeks
Zhang et al., J Biol Chem. 2013	Immuno-histochemistry	17.6% BrdU(+) (n = 70/397 hematoxylin(+) cells; Figure 5A)	4 days post-injury (once, 100 mg/kg, 18 h prior to sacrifice)	WT	NA	30 µL of 10 uM cardiotoxin	C57BL6	12–16 weeks