Morphology-based analysis of myoblasts for prediction of myotube formation

Kiyoshi Ishikawa[1,2†], Kei Yoshida[1†], Kei Kanie[1], Kenji Omori[2], Ryuji Kato[1]

[1]Graduate School of Pharmaceutical Sciences, Nagoya University, Furocho, Chikusa-ku, Nagoya, 464-8601, Japan

[2]Mitsubishi Tanabe Pharma Corporation, 1000, Shida-cho, Aoba-ku, Yokohama, 227-0033, Japan

[†]Authors contributed equally

Corresponding Author: Dr. Ryuji Kato, Graduate School of Pharmaceutical Sciences, Nagoya University, Furocho, Chikusa-ku, Aichi, 464-8601, Japan.

Tel.: +81-52-747-6811

Fax: +81-52-747-6813

E-mail: kato-r@ps.nagoya-u.ac.jp

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Supplementary Information

Figure Legends

Figure S1. The scheme of morphological analysis of confluent myotube cells.

Starting with the phase contrast image of myotube confluence status, the image was next processed through five filters to grasp recognisable objects that reflect the status of the myotubes. In this processing procedure, the ultimate goal was to extract the phenotypic morphological character of the confluent cellular status, rather than to segment the details of objects. Because there is no clear definition of myotube size, our approach was to recognise the candidate cellular objects, which may include all stages of myoblasts differentiating toward the myotube, and to statistically represent the morphological feature of a ‘group of cells in the image’ by means of the average and standard deviation of six individual morphological parameters. By accumulating these parameters throughout the time course of the study, our morphological parameters represent not only each stage of the morphological pattern but also the time course transitions of morphological patterns of ‘differentiating myoblasts’.

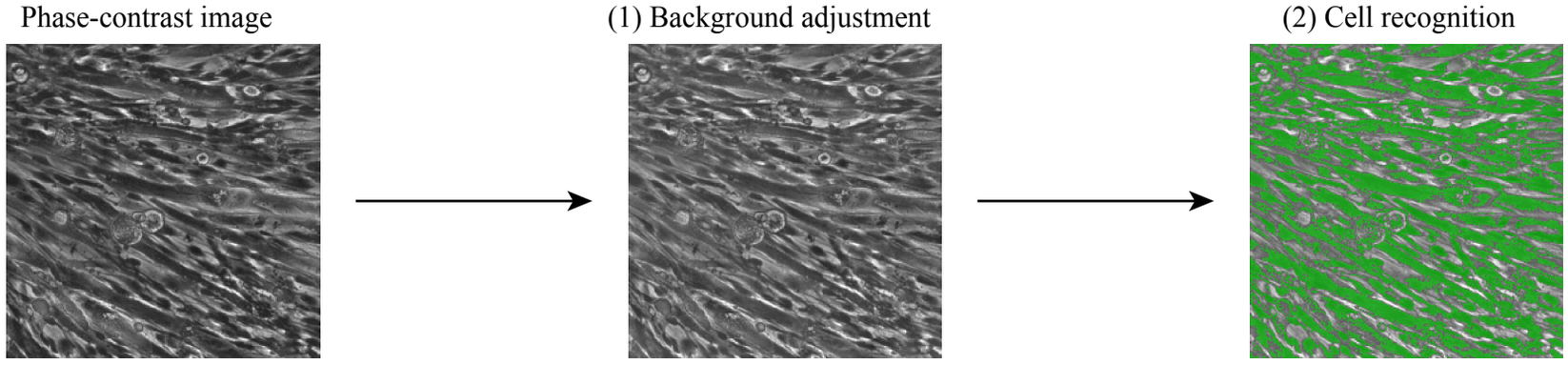
Figure S2. Prediction of an MHC-positive cellular area on days 10, 18, and 22.

(A) Representative phase contrast images and immunofluorescent images of C2C12 cells stained with MHC (green) and nuclei with SYTOX Blue (blue) on days 10, 18, and 22. The cells were

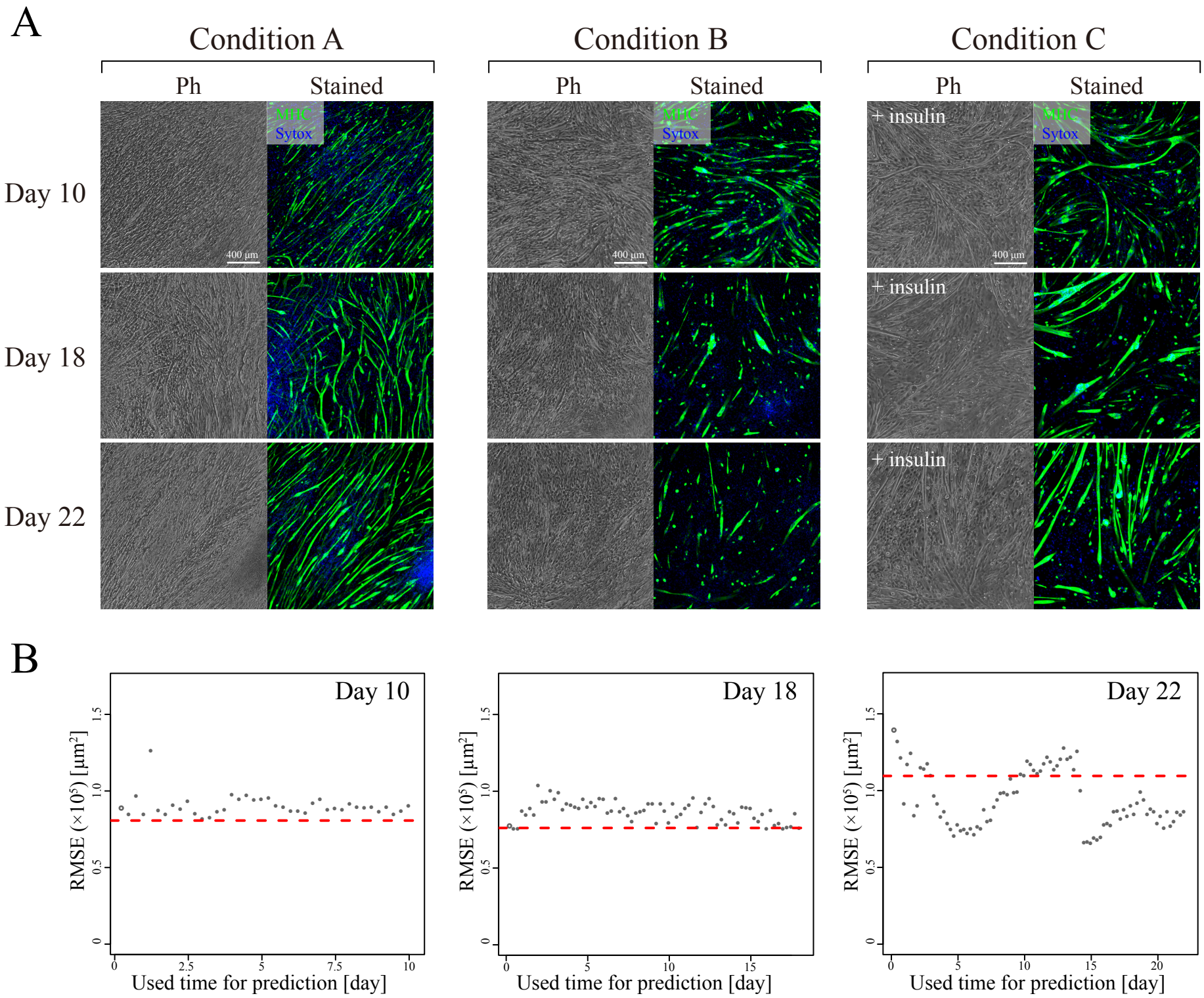
maintained under three types of culture conditions (A, B, and C). Scale bar, 400 μm. (B) Performance on the prediction of an MHC-positive area. Prediction of days 10, 18, and 22 was trained by morphological profiling from 0–10, 0–18, and 0–22 days, respectively. The dots indicate root mean square error (RMSE) throughout the analysis using different time periods of morphological information. For example, a blank dot indicates the RMSE of the prediction model trained with 0–6 hours of images. X-axis: duration of collecting the data for a morphological profile for prediction; Y-axis: RMSE values. The dotted line indicates the predictive performance of the NULL model, which is the negative control.

Figure S3. Analysis of a morphological response to the cGMP addition (on day 18) in condition B.

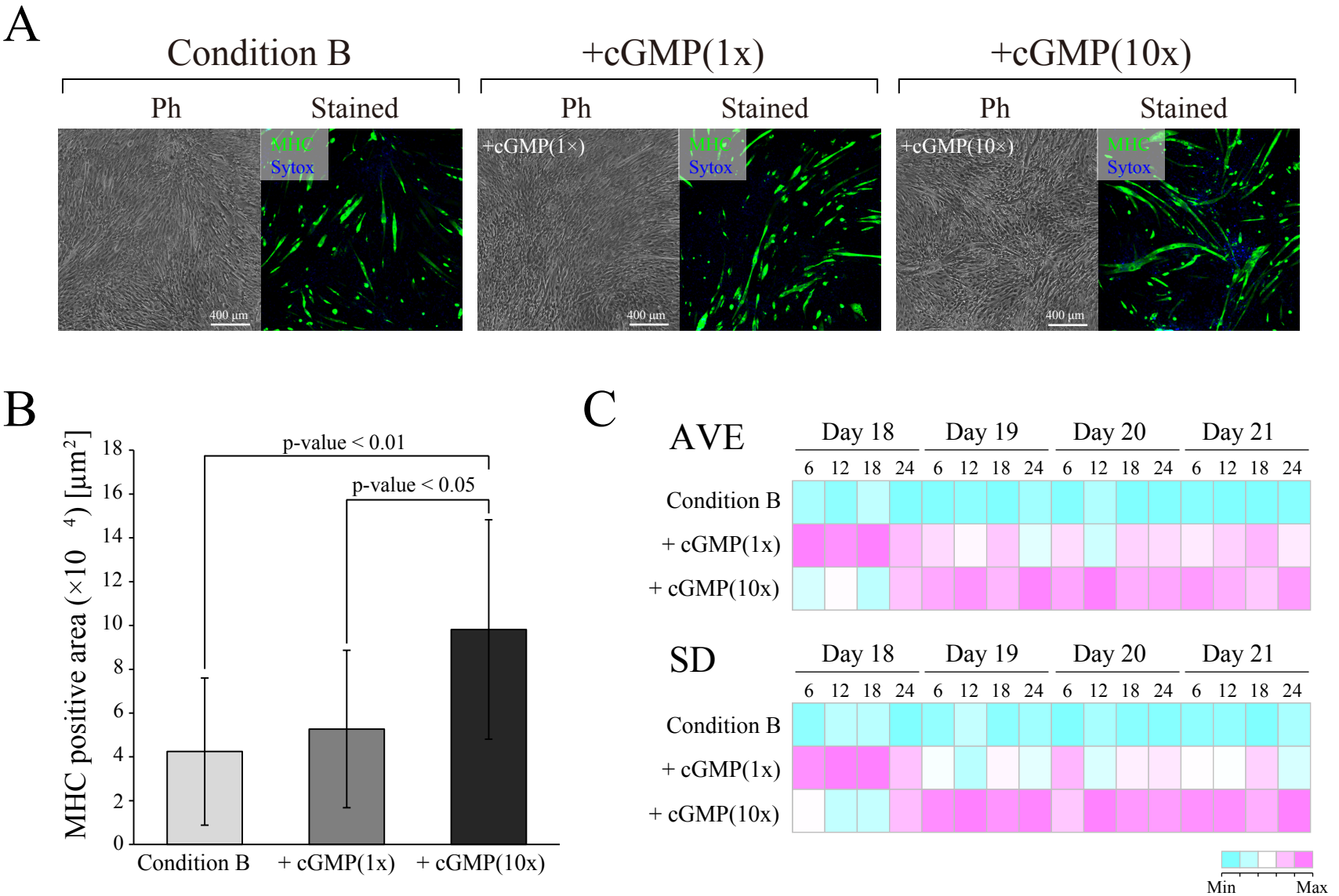
(A) Representative phase contrast images and immunofluorescent images of C2C12 cells stained for MHC (green) and nuclei by SYTOX Blue fluorescent dye staining (blue) on day 22. Scale bar, 400 μm. (B) The effect of cGMP addition on day 18 as quantified by means of the MHC-positive area. Error bars indicate means ± SD for four independent images per C2C12 lot (total number of images = 12). (C) A morphological profile indicating the cellular response of myotubes to cGMP. The heatmap shows the AVE and SD values of the l/w ratio at each time point (6 h intervals).



Supplementary Figure S1



Supplementary Figure S2


A
Condition B
+cGMP(1x)
+cGMP(10x)
Ph
Stained
MHC
Sytox
+cGMP(1×)
+cGMP(10×)
400 μm
B
MHC positive area (×10 4) [μm2]
p-value < 0.01
p-value < 0.05
Condition B
+ cGMP(1x)
+ cGMP(10x)
C
AVE
SD
Day 18
Day 19
Day 20
Day 21
6 12 18 24
Min
Max