Electronic Supplementary Material (ESI) for Integrative Biology.
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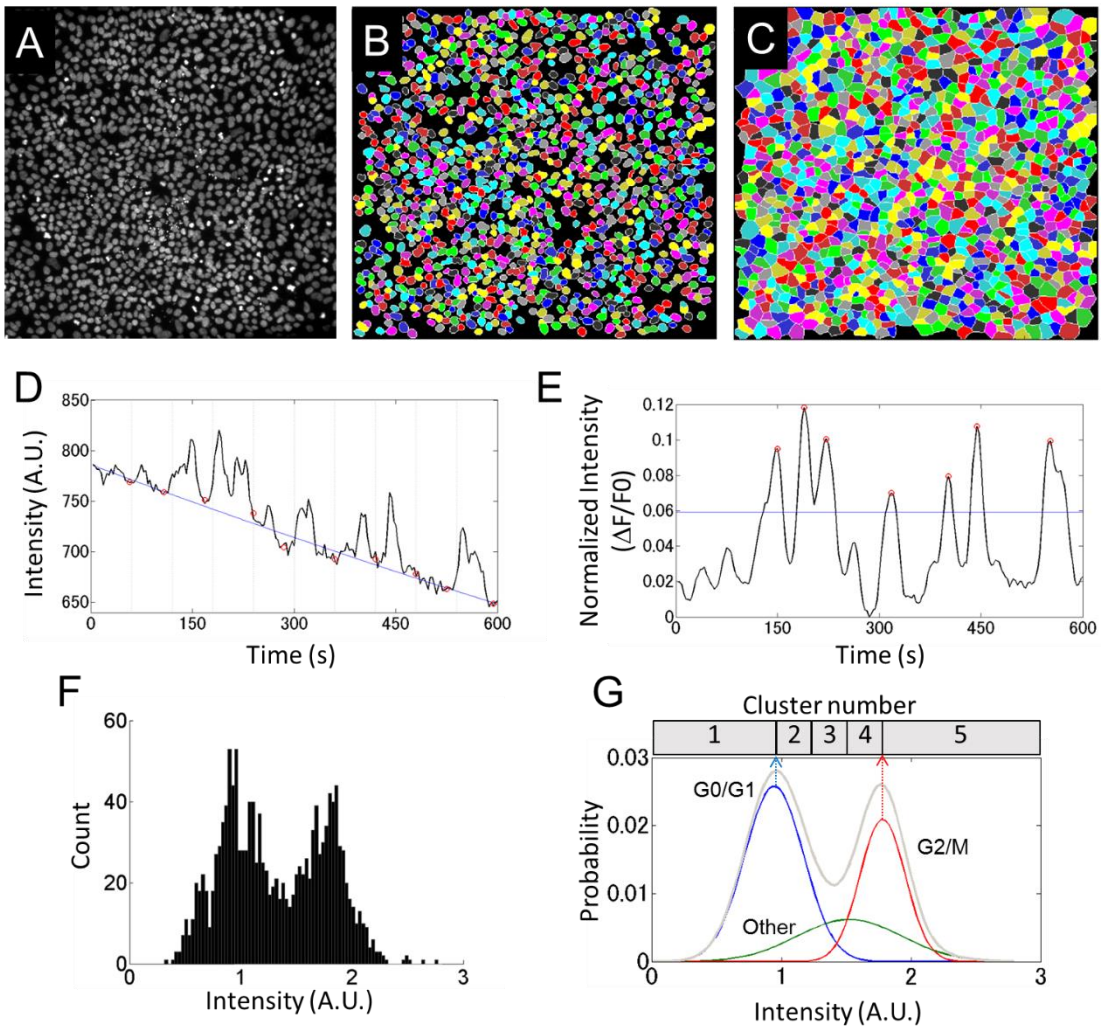
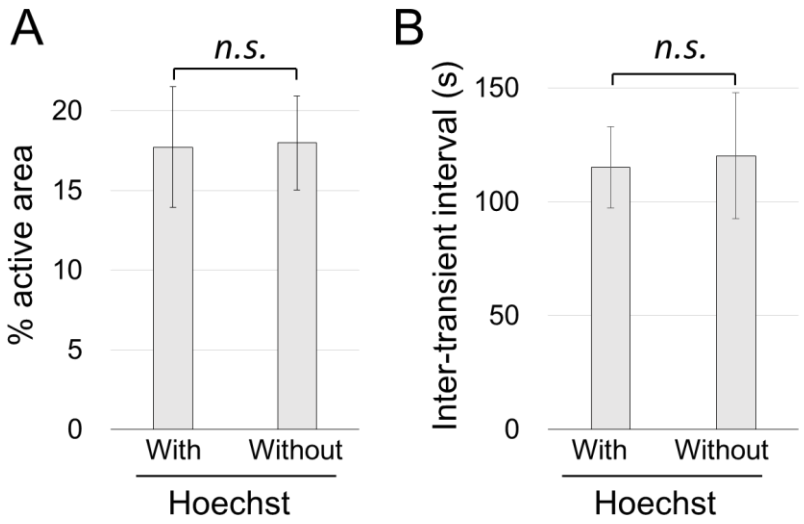
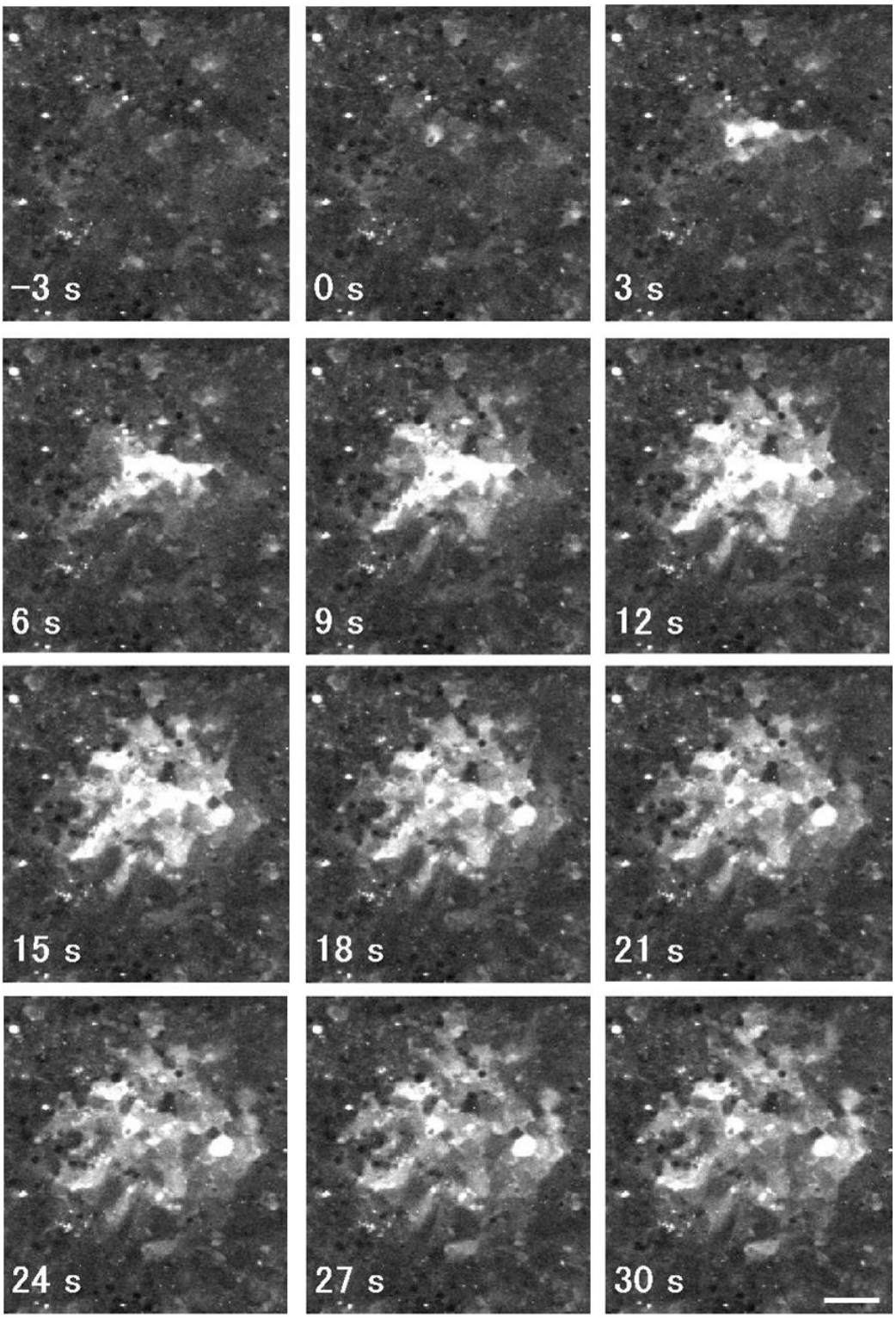


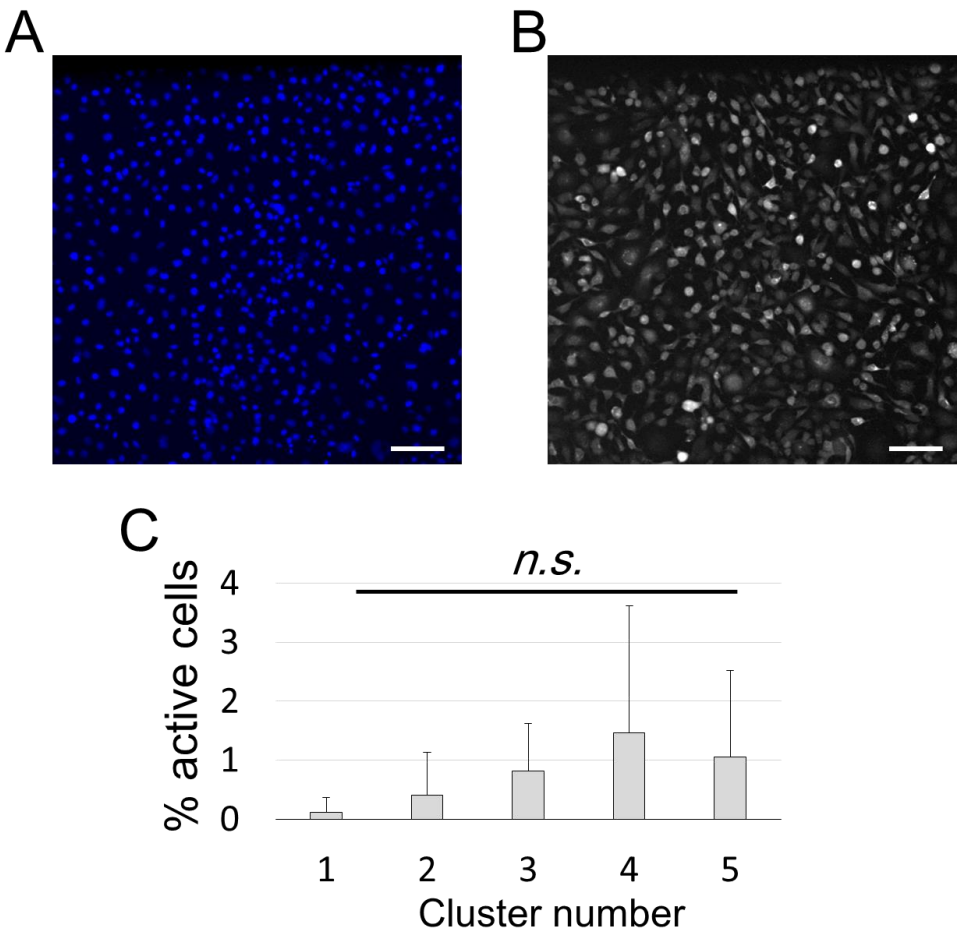
Supplementary figure S1. Procedure of image analysis. Cell nuclei (B) were recognized from nuclei images (A), and then cell areas (C) were segregated by expanding the nuclei areas. Ca^{2+} intensity (D) was normalized by fitting a second-order polynomial (blue line). Ca^{2+} transients beyond a threshold (blue line) were detected from the normalized Ca^{2+} trace (E). Three Gaussian distributions were fitted to histogram of integrated intensity of DNA-binding dye (F). Cells were partitioned into five clusters based on the G0/G1 and G2/M peaks (G).



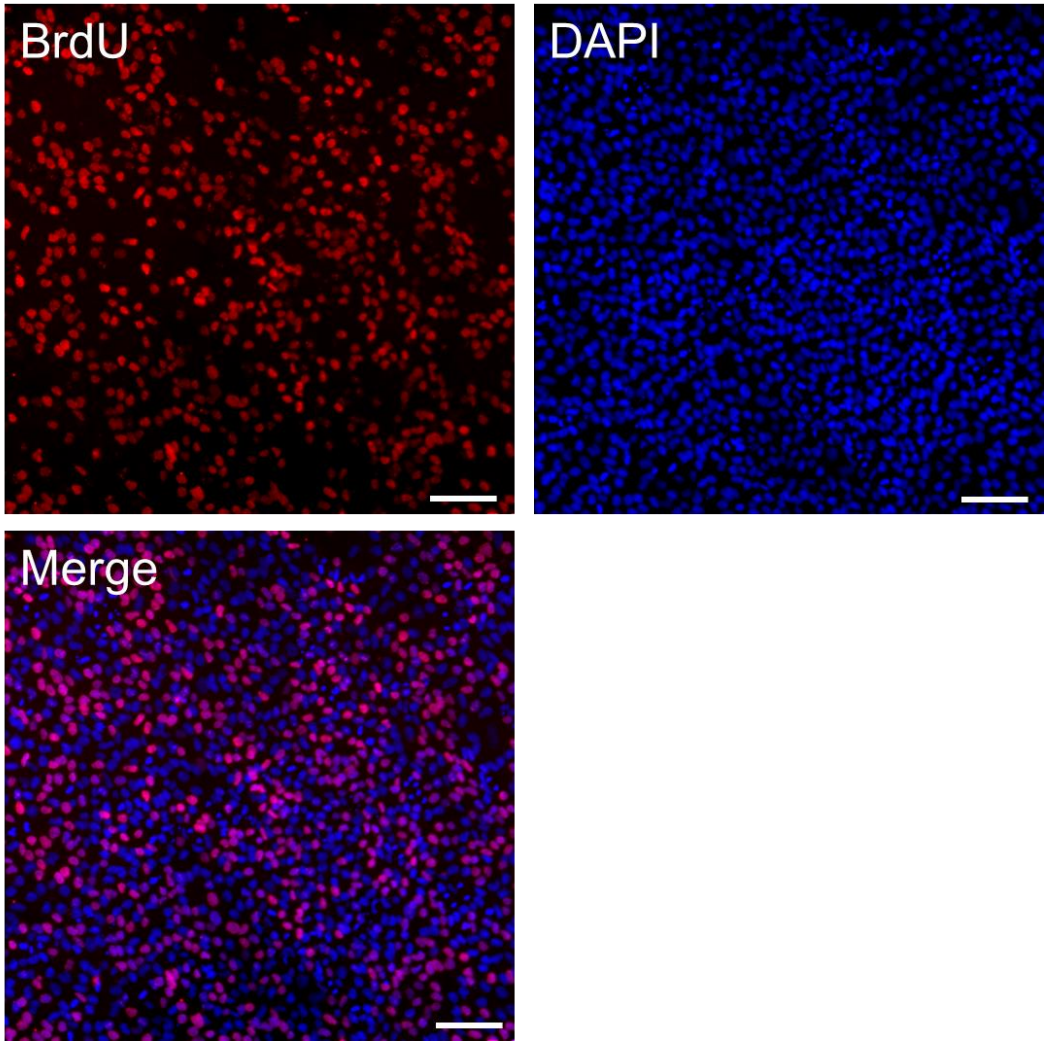
Supplementary figure S2. Effects of Hoechst incorporation into DNA. Images were separated into 2500 patches and Ca^{2+} transients were detected from each patch. (A) Ratios of cells exhibiting Ca^{2+} transients. (B) Inter-transients intervals of Ca^{2+} transients. There are no significant differences with and without Hoechst incorporation, indicating that Hoechst did not change the properties of the Ca^{2+} transients. Error bars represent SD; $n = 22$ for with Hoechst, $n = 5$ for without Hoechst; *n.s.*: $p > 0.1$; Welch's t test.



Supplementary figure S3. Ca wave in human iPS cells. Elevation of intracellular Ca^{2+} concentration in single cell propagated through multiple cells. Ca wave lasted for 57 ± 22 s. Scale bar indicates 50 μm.



Supplementary figure S4. Relationship between cell cycle phase and Ca^{2+} transients in SNL 76/7 cells. (A) Image of nuclei. (B) Representative image of Ca imaging. Scale bar indicates 100 μm. (C) Relationship between cluster number and ratio of cells with Ca^{2+} transients. Ca^{2+} transients of SNL 76/7 cells were observed from only 0.53 ± 0.32% of cells. Any relationship between cell cycle phase and Ca^{2+} transients was observed in SNL 76/7 cells. Error bars represent SD; $n = 5$; *n.s.*: $p > 0.1$; Paired t test.



Supplementary figure S5. Representative images with BrdU staining. Cell cycle of human iPS cells was examined with BrdU pulse labeling assay. Cells in S phase were labeled with BrdU (red). Nuclei were stained with DAPI. Scale bars indicate 100 μm.

Supplementary table S1. Parameters in the CellProfiler.

IdentifyPrimaryObjects	
Typical diameter of objects	7 – 15
Discard objects outside the diameter	Yes
Discard objects touching the border	Yes
Threshold strategy	Adaptive
Thresholding method	Otsu
Two-class or three class thresholding	Two classes
The weighted variance or the entropy	Weighted variance
Smoothing method for thresholding	Automatic
Threshold correction factor	1 for iPS cells, 1.1 for SNL 76/7 cells
Lower and upper bounds on threshold	0.0 - 1.0
Methods to calculate adaptive window size	Custom
Size of adaptive window	20
Method to distinguish clumped objects	Laplacian of Gaussian
Automatically calculate the threshold	No
Laplacian of Gaussian threshold	0.2
Automatically calculate the size of objects	No
LoG filter diameter	8
Method to draw dividing lines	Intensity
Automatically calculate smoothing filter	No
Size of smoothing filter	5
Automatically calculate minimum distance	No
minimum allowed distance	3
Speed up by using lower-resolution image	Yes
Retain outlines of the identified objects	Yes

IdentifySecondaryObjects	
Select the method to identify the secondary objects	Distance - N
Number of pixels by which to expand	10
Fill holes in identified objects	Yes
Discard secondary objects touching the border	No
Retain outlines of the identified objects	Yes