Legends for Supplementary Figures

Figure S1. GSK126 facilitates iCM formation from mouse embryonic fibroblasts with MM_3-GHT.

(A) Western blotting demonstrating a decrease of H3K27me3 with 1 μM GSK126 added to the culture medium of non-transduced fibroblasts for 2 days, in comparison to the culture without GSK126. Histone H3 was used as a loading control.

(B) Temporal profile of the number of iCM clusters (mean ± SD) after the addition of GSK126 to the culture medium from Day 1 to Day 4 (red line), compared with the culture without GSK126 (gray line). 10,000 fibroblasts were seeded in each well of 48-well plates on Day –2. Total number of iCM clusters in each well was counted each day.

(C) Oscillation of green fluorescence reflecting the oscillation of the intracellular Ca^{2+} concentration in fibroblasts on Day 14. The cells were transduced with MM_3-GHT and treated with GSK126 from Day 1 to Day 4. The imaging time point is depicted above each panel. The fluorescence signal oscillated in the cells encircled by dashed lines in the 5.5 second panel. The cells encircled by dotted lines in the phase contrast and Hoechst panels displayed spontaneous beating. Hoechst stain is shown to indicate nuclei. Videos of these panels are available in Supplementary Video 1A (fluorescence) and 1B (phase contrast). Bar, 100 μm.

(D) Frequency of oscillating cells on Day 14 after the addition of GSK126 from Day 1 to Day 4. The percentage of these cells among more than 500 Hoechst-positive cells is shown.

Figure S2. UNC0638 promotes iCM formation from mouse embryonic fibroblasts with MM_3-GHT.

(A) Western blotting showing a decrease of H3K9me2 with 0.25 μM UNC0638 added to the culture medium of non-transduced fibroblasts for 2 days.

(B) Temporal profile of the number of iCM clusters (mean ± SD) per well of a 48-well plate after the addition of UNC0638 to the culture medium from Day 3 to Day 7 (black line) compared with the culture without UNC0638 (gray line). 10,000 fibroblasts were seeded in each well of 48-well plates

on Day –2. Total number of iCM clusters in each well was counted each day.

(C) Maximum numbers of iCM clusters after the addition of the indicated inhibitor(s) to fibroblasts transduced with MM_3-GHT from Day 1 to Day 4.

(D) Maximum numbers of iCM clusters after the addition of the indicated inhibitor(s) to fibroblasts transduced with MM_3-GHT from Day 1 to Day 7.

(E) Maximum numbers of iCM clusters after the sequential addition of GSK126 from Day 1 to Day 4 and UNC0638 from Day 4 to Day 7 to cells transduced with MM_3-GHT.

Figure S3. GSK126 and UNC0638 promote iCM formation with M-GHT.

Maximum numbers of iCM clusters in a well of a 48-well plate with the addition of the indicated inhibitors to fibroblasts transduced with M-GHT.

(A) 1 μM GSK126 and/or 0.25 μM UNC0638 were used from Day 1 to Day 4. **P* < 0.05, ***P* < 0.01 (Student's *t*-test).

(B) 1 μM GSK126 and different concentrations of UNC0638 were used from Day 1 to Day 4.

(C) 1 μM GSK126 and/or 0.25 μM UNC0638 were used from Day 1 to Day 7.

(D) 1 μM GSK126 was used from Day 1 to Day 4, and different concentrations of UNC0638 were used from Day 4 to Day 7.

(E) Different concentrations of UNC0638 were used from Day 4 to Day 7.

Figure S4. Expression of cTnT and Hcn4 on Day 14 after gene transduction and addition of inhibitors.

(A) Frequency of cells positive for cardiac troponin T (cTnT) on Day 14. 1 μM GSK126 and/or 0.25 μM UNC0638 were used from Day 1 to Day 4.

(B) Immunofluorescence staining of cTnT and Hcn4 on Day 14. Cells were transduced with MM_3-GHT, and 1 μM GSK126 and 0.25 μM UNC0638 were added from Day 1 to Day 4. DNA was

counterstained with Hoechst 33342. The area surrounded by a white rectangle is shown at a larger magnification in the “Magnified” panel. Bars, 50 μm. The top panel, which is identical to Figure 1F, is shown here again for easy comparison.

Legends for Videos

Supplementary Video 1. Ca^{2+} oscillations and beating of the cells on Day 14 after transduction with MM_3-GHT (Preparation 1).

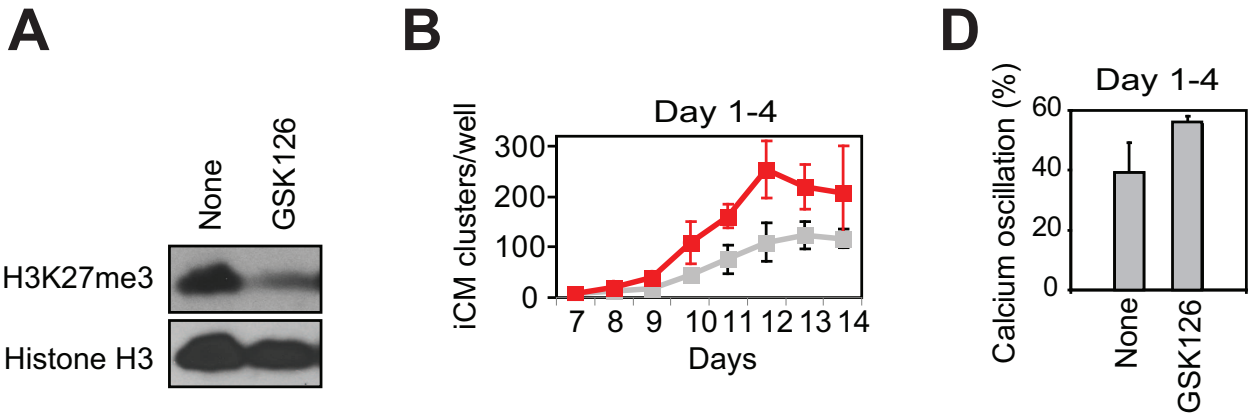
(A) Fluorescence video demonstrating oscillation of the intensity of green fluorescence, which represents the fluctuation of the intracellular Ca^{2+} concentration. 1 μM GSK126 was added to the culture medium from Day 1 to Day 4. This video is presented 10 times faster than real time speed for a technical reason.

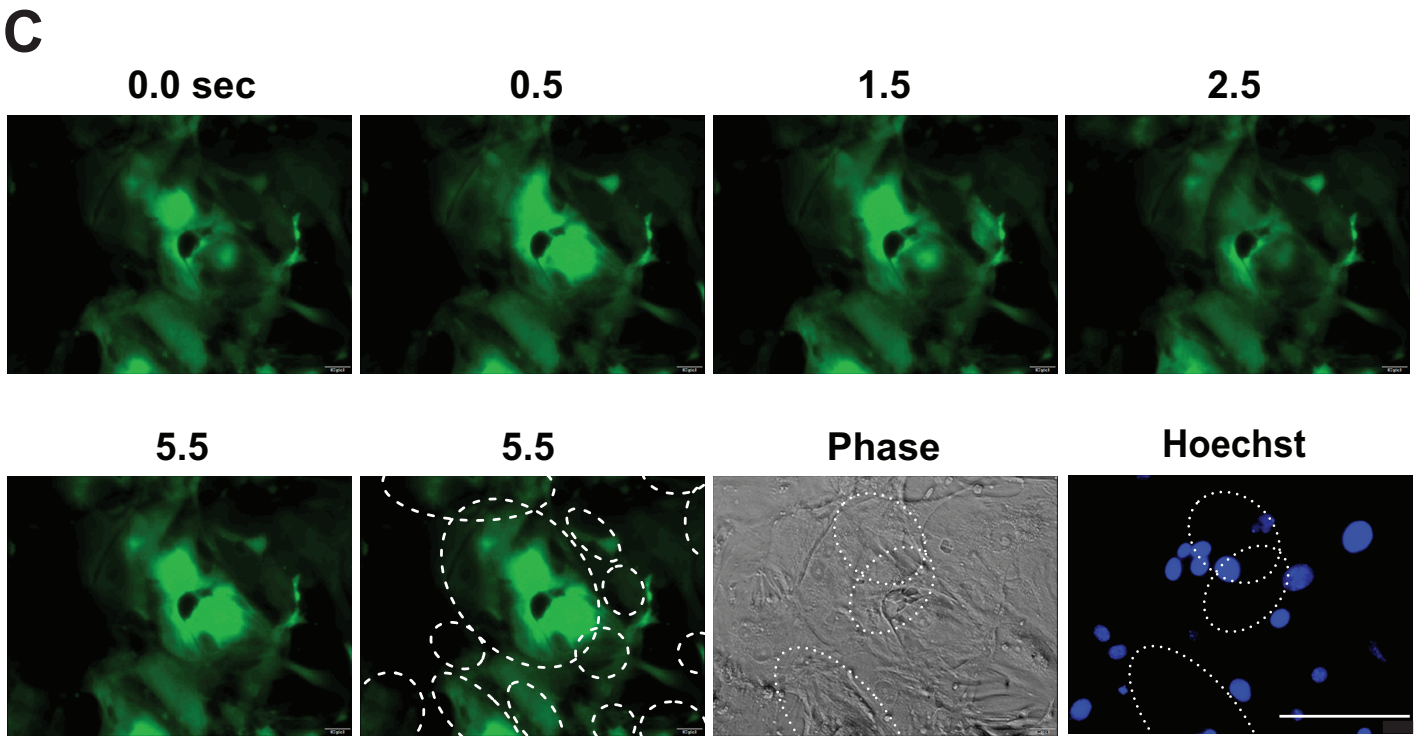
(B) Phase contrast video of the same field as in (A) showing spontaneous beating. This video is presented at real time speed.

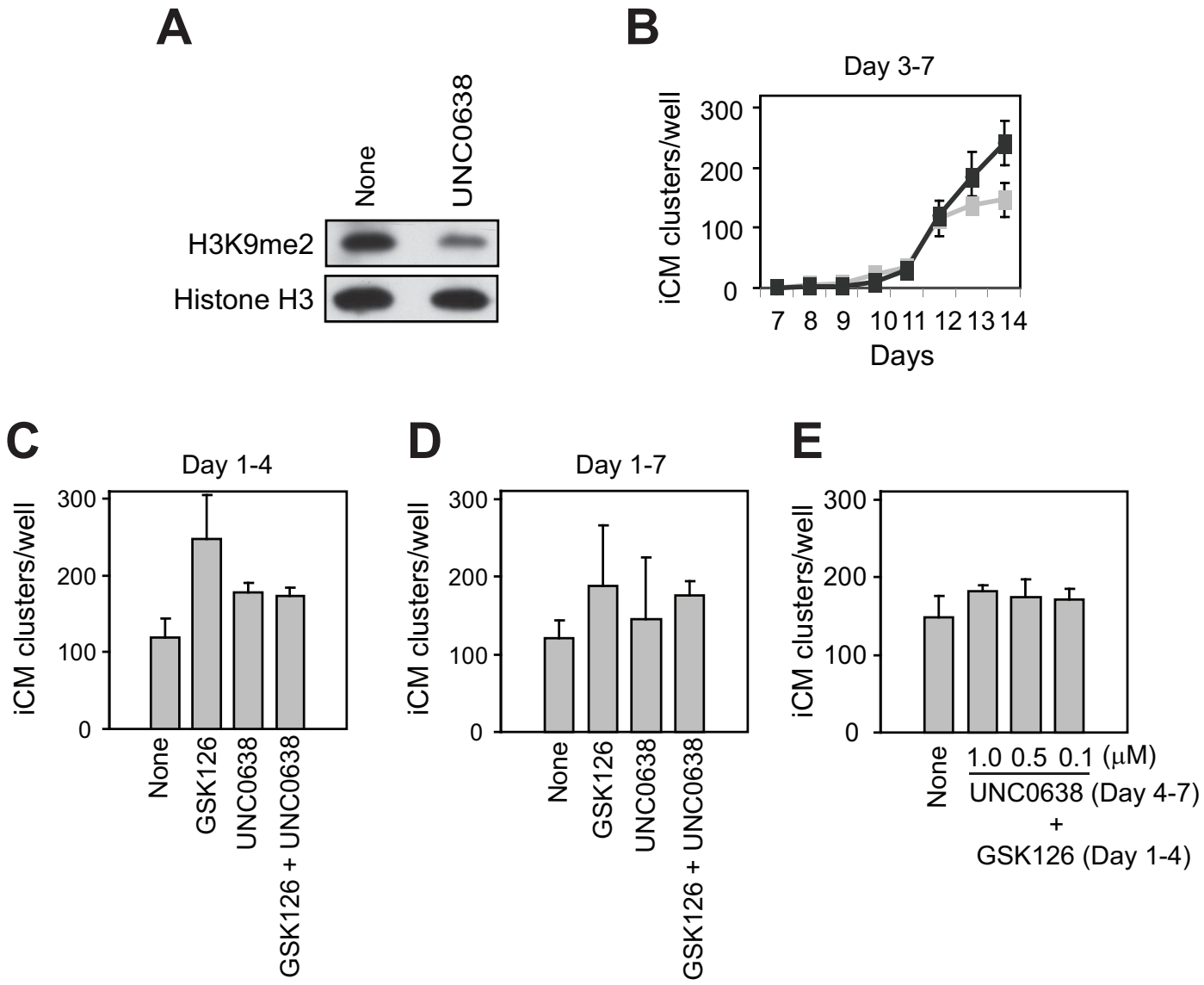
Supplementary Video 2. Ca^{2+} oscillations and beating of the cells on Day 14 after transduction with MM_3-GHT (Preparation 2).

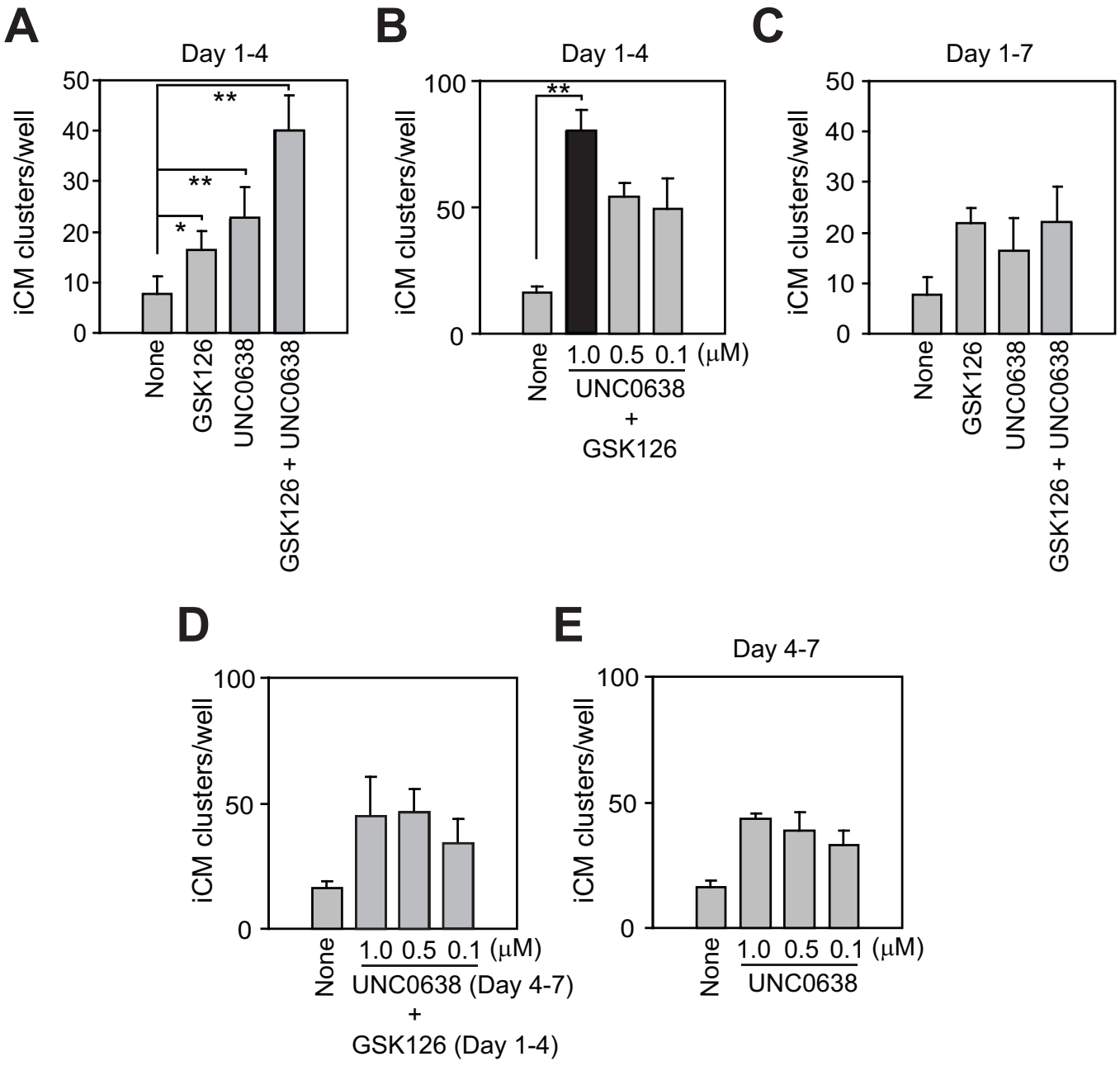
(A) Green fluorescence video representing the oscillation of intracellular Ca^{2+} concentration. 1 μM GSK126 was added to the culture medium from Day 1 to Day 4. This video is 10 times faster than real time speed for a technical reason.

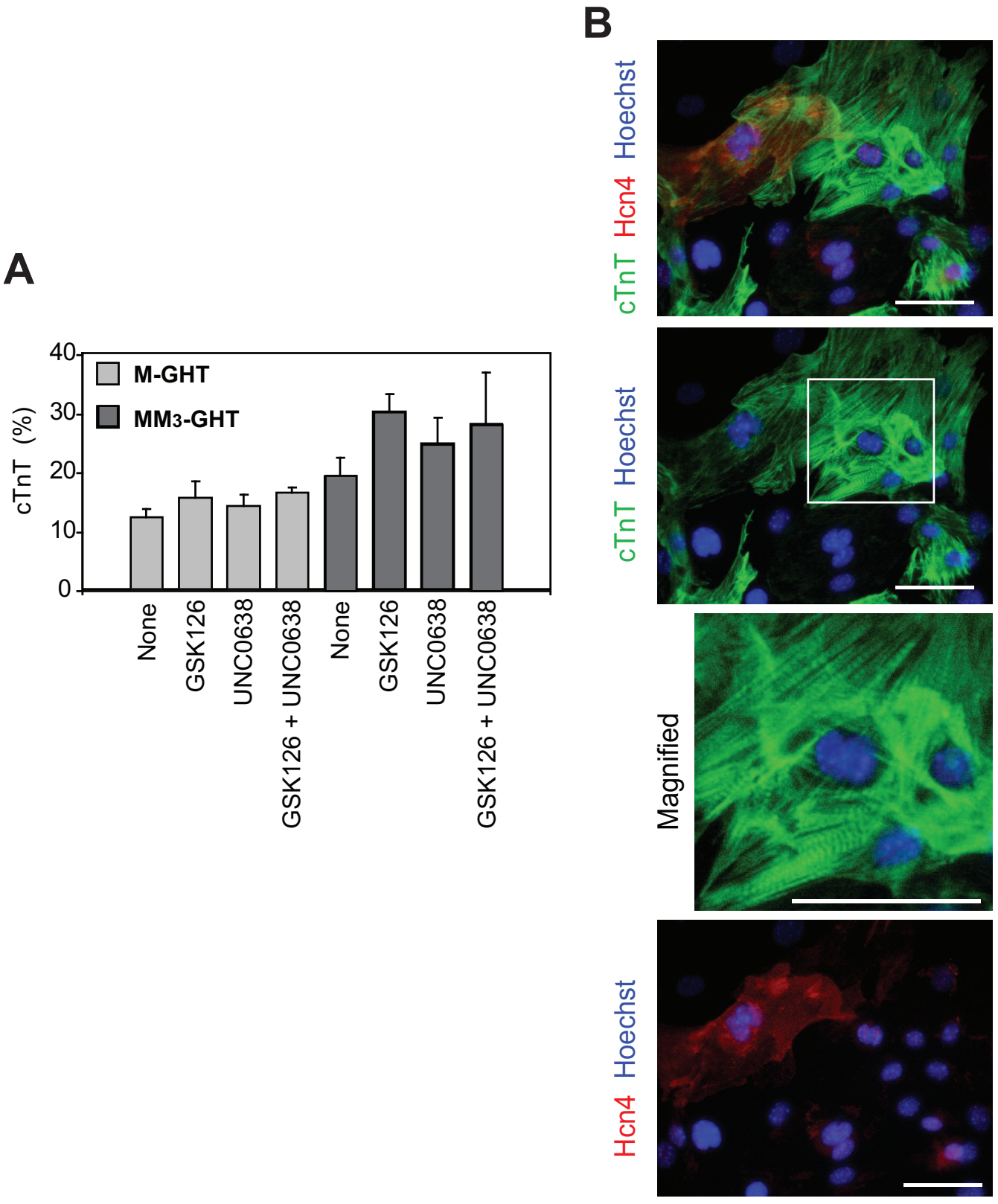
(B) Phase contrast video of the same field as (A). This video is presented at real time speed.


A
None
GSK126
H3K27me3
Histone H3
B
Day 1-4
iCM clusters/well
300
200
100
0
7 8 9 10 11 12 13 14
Days
D
Day 1-4
Calcium oscillation (%)
60
40
20
0
None
GSK126


C
0.0 sec
0.5
1.5
2.5
5.5
5.5
Phase
Hoechst


A
None
UNC0638
H3K9me2
Histone H3
B
Day 3-7
iCM clusters/well
300
200
100
0
7 8 9 10 11 12 13 14
Days
C
Day 1-4
iCM clusters/well
300
200
100
0
None
GSK126
UNC0638
GSK126 + UNC0638
D
Day 1-7
iCM clusters/well
300
200
100
0
None
GSK126
UNC0638
GSK126 + UNC0638
E
iCM clusters/well
300
200
100
0
None
1.0 0.5 0.1 (μM)
UNC0638 (Day 4-7)
+
GSK126 (Day 1-4)


A
Day 1-4
iCM clusters/well
50
40
30
20
10
0
**
**
*
None
GSK126
UNC0638
GSK126 + UNC0638
B
Day 1-4
iCM clusters/well
100
50
0
**
None
1.0 0.5 0.1 (μM)
UNC0638
+
GSK126
C
Day 1-7
iCM clusters/well
50
40
30
20
10
0
None
GSK126
UNC0638
GSK126 + UNC0638
D
iCM clusters/well
100
50
0
None
1.0 0.5 0.1 (μM)
UNC0638 (Day 4-7)
+
GSK126 (Day 1-4)
E
Day 4-7
iCM clusters/well
100
50
0
None
1.0 0.5 0.1 (μM)
UNC0638


A
cTnT (%)
40
30
20
10
0
M-GHT
MM3-GHT
None
GSK126
UNC0638
GSK126 + UNC0638
None
GSK126
UNC0638
GSK126 + UNC0638
B
cTnT Hcn4 Hoechst
cTnT Hoechst
Magnified
Hcn4 Hoechst