

Electronic Supplementary Information

Modulating cardiomyocyte and fibroblast interaction using layer-by-layer deposition facilitates synchronisation of cardiac macro tissues

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Contents:

Figure S1-S8

Table S1-S4

Movie S1-S4 (please see the separated zip files)

Movie S1-S4. Corresponding movie for Figure 4 at day 6. Movie S1 (mixture GF-), Movie S2 (LBL GF-), Movie S3 (mixture GF+), and Movie S4 (LBL GF+)

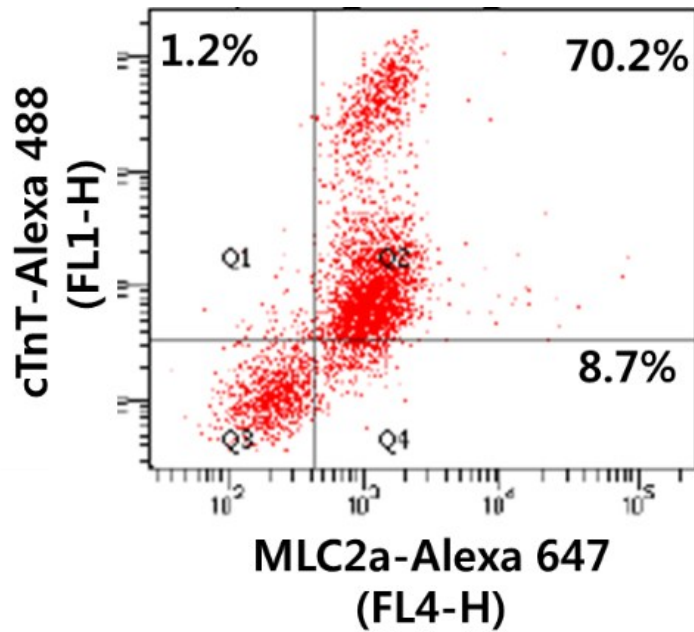


Figure S1. Flow cytometry analysis of cardiomyocytes differentiated from hESCs via small-molecule modulation of Wnt signaling. Cardiomyocytes were generated from hESCs (H9) with 6 μ M CHIR99021 treatment at day 0 for 48 h and 5 μ M IWP2 treatment at day 3 for 48 h. At day 11, cells were analyzed for cTnT/MLC2a expression by flow cytometry. Cardiomyocytes were analyzed by flow cytometry using a FACS Canto II. Note that cTnT+ cardiomyocytes: 71.4% and MLC2a+ cardiomyocytes: 78.9%

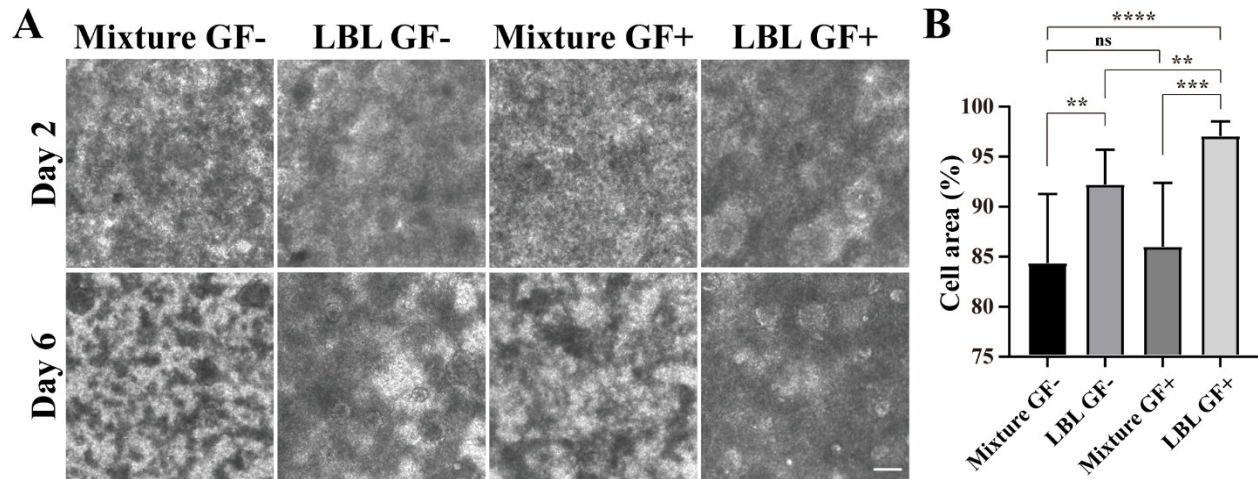


Figure S2. Bright field microscope images of CMTs in molds (day 2) and detached CMTs (day 6) (A). Quantitative analysis of cell area (%) in CMTs (B). Note that cells are placed in dark area. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, unpaired two-tailed Student's t-test. Scale bar: 500 μm . Note that the error bars were estimated by the standard deviation of 9 samples.

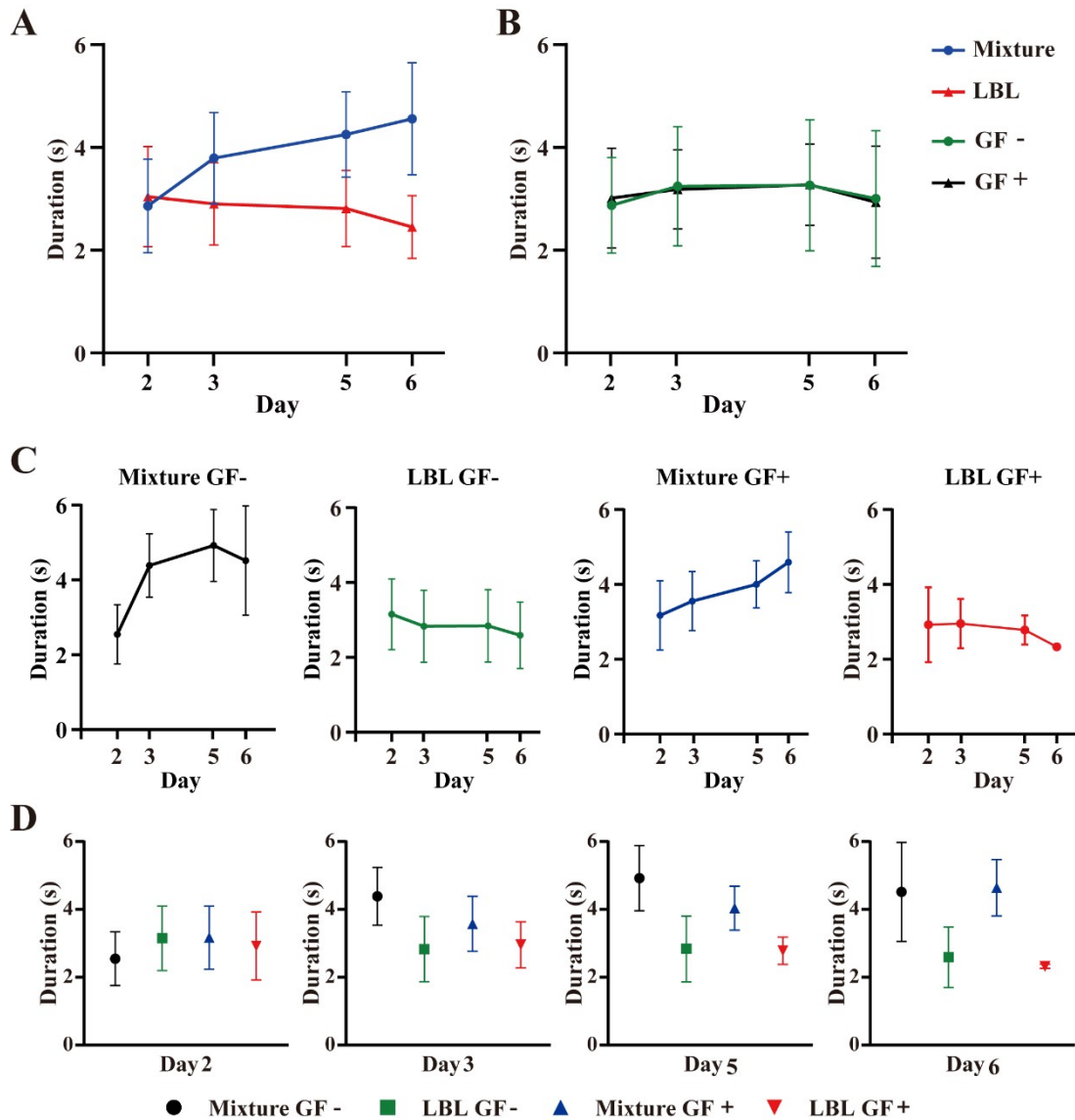


Figure S3. Statistical analysis of peak to peak (PTP) duration in cardiac beating (Figure 4) using ANOVA. Univariate analysis was performed to determine influence of cell deposition methods (A) and growth factors (B) on PTP duration (Day2: F-value(P) = 8.677(0.004), Day3: F-value(P) = 8.329(0.005), Day5: F-value(P) = 8.519(0.004), Day6: F-value(P) = 1.166(0.282)). The changes in PTP duration by incubation time for each group (C) and by all the groups at each day (D) are shown. Please see the Table S1, S2 and S3 for statistical analysis for each case. Note that N = 5 per each group and the error bars were estimated by the standard deviations of 5 samples.

Table S1. Univariate analysis of PTP duration for influence of cell deposition methods and growth factors

Univariate analysis (Day2)					Univariate analysis (Day3)				
Deposition	Cytokine	Average	SD	N	Deposition	Cytokine	Average	SD	N
Mixture	GF-	2.55	0.79	38	Mixture	GF-	4.39	0.82	12
	GF+	3.17	0.93	37		GF+	3.55	0.79	30
	All	2.86	0.91	75		All	3.79	0.89	42
LBL	GF-	3.15	0.95	47	LBL	GF-	2.83	0.96	32
	GF+	2.92	1.00	45		GF+	2.95	0.66	43
	All	3.04	0.98	92		All	2.90	0.80	75
All	GF-	2.89	0.93	85	All	GF-	3.26	1.16	44
	GF+	3.03	0.97	82		GF+	3.20	0.77	73
F-value (P)		8.677**(0.004)			F-value (P)		8.329**(0.005)		

Univariate analysis (Day5)					Univariate analysis (Day6)				
Deposition	Cytokine	Average	SD	N	Deposition	Cytokine	Average	SD	N
Mixture	GF-	4.92	0.96	12	Mixture	GF-	4.52	1.46	14
	GF+	4.00	0.63	32		GF+	4.59	0.81	22
	All	4.25	0.83	44		All	4.56	1.09	36
LBL	GF-	2.84	0.97	45	LBL	GF-	2.59	0.89	48
	GF+	2.78	0.39	44		GF+	2.33	0.06	59
	All	2.81	0.74	89		All	2.45	0.61	107
All	GF-	3.28	1.28	57	All	GF-	3.02	1.32	62
	GF+	3.29	0.79	76		GF+	2.95	1.09	81
F-value (P)		8.519**(0.004)			F-value (P)		1.162(0.282)		

*P < 0.05, **P < 0.01, ***P < 0.001

Table S2. One-way ANOVA analysis of PTP

duration by incubation time for each group.

Mixture GF-			
Group	N	Average	SD
Day 2	38	2.55	0.79
Day 3	12	4.39	0.85
Day 5	12	4.92	0.96
Day 6	14	4.52	1.46

Dunnette T3 test (Levene, P=0.003)

Day 2	Day 3	P = 0.000	***
	Day 5	P = 0.000	***
	Day 6	P = 0.001	**
Day 3	Day 5		
	Day 6		
Day 5	Day 6		
F-value(P)		28.531***(0.000)	

LBL GF-			
Group	N	Average	SD
Day 2	47	3.15	0.95
Day 3	32	2.83	0.96
Day 5	45	2.84	0.97
Day 6	48	2.59	0.89

Scheffe's test (Levene, P=0.721)

Day 2	Day 3		
	Day 5		
	Day 6	P = 0.038	*
Day 3	Day 5		
	Day 6		
Day 5	Day 6		
F-value(P)		2.89*(0.037)	

Mixture GF+			
Group	N	Average	SD
Day 2	37	3.17	0.93
Day 3	30	3.55	0.79
Day 5	32	4.00	0.63
Day 6	22	4.59	0.81

Dunnette T3 test (Levene, P=0.025)

Day 2	Day 3		
	Day 5	P = 0.000	***
	Day 6	P = 0.000	***
Day 3	Day 5		
	Day 6	P = 0.000	***
Day 5	Day 6	P = 0.040	*
F-value(P)		16.109***(0.000)	

LBL GF+			
Group	N	Average	SD
Day 2	45	2.92	1.00
Day 3	43	2.95	0.66
Day 5	44	2.78	0.39
Day 6	59	2.33	0.06

Dunnette T3 test (Levene, P=0.000)

Day 2	Day 3		
	Day 5		
	Day 6	P = 0.002	**
Day 3	Day 5		
	Day 6	P = 0.000	***
Day 5	Day 6	P = 0.000	***
F-value(P)		11.854***(0.000)	

*P < 0.05, **P < 0.01, ***P < 0.001

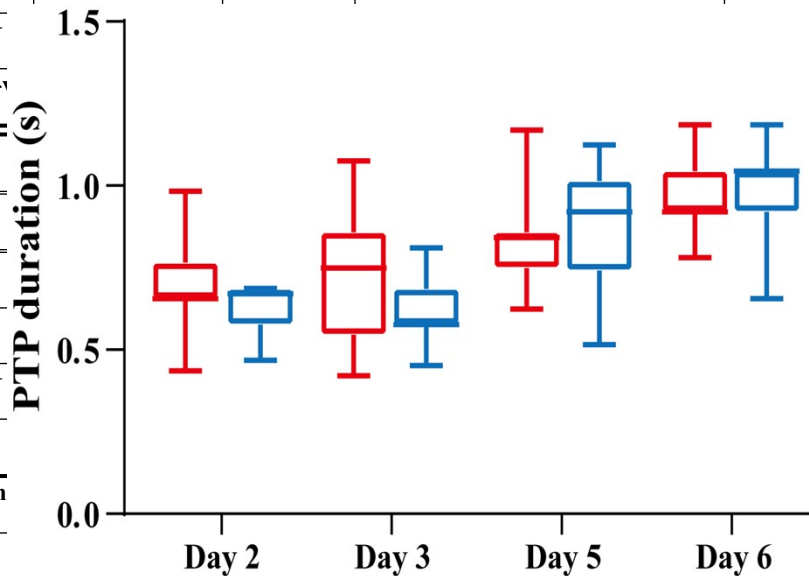
Table S3. One-way ANOVA analysis of PTP duration

Day 2			
Group	N	Average	SD
Mixture GF-	38	2.55	0.79
LBL GF-	47	3.15	0.95
Mixture GF+	37	3.17	0.93
LBL GF+	45	2.92	1.00

Scheffe's test (Levene, P=0.562)

Mixture GF-	LBL GF-	P = 0.034	*
	Mixture GF+	P = 0.044	*
	LBL GF+		
LBL GF-	Mixture GF+		
	LBL GF+		

Mixture GF+	
F _{1,1}	
Group	
Mixture GF-	
LBL GF-	
Mixture GF+	
LBL GF+	
Dun	



Mixture GF-	Mixture GF+	P = 0.043	*
	LBL GF+	P = 0.000	***
LBL GF-	Mixture GF+	P = 0.000	***
	LBL GF+		
Mixture GF+	LBL GF+	P = 0.000	***
F-value(P)		41.915***(0.000)	

Figure S4. Beat duration

Day 3			
Group	N	Average	SD
Mixture GF-	12	4.39	0.85
LBL GF-	32	2.83	0.96
Mixture GF+	30	3.55	0.79
LBL GF+	43	2.95	0.66

Scheffe's test (Levene, P=0.05)

Mixture GF-	LBL GF-	P = 0.000	***
	Mixture GF+	P = 0.028	*
	LBL GF+	P = 0.000	***
LBL GF-	Mixture GF+	P = 0.008	**
	LBL GF+		

Mixture GF+	
F _{1,1}	
Group	
Mixture GF-	
LBL GF-	
Mixture GF+	
LBL GF+	
Dun	

Mixture GF-	Mixture GF+		
	LBL GF+	P = 0.001	**
LBL GF-	Mixture GF+	P = 0.000	***
	LBL GF+		
Mixture GF+	LBL GF+	P = 0.000	***
F-value(P)		71.188***(0.000)	

analysis of CM only CMTs. Peak to peak (PTP) duration of CM GF-group (red), CM GF+ (blue) at day

2, 3, 5 and 6, respectively. Note that the error bars were estimated by the standard deviation of five samples. Note that $N = 5$ per each group.

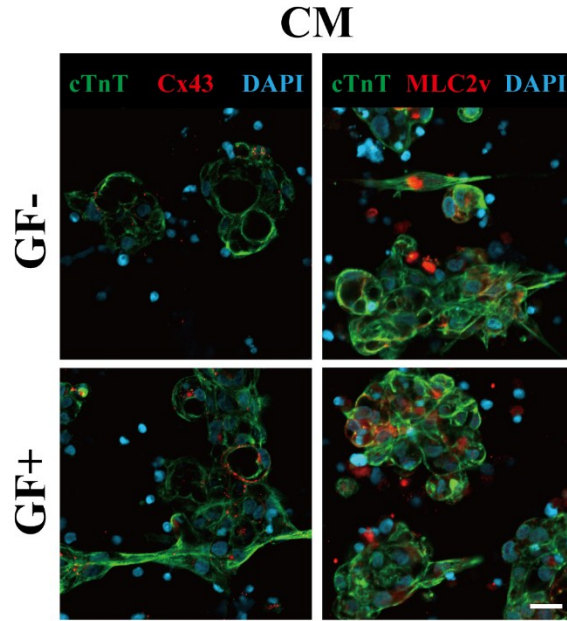


Figure S5. Assessment of Cx43 and MLC2v expression in CM only CMTs. Fluorescence microscope images of CMTs stained for Cx43 (red) and cTnT (green) (left column), and MLC2v (red) and cTnT (green) (right column). Scale bar: 20 μ m.

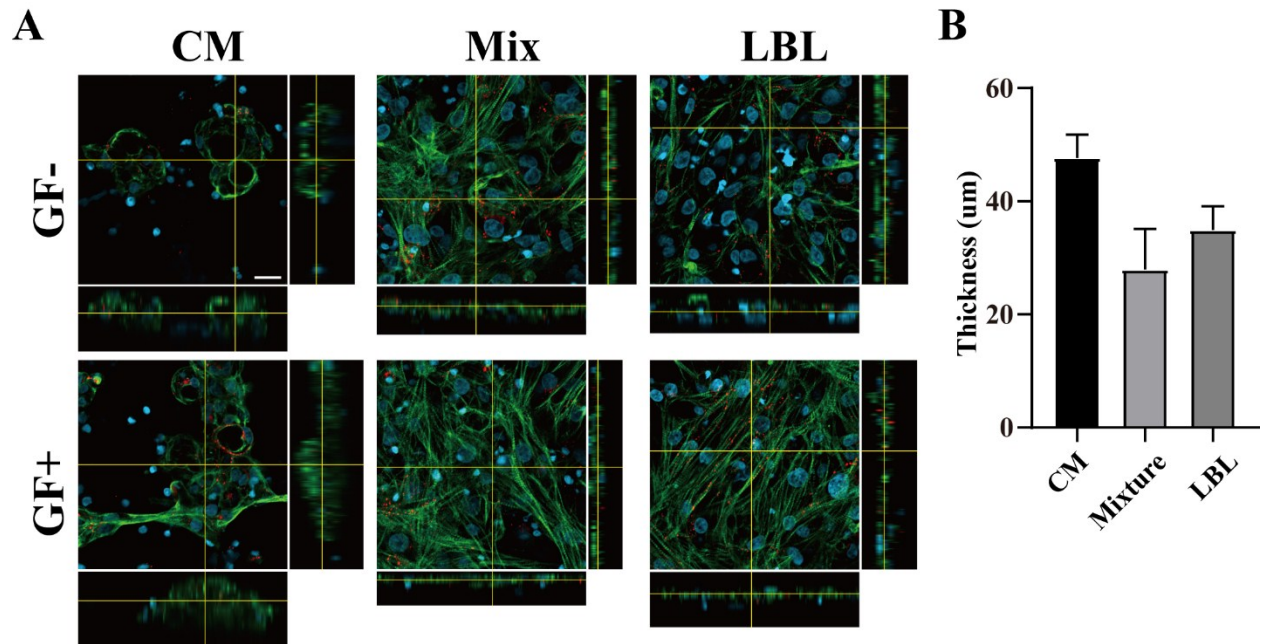


Figure S6. Z-stack scanning image of CMTs (A) and quantitative analysis of CMT thickness (B). Scale bar: 20 μm . Note that $N = 2$ per each group and the error bars were estimated by the standard deviation of 2 samples.

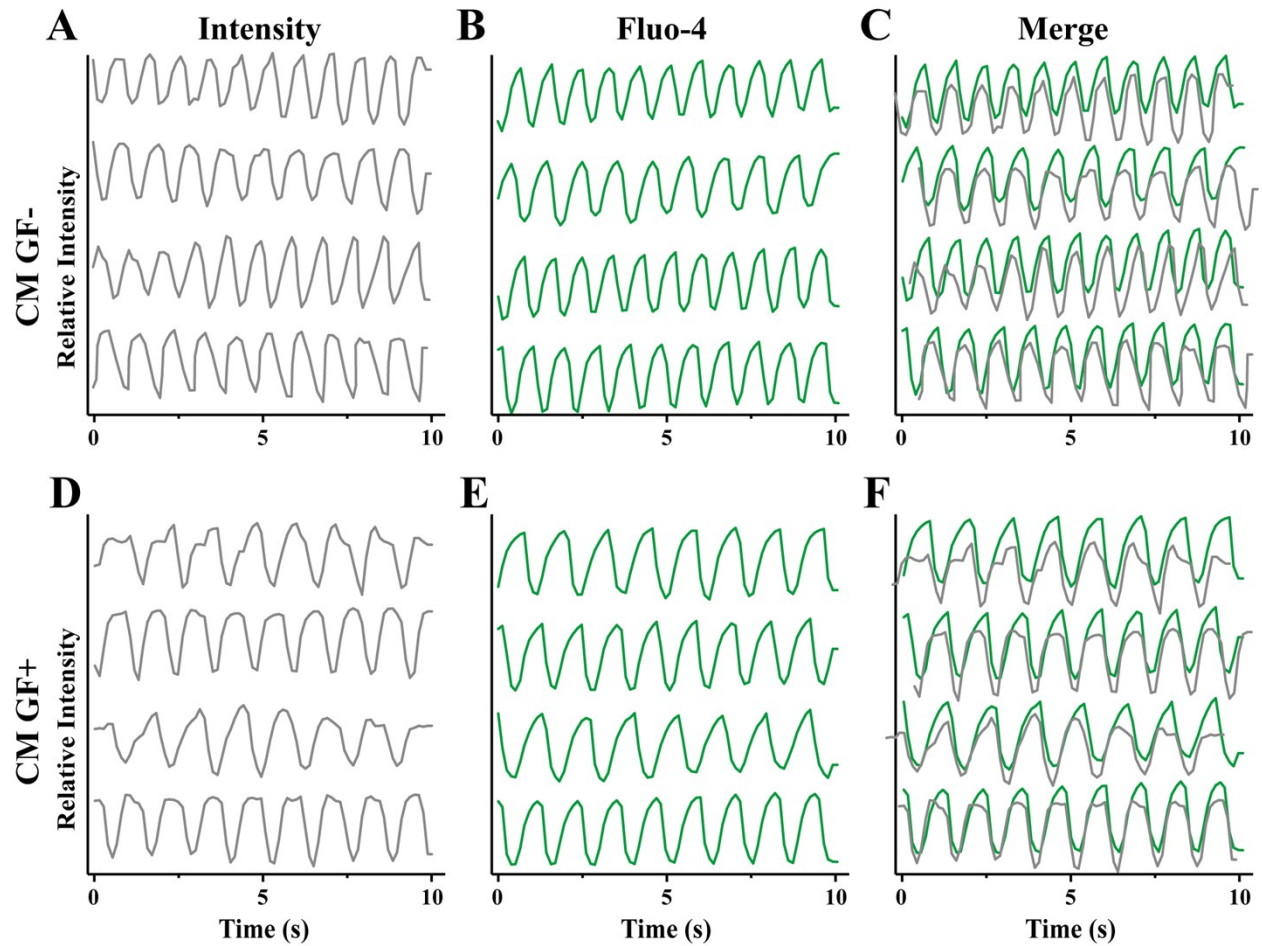


Figure S7. Comparison of video-based and calcium transient methods for beating analysis on the day 6 of CM GF- group and CM GF+ group. Beating analysis by video-based intensity (A, D), calcium transient using fluo-4 intensity (B, E) and the their merged traces for CM GF- (C) and CM GF+ (F), respectively.

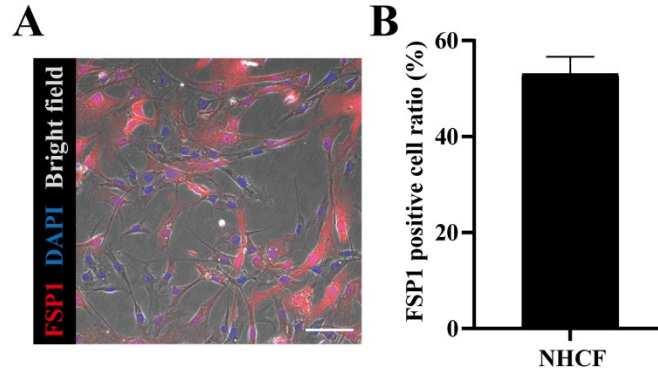


Figure S8. FSP1-positive cell ratio of CFBs using fluorescence staining. A) Merged image of FSP1 (red), DAPI (blue) and bright field image. B) Quantitative data of the proportion of FSP1 positive cells in CFBs. Scale: 100 μ m. Note that N = 3 for graph B and the error bar was estimated by the standard deviation of three samples.

Table S4. List of primers used for quantitative PCR analysis

Gene	Forward primer	Reverse primer	Company
β -actin	GGACCTGACTGACTACCTCAT	CGTAGCACAGCTTCTCCTTAAT	Integrated DNA Technologies (USA)
MLC2v	CGGAGAAGAGAAGGACTAGGA	ACAGACAAGGTAGGGACAGA	Integrated DNA Technologies (USA)
cTnI	GACAAGGTGGATGAAGAGAGATAC	CTTGCCTCGAAGGTCAAAGA	Integrated DNA Technologies (USA)
TBX18	CTGGATGACCAAGGCCATATTA	ACAGGCTTGATGGGAGAAAG	Integrated DNA Technologies (USA)
cTnT	CGATGGATTCCAGTTCGAGTATG	CTTGCAGTGGTAGGTGATGTT	Integrated DNA Technologies (USA)
HCN4	CTGAGAACTGGAAGGACTTAGC	CAGGACAAGACTGTGGGTTT	Integrated DNA Technologies (USA)
GJA1	GGTGACTGGAGCGCCTTAG	GCGCACATGAGAGATTGGGA	Integrated DNA Technologies (USA)