Electronic Supplementary Information

Multidimensional assembly using layer-by-layer deposition for synchronized cardiac macro tissues

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Contents:

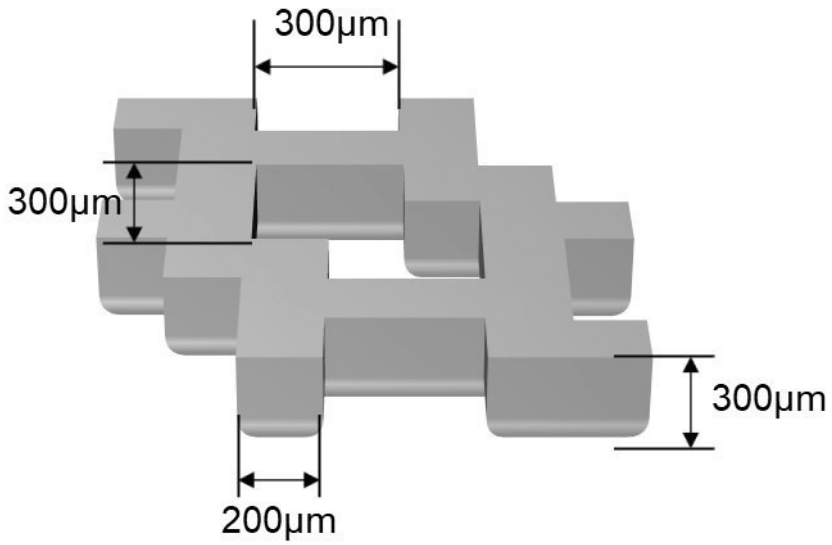

Figure S1. Geometric detail of accordion like honeycomb scaffold.

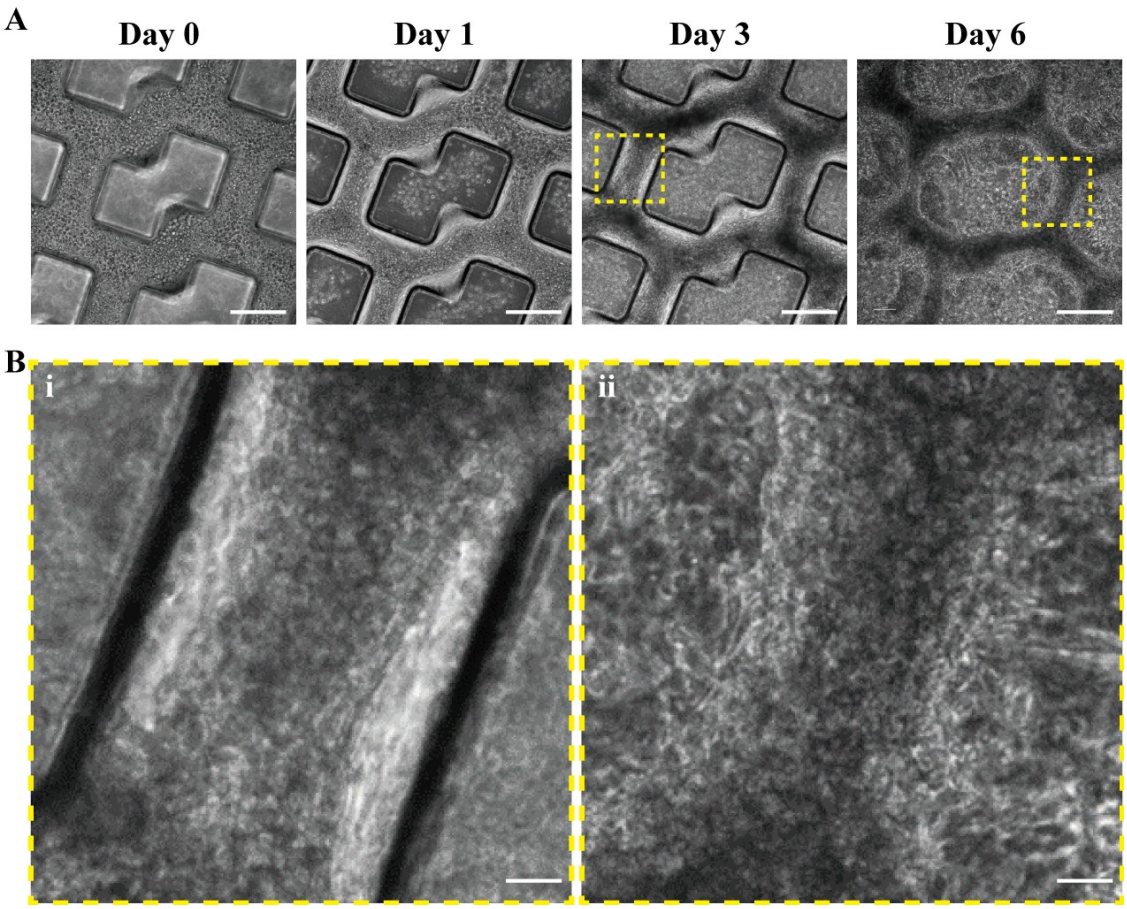

Figure S2. Bright field microscope image of cardiac patches by date. A) The cardiac patch in the mold on day 0, 1 and 2, and the detached cardiac patch on day 6. B) Magnified images of cardiac patches on day 3 (B i) and day 6 (B ii). Scale bars: 250 μm in A, 1000 μm in B.

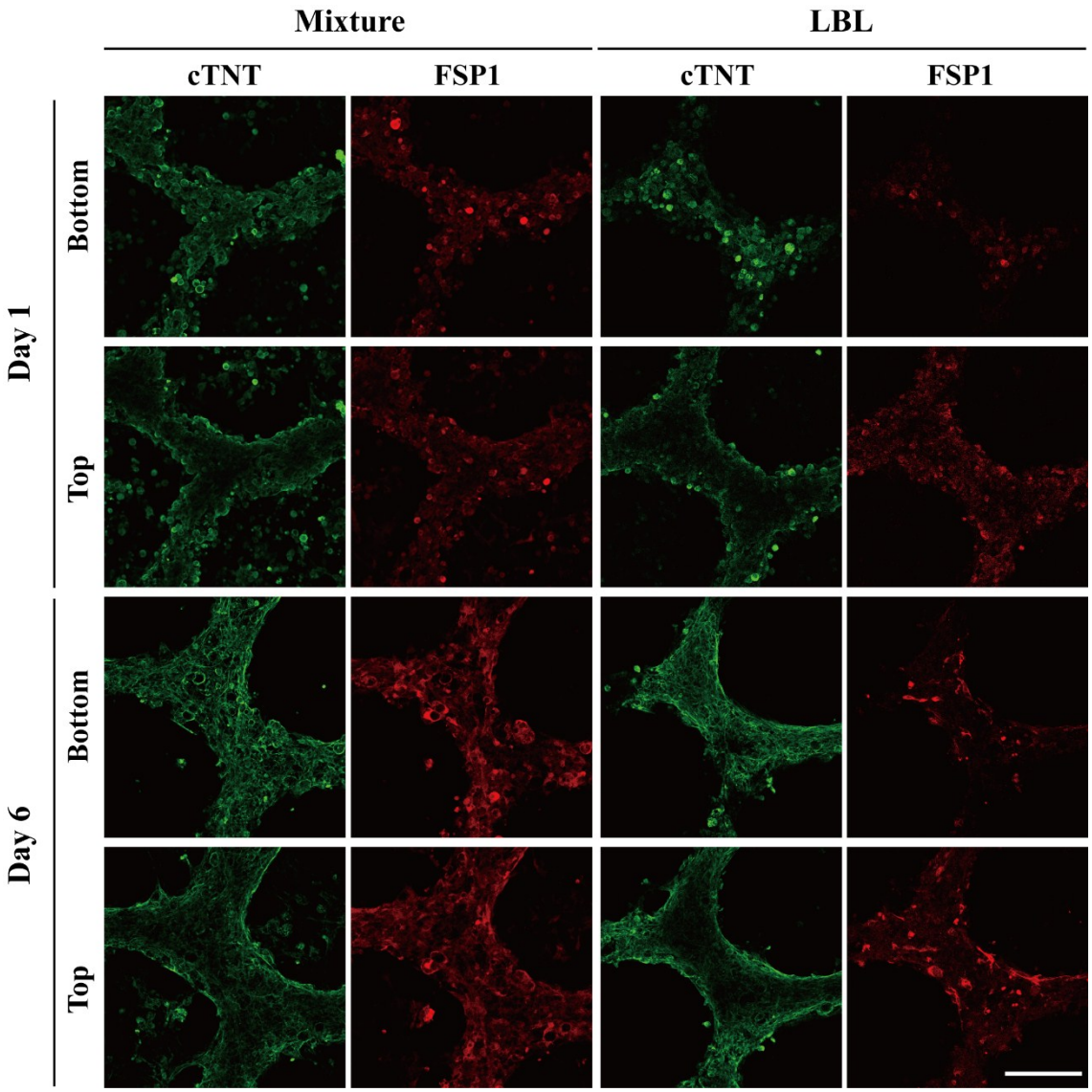

Figure S3. The fluorescence images of the cardiac and the fibroblast specific markers. The distribution of hPSC-derived CMs and CFBs in mCMT were shown for LBL and mixture deposition, respectively. Cardiac troponin T (cTNT; green) and fibroblast-specific protein 1 (FSP1; red) were immunohistochemically stained and imaged with separate channels, respectively. Scale bars are 200 μm.

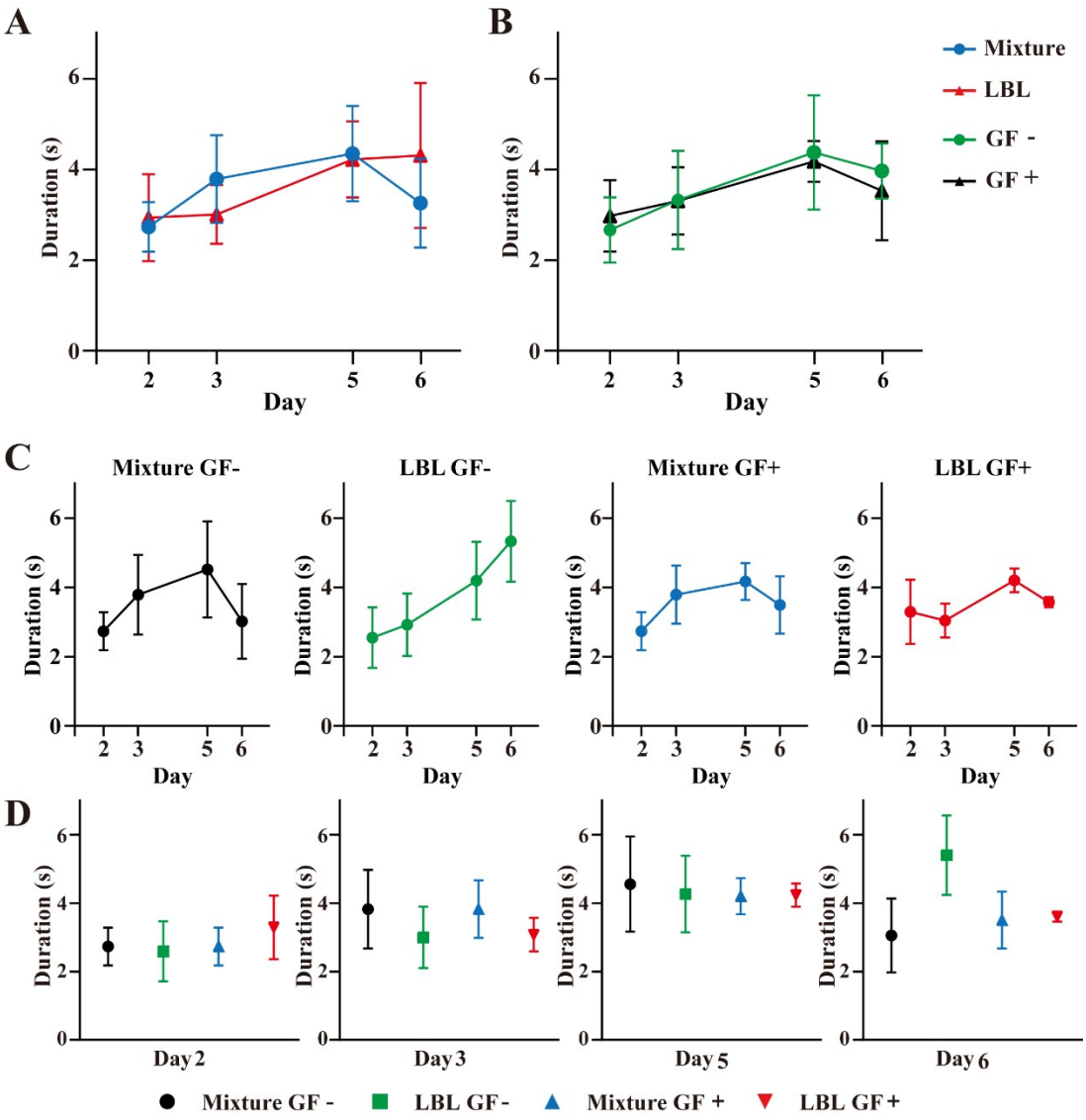

Figure S4. Statistical analysis of peak to peak (PTP) duration in cardiac beating (Figure 4) using ANOVA. Univariate analysis was performed to determine influence of cell deposition methods (A) and growth factors (B) on PTP duration (Day2: F-value (P) = 9.343 (0.003), Day3: F-value (P) = 0.080 (0.777), Day5: F-value (P) = 0.659 (0.419), Day6: F-value (P) = 47.041 (0.000)). 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 the error bars were estimated by the standard deviations of 5 samples.

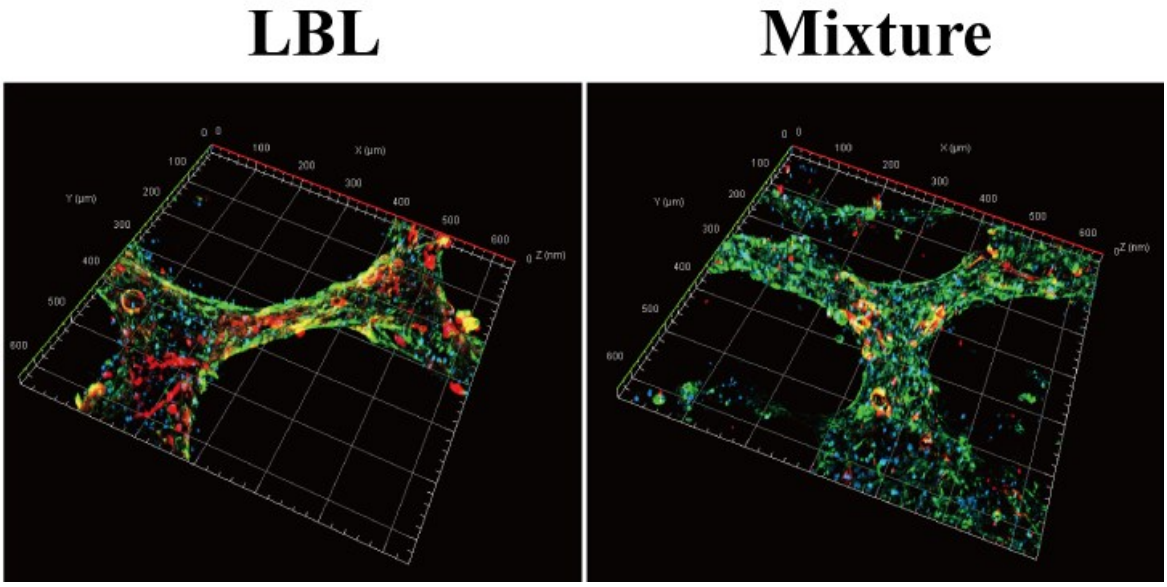

Figure S5. 3D reconstruction image of mCMTs by z-stack scanning using confocal microscope (Green: cTNT, Red: MLC2v, Blue: DAPI).

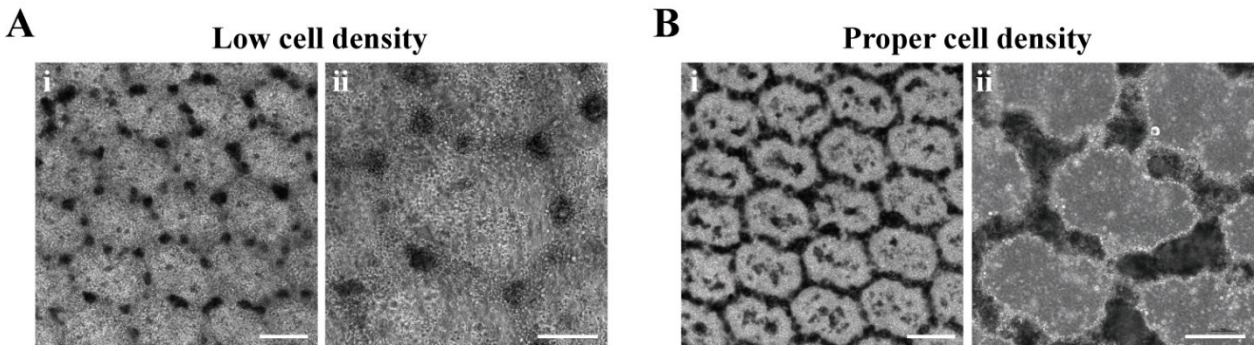

Figure S6. The cardiac patch assembly by cell density. The image of cardiac patch using two different cell densities (A for 8 x 10^5 cells/patch and B for 1.2 x 10^6 cells/patch). Scale bars: 500 μm in A (i) and B (i), 250 μm in A (ii) and B (ii).

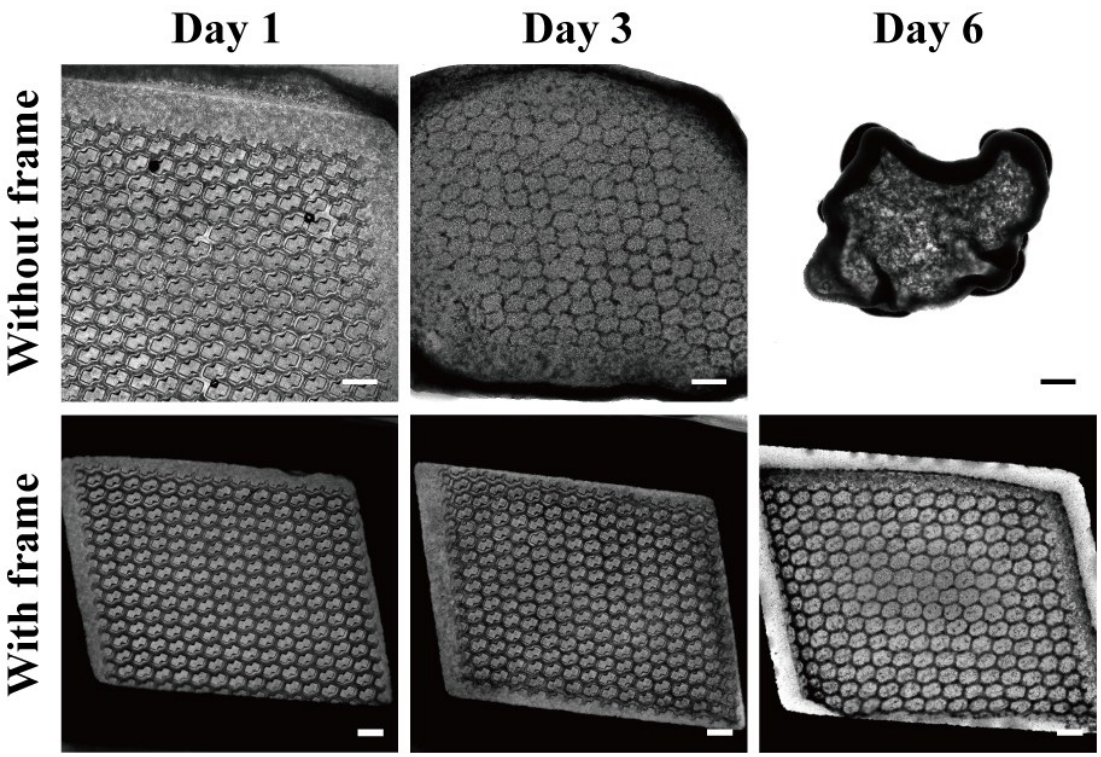

Figure S7. Bright field microscope images of mCMTs at day 1, 3, and 6 according to the presence or absence of the frame. Scale bars are 1000 μm.

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

Univariate analysis (Day2)				
Deposition	**Cytokine**	**Average**	**SD**	**N**
Mixture	GF-	2.73	0.55	44
	GF+	2.73	0.55	45
	All	2.73	0.55	89
LBL	GF-	2.59	0.88	37
	GF+	3.29	0.93	37
	All	2.94	0.96	74
All	GF-	2.67	0.72	81
	GF+	2.98	0.79	82
F-value (P)		9.343**(0.003)		

Univariate analysis (Day3)				
Deposition	**Cytokine**	**Average**	**SD**	**N**
Mixture	GF-	3.79	1.15	23
	GF+	3.79	0.84	33
	All	3.79	0.97	56
LBL	GF-	2.96	0.90	29
	GF+	3.04	0.49	57
	All	3.01	0.65	86
All	GF-	3.33	1.09	52
	GF+	3.31	0.74	90
F-value (P)		0.080(0.777)		

Univariate analysis (Day5)				
Deposition	**Cytokine**	**Average**	**SD**	**N**
Mixture	GF-	4.52	1.39	27
	GF+	4.17	0.53	27
	All	4.35	1.05	54
LBL	GF-	4.23	1.12	25
	GF+	4.20	0.34	22
	All	4.22	0.84	47
All	GF-	4.38	1.26	52
	GF+	4.18	0.45	49
F-value (P)		0.659(0.419)		

Univariate analysis (Day6)				
Deposition	**Cytokine**	**Average**	**SD**	**N**
Mixture	GF-	3.02	1.08	31
	GF+	3.49	0.83	34
	All	3.26	0.98	65
LBL	GF-	5.37	1.17	21
	GF+	3.57	0.15	30
	All	4.31	1.60	51
All	GF-	3.97	0.61	52
	GF+	3.53	1.09	64
F-value (P)		47.041***(0.000)		

*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	44	2.73	0.55
Day 3	23	3.79	1.15
Day 5	27	4.52	1.39
Day 6	31	3.02	1.08
Dunnette T3 test (Levene, P=0.000)			
Day 2	Day 3	P = 0.002	**
	Day 5	P = 0.000	***
	Day 6		
Day 3	Day 5		
	Day 6		
Day 5	Day 6	P = 0.000	***
F-value(P)		19.498***(0.000)	

LBL GF-			
Group	**N**	**Average**	**SD**
Day 2	37	0.88	0.14
Day 3	29	0.90	0.17
Day 5	25	1.12	0.22
Day 6	21	1.17	0.26
Dunnette T3 test (Levene, P=0.003)			
Day 2	Day 3		
	Day 5	P = 0.000	***
	Day 6	P = 0.000	***
Day 3	Day 5	P = 0.000	***
	Day 6	P = 0.000	***
Day 5	Day 6	P = 0.003	**
F-value(P)		28.531***(0.000)	

Mixture GF+			
Group	**N**	**Average**	**SD**
Day 2	45	2.73	0.08
Day 3	33	3.79	0.15
Day 5	27	4.17	0.10
Day 6	34	3.49	0.14
Dunnette T3 test (Levene, P=0.015)			
Day 2	Day 3	P = 0.000	***
	Day 5	P = 0.000	***
	Day 6	P = 0.000	***
Day 3	Day 5		
	Day 6		
Day 5	Day 6	P = 0.003	**
F-value(P)		28.284***(0.000)	

LBL GF+			
Group	**N**	**Average**	**SD**
Day 2	37	3.29	0.93
Day 3	57	3.04	0.49
Day 5	22	4.20	0.34
Day 6	30	3.57	0.15
Dunnette T3 test (Levene, P=0.003)			
Day 2	Day 3		
	Day 5	P = 0.000	***
	Day 6		
Day 3	Day 5	P = 0.000	
	Day 6	P = 0.000	***
Day 5	Day 6	P = 0.000	***
F-value(P)		22.488***(0.000)	

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

Table S3. One-way ANOVA analysis of PTP duration of all the groups at each day. *P < 0.05, **P < 0.01, ***P < 0.001

Day 2			
Group	**N**	**Average**	**SD**
Mixture GF-	44	2.73	0.55
LBL GF-	37	2.59	0.88
Mixture GF+	45	2.73	0.55
LBL GF+	37	3.29	0.93
Scheffe's test (Levene, P=0.005)			
Mixture GF-	LBL GF-		
	Mixture GF+		
	LBL GF+	P = 0.014	*
LBL GF-	Mixture GF+		
	LBL GF+	P = 0.009	**
Mixture GF+	LBL GF+	P = 0.012	*
F-value(P)		6.695***(0.000)	

Day 3			
Group	**N**	**Average**	**SD**
Mixture GF-	23	3.79	1.15
LBL GF-	29	2.96	0.90
Mixture GF+	33	3.79	0.84
LBL GF+	57	3.04	0.49
Scheffe's test (Levene, P=0.000)			
Mixture GF-	LBL GF-	P = 0.041	*
	Mixture GF+		
	LBL GF+	P = 0.033	*
LBL GF-	Mixture GF+	P = 0.003	**
	LBL GF+		
Mixture GF+	LBL GF+	P = 0.000	***
F-value(P)		10.729***(0.000)	

Day 5			
Group	**N**	**Average**	**SD**
Mixture GF-	27	4.52	1.39
LBL GF-	25	4.23	1.12
Mixture GF+	27	4.17	0.53
LBL GF+	22	4.20	0.34
Dunnett T3 test (Levene, P=0.000)			
Mixture GF-	LBL GF-		
	Mixture GF+		
	LBL GF+		
LBL GF-	Mixture GF+		
	LBL GF+		
Mixture GF+	LBL GF+		
F-value(P)		0.759(0.520)	

Day 6			
Group	**N**	**Average**	**SD**
Mixture GF-	31	3.02	1.08
LBL GF-	21	5.37	1.16
Mixture GF+	33	3.47	0.83
LBL GF+	30	3.57	0.15
Dunnett T3 test (Levene, P=0.000)			
Mixture GF-	LBL GF-	P = 0.000	***
	Mixture GF+		
	LBL GF+	P = 0.045	*
LBL GF-	Mixture GF+	P = 0.000	***
	LBL GF+	P = 0.000	***
Mixture GF+	LBL GF+		
F-value(P)		32.927***(0.000)	

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)
cTNT	CGATGGATTCCAGTTCGAGTATG	CTTGCAGTGGTAGGTGATGTT	Integrated DNA Technologies (USA)
Piezo1	GAGGGTGGAGGTGGAACAACCA TGGTGAGCAAGGGCGCC	TCCACCTCCACCCTCCTTCTCA CGAGTCCAC	Integrated DNA Technologies (USA)
Pre-Mir21	TGTCGGGTAGCTTATCAGAC	TGTCAGACAGCCCATCGACT	Integrated DNA Technologies (USA)
GJA1	GGTGACTGGAGCGCCTTAG	GCGCACATGAGAGATTGGGA	Integrated DNA Technologies (USA)