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Supplemental Information

Fibroblast Growth Factors and Vascular Endothelial Growth Factor Promote Cardiac Reprogramming under Defined Conditions

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Supplementary Figure 1

A

Number of beating cells

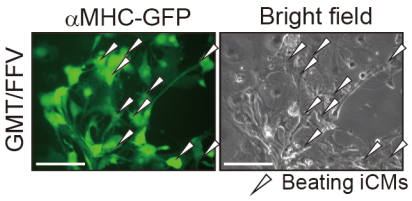
		FGF2 [ng/mL]			
		0	1	10	50
VEGF [ng/mL]	0	0	0	1	0
	1	0	1	4	3
	5	1	1	5	5

B

Number of beating cells

	FGF10 [ng/mL]			
	0	1	25	100
FGF2 [10ng/mL] VEGF [5ng/mL]	4	3	32	18
FGF2 [50ng/mL] VEGF [5ng/mL]	3	1	24	27

C



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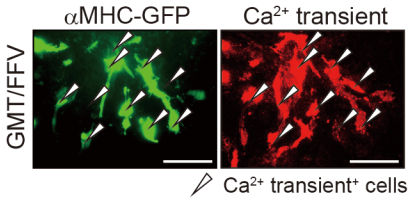
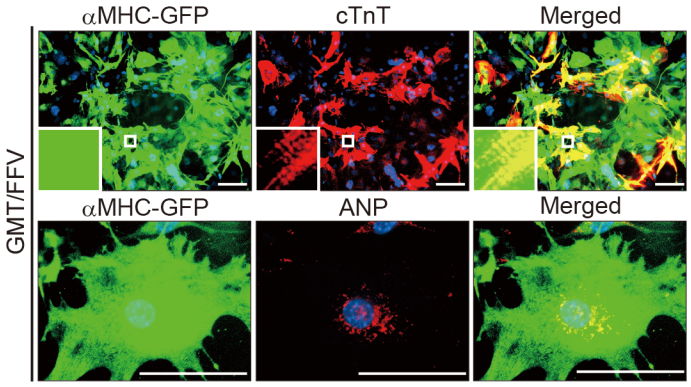
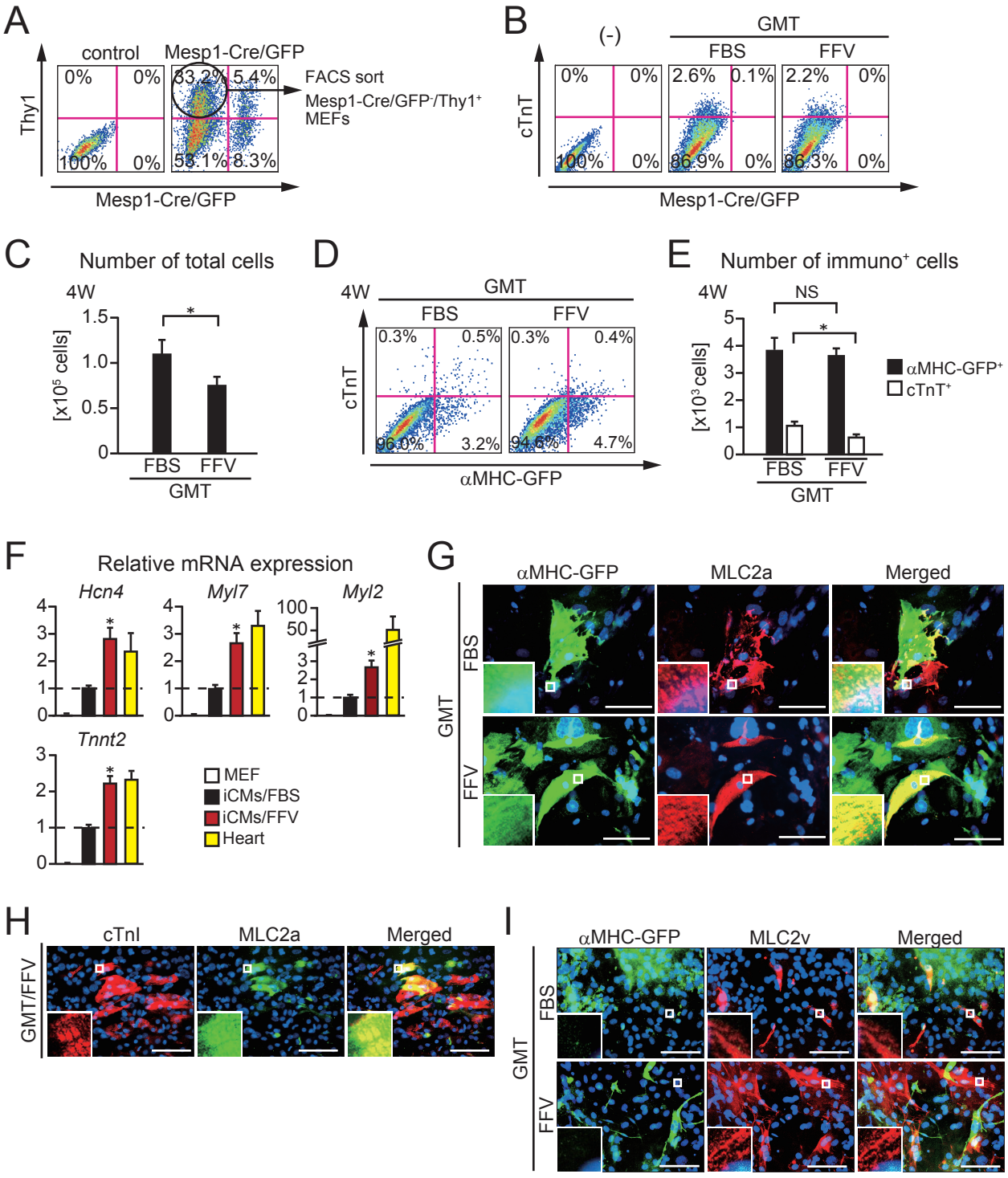


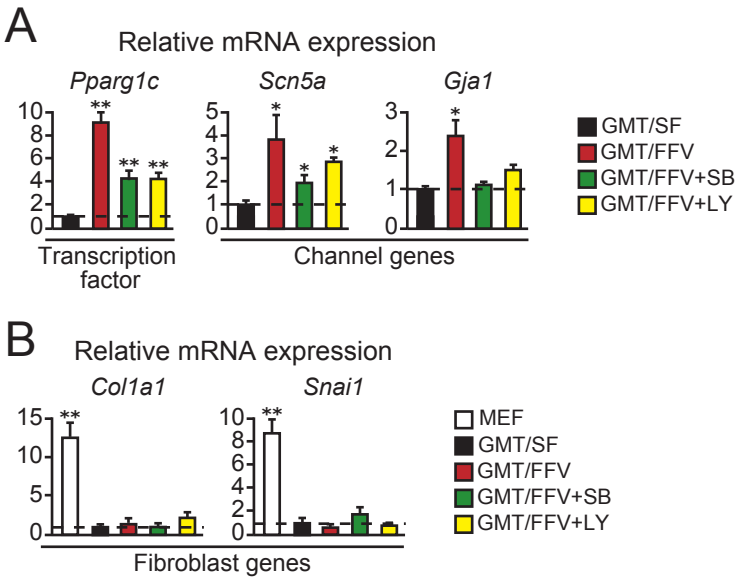
E



Supplementary Figure 2

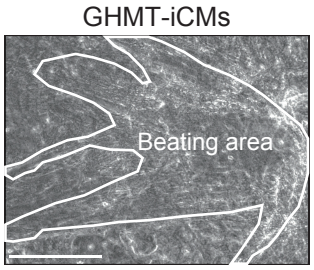


Supplementary Figure 3



Supplementary Figure 4

A



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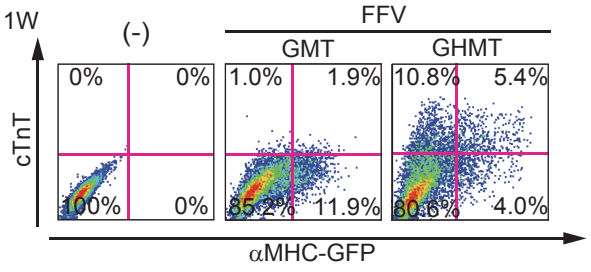

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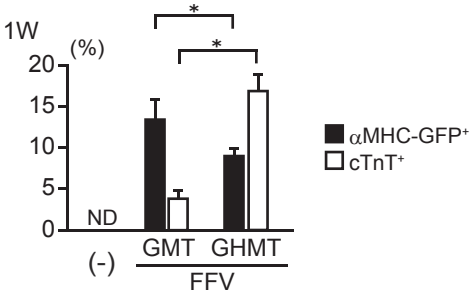

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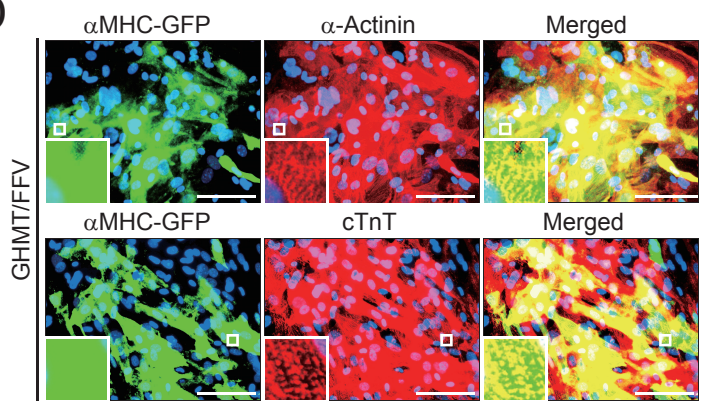

E

GO terms including “heart” or “cardiac” enriched in GMTMM-iCMs/FFV upregulated genes

GO term	Genes	Size	p-value
Ventricular cardiac muscle tissue morphogenesis	9	25	3.2E-8
Cardiac myofibril assembly	5	7	5.5E-7
Heart development	17	151	4.5E-6
Cardiac muscle cell proliferation	5	11	1.1E-5
Cardiac muscle contraction	6	23	5.4E-5
Heart morphogenesis	7	42	0.00027
Adult heart development	4	13	0.00051
Cardiac muscle tissue morphogenesis	3	7	0.00093
Regulation of the force of heart contraction	4	18	0.0019
Cardiac left ventricle morphogenesis	2	4	0.0055
Positive regulation of cardiac muscle hypertrophy	2	4	0.0055
Regulation of cardiac muscle contraction	2	5	0.0089
Negative regulation of heart rate	2	5	0.0089
Atrial cardiac muscle tissue morphogenesis	2	5	0.0089

Supplemental Information

Supplemental Figure Legends

Figure S1. GMT-transduced cells expressed several cardiac markers in FFV culture

(A, B) Dose dependency of FFV-mediated cardiac reprogramming with GMT transduction. The number of beating cells in each well is shown.

(C) Spontaneously beating GMT-iCMs cultured with FFV for 4 weeks (arrowheads), corresponding to **Movie S2**.

(D) Spontaneous Ca^{2+} oscillations observed in GMT-iCMs (arrowheads) after 4 weeks of FFV treatment.

(E) Immunocytochemistry for αMHC-GFP, cTNT, ANP, and DAPI. GMT-iCMs treated with FFV express cardiac proteins. Insets indicate high-magnification. Scale bars represent 100 μm.

Figure S2. FFV treatment promoted cardiac reprogramming without stimulating cell proliferation

(A, B) Mesp1-GFP-/$Thy1^+$ MEFs were sorted (A), transduced with GMT, and cultured with FBS or FFV. All $cTNT^+$ cells were negative for Mesp1-GFP (B).

(C-E) The total number of cells in each well was counted after 4 weeks (C) (n = 3 independent triplicate experiments). FACS analysis of αMHC-GFP^+ and $cTNT^+$ cells 4 weeks after GMT transduction and culture with FBS or FFV (D). The number of αMHC-GFP^+ and $cTNT^+$ cells in each well was calculated as the total number of cells times the percentage of cardiac marker+ cells, and quantitative data are shown in (E) (n = 3 independent triplicate experiments).

(F) Relative mRNA expression of *Hcn4*, *Myl7*, *Myl2*, and *TnnT2* in MEFs, iCMs with FBS or FFV, and postnatal hearts (n = 3 independent triplicate experiments).

(G-I) Immunocytochemistry for αMHC-GFP, MLC2a, cTNI, MLC2v, and DAPI in iCMs cultured with FBS or FFV for 4 weeks. Quantitative data are shown in **Figure 2I**.

All data are presented as mean±SD. *, $P < 0.05$ vs. the relevant control. NS, not significant. Scale bars represent 100 μm.

Figure S3. FFV induced cardiac gene expression through p38MAPK and PI3K/AKT pathways

(A, B) Relative mRNA expression of the indicated genes in MEFs (white bars) and GMT-transduced cells cultured with SF (black bars), FFV without inhibitors (red bars), or FFV with SB203580 (green bars) or LY294002 (yellow bars) was determined by qRT-PCR after 4 weeks (n = 3 independent triplicate experiments). Note that FFV did not alter fibroblast gene expression (B). Data were normalized to the values of GMT-transduced cells in SF. See also **Figure 3F**.

All data are presented as mean±SD. *, $P < 0.05$ vs. the relevant control.

Figure S4. Hand2 increased cardiac marker expressing cells at early stage of reprogramming

(A) Spontaneously beating GHMT-iCMs cultured with FFV for 17 weeks, corresponding to **Movie S5**. The iCMs beat like contractile sheets (white line).

(B, C) FACS analysis of αMHC-GFP$^+$ and cTNT$^+$ cells 1 week after GMT or GHMT transduction with FFV. Quantitative data are shown in panel (C) (n = 6 independent triplicate experiments).

(D) Immunocytochemistry for αMHC-GFP, α-actinin, cTNT, and DAPI. GHMT transduction induced cardiac protein expression 4 weeks after transduction. High-magnification views in insets show sarcomeric organization.

(E) GO terms including “heart” or “cardiac” in the upregulated genes in GMTMM-iCMs cultured with FFV. See also **Figure 5H**.

All data are presented as mean±SD. **, $P < 0.01$; *, $P < 0.05$ vs. the relevant control. Scale bars represent 100 μm.

Movie S1.

Spontaneously beating GMT-iCMs cultured with FFV for 4 weeks, corresponding to **Figure 1E**.

Movie S2.

Spontaneously beating GMT-iCMs cultured with FFV for 4 weeks, corresponding to **Figure S1C.**

Movie S3.

Spontaneous Ca^{2+} oscillations in GMT-iCMs cultured with FFV for 4 weeks, corresponding to **Figure 1G.**

Movie S4.

Spontaneously beating MT-iCMs cultured with FFV for 4 weeks, corresponding to **Figure 4D.**

Movie S5.

Spontaneously beating GHMT-iCMs cultured with FFV for 17 weeks, corresponding to **Figure S4A.** Cells are beating as contractile sheets.

Movie S6.

Spontaneously beating GMTMM-iCMs derived from TTFs cultured with FFV for 8 weeks, corresponding to **Figure 5D.**