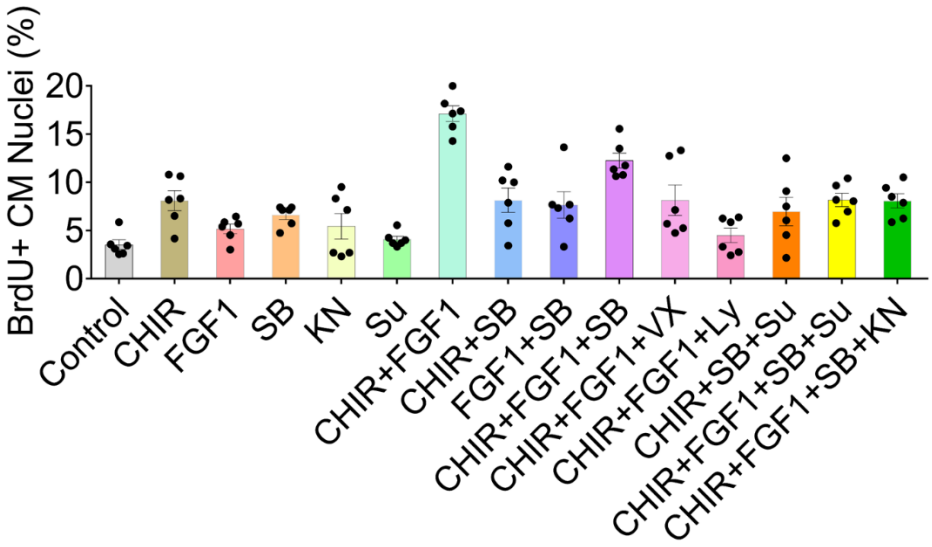
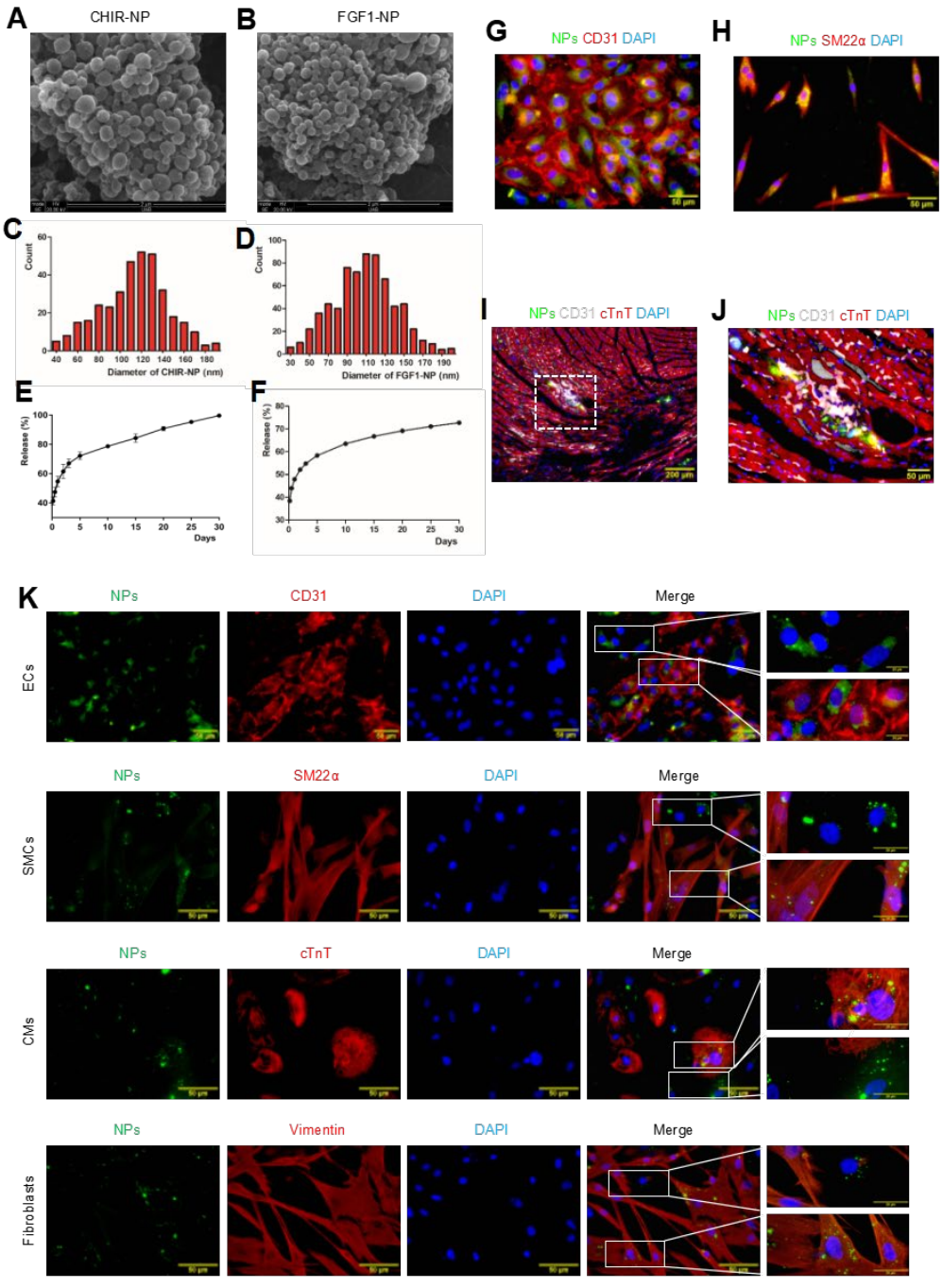
Supplemental figures and figure legends

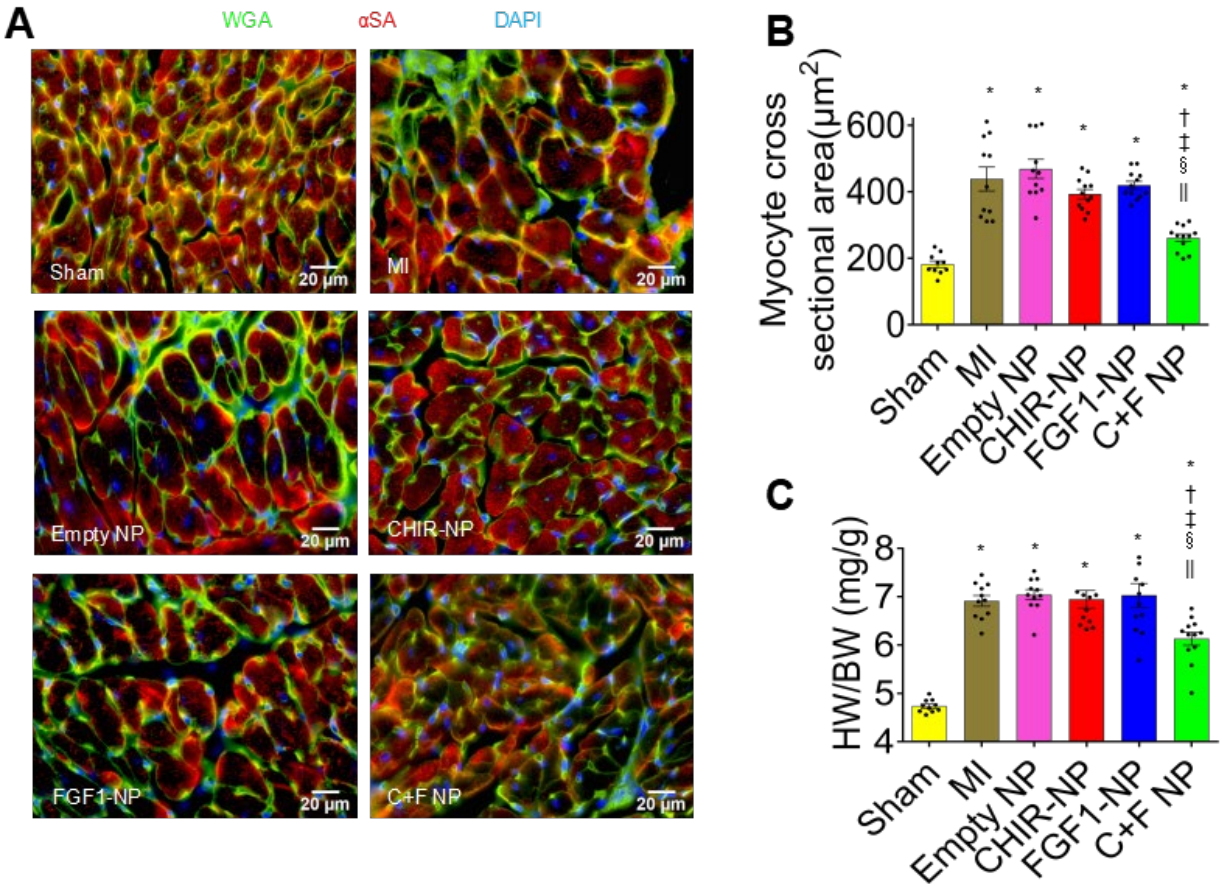


Supplemental Figure 1. Screening of chemicals that promote cell cycle of hiPSC-CMs. At 28 days after initiation of cardiac differentiation, the hiPSC-CMs were treated with CHIR99021, FGF1, SB203580, VX702, KN93, Su1498, or different combination of these chemicals for 24 hours. BrdU (15 μM final concentration) incorporation assay was used to mark the hiPSC-CMs undergoing DNA synthesis (S phase). Anti-BrdU immunostaining was used to pick the cardiomyocytes with active cell cycle, and all cardiomyocyte nuclei were counterstained with anti-human Nkx2.5 antibody. The number of BrdU- positive cardiomyocyte nuclei was normalized to the total number of cardiomyocyte nuclei and the results were expressed as percentage (%). A combination of CHIR and FGF1 treatment resulted in the highest cell cycle activity. All experiments were repeated for 5 times or more.

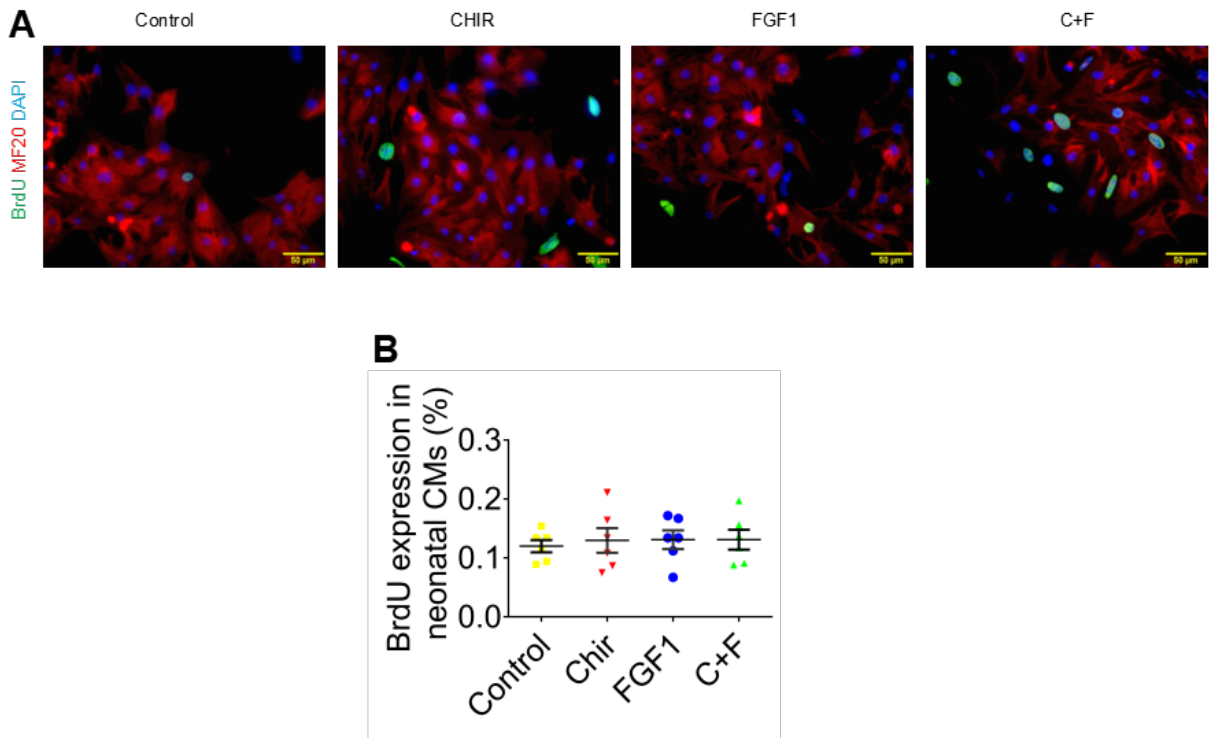


Supplemental Figure 2. Characterization of poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs). The size of poly (lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) was measured using scanning electron microscopy (SEM), for both CHIR99021- or FGF1-loaded NPs (**A** and **B**; scale bar = 2 μm). Quantification of particle diameter was performed using a NIH ImageJ software, for CHIR-NPs (**C**) and FGF1-NPs (**D**). Determination of release kinetics of CHIR- or FGF-1-loaded NPs as a function of time, and cumulative percentage of CHIR or FGF1 released from NPs (**E** and **F**). Fluorescent

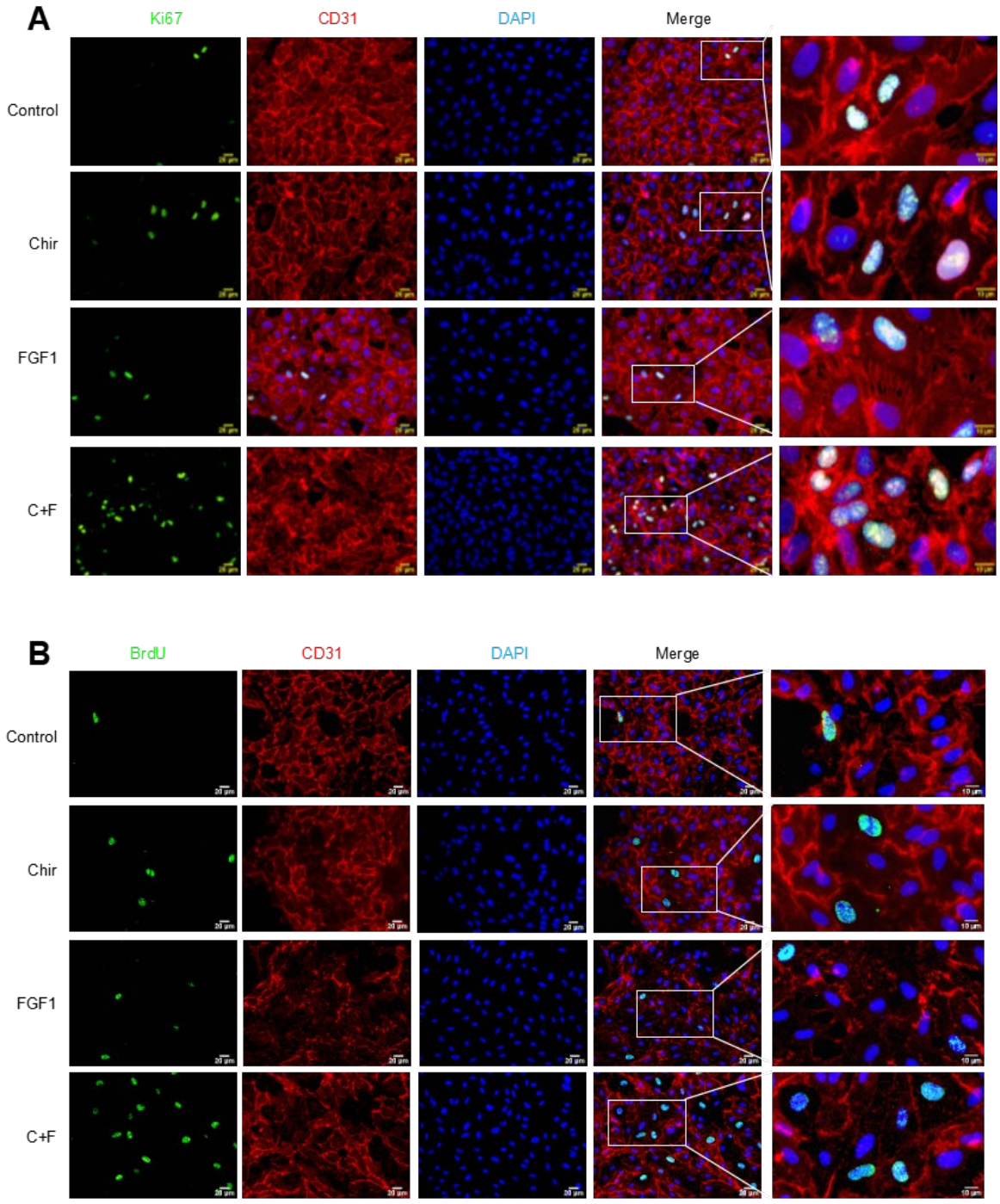
microscopic analysis revealed cellular localization and distribution of NPs. The endothelial (red, CD31) and smooth muscle (red, SM22α) cells were able to uptake the engineered NPs (**G** and **H;** scale bar = 50 μm). The left ventricular border zone tissue sections 24 hours after injection were subjected to dual immunostaining, viz., a cardiac specific regulatory protein, cardiac troponin T (red, cTnT) and a endothelium associated protein, Pecam-1/CD31 (white, CD31) (**I** and **J;** scale bar =200 and 50 μm respectively). Fluorescent microscopic analysis clearly revealed localization and distribution of NPs (green, NPs) within the ventricular tissue (**J**). Cells were also counterstained for nuclei (blue, DAPI). (K) Next, we investigated the cellular uptake and biodistribution of engineered NPs in the mixture of HUVECs, HVSMCs, hiPSC-CMs and fibroblasts in vitro by fluorescence microscopy. The endothelial (red, CD31), smooth muscle (red, SM22α) cells, cardiomyocytes (red, cTnT) and fibroblasts (red, vimentin) were able to consistently uptake and internalize the engineered NPs.

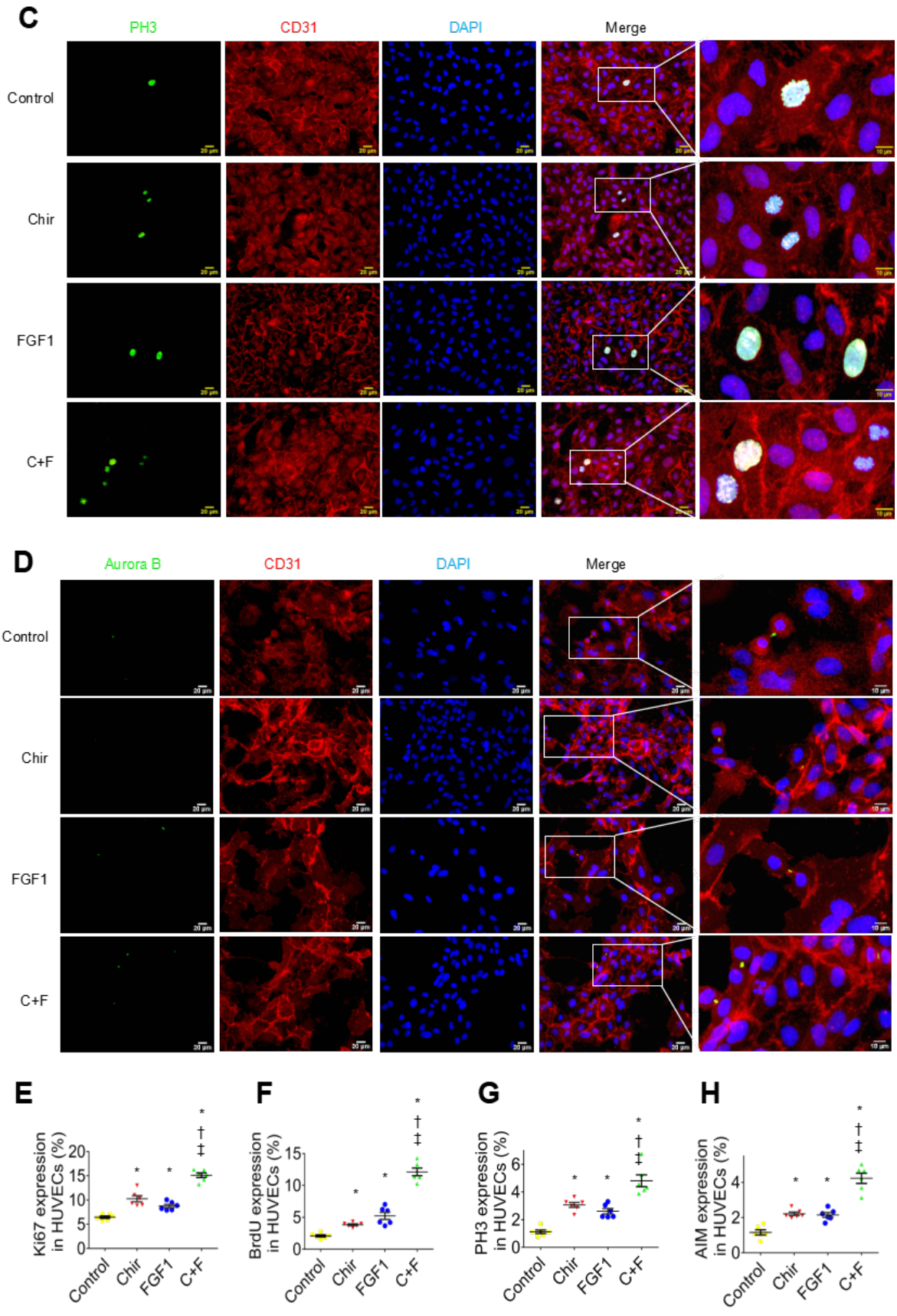


Supplemental Figure 3. CHIR99021 and/or FGF1 nanoparticles (NPs) treatment: assessment of hypertrophy in a mouse model of myocardial infarction (MI). In post MI day 28 left ventricular (LV) peri-infarct border zone sections, Immunolocalization of a cardiac contractile protein, α-sarcomeric protein (red, α-SA) and a plasma membrane marker, wheat germ agglutinin (green, WGA) in the peri-infarct border zone at 28 days post MI (**A**). Myocyte cross-sectional surface area in the peri-infarct border zone (**B**). The MI and NPs-treated groups showed significantly higher myocyte cross-sectional areas compared with sham operated animals. Besides, the myocyte cross-sectional area was significantly lower in the case of CHIR+FGF1-NPs treatment group compared with other treatment groups (**B**). The heart weight to body weight (HW/BW) ratio was also determined (**C**). The data suggest that CHIR+FGF1-NPs reasonably reduced LV hypertrophy. Cells were also counterstained for nuclei (blue, DAPI). Scale bar = 20 μm (Panels in **A**). Data are given as means ± SE. 10-12 animals per group. Statistical analysis: One-way ANOVA with Dunn's multiple comparisons test. *p< 0.01 vs. sham; †p < 0.01 vs. MI; ‡p < 0.01 vs. empty NPs; §p < 0.01 vs. CHIR-NPs; ||p < 0.01 vs. FGF1-NPs.



Supplemental Figure 4. Expression pattern of a cell cycle associated marker, bromodeoxyuridine (BrdU), in mouse neonatal cardiac myocytes (NCMs) treated with CHIR99021 and/or FGF1. Immunostaining and fluorescence microscopic analyses of synchronized *in vitro* mouse neonatal cardiac myocytes (NCMs) cultures, treated with CHIR99021 (5 μM), FGF1 (100 ng/ml), or CHIR+FGF1 (5 μM, 100 ng/ml, respectively) for 24 hours, demonstrated the expression of bromodeoxyuridine, (green, BrdU) (**A**). Scale bar = 50 μm. Quantification of the percentage of BrdU-positive NCMs expressing myosin heavy chain II did not show significant differences between the CHIR, FGF1, and CHIR+FGF1 groups compared to controls (**B**). Cells were also counterstained for nuclei (blue, DAPI). The values are presented as means ± SE. Six independent experiments. Statistical analysis: One-way ANOVA with Dunn's multiple comparisons test. $p > 0.05$. No significant difference was found among groups.


A
Ki67
CD31
DAPI
Merge
Control
Chir
FGF1
C+F
B
BrdU
CD31
DAPI
Merge
Control
Chir
FGF1
C+F


C
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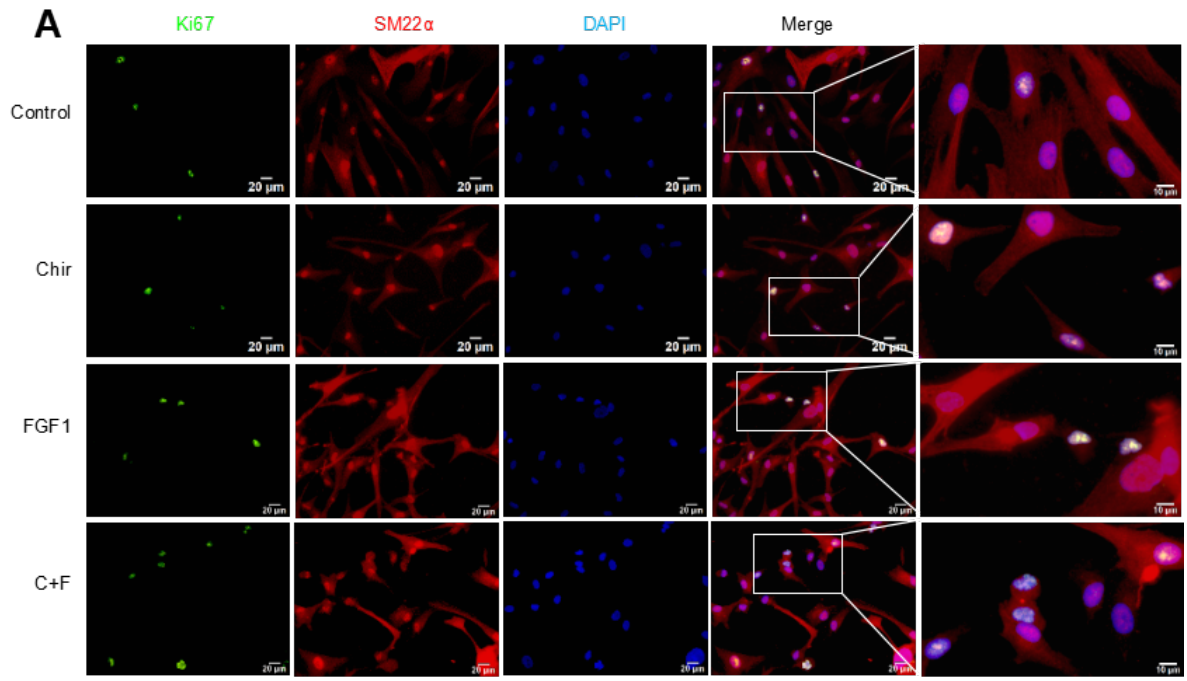
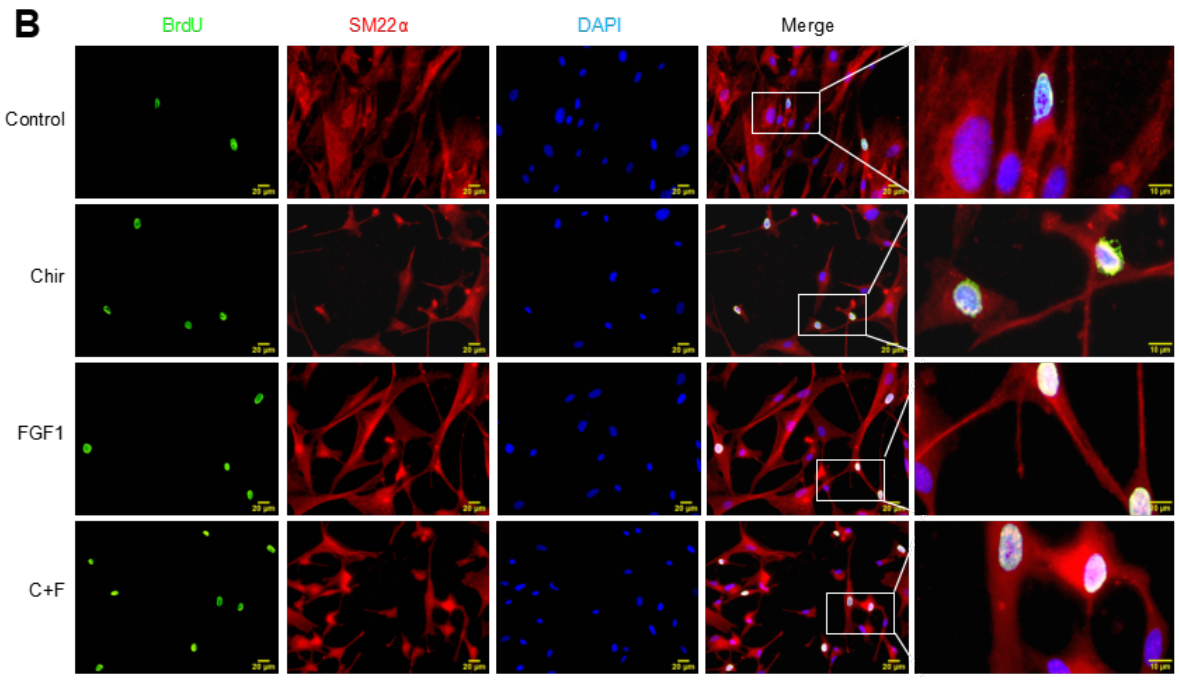
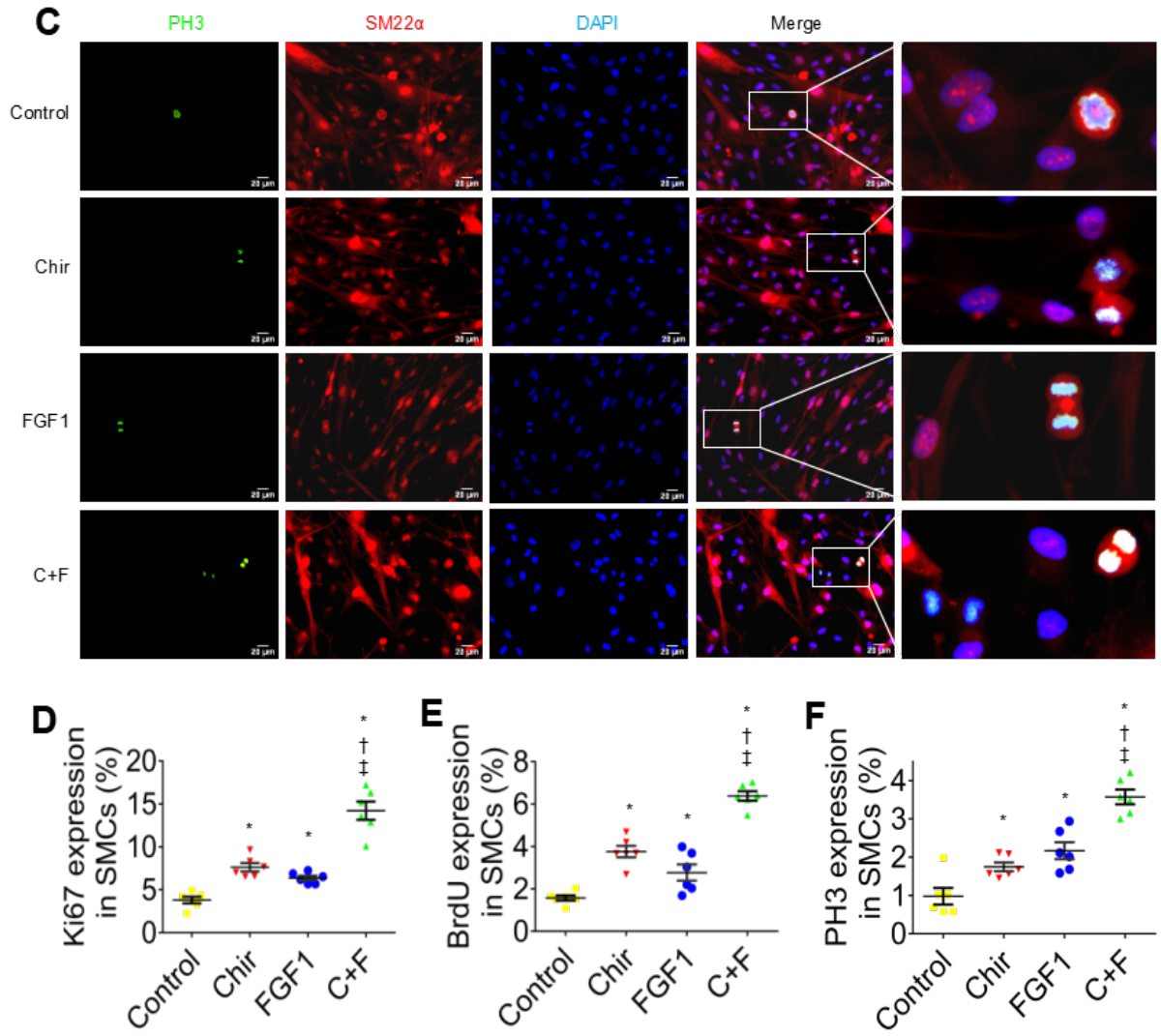
D
Aurora B
CD31
DAPI
Merge
Control
Chir
FGF1
C+F
E
Ki67 expression in HUVECs (%)
Control
Chir
FGF1
C+F
F
BrdU expression in HUVECs (%)
Control
Chir
FGF1
C+F
G
PH3 expression in HUVECs (%)
Control
Chir
FGF1
C+F
H
AIM expression in HUVECs (%)
Control
Chir
FGF1
C+F

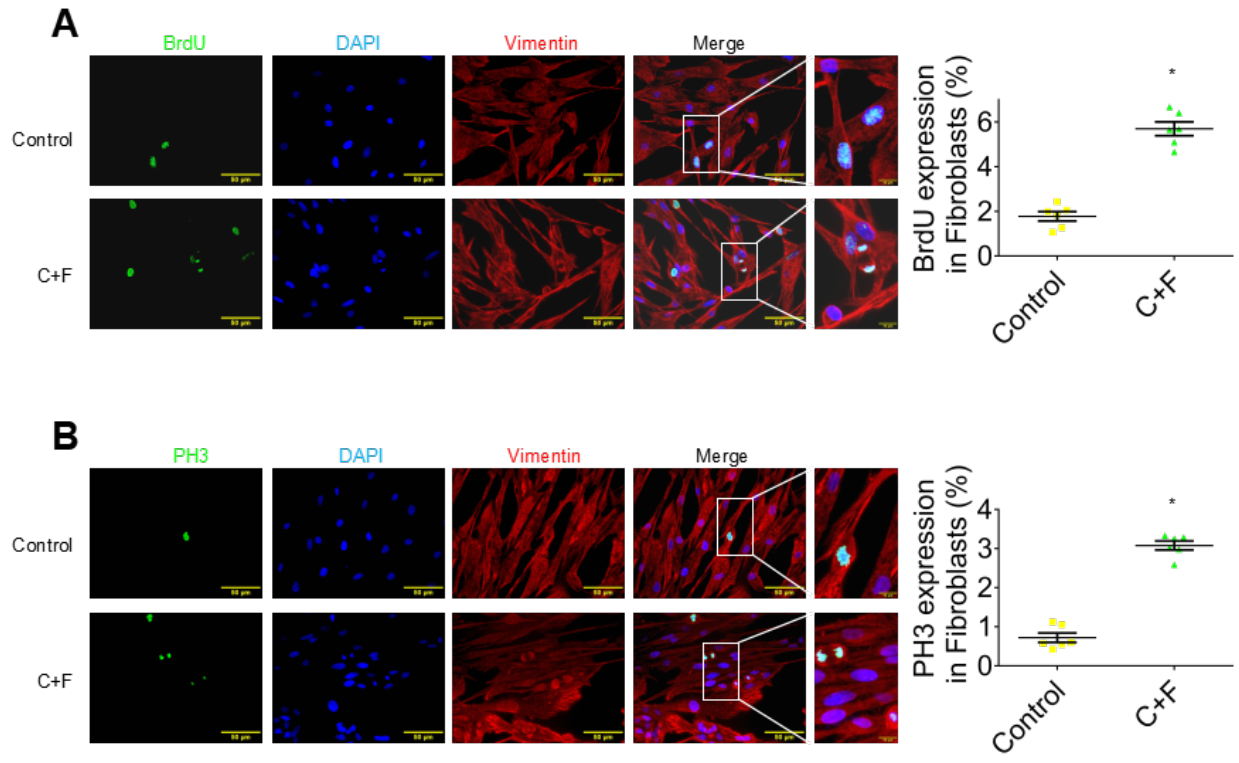
Supplemental Figure 5. Assessment of cell cycle in human umbilical vein endothelial cells (HUVECs) treated with CHIR99021 (CHIR) and/or FGF1.
Immunostaining and fluorescent microscopic analyses of synchronized *in vitro* human umbilical vein endothelial cell (HUVEC) cultures, exposed to CHIR (5 μM), FGF1 (100 ng/ml), or CHIR+FGF1 (5 μM, 100 ng/ml, respectively) for 24 hours, revealed the expression of a broad cell cycle marker (green, Ki67), bromodeoxyuridine (green, BrdU), the mitosis marker, phosphorylated histone H3 (green, PH3) and Aurora B kinase (green, Aurora B) (**A**-**D** Scale bar = 20 μm). Quantification of the percentage of CD31-positive (red, CD31) HUVECs demonstrated that the expression levels of Ki67 (**E**), BrdU (**F**), PH3 (**G**) and Aurora B (**H**) were significantly higher in the CHIR+FGF1-treated group compared with other treatments (CHIR or FGF1) and control groups. Cells were also counterstained for nuclei (blue, DAPI). The values are presented as means ± SE. Six independent experiments. Statistical analysis: One-way ANOVA with Dunn's multiple comparisons test. *$p < 0.01$ vs. Control; †$p < 0.05$ vs. CHIR; ‡$p < 0.01$ vs. FGF1.


A
Ki67
SM22α
DAPI
Merge
Control
Chir
FGF1
C+F
20 μm
10 μm

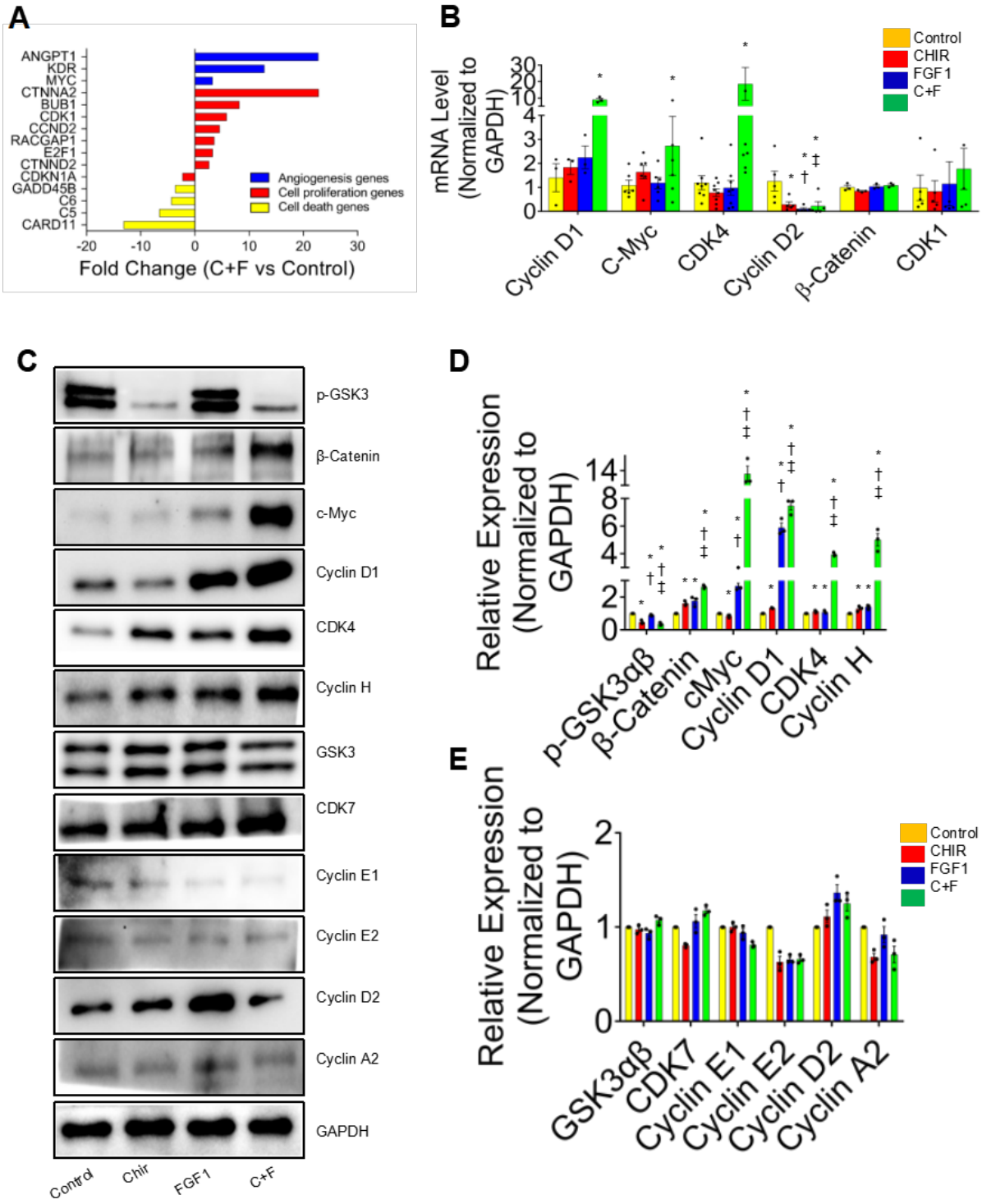

B
BrdU
SM22α
DAPI
Merge
Control
Chir
FGF1
C+F



Supplemental Figure 6. Assessment of cell cycle in human vascular smooth muscle cells (HVSMCs) treated with CHIR99021 (CHIR) and/or FGF1. Immunostaining and fluorescent microscopic analyses of synchronized *in vitro* human vascular smooth muscle cells (HVSMCs) cultures, exposed to CHIR (5 μM), FGF1 (100 ng/ml), or CHIR+FGF1 (5 μM, 100 ng/ml, respectively) for 24 hours, revealed the expression of a broad cell cycle marker (Ki67), bromodeoxyuridine (BrdU), and the mitosis marker, phosphorylated histone H3 (PH3) (**A-C**; Scale bar = 20 μm). Quantification of the percentage of smooth muscle protein 22-alpha-positive (red, SM22α) HVSMCs revealed that the expression levels of both Ki67, BrdU and PH3 were significantly higher in the case of CHIR+FGF1 treatment group compared with other treatments (CHIR or FG1) and control groups (**D-F**). Cells were also counterstained for nuclei (blue, DAPI). The values are presented as means ± SE. Six independent experiments. Statistical analysis: One-way ANOVA with Dunn's multiple comparisons test. **p* < 0.01 vs. Control; †*p* < 0.05 vs. CHIR; ‡*p* < 0.01 vs. FGF1.

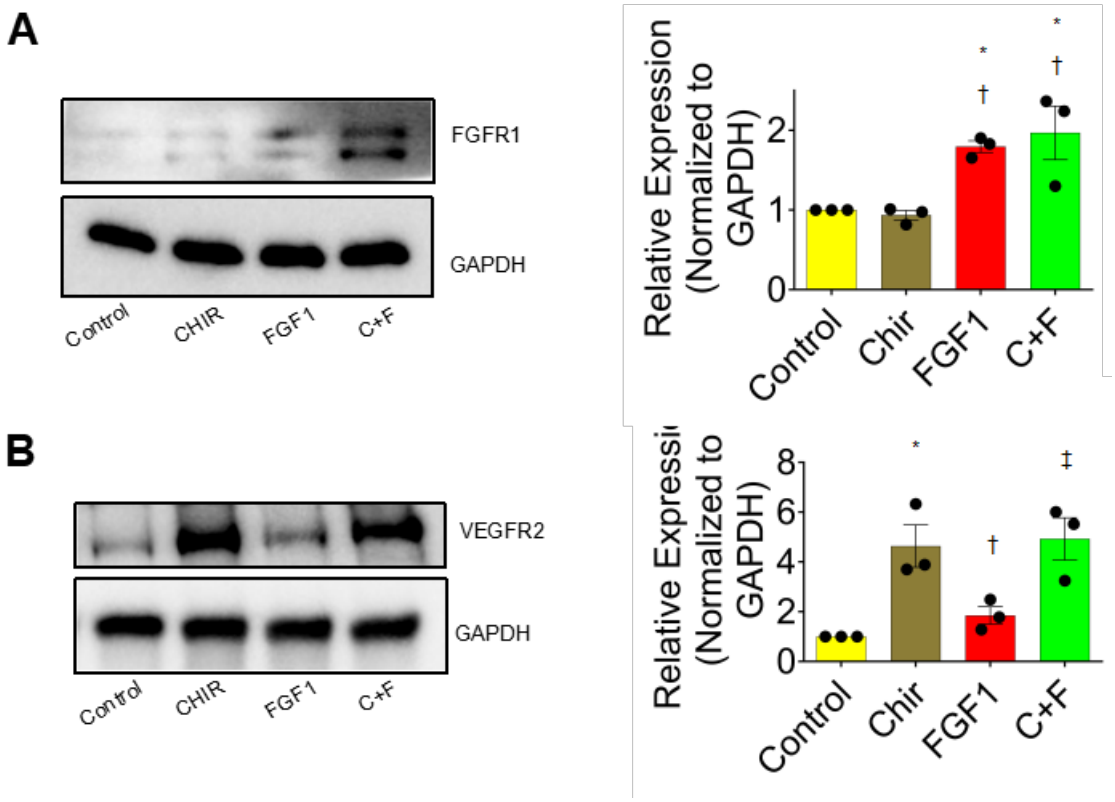


Supplemental Figure 7. Assessment of cell cycle in cardiac fibroblasts in vitro. Immunostaining and fluorescent microscopic analyses of synchronized in vitro primary porcine cardiac fibroblast cultures, exposed to CHIR+FGF1 (5 μM, 100 ng/ml, respectively) for 24 hours. Quantification of the percentage of Vimentin positive (red) fibroblasts revealed that the incorporation rate of BrdU **(A)** and the expression levels of PH3 **(B)** were significantly higher in the CHIR+FGF1 treatment group compared with control group. Cells were also counterstained for nuclei (blue, DAPI). Scale bar = 50 μm. The values are presented as means ± SE. Six independent experiments. Statistical analysis: Student test. *p < 0.01 vs. Control.

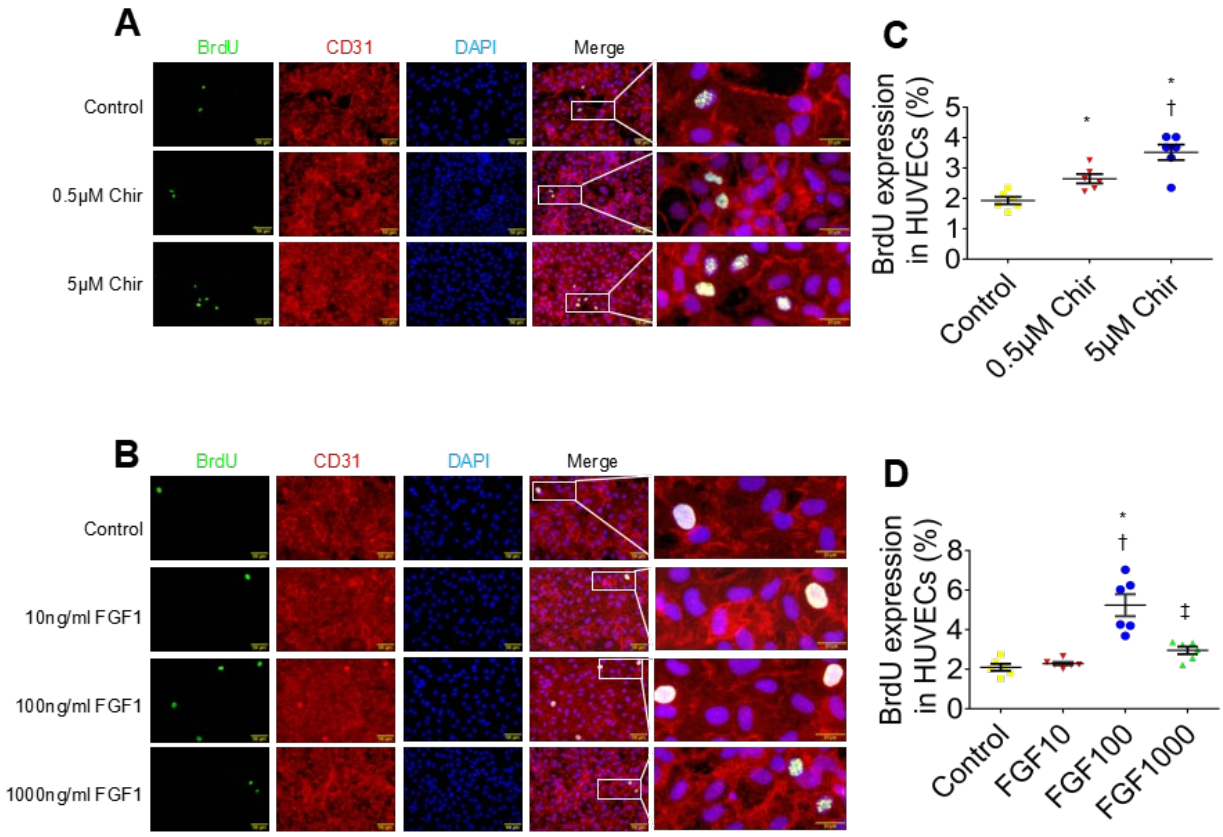


Supplemental Figure 8. Expression profiling of cell cycle regulatory genes, in human umbilical vein endothelial cells (HUVECs) treated with CHIR99021 (CHIR, 5 μM**) and/or FGF1 (**100 ng/ml**).** (**A**) Relative mRNA expression of genes related to angiogenesis, cell proliferation, and cell death. (**B**) Quantitative polymerase chain reaction (qPCR) confirming the upregulation of cyclin D1, CDK1, CDK4, and cMyc in the

CHIR+FGF1 treatment group compared with other treatments and control groups. (**C**) Western blot analyses revealing the expression pattern of various cell cycle regulatory proteins, such as cyclins, CDKs, cMyc, GSK3, and β-catenin. (**D** and **E**) Semi-quantitative Western blot analyses demonstrating a significantly upregulated expression levels of various cyclins in the CHIR+FGF1 treatment group compared with other treatments and control groups. The house keeping protein GAPDH, was used for Western blot normalization. The values were presented as means ± SE. Three independent experiments. Statistical analysis: One-way ANOVA with Dunn's multiple comparisons test. *$p < 0.01$ vs. control; †$p < 0.05$ vs. CHIR; ‡$p < 0.05$ vs. FGF1.



Supplemental Figure 9. Expression profiling of FGF and VEGF receptors, in human umbilical vein endothelial cells (HUVECs) treated with CHIR99021 (CHIR, 5 μM) and/or FGF1 (100 ng/ml). Western blot analyses revealing the expression of the FGF receptor 1 (**A**) and VEGF receptor 2 (**B**). The house keeping protein, GAPDH, was used for Western blot normalization. The values were presented as means ± SE. Three independent experiments. Statistical analysis: One-way ANOVA with Dunn's multiple comparisons test. *$p < 0.01$ vs. control; †$p < 0.05$ vs. CHIR; ‡$p < 0.05$ vs. FGF1.



Supplemental Figure 10. Expression pattern of a cell cycle associated marker, Bromodeoxyuridine (BrdU), in human umbilical vein endothelial cells (HUVECs) treated with various concentrations of CHIR99021 (CHIR) or FGF1. Immunostaining and fluorescent microscopic analyses of synchronized *in vitro* human umbilical vein endothelial cell (HUVEC) cultures, exposed to various concentrations of CHIR99021 (0.5 μM, 5 μM, or 50 μM) or FGF1 (10 ng/ml, 100 ng/ml, or 1000 ng/ml) for 24 hours, revealed the expression of bromodeoxyuridine (BrdU) (**A** and **B**). Quantification of the percentage of CD31-positive (red, CD31) HUVECs showed that the expression levels of BrdU were significantly higher in the 5 μM CHIR-treated group compared with 0.5 μM CHIR-treated or control groups (**C**). Similarly, quantification of the percentage of CD31-positive HUVECs revealed that the expression levels of BrdU were significantly greater in the 100 ng/ml FGF1 treatment group compared with other treatments (10 ng/ml or 1000 ng/ml) and control groups (**D**). Cells were also counterstained for nuclei (blue, DAPI). Scale bar = 50 μm **(**Panels in **A, B)**. The values were presented as means ± SE.Six independent experiments. Statistical analysis: One-way ANOVA with Dunn's multiple comparisons test. Panel **C**:*$p < 0.01$ vs. control; †$p < 0.05$ vs. CHIR (0.5 μM). Panel **D**:*$p < 0.01$ vs. Control; †$p < 0.01$ vs. FGF 10; ‡$p < 0.01$ vs. FGF1000.

SUPPLEMENTAL TABLE 1. RT-qPCR primer sequences used in this study

Genes	Forward Primer	Reverse Primer	Product Length (bp)	Annealing Temperature (°C)	GenBank accession No.
CCND1	5'-GGCGGATTGGAAATGAACTT-3'	5'-TCCTCTCCAAAATGCCAGAG-3'	109	61	NM_053056.2
CCND2	5'-CCTCCAAACTCAAAGAGACCAG-3'	5'-TTCCACTTCAACTTCCCCAG-3'	124	60	NM_001759.3
CDK1	5'-AAACCAGGAAGCCTAGCATC-3'	5'-ATGATTCAGTGCCATTTTGCC-3'	120	58	NM_001170406.1
CDK4	5'-TGAGGGGGCCTCTCTAGCTT-3'	5'-CAAGGGAGACCCTCACGCC-3'	162	60	NM_000075.3
CTNNB1	5'-GCCACAAGATTACAAGAAACGG-3'	5'-TGGGCACCAATATCAAGTCC-3'	111	61	NM_001098209.1
c-Myc	5'-CACCGAGTCGTAGTCGAGGT-3'	5'-TTTCGGGTAGTGGAAAACCA-3'	93	60	NM_001354870.1
GAPDH	5'-GGTTTACATGTTCCAATATG-3'	5'-GAGGGATCTCGCTCCTGGAA-3'	120	60	NM_002046.7