

**Table S1.** Primers used for qRT-PCR

| Gene     | Forward primer (5'–3')      | Reverse primer (5'–3')     | Product (bp) |
|----------|-----------------------------|----------------------------|--------------|
| ANP      | GAGCGGACTGGGCTGTAAC         | GGAGCCTCTTGCACTCTGTC       | 93           |
| BIRC5    | TCTGCTTCAAGGAGCTGGA         | AAAGTGCTGGTATTACAGGCGTA    | 88           |
| BIRC7    | GCGCACCTTCCTGTCCTA          | GGCCCAGAACACAGTCCA         | 100          |
| BNIP3    | TGCTGCTCTCTCATTGCTG         | GACTCCAGTTCTTCATCAAAAGGT   | 95           |
| BNIP3L   | AATGTCGTCCCACCTAGTCG        | AGCTCCACCCAGGAAGTGT        | 117          |
| BUB1     | AGCCCAGACAGTAACAGACTCC      | GCTCCACTCTGTGGCTTGA        | 96           |
| BUB1B    | CAGTCAGACTCTCAGCATCAAGA     | CGAGGCAGAAGAACCAGAGA       | 94           |
| CASP6    | GATGCAGCCTCCGTTTACA         | CACAGTTTCCCGGTGAGAATA      | 93           |
| CASP9    | AAGCCCAAGCTCTTTTTCATC       | ACTCGTCTTCAGGGGAAGTG       | 88           |
| CCNB1    | CATGGTGCACCTTTCCTCCTT       | AGGTAATGTTGTAGAGTTGGTGTCC  | 102          |
| CD31     | GAGTCCTGCTGACCCTTCTG        | TCAGGTTCTTCCCATTTTG        | 120          |
| CDK1     | TGGATCTGAAGAAATACTTGGATTCTA | CAATCCCCTGTAGGATTG         | 96           |
| cTnI     | GCAGATGCCATGATGCAG          | CACCTCCCGTTTTTCCTT         | 114          |
| cTnT     | GTCGGCAGCTGCTGTTCT          | TCCTCTCTCCAGTCTCCTCT       | 124          |
| CXCR4    | CCTGCCTGGTATTGTCATCC        | AGGATGACTGTGGTCTTGAGG      | 105          |
| DDIT4    | CTGGAGAGCTCGGACTGC          | TCCAGGTAAGCCGTGTCTTC       | 77           |
| DDX41    | GGGGATGAGTCCATGCTG          | GGCAGTCAGTGATCCGATG        | 82           |
| EGLN1    | CGACCTGATACGCCACTGT         | GTTCCATTGCCCGGATAAC        | 93           |
| EGLN3    | ATCGACAGGCTGGTCTCTA         | GATAGCAAGCCACCATTGC        | 85           |
| ENG      | AATGCCATCCTTGAAGTCCA        | GTGCCATTTTGCTTGGATG        | 95           |
| ENO1     | TCCCAACATCCTGGAGAATAA       | ATGCCGATGACCACCTTATC       | 90           |
| ERO1A    | GGATTCTTGTTTGGCCTCCT        | CAGGTACAATCATCCAAGTAACCA   | 128          |
| FAM162A  | CGAAGTCTGCGCTGGTC           | GGAAACATCTCTTTCACATAACCTAA | 106          |
| FOXO3    | CTGTGAATGAATCTGAGCTTGG      | TCCCATTAACAGGAGTGACAAG     | 103          |
| GAPDH    | GAGTCCACTGGCGTCTTCAC        | TTCACACCCATGACGAACAT       | 119          |
| HCN4     | GCCGACGGCTCCTACTTT          | GTAGAGGCGGCAGTAGGTGT       | 90           |
| HK1      | GACCAAGTTTCTCTCTCAGATCG     | CCTAGCTGCTGGAGGATAGC       | 75           |
| HMOX1    | TGAACTCCCTGGAGATGACTC       | CCTGCAACTCCTCAAAGAGC       | 99           |
| ITGA2    | GCTGATAATTTGGTCAACCTCA      | GAACATTCCCATCCGAAGAG       | 109          |
| LDHA     | TCTCTGTAGCAGATTTGGCAGA      | AAGACATCATCCTTTATTCCGTAAA  | 103          |
| MELK     | AAACCAGTTCGTTTAAGGCTTTC     | GCGGTACATCTTCCAGACT        | 104          |
| MESP1    | CCGAGTCTTGATGCTCTC          | AGTCTGGGGACGAGACGAG        | 96           |
| MIXL1    | GGTACCCCGACATCCACTT         | GCCTGTCTGGAACCATACTT       | 87           |
| MLC2a    | GGGTGGTGAACAAGGATGAG        | GTGTCAGGGCGAACATCTG        | 93           |
| MLC2v    | GCAGGCGGAGAGGTTTTT          | AGTTGCCAGTCACGTCAGG        | 74           |
| NANOG    | GATTTGTGGGCTGAAGAAA         | AAGTGGGTGTTTGCCTTTG        | 155          |
| NDRG1    | GTTTCTTGGCGTCGTCTC          | CATGTCCCTGCTGTACCTT        | 100          |
| P4HB     | CCACGGAGGAGTCTGACCT         | TCACGATGTCATCAGCCTCT       | 129          |
| PDK1     | TCCGTTCAATTGGTACAAAGC       | CCATGTTCTTCTAGGCCTTTCA     | 114          |
| PFKFB3   | AGCTTGTGCCAAAGGTCACCT       | TCTAAATCTCCCACCCTCACC      | 104          |
| PGK1     | GCTGGTGGGTTTTTGATGA         | TTTAGCTCCGCCAGGAT          | 96           |
| POU5F1   | AGTGAGAGGCAACCTGGAGA        | ACACTCGGACCACATCCTTC       | 110          |
| PSME3    | CCGGCTGTTGATTGAGAAAT        | TCTGCAACTGTTCTCTCTG        | 114          |
| RORA     | TCCCTACTGTTTCGTTACCA        | CAGGTTTCCAGATGCGATTT       | 102          |
| SERPINE1 | GCAGCAGGACCGACAAGT          | TGTCTTATGGCATCCAGTTAAGC    | 91           |
| SIAH2    | GGCAGTCTGTTTCCCTGT          | CACTTGACAGGAAGCACCAG       | 135          |
| SLC2A1   | GCCCATGTATGTGGGTGAA         | AGTCCAGGCCGAACACCT         | 113          |
| SLC8A3   | CCGCATGGTGGATATGAGTT        | TCACCCAATACTGGCTTTCC       | 117          |
| SM22     | GGCCAAGGCTCTACTGTCTG        | CCCTTGTTGGCCATGTCT         | 74           |
| SMA      | ATCCCCGGGACTAAGACG          | CAAAGCCGGCCTTACAGAG        | 113          |
| STAT1    | TTGGCACCTAACGTGCTGT         | AGTTCGTACCACTGAGACATCCT    | 96           |
| STC1     | GAGGCGGAGCAGAATGACT         | GTTGAGGCAACGAACCACTT       | 84           |
| STC2     | CCTACGTGGACCTCGTGAAC        | CCCAGTTCTGCTCACACTGA       | 96           |
| T        | GCTGTGACAGGTACCCAACC        | CATGCAGGTGAGTTGTCAGAA      | 106          |
| TBX18    | CCTGGAATTCCCAAGCAAG         | GAGGAGCCAGACAAAAGGTG       | 101          |

|                |                             |                          |     |
|----------------|-----------------------------|--------------------------|-----|
| TFRC           | CTTTGGAGTTATTAAAGGCTTTGTAGA | CTGTGCCTACACCGGATTTT     | 104 |
| TPX2           | CCATTCCGTCAAATGCTTG         | TCCTTCTGAGCAGAAAGCCTA    | 106 |
| VCAM1          | TGCACAGTGACTTGTGGACAT       | CCACTCATCTCGATTTCTGGA    | 92  |
| VEGFA          | TGCCCCGCTGCTGTCTAAT         | TCTCCGCTCTGAGCAAGG       | 70  |
| VEGFR2         | ATGACATTTTGATCATGGAGC       | CCCAGATGCCGTGCATGAG      | 193 |
| $\beta$ -ACTIN | CTTCTACAATGAGCTGCGTGTGGCTC  | GTACATGGCTGGGGTGTGAAGGTC | 108 |

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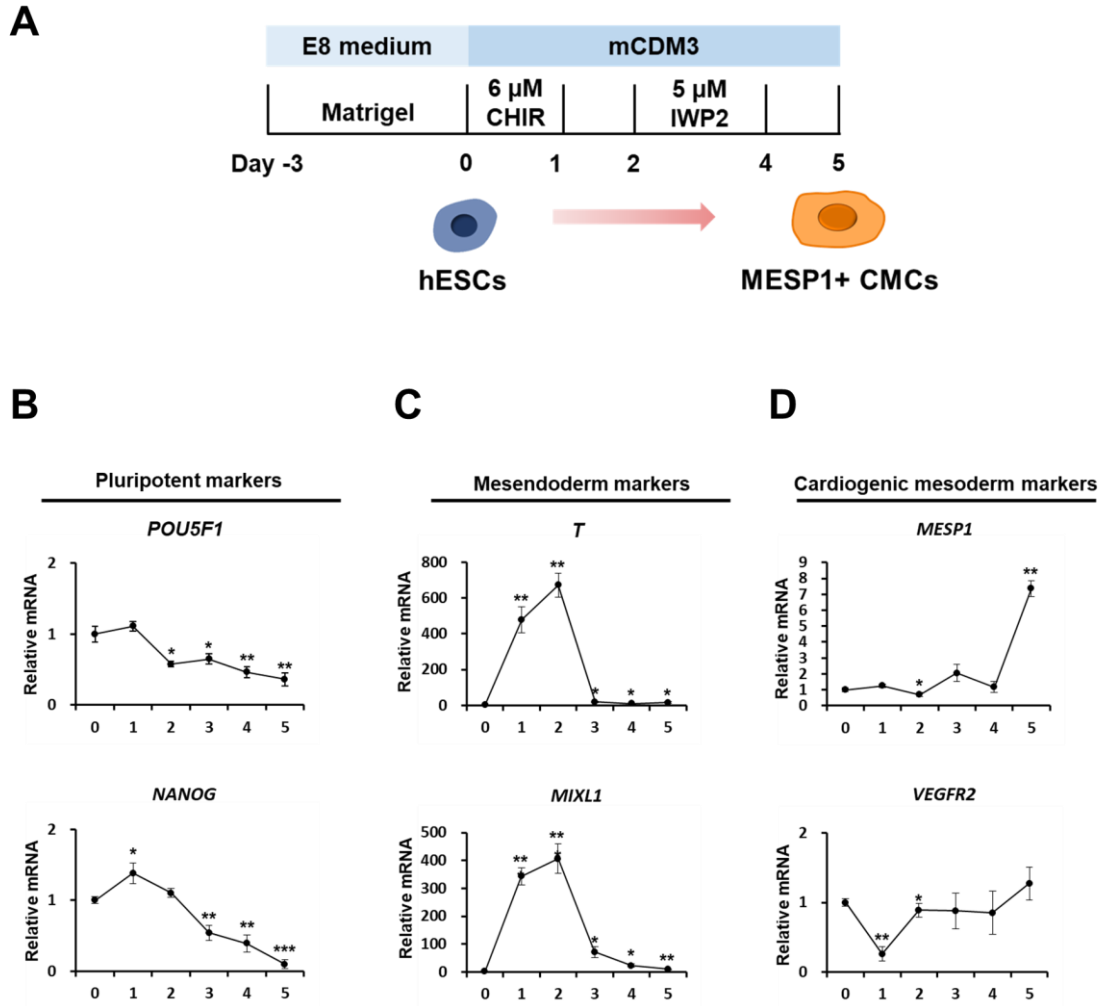


**Figure S1.** Temporal expression patterns of cell lineage markers during cardiogenic mesoderm differentiation of BG01 hESCs. (A) Schematic diagram of cardiogenic mesoderm differentiation protocol for BG01 hESCs along with Wnt modulation in mCDM3 medium. CHIR, CHIR99021; CMC, cardiogenic mesoderm cell; mCDM3, modified chemically defined medium consisting of three components; E8, essential 8. Gene expression in samples undergoing cardiogenic mesoderm differentiation from BG01 hESCs in mCDM3 at the indicated time points was analyzed with a focus on genes associated with (B) pluripotency (*POU5F1*, *NANOG*), (C) mesendoderm (*T*, *MIXL1*), and (D) cardiogenic mesoderm (*MESP1*, *VEGFR2*). Data were normalized to *GAPDH* level and expressed as relative values. Three independent experiments were performed. Values represent means  $\pm$  SDs.  $n = 3$ . \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  versus day 1. Two-tailed unpaired Student's *t*-test.

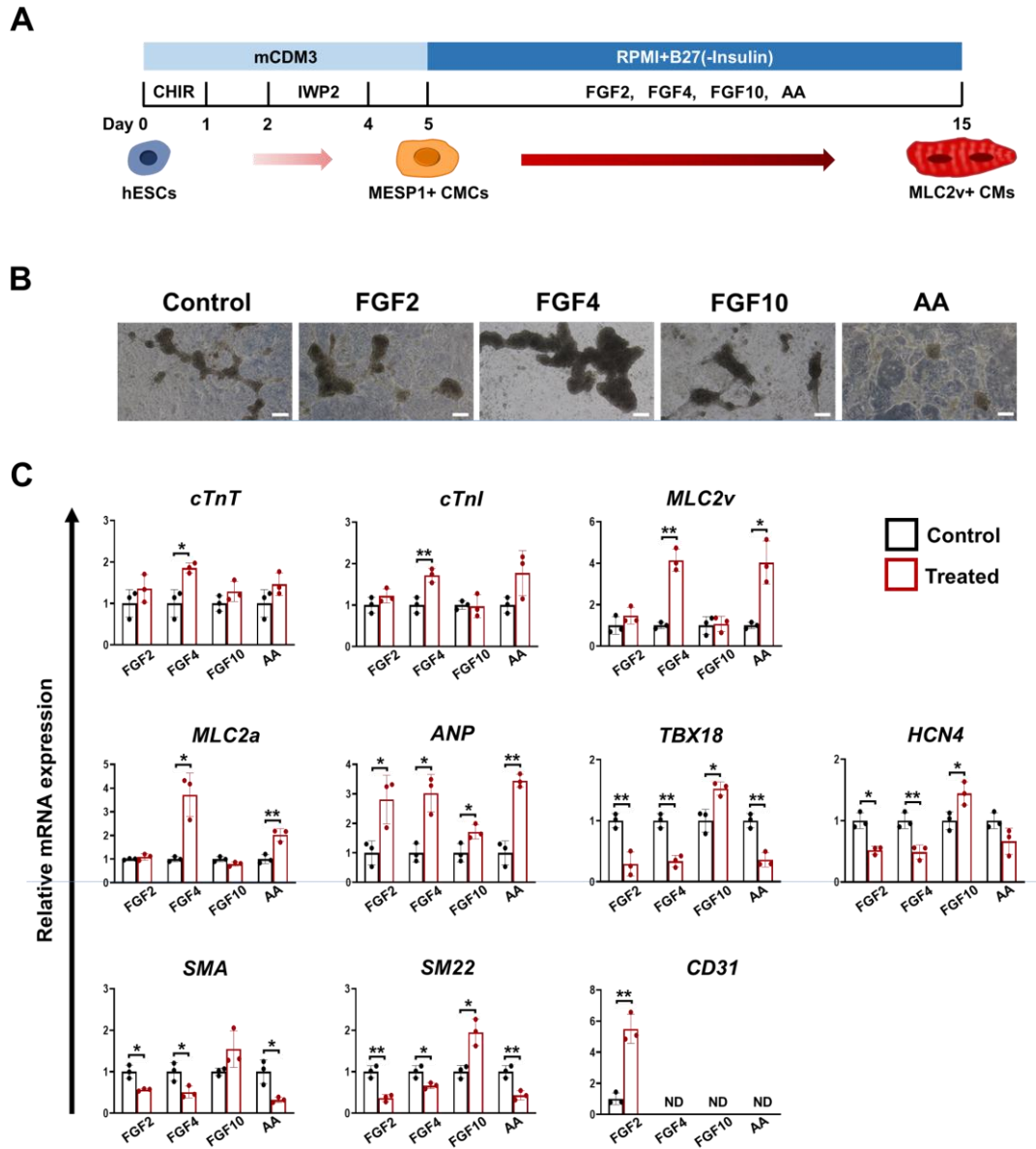


**Figure S2.** FGF4 and AA enhance gene expression of mature, ventricular, and atrial CM subtype markers, but reduce gene expression of nodal CM and smooth muscle cell markers in BG01 hESC-CMs. **(A)** Schematic diagram of CM subtype differentiation protocol from BG01 hESC along with Wnt modulation followed by 10 ng/mL FGF2, 10 ng/mL FGF4, 10 ng/mL FGF10, or 200  $\mu$ g/mL AA between days 5 and 15 of differentiation in mCDM3 and RPMI/B27(-Insulin) media. CHIR: CHIR99021; CM: cardiomyocyte; CMC: cardiogenic mesoderm cell; mCDM3: modified chemically defined medium consisting of three components. **(B)** Morphology of hESC-CMs treated with 10 ng/mL FGF2, 10 ng/mL FGF4, 10 ng/mL FGF10, or 200  $\mu$ g/mL AA. Scale bars = 200  $\mu$ m. **(C)** qRT-PCR analysis of a total CM marker (*cTnT*), a mature CM marker (*cTnI*), a ventricular CM marker (*MLC2v*), atrial CM markers (*MLC2a*, *ANP*), nodal CM markers (*TBX18*, *HCN4*), smooth muscle cell markers (*SMA*, *SM22*), and an endothelial cell marker (*CD31*) in BG01 hESC-CMs treated with 10 ng/mL FGF2, 10 ng/mL FGF4, 10 ng/mL FGF10, or 200  $\mu$ g/mL AA in mCDM3 and RPMI/B27(-Insulin) media at differentiation day 15. Data were normalized to GAPDH level and expressed as relative values. Values represent means  $\pm$  SDs.  $n = 3$ . ND: not detected. \* $p < 0.05$  and \*\* $p < 0.01$  versus controls. Two-tailed unpaired Student's t-test.

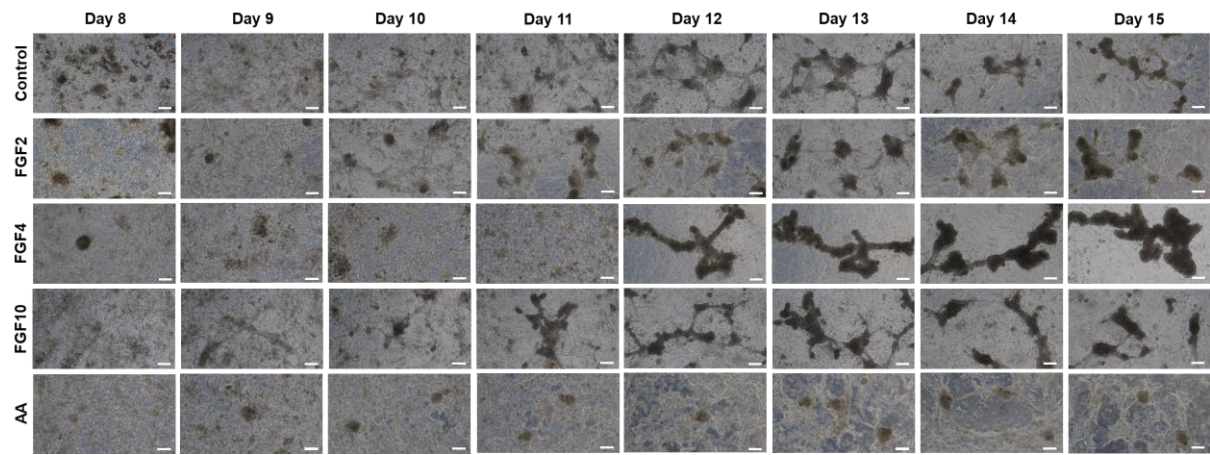


**Figure S3.** Sequential morphological changes during cardiac differentiation of BG01 hESC-CMCs. Morphological changes during hESC-CMC differentiation toward a cardiac lineage after treatment with FGF2, FGF4, FGF10, or AA in mCDM3 and RPMI/B27(-Insulin) media from days 8 to 15. Scale bar, 200  $\mu$ m.

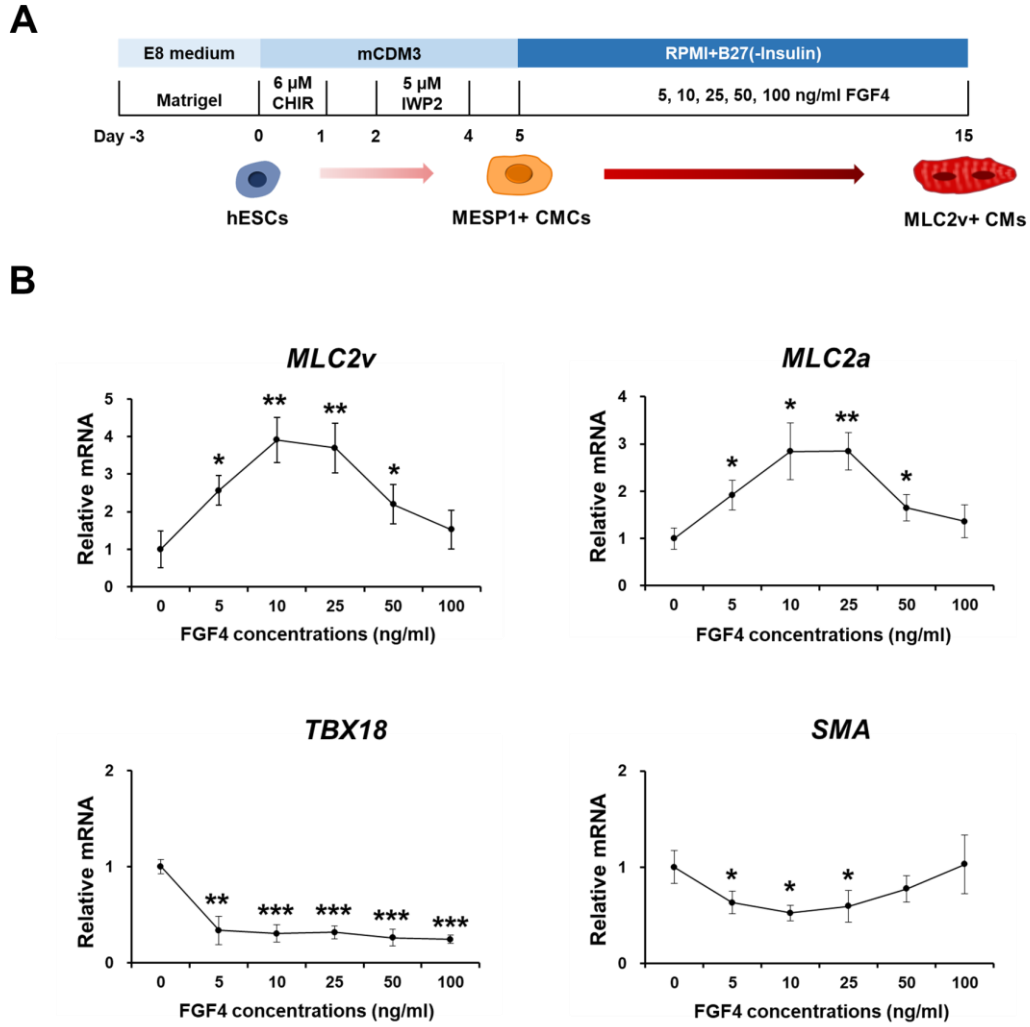


**Figure S4.** Effective FGF4 concentrations at inducing differentiation of BG01 hESC-CMCs into ventricular and atrial CMs. (A) Schematic diagram of the protocol used for CM subtype differentiation from hESC-CMCs treated with different concentrations of FGF4 in mCDM3 and RPMI/B27(-Insulin) media. CHIR, CHIR99021; CMC, cardiogenic mesoderm cell; mCDM3, modified chemically defined medium consisting of three components; E8, essential 8. (B) qRT-PCR analysis for a ventricular CM marker (*MLC2v*), an atrial CM marker (*MLC2a*), a nodal CM marker (*TBX18*), and a smooth muscle cell marker (*SMA*) in BG01 hESC-CMCs treated with different concentrations of FGF4 in mCDM3 and RPMI/B27(-Insulin) media at differentiation day 15. Data were normalized to *GAPDH* level and expressed as relative values. Values represent means  $\pm$  SDs.  $n = 3$ . \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  versus controls. Two-tailed unpaired Student's *t*-test.

**A****cTnI**

|        | Control  |         |          |         | FGF4+AA  |         |          |         |
|--------|----------|---------|----------|---------|----------|---------|----------|---------|
|        | AVE      |         | SD       |         | AVE      |         | SD       |         |
|        | Normoxia | Hypoxia | Normoxia | Hypoxia | Normoxia | Hypoxia | Normoxia | Hypoxia |
| Day 11 | 0.002    | 0.002   | 0.002    | 0.000   | 0.003    | 0.002   | 0.001    | 0.002   |
| Day 13 | 0.003    | 0.003   | 0.001    | 0.003   | 0.005    | 0.007   | 0.001    | 0.001   |
| Day 15 | 0.005    | 0.005   | 0.003    | 0.001   | 0.006    | 0.007   | 0.002    | 0.001   |
| Day 17 | 0.007    | 0.008   | 0.005    | 0.002   | 0.005    | 0.019   | 0.002    | 0.003   |
| Day 19 | 0.006    | 0.007   | 0.002    | 0.001   | 0.007    | 0.015   | 0.002    | 0.003   |
| Day 21 | 0.006    | 0.004   | 0.003    | 0.000   | 0.007    | 0.013   | 0.002    | 0.001   |

**B****CK-MB**

|        | Control  |         |          |         | FGF4+AA  |         |          |         |
|--------|----------|---------|----------|---------|----------|---------|----------|---------|
|        | AVE      |         | SD       |         | AVE      |         | SD       |         |
|        | Normoxia | Hypoxia | Normoxia | Hypoxia | Normoxia | Hypoxia | Normoxia | Hypoxia |
| Day 11 | 0.208    | 0.265   | 0.042    | 0.151   | 0.383    | 0.378   | 0.197    | 0.155   |
| Day 13 | 0.269    | 0.347   | 0.082    | 0.185   | 0.289    | 0.564   | 0.111    | 0.099   |
| Day 15 | 0.433    | 0.636   | 0.159    | 0.307   | 0.721    | 1.055   | 0.623    | 0.452   |
| Day 17 | 0.249    | 0.944   | 0.054    | 0.451   | 0.595    | 2.693   | 0.511    | 0.231   |
| Day 19 | 0.267    | 0.603   | 0.089    | 0.129   | 0.400    | 1.448   | 0.238    | 0.203   |
| Day 21 | 0.226    | 0.441   | 0.084    | 0.162   | 0.251    | 1.322   | 0.140    | 0.412   |

**C****myoglobin**

|        | Control  |         |          |         | FGF4+AA  |         |          |         |
|--------|----------|---------|----------|---------|----------|---------|----------|---------|
|        | AVE      |         | SD       |         | AVE      |         | SD       |         |
|        | Normoxia | Hypoxia | Normoxia | Hypoxia | Normoxia | Hypoxia | Normoxia | Hypoxia |
| Day 11 | 0.031    | 0.003   | 0.029    | 0.006   | 0.000    | 0.011   | 0.000    | 0.018   |
| Day 13 | 0.031    | 0.037   | 0.046    | 0.033   | 0.000    | 0.000   | 0.000    | 0.000   |
| Day 15 | 0.039    | 0.066   | 0.068    | 0.114   | 0.016    | 0.025   | 0.028    | 0.034   |
| Day 17 | 0.032    | 0.053   | 0.055    | 0.079   | 0.015    | 0.131   | 0.020    | 0.004   |
| Day 19 | 0.085    | 0.004   | 0.027    | 0.007   | 0.000    | 0.153   | 0.000    | 0.061   |
| Day 21 | 0.013    | 0.068   | 0.022    | 0.048   | 0.003    | 0.185   | 0.005    | 0.091   |

**Figure S5.** Non-invasive and continuous detection of AMI biomarkers from the culture medium of BG01 hESC-CMs. Culture media from normoxia- or hypoxia-treated BG01 hESC-CMs cultured for 24 h with or without FGF4+AA were collected on days 11, 13, 15, 17, 19, and 21 of differentiation. AMI specific biomarkers (cTnI, CK-MB, and myoglobin) were evaluated using PATHFAST.

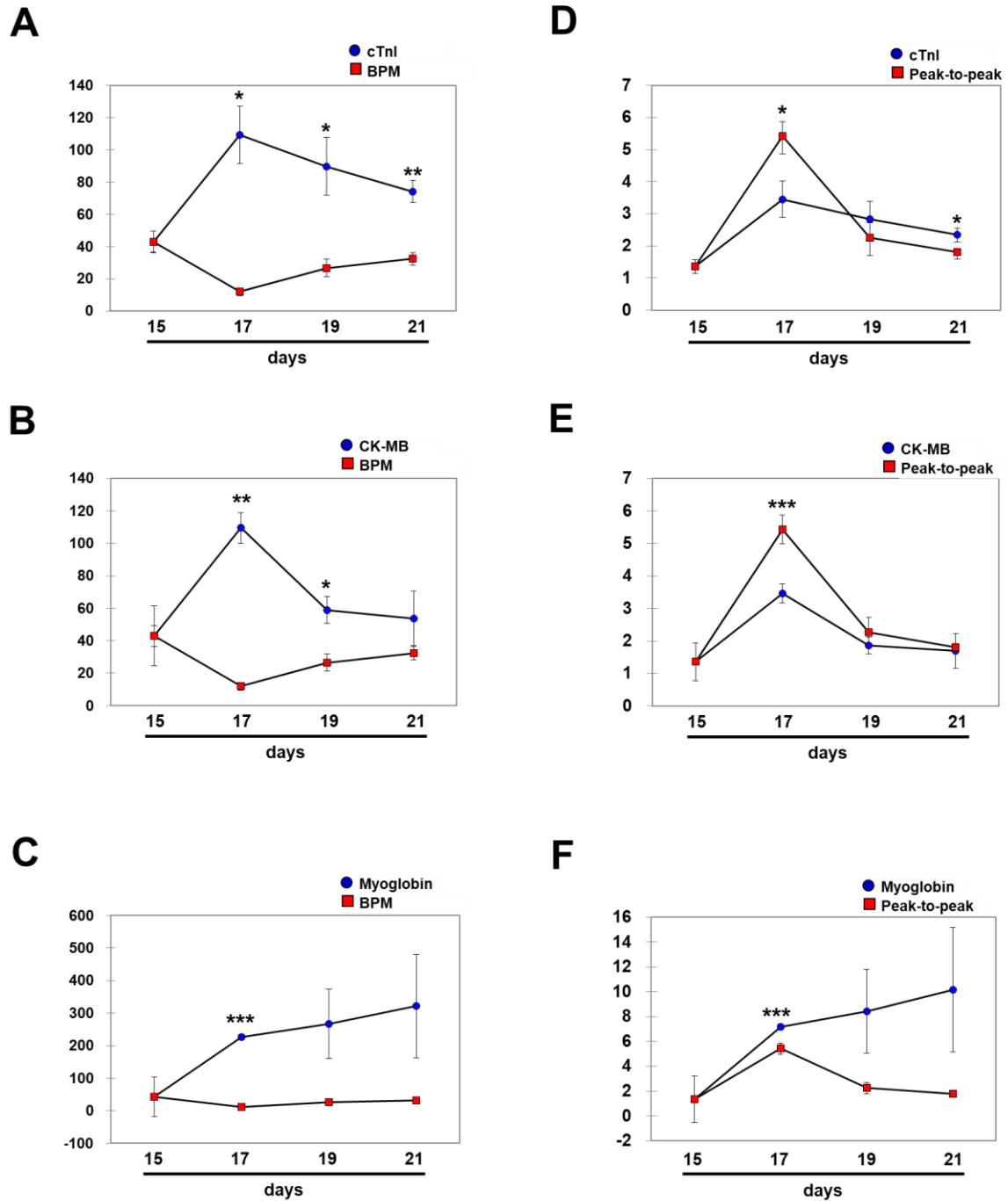


**Figure S6.** Relationship between AMI biomarkers and contractile properties of BG01 hESC-CMs. Relationships between normalized cTnI (A, D), CK-MB (B, E), myoglobin (C, F) release, and contractile properties (beat-rate and peak-to-peak duration) of BG01 hESC-CMs during hypoxic injury are shown. Values represent means  $\pm$  SDs. \* $p < 0.05$  and \*\* $p < 0.01$  versus controls. Two-tailed unpaired Student's t-test.

**A****Normalized cTnI (fold-change)**

|        | BPM             |        |                 |       | Peak-to-peak    |              |                 |              |
|--------|-----------------|--------|-----------------|-------|-----------------|--------------|-----------------|--------------|
|        | AVE             |        | SD              |       | AVE             |              | SD              |              |
|        | Normalized cTnI | BPM    | Normalized cTnI | BPM   | Normalized cTnI | Peak-to-peak | Normalized cTnI | Peak-to-peak |
| Day 15 | 42.932          | 42.932 | 6.760           | 6.463 | 1.357           | 1.357        | 0.214           | 0.054        |
| Day 17 | 109.281         | 11.957 | 17.885          | 2.407 | 3.453           | 5.430        | 0.565           | 0.446        |
| Day 19 | 89.767          | 26.560 | 17.885          | 5.387 | 2.837           | 2.264        | 0.565           | 0.457        |
| Day 21 | 74.155          | 32.331 | 6.760           | 3.969 | 2.343           | 1.809        | 0.214           | 0.113        |

**B****Normalized CK-MB (fold-change)**

|        | BPM              |        |                  |       | Peak-to-peak     |              |                  |              |
|--------|------------------|--------|------------------|-------|------------------|--------------|------------------|--------------|
|        | AVE              |        | SD               |       | AVE              |              | SD               |              |
|        | Normalized CK-MB | BPM    | Normalized CK-MB | BPM   | Normalized CK-MB | Peak-to-peak | Normalized CK-MB | Peak-to-peak |
| Day 15 | 42.931           | 42.932 | 18.399           | 6.463 | 1.357            | 1.357        | 0.582            | 0.054        |
| Day 17 | 109.565          | 11.957 | 9.395            | 2.407 | 3.464            | 5.430        | 0.297            | 0.446        |
| Day 19 | 58.905           | 26.560 | 8.277            | 5.387 | 1.862            | 2.264        | 0.262            | 0.457        |
| Day 21 | 53.779           | 32.331 | 16.750           | 3.969 | 1.700            | 1.809        | 0.530            | 0.113        |

**C****Normalized myoglobin (fold-change)**

|        | BPM                  |        |                      |       | Peak-to-peak         |              |                      |              |
|--------|----------------------|--------|----------------------|-------|----------------------|--------------|----------------------|--------------|
|        | AVE                  |        | SD                   |       | AVE                  |              | SD                   |              |
|        | Normalized myoglobin | BPM    | Normalized myoglobin | BPM   | Normalized myoglobin | Peak-to-peak | Normalized myoglobin | Peak-to-peak |
| Day 15 | 42.932               | 42.932 | 59.922               | 6.463 | 1.357                | 1.357        | 1.894                | 0.054        |
| Day 17 | 227.423              | 11.957 | 7.246                | 2.407 | 7.187                | 5.430        | 0.229                | 0.446        |
| Day 19 | 266.874              | 26.560 | 106.743              | 5.387 | 8.433                | 2.264        | 3.373                | 0.457        |
| Day 21 | 321.409              | 32.331 | 158.769              | 3.969 | 10.157               | 1.809        | 5.017                | 0.113        |

**Figure S7.** Relationship between AMI biomarkers and contractile properties of BG01 hESC-CMs. Relationships between normalized cTnI (**A**), CK-MB (**B**), myoglobin (**C**) release, and contractile properties (beat-rate and peak-to-peak duration) of BG01 hESC-CMs during hypoxic injury are shown.

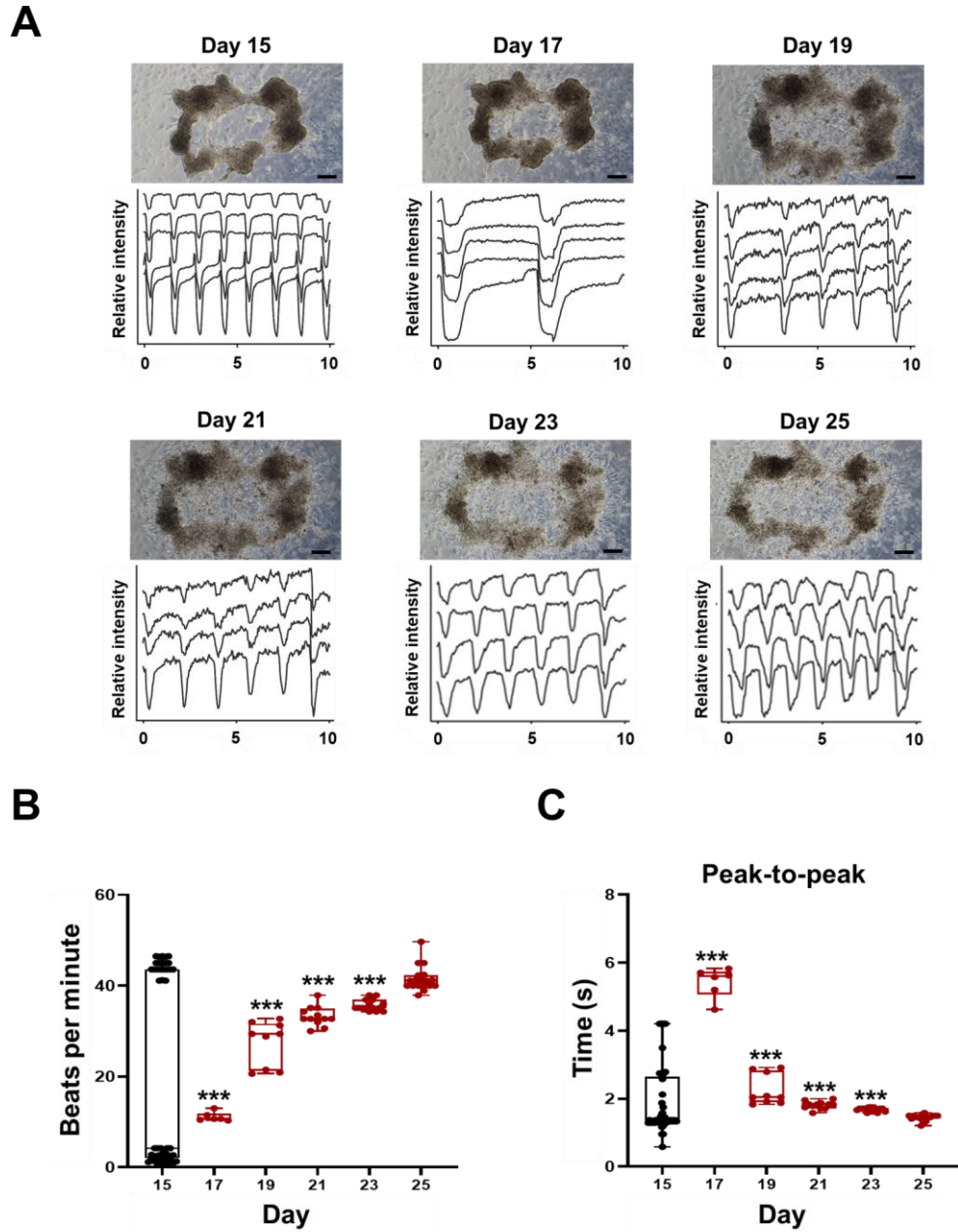


**Figure S8.** Sequential changes in the beating properties of BG01 hESC-CMs exposed to hypoxia. Temporal changes in morphology (A), beats per minutes (B), and peak-to-peak (C) of FGF4+AA-treated hESC-CMs cultured under hypoxia (2% O<sub>2</sub>) were video-recorded every 2 days between days 15 and 25 of differentiation. Scale bars = 200  $\mu$ m. Values represent means  $\pm$  SDs. \*\*\* $p$  < 0.001 versus day 15. Significant differences between the means were analyzed by a one-way ANOVA followed by the Student–Newman–Keuls test.  $n$  = 21, 6, 9, 12, 15, and 18 for beating analysis on days 15, 17, 19, 21, 23, and 25, respectively.

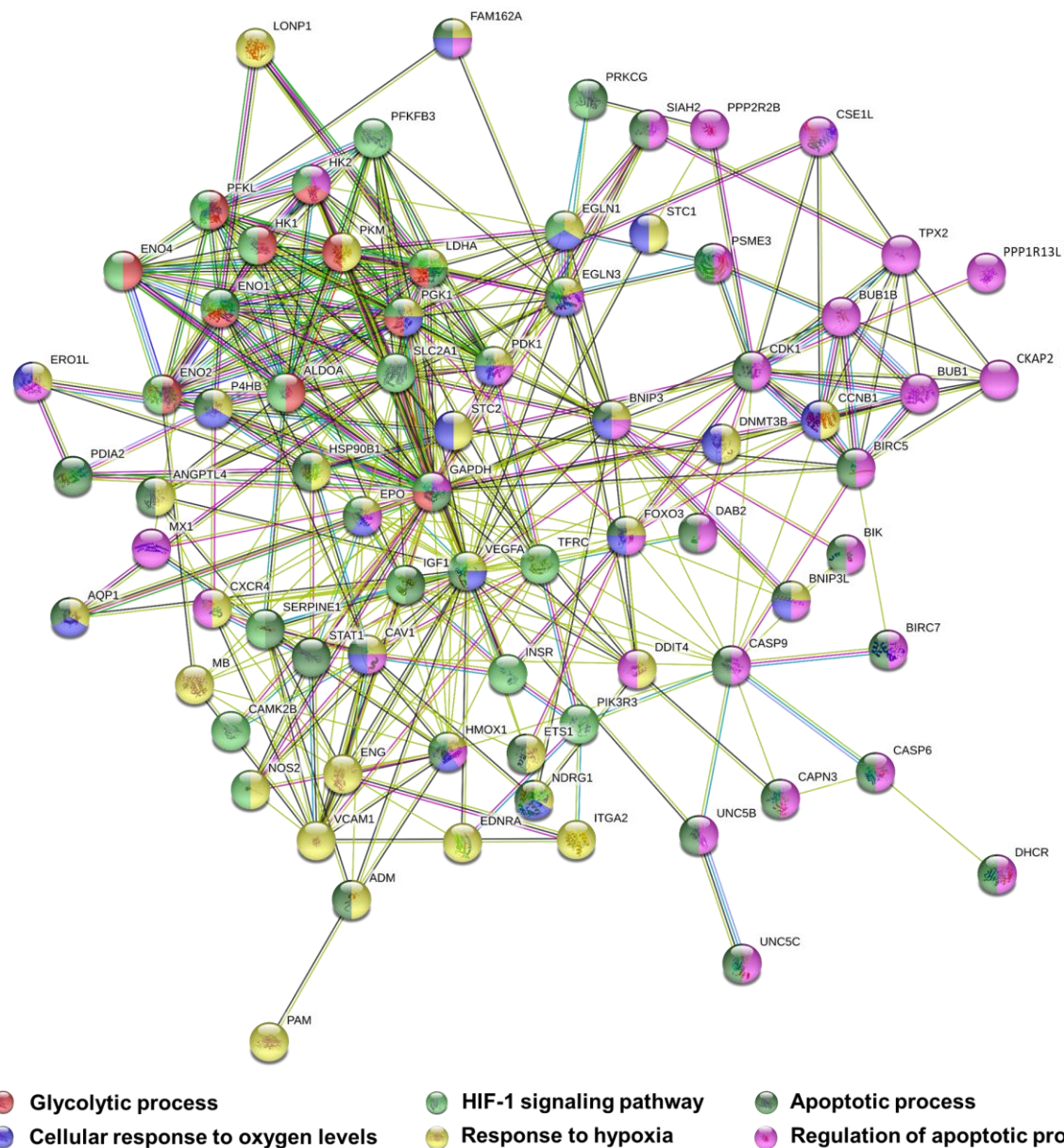


**Figure S9.** Protein-protein interaction networks among differentially expressed genes in hypoxia-induced BG01 hESC-CMs. The STRING search tool was used to generate protein-protein interaction networks in differentially expressed genes related to glycolytic process, cellular response to oxygen levels, and HIF-1 signaling pathway, response to hypoxia, apoptotic process, and regulation of apoptotic process in BG01 hESC-CMs cultured under hypoxia for 24 h.

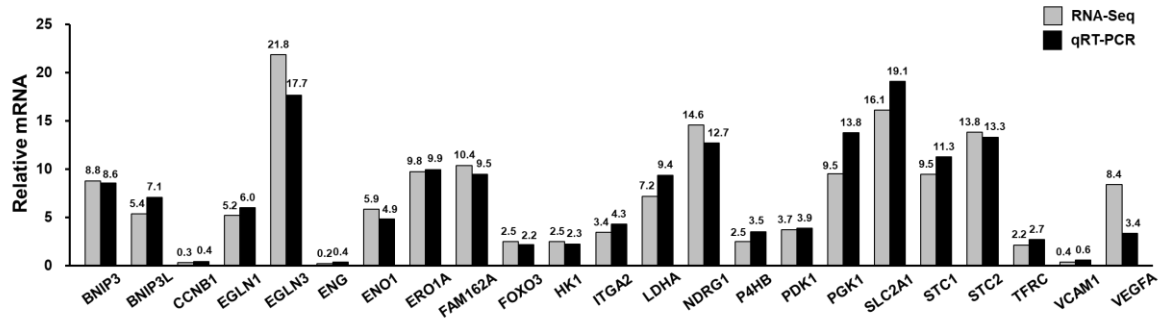
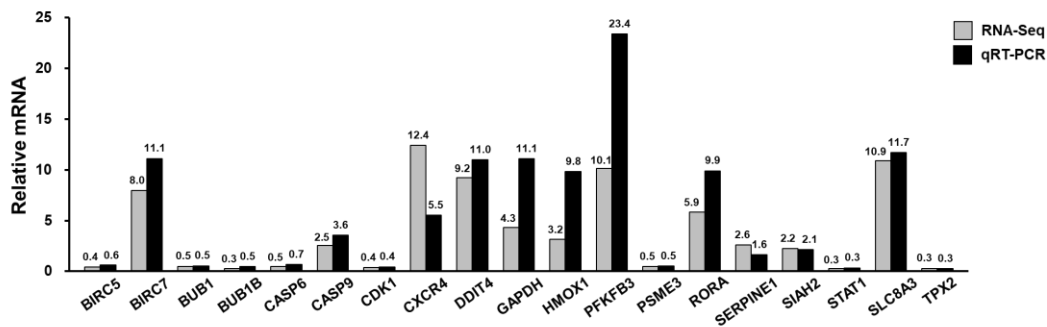
**A****B**

**Figure S10.** Validation of differentially expressed genes in hypoxia-induced BG01 hESC-CMs with qRT-PCR. Relative expression of genes involved in (A) responses to hypoxia including glycolytic process, cellular response oxygen levels, and HIF-1 signaling pathway, and (B) apoptotic processes was analyzed by qRT-PCR using the same samples as used for RNA-Seq. Data were normalized to  $\beta$ -ACTIN level and expressed as relative values. Values represent means  $\pm$  SDs from triplicate data.

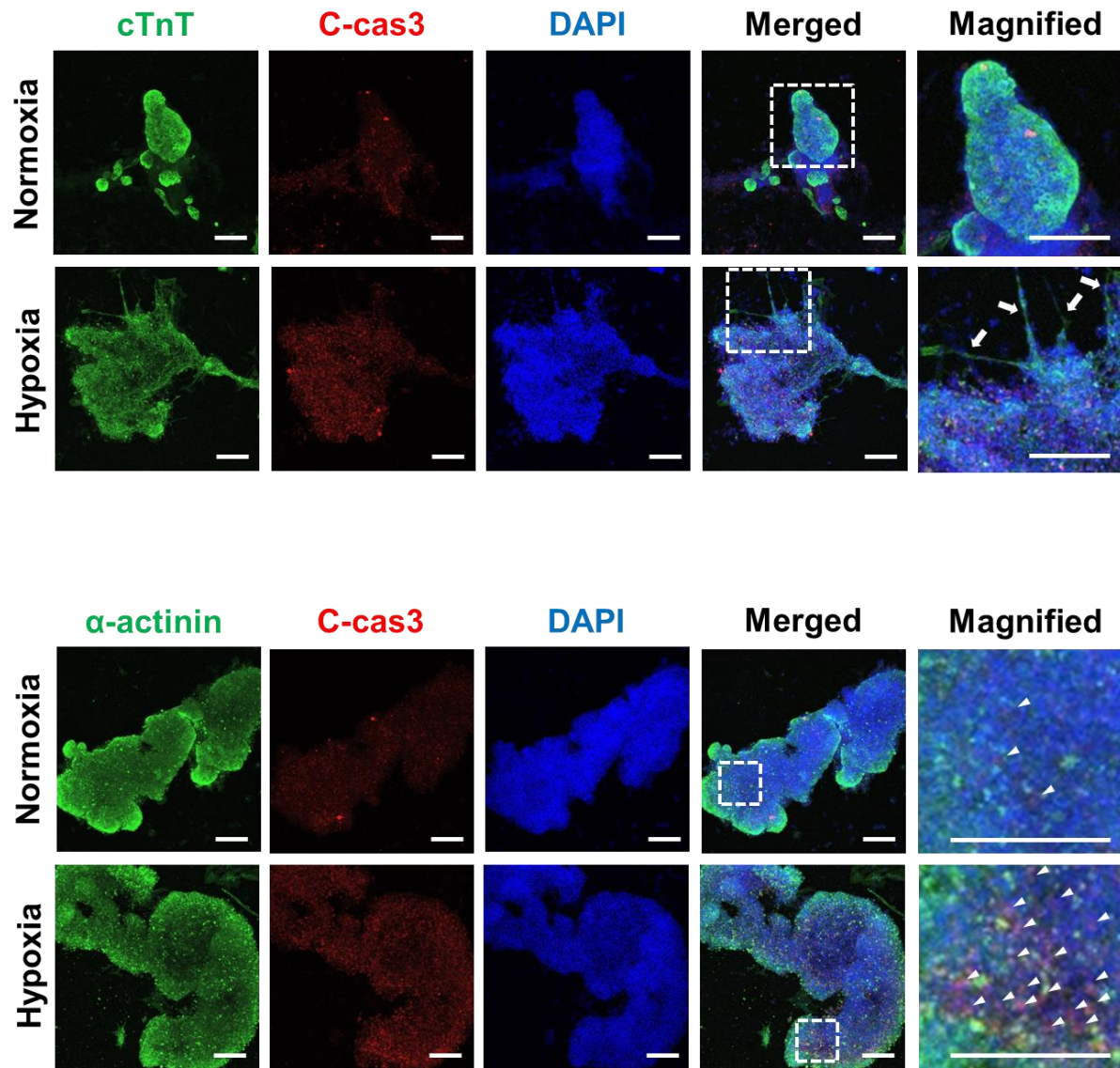


**Figure S11.** Expression of an apoptotic marker, cleaved caspase 3 (C-cas3), was increased in hypoxia-treated BG01 hESC-CMs. Immunofluorescence images of hESC-CMs treated with FGF4+AA at day 15. Primary antibody staining followed by cTnT (green) or  $\alpha$ -actinin (green) and C-cas3 (red). Nuclei were stained with DAPI (blue). White arrows indicate damage of cell membranes. White arrowheads indicate C-cas3-positive cells. Scale bar, 100  $\mu$ m.

## **Legends to Supplemental Videos**

**Video S1.** BG01 hESC-CMs (control) untreated between days 5 and 15 of differentiation in mCDM3 and RPMI/B27(-Insulin) media.

**Video S2.** BG01 hESC-CMs treated with FGF2 between days 5 and 15 of differentiation in mCDM3 and RPMI/B27(-Insulin) media. .

**Video S3.** BG01 hESC-CMs treated with FGF4 between days 5 and 15 of differentiation in mCDM3 and RPMI/B27(-Insulin) media.

**Video S4.** BG01 hESC-CMs treated with FGF10 between days 5 and 15 of differentiation in mCDM3 and RPMI/B27(-Insulin) media.

**Video S5.** BG01 hESC-CMs treated with AA between days 5 and 15 of differentiation in mCDM3 and RPMI/B27(-Insulin) media.

**Video S6.** BG01 hESC-CMs (control) untreated between days 5 and 15 of differentiation in mCDM3 and RPMI/B27(-Insulin) media.

**Video S7.** BG01 hESC-CMs treated with FGF4+AA between days 5 and 15 of differentiation in mCDM3 and RPMI/B27(-Insulin) media.