

# **SUPPLEMENTAL MATERIAL**

**Data S1.**

## **SUPPLEMENTAL METHODS**

### **Characterization of hiPSC-derived cardiomyocytes**

Cells were cultured in Matrigel-coated plates with mTeSR-1 culture medium (Stemcell Tech, USA) and passaged every 4-5 days. The protocol used to differentiate hiPSCs into CMs has been described previously<sup>1, 2</sup>. Briefly, undifferentiated hiPSCs were expanded on a Matrigel-coated dish for 4 days; then, differentiation was induced by culturing the cells with CHIR99021 in RPMI basal medium plus B27 without insulin (B27-). Twenty-four hours later, the cells were recovered and cultured with RPMI basal medium plus B27- for 2 days, in RPMI basal medium with B27- and the Wnt-signaling inhibitor IWR-1 for 48 hrs, and in RPMI/B27- alone for 48 hrs; then, 5  $\mu$ M insulin was added. Beating cardiomyocytes usually appeared on day 8 of the differentiation protocol, and hiPSC-CMs were characterized via immunostaining for the expression of cardiac troponin T (cTnT), Connexin 43, and MLC2v.

### **Characterization of hiPSC-derived endothelial cells**

hiPSCs were differentiated into ECs performed as previously described<sup>3, 4</sup>. Briefly, the undifferentiated cells were treated with a GSK-3 $\beta$  inhibitor and ascorbic acid to induce mesoderm differentiation. Five days later, cells that expressed CD34 (i.e., hiPSC-derived vascular progenitor cells [hiPSC-VPCs]) were collected via magnetic nanoparticle selection; then, the hiPSC-VPCs were differentiated into ECs by culturing them on fibronectin-coated flasks with EC-developmental medium (EGM2-MV; Lonza, Basel, Switzerland). ECs were characterized via the expression of CD31, CD144.

### **Telomerase activity**

Telomerase activity performed as previously described<sup>5</sup>. Briefly, Telomerase activity was measured with a TRAPEze Telomerase Detection Kit (Cat# S7700, Millipore, Inc.); the lysis buffer and inactivated hiPSCs were used as negative controls. Briefly,  $1 \times 10^6$  cells were added to 200  $\mu$ L of 1 $\times$  Chaps cell lysis buffer, incubated at 4 $^{\circ}$ C for 30 minutes, and centrifuged at 14,000 rpm for 20 minutes; then, the supernatants were collected and stored at -80  $^{\circ}$ C. Protein concentrations were determined by the Bradford method and adjusted to 1  $\mu$ g/ $\mu$ L; then, 1  $\mu$ L of the protein sample was incubated at 30 $^{\circ}$ C with 49  $\mu$ L of Telomeric Repeat Amplification Protocol Reaction Mix (20 mM Tris-HCl, pH 8.3, 1.5 mM MgCl<sub>2</sub>, 63 mM KCl, 0.05% Tween-20, 1 mM EGTA, 0.05 mM dNTP, and diethylpyrocarbonate-treated water) for 30 minutes and heated at 95 $^{\circ}$ C for 5 minutes to inactivate the telomerase. The samples were amplified for 30 PCR cycles, and each cycle consisted of denaturation at 95 $^{\circ}$ C for 30 seconds, annealing at 59 $^{\circ}$ C for 30 seconds, and extension at 72 $^{\circ}$ C for 1 minute. The cycling period was followed by a single extension period at 72 $^{\circ}$ C for 7 minutes. The PCR products were separated on 12% non-denaturing polyacrylamide gels and stained with 1:10,000 GelRed nucleic acid gel staining reagent (Cat# 41008, Biotium Inc), and the signal was visualized with a Chemidoc imaging system (Cat# 17001402, Bio-Rad Laboratories Inc). Relative telomerase activity was calculated as the ratio of the intensity of the telomerase product ladder to that of the 36-base pair (bp) internal control band and was normalized to the

protein concentration in each sample. Quantification was performed with NIH Image-J1.36b software.

### **Western blotting**

Cells were lysed with RIPA buffer (Sigma) and protease inhibitors; then, proteins (10 µg) were separated via 4-20% SDS-PAGE and Western-blotted with the corresponding antibodies (Supplemental Table 1), Precast Protein Gels (Bio-Rad), and Trans-Blot Turbo Mini PVDF Transfer Packs (Bio-Rad) as described previously<sup>5</sup>. GAPDH protein levels were also determined to confirm equal loading.

### **Transmission electron microscopy (TEM)**

Spheroids were fixed with 2.5% (vol/vol) glutaraldehyde over night at 4 °C, followed with three washes with PBS (15 min each) and post-fixed with 1% osmium tetroxide for 3 h. The samples were washed in PBS, dehydrated in graded ethanol (50%, 70%, 90% and 100%) and then in propylene oxide for 10 min. Next, samples were embedded, sectioned at about 70-nm thickness and stained with lead citrate. Micrographs were captured using a Morgagni 268 transmission electron microscope (FEI Company, The Netherlands) at 80 kV.

### **Mutation analysis**

Multiple alignments of B2M and CIITA amino acid sequences from WT and KO were done by Clustal<sup>6</sup>. Pathogenic potential of identified indel mutations WT and KO hiPSC was predicted by different webserver such as ExPASy - Translate tool and ATGpr<sup>7</sup>.

### **Statistical Analysis**

All values are expressed as mean ± standard error of mean (SEM), and were tested for significance level of type I error ( $P < 0.05$ ) via the Student's t test or ANOVA for differences between the values.

**Table S1. Antibodies.**

<b>Antigen</b>	<b>Vendor</b>	<b>Catalog#</b>	<b>Type</b>	<b>WB</b>	<b>IF</b>	<b>FC</b>	<b>Stim</b>	<b>PS</b>
<b>GAPDH</b>	Cell Signaling Technology	3683S	Rabbit mAb	Yes				
<b>Anti-beta 2 Microglobulin</b>	Abcam	ab75853	Rabbit mAb	Yes	Yes			
<b>Anti-CIITA antibody</b>	Abcam	ab7541	Rabbit polyclonal	Yes				
					Yes			
<b>Human Cardiac Troponin T</b>	Abcam	ab91605	Rabbit mAb		Yes			
<b>NKX2.5</b>	Thermo Fisher	PA5-47322	Goat Polyclonal		Yes			
<b>Anti-Human Nuclear Antigen antibody</b>	Abcam	ab191181	Mouse mAb		Yes			
<b>Anti-CD31</b>	Abcam	ab28364	Rabbit polyclonal		Yes			
<b>Connexin 43</b>	Abcam	Ab11370	Rabbit polyclonal		Yes			
<b>MLC2-Ventricular</b>	Proteintech	10906-1-AP	Rabbit Polyclonal		Yes			

<b>Fitc-donkey anti-mouse</b>	Jackson ImmunoResearch Laboratory	715-095-150	Polyclonal		Yes			
<b>Cy™3 -donkey antirabbit</b>	Jackson ImmunoResearch Laboratory	711-165-152	Polyclonal		Yes			
<b>Cy5-donkey anti-mouse</b>	Jackson ImmunoResearch Laboratory	715-175-150	Polyclonal		Yes			
<b>CD28/49d</b>	BD Biosciences	346049					Yes	
<b>CD3</b>	BD Biosciences	346049				Yes		
<b>CD69</b>	BD Biosciences	346049				Yes		
<b>CD8</b>	Stemcell Technologies	187853						Yes
<b>CD4</b>	Stemcell Technologies	17852						Yes

WB, Western Blotting; IF, Immunofluorescence Microscopy<sup>2</sup>; FC, Flow Cytometry; Stim, T-cell Stimulation; PS, Positive Selection.

**Figure S1.** DNA sequence alignment of A) wild type B2M 4bp (B2M\_WT\_4bp) and Knockout B2M 4bp insertion (B2M\_KO\_4bp); B) wild type B2M 14bp (B2M\_WT\_14bp) and Knockout B2M 4bp deletion (B2M\_KO\_14bp). C) sgRNA 1 targeting B2M-KO sequence chromatogram and red arrows indicate 10 bp deletion . D) ATGpr used to predict which ATG is the initiation codon for) wild type B2M 10bp (B2M\_WT\_10bp) and Knockout B2M 10bp deletion (B2M\_KO\_10bp). \* aligned nucleotide bases.

**A**

```

B2M_WT      ATGTCTCGCTCCGTGG----CCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCTGGA
B2M_KO_4bp  ATGTCTCGCTCCGTGGCGCTCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCTGGA
*****

B2M_WT      GGCTATCCAGCGTACTCCAAAGATTTCAGGTTTACTCACGTCATCCAGCAGAGAATGGAAA
B2M_KO_4bp  GGCTATCCAGCGTACTCCAAAGATTTCAGGTTTACTCACGTCATCCAGCAGAGAATGGAAA
*****

B2M_WT      GTCAAATTTCTGAATTGCTATGTGTCTGGGTTTTCATCCATCCGACATTGAAGTTGACTT
B2M_KO_4bp  GTCAAATTTCTGAATTGCTATGTGTCTGGGTTTTCATCCATCCGACATTGAAGTTGACTT
*****

B2M_WT      ACTGAAGAATGGAGAGAGAATTGAAAAAGTGGAGCATTTCAGACTTGTCTTTCAGCAAGGA
B2M_KO_4bp  ACTGAAGAATGGAGAGAGAATTGAAAAAGTGGAGCATTTCAGACTTGTCTTTCAGCAAGGA
*****

B2M_WT      CTGGTCTTTCTATCTCTTGTACTACACTGAATTCACCCCACTGAAAAAGATGAGTATGC
B2M_KO_4bp  CTGGTCTTTCTATCTCTTGTACTACACTGAATTCACCCCACTGAAAAAGATGAGTATGC
*****

B2M_WT      CTGCCGTGTGAACCATGTGACTTTGTACAGCCCAAGATAGTTAAGTGGGATCGAGACAT
B2M_KO_4bp  CTGCCGTGTGAACCATGTGACTTTGTACAGCCCAAGATAGTTAAGTGGGATCGAGACAT
*****

B2M_WT      GTAA
B2M_KO_4bp  GTAA
*****

```

**B**

```
B2M_WT_14bp      GGCATTCCCTGAAGCTGACAGCATTCCGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTG
B2M_KO_14bp      GGCATTCCCTGAAGCTGACAGCATTCCGGGCCGAGATGTC-----TAGCTGTG
*****

B2M_WT_14bp      CTCGCGCTACTCTCTCTTTCTGGCCTGGAGGCTATCCAGCGTACTCCAAAGATTCAGGTT
B2M_KO_14bp      CTCGCGCTACTCTCTCTTTCTGGCCTGGAGGCTATCCAGCGTACTCCAAAGATTCAGGTT
*****

B2M_WT_14bp      TACTCACGTCATCCAGCAGAGAATGGAAAGTCAAATTTCTGAATTGCTATGTGTCTGGG
B2M_KO_14bp      TACTCACGTCATCCAGCAGAGAATGGAAAGTCAAATTTCTGAATTGCTATGTGTCTGGG
*****

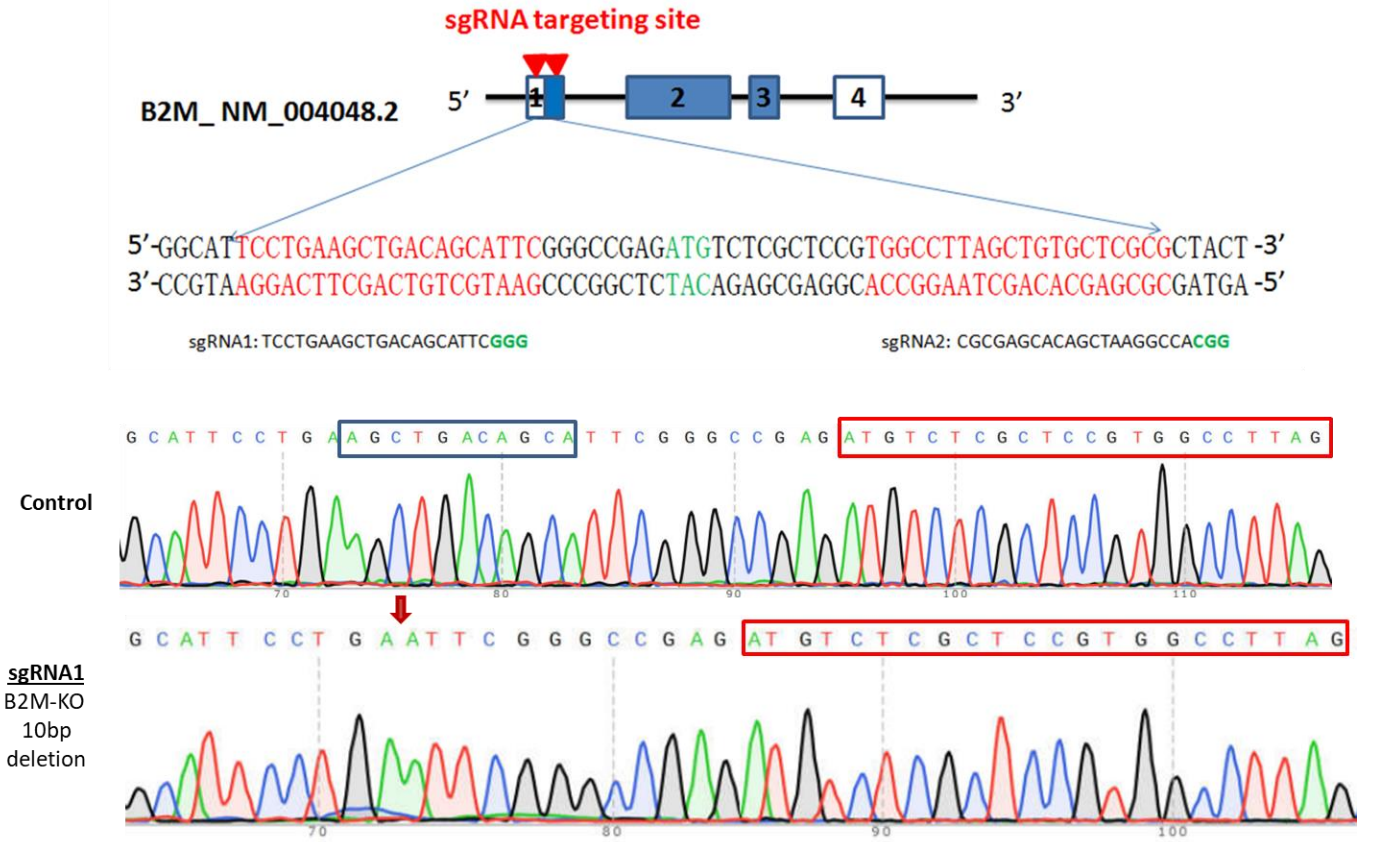
B2M_WT_14bp      TTTTCATCCATCCGACATTGAAGTTGACTTACTGAAGAATGGAGAGAGAATTGAAAAAGTG
B2M_KO_14bp      TTTTCATCCATCCGACATTGAAGTTGACTTACTGAAGAATGGAGAGAGAATTGAAAAAGTG
*****

B2M_WT_14bp      GAGCATTTCAGACTTGTCTTTCAGCAAGGACTGGTCTTTCTATCTCTTGTACTACACTGAA
B2M_KO_14bp      GAGCATTTCAGACTTGTCTTTCAGCAAGGACTGGTCTTTCTATCTCTTGTACTACACTGAA
*****

B2M_WT_14bp      TTCACCCCACTGAAAAAGATGAGTATGCCTGCCGTGTGAACCATGTGACTTTGTCACAG
B2M_KO_14bp      TTCACCCCACTGAAAAAGATGAGTATGCCTGCCGTGTGAACCATGTGACTTTGTCACAG
*****

B2M_WT_14bp      CCCAAGATAGTTAAGTGGGATCGAGACATGTAA
B2M_KO_14bp      CCCAAGATAGTTAAGTGGGATCGAGACATGTAA
*****
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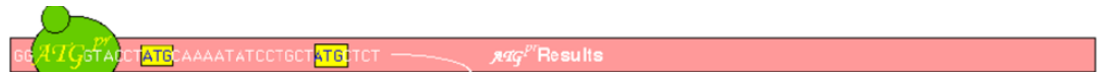
C





D

**i. sgRNA1**  
**B2M-WT**  
 10bp



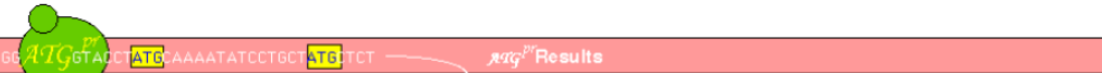
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1 GGCATTCTCT AAGCTGACAG CATTCTGGCC GAGATGTCT GCTCCGTGGC CTTAGCTGTG
61 CTCGCGTAC TCTCTCTTC TGGCTGGAG GCTATCCAGC GACTCCAAA GATTCAGGTT
121 TACTCACGTC ATCCAGCAGA GAATGGAAAG TCAAATTTCC TGAATTGCTA TGTGTCTGGG
181 TTTTCATCCAT CCGACATTGA AGTTGACTTA CTGAAGAATG GAGAGAGAAT TGAAAAAGTG
241 GAGCATTGAG ACTTGTCTTT CAGCAAGGAC TGGTCTTTCT ATCTCTTGTA CTACACTGAA
301 TTCACCCCA CTGAAAAAGA TGAGTATGCC TGCCGTGTGA ACCATGTGAC TTTGTCACAG
361 CCCAAGATAG TTAAGTGGGA TCGAGACATG TAA
  
```

No. of ATG from 5' end	Reliability	Frame	Identity to Kozak rule A/GXXATGG	Start (bp)	Finish (bp)	ORF Length (aa)	Stop codon found?	Sequence
1	0.47	1	GXXATgt	34	390	119	Yes	MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGF HPSDIEVDLLKNGERIEKVEHSDFSFKDWSFYLLYYTEFTPEKDEYAC RVNHVTLSPKIVKWRDM
2	0.04	2	AXXATGG	143	160	6	Yes	MESQIS
3	0.04	2	GXXATgt	170	211	14	Yes	MCLGFIHPTLKLTY
4	0.04	2	AXXATGG	218	337	40	Yes	MERELKKWSIQTCLARTGLSISCTTLNSPPLKKMSPAV
5	0.04	2	AXXATGa	320	337	6	Yes	MSMPAV

**ii. sgRNA1**  
**B2M-KO**  
 10bp  
 deletion



Query Sequence : QUERY

```

1 GGCATTCTCT AAGCTGACAG CATTCTGGCC GAGATGTCT GCTCCGTGGC CTTAGCTGTG
61 CTCGCGTAC TCTCTCTTC TGGCTGGAG GCTATCCAGC GACTCCAAA GATTCAGGTT
121 TACTCACGTC ATCCAGCAGA GAATGGAAAG TCAAATTTCC TGAATTGCTA TGTGTCTGGG
181 TTTTCATCCAT CCGACATTGA AGTTGACTTA CTGAAGAATG GAGAGAGAAT TGAAAAAGTG
241 GAGCATTGAG ACTTGTCTTT CAGCAAGGAC TGGTCTTTCT ATCTCTTGTA CTACACTGAA
301 TTCACCCCA CTGAAAAAGA TGAGTATGCC TGCCGTGTGA ACCATGTGAC TTTGTCACAG
361 CCCAAGATAG TTAAGTGGGA TCGAGACATG TAA
  
```

No. of ATG from 5' end	Reliability	Frame	Identity to Kozak rule A/GXXATGG	Start (bp)	Finish (bp)	ORF Length (aa)	Stop codon found?	Sequence
1	0.47	1	GXXATgt	34	390	119	Yes	MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGF HPSDIEVDLLKNGERIEKVEHSDFSFKDWSFYLLYYTEFTPEKDEYAC RVNHVTLSPKIVKWRDM
2	0.04	2	AXXATGG	143	160	6	Yes	MESQIS
3	0.04	2	GXXATgt	170	211	14	Yes	MCLGFIHPTLKLTY
4	0.04	2	AXXATGG	218	337	40	Yes	MERELKKWSIQTCLARTGLSISCTTLNSPPLKKMSPAV
5	0.04	2	AXXATGa	320	337	6	Yes	MSMPAV

**Figure S2.** DNA sequence alignment of A) wild type CIITA 1bp (CIITA\_WT\_1bp) and Knockout CIITA 1bp insertion (B2M\_KO\_1bp); Protein sequence alignment of B) wild type 3 different isoforms of CIITA of 1bp (CIITA\_WT\_1bp). C) CRISPR-Cas9 design of CIITA gene knockout sgRNA 2&3. D) DNA sequence alignment of wild type 3 different isoforms of CIITA of 1bp (CIITA\_WT\_1bp). \* aligned nucleotide and protein sequence.

**A**

```

CIITA_WT          ATGCGTT-GCCTGGCTCCACGCCCTGCTGGGTCTACCTGTCAGAGCCCCAAGGCAGCTC
CIITA_KO_1bp     ATGCGTTTGCCTGGCTCCACGCCCTGCTGGGTCTACCTGTCAGAGCCCCAAGGCAGCTC
*****

CIITA_WT          ACAGTGTGCCACCATGGAGTTGGGGCCCCCTAGAAGGTGGCTACCTGGAGCTTCTTAACAG
CIITA_KO_1bp     ACAGTGTGCCACCATGGAGTTGGGGCCCCCTAGAAGGTGGCTACCTGGAGCTTCTTAACAG
*****

CIITA_WT          CGATGCTGACCCCTGTGCCTCTACCCTTCTATGACCAGATGGACCTGGCTGGAGAAGA
CIITA_KO_1bp     CGATGCTGACCCCTGTGCCTCTACCCTTCTATGACCAGATGGACCTGGCTGGAGAAGA
*****

CIITA_WT          AGAGATTGAGCTCTACTCAGAACCCGACACAGACACCATCAACTGCGACCAGTTCAGCAG
CIITA_KO_1bp     AGAGATTGAGCTCTACTCAGAACCCGACACAGACACCATCAACTGCGACCAGTTCAGCAG
*****

CIITA_WT          GCTGTTGTGTGACATGGAAGGTGATGAAGAGACCAGGGAGGCTTATGCCAATATCGCGGA
CIITA_KO_1bp     GCTGTTGTGTGACATGGAAGGTGATGAAGAGACCAGGGAGGCTTATGCCAATATCGCGGA
*****

CIITA_WT          ACTGGACCAGTATGTCTTCCAGGACTCCCAGCTGGAGGGCCTGAGCAAGGACATTTTCAT
CIITA_KO_1bp     ACTGGACCAGTATGTCTTCCAGGACTCCCAGCTGGAGGGCCTGAGCAAGGACATTTTCAT
*****

CIITA_WT          AGAGCACATAGGACCAGATGAAGTGATCGGTGAGAGTATGGAGATGCCAGCAGAAGTTGG
CIITA_KO_1bp     AGAGCACATAGGACCAGATGAAGTGATCGGTGAGAGTATGGAGATGCCAGCAGAAGTTGG
*****

CIITA_WT          GCAGAAAAGTCAGAAAAGACCCTTCCCAGAGGAGCTTCCGGCAGACCTGAAGCACTGGAA
CIITA_KO_1bp     GCAGAAAAGTCAGAAAAGACCCTTCCCAGAGGAGCTTCCGGCAGACCTGAAGCACTGGAA
*****

CIITA_WT          GCCAGCTGAGCCCCCACTGTGGTGACTGGCAGTCTCCTAGTGGGACCAGTGAAGCGACTG
CIITA_KO_1bp     GCCAGCTGAGCCCCCACTGTGGTGACTGGCAGTCTCCTAGTGGGACCAGTGAAGCGACTG
*****

CIITA_WT          CTCCACCCTGCCTGCCTGCCACTGCCTGCGCTGTTCAACCAGGAGCCAGCCTCCGGCCA
CIITA_KO_1bp     CTCCACCCTGCCTGCCTGCCACTGCCTGCGCTGTTCAACCAGGAGCCAGCCTCCGGCCA
*****

CIITA_WT          GATGCGCCTGGAGAAAACCGACCAGATTTCCCATGCCTTTCTCCAGTTCCTCGTTGAGCTG
CIITA_KO_1bp     GATGCGCCTGGAGAAAACCGACCAGATTTCCCATGCCTTTCTCCAGTTCCTCGTTGAGCTG
*****

CIITA_WT          CCTGAATCTCCCTGAGGGACCCATCCAGTTTGTCCCCACCATCTCCACTCTGCCCCATGG
CIITA_KO_1bp     CCTGAATCTCCCTGAGGGACCCATCCAGTTTGTCCCCACCATCTCCACTCTGCCCCATGG
*****

CIITA_WT          GCTCTGGCAAATCTCTGAGGCTGGAACAGGGGTCTCCAGTATATTCATCTACCATGGTGA
CIITA_KO_1bp     GCTCTGGCAAATCTCTGAGGCTGGAACAGGGGTCTCCAGTATATTCATCTACCATGGTGA
*****

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CIITA_WT	GGTGCCCCAGGCCAGCCAAGTACCCCCCTCCCAGTGGATTCACTGTCCACGGCCTCCCAAC
CIITA_KO_1bp	GGTGCCCCAGGCCAGCCAAGTACCCCCCTCCCAGTGGATTCACTGTCCACGGCCTCCCAAC *****
CIITA_WT	ATCTCCAGACCGGCCAGGCTCCACCAGCCCCCTTCGCTCCATCAGCCACTGACCTGCCCAG
CIITA_KO_1bp	ATCTCCAGACCGGCCAGGCTCCACCAGCCCCCTTCGCTCCATCAGCCACTGACCTGCCCAG *****
CIITA_WT	CATGCCTGAACCTGCCCTGACCTCCCCGAGCAAACATGACAGAGCACAAGACGTCCCCAC
CIITA_KO_1bp	CATGCCTGAACCTGCCCTGACCTCCCCGAGCAAACATGACAGAGCACAAGACGTCCCCAC *****
CIITA_WT	CCAATGCCCCGGCAGCTGGAGAGGTCTCCAACAAGCTTCCAAAATGGCCTGAGCCGGTGGA
CIITA_KO_1bp	CCAATGCCCCGGCAGCTGGAGAGGTCTCCAACAAGCTTCCAAAATGGCCTGAGCCGGTGGA *****
CIITA_WT	GCAGTTCTACCGCTCACTGCAGGACACGTATGGTGCCGAGCCCCGAGGCCCGGATGGCAT
CIITA_KO_1bp	GCAGTTCTACCGCTCACTGCAGGACACGTATGGTGCCGAGCCCCGAGGCCCGGATGGCAT *****
CIITA_WT	CCTAGTGGAGGTGGATCTGGTGCAGGCCAGGCTGGAGAGGAGCAGCAGCAAGAGCCTGGA
CIITA_KO_1bp	CCTAGTGGAGGTGGATCTGGTGCAGGCCAGGCTGGAGAGGAGCAGCAGCAAGAGCCTGGA *****
CIITA_WT	GCGGGAAC TGGCCACCCCGGACTGGGCAGAACGGCAGCTGGCCCAAGGAGGCCTGGCTGA
CIITA_KO_1bp	GCGGGAAC TGGCCACCCCGGACTGGGCAGAACGGCAGCTGGCCCAAGGAGGCCTGGCTGA *****
CIITA_WT	GGTGCTGTTGGCTGCCAAGGAGCACCGGCGGCCGCTGAGACACGAGTGATTGCTGTGCT
CIITA_KO_1bp	GGTGCTGTTGGCTGCCAAGGAGCACCGGCGGCCGCTGAGACACGAGTGATTGCTGTGCT *****
CIITA_WT	GGGCAAAGCTGGTCAAGGCAAGAGCTATTGGGCTGGGGCAGTGAGCCGGGCCTGGGCTTG
CIITA_KO_1bp	GGGCAAAGCTGGTCAAGGCAAGAGCTATTGGGCTGGGGCAGTGAGCCGGGCCTGGGCTTG *****
CIITA_WT	TGGCCGGCTTCCCCAGTACGACTTTGTCTTCTCTGTCCCCTGCCATTGCTTGAACCGTCC
CIITA_KO_1bp	TGGCCGGCTTCCCCAGTACGACTTTGTCTTCTCTGTCCCCTGCCATTGCTTGAACCGTCC *****
CIITA_WT	GGGGGATGCCTATGGCCTGCAGGATCTGCTCTTCTCCCTGGGCCCACAGCCACTCGTGGC
CIITA_KO_1bp	GGGGGATGCCTATGGCCTGCAGGATCTGCTCTTCTCCCTGGGCCCACAGCCACTCGTGGC *****
CIITA_WT	GGCCGATGAGGTTTTTCAGCCACATCTTGAAGAGACCTGACCGCGTTCGTCTCATCTAGA
CIITA_KO_1bp	GGCCGATGAGGTTTTTCAGCCACATCTTGAAGAGACCTGACCGCGTTCGTCTCATCTAGA *****
CIITA_WT	CGGCTTCGAGGAGCTGGAAGCGCAAGATGGCTTCTTGCACAGCACGTGCGGACCGGCACC
CIITA_KO_1bp	CGGCTTCGAGGAGCTGGAAGCGCAAGATGGCTTCTTGCACAGCACGTGCGGACCGGCACC *****
CIITA_WT	GGCGGAGCCCTGCTCCCTCCGGGGGCTGCTGGCCGGCCTTTTCCAGAAGAAGCTGCTCCG
CIITA_KO_1bp	GGCGGAGCCCTGCTCCCTCCGGGGGCTGCTGGCCGGCCTTTTCCAGAAGAAGCTGCTCCG *****
CIITA_WT	AGGTTGCACCCTCCTCCTCACAGCCCCGGCCCCGGGCGCCTGGTCCAGAGCCTGAGCAA
CIITA_KO_1bp	AGGTTGCACCCTCCTCCTCACAGCCCCGGCCCCGGGCGCCTGGTCCAGAGCCTGAGCAA *****

CIITA\_WT GGCCGACGCCCTATTTGAGCTGTCCGGCTTCTCCATGGAGCAGGCCAGGCATACGTGAT  
CIITA\_KO\_1bp GGCCGACGCCCTATTTGAGCTGTCCGGCTTCTCCATGGAGCAGGCCAGGCATACGTGAT  
\*\*\*\*\*

CIITA\_WT GCGCTACTTTGAGAGCTCAGGGATGACAGAGCACCAAGACAGAGCCCTGACGCTCCTCCG  
CIITA\_KO\_1bp GCGCTACTTTGAGAGCTCAGGGATGACAGAGCACCAAGACAGAGCCCTGACGCTCCTCCG  
\*\*\*\*\*

CIITA\_WT GGACCGGCCACTTCTTCTCAGTCACAGCCACAGCCCTACTTTGTGCCGGGCAGTGTGCCA  
CIITA\_KO\_1bp GGACCGGCCACTTCTTCTCAGTCACAGCCACAGCCCTACTTTGTGCCGGGCAGTGTGCCA  
\*\*\*\*\*

CIITA\_WT GCTCTCAGAGGCCCTGCTGGAGCTTGGGGAGGACGCCAAGCTGCCCTCCACGCTCACGGG  
CIITA\_KO\_1bp GCTCTCAGAGGCCCTGCTGGAGCTTGGGGAGGACGCCAAGCTGCCCTCCACGCTCACGGG  
\*\*\*\*\*

CIITA\_WT ACTCTATGTCGGCCTGCTGGGCCGTGCAGCCCTCGACAGCCCCCGGGGCCCTGGCAGA  
CIITA\_KO\_1bp ACTCTATGTCGGCCTGCTGGGCCGTGCAGCCCTCGACAGCCCCCGGGGCCCTGGCAGA  
\*\*\*\*\*

CIITA\_WT GCTGGCCAAGCTGGCCTGGGAGCTGGGCCGAGACATCAAAGTACCCTACAGGAGGACCA  
CIITA\_KO\_1bp GCTGGCCAAGCTGGCCTGGGAGCTGGGCCGAGACATCAAAGTACCCTACAGGAGGACCA  
\*\*\*\*\*

CIITA\_WT GTTCCCATCCGACAGCTGAGGACCTGGGCGATGGCCAAAGGCTTAGTCCAACACCCACC  
CIITA\_KO\_1bp GTTCCCATCCGACAGCTGAGGACCTGGGCGATGGCCAAAGGCTTAGTCCAACACCCACC  
\*\*\*\*\*

CIITA\_WT GCGGGCCGACAGTCCGAGCTGGCCTTCCCCAGCTTCTCTGCAATGCTTCTGGGGGC  
CIITA\_KO\_1bp GCGGGCCGACAGTCCGAGCTGGCCTTCCCCAGCTTCTCTGCAATGCTTCTGGGGGC  
\*\*\*\*\*

CIITA\_WT CCTGTGGCTGGCTCTGAGTGGCGAAATCAAGGACAAGGAGCTCCCGCAGTACCTAGCATT  
CIITA\_KO\_1bp CCTGTGGCTGGCTCTGAGTGGCGAAATCAAGGACAAGGAGCTCCCGCAGTACCTAGCATT  
\*\*\*\*\*

CIITA\_WT GACCCCAAGGAAGAAGAGGCCCTATGACAACCTGGCTGGAGGGCGTGCCACGCTTCTGGC  
CIITA\_KO\_1bp GACCCCAAGGAAGAAGAGGCCCTATGACAACCTGGCTGGAGGGCGTGCCACGCTTCTGGC  
\*\*\*\*\*

CIITA\_WT TGGGCTGATCTTCCAGCCTCCCGCCCGCTGCCTGGGAGCCCTACTCGGGCCATCGGCGGC  
CIITA\_KO\_1bp TGGGCTGATCTTCCAGCCTCCCGCCCGCTGCCTGGGAGCCCTACTCGGGCCATCGGCGGC  
\*\*\*\*\*

CIITA\_WT TGCCTCGGTGGACAGGAAGCAGAAGGTGCTTGCGAGGTACCTGAAGCGGCTGCAGCCGGG  
CIITA\_KO\_1bp TGCCTCGGTGGACAGGAAGCAGAAGGTGCTTGCGAGGTACCTGAAGCGGCTGCAGCCGGG  
\*\*\*\*\*

CIITA\_WT GACACTGCGGGCGGGCAGCTGCTGGAGCTGCTGCACTGCGCCACGAGGCCGAGGAGGC  
CIITA\_KO\_1bp GACACTGCGGGCGGGCAGCTGCTGGAGCTGCTGCACTGCGCCACGAGGCCGAGGAGGC  
\*\*\*\*\*

CIITA\_WT TGGAATTTGGCAGCACGTGGTACAGGAGCTCCCCGGCCGCTCTCTTTTCTGGGCACCCG  
CIITA\_KO\_1bp TGGAATTTGGCAGCACGTGGTACAGGAGCTCCCCGGCCGCTCTCTTTTCTGGGCACCCG  
\*\*\*\*\*

CIITA\_WT CCTCACGCCTCCTGATGCACATGTACTGGGCAAGGCCTTGGAGGGCGGGCCAAAGACTT  
CIITA\_KO\_1bp CCTCACGCCTCCTGATGCACATGTACTGGGCAAGGCCTTGGAGGGCGGGCCAAAGACTT  
\*\*\*\*\*

CIITA\_WT CTCCCTGGACCTCCGCAGCACTGGCATTGCCCCCTCTGGATTGGGGAGCCTCGTGGGACT  
CIITA\_KO\_1bp CTCCCTGGACCTCCGCAGCACTGGCATTGCCCCCTCTGGATTGGGGAGCCTCGTGGGACT  
\*\*\*\*\*

CIITA\_WT CAGCTGTGTACCCGTTTCAGGGCTGCCTTGAGCGACACGGTGGCGCTGTGGGAGTCCCT  
CIITA\_KO\_1bp CAGCTGTGTACCCGTTTCAGGGCTGCCTTGAGCGACACGGTGGCGCTGTGGGAGTCCCT  
\*\*\*\*\*

CIITA\_WT GCAGCAGCATGGGGAGACCAAGCTACTTCAGGCAGCAGAGGAGAAGTTCACCATCGAGCC  
CIITA\_KO\_1bp GCAGCAGCATGGGGAGACCAAGCTACTTCAGGCAGCAGAGGAGAAGTTCACCATCGAGCC  
\*\*\*\*\*

CIITA\_WT TTTCAAAGCCAAGTCCCTGAAGGATGTGGAAGACCTGGGAAAGCTTGTGCAGACTCAGAG  
CIITA\_KO\_1bp TTTCAAAGCCAAGTCCCTGAAGGATGTGGAAGACCTGGGAAAGCTTGTGCAGACTCAGAG  
\*\*\*\*\*

CIITA\_WT GACGAGAAGTTCCTCGGAAGACACAGCTGGGGAGCTCCCTGCTGTTCCGGGACCTAAAGAA  
CIITA\_KO\_1bp GACGAGAAGTTCCTCGGAAGACACAGCTGGGGAGCTCCCTGCTGTTCCGGGACCTAAAGAA  
\*\*\*\*\*

CIITA\_WT ACTGGAGTTTGCCTGGGCCCTGTCTCAGGCCCCAGGCTTTCCCAAACCTGGTGC GGAT  
CIITA\_KO\_1bp ACTGGAGTTTGCCTGGGCCCTGTCTCAGGCCCCAGGCTTTCCCAAACCTGGTGC GGAT  
\*\*\*\*\*

CIITA\_WT CCTCACGGCCTTTTCCCTCCCTGCAGCATCTGGACCTGGATGCGCTGAGTGAGAACAAGAT  
CIITA\_KO\_1bp CCTCACGGCCTTTTCCCTCCCTGCAGCATCTGGACCTGGATGCGCTGAGTGAGAACAAGAT  
\*\*\*\*\*

CIITA\_WT CGGGGACGAGGGTGTCTCGCAGCTCTCAGCCACCTTCCCCCAGCTGAAGTCCTTGAAAC  
CIITA\_KO\_1bp CGGGGACGAGGGTGTCTCGCAGCTCTCAGCCACCTTCCCCCAGCTGAAGTCCTTGAAAC  
\*\*\*\*\*

CIITA\_WT CCTCAATCTGTCCAGAACAACATCACTGACCTGGGTGCCTACAAACTCGCCGAGGCCCT  
CIITA\_KO\_1bp CCTCAATCTGTCCAGAACAACATCACTGACCTGGGTGCCTACAAACTCGCCGAGGCCCT  
\*\*\*\*\*

CIITA\_WT GCCTTCGCTCGCTGCATCCCTGCTCAGGCTAAGCTTGTACAATAACTGCATCTGCGACGT  
CIITA\_KO\_1bp GCCTTCGCTCGCTGCATCCCTGCTCAGGCTAAGCTTGTACAATAACTGCATCTGCGACGT  
\*\*\*\*\*

CIITA\_WT GGGAGCCGAGAGCTTGGCTCGTGTGCTTCCGGACATGGTGTCCCTCCGGGTGATGGACGT  
CIITA\_KO\_1bp GGGAGCCGAGAGCTTGGCTCGTGTGCTTCCGGACATGGTGTCCCTCCGGGTGATGGACGT  
\*\*\*\*\*

CIITA\_WT CCAGTACAACAAGTTCACGGCTGCCGGGGCCCAGCAGCTCGCTGCCAGCCTTCGGAGGTG  
CIITA\_KO\_1bp CCAGTACAACAAGTTCACGGCTGCCGGGGCCCAGCAGCTCGCTGCCAGCCTTCGGAGGTG  
\*\*\*\*\*

CIITA\_WT TCCTCATGTGGAGACGCTGGCGATGTGGACGCCACCATCCCATTCAGTGTCCAGGAACA  
CIITA\_KO\_1bp TCCTCATGTGGAGACGCTGGCGATGTGGACGCCACCATCCCATTCAGTGTCCAGGAACA  
\*\*\*\*\*

CIITA\_WT CCTGCAACAACAGGATTCACGGATCAGCCTGAGATGA  
CIITA\_KO\_1bp CCTGCAACAACAGGATTCACGGATCAGCCTGAGATGA  
\*\*\*\*\*

**B**

CIITA\_Iso1 MRCLAPRPAGSYLSEPOGSSQCATMELGPLEGGYLELLNSDADPLCLYHFYDQMDLAGEE  
CIITA\_Iso2 MRCLAPRPAGSYLSEPOGSSQCATMELGPLEGGYLELLNSDADPLCLYHFYDQMDLAGEE  
CIITA\_Iso3 MRCLAPRPAGSYLSEPOGSSQCATMELGPLEGGYLELLNSDADPLCLYHFYDQMDLAGEE  
\*\*\*\*\*

CIITA\_Iso1 EIELYSEPDTDTINCDQFSRLLCDMEGDEETREAYANIAELDQYVFQDSQLEGLSKDIFI  
CIITA\_Iso2 EIELYSEPDTDTINCDQFSRLLCDMEGDEETREAYANIAELDQYVFQDSQLEGLSKDIFK  
CIITA\_Iso3 EIELYSEPDTDTINCDQFSRLLCDMEGDEETREAYANIAELDQYVFQDSQLEGLSKDIFK  
\*\*\*\*\*

CIITA\_Iso1 EHIGPDEVIGESMEMPAEVLGQKSQKRPFPPEELPADLKHWPAPPTVVTGSLVGPVSDC  
CIITA\_Iso2 HIG-PDEVIGESMEMPAEVLGQKSQKRPFPPEELPADLKHWPAPPTVVTGSLVGPVSDC  
CIITA\_Iso3 HIG-PDEVIGESMEMPAEVLGQKSQKRPFPPEELPADLKHWP-----  
. \*\*\*\*\*

CIITA\_Iso1 STLPLCLPLPALFNQEPASGQMRLEKTDQIPMPFSSSSLSCLNLPEGPIQFVPTISTLPHG  
CIITA\_Iso2 STLPLCLPLPALFNQEPASGQMRLEKTDQIPMPFSSSSLSCLNLPEGPIQFVPTISTLPHG  
CIITA\_Iso3 -----PVPFSSSSLSCLNLPEGPIQFVPTISTLPHG  
\* : \*\*\*\*\*

CIITA\_Iso1 LWQISEAGTGVSSIFIYHGEVPPQASQVPPPSGFTVHGLPTSPDRPGSTSPFAPSATDLPS  
CIITA\_Iso2 LWQISEAGTGVSSIFIYHGEVPPQASQVPPPSGFTVHGLPTSPDRPGSTSPFAPSATDLPS  
CIITA\_Iso3 LWQISEAGTGVSSIFIYHGEVPPQASQVPPPSGFTVHGLPTSPDRPGSTSPFAPSATDLPS  
\*\*\*\*\*

CIITA\_Iso1 MPEPALTSRANMTEHKTSPTQCPAAGEVSNKLPKWPEPVEQFYRSLQDTYGAEPAGPDGI  
CIITA\_Iso2 MPEPALTSRANMTEHKTSPTQCPAAGEVSNKLPKWPEPVEQFYRSLQDTYGAEPAGPDGI  
CIITA\_Iso3 MPEPALTSRANMTEHKTSPTQCPAAGEVSNKLPKWPG-----  
\*\*\*\*\*

CIITA\_Iso1 LVEVDLVQARLERSSSKSLERELATPDWAERQLAQGGLAEVLLAAKEHRRPRETRVIAVL  
CIITA\_Iso2 LVEVDLVQARLERSSSKSLERELATPDWAERQLAQGGLAEVLLAAKEHRRPRETRVIAVL  
CIITA\_Iso3 -----

CIITA\_Iso1 GKAGQGKSYWAGAVSRAWACGRLPQYDFVFSVPCHCLNRPDAYGLQDLLFSLGPQPLVA  
CIITA\_Iso2 GKAGQGKSYWAGAVSRAWACGRLPQYDFVFSVPCHCLNRPDAYGLQDLLFSLGPQPLVA  
CIITA\_Iso3 -----

CIITA\_Iso1 ADEVFSHILKRPDRVLLILDGFEELEAQDGFLHSTCGPAPAEPCLRGLLAGLFQKKLLR  
CIITA\_Iso2 ADEVFSHILKRPDRVLLILDGFEELEAQDGFLHSTCGPAPAEPCLRGLLAGLFQKKLLR  
CIITA\_Iso3 -----

CIITA\_Iso1 GCTLLLTARPRGRLVQSLSKADALFELSGFSMEQAQAYVMRYFESSGMTEHQDRALTLLR  
CIITA\_Iso2 GCTLLLTARPRGRLVQSLSKADALFELSGFSMEQAQAYVMRYFESSGMTEHQDRALTLLR  
CIITA\_Iso3 -----

CIITA\_Iso1 DRPLLLSHSHSPTLCRAVCQLSEALLELGEDAKLPSTLTGLYVGLLGRAALDSPPGALAE  
CIITA\_Iso2 DRPLLLSHSHSPTLCRAVCQLSEALLELGEDAKLPSTLTGLYVGLLGRAALDSPPGALAE  
CIITA\_Iso3 -----

CIITA\_Iso1 LAKLAWELGRRHQSTLQEDQFPSADVRTWAMAKGLVQHPPRAAESELAFFSFLQCFGLGA  
CIITA\_Iso2 LAKLAWELGRRHQSTLQEDQFPSADVRTWAMAKGLVQHPPRAAESELAFFSFLQCFGLGA  
CIITA\_Iso3 -----

CIITA\_Iso1 LWLALSGEIKDKELPQYLALTPRKKRPYDNWLEGVPRFLAGLIFQPPARCLGALLGPSAA  
CIITA\_Iso2 LWLALSGEIKDKELPQYLALTPRKKRPYDNWLEGVPRFLAGLIFQPPARCLGALLGPSAA  
CIITA\_Iso3 -----

CIITA\_Iso1 ASVDRKQKVLARYLKRLQPGTLRARQLLELLHCAHEAEEAGIWQHVVQELPGRLSFLGTR  
CIITA\_Iso2 ASVDRKQKVLARYLKRLQPGTLRARQLLELLHCAHEAEEAGIWQHVVQELPGRLSFLGTR  
CIITA\_Iso3 -----

CIITA\_Iso1 LTPPDAHVLGKALEAAGQDFSLDLRSTGICPSGLGSLVGLSCVTRFRAALSDTVALWESL  
CIITA\_Iso2 LTPPDAHVLGKALEAAGQDFSLDLRSTGICPSGLGSLVGLSCVTRFRAALSDTVALWESL  
CIITA\_Iso3 -----LAWSPCLGLRPSLHRAALSDTVALWESL  
\* : .\*\*\*\*\*

CIITA\_Iso1 QQHGETKLLQAAEEKFTIEPFKAKSLKDVEDLGKLVQTQRTRSSSEDTAGELPAVRDLKK  
CIITA\_Iso2 QQHGETKLLQAAEEKFTIEPFKAKSLKDVEDLGKLVQTQRTRSSSEDTAGELPAVRDLKK  
CIITA\_Iso3 QQHGETKLLQAAEEKFTIEPFKAKSLKDVEDLGKLVQTQRTRSSSEDTAGELPAVRDLKK  
\*\*\*\*\*

CIITA\_Iso1 LEFALGPVSGPQAFPKLVRIILTAFFSSLQHLDLDALESENKIGDEGVSQLSATFPQLKSLET  
CIITA\_Iso2 LEFALGPVSGPQAFPKLVRIILTAFFSSLQHLDLDALESENKIGDEGVSQLSATFPQLKSLET  
CIITA\_Iso3 LEFALGPVSGPQAFPKLVRIILTAFFSSLQHLDLDALESENKIGDEGVSQLSATFPQLKSLET  
\*\*\*\*\*

CIITA\_Iso1 LNLSQNNITDLGAYKLAEALPSLAASLLRSLYNNCICDVGAESLARVLPDMVSLRVMDV  
CIITA\_Iso2 LNLSQNNITDLGAYKLAEALPSLAASLLRSLYNNCICDVGAESLARVLPDMVSLRVMDV  
CIITA\_Iso3 LNLSQNNITDLGAYKLAEALPSLAASLLRSLYNNCICDVGAESLARVLPDMVSLRVMDV  
\*\*\*\*\*

CIITA\_Iso1 QYNKFTAAGAQQLAASLRRCPHVETLAMWTPTIPFSVQEHLOQDSTRISLR  
CIITA\_Iso2 QYNKFTAAGAQQLAASLRRCPHVETLAMWTPTIPFSVQEHLOQDSTRISLR  
CIITA\_Iso3 QYNKFTAAGAQQLAASLRRCPHVETLAMWTPTIPFSVQEHLOQDSTRISLR  
\*\*\*\*\*





\*\*\*\*\*

CIITA\_Iso1  
CIITA\_Iso3  
CIITA\_Iso2

CCAGGAACACCTGCAACAACAGGATTCACGGATCAGCCTGAGATGA  
CCAGGAACACCTGCAACAACAGGATTCACGGATCAGCCTGAGATGA  
CCAGGAACACCTGCAACAACAGGATTCACGGATCAGCCTGAGATGA  
\*\*\*\*\*

## **Supplemental Video Legends:**

**Video S1.** WT hiPSC differentiated cardiomyocytes derived spheroid synchronous contractile activity of individual spheroids in culture. Best viewed with Windows Media Player.

**Video S2.** KO hiPSC differentiated cardiomyocytes derived spheroid synchronous contractile activity of individual spheroids in culture. Best viewed with Windows Media Player.

**Video S3.** WT spheroid co-culture with PBMC for 5 days will result in the loss of spontaneous and synchronous contractile activity of individual spheroids in culture. Best viewed with Windows Media Player.

**Video S4.** KO spheroid co-culture with PBMC for 5 days will result in no change of spontaneous and synchronous contractile activity of individual spheroids in culture. Best viewed with Windows Media Player.

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