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Supplemental Information

Evaluation of *MYBPC3 trans*-Splicing and Gene Replacement as Therapeutic Options in Human iPSC-Derived Cardiomyocytes

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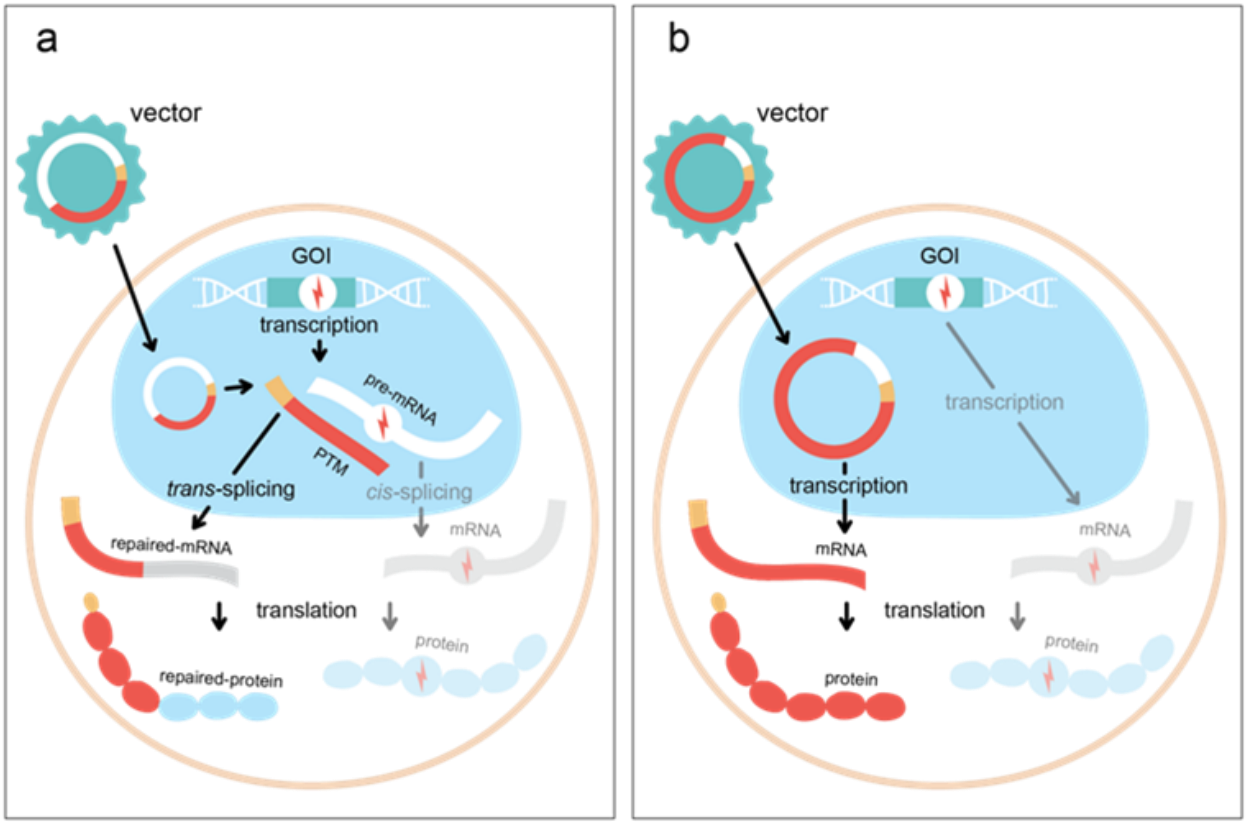

Figure S1: Schematic representation of two gene therapy approaches. (a) RNA *trans*-splicing. After virus-mediated delivery, pre-*trans*-splicing molecules (PTMs) are transcribed in the nucleus. PTMs target the pre-mRNA of the gene of interest (GOI) and produce via *trans*-splicing a repaired, chimeric mRNA, without mutation. Translation of the repaired mRNA leads to a corrected fully functional protein. This process is competing with *cis*-splicing, the classical splicing mechanism, by which endogenous mRNA and proteins are produced. **(b)** Gene replacement. The mutation in the GOI results in low level or the absence of corresponding protein. After virus-mediated delivery, a full-length wild-type cDNA of GOI is transcribed in the nucleus. The resulting mRNA is translated into functional protein that replaces the missing mutant endogenous protein. GOI: gene of interest; yellow tag: i.e. FLAG to discriminate exogenous molecules; red bolt: gene mutation.

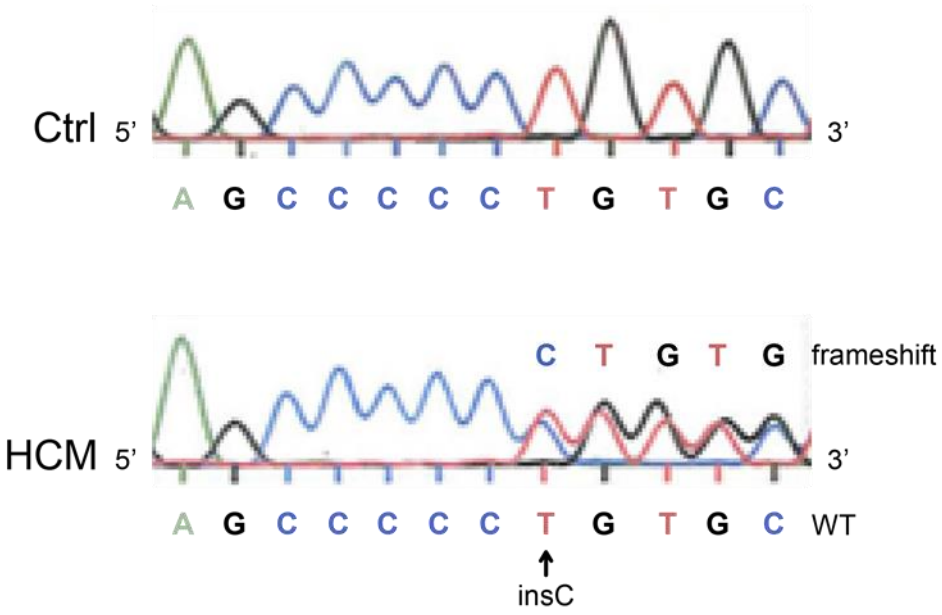

Figure S2: Validation of the *MYBPC3* mutation at the genomic level. PCR was performed on genomic DNA from blood cells from the HCM patient using intronic primers around *MYBPC3* exon 16 and compared to a human control sequence. Sanger sequencing confirmed the presence of an insertion of a C (c.1358_1359insC) at the heterozygous state in the HCM patient. Wild-type and frameshift sequences are also shown.

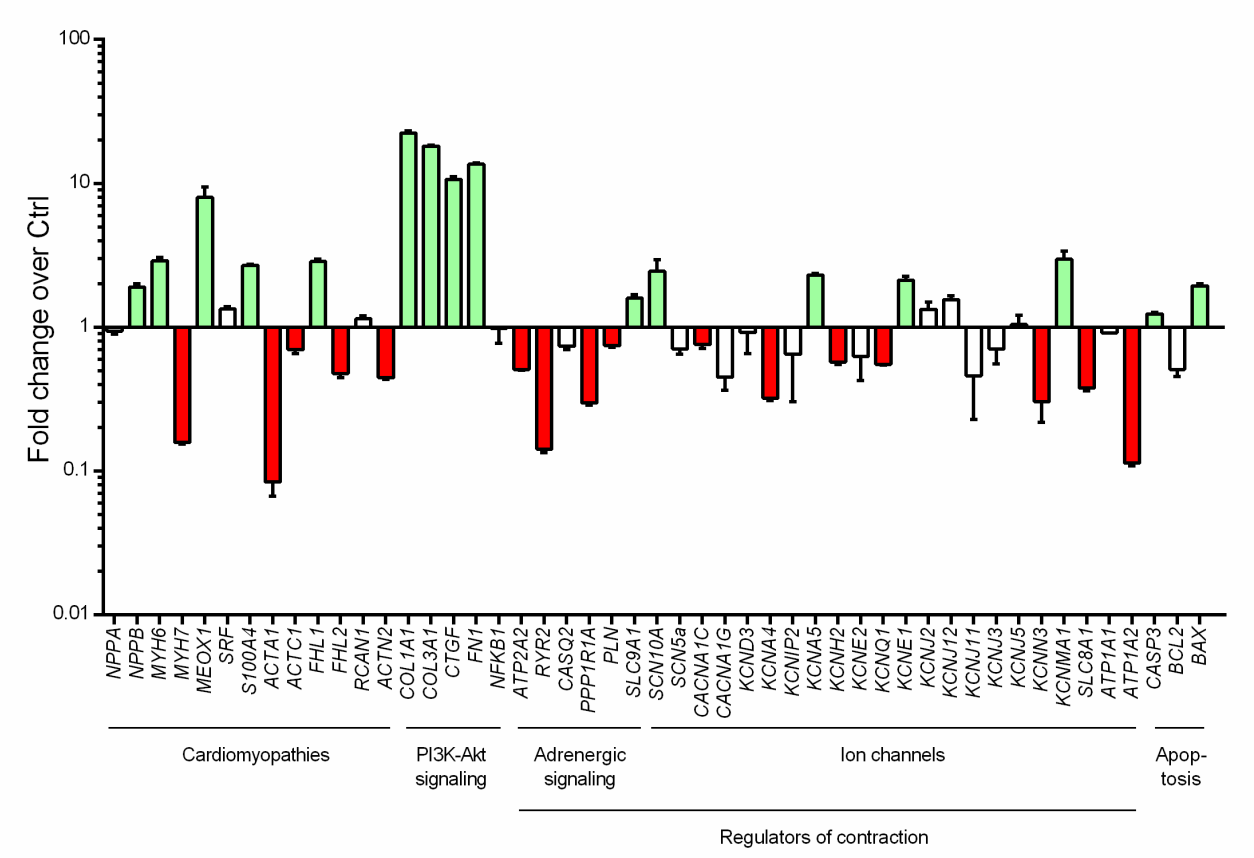

Figure S3: Gene expression analysis of Ctrl and HCM hiPSC-derived cardiomyocytes. Evaluation of mRNA levels determined by nCounter NanoString technology in Ctrl and HCM hiPSC-derived CM (n=3-6, with n=number of wells from one transduction experiment). Green bars are significantly upregulated and red bars are significantly downregulated. Data are expressed as mean ± SEM. $P<0.05$, unpaired Students *t*-test.

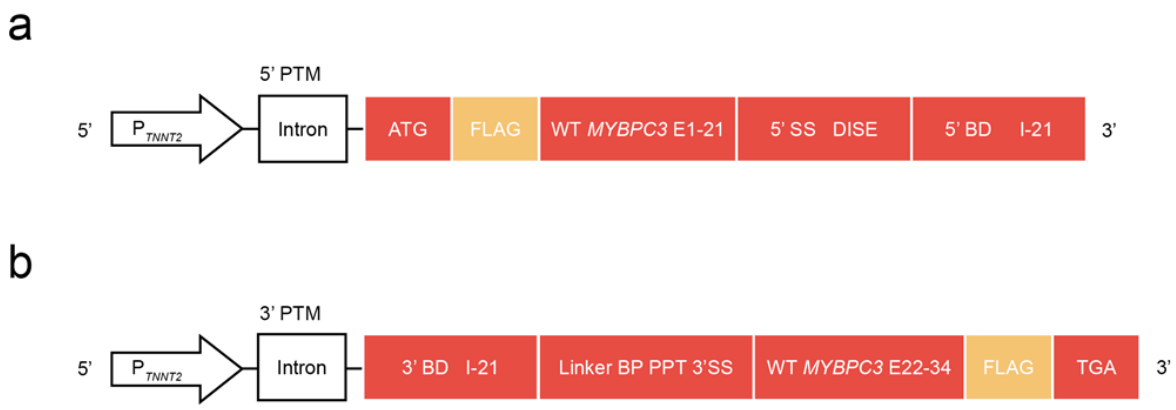

Figure S4: Schematic illustration of PTMs. (a) The 5'PTM plasmid carries the 5'-FLAG-tagged wild-type (WT) *MYBPC3* coding sequence of exons 1 to 21 under the control of the human cardiac troponin T promoter (*TNNT2*). The 5'PTM also included a chimeric intron, containing sequences from the human β-globin and immunoglobulin (IgG) genes, conserved splice donor site (5'SS) followed by an intronic region (DISE) from the rat fibroblast growth factor receptor 2 gene and 120 nucleotides for binding to *MYBPC3* intron 21 (5' BD I-21). **(b)** The 3'PTM plasmid carries wild-type (WT) *MYBPC3* coding sequence of exons 22 to 34 under the control of the human cardiac troponin T promoter (*TNNT2*). The sequence is FLAG-tagged at the 3' end before the stop codon (TGA). The 3'PTM includes the same intron as in (a) and the binding domain targets the same sequence in *MYBPC3* intron 21 as 5'PTM. In addition, conserved 3' splicing sequences such as branch point (BP) and polypyrimidine tract (PTT) are present. ATG, start codon; BP, branch point; DISE, downstream intronic splicing enhancer element; P_{TNNT2}, cardiac troponin T promoter; PPT, polypyrimidine tract; TGA, stop codon; 5´SS, 5´-splice site; 3´SS, 3´-splice site.

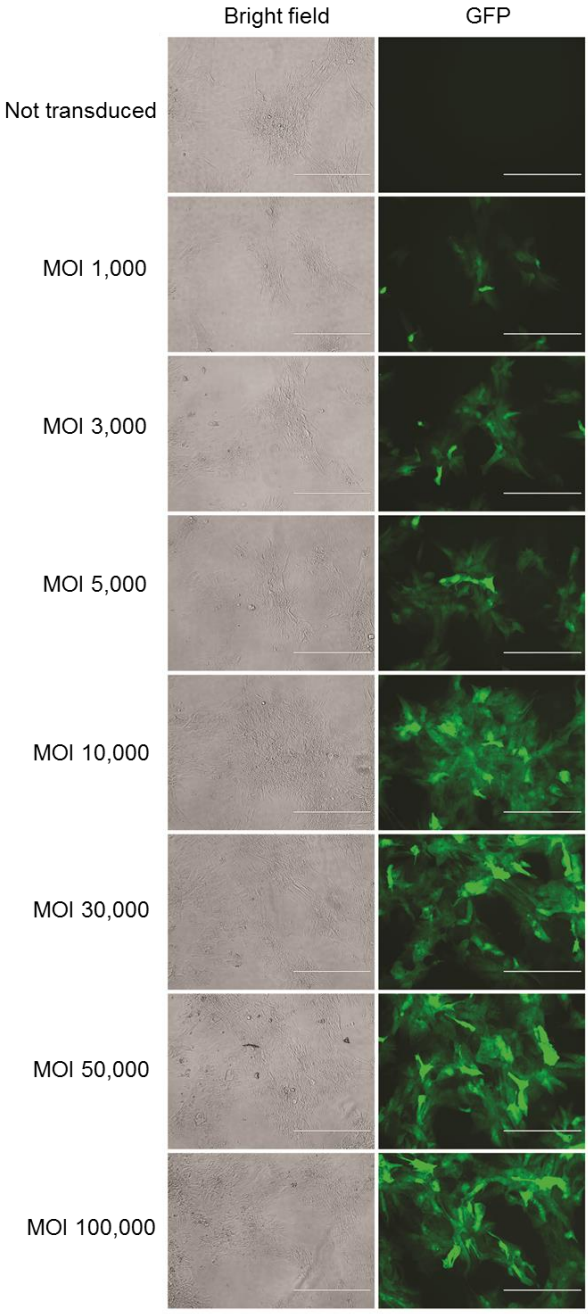

Figure S5: Efficiency of AAV-*TNNT2*-GFP-mediated transduction in control hiPSC-derived cardiomyocytes. hiPSC-CMs were transduced with MOIs of 1,000 up to 100,000 and cultured in 2D for seven days. GFP expression was evaluated by epifluorescence microscopy. Corresponding bright field images are also shown. Scale bars, 400 µm. GFP, green fluorescent protein; MOI, multiplicity of infection.

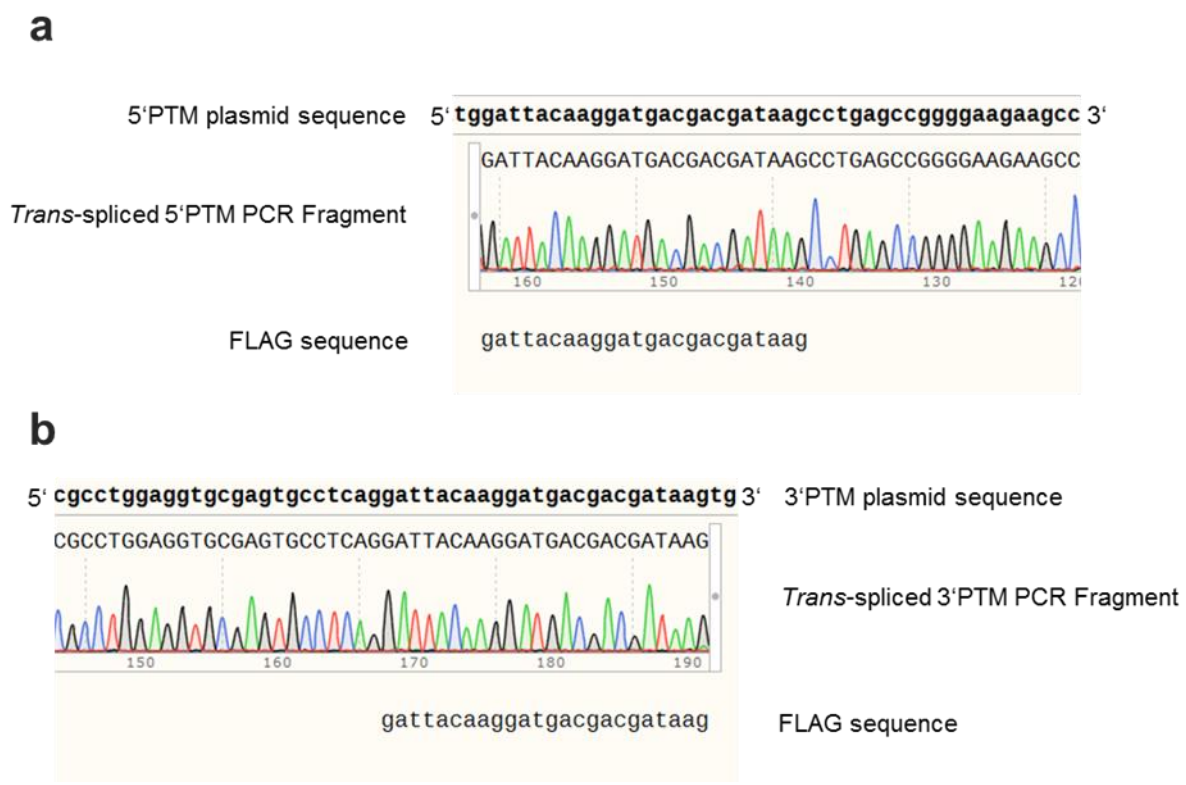

Figure S6: Validation of *trans*-spliced *MYBPC3* mRNA after 5’- and 3’-mode of *trans*-splicing. (a) Sequencing of the gel-extracted 2,180-bp fragment amplified by RT-PCR with primers FLAG-F/E23-R in the AAV-5’PTM-transduced hiPSC-CMs sample validated the presence of the FLAG sequence after alignment with the 5’PTM plasmid. **(b)** Sequencing of the gel-extracted 1,874-bp fragment amplified by RT-PCR with primers E21-F/FLAG-R in AAV-3’PTM-transduced hiPSC-CMs sample validated the presence of the FLAG sequence after alignment with the 3’PTM. Alignment was performed with SnapGene® software. E, exon; RT-PCR, reverse transcriptase PCR.

Supplemental Table S1. Acronyms and names of genes evaluated with the nanoString nCounter® Elements technology.

Acronym	Name	Accession number (NCBI)
ABCF1	ATP binding cassette subfamily F member 1	NM_001090.2
ACTA1	Actin, alpha, skeletal muscle	NM_001100.3
ACTC1	Actin, alpha, cardiac muscle 1	NM_005159.4
ACTN2	Alpha-Actinin 2	NM_001103.2
ATP1A1	Na/K-ATPase α1 subunit	NM_000702.3
ATP1A2	Na/K-ATPase α2 subunit	NM_001681.3
ATP2A2	ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 2	NM_001681.3
BAX	BCL2 associated X, apoptosis regulator	NM_138761.3
BCL2	BCL2, apoptosis regulator	NM_000657.2
CACNA1C	Calcium voltage-gated channel subunit alpha1 C (L-Type Ca2+ channel)	NM_199460.2
CACNA1G	Calcium voltage-gated channel subunit alpha1 G (T type Ca2+ channel)	NM_198397.1
CASP3	Caspase 3	NM_032991.2
CASQ2	Calsequestrin-2	NM_001232.3
CLTC	Clathrin heavy chain	NM_004859.2
COL1A1	Collagen type I alpha 1	NM_000088.3
COL3A1	Collagen type III alpha 1	NM_000090.3
CTGF	Connective tissue growth factor	NM_001901.2
FHL1	Four-and-a-half-LIM-domains 1	NM_001449.4
FHL2	Four-and-a-half-LIM-domains 2	NM_001039492.2
FN1	Fibronectin 1	NM_212482.1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046.3
KCNA4	Potassium voltage-gated channel subfamily A member 4 (Ito,s K+ channel)	NM_002233.3
KCNA5	Potassium voltage-gated channel subfamily A member 5 (IKUR)	NM_002234.2
KCND3	Potassium voltage-gated channel subfamily D member 3 (Ito, f beta subunit, Kv4.3)	NM_004980.4
KCNE1	Potassium voltage-gated channel subfamily E regulatory subunit 1 (MinK, Iks)	NM_001127670.1
KCNE2	Potassium voltage-gated channel subfamily E regulatory subunit 2 (MIRP2, Ikr)	NM_172201.1
KCNH2	Potassium voltage-gated channel subfamily H member 2 (Ikr K+ channel)	NM_172057.2
KCNIP2	Potassium voltage-gated channel interacting protein 2 (KChiP2 beta subunit, Ito)	NM_014591.4
KCNJ11	Potassium voltage-gated channel subfamily J member 11 (Kir 6.2, IkATP)	NM_000525.3
KCNJ12	Potassium voltage-gated channel subfamily J member 12 (Ik1 channel subunit 2)	NM_021012.4
KCNJ2	Potassium voltage-gated channel subfamily J member 2 (Ik1 channel)	NM_000891.2
KCNJ3	Potassium voltage-gated channel subfamily J member 3 (IKACH)	NM_001260508.1
KCNJ5	Potassium voltage-gated channel subfamily J member 5 (IKACH)	NM_000890.3
KCNMA1	Potassium calcium-activated channel subfamily M alpha 1 (BK Channel)	NM_001014797.2
KCNN3	Potassium calcium-activated channel subfamily N member 3 (SK3)	NM_002249.4
KCNQ1	Potassium voltage-gated channel subfamily Q member 1 (Iks K+ channel)	NM_181798.1
MEOX1	Mesenchyme homeobox 1	NM_001040002.1
MYH6	Myosin heavy chain 6	NM_002471.3
MYH7	Myosin heavy chain 7	NM_000257.2
NFKB1	Nuclear factor kappa B subunit 1	NM_003998.2
NPPA	Natriuretic peptide A	NM_006172.2
NPPB	Natriuretic peptide B	NM_002521.2
PGK1	Phosphoglycerate kinase 1	NM_000291.2
PLN	Phospholamban	NM_002667.3
PPP1R1A	Protein phosphatase 1, regulatory (inhibitor) subunit 1A (I-1)	NM_006741.3
RCAN1	Regulator of calcineurin 1	NM_004414.5
RYR2	Ryanodine receptor 2	NM_001035.2
S100A4	S100 calcium binding protein A4 (=FSP1)	NM_002961.2
SCN10A	Sodium voltage-gated channel alpha subunit 10 (Na+ channel, Nav1.8)	NM_006514.2
SCN5A	Sodium voltage-gated channel alpha subunit 5 (Na+ channel, Nav1.5)	NM_198056.2
SLC8A1	Solute carrier family 8 member A1 (Natrium-Calcium Exchanger, NCX)	NM_021097.1
SLC9A1	Solute carrier family 9 member A1 (Na+/H+ exchanger)	NM_003047.4
SRF	Serum response factor	NM_003131.3
TUBB	Tubulin beta class 1	NM_178014.3

Supplemental Table S2. Sequences of PCR primers

Primer	Sequence (5' to 3')
E1-F	GCCAGTCTCAGCTTTTAGCAA
E2-R	CAGGCCGTACTTGTTGCTG
I15-F	CTGGGACCTGAGGATGTGGG
I16-R	GGTGGGTGGGTGGCAAGTG
E15-F	CCAAGCGTACCCTGACCA
E16-R	CCCTCCTCCGATACTTCACA
E21-F	CCATTGTGGTTGTAGCTGGA
E23-R	CACACAGCAGCTTCTTGTCA
E33-F	CCCAAGATTTCCTGGTTCAAA
E33-R	CCTCGCCCTGTAAGTTGGT
FLAG-F	GGATTACAAGGATGACGACGA
FLAG-R	CTTATCGTCGTCATCCTTGTAATC
GAPDH-F	ATGTTCGTCATGGGTGTGAA
GAPDH-R	TGAGTCCTTCCACGATACCA
F3-1	TTCGACGCTAGCACCCACACTGCCCACCTT
R3-1	TGTTCCGCGGGGATCCTGTGTGGAACCAGCCAAG
F3-2	GTTCCACACAGGATCCCCGCGGAACATTATTATAAC
R3-2	CCTTATTCCCCTGTTTTCCGGAAA
F3-3	TTCCGGAAAACAGGGGAATAAGGC
R3-3	TTCGACGCGGCCGCTCACTTATCGTCGTCATCCTTGTAATCCTGAGGCACTCG
F5-1	TTCGACGCTAGCATGGATTACAAGGATGACGACGATAAGCCTGAGCCGGGGAAGA
R5-1	AGTGTGGGTGGATCCAGGCCAACCCATGGAAAGAAAGAGCTGTACTCACCTGCGTGATAGCCTTCTG
F5-2	GGGTTGGCCTGGATCCACCCACACTGCCCACCTT
R5-2	TTCGACGCGGCCGCTGTGTGGAACCAGCCAAG