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Supplemental Information

miR-25 Promotes Cardiomyocyte Proliferation by Targeting *FBXW7*

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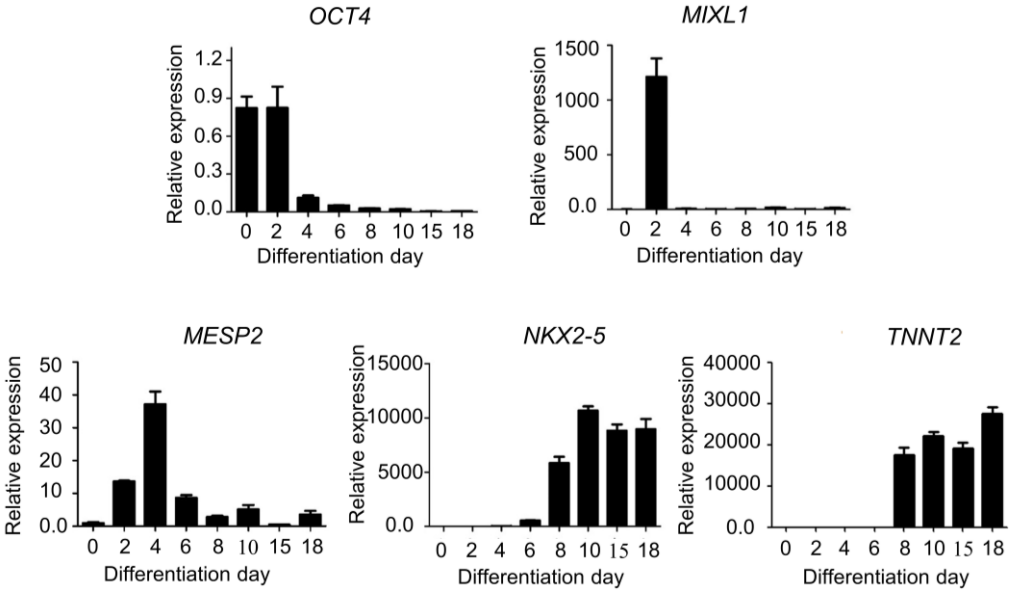
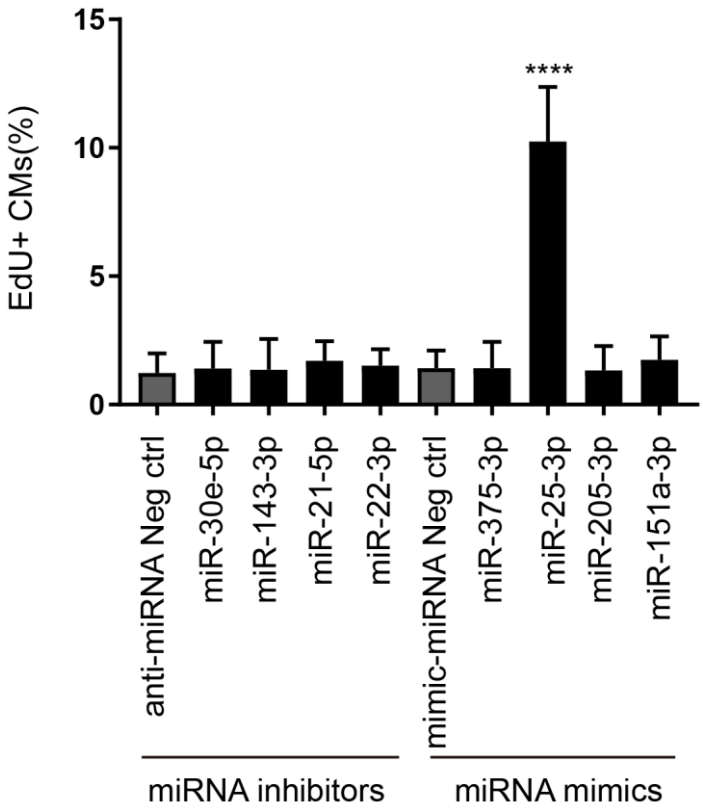
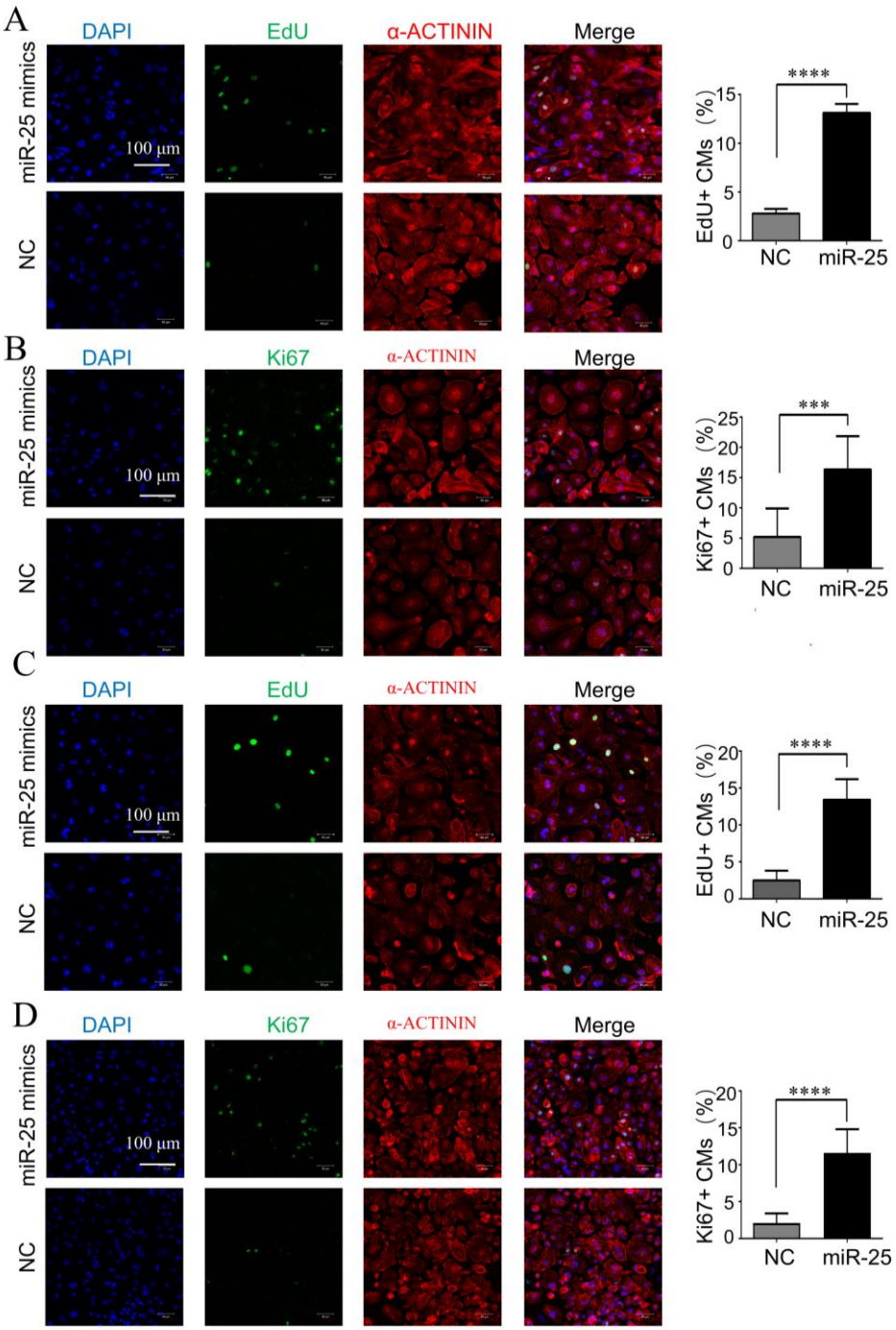


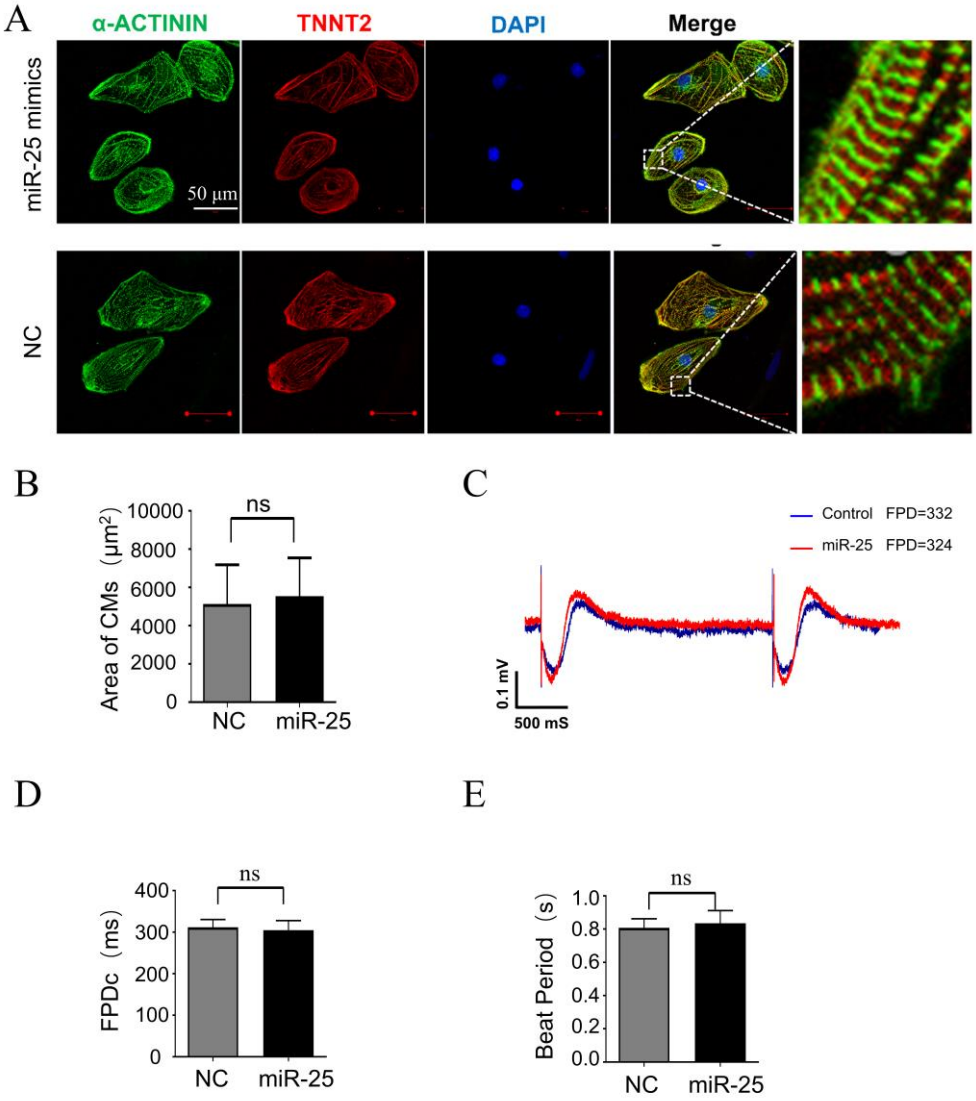
Supplemental Figure 1. Relative mRNA expression levels of marker genes for pluripotent stem cells (*OCT4*), mesendoderm (*MIXL1*), cardiac mesoderm (*MESP1*), cardiac progenitors (*NKX2-5*) and cardiomyocytes (*TNNT2*) were analyzed by qRT-PCR during CM differentiation (n=3, error bars show the mean ± SEM).



Supplemental Figure 2. Anti-miRNA and mimic-miRNA screen for hESC-CMs proliferation. The percentage of EdU+ and α-ACTININ+ cells were quantificated using ImageJ. (n=3 per group, error bars showed mean± SD).

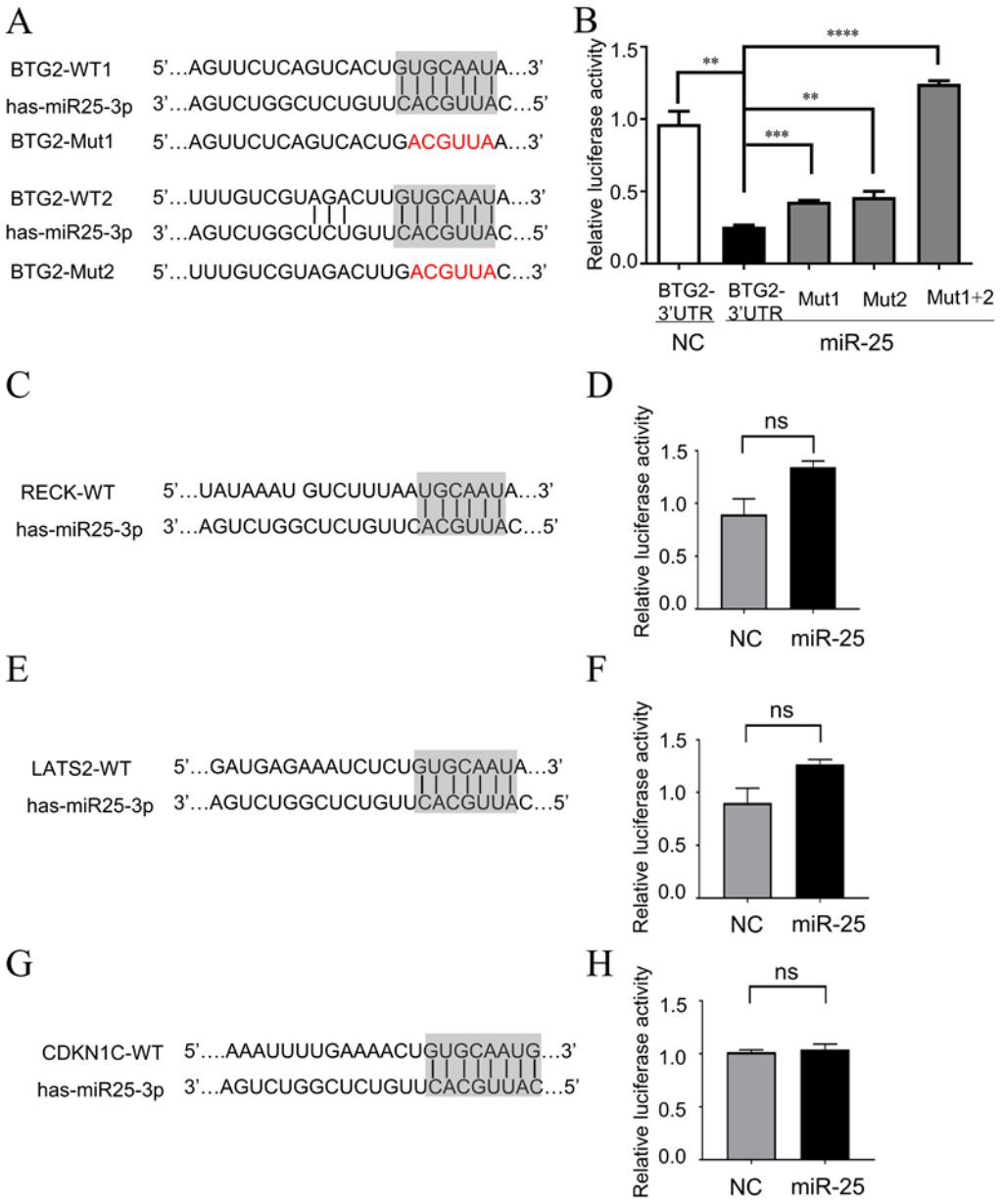


Supplemental Figure 3. miR-25 overexpression promotes the proliferation of CMs derived from two hiPSC lines. (A) EdU staining (green) revealed that miR-25 transfection increased the proliferation of CMs derived from hiPSC line 1. (B) Immunostaining of Ki-67 revealed that miR-25 transfection increased the proliferation of CMs derived from hiPSC line 1. (C) EdU staining (green) revealed that miR-25 transfection increased the proliferation of CMs derived from hiPSC line 2. (D) Immunostaining of Ki-67 revealed that miR-25 transfection increased the proliferation of CMs derived from hiPSC line 2. Nuclei were stained with DAPI (blue); CMs were stained with an antibody against α-ACTININ (red). At least 2000 cells were quantified in each group.

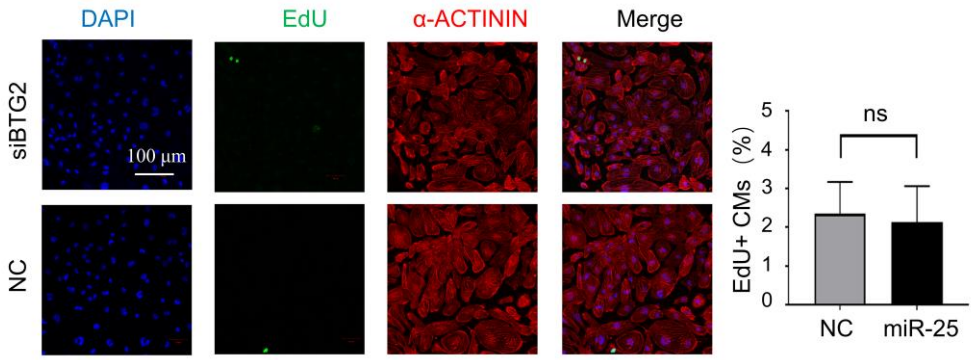


Supplemental Figure 4. Overexpression of miR-25 did not influence CM morphology or electrical activity. (A) Representative images of hESC-CMs transfected with miR-25 mimics or NC stained for α-ACTININ (green), TNNT2 (red) and DAPI (blue). (B) Quantification of cell size. Approximately 60 cells for each group were analyzed. (C) Representative traces of average FPD recordings using the MEA system. (D, E) The results of field potential duration (FPD, Fridericia corrected) and beat period analysis (n=3, error bars show the mean ± SEM).

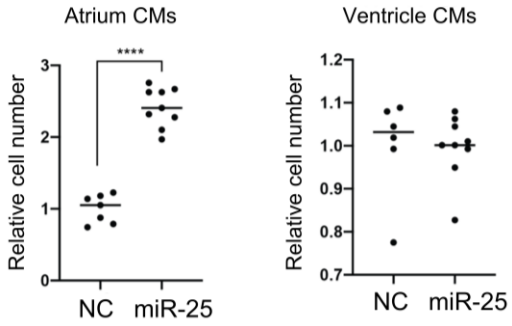
Supplemental Figure 5. RNA-seq analysis revealed that miR-25 overexpression influenced the expression of multiple genes in hESC-CMs. (A) KEGG classification enrichment analysis of the differentially expressed genes in CMs transfected with miR-25. (B) GO enrichment analysis of the differentially expressed genes in CMs transfected with miR-25.



Supplemental Figure 6. The potential targets of miR-25 were tested with a dual-luciferase assay. The potential target sites (highlighted in gray) of miR-25 on the 3'-UTR of *BTG2* (A), *RECK* (C), *LATS2* (E) and *CDKN1C* (G) were predicted by TargetScan. The mutated target sequences for *BTG2* are marked in red. (B, D, F, H) Results of luciferase reporter assays for each gene (n=3, error bars show the mean ± SEM); **P<0.01; ***P<0.001; ****P<0.0001.



Supplemental Figure 7. Knockdown of *BTG2* had no influence on CM proliferation. EdU staining revealed that *BTG2* knockdown by siBTG2 had no influence on CM proliferation. Nuclei were stained with DAPI (blue); CMs were stained with an antibody against α-ACTININ (red). At least 2000 cells were quantified in each group.



Supplemental Figure 8. miR-25 shows more significant effect on the proliferation of atrium CMs. miR-25 injection increased the atrium CMs number. A transgenic zebrafish line with myocardium-specific RFP expression in the nuclei was employed.

Table S1. Primers and oligonucleotides

Name	Sequence (5'-3')	Description
q-OCT4-F	CCTGAAGCAGAAGAGGATCACC	Primers for qPCR
q-OCT4-R	AAAGCGGCAGATGGTCGTTTGG	
q-MIXL1-F	CCCGACATCCACTTGCGCGAG	
q-MIXL1-R	GGAAGGATTTCCCACTCTGACG	
q-MESP2-F	GAACCCACCAGTGCCCTGGAC	
q-MESP2-R	TGCAGTCTCTGGCATGATGGGT	
q-NKX2-5-F	CTGTCTTCTCCAGCTCCACC	
q-NKX2-5-R	TTCTATCCACGTGCCTACAGC	
q-TNNT2-F	AAGAGGCAGACTGAGCGGGAAA	
q-TNNT2-R	AGATGCTCTGCCACAGCTCCTT	
q-FBXW7-F	GTTTGGTCAGCAGTCACAGGCA	
q-FBXW7-R	CCACACTTTGAGTGTCCGATCTG	
q-GAPDH-F	GAAGGTGAAGGTCGGAGTC	
q-GAPDH-R	GAAGATGGTGATGGGATTTC	
q-PCNA-F	CAAGTAATGTCGATAAAGAGGAGG	
q-PCNA-R	GTGTCACCGTTGAAGAGAGTGG	
q-BRCA2-F	GGCTTCAAAAAGCACTCCAGATG	
q-BRCA2-R	GGATTCTGTATCTCTTGACGTTCC	
q-NUSAP1-F	CTGACCAAGACTCCAGCCAGAA	
q-NUSAP1-R	GAGTCTGCGTTGCCTCAGTTGT	
q-RACGAP1-F	ATGCTGGCAGACTTTGTGTCCC	
q-RACGAP1-R	CAGCCAGAGATCCTATACAGGC	
q-CKS2-F	GAGGAGACTTGGTGTCCAACAG	
q-CKS2-R	GATTTGACGATCCCCAGATAAACT	
q-CCNA2-F	CTCTACACAGTCACGGGACAAAG	
q-CCNA2-R	CTGTGGTGCTTTGAGGTAGGTC	
q-CCNB-F	GACCTGTGTCAGGCTTTCTCTG	
q-CCNB-R	GGTATTTTGGTCTGACTGCTTGC	
q-CCND1-F	TCTACACCGACAACTCCATCCG	
q-CCND1-R	TCTGGCATTTTGGAGAGGAAGTG	
q-CCND2-F	GAGAAGCTGTCTCTGATCCGCA	
q-CCND2-R	CTTCCAGTTGCGATCATCGACG	
q-CDK2-F	ATGGATGCCTCTGCTCTCACTG	
q-CDK2-R	CCCGATGAGAATGGCAGAAAGC	
q-E2F2-F	CTCTCTGAGCTTCAAGCACCTG	
q-E2F2-R	CTTGACGGCAATCACTGTCTGC	
FBXW7-F	GTAATTCTAGGCGATCGCTCGAGTCAGTG GTGCAGGATGTTGG	Primers for luciferase reporter assay
FBXW7-R	TTTTATTGCGGCCAGCGGCCGCGCCCAAT GACCACTGGAGAA	
BTG2-F	GTAATTCTAGGCGATCGCTCGAGTGCTAGT	

	GCTGCTTTGTGTG	
BTG2-R	TTTTATTGCGGCCAGCGGCCGCCATCCTGG CCAAATGCCCTA	
RECK-F	GTAATTCTAGGCGATCGCTCGAG GTGCTGATGTAGCATGCTTGT	
RECK-R	TTTTATTGCGGCCAGCGGCCGC GGAAGGCCTGAAGCTCTCTC	
LATS2-F	CCGCTCGAGTACTGACAGACTGCCCACAC	
LATS2-R	GAATGCGGCCGCAGAGCGCTGTGAGTAAC AACA	
CDKN1C-F	GTAATTCTAGGCGATCGCTCGAG CGGTGAGCCAATTTAGAGCC	
CDKN1C-R	TTTTATTGCGGCCAGCGGCCGC ATAACCGAGCTAGTGCGTGG	
MT-FBXW7-F	CACGTTAAATTTCTTTATTTTCTTCTCCAG	
MT-FBXW7-R	CTGGTTTGATTTAGAAAGTCTC	
MT1-BTG2-F	ACATTGAATGCCCCCTGGGTCCCAGGA	
MT1-BTG2-R	AGTGACTGAGAACTCTTGTCC	
MT2-BTG2-F	ACATTGAATATACTGTTGTGGGTTGGA	
MT2-BTG2-R	AAGTCTACGACAAATTTGGA	
hsa-miR-25-3p mimics	5'-CAUUGCACUUGUCUCGGUCUGA-3' 5'-AGACCGAGACAAGUGCAAUGUU-3'	Oligonucleotide sequences of microRNA mimics and siRNA
mimic negative control	5'-UUCUCCGAACGUGUCACGUTT-3' 5'-ACGUGACACGUUCGGAGAATT-3'	
siFBXW7	5'-CCAAUUGUGUAGACGAUAUAC-3' 5'-GUAUAUCGUCUACACAAUUGG-3'	
siBTG2	5'-GGUCAUAGAGCUACCGUAUTT-3' 5'-AUACGGUAGCUCUAUGACCTT-3'	
siNC	5'-UUCUCCGAACGUGUCACGUTT-3' 5'-ACGUGACACGUUCGGAGAATT-3'	