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Supplemental Information

Hepatocyte Growth Factor Improves the Therapeutic Efficacy of Human Bone Marrow Mesenchymal Stem Cells via RAD51

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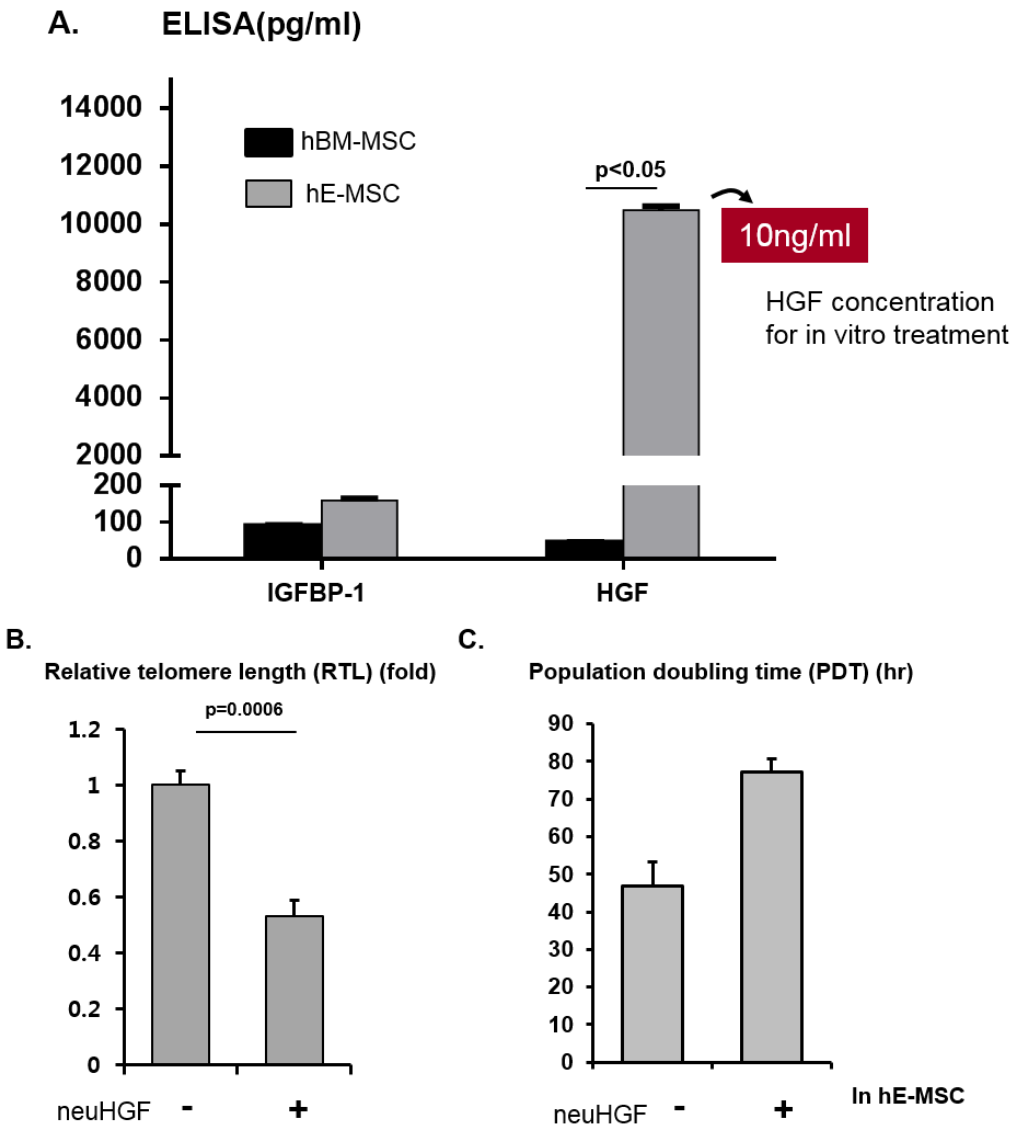
Figure S 1

Determine of HGF concentration and validation of HGF effect in hE-MSC



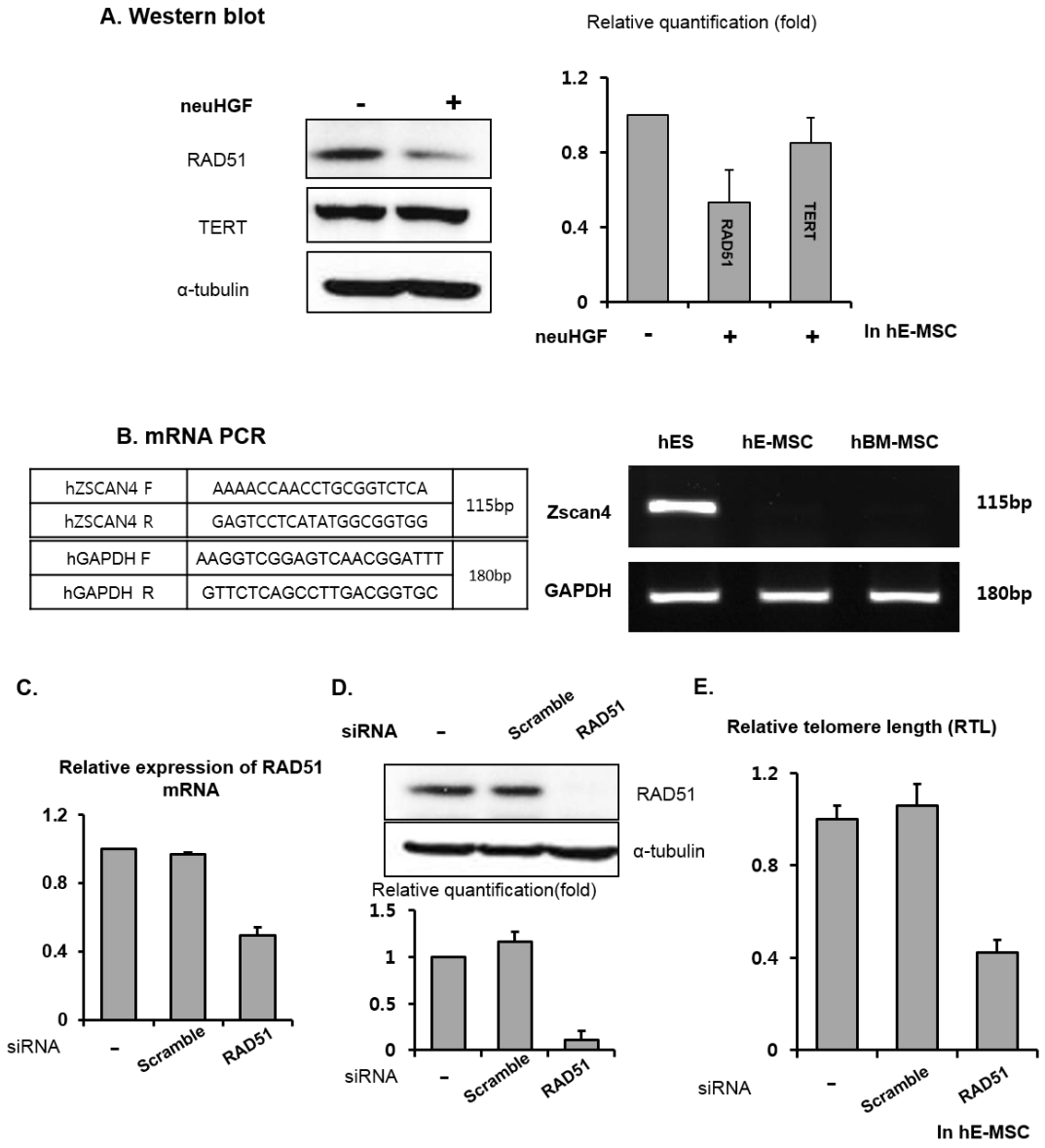
A ELISAs of HGF and IGFBP1 secretion by hBM-MSCs and hE-MSCs. The HGF concentration in the culture supernatant of hE-MSCs was 10 ng/mL, which was significantly higher than that in the culture supernatant of hBM-MSCs. The IGFBP1 concentration did not significantly differ between the two cell types.

B Determination of RTL by real-time PCR. Loss of HGF function in hE-MSCs by treatment with a HGF neutralizing antibody decreased telomere length and the cell number.

C PDT was increased in hE-MSCs treated with a HGF neutralizing antibody.

Figure S 2

Validation of RAD51 in hE-MSC.



A Western blotting demonstrated that protein expression of RAD51 in hE-MSCs was reduced by neutralization of HGF, while that of TERT was not changed.

B RT-PCR to check Zscan4 expression in hE-MSCs and hBM-MSCs. Human embryonic stem cells (hES) were used as a positive control.

C Real-time PCR analysis of RAD51 mRNA expression in siRAD51-transfected hE-MSCs.

D Western blot analysis of RAD51 protein expression in siRAD51-transfected hE-MSCs.

E RTL was decreased following RAD51 knock-down.

*Figure S*3

Promoter analysis of RAD51 and validation of IKF1 and RUNX1 in hE-MSC

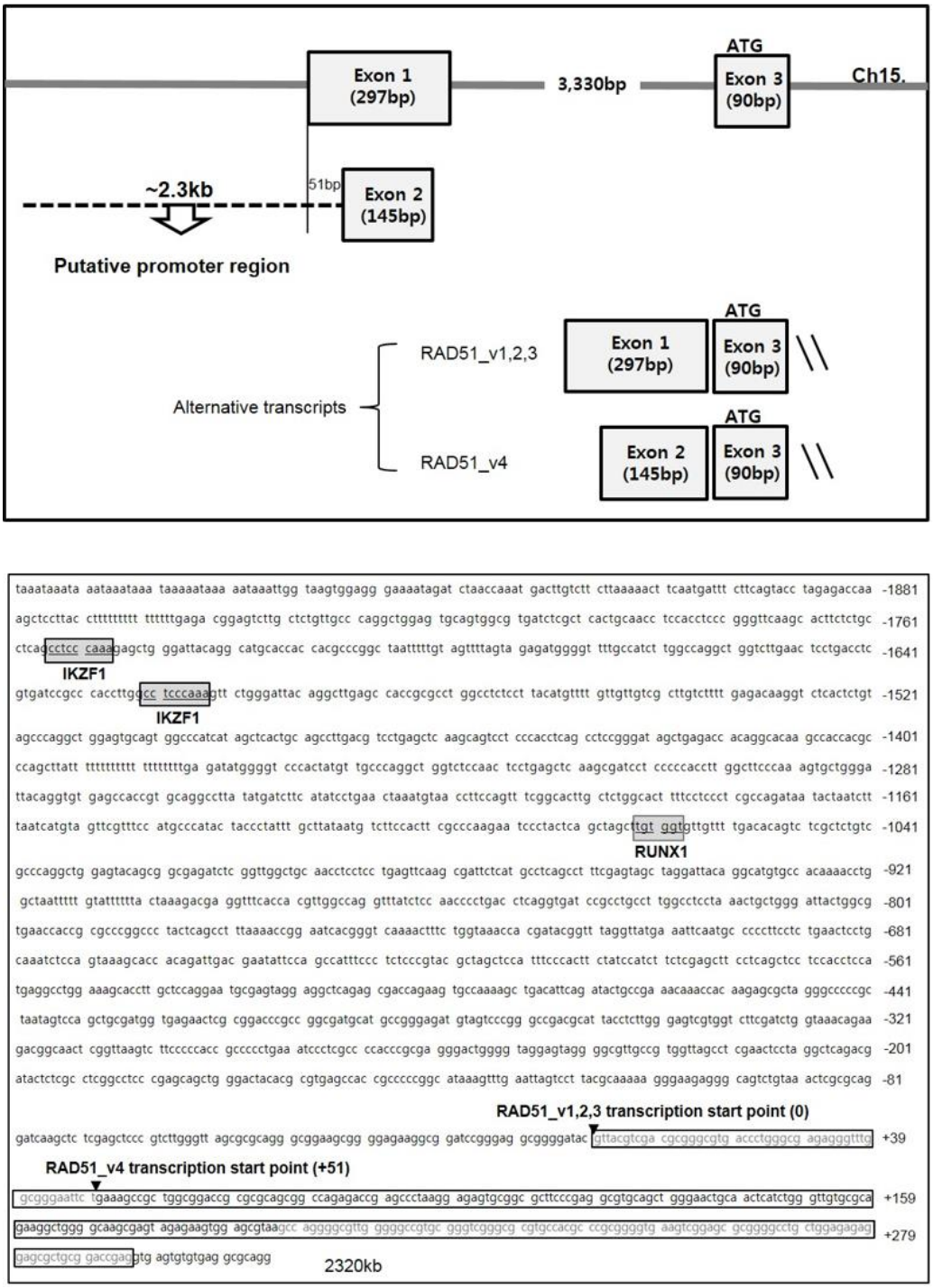
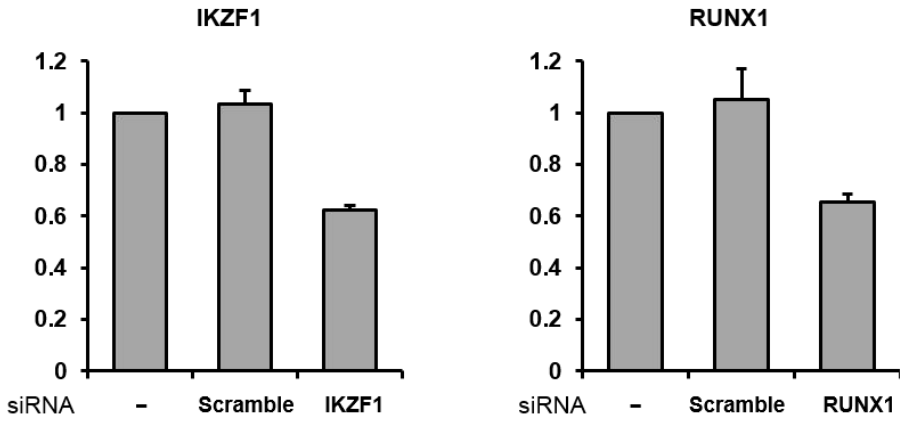


Figure S 3**continued**

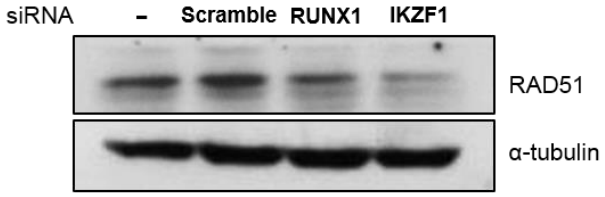
B.

Real time cDNA PCR



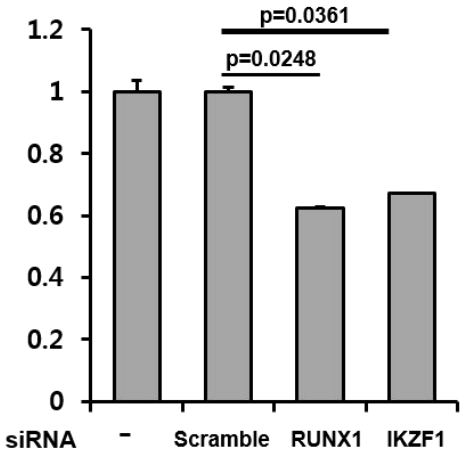
C.

Western blot



D.

Relative telomere length (RTL) by Real time gDNA PCR



In hE-MSC

A Exon-intron organization of human RAD51. Four alternative transcripts of RAD51 were arranged and the putative promoter region of RAD51 was identified. Screening for transcriptional activators that bind to the putative promoter region of RAD51. IKZF1- and RUNX1-binding sites were identified using a transcription factor-binding site analysis program (TFSEARCH V1.3 database).

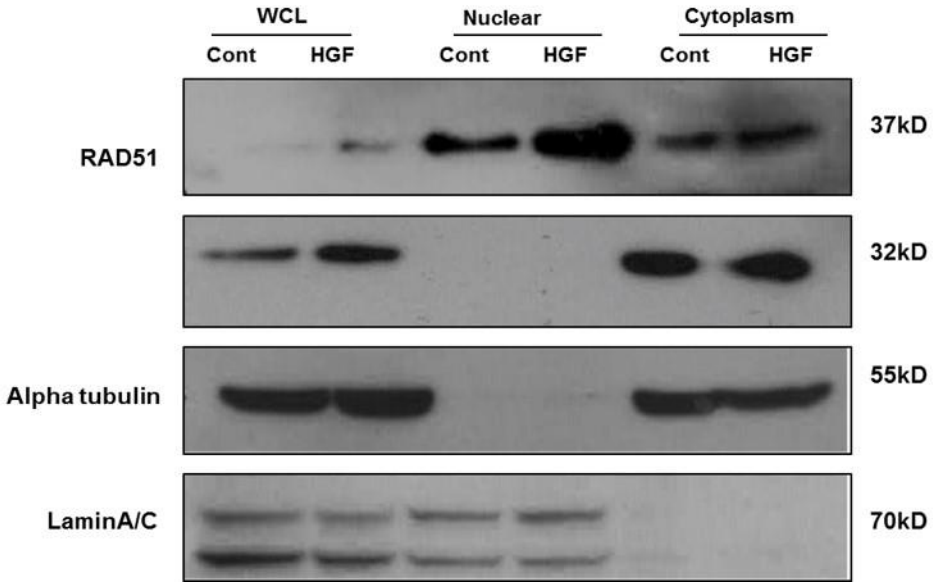
B IKZF1 or RUNX1 mRNA knock-down was validated by real-time PCR.

C Western blot analysis of the RAD51 protein level following knock-down of each gene. α-tubulin was used as an endogenous control.

D RTL was decreased following RUNX1 or IKZF1 knock-down.

Figure S 4 In BM-MSC

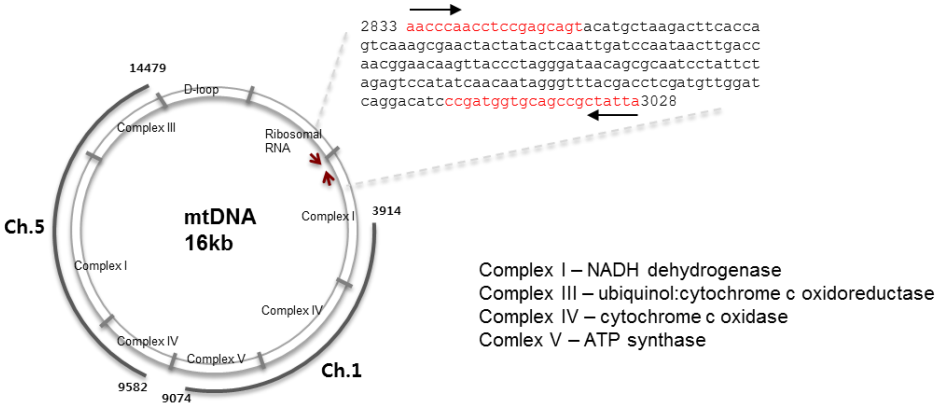
A. Western blotting



A Western blotting of cytosol and nuclear fractionation after HGF treatment.

Figure S 5 In BM-MSC

A. Diagram showing the binding sites of mtDNA-specific primers.



	Primer	Sequence 5′-3′
mtDNA	mtDNA 2833 Forward	aacccaacctccgagcagt
	mtDNA 3028 Reverse	taatagcggctgcaccatcgg
nuclearDNA (one copy gene)#	AIB1 Forward	gagtttcctggacaaatgag
	AIB1 Reverse	cattgtttcatatctctggcg

B. Scheme of the experimental procedure used for CytoChIP

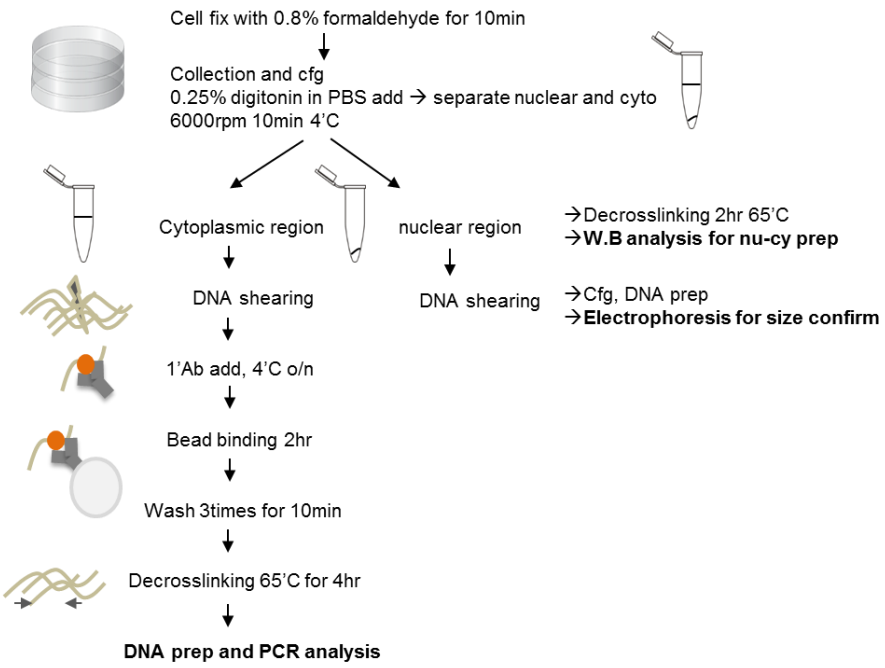
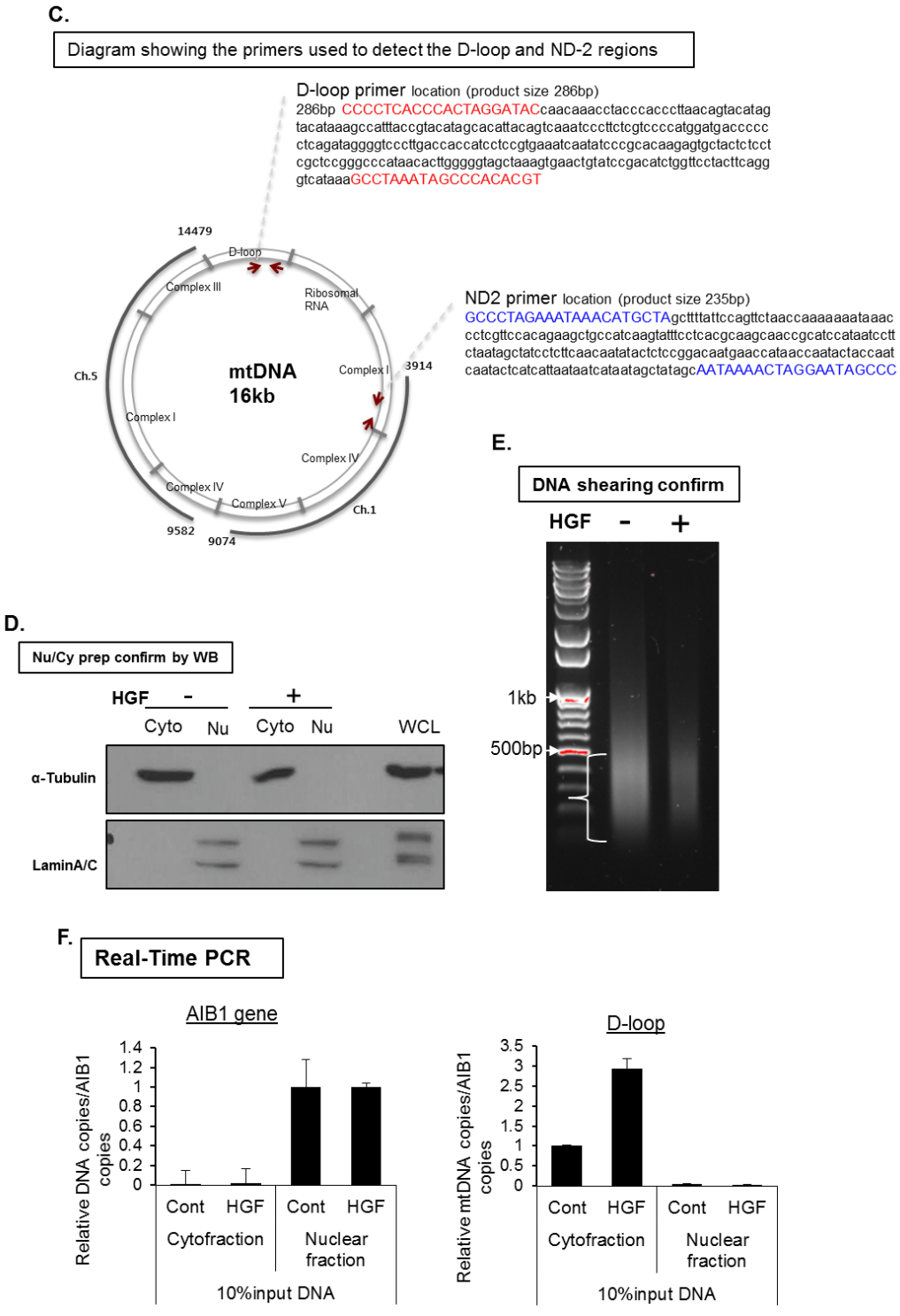


Figure S 5 **continued** In BM-MSC



A Diagram showing the binding sites of mtDNA-specific primers. mtDNA-specific primer designed on the region except of matching with chromosomal DNA. Their sequences are showed in the table. AIB1 was chosen as a typical single copy in genomic DNA.

B Scheme of the experimental procedure used for CytoChIP.

C Diagram showing the primers used to detect the D-loop and ND-2 regions.

D Western blotting confirmed the generation of cytoplasmic and nuclear fractions. WCL, whole cell lysate.

E DNA was sheared into fragments of 100–500 bp(denoted by the bracket), which covered length of PCR product.

F Real-time PCR analysis of nuclear and mtDNA markers to validate separation of the cytoplasmic and nuclear fractions using 10% of each fraction. AIB1 was used as the nuclear marker and the D-loop region was used as the cytoplasmic and mtDNA marker.