Supplemental Material:

Modification of hCPCs:

For lentiviral infection, hCPC were plated in 6-well dish (50,000 cells/ well), serially transduced with lentivirus (multiplicity of infection (MOI) of 20 followed by MOI of 10 two days after first infection) to obtain hCPC expressing enhanced green fluorescent protein (eGFP) together with Pim-1 or eGFP alone. Engineered hCPCs were expanded and analyzed using flow cytometry to validate high percentage eGFP expression.

Telomere Length Measurements (Q-FISH)

Telomere length was analyzed by quantitative in situ hybridization (Q-FISH) and confocal microscopy. PNA probe was purchased from DAKO (K5325) and measurements were done according to manufacturer's protocol. Briefly, slides were deparaffinized and rehydrated, followed by antigen retrieval using 10mM Citrate buffer pH 6.0. Slides were subsequently washed with PBS and treated for 9 minutes with Proteinase K (Dako, S3020). Following another PBS wash slides were dehydrated with a series of cold ethanol then allowed to dry. PNA probe was placed on the section and denatured at 80°C for 10 minutes and hybridized at 37°C overnight. Slides were washed two times in Wash Buffer at 65°C and counterstained with sytox blue. Telomere signal was acquired in each nucleus using Leica software and divided by sytox blue signal to account for differences in nuclear size.

Telomere length (Rt-PCR)

Telomere lengths measured by modified monochrome multiplex Quantitative PCR method on a Real time PCR machine. In this PCR method Albumin is amplified with the telomere template at the same time as described in Weischer M *et al.* Briefly, 20ng of DNA, 1X syber green with albumin and telomere primer were combined in a reaction volume of 10ul and the samples were atleast run in a set of 6 replicates. The thermal cycle used Stage 1:15 min at 95°C, Stage 2: 2 cycles of 15 s at 94°C, 15s at 49°C and stage 3: 32 cycles of 15 s at 94°C, 10 s at 62°C, 15s at 73°C with signal acquisition, 10 s at 84°C, 15 s at 87°C with signal acquisition, stage 4: 1 cycle of 0.05 s at 65°C with signal acquisition.

Telomerase Activity

Telomerase activity was assessed by quantitative PCR according to the manufacturer's protocol (TRAPeze RT, Chemicon S7710). Briefly, hCPCs were harvested in CHAPS buffer and centrifuged at 4°C. 1 µg of lysate was obtained then incubated in a solution containing reverse transcriptase reaction mix and Taq polymerase at 30°C for 30 minutes. Control cells provided by the manufacturer were used as positive control and serial dilutions of control template TSR8 was employed for quantification. CHAPS buffer in the absence of protein lysates and heat-inactivated lysates were used as negative control. PCR cycling conditions and data analysis were performed according to the manufacturer's protocol.

Online Figure I:

Human CPCs over expressing Pim-1 A) c-kit expression in hCPC-S and hCPC-F by FACS analysis. B) lentiviral vectors for eGFP and eGFP Pim-1. C) Percentage GFP in hCPC (eGFP) and hCPC (Pim) D) Immunoblot analysis of hCPC for over expression of Pim-1 after lentiviral transduction in hCPC-S and hCPC-F.

Online Table I:

List of primers

P53 (Fwd)	GCAGCGCCTCACAACCTCCG
P53 (Rev)	TGATTCCACACCCCCGCCCG
P16 (Fwd)	AGCATGGAGCCTTCGGCTGA
P16 (Rev)	CCATCATCATGACCTGGATCG
Cyclin D1 (Fwd)	CTGGCCATGAACTACCTGGA
Cyclin D1 (Rev)	TCACACTTGATCACTCTCG
Pim-1 (Fwd)	TGCCATTAGGCAGCTCTCCCCA
Pim-1 (Rev)	GCGGCTTCGGCTCGGTCTACT
Albumin (Fwd)	CGGCGGCGGGGCGCGGGGCTGGGCGGAAATGCTGCACAGAATCCTTG
Albumin (Rev)	GCCCGGCCCGCCGCCGTCCCGCCGAAAAGCATGGTCGCCTGTT
Tert (Fwd)	ACACTAAGGTTTGGGTTTGGGTTTGGGTTAGTGT
Tert (Rev)	TGTTAGGTATCCCTATCCCTATCCCTATCCCTATAACA

Online Table II:

Western blot	Antibody	Dilution	Amplify	Company
Western blot	p53	1:500	No	Santa Cruz
Western blot	p16	1:100	No	Santa Cruz
Western blot	Cyclin D1	1:500	No	Cell Signaling technology
Western blot	Pim-1	1:100	yes	Zymed
Western blot	B tubulin	1:1000	No	Santa Cruz
FACS	c-kit	1:50	No	ABCAM

List of antibodies

Α

С



Online Figure I:

Human CPCs over expressing Pim-1 A) c-kit expression in hCPC-S and hCPC-F by FACS analysis B) lentiviral vectors for eGFP and eGFP Pim-1. C) Percentage GFP in hCPC-S and hCPC-F after modification with (eGFP) and (Pim-1) D) Immunoblot analysis of hCPC for over expression of Pim-1 after lentiviral transduction in hCPC-S and hCPC-F.

В