

Fig. S1 Alves et al

Figure S1. hMSC morphology and differentiation is affected by in vitro expansion

A) Phase-contrast photos of hMSCs at different RPD, x40 magnification. An evident change in cell size and morphology can be observed with increased time of culture. B) Change in the mineralization potential after in vitro expansion. Relative calcium accumulation by hMSCs in MM after 28 days, expressed as percentage reduction in calcium deposition ability over passages compared to RPD 6. No detectable calcium was measured in hMSCs cultured in BM (data not shown). C) Phase-contrast images of hMSC in adipogenic medium at different RPD. Adipocytes are stained with Oil red O and visualized at x40 magnification. Error bars represent standard deviation. Statistical analysis was performed using one-way ANOVA and Tukey post test with a significance level of 0.05. * represents $p < 0.01$ to 0.05 and *** $p < 0.001$.

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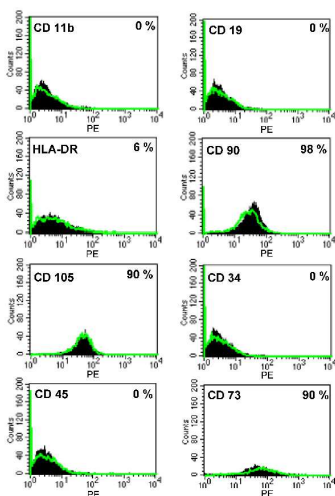


Fig. S2 Alves et al

Figure S2. Immunophenotype of hMSCs (RPD 6 vs RPD 20). Representative overlay of flow cytometry histograms of hMSCs from human bone marrow are presented. Black histogram, RPD 6 and green line, histogram of RPD 20.
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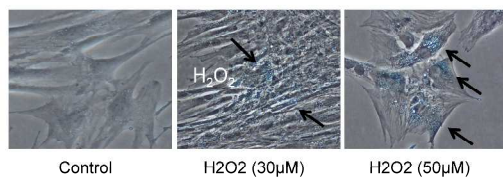


Fig. S3 Alves et al

Figure S3. Senescence-Associated β -galactosidase expression after DNA damage induction. RPD 6 cells were exposed to 0, 30 or 50 μ M of H₂O₂ for 6 days after which cells were stained for SA- β -galactosidase.
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Donor number:	24	26	29	36	41	42	46
Gender:	Female	Male	Male	Female	Female	Female	Female
Year birth:	1945	1955	1939	1967	1967	1978	1944
Aspiration site:	Acetabulum	Acetabulum	Acetabulum	Iliac Crest	Acetabulum	Iliac Crest	Acetabulum

Table S4 Alves et al.

Table S4. Information relative to the hMSCs donors used on this manuscript.
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