Supplementary Materials and Methods

ChIP-Seq: Nucleosomal ChIP and SOLiD Library Preparation

***Preparation of nucleosomal lysates*:** 3 x 10^5 SR and SEN ADSCs were washed with PBS, and crosslinked in 1% formaldehyde. The cells were scraped from the plate in CSK buffer with 0.5% Triton X-100, washed, and sonicated in micrococcal nuclease buffer (50 mM Tris-HCl, pH 7.9, 5 mM CaCl; NEB) with a Covaris S2 Sonicator (frequency sweep at 1% duty cycle, 5 intensity and 50 cycles per burst for 12 cycles of 10 sec each at 4ºC). Sonicates were cenrtrifuged to get rid of the cellular debris, and supernatants were incubated with 25U/μl of micrococcal nuclease (NEB).

Immunoprecipitation: Nucleosomal lysates were adjusted to antibody binding buffer (25mM Tris-HCl pH 7.9, 0.15% SDS, 1% Triton X-100, 150mM NaCl), and immunoprecipitated with 10 μg γH2AX antibody overnight at 4ºC. Protein A Sepharose-beads were blocked with 10 μg/ml glycogen, 10 μg/ml lysozyme, 5 μg/ml tRNA and 20 μg/ml BSA for 1 hour at 4ºC before binding to the immuno-complexes for 1hour at 4ºC. Beads were washed with Binding buffer and again with the same buffer containing 0.5M NaCl and finally in TE (10mM Tris-HCl pH 7.6, 1mM EDTA). Immuno-complexes bound to beads were re-suspended in TE buffer, de-crosslinked, and purified by Proteinase K treatment, phenol:chloroform extraction, and isopropanol precipitation. DNA fragments were prepared for adapter ligation by filling-in ends by DNA polymerase I (Klenow fragment) and phosphorylating 5' ends of PCR primers by Polynucleotide kinase (NEB), and ligated to 30-fold molar excess of SOLiD™ System 2.0 (Applied Biosystems) library adapters according to manufacturer's protocol. DNA libraries were amplified by PCR using cloned Pfu DNA Polymerase (Stratagene, Agilent Technologies. Mononucleosomal size DNA fragments were size-selected in a 2% agarose gel, cut around 200 bp size, and purified with QIAquick® Gel Extraction Kit (Qiagen). Emulsion PCR (ePCR) for sequencing bead preparations were done with 0.05 and 0.1 pg/μl of library DNA for each sample. Samples were sequenced on SOLiD™ System 2.0 (Applied Biosystems) according to manufacturer's protocol.

Expression Profiling

Total RNAs from ~1.5 x 10^6 SR and SEN ADSCs were prepared by TRIzol® method (Invitrogen). Human Cell Cycle qRCR Array (SABiosciences #PAHS-020C-2) was used for the

profiling (7500 Fast PCR System, Applied Biosystems). Three RNA samples from SR ADSCs were matched to RNA samples for their replicatively SEN counterparts. Statistical significance of the transcriptional differences was calculated by T-test as described in SABiosciences manual and http://www.sabiosciences.com/RTPCR. The model-based expression values were calculated and filtered for the genes with higher or lower than two-fold differences in expression between SEN and SR samples. Categories of the genes were annotated using the PANTHER (protein analysis through evolutionary relationships) classification system.

Immunofluorescence

Epifluorescence images were acquired on an Olympus BX60 fluorescence microscope with Spotfire 3.2.4 software (Diagnostics Instruments). Confocal images (z-series slice thickness 0.39μm) were acquired on Zeiss LSM 510 NLO with 488nm Argon, 543 nm HeNe, and Coherent Chameleon 2-photon lasers using a 63x plan-apo objective and 0.08x0.08x0.39um voxel dimensions. Image stacks were deconvolved using Huygens Professional 3.4.0 (Scientific Volume Imaging, Netherlands) and visualized in Bitplane Imaris 6.3.1 (Bitplane Inc., Saint Paul, MN).

Bioinformatic Analysis

ChIP-seq was performed on four replicates each of SR and SEN hADSC cultures.

The ChIP-seq experimental protocol and data merging we employed were supported by the highly consistent mapping results seen for the replicate experiments (**Suppl. Fig. 4**). Having mapped and processed the ChIP-seq tags, we evaluated tag counts with respect to an empirically-derived count threshold for approximately mono-nucleosomal-sized windows of 200bp in order to identify individual γH2AX modified nucleosomes on the human genome. Outliers removal, noise reduction and data merging is published elsewhere.

Comparison with genomic features

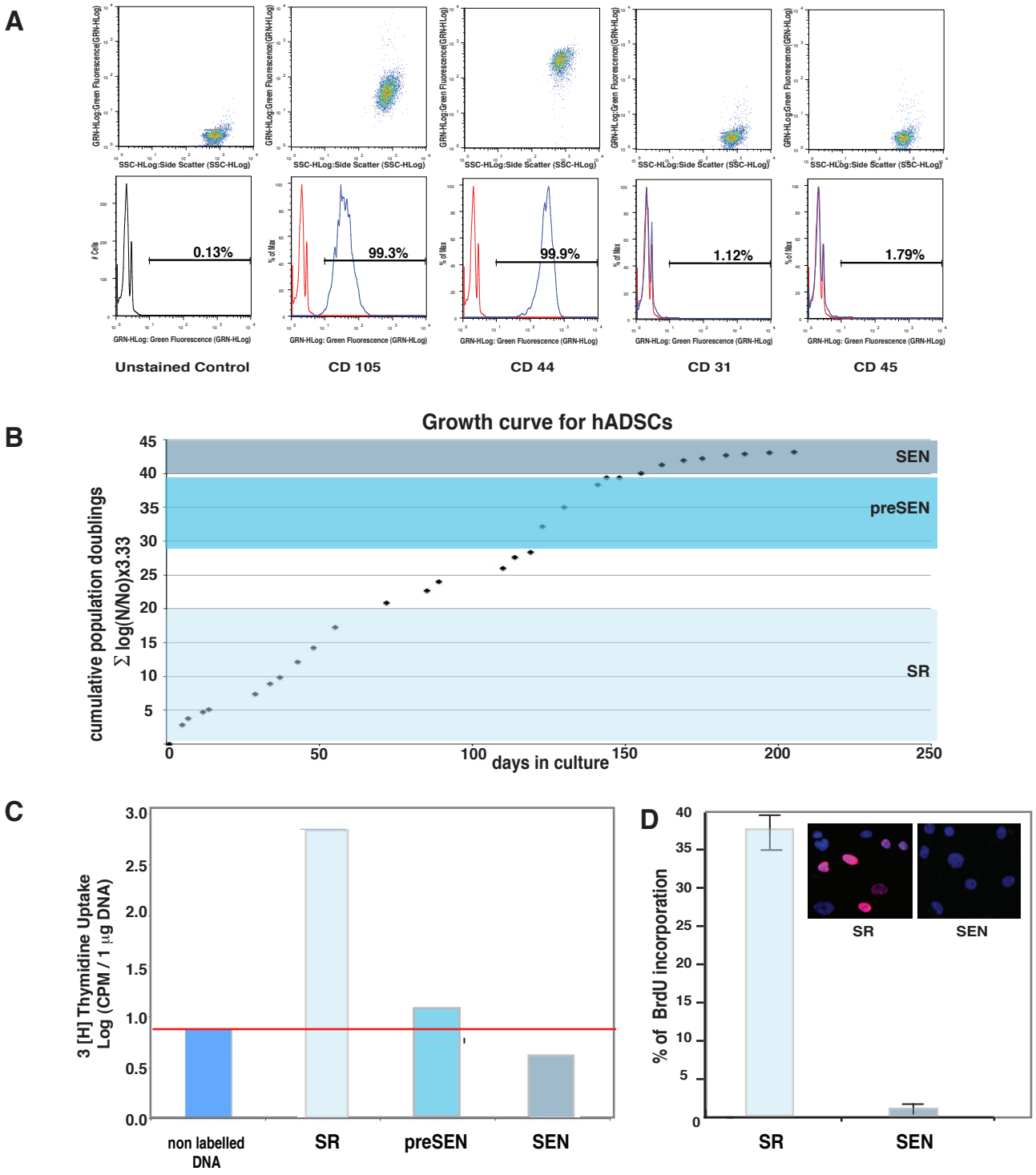
The tag-to-genome mapping and the quality control procedures yielded maps of nucleosome positions in the human genome that can be confidently considered to be modified by γH2AX for self-renewing (SR) and senescent (SEN) hADSCs. These maps were used to compare the locations of γH2AX modified nucleosomes to various genomic features of interest within and

between SR and SEN cells. For instance, we compared the locations of modified nucleosomes in the genome to the locations of transcription start sites, exons, introns, repetitive sequence elements, pericentric regions and peri-telomeric regions. These comparisons were done using the UCSC Table Browser tool (Karolchik et al., 2004), which is a part of the UCSC Genome Browser suite of tools and genome annotations (Karolchik et al., 2003). In addition, custom Perl scripts were used to modify UCSC Genome Browser tracks, such as the gene annotation tracks, to pull out specific features of interest including transcription start site locations and exon/intron boundaries for further analysis.

Karlin, S., and Altschul, S.F. (1990). Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proc Natl Acad Sci U S A *87*, 2264-2268.

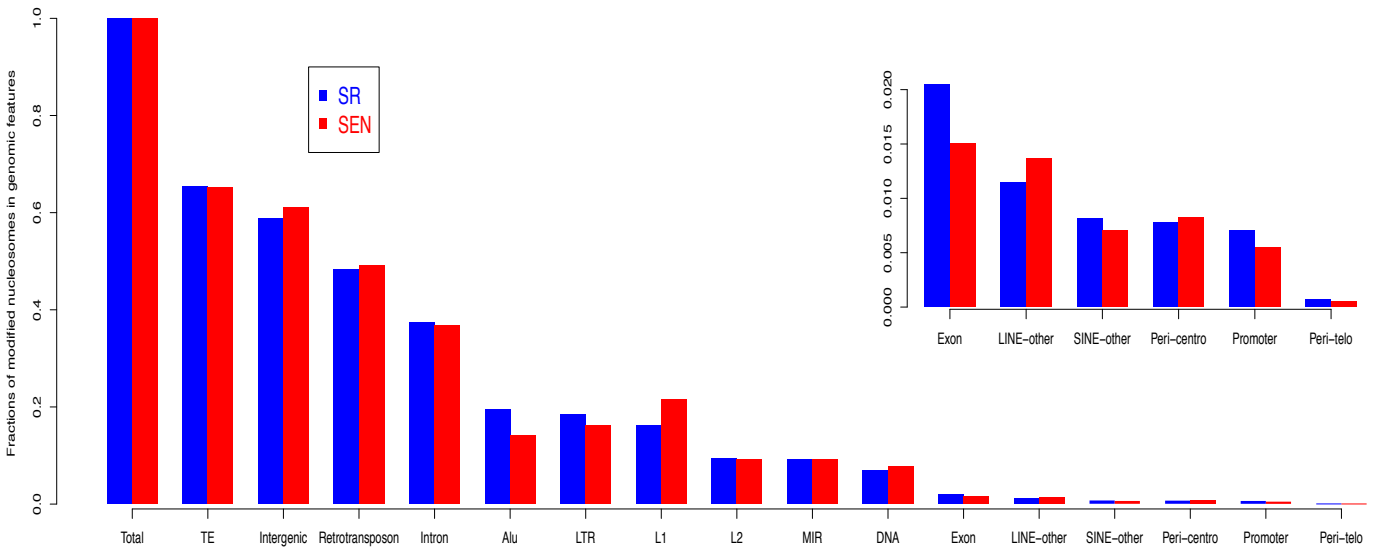
Karolchik, D., Baertsch, R., Diekhans, M., Furey, T.S., Hinrichs, A., Lu, Y.T., Roskin, K.M., Schwartz, M., Sugnet, C.W., Thomas, D.J.*, et al.* (2003). The UCSC Genome Browser Database. Nucleic Acids Res *31*, 51-54.

Karolchik, D., Hinrichs, A.S., Furey, T.S., Roskin, K.M., Sugnet, C.W., Haussler, D., and Kent, W.J. (2004). The UCSC Table Browser data retrieval tool. Nucleic Acids Res *32*, D493-496.

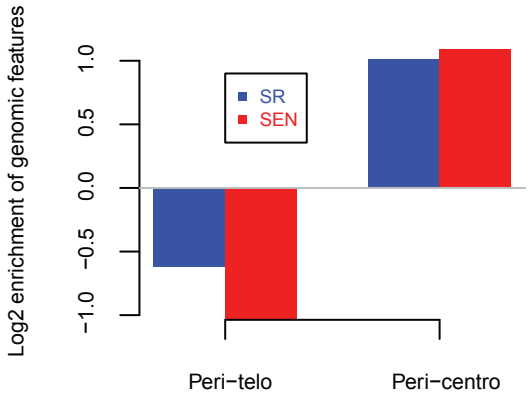


Supplemental Figure 1. FACS analysis and proliferative properties of of hADSCs.

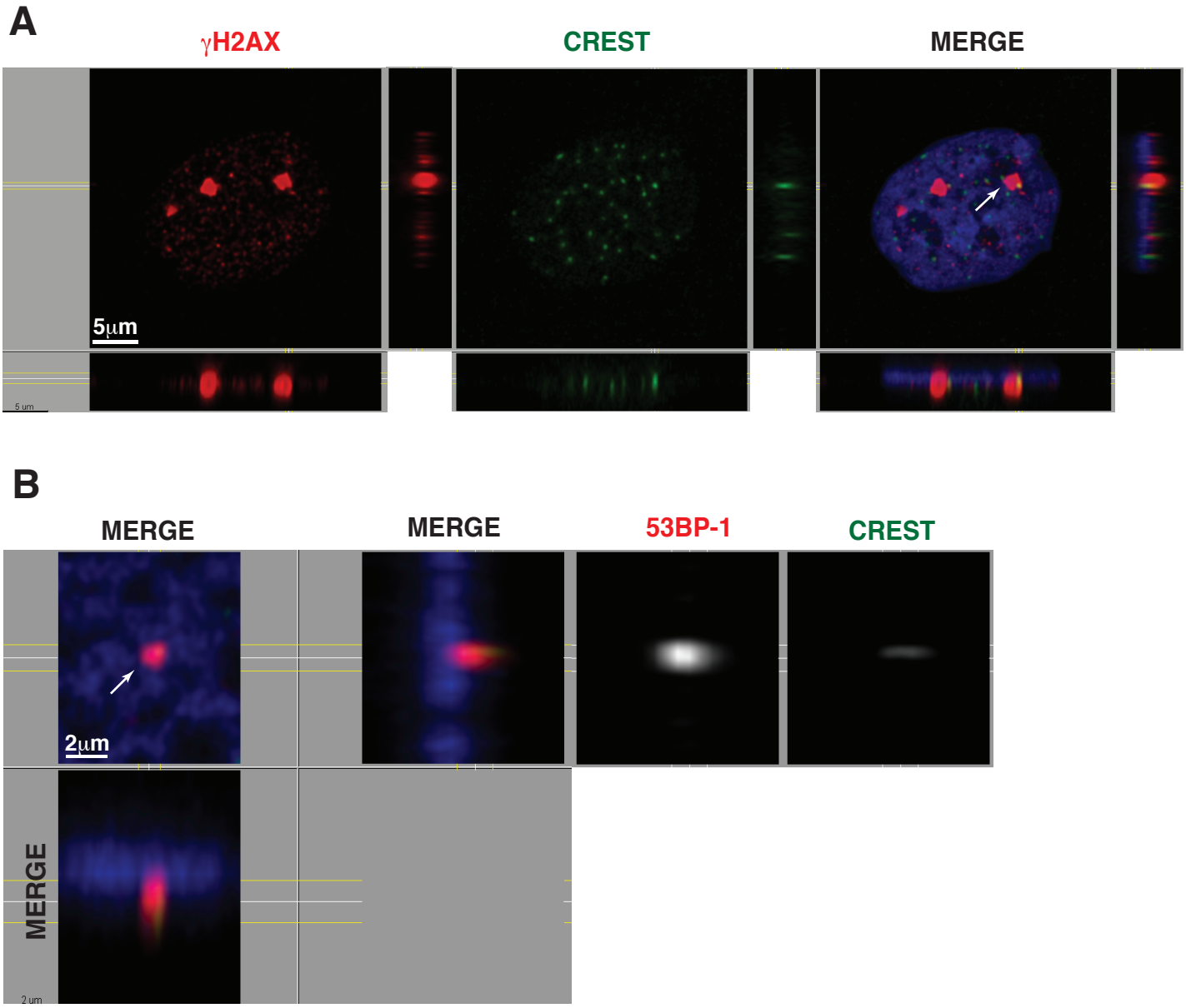
(**A**) FACS analysis of hADSCs. hADSCs were stained with FITC (CD 31, CD44 and CD 45) or AlexaFlour-488 (CD105) conjugated antibodies against cell surface markers and subjected to flow cytometric analysis. The cell populations are shown as fluorescence to side scatter graphs (top), and the histograms (bottom) of stained cells (blue line) compared to un-stained cells (red line); with percentage of positive cells indicated. (**B**) Representative growth curve of hADSCs. Three distinct states are shown: SR- self-renewing (population doubling <17); preSEN- presenescent (population doubling 29-38); SEN- senescent (population doubling >39).(**C**) Replication capacity of hADSCs declines with ex-vivo aging. Proliferation in self-renewing (SR), pre-senescent (preSEN) and senescent cells (SEN) hADSCs was measured by 3[H]-thymidine uptake. Results are presented as the amount of 3[H]-thymidine (cpm) incorporated during DNA synthesis per 1μg of isolated DNA. DNA from cells not exposed to 3[H]-thymidine was used as background radiation control.**D**) Immunohistochemical detection of 5'-bromo-2'deoxyuridine (BrdU) incorporation. Examples of hADSCs' BrdU incorporation upon replication (10X magnification) are shown in inserts. Bar graphs correspond to percentage of BrdU positive cells with progressive ex-vivo hADSC expansion, based on three independent experiments. Error bars are standard deviations from the mean .



Supplemental Figure 2. Fractions of γH2AX modified nucleosomes that map to different genomic features for self-renewing (SR in blue) and senescent (SEN in red) adult adipose derived mesenchymal stem cells (hADSCs). The right tail of the distribution is enlarged and shown as an inset for clarity.

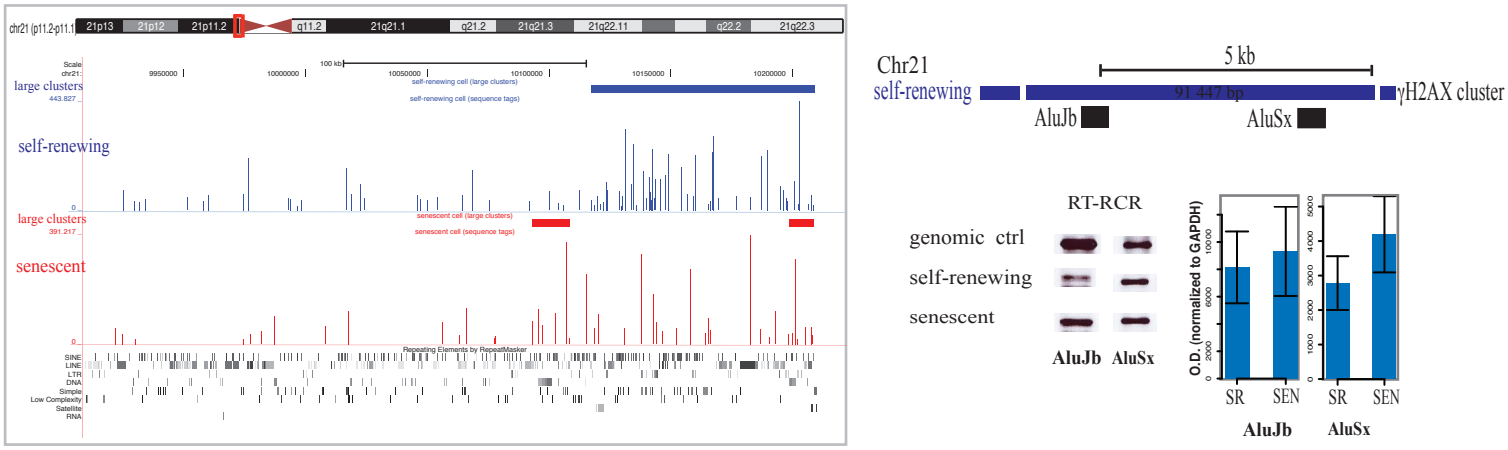


Supplemental Figure 3. Relative enrichment of γH2AX modified nucleosomes in peri-telomeric versus pericentric genomic regions.
Enrichment values were calculated as log2 normalized ratios of the gH2AX ChIP-seq tag counts per position in each region divided the genomic background tag counts per position. Values for self-renewing (SR) cells are shown in blue and for senescent (SEN) cells in red. Peri-telomeric regions are depleted for gH2AX, whereas pericentric regions are enriched.



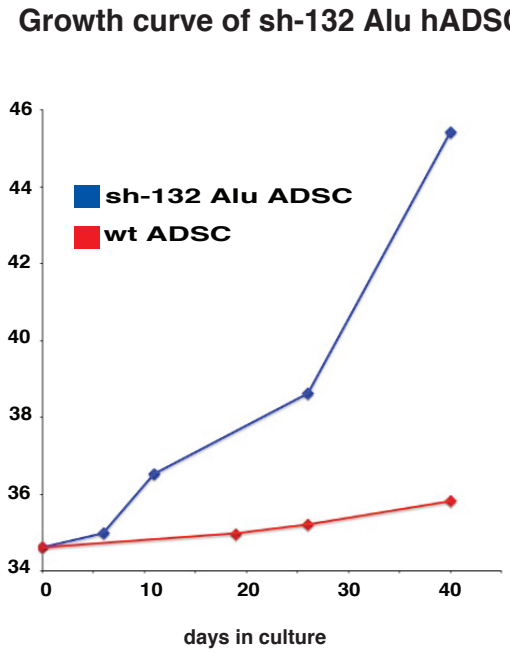
Supplemental Figure 4. Co-localization of senescence-associated persistent γH2AX/53BP1 foci with inner kinetochore in senescent hADSC.

Double immunostaining of senescent hADSCs with antibodies against of γH2AX (**A**) or 53BP1 (**B**) and antisera to the inner kinetochore from patients with calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia (collectively abbreviated as CREST). Scale bars are shown for each individual image. Black and white images of the separate channels are given in (**B**) for the convenience of visualization. Image was analyzed by Imaris software.



Supplemental Figure 5. Transcriptional activity of SINE/Alu retrotransposons from pericentric area of chromosome 21

On the left, peri-centric region of chromosome 21. Genomic tracks of γH2AX nucleosomal distribution are given for the location Chr21:9,950,000-10,200,000. On the right, schematic representation of large repairable cluster of γH2AX chromatin in self-renewing hADSCs. The positions of *AluJb* and *AluSx* relative to the γH2AX cluster are given in scale. Strand-specific RT-PCR analysis of these repeats did not reveal statistically significant up-regulation of these transcripts in senescent cells. No γH2AX accumulation was observed in this location in senescent hADSCs. Graphs are a quantitative representation of the results from 3 independent experiments. Data shown are mean ± SEM



Supplemental Figure 6. Growth curve of hADSCs demonstrate stable reversal of the senescent phenotype in the prior senescent cell (wt) by lentivirus mediated knockdown of generic SINE/Alu (sh-132Alu)

Supplemental Table 3. Total amount and fractions of γH2AX clusters occupied by genomic features. Figures cited in the text of the manuscript are shown in bold.

features	Mono-nucleosomal		Mid-size		Large	
	SR	SEN	SR	SEN	SR	SEN
Total amount bp occupied by γH2AX						
Whole genome	21,362,400	21,488,800	86,537,705	69,402,159	**37,467,892**	**11,243,429**
Genic Regions						
All genic regions	41.5%	41.0%	42.9%	39.7%	**47.9%**	**35.2%**
Promoter	0.75%	0.63%	0.83%	0.61%	0.94%	0.56%
Exon	2.86%	2.43%	3.20%	2.14%	4.02%	1.78%
Intron	37.9%	38.0%	38.9%	36.9%	42.9%	32.9%
Exon-intron Junction	0.19%	0.16%	0.21%	0.14%	0.28%	0.11%
Intergenic Regions						
All Intergenic regions	59.6%	59.9%	57.8%	61.4%	52.5%	66.8%
All Transposable Elements (TEs)						
All TEs	72.7%	70.6%	69.6%	70.2%	64.4%	65.6%
Retrotransposons						
All Retrotransposons	**67.3%**	**65.8%**	66.0%	65.8%	63.5%	61.4%
SINE Elements						
All SINEs	**34.9%**	**30.0%**	36.3%	30.9%	37.5%	28.6%
Alu	23.4%	17.8%	22.6%	18.8%	20.8%	16.9%
MIR	10.4%	11.2%	12.7%	11.2%	15.8%	11.0%
SINE-other	1.06%	0.97%	1.03%	0.88%	0.91%	0.71%

LINE Elements						
All LINEs	**32.4%**	**35.8%**	29.7%	34.9%	26.0%	32.8%
L1	20.3%	23.6%	17.2%	22.8%	12.9%	21.9%
L2	10.7%	10.5%	10.9%	10.4%	11.5%	9.19%
LINE-other	1.42%	1.68%	1.55%	1.65%	1.64%	1.75%
Other Transposable Elements						
LTR	20.8%	17.7%	18.6%	17.1%	14.9%	15.9%
DNA	8.50%	9.15%	8.75%	9.35%	8.70%	8.57%

Supplementary Table 4. γH2AX tag density compared to chromosome gene density and GC content.

SR			SEN		
Slope	Correlation	P-value	Slope	Correlation	P-value
Correlation: γH2AX tag density x chromosome gene density [$R_{\gamma H2AX|gene\ density}$]					
0.48	0.64	5.2E-04	-0.19	-0.22	0.16
Correlation: γH2AX tag density x chromosome GC content [$R_{\gamma H2AX|GC}$]					
2366	0.67	2.6E-04	2429	0.54	3.9E-03
Partial correlation: γH2AX tag density x chromosome gene density controlling for GC content [$r_{\gamma H2AX|gene\ density.GC}$]					
N/A	0.49	0.01	N/A	-0.65	1.1E-04

Supplemental Table 5. RT-PCR and qPCR primers

MIR	Forward:	5’-AGGATTTAAAGCTCTCTCTGCAGG-3’
	Reverse:	5’- ATGACTGAACTCTAAGATAAAGATCACAGC-3’
Alu	Forward:	5’-AGACAATCCTGGCCAACTTGG-3’
	Reverse:	5’-GCATTCCTGGACTGTGATGTGG-3’
AluJb	Forward:	5’-TGTGTGCCTGTAGTCCTAGCTACTAGG-3’
	Reverse:	5’-TTCAGGTTAGAGCTCTGAAGTCACG-3’
AluSx	Forward:	5’-TCTGCTCGGGAGGCTGAGG-3’
	Reverse:	5’-CCACCCACGAAGAATACATTTGC-3’
RPL13A	Forward:	5'-AAGGTGTTTGACGGCATCC-3'
	Reverse:	5'-GTTCTTCTCGGCCTGTTTCC-3'
Oct4	Forward:	5'- CTTCGCAAGCCCTCATTTC-3'
	Reverse:	5'-GAGAAGGCGAAATCCGAAG-3'
Nanog	Forward:	5'-CCCCAGCCTTTACTCTTCCT-3'
	Reverse:	5'-CTGGTTGCTCCAGGTTGAAT-3'
β-actin	Forward:	5'-CAACTCCATCATGAAGTGTGAC-3'
	Reverse:	5'-GCCATGCCAATCTCATCTTG-3'