Supplementary Figures

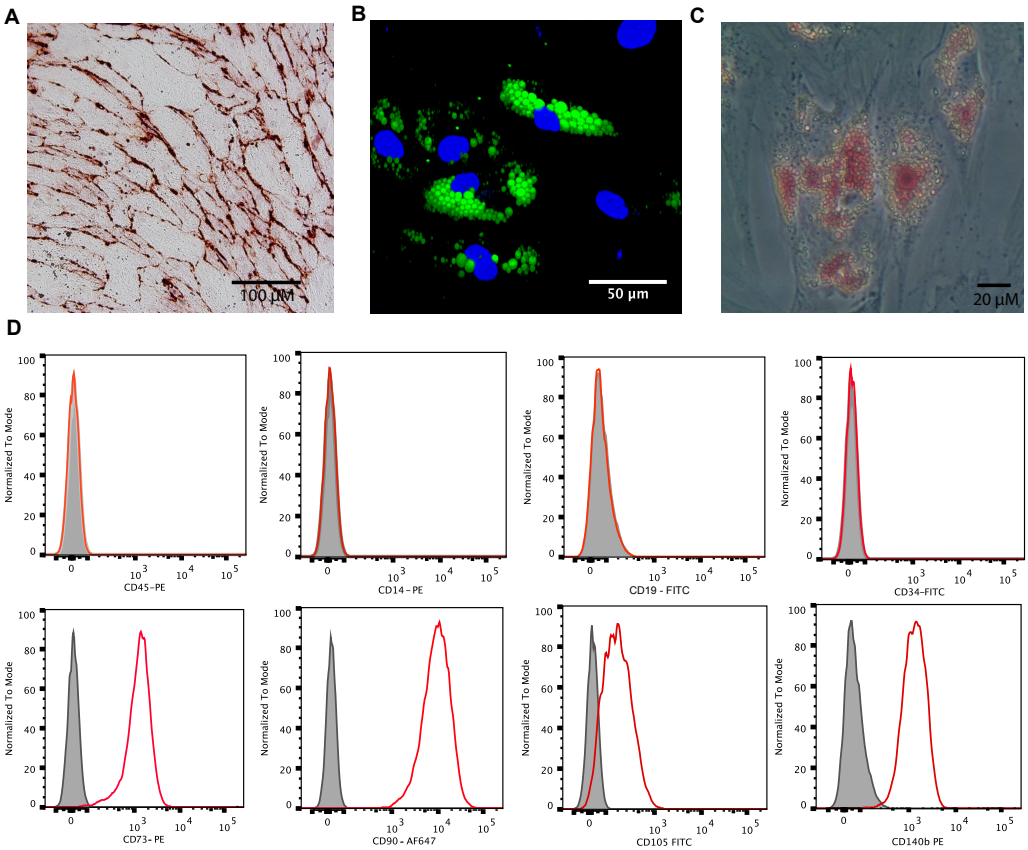

Figure 1: **A:** Alizarin red staining in MSCs after osteogenic differentiation. **B:** Confocal Z-stack of Lipidtox green staining after adipogenic differentiation, nuclei are stained blue with DAPI. **C:** Oil red staining of adipogenically differentiated MSCs **D:** MSC Characterization by Flow Cytometry. The red histograms represent MSCs stained with fluorochorme conjugated antibodies as listed under each graph, the shaded gray histograms are isotype controls for the fluorophore.

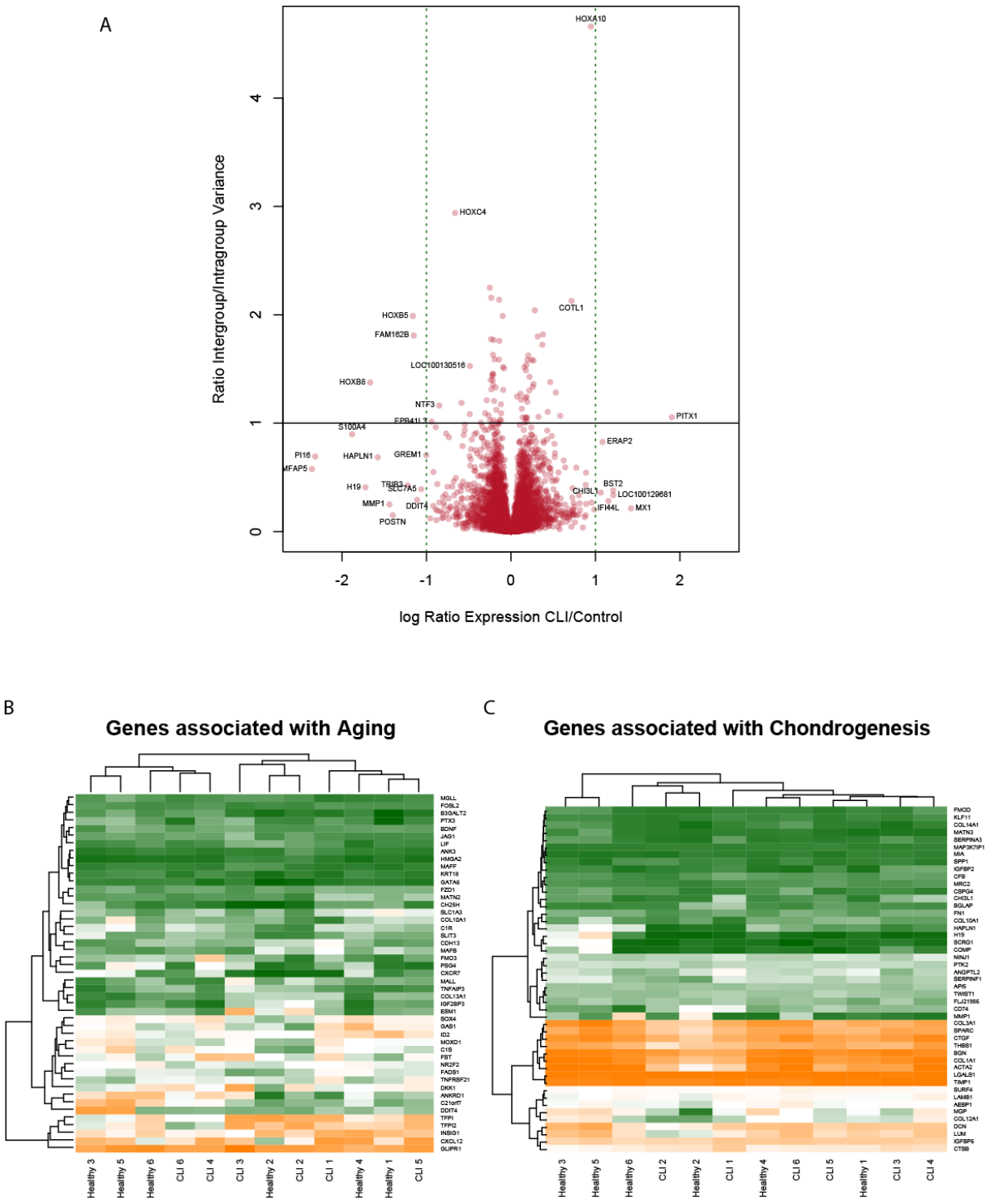

Figure 2: **A:** Modified Volcano plot showing intergroup vs intragroup variance for CLI-MSCs and Control MSCs. **B:** Heatmap showing genes associated with MSC donor aging, note the large variation in expression. **C:** Heatmap showing genes associated with chondrogenic differentation.

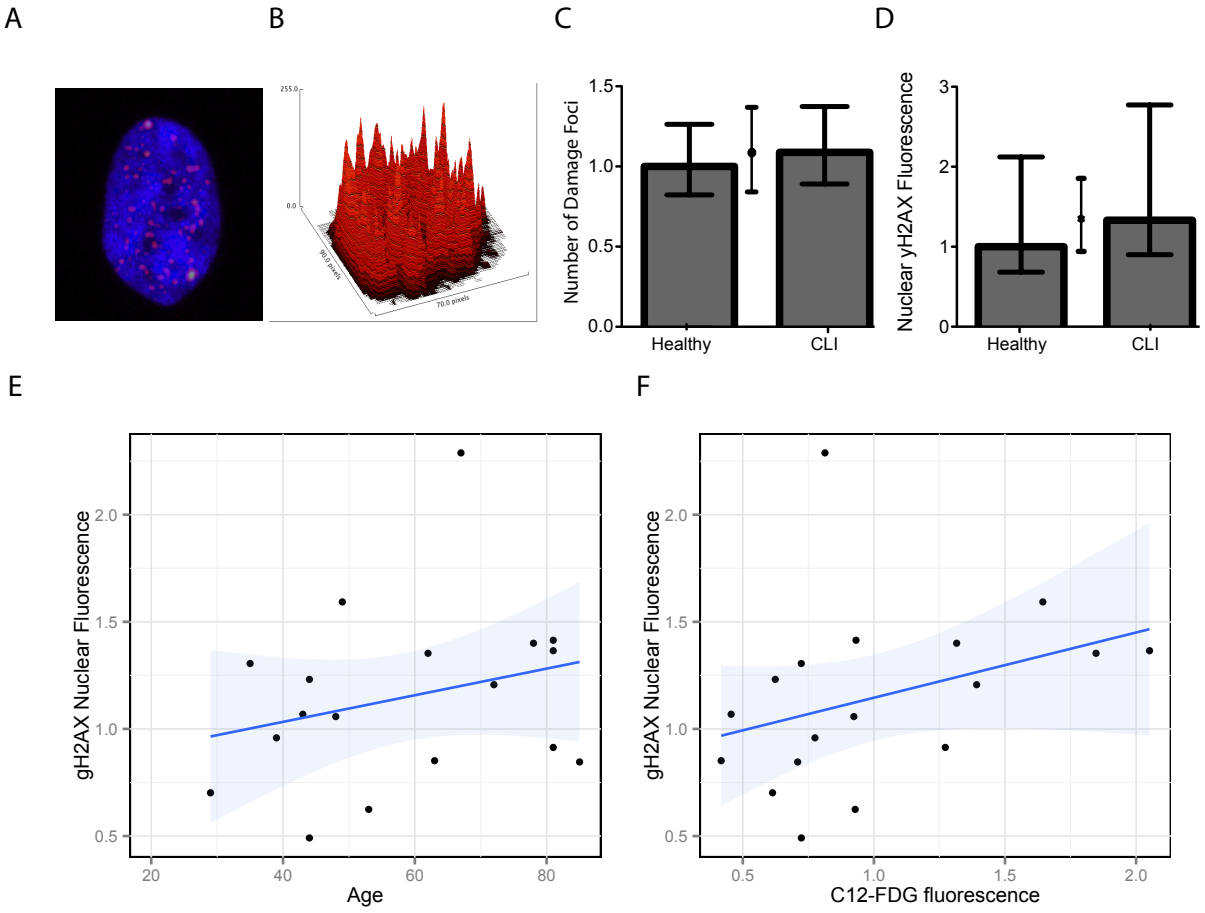

Figure 3: **A:** Representative nucleus showing γH2AX damage Foci **B:** Maxima as detected by software algorithm **C:** Total nuclear fluorescent signal γH2AX in nuclei of CLI-MSCs and controls (error bars indicate S.E.M.) **D:** Number of DNA damage foci in nuclei of CLI-MSCs and controls **E:** Association of γH2AX signal and age (R=0.27) **F:** Association of γH2AX signal and C12FDG Fluorescence (R=0.34), bands indicate 95% CI.

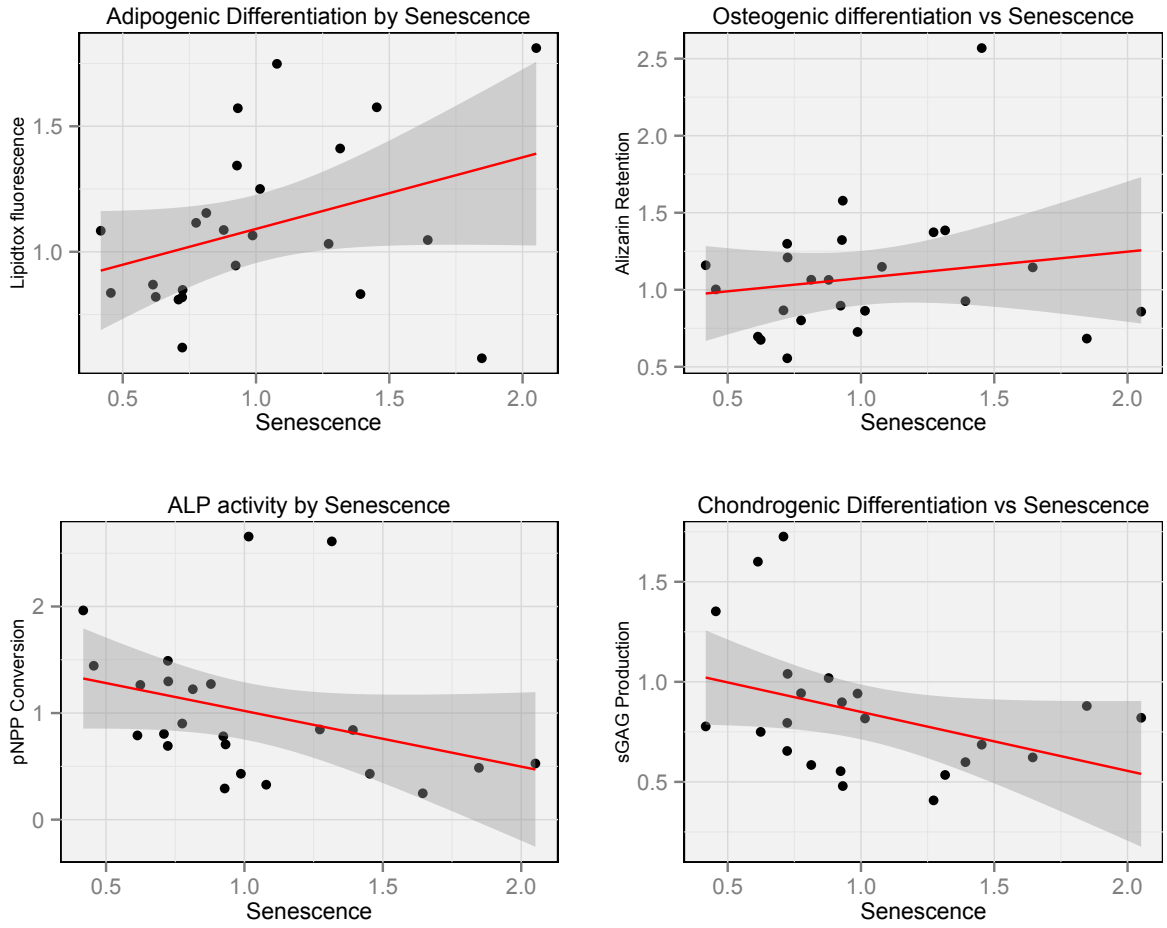

Figure 4: **Associations of Differentiation Capacity with Donor Senescence**. For Adipogenic Differentiation $R_{Senescence} = 0.41$ (p=0.04). For ALP activity (early osteogenic differentiation) $R_{Senescence} = -0.34$, (p=0.10). For Alizarin retention (late osteogenic differentation $R_{Senescence} = 0.18$ (p=0.4). For chondrogenic differentiation $R_{Senescence} = -0.34$ (p=0.10). Bands indicate 95% CI.

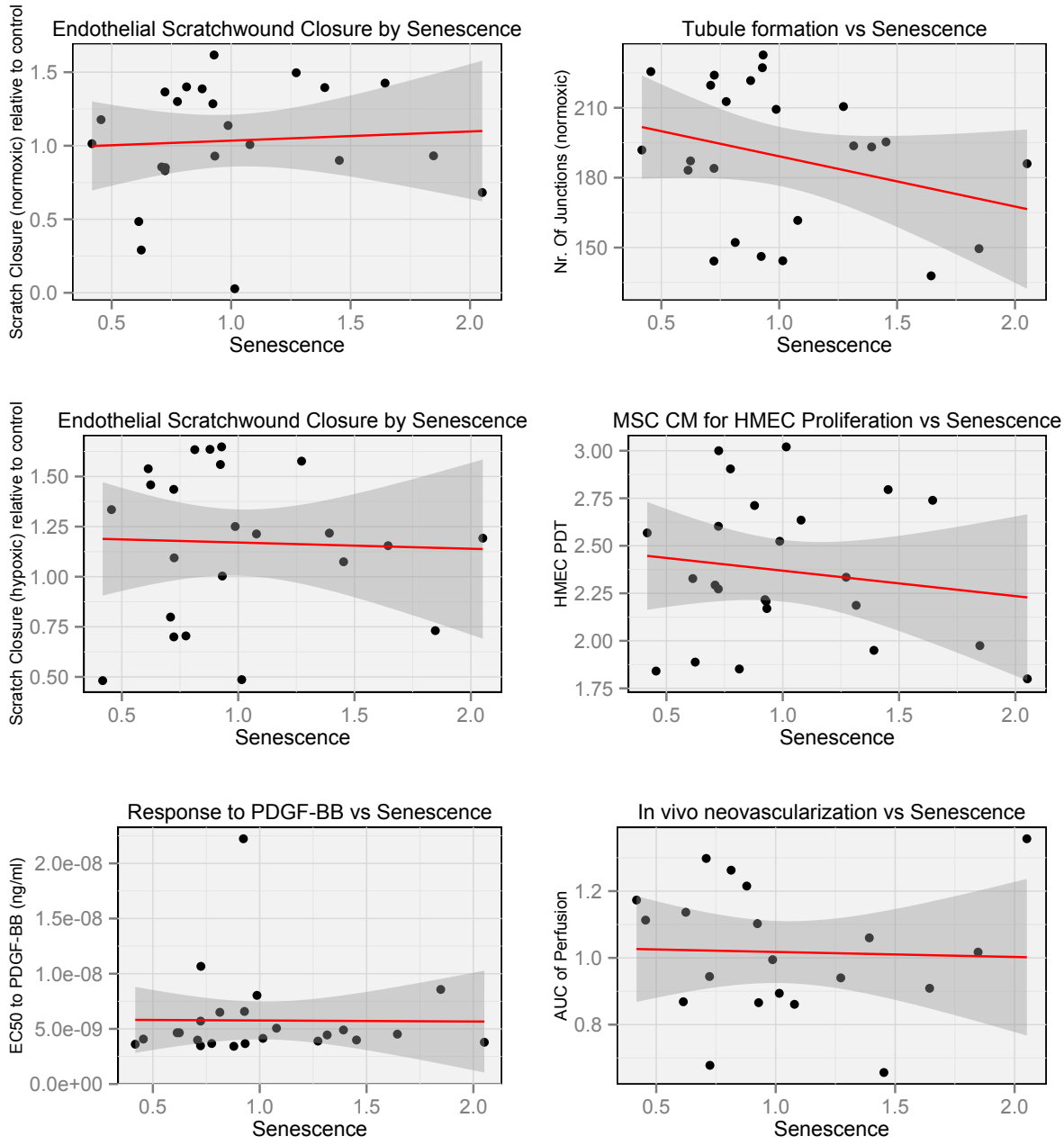

Figure 5: **Associations of Measures of Proangiogenic Capacity with Senescence.** Graphs showing the association of Endothelial Scratch wound closure in the presence of MSC-conditioned medium collected in normoxic R=0.07 and hypoxic conditions (R=-0.04), Donor EC_{50} to PDGF-BB (R=0.02), Junctions in Tubule formation of Endothelial Cells with MSC CM (R=-0.24), Endothelial Proliferation with MSC CM (R=-0.15), and neovascularization *in vivo* (R=-0.155) in relationship to senescence by MSC SA-β-activity per donor. Bands indicate 95% CI.

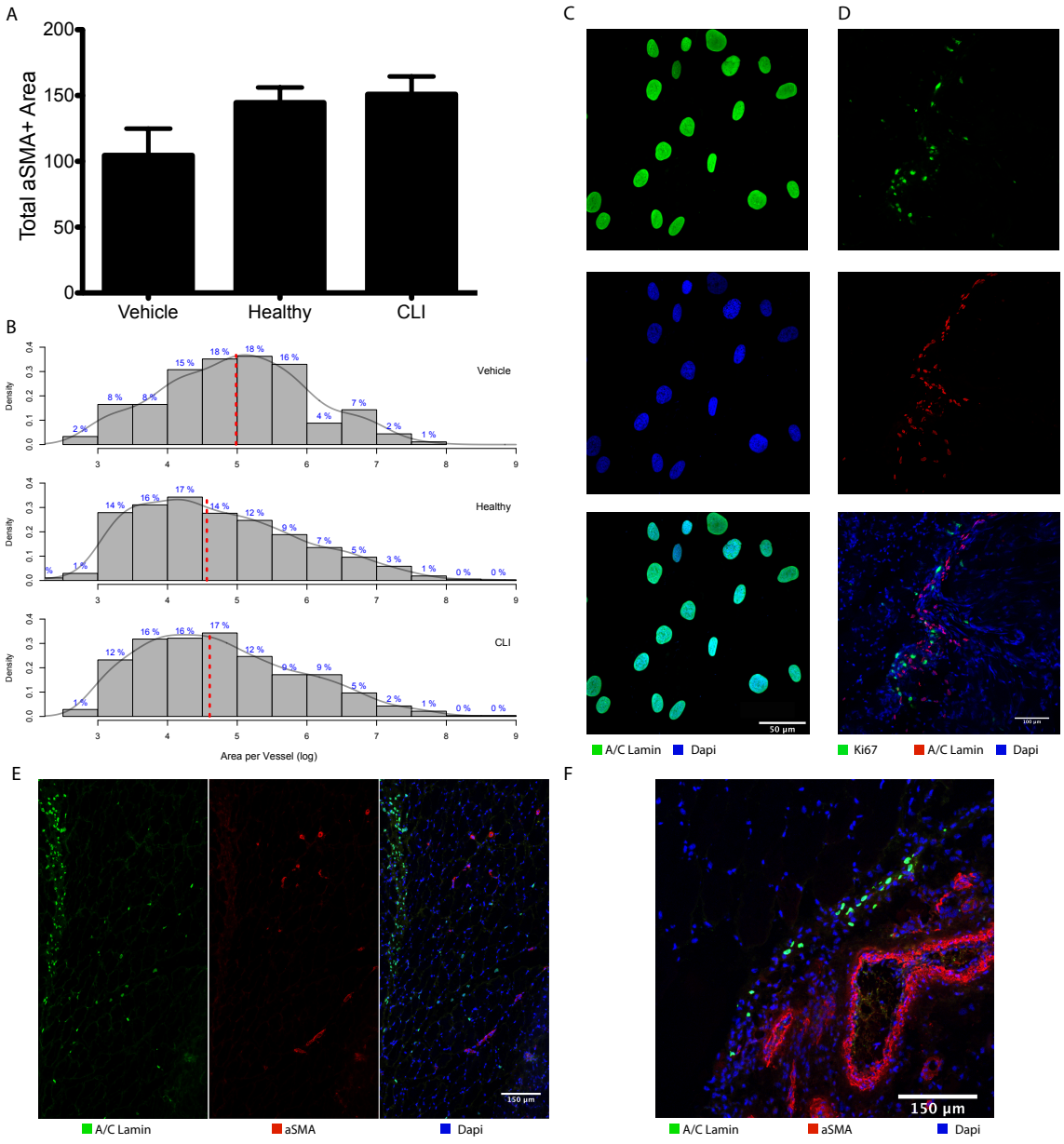

Figure 6: **Supplemental histology: A:** Barplot showing total aSMA+ Area per High Powered Field. **B:** Histograms showing the size distribution of aSMA+ Vessels. In the MSC treated groups an enrichment of of smaller aSMA+ vessels was observed (p=0.02). In conjunction with the trend towards an increased number of vessels in MSC treated animals, this indicates that MSC treatment mainly promotes early arteriogenesis. **C:** Human specific A/C Lamin staining on cultured MSCs **D:** Representative section of staining for proliferating cells (Ki67 - Green) and human nuclei (A/C Lamin - Red), no double staining nuclei were observed. **E:** Confocal photomicrograph showing lack of co-localization of human MSCs (green nuclei) with αSMA positive vessels. **F:** MSC do not appear to integrate into the adventitia of larger vessels when injected in close proximity.

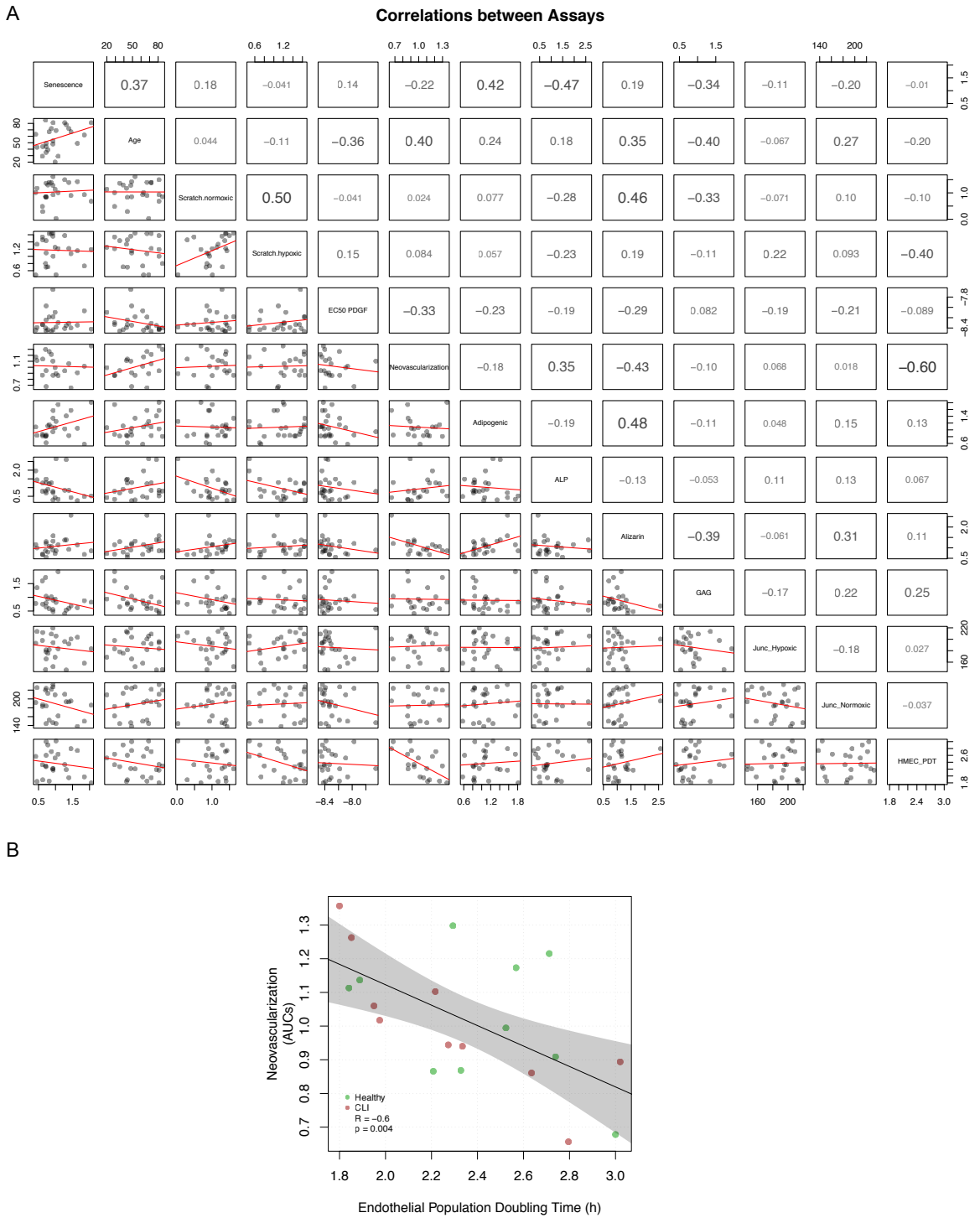

Figure 7: **Correlations between Assays: A:** Correlation matrix between all assays performed in this paper. The upper right half shows the spearman rank correlation coefficient (ρ) between assays. The lower left half shows the bivariate scatterplot with linear regression line. **B:** Graph showing the inverse correlation between the population doubling time of endothelial cells cultured in the presence of conditioned medium from a given MSC donor and the angiogenic effect of the same MSC donor in the hindlimb ischemia model. The confidence bands indicate 95% CI.

Supplementary Table 1

Top 50 of most differentially expressed genes. Fold Change values are CLI-MSC/Controls

Gene ID	logFC	AveExpr	adj.P.Val
HOXA10	0.94288377	7.57252979	0.0767918
HOXC4	-0.6594873	7.58156994	0.44694563
COTL1	0.71622031	8.22118014	0.77842982
HOXB5	-1.1614513	8.51924989	0.77842982
FAM162B	-1.1450439	7.27933693	0.93750713
MMS19	0.37761764	8.48965826	0.99998412
HOXB8	-1.6599107	7.40213098	0.99998412
FCHSD2	0.37101766	7.5905157	0.99998412
LOC100130516	-0.486479	12.4017821	0.99998412
LOC729466	0.28239049	12.4037808	0.99998412
ENDOD1	0.31549811	7.73883816	0.99998412
TMEM144	0.28015964	6.73183879	0.99998412
LOC100133517	-0.2495336	7.34863085	0.99998412
NLGN4X	0.40076781	7.0130284	0.99998412
CTNNAL1	0.46395048	9.46482092	0.99998412
LOC646483	0.53081555	11.2920232	0.99998412
NTF3	-0.8441367	7.33593998	0.99998412
MDFIC	-0.2330793	7.57678412	0.99998412
MSX1	-0.5854864	8.26462333	0.99998412
PITX1	1.89510127	7.99626955	0.99998412
C12orf51	0.26561028	7.36700007	0.99998412
MEMO1	-0.235202	7.83973898	0.99998412
HLA-H	0.58314878	10.7793849	0.99998412
EPB41L3	-0.9320435	6.92621386	0.99998412
STT3B	-0.4883526	8.05462192	0.99998412
LOC440589	0.24467132	13.978676	0.99998412
ISG20L1	0.43442876	8.20457094	0.99998412
HOXC8	-0.8901607	9.65277126	0.99998412
LOC100132439	0.26381908	6.81315882	0.99998412
GNG12	-0.5467546	8.82498482	0.99998412
CCNI2	0.25455825	6.77441999	0.99998412
LOC729970	-0.2855261	7.05040725	0.99998412
S100A4	-1.8803412	9.62178178	0.99998412
GPR89B	-0.2376861	7.32458817	0.99998412
RWDD1	-0.2040496	10.3629303	0.99998412
NIT2	-0.2130933	8.38670539	0.99998412
SCG5	-0.7683295	8.63124687	0.99998412
CPE	-0.5422083	6.83635888	0.99998412
NKX3-1	0.32497377	7.68010543	0.99998412
STAM2	-0.3046272	7.70290031	0.99998412
LOC404266	-0.3541471	6.90002896	0.99998412

HTRA2	0.20421374	7.70437432	0.99998412
LOC730996	-0.219369	6.9582107	0.99998412
C14orf147	-0.2165897	7.41689851	0.99998412
RAB11A	0.21940206	10.3500712	0.99998412
NFIB	-0.7350922	8.67198173	0.99998412
SGCE	-0.5602231	9.86945036	0.99998412
TMEM183A	-0.2318937	9.03825187	0.99998412
LOC100133565	0.42865646	7.79386441	0.99998412
LOC100128115	-0.210383	7.04753602	0.99998412

Extended Methods

CLI patients and healthy controls

Patient BM was harvested during the JUVENTAS trial, which investigates the efficacy of repeated intra-arterial BM-MNCs injections in patients with CLI [1] (Trial identifier: NCT00371371). Inclusion criteria for the trial consist of severe infra-popliteal arterial occlusive disease and ineligibility for surgical or endovascular revascularization procedures. For this study we cultured BM-MSCs from 12 sequentially included patients from March to August 2011. Control BM of 12 donors without PAD was collected during elective orthopedic interventions. Procedures were approved by the local ethics committee, and are in accordance with the Declaration of Helsinki.

Reagents

Reagents were purchased from Sigma-Aldrich, St. Louis, MO, USA, unless otherwise specified.

MSC isolation and culture

BM from all donors was obtained from the iliac crest by needle aspiration. MNCs were isolated using density-gradient centrifugation and counted using a CBC counter (Abbott CellDyn 1800). For each donor 10 x 10^6 MNCs were suspended in MEM alpha (Gibco, Grand Island, NY, USA), 10% Fetal Calf Serum (FCS) and were left to adhere for 24h in a 10cm^2 tissue culture well. Adherent cells were then washed once with phosphate buffered saline (PBS) on day 1 and medium was changed thrice weekly thereafter. Cells were expanded for three passages until they covered four 75cm^2 tissue culture flasks and were then cryopreserved in MEM alpha containing 20% FCS and 10% dimethylsulfoxide (DMSO), using an isopropanol-filled freezing container (Mr. Frosty, Thermo Fisher) for controlled-rate cooling. For long term storage cells were transferred to the vapor phase of liquid nitrogen, in a specialized container.

Cells were thawed by placing cryovials in a water bath set to 37°C and were resuspended in fresh medium immediately after the frozen suspension had melted. Cells were then washed once in fresh medium and seeded in culture flasks. All further experiments were conducted with cells in passage 3+1 and were started 48h after thawing and seeding the cryopreserved cells.

Flow Cytometric Analysis

Cultured MSCs were lifted by 3 min incubation with TrypLE® Express (Gibco) recombinant trypsin and washed with serum-containing medium. Cells were then incubated for 30 min at 4°C with human FcR blocking reagent (Miltenyi, Leiden, NLD) and the following antibodies: CD45-PE (#560975 BD Pharmigen, Breda, NLD), CD14 (#R0864, Dako, Heverlee, BEL), CD19 (130-091-328, Miltenyi), CD34 (BD #555821), CD73 (BD #550257), CD90 (#B113673 Biolegend, Fell, DE), CD105-Fitc (FAB 10971F, R&D, Minneapolis, MN, USA) and CD140b (BD #558821). After staining cells were washed with PBS and cell fluorescence was measured in 10 000 viable cells using a FACSCanto II flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). SytoxBlue (Molecular Probes/Invitrogen, Eugene, OR, USA) was used for exclusion of dead cells.

Whole-genome gene expression profiling

Total RNA was isolated using spin columns (Qiagen, Venlo, NLD). 1 µg of RNA from each cell sample was amplified and labelled cRNA was hybridized to an Illumina WT-12 v4.0 Expression Beadchip. Samples included RNA from the MSCs of 6 patients and 6 controls. Gene expression data obtained from Illumina Beadstudio was normalized using 'R' bioconductor with the 'lumi' package[2].

Differentiation protocols

For osteogenic and adipogenic differentiation, MSCs were seeded and grown until confluency on 24-well plates and then differentiated using StemPro Differentiation Kits

(Gibco) for 14 days. For chondrogenic differentiation, cells were trypsinized and $1x10^5$ cells were resuspended in 500µl chondrogenic differentiation medium (Gibco). Cells were subsequently centrifuged at 500 RCF for 15 min in 15ml polypropylene conical tubes[3]. Pelleted cells were incubated at 37°C under 5% CO_2 in a humidified incubator with loosened caps to permit gas exchange. Within 24h of incubation, the sedimented cells formed a spherical aggregate at the bottom of each tube. The cells were further cultured in the conical tubes for the remainder of the experiment; medium was changed every 3 days and cells were harvested at day 28.

Measurement of Alkaline Phosphatase activity
Alkaline Phosphatase (ALP) activity was measured by p-Nitrophenyl Phosphate (pNPP) conversion. Cells were lysed using Tris Buffered Saline with 0.1% Triton X-100 and incubated with a ready-made pNPP solution (Sigma-Aldrich) for 30 min. Absorption was measured at 405 nm in a spectrophotometric plate reader. Values were corrected for a cell number obtained by incubating the cells with PrestoBlue® (Molecular Probes) for 15 minutes and measuring fluorescence at 575 nm directly prior to the experiment.

Staining and Measurement of Calcium deposition by Alizarin Red retention
MSCs were fixed with 4% (w/v) paraformaldehyde for 15 min at room temperature. Cells were then stained with 40mM Alizarin Red staining solution for 20 min. Cells were washed 4 times with PBS and pictures were taken using an inverted microscope (Olympus, Zoeterwoude, NLD). For quantification of staining 200µl 10% (v/v) acetic acid was added and cells were incubated for 30 min as previously described [4]. The monolayer was subsequently transferred to a microcentrifuge tube and incubated at 85°C for 10 min. After neutralization with 10% (v/v) ammonium hydroxide, the absorbance was measured at 405nm. Cell number was corrected with PrestoBlue as described.

Fat Droplet formation Measurement by Oil Red O and Lipitox Green Staining
Cells were fixed for 20 min at room temperature using 4% paraformaldehyde and washed 3 times with PBS. Cells were subsequently stained with Lipidtox Green (Molecular Probes) or Oil Red O for 30 min at room temperature and pictures were taken using an inverted (fluorescent) microscope. Lipidtox Green staining was further quantified using a fluorescent plate reader (ThermoFisher, Waltham, MA, USA) with excitation and emission wavelengths of 488 and 538 nm respectively. Values are corrected for cell number with PrestoBlue as described.

Measurement of GAG production
Glycosaminoglycans were quantified using a commercial assay (Blyscan, Biocolor, Carrickfergus, UK). Briefly cell pellets were digested in a 20 mg/ml papain solution (Sigma) overnight and the resulting supernatant was analyzed for sGAGs by 1,9-dimethyl-methylene blue precipitation. The resulting precipitant was resuspended and absorption was measured at 650 nm.

Gamma-H2AX staining
As an additional marker of senescence we examined the number of spontaneous double strand breaks, by staining for phosphorylation of Histone 2AX at serine 139 (gamma-H2AX). MSCs were grown until confluency on Labtek II chamber slides. Cells were subsequently fixed in 4% paraformaldehyde and permeabilized with 0.5% Triton. Cells were stained overnight with anti-Ser139 H2AX (gH2AX) (Millipore). As secondary antibody anti-mouse IgG AlexaFluor 555 was used and nuclei were visualized using Hoechst 33342. Images were taken on an Olympus BX61 fluorescent microscope. Analysis of the images was performed using ImageJ; images were segmented into regions of interest on the basis of the nuclei in the Hoechst image. For each individual nucleus the total fluorescence of the gH2AX signal was recorded, as well as the number of damage foci. A minimum of 50 (mean 112 ± 37) nuclei per donor was counted.

Fluorescent staining for senescence with C12 FDG
8 x 10^4 cells per donor were seeded in a 6-well plate and cells were left to adhere overnight. Cells were then incubated with 100nM bafilomycin A1 (Sigma) for 1 h to induce lysosomal alkalinization[5]. Next 5-dodecanoylaminofluorescein di-β-D-galactopyranoside (C12FDG, Invitrogen) was added to a final concentration of 30μM and cells were incubated for an additional hour. Cells were trypsinized and median cellular fluorescence was quantified using Flow Cytometry. SytoxBlue ® (Invitrogen) was added just before measurement to identify dead cells, which were excluded from analysis.

Migration assays
Real-time measurement of cell migration was performed using the xCELLigence RTCA DP device from Roche Diagnostics (Mannheim, DEU). This system is a variant of the two-chamber trans-well in which vertical cell migration through a microporous membrane is recorded by measuring impedance on the underside of the membrane. In the lower wells of CIM-Plates 16 with 8μm pore-size, a short serial dilution of (Platelet-derived growth factor subunit B homodimer) PDGF-BB was prepared in serum-free medium, for each donor. In the upper wells 40000 MSCs were seeded in serum-free medium and allowed to settle for 15 min prior to the beginning of the experiment. All conditions were conducted in duplicate. Data acquisition and analysis was performed with the RTCA software (version 1.2, Roche Diagnostics).
For each well, the end-point migration was calculated by subtracting the baseline value from the point of maximum impedance. A migration index was calculated by subtracting the cumulative migration from the chemo-attractant-free control. MSCs from each donor were allowed to migrate towards a dose-range of 0, 5, 10 and 20 ng/ml PDGF-BB. The half-maximal effective dose was calculated for each donor by fitting a four-parameter log-logistic function.

Collection of Conditioned Medium and Endothelial Scratch-wound Assay
8 x 10^4 Cells per donor were seeded in a 6-well plate and cells were left to adhere for 24 hours. Conditioned medium (CM) was then collected in serum free aMEM for 24 hours. For each donor CM was collected both under normoxic and hypoxic (2% O_2) culturing conditions and stored at -80°C for further experiments.
To assess the ability of the CM to induce endothelial migration, a scratch-wound assay on immortalized human microvascular endothelial cells (HMEC-1) was used [6]. HMEC1 cells were grown until they formed a confluent monolayer on 24-well plates. A scratch in the monolayer was made across the well using a pipette tip, detached cells were washed off and MSC CM or control stimuli were added to the wells. Photographs were made on demarcated locations in the well at baseline and after 6 hours. Average scratch width per high powered field was calculated by dividing the area of the scratch by the length and migration was subsequently calculated by subtracting width at t=0 by width at t=6h.

Endothelial Proliferation
2000 HMEC-1 Endothelial Cells were seeded on a collagen I (Rat Tail, BD, Biosciences) coated E-Plate 16 (ACEA Biosciences, San Diego) and allowed to attach for 3 hours, at which point a stable impedance signal had been achieved. Cells were then washed once with serum free aMEM, and MSC Conditioned Medium was added.
Cell proliferation was then quantified in real time using Cell Impedance Measurement using an xCELLigence RTCA DP device from Roche Diagnostics (Mannheim, DEU). Cell Index (derived from impedance measurements at 10, 25 and 50 kHz and corrected for unseeded baseline) was recorded every 15 minutes for 72 hours. Population Doubling Time (PDT) was subsequently calculated using the linear part of the Gompertz Curve.

Tubule Formation
Tubule formation assays were performed in ibidi µ-Slide angiogenesis slides (ibidi, Martinsried, Germany). 10µl of Growth Factor Reduced Matrigel (BD Biosciences) was added into the lower compartment of the slide and allowed to solidify for 45 minutes. HMEC-1 cells were lifted and washed twice with serum free aMEM. Cells were then suspended in MSC-CM and 10000 cells were added per well (all conditions were repeated in triplicate), Endothelial Growth Medium 2 (EGM-2) with 10% FCS, was used as positive control. Cells were then allowed to form tubules for 24h, after which photomicrographs were taken using an Olympus BX53 microscope. Images were analyzed using an automated imageJ plugin [7]

Hind-limb ischemia Model
All animal experiments were conducted after approval of the local ethical committee in accordance with the Dutch law on animal experimentation (Protocol # 2012.II.04.070). Hind-limb ischemia was induced in 8-10 week old male nude NMRI FoxN1$^{nu/nu}$ mice (Harlan, Horst, NLD). Prior to surgery mice were anesthetized using a mixture of medetomidine (Eurovet, Bladel, NLD) and midazolam (Roche, Woerden, NL) and given perioperative analgesia through fentanyl (Janssen-Cilag, Tilburg, NLD). The femoral artery was occluded with the use of an electrocauterization device (Bovie, Clearwater, FL) directly as it emerged from under the femoral ligament, closing off both superficial and deep branches. The femoral vein and nerve were left intact. The segment of the superficial femoral artery distal to the occlusion was then carefully stripped away from the adjacent vein until the popliteal bifurcation was reached. After removal of the arterial segment, the overlaying skin was closed using surgical staples and anesthesia was antagonized using atipamezole (Eurovet) and flumazenil (Roche).

Cell administration and Laser Doppler Perfusion Imaging
Cell injections were performed 24 hours after femoral ligation. For the dose ranging 1, 3.3, and 10 x 10^5 MSCs were injected intramuscularly divided over 5 different sites in the adductor muscle (10µL/injection). In subsequent experiments using patient derived cells 1 x 10^5 MSCs were used.
MSCs from 10 donors in each group were injected in 3 mice per donor; in addition a total of 21 vehicle controls was included. Relative perfusion of the ischemic limb compared to the control limb was measured using Laser Doppler Perfusion Imaging, with moorLDI2-HR (Moor Instruments, Devon, UK) imager with an 830nm Helium-Neon laser. For measurements mice were anesthetized using a mixture of medetomidine and midazolam and kept warm on a heating pad. After measurement anesthesia was antagonized by flumazenil and atipamezole. The dorsal surface of each foot was measured and regions of interests were drawn around the foot and digits on basis of the optical Laser image. The median pixel intensity per ROI of ischemic/control limb was used as readout for relative perfusion. Relative perfusion was followed over time by measurements on Day 0, 1, 4, 7, 10 and 14.

Histology
Mice were sacrificed 14 days after cell injection by cervical dislocation under anesthesia. Capillary density was determined in 8µM frozen sections of the adductor muscles, collected 14 days after cell injection. Endothelial cells were stained for CD31 with Goat-anti-Mouse Pecam (Santa Cruz sc-1506) and muscle fiber perimysia were stained with FITC conjugated Triticum vulgaris lectin (Sigma). The number of capillaries is given as ratio of vessels/muscle fibers. Human MSCs were visualized with anti-human nuclear A/C lamin (Vector labs cat #. VP-L550), using an AlexaFluor 488 conjugated secondary antibody (Invitrogen). Nuclei of proliferating cells were stained with anti Ki67 (Thermo Scientific # RM 9106). Anti-∝ Smooth Muscle Actin staining was performed using Cy3-conjugated anti aSMA (Sigma, Clone 1A4).

Design and Statistics

In order to provide an easily interpretable value to compare CLI- to Control MSCs in the various assays performed in this study, values are presented as relative to the Healthy MSC donor group. As indicator of precision the Bayesian 95% credibility interval is given. Both *in vitro* and *in vivo* experiments were divided into balanced cohorts of 3 vs 3 donors, as was dictated by the number of slots on the xCelligence machine (the limited number of cells at P3+1 required a measure of coordination in the experiments). Data were analyzed by using generalized linear mixed models, using a restricted maximum likelihood (REML) approach to estimate model parameters. Cohort differences were modeled as random intercept. P-values and (highest posterior density, HPD) credibility intervals were estimated by means of Markov Chain Monte Carlo (MCMC) sampling from the posterior distribution of parameter values (10000 iterations). In the analysis of the HLI experiments a hierarchical linear mixed model was used, in which mice were nested within MSC donors. Relative perfusion was modeled as function of treatment group and time, using both random intercepts and random slopes at the mouse and donor level. To create a meaningful summary measure for equivalency we calculated areas under curve (AUCs) of relative perfusion over time. Analyses were conducted in 'R' software [8] (version 2.15.3), using the lmer function in the lme4 package [9]. Groups were considered equivalent when 95% of the posterior distribution was within a pre-defined threshold. As threshold a range of 0.8-1.25, taken from bioequivalency guidelines set forth by drug regulatory agencies such as the FDA or EMA [10], was used.
In the microarray experiment, a moderated T-test approach [33] as employed in the 'limma' package was used to test for differences between groups. As a lack of a statistically significant difference may for some genes be attributable to a lack of power in the experiment, we took a reverse approach to analysis by excluding genes that with 95% confidence did not differ by more than a factor 2 in expression and are thus considered equivalently expressed (EE), as is proposed in [11]. Genes were considered to be equivalently expressed if $\frac{C-|D_g|}{s_{\overline{X_1}-\overline{X_2}}} > t_{0.95,Df}$ where C is a cut-off value of a factor 2 differential gene expression, D_g is the absolute difference in log intensity between the group averages.

Suppl. References

1. Sprengers, RW, Moll, FL, Teraa, M, Verhaar, MCJUVENTAS Study Group (2010). Rationale and design of the JUVENTAS trial for repeated intra-arterial infusion of autologous bone marrow-derived mononuclear cells in patients with critical limb ischemia. *J. Vasc. Surg.* **51**: 1564–1568.
2. Du, P, Kibbe, WA and Lin, SM (2008). lumi: a pipeline for processing Illumina microarray. *Bioinformatics* **24**: 1547–1548.
3. García-Álvarez, F, Alegre-Aguarón, E, Desportes, P, Royo-Cañas, M, Castiella, T, Larrad, L, *et al.* (2011). Chondrogenic differentiation in femoral bone marrow-derived mesenchymal cells (MSC) from elderly patients suffering osteoarthritis or femoral fracture. *Archives of Gerontology and Geriatrics* **52**: 239–242.
4. Gregory, CA, Grady Gunn, W, Peister, A and Prockop, DJ (2004). An Alizarin red-based assay of mineralization by adherent cells in culture: comparison with cetylpyridinium chloride extraction. *Anal. Biochem.* **329**: 77–84.
5. Debacq-Chainiaux, F, Erusalimsky, JD, Campisi, J and Toussaint, O (2009). Protocols to detect senescence-associated beta-galactosidase (SA-βgal) activity, a biomarker of senescent cells in culture and in vivo. *Nat Protoc* **4**: 1798–1806.
6. van Balkom, BWM, de Jong, OG, Smits, M, Brummelman, J, Ouden, den, K, de Bree, PM, *et al.* (2013). Endothelial cells require miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells. *Blood* **121**: 3997–4006.
7. Carpentier, G, Martinelli, M, Courty, J and Cascone, I (2012). Angiogenesis Analyzer

for ImageJ. *4th ImageJ User and Developer Conference proceedings*, Mondorf-les-Bains, Luxembourg, 4pp: pp 198–201.
8. R Foundation for Statistical Computing. R: A language and environment for statistical computingat <http://www.R-project.org/>.
9. Bates, D, Maechler, M and Bolker, B. lme4: Linear mixed-effects models using S4 classes.at <http://CRAN.R-project.org/package=lme4>.
10. Karalis, V, Symillides, M and Macheras, P (2011). Bioequivalence of Highly Variable Drugs: A Comparison of the Newly Proposed Regulatory Approaches by FDA and EMA. *Pharm Res* **29**: 1066–1077.
11. Qiu, J and Cui, X (2010). Evaluation of a Statistical Equivalence Test Applied to Microarray Data. *J Biopharm Stat* **20**: 240–266.