Supplemental Material

Detailed Methods

Study protocol and statistics

Primary endpoints of the study were BM capillary density and abundance of BM $CD34^{pos}$ cells. Secondary endpoints comprise sinusoid and arteriole density and antigenic and transcriptional characteristics of $CD34^{pos}$ cells. In pilot studies, we determined that a sample size of 7 in each of the 3 groups would have an 80% power to detect a difference between means of 13.6 capillaries per mm^2 with an expected average standard deviation of 8.3 and a significance level (alpha) of 0.05 (two-tailed). The same group size would have a 90% power to detect a difference between means of 1.1 $CD34^{pos}$ cells/100 BM mononuclear cells (BM-MNCs) with an expected average standard deviation of 0.58. Recruitment of patients was continued until reaching the requested sample size as by power calculation for primary endpoints. Since patients were screened consecutively, the actual sample size eventually exceeded the minimum requested in 2 of the 3 groups. Moreover, depending on the dimensions of bone leftovers, we attempted to perform all the analyses on the same sample. When this was not possible, priority was given to histological analyses until reaching the requested group size and then to flow cytometry. **Online Table II** summarizes the samples attribution to different assays.

In order to verify the direct effect of hyperglycemia on molecular mechanisms, $CD34^{pos}$-PCs were immunosorted from the BM of 5 non-diabetic patients undergoing hip replacement surgery and used for expressional studies and colony forming unit (CFU) assays, following exposure to HG with or without miR-155 forced expression.

Results are presented as means±SEM. Normal distribution of the variables of interest was verified with D'agostino and Pearson test. data failed to pass normality or equal variance tests, non-parametric analysis was applied and results were expressed as median with 5-95 percentile distribution. For gene expression studies, logarithm transformation of the data was used followed by parametric tests. Multiple groups were compared by parametric analysis of variance (ANOVA), followed by Bonferroni t test, or non-parametric analysis of variance on ranks, followed by Tukey pairwise comparison or Dunnett's test for multiple comparisons against a single control group. Comparison of 2 groups was carried out by paired or unpaired Student t test or Mann-Whitney rank sum test. Comparison of categorical variables was made by chi-square test or Fisher's exact test. In addition, the Cohen's *d* index was calculated to determine if a statistically significant difference was of biological importance. The results of Cohen's *d* are considered to be small (< 0.5), medium (0.5 – 0.8), or large (>0.8).

In order to control the effects of background factors, means of dependent variables were compared and adjusted across the study groups by analysis of covariance (ANCOVA) (**Online Tables III** and **IV**). Furthermore, multiple linear regression analyses were performed to verify if a given endpoint is predicted by combination of independent variables, including group, duration of diabetes, fasting glycemia, age, gender, coronary artery disease (CAD), stroke, hypertension, body mass index (BMI), smoking and treatment (**Online Table V**). Since grouping factor, duration of diabetes and fasting glucose showed a high collinearity index (~2), each of the 3 independent variables was computed distinct from the other 2 in multiple linear regression analyses. The relationship between miR-155 and FOXO3a expression in $CD34^{pos}$ cells was calculated using the Spearman correlation coefficient. A p value <0.05 was considered significant. Stated *n* values represent biological replicates.

Histomorphometry, immunohistochemistry, and immunofluorescence analyses

A femoral bone fragment of at least 0.5 cm^3 was fixed in 10% neutral buffered formalin, decalcified in 10% formic acid, and embedded in paraffin. Analyses were conducted on 3 micrometer-thick BM sections mounted on silane-coated slides (Dako, Denmark). Briefly, for histomorphometric studies, BM sections were stained with Hematoxylin-Eosin (HE) and images captured with a Nikon E800light microscope (Nikon, The Netherlands) were analyzed to assess the marrow composition using Image J, a Java-based processing program from the National Institutes of Health (NIH) (3 sections and 4-field/section for each patient).

For immunohistochemistry, BM staining was conducted with primary antibodies followed by appropriate secondary antibody (**Online Table VI**). A Peroxidase/DAB commercial kit

(EnVision Detection Systems, Dako, Denmark) was used as revelation system. Counterstaining of nuclei was performed using Mayer's Hematoxylin (Sigma, St. Louis, USA). Images were captured with a Nikon E800light microscope as mentioned above.
Immunofluorescence microscopy was performed by incubating BM sections with appropriately diluted primary antibodies overnight at 4°C, followed by secondary fluorescence-conjugated antibodies for 1 hour at room temperature. Image acquisition was carried out with a confocal laser microscope (Leica TCS-SP2, Leica Microsystems, Germany).
In addition, percentage of apoptotic BM cells was measured as function of FITC-positive nuclei that bear DNA breaks labeled by TUNEL assay (Calbiochem, Germany). Fluorescence was visualized and captured using AXIO OBSERVER A1 microscope equipped with digital image processing software (AxioVision Imaging System), both from Zeiss (Germany).

Cell isolation and culture
MNCs were isolated from total BM single cell suspension by centrifugation on Histopaque-1077 density medium (Sigma, St. Louis, USA). $CD34^{pos}$ cells were separated from BM-MNCs by magnetic bead-assisted cell sorting (MACS, MiltenyiBiotec, Germany). CD34 purity was ~80% as confirmed by flow cytometry.
Cells were then used for molecular biology studies either immediately after sorting or following exposure to high glucose (HG). To the latter aim, $CD34^{pos}$ cells were cultured for 24 hours in standard medium (EBM) (LONZA, Switzerland), 10% fetal bovine serum (FBS), human Stem Cell Factor (hSCF, 100ng/mL) (RD, USA), interleukin 3 (IL3, 20 ng/mL), and FMS Like Tyrosine Kinase 3 Ligand (Flt3-L, 100ng/mL) (both from Provitro Gmbh, Germany) containing 5 mM D-glucose (normal glucose, NG), or 25mM D-glucose (HG). Preservation of the antigenic profile after cell culture was confirmed by flow cytometry.

miR-155 Transfection
$CD34^{pos}$ were transfected with 50 nmol/L pre-miR-155 or negative control (a non-targeting sequence, also identified as scramble, SCR, throughout the manuscript) (all from Applied Biosystems) using GeneSilencer (Dharmacon) following the manufacturer's instructions. Changes in relative miR-155 expression were measured by TaqMan PCR (*vide infra*).

Colony Forming Unit Assay
$CD34^{pos}$ cells were transduced with miR-155or scramble control (Applied Biosystem) as mentioned above and used in standard colony-forming unit (CFU) assays for myeloid and erythroid differentiation following the manufacturer's instructions (Stem Cells Technologies). Colonies were harvested at day 14 and isolated cells were placed on glass slides for staining with specific markers. In particular, myeloperoxidase (MPO), a marker of granulocytes, was used to stain cells in CFU-GEMM colonies, CD68, a marker of macrophages, to stain cells from CFU-GM colonies, and glyphorin, a marker of erythrocutes to characterize cells from CFU-E colonies.

Flow cytometry
The following cell populations were identified within BM-MNCs and PB-MNCs as previously described in detail:[1-5] HSCs ($CD45^{dim}CD34^{pos}$ and $CD45^{dim}CD34^{pos}CD133^{pos}$), $CD34^{pos}CD14^{pos}CD45^{dim}KDR^{pos}CXCR4^{pos}$ and $CD34^{pos}CD14^{neg}CD45^{neg}KDR^{pos}CXCR4^{pos}$ cells, Natural Killer cells (NKs, $CD3^{neg}CD56^{pos}CD16^{pos}$), T-lymphocytes ($CD45^{pos}CD3^{pos}$), B-lymphocytes ($CD45^{pos}CD19^{pos}$), non-hematopoietic SCs ($CXCR4^{pos}CD34^{pos}CD45^{neg}$ and c-Kit^{pos} cells) and endothelial cells (ECs, $CD45^{neg}CD31^{pos}CD144^{pos}$).
Cell cycle was analyzed on $CD34^{pos}$ cells fixed in 70% ethanol solution for 18 hours at -20°C. Cells were then washed twice with cold PBS, incubated in a 0.1% TritonX-100, 0.1% sodium citrate, 50μg/ml propidium iodide solution for 40min at 37°C in the dark, and analyzed by flow cytometry within 45 min.
For each test, $1X10^5$ to $5X10^6$ total events were analyzed in a FACSCanto flow cytometer using the FACSDiva software (both from BD Biosciences, New Jersey, USA).

RT-PCR analysis on sorted $CD34^{pos}$ BM-MNCs

RNA extraction and cDNA synthesis was performed using Power SYBR® Green Cells-to-CT™ Kit(Applied Biosystems, California, USA).
PCR primers for gene expression were designed with Primer3 software. CDKN1A-p21 (sense: 5'-GACACCACTGGAGGGTGACT-3'; antisense: 5'-CAGGTCCACATGGTCTTCCT-3'); CDKN1B-$p27^{kip1}$ (sense: 5'-GAGTGGCAAGAGGTGGAGAA-3'; antisense: 5'-GCGTGTCCTCAGAGTTAGCC-3'); FOXO3A (sense: 5'- ACAAACGGCTCACTCTGTCC-3'; antisense: 5'- ATTCTGGACCCGCATGAAT 3'); 18S (sense: 5'-CGCAGCTAGGAATAATGGAATAGG-3'; antisense: 5'-CATGGCCTCAGTTCCGAAA-3').
RNA reverse transcription and PCR to measure miR expression was performed using commercially available TaqManmiRNA reverse transcription kit and miR-specific primers, according the manufacturer's instructions (Applied Biosystems, California, USA). Each Real time PCR reaction was performed in triplicate, and relative expression of mRNAs and miRs was calculated by the $2^{-\Delta\Delta Ct}$ method [6] using 18S ribosomal RNA or the U6 small nucleolar RNA (snRU6) as endogenous control respectively.

Supplemental References

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Online Table I: Characteristics of study subjects

	Controls (n=49)	T2D (n=10)	T2D+CLI (n=23)	Test	p=
Age, years	70 (42-85)	73 (55-78)	76 (37-88)	ANOVA	0.14
Male, %	57	90	57	Chi-square	0.13
BMI, kg/m^2	27 (20-36)	33 (24-38)	26 (21-35)	ANOVA	<0.01
Diabetes duration, years	-	9 (1-15)	20 (14-36)	Unpaired t test	<0.001
Fasting glucose, mg/dL	91 (72-129)	122 (102-170)	182 (91-277)	Kruskal-Wallis test	<0.001
HbA1c , %Hb	ND	6.6 (6-8.2)	7.4 (6.0-12.1)	Unpaired t test	0.13
Smoking, %	11	0	65	Chi-square	<0.001
Hypertension, %	47	90	96	Chi-square	<0.001
Complications, %					
Coronary artery disease	14.8	20.0	56.5	Chi-square	0.001
Stroke	2.1	0	39.1	Chi-square	<0.001
Retinopathy	-	10	34.8	Fisher exact test	0.41
Neuropathy	-	10	14	Fisher exact test	0.94
Nephropathy	-	10	39.1	Fisher exact test	0.25
Laboratory tests					
LDL, mg/dL	78 (21-190)	104 (19-126)	ND	Unpaired t test	0.74
HDL, mg/d L	43 (20-84)	48 (28-62)	ND	Unpaired t test	0.56
Triglycerides, mg/dL	107 (53-311)	119 (51-171)	125 (73-314)	Kruskal-Wallis test	0.58
Creatinine, mg/dL	0.8 (0.5-1.6)	1.0 (0.7-2.1)	1.1 (0.6-2.6)	Kruskal-Wallis test	0.01
Medications					
Insulin, %	-	11	90.4	Fisher exact test	<0.001
Oral anti-diabetic drugs, %	-	100	4.7	Fisher exact test	<0.001
Glitazones, %	-	33	0	Fisher exact test	<0.05
Statin, %	4.3	40.0	52.3	Chi-square	<0.001
Anti-hypertensive drugs, n.	1 (0-2)	2 (0-4)	2 (0-2)	Chi-square	<0.001

Quantitative data are expressed as median with minimum and maximum. T2D: Type 2 diabetic patients, CLI: Critical Limb Ischemia, CAD: Coronary Artery Disease, ND: not determined

Online Table II. Samples distribution to different assays

Number	Group	Morphometry	IHC	FACS	PCR
1	Control	x	x		
2	Control	x	x		
3	Control	x	x		
4	Control	x	x	x	
5	Control	x	x	x	
6	Control	x	x	x	
7	Control	x	x	x	
8	Control	x	x	x	
9	Control	x	x	x	
10	Control	x	x		x
11	Control			x	
12	Control			x	
13	Control			x	
14	Control			x	
15	Control			x	
16	Control			x	
17	Control			x	
18	Control			x	
19	Control			x	
20	Control			x	
21	Control			x	
22	Control			x	
23	Control			x	
24	Control			x	
25	Control			x	x
26	Control			x	x
27	Control			x	x
28	Control			x	x

29	Control			x	x
30	Control				x
31	Control				x
32	Control				x
33	Control				x
34	Control				x
35	Control				x
36	Control				x
37	Control				x
38	Control				x
39	Control				x
40	Control				x
41	Control				x
42	Control				x
43	Control				x
44	Control				x
45	Control				x
46	Control				x
47	Control				x
48	Control				x
49	Control				x
Number	**Group**	**Morphometry**	**IHC**	**FACS**	**PCR**
1	T2D	x	x	x	
2	T2D	x	x	x	
3	T2D	x	x	x	
4	T2D	x	x	x	x
5	T2D	x	x	x	x
6	T2D	x	x	x	x
7	T2D	x	x	x	x
8	T2D			x	x

9	T2D			x	x
10	T2D				x
Number	**Group**	**Morphometry**	**IHC**	**FACS**	**PCR**
1	T2D+CLI	x	x	x	
2	T2D+CLI	x	x	x	
3	T2D+CLI	x	x	x	
4	T2D+CLI	x	x	x	
5	T2D+CLI	x	x	x	
6	T2D+CLI	x	x		
7	T2D+CLI	x	x	x	
8	T2D+CLI	x	x	x	x
9	T2D+CLI	x	x		x
10	T2D+CLI	x	x		
11	T2D+CLI		x		
12	T2D+CLI		x		
13	T2D+CLI		x		
14	T2D+CLI		x		
15	T2D+CLI			x	
16	T2D+CLI			x	
17	T2D+CLI			x	
18	T2D+CLI			x	x
19	T2D+CLI			x	
20	T2D+CLI			x	
21	T2D+CLI			x	x
22	T2D+CLI				x
23	T2D+CLI				x

Online Table III: Analysis of covariance for histological and cellular endpoints.

		Dependent Variable	Group mean values			Pairwise Comparison, p=			Between-group p=
			C	T2D	T2D+CLI	C vs. T2D	C vs. T2D+CLI	T2D vs.T2D+CLI	
Covariates: Age, Gender, BMI	Histology/IHC	*Hematopoietic fraction*	50.2±4.1	33.5±5.3	21.5±3.9	0.0729	0.0002	0.2807	<0.001
		Fat fraction	38.1±3.5	60.7±4.5	76.1±3.4	0.0031	<0.0001	0.0466	<0.001
		Capillaries	25.3±2.4	11.3±3.1	11.8±2.3	0.0075	0.0022	1.0000	0.001
		Sinusoids	16.0±1.7	11.6±2.2	5.5±1.6	0.4160	0.0009	0.1246	0.001
		Arterioles	23.4±2.4	19.2±3.1	7.7±2.3	0.9016	0.0004	0.0256	<0.001
		$CD34^+CD45^+$ cells	62±11	60±14	18±12	1.0000	0.0449	0.1082	0.030
		$CD34^-CD45^+$ cells	141±13	82±17	34±15	0.0571	0.0002	0.1477	<0.001
	FACS	*BM $CD34^+CD45^{dim}$ cells*	1.04±0.14	0.45±0.23	0.42±0.16	0.1477	0.0352	0.1477	0.023
		PB $CD34^+CD45^{dim}$ cells	0.052±0.016	0.035±0.021	0.047±0.017	1.0000	1.0000	1.0000	0.978
		BM $CD34^+CD133^+$ cells	0.007±0.001	0.002±0.001	0.001±0.001	0.0851	0.0130	1.0000	0.012
		PB $CD34^+CD133^+$ cells	0.004±0.001	0.002±0.001	0.001±0.001	1.0000	0.3228	1.0000	0.257
		BM $CD34^+KDR^+CD45^{dim}$ cells	0.031±0.005	0.004±0.011	0.010±0.001	0.0606	0.1191	0.8904	0.025
		PB $CD34^+KDR^+CD45^{dim}$ cells	0.007±0.001	0.002±0.001	0.001±0.006	0.0851	0.0130	0.8904	0.041
		BM $CD34^+CD14^+CD45^{dim}KDR^+CXCR4^+$ cells	0.051±0.011	0.006±0.008	0.024±0.010	0.2368	0.3393	1.0000	0.134
		PB $CD34^+CD14^+CD45^{dim}KDR^+CXCR4^+$ cells	0.019±0.005	0.006±0.001	0.0001±0.005	0.6655	0.0568	1.0000	0.054
Covariates: Glitazone, Statin, Anti-hypertensive Drugs	Histology/IHC	*Hematopoietic fraction*	50.2±4.6	28.5±5.5	26.3±4.4	0.0319	0.0097	1.0000	0.007
		Fat fraction	38.4±3.8	63.3±4.5	72.1±3.7	0.0027	0.0001	0.4909	<0.001
		Capillaries	21.3±1.8	12.9±2.1	15.2±1.7	0.0316	0.1094	1.0000	0.025
		Sinusoids	14.5±1.8	12.9±2.2	6.4±1.8	1.0000	0.0335	0.1225	0.025
		Arterioles	23.7±2.8	19.8±3.4	7.5±2.8	1.0000	0.0050	0.0452	0.004
		$CD34^+CD45^+$ cells	60±11	57±14	22±12	1.0000	0.1268	0.2131	0.077
		$CD34^-CD45^+$ cells	144±15	74±19	36±14	0.0500	0.0010	0.4695	0.001
	FACS	*BM $CD34^+CD45^{dim}$ cells*	1.09±0.17	0.36±0.26	0.36±0.21	0.1309	0.0081	1.0000	0.005
		PB $CD34^+CD45^{dim}$ cells	0.043±0.016	0.051±0.017	0.051±0.016	1.0000	1.0000	1.0000	0.932
		BM $CD34^+CD133^+$ cells	0.008±0.002	0.003±0.002	0.001±0.002	0.1895	0.0434	1.0000	0.042
		PB $CD34^+CD133^+$ cells	0.004±0.002	0.002±0.002	0.002±0.002	1.0000	0.9637	1.0000	0.699
		BM $CD34^+KDR^+CD45^{dim}$ cells	0.037±0.007	0.000±0.011	0.002±0.008	0.0938	0.0761	1.0000	0.047
		PB $CD34^+KDR^+CD45^{dim}$ cells	0.019±0.004	0.000±0.004	0.003±0.005	0.0207	0.064	1.0000	0.016
		BM $CD34^+CD14^+CD45^{dim}KDR^+CXCR4^+$ cells	0.054±0.014	0.002±0.019	0.024±0.013	0.2593	0.6411	0.9766	0.217
		PB $CD34^+CD14^+CD45^{dim}KDR^+CXCR4^+$ cells	0.020±0.006	0.002±0.008	0.003±0.007	0.3576	0.2903	1.0000	0.191

Covariate: Creatinine	**Histology/IHC**	*Hematopoietic fraction*	50.4±3.9	32.7±4.8	20.9±4.0	0.0297	0.0001	0.2345	<0.001
		Fat fraction	37.7±3.3	60.9±3.9	76.8±3.3	0.0006	<0.0001	0.0192	<0.001
		Capillaries	25.9-±2.4	9.8±2.9	12.7±2.4	0.0012	0.0034	1.0000	0.001
		Sinusoids	15.8±1.7	10.9±2.0	5.7±1.7	0.2320	0.0014	0.1986	0.002
		Arterioles	23.2±2.3	18.4±2.8	7.9±2.3	0.6182	0.0006	0.0312	0.001
		$CD34^{+}CD45^{+}$ cells	58±10	58±12	23±13	1.0000	0.1612	0.2066	0.109
		$CD34^{-}CD45^{+}$ cells	134±13	83±17	42±17	0.0803	0.0019	0.3166	0.002
	FACS	*BM $CD34^{+}CD45^{dim}$ cells*	1.16±0.14	0.29±0.22	0.32±0.17	0.0089	0.0042	1.0000	0.001
		PB $CD34^{+}CD45^{dim}$ cells	0.051±0.014	0.039±0.016	0.047±0.018	1.0000	1.0000	1.0000	0.854
		BM $CD34^{+}CD133^{+}$ cells	0.008±0.001	0.003±0.001	0.001±0.001	0.0573	0.0227	1.0000	0.015
		PB $CD34^{+}CD133^{+}$ cells	0.004±0.001	0.002±0.001	0.001±0.002	1.0000	0.9637	1.0000	0.538
		BM $CD34^{+}KDR^{+}CD45^{dim}$ cells	0.031±0.005	0.002±0.008	0.005±0.007	0.0388	0.0069	1.0000	0.013
		PB $CD34^{+}KDR^{+}CD45^{dim}$ cells	0.016±0.004	0.001±0.005	0.004±0.005	0.0796	0.3579	1.0000	0.061
		BM $CD34^{+}CD14^{+}CD45^{dim}KDR^{+}CXCR4^{+}$ cells	0.052±0.010	0.002±0.015	0.026±0.010	0.0422	0.3102	0.6397	0.039
		PB $CD34^{+}CD14^{+}CD45^{dim}KDR^{+}CXCR4^{+}$ cells	0.019±0.006	0.005±0.007	0.002±0.007	0.5793	0.4106	1.0000	0.248
Covariates: Previous Myocardial Infarction, Stroke, Hypertension	**Histology/IHC**	*Hematopoietic fraction*	51.4±3.9	36.03±4.5	20.8±4.0	0.0497	0.0002	0.0795	<0.001
		Fat fraction	38.1±3.3	57.4±3.8	76.6±3.4	0.0024	<0.0001	0.0051	<0.001
		Capillaries	24.2±2.5	15.6±2.9	11.4±2.6	0.1033	0.0111	0.9693	0.010
		Sinusoids	14.9±1.7	12.9±1.9	6.0±4.2	1.0000	0.0079	0.0582	0.008
		Arterioles	24.7±2.3	21.2±2.7	5.9±2.4	1.0000	0.0001	0.0015	<0.001
		$CD34^{+}CD45^{+}$ cells	65±9	51±12	18±12	1.0000	0.0248	0.1846	0.027
		$CD34^{-}CD45^{+}$ cells	136±13	83±16	37±16	0.0534	0.0006	0.1839	0.001
	FACS	*BM $CD34^{+}CD45^{dim}$ cells*	1.34±0.17	0.37±0.22	0.32±0.20	0.0373	0.0379	1.0000	0.018
		PB $CD34^{+}CD45^{dim}$ cells	0.047±0.017	0.044±0.017	0.055±0.017	1.0000	1.0000	1.0000	0.905
		BM $CD34^{+}CD133^{+}$ cells	0.007±0.001	0.003±0.001	0.002±0.001	0.0955	0.0398	1.0000	0.033
		PB $CD34^{+}CD133^{+}$ cells	0.004±0.001	0.003±0.001	0.002±0.001	1.0000	1.0000	1.0000	0.491
		BM $CD34^{+}KDR^{+}CD45^{dim}$ cells	0.030±0.006	0.000±0.008	0.005±0.006	0.0544	0.0912	1.0000	0.040
		PB $CD34^{+}KDR^{+}CD45^{dim}$ cells	0.018±0.004	0.000±0.004	0.002±0.004	0.0097	0.0300	1.0000	0.007
		BM $CD34^{+}CD14^{+}CD45^{dim}KDR^{+}CXCR4^{+}$ cells	0.054±0.014	0.017±0.015	0.023±0.012	0.3917	0.5432	1.0000	0.287
		PB $CD34^{+}CD14^{+}CD45^{dim}KDR^{+}CXCR4^{+}$ cells	0.018±0.006	0.004±0.006	0.000±0.005	0.4268	0.2241	1.0000	0.174

Values are estimated marginal means ± Std. Error. Insulin, Oral Anti-diabetic Drugs, HDL, LDL and Triglycerides not included because the statistical software cannot solve equation.

Online Table IV: Effect of diabetes on the expression of miR-155, FOXO3a, p21 and $p27^{kip1}$ before and after adjustment for background covariates by ANCOVA.

Covariate	Dependent Variable	p=	Adjusted p=
Age, Gender, BMI	*miR-155*	0.019	0.013
	FOXO3a	0.004	0.005
	p21	0.001	0.006
	$p27^{kip1}$	0.006	0.035
Glitazone, Statin, Anti-hypertensive Drugs	*miR-155*	0.019	0.050
	FOXO3a	0.004	0.031
	p21	0.001	0.030
	$p27^{kip1}$	0.006	0.196
Creatinine	*miR-155*	0.019	0.004
	FOXO3a	0.004	0.010
	p21	0.001	0.001
	$p27^{kip1}$	0.006	0.004
Previous Myocardial Infarction, Stroke, Hypertension	*miR-155*	0.019	0.041
	FOXO3a	0.004	0.017
	p21	0.001	0.008
	$p27^{kip1}$	0.006	0.072

Online Table V: Multiple Regression Analysis

Dependent Variable		Independent Variable	p=	Independent Variable	p=	Independent Variable	p=
Histology/IHC	*Hematopoietic fraction*	Group	*<0.001*	Diabetes duration	*0.009*		
	Fat fraction	Group	*<0.001*	Diabetes duration BMI	*<0.001* *0.040*	Fasting glucose	*0.017*
	Capillaries	Group Hypertension	*<0.001* *<0.001*	Diabetes duration Hypertension	*<0.001* *<0.001*		
	Sinusoids	Group Hypertension	*0.005* *0.048*	Diabetes duration	*0.004*	Fasting glucose Hypertension	*0.022* *0.031*
	Arterioles	Group	*<0.001*	Diabetes duration	*<0.001*	Fasting glucose	*0.038*
	$CD34^+CD45^+$ cells	Group	*0.020*				
	$CD34^-CD45^+$ cells	Group	*<0.001*	Diabetes duration	*0.003*	Fasting glucose	*0.032*
FACS	*BM $CD34^+CD45^{dim}$ cells*	Group	*<0.001*	Diabetes duration	*0.011*	Fasting glucose	*0.003*
	BM $CD34^+CD133^+$ cells	Group	*0.003*	Diabetes duration	*0.012*	Fasting glucose	*0.016*
	BM $CD34^+KDR^+CD45^{dim}$ cells	Group	*0.017*	Diabetes duration	*0.050*	Fasting glucose	*0.028*
	PB $CD34^+KDR^+CD45^{dim}$ cells	Group	*0.050*	Diabetes duration	*0.043*		
	PB $CD34^+CD14^+CD45^{dim}KDR^+CXCR4^+$ cells	Group	*0.049*				

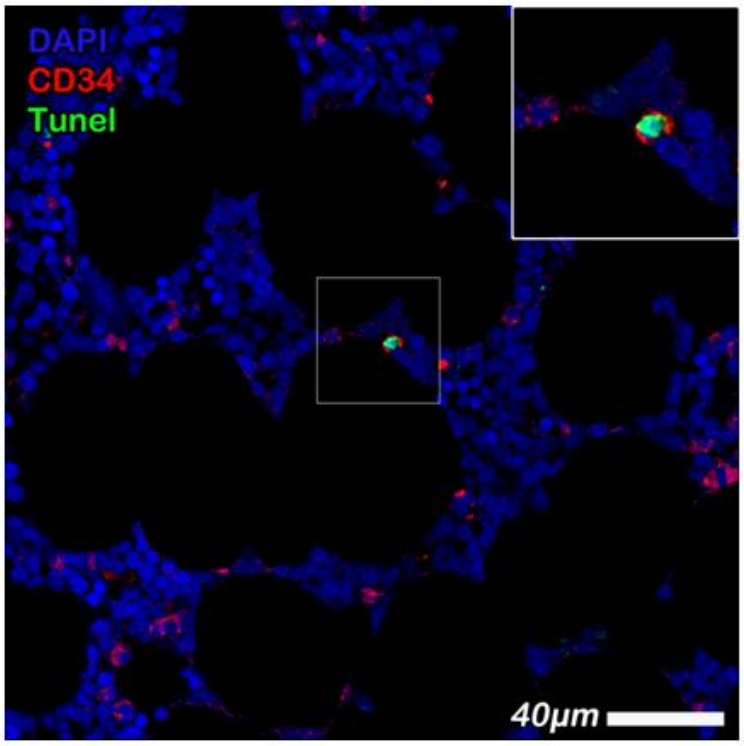
Results indicate the independent variables that, alone or in linear combination with other independent variables, predict the dependent variable under study

Online Table VI. Monoclonal and polyclonal antibodies used in this study

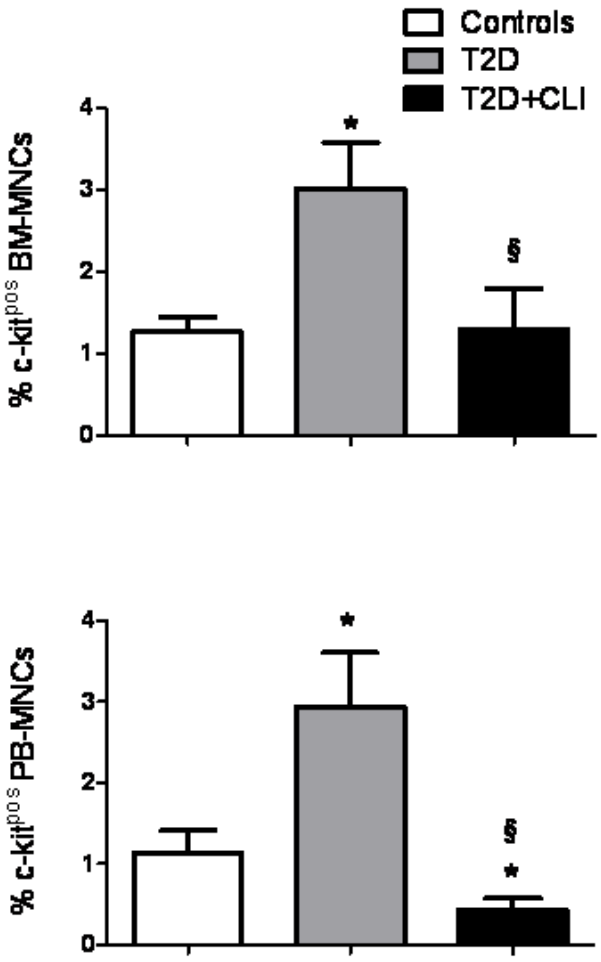
Primary antibody	Dilution	Incubation Time	Source
Anti-CD34 (Code:M7165, mc)	1:10	O/N	DAKO
Anti-CD45 (Code ab10559, pc)	1:50	60 min	Abcam
Anti-CD31 (Code: M0823, mc)	1:10	60 min	DAKO
Anti-alpha-SMA (Code: M0851, mc)	1:20	60 min	DAKO
Anti-CD68 (mc)	-	30 min	Ventana Medical Systems
Anti-Myeloperoxidase (pc)	-	30 min	Ventana Medical Systems
Anti-Gycophoryn (mc)	-	30 min	Ventana Medical Systems

mc: monoclonal; pc: polyclonal ; O/N : overnight

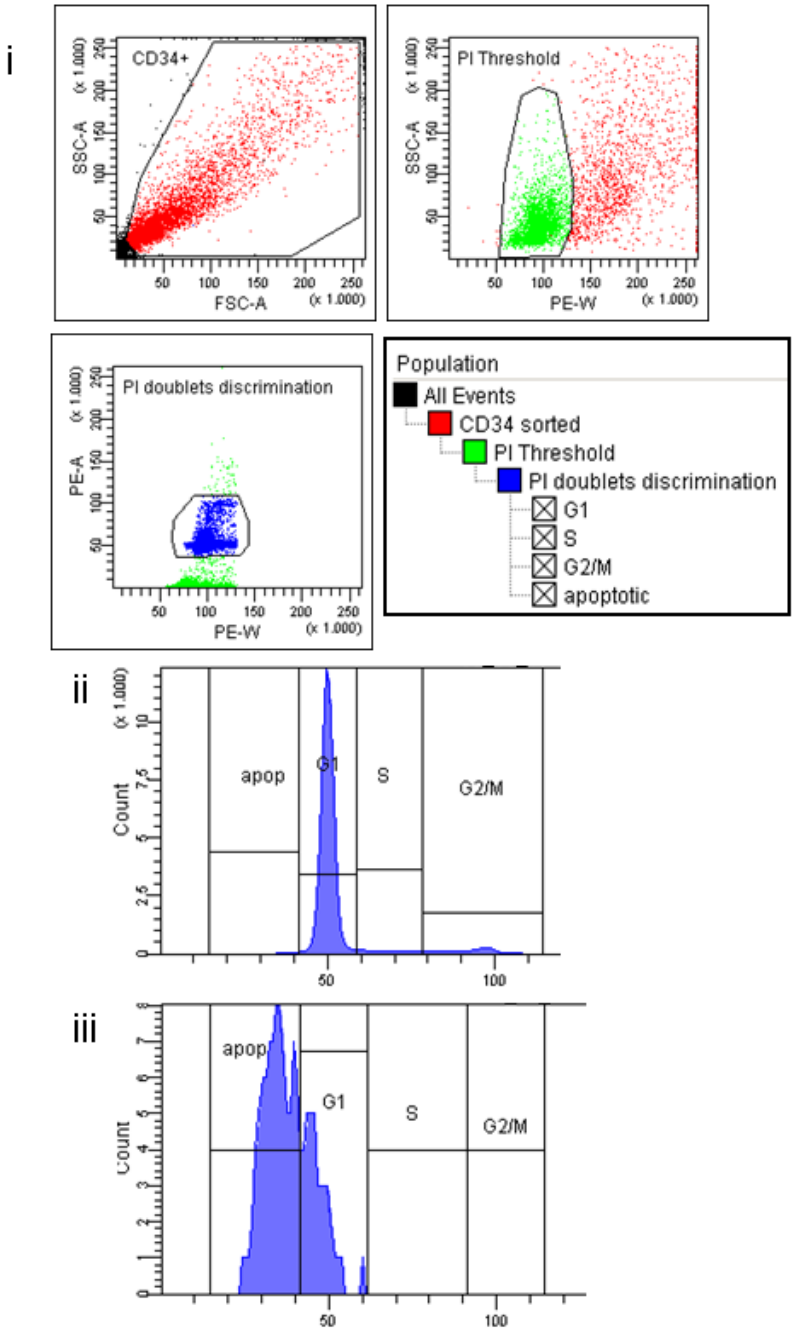
Supplemental Figures and Figure legends



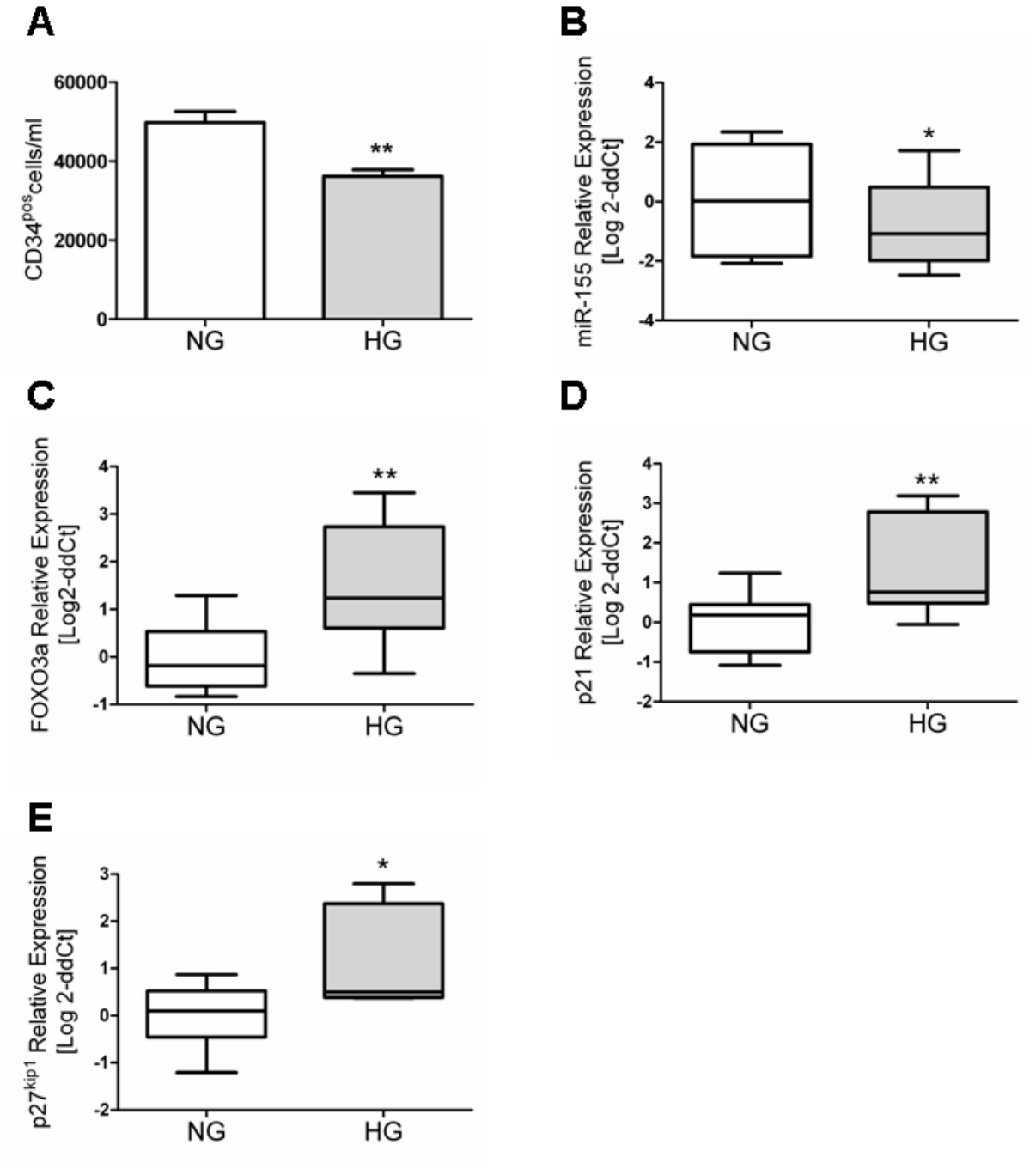
Online Figure I. Immunohistochemical assessment of apoptosis in CD34^{pos} BM cells. Representative microphotograph of human BM stained with anti-CD34 antibody in combination with TUNEL reaction to show apoptosis.



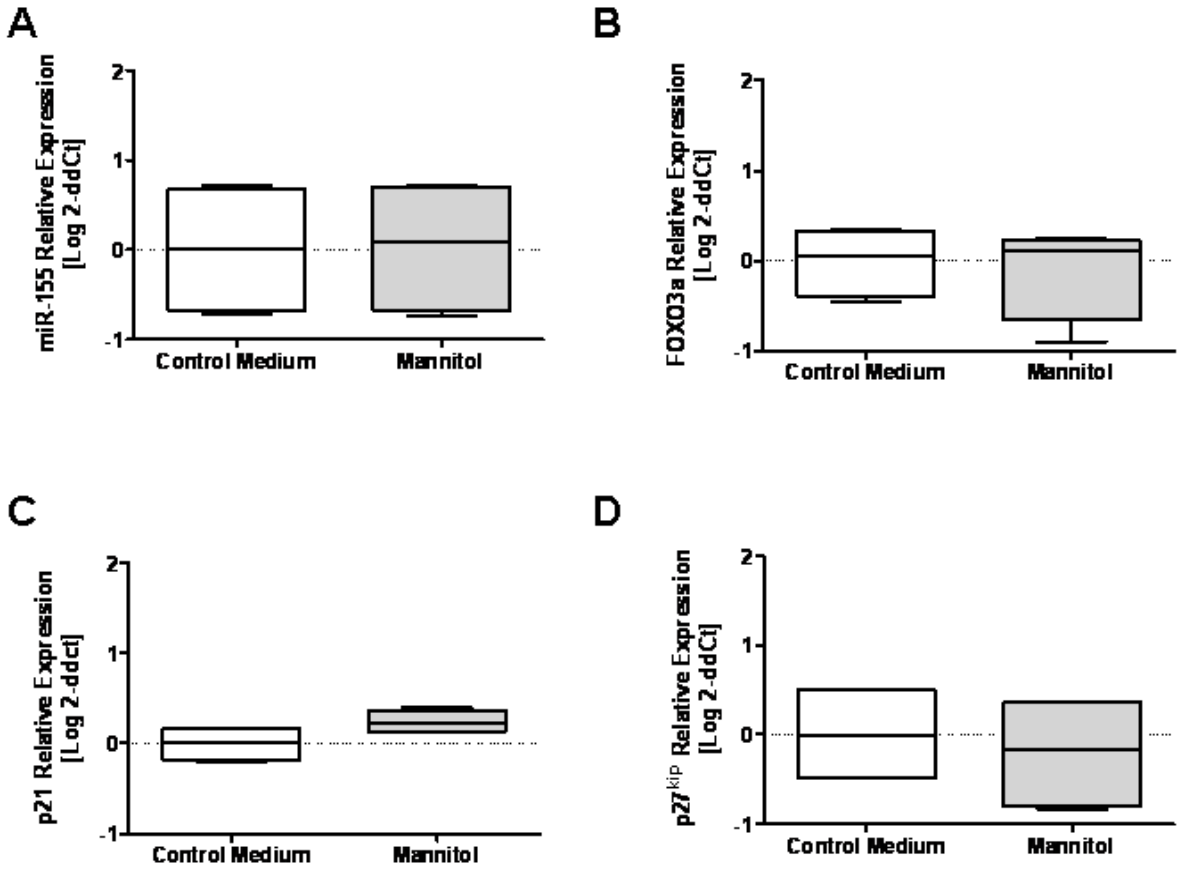
Online Figure II. Flow cytometry analysis of c-kit^{pos}MNCs. Bar graphs showing the percentage of cKit^{pos} cells. *p<0.05 vs. controls, §p<0.05 vs. T2D. Controls, n=11; T2D, n=6 to 9; T2D+CLI, n=8 to 9.



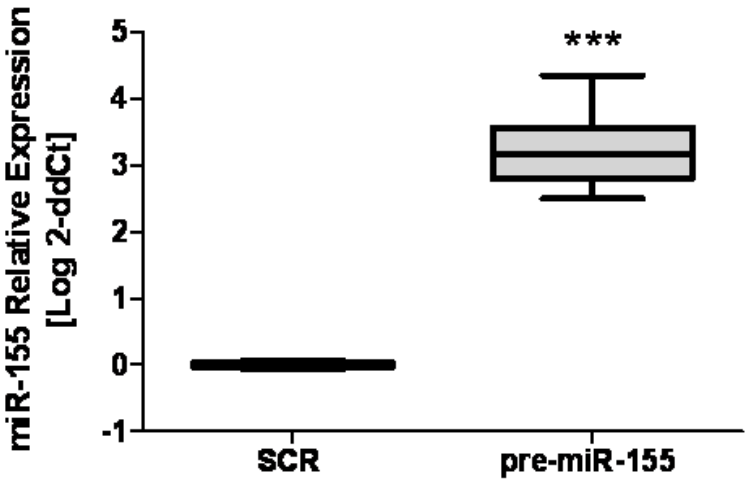
Online Figure III. Flow cytometry analysis of cell cycle in freshly-sorted CD34^{pos} cells: (**i**) gating strategy, (**ii**) control, (**iii**) T2D.



Online Figure IV. Effect of high glucose on cell counts and gene expression. A to **E**) Bar graphs showing the effect of high glucose (25mM D-glucose, HG) on BM $CD34^{pos}$ cell counts (**A**), levels of miR-155 (**B**), FOXO3a (**C**), CDKN1A/p21 (**D**) and CDKN1B/$p27^{kip1}$ (**E**). Normal glucose (5mM D-glucose, NG). *p<0.05 and **p<0.01 vs. NG. n=7 per group.



Online Figure V. The osmotic control mannitol does not influence the expression of miR-155 and downstream targets in CD34^{pos}-PCs from non-diabetic patients. Bar graphs of real time PCR showing levels of miR-155 (**A**), FOXO3a (**B**), CDKN1A/p21 (**C**) and CDKN1B/p27^{kip1} (**D**) in the presence or absence of 25mM mannitol. N=2 donors assayed in duplicate.



Online Figure VI. miR-155 expression after transfection of CD34^{pos}-PCs with pre-miR or scramble (SCR). N=5 donors assayed in duplicate, ***p<0.01 vs. SCR.