List of Supplementary Material

Supplementary Materials and Methods

Supplementary Figure 1: Recovery of patients following stem cell tranplantation.

Supplementary Figure 2: Diabetes-induced changes in stem cells and their microenviroment.

Supplementary Figure 3: Detection of nestin+ cells in human bone marrow biopsies.

Supplementary Table 1: Details of the human caseload included in the study.

References

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Human caseload

Complete data were obtained for 62 total patients. 19 cases of diabetes mellitus were identified (mean 57±7 yrs of age; 10 males) following the guidelines of the American Diabetes Association[50]. The interval from the documentation of diabetes to transplantation ranged from 6 months to 5.3 years. High levels of blood glucose, HbA1c > 7 gm% (PID 5 HBA1C=6.8, PID17 HBA1C=6.7, PID1,8,9,12 HBA1C missing) and need for insulin treatment were documented. In two cases (11%), diabetes mellitus was associated with the use of corticosteroids, whereas in the remaining cases type I and II diabetes were equally present. Controls consisted of 43 non-diabetic subjects (52±12 yrs; 24 males). The principal needs for autologous peripheral blood stem cell transplantation were clinical remission of multiple myeloma (42%), lymphoma (32%) and acute myeloid leukemia (26%).

Stem Cell Mobilization

$CD34^+$ cells were counted using a Becton-Dickinson FACSCalibur after erythrocyte lysis (EDTA-ammonium chloride solution) and direct incubation for 30 minutes with phycoerythrin (PE) conjugated monoclonal anti-CD34 (HPCA2, Becton-Dickinson, San Jose, Ca, USA). $CD34^+$ cell counts were performed following ISHAGE logical gate protocol. A negative control for non specific fluorescence (Simultest; Becton Dickinson, Erembodegem-Aalsi, Belgium) was used with unstained cells; the minimal number of events acquired for each determination was 70,000 and the entire procedure was performed at 4 °C. In all cases only the $CD34^+$ cell count performed in the

lymphomonocyte gate was considered for data analysis. Chemotherapy included high-dose of cyclophosphamide (7 g/m^2), ifosfamide, carboplatin, etoposide (ICE)[51] or high-dose Ara-C and dexamethasone (DHAP)[52] regimens, which were equally employed in the two groups (See supplementary table 1). Starting 24 hrs after the discontinuation of chemotherapy, G-CSF (10 ug/kg/day, Roche, Basel, Switzerland) was subcutaneously given until leukapheresis. From day 9 after priming, circulating CD34$^+$ cells were monitored daily.

The following parameters were used as criteria for the beginning of leukapheresis: CD34$^+$ cells $\geq$ 20/uL and/or PMNs $\geq$ 3000/uL, leukocyte count $\geq$ 5,000/uL, platelet count $\geq$ 50,000/uL.

Human Bone Marrow Biopsies

Informed consent was obtained from patients according to the National ethical guidelines of the Italian Ministry of Health: DL 116, January 1992. Bone marrow biopsies were performed on the posterior iliac crest using a stainless steel needle, provided with ergonomic handle (ISO 9626 standard) and specimens were fixed in formalin sublimate for 2-4 hr according to the size. After a short decalcification, samples were embedded in paraffin. Sections of 4-5 μm thickness were employed for histochemical and immunohistochemical studies.

Retrospective analysis involved 1,171 bone marrow biopsies performed at the University-Hospital of Parma by the Department of Internal Medicine and Hematology from January 2002 to May 2008 and collected from the database of the Department of Pathology. Non Hodgkin Lymphoma (NHL), Hodgkin Lymphoma (HL), Multiple Myeloma (MM),

Monoclonal Gammopathies of Uncertain Significance (MGUS), Myelodysplastic/Myeloproliferative diseases (MD/MP) including Acute Leukemia (AL), undetermined cytopenias and fever of unknown origin (FUO) were the major indications for bone marrow examination. Clinical records were available on most patients to ensure the presence or absence of diabetes (according the guidelines of the American Diabetes Association) and its time course. Diabetes involved 65 patients in whom 10% (n=7) was type I, 85% (n=55) type II and remaining 5% (n=3) was secondary to corticosteroids treatment.

Normal bone marrow findings, as defined by the absence of neoplastic infiltrates or morphologic or quantitative changes in bone marrow hematopoietic compartments compatible with hematologic disorders, were present in 309 cases and type II diabetes involved 19 (mean age 57±7, 10 males) of these patients. Due to the lack of detailed clinical information or technical artifacts of the specimens, the analysis of control bone marrow samples was restricted to 43 (mean age 52±12, 24 males) non-diabetic cases. On immunostaining we assessed the frequencies and percentage of nestin^{+} cells.

In order to reduce non-specific antibody binding, bone marrow sections were exposed to 10-20% serum of the species in which secondary antibodies were developed. Sections were then incubated with isotype-matched controls or specific primary antibodies. Confocal microscopy (Zeiss LSM510 Meta-Axiovert 200) and multiple probes were used to detect by immunofluorescence: 1) nestin (clone P48681, Millipore) expression in individual cells and in vascular profiles including α-smooth muscle actin (α-SMA, Sigma) positive arteries. The reactions were visualized by FITC -Ig, TRITC-Ig and Cy5-Ig conjugated secondary antibodies (Jackson Laboratories). Nuclei were stained by DAPI

(4',6-diamidine-2-phenyndole, SIGMA). Quenching of autofluorescence was improved by immersion of sections in an alcoholic solution of Sudan Black.

Tissue sampling involved the entire valuable area included in bone marrow biopsies (range: 20-90 mm^2) for morphometric parameters and counting a minimum of 120,000 to a maximum of 210,000 cells for the quantification of each investigated antigen. Data were expressed as the incidence of cells per unit area of tissue and by calculating the fraction of cells labelled by the antigen over the total number of counted cells.

Stromal co-culture

Bone marrow stromal cells were obtained expanding the adherent fraction of cells. The expanded cells were then plated at 2200 cells/well in 384-well tissue culture plates (Millipore; FC010) in αMEM plus 10%FCS. After four days of culture, 250 LSK 48^- cells from 8-12 week old actin-DsRed positive mice (Jackson Laboratory; 005441) were plated in each well. After 5 days of co-culture the number of Ds-Red^+ cells was assessed using a fluorescent microscope (Leica) and the number of fluorescent red cells was counted using Image J software.

Supplementary table 1: Details of the human caseload included in the study. Columns represent (from left to right): age; sex; glucose levels at the time of admission; years from the onset of the diabetes; HBA1C: glycate hemoglobin levels (%); hematologic disease; No preTx: number of chemotherapy cycles administered to the patient before the mobilization; type of mobilization regimen; amount of $CD34^+$ cells collected; amount of mononuclear cells harvested from the BM (data applicable just for the poor mobilizers); daily dose of insulin in diabetic subjects.

Patient	Age	Sex	Glucose (mg/dL)	Years of diabetes	HBA1C	Disease	No preTx	Mobilization regimen	$CD34^+$ ($x10^6$/kg)	BM ($x10^6$/kg)	Insulin (U/day)
Diabetic	67	M	100	0.5		MM	2	ICE	7.29		20
	67	F	120	2	8	MM	2	ICE	12		28
	57	F	185	1.4	9.1	MM	2	Cyclophosphamide	4.4		14
	63	M	137	2.2	8.9	MM	2	Cyclophosphamide	16.7		8
	43	M	177	0.8	6.8	MM	2	Cyclophosphamide	7.5		24
	58	M	200	3.4	8.3	MM	2	Cyclophosphamide	3.53		30
	61	M	111	2.8	8.5	L	4	RDHAP	3		20
	49	M	258	0.9		MM	2	ICE	4.1		16
	56	F	290	0.7		L	4	RDHAP	4.75		22
	55	F	213	5.2	8.4	MM	2	Cyclophosphamide	5.05		18
	39	F	165	3.2	7.9	MM	2	Cyclophosphamide	10		14
	62	F	201	5.1		MM	1	Cyclophosphamide	8.7		12
	56	F	110	4	7.6	AL	2	FLAG	FAILURE	180	24
	55	M	230	2.4	8.1	AL	2	DNR-ARAC	FAILURE	214	28
	58	M	140	3.3	8.4	MM	2	Cyclophosphamide	FAILURE	112.5	22
	54	F	134	3.9	7.7	AL	2	DNR-ARAC	FAILURE	160	18
	61	M	245	0.6	6.7	AL	3	DNR-ARAC	FAILURE	257.2	12
	62	F	303	5.3	8.2	AL	2	DNR-ARAC	FAILURE	150	24
	59	M	98	4.8	9.1	AL	3	DNR-ARAC	FAILURE	211	20
Non diabetic	28	M	80			AL	2	DNR-ARAC	28.3		
	51	F	84			MM	2	Cyclophosphamide	8.5		
	60	F	76			MM	2	Cyclophosphamide	15.6		
	69	M	90			AL	2	DNR-ARAC	3.3		
	64	M	80			MM	2	Cyclophosphamide	12.1		
	69	F	92			L	4	DNR-ARAC	10		

Patient	AGE	SEX	Glucose (mg/dL)	Years of diabetes	HBA1C	Disease	No of preTx	Mobilization regimen	$CD34^+$ ($x10^6$/kg)	BM ($x10^6$/kg)	Insulin (U/day)
Non diabetic	55	F	91			MM	2	IEV	19.1		
	60	F	100			AL	3	DNR-ARAC	5		
	70	F	99			MM	2	Cyclophosphamide	10.8		
	46	M	68			AL	3	DNR-ARAC	7.8		
	69	M	79			MM	3	Cyclophosphamide	12.5		
	32	M	145			L	6	Cyclophosphamide	18.7		
	60	F	88			L	4	Cyclophosphamide	4.8		
	40	M	83			AL	3	DNR-ARAC	20		
	62	F	84			MM	2	Cyclophosphamide	12		
	58	M	84			L	4	R-DHAP-R	7.3		
	48	F	80			MM	1	Cyclophosphamide	26.3		
	51	M	95			L	6	Cyclophosphamide	8.2		
	52	M	92			MM	2	cyclophosphamide	16.1		
	39	F	97			L	4	ICE	4.4		
	42	F	72			L	4	IV	3.4		
	51	M	81			L	4	R-DHAP-R	11.6		
	29	F	74			L	4	Cyclophosphamide	6		
	35	M	89			MM	2	Cyclophosphamide	7.4		
	50	M	94			MM	2	Cyclophosphamide	10		
	41	F	110			L	4	DNR-ARAC	11.17		
	67	M	69			L	4	DNR-ARAC	17.8		
	43	F	113			L	4	Cyclophosphamide+R	35.7		
	49	F	80			L	4	R-DHAP-R	26.5		
	48	F	79			L	4	ICE	11.6		
	46	F	82			AL	3	DNR-ARAC	21		
	54	M	80			L	4	DHAP	40		
	45	M	77			MM	2	Cyclophosphamide	6.3		
	34	M	93			AL	3	Cyclophosphamide	4.5		
	55	M	90			MM	3	Cyclophosphamide	5.6		
	57	M	109			MM	2	Cyclophosphamide	8.9		
	57	F	98			AL	3	DNR-ARAC	FAILURE	277	
	58	F	102			L	4	DHAP	FAILURE	200	

Patient	AGE	SEX	Glucose (mg/dL)	Years of diabetes	HBA1C	Disease	No of preTx	Mobilisation regimen	$CD34^+$ ($x10^6$/kg)	BM ($x10^6$/kg)	Insulin (U/day)
Non diabetic	48	M	142			L	4	RDHAP	FAILURE	193	
	50	M	99			AL	3	DNR-ARAC	FAILURE	154	
	43	M	105			L	4	RDHAP	FAILURE	139	
	71	M	110			AL	3	DNR-ARAC	FAILURE	238	
	69	M	132			MM	2	Cyclophosphamide	FAILURE	190	

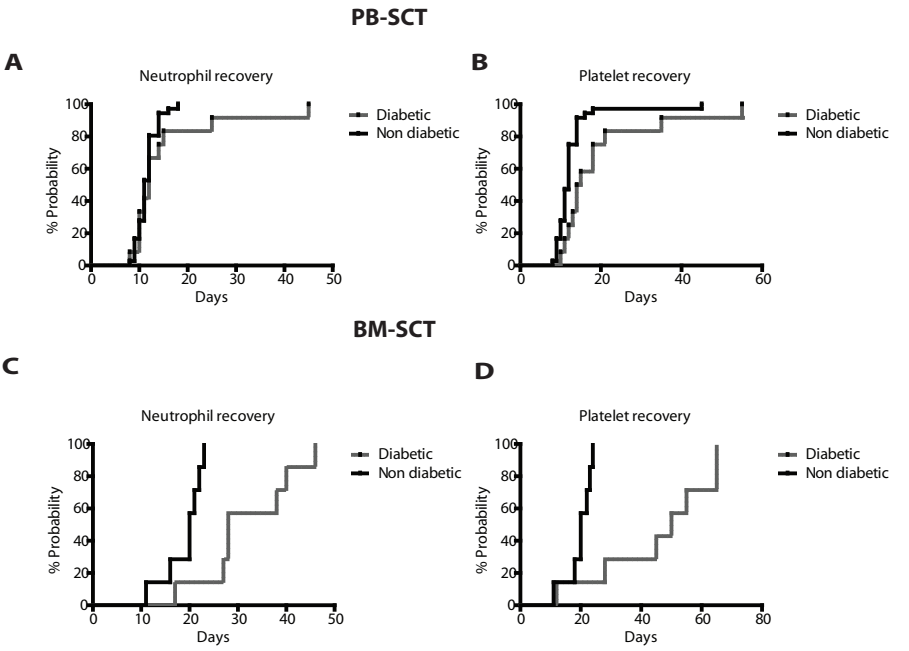
Abbreviations: M: male; F: female; MM: multiple myeloma; L: lymphoma; AL: acute leukemia; ICE: Ifosfamide, Carboplatin, Etoposide; R-DHAP: Rituximab, Dexamethazone, Cytarabine, Cisplatin; DNR-ARAC: Daunorubicin, standard doses- Cytarabine high doses; IEV: Ifosfamide, Epirubicin, Etoposide; R-DHAP-R: Rituximab-Dexamethazone, Cytarabine,Cisplatin-Rituximab; DHAP: Dexamethazone, Cytarabine, Cisplatin, standard doses; IV: Ifosfamide, Etoposide; BUCY: Busulphan, Cyclophosfamide; FLAG: G-CSF, Fludarabine, Cytarabine.

The following drugs were equally used by both groups of patients:Angitensin Converting Enzyme Inhibitors, beta-Blockers, alpha blockers, Calcium antagonist, Clonidine, Thiazide, Thyroxine, Gabapentin, Sartans, Statins, Aspirin, Valproic acid Paroxetine, Sertraline, Serotonine reuptake inhibitors, Tiamazol

In not diabetic patients: Insulin doses were only occasionally required.

In diabetic patients: Oral hypoglycemic drugs were used by 30% of patients and daily Insulin doses (25-40 U) were employed in all cases.

Supplementary Fig. 1

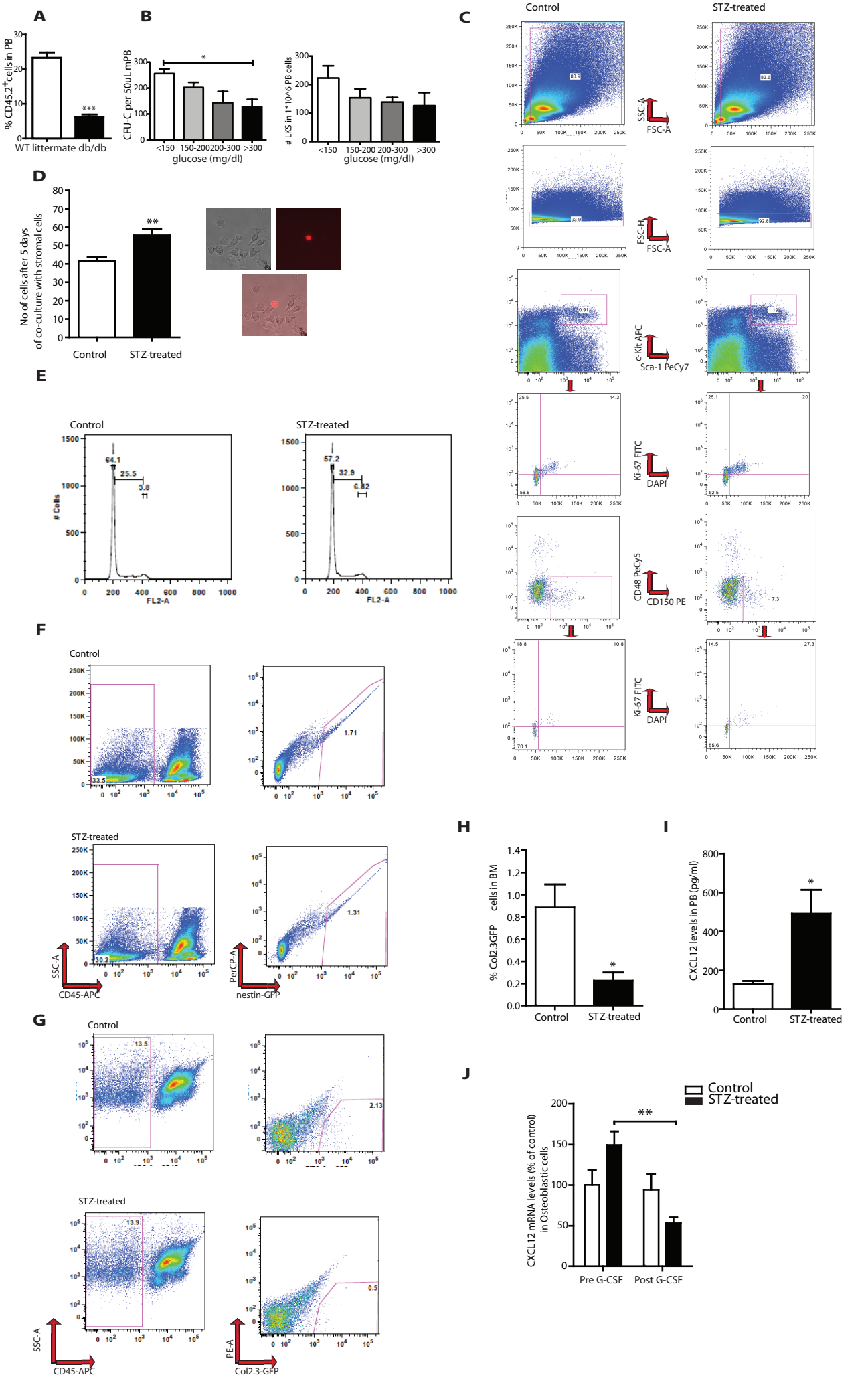


E

	Non-diabetic	Diabetic	p
PB-SCT			
PMNs recovery Median days (range)	11 (8-18)	12 (8-45)	0.146
PLTs recovery Median days (range)	12 (8-45)	14.5 (10-55)	0.006
BM-SCT			
PMNs recovery Median days (range)	20 (11-23)	28 (17-46)	0.002
PLTs recovery Median days (range)	20 (11-24)	50 (12-65)	0.003

Supplementary figure 1:

Curves representing probability of (**A**) neutrophil and (**B**) platelet engraftment in patients receiving mobilized $CD34^{+}$ autologous cells. Diabetic patients exhibit trend towards longer neutrophil recovery (n=12, p= 0.146) and longer time to platelet engraftment (p=0.006). Curves representing probability of (**C**) neutrophil and (**D**) platelet engraftment in patients receiving BM harvested autologous cells. Diabetes is associated with slower engraftment of both neutrophil and platelet (n=7, p=0.002 and p=0.003 respectively) (**E**) Table showing median days (range) of platelets and neutrophil recovery in both groups of patients.

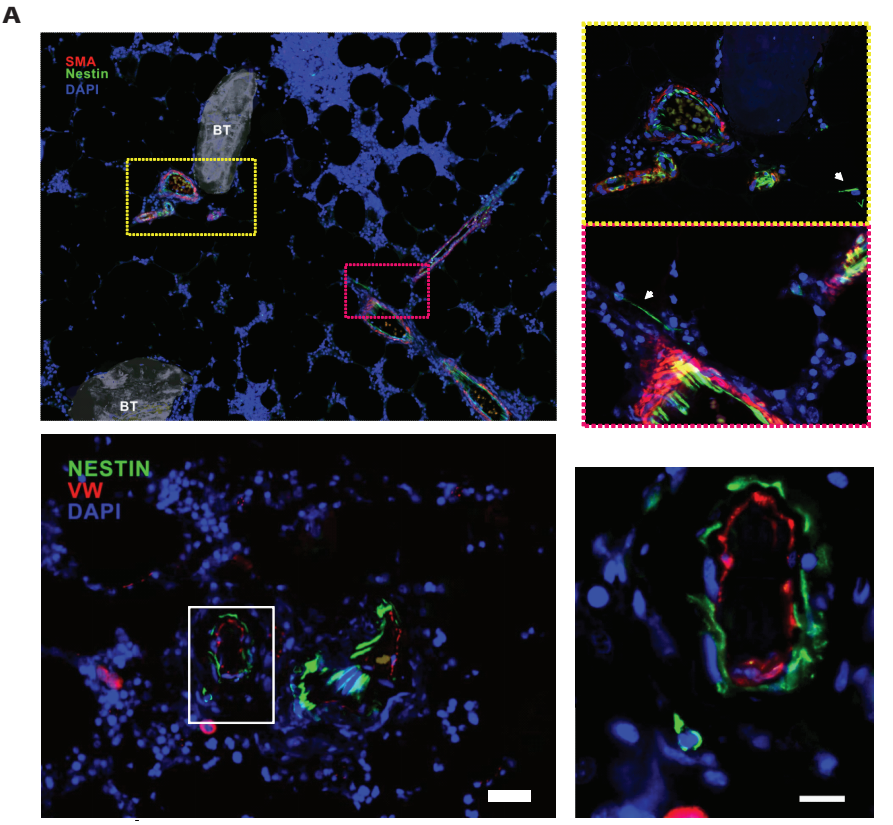

A
% CD45.2+cells in PB
WT littermate db/db
***
B
CFU-C per 50uL mPB
glucose (mg/dl)
<150
150-200
200-300
>300
*
LKS in 1*10^6 PB cells
C
Control
STZ-treated
SSC-A
FSC-A
FSC-H
c-Kit APC
Sca-1 PeCy7
Ki-67 FITC
DAPI
CD48 PeCy5
CD150 PE
D
No of cells after 5 days of co-culture with stromal cells
**
E
Cells
FL2-A
F
CD45-APC
nestin-GFP
PerCP-A
G
Col2.3-GFP
PE-A
H
% Col2.3GFP cells in BM
I
CXCL12 levels in PB (pg/ml)
J
CXCL12 mRNA levels (% of control) in Osteoblastic cells
Pre G-CSF
Post G-CSF

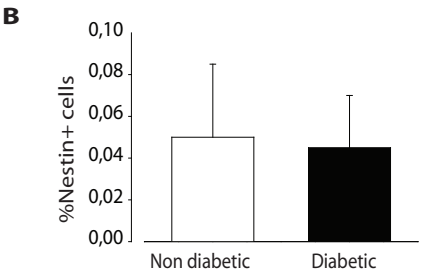
Supplementary figure 2:

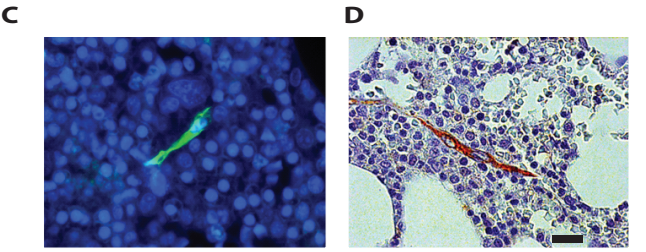
(**A**) Percentages of total CD45.2^{+} donor derived cells in the peripheral blood of lethally irradiated SJL recipients transplanted with 150 ul G-CSF mobilized PB from db/db or wild type littermates as assessed by FACS analysis four weeks post-transplantation. Columns represent mean ± s.e.m., n=11 control and n=12 db/db, ***p<0.001. (**B**) Bars are mean number ± s.e.m of CFU-C (left graph) and LSK (right panel) of *db/db* mice grouped in four categories based upon glycemic levels (<150, 150-200, 200-300, >300 mg/dl), * p<0.05. (**C**) Representative FACS plots of gating strategies used in cell cycle analysis of LSK and LT-HSC. (**D**) Columns are mean ± s.e.m. of Lin^{-}Sca^{+}cKit^{+}CD48^{-} after 5 days co-culture onto control or STZ-treated BM-originating stroma cells. Representative photos showing a DsRed^{+} (top left panel, red dot) Lin^{-}Sca^{+}cKit^{+}CD48^{-} cell on top of stromal cells (top right panel, bright field). Bottom panel: merged fluorescent and bright field. (**E**) Representative FACS plots of propidium iodide staining in whole BM control and STZ-treated mice to determine cell cycle status. STZ-treated mice exhibit lower fractions of cells in G0-G1 phase compared to control mice (n=5, *p<0.05). (**F**) FACS plots showing percentages of nestinGFP^{+} cells in collagenase treated bones from STZ-treated and control mice. No difference in the percentages between the two groups was found. (**G**) FACS plots showing the difference in col2.3GFP cells percentages in diabetic and control mice. (H) Histograms showing statistical significant decrease in the percentages of col2.3 GFP cells in diabetic mice (*p<0.05, n=6). (**I**) Quantification of CXCL12 levels in PB of STZ-treated versus control mice. Diabetic mice have higher CXCL12 circulating levels (*p<0.05, n=6). (**J**) Percentage difference

in *Cxcl12* mRNA levels in osteoblastic cells under steady state condition and after G-CSF administration. White bars are controls and black bars represent STZ-treated mice, n=6, **$p<0.01$.

Supplementary Fig. 3







Supplementary figure 3:

The number of nestin cells was analysed in disease free bone marrow biopsies from 19 diabetic patients and 32 non diabetic individuals (see methods). (**A**) Immunohistochemical detection of nestin in human bone marrow. Upper panel: low power confocal image of nestin (green fluorescence) labelling on the subendothelial layer of α-smooth muscle actin positive (α-SMA, red fluorescence) arterioles. Phase contrast images of bone trabeculae (BT) were superimposed. Adiacent yellow and red rectangles include microscopic fields shown at higher magnification. Lower panels: irregularly shaped nestin^{+} a-SMA^{-} cells are shown in proximity of arterial profiles. Blue fluorescence corresponds to DAPI staining of nuclei. (**B**) Bar graphs of the percentages of nestin^{+} cells in BM of control and diabetic patients. P=NS. Two examples of individual nestin^{+} cells with an elongated shape are shown by (**C**) immunofluorescence (green) and (**D**) immunoperoxidase (brownish). Scale bars = 10 μm.