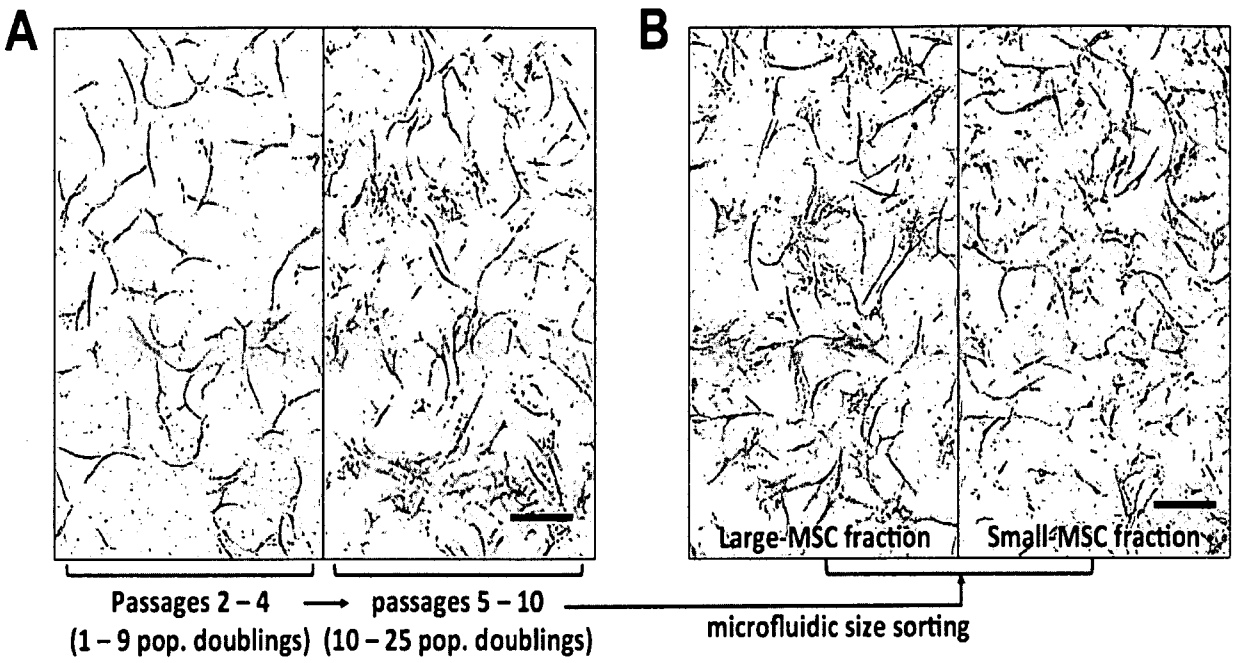
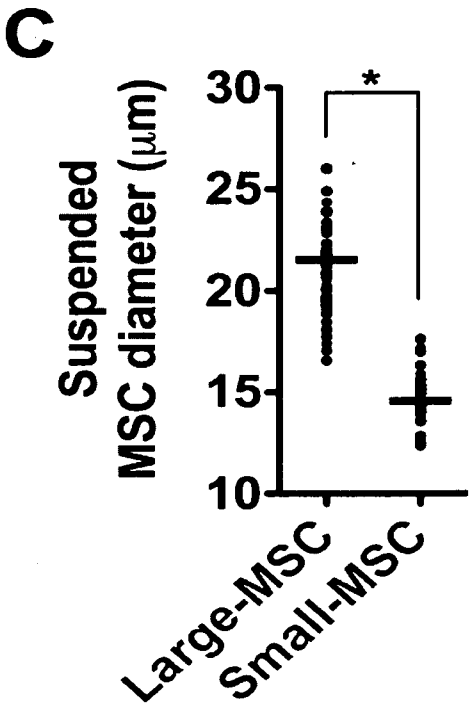
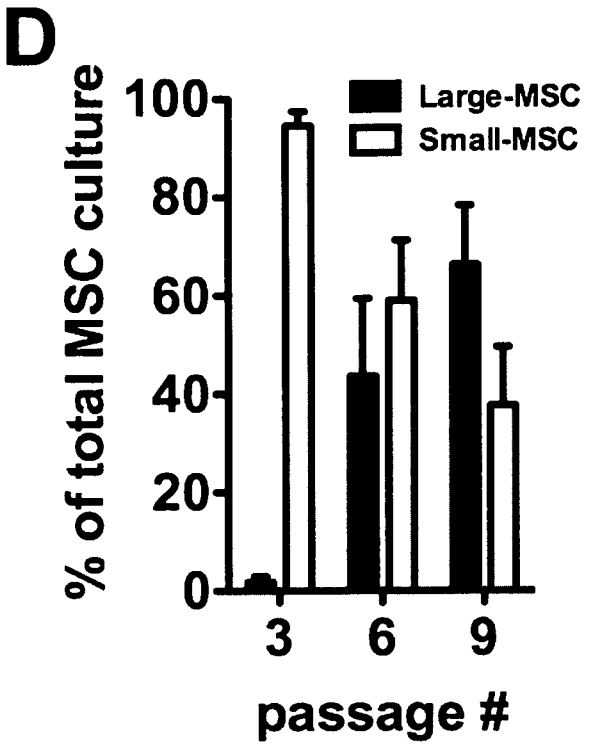
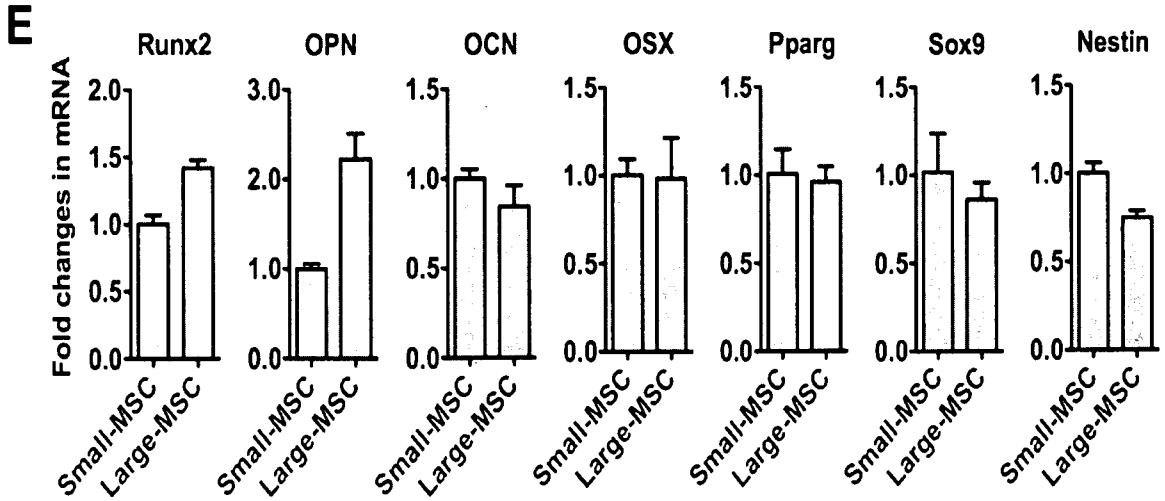

A
B
Large-MSC fraction
Small-MSC fraction
Passages 2 – 4
(1 – 9 pop. doublings)
passages 5 – 10
(10 – 25 pop. doublings)
microfluidic size sorting


C
Suspended MSC diameter (μm)
30
25
20
15
10
*
Large-MSC
Small-MSC

FIGS. 1A – 1C


D
% of total MSC culture
100
80
60
40
20
0
Large-MSC
Small-MSC
3
6
9
passage #


E
Fold changes in mRNA
Runx2
OPN
OCN
OSX
Pparg
Sox9
Nestin
Small-MSC
Large-MSC

FIGS. 1D - 1E

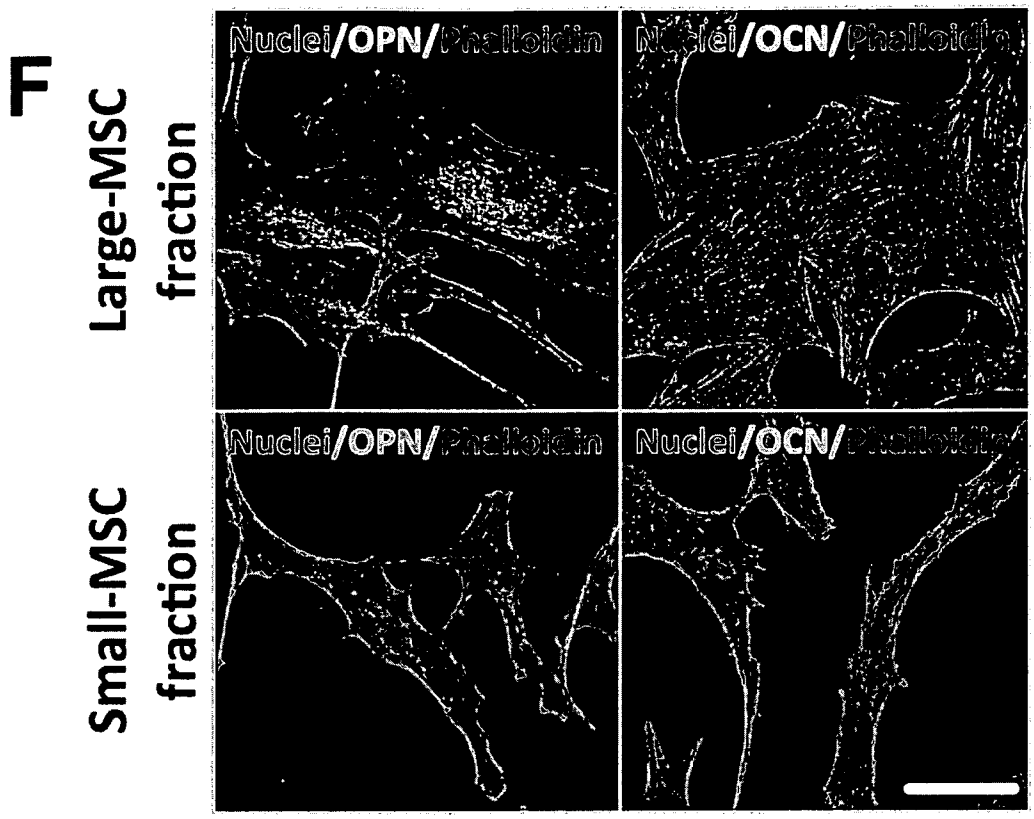

F
Large-MSC fraction
Small-MSC fraction
Nuclei/OPN/Phalloidin
Nuclei/OCN/Phalloidin
Nuclei/OPN/Phalloidin
Nuclei/OCN/Phalloidin

FIG. 1F

G

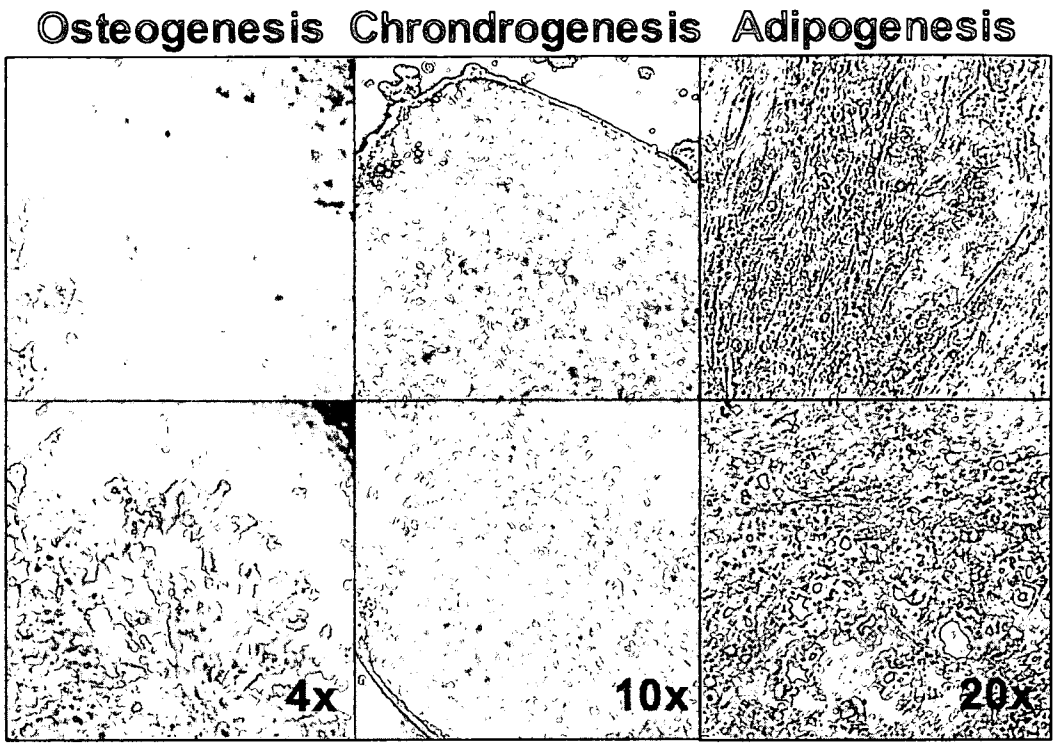
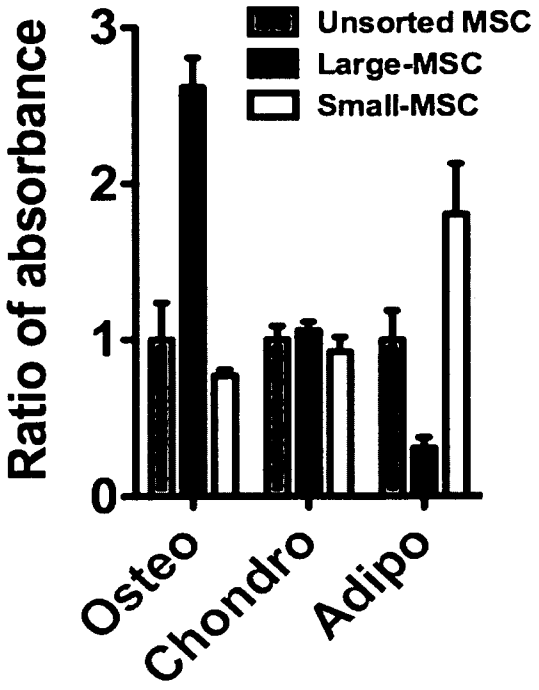
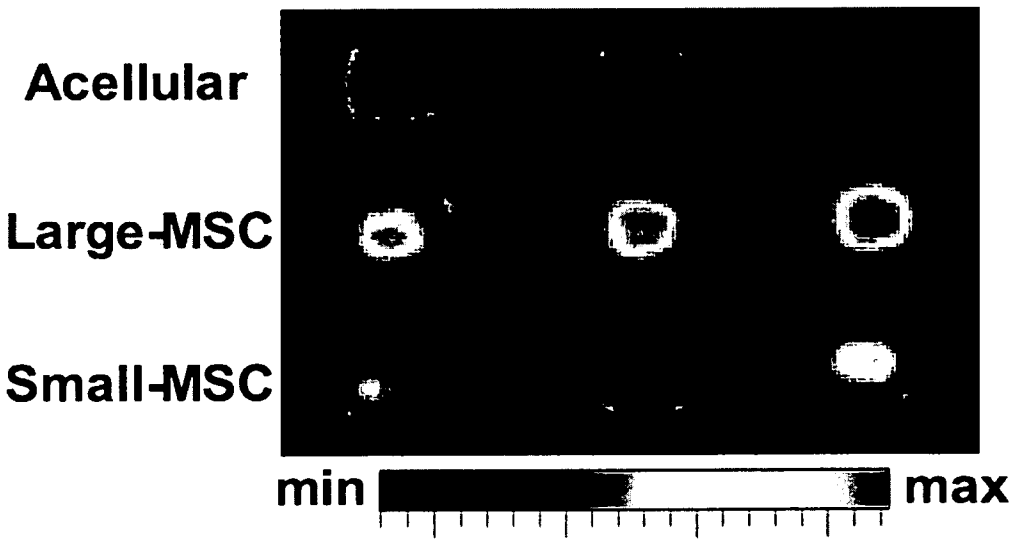


FIG. 1G

H


Ratio of absorbance
3
2
1
0
Unsorted MSC
Large-MSC
Small-MSC
Osteo
Chondro
Adipo

I


Acellular
Large-MSC
Small-MSC
min
max

FIGS. 1H - 1I

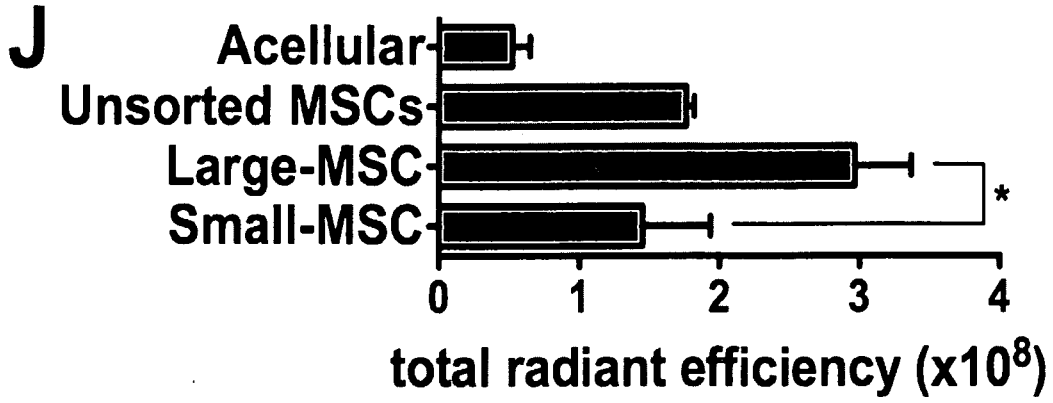
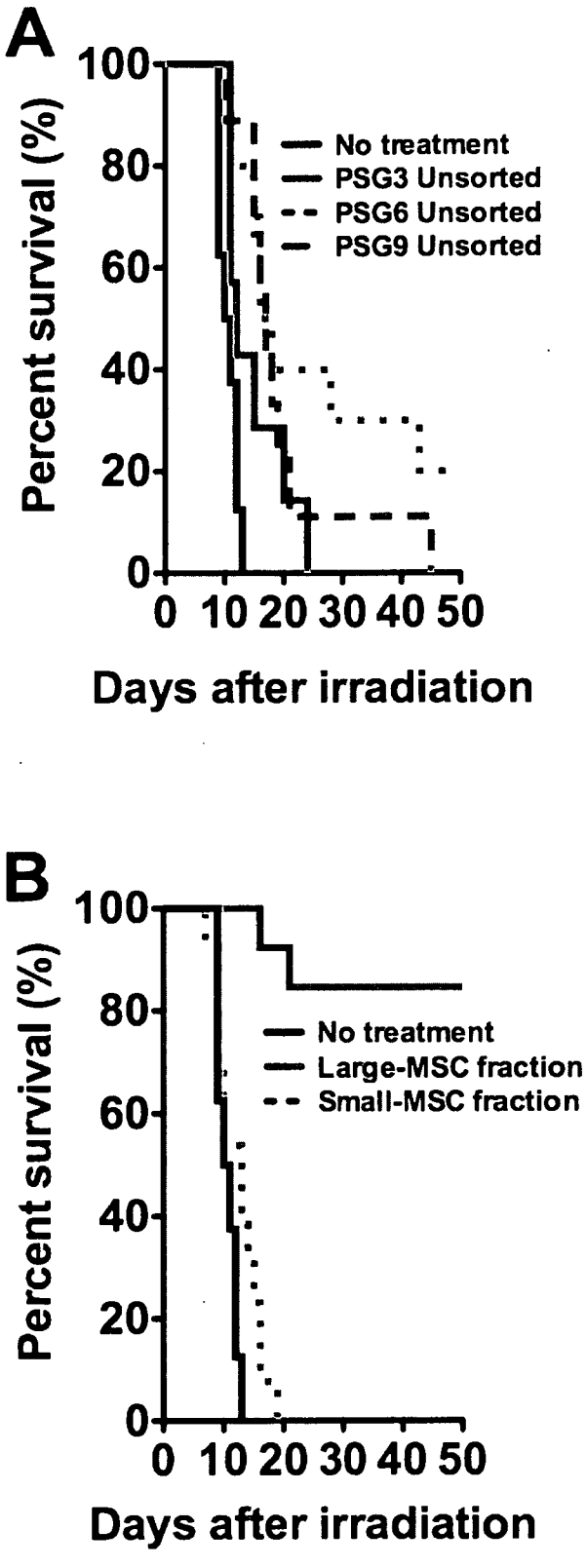
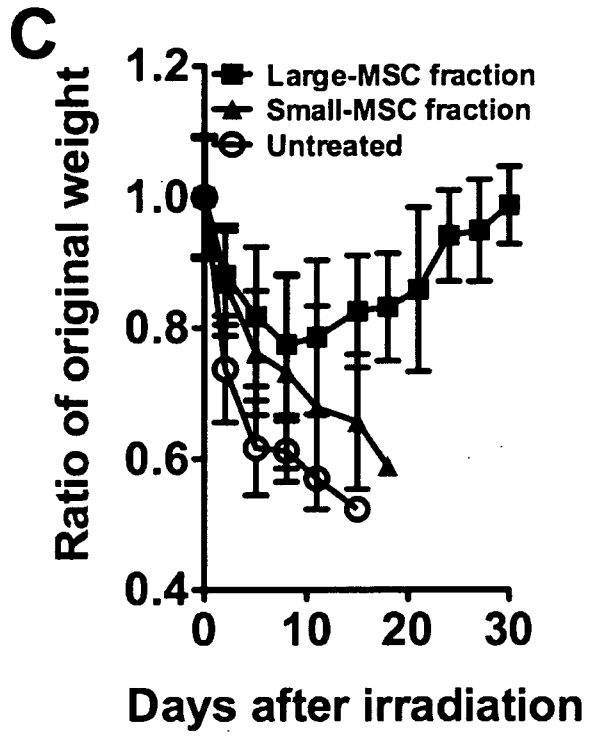
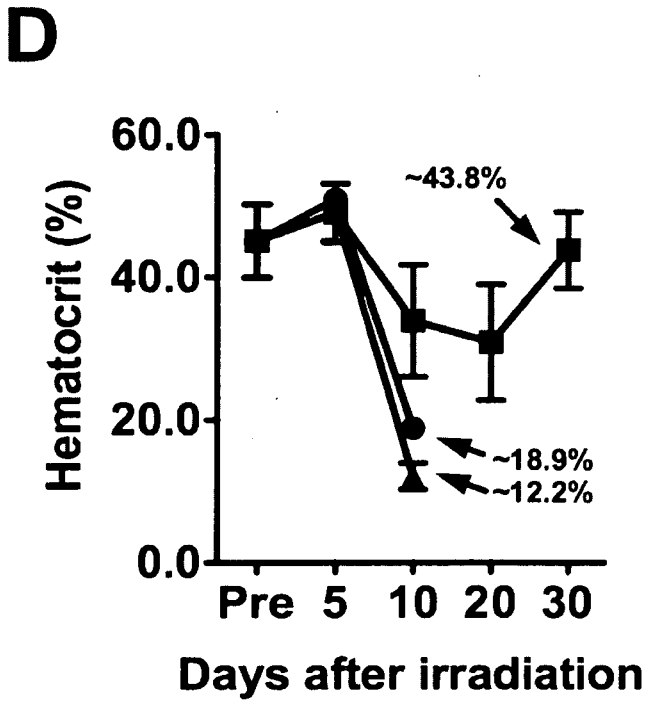

J
Acellular
Unsorted MSCs
Large-MSC
Small-MSC
*
0
1
2
3
4
total radiant efficiency (x10^8)

FIG. 1J

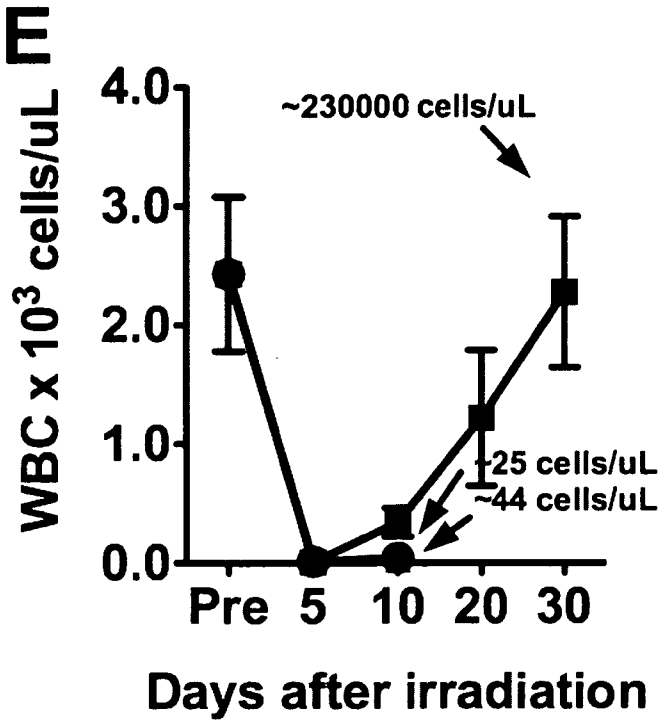

A
100
80
60
40
20
0
Percent survival (%)
No treatment
PSG3 Unsorted
PSG6 Unsorted
PSG9 Unsorted
0 10 20 30 40 50
Days after irradiation
B
100
80
60
40
20
0
Percent survival (%)
No treatment
Large-MSC fraction
Small-MSC fraction
0 10 20 30 40 50
Days after irradiation

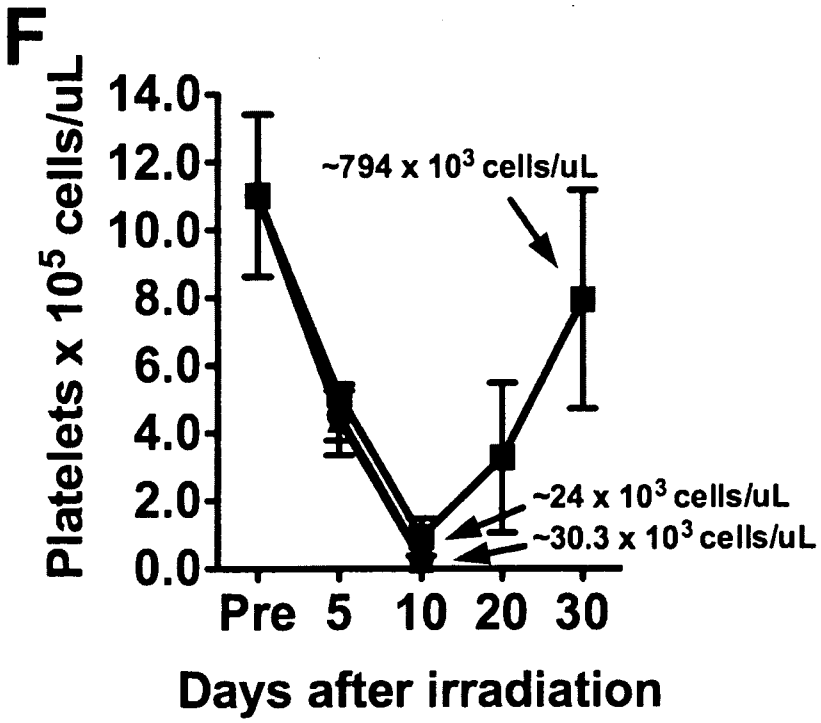
FIGS. 2A - 2B


C
Large-MSC fraction
Small-MSC fraction
Untreated
Ratio of original weight
1.2
1.0
0.8
0.6
0.4
0
10
20
30
Days after irradiation

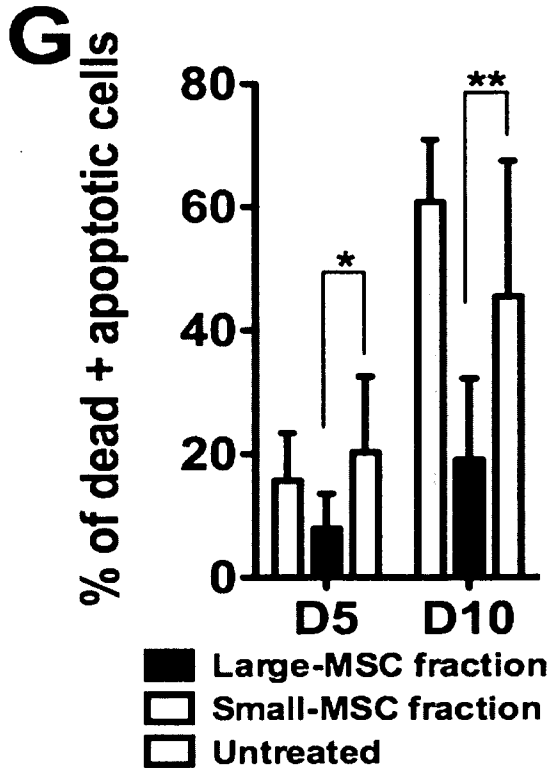

D
Hematocrit (%)
60.0
40.0
20.0
0.0
~43.8%
~18.9%
~12.2%
Pre
5
10
20
30
Days after irradiation

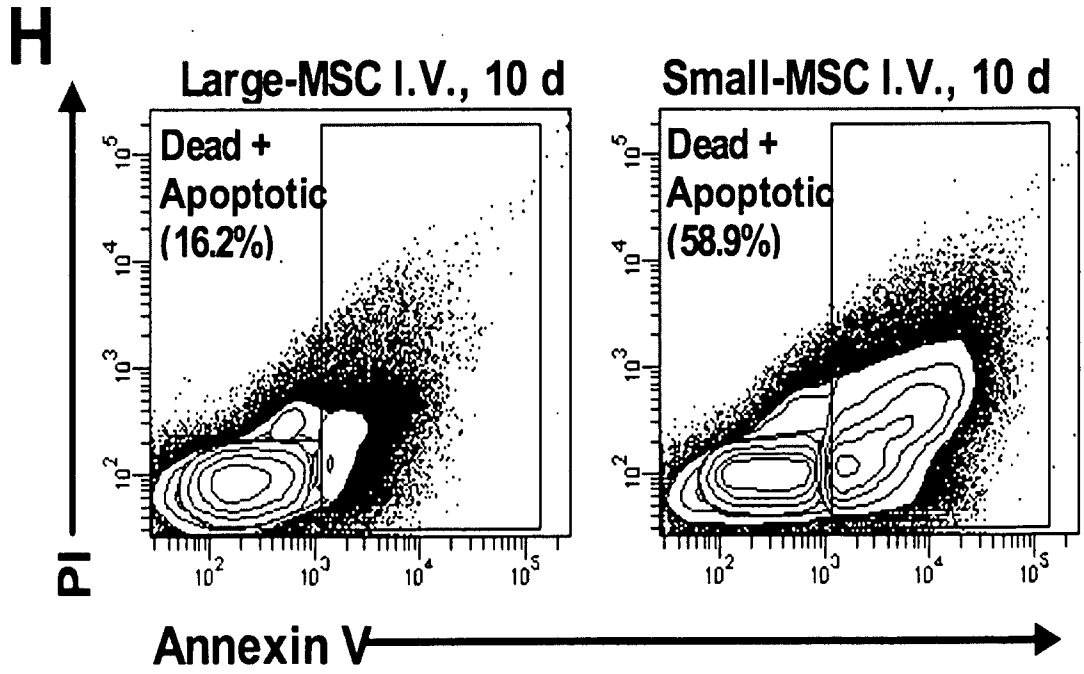
FIGS. 2C - 2D


E
WBC x 10^3 cells/uL
4.0
3.0
2.0
1.0
0.0
~230000 cells/uL
~25 cells/uL
~44 cells/uL
Pre 5 10 20 30
Days after irradiation


F
Platelets x 10^5 cells/uL
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
~794 x 10^3 cells/uL
~24 x 10^3 cells/uL
~30.3 x 10^3 cells/uL
Pre 5 10 20 30
Days after irradiation

FIGS. 2E - F


G
% of dead + apoptotic cells
80
60
40
20
0
*
**
D5
D10
Large-MSC fraction
Small-MSC fraction
Untreated


H
Large-MSC I.V., 10 d
Small-MSC I.V., 10 d
Dead +
Apoptotic
(16.2%)
Dead +
Apoptotic
(58.9%)
PI
Annexin V

FIGS. 2G - H

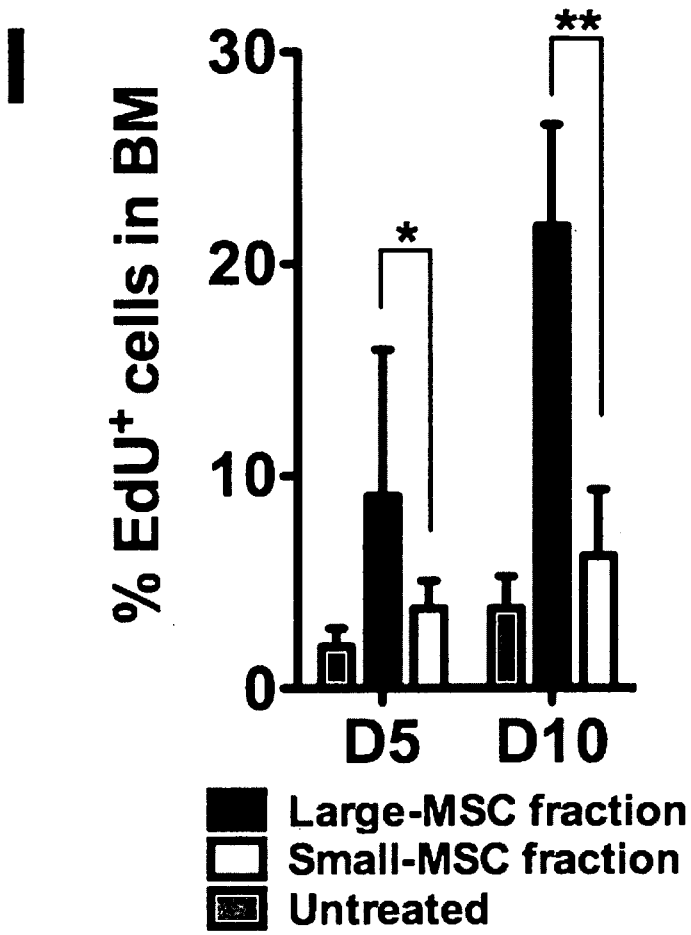

I
% EdU+ cells in BM
30
20
10
0
*
**
D5
D10
Large-MSC fraction
Small-MSC fraction
Untreated

FIG. 2I

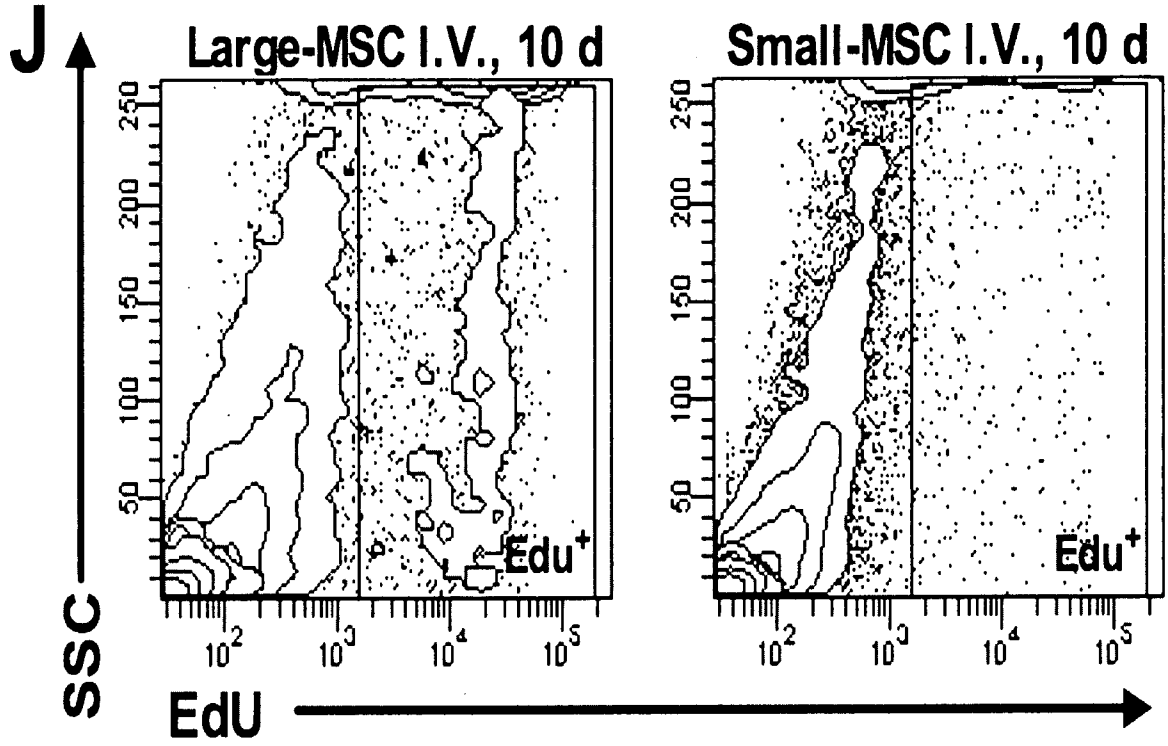

J
Large-MSC I.V., 10 d
Small-MSC I.V., 10 d
SSC
EdU
Edu+
Edu+
50
100
150
200
250
10^2
10^3
10^4
10^5

FIG. 2J

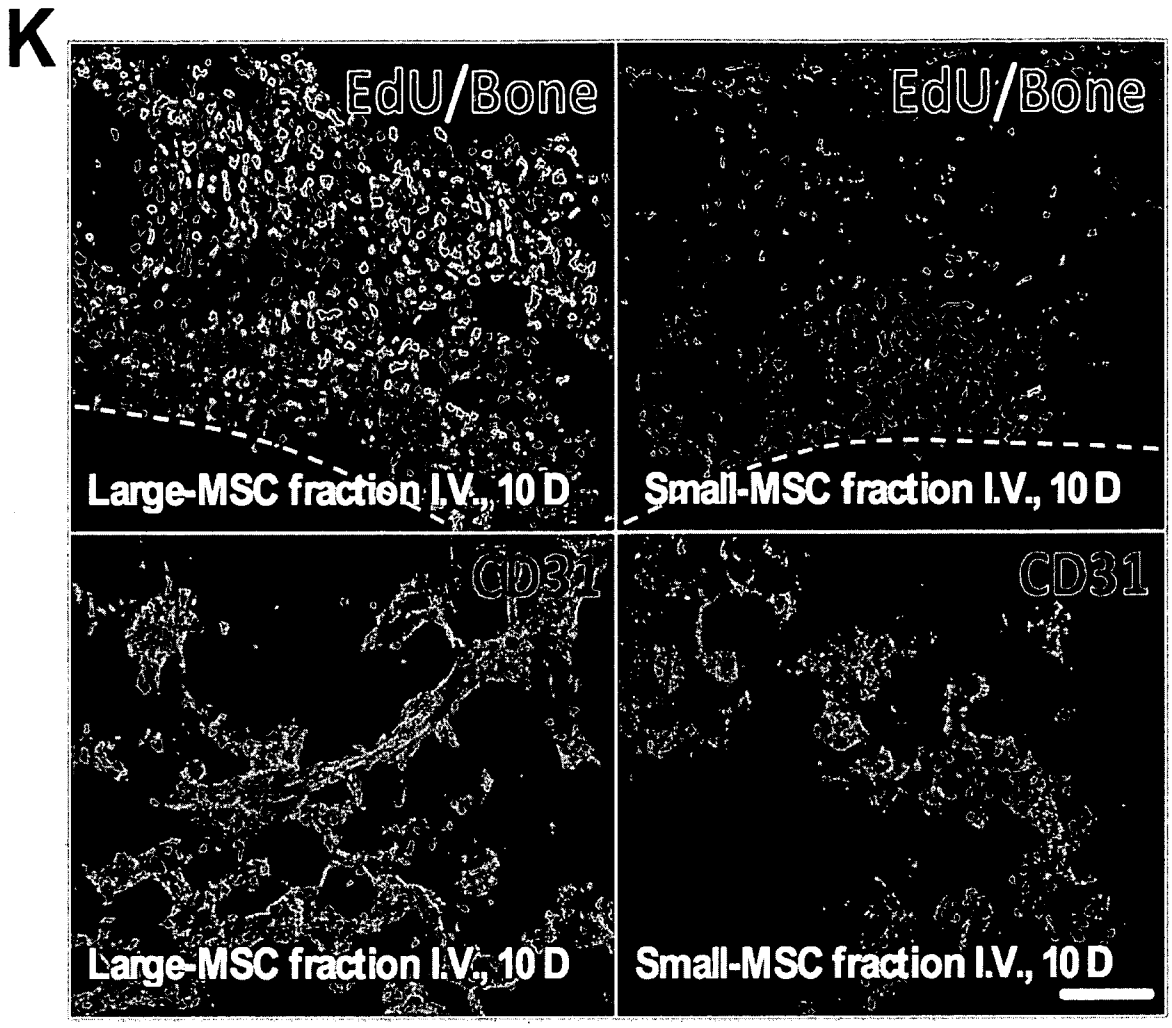

K
EdU/Bone
Large-MSC fraction I.V., 10 D
EdU/Bone
Small-MSC fraction I.V., 10 D
CD31
Large-MSC fraction I.V., 10 D
CD31
Small-MSC fraction I.V., 10 D

FIG. 2K

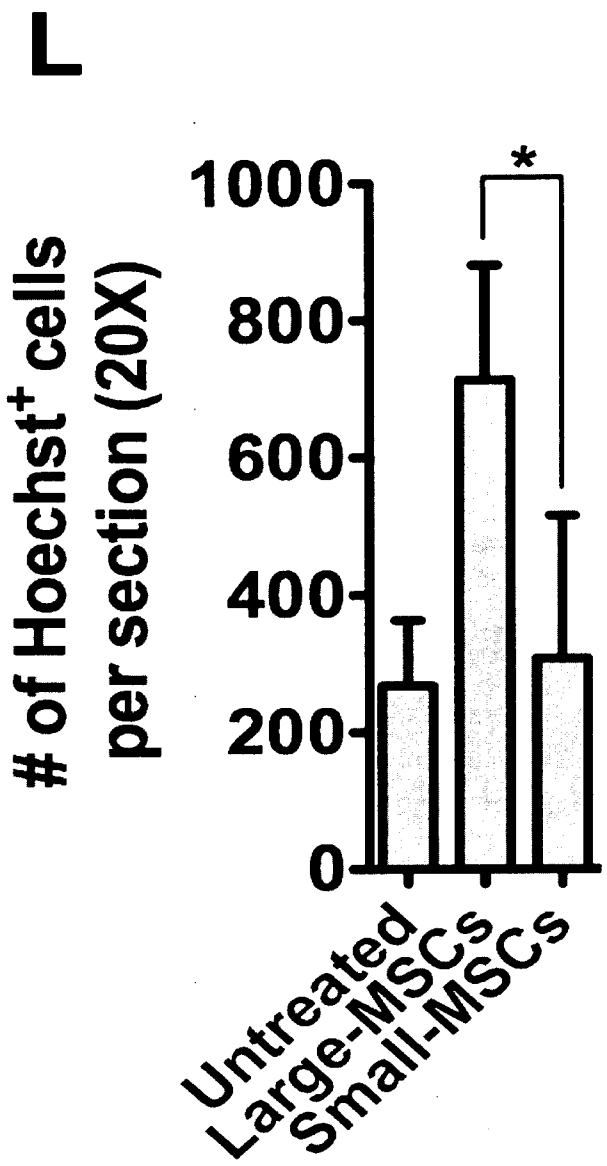

L
# of Hoechst+ cells per section (20X)
1000
800
600
400
200
0
*
Untreated
Large-MSCs
Small-MSCs

FIG. 2L

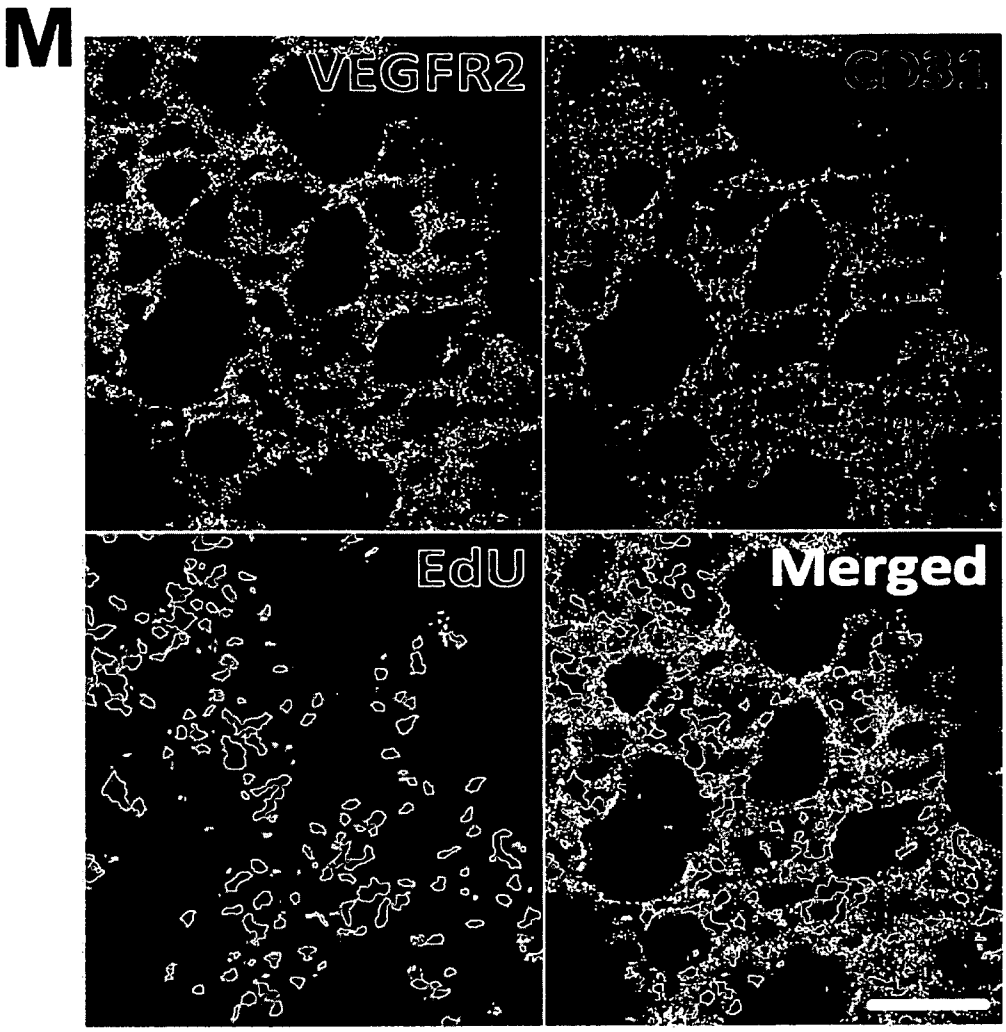


FIG. 2M

N

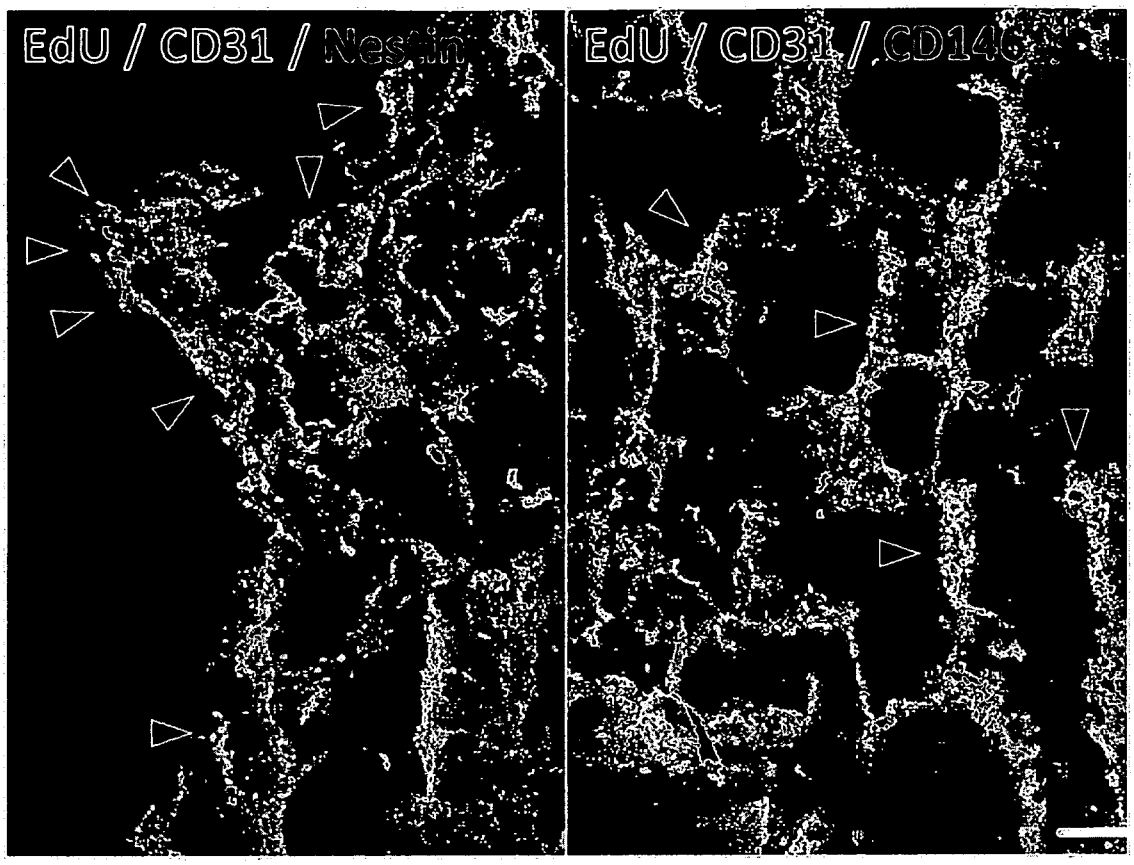
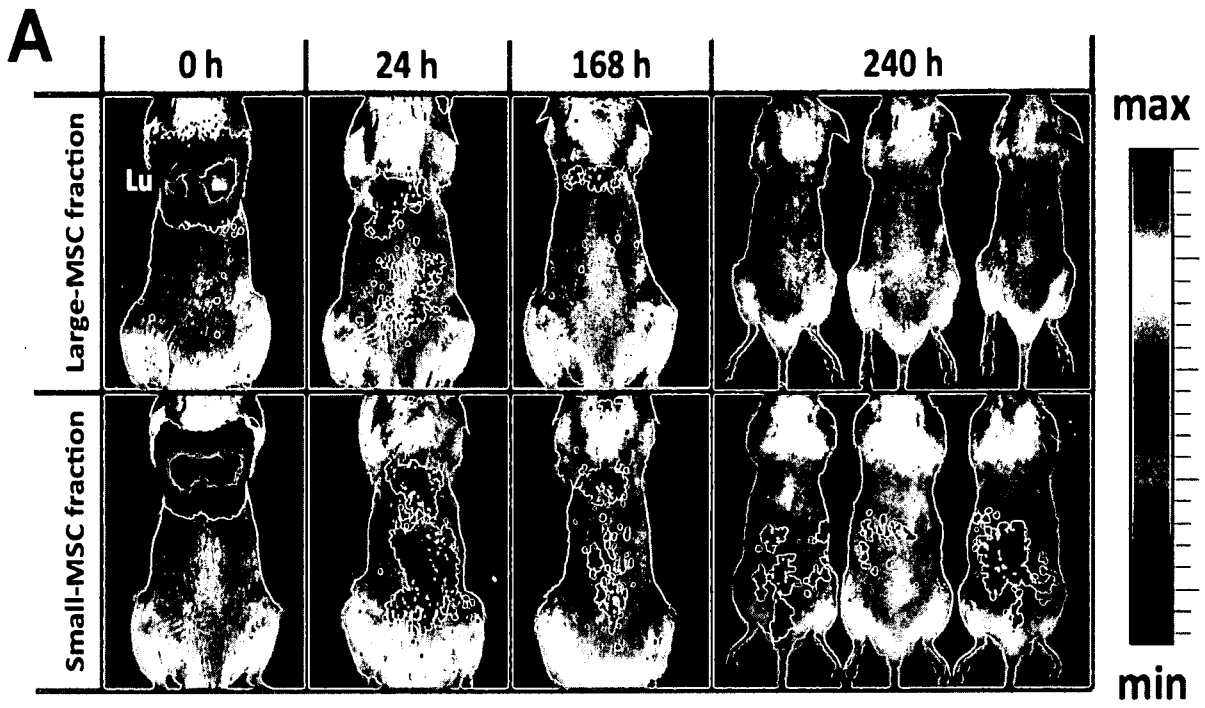
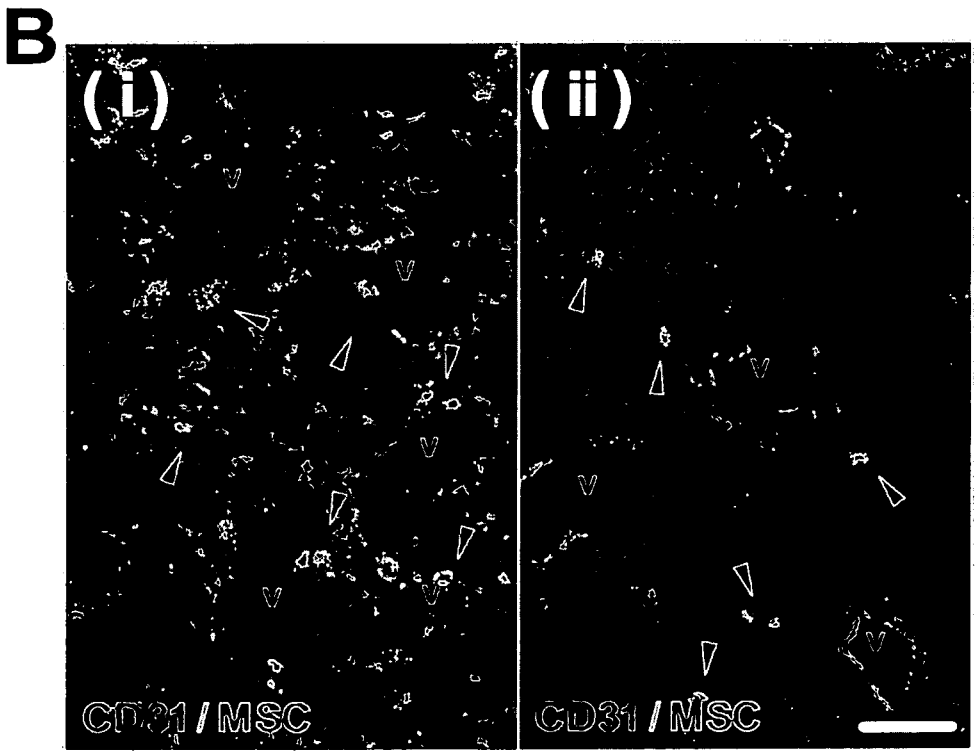
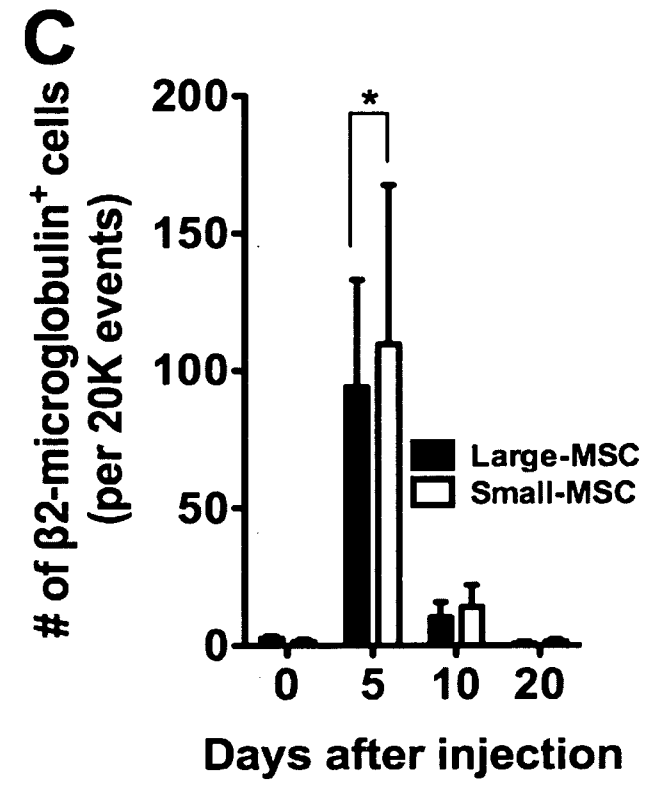


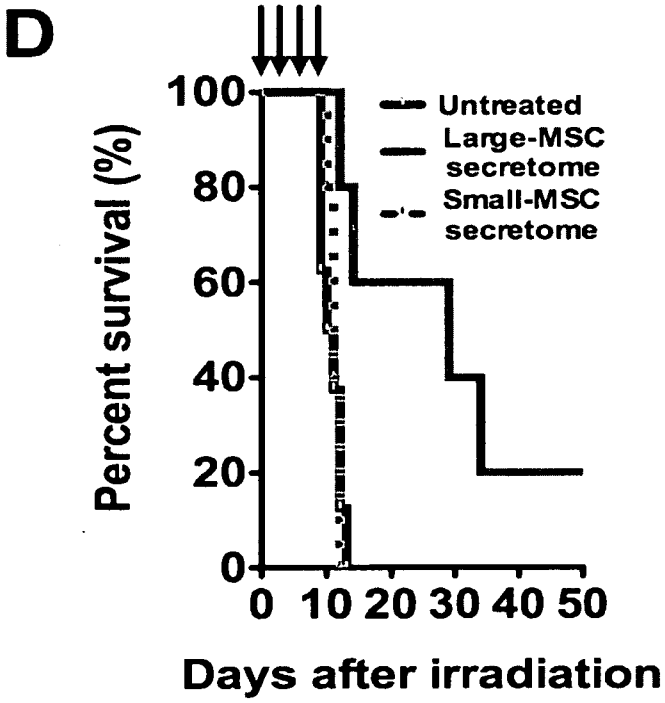
FIG. 2N


A
0 h
24 h
168 h
240 h
Large-MSC fraction
Small-MSC fraction
Lu
max
min


B
(i)
(ii)
CD31 / MSC
CD31 / MSC

FIGS. 3A - 3B


C
# of β2-microglobulin+ cells (per 20K events)
200
150
100
50
0
*
Large-MSC
Small-MSC
0 5 10 20
Days after injection


D
Percent survival (%)
100
80
60
40
20
0
Untreated
Large-MSC secretome
Small-MSC secretome
0 10 20 30 40 50
Days after irradiation

FIGS. 3C - 3D

E

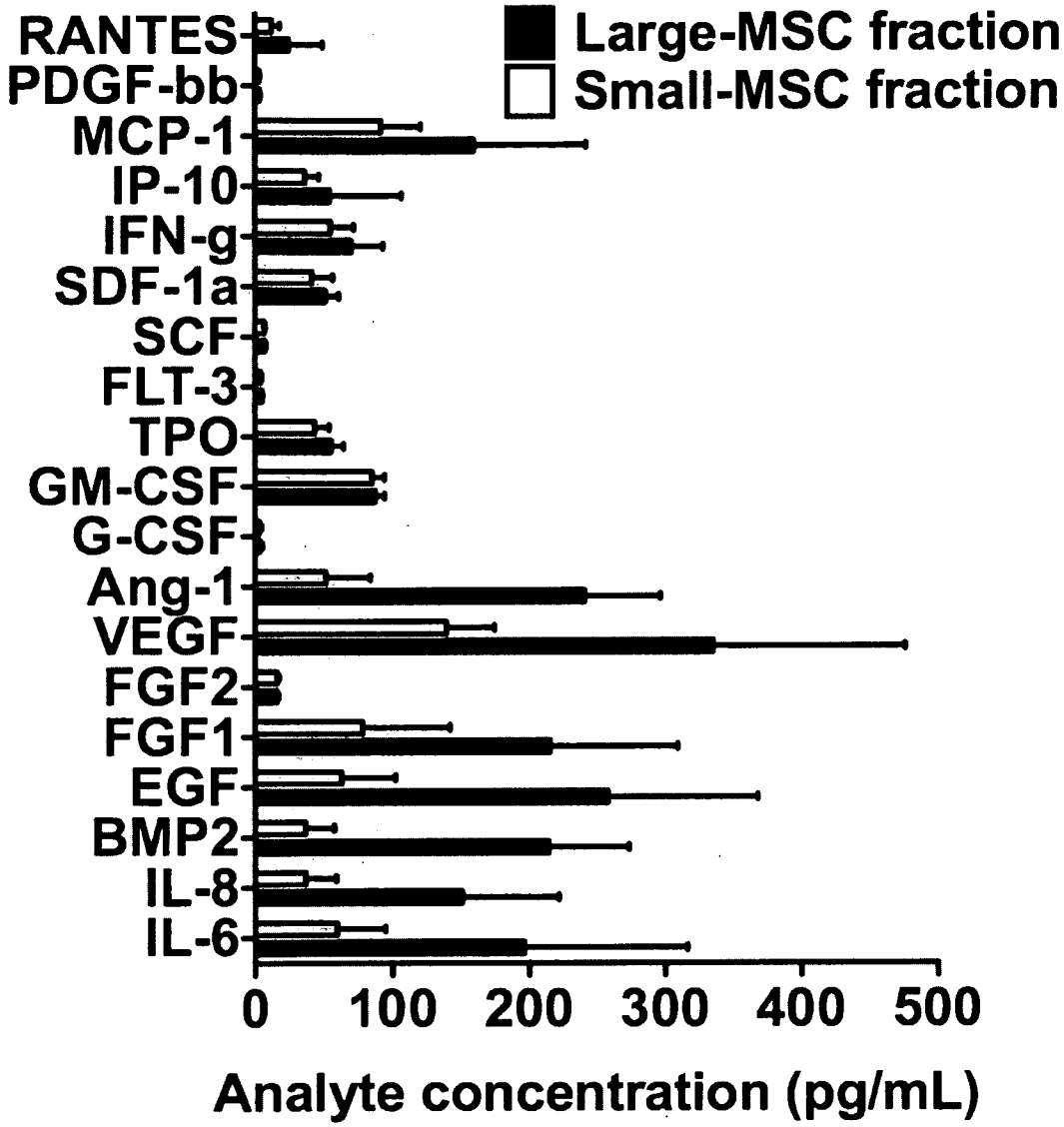

RANTES
PDGF-bb
MCP-1
IP-10
IFN-g
SDF-1a
SCF
FLT-3
TPO
GM-CSF
G-CSF
Ang-1
VEGF
FGF2
FGF1
EGF
BMP2
IL-8
IL-6
Large-MSC fraction
Small-MSC fraction
0
100
200
300
400
500
Analyte concentration (pg/mL)

FIG. 3E

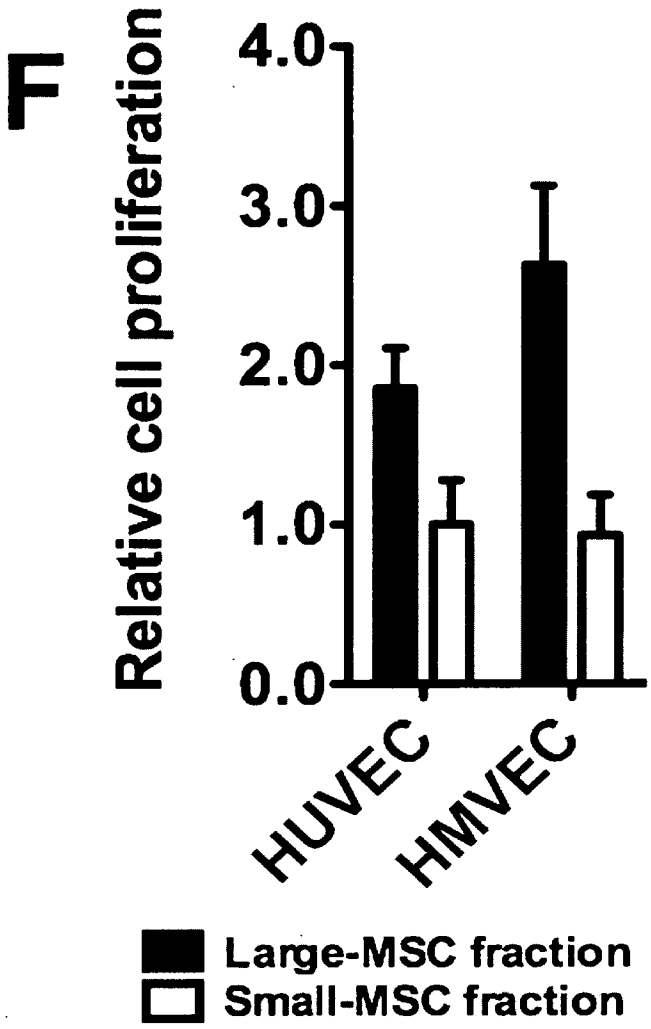

F
Relative cell proliferation
4.0
3.0
2.0
1.0
0.0
HUVEC
HMVEC
Large-MSC fraction
Small-MSC fraction

FIG. 3F

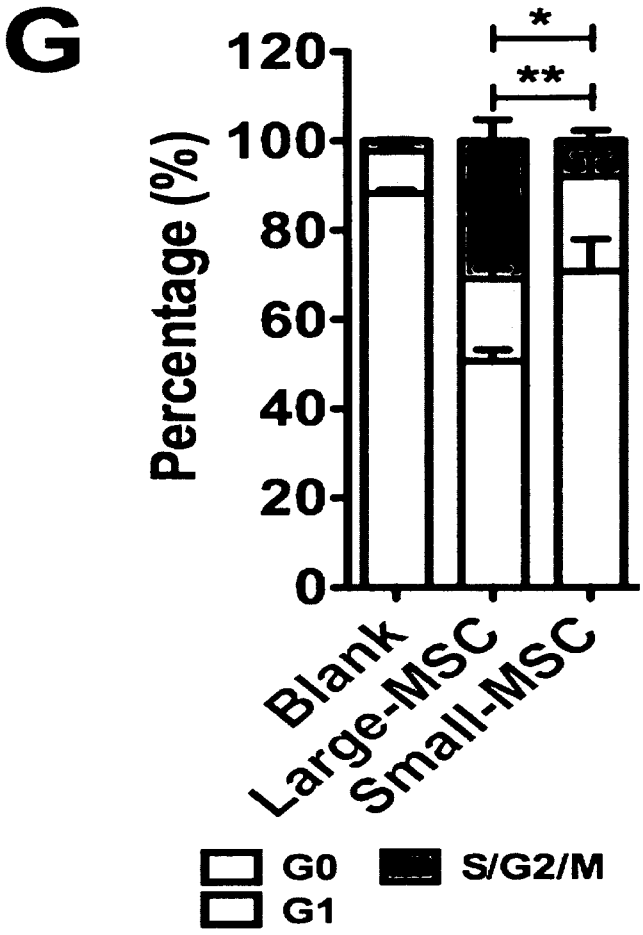

G
120
100
80
60
40
20
0
Percentage (%)
*
**
Blank
Large-MSC
Small-MSC
G0
G1
S/G2/M

FIG. 3G

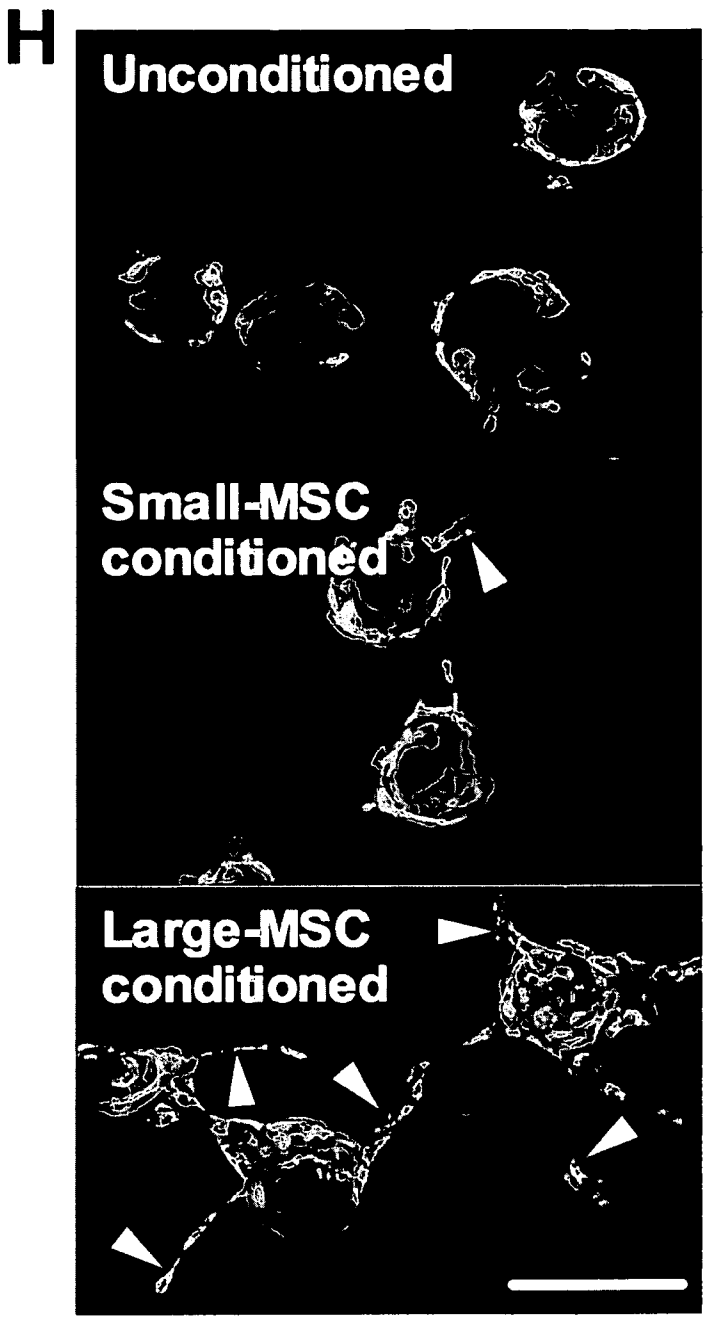

H
Unconditioned
Small-MSC conditioned
Large-MSC conditioned

FIG. 3H

I

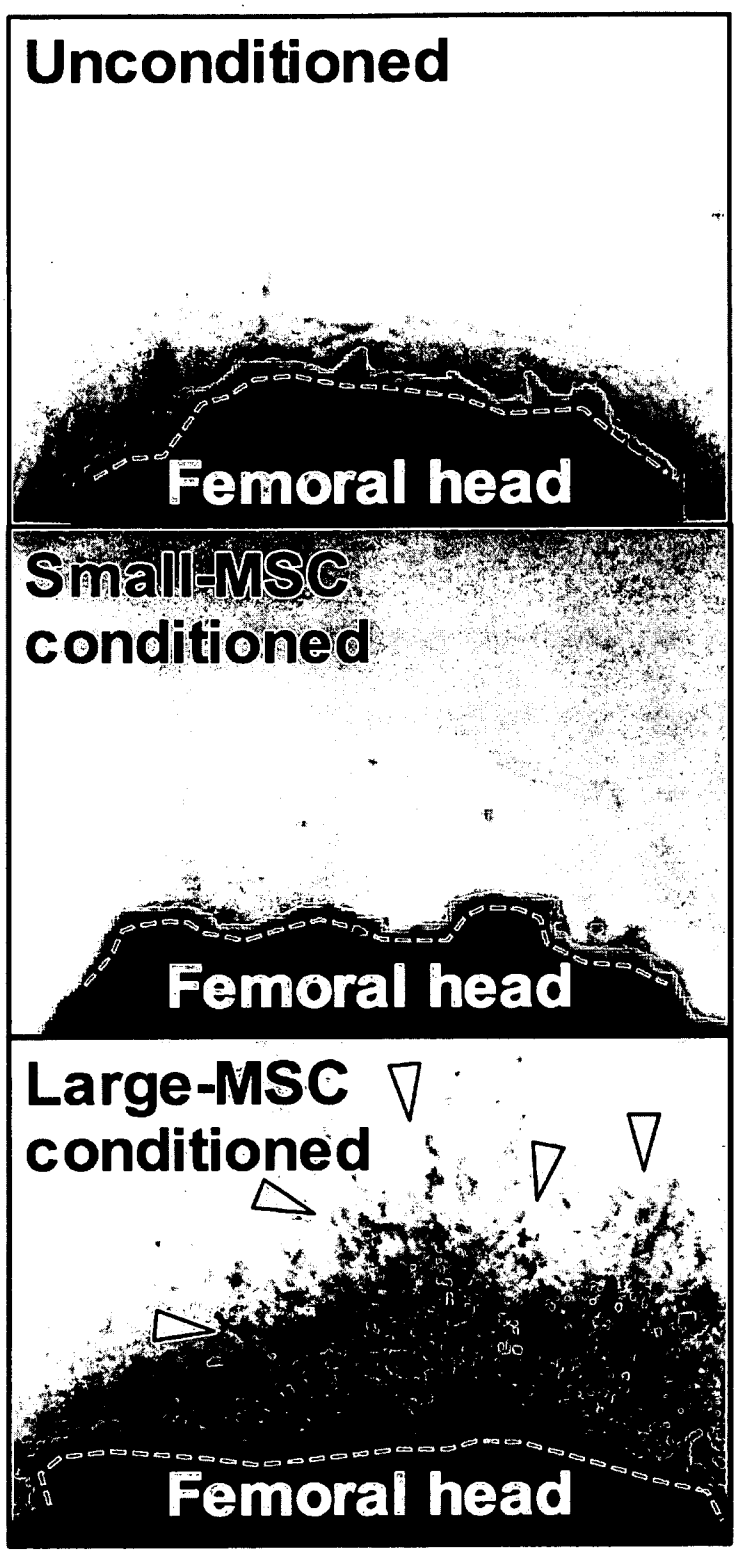

Unconditioned
Femoral head
Small-MSC conditioned
Femoral head
Large-MSC conditioned
Femoral head

FIG. 3I

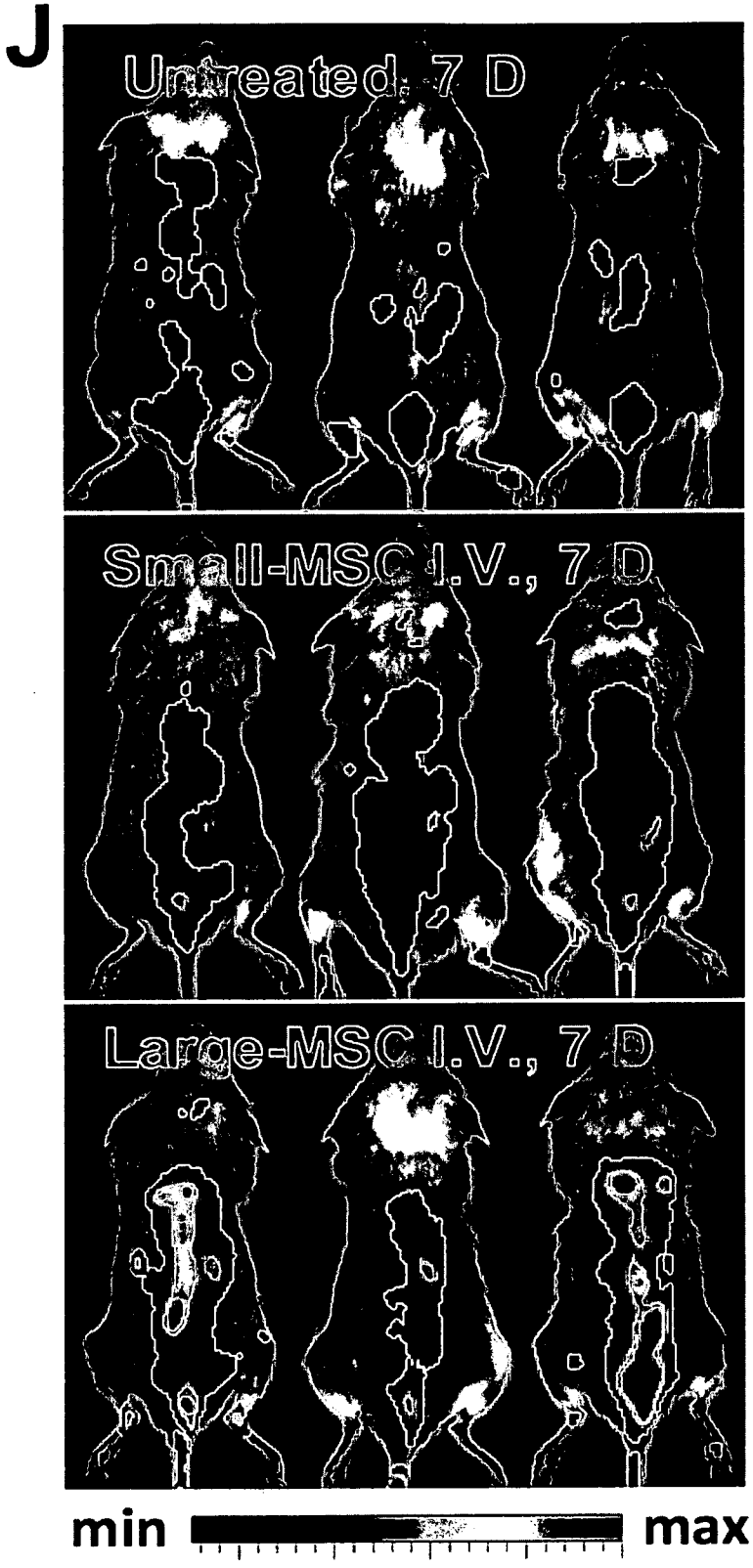
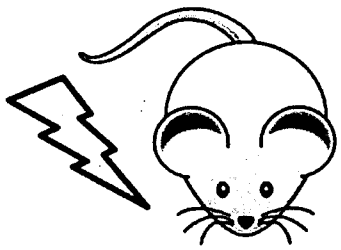


FIG. 3J

A



Day 0:
Sublethal irraidation

Treatment 1:
MSC infusion on day 1
HSC infusion on day 7
Analysis on day 8

Treatment 2:
MSC + HSC co-infusion on day 1
Analysis on day 8

FIG. 4A

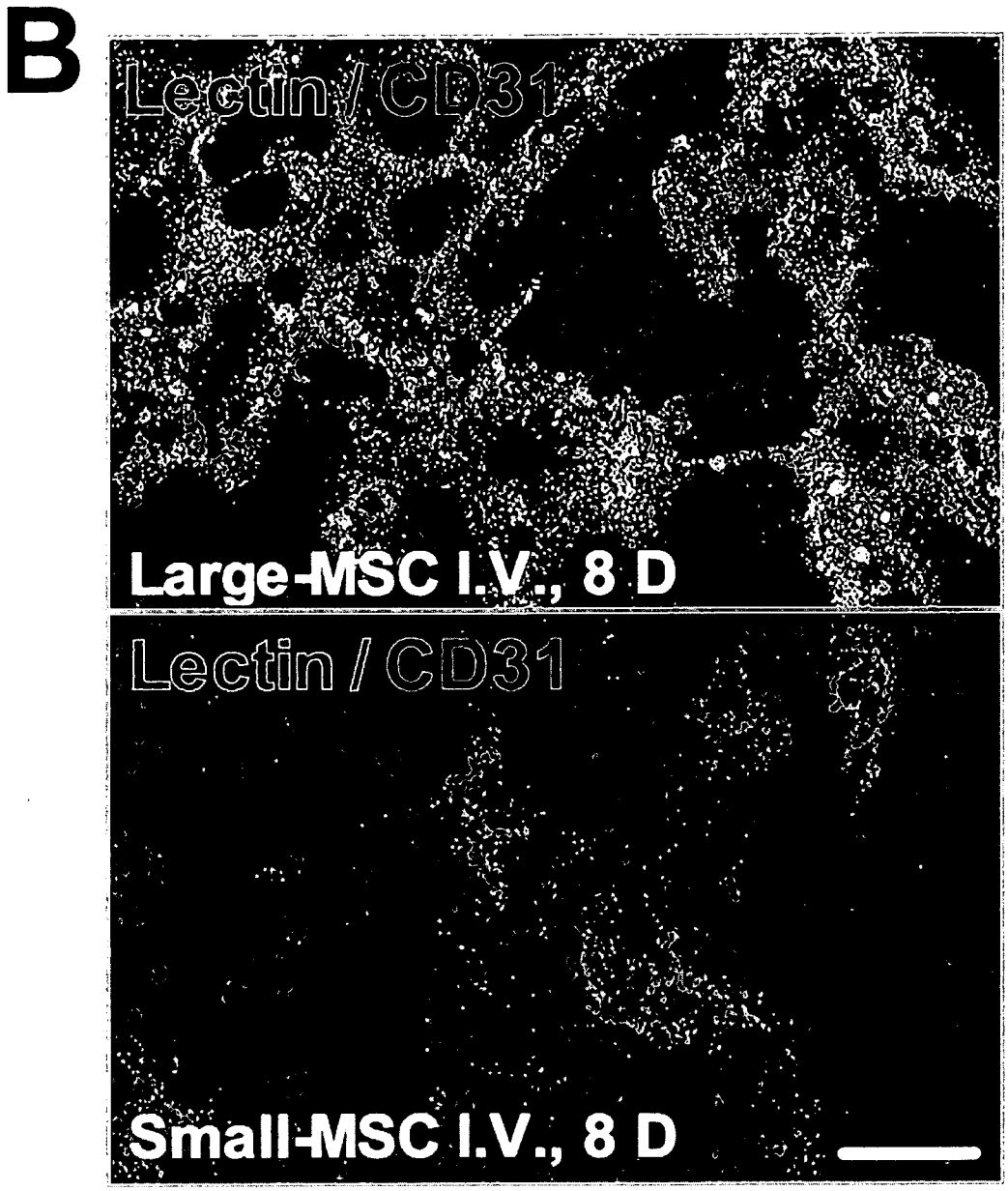


FIG. 4B

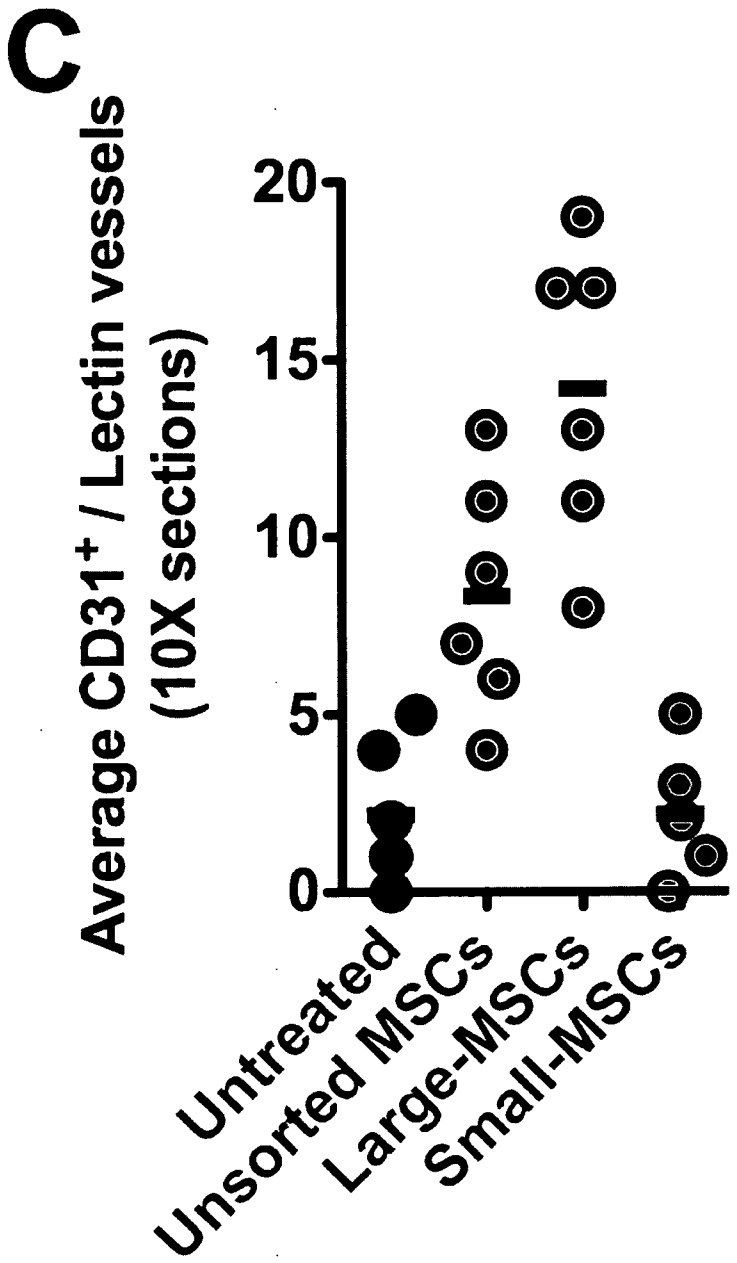

C
Average CD31+ / Lectin vessels
(10X sections)
20
15
10
5
0
Untreated
Unsorted MSCs
Large-MSCs
Small-MSCs

FIG. 4C

D

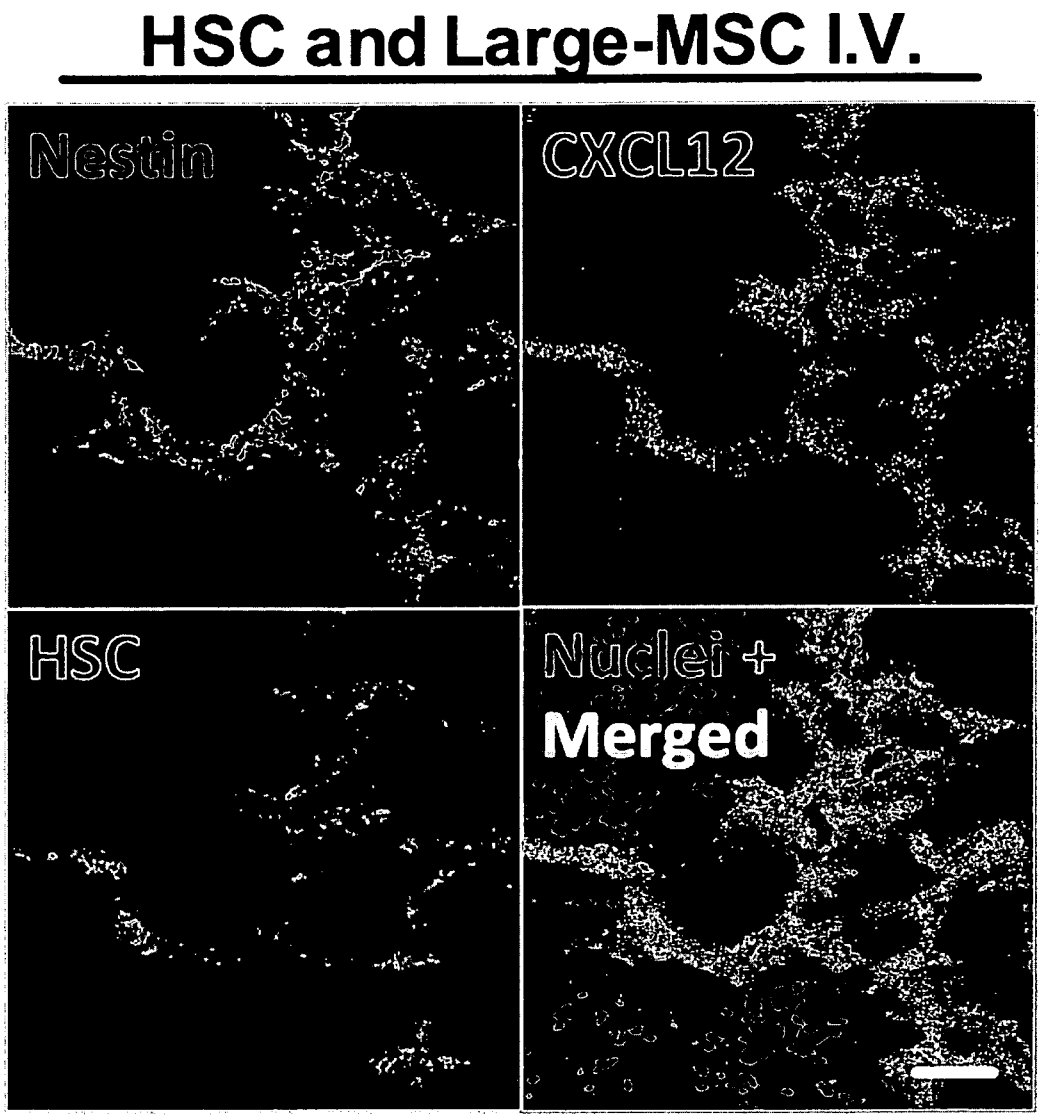


FIG. 4D

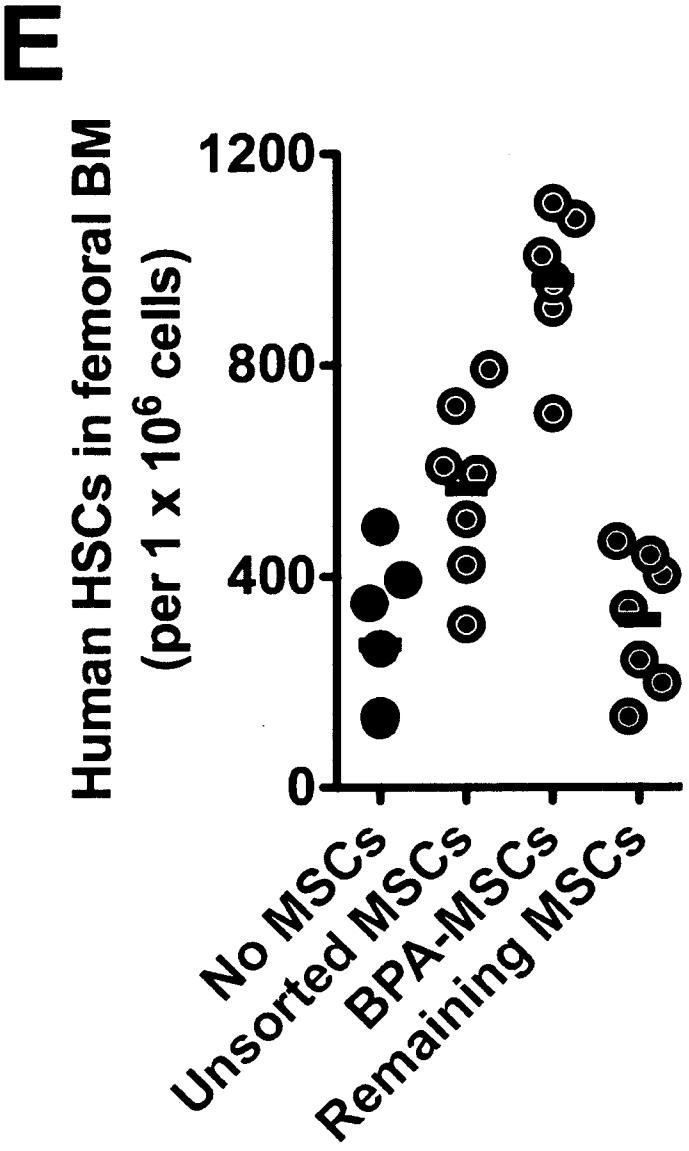

E
Human HSCs in femoral BM
(per 1 x 10^6 cells)
1200
800
400
0
No MSCs
Unsorted MSCs
BPA-MSCs
Remaining MSCs

FIG. 4E

F

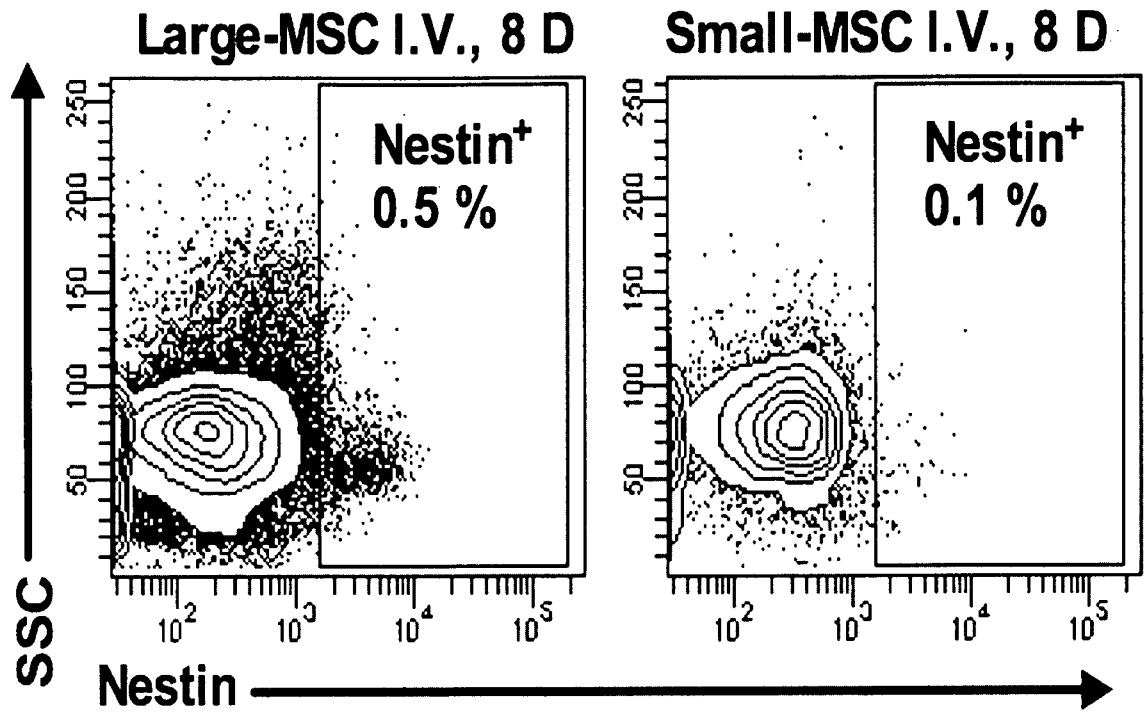
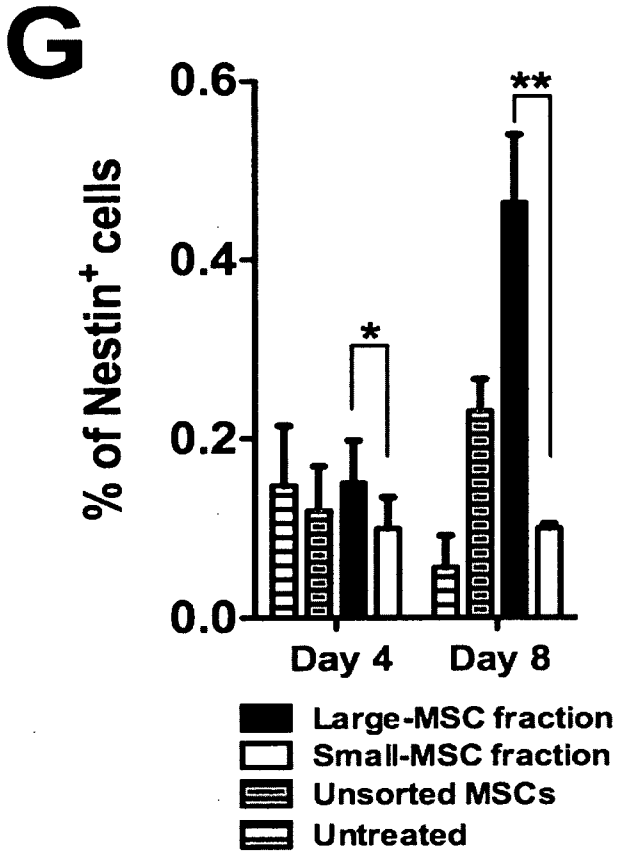
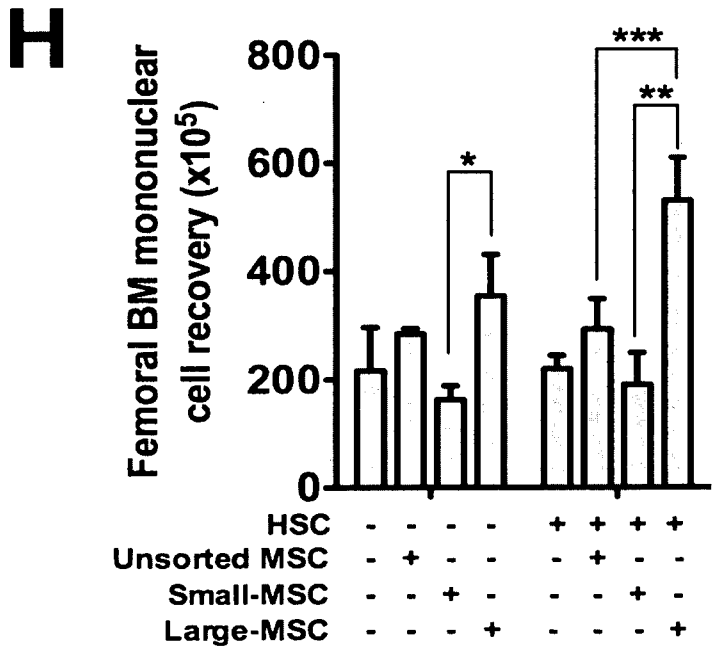
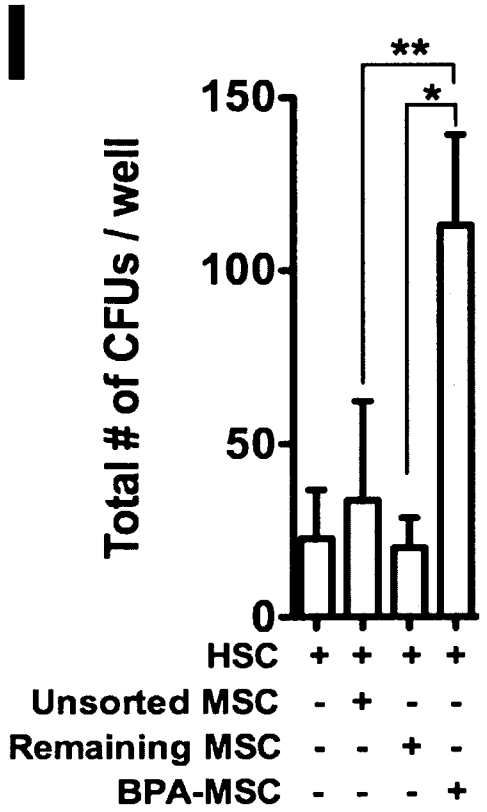

Large-MSC I.V., 8 D
Small-MSC I.V., 8 D
Nestin+
0.5 %
Nestin+
0.1 %
SSC
Nestin
50
100
150
200
250
10^2
10^3
10^4
10^5

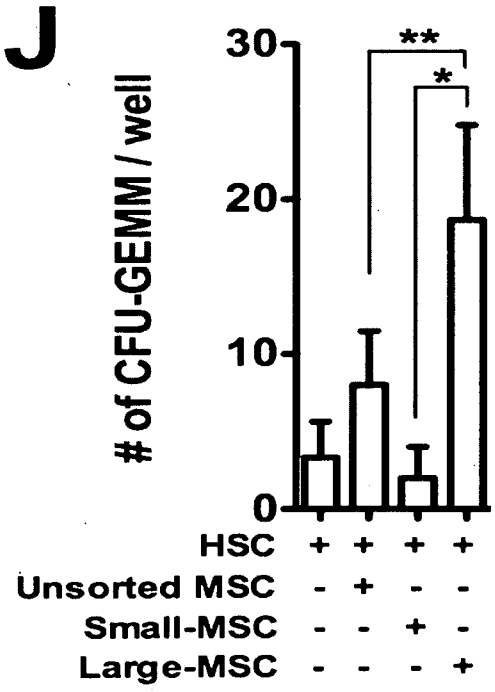
FIG. 4F


G
0.6
0.4
0.2
0.0
% of Nestin+ cells
*
**
Day 4
Day 8
Large-MSC fraction
Small-MSC fraction
Unsorted MSCs
Untreated


H
800
600
400
200
0
Femoral BM mononuclear cell recovery (x10^5)
*
***
**
HSC - - - - + + + +
Unsorted MSC - + - - - + - -
Small-MSC - - + - - - + -
Large-MSC - - - + - - - +

FIGS. 4G - 4H


I
Total # of CFUs / well
150
100
50
0
**
*
HSC + + + +
Unsorted MSC - + - -
Remaining MSC - - + -
BPA-MSC - - - +


J
# of CFU-GEMM / well
30
20
10
0
**
*
HSC + + + +
Unsorted MSC - + - -
Small-MSC - - + -
Large-MSC - - - +

FIGS. 4I - 4J

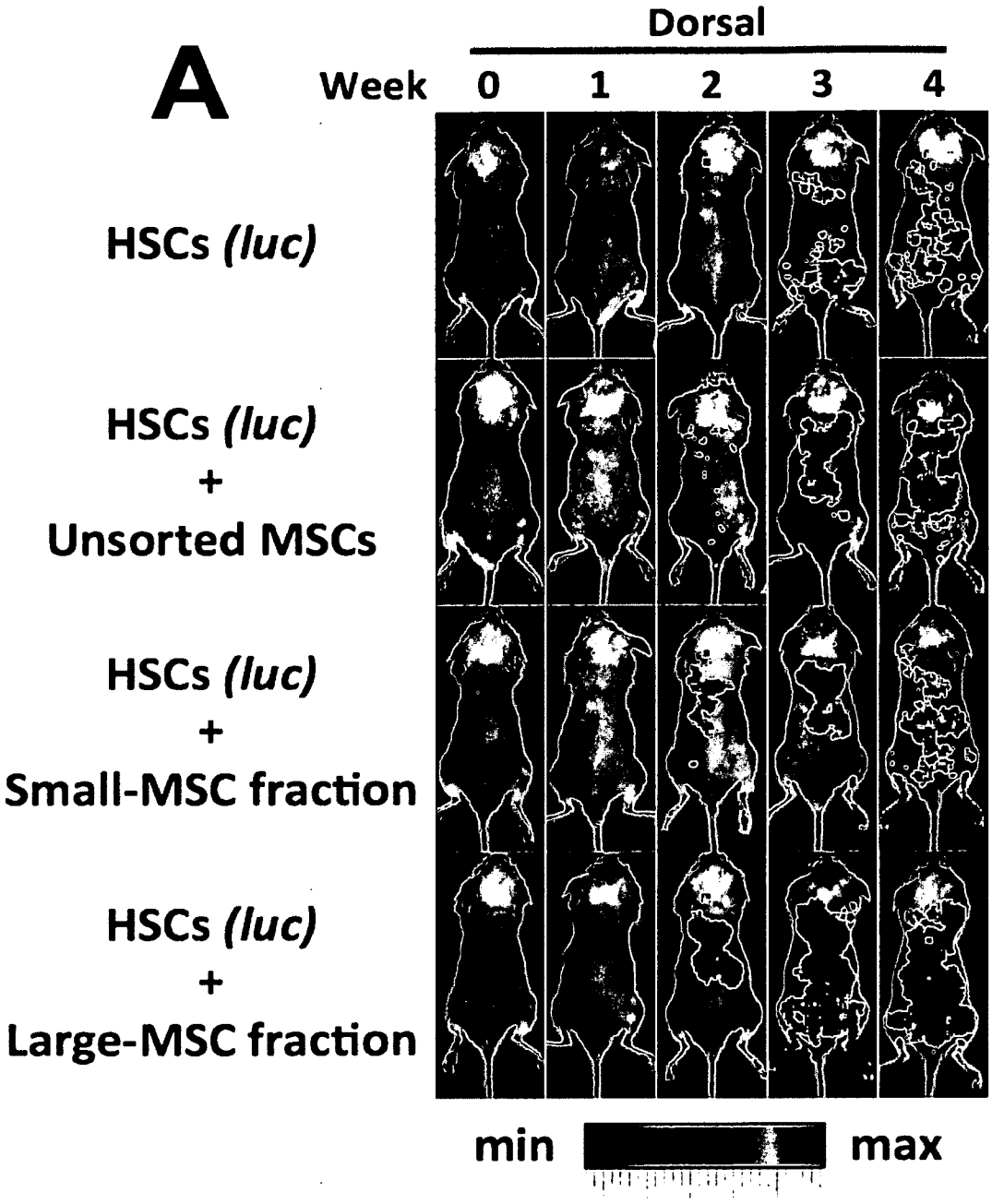
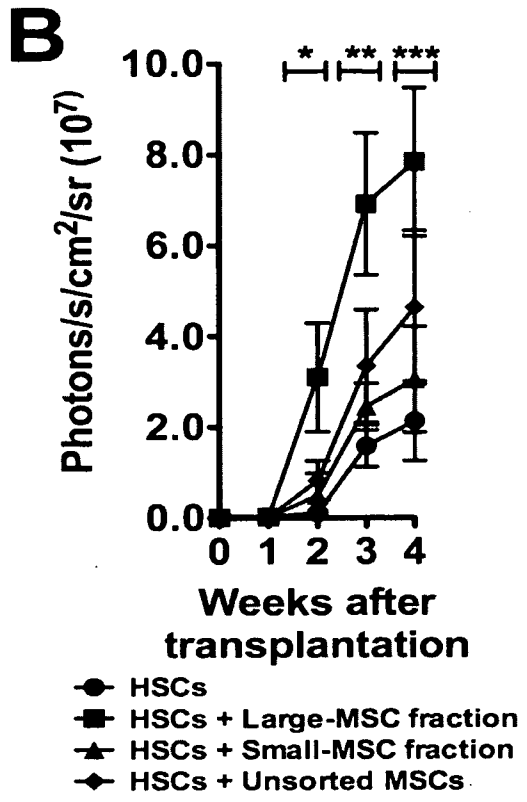
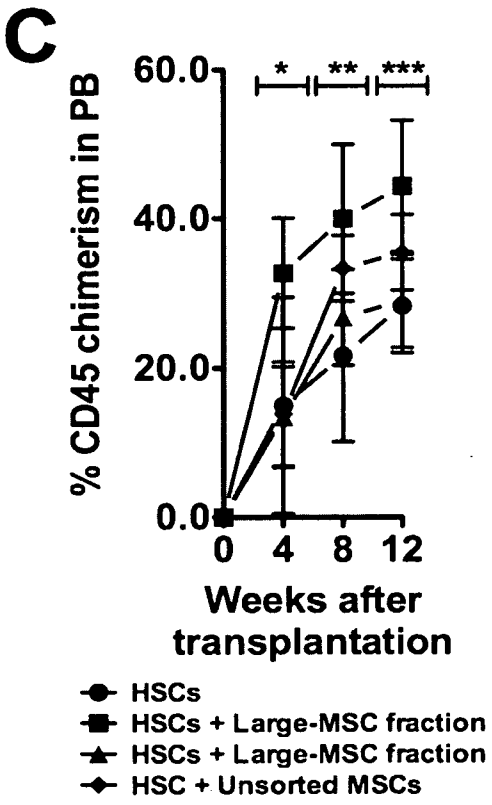

A
Dorsal
Week 0 1 2 3 4
HSCs (luc)
HSCs (luc) + Unsorted MSCs
HSCs (luc) + Small-MSC fraction
HSCs (luc) + Large-MSC fraction
min
max

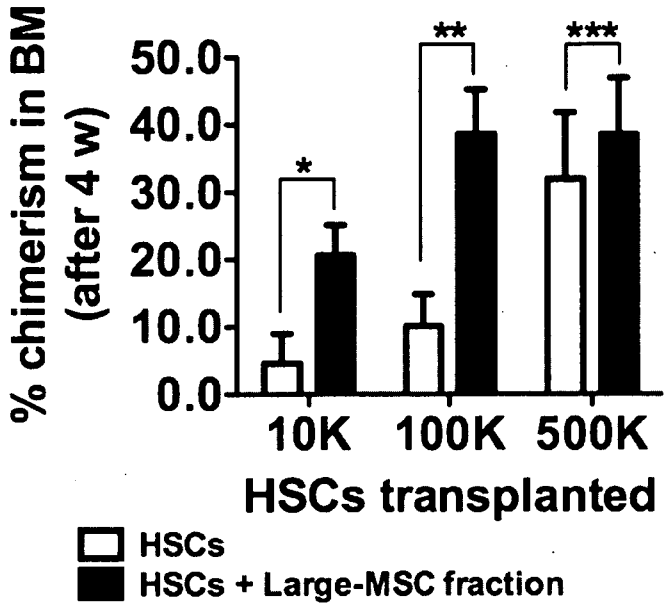
FIG. 5A


B
10.0
8.0
6.0
4.0
2.0
0.0
Photons/s/cm2/sr (10^7)
*
**
***
0 1 2 3 4
Weeks after transplantation
HSCs
HSCs + Large-MSC fraction
HSCs + Small-MSC fraction
HSCs + Unsorted MSCs

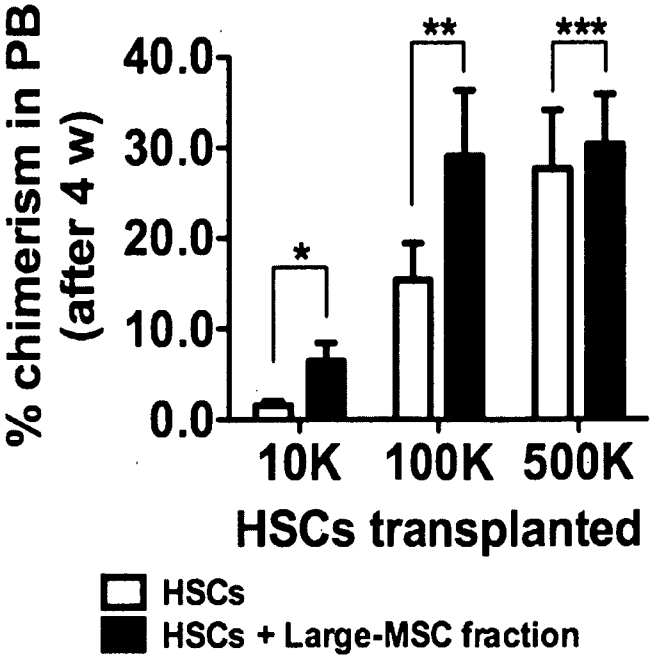

C
60.0
40.0
20.0
0.0
% CD45 chimerism in PB
*
**
***
0 4 8 12
Weeks after transplantation
HSCs
HSCs + Large-MSC fraction
HSCs + Large-MSC fraction
HSC + Unsorted MSCs

FIGS. 5B - 5C

D


% chimerism in BM
(after 4 w)
50.0
40.0
30.0
20.0
10.0
0.0
*
**
***
10K
100K
500K
HSCs transplanted
HSCs
HSCs + Large-MSC fraction

E


% chimerism in PB
(after 4 w)
40.0
30.0
20.0
10.0
0.0
*
**
***
10K
100K
500K
HSCs transplanted
HSCs
HSCs + Large-MSC fraction

FIGS. 5D - 5E

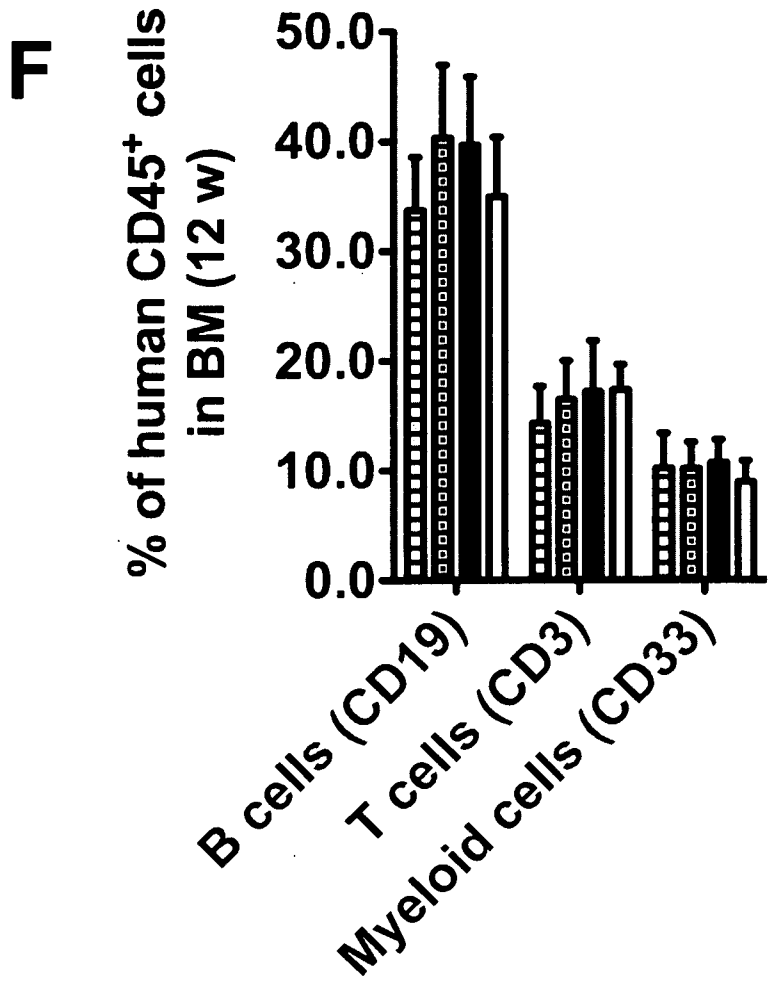

F
% of human CD45+ cells in BM (12 w)
50.0
40.0
30.0
20.0
10.0
0.0
B cells (CD19)
T cells (CD3)
Myeloid cells (CD33)

FIG. 5F

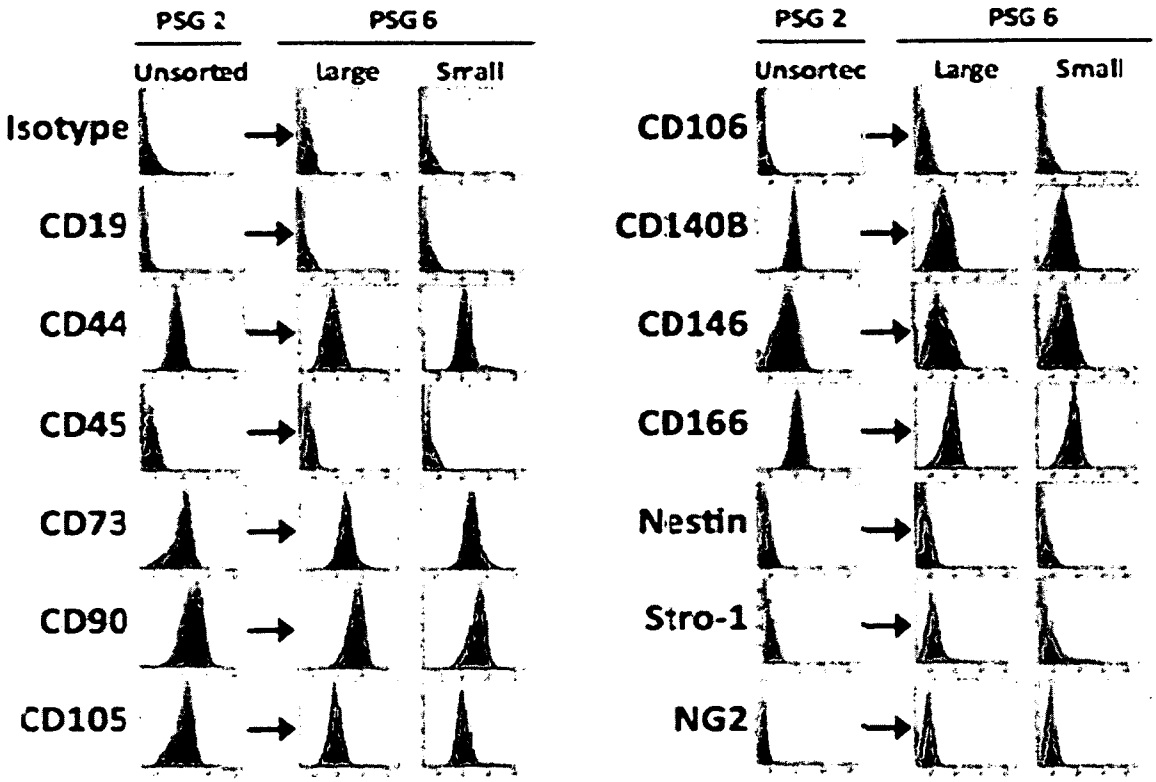
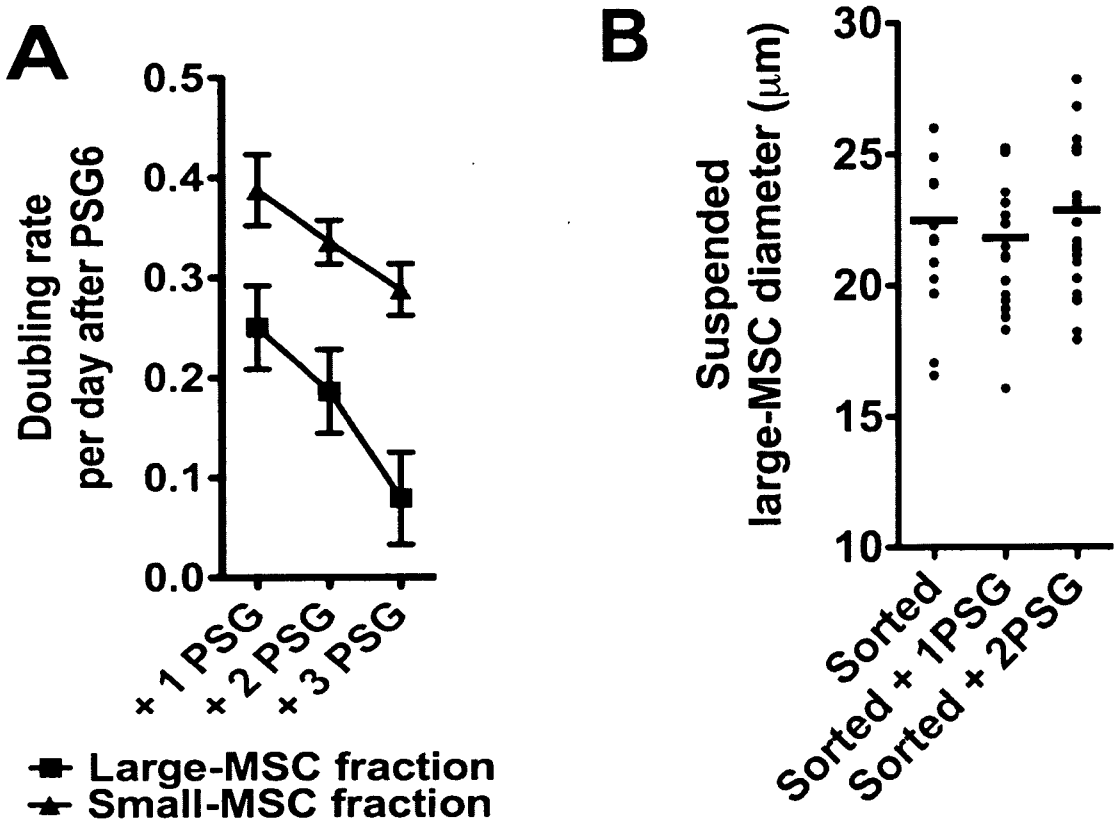

PSG 2
PSG 6
Unsorted
Large
Small
Isotype
CD19
CD44
CD45
CD73
CD90
CD105
PSG 2
PSG 6
Unsortec
Large
Small
CD106
CD140B
CD146
CD166
Nestin
Stro-1
NG2

FIG. 6


A
Doubling rate per day after PSG6
0.5
0.4
0.3
0.2
0.1
0.0
x 1 PSG
x 2 PSG
x 3 PSG
Large-MSC fraction
Small-MSC fraction
B
Suspended large-MSC diameter (μm)
30
25
20
15
10
Sorted
Sorted + 1PSG
Sorted + 2PSG

FIGS. 7A - 7B

C

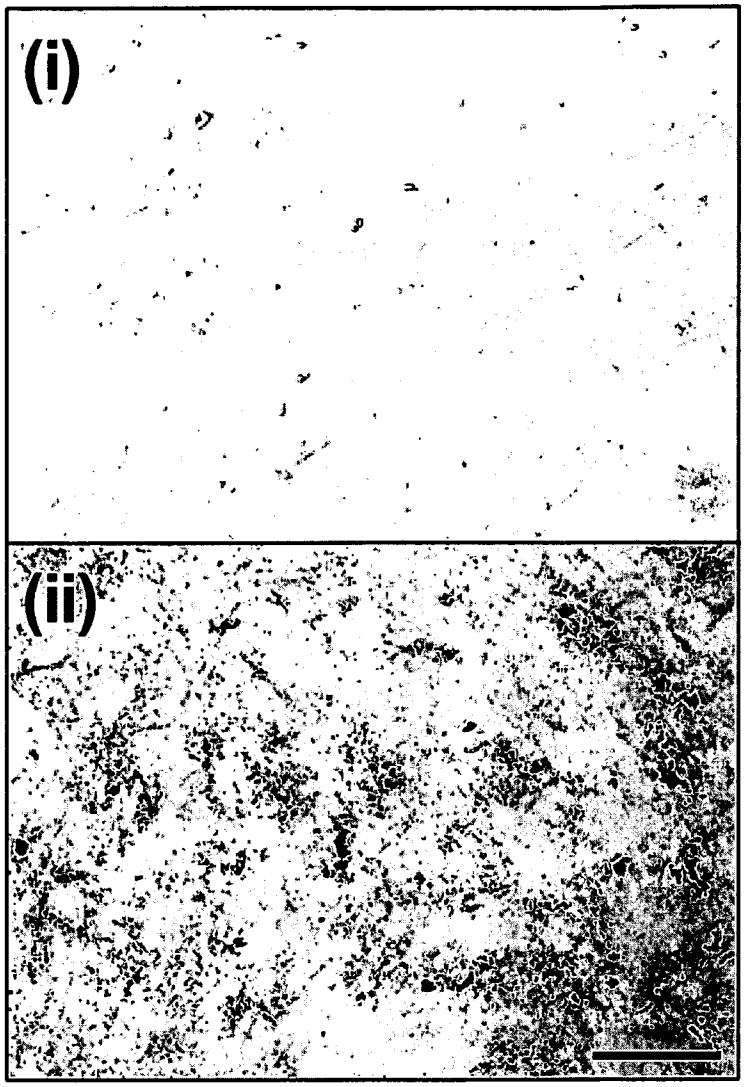
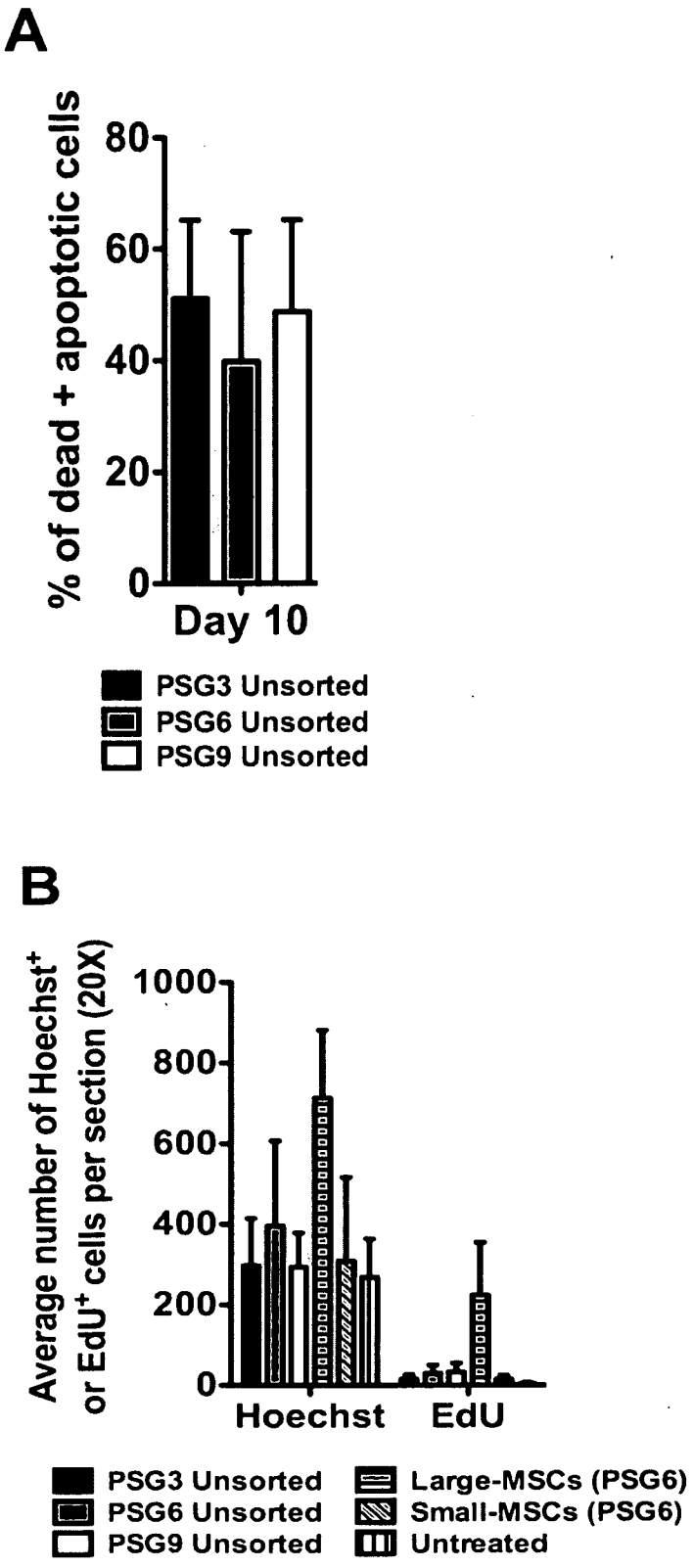


FIG. 7C


A
% of dead + apoptotic cells
80
60
40
20
0
Day 10
PSG3 Unsorted
PSG6 Unsorted
PSG9 Unsorted
B
Average number of Hoechst+ or EdU+ cells per section (20X)
1000
800
600
400
200
0
Hoechst
EdU
PSG3 Unsorted
PSG6 Unsorted
PSG9 Unsorted
Large-MSCs (PSG6)
Small-MSCs (PSG6)
Untreated

FIGS. 8A - 8B

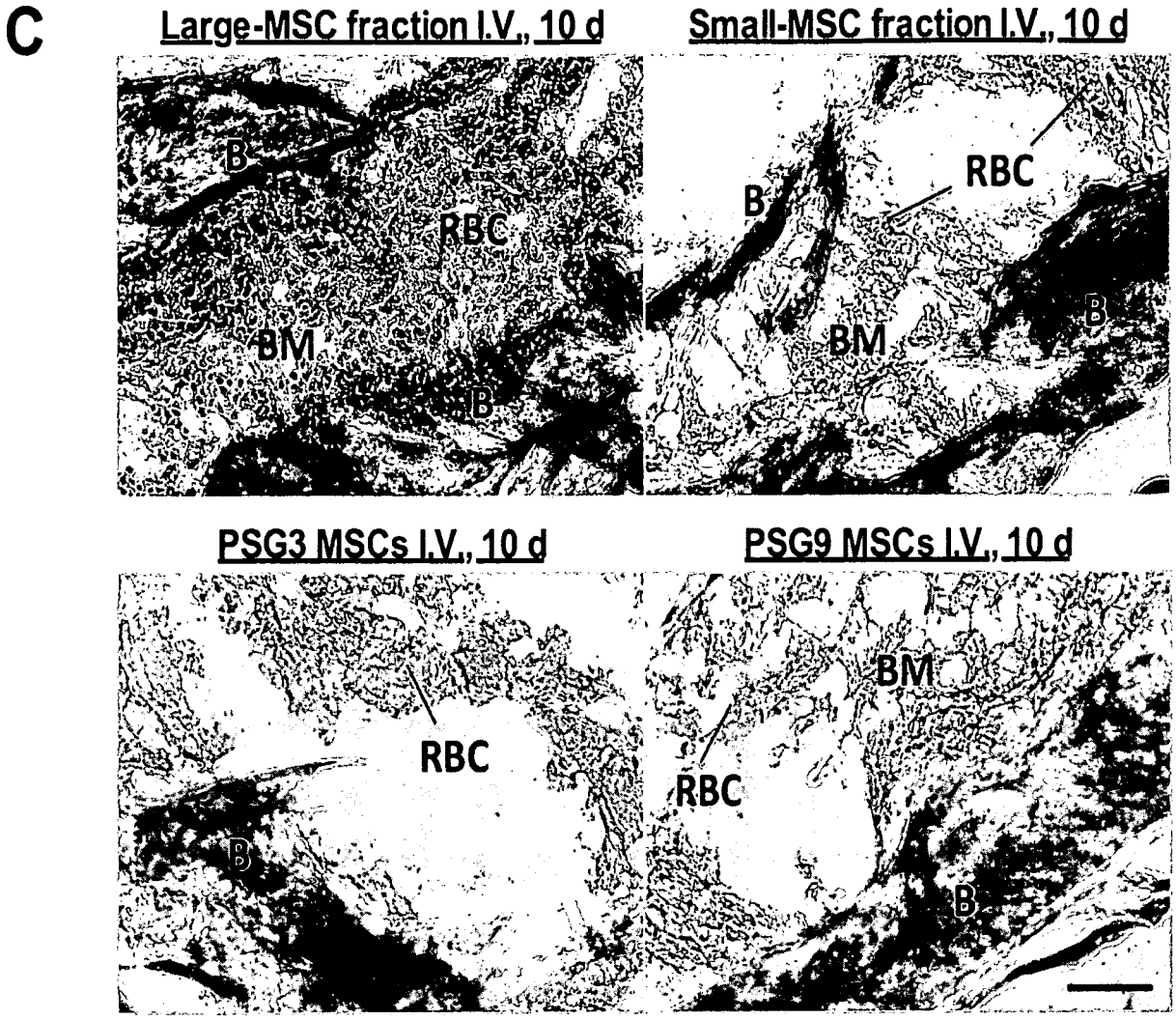

C
Large-MSC fraction I.V., 10 d
Small-MSC fraction I.V., 10 d
B
RBC
BM
B
RBC
B
BM
B
PSG3 MSCs I.V., 10 d
PSG9 MSCs I.V., 10 d
RBC
B
BM
RBC
B

FIG. 8C

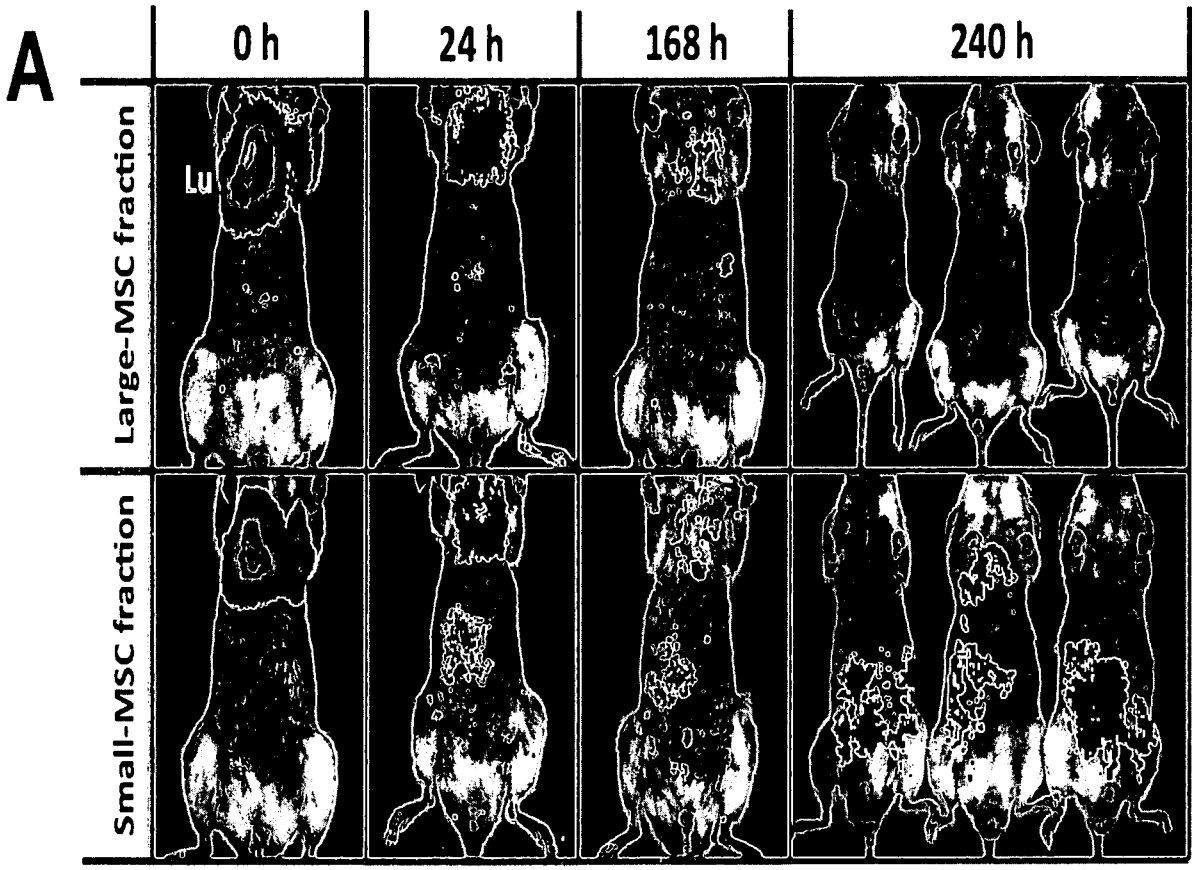


FIG. 9A

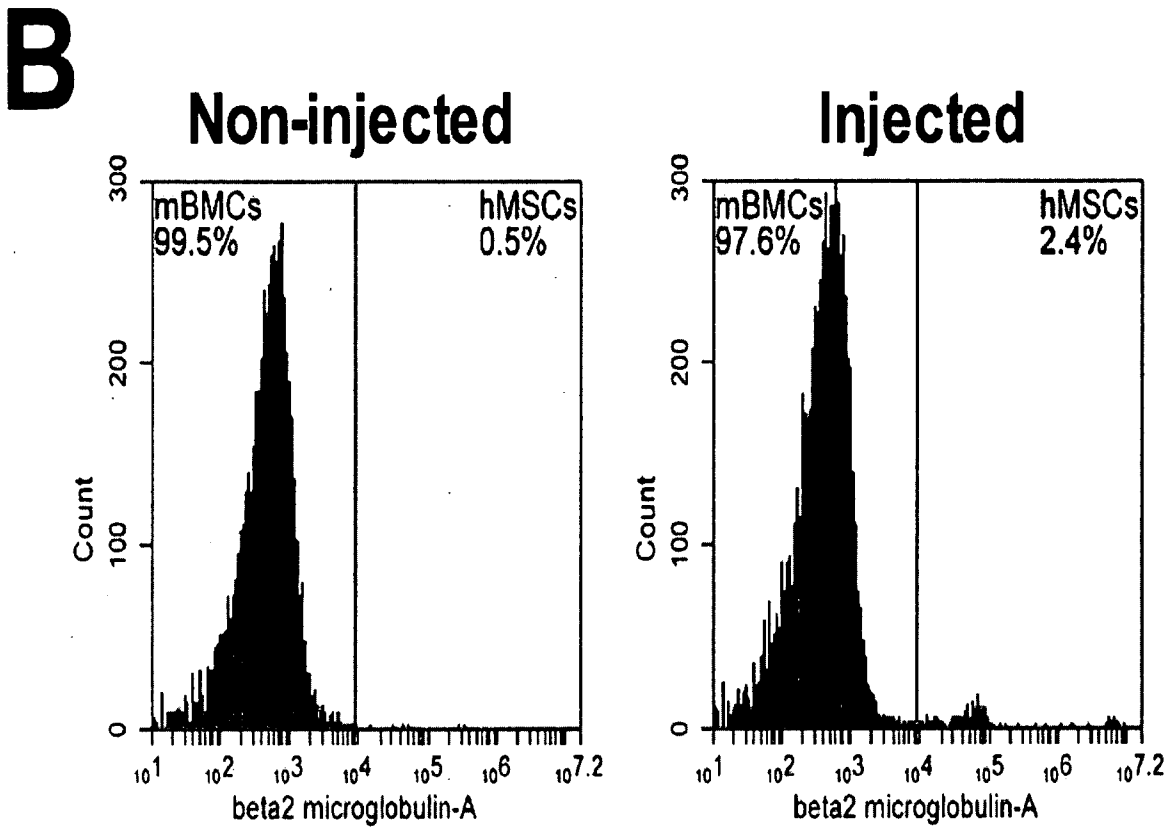

B
Non-injected
mBMCs
99.5%
hMSCs
0.5%
Count
300
200
100
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
beta2 microglobulin-A
Injected
mBMCs
97.6%
hMSCs
2.4%
Count
300
200
100
0
10^1 10^2 10^3 10^4 10^5 10^6 $10^{7.2}$
beta2 microglobulin-A

FIG. 9B

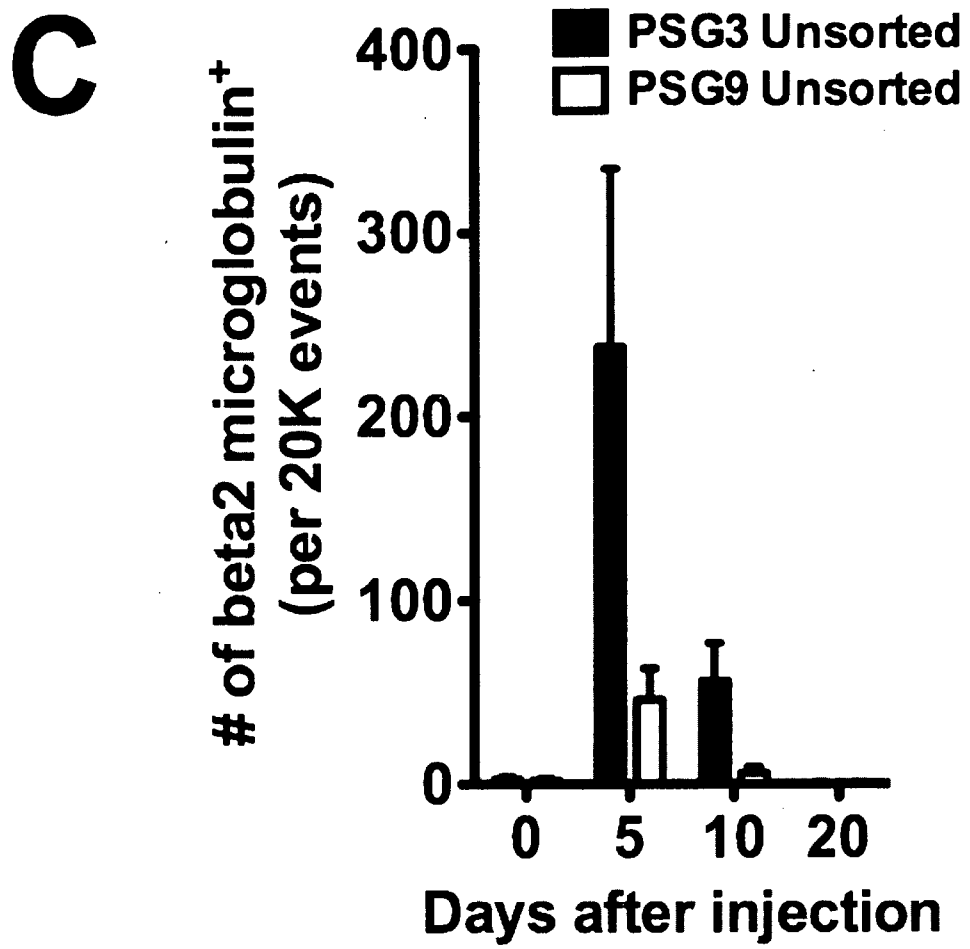

C
PSG3 Unsorted
PSG9 Unsorted
# of beta2 microglobulin+ (per 20K events)
400
300
200
100
0
0
5
10
20
Days after injection

FIG. 9C

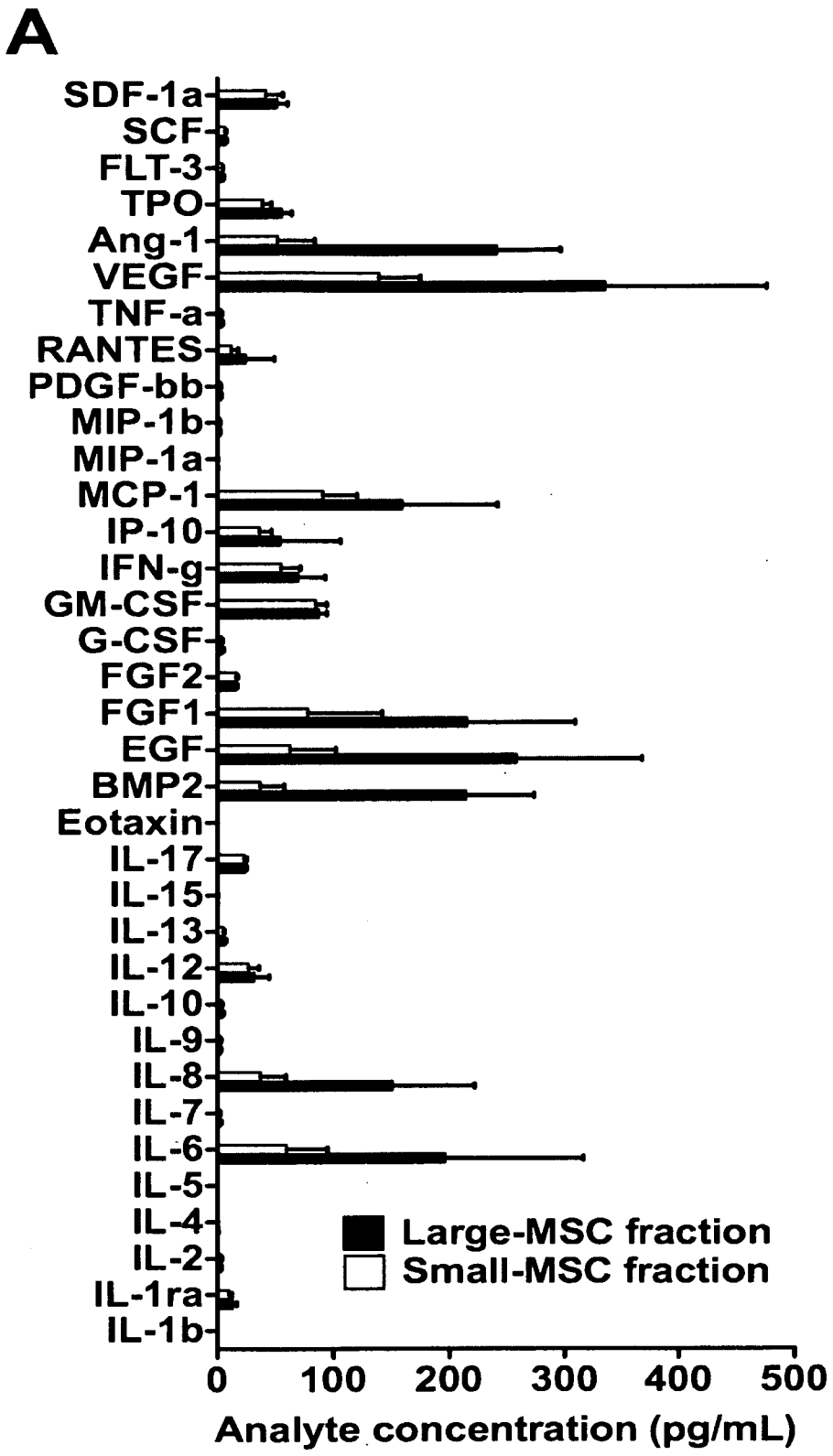

A
SDF-1a
SCF
FLT-3
TPO
Ang-1
VEGF
TNF-a
RANTES
PDGF-bb
MIP-1b
MIP-1a
MCP-1
IP-10
IFN-g
GM-CSF
G-CSF
FGF2
FGF1
EGF
BMP2
Eotaxin
IL-17
IL-15
IL-13
IL-12
IL-10
IL-9
IL-8
IL-7
IL-6
IL-5
IL-4
IL-2
IL-1ra
IL-1b
Large-MSC fraction
Small-MSC fraction
0
100
200
300
400
500
Analyte concentration (pg/mL)

FIG. 10A

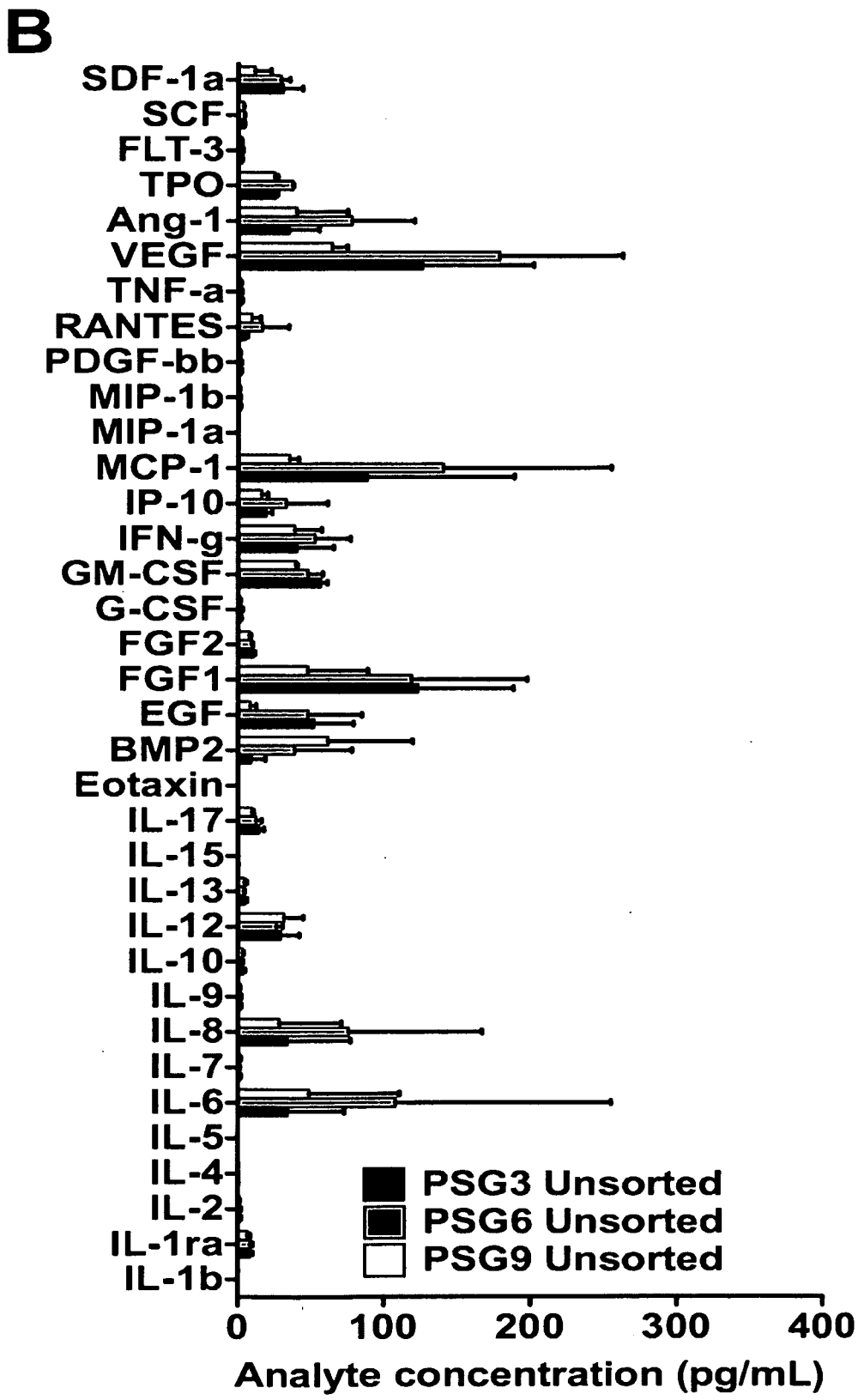

B
SDF-1a
SCF
FLT-3
TPO
Ang-1
VEGF
TNF-a
RANTES
PDGF-bb
MIP-1b
MIP-1a
MCP-1
IP-10
IFN-g
GM-CSF
G-CSF
FGF2
FGF1
EGF
BMP2
Eotaxin
IL-17
IL-15
IL-13
IL-12
IL-10
IL-9
IL-8
IL-7
IL-6
IL-5
IL-4
IL-2
IL-1ra
IL-1b
PSG3 Unsorted
PSG6 Unsorted
PSG9 Unsorted
0
100
200
300
400
Analyte concentration (pg/mL)

FIG. 10B

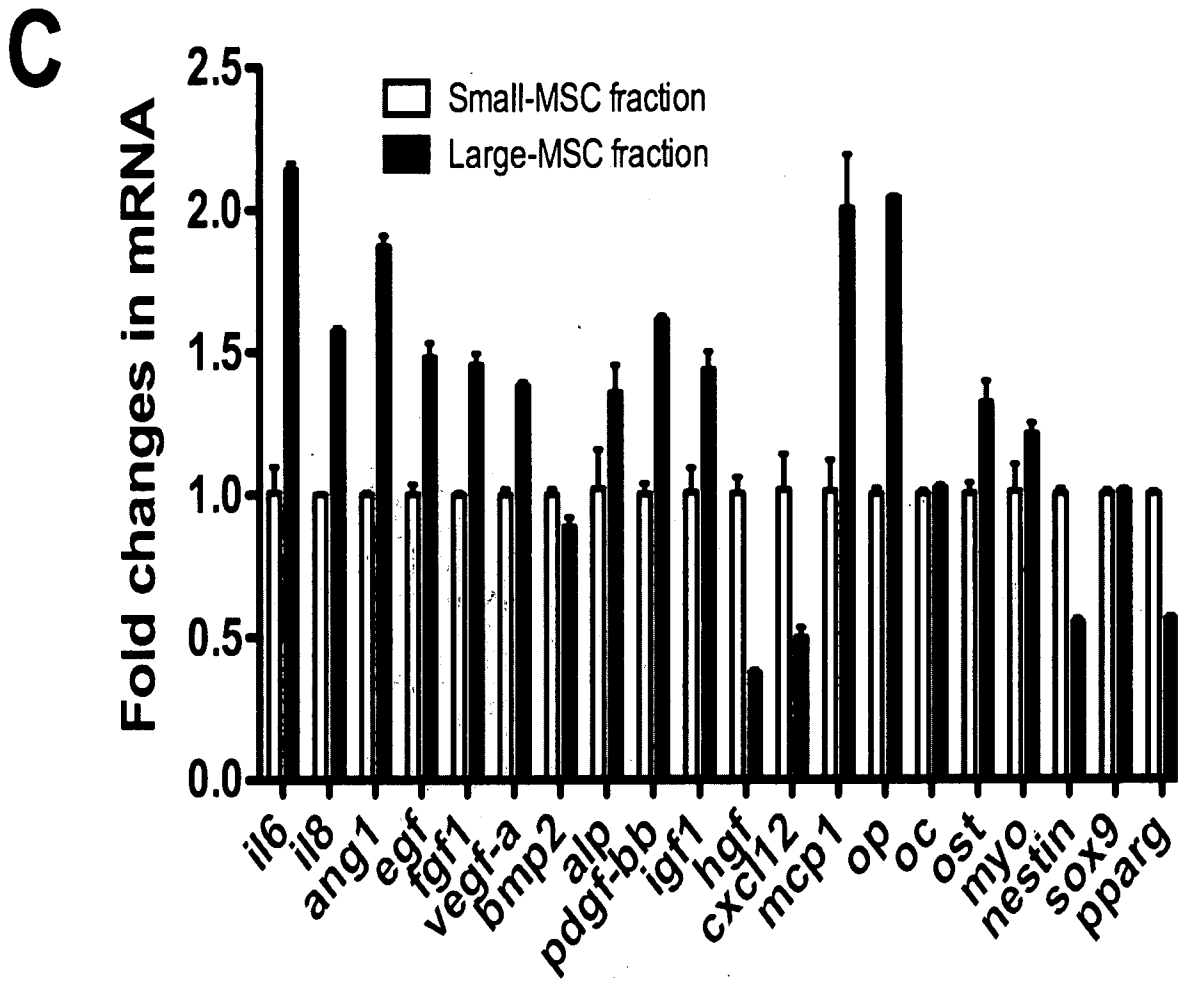

C
Fold changes in mRNA
2.5
2.0
1.5
1.0
0.5
0.0
Small-MSC fraction
Large-MSC fraction
il6
il8
ang1
egf
fgf1
vegf-a
bmp2
alp
pdgf-bb
igf1
hgf
cxcl12
mcp1
op
oc
ost
myo
nestin
sox9
pparg

FIG. 10C

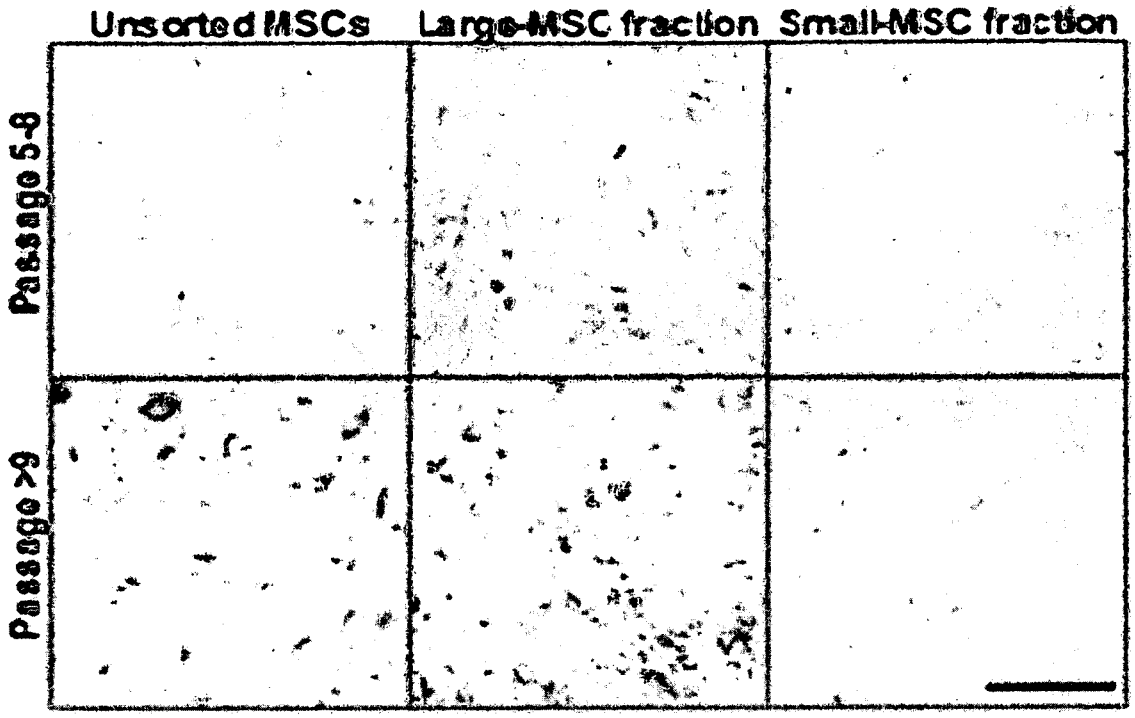
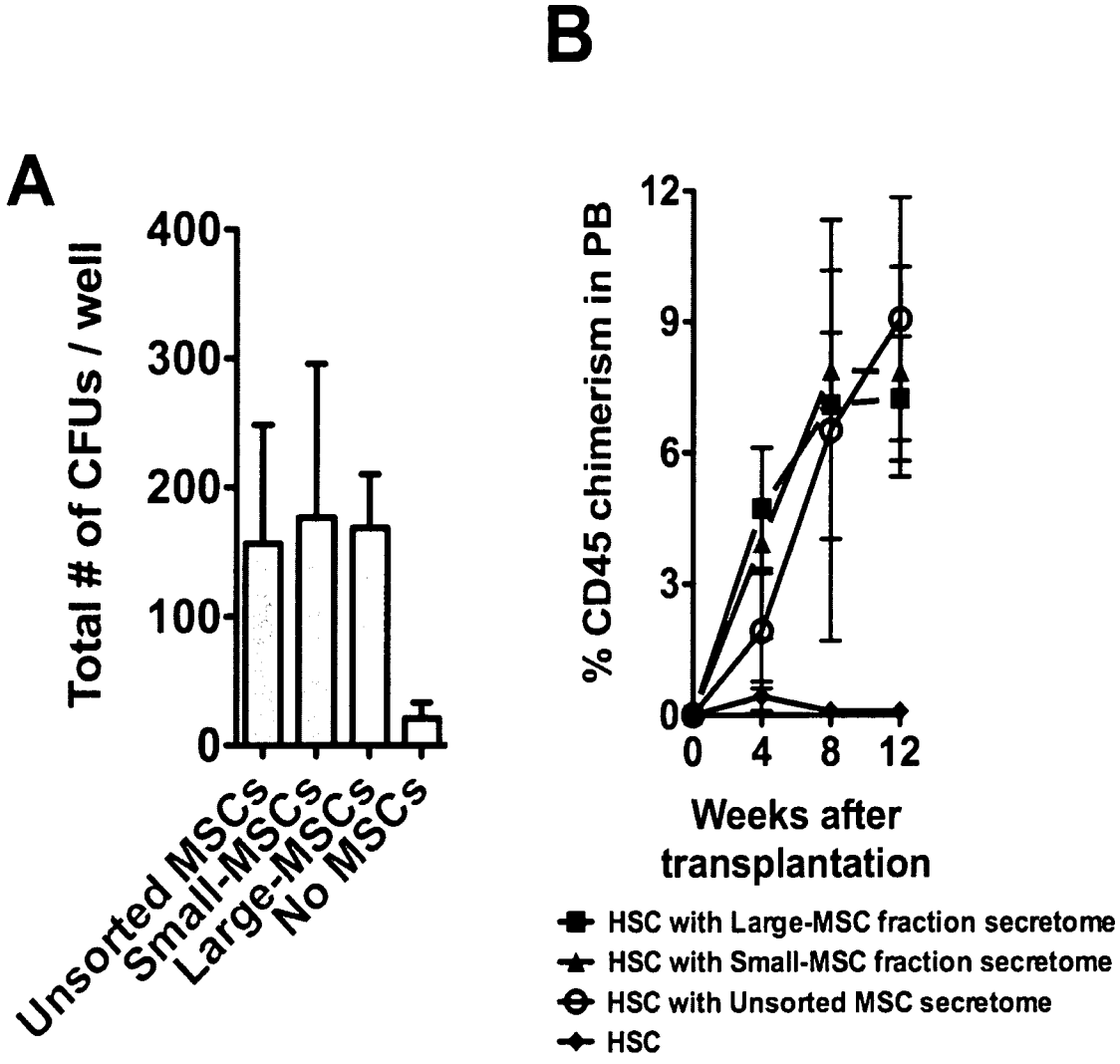

Unsorted MSCs
Large-MSC fraction
Small-MSC fraction
Passage 5-8
Passage >9

FIG. 11


A
Total # of CFUs / well
400
300
200
100
0
Unsorted MSCs
Small-MSCs
Large-MSCs
No MSCs
B
% CD45 chimerism in PB
12
9
6
3
0
0 4 8 12
Weeks after transplantation
HSC with Large-MSC fraction secretome
HSC with Small-MSC fraction secretome
HSC with Unsorted MSC secretome
HSC

FIGS. 12A - 12B

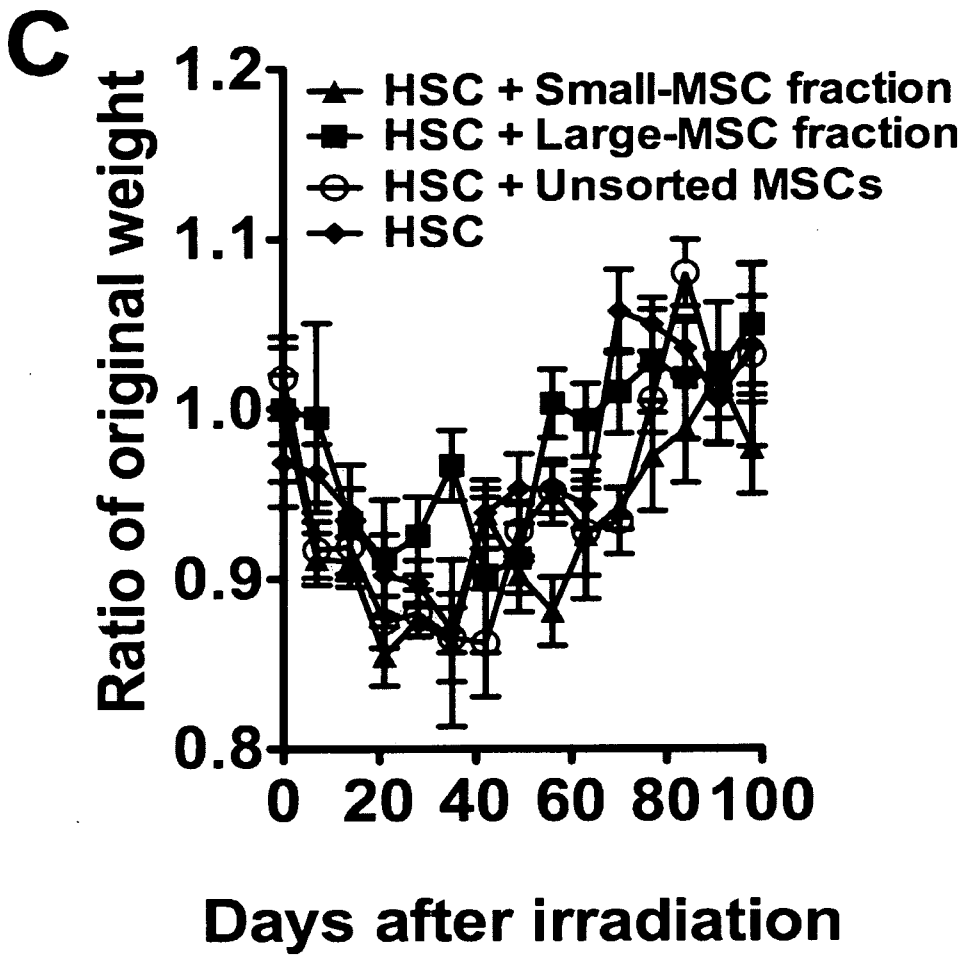

C
HSC + Small-MSC fraction
HSC + Large-MSC fraction
HSC + Unsorted MSCs
HSC
Ratio of original weight
1.2
1.1
1.0
0.9
0.8
0 20 40 60 80 100
Days after irradiation

FIG. 12C

Gene	Primer (5' to 3')	Accession Number	SEQ ID NO:
GAPDH forward	CAAGGCTGTGGGCAAGGT	NM_002046.4	SEQ ID NO:1
GAPDH reverse	GGAAGGCCATGCCAGTGA		SEQ ID NO:2
Runx2 forward	CGAATGGCAGCACGCTATTA	NM_001024630.3	SEQ ID NO:3
Runx2 reverse	TGGCTTCCATCAGCGTCAA		SEQ ID NO:4
Osteopontin forward	CGGGACCAGACTCGTCTCA	NM_000582.2	SEQ ID NO:5
Osteopontin reverse	TTCCTTGGTCGGCGTTTG		SEQ ID NO:6
Osteocalcin forward	TCCACAGCCTTTGTGTCCAA	NM_199173.4	SEQ ID NO:7
Osteocalcin reverse	GCGCCTGGGTCTCTTCACTA		SEQ ID NO:8
Pparg forward	TCAGGGCTGCCAGTTTCG	NM_138712.3	SEQ ID NO:9
Pparg reverse	GCTTTTGGCATACTCTGTGATCTC		SEQ ID NO:10
Sox9 forward	CCGGGCCCGCGTAT	NM_000346.3	SEQ ID NO:11
Sox9 reverse	TCCTGCTCGTCGGTCATCTT		SEQ ID NO:12
IL6 forward	AGGGCTCTTCGGCAAATGTA	NM_000600.3	SEQ ID NO:13
IL6 reverse	GAAGGAATGCCCATTAACAACAA		SEQ ID NO:14
IL8 forward	CACCGGAAGGAACCATCTCA	NM_000584.3	SEQ ID NO:15
IL8 reverse	AGAGCCACGGCCAGCTT		SEQ ID NO:16
VEGF-A forward	CGAGGGCCTGGAGTGTGT	NM_001204385.1	SEQ ID NO:17
VEGF-A reverse	CGCATAATCTGCATGGTGATG		SEQ ID NO:18
Bmp2 forward	AAAGGGCATCCTCTCCACAA	NM_001200.2	SEQ ID NO:19
Bmp2 reverse	AGGCGTTTCCGCTGTTTG		SEQ ID NO:20
FGF1 forward	GTTTAATCTGCCTCCAGGGAA TT	NM_001257212.1	SEQ ID NO:21
FGF1 reverse	AGTGGCCCCCGTTGCTA		SEQ ID NO:22
IGFBP2 forward	TGACAAGCATGGCCTGTACAA	NM_000597.2	SEQ ID NO:23
IGFBP2 reverse	CACGCTGCCCGTTCAGA		SEQ ID NO:24
ANGPTL2 forward	CCTCCCCCCACATTTTCAT	NM_012098.2	SEQ ID NO:25
ANGPTL2 reverse	GGATAAGTGGGTGATGGATGGT		SEQ ID NO:26
ANGPTL3 forward	CCAATGCAATCCCGGAAA	NM_014495.3	SEQ ID NO:27
ANGPTL3 reverse	TGAAGTGTCCTTTTGCTTTGTGA		SEQ ID NO:28
ANGPTL5 forward	TGGTCAATGGTCAGTCTGTGAAG	NM_178127.4	SEQ ID NO:29
ANGPTL5 reverse	AACCACCAGCCGGTCTTGT		SEQ ID NO:30
Nestin forward	AGCCCTGACCACTCCAGTTTAG	NM_006617.1	SEQ ID NO:31
Nestin reverse	CCCTCTATGGCTGTTTCTTTCTCT		SEQ ID NO:32
Ang1 forward	TCCAGGTTATCCCAGAGATTTAATG	NM_004673.3	SEQ ID NO:33
Ang1 reverse	GGGCTTTTGGTGGGAGAAGT		SEQ ID NO:34
EGF forward	TGTAAAAACACCCCTGGATCCTA	NM_001178131.1	SEQ ID NO:35
EGF reverse	CCCATCAGGAAGCAGAACAAA		SEQ ID NO:36
FGF2 forward	AATCAAAAGTTCGGCATGTAGCT	NM_002006.4	SEQ ID NO:37
FGF2 reverse	CTGAGCAGGGCAGATTTGCT		SEQ ID NO:38
MCP1 forward	CAAGCAGAAGTGGGTTCAGGAT	NM_002982.3	SEQ ID NO:39
MCP1 reverse	TCTTCGGAGTTTGGGTTTGC		SEQ ID NO:40
PDGFB forward	ATCGCCATCTTCTTCCCTTAACT	NM_033016.2	SEQ ID NO:41
PDGFB reverse	AGAGCGACCCCATCAGTCTCT		SEQ ID NO:42

FIG. 13

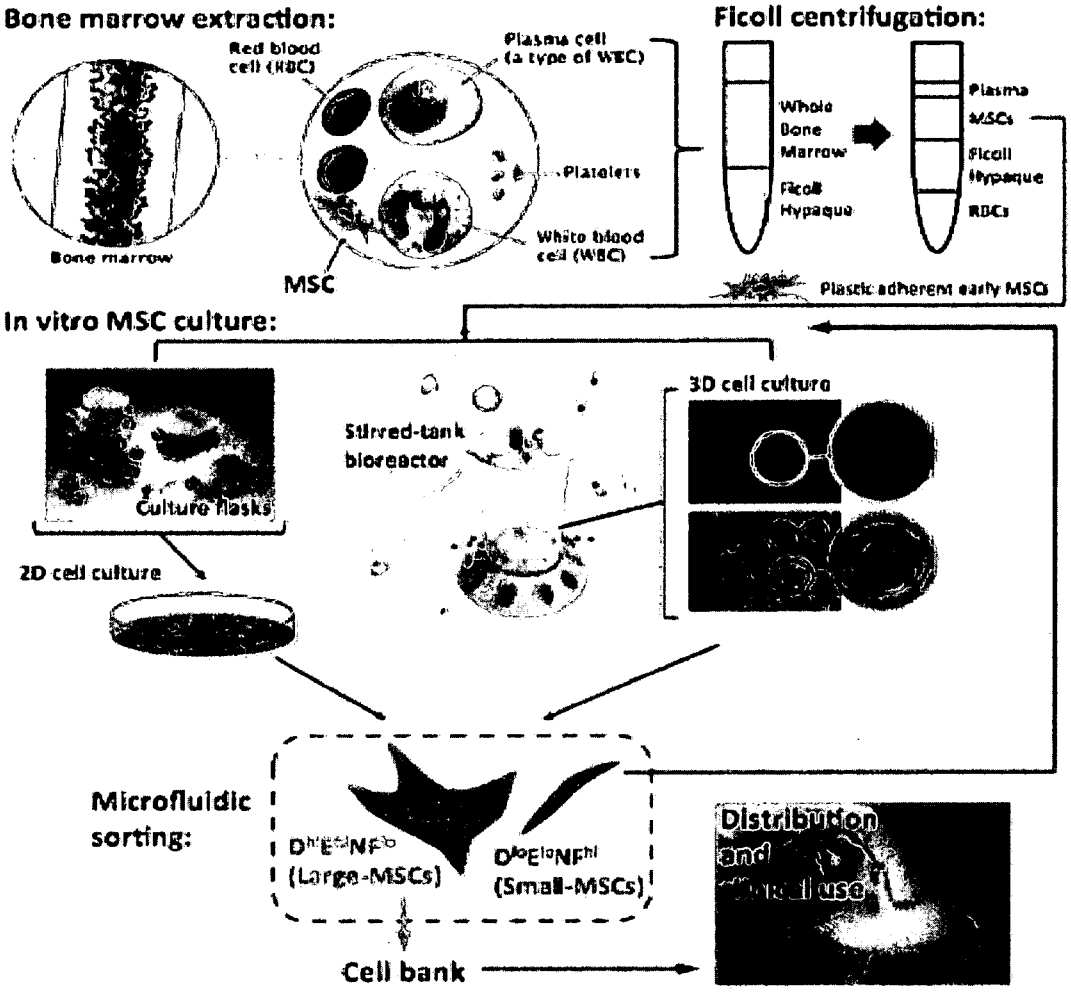

Bone marrow extraction:
Red blood cell (RBC)
Plasma cell (a type of WBC)
Platelets
White blood cell (WBC)
Bone marrow
MSC
Ficoll centrifugation:
Whole Bone Marrow
Ficoll Hypaque
Plasma
MSCs
Ficoll Hypaque
RBCs
Plastic adherent early MSCs
In vitro MSC culture:
Culture flasks
2D cell culture
Stirred-tank bioreactor
3D cell culture
Microfluidic sorting:
(Large-MSCs)
(Small-MSCs)
Cell bank
Distribution and

FIG. 14

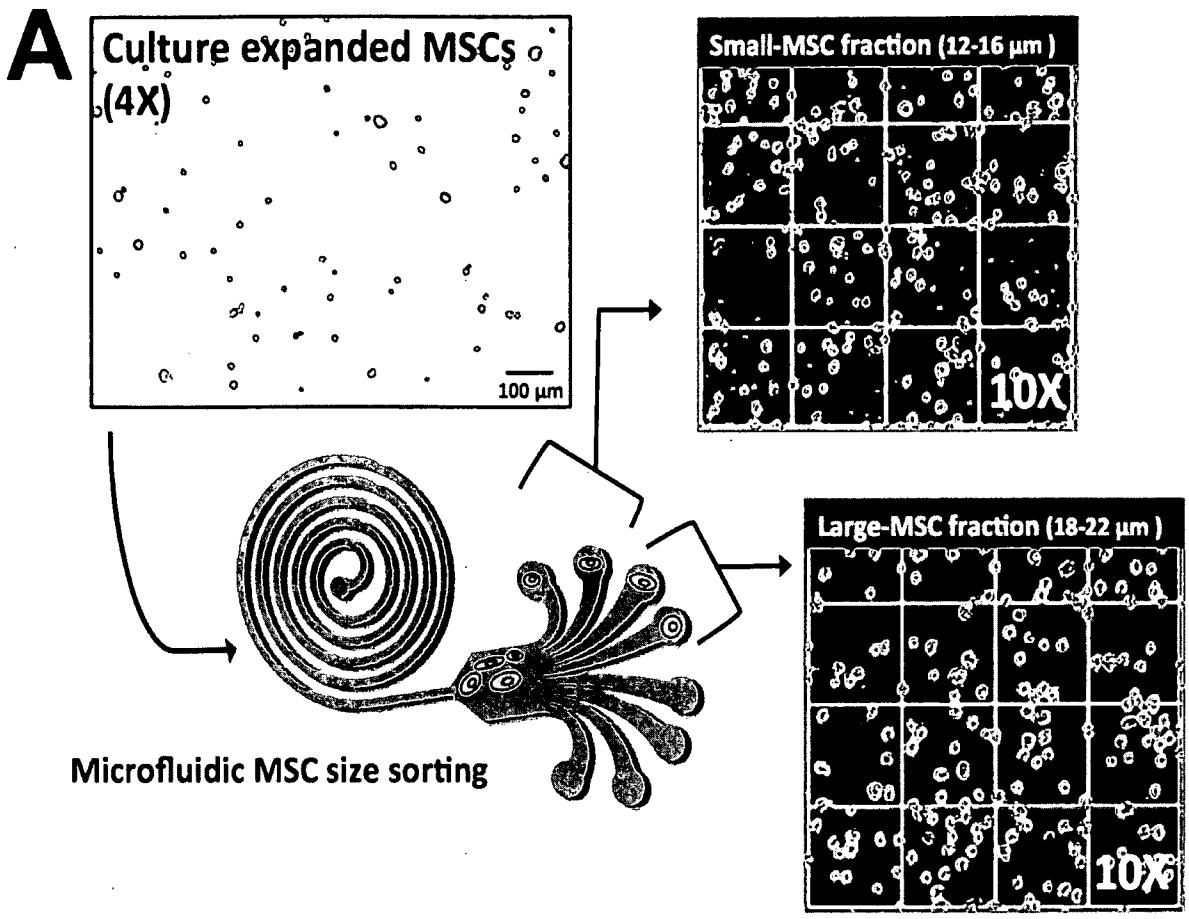
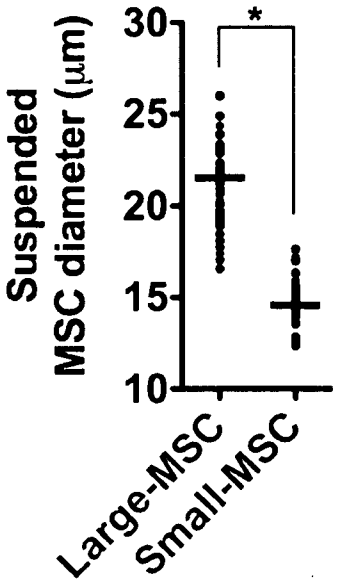
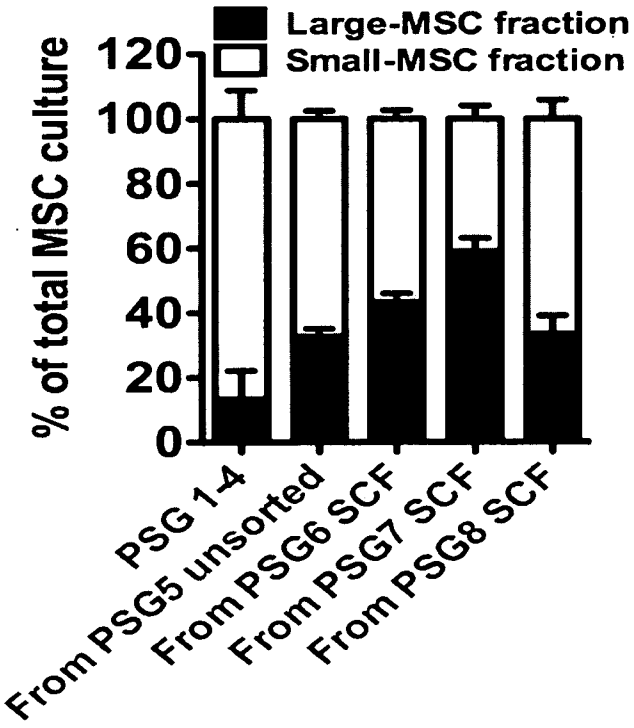

A
Culture expanded MSCs
(4X)
100 µm
Small-MSC fraction (12-16 µm)
10X
Large-MSC fraction (18-22 µm)
10X
Microfluidic MSC size sorting

FIG. 15A

B



C



FIGS. 15B - 15C

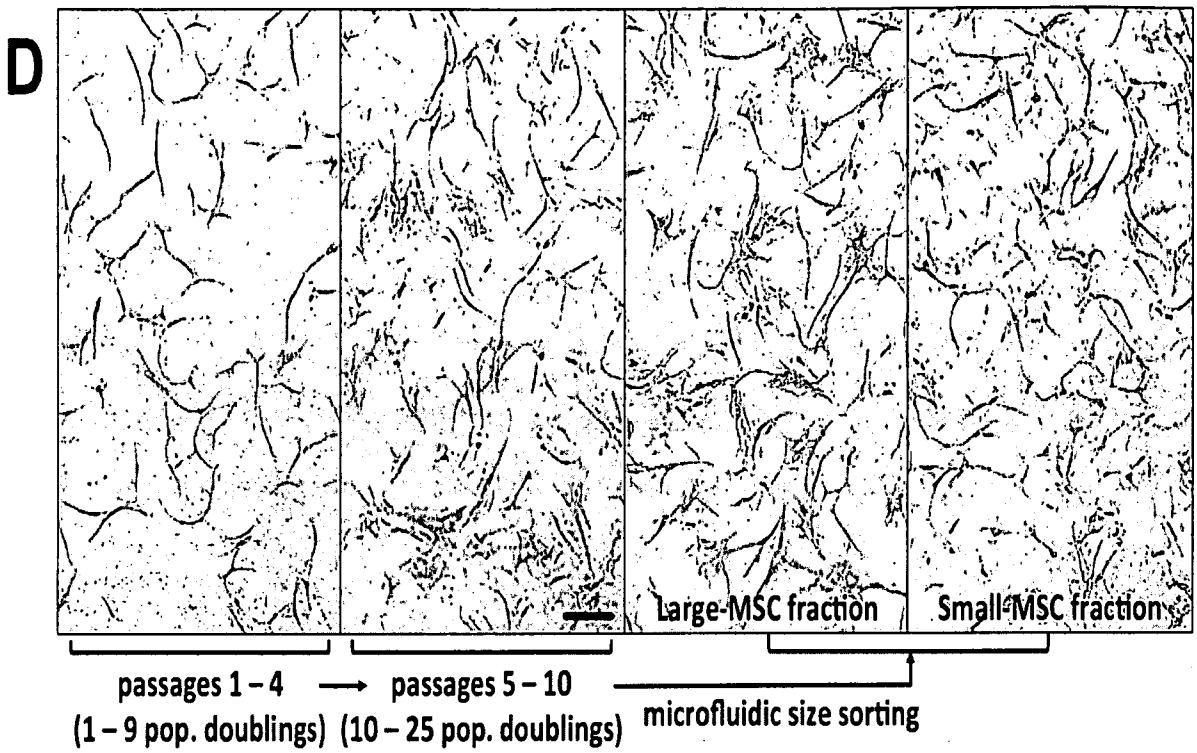
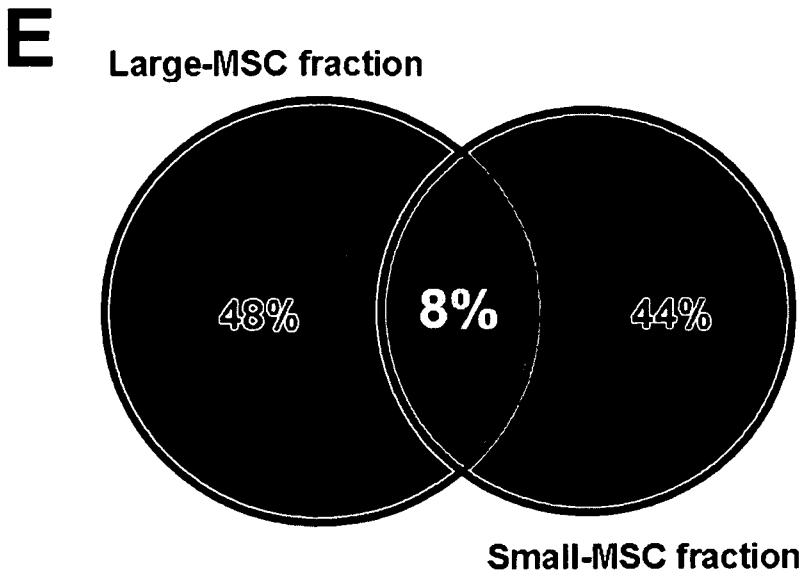
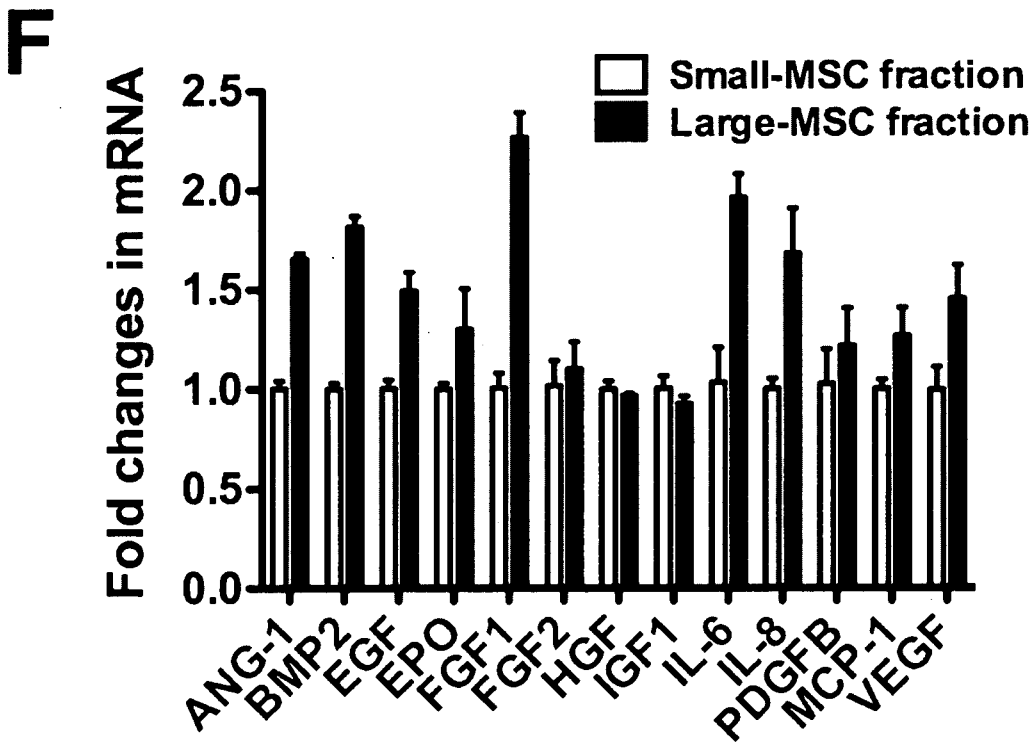

D
Large-MSC fraction
Small-MSC fraction
passages 1 – 4
(1 – 9 pop. doublings)
passages 5 – 10
(10 – 25 pop. doublings)
microfluidic size sorting

FIG. 15D


E
Large-MSC fraction
48%
8%
44%
Small-MSC fraction


F
Small-MSC fraction
Large-MSC fraction
Fold changes in mRNA
2.5
2.0
1.5
1.0
0.5
0.0
ANG-1
BMP2
EGF
EPO
FGF1
FGF2
HGF
IGF1
IL-6
IL-8
PDGFB
MCP-1
VEGF

FIGS. 15E - 15F

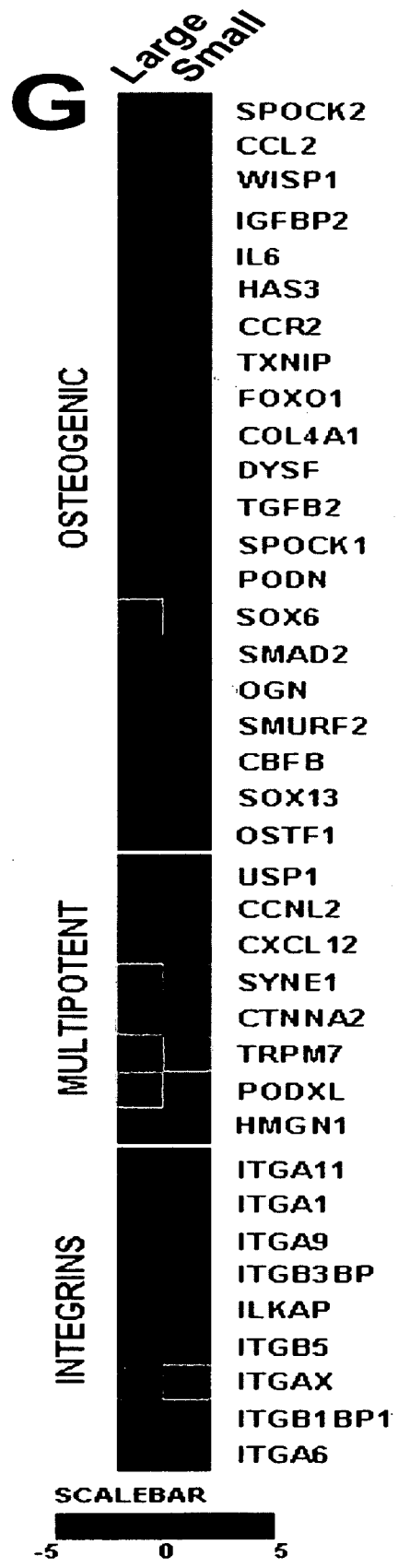

G
Large
Small
OSTEOGENIC
SPOCK2
CCL2
WISP1
IGFBP2
IL6
HAS3
CCR2
TXNIP
FOXO1
COL4A1
DYSF
TGFB2
SPOCK1
PODN
SOX6
SMAD2
OGN
SMURF2
CBFB
SOX13
OSTF1
MULTIPOTENT
USP1
CCNL2
CXCL12
SYNE1
CTNNA2
TRPM7
PODXL
HMGN1
INTEGRINS
ITGA11
ITGA1
ITGA9
ITGB3BP
ILKAP
ITGB5
ITGAX
ITGB1BP1
ITGA6
SCALEBAR
-5
0
5

FIG. 15G

A

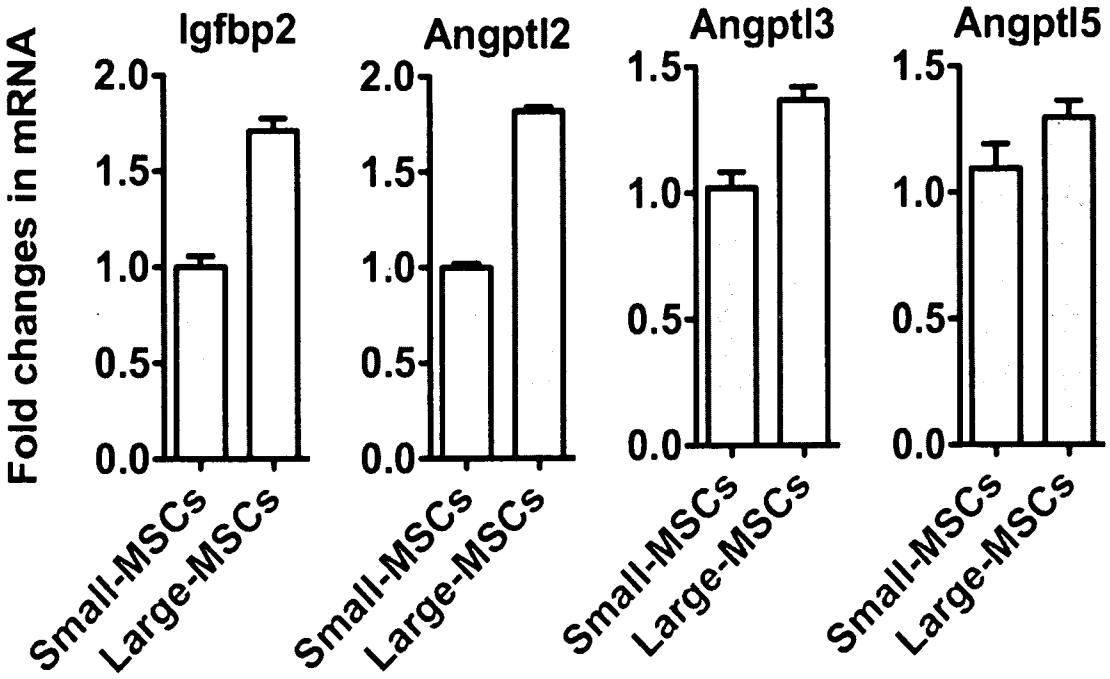

Fold changes in mRNA
Igfbp2
Angptl2
Angptl3
Angptl5
2.0
1.5
1.0
0.5
0.0
Small-MSCs
Large-MSCs

FIG. 16A