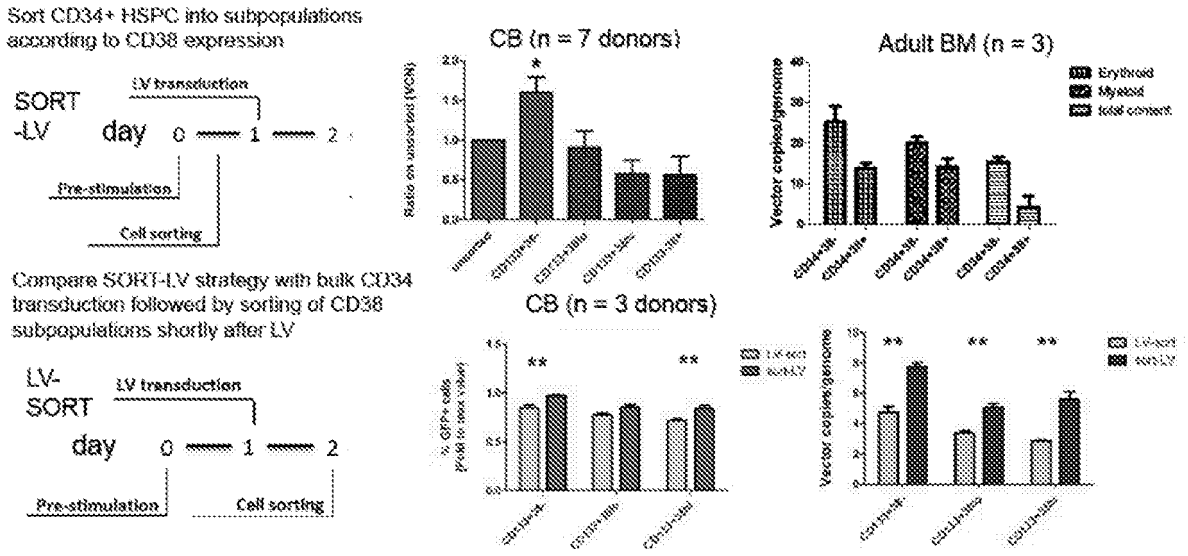
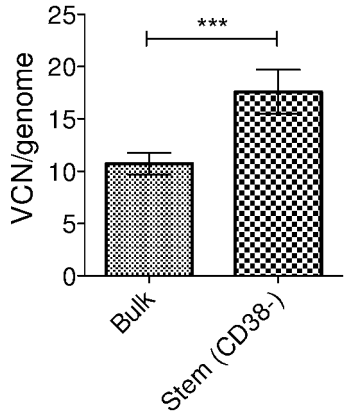
DRAWINGS

A



B



C

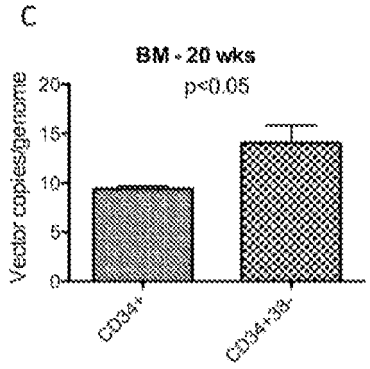


Figure 1

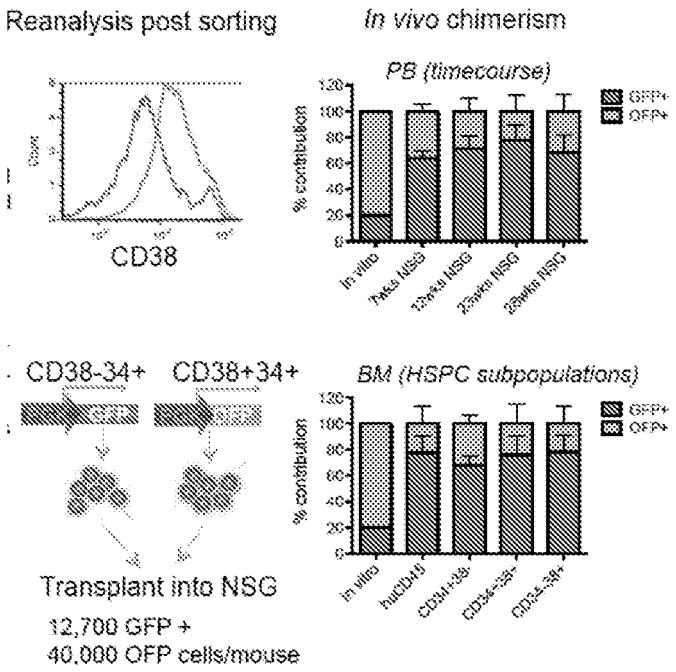

Reanalysis post sorting
In vivo chimerism
PB (timecourse)
CD38
% contribution
GFP+
OFP+
In vitro
CD38-34+
CD38+34+
BM (HSPC subpopulations)
Transplant into NSG
12,700 GFP +
40,000 OFP cells/mouse

Figure 2

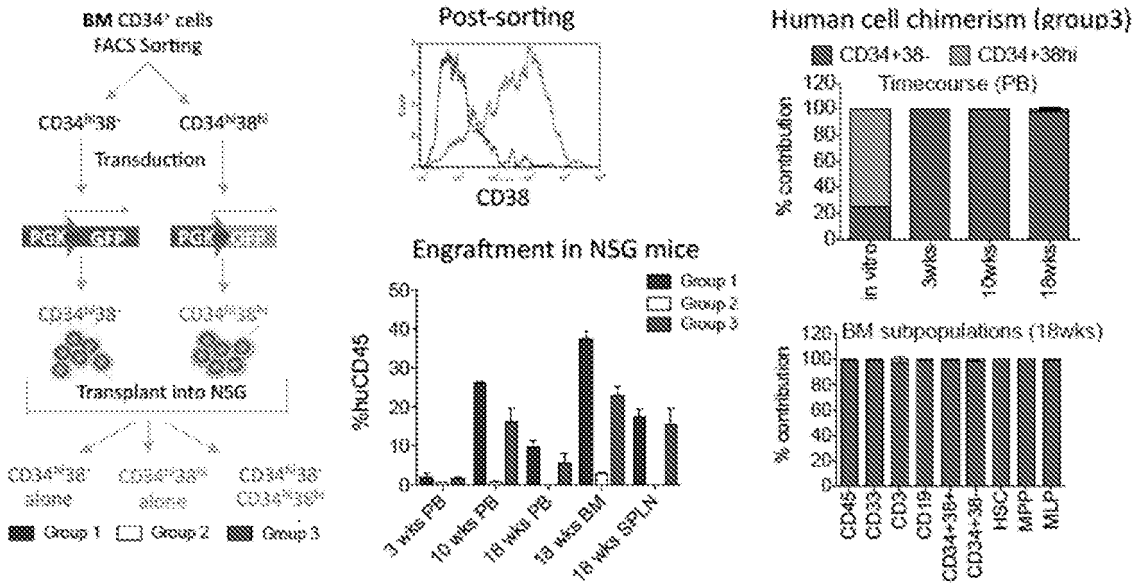
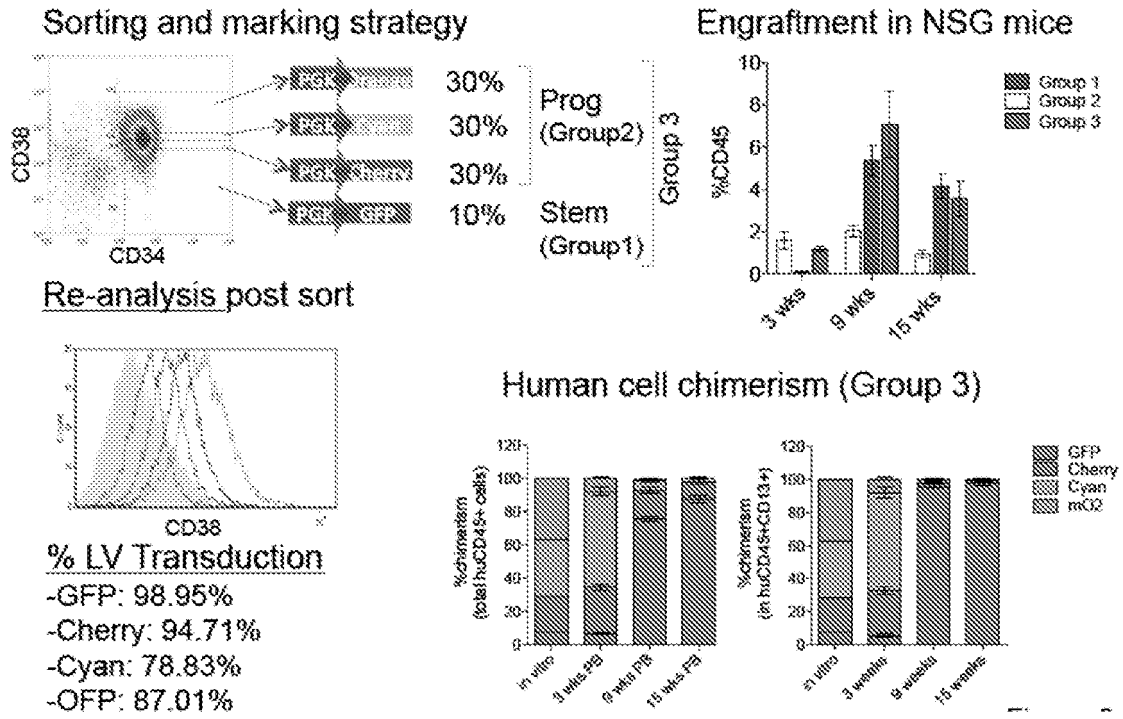

BM CD34+ cells
FACS Sorting
Transduction
Transplant into NSG
Group 1
Group 2
Group 3
Post-sorting
CD38
Engraftment in NSG mice
%huCD45
3 wks PB
10 wks PB
18 wks PB
18 wks BM
18 wks SPLN
Human cell chimerism (group3)
CD34+38-
CD34+38hi
Timecourse (PB)
% contribution
in vitro
3wks
10wks
18wks
BM subpopulations (18wks)
CD45
CD33
CD3
CD19
CD34+38+
CD34+38-
HSC
MPP
MLP

Figure 3

A


Sorting and marking strategy
CD38
CD34
30%
30%
30%
10%
Prog
(Group2)
Stem
(Group1)
Group 3
Re-analysis post sort
CD38
% LV Transduction
-GFP: 98.95%
-Cherry: 94.71%
-Cyan: 78.83%
-OFP: 87.01%
Engraftment in NSG mice
%CD45
Group 1
Group 2
Group 3
3 wks
9 wks
15 wks
Human cell chimerism (Group 3)
GFP
Cherry
Cyan
mO2

B

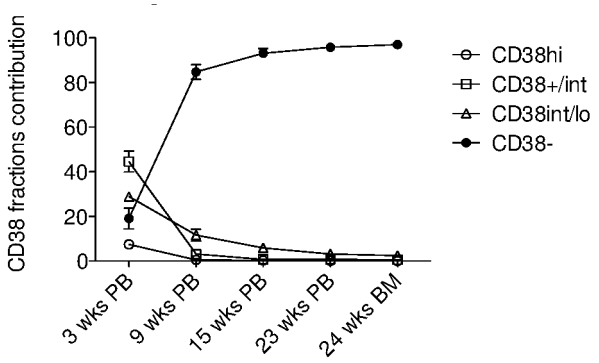

CD38 fractions contribution
100
80
60
40
20
0
3 wks PB
9 wks PB
15 wks PB
23 wks PB
24 wks BM
CD38hi
CD38+/int
CD38int/lo
CD38-

Figure 4

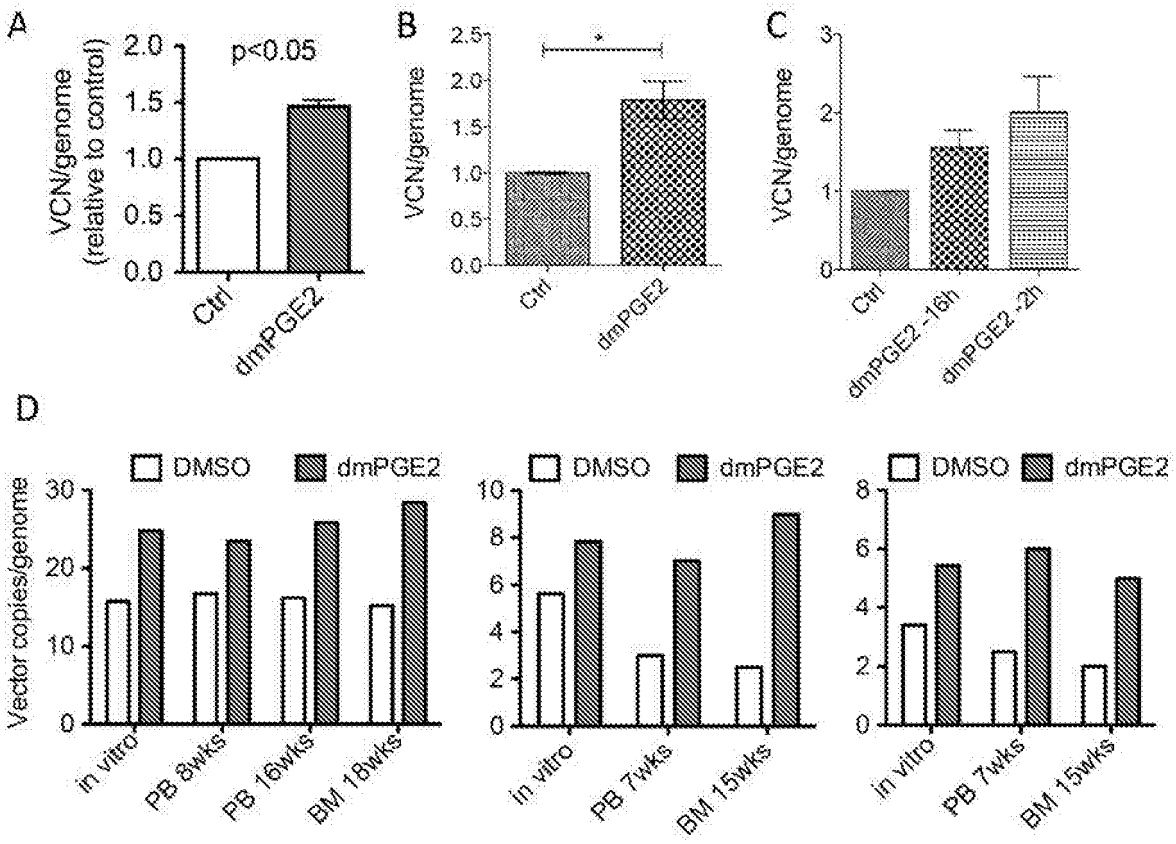

A
VCN/genome (relative to control)
2.0
1.5
1.0
0.5
0.0
p<0.05
Ctrl
dmPGE2
B
VCN/genome
2.5
2.0
1.5
1.0
0.5
0.0
*
Ctrl
dmPGE2
C
VCN/genome
3
2
1
0
Ctrl
dmPGE2 -16h
dmPGE2 -2h
D
Vector copies/genome
DMSO
dmPGE2
30
20
10
0
in vitro
PB 8wks
PB 16wks
BM 18wks
DMSO
dmPGE2
10
8
6
4
2
0
in vitro
PB 7wks
BM 15wks
DMSO
dmPGE2
8
6
4
2
0
in vitro
PB 7wks
BM 15wks

Figure 5(A-D)

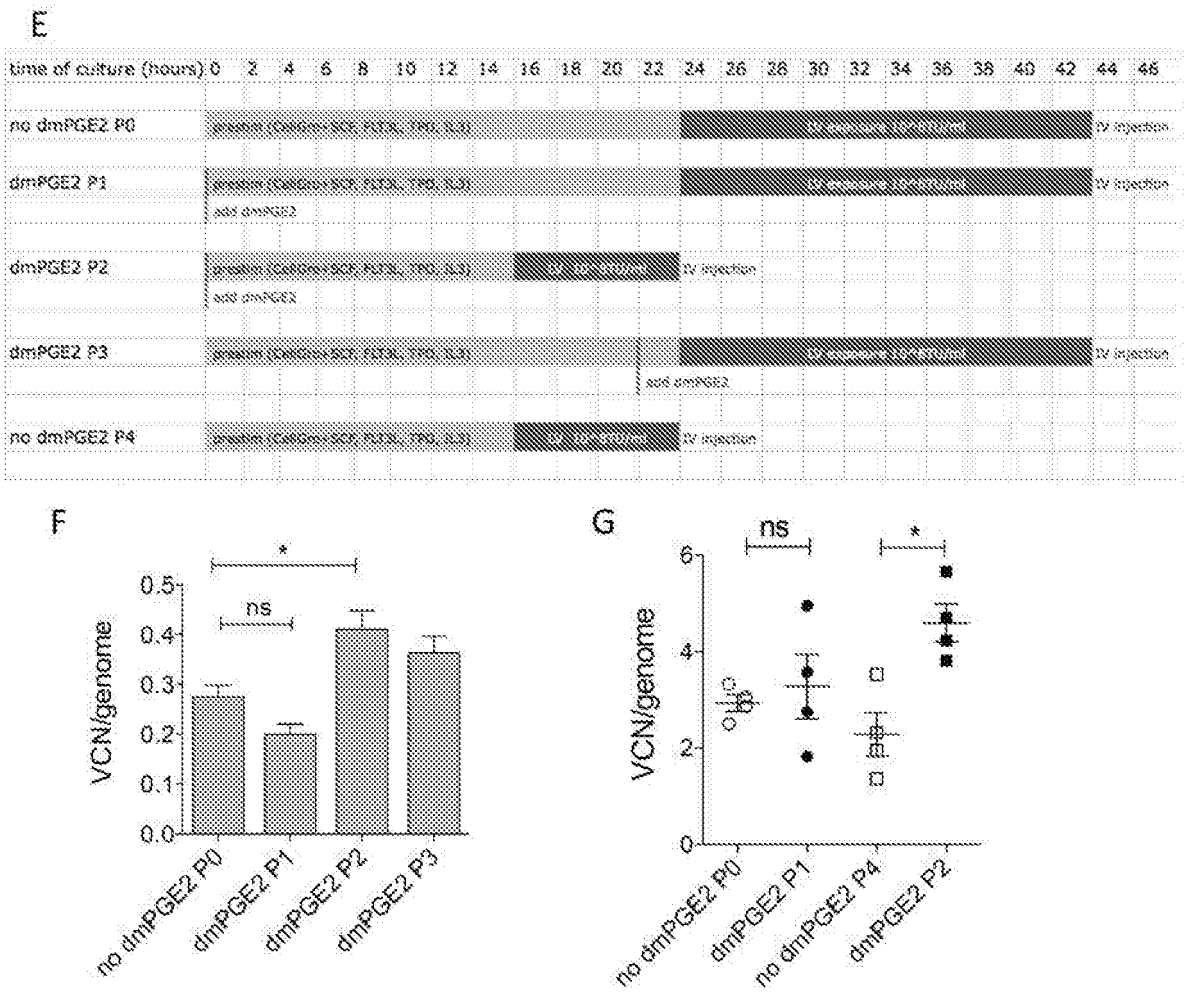

E
time of culture (hours) 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
no dmPGE2 P0
dmPGE2 P1
add dmPGE2
dmPGE2 P2
add dmPGE2
dmPGE2 P3
add dmPGE2
no dmPGE2 P4
IV injection
F
VCN/genome
0.0
0.1
0.2
0.3
0.4
0.5
*
ns
no dmPGE2 P0
dmPGE2 P1
dmPGE2 P2
dmPGE2 P3
G
VCN/genome
0
2
4
6
ns
*
no dmPGE2 P0
dmPGE2 P1
no dmPGE2 P4
dmPGE2 P2

Figure 5(E-G)

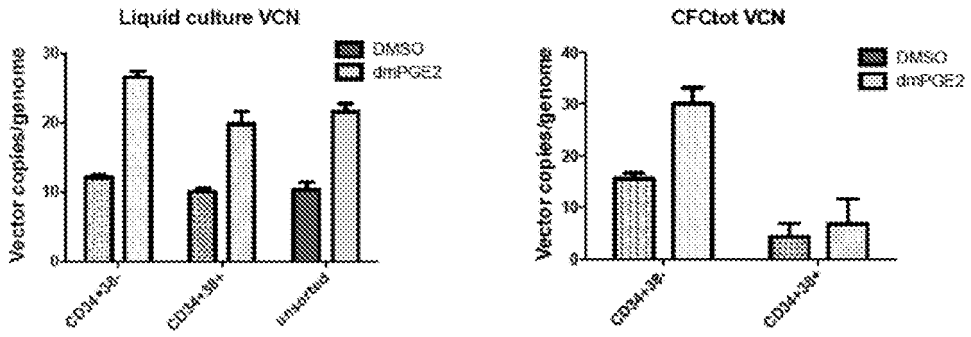

Liquid culture VCN
DMSO
dmPGE2
Vector copies/genome
30
20
10
0
CD34+38-
CD34+38+
unsorted
CFCtot VCN
DMSO
dmPGE2
Vector copies/genome
40
30
20
10
0
CD34+38-
CD34+38+

Figure 6

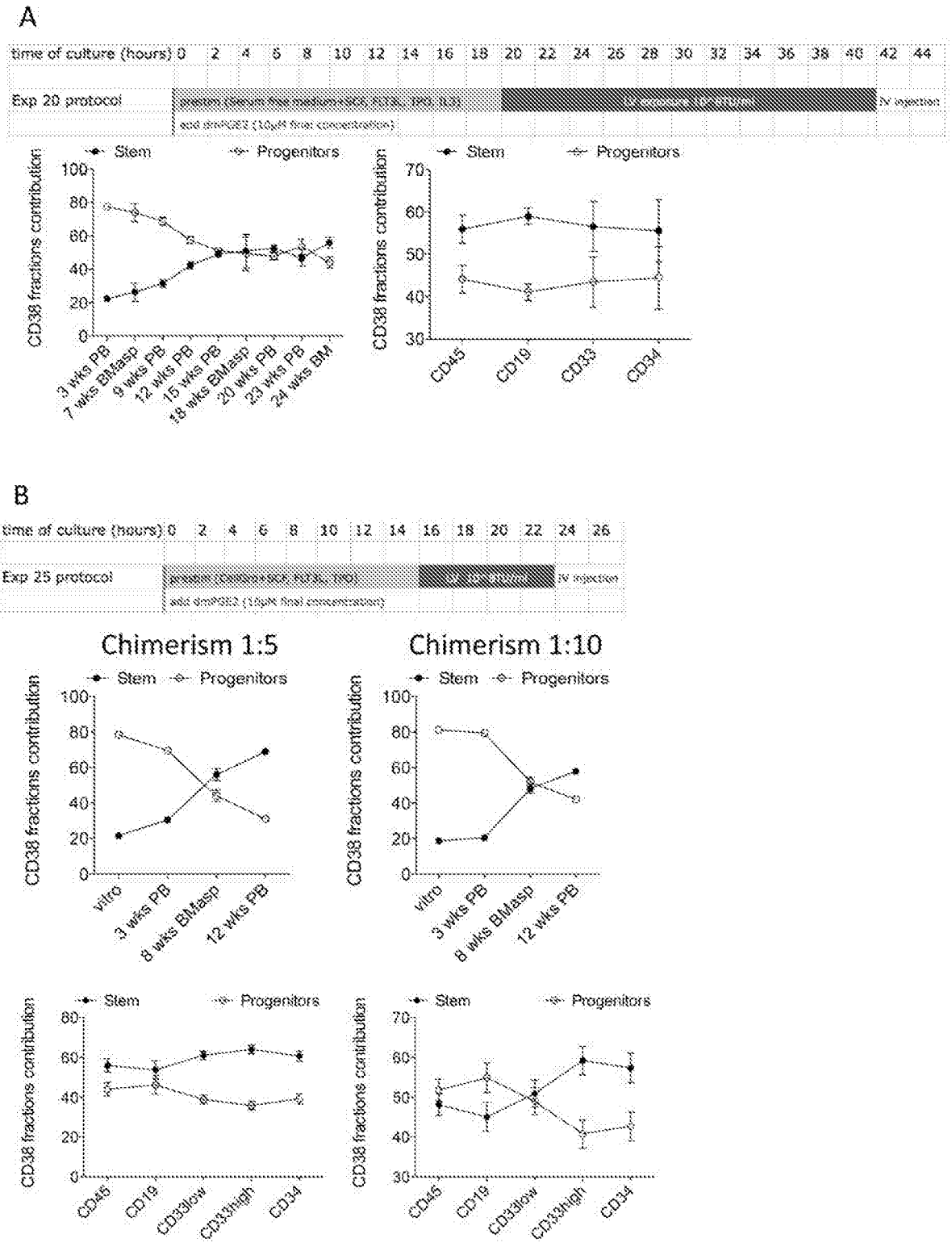
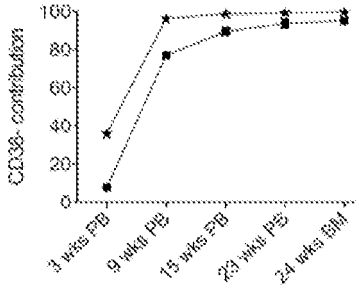

A
time of culture (hours) 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44
Exp 20 protocol
IV injection
Stem
Progenitors
CD38 fractions contribution
3 wks PB
7 wks BMasp
9 wks PB
12 wks PB
15 wks BMasp
18 wks BMasp
20 wks PB
23 wks PB
24 wks BM
CD45
CD19
CD33
CD34
B
time of culture (hours) 0 2 4 6 8 10 12 14 16 18 20 22 24 26
Exp 25 protocol
IV injection
Chimerism 1:5
Chimerism 1:10
Stem
Progenitors
CD38 fractions contribution
vitro
3 wks PB
8 wks BMasp
12 wks PB
CD45
CD19
CD33low
CD33high
CD34

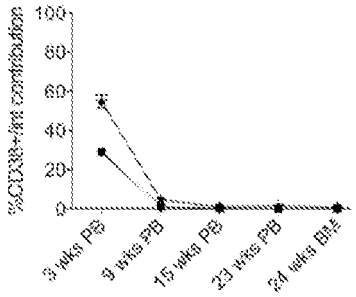
Figure 7

5

A


CD38- contribution
100
80
60
40
20
0
3 wks PB
9 wks PB
15 wks PB
23 wks PB
24 wks BM

B


%CD38+/int contribution
100
80
60
40
20
0
3 wks PB
9 wks PB
15 wks PB
23 wks PB
24 wks BM

C

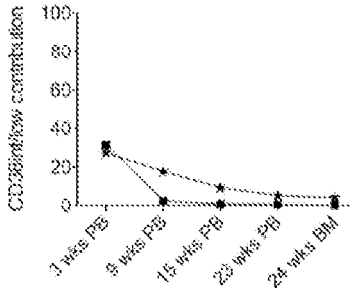

CD38bright contribution
100
80
60
40
20
0
3 wks PB
9 wks PB
15 wks PB
23 wks PB
24 wks BM

Figure 8

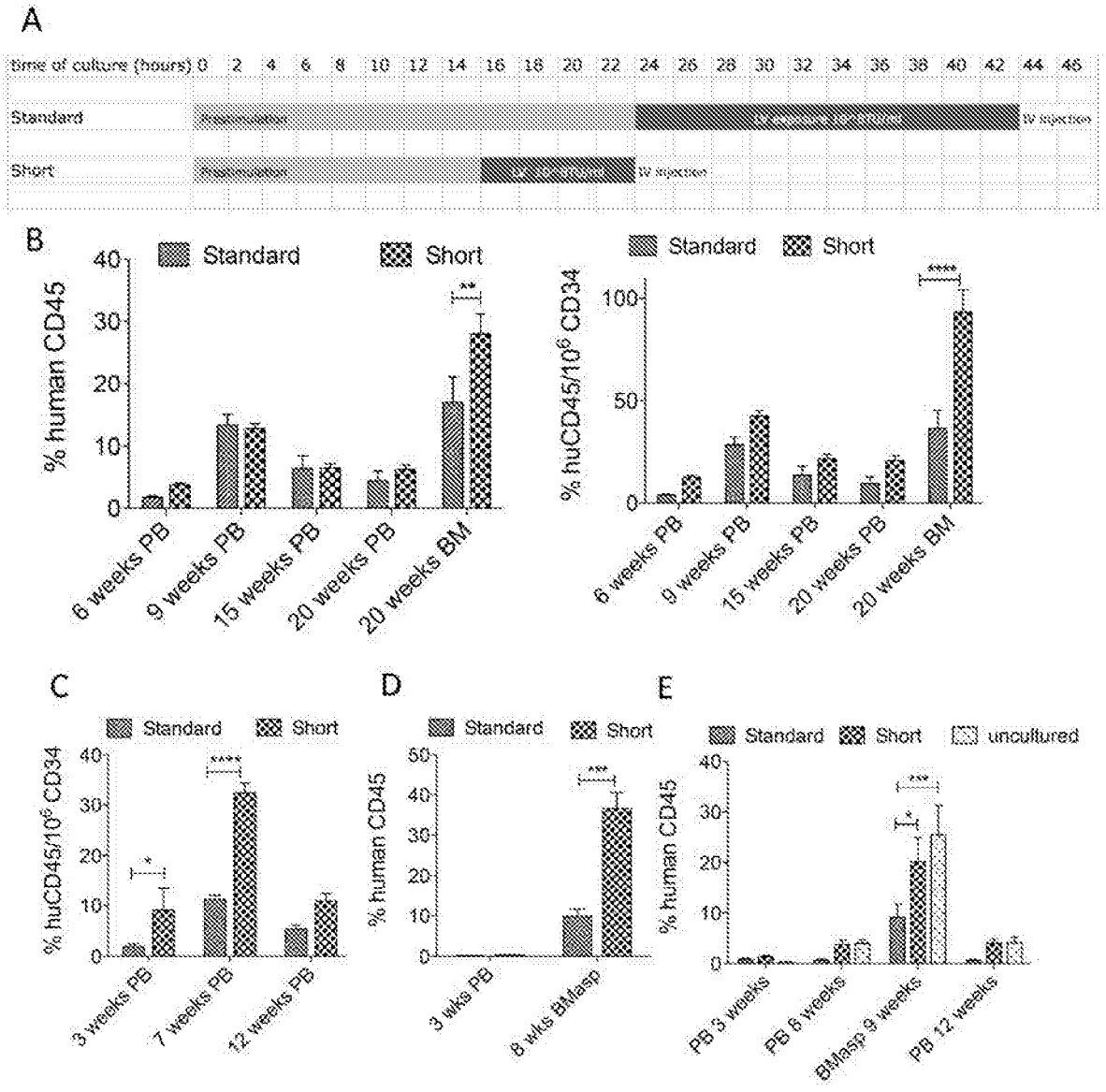

A
Time of culture (hours) 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Standard
Short
IV injection
B
Standard
Short
% human CD45
% huCD45/10^6 CD34
6 weeks PB
9 weeks PB
15 weeks PB
20 weeks PB
20 weeks BM
C
% huCD45/10^6 CD34
3 weeks PB
7 weeks PB
12 weeks PB
D
% human CD45
3 wks PB
8 wks BMasp
E
uncultured
% human CD45
PB 3 weeks
PB 6 weeks
BMasp 9 weeks
PB 12 weeks

Figure 9

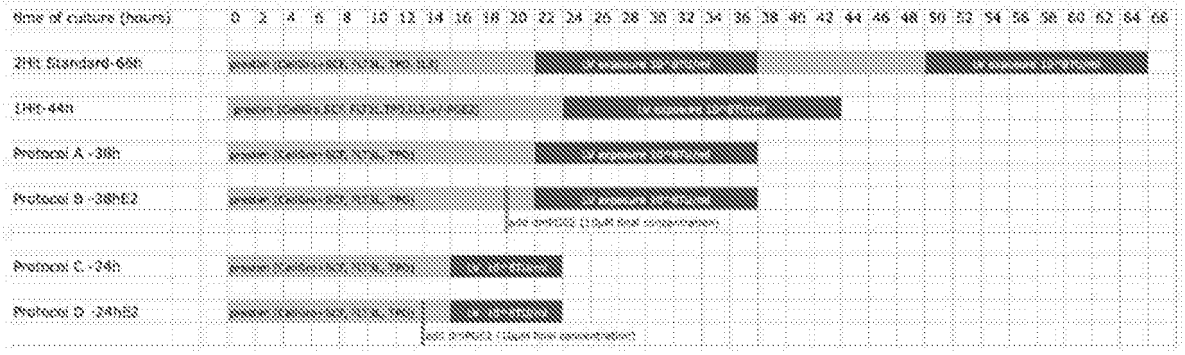

time of culture (hours)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60 62 64 66
ZHR Standard-68h
ZHR-44h
Protocol A -38h
Protocol B -38h62
Protocol C -24h
Protocol D -24h62

Figure 10