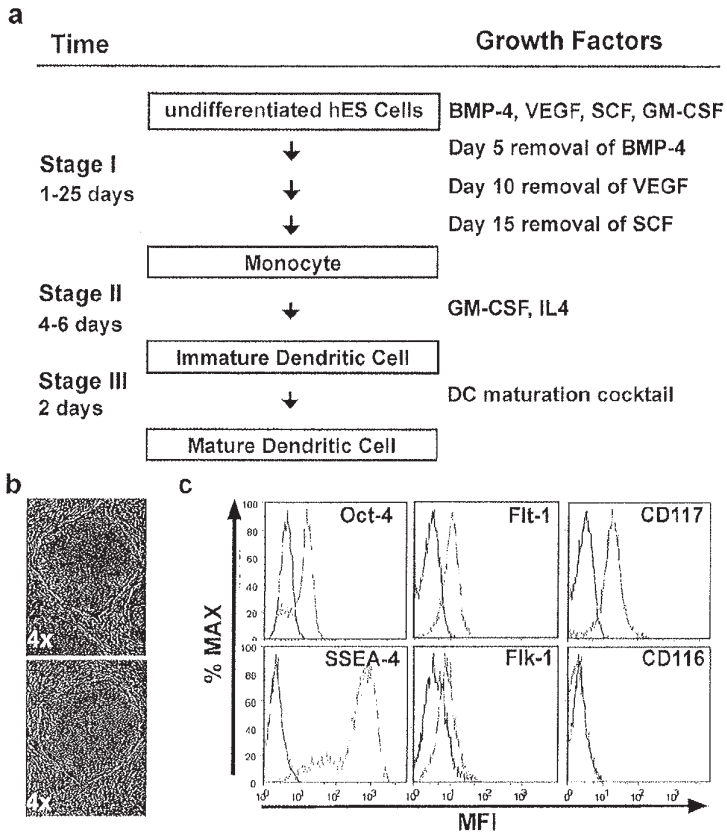

a
Time
Growth Factors
undifferentiated hES Cells
BMP-4, VEGF, SCF, GM-CSF
Stage I
1-25 days
Day 5 removal of BMP-4
Day 10 removal of VEGF
Day 15 removal of SCF
Monocyte
Stage II
4-6 days
GM-CSF, IL4
Immature Dendritic Cell
Stage III
2 days
DC maturation cocktail
Mature Dendritic Cell
b
4x
4x
c
Oct-4
Flt-1
CD117
SSEA-4
Flk-1
CD116
% MAX
MFI

Figure 1

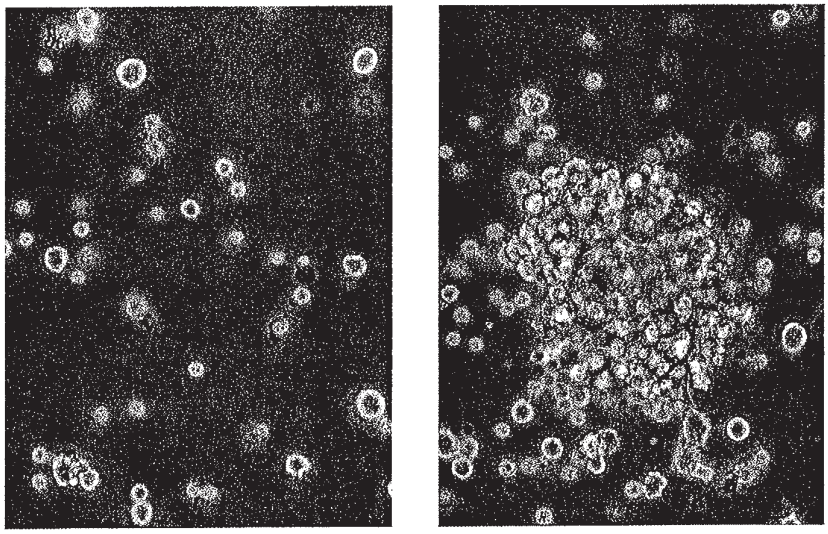


FIG. 2

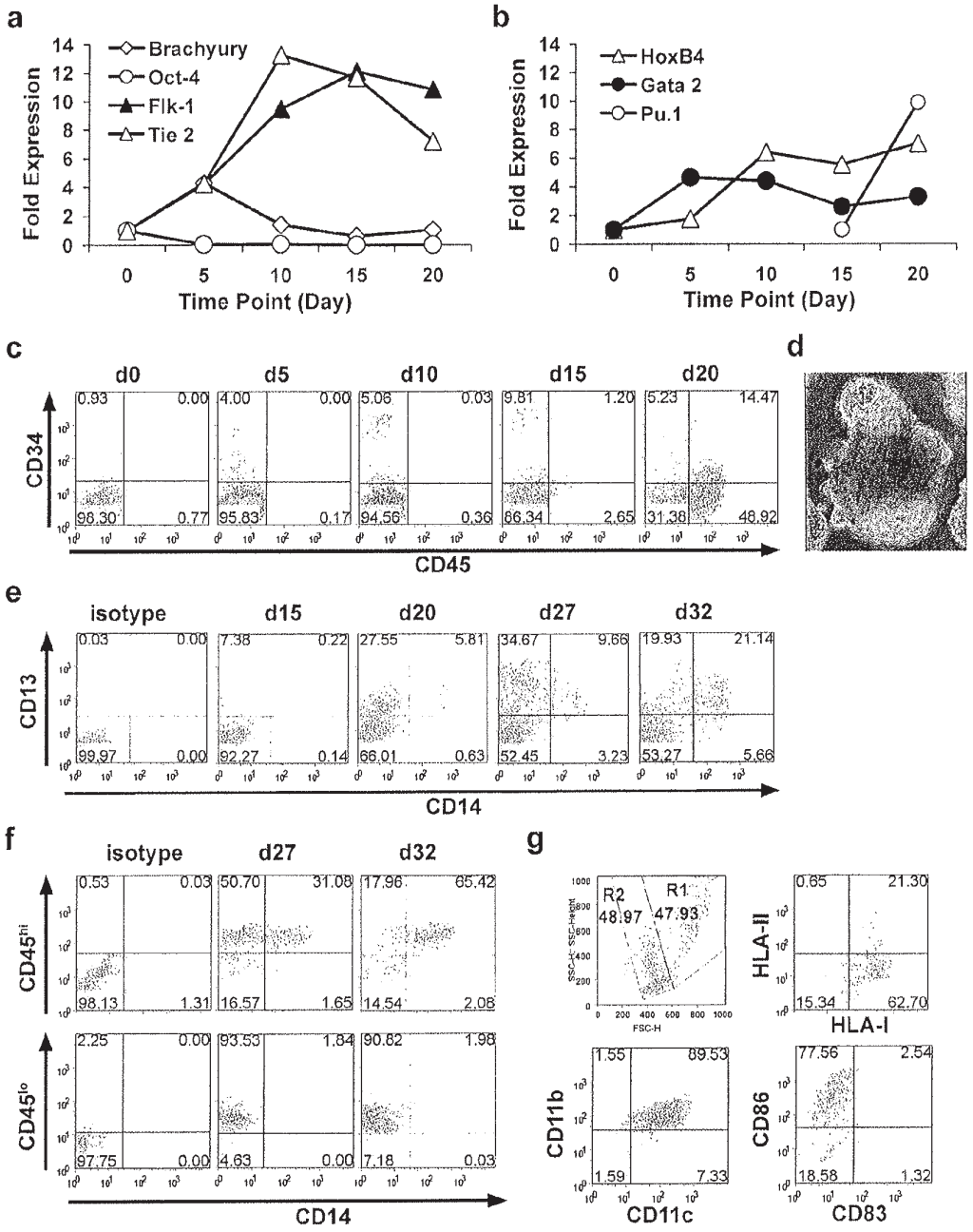

a
Brachyury
Oct-4
Flk-1
Tie 2
Fold Expression
Time Point (Day)
b
HoxB4
Gata 2
Pu.1
Fold Expression
Time Point (Day)
c
d0
d5
d10
d15
d20
CD34
CD45
d
e
isotype
d15
d20
d27
d32
CD13
CD14
f
isotype
d27
d32
CD45hi
CD45lo
CD14
g
R1
R2
HLA-II
HLA-I
CD11b
CD11c
CD86
CD83

Figure 3

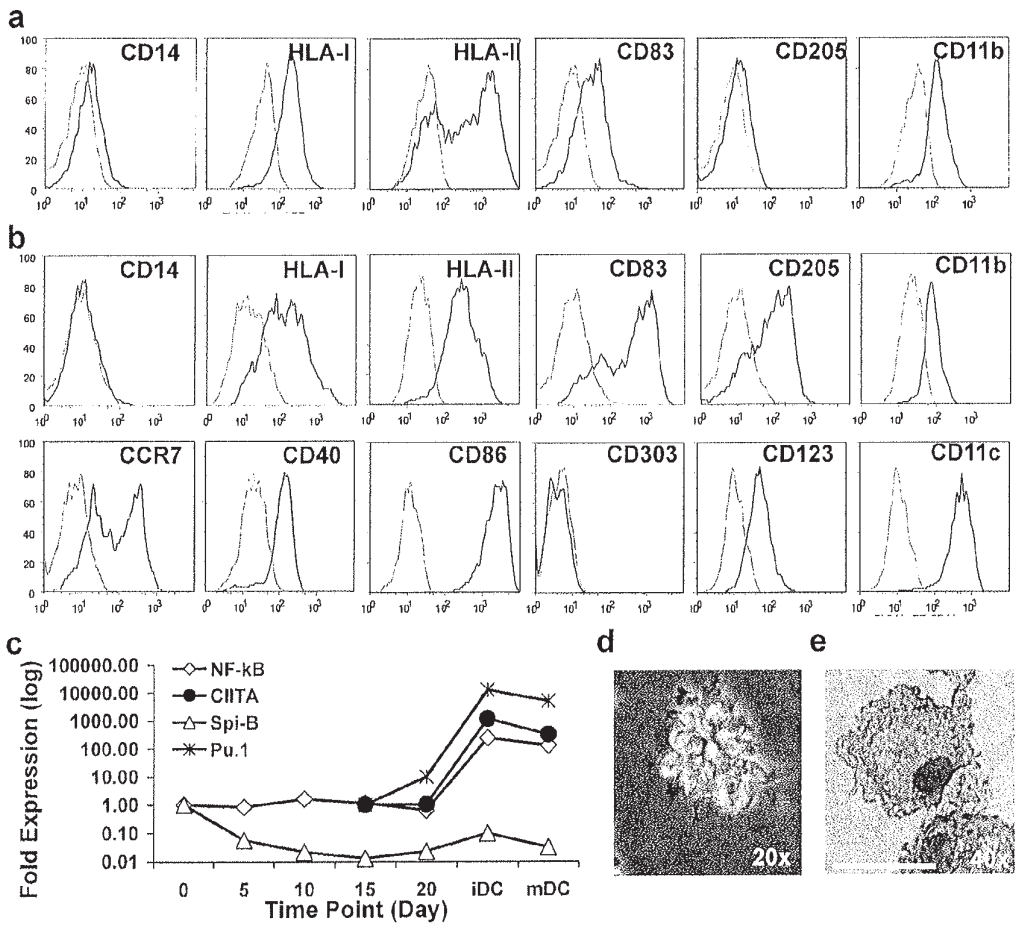

a
CD14
HLA-I
HLA-II
CD83
CD205
CD11b
b
CD14
HLA-I
HLA-II
CD83
CD205
CD11b
CCR7
CD40
CD86
CD303
CD123
CD11c
c
Fold Expression (log)
100000.00
10000.00
1000.00
100.00
10.00
1.00
0.10
0.01
NF-kB
CIITA
Spi-B
Pu.1
0
5
10
15
20
iDC
mDC
Time Point (Day)
d
20x
e

Figure 4

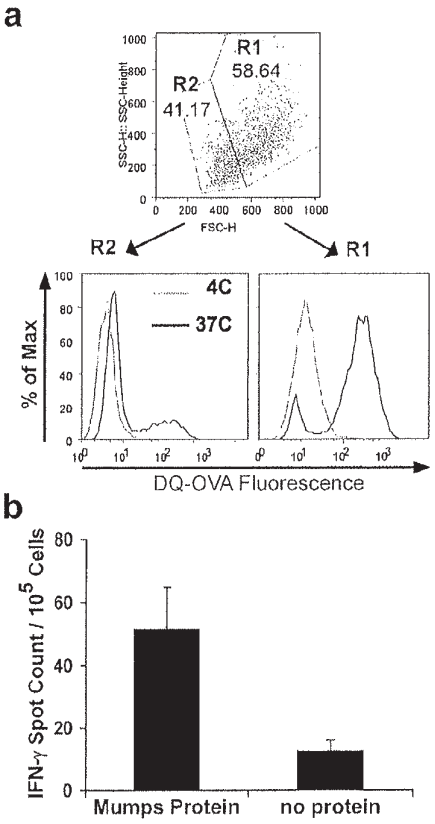

a
R1
58.64
R2
41.17
SSC-H: SSC-Height
FSC-H
R2
R1
4C
37C
% of Max
DQ-OVA Fluorescence
b
IFN-γ Spot Count / 10^5 Cells
Mumps Protein
no protein

Figure 5

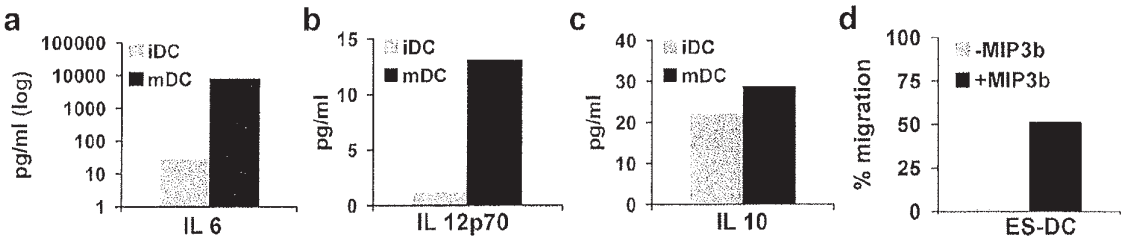

a
100000
10000
1000
100
10
1
pg/ml (log)
iDC
mDC
IL 6
b
15
10
5
0
pg/ml
iDC
mDC
IL 12p70
c
40
30
20
10
0
pg/ml
iDC
mDC
IL 10
d
100
75
50
25
0
% migration
-MIP3b
+MIP3b
ES-DC

Figure 6

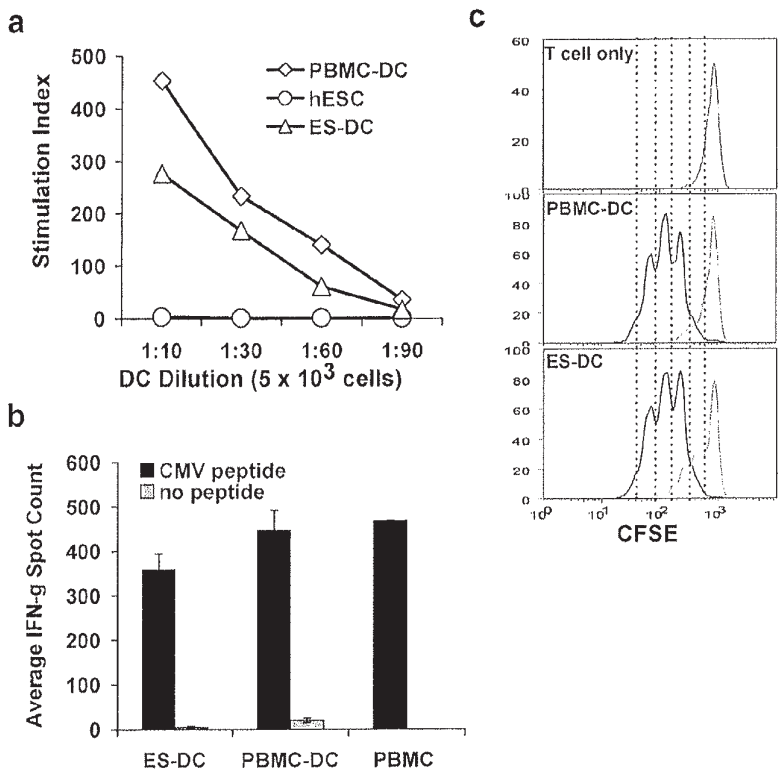
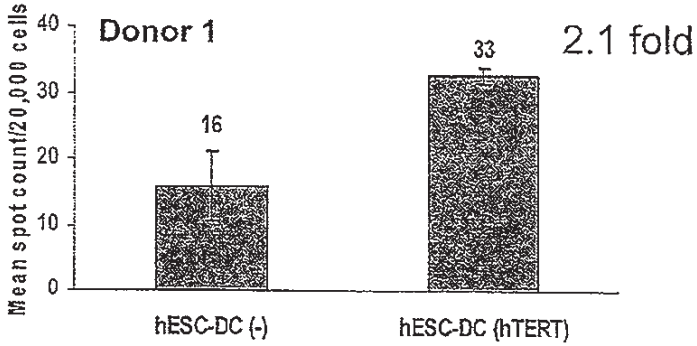
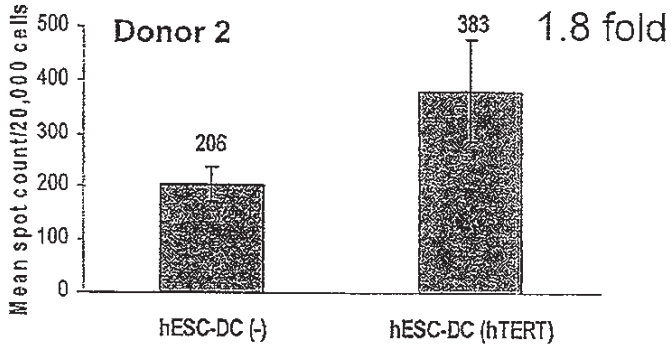
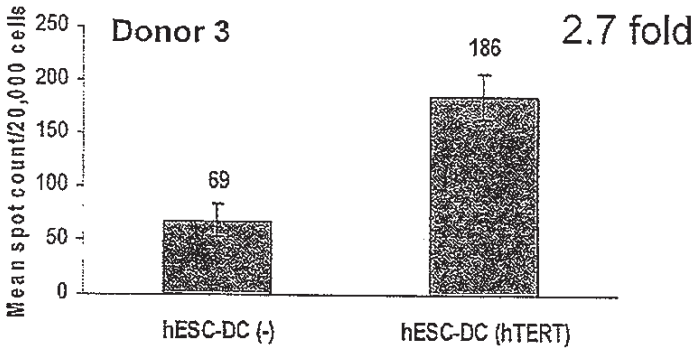

a
Stimulation Index
500
400
300
200
100
0
PBMC-DC
hESC
ES-DC
1:10
1:30
1:60
1:90
DC Dilution (5 x 10^3 cells)
b
Average IFN-g Spot Count
600
500
400
300
200
100
0
CMV peptide
no peptide
ES-DC
PBMC-DC
PBMC
c
T cell only
PBMC-DC
ES-DC
CFSE

Figure 7

Mature hESC-DC present hTERT antigen on HLA-A2 and stimulate IFN-g secretion from T cells







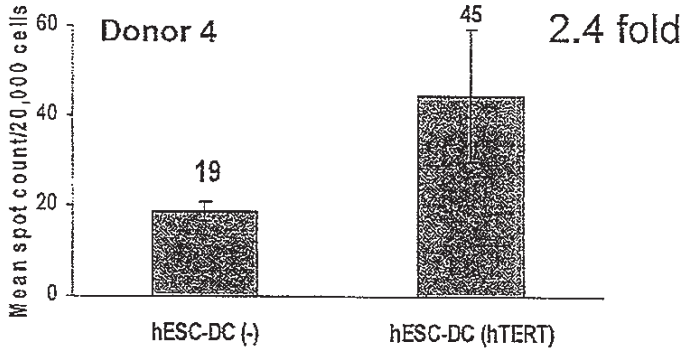


Figure 8

Mature hESC-DC present hTERT antigen on HLA-A2 and stimulate T cell proliferation

Proliferation was measured using CFSE dye dilution and hTERT pentamer which identifies antigen-specific cells

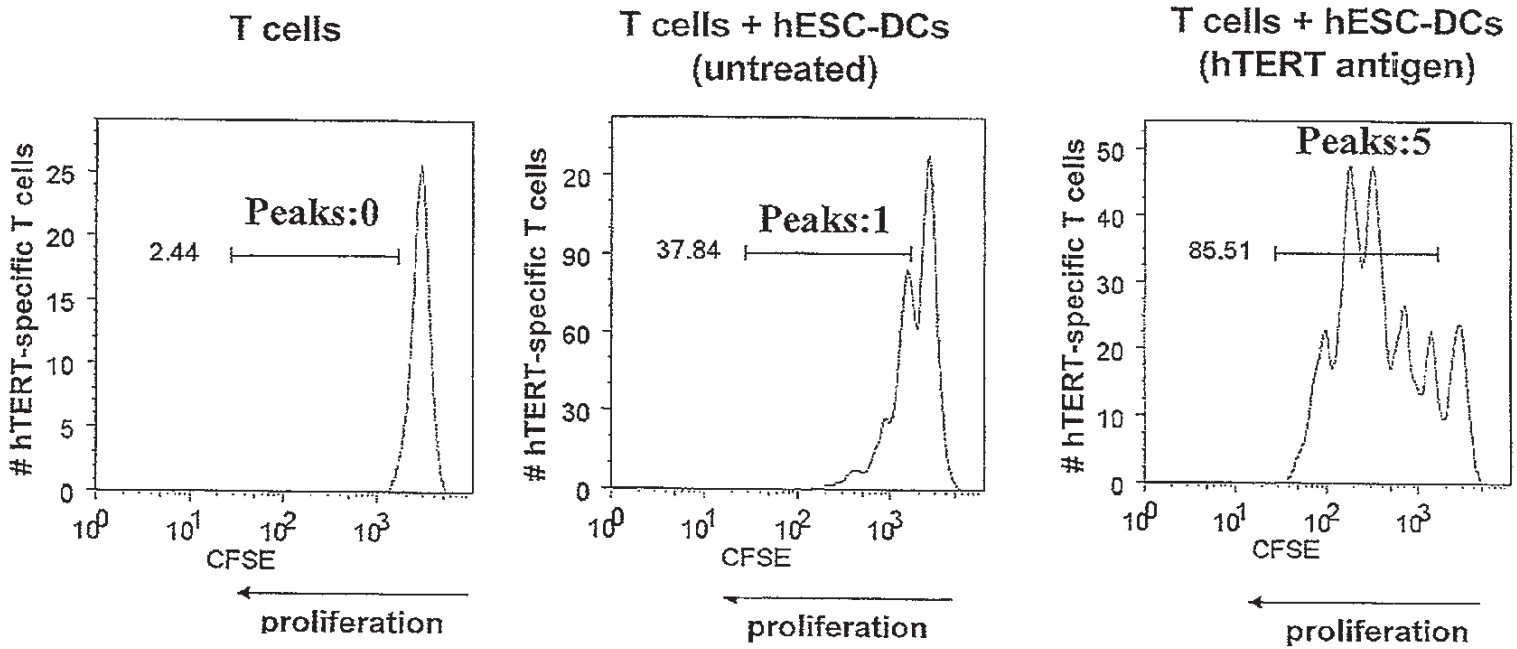


Figure 9

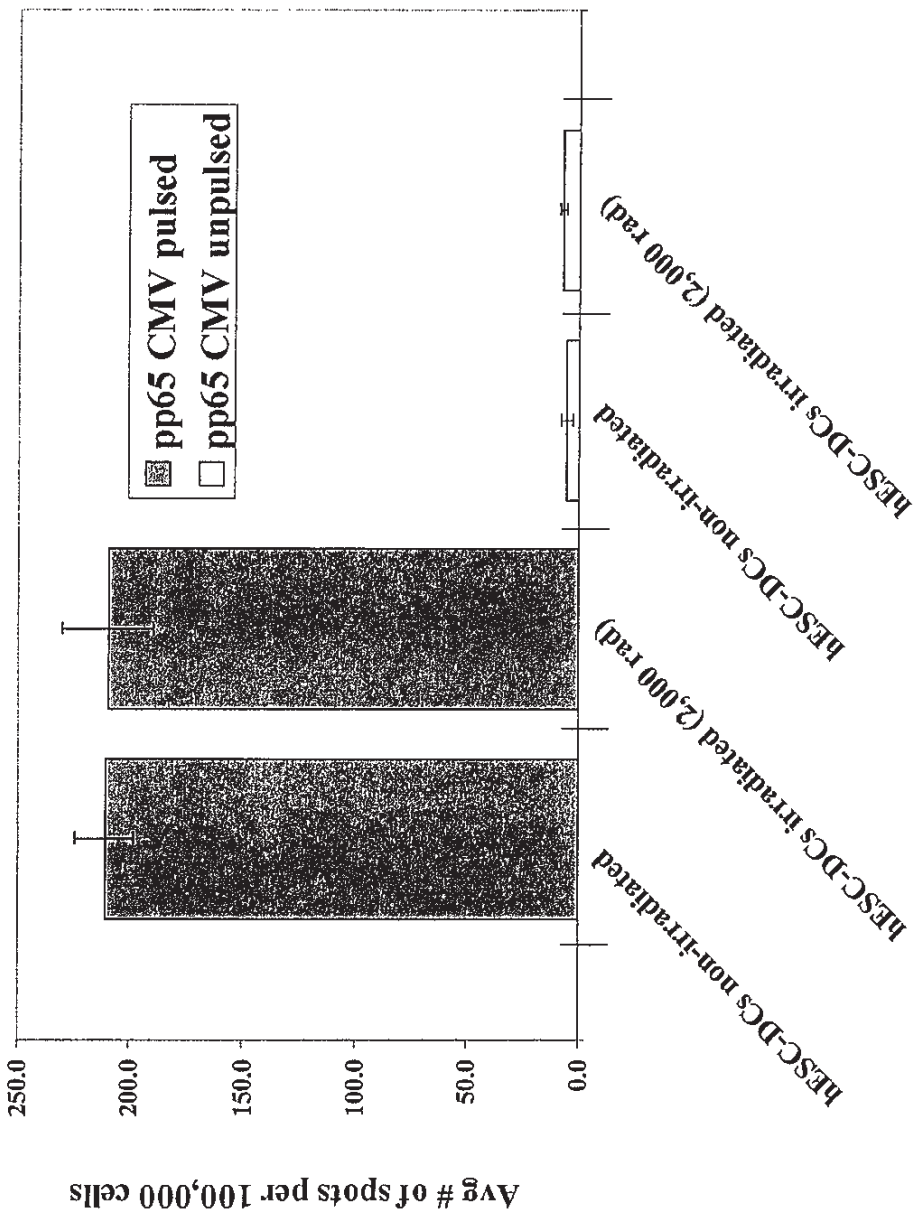

Avg # of spots per 100,000 cells
250.0
200.0
150.0
100.0
50.0
0.0
pp65 CMV pulsed
pp65 CMV unpulsed
hESC-DCs non-irradiated
hESC-DCs irradiated (2,000 rad)
hESC-DCs non-irradiated
hESC-DCs irradiated (2,000 rad)

Figure 10

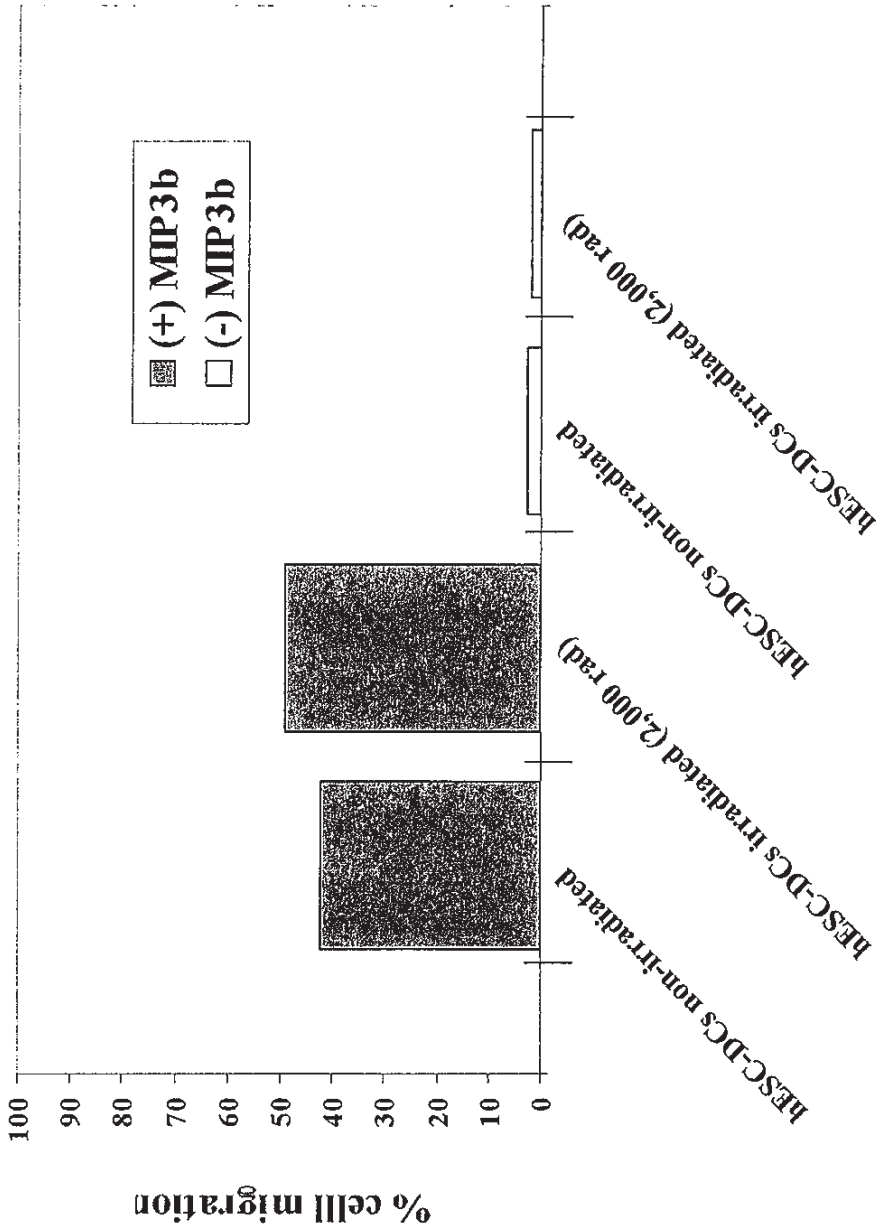

% cell migration
100
90
80
70
60
50
40
30
20
10
0
(+) MIP3b
(-) MIP3b
hESC-DCs non-irradiated
hESC-DCs irradiated (2,000 rad)
hESC-DCs non-irradiated
hESC-DCs irradiated (2,000 rad)

Figure 11

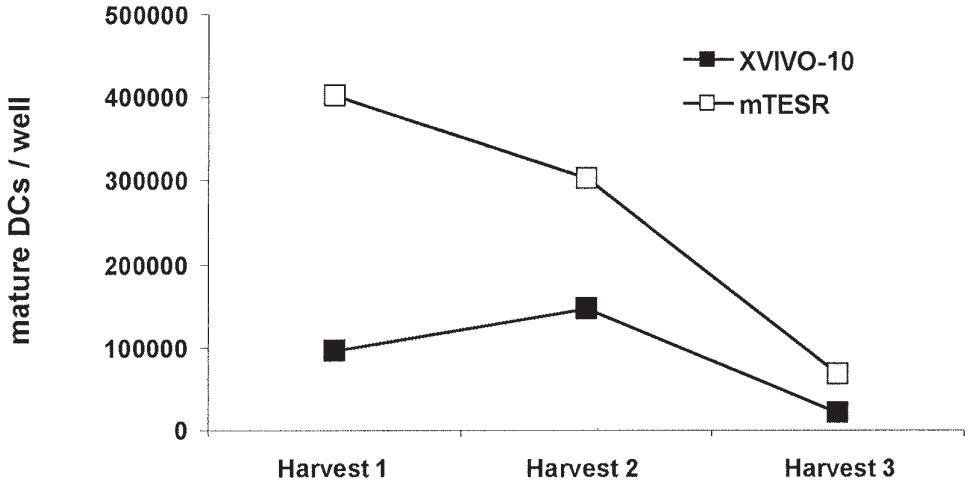


Figure 12

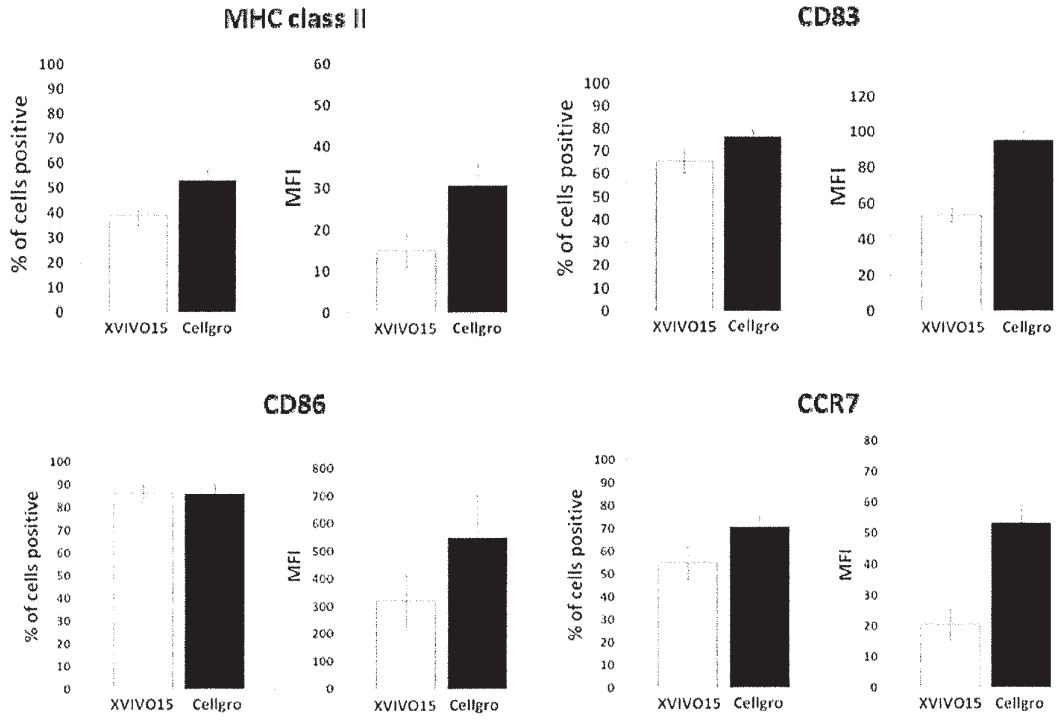


Figure 13

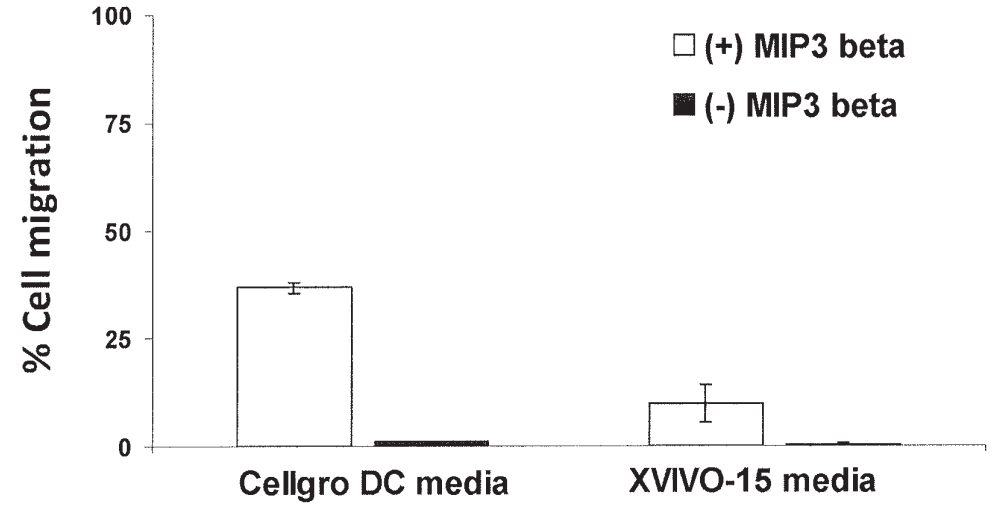


Figure 14

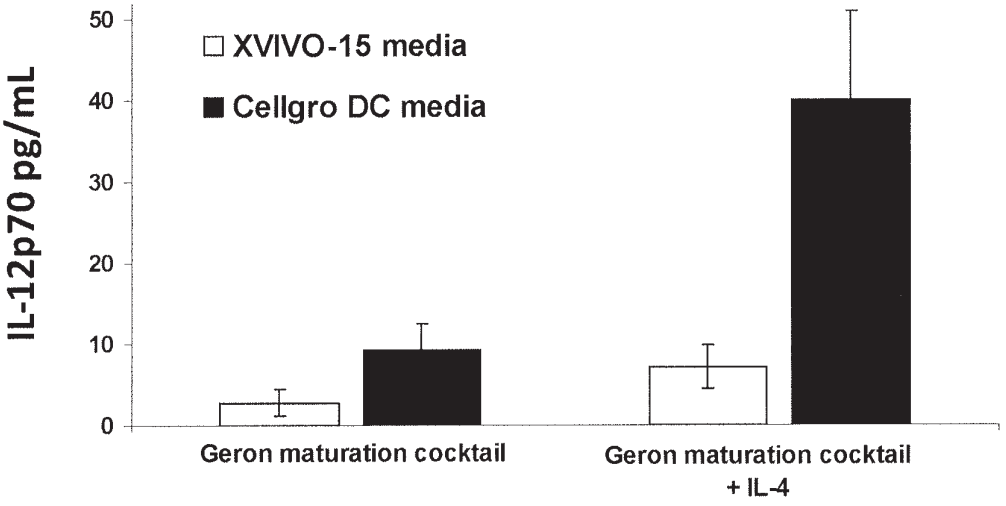


Figure 15

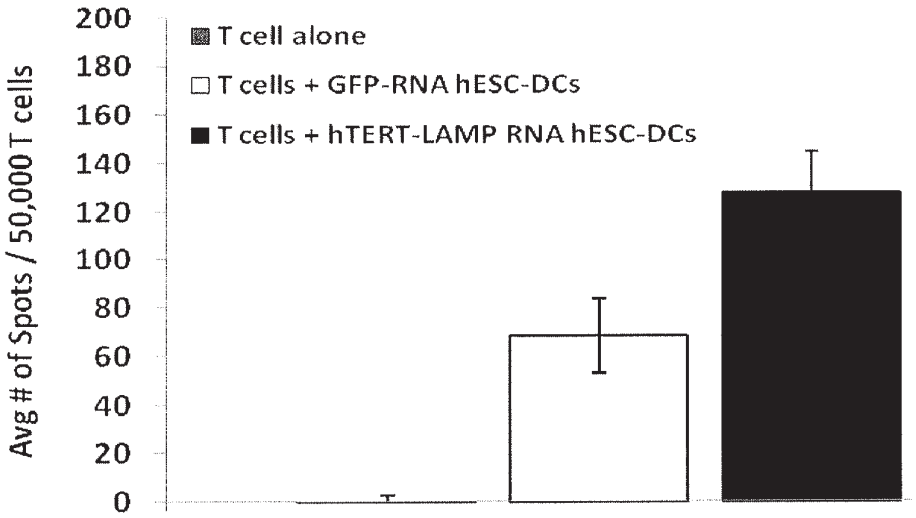

200
180
160
140
120
100
80
60
40
20
0
Avg # of Spots / 50,000 T cells
T cell alone
T cells + GFP-RNA hESC-DCs
T cells + hTERT-LAMP RNA hESC-DCs

Figure 16