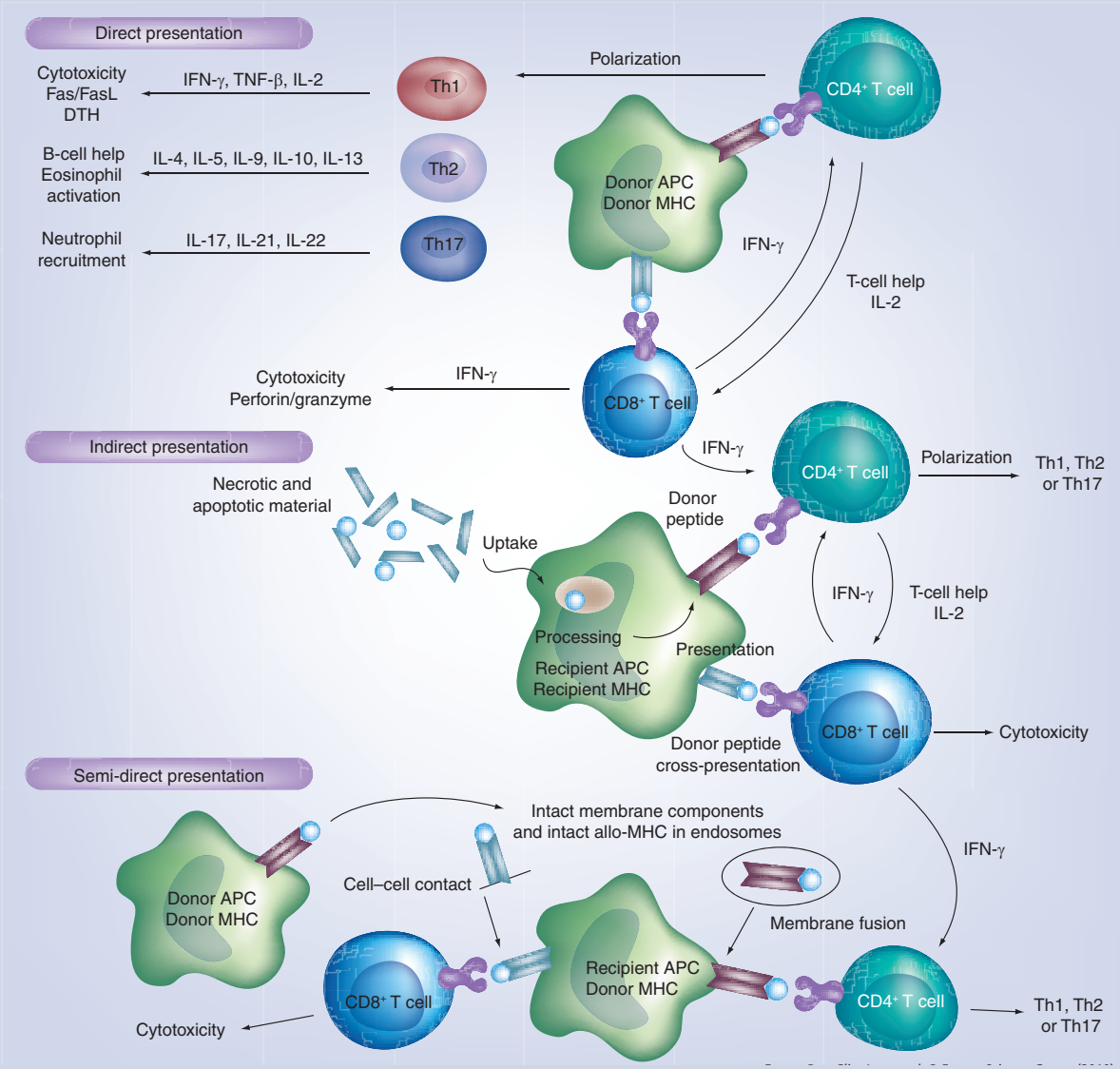
Figure 1.1



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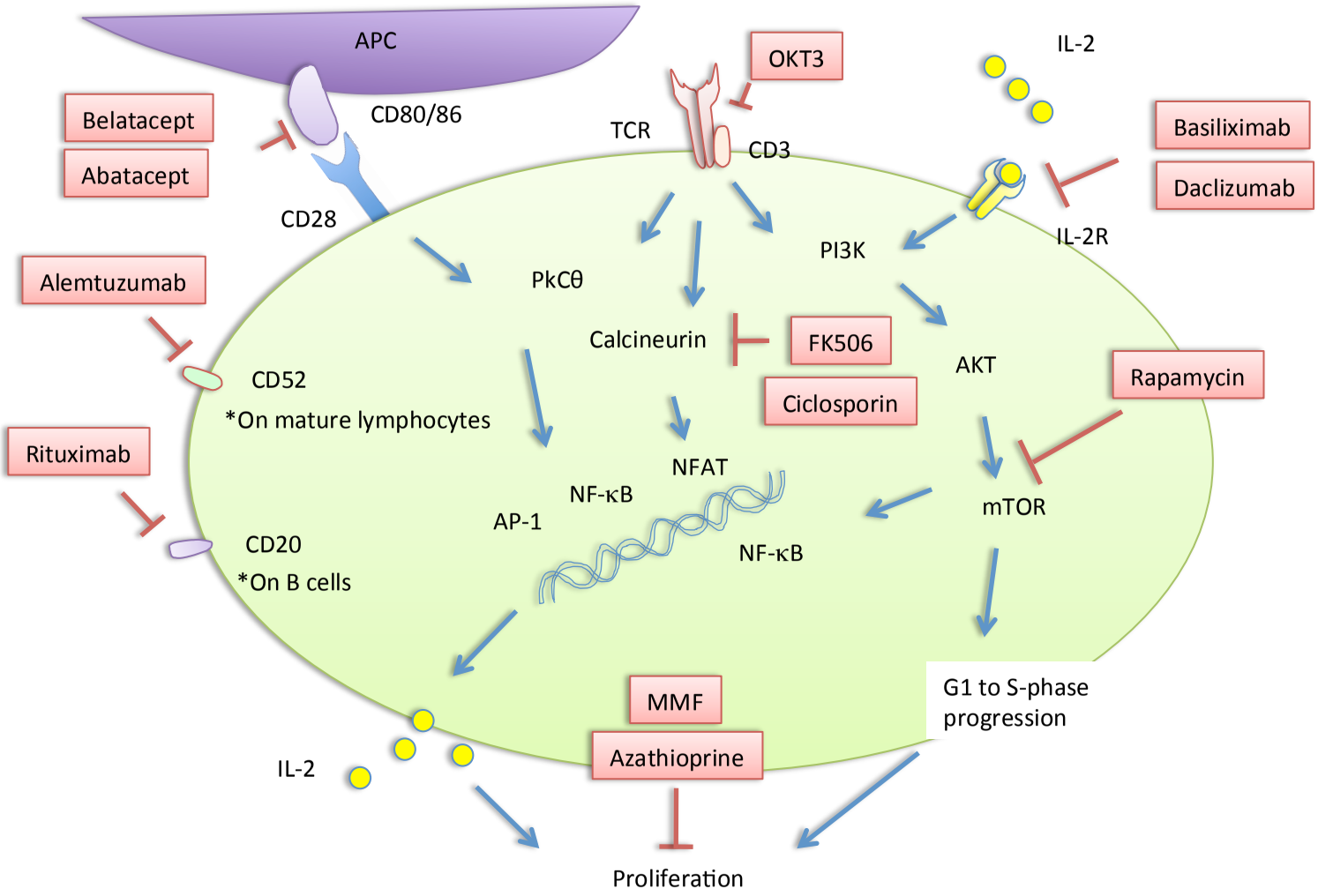
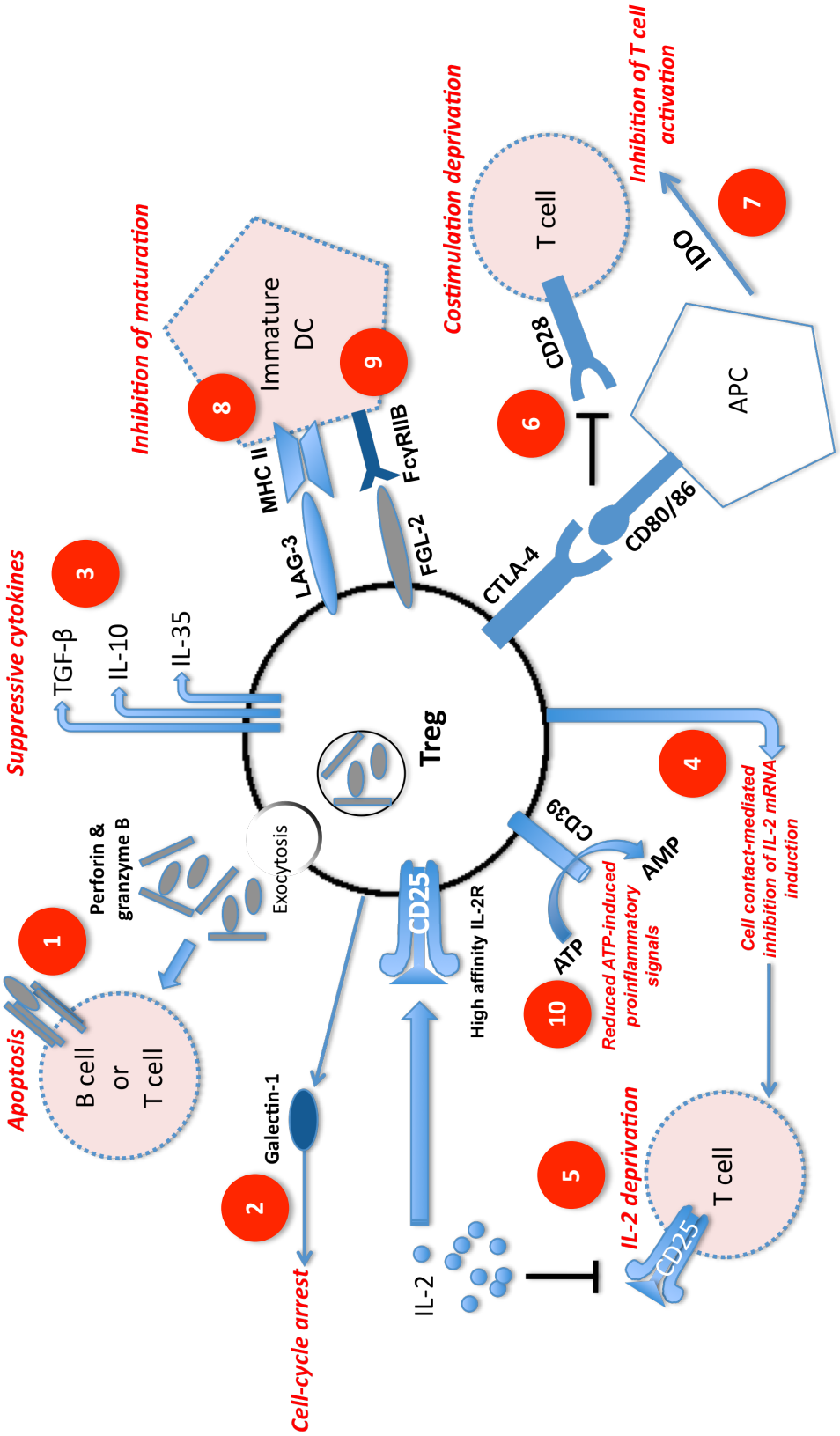


Figure 1.2

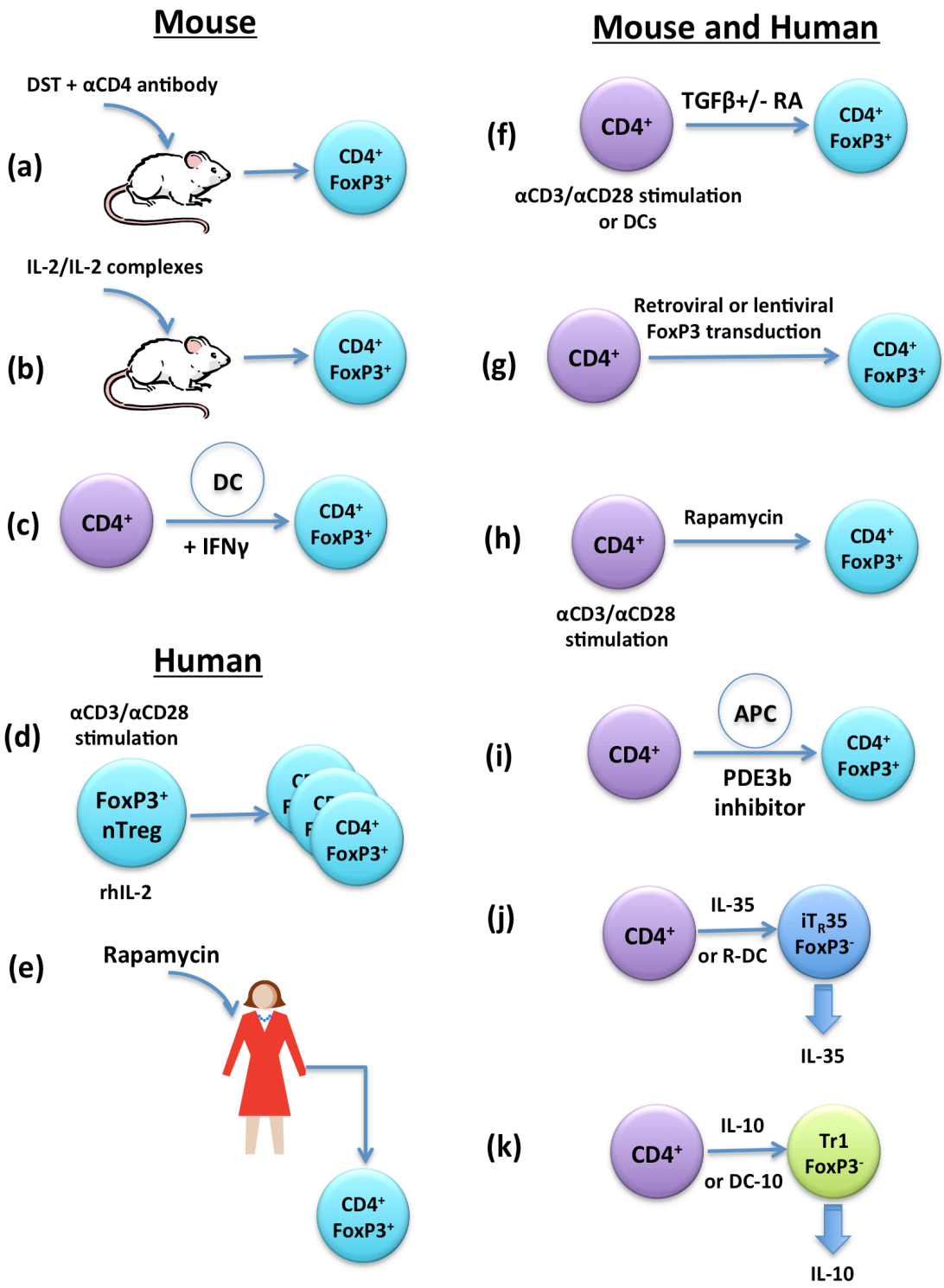
Reproduced with permission from Issa et al. *Principles of Immunology* in *A Clinical Guide to Organ Transplantation* (2011)

Figure 1.3



Reproduced with permission from Issa et al. *Curr Opin Org Transp* (2010)

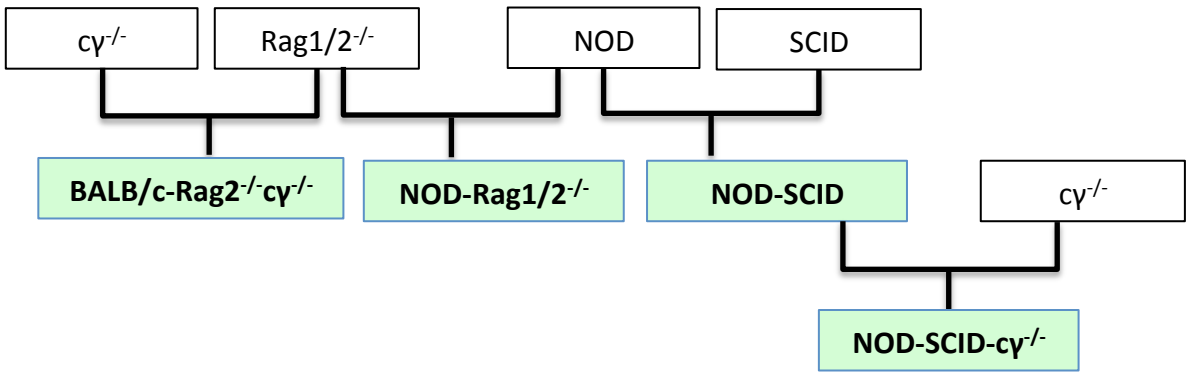
Figure 1.4



Reproduced with permission from Issa et al. Curr Opin Imm (2011)

Figure 1.5

(a)



(b)

Mouse strain	Phenotype
NOD-SCID[19]	1. No mature T and B cells 2. Decreased innate immunity
SCID/beige[25, 61, 68]	1. No mature T and B cells 2. Decreased NK activity
Balb/c-Rag1/2$^{-/-}$*γc*$^{-/-}$[60, 61]	1. Rag mutation causes lack of mature T and B cells 2. IL-2Rγ-chain deficiency leads to many innate immune defects
NOD/SCID-γc/Il2rγ$^{-/-}$[61, 69-71]	1. Lack mature T and B cells 2. IL-2Rγ-chain deficiency leads to many innate immune defects

Figure 2.1

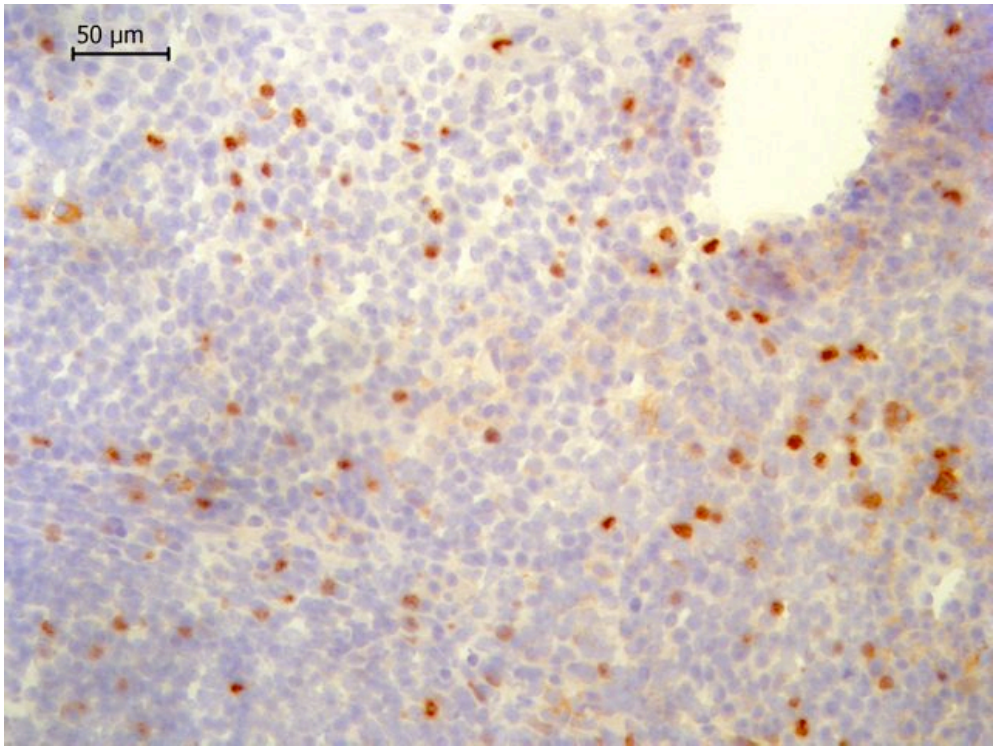


Figure 3.1

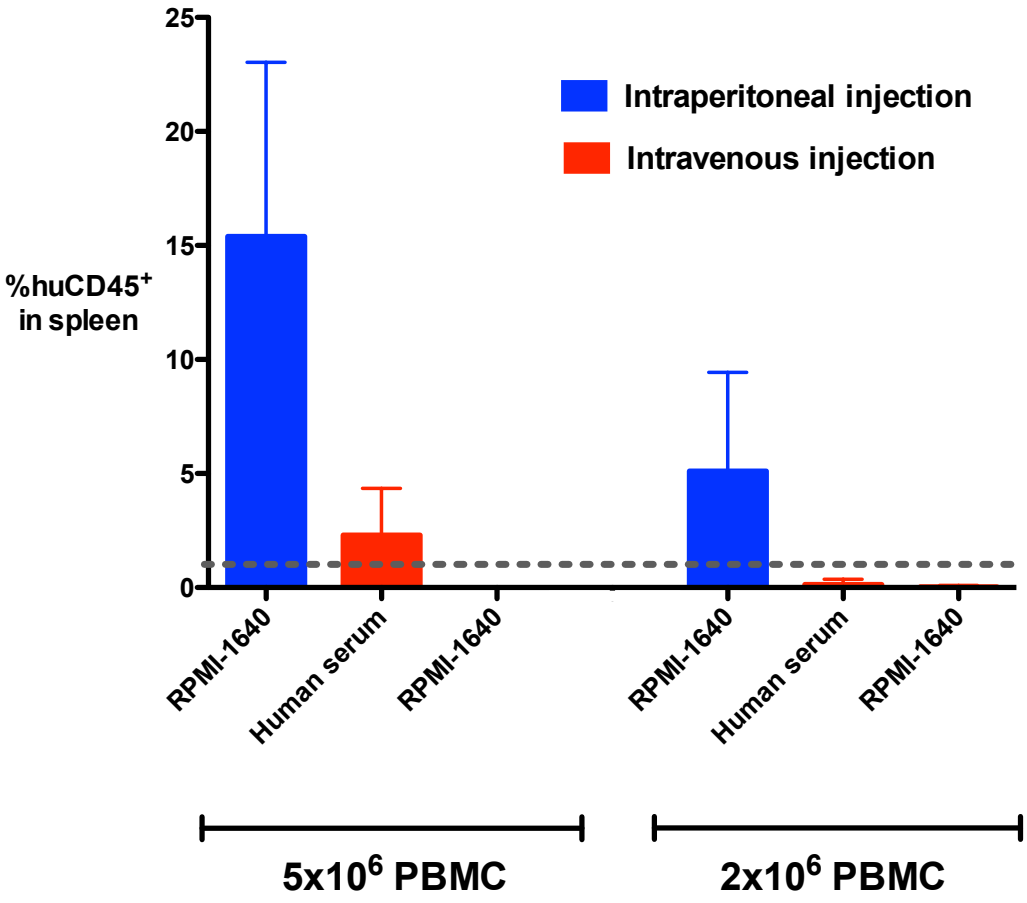
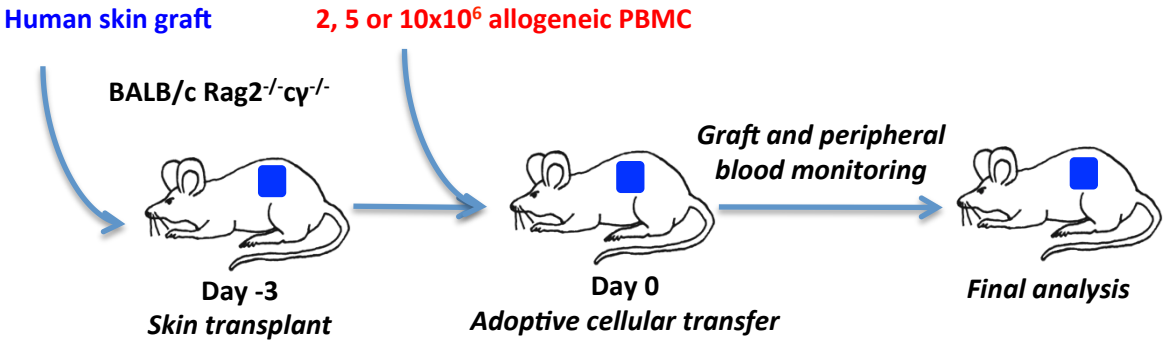
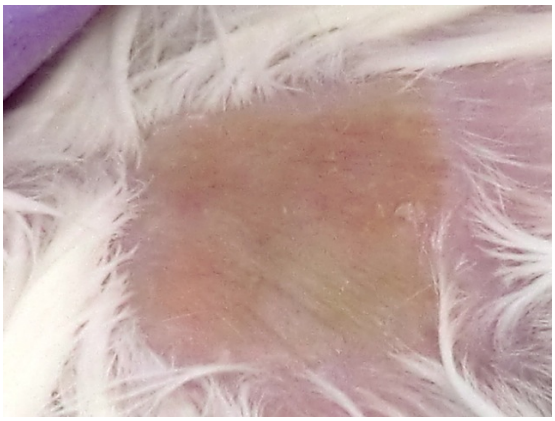

25
20
15
10
5
0
%huCD45+ in spleen
Intraperitoneal injection
Intravenous injection
RPMI-1640
Human serum
RPMI-1640
RPMI-1640
Human serum
RPMI-1640
5x10^6 PBMC
2x10^6 PBMC

Figure 3.2

(a)



(b)



(c)

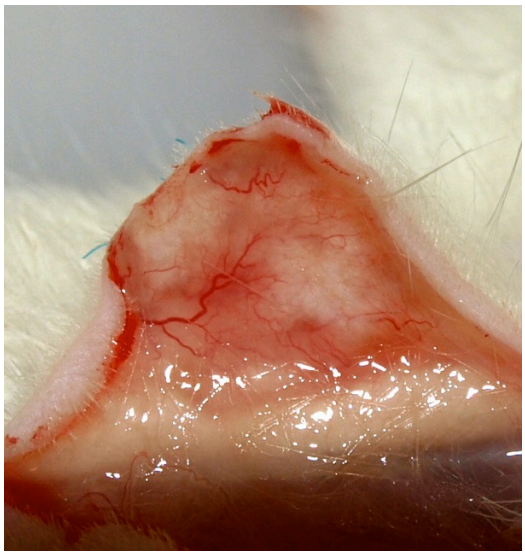
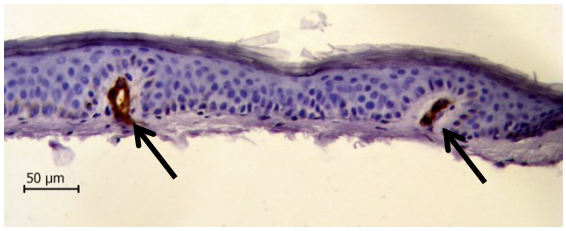


Figure 3.3

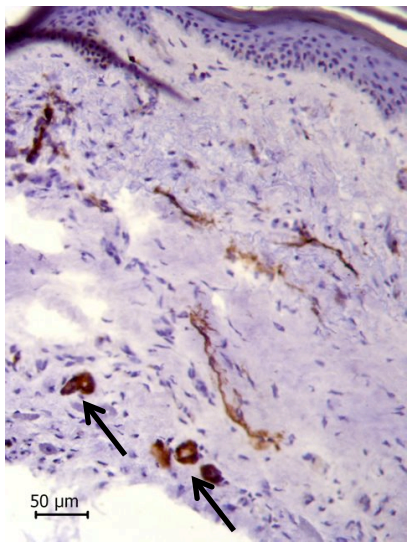
Human CD31

(a)

Pre-transplantation



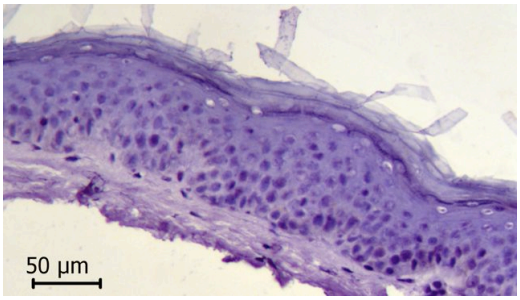
Day 100 post-transplantation



Mouse CD31

(b)

Pre-transplantation



Day 100 post-transplantation

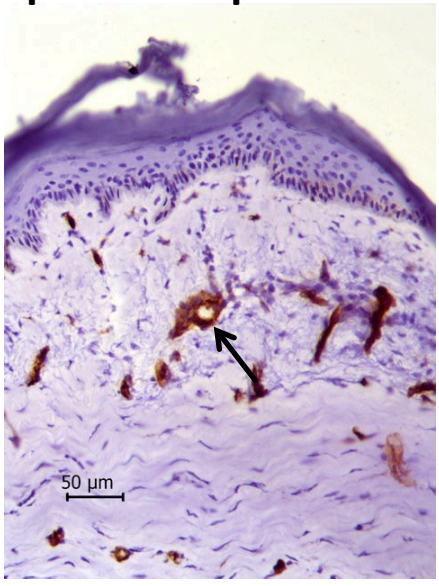
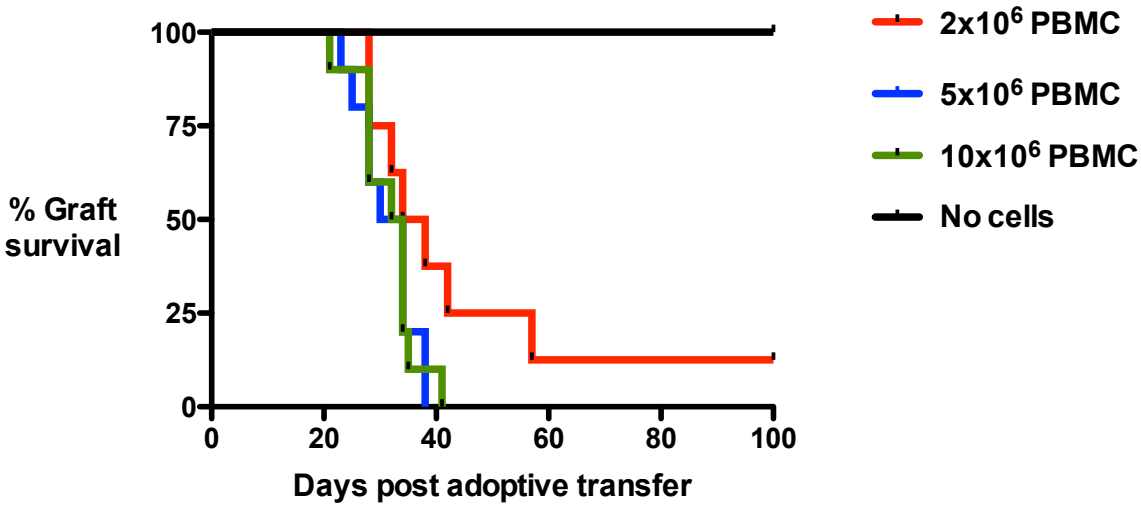


Figure 3.4

(a)



(b)

Group	Group size	Graft loss days	MST (days)
2x10^6 PBMC	8	28, 28, 32, 34, 38, 42, 57, >100	36
5x10^6 PBMC	10	23, 25, 28, 28, 30, 34, 34, 34, 38, 38	32
10x10^6 PBMC	10	21, 28, 28, 28, 32, 34, 34, 34, 35, 41	33
No cells	5	>100, >100, >100, >100, >100	>100

(c)

No cells



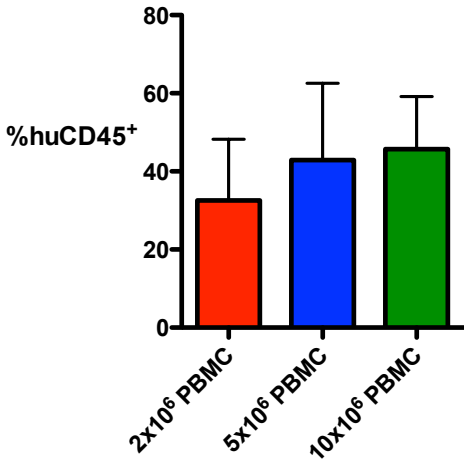
(d)

5x10^6 PBMC

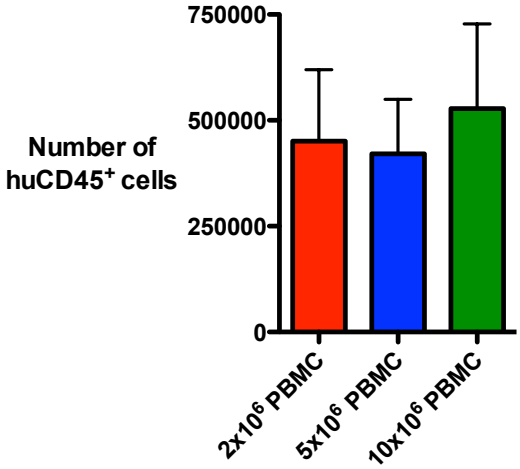


Figure 3.5

(a)



(b)



(c)

Rate of failure of reconstitution

$2x10^6$ PBMC	$5x10^6$ PBMC	$10x10^6$ PBMC
33%	17%	0%

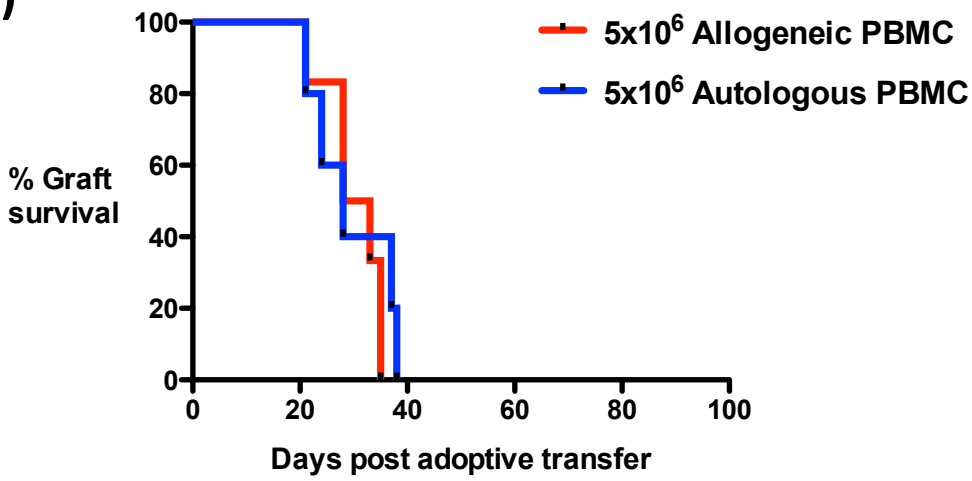
(d)

Rate of XvHD development

$2x10^6$ PBMC	$5x10^6$ PBMC	$10x10^6$ PBMC
12.5%	20%	50%

Figure 3.6

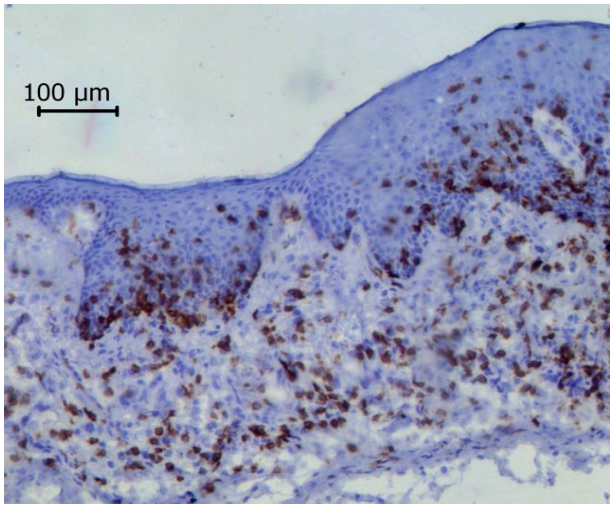
(a)



(b)

Group	Group size	Graft loss days	MST (days)
$5x10^6$ Allogeneic PBMC	6	21, 28, 28, 33, 35, 35	30.5
$5x10^6$ Autologous PBMC	5	21, 24, 28, 37, 38	28

(c)



(d)

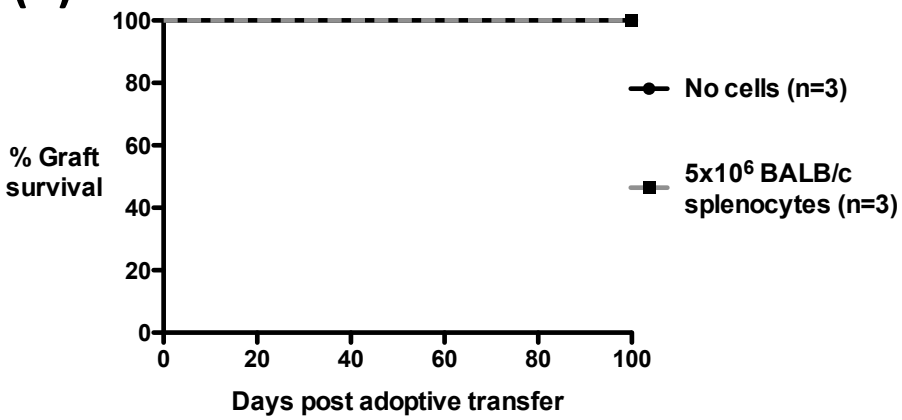


Figure 3.7

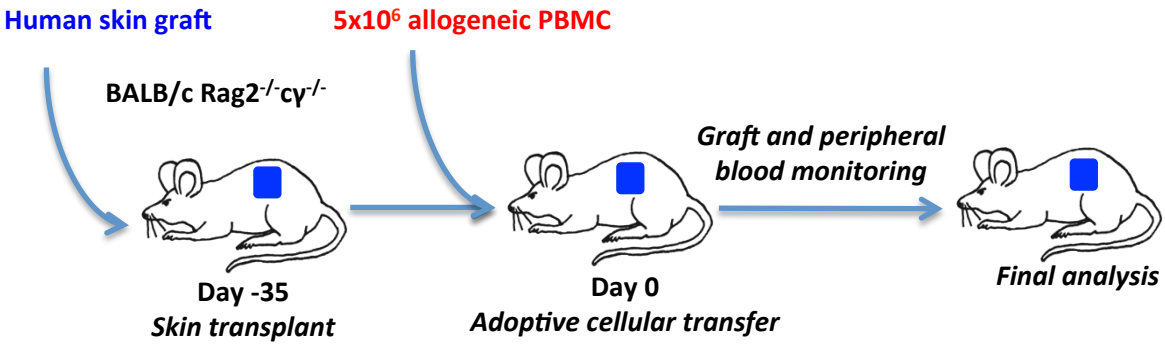
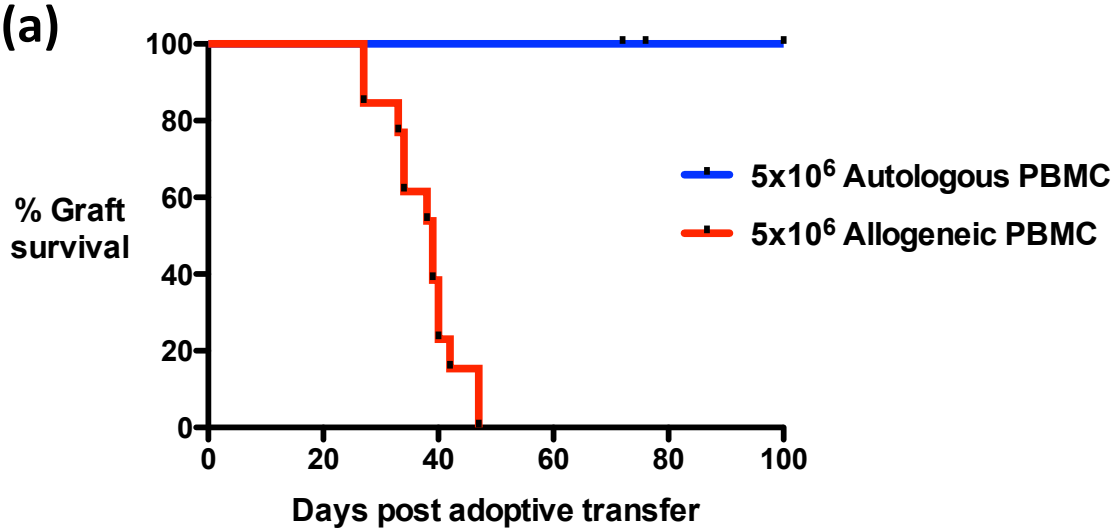

Human skin graft
5x10⁶ allogeneic PBMC
BALB/c Rag2-/-cγ-/-
Graft and peripheral blood monitoring
Day -35
Skin transplant
Day 0
Adoptive cellular transfer
Final analysis

Figure 3.8

(a)



(b)

Group	Group size	Graft loss days	MST (days)
5x10^6 Autologous PBMC	10	>100, >100, >100, >100, >100, >100, >100, two sacrificed at days 72 and 76 due to XvHD	>100
5x10^6 Allogeneic PBMC	13	27, 33, 34, 34, 38, 39, 39, 40, 40, 42, 47, 47	39

(c)

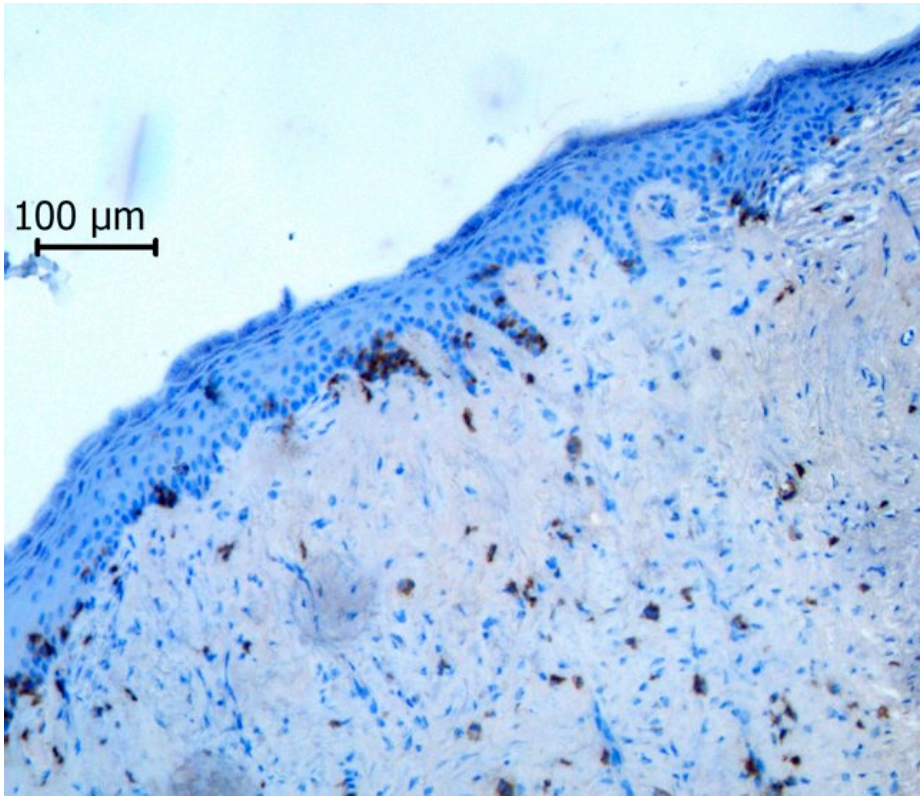
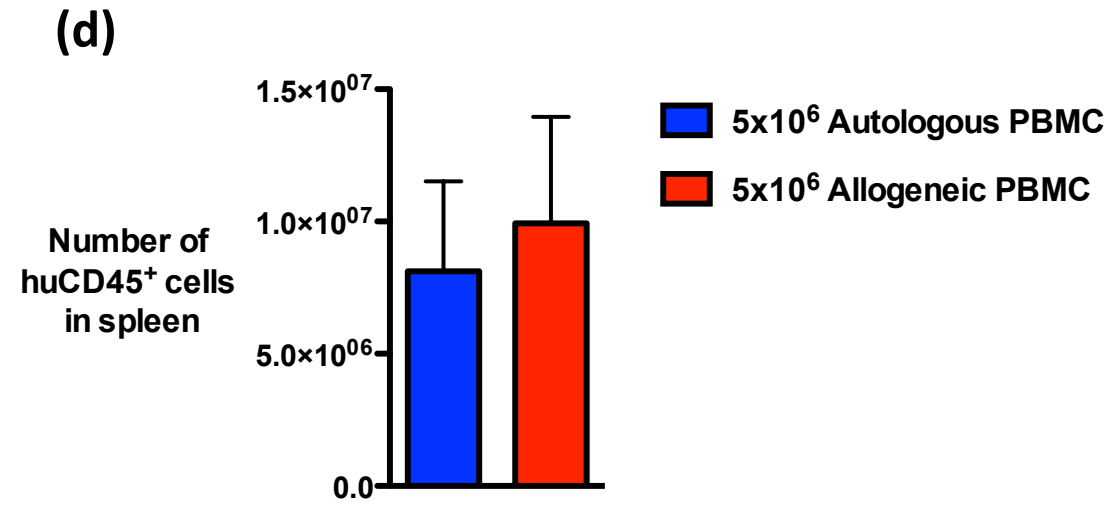


Figure 3.8 cont.



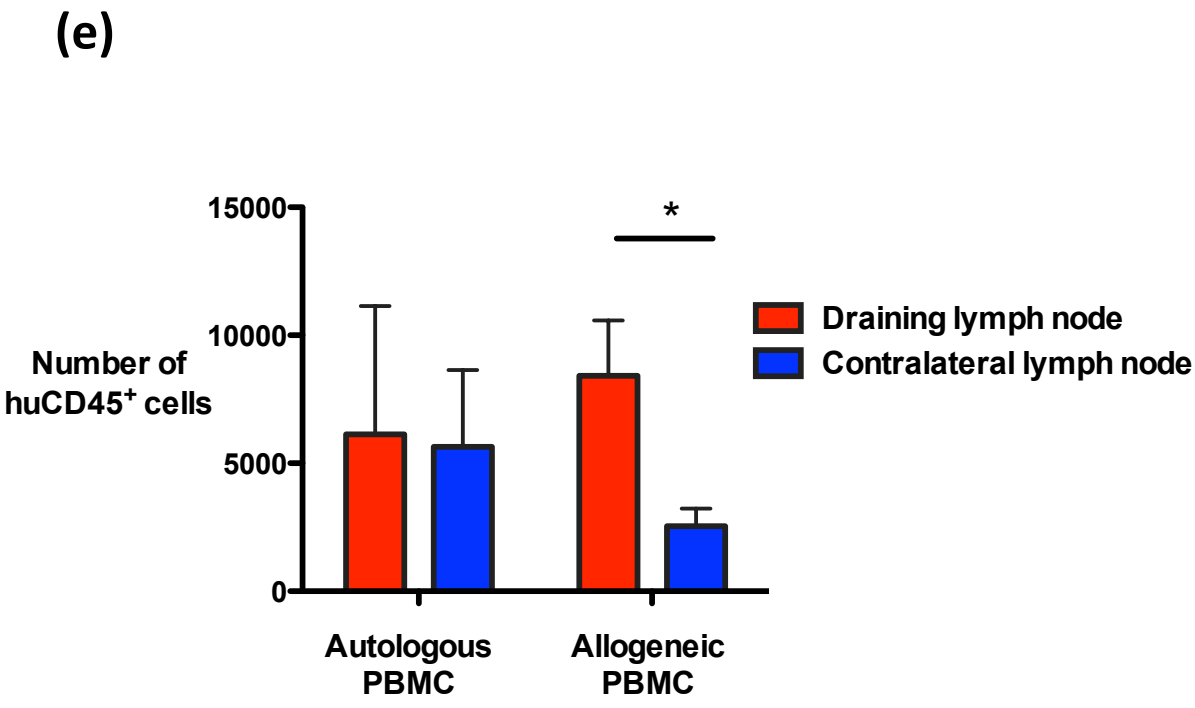
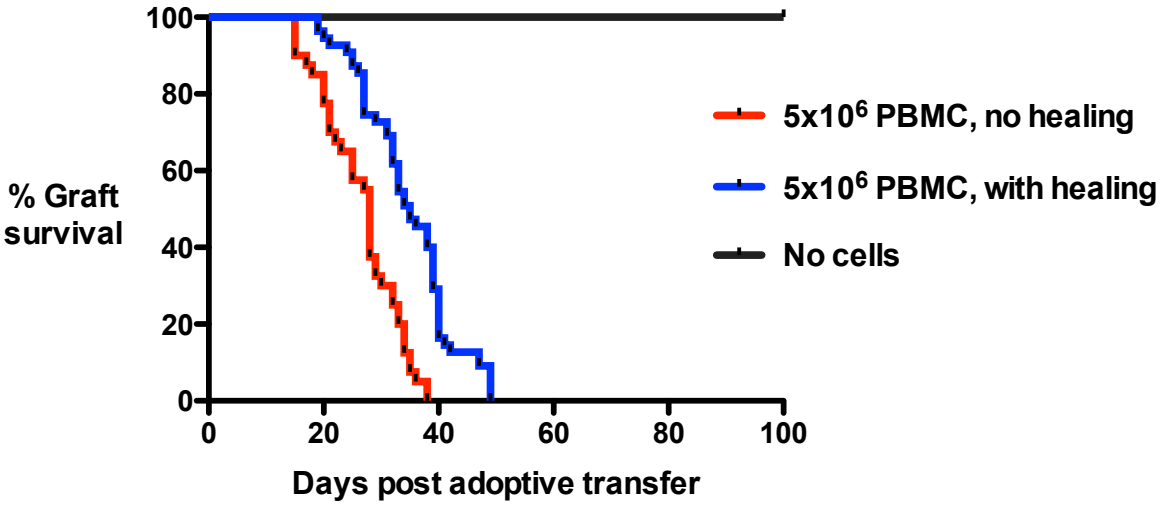


Figure 3.9

(a)



(b)

Group	Group size	Graft loss days	MST (days)
5x10^6 PBMC, no healing	40	15, 15, 15, 15, 17, 18, 20, 20, 20, 21, 21, 21, 22, 23, 25, 25, 25, 27, 28, 28, 28, 28, 28, 28, 28, 29, 29, 30, 32, 32, 33, 33, 34, 34, 34, 35, 35, 36, 38, 38,	28
5x10^6 PBMC, with healing	55	19, 19, 20, 21, 24, 25, 25, 26, 27, 27, 27, 27, 27, 27, 29, 31, 31, 32, 32, 32, 32, 33, 33, 33, 33, 34, 34, 35, 35, 36, 38, 38, 38, 39, 39, 39, 39, 39, 39, 40, 40, 40, 40, 40, 40, 40, 41, 42, 47, 47, 49, 49, 49, 49, 49	35
No cells	10	>100, >100, >100, >100, >100, >100, >100, >100, >100, >100,	>100

(c)

No cells



(d)

Allogeneic PBMC



Figure 3.9 (cont.)

(e)

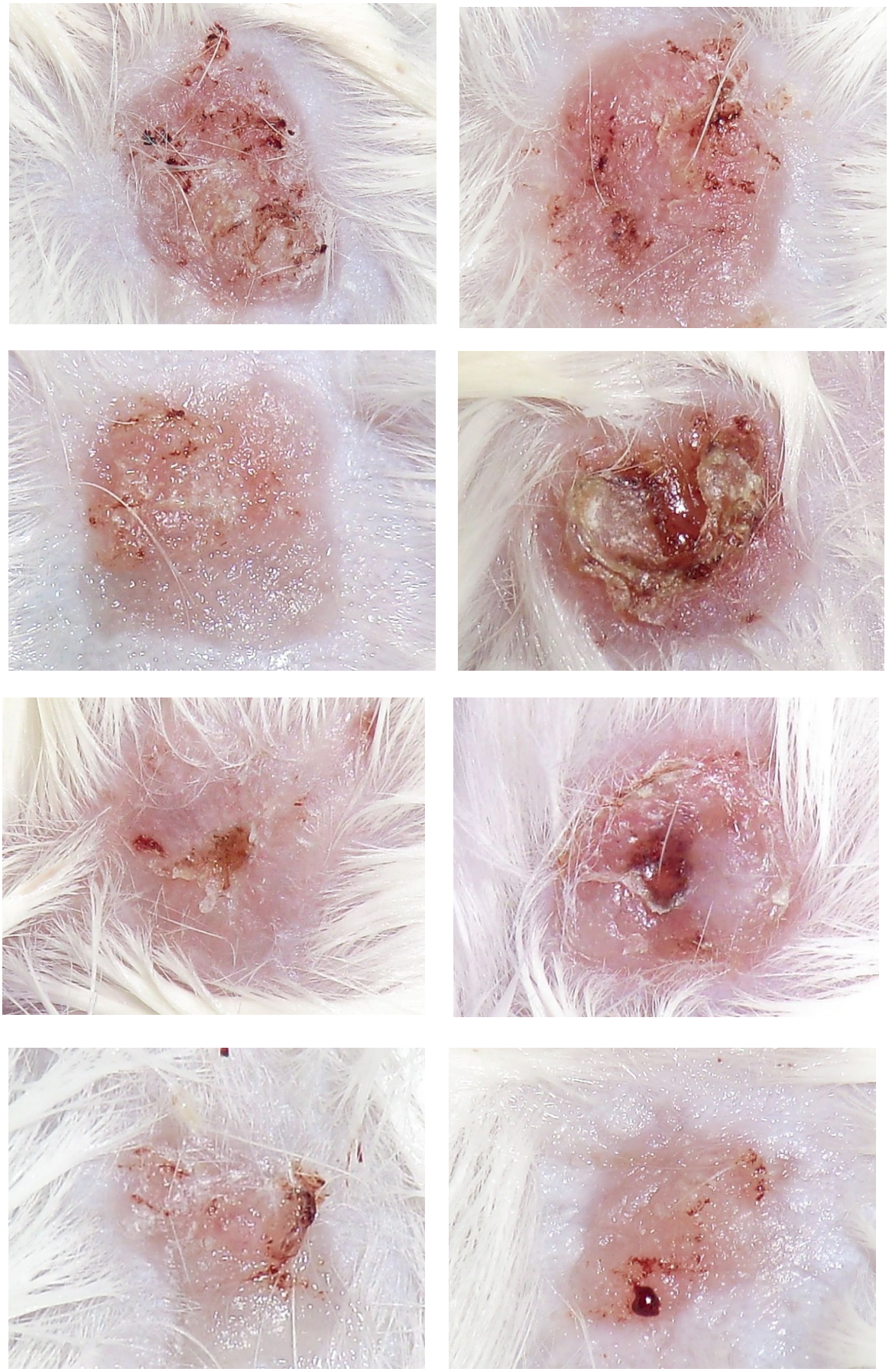


Figure 3.10

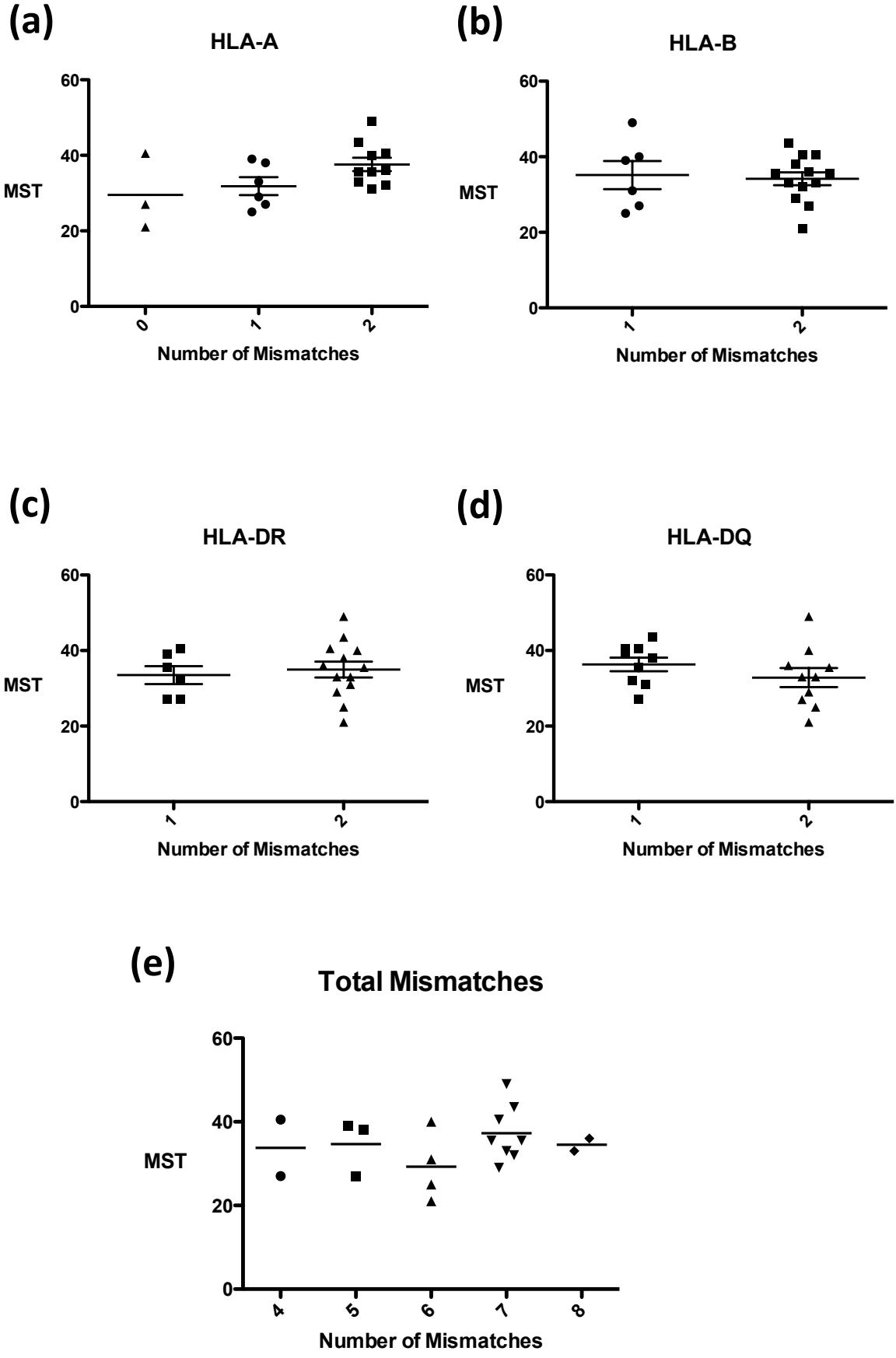
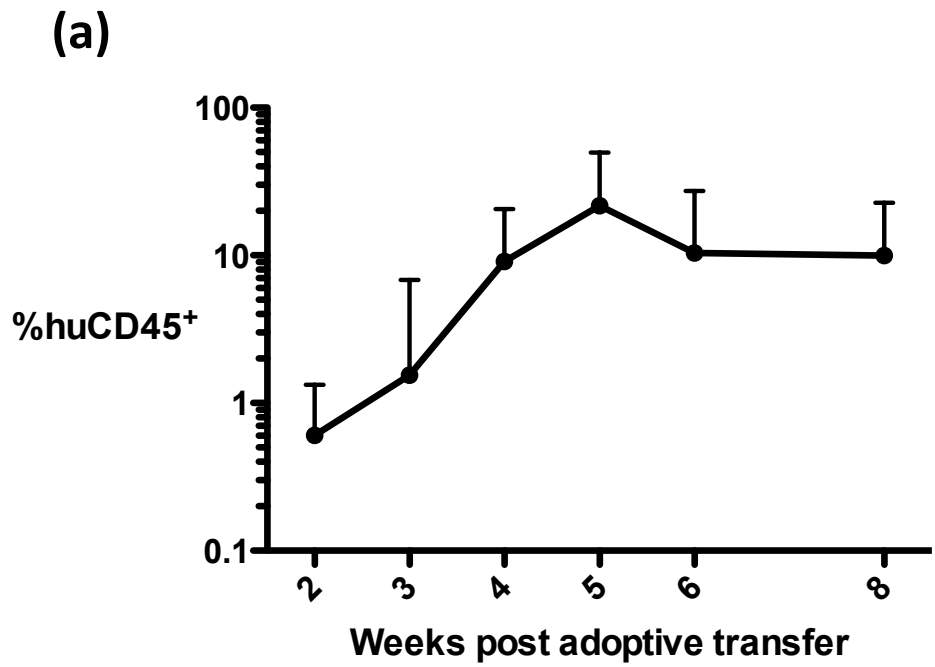

(a)
HLA-A
MST
60
40
20
0
0
1
2
Number of Mismatches
(b)
HLA-B
MST
60
40
20
0
1
2
Number of Mismatches
(c)
HLA-DR
MST
60
40
20
0
1
2
Number of Mismatches
(d)
HLA-DQ
MST
60
40
20
0
1
2
Number of Mismatches
(e)
Total Mismatches
MST
60
40
20
0
4
5
6
7
8
Number of Mismatches

Figure 3.11


(a)
100
10
1
0.1
%huCD45+
2
3
4
5
6
8
Weeks post adoptive transfer

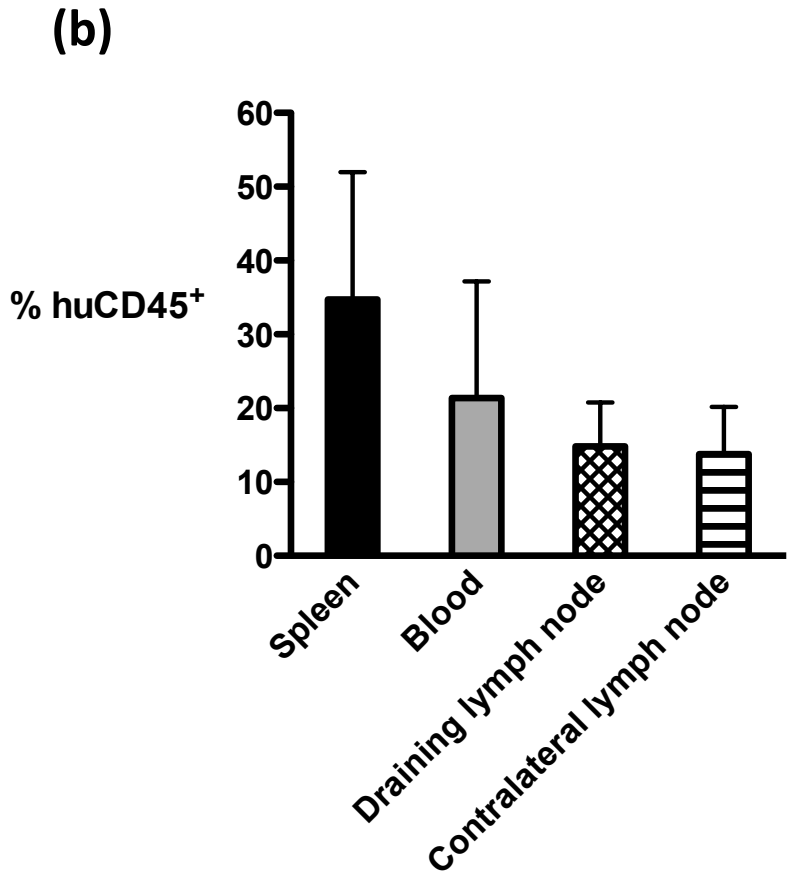
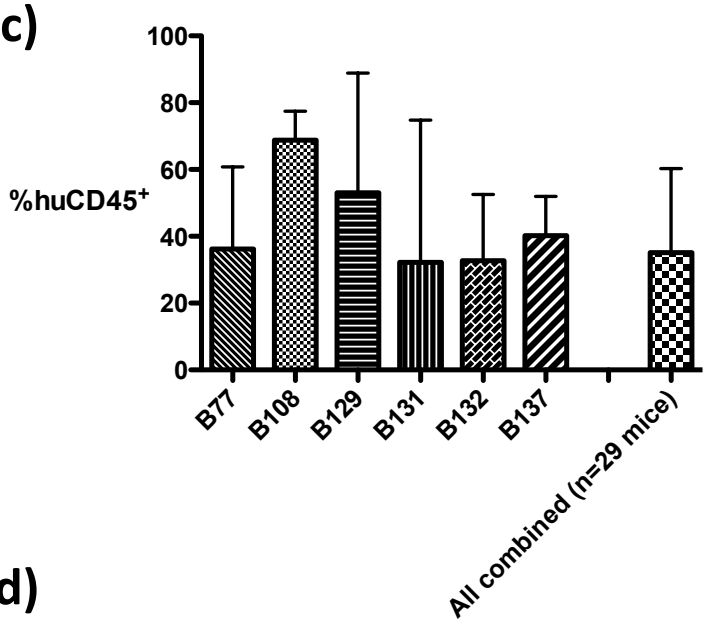
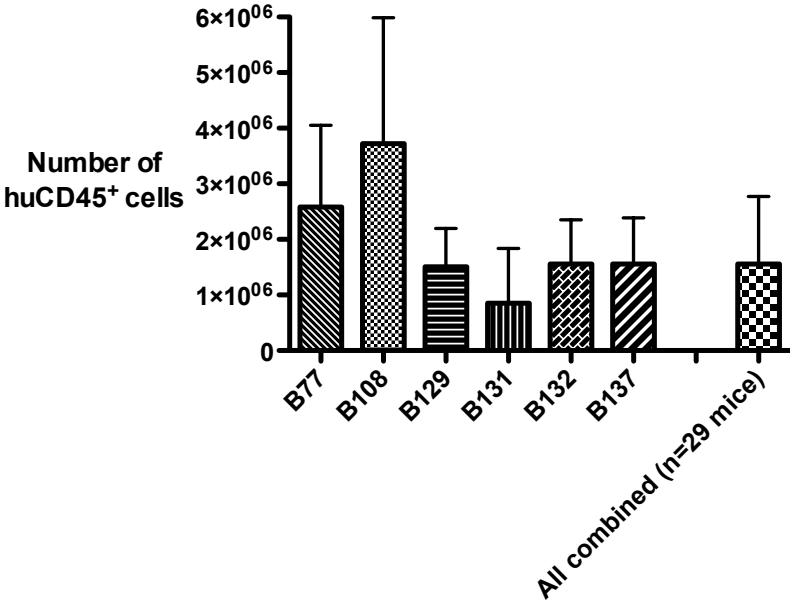

(b)
60
50
40
30
20
10
0
% huCD45+
Spleen
Blood
Draining lymph node
Contralateral lymph node

Figure 3.11 cont.

(c)



(d)



(e)

PBMC donor	MST (days)	HLA mismatches
B77	41	7
B108	31	6
B129	35.5	8
B131	35.5	7
B132	33	7
B137	27	5

Figure 3.12

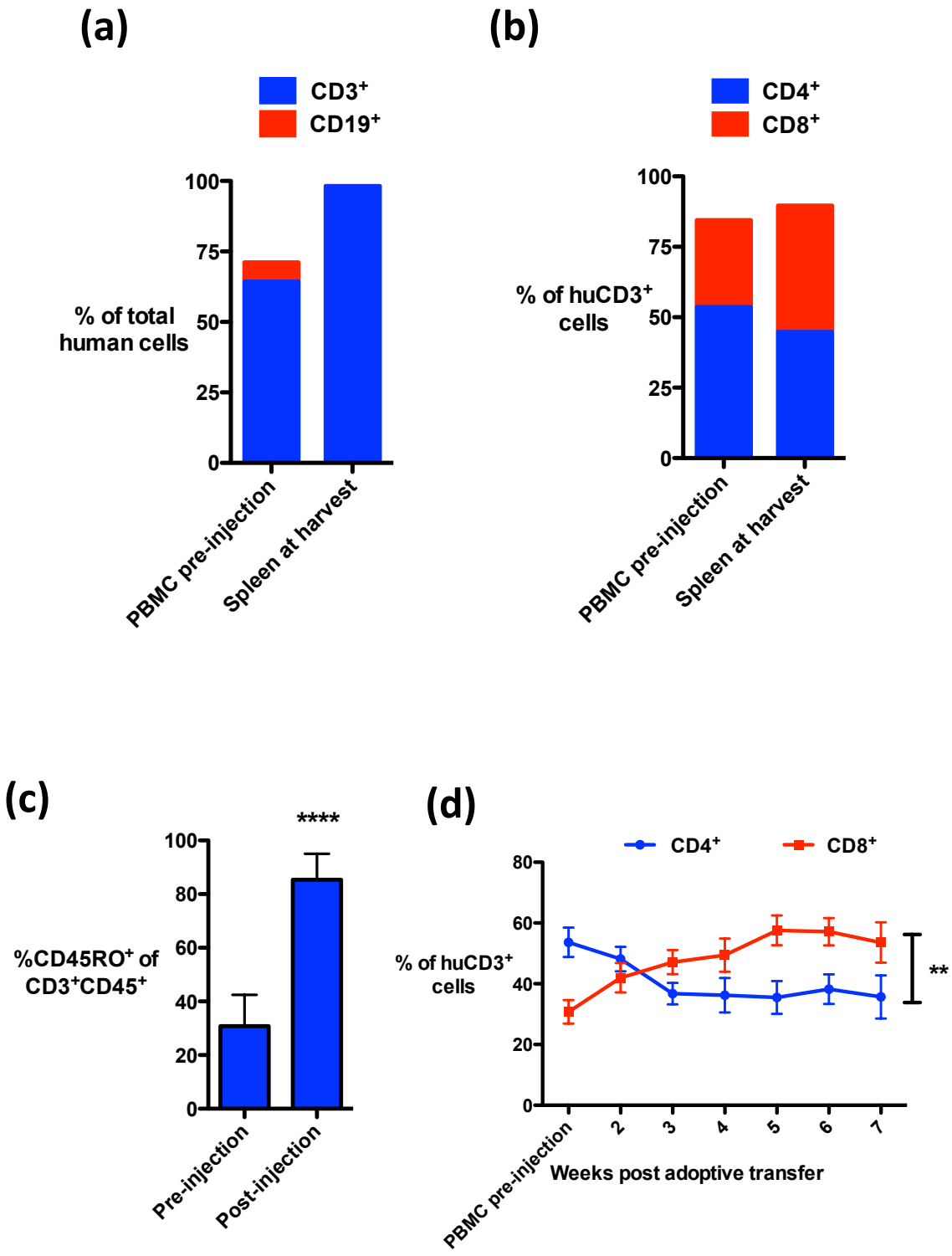

(a)
CD3+
CD19+
100
75
50
25
0
% of total human cells
PBMC pre-injection
Spleen at harvest
(b)
CD4+
CD8+
100
75
50
25
0
% of huCD3+ cells
PBMC pre-injection
Spleen at harvest
(c)
****
100
80
60
40
20
0
%CD45RO+ of CD3+CD45+
Pre-injection
Post-injection
(d)
CD4+
CD8+
80
60
40
20
0
% of huCD3+ cells
**
PBMC pre-injection
2
3
4
5
6
7
Weeks post adoptive transfer

Figure 3.13

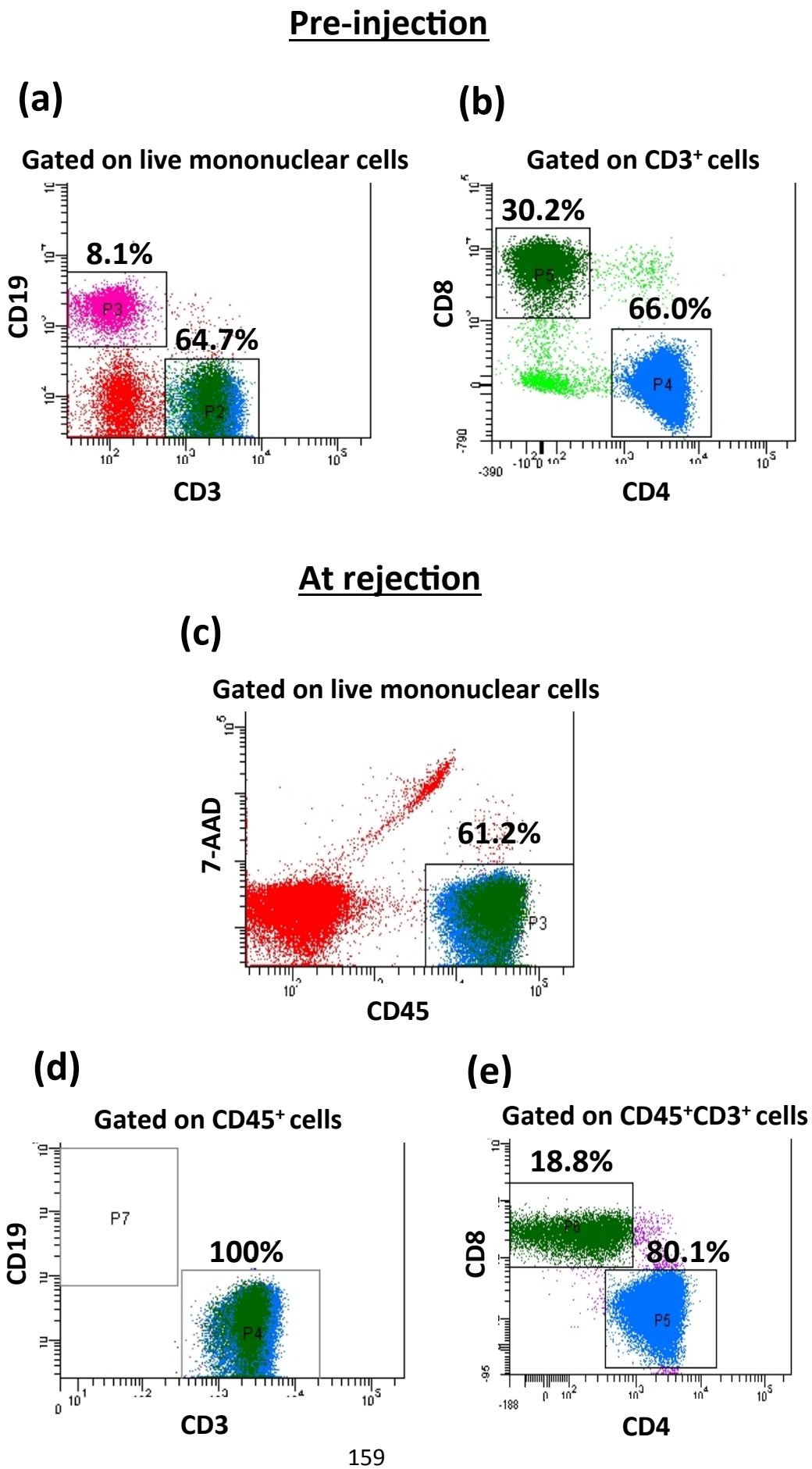

Pre-injection
(a)
Gated on live mononuclear cells
8.1%
64.7%
P3
P2
CD19
CD3
(b)
Gated on CD3+ cells
30.2%
66.0%
P5
P4
CD8
CD4
At rejection
(c)
Gated on live mononuclear cells
61.2%
P3
7-AAD
CD45
(d)
Gated on CD45+ cells
P7
100%
P4
CD19
CD3
(e)
Gated on CD45+CD3+ cells
18.8%
80.1%
P5
CD8
CD4

Figure 4.1

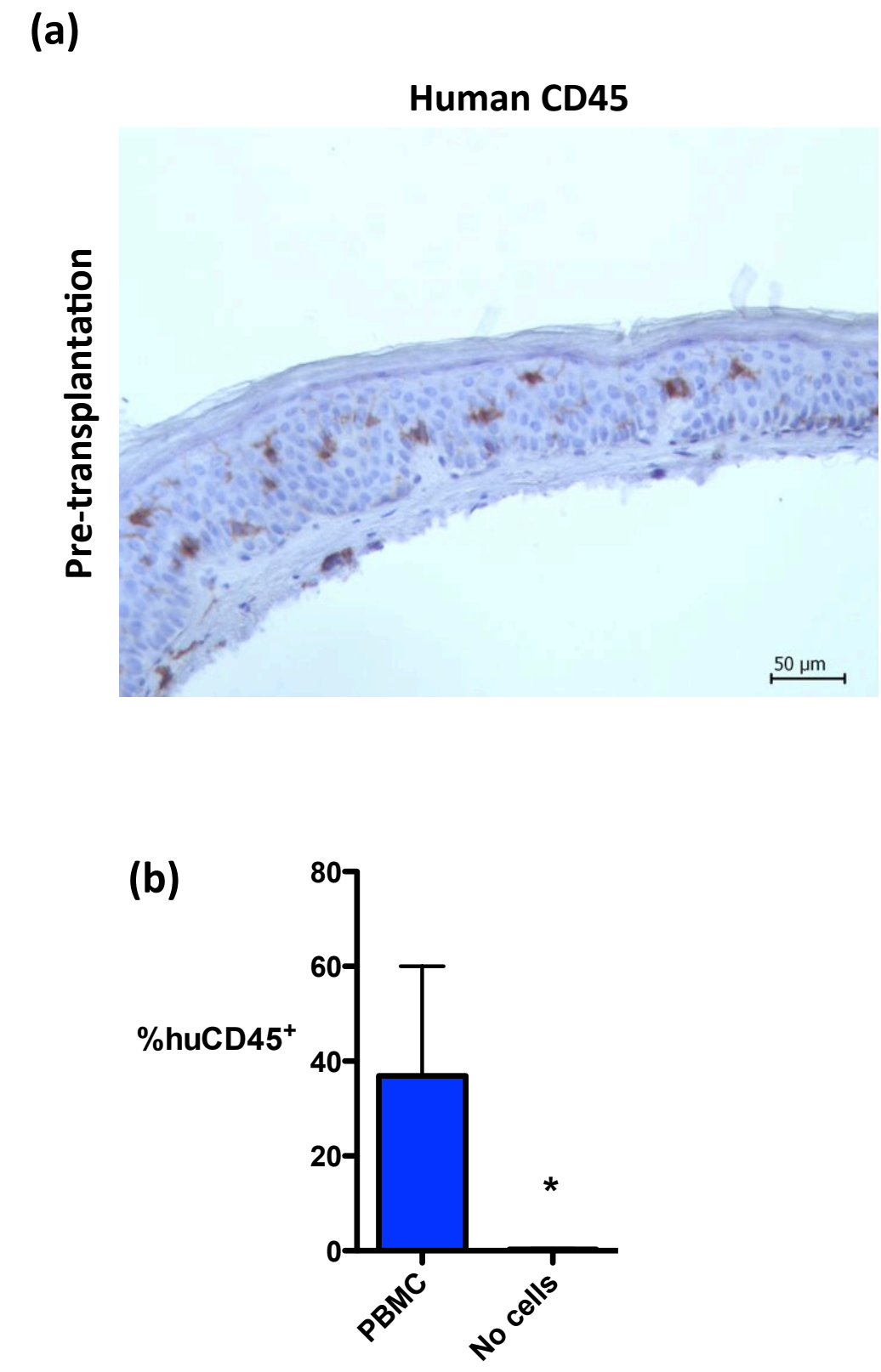

(a)
Human CD45
Pre-transplantation
50 µm
(b)
%huCD45+
80
60
40
20
0
*
PBMC
No cells

Figure 4.2

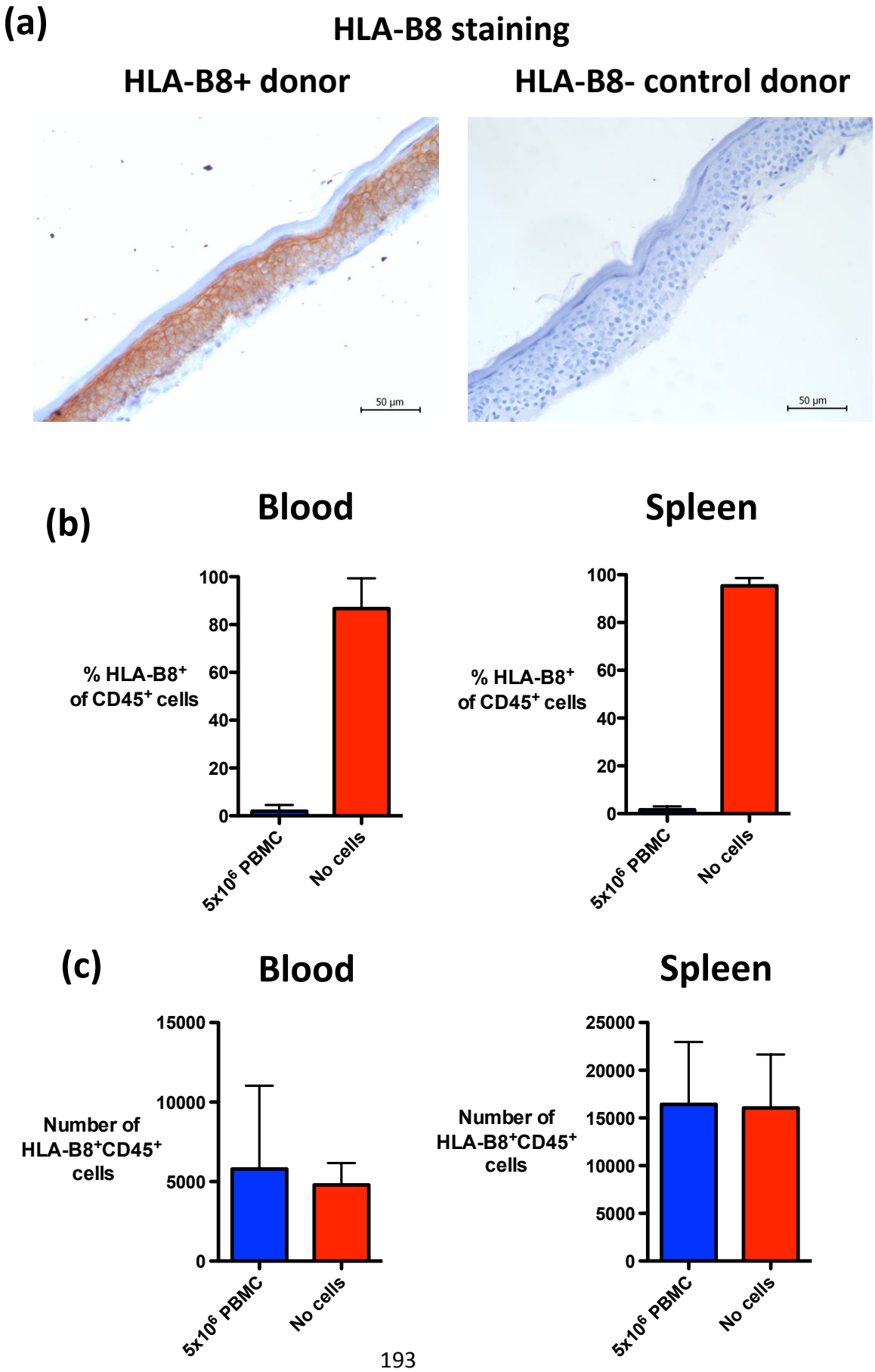
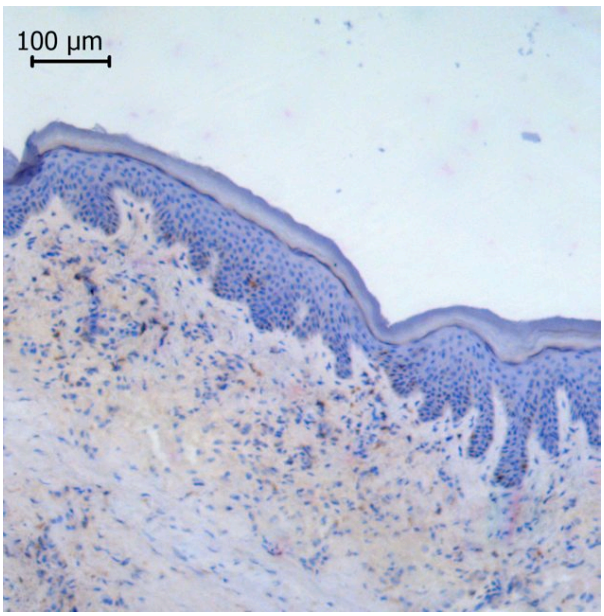
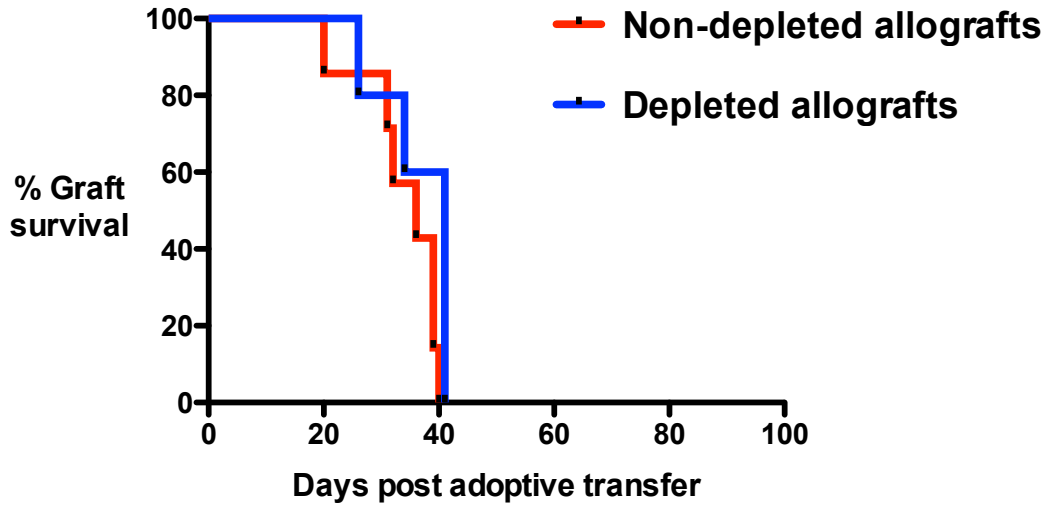

(a)
HLA-B8 staining
HLA-B8+ donor
HLA-B8- control donor
50 µm
50 µm
(b)
Blood
Spleen
% HLA-B8+ of CD45+ cells
100
80
60
40
20
0
5x10^6 PBMC
No cells
(c)
Blood
Spleen
Number of HLA-B8+CD45+ cells
15000
10000
5000
0
25000
20000
5x10^6 PBMC
No cells

Figure 4.2 cont.

(d)



(e)



(f)

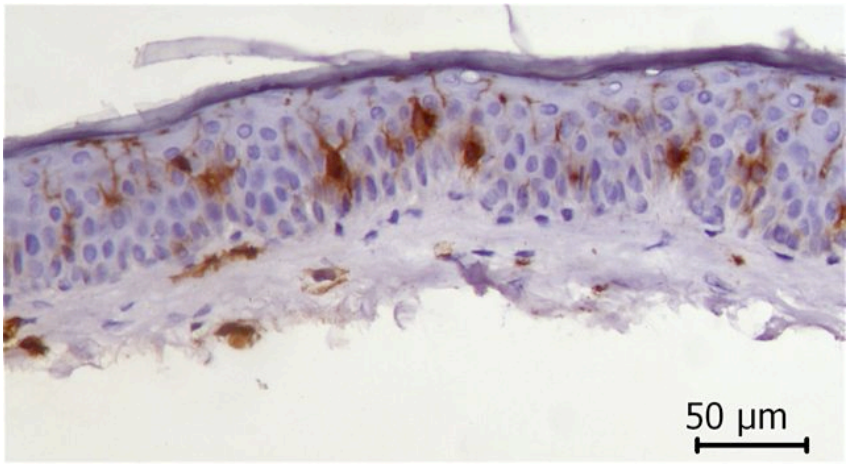
Group	Group size	Graft loss days	MST (days)
Non-depleted allografts	7	20, 31, 32, 36, 39, 39, 40	36
Depleted allografts	5	26, 34, 41, 41, 41	41

Figure 4.3

HLA Class II Expression

(a)

Pre-transplantation: HLA-DR antibody



(b)

Pre-transplantation: Control antibody

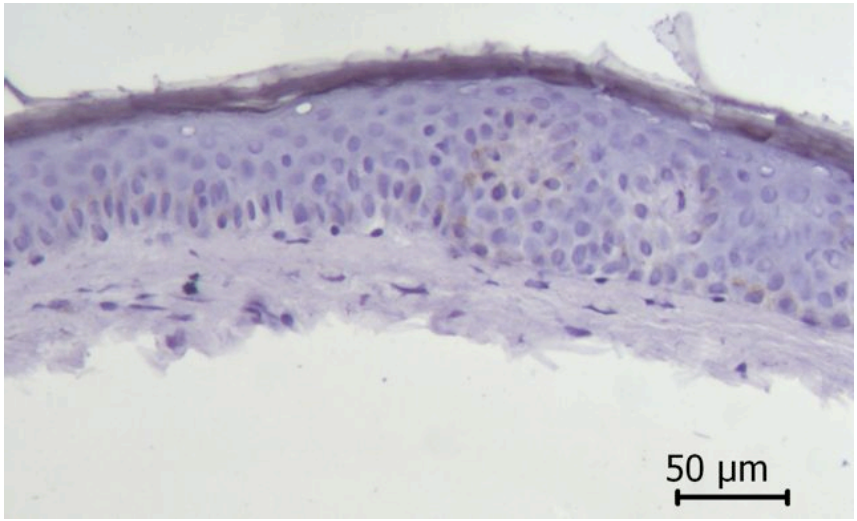
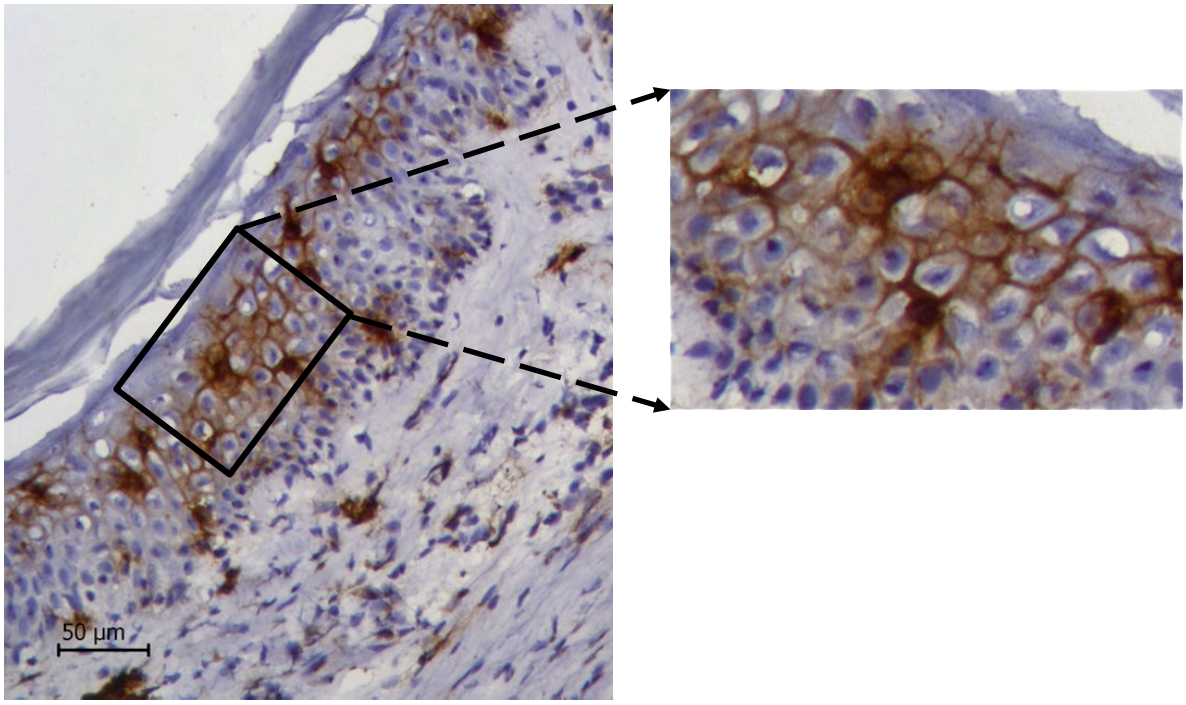


Figure 4.3 cont.

(c)

21 days after injection of medium only



(d)

21 days after injection of $5x10^6$ PBMC

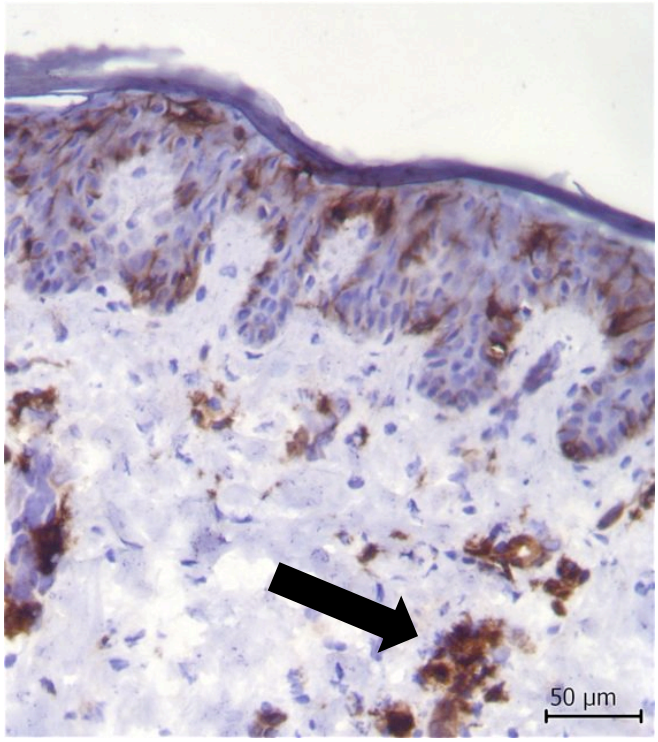
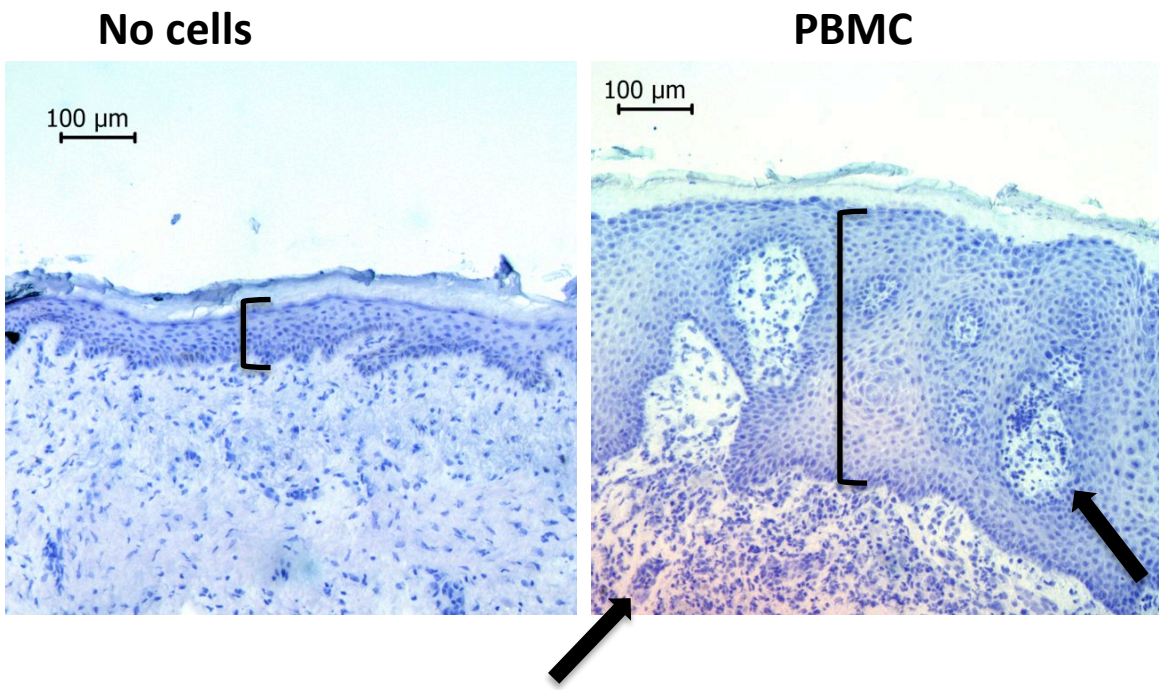


Figure 4.4

(a)



(b)

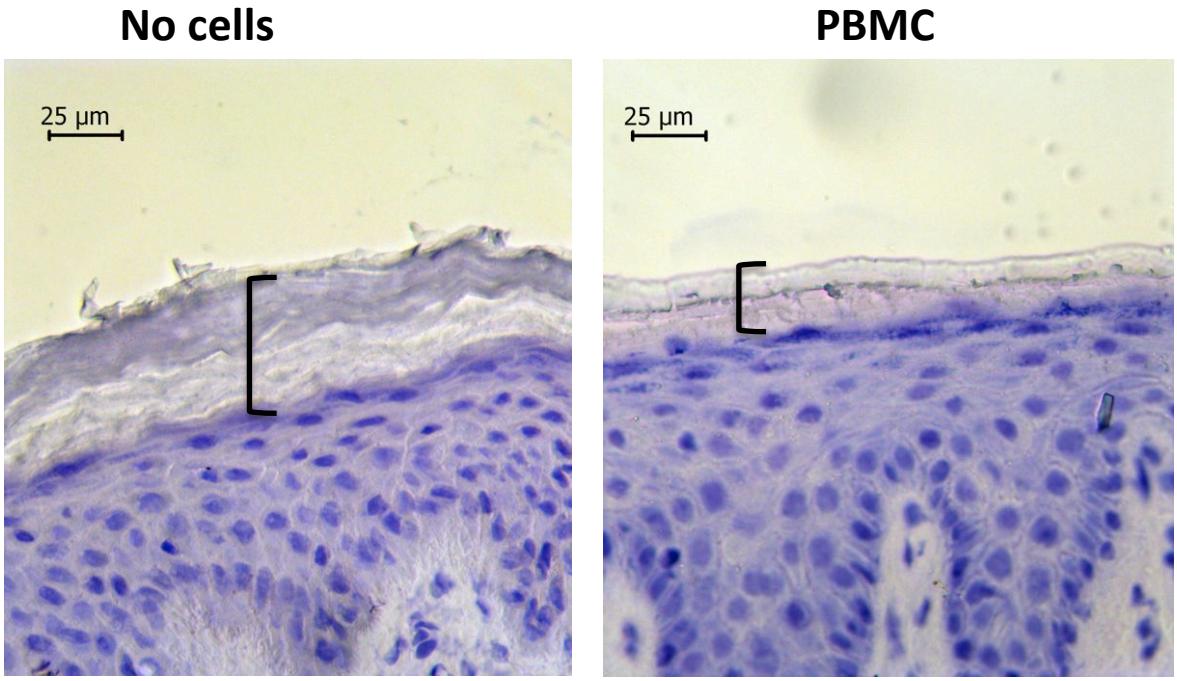
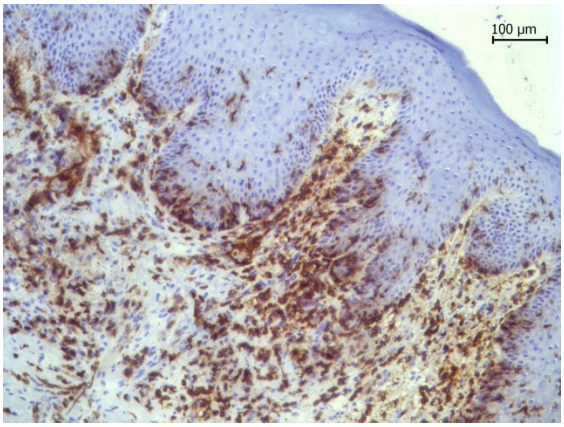
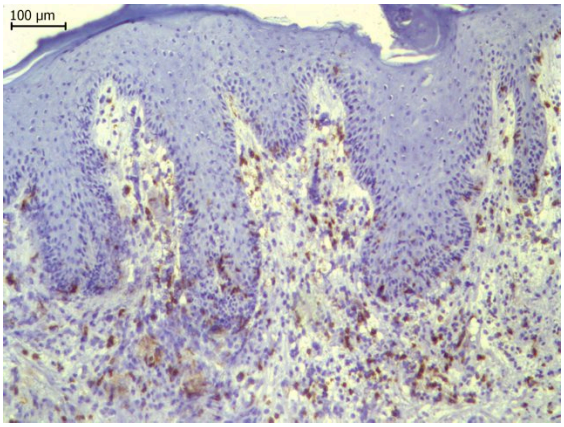


Figure 4.5

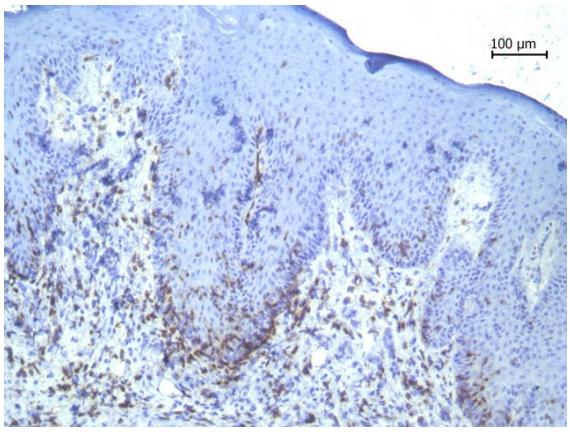
(a) huCD45



(b) huCD4



(c) huCD8



(d) huCD45 (No PBMC)

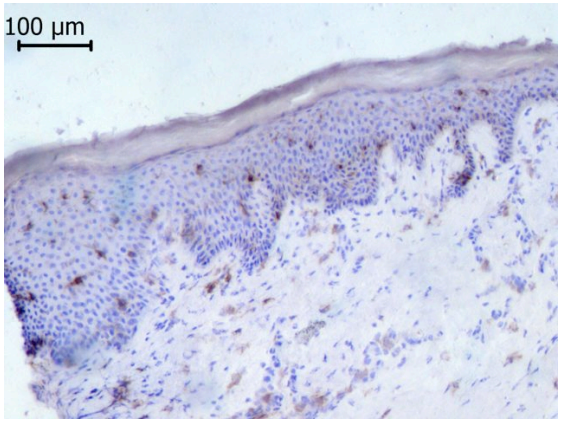


Figure 4.5 (cont.)

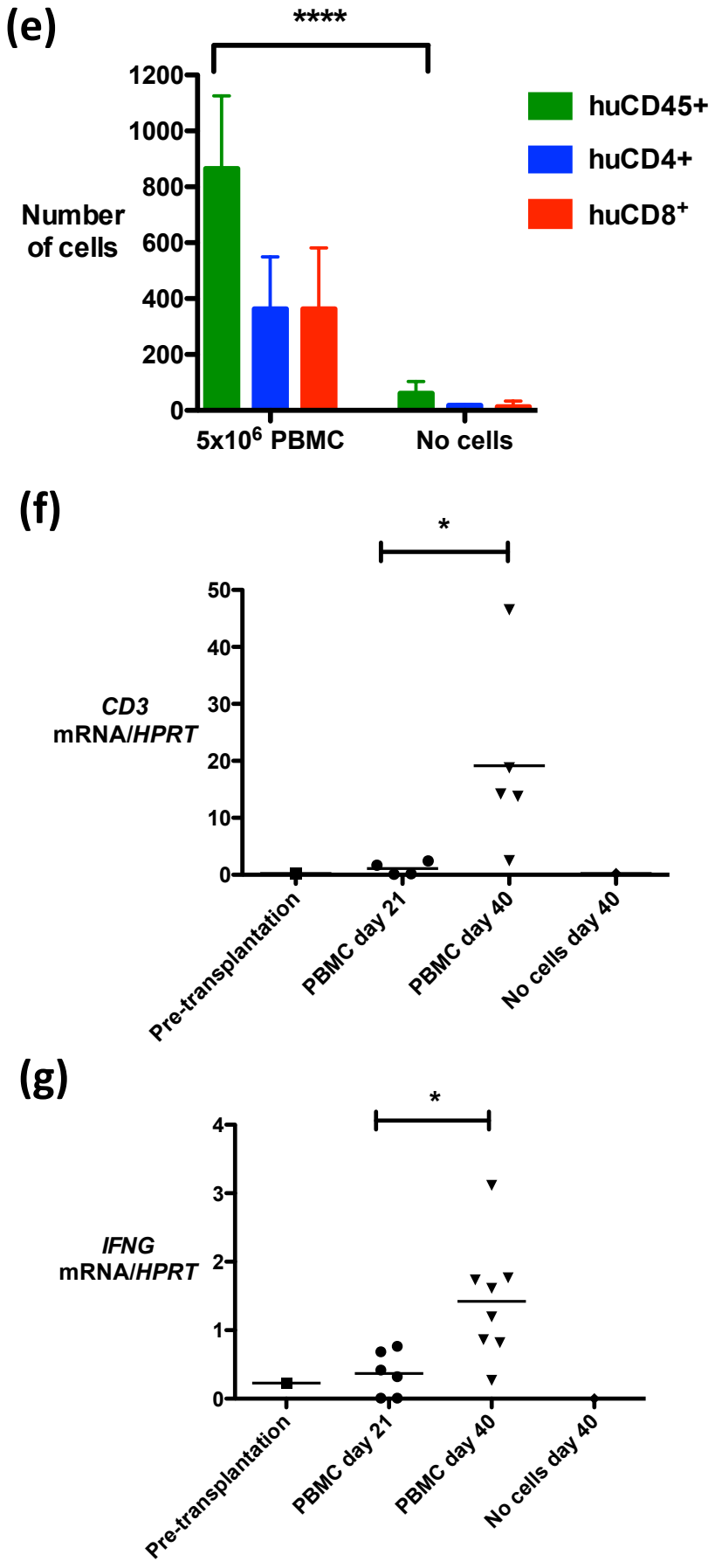
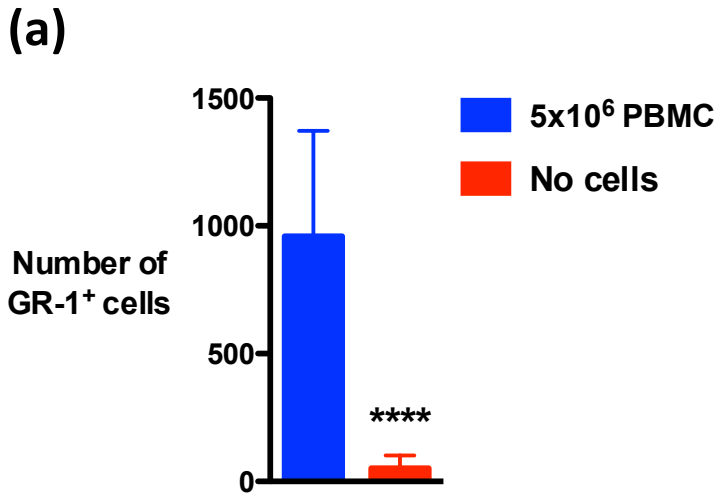
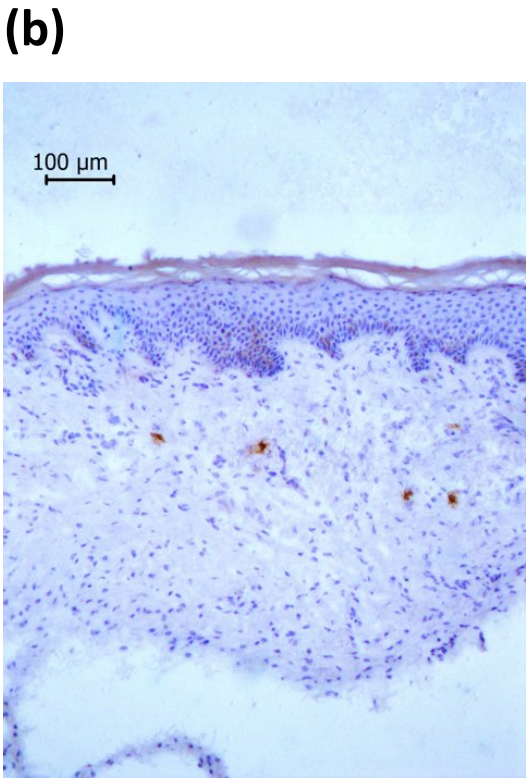


Figure 4.6


(a)
1500
1000
500
0
Number of
GR-1+ cells
5x10^6 PBMC
No cells
****


(b)
100 µm

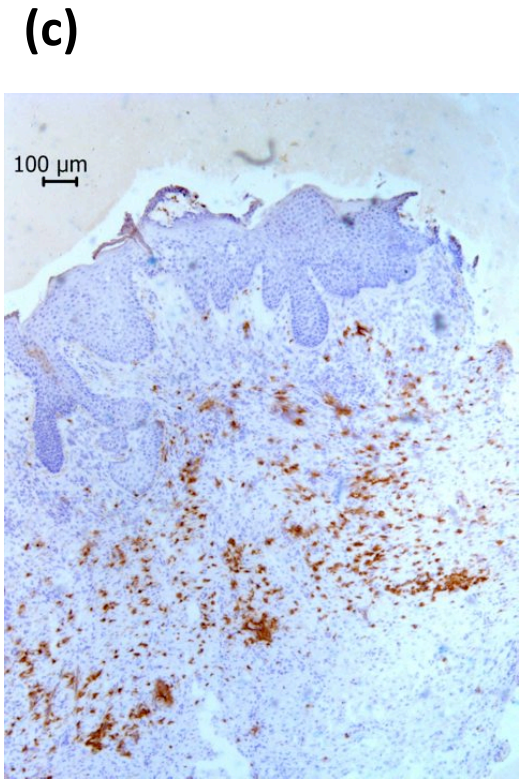

(c)
100 µm

Figure 4.7

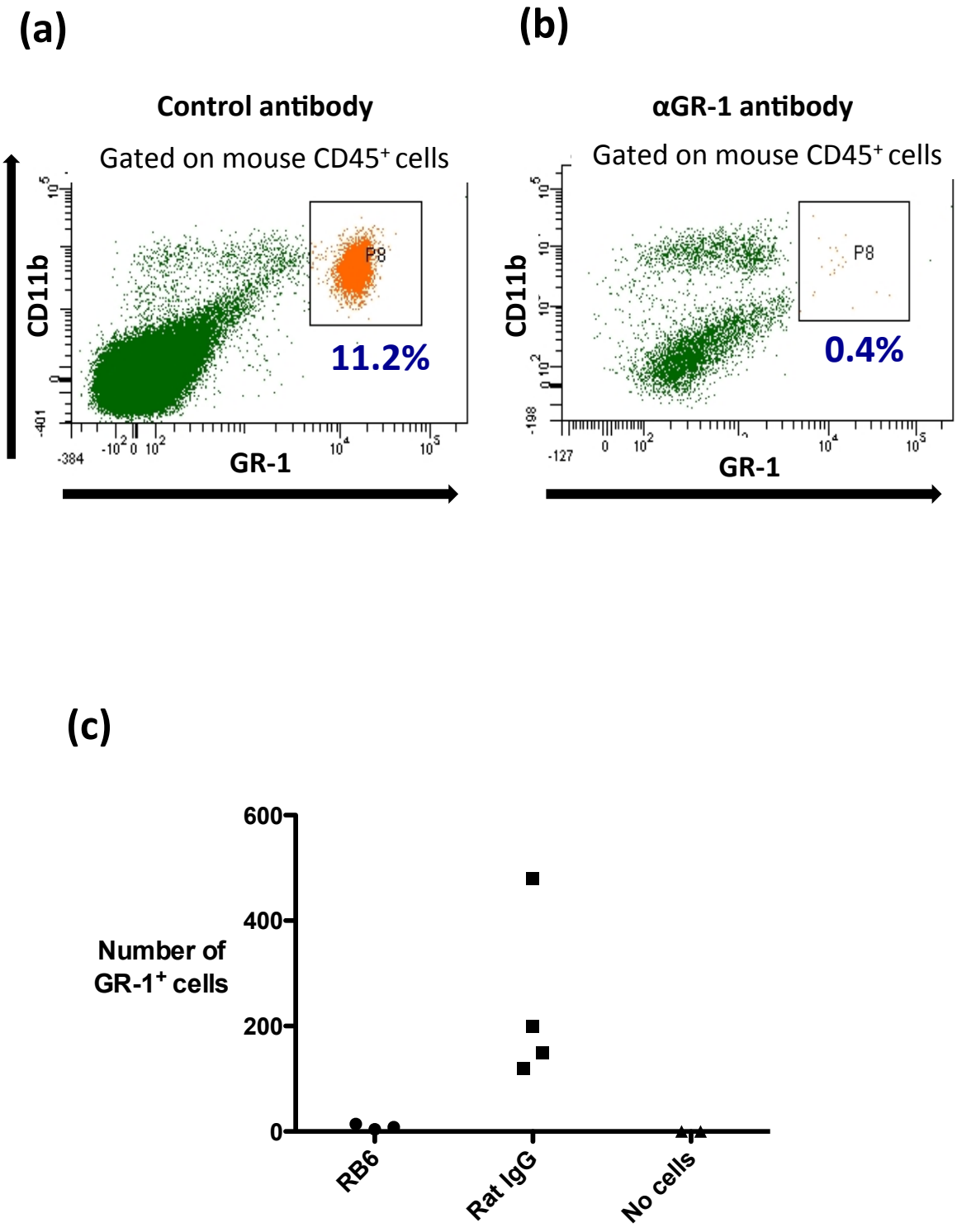
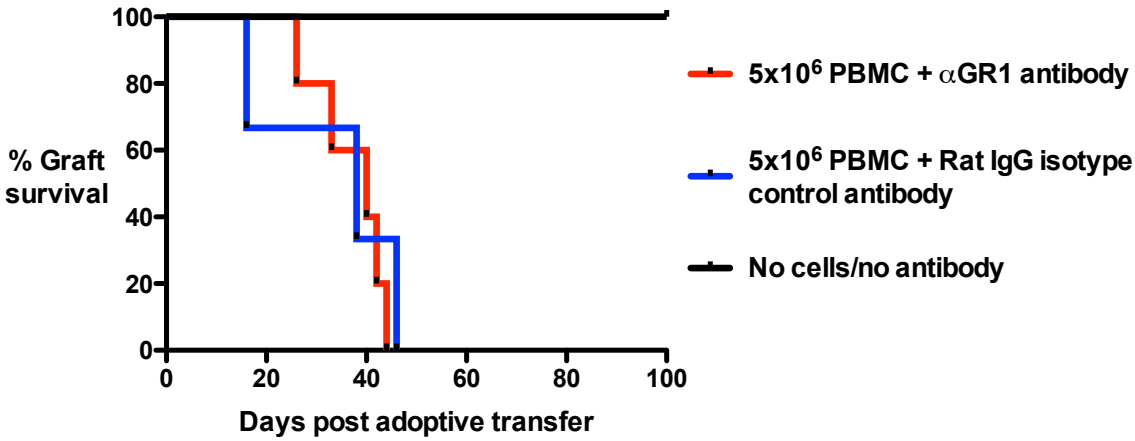

(a)
(b)
Control antibody
Gated on mouse CD45+ cells
αGR-1 antibody
Gated on mouse CD45+ cells
CD11b
GR-1
P8
11.2%
0.4%
(c)
Number of GR-1+ cells
600
400
200
0
RB6
Rat IgG
No cells

Figure 4.7 (cont.)

(d)

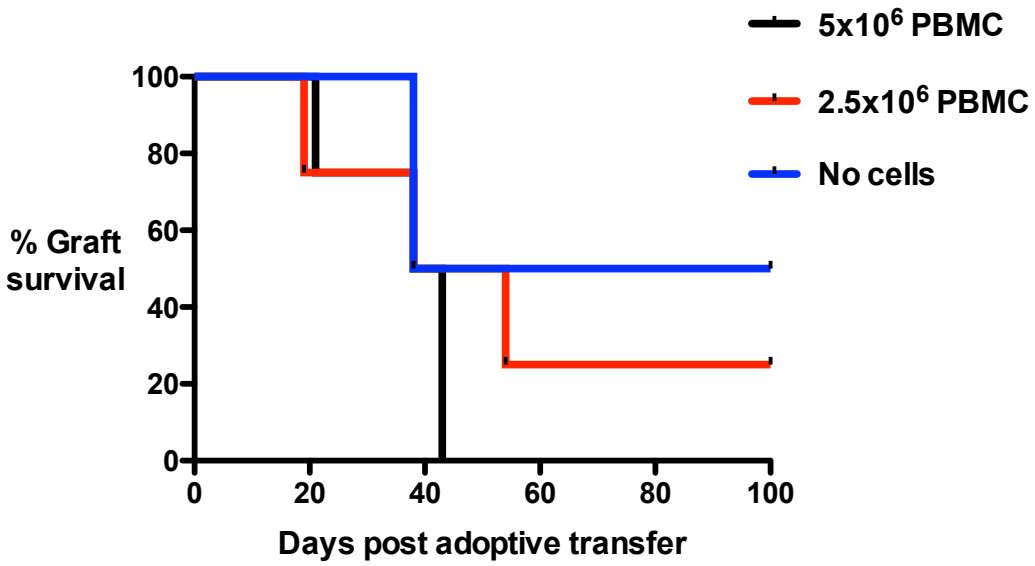


(e)

Group	Group size	Graft loss days	MST (days)
5x10^6 PBMC + αGR-1 antibody	5	26, 33, 40, 42, 44	40
5x10^6 PBMC + Rat IgG isotype control antibody	3	16, 38, 46	38
No cells, no antibody	2	>100, >100	>100

Figure 4.8

(a)

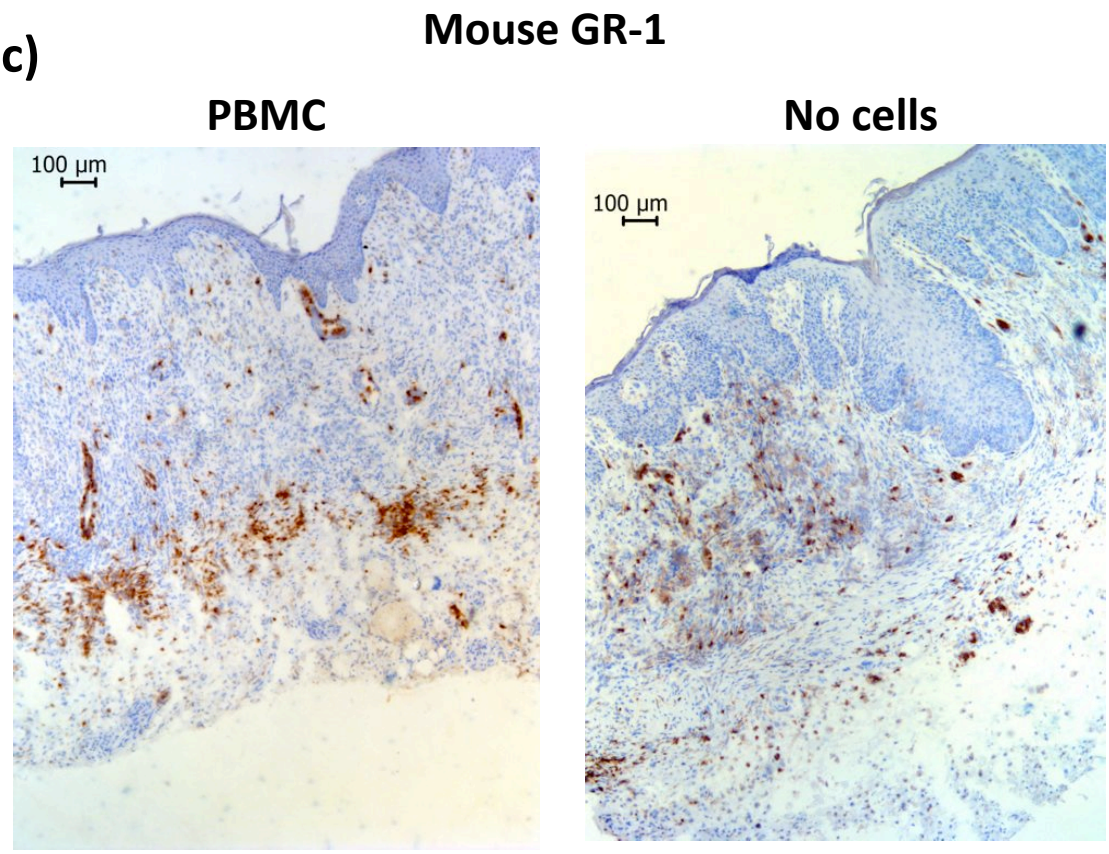


(b)

Group	Group size	Graft loss days	MST (days)
$5x10^6$ PBMC	4	21, 38, 43, 43	40.5
$2.5x10^6$ PBMC	4	19, 38, 54, >100	46
No cells	2	38, >100	69

Figure 4.8 (cont.)

(c)



(d)

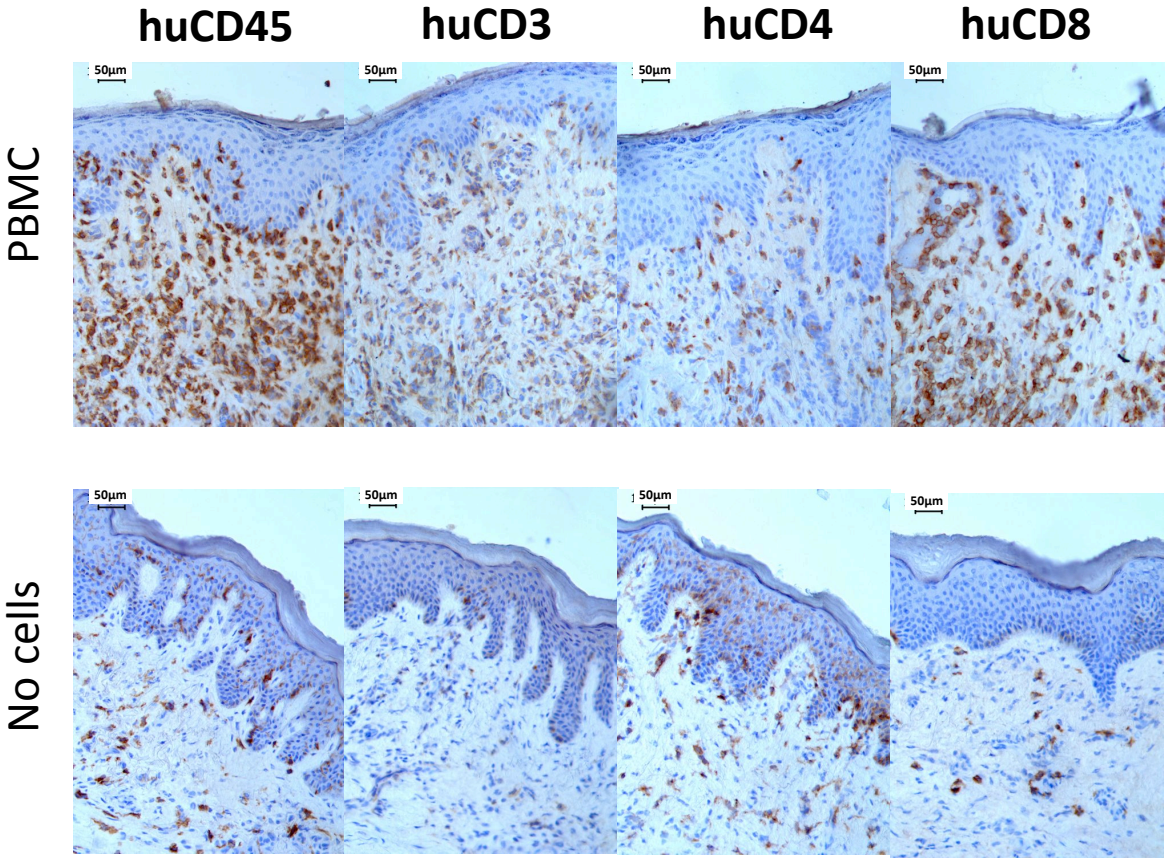
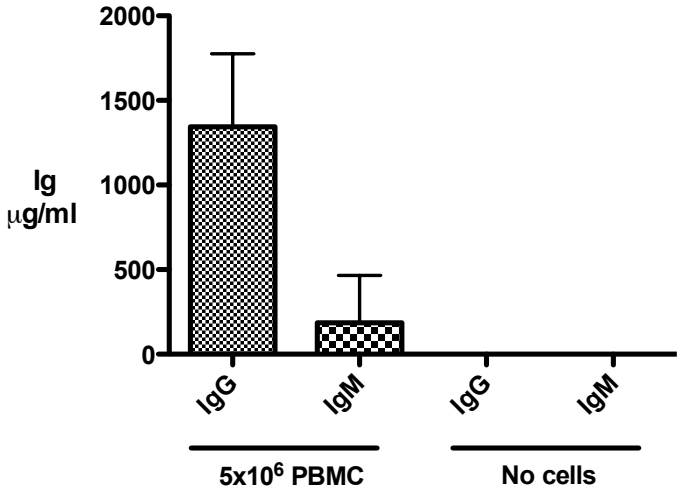
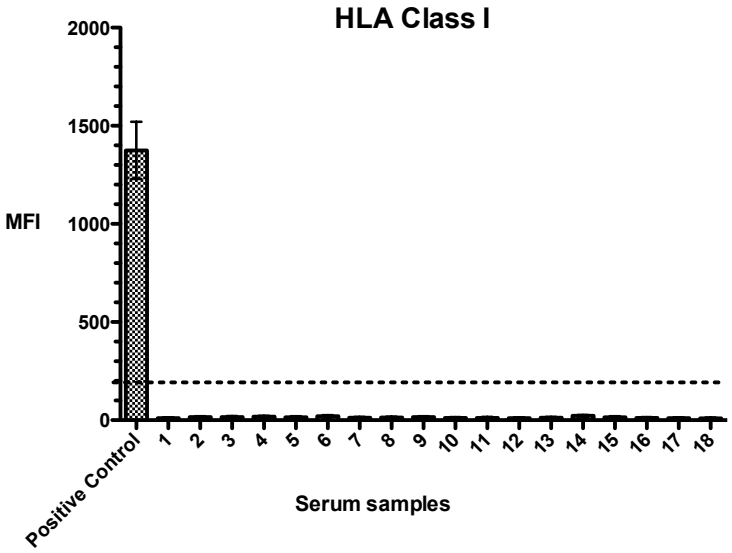


Figure 4.9

(a)



(b)



(c)

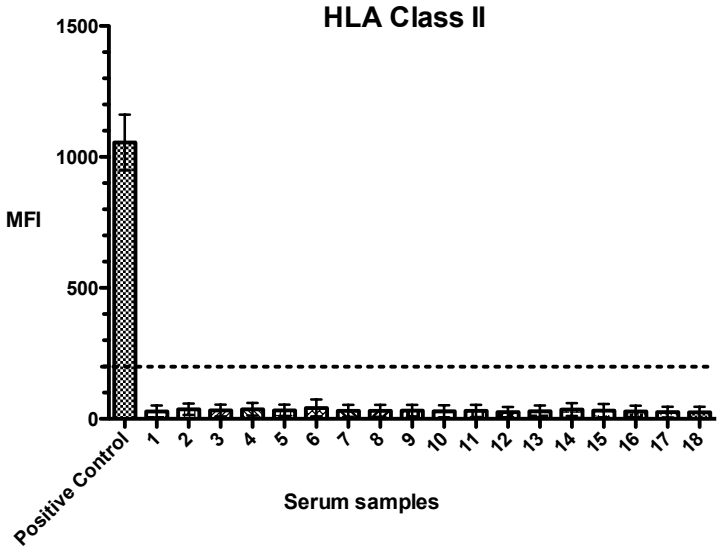
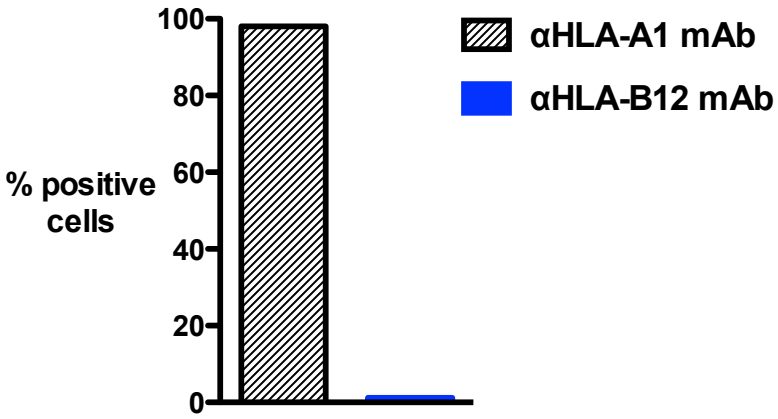


Figure 4.10

(a)



(b)

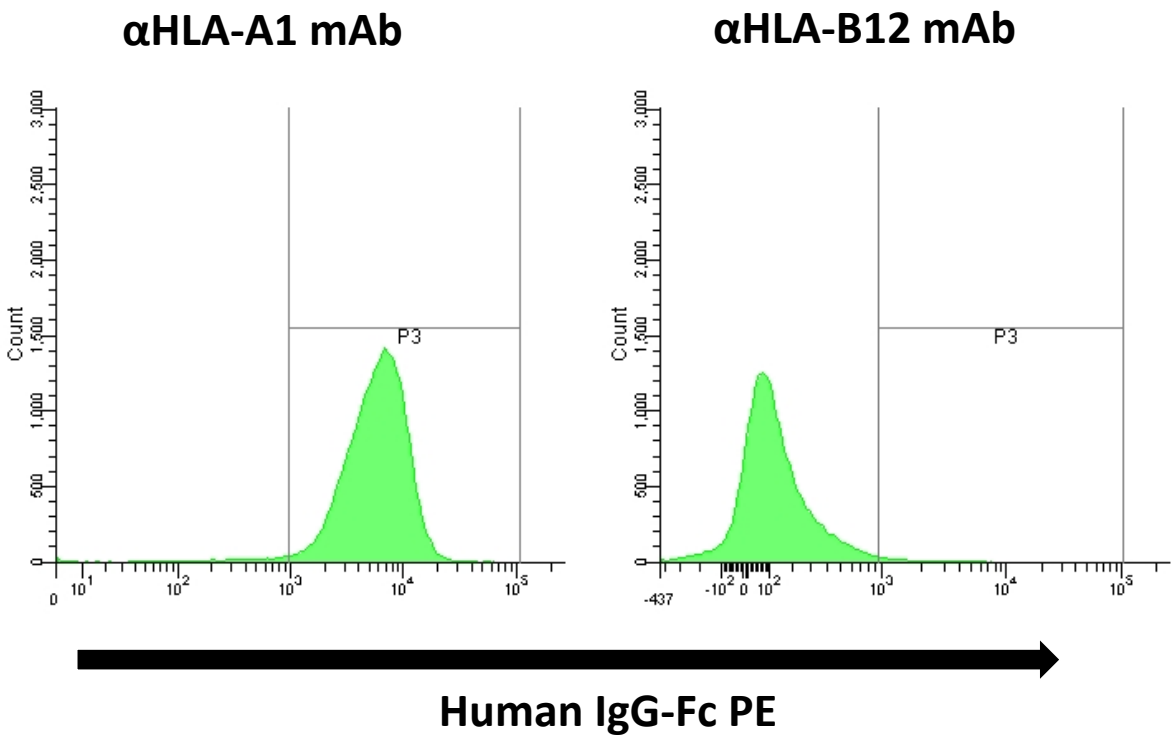
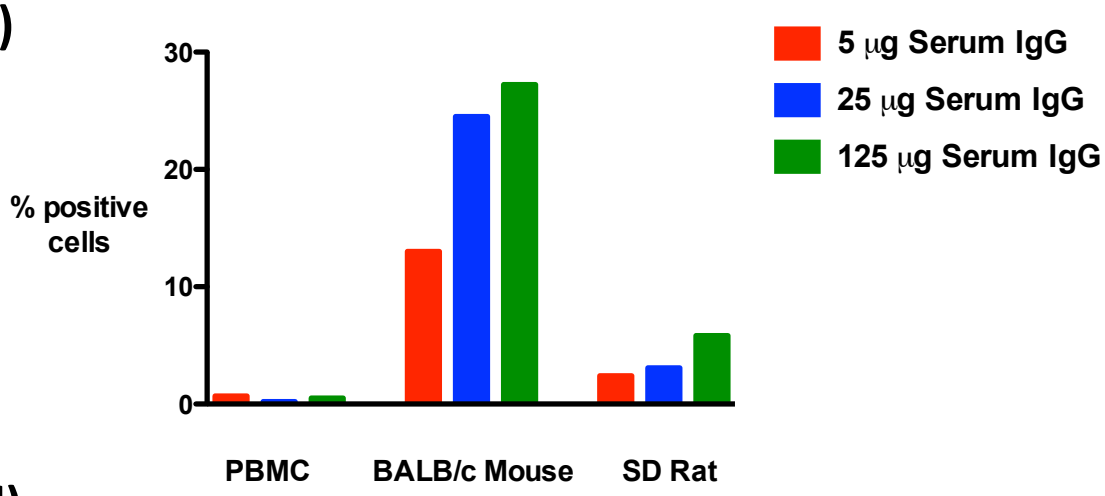


Figure 4.10 (cont.)

(c)



(d)

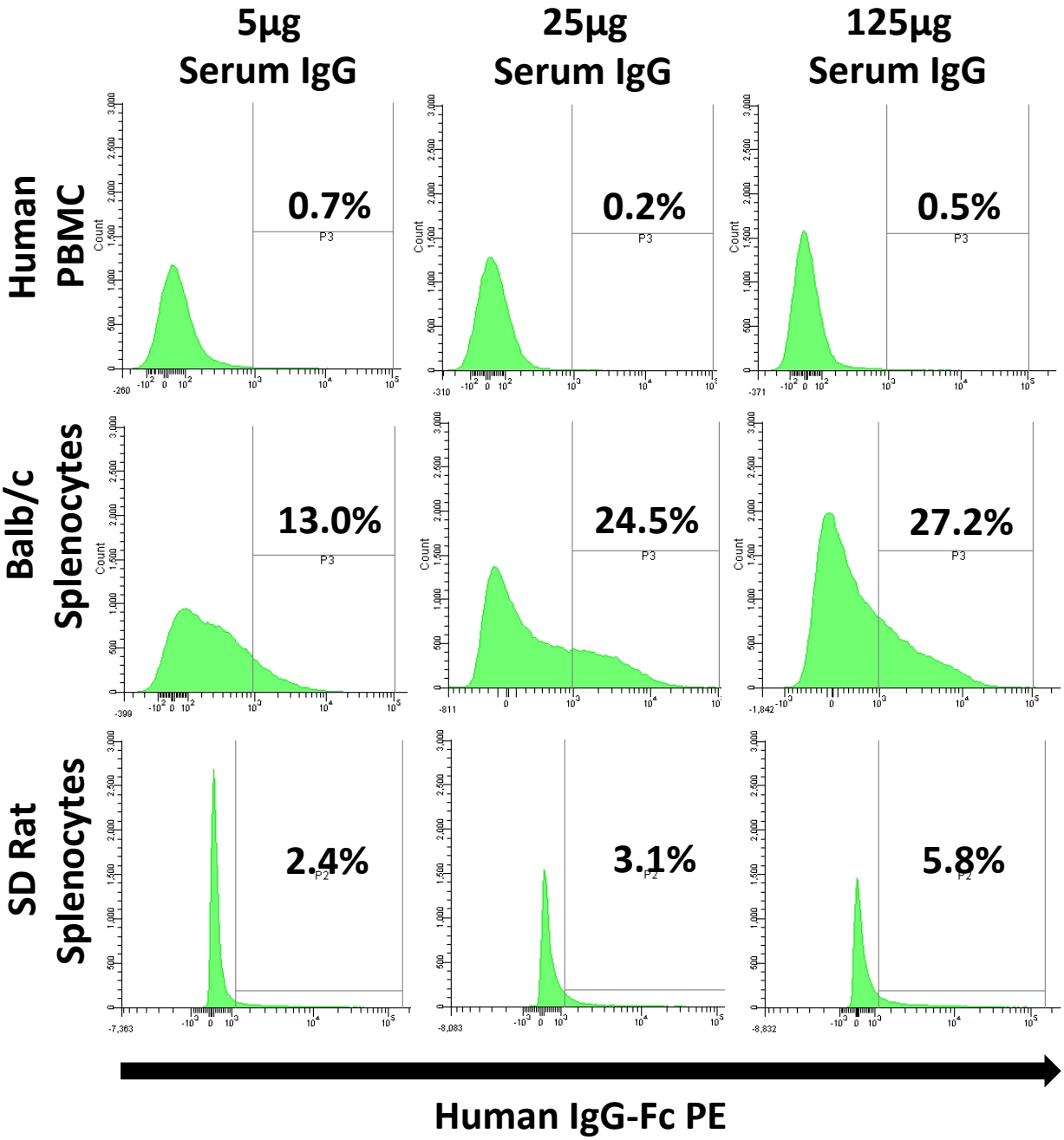
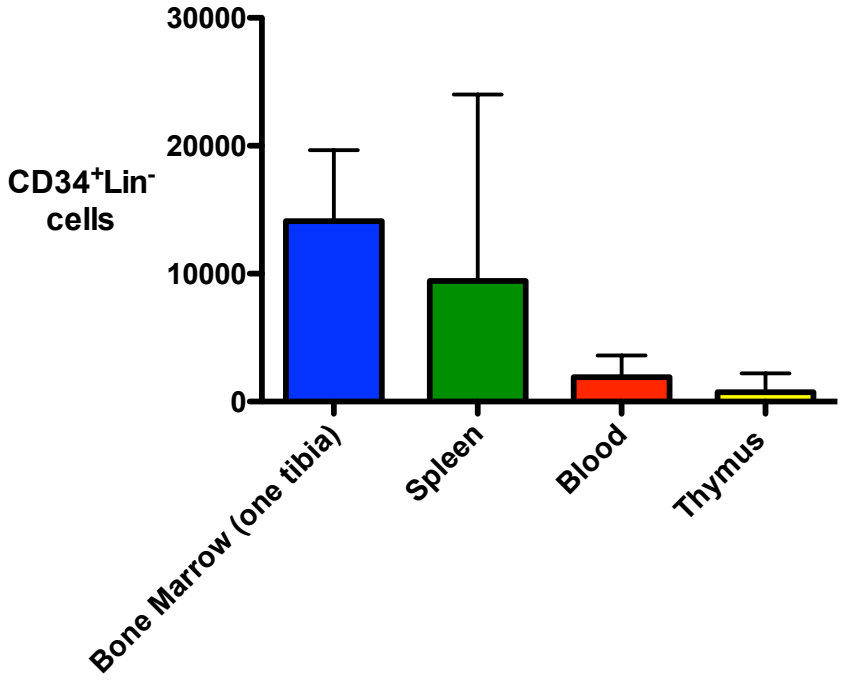


Figure 4.11

(a)



(b)

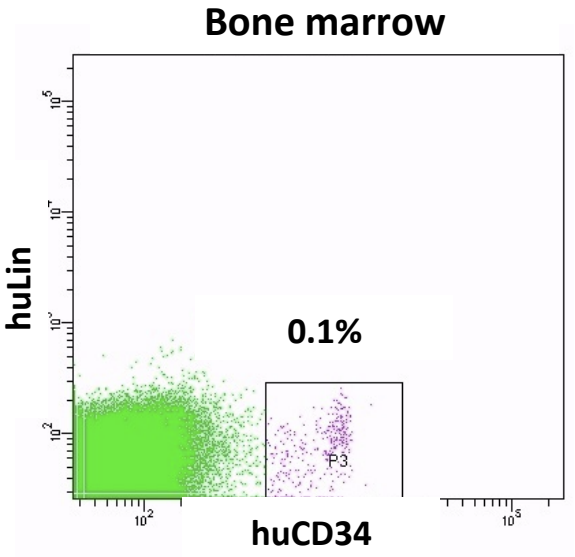
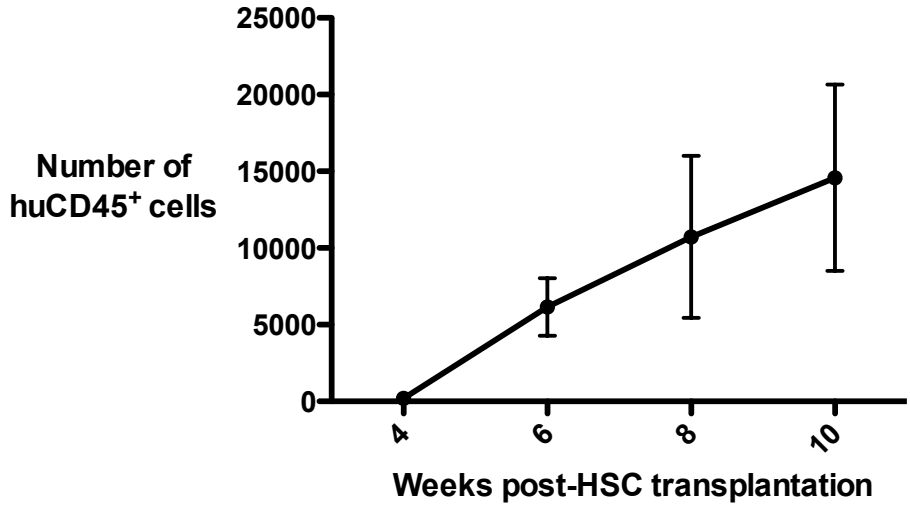


Figure 4.11 cont.

(c)



(d)

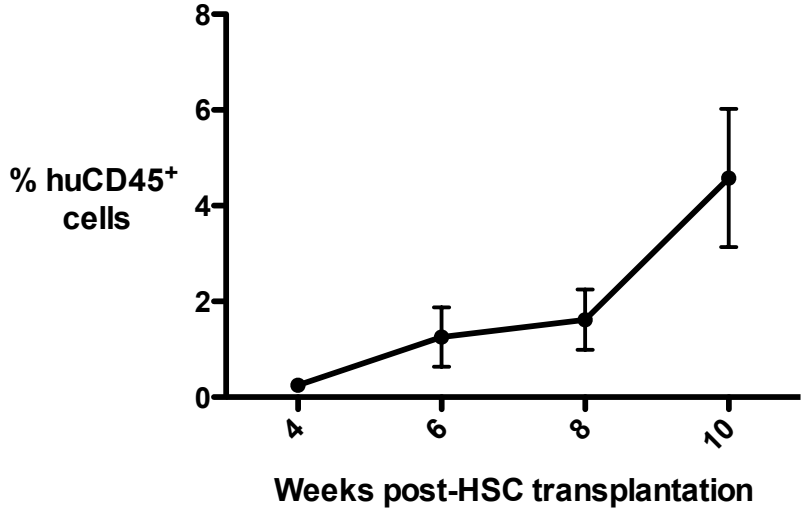


Figure 4.12

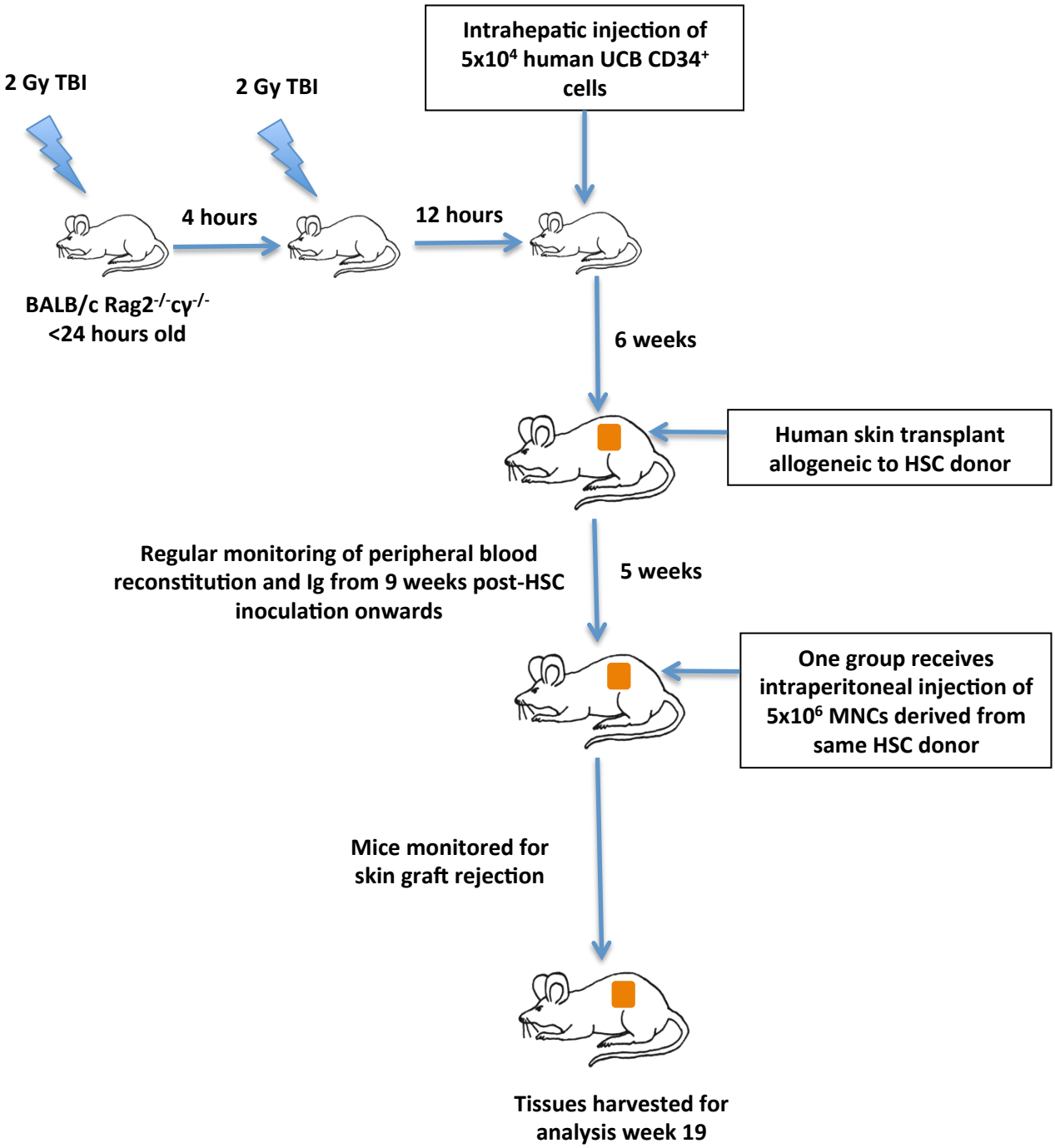

2 Gy TBI
2 Gy TBI
Intrahepatic injection of 5x10⁴ human UCB CD34⁺ cells
4 hours
12 hours
BALB/c Rag2⁻/⁻cγ⁻/⁻
<24 hours old
6 weeks
Human skin transplant allogeneic to HSC donor
Regular monitoring of peripheral blood reconstitution and Ig from 9 weeks post-HSC inoculation onwards
5 weeks
One group receives intraperitoneal injection of 5x10⁶ MNCs derived from same HSC donor
Mice monitored for skin graft rejection
Tissues harvested for analysis week 19

Figure 4.13

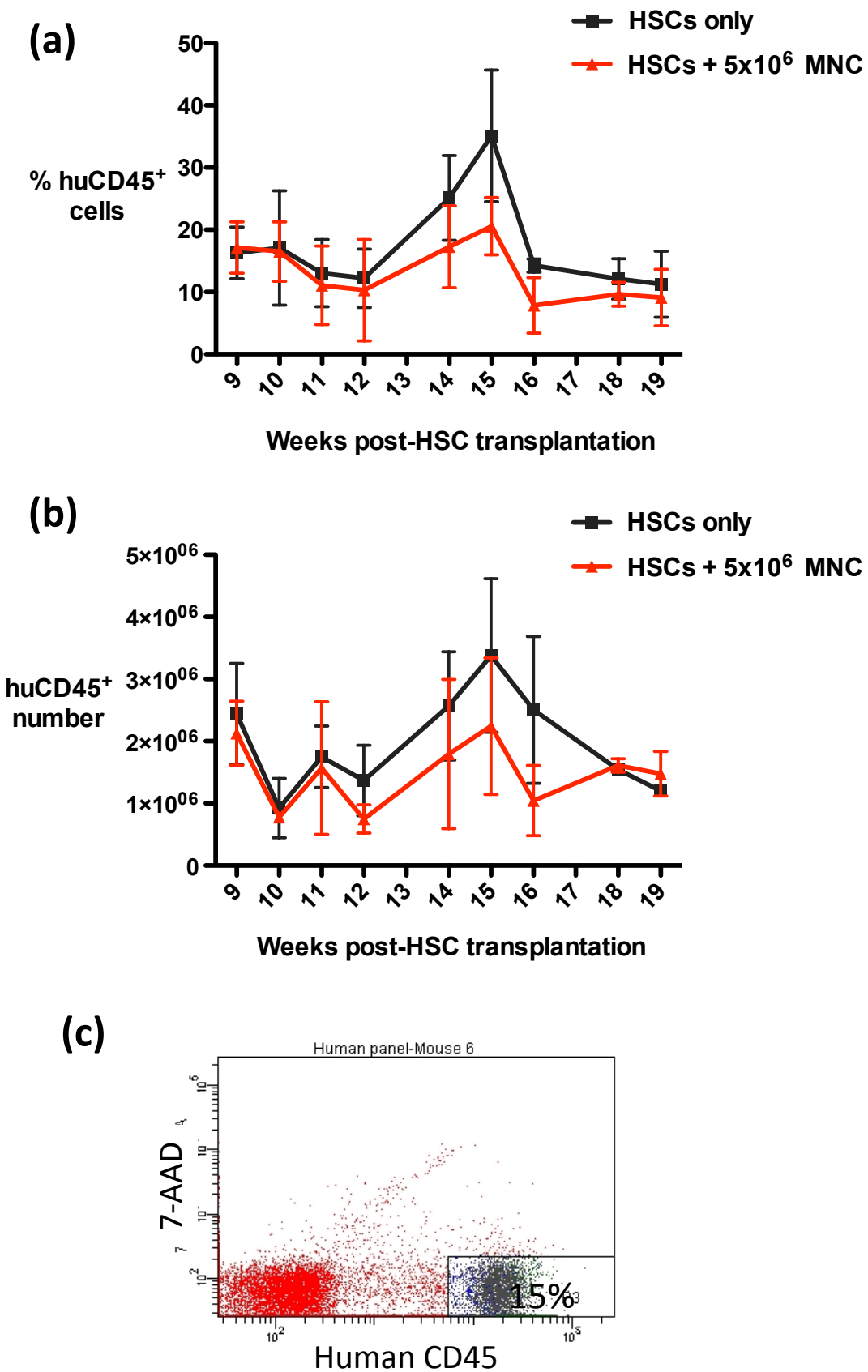
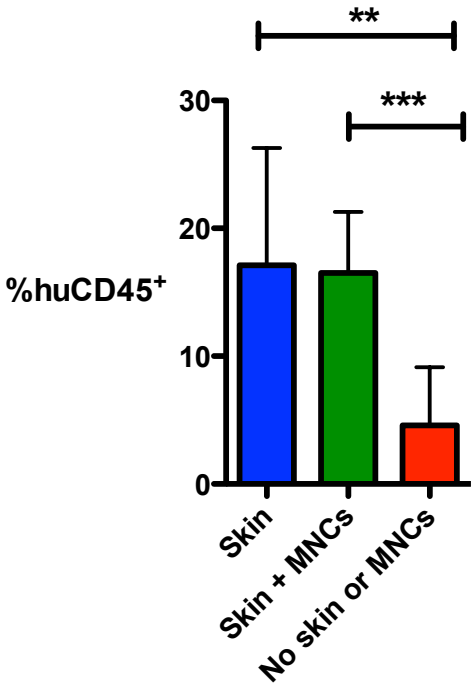

(a)
HSCs only
HSCs + 5x10^6 MNC
% huCD45+ cells
50
40
30
20
10
0
9
10
11
12
13
14
15
16
17
18
19
Weeks post-HSC transplantation
(b)
HSCs only
HSCs + 5x10^6 MNC
huCD45+ number
5×10^06
4×10^06
3×10^06
2×10^06
1×10^06
0
9
10
11
12
13
14
15
16
17
18
19
Weeks post-HSC transplantation
(c)
Human panel-Mouse 6
7-AAD
15%
Human CD45

Figure 4.13 cont.

(d)



(e)

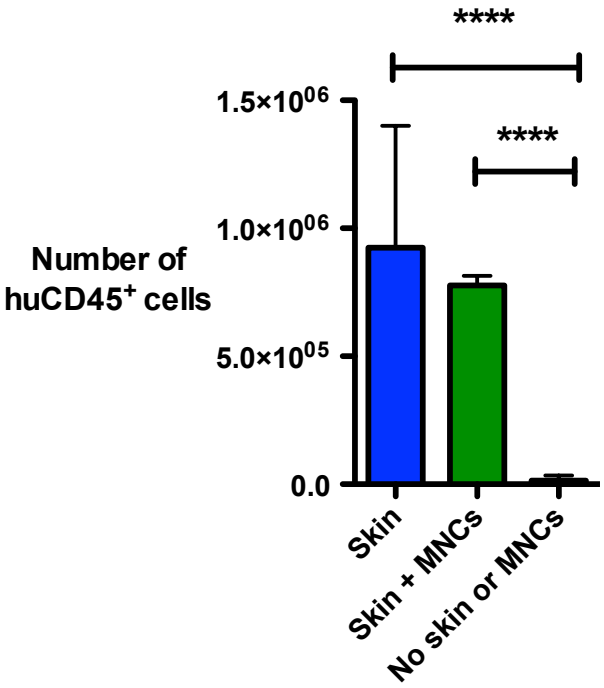
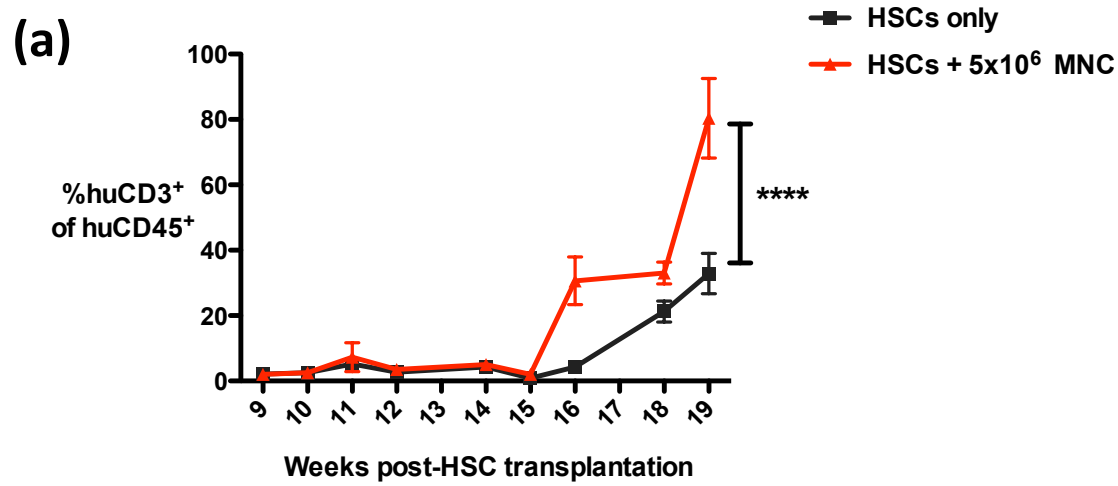
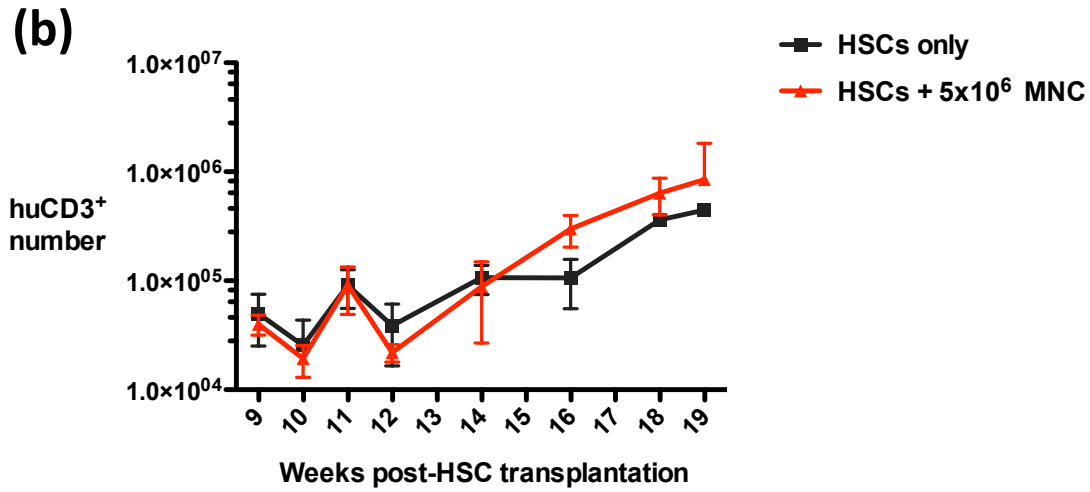


Figure 4.14


(a)
HSCs only
HSCs + 5x10^6 MNC
%huCD3+ of huCD45+
100
80
60
40
20
0
****
9
10
11
12
13
14
15
16
17
18
19
Weeks post-HSC transplantation


(b)
HSCs only
HSCs + 5x10^6 MNC
huCD3+ number
1.0×10^07
1.0×10^06
1.0×10^05
1.0×10^04
9
10
11
12
13
14
15
16
17
18
19
Weeks post-HSC transplantation

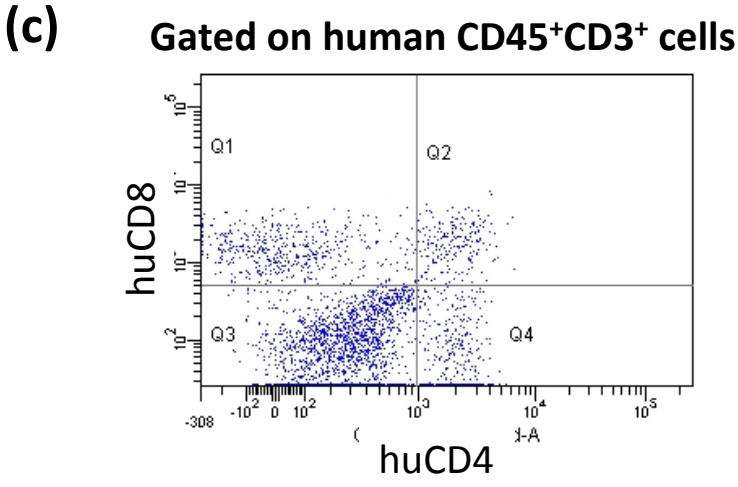
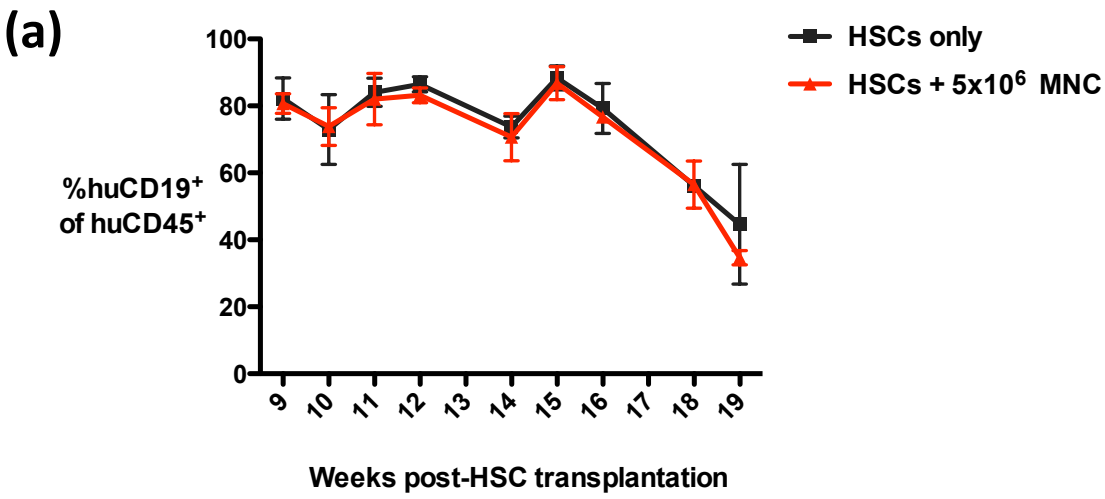
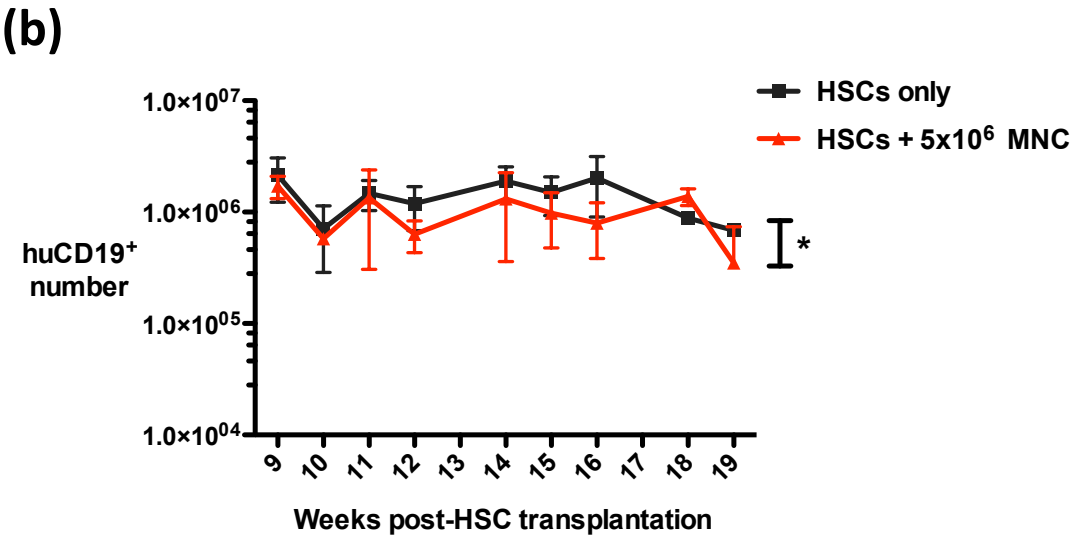

(c)
Gated on human CD45+CD3+ cells
huCD8
Q1
Q2
Q3
Q4
huCD4

Figure 4.15


(a)
HSCs only
HSCs + 5x10^6 MNC
%huCD19+ of huCD45+
100
80
60
40
20
0
9
10
11
12
13
14
15
16
17
18
19
Weeks post-HSC transplantation


(b)
HSCs only
HSCs + 5x10^6 MNC
huCD19+ number
1.0×10^07
1.0×10^06
1.0×10^05
1.0×10^04
*
9
10
11
12
13
14
15
16
17
18
19
Weeks post-HSC transplantation

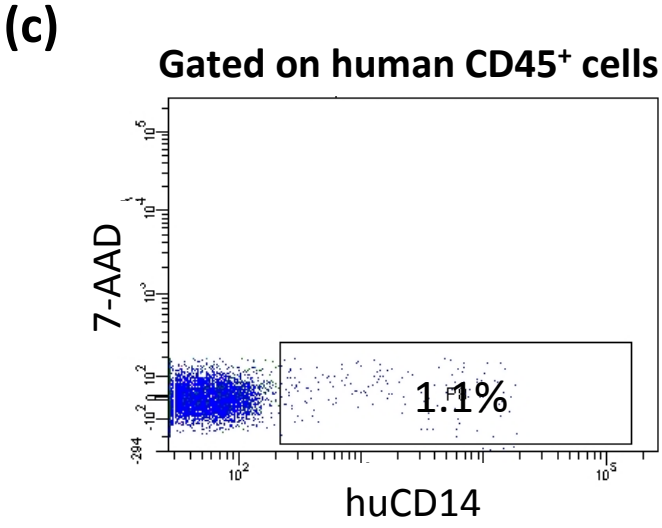
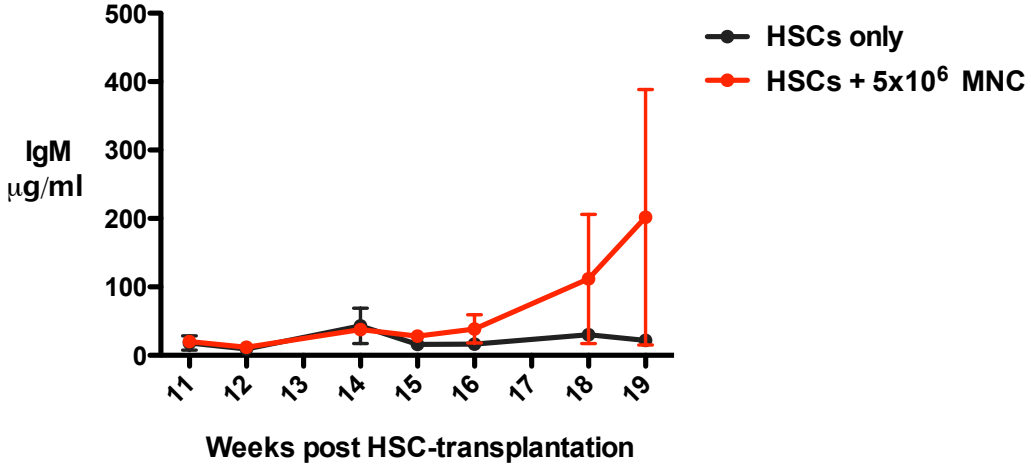

(c)
Gated on human CD45+ cells
7-AAD
1.1%
huCD14

Figure 4.16

(a)



(b)

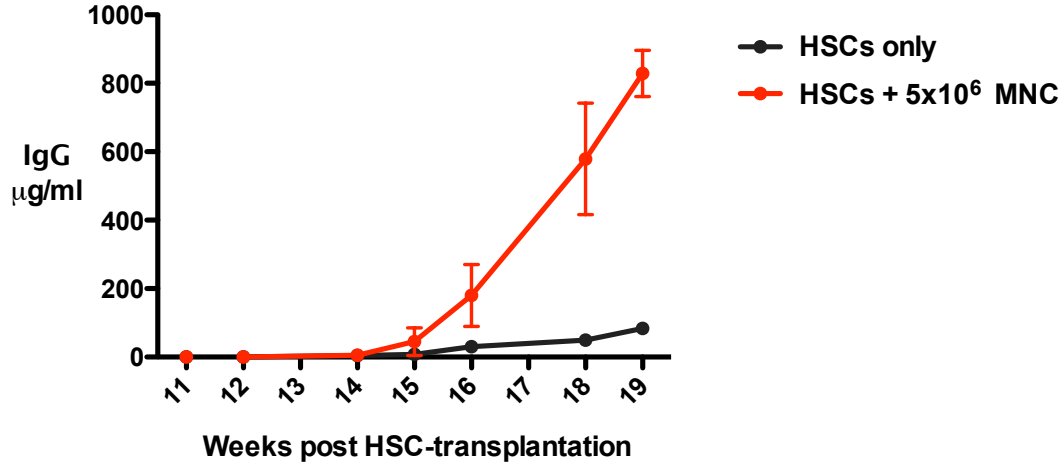
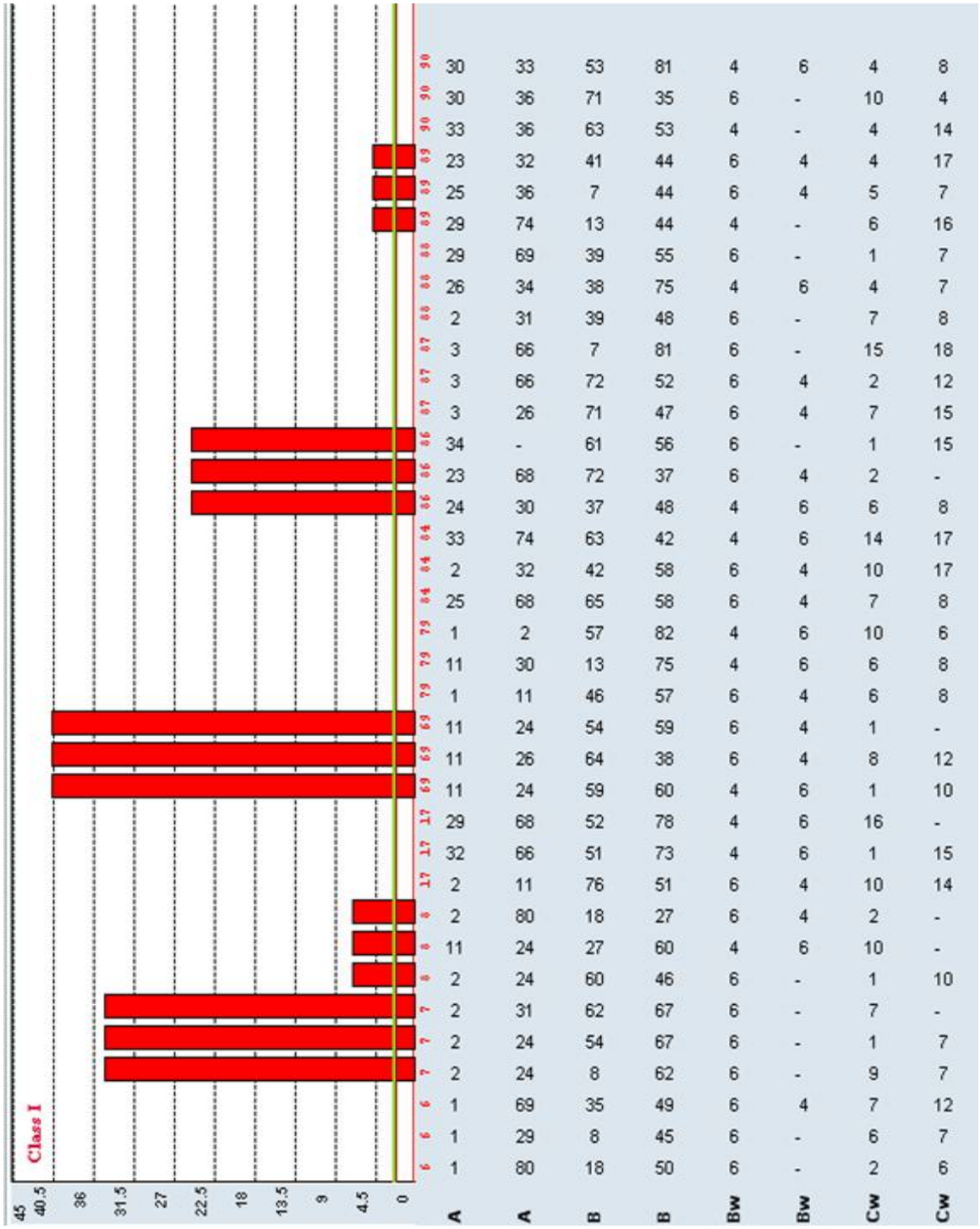


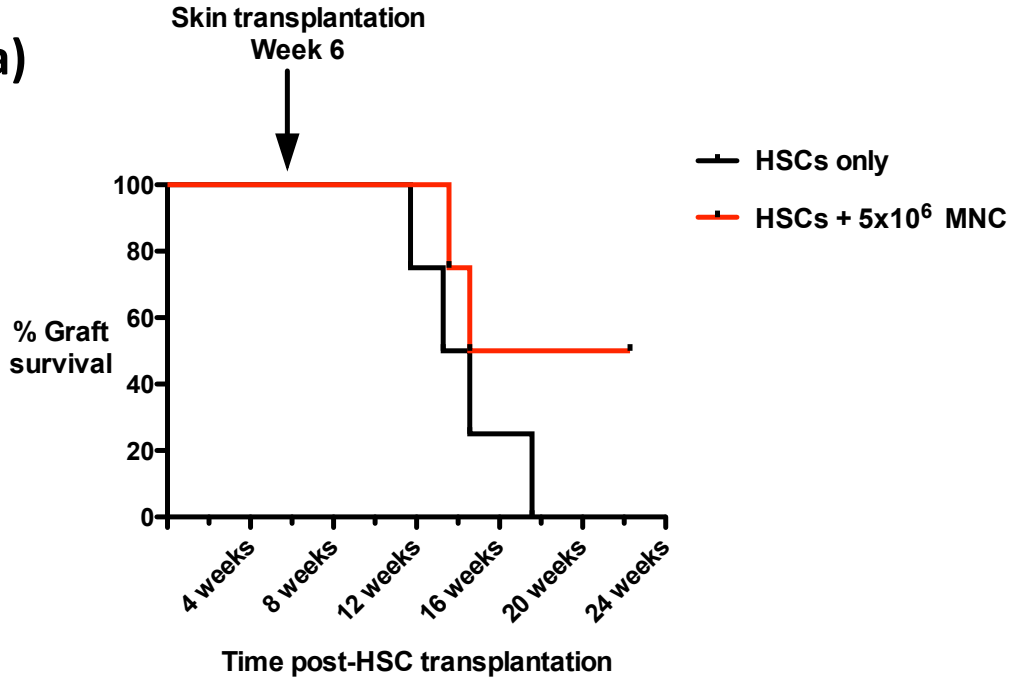
Figure 4.17



30	33	53	81	4	6	4	8	90
30	36	71	35	6	-	10	4	90
33	36	63	53	4	-	4	14	90
23	32	41	44	6	4	4	17	89
25	36	7	44	6	4	5	7	89
29	74	13	44	4	-	6	16	89
29	69	39	55	6	-	1	7	88
26	34	38	75	4	6	4	7	88
2	31	39	48	6	-	7	8	88
3	66	7	81	6	-	15	18	87
3	66	72	52	6	4	2	12	87
3	26	71	47	6	4	7	15	87
34	-	61	56	6	-	1	15	86
23	68	72	37	6	4	2	-	86
24	30	37	48	4	6	6	8	86
33	74	63	42	4	6	14	17	84
2	32	42	58	6	4	10	17	84
25	68	65	58	6	4	7	8	84
1	2	57	82	4	6	10	6	79
11	30	13	75	4	6	6	8	79
1	11	46	57	6	4	6	8	79
11	24	54	59	6	4	1	-	69
11	26	64	38	6	4	8	12	69
11	24	59	60	4	6	1	10	69
29	68	52	78	4	6	16	-	17
32	66	51	73	4	6	1	15	17
2	11	76	51	6	4	10	14	17
2	80	18	27	6	4	2	-	8
11	24	27	60	4	6	10	-	8
2	24	60	46	6	-	1	10	8
2	31	62	67	6	-	7	-	7
2	24	54	67	6	-	1	7	7
2	24	8	62	6	-	9	7	7
1	69	35	49	6	4	7	12	6
1	29	8	45	6	-	6	7	6
1	80	18	50	6	-	2	6	6
A	A	B	B	Bw	Bw	Cw	Cw	

Figure 4.18

(a)



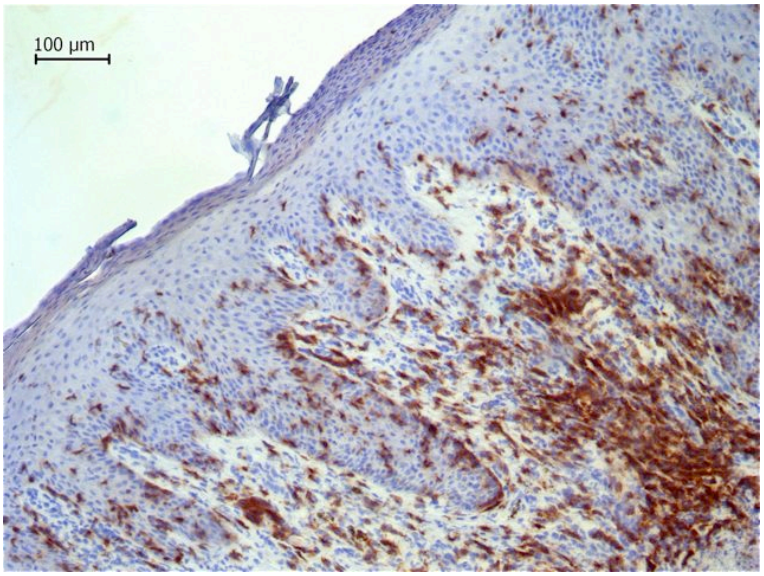
(b)

Group	Group size	Graft loss days	MST (days)
HSCs only	4	40, 51, 60, 81	55.5
HSCs + 5x10^6 MNCs	4	53, 60, >100, >100	87

(c)

Group	Group size	Graft loss days	MST (days)
5x10^6 MNCs	5	35, 61, 83, 89, >100	83

(d)

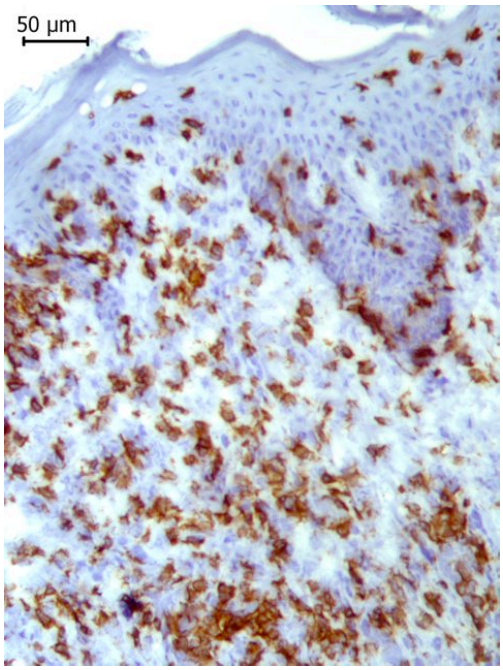


huCD45

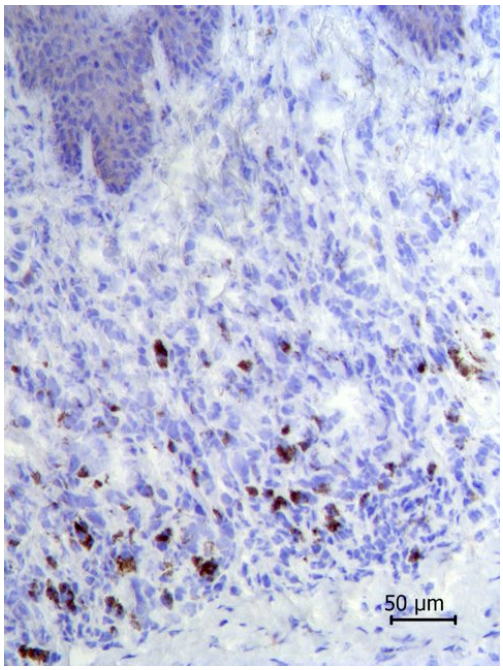
Figure 4.18 (cont.)

(e)

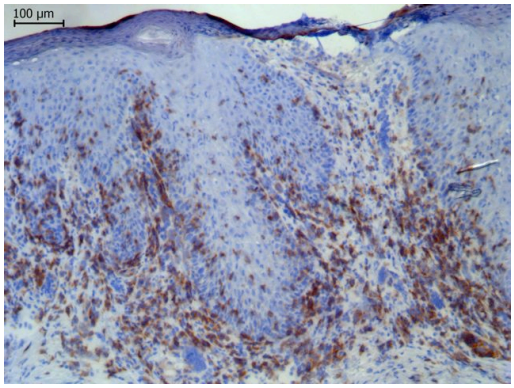
huCD3



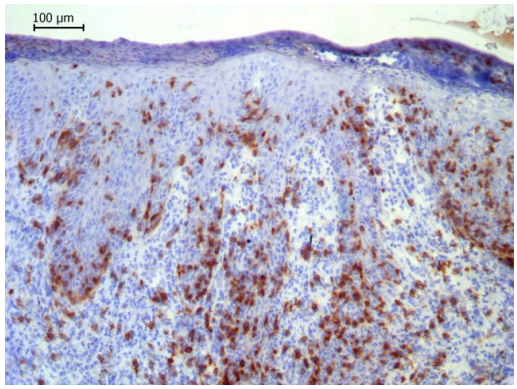
huCD19



huCD4



huCD8



huCD68

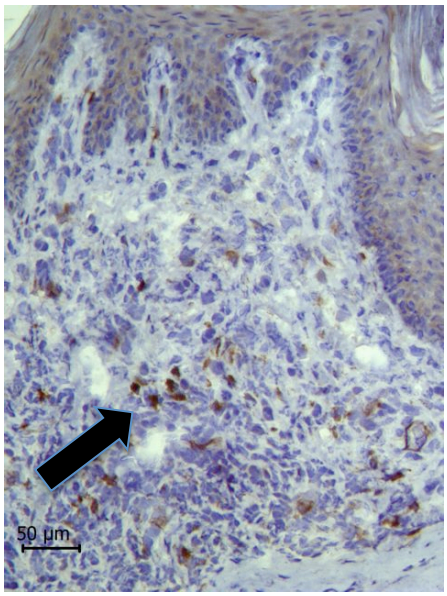


Figure 5.1

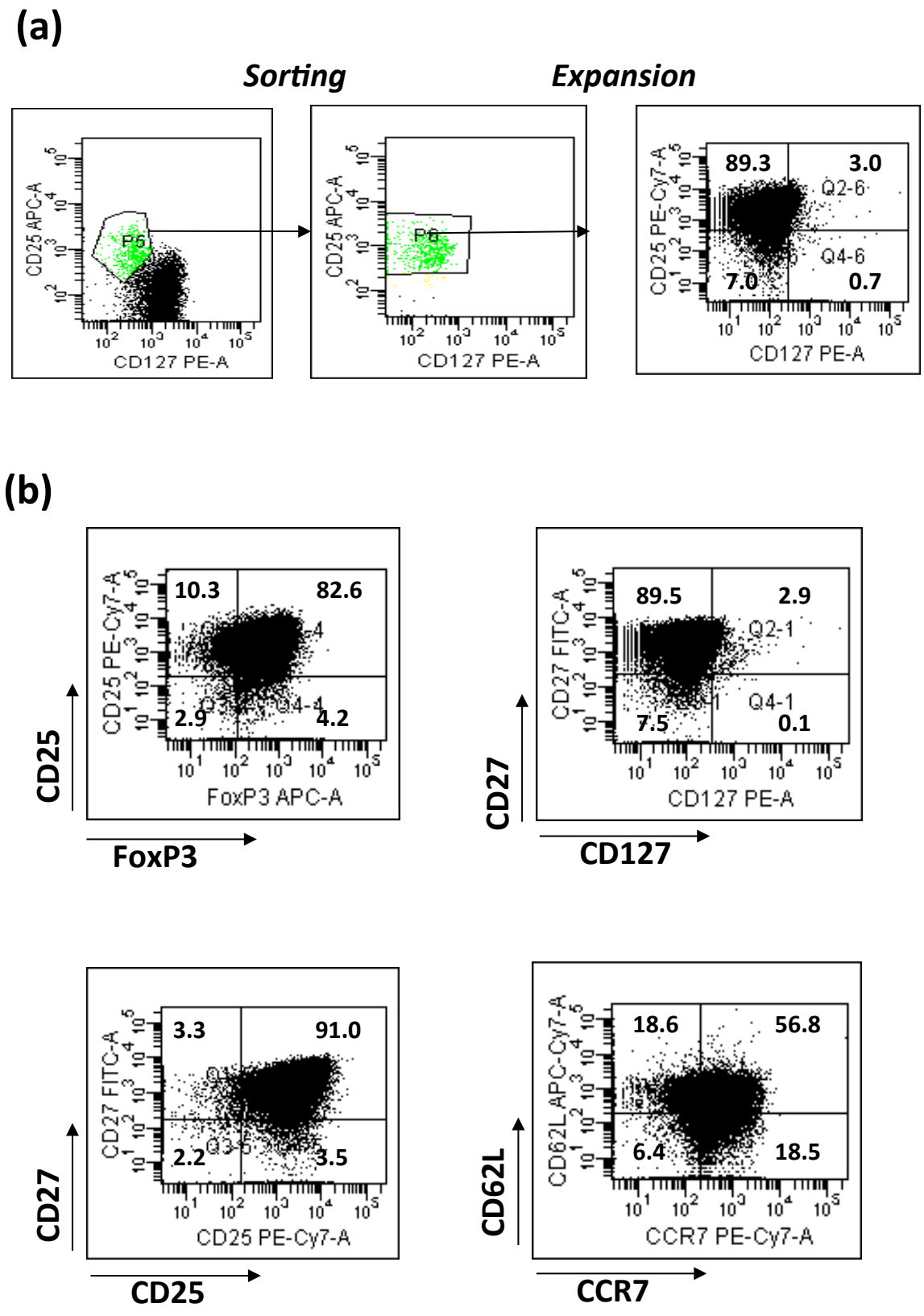

(a)
Sorting
Expansion
CD25 APC-A
CD127 PE-A
P6
CD25 PE-Cy7-A
89.3
3.0
Q2-6
Q4-6
7.0
0.7
(b)
CD25
FoxP3
CD25 PE-Cy7-A
FoxP3 APC-A
10.3
82.6
2.9
4.2
CD27
CD127
CD27 FITC-A
CD127 PE-A
89.5
2.9
Q2-1
Q4-1
7.5
0.1
CD27
CD25
CD27 FITC-A
CD25 PE-Cy7-A
3.3
91.0
2.2
3.5
CD62L
CCR7
CD62L APC-Cy7-A
CCR7 PE-Cy7-A
18.6
56.8
6.4
18.5

Figure 5.2

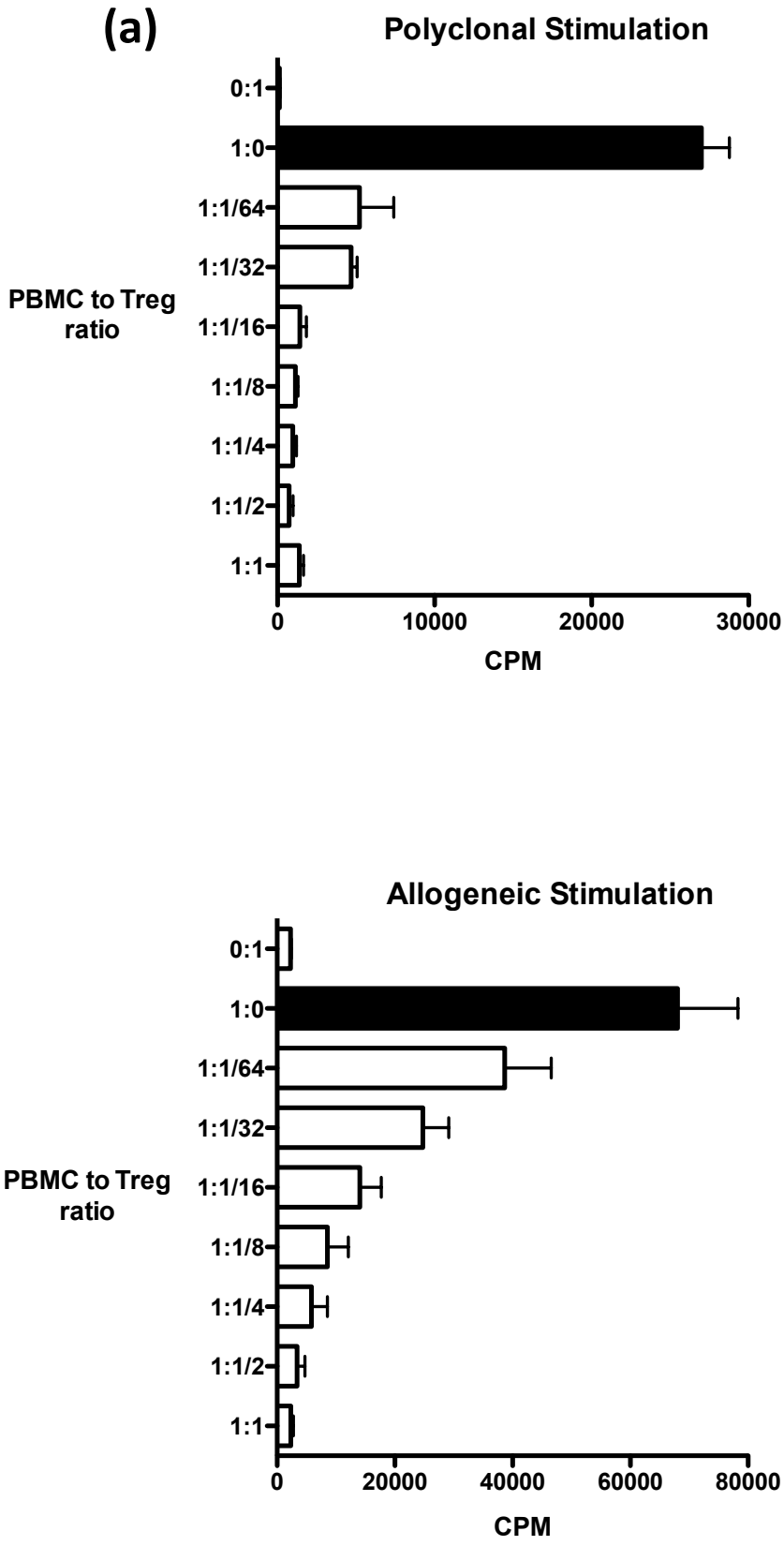
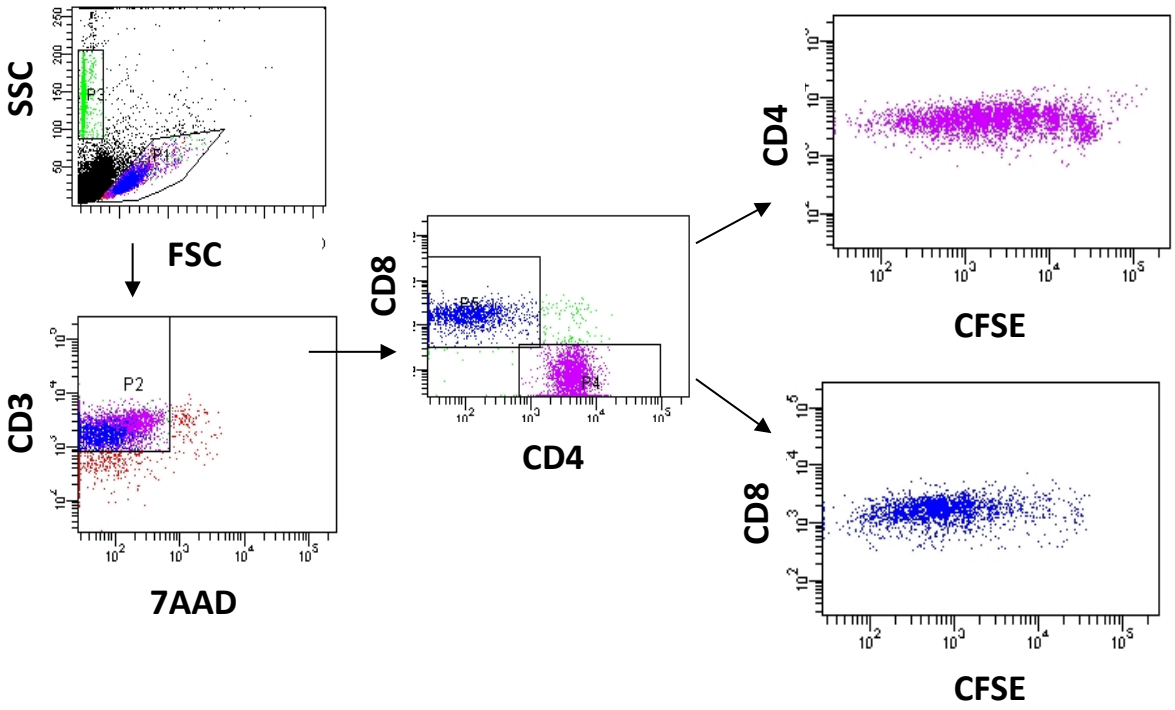

(a)
Polyclonal Stimulation
0:1
1:0
1:1/64
1:1/32
1:1/16
1:1/8
1:1/4
1:1/2
1:1
PBMC to Treg ratio
0
10000
20000
30000
CPM
Allogeneic Stimulation
0:1
1:0
1:1/64
1:1/32
1:1/16
1:1/8
1:1/4
1:1/2
1:1
PBMC to Treg ratio
0
20000
40000
60000
80000
CPM

Figure 5.2 (cont.)

(b)



(c)

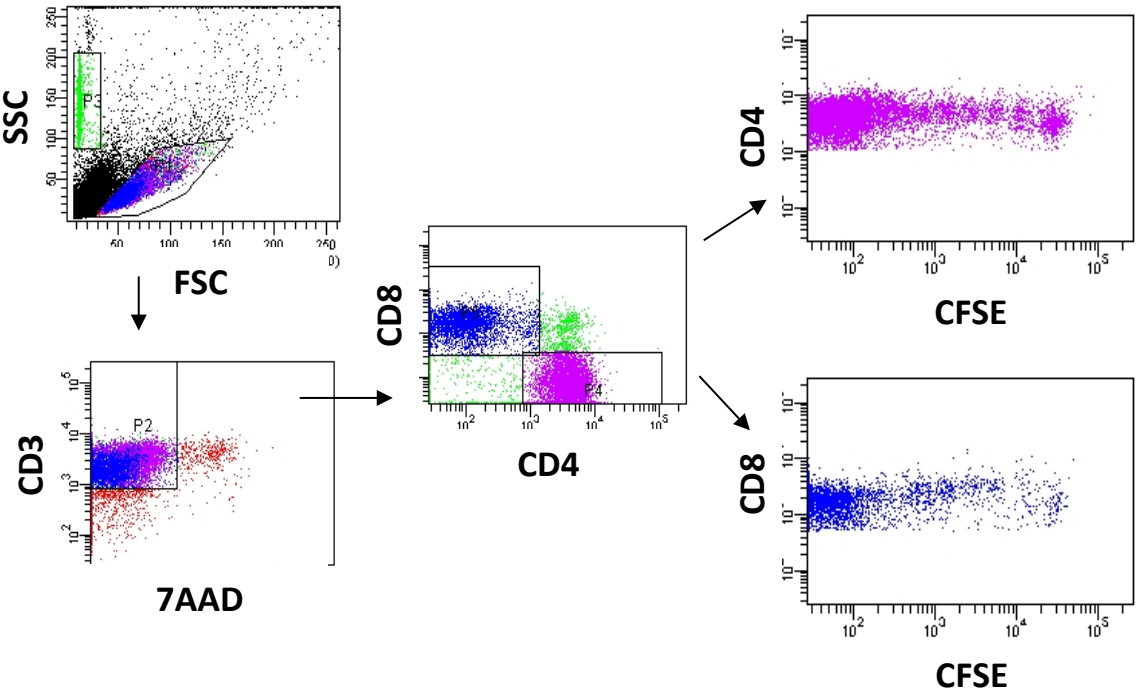
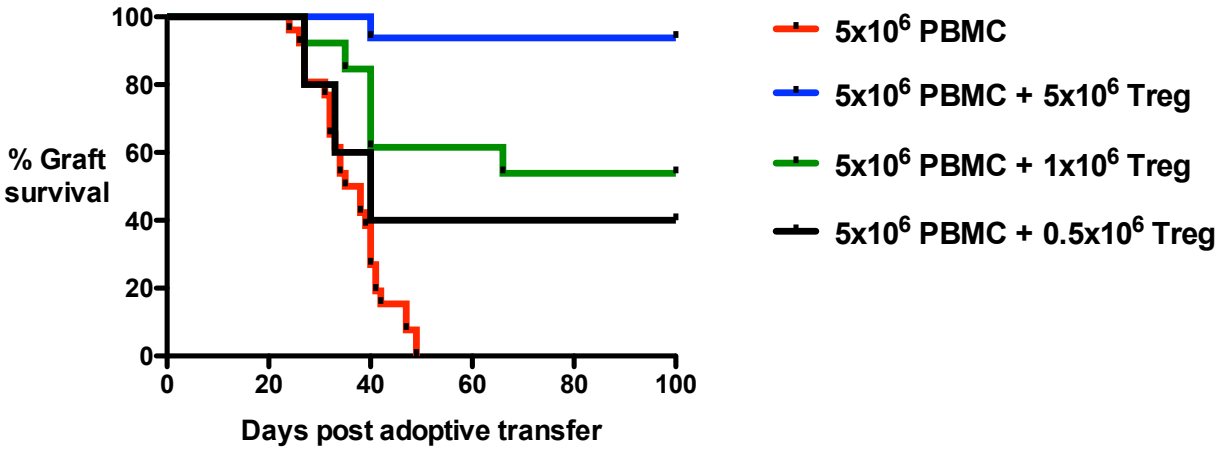


Figure 5.3

(a)



(b)

Group	Group size	Graft loss days	MST (days)
5x10^6 PBMC	26	24, 26, 27, 27, 27, 31, 32, 32, 32, 33, 34, 34, 35, 38, 38, 39, 40, 40, 40, 41, 41, 42, 47, 47, 49, 49	36.5
5x10^6 PBMC + 5x10^6 Treg	16	40, >100, >100, >100, >100, >100, >100, >100, >100, >100, >100, >100, >100, >100, >100, >100	>100
5x10^6 PBMC + 1x10^6 Treg	13	27, 35, 40, 40, 40, 66, >100, >100, >100, >100, >100, >100, >100	>100
5x10^6 PBMC + 0.5x10^6 Treg	5	27, 33, 40, >100, >100	40

(c)

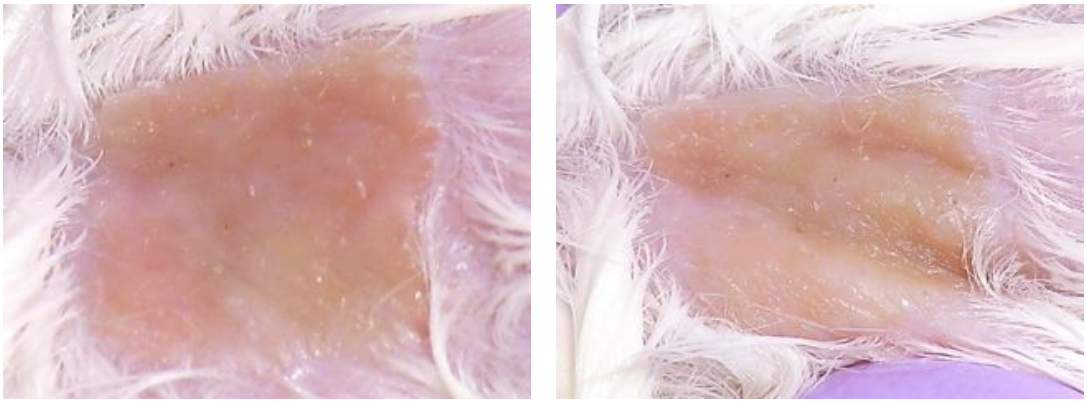
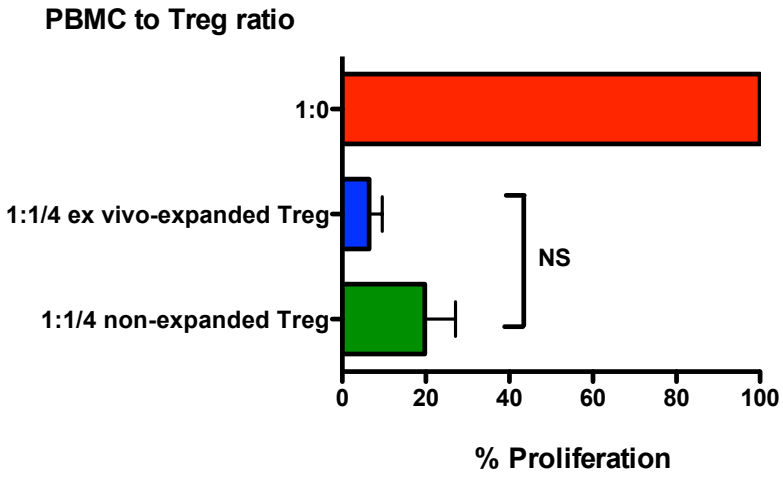
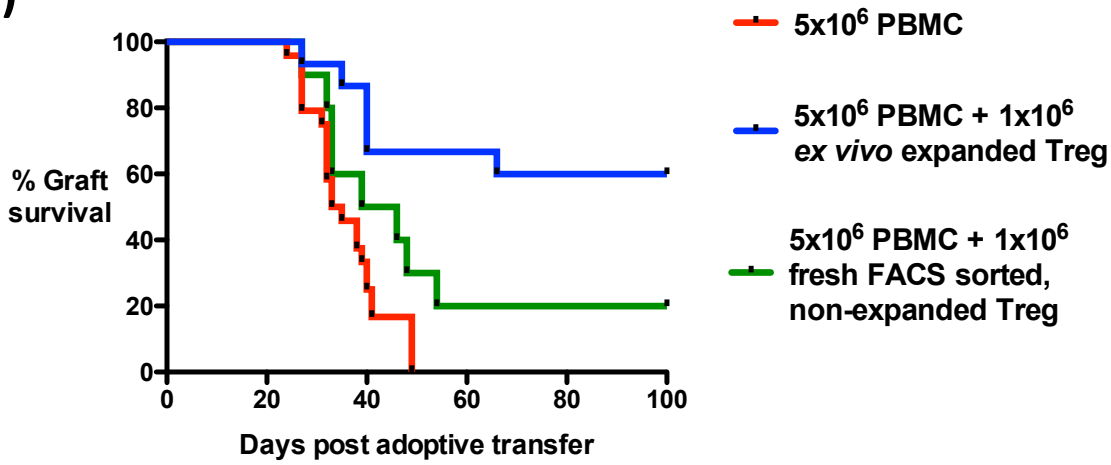


Figure 5.4

(a)



(b)



(c)

Group	Group size	Graft loss days	MST (days)
5x10^6 PBMC	24	24, 27, 27, 27, 27, 31, 32, 32, 32, 32, 33, 33, 35, 38, 38, 39, 40, 40, 41, 41, 49, 49, 49, 49	34
5x10^6 PBMC + 1x10^6 *ex vivo* expanded Treg	15	27, 35, 40, 40, 40, 66, >100, >100, >100, >100, >100, >100, >100, >100, >100	>100
5x10^6 PBMC + 1x10^6 fresh FACS sorted, non-expanded Treg	10	27, 32, 33, 33, 39, 46, 48, 54, >100, >100,	42.5

Figure 5.5

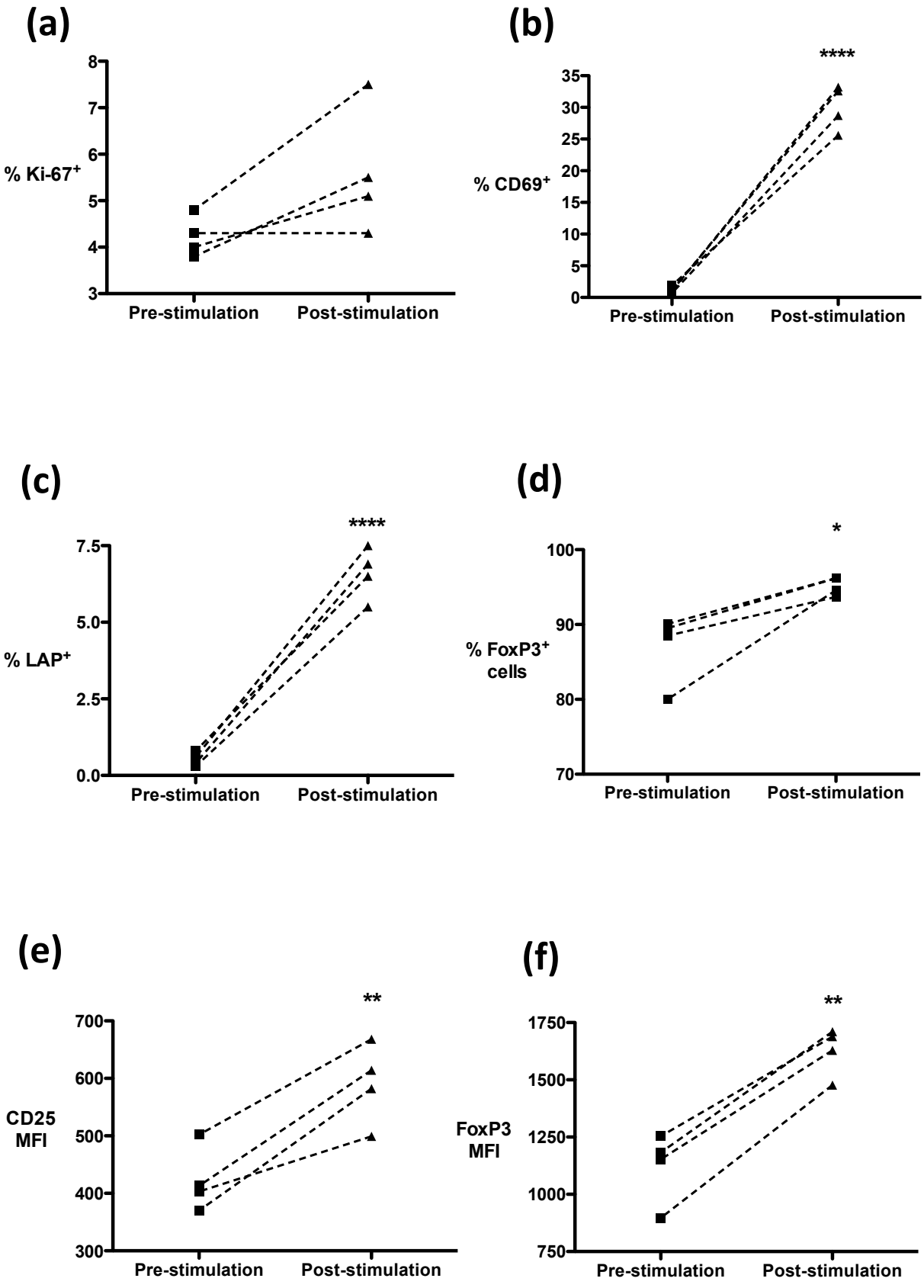

(a)
% Ki-67+
8
7
6
5
4
3
Pre-stimulation
Post-stimulation
(b)
****
% CD69+
35
30
25
20
15
10
5
0
Pre-stimulation
Post-stimulation
(c)
****
% LAP+
7.5
5.0
2.5
0.0
Pre-stimulation
Post-stimulation
(d)
*
% FoxP3+ cells
100
90
80
70
Pre-stimulation
Post-stimulation
(e)
**
CD25 MFI
700
600
500
400
300
Pre-stimulation
Post-stimulation
(f)
**
FoxP3 MFI
1750
1500
1250
1000
750
Pre-stimulation
Post-stimulation

Figure 5.5 cont.

(g)

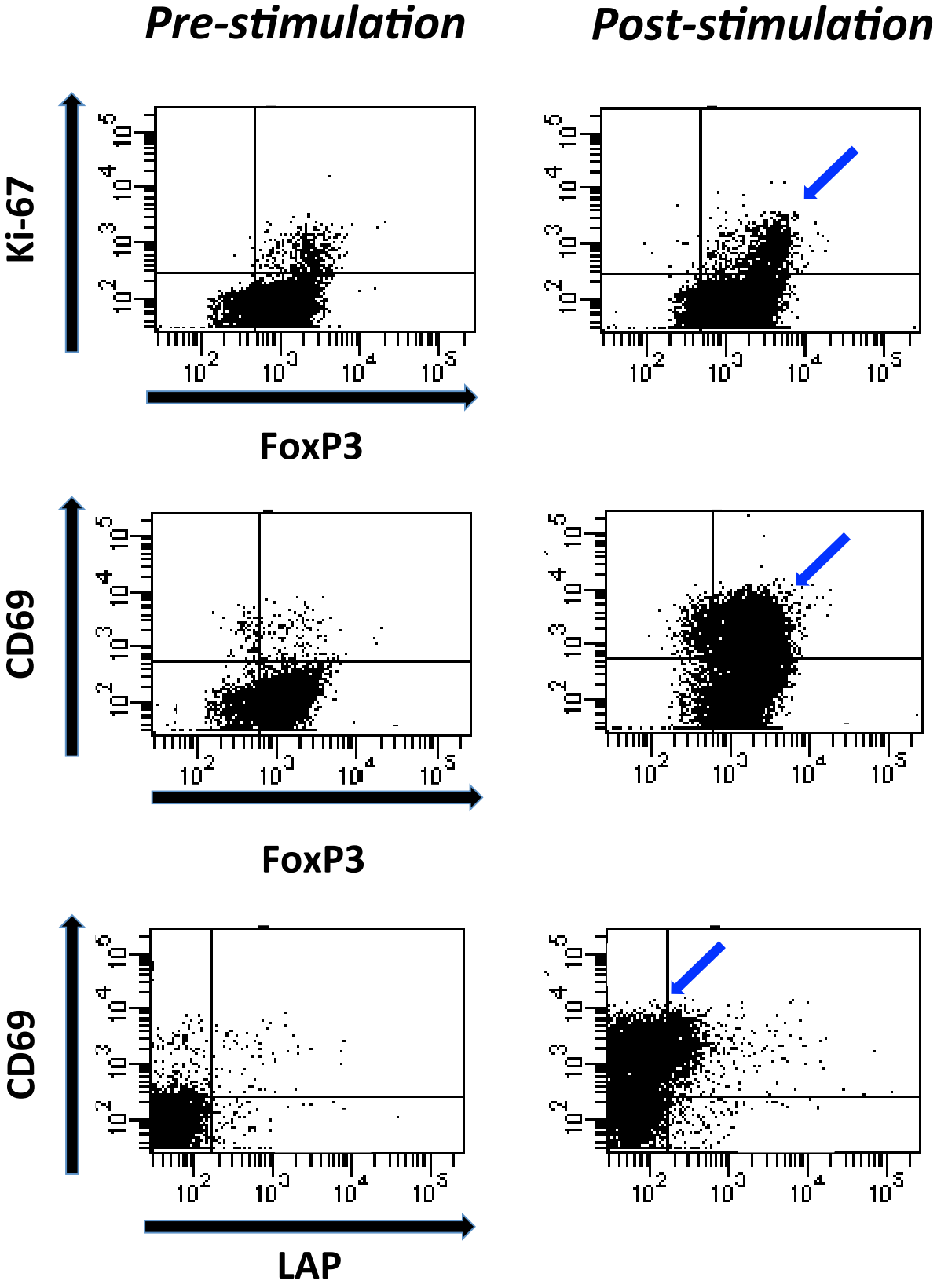
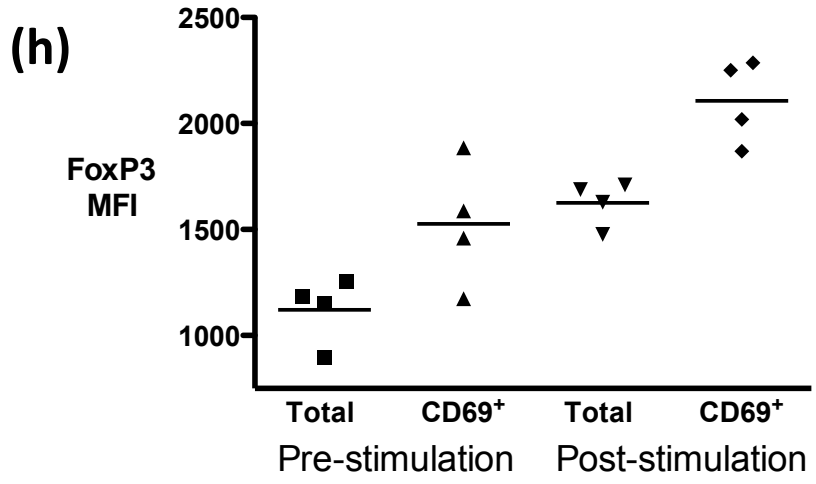
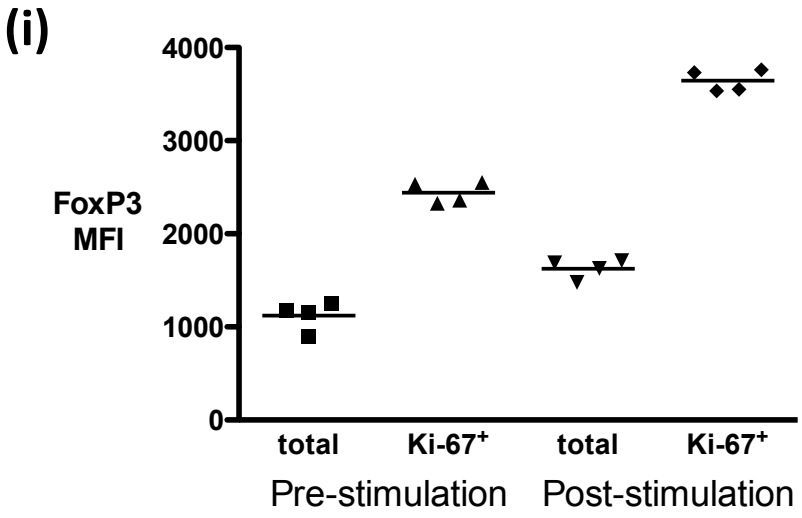


Figure 5.5 cont.





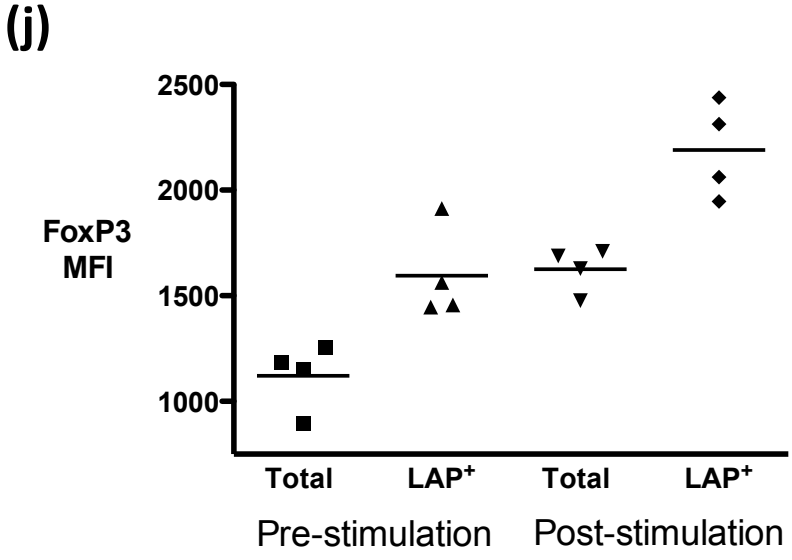
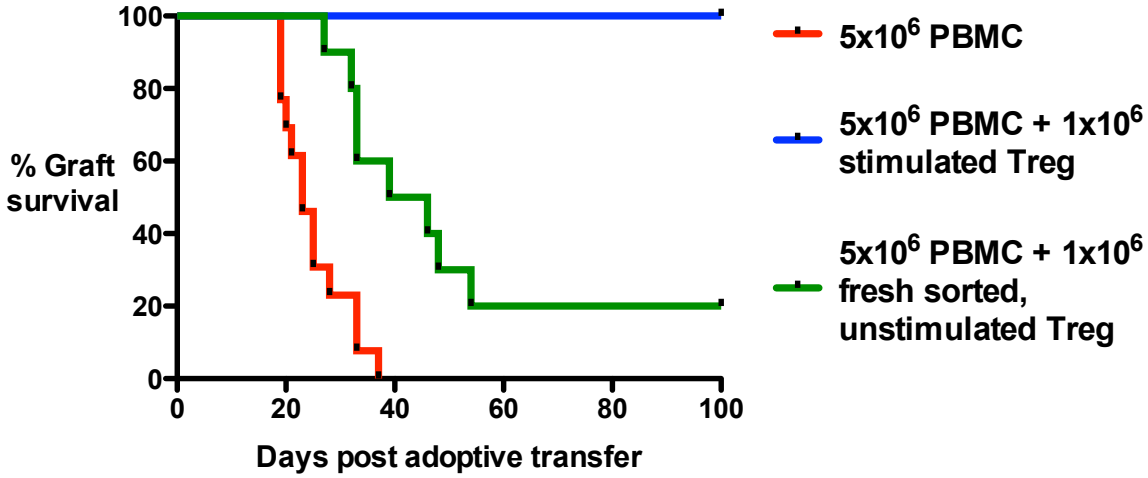


Figure 5.6

(a)

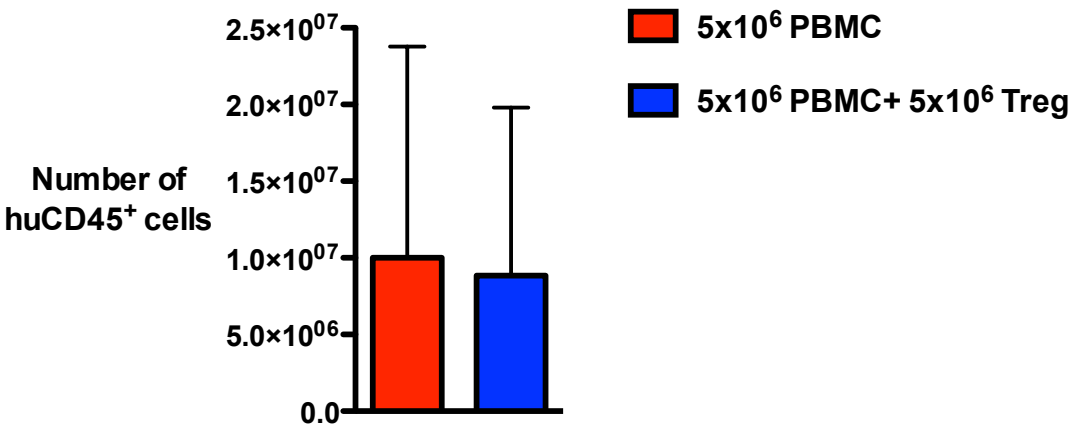


(b)

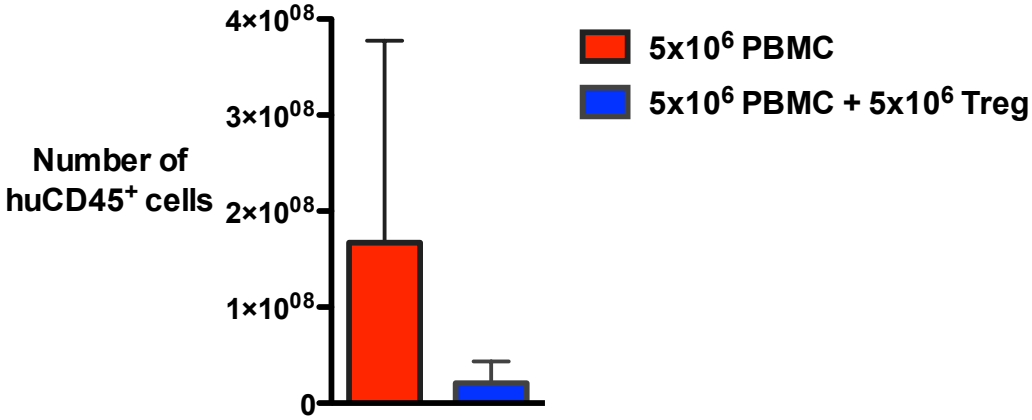
Group	Group size	Graft loss days	MST (days)
5x10^6 PBMC	13	19, 19, 19, 20, 21, 23, 23, 25, 25, 28, 33, 33, 37	23
5x10^6 PBMC + 1x10^6 stimulated Treg	4	>100, >100, >100, >100	>100
5x10^6 PBMC + 1x10^6 fresh sorted , unstimulated Treg	10	27, 32, 33, 33, 39, 46, 48, 54, >100, >100	42.5

Figure 5.7

(a)



(b)



(c)

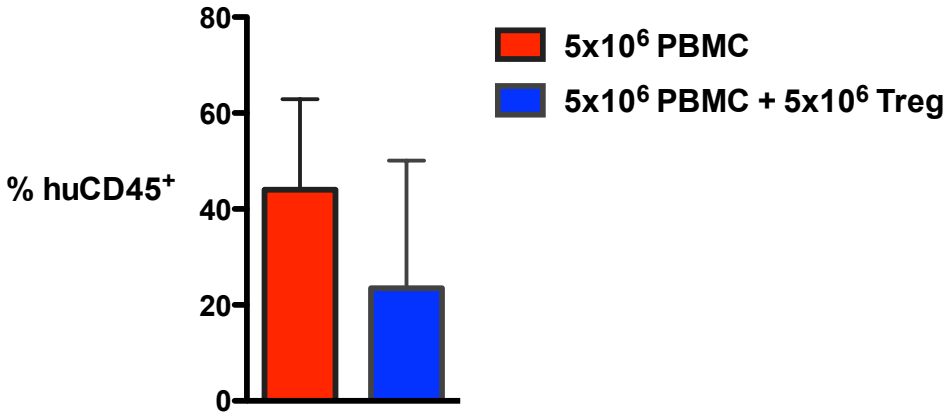
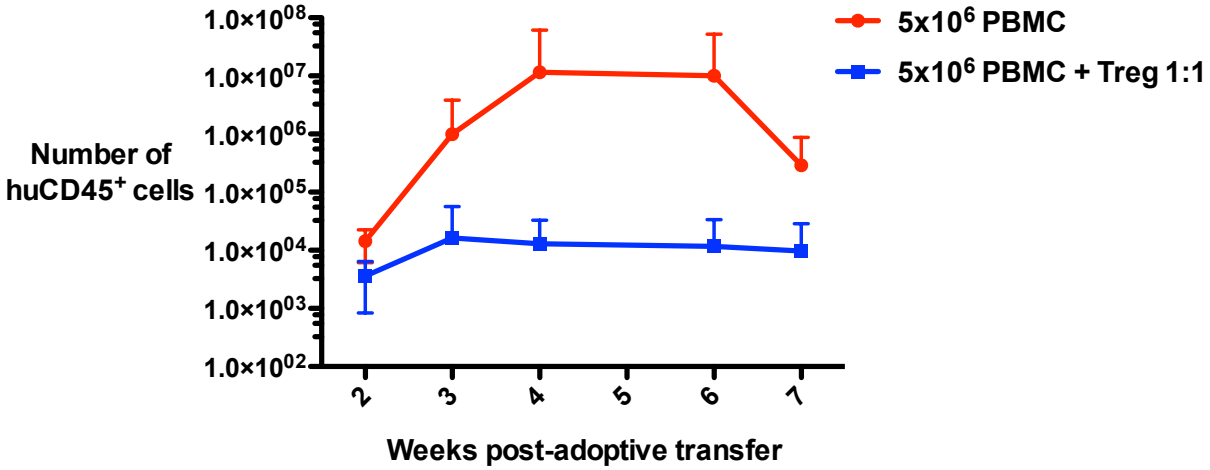


Figure 5.8

(a)


Number of huCD45+ cells
1.0×10^08
1.0×10^07
1.0×10^06
1.0×10^05
1.0×10^04
1.0×10^03
1.0×10^02
2
3
4
5
6
7
Weeks post-adoptive transfer
5x10^6 PBMC
5x10^6 PBMC + Treg 1:1

(b)

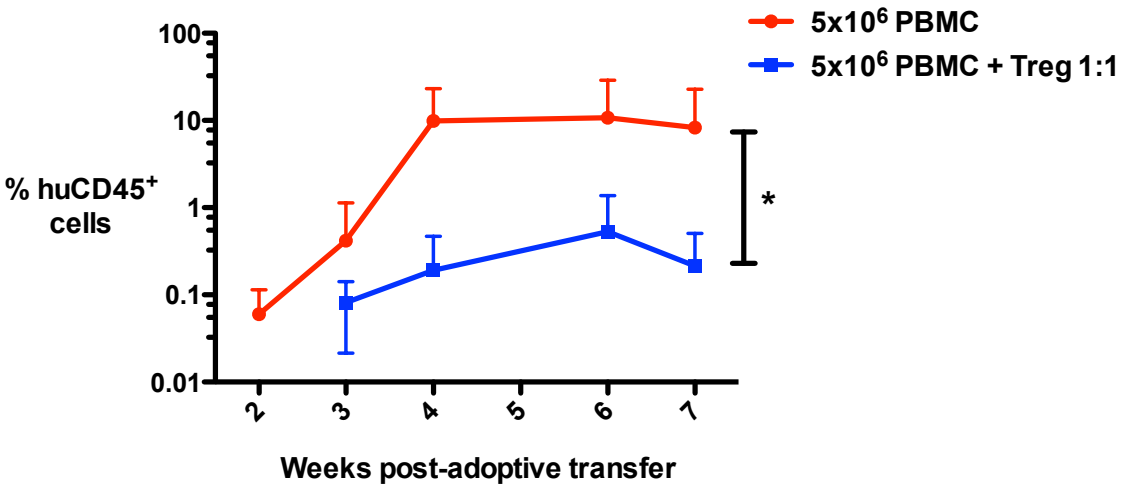

% huCD45+ cells
100
10
1
0.1
0.01
2
3
4
5
6
7
Weeks post-adoptive transfer
5x10^6 PBMC
5x10^6 PBMC + Treg 1:1
*

Figure 5.9

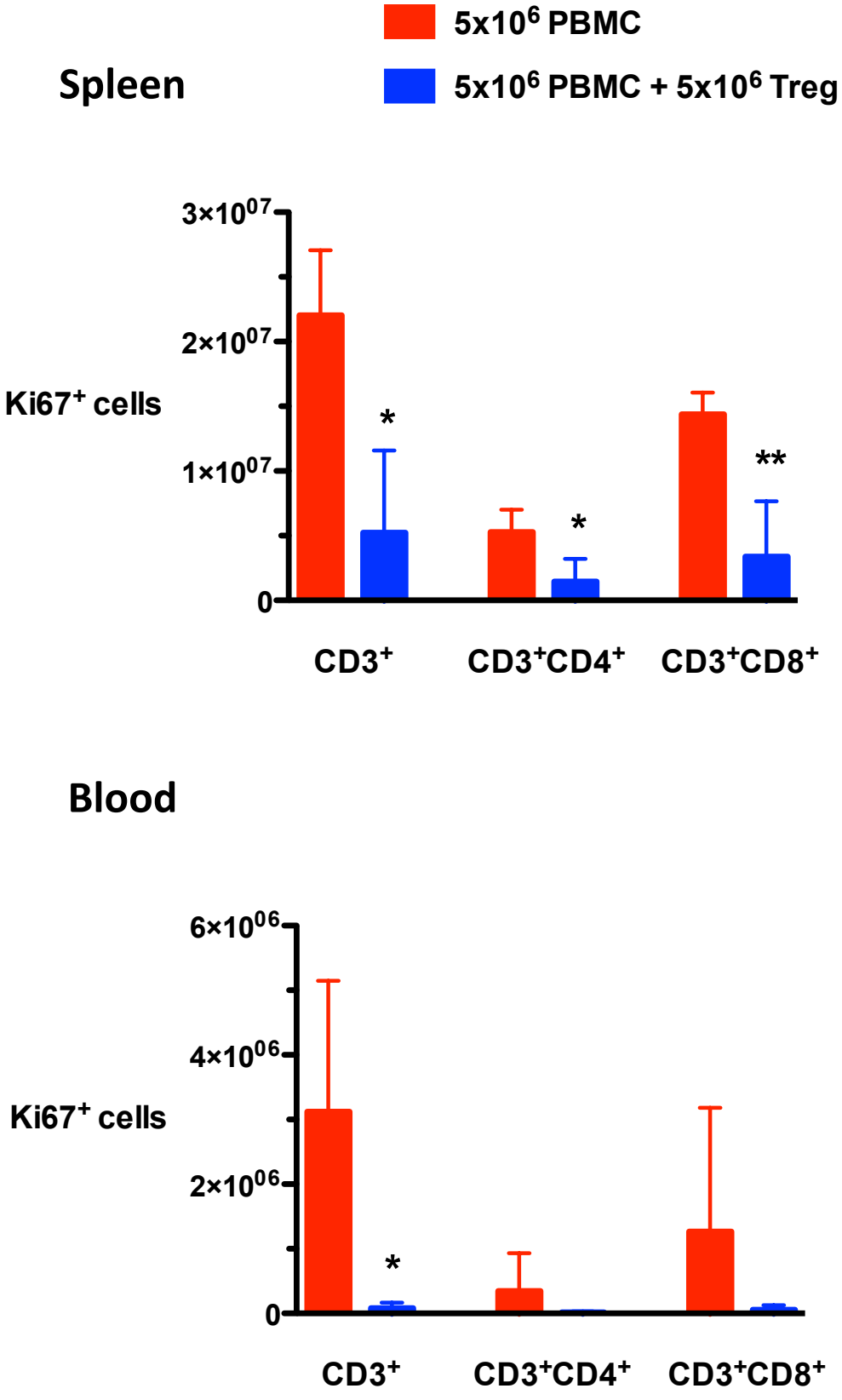
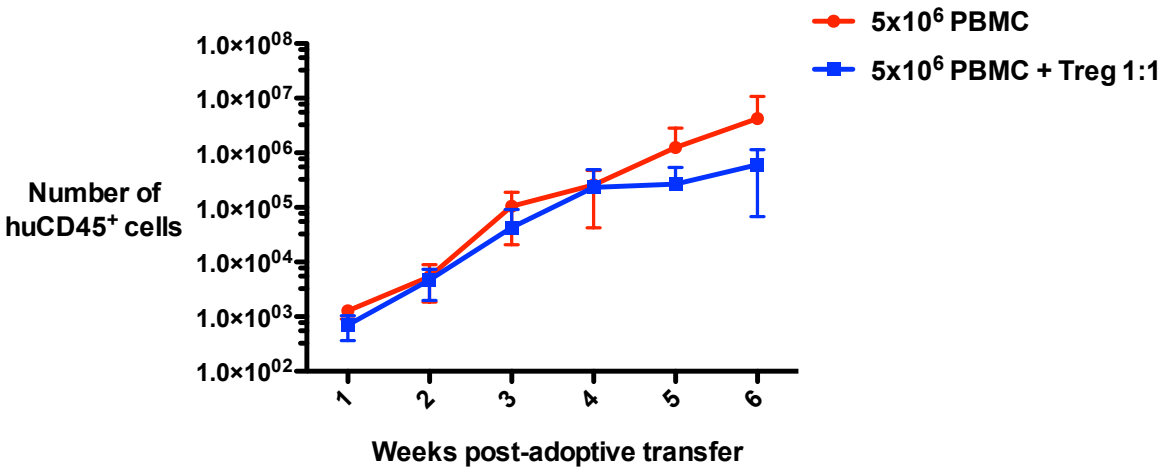

5x10^6 PBMC
5x10^6 PBMC + 5x10^6 Treg
Spleen
Ki67+ cells
3×10^07
2×10^07
1×10^07
0
*
*
**
CD3+
CD3+CD4+
CD3+CD8+
Blood
Ki67+ cells
6×10^06
4×10^06
2×10^06
0
*
CD3+
CD3+CD4+
CD3+CD8+

Figure 5.10

(a)



(b)

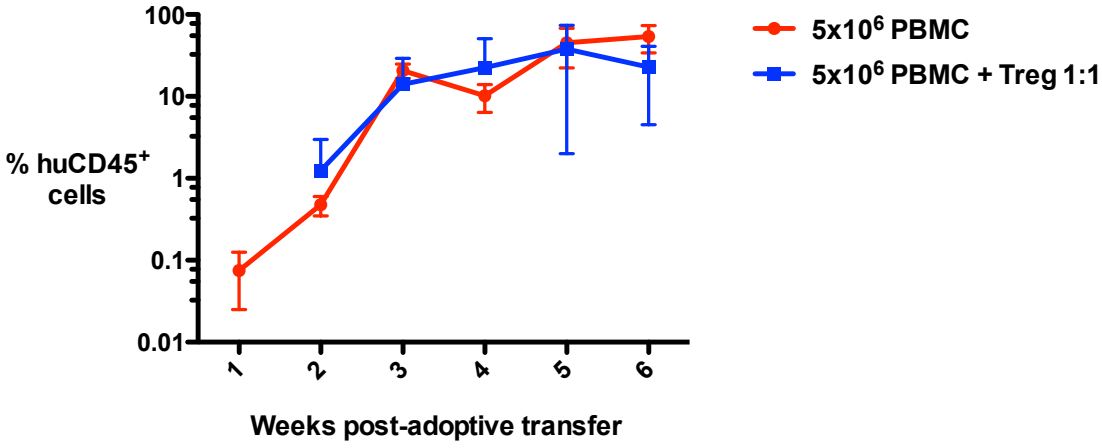


Figure 5.11

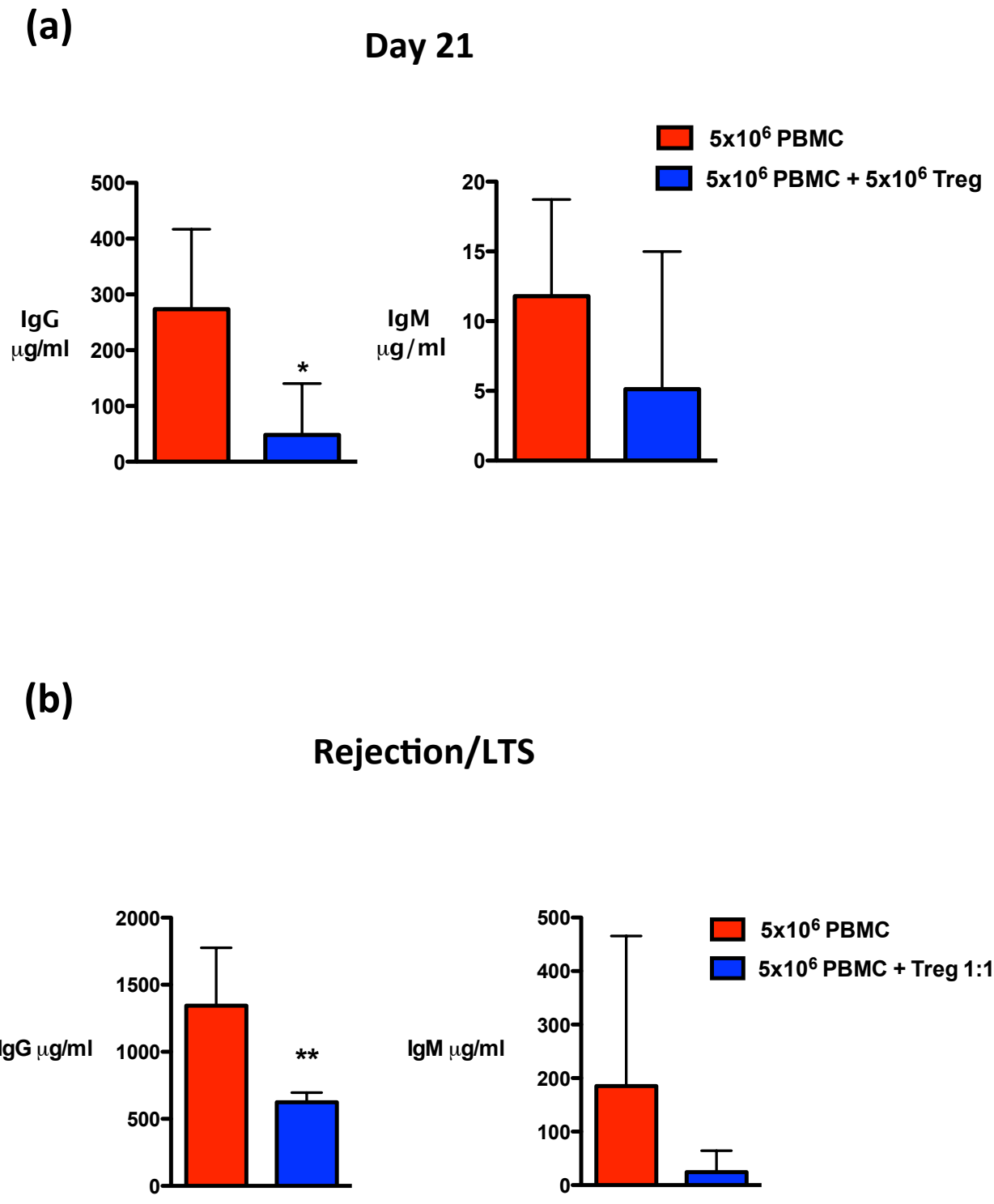

(a)
Day 21
5x10^6 PBMC
5x10^6 PBMC + 5x10^6 Treg
IgG μg/ml
500
400
300
200
100
0
*
IgM μg/ml
20
15
10
5
0
(b)
Rejection/LTS
IgG μg/ml
2000
1500
1000
500
0
**
IgM μg/ml
500
400
300
200
100
0
5x10^6 PBMC
5x10^6 PBMC + Treg 1:1

Figure 5.12

(a)

5x10^6 PBMC



(b)

5x10^6 PBMC + 5x10^6 Treg

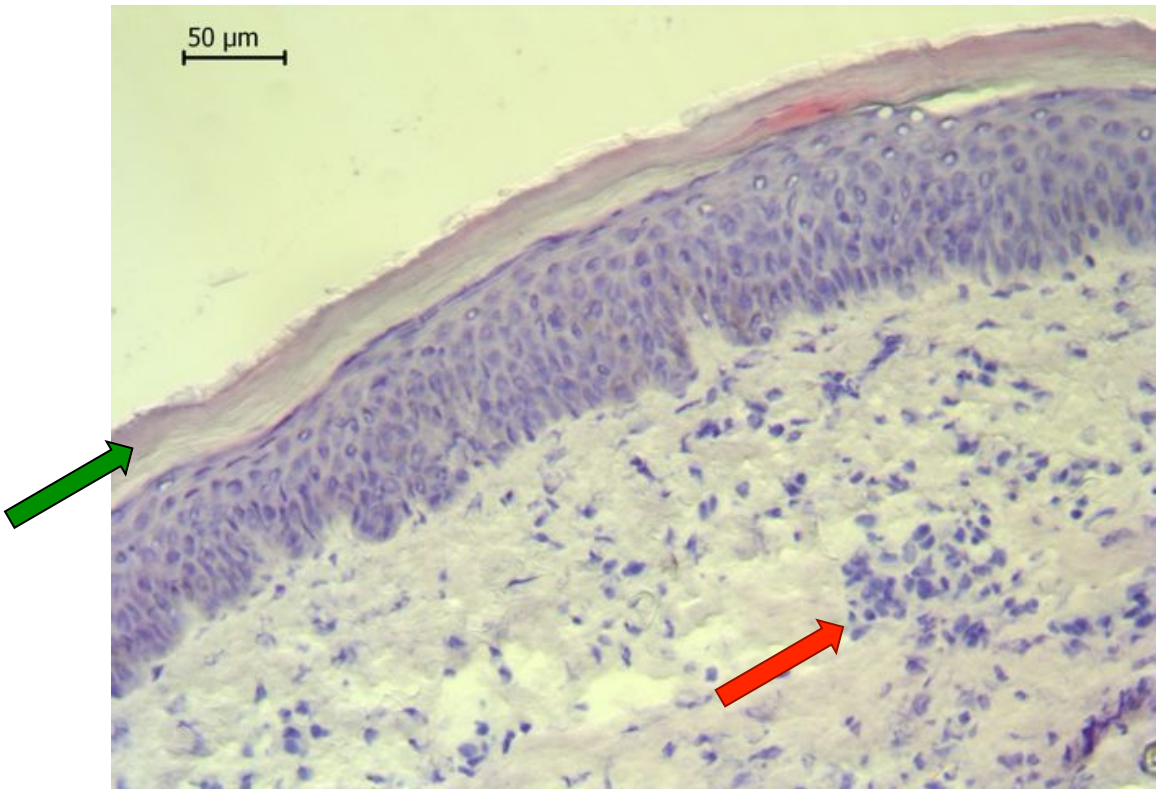


Figure 5.13

(a)

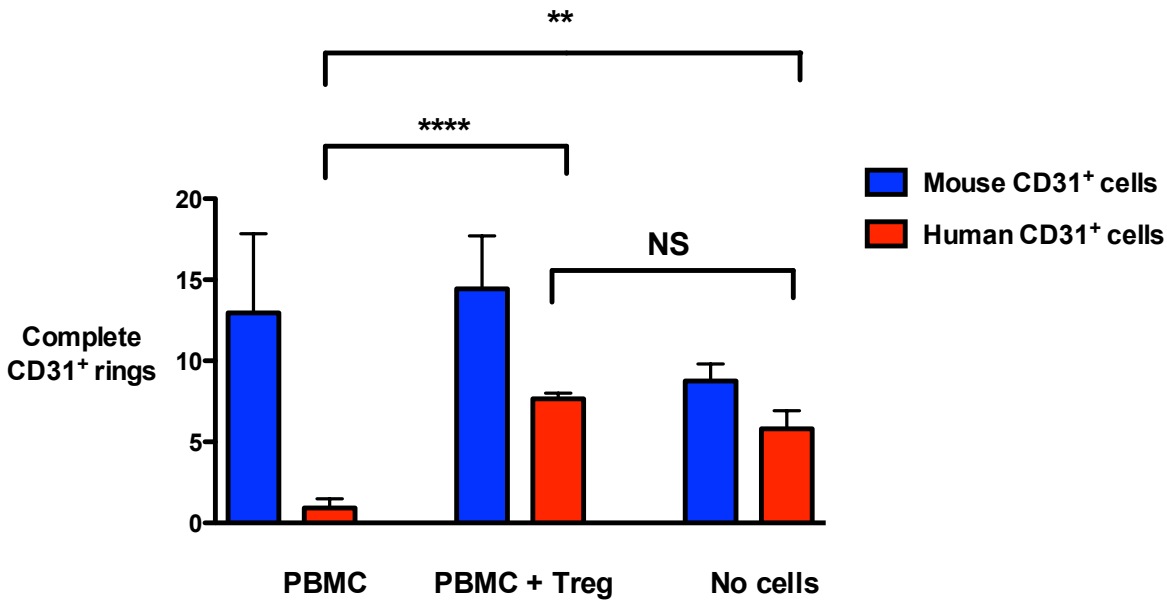
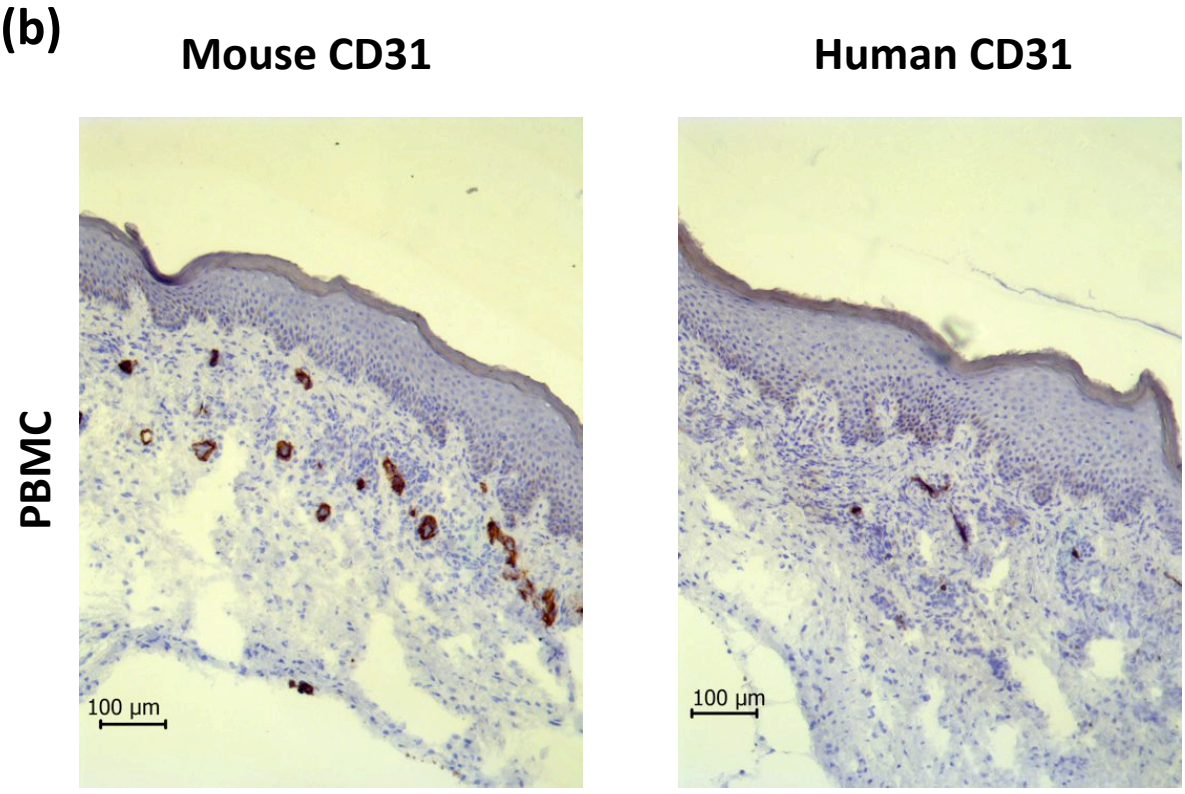

**
****
NS
Mouse CD31+ cells
Human CD31+ cells
Complete CD31+ rings
20
15
10
5
0
PBMC
PBMC + Treg
No cells

Figure 5.13 cont.

(b)



(c)

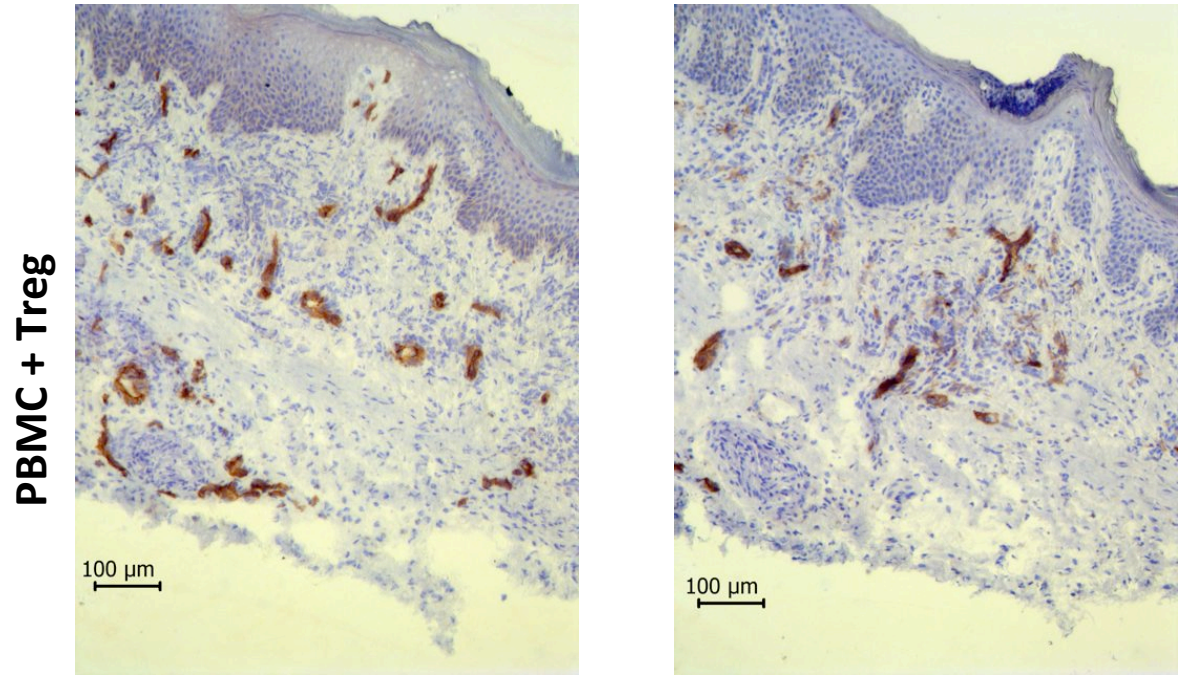
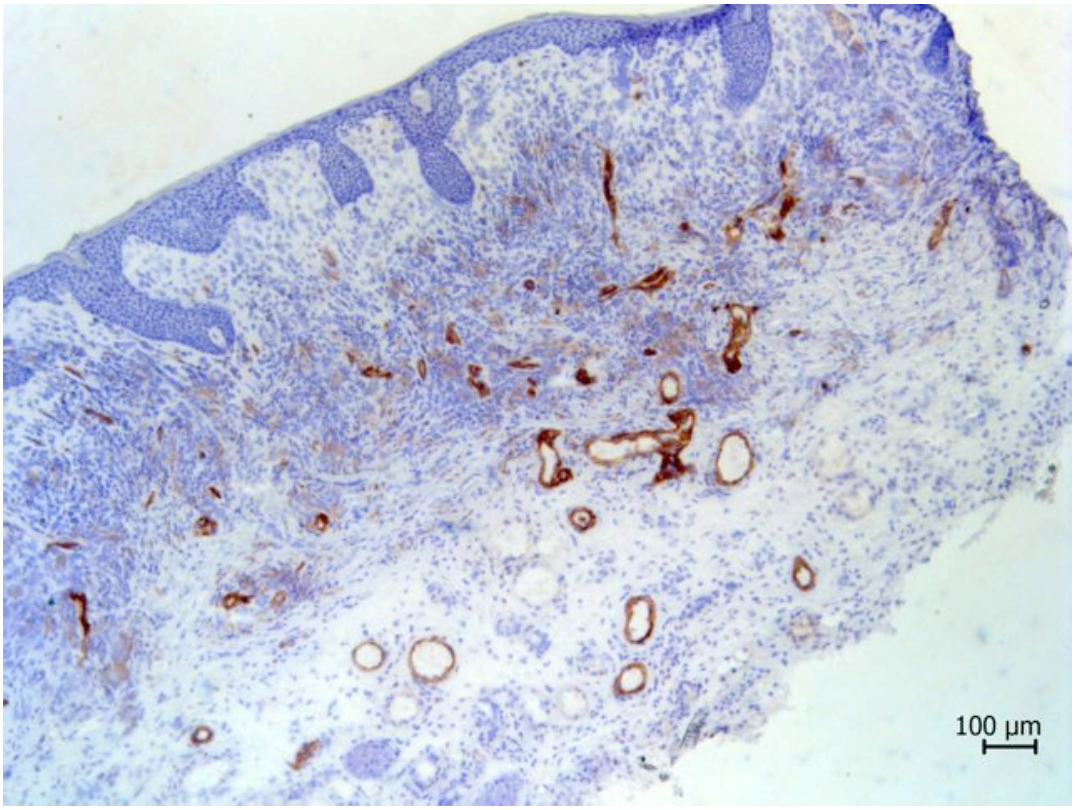


Figure 5.13 cont.

(d)

Human CD31



(e)

Mouse CD31

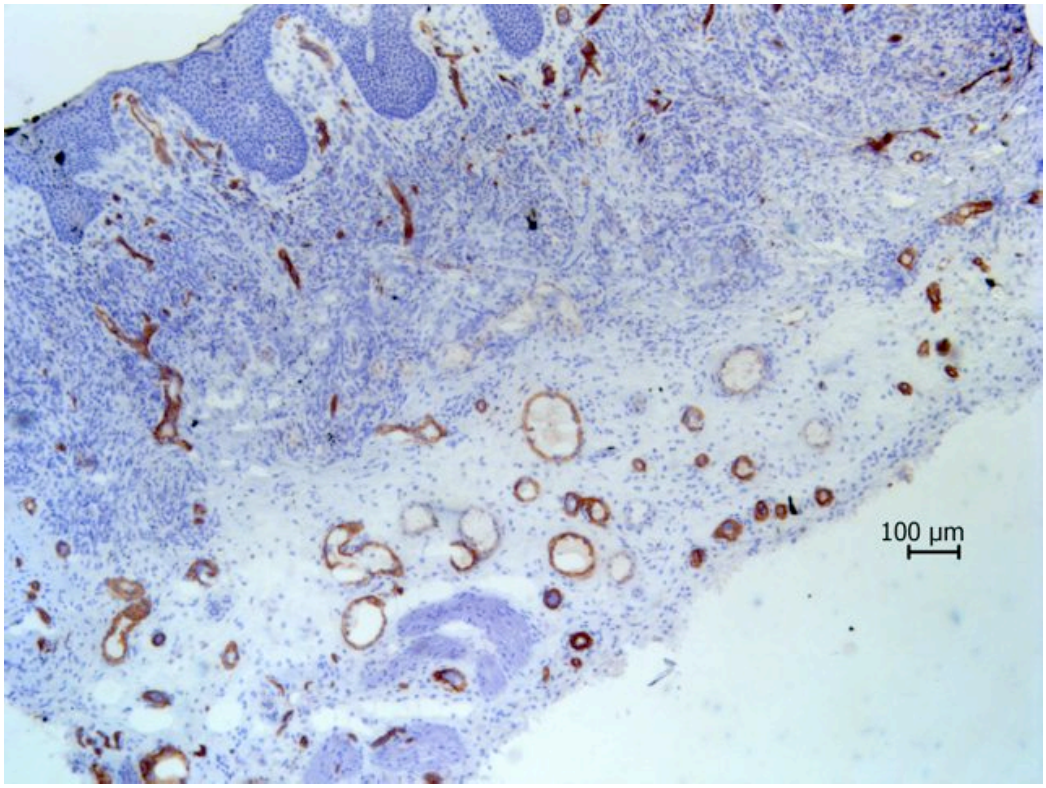


Figure 5.14

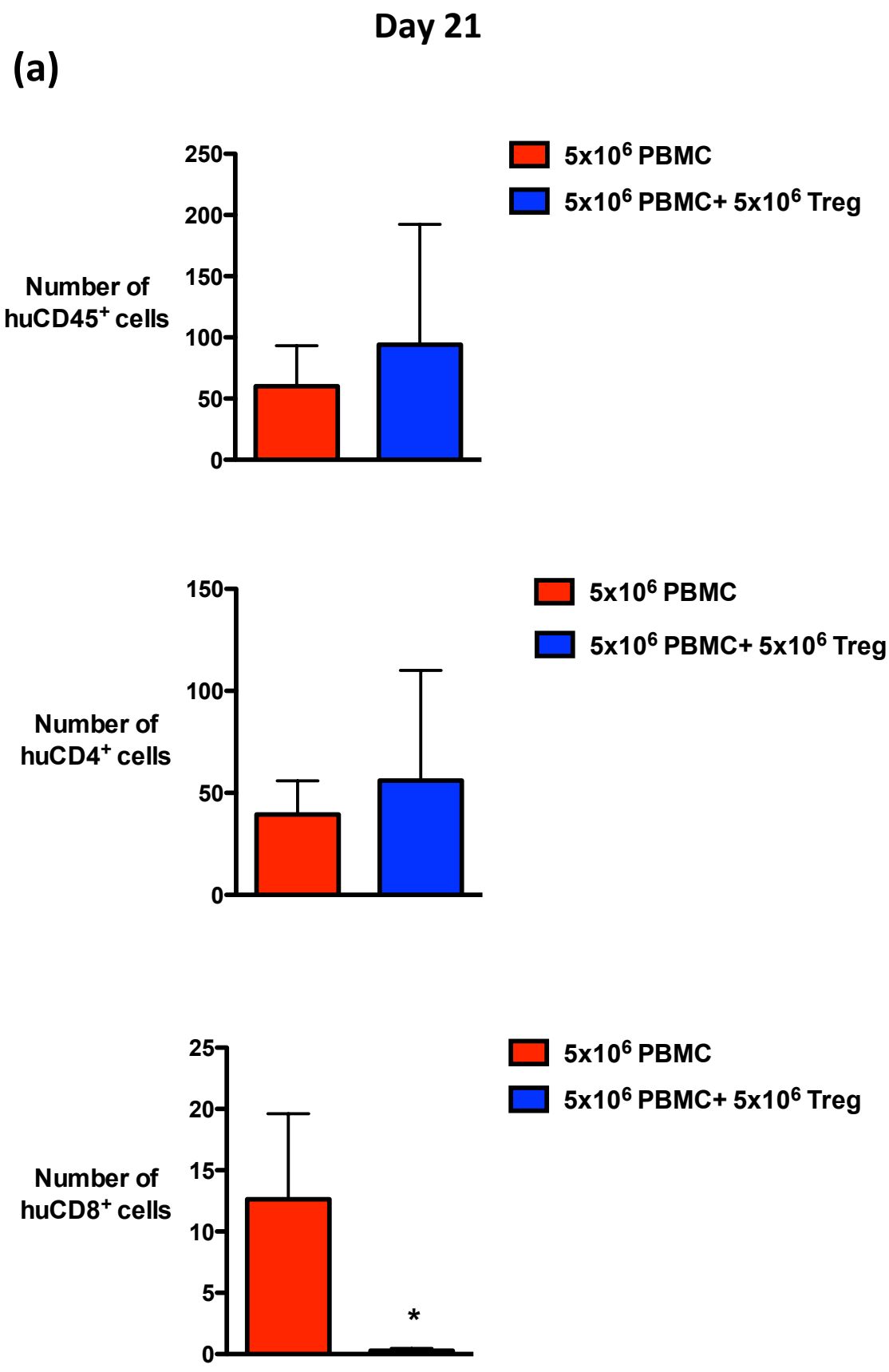

Day 21
(a)
Number of huCD45+ cells
250
200
150
100
50
0
5x10^6 PBMC
5x10^6 PBMC+ 5x10^6 Treg
Number of huCD4+ cells
150
100
50
0
5x10^6 PBMC
5x10^6 PBMC+ 5x10^6 Treg
Number of huCD8+ cells
25
20
15
10
5
0
*
5x10^6 PBMC
5x10^6 PBMC+ 5x10^6 Treg

Figure 5.14 cont.

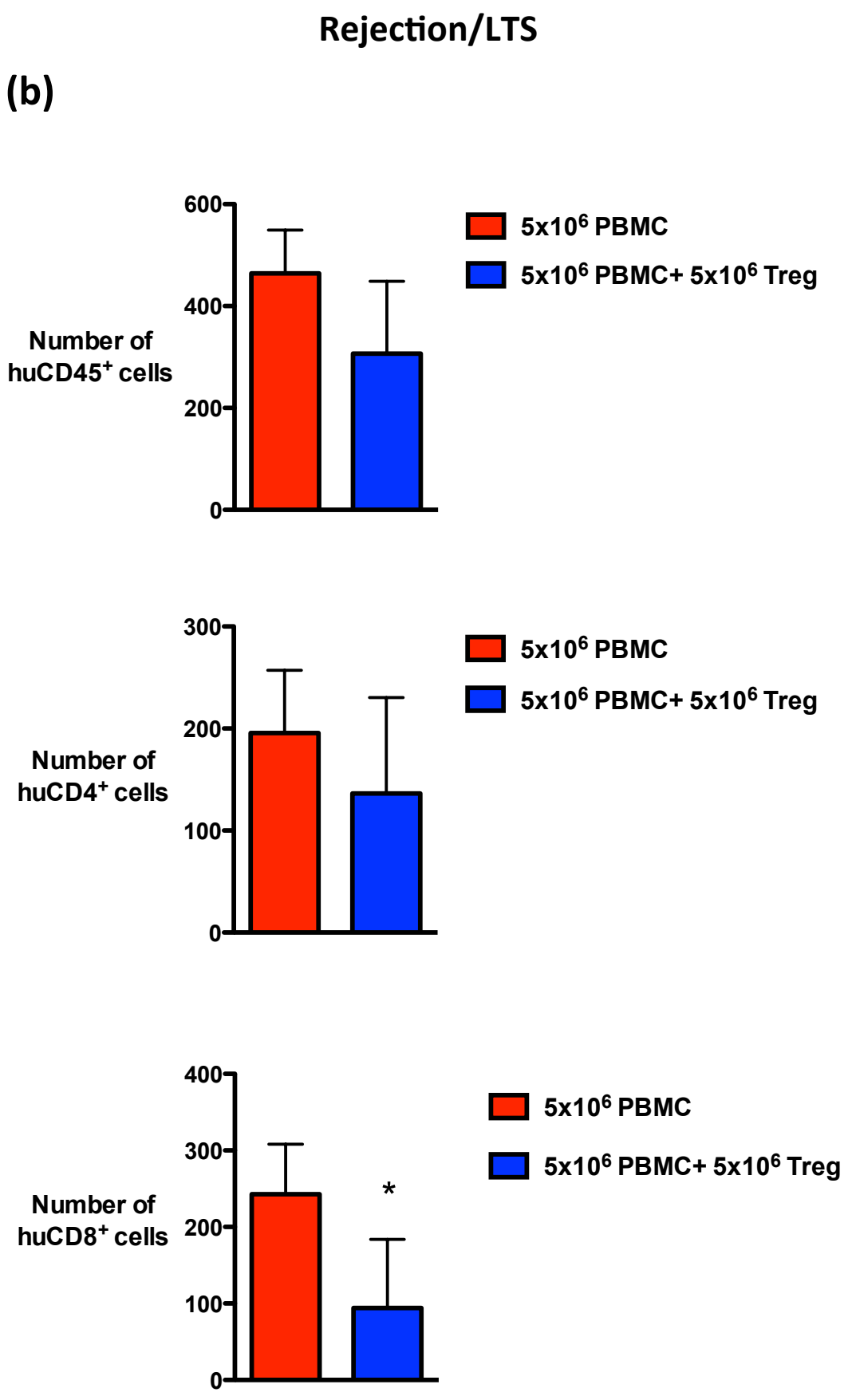


Figure 5.14 cont.

(c)

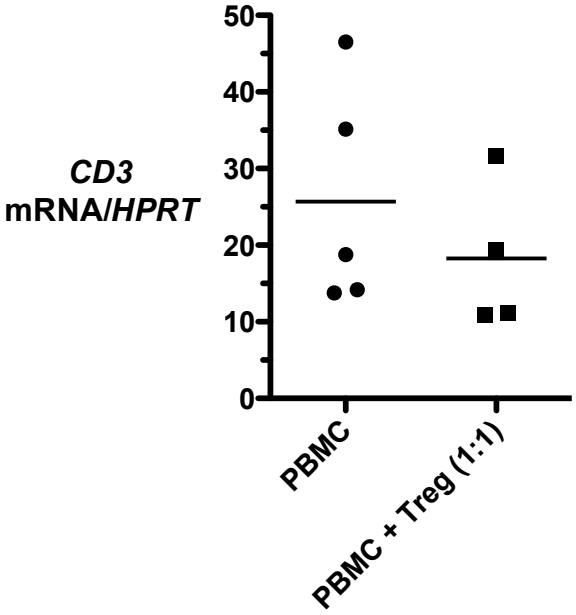


Figure 5.15

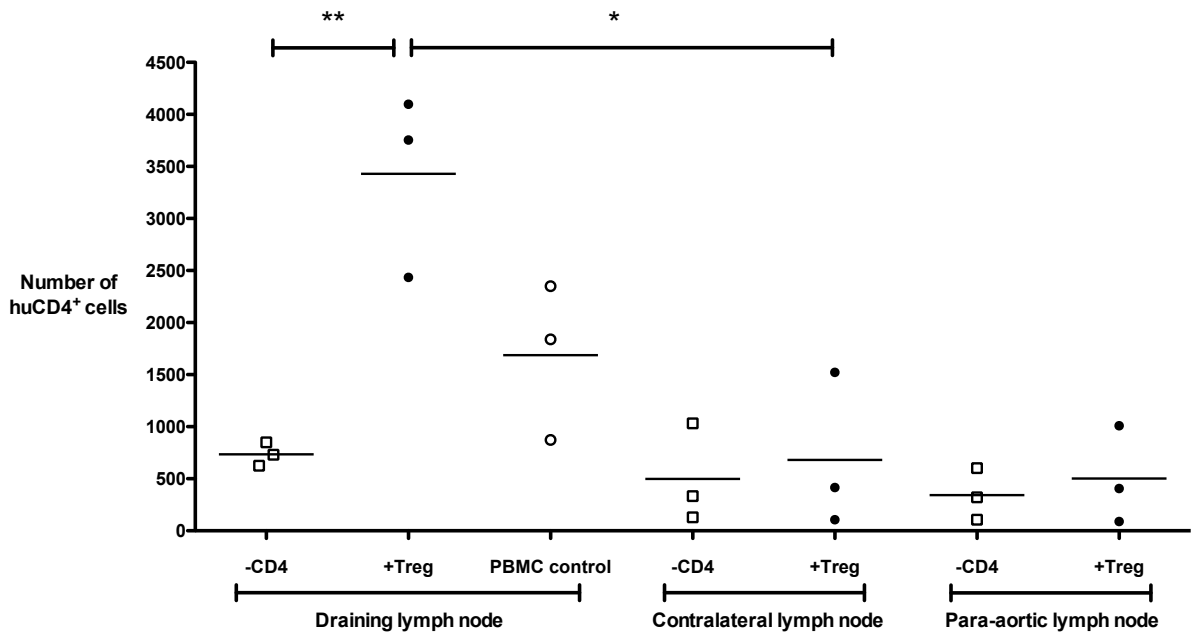
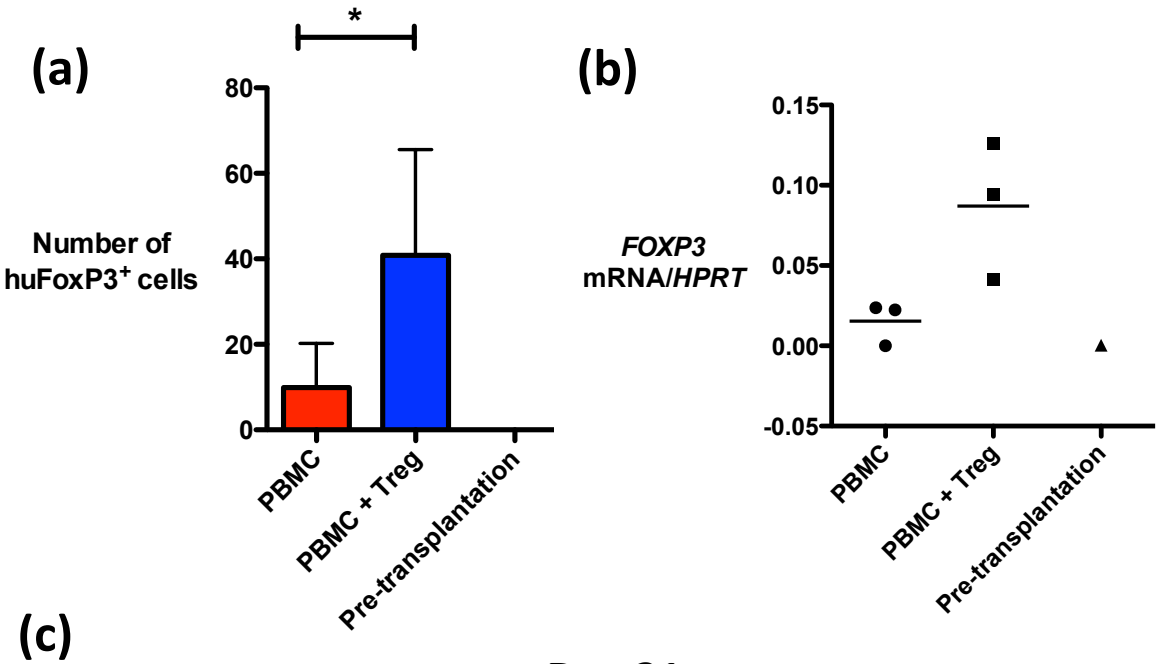

**
*
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Number of huCD4+ cells
-CD4
+Treg
PBMC control
-CD4
+Treg
-CD4
+Treg
Draining lymph node
Contralateral lymph node
Para-aortic lymph node

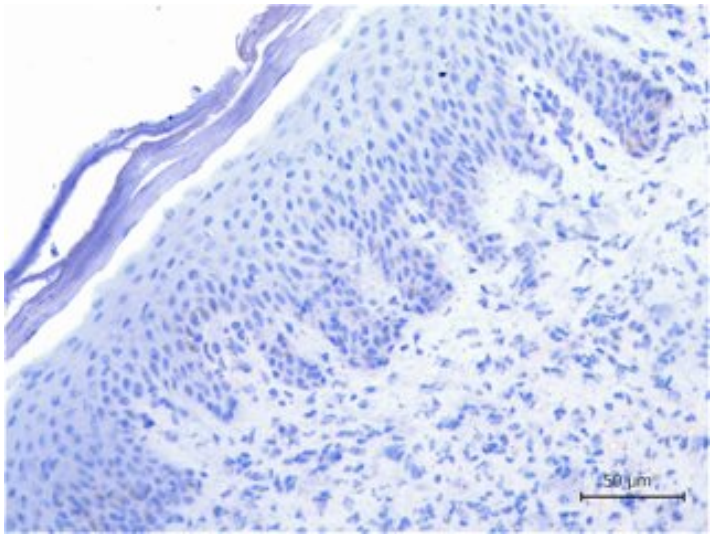
Figure 5.16



(c)

Day 21

PBMC



PBMC + Treg

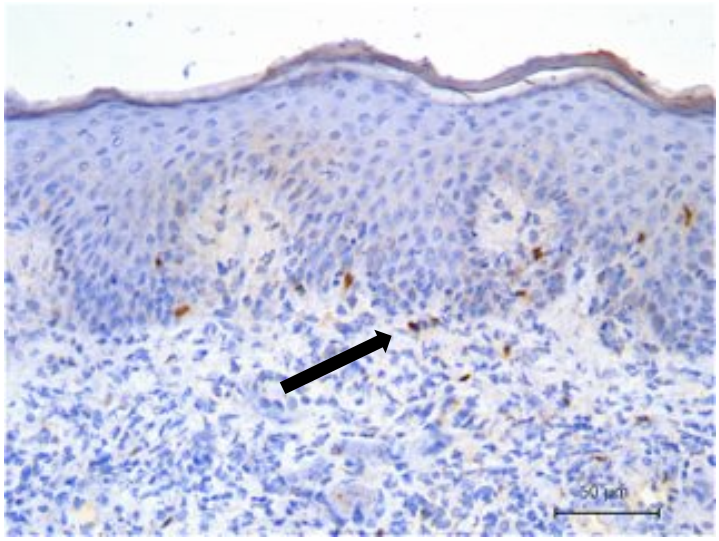
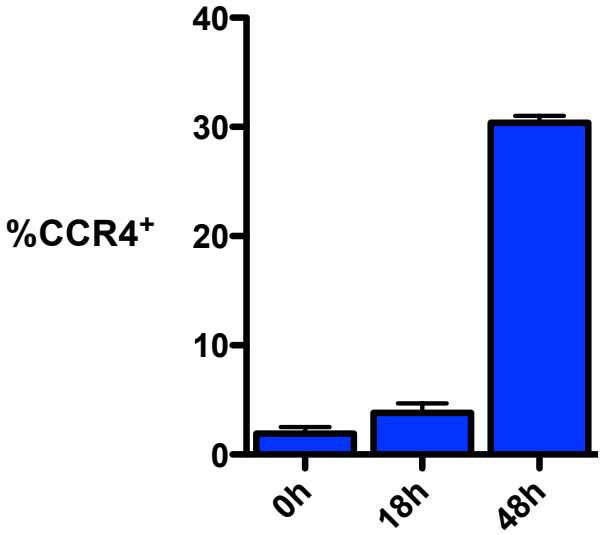


Figure 5.16 cont.

(d)



(e)

Day 100

PBMC + Treg

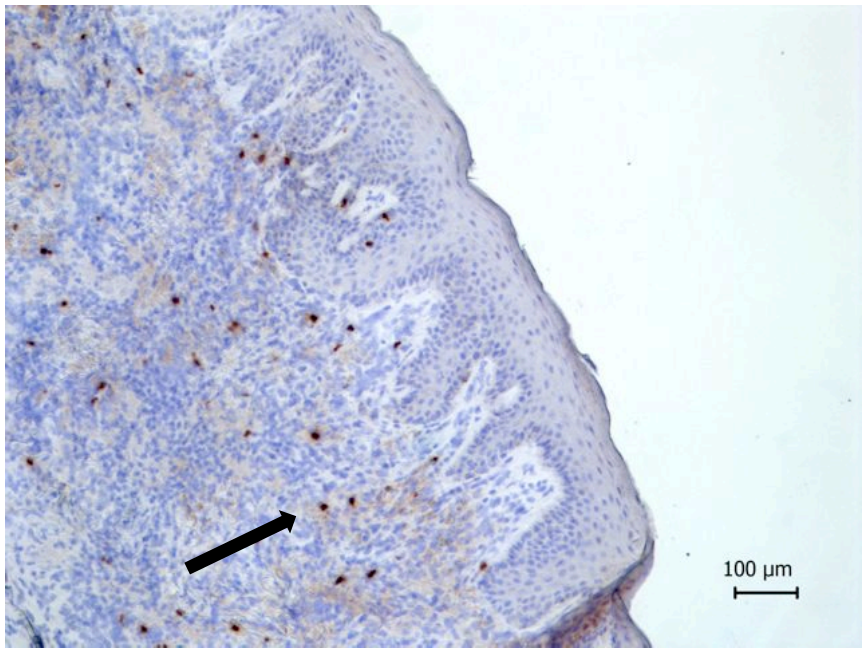
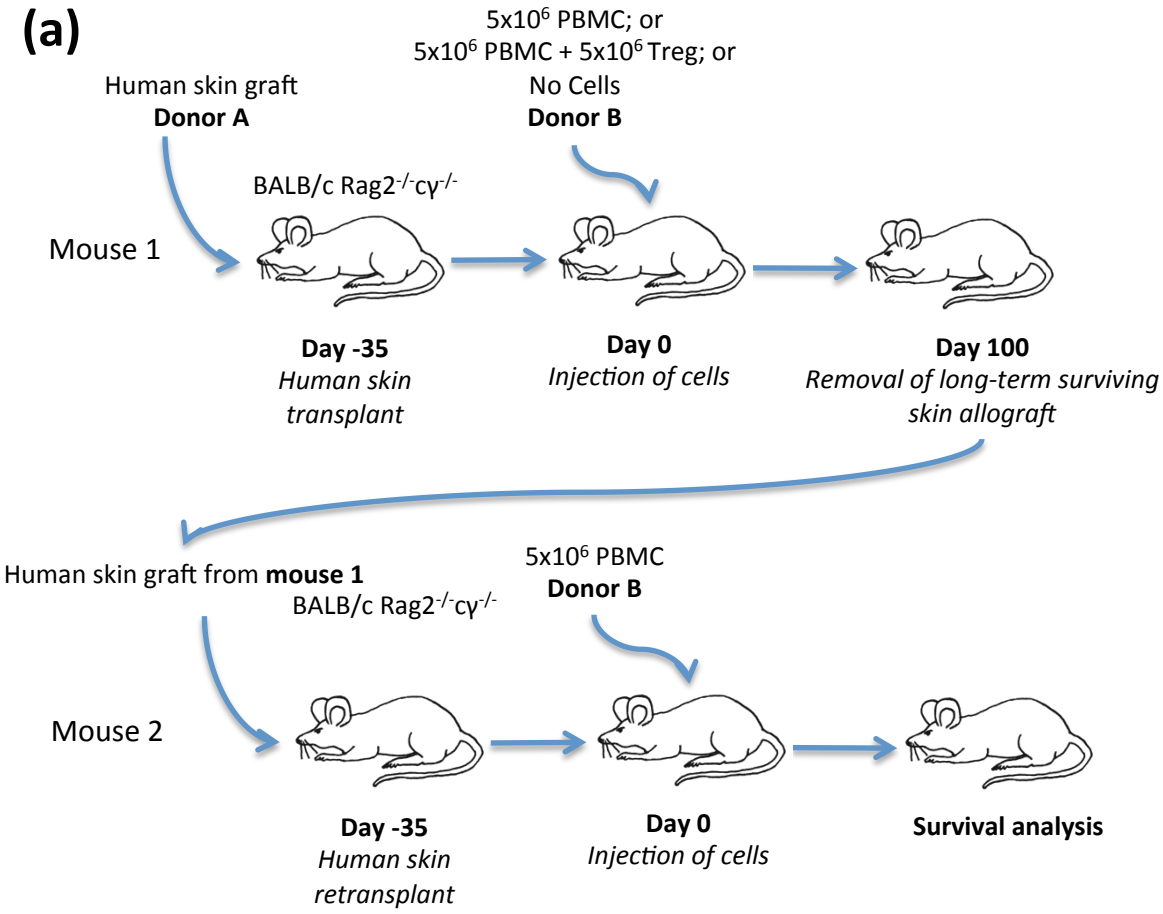
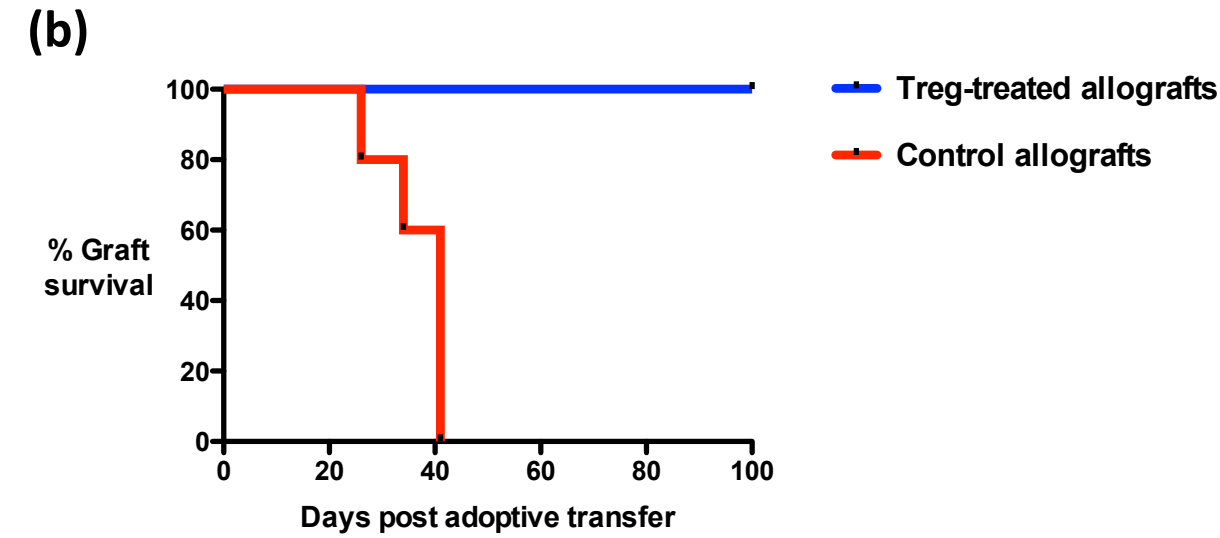


Figure 5.17

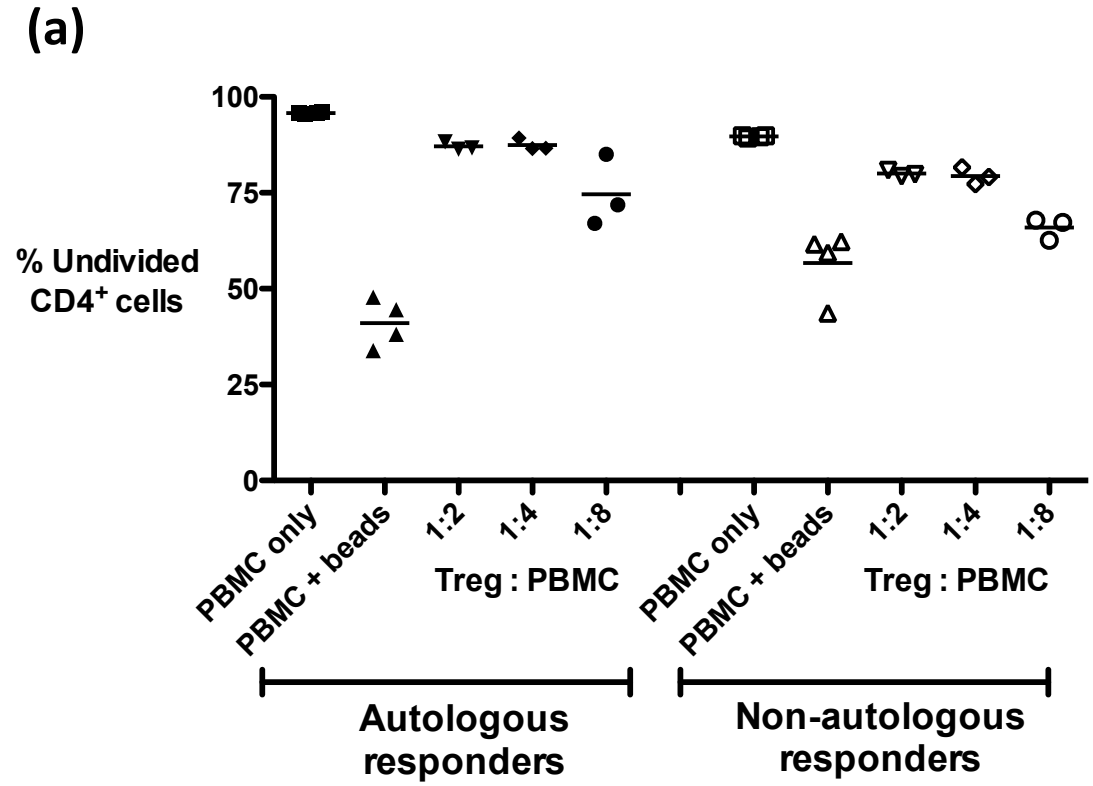




(c)

Group	Group size	Graft loss days	MST (days)
Treg-treated allografts	3	>100, >100, >100	>100
Control allografts	5	26, 34, 41, 41, 41	41

Figure 5.18


(a)
% Undivided CD4+ cells
100
75
50
25
0
PBMC only
PBMC + beads
1:2
1:4
1:8
Treg : PBMC
PBMC only
PBMC + beads
1:2
1:4
1:8
Treg : PBMC
Autologous responders
Non-autologous responders

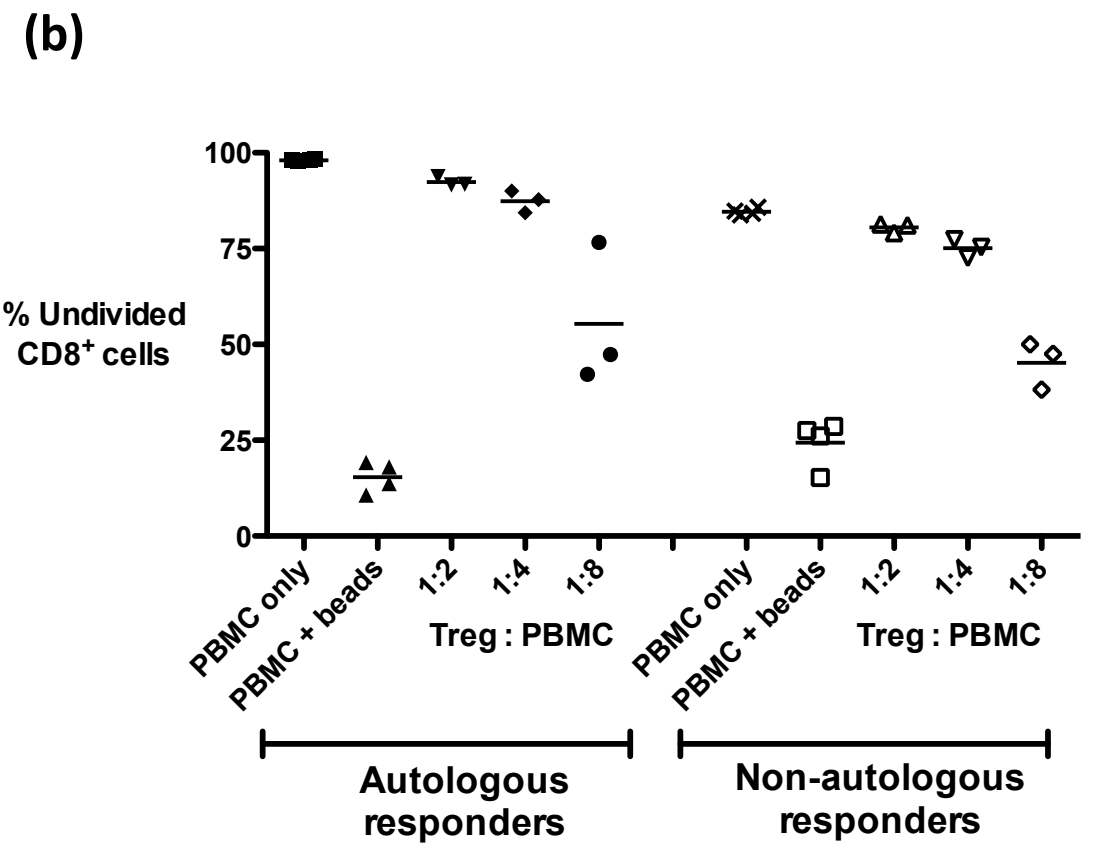

(b)
% Undivided CD8+ cells
100
75
50
25
0
PBMC only
PBMC + beads
1:2
1:4
1:8
Treg : PBMC
PBMC only
PBMC + beads
1:2
1:4
1:8
Treg : PBMC
Autologous responders
Non-autologous responders

Figure 5.18 cont.

(c)

Gated on CD4⁺ cells

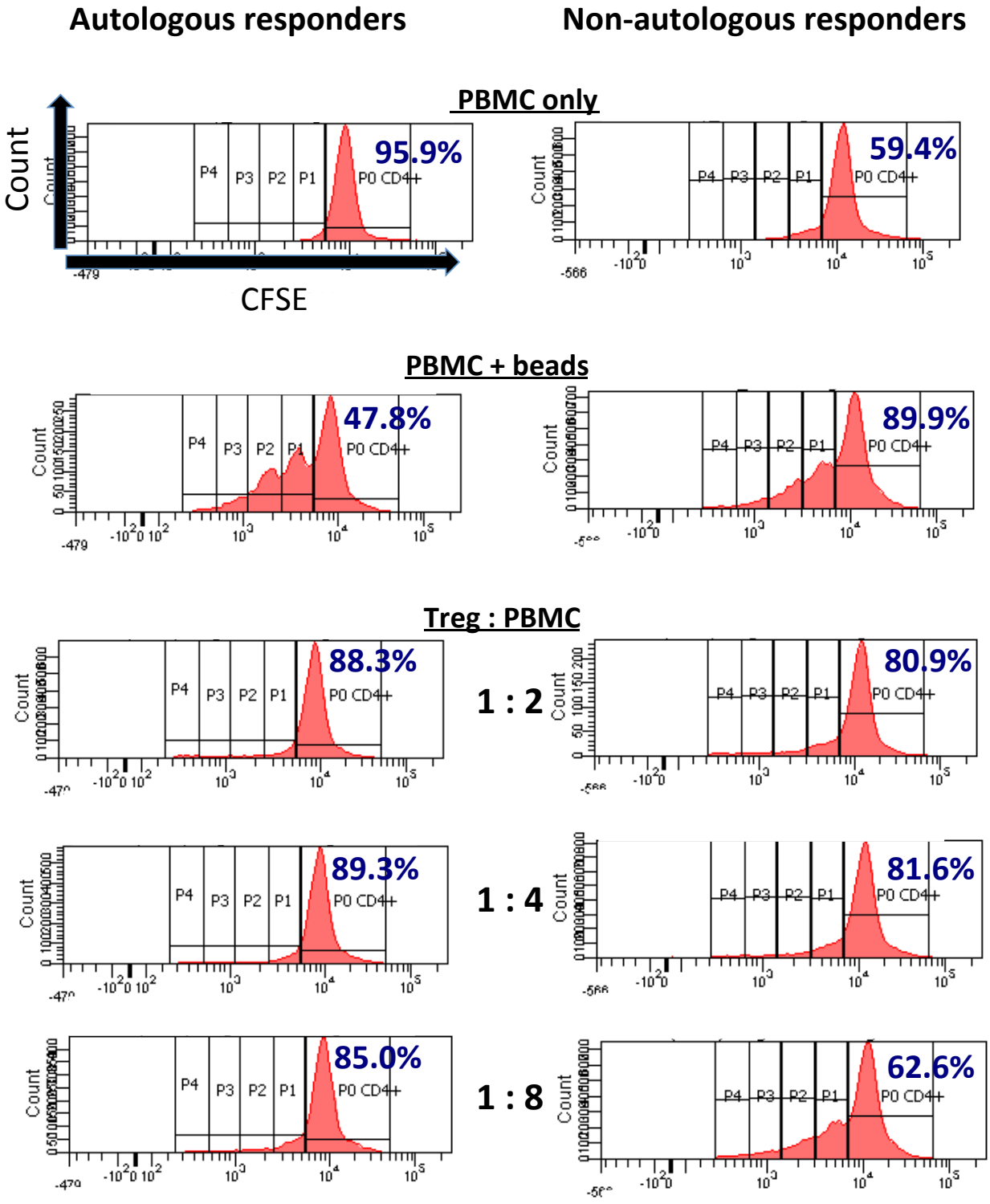


Figure 5.18 cont.

(d)

Gated on CD8⁺ cells

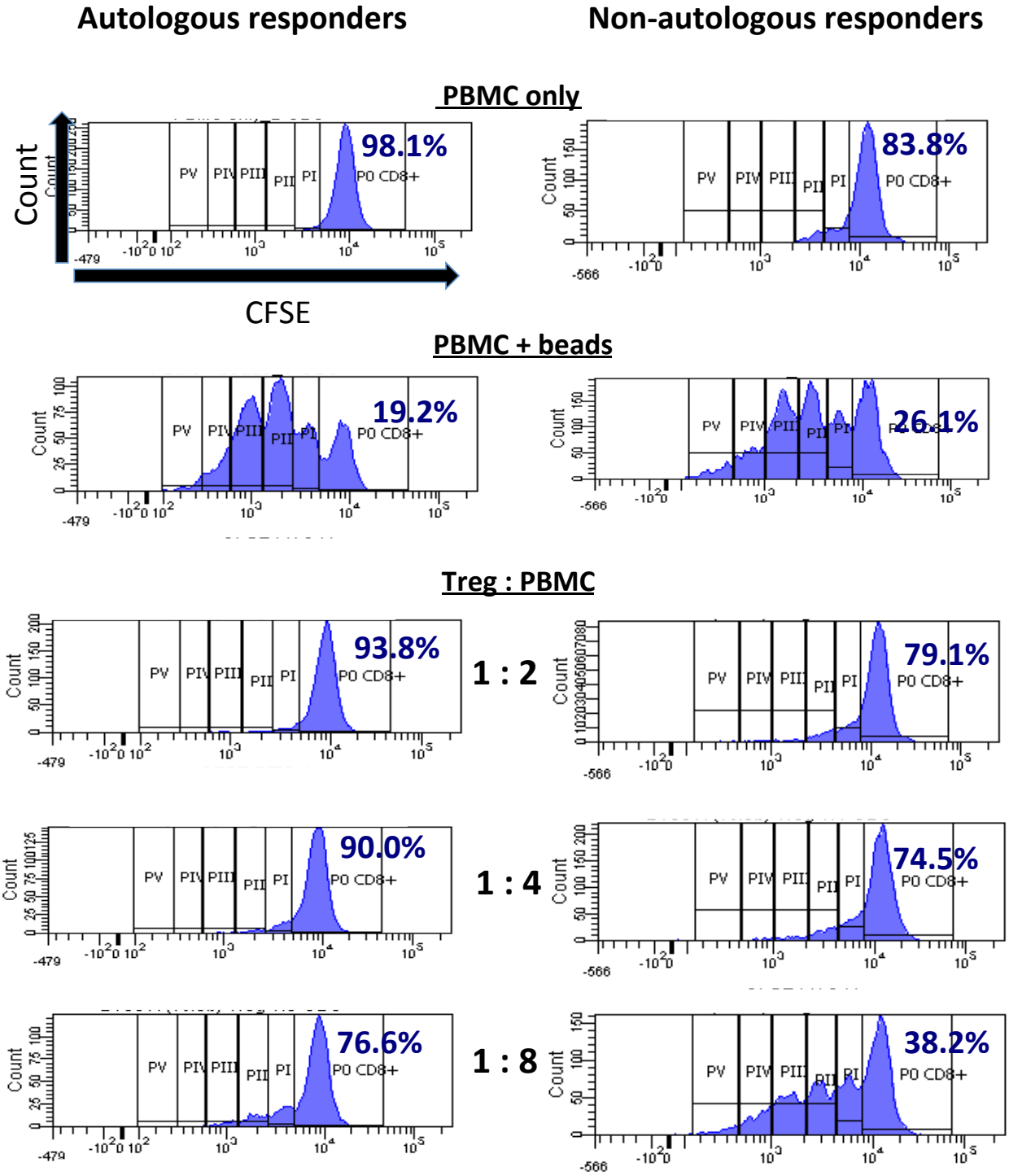
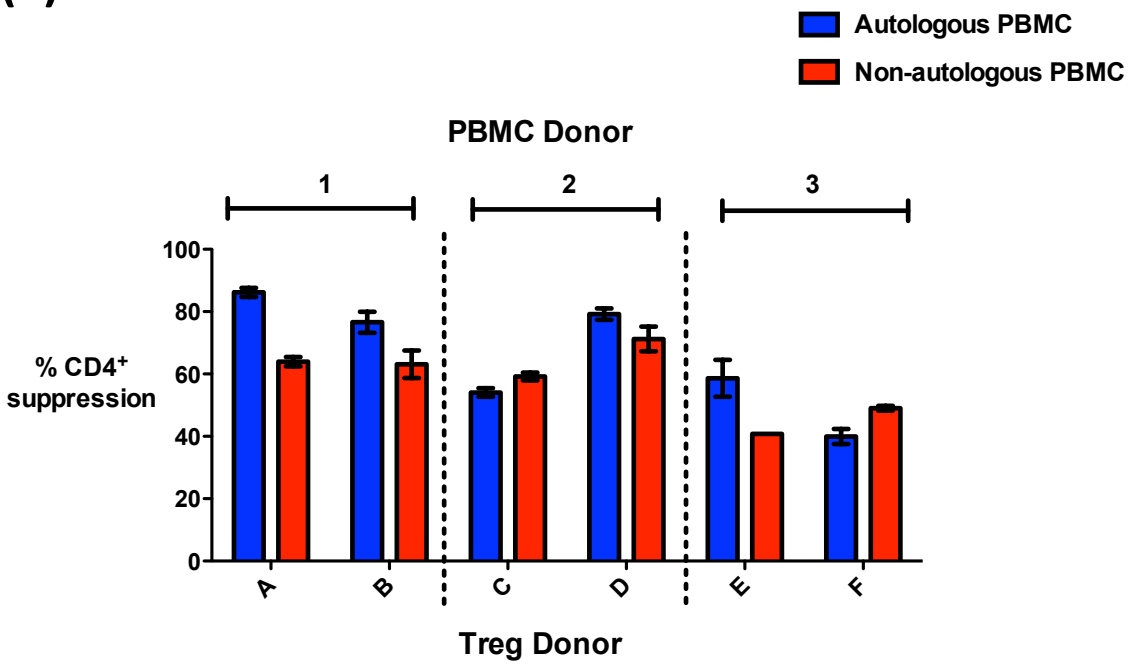


Figure 5.18 cont.

(e)



(f)

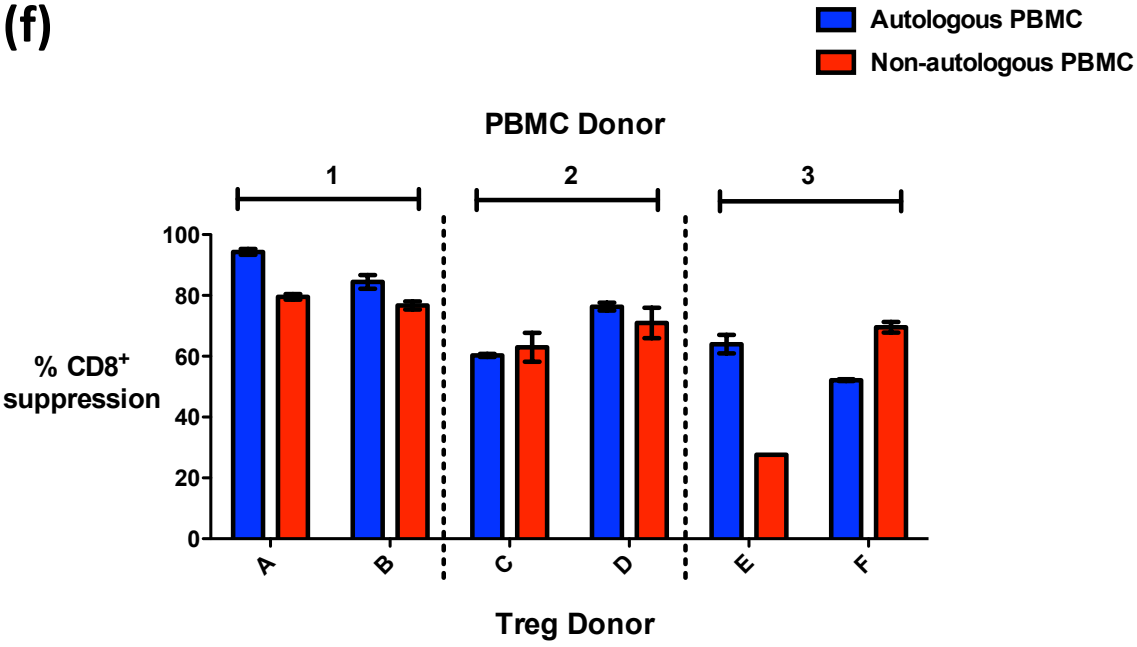
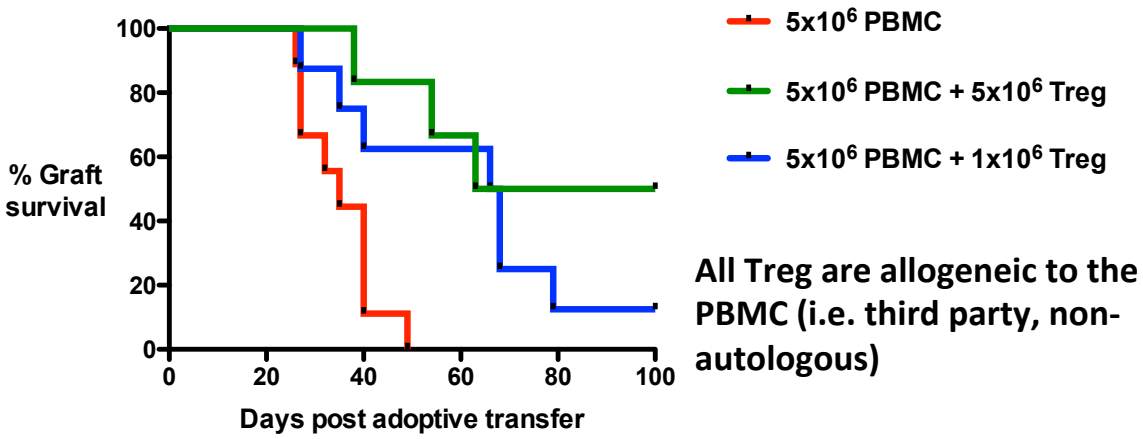


Figure 5.19

(a)



(b)

Group	Group size	Graft loss days	MST (days)
$5x10^6$ PBMC	9	26, 27, 27, 32, 35, 40, 40, 40, 49	35
$5x10^6$ PBMC + $5x10^6$ Treg	6	38, 54, 63, >100, >100, >100	81.5
$5x10^6$ PBMC + $1x10^6$ Treg	8	27, 35, 40, 66, 68, 68, 79, >100	67

(c)

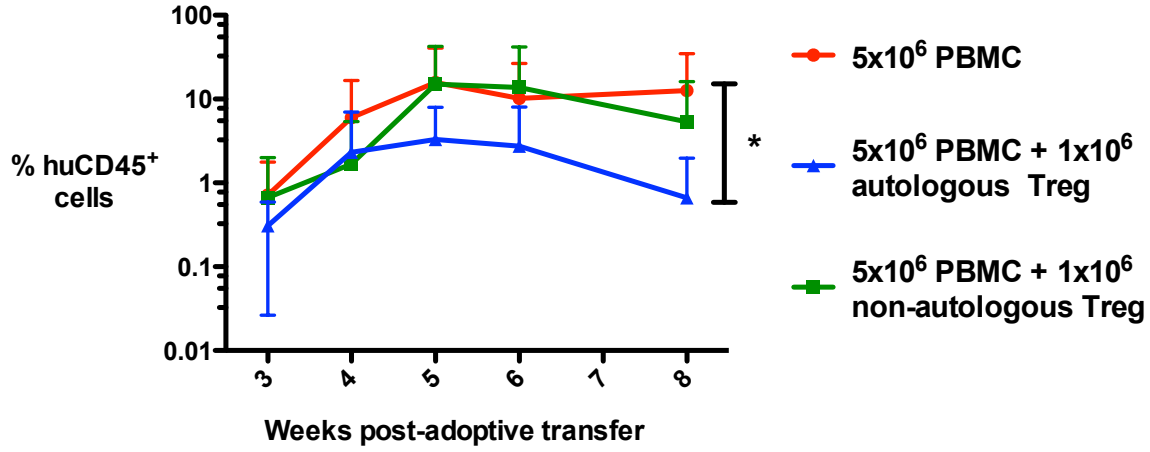
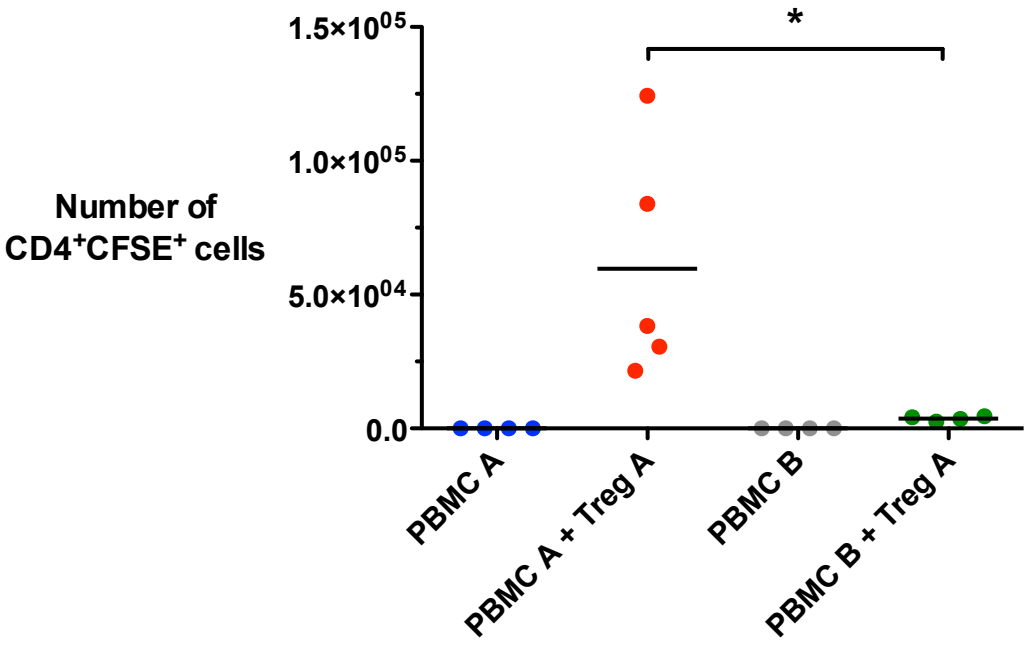


Figure 5.20

(a)



(b)

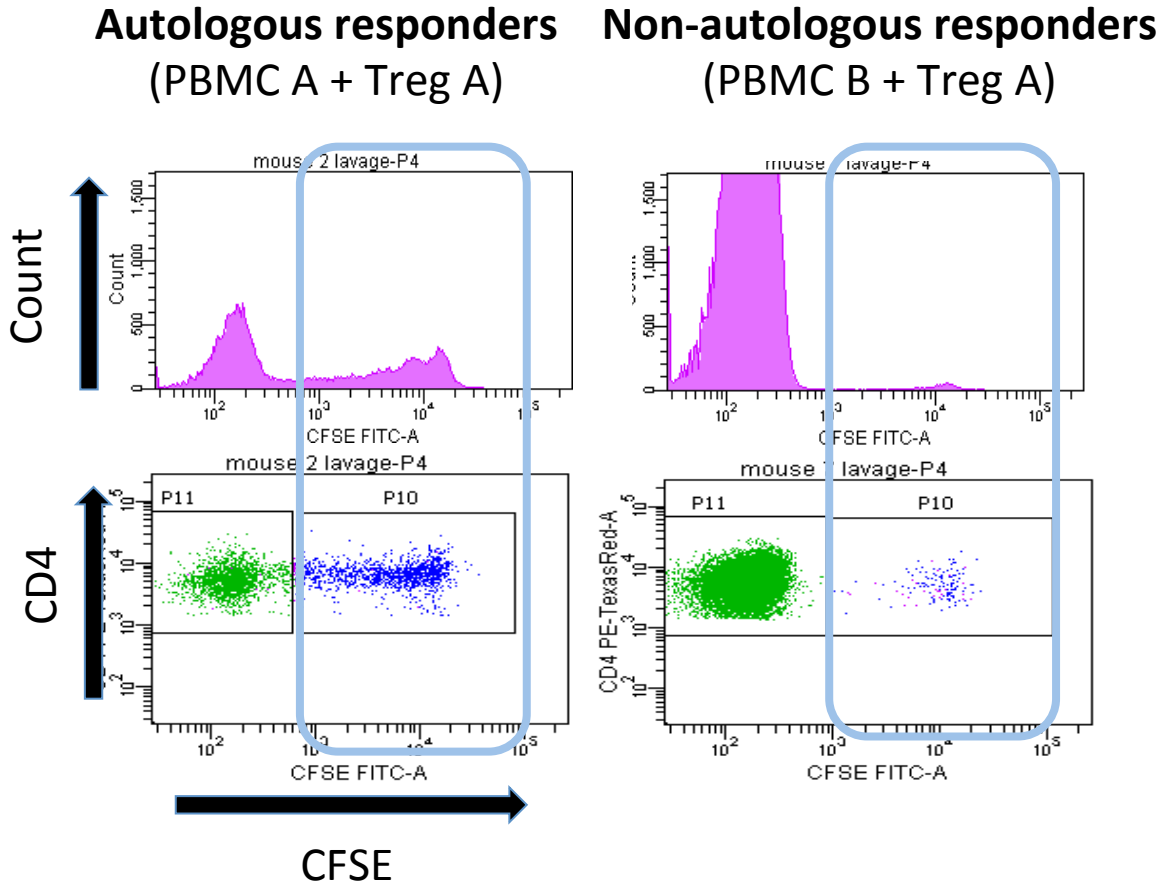
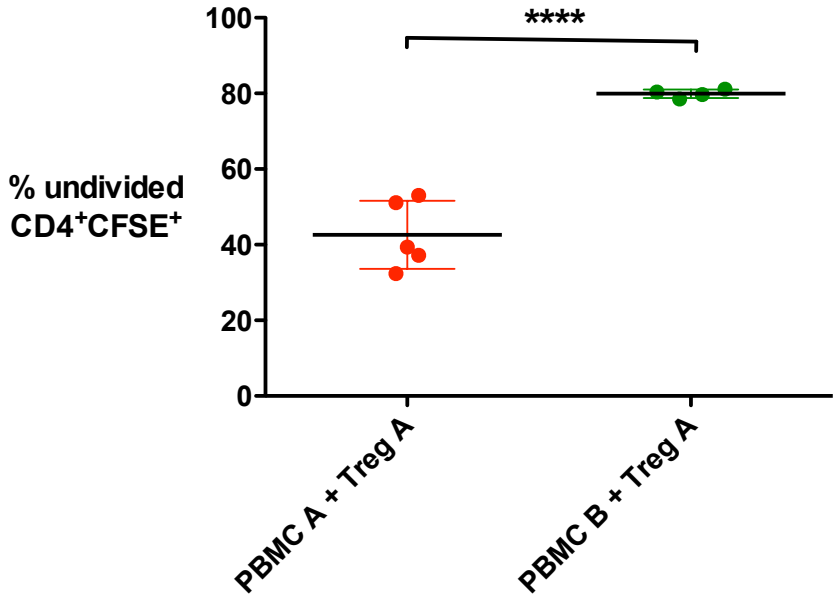


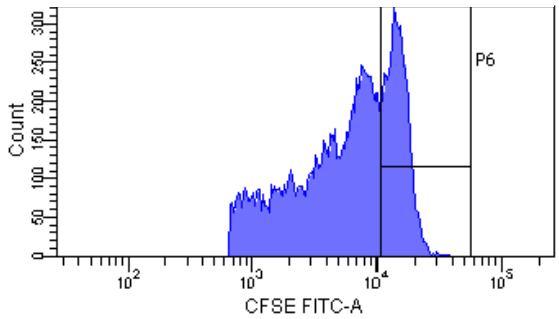
Figure 5.20 cont.

(c)



(d)

Autologous responders
(PBMC A + Treg A)



Non-autologous responders
(PBMC B + Treg A)

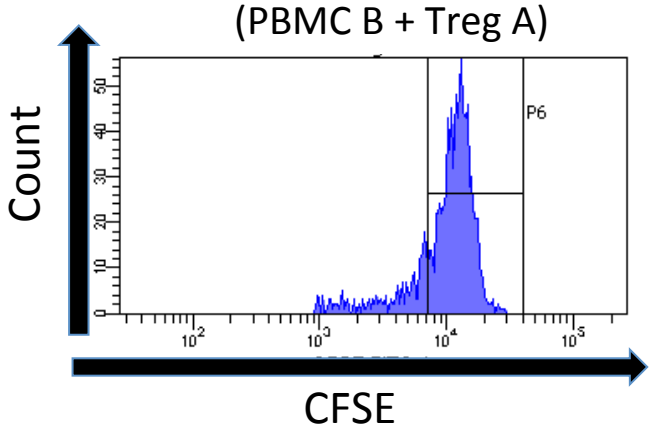
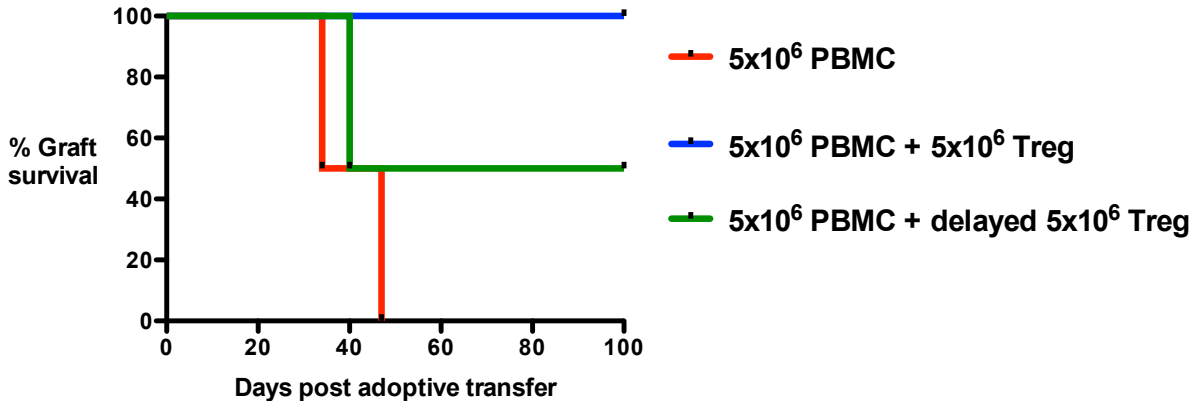


Figure 5.21

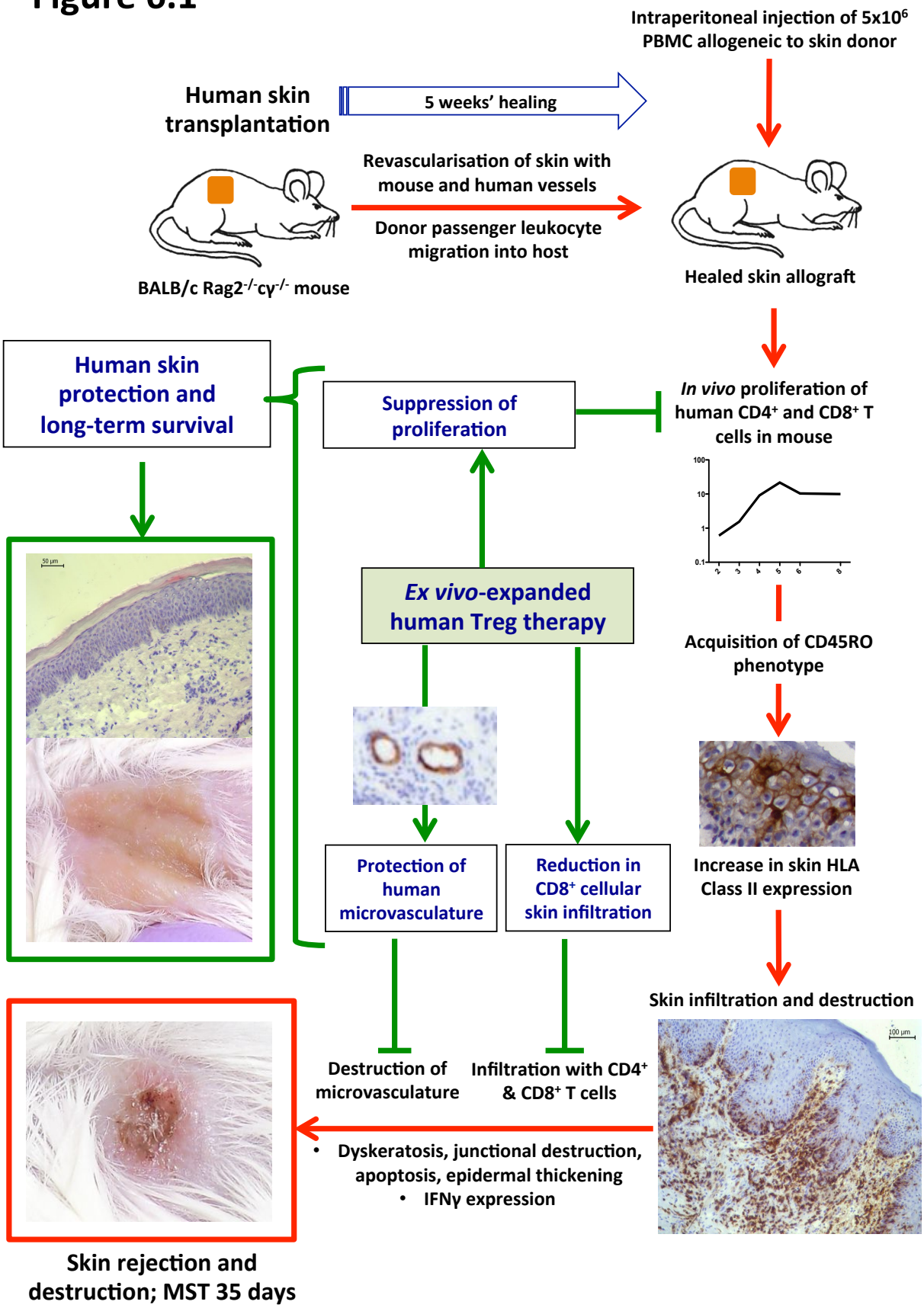
(a)



(b)

Group	Group size	Graft loss days	MST (days)
5x10^6 PBMC	4	34, 34, 47, 47	40.5
5x10^6 PBMC + 5x10^6 Treg	4	>100, >100, >100, >100	>100
5x10^6 PBMC + delayed 5x10^6 Treg	4	40, 40, >100, >100	70

Figure 6.1


Intraperitoneal injection of 5x10⁶ PBMC allogeneic to skin donor
Human skin transplantation
5 weeks' healing
Revascularisation of skin with mouse and human vessels
Donor passenger leukocyte migration into host
BALB/c Rag2-/-cγ-/- mouse
Healed skin allograft
Human skin protection and long-term survival
Suppression of proliferation
In vivo proliferation of human CD4+ and CD8+ T cells in mouse
Ex vivo-expanded human Treg therapy
Acquisition of CD45RO phenotype
Protection of human microvasculature
Reduction in CD8+ cellular skin infiltration
Increase in skin HLA Class II expression
Skin infiltration and destruction
Destruction of microvasculature
Infiltration with CD4+ & CD8+ T cells
• Dyskeratosis, junctional destruction, apoptosis, epidermal thickening
• IFNγ expression
Skin rejection and destruction; MST 35 days