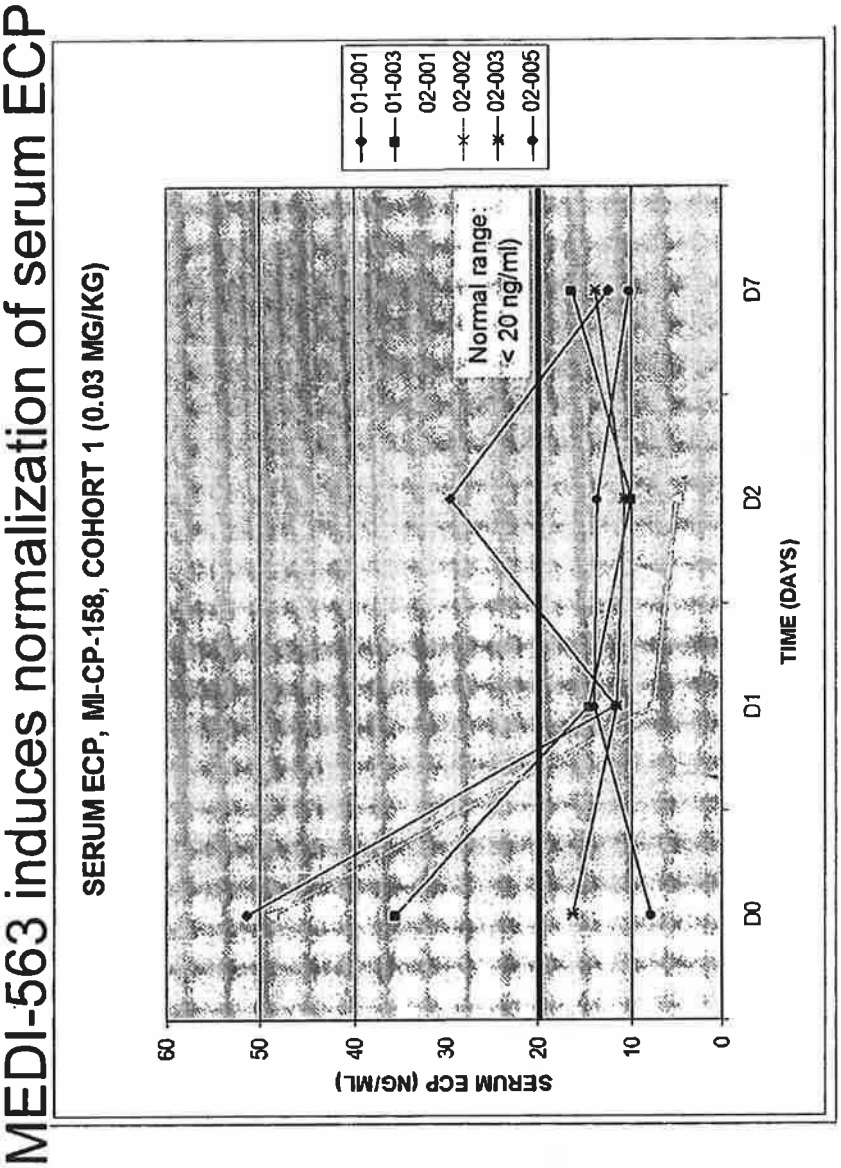

MEDI-563 induces normalization of serum ECP
SERUM ECP, MI-CP-158, COHORT 1 (0.03 MG/KG)
SERUM ECP (NG/ML)
60
50
40
30
20
10
0
Normal range:
< 20 ng/ml)
01-001
01-003
02-001
02-002
02-003
02-005
D0
D1
D2
D7
TIME (DAYS)

Fig. 1

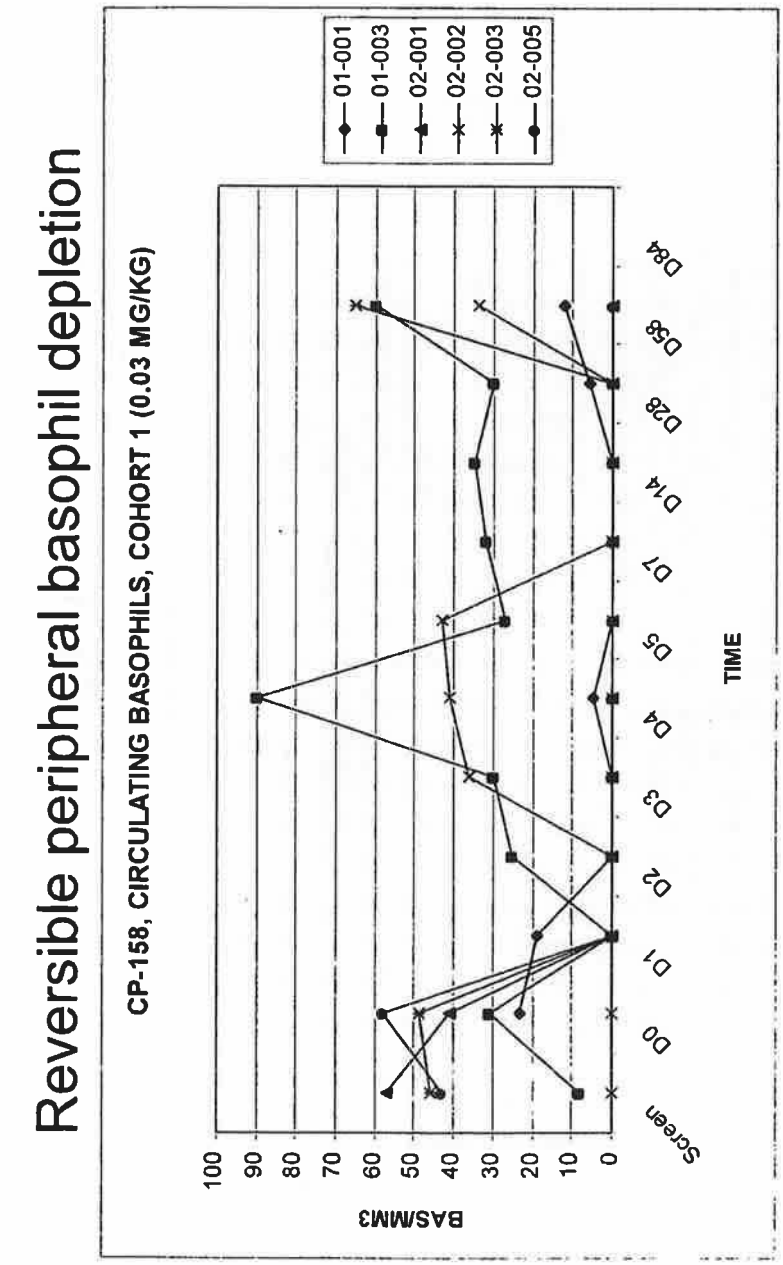
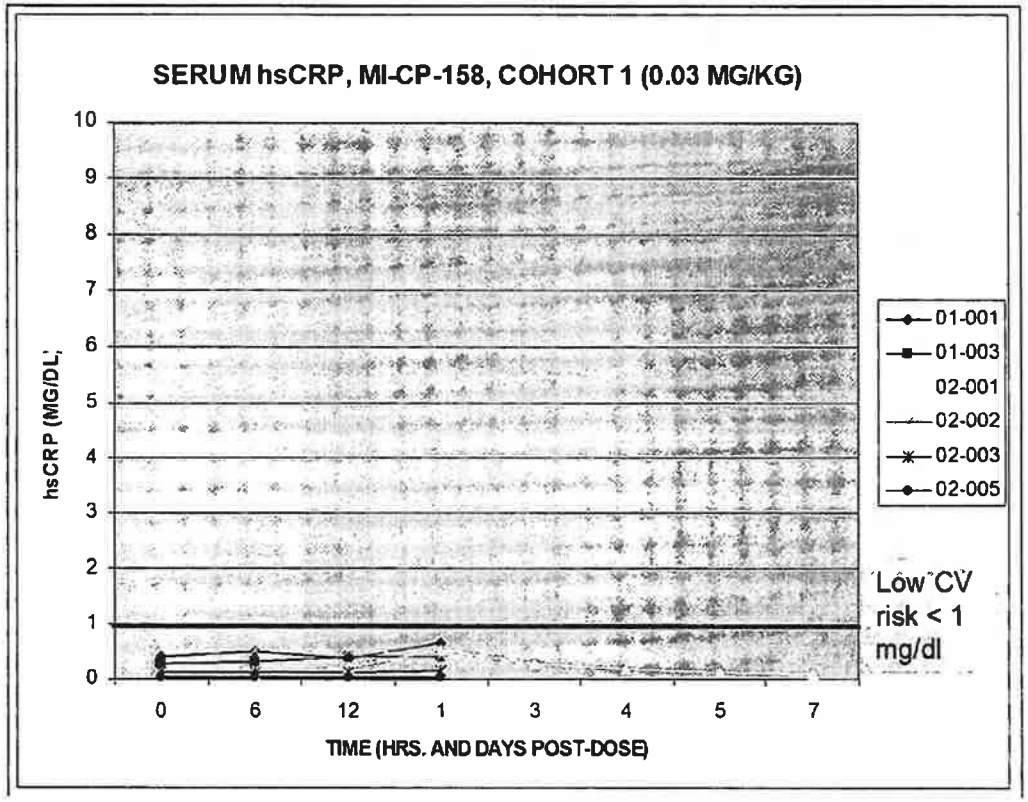

Reversible peripheral basophil depletion
CP-158, CIRCULATING BASOPHILS, COHORT 1 (0.03 MG/KG)
01-001
01-003
02-001
02-002
02-003
02-005
BASMM3
100
90
80
70
60
50
40
30
20
10
0
Screen
D0
D1
D2
D3
D4
D5
D7
D14
D28
D56
D84
TIME

Fig. 2

No increase in serum hsCRP with 0.03 mg/kg of Medi-563



hsCRP:
>10 mg/dl,
inflammation

CV risk (mg/dl):
Low <1
Med 1-3
High 3-10

Cytokine storm:
hsCRP,
10-25 mg/dL

Fig. 3

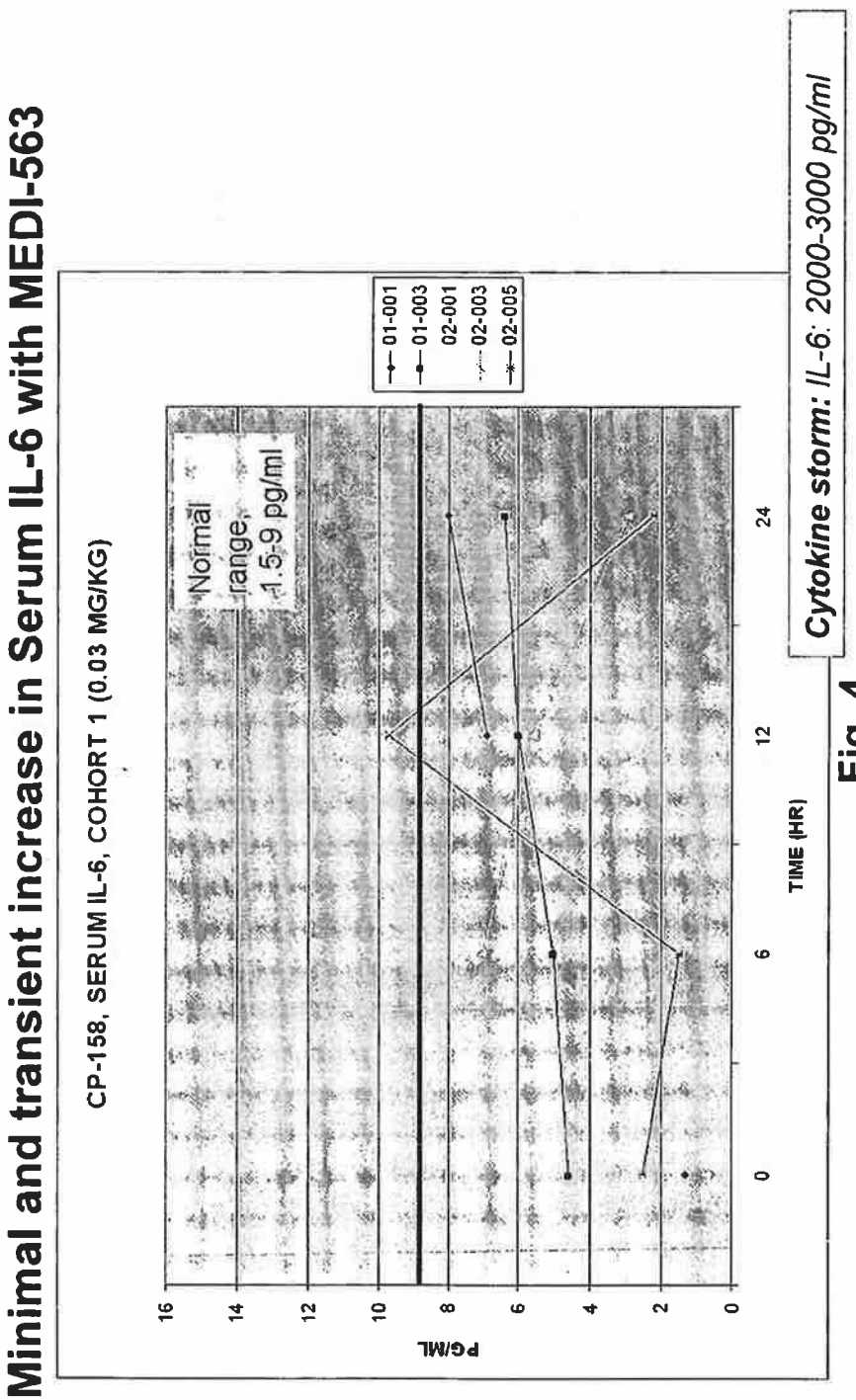

Minimal and transient increase in Serum IL-6 with MEDI-563
CP-158, SERUM IL-6, COHORT 1 (0.03 MG/KG)
Normal range, 1.5-9 pg/ml
01-001
01-003
02-001
02-003
02-005
PG/ML
0
2
4
6
8
10
12
14
16
0
6
12
24
TIME (HR)
Cytokine storm: IL-6: 2000-3000 pg/ml

Fig. 4

Variable decrease of circulating neutrophils

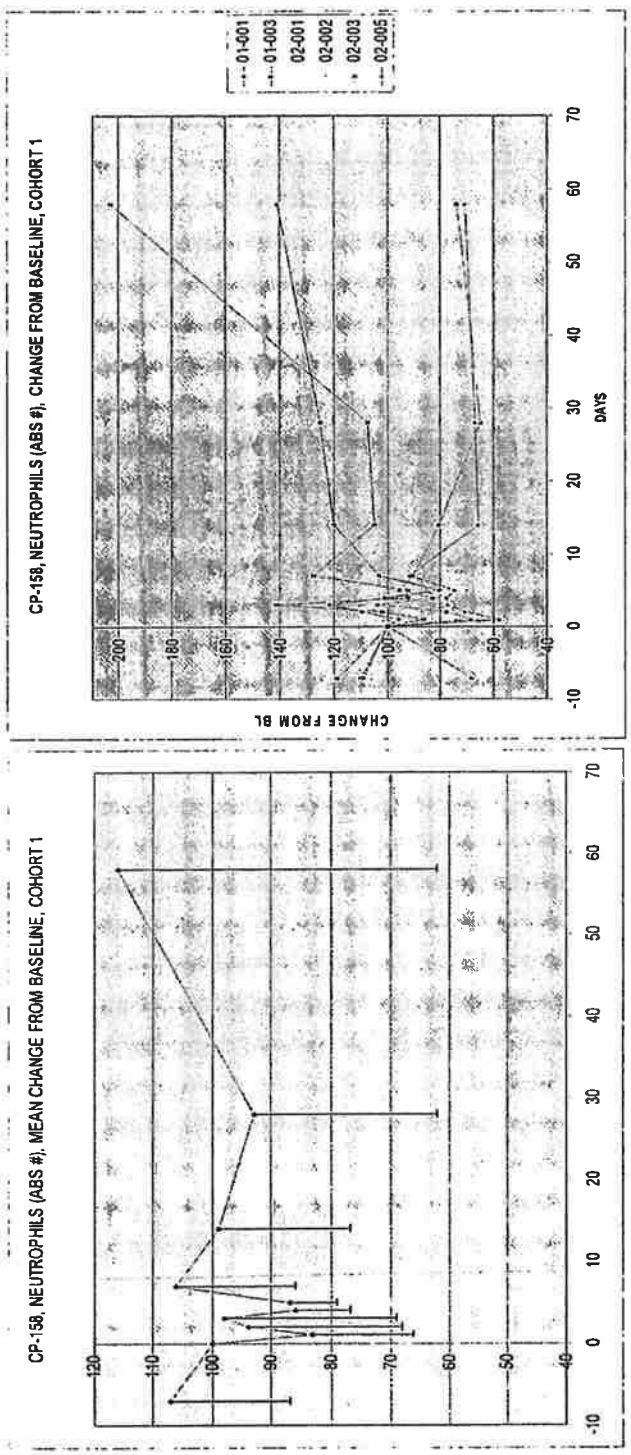


Fig. 5

Variable decrease of circulating lymphocytes

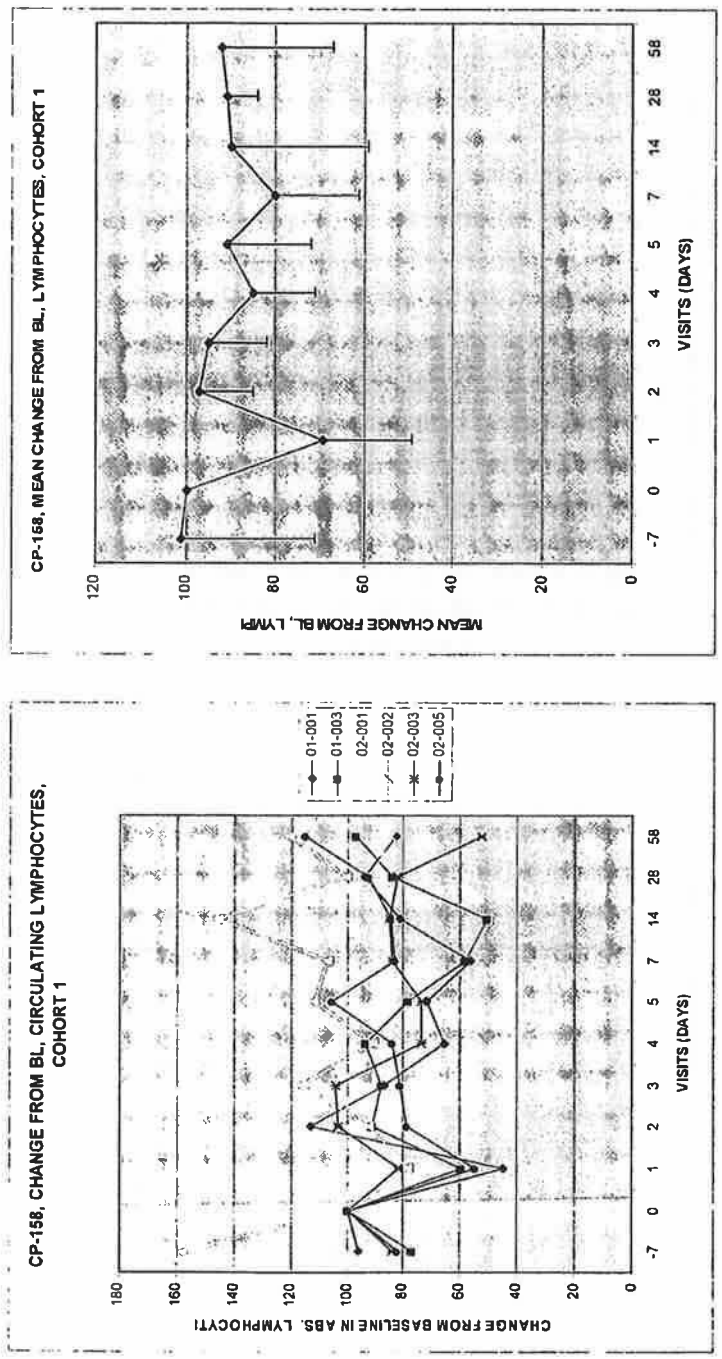


Fig. 6

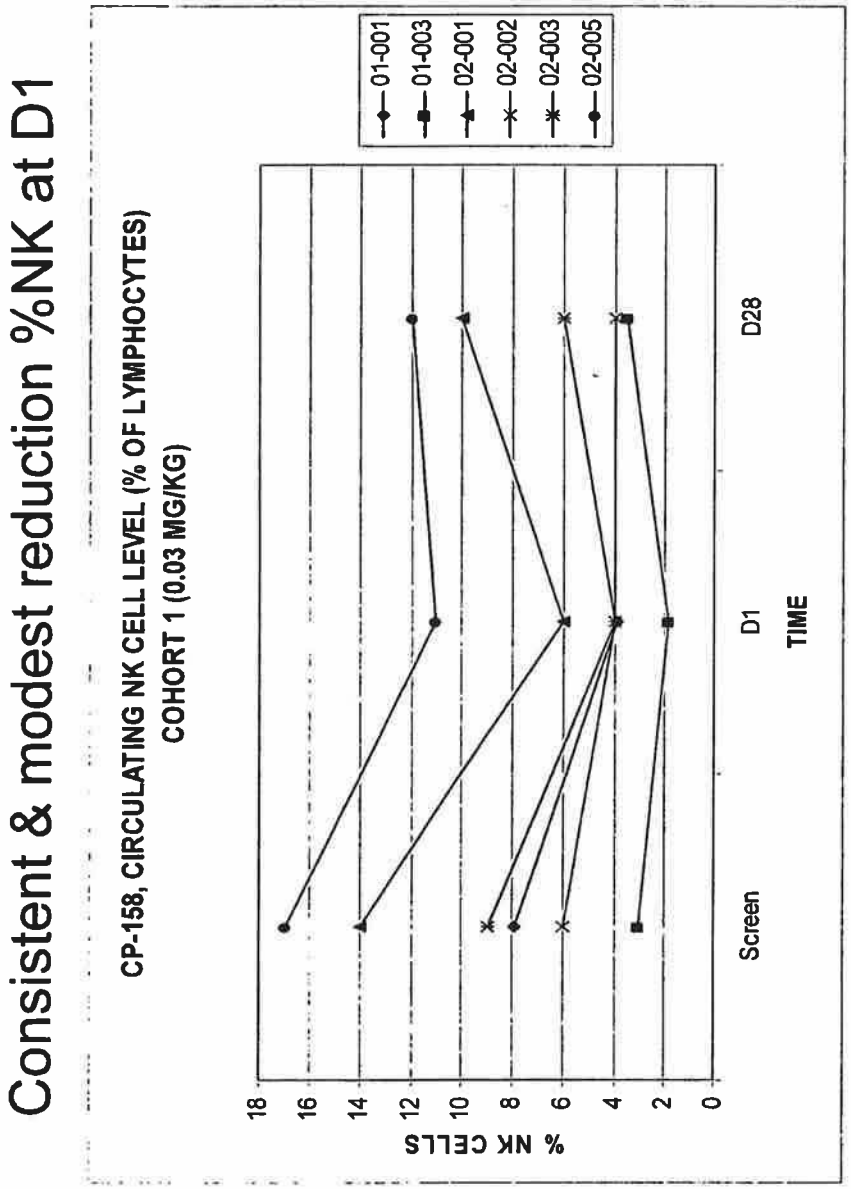

Consistent & modest reduction %NK at D1
CP-158, CIRCULATING NK CELL LEVEL (% OF LYMPHOCYTES)
COHORT 1 (0.03 MG/KG)
01-001
01-003
02-001
02-002
02-003
02-005
% NK CELLS
0
2
4
6
8
10
12
14
16
18
Screen
D1
D28
TIME

Fig. 7

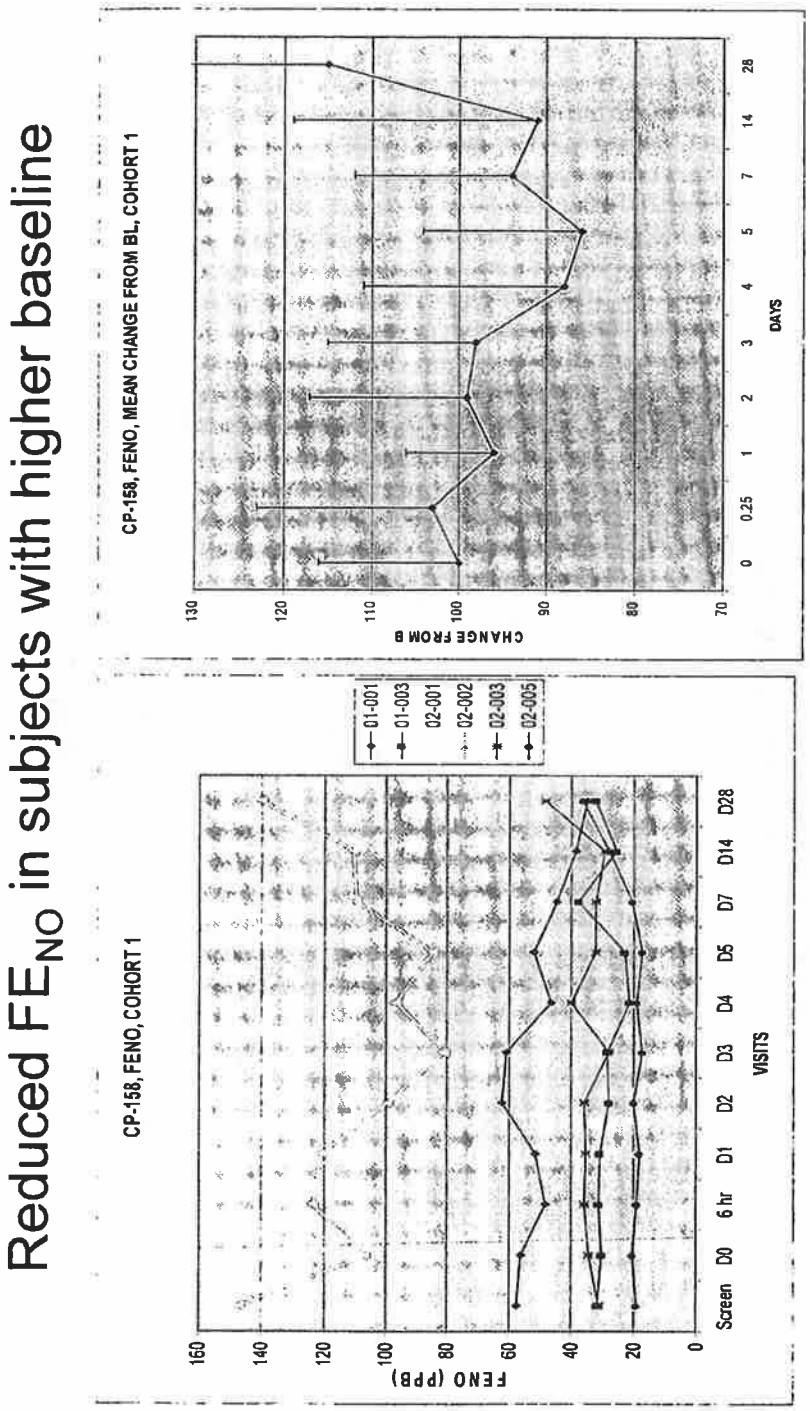

Reduced FE_{NO} in subjects with higher baseline
CP-158, FENO, COHORT 1
FENO (PPB)
160
140
120
100
80
60
40
20
0
Screen
D0
6 hr
D1
D2
D3
D4
D5
D7
D14
D28
VISITS
01-001
01-003
02-001
02-002
02-003
02-005
CP-158, FENO, MEAN CHANGE FROM BL, COHORT 1
CHANGE FROM B
130
120
110
100
90
80
70
0
0.25
1
2
3
4
5
7
14
28
DAYS

Fig. 8

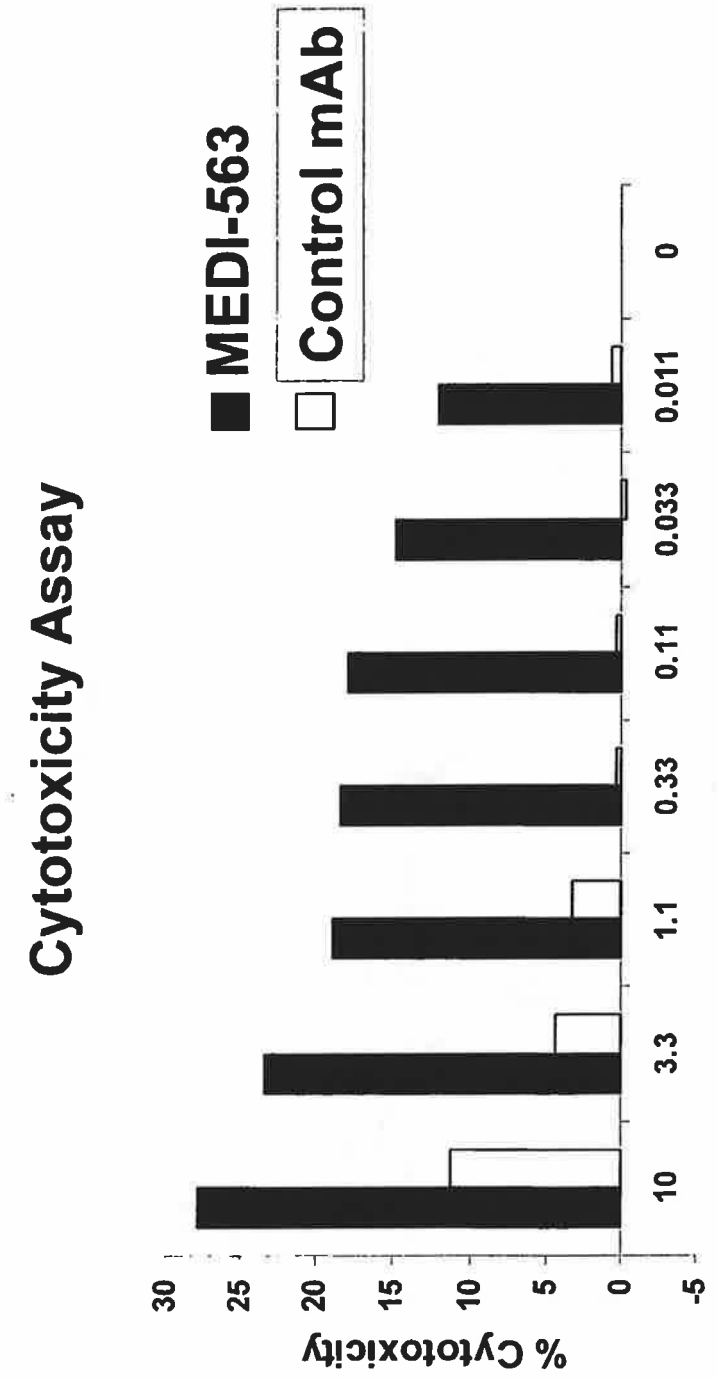

Cytotoxicity Assay
MEDI-563
Control mAb
% Cytotoxicity
30
25
20
15
10
5
0
-5
10
3.3
1.1
0.33
0.11
0.033
0.011
0
µg/ml Ab
KC1333 effector : CTLL2 target in 5 : 1 ratio
LDH readout at four hour timepoint

Fig. 9A

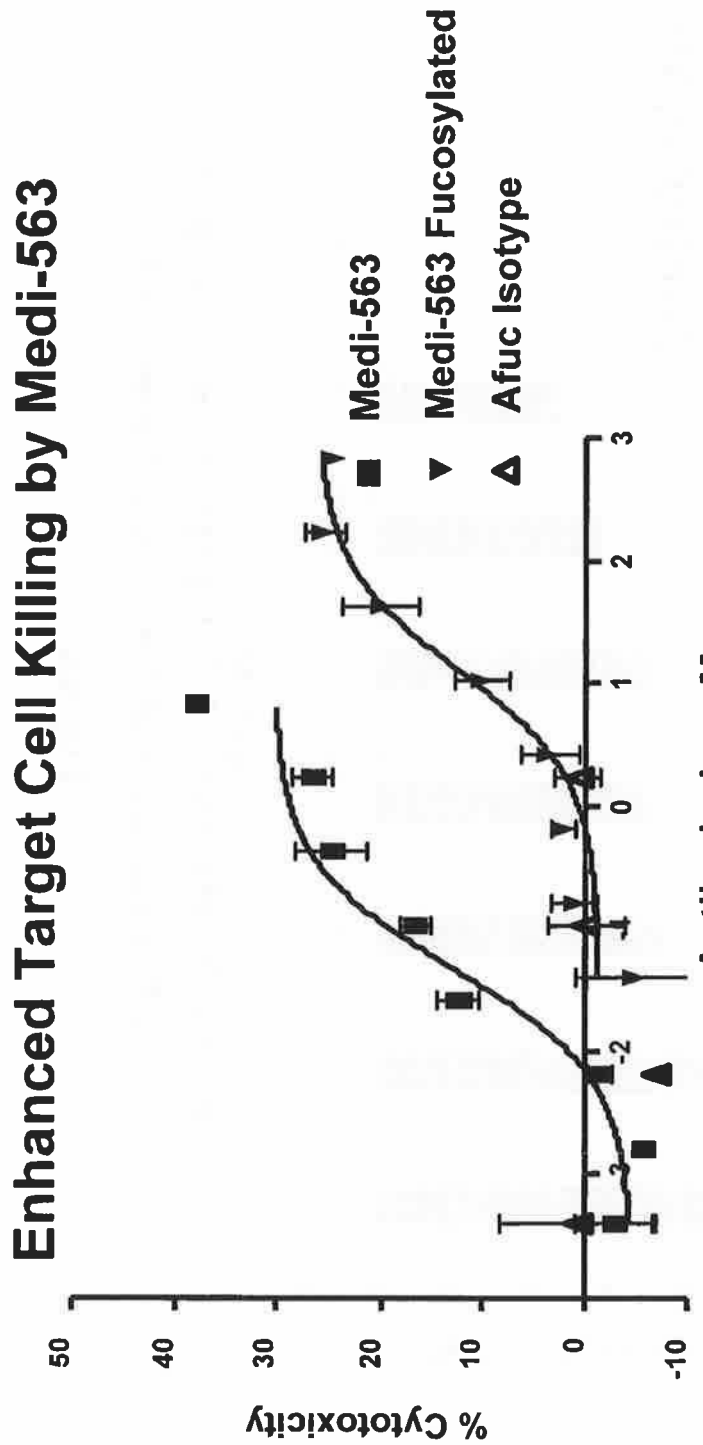

Enhanced Target Cell Killing by Medi-563
% Cytotoxicity
50
40
30
20
10
0
-10
-2
0
1
2
3
Antibody, log nM
Medi-563
Medi-563 Fucosylated
Afuc Isotype
Medi-563 EC50 = 40 pM
Fucosylated EC50 = 10 nM
~ 250 fold

Fig. 9B

MEDI-563 Binding to rhuIL-5Ra

	Format	Instrument	kon (1/Ms)	koff (1/sec)	K_D (nM)
			(xe+5)	(xe-3)	
Experiment 0	Capture	3000	7.4	2.1	2.9
Experiment 1	rhIL-5Ra-down	3000	5.16* (5.06/5.26)	1.11* (0.79/1.42)	2.13* (1.57/2.69)
Experiment 2	Capture	T100	3.03* (2.79/3.27)	1.92* (1.82/2.02)	6.35* (6.52/6.18)

Fig. 10

MEDI-563 Binding to rhuFcγRs

Test Reagent	[Batch]	Fc Receptor	Fc Lot	K_D (nM)
BIW8405	5mg/mL	huFcgRIIb -	5/7/07	5730
BIW8405	5mg/mL	huFcgRIIIA - 158F	5/1/06	4760
BIW8405	5mg/mL	huFcgRIIIA - 158V	R22K ZZ3401-03	46
BIW8405	5mg/mL	huFcRI -	3/7/06	16
BIW8405	5mg/mL	huFcRIIA -	3/7/06	1290
BIW8405	5mg/mL	muFcgRIIb -	KB031407	1100
BIW8405	5mg/mL	muFcgRIII -	KS032307	4930
BIW8405	5mg/mL	muFcgRIV -	KS032307	32
Control	5mg/mL	huFcgRIIb -	5/7/07	14500
Control	5mg/mL	huFcgRIIIA - 158F	5/1/06	0
Control	5mg/mL	huFcgRIIIA - 158V	R22K ZZ3401-03	574
Control	5mg/mL	huFcRI -	3/7/06	19
Control	5mg/mL	huFcRIIA -	3/7/06	1280
Control	5mg/mL	muFcgRIIb -	KB031407	1470
Control	5mg/mL	muFcgRIII -	KS032307	6360
Control	5mg/mL	muFcgRIV -	KS032307	329

Fig. 11

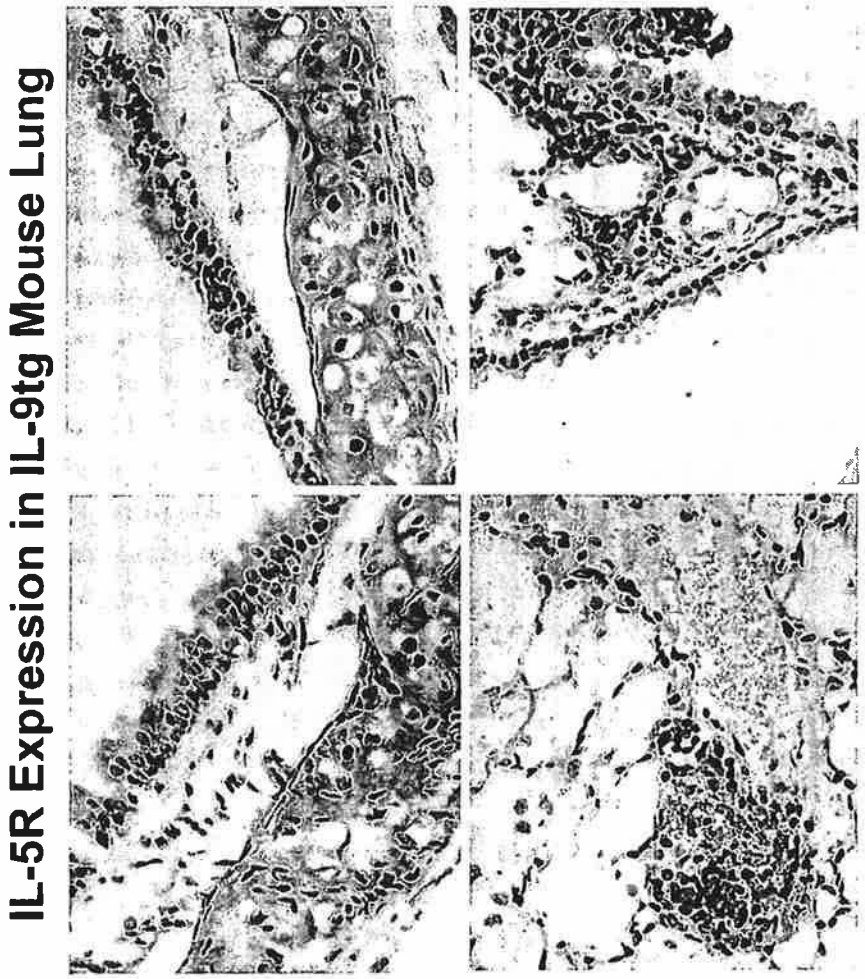


Fig. 12

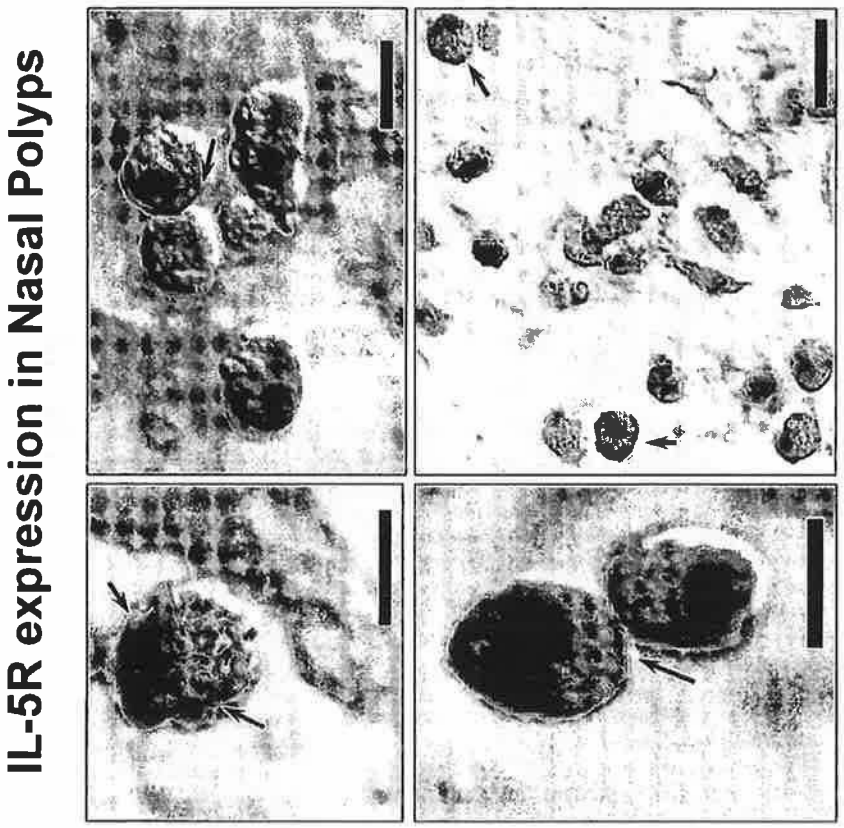


Fig. 13

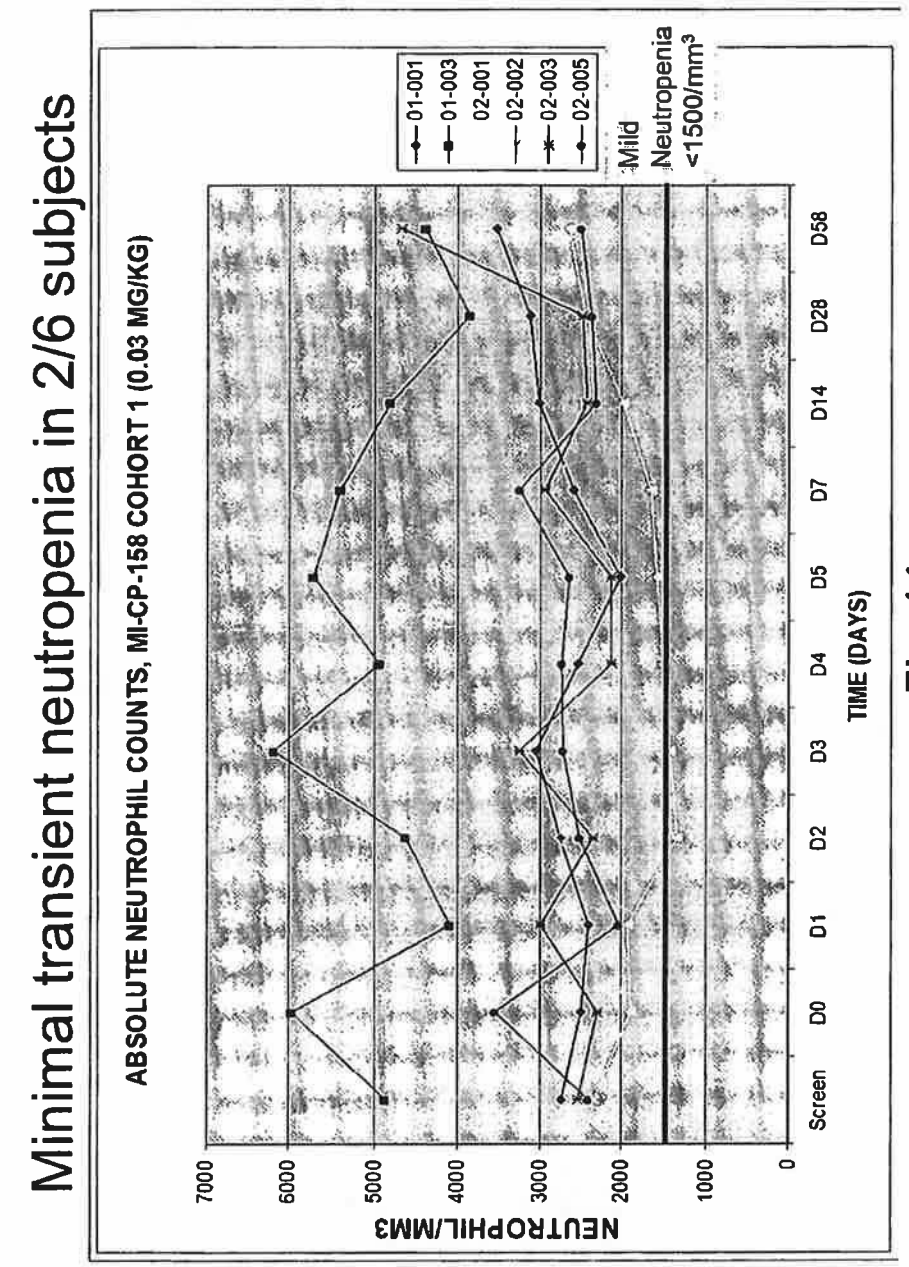

Minimal transient neutropenia in 2/6 subjects
ABSOLUTE NEUTROPHIL COUNTS, MI-CP-158 COHORT 1 (0.03 MG/KG)
NEUTROPHIL/MM3
7000
6000
5000
4000
3000
2000
1000
0
Screen
D0
D1
D2
D3
D4
D5
D7
D14
D28
D58
TIME (DAYS)
01-001
01-003
02-001
02-002
02-003
02-005
Mild Neutropenia <1500/mm3

Fig. 14

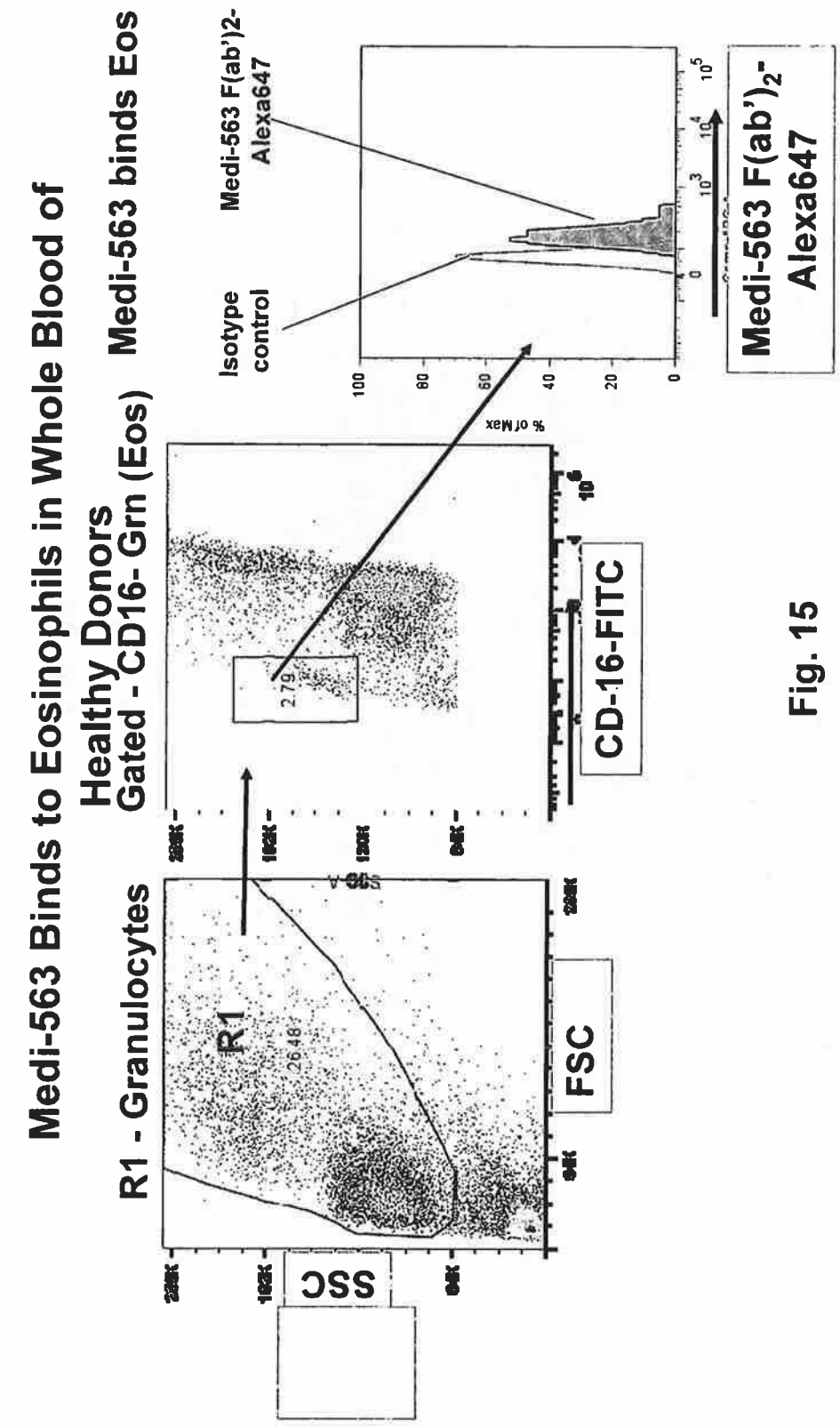

Medi-563 Binds to Eosinophils in Whole Blood of Healthy Donors
R1 - Granulocytes
Gated - CD16- Grn (Eos)
Medi-563 binds Eos
SSC
FSC
R1
2648
CD-16-FITC
2.79
% of Max
Isotype control
Medi-563 F(ab')2-Alexa647
Medi-563 F(ab')2-Alexa647

Fig. 15

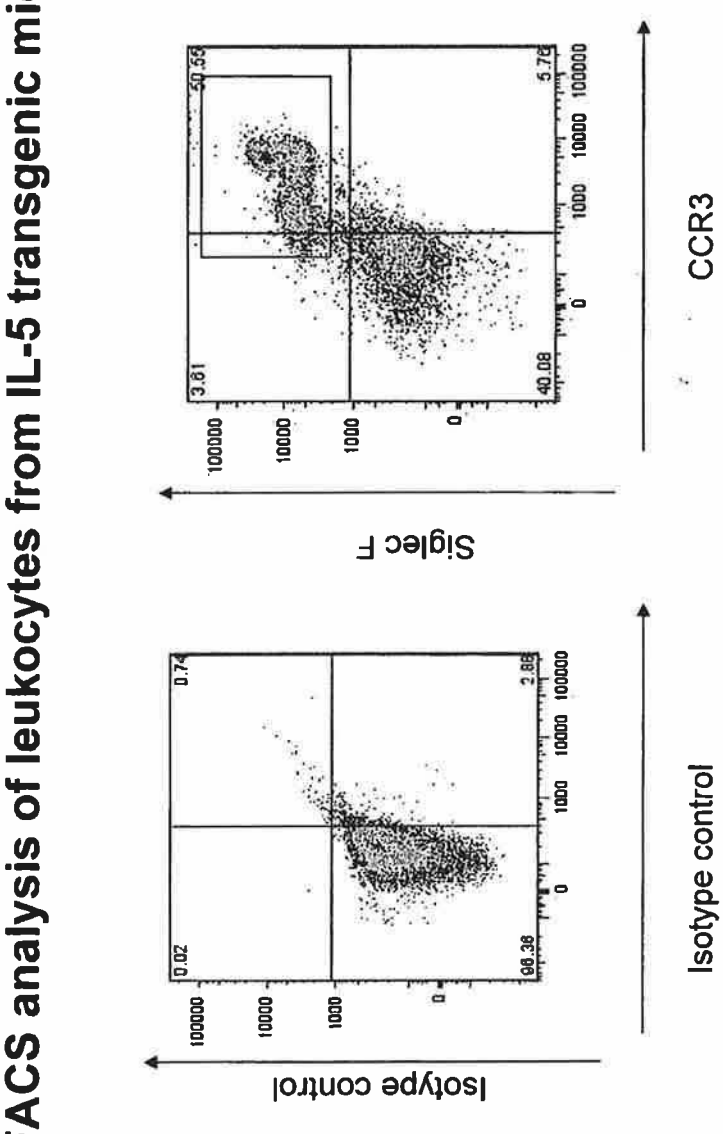

FACS analysis of leukocytes from IL-5 transgenic mice
Isotype control
Siglec F
CCR3
0.02
0.74
99.38
2.88
3.61
50.55
40.08
5.76
100000
10000
1000
0
1000
10000
100000

Fig. 16A

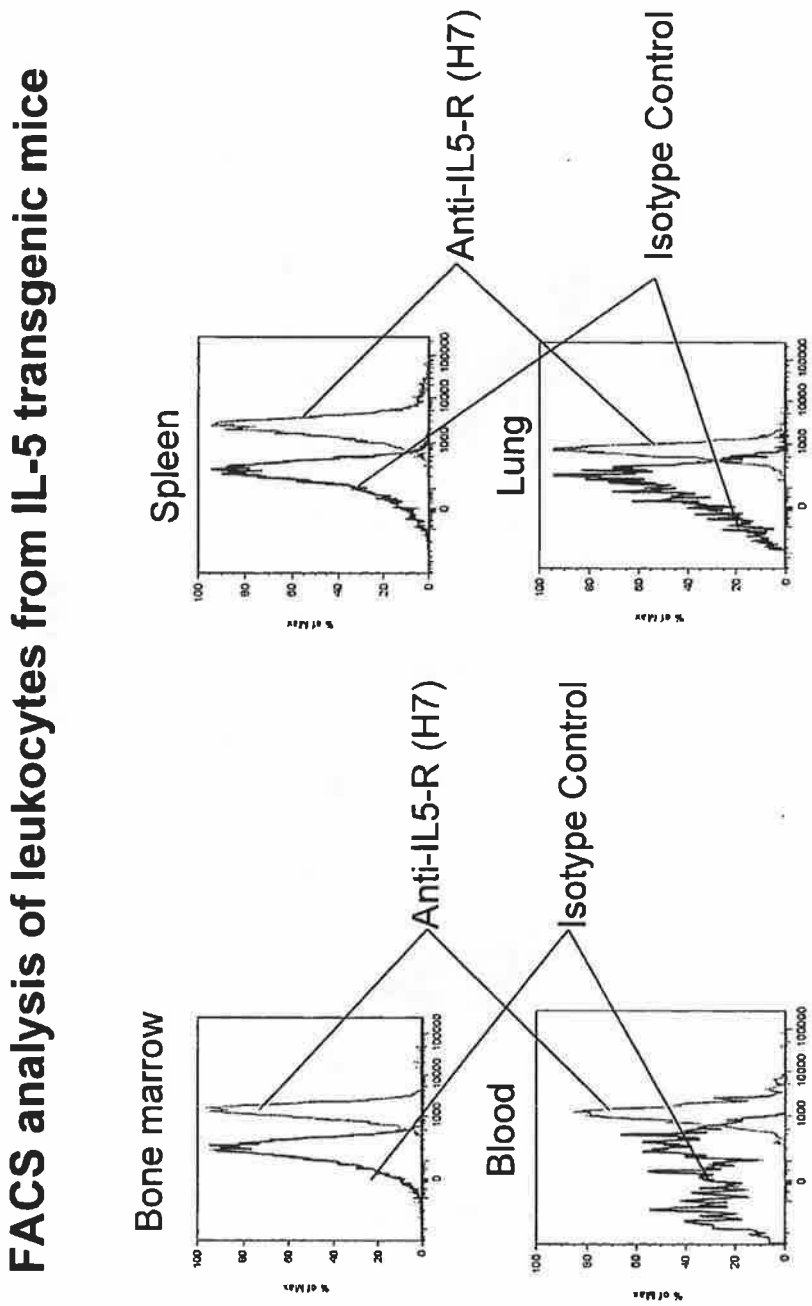

FACS analysis of leukocytes from IL-5 transgenic mice
Spleen
Anti-IL5-R (H7)
Lung
Isotype Control
Bone marrow
Anti-IL5-R (H7)
Blood
Isotype Control

Fig. 16B

Medi-563 Depletes IL-5Ra Positive Mononuclear Cells From Bone Marrow

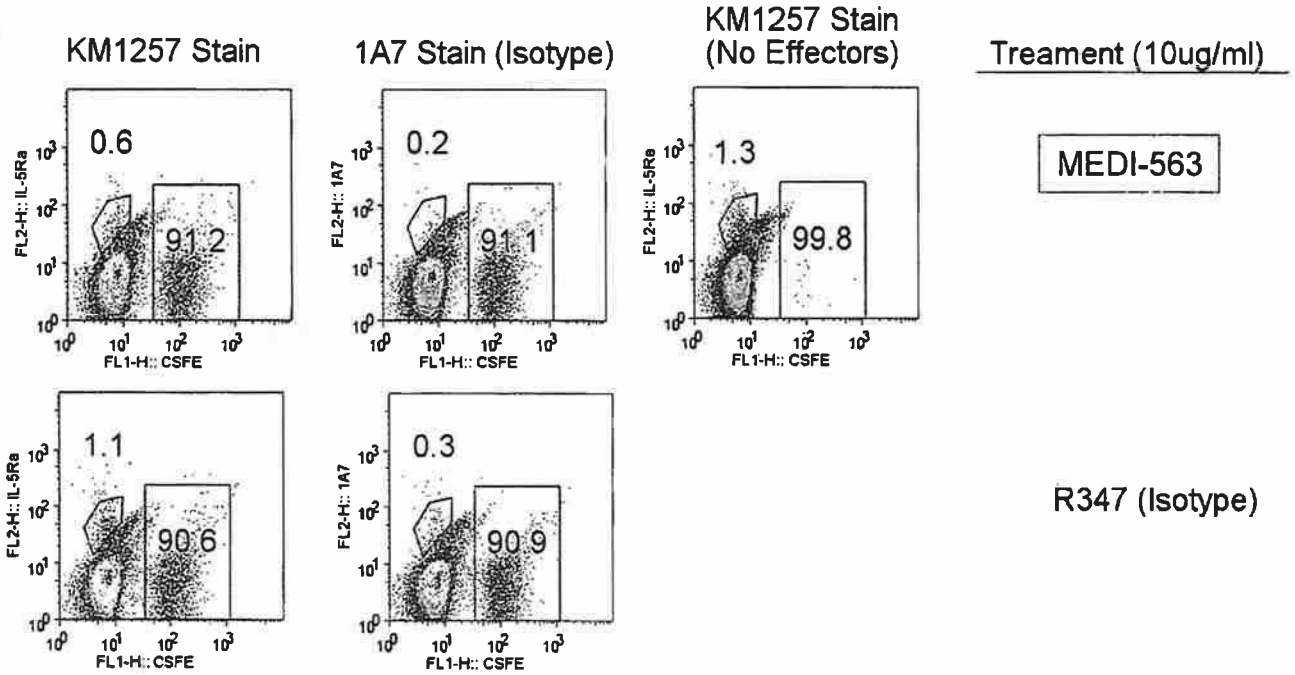
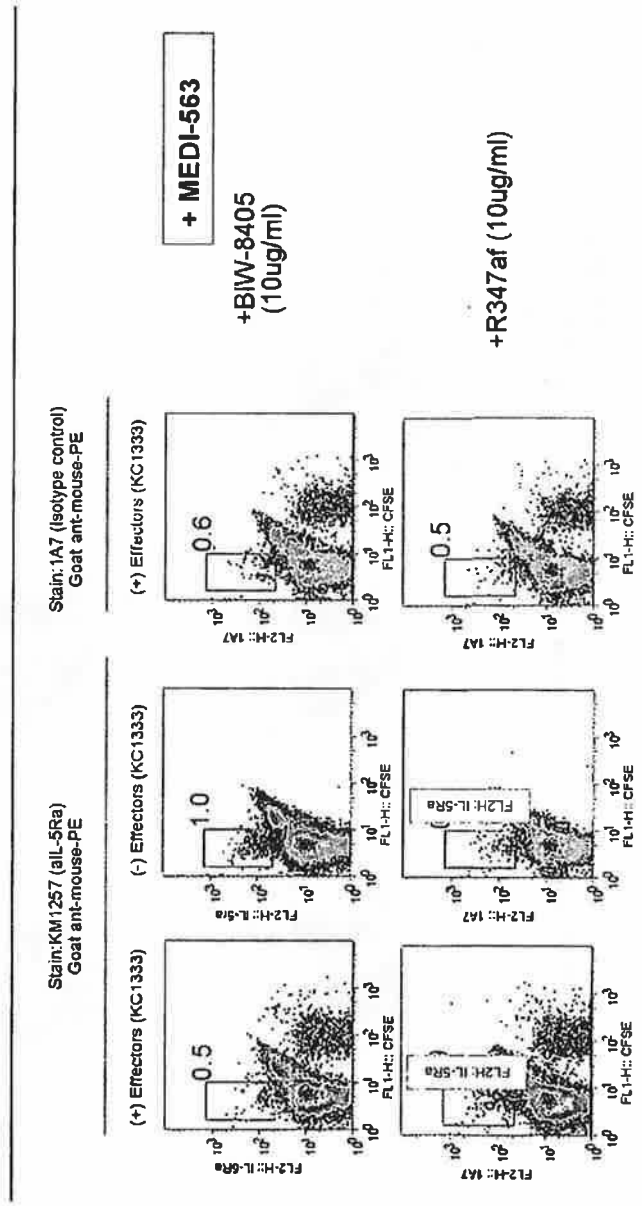


Fig. 17A

Medi-563 Depletes IL-5Ra Positive Mononuclear Cells From Bone Marrow

BM MNC ADCC Assay #2
(MEDI-563)



50,000 CFSE Labeled KC1333
100,000 BM MNC Targets

Fig. 17B

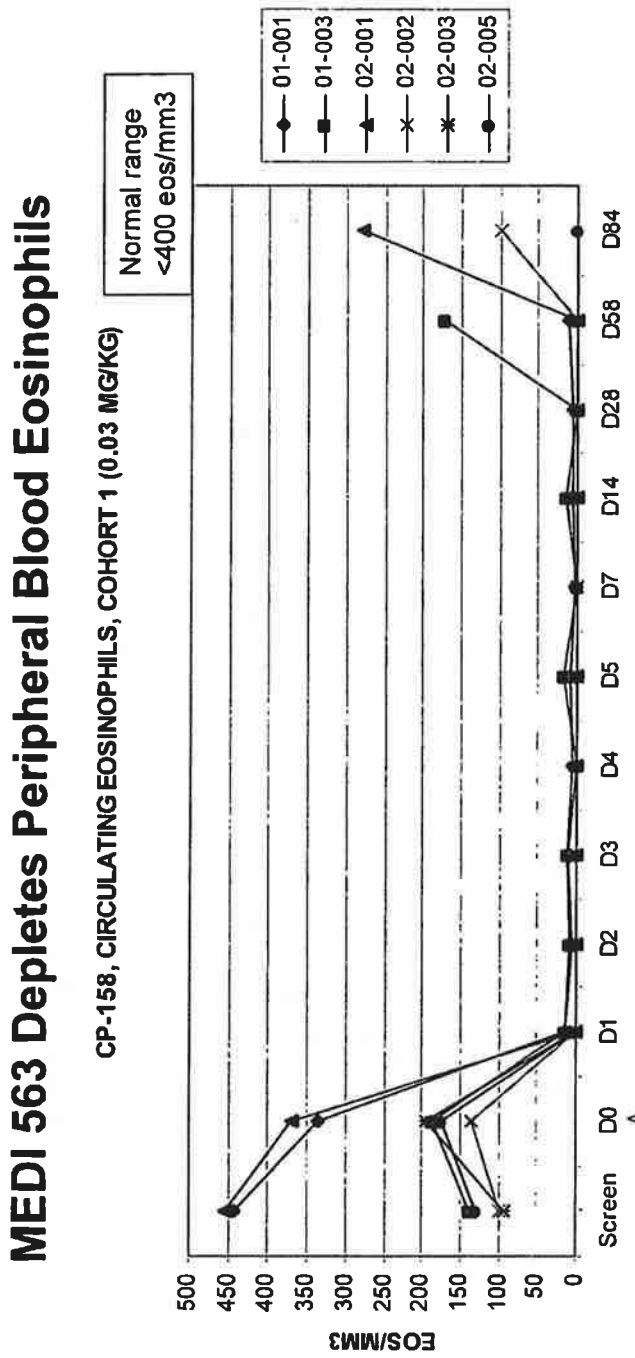

MEDI 563 Depletes Peripheral Blood Eosinophils
CP-158, CIRCULATING EOSINOPHILS, COHORT 1 (0.03 MG/KG)
Normal range
<400 eos/mm3
01-001
01-003
02-001
02-002
02-003
02-005
EOS/MM3
500
450
400
350
300
250
200
150
100
50
0
Screen
D0
D1
D2
D3
D4
D5
D7
D14
D28
D58
D84
TIME
SD Medi-563 0.03 mg/kg

Fig. 18A

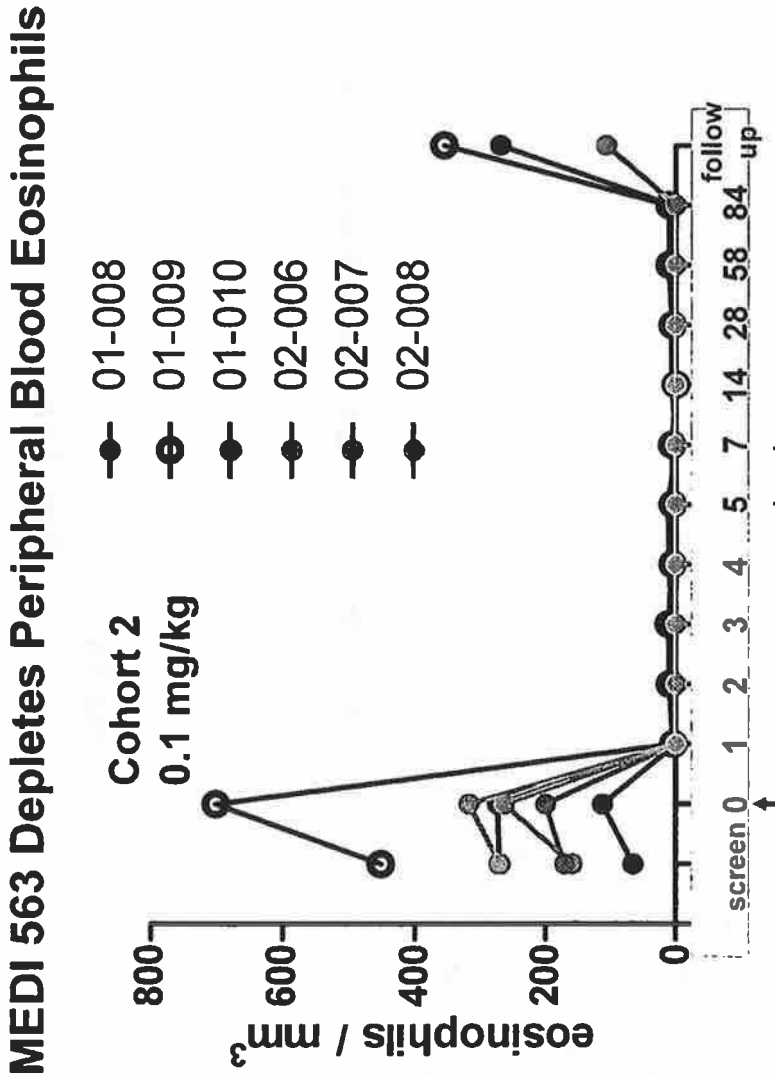

MEDI 563 Depletes Peripheral Blood Eosinophils
Cohort 2
0.1 mg/kg
01-008
01-009
01-010
02-006
02-007
02-008
eosinophils / mm³
800
600
400
200
0
screen 0
1
2
3
4
5
7
14
28
58
84
follow up
study day
MEDI-563

Fig. 18B

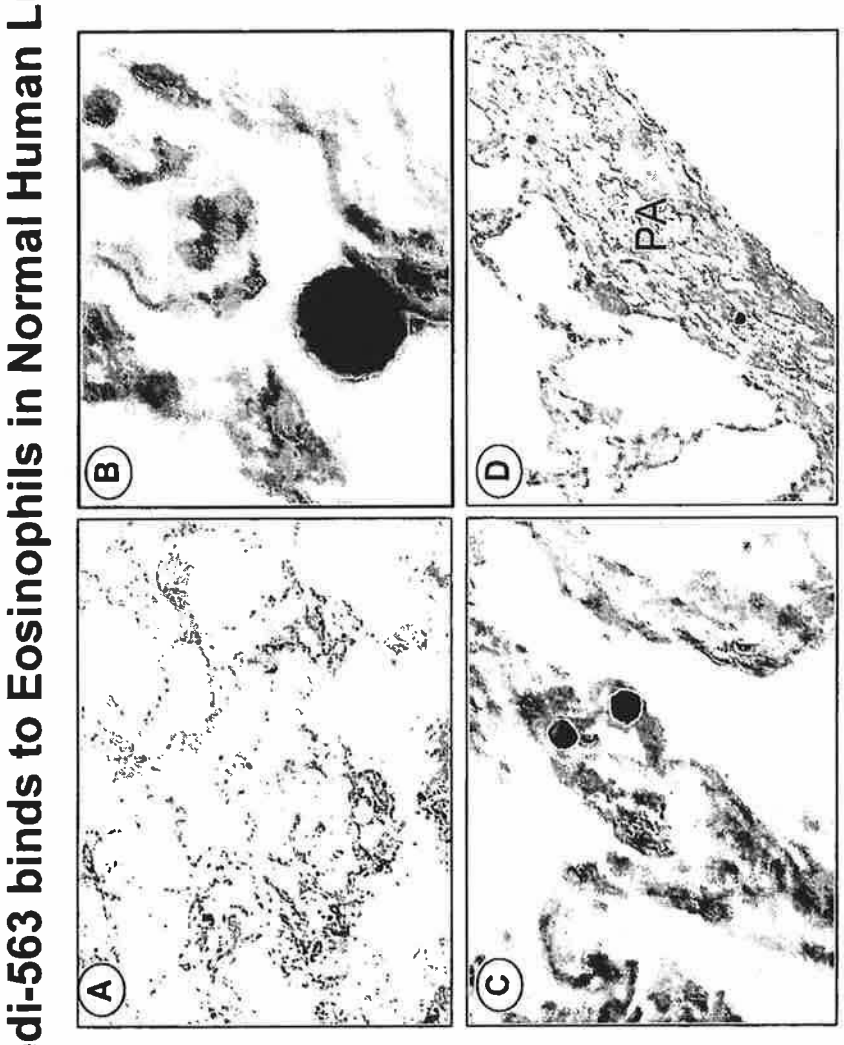


Fig. 19

MEDI-563 Stains all Eosinophils in Asthmatic Lung Biopsies

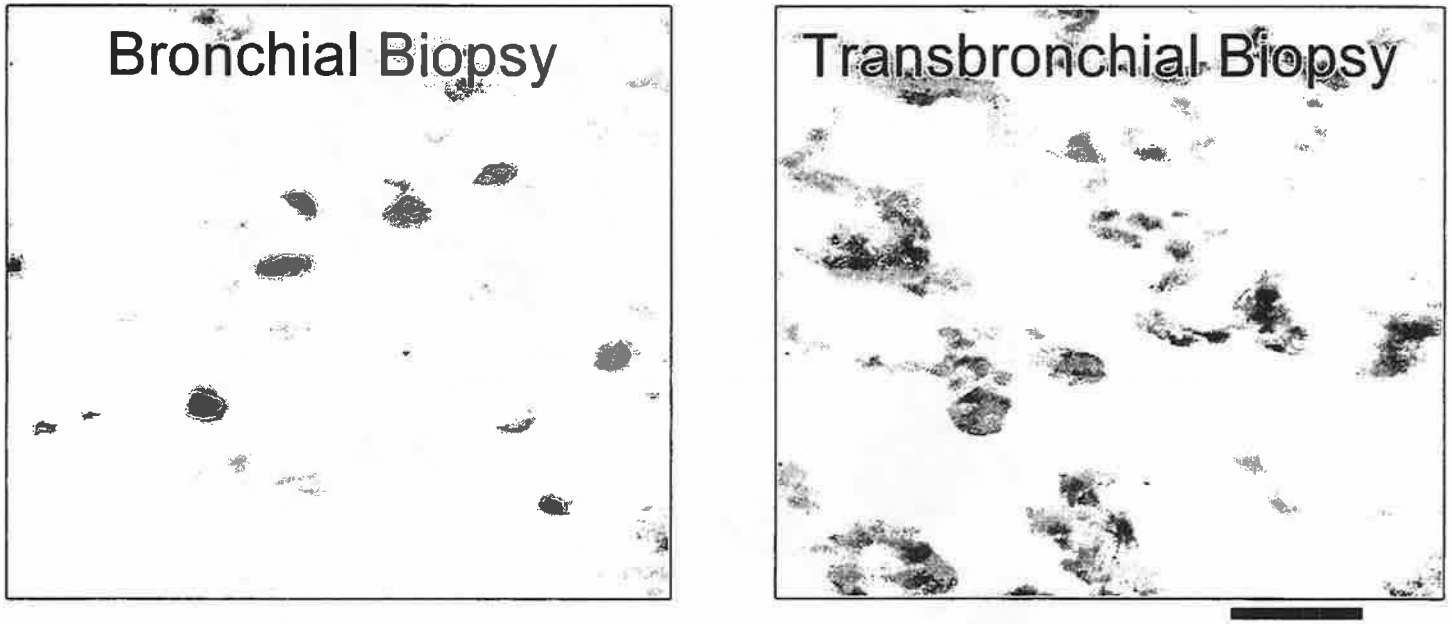
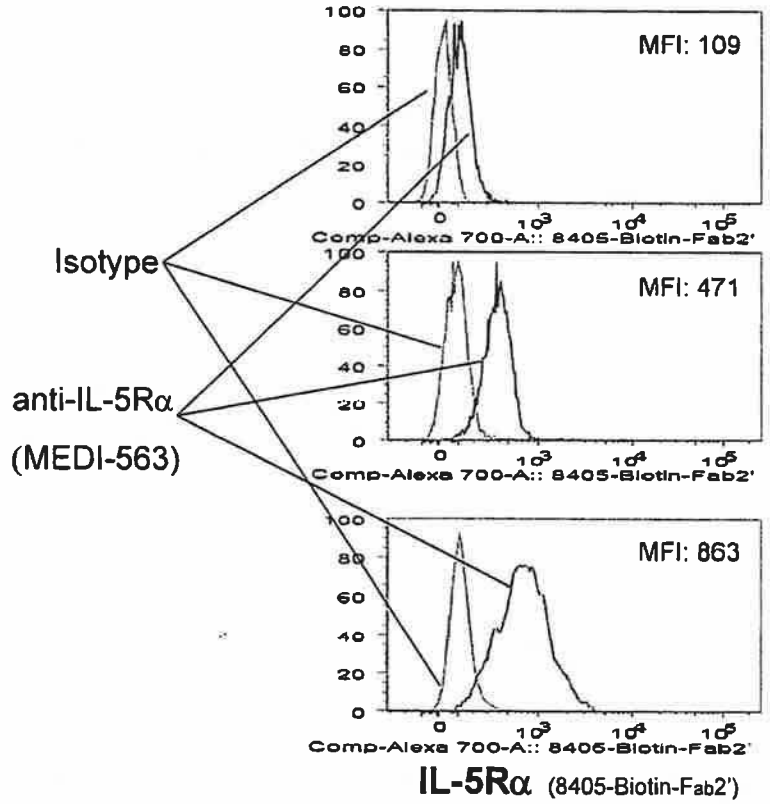


Fig. 20

IL-5Ra Expression on Purified 1° Cells from Healthy Donors



Basophils

Eosinophils

CTLLh5r(IL-5Rα/β transfected tumor cell line)

Fig. 21

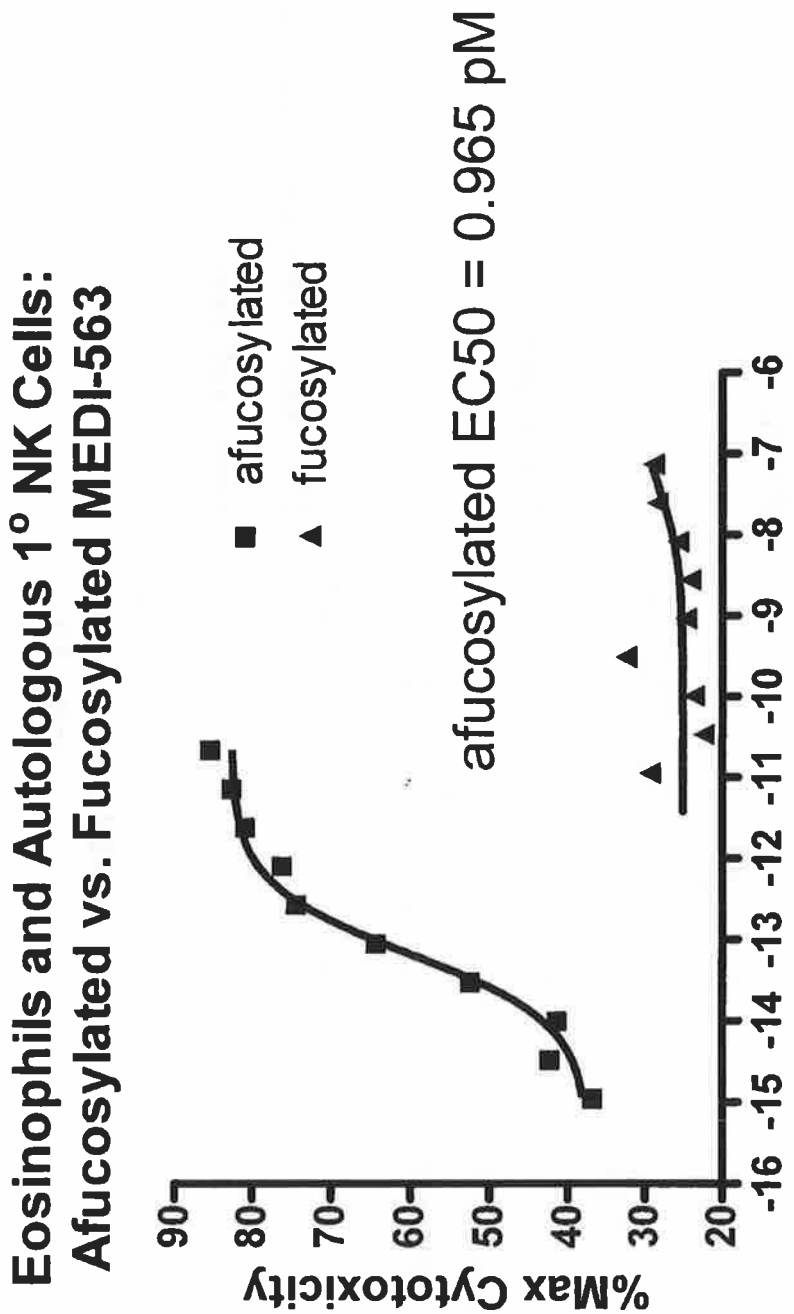

Eosinophils and Autologous 1° NK Cells:
Afucosylated vs. Fucosylated MEDI-563
afucosylated
fucosylated
afucosylated EC50 = 0.965 pM
%Max Cytotoxicity
90
80
70
60
50
40
30
20
-16 -15 -14 -13 -12 -11 -10 -9 -8 -7 -6
Log10[pM]

Fig. 22

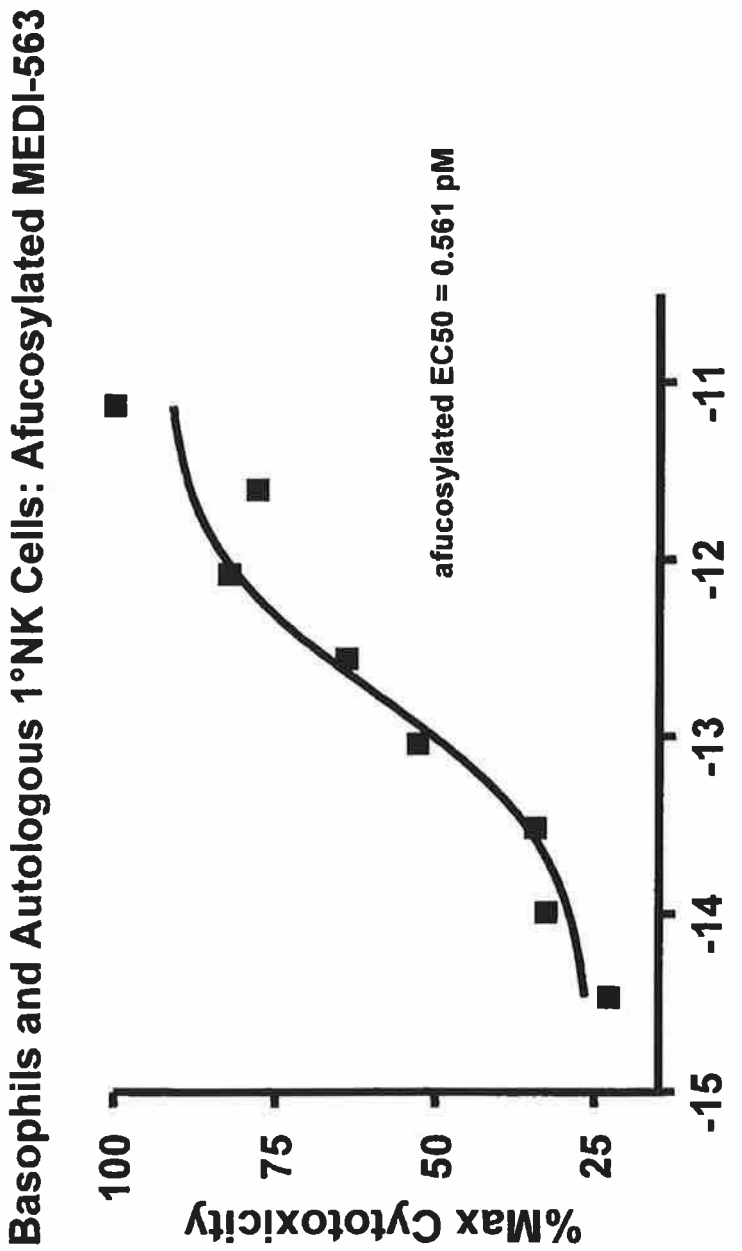

Basophils and Autologous 1°NK Cells: Afucosylated MEDI-563
%Max Cytotoxicity
100
75
50
25
afucosylated EC50 = 0.561 pM
-15
-14
-13
-12
-11
Log10[pM]

Fig. 23

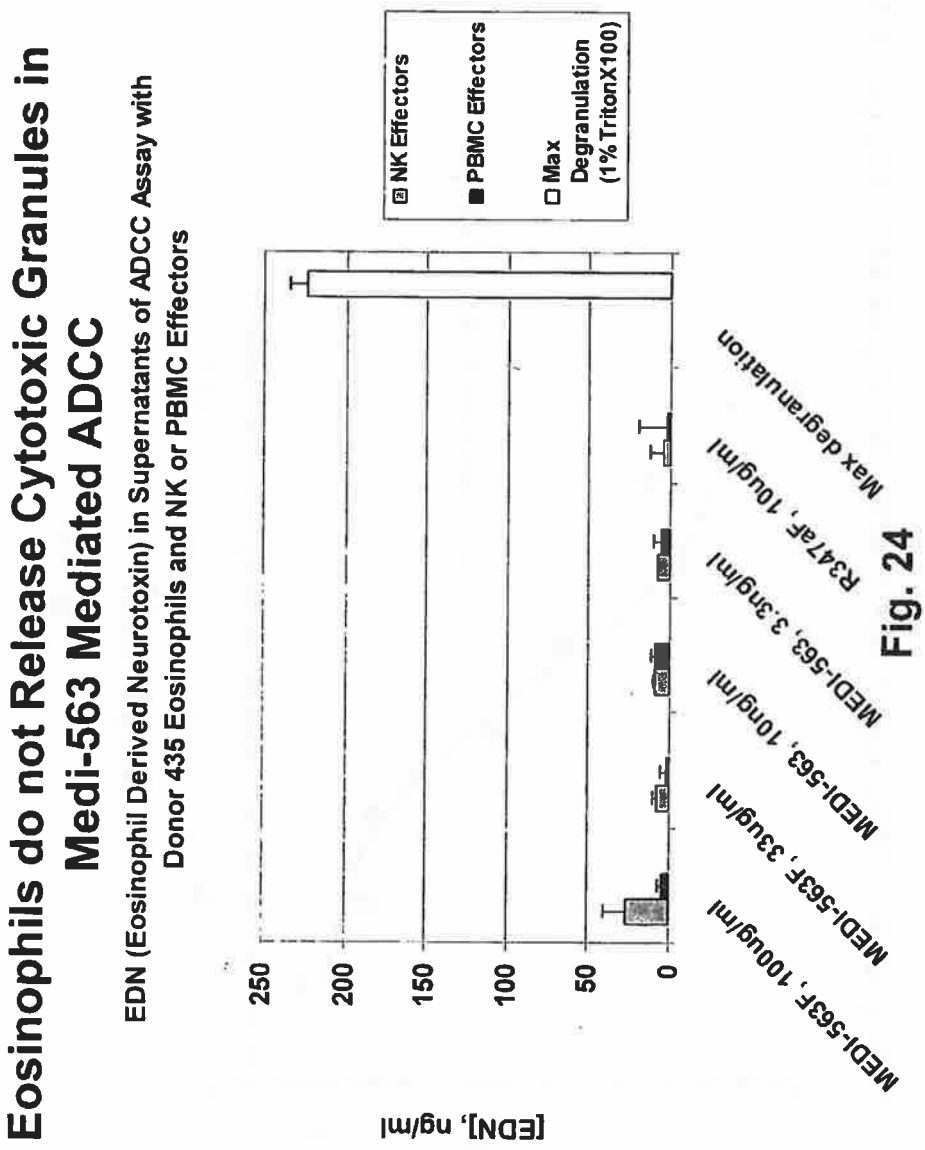

Eosinophils do not Release Cytotoxic Granules in Medi-563 Mediated ADCC
EDN (Eosinophil Derived Neurotoxin) in Supernatants of ADCC Assay with Donor 435 Eosinophils and NK or PBMC Effectors
NK Effectors
PBMC Effectors
Max Degranulation (1% TritonX100)
[EDN], ng/ml
250
200
150
100
50
0
MEDI-563F, 100ug/ml
MEDI-563F, 33ug/ml
MEDI-563, 10ng/ml
MEDI-563, 3.3ng/ml
R347aF, 10ug/ml
Max degranulation

Fig. 24

Engineering Swap Mutants to Localize the MEDI-563 Binding Epitope

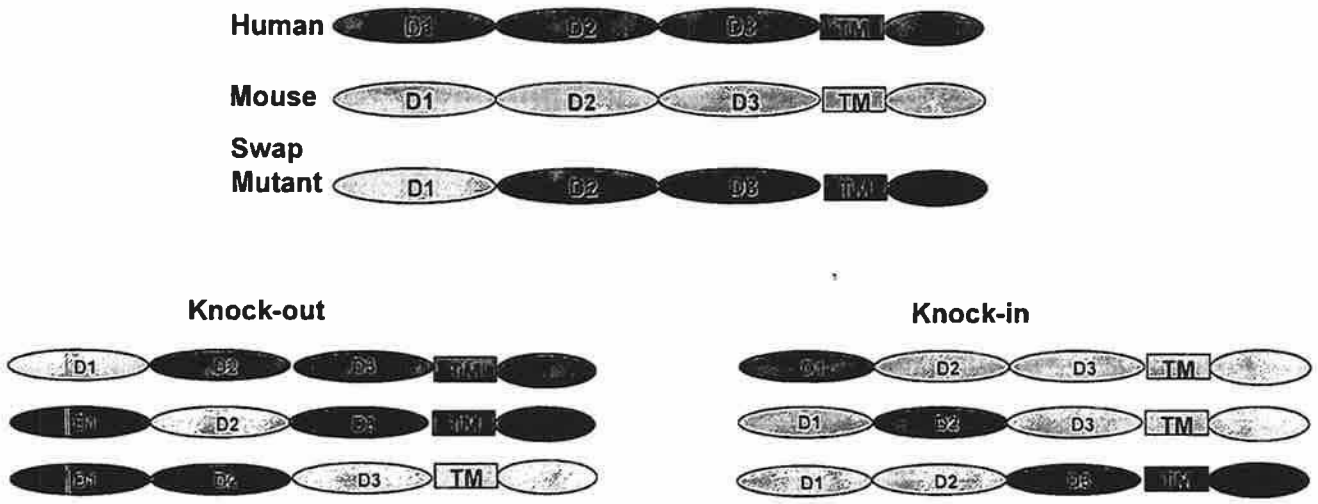


Fig. 25A

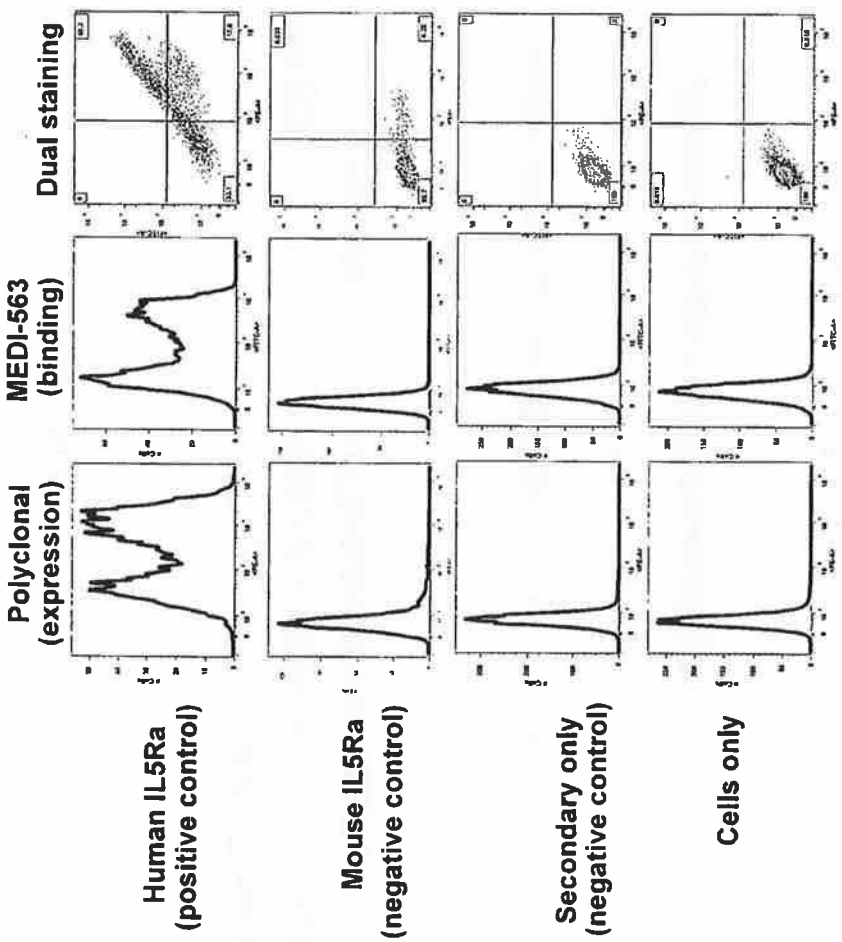

Polyclonal (expression)
MEDI-563 (binding)
Dual staining
Human IL5Ra (positive control)
Mouse IL5Ra (negative control)
Secondary only (negative control)
Cells only

Fig. 25B

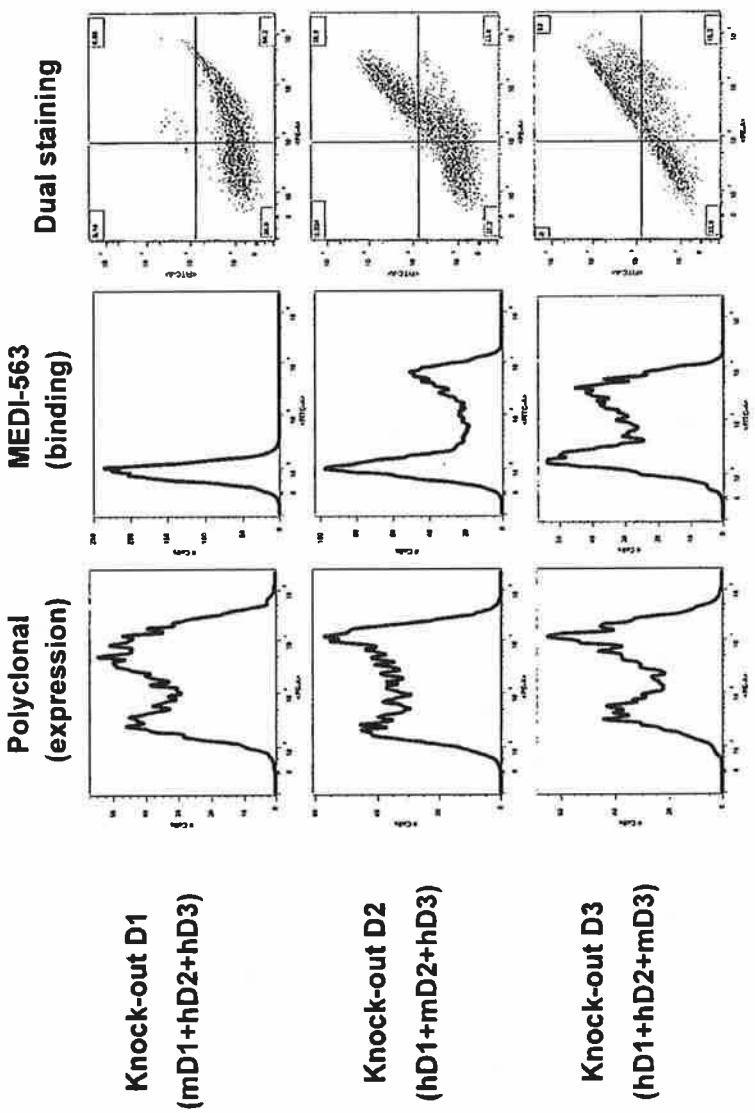

Polyclonal (expression)
MEDI-563 (binding)
Dual staining
Knock-out D1 (mD1+hD2+hD3)
Knock-out D2 (hD1+mD2+hD3)
Knock-out D3 (hD1+hD2+mD3)

Fig. 25C

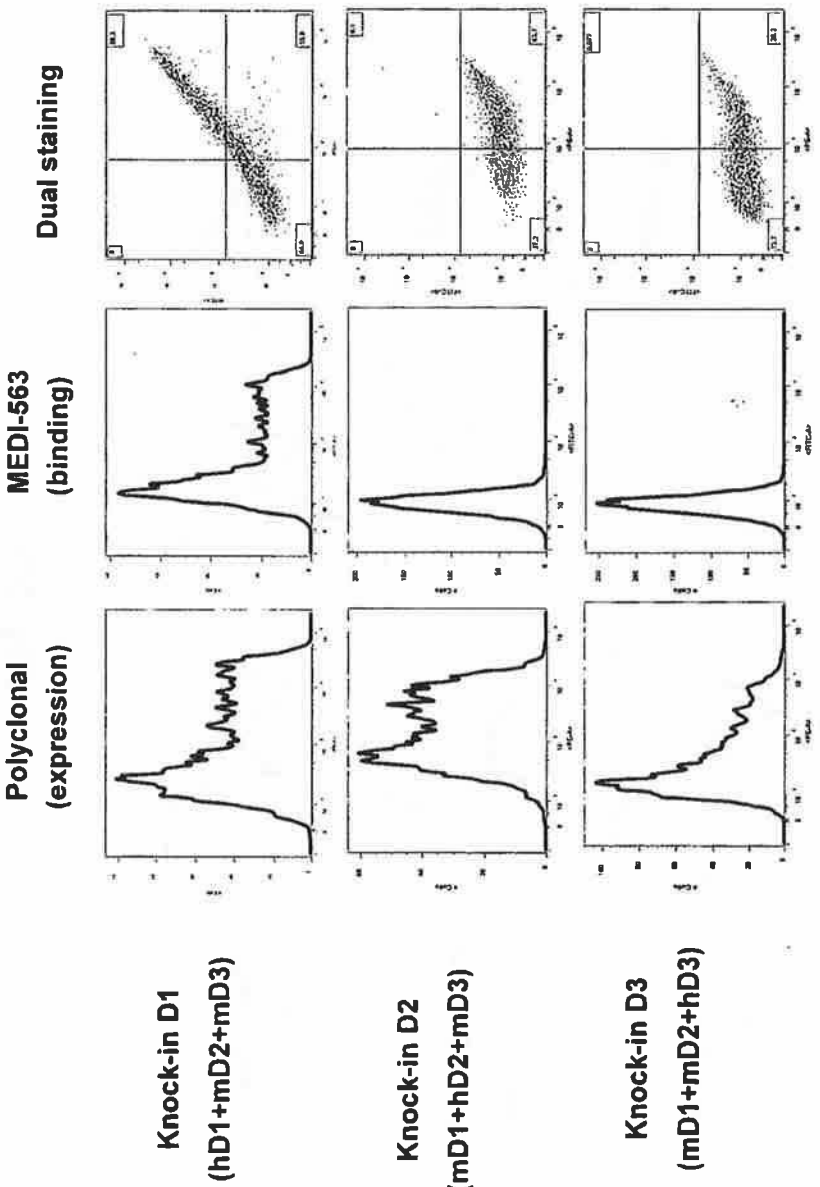

Polyclonal (expression)
MEDI-563 (binding)
Dual staining
Knock-in D1 (hD1+mD2+mD3)
Knock-in D2 (mD1+hD2+mD3)
Knock-in D3 (mD1+mD2+hD3)

Fig. 25D

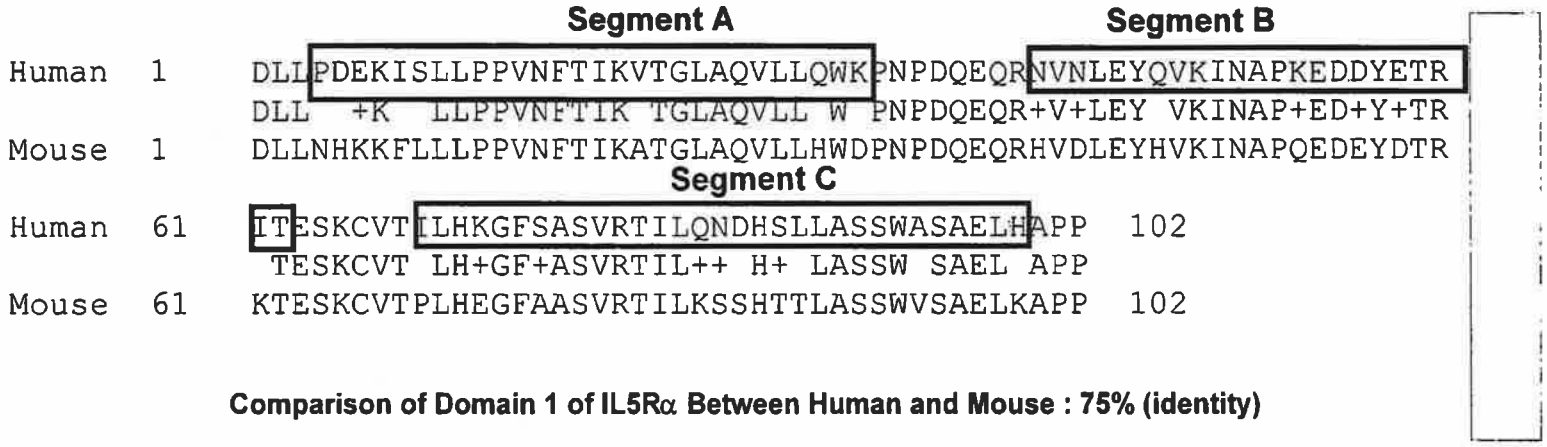

Segment A
Segment B
Human 1 DLLPDEKISLLPPVNFTIKVTGLAQVLLQWKPNPDQEQRNVNLEYQVKINAPKEDDYETR
DLL +K LLPPVNFTIK TGLAQVLL W PNPDQEQR+V+LEY VKINAP+ED+Y+TR
Mouse 1 DLLNHKKFLLLPPVNFTIKATGLAQVLLHWDPNPDQEQRHVDLEYHVKINAPQEDEYDTR
Segment C
Human 61 ITESKCVTILHKGFSASVRTILQNDHSLLASSWASAELHAPP 102
TESKCVT LH+GF+ASVRTIL++ H+ LASSW SAEL APP
Mouse 61 KTESKCVTPLHEGFAASVRTILKSSHTTLASSWVSAELKAPP 102
Comparison of Domain 1 of IL5Rα Between Human and Mouse : 75% (identity)

Fig. 26A

Knock-out mutants -Substitute human (*hu*) residues in segment A,B,C with mouse counterparts

- **muA** *huB* *huC*
- *huA* **muB** *huC*
- *huA* *huB* **muC**

Knock-in mutants -Substitute murine (**mu**) residues in segment A,B,C with human counterparts

- *huA* **muB** **muC**
- **muA** *huB* **muC**
- **muA** **muB** *huC*

Fig. 26B

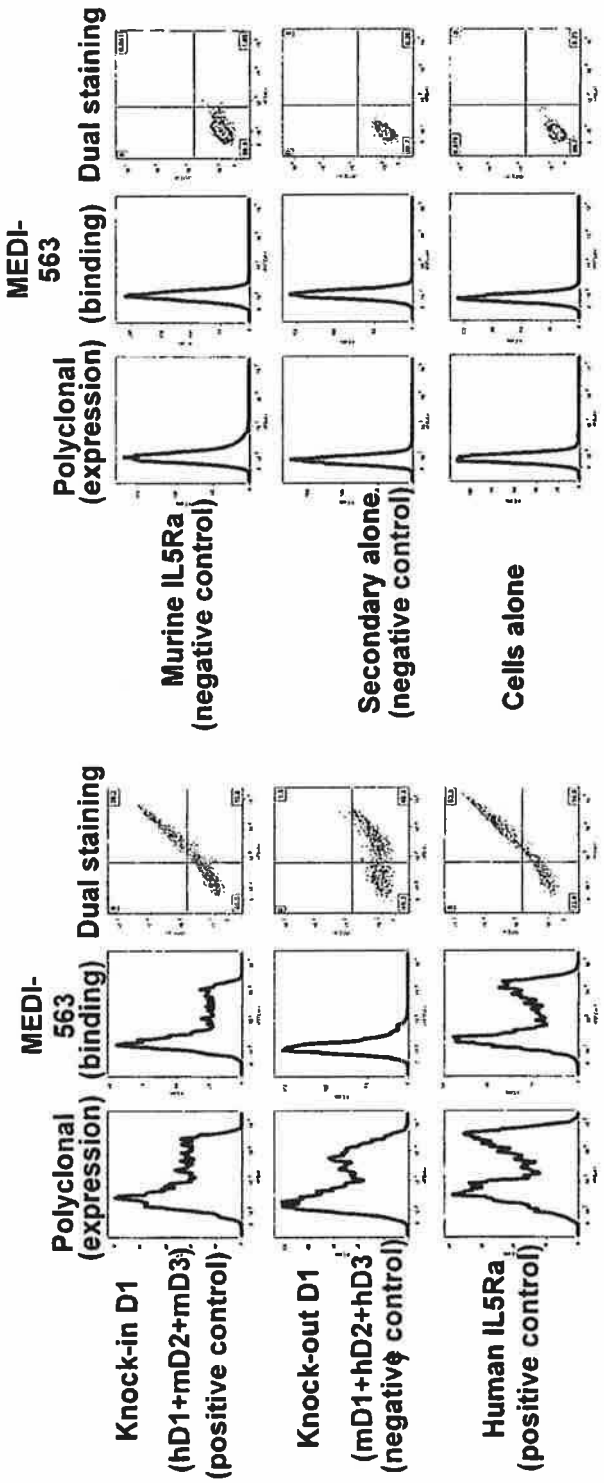

Polyclonal (expression)
MEDI-563 (binding)
Dual staining
Knock-in D1 (hD1+mD2+mD3) (positive control)
Knock-out D1 (mD1+hD2+hD3 (negative control)
Human IL5Ra (positive control)
Murine IL5Ra (negative control)
Secondary alone (negative control)
Cells alone

Fig. 26C

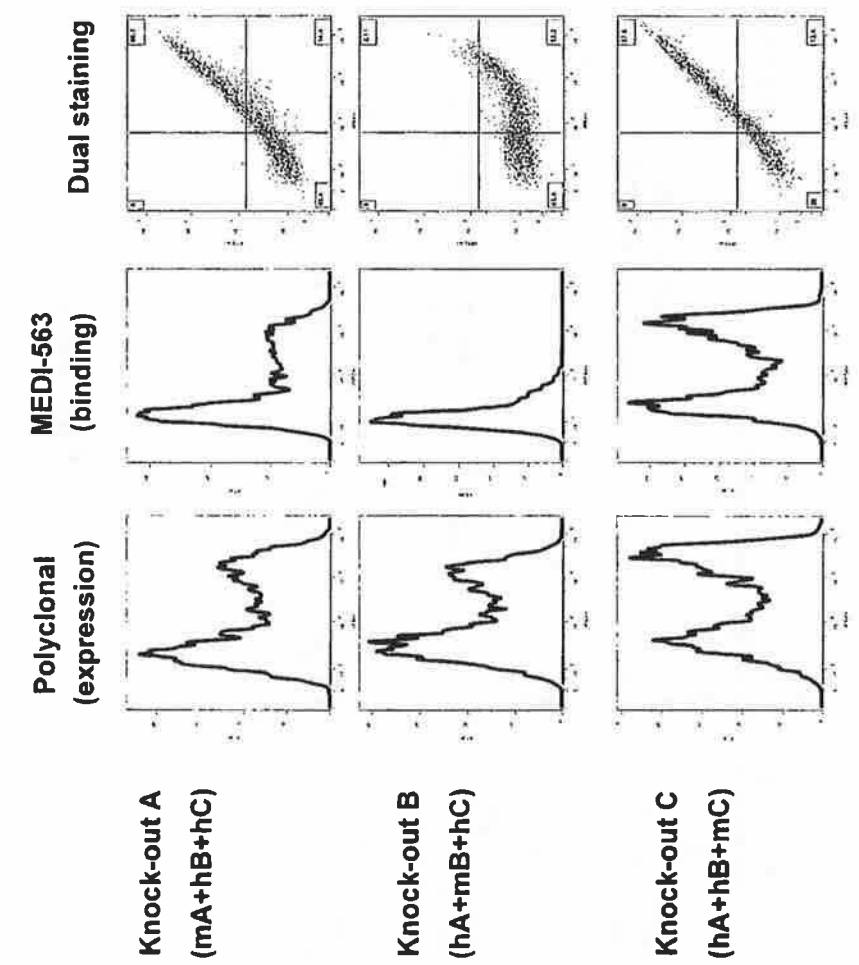

Polyclonal (expression)
MEDI-563 (binding)
Dual staining
Knock-out A (mA+hB+hC)
Knock-out B (hA+mB+hC)
Knock-out C (hA+hB+mC)

Fig. 26D

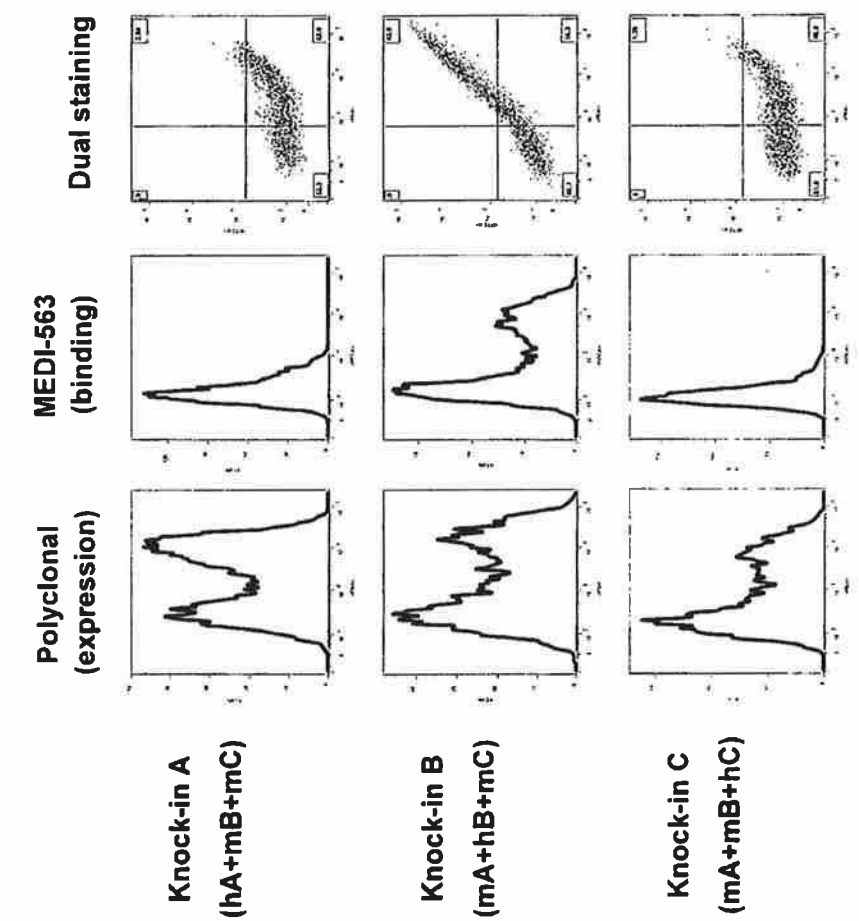
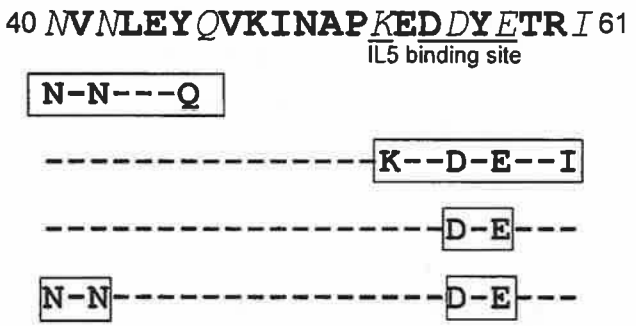

Polyclonal (expression)
MEDI-563 (binding)
Dual staining
Knock-in A (hA+mB+mC)
Knock-in B (mA+hB+mC)
Knock-in C (mA+mB+hC)

Fig. 26E



Knock-in & Knock-out constructs

= NNQ

= KDEI

= DE

= NNDE

- Amino acid sequence of human Segment B1 of the D1 extracellular domain of IL-5Ralpha is shown.
- Residues in *italics* are different beween human and mouse.
- Underlined residues are contributing to ligand binding (Chaiken I., et al. JBC 2004,279).

Fig. 27A

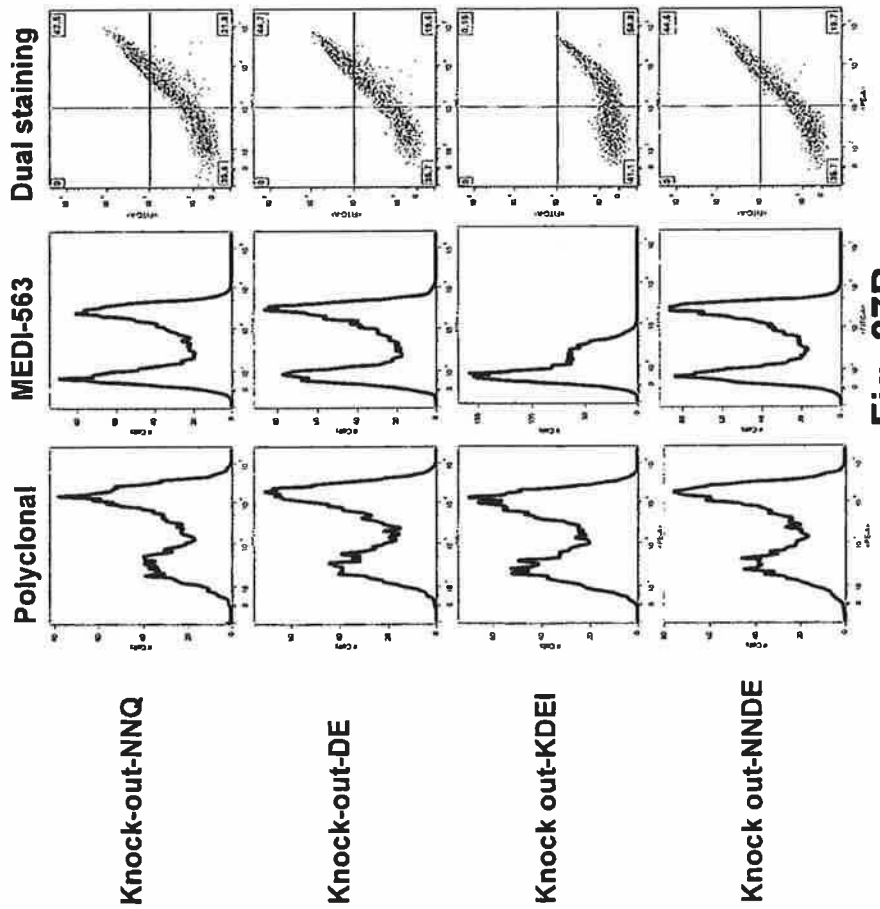

Polyclonal
MEDI-563
Dual staining
Knock-out-NNQ
Knock-out-DE
Knock out-KDEI
Knock out-NNDE

Fig. 27B

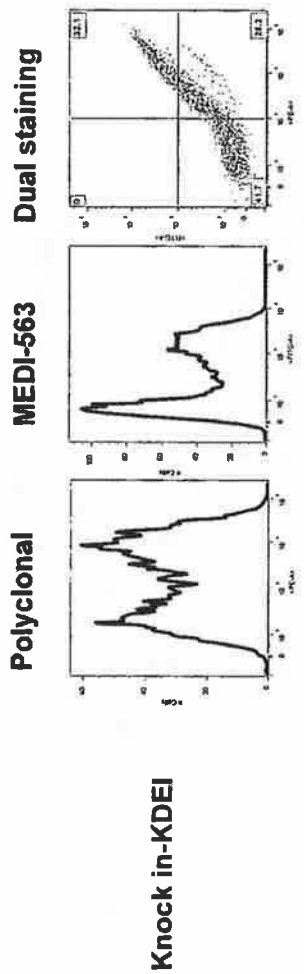


Fig. 27C

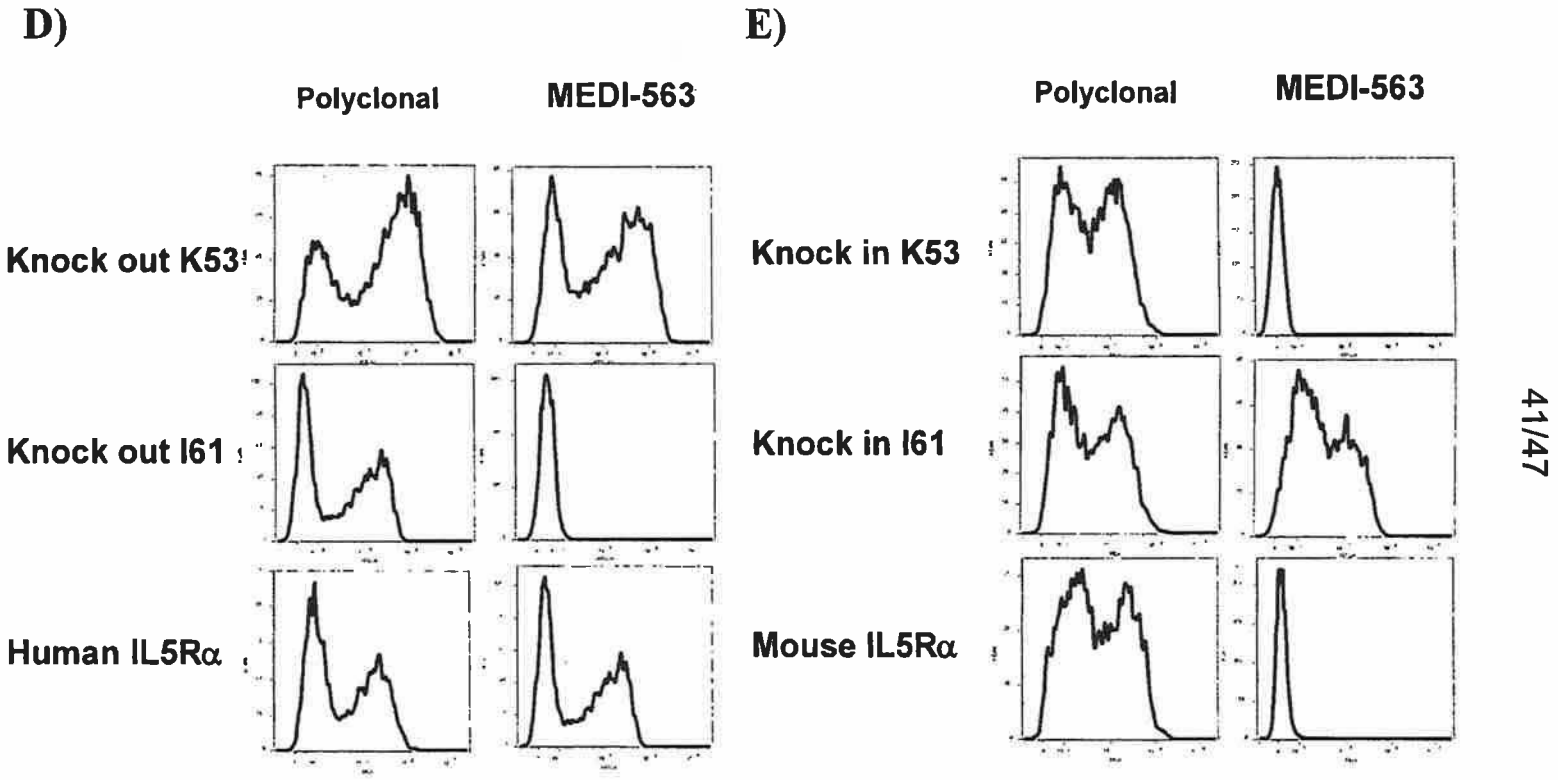

D)
Polyclonal
MEDI-563
Knock out K53
Knock out I61
Human IL5Rα
E)
Polyclonal
MEDI-563
Knock in K53
Knock in I61
Mouse IL5Rα

Fig. 27DE

	muFcgRIV	muFcgRIII	muFcgRIIb	muFcgRI
Hu IgG_1	253	3660	1240	280
Hu IgG_1 afuc	43	3960	1260	397

Fig. 28

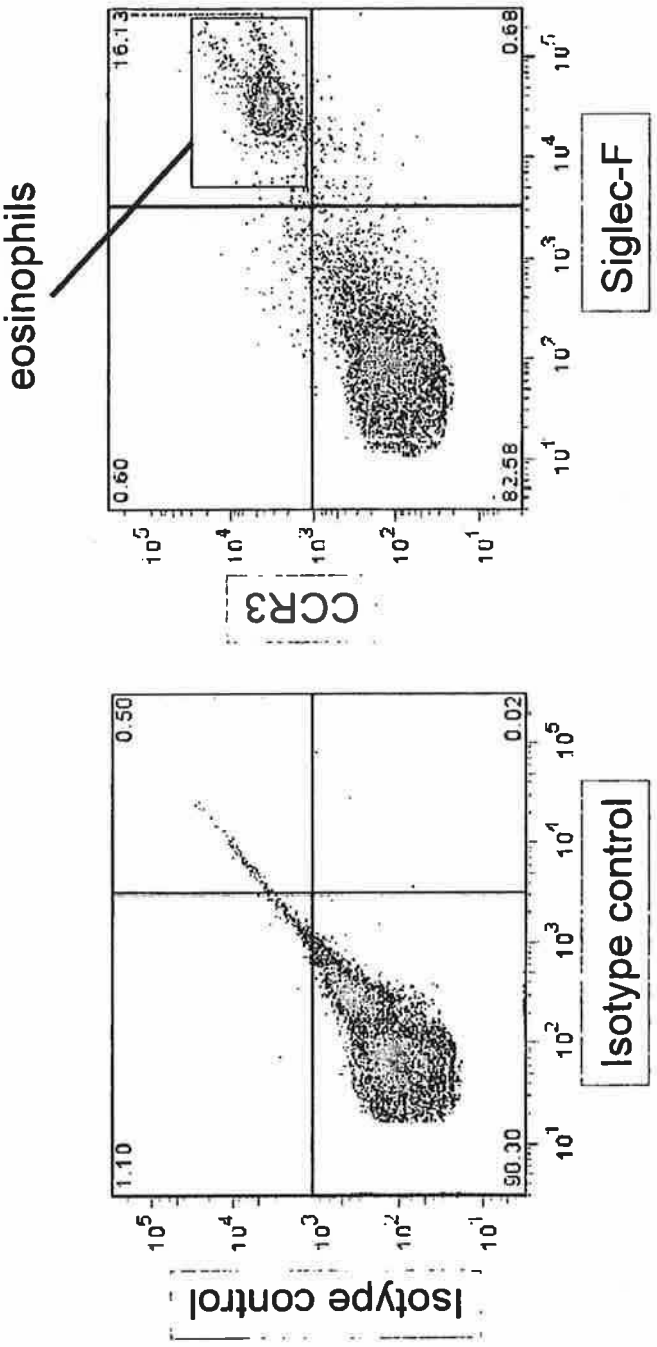

eosinophils
CCR3
Siglec-F
Isotype control
0.60
16.13
82.58
0.68
1.10
0.50
90.30
0.02

Fig. 29A

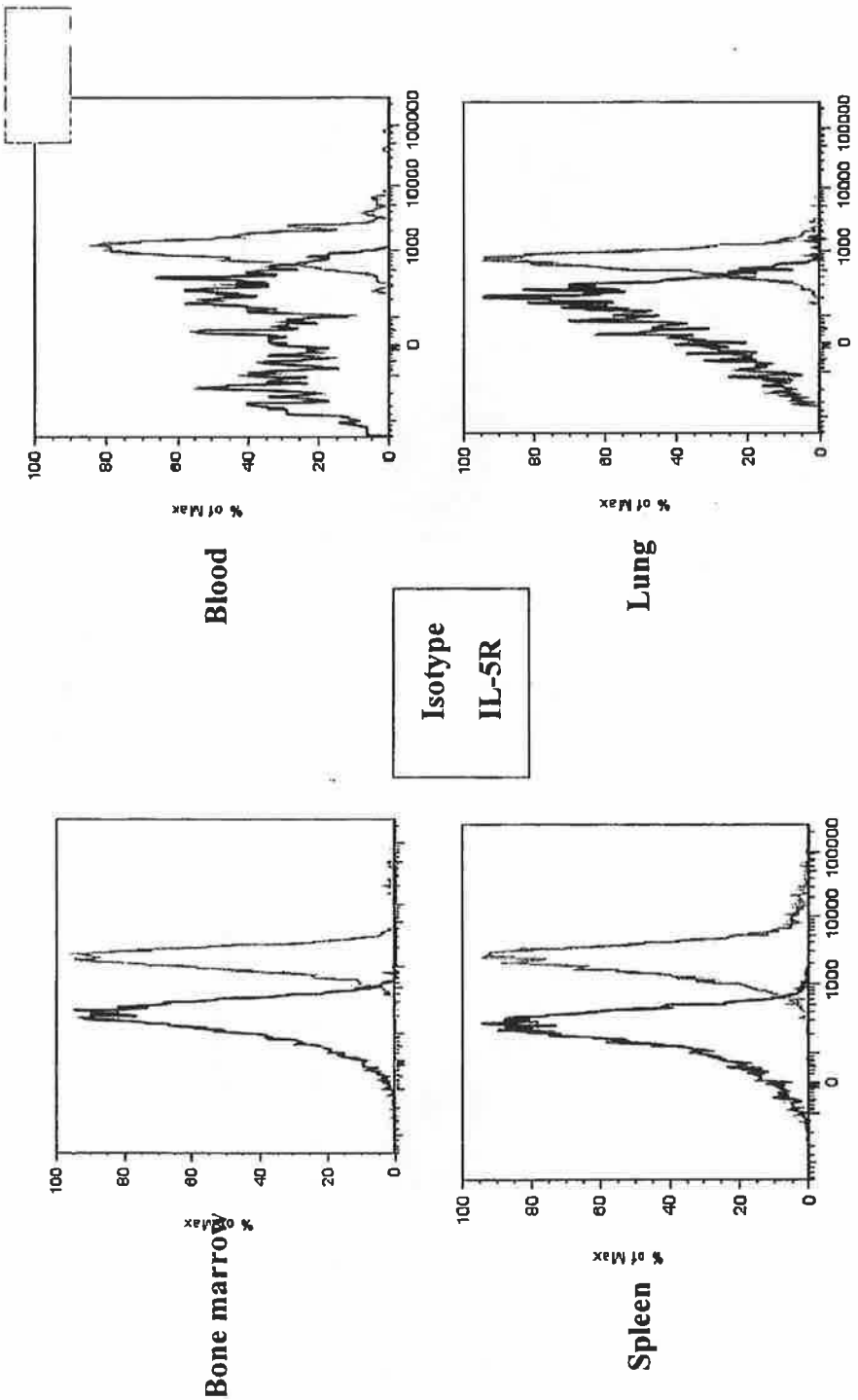

Blood
Lung
Isotype
IL-5R
Bone marrow
Spleen
% of Max
0
20
40
60
80
100
0
1000
10000
100000

Fig. 29B

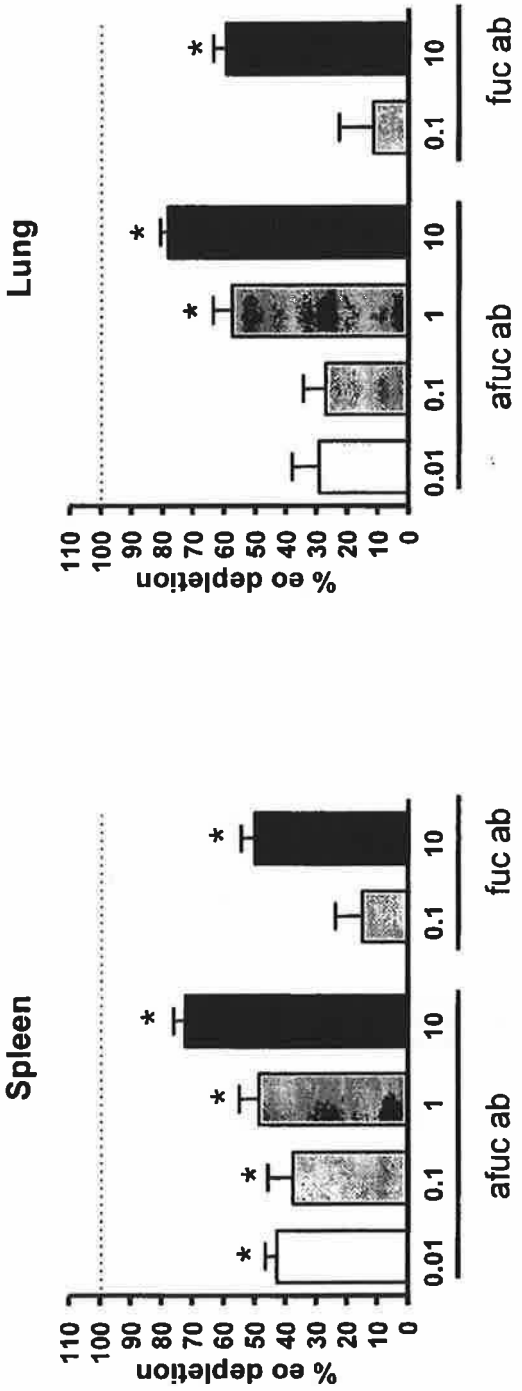

Lung
% eo depletion
110
100
90
80
70
60
50
40
30
20
10
0
*
*
*
0.01
0.1
1
10
afuc ab
0.1
10
fuc ab
Spleen
% eo depletion
110
100
90
80
70
60
50
40
30
20
10
0
*
*
*
*
0.01
0.1
1
10
afuc ab
0.1
10
fuc ab

Fig. 30A

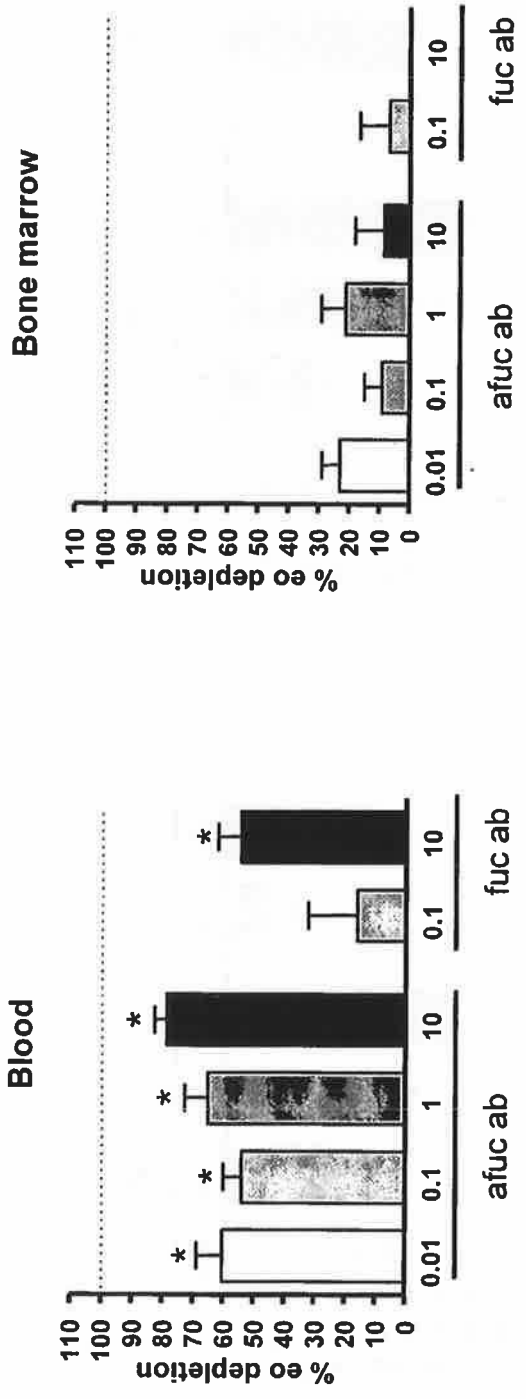

Bone marrow
% eo depletion
110
100
90
80
70
60
50
40
30
20
10
0
0.01
0.1
1
10
0.1
10
afuc ab
fuc ab
Blood
% eo depletion
*
*
*
*
*
afuc ab
fuc ab

Fig. 30B

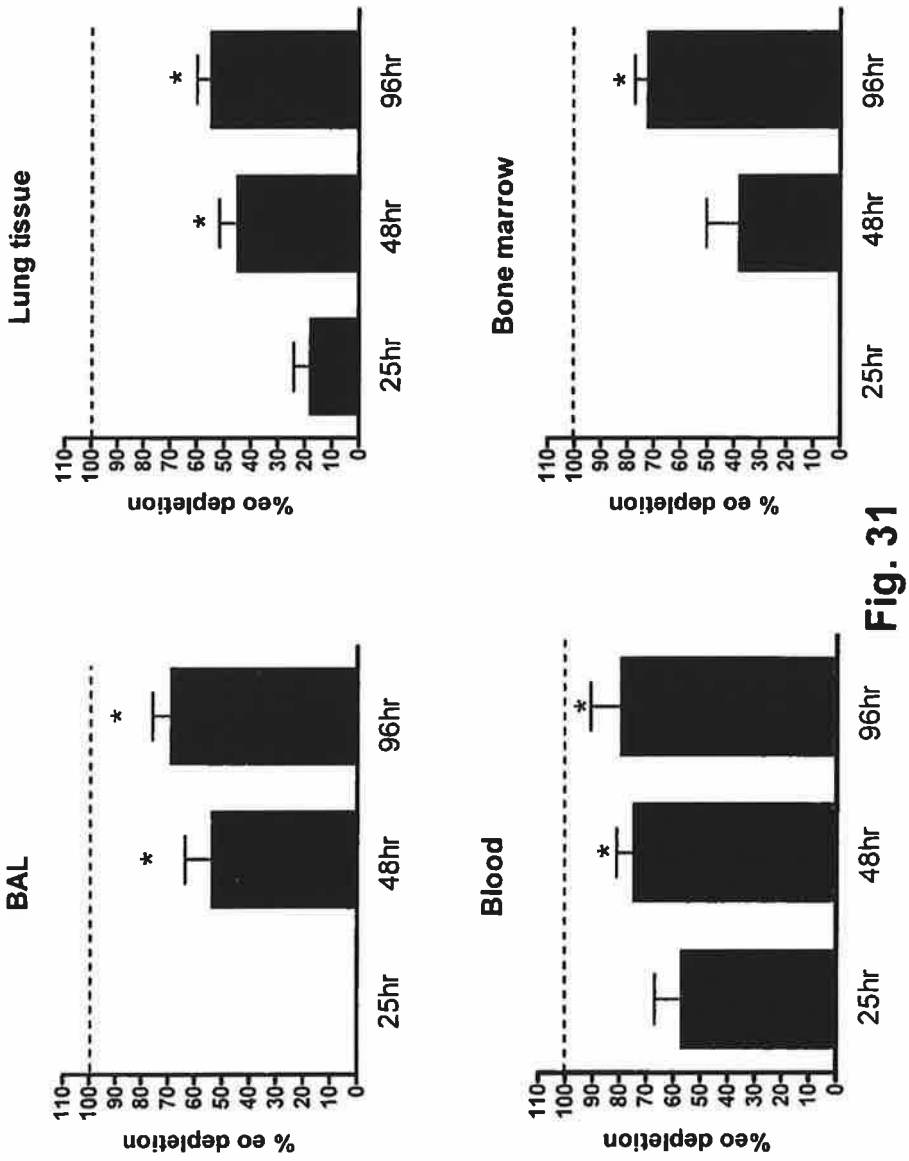

Lung tissue
%eo depletion
110
100
90
80
70
60
50
40
30
20
10
0
*
*
25hr
48hr
96hr
Bone marrow
% eo depletion
BAL
% eo depletion
Blood
%eo depletion

Fig. 31