Figure 1

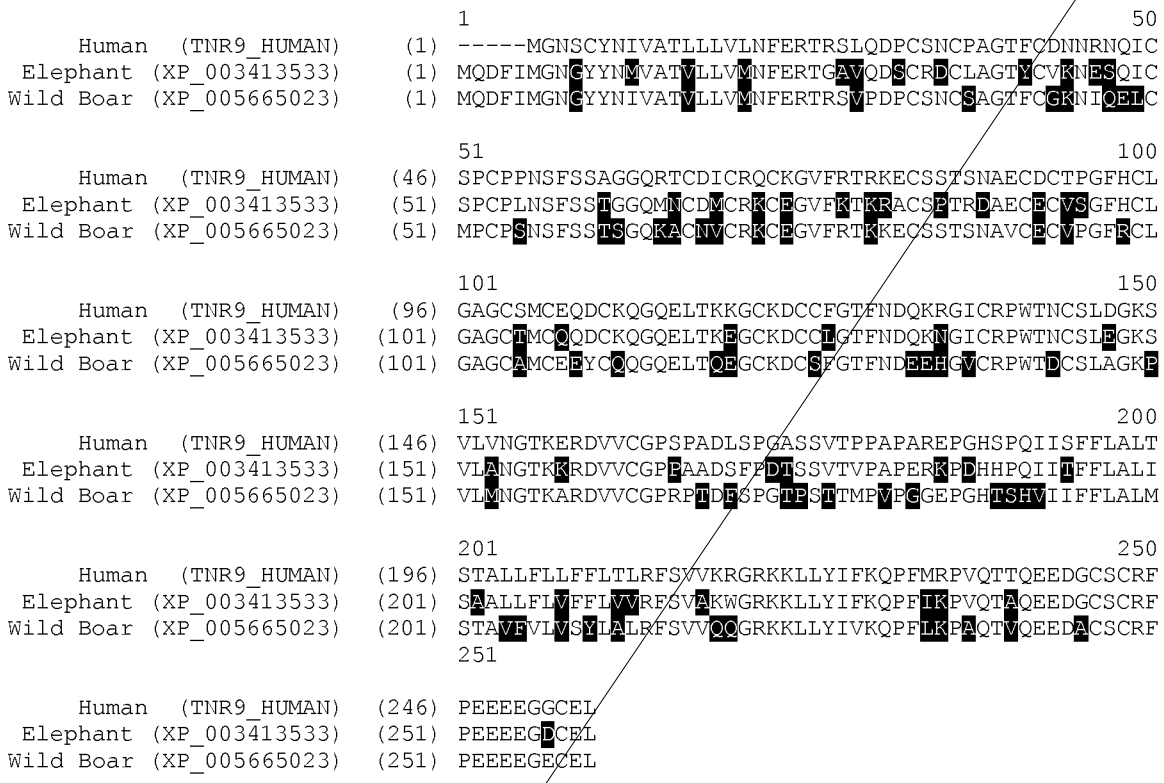
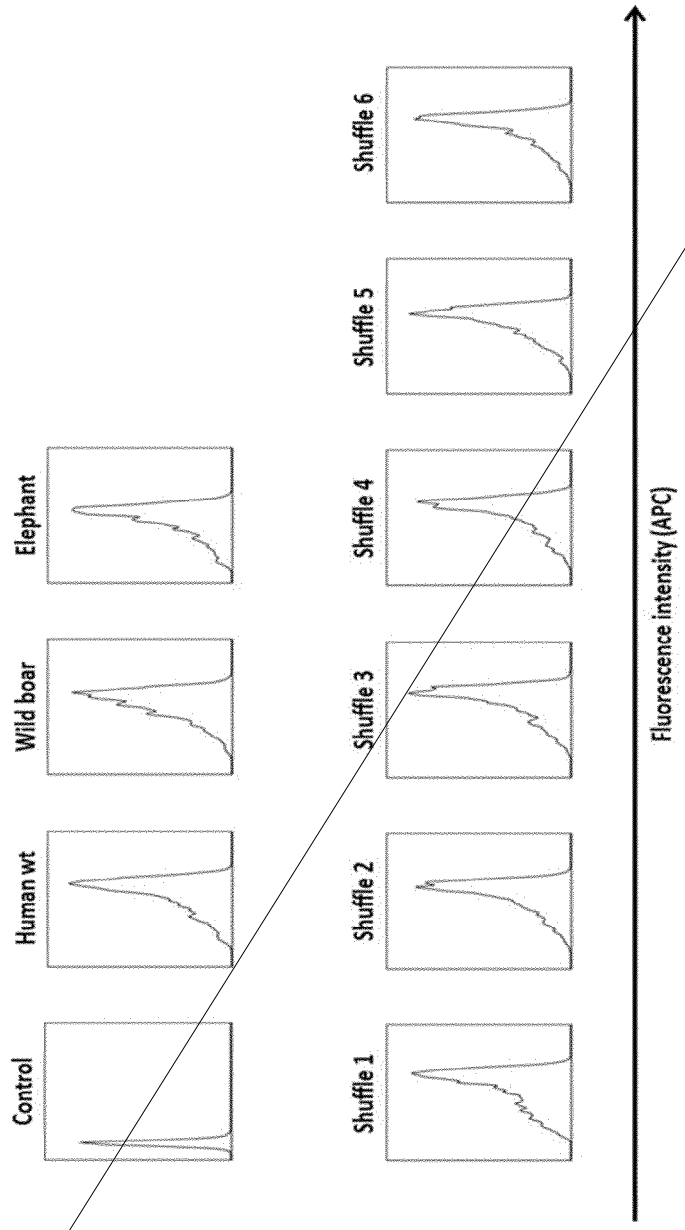

1 50
Human (TNR9_HUMAN) (1) -----MGNSCYNIVATLLLVLNFERTRSLQDPCSNCPAGTFCDNNRNQIC
Elephant (XP_003413533) (1) MQDFIMGNGYYNMVATVLLVMNFERTGAVQDSCRDCLAGTYCVKNESQIC
Wild Boar (XP_005665023) (1) MQDFIMGNGYYNIVATVLLVMNFERTRSVPDPCSNCSAGTFCGKNIQELC
51 100
Human (TNR9_HUMAN) (46) SPCPPNSFSSAGGQRTCDICRQCKGVFRTRKECSSTSNAECDCTPGFHCL
Elephant (XP_003413533) (51) SPCPLNSFSSTGGQMNCDMCRKCEGVFKTKRACSPTRDAECECVSGFHCL
Wild Boar (XP_005665023) (51) MPCPSNSFSSTSGQKACNVCRKCEGVFRTKKECSSTSNAVCECVPGFRCL
101 150
Human (TNR9_HUMAN) (96) GAGCSMCEQDCKQGQELTKKGCKDCCFGTFNDQKRGICRPWTNCSLDGKS
Elephant (XP_003413533) (101) GAGCTMCQQDCKQGQELTKEGCKDCCLGTFNDQKNGICRPWTNCSLEGKS
Wild Boar (XP_005665023) (101) GAGCAMCEEYCQQGQELTQEGCKDCSFGTFNDEEHGVCRPWTDCSLAGKP
151 200
Human (TNR9_HUMAN) (146) VLVNGTKERDVVCGPSPADLSPGASSVTPPAPAREPGHSPQIISFFLALT
Elephant (XP_003413533) (151) VLANGTKKRDVVCGPPAADSFPDTSSVTVPAPERKPDHHPQIITFFLALI
Wild Boar (XP_005665023) (151) VLMNGTKARDVVCGPRPTDESPGTPSTTMPVPGGEPGHTSHVIIFFLALM
201 250
Human (TNR9_HUMAN) (196) STALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF
Elephant (XP_003413533) (201) SAALLFLVFFLVVRFSVAKWGRKKLLYIFKQPFIKPVQTAQEEDGCSCRF
Wild Boar (XP_005665023) (201) STAVFVLVSYLALRFSVVQQGRKKLLYIVKQPFLKPAQTVQEEDACSCRF
251
Human (TNR9_HUMAN) (246) PEEEEGGCEL
Elephant (XP_003413533) (251) PEEEEGDCEL
Wild Boar (XP_005665023) (251) PEEEEGECEL

Figure 2

Figure 3


Control
Human wt
Wild boar
Elephant
Shuffle 1
Shuffle 2
Shuffle 3
Shuffle 4
Shuffle 5
Shuffle 6
Fluorescence intensity (APC)

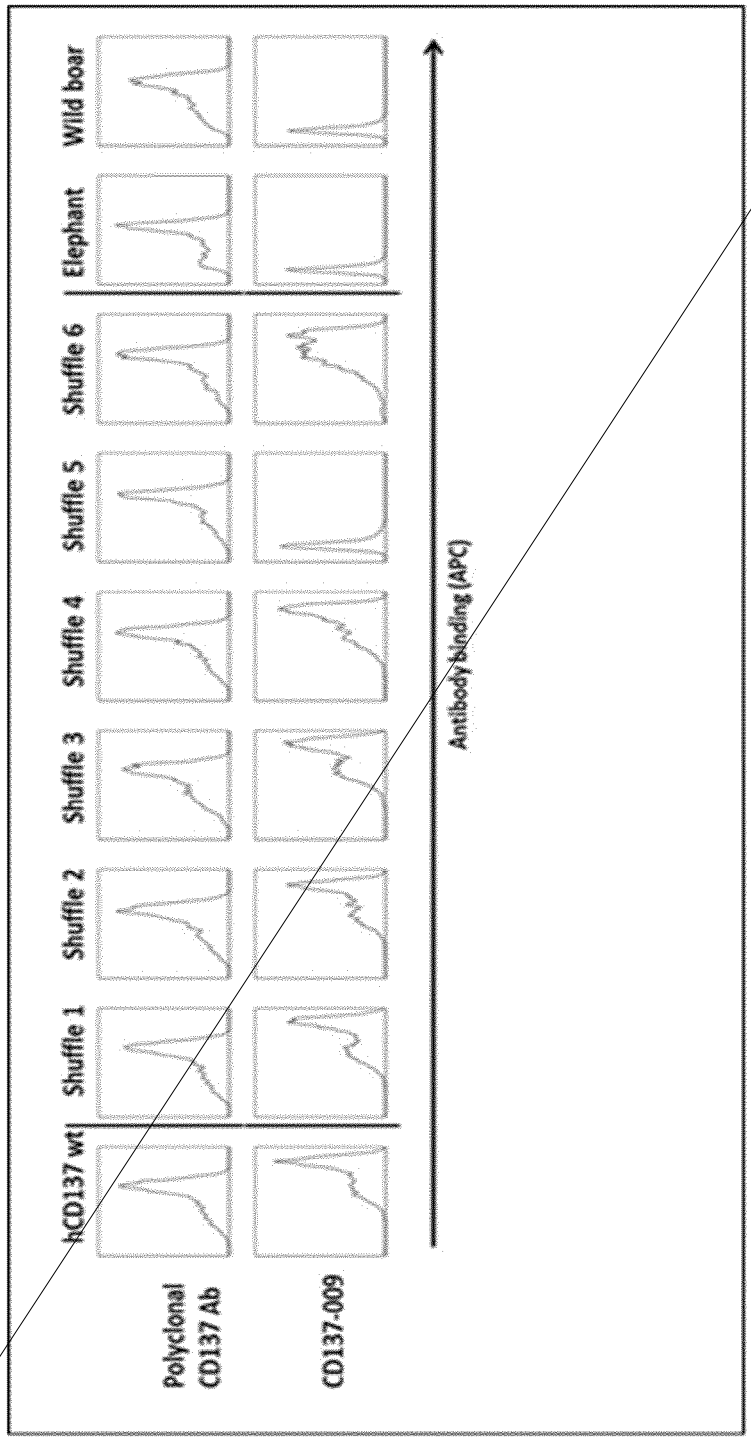

hCD137 wt
Shuffle 1
Shuffle 2
Shuffle 3
Shuffle 4
Shuffle 5
Shuffle 6
Elephant
Wild boar
Polyclonal CD137 Ab
CD137-009
Antibody binding (APC)

Figure 4

Figure 5

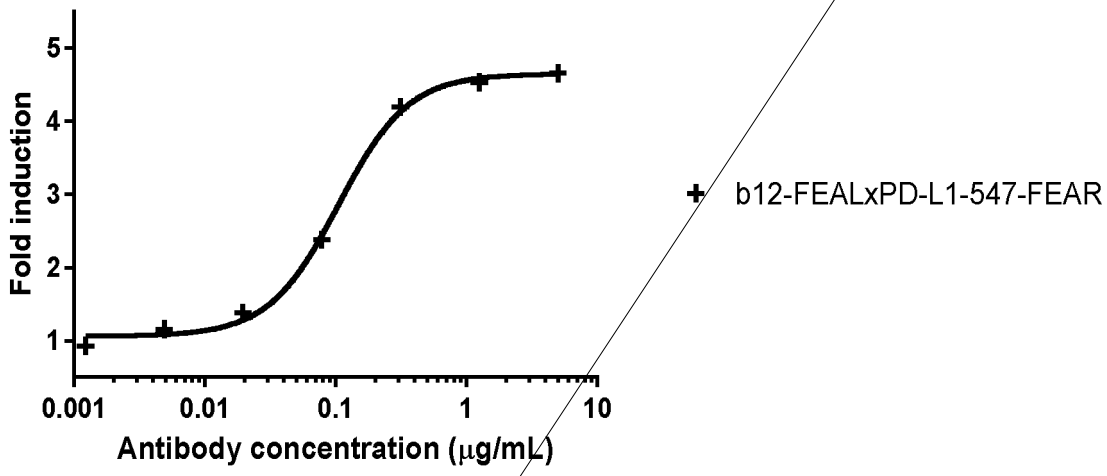

Fold induction
5
4
3
2
1
0.001
0.01
0.1
1
10
Antibody concentration (μg/mL)
b12-FEALxPD-L1-547-FEAR

Figure 6

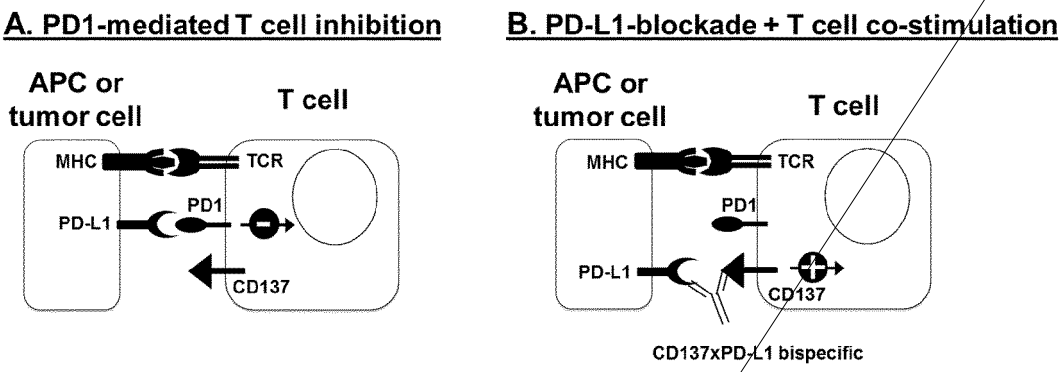

A. PD1-mediated T cell inhibition
APC or tumor cell
T cell
MHC
TCR
PD1
PD-L1
CD137
B. PD-L1-blockade + T cell co-stimulation
APC or tumor cell
T cell
MHC
TCR
PD1
PD-L1
CD137
CD137xPD-L1 bispecific

Figure 7

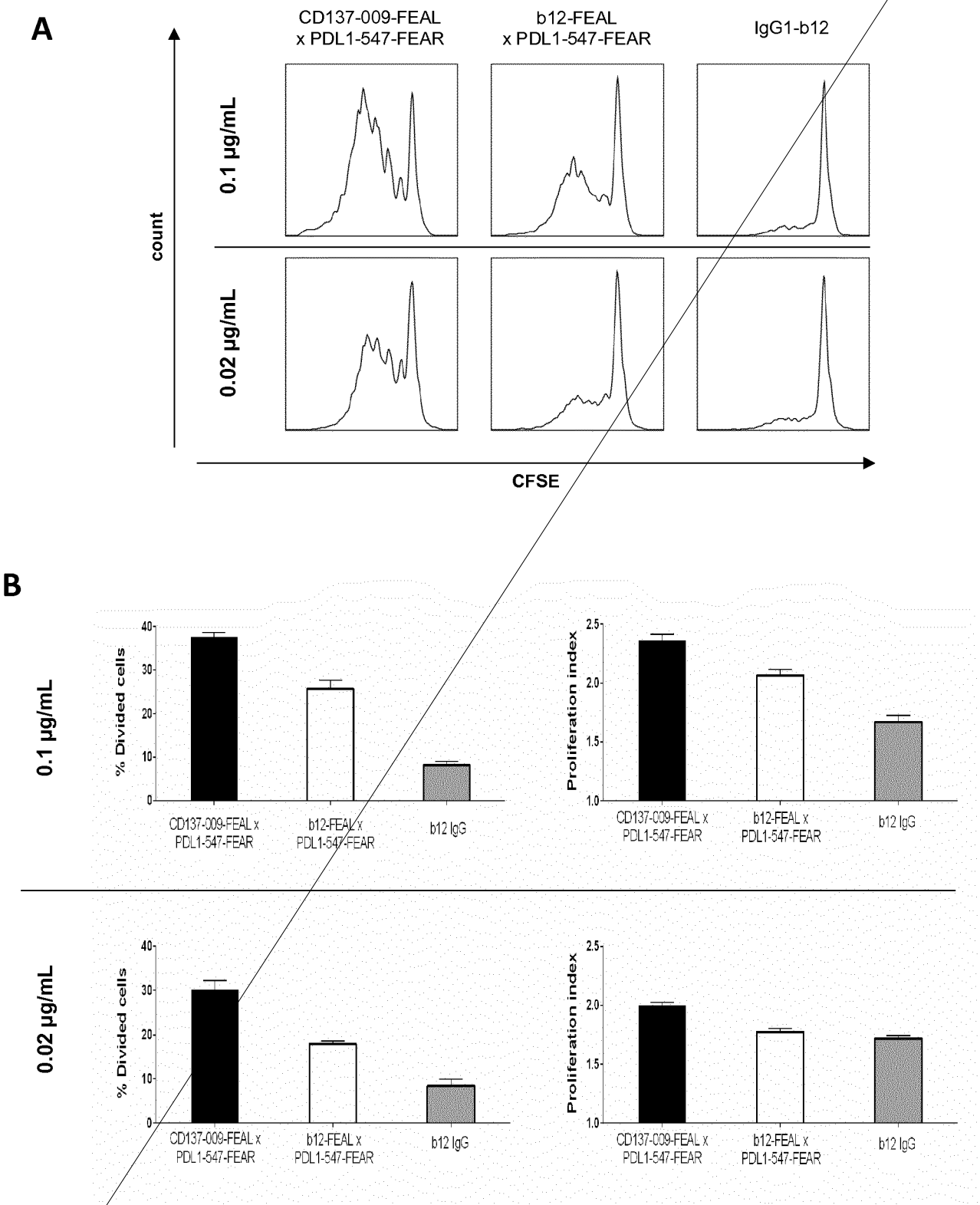

A
CD137-009-FEAL x PDL1-547-FEAR
b12-FEAL x PDL1-547-FEAR
IgG1-b12
0.1 µg/mL
0.02 µg/mL
count
CFSE
B
0.1 µg/mL
0.02 µg/mL
% Divided cells
Proliferation index
CD137-009-FEAL x PDL1-547-FEAR
b12-FEAL x PDL1-547-FEAR
b12 IgG

Figure 7 continued

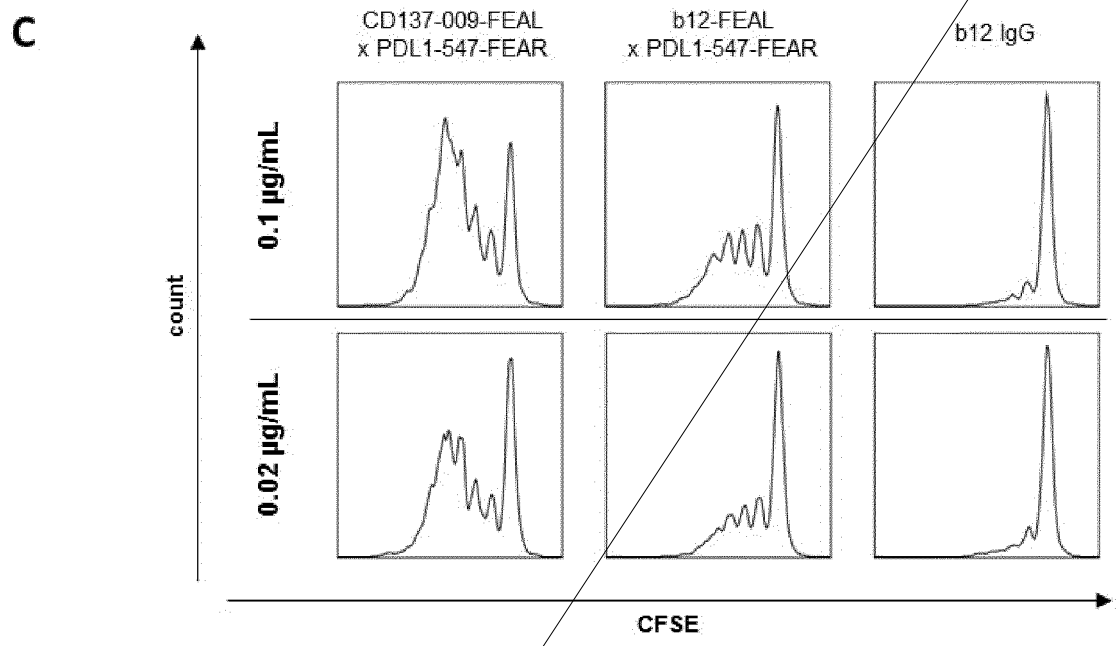


Figure 7 Continued

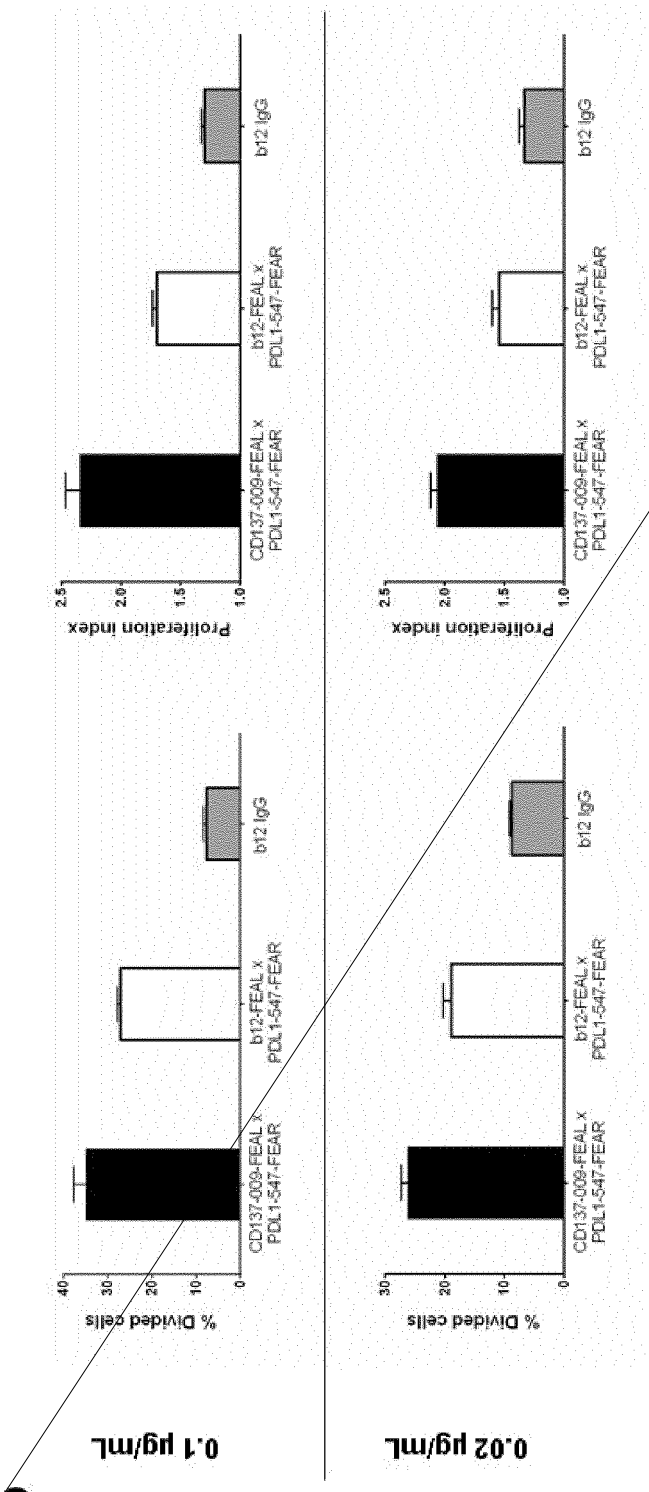
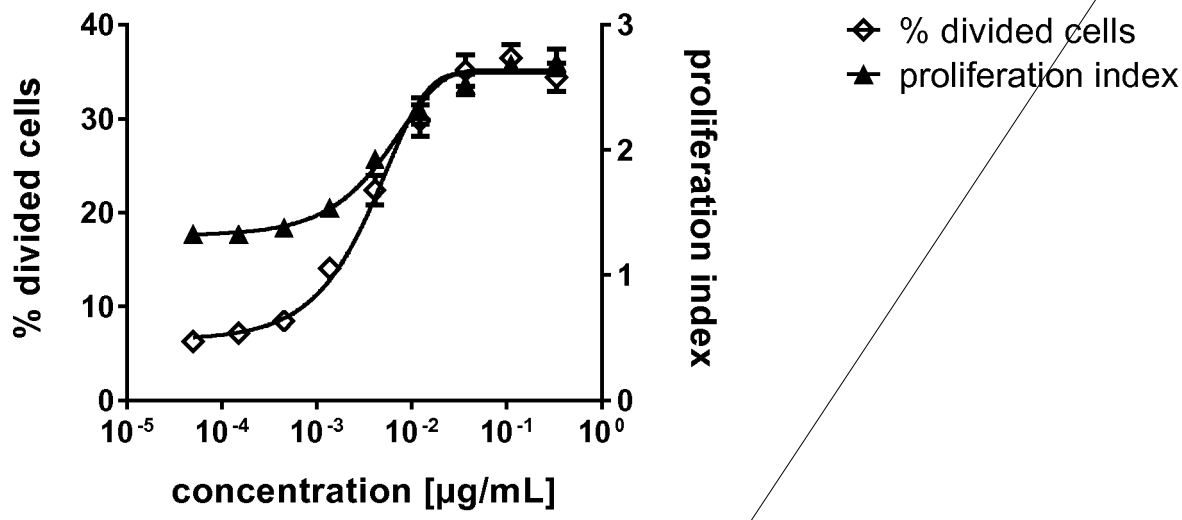


Figure 8



	% Divided cells	Proliferation index
EC_{50}	0.003492 µg/mL	0.005388 µg/mL

Figure 9

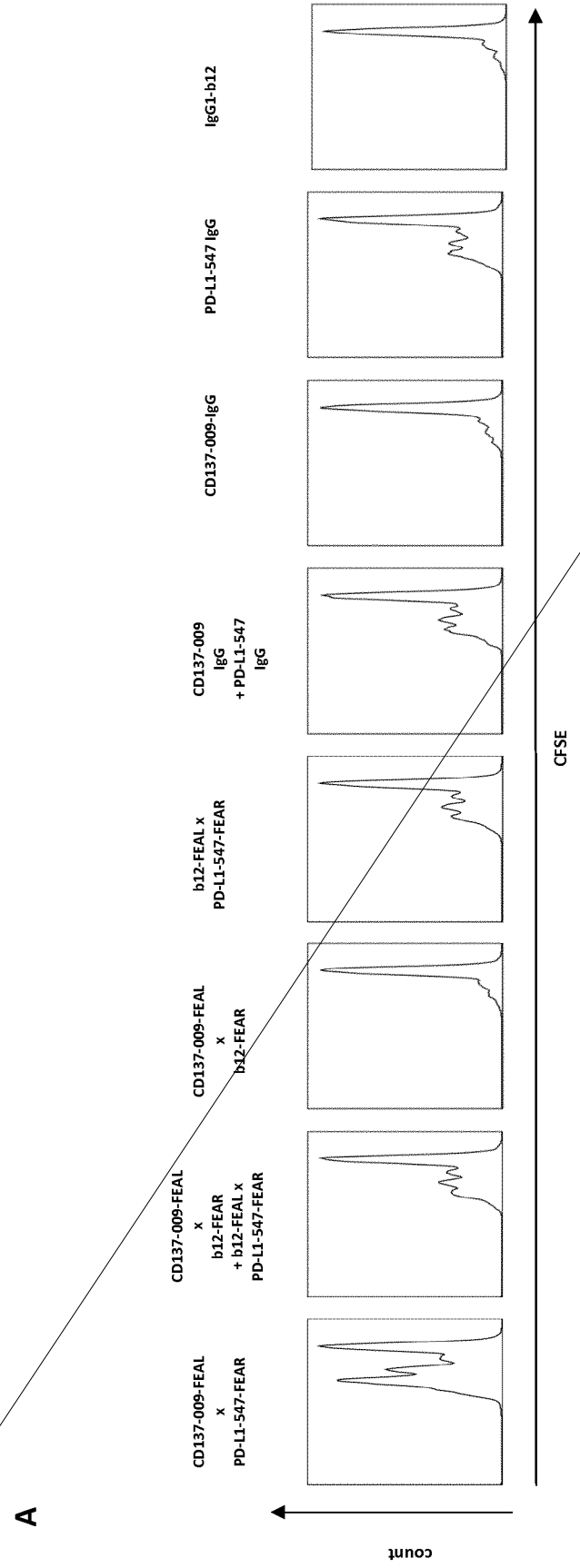
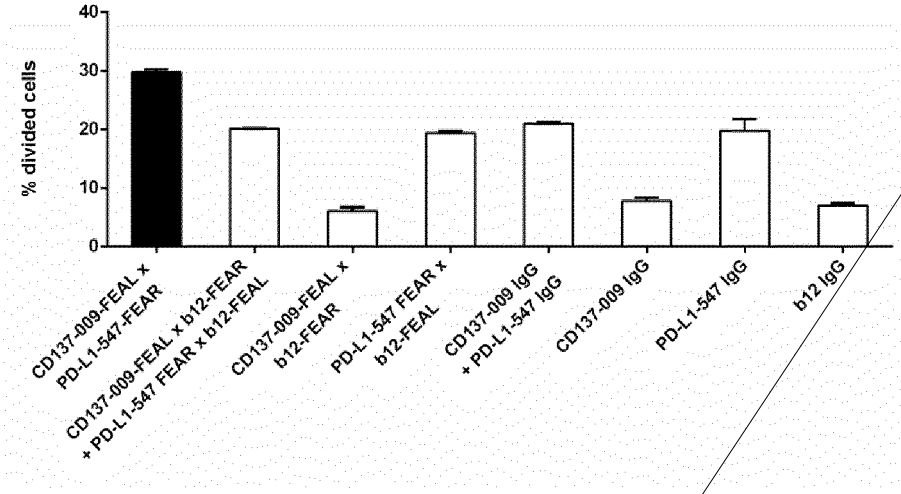

A
count
CFSE
CD137-009-FEAL x PD-L1-547-FEAR
CD137-009-FEAL x b12-FEAR + b12-FEAL x PD-L1-547-FEAR
CD137-009-FEAL x b12-FEAR
b12-FEAL x PD-L1-547-FEAR
CD137-009 IgG + PD-L1-547 IgG
CD137-009-IgG
PD-L1-547 IgG
IgG1-b12

Figure 9 continued

B



C

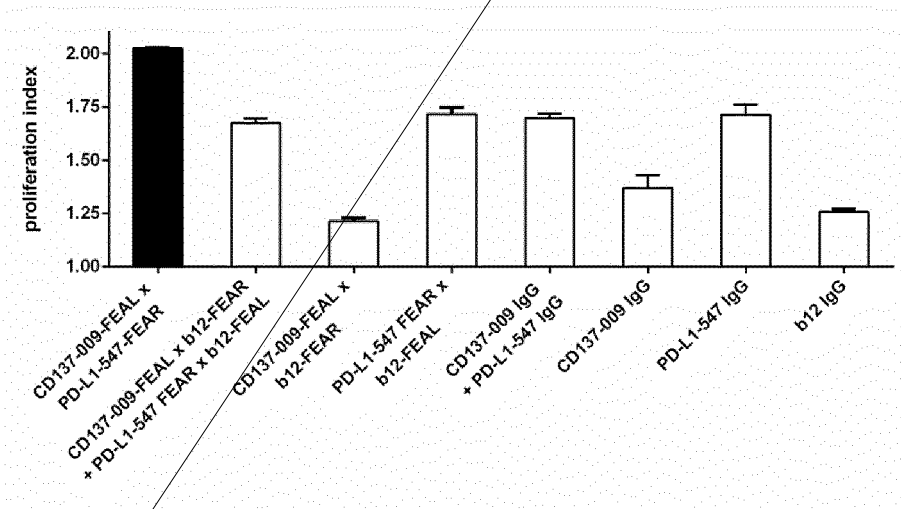


Figure 10

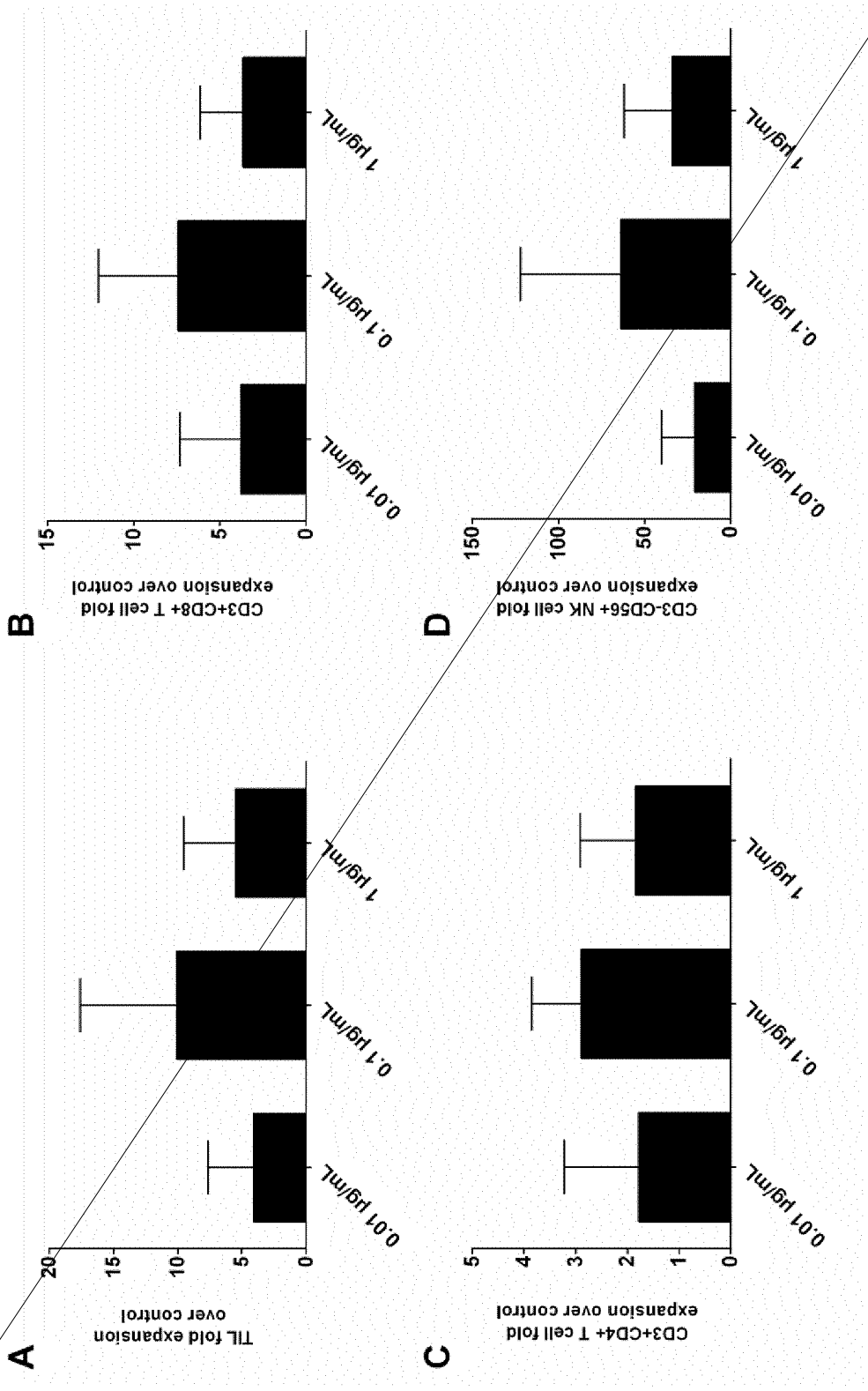

A
TIL fold expansion over control
0
5
10
15
20
0.01 µg/mL
0.1 µg/mL
1 µg/mL
B
CD3+CD8+ T cell fold expansion over control
0
5
10
15
0.01 µg/mL
0.1 µg/mL
1 µg/mL
C
CD3+CD4+ T cell fold expansion over control
0
1
2
3
4
5
0.01 µg/mL
0.1 µg/mL
1 µg/mL
D
CD3-CD56+ NK cell fold expansion over control
0
50
100
150
0.01 µg/mL
0.1 µg/mL
1 µg/mL

Figure 11

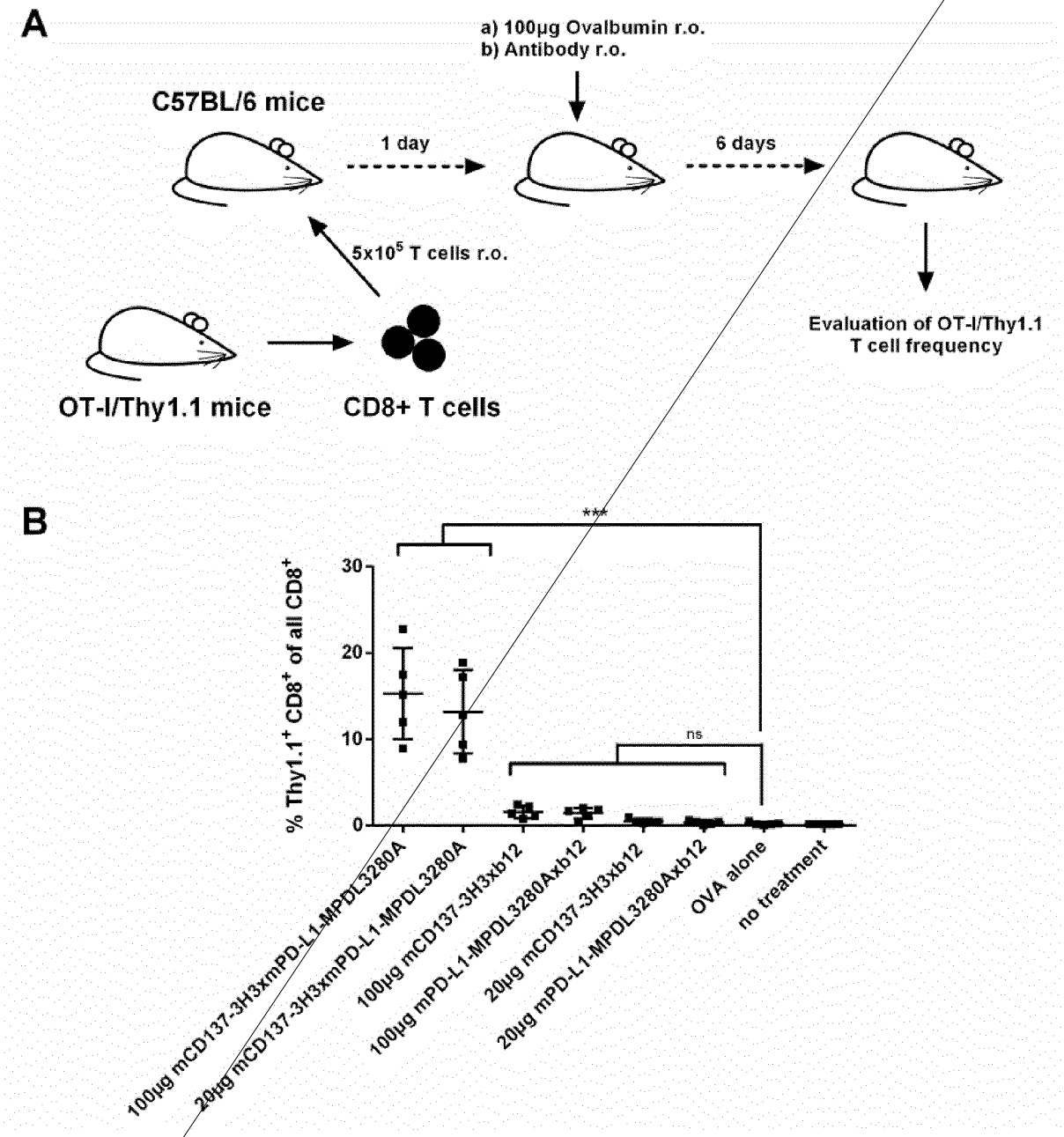

A
a) 100µg Ovalbumin r.o.
b) Antibody r.o.
C57BL/6 mice
1 day
6 days
5x10^5 T cells r.o.
Evaluation of OT-I/Thy1.1 T cell frequency
OT-I/Thy1.1 mice
CD8+ T cells
B
***
ns
% Thy1.1+ CD8+ of all CD8+
30
20
10
0
100µg mCD137-3H3xmPD-L1-MPDL3280A
20µg mCD137-3H3xmPD-L1-MPDL3280A
100µg mCD137-3H3xb12
100µg mPD-L1-MPDL3280Axb12
20µg mCD137-3H3xb12
20µg mPD-L1-MPDL3280Axb12
OVA alone
no treatment

Figure 12

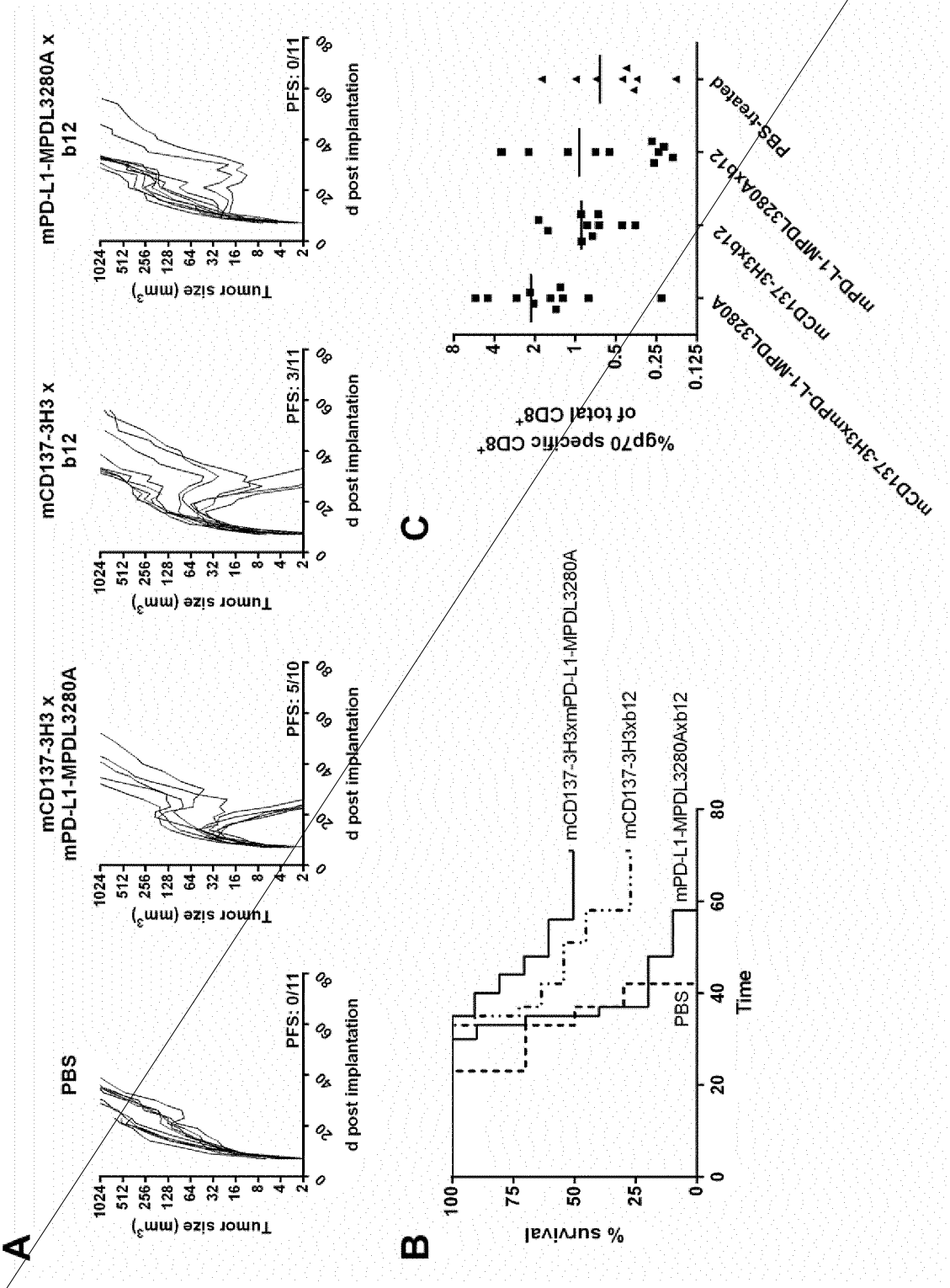

A
PBS
mCD137-3H3 x mPD-L1-MPDL3280A
mCD137-3H3 x b12
mPD-L1-MPDL3280A x b12
Tumor size (mm³)
d post implantation
PFS: 0/11
PFS: 5/10
PFS: 3/11
PFS: 0/11
B
% survival
Time
mCD137-3H3xmPD-L1-MPDL3280A
mCD137-3H3xb12
mPD-L1-MPDL3280Axb12
PBS
C
%gp70 specific CD8+ of total CD8+
mCD137-3H3xmPD-L1-MPDL3280A
mCD137-3H3xb12
mPD-L1-MPDL3280Axb12
PBS-treated

Figure 13

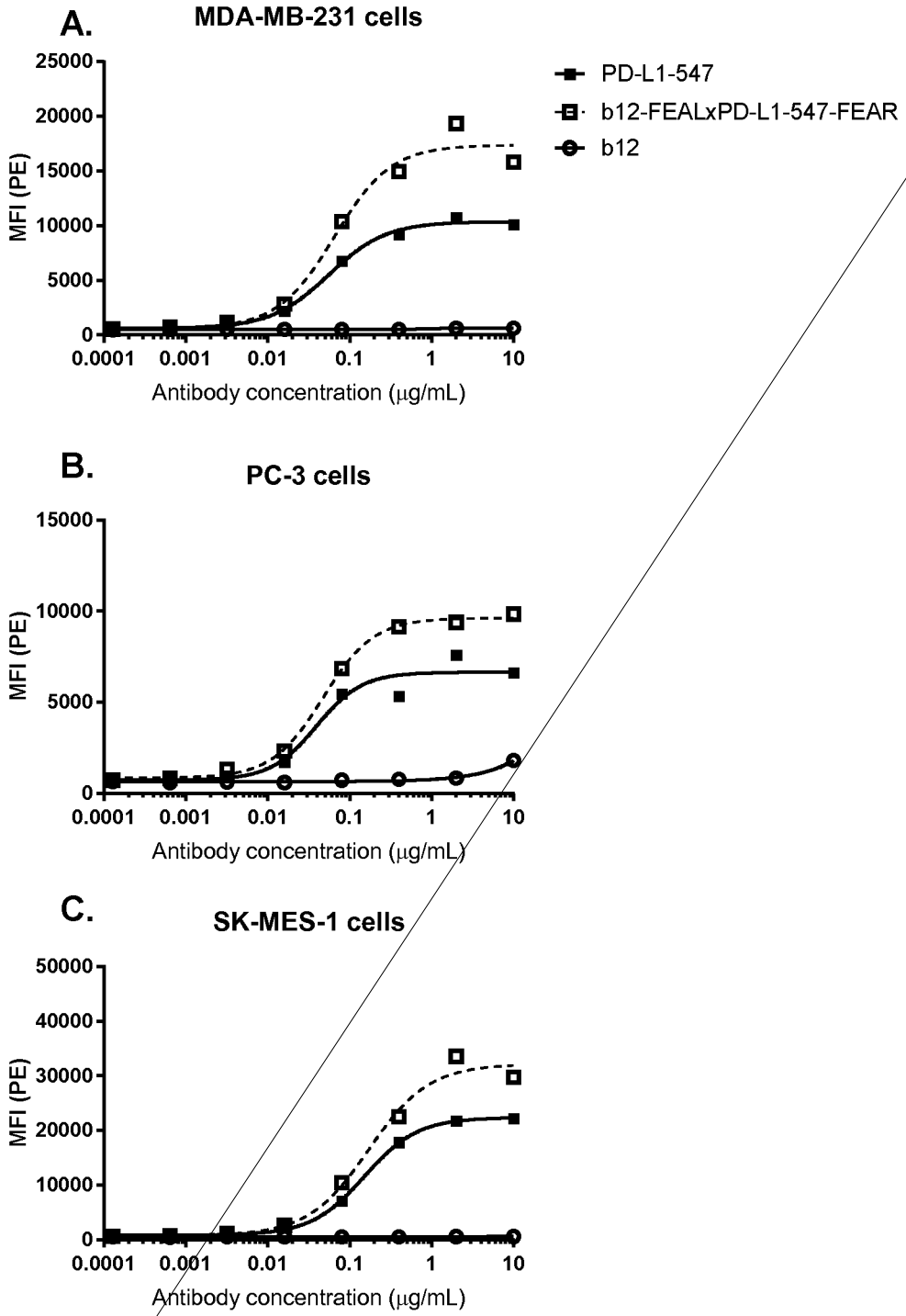

A.
MDA-MB-231 cells
MFI (PE)
25000
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (μg/mL)
PD-L1-547
b12-FEALxPD-L1-547-FEAR
b12
B.
PC-3 cells
MFI (PE)
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (μg/mL)
C.
SK-MES-1 cells
MFI (PE)
50000
40000
30000
20000
10000
0
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (μg/mL)

Figure 14

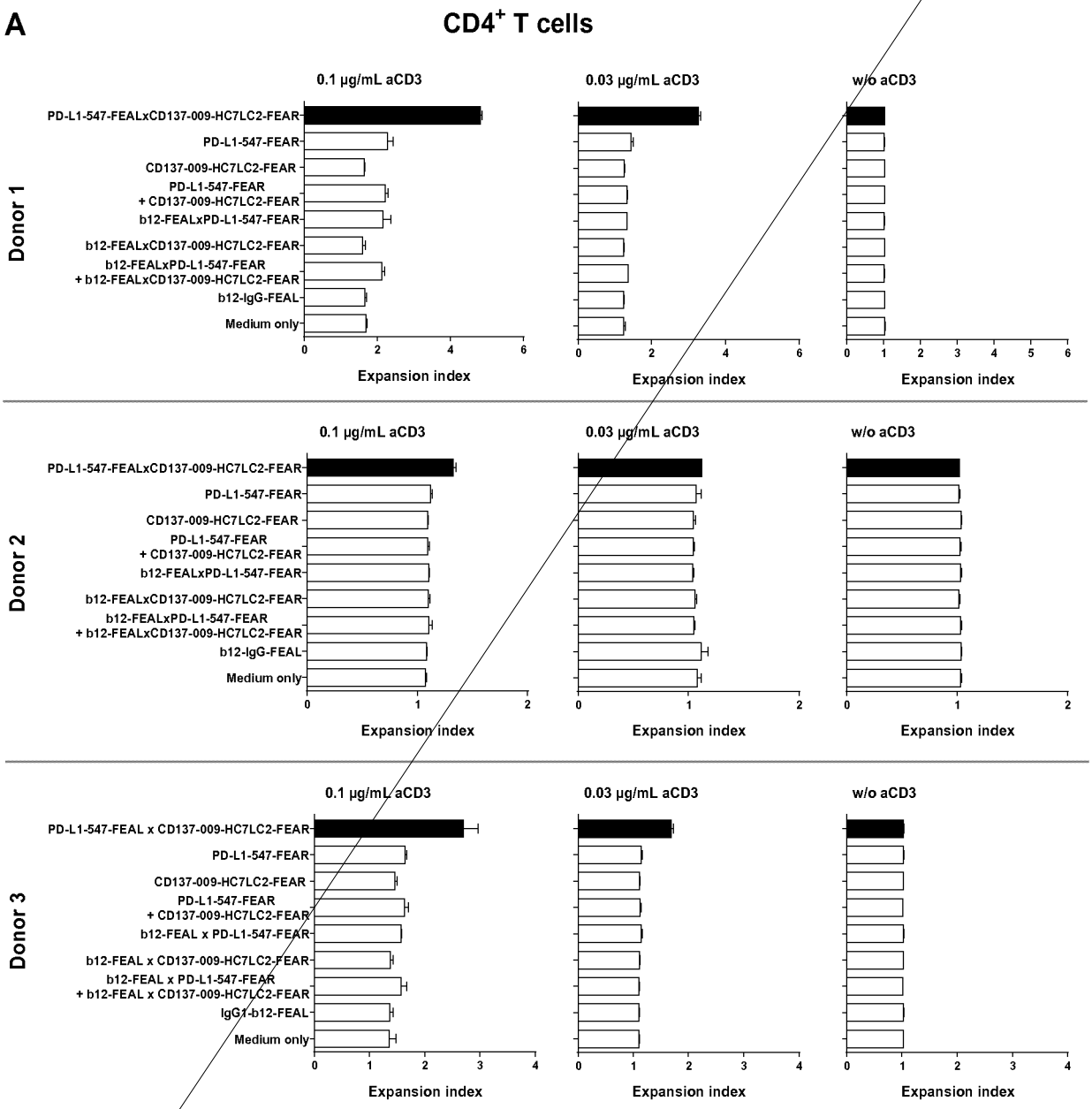

A
CD4+ T cells
Donor 1
Donor 2
Donor 3
0.1 µg/mL aCD3
0.03 µg/mL aCD3
w/o aCD3
PD-L1-547-FEALxCD137-009-HC7LC2-FEAR
PD-L1-547-FEAR
CD137-009-HC7LC2-FEAR
PD-L1-547-FEAR + CD137-009-HC7LC2-FEAR
b12-FEALxPD-L1-547-FEAR
b12-FEALxCD137-009-HC7LC2-FEAR
b12-FEALxPD-L1-547-FEAR + b12-FEALxCD137-009-HC7LC2-FEAR
b12-IgG-FEAL
Medium only
PD-L1-547-FEAL x CD137-009-HC7LC2-FEAR
b12-FEAL x PD-L1-547-FEAR
b12-FEAL x CD137-009-HC7LC2-FEAR
b12-FEAL x PD-L1-547-FEAR + b12-FEAL x CD137-009-HC7LC2-FEAR
IgG1-b12-FEAL
Expansion index

Figure 14 continued

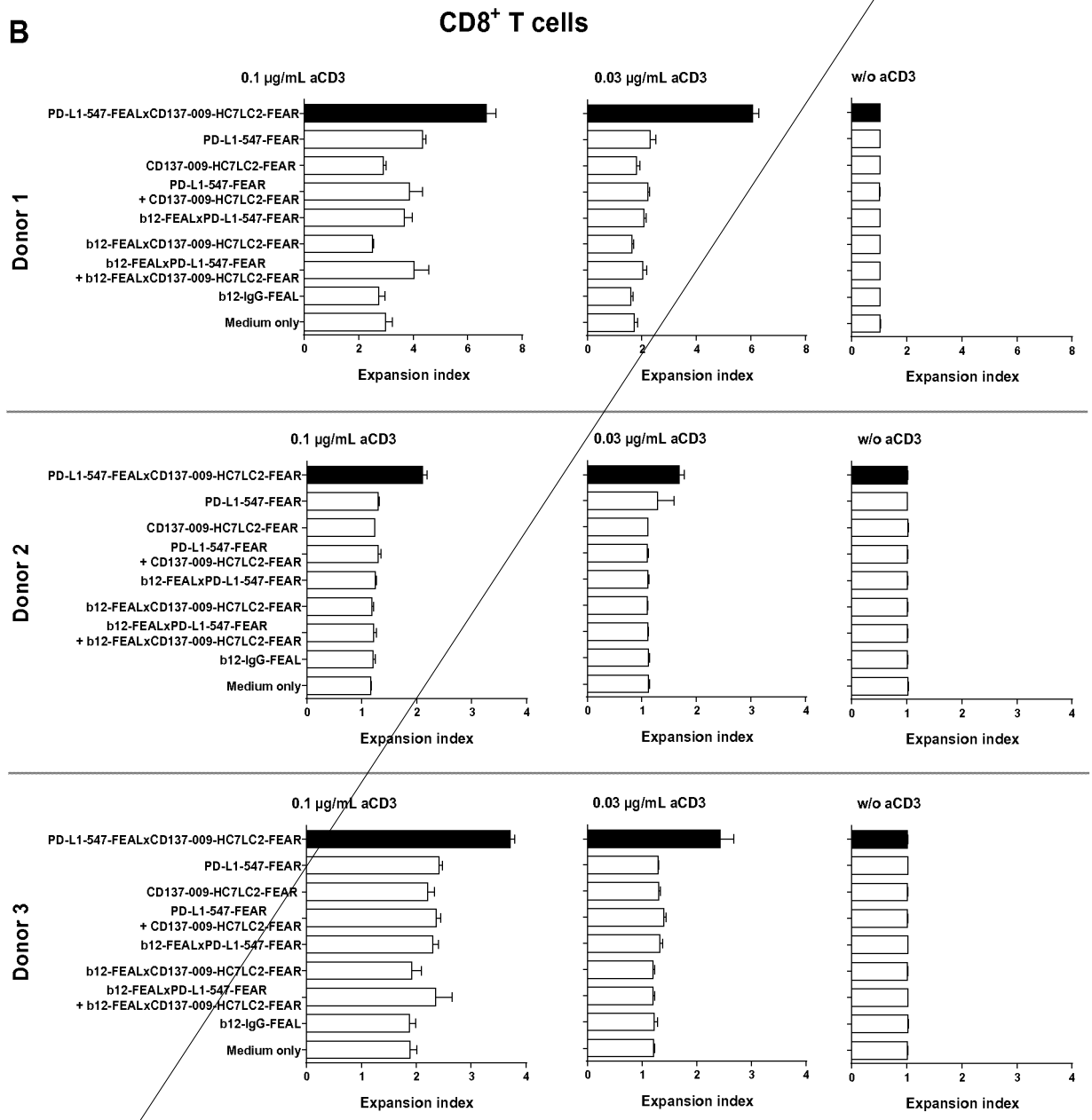


Figure 15

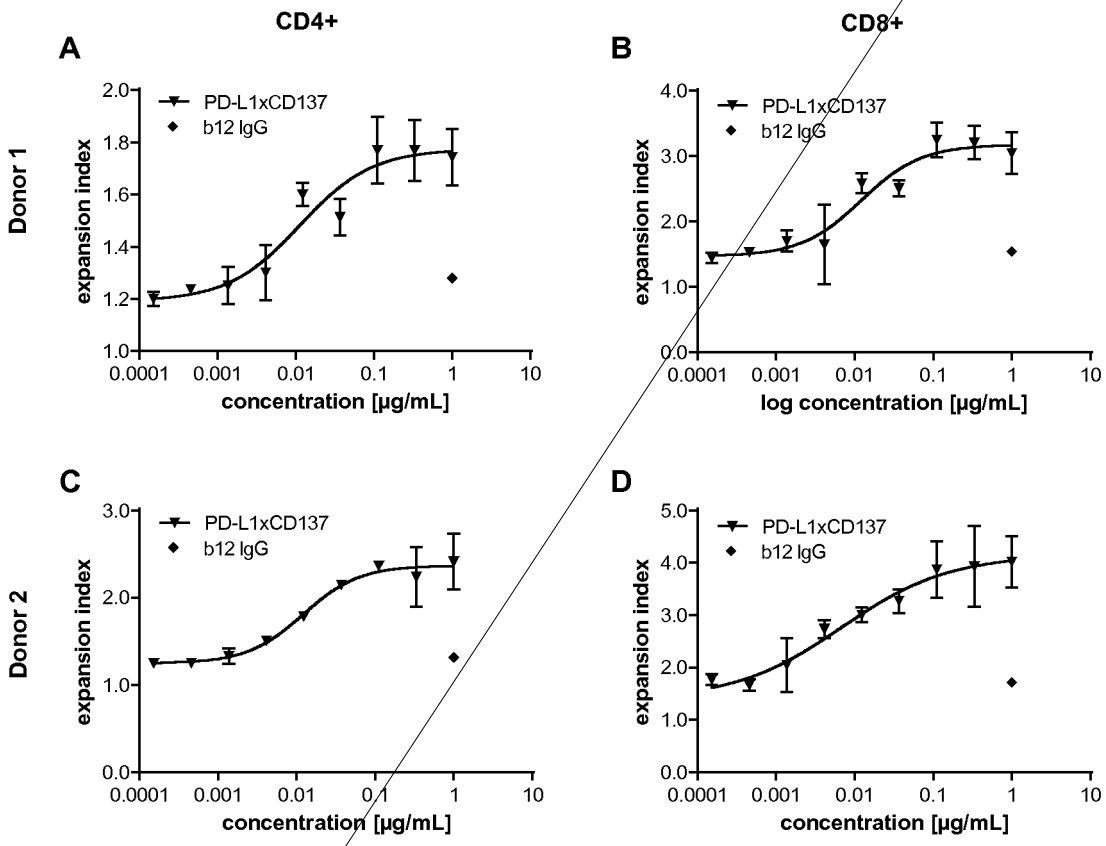

CD4+
CD8+
A
B
C
D
Donor 1
Donor 2
PD-L1xCD137
b12 IgG
expansion index
concentration [µg/mL]
log concentration [µg/mL]
0.0001
0.001
0.01
0.1
1
10

Figure 16

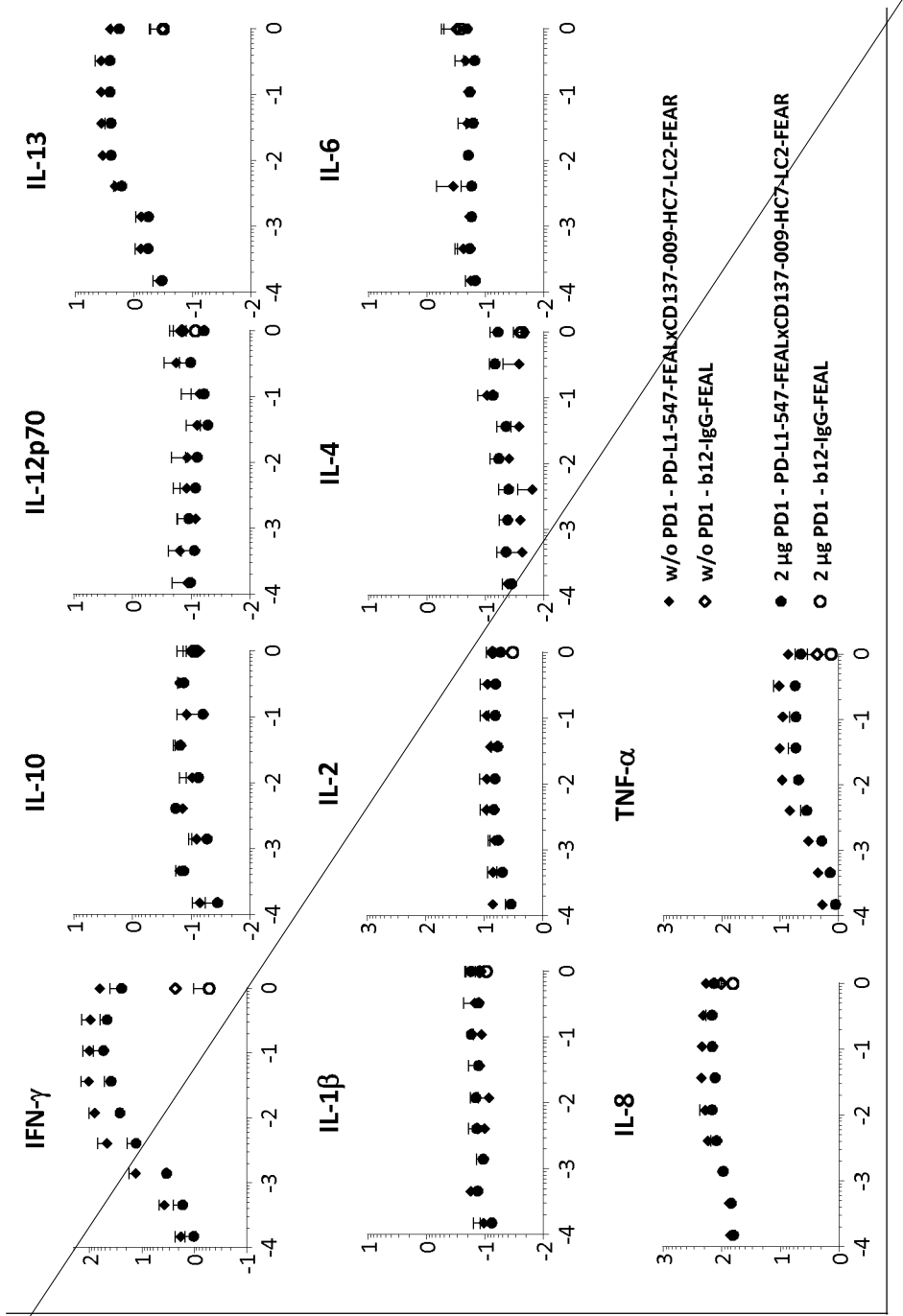

IFN-γ
IL-10
IL-12p70
IL-13
IL-1β
IL-2
IL-4
IL-6
IL-8
TNF-α
w/o PD1 - PD-L1-547-FEALxCD137-009-HC7-LC2-FEAR
w/o PD1 - b12-IgG-FEAL
2 µg PD1 - PD-L1-547-FEALxCD137-009-HC7-LC2-FEAR
2 µg PD1 - b12-IgG-FEAL
Log concentration of cytokine [pg/mL]
Log concentration [µg/mL]

Figure 17

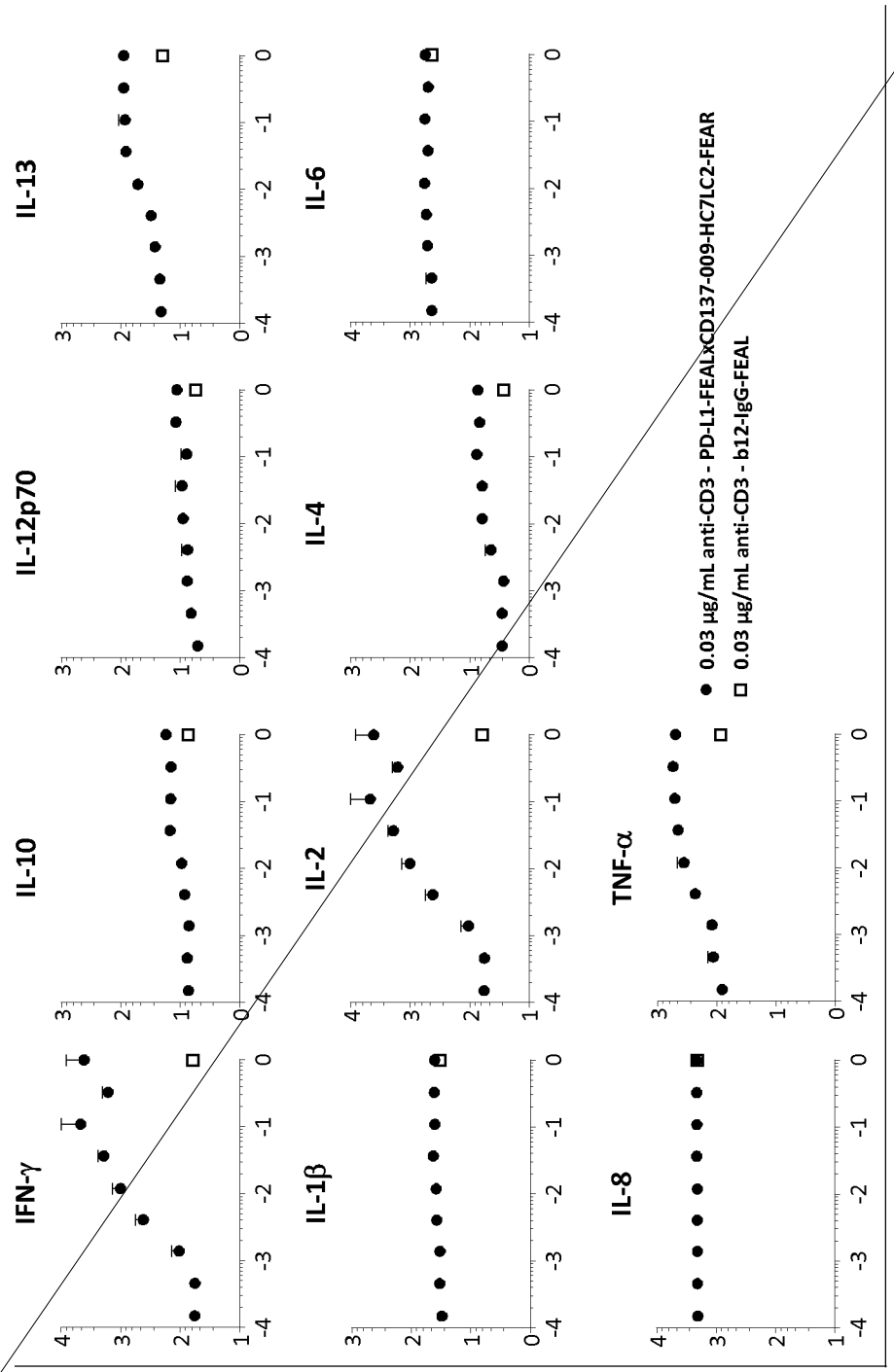

IFN-γ
IL-10
IL-12p70
IL-13
IL-1β
IL-2
IL-4
IL-6
IL-8
TNF-α
0.03 µg/mL anti-CD3 - PD-L1-FEALxCD137-009-HC7LC2-FEAR
0.03 µg/mL anti-CD3 - b12-IgG-FEAL
Log concentration of cytokine [pg/mL]
Log concentration [µg/mL]

Figure 1

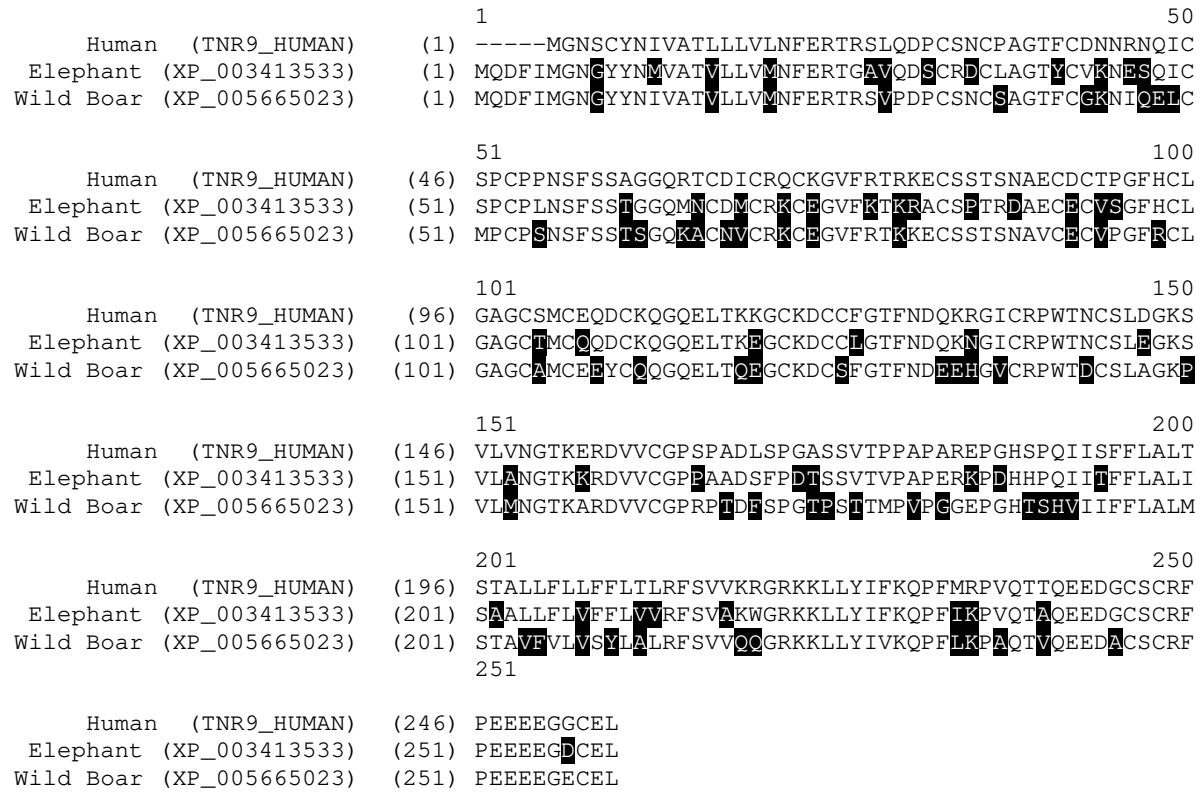

1 50
Human (TNR9_HUMAN) (1) -----MGNSCYNIVATLLLVLNFERTRSLQDPCSNCPAGTFCDNNRNQIC
Elephant (XP_003413533) (1) MQDFIMGNGYYNMVATVLLVMNFERTGAVQDSCRDCLAGTYCVKNESQIC
Wild Boar (XP_005665023) (1) MQDFIMGNGYYNIVATVLLVMNFERTRSVPDPCSNCSAGTFCGKNIQELC
51 100
Human (TNR9_HUMAN) (46) SPCPPNSFSSAGGQRTCDICRQCKGVFRTRKECSSTSNAECDCTPGFHCL
Elephant (XP_003413533) (51) SPCPLNSFSSTGGQMNCDMCRKCEGVFKTKRACSPTRDAECECVSGFHCL
Wild Boar (XP_005665023) (51) MPCPSNSFSSTSGQKACNVCRKCEGVFRTKKECSSTSNAVCECVPGFRCL
101 150
Human (TNR9_HUMAN) (96) GAGCSMCEQDCKQGQELTKKGCKDCCFGTFNDQKRGICRPWTNCSLDGKS
Elephant (XP_003413533) (101) GAGCTMCQQDCKQGQELTKEGCKDCCLGTFNDQKNGICRPWTNCSLEGKS
Wild Boar (XP_005665023) (101) GAGCAMCEEYCQQGQELTQEGCKDCSFGTFNDEEHGVCRPWTDCSLAGKP
151 200
Human (TNR9_HUMAN) (146) VLVNGTKERDVVCGPSPADLSPGASSVTPPAPAREPGHSPQIISFFLALT
Elephant (XP_003413533) (151) VLANGTKKRDVVCGPPAADSFPDTSSVTVPAPERKPDHHPQIITFFLALI
Wild Boar (XP_005665023) (151) VLMNGTKARDVVCGPRPTDFSPGTPSTTMPVPGGEPGHTSHVIIFFLALM
201 250
Human (TNR9_HUMAN) (196) STALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF
Elephant (XP_003413533) (201) SAALLFLVFFLVVRFSVAKWGRKKLLYIFKQPFIKPVQTAQEEDGCSCRF
Wild Boar (XP_005665023) (201) STAVFVLVSYLALRFSVVQQGRKKLLYIVKQPFLKPAQTVQEEDACSCRF
251
Human (TNR9_HUMAN) (246) PEEEEGGCEL
Elephant (XP_003413533) (251) PEEEEGDCEL
Wild Boar (XP_005665023) (251) PEEEEGECEL

Figure 2

R9_HUMAN
huffle
R9_HUMAN shuffle 1
huffle
R9_HUMAN shuffle 2
huffle
R9_HUMAN shuffle 3
huffle
R9_HUMAN shuffle 4
huffle
R9_HUMAN shuffle 5
huffle
R9_HUMAN shuffle 6
huffle
_003413533
_005665023
N
N shuffle 1
N shuffle 2
N shuffle 3
N shuffle 4
N shuffle 5
N shuffle 6
Shuffle 1
Shuffle 2
Shuffle 3
Shuffle 4
Shuffle 5
Shuffle 6

Figure 3

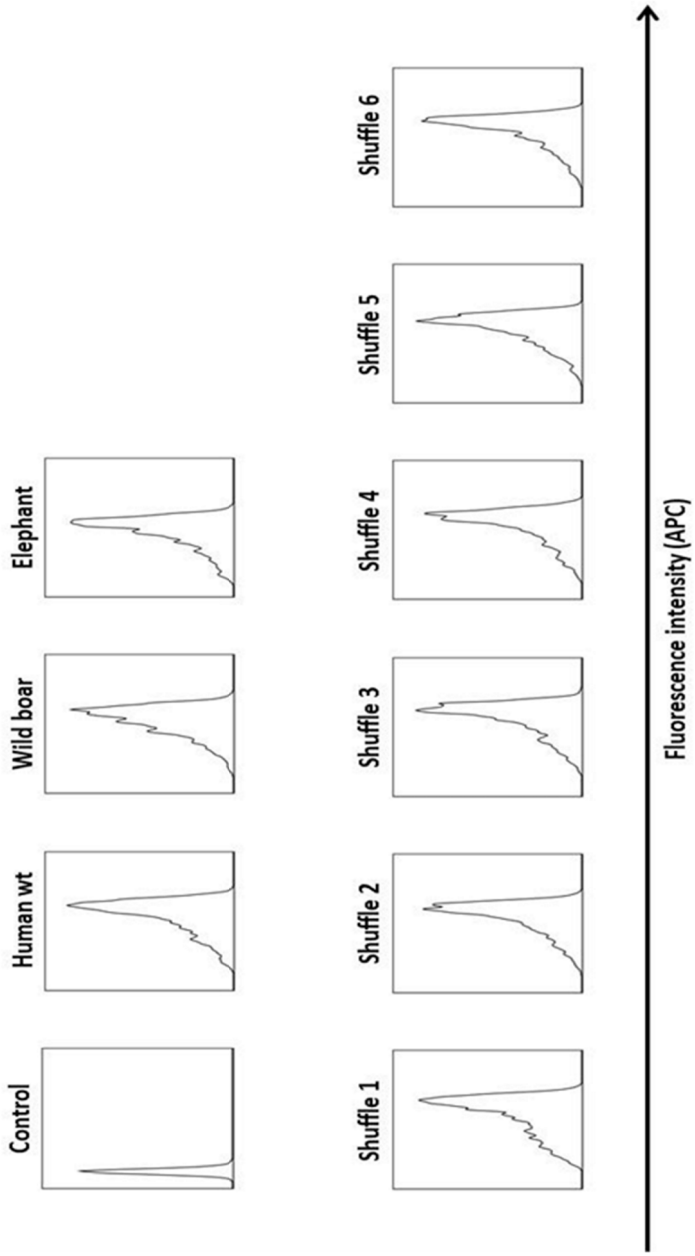

Control
Human wt
Wild boar
Elephant
Shuffle 1
Shuffle 2
Shuffle 3
Shuffle 4
Shuffle 5
Shuffle 6
Fluorescence intensity (APC)

Figure 4

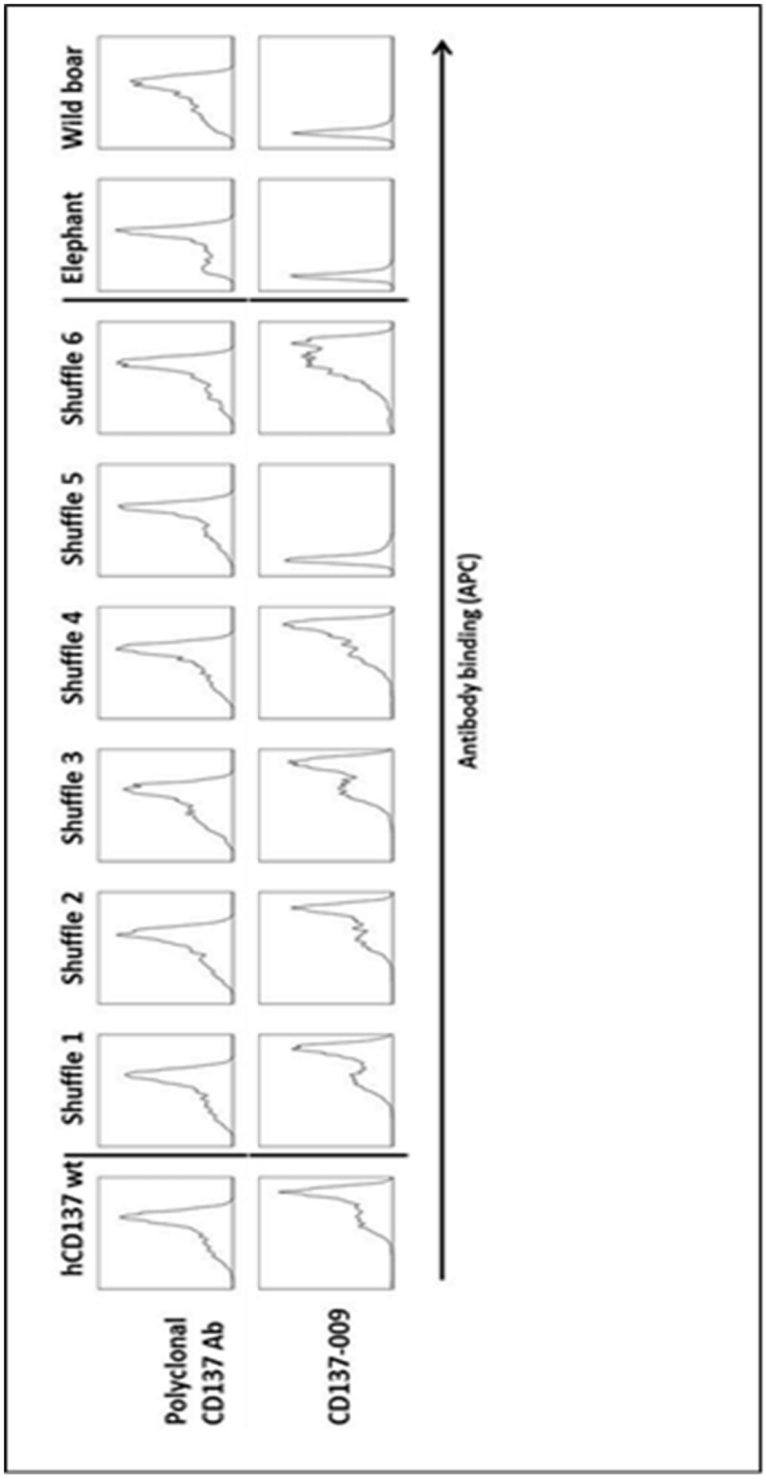

hCD137 wt
Shuffle 1
Shuffle 2
Shuffle 3
Shuffle 4
Shuffle 5
Shuffle 6
Elephant
Wild boar
Polyclonal CD137 Ab
CD137-009
Antibody binding (APC)

Figure 5

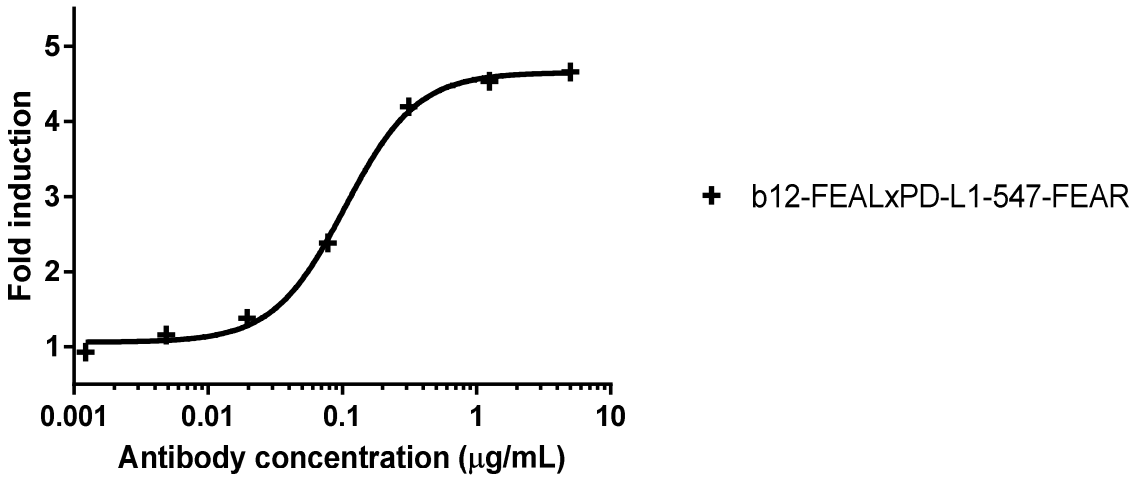

Fold induction
5
4
3
2
1
0.001
0.01
0.1
1
10
Antibody concentration (μg/mL)
b12-FEALxPD-L1-547-FEAR

Figure 6

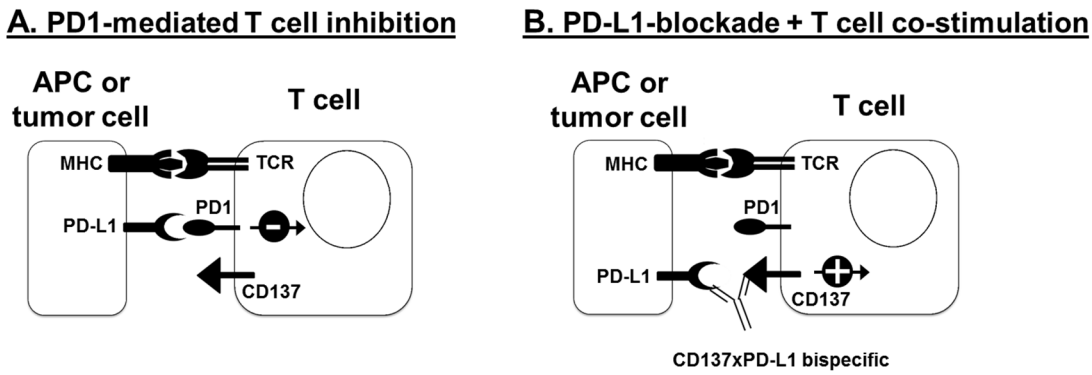
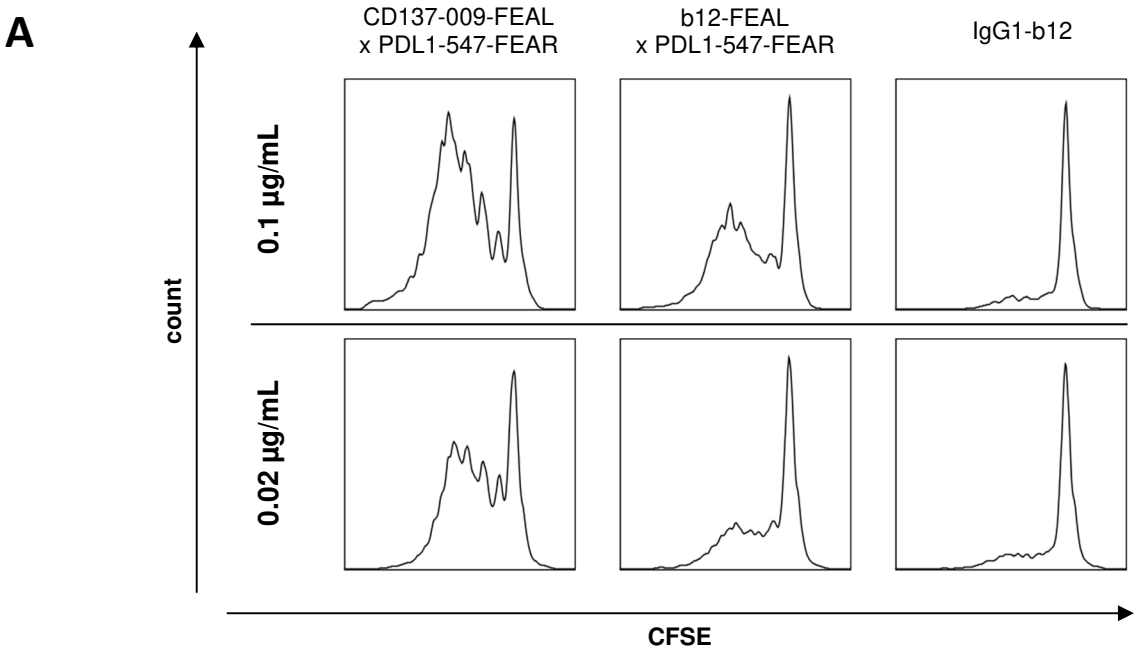

A. PD1-mediated T cell inhibition
APC or tumor cell
T cell
MHC
TCR
PD1
PD-L1
CD137
B. PD-L1-blockade + T cell co-stimulation
APC or tumor cell
T cell
MHC
TCR
PD1
PD-L1
CD137
CD137xPD-L1 bispecific

Figure 7


A
CD137-009-FEAL x PDL1-547-FEAR
b12-FEAL x PDL1-547-FEAR
IgG1-b12
0.1 µg/mL
0.02 µg/mL
count
CFSE

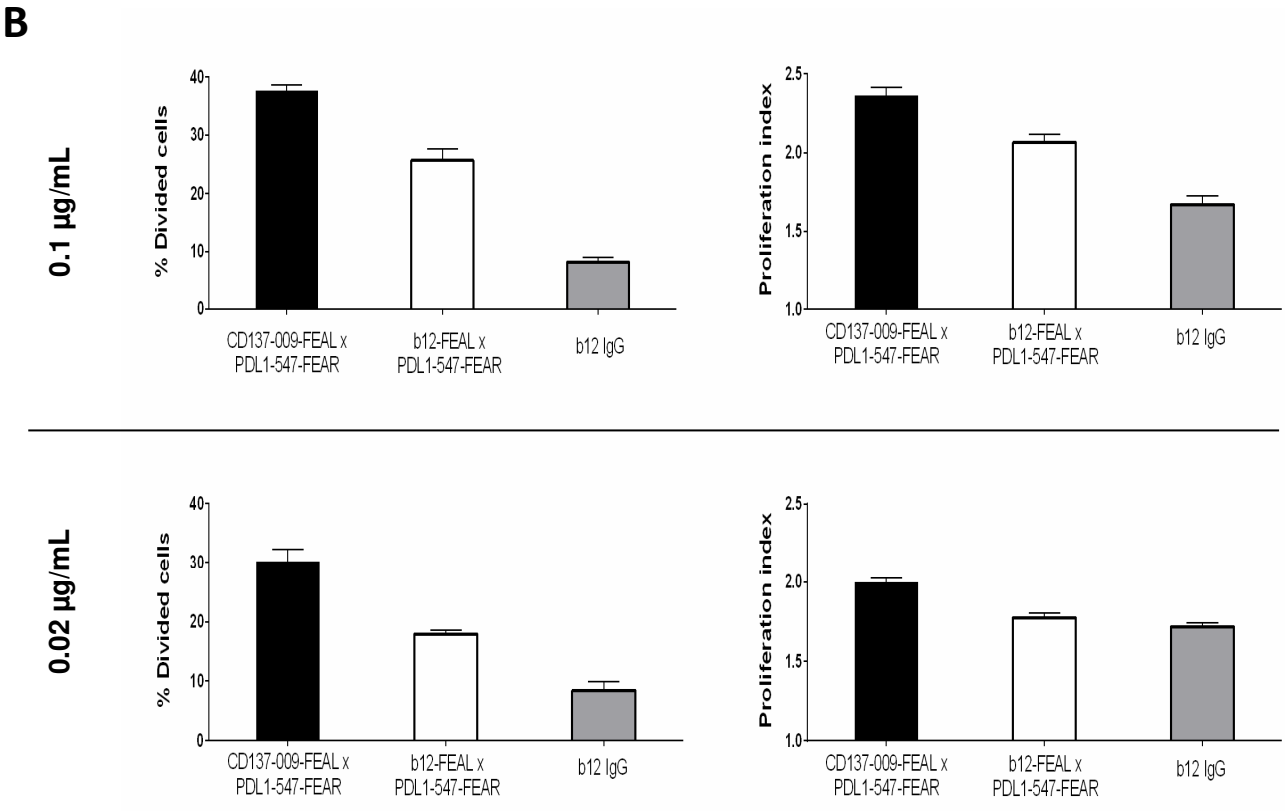

B
0.1 µg/mL
% Divided cells
Proliferation index
CD137-009-FEAL x PDL1-547-FEAR
b12-FEAL x PDL1-547-FEAR
b12 IgG
0.02 µg/mL

Figure 7 continued

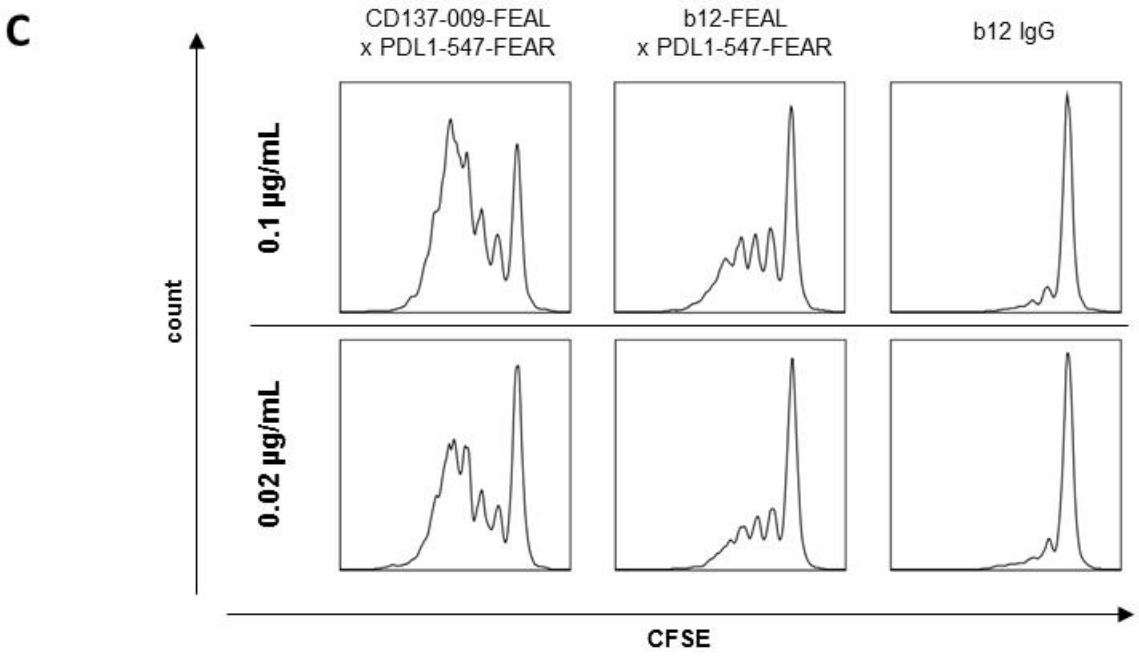


Figure 7 Continued

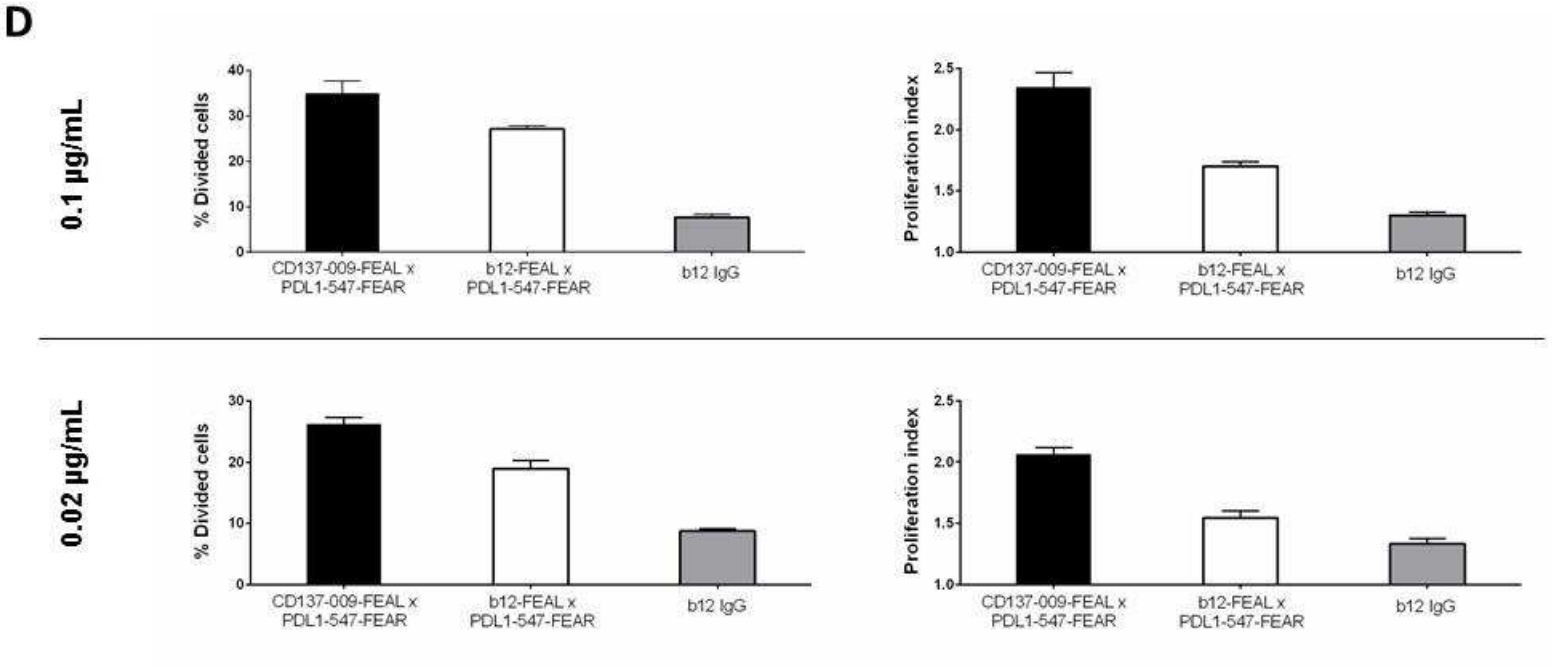
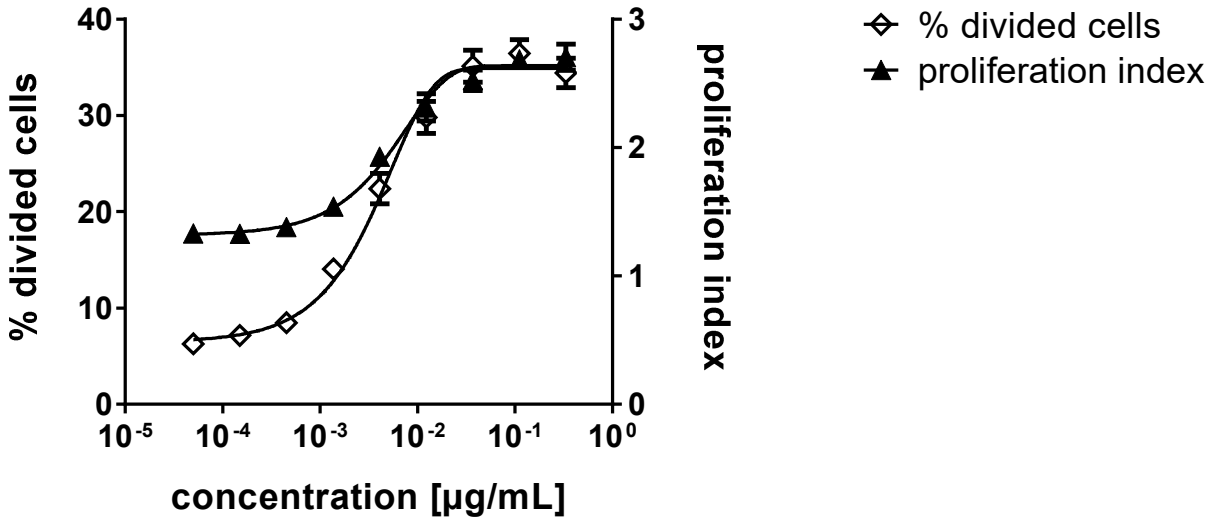


Figure 8



	% Divided cells	Proliferation index
EC_{50}	0.003492 µg/mL	0.005388 µg/mL

Figure 9

A

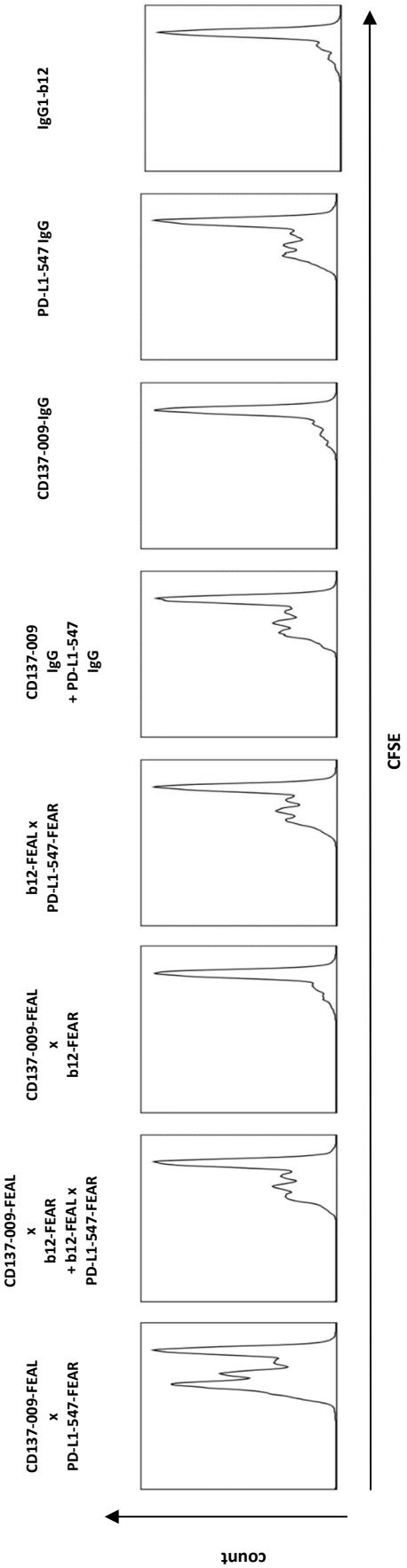
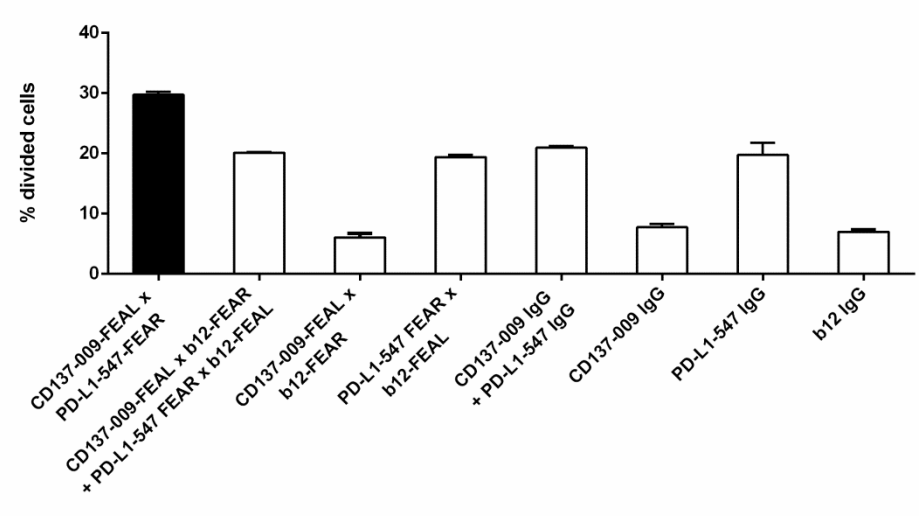

CD137-009-FEAL x PD-L1-547-FEAR
CD137-009-FEAL x b12-FEAR + b12-FEAL x PD-L1-547-FEAR
CD137-009-FEAL x b12-FEAR
b12-FEAL x PD-L1-547-FEAR
CD137-009 IgG + PD-L1-547 IgG
CD137-009-IgG
PD-L1-547 IgG
IgG1-b12
count
CFSE

Figure 9 continued

B



C

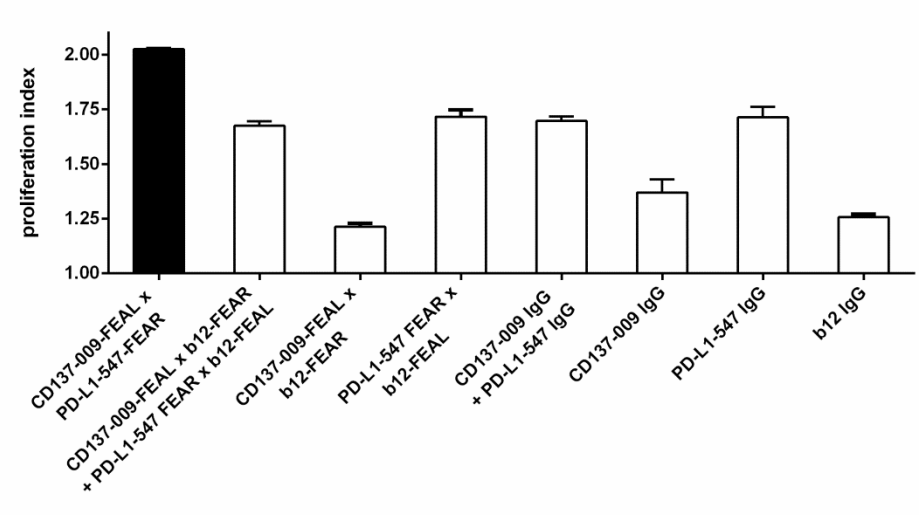


Figure 10

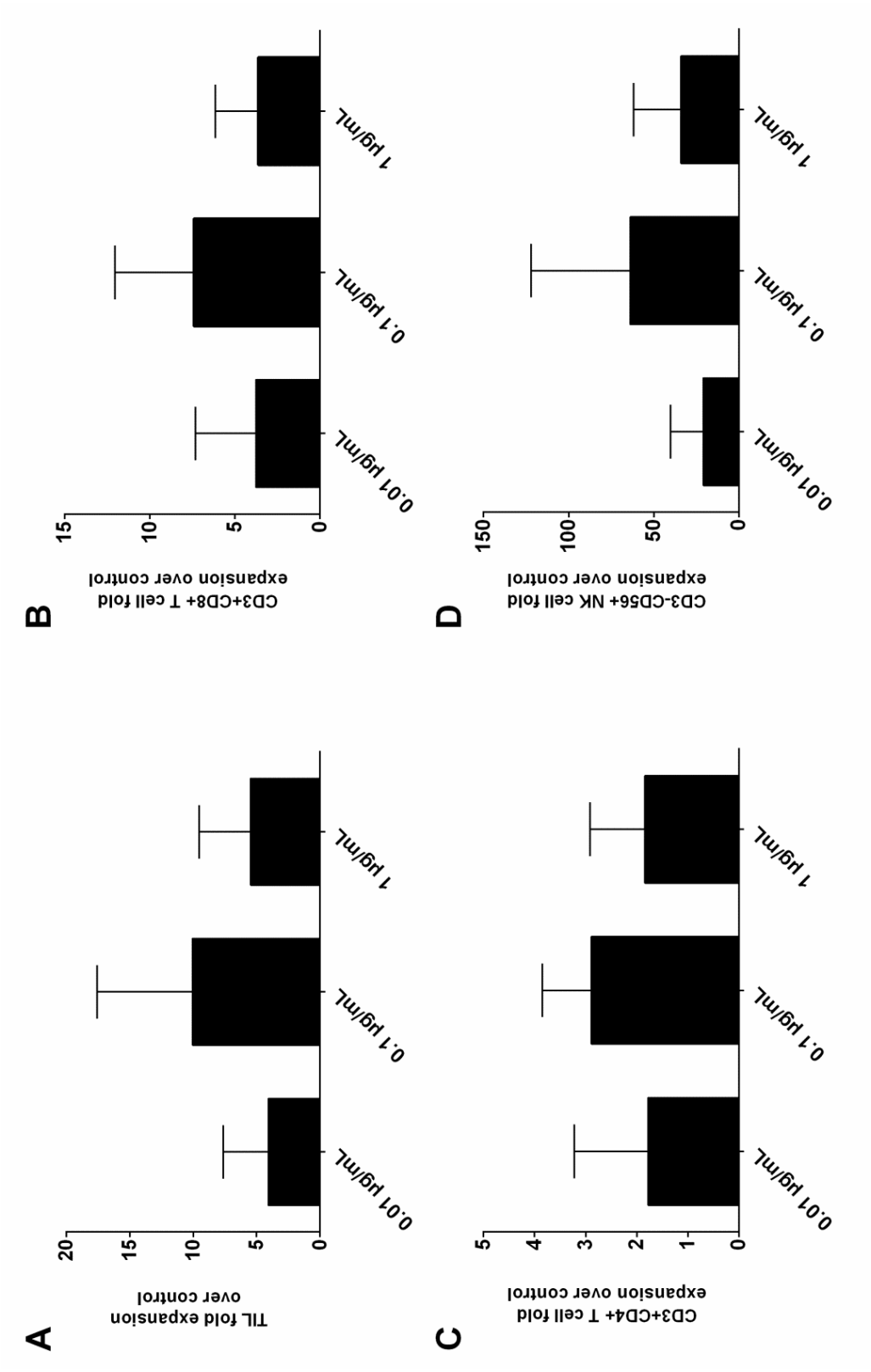

A
TIL fold expansion over control
0
5
10
15
20
0.01 µg/mL
0.1 µg/mL
1 µg/mL
B
CD3+CD8+ T cell fold expansion over control
0
5
10
15
0.01 µg/mL
0.1 µg/mL
1 µg/mL
C
CD3+CD4+ T cell fold expansion over control
0
1
2
3
4
5
0.01 µg/mL
0.1 µg/mL
1 µg/mL
D
CD3-CD56+ NK cell fold expansion over control
0
50
100
150
0.01 µg/mL
0.1 µg/mL
1 µg/mL

Figure 11

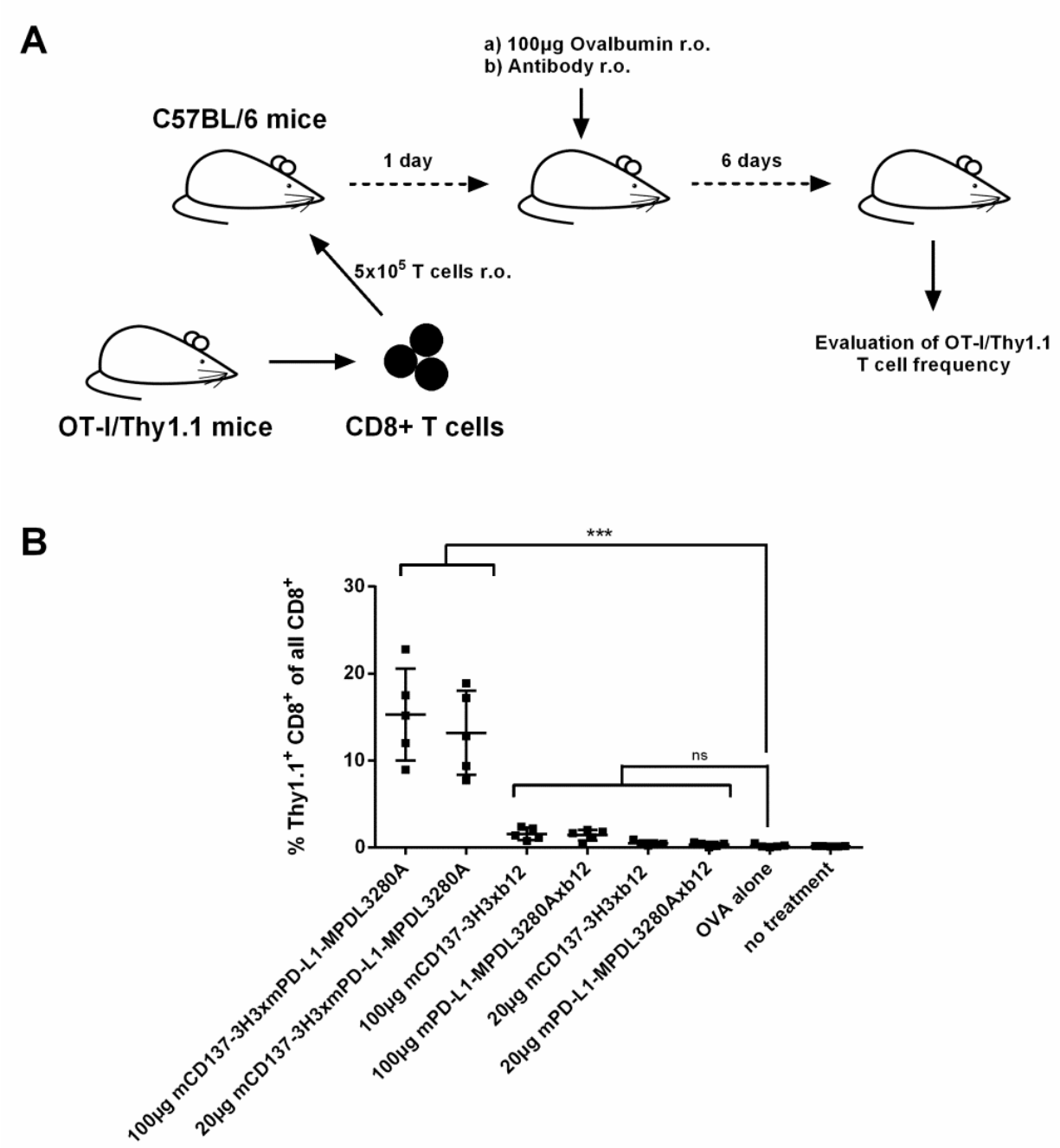

A
a) 100µg Ovalbumin r.o.
b) Antibody r.o.
C57BL/6 mice
1 day
6 days
5x10^5 T cells r.o.
Evaluation of OT-I/Thy1.1 T cell frequency
OT-I/Thy1.1 mice
CD8+ T cells
B
***
ns
% Thy1.1+ CD8+ of all CD8+
30
20
10
0
100µg mCD137-3H3xmPD-L1-MPDL3280A
20µg mCD137-3H3xmPD-L1-MPDL3280A
100µg mCD137-3H3xb12
100µg mPD-L1-MPDL3280Axb12
20µg mCD137-3H3xb12
20µg mPD-L1-MPDL3280Axb12
OVA alone
no treatment

Figure 12

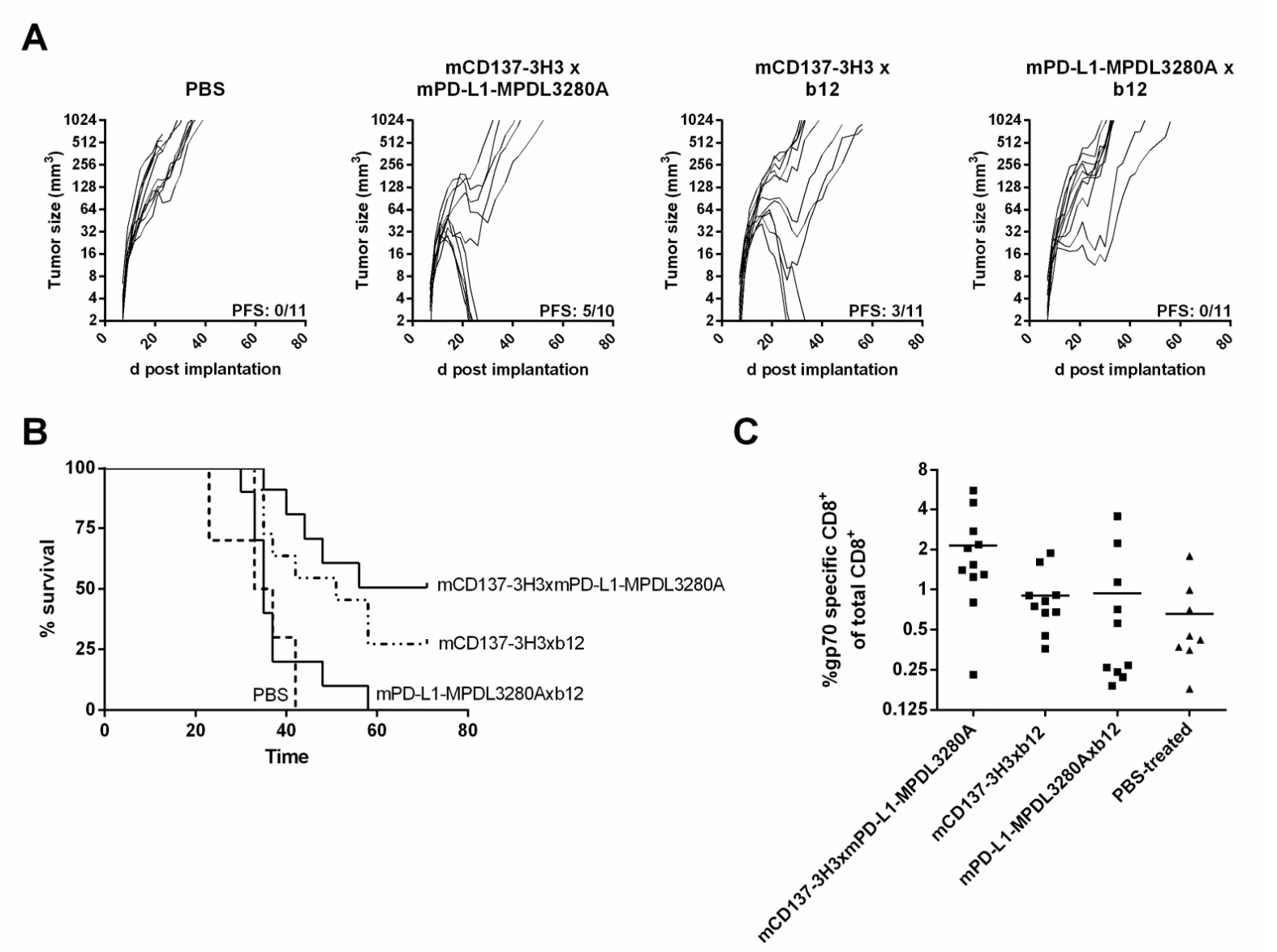

A
PBS
mCD137-3H3 x mPD-L1-MPDL3280A
mCD137-3H3 x b12
mPD-L1-MPDL3280A x b12
Tumor size (mm3)
d post implantation
PFS: 0/11
PFS: 5/10
PFS: 3/11
PFS: 0/11
B
% survival
Time
mCD137-3H3xmPD-L1-MPDL3280A
mCD137-3H3xb12
PBS
mPD-L1-MPDL3280Axb12
C
%gp70 specific CD8+ of total CD8+
mCD137-3H3xmPD-L1-MPDL3280A
mCD137-3H3xb12
mPD-L1-MPDL3280Axb12
PBS-treated

Figure 13

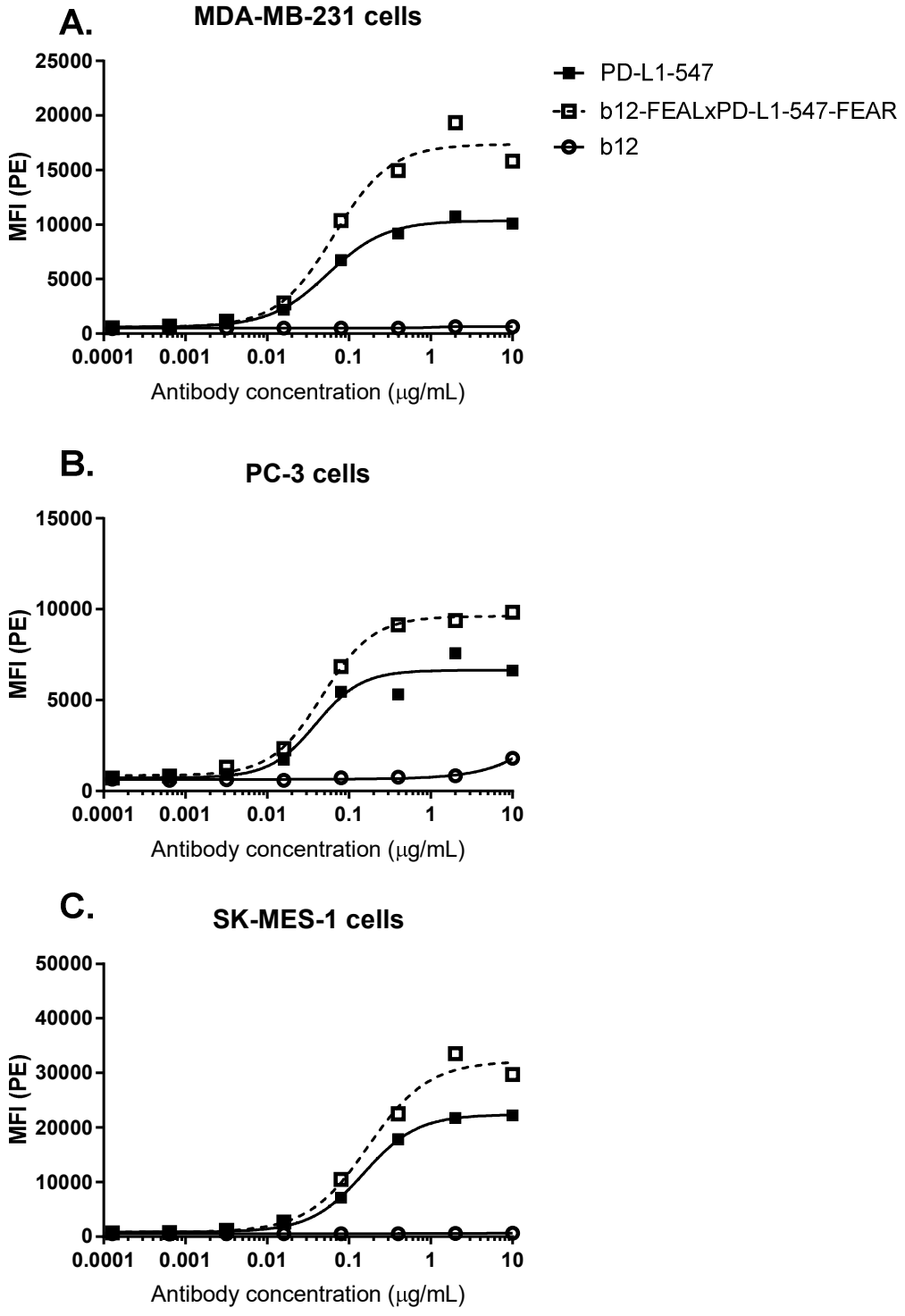

A.
MDA-MB-231 cells
MFI (PE)
25000
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (μg/mL)
PD-L1-547
b12-FEALxPD-L1-547-FEAR
b12
B.
PC-3 cells
MFI (PE)
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (μg/mL)
C.
SK-MES-1 cells
MFI (PE)
50000
40000
30000
20000
10000
0
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (μg/mL)

Figure 14

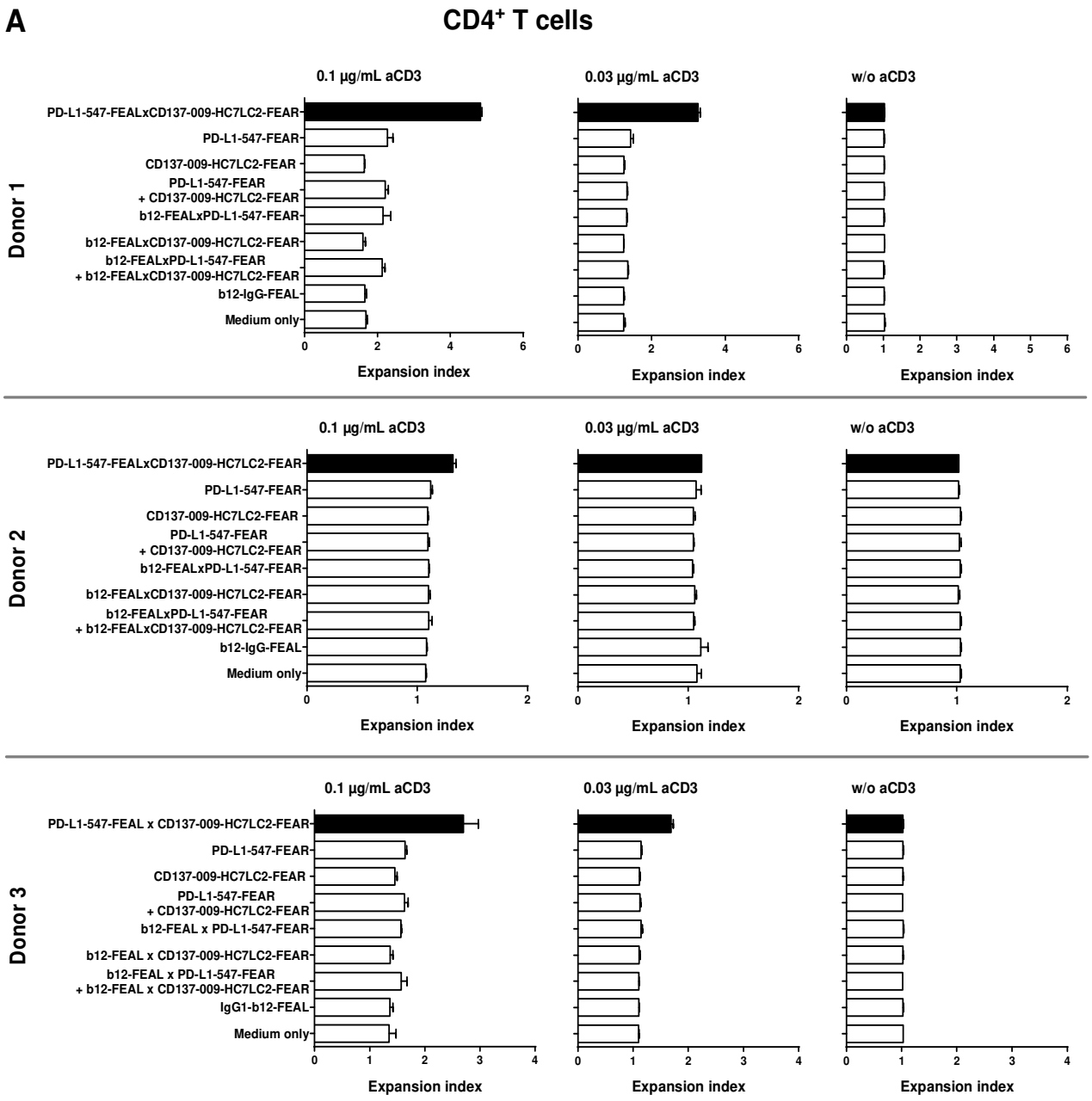

A
CD4+ T cells
Donor 1
Donor 2
Donor 3
0.1 µg/mL aCD3
0.03 µg/mL aCD3
w/o aCD3
PD-L1-547-FEALxCD137-009-HC7LC2-FEAR
PD-L1-547-FEAR
CD137-009-HC7LC2-FEAR
PD-L1-547-FEAR + CD137-009-HC7LC2-FEAR
b12-FEALxPD-L1-547-FEAR
b12-FEALxCD137-009-HC7LC2-FEAR
b12-FEALxPD-L1-547-FEAR + b12-FEALxCD137-009-HC7LC2-FEAR
b12-IgG-FEAL
Medium only
PD-L1-547-FEAL x CD137-009-HC7LC2-FEAR
b12-FEAL x PD-L1-547-FEAR
b12-FEAL x CD137-009-HC7LC2-FEAR
b12-FEAL x PD-L1-547-FEAR + b12-FEAL x CD137-009-HC7LC2-FEAR
IgG1-b12-FEAL
Expansion index

Figure 14 continued

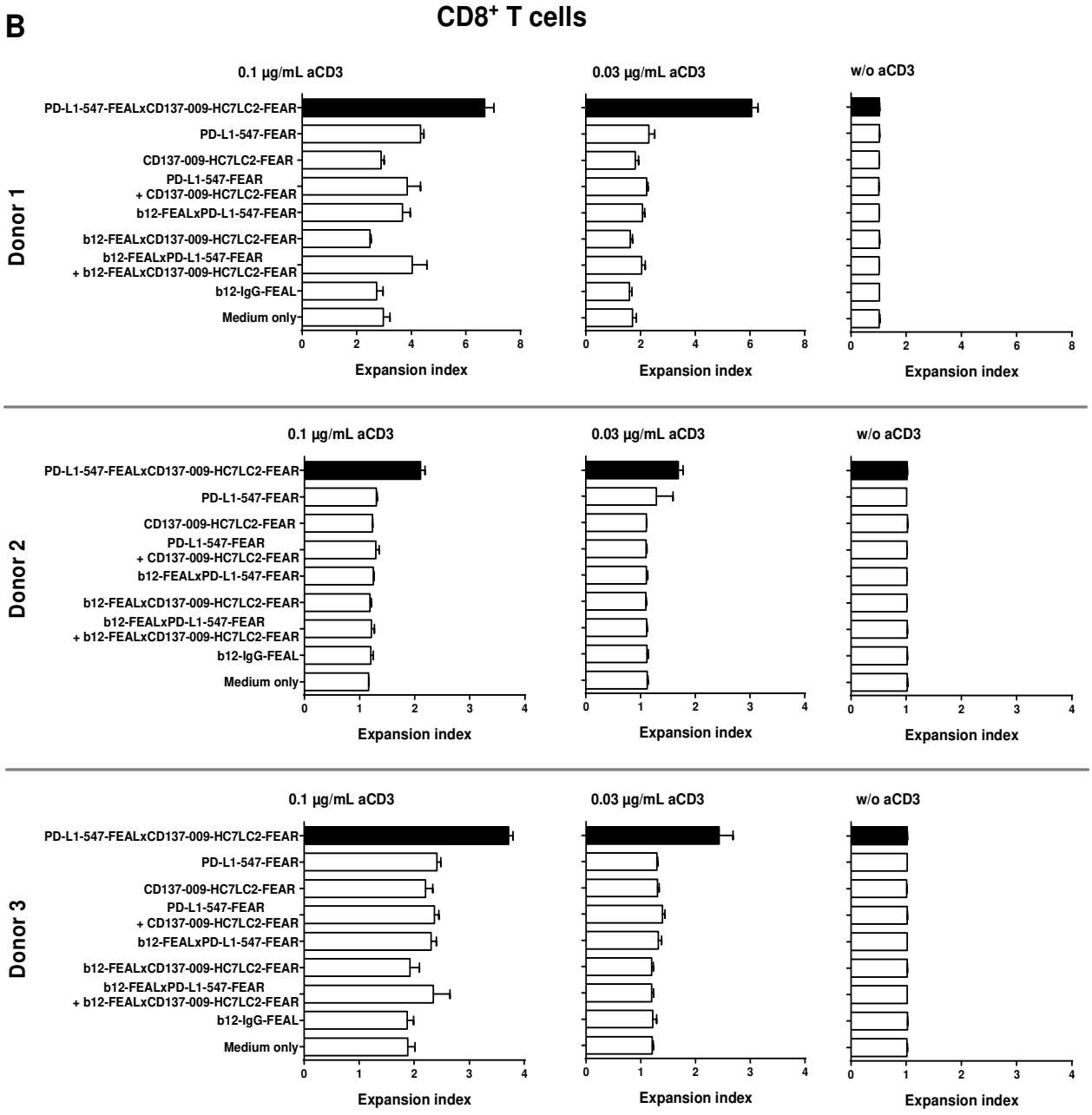


Figure 15

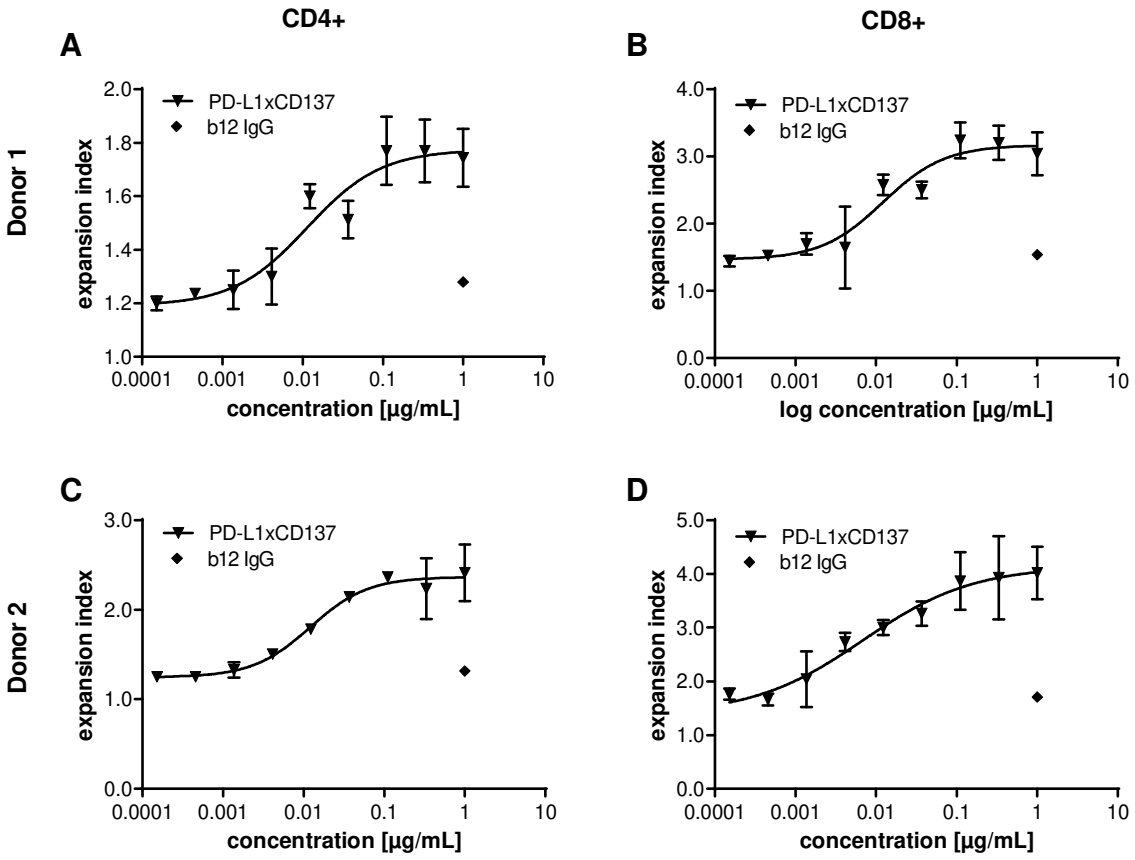

CD4+
CD8+
A
B
C
D
Donor 1
Donor 2
PD-L1xCD137
b12 IgG
expansion index
concentration [µg/mL]
log concentration [µg/mL]

Figure 16

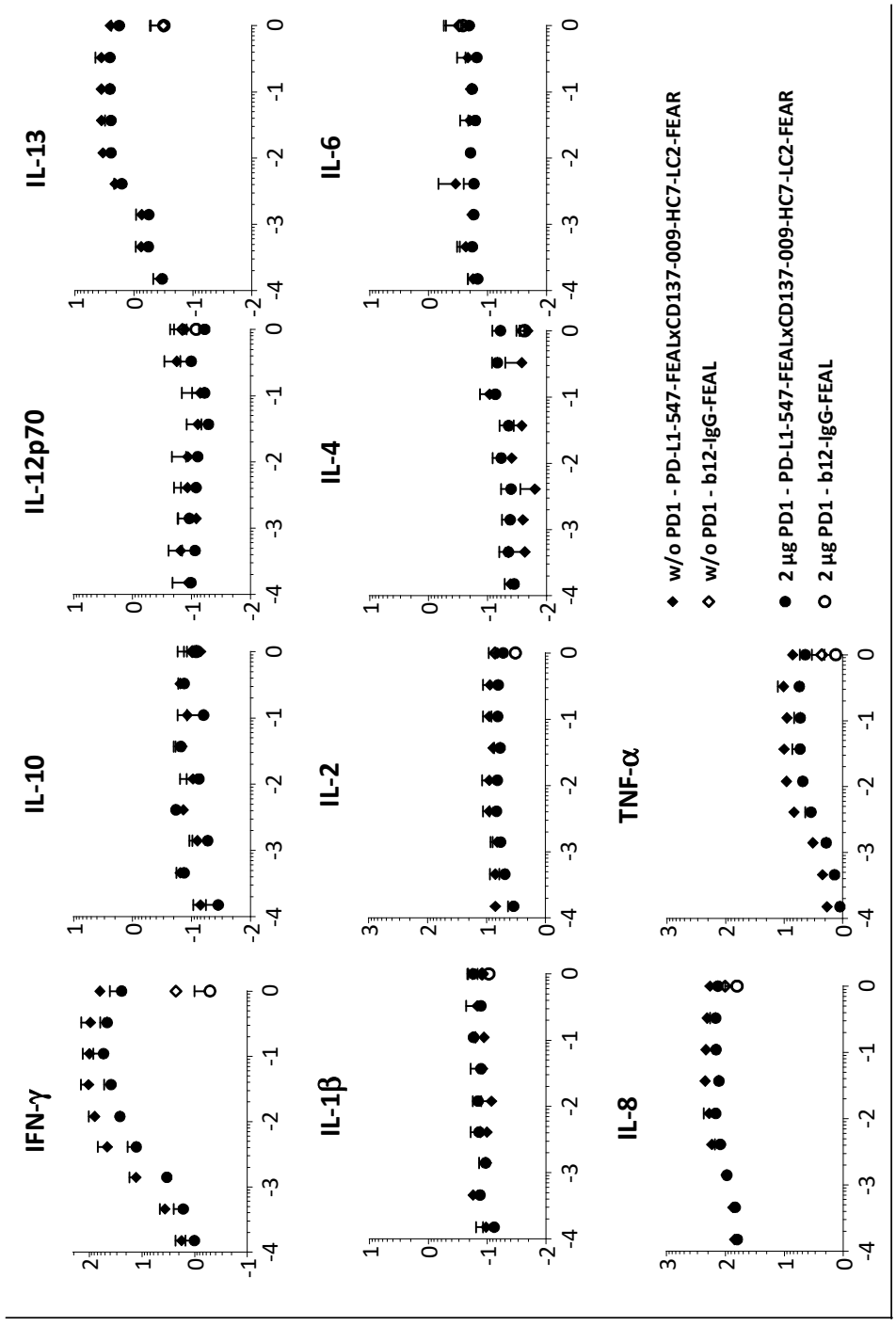
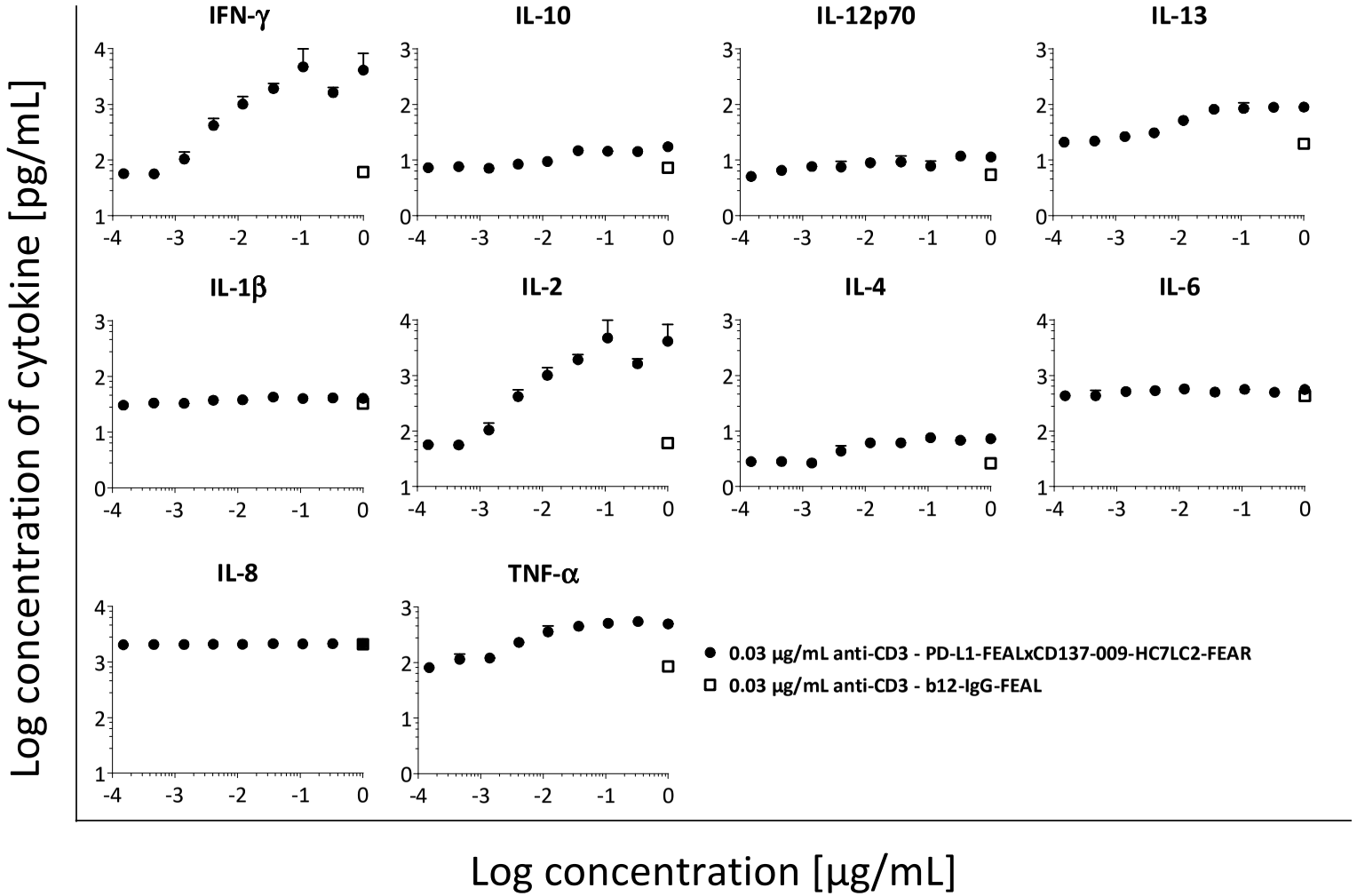

IFN-γ
IL-10
IL-12p70
IL-13
IL-1β
IL-2
IL-4
IL-6
IL-8
TNF-α
w/o PD1 - PD-L1-547-FEALxCD137-009-HC7-LC2-FEAR
w/o PD1 - b12-IgG-FEAL
2 µg PD1 - PD-L1-547-FEALxCD137-009-HC7-LC2-FEAR
2 µg PD1 - b12-IgG-FEAL
Log concentration of cytokine [pg/mL]
Log concentration [µg/mL]

Figure 17


IFN-γ
IL-10
IL-12p70
IL-13
IL-1β
IL-2
IL-4
IL-6
IL-8
TNF-α
Log concentration of cytokine [pg/mL]
Log concentration [µg/mL]
0.03 µg/mL anti-CD3 - PD-L1-FEALxCD137-009-HC7LC2-FEAR
0.03 µg/mL anti-CD3 - b12-IgG-FEAL