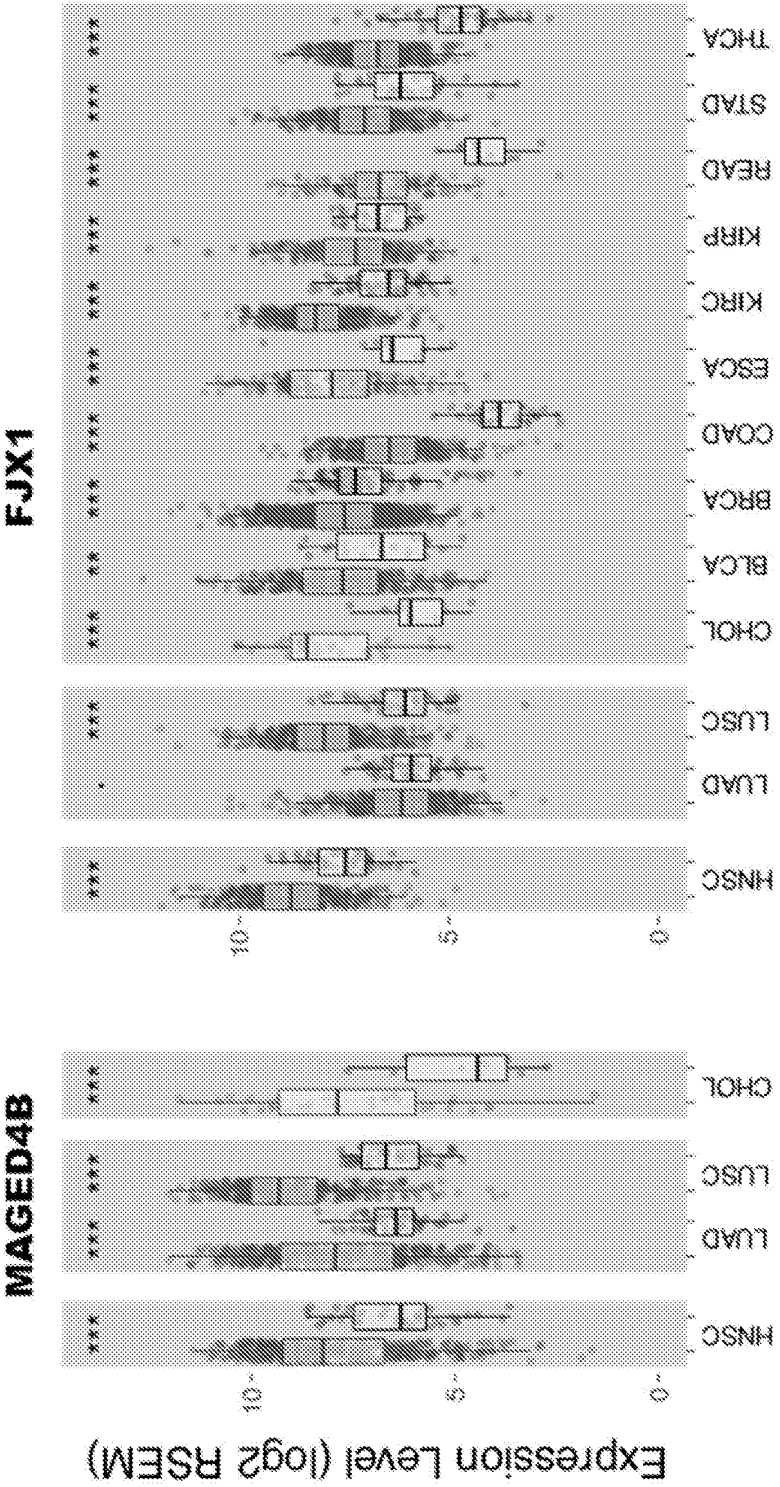

FJX1
MAGED4B
THCA
STAD
READ
KIRP
KIRC
ESCA
COAD
BRCA
BLCA
CHOL
LUSC
LUAD
HNSC
Expression Level (log2 RSEM)

Figure 1

Figure 2

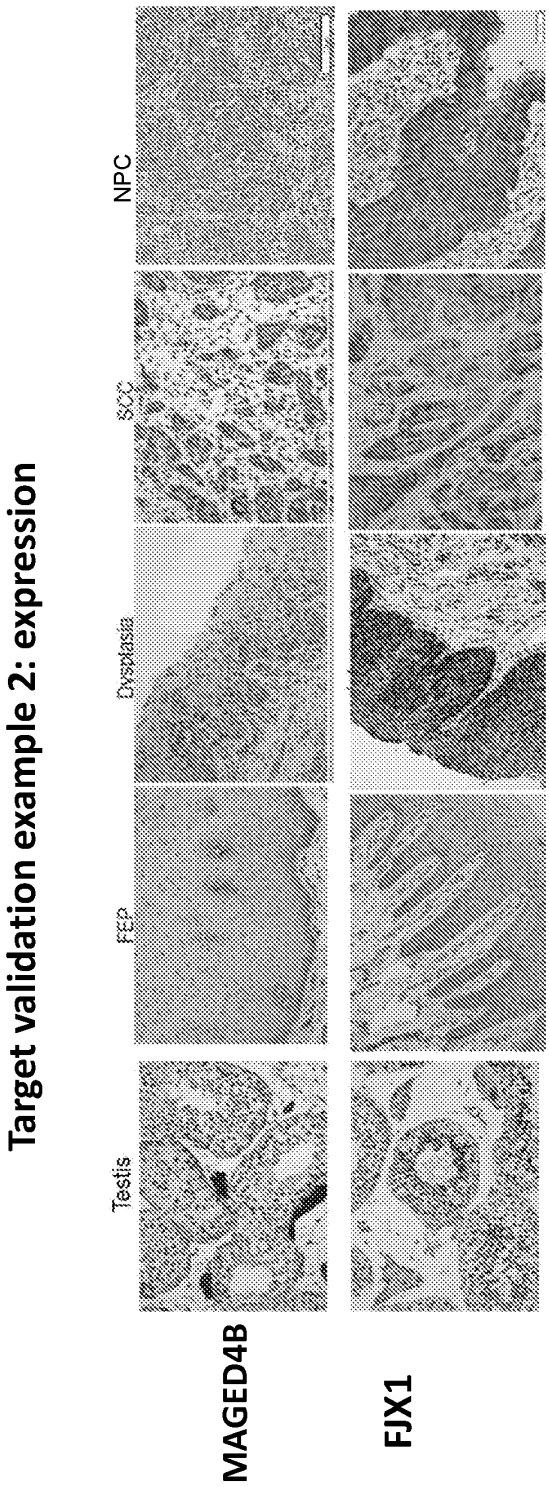

Target validation example 2: expression
Testis
FEP
Dysplasia
SCC
NPC
MAGED4B
FJX1

Figure 3

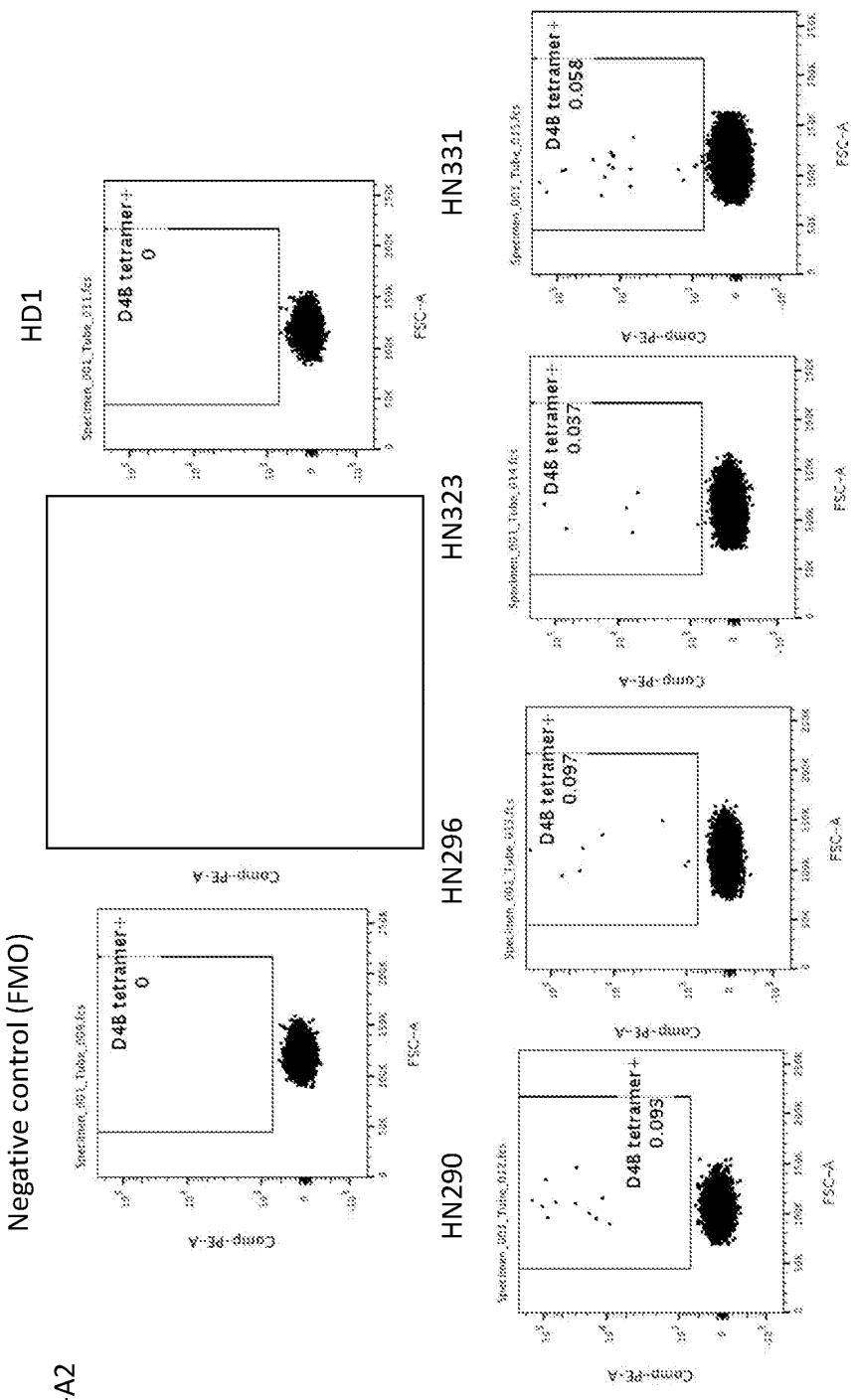

D4B/HLA-A2 tetramer
Negative control (FMO)
HD1
HN290
HN296
HN323
HN331
D4B tetramer+
0
0.093
0.097
0.037
0.058
Comp-PE-A
FSC-A

Figure 4

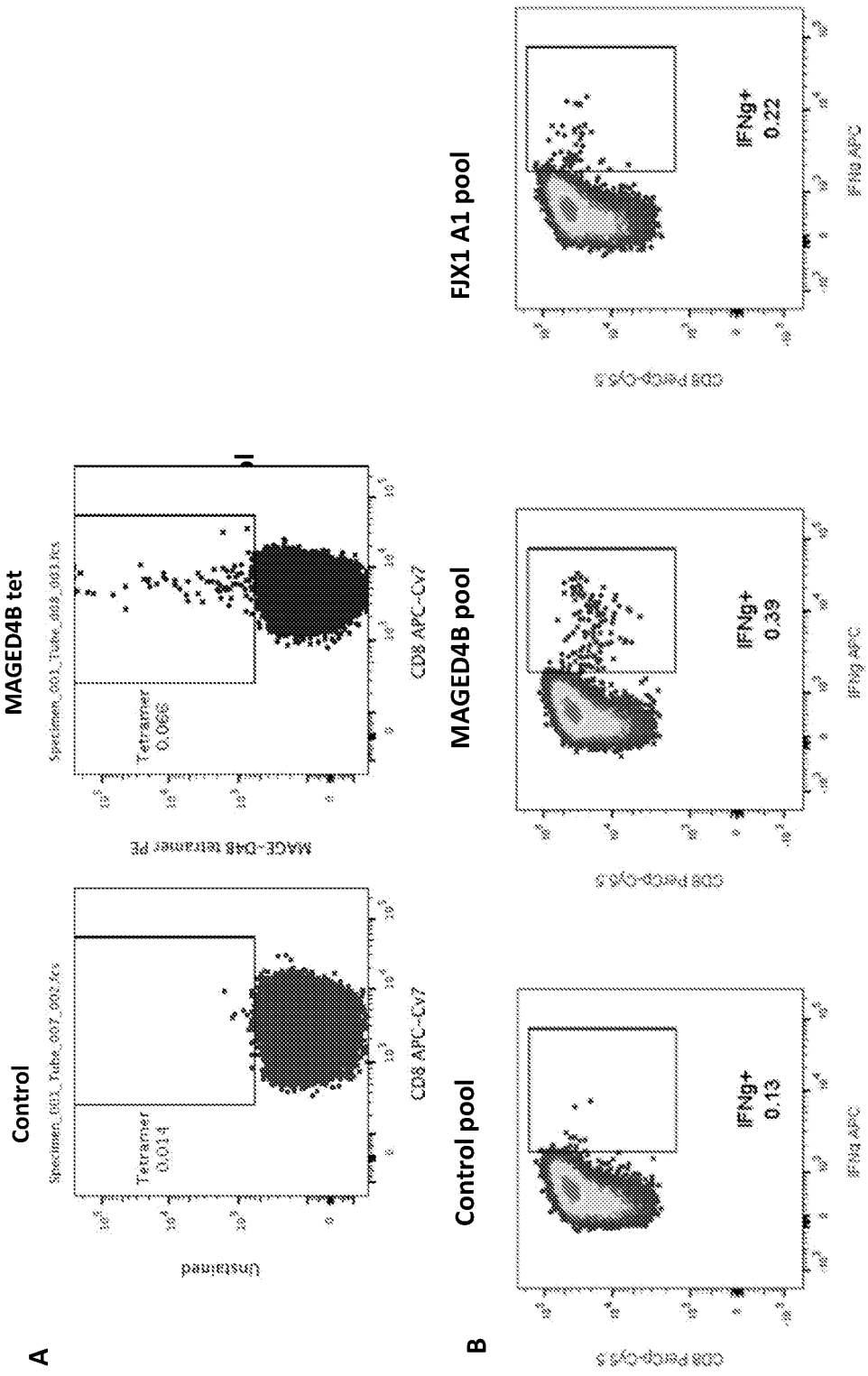

A
Control
Unstained
Tetramer
0.014
CD8 APC-Cy7
MAGED4B tet
MAGE-D4B tetramer PE
Tetramer
0.066
CD8 APC-Cy7
B
Control pool
IFNg+
0.13
CD8 PerCp-Cy5.5
IFNg APC
MAGED4B pool
IFNg+
0.39
CD8 PerCp-Cy5.5
IFNg APC
FJX1 A1 pool
IFNg+
0.22
CD8 PerCp-Cy5.5
IFNg APC

Figure 5

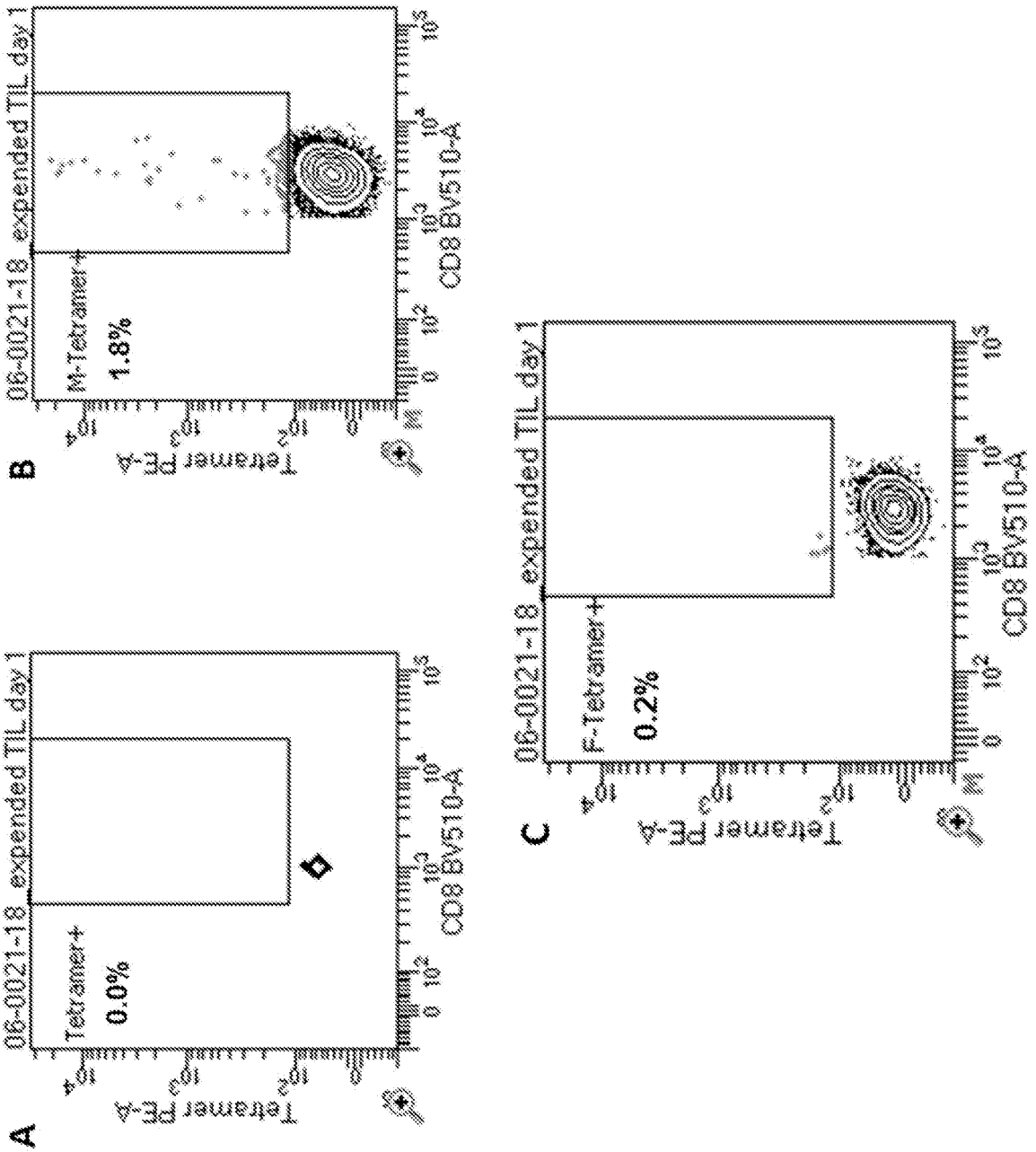
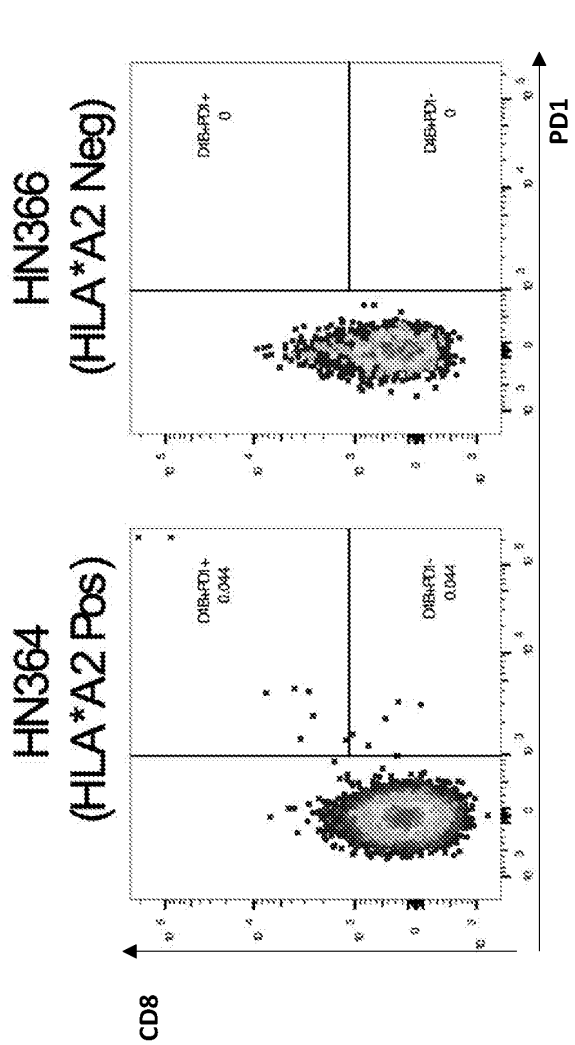

A
06-0021-18_expended TIL day 1
Tetramer+
0.0%
Tetramer PE-A
CD8 BV510-A
B
06-0021-18_expended TIL day 1
M-Tetramer+
1.8%
Tetramer PE-A
CD8 BV510-A
C
06-0021-18_expended TIL day 1
F-Tetramer+
0.2%
Tetramer PE-A
CD8 BV510-A

Figure 6



	CD3 Count	% CD8+	% CD4+	% PD1+	%D4B+	% D4B+/PD1+
HN364	46290	34.3	59.5	1.07	0.076	0.015
HN366	23938	9.57	83.4	6.21	0.042	0

Figure 7

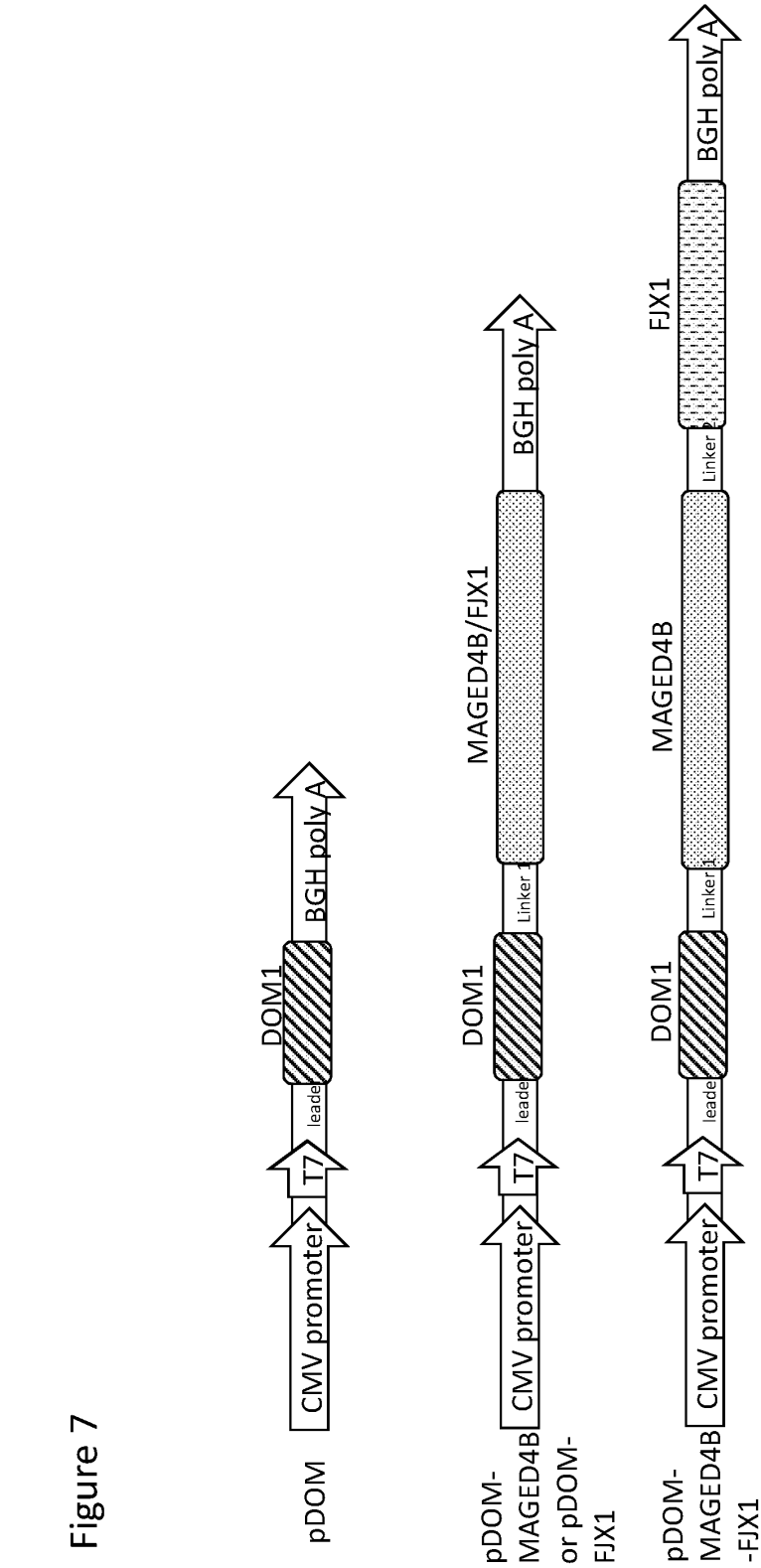

pDOM
CMV promoter
T7
leader
DOM1
BGH poly A
pDOM-MAGED4B or pDOM-FJX1
CMV promoter
T7
leader
DOM1
Linker 1
MAGED4B/FJX1
BGH poly A
pDOM-MAGED4B-FJX1
CMV promoter
T7
leader
DOM1
Linker 1
MAGED4B
Linker 2
FJX1
BGH poly A

Figure 8

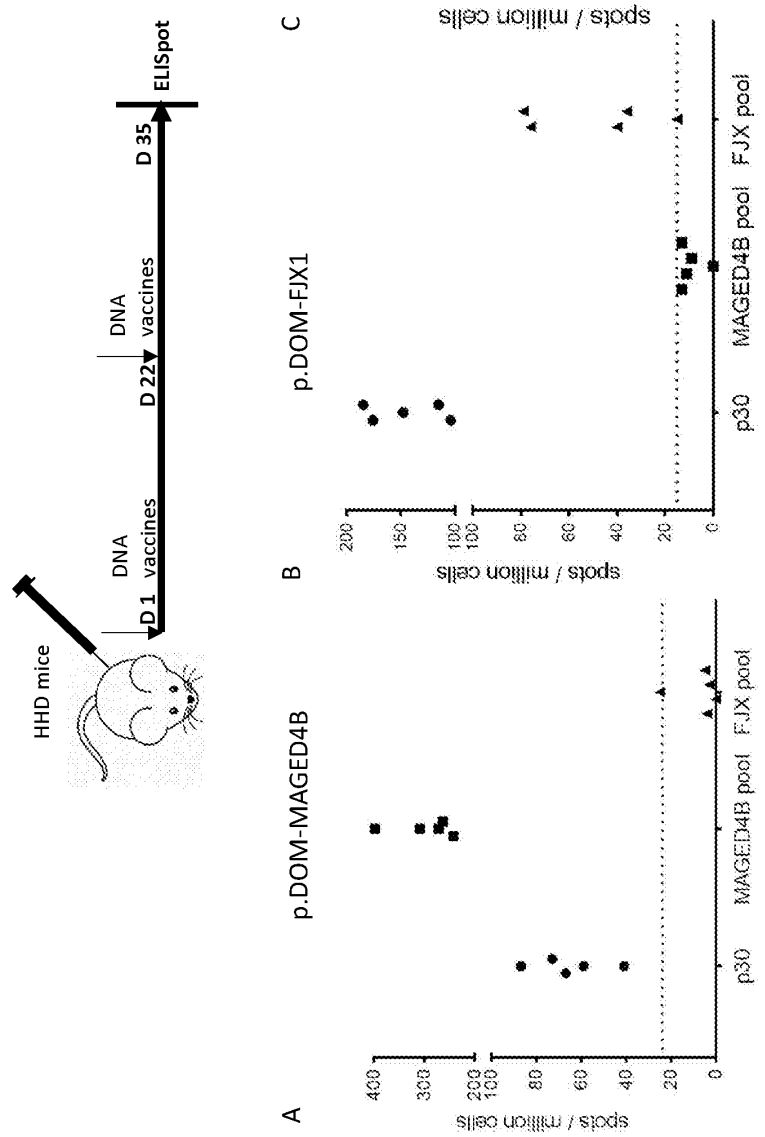

HHD mice
DNA vaccines
D 1
DNA vaccines
D 22
D 35
ELISpot
A
p.DOM-MAGED4B
B
p.DOM-FJX1
C
p.DOM
spots / million cells
p30
MAGED4B pool
FJX pool

Figure 9

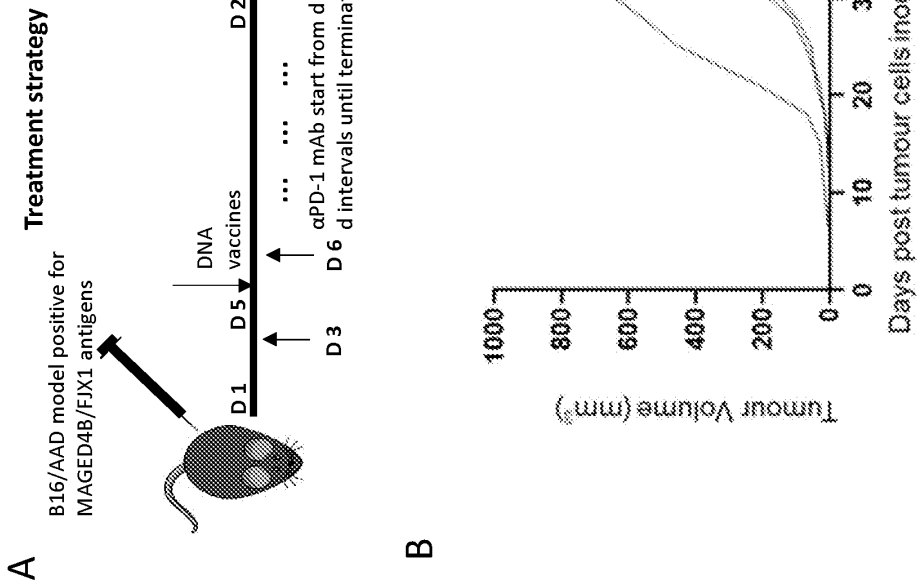

A
Treatment strategy
B16/AAD model positive for MAGED4B/FJX1 antigens
D 1
D 3
DNA vaccines
D 5
D 6
αPD-1 mAb start from d 3, with 3 d intervals until termination
DNA vaccines
D 26
D 35
efficacy graph, TIL/ ELISpot
B
Tumour Volume (mm³)
1000
800
600
400
200
0
0
10
20
30
40
Days post tumour cells inoculation
Control
DNA vaccine
α-PD-1
α-PD1+ DNA vaccine
n.s.
*
*
*

Figure 9 continued

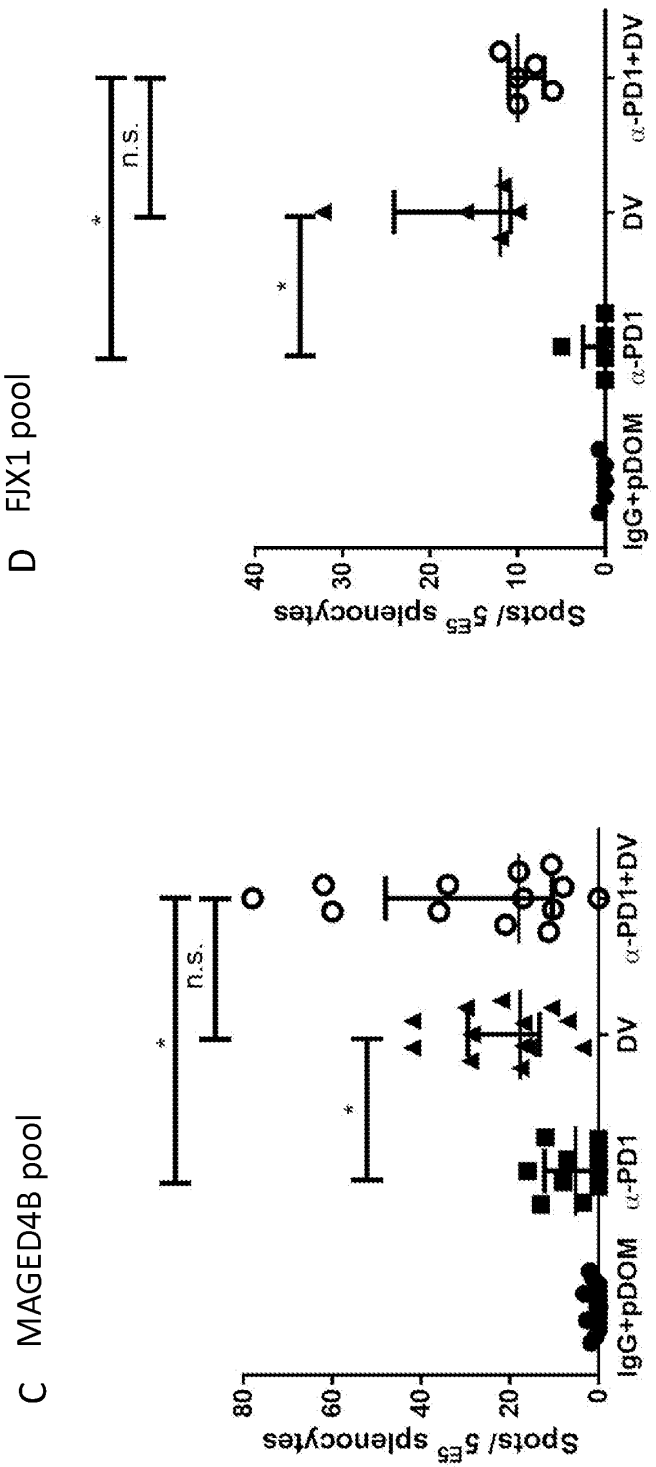


Figure 10

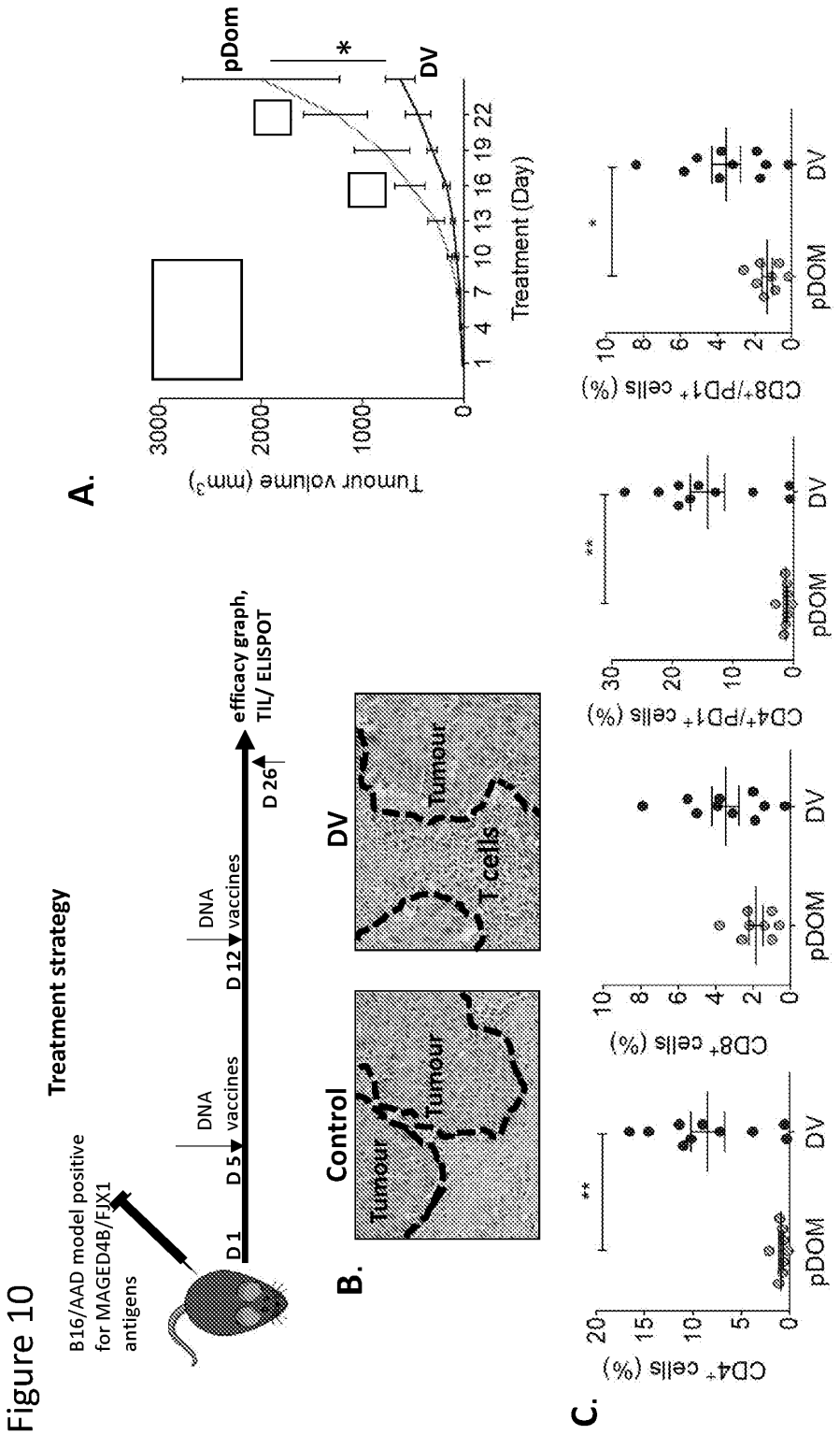

Treatment strategy
B16/AAD model positive for MAGED4B/FJX1 antigens
D 1
D 5
DNA vaccines
D 12
DNA vaccines
D 26
efficacy graph, TIL/ ELISPOT
A.
Tumour volume (mm3)
3000
2000
1000
0
1 4 7 10 13 16 19 22
Treatment (Day)
pDom
DV
*
B.
Control
DV
Tumour
Tumour
Tumour
T cells
C.
CD4+ cells (%)
CD8+ cells (%)
CD4+/PD1+ cells (%)
CD8+/PD1+ cells (%)
pDOM
DV
**
*

Figure 11

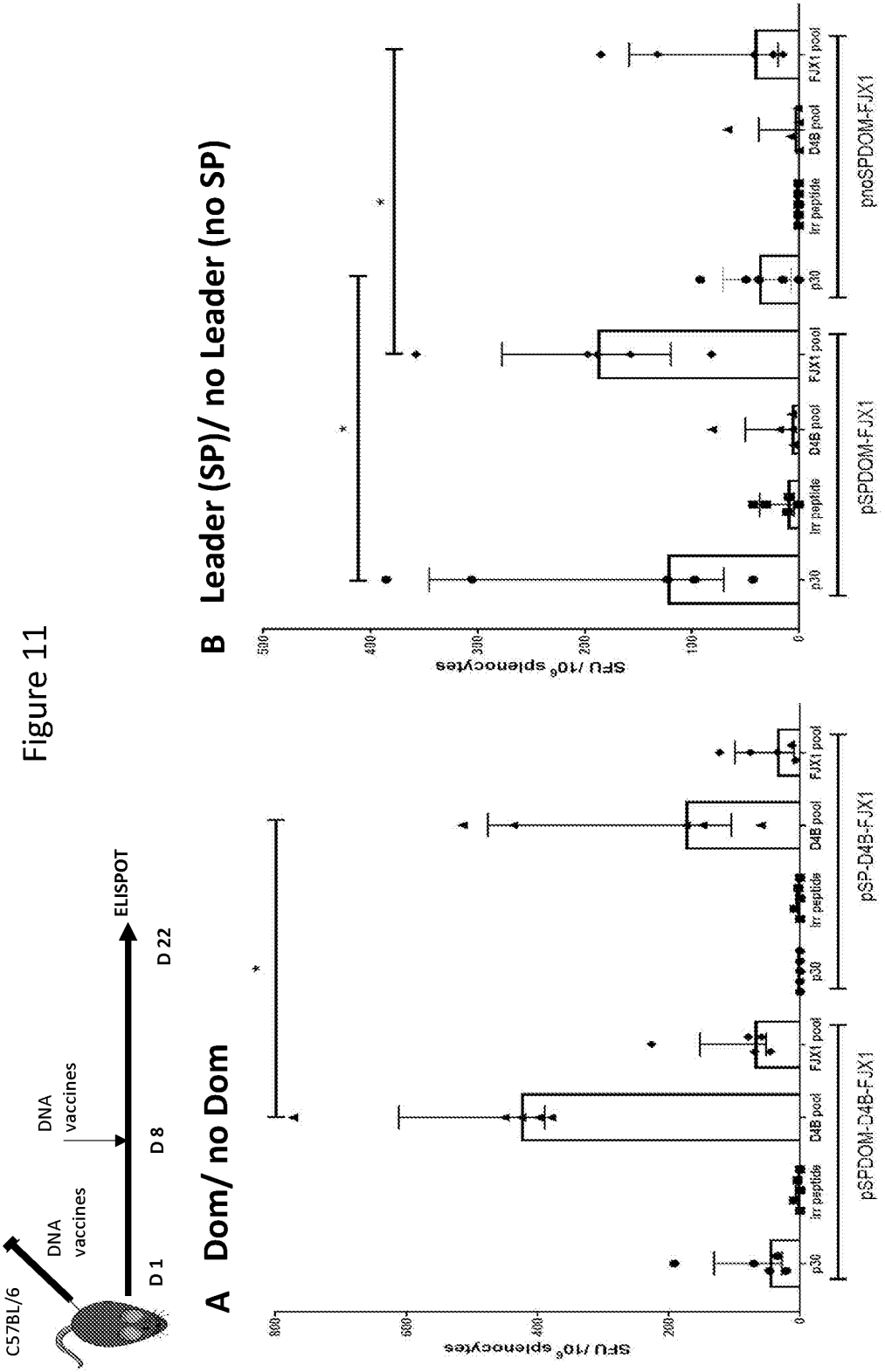

C57BL/6
DNA vaccines
DNA vaccines
D 1
D 8
D 22
ELISPOT
A Dom/ no Dom
B Leader (SP)/ no Leader (no SP)
SFU /10^6 splenocytes
p30
Irr peptide
D4B pool
FJX1 pool
pSPDOM-D4B-FJX1
pSP-D4B-FJX1
pSPDOM-FJX1
pnoSPDOM-FJX1

Fig. 12

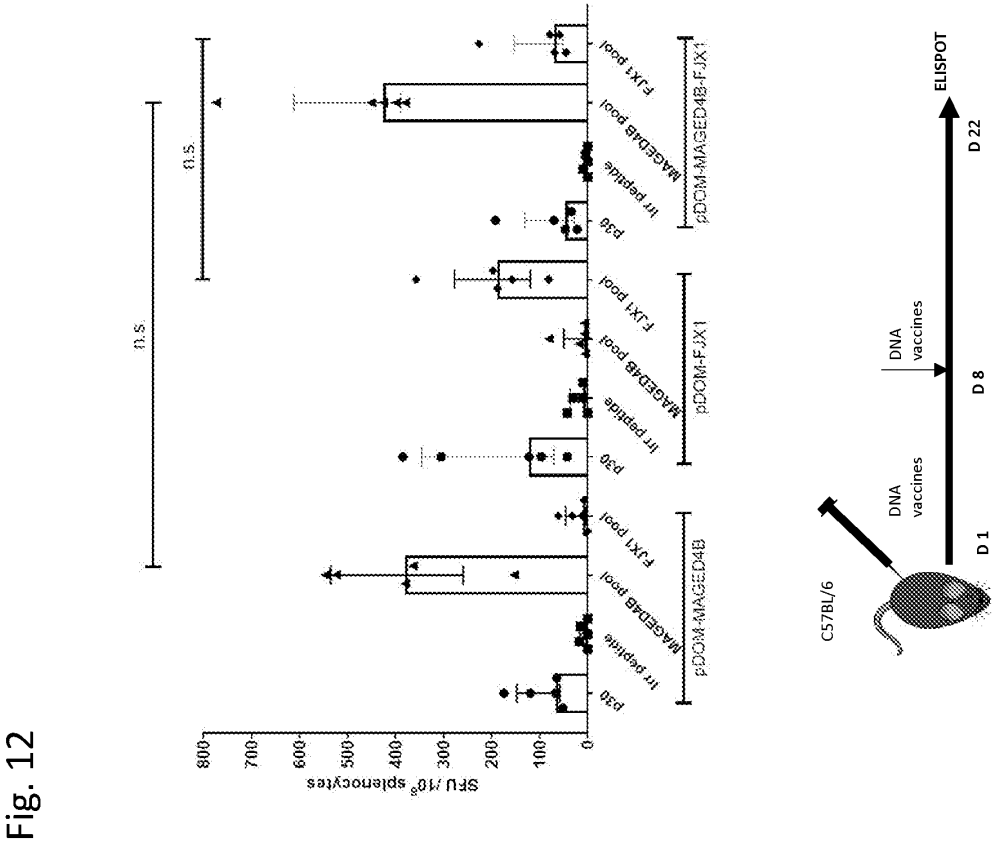
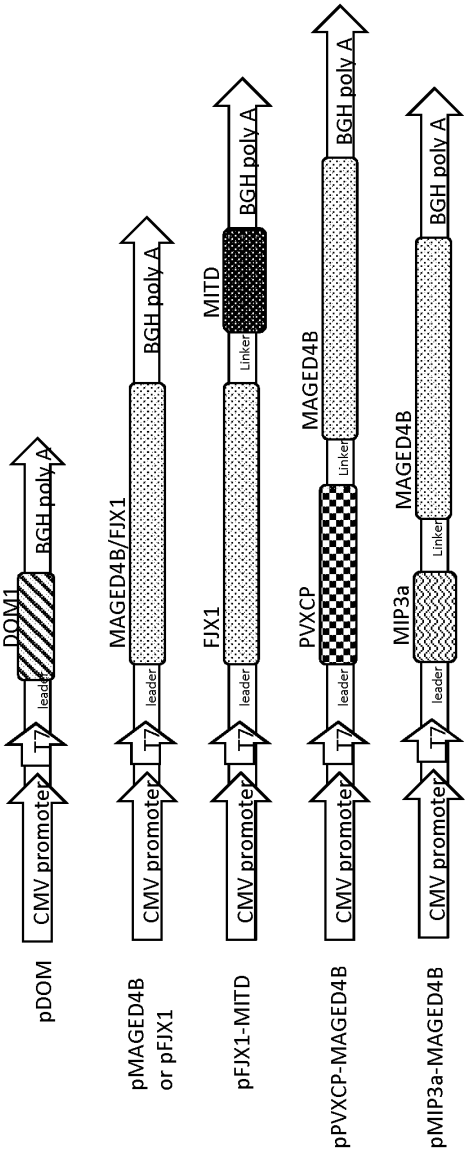

SFU /10^6 splenocytes
0
100
200
300
400
500
600
700
800
n.s
n.s
p30
Ir peptide
MAGED4B pool
F-JX1 pool
pDOM-MAGED4B
p30
Ir peptide
MAGED4B pool
F-JX1 pool
pDOM-FJX1
p30
Ir peptide
MAGED4B pool
F-JX1 pool
pDOM-MAGED4B-FJX1
C57BL/6
DNA vaccines
DNA vaccines
D 1
D 8
D 22
ELISPOT

Fig 13


CMV promoter
T7
leader
DOM1
BGH poly A
pDOM
CMV promoter
T7
leader
MAGED4B/FJX1
BGH poly A
pMAGED4B or pFJX1
CMV promoter
T7
leader
FJX1
Linker
MITD
BGH poly A
pFJX1-MITD
CMV promoter
T7
leader
PVXCP
Linker
MAGED4B
BGH poly A
pPVXCP-MAGED4B
CMV promoter
T7
leader
MIP3a
Linker
MAGED4B
BGH poly A
pMIP3a-MAGED4B

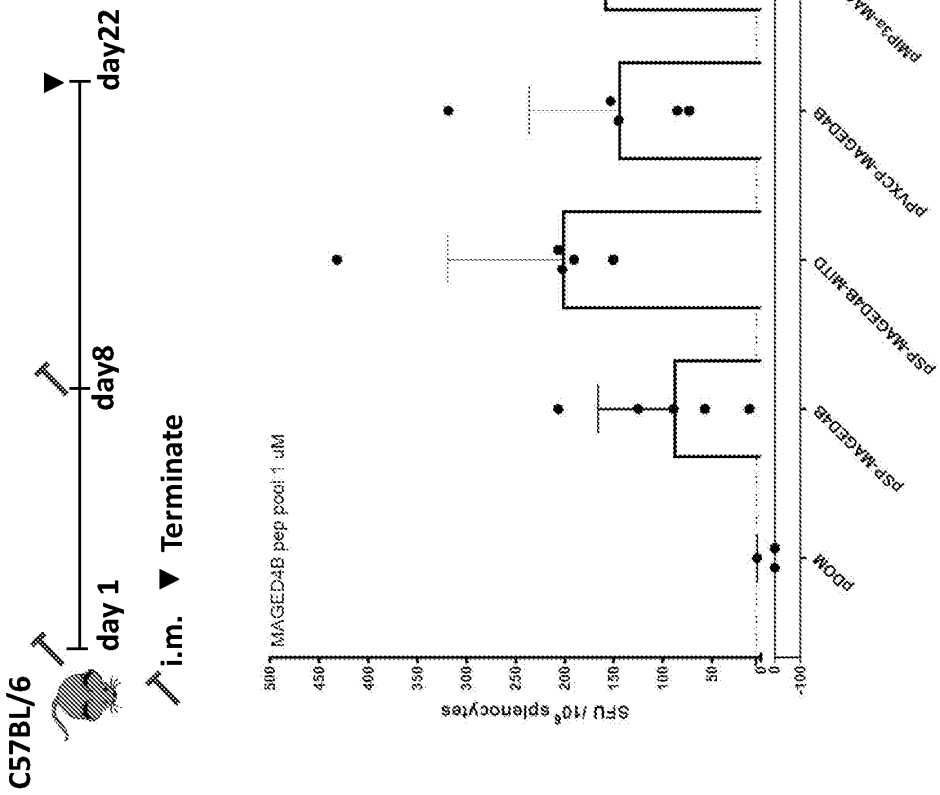

C57BL/6
day 1
day8
day22
i.m.
Terminate
MAGED4B pep pool 1 uM
SFU /10^6 splenocytes
pDOM
pSP-hMAGED4B
pSP-hMAGED4B-hITD
pPVXCP-MAGED4B
pMIP3a-MAGED4B
FJX1 pep pool 1 uM
SFU /10^6 splenocytes
pCTRL
pSP-F-JX1
pSP-F-JX1-hITD

Fig. 14

Fig. 15

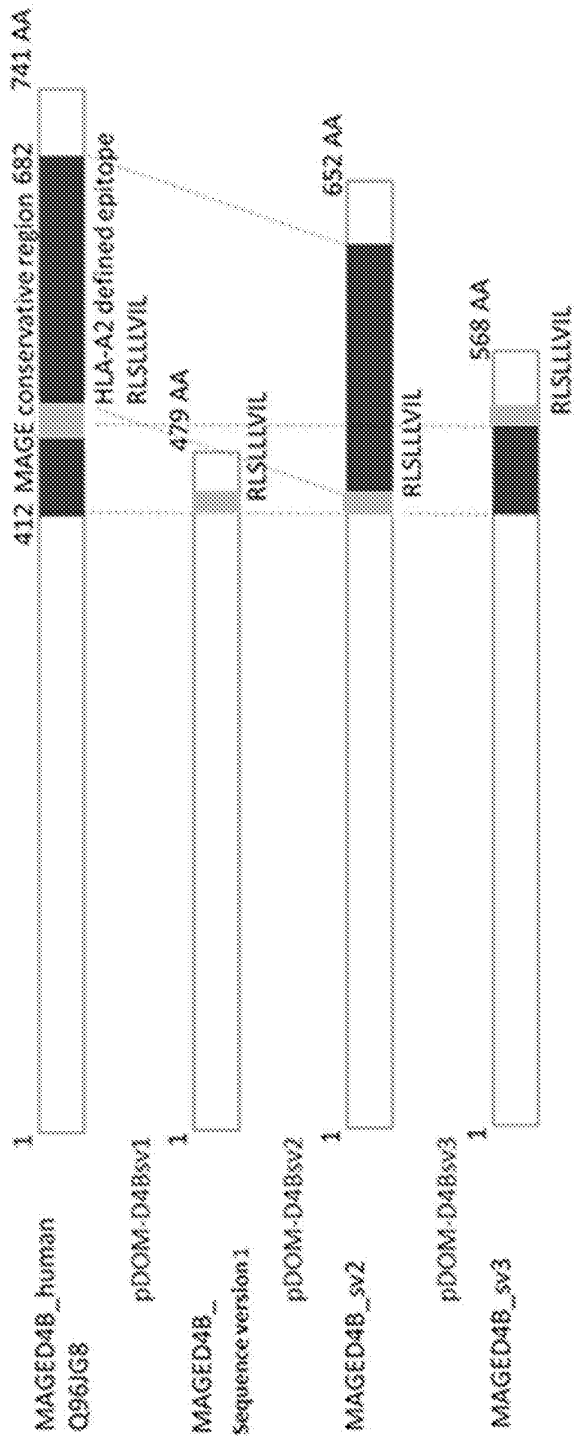
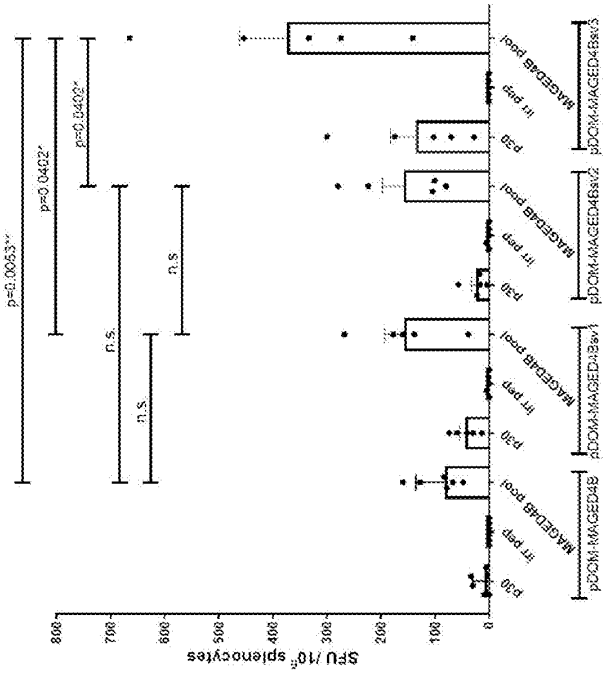

MAGED4B_human
Q96JG8
1
412 MAGE conservative region 682
741 AA
HLA-A2 defined epitope
RLSLLIVIL
pDOM-D4Bsv1
1
479 AA
MAGED4B_
Sequence version 1
RLSLLIVIL
pDOM-D4Bsv2
1
652 AA
MAGED4B_sv2
RLSLLIVIL
pDOM-D4Bsv3
1
568 AA
MAGED4B_sv3
RLSLLIVIL

Fig. 16


SFU /10^6 splenocytes
0
100
200
300
400
500
600
700
800
p30
IT pep
MAGED4B pcor
p30
IT pep
MAGED4B pcor
pDOM-MAGED4B
p30
IT pep
MAGED4B pcor
pDOM-MAGED4Bsv1
p30
IT pep
MAGED4B pcor
pDOM-MAGED4Bsv2
p30
IT pep
MAGED4B pcor
pDOM-MAGED4Bsv3
p=0.0053**
p=0.0422*
p=0.0402*
n.s.
n.s.
n.s.

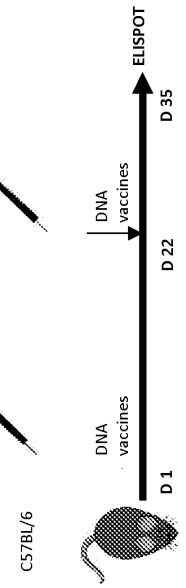

C57BL/6
DNA vaccines
DNA vaccines
D 1
D 22
D 35
ELISPOT

Fig. 17

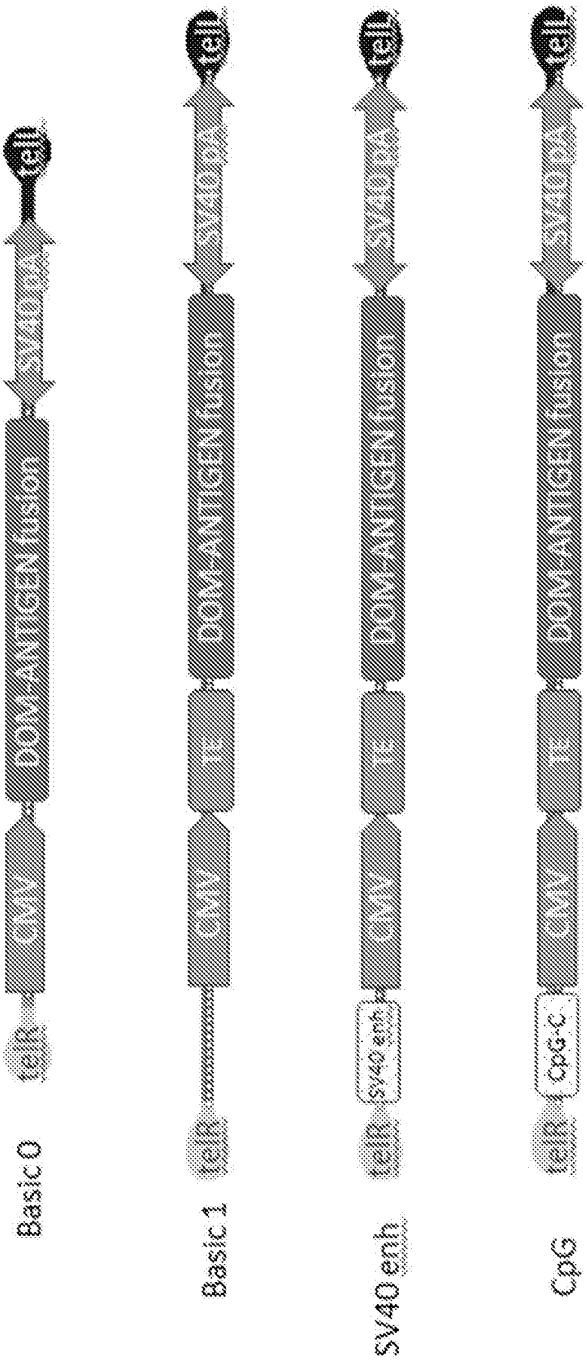

Basic 0
telR
CMV
DOM ANTIGEN fusion
SV40 pA
tell
Basic 1
telR
CMV
TE
DOM ANTIGEN fusion
SV40 pA
tell
SV40 enh
telR
SV40 enh
CMV
TE
DOM ANTIGEN fusion
SV40 pA
tell
CpG
telR
CpG-C
CMV
TE
DOM ANTIGEN fusion
SV40 pA
tell

Fig. 18

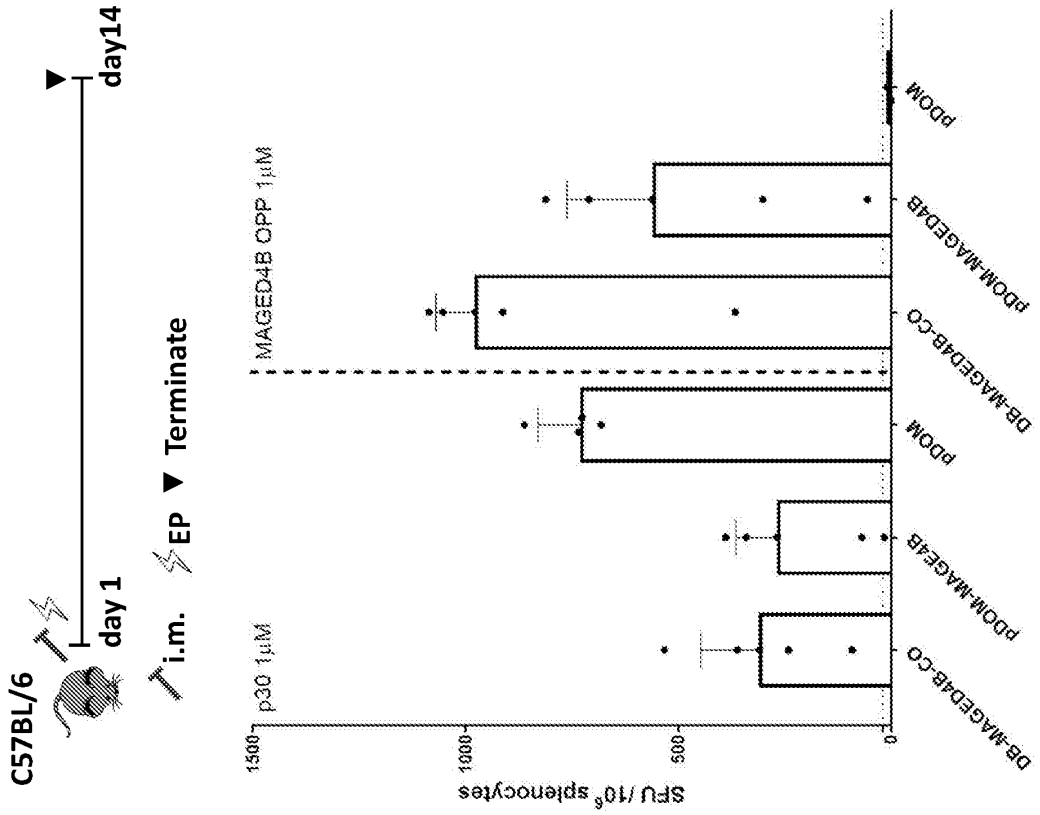

C57BL/6
day 1
day14
i.m.
EP
Terminate
p30 1μM
MAGED4B OPP 1μM
SFU /10^5 splenocytes
1500
1000
500
0
DB-MAGED4B-Co
pDOM-MAGE-4B
pDOM
DB-MAGED4B-Co
pDOM-MAGED4B
pDOM

Fig. 19

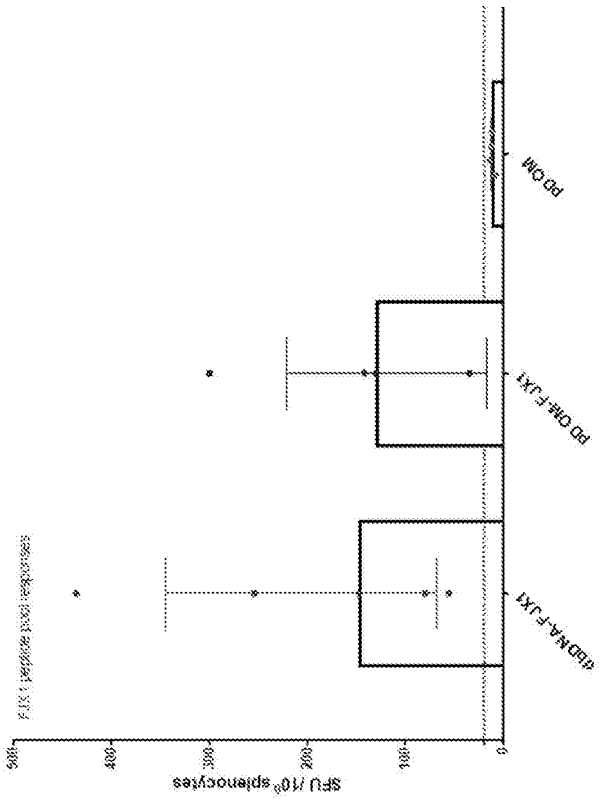


Fig. 20a

Fig. 20b

Fig. 21

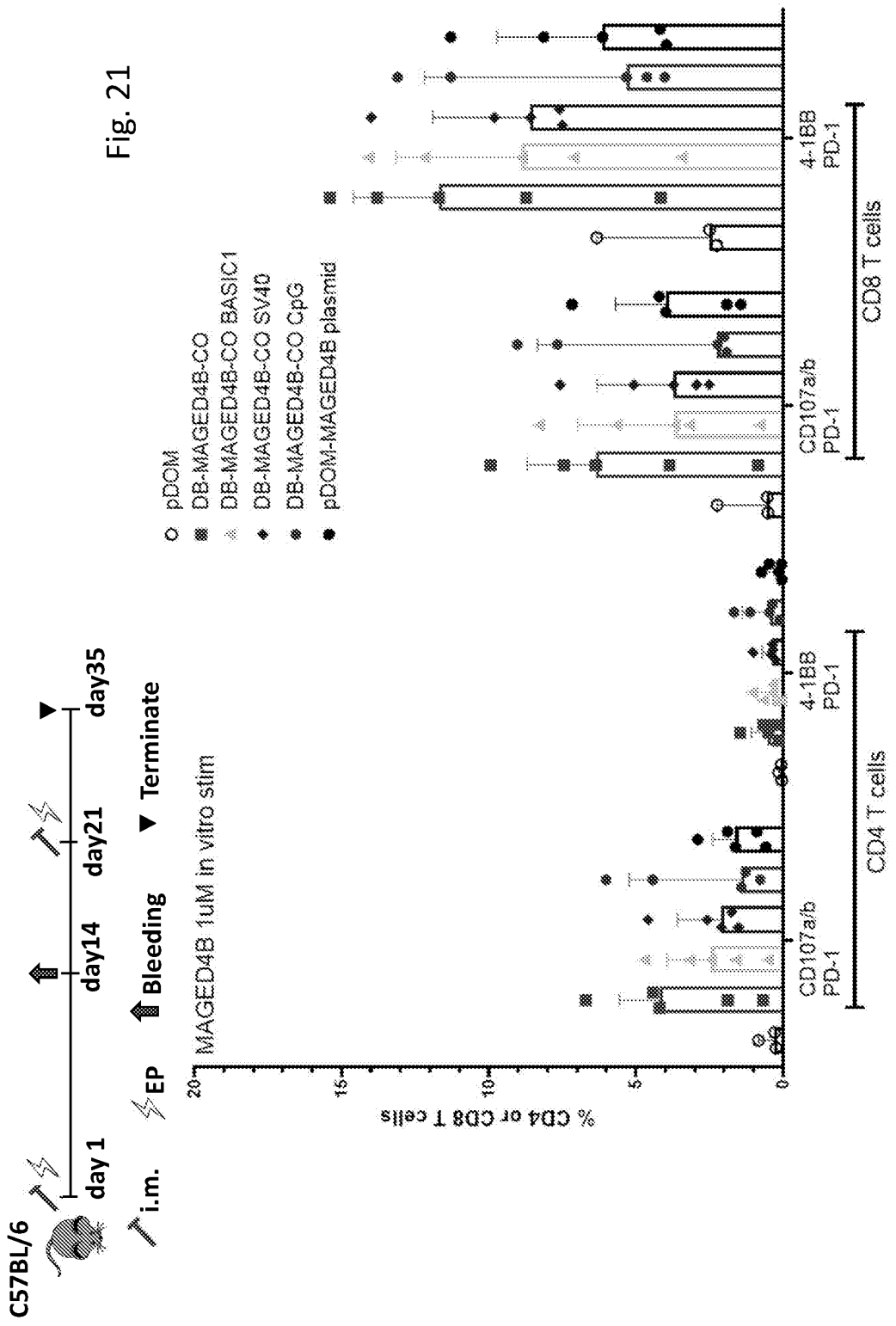

C57BL/6
i.m.
day 1
EP
day14
Bleeding
day21
Terminate
day35
MAGED4B 1uM in vitro stim
% CD4 or CD8 T cells
pDOM
DB-MAGED4B-CO
DB-MAGED4B-CO BASIC1
DB-MAGED4B-CO SV40
DB-MAGED4B-CO CpG
pDOM-MAGED4B plasmid
CD107a/b
PD-1
4-1BB
PD-1
CD4 T cells
CD8 T cells

Fig. 22

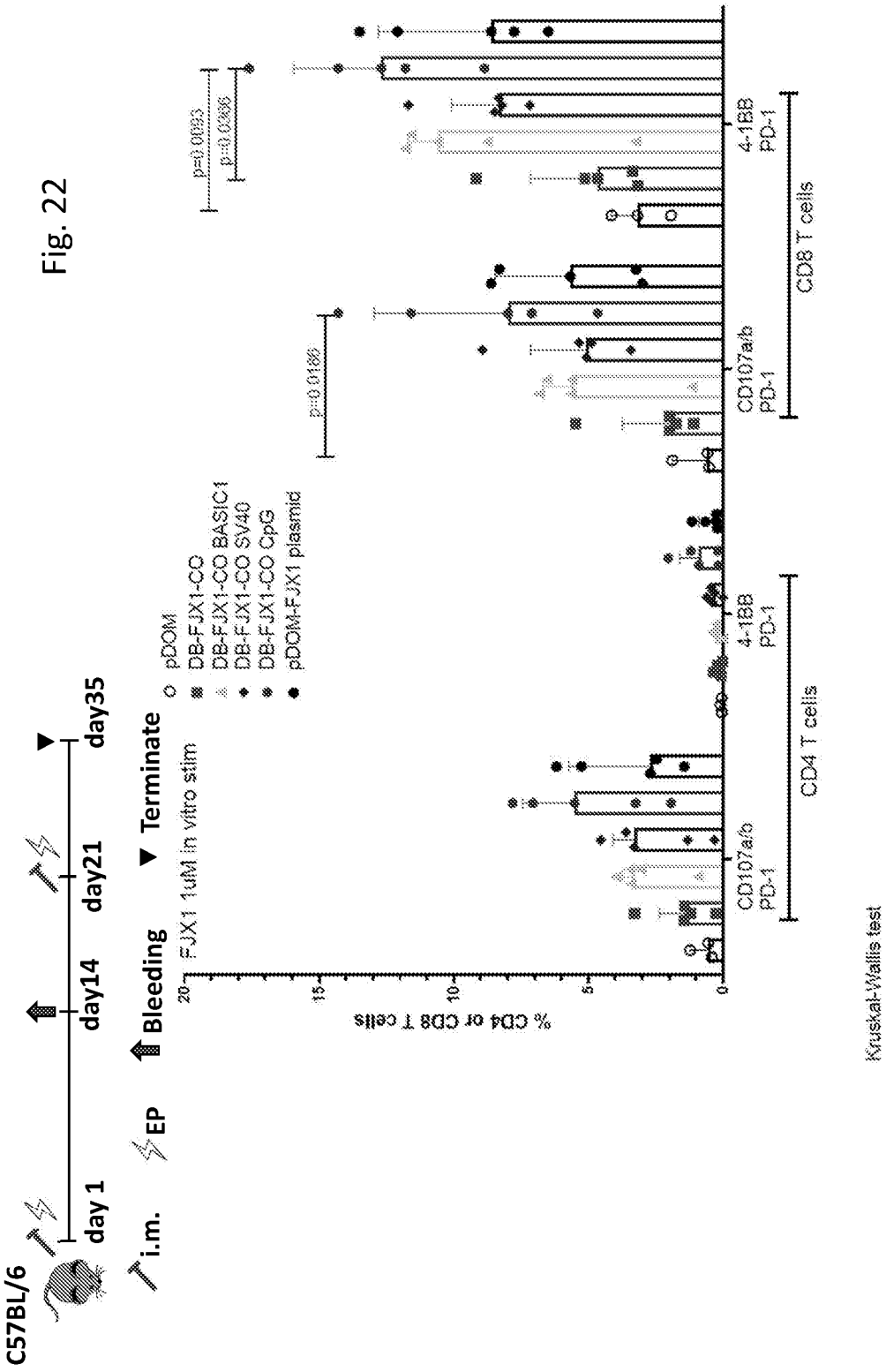
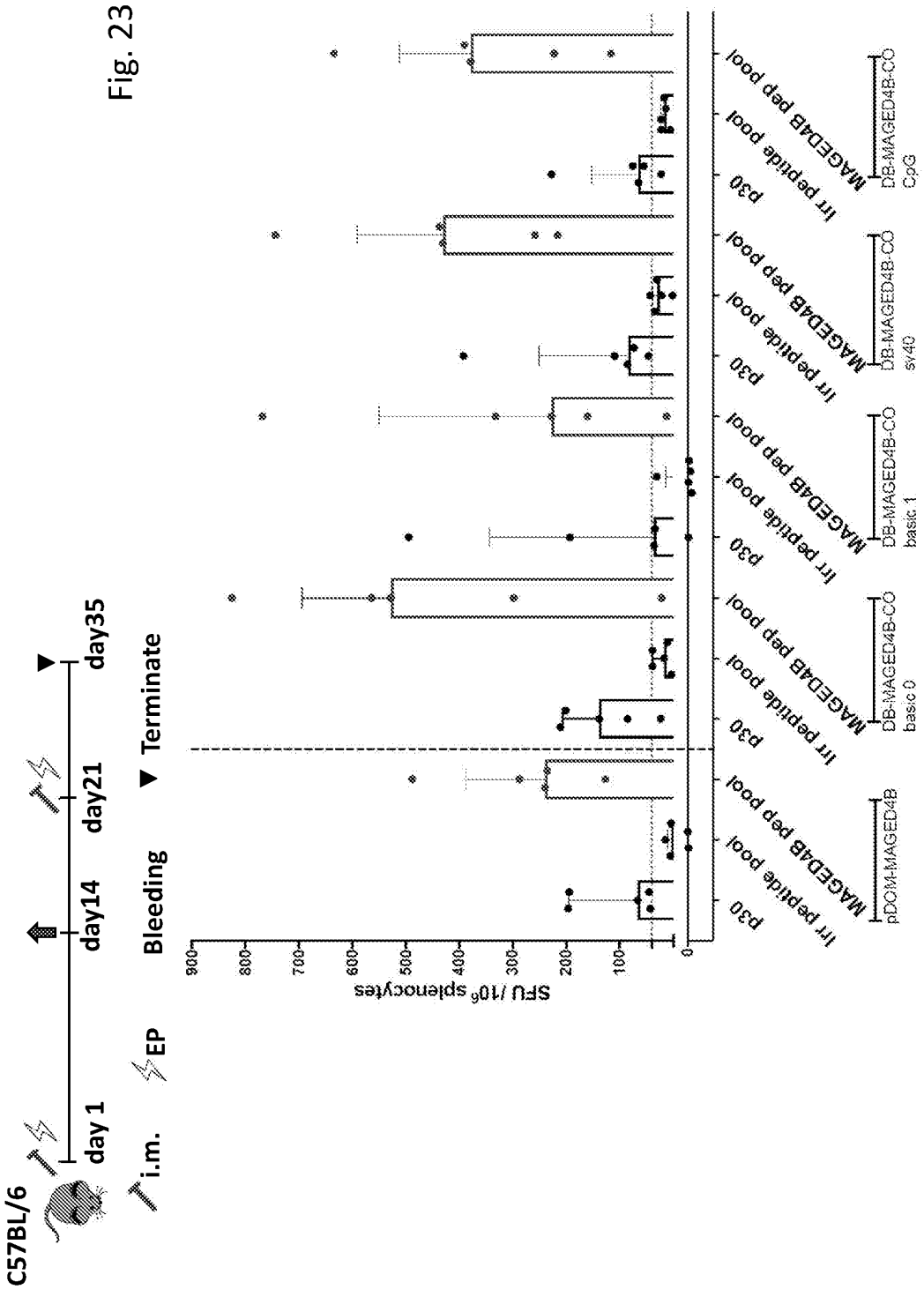

C57BL/6
i.m.
EP
day 1
day14
day21
day35
Bleeding
Terminate
FJX1 1uM in vitro stim
pDOM
DB-FJX1-CO
DB-FJX1-CO BASIC1
DB-FJX1-CO SV40
DB-FJX1-CO CpG
pDOM-FJX1 plasmid
% CD4 or CD8 T cells
p=0.0093
p=0.0286
p=0.0186
CD107a/b
PD-1
4-1BB
PD-1
CD4 T cells
CD8 T cells
Kruskal-Wallis test

Fig. 23


C57BL/6
day 1
day14
day21
day35
i.m.
EP
Bleeding
Terminate
SFU /10^6 splenocytes
100
200
300
400
500
600
700
800
900
0
p30
lrr peptide pool
MAGED4B pep pool
pDOM-MAGED4B
DB-MAGED4B-CO
basic 0
basic 1
sv40
CpG

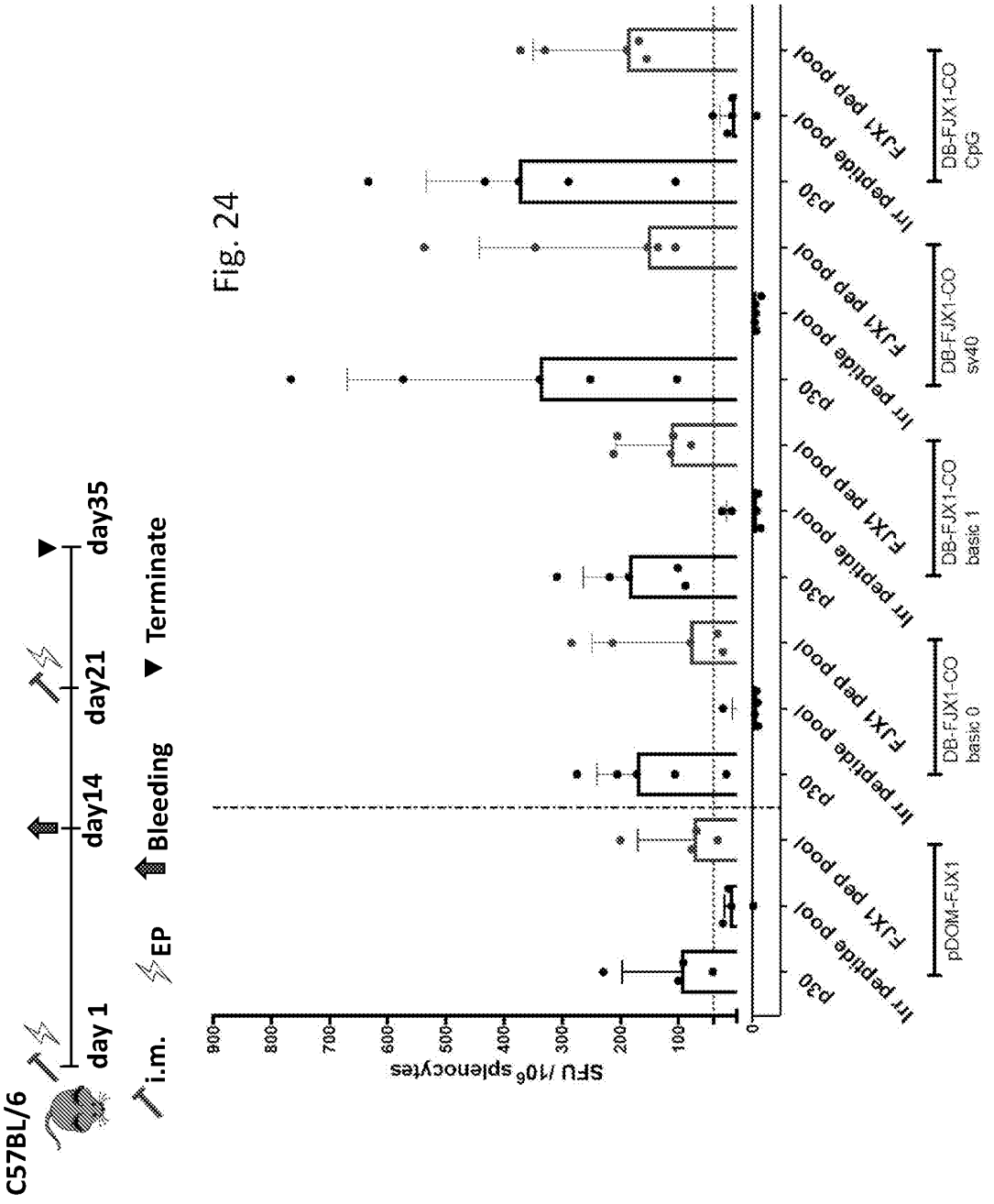

C57BL/6
day 1
day14
day21
day35
i.m.
EP
Bleeding
Terminate
SFU /10^6 splenocytes
0
100
200
300
400
500
600
700
800
900
p30
FJX1 peptide pool
Irr peptide pool
pDOM-FJX1
DB-FJX1-CO basic 0
DB-FJX1-CO basic 1
DB-FJX1-CO sv40
DB-FJX1-CO CpG

Fig. 24

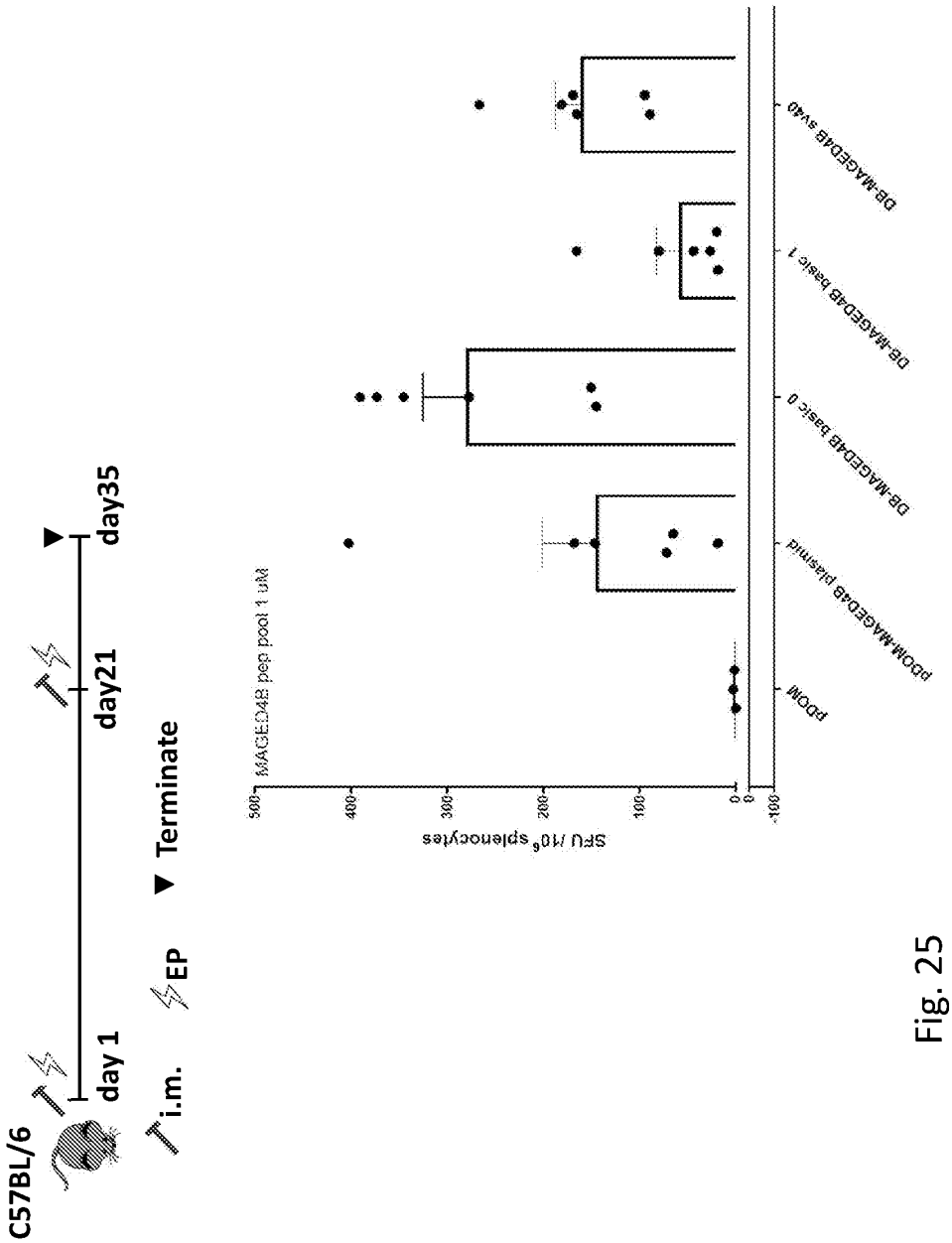
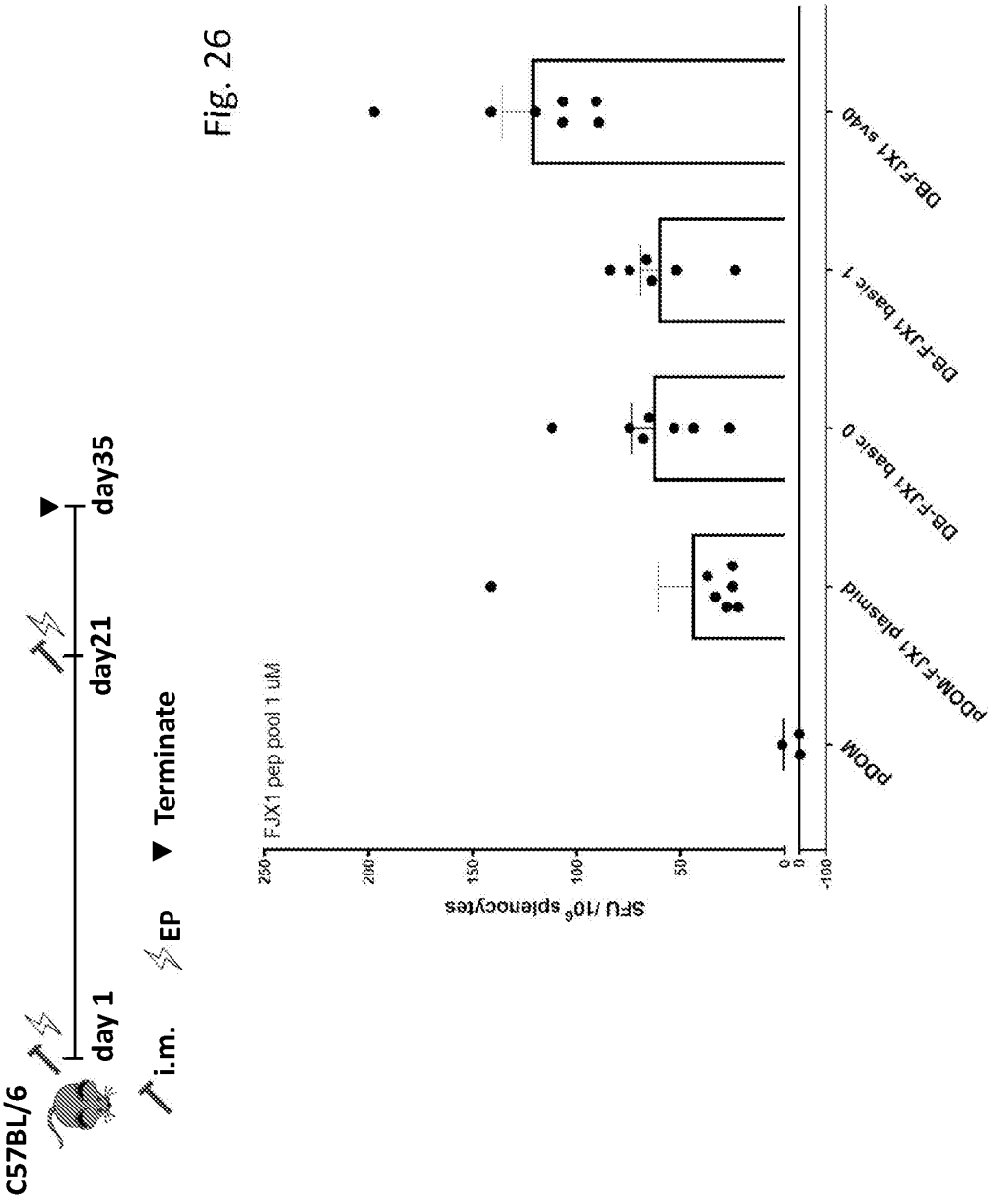

C57BL/6
day 1
day21
day35
i.m.
EP
Terminate
MAGED4B pep pool 1 uM
SFU /10^6 splenocytes
500
400
300
200
100
0
-100
pDOM
pDOM-hMAGED4B plasmid
DB-hMAGED4B basic 0
DB-hMAGED4B basic 1
DB-hMAGED4B sv40

Fig. 25

Fig. 26


C57BL/6
day 1
day21
day35
i.m.
EP
Terminate
FJX1 pep pool 1 uM
SFU /10^6 splenocytes
250
200
150
100
50
0
-100
pDOM
pDOM-FJX1 plasmid
DB-FJX1 basic 0
DB-FJX1 basic 1
DB-FJX1 sv40

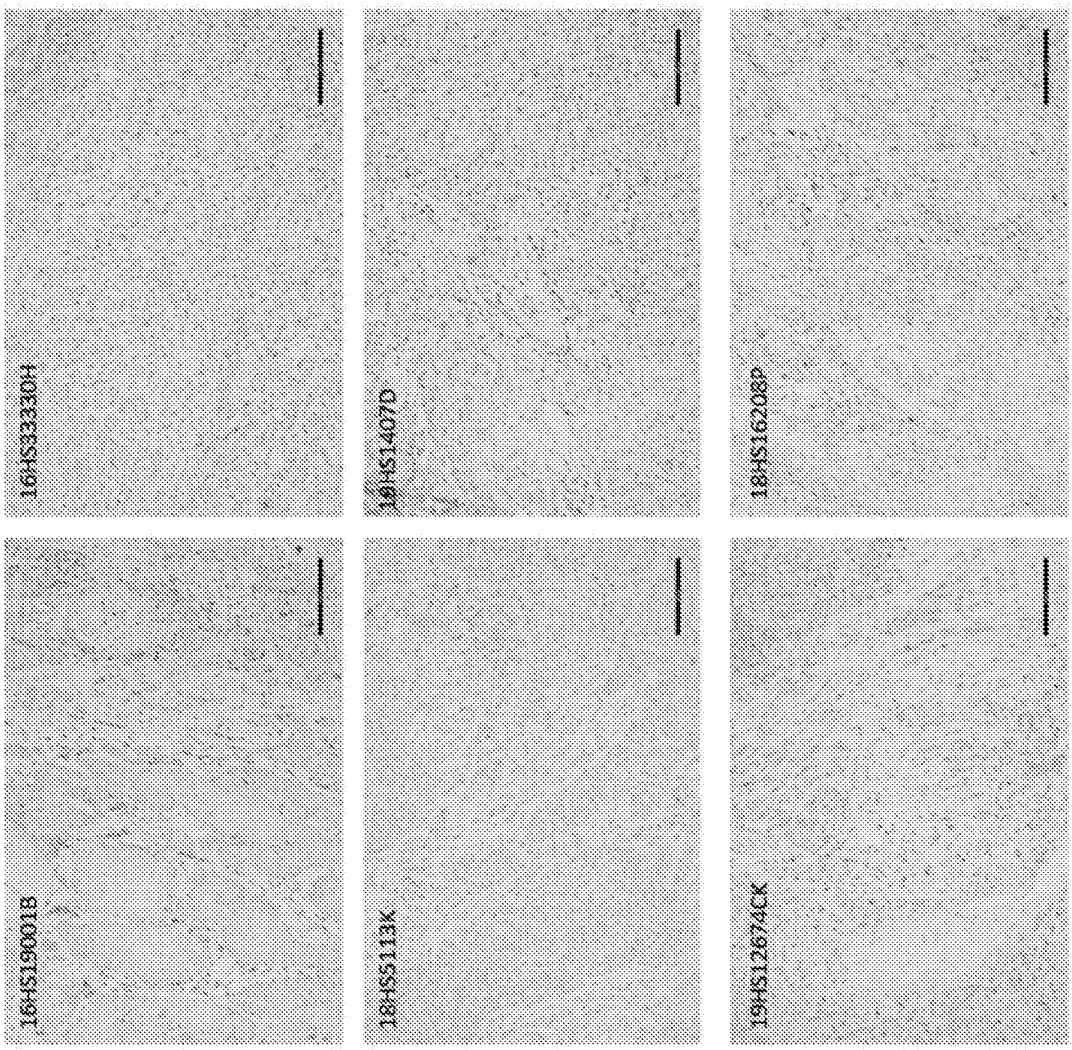

16HS3333OH
16HS1407D
18HS16208P
16HS19001B
18HS5113K
19HS12674CK

Fig. 27

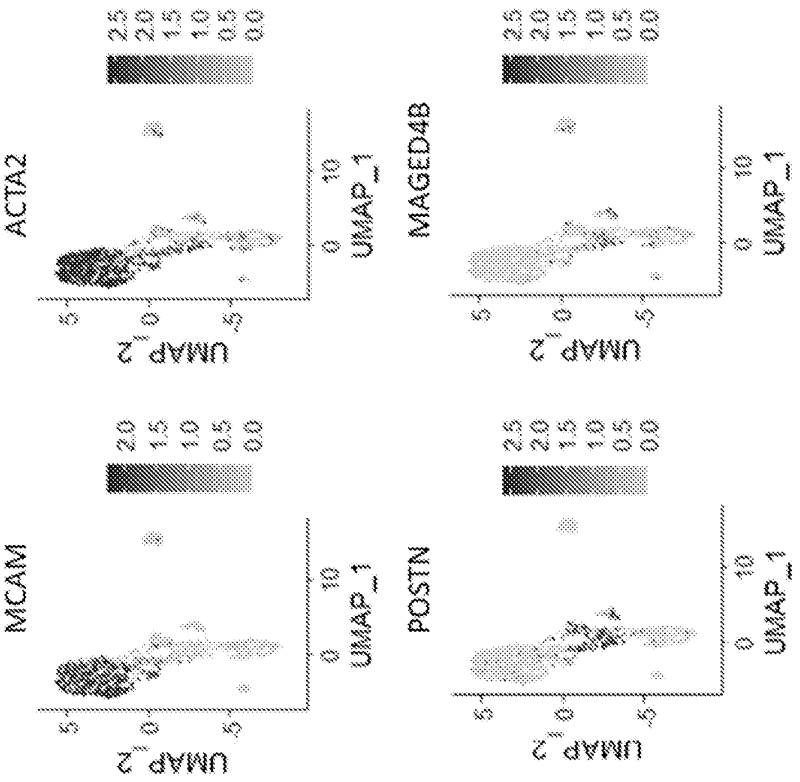


Fig. 28b

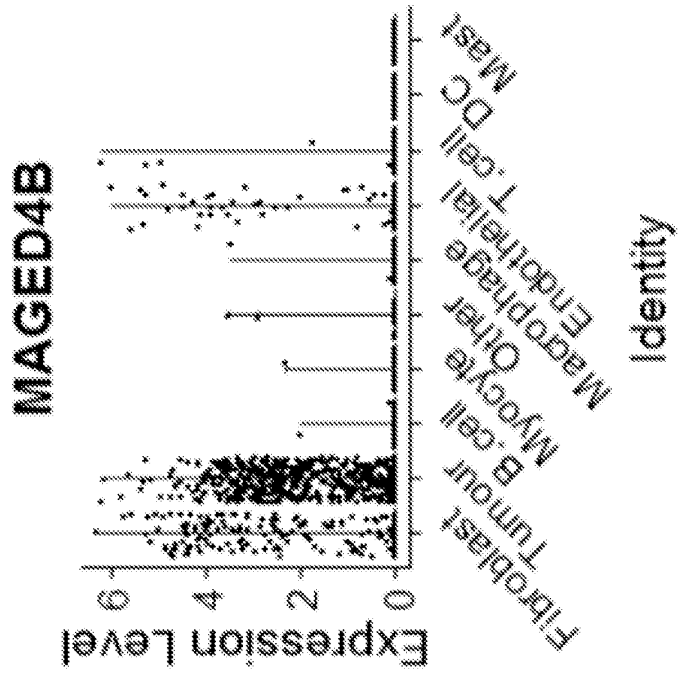


Fig. 28a

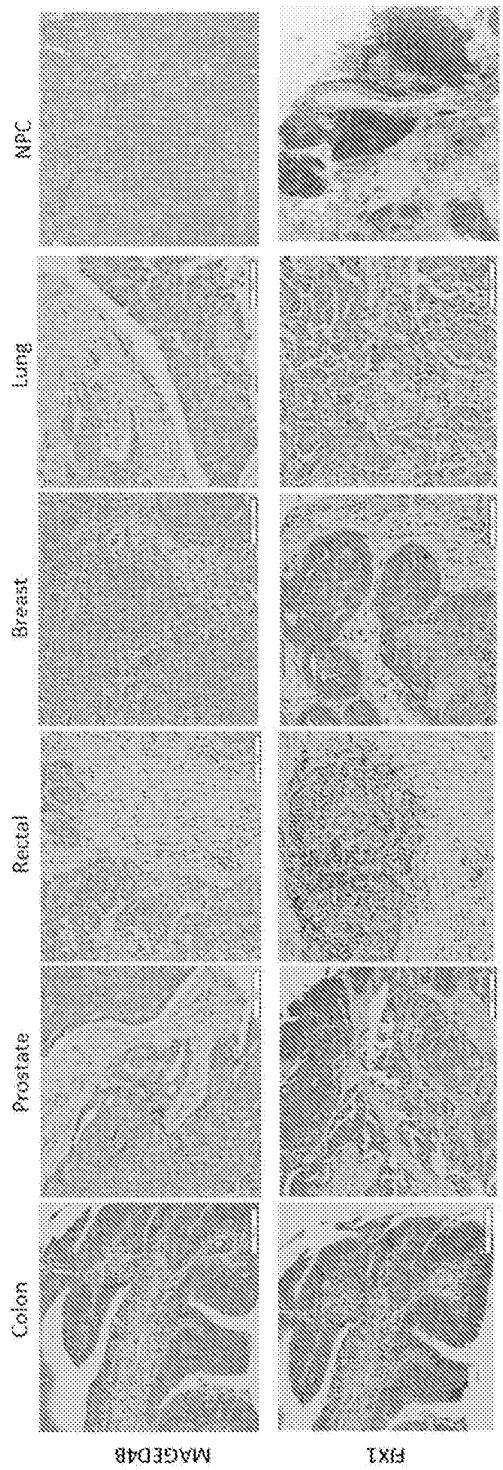


Fig. 29