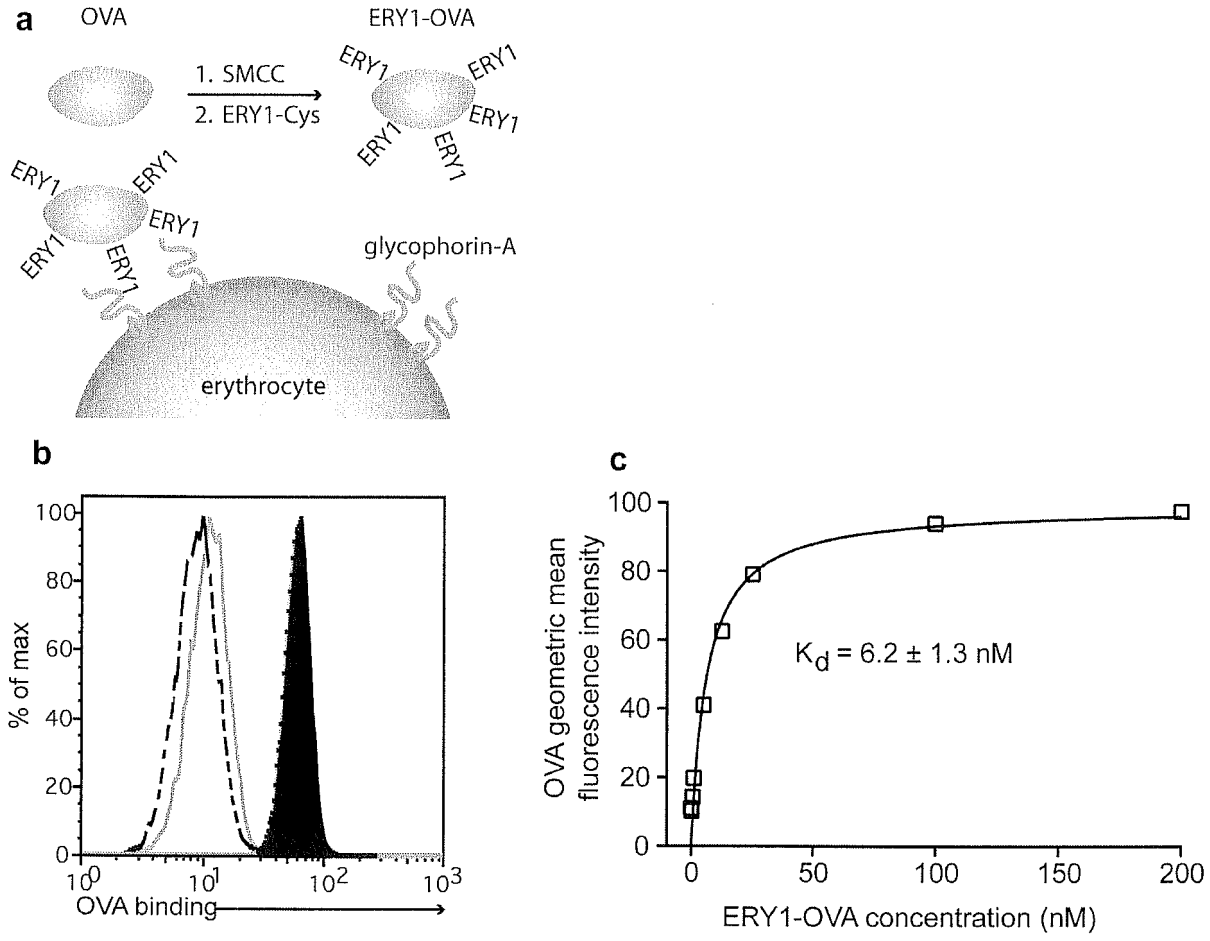

a
OVA
ERY1-OVA
1. SMCC
2. ERY1-Cys
ERY1
glycophorin-A
erythrocyte
b
% of max
OVA binding
c
OVA geometric mean fluorescence intensity
K_d = 6.2 ± 1.3 nM
ERY1-OVA concentration (nM)

Fig. 1

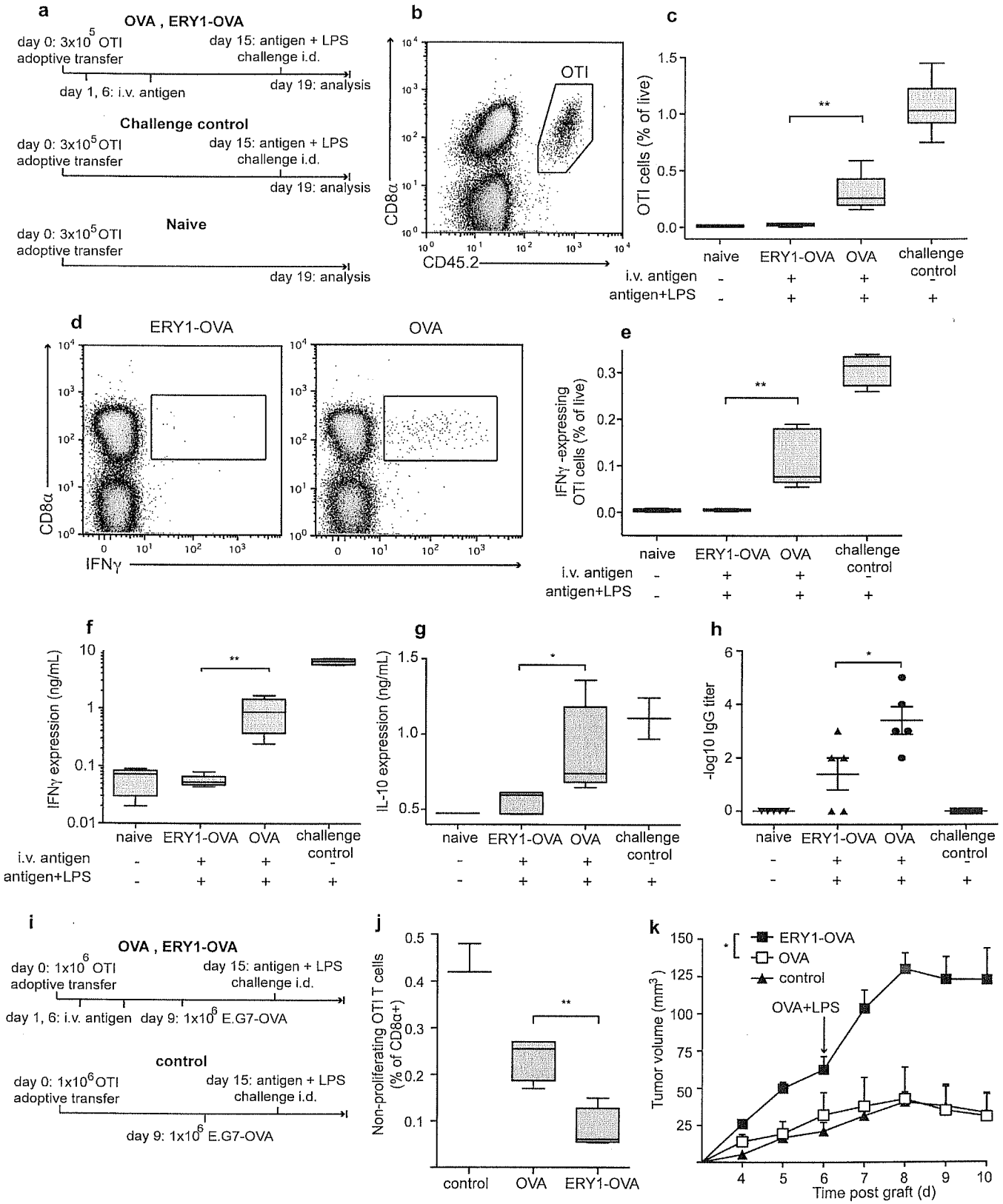

a
OVA , ERY1-OVA
day 0: 3x10^5 OTI adoptive transfer
day 15: antigen + LPS challenge i.d.
day 1, 6: i.v. antigen
day 19: analysis
Challenge control
day 0: 3x10^5 OTI adoptive transfer
day 15: antigen + LPS challenge i.d.
day 19: analysis
Naive
day 0: 3x10^5 OTI adoptive transfer
day 19: analysis
b
OTI
CD8α
CD45.2
c
OTI cells (% of live)
naive ERY1-OVA OVA challenge control
i.v. antigen - + + -
antigen+LPS - + + +
d
ERY1-OVA
OVA
CD8α
IFNγ
e
IFNγ -expressing OTI cells (% of live)
naive ERY1-OVA OVA challenge control
i.v. antigen - + + -
antigen+LPS - + + +
f
IFNγ expression (ng/mL)
naive ERY1-OVA OVA challenge control
i.v. antigen - + + -
antigen+LPS - + + +
g
IL-10 expression (ng/mL)
naive ERY1-OVA OVA challenge control
h
-log10 IgG titer
naive ERY1-OVA OVA challenge control
i
OVA , ERY1-OVA
day 0: 1x10^6 OTI adoptive transfer
day 15: antigen + LPS challenge i.d.
day 1, 6: i.v. antigen day 9: 1x10^6 E.G7-OVA
control
day 0: 1x10^6 OTI adoptive transfer
day 15: antigen + LPS challenge i.d.
day 9: 1x10^6 E.G7-OVA
j
Non-proliferating OTI T cells (% of CD8α+)
control OVA ERY1-OVA
k
ERY1-OVA
OVA
control
OVA+LPS
Tumor volume (mm^3)
Time post graft (d)

Fig. 2

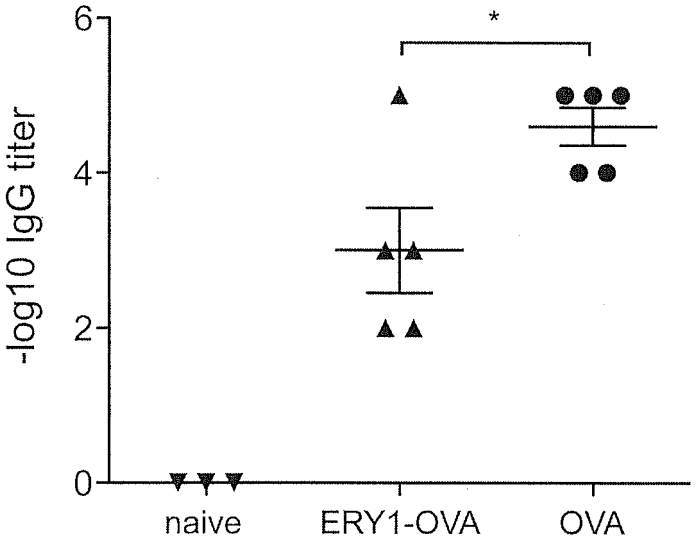

6
4
2
0
-log10 IgG titer
*
naive
ERY1-OVA
OVA

Fig. 3

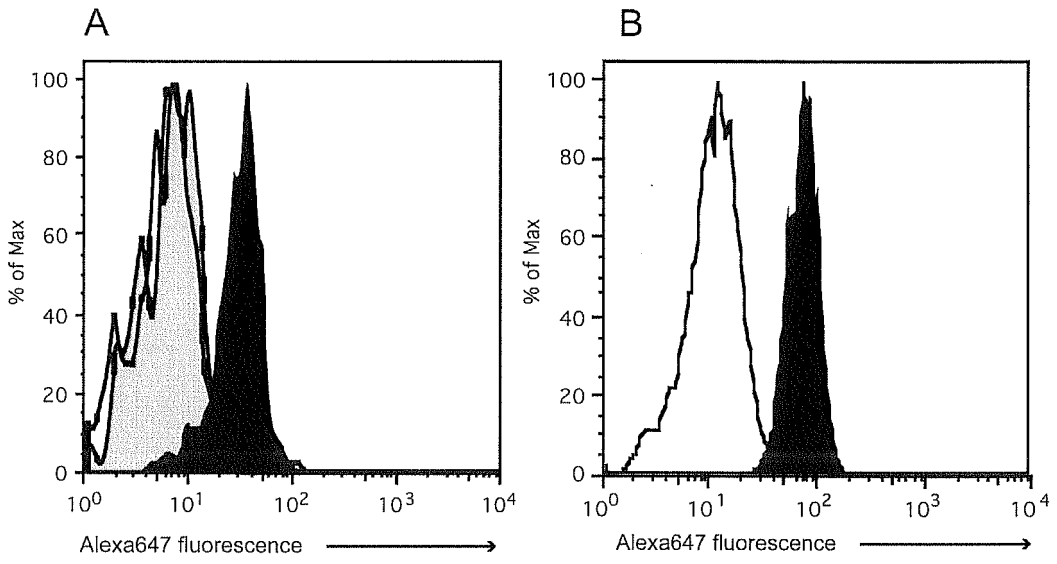

A
B
100
80
60
40
20
0
% of Max
10^0
10^1
10^2
10^3
10^4
Alexa647 fluorescence

Fig. 4

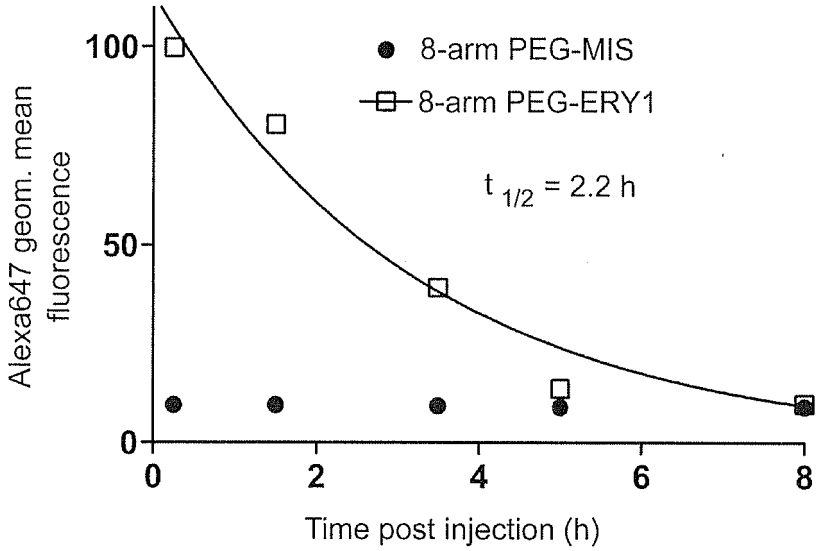

100
50
0
0
2
4
6
8
Alexa647 geom. mean fluorescence
Time post injection (h)
8-arm PEG-MIS
8-arm PEG-ERY1
t 1/2 = 2.2 h

Fig. 5

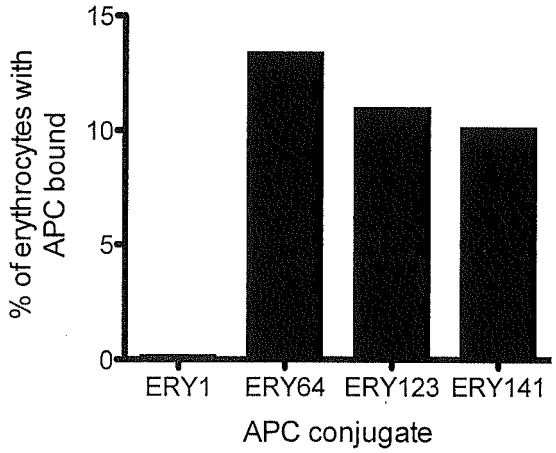

15
10
5
0
% of erythrocytes with APC bound
ERY1
ERY64
ERY123
ERY141
APC conjugate

Fig. 6

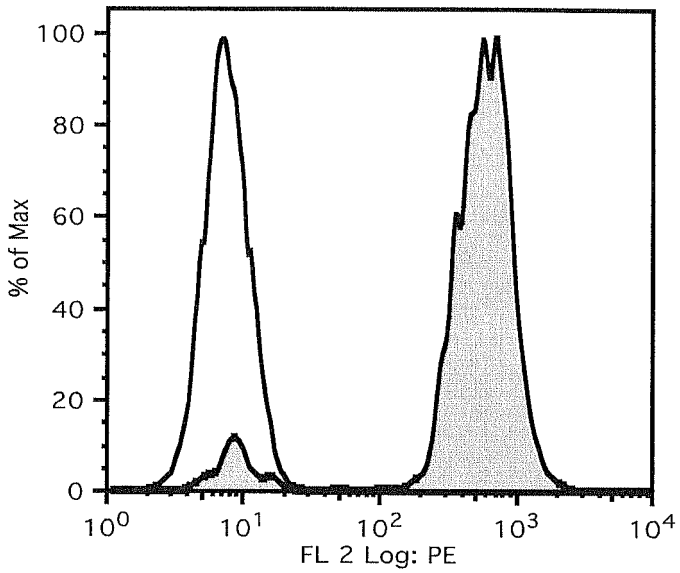

100
80
60
40
20
0
% of Max
10^0
10^1
10^2
10^3
10^4
FL 2 Log: PE

Fig. 7

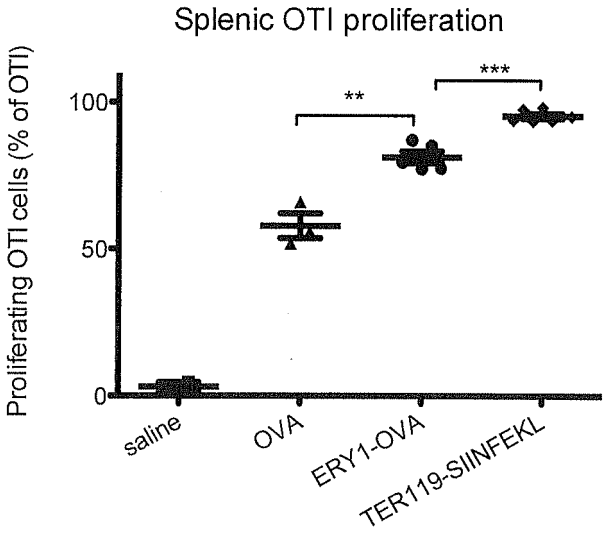

Splenic OTI proliferation
Proliferating OTI cells (% of OTI)
100
50
0
**
***
saline
OVA
ERY1-OVA
TER119-SIINFEKL

Fig. 8

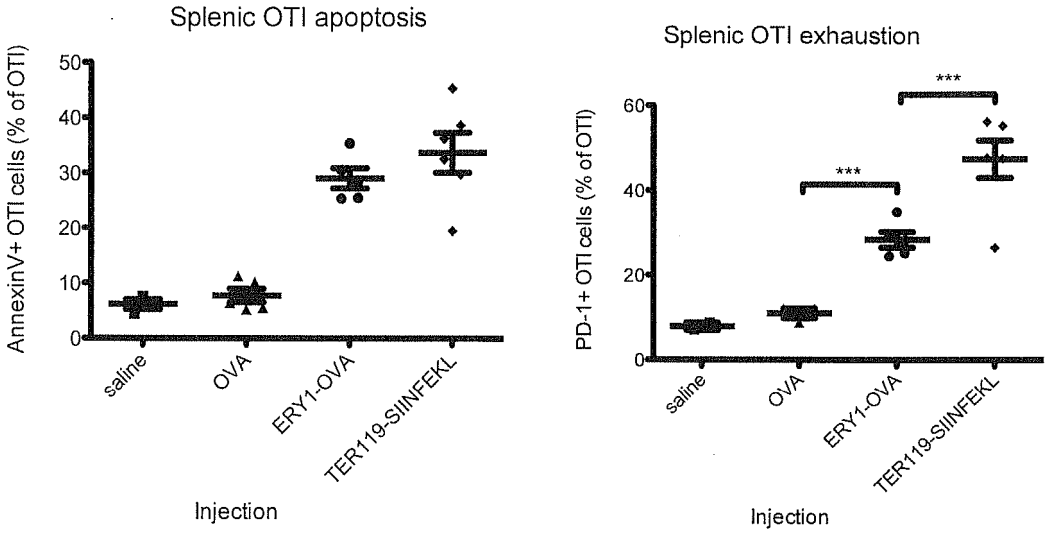

Splenic OTI apoptosis
AnnexinV+ OTI cells (% of OTI)
50
40
30
20
10
0
saline
OVA
ERY1-OVA
TER119-SIINFEKL
Injection
Splenic OTI exhaustion
PD-1+ OTI cells (% of OTI)
60
40
20
0
***
***
saline
OVA
ERY1-OVA
TER119-SIINFEKL
Injection

Fig. 9

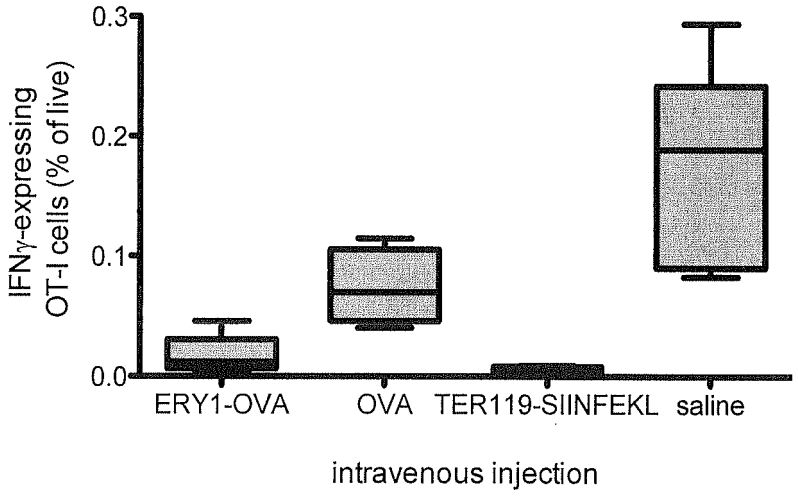

IFNγ-expressing OT-I cells (% of live)
0.3
0.2
0.1
0.0
ERY1-OVA
OVA
TER119-SIINFEKL
saline
intravenous injection

Fig. 10

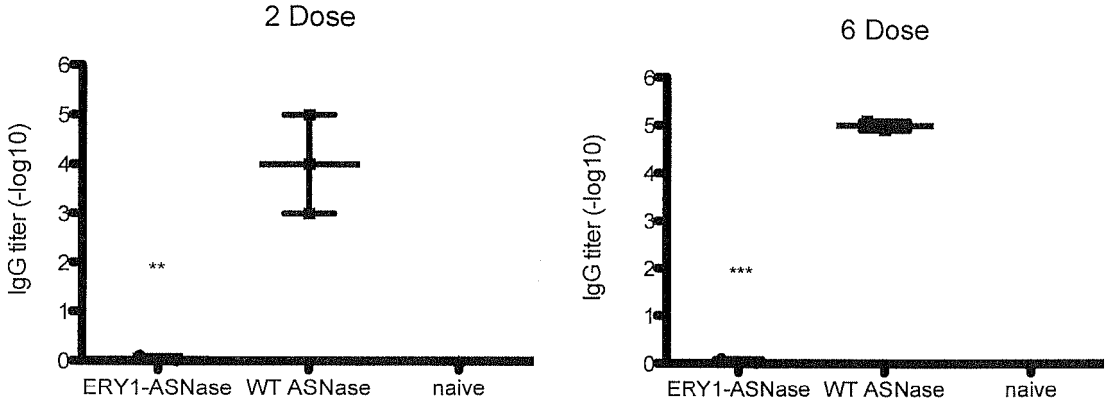

2 Dose
6 Dose
IgG titer (-log10)
**
***
ERY1-ASNase
WT ASNase
naive

Fig. 11

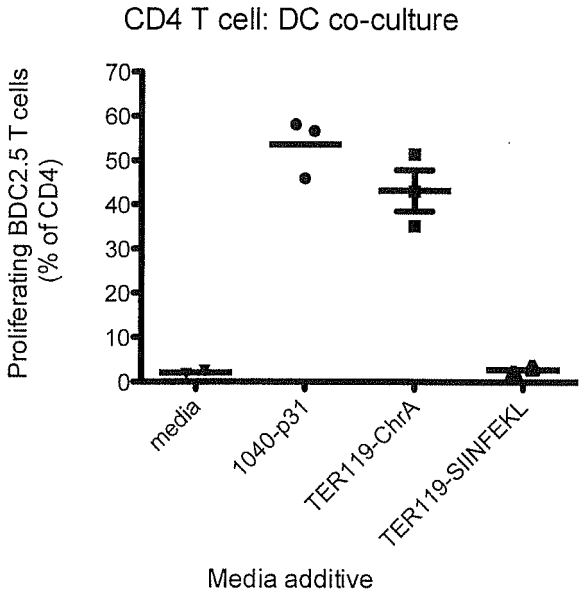

CD4 T cell: DC co-culture
Proliferating BDC2.5 T cells (% of CD4)
media
1040-p31
TER119-ChrA
TER119-SIINFEKL
Media additive

Fig. 12

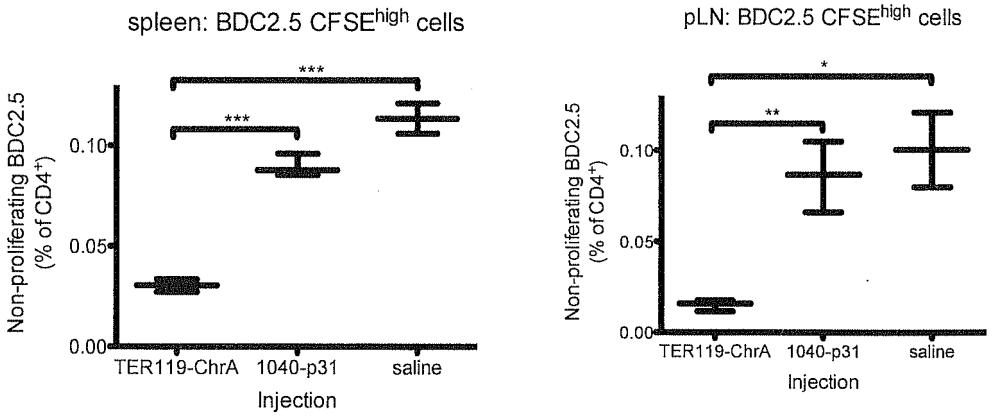

spleen: BDC2.5 CFSE^high cells
Non-proliferating BDC2.5 (% of CD4+)
0.10
0.05
0.00
***
***
TER119-ChrA
1040-p31
saline
Injection
pLN: BDC2.5 CFSE^high cells
Non-proliferating BDC2.5 (% of CD4+)
0.10
0.05
0.00
*
**
TER119-ChrA
1040-p31
saline
Injection

Fig. 13