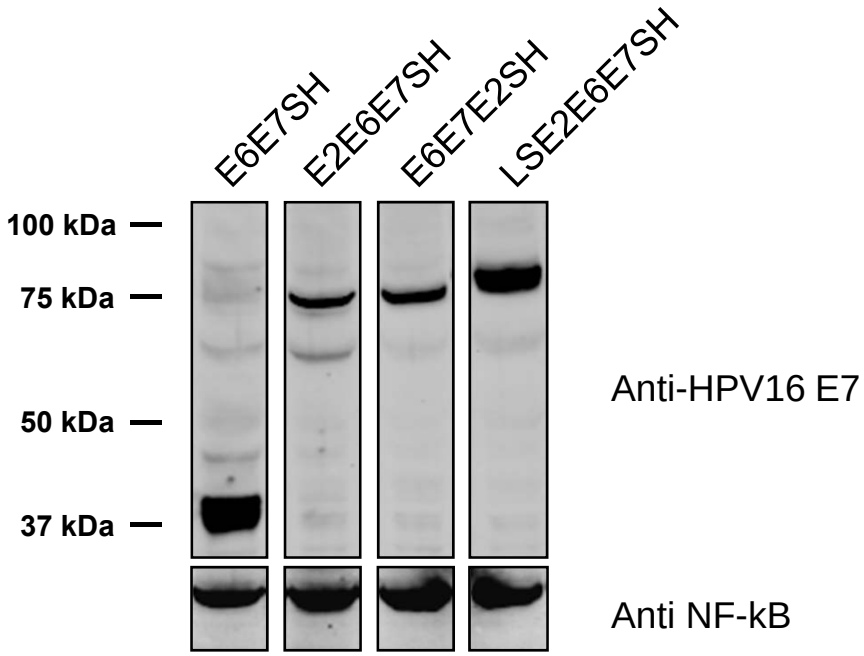
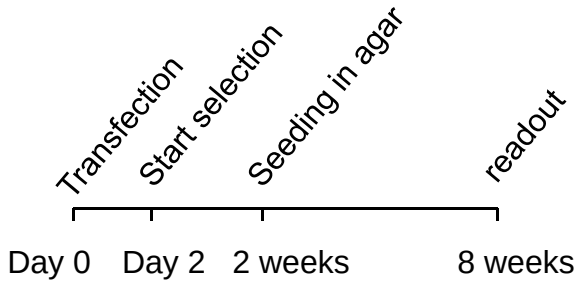
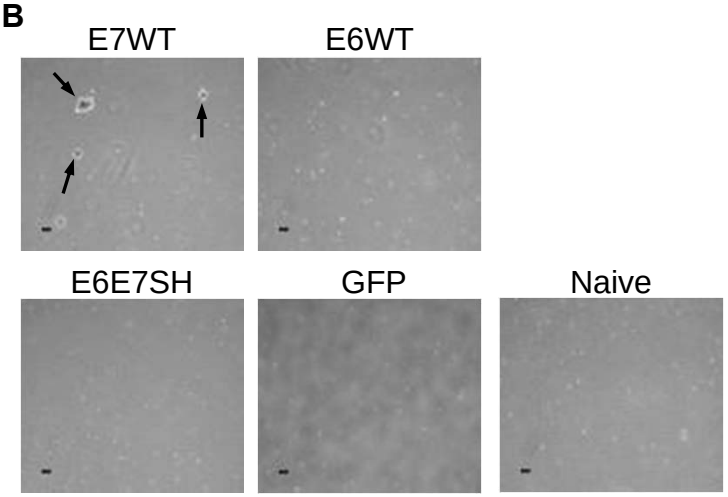

E6E7SH
E2E6E7SH
E6E7E2SH
LSE2E6E7SH
100 kDa
75 kDa
50 kDa
37 kDa
Anti-HPV16 E7
Anti NF-kB

**Fig. 1**

A


Transfection
Start selection
Seeding in agar
readout
Day 0
Day 2
2 weeks
8 weeks

B


E7WT
E6WT
E6E7SH
GFP
Naive

C

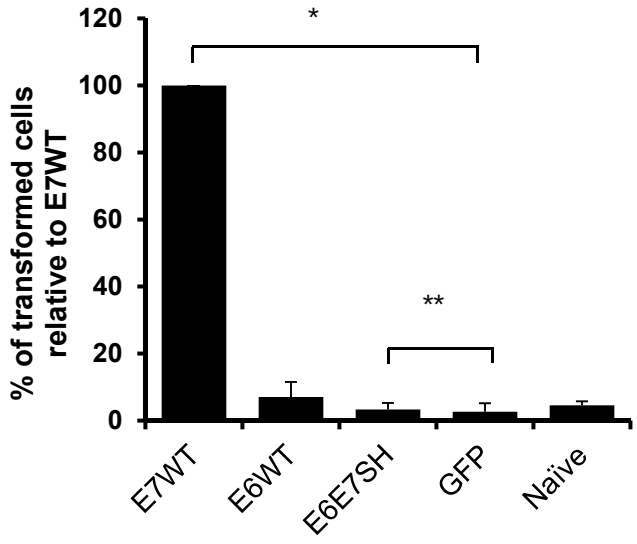
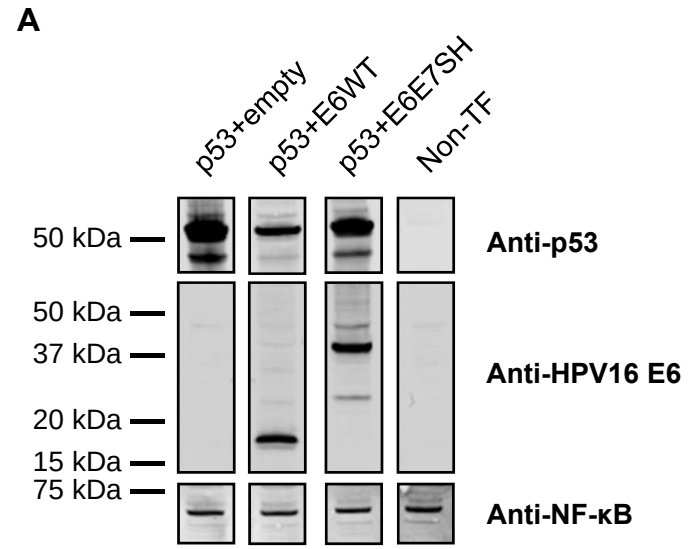

% of transformed cells relative to E7WT
120
100
80
60
40
20
0
*
**
E7WT
E6WT
E6E7SH
GFP
Naïve

Fig. 2


A
p53+empty
p53+E6WT
p53+E6E7SH
Non-TF
50 kDa
Anti-p53
50 kDa
37 kDa
20 kDa
15 kDa
Anti-HPV16 E6
75 kDa
Anti-NF-κB

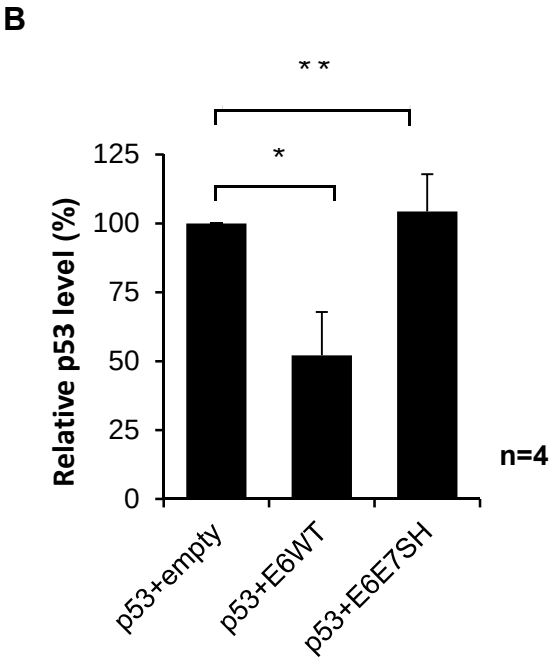
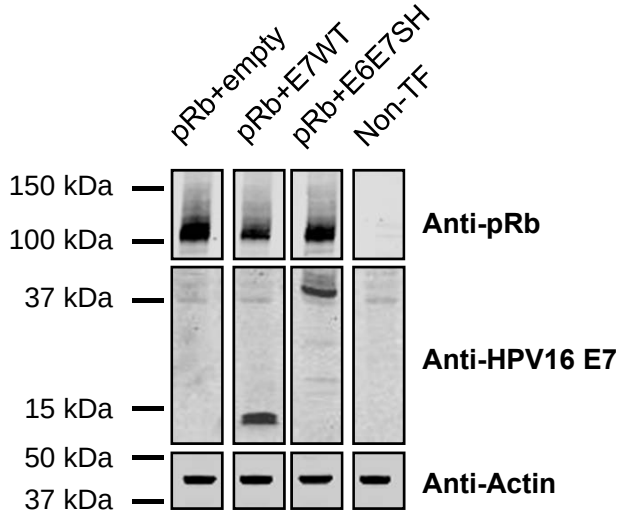

B
* *
*
125
100
75
50
25
0
Relative p53 level (%)
n=4
p53+empty
p53+E6WT
p53+E6E7SH

Fig. 3

C




D

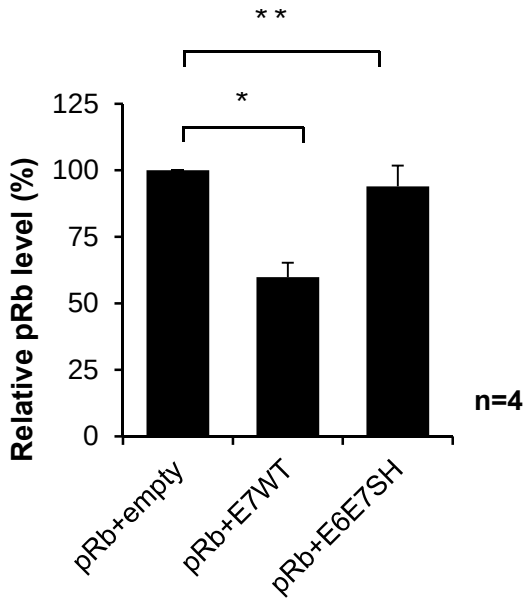



**Fig. 3 - continued**

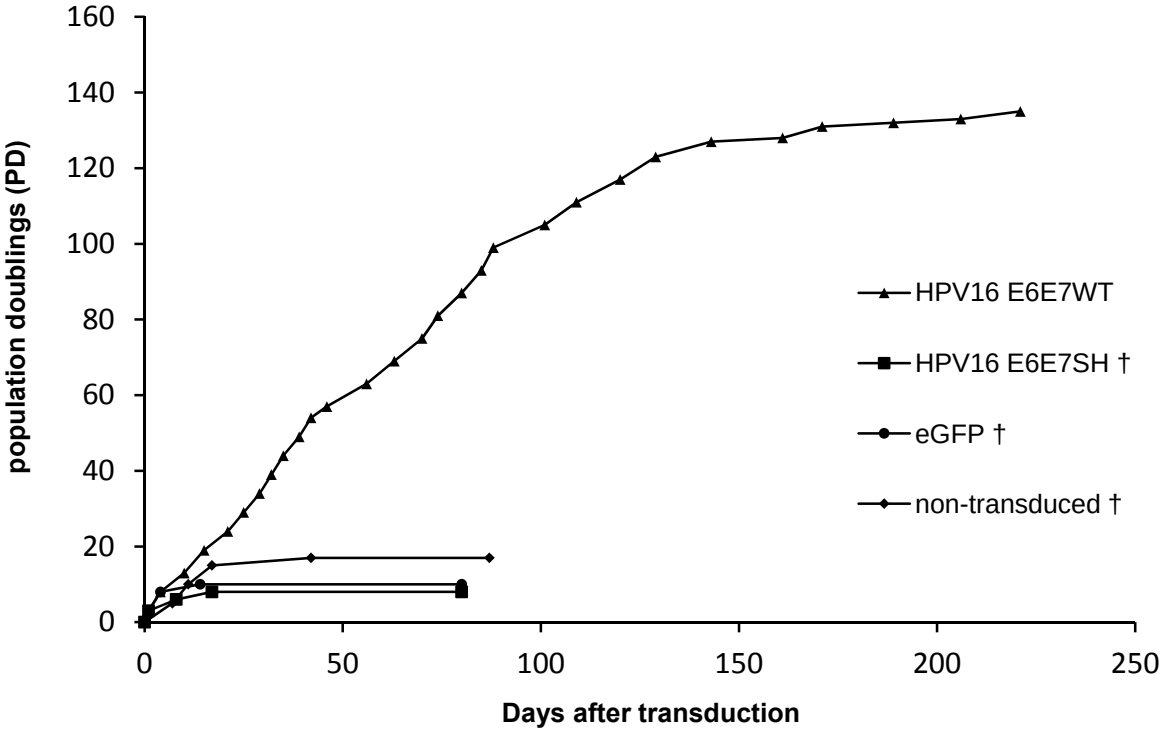
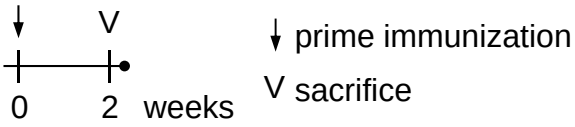

160
140
120
100
80
60
40
20
0
population doublings (PD)
0
50
100
150
200
250
Days after transduction
HPV16 E6E7WT
HPV16 E6E7SH †
eGFP †
non-transduced †

Fig. 4

**A**


V
0
2
weeks
prime immunization
V sacrifice

**B**

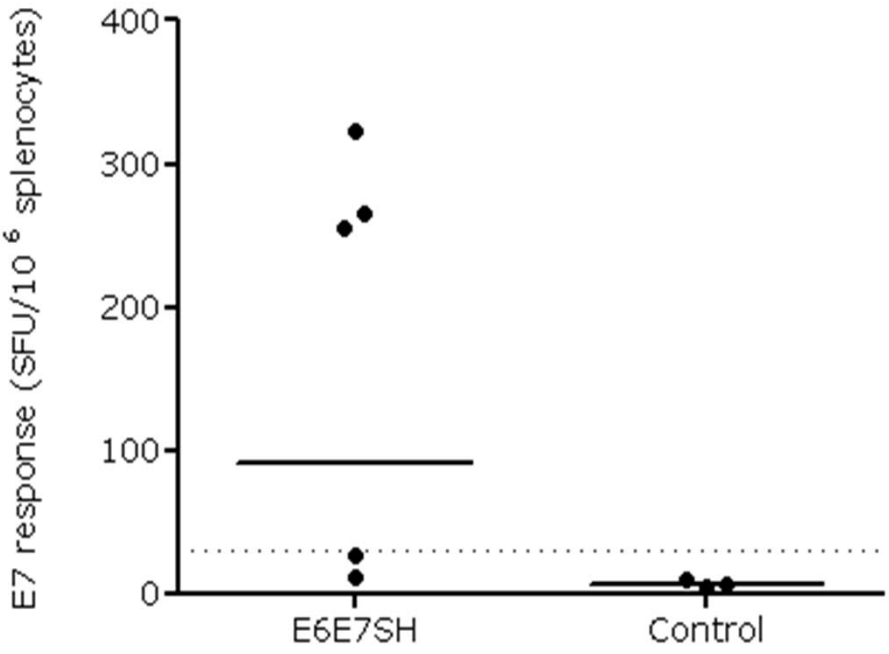
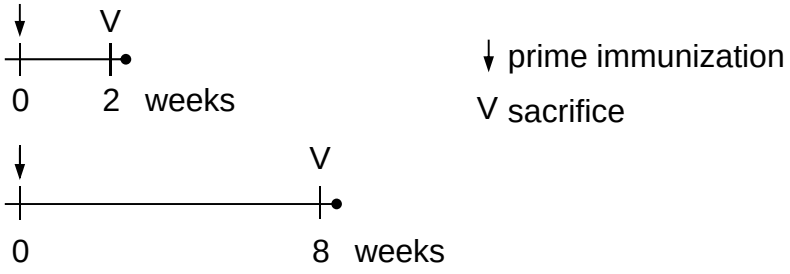
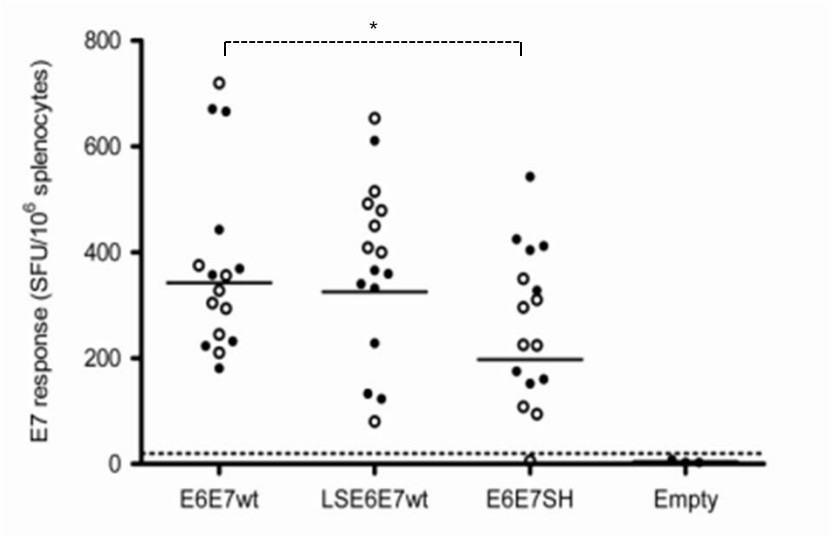

E7 response (SFU/10 6 splenocytes)
400
300
200
100
0
E6E7SH
Control

**Fig. 5**

**A**


V
0
2 weeks
prime immunization
V sacrifice
V
0
8 weeks

**B**


*
800
600
400
200
0
E7 response (SFU/10^6 splenocytes)
E6E7wt
LSE6E7wt
E6E7SH
Empty

**C**

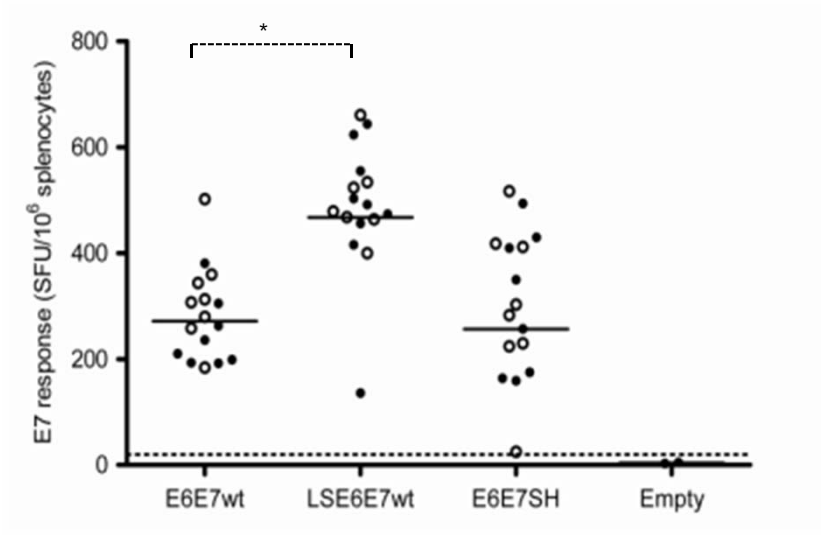
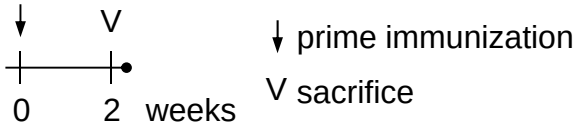

*
800
600
400
200
0
E7 response (SFU/10^6 splenocytes)
E6E7wt
LSE6E7wt
E6E7SH
Empty

**Fig. 6**

**A**


V
0
2
weeks
prime immunization
V sacrifice

**B**

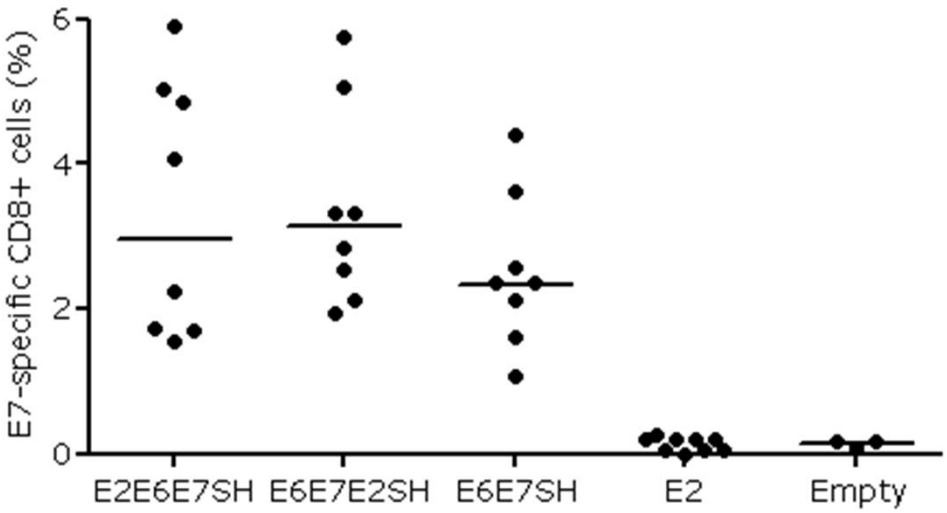
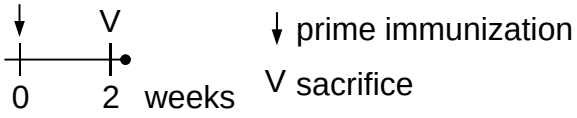
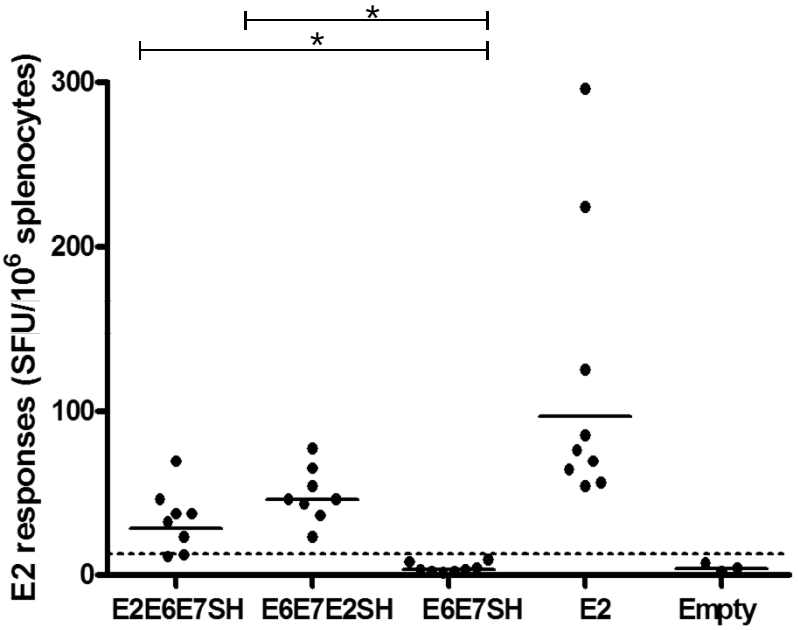

E7-specific CD8+ cells (%)
6
4
2
0
E2E6E7SH
E6E7E2SH
E6E7SH
E2
Empty

**Fig. 7**

A


V
prime immunization
V sacrifice
0
2
weeks

B


*
*
E2 responses (SFU/10^6 splenocytes)
300
200
100
0
E2E6E7SH
E6E7E2SH
E6E7SH
E2
Empty

C

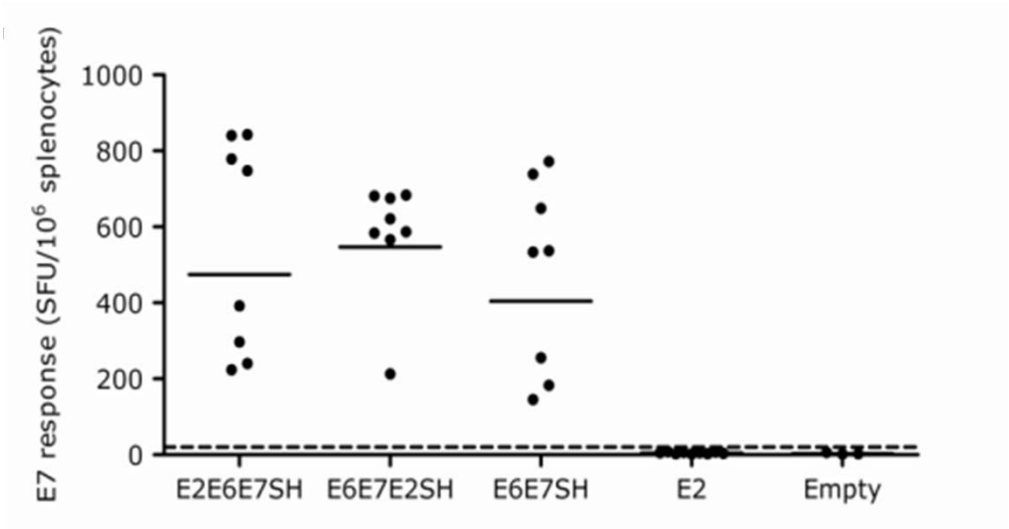

E7 response (SFU/10^6 splenocytes)
1000
800
600
400
200
0
E2E6E7SH
E6E7E2SH
E6E7SH
E2
Empty

Fig. 8

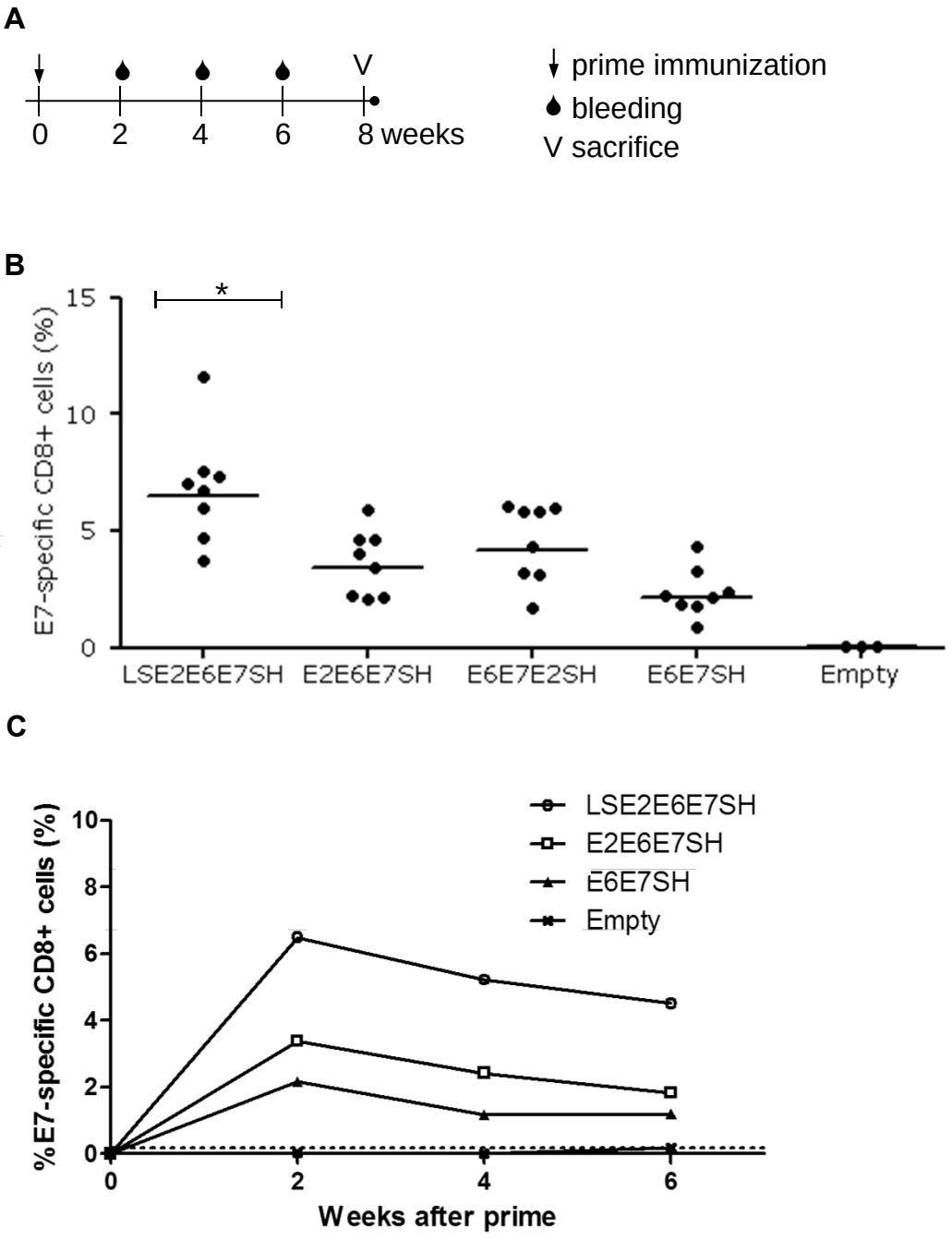
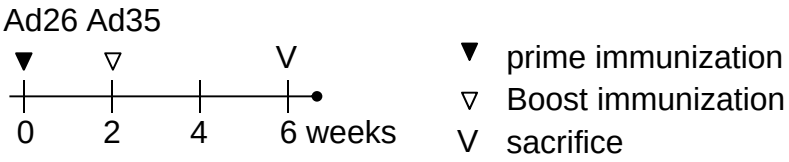

A
V
0
2
4
6
8 weeks
prime immunization
bleeding
V sacrifice
B
*
E7-specific CD8+ cells (%)
15
10
5
0
LSE2E6E7SH
E2E6E7SH
E6E7E2SH
E6E7SH
Empty
C
%E7-specific CD8+ cells (%)
10
8
6
4
2
0
0
2
4
6
Weeks after prime
LSE2E6E7SH
E2E6E7SH
E6E7SH
Empty

**Fig. 9**

A


Ad26 Ad35
V
0
2
4
6 weeks
prime immunization
Boost immunization
V sacrifice

B

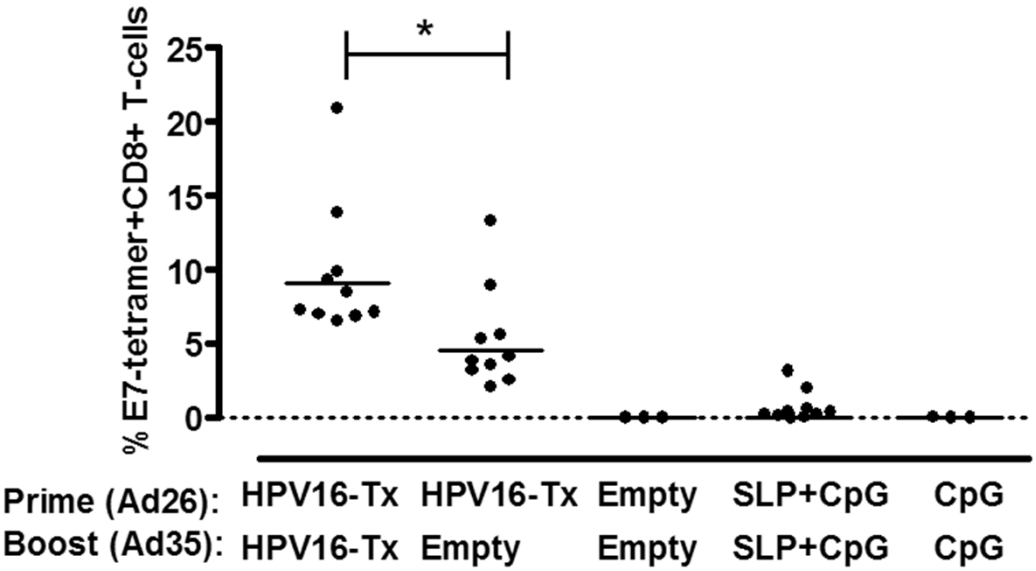
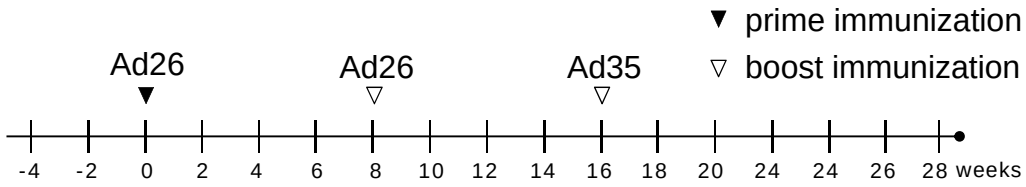

% E7-tetramer+CD8+ T-cells
25
20
15
10
5
0
*
Prime (Ad26): HPV16-Tx HPV16-Tx Empty SLP+CpG CpG
Boost (Ad35): HPV16-Tx Empty Empty SLP+CpG CpG

Fig. 10

A


prime immunization
boost immunization
Ad26
Ad26
Ad35
-4 -2 0 2 4 6 8 10 12 14 16 18 20 24 24 26 28 weeks

B

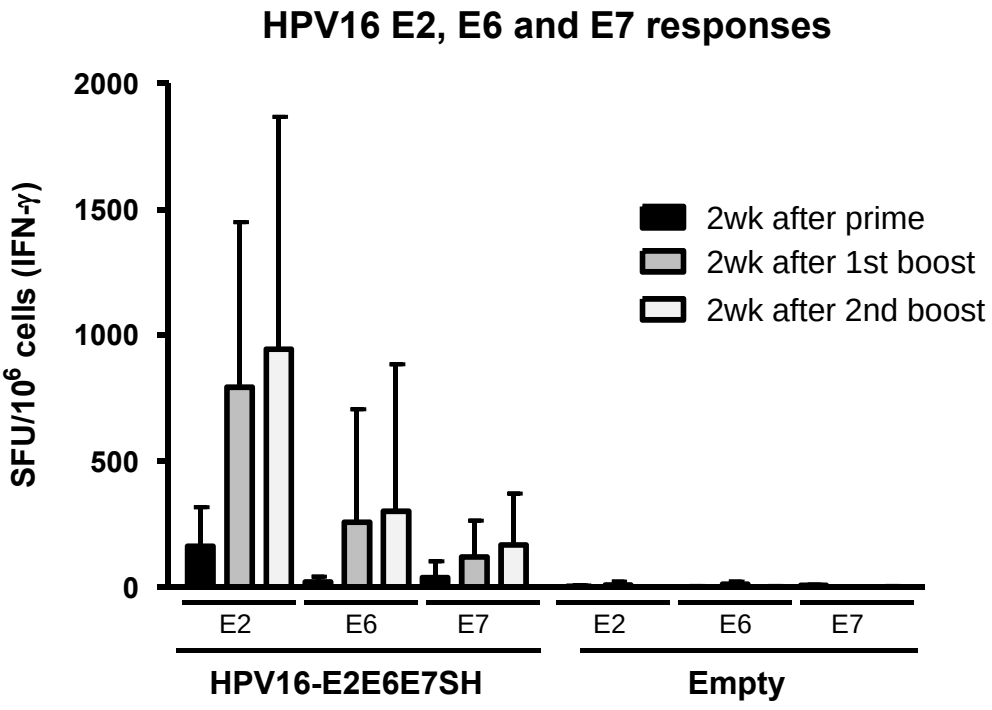

HPV16 E2, E6 and E7 responses
SFU/10^6 cells (IFN-γ)
2000
1500
1000
500
0
2wk after prime
2wk after 1st boost
2wk after 2nd boost
E2
E6
E7
E2
E6
E7
HPV16-E2E6E7SH
Empty

Fig. 11

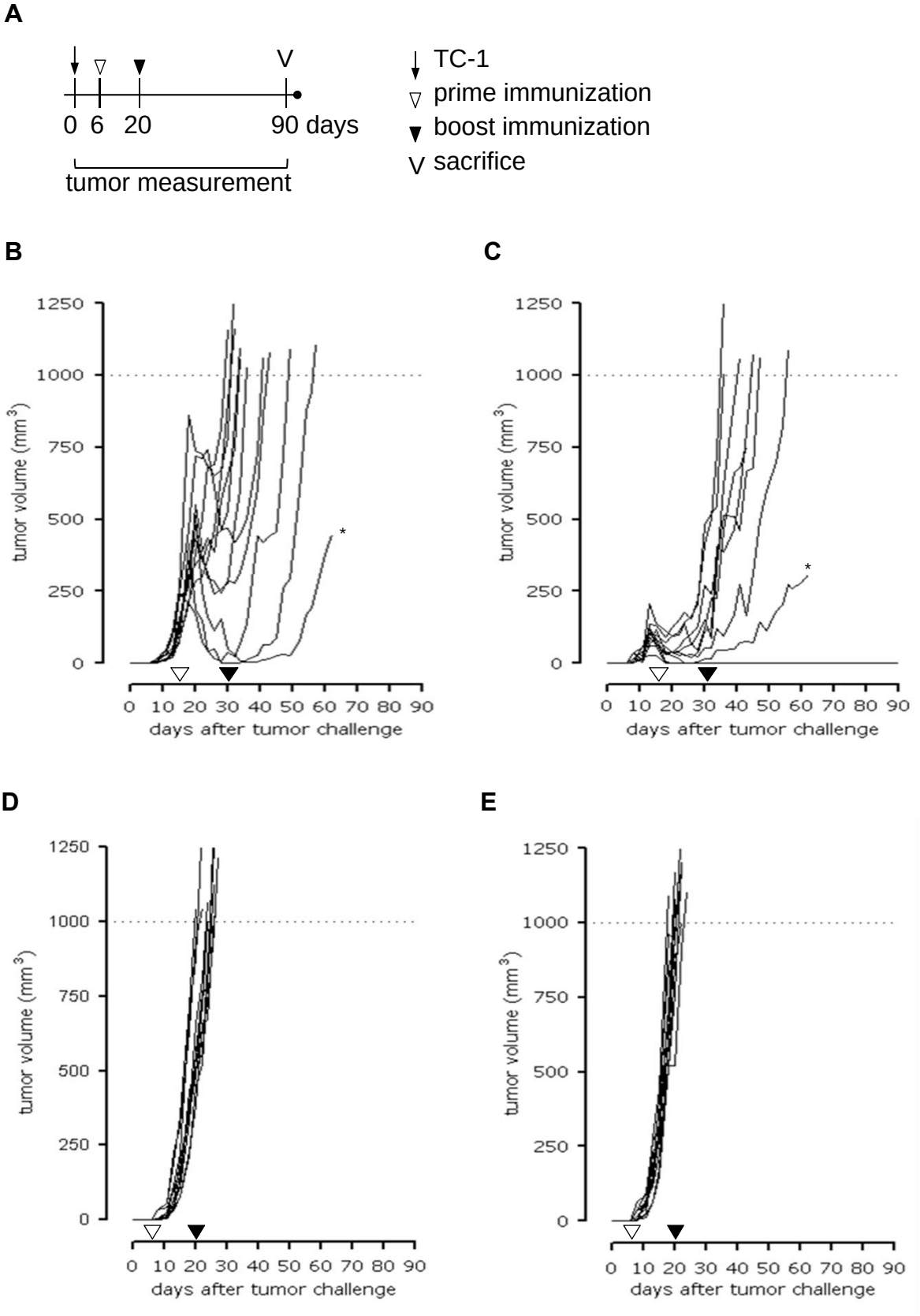
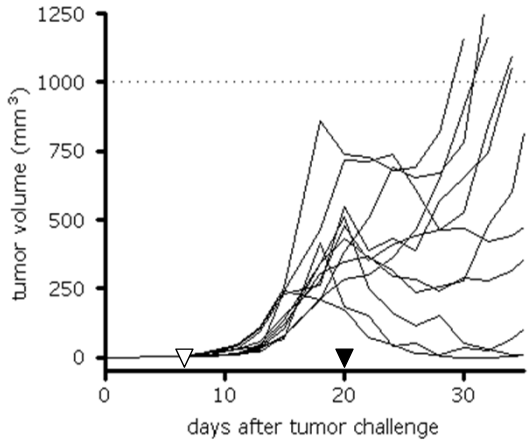
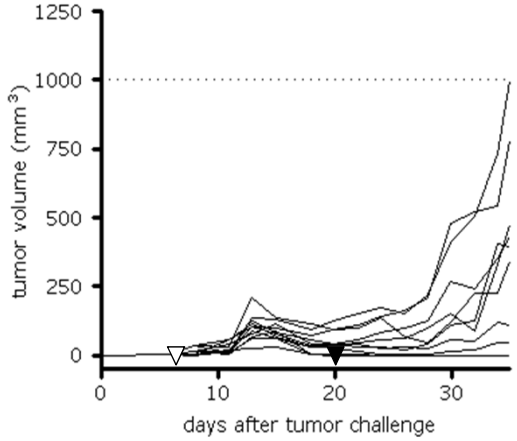

A
V
0 6 20 90 days
tumor measurement
TC-1
prime immunization
boost immunization
sacrifice
B
C
D
E
tumor volume (mm3)
1250
1000
750
500
250
0
0 10 20 30 40 50 60 70 80 90
days after tumor challenge
*

Fig. 12

F




G




H

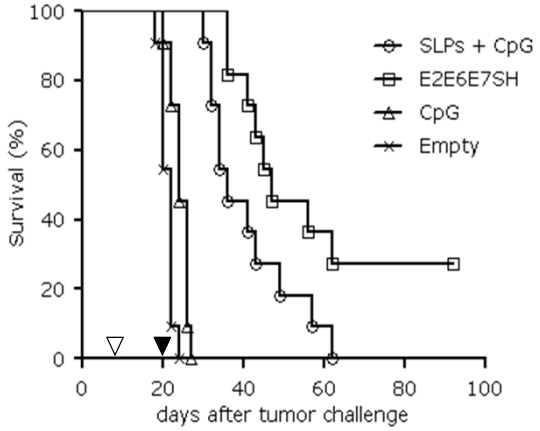



**Fig. 12 - continued**

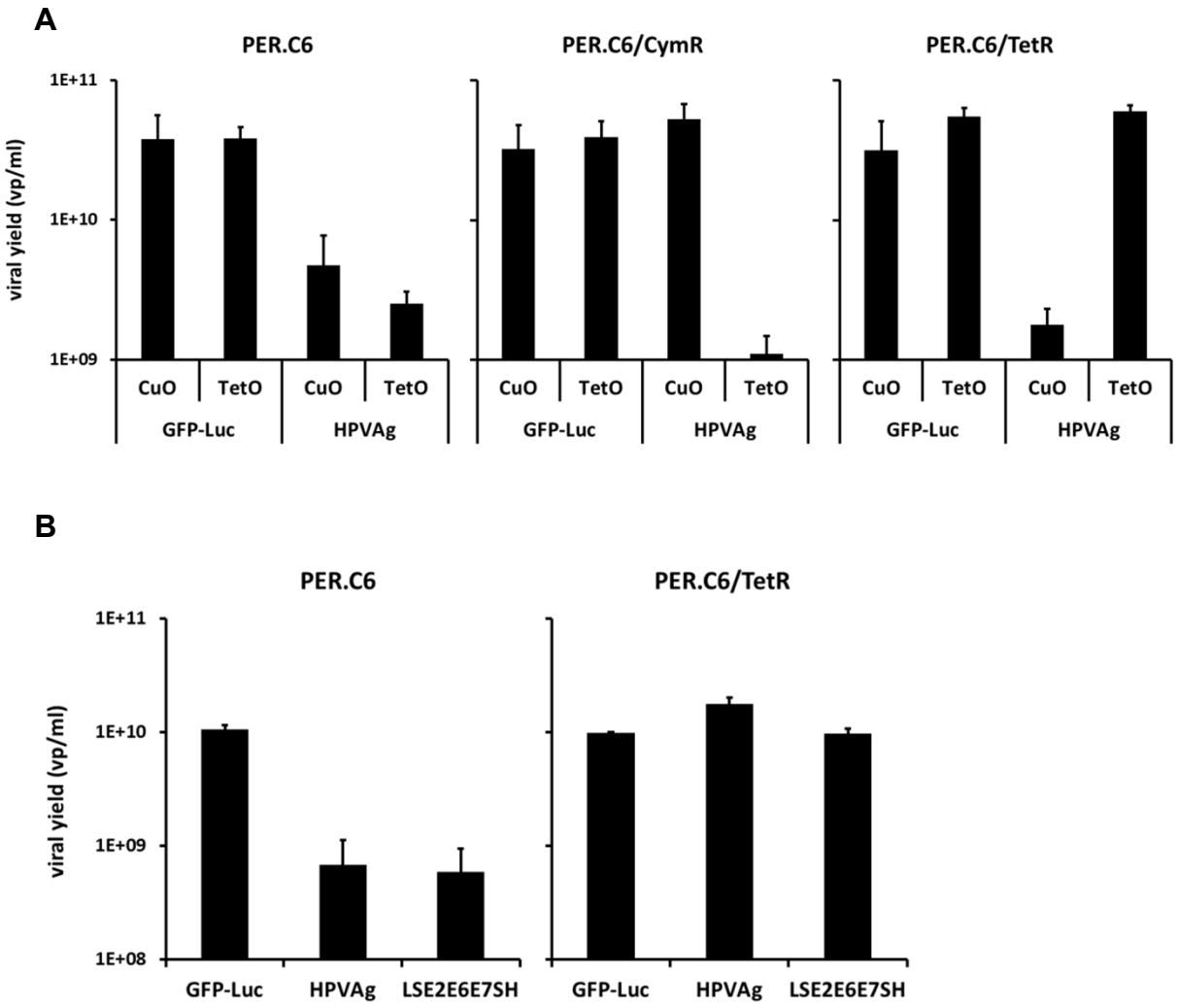

A
PER.C6
PER.C6/CymR
PER.C6/TetR
viral yield (vp/ml)
1E+11
1E+10
1E+09
CuO
TetO
CuO
TetO
GFP-Luc
HPVAg
B
PER.C6
PER.C6/TetR
viral yield (vp/ml)
1E+11
1E+10
1E+09
1E+08
GFP-Luc
HPVAg
LSE2E6E7SH

Fig. 13

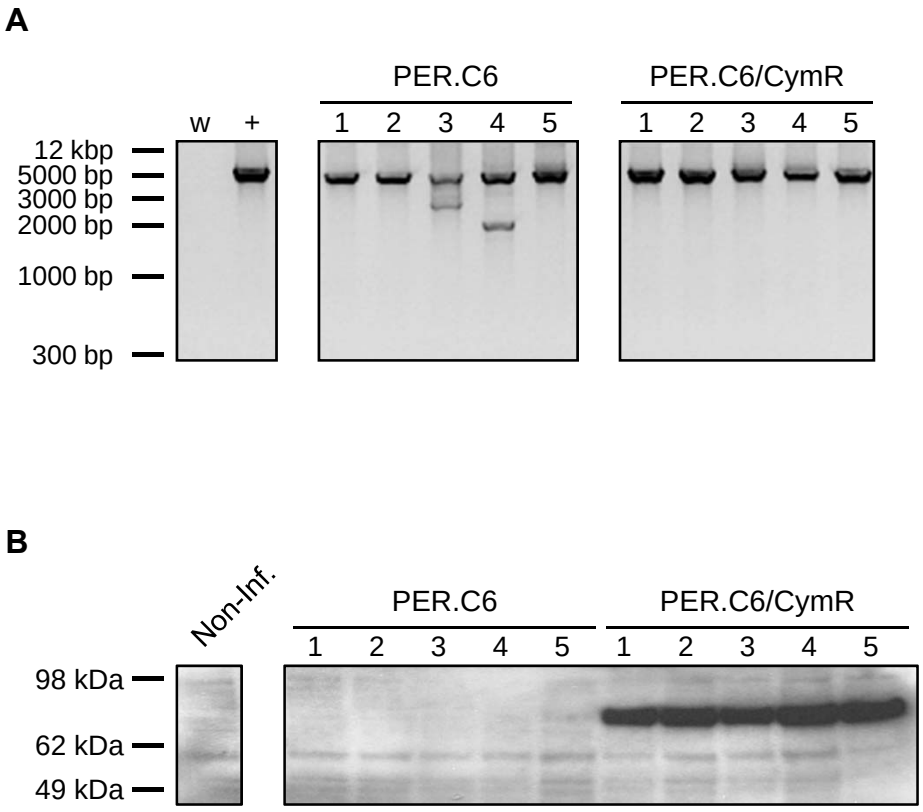

A
PER.C6
PER.C6/CymR
w
+
1
2
3
4
5
1
2
3
4
5
12 kbp
5000 bp
3000 bp
2000 bp
1000 bp
300 bp
B
Non-Inf.
PER.C6
PER.C6/CymR
1
2
3
4
5
1
2
3
4
5
98 kDa
62 kDa
49 kDa

Fig. 14

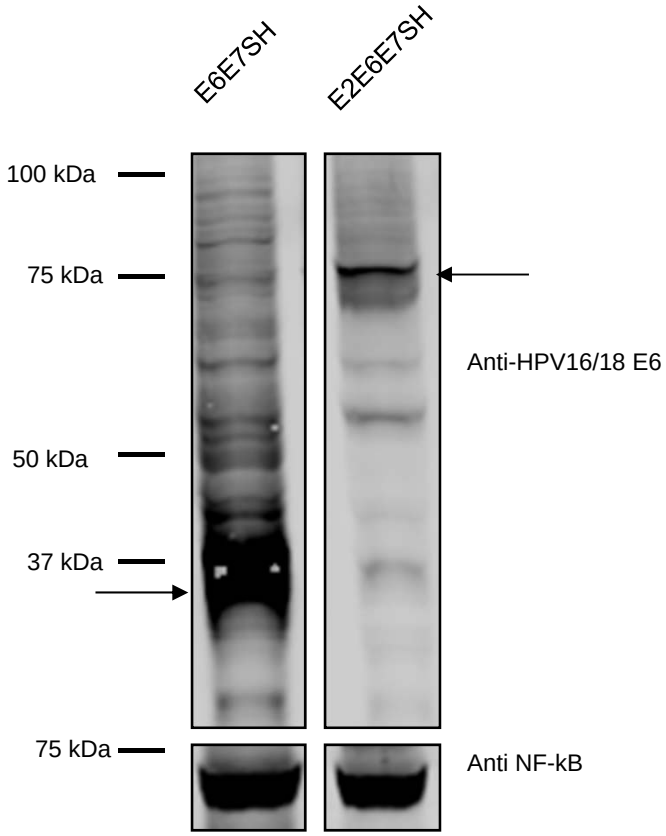

E6E7SH
E2E6E7SH
100 kDa
75 kDa
50 kDa
37 kDa
75 kDa
Anti-HPV16/18 E6
Anti NF-kB

Fig. 15

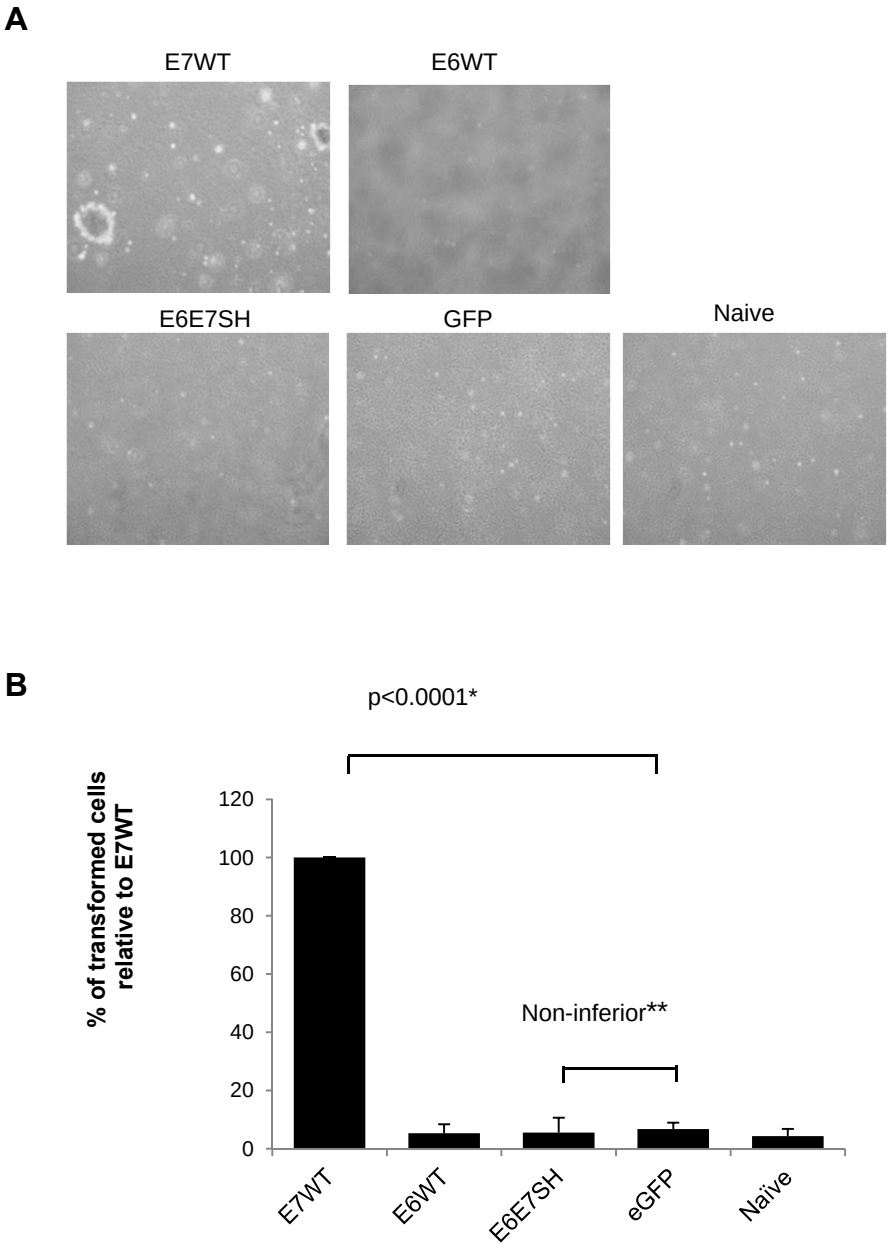

A
E7WT
E6WT
E6E7SH
GFP
Naive
B
p<0.0001*
% of transformed cells relative to E7WT
120
100
80
60
40
20
0
Non-inferior**
E7WT
E6WT
E6E7SH
eGFP
Naïve

Fig. 16

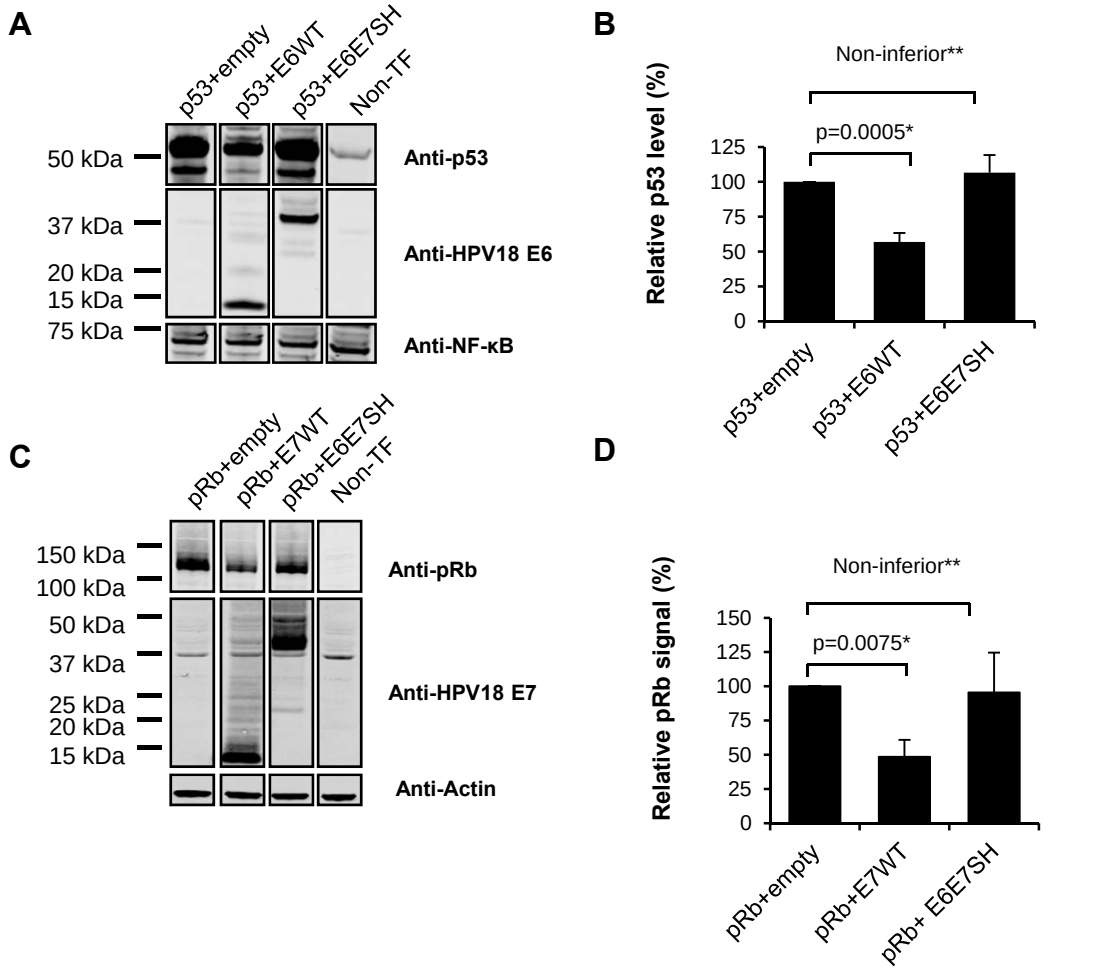

A
p53+empty
p53+E6WT
p53+E6E7SH
Non-TF
50 kDa
37 kDa
20 kDa
15 kDa
75 kDa
Anti-p53
Anti-HPV18 E6
Anti-NF-κB
B
Non-inferior**
p=0.0005*
Relative p53 level (%)
125
100
75
50
25
0
p53+empty
p53+E6WT
p53+E6E7SH
C
pRb+empty
pRb+E7WT
pRb+E6E7SH
Non-TF
150 kDa
100 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
Anti-pRb
Anti-HPV18 E7
Anti-Actin
D
Non-inferior**
p=0.0075*
Relative pRb signal (%)
150
125
100
75
50
25
0
pRb+empty
pRb+E7WT
pRb+ E6E7SH

**Fig. 17**

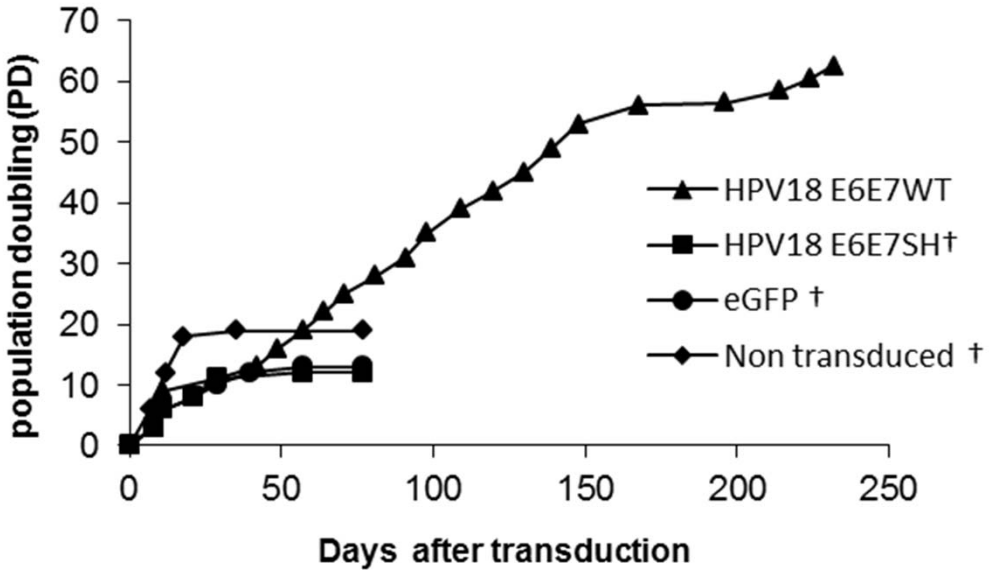

population doubling (PD)
70
60
50
40
30
20
10
0
0
50
100
150
200
250
Days after transduction
HPV18 E6E7WT
HPV18 E6E7SH†
eGFP †
Non transduced †

Fig. 18

A

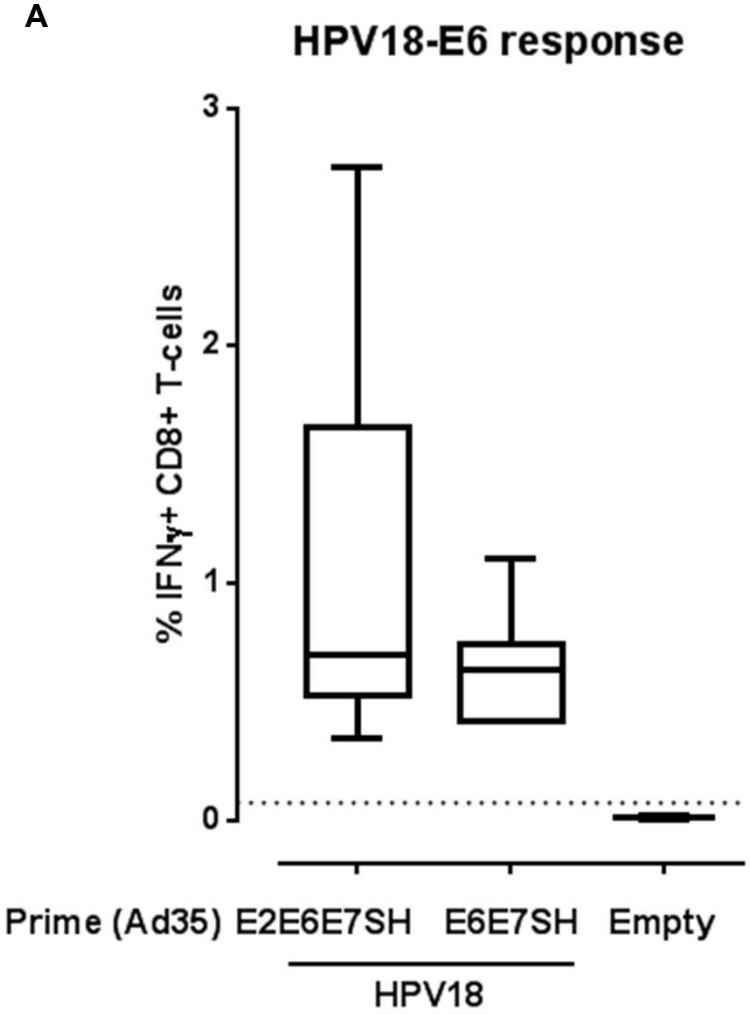

HPV18-E6 response
% IFNγ+ CD8+ T-cells
3
2
1
0
Prime (Ad35)
E2E6E7SH
E6E7SH
Empty
HPV18

Fig. 19

B

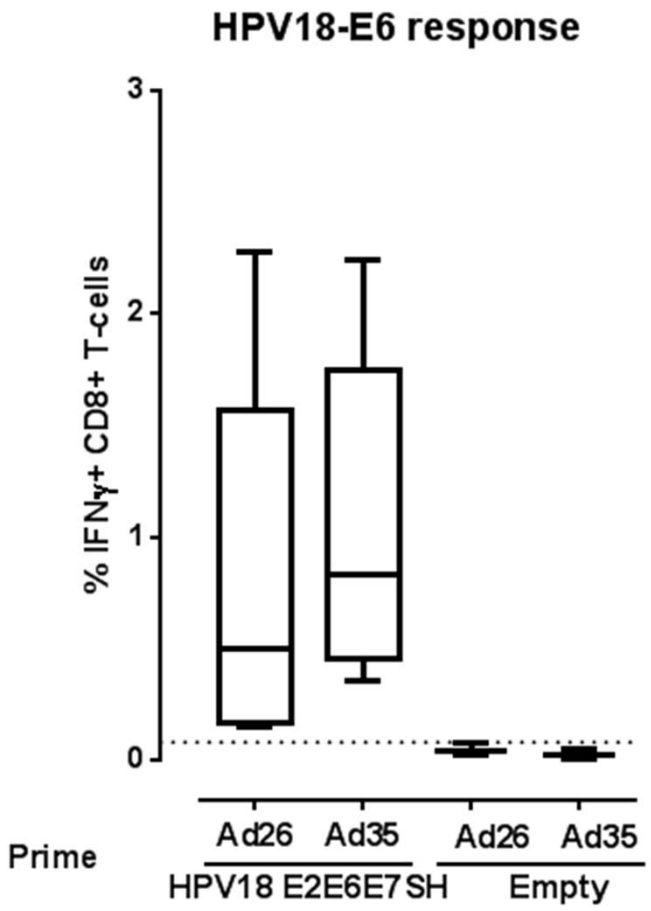



Fig. 19 - continued

A

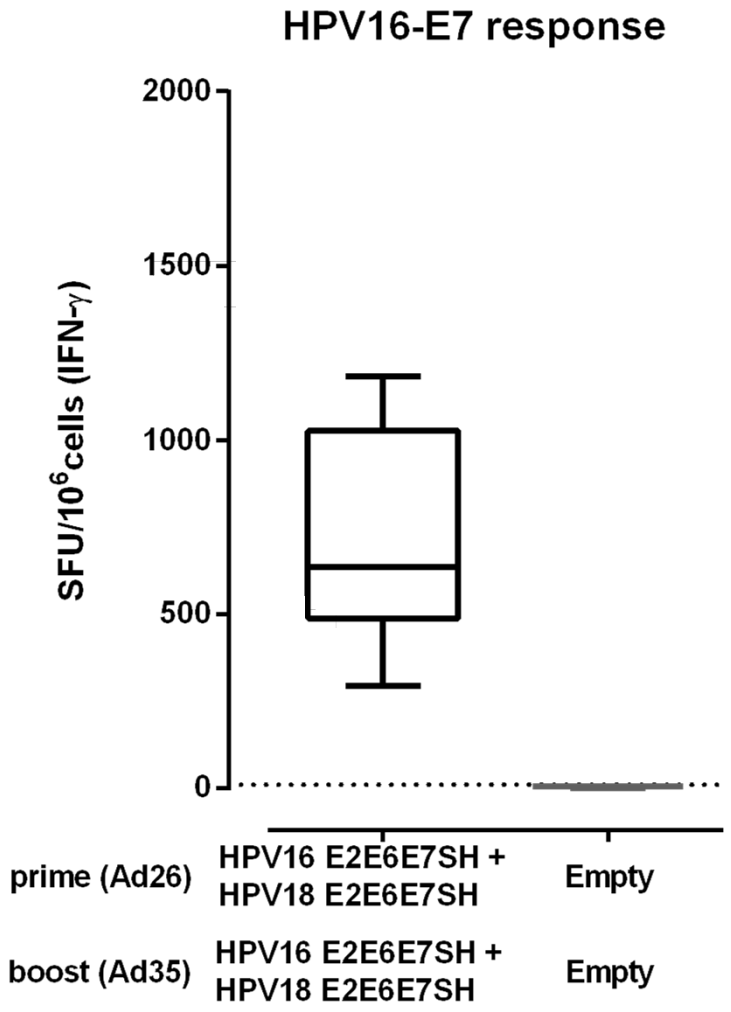

HPV16-E7 response
2000
1500
1000
500
0
SFU/10^6cells (IFN-γ)
prime (Ad26)
HPV16 E2E6E7SH + HPV18 E2E6E7SH
Empty
boost (Ad35)
HPV16 E2E6E7SH + HPV18 E2E6E7SH
Empty

Fig. 20

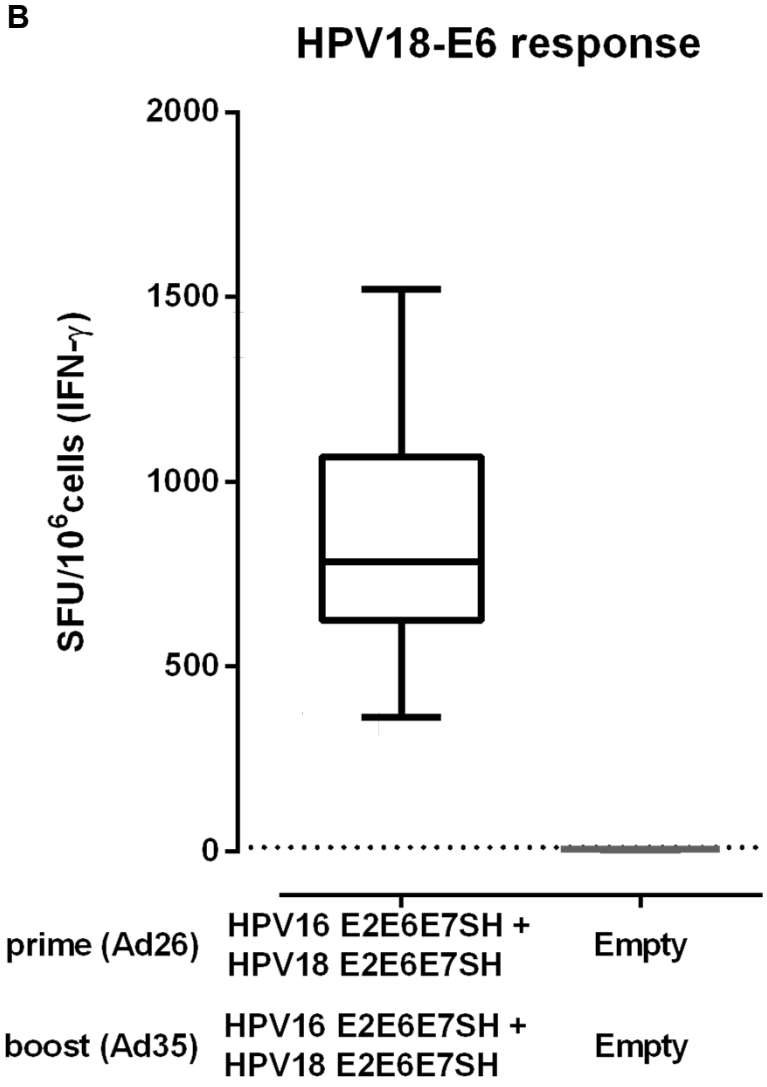



**Fig. 20 – continued**

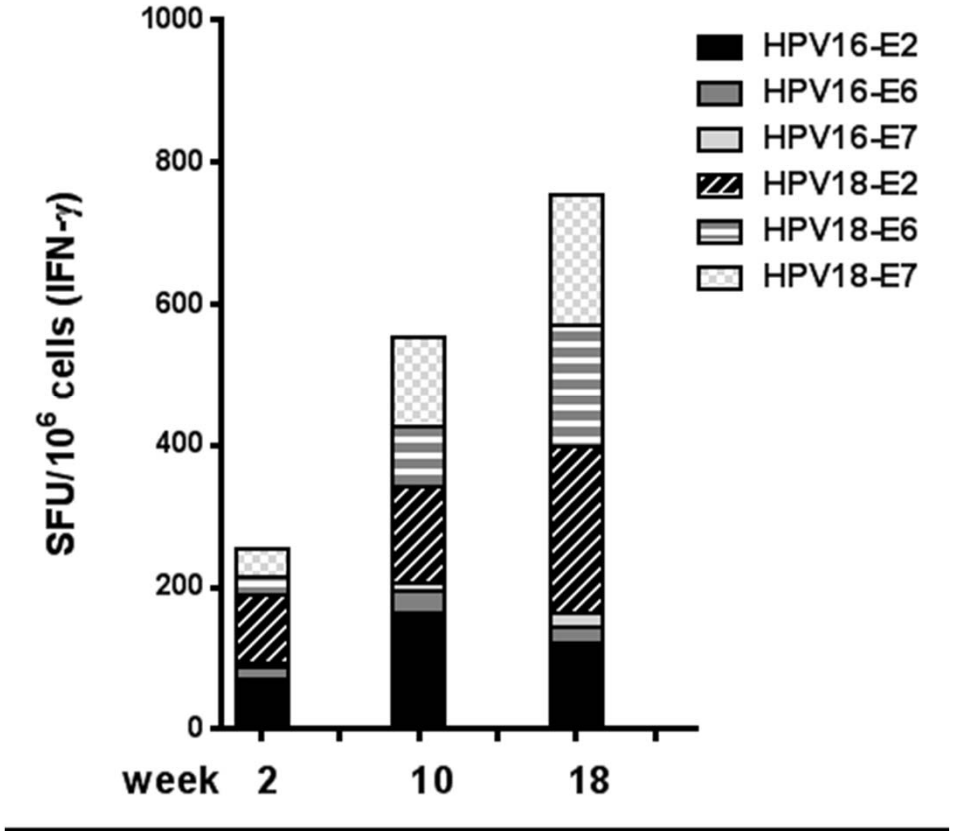

1000
800
600
400
200
0
SFU/10^6 cells (IFN-γ)
week 2
10
18
HPV16-E2
HPV16-E6
HPV16-E7
HPV18-E2
HPV18-E6
HPV18-E7

Fig. 21

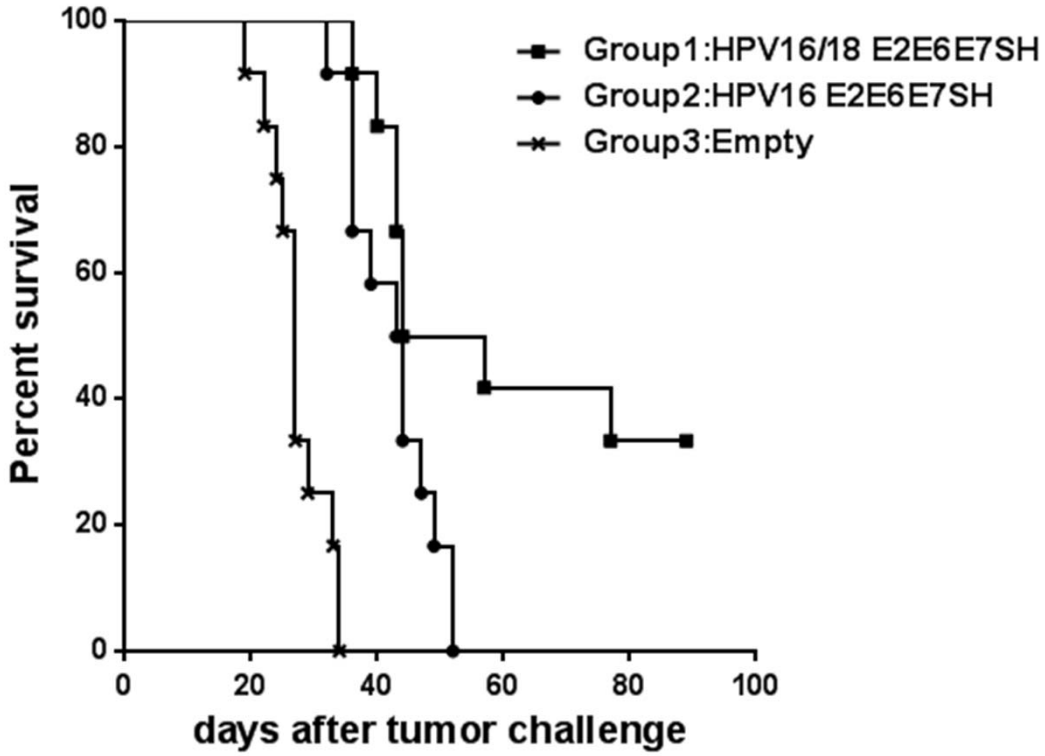
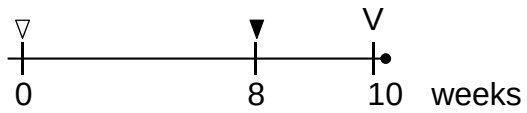

Group1:HPV16/18 E2E6E7SH
Group2:HPV16 E2E6E7SH
Group3:Empty
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
days after tumor challenge

Fig. 22

**A**




▽ prime immunization
▼ boost immunization
V sacrifice

**B**

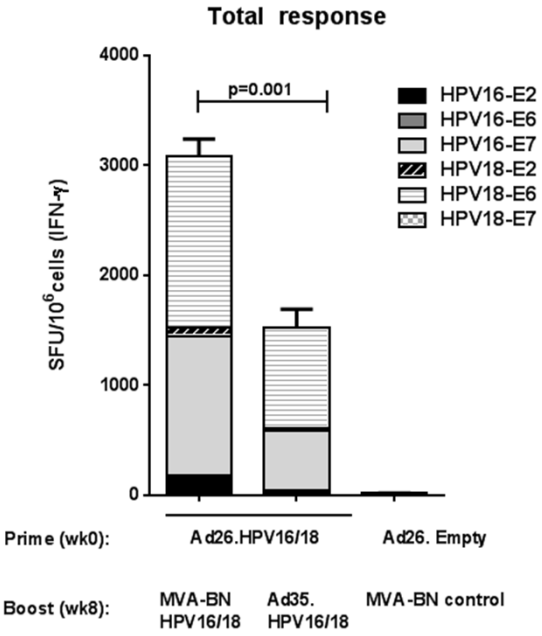



**Fig. 23**

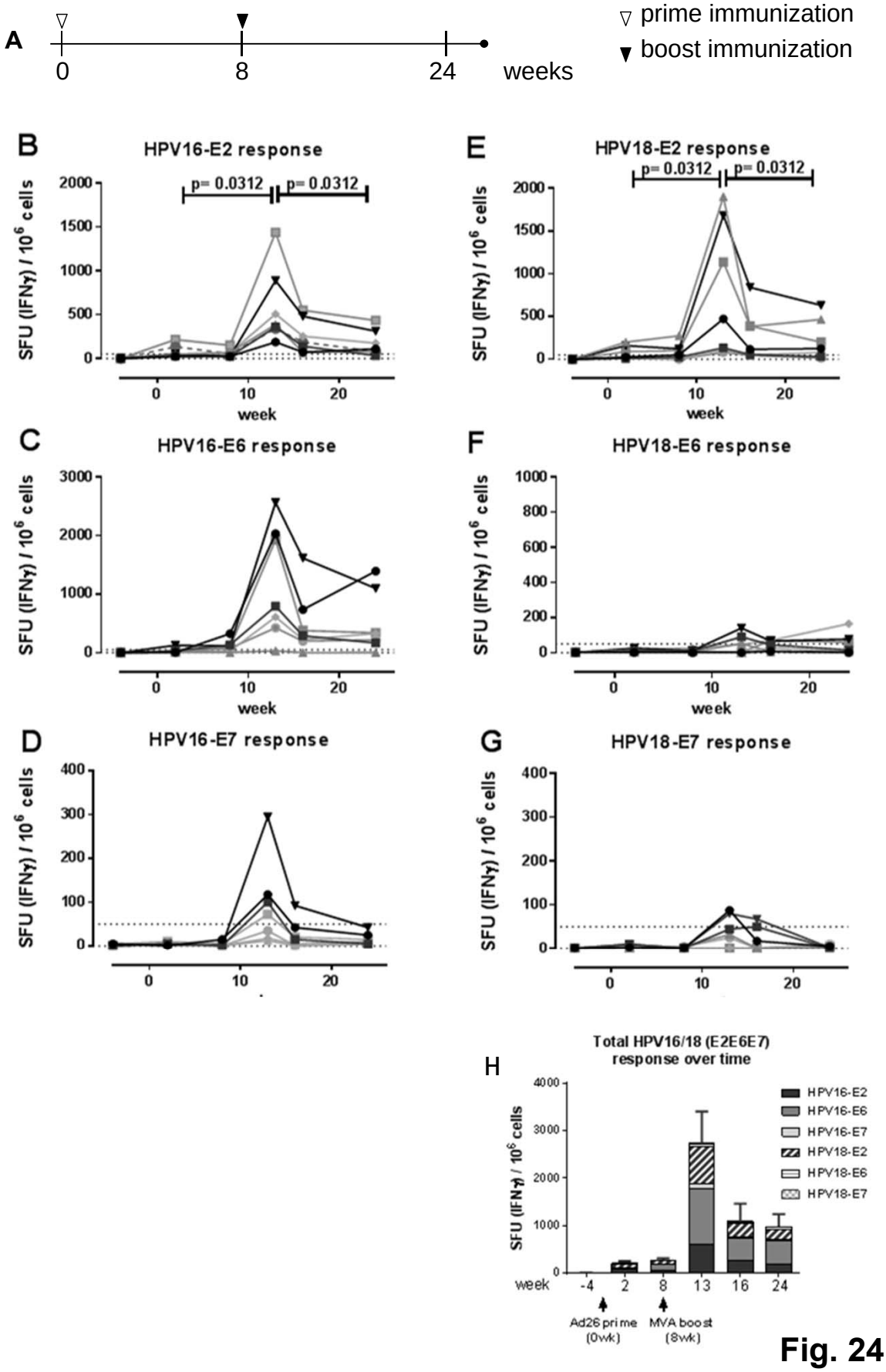

A
0
8
24
weeks
prime immunization
boost immunization
B
HPV16-E2 response
p= 0.0312
p= 0.0312
SFU (IFNγ) / 10^6 cells
week
E
HPV18-E2 response
p= 0.0312
p= 0.0312
C
HPV16-E6 response
F
HPV18-E6 response
D
HPV16-E7 response
G
HPV18-E7 response
H
Total HPV16/18 (E2E6E7) response over time
HPV16-E2
HPV16-E6
HPV16-E7
HPV18-E2
HPV18-E6
HPV18-E7
week
-4
2
8
13
16
24
Ad26 prime (0wk)
MVA boost (8wk)

Fig. 24

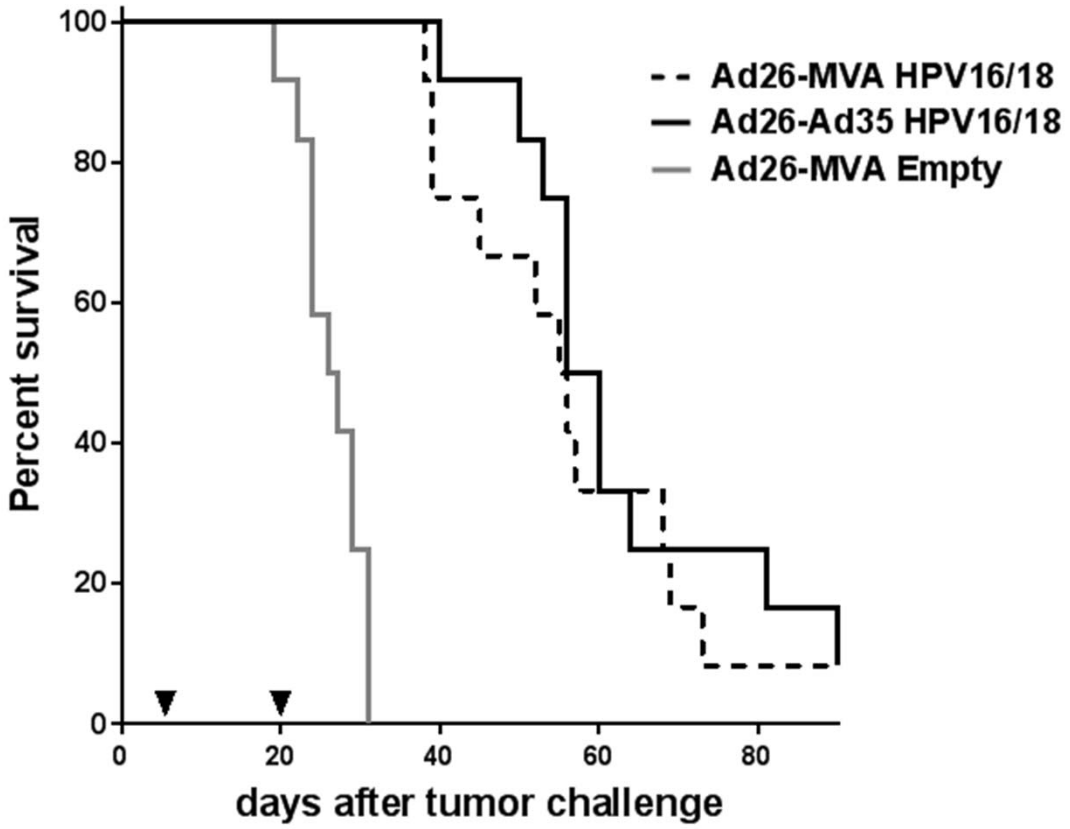

Ad26-MVA HPV16/18
Ad26-Ad35 HPV16/18
Ad26-MVA Empty
Percent survival
100
80
60
40
20
0
0
20
40
60
80
days after tumor challenge

Fig. 25