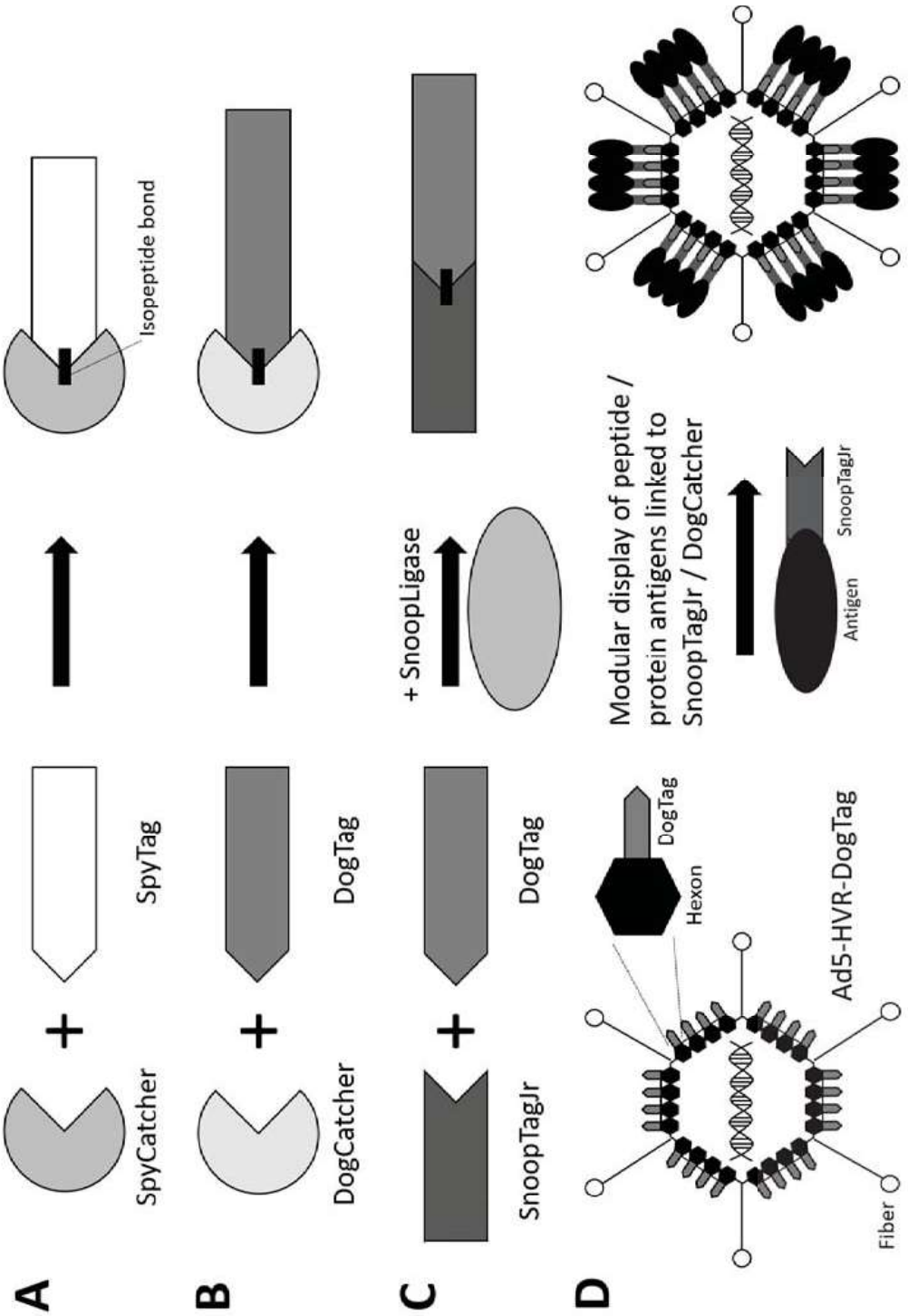

A
SpyCatcher
SpyTag
Isopeptide bond
B
DogCatcher
DogTag
C
SnoopTagJr
DogTag
+ SnoopLigase
D
Ad5-HVR-DogTag
Hexon
DogTag
Fiber
Modular display of peptide / protein antigens linked to SnoopTagJr / DogCatcher
Antigen
SnoopTagJr

Fig. 1

**E**

= HVR regions (Rux *et al*, 2003)

= Deleted in hexon HVR modified vectors

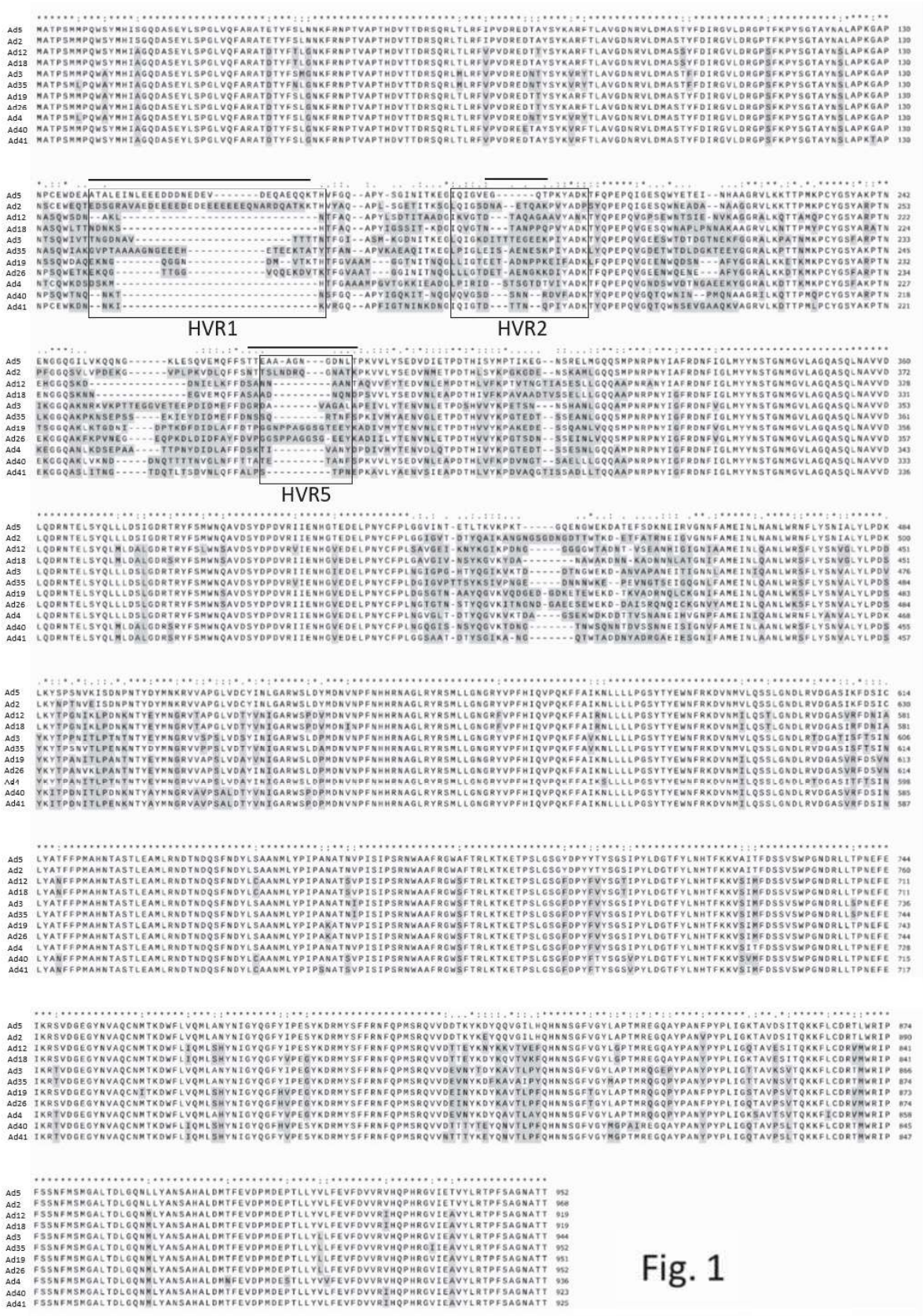



Fig. 1

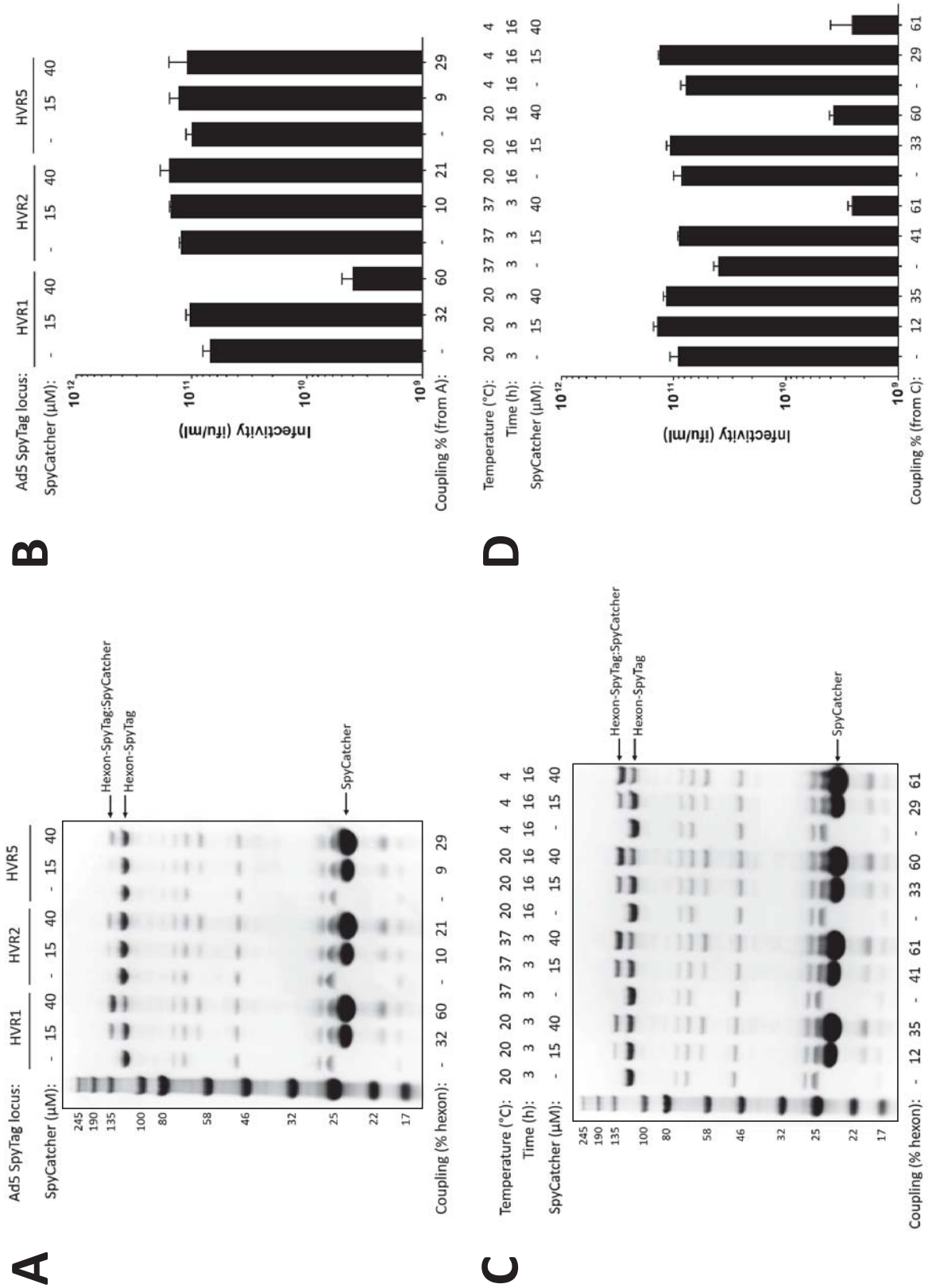
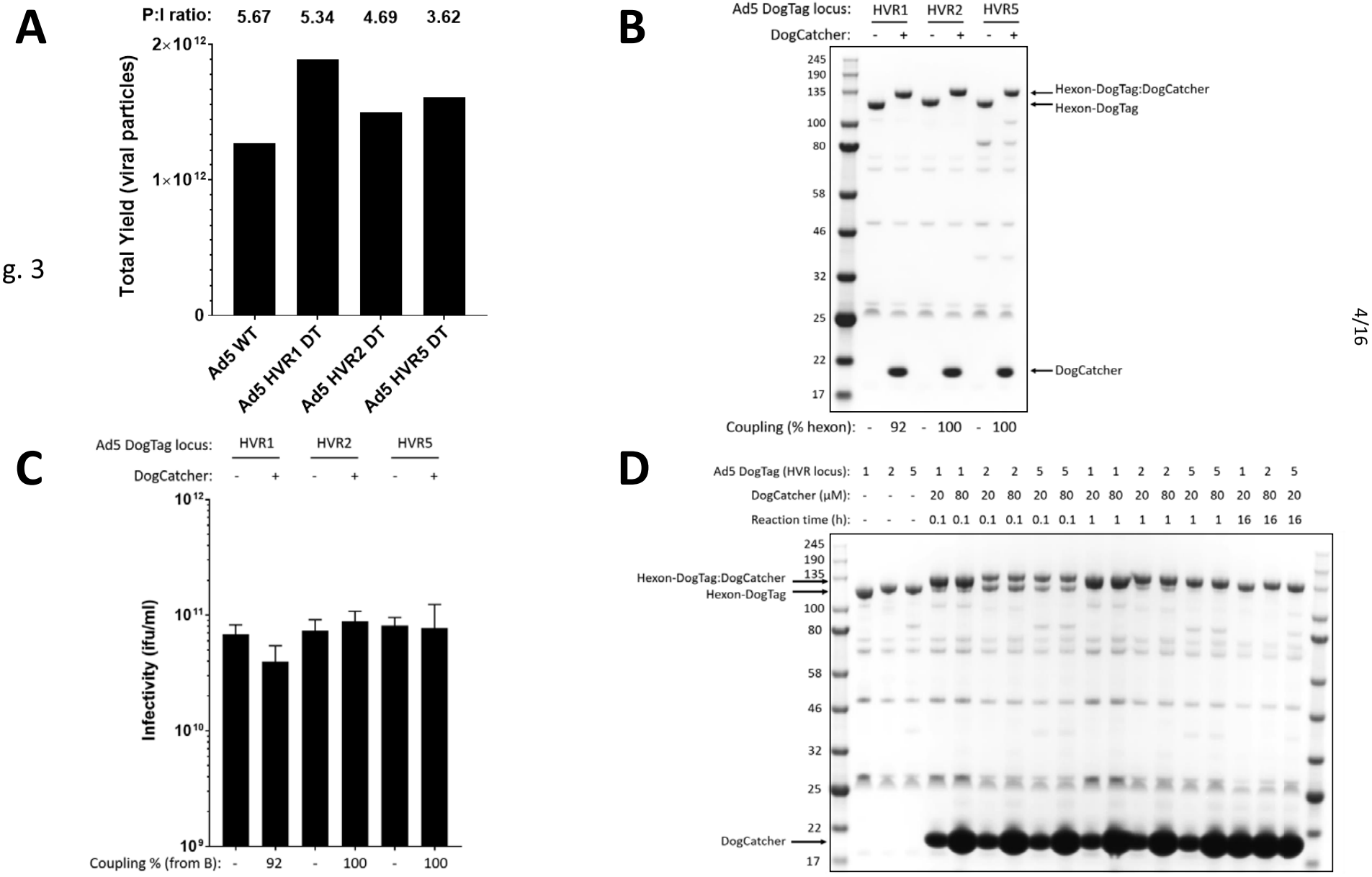

A
Ad5 SpyTag locus:
HVR1
HVR2
HVR5
SpyCatcher (μM):
Hexon-SpyTag:SpyCatcher
Hexon-SpyTag
SpyCatcher
Coupling (% hexon):
B
Infectivity (ifu/ml)
Coupling % (from A):
C
Temperature (°C):
Time (h):
D
Coupling % (from C):

Fig. 2

Fig. 3


A
P:I ratio: 5.67 5.34 4.69 3.62
Total Yield (viral particles)
2×10^12
1×10^12
0
Ad5 WT
Ad5 HVR1 DT
Ad5 HVR2 DT
Ad5 HVR5 DT
B
Ad5 DogTag locus: HVR1 HVR2 HVR5
DogCatcher: - + - + - +
245
190
135
100
80
58
46
32
25
22
17
Hexon-DogTag:DogCatcher
Hexon-DogTag
DogCatcher
Coupling (% hexon): - 92 - 100 - 100
C
Ad5 DogTag locus: HVR1 HVR2 HVR5
DogCatcher: - + - + - +
Infectivity (ifu/ml)
10^12
10^11
10^10
10^9
Coupling % (from B): - 92 - 100 - 100
D
Ad5 DogTag (HVR locus): 1 2 5 1 1 2 2 5 5 1 1 2 2 5 5 1 2 5
DogCatcher (μM): - - - 20 80 20 80 20 80 20 80 20 80 20 80 20 80 20
Reaction time (h): - - - 0.1 0.1 0.1 0.1 0.1 0.1 1 1 1 1 1 1 16 16 16
245
190
135
100
80
58
46
32
25
22
17
Hexon-DogTag:DogCatcher
Hexon-DogTag
DogCatcher

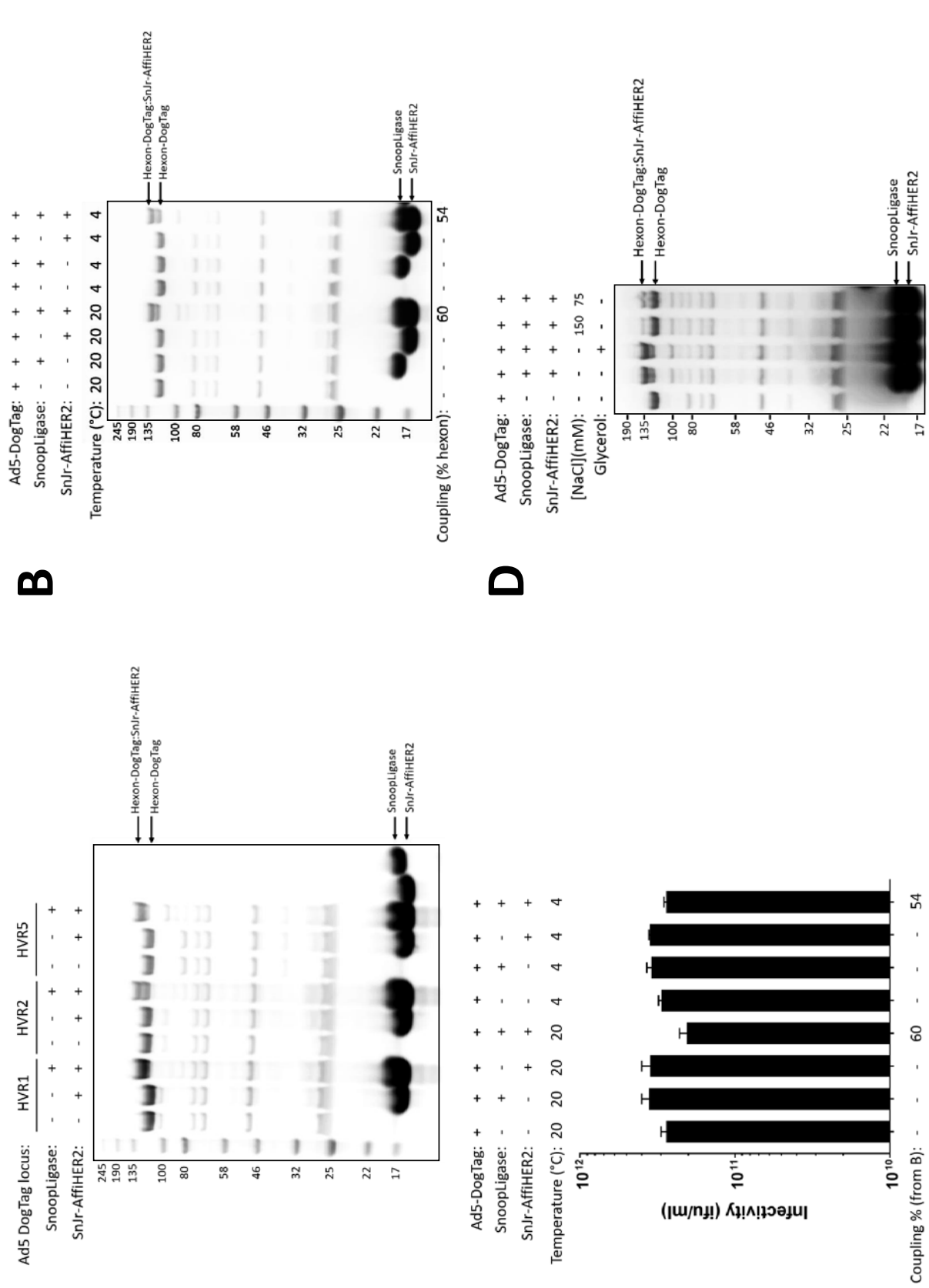

A
Ad5 DogTag locus:
HVR1
HVR2
HVR5
SnoopLigase:
SnJr-AffiHER2:
Hexon-DogTag:SnJr-AffiHER2
Hexon-DogTag
SnoopLigase
SnJr-AffiHER2
245
190
135
100
80
58
46
32
25
22
17
B
Ad5-DogTag:
SnoopLigase:
SnJr-AffiHER2:
Temperature (°C):
Coupling (% hexon):
C
Ad5-DogTag:
SnoopLigase:
SnJr-AffiHER2:
Temperature (°C):
Infectivity (ifu/ml)
Coupling % (from B):
D
Ad5-DogTag:
SnoopLigase:
SnJr-AffiHER2:
[NaCl](mM):
Glycerol:

Fig. 4

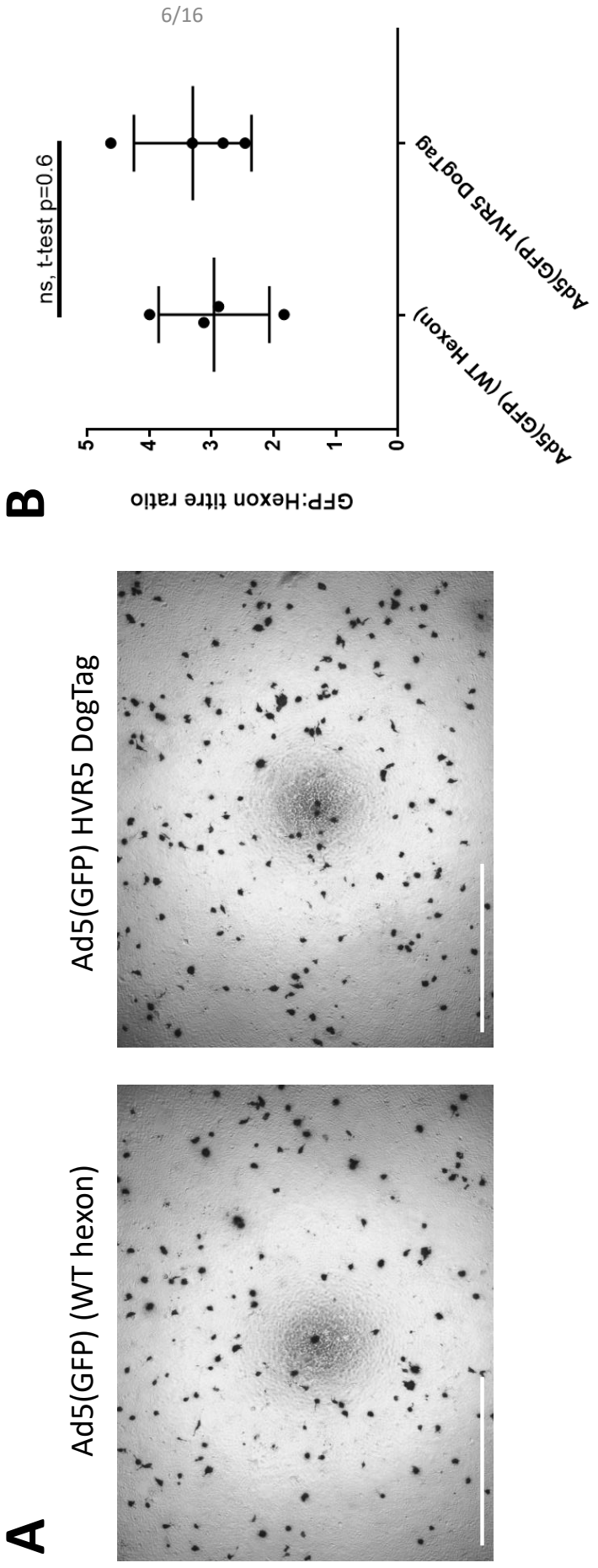

A
Ad5(GFP) (WT hexon)
Ad5(GFP) HVR5 DogTag
B
GFP:Hexon titre ratio
0
1
2
3
4
5
ns, t-test p=0.6
Ad5(GFP) (WT Hexon)
Ad5(GFP) HVR5 DogTag

Fig. 5

Fig. 6

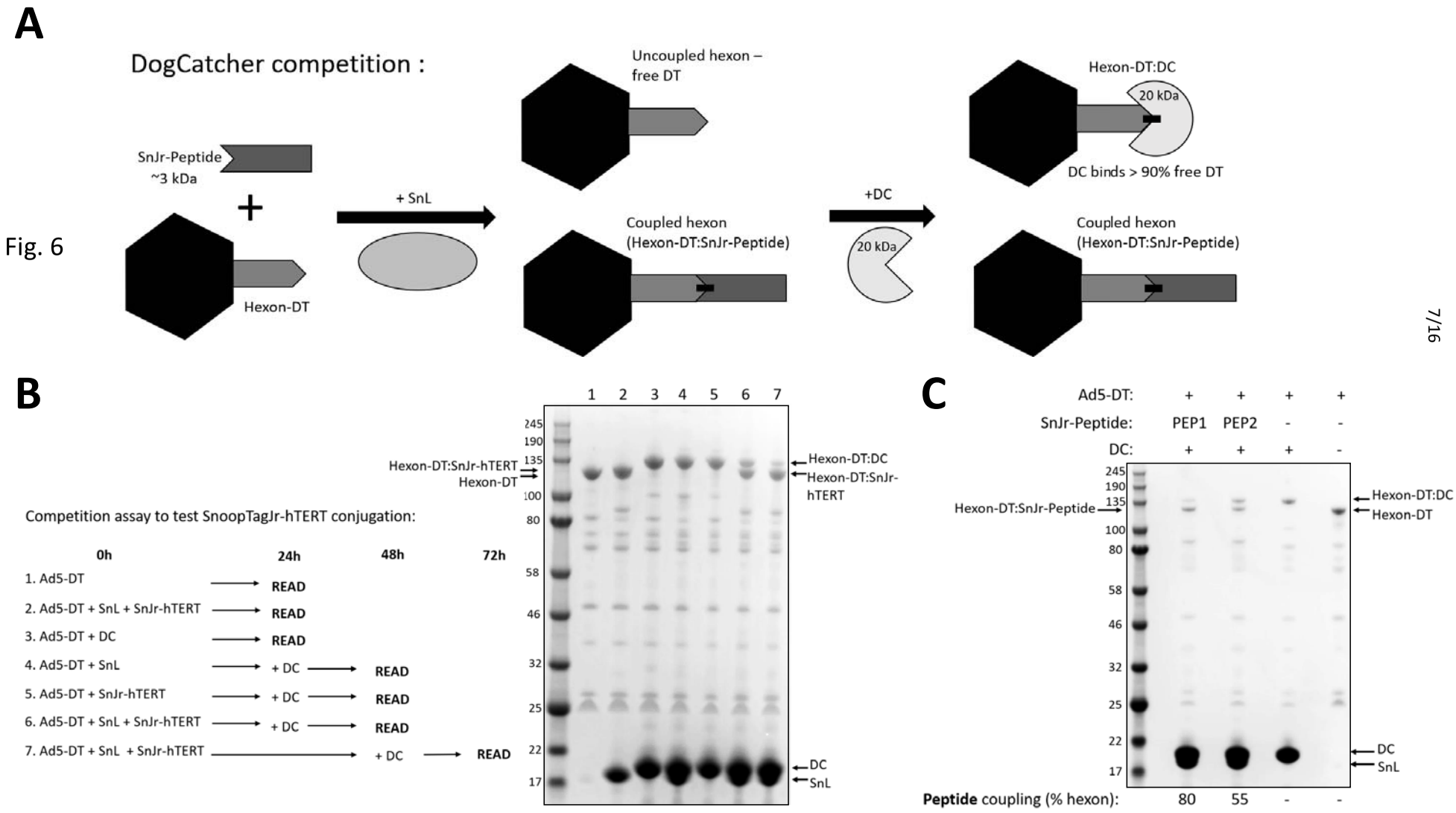
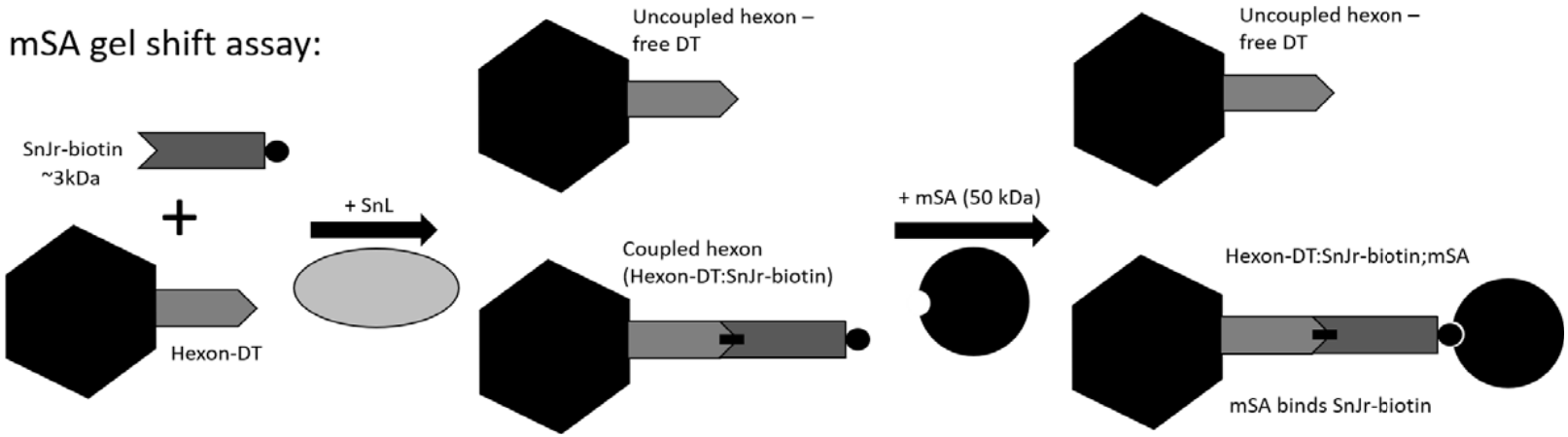
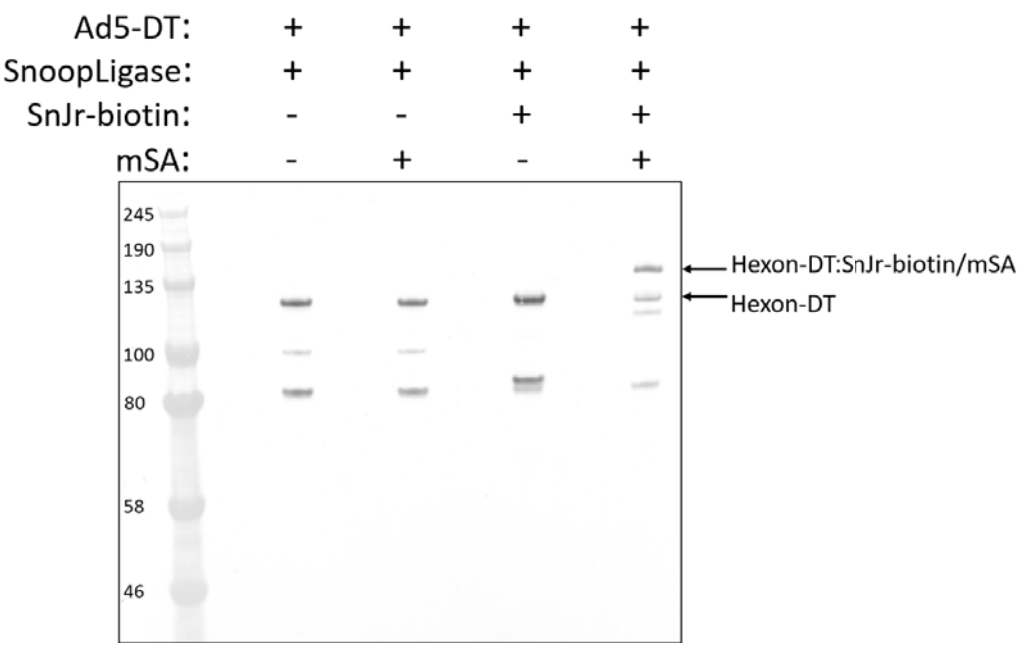

A
DogCatcher competition :
SnJr-Peptide
~3 kDa
Hexon-DT
+ SnL
Uncoupled hexon –
free DT
Coupled hexon
(Hexon-DT:SnJr-Peptide)
+DC
20 kDa
Hexon-DT:DC
20 kDa
DC binds > 90% free DT
Coupled hexon
(Hexon-DT:SnJr-Peptide)
B
Competition assay to test SnoopTagJr-hTERT conjugation:
0h
24h
48h
72h
1. Ad5-DT
READ
2. Ad5-DT + SnL + SnJr-hTERT
READ
3. Ad5-DT + DC
READ
4. Ad5-DT + SnL
+ DC
READ
5. Ad5-DT + SnJr-hTERT
+ DC
READ
6. Ad5-DT + SnL + SnJr-hTERT
+ DC
READ
7. Ad5-DT + SnL + SnJr-hTERT
+ DC
READ
1 2 3 4 5 6 7
245
190
135
100
80
58
46
32
25
22
17
Hexon-DT:SnJr-hTERT
Hexon-DT
Hexon-DT:DC
Hexon-DT:SnJr-
hTERT
DC
SnL
C
Ad5-DT: + + + +
SnJr-Peptide: PEP1 PEP2 - -
DC: + + + -
245
190
135
100
80
58
46
32
25
22
17
Hexon-DT:SnJr-Peptide
Hexon-DT:DC
Hexon-DT
DC
SnL
Peptide coupling (% hexon): 80 55 - -

Fig. 6

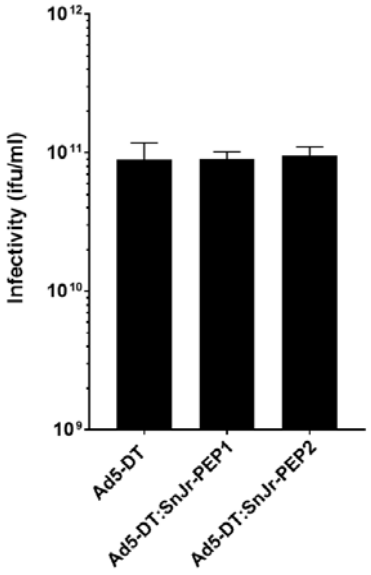
**D**


mSA gel shift assay:
SnJr-biotin
~3kDa
+
Hexon-DT
+ SnL
Uncoupled hexon –
free DT
Coupled hexon
(Hexon-DT:SnJr-biotin)
+ mSA (50 kDa)
Uncoupled hexon –
free DT
Hexon-DT:SnJr-biotin;mSA
mSA binds SnJr-biotin

**E**


Ad5-DT: + + + +
SnoopLigase: + + + +
SnJr-biotin: - - + +
mSA: - + - +
245
190
135
100
80
58
46
Hexon-DT:SnJr-biotin/mSA
Hexon-DT

**F**


Infectivity (ifu/ml)
10^12
10^11
10^10
10^9
Ad5-DT
Ad5-DT:SnJr-PEP1
Ad5-DT:SnJr-PEP2

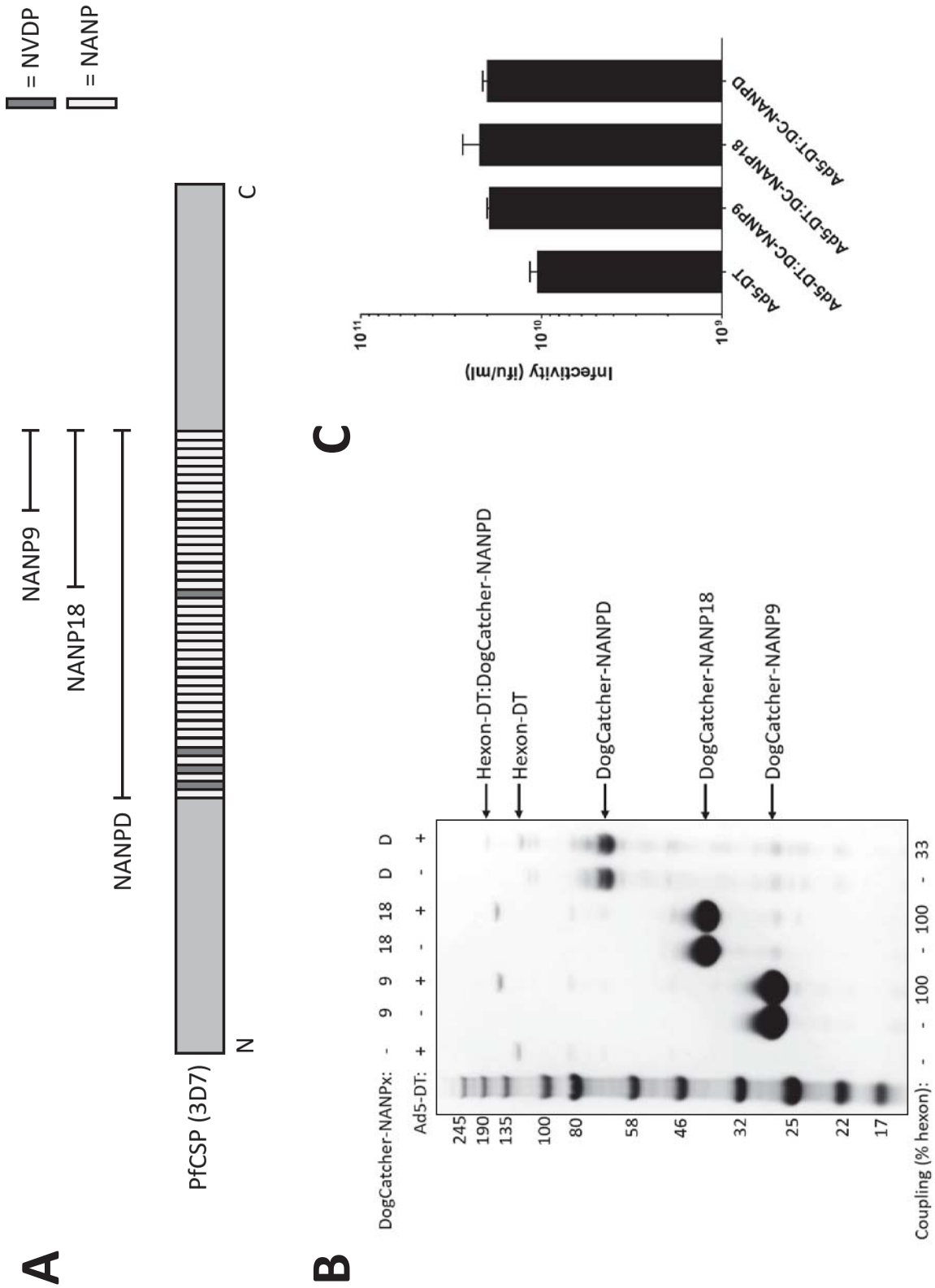

A
= NVDP
= NANP
NANP9
NANP18
NANPD
PfCSP (3D7)
N
C
B
DogCatcher-NANPx: - 9 9 18 18 D D
Ad5-DT: + - + - + - +
245
190
135
100
80
58
46
32
25
22
17
Hexon-DT:DogCatcher-NANPD
Hexon-DT
DogCatcher-NANPD
DogCatcher-NANP18
DogCatcher-NANP9
Coupling (% hexon): - - 100 - 100 - 33
C
Infectivity (ifu/ml)
$10^{11}$
$10^{10}$
$10^{9}$
Ad5-DT
Ad5-DT:DC-NANP9
Ad5-DT:DC-NANP18
Ad5-DT:DC-NANPD

Fig. 7

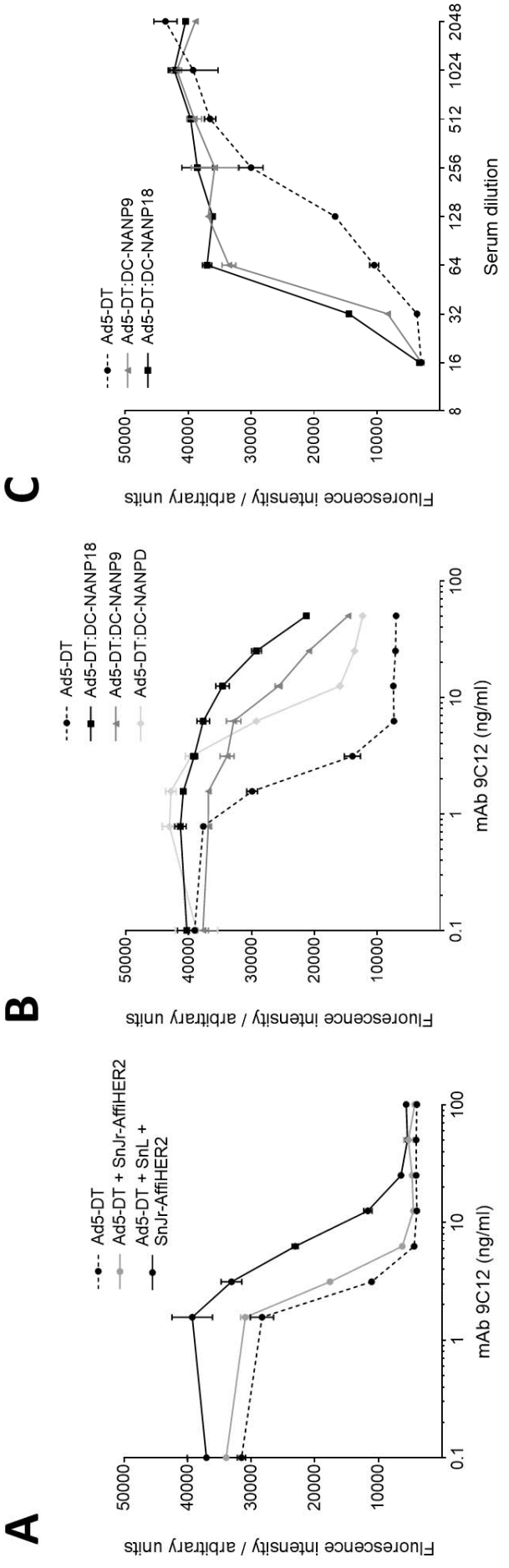

A
Fluorescence intensity / arbitrary units
50000
40000
30000
20000
10000
0.1
1
10
100
mAb 9C12 (ng/ml)
Ad5-DT
Ad5-DT + SnJr-AffiHER2
Ad5-DT + SnL + SnJr-AffiHER2
B
Fluorescence intensity / arbitrary units
50000
40000
30000
20000
10000
0.1
1
10
100
mAb 9C12 (ng/ml)
Ad5-DT
Ad5-DT:DC-NANP18
Ad5-DT:DC-NANP9
Ad5-DT:DC-NANPD
C
Fluorescence intensity / arbitrary units
50000
40000
30000
20000
10000
8
16
32
64
128
256
512
1024
2048
Serum dilution
Ad5-DT
Ad5-DT:DC-NANP9
Ad5-DT:DC-NANP18

Fig. 8

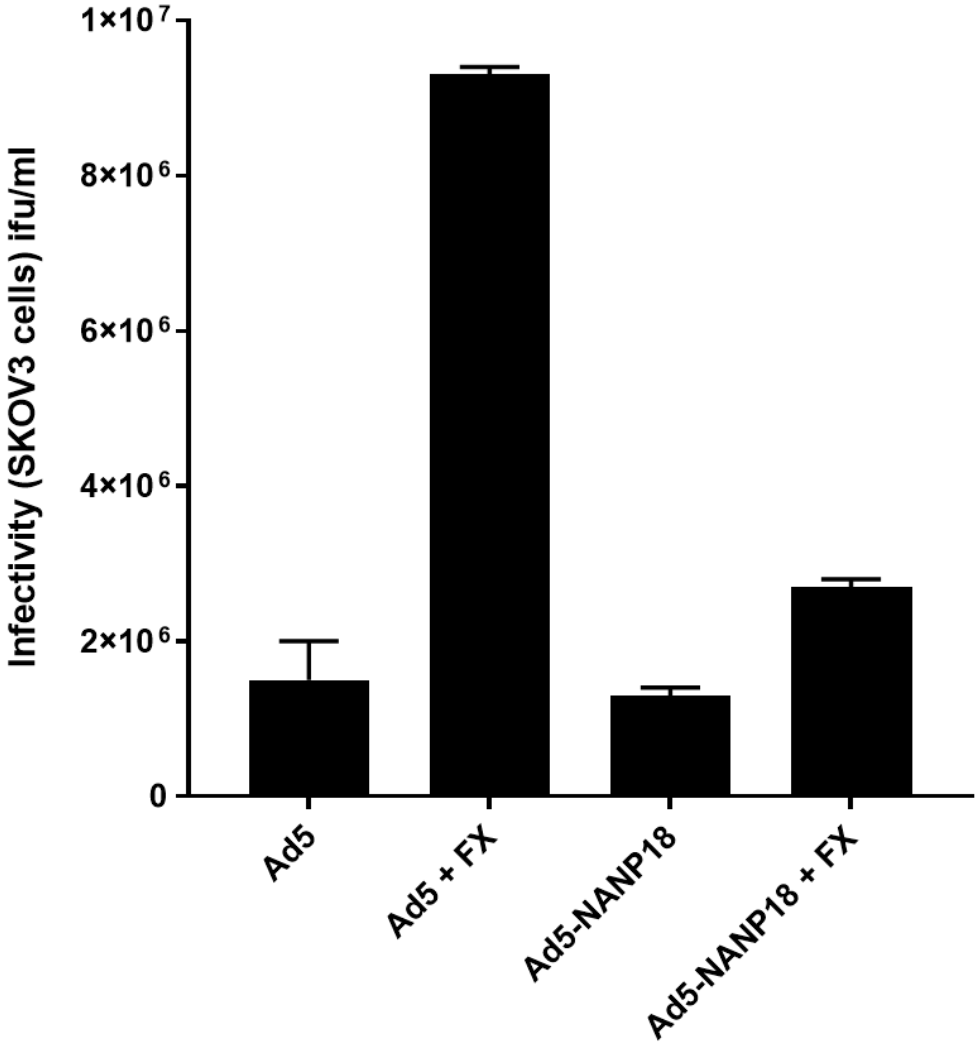

Infectivity (SKOV3 cells) ifu/ml
1×10^7
8×10^6
6×10^6
4×10^6
2×10^6
0
Ad5
Ad5 + FX
Ad5-NANP18
Ad5-NANP18 + FX

Fig. 9

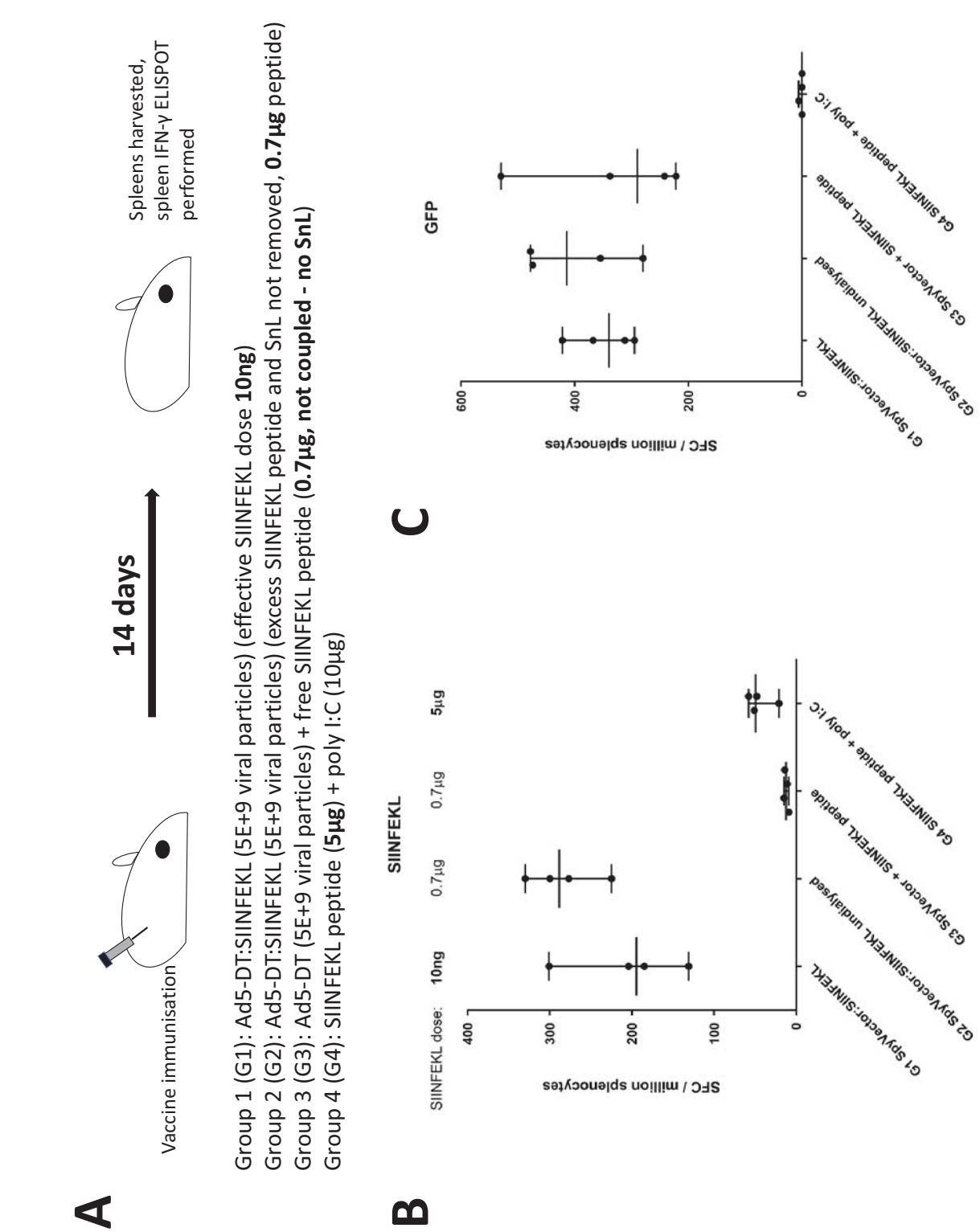
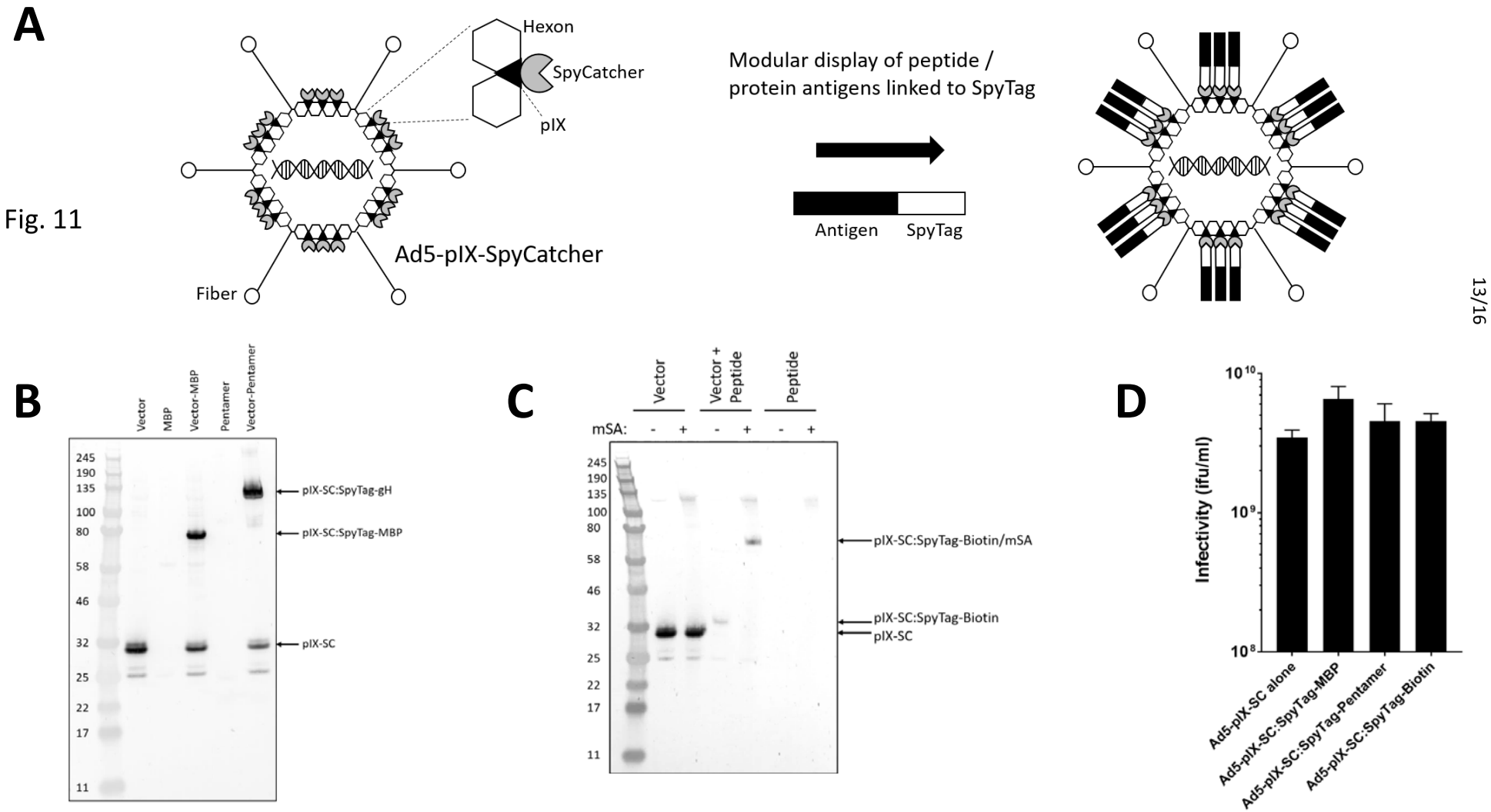

A
Vaccine immunisation
14 days
Spleens harvested, spleen IFN-γ ELISPOT performed
Group 1 (G1): Ad5-DT:SIINFEKL (5E+9 viral particles) (effective SIINFEKL dose 10ng)
Group 2 (G2): Ad5-DT:SIINFEKL (5E+9 viral particles) (excess SIINFEKL peptide and SnL not removed, 0.7µg peptide)
Group 3 (G3): Ad5-DT (5E+9 viral particles) + free SIINFEKL peptide (0.7µg, not coupled - no SnL)
Group 4 (G4): SIINFEKL peptide (5µg) + poly I:C (10µg)
B
SIINFEKL
SIINFEKL dose: 10ng 0.7µg 0.7µg 5µg
SFC / million splenocytes
400
300
200
100
0
G1 SpyVector:SIINFEKL
G2 SpyVector:SIINFEKL undialysed
G3 SpyVector + SIINFEKL peptide
G4 SIINFEKL peptide + poly I:C
C
GFP
SFC / million splenocytes
600
400
200
0
G1 SpyVector:SIINFEKL
G2 SpyVector:SIINFEKL undialysed
G3 SpyVector + SIINFEKL peptide
G4 SIINFEKL peptide + poly I:C

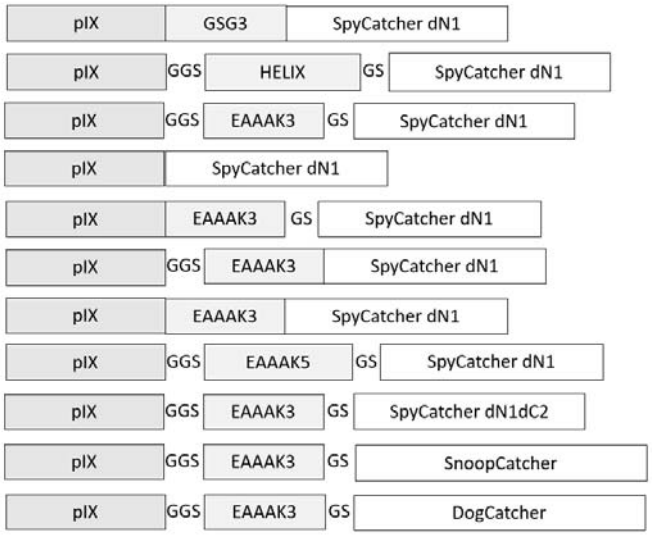
Fig. 10

Fig. 11


A
Hexon
SpyCatcher
pIX
Fiber
Ad5-pIX-SpyCatcher
Modular display of peptide / protein antigens linked to SpyTag
Antigen
SpyTag
B
Vector
MBP
Vector-MBP
Pentamer
Vector-Pentamer
245
190
135
100
80
58
46
32
25
22
17
11
pIX-SC:SpyTag-gH
pIX-SC:SpyTag-MBP
pIX-SC
C
Vector
Vector + Peptide
Peptide
mSA: - + - + - +
245
190
135
100
80
58
46
32
25
22
17
11
pIX-SC:SpyTag-Biotin/mSA
pIX-SC:SpyTag-Biotin
pIX-SC
D
Infectivity (ifu/ml)
10^10
10^9
10^8
Ad5-pIX-SC alone
Ad5-pIX-SC:SpyTag-MBP
Ad5-pIX-SC:SpyTag-Pentamer
Ad5-pIX-SC:SpyTag-Biotin

**A**

**pIX-Catcher construct**




| Viability | Genetic stability (>3 passages) |
|---|---|
| No | - |
| No | - |
| **Yes** | No |
| No | - |
| No | - |
| Poor | - |
| No | - |
| No | - |
| No | - |
| **Yes** | **Yes** |
| **Yes** | **Yes** |

**B**

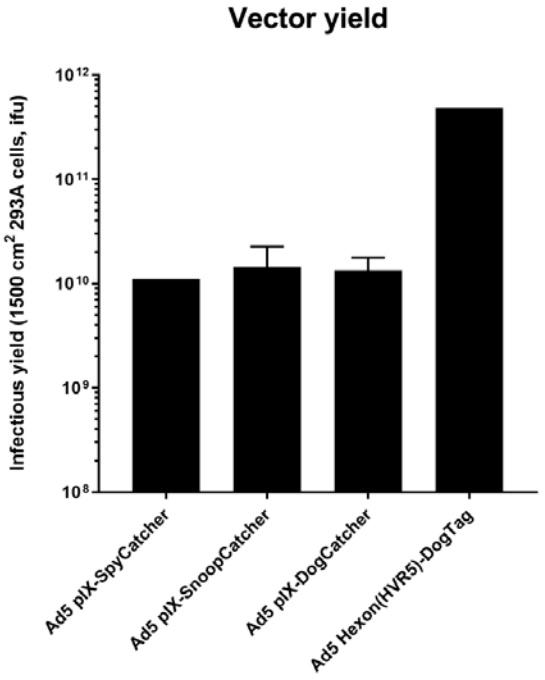



Fig. 12

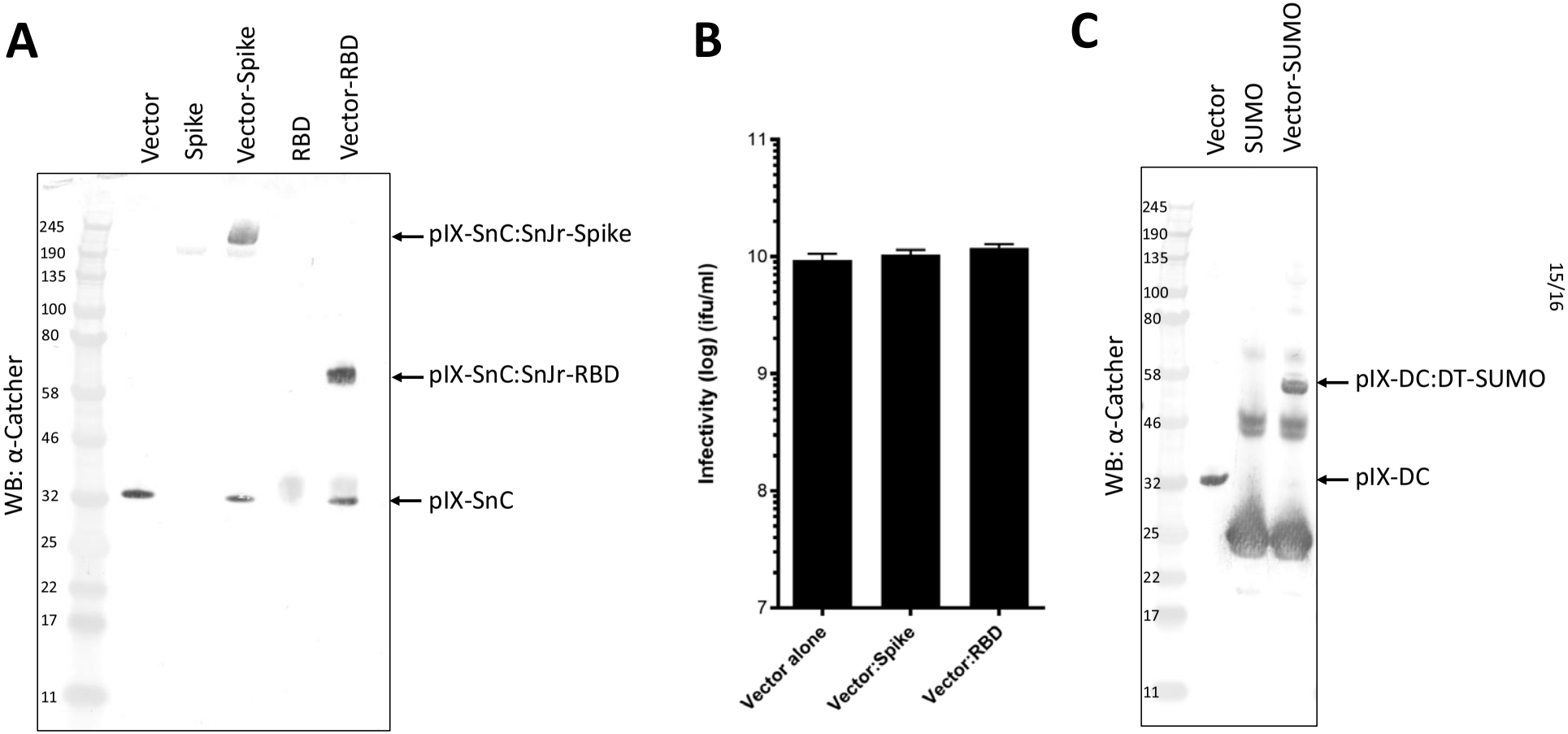

A
Vector
Spike
Vector-Spike
RBD
Vector-RBD
WB: α-Catcher
245
190
135
100
80
58
46
32
25
22
17
11
pIX-SnC:SnJr-Spike
pIX-SnC:SnJr-RBD
pIX-SnC
B
Infectivity (log) (ifu/ml)
11
10
9
8
7
Vector alone
Vector:Spike
Vector:RBD
C
Vector
SUMO
Vector-SUMO
WB: α-Catcher
245
190
135
100
80
58
46
32
25
22
17
11
pIX-DC:DT-SUMO
pIX-DC

Fig. 13

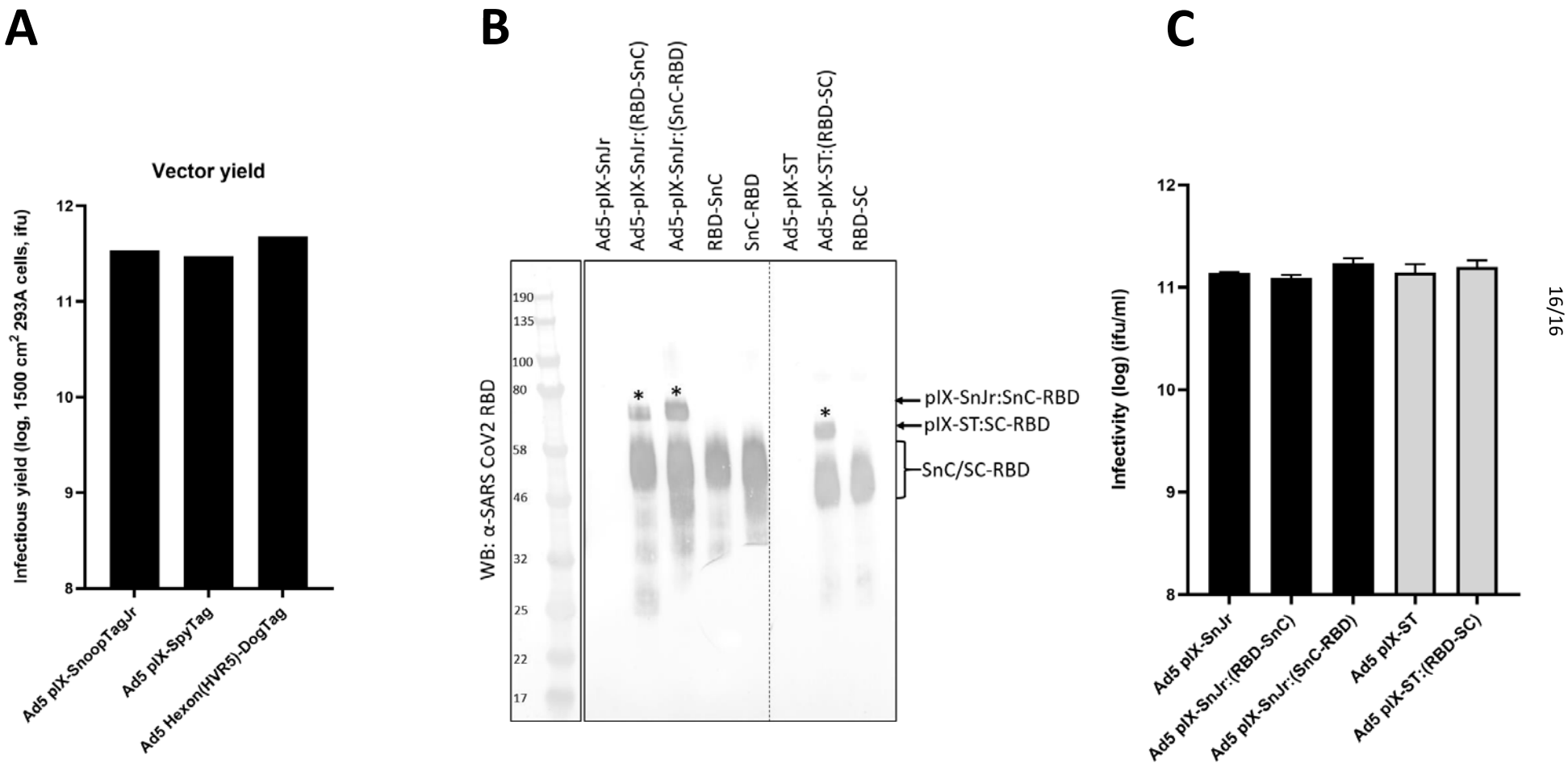

A
Vector yield
Infectious yield (log, 1500 cm2 293A cells, ifu)
12
11
10
9
8
Ad5 pIX-SnoopTagJr
Ad5 pIX-SpyTag
Ad5 Hexon(HVR5)-DogTag
B
Ad5-pIX-SnJr
Ad5-pIX-SnJr:(RBD-SnC)
Ad5-pIX-SnJr:(SnC-RBD)
RBD-SnC
SnC-RBD
Ad5-pIX-ST
Ad5-pIX-ST:(RBD-SC)
RBD-SC
WB: α-SARS CoV2 RBD
190
135
100
80
58
46
32
25
22
17
pIX-SnJr:SnC-RBD
pIX-ST:SC-RBD
SnC/SC-RBD
C
Infectivity (log) (ifu/ml)
12
11
10
9
8
Ad5 pIX-SnJr
Ad5 pIX-SnJr:(RBD-SnC)
Ad5 pIX-SnJr:(SnC-RBD)
Ad5 pIX-ST
Ad5 pIX-ST:(RBD-SC)

Fig. 14