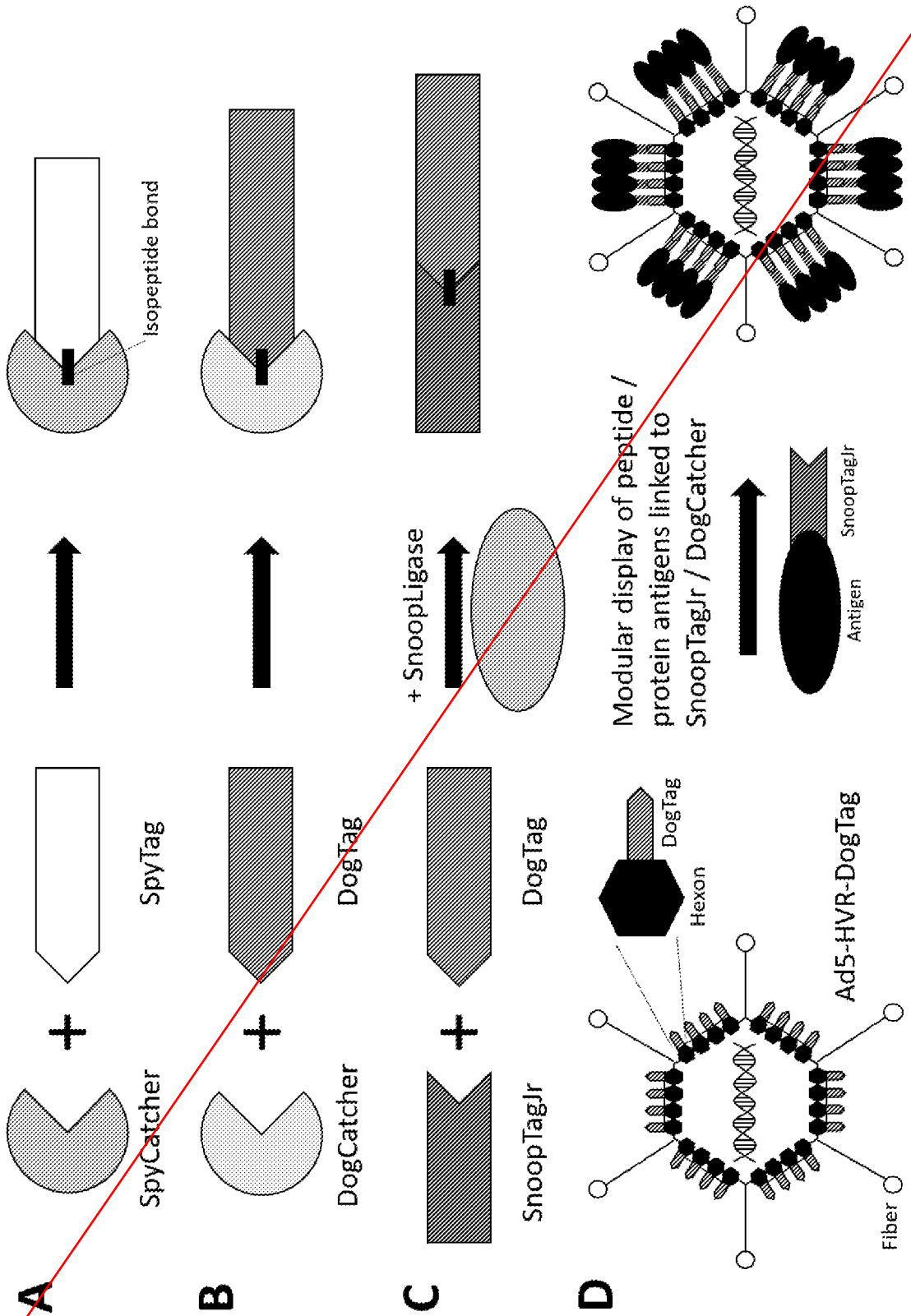

A
SpyTag
SpyCatcher
Isopeptide bond
B
DogTag
DogCatcher
C
DogTag
SnoopTagJr
+ SnoopLigase
D
DogTag
Hexon
Ad5-HVR-DogTag
Fiber
Modular display of peptide / protein antigens linked to SnoopTagJr / DogCatcher
SnoopTagJr
Antigen

Fig. 1

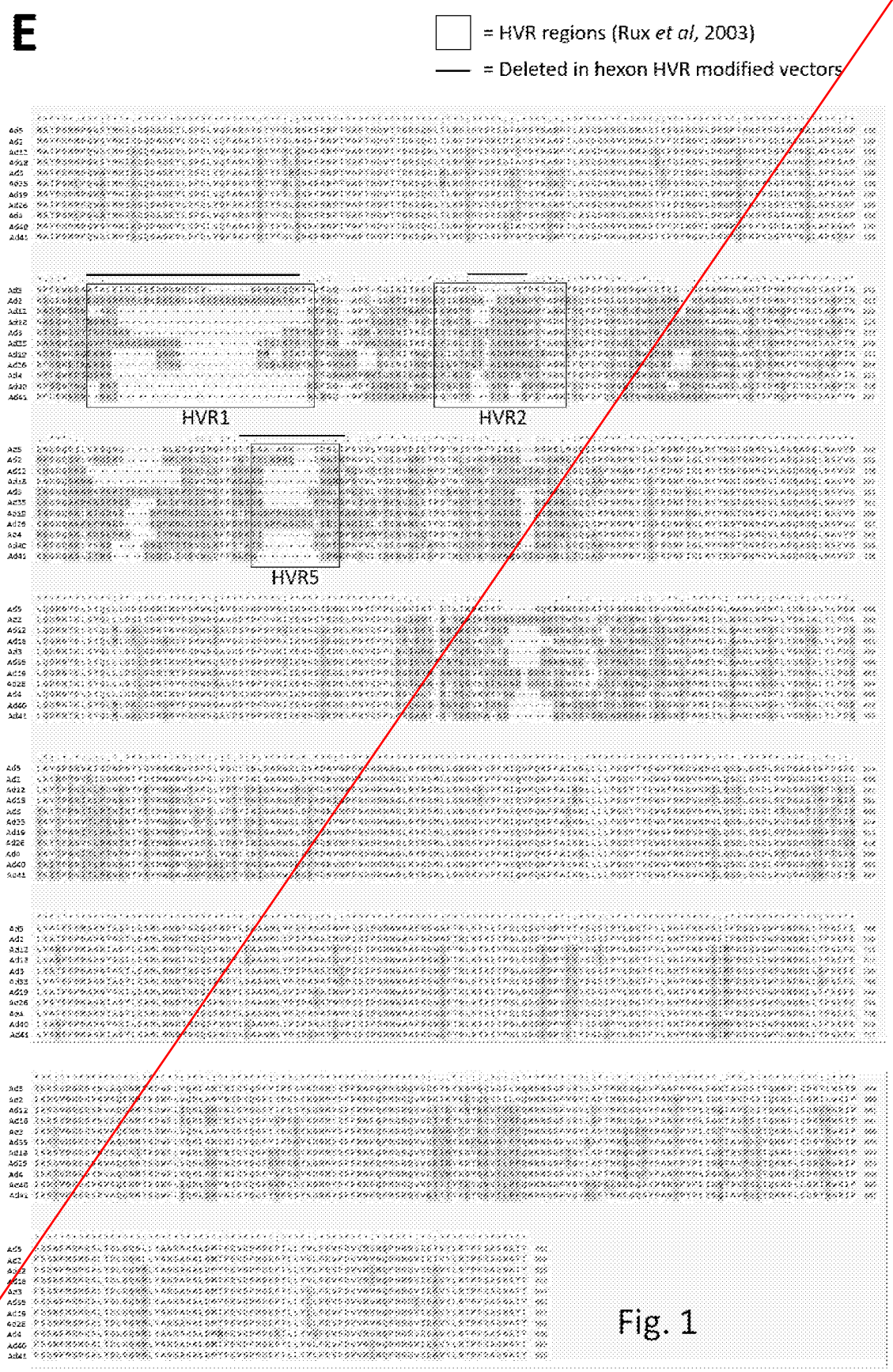
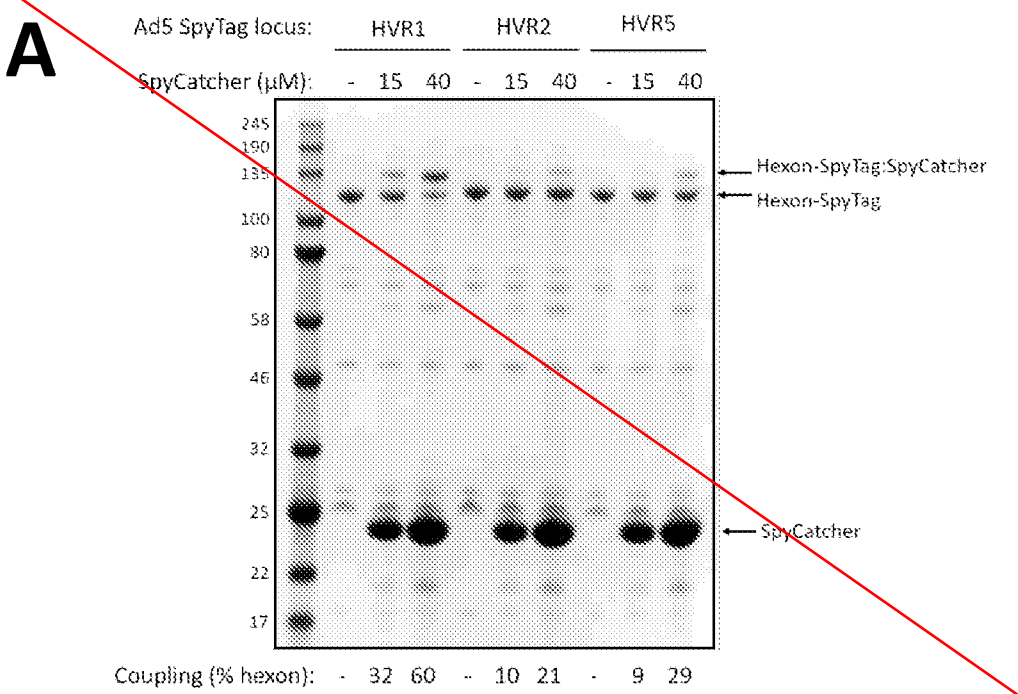
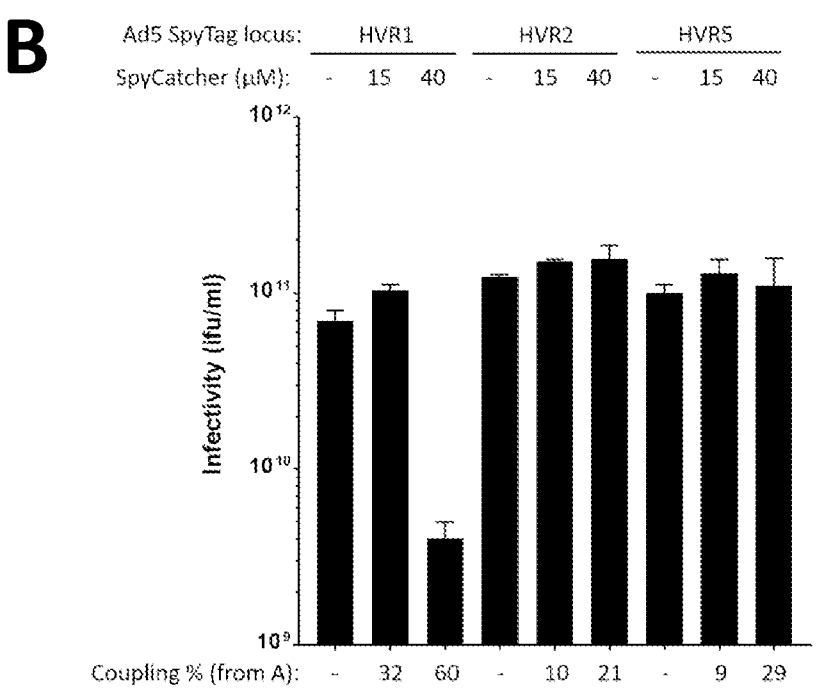
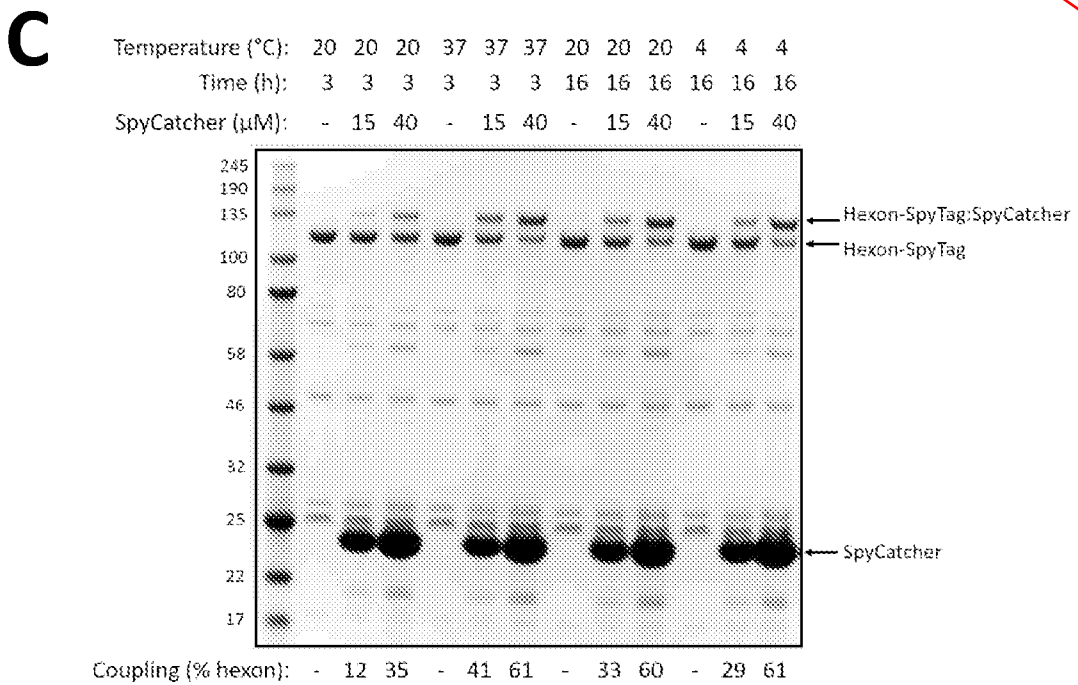

E
= HVR regions (Rux et al, 2003)
= Deleted in hexon HVR modified vectors
HVR1
HVR2
HVR5

Fig. 1


A
Ad5 SpyTag locus: HVR1 HVR2 HVR5
SpyCatcher (μM): - 15 40 - 15 40 - 15 40
245
190
135
100
80
58
46
32
25
22
17
Hexon-SpyTag:SpyCatcher
Hexon-SpyTag
SpyCatcher
Coupling (% hexon): - 32 60 - 10 21 - 9 29


B
Ad5 SpyTag locus: HVR1 HVR2 HVR5
SpyCatcher (μM): - 15 40 - 15 40 - 15 40
Infectivity (ifu/ml)
10^12
10^11
10^10
10^9
Coupling % (from A): - 32 60 - 10 21 - 9 29


C
Temperature (°C): 20 20 20 37 37 37 20 20 20 4 4 4
Time (h): 3 3 3 3 3 3 16 16 16 16 16 16
SpyCatcher (μM): - 15 40 - 15 40 - 15 40 - 15 40
245
190
135
100
80
58
46
32
25
22
17
Hexon-SpyTag:SpyCatcher
Hexon-SpyTag
SpyCatcher
Coupling (% hexon): - 12 35 - 41 61 - 33 60 - 29 61

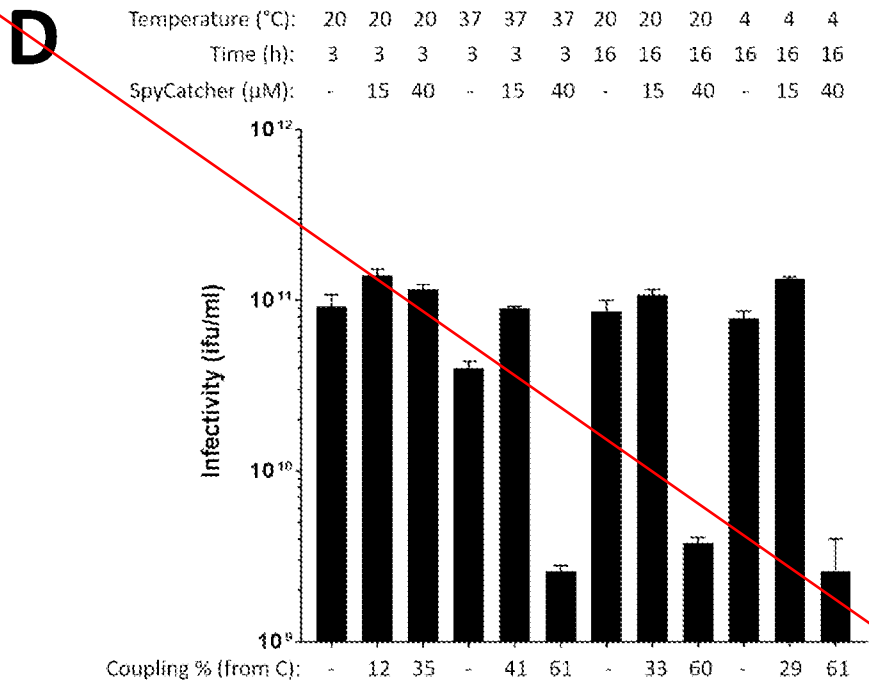
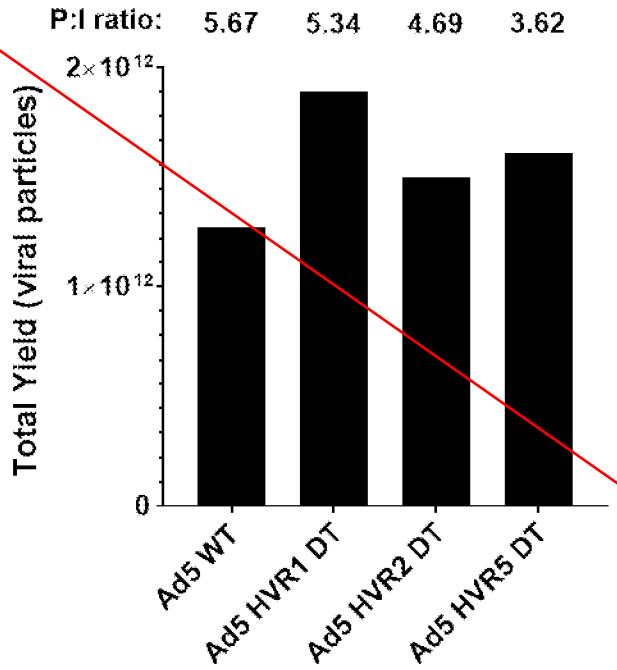
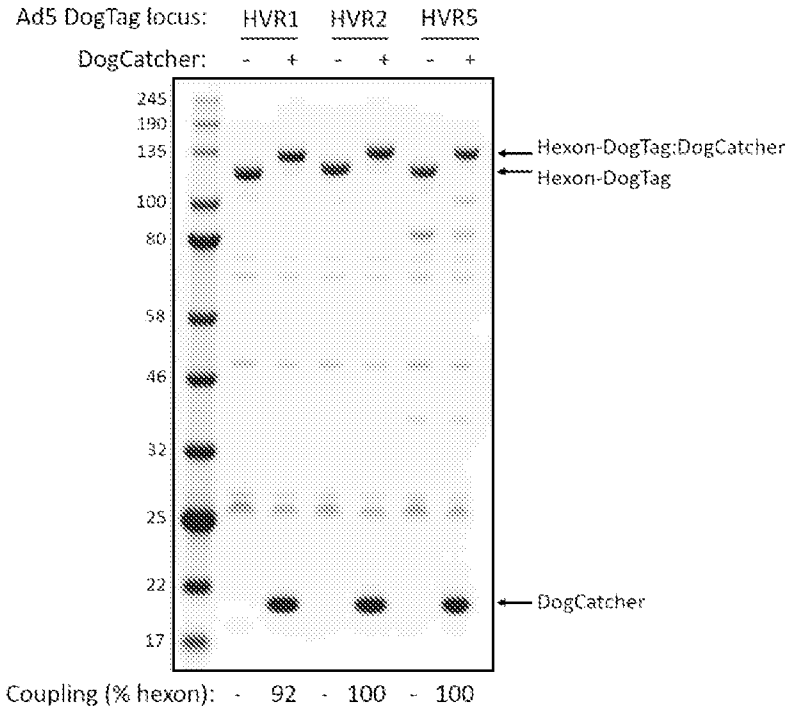

D
Temperature (°C): 20 20 20 37 37 37 20 20 20 4 4 4
Time (h): 3 3 3 3 3 3 16 16 16 16 16 16
SpyCatcher (μM): - 15 40 - 15 40 - 15 40 - 15 40
Infectivity (ifu/ml)
10^12
10^11
10^10
10^9
Coupling % (from C): - 12 35 - 41 61 - 33 60 - 29 61

Fig. 2

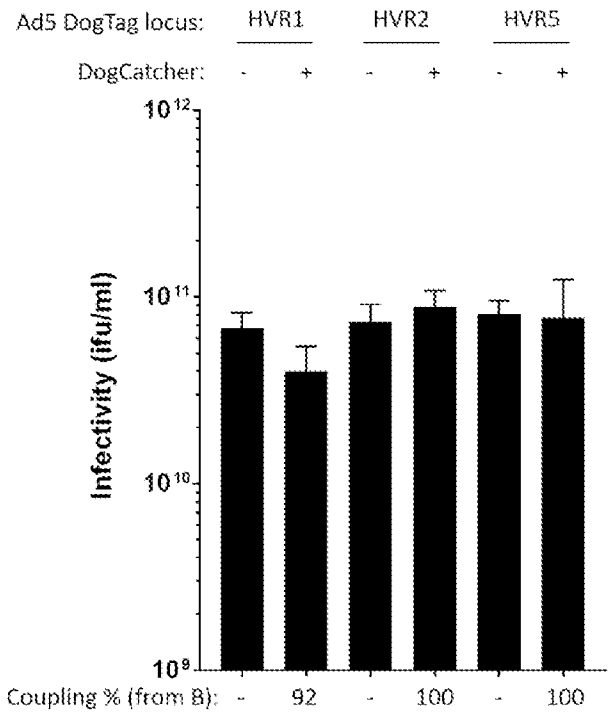
A


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

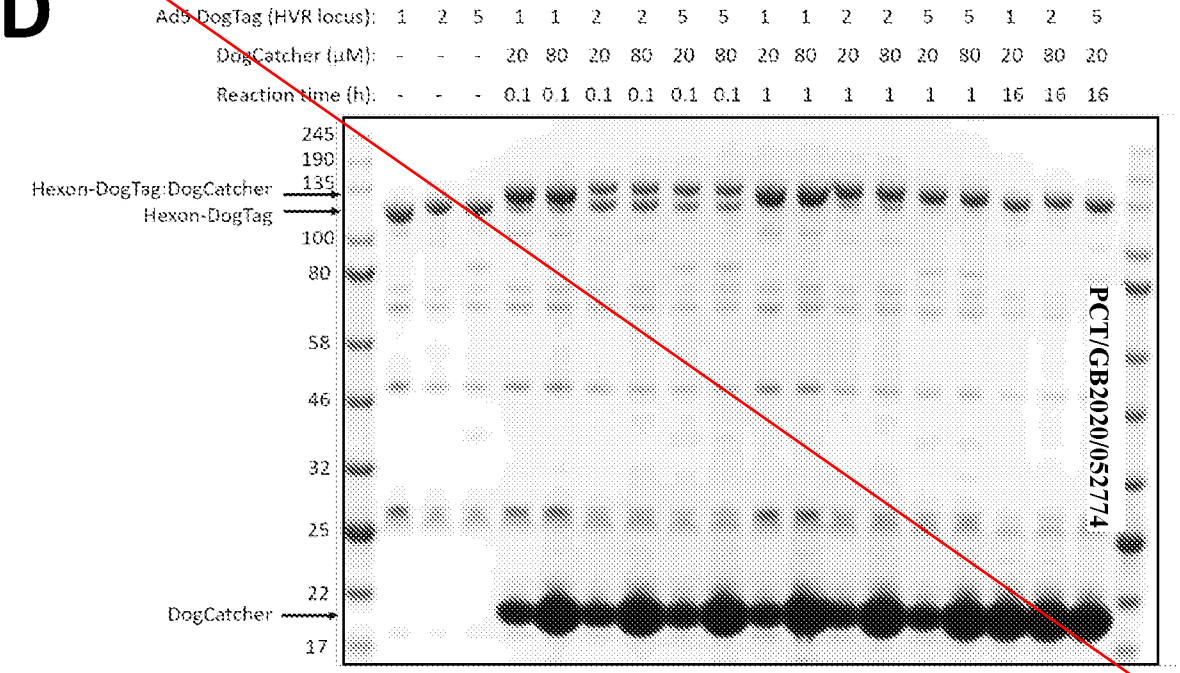
B

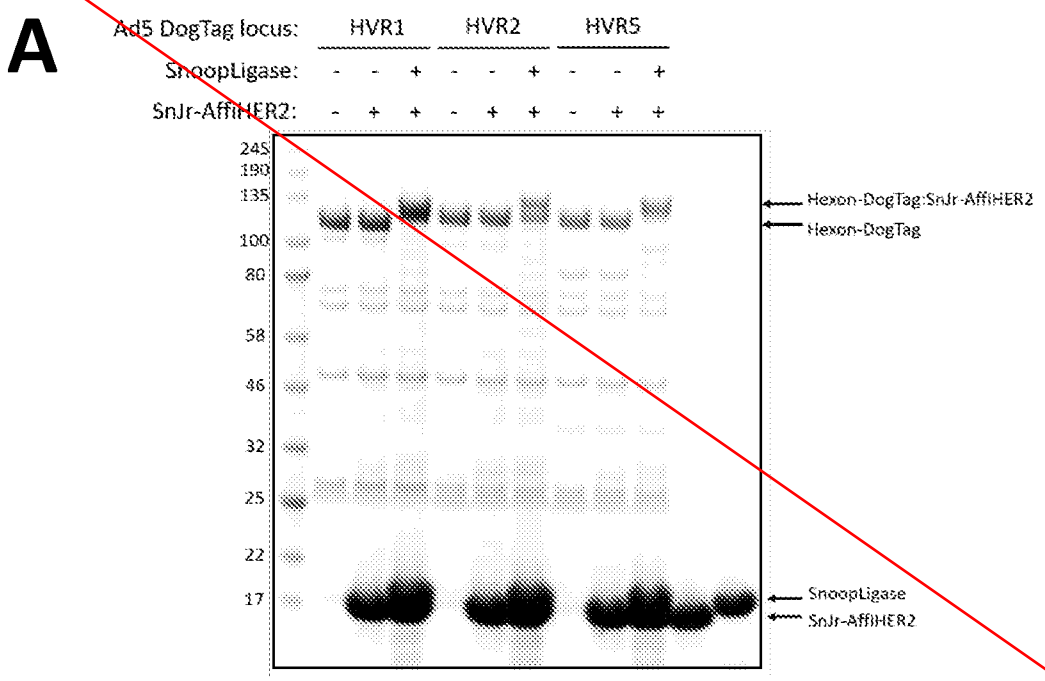

Ad5 DogTag locus: HVR1 HVR2 HVR5
DogCatcher: - + - + - +
245
190
135
100
80
58
46
32
25
22
17
Hexon-DogTag:DogCatcher
Hexon-DogTag
DogCatcher
Coupling (% hexon): - 92 - 100 - 100

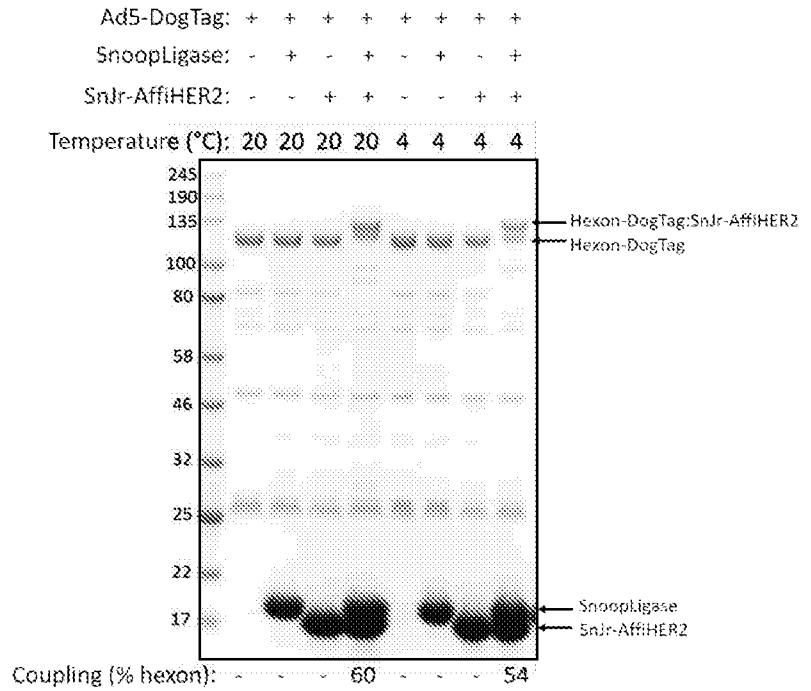
C

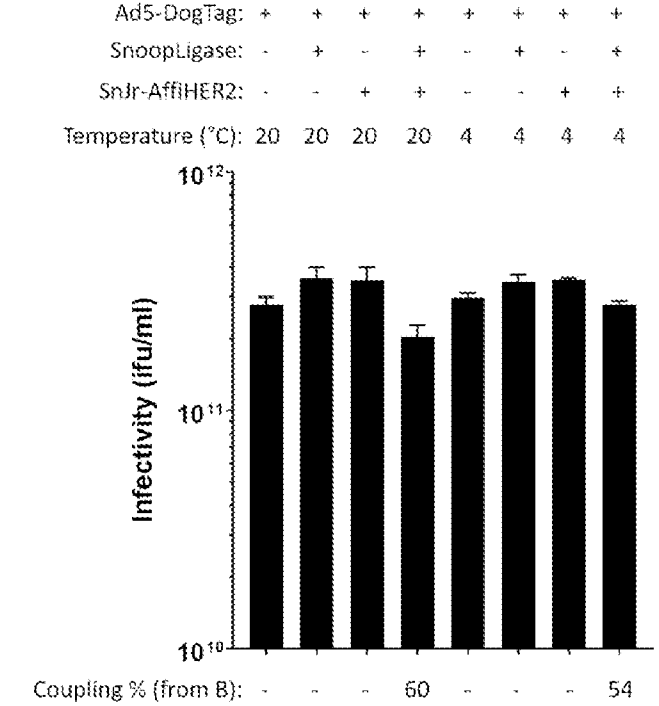

Ad5 DogTag locus: HVR1 HVR2 HVR5
DogCatcher: - + - + - +
Infectivity (ifu/ml)
10^12
10^11
10^10
10^9
Coupling % (from B): - 92 - 100 - 100

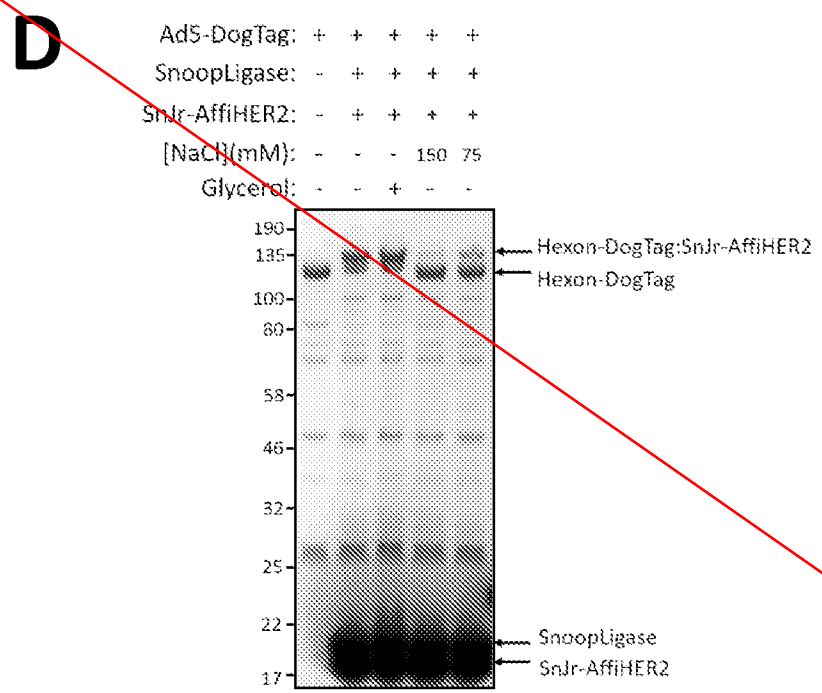
D

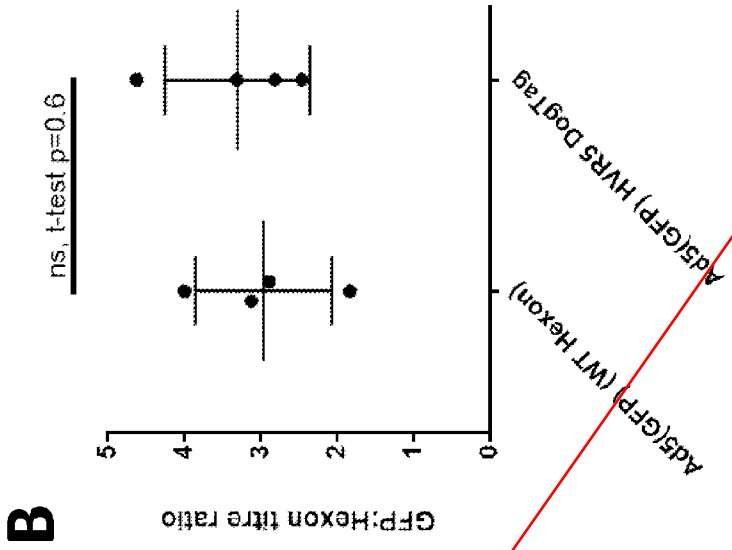

Ad5 DogTag (HVR locus): 1 2 5 1 1 2 2 5 5 1 1 2 2 5 5 1 2 5
DogCatcher (μM): - - - 20 80 20 80 20 80 20 80 20 80 20 80 20 80 20
Reaction time (h): - - - 0.1 0.1 0.1 0.1 0.1 0.1 1 1 1 1 1 1 16 16 16
245
190
135
100
80
58
46
32
25
22
17
Hexon-DogTag:DogCatcher
Hexon-DogTag
DogCatcher


A
Ad5 DogTag locus: HVR1 HVR2 HVR5
SnoopLigase:
SnJr-AffiHER2:
245
190
135
100
80
58
46
32
25
22
17
Hexon-DogTag:SnJr-AffiHER2
Hexon-DogTag
SnoopLigase
SnJr-AffiHER2


B
Ad5-DogTag:
SnoopLigase:
SnJr-AffiHER2:
Temperature (°C): 20 20 20 20 4 4 4 4
245
190
135
100
80
58
46
32
25
22
17
Hexon-DogTag:SnJr-AffiHER2
Hexon-DogTag
SnoopLigase
SnJr-AffiHER2
Coupling (% hexon): 60 54


C
Ad5-DogTag:
SnoopLigase:
SnJr-AffiHER2:
Temperature (°C): 20 20 20 20 4 4 4 4
Infectivity (ifu/ml)
10^12
10^11
10^10
Coupling % (from B): 60 54


D
Ad5-DogTag:
SnoopLigase:
SnJr-AffiHER2:
[NaCl](mM): 150 75
Glycerol:
190
135
100
80
58
46
32
25
22
17
Hexon-DogTag:SnJr-AffiHER2
Hexon-DogTag
SnoopLigase
SnJr-AffiHER2


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

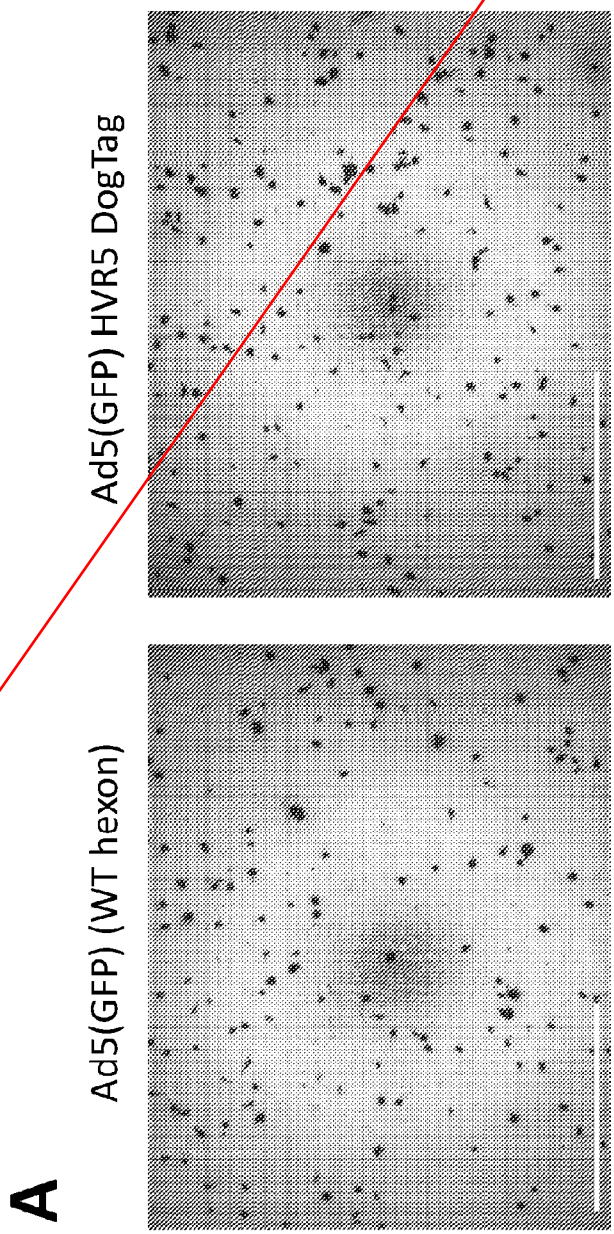
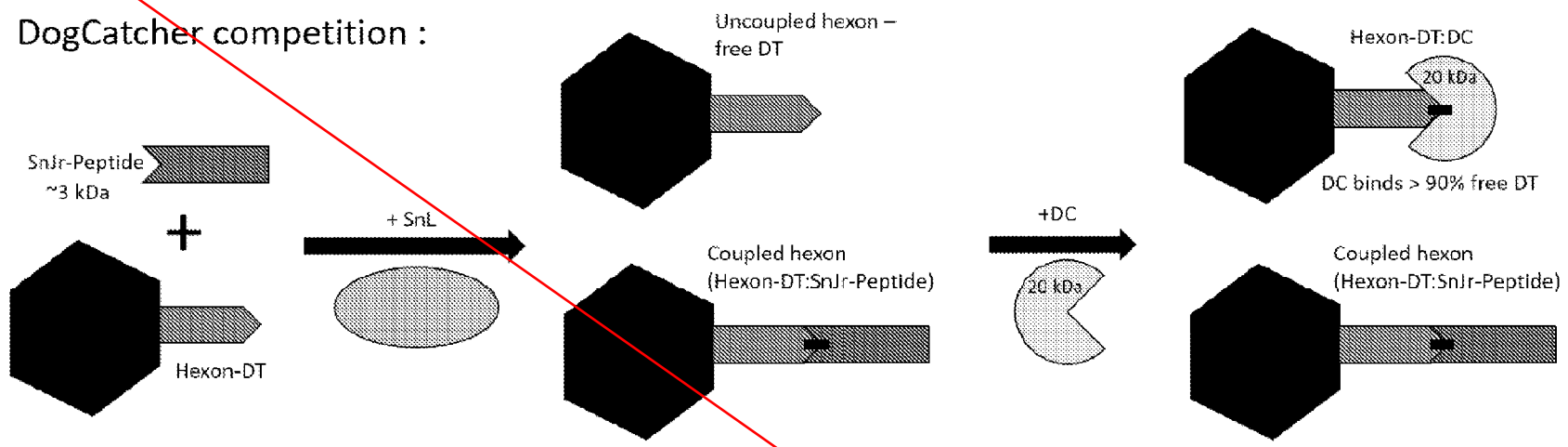
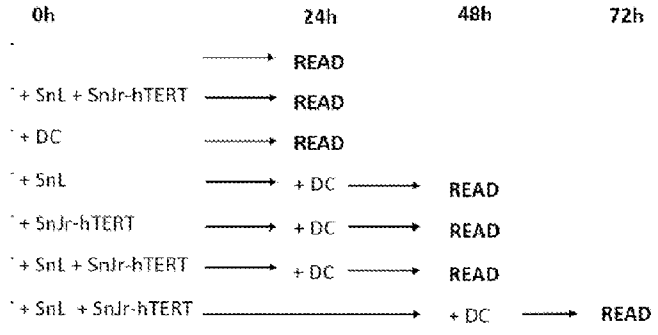

A
Ad5(GFP) (WT hexon)
Ad5(GFP) HVR5 DogTag

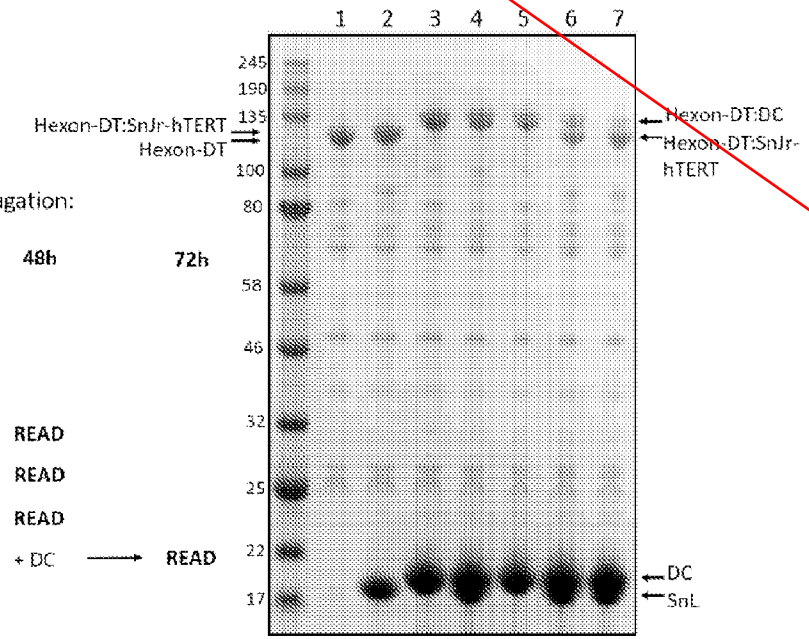
Fig. 5

Fig. 7

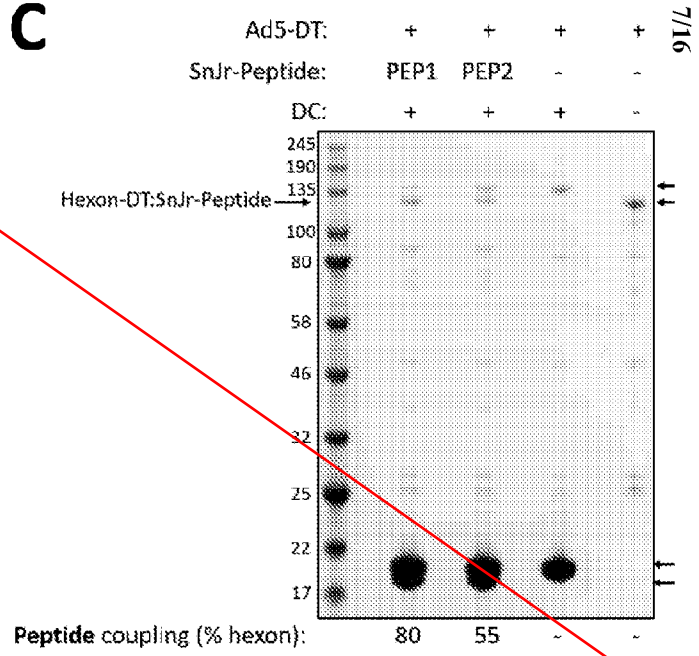


tion assay to test SnoopTagJr-hTERT conjugation:



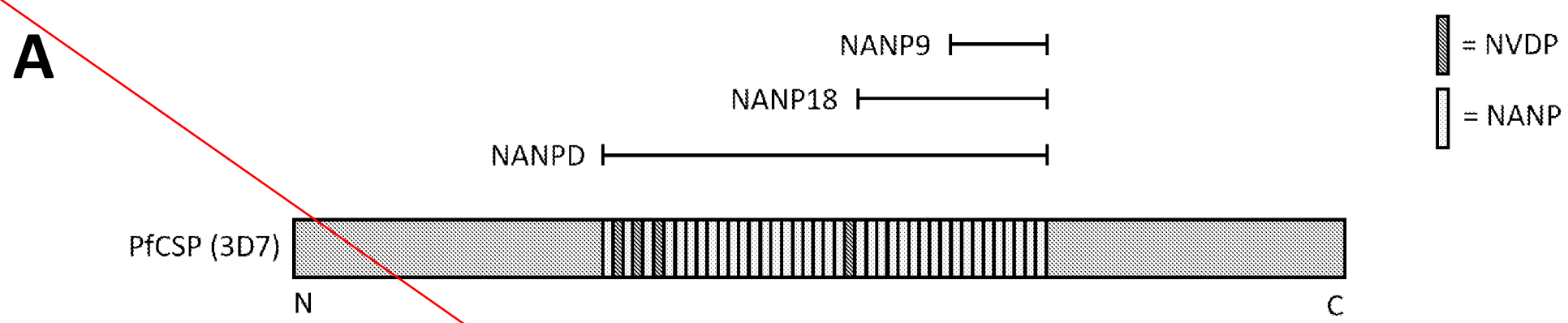


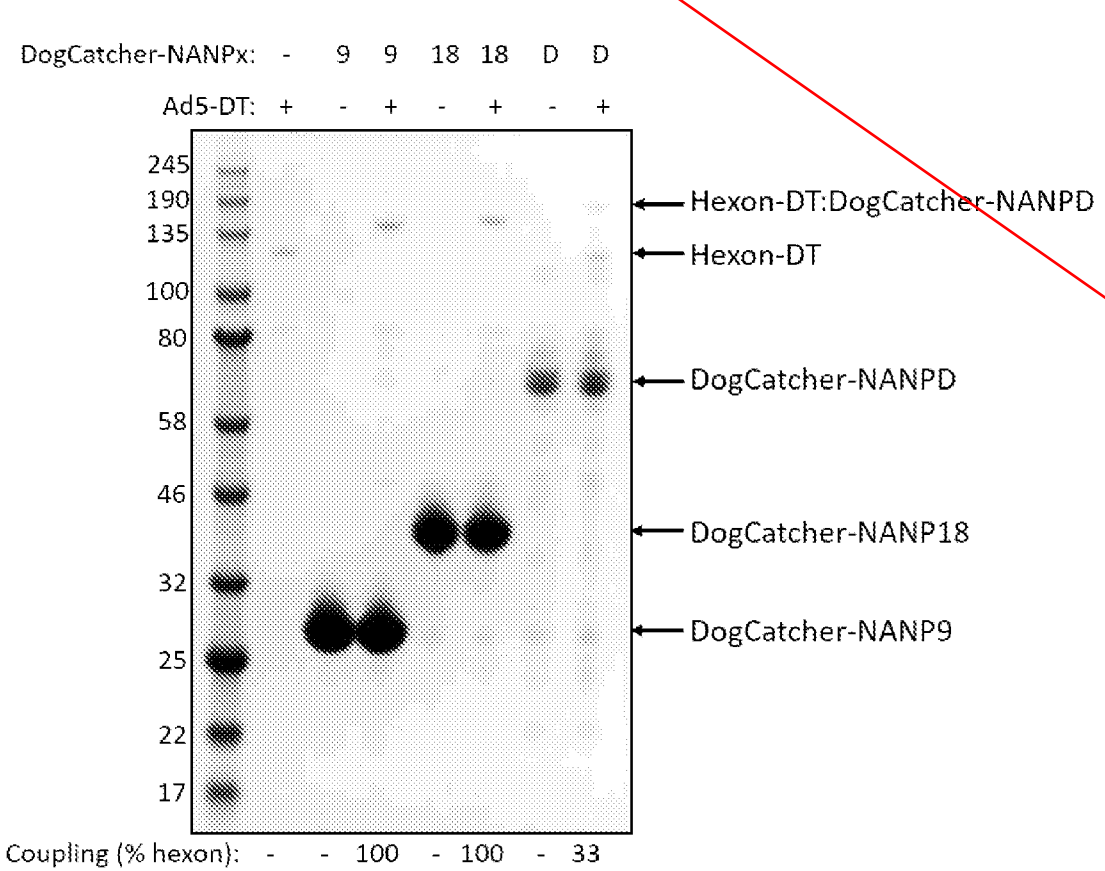
C



mSA gel shift assay:
SnJr-biotin ~3kDa
Hexon-DT
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
F
Ad5-DT:
SnoopLigase:
SnJr-biotin:
mSA:
Hexon-DT:SnJr-biotin/mSA
Hexon-DT
245
190
135
100
80
58
46
Infectivity (ifu/ml)
Ad5-DT
Ad5-DT:SnJr-PEPI
Ad5-DT:SnJr-PEP2

Fig. 6


A
NANP9
NANP18
NANPD
= NVDP
= NANP
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

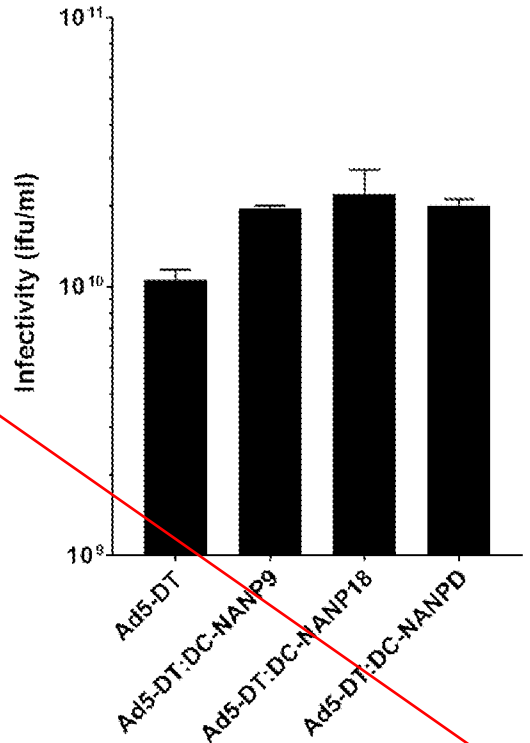

C
Infectivity (ifu/ml)
10^{11}
10^{10}
10^{9}
Ad5-DT
Ad5-DT:DC-NANP9
Ad5-DT:DC-NANP18
Ad5-DT:DC-NANPD

Fig. 7

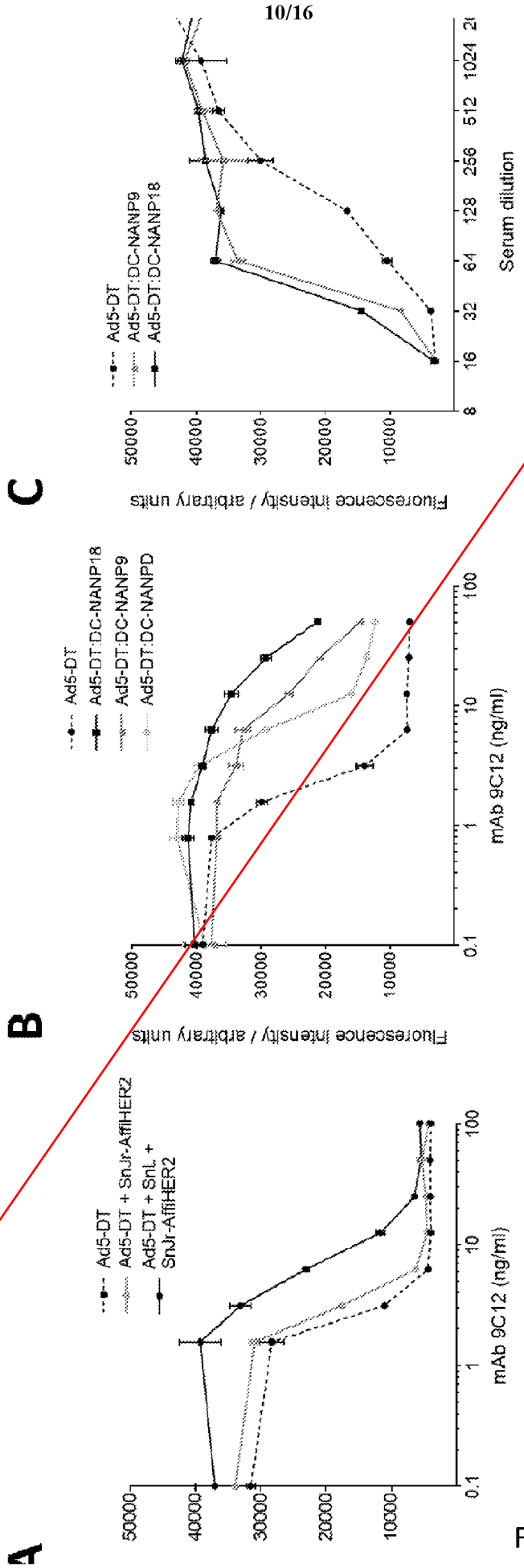

A
B
C
Ad5-DT
Ad5-DT + SnJr-AffiHER2
Ad5-DT + SnL + SnJr-AffiHER2
Ad5-DT:DC-NANP18
Ad5-DT:DC-NANP9
Ad5-DT:DC-NANPD
Fluorescence intensity / arbitrary units
mAb 9C12 (ng/ml)
Serum dilution
50000
40000
30000
20000
10000
0.1
1
10
100
8
16
32
64
128
256
512
1024

Fig. 8

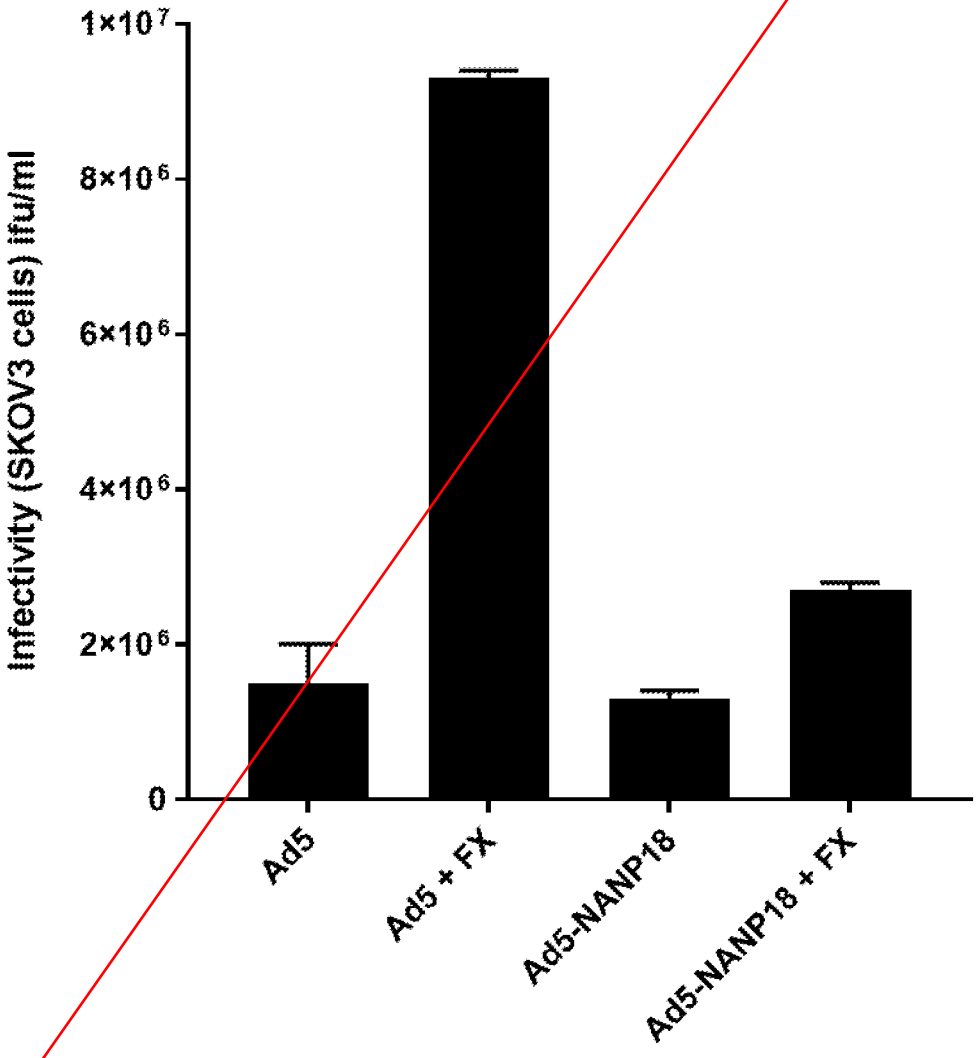
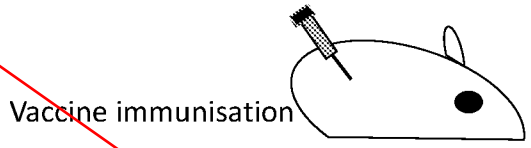

Infectivity (SKOV3 cells) ifu/ml
1×10⁷
8×10⁶
6×10⁶
4×10⁶
2×10⁶
0
Ad5
Ad5 + FX
Ad5-NANP18
Ad5-NANP18 + FX

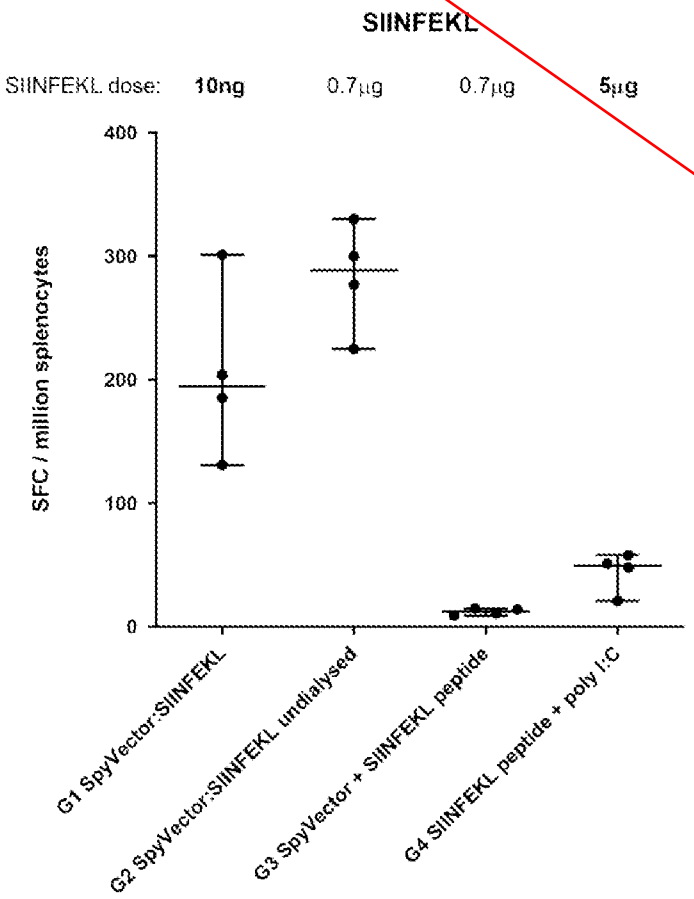
Fig. 9

A



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



C

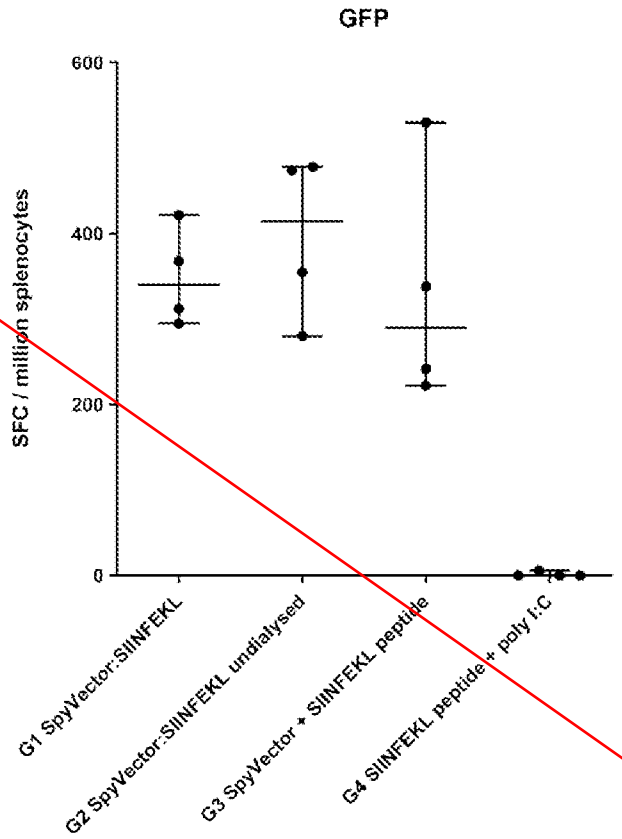
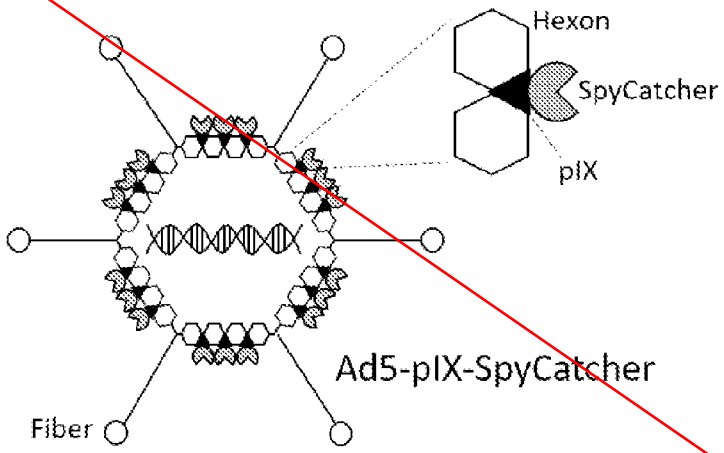
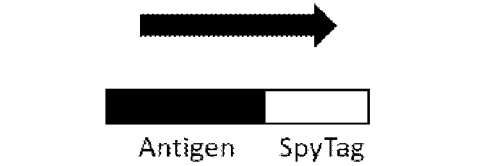
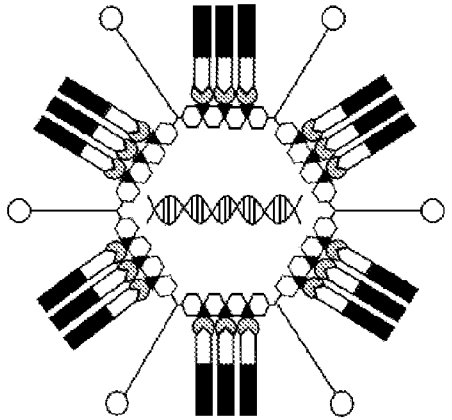


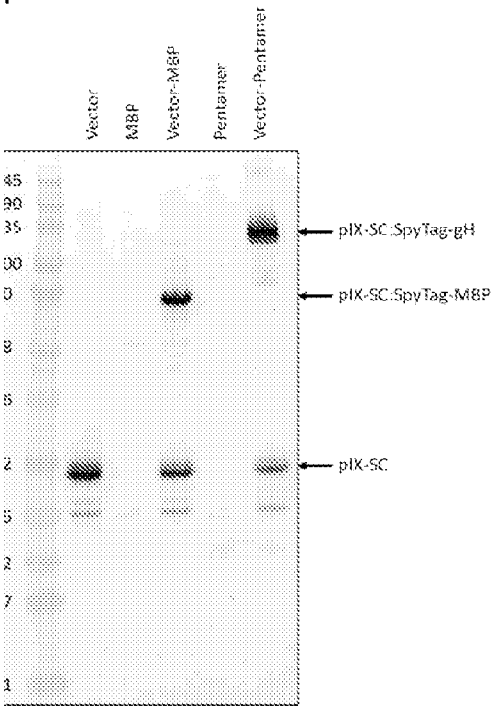
Fig. 10

Fig. 11

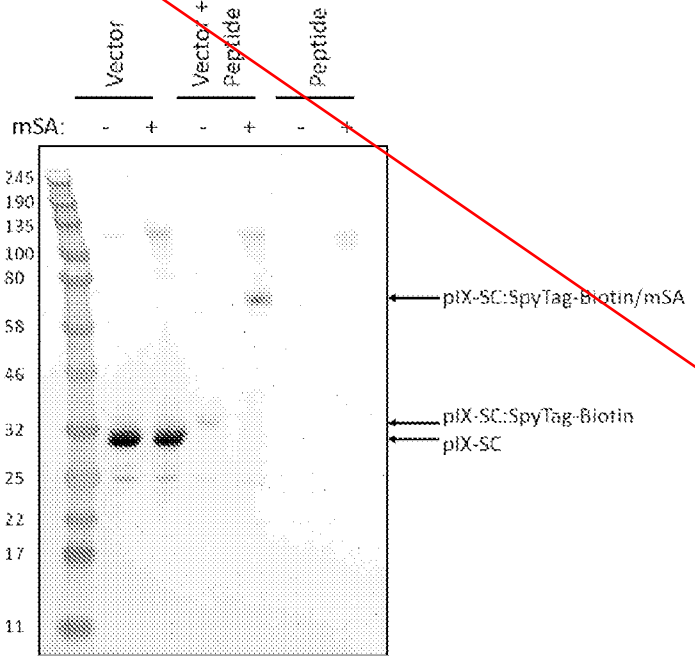

Hexon
SpyCatcher
pIX
Fiber
Ad5-pIX-SpyCatcher


Modular display of peptide / protein antigens linked to SpyTag
Antigen
SpyTag

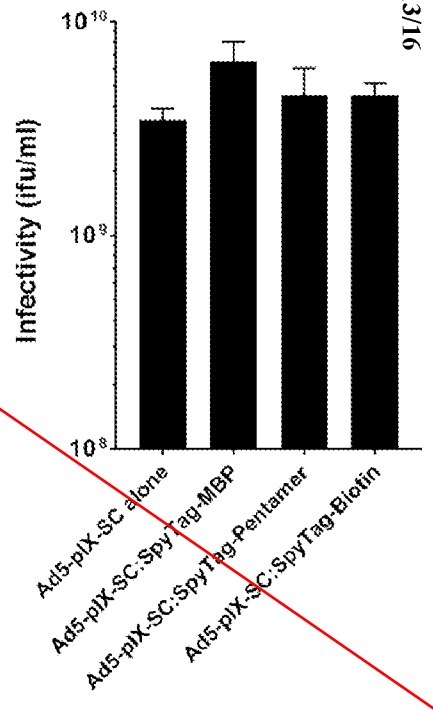



Vector
MBP
Vector-MBP
Pentamer
Vector-Pentamer
pIX-SC:SpyTag-gH
pIX-SC:SpyTag-MBP
pIX-SC

C


Vector
Vector + Peptide
Peptide
mSA:
pIX-SC:SpyTag-Biotin/mSA
pIX-SC:SpyTag-Biotin
pIX-SC

D


Infectivity (ifu/ml)
Ad5-pIX-SC alone
Ad5-pIX-SC:SpyTag-MBP
Ad5-pIX-SC:SpyTag-Pentamer
Ad5-pIX-SC:SpyTag-Biotin

A

IX-Catcher construct

pIX	GSG3	SpyCatcher dN1		
pIX	GGS	HELIX	GS	SpyCatcher dN1
pIX	GGS	EAAAK3	GS	SpyCatcher dN1
pIX	SpyCatcher dN1			
pIX	EAAAK3	GS	SpyCatcher dN1	
pIX	GGS	EAAAK3	SpyCatcher dN1	
pIX	EAAAK3	SpyCatcher dN1		
pIX	GGS	EAAAK5	GS	SpyCatcher dN1
pIX	GGS	EAAAK3	GS	SpyCatcher dN1dC2
pIX	GGS	EAAAK3	GS	SnoopCatcher
pIX	GGS	EAAAK3	GS	DogCatcher

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

B

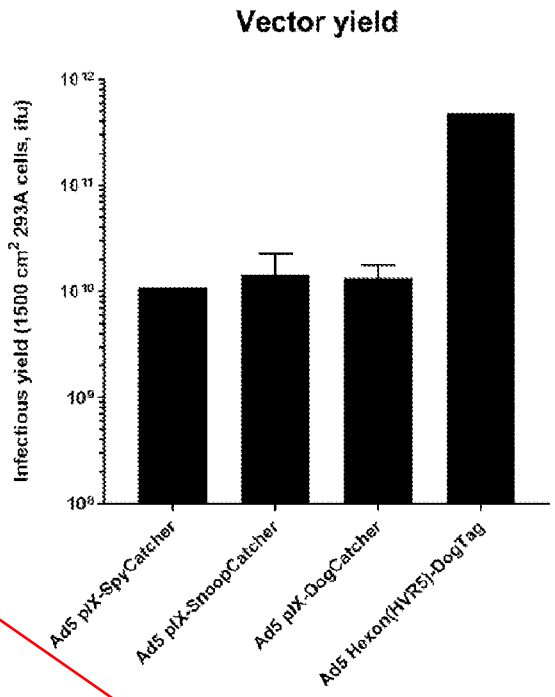


Fig. 12

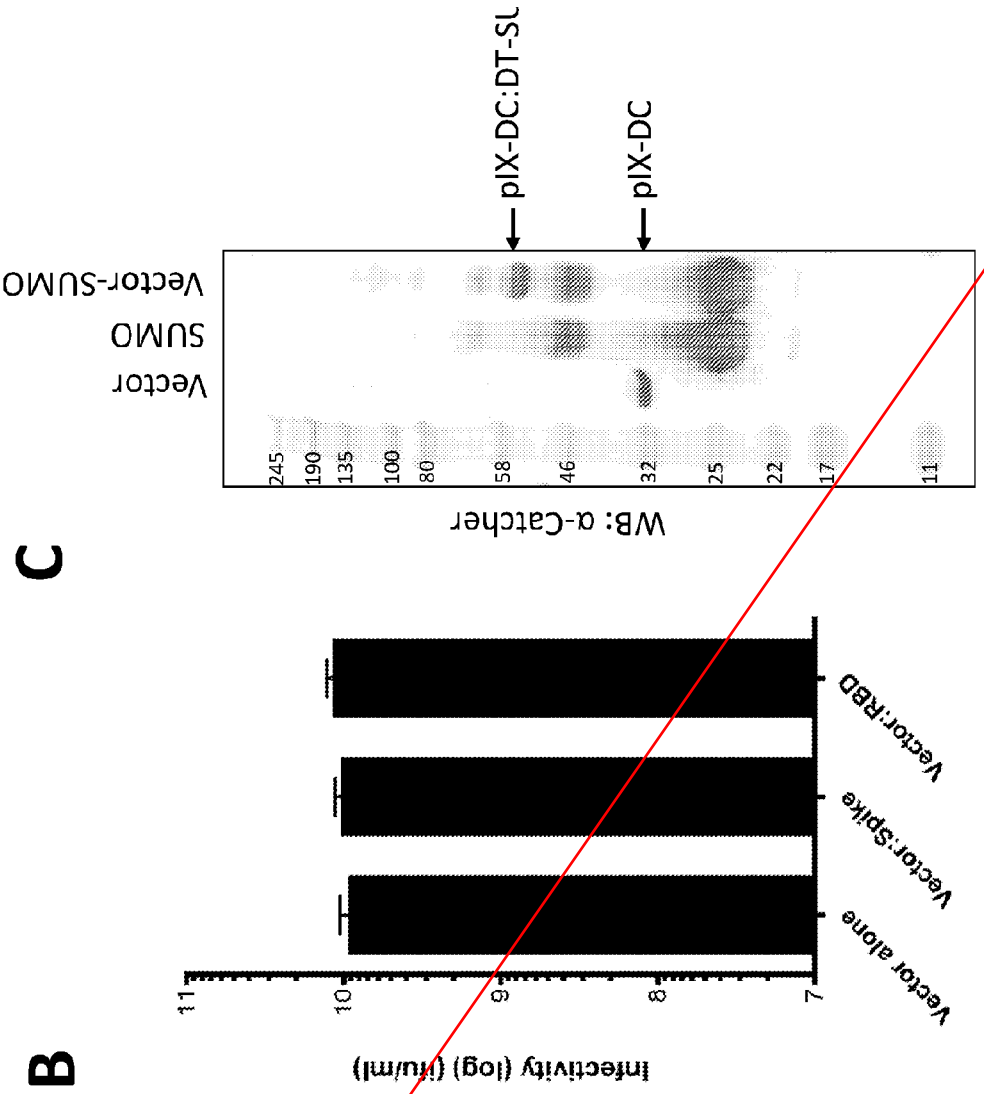

C
pIX-DC:DT-SL
pIX-DC
Vector
SUMO
Vector-SUMO
245
190
135
100
80
58
46
32
25
22
17
11
WB: α-Catcher
B
pIX-SnC:SnJr-Spike
pIX-SnC:SnJr-RBD
pIX-SnC
Vector
Spike
Vector-Spike
RBD
Vector-RBD
Infectivity (log) (ffu/ml)
11
10
9
8
7
Vector alone
Vector:Spike
Vector:RBD

Fig. 13

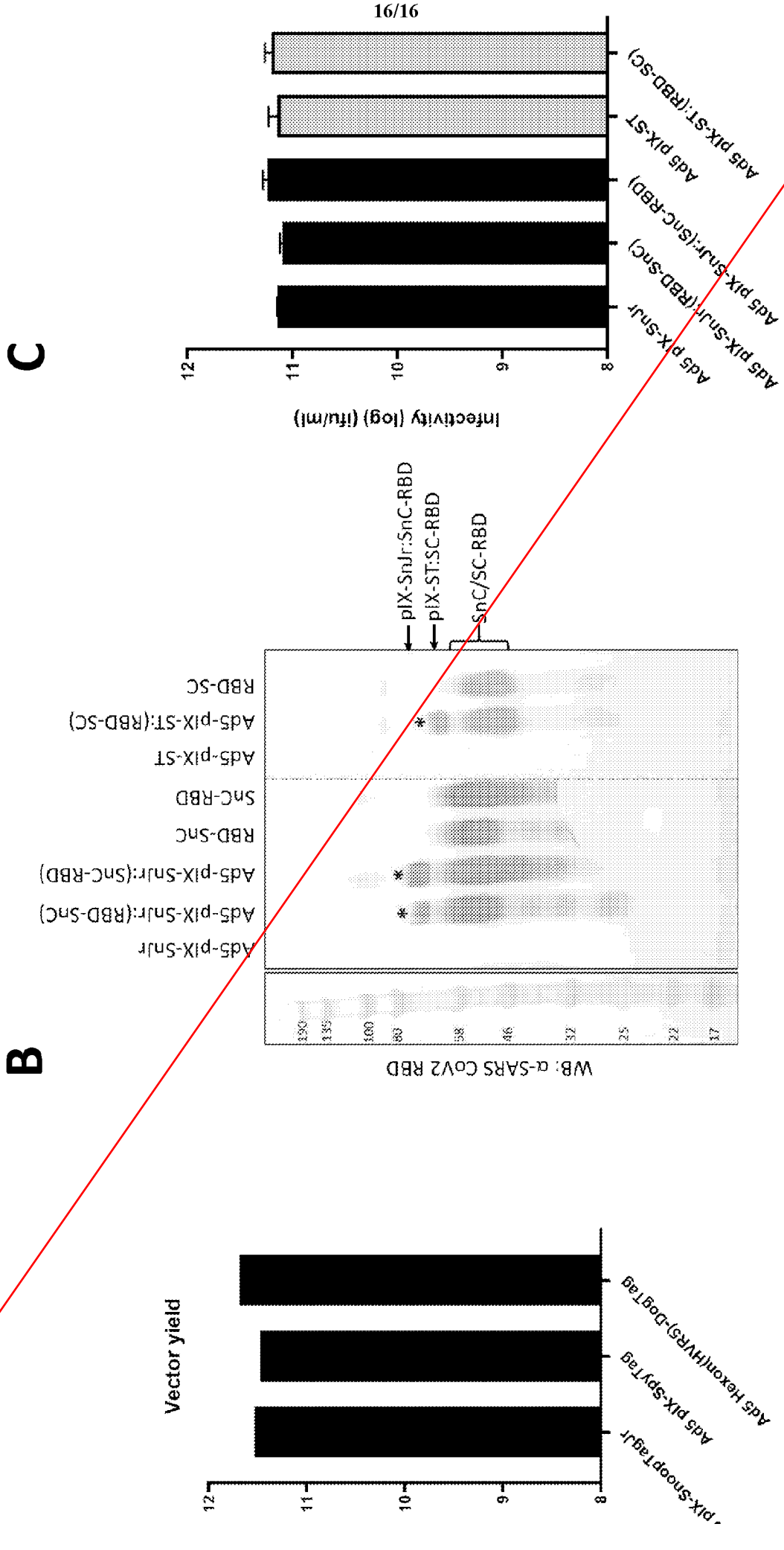

C
Infectivity (log) (ifu/ml)
12
11
10
9
8
Ad5 pIX-SnJr
Ad5 pIX-SnJr:(RBD-SnC)
Ad5 pIX-SnJr:(SnC-RBD)
Ad5 pIX-ST
Ad5 pIX-ST:(RBD-SC)
B
WB: α-SARS CoV2 RBD
190
135
100
80
58
46
32
25
22
17
pIX-SnJr:SnC-RBD
pIX-ST:SC-RBD
SnC/SC-RBD
Ad5-pIX-SnJr
Ad5-pIX-SnJr:(RBD-SnC)
Ad5-pIX-SnJr:(SnC-RBD)
RBD-SnC
SnC-RBD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

Fig. 14

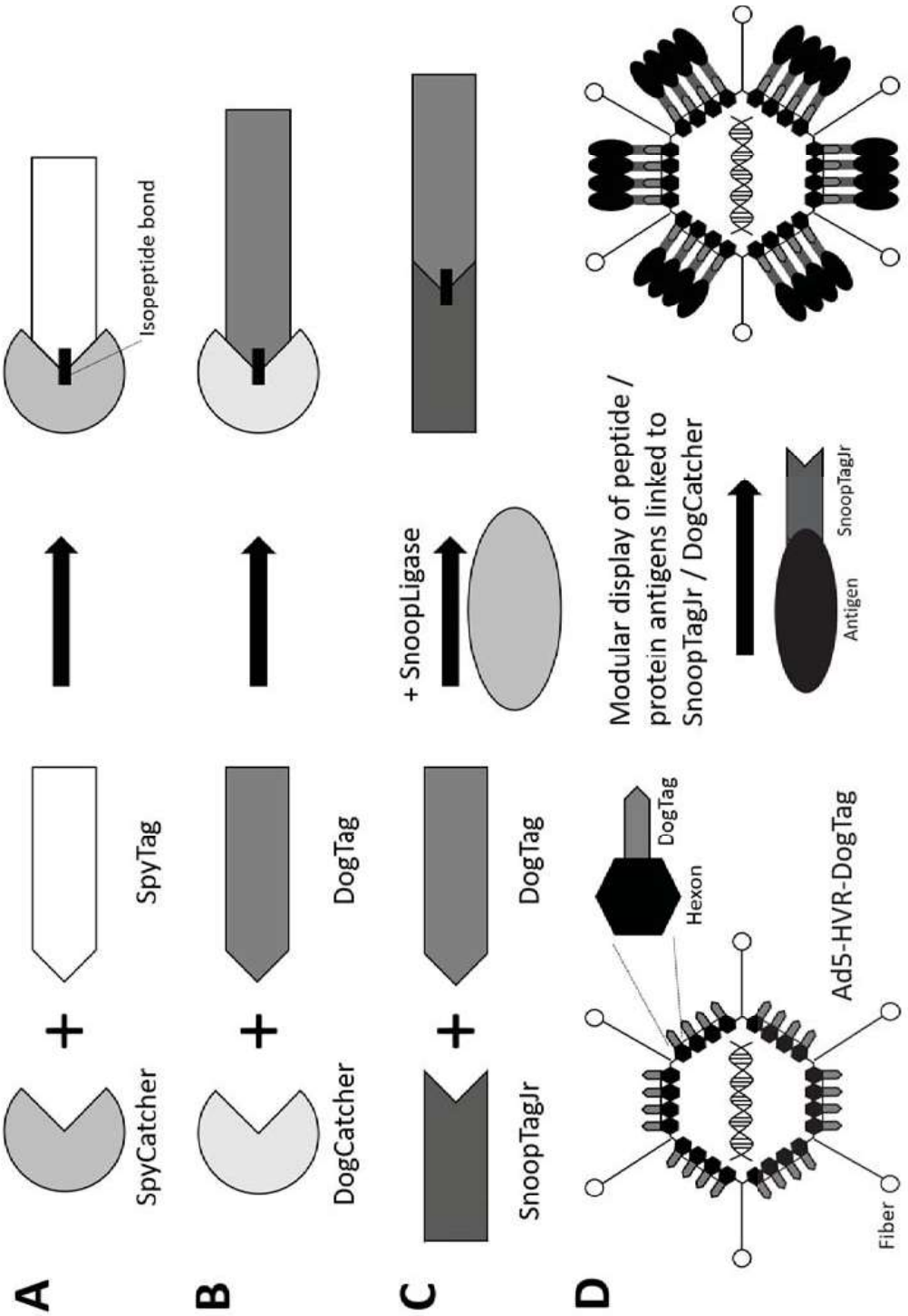

A
SpyCatcher
SpyTag
Isopeptide bond
B
DogCatcher
DogTag
C
SnoopTagJr
DogTag
+ SnoopLigase
D
Ad5-HVR-DogTag
Hexon
DogTag
Fiber
Modular display of peptide / protein antigens linked to SnoopTagJr / DogCatcher
Antigen
SnoopTagJr

Fig. 1

E

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

= Deleted in hexon HVR modified vectors

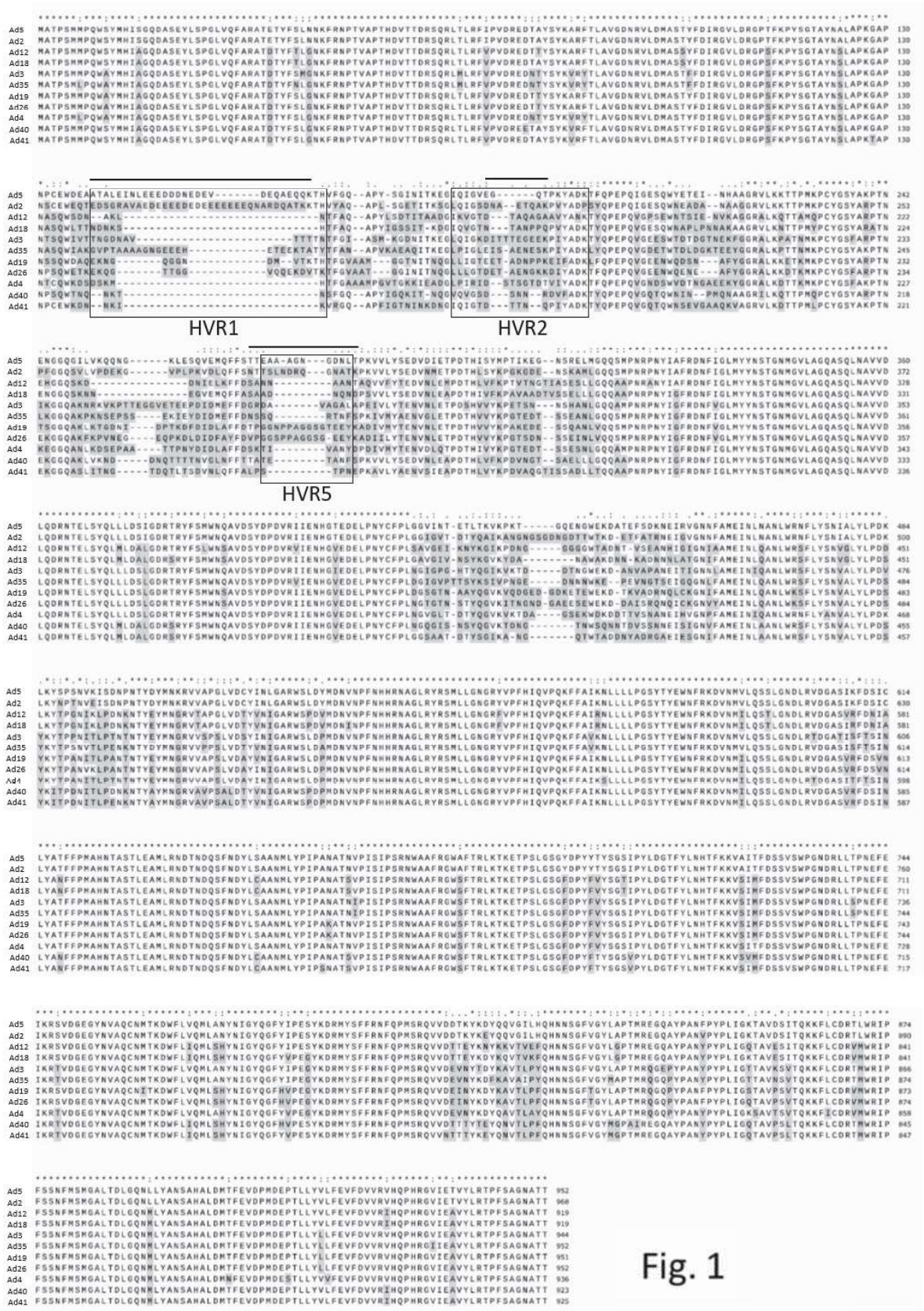


Fig. 1

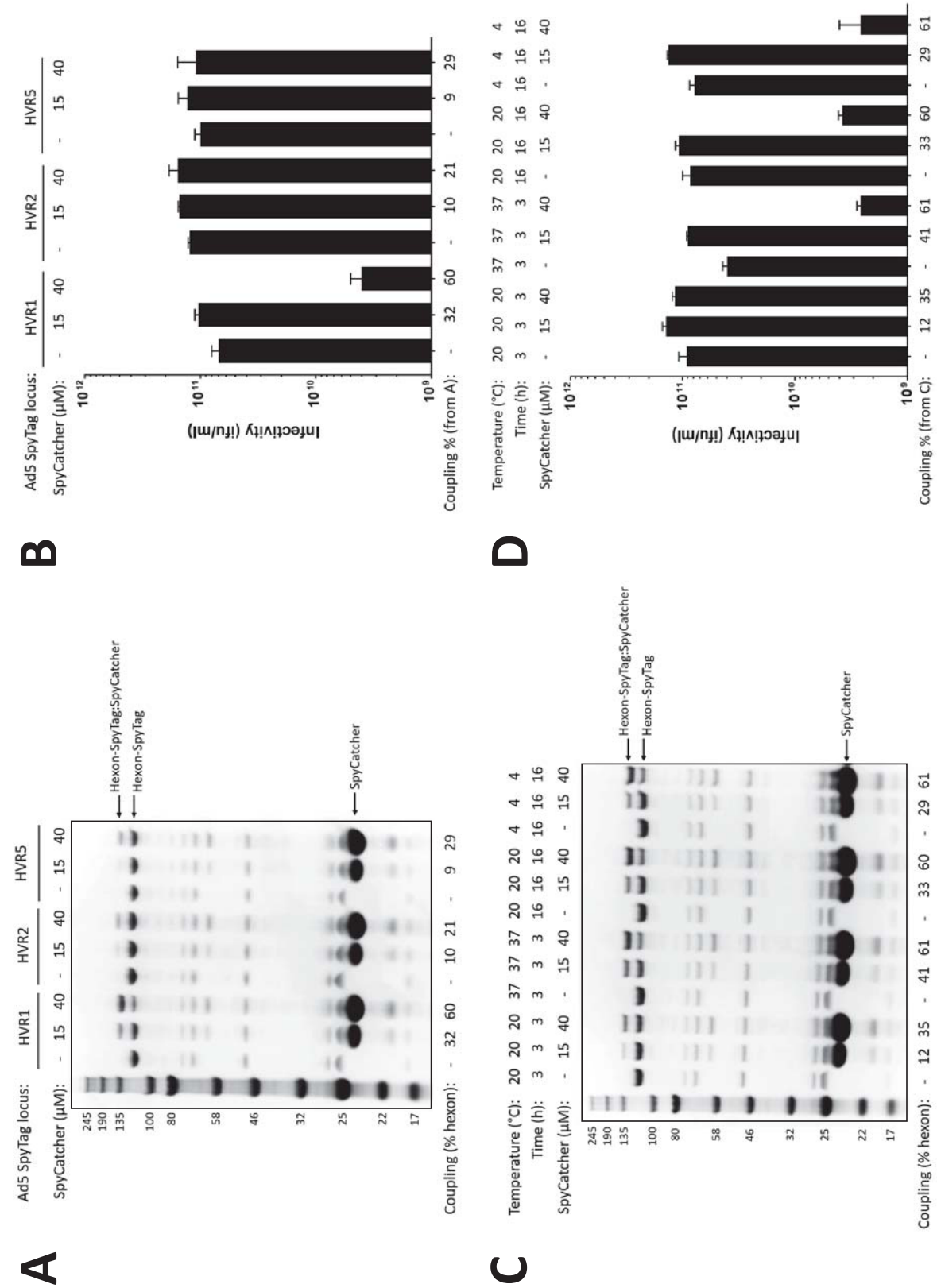

A
Ad5 SpyTag locus:
HVR1
HVR2
HVR5
SpyCatcher (μM):
Hexon-SpyTag:SpyCatcher
Hexon-SpyTag
SpyCatcher
Coupling (% hexon):
B
Infectivity (ifu/ml)
Coupling % (from A):
C
Temperature (°C):
Time (h):
D
Coupling % (from C):

Fig. 2

Fig. 3

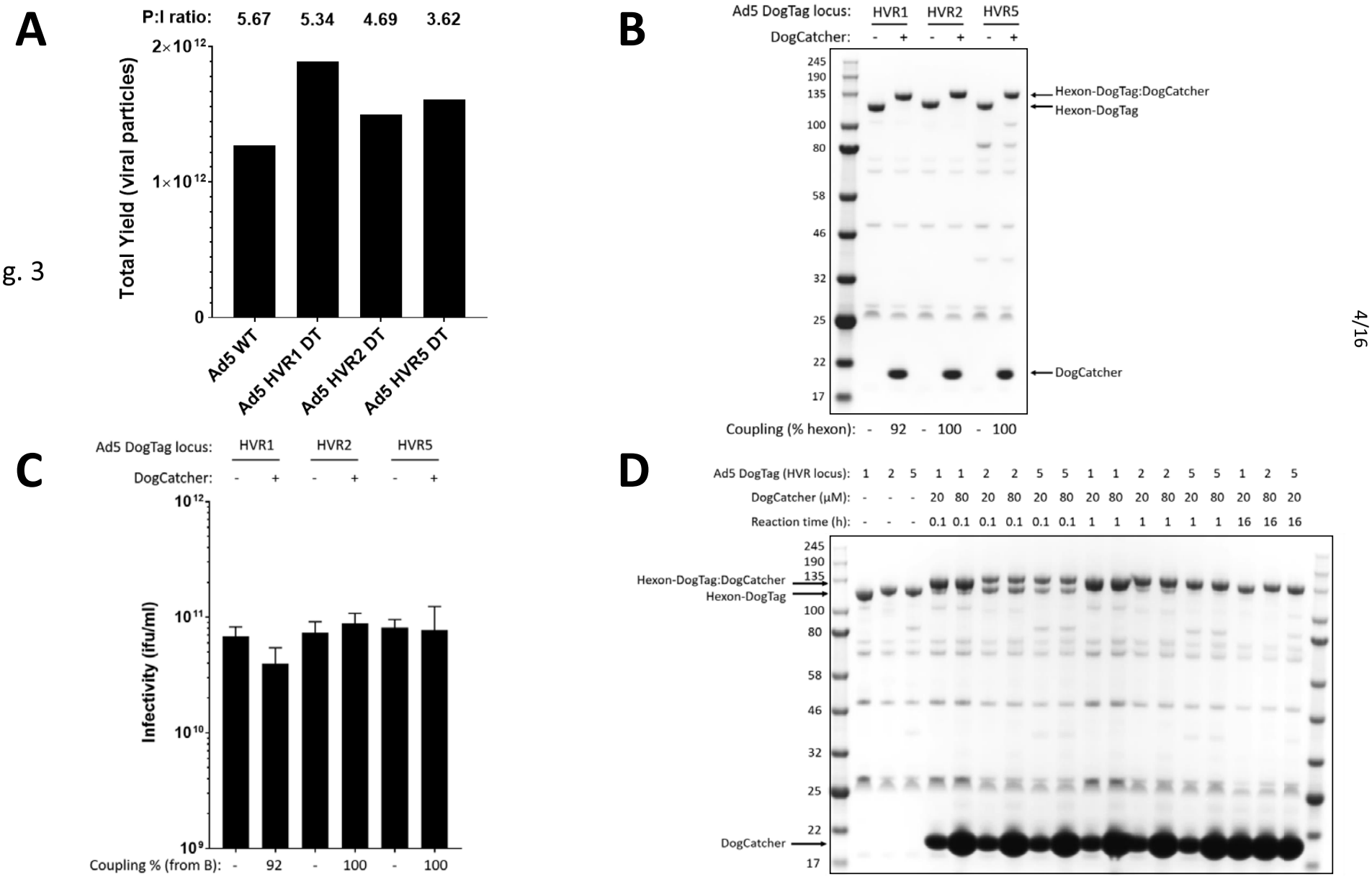
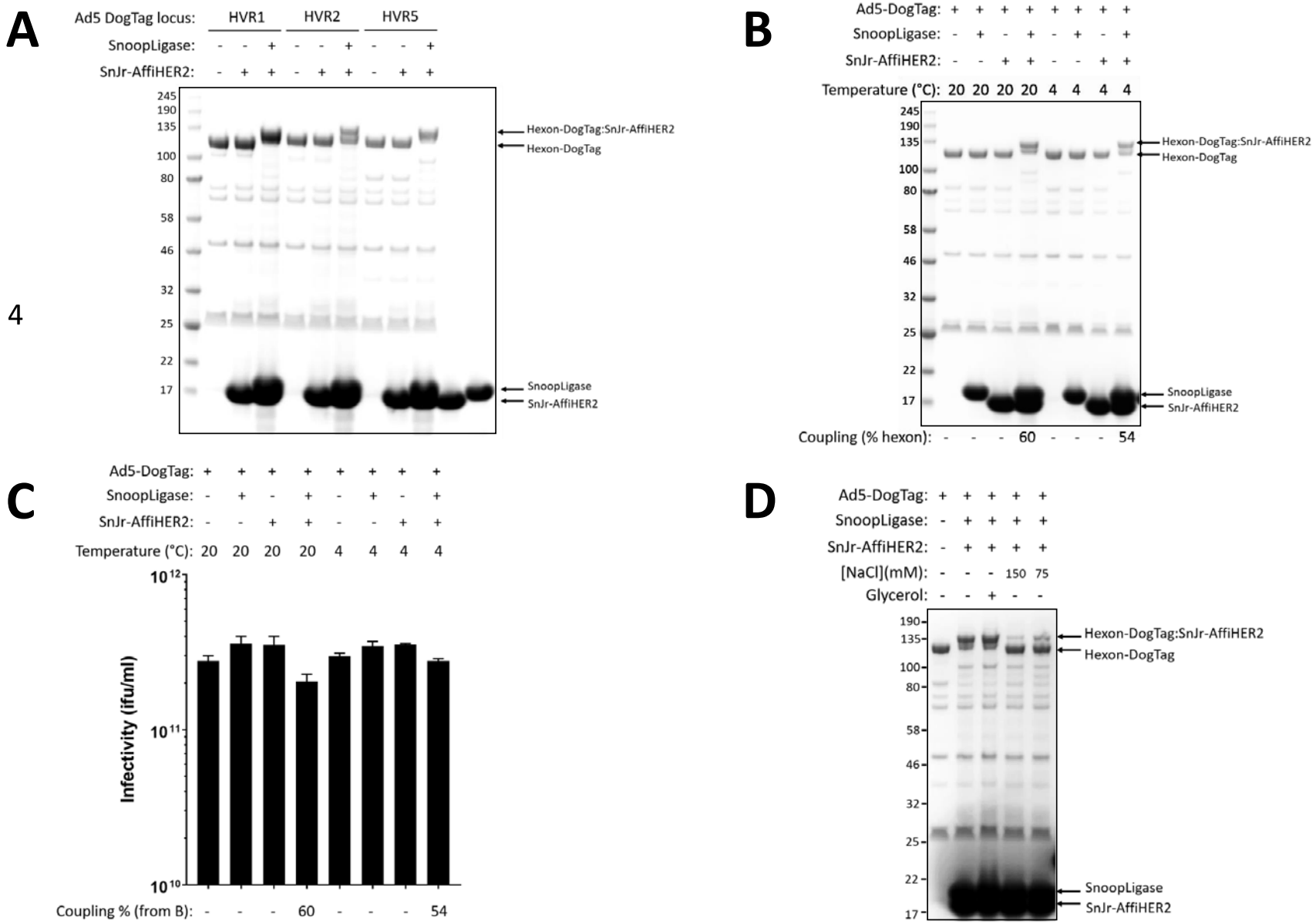

A
P:I ratio: 5.67 5.34 4.69 3.62
Total Yield (viral particles)
2×10^12
1×10^12
0
Ad5 WT
Ad5 HVR1 DT
Ad5 HVR2 DT
Ad5 HVR5 DT
B
Ad5 DogTag locus: HVR1 HVR2 HVR5
DogCatcher: - + - + - +
245
190
135
100
80
58
46
32
25
22
17
Hexon-DogTag:DogCatcher
Hexon-DogTag
DogCatcher
Coupling (% hexon): - 92 - 100 - 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

Fig. 4


A
Ad5 DogTag locus: HVR1 HVR2 HVR5
SnoopLigase: - - + - - + - - +
SnJr-AffiHER2: - + + - + + - + +
245
190
135
100
80
58
46
32
25
22
17
Hexon-DogTag:SnJr-AffiHER2
Hexon-DogTag
SnoopLigase
SnJr-AffiHER2
B
Ad5-DogTag: + + + + + + + +
SnoopLigase: - + - + - + - +
SnJr-AffiHER2: - - + + - - + +
Temperature (°C): 20 20 20 20 4 4 4 4
Coupling (% hexon): - - - 60 - - - 54
C
Ad5-DogTag: + + + + + + + +
SnoopLigase: - + - + - + - +
SnJr-AffiHER2: - - + + - - + +
Temperature (°C): 20 20 20 20 4 4 4 4
Infectivity (ifu/ml)
Coupling % (from B): - - - 60 - - - 54
D
Ad5-DogTag: + + + + +
SnoopLigase: - + + + +
SnJr-AffiHER2: - + + + +
[NaCl](mM): - - - 150 75
Glycerol: - - + - -

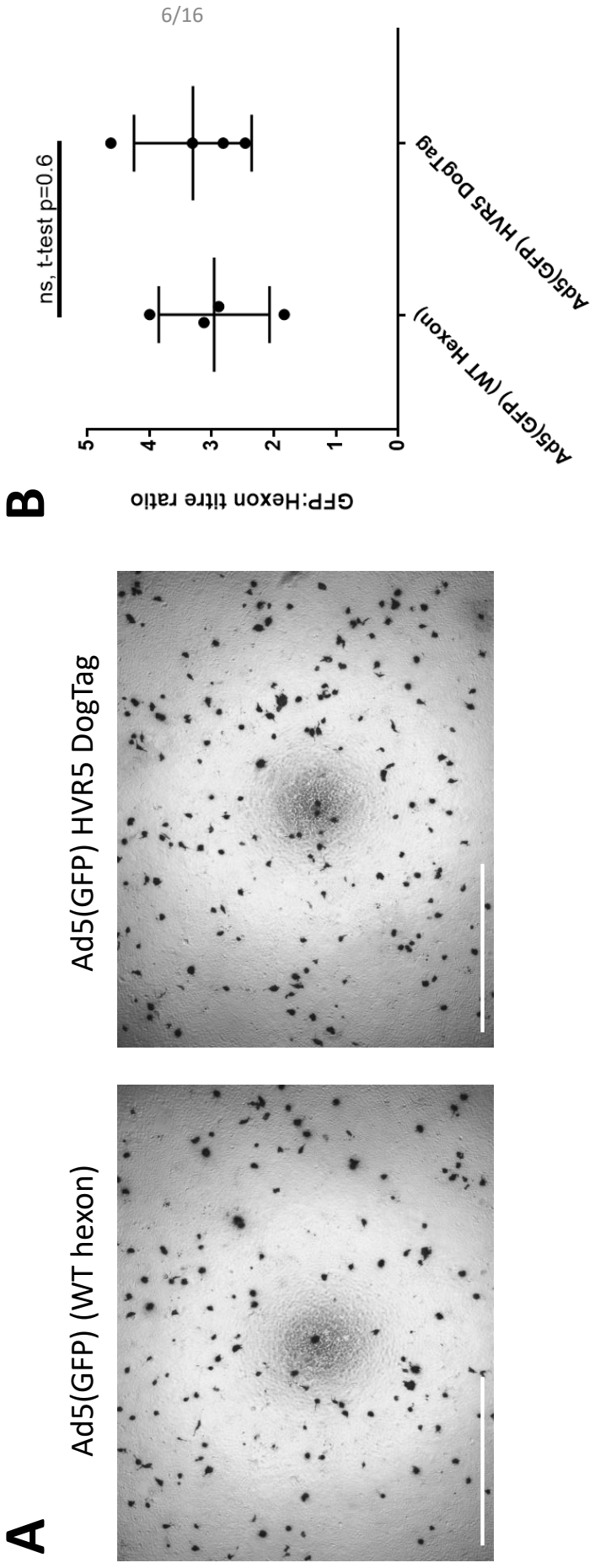
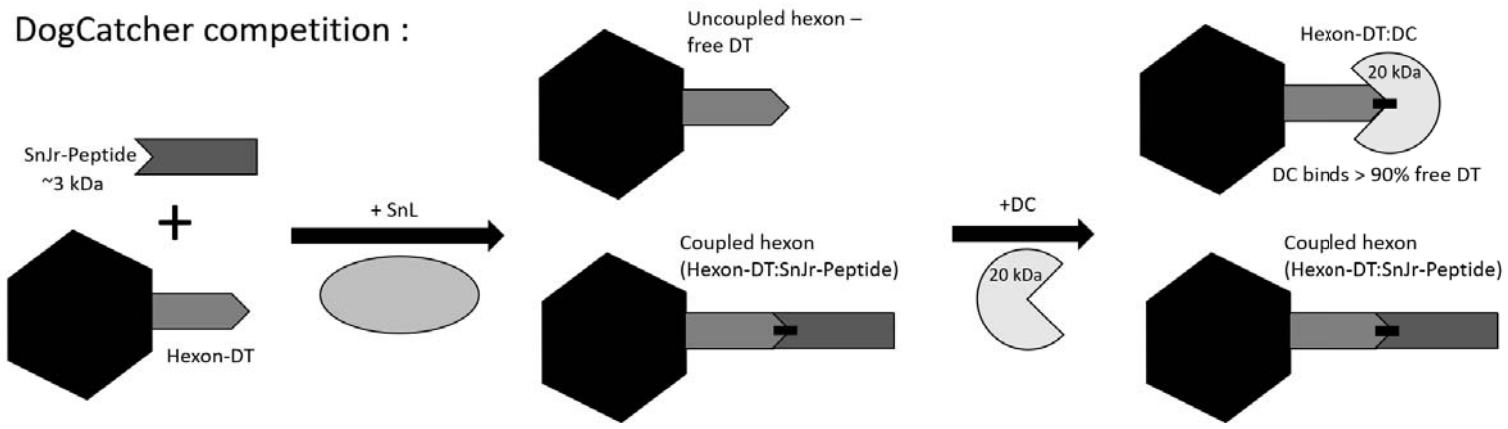
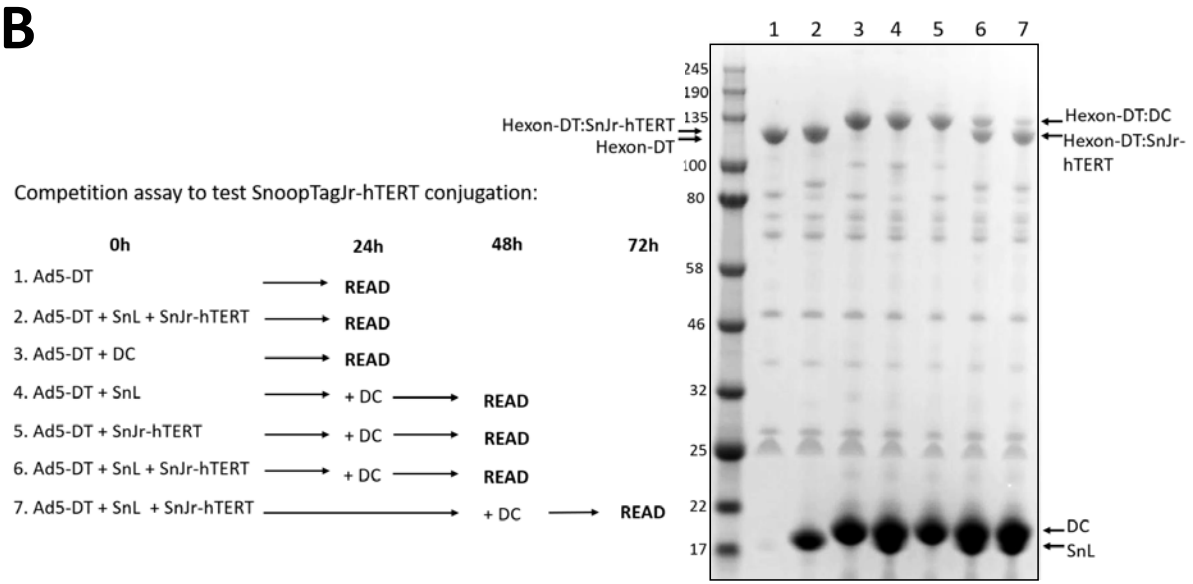

A
Ad5(GFP) (WT hexon)
Ad5(GFP) HVR5 DogTag
B
GFP:Hexon titre ratio
0
1
2
3
4
5
ns, t-test p=0.6
Ad5(GFP) (WT Hexon)
Ad5(GFP) HVR5 DogTag

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

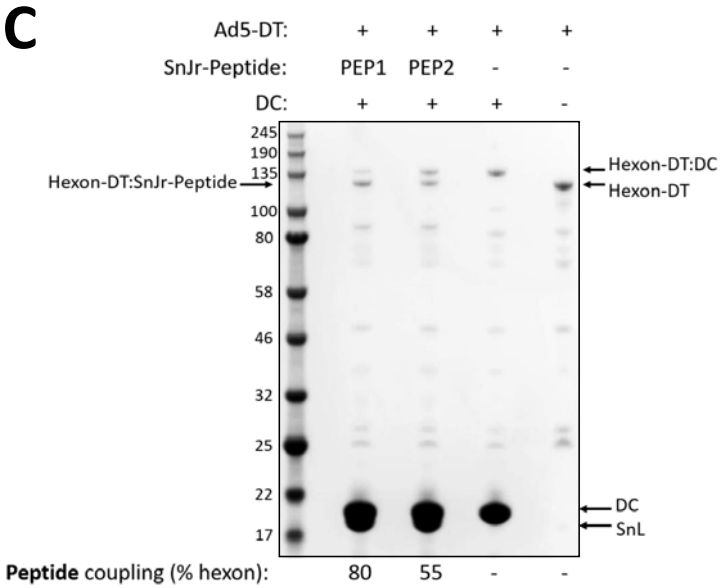
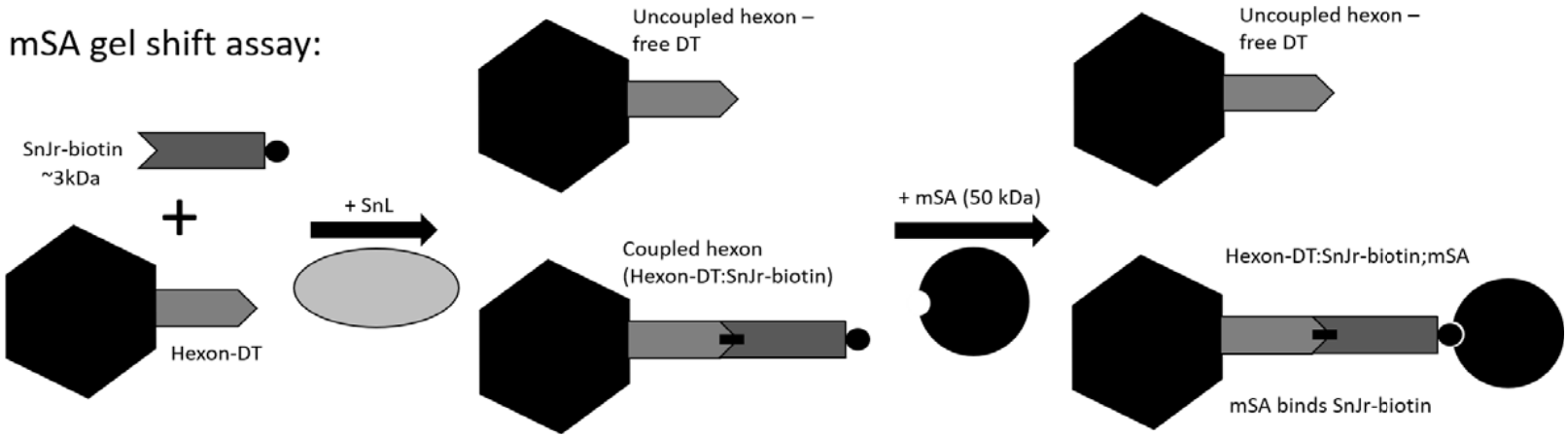
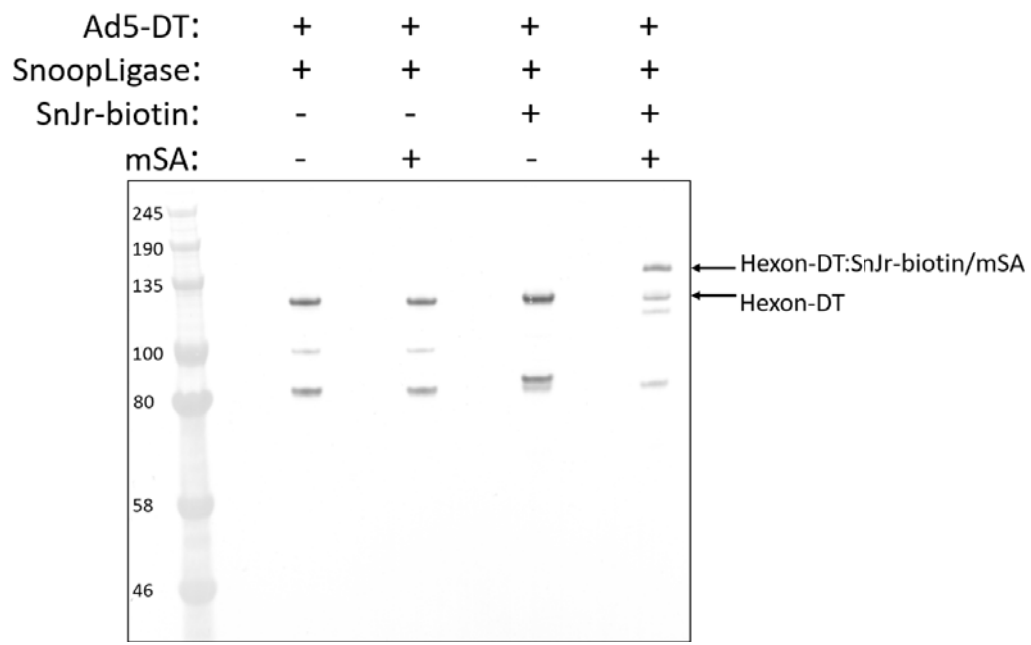

C
Ad5-DT: + + + +
SnJr-Peptide: PEP1 PEP2 - -
DC: + + + -
245
190
135
100
80
58
46
32
25
22
17
Hexon-DT:SnJr-Peptide
Hexon-DT:DC
Hexon-DT
DC
SnL
Peptide coupling (% hexon): 80 55 - -

Fig. 6

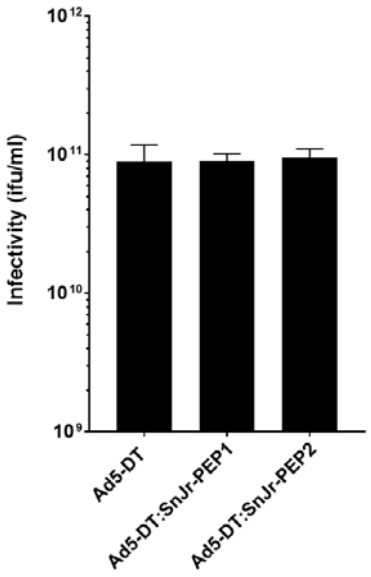
D

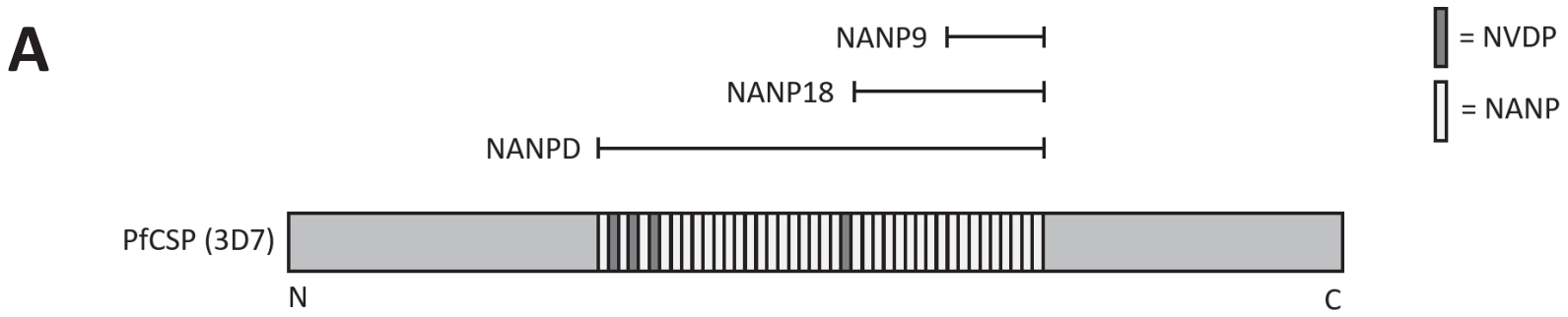

mSA gel shift assay:
SnJr-biotin
~3kDa
Hexon-DT
+ SnL
Uncoupled hexon –
free DT
Coupled hexon
(Hexon-DT:SnJr-biotin)
+ mSA (50 kDa)
Uncoupled hexon –
free DT
Hexon-DT:SnJr-biotin;mSA
mSA binds SnJr-biotin

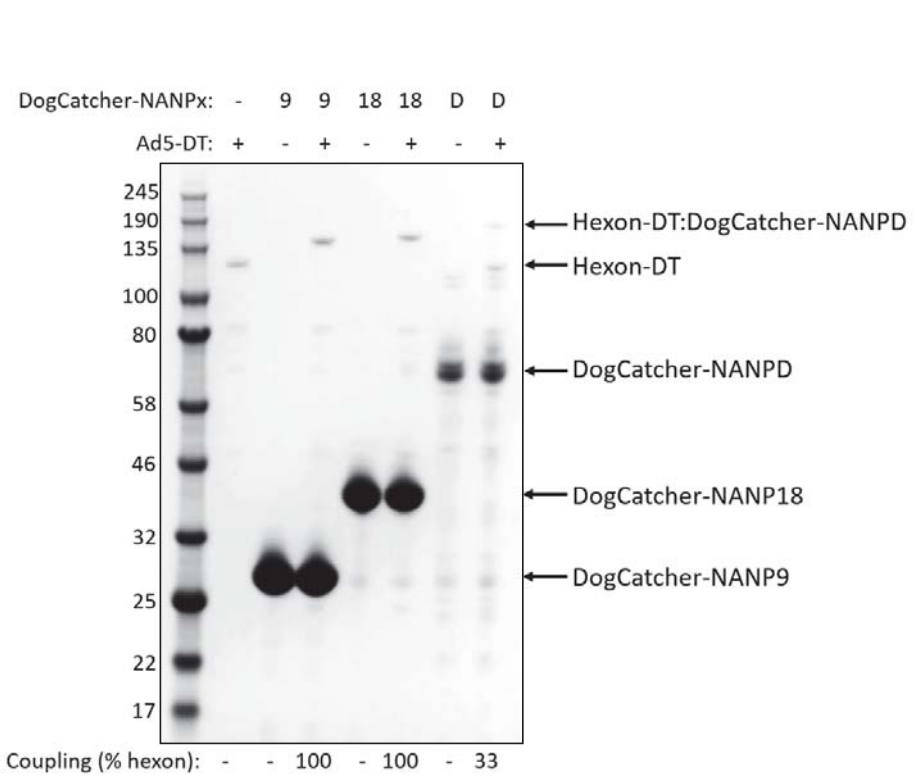
E


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
NANP9
NANP18
NANPD
= NVDP
= NANP
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

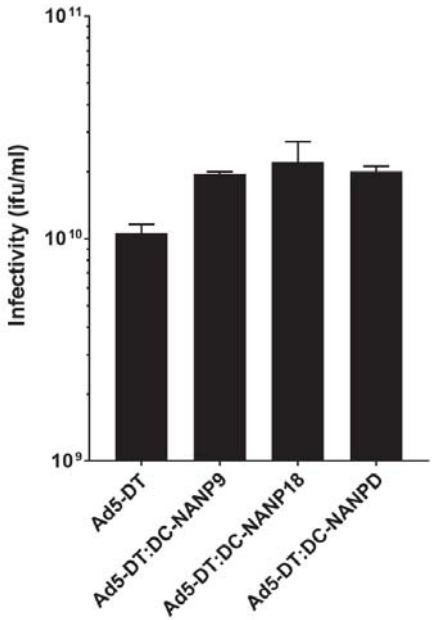

C
Infectivity (ifu/ml)
10^{11}
10^{10}
10^{9}
Ad5-DT
Ad5-DT:DC-NANP9
Ad5-DT:DC-NANP18
Ad5-DT:DC-NANPD

Fig. 7

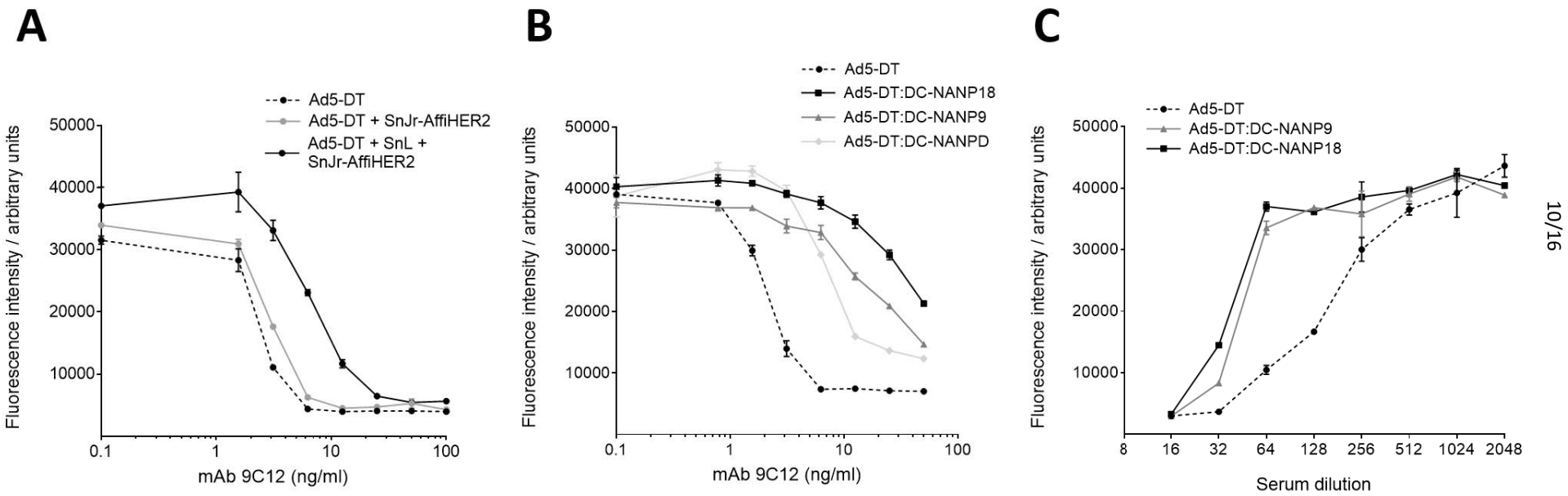

A
Ad5-DT
Ad5-DT + SnJr-AffiHER2
Ad5-DT + SnL + SnJr-AffiHER2
Fluorescence intensity / arbitrary units
50000
40000
30000
20000
10000
0.1
1
10
100
mAb 9C12 (ng/ml)
B
Ad5-DT
Ad5-DT:DC-NANP18
Ad5-DT:DC-NANP9
Ad5-DT:DC-NANPD
Fluorescence intensity / arbitrary units
50000
40000
30000
20000
10000
0.1
1
10
100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

Fig. 8

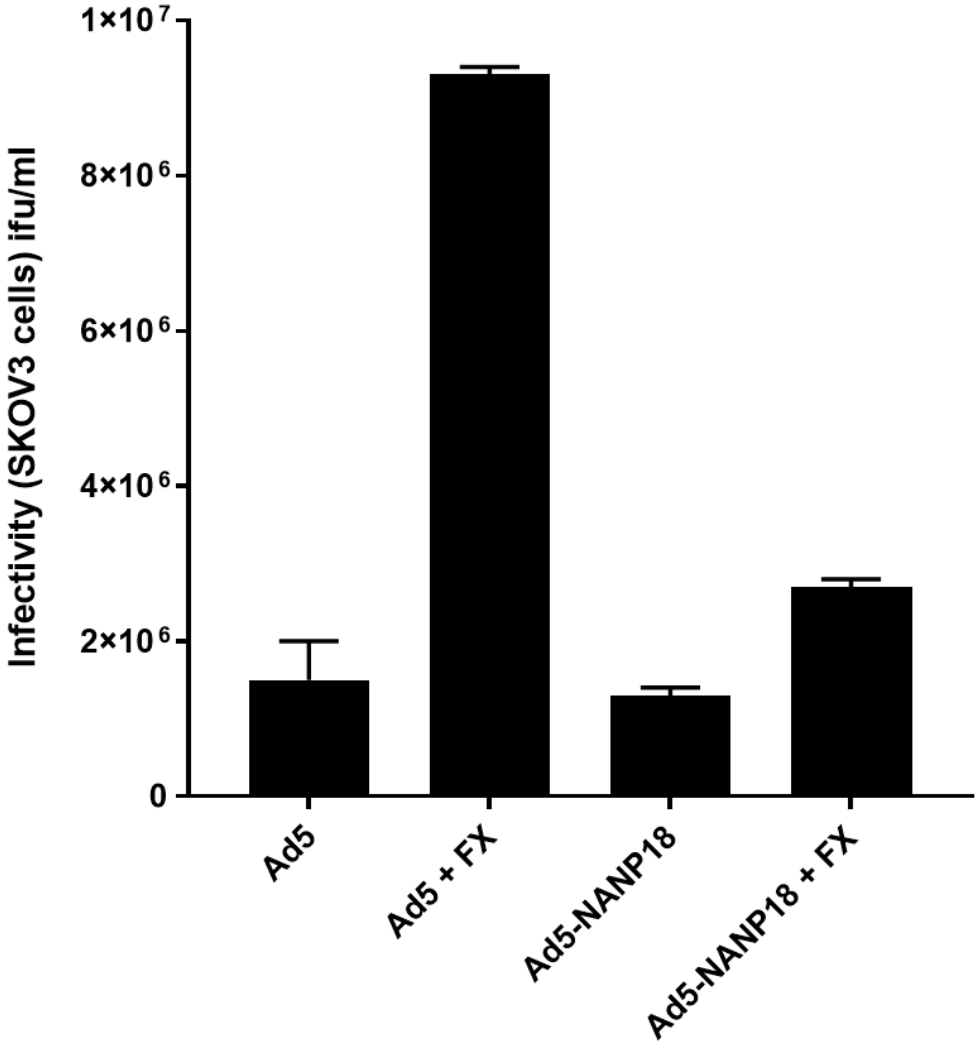

Infectivity (SKOV3 cells) ifu/ml
1×10^7
8×10^6
6×10^6
4×10^6
2×10^6
0
Ad5
Ad5 + FX
Ad5-NANP18
Ad5-NANP18 + FX

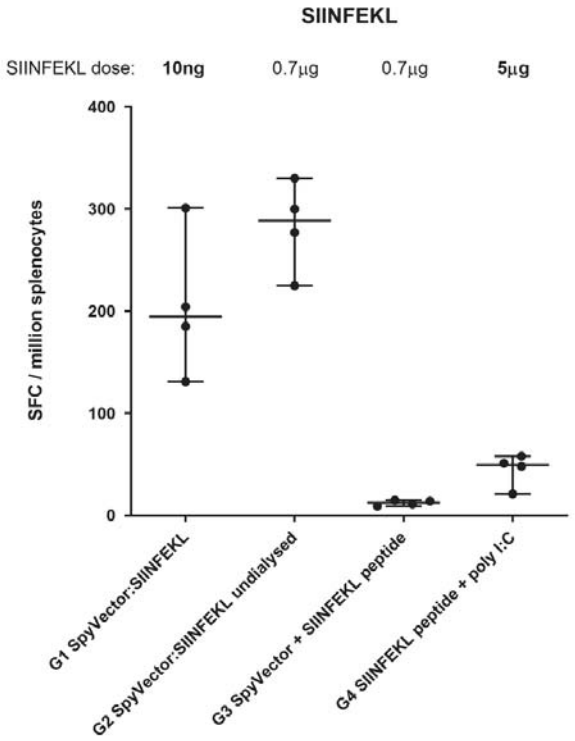
Fig. 9

A



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



C

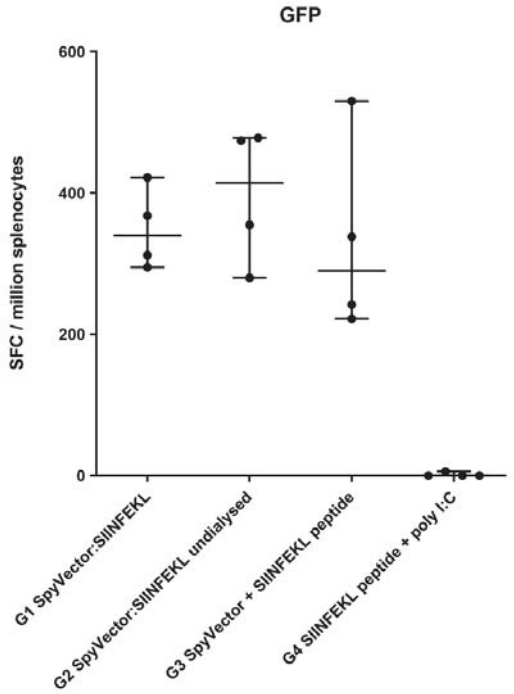
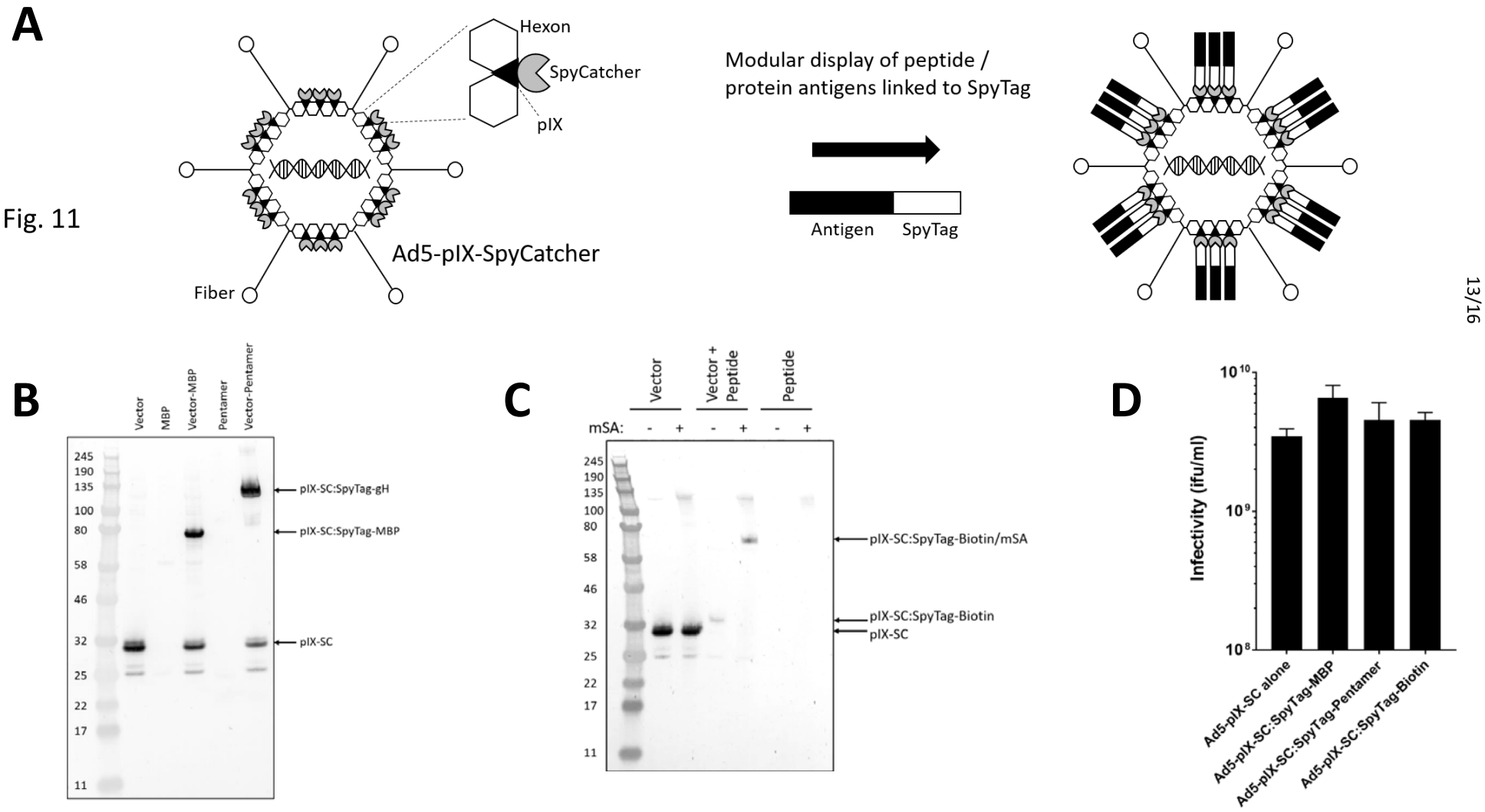


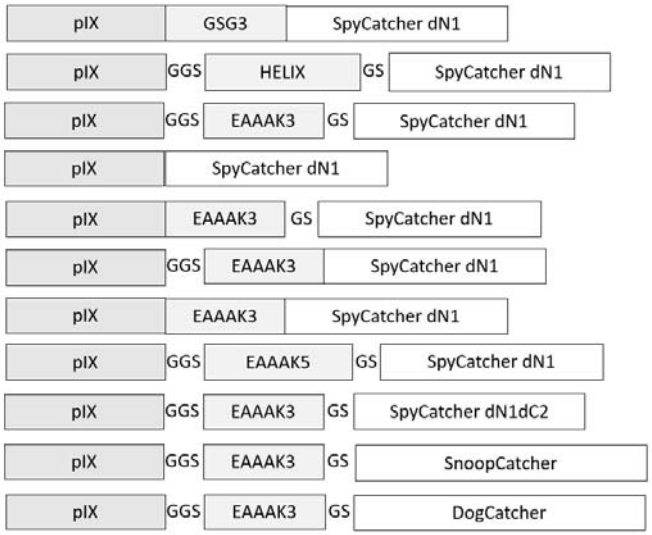
Fig. 10

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

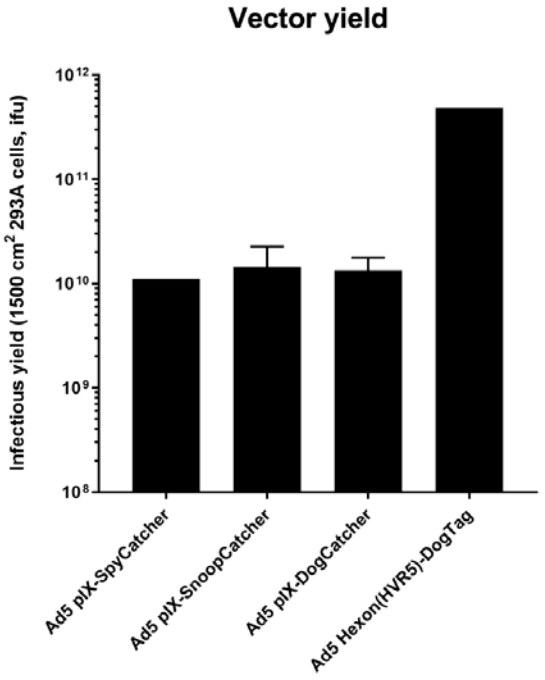


Fig. 12

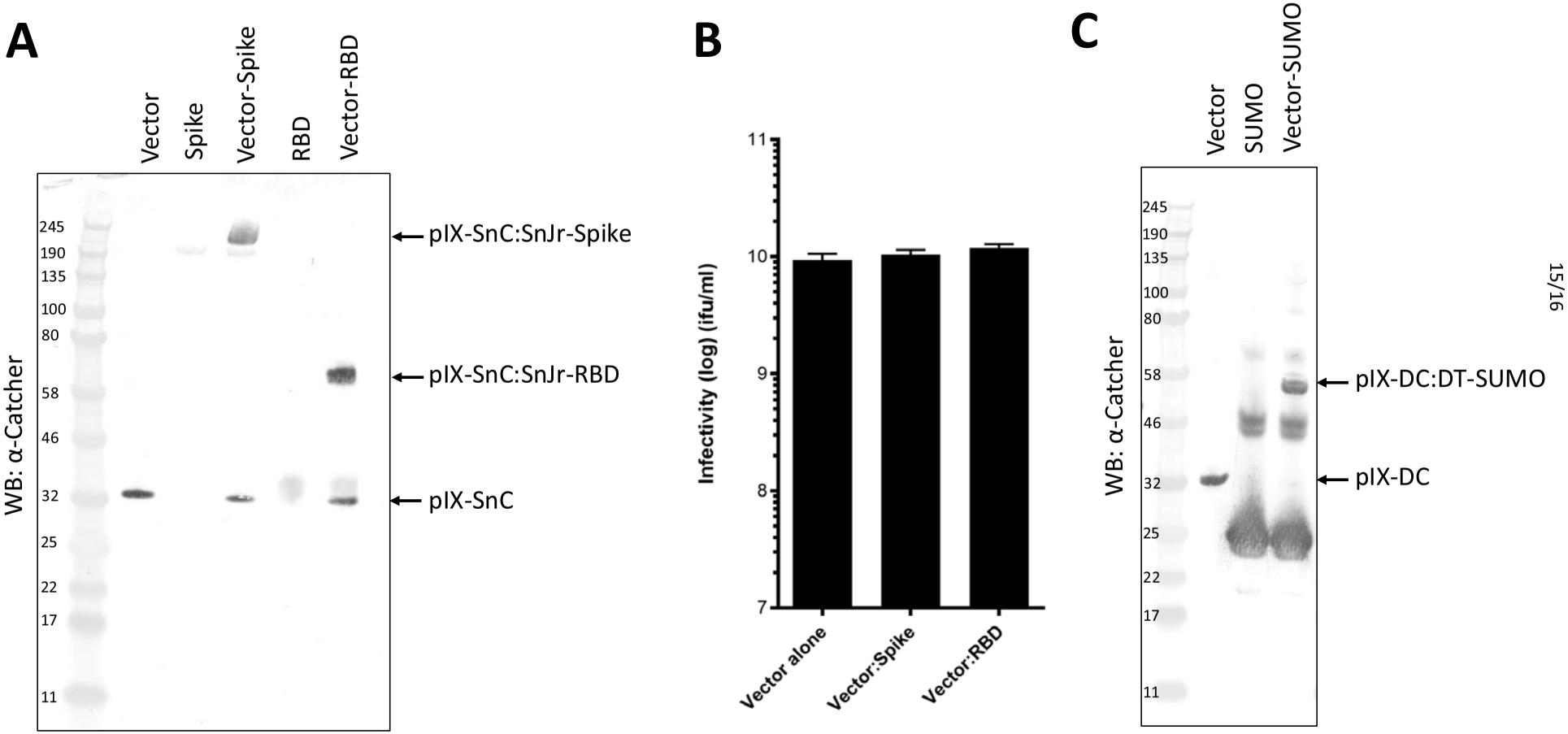

A
Vector
Spike
Vector-Spike
RBD
Vector-RBD
WB: α-Catcher
245
190
135
100
80
58
46
32
25
22
17
11
pIX-SnC:SnJr-Spike
pIX-SnC:SnJr-RBD
pIX-SnC
B
Infectivity (log) (ifu/ml)
11
10
9
8
7
Vector alone
Vector:Spike
Vector:RBD
C
Vector
SUMO
Vector-SUMO
WB: α-Catcher
245
190
135
100
80
58
46
32
25
22
17
11
pIX-DC:DT-SUMO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

Fig. 13

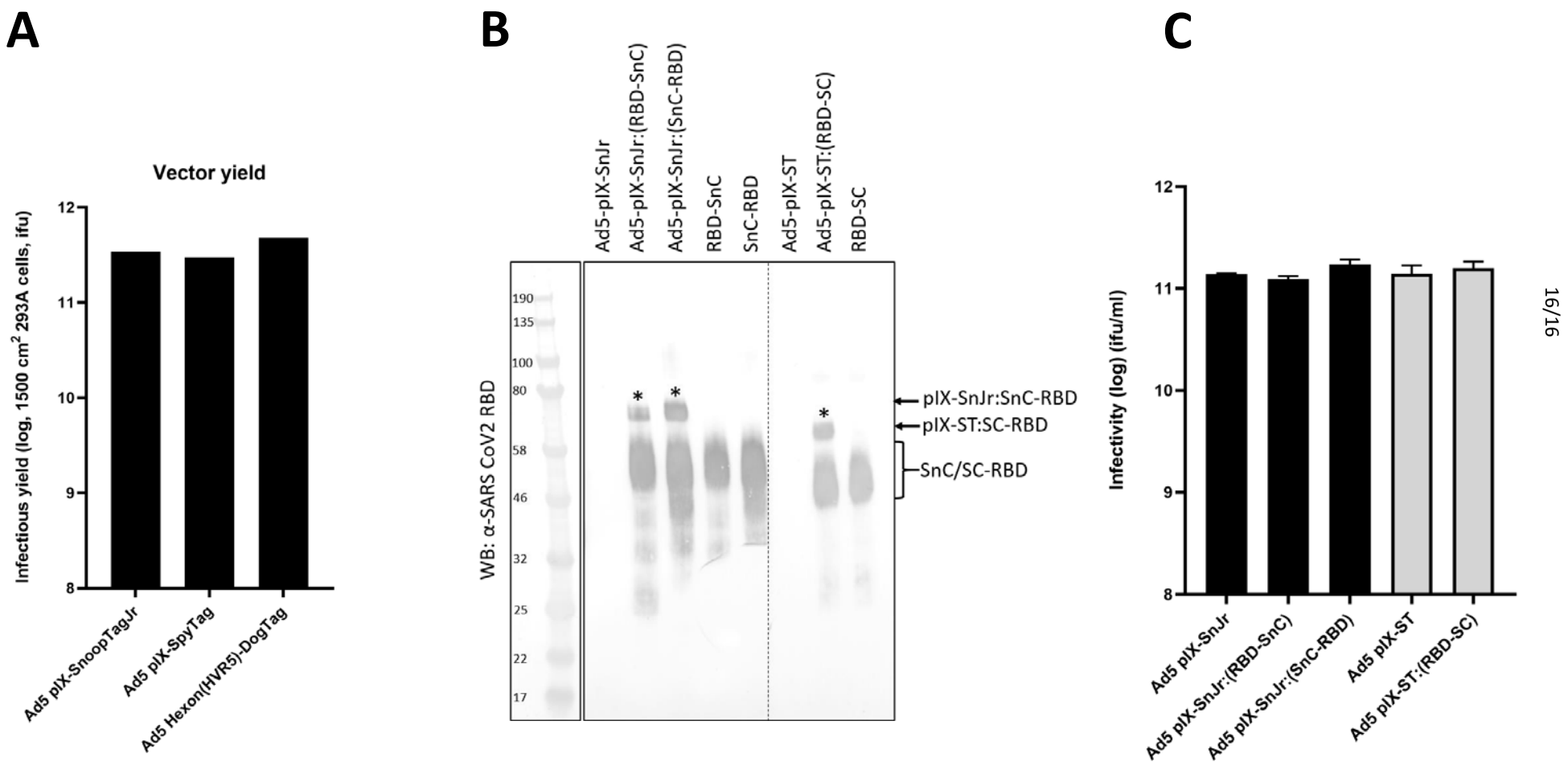

A
Vector yield
Infectious yield (log, 1500 cm2 293A cells, ifu)
12
11
10
9
8
Ad5 pIX-SnoopTagJr
Ad5 pIX-SpyTag
Ad5 Hexon(HVR5)-DogTag
B
Ad5-pIX-SnJr
Ad5-pIX-SnJr:(RBD-SnC)
Ad5-pIX-SnJr:(SnC-RBD)
RBD-SnC
SnC-RBD
Ad5-pIX-ST
Ad5-pIX-ST:(RBD-SC)
RBD-SC
WB: α-SARS CoV2 RBD
190
135
100
80
58
46
32
25
22
17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