Figure 1A

Unmodified HPXV genome (strain MNR-76; GI DQ792504)

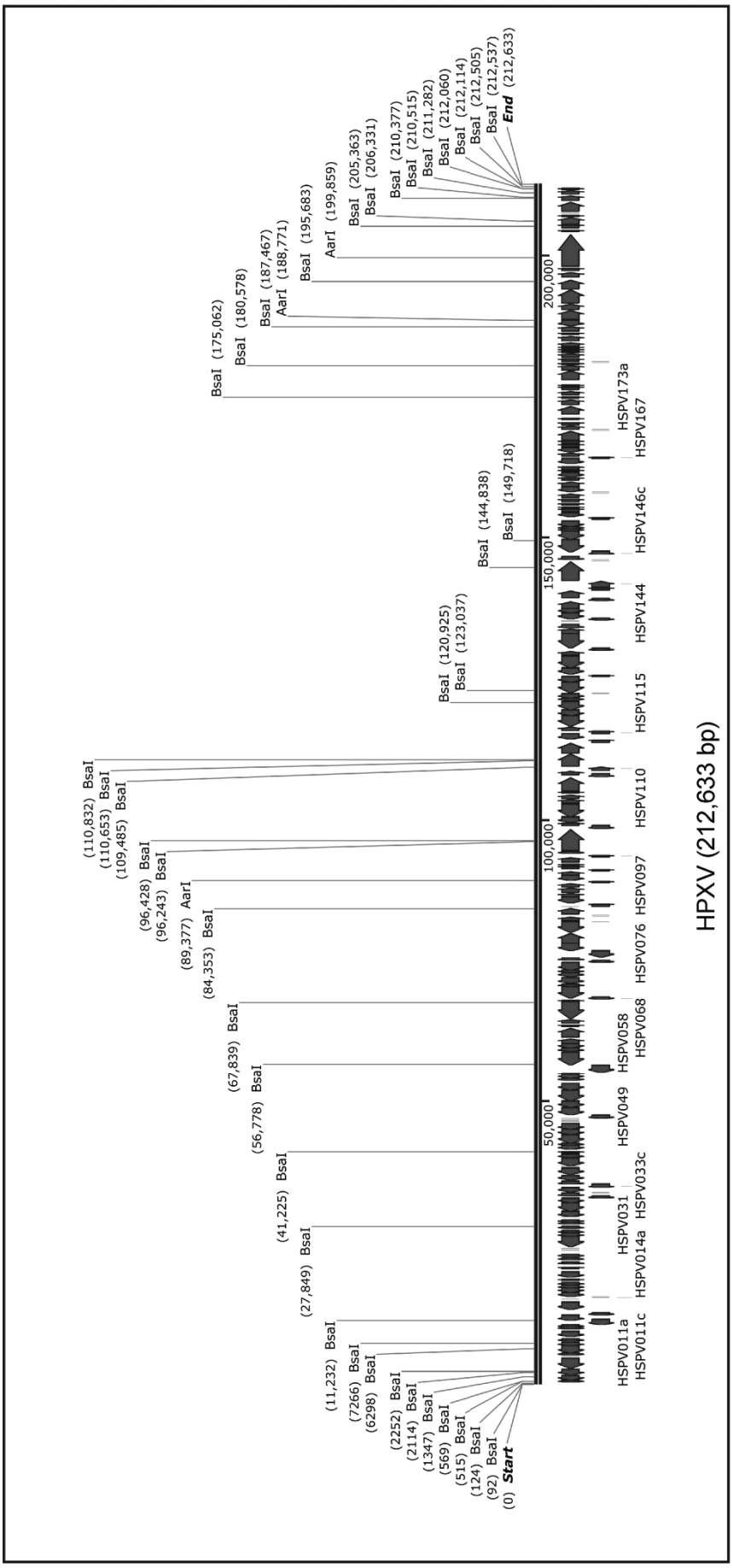


Figure 1B

scHPXV genome used for DNA synthesis

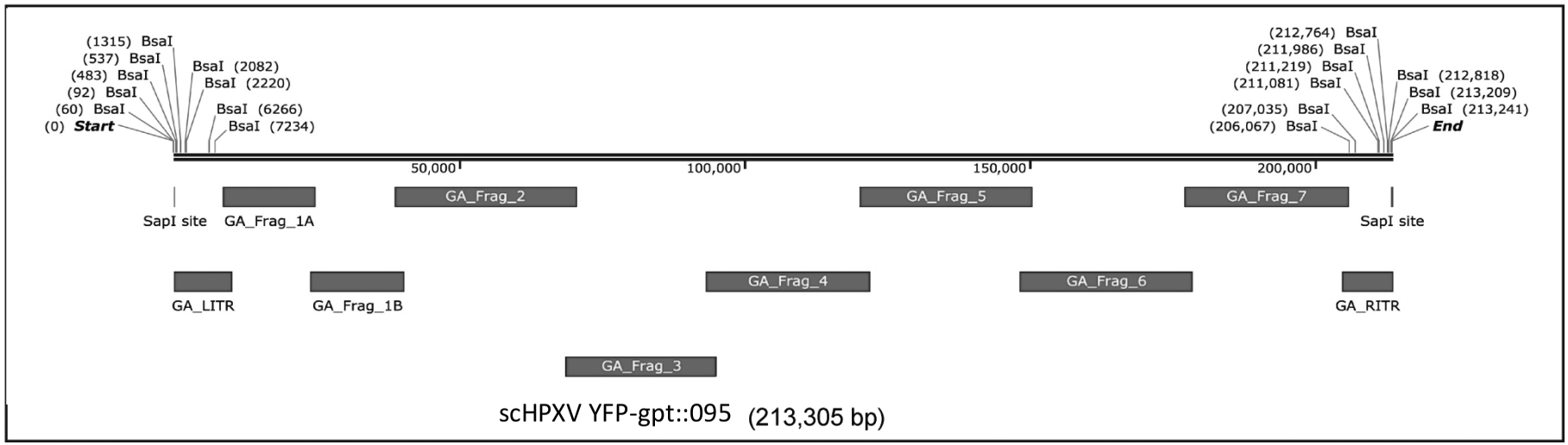
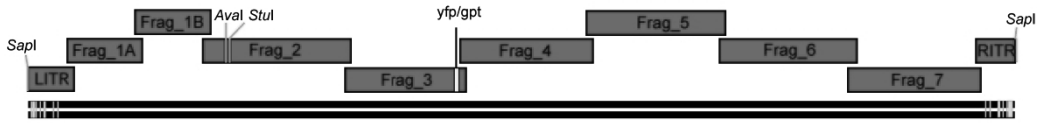


Figure 2

A **Modified scHPXV genome**



scHPXV YFP-gpt::095 genome
213, 305 bp

B Nucleotide sequence of VACV (WR strain) terminal hairpin loops with concatamer resolution sites

S Form

5′-ACATTTTTTTCTAGACACTAAATAAAAATTTAAAATATAATATTAATGTACTAAAACTATATATTA
TTAATTTATCTAACTAAAGTTAGTAAATTATATATATAATTTTATAATTAATTTAATTTTACTATATTT
TATTTAGTGTCTAGAAAAAAA-3′

F Form

5′-ACATTTTTTTCTAGACACTAAATAAAATTAGTAAAATTAAATTAATTATAAAATTATATATATAAT
TTACTAACTTTAGTTAGATAAATTAATAATATATAAGTTTTAGTACATTAATATTATATTTTAAATATT
TTATTTAGTGTCTAGAAAAAAA-3′

C Secondary structure of VACV (WR) terminal loops

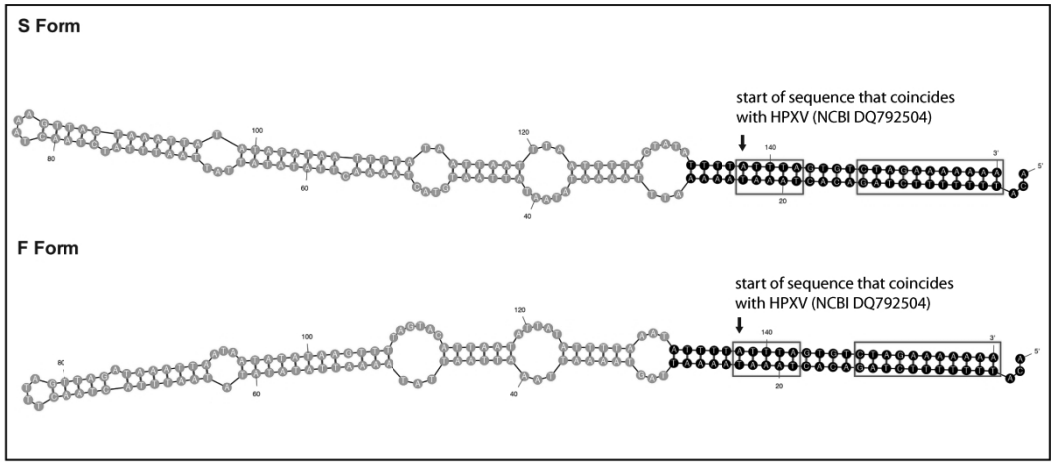


Figure 3A

Schematic diagram of scHPXV YFP-gpt::095 left and right ITR digested with *PvuII*

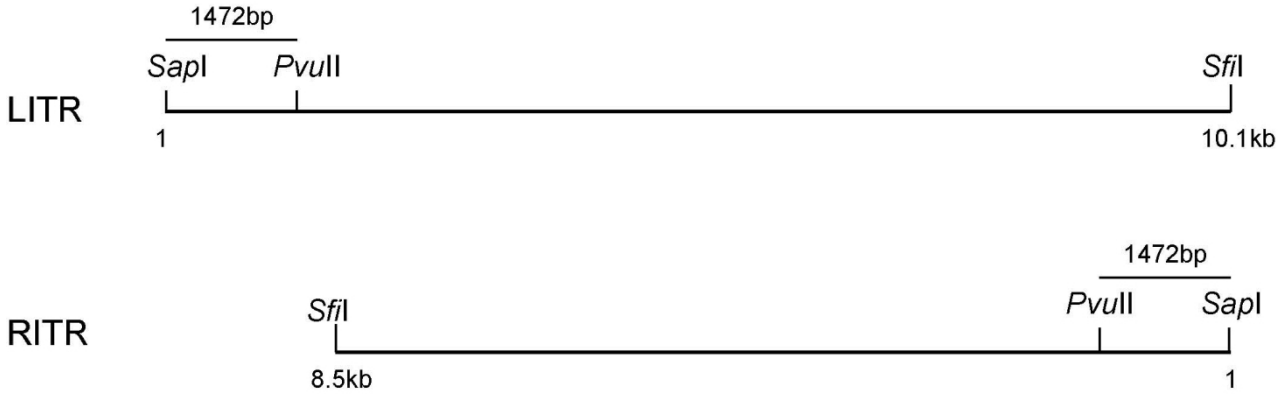


Figure 3B

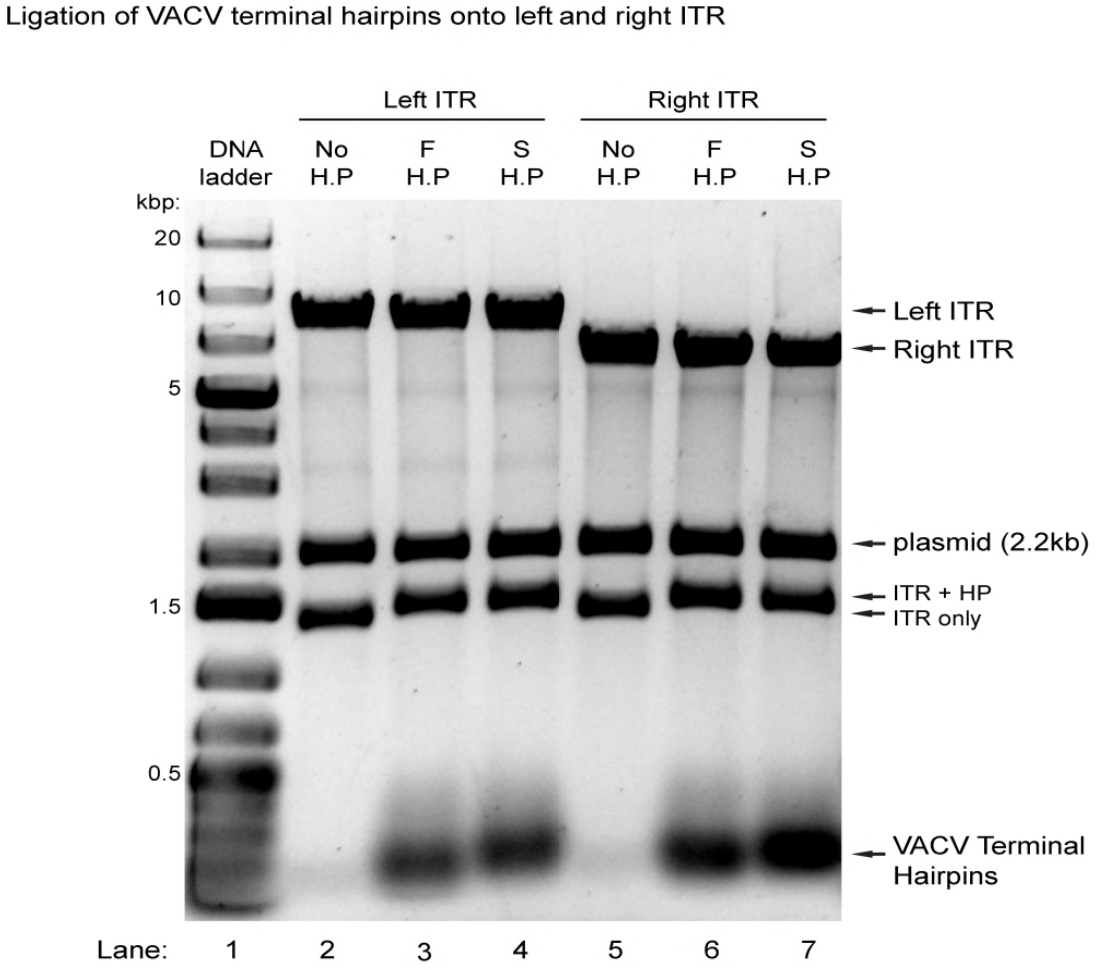

Ligation of VACV terminal hairpins onto left and right ITR
Left ITR
Right ITR
DNA ladder
No H.P
F H.P
S H.P
No H.P
F H.P
S H.P
kbp:
20
10
5
1.5
0.5
Left ITR
Right ITR
plasmid (2.2kb)
ITR + HP
ITR only
VACV Terminal Hairpins
Lane:
1
2
3
4
5
6
7

Figure 4A

PCR analysis of *BsaI* sites in VACV and scHPXV YFP-gpt::095 (plaque pick 1 and 3)

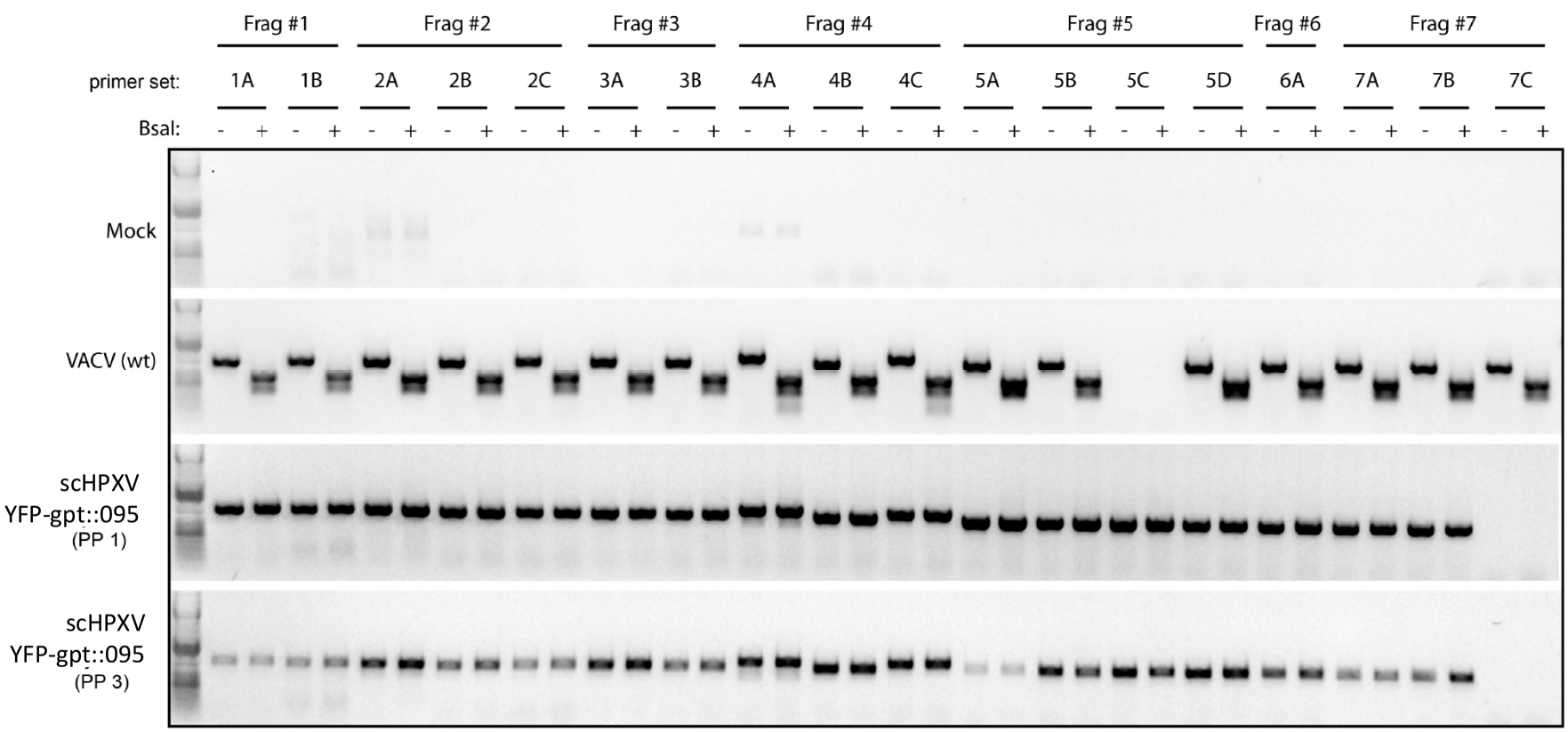


Figure 4B

PFGE of scHPXV YFP-gpt::095 following *BsaI* and *HindIII* digest

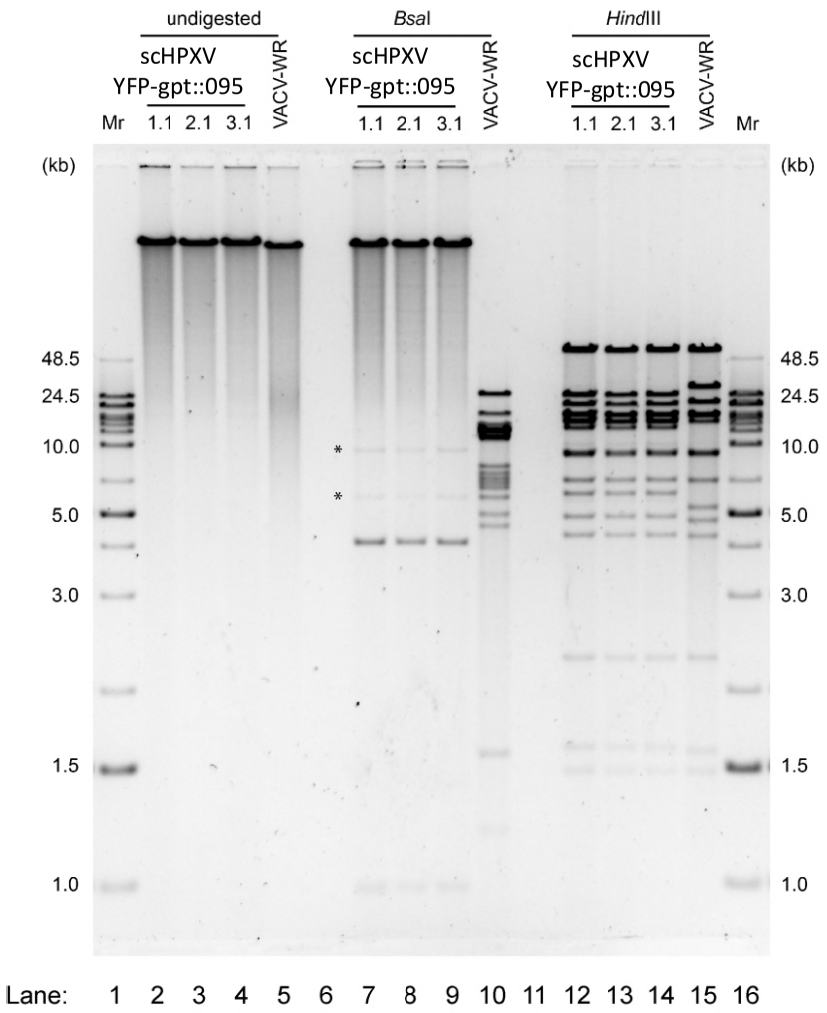


Figure 4C

Agarose electrophoresis of scHPXV YFP-gpt::095 following *BsaI* and *HindIII* digest

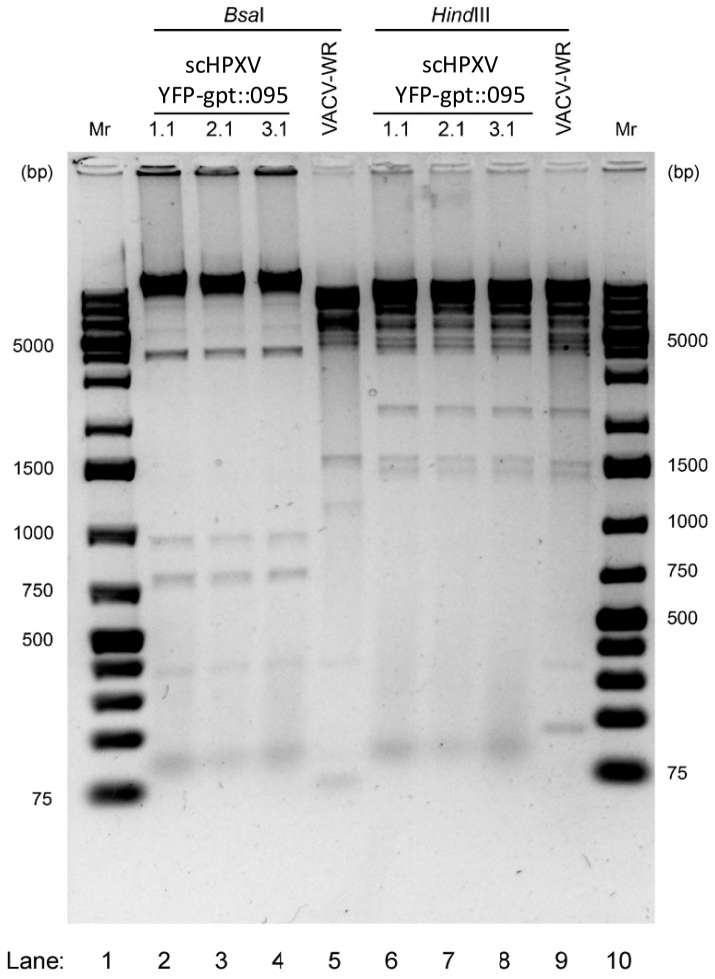


Figure 5A


scHPXV
YFP-gpt::095
(Cs on end)
Consensus
Coverage
Illumina Reads
SapI
Terminal Loop
Concatamer Resolution site
VACV WR terminal hairpin loop

Figure 5B

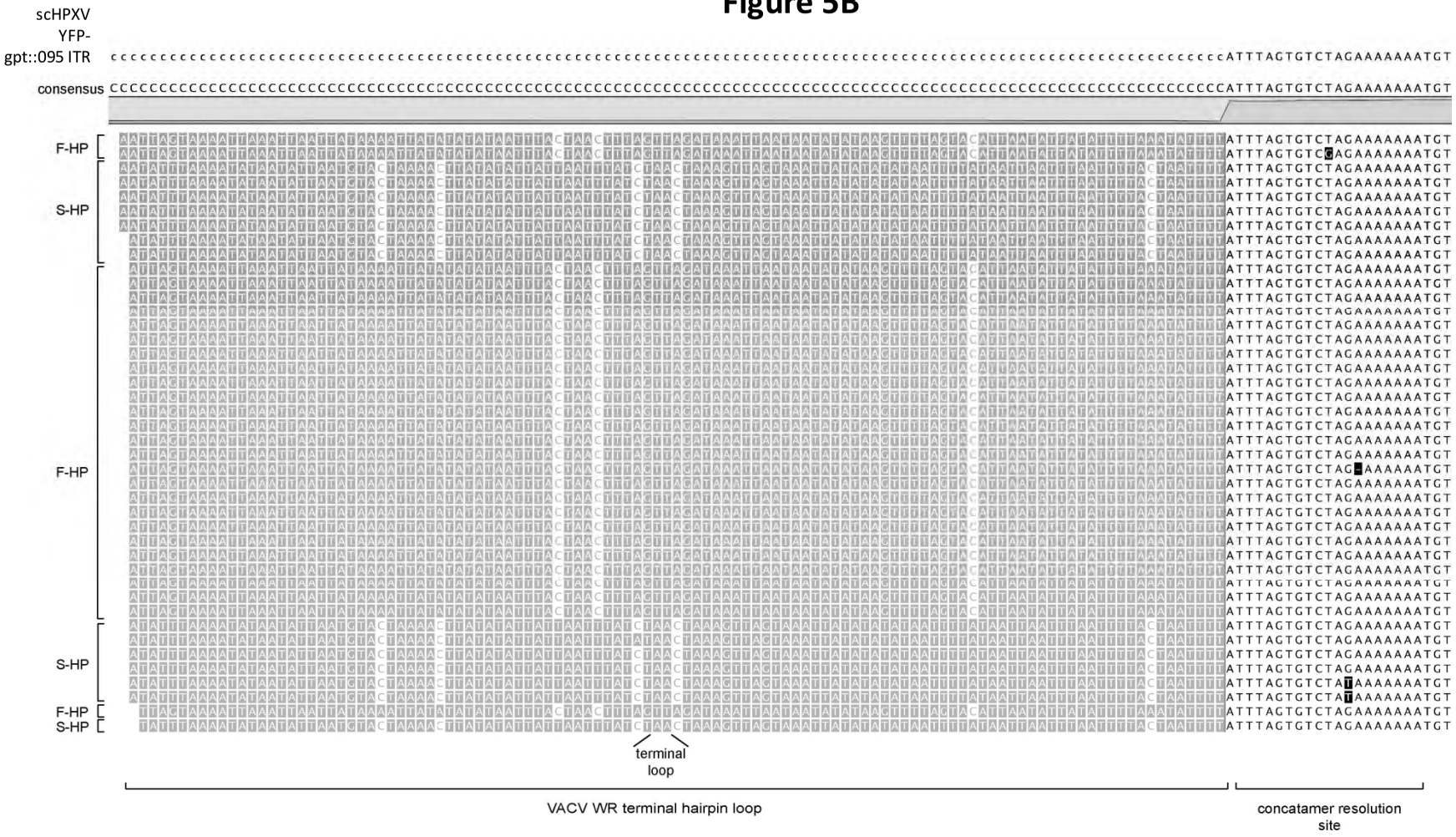

scHPXV YFP-gpt::095 ITR
consensus
F-HP
S-HP
F-HP
S-HP
F-HP
S-HP
terminal loop
VACV WR terminal hairpin loop
concatamer resolution site

Figure 6A

Overall Illumina read coverage in scHPXV YFP-gpt::095 region 96,050 to 96,500

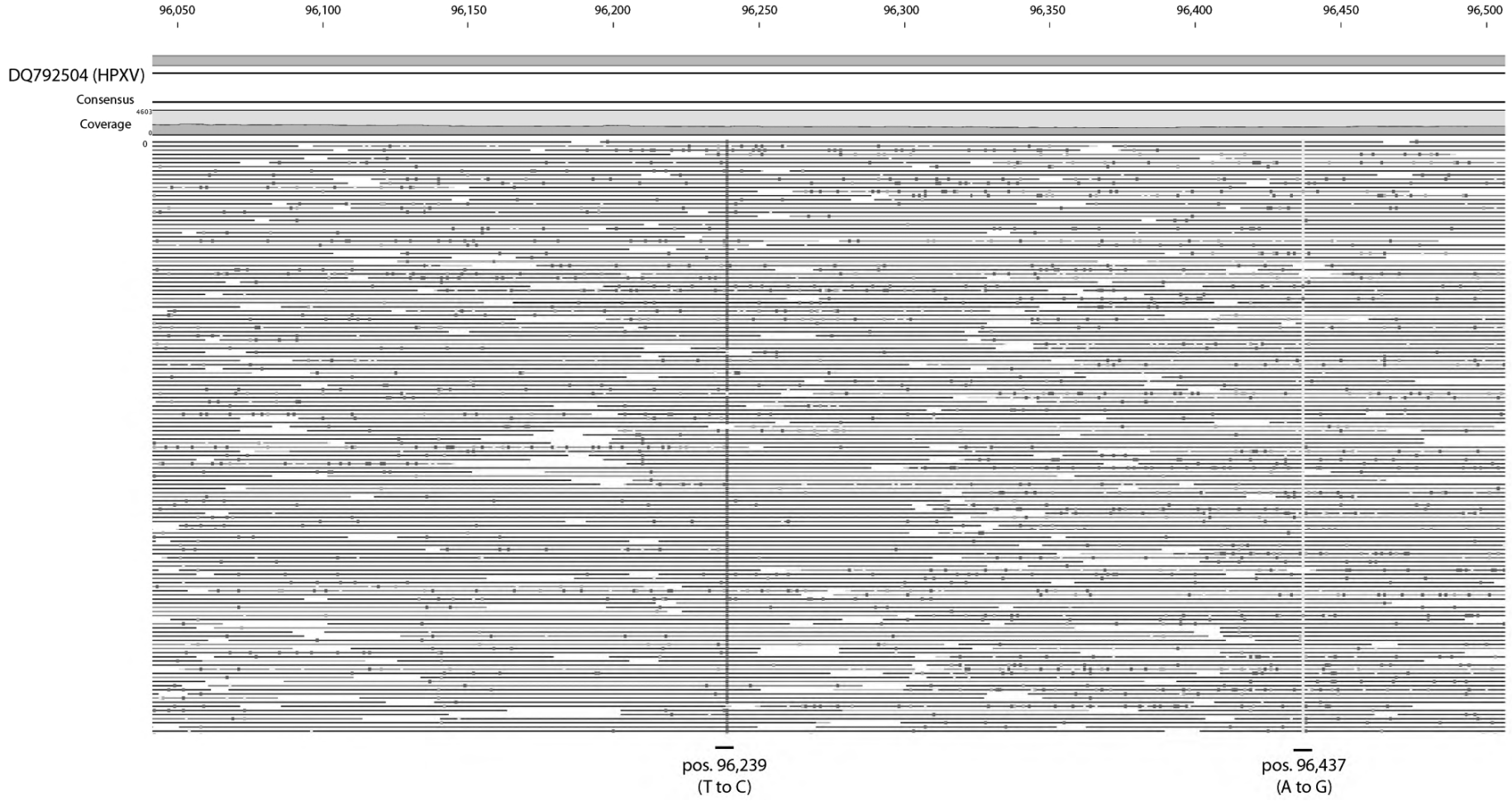


Figure 6B

*Bsa*I mutation at pos. 96239

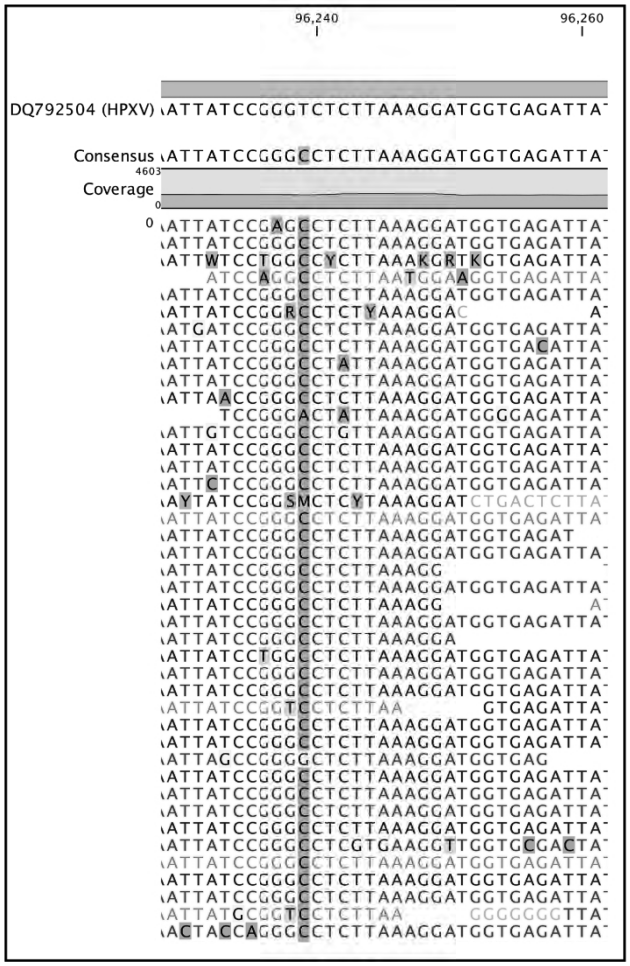


Figure 6C

*Bsa*I mutation at pos. 96437

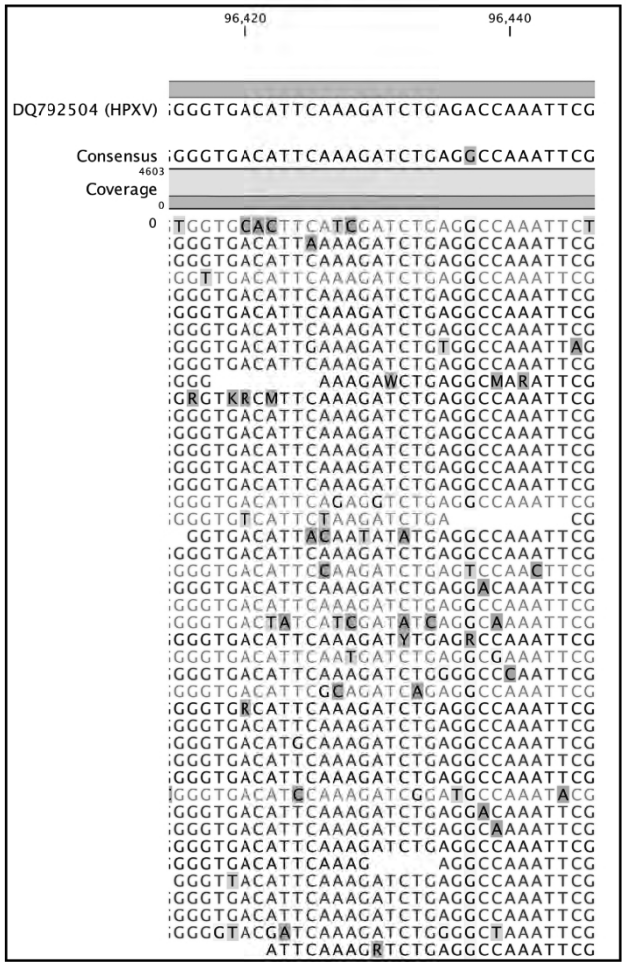


Figure 7A

Nucleotide changes in HPXV044 to create unique restriction sites in scHPXV YFP-gpt::095

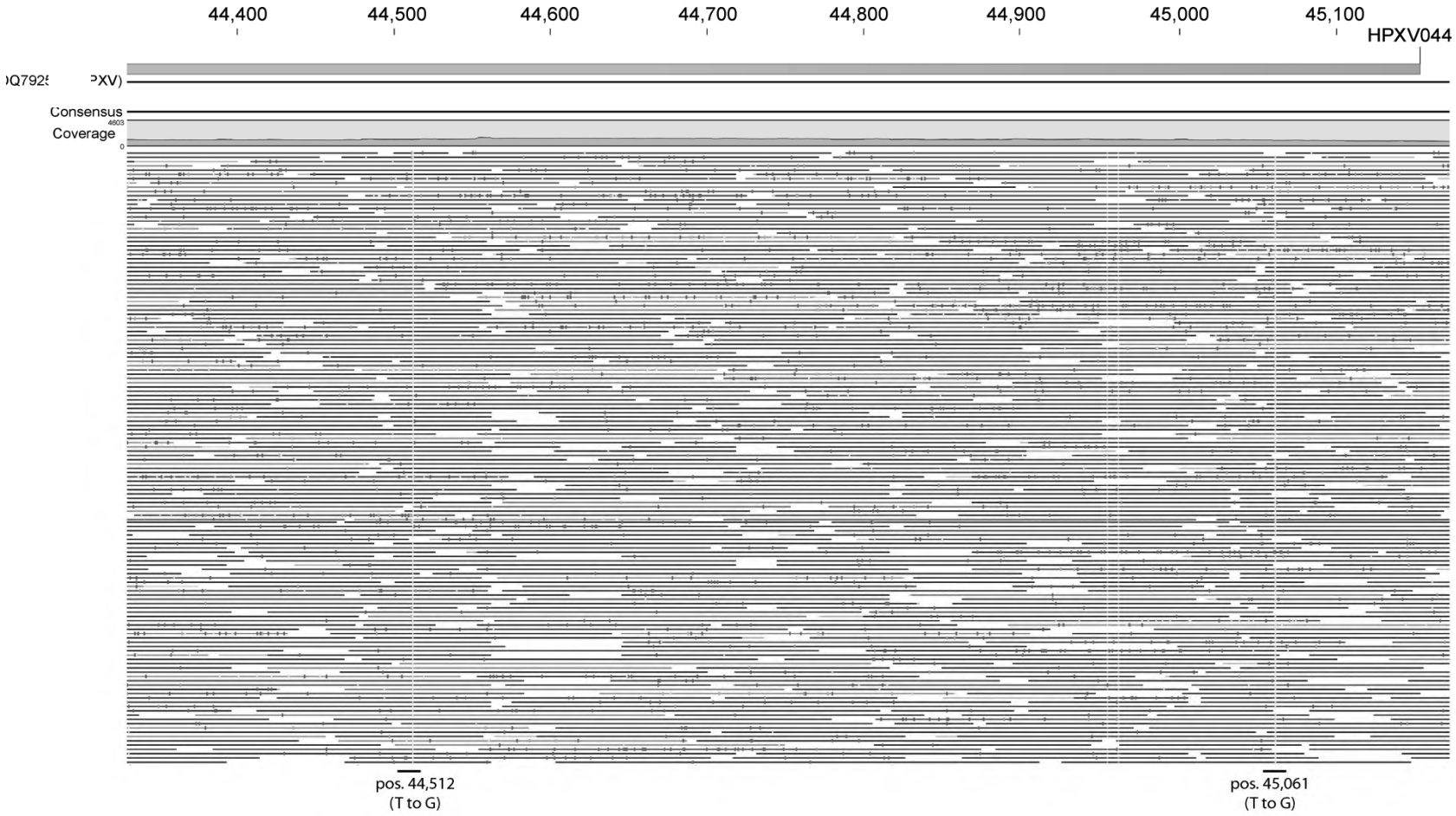


Figure 7B

Nucleotide change in HPXV044 at pos. 44,512
(Creation of *Ava*I restriction site)

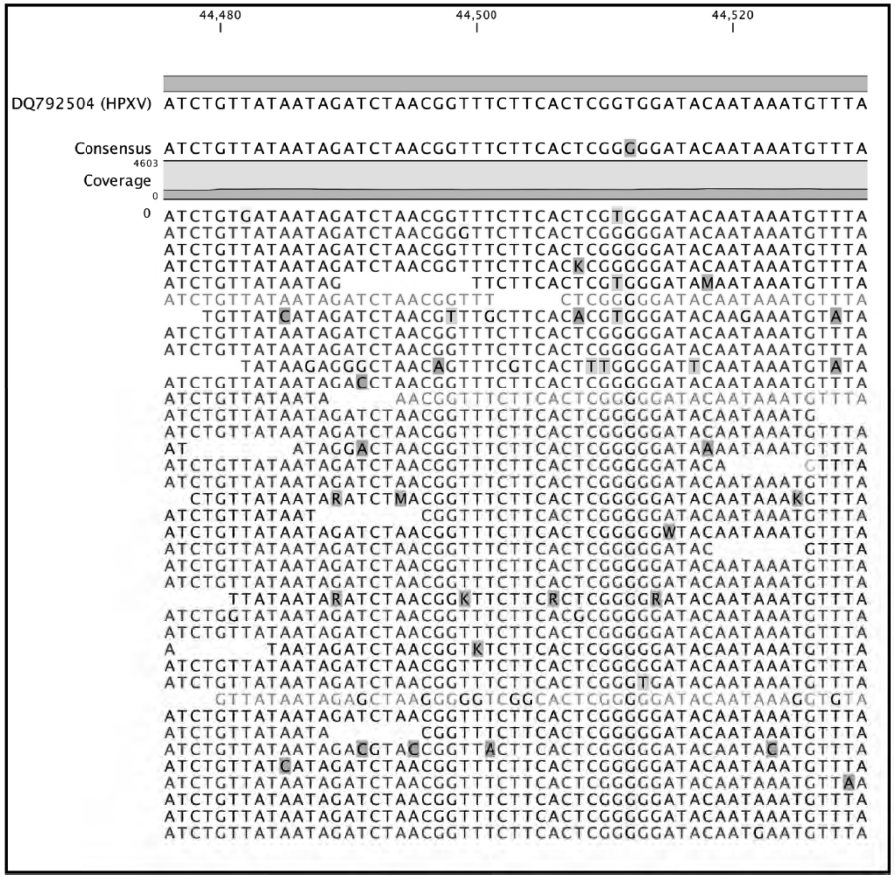


Figure 7C

Nucleotide change in HPXV044 at pos. 45,061
(Creation of *Stu*I restriction site)

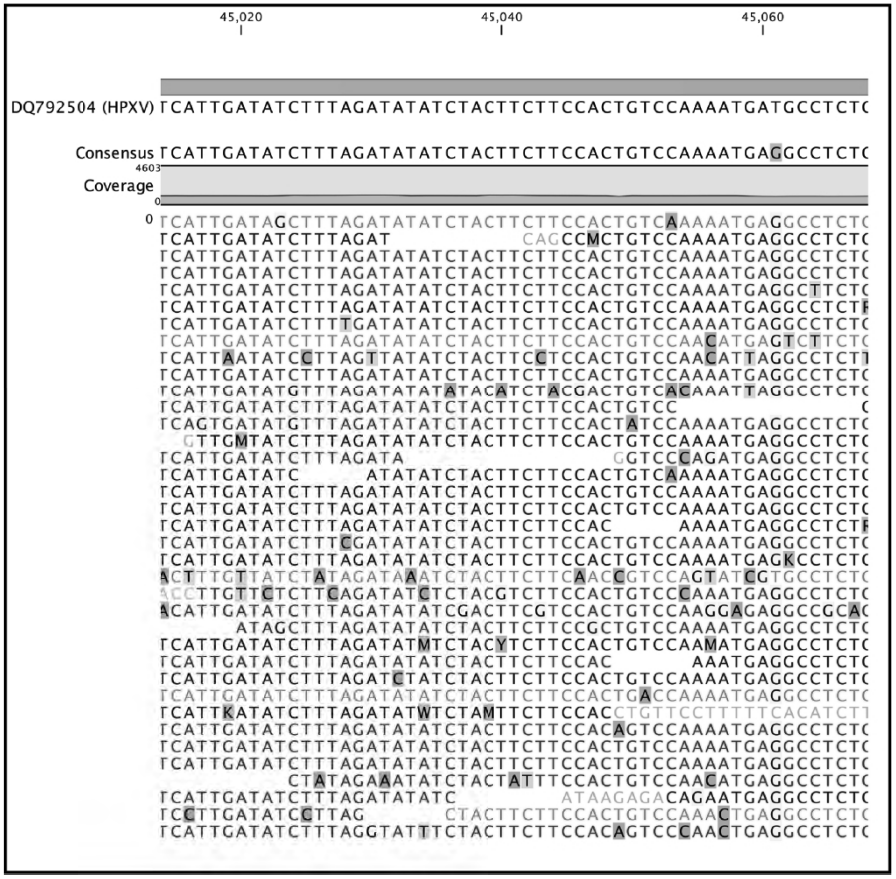


Figure 8A

Growth of scHPXV YFP-gpt::095 in cell lines

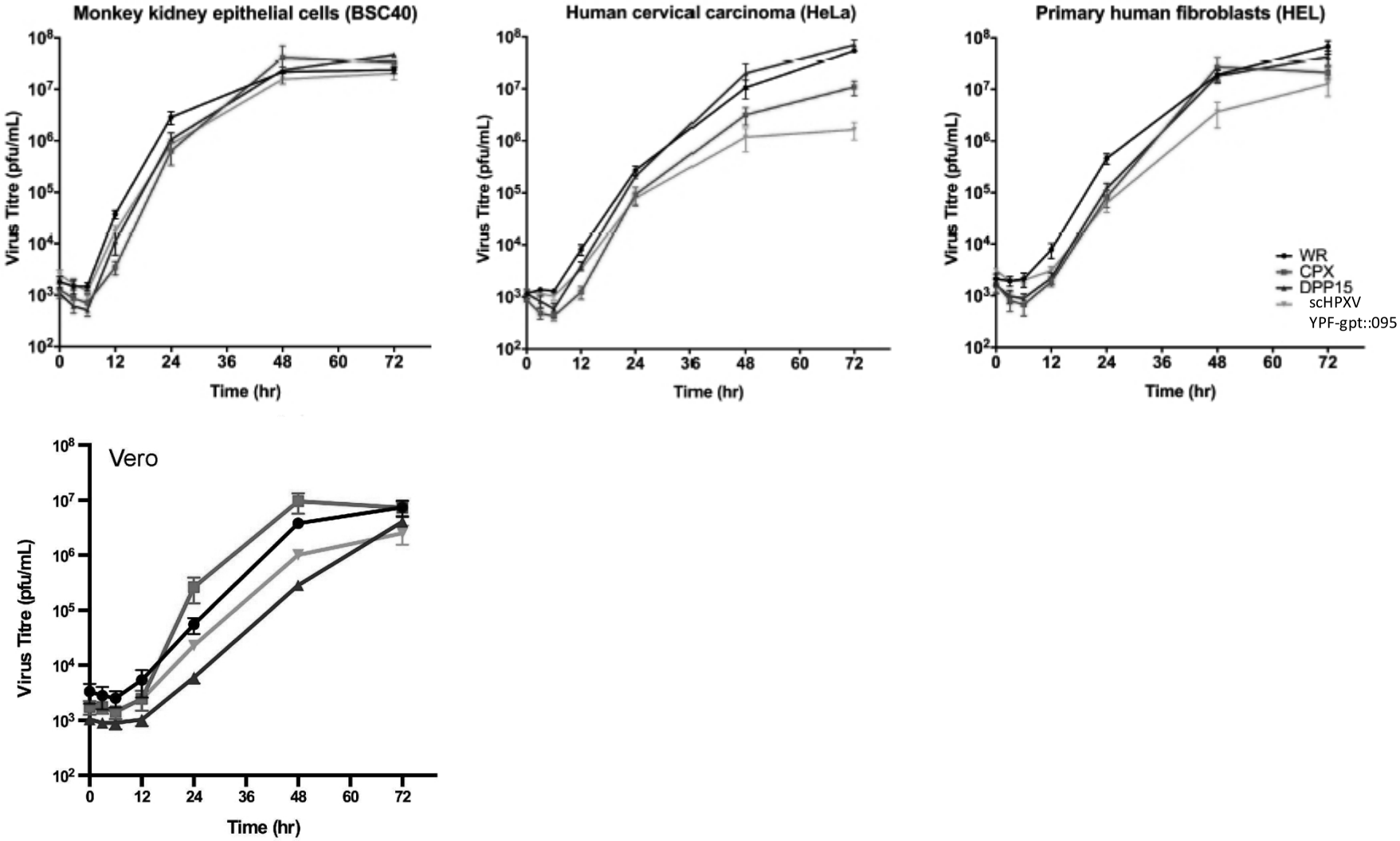


Figure 8B

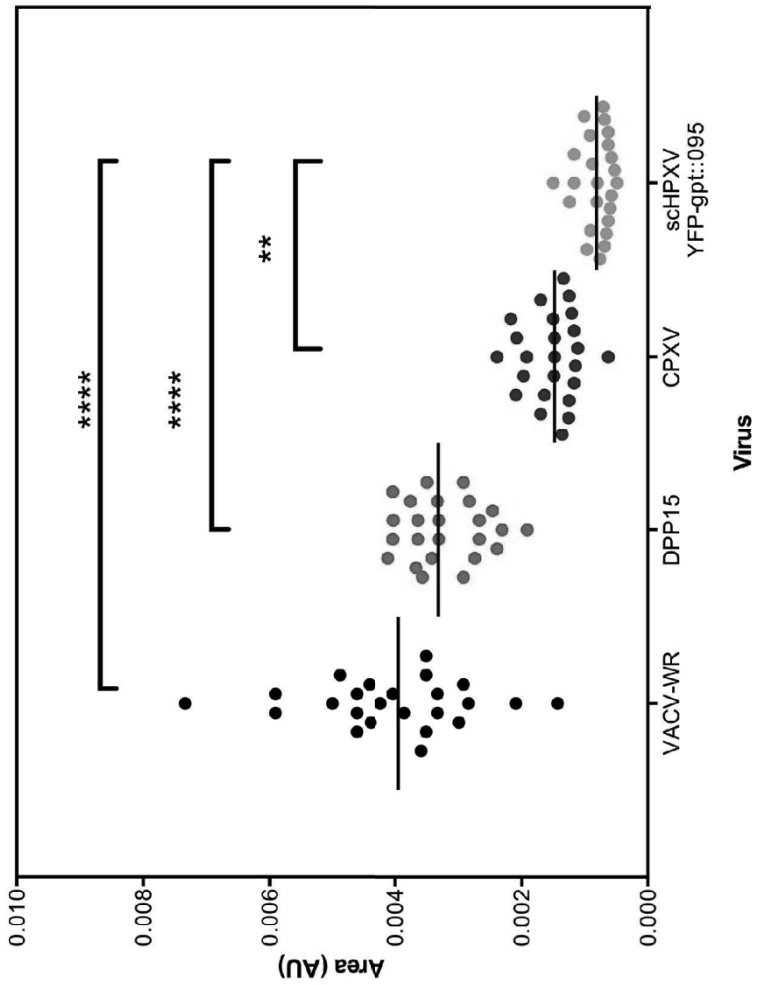

Plaque size of scHPXV YFP-gpt::095 in BSC-40 cells
Area (AU)
0.010
0.008
0.006
0.004
0.002
0.000
****
****
**
VACV-WR
DPP15
CPXV
scHPXV YFP-gpt::095
Virus

Figure 8C

Plaque morphology of scHPXV YFP-gpt::095 in BSC-40 cells

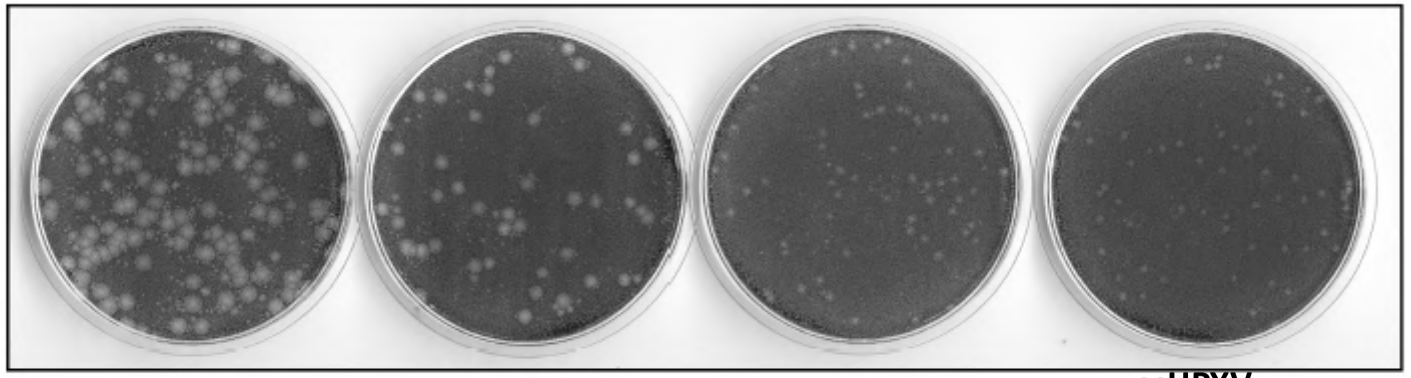


Figure 9

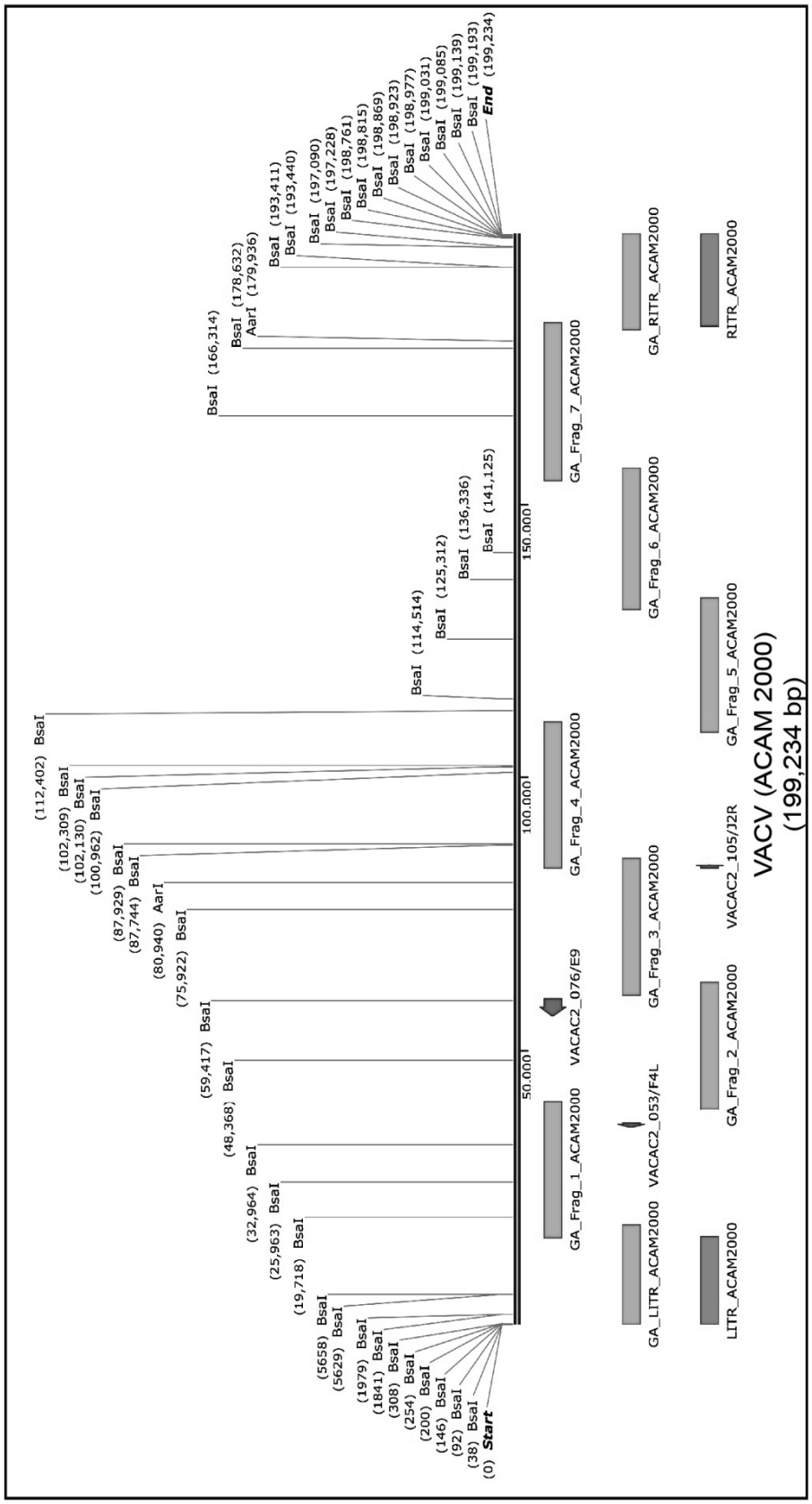

VACV (ACAM 2000)
(199,234 bp)
(0) Start
(38) BsaI
(92) BsaI
(146) BsaI
(200) BsaI
(254) BsaI
(308) BsaI
(1841) BsaI
(1979) BsaI
(5629) BsaI
(5658) BsaI
(19,718) BsaI
(25,963) BsaI
(32,964) BsaI
(48,368) BsaI
(59,417) BsaI
(75,922) BsaI
(80,940) AarI
(87,744) BsaI
(87,929) BsaI
(100,962) BsaI
(102,130) BsaI
(102,309) BsaI
(112,402) BsaI
(114,514) BsaI
(125,312) BsaI
(136,336) BsaI
(141,125) BsaI
(166,314) BsaI
(178,632) BsaI
(179,936) AarI
(193,411) BsaI
(193,440) BsaI
(197,090) BsaI
(197,228) BsaI
(198,761) BsaI
(198,815) BsaI
(198,869) BsaI
(198,923) BsaI
(198,977) BsaI
(199,031) BsaI
(199,085) BsaI
(199,139) BsaI
(199,193) BsaI
End (199,234)
50.000
100.000
150.000
GA_LITR_ACAM2000
LITR_ACAM2000
VACAC2_053/F4L
GA_Frag_1_ACAM2000
GA_Frag_2_ACAM2000
VACAC2_076/E9
GA_Frag_3_ACAM2000
GA_Frag_4_ACAM2000
VACAC2_105/J2R
GA_Frag_5_ACAM2000
GA_Frag_6_ACAM2000
GA_Frag_7_ACAM2000
GA_RITR_ACAM2000
RITR_ACAM2000

Figure 10

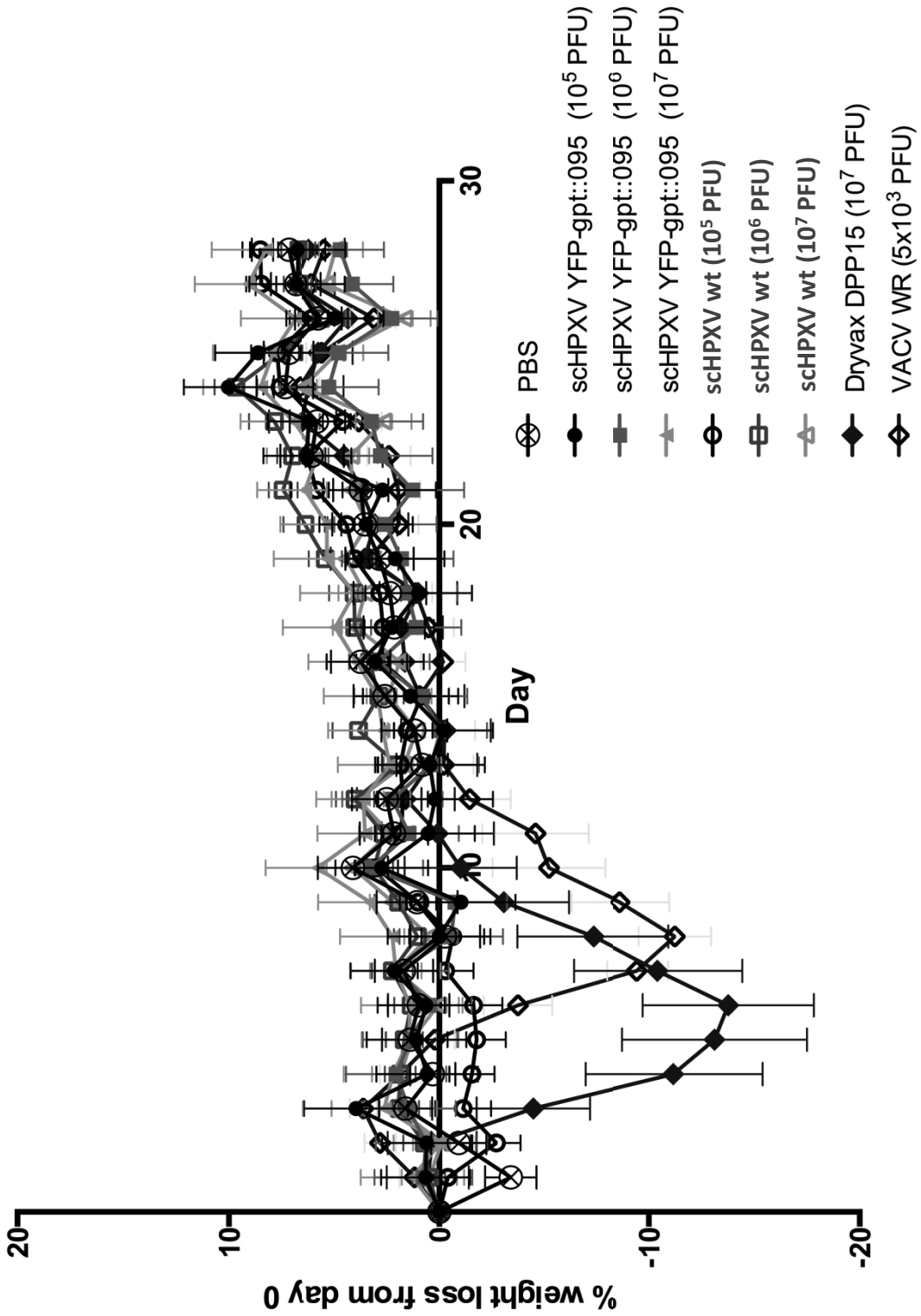

% weight loss from day 0
20
10
0
-10
-20
0
10
20
30
Day
PBS
scHPXV YFP-gpt::095 (10^5 PFU)
scHPXV YFP-gpt::095 (10^6 PFU)
scHPXV YFP-gpt::095 (10^7 PFU)
scHPXV wt (10^5 PFU)
scHPXV wt (10^6 PFU)
scHPXV wt (10^7 PFU)
Dryvax DPP15 (10^7 PFU)
VACV WR (5x10^3 PFU)

Figure 11A

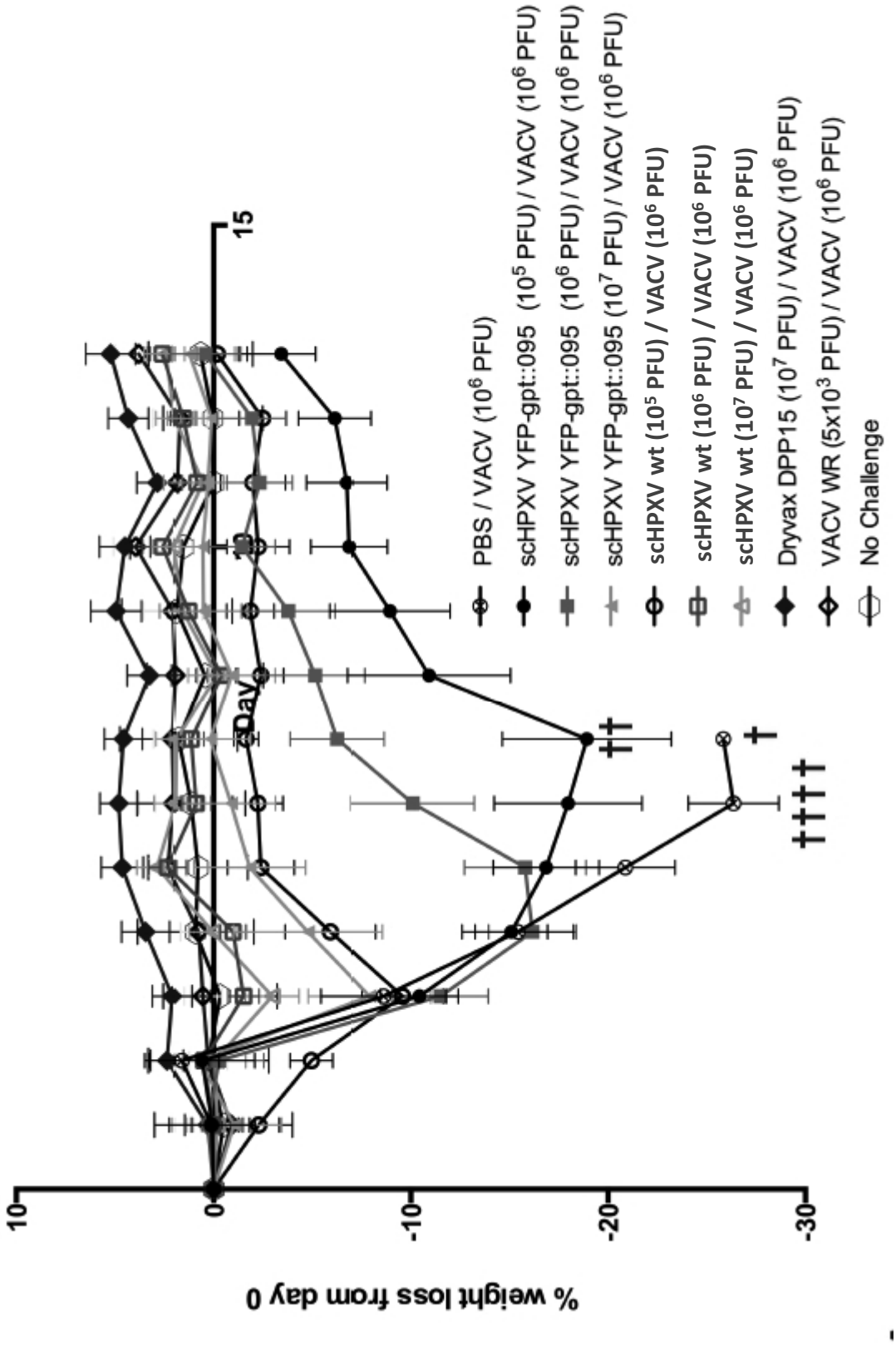

% weight loss from day 0
10
0
-10
-20
-30
Day
15
PBS / VACV (10^6 PFU)
scHPXV YFP-gpt::095 (10^5 PFU) / VACV (10^6 PFU)
scHPXV YFP-gpt::095 (10^6 PFU) / VACV (10^6 PFU)
scHPXV YFP-gpt::095 (10^7 PFU) / VACV (10^6 PFU)
scHPXV wt (10^5 PFU) / VACV (10^6 PFU)
scHPXV wt (10^6 PFU) / VACV (10^6 PFU)
scHPXV wt (10^7 PFU) / VACV (10^6 PFU)
Dryvax DPP15 (10^7 PFU) / VACV (10^6 PFU)
VACV WR (5x10^3 PFU) / VACV (10^6 PFU)
No Challenge

Figure 11B

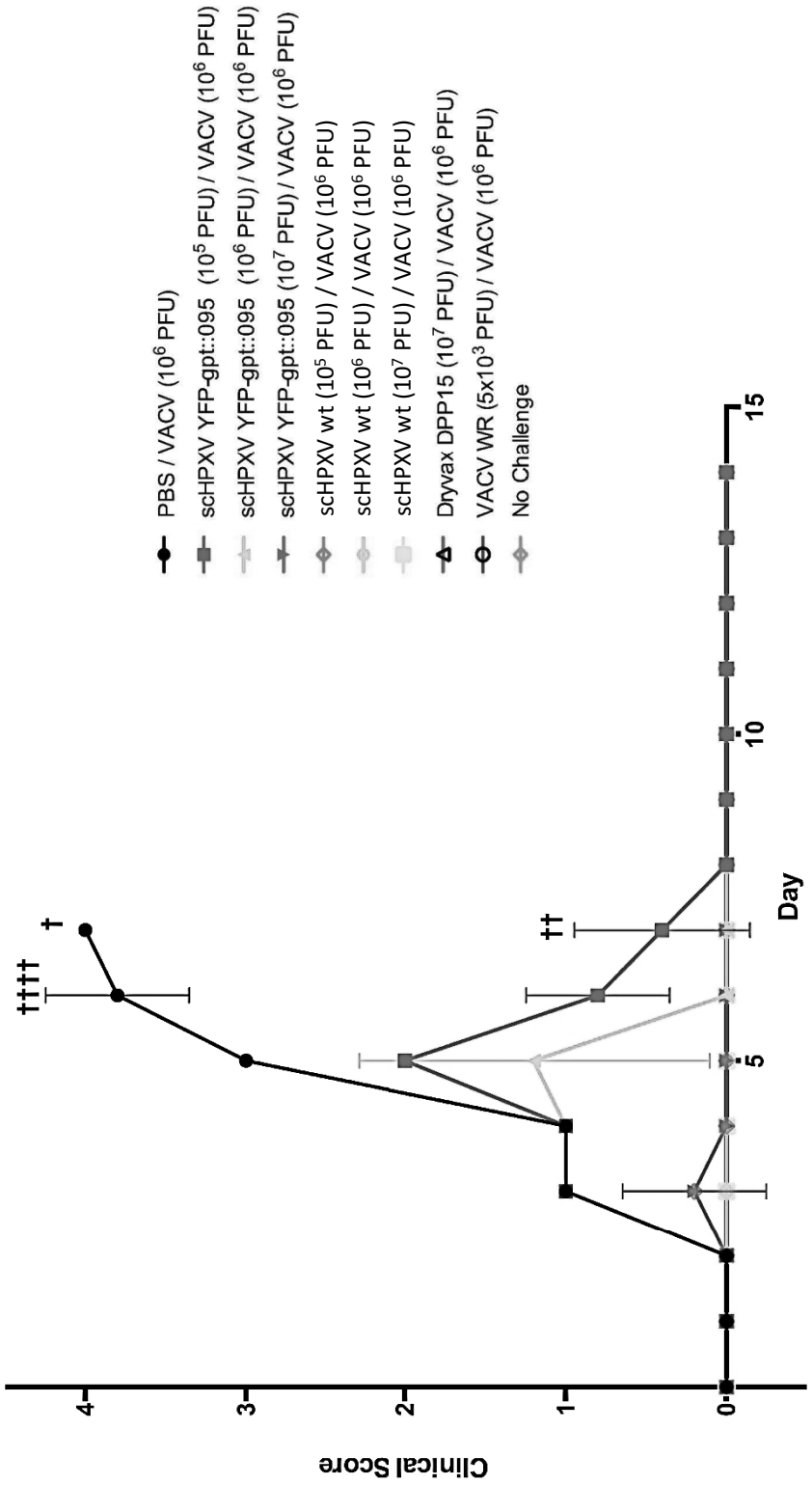

PBS / VACV (10^6 PFU)
scHPXV YFP-gpt::095 (10^5 PFU) / VACV (10^6 PFU)
scHPXV YFP-gpt::095 (10^6 PFU) / VACV (10^6 PFU)
scHPXV YFP-gpt::095 (10^7 PFU) / VACV (10^6 PFU)
scHPXV wt (10^5 PFU) / VACV (10^6 PFU)
scHPXV wt (10^6 PFU) / VACV (10^6 PFU)
scHPXV wt (10^7 PFU) / VACV (10^6 PFU)
Dryvax DPP15 (10^7 PFU) / VACV (10^6 PFU)
VACV WR (5x10^3 PFU) / VACV (10^6 PFU)
No Challenge
Clinical Score
Day
0
1
2
3
4
5
10
15
†
††††
††

Figure 12

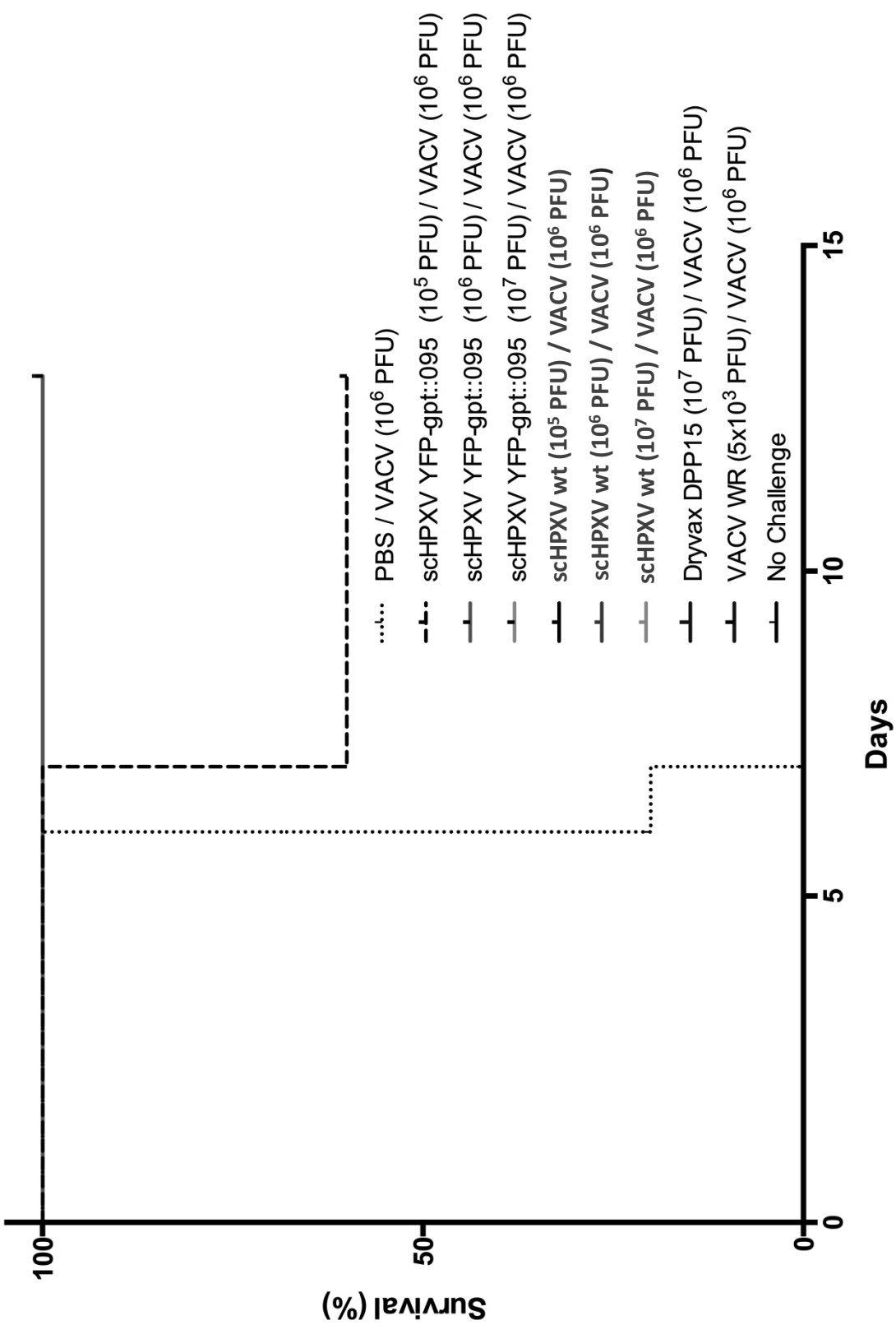

Survival (%)
100
50
0
0
5
10
15
Days
PBS / VACV (10^6 PFU)
scHPXV YFP-gpt::095 (10^5 PFU) / VACV (10^6 PFU)
scHPXV YFP-gpt::095 (10^6 PFU) / VACV (10^6 PFU)
scHPXV YFP-gpt::095 (10^7 PFU) / VACV (10^6 PFU)
scHPXV wt (10^5 PFU) / VACV (10^6 PFU)
scHPXV wt (10^6 PFU) / VACV (10^6 PFU)
scHPXV wt (10^7 PFU) / VACV (10^6 PFU)
Dryvax DPP15 (10^7 PFU) / VACV (10^6 PFU)
VACV WR (5x10^3 PFU) / VACV (10^6 PFU)
No Challenge

Figure 13A

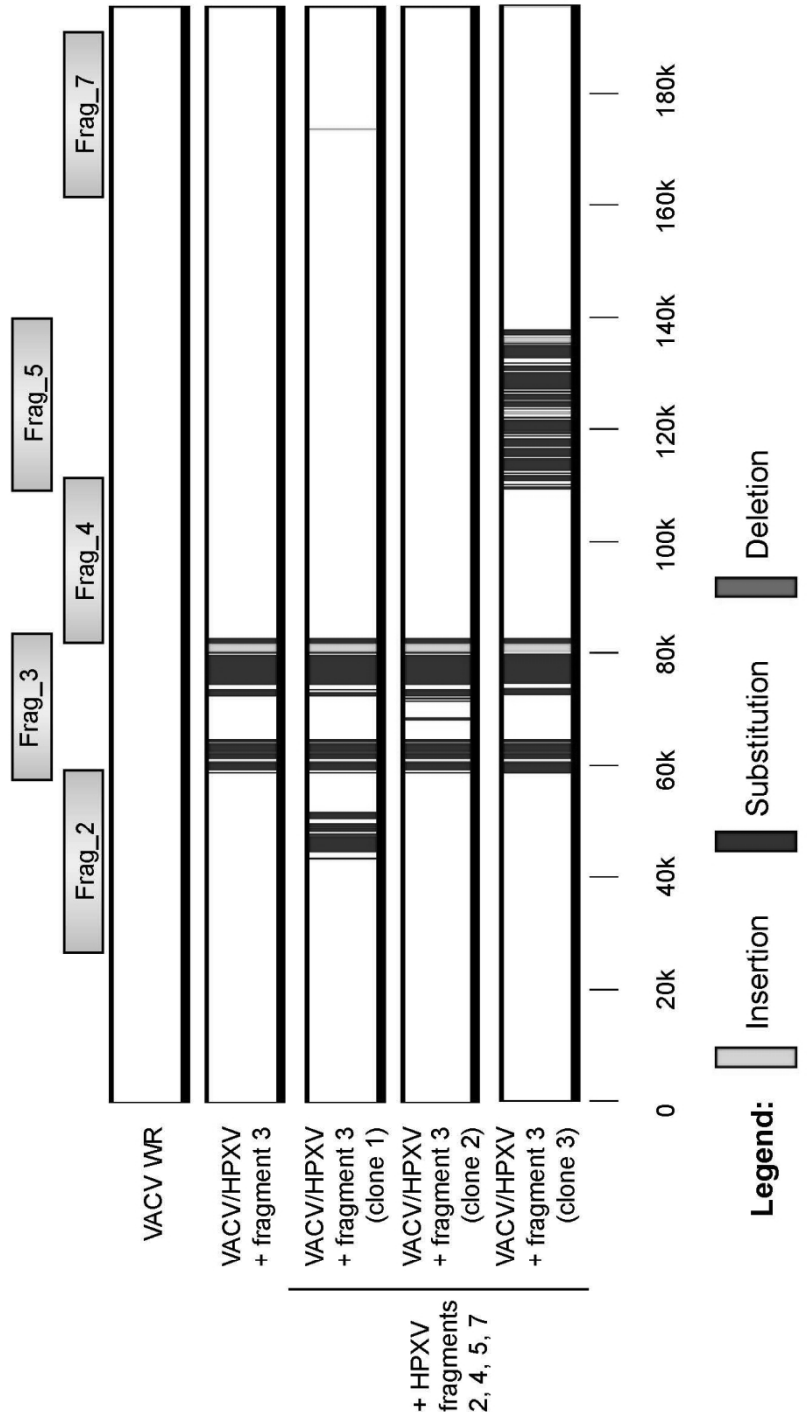

Frag_2
Frag_3
Frag_4
Frag_5
Frag_7
VACV WR
VACV/HPXV + fragment 3
VACV/HPXV + fragment 3 (clone 1)
VACV/HPXV + fragment 3 (clone 2)
VACV/HPXV + fragment 3 (clone 3)
+ HPXV fragments 2, 4, 5, 7
0
20k
40k
60k
80k
100k
120k
140k
160k
180k
Legend:
Insertion
Substitution
Deletion

Figure 13B

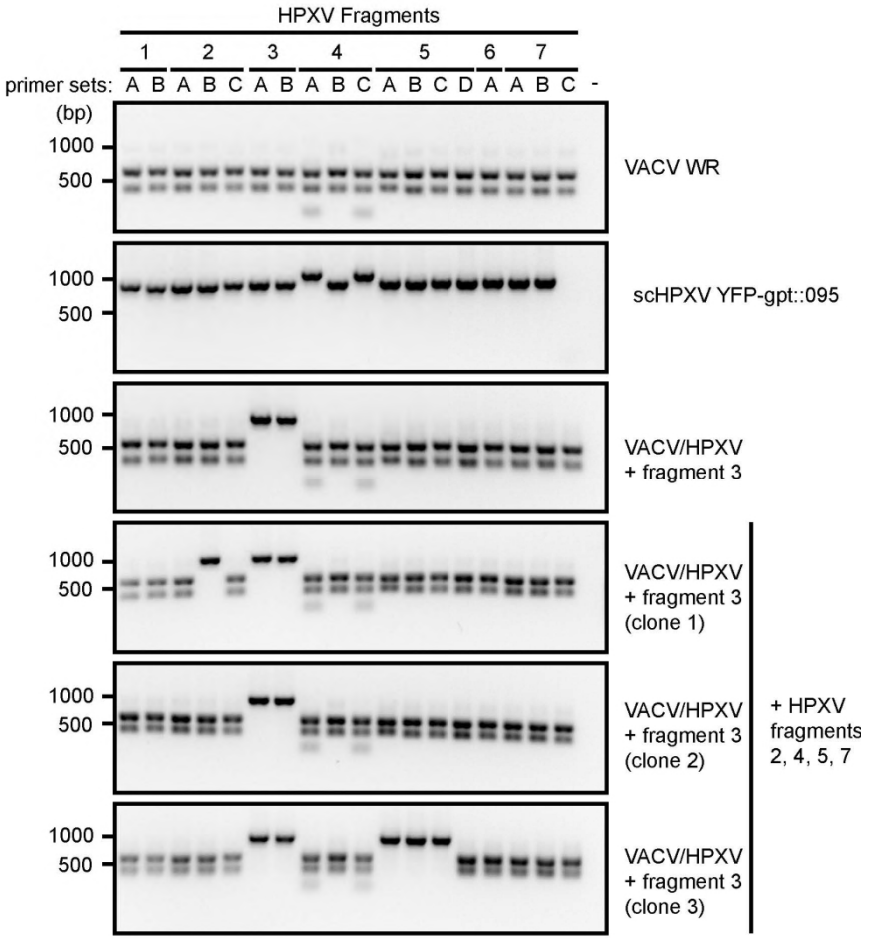

HPXV Fragments
1
2
3
4
5
6
7
primer sets: A B A B C A B A B C A B C D A A B C -
(bp)
1000
500
VACV WR
scHPXV YFP-gpt::095
VACV/HPXV + fragment 3
VACV/HPXV + fragment 3 (clone 1)
VACV/HPXV + fragment 3 (clone 2)
VACV/HPXV + fragment 3 (clone 3)
+ HPXV fragments 2, 4, 5, 7

Figure 14A

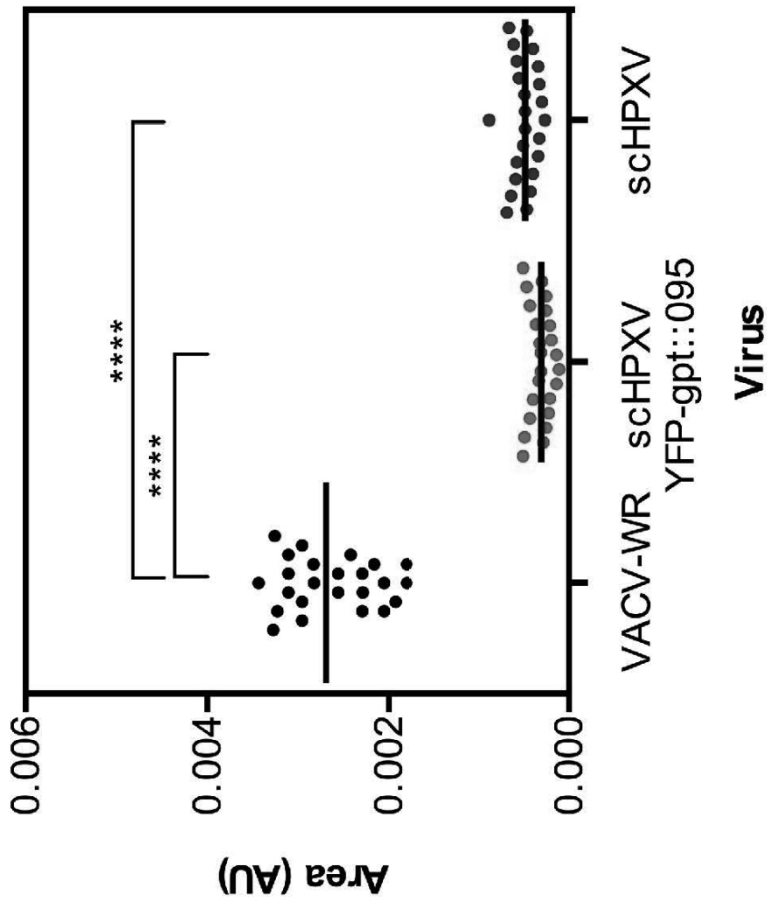

Area (AU)
0.006
0.004
0.002
0.000
****
****
VACV-WR
scHPXV YFP-gpt::095
scHPXV
Virus

Figure 14B

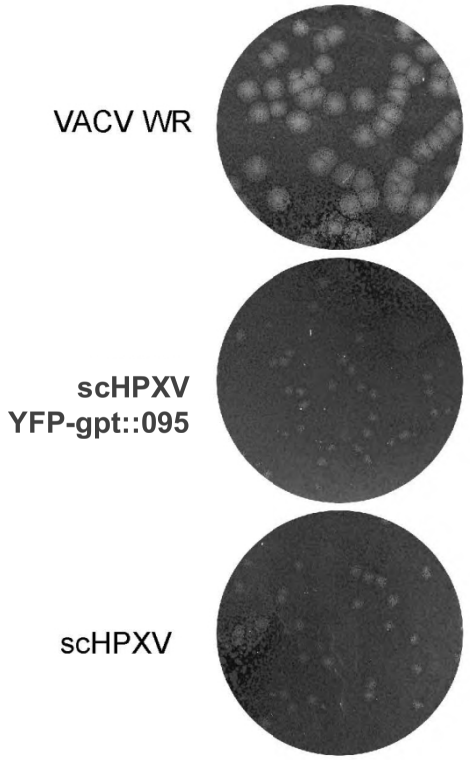
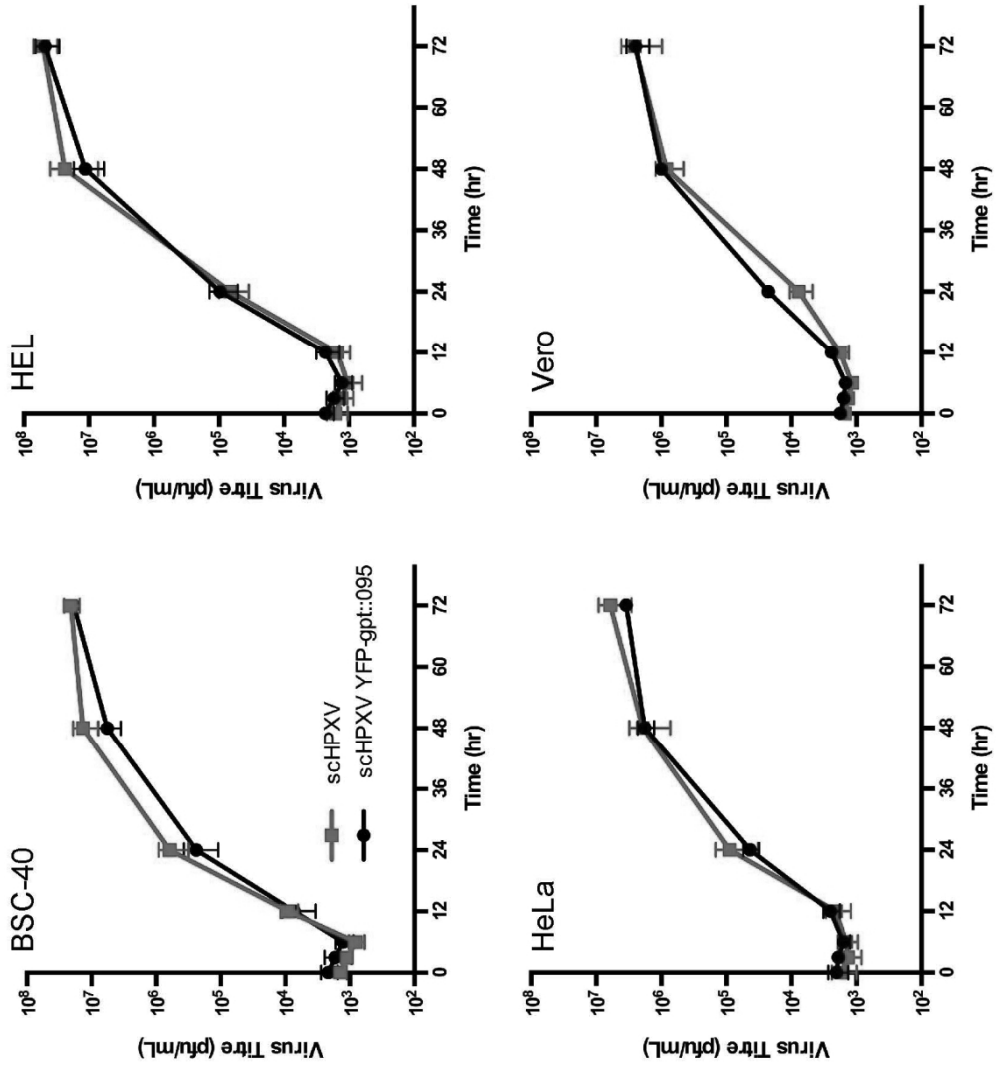


Figure 14C


BSC-40
HEL
HeLa
Vero
scHPXV
scHPXV YFP-gpt::095
Virus Titre (pfu/mL)
Time (hr)
10^8
10^7
10^6
10^5
10^4
10^3
10^2
0
12
24
36
48
60
72