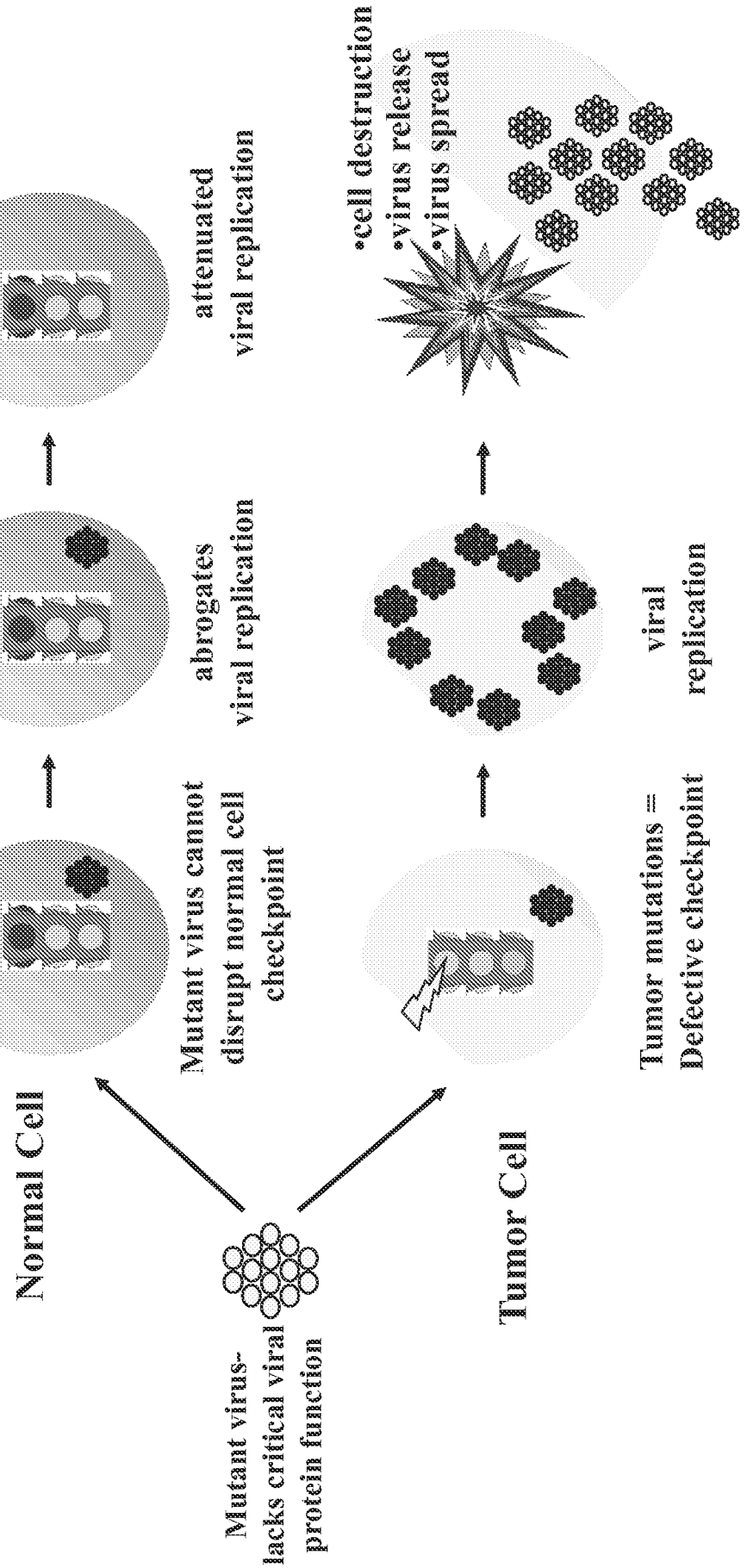

Normal Cell
Mutant virus-
lacks critical viral
protein function
Mutant virus cannot
disrupt normal cell
checkpoint
abrogates
viral replication
attenuated
viral replication
Tumor Cell
Tumor mutations =
Defective checkpoint
viral
replication
•cell destruction
•virus release
•virus spread

Fig. 1

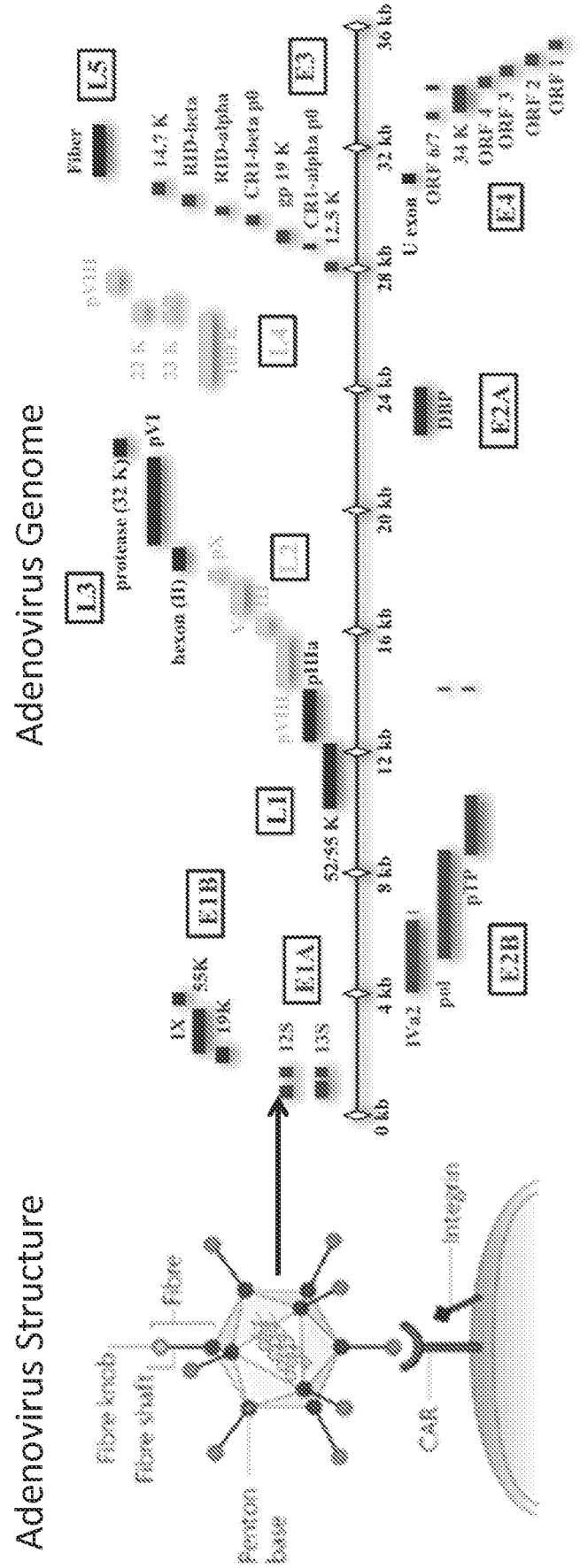

Adenovirus Structure
Adenovirus Genome
fibre knob
fibre shaft
fibre
penton base
CAR
integrin
E1A
E1B
E2B
E2A
E3
E4
L1
L2
L3
L4
L5
Fiber
protease (32 K)
pVI
hexon (II)
pVII
pVIII
52/55 K
pIIIa
pTP
pol
DBP
14.7 K
RID-beta
RID-alpha
CR1-beta p8
gp 19 K
CR1-alpha p8
11.5 K
U exon
ORF 6/7
34 K
ORF 4
ORF 3
ORF 2
ORF 1
0 kb
4 kb
8 kb
12 kb
16 kb
20 kb
24 kb
28 kb
32 kb
36 kb

Fig. 2

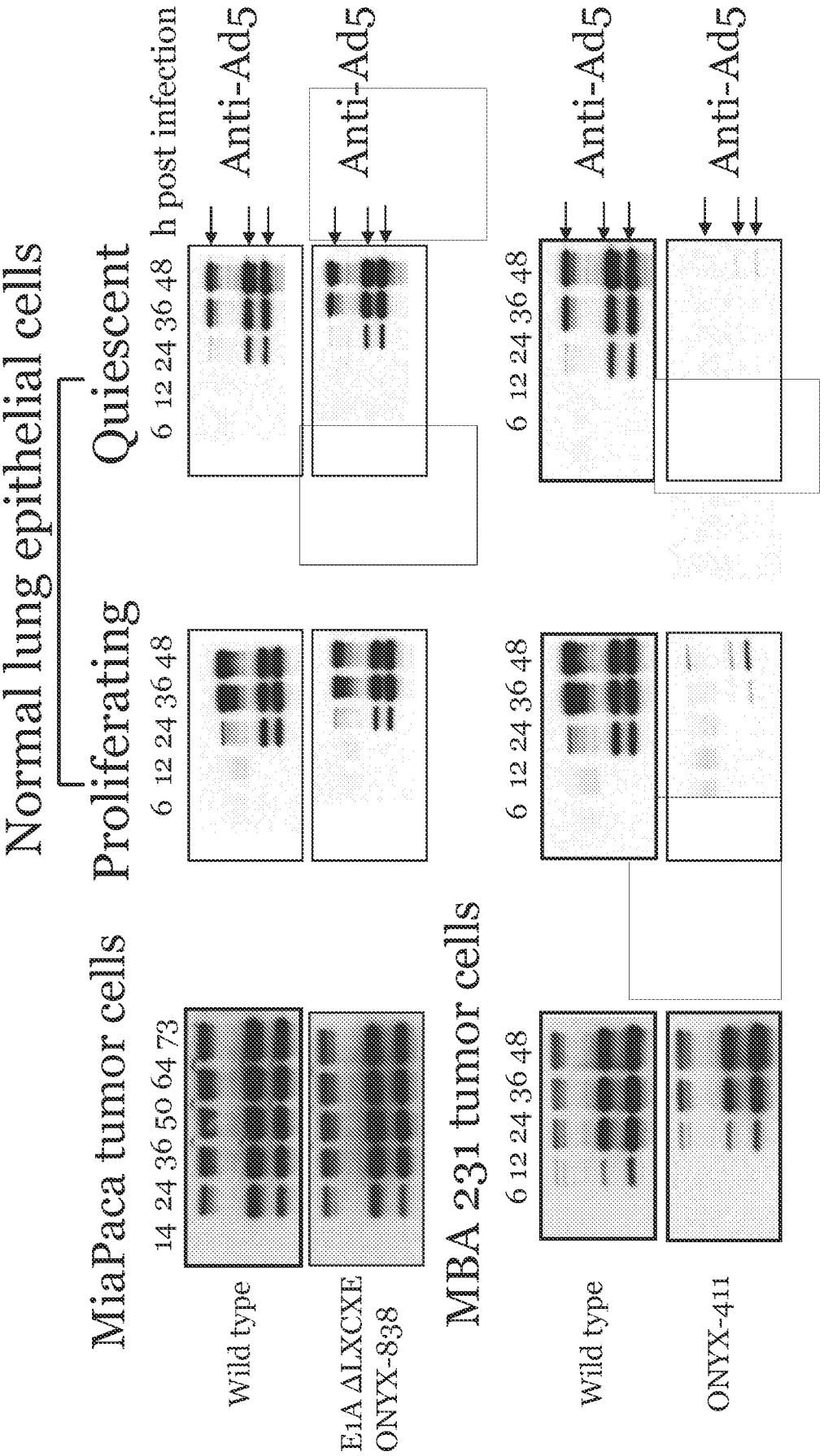

Normal lung epithelial cells
Proliferating
Quiescent
MiaPaca tumor cells
14 24 36 50 64 73
Wild type
E1A ΔLXCXE
ONYX-838
MBA 231 tumor cells
6 12 24 36 48
Wild type
ONYX-411
6 12 24 36 48 h post infection
Anti-Ad5
Anti-Ad5
6 12 24 36 48
Anti-Ad5
Anti-Ad5

Fig. 3

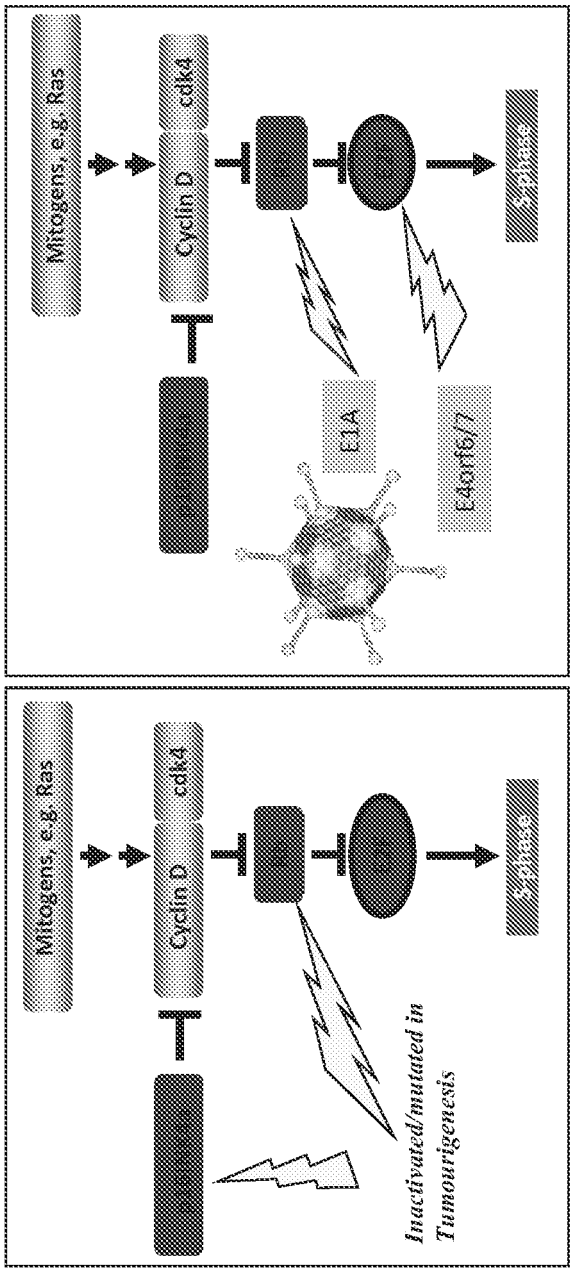

Mitogens, e.g. Ras
Cyclin D
cdk4
E1A
E4orf6/7
Inactivated/mutated in Tumourigenesis

Fig. 4B

Fig. 4A

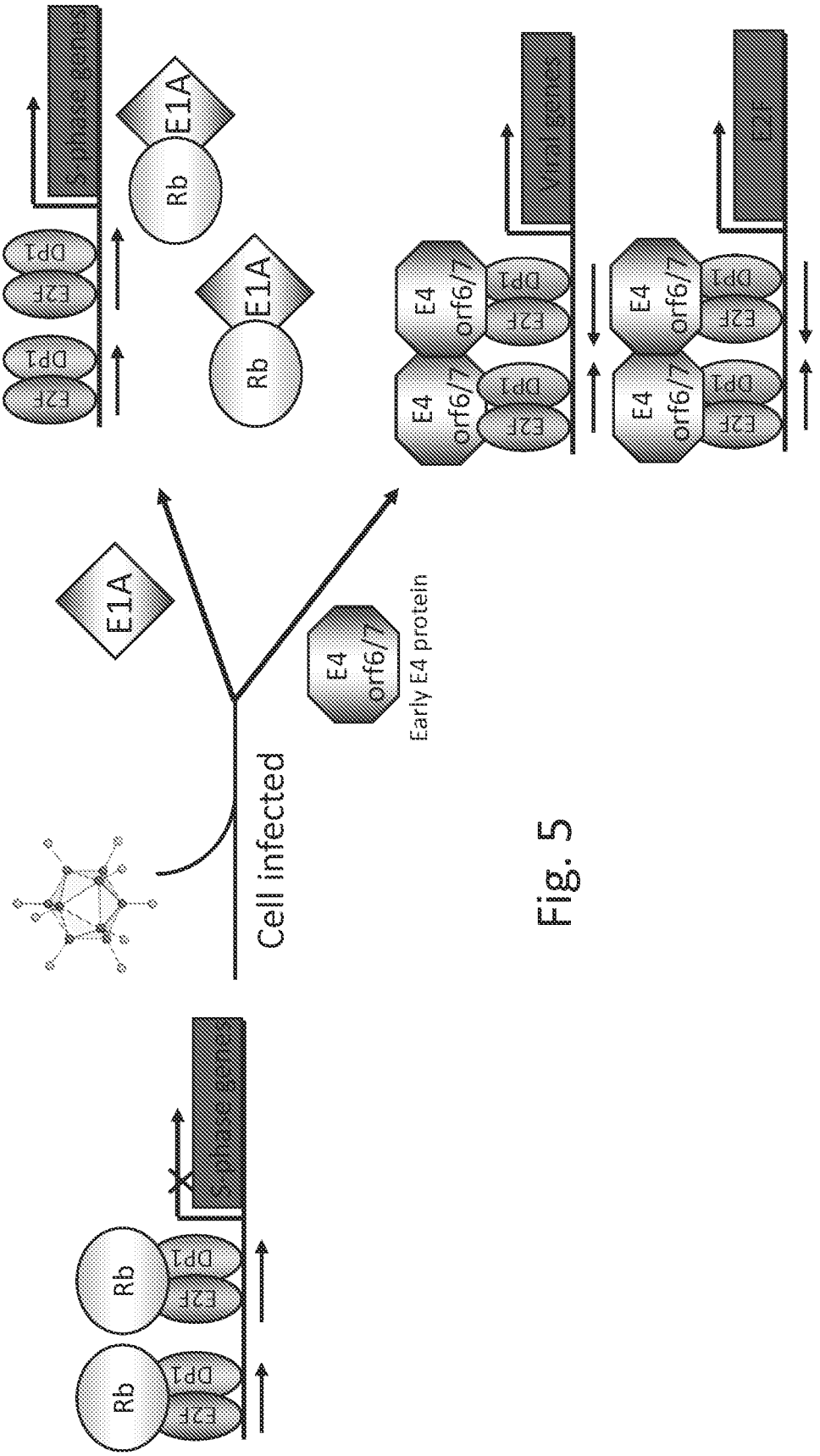

Cell infected
E1A
E4 orf6/7
Early E4 protein
Rb
E2F
DP1
E1A
E4 orf6/7

Fig. 5

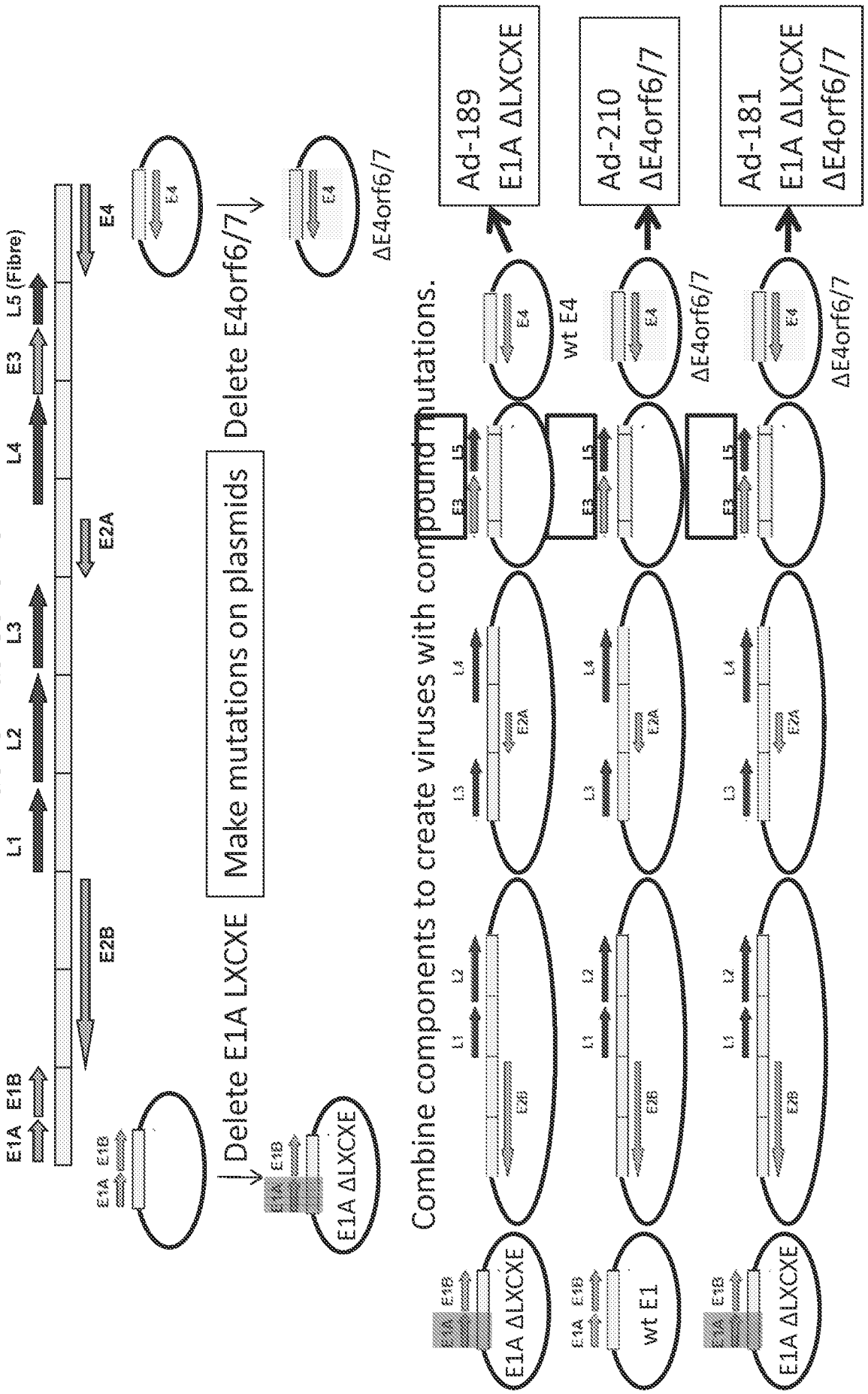

Adenovirus Genome
E1A E1B
L1
L2
L3
L4
E3
L5 (Fibre)
E4
E2B
E2A
Delete E1A LXCXE
Make mutations on plasmids
Delete E4orf6/7
ΔE4orf6/7
Combine components to create viruses with compound mutations.
E1A ΔLXCXE
wt E1
wt E4
ΔE4orf6/7
Ad-189
E1A ΔLXCXE
Ad-210
ΔE4orf6/7
Ad-181
E1A ΔLXCXE
ΔE4orf6/7

Fig. 6

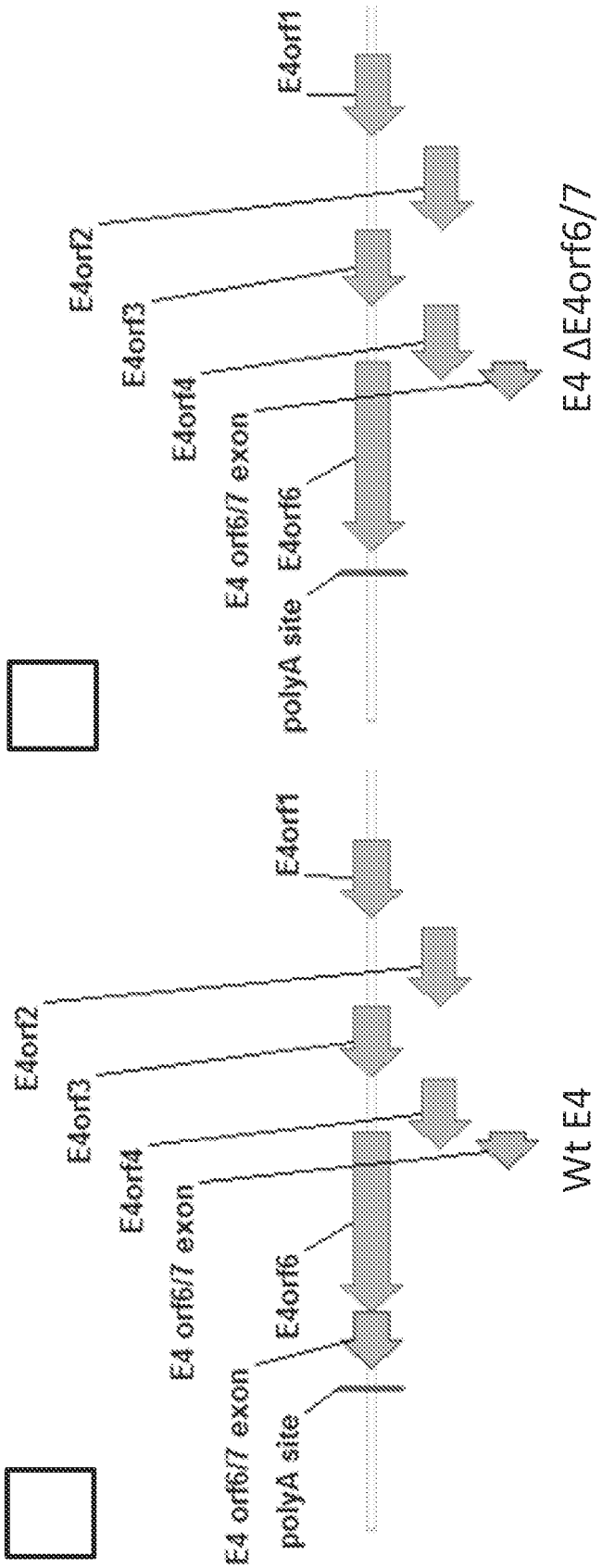

E4orf1
E4orf2
E4orf3
E4orf4
E4 orf6/7 exon
E4orf6
polyA site
E4 ΔE4orf6/7
E4orf1
E4orf2
E4orf3
E4orf4
E4 orf6/7 exon
E4orf6
E4 orf6/7 exon
polyA site
Wt E4

Fig. 7B

Fig. 7A

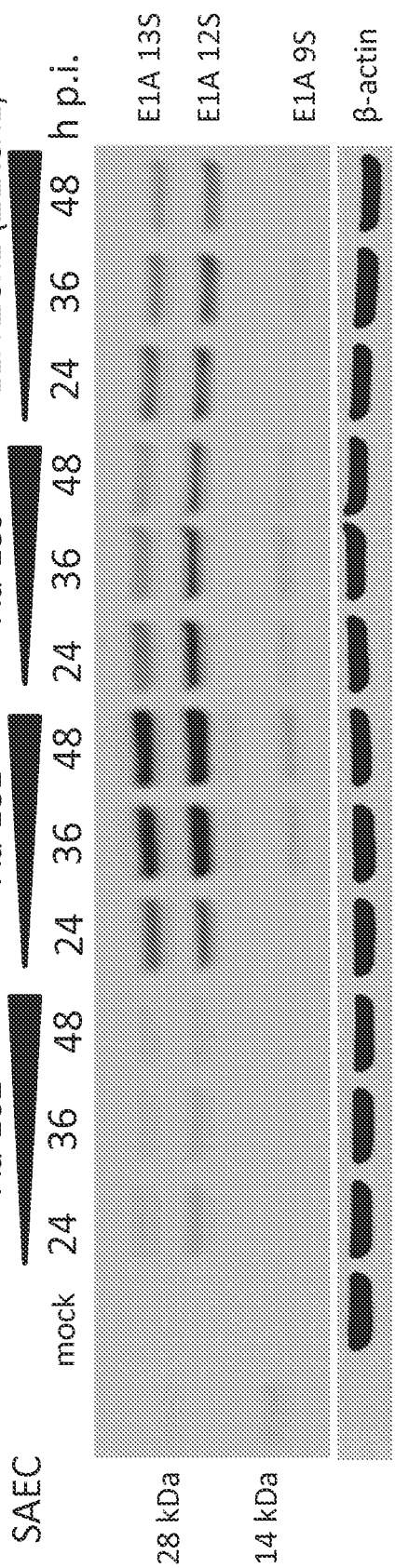

SAEC
ONYX-838
E1A ΔCR2 (ΔLXCXE)
Ad-102
Ad-181
Ad-189
mock 24 36 48 24 36 48 24 36 48 h p.i.
E1A 13S
E1A 12S
E1A 9S
β-actin
28 kDa
14 kDa

Fig. 8A

A549
ONYX-838
E1A ΔCR2 (ΔLXCXE)
Ad-102
Ad-181
Ad-189
mock 24 36 48 24 36 48 24 36 48 h p.i.
E1A 13S
E1A 12S
E1A 9S
β-actin
28 kDa
14 kDa

Fig. 8B

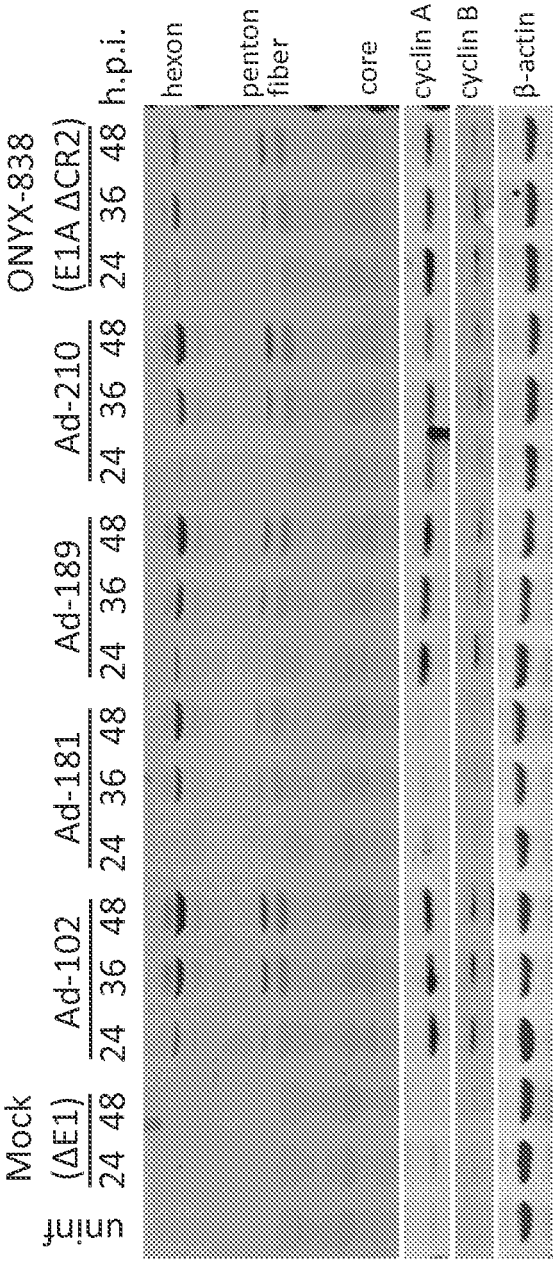

SAEC
mock (ΔE1)
Ad-102
Ad-181
Ad-189
Ad-210
ONYX-838 (E1A ΔCR2)
24 48
24 36 48
h.p.i.
hexon
penton fiber
core
cyclin A
cyclin B
β-actin

Fig. 9A

A549
mock (ΔE1)
Ad-102
Ad-181
Ad-189
Ad-210
ONYX-838 (E1A ΔCR2)
24 48
24 36 48
h.p.i.
hexon
penton fiber
core
cyclin A
cyclin B
β-actin

Fig. 9B

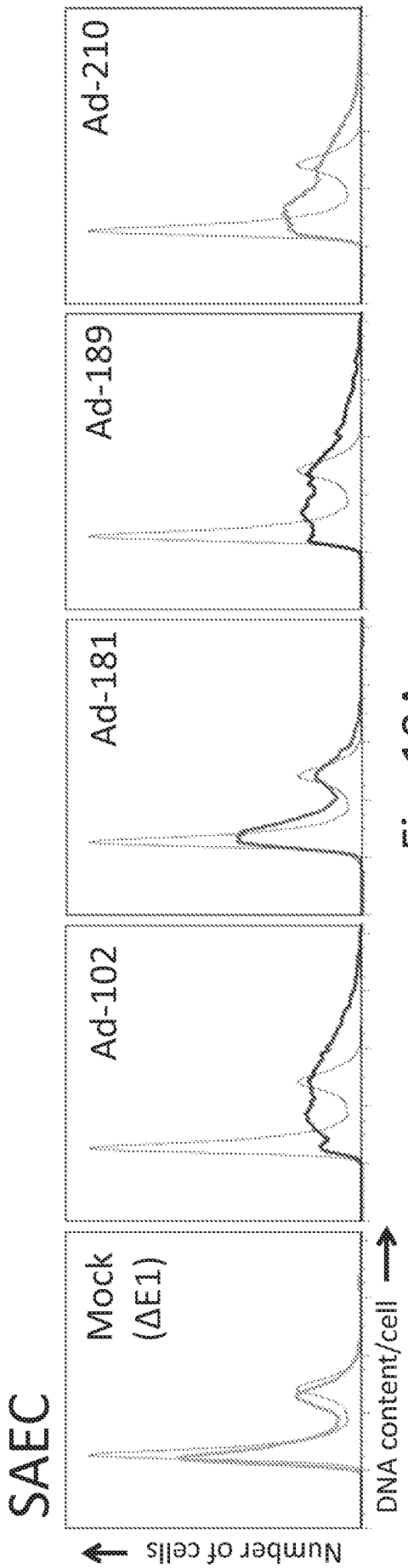


Fig. 10A

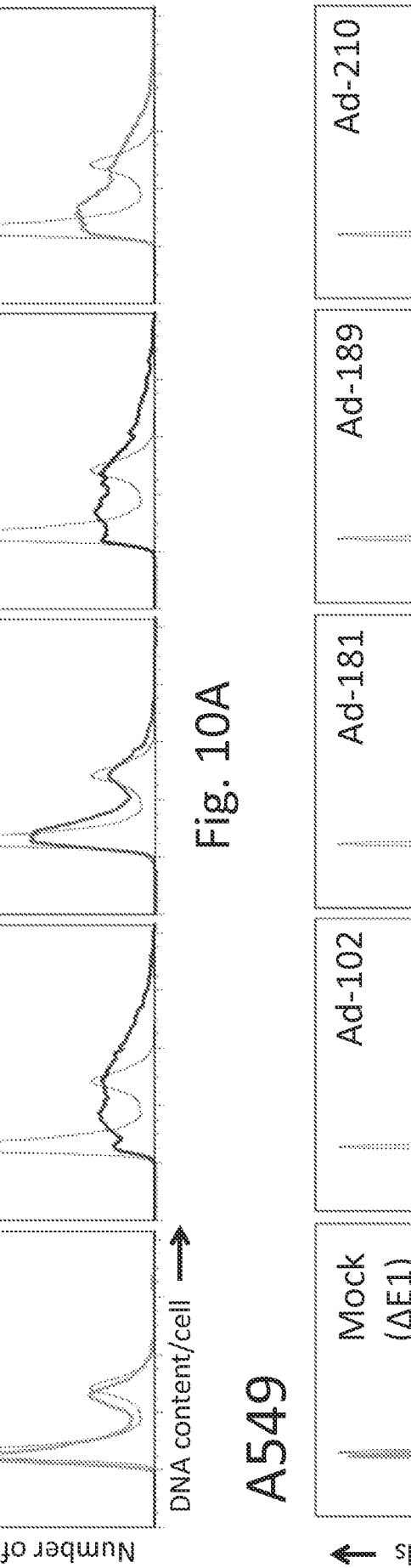


Fig. 10B

Fig. 11A

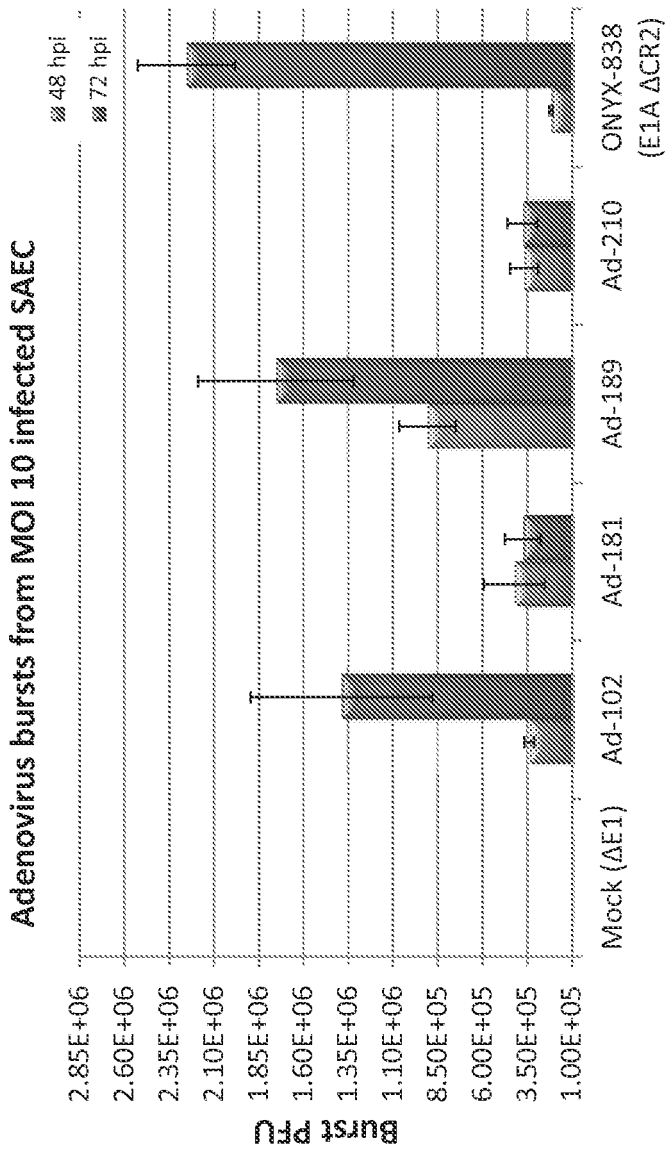


Fig. 11B

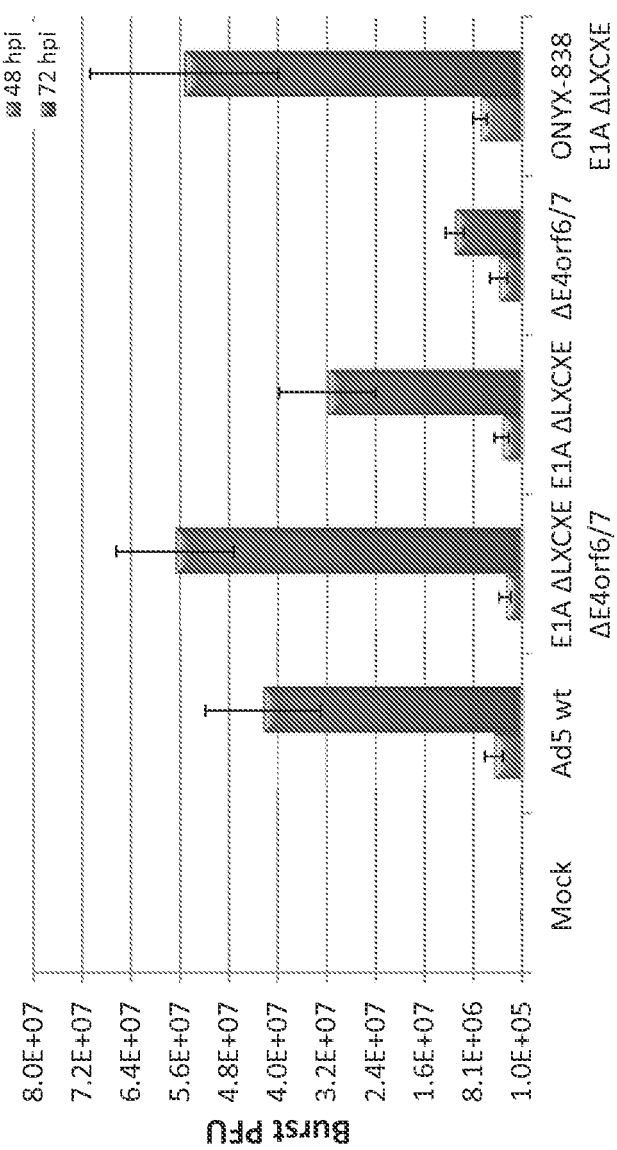


Fig. 12A

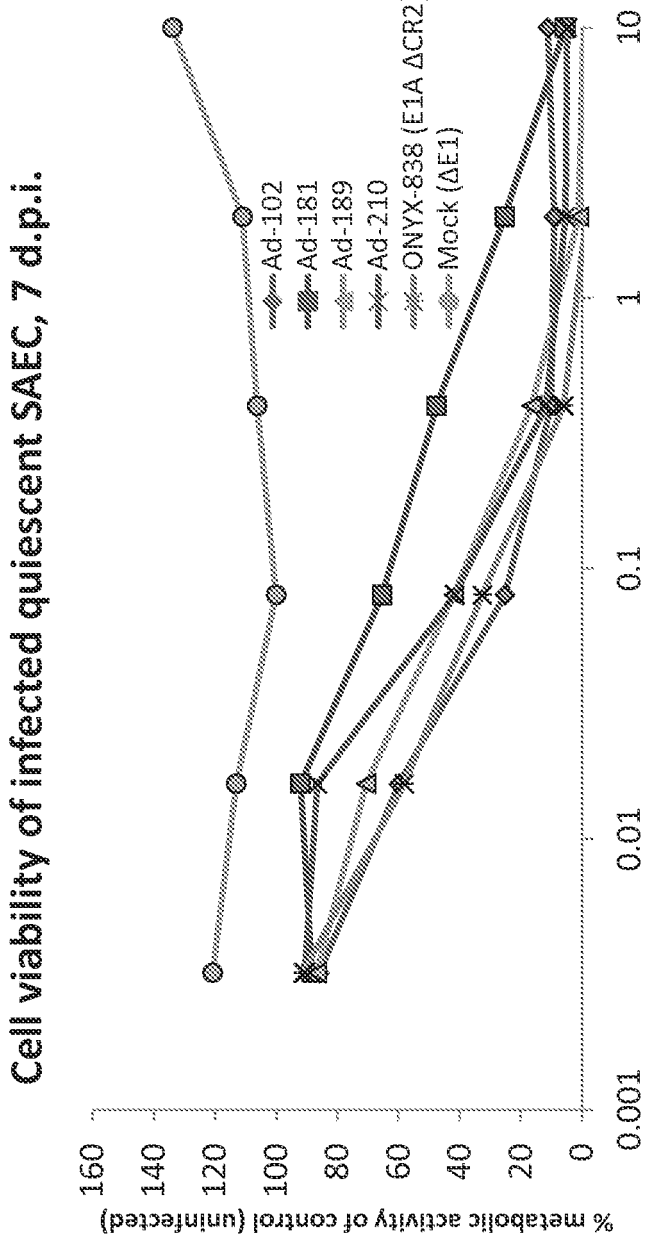
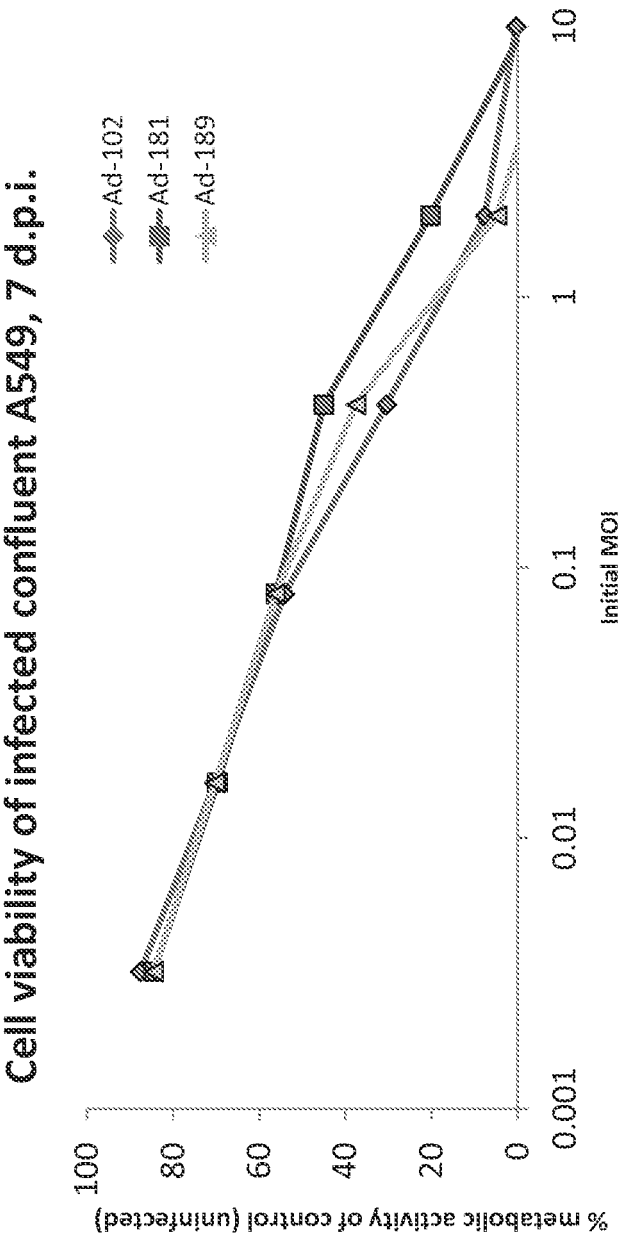


Fig. 12B



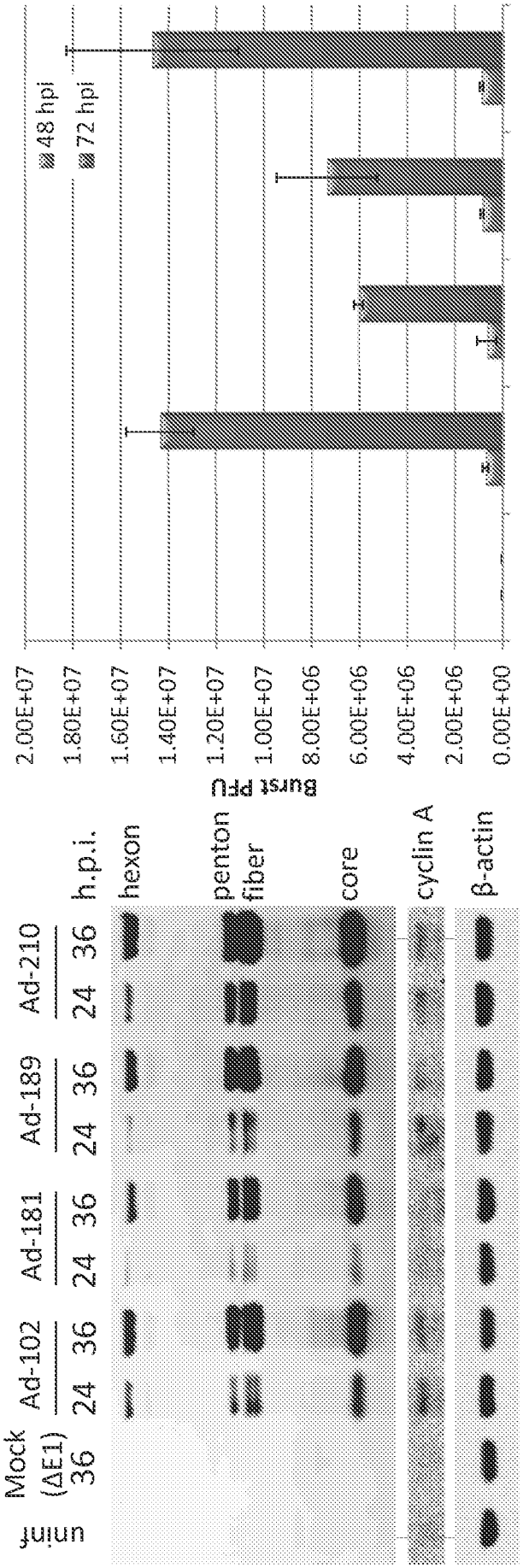


Fig. 13A

Fig. 13B

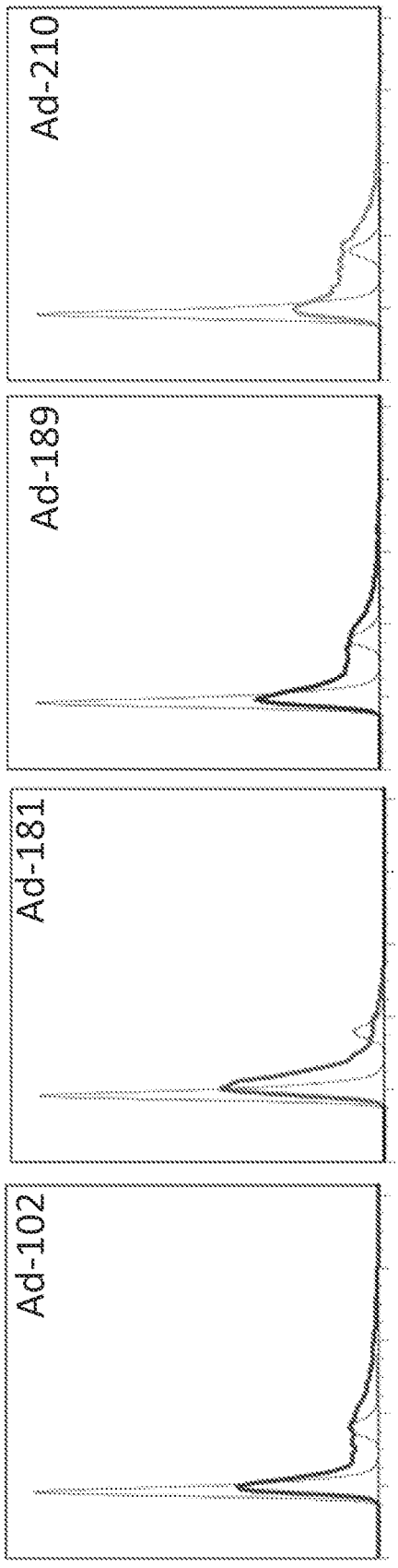


Fig. 13C

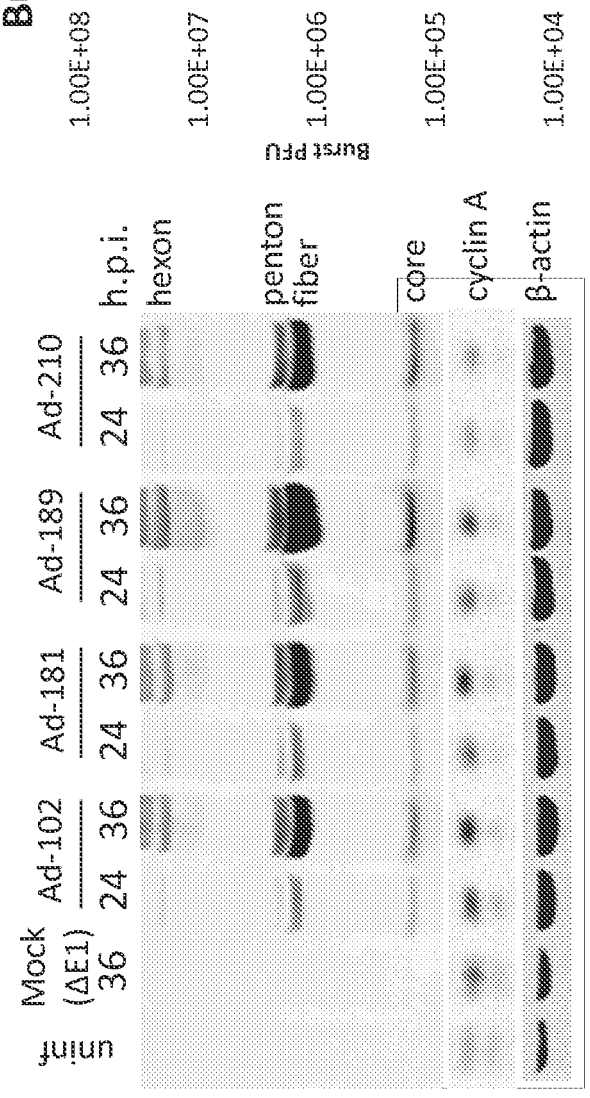


Fig. 14A

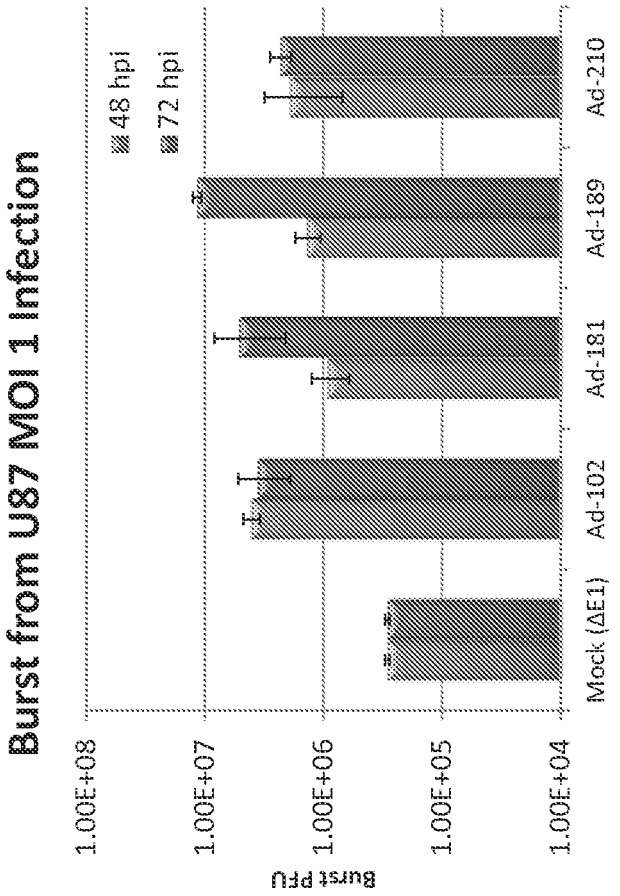


Fig. 14B

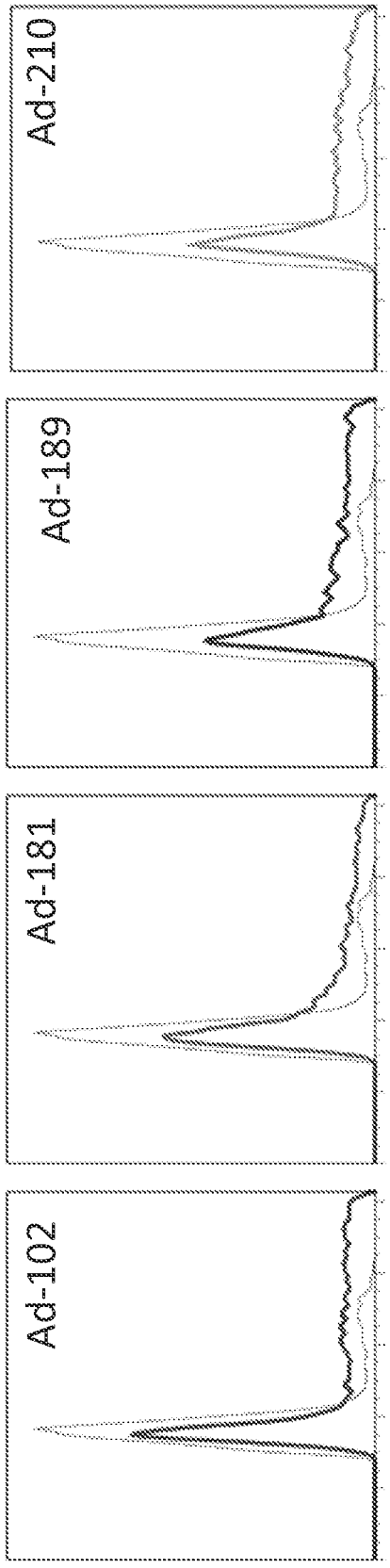


Fig. 14C

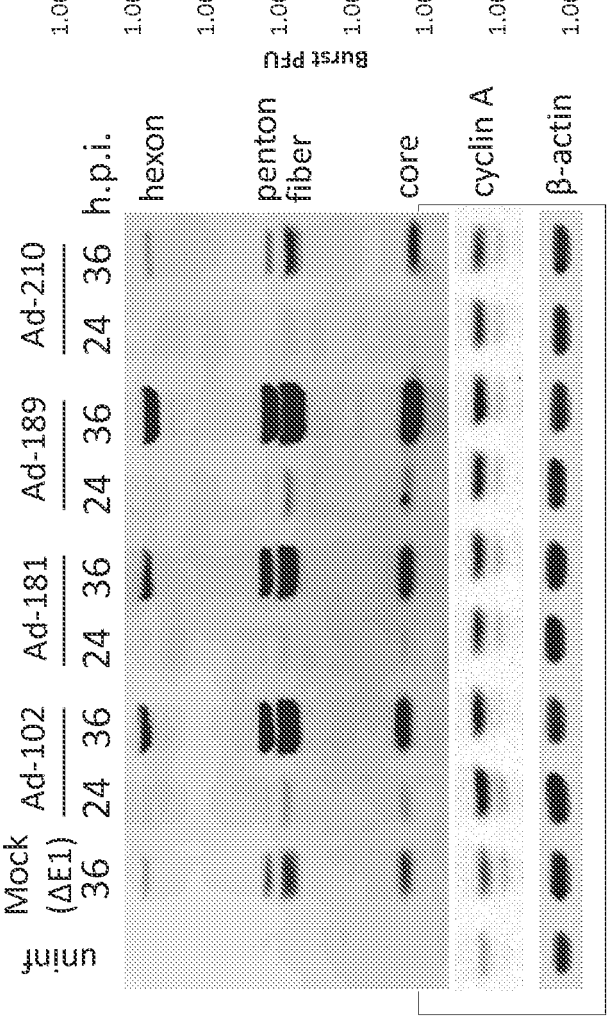


Fig. 15A

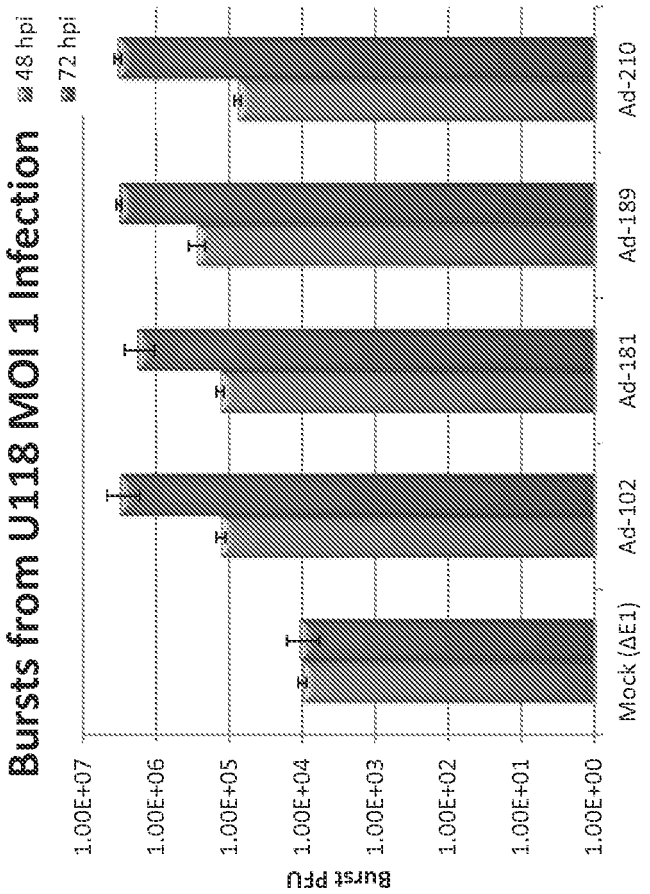


Fig. 15B

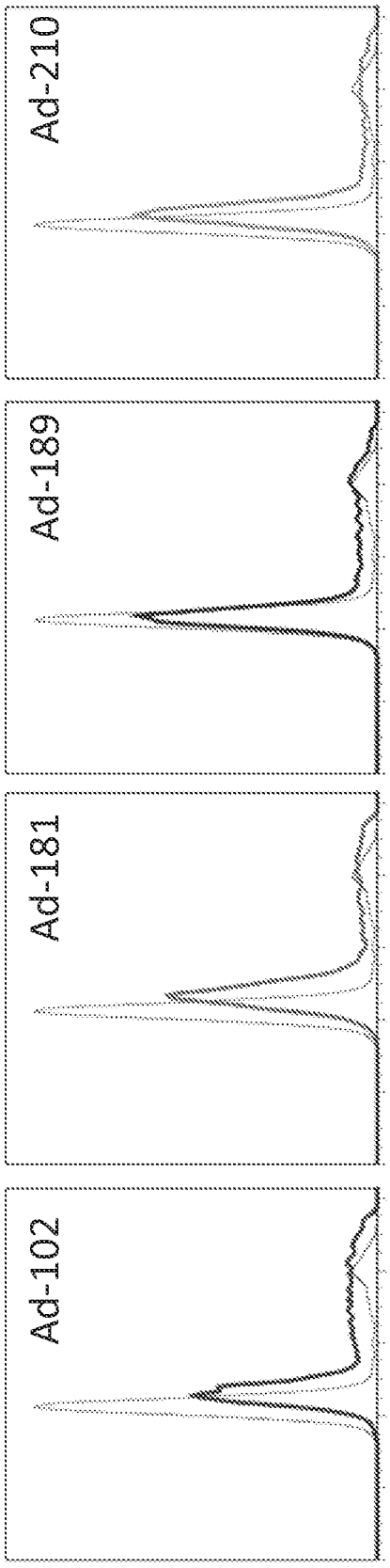


Fig. 15C

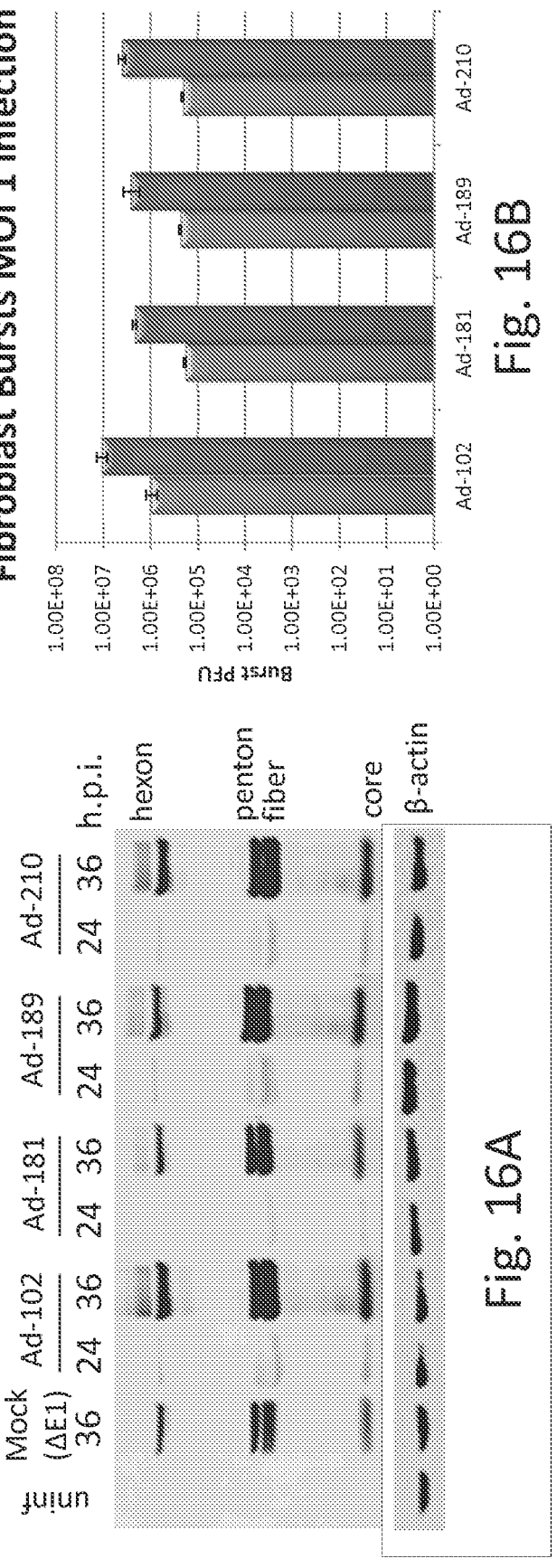


Fig. 16A

Fig. 16B

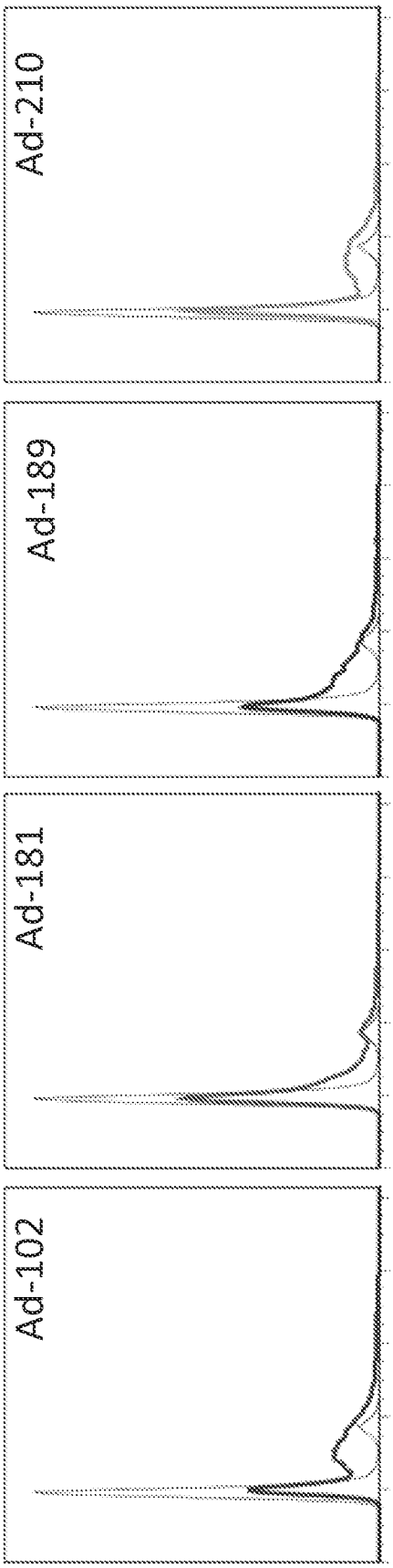


Fig. 16C

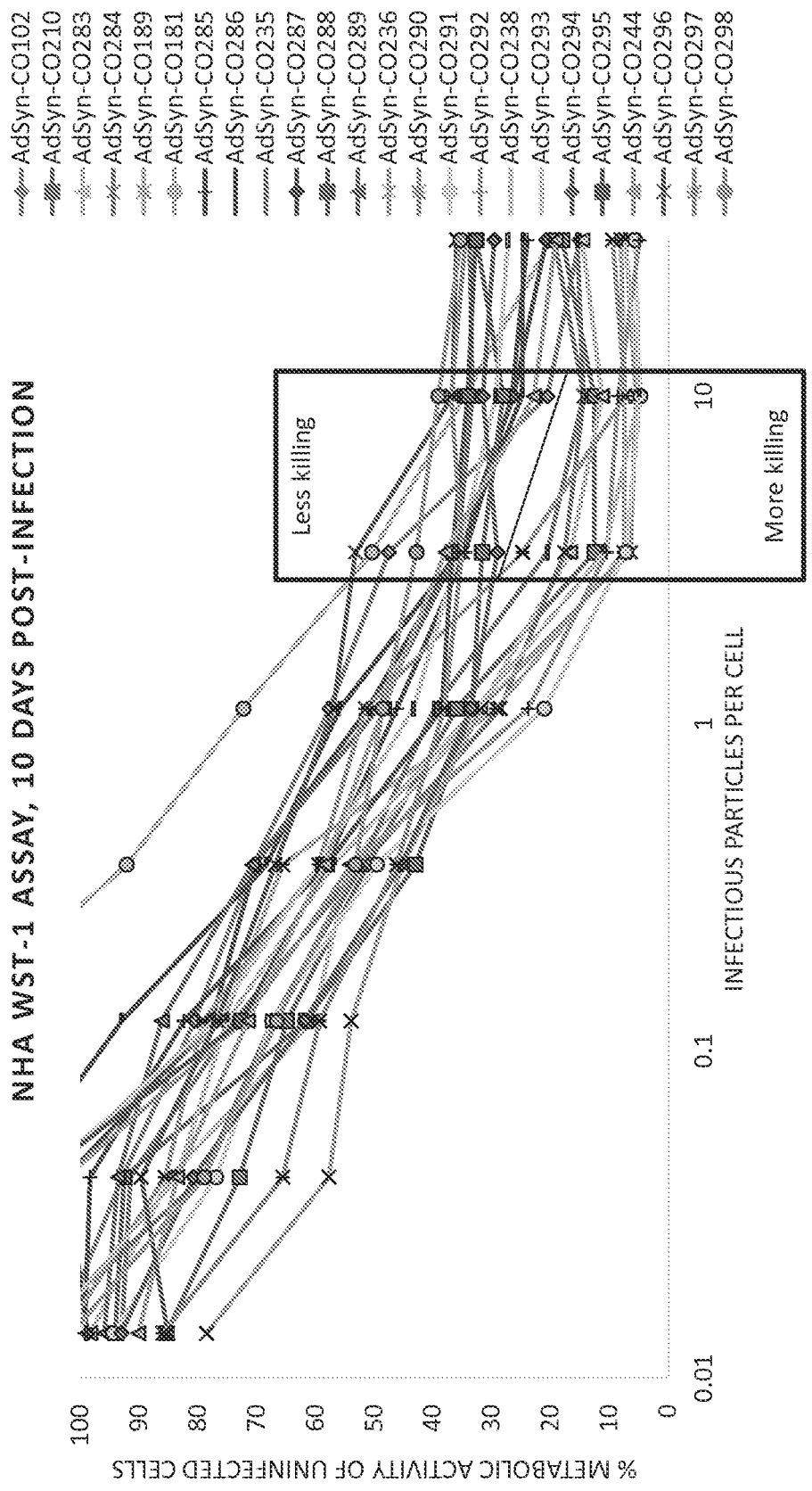

NHA WST-1 ASSAY, 10 DAYS POST-INFECTION
AdSyn-CO102
AdSyn-CO210
AdSyn-CO283
AdSyn-CO284
AdSyn-CO189
AdSyn-CO181
AdSyn-CO285
AdSyn-CO286
AdSyn-CO235
AdSyn-CO287
AdSyn-CO288
AdSyn-CO289
AdSyn-CO236
AdSyn-CO290
AdSyn-CO291
AdSyn-CO292
AdSyn-CO238
AdSyn-CO293
AdSyn-CO294
AdSyn-CO295
AdSyn-CO244
AdSyn-CO296
AdSyn-CO297
AdSyn-CO298
Less killing
More killing
INFECTIOUS PARTICLES PER CELL
0.01
0.1
1
10
% METABOLIC ACTIVITY OF UNINFECTED CELLS
100
90
80
70
60
50
40
30
20
10
0

Fig. 17

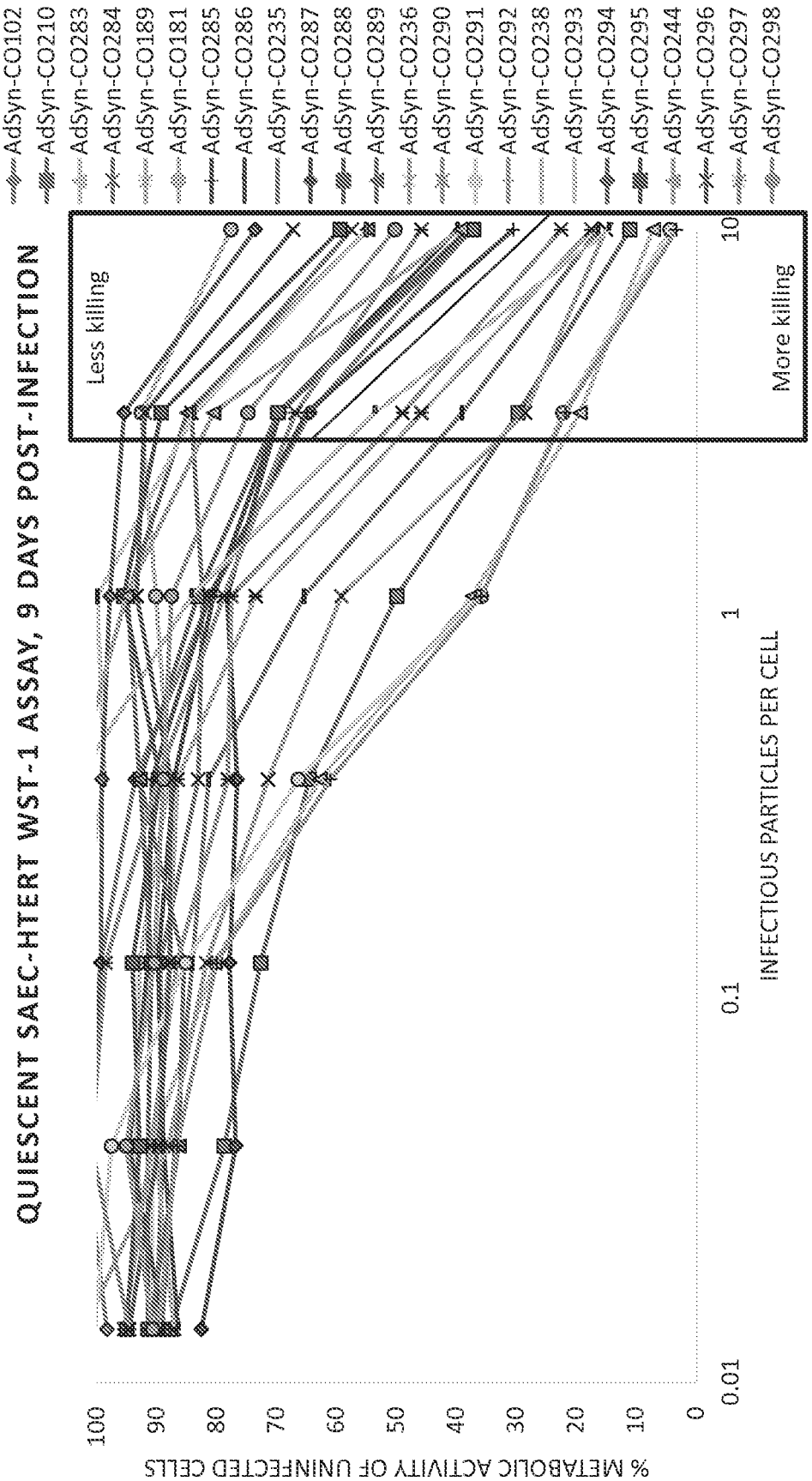

QUIESCENT SAEC-HTERT WST-1 ASSAY, 9 DAYS POST-INFECTION
% METABOLIC ACTIVITY OF UNINFECTED CELLS
INFECTIOUS PARTICLES PER CELL
Less killing
More killing
AdSyn-CO102
AdSyn-CO210
AdSyn-CO283
AdSyn-CO284
AdSyn-CO189
AdSyn-CO181
AdSyn-CO285
AdSyn-CO286
AdSyn-CO235
AdSyn-CO287
AdSyn-CO288
AdSyn-CO289
AdSyn-CO236
AdSyn-CO290
AdSyn-CO291
AdSyn-CO292
AdSyn-CO238
AdSyn-CO293
AdSyn-CO294
AdSyn-CO295
AdSyn-CO244
AdSyn-CO296
AdSyn-CO297
AdSyn-CO298

Fig. 18

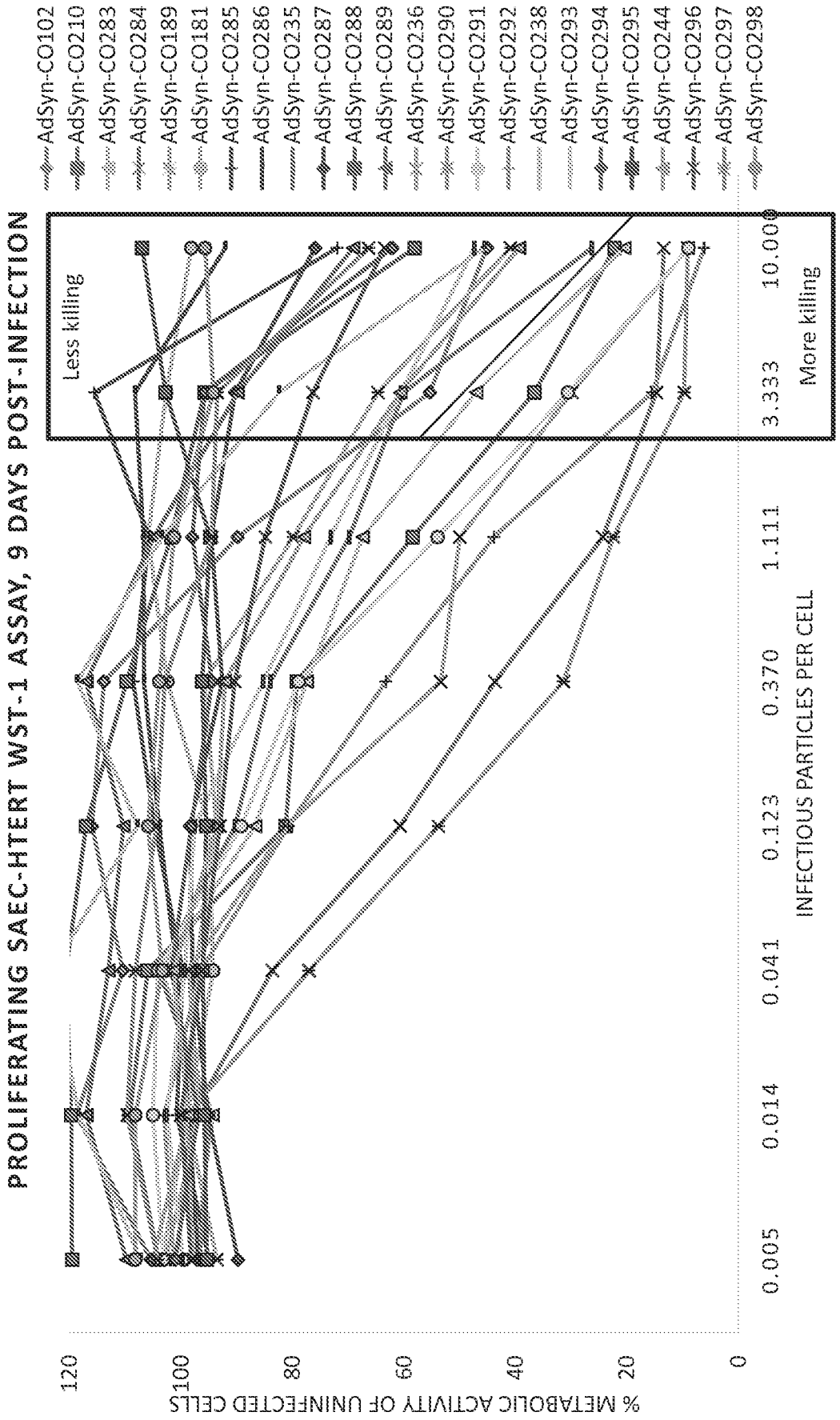

PROLIFERATING SAEC-HTERT WST-1 ASSAY, 9 DAYS POST-INFECTION
% METABOLIC ACTIVITY OF UNINFECTED CELLS
120
100
80
60
40
20
0
INFECTIOUS PARTICLES PER CELL
0.005
0.014
0.041
0.123
0.370
1.111
3.333
10.000
Less killing
More killing
AdSyn-CO102
AdSyn-CO210
AdSyn-CO283
AdSyn-CO284
AdSyn-CO189
AdSyn-CO181
AdSyn-CO285
AdSyn-CO286
AdSyn-CO235
AdSyn-CO287
AdSyn-CO288
AdSyn-CO289
AdSyn-CO236
AdSyn-CO290
AdSyn-CO291
AdSyn-CO292
AdSyn-CO238
AdSyn-CO293
AdSyn-CO294
AdSyn-CO295
AdSyn-CO244
AdSyn-CO296
AdSyn-CO297
AdSyn-CO298

Fig. 19

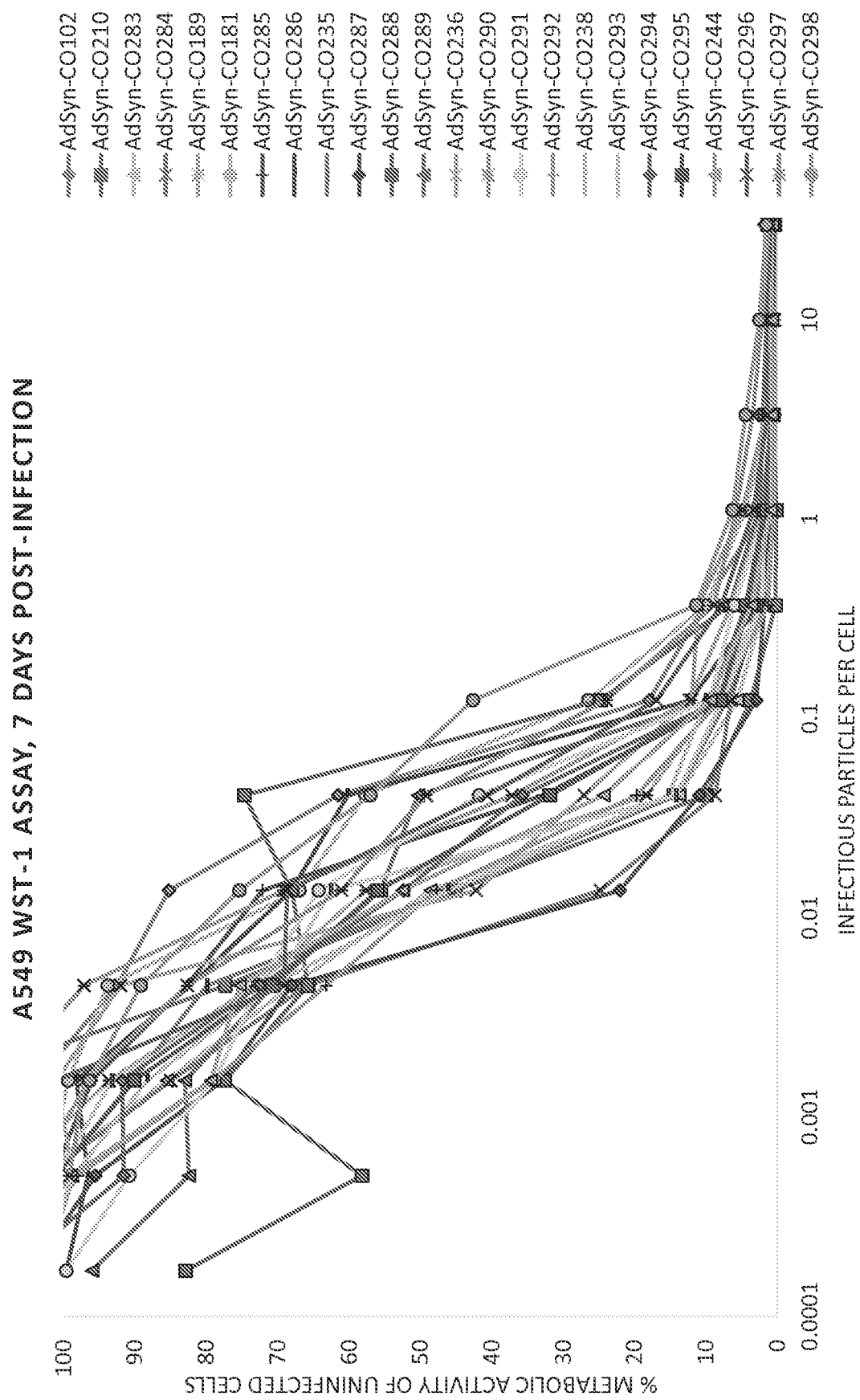

A549 WST-1 ASSAY, 7 DAYS POST-INFECTION
% METABOLIC ACTIVITY OF UNINFECTED CELLS
INFECTIOUS PARTICLES PER CELL
AdSyn-CO102
AdSyn-CO210
AdSyn-CO283
AdSyn-CO284
AdSyn-CO189
AdSyn-CO181
AdSyn-CO285
AdSyn-CO286
AdSyn-CO235
AdSyn-CO287
AdSyn-CO288
AdSyn-CO289
AdSyn-CO236
AdSyn-CO290
AdSyn-CO291
AdSyn-CO292
AdSyn-CO238
AdSyn-CO293
AdSyn-CO294
AdSyn-CO295
AdSyn-CO244
AdSyn-CO296
AdSyn-CO297
AdSyn-CO298

Fig. 20

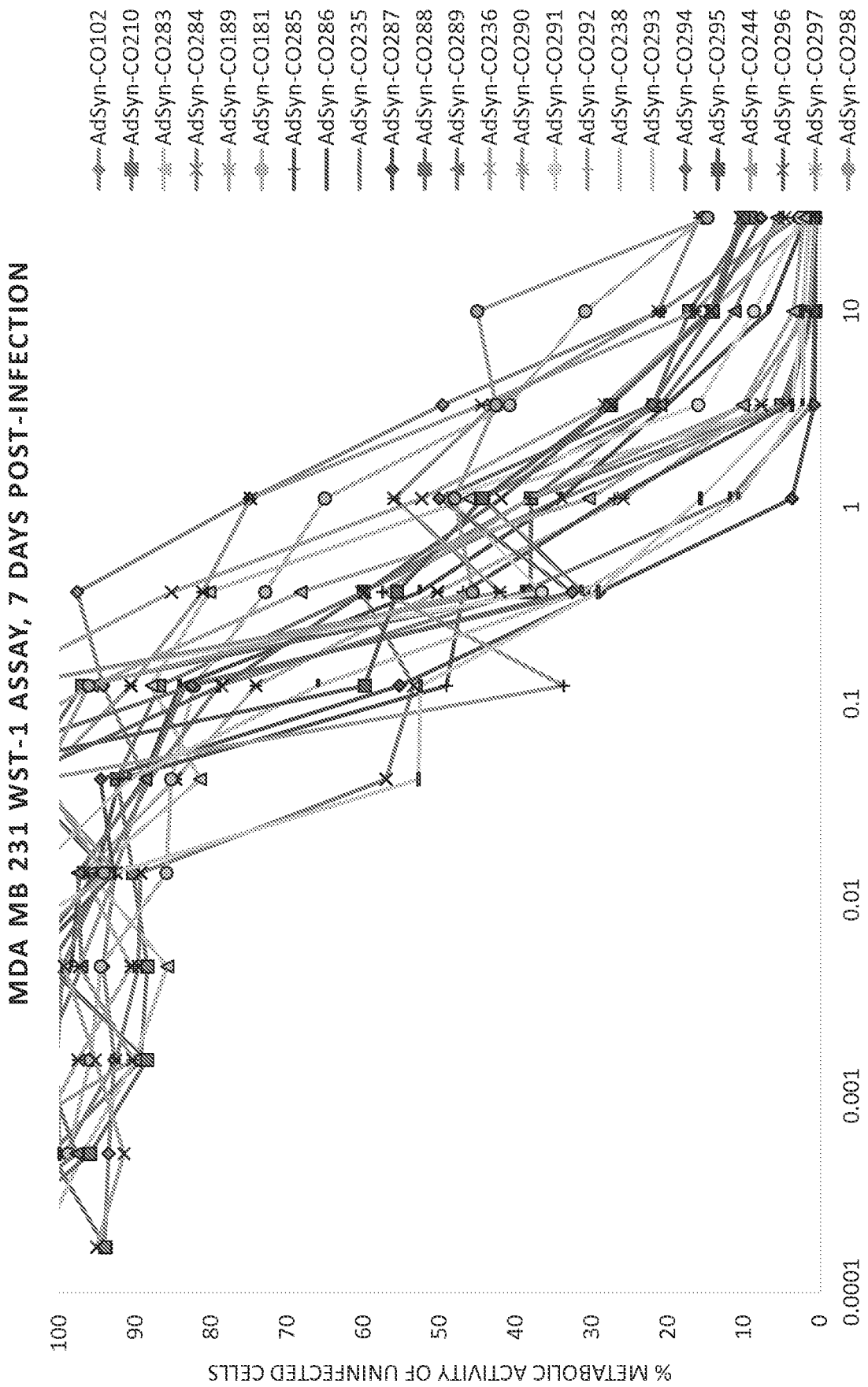

MDA MB 231 WST-1 ASSAY, 7 DAYS POST-INFECTION
% METABOLIC ACTIVITY OF UNINFECTED CELLS
INFECTIOUS PARTICLES PER CELL
0.0001
0.001
0.01
0.1
1
10
0
10
20
30
40
50
60
70
80
90
100
AdSyn-CO102
AdSyn-CO210
AdSyn-CO283
AdSyn-CO284
AdSyn-CO189
AdSyn-CO181
AdSyn-CO285
AdSyn-CO286
AdSyn-CO235
AdSyn-CO287
AdSyn-CO288
AdSyn-CO289
AdSyn-CO236
AdSyn-CO290
AdSyn-CO291
AdSyn-CO292
AdSyn-CO238
AdSyn-CO293
AdSyn-CO294
AdSyn-CO295
AdSyn-CO244
AdSyn-CO296
AdSyn-CO297
AdSyn-CO298

Fig. 21

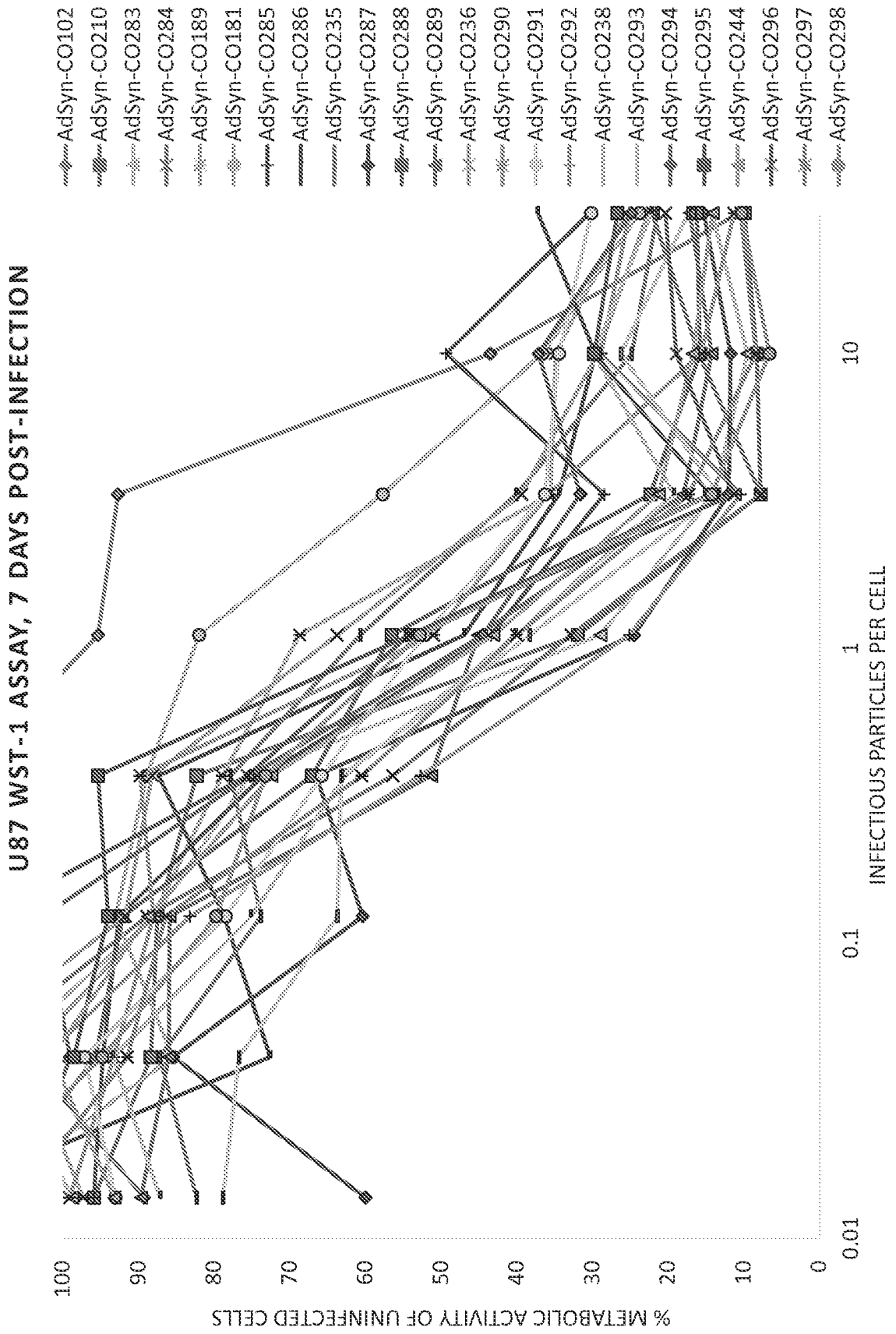

U87 WST-1 ASSAY, 7 DAYS POST-INFECTION
% METABOLIC ACTIVITY OF UNINFECTED CELLS
100
90
80
70
60
50
40
30
20
10
0
0.01
0.1
1
10
INFECTIOUS PARTICLES PER CELL
AdSyn-CO102
AdSyn-CO210
AdSyn-CO283
AdSyn-CO284
AdSyn-CO189
AdSyn-CO181
AdSyn-CO285
AdSyn-CO286
AdSyn-CO235
AdSyn-CO287
AdSyn-CO288
AdSyn-CO289
AdSyn-CO236
AdSyn-CO290
AdSyn-CO291
AdSyn-CO292
AdSyn-CO238
AdSyn-CO293
AdSyn-CO294
AdSyn-CO295
AdSyn-CO244
AdSyn-CO296
AdSyn-CO297
AdSyn-CO298

Fig. 22

SUBSTITUTE SHEET (RULE 26)

	NHA			Quiescent SAEC-hTERT			Proliferating SAEC-hTERT			A549			MDA MB 231			U87		
Adenovirus, Initial MOI	10	3.33	1.11	10	3.33	1.11	10	3.33	1.11	0.37	0.12	0.04	3.33	1.11	0.37	3.33	1.11	0.37
AdSyn-CO102*	21	47	68	38	64	81	45	55	90	10	18	62	50	75	96	93	95	113
AdSyn-CO210	13	13	36	11	30	50	22	37	59	0	4	10	5	38	60	8	32	82
AdSyn-CO283	11	17	32	7	19	37	21	47	67	2	6	14	4	46	80	14	29	75
AdSyn-CO284	14	18	29	57	84	82	13	15	24	3	7	9	5	34	60	8	33	56
AdSyn-CO189	**5**	**6**	**32**	**15**	**29**	**59**	**9**	**30**	**50**	**6**	**12**	**27**	**28**	**52**	**85**	**39**	**64**	**88**
AdSyn-CO181	7	25	51	23	49	78	9	10	22	11	12	37	44	75	81	36	51	90
AdSyn-CO285	5	7	21	4	22	36	9	31	54	6	10	42	16	49	37	36	52	66
AdSyn-CO286	8	10	24	3	22	36	6	16	44	1	8	20	10	38	58	10	25	53
AdSyn-CO235	7	7	29	17	46	74	41	65	80	2	9	18	8	26	50	36	69	79
AdSyn-CO287	35	50	72	78	93	90	98	103	106	10	27	58	41	65	73	58	82	89
AdSyn-CO288	27	35	46	31	65	81	72	116	106	2	7	33	4	27	47	28	43	74
AdSyn-CO289	25	36	56	40	70	81	92	108	107	3	12	60	20	34	53	34	47	87
AdSyn-CO236	14	21	40	16	39	65	26	60	70	3	7	14	1	12	39	40	61	78
AdSyn-CO290	26	36	51	38	69	78	76	90	95	2	3	11	1	4	29	12	24	66
AdSyn-CO291	28	32	33	37	70	87	58	96	102	7	25	75	22	38	38	22	57	67
AdSyn-CO292	33	36	49	55	83	95	69	90	105	4	9	50	21	44	31	18	45	51
AdSyn-CO238	5	11	33	15	54	84	46	82	103	2	10	15	2	11	31	19	40	63
AdSyn-CO293	29	36	43	55	84	103	47	61	73	2	8	14	4	16	29	13	38	63
AdSyn-CO294	31	29	34	74	95	98	62	96	98	4	8	36	22	50	33	32	44	75
AdSyn-CO295	34	37	38	59	89	96	107	103	95	5	8	32	28	44	56	14	54	95
AdSyn-CO244	23	38	48	39	81	95	39	61	78	4	9	24	10	30	68	21	43	72
AdSyn-CO296	37	53	67	67	92	93	64	76	85	8	17	41	27	42	60	11	54	76
AdSyn-CO297	37	35	38	46	67	79	66	94	95	9	24	49	43	56	42	17	40	60
AdSyn-CO298	39	43	49	50	75	88	96	94	101	11	43	57	43	48	46	14	53	73

Min		Max	Min		Max	Min		Max	Min		Max	Min		Max	Min		Max
5	38	72	3	52	106	6	61	116	0	38	75	1	49	96	8	60	113

Fig. 23

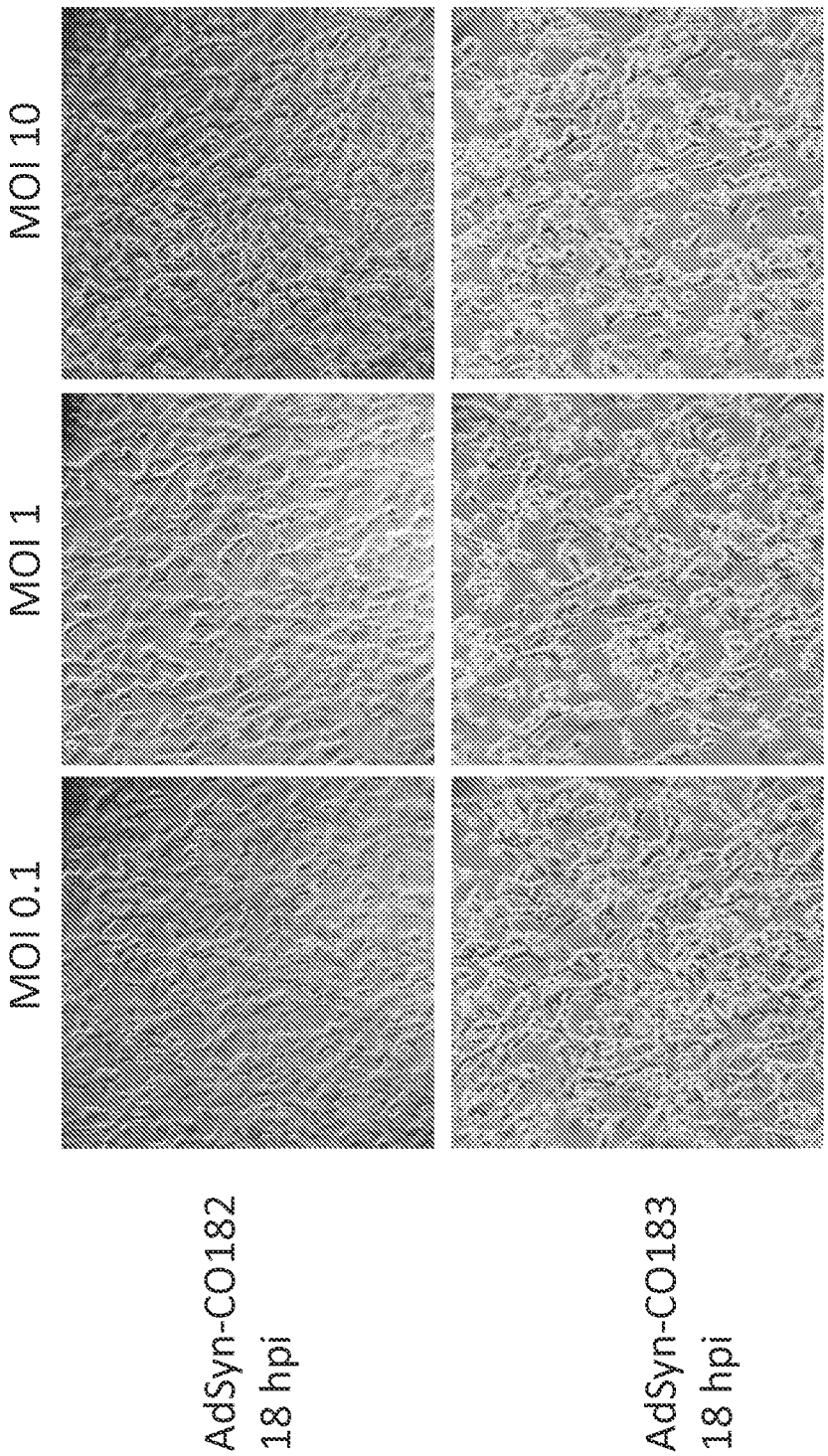


Fig. 24