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Myxoma viruses that are deficient in the activity of a Myxoma virus protein selected from the group consisting of M11L, M063, M 136, M-T4 and M-T7 are useful for treating cancer.

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(54) Title: MYXOMA VIRUS MUTANTS FOR CANCER TREATMENT

(57) Abstract: Myxoma viruses that are deficient in the activity of a Myxoma virus protein selected from the group consisting of M11L, M063, M 136, M-T4 and M-T7 are useful for treating cancer.

MYXOMA VIRUS MUTANTS FOR CANCER TREATMENT

BACKGROUND OF THE INVENTION

The use of certain genetically modified myxoma viruses for treating cancer is disclosed in WO 04/078206 (Robarts Research Institute).

SUMMARY OF THE INVENTION

This invention relates to Myxoma viruses (MV) that are deficient in the activity of a Myxoma virus protein selected from the group consisting of M11L, M063, M136, M-T4 and M-T7. Such viruses are used in a method for and in the manufacture of a medicament for, inhibiting a cancer cell, which method comprises administering to the cell an effective amount of the Myxoma virus. They are also used in a method for and in the manufacture of a medicament for, treating a human subject having cancer, comprising administering to the patient an effective amount of the Myxoma virus. This invention also provides a pharmaceutical composition comprising such Myxoma viruses and a pharmaceutically acceptable carrier, as well as a kit comprising such Myxoma viruses and instructions for treating a cancer patient.

DESCRIPTION OF THE FIGURES

Figure 1. Endogenous activated Akt levels in human glioma cells

Figure 2. Viral replication efficiency of the various vMyx-hrKOs and controls in human glioma cell lines.

Figure 3. Secreted early and late viral gene expression indicates that some of the vMyx-hrKO are unable to transit from early to late gene expression.

Figure 4. Selected single step growth curves.

Figure 5. Cell-based cytotoxicity assay

DETAILED DESCRIPTION OF THE INVENTION

WO 04/078206 (Robarts Research Institute) discloses the use of certain genetically modified myxoma viruses for treating cancer. This invention represents an advance by providing more specific modified myxoma viruses for such uses. The techniques disclosed therein are applicable generally to the myxoma viruses of this invention.

As used herein "deficient in the activity of" a given Myxoma virus protein means that the virus has less of the activity in question than wild-type Myxoma virus. "Substantially no activity" of a given viral protein means that the virus has no detectable level of such activity. Examples of Myxoma viruses having substantially no activity of a given viral protein include mutants in which the gene for such protein has been deleted or otherwise knocked-out.

In accordance with this invention, any kind of cancer or cancer cell can be inhibited or treated. In an embodiment of this invention, the cancer cell is a mammalian cancer cell. In a more specific embodiment, the cancer cell is a human cancer cell. Examples of such human cancer cells include gliomas.

It has been demonstrated that wild-type myxoma virus (vMyxgfp) can produce a productive, long-lived infection, and destroy and clear implanted tumor tissue when injected intratumorally into human gliomas implanted in murine brains (Lun et al, 2005 Cancer Research 65:9982-9990). As well, a screen of the NCI-60 reference collection indicated that MV productively infects the majority (15/21) of human tumor cells tested (Sypula et al. 2004 Gene Ther. Mol. Biol. 8:103-114). To expand understanding of MV tropism in cancer cells, a series of human glioma cells (U87, U118, U251, U343, U73) that were previously tested for wild-type MV permissiveness were screened. These findings have been extended in the

following Examples by testing the infection and replication of several MV viruses in which specific host range genes, identified as having a role in defining MV tropism in rabbit cells, have been deleted. These viruses are collectively referred to as host range knockouts (vMyx-hrKO). Variation was observed in the ability of various vMyx-hrKOs to replicate and spread in the human glioma cells. vMyxT2(U251), vMyxT4KO (U87, U118, U251 and U373) and vMyxT5KO (U251, U373) exhibited some restriction in specific human gliomas. In contrast vMyx63KO and vMyx135KO appeared to replicate and kill more effectively in several of the gliomas.

The invention will be better understood by reference to the following examples, which illustrate but do not limit the invention described herein.

EXAMPLES

EXAMPLE 1

Fifty micrograms of whole cell lysates were probed with antibodies against phosphorylated Akt at positions threonine 308 (P-Akt T308) and serine 473 (P-Akt S473) or total Akt. Based on the levels of activated Akt U87 and U343 would be expected to be infectable and U118, U251 and U373 to be more resistant to MV infection. (Figure 1).

EXAMPLE 2

Virus stocks were titrated on the various glioma cells and control BGMK or RK13 cells. Virus titres, derived from the gliomas, were compared to the control levels and a value of viral replication efficiency was estimated. Based on these results none of the gliomas supported viral growth to the levels observed in the control lines. However some viruses (vMyx135KO, vMyx63KO and vMyx136KO) were more efficient than other knockouts. As well, some glioma lines supported more replication (U87, U343 and U373). (Figure 2)

EXAMPLE 3

Various human gliomas were infected with a range of vMyx-hrKOs. Twenty hpi the infected supernatants were collected and concentrated 10x. Fifteen microlitres

of concentrated sups were separated on a 12% SDS-PAGE gel and probed with anti-Serp1 (mAB; late MV gene product). The blots were stripped and probed for early gene expression with anti-M-T7 (pAB; early MV gene product). The results suggest that several vMyx-hrKOs are restricted in their transit from early to late gene expression including vMyxT2KO, vMyxT4KO and vMyxT5KO. And in two glioma lines (U87 and U37), vMyxT4KO does not even undergo early gene expression. (Figure 3).

EXAMPLE 4

Cells were seeded in 48 well dishes and infected cells were collected at the times indicated. Virus was released from the collected cell pellets and titrated back onto BGMK cells. Although there was replication of the tested viruses, the best amplification appeared to occur in the U87 and U343 cells. (Figure 4).

EXAMPLE 5

The ability of the various vMyx-hrKOs and control viruses to have a killing effect in the panel of human gliomas was tested by a cytotoxicity assay. The appropriate cells were seeded in 96 well dishes and 24 h later were infected with the viruses at various MOIs. Seventy-two hours post infection the infected cells were treated with the WTS reagent (Roche) to measure cell viability. Colour changes were measured at 450nm every 60 minutes for 4 hours. Uninfected control wells were used to determine normal proliferation and a blank well served as a background control. (Figure 5).

What is claimed is:

1. An *in vitro* method for inhibiting a cancer cell comprising administering to the cell an effective amount of a Myxoma virus that is deficient in the activity of Myxoma virus protein M11L.

2. The method of claim 1, wherein the gene for the Myxoma virus protein M11L has been deleted or otherwise knocked out in said Myxoma virus.

3. The method of claim 1 or 2, wherein the Myxoma virus has substantially no activity of the Myxoma virus protein M11L.

4. The method of any one of claims 1 to 3, wherein the cell is a mammalian cancer cell.

5. The method of claim 4, wherein the cell is a human cancer cell.

6. The method of claim 5, wherein the cell is a glioma cell.

7. Use of an effective amount of a Myxoma virus for inhibiting a cancer cell, wherein the Myxoma virus is deficient in the activity of Myxoma virus protein M11L.

8. Use of an effective amount of a Myxoma virus in the manufacture of a medicament for inhibiting a cancer cell, wherein the Myxoma virus is deficient in the activity of Myxoma virus protein M11L.

9. The use of claim 7 or 8, wherein the gene for the Myxoma virus protein M11L has been deleted or otherwise knocked out in said Myxoma virus.

10. The use of any one of claims 7 to 9, wherein the Myxoma virus has substantially no activity of the Myxoma virus protein M11L.

11. The use of any one of claims 7 to 10, wherein the cell is a mammalian cancer cell.

12. The use of claim 11, wherein the cell is a human cancer cell.

13. The use of claim 12, wherein the cell is a glioma cell.

14. Use of an effective amount of a Myxoma virus for treating a human subject having cancer, wherein the Myxoma virus is deficient in the activity of Myxoma virus protein M11L.

15. Use of an effective amount of a Myxoma virus in the manufacture of a medicament for treating a human subject having cancer, wherein the Myxoma virus is deficient in the activity of Myxoma virus protein M11L.

16. The use of claim 14 or 15, wherein the gene for the Myxoma virus protein M11L has been deleted or otherwise knocked out in said Myxoma virus.

17. The use of any one of claims 14 to 16, wherein the Myxoma virus has substantially no activity of the Myxoma virus protein M11L.

18. The use of any one of claims 14 to 17, wherein the cancer is a glioma.

19. A pharmaceutical composition comprising a Myxoma virus and a pharmaceutically acceptable carrier for use in treating cancer, wherein the Myxoma virus is deficient in the activity of Myxoma virus protein M11L.

20. The pharmaceutical composition of claim 19, wherein the gene for the Myxoma virus protein M11L has been deleted or otherwise knocked out in said Myxoma virus.

21. The pharmaceutical composition of claim 19 or 20, wherein the Myxoma virus has substantially no activity of the Myxoma virus protein M11L.

22. The pharmaceutical composition of any one of claims 19 to 21, wherein the cancer is a glioma.

FIGURE 1

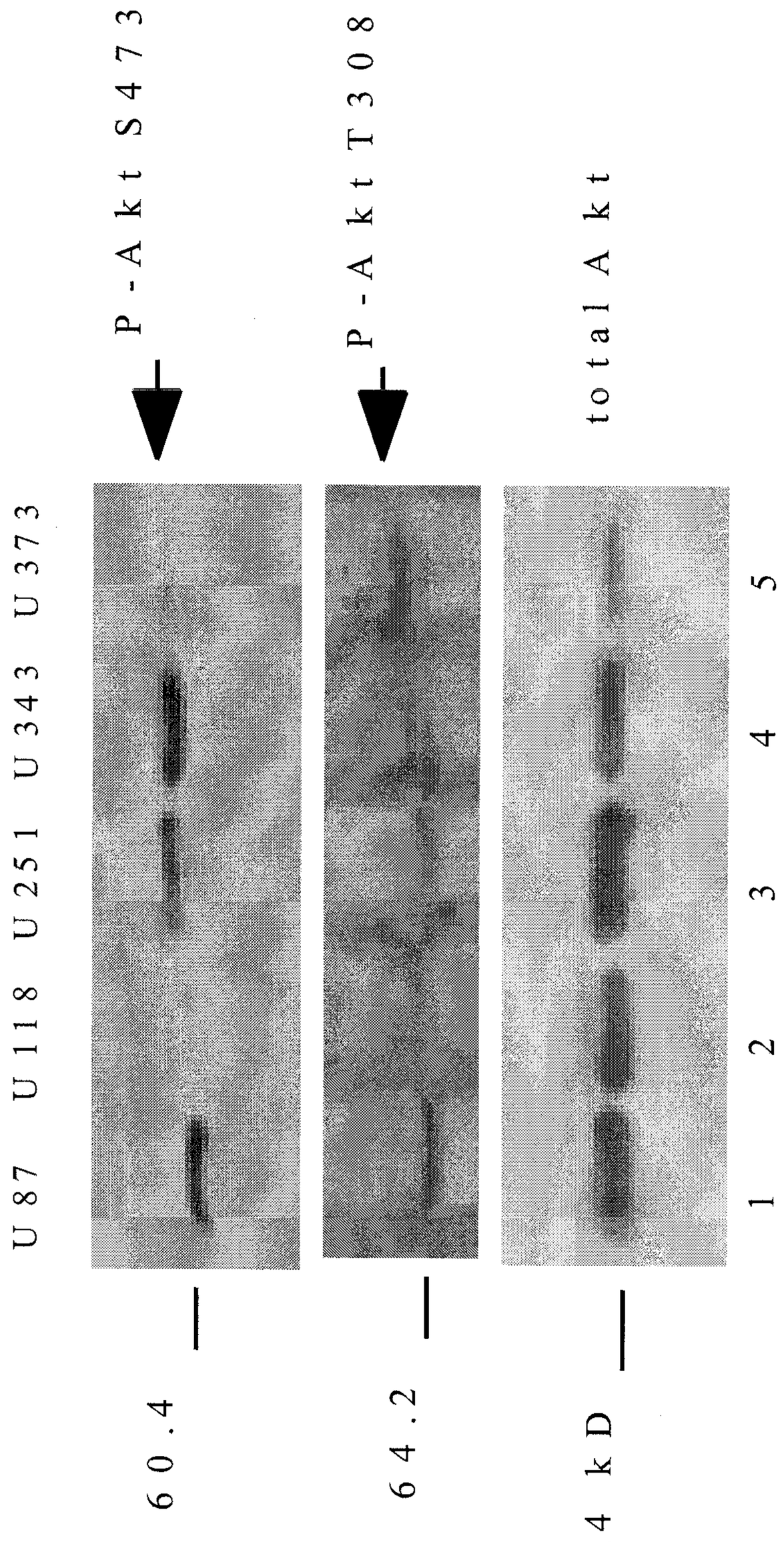
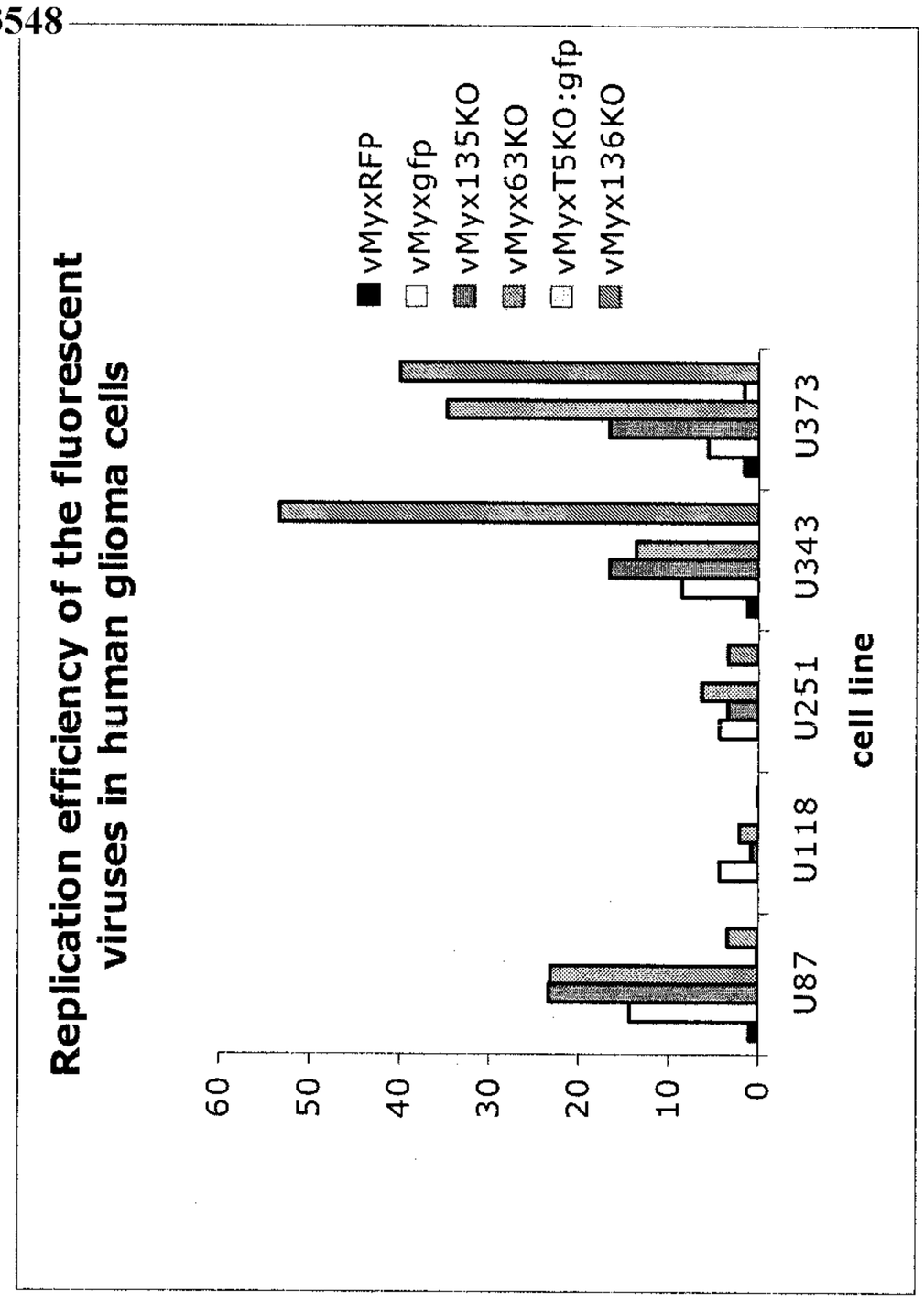

U251
U343
U373
U87
U118
60.4
64.2
.4 kD
1
2
3
4
5
6
7
8
9
0
P-Akt S473
P-Akt T308
Total Akt

FIGURE 2


Replication efficiency of the fluorescent viruses in human glioma cells
vMyxRFP
vMyxgfp
vMyx135KO
vMyx63KO
vMyxT5KO:gfp
vMyx136KO
60
50
40
30
20
10
0
U87
U118
U251
U343
U373
cell line

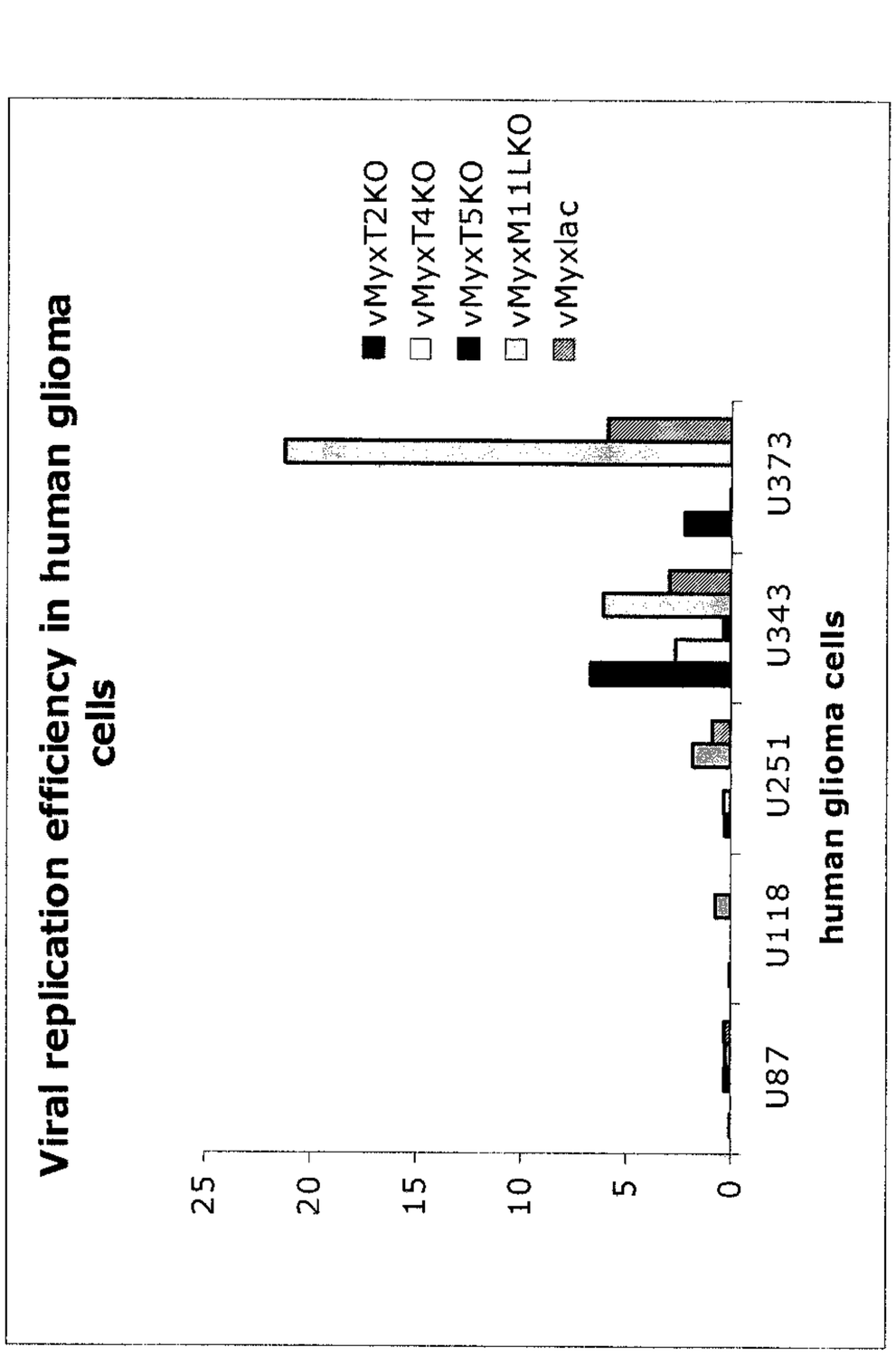

Viral replication efficiency in human glioma cells
vMyxT2KO
vMyxT4KO
vMyxT5KO
vMyxM11LKO
vMyxlac
25
20
15
10
5
0
U87
U118
U251
U343
U373
human glioma cells

FIGURE 3

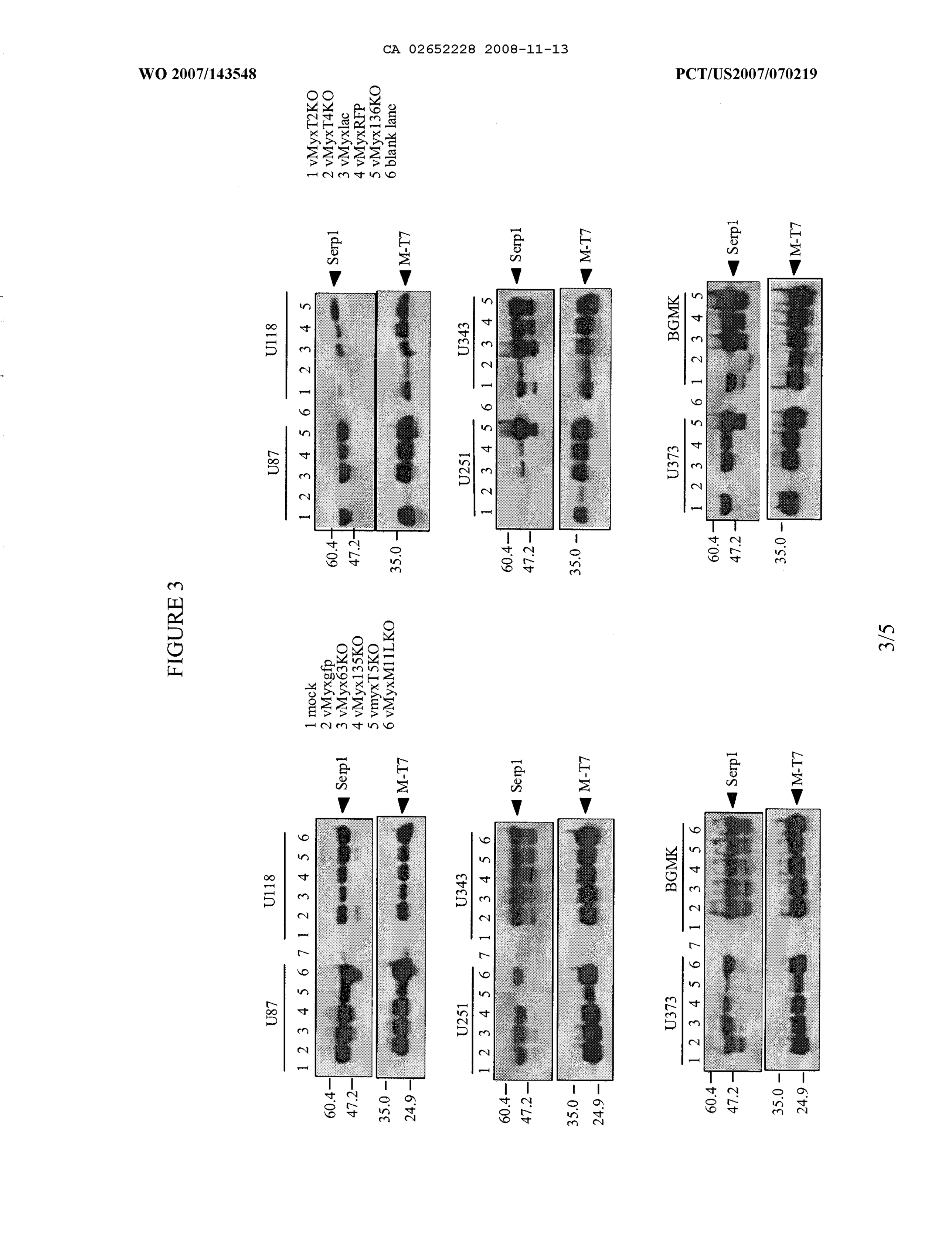

1 vMyxT2KO
2 vMyxT4KO
3 vMyxlac
4 vMyxRFP
5 vMyx136KO
6 blank lane
mock
vMyxgfp
vMyx63KO
vMyx135KO
vmyxT5KO
vMyxM11LKO
Serp1
M-T7
U87
U118
U251
U343
U373
BGMK
1 2 3 4 5 6 7
60.4
47.2
35.0
24.9

FIGURE 4

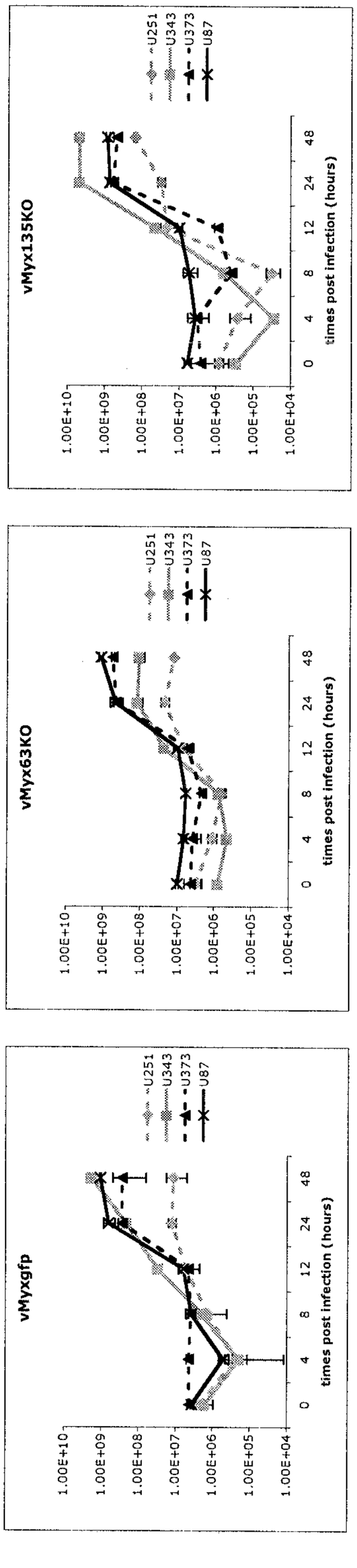
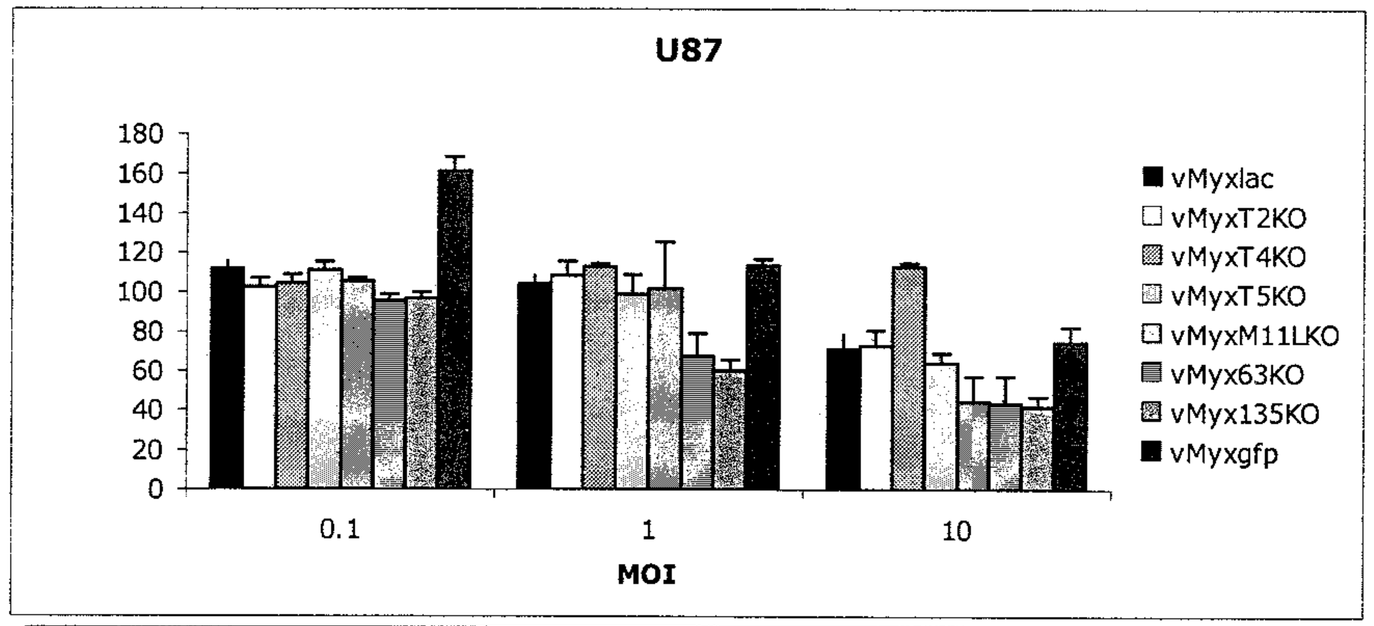
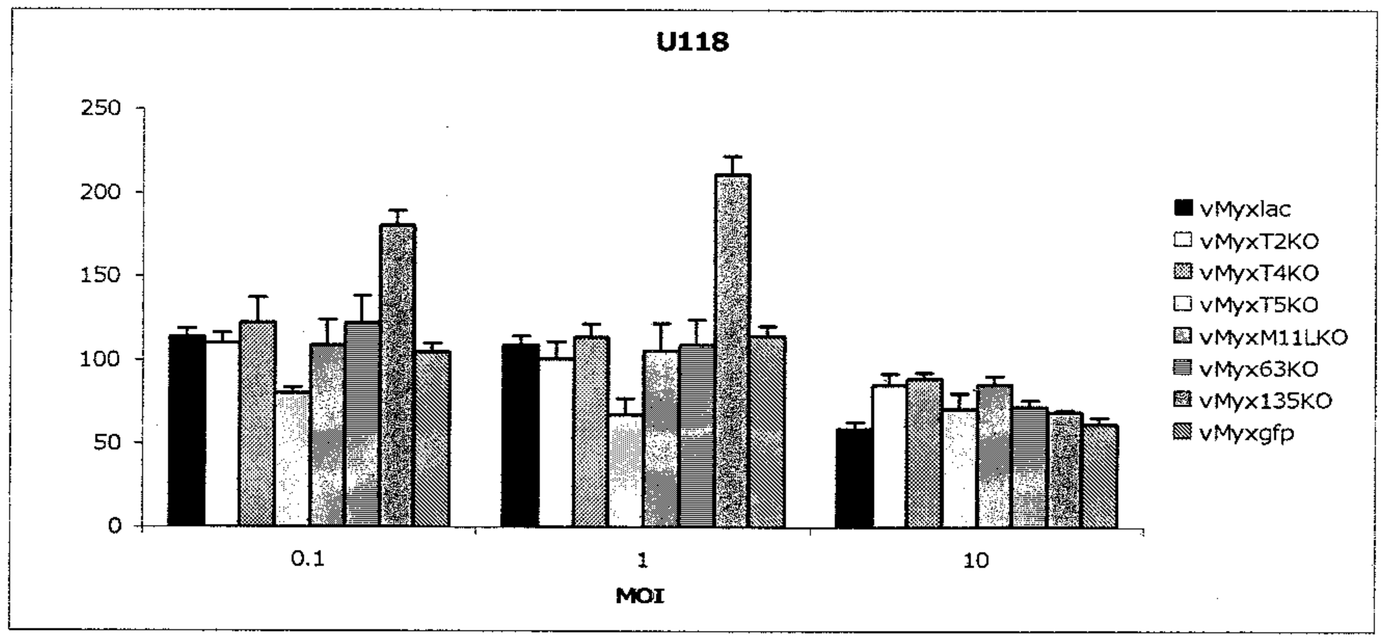

vMyx135KO
vMyx63KO
vMyxgfp
U251
U343
U373
U87
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
0
4
8
12
24
48
times post infection (hours)

FIGURE 5


U87
180
160
140
120
100
80
60
40
20
0
0.1
1
10
MOI
vMyxlac
vMyxT2KO
vMyxT4KO
vMyxT5KO
vMyxM11LKO
vMyx63KO
vMyx135KO
vMyxgfp


U118
250
200
150
100
50
0
0.1
1
10
MOI
vMyxlac
vMyxT2KO
vMyxT4KO
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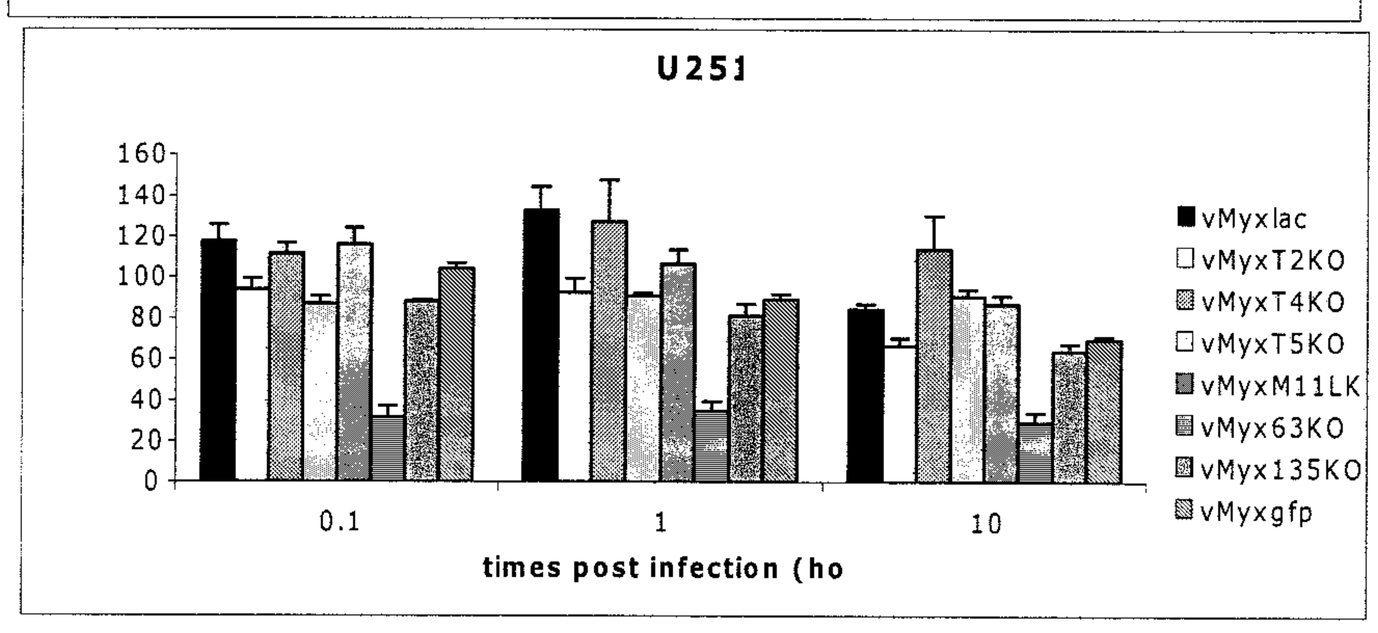
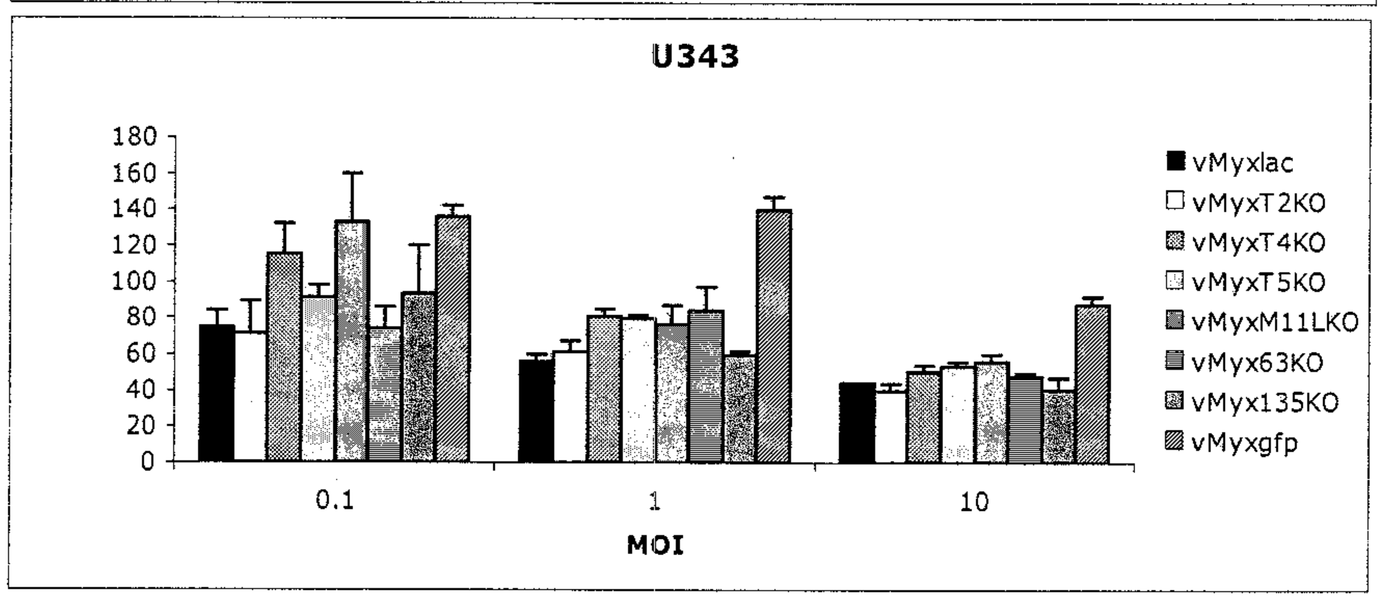
vMyxM11LKO
vMyx63KO
vMyx135KO
vMyxgfp


U251
160
140
120
100
80
60
40
20
0
0.1
1
10
times post infection (ho
vMyxlac
vMyxT2KO
vMyxT4KO
vMyxT5KO
vMyxM11LK
vMyx63KO
vMyx135KO
vMyxgfp


U343
180
160
140
120
100
80
60
40
20
0
0.1
1
10
MOI
vMyxlac
vMyxT2KO
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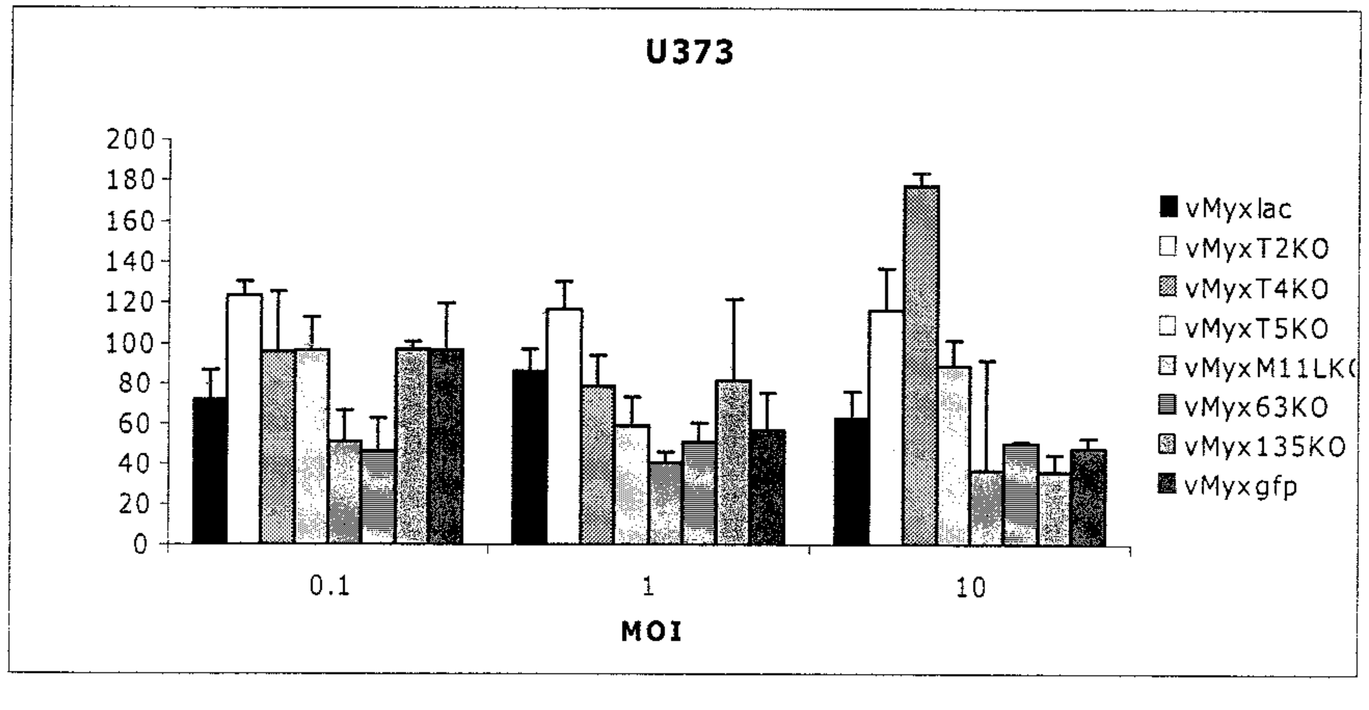
vMyxM11LKO
vMyx63KO
vMyx135KO
vMyxgfp


U373
200
180
160
140
120
100
80
60
40
20
0
0.1
1
10
MOI
vMyxlac
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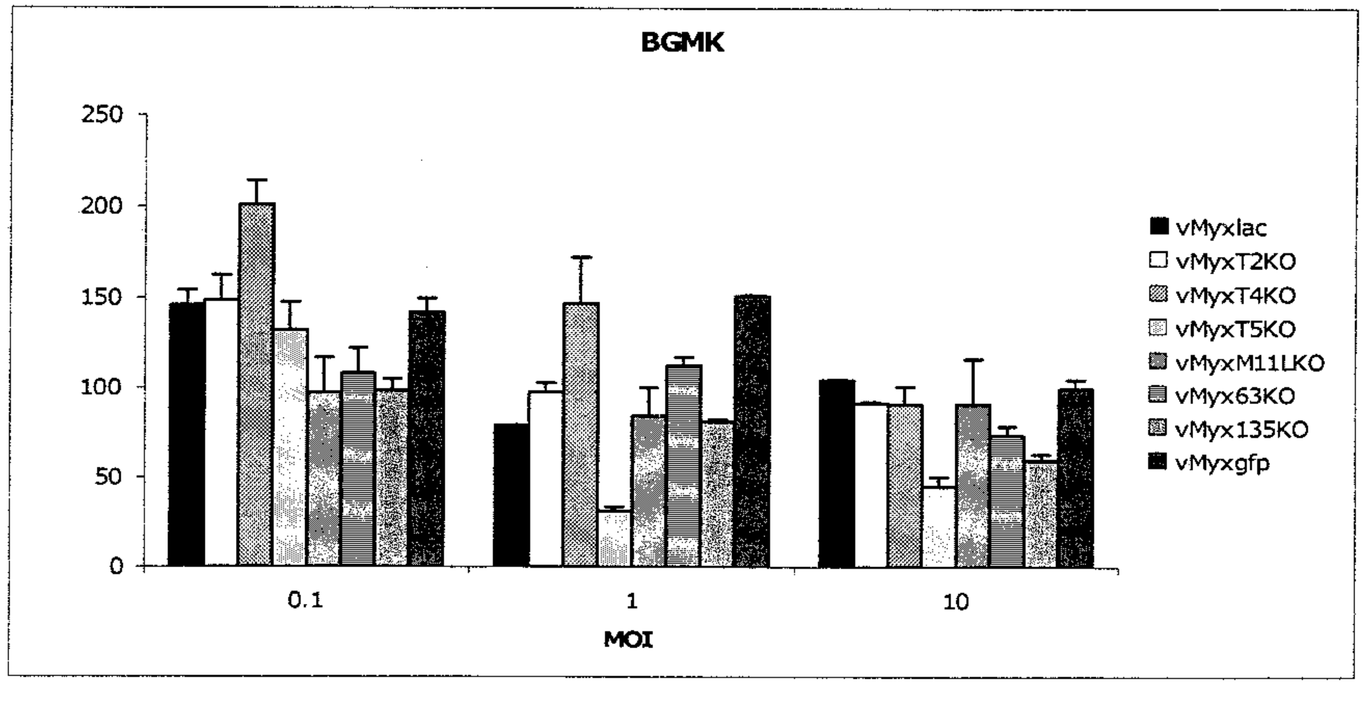
vMyxgfp


BGMK
250
200
150
100
50
0
0.1
1
10
MOI
vMyxlac
vMyxT2KO
vMyxT4KO
vMyxT5KO
vMyxM11LKO
vMyx63KO
vMyx135KO
vMyxgfp