Figure S1

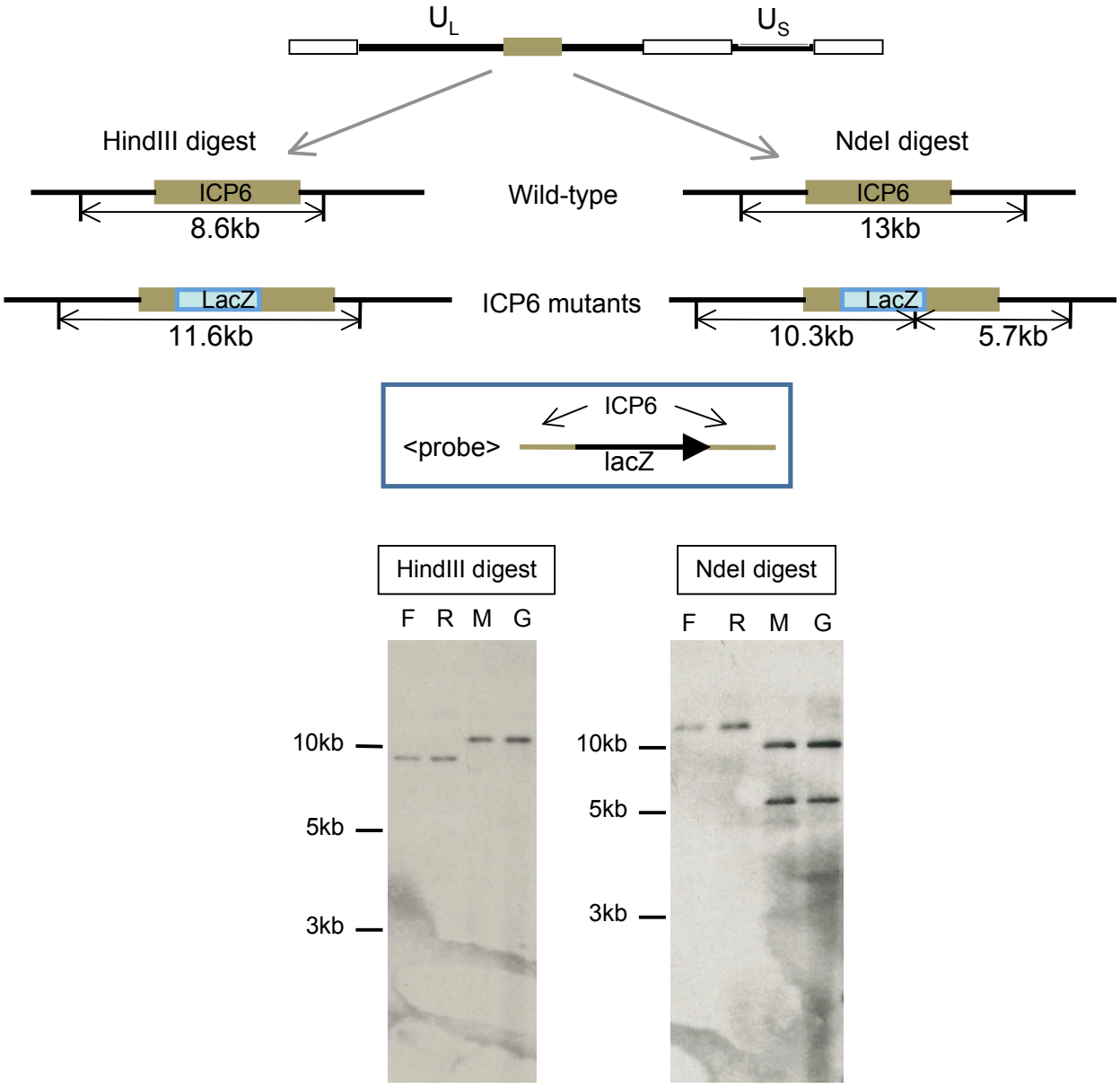

U_L
U_S
HindIII digest
NdeI digest
ICP6
8.6kb
Wild-type
ICP6
13kb
LacZ
11.6kb
ICP6 mutants
LacZ
10.3kb
5.7kb
ICP6
<probe>
lacZ
HindIII digest
F R M G
10kb
5kb
3kb
NdeI digest
F R M G
10kb
5kb
3kb

Figure S2

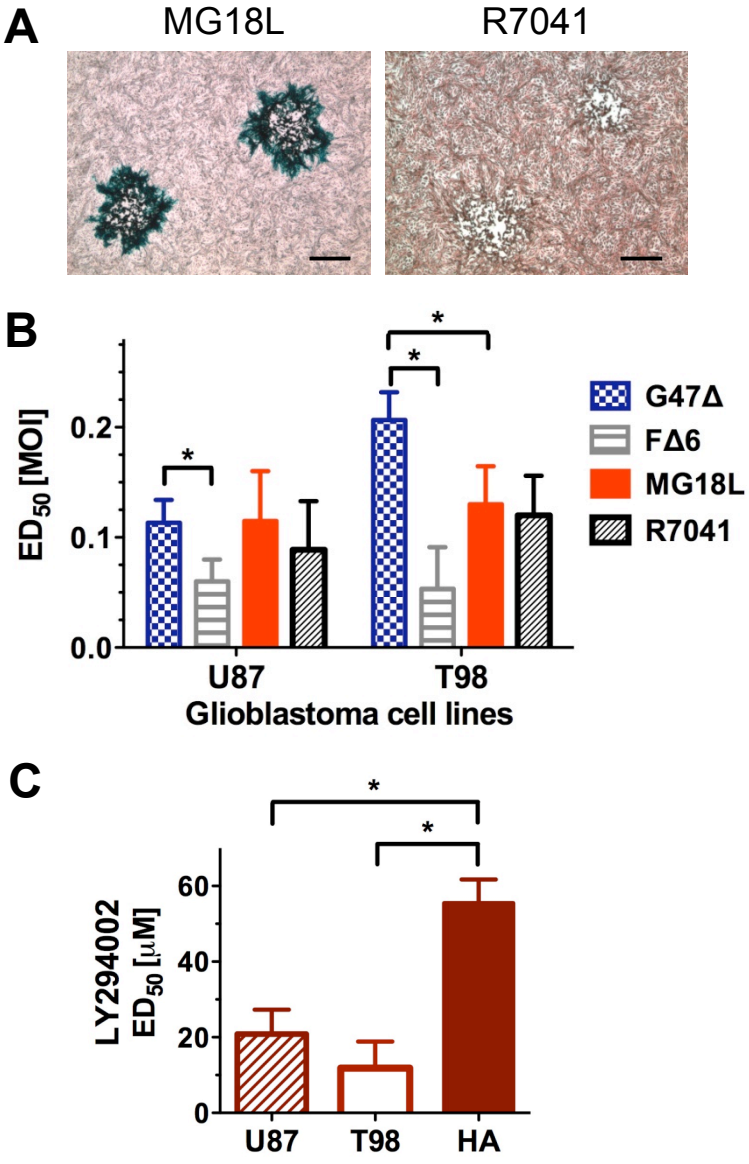

A
MG18L
R7041
B
ED50 [MOI]
0.2
0.1
0.0
*
*
*
G47Δ
FΔ6
MG18L
R7041
U87
T98
Glioblastoma cell lines
C
LY294002
ED50 [μM]
60
40
20
0
*
*
U87
T98
HA

Figure S3

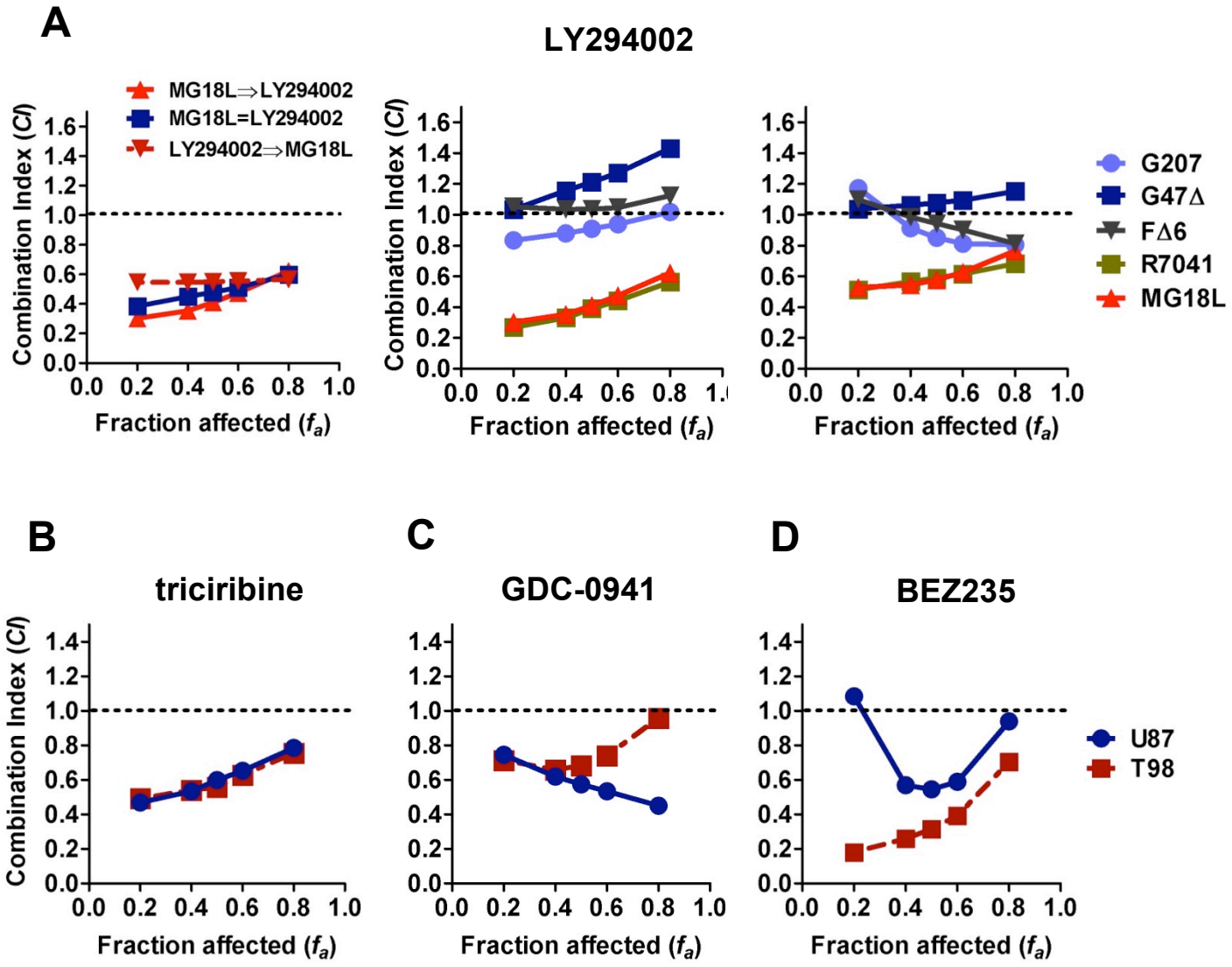

A
LY294002
MG18L⇒LY294002
MG18L=LY294002
LY294002⇒MG18L
Combination Index (CI)
Fraction affected (fa)
G207
G47Δ
FΔ6
R7041
MG18L
B
triciribine
C
GDC-0941
D
BEZ235
U87
T98

Figure S4

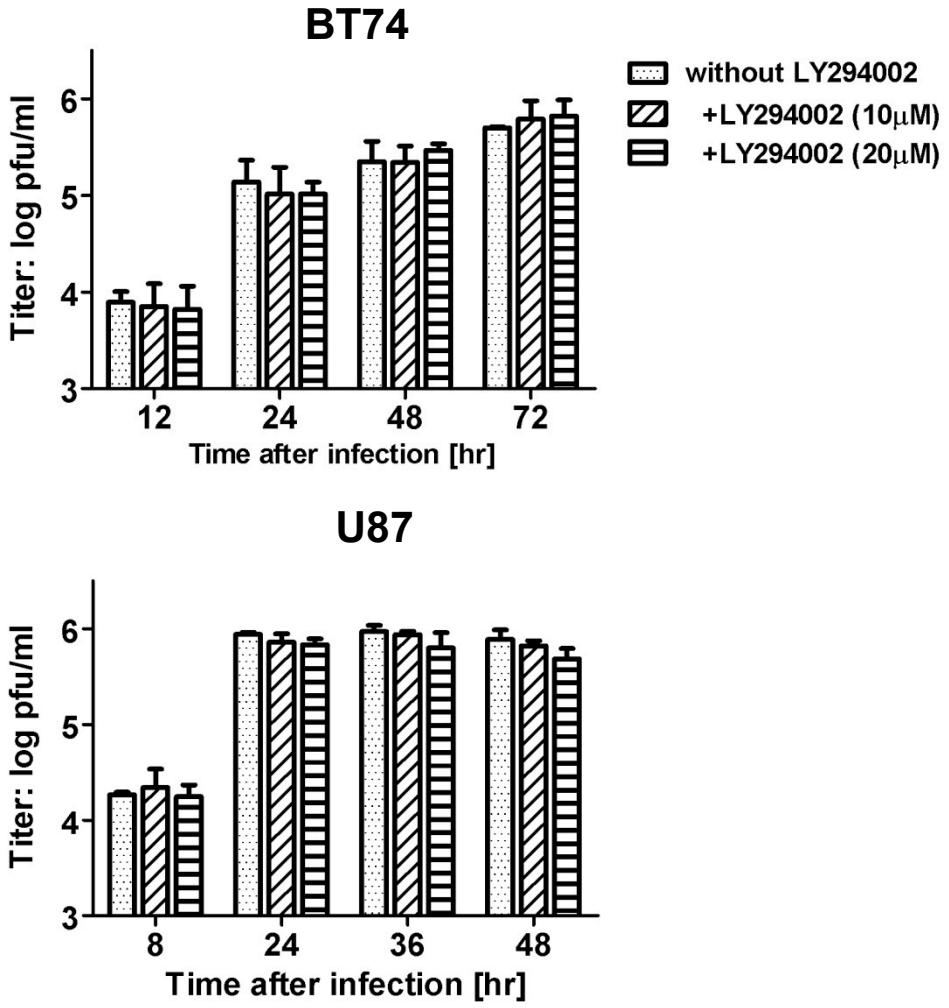

BT74
Titer: log pfu/ml
6
5
4
3
12
24
48
72
Time after infection [hr]
without LY294002
+LY294002 (10μM)
+LY294002 (20μM)
U87
Titer: log pfu/ml
6
5
4
3
8
24
36
48
Time after infection [hr]

Figure S5

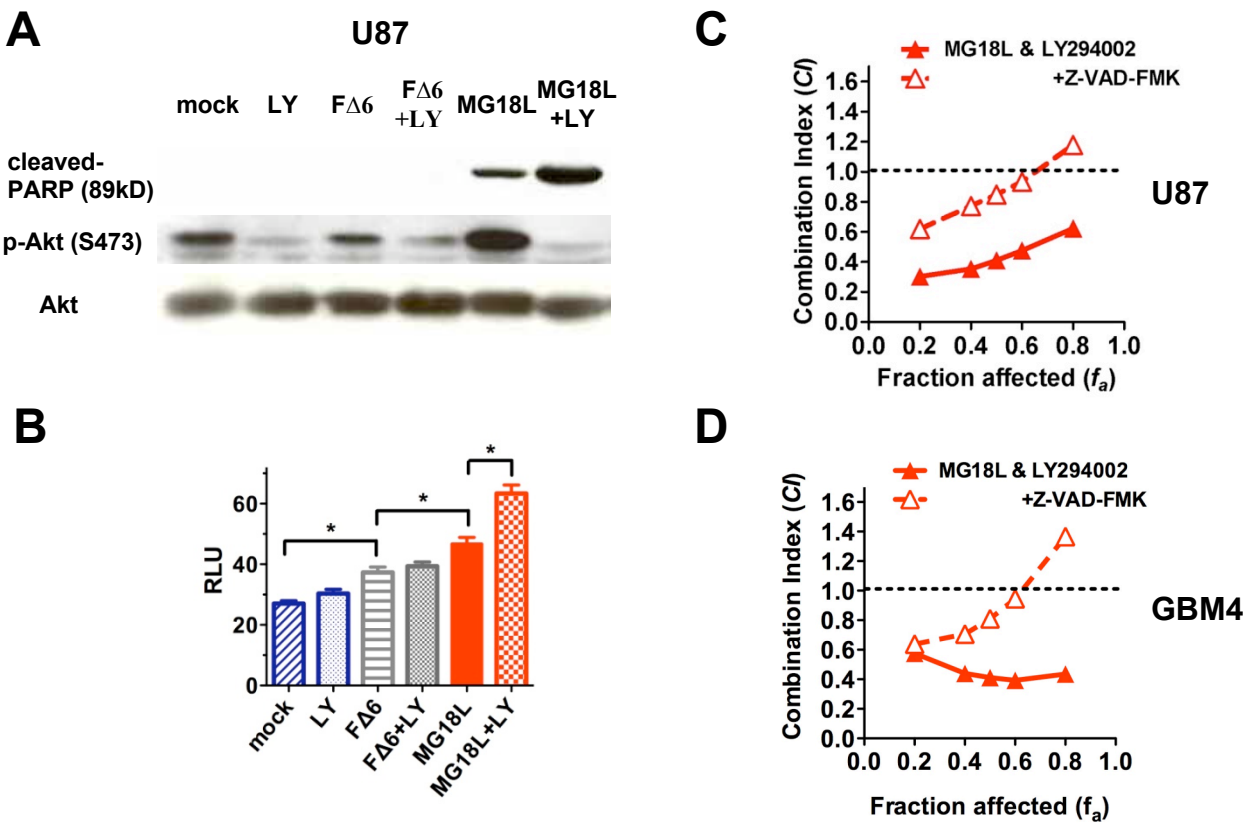

A
U87
mock
LY
FΔ6
FΔ6 +LY
MG18L
MG18L +LY
cleaved-PARP (89kD)
p-Akt (S473)
Akt
B
RLU
mock
LY
FΔ6
FΔ6+LY
MG18L
MG18L+LY
C
MG18L & LY294002
+Z-VAD-FMK
Combination Index (CI)
Fraction affected (fa)
U87
D
MG18L & LY294002
+Z-VAD-FMK
Combination Index (CI)
Fraction affected (fa)
GBM4

Figure S6

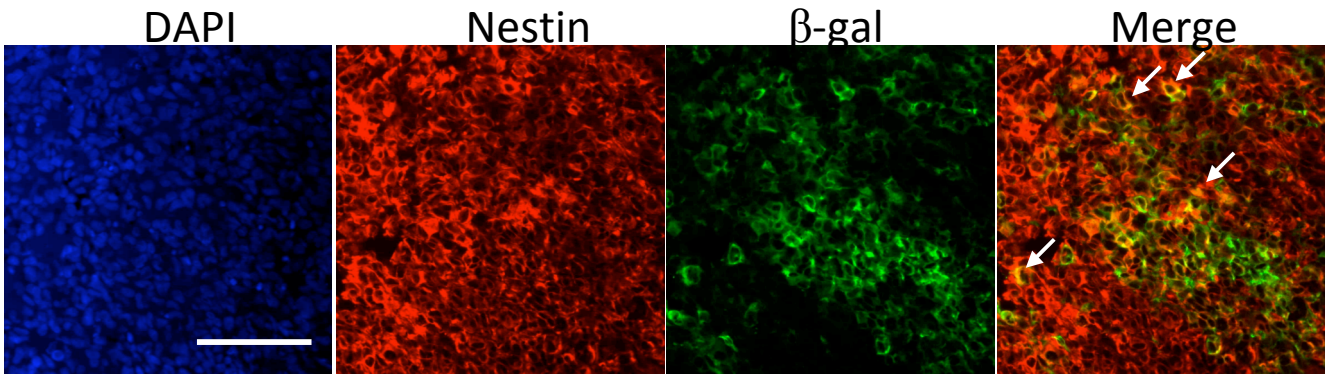
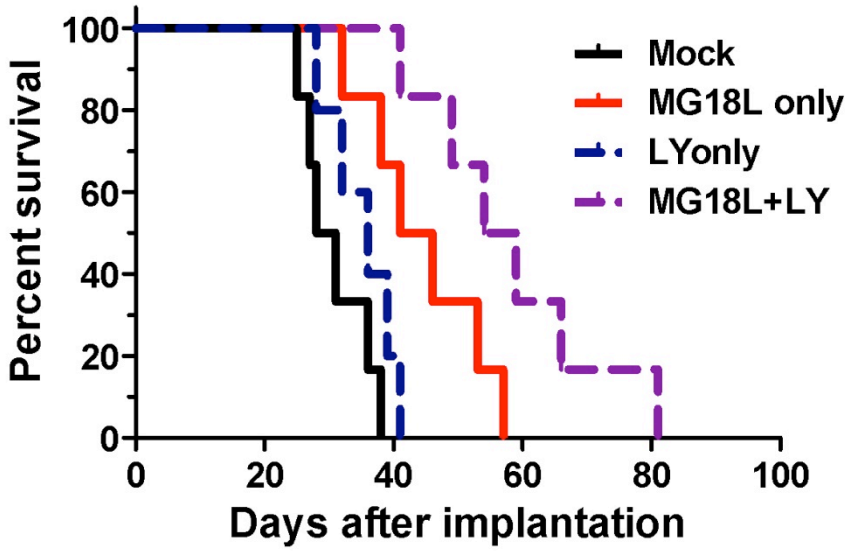


Figure S7


Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
Days after implantation
Mock
MG18L only
LYonly
MG18L+LY

Supplementary Tables

Cells	LY294002	triciribine	GDC-0941	BEZ235
U87	20.9	178.1	2.17	0.022
T98	11.9	188.8	1.34	0.035

Supplementary Table 1. Median-effect doses (ED_{50}) of PI3K/Akt pathway inhibitors for glioblastoma cell lines (U87, T98) *in vitro.* Sensitivity to PI3K/Akt inhibitors (μM) was determined 3 days after treatment and virus 3.5 days post-infection (MOI). Dose–response curves were obtained and ED_{50} values calculated.

Legends for Supplementary Figures

Supplementary Figure 1. Characterization of MG18L structure

The multi-mutated virus, MG18L, was constructed by recombining the *Escherichia coli lacZ* coding sequence into the U_L39 locus (*ICP6* gene) of R7041, which contains deletion at Us3 loci. Recombinant viruses were isolated by picking blue plaques after staining for 5-bromo-4-chloro-3-indoyl-β-galactosidase (X-gal). The DNA structure of the plaque-purified, recombinant virus, MG18L (Fig 1A) was confirmed by restriction endonuclease mapping and Southern blot hybridization. DNA transferred to a solid substrate was hybridized with labeled probes for the presence of ICP6, lacZ genes. The *lacZ* insertion in *ICP6* gene was confirmed by hybridization of HindIII-digested or NdeI-digested viral DNAs with a probe containing *lacZ* sequence flanked by *ICP6* gene sequence. Hybridization of HindIII-digested viral DNAs detects the 8.6-kb fragments of strain F and of R7041, and the 11.6-kb fragments of MG18L, expected with the lacZ insertion, as in G207. Hybridization of NdeI-digested viral DNAs detects the 13-kb fragments of strain F and of R7041, and the expected 5.7-kb and 10.3-kb fragments of MG18L, as in G207. F; strain F (wild-type), R; R7041, M; MG18L, G; G207.

Supplementary Figure 2. *In vitro* efficacy of oHSV on glioma cell lines.

A. Representative plaques on Vero cells. Vero cells infected with MG18L (left) and R7041 (right) were fixed on day 3 post-infection, stained with X-gal, counterstained with neutral red. Scale bar = 400μm. **B.** Median-effect doses (ED_{50}) for oHSV (G47Δ, FΔ6, MG18L, R7041) killing of glioma cell lines (U87, T98G) *in vitro*, 3.5 days post-infection. Cell viability was determined by MTS assay and ED_{50} calculated from dose-response curves. **C**. Median-effect doses (ED_{50}) for LY294002 killing of

glioma cell lines (U87, T98G) and human astrocytes *in vitro*, 3 days post-treatment. * $p<0.05$, unpaired t test.

Supplementary Figure 3. The interaction between oHSV and PI3K/Akt pathway inhibitors on glioma cell (U87, T98G) killing.

The interaction was determined using the median effect method of Chou-Talalay. Data are shown as Fraction affected–Combination Index (f_a-*CI*) plot. CI<1, =1, >1 represent synergistic, additive and antagonistic interactions respectively. **A.** The interaction between oHSV and LY294002 in killing glioma cell lines. **Left**; Effect of order of addition in U87; MG18L followed by LY294002 12 hours later (MG18L⇒LY294002), MG18L simultaneously with LY294002 (MG18L=LY294002), and LY294002 followed by MG18L 12 hours later (LY294002⇒MG18L). **Middle**; Interactions in U87. **Right**; Interactions in T98G. **B**. Interactions between MG18L and triciribine. **C**. Interactions between MG18L and GDC-0941. **D**. Interactions between MG18L and BEZ235.

Supplementary Figure 4. LY294002 does not affect MG18L replication in glioblastoma cells.

Cells (BT74, upper; U87, lower) were treated with LY294002 6 hours after infection with MG18L at MOI of 1.5. At indicated times after infection, cells and media were collected and the titers of infectious virus determined by plaque assay on Vero cells.

Supplementary Figure 5. Combined treatment with MG18L and LY294002 results in enhanced apoptosis in U87 cells and synergy was impaired by apoptosis inhibitor.

A. U87 cells were infected with FΔ6 or MG18L at MOI of 1.5, or mock infected, 14 hours later, cells were treated with or without LY294002 (20μM) and 6 hours later cells were collected and processed for western blotting. Blots were processed with antibodies to cleaved PARP, phospho-Akt

(S473), and Akt. **B.** U87 cells were infected with FΔ6 or MG18L at MOI of 1.5, or mock infected, 10 hours later cells were treated with or without LY294002 (20μM) and 6 hours later activity of caspase-3 and -7 were measured with caspase Glo-3/7 asssay kit (RLU, relative luminescence units). **C.** Synergy between MG18L and LY294002 in U87 was impaired by the addition of pan-caspase inhibitor Z-VAD-FMK (50μM) (f_a-*CI* plot). **D.** Synergy between MG18L and LY294002 in GBM4 was impaired by the addition of pan-caspase inhibitor Z-VAD-FMK (50μM) (f_a-*CI* plot).

Supplementary Figure 6. MG18L infects nestin-positive tumor cells. Sections from BT74 tumor 12hrs after MG18L injection were co-immunostained with anti-nestin Ab (red; Santa Cruz) and anti-ß-gal, and DAPI (blue). Examples of double-stained cells are indicated with white arrows.

Supplementary Figure 7. Combination therapy extends survival of mice bearing intracerbral U87 tumors. Kaplan-Meier survival curve of mice bearing U87 tumors after treatment with intratumoral MG18L (5×10^5 pfu) or Mock on day 10 post-implantation, and daily intraperitoneal LY294002 (LY; 25mg/Kg/day) or vehicle (Mock, n=6; MG18L, n=6; LY294002, n=5; Combination n=6) from days 10-14. $p<0.05$ (mock vs MG18L, MG18L vs Combination, LY294002 versus Combination) (Log-rank test).