Supplementary Figure Legends

Supplementary Figure S1. Schematic chart describing the construction of the double targeted RGD4C/*Grp78* vector. A) Plasmid maps of the pDrive-rGRP78 and pBluescript II SK (+/-). Restriction sites used for the subcloning of *Grp78* promoter into pBluescript plasmid are shown. The *Grp78* promoter was released by *Pst*I and *Nco*I double restriction digestion, ligated to *Xba*I linkers, then inserted into *Xba*I site of the pBluescript. B) *Grp78* promoter flanked by *Spe*I and *Not*I was released from pBluescript and ligated to the phage plasmid digested with *Nhe*I and *Not*I as *Spe*I can liaget to *Nhe*I. (Shble: zeocin-resistance gene, amp: ampicillin resistance gene, β-gal: β-galactosidase gene, TetR: tetracycline resistance gene, ITR: inverted terminal repeat of AAV2).

Supplementary Figure S2. Efficacy of gene delivery by RGD4C/*Grp78* vector in HEK293 cells upon stress treatment. Human HEK293 cells (70-80% confluent in 48 -well plates) were incubated with RGD4C/*Grp78-GFP* or negative control insertless vector. Five days later, cells were treated with either 300nM TG for 16 hr or 0.5 μM A23187 for 5 hr. A control condition of non-treated cells was also included for comparison. GFP expression was observed under a Nikon Eclipse fluorescence microscope by using a 20x objective and photographed.

Supplementary Figure S3. Characterisation of 9L and U87 tumor cells. A) Relative proliferation rate and B) cell survival in low serum conditions of the 9L and U87 glioblastoma cells. Cells were seeded in 24 well plates with 10,000 cells/well and counted daily over 6 days. Experiment was performed in triplicate and repeated three times. C) Endogenous Grp78 expression in tumor cells. 9L and U87 cells were grown to 70-80% confluent, followed by western blot analyses of their endogenous Grp78 protein. The intensity of Grp78 protein bands obtained by western blot was measured and normalised to that of GAPDH. Results represent the average of 3 independent western

blots. D) Semi-quantitative RT-PCR of *XBP1* transcript in 9L and U87 cells incubated with or without 300 nM TG for16 hr.

Supplementary Figure S4. Evaluation of *Grp78* and *CMV* promoter activity in stably transduced 9L cells grown *in vivo*. A) *In vivo* bioluminescent imaging (BLI) of immunodeficient nude mice bearing tumors derived from 9L cells stably transduced with either RGD4C/*Grp78-Luc-puro*^{R} or RGD4C/*CMV-Luc-puro*^{R} overtime. Shown are representative mice at the indicated time points. B) Quantification of the Luc tumor activity at the indicated times.

Supplementary Figure S5. Long-term cell killing efficiency of RGD4C/*Grp78-HSVtk* and RGD4C/*CMV-HSVtk* in U87 cells. A) To evaluate killing efficacy on U87 cells over time after GCV treatment, stably transduced U87 cells with either RGD4C/*Grp78-HSVtk-puro*^{R} or RGD4C/*CMV-HSVtk-puro*^{R} were plated at 70,000 cells/well of 24-well plates. Treatment with GCV commenced 48 hr later. Cells were counted in triplicate for each condition in 24 hr intervals over 96 hr post GCV treatment. Data were calculated by dividing the per-cent conversion under GCV condition by that under non-GCV condition. Data represent the average obtained from triplicate wells and are representative of two independent experiments. Abbreviations: t1= 66 days post vector transduction, t2= 137 days post vector transduction. B) and C) Representative phase contrast images post GCV treatment (24 hr and 96 hr) of stably transduced U87 cells at t1 and t2, respectively (Scale bar = 45μm).

Supplementary Figure S6. Efficacy of RGD4C/*Grp78* in transduced but non-selected 9L and U87 tumor cells. A) Quantitative analyses of *Luc*-mediated reporter transgene expression. Cells (70-80% confluent in 48-well plates) were incubated with either RGD4C/*Grp78-Luc* or RGD4C/*CMV-Luc.* Insertless vectors containing either *Grp78* or *CMV* promoter were used as negative controls. TG (300 nM for 16 hr) was added to 9L cells at day 5 and to U87 cells at day 3 post-vector transduction. *Luc* measurement assay was performed at the indicated days and normalised to protein concentration determined by the Bradford assay. Results represent the average Relative Luminescence Units (RLU)

/µg of protein from triplicate wells. *Luc* quantification of RGD4C/*Grp78-Luc* and RGD4C/*CMV-Luc* was normalised to control insertless *Grp78-Luc* and *CMV-Luc*, respectively. B) and C) Cell killing efficacy *in vitro* of the double targeted RGD4C/*Grp78-HSVtk* plus GCV in 9L and U87 cells, respectively. Cells (70-80% confluent in 48-well plates) were incubated with either RGD4C/*Grp78-HSVtk* or RGD4C /*CMV-HSVtk.* Insertless vectors containing either *Grp78* or *CMV* promoter were used as negative controls. At day 3 post- vector transduction, cells were treated with TG (300nM for 16hr), followed by GCV treatment for 5 days. Viable cells were counted by using the Trypan blue method.

Supplementary Figure S7. Efficacy of double targeted RGD4C/*Grp78* in a model of human breast cancer. A) The MCF7 human breast adenocarcinoma cells (70-80% confluent in 48-well plate) were incubated with either RGD4C/*Grp78-Luc* or insertless non-targeted vector as negative control. Luciferase assay was performed at the indicated days and was normalised to protein concentration using the Bradford assay. Results represent the average RLU/µg protein of triplicate wells. Luciferase quantification of RGD4C/*Grp78-Luc* was normalised to that of control insertless vector. Treatment with TG was performed at day 5 post-vector transduction. B) Western blot analysis of endogenous *Grp78* gene expression in MCF7 cells, in the absence and presence of TG treatment. The intensity of Grp78 protein bands obtained by western blot was measured and normalised to that of GAPDH.

Supplementary Figure S8. Effect of *HSVtk* expression and GCV on the spliced *XBP1* mRNA. 9L cells stably transduced with RGD4C/*Grp78-HSVtk-puro*^{R} were treated with GCV over a time course, then subjected to semi-quantitative RT-PCR for *XBP1* transcript. Addition of TG for 16 hr was included in this experiment as a positive control.