Supporting Information

Enhanced caspase-mediated abrogation of autophagy by temozolomide-loaded and panitumumab-conjugated PLGA nanoparticles in EGFR overexpressing glioblastoma cells

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Additional methods.

Determination of EGFR expression in GBM cell lines. The EGFR expression on U-87 MG and LN229 cell line was evaluated using western blot assay. Briefly, 1×10^5 U-87 MG and LN229 cells were harvested, lysed with lysis buffer, and the protein concentration was determined with the aid of the Pierce BCA protein assay kit. Then the protein was separated using 8% Bis-Tris polyacrylamide gel operated at 100 V for 90 min and then transferred to a polyvinylfluoride membrane. Then the membrane was blocked with 5% BSA in TBST, incubated overnight with primary antibodies targeting EGFR and GAPDH. Finally, the membrane was incubated with a 2º antibody for 1 h, soaked in a chemiluminescent substrate, and was photographed using Image Quant LAS 4000 (Taunton, MA, USA).

FACS analysis for the determination of Beclin-1 and Caspase-9 after the treatment of different formulations. Briefly, U-87 MG and LN229 cells (1×10^5 cells/well) were seeded overnight in 12-well plates followed by the incubation with PmAb-TMZ-PLGA-NPs for 30 h, 36 h, 42 h. Cells were incubated with Beclin-1 and caspase-9, as per the manufacturer's instructions, and analyzed using flow cytometry.

Western blot assay for the determination of LC3B marker. U-87 MG and LN229 cell lines. (1×10^5 cells/well) were seeded in 12-well plates. Cells were then treated with PLGA-NPs, TMZ, TMZ-PLGA-NPs, and PmAb-TMZ-PLGA-NPs for 24 h. After 24 h, cells were lysed with lysis buffer and protein concentration was determined with Pierce BCA protein assay kit. Then the proteins were separated on 13% Bis-Tris polyacrylamide gel operated at 100 V for 90 min and then transferred to a polyvinylfluoride membrane. Afterward, the membrane was blocked with 5% BSA in TBST and further incubated with primary antibodies

overnight targeting LC3B and GAPDH. Ultimately, incubation of the membrane with 2º antibody for 1 h, and soaking in a chemiluminescent substrate was carried out to visualize the band using Image Quant LAS 4000.

Additional Results.

FACS analysis for the determination of Beclin-1 and Caspase-9 after the treatment of different formulations. FACS analysis showed that there was decresase decrease in Beclin-1/caspase-9 expression from 30 h time point to 42 h time point following the treatment of PmAb-TMZ-PLGA-NPs (Figure S8). More significant decline in Beclin/caspase-9 ratio was observed in U-87 MG cell line. This result suggested that PmAb-TMZ-PLGA-NPs enhanced the expression of caspase-9 which in turn downregulate the expression of autophagic marker (Beclin-1).

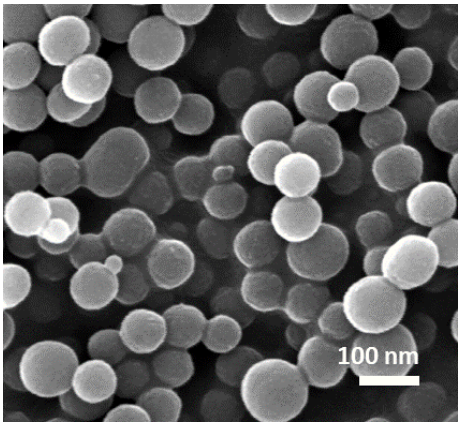

Figure S1. SEM images of PLGA-NPs (scale bar: 100 nm)

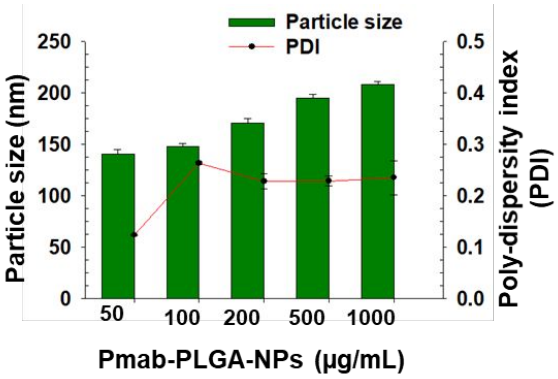

Figure S2. Optimization of particle size and PDI of different amount of antibody (50, 100, 200, 500, 1000 μg) conjugated on the surface of PLGA NPs through DLS.

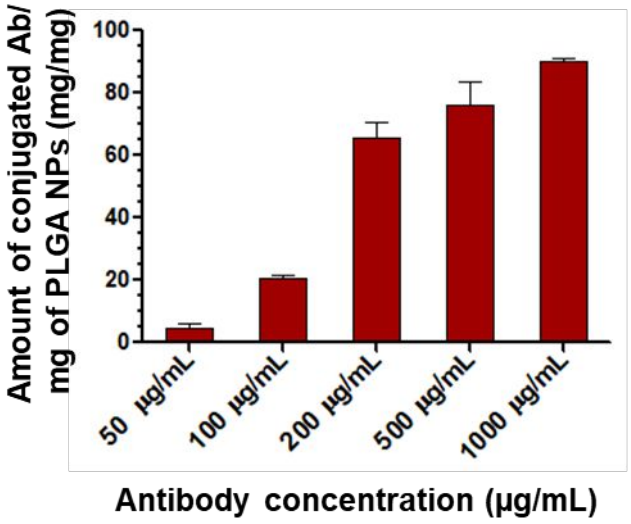

Figure S3. Amount of antibody conjugated per mg of PLGA NPs determined by UV spectroscopy.

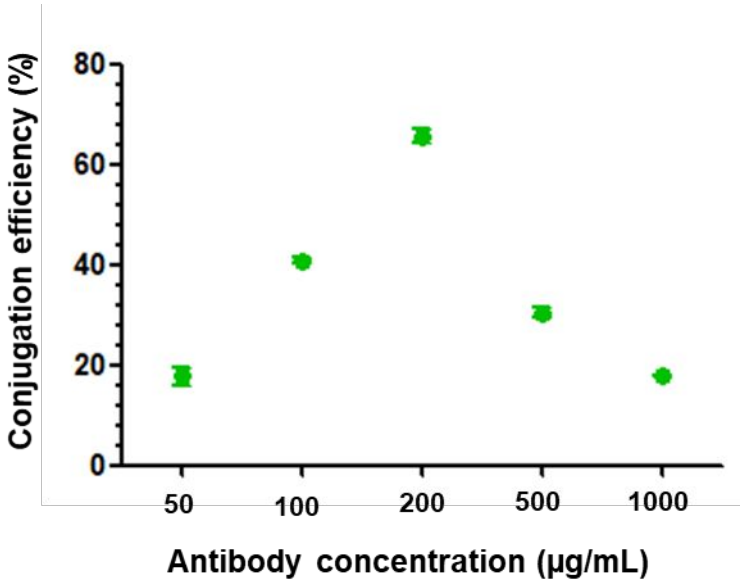

Figure S4. Determination of conjugation efficiency of different amount of panitumumab antibody (50, 100, 200, 500, 1000 μg) on the surface of 1mg of PLGA NPs.

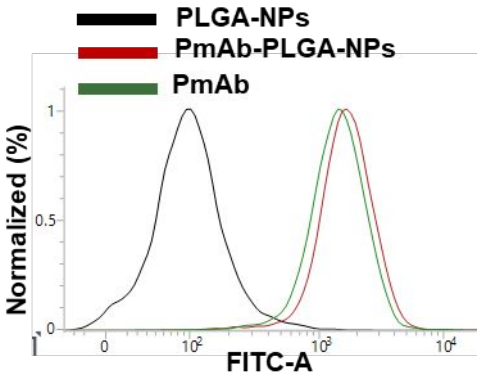

Figure S5. Flow cytometry histogram plot illustrating the immunoreactivity of PmAb-PLGA NPs on U-87 MG glioblastoma cell line and their respective quantitative fluorescence analysis.

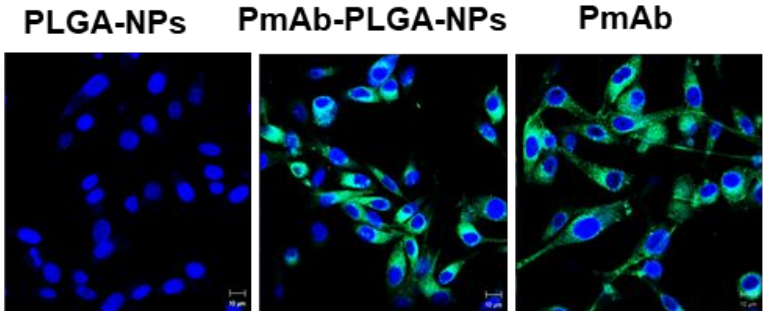

Figure S6. Confocal fluorescence microscopy images showing the binding and immunoreactivity of PmAb-PLGA NPs by U-87 MG glioblastoma cell line. The cells were incubated with AlexaFluor 488 anti-human IgG (green) to visualize the anti-EGFR antibody. Counterstaining of cell nucleus (blue) color was carried out using DAPI dye; scale bar (10 µm).

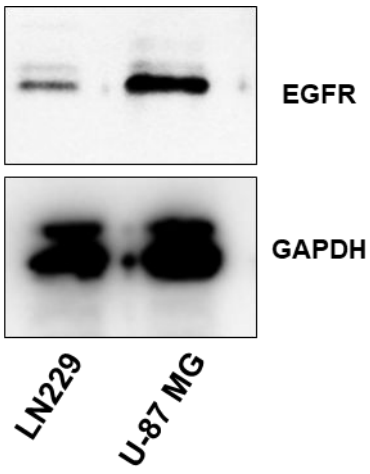

Figure S7. Western blot analysis for the determination of EGFR expression in LN229 and U-87 MG cell lines.

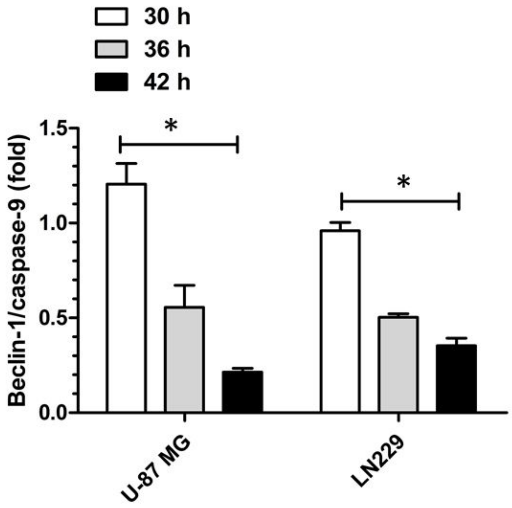

Figure S8. FACS analysis for the determination of Beclin-1/caspase-9 expression for 30 h, 36 h and 42 h following the treatment of PmAb-TMZ-PLGA-NPs at an equivalent concentration of TMZ (250 μM) in U-87 MG and LN229 cell lines.

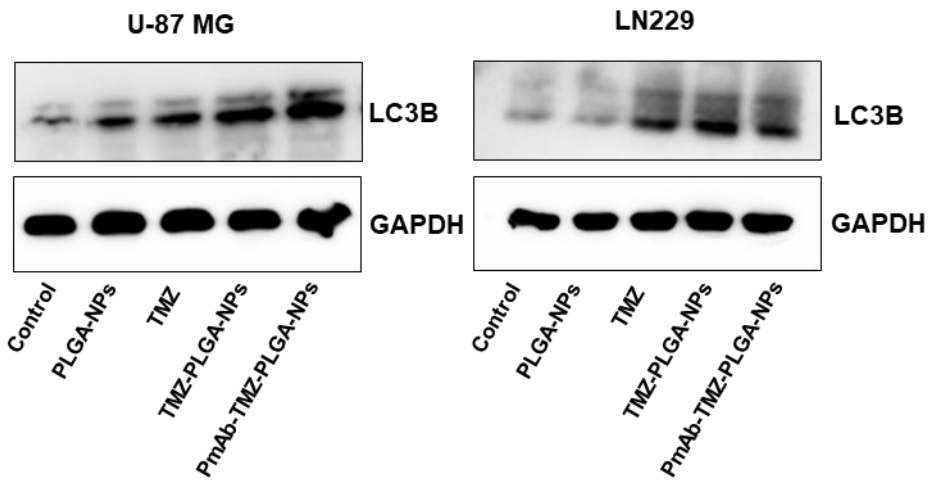

Figure S9. Western blot analysis for the determination of autophagic marker (LC3B) for 24 h following the treatment with PLGA-NPs, TMZ, TMZ-PLGA-NPs and PmAb-TMZ-PLGA-NPs at an equivalent concentration of TMZ (250 μM) in U-87 MG and LN229 cell lines.