Supporting Information

Large Aminoacid Transporter 1 Selective Liposomes of L-DOPA Functionalized Amphiphile for Combating Glioblastoma

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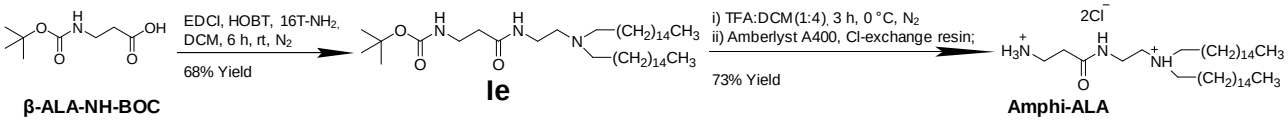

Figure S1. Synthetic scheme for preparation of Amphi-ALA

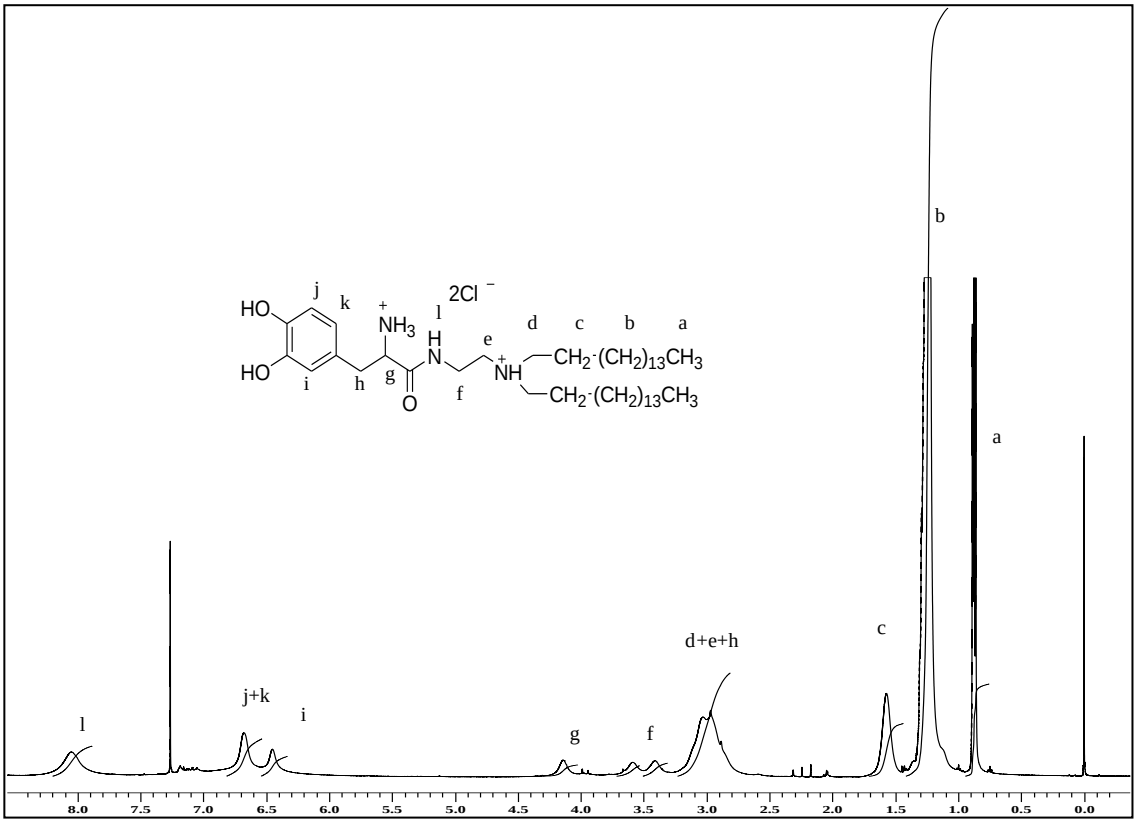

Figure S2. ^{1}H-NMR spectrum of Amphi-DOPA

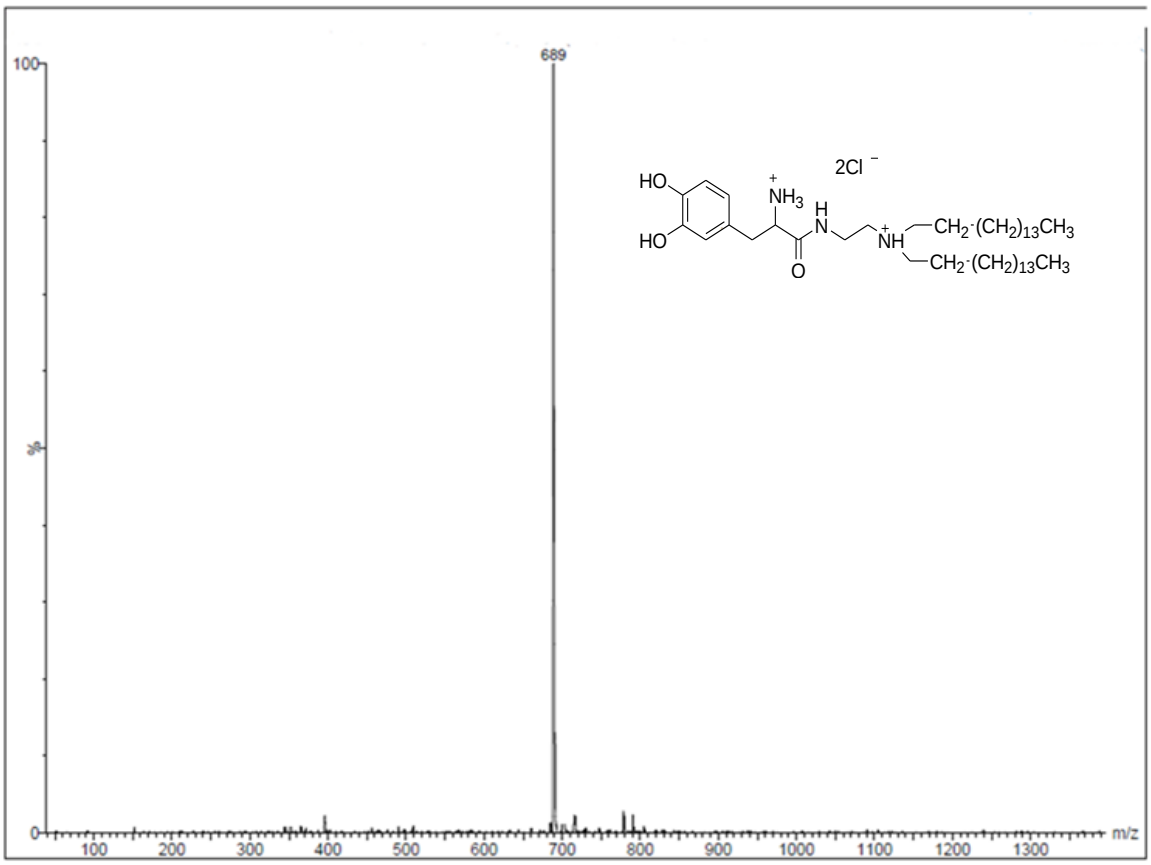

Figure S3. ESI-Mass spectrum of Amphi-DOPA

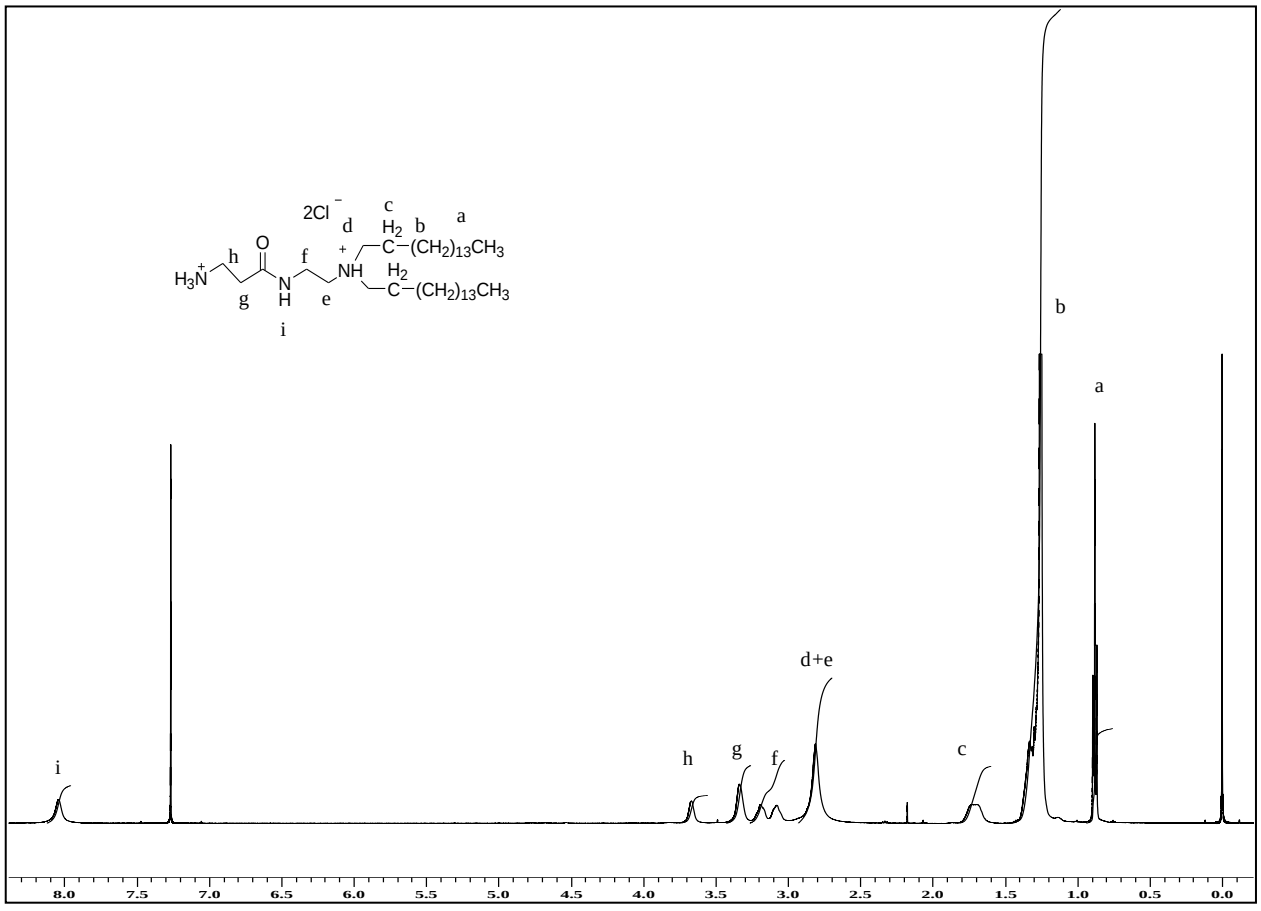

Figure S4. ^{1}H-NMR spectrum of Amphi-ALA

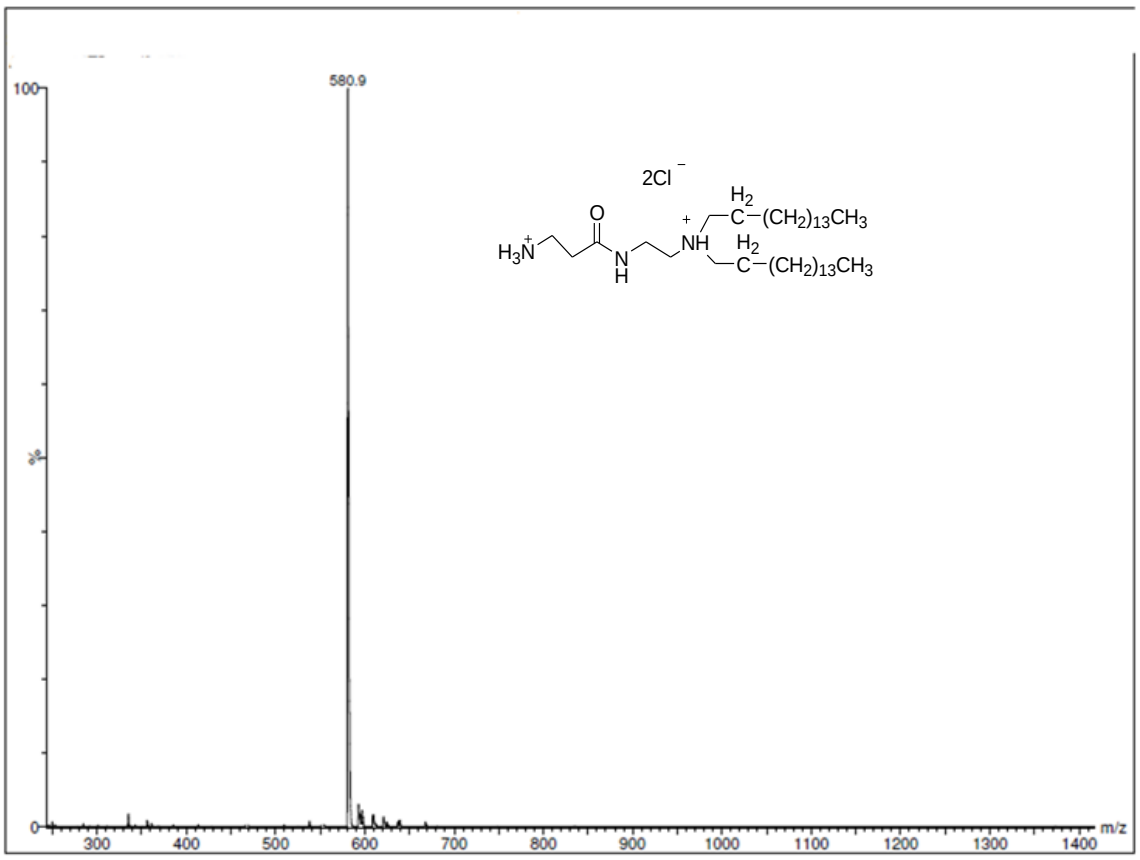

Figure S5. ESI-Mass spectrum of Amphi-ALA

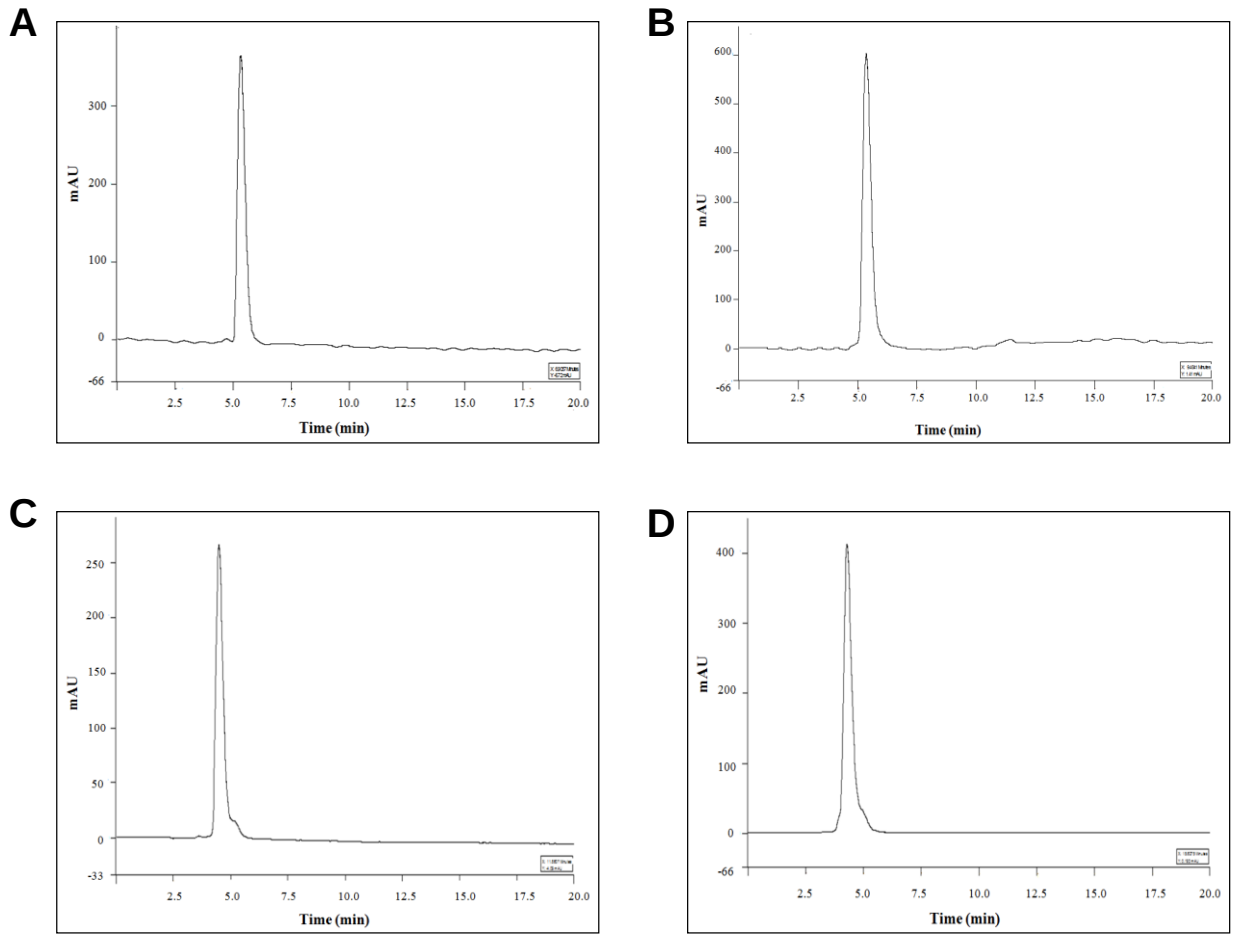

Figure S6. HPLC Chromatograms of: Amphi-DOPA using pure Methanol (A) & Methanol:Water, 95:5 (v/v) as mobile phases (B). HPLC Chromatograms of: Amphi-ALA in Mobile Phases: pure Methanol (C) and Methanol:Water, 95:5 (v/v) (D).

HPLC Conditions:

System: Varian Prostar 210
Column: Metasil AQ 10U C18 120A, 250 x 10 mm
Flow Rate: 1.0 mL/min (0-20 min)
Typical Column Pressure: 60-65 Bars
Temperature: 25 ^{o}C; Detection: UV at 260 nm

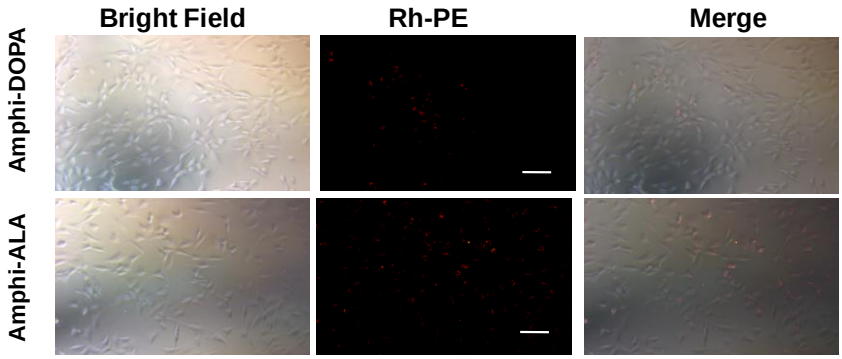

Figure S7. Epifluorescence micrographs (10X) of NIH-3T3 cells treated with Rh-PE labeled liposome of Amphi-DOPA (upper panel) & Amphi-ALA (lower panel). Bar = 100 μm.

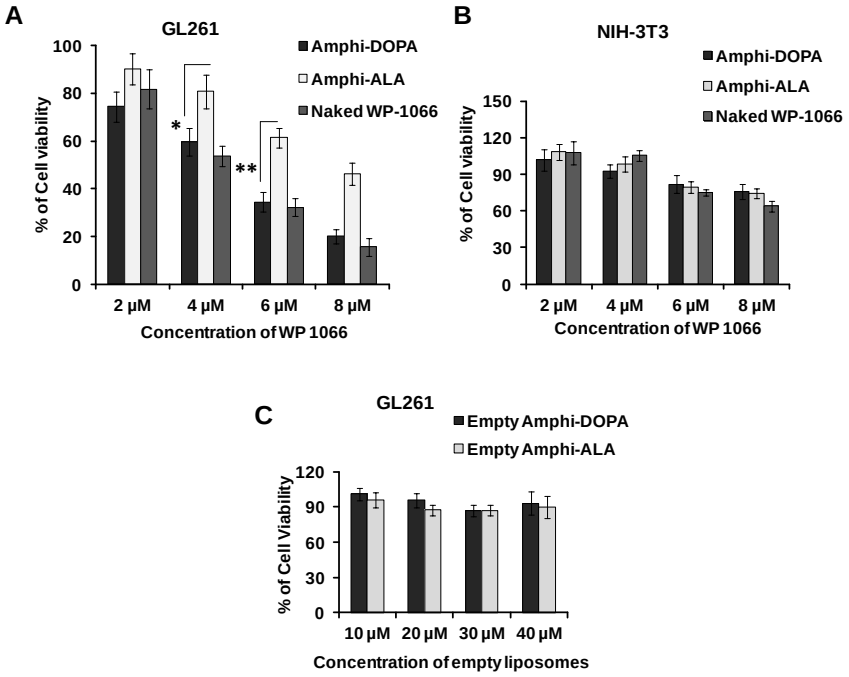

Figure S8. WP1066 loaded liposomes of Amphi-DOPA preferably kill glioblastoma cells without affecting healthy cells. Percentage cell viability profiles of: (A) GL261 cells and (B) healthy NIH-3T3 cells separately treated with WP1066 loaded liposomes of Amphi-DOPA & Amphi-ALA. (C) Percentage cell viability profiles of GL261 cells treated with empty liposomes of Amphi-DOPA & Amphi-ALA. * denotes $P<0.05$ & ** denotes $P<0.01$ as determined by student's t-test. The results shown are average of triplicate experiments performed on the same day.

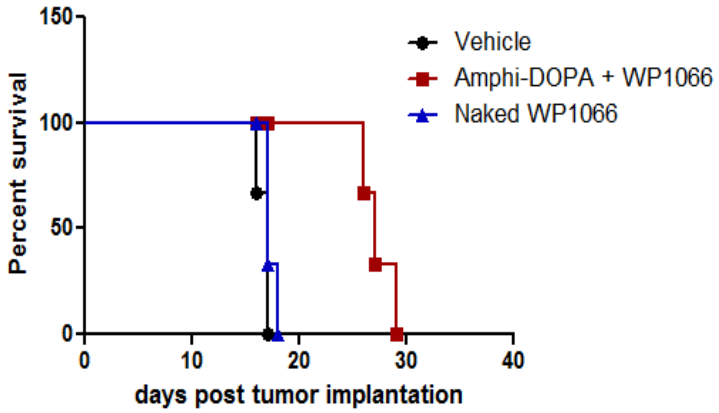

Figure S9. Survivability graph of brain tumor bearing C57/BL6J mice (n = 3) *i.v.* treated with: vehicle (5% aqueous glucose, black); naked WP1066 (10 mg/kgBW) dissolved in 0.5% DMSO (blue) and liposomes of Amphi-DOPA (red) containing encapsulated WP1066 (10 mg/kgBW) on day 7, 9, 11, 13 and 15. Survivability of all the mice groups was monitored from day 16 onward.

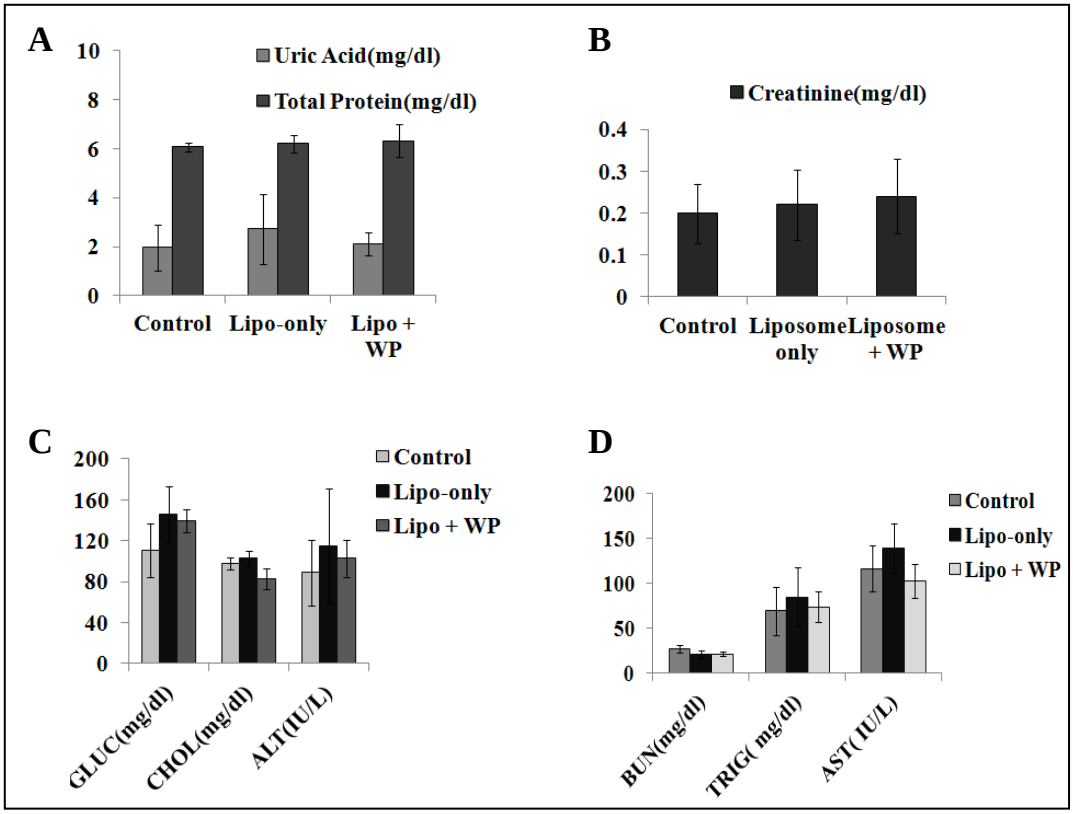

Figure S10. Intravenously administered liposomes of Amphi-DOPA do not alter serum biochemical profile significantly in healthy mice. Serum analysis were performed to estimate (A) uric acid & total protein (TP); (B) creatinine (CRE); (C) total glucose (GLUC), cholesterol (CHOL), alanine transaminase (ALT); (D) blood urea nitrogen (BUN), triglycerides (TRIG), aspartate transaminase (AST).

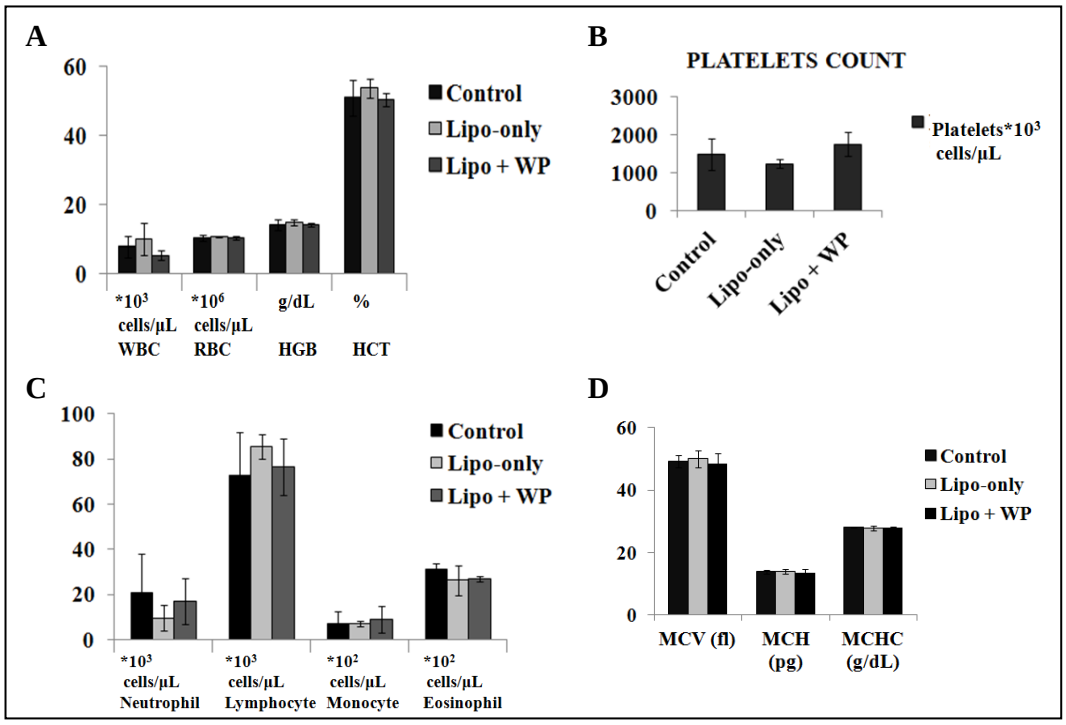

Figure S11. Blood parameters of healthy mice do not change significantly upon *i.v.* administration of liposomal formulations of Amphi-DOPA. (A) White blood cell (WBC), Red blood cell (RBC), Hemoglobin (HGB), Hematocrit (HCT); (B) Platelets; (C) Neutrophil, Lymphocye, Monocyte & Eosinophil; and (D) mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC).

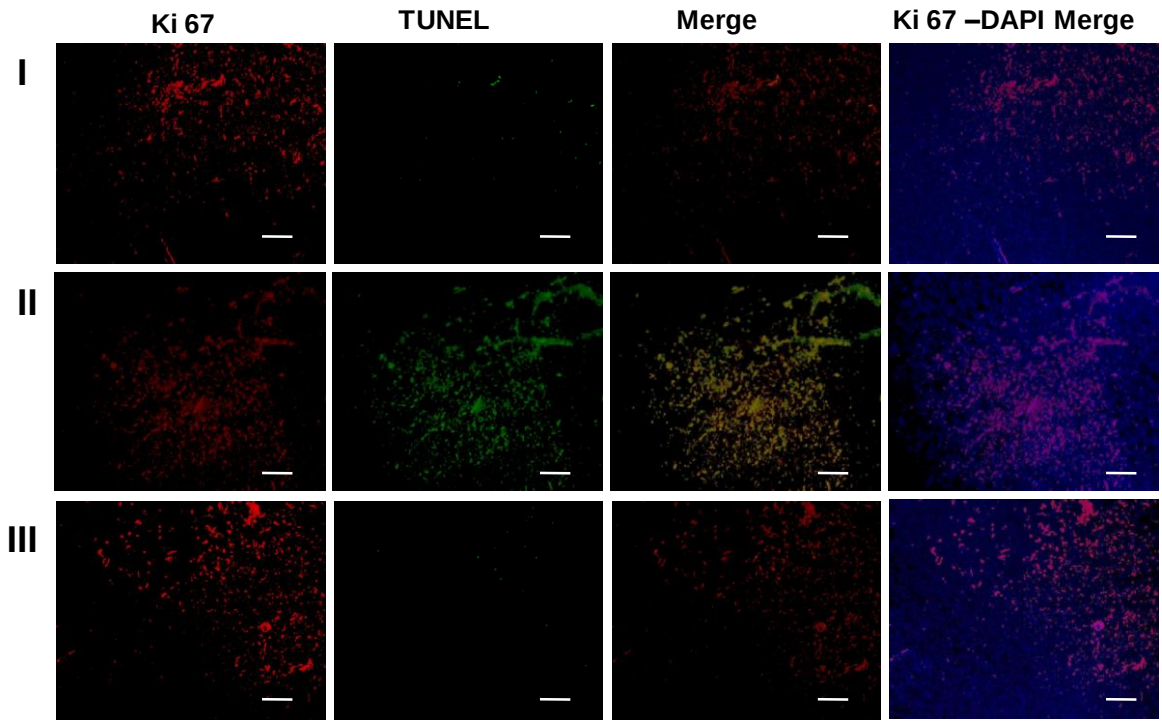

Figure S12. WP1066-loaded liposomes of Amphi-DOPA induce apoptosis in glioblastoma tissue. Mice bearing orthotopic glioblastoma (n = 3) were separately administered (*i.v.*) with 5% aqueous glucose solution (Group-I), WP1066 (10 mg/kg B.W.)-loaded liposomal formulations of: Amphi-DOPA (Group-2) and Amphi-ALA (Group-3) on day 7, 9, 11, 13 and 15 post tumor implantation. On day 16 brains were excised from each group, cryosectioned and immunostained with anti-Ki67 antibody (markers of proliferative cells, red) and TUNEL kit (markers for apoptotic cells, green). Representative epifluorescence micrographs (10X) of immunostained cryosections prepared from Group 1 (I), Group 2 (II) and Group 3 (III).Bar in part A = 100 μm. Results shown here are the representative figures of three independent experiments performed for both *in vitro* and *in vivo*.

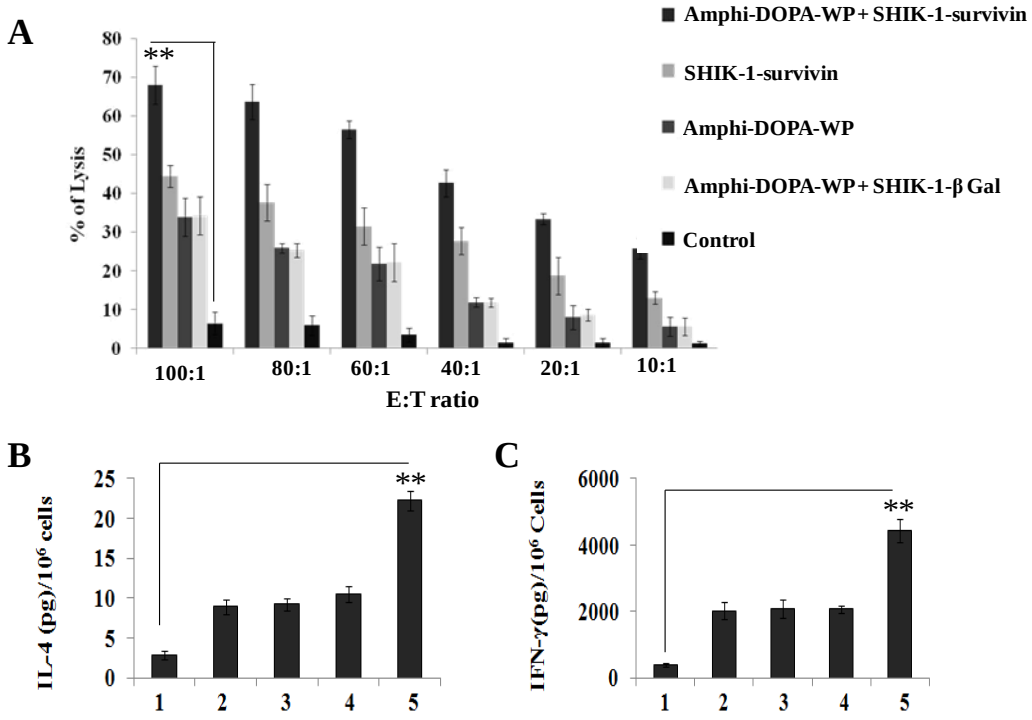

Figure S13. Enhanced overall survivability of brain tumor bearing mice observed in combination therapy mode presumably originates from increased cellular and humoral immune responses. C57BL/6J mice bearing orthotopic glioblastoma (GL261) were sorted into five groups (n = 5 in each group). *In vivo* DC-targeted DNA vaccination (s.c.) were performed on day 6, 8, & 16 post tumor implantation and WP1066-loaded liposomes (10 mg WP1066/kg BW) were *i.v.* administered on day 7, 9, 11, 13 & 15. SHIK-1-Survivin group: mice s.c. immunized with lipoplexes of *in vivo* DC-targeting liposomes of SHIK-1 and p-CMV-survivin; Amphi-DOPA-WP + SHIK-1-Survivin group: mice s.c. immunized with lipoplexes of SHIK-1 liposome & p-CMV-survin and simultaneously received *i.v.* dose of WP1066-loaded liposomes of Amphi-DOPA; Amphi-DOPA-WP + SHIK-1-β-gal group: mice s.c. immunized with lipoplexes of SHIK-1 liposomes & β-gal plasmid DNA and simultaneously treated (*i.v.*) with WP1066-loaded liposomes of Amphi-DOPA; Amphi-DOPA-WP group: mice received only *i.v.* dose of WP1066-loaded liposomes of Amphi-DOPA; and Control group: mice received 5% aqueous glucose on day 7, 9, 11, 13 & 15 post tumor cell inoculation. 12 days after the last immunization

splenocytes were isolated from each group, co-stimulated with GL261 cells (Target cells) for 72 h, and CTL response (A); IL-4 level (B) & IFN-γ levels (C) were measured as described in text. Results shown here are the averages of triplicate experiments performed on the same day. ** denotes $P<0.01$.