Low-intensity pulsed ultrasound-mediated blood-brain barrier opening increases anti-programmed death-ligand 1 delivery and efficacy in Gl261 mouse model

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Material and Methods

Samples were acquired on a spectral flow cytometer (Aurora, Cytek) and analyzed by FlowJo software (FlowJo, LLC). Briefly, cells were selected based on their morphology, doublets, and dead cells were excluded using (Biolegend, #423107) while tumor cells were excluded based on their *mKate* expression. Monocytes ($Ly6C^+$ $Ly6G^-$) and neutrophils ($Ly6C^+$ $Ly6G^+$) were excluded from non-tumoral live cells using Ly-6C (Biolegend, #128036) and Ly-6G (Biolegend, #127617). Microglia were identified based on their expression of $CD11b^+$ and $CD45^{low}$ using CD45 (Biolegend, #103131) and CD11b (Biolegend, #101255). Activated microglia were identified as $CD68^+$ using (Biolegend, #137003). F4/80 marker (Biolegend, #123117) was used to determine macrophages in the $CD45^{high}$ $CD11b^+$ cell population. CD206 marker (Biolegend, #141729) was used to distinguish between subpopulations of macrophages. Lymphocytes $CD4^+$ (Biolegend, #100541) and $CD8^+$ (Biolegend, #100737) were identified on the $CD45^+$ $CD11b^-$ fraction of non-tumoral live cells. The percentage of each subpopulation was calculated and using in our flow cytometry analyses.

Supplemental Figure 1: Schematic representation of LIPU procedure. (A) Graphical representation of LIPU generator set up. (B) Experimental timeline for anti-PD-1 treatment with BBB disruption. (C) IHC of tumor size in UMBO plus anti-PD-L1 (the right image) at day 45 compared to anti-PD-L1 (the left image) treated mice. (D) A representative gating strategy illustrating immune cell population being subgated to the levels of microglia, lymphocytes and macrophages.