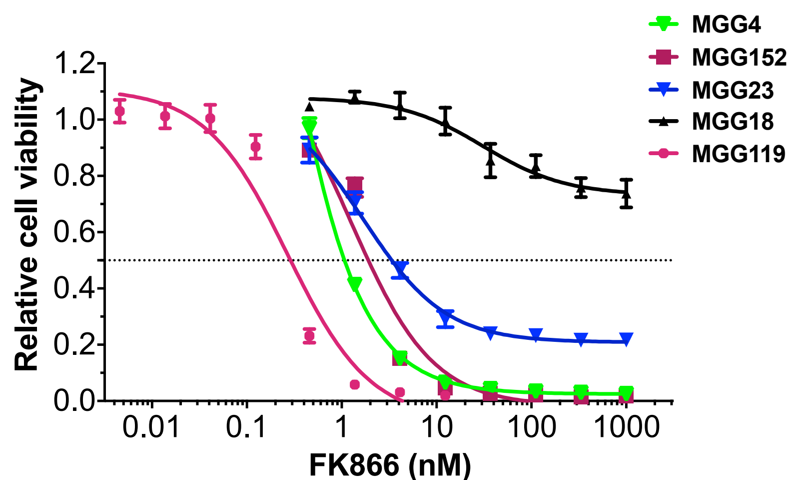
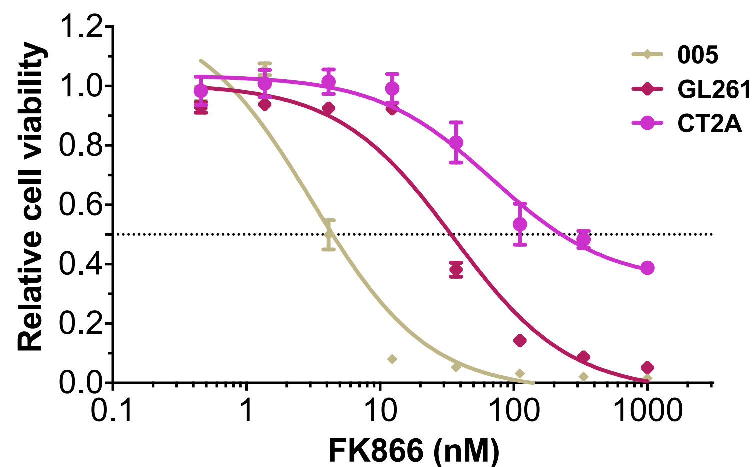
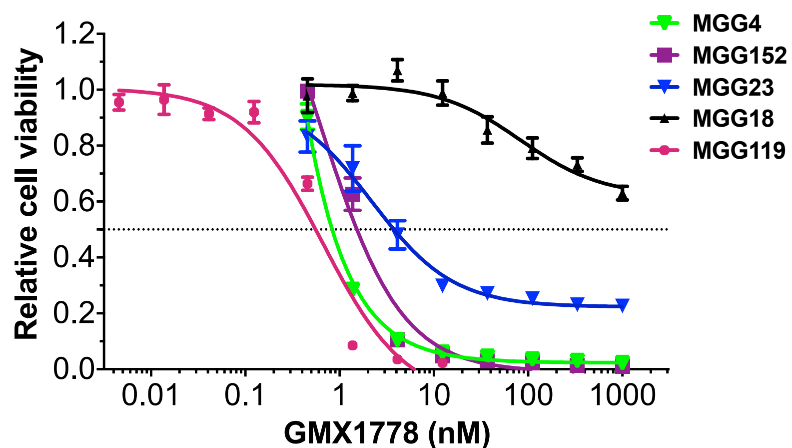
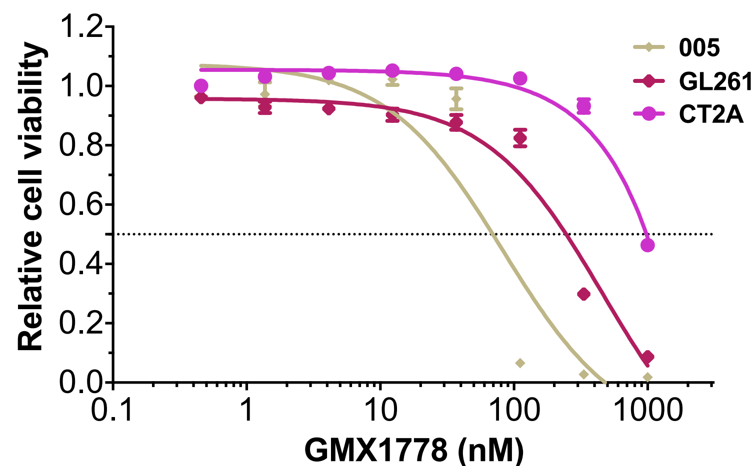
Supplementary Figure S1



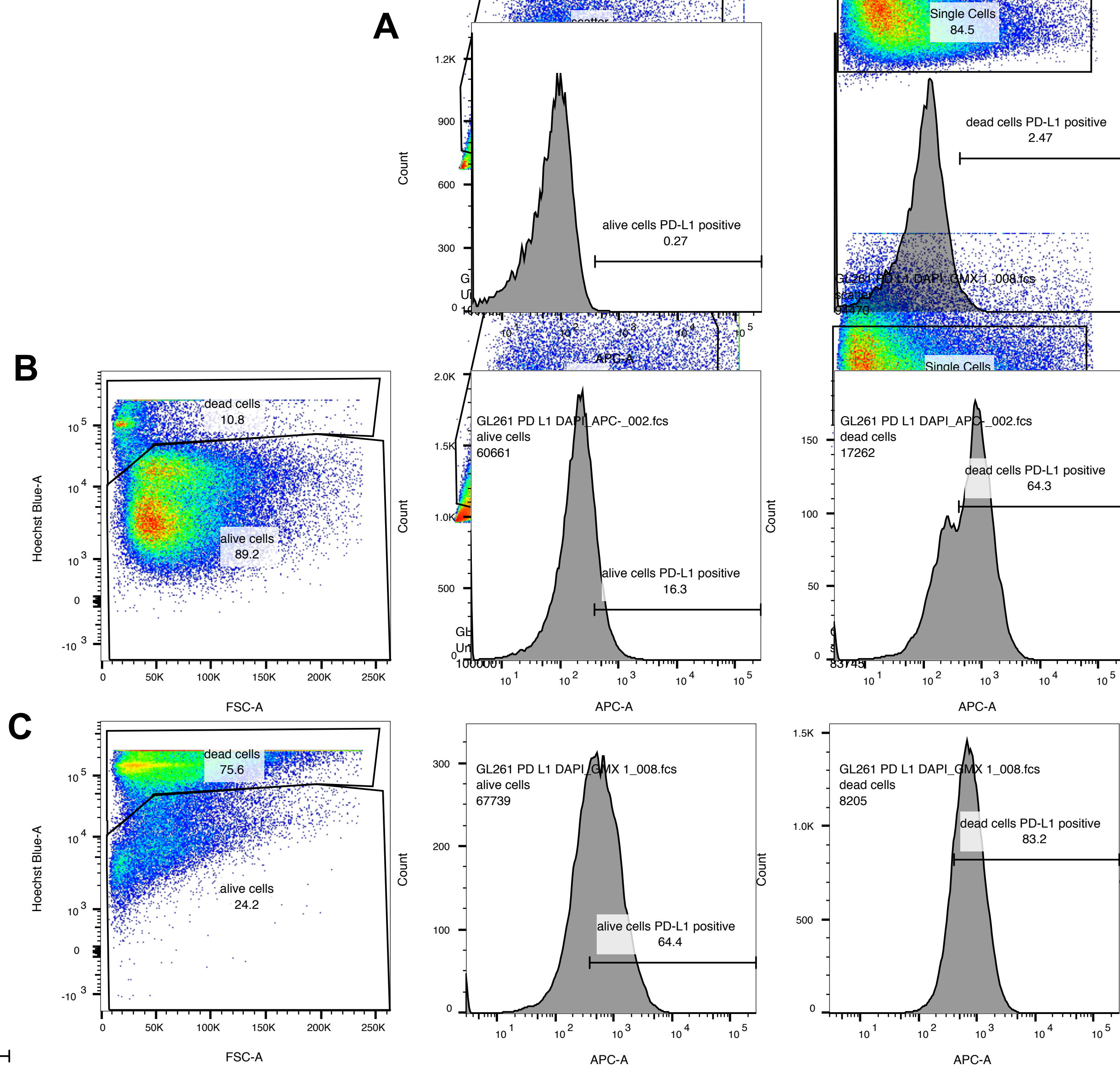






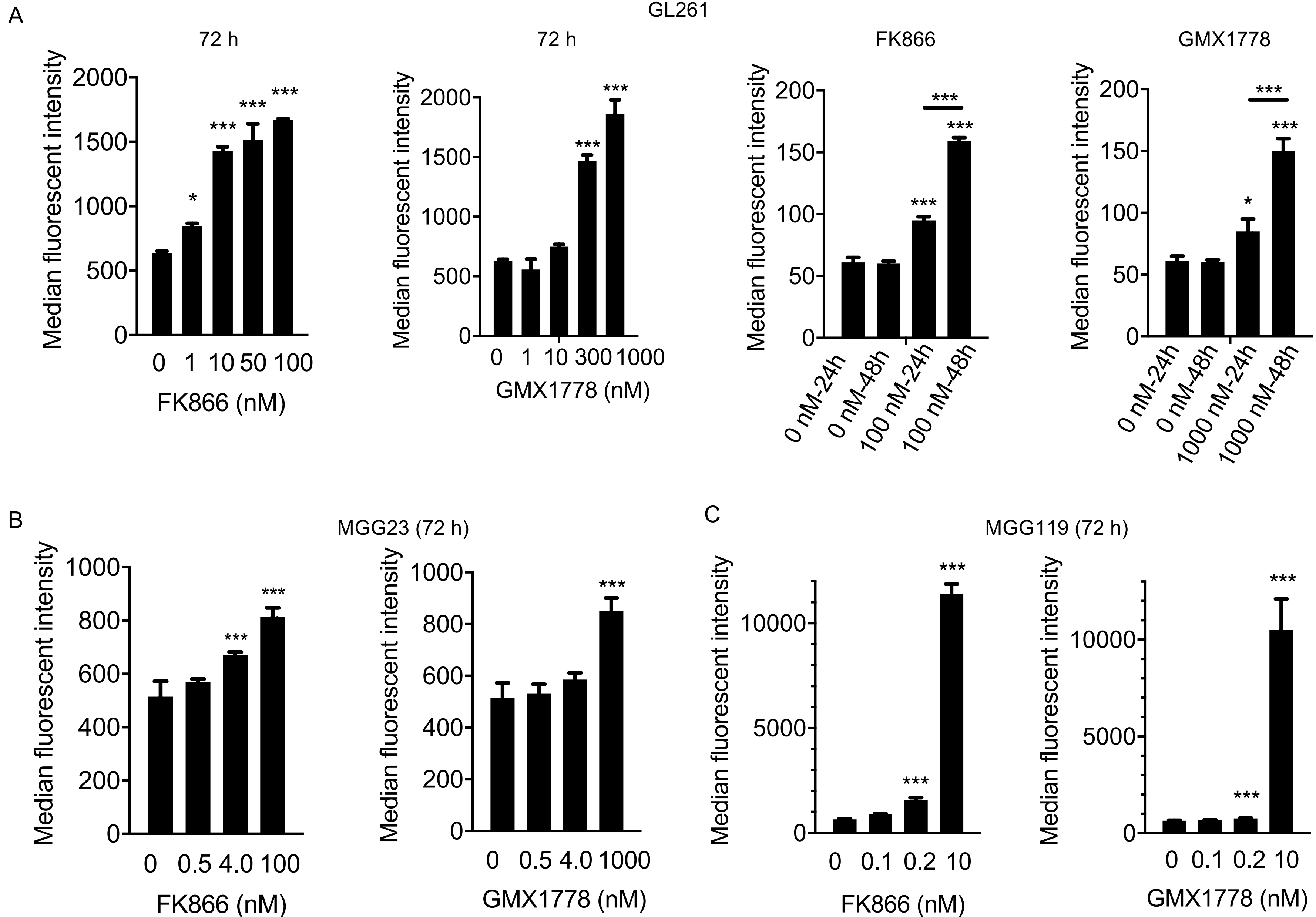
Supplementary Figure S1. Cell viability assay with NAMPT inhibitors. Cytotoxic effects of NAMPT inhibitors FK866 **(A, C)** and GMX1778 **(B, D)** in patient-derived glioblastoma (GBM) cells **(A, B)** and murine GBM cells **(C, D)**. Assay was done three days after drug treatment.

Supplementary Figure S2



Supplementary Figure S2. NAMPT inhibitor induces PD-L1 upregulation on alive cells. Flow cytometry assay for PD-L1 on GL261 cells. **A,** Untreated GL261 cells unstained with APC-conjugated anti-PD-L1 antibody (negative control). **B and C,** GL261 cells exposed to GMX1778 (1 μM) for 3 days (**C**) or control (**B**) were stained with DAPI for alive and dead cell discrimination and APC-conjugated anti-PD-L1, followed by flow cytometry.

Supplementary Figure S3



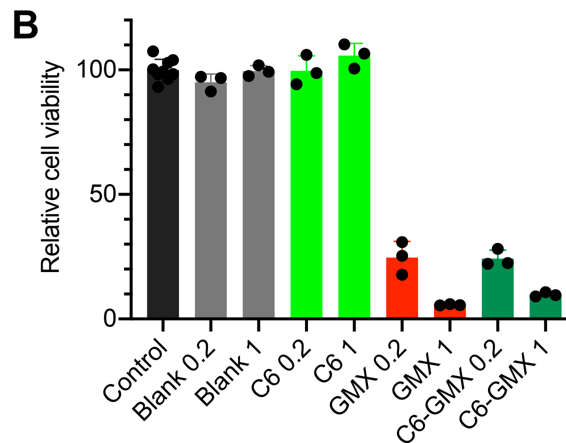
Supplementary Figure S3. NAMPT inhibitor induces PD-L1 upregulation. Median fluorescent intensity of PD-L1 in flow cytometry assay of GBM cells after treatment with NAMPT inhibitors. **A,** GL261 cells. Left two panels, dose-dependent effect. Right two panels, time-dependent effect. **B,** MGG23 cells. **C,** MGG119 cells. Error bars, SEM. *p＜0.05, ***p＜0.01 compared with base line control or between the two groups.

Supplementary Figure S4

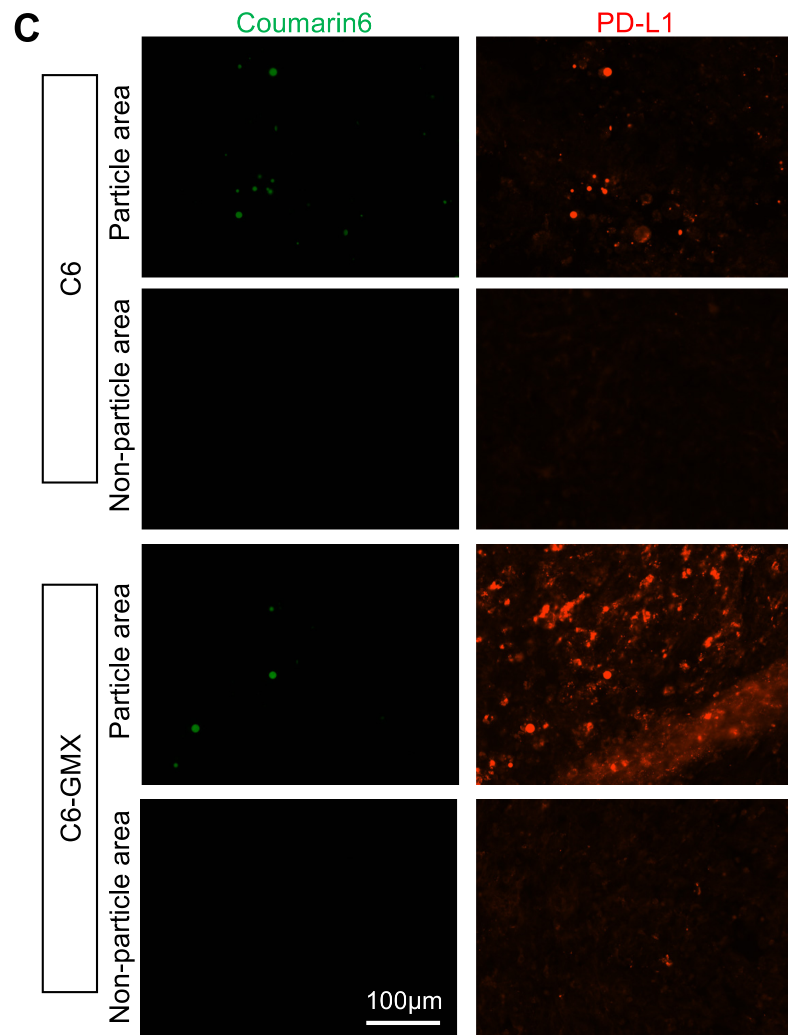
A

Formulation	GMX1778 concentration (μg/ml)
Blank	0.0
Coumarin6	0.0
GMX1778	3.4
Coumarin6+GMX1778	1.8

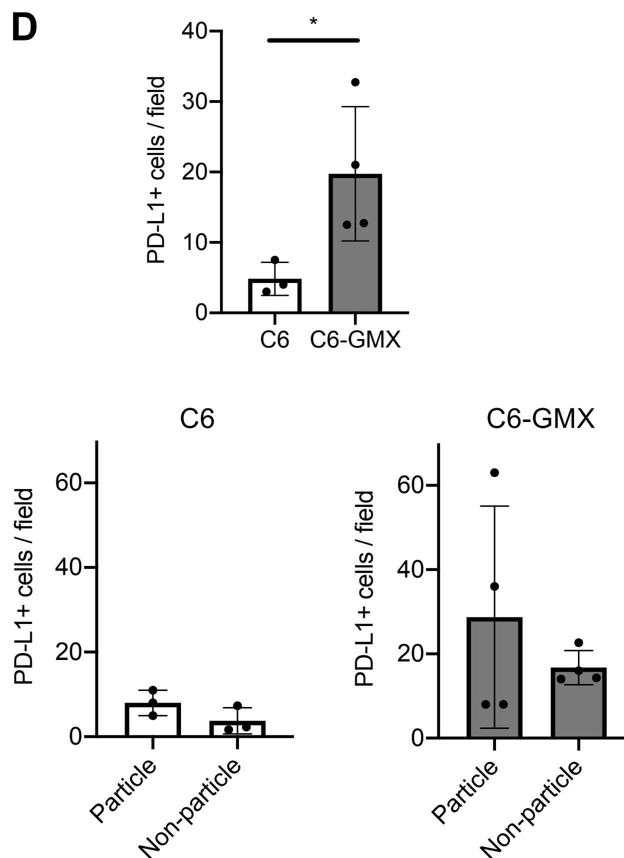
B



C

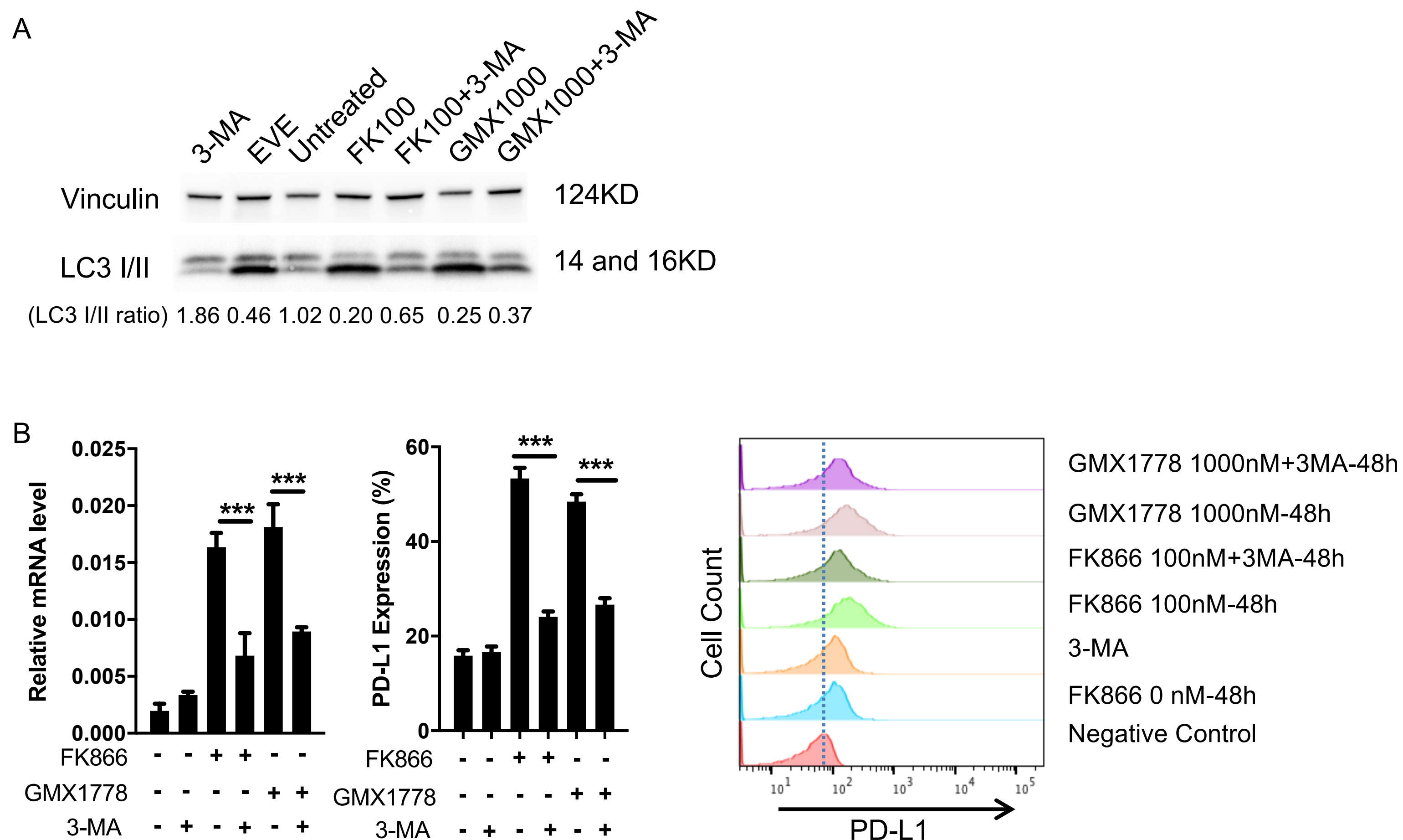


D



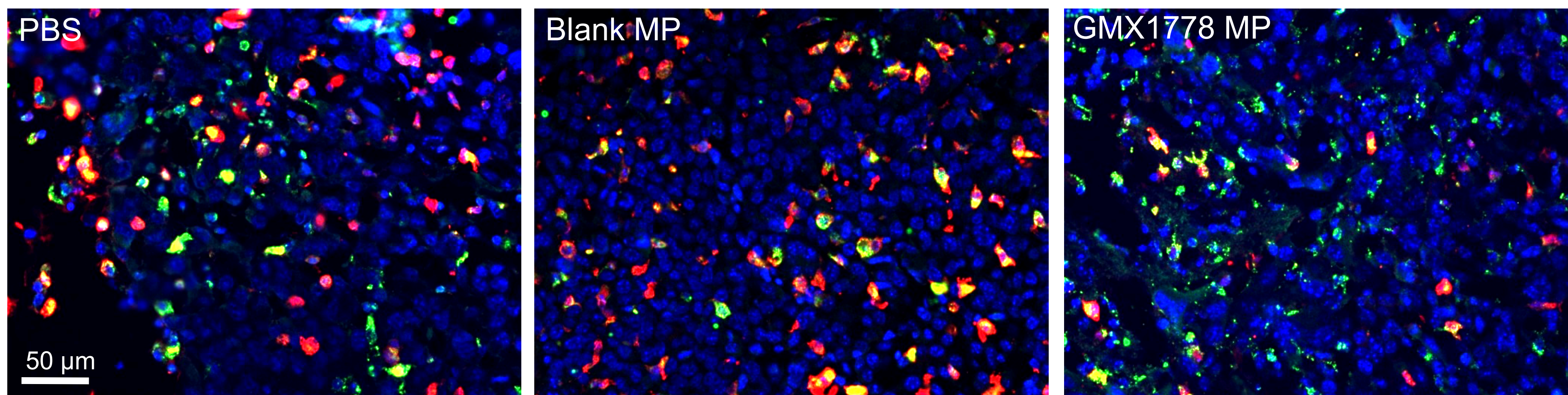
Supplementary Figure S4. Tumor PD-L1 upregulation after injection of coumarin6-GMX1778 co-loaded microparticles. A, Measurement of GMX1778 concentrations released from different MP formulations into media. **B,** Cytotoxicity of GMX1778 released from GMX1778 microparticles (GMX) and coumarin6-GMX1778 microparticles (C6-GMX), measured by MTS cell viability assay 72 hours after exposure to GL261 cells. 0.2=0.2 μM. 1=1 μM. For blank and coumarin6 particles, amount of media comparable to GMX microparticles was used as negative control. **C**, Representative microscopic pictures of C6 fluorescent signals (green) and PD-L1 immunofluorescence (red) in GL261 intracerebral tumors, 4 days after intratumoral injection of C6 and C6-GMX microparticles. Tumor areas with (Particle area) and without (Non-particle area) detectable C6 signals are presented. **D**, Quantification of **C**. Number of PD-L1+ cells in GL261 treated with C6 and C6-GMX microparticles. N=3 for C6 and N=4 for C6-GMX microparticles-treated animals. Error bars, SD. *$p < 0.05$,

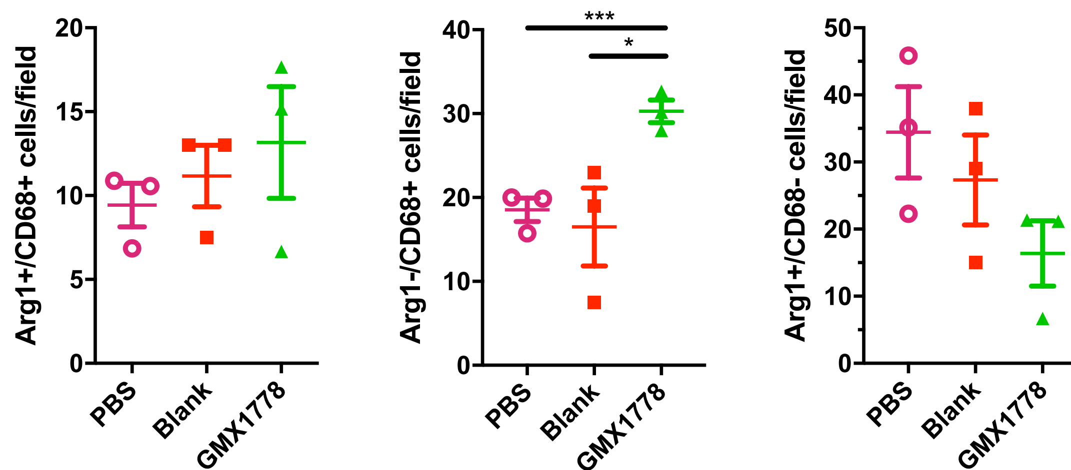
Supplementary Figure S5



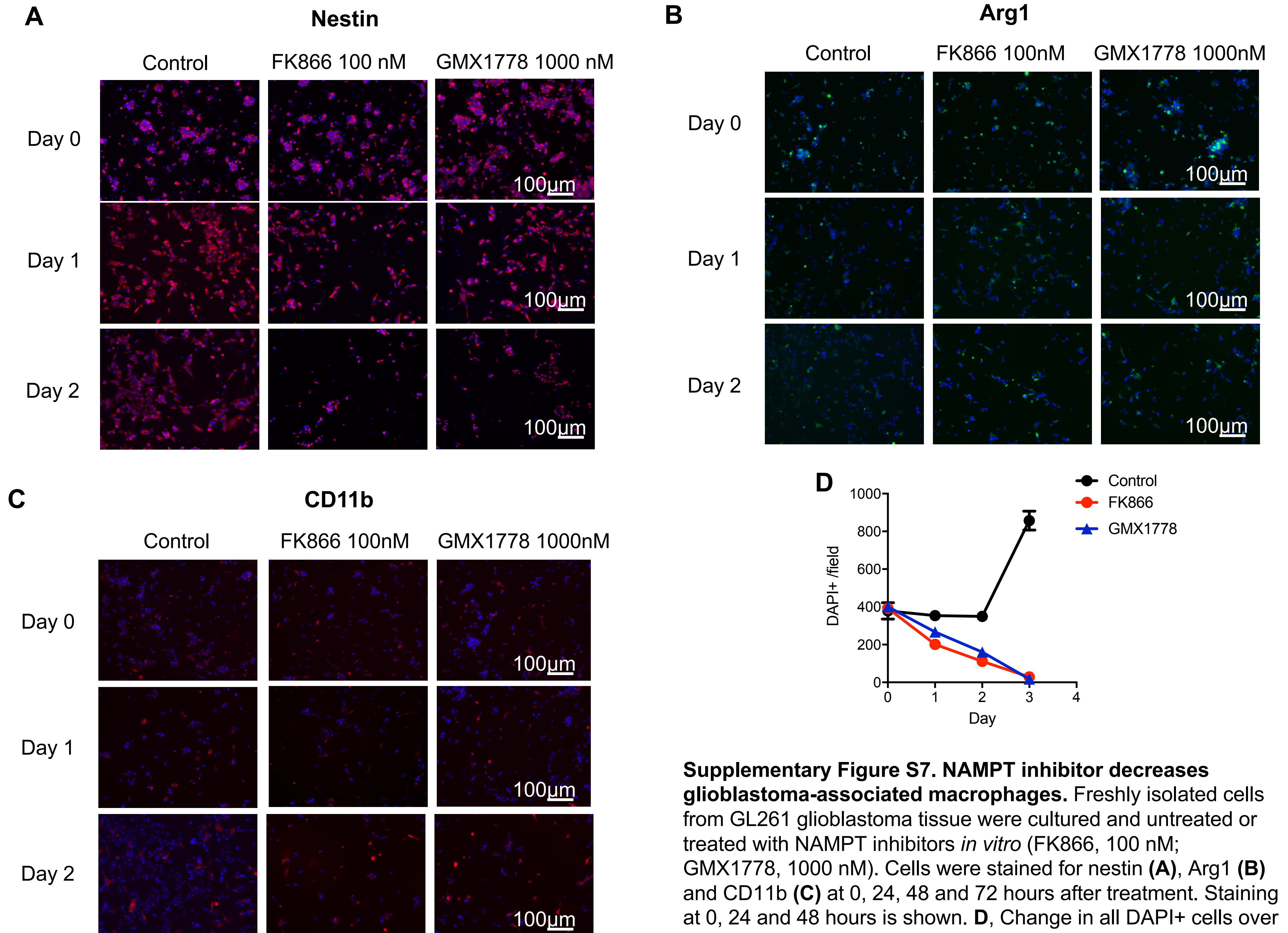
Supplementary Figure S5. NAMPT inhibitor-induced autophagy underlies an increase in PD-L1 mRNA and protein levels. A, Western blot for autophagy marker LC3ALC3-I/B II showing induction of autophagy by NAMPT inhibitors, FK866 and GMX1778, in GL261 cells. Everolimus (EVE, mTORC1 inhibitor) served as a positive control of autophagy inducer. 3-MA, 3-methyladenine. The ratio of LC3 I / LC3II band intenstity is shown underneath the blot. **B,** Left, quantitative RT-PCR showing upregulation of PD-L1 (*Cd274*) mRNA by NAMPT inhibitors was partially abrogated by 3-MA (1 mM). Middle and right, Flow cytometry showing 3-MA inhibition of NAMPT inhibitor-induced PD-L1 upregulation. Cells were exposed to drugs for 48 hours. Error bars, SEM. ***p＜0.01 for the difference between two groups.

Supplementary Figure S6



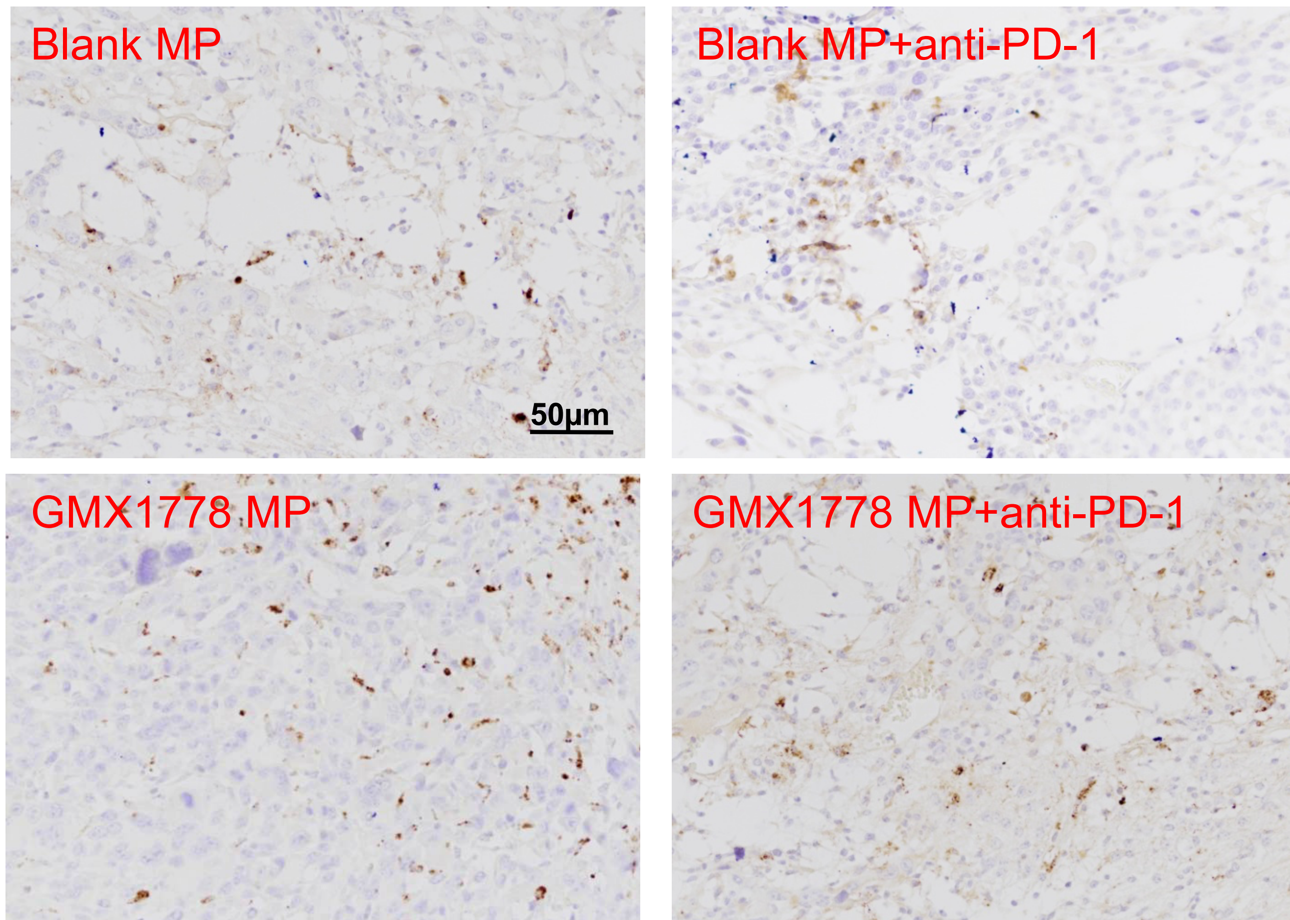


Supplementary Figure S6. Impact of local treatment with GMX1778 microparticles on cells labeled with Arg1 and CD68 in murine glioblastoma. Top, Double immunofluorescence for Arg1 (red) and CD68 (green) in orthotopic GL261 glioblastoma treated with PBS, blank microparticles (MP) and GMX1778 MP. Brains were removed 4 days after treatment of 16-day-old tumors. Bottom, Arg1-single positive, CD68-single positive, and Arg1/CD68-double positive cells were enumerated. *, P<0.05; ***p＜0.01.

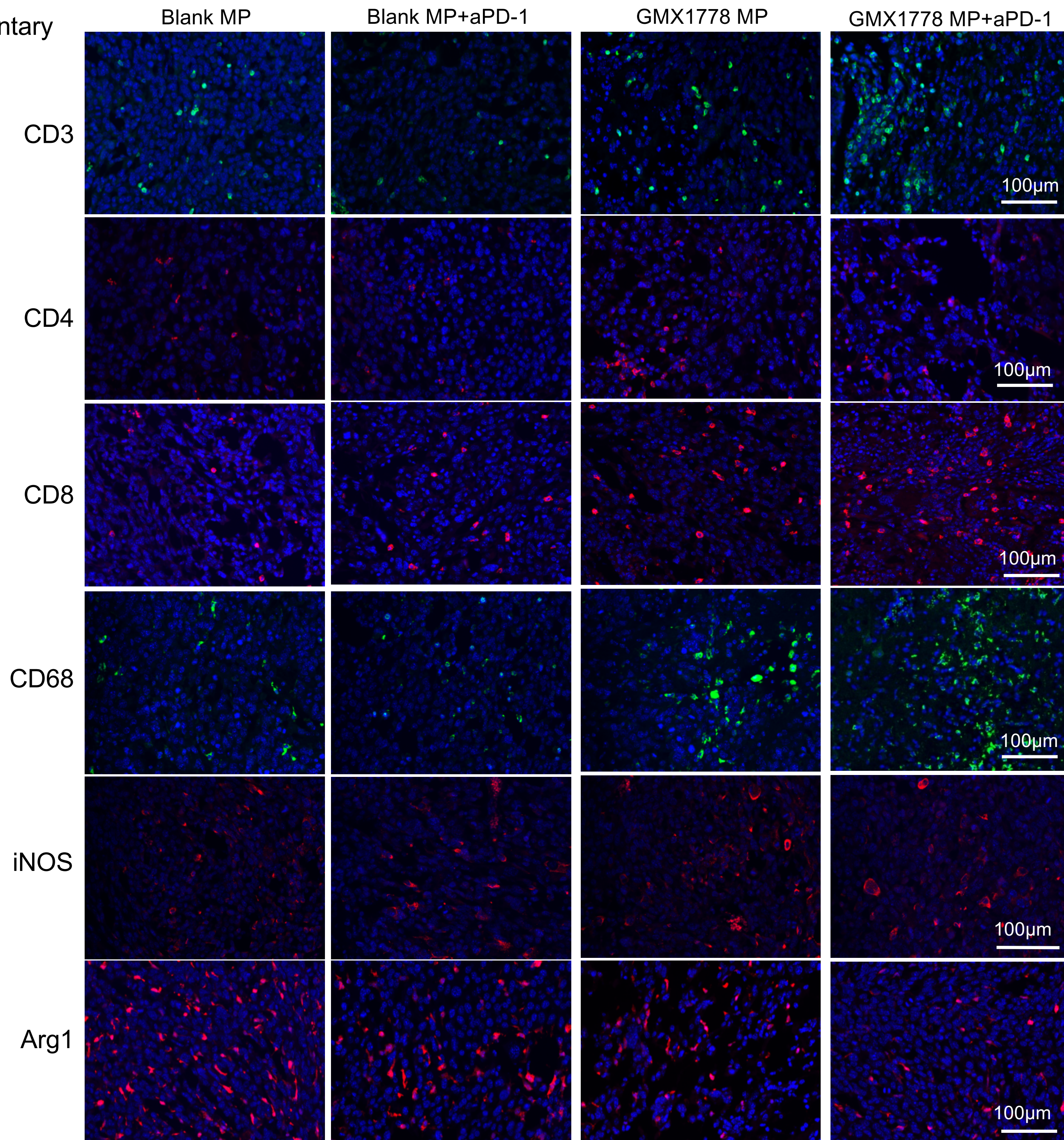


Supplementary Figure S7. NAMPT inhibitor decreases glioblastoma-associated macrophages. Freshly isolated cells from GL261 glioblastoma tissue were cultured and untreated or treated with NAMPT inhibitors *in vitro* (FK866, 100 nM; GMX1778, 1000 nM). Cells were stained for nestin **(A)**, Arg1 **(B)** and CD11b **(C)** at 0, 24, 48 and 72 hours after treatment. Staining at 0, 24 and 48 hours is shown. **D**, Change in all DAPI+ cells over time.

Supplementary Figure S8

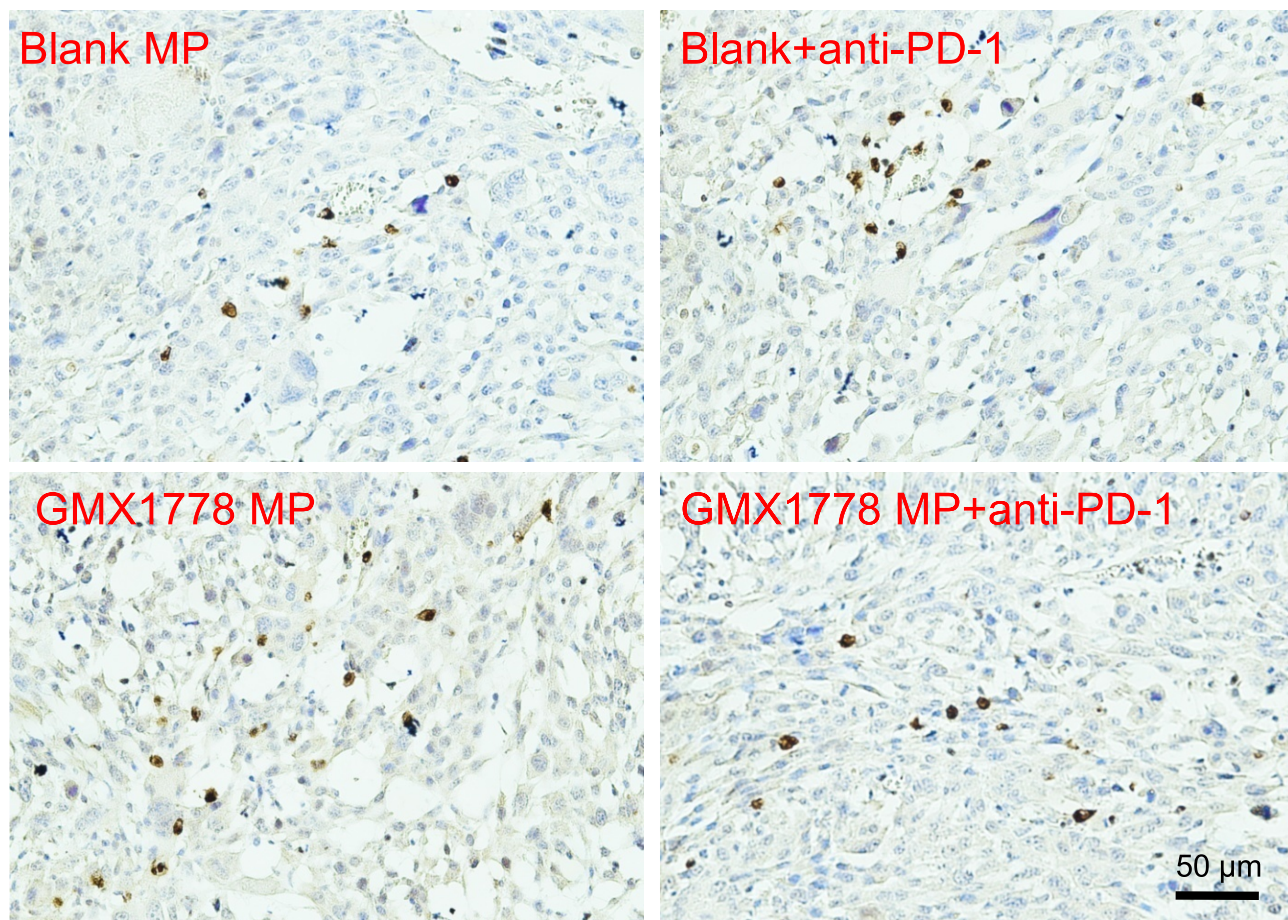


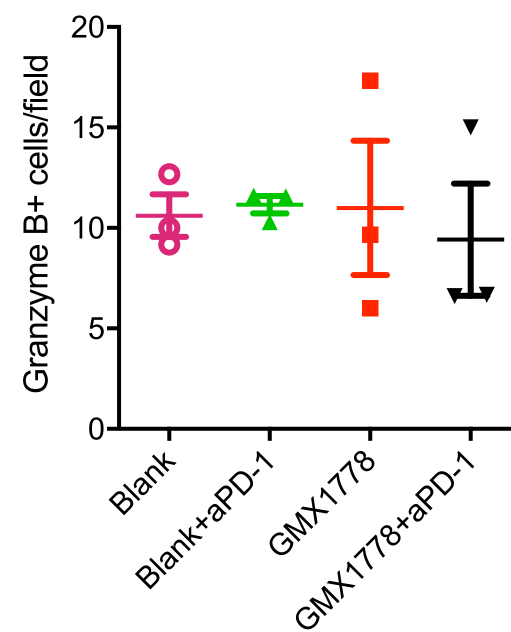
Supplementary Figure S8. PD-L1 immunohistochemistry (brown) of GL261 glioblastoma after treatment with blank micro-particles (MP), blank MP and anti-PD-1, GMX1778 MP, and combination of GMX1778 MP and anti-PD-1. Quantitation is shown in Fig. 5E.



Supplementary Figure S9. Immunoflurescence of GL261 glioblastoma after treatment with blank micro-particles, anti-PD-1, GMX1778 micro-particles, and combination. Representative microscopic pictures of staining for T cells (CD3, CD4, and CD8), and macrophages (CD68, iNOS, and Arg1) are shown. See Figure 7D, F-K for experimental scheme and quantification.

Supplementary Figure S10





Supplementary Figure S10. GranzymeB immunohistochemistry (brown) of GL261 glioblastoma after treatment with blank micro-particles (MP), blank MP and anti-PD-1, GMX1778 MP, and combination of GMX1778 MP and anti-PD-1.