

Supplementary Materials: EphA3 Pay-Loaded Antibody Therapeutics for the Treatment of Glioblastoma

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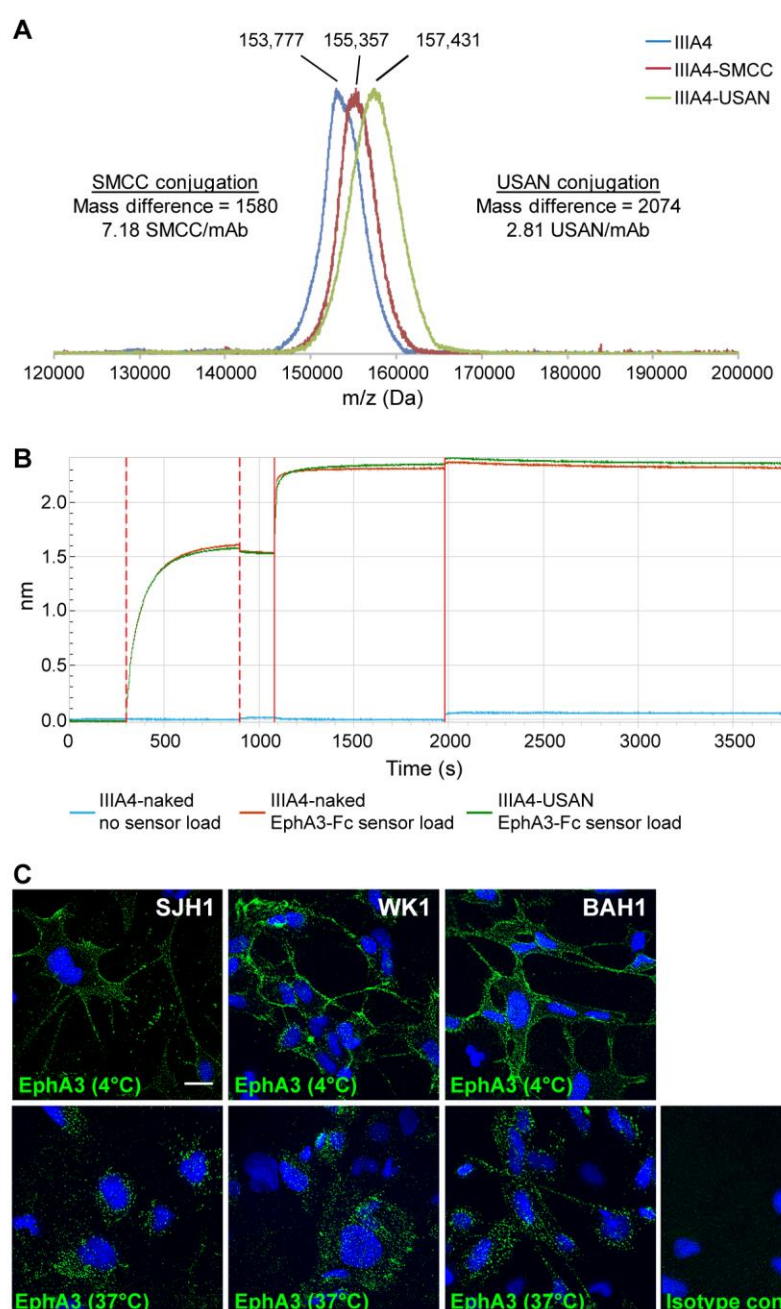


Figure S1. Validation of the EphA3-maytansine ADC IIIA4-USAN. **(A)** MALDI-ToF MS spectra of IIIA4 (blue trace), IIIA4-SMCC (red trace), and IIIA4-USAN (green trace). Observed shifts of the peak molecular weights were used to determine conjugation of both SMCC and USAN; the drug-antibody ratio (DAR) was calculated to be 2.81 USAN/mAb. **(B)** Binding kinetics of unconjugated

IIIA4 (IIIA4-naked) and IIIA4 conjugated to maytansine (IIIA4-USAN) using Bio-Layer Interferometry (BLI) technology. Biotinylated EphA3-Fc was immobilized onto streptavidin biosensors. Real-time binding charts were then acquired with the Octet® system to compare the binding kinetics of IIIA4-naked and IIIA-USAN (30 µg/mL) to the biosensors. (C) Representative immunofluorescence images of EphA3-labelling with IIIA4 mAbs (shown in green) on the cell surface (top panel) and after antibody internalization (bottom panel) in the patient-derived GBM cell line WK1, SJH1 and BAH1. An IgG1 antibody was used as an isotype control. Scale bar represents 10 µm.

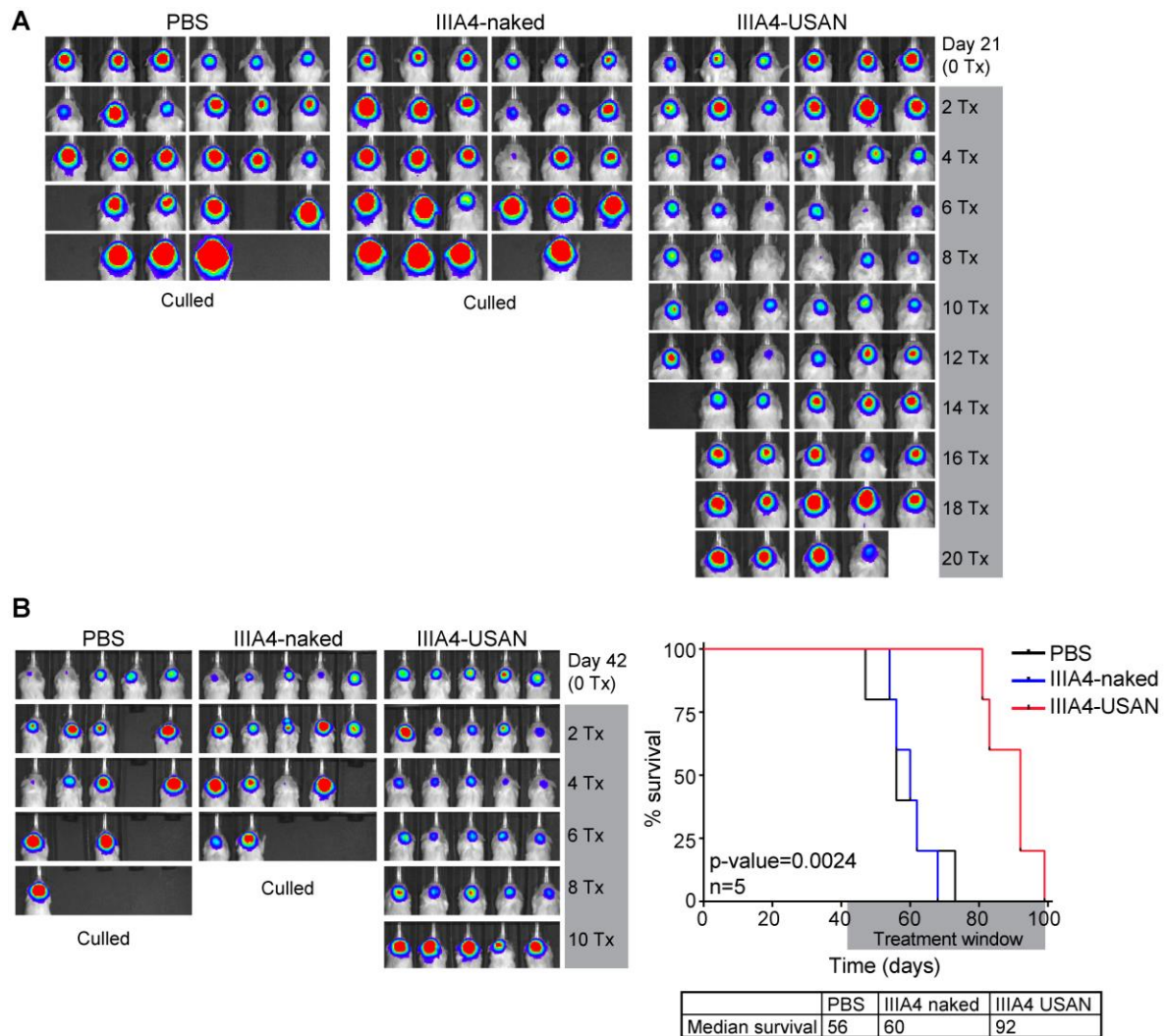


Figure S2. In vivo efficacy of the EphA3-maytansine ADC IIIA4-USAN. (A) Complete imaging data for the experiment shown in Figure 4A. Luciferase-expressing U251 cells were engrafted intracranially. Starting from day 21, animals received twice weekly IV injections of IIIA4-USAN (10 mg/kg), IIIA4-naked (10 mg/kg) or PBS vehicle control. (B) Luciferase-expressing U251 cells were injected intracranially. On day 42, mice were randomized and tumour-matched based on bioluminescence imaging. Mice subsequently received twice weekly intravenous injections of 10 mg/kg IIIA4-USAN, IIIA4-naked or PBS vehicle control. Intravital bioluminescence imaging of luciferase activity in tumour cells and Kaplan-Meier survival plot are shown.

