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Supplementary Materials for

Identification of variable lymphocyte receptors that can target therapeutics to pathologically exposed brain extracellular matrix

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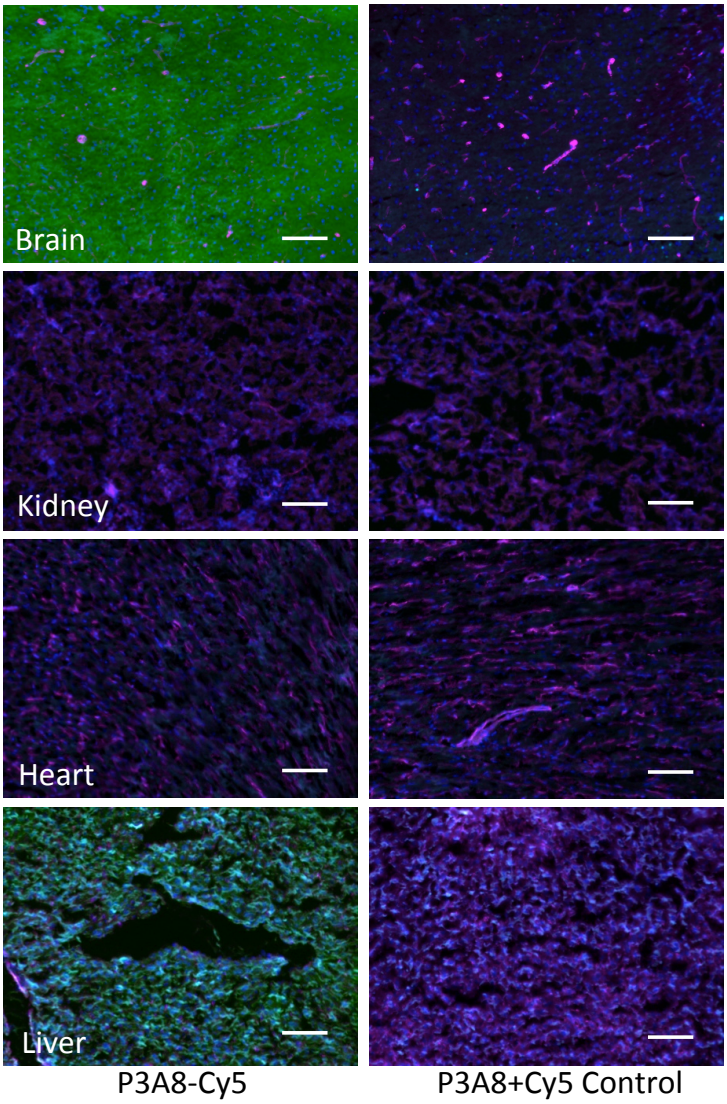

Fig. S1. P3A8 binding to murine tissue sections. Images on the left side are stained with Hoechst 33342 for DNA (blue) and labeled with GS-IB4-AF488 for microvessels (magenta) and P3A8-Cy5 (green). Images on the right are labeled as the left except the P3A8 VLR and Cy5 are not chemically conjugated to serve as a negative control. Origin of the tissue sections are labeled for each pair of images. Scale bars = 100 μm.

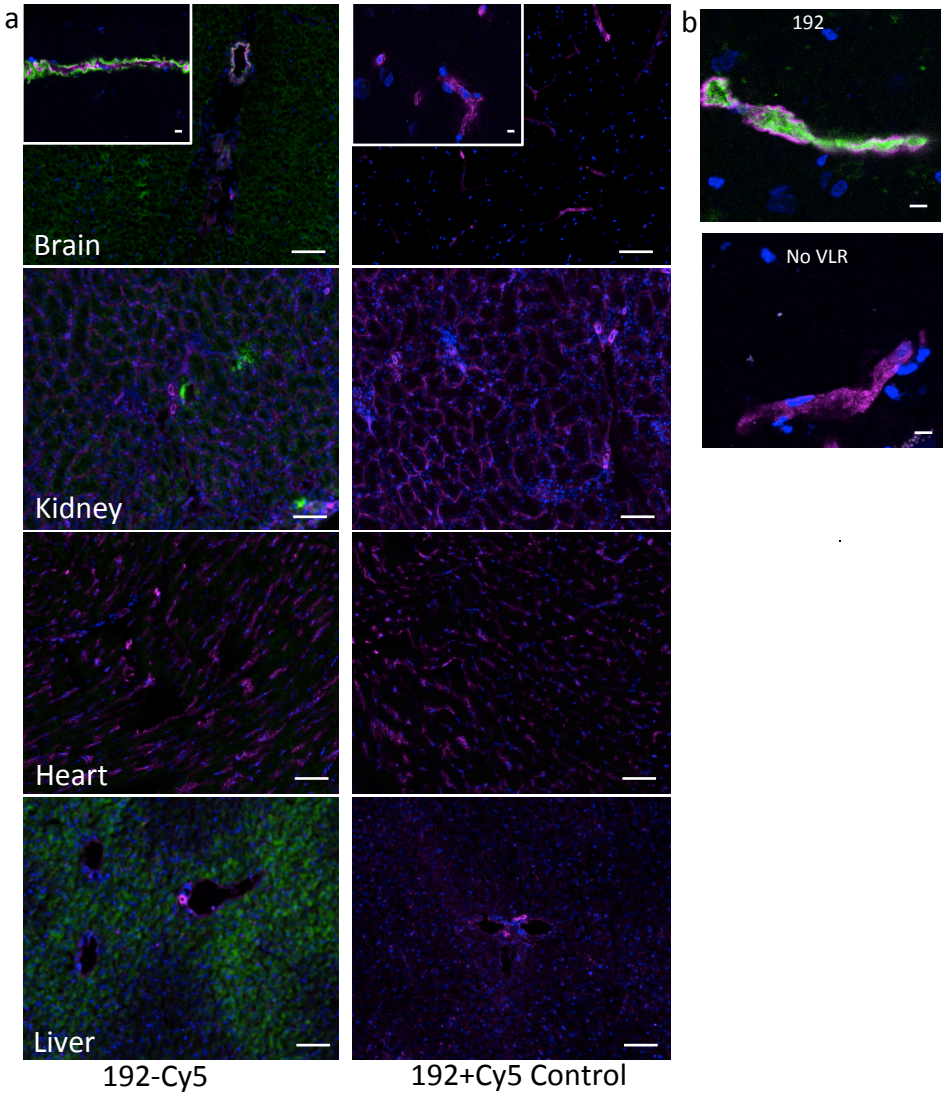

Fig. S2. VLR 192 binding to tissue sections. a) Images in the left column are stained with Hoechst 33342 for DNA (blue) and labeled with GS-IB4-AF488 for microvessels (magenta) and 192-Cy5 (green). Images on the right are stained as the left except the 192 VLR and Cy5 are not chemically conjugated to serve as a negative control. Scale bars = 100 μm. Inset images are higher magnification images of the same groups. Scale bars = 20 μm Origin of the tissue sections are labeled for each pair of images. b) human brain sections labeled with 192-Cy5 as in a) microvessels are immunolabeled with an anti-Cd31 antibody (magenta). Scale bars = 20 μm

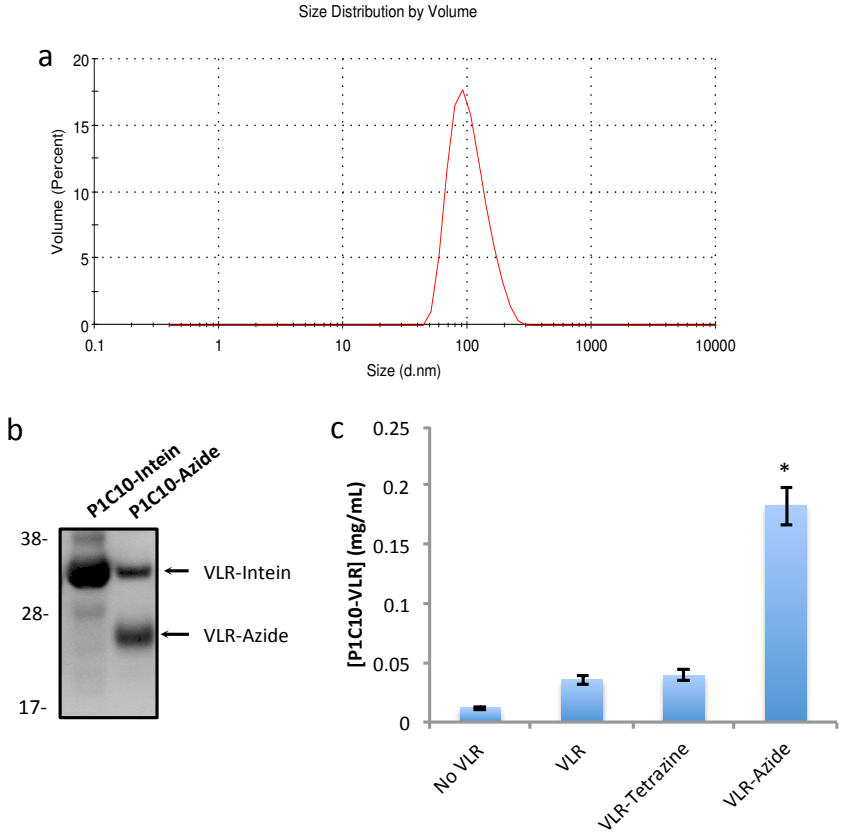

Fig. S3. Characterization of doxorubicin-loaded liposomes. a) Doxorubicin-loaded liposomes were sized using dynamic light scattering. Formed liposomes demonstrated a narrow size distribution with mean diameter of 94.2 nm. **b)** Anti-Flag western blot of P1C10-Intein fusion in lane 1 and P1C10 that has undergone EPL to append an azide at the carboxy-terminus in lane 2. **c)** VLRs are incubated with liposomes that contain DSPE-PEG2000-DBCO. Compared are a no VLR group, a VLR with no chemically reactive carboxy-terminal group, a VLR with a non-DBCO reactive tetrazine at its carboxy-terminus and the VLR with a DBCO-reactive azide at its carboxy-terminus. After removal of excess protein, the amount of protein present in each liposome group is quantified using a BCA assay (* $p < 0.05$ using ANOVA).

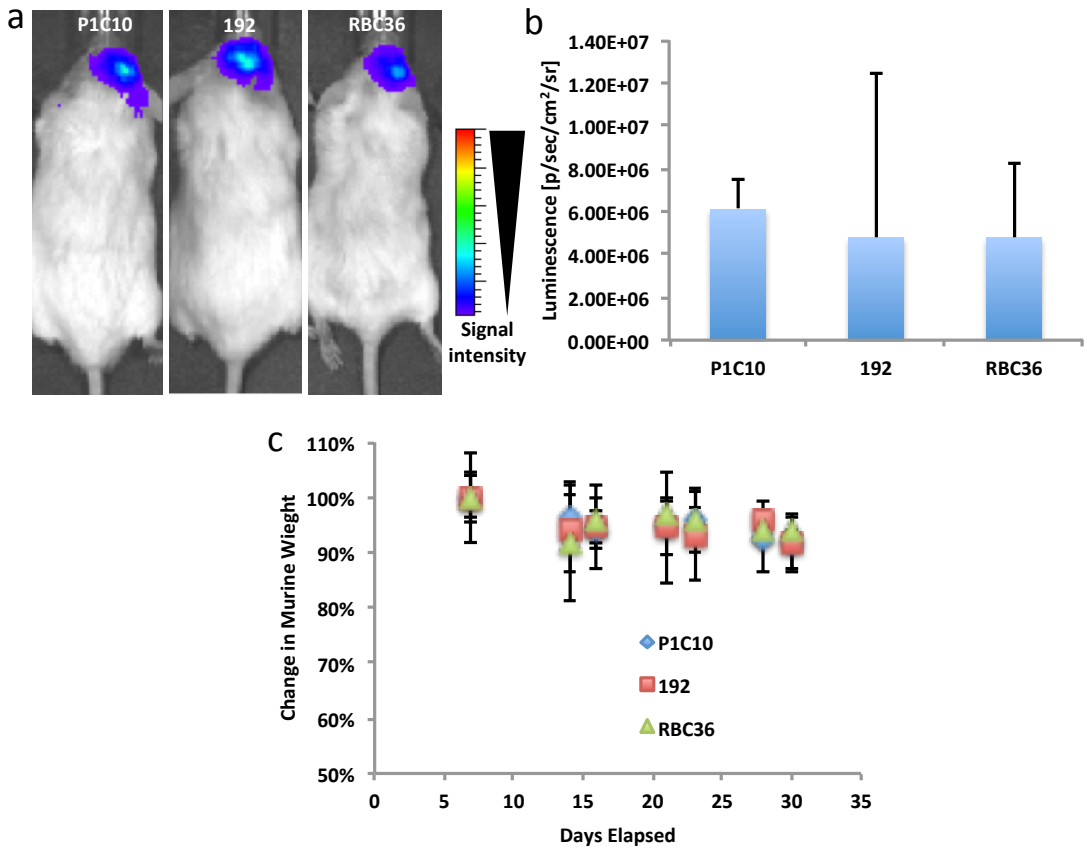

Fig. S4. Luminescence and Weights from U87 Tumor Survival Experiment. **a)** Representative, 7-day post implantation IVIS imager images of mice bearing luciferase-expressing U87 intracranial tumors. **b)** Mean luminescence signal and standard deviation of IVIS image signal from day 7 brain tumors (ANVOA, $p>0.05$). **c)** Murine weights are plotted as percentage of weight change from day 7 for each group, Mean± S.D. There are no statistically significant differences in weight between groups over time ($p>0.05$, ANOVA).

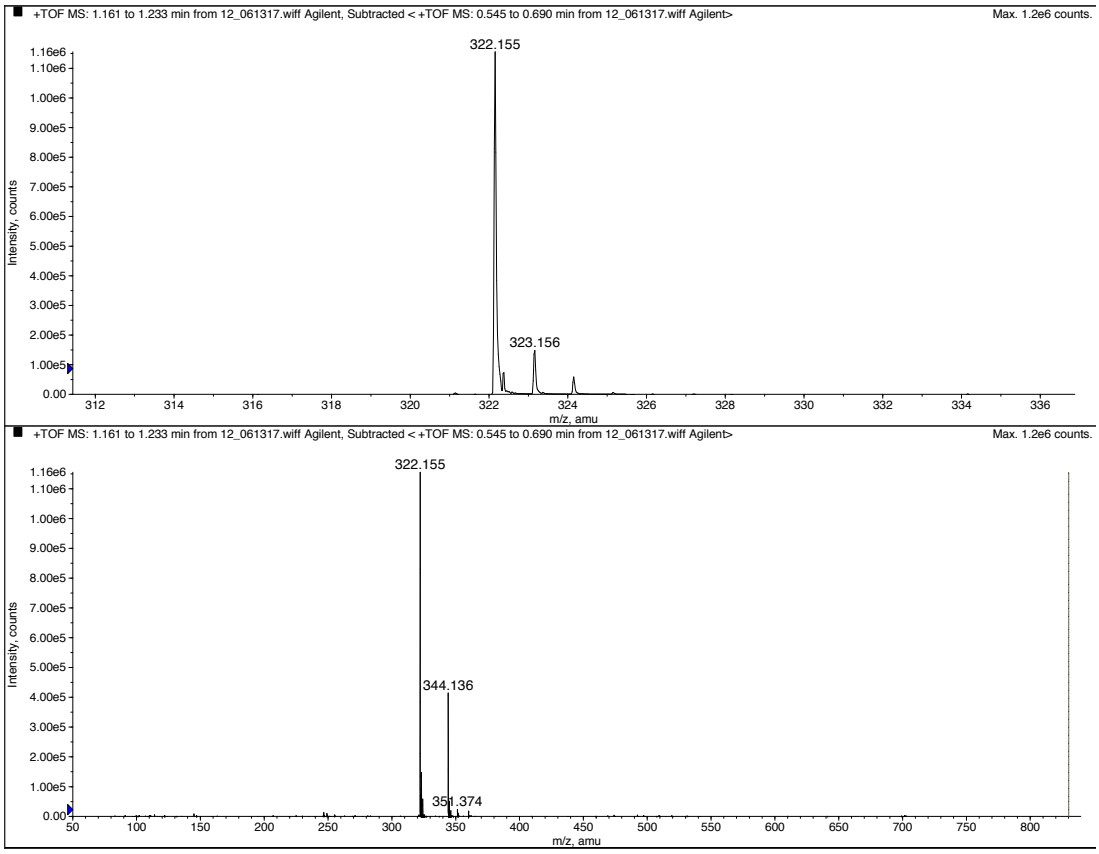

Fig. S5. Mass Spectrometry traces for Manufacture of Cys-PEG3-Azide. Amine-PEG3-Azide (Santa Cruz) was conjugated to the c-terminus of Cysteine using FMOC-Cys (Sassrin resin) OH (Bachem) using standard SPPS techniques. The peptide was purified to 90% purity by HPLC and Mass Spectrometry used determine formation of the desired product. Top panel shows the narrow mass range while bottom panel shows wide mass range. Expected mass for Cys-PEG3-Azide is 321 daltons.