Supplementary Information

Serum Stability and Affinity Optimization of M2 Macrophage-Targeting Peptide (M2pep)

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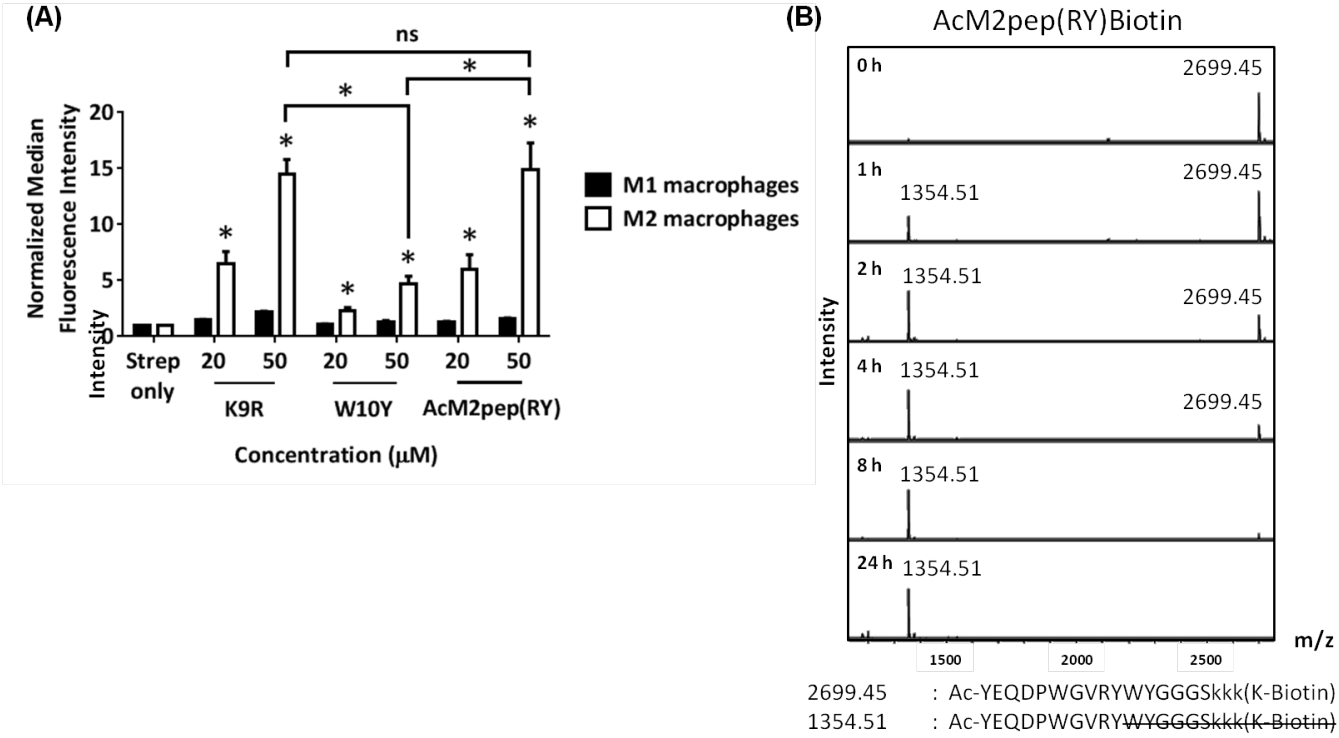

Fig. S1. (A) Binding of AcM2pep(RY)Biotin, K9R, and W10Y analogs to M1 and M2 macrophages. (B) MALDI-TOF MS spectra of AcM2pep(RY)Biotin at different serum incubation times. Adjacent to the primary peaks are their respective Na^+ adducts. Unless labeled in pairs, stars denote statistical significance between M1 and M2 macrophages in the same treatment group. * $P < 0.05$, ns = not statistically significant.

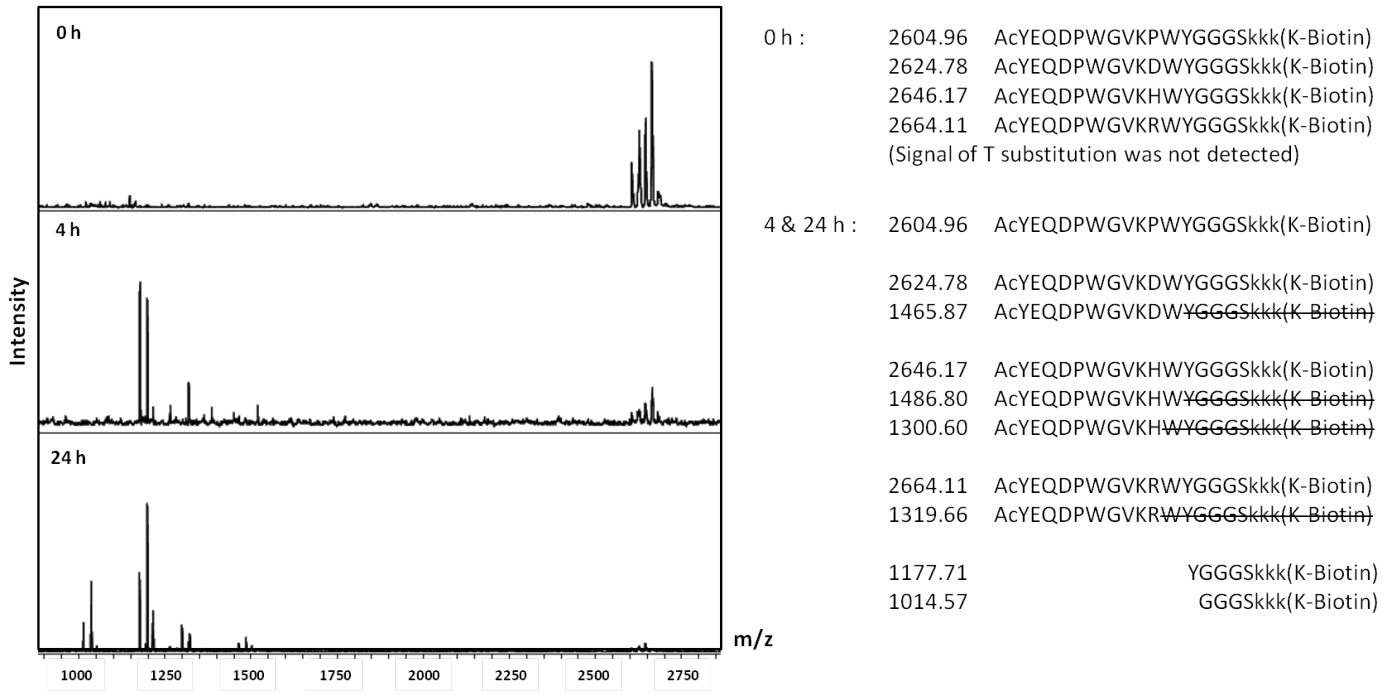

Fig. S2. MALDI-TOF MS spectra of crude W10(P,D,T,R,H) analog at different serum incubation times. The crude mixture was synthesized using the split-and-mix strategy at the W10 position. Briefly, WYGGGSkkk(K-Biotin) was synthesized on the automatic peptide synthesizer. The peptide on resin was then split into 5 portions, separately coupled with Pro, Asp, Thr, Arg, and His, manually pooled together, and put on the peptide synthesizer to continue the synthesis of the remaining sequence. The crude mixture was used without RP-HPLC purification.

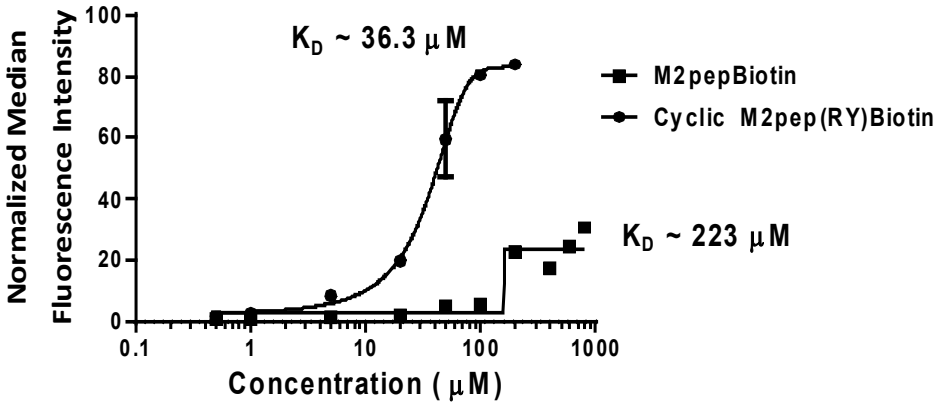

Fig. S3. Binding curves of M2pepBiotin and cyclic M2pep(RY)Biotin on M2 macrophages. The K_D value for M2pepBiotin reported here is slightly higher than the previously reported value due to some differences in experimental methodology. In this study, M2 macrophages were not fixed after incubation with peptides and were stained with propidium idodide to only quantify binding on live cells.

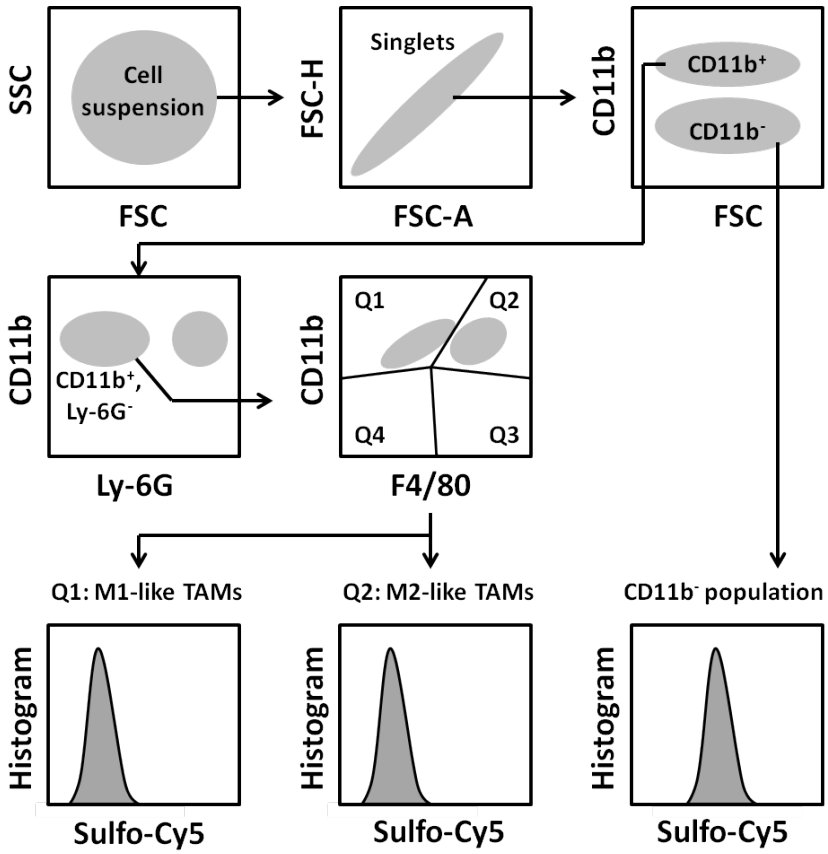

Fig. S4. Schematics elucidating the gating strategy for analysis of intratumoral biodistribution of M2pep analogs in 1) $CD11b^-$ population, 2) M1-likeTAMs, and 3) M2-like TAMs.