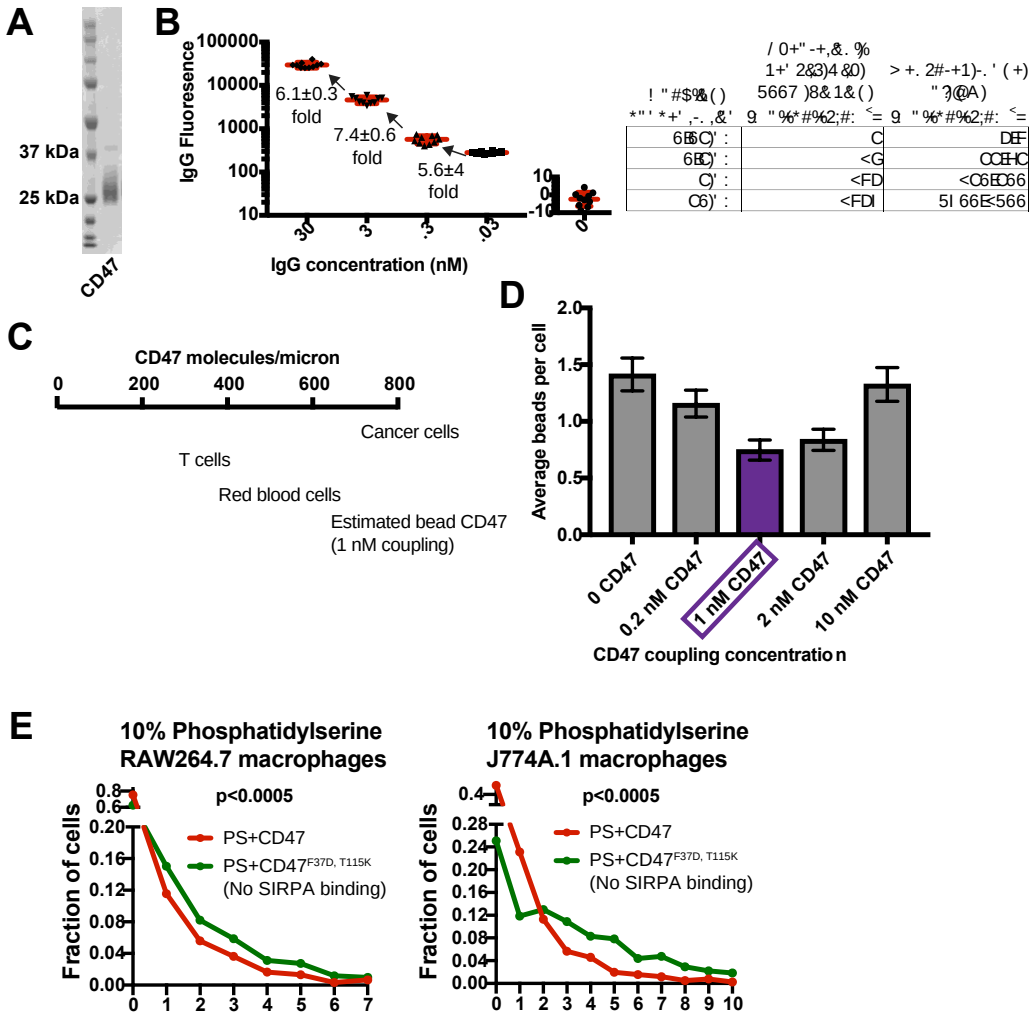

Figure S1, related to Figure 1: A reconstitution system for studying CD47-SIRPA signaling

(A) SDS page gel shows the N-terminal extracellular domain of murine CD47 purified from insect cells using a C-terminal His_{10}. (B) Beads coated in supported lipid bilayers were incubated with the indicated concentration of anti-biotin IgG. The fluorescent intensity of Alexa Fluor 647-IgG on the bead was measured to ensure that the binding of IgG increased with higher coupling concentrations. To estimate the IgG density, the Alexa Fluor 647 fluorescent intensity on each bead was compared to fluorescent bead standards. The estimated IgG density is presented in table on the right, alongside a theoretical prediction for IgG density assuming 100% binding. (C) The estimated surface density of CD47 on red blood cells (Gardner et al., 1991; Mouro-Chanteloup et al., 2003), T cells (Subramanian et al., 2006), cancer cells (Dheilly et al., 2017; Jaiswal et al., 2009; Michaels et al., 2017) and the beads used in this study. (D) IgG surface density was held constant while CD47 density was titrated. The 1 nM CD47 coupling concentration was selected for use throughout this study. (E) Graph shows the distribution of number of beads engulfed by single cells. Beads were coated in a supported lipid bilayer containing 10% phosphatidylserine and CD47 (red) or the signaling-defective $CD47^{F37D,T115K}$ (green) and fed to RAW264.7 or J774A.1 macrophages. The RAW264.7 data correspond to the replicates depictured in Figure 1E. RAW264.7 engulfment was measured after 30 min and J774A.1 was measured after 90 min.

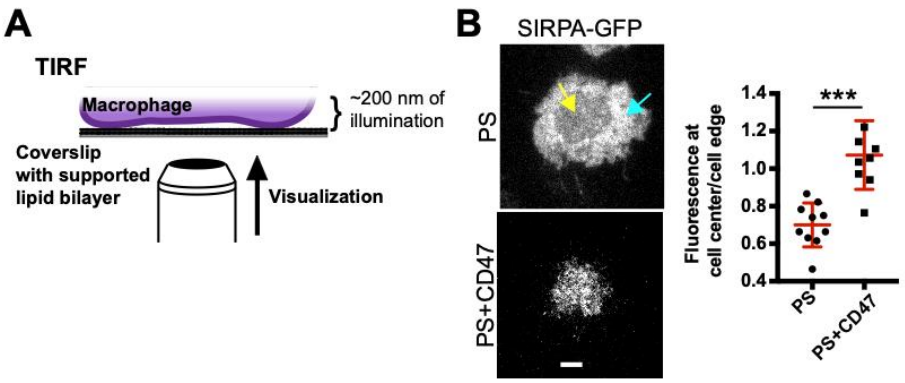

Figure S2, related to Figure 2: SIRPA is at the periphery of the phagocytic synapse with a phosphatidylserine bilayer

(A) Schematic depicts TIRF imaging. (B) TIRF microscopy of J774A.1 macrophages encountering a 10% phosphatidylserine bilayer reveals that SIRPA-GFP is depleted at the center off the cell-bilayer synapse (top; yellow arrow compared to cyan arrow). Macrophages did not form this zone of depletion when encountering a bilayer containing both phosphatidylserine and CD47 (bottom). The ratio of SIRPA-GFP fluorescent intensity at the cell center/cell edge is quantified on the right. Each dot represents an individual cell and data is pooled from 3 independent experiments. Red lines denote the mean ± SD. *** denotes $p<0.0005$ by Student's T test.

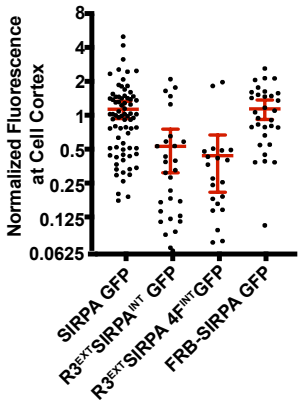

Figure S3, related to Figure 3: Expression levels of SIRPA chimeras

SIRPA-GFP and the chimeric receptors used in Figure 2 are expressed at similar levels. Fluorescent intensity was normalized to the average intensity of SIRPA-GFP in that experiment. Each dot represents an individual cell and data is pooled from 3 independent experiments. Lines denote the mean ± SD.

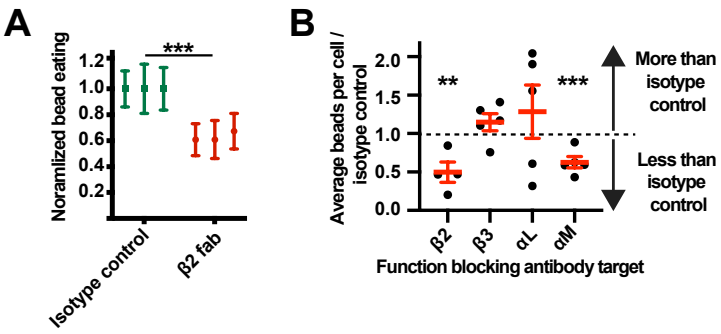

Figure S4, related to Figure 5: Blockade of β2 or αM integrins disrupts engulfment

(A) Macrophages were incubated with a Fab generated from the β2 function-blocking antibody (2E6, red) or from an isotype control (green). Three independent replicates are graphed with error bars denoting SEM. *** indicates $p<0.0005$ by Student's T-test. (B) Macrophages were incubated with a function blocking antibody targeting the indicated integrin subunit, or the relevant isotype control. To remove any potential non-specific integrin ligands, this assay was performed in protein free HEPES-based imaging buffer. The average number of beads eaten per cell was counted and divided by the average beads per cell in the isotype control. Lines indicate the mean ± SEM. *** indicates $p<0.0005$ and ** indicates $p<0.005$ by Student's T-test comparing the blocking antibody to the isotype control.

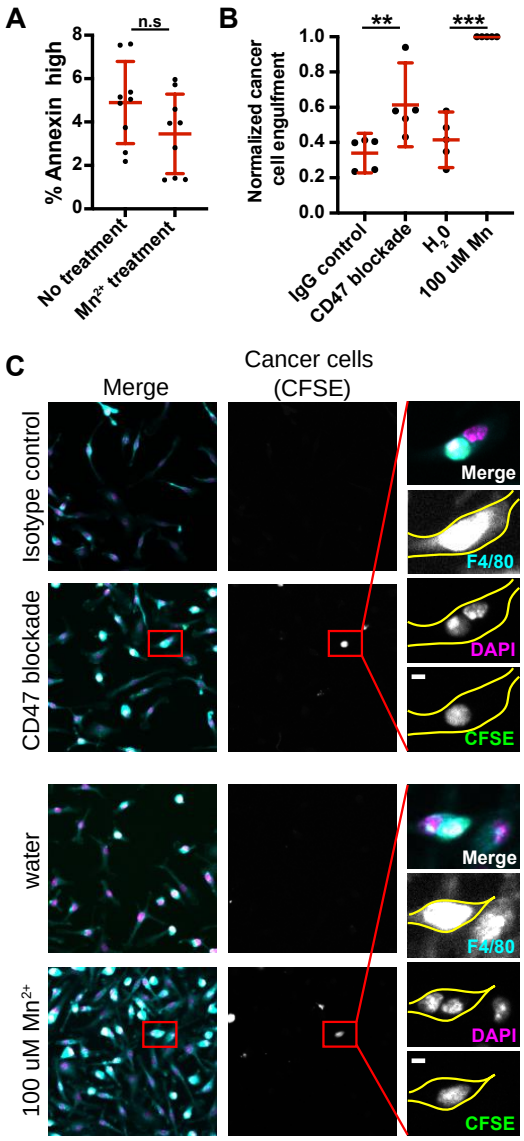

Figure S5, related to Figure 6: Manganese drives engulfment of viable cancer cells

(A) L1210 cells were serum starved for 2 hrs, then treated with 100 μM manganese for 6 hrs as in Figure 4. The percent of cells that bound high levels of annexin, indicating phosphatidylserine exposure and the initiation of apoptosis, was measured by flow cytometry. (B) L1210 cancer cells were dyed with CFSE and incubated with primary bone marrow derived macrophages for 4 hours at a 2:1 cancer cell:macrophage ratio. Cells were then fixed and stained for F4/80 to label the macrophages and DAPI to label nuclei. Cells that were CFSE and F4/80 double positive, and contained 2 nuclei were scored as an engulfment event. Representative images are shown in (C). The red box indicates an engulfment event and is shown at higher magnification on the right. The macrophage cortex is outlined in yellow.