Supplementary Figures

Figure S1: SRF231 full-length IgG and F(ab')2 bind equivalently to Jurkat cells.

Jurkat cells were incubated with increasing doses of either unlabeled SRF231 or SRF231 F(ab')2 for 30 minutes at 4°C. Cells were washed and incubated with a saturating dose of AF647-labeled SRF231 for an additional 30 minutes at 4°C. AF647 mean fluorescence intensity (MFI) was evaluated by flow cytometry and inversely correlates with the degree of on-cell binding of unlabeled SRF231 or SRF231 F(ab')2.
Figure S2: Pure blockade of CD47/SIRPα is sufficient to induce phagocytosis by mouse macrophages.

Mouse bone marrow-derived macrophages were prepared from either Balb/c (left panels) or NOD (right panels) mice and used as effector cells in a phagocytosis assay. CFSE-labeled human Jurkat (A) or mouse TK-1 (B) cells were targets and cocultures were exposed to the indicated concentrations of antibody (full-length or F(ab’)2). Phagocytosis is represented as the percent of CD11b+ macrophages that became CFSE+ after co-culture. (C) CB.17 SCID mice were subcutaneously engrafted with 10 × 10^6 Raji B cells. When tumors averaged 100-150 mm^3, mice were randomized and treated IP with 200 μg/mouse of isotype control (polyclonal human IgG; hIgG), SRF231 or SRF231mut 3 times/week for 3 weeks. (D) NOD.SCID or CB17.SCID (Balb/c background) mice were inoculated with 10 × 10^6 Raji B cells subcutaneously. When tumors averaged 100-150 mm^3, mice were randomized, and treated IP with 30 μg/mouse of SRF231 or 100 μg/mouse of isotype control (polyclonal human IgG; hIgG), 2 times/week for 3 weeks. Data are shown as mean tumor volumes ± SEM.
Figure S3: Macrophage-expressed CD32a and tumor target-expressed CD47 are necessary for SRF231-mediated induction of phagocytosis.

(A) Primary human macrophages, Jurkat cells, and U937 cells were stained with either isotype control (dotted lines; mlG2b) or FITC-conjugated anti-CD32a antibody (clone IV.3, closed lines) and CD32a cell surface expression was analyzed by flow cytometry. (B) Jurkat wild-type (WT) and CD47 KO lines were CFSE-labeled and used as targets in the human macrophage-driven phagocytosis assay, in the presence of the indicated concentrations of SRF231 or isotype control (hlgG4). Phagocytosis was assessed by flow cytometry and data are presented as the percent of CD14+ events that were CFSE+ after coculture. (C) Activation of CD32a in these cultures was measured using chemiluminescence phospho-array imaging and the mean pixel intensity of phospho-CD32a levels in the presence of either hlgG4 or SRF231 is graphed.
Figure S4: SRF231-mediated induction of cell death is CD47-dependent and is translatable to several tumor cell types.

(A) Induction of Annexin V positivity in the context of the mouse phagocytosis assay. Non-phagocyted targets were gated on and analyzed for Annexin V staining. (B) Induction of Annexin V positivity using protein G-bound antibody and WT versus CD47 KO Jurkat cells. (C) Cell death induction evaluated across multiple cancer cell lines in culture with protein G-bound SRF231 or isotype control (hIgG4) for 24 hours. (D) A variety of primary, non-malignant cell types were evaluated for sensitivity to protein G-bound SRF231 cell death induction and results were compared to Jurkat cells and correlated with relative CD47 expression levels.