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')$_2$.

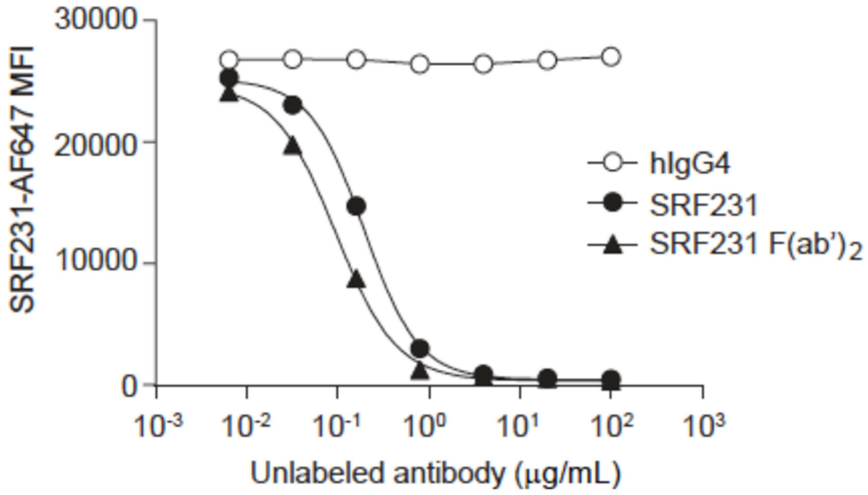

Figure S2: Pure blockade of CD47/SIRPα is sufficient to induce phagocytosis by mouse macrophages.

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.

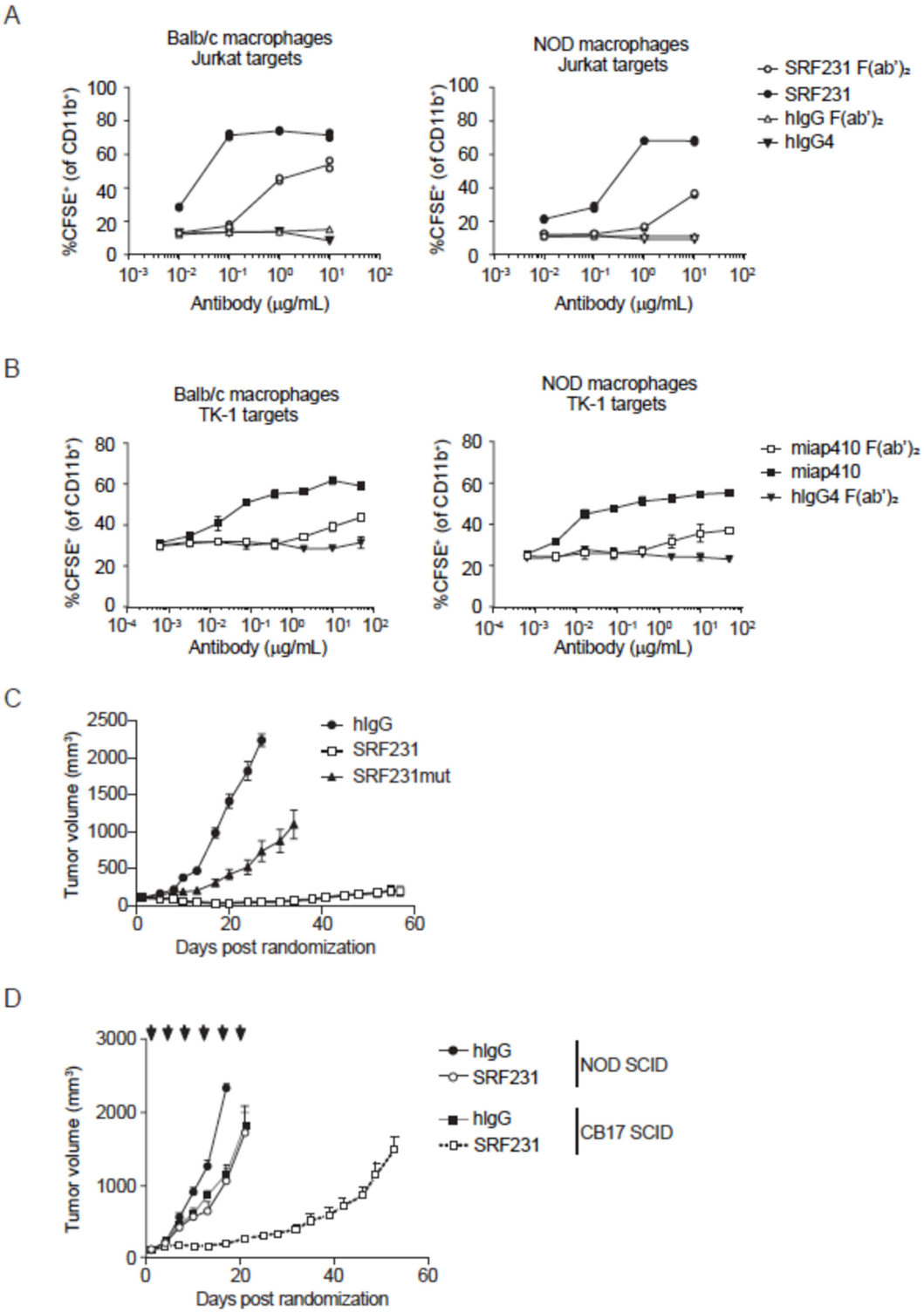
A
Balb/c macrophages
Jurkat targets
NOD macrophages
Jurkat targets
%CFSE+ (of CD11b+)
Antibody (µg/mL)
SRF231 F(ab')2
SRF231
hIgG F(ab')2
hIgG4
B
Balb/c macrophages
TK-1 targets
NOD macrophages
TK-1 targets
miap410 F(ab')2
miap410
hIgG4 F(ab')2
C
Tumor volume (mm3)
Days post randomization
hIgG
SRF231
SRF231mut
D
hIgG
SRF231
NOD SCID
CB17 SCID

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; mIgG2b) 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 (hIgG4). 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 hIgG4 or SRF231 is graphed.

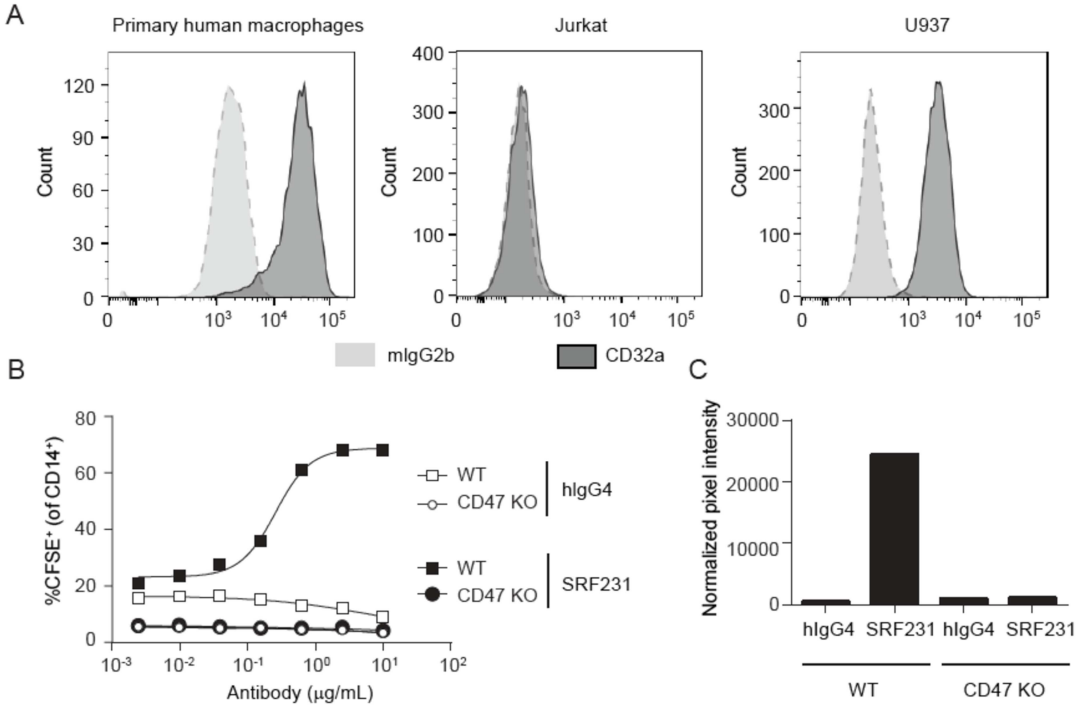

Figure S4: SRF231-mediated induction of cell death is CD47-dependent and is translatable to several tumor cell types.

(A) Induction of Annexin V positivity in the context of the mouse phagocytosis assay. Non-phagocytosed 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.