Supporting Information

Ring et al. 10.1073/pnas.1710877114

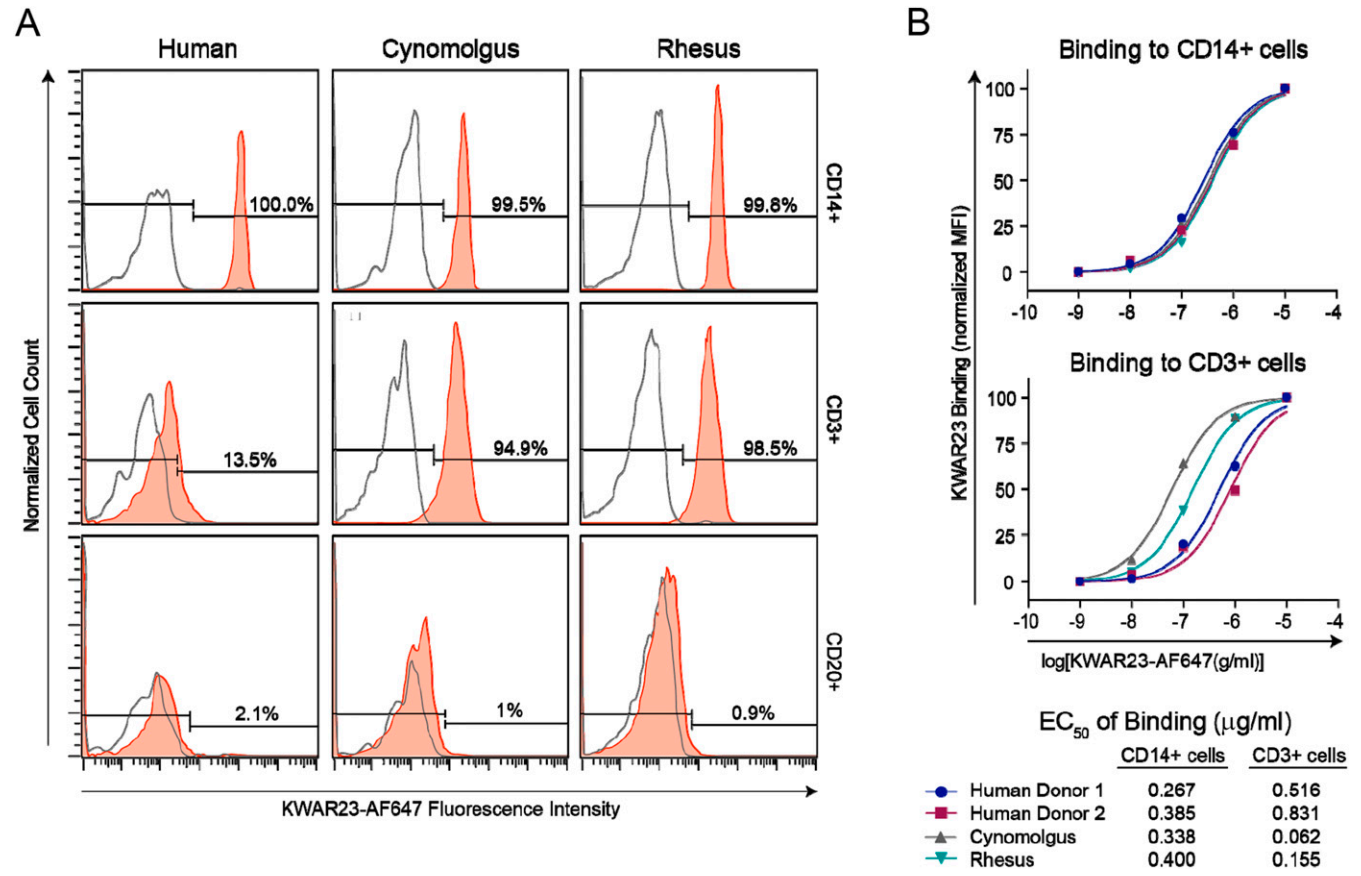

Fig. S1. Antihuman SIRPα antibody KWAR23 also binds to nonhuman primate SIRPα. (*A* and *B*) KWAR23 binds cynomolgus and rhesus SIRPα expressed on $CD14^+$ monocytes and $CD3^+$ T cells, but not $CD20^+$ B cells.

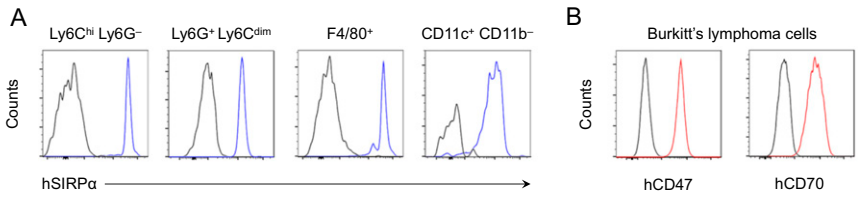

Fig. S2. Expression of human SIRPα (hSIRPα) in human *SIRPA* knockin *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ (SRG) mice, and expression of hCD47 and hCD70 on Burkitt's lymphoma cells. (*A*) Exons 2–4 of the mouse *Sirpa* (extracellular domain) were replaced by the human *SIRPA* sequence using VelociGene Technology (Regeneron Pharmaceuticals) (18). Expression of hSIRPα in circulating Ly6C^{hi} monocytes, neutrophils (Ly6G$^+$ Ly6C^{dim}), macrophages (F4/80$^+$), and dendritic cells (CD11c$^+$CD11b$^-$) in RG (black) and SRG mice (blue) analyzed by flow cytometry. Histograms are representative of two RG and six SRG mice. (*B*) Histograms showing expression of hCD47 and hCD70 in stained (red) and unstained (black) Burkitt's lymphoma cells. One representative experiment is shown.

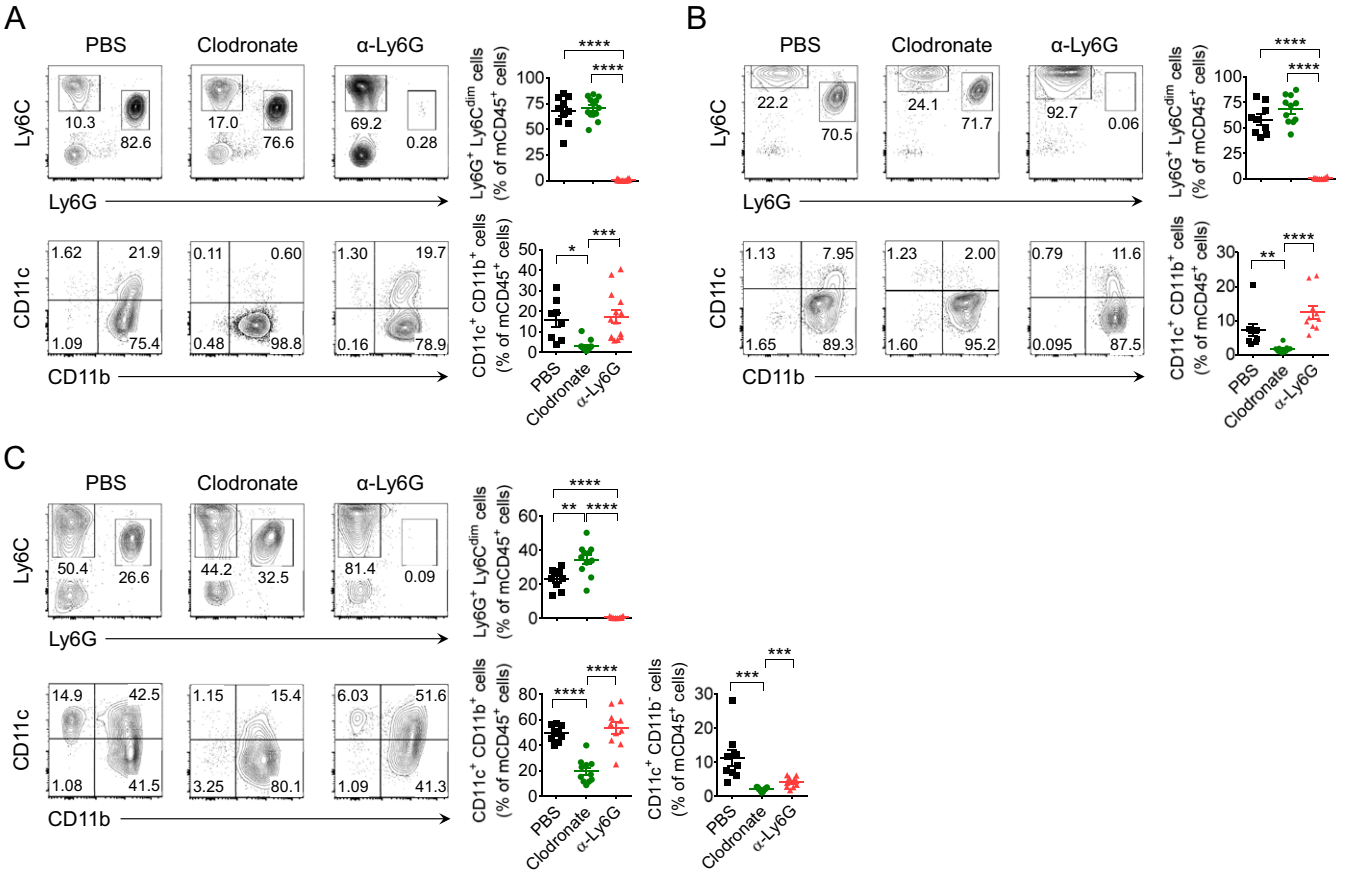

Fig. S3. In vivo depletion of myeloid cell lineages in SRG mice. Efficacy of the depletion of myeloid cell lineages in the peripheral blood (*A* and *B*) and tumor (*C*) of SRG mice treated with PBS, clodronate, or α-Ly6G before initiation of therapeutic antibody treatment (*A*) or at the end of the experiment (*B* and *C*) is shown. Mean ± SEM is shown. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ (unpaired two-tailed Student's *t* test).

Table S1. Data collection and refinement statistics

Data collection and refinement parameters	KWAR23 Fab/SIRPα IgV complex
Data collection	
Wavelength	0.9795
Space group	P 3_2 2 1
Cell dimensions	
a, *b*, *c*, Å	164.97, 164.97, 96.72
α, β, γ, °	90, 90, 120
Resolution, Å	47.52–2.19 (2.27–2.19)*
R_{sym} or R_{merge}	0.086 (0.853)
$I/\sigma I$	21.5 (1.6)
Completeness, %	99.2 (87.9)
Redundancy	6.0 (4.3)
Refinement	
Resolution, Å	2.20
No. of reflections	76,965 (6,916)
R_{work}/R_{free}	0.18/0.22
No. of atoms	8,806
Macromolecules	8,166
Solvent	640
B-factors, Å^2	50.62
Macromolecules	50.90
Solvent	47.04
rmsd	
Bond lengths, Å	0.003
Bond angles, °	0.87
Ramachandran plot	
Residues in most favored regions, %	97.1
Residues in allowed regions, %	2.9
Residues in disallowed regions, %	0.0

*Values in parentheses are for highest resolution shell.

Table S2. Amino acid sequences of antibody V-regions

Antibody name	V-region sequence
KWAR23 heavy chain	EVQLQQSGAELVKPGASVKLSCTASGFNIKDYYIHWVQQRTEQ GLEWIGRIDPEDGETKYAPKFQDKATITADTSSNTAYLHLSSLT SEDTAVYYCARWGAYWGQGTLVTVSA
KWAR23 light chain	QIVLTQSPAIMSASPGEKVTLTCSASSSVSSSYLYWYQQKPGSSP KLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAASYFCH QWSSYPRTFGAGTKLELK
Vorsetuzumab heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAP GQGLKWMGWINTYTGEPTYADAFKGRVTMTRDTSISTAYMEL SRLRSDDTAVYYCARDYGDYGMDYWGQGTTVTVSS
Vorsetuzumab light chain	DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSFMHWYQQK PGQPPKLLIYLASNLESGVPDRFSGSGSGTDFTLTISSLQAEDVA VYYCQHSREVPWTFGQGTKVEIK
CD70/KWAR23 bispecific heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAP GQGLKWMGWINTYTGEPTYADAFKGRVTMTRDTSISTAYMEL SRLRSDDTAVYYCARDYGDYGMDYWGQGTTVTVSSASTKGPE VQLQQSGAELVKPGASVKLSCTASGFNIKDYYIHWVQQRTEQG LEWIGRIDPEDGETKYAPKFQDKATITADTSSNTAYLHLSSLTSE DTAVYYCARWGAYWGQGTLVTVSS
CD70/KWAR23 bispecific light chain	DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSFMHWYQQK PGQPPKLLIYLASNLESGVPDRFSGSGSGTDFTLTISSLQAEDVA VYYCQHSREVPWTFGQGTKVEIKRTVAAPQIVLTQSPAIMSASP GEKVTLTCSASSSVSSSYLYWYQQKPGSSPKLWIYSTSNLASGV PARFSGSGSGTSYSLTISSMEAEDAASYFCHQWSSYPRTFGAGT KLELK