To overcome this obstacle, we identified a CD47-targeting mAb and evaluated its enhanced macrophage-mediated phagocytosis capacity and tumor suppression efficacy. We screened a panel of 50 murine mAbs from mice immunized with human CD47 and identified an efficient CD47/SIRPα-blocking mAb, m4C1 (Fig. S1a). m4C1 was subsequently humanized based on sequence homology and computational prediction (Fig. S1c). Humanized 4C1 (h4C1) blocked the CD47/SIRPα interaction as efficiently as m4C1 (Fig. S1e). Surface plasmon resonance (SPR)-binding assays demonstrated that h4C1 bound to the human CD47-ECD antigen with a $K_D$ of 0.85 nM (Fig. S1d), which is comparable to that of m4C1 (Fig. S1b, Table S1). To investigate the macrophage-mediated phagocytosis activity of h4C1, a tumor cell phagocytosis assay was conducted with bone marrow-derived macrophages (BMDMs). We found that h4C1 exhibited significant phagocytosis in Raji cells, with an $EC_{50}$ of 7.30 ng/mL (Fig. 1a).

We further assessed the in vivo tumor suppression efficacy of the CD47-targeting antibody, h4C1, in a mouse model. We subcutaneously engrafted luciferase-labeled Raji cells into the backs of immune-compromised NCG mice. Seven days after the engraftment, the mice were administered h4C1, Hu5F9 (a validated CD47-blocking mAb under clinical investigation$^1$), or isotype control mAb daily for 2 weeks (Fig. S2a). The average luciferase intensity in the h4C1-treated group was significantly lower than that of the isotype mAb-treated group (Figs. 1b, c and S2b-d). Treatment with h4C1 also resulted in a substantially improved survival of the tumor-bearing mice (Fig. S2e). Together, these results suggest that h4C1 represents a potential therapeutic mAb for lymphoma.

The binding characteristics of h4C1 to CD47 were subsequently explored through the determination of the h4C1/CD47 complex structure (Fig. S3a, b, Table S2). The overall structure reveals that h4C1 binds to CD47 with a buried surface area of 688 Å$^2$. All three CDRs of heavy chain (VH) and LCDR2 provide contacts with CD47-ECD, while LCDR1 and LCDR3 are not engaged (Fig. S4). The findings indicate that h4C1 exhibits a VH-dominated interaction with CD47, while the binding of h4C1 is mainly located on three loops of CD47 (FG, BC, and CC)$^5$.

To analyze the mechanism of CD47 antagonism by h4C1, the structure of the h4C1-Fab/CD47-ECD complex was superimposed onto the CD47-ECD/SIRPα complex (PDB ID: 2JJS). Detailed comparison of reciprocal binding areas between the two complexes revealed multiple striking parallels. Specifically, the FG loop of CD47-ECD inserts into a groove on the surface of domain I of SIRPα and contributes more than half of the interfacial surface$^4$. Similarly, the FG loop of CD47-ECD dominates the interaction with VH of h4C1. The binding of h4C1 to CD47 displayed substantial stereospecific hindrance to the binding of h4C1 to SIRPα (Fig. 1d).

There are five potential N-linked glycosylation sites on CD47-ECD. In the complex structure of h4C1/CD47, glycosylation modifications were observed at all five of these sites in CD47 (Fig. S5a). Notably, the glycan chain on N32 substantially stretches toward the h4C1/CD47 interface and contacts h4C1 (Table S3 and Fig. S3c). Additionally, glycosylated N55 is also near the h4C1/CD47 interface. The binding profiles of h4C1 to N32A-mutated or N55A-mutated CD47 proteins were further analyzed with SPR to investigate the glycosylation dependency of the h4C1/CD47 interaction. SDS–PAGE analysis revealed that N32A-mutated or N55A-mutated CD47 proteins with a reduction in molecular weight indicates the existence of the N-glycosylation modification at these sites (Fig. S3d). The SPR assays demonstrated that no substantial differences were observed in the binding affinity of WT CD47-ECD (Fig. S1d) and N32A-mutated or N55A-mutated CD47 proteins with a reduction in molecular weight indicates the existence of the N-glycosylation modification at these sites (Fig. S3d). The SPR assays demonstrated that no substantial differences were observed in the binding affinity of WT CD47-ECD (Fig. S1d) and N32A-mutated or N55A-mutated CD47 proteins with a reduction in molecular weight indicates the existence of the N-glycosylation modification at these sites (Fig. S3d). The SPR assays demonstrated that no substantial differences were observed in the binding affinity of WT CD47-ECD (Fig. S1d) and N32A-mutated or N55A-mutated CD47 proteins with a reduction in molecular weight indicates the existence of the N-glycosylation modification at these sites (Fig. S3d). The SPR assays demonstrated that no substantial differences were observed in the binding affinity of WT CD47-ECD (Fig. S1d) and N32A-mutated or N55A-mutated CD47 proteins with a reduction in molecular weight indicates the existence of the N-glycosylation modification at these sites (Fig. S3d). The SPR assays demonstrated that no substantial differences were observed in the binding affinity of WT CD47-ECD (Fig. S1d) and N32A-mutated or N55A-mutated CD47 proteins with a reduction in molecular weight indicates the existence of the N-glycosylation modification at these sites (Fig. S3d). The SPR assays demonstrated that no substantial differences were observed in the binding affinity of WT CD47-ECD (Fig. S1d) and N32A-mutated or N55A-mutated CD47 proteins with a reduction in molecular weight indicates the existence of the N-glycosylation modification at these sites (Fig. S3d).
structure of CD47 and a murine mAb that showed potential tumor suppression efficacy. B6H12.2, also exhibited FG loop-dominated binding to the mAb. The complex structure of C47B222/CD47 shows that the FG loop of CD47 also plays a critical role in binding to C47B222 (Fig. 1e). Therefore, the FG loop of CD47 dominates the interactions with these mAbs and could serve as a blocking "hotspot" for the design of next-generation anti-CD47 drugs in the future.

To reduce the hemagglutination of CD47-targeting mAb, we constructed a bispecific antibody (BsAb) in a dual-variable-domain construct.
immunoglobulin (DVD-lg) format, and this antibody was expected to reduce the binding affinity to CD47. We introduced the variable domains from a validated PD-L1-targeting mAb #18 (CN Patent: 201810952740.3) onto h4C1 to enhance both innate and adaptive antitumor immune responses (Fig. 1f). A cell-based blocking assay showed that the BsAb maintained the blocking efficacy for both the CD47/SIRPa and PD-1/PD-L1 interactions (Fig. 1g). SPR was used to characterize the binding of the BsAb to PD-L1 or CD47, and a substantially reduced binding affinity was observed between BsAb and CD47; however, the binding affinity remained comparable to the binding of PD-L1 (Fig. 1h). Furthermore, a hemagglutination assay revealed that compared to h4C1 mAb, the CD47/PD-L1 BsAb exhibited substantially reduced hemagglutination in a wide range of concentrations in vitro (Fig. 1i). Therefore, the design of a CD47/PD-L1 dual-targeting BsAb with limited hemagglutination provides insight into next-generation anti-CD47 drugs to improve tumor ICT.

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AUTHOR CONTRIBUTIONS
J.Y., J.Z. and G.F.G. initiated and coordinated the project. R.S., S.T., J.Y. and G.F.G. designed the experiments. R.S. and Y.C. conducted the experiments with the assistance of X.D. and X.B. R.S. and Y.C. obtained diffractable complex crystals of CD47 and h4C1 and solved the crystal structures. R.S., Q.W., Q.H., J.Y. and G.F.G. analyzed the data. R.S. wrote the manuscript. S.T., Y.C., J.Z., J.Y. and G.F.G. revised the manuscript.

ADDITIONAL INFORMATION
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