Treatment of anti-SIRPα in combination with anti-TAA* exerts superior anti-tumor activity

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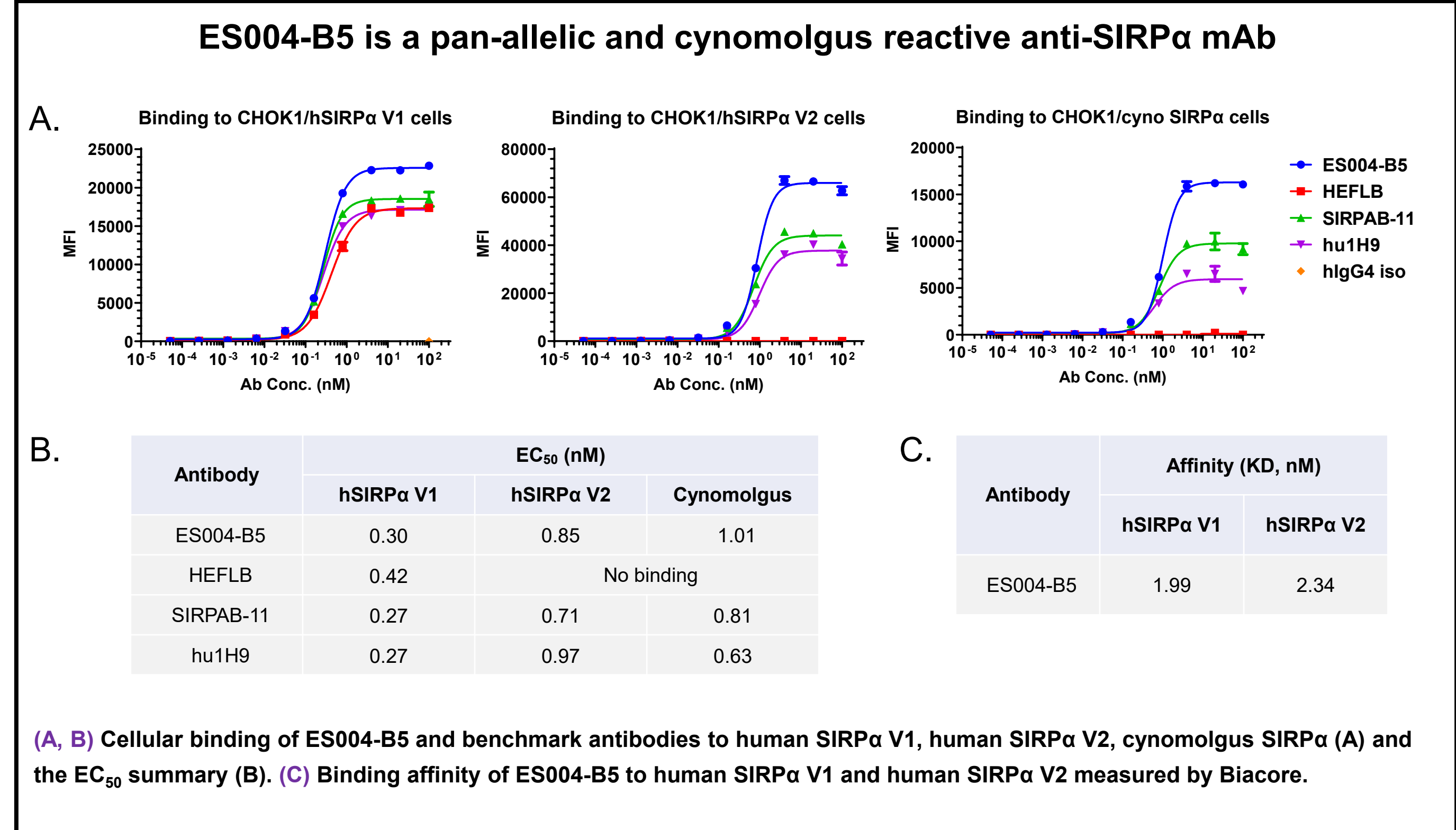
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*TAA: tumor associated antigen

BACKGROUND

Signal-regulatory protein alpha (SIRPα), is an inhibitory receptor expressed on myeloid cells and dendritic cells. Ligation of CD47 to SIRPα delivers a "don't eat me" signal to suppress phagocytosis. Tumor cells frequently overexpress CD47 to evade macrophage-mediated destruction. Currently, agents targeting CD47 have proceeded to clinical trials and demonstrated promising anti-tumor results. However, these agents have been associated with hemolytic anemia and thrombocytopenia. In addition, universal expression of CD47 causes antigen sink, which leads to reduced efficacy. We therefore consider targeting CD47 receptor SIRPα in order to achieve improved efficacy with better safety profile. We have developed a pan-allele anti-SIRPα competitive functional antibody ES004-B5 that potently potentiates antibody dependent cellular phagocytosis (ADCP) activity of antibodies against tumor associated antigens (TAAs) *in vitro* and *in vivo*.
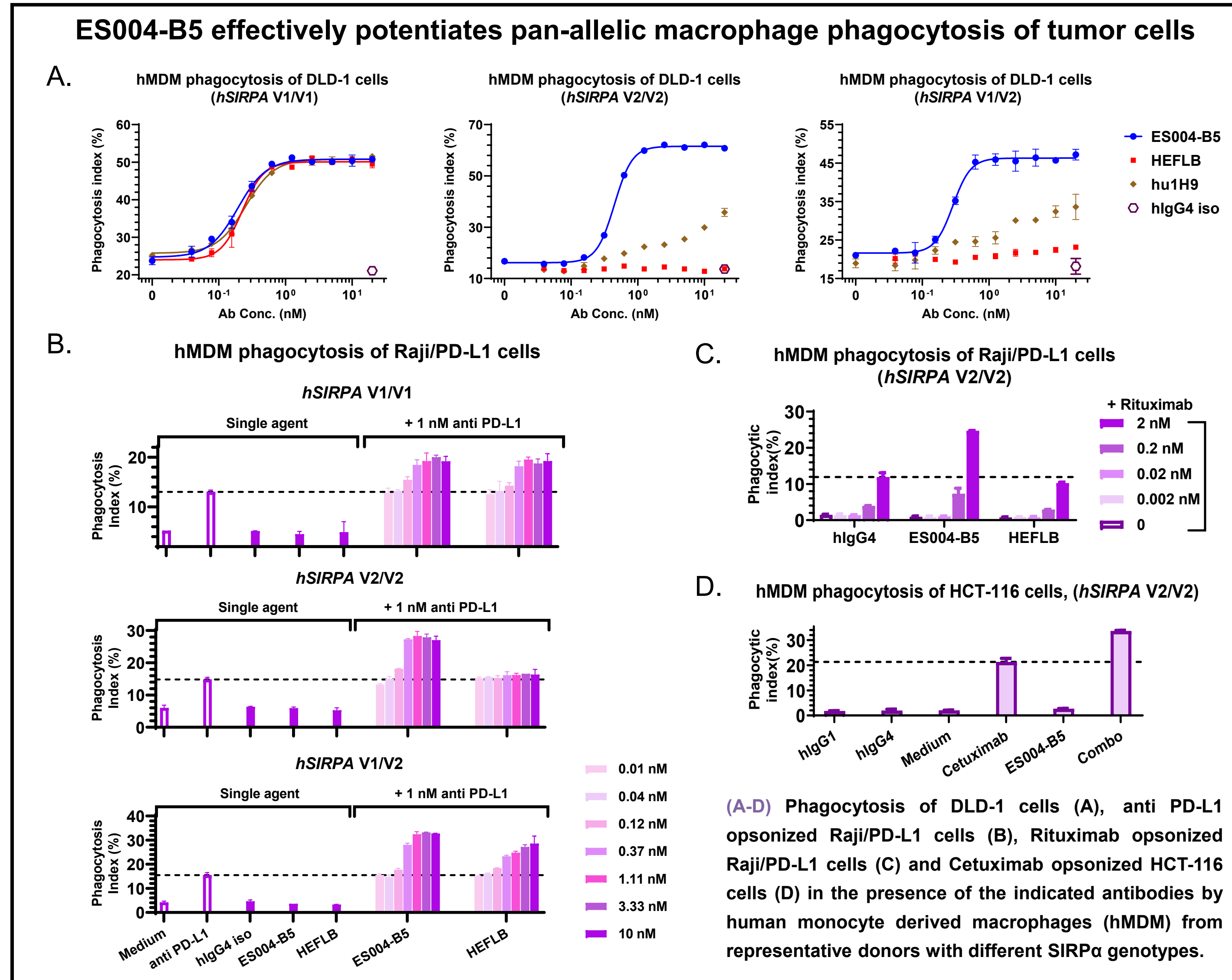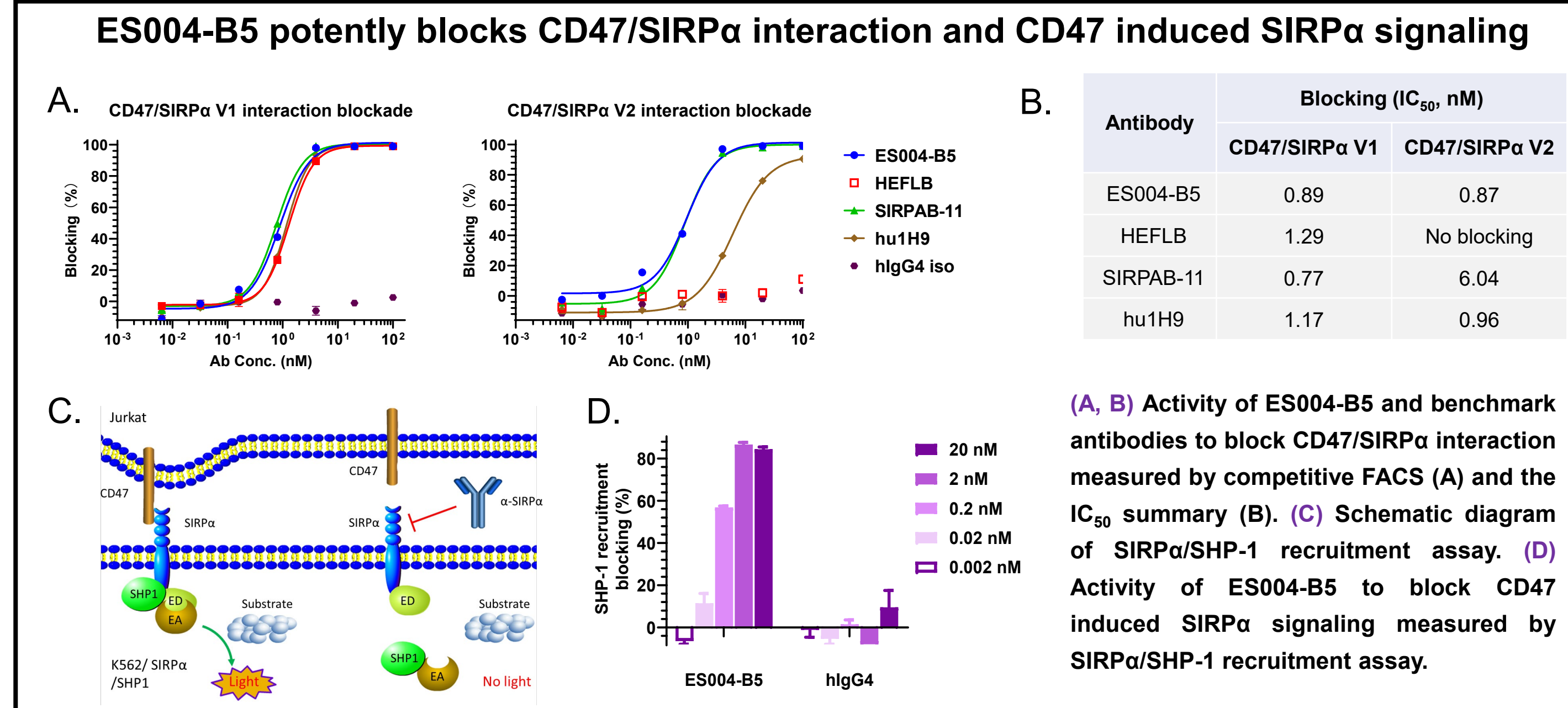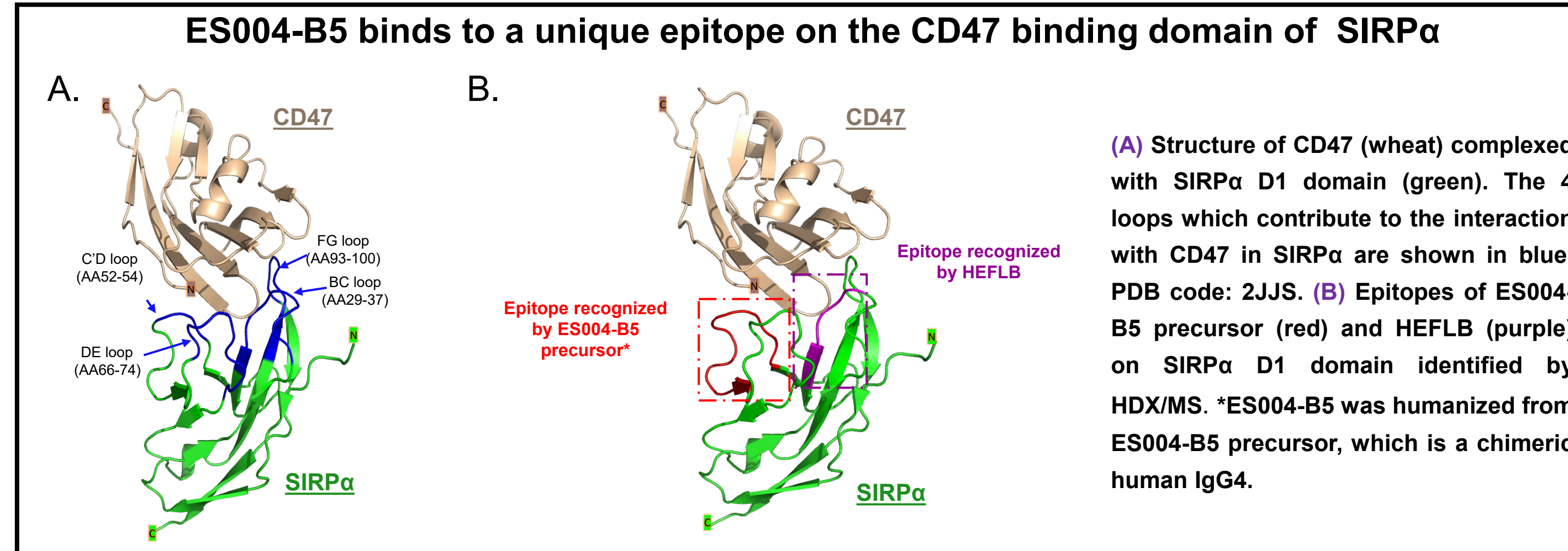
METHODS

Pan-allele SIRPα reactivities, SIRP family homologue binding properties, and cross-species reactivity were evaluated by ELISA and FACS. Antigen binding affinity was determined by surface plasmon resonance system (Biacore). Blocking activity was determined by competition assay and SHP-1 recruitment assay. *In vitro* functional activity was determined by phagocytosis assay. The impact on T cell function was evaluated by mixed lymphocyte reaction (MLR). *In vivo* efficacy was evaluated in a syngeneic mouse tumor model with human CD47/human SIRPα double knock-in mice. Epitope analysis was performed by competitive ELISA and hydrogen deuterium exchange mass spectrometry (HDX-MS). Lead clone was humanized via CDR grafting and back mutation screening. Non-human primates (NHPs) models were used to assess the safety and pharmacokinetics of the humanized candidate.
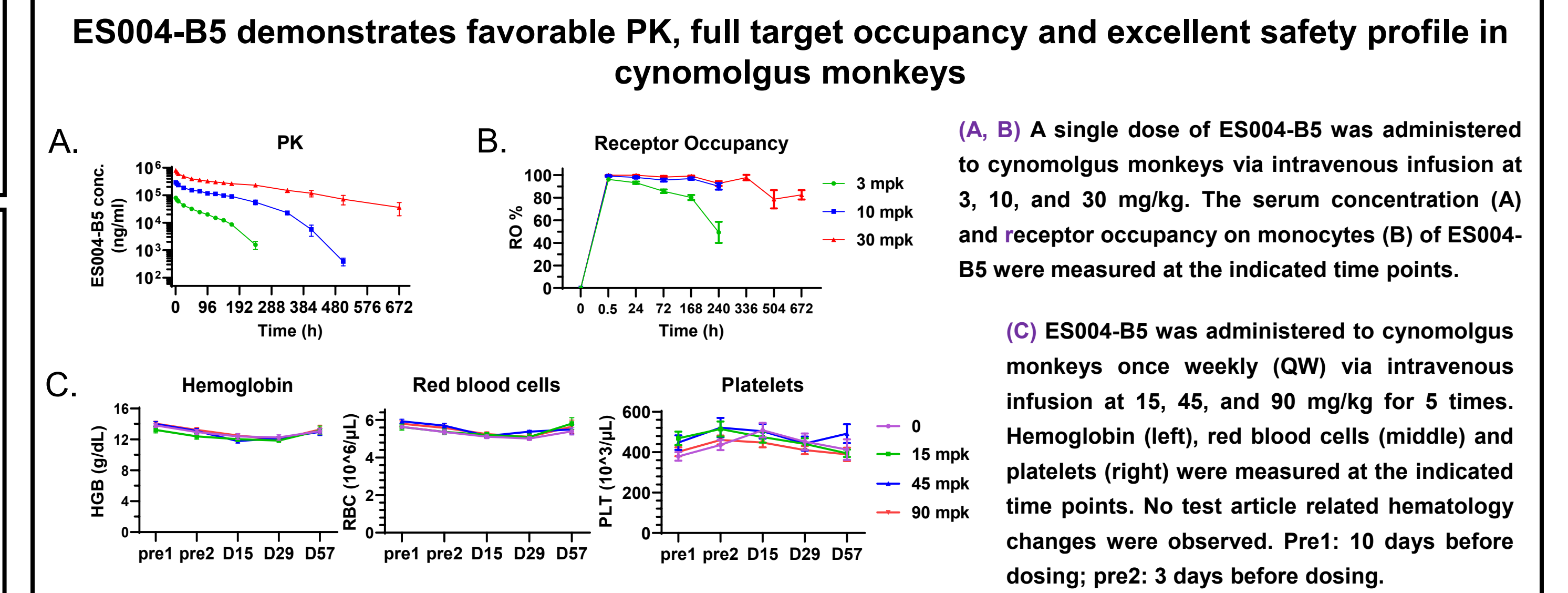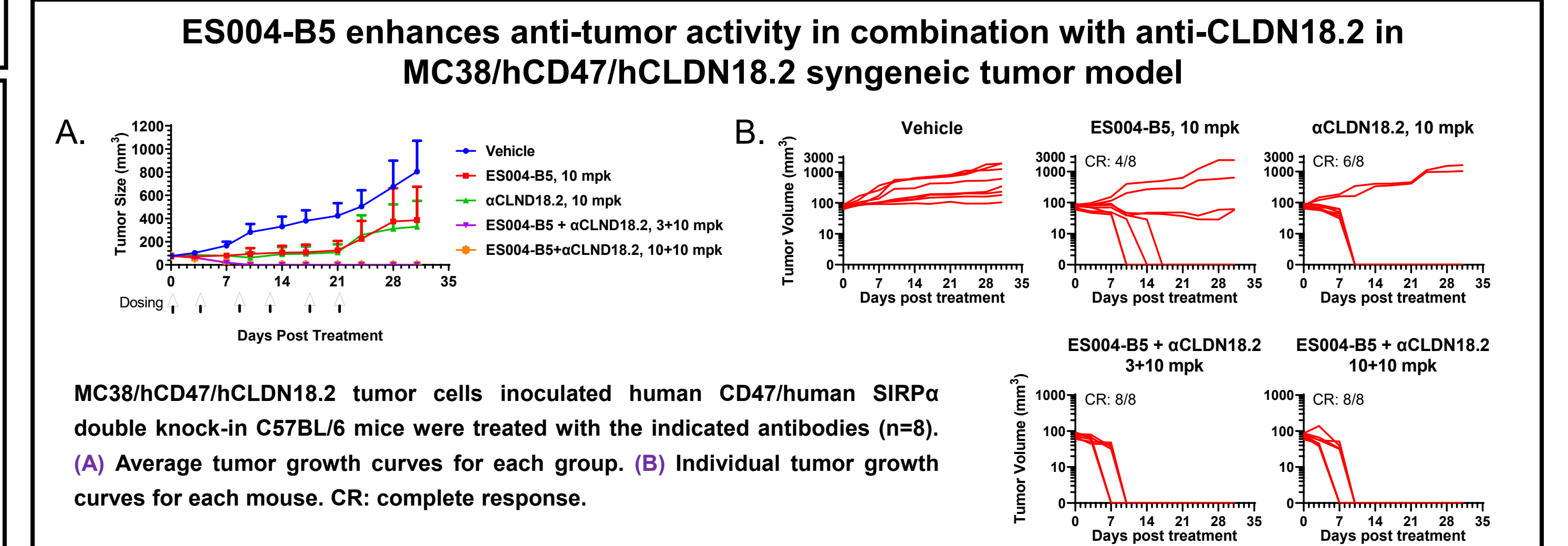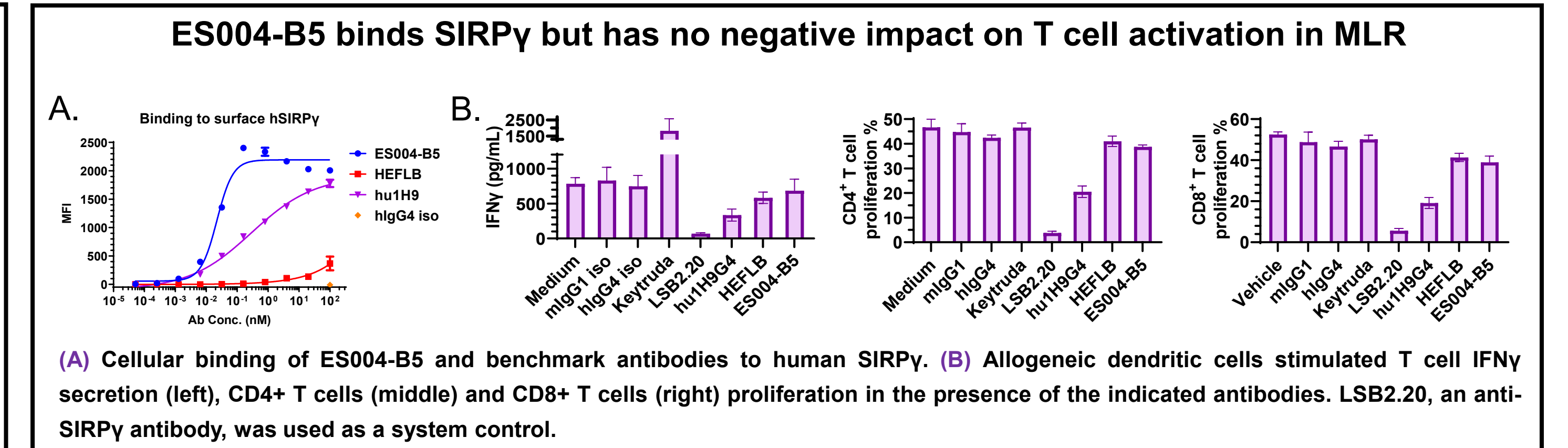
RESULTS

ES004-B5 is a pan-allelic and cynomolgus reactive anti-SIRPα mAb

A.
Binding to CHOK1/hSIRPα V1 cells
Binding to CHOK1/hSIRPα V2 cells
Binding to CHOK1/cyno SIRPα cells

- ES004-B5
- HEFLB
- SIRPAB-11
- hu1H9
- hIgG4 iso

B.

Antibody	EC50 (nM)		
	hSIRPα V1	hSIRPα V2	Cynomolgus
ES004-B5	0.30	0.85	1.01
HEFLB	0.42	No binding	
SIRPAB-11	0.71	0.71	0.81
hu1H9	0.27	0.97	0.63

C.

Antibody	Affinity (KD, nM)	
	hSIRPα V1	hSIRPα V2
ES004-B5	1.99	2.34

(A, B) Cellular binding of ES004-B5 and benchmark antibodies to human SIRPα V1, human SIRPα V2, cynomolgus SIRPα (A) and the EC50 summary (B). (C) Binding affinity of ES004-B5 to human SIRPα V1 and human SIRPα V2 measured by Biacore.

RESULTS

ES004-B5 binds to a unique epitope on the CD47 binding domain of SIRPα

A.
CD47
C'D loop (AA52-54)
FG loop (AA95-100)
BC loop (AA29-37)
DE loop (AA66-74)
SIRPα
Epitope recognized by ES004-B5 precursor*

B.
CD47
Epitope recognized by HEFLB
SIRPα

(A) Structure of CD47 (wheat) complexed with SIRPα D1 domain (green). The 4 loops which contribute to the interaction with CD47 in SIRPα are shown in blue. PDB code: 2JJS. (B) Epitopes of ES004-B5 precursor (red) and HEFLB (purple) on SIRPα D1 domain identified by HDX/MS. *ES004-B5 was humanized from ES004-B5 precursor, which is a chimeric human IgG4.

ES004-B5 potently blocks CD47/SIRPα interaction and CD47 induced SIRPα signaling

A.
CD47/SIRPα V1 interaction blockade
CD47/SIRPα V2 interaction blockade

- ES004-B5
- HEFLB
- SIRPAB-11
- hu1H9
- hIgG4 iso

B.

Antibody	Blocking (IC50, nM)	
	CD47/SIRPα V1	CD47/SIRPα V2
ES004-B5	0.89	0.87
HEFLB	1.29	No blocking
SIRPAB-11	0.77	6.04
hu1H9	1.17	0.96

C.
Jurkat
CD47 / SIRPα / Substrate / K562/ SIRPα /SHP1 / α-SIRPα / No light

D.
SHP-1 recruitment blocking

- 20 nM
- 2 nM
- 0.2 nM
- 0.02 nM
- 0.002 nM

ES004-B5   hIgG4

(A, B) Activity of ES004-B5 and benchmark antibodies to block CD47/SIRPα interaction measured by competitive FACS (A) and the IC50 summary (B). (C) Schematic diagram of SIRPα/SHP-1 recruitment assay. (D) Activity of ES004-B5 to block CD47 induced SIRPα signaling measured by SIRPα/SHP-1 recruitment assay.

ES004-B5 effectively potentiates pan-allelic macrophage phagocytosis of tumor cells

A.
hMDM phagocytosis of DLD-1 cells (hSIRPA V1/V1)
hMDM phagocytosis of DLD-1 cells (hSIRPA V2/V2)
hMDM phagocytosis of DLD-1 cells (hSIRPA V1/V2)

- ES004-B5
- HEFLB
- hu1H9
- hIgG4 iso

B.
hMDM phagocytosis of Raji/PD-L1 cells

hSIRPA V1/V1
Single agent / + 1 nM anti PD-L1

hSIRPA V2/V2
Single agent / + 1 nM anti PD-L1

hSIRPA V1/V2
Single agent / + 1 nM anti PD-L1

- 0.01 nM
- 0.04 nM
- 0.12 nM
- 0.37 nM
- 1.11 nM
- 3.33 nM
- 10 nM

C.
hMDM phagocytosis of Raji/PD-L1 cells (hSIRPA V2/V2)
+ Rituximab

- 2 nM
- 0.2 nM
- 0.02 nM
- 0.002 nM
- 0

hIgG4   ES004-B5   HEFLB

D.
hMDM phagocytosis of HCT-116 cells, (hSIRPA V2/V2)

hIgG1   hIgG4   Medium   Cetuximab   ES004-B5   Combo

(A-D) Phagocytosis of DLD-1 cells (A), anti PD-L1 opsonized Raji/PD-L1 cells (B), Rituximab opsonized Raji/PD-L1 cells (C) and Cetuximab opsonized HCT-116 cells (D) in the presence of the indicated antibodies by human monocyte derived macrophages (hMDM) from representative donors with different SIRPα genotypes.

RESULTS

ES004-B5 binds SIRPγ but has no negative impact on T cell activation in MLR

A.
Binding to surface hSIRPγ

- ES004-B5
- HEFLB
- hu1H9
- hIgG4 iso

B.
IFNγ secretion (left), CD4+ T cells (middle), CD8+ T cells (right)

(A) Cellular binding of ES004-B5 and benchmark antibodies to human SIRPγ. (B) Allogeneic dendritic cells stimulated T cell IFNγ secretion (left), CD4+ T cells (middle) and CD8+ T cells (right) proliferation in the presence of the indicated antibodies. LSB2.20, an anti-SIRPγ antibody, was used as a system control.

ES004-B5 enhances anti-tumor activity in combination with anti-CLDN18.2 in MC38/hCD47/hCLDN18.2 syngeneic tumor model

A.
Tumor Size (mm³) vs Days Post Treatment

- Vehicle
- ES004-B5, 10 mpk
- αCLDN18.2, 10 mpk
- ES004-B5 + αCLDN18.2, 3+10 mpk
- ES004-B5 + αCLDN18.2, 10+10 mpk

B.
Individual tumor growth curves:
Vehicle / ES004-B5, 10 mpk (CR: 4/8) / αCLDN18.2, 10 mpk (CR: 6/8) / ES004-B5 + αCLDN18.2 3+10 (CR: 8/8) / ES004-B5 + αCLDN18.2 10+10 (CR: 8/8)

MC38/hCD47/hCLDN18.2 tumor cells inoculated human CD47/human SIRPα double knock-in C57BL/6 mice were treated with the indicated antibodies (n=8). (A) Average tumor growth curves for each group. (B) Individual tumor growth curves for each mouse. CR: complete response.

ES004-B5 demonstrates favorable PK, full target occupancy and excellent safety profile in cynomolgus monkeys

A.
PK
ES004-B5 conc. (ng/ml) vs Time (h)

B.
Receptor Occupancy
RO % vs Time (h)

- 3 mpk
- 10 mpk
- 30 mpk

C.
Hemoglobin / Red blood cells / Platelets
HGB (g/dL) / RBC (10^6/μL) / PLT (10^3/μL) vs pre1 pre2 D15 D29 D57

- 0
- 15 mpk
- 45 mpk
- 90 mpk

(A, B) A single dose of ES004-B5 was administered to cynomolgus monkeys via intravenous infusion at 3, 10, and 30 mg/kg. The serum concentration (A) and receptor occupancy on monocytes (B) of ES004-B5 were measured at the indicated time points.

(C) ES004-B5 was administered to cynomolgus monkeys once weekly (QW) via intravenous infusion at 15, 45, and 90 mg/kg for 5 times. Hemoglobin (left), red blood cells (middle) and platelets (right) were measured at the indicated time points. No test article related hematology changes were observed. Pre1: 10 days before dosing; pre2: 3 days before dosing.

SUMMARY

ES004-B5 recognizes pan-allele human SIRPα with high affinity, and cross reacts well with cynomolgus SIRPα. It binds a unique epitope on SIRPα that is distinct from known competitor molecules. Although ES004-B5 binds SIRPγ expressed on T cell surface, it doesn't cause negative impact on T cell activation. ES004-B5 potently blocks CD47/SIRPα interaction as well as CD47 mediated SHP-1 recruitment to phosphorylated SIRPα intracellular tail. Through blocking CD47-induced inhibitory "don't eat me" signals, ES004-B5 effectively potentiates ADCP activity of anti-TAA antibodies such as Rituximab and Cetuximab. In a syngeneic mouse tumor model, the combination of ES004-B5 plus anti-Claudin18.2 significantly inhibited tumor growth *in vivo*. ES004-B5 has favorable pharmacokinetics and safety profile in NHPs.

⊖ Don't eat me!
Tumor cell / CD47 / TAA / Don't eat me / SIRPα / Macrophage / FcγR

⊕ Eat me!
Tumor cell / CD47 / α-SIRPα / TAA / SIRPα / Macrophage / ADCP / FcγR / α-TAA