Supplementary Methods

Establishment of stable cell lines

The PD-1 gene coding sequence was ligated into the pLVX-Puro vector to construct the PD-1-over-expression plasmid. The primers used for human PD-1 were as follows: forward, 5′- CCAGCATGGTTCTTAGACTC-3′; reverse, 5′-TTATTGGAACTGGCCGGCTGG-3′. The primers used for mouse PD-1 were as follows: forward, 5′-GCCACCATGTGGGTCCGG-3′; reverse, 5′-TCAAAGAGGCCAAGAACAATGCC-3′. CHO-K1 cells were cultured and then transfected with pLVX-puro-hPD-1 or pLVX-puro-mPD-1 via Lipofectamine 3000 (Invitrogen), while the control cells were transfected with pLVX-Puro empty vector. The establishment of the stable cell lines was achieved by selection with puromycin, and transfected efficiencies were measured using flow cytometry (FACS Calibur, BD Biosciences).

Blocking assay

For CD24 blocking assay, CSBP was incubated with $CD24^+$ HepG2 cells or $CD24^+$ 4T1 cells (2×10^5 cells/well), human Siglec-10-Fc protein (ACROBiosystems, USA) was added in the mixture. For PD-L1 blocking experiments, CSBP was mixed with human or mouse PD-L1-Fc protein (Sino Biological, China). The mixture was incubated with CHO-K1-hPD-1 cells or CHO-K1-mPD-1 cells (2×10^5 cells/well), finally followed incubation with PE goat anti-human IgG (eBioscience, USA). The peptide CSBP was synthesized by Nanjing ChenPeptide Biotech Co. Ltd. (Nanjing,

China). Flow cytometry was used and MFI was calculated accordingly.

Microscale thermophoresis (MST)

The peptide was dissolved in PBS buffer containing DMSO at 5% (v/v). For MST measurements, the PBS buffer as the MST buffer containing Tween20 at 0.05% (v/v) and DMSO at 5% (v/v) was used for reactant preparation. Fluorescent labeling approach was used to label the target proteins according to the manufacturer's protocol, including human/mouse PD-L1-His protein (Sino Biological, China), human/mouse CD24-His protein (ACROBiosystems, USA). The peptide was diluted, and 5 μl of peptide was incubated with 5 μl of 100 nM labelled human/mouse CD24-His protein, 400 nM labelled human PD-L1-His protein or 200 nM labelled mouse PD-L1-His protein, the mixture was filled into capillaries through capillary action. The Monolith NT.115 MST instrument was performed for quantitative analysis of protein interactions.

In vitro phagocytosis assay

Bone marrow cells were isolated from mouse long bones and induced to differentiate into macrophages by 20 ng/mL GM-CSF (PeproTech, USA). Carboxyfluorescein succinimidyl esteractivated (CFSE)-labeled (Thermo Fisher Scientific) 4T1 or MC38 tumor cells were treated with CSBP or anti-CD47 antibody (miap301). Bone marrow-derived macrophages (BMDM) were incubated with treated or untreated 4T1 or MC38 tumor cells (ratio 1:4) for 2 h. Cells were harvested and stained with rat anti-

mouse F4/80 antibody (eBioscience) for 30 min. In addition, the splenocytes from 4T1 tumor-bearing mice were co-cultured with 4T1 tumor cells (ratio 1:1) for 2 h. Phagocytosis was assessed as the frequency of $CD11b^+$ $F4/80^+$ $FITC^+$ events out of all $CD11b^+$ $F4/80^+$ events, or the frequency of $CD11b^+$ $Ly6G^-$ $Ly6C^+$ $FITC^+$ events out of all $CD11b^+$ $Ly6G^-$ $Ly6C^+$ events via flow cytometry (FACS Calibur, BD Biosciences).

T-cell proliferation assay

BMDM were cocultured overnight with equal numbers of 4T1 cells. The next day, peripheral lymph nodes were harvested from mice and labeled with 0.5 μM CFSE. CFSE-labeled $CD8^+$ T cells were cultured with 100 U/mL murine IL-2 (PeproTech, USA) and stimulated by 0.5 μg/mL anti-CD3 (17A2, BioGems) plus 0.5 μg/mL anti-CD28 (37.51, BioGems) in the cultures for 3 days. The proliferation of $CD8^+$ T cells was tested.

Co-culture assay

Human peripheral blood mononuclear cells (PBMCs) from healthy honors were isolated by lymphocyte separation solution (TBD, LTS1077, China) according to the manufacture's instruction. PBMCs were labeled with 0.2 μM CFSE. CFSE-labeled PBMCs were cultured with 100 U/mL human IL-2 (PeproTech, USA) and stimulated by 1 μg/mL anti-CD3 (OKT3, BioGems) plus 1 μg/mL anti-CD28 (CD28.2, BioGems). Then, the PBMCs were co-cultured with CHO-K1-hPD-L1 or CHO-K1-Vec with or without CSBP. After cells were cultured for 3 days. The proliferation of

67 $CD8^+$ T cells and proportion of IFN-γ^+ $CD8^+$ T cells were tested.