Supplementary Figure 1. Pan-Cancer analysis identification of S1PR3 as a critical regulator of PD-1 inhibitor resistance and Poor prognostic biomarker.

(A) Venn diagram analysis across two groups of genes. Differential expressed genes (DEGs) in renal cell carcinoma (RCC), response versus no response (n = 408); DEGs in melanoma, response versus no response (n = 1371); Common DEGs in RCC and melanoma (n = 25). DEGs were defined with $|\log FC| \geq 1.0$ and adj. $P < 0.05$.

(B) Heatmap showing the expression intensity of 25 common DEGs, response versus no response to Nivolumab. Red represents high expression, and green represents low expression. DEGs were defined with $|\log FC| \geq 1.0$ and adj. $P < 0.05$.

(C) Principal component analysis (PCA) based on 25 common DEGs of RCC and melanoma showed separation between different treatment outcomes.

(D) KEGG pathway enrichment analysis of DEGs in melanoma and RCC. P-value < 0.05 was set as the threshold.

(E, F) Differential expression of S1PR3 between tumor and paired normal tissue samples in TCGA pan-cancer dataset(D) and GEPIA database(E). Association between S1PR3 expression and tumor stage in Adrenocortical carcinoma (ACC), Colon adenocarcinoma (COAD), Liver hepatocellular carcinoma (LIHC), Skin Cutaneous Melanoma (SKCM).

(G) Kaplan-Meier survival analysis comparing DFS and OS between patients with high and low S1PR3 expression subgroups of patients with ACC, COAD, Lung adenocarcinoma (LUAD) and Kidney renal papillary cell carcinoma (KIRP). Log-rank test was used for statistical analysis.

Supplementary Figure 2. Correlation analysis between other four S1PRs expression with immune related gene signature

(A) Correlation analysis between other four S1PRs expression with T cell exhaustion signature (e.g., HAVCR2, TIGIT, LAG3, PDCD1, CXCL13 and LAYN) in breast cancer(BRCA), colon adenocarcinoma (COAD), kidney renal clear cell carcinoma(KIRC) and lung adenocarcinoma (LUAD).

(B) Correlation analysis between other four S1PRs expression with negative immune receptor genes (FOXP3,IDO2,IL10 and TGFB1) in melanoma.

Supplementary Figure 3. Selective S1PR3 antagonist TY-52156 inhibit S1PR3 expression and S1P level in tumor cells and mouse T cells.

(A) Representative flow cytometry plot showing percentage of $S1PR3^{+}$cells in mouse lymph nodes derived T cells and mouse tumor cells when treated with or without selective S1PR3 antagonist TY-52156 for 24h (gated on live cells).

(B) Concentration of S1P in the culture supernatants were measured by ELISA from different tumor cells after treating with or without selective S1PR3 antagonist TY-52156 for 24h (n=3).

(C) Concentration of S1P in the culture supernatants collected from EpCAM CAR-T cells co-cultured with 4T1 cells at effector to target cell (E:T) ratios of 4:1 in the presence or absence of TY-52156(10uM). Culture supernatant was collected 24h after incubation.

Supplementary Figure 4. Promoted tumor regression by treatment with combination of S1PR3 antagonist and anti-PD1.

(A) Schematic of the treatment schedule for the TY-52156 combination with anti-PD-1. Balb/c mice were injected with CT26 cells (5×10^5 cells) in the right flank. C57BL/6J mice were subcutaneously injected with B16-F10 cells (5×10^5 cells) in the right flank.when the tumor volume come to 70-80mm^3 on day 7, mice were treated with PBS or S1PR3 antagonist TY-52156 at 10mg/kg orally every other day and injected with anti-PD-1 200μg intraperitoneally. ig. intragastric administration. i.p. intraperitoneal injection.

(B) Promoted tumor regression by treatment with combination of S1PR3 antagonist and anti-PD1. C57/BL mice were injected with 4T1 cells (1×10^6 cells) in the right flank. When the tumor volume come to 70-80mm^3 on day 7, mice were treated with PBS or S1PR3 antagonist TY-52156 at 10mg/kg orally every other day and injected with anti-PD-1 200μg intraperitoneally. Tumor volumes were monitored and recorded by caliper every two days. Compound dosing was discontinued on day 20. Tumor growth was plotted starting from the day before initial treatment (n= 8-10 mice pooled). Mixed-effects analysis.

Statistical significance was determined at the levels of *P<0.05; **P<0.01; ***P<0.005 and ****P<0.001.

Supplementary Figure 5. S1PR3 inhibitor promoted the killing activity of CAR-T cells in vitro

(A) Enzyme-linked immunosorbent assay (ELISA) analysis of IFN-γ, IL-2 and TNF-α production in supernatants collected from CAR-T or control T cells co-cultured with 4T1 cells in 96-well plates at increasing effector to target cell (E:T) ratios in the presence of various concentrations of TY-52156(5μM,10μM,15μM,20μM). Culture supernatant was collected 24h after incubation.

(B) EpCAM CAR-T cells were preincubated with or without TY-52156(10μM) for 24h, then EpCAM CAR-T cells were cocultured with 4T1 cells in the absence of TY-52156 at a 4:1 E:T ratio for 24h. IFN-γ, IL-2 and TNF-α cytokine secretion was detected by ELISA. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.

(C) Enzyme-linked immunosorbent assay (ELISA) analysis of IFN-γ, IL-2 and TNF-α production in supernatants collected from CAR-T or control T cells co-cultured with B16-F10 cells in 96-well plates at increasing effector to target cell (E:T) ratios in the presence of TY-52156(10μM). Culture supernatant was collected 24h after incubation.

Supplementary Figure 6. Anti-tumor efficacy of combination therapy of S1PR3 inhibitor CAY10444 and EpCAM CAR-T cells in immunocompetent murine tumor model.

(A) Enzyme-linked immunosorbent assay (ELISA) analysis of IFN-γ, IL-2 and TNF-α production in supernatants collected from CAR-T or control T cells co-cultured with 4T1 cells in 96-well plates at effector to target cell (E:T) ratio of 4:1 in the presence of CAY10444(10μM). Culture supernatant was collected 24h after incubation. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.

(B) Left, representative flow cytometry plot of $CD69^+$ T cells(gated on $CD3^+$ $CD8^+$ T cells). Right, quantification showing the individual values of $CD69^+$ T cells. EpCAM CAR-T cells were cocultured with 4T1 cells in the presence or absence of CAY10444(10μM) at a 4:1 E:T ratio for 48h. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.

(C) Up, representative flow cytometry plot of PD-1^+ T cells, LAG-3^+ T cells and TIM-3^+ T cells (gated on $CD3^+$ $CD8^+$ T cells). Down, quantification showing the individual values of PD-1^+ T cells, LAG-3^+ T cells and TIM-3^+ T cells. EpCAM CAR-T cells were cocultured with 4T1 cells in the presence or absence of CAY10444(10μM) at a 4:1 E:T ratio for 48h. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.

(D) Schematic of the treatment schedule for the CAY10444 and EpCAM CAR-T cells combination therapy. Balb/c mice were injected with 4T1 cells (5×10^5 cells) in situ into the fourth inguinal mammary fat pads and treated with CTX(2.5mg/kg) when the tumor volume come to ~80mm^3 on day 0. On day 1 and 3, 1×10^6 EpCAM CAR-T cells or control T cells were intravenously injected into tumor-bearing mice. On day 0,2,4,6 and 8, PBS or S1PR3 antagonist CAY10444 at 10mg/kg was administered orally for 4 times. CTX, cyclophosphamide. i.p. intraperitoneal injection. i.v. intravenously injection. ig. intragastric administration.

(E) 4T1 tumor volume was measured and recorded by caliper every two days. The left side is the tumor volume data of all groups, and the right side is the line graph of the tumor volume in the EpCAM CAR-T cells(green) and EpCAM CAR-T cells combination with TY-52156 group(red). Tumor growth was plotted starting from the day before initial dose of CTX (n= 7-10 mice pooled). Mixed-effects analysis.

(F) Left, Representative images showing tumors harvested from mice bearing 4T1 tumors given CAY10444,EpCAM CAR-T cells or the combination. Right,

inhibition rate of 4T1 tumors in mice given different treatment.

(G) Quantification showing the individual values of $CD3^+$T cells, $CD4^+$T cells, and $CD8^+$ T cells in the tumor of mice bearing 4T1 tumors. n = 5 mice per group. Unpaired Student's t test. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.

(H) Quantification showing the individual values of $CD3^+$T cells, $CD4^+$T cells, and $CD8^+$ T cells in blood of mice bearing 4T1 tumors. n = 5 mice per group. Unpaired Student's t test. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.

Supplementary Figure 7. S1PR3 antagonist inhibits tumor cells secret IL-6 and TGF-β

(A) MC38, CT26, 4T1, B16-F10 and LLC tumor cells were treated with TY-52156(10μM) in 24-well plates for 24h. Culture supernatant was collected for Enzyme-linked immunosorbent assay (ELISA) analysis of TGF-β, IL-6 and IL-1β production. Unpaired Student's t test. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.

Supplementary Figure 8. S1PR3 inhibition upregulates T-cell activation, cytotoxicity, and proliferation related gene expressions in EpCAM CAR-T cells

(A) EpCAM CAR-T cells were stimulated with S1PR3 antagonist(10μM) and prolonged 4T1 tumor cells antigen stimulation at three time points of 24h、48h、72h. CAR-T cells were sorted for RNA-sequencing (RNA-seq) analysis on 72h.

(B) RNA-seq analysis of EpCAM CAR-T cells and EpCAM CAR-T cells+TY-52156 at 72h after stimulation with 4T1 cells, and the differentially expressed genes (DEGs) were shown in volcano plot (|logFC|≥1, Q≤0.05). FC, foldchange.

(C) Significant enriched KEGG pathway overview terms of differentially expressed genes (DEGs) between EpCAM CAR-T cells +TY-52156 group and EpCAM CAR-T cells group at 72h post-stimulation with 4T1 cells(|logFC|≥1, Q≤0.05). FC, foldchange.

(D) Significant enriched KEGG signal transduction pathway terms of differentially expressed genes (DEGs) between EpCAM CAR-T cells +TY-52156 group and EpCAM CAR-T cells group at 72h post-stimulation with 4T1 cells(|logFC|≥1, Q≤0.05). FC, foldchange.

(E) Significant enriched KEGG immune system pathway terms of differentially expressed genes (DEGs) between EpCAM CAR-T cells +TY-52156 group and EpCAM CAR-T cells group at 72h post-stimulation with 4T1 cells(|logFC|≥1, Q≤0.05). FC, foldchange.

(F-I) Gene-set enrichment analysis (GSEA) of IFN-γ(F), IL-2(G), T cell proliferation(H) and T cell receptor signaling(I) pathways was performed on all gene sets. NES, normalized enrichment score;FDR, false discovery rate.

Supplementary Figure 9. No severe toxicity caused by CAR-T cells and TY-52156 treatment in vivo

(A,B) The H&E staining results of heart, liver, spleen,lung ,kidney and intestine of MC38 bearing mice(A) and 4T1 bearing mice (B) from each group. Scale bars, 100μm.

Supplementary Figure 10. S1PR3 inhibition promotes systemic changes of T cells in TME.

(A) Left, representative flow cytometry plot identifying $CD3^+CD8^+$Granzyme B^+ T cells in blood of mice bearing MC38 tumors on day 20 after treatment (gated on live cells). Right, quantification showing the individual values of $CD3^+$T cells, $CD8^+$T cells, and $CD8^+$Granzyme B^+T cells in blood of mice bearing MC38 tumors and individual values of $CD3^+$T cells, $CD8^+$T cells in blood of mice bearing 4T1 tumors. n = 3 mice per group. Unpaired Student's t test. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.

(B) Left, representative flow cytometry plot identifying $CD3^+CD8^+$Granzyme B^+ T cells in spleen of mice bearing MC38 tumors on day 20 after treatment (gated on live cells). Right, quantification showing the individual values of $CD3^+$T cells, $CD8^+$T cells, and $CD8^+$Granzyme B^+T cells in spleen of mice bearing MC38 tumors and individual values of $CD3^+$T cells, $CD8^+$T cells in spleen of mice bearing 4T1 tumors. n=3 mice per group. Unpaired Student's t test. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.

(C) Left, representative flow cytometry plot identifying $CD3^+CD8^+$ T cells in tumor-draining lymph nodes(TDLNs) of mice bearing 4T1 tumors on day 20 after treatment (gated on live cells). Right, quantification showing the individual values of $CD3^+$T cells, $CD8^+$T cells in TDLNs of mice bearing 4T1 tumors. n=5 mice per group. Unpaired Student's t test. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.

Supplementary Figure 11. S1PR3 inhibition promotes systemic changes of macrophages in TME.

(A) Left, representative flow cytometry plot. Right, quantification showing the individual values of $CD11b^+F4/80^+$ macrophages in bone marrow of mice bearing MC38 and 4T1 tumors on day 20 after treatment (gated on $CD45^+$ cells). n = 3 mice per group. Unpaired Student's t test. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.

Left, representative flow cytometry plot. Right, quantification showing the individual values of $CD11b^+F4/80^+$ macrophages in spleen of mice bearing MC38 and 4T1 tumors on day 20 after treatment (gated on $CD45^+$ cells). n = 3 mice per group. Unpaired Student's t test. ns, P>0.05, *P<0.05; **P<0.01; ***P<0.005.