Supporting Information for

Original article

A phosphoglycerate mutase 1 allosteric inhibitor restrains TAM-mediated colon cancer progression

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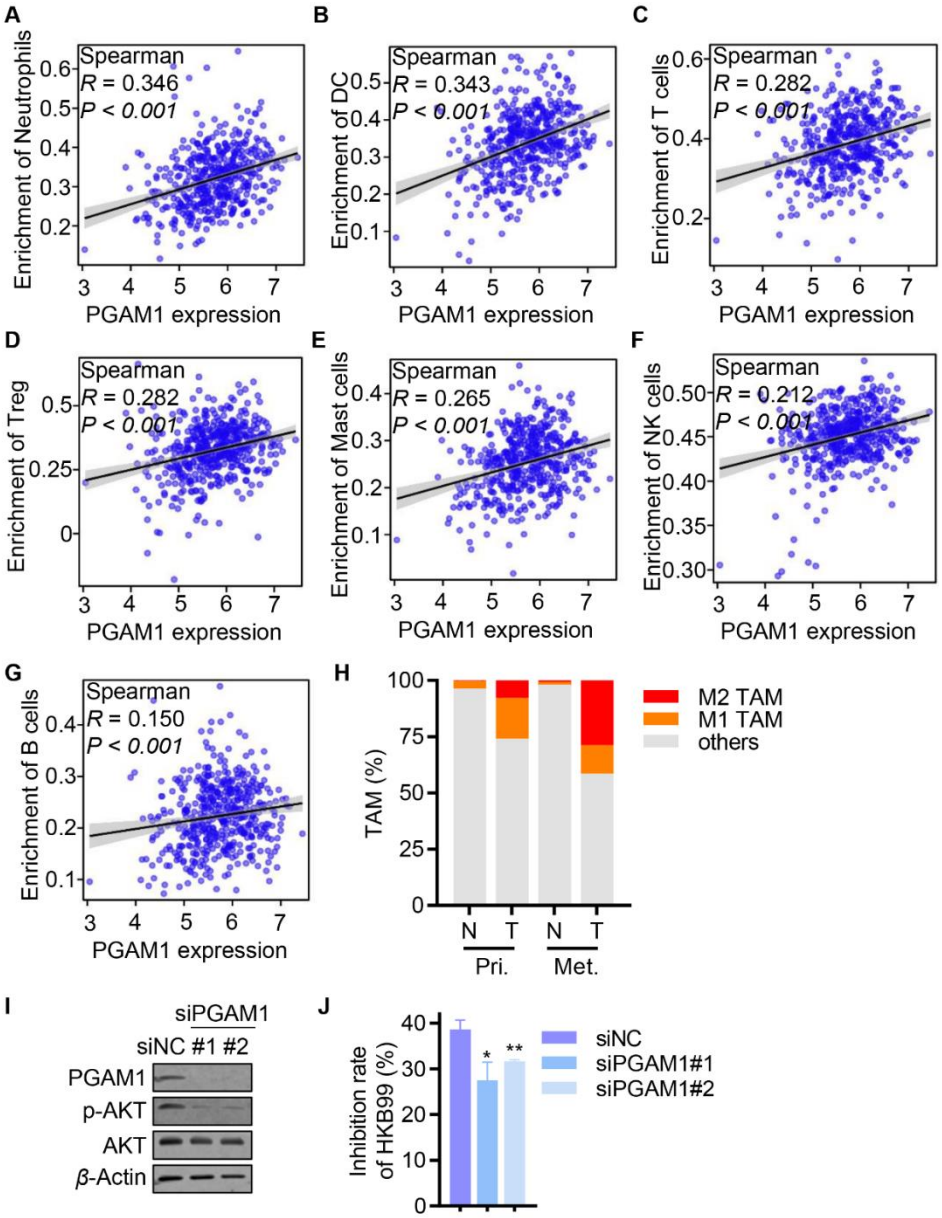

Figure S1. M2-TAMs were enriched in liver metastasis of colon cancer. (A–G) Correlation analysis between *PGAM1* and enrichment of immune cells in TCGA colon adenocarcinoma database (n = 480). (H) Proportion of macrophages in colon cancer and liver metastases from the single-cell RNA-seq dataset GSE164522 (n = 17). (I) The proteins as indicated were immunoblotted with β-actin as a loading control after MC38 cells were transfected with 25 nmol/L siPGAM1#1 and siPGAM1#2 for 72 h. (J) MC38 cells were treated with 1 μmol/L HKB99 for 48 h after 24 h transfection of 25 nmol/L siPGAM#1 and siPGAM1#2. Cell viability was then detected by CCK8 assays. Data are shown as mean ± SD. *P < 0.05; **P < 0.01.

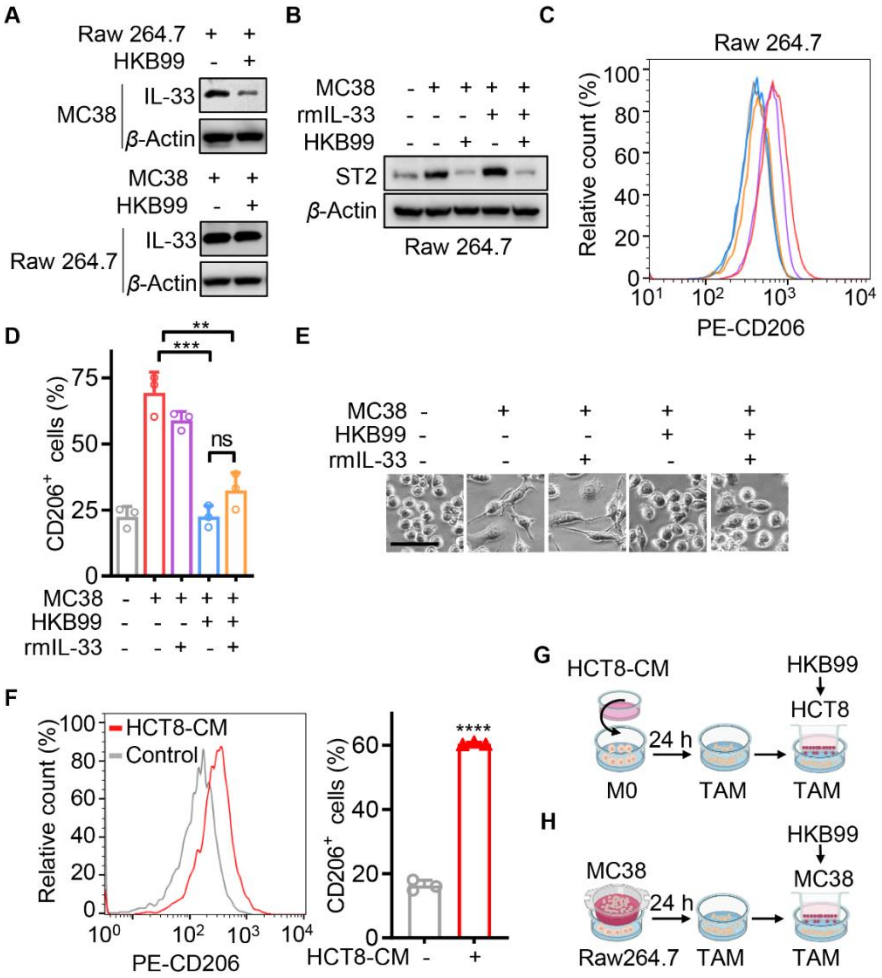

Figure S2. HKB99 suppresses M2-like polarization of TAMs in colon cancer cells. (A) Co-culture of MC38 cells and Raw 264.7 macrophages were established and treated with 1.0 μmol/L HKB99 for 18 h. The IL-33 protein level was immunoblotted with *β*-actin as a loading control in MC38 cells and Raw 264.7 macrophages respectively. (B) Raw 264.7 macrophages were pretreated with or without 30 ng/mL recombinant murine IL-33 (rmIL-33) for 12 h, and then co-cultured with MC38 cells in the presence of 1.0 μmol/L HKB99 for another 18 h. IL-33 and ST2 protein levels were immunoblotted with *β*-actin as a loading control in Raw 264.7 macrophages. (C) Raw 264.7 macrophages were pretreated with or without 100 ng/mL rmIL-33 for 12 h, and then co-cultured with MC38 cells in the presence of 1.0 μmol/L HKB99 for another 24 h. CD206 positive fraction of Raw 264.7 macrophages was evaluated by flow cytometry. (D) The statistical results of (C). (E) Cell morphology of Raw 264.7 macrophages was detected under the same condition as (C). (F) CD206 positive fraction of macrophages was evaluated by flow cytometry. (G) Human THP-1 monocytes were cultured with 200 nmol/L PMA for 24 h and exposed to conditioned media (CM) produced by HTC8

cells for another 24 h, finally co-cultured with HCT8 cells in the presence of 0.5 μmol/L HKB99 for 48 h. Trans-well assays were performed to evaluate the HCT8 cell migration. (H) Mouse Raw 264.7 macrophages were co-cultured with MC38 cells for 24 h to differentiate into M2-TAMs. Then M2-TAMs were co-cultured with MC38 cells in the presence of HKB99 at increasing concentrations of 0.25 μmol/L, 0.5 μmol/L, and 1 μmol/L for another 24 h. Trans-well assays were performed to detect the MC38 cell migration. Data are shown as mean ± SD. ns, non-significant. $^{**}P < 0.01$. $^{***}P < 0.001$. ****, $P < 0.0001$.

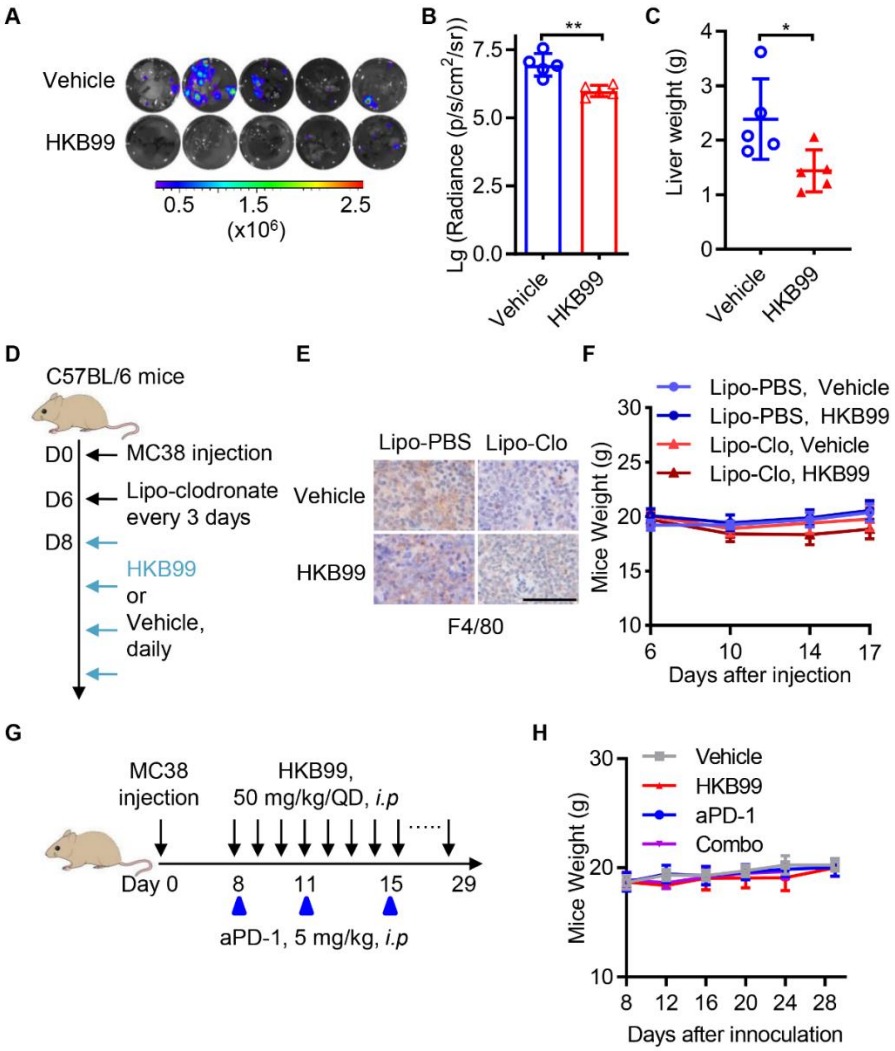

Figure S3. HKB99 suppresses tumor growth and metastasis *in vivo*. (A) The bioluminescence signal value of the dissected liver was measured via the IVIS spectrum in a murine intrasplenic liver metastasis model on Day 19 after CT26-luc cell injection. HKB99 was injected at a dose of 50 mpk daily starting on Day 8 after the CT26-luc injection. (B) Statistical results of (A) (n = 5). (C) Weight of dissected liver in (A). (D) Scheme of a macrophage depletion model with MC38 subcutaneous tumor. Lipo-Clo was administered at 50 mpk every 3 days starting 2 days before HKB99 treatment. HKB99 was injected at a dose of 50 mpk daily. Lipo-PBS and Vehicle were used as Lipo-Clo and HKB99's control respectively (n = 6 for each group). (E) The IHC staining of the spleen section with F4/80 macrophage marker. Scale bars, 50 μm. (F) The body weights of mice were measured during the treatment period as indicated in (D). (G) Scheme of MC38 subcutaneous tumor for HKB99 and anti-PD-1 combination model (n = 7 for each group). (H) The body weight of mice was measured during the administration as indicated in (G). Data, mean ± SD. *P < 0.05; **P < 0.01.

Table S1. Primers used for qRT-PCR.

Gene	Forward primer	Reverse primer
CD163	TTTGTCAACTTGAGTCCCTTCAC	TCCCGCTACACTTGTTTTCAC
CD206	GGGTTGCTATCACTCTCTATGC	TTTCTTGTCTGTTGCCGTAGTT
TGF-β	AAGGACCTCGGCTGGAAGTGC	CCGGGTTATGCTGGTTGTA
SPP1	ATCTCCTAGCCCCACAGACCCTT	TCGGTTGCTGGCAGGTCCGT
GAPDH	GTCCACTGGCGTCTTCACCACC	AGGCATTGCTGATGATCTTGAGGC