Supplemental Figure S1. L-5-HTP does not affect the constitutive PD-L1 expression in cancer cells. (A) BXPC3 were treated with L-5-HTP or D-5-HTP for 48h. Surface PD-L1 expression on BXPC3 was detected by flow cytometry. The constitutive PD-L1 expression of (B) BXPC3, (C) A549 and (D) SKOV3 cancer cells was measured by flow cytometry on 48h and 72h. (n=3 independent experiments). Data are shown as mean ± SD. ns P>0.05, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 vs DMSO group.
Supplemental Figure S2. L-5-HTP transcriptionally suppresses inducible PD-L1 expression and does not affect protein stability of PD-L1. (A) qPCR analysis of L-5-HTP treatment on IFN-γ-stimulated MC38, CT26 for 48h. (B) BXPC3 was treated with IFN-γ and L-5-HTP for 48h which was followed by 6h treatment of 10μM proteasome inhibitor MG132. Quantification analysis was shown (right). Relative fold changes of PD-L1 are normalized to mock group. (n=3 independent experiments). Data are shown as mean ± SD. ns P>0.05, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 vs IFN-γ group.
Supplemental Figure S3. The inhibitory effects of L-5-HTP on IFN-γ stimulated PD-L1 induction are mediated by a reduction in upstream RTK ligands in RTK receptors/MEK/ERK/c-JUN pathway cascade. (A) BXPC3 and A549 were treated with IFN-γ and L-5-HTP for 24h and then lysed. JAK1, p-JAK1, STAT1, p-STAT1, GAPDH were determined by western blot. (B) Volcano plot of significant differential expression genes
analysis of L-5-HTP vs DMSO group. (C) BXPC3 and A549 were treated with IFN-γ and L-5-HTP for 24h and then lysed. P65, p-P65, GAPDH were determined by western blot.

(D) BXPC3 and A549 were treated with IFN-γ and L-5-HTP for 24h and then lysed. MEK, p-MEK, ERK1/2, p-ERK1/2, c-JUN, p-c-JUN and GAPDH were determined by western blot. Quantification of western blot results for p-MEK/MEK, p-ERK1/2/ERK1/2, p-c-JUN/c-JUN are also shown. Relative fold changes of p-MEK/MEK, p-ERK1/2/ERK1/2, p-c-JUN/c-JUN are normalized to DMSO group. (n=3 independent experiments). (E) Analysis of PD-L1 mRNA expression in BXPC3 transfected with siRNA targeting c-JUN siRNA control and treated with IFN-γ and/or L-5-HTP. The knock down efficiency of siRNA targeting c-JUN in BXPC3 is shown. (F) BXPC3 are treated with IFN-γ and L-5-HTP for 24h. AREG and ANGPT4 protein level in culture supernatant were determined by ELISA assay. (G) BXPC3 were treated with IFN-γ and L-5-HTP for 24h and then lysed. c-MET, p-c-MET, EGFR, p-EGFR, GAPDH are determined by western blot. Quantification of western blot results for p-c-MET/c-MET, p-EGFR/EGFR are shown. Relative fold changes of p-c-MET/c-MET, p-EGFR/EGFR are normalized to DMSO group. (n=3 independent experiments). Data are shown as mean ± SD. ns P>0.05, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.
Supplemental Figure S4. L-5-HTP reduces PD-L1 expression and tumor growth in vivo. (A) L-5-HTP was added to assess the impact on the proliferation of OVA activated T cells isolated from OT-1 mouse. (n=3 independent experiments). Wild type C57BL/6 mice and wild type BALB/c mice were incubated with MC38 tumors (B) and CT26 tumors (C)
and were treated with 100mg/kg L-5-HTP or PBS intraperitoneally until tumor reached about 5 mm x 5 mm. Tumor volume and body weight were recorded once every three days. Median tumor volume of MC38 tumors (B, left) and CT26 tumors (C, left), tumor weight of MC38 tumors (B, middle), CT26 tumors (C, middle) and representative image of MC38 tumors (B, right), CT26 tumors (C right) are shown. Body weight of mice in (B) and (C) are shown in (D). (E) Representative plot and PD-L1 expression quantification of CD11c+ MHC-II+ DC cells and CD11b+F4/80+ macrophages from MC38 tumors were detected by flow cytometry. (F) Pd-l2 mRNA expression of MC38 tumors was analyzed by qPCR (n=5). (G) BXPC3 and A549 were treated with IFN-γ and L-5-HTP for 48h, PD-L2 mRNA expression was detected by qPCR. (H) CD45 IHC staining was performed from the resected MC38 tumor tissues and CT26 tumor tissues. Data are shown as mean ± SD. ns P>0.05, *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. Scale bar=50 μm.
Supplemental Figure S5. L-5-HTP enhances anti-tumor immunity response. (A) Flow cytometry gating strategy for intra-tumoral immune cells in MC38 tumors. (B) CT26 tumors were harvested at the endpoint and the IHC staining was performed. CD8, granzyme B IHC staining were performed. Representative images of staining intensity and
quantification are shown in (B) and (C) respectively. The number of CD8, granzyme B
IHC-positively stained cells of each view were analyzed. (D) PD-1 expression of CD8+ T
cells in MC38 tumors and CT26 tumors were detected by flow cytometry. (E) With the
treatment of L-5-HTP for 24h and 72h, PD-1 expression of OVA-activated primary T cells
isolated from OT-I mouse was detected by flow cytometry. (n=3 independent
experiments). Data are shown as mean ± SD. ns P>0.05, *P<0.05, **P<0.01, ***P<0.001
and ****P<0.0001. Scale bar=50 μm.
Supplemental Figure S6. An intact immune system and PD-L1 axis are indispensable for L-5-HTP to reduce tumor growth. L-5-HTP concentration within MC38 tumors (A) and serum (B) with 100mg/kg L-5-HTP treatment (i.p) were detected by
UHPLC-MS/MS (n=3 independent experiments). Tumor volume were about 1000 mm$^3$.

Different concentrations of L-5-HTP on the proliferation effect on MC38 (C) and CT26 (D) for 72h were detected by CellTiter-Glo luminescent assay. (n=3 independent experiments).

Tumor weight (related to figure 6A) and representative images (related to figure 6A) are shown in (E) and (F) respectively. (G) Proliferation rates of MC38 $^{WT}$ and MC38 $^{Pd-l1/-}$ were detected by CellTiter-Glo luminescent assay. (n=3 independent experiments). (H) $1\times10^6$ per mouse MC38 $^{WT}$ cell line and $1\times10^6$ per mouse MC38 $^{Pd-l1/-}$ cell line were implanted in nude mice. Tumor volume was measured once every three days. Median tumor volume is shown in (up panel) and the image of tumor harvested at the endpoint is shown in (down panel). Data are shown as mean ± SD. ns $P>0.05$, *$P<0.05$, **$P<0.01$, ***$P<0.001$ and ****$P<0.0001$. 

Supplemental material

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Supplemental Figure S7. L-5-HTP relieves depression-like behaviors in a CSDS-conditioned tumor mouse model. (A) A schematic shows the CSDS-conditioned tumor model. (B) Median tumor volumes of mice under stress and tumor burden were measured once every three days. Behavioral tests were performed at the end of day 22. (C) Immobility time in the tail suspension test and (D) sucrose consumption ratio in the sucrose preference test are shown. Control group (n=8), Tumor-PBS group (n=6), Tumor-5-HTP group (n=7), Tumor-CSDS-PBS group (n=6), Tumor-CSDS-5-HTP (n=8). Data are shown as mean ± SD. ns, P>0.05; *P<0.05; **P<0.01; ***P<0.001; and ****P<0.0001.