**Supplementary Figures**

# Targeting cathepsin B by cycloastragenol enhances antitumor immunity of CD8 T cells via inhibiting MHC-1 degradation

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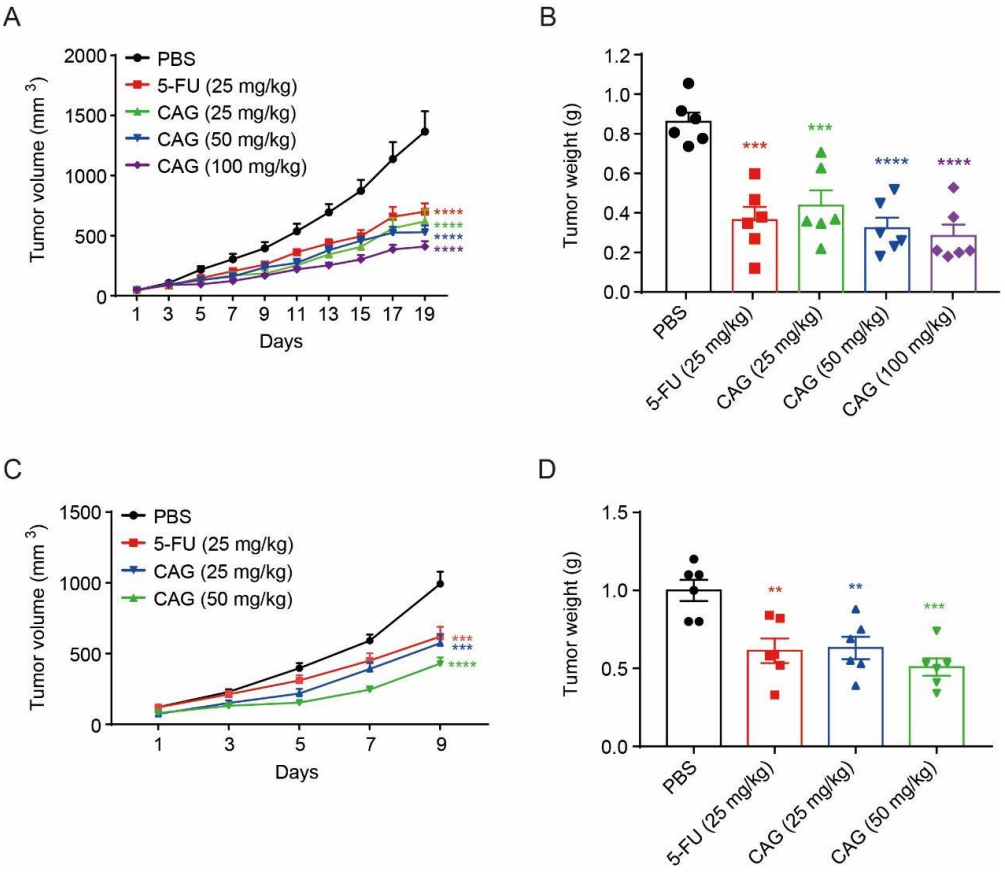
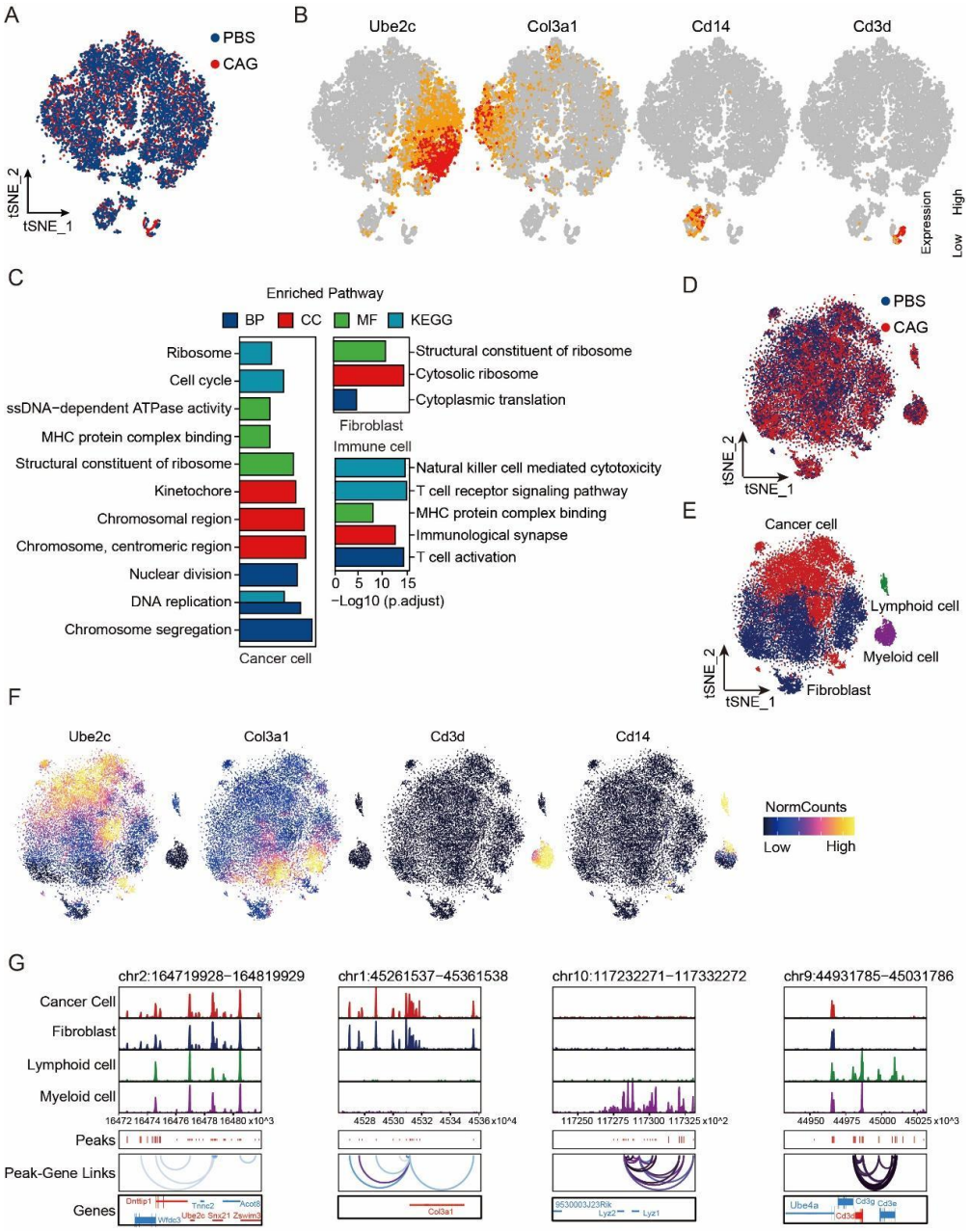
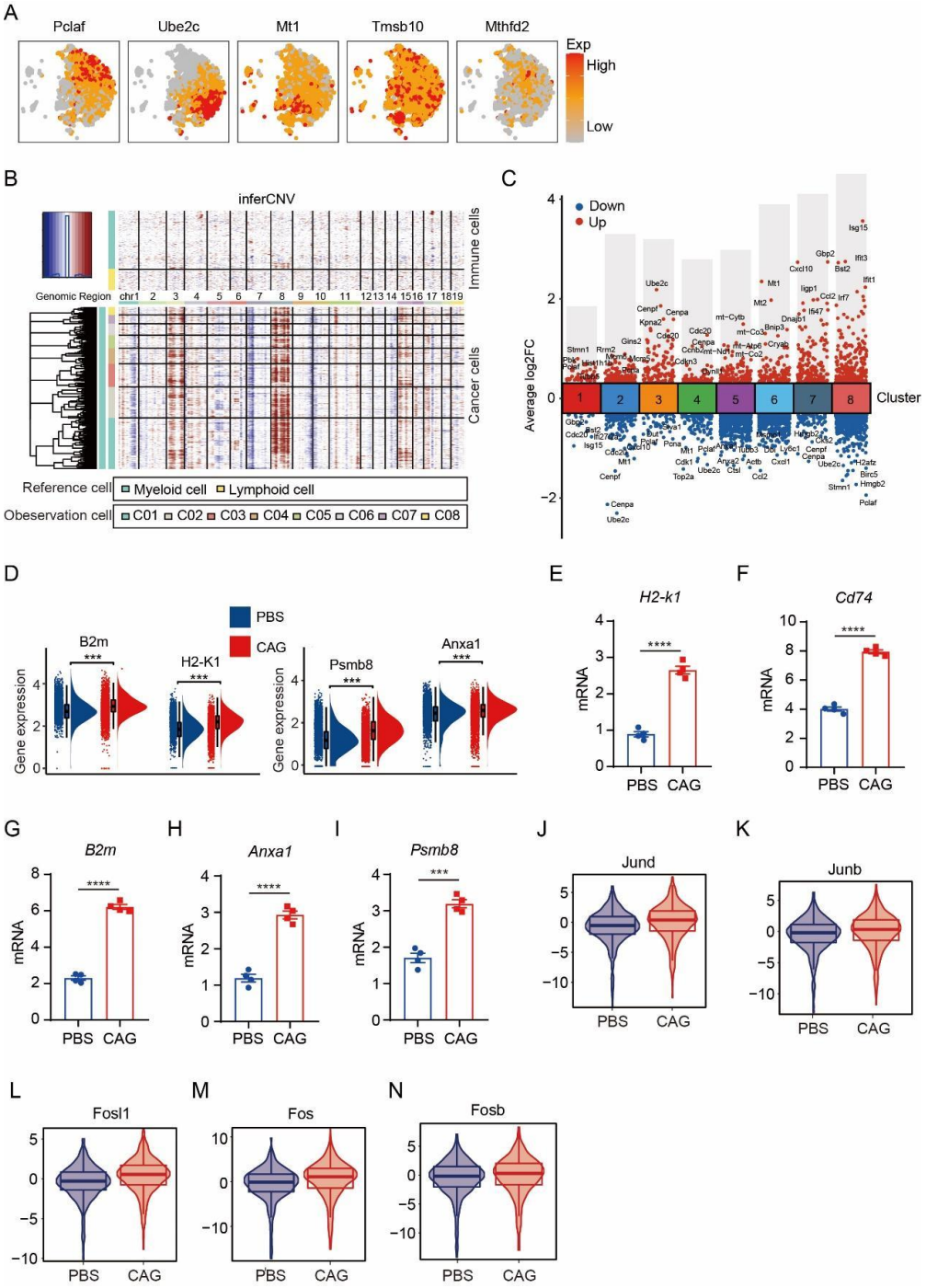
Supplementary Figure 1-7



Supplementary Figure 1. CAG inhibits the growth of transplanted colon cancer in mice. MC38 cancer cells ($1\times10^6$) were inoculated subcutaneously into each mouse (n = 6). When the tumor grew to 100 $mm^3$, the mice were randomly divided into the PBS group (ig, once a day), 5-FU group (ip, once every other day) and CAG different dose group (ig, once a day) for 19 days, and the mouse tumor was removed. (A) Tumor growth curve. (B) Tumor weight. CT26 cancer cells ($1\times10^6$) were inoculated subcutaneously into each mouse (n = 6). When the tumor grew to 100 mm, the mice were randomly divided into the PBS group (ig, once a day), 5-FU group (ig, once every other day) and CAG different dose group (ig, once a day) for 9 days, following which the mouse tumor was removed. (C) Tumor growth curve. (D) Tumor weight. Data are represented as mean ± SEM. *P*-values are determined by two-tailed Student's *t* test. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

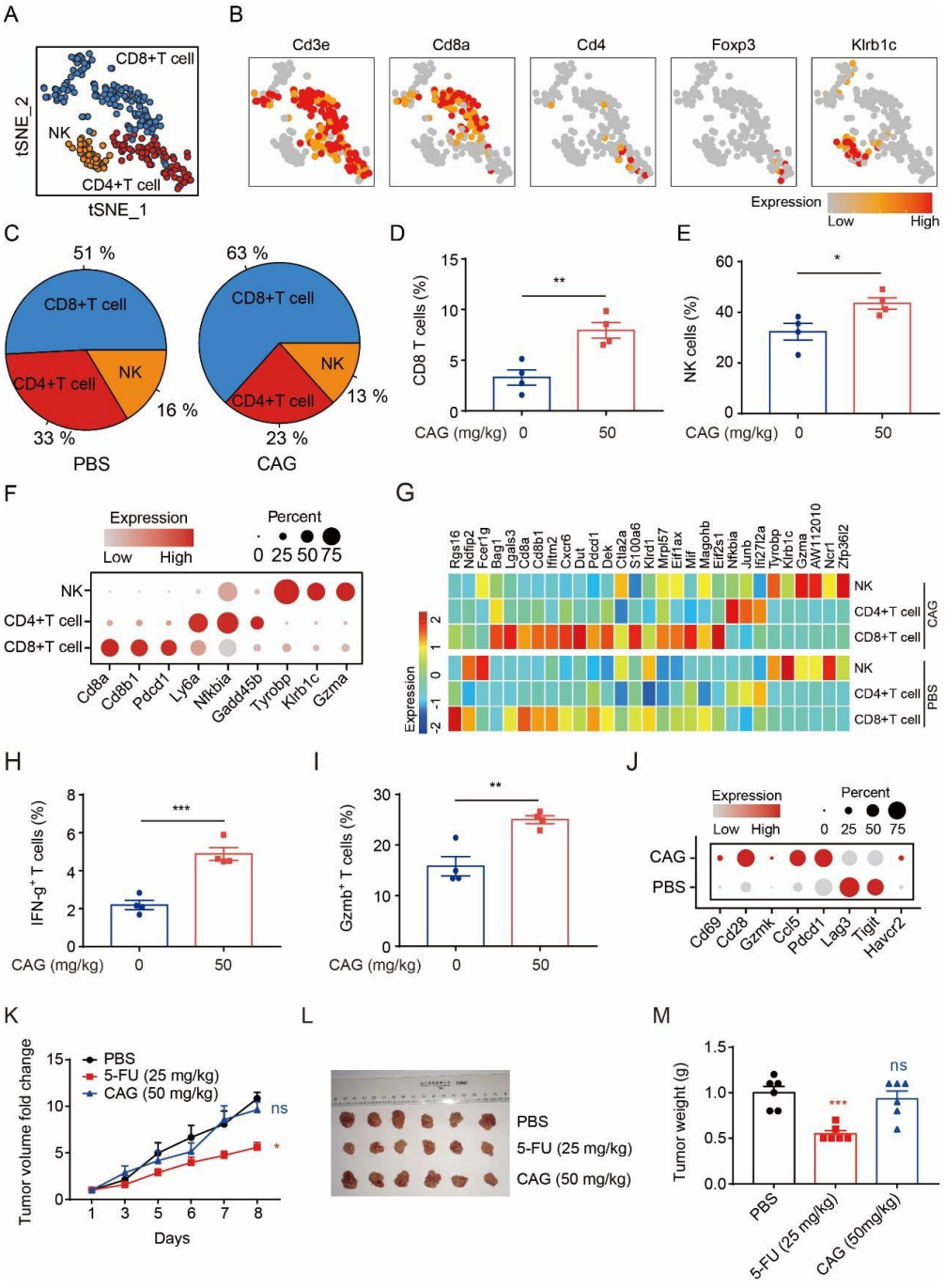



Supplementary Figure 2. Single cell multiomics analysis showed that CAG inhibited tumor growth. (A) Cell integration diagram of the PBS and CAG groups with scRNA-seq analysis. (B) Marker genes for clustering. (C) Enrichment analysis of cancer cells, fibroblasts and immune cells. (D) Cell integration diagram of the PBS and CAG groups with scATAC-seq analysis. (E) scATAC-seq analysis clustering cell population. (F) Expression of example marker genes. (G) Transcription factor validation of clustering.



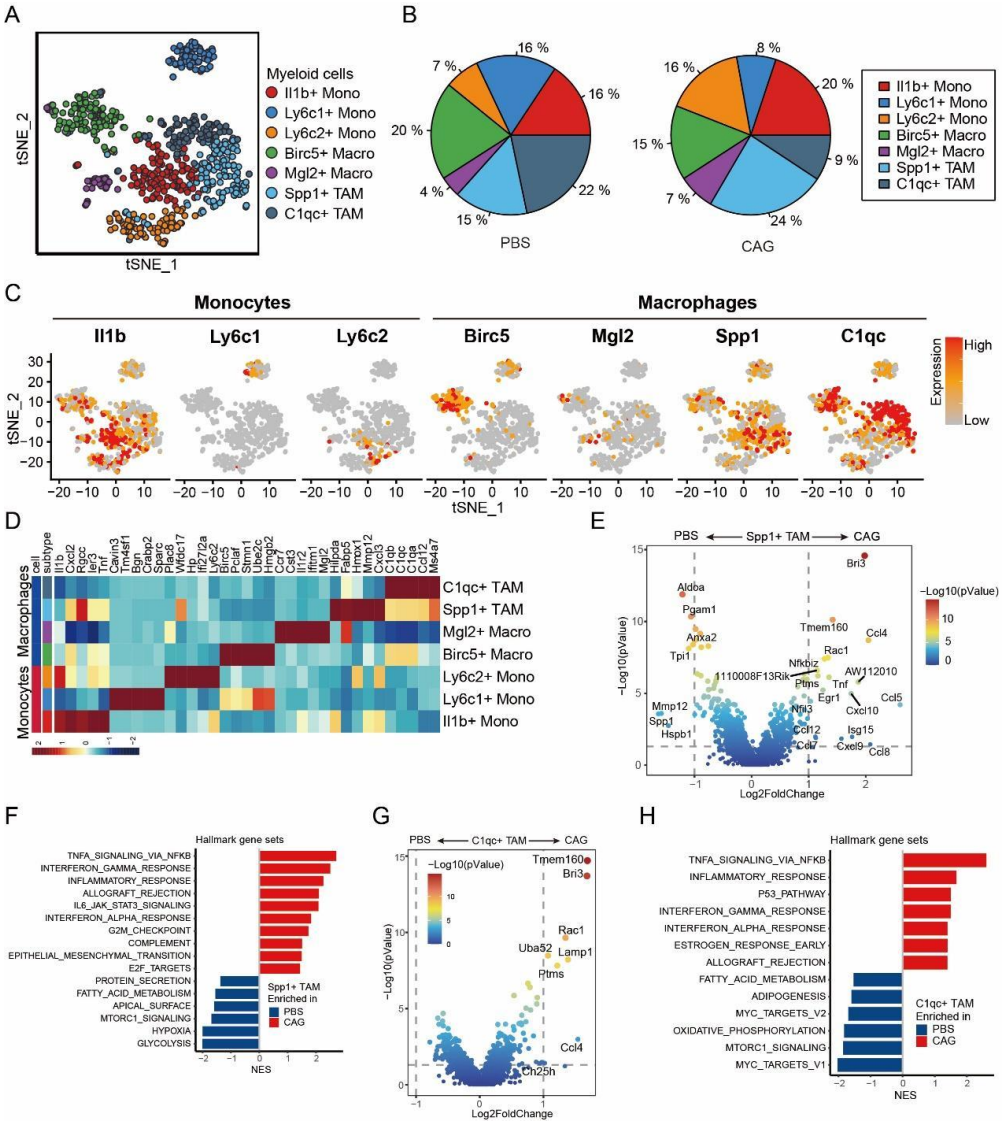

Supplementary Figure 3. CAG promotes the expression of antigen presentation related genes in cancer cells. (A) Featureplot showing marker gene expression of tumor cell subsets. (B) Landscape of inferred large-scale chromosomal CNVs distinguishing cancer cells from non-cancer cells. The reference cells were lymphocytes and myeloid cells. (C) Differential gene expression analysis showing up-

and down-regulated genes across all 10 clusters. (D) Expression of tumor antigen-related genes. (E-I) Transcription factors significantly enriched in tumor tissues. Data are represented as mean ± SEM. *P*-values are determined by two-tailed Student's *t* test. ***$P < 0.001$.

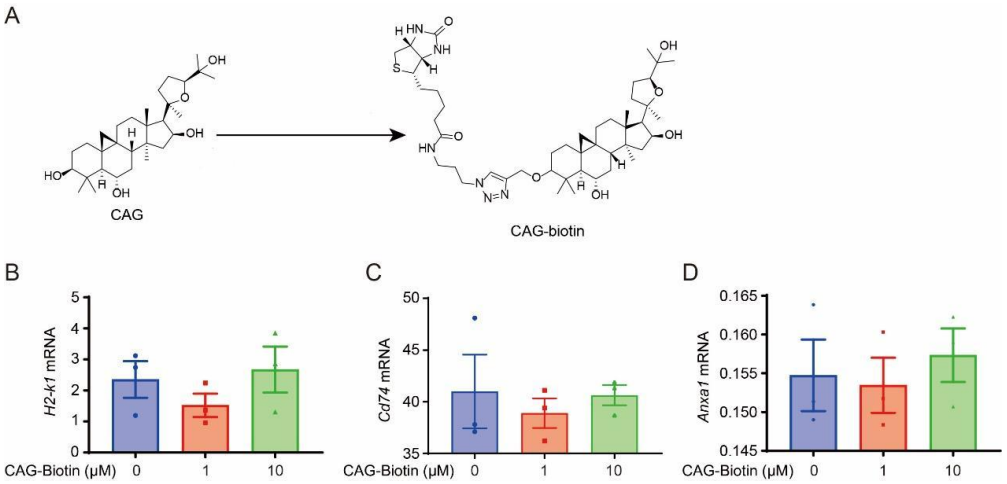



Supplementary Figure 4. CAG enhances the killing function of $CD8^+$ T cells. (A) Lymphocyte clustering. (B) Marker genes of each subgroup. (C) Proportion of subpopulations of cells between the two groups. (D) $CD8^+$ T cells and (E) $NK^+$ cells infiltrated in tumor tissues analyzed by flow cytometry. (F) The bubble chart showing the highly expressed genes in each subgroup. (G) Heatmap showing the different

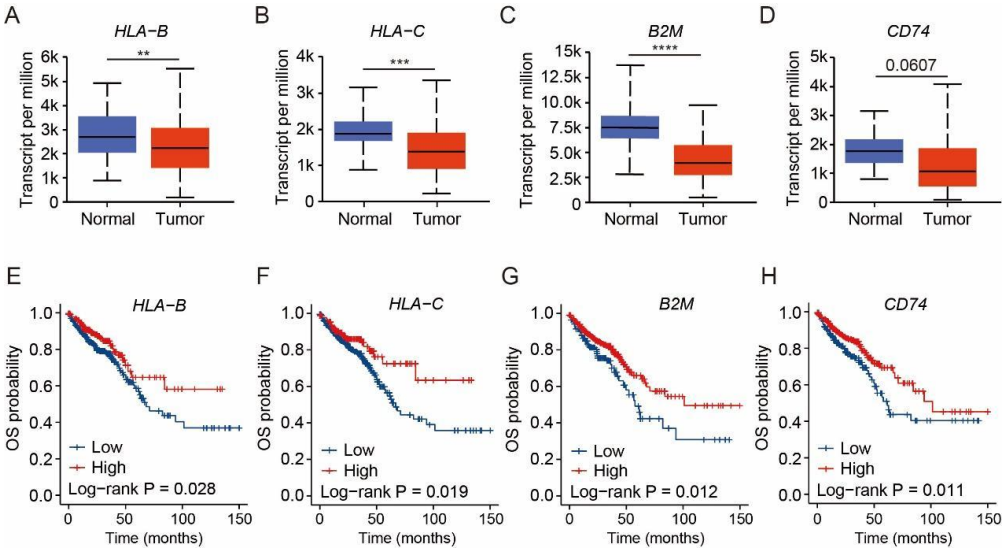
genes of each subgroup in the two groups. (H) $CD8^+$ IFN-$g^+$ T cells and (I) $CD8^+$ $Gzmb^+$ T cells infiltrated in tumor tissues were analyzed by flow cytometry. (J) $CD8^+$ T cell depletion and functional genotype gene display. (K) CT26 cancer cells ($1\times10^6$) were inoculated subcutaneously into each nude mouse (n = 6). After 5 days, the mice were randomly divided into the PBS group (ig, once a day) and CAG group (ig, once a day), On the 13th day, the mouse tumor was removed. (L) Tumor photos. (M) Tumor weight. Data are represented as mean ± SEM. *P*-values are determined by two-tailed Student's *t* test. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.



Supplementary Figure 5. CAG enhances the pro-inflammatory function of tumor associated macrophages. (A and B) Myeloid cell grouping and cell proportion. (C) Clustered cell marker genes. (D) Heatmap showing the highly expressed genes in each group of cells. (E and F) Differential gene expression and gene-set enrichment analysis (GSEA) of $Spp1^+$ TAM cells in the two groups. (G and H) Differential gene expression and GSEA of $C1qc^+$ TAM cells in the two groups.




Supplementary Figure 6. CAG-biotin had no effect on tumor antigen presentation. (A) CAG biotin structural formula. (B-D) Effect of CAG-biotin on antigen presentation related genes in cancer cells. MC38 cells ($1\times10^6$/well) were inoculated in 6-well plates for 6 h, then incubated with CAG-biotin (10 μM) for 24h, and the mRNA expression was detected.




Supplementary Figure 7. Relationship between expression of antigen presentation related genes and survival rate of colon cancer. (A-D) Analysis of *HLA-B*, *HLA-C*, *B2M*, and *CD74* gene expression in colon cancer by TCGA database. N = Normal, T = Tumor. (E-F) Relationship between high and low expression of *HLA-B*, *HLA-C*, *B2M* and *CD74* gene and survival rate in colon cancer analyzed by TCGA database. Data are represented as mean ± SEM. *P*-values are determined by two-tailed Student's *t* test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.