iScience, Volume *26*

Supplemental information

STING-IRG1 inhibits liver metastasis of colorectal cancer by regulating the polarization of tumor-associated macrophages

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Figure S1. Inducing M1 or M2 macrophages and Cell viability with STING agonist treatment, related to Figure 5. (A)With or without DMXAA, RAW264.7 cells were induced into M1 or M2 macrophages with LPS/IFN-γ or IL4, and qPCR was done to assess the mRNA levels of markers of RAW264.7 (*Arg1, Fizz1, IRG1, CXCL10, iNOS* and *TNF-α*). (B) Cell viability of colon cancer cells (MC38 and CT26) at different DMXAA treatment concentrations, CCK8 assay. (C) Cell viability of RAW264.7 cells at different DMXAA treatment concentrations, CCK8 assay.

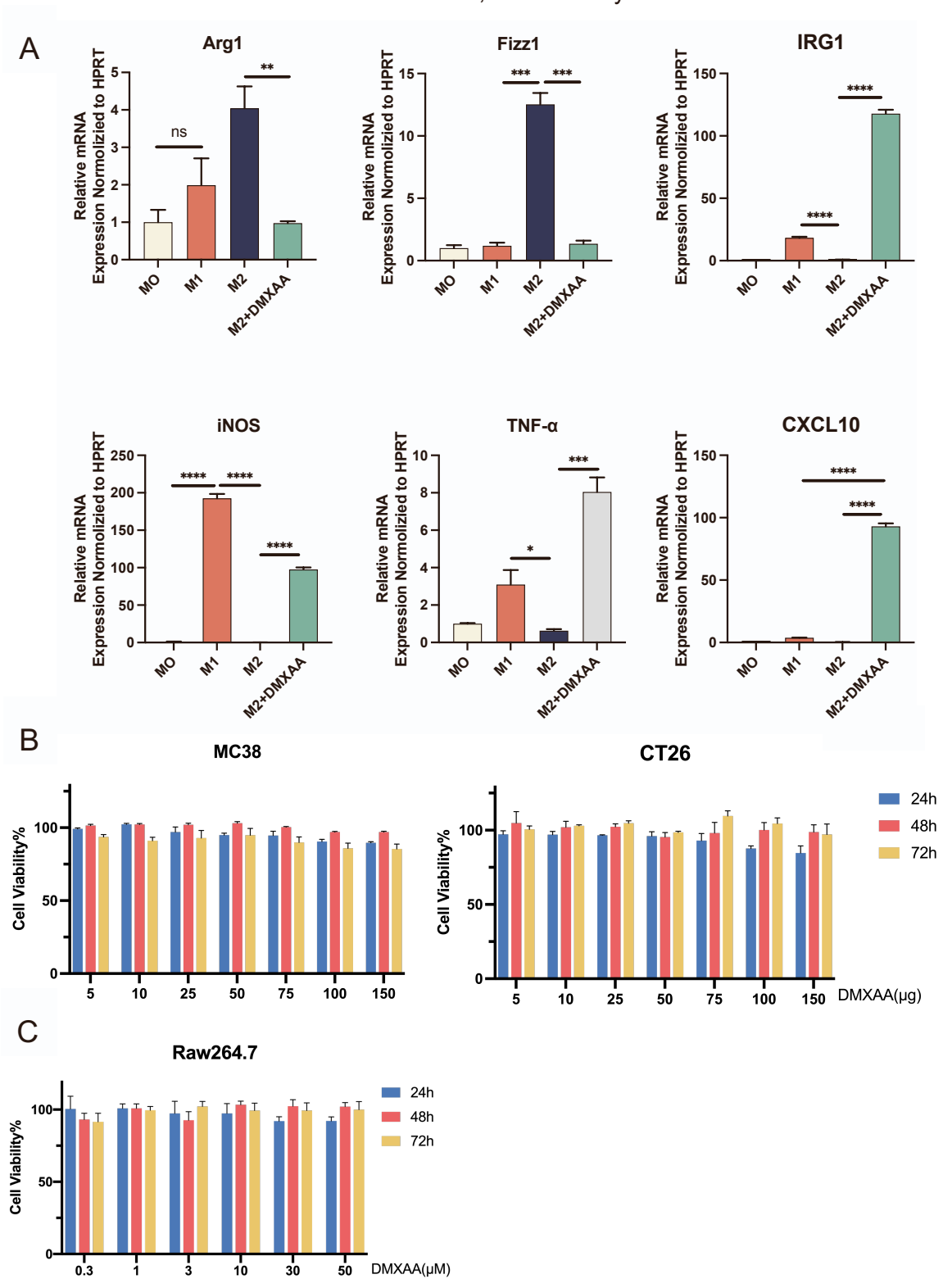

Figure S2. T cell subtypes in peripheral blood and tumor tissues of mice with colorectal cancer liver metastases, related to Figure 3.Flow cytometry to identify the proportion of cytotoxic T cells ($CD45^+CD3^+CD8^+$), helper T cells ($CD45^+CD3^+CD4^+$) and Treg cell subsets ($CD4^+CD127^{low}CD25^{hi}$) in peripheral blood and tumor tissues of mice.

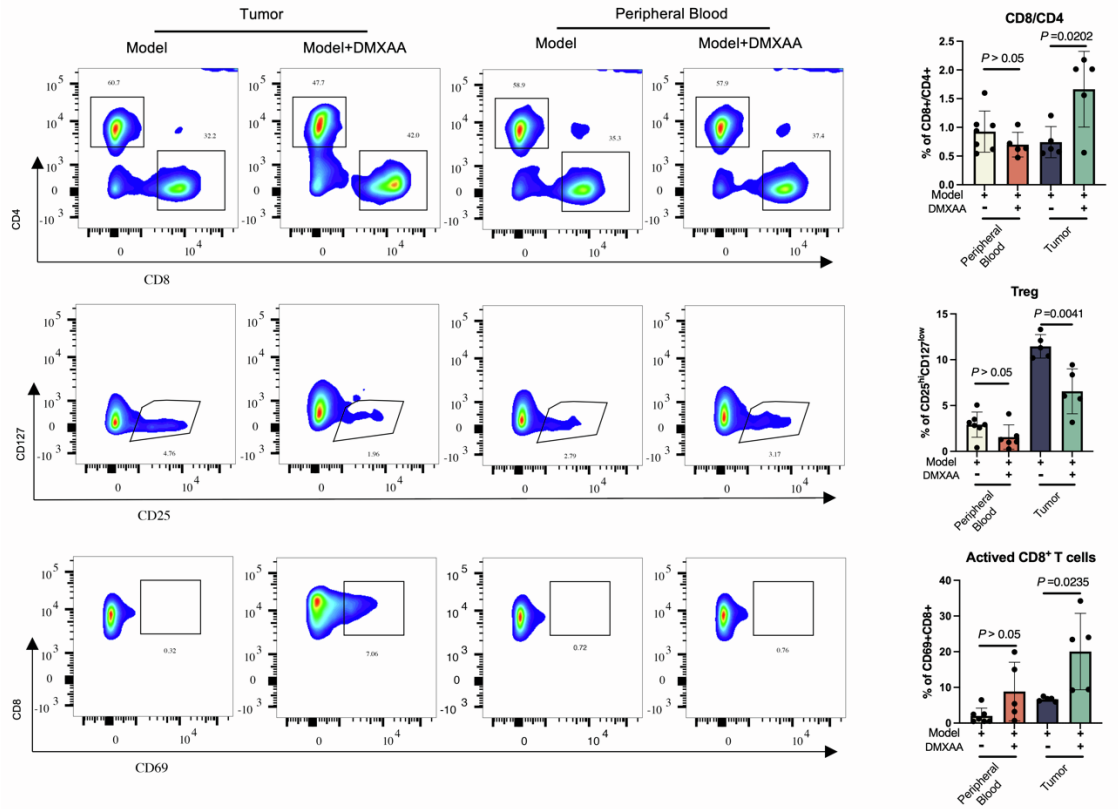

Figure S3. Macrophage subtypes in liver metastases from colorectal cancer in mice, related to Figure 9.Immunofluorescence analysis of liver metastatic tumor tissues in colorectal cancer liver metastasis mice treated with pbs or OI, $CD206^{+}$ (green), $CD86^{+}$ (red) and DAPI (blue). (B) Analysis of STING expression in primary CRC with metastasis tissues in CRC microarray profile(GSE41258). (C) MC38-luciferase cells were injected to construct a transfer model and treated with DMXAA (i.p.) (10mg/kg/3days) or PBS (i.p.) for three weeks. Western blot to detect the protein levels of IRG1 in mouse liver using GAPDH as the loading control.

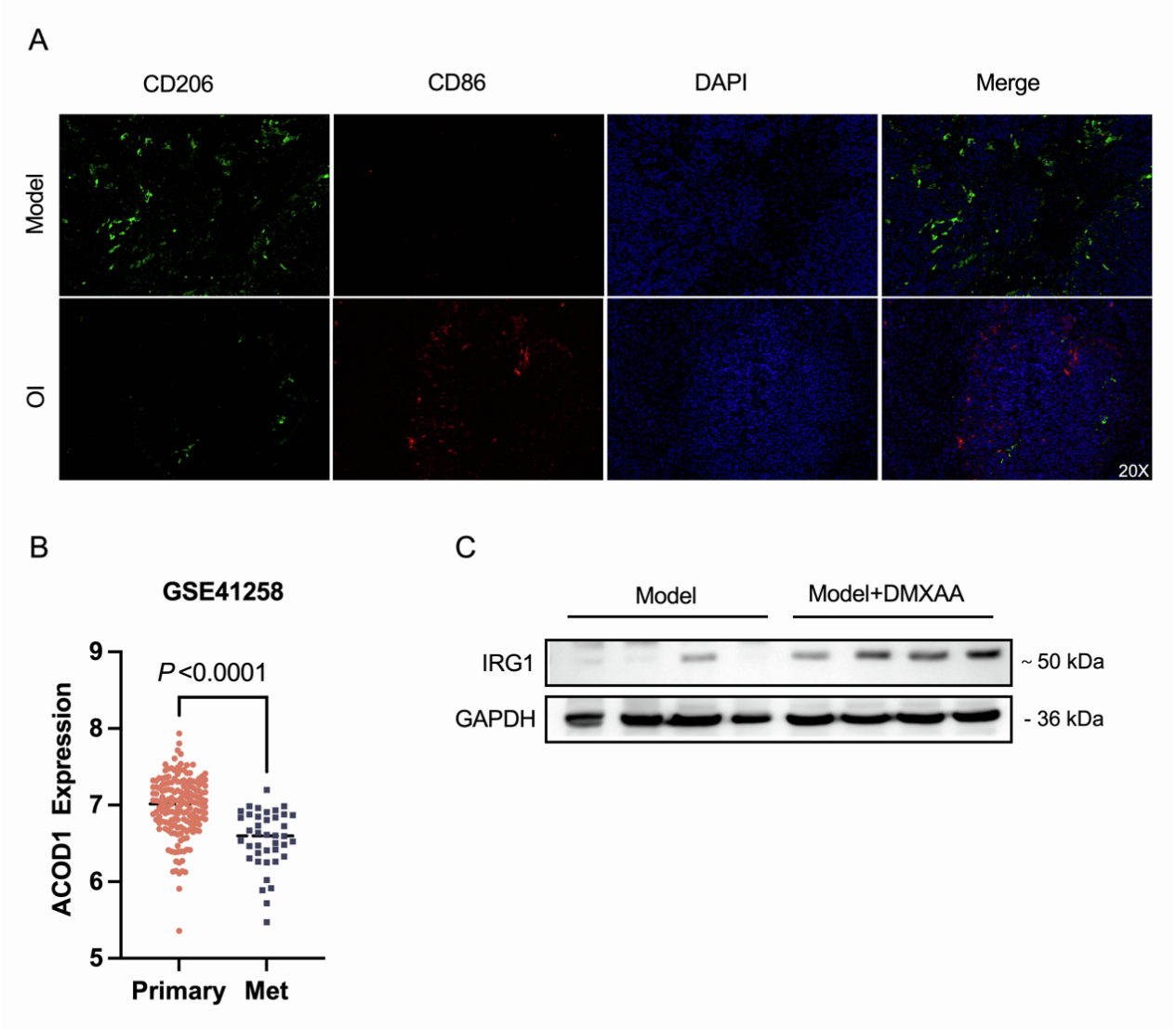

Figure S4. Co-localization of IRG1 and STING in macrophages, related to Figure 7. Macrophages were treated with PBS or DMXAA for 24h, and co-localization between IRG1 and STING was analyzed to determine the relationship between IRG1 and STING by immunofluorescence.

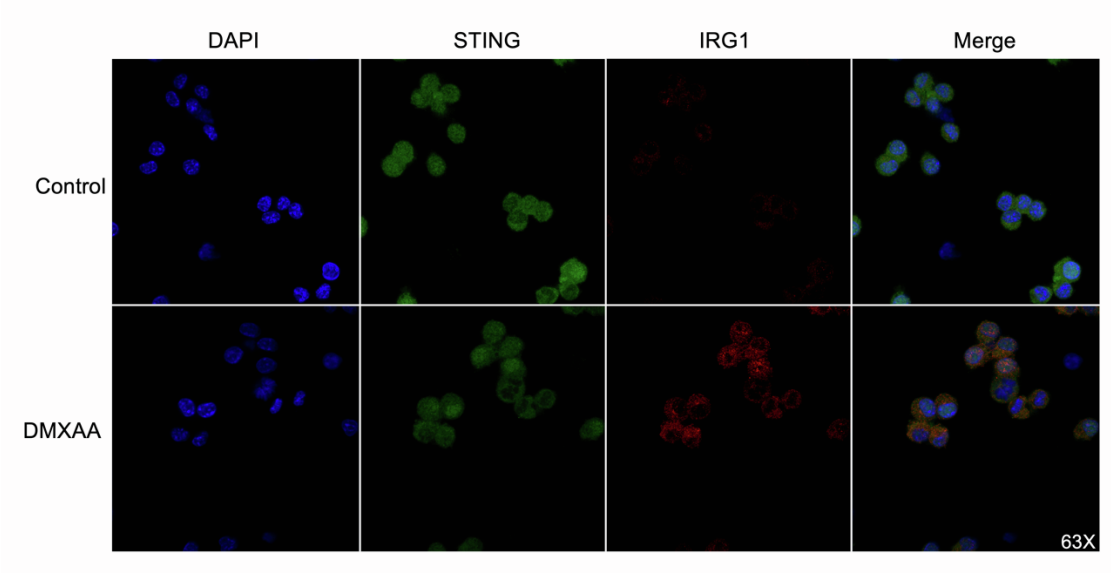

Figure S5. STING inhibition of colorectal cancer liver metastasis was mediated by macrophages, related to Figure 3. (A) After depletion of macrophages by clodronate liposomes(CLO), DMXAA (i.p) (10mg/kg/3day) was given for 3 weeks after the construction of colorectal cancer liver metastasis mice. Bioluminescence signal imaging of representative mice (left) and quantitation analyses of bioluminescence (right) were done following treatments,n≥5. (B) Flow cytometry analysis of macrophages in colorectal cancer liver metastasis tumor tissue and peripheral blood ($CD45^+CD11b^+F4/80^+$).

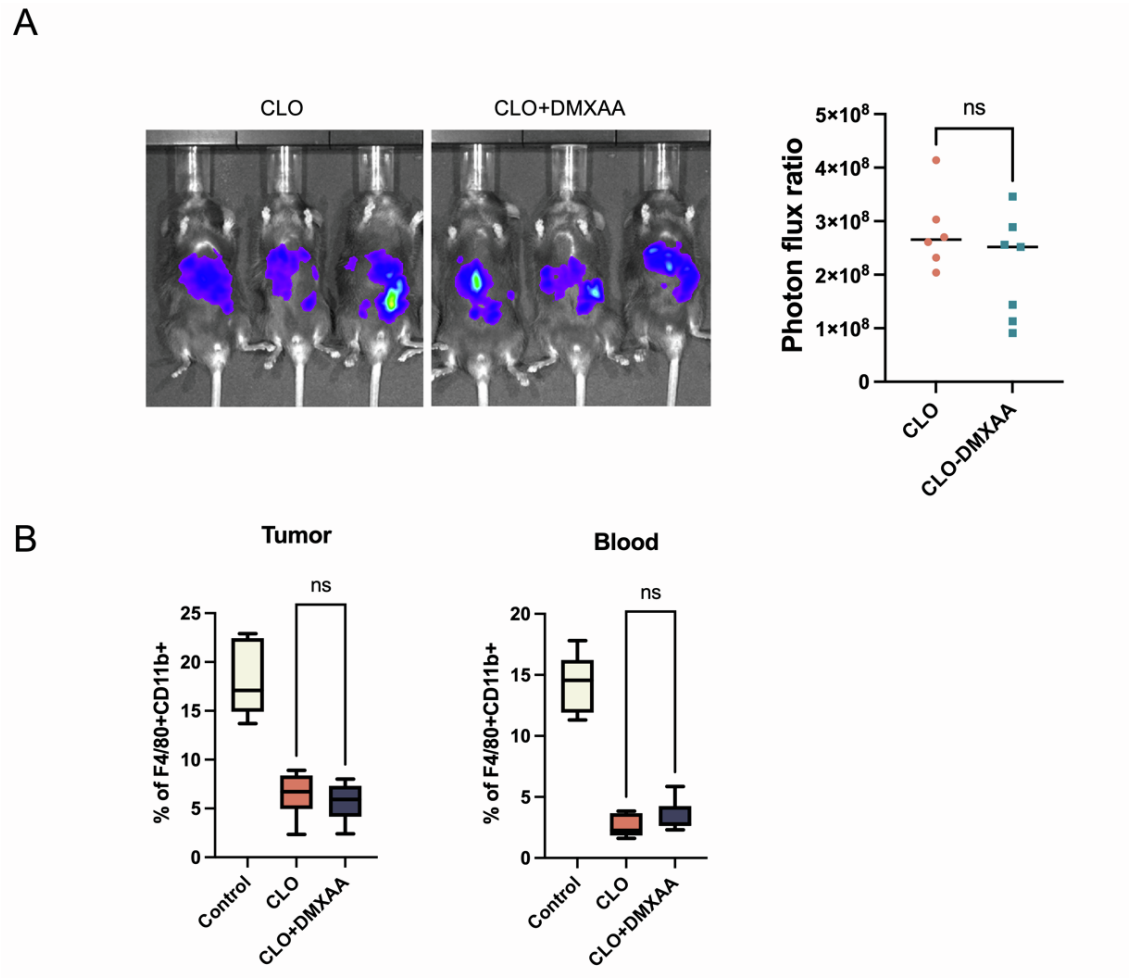

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Figure S6. Gating strategy for flow cytometry, related to Figure 2. Gating strategy for flow cytometry of macrophages and T cells in tumor tissue. Immune cells were isolated from tumor tissues and flow cytometry was used to analyze the degree of infiltration of macrophages (A) and T cells (B) immune cells in tumor tissues to characterize the infiltration and activation of macrophages and T cells.

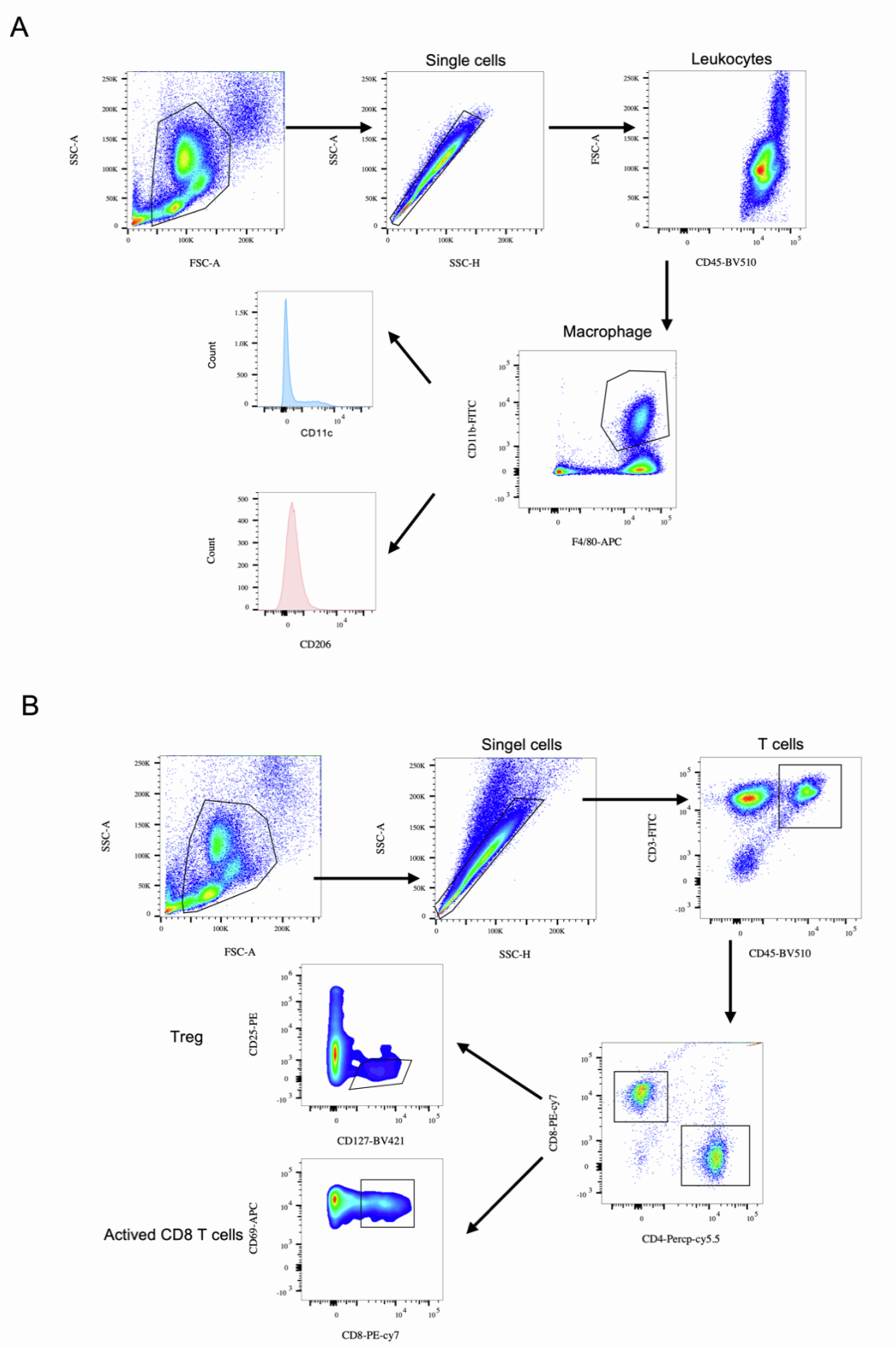

Figure S7. Expression levels of STING in different cell lines, related to Figure 1. Western blotting analysis of STING in CRC cell lines and normal intestinal epithelial cell lines.