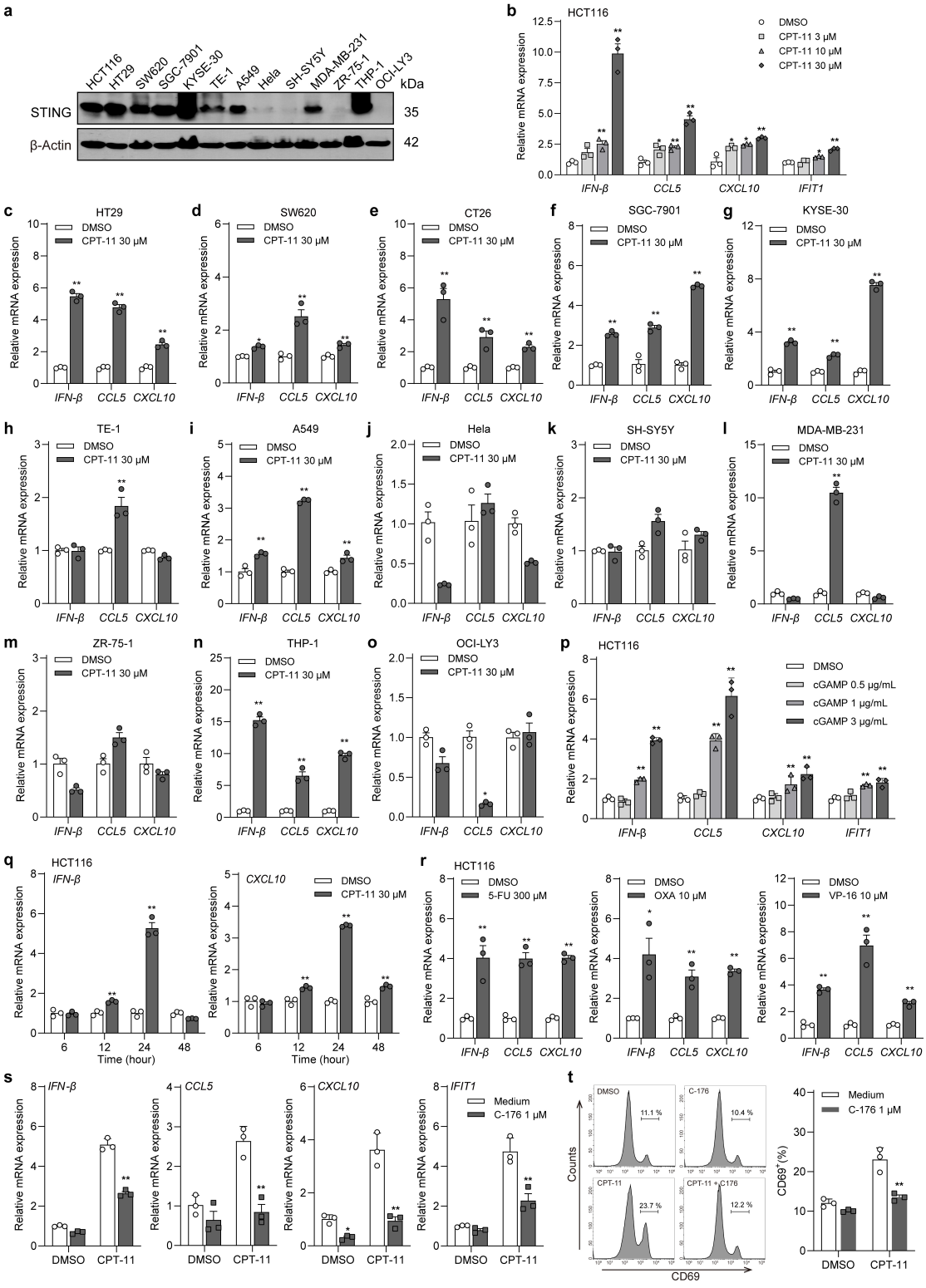
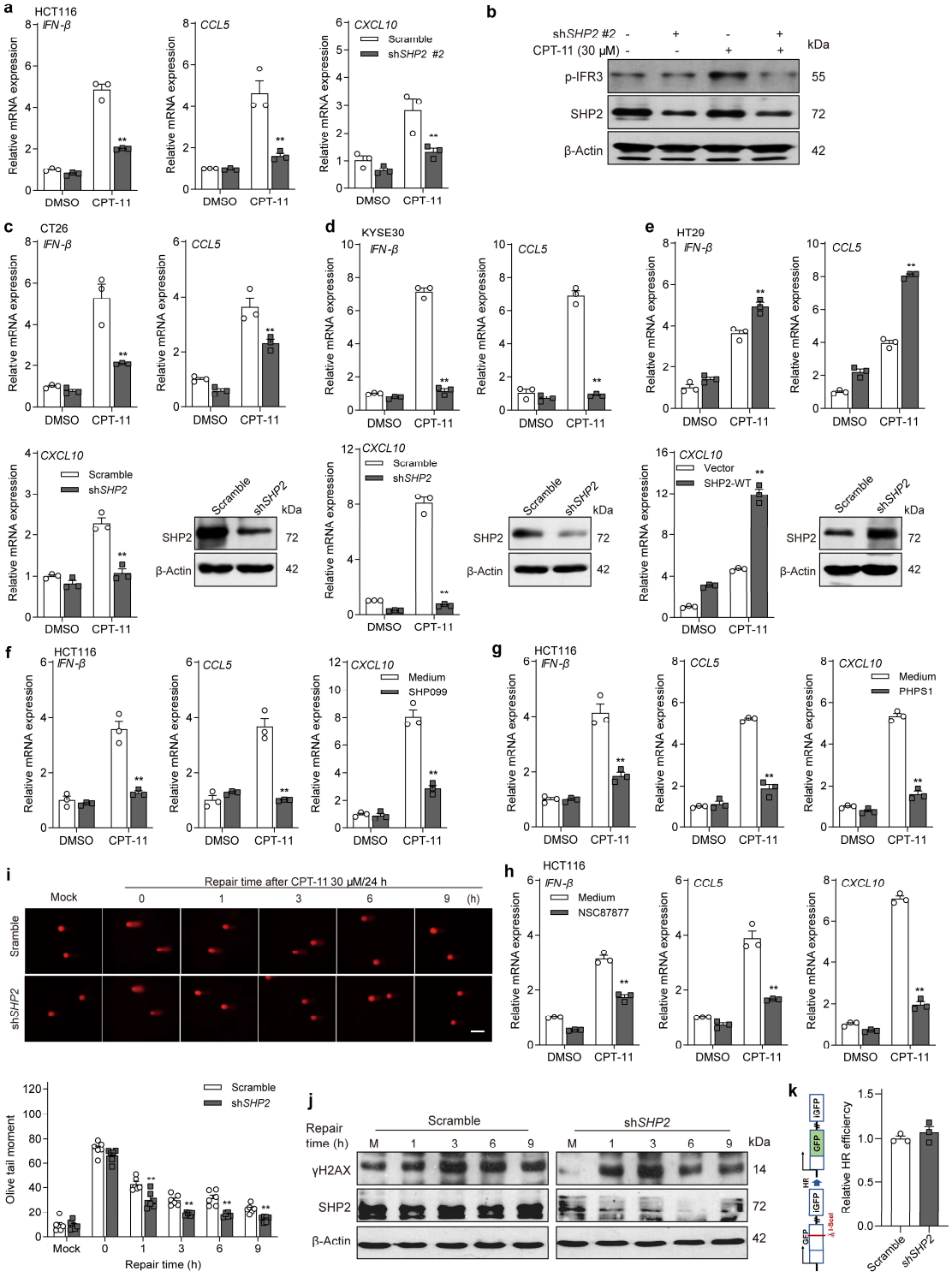
Supplementary Figure S1



Supplementary Figure S1. DNA damage chemotherapeutic drugs activated STING pathway in STING intact cancer cells. (a) Cell lysis of HCT116, HT29, SW620, SGC-7901, KYSE-30, TE-1, A549, Hela, SH-SY5Y, MDA-MB-231, ZR-75-1, THP-1, and OCI-LY3 was analyzed via western blot using the indicated

antibodies. (b) HCT-116 cells were treated with CPT-11 (3, 10, 30 μM) or DMSO for 24 h, and the expressions of *IFN-β*, *CCL5*, *CXCL10*, and *IFIT1* were measured via qPCR. *P < 0.05, **P < 0.01; one-way ANOVA. (c-o) HCT116, HT29, SW620, CT26, SGC-7901, KYSE-30, TE-1, A549, Hela, SH-SY5Y, MDA-MB-231, ZR-75-1, THP-1 and OCI-LY3 cells were treated with CPT-11 (30 μM), and the expressions of *IFN-β*, *CCL5*, and *CXCL10* were measured via qPCR. *P < 0.05, **P < 0.01; Student's t test. (p) HCT-116 cells were treated with cGAMP (0.5, 1, 3 μg/mL) or DMSO for 24 h, and the expressions of *IFN-β*, *CCL5*, *CXCL10*, and *IFIT1* were measured via qPCR. *P < 0.05, **P < 0.01; one-way ANOVA. (q) HCT-116 cells were treated with CPT-11 (30 μM) or DMSO for indicated time, and the expressions of *IFN-β* and *CXCL10* were measured via qPCR. *P < 0.05, **P < 0.01; one-way ANOVA. (r) HCT-116 cells were treated with 5-Fu (300 μM), OXA (10 μM), or VP-16 (10 μM) for 24 h, and the expressions of *IFN-β*, *CCL5*, and *CXCL10* were measured via qPCR. *P < 0.05, **P < 0.01; Student's t test. (s) HCT-116 cells were pretreated with C-176 (1 μM) for 6 h and stimulated with CPT-11 (30 μM). The expressions of *IFN-β*, *CCL5*, *CXCL10*, and *IFIT1* were measured via qPCR. *P < 0.05, **P < 0.01; two-way ANOVA. (t) HCT-116 cells were pretreated with C-176 (1 μM, 6 h) and exposed to CPT-11 (30 μM, 24 h). Then, they were co-cultured with Jurkat cells for an additional 24 h, and T cell activation was measured according to surface expression of CD69. Quantitative analysis of the $CD69^+$ T cells. **P < 0.01; two-way ANOVA. Data shown in (b-t) are mean ± SEM of 3 different experiments.

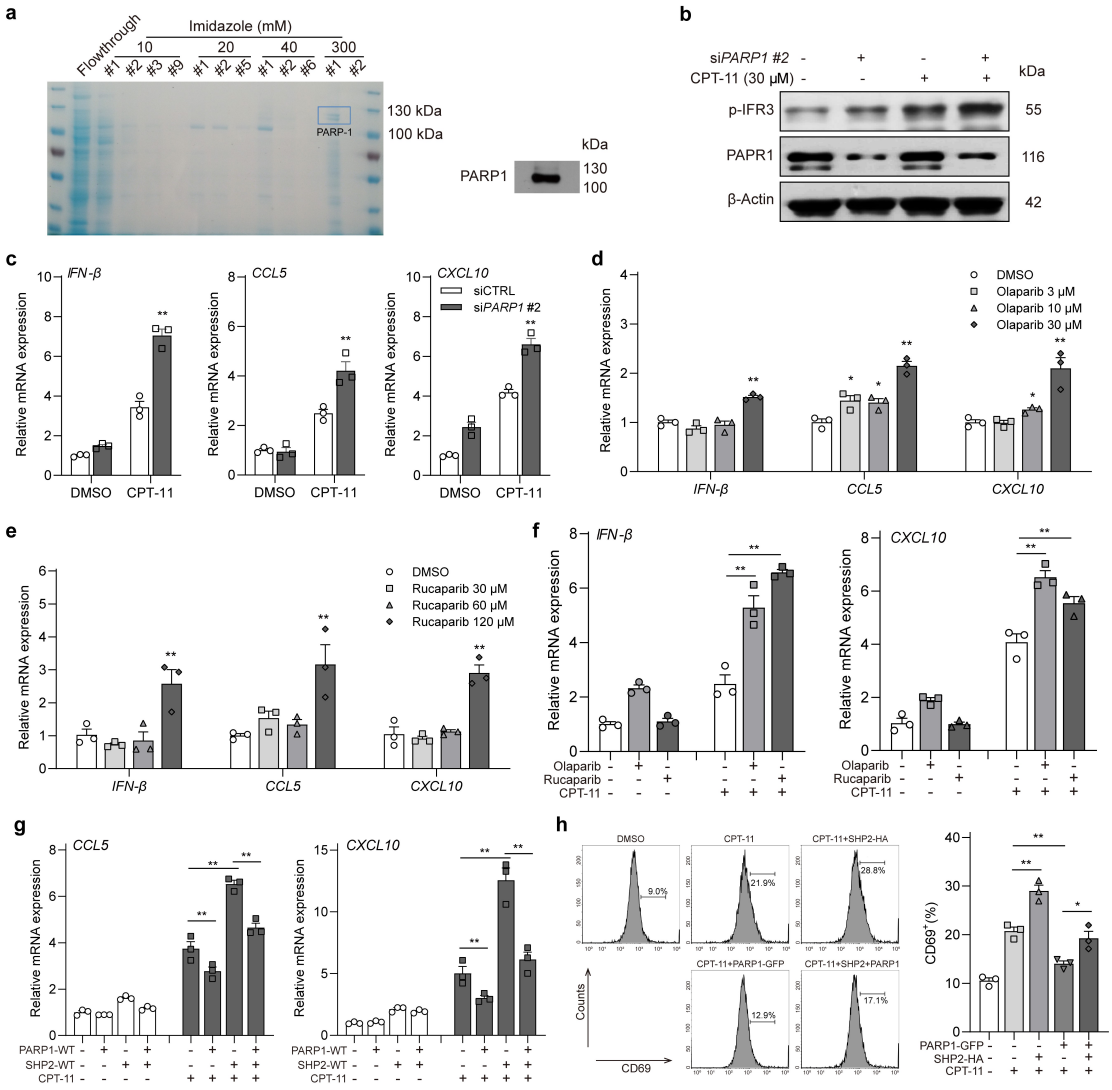
Supplementary Figure S2



Supplementary Figure S2. SHP2 regulated the STING signaling and DDR in colon cancer. (a-b) HCT-116 cells transfected with scramble or *shSHP2 #2* lentivirus were stimulated with CPT-11 (30 μM). The expressions of *IFN-β*, *CCL5*, *CXCL10*, and *IFIT1* mRNA were measured via qPCR (a). $^{**}P < 0.01$; two-way ANOVA. Cell lysis was analyzed via western blot using the indicated antibodies (b). (c, d) CT26 and

KYSE30 cells transfected with scramble or sh*SHP2* lentivirus were stimulated with CPT-11 (30 μM). Induction of *IFN-β*, *CCL5*, and *CXCL10* mRNA expression was measured via qPCR. Cell lysis was analyzed via western blot using the indicated antibodies. $^{**}P < 0.01$; two-way ANOVA. (e) HT-29 cells transfected with Vector or SHP2-WT plasmid were stimulated with CPT-11 (30 μM). Induction of *IFN-β*, *CCL5*, and *CXCL10* mRNA was measured via qPCR. Cell lysis was analyzed using western blot using the indicated antibodies. $^{**}P < 0.01$; two-way ANOVA. (f-h) Quantitative PCR of *IFN-β*, *CCL5*, and *CXCL10* in HCT-116 cells untreated or treated with the SHP2 inhibitor SHP099 (10 μM), PHPS1 (10 μM) or NSC87877 (10 μM) for 3 h, followed by CPT-11 treatment. $^{**}P < 0.01$; two-way ANOVA. (i, j) CT-26 cells with scramble or sh*SHP2* lentivirus were treated with CPT-11 and allowed to repair DSBs for different time intervals. (i) The repair kinetics of DSBs were detected using comet assay. Scale bar, 50 μm. The olive tail moment was determined as the end point of DSBs. Data represent the mean ± SEM (n = 6). $^{**}P < 0.01$; two-way ANOVA. (j) Cell lysis was analyzed via western blot using the indicated antibodies. (k) Schematic of cell-based repair assay. The results showed that SHP2 knockdown did not affect the HR repair efficiency in HCT-116 cells. Representative FACs analysis of GFP-positive cells. Student's t test. Data shown in (a, c-h, and k) are mean ± SEM of 3 different experiments.

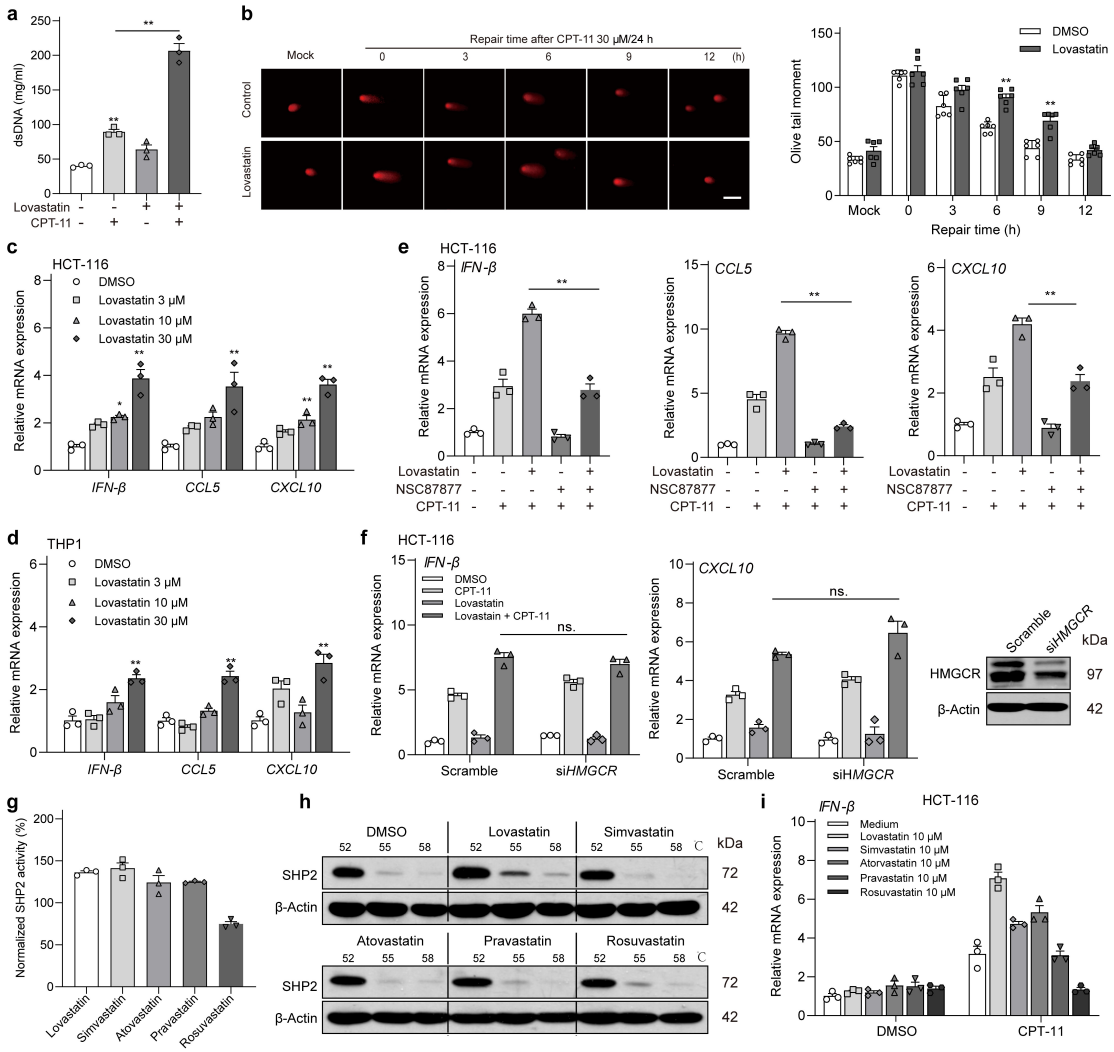
Supplementary Figure S3



Supplementary Figure S3. PARP1 inhibition amplified CPT-11-induced STING signaling. (a) Expression, purification and identification of PARP1. (b, c) Two groups of HCT-116 cells with scramble or si*PARP1* #2 were stimulated with CPT-11 (30 μM, 24 h). Cell lysis was analyzed via western blot using the indicated antibodies (b). Quantitative PCR of *IFN-β*, *CCL5*, and *CXCL10* (c). $^{**}P < 0.01$; two-way ANOVA. (d, e) HCT-116 cells were treated with olaparib (3, 10, and 30 μM) or rucaparib (30, 60, and 120 μM) for 24 h, and the expressions of *IFN-β*, *CCL5*, and *CXCL10* were measured via qPCR. $^{*}P < 0.05$, $^{**}P < 0.01$; one-way ANOVA. (f) HCT-116 cells were treated with olaparib (30 μM) or rucaparib (60 μM) with CPT-11 (30 μM) for 24 h, then the expression of *IFN-β and CXCL10* was measured via qPCR. $^{**}P < 0.01$; two-way ANOVA. (g, h) SHP2 or PARP1 overexpression and double overexpression

HCT-116 cells were stimulated with CPT-11 (30 μM). (g) Induction of *CCL5* and *CXCL10* mRNA was measured via qPCR. $^{**}P < 0.01$; two-way ANOVA. (h) After exposure to CPT-11 for 24 h, the HCT-116 cells were co-cultured with Jurkat cells for an additional 24 h, and the T cell activation was measured according to the surface expression of CD69. Quantitative analysis of the $CD69^{+}$ T cells. $^{*}P < 0.05$, $^{**}P < 0.01$; two-way ANOVA. Data shown in (c-h) are mean ± SEM of 3 different experiments.

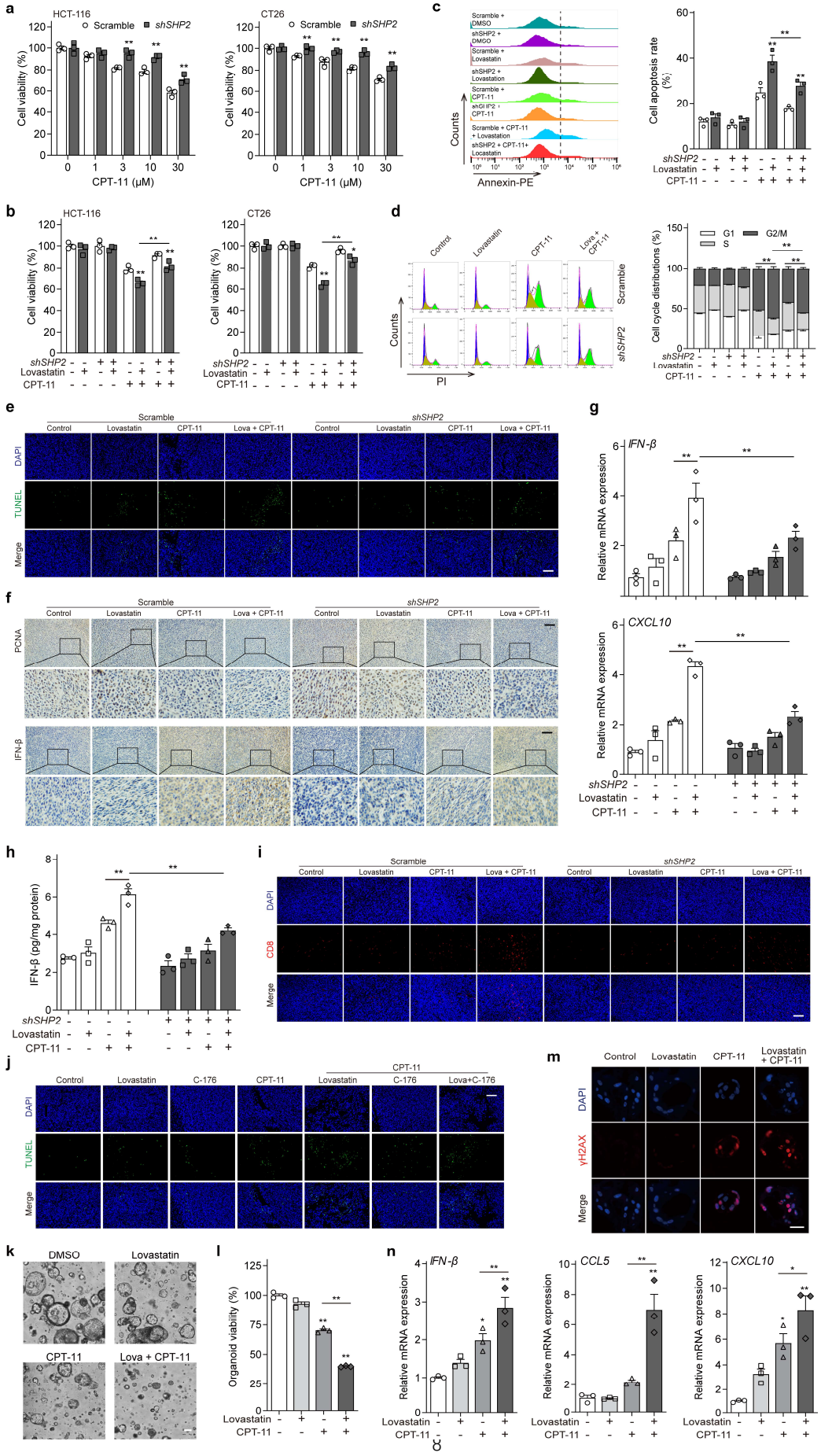
Supplementary Figure S4



Supplementary Figure S4. Lovastatin regulated the DDR and activated the STING pathway independent of HMGCR. (a) HCT-116 cells were treated with lovastatin (10 μM, 24 h) followed by CPT-11 (30 μM, 24 h) treatment. The

concentration of extracellular dsDNA in the culture supernatant was detected using the PicoGreen dsDNA kit. $^{**}P < 0.01$; two-way ANOVA. (b) HCT-116 cells were treated with CPT-11 (30 μM) for 24 h to induce DNA damage and then allowed to repair DSBs for different time intervals under DMSO or lovastatin (10 μM) treatment. The repair kinetics of DSBs were detected using a comet assay. Scale bar, 50 μm. The olive tail moment was determined as the end point of DSBs. Data represent the mean ± SEM (n = 6). $^{**}P < 0.01$; two-way ANOVA. (c) HCT-116- and (d) PMA-activated THP1 cells were treated with lovastatin (3, 10, and 30 μM) for 24 h, and then the expressions of *IFN-β*, *CCL5*, and *CXCL10* were measured via qPCR. $^{*}P < 0.05$, $^{**}P < 0.01$; one-way ANOVA. (e) Quantitative PCR of *IFN-β*, *CCL5*, and *CXCL10* in HCT-116 pretreated with the SHP2 inhibitor NSC87877 (10 μM, 4 h) or lovastatin (10 μM, 4 h), followed by CPT-11 (30 μM, 24 h) treatment. $^{**}P < 0.01$; two-way ANOVA. (f) Quantitative PCR of *IFN-β* and *CXCL10* in scramble or si*HMGCR* HCT-116 pretreated with lovastatin (10 μM, 4 h), followed by CPT-11 (30 μM, 24 h) treatment. ns. represents no significance; two-way ANOVA. (g) The effects of statins (lovastatin, simvastatin, atorvastatin, pravastatin, or rosuvastatin) on SHP2 enzyme activity were examined. (h) HCT-116 cells were incubated with or without indicated compound (30 μM), the cells were then collected and subjected to CETSA assay. (i) Quantitative PCR of *IFN-β* in HCT-116 pretreated with stains (10 μM, 4 h), followed by CPT-11 (30 μM, 24 h) treatment. Data shown in (a, c-g, and i) are mean ± SEM of 3 different experiments.

Supplementary Figure S5



Supplementary Figure S5. Lovastatin potentiates SHP2-mediated anti-tumor immunity. (a) HCT-116 or CT26 cells transfected with scramble or sh*SHP2* lentivirus were treated with CPT-11 (0, 1, 3, 10, and 30 μM) for 24 h. Cell viability was detected using a CCK-8 kit. $^{**}P < 0.01$; two-way ANOVA. (b) Inhibition of cell proliferation in HCT-116 or CT26 cells transfected with scramble or sh*SHP2* lentivirus was detected using a CCK-8 kit after CPT-11 (10 μM) treatment with or without lovastatin (10 μM) for 24 h. $^{*}P < 0.05$, $^{**}P < 0.01$; two-way ANOVA. (c, d) Cell apoptosis and cycle distribution were measured by FACS after treatment with or without lovastatin (10 μM) and CPT-11(30 μM) transfected with scramble or sh*SHP2* lentivirus for 24 h. $^{**}P < 0.01$, two-way ANOVA. Mice with established WT or sh*SHP2* CT26 tumors were treated with CPT-11 or lovastatin. (e) TUNEL activity in the indicated groups was detected via immunofluorescence. Scale bar, 50 μm. (f) PCNA and IFN-β staining in the indicated groups was detected using immunohistochemistry. Scale bar, 50 μm. (g) Induction of *IFN-β* and *CXCL10* mRNA was measured via qPCR. Values are mean ± SEM of 3 mice/group. $^{**}P < 0.01$; two-way ANOVA. (h) Induction of IFN-β protein in tumors was measured via ELISA. $^{**}P < 0.01$; two-way ANOVA. (i) Tumor-infiltrating $CD8^+$ T lymphocytes were detected via immunofluorescence. Scale bar, 50 μm. (j) Mice inoculated with CT26 tumors were treated with C176, lovastatin, and CPT-11 alone or in combination. TUNEL activity in the indicated groups was detected via immunofluorescence. Scale bar, 50 μm. The colon cancer-derived organoids from a colon cancer patient were treated with DMSO vehicle, lovastatin (30 μM), CPT-11(30 μM), or lovastatin plus CPT-11. (k) Bright-field microscopy images of PDOs cultured for 5 days. Scale bar, 50 μm. (l) The organoid viability was measured via luminescence signal intensities. $^{**}P < 0.01$; two-way ANOVA. (m) γH2AX staining in the indicated groups was detected via immunofluorescence. Scale bar, 25 μm. (n) The mRNA expressions of *IFN-β*, *CCL5* and *CXCL10* were measured via qPCR. $^{*}P < 0.05$, $^{**}P < 0.01$; two-way ANOVA. Data shown in (a-d, l, and n) are mean ± SEM of 3 different experiments.