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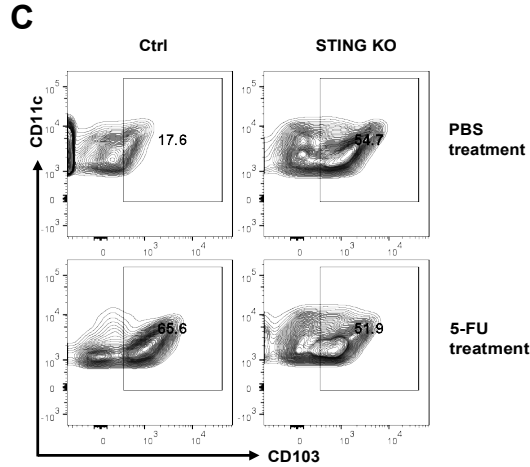
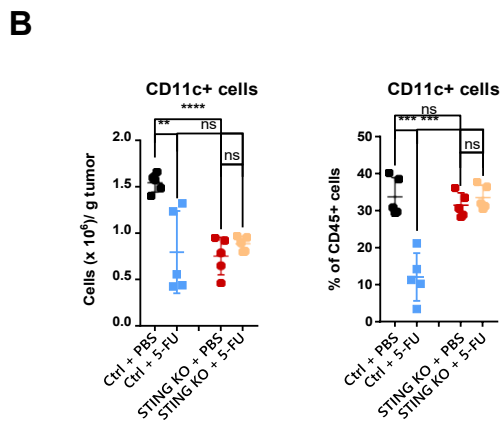
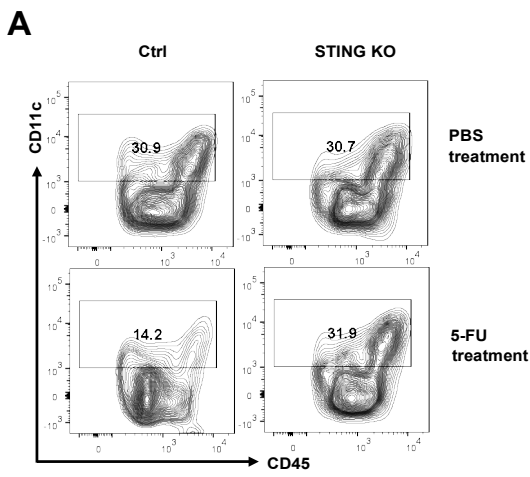
**Appendix Figure S3:** Effect of 5-FU on surface antigens in intratumoral CD45<sup>-</sup> cells

<b>sgRNA sequences</b>		
STING sgRNA 1	mouse	GTAGAATCATAGCCATACAG
STING sgRNA 2	mouse	GAAGGCCAAACATCCAAGT
STING sgRNA 3	mouse	CAGTAGTCCAAGTTCGTGCG
cGAS sgRNA 1	mouse	CAGAATGCAGAAACGGGAGT
cGAS sgRNA 2	mouse	GAGGCGCGGAAAGTCGTAAG
Ifnb1 sgRNA 1	mouse	GAAACAATTTCTCCAGCACT
Ifnb1 sgRNA 2	mouse	GTTTCTGAAGACAGTACTAG
Ifna1 sgRNA 1	mouse	TCACATCCTAGAGAGCAGGT
Ifna1 sgRNA 2	mouse	GAAGATGTTTCAGGATCTGCT
Ifnar1 sgRNA 1	mouse	TTCAGCAGAATATCGAACGT
Ifnar1 sgRNA 2	mouse	CTTCTAAACGTAAGTCTGGG

**Appendix Table S1. sgRNA sequences used in this study.**

<b>RT primers</b>		
lfnb1-f	mouse	CCCTATGGAGATGACGGAGA
lfnb1-r	mouse	CCCAGTGCTGGAGAAATTGT
lrf7-f	mouse	AGAGGGCGTTTTATCTTGCG
lrf7-r	mouse	TGGAGCCCAGCATTTTCTCT
Stat1-f	mouse	CACGCTGCCTATGATGTCTC
Stat1-r	mouse	ACGCTTGCTTTTCCGTATGT
lfit1-f	mouse	CAGAAGCACACATTGAAGAA
lfit1-r	mouse	TGTAAGTAGCCAGAGGAAGG
Gapdh-f	mouse	ATGTGTCCGTCGTGGATCTGAC
Gapdh-r	mouse	AGACAACCTGGTCCTCAGTGTAG

**Appendix Table S2. Primers used in this study.**



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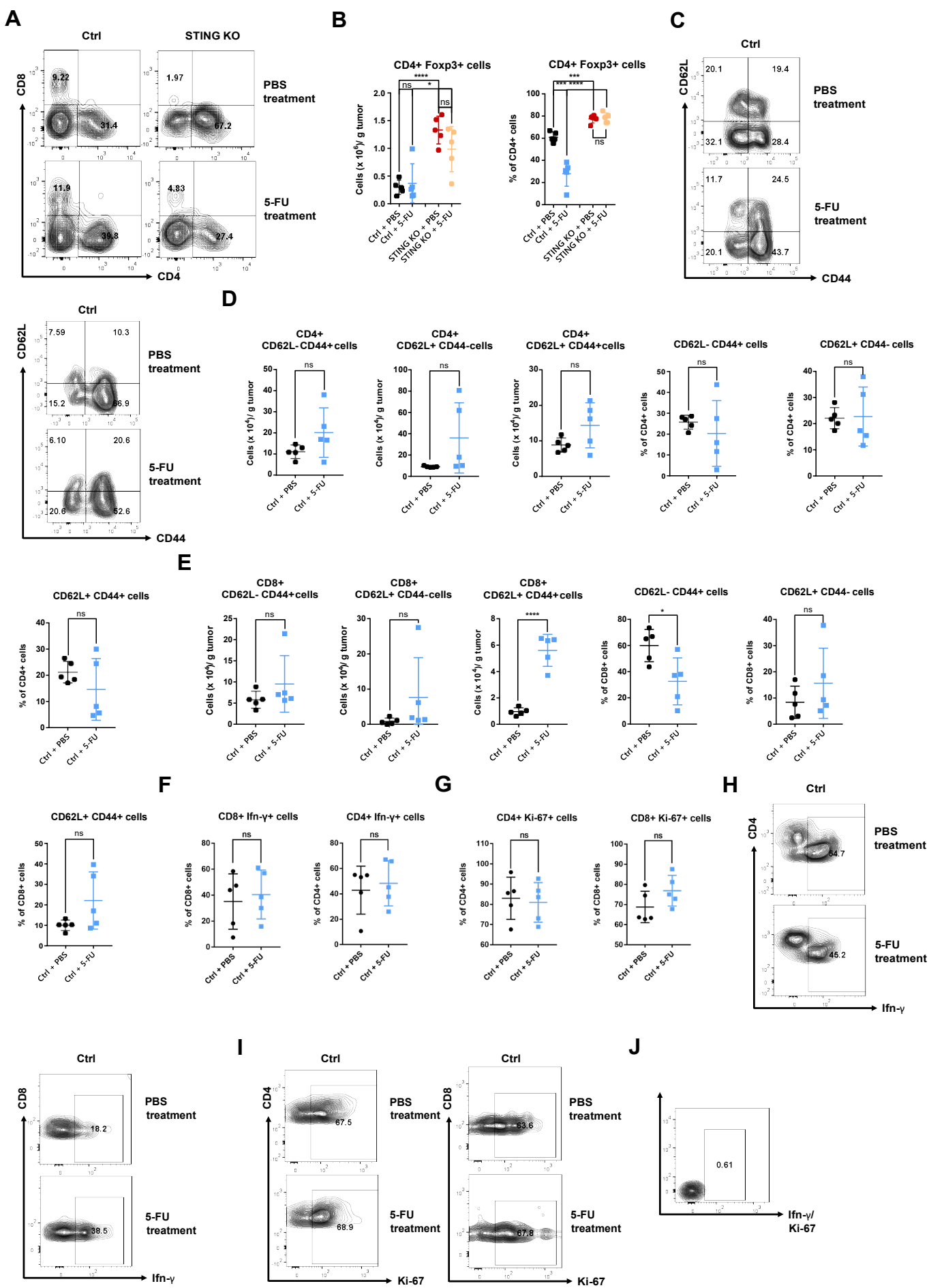
**Appendix Figure S1. Effect of 5-FU treatment on intratumoral dendritic cells.**

**A.** Representative flow cytometry plots showing the percentages of intratumoral CD11c<sup>+</sup> cells, after gating on CD45<sup>+</sup> cells.

**B.** The counts of intratumoral CD11c<sup>+</sup> cells per gram of tumor were quantified (left panel). The percentages of CD11c<sup>+</sup> cells within intratumoral CD45<sup>+</sup> cells (right panel). Each dot represents one mouse. N=5.

**C.** Representative flow cytometry plots showing the percentages of CD103<sup>+</sup> cells within intratumoral CD11c<sup>+</sup> cells.

For all panels, error bars represent s.d., and center values represent mean. Two-tailed unpaired student's t-test was used. \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ; \*\*\*\*:  $p < 0.0001$ ; ns: not significant.



## Appendix Figure S2. Analysis of intratumoral T cells after 5-FU treatment

**A.** Representative flow cytometry plots showing the percentages of CD4<sup>+</sup> or CD8<sup>+</sup> T cells within intratumoral CD3<sup>+</sup> cells.

**B.** (Left) The counts of intratumoral Tregs (CD4<sup>+</sup>FoxP3<sup>+</sup>) per gram of tumor were quantified. (Right) The percentages of Tregs among CD4<sup>+</sup> cells were quantified. N=5. Each dot represents one mouse.

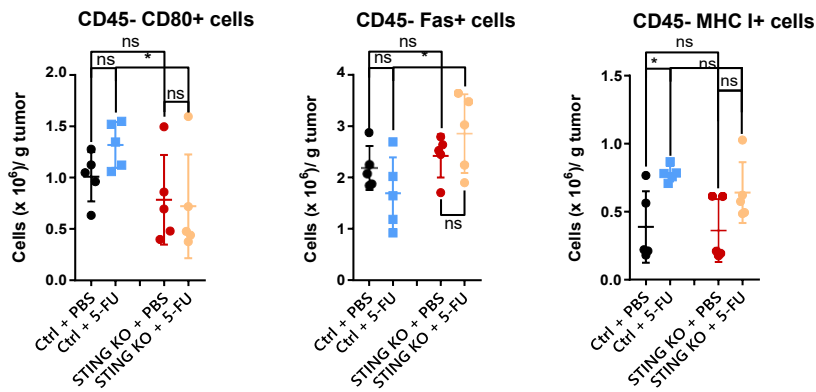
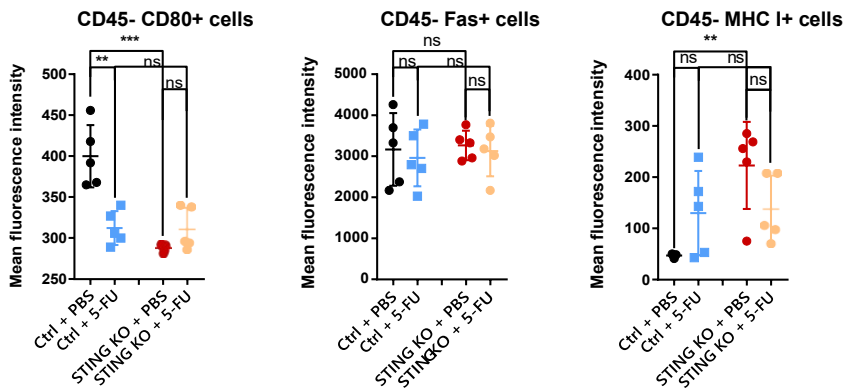
**C.** Representative flow cytometry plots analyzing intratumoral CD4<sup>+</sup> (left panel) or CD8<sup>+</sup> (right panel) T cells with CD62L and CD44.

**D.** The counts of CD4<sup>+</sup> intratumoral CD62L<sup>-</sup>CD44<sup>+</sup>, CD62L<sup>+</sup>CD44<sup>-</sup>, and CD62L<sup>+</sup>CD44<sup>+</sup> cells per gram of tumor were quantified. Additionally, the corresponding percentage of each population among CD4<sup>+</sup> T cells were quantified. N=5. Each dot represents one mouse.

**E.** The counts of CD8<sup>+</sup> intratumoral CD62L<sup>-</sup>CD44<sup>+</sup>, CD62L<sup>+</sup>CD44<sup>-</sup>, and CD62L<sup>+</sup>CD44<sup>+</sup> cells per gram of tumor were quantified. Additionally, the corresponding percentage of each population among CD8<sup>+</sup> T cells were quantified. N=5. Each dot represents one mouse.

**F-J.** Control MC38 cells were injected into mice and mice were treated with 5-FU or PBS. Tumors were harvested at 2 weeks post cancer cell injection. **(F)** The percentages of intratumoral Ifn- $\gamma$ <sup>+</sup> cells within CD4<sup>+</sup> (left panel) or CD8<sup>+</sup> (right panel) cells were quantified. **(G)** The percentages of intratumoral Ki67<sup>+</sup> cells within CD4<sup>+</sup> (left panel) or CD8<sup>+</sup> (right panel) cells were quantified. Each dot represents one mouse. **(H)** Representative flow cytometry plots showing the percentages of Ifn- $\gamma$ <sup>+</sup> cells within CD4<sup>+</sup> and CD8<sup>+</sup> cells. **(I)** Representative flow cytometry plots showing the percentages of Ki67<sup>+</sup> cells within CD4<sup>+</sup> and CD8<sup>+</sup> cells. **(J)** Flow cytometry plots showing the negative control of staining for (H) and (I).

For all panels, error bars represent s.d., and center values represent mean. Two-tailed unpaired student's t-test was used. \*: p < 0.05; \*\*\*\*: p < 0.0001; ns: not significant.

**A****B**

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**Appendix Figure S3. Effect of 5-FU on surface antigens in intratumoral CD45<sup>+</sup> cells.**

**A-B.** Mice were injected with control (Ctrl) or STING-KO MC38 cells and treated with PBS or 5-FU. Tumors were harvested 2 weeks after cancer cell injection and intratumoral CD45<sup>+</sup> cells were examined by flow cytometry. **(A)** The counts of CD45<sup>+</sup>CD80<sup>+</sup>, CD45<sup>+</sup>Fas<sup>+</sup>, and CD45<sup>+</sup>MHC-I<sup>+</sup> cells per gram of tumor were quantified. Each dot represents one mouse. N=5. **(B)** The mean fluorescence intensities of CD80, Fas and MHC-I in CD45<sup>+</sup>CD80<sup>+</sup>, CD45<sup>+</sup>Fas<sup>+</sup>, and CD45<sup>+</sup>MHC-I<sup>+</sup> cells, respectively, were quantified. Each dot represents one mouse. N=5.

For all panels, error bars stand for s.d., and center values represent mean. Two-tailed unpaired student's t-test was used. \*: p < 0.05; ns: not significant. Data are representative of two independent experiments.