Supporting Information
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Sulfisoxazole elicits robust antitumour immune response along with immune checkpoint therapy by inhibiting exosomal PD-L1
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**Sulfisoxazole elicits robust antitumour immune response along with immune checkpoint therapy by inhibiting exosomal PD-L1**

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**Figure S1.** PD-L1, ETA, and ETB expression in murine cancer cell lines (4T1: breast cancer, B16F10: melanoma, CT26: colon cancer).
Figure S2. Sulfisoxazole (SFX) inhibits cancer EXO biogenesis and suppresses exosomal PD-L1 from CT26 murine colon cancer cell line. (a) Immunoblot for the indicated proteins in cell lysates and exosomes from CT26 cells treated with or without SFX. Beta-actin was used as loading controls for cell lysates. Exosomal proteins obtained from equal cell numbers (1 x 10^7) were loaded per lanes. (b) Quantification of PD-L1 and Rab27 in whole cell lysates (n = 3). (c) Quantification of exosomal proteins (n = 3). Significance was determined using an
unpaired two-tailed Student’s *t*-test. ***$p<0.001$, **$p<0.01$ and *$p<0.05$. Error bar, standard deviation (SD).

**Figure S3.** Quantification of exosomal PD-L1 in plasma from CT26 tumor-bearing mice. (a) Experimental regime of exosomal PD-L1 isolation. (b) Relative exosomal PD-L1 in plasma from WT and CT26 tumor-bearing mice ($n = 7$ and 11 for WT and CT26, respectively). Significance was determined using an unpaired two-tailed Student’s *t*-test. ***$p<0.001$, **$p<0.01$ and *$p<0.05$. Error bar, standard deviation (SD).
Figure S4. Antitumor efficacy of combinational treatment of SFX and αPD-L1 in CT26 tumor-bearing mice. (a) Experimental scheme for antitumor efficacy. (b) Average tumor volume ($n = 7$). (c) Individual tumor volume ($n = 7$). Significance was determined using an ANOVA with Tukey correction. ***$p<0.001$, **$p<0.01$ and *$p<0.05$. Error bar, standard deviation (SD).
Figure S5. Antitumor efficacy of SFX, αPD-1, and SFX + αPD-1. (a) Individual tumor volume (n = 9). (b) Tumor weight after treatment (n = 9). (c) Changes in body weights (n = 9). (d) Photographs of the tumors harvested at 23 d (n = 9). Significance was determined using an ANOVA with Tukey correction. ***p<0.001, **p<0.01 and *p<0.05. Error bar, standard deviation (SD).
Figure S6. (a) Immunofluorescence microscopy images of CD8$^+$ (upper) and CD4$^+$ (lower) cells in the tumor microenvironment. Scale bars, 200 nm. Blue, cell nuclei. The images are representative of three independent experiments. (b) Relative value of fluorescence intensity to DAPI for CD8-FITC (left) and CD4-FITC (right), corresponding to data in a ($n = 3$). Significance was determined using an ANOVA with Tukey correction. $***p<0.001$, $**p<0.01$ and $*p<0.05$. Error bar, standard deviation (SD).
Figure S7. CD4/CD8 ratio of tumor-infiltrated lymphocytes in tumor microenvironment
(DPBS; n = 5, SFX; n = 5, αPD-1; n = 4 and SFX+ αPD-1; n = 5).