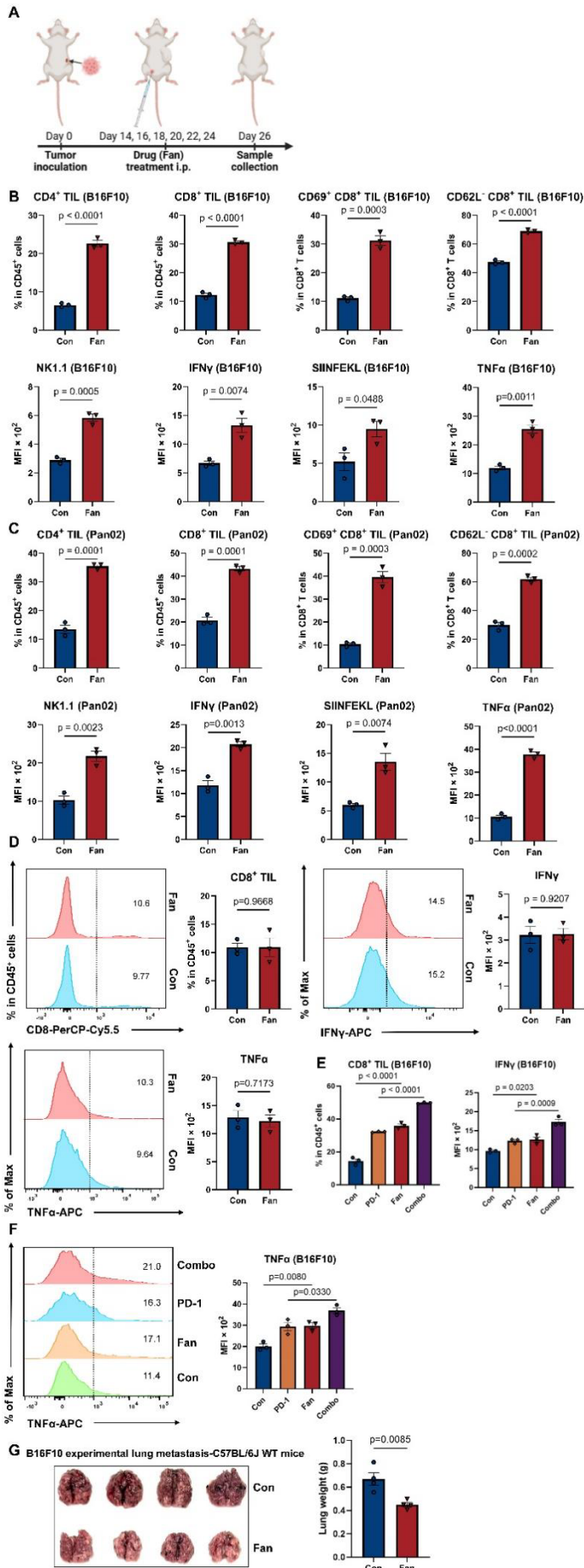


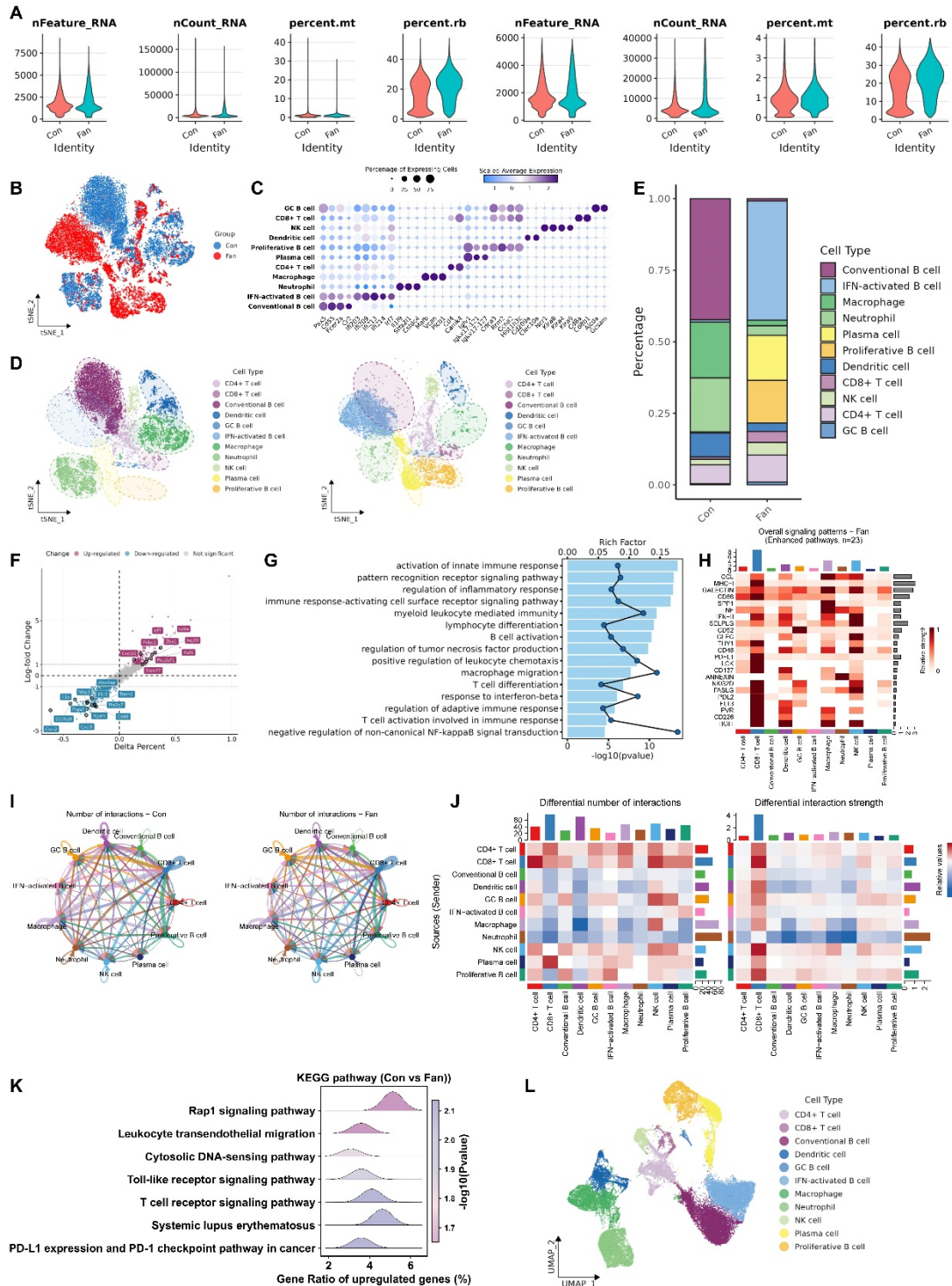
### Supplementary Figure 1

(A) Secreted type I IFN measured by ELISA in the same THP1, BMDMs, RAW264.7 and HT1080 cells and treatments as in Figure 1A-1D. Ordinary one-way ANOVA test. (B) Secreted type I IFN measured by ELISA in mice serum and targeted organs. Six-week-old C57BL/6J mice 6 hours after a single intraperitoneal dose of Fan (20 mg/kg). Unpaired t test. (C) Secreted type I IFN measured by ELISA in the same cell lines and treatments as in Figure 1F. Two-way ANOVA test. (D) Dose-response analysis of Fan in RAW 264.7 cells. The apparent  $EC_{50}$  was determined based on *IFNβ1* mRNA induction after treatment with the indicated concentrations of Fan. (E) The  $CC_{50}$  was determined in parallel using a cell viability assay under the same treatment conditions. (F) Body weight monitoring of C57BL/6J mice during two weeks of repeated intraperitoneal administration of Fan or vehicle control under the indicated dosing schedule. Two-way ANOVA test. Data are shown as mean  $\pm$  SEM.



## Supplementary Figure 2

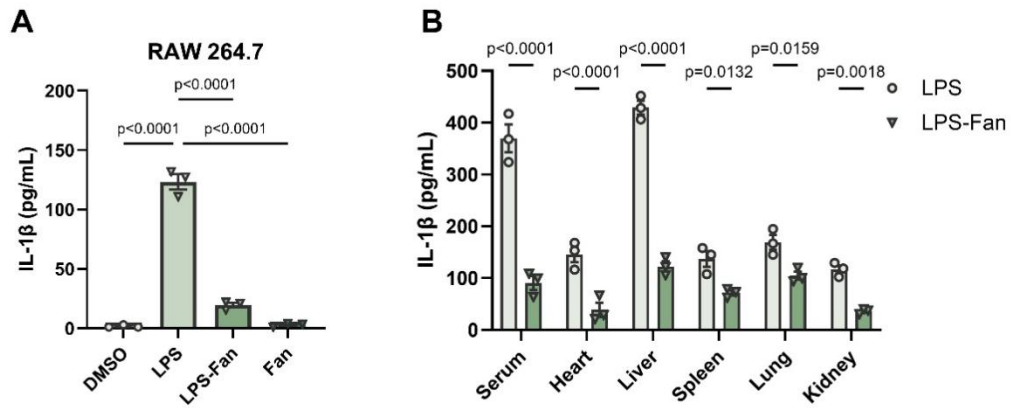
(A) Schematic illustration of the experimental design for evaluating the therapeutic efficacy of Fan in ectopic tumor models. (B) Quantification of tumor infiltrating CD45<sup>+</sup> CD4<sup>+</sup> TILs, CD45<sup>+</sup> CD8<sup>+</sup> TILs, CD69<sup>+</sup> CD8<sup>+</sup> TILs, CD62L<sup>-</sup> CD8<sup>+</sup> TILs, NK1.1<sup>+</sup> TILs, IFN $\gamma$ <sup>+</sup> TILs, SIINFEKL<sup>+</sup> TILs and TNF $\alpha$ <sup>+</sup> TILs of mice as in Figure 2A. Unpaired t test. (C) Quantification of tumor infiltrating CD45<sup>+</sup> CD4<sup>+</sup> TILs, CD45<sup>+</sup> CD8<sup>+</sup> TILs, CD69<sup>+</sup> CD8<sup>+</sup> TILs, CD62L<sup>-</sup> CD8<sup>+</sup> TILs, NK1.1<sup>+</sup> TILs, IFN $\gamma$ <sup>+</sup> TILs, SIINFEKL<sup>+</sup> TILs and TNF $\alpha$ <sup>+</sup> TILs of mice as in Figure 2D. Unpaired t test. (D) Representative FACS data and quantification of tumor infiltrating CD45<sup>+</sup> CD8<sup>+</sup> TILs, IFN $\gamma$ <sup>+</sup> TILs and TNF $\alpha$ <sup>+</sup> TILs of mice as in Figure 2G. Unpaired t test. (E) Quantification of tumor infiltrating CD45<sup>+</sup> CD8<sup>+</sup> TILs, IFN $\gamma$ <sup>+</sup> TILs of mice as in Figure 2I. Ordinary one-way ANOVA test. (F) Representative FACS data and quantification of tumor infiltrating TNF $\alpha$ <sup>+</sup> TILs of mice as in Figure 2I. Ordinary one-way ANOVA test. (G) Representative images of lungs and lung weights at the experimental endpoint in the B16F10 lung metastasis model. Unpaired t test. Data are shown as mean  $\pm$  SEM.



### Supplementary Figure 3

(A) The panel showed pre-QC distributions of key quality metrics and the corresponding post-QC distributions following application of our filtering criteria. (B) t-SNE visualization of scRNA-seq data showing the distribution of immune cells from Con and Fan treatment groups. (C) Marker gene expression across the cell clusters was shown as a dot plot. The size and color of the dots respectively represent the proportion of cells expressing the corresponding genes and the average scaling value

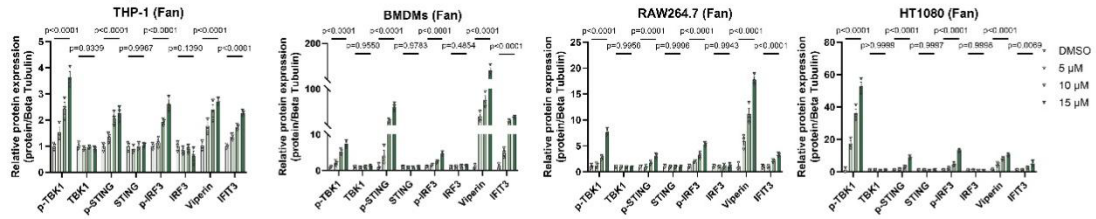
of their logarithmic transformation expression. (D) t-SNE projection of scRNA-seq data annotated by major immune cell types. (E) Bar chart comparing the proportions of major cell types between Fan- and PBS-treated groups in CD45<sup>+</sup> B16F10 tumors. (F) Differential cell-type abundance analysis comparing Fan and Con groups. (G) Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs) in CD45<sup>+</sup> cells following Fan treatment compared to Con. (H) Heatmap of gene expression across various cell types in the Fan- and PBS-treated groups. (I) Interaction network between different cell types in the tumor microenvironment, showing predicted interactions. Line thickness indicated the number of interactions or the strength of interactions. (J) Heatmap of differential number of interactions across various cell types in the Fan and Con groups. (K) KEGG pathway analysis of DEGs. (L) UMAP visualization of scRNA-seq data showing the distribution of immune cells from Con and Fan treatment groups.



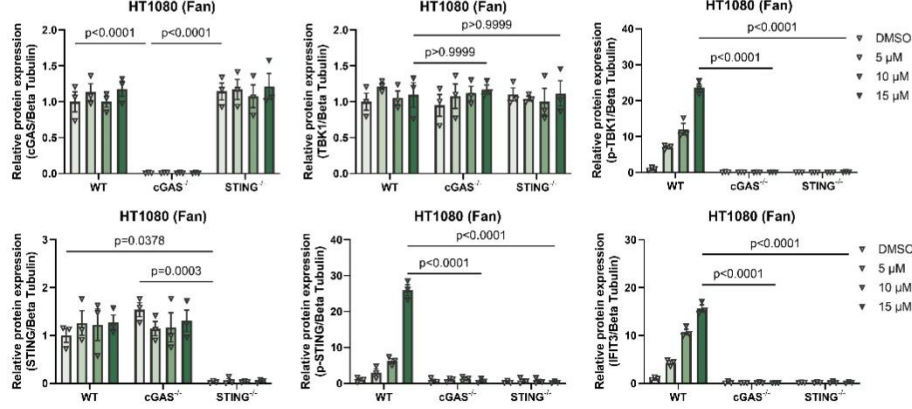
#### Supplementary Figure 4

(A) ELISA quantification of secreted IL-1 $\beta$  in the culture supernatants of RAW264.7 cells following treatment with DMSO, LPS, LPS+Fan, or Fan alone. Ordinary one-way ANOVA test. (B) ELISA quantification of IL-1 $\beta$  protein levels in serum, heart, liver, spleen, lung, and kidney from C57BL/6J mice following LPS challenge with or without Fan treatment. Unpaired t test. Data are shown as mean  $\pm$  SEM.

**A Related to Figure 1A-1D**



**B Related to Figure 1G**



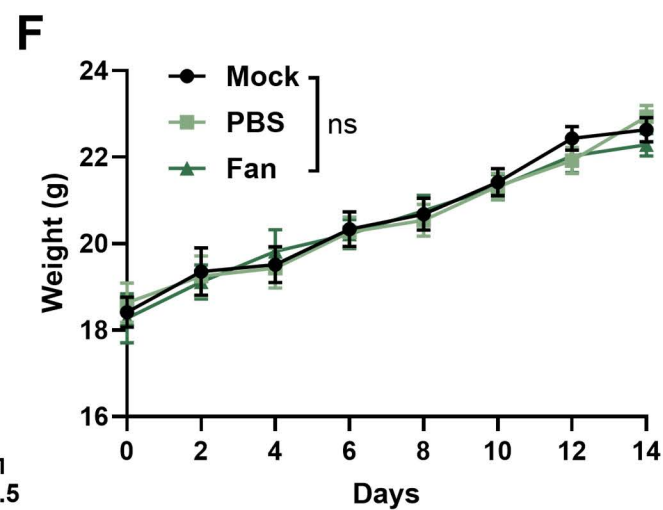
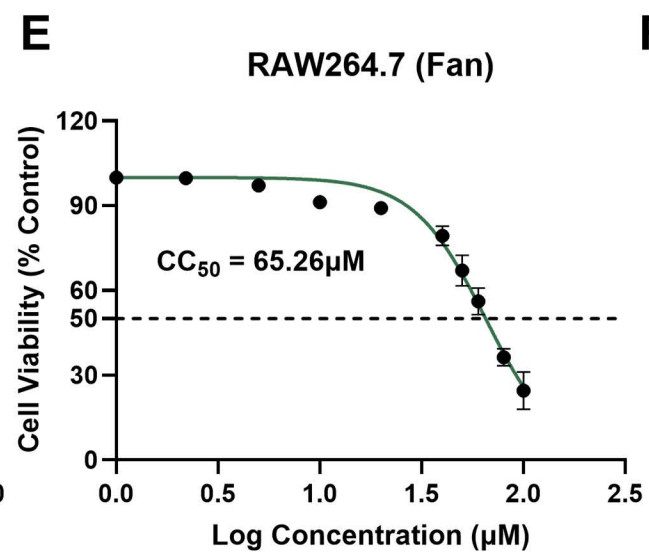
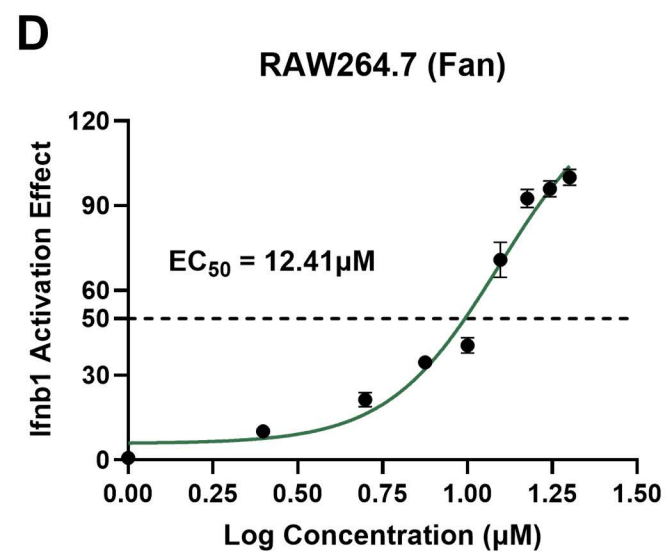
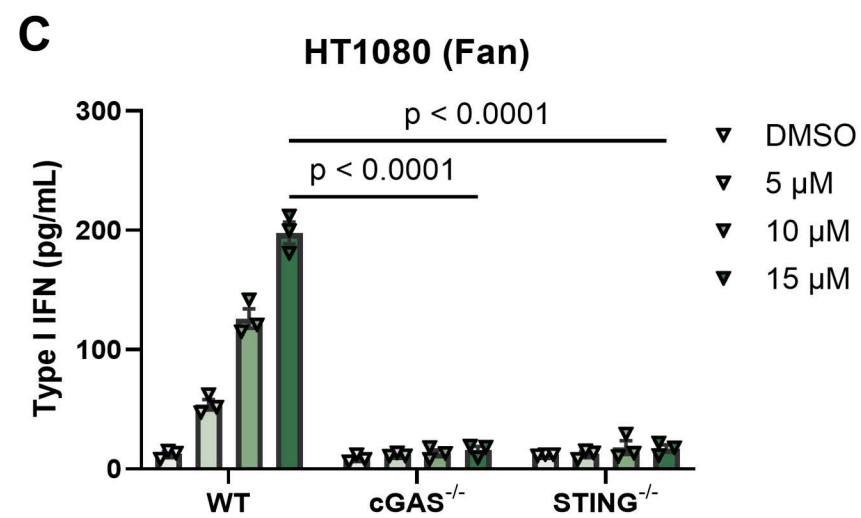
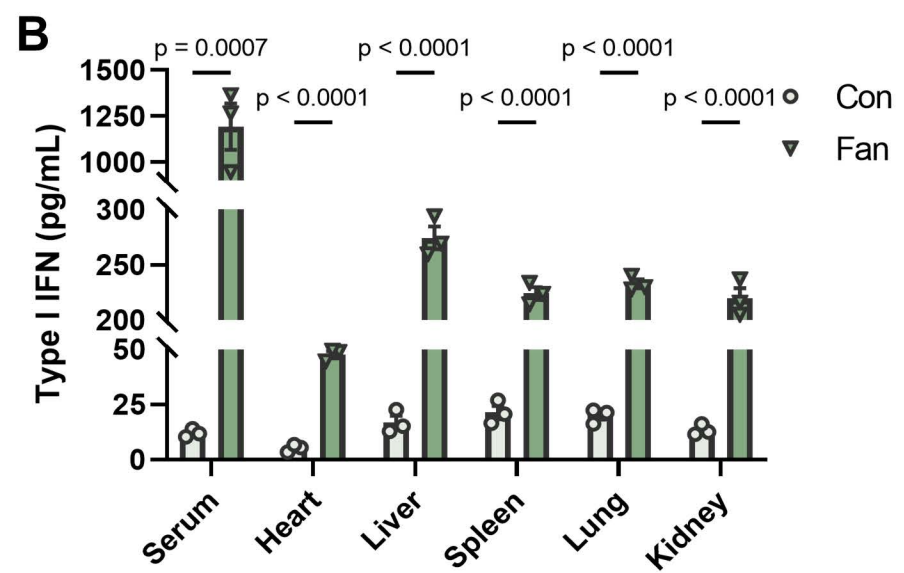
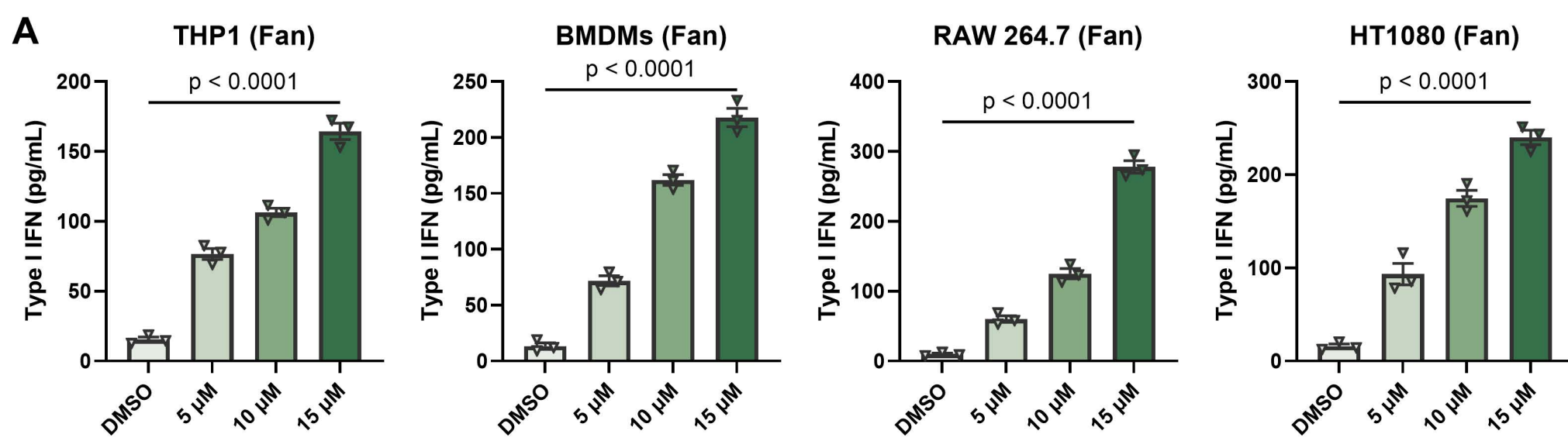
**Supplementary Figure 5**

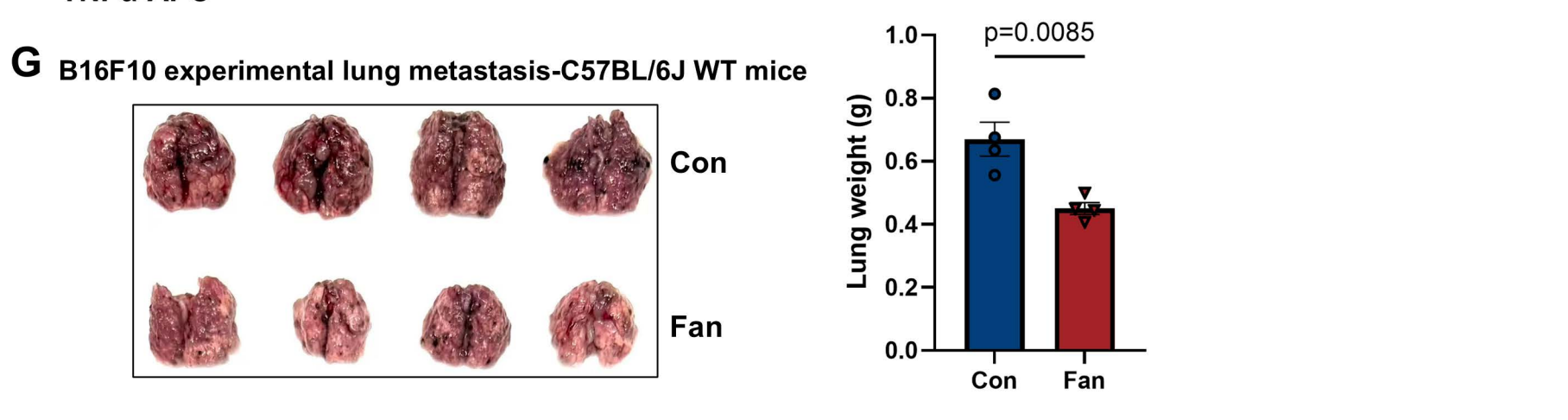
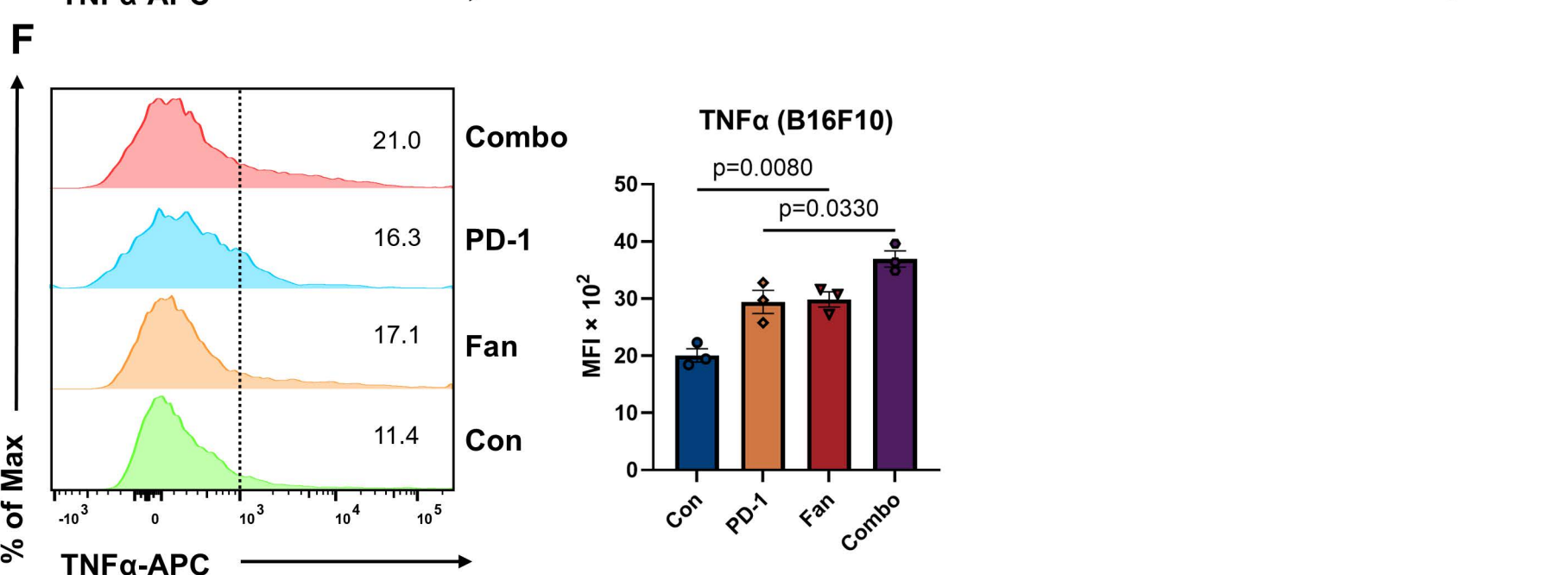
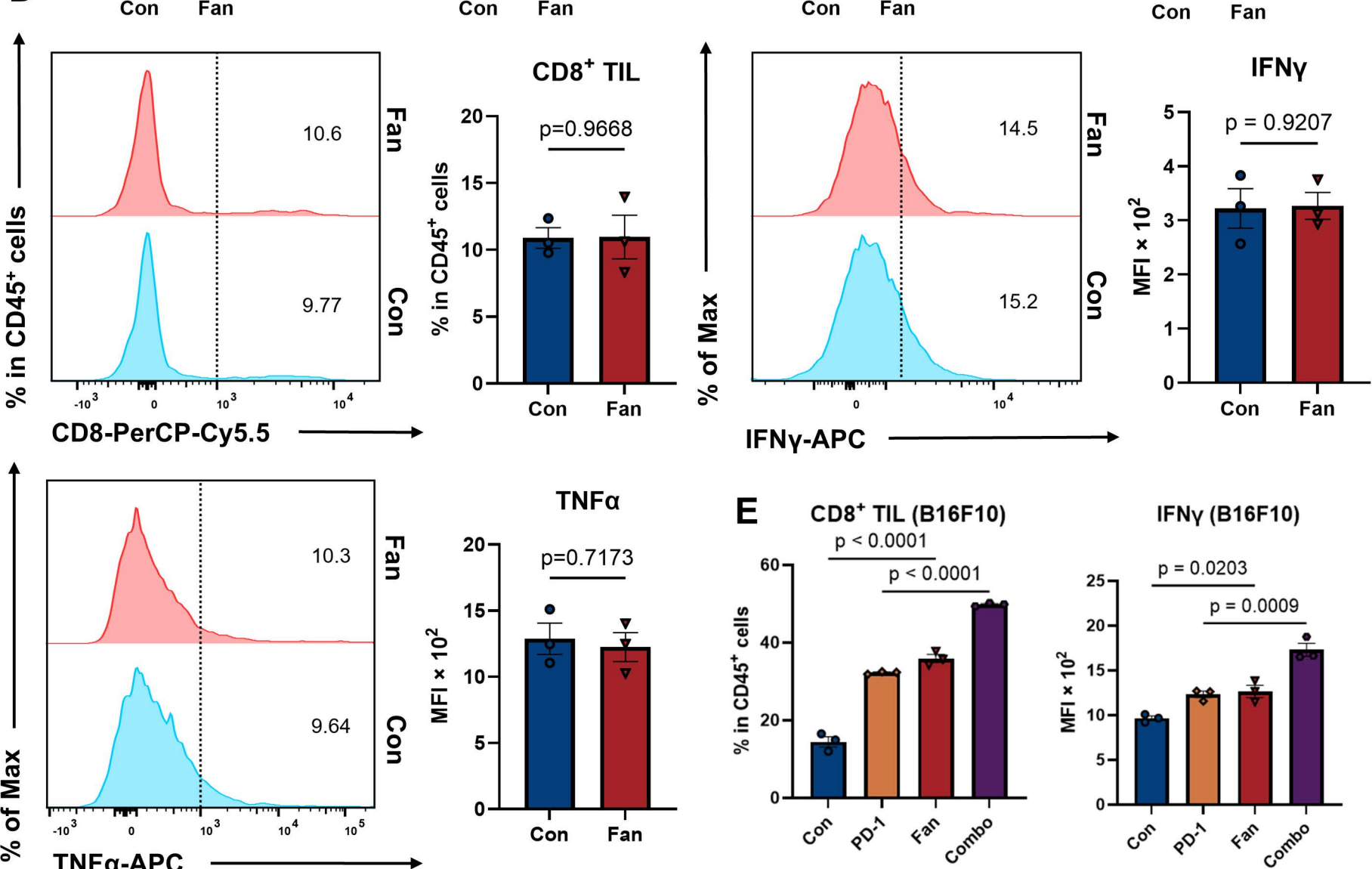
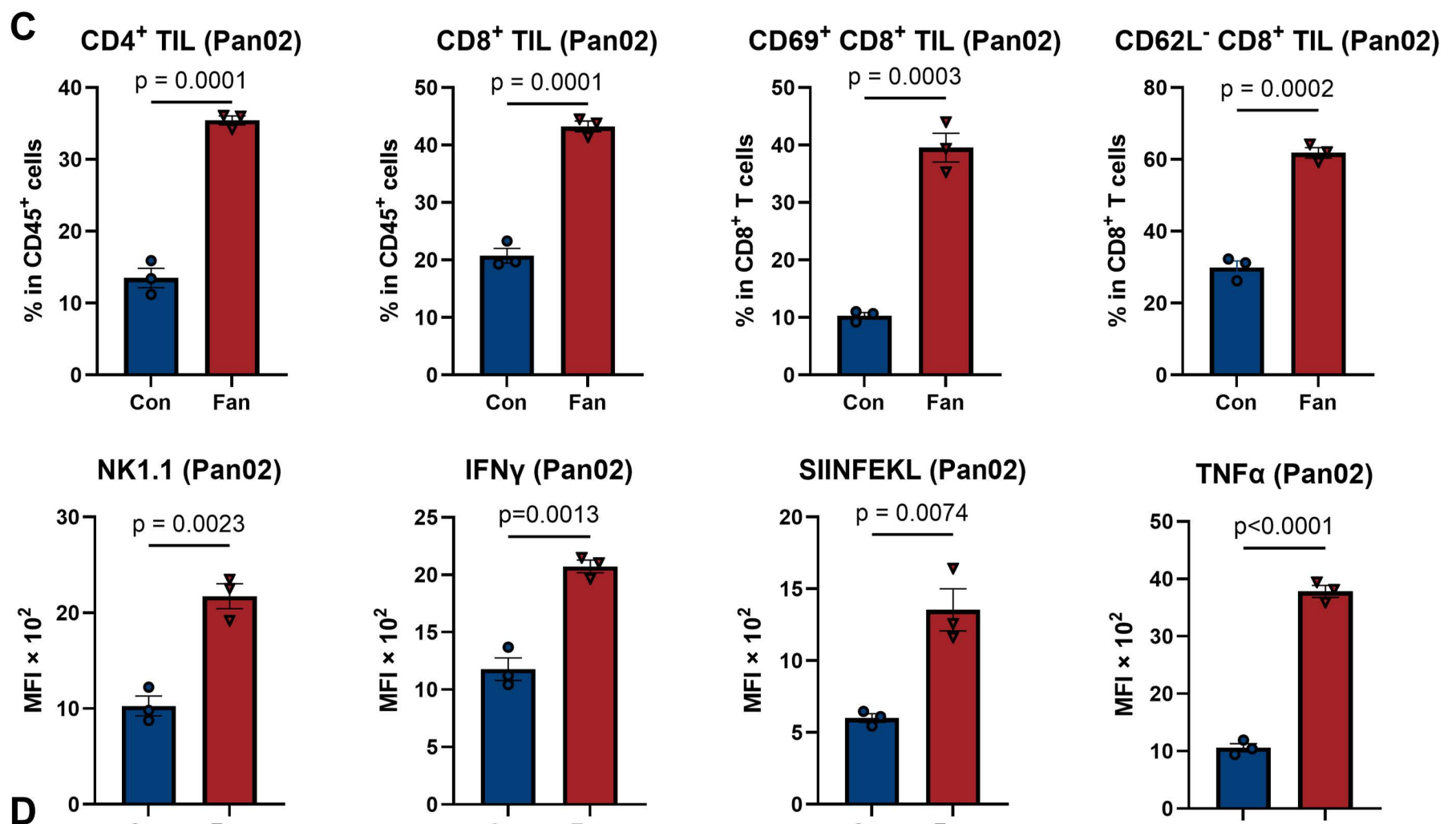
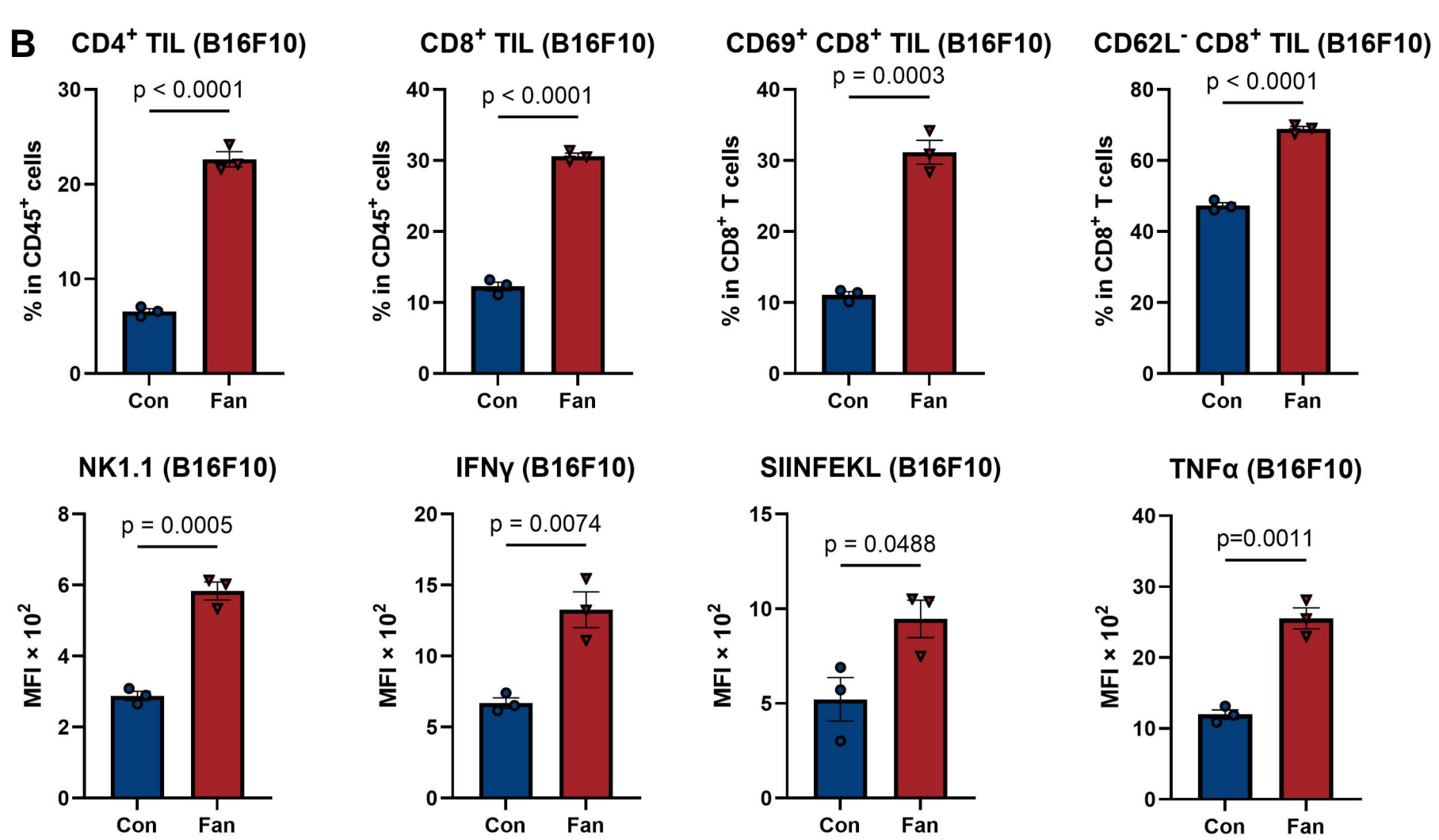
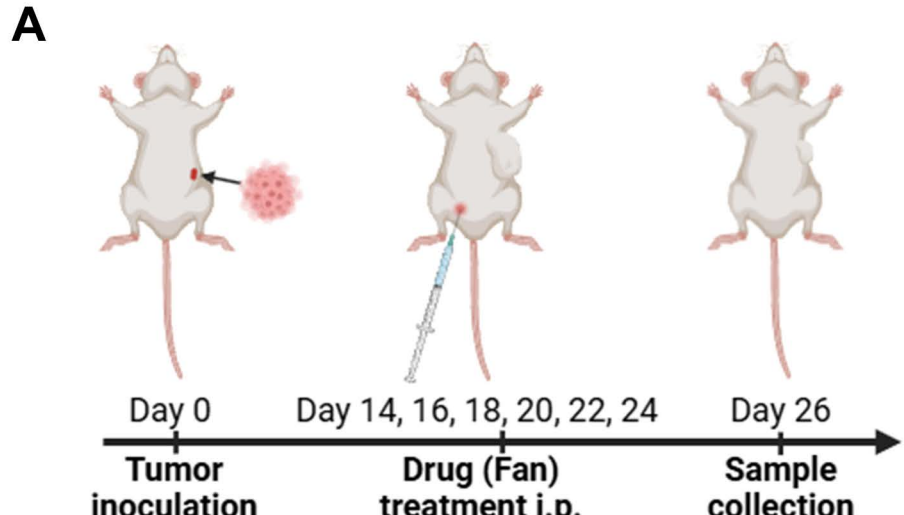
(A-B) quantification of band intensities in Figure 1A-1D (A) and Figure 1G (B). Densitometry was performed in ImageJ 1.51 using fixed rectangular ROIs with local background subtraction. Target proteins were normalized to loading control and then to the vehicle group, which was set to 1.0. Data are shown as mean ± SEM (n = 3 independent experiments). Two-way ANOVA test. Data are shown as mean ± SEM.

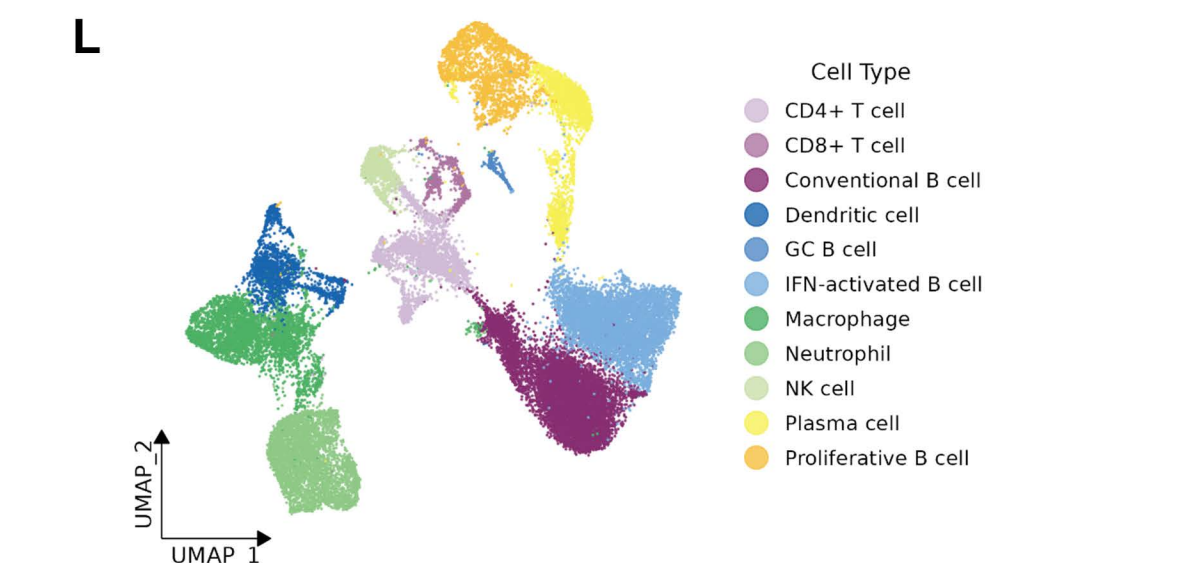
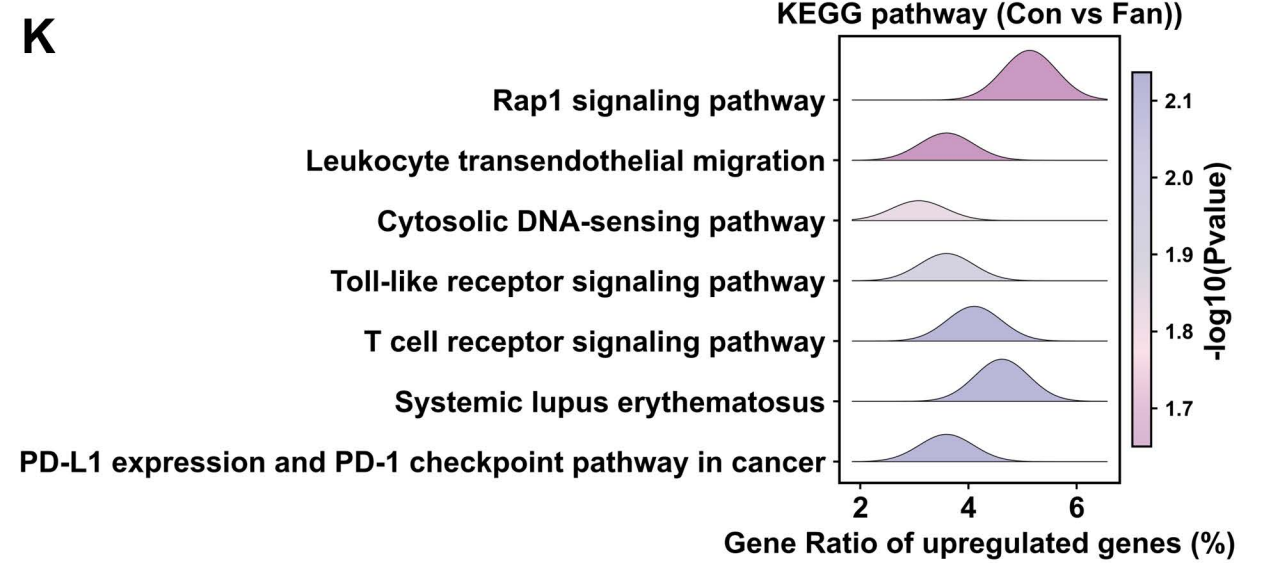
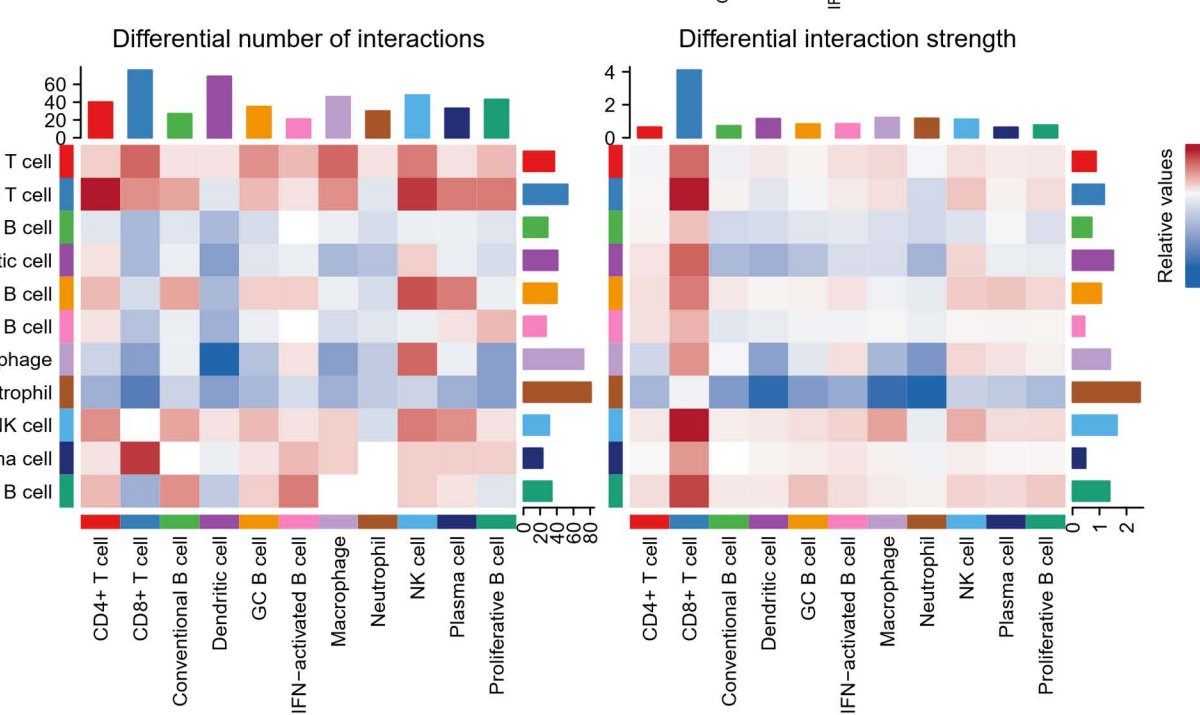
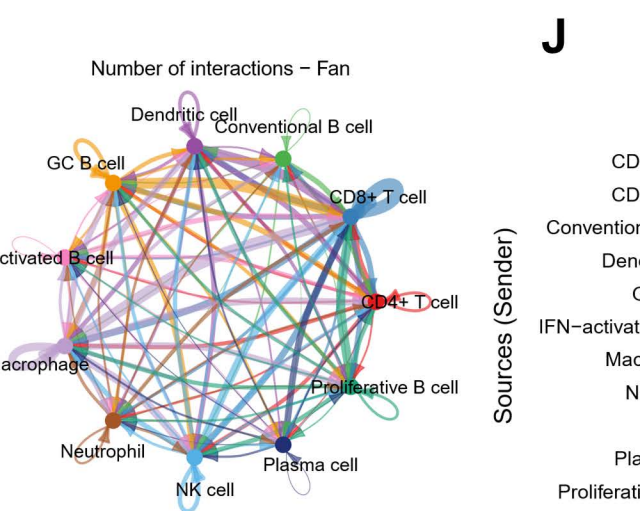
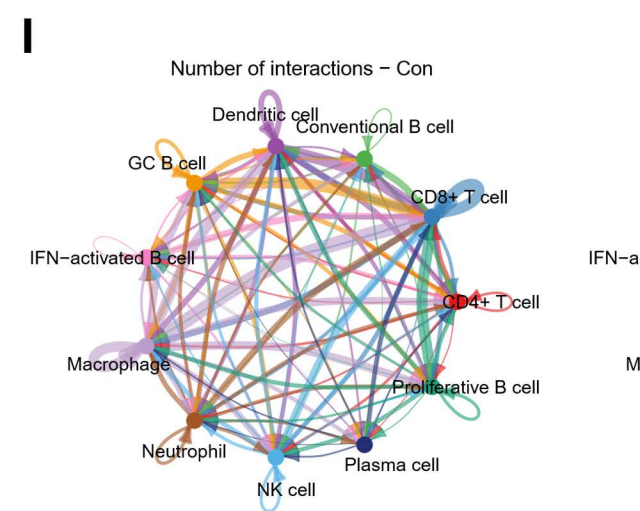
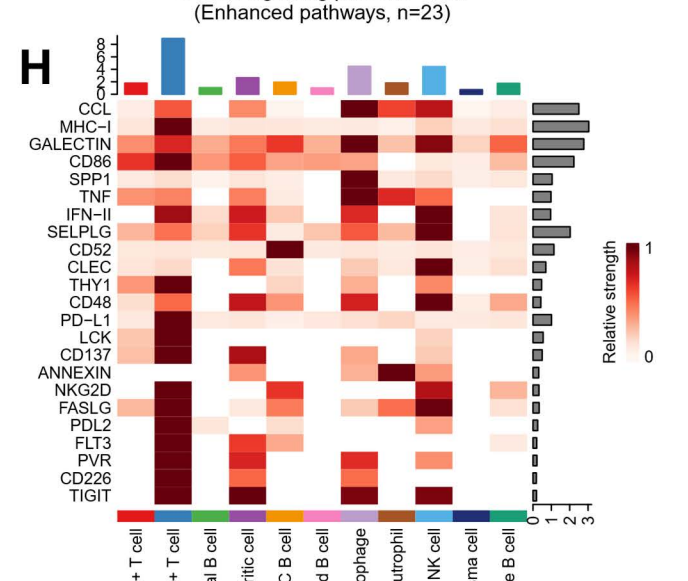
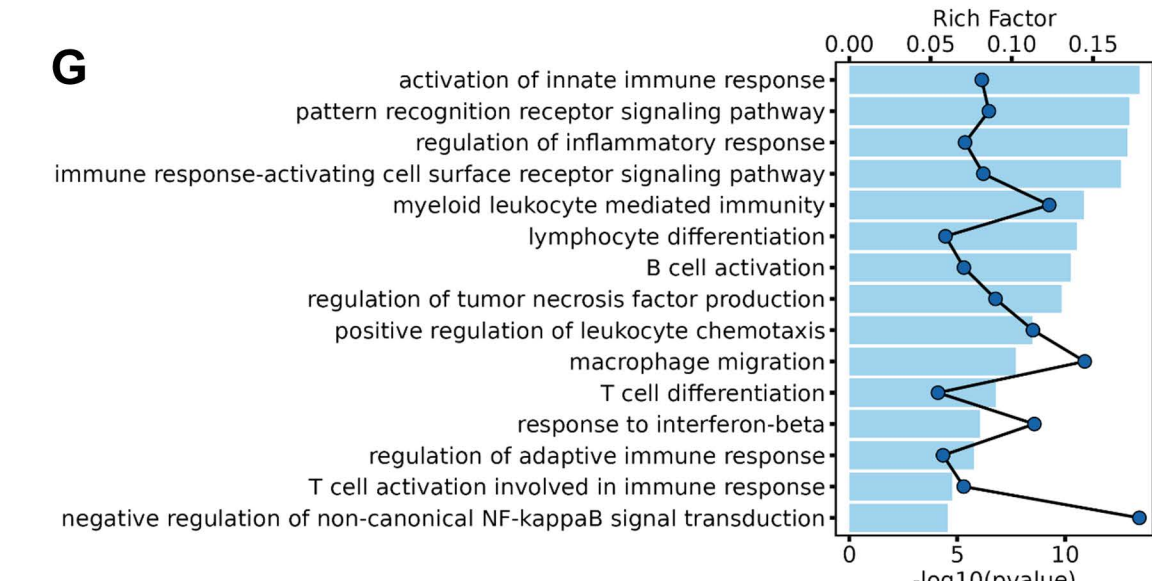
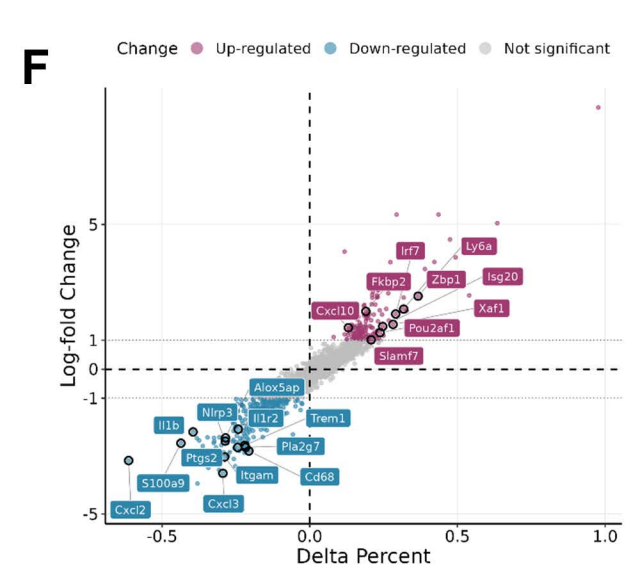
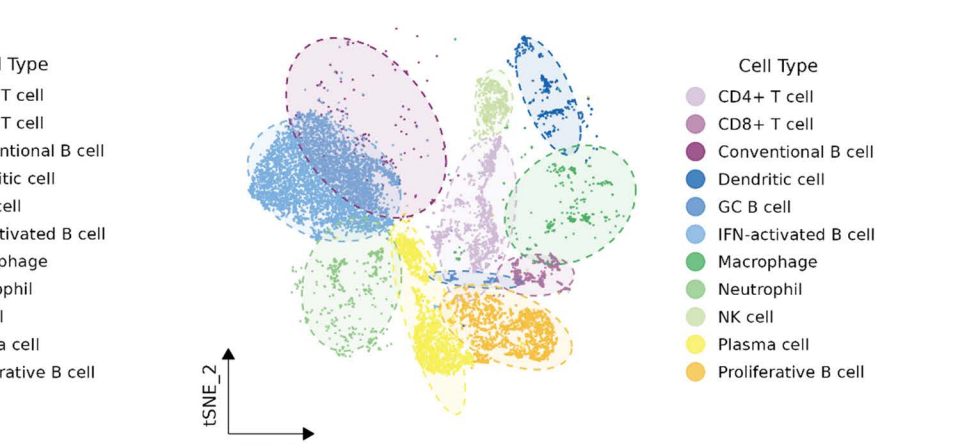
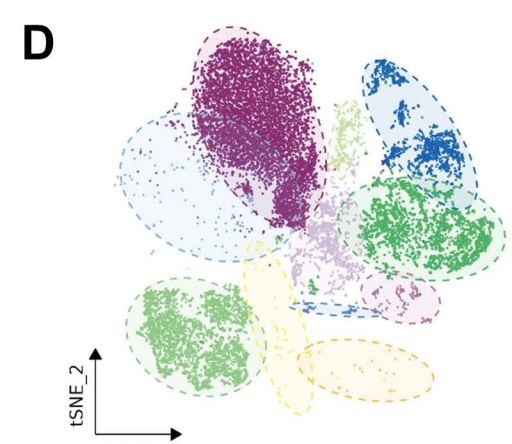
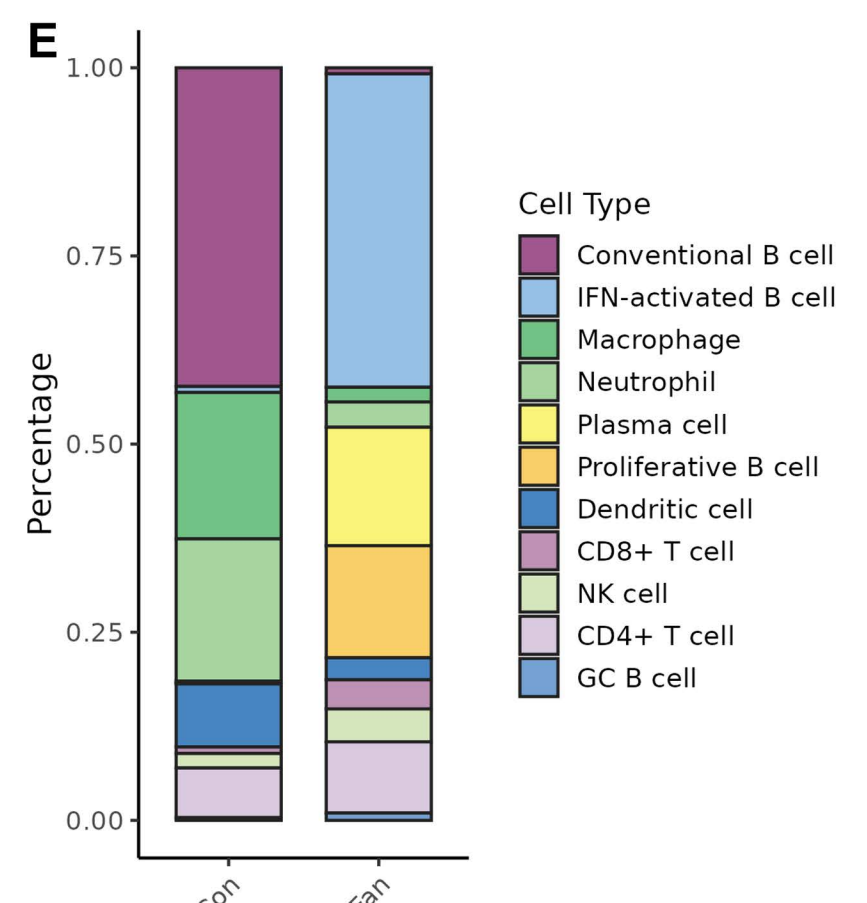
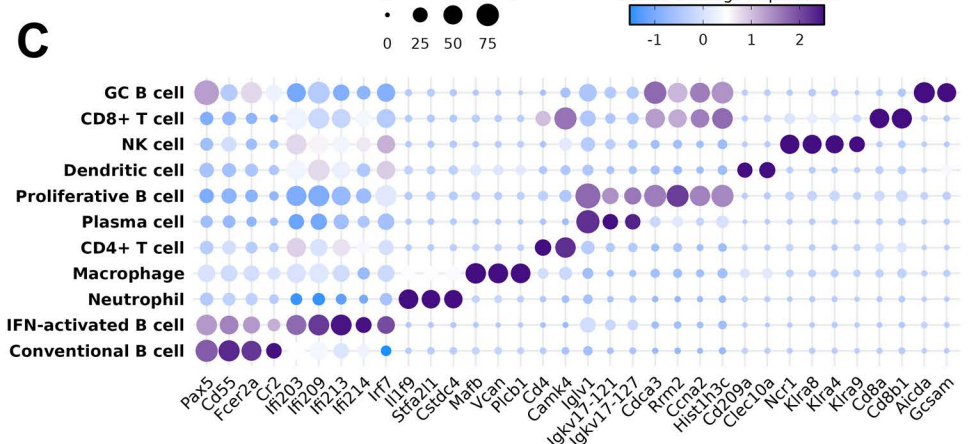
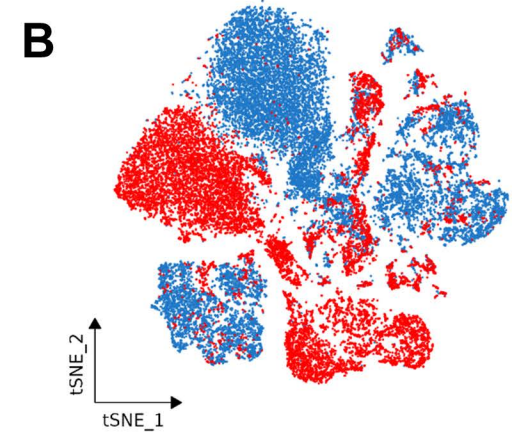
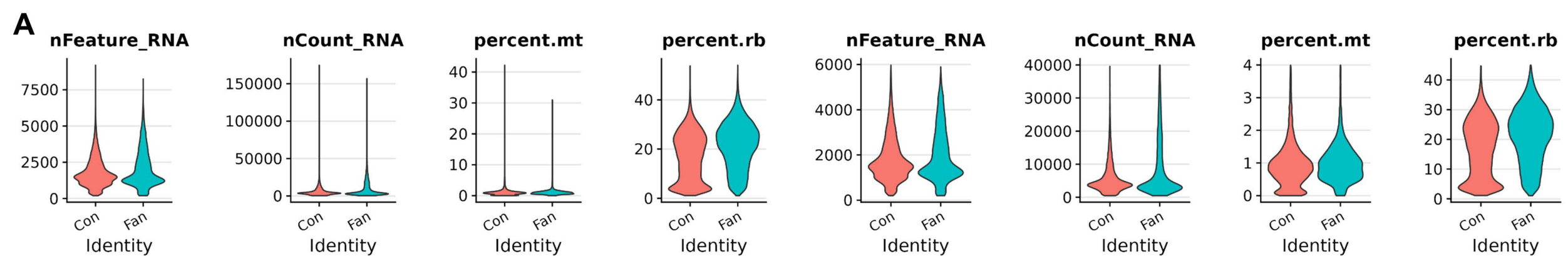
**Supplementary Table 1. Primer sequences used for RT-qPCR.**

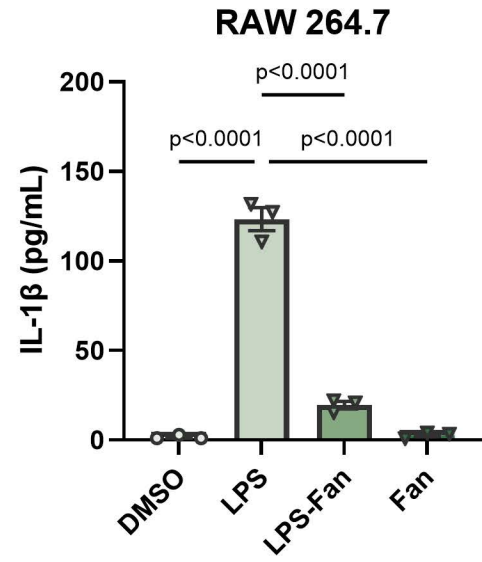
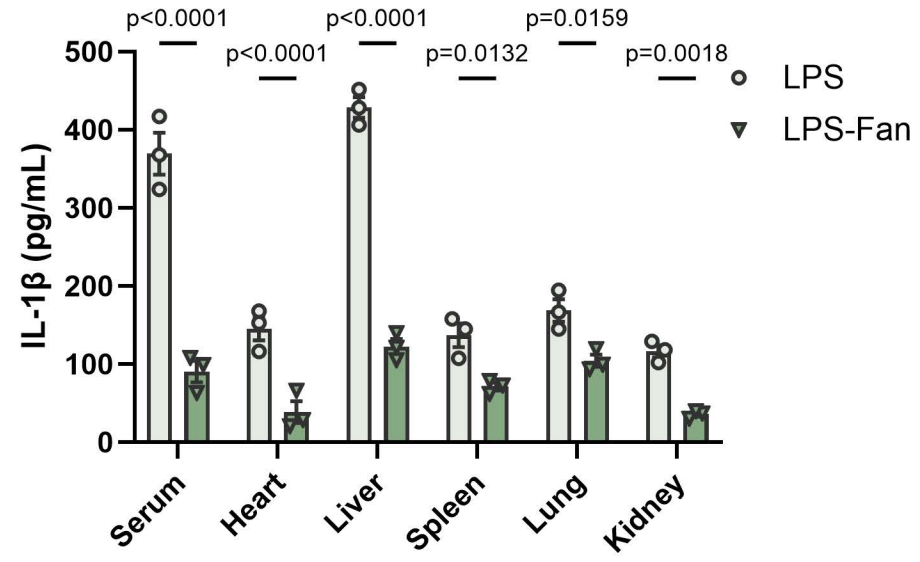
Forward and reverse primer sequences (5'→3') for all genes analyzed in this study.

<b>Gene</b>	<b>Primer sequences</b>
<b>hIFNB</b>	F: 5-GCTTGGATTCCTACAAAGAAGCA-3 R: 5-ATAGATGGTCAATGCGGCGTC-3
<b>mIl1b</b>	F: 5-AGTTGACGGACCCCAAA-3 R: 5-TCTTGTTGATGTGCTGCTG-3
<b>mIl6</b>	F: 5-CTGCAAGAGACTTCCATCCAG-3 R: 5-AGTGGTATAGACAGGTCTGTTGG-3
<b>mIfnb</b>	F: 5-TCCGAGCAGAGATCTTCAGGAA-3 R: 5-TGCAACCACCACTCATTCTGAG-3
<b>hACTB</b>	F: 5-CCTGGCACCCAGCACAAT-3 R: 5-GCCGATCCACACGGAGTACT-3
<b>mActb</b>	F: 5-GGCTGTATTCCCCTCCATCG-3 R: 5-CCAGTTGGTAACAATGCCATGT-3
<b>mNos2</b>	F: 5-GTTCTCAGCCCAACAATAACAAGA-3 R: 5-GTGGACGGGTCGATGTCAC-3
<b>mPtgs2</b>	F: 5-TTCAACACACTCTATCACTGGC-3 R: 5-AGAAGCGTTTGCGGTACTCAT-3
<b>mLta</b>	F: 5-CCACCTCTTGAGGGTGCTTG-3 R: 5-CATGTCGGAGAAAGGCACGAT-3

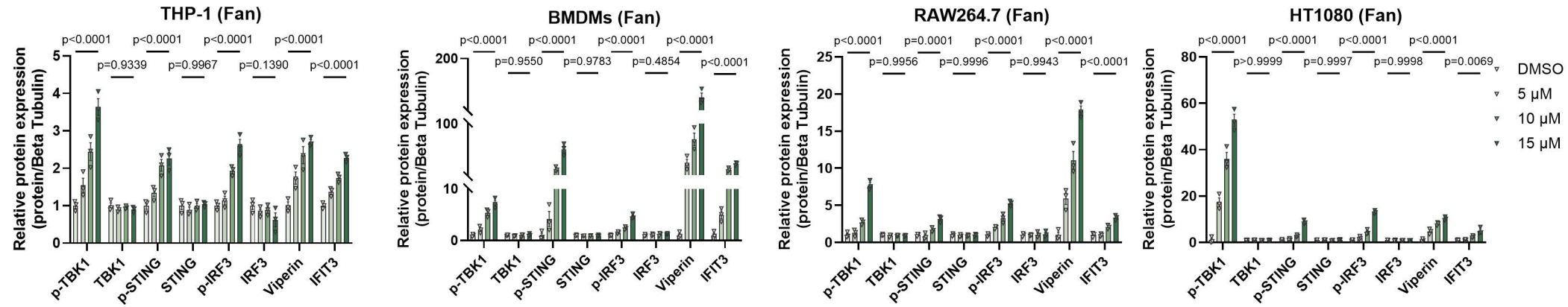




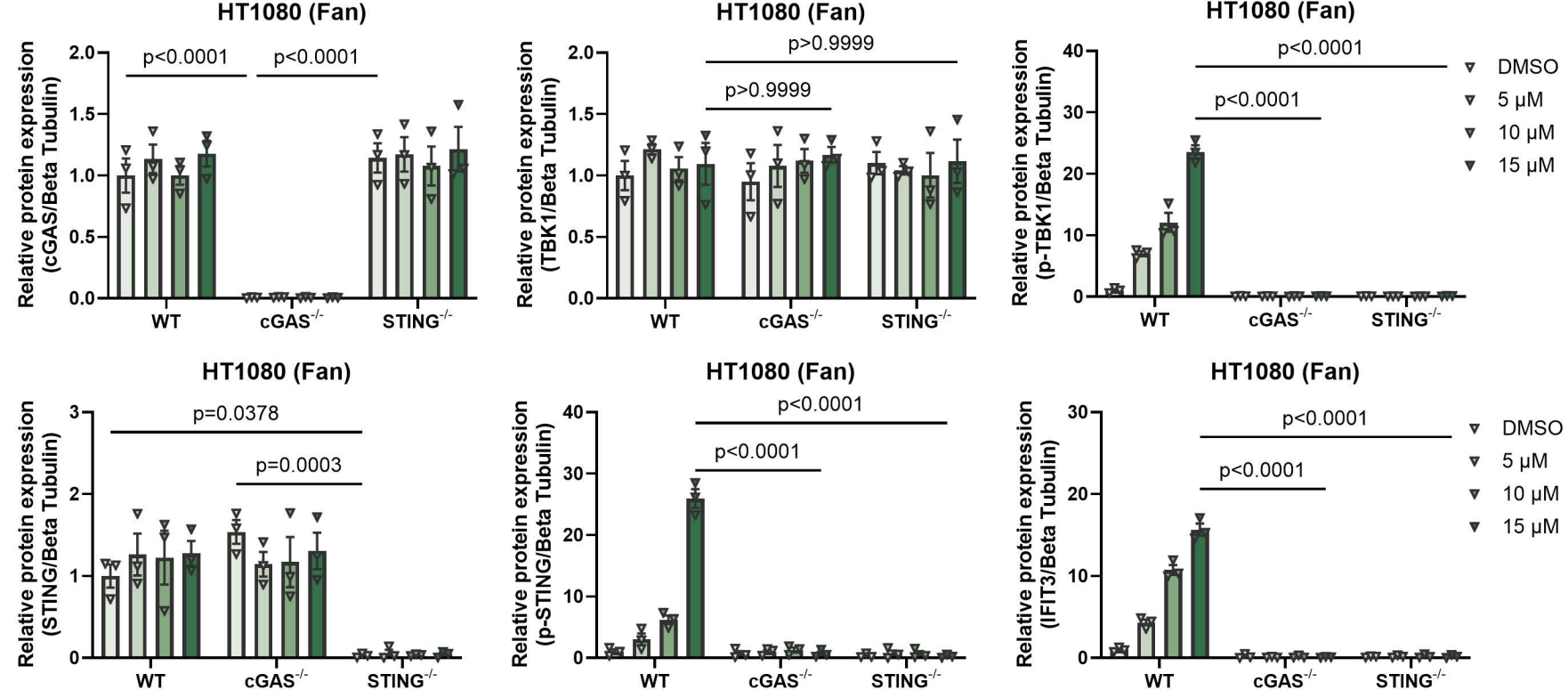


**A****B**

## A Related to Figure 1A-1D



## B Related to Figure 1G



**Supplementary Table 1. Primer sequences used for RT-qPCR.**

gene	primer sequences
hIFNB-F	GCTTGGATTCCTACAAAGAAGCA
hIFNB-R	ATAGATGGTCAATGCGGCGTC
mI11b-F	AGTTGACGGACCCCAAA
mI11b-R	TCTTGTTGATGTGCTGCTG
mI16-F	CTGCAAGAGACTCCATCCAG
mI16-R	AGTGGTATAGACAGGTCTGTTGG
mIfnb-F	TCCGAGCAGAGATCTTCAGGAA
mIfnb-R	TGCAACCACCACTCATTCTGAG
hACTB-F	CCTGGCACCCAGCACAAT
hACTB-R	GCCGATCCACACGGAGTACT
mActb-F	GGCTGTATTCCCCTCCATCG
mActb-R	CCAGTTGGTAACAATGCCATGT
mNos2_F	GTTCTCAGCCCAACAATACAAGA
mNos2_F	GTGGACGGGTTCGATGTCAC
mPtgs2_f	TTCAACACACTCTATCACTGGC
mPtgs2_f	AGAAGCGTTTGCGGTACTCAT
mLta_F	CCACCTCTTGAGGGTGCTTG
mLta_R	CATGTCGGAGAAAGGCACGAT