

FIGURE LEGENDS FOR SUPPLEMENTARY FIGURES

Supplemental figure 1. TH1579 elevates the expression of PD-L1 at mRNA level in human colon cancer and uveal melanoma cells.

A. Dose-response curve of NTUB1, UMUC3 and A549 cells treated with TH1579 for 72 hours. Viability in different concentrations was averaged from four independent experiments. Expression of *PDL1* in **B.** HCT116 and **C.** MP41 cells was assessed by qPCR. Cells were treated with different concentrations of TH1579 or cisplatin for 48 hours. The fold change in relative mRNA expression was averaged from one experiment for HCT116 and two independent experiments for MP41. * $p < 0.05$, ** $p < 0.01$, Student's *t* test. **D.** Representative flow cytometry gating strategy for the identification of tumour cells. Isolated tumour cells were gated on size, singularity, viability. Median fluorescence intensity (MFI) of APC was analysed in live cells.

Supplemental figure 2. PD-L1 expression at transcriptional level by treated with TH1579.

A. Dose-response curve of CT26, LL/2, RenCa, 4T1 and B16F10 cells treated with different concentrations of TH1579 for 72 hours. Viability in different concentrations was averaged from four independent experiments. **B.** Expression of *Pdl1* in 4T1 cells was assessed by qPCR. Cells were treated with different concentrations of TH1579 or cisplatin for 24 hours. The fold change in relative mRNA expression was averaged from two independent experiments. * $p < 0.05$, Student's *t* test. **C.** Representative blot of B16F10 and 4T1 cells. Cells were cultured in DMSO or 1.0 μ M TH1579 for 72 hours and lysates prepared for Western Blot analysis with indicated antibodies, $n=2$. **D.** Representative blot of NTUB1, UMUC3, A549, B16F10, 4T1 cells. Cells were cultured in media and lysates prepared for Western Blot analysis with indicated antibodies, $n=2$. **E** and **F.** NTUB1, UMUC3, A549,

27 B16F10 and 4T1 cells were treated with TH1579 for 24 hours. Genomic levels of 8-oxo-dG
28 were analysed using modified comet assay. **E.** Representative images of NTUB1. **F.**
29 Quantification of Tail moment, at least 200 cells in two independent experiments were
30 analysed. **** $p < 0.0001$, One-Way ANOVA.

31

32 **Supplemental figure 3. Expression of cytokines and chemokines at transcriptional**
33 **level in murine cells.**

34 **A-C.** Histograms of individual gene expression fold changes in **B.** NTUB1, **C.** UMUC3 and **D.**
35 A549 from Figure 3A. Transcriptional level expression of *Ccl5*, *Cxcl10*, and *Ifnb* in **D.** B16F10
36 and **E.** 4T1 cells was assessed by qPCR. Cells were treated with different concentrations of
37 TH1579 or cisplatin for 24 hours. The fold change in relative mRNA expression was averaged
38 from two independent experiments. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, Student's t test.

39

40 **Supplemental figure 4. TH1579 activates cGAS-STING pathway after 48-hour treatment**
41 NTUB1 cells were cultured in 0.5 μ M TH1579 for 0, 24, 48 or 72 hours and lysates
42 prepared for Western Blot analysis with indicated antibodies.

43

44 **Supplemental figure 5. PD-L1 and cytokine response by TH1579 is cGAS-STING**
45 **dependent in NTUB1 cells**

46 NTUB1 cells were transferred with siCGAS for 48 hours followed by DMSO or 0.5 μ M TH1579
47 treatment for 48 hours. **A.** *CGAS*, **B.** *CCL5*, **C.** *CXCL10*, **D.** *IFNB* and **E.** *PDL1* were detected
48 at mRNA level measured by qPCR. The fold change in relative mRNA expression was
49 averaged from three independent experiments. **F.** NTUB1 cells were transferred with siCGAS
50 for 48 hours followed by DMSO or 0.5 μ M TH1579 treatment for 72 hours then measured
51 PD-L1 expression by flow cytometry. The fold change in median fluorescence intensity (MFI)

52 was averaged from three independent experiments. *p < 0.05, ***p < 0.001, ****p < 0.0001,
53 Student's t test.

54

55 **Supplemental figure 6. Gating strategy for *in vivo* study (relates to Figure 3)**

56 **A.** Representative flow cytometry gating strategy for the identification of CD8⁺ T cell
57 populations in tumours from B16F10 model. Isolated CD8⁺ T cells were first gated on size,
58 singularity, viability and positive expression of CD45 and CD3. **B.** Representative flow
59 cytometry gating strategy for the identification of B16F10 cell populations in tumours from
60 B16F10 model. Isolated tumour cells were first gated on size, singularity, viability, negative
61 expression of CD45 and positive expression of gp-100.

62

63 **Supplemental figure 7. Isotype control validation and basal of PD-L1 in different cell**
64 **lines (relates to Figure 1)**

65 Representative histograms for PD-L1 expression in NTUB1, UMUC3 and A549 with different
66 treatments. Cells were seeded 200,000 in each well and treated with indicated compounds
67 for 72 hours. After that, cells were collected and stained with PD-L1 antibody and isotype
68 control with 1:40 dilution. Cells were gated according to the gating strategy in **Figure S1D**.
69 N=2, for cisplatin group, n=1

Figure S1

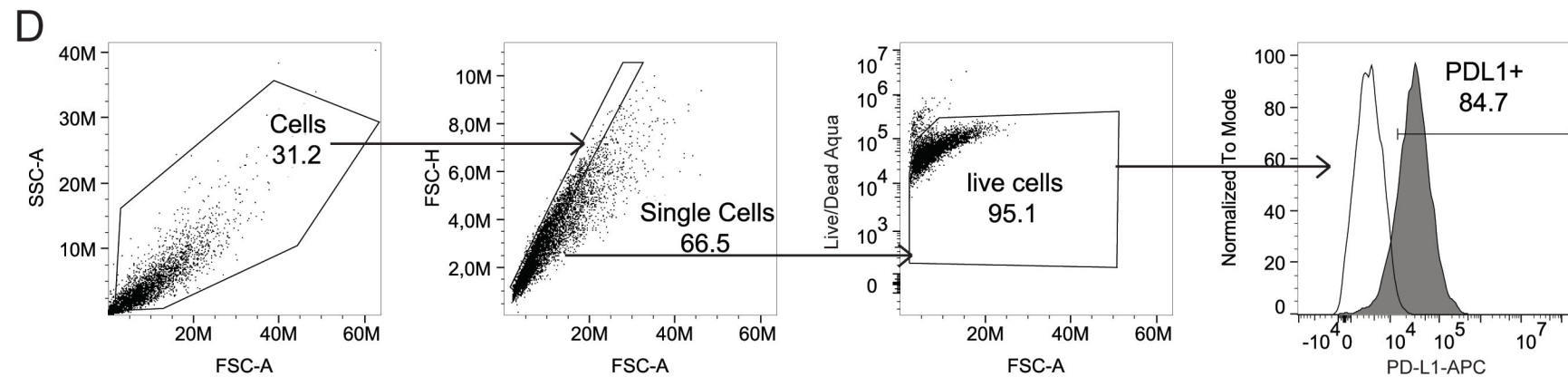
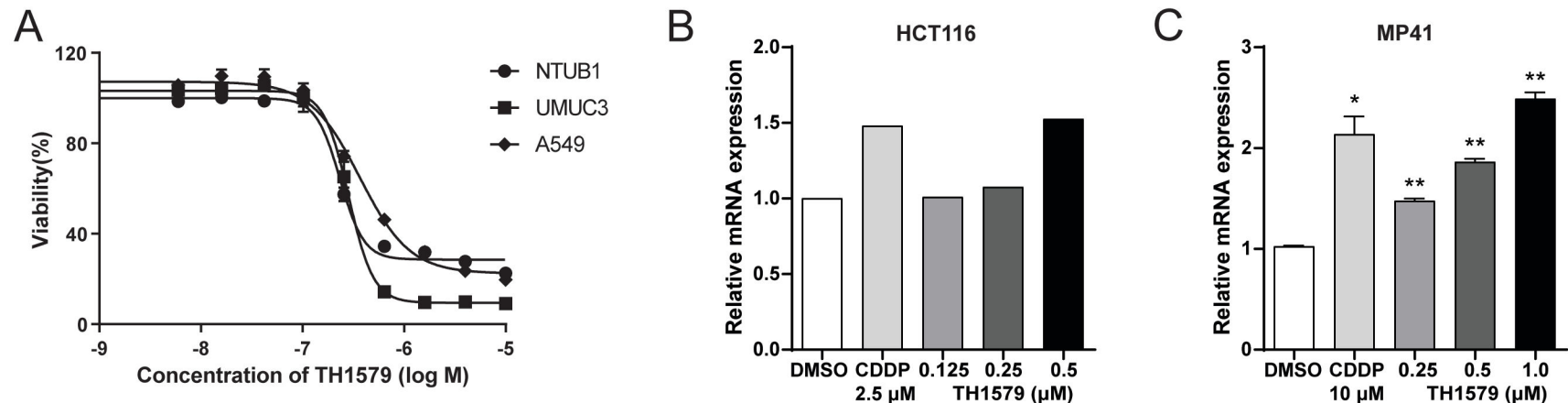
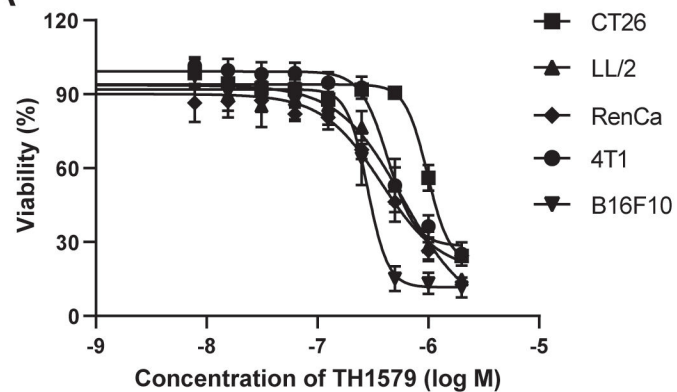
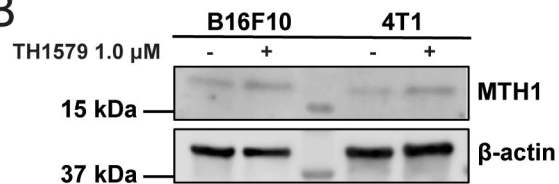


Figure S2

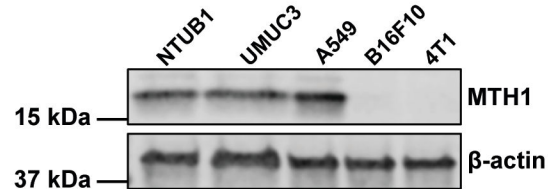
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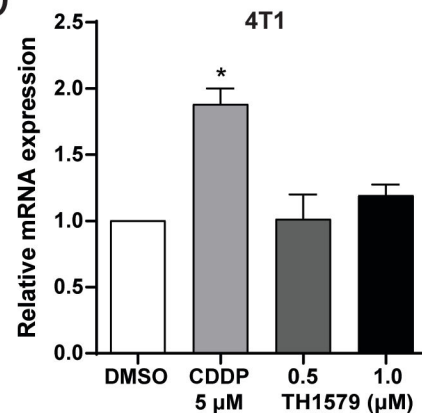
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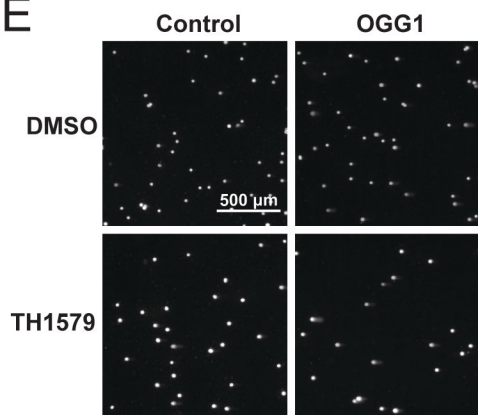
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D



E



F

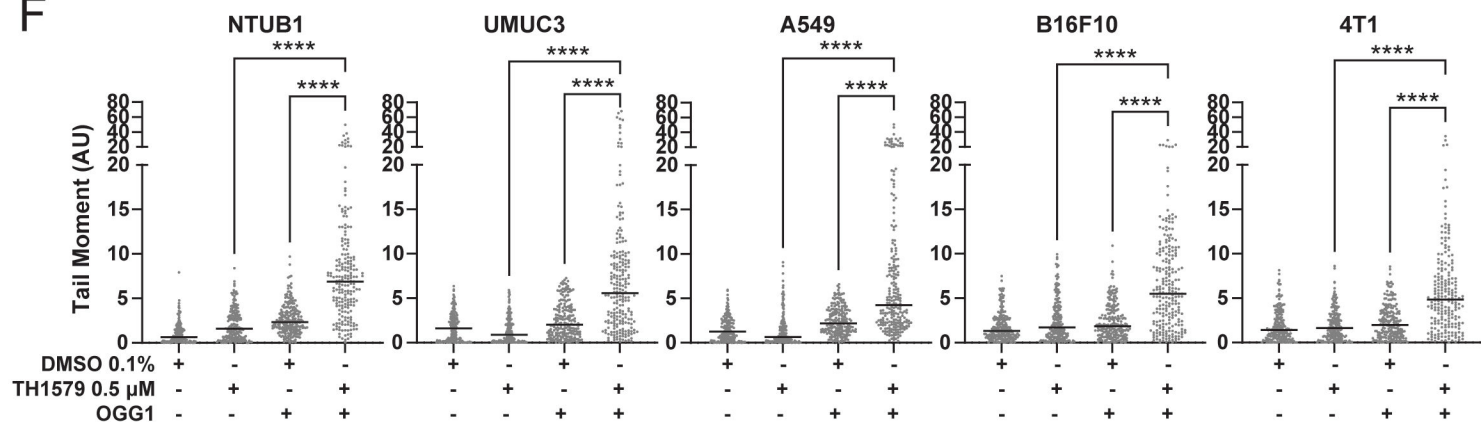


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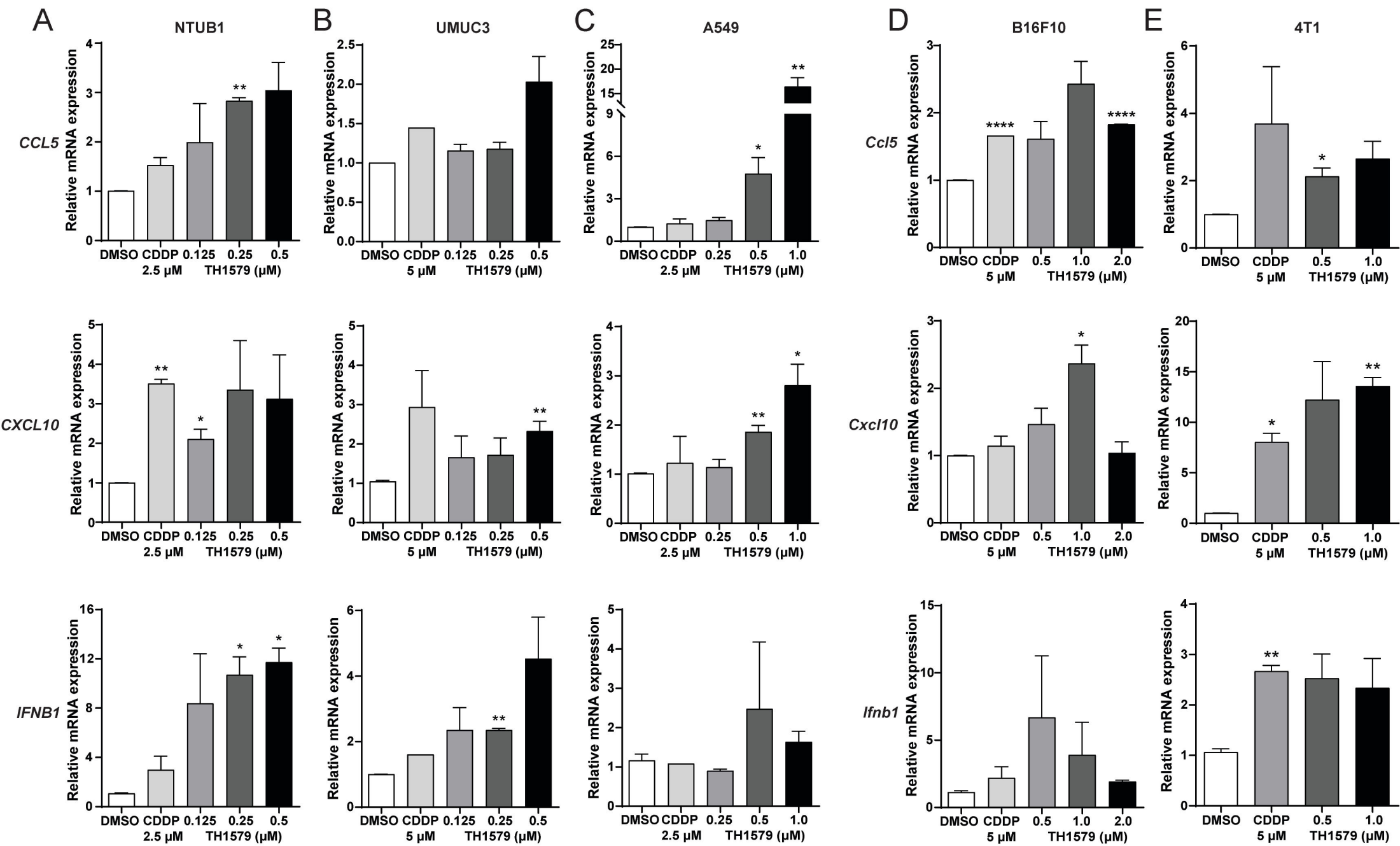


Figure S4

A

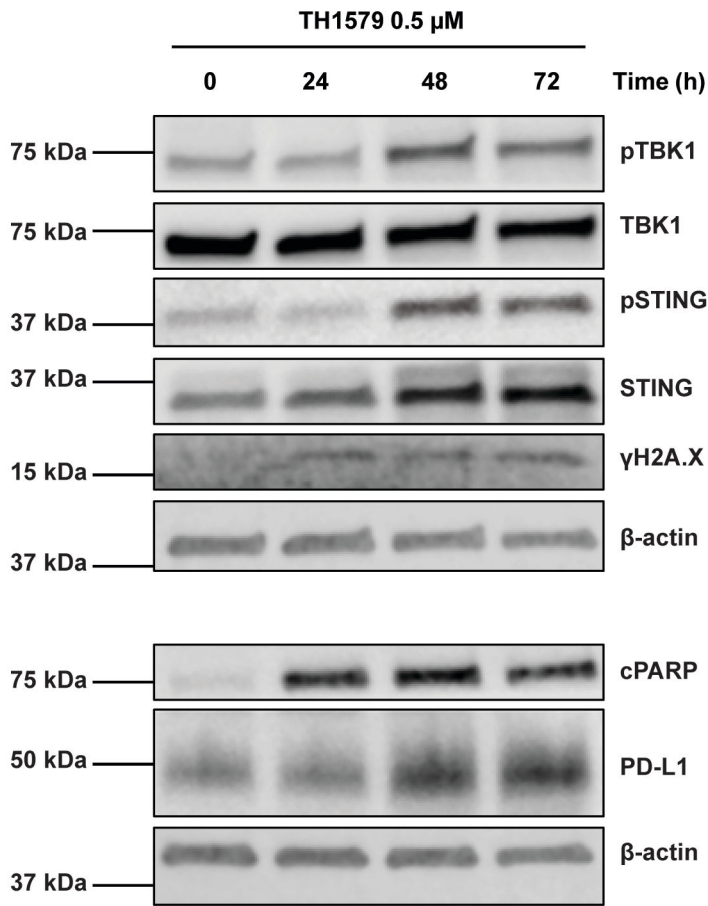
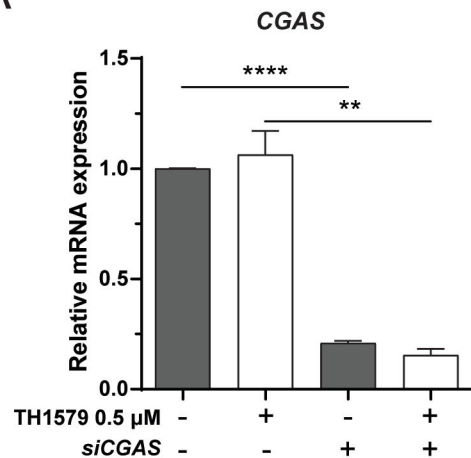
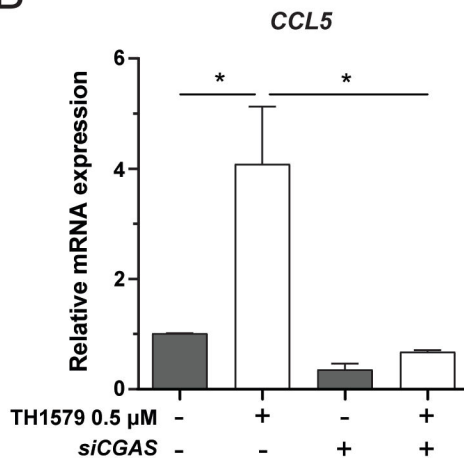


Figure S5

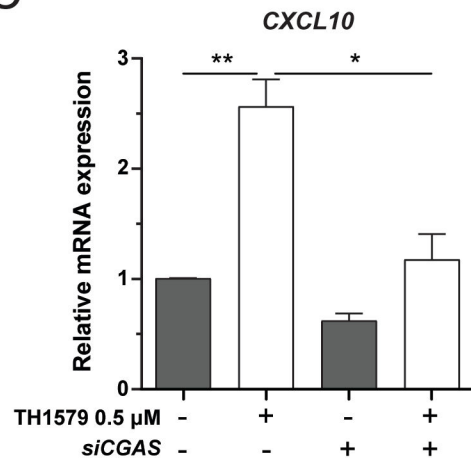
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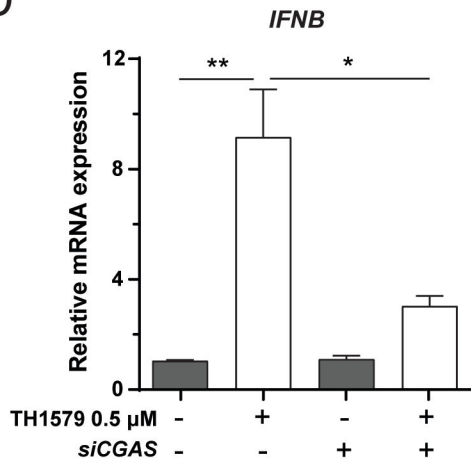
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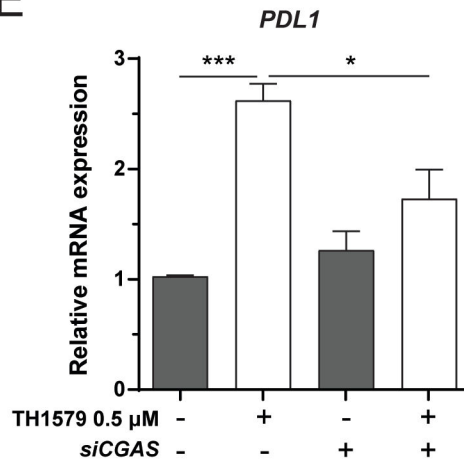
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D



E



F

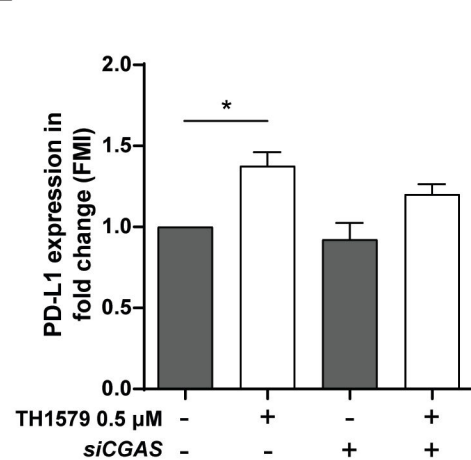
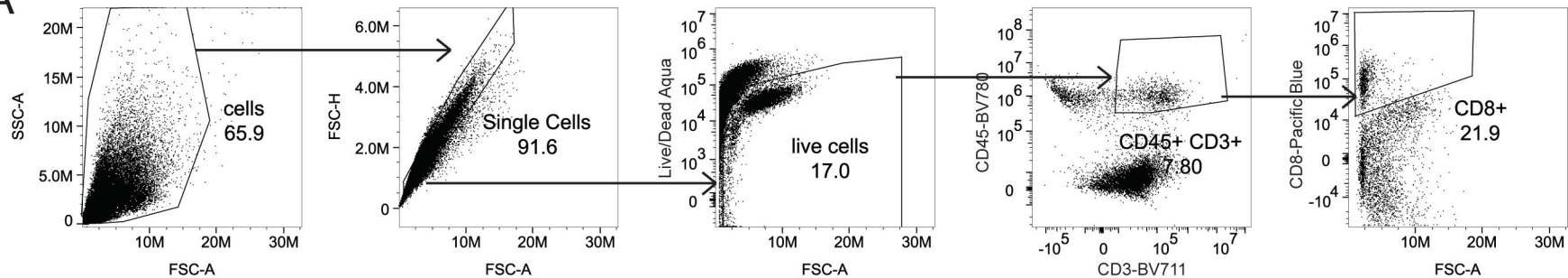


Figure S6

A



B

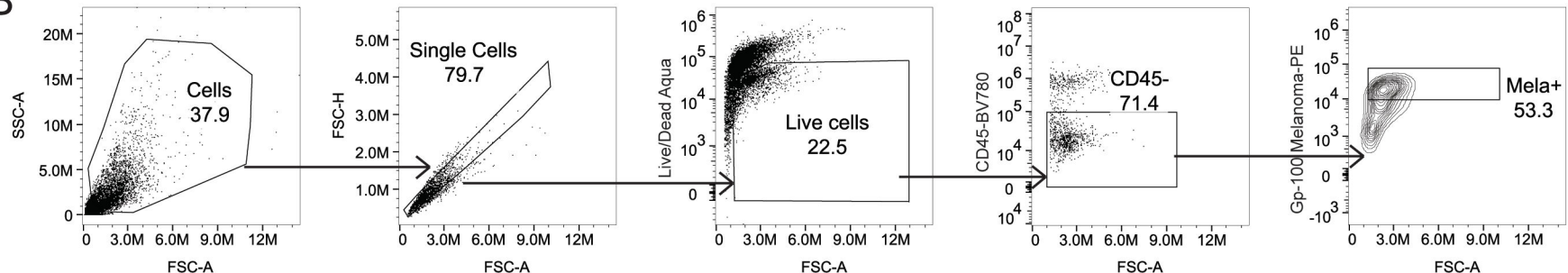


Figure S7

