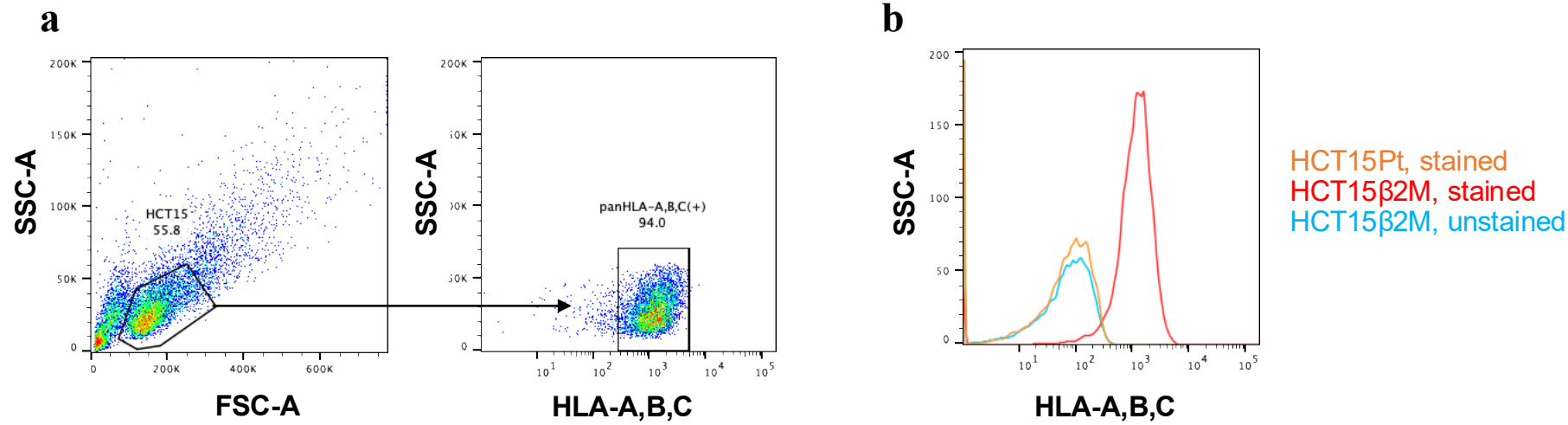


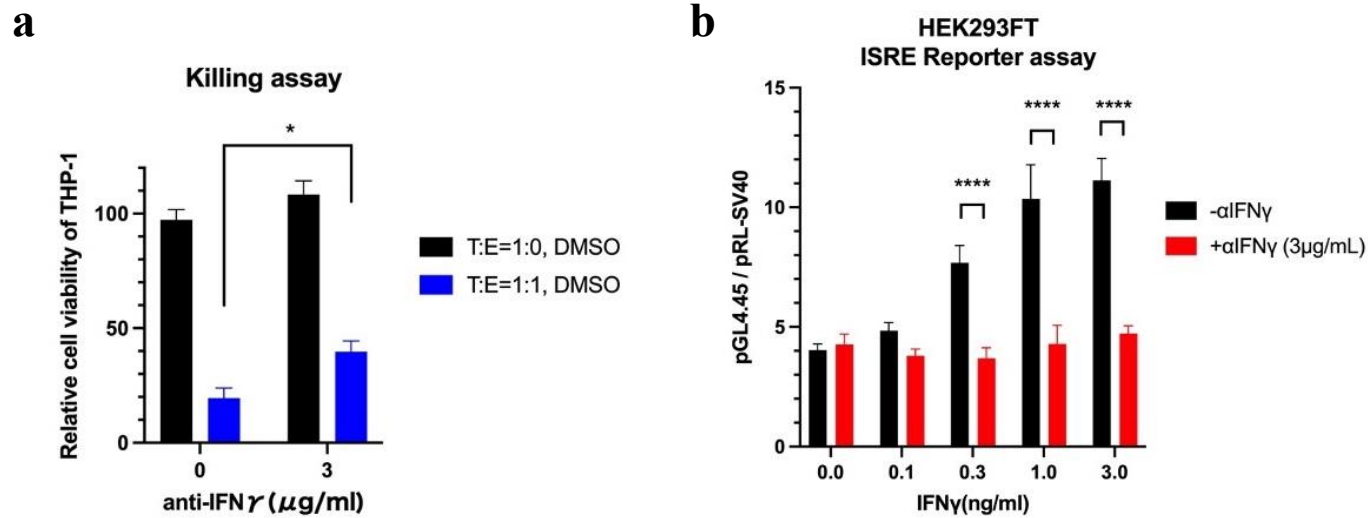
Supplementary Fig. S1



Supplementary Fig. S1 Flow cytometric analysis of HLA-A,B,C expression in HCT15 cells

- a. Gating strategy for flow cytometry analysis. HCT15 were selected from a forward scatter-area (FSC-A) vs side scatter-area (SSC-A) dot plot. HLA-A,B,C expression was analyzed within this gated population.
- b. Offset histogram overlay of HLA-A,B,C expression within the HCT15 cell population.

Supplementary Fig. S2



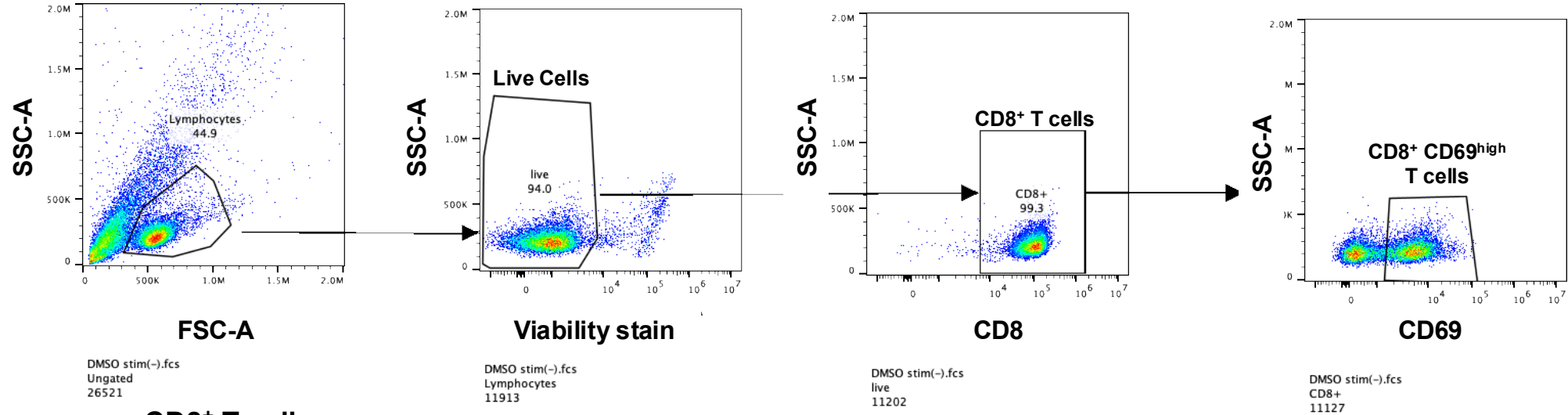
Supplementary Fig. S2 IFN- γ contributes to T cell mediated killing

a. THP1 cells and WT1#3-3 were co-cultured with 10 nM WT1 peptide with or without 3 μ g/mL anti-IFN- γ antibody for 48 h. Anti-IFN- γ antibody partially rescued target cell viability by attenuating T-cell mediated killing. Statistical significance was determined by two-tailed Student's t-test. *: $P < 0.05$.

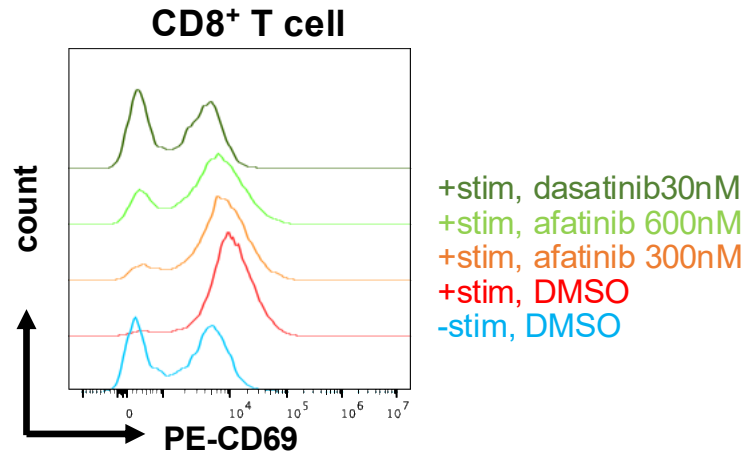
b. ISRE reporter assay with IFN- γ and anti-IFN- γ antibody. HEK293FT cells were transfected with pGL4.45 (Promega #E414A) and pRL-SV40 (Promega E223A) for 24h. Cells were pretreated with 3 μ g/mL anti-IFN- γ antibody for 1h, followed by stimulation with IFN- γ (0, 0.1, 0.3, 1, 3 ng/mL) for 24 h. Luciferase activity was measured using the Dual Glo Luciferase Assay System (Promega #2920). Firefly luciferase (pGL4.45) was normalized to Renilla luciferase (pRL-SV40). Anti-IFN- γ antibody at 3 μ g/mL neutralized IFN- γ -induced ISRE activation. Statistical significance was determined by two-way ANOVA. ****: $P < 0.0001$.

Supplementary Fig. S3

a



b

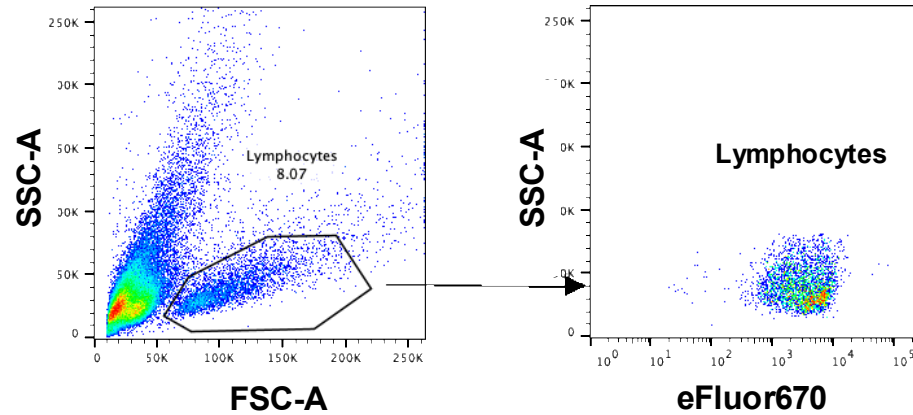


Supplementary Fig. S3 Original data of Flow cytometric analysis for Fig. 4b

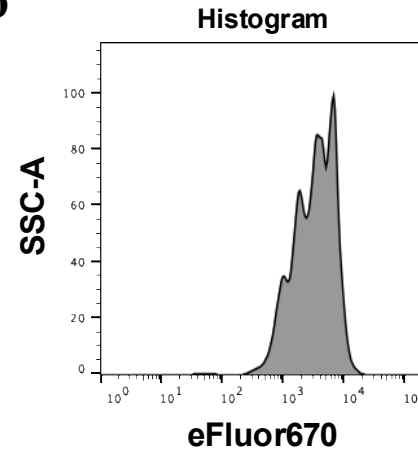
- a. Gating strategy for flow cytometry analysis. Lymphocytes were first selected from a forward scatter-area (FSC-A) vs side scatter-area (SSC-A) dot plot. Live cells were gated as the viability dye-negative population. CD8⁺ T cells were then selected, and CD69 expression was analyzed within this gated population.
- b. Offset histogram overlay of CD69 expression within CD8⁺ T cell population.

Supplementary Fig. S4

a



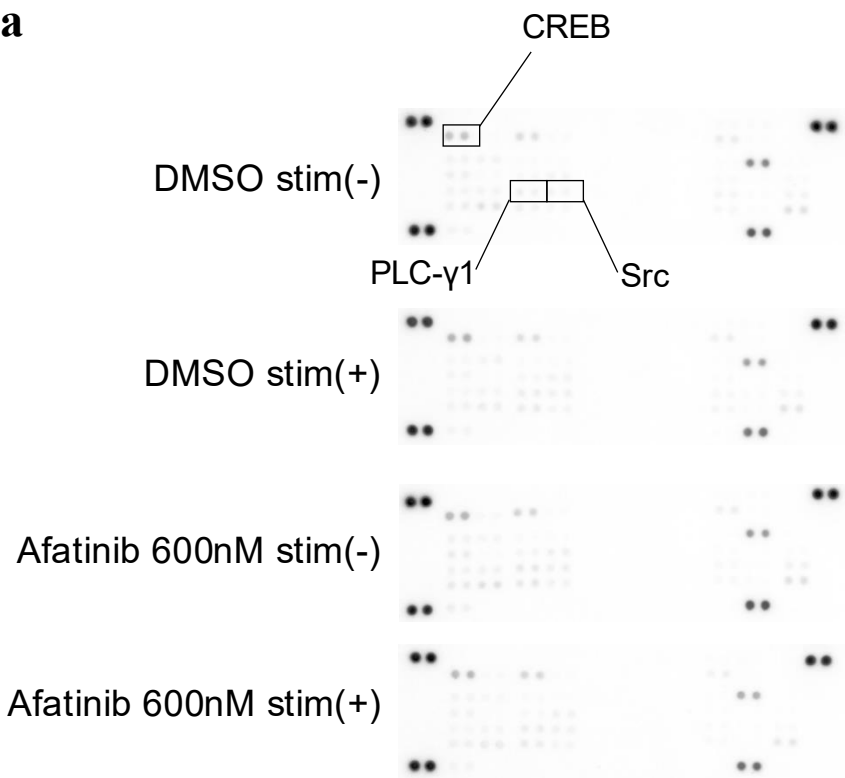
b



Supplementary Fig. S4 Original data of Flow cytometric analysis for Fig. 5

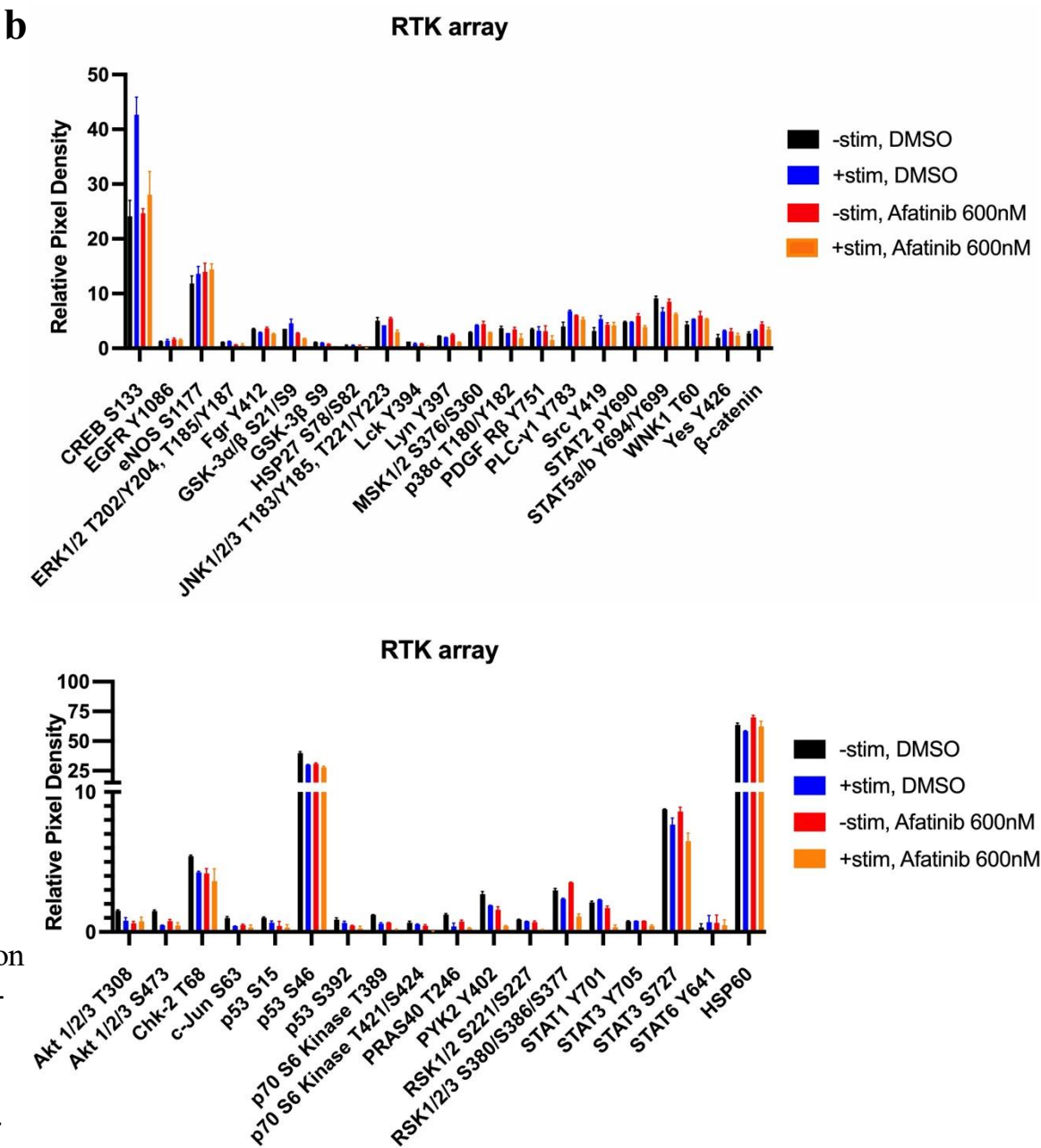
- a. Gating strategy for flow cytometry analysis. Lymphocytes were selected from a forward scatter-area (FSC-A) vs side scatter-area (SSC-A) dot plot. eFluor670 expression was analyzed within this gated population.
- b. Histogram of eFluor670 expression within lymphocytes.

Supplementary Fig. S5

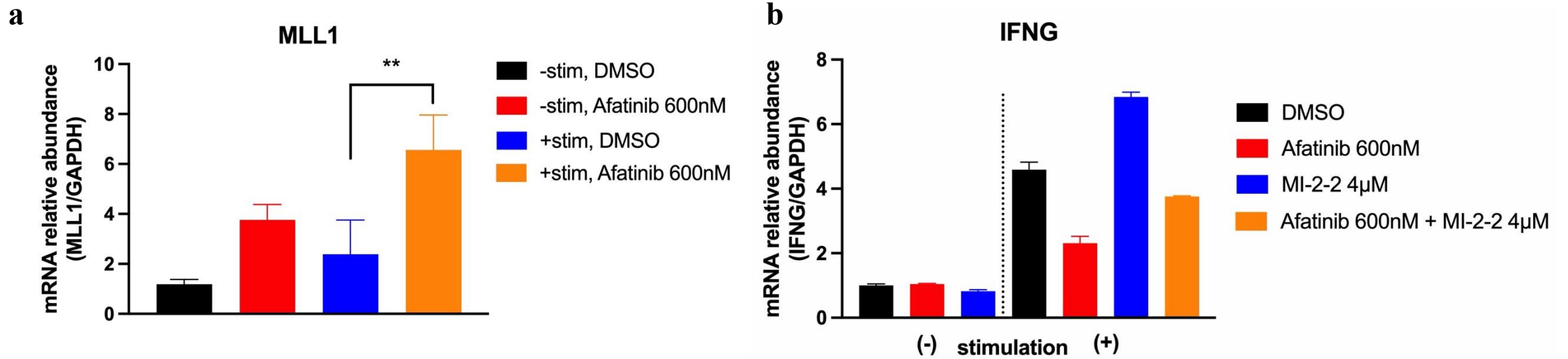


Supplementary Fig. S5 Original data of phospho-RTK array for Fig. 6a
a. Human phospho-RTK array contained immobilized antibodies specific for the phosphorylated forms of 37 receptor tyrosine kinases and 2 related total proteins. CREB(S133), PLC-γ1(Y783), Src(Y419) were enhanced upon anti-CD3/CD28 stimulation in the DMSO-treated group, whereas this phosphorylation was attenuated in the Afatinib-treated group.

b. Signal intensity is presented as relative pixel density normalized to the reference spots.



Supplementary Fig. S6

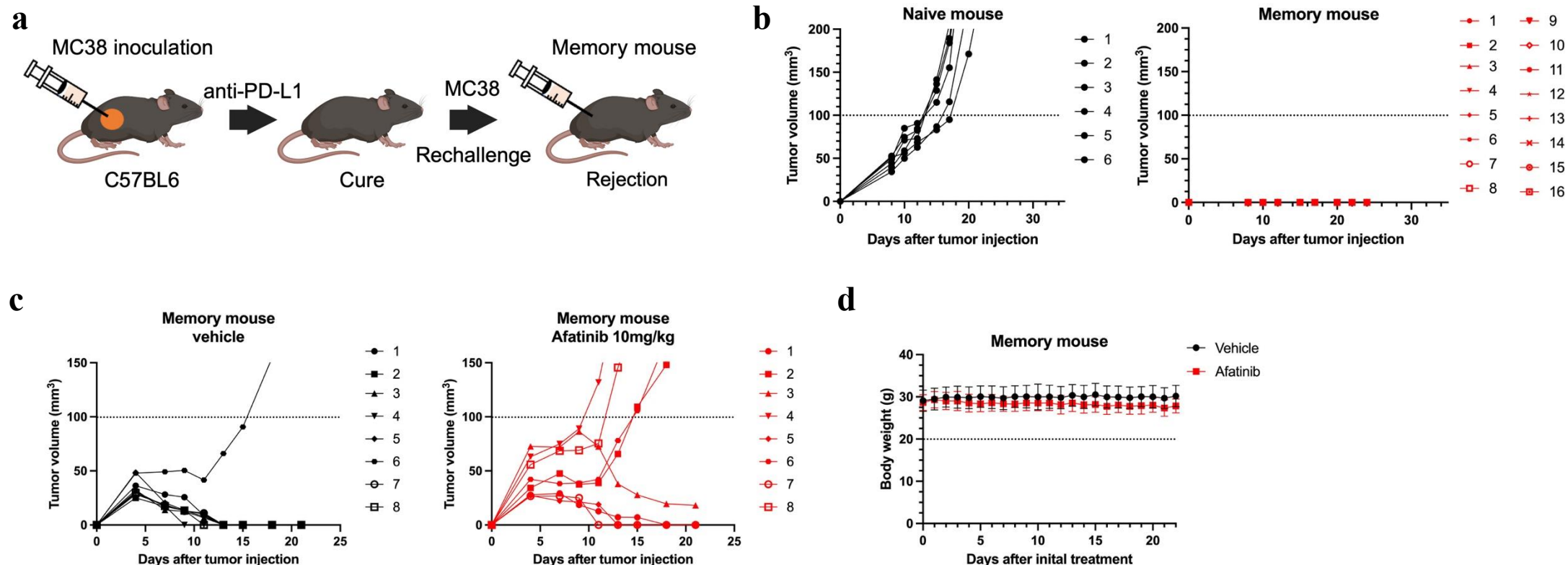


Supplementary Fig. S6 MLL inhibitor mitigated afatinib-induced suppression of IFN- γ gene suppression

a. WT1#3-3 cells were stimulated with anti-CD3/CD28 beads at a 1:6 (beads:cells) ratio in the presence or absence of afatinib (600 nM) for 24 h. RT-qPCR was performed in triplicate for each sample, with three independent biological replicates. Afatinib treatment significantly upregulated *MLL1* expression upon CD3/CD28 stimulation. Statistical differences were determined by one-way ANOVA. **: $P < 0.01$.

b. WT1#3-3 cells were stimulated with anti-CD3/CD28 beads at a 1:6 (beads:tumor cells) ratio in the presence or absence of afatinib (600 nM) and/or MI-2-2 (4 μ M) for 24 h. RT-qPCR was performed in triplicate for each sample. Representative RT-qPCR data of *IFNG* expression was shown as bar graph. Error bars represent the standard deviation of triplicate wells. Co-treatment with MI-2-2 (4 μ M) mitigated the afatinib-induced suppression of *IFNG* transcription.

Supplementary Fig. S7



Supplementary Fig. S7 Afatinib suppressed tumor rejection in immunological memory mice

a. Schematic representation of the establishment of immunological memory mouse.

b. Cured mice were rechallenged subcutaneously with 1×10^6 MC38 cells to evaluate the acquisition of immunological memory against MC38. All cured mice rejected the rechallenged MC38 cells, indicating that they had developed immunological memory (referred to as memory mouse), whereas naïve mice did not reject the tumors.

c. Memory mice were re-rechallenged with 1×10^7 MC38 cells, and treated orally once daily with either vehicle (1% Tween 80) or afatinib (10 mg/kg), starting one day before tumor inoculation. Four out of eight mice (50%) failed to reject tumor engraftment in the afatinib-treated group, whereas only one out of eight mice (12.5%) failed to reject engraftment in the vehicle group. Individual tumor volumes are shown.

d. Body weight was measured daily. Afatinib treatment did not induce severe weight loss.