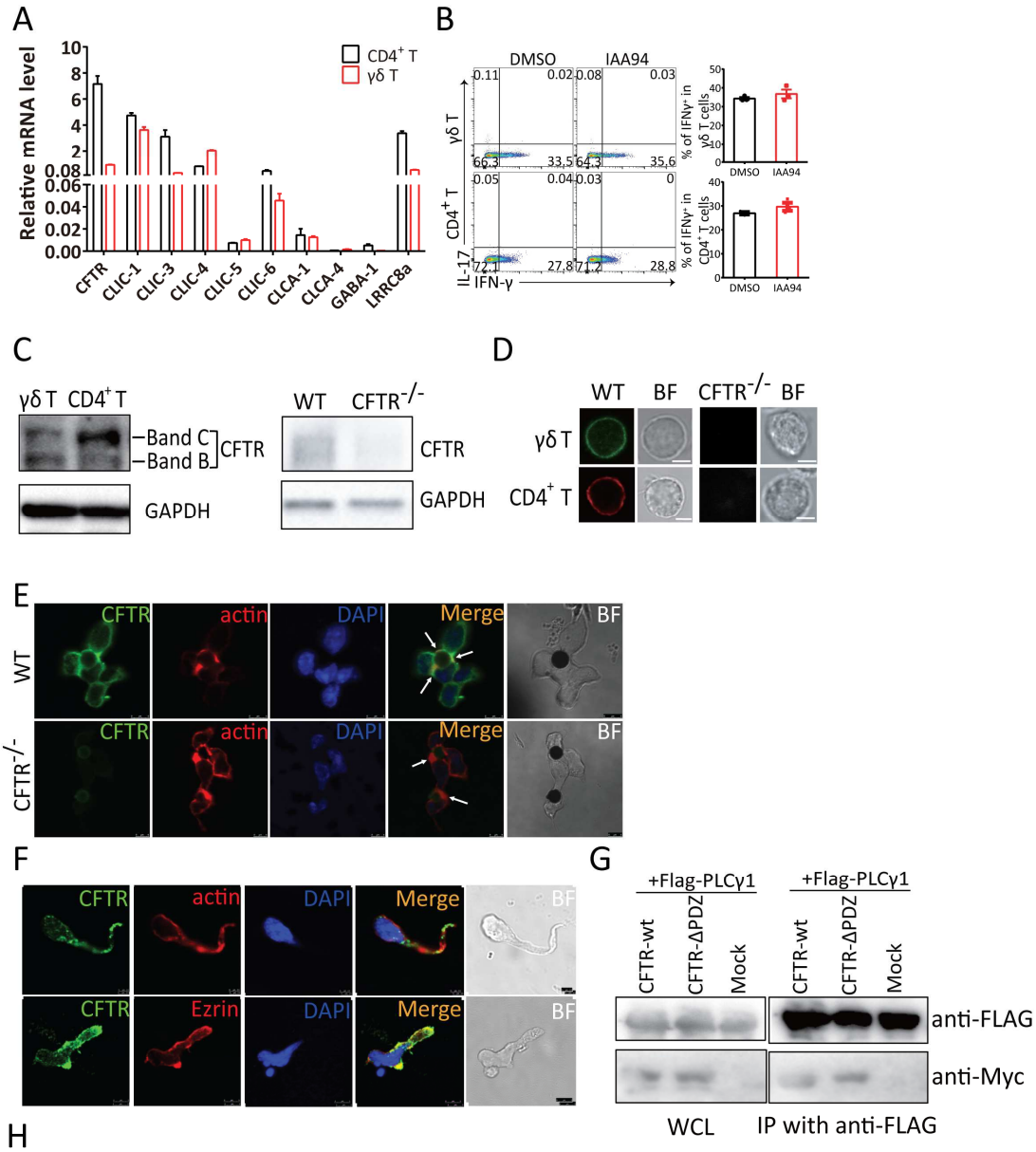
Supplementary Information for

CFTR is a negative regulator of γδ T cell IFN-γ production and anti-tumor immunity.

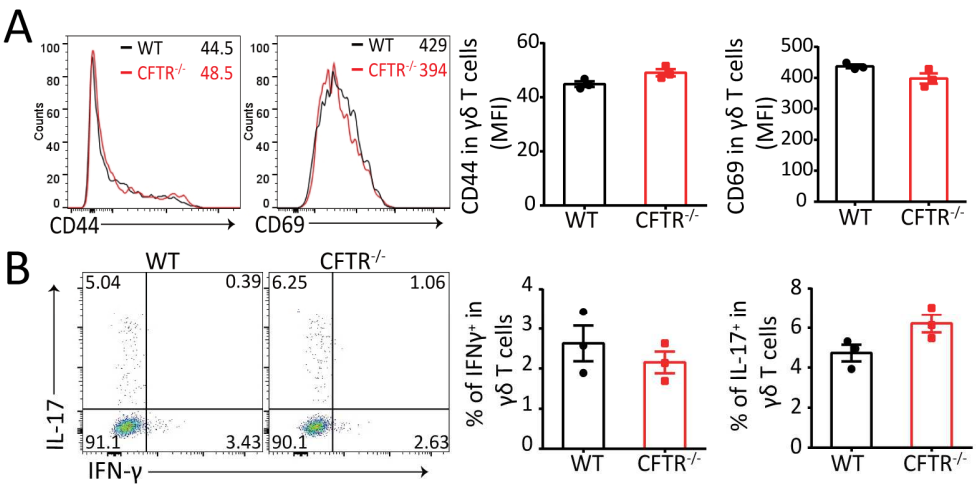
Duan et al.



Duan et al. Fig.S1

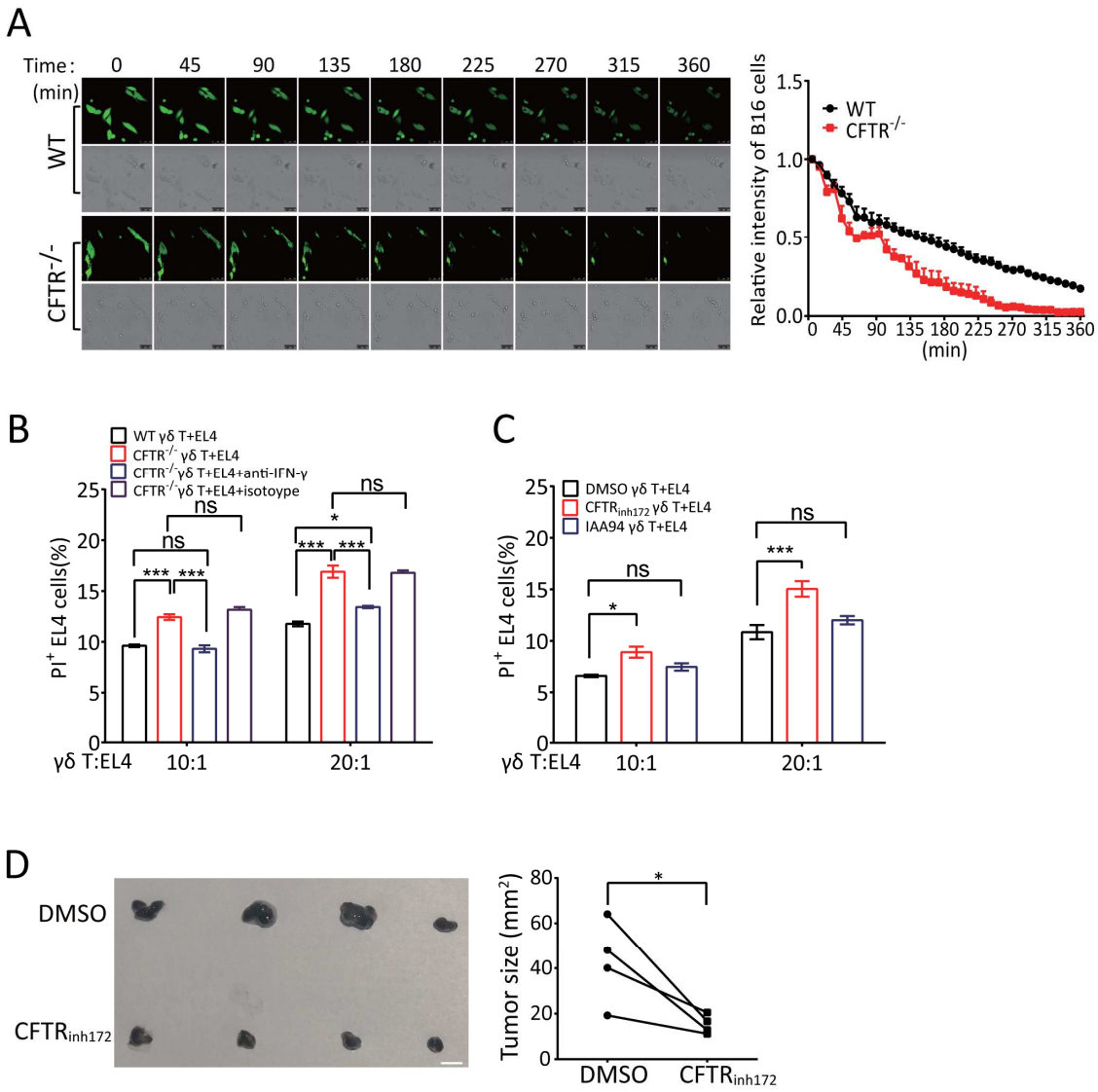
Fig S1. CFTR was highly expressed in the cell surface of γδ T and CD4$^+$ T cells, and CFTR polarized towards to immunological synapse via cytoskeleton in γδ T cells. A) Quantitative PCR analysis of different types of chloride ion channels in splenic CD4$^+$ and γδ T cells (n=3); B) Flow cytometry of splenic γδ T/CD4$^+$ T cells with PMA (50 ng/ml) and ionomycin (1 μg/ml) stimulation for 6 h in the presence of CLICs inhibitor IAA94 (50 μM) or its vehicle DMSO to detect IFN-γ and IL-17 production (n=3); C) Left: Immunoblot analysis of CFTR expression in mouse splenic γδ and CD4$^+$ T cells on the 10% SDS-PAGE gel. Doublets at ~140 kDa and ~180 kDa correspond to the nonglycosylated and glycosylated forms of CFTR. GAPDH was detected as loading control; Right: Immunoblot analysis of CFTR expression in mouse splenic WT and CFTR$^{-/-}$ γδ T cells on the 10% SDS-PAGE gel; D) Confocal microscopy of the expression of CFTR in splenic WT or CFTR$^{-/-}$ CD4$^+$ and γδ T cells stained with Alexa Fluor 488-conjugated anti-mouse IgG (γδ T) or mouse anti-CFTR followed by Alexa Fluor 594-conjugated anti-mouse IgG (CD4$^+$ T) (scale bar: 2.5 μm); E) Confocal microscopy of the stimulated WT or CFTR$^{-/-}$ γδ T cells with Dynabeads® Mouse T-Activator CD3/CD28, and stained with mouse anti-CFTR antibody followed by Alexa Fluor 488-conjugated anti-mouse IgG, and with Alexa Fluor 594-conjugated phalloidin (arrowheads indicate the immunosynapse area) (scale bar: 5 μm); F) Confocal microscopy of the stimulated γδ T cells with Dynabeads® Mouse T-Activator CD3/CD28, and stained with mouse anti-CFTR followed by Alexa Fluor 488-conjugated anti-mouse IgG, and with Alexa Fluor 594-conjugated phalloidin or with rabbit anti-ezrin followed by Alexa Fluor 594-conjugated anti-rabbit IgG (scale bar: 5 μm); G) Immunoblot analysis of HEK293T cells transfected with Flag-tagged PLCγ-1 and Myc-tagged CFTR-wt, Myc-tagged CFTR-ΔPDZ, or Myc empty vector (Mock). Cells were immunoprecipitated with anti-FlAG M2 magnetic beads and probed with anti-Myc or anti-Flag antibodies. Data are representative of at least three experiments; H) Current-voltage relationship of the Forskolin (FSK, 10 μM)-induced current (P=0.002, n=7) (left) and the GlyH101(10 μM)-inhibited current (P=0.002, n=7)

(middle), and inhibition by GlyH101 to FSK-induced current (FSK: P=0.042, GlyH101: P=0.007, n=5) in γδ T cells.



Duan et al. Fig.S2

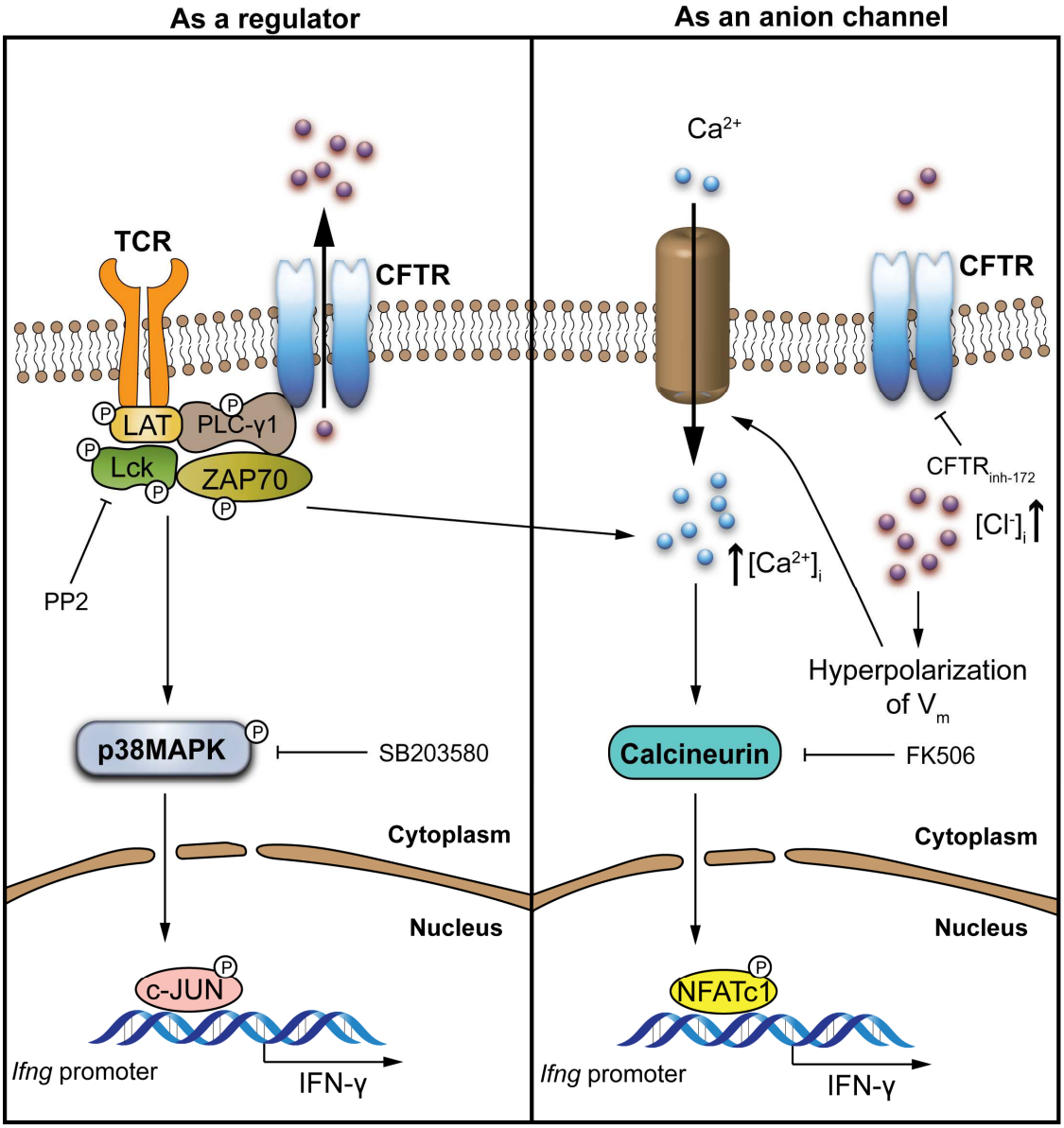
Fig S2. CFTR exerted no effect on the thymic programming. A) representative flow cytometry and statistical analysis of CD44 and CD69 in WT or CFTR$^{-/-}$ thymic γδ T cells (n=3). B) representative flow cytometry and statistical analysis of WT or CFTR$^{-/-}$ thymic γδ T cells with the PMA (50 ng/ml) and ionomycin (1 μg/ml) stimulation for 6 h to detect the IFN-γ and IL-17 production (n=3).



Duan et al. Fig.S3

Fig S3. CFTR deficiency and dysfunction in γδ T cells displayed higher killing ability against B16 melanoma and EL4 lymphoma. A) The real-time confocal microscopy and statistical analysis of coculture of the in vitro-expanded WT or CFTR$^{-/-}$ γδ T cells with calcein AM-labeled B16 cells in 5:1 ratio for 6 h (n=5); B) Co-culture of the in vitro-expanded WT or CFTR$^{-/-}$ γδ T cells with CFSE labeled EL4 cells in indicated ratio, anti-IFN-γ (10 μg/ml) was added to neutralize the cytotoxicity and isotype control antibody was added. 6 h later, dead EL4 cells were assessed by PI staining (***, p<0.001; *, p<0.05; ns, not significant; n=3); C)

In vitro-expanded γδ T cells were pretreated with CFTR inhibitor $CFTR_{inh172}$ (5 μM), CLICs inhibitor IAA94 (50 μM) or its vehicle DMSO for 2 h, and then co-cultured with CFSE labeled EL4 cells in indicated ratio. 6 h later, the dead EL4 cells were assessed by PI staining (***, $p<0.001$; *, $p<0.05$; ns, not significant; n=3); D) B16 cells (2×10^5 cells/mouse) were mixed with *in vitro*-expanded and purified WT or $CFTR_{inh172}$- treated γδ T cells (0.5×10^5 cells/mouse) and s.c. injected into B6 TCR $\delta^{-/-}$ mice (n = 4 per each group). At day 12 post tumor injection, tumors were isolated (left panel, scale bar=5mm) and tumor sizes were measured (right panel, paired t test; *, $p< 0.05$).



Duan et al. Fig.S4

Fig S4. Proposed model for regulating IFN-γ production in γδ T cells. We observed that molecular mechanisms underline the regulation of CFTR on IFN-γ production by γδ T cells were either TCR dependent or Ca^{2+} influx in γδ T cells. In one hand, CFTR, which was served as a part of TCR signaling cascade and a regulator, suppresses Lck-P38-c-Jun pathway as well as TCR-stimulated Ca^{2+} influx and decreases IFN-γ expression. On the other hand, CFTR functions as a chloride channel via TCR-independent manner. Once CFTR channel activity is

blocked, the resultant elevated $[Cl^-]_i$ hyperpolarizes V_m and triggers Ca^{2+} influx, which in turn leads to downstream Calcineurin-NFATc1 signaling pathway and IFN-γ production of γδ T cells. Proper inactivation of CFTR could enhance anti-tumor immunity in γδ T cell-mediated cancer immunotherapy.