Supplementary Materials

Fig. S1. Graded TCR signaling drives varying outcomes in Nur77 and CD25 expression.

Fig. S2. PMA and Ionomycin together drive graded IRF4 expression while Nur77 and CD69 have some expression with PMA alone.

Fig. S3. NFAT1 and NFAT2 have similar responses in activated CD8+ T cells and hyper-activable NFAT partially rescues IRF4 expression in ITK-inhibited $CD8^+$ T cells.

Fig. S4. ITK inhibition increases the coefficient of variance (CV) for IRF4 protein and mRNA in activated $CD8^+$ T cells.

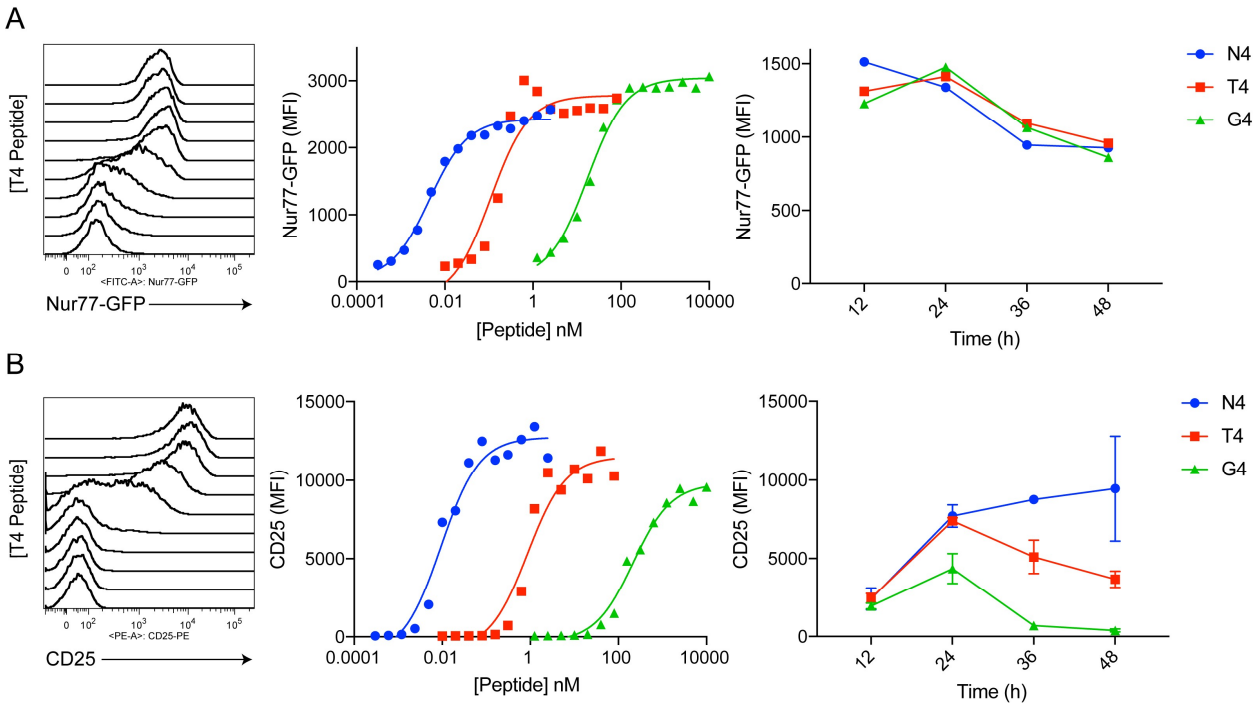


Figure S1. Graded TCR signaling induces distinct patterns of Nur77 and CD25 expression.

(**A**) OT-I Nur77-GFP cells were treated with varying doses of N4, T4, or G4 peptides for 24h. Representative histograms for Nur77-GFP are shown for the T4 peptide (left). GFP MFI is plotted for each peptide dose (middle). Cells were treated with 1nM N4, 100nM T4, or 1µM G4 from 12-48h and MFI for each peptide is plotted over time.

(**B**) OT-I cells were treated with varying doses of N4, T4, or G4 peptides for 24h. Representative histograms of CD25 staining are shown for the T4 peptide (left). CD25 MFI is plotted for each peptide dose (middle). Cells were treated with 1nM N4, 100nM T4, or 1µM G4 from 12-48h and MFI for each peptide is plotted over time.

Data are representative of three experiments.

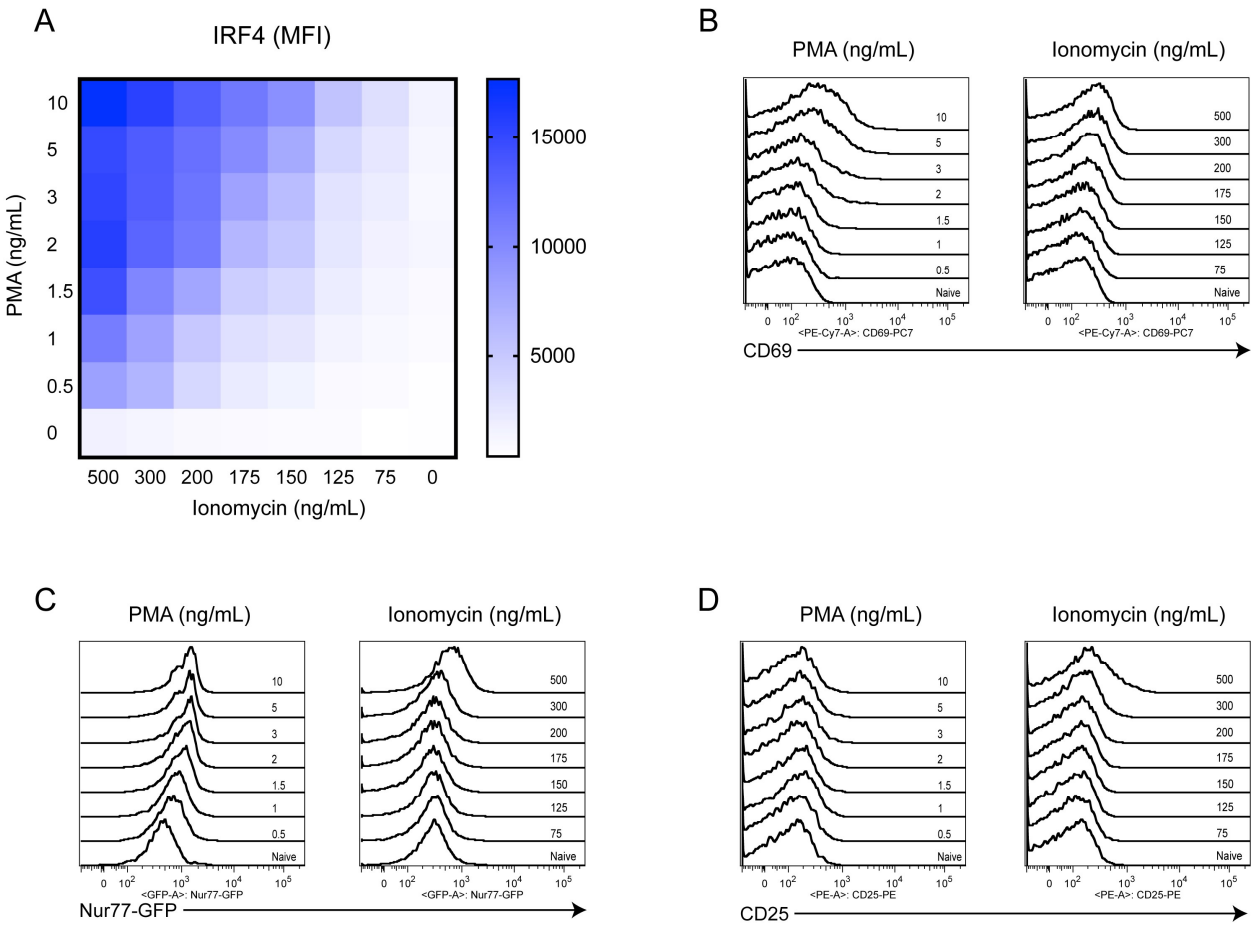
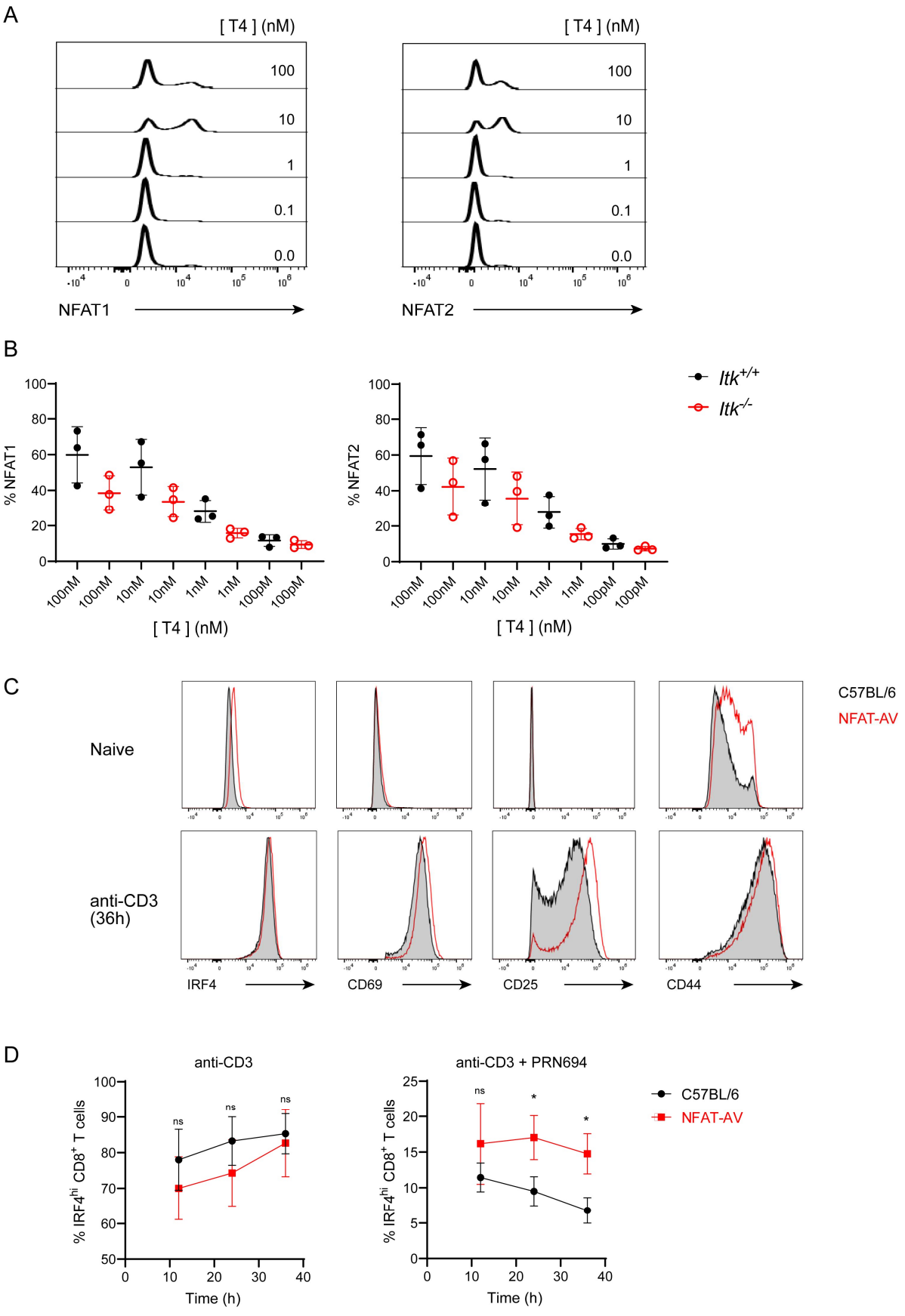


Figure S2. PMA and Ionomycin together drive graded IRF4 expression while Nur77 and CD69 show modest expression with PMA alone.

(**A**) Representative heat map of IRF4 MFI values for OT-I T cells treated with various doses of PMA and Ionomycin in combination for 24h.

(**B-D**) Representative histograms for CD69 (B), Nur77-GFP (C), and CD25 (D) after treatment with PMA or Ionomycin for 24h.

Data are representative of three experiments.


A
[T4] (nM)
100
10
1
0.1
0.0
NFAT1
[T4] (nM)
100
10
1
0.1
0.0
NFAT2
B
Itk+/+
Itk-/-
% NFAT1
% NFAT2
100nM
100nM
10nM
10nM
1nM
1nM
100pM
100pM
[T4] (nM)
C
C57BL/6
NFAT-AV
Naive
anti-CD3
(36h)
IRF4
CD69
CD25
CD44
D
anti-CD3
anti-CD3 + PRN694
% IRF4hi CD8+ T cells
Time (h)
ns
*
C57BL/6
NFAT-AV

30

Figure S3. NFAT1 and NFAT2 have similar responses in activated CD8$^+$ T cells and hyper-activable NFAT partially rescues IRF4 expression in ITK-inhibited CD8$^+$ T cells.

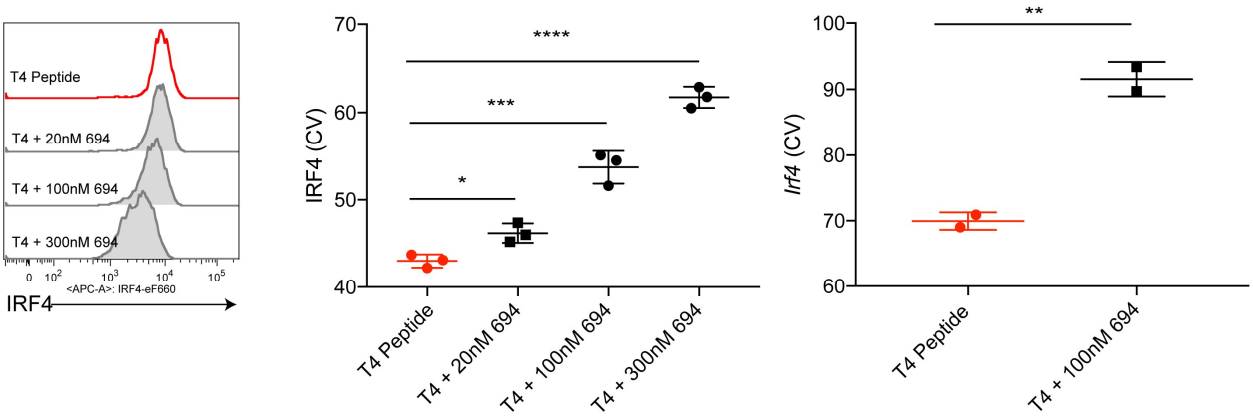
(**A**) Representative histograms of NFAT1 and NFAT2 fluorescence in OT-I nuclei isolated from T cells stimulated with B6 splenocytes pulsed with the indicated doses of T4 peptide for 30m.

(**B**) Dot plots of %NFAT1$^+$ and % NFAT2$^+$ nuclei after 30m of stimulation of wild-type or *Itk*$^{-/-}$ OT-I T cells with B6 splenocytes pulsed with the indicated doses of T4 peptide. OT-I nuclei were identified as CellTrace Violet$^+$ events.

(**C**) Representative histograms of IRF4, CD69, CD25, and CD44 in Naïve and anti-CD3 stimulated WT and NFAT-AV CD8$^+$ T cells. Fractionated CD8$^+$ T cells from bulk splenocytes were stimulated with 1μg/mL anti-CD3ε antibody for 36h and stained for flow cytometry. Cells were gated on live CD8$^+$ events.

(**D**) Line plots comparing %IRF4^{hi}CD8$^+$ in WT and NFAT-AV T cells stimulated for 12-36h with anti-CD3ε antibody (left panel) or with anti-CD3ε antibody in the presence of 50nM PRN694 (right panel). * $p \leq 0.1$ (Unpaired student *t* test)

Data are representative of three experiments.



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49 **Figure S4. ITK inhibition increases the coefficient of variance (CV) for IRF4 protein and**

50 **mRNA in activated CD8$^+$ T cells.**

51 (**A**) Representative histograms for OT-I T cells stimulated with 100nM T4 peptide in the absence

52 or presence of various doses of PRN694 treatment for 24h, and cells were stained for

53 intracellular IRF4. The coefficient of variance (CV) of IRF4 staining was plotted for each

54 treatment group. * $p \leq 0.1$ ** $p \leq 0.01$ *** $p \leq 0.001$ **** $p \leq 0.0001$ (one-way ANOVA followed by

55 Dunnett's test for the left panel and unpaired student *t* test for the right panel)

56 Data are representative of three experiments.

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