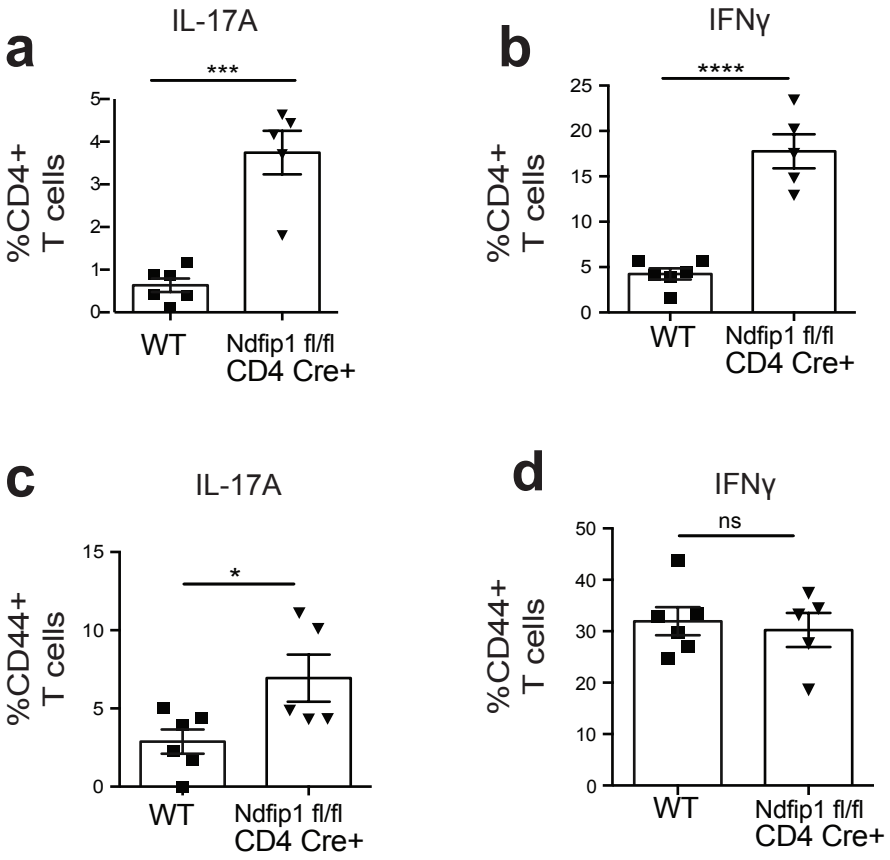
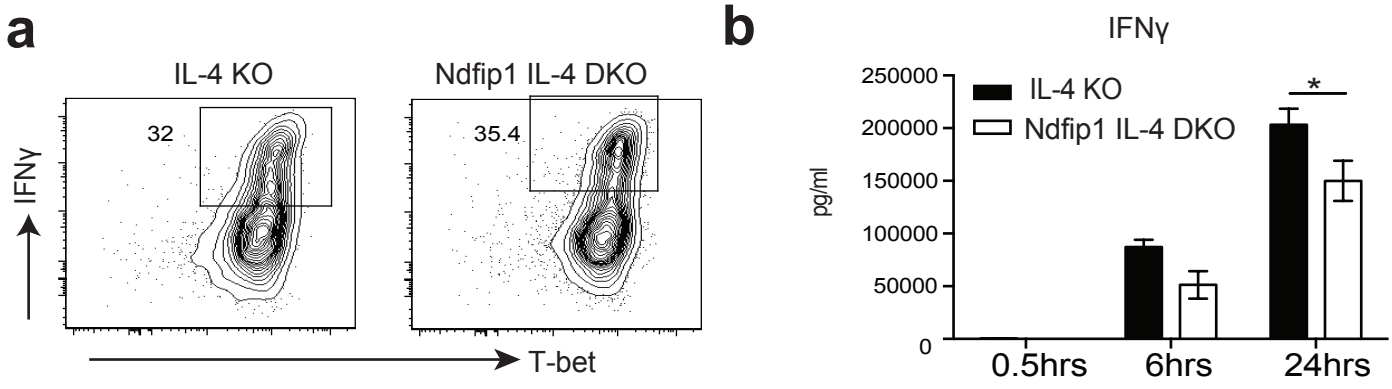
Supplementary figures

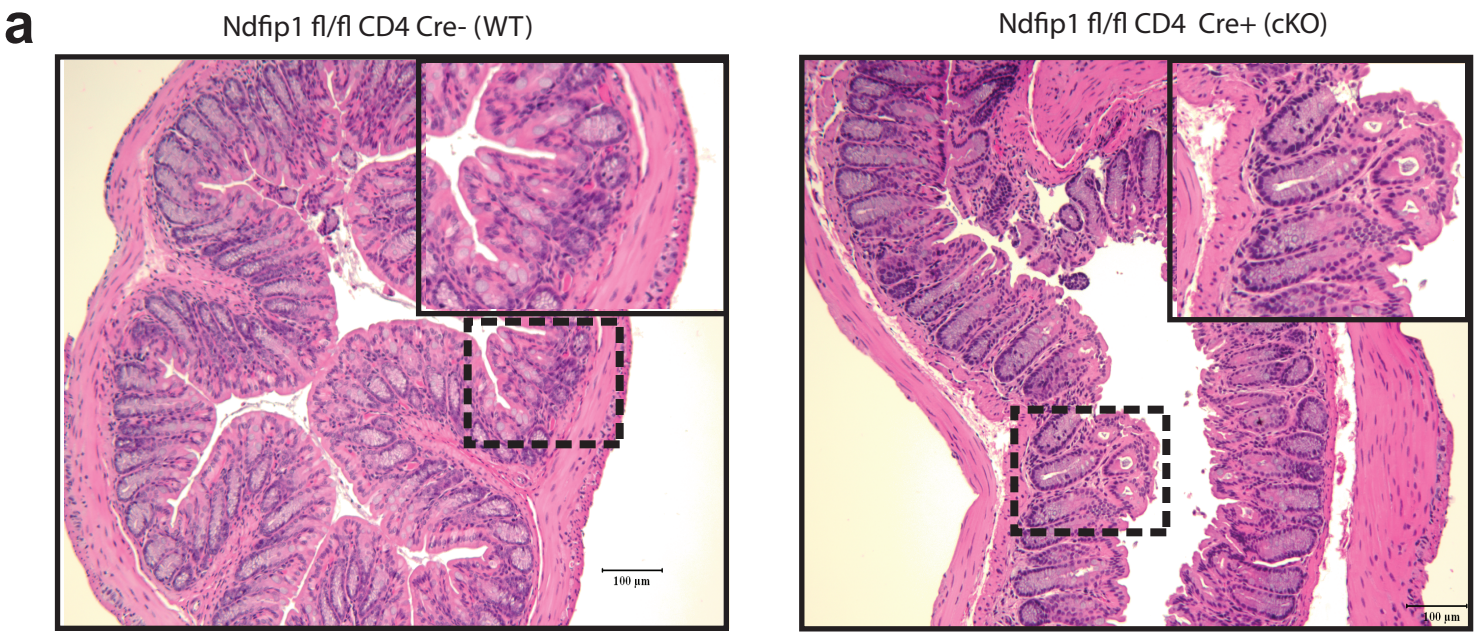
Ndfip1 restricts Th17 cell potency by limiting lineage stability and proinflammatory cytokine production.

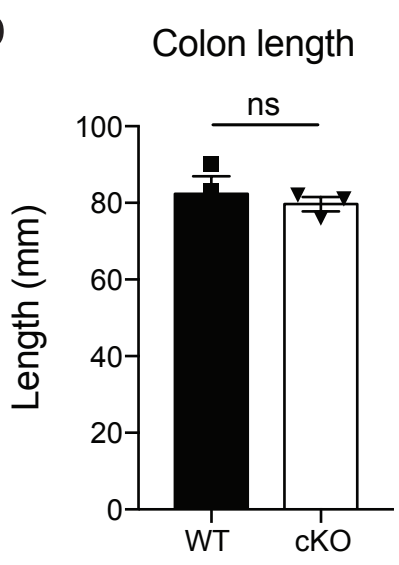
Awo Akosua Kesewa Layman[1], Stephanie Sprout[2], Dylan Phillips[2] and Paula M. Oliver [2,3,*]

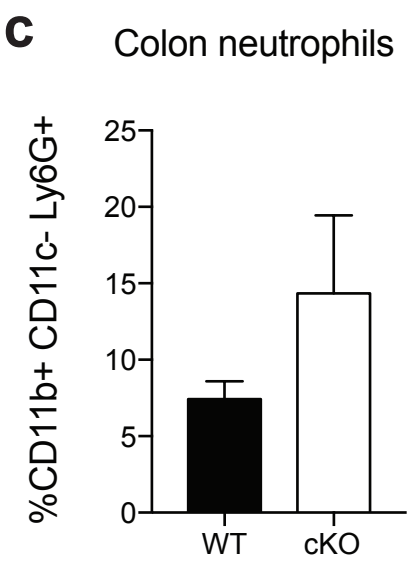
[1]Medical Scientist Training Program, Perelman School of Medicine and Biomedical Graduate Studies at the University of Pennsylvania, [2]The Children's Hospital of Philadelphia, Cell Pathology Division, [3]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania.

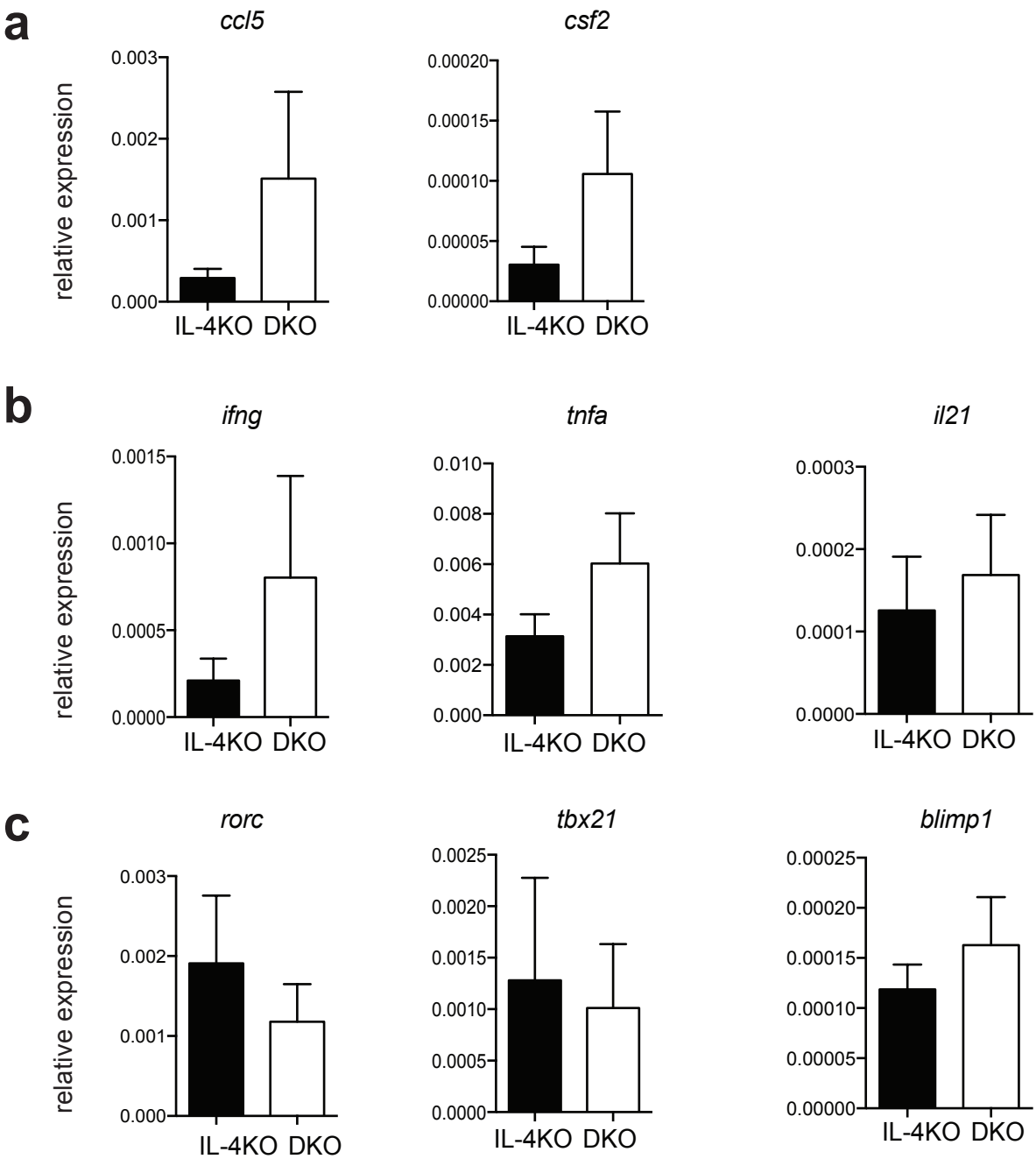

a
IL-17A
***
%CD4+ T cells
0
1
2
3
4
5
WT
Ndfip1 fl/fl CD4 Cre+
b
IFNγ
****
%CD4+ T cells
0
5
10
15
20
25
WT
Ndfip1 fl/fl CD4 Cre+
c
IL-17A
*
%CD44+ T cells
0
5
10
15
WT
Ndfip1 fl/fl CD4 Cre+
d
IFNγ
ns
%CD44+ T cells
0
10
20
30
40
50
WT
Ndfip1 fl/fl CD4 Cre+


a
IL-4 KO
Ndfip1 IL-4 DKO
32
35.4
IFNγ
T-bet
b
IFNγ
IL-4 KO
Ndfip1 IL-4 DKO
*
pg/ml
250000
200000
150000
100000
50000
0
0.5hrs
6hrs
24hrs


a
Ndfip1 fl/fl CD4 Cre- (WT)
Ndfip1 fl/fl CD4 Cre+ (cKO)
100 µm
100 µm


b
Colon length
ns
Length (mm)
100
80
60
40
20
0
WT
cKO


c
Colon neutrophils
%CD11b+ CD11c- Ly6G+
25
20
15
10
5
0
WT
cKO


a
ccl5
csf2
relative expression
0.003
0.002
0.001
0.000
0.00020
0.00015
0.00010
0.00005
0.00000
IL-4KO DKO
b
ifng
tnfa
il21
relative expression
0.0015
0.0010
0.0005
0.0000
0.010
0.008
0.006
0.004
0.002
0.000
0.0003
0.0002
0.0001
0.0000
IL-4KO DKO
c
rorc
tbx21
blimp1
relative expression
0.003
0.002
0.001
0.000
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
0.00025
0.00020
0.00015
0.00010
0.00005
0.00000
IL-4KO DKO

Supplementary Figure S1. Ndfip1 limits the abundance of Th17 cells.
Flow cytometric analysis of CD3+ CD4+ T cells among cells isolated from lungs of the wild type (Cre-) or Ndfip1fl/fl CD4-Cre + (cKO) animals shown in Figure 1, but focused only on litter-matched Cre- and Cre+ controls (i.e. litters in which Cre- and Cre+ mice came from the same mothers). (a-b) Percentages of lung CD4+ T cells that are IL-17A+ (a) or IFNγ+ (b). (c-d) Percentages of CD44+ CD4+ cells that are IL-17A+ (c) or IFNγ+ (d). n= 4 Cre- and n=5 Cre+ in two independent experiments. *p<0.05, **p<0.01,****p<0.0001. p values were calculated by unpaired two-tailed T tests. All error bars represent mean +/- SEM

Supplementary Figure S2. Ndfip1 does not restrain IFNγ secretion from restimulated Th1 cells. (a) Representative plot showing Tbet+ IFNγ+ Th1 cells after Th1 polarization and IL-2 expansion of IL-4-/- or Ndfip1 IL-4 DKO CD4+ T cells. (b) Summary of IFNγ ELISAs from plated Th1 cells. Data is pooled for n=2 Ndfip1 IL-4 DKO and n=3 IL-4-/- animals and is analyzed by 2-way ANOVA. *p<0.05, **p<0.01,***p<0.001. Error bars represent mean +/- SEM

Supplementary Figure S3. Loss of Ndfip1 in T cells drives a spontaneous colitis in mice
(a) Colon histology of ten week old Ndfip1 fl/fl CD4 Cre+ (cKO) or WT (Ndfip1 fl/fl Cre- or Ndfip1 fl/+ Cre-) animals showing evidence of spontaneous colon inflammation in cKO animals. Bars represent 100um and inset shows enlarged version of the boxed section of the image. (b) Ndfip1 fl/fl CD4 Cre+ (cKO) and WT animals do not show differences in colon length. (c) Flow cytometry data indicating a trend towards increased CD11b+ CD11c- Ly6G+ neutrophils in the colons of Ndfip1 fl/fl CD4 Cre+ (cKO) versus WT animals. Data is shown for an n= 3 Ndfip1 fl/fl CD4 Cre + and n=3 WT (Ndfip1 fl/fl Cre- or Ndfip1 fl/+ Cre-) animals at ten weeks old. All error bars represent mean +/- SEM

Supplementary Figure S4. Th17 cells lacking Ndfip1 show a gene expression profile associated with Th17 pathogenicity.
Naïve sorted CD4+ T cells were differentiated into Th17 cells for 5 days. Cells were harvested and expanded in IL-2 for 3 days and then used for qPCR gene expression analysis shown here and also used in the ELISA studies in Figure 4. Data is shown for n=4 mice per genotype in 2 independent differentiation experiments. All error bars represent mean +/- SEM