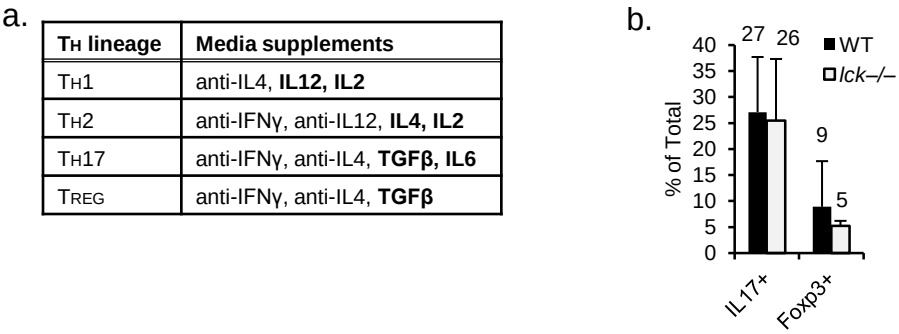
Gene	Gene accession number	Forward primer (5' → 3')	Reverse primer (5' → 3')
β-actin	RefSeq: NM_007393.3	ggc tcc tag cac cat gaa ga	gaa agg gtg taa aac gca gc
Il17 (Il17A)	RefSeq: NM_010552.3	gct cca gaa ggc cct cag a	ctt tcc ctc cgc att gac a
Il17f	RefSeq: NM_013646.1	cccatgggattacaacatcactc	cactgggcctcagcgatc
Foxp3	RefSeq: NM_054039.1	tcc ttc cca gag ttc ttc ca	gtc cac act gct ccc ttc tc
Rorc(γ)t	GI: 5679306	ccg ctg aga ggg ctt cac	tgc agg agt agg cca cat tac a
Rora (isoform 4)	RefSeq: NM_013646.1	tct ccc tgc gct ctc cgc ac	tcc aca gat ctt gca tgg a
Socs3	RefSeq: NM_007707.3	ccc tcg ccc agg tcc ttt gc	gga gcc agc gtg gat ctg cg
Irf4	RefSeq: NM_013674.1	tga aaa tgg ttg cca ggt gac agg	gca gcc ttc agg gct cgt cg
Il21	RefSeq: NM_021782.2	atc ctg aac ttc tat cag ctc cac	gca ttt agc tat gtg ctt ctg ttt c
Il23r	RefSeq: NM_144548.1	gtc cac caa act tcc caa ga	ccc gac aaa agt cca atg tc
Il6Rα	RefSeq: NM_010559.2	aaa gga gtt cac ggt gtt gct	ggt tgg cag agt ctt caa cag
Tgfβr1	RefSeq: NM_009370.2	ata tct gcc ata acc gca ctg	gtc ctg gca att gtt ctt tga

Supplementary Table I. Primer sequences for quantitative RT-PCR. The sequences of primers used in SYBR Green-based qRT-PCR analyses.

a.

TH lineage	Media supplements
TH1	anti-IL4, **IL12, IL2**
TH2	anti-IFNγ, anti-IL12, **IL4, IL2**
TH17	anti-IFNγ, anti-IL4, **TGFβ, IL6**
TREG	anti-IFNγ, anti-IL4, **TGFβ**



Supplementary Figure 1. Fyn deletion alters T_H17 polarization without affecting IL6 or TGFβ receptor expression.

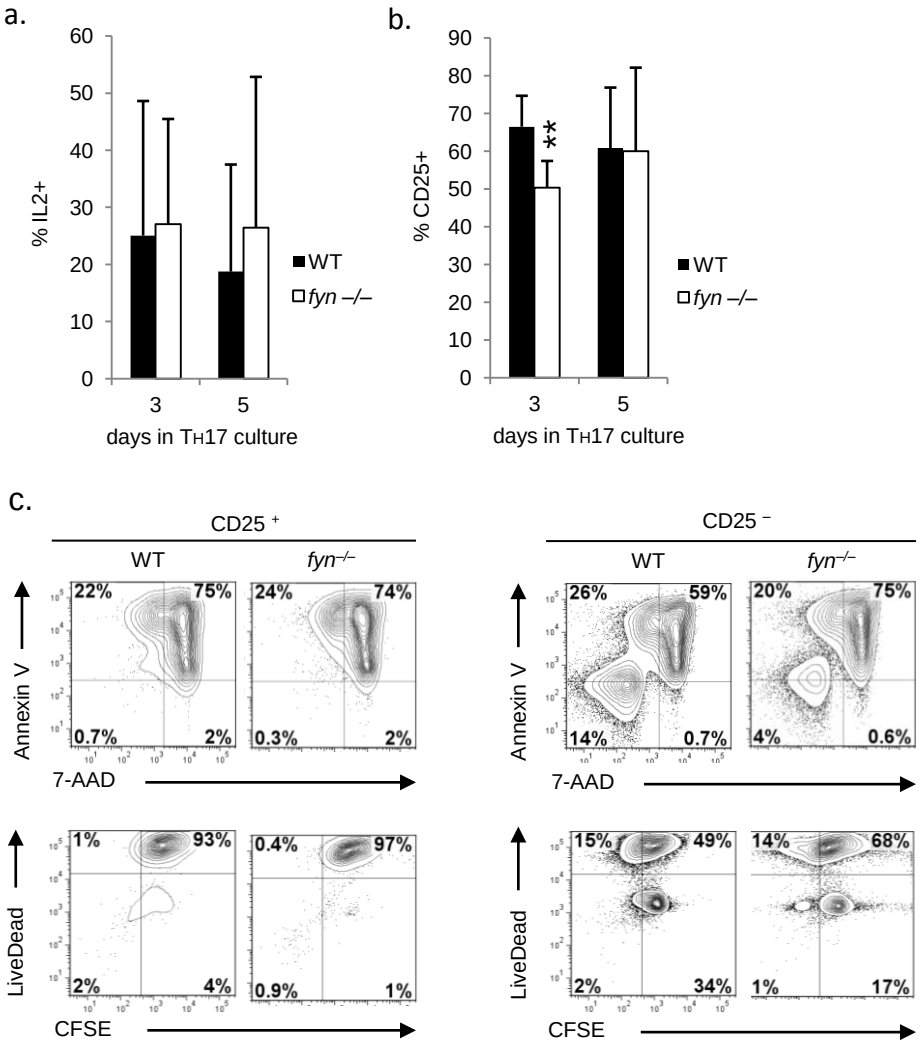
Supplementary Figure 1. Fyn deletion alters T_H17 polarization without affecting IL6 or TGFβ receptor expression (continued).

a) Unless otherwise indicated, T_H17 and T_{REG} cultures were polarized in the indicated cytokine milieu for 5 days in the presence of plate-bound anti-TCRβ and anti-CD28. Cells under T_H1 and T_H2 conditions were removed from TCRβ/CD28 stimulation on day 4, and cultured for 2 additional days in fresh cytokine-containing media without TCRβ/CD28 stimulation.

b) $CD4^+$ T-cells lacking Lck produce normal levels of Foxp3 and IL17 under T_H17-polarizing conditions. WT or *lck*$^{-/-}$ $CD4^+$ splenocytes were skewed in vitro under T_H17-polarizing conditions. IL17 and Foxp3 expression was analyzed by flow cytometry. Plots are gated on viable singlet $CD4^+$ events. The quantitation of results from at least six experiments is shown.

c, d) *fyn*$^{-/-}$ T_H17 cells express higher levels of Foxp3 and lower levels of IL17 despite changes in IL6 and TGFβ signal strength. IL6 or TGFβ concentrations were titrated in the T_H17 skewing of WT or *fyn*$^{-/-}$ CD25-depleted $CD4^+$ splenocytes. During the titration of one cytokine, the concentration of the other cytokine was kept constant at 20ng/ml or 1ng/ml for IL6 or TGFβ, respectively. After 5 days, Foxp3 (d) and IL17 (e) expression was analyzed by intracellular staining and flow cytometry in the viable singlet $CD4^+$ gate. Results are representative of three experiments.

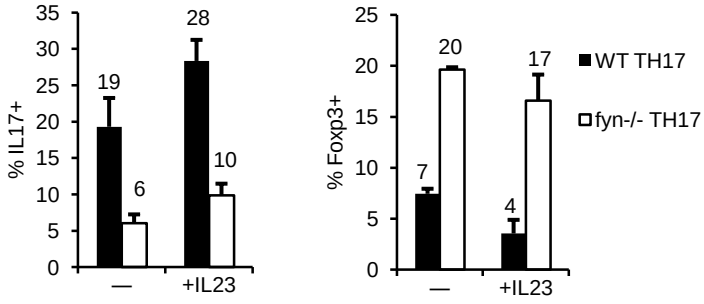
e) Expression of IL6 and TGFβ receptors is comparable in WT and *fyn*$^{-/-}$ T_H17 cells. At 48 hours (left) and 5 days (right) after the initiation of T_H17 skewing, the expression of *Il6rα* and *TgfβrI* in WT and *fyn*$^{-/-}$ $CD4^+$ T-cells was determined by qRT-PCR. The average of three experiments is shown; error bars denote one standard deviation from the mean. n.s.: not significant, two-tailed paired Student's t-test.



Supplentary Figure 2. *fyn*$^{-/-}$ T_H17 cells do not have elevated expression of IL2 or the high-affinity IL2-receptor subunit CD25, and exhibit proliferation and survival comparable to WT T_H17.

a, b) CD25-depleted CD4$^+$ splenocytes from WT or *fyn*$^{-/-}$ mice were skewed under T_H17-polarizing conditions. At the indicated timepoints, cells were stimulated with ionomycin and PMA in the presence of a protein transport inhibitor, and IL2 expression was determined by intracellular staining (a). Alternatively, the surface expression of CD25 was assessed on unstimulated cells (b). Plots are gated on viable singlet CD4$^+$ events. Data is representative of 4 (IL2) or 3 (CD25) independent experiments. Statistical significance between WT and *fyn*$^{-/-}$ means was determined by a two-tailed paired Student's t-test; **: $p \leq 0.01$.

c) MACS-purified CD25$^+$ or CD25$^-$ CD4$^+$ splenocytes were stained with CFSE and cultured under T_H17-skewing conditions for 2 days. Apoptosis and cell death were assessed by AnnexinV and 7-AAD staining. Alternatively, viable cells were identified as those with little staining by LiveDead reagent (Invitrogen). Plots are gated on total singlet events. Data is representative of 2 independent experiments.



Supplementary Figure 3. *fyn*$^{-/-}$ T_H17 cells retain IL23 responsiveness.
The T_H17-skewing culture of WT and *fyn*$^{-/-}$ CD4$^+$ T-cells was performed with or without the addition of exogenous IL23 (10ng/ml). IL17 and Foxp3 expression was determined by intracellular staining and flow cytometry. Plots are gated on viable singlet CD4$^+$ events. Numbers above the bars represent the mean value from two experiments, and the error bars denote one standard deviation from the mean.