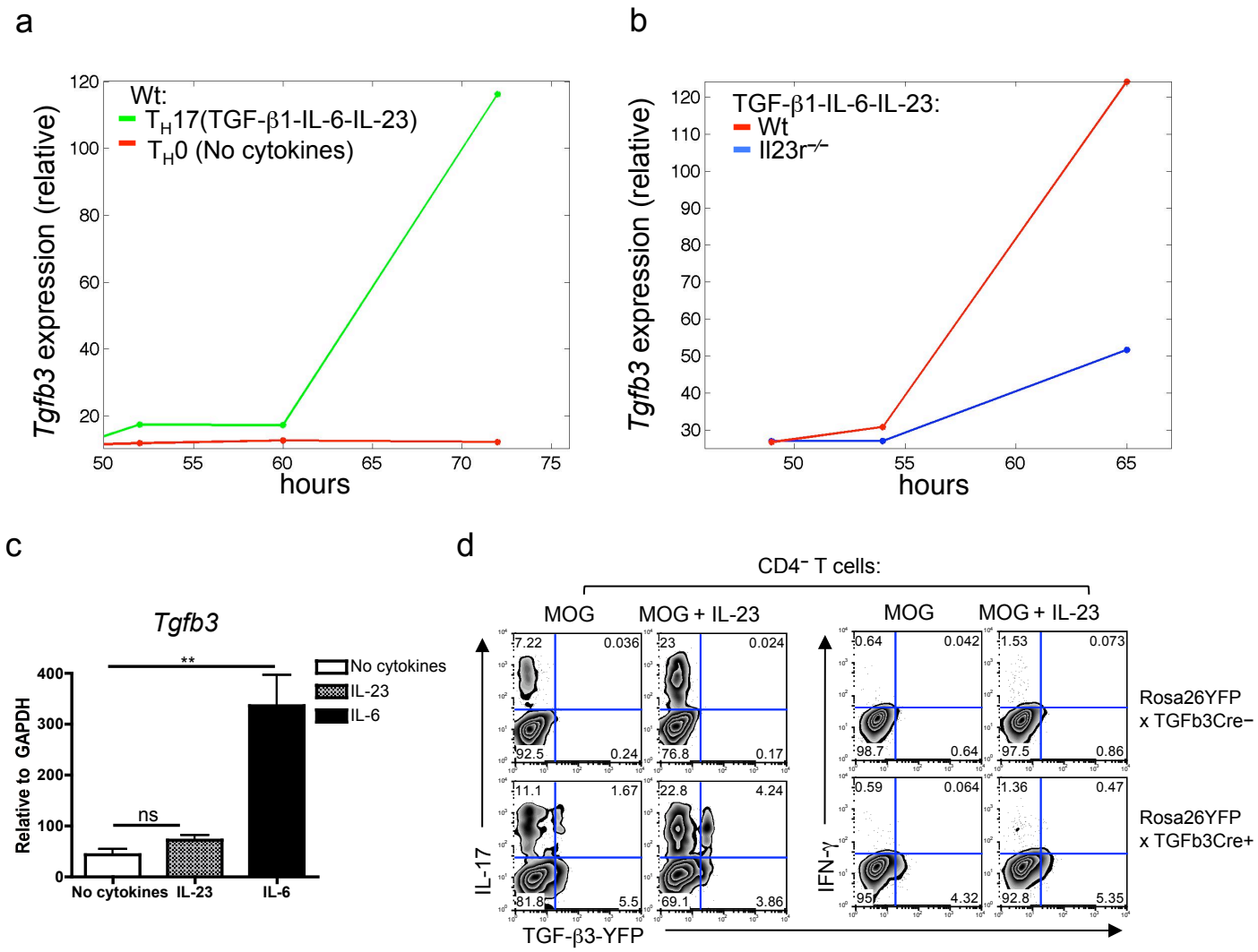
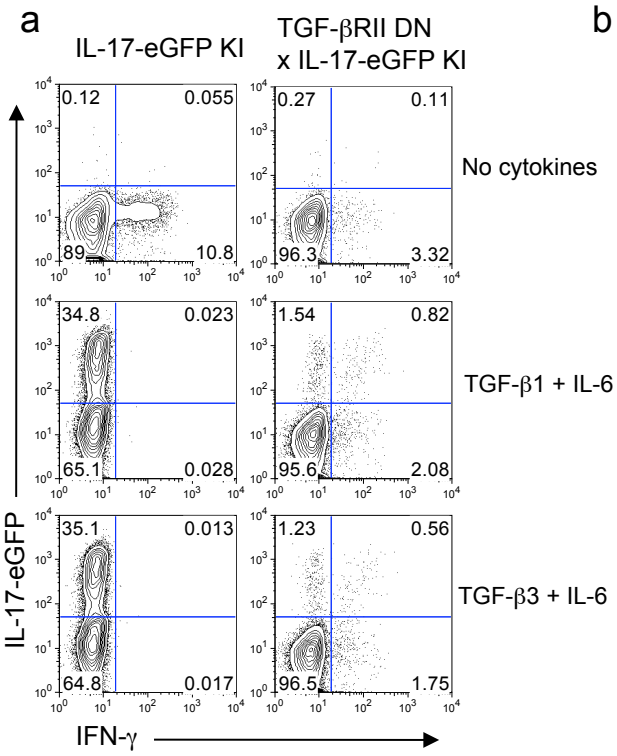
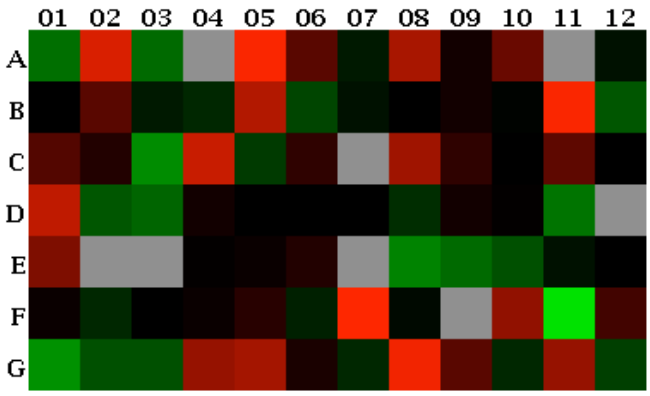
Induction and molecular signature of pathogenic T_H17 cells

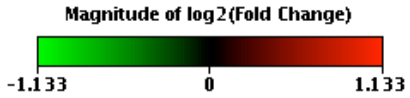
Youjin Lee, Amit Awasthi, Nir Yosef, Francisco J. Quintana, Sheng Xiao, Anneli Peters, Chuan Wu , Markus Kleinewietfeld, Sharon Kunder, David Hafler, Raymond A. Sobel, Aviv Regev, Vijay K. Kuchroo



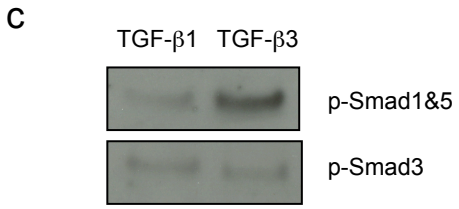
Supplementary figure 1: The induction of TGF-β3 in IL-17 producing cells (**a,b**) Microarray analysis showing *Tgfb3* mRNA expression of naïve $CD4^+$ T cells from wt or $Il23r^{-/-}$mice differentiated *in vitro* with TGF-β1-IL-6-IL-23 or no cytokines at various time points. (**c**) Quantitative RT-PCR showing *Tgfb3* mRNA expression of naïve $CD4^+$ T cells from wt mice activated *in vitro* with IL-6, IL-23, or no cytokines for four days. (**d**) Intracellular cytokine staining showing co-expression of TGF-β3-YFP with either IL-17 or IFN-γ in naïve $CD4^-$ T cells from MOG_{35-55}+CFA immunized TGF-$b3^{cre}$x$Rosa26^{yfp}$ mice stimulated with PMA+ionomycin four days post *in vitro* culture with or without IL-23. Data is a representation of at least two independent experiments.



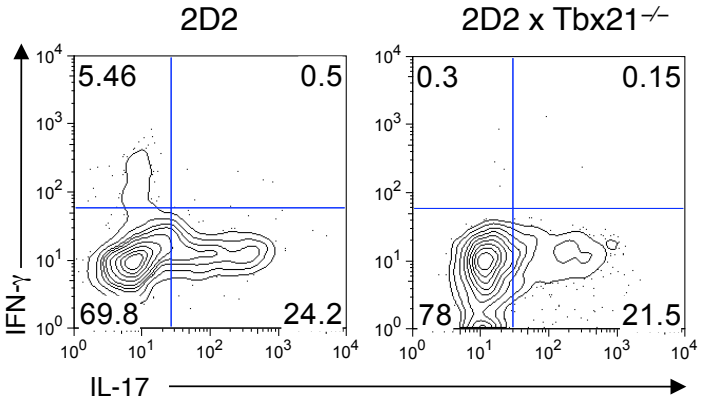




Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	Acta2 -1.35 B	Acvr1 1.91 A	Acvrl1 -1.33 OKAY	Agt -1.69 C	Aipl1 2.14 B	Ar 1.25 B	Atf3 -1.08 OKAY	Atf4 1.61 OKAY	Bach1 1.05 OKAY	Bcl2l1 1.31 OKAY	Bdnf -1.69 C	Bhlhe40 -1.06 OKAY
B	Brd2 -1.00 OKAY	Cdc6 1.26 OKAY	Cdkn1b -1.07 OKAY	Cebpb -1.11 OKAY	Creb1 1.66 B	Crebbp -1.19 OKAY	Cryab -1.05 B	Ctnnb1 -1.00 OKAY	Dnaja1 1.06 OKAY	E2f4 -1.02 OKAY	Emp1 2.17 OKAY	Eng -1.24 OKAY
C	Ep300 1.23 OKAY	Ephb2 1.09 B	Fn1 -1.49 B	Fos 1.77 OKAY	Furin -1.16 OKAY	Gadd45b 1.12 OKAY	Gli2 -1.69 C	Gtf2i 1.56 OKAY	Herpud1 1.12 OKAY	Hes1 1.01 A	Hey1 1.26 A	Hmox1 -1.00 OKAY
D	Id1 1.74 OKAY	Id2 -1.26 OKAY	Id3 -1.31 OKAY	Ifrd1 1.05 OKAY	Il10 1.01 OKAY	Klf10 -1.01 OKAY	Map3k7 1.01 OKAY	Mapk14 -1.12 OKAY	Mapk8 1.06 OKAY	Mbd1 1.02 OKAY	Mmp2 -1.38 B	Msx2 -1.69 C
E	Myc 1.39 OKAY	Myod1 -1.69 C	Nfib -1.69 C	Nfkbia 1.02 OKAY	Notch1 1.03 OKAY	Pdgfa 1.08 B	Plg -1.69 C	Ppara -1.44 B	Ptgs2 -1.33 OKAY	Pthlh -1.23 B	Ptk2 -1.06 OKAY	Ptk2b 1.01 OKAY
F	Rad21 1.05 OKAY	Rara -1.11 OKAY	Rbl1 1.01 OKAY	Rhoa 1.04 OKAY	Rhob 1.10 OKAY	Runx1 -1.08 OKAY	S100a8 2.19 B	Serpine1 -1.03 B	Shh -1.69 C	Smad1 1.49 OKAY	Smad3 -2.02 OKAY	Smad5 1.16 OKAY
G	Smad6 -1.50 OKAY	Snai1 -1.24 OKAY	Sox4 -1.23 OKAY	Sp1 1.50 OKAY	Srebf2 1.57 OKAY	Tgfb2 1.07 B	Tgfbr2 -1.10 OKAY	Thbs1 2.09 A	Tnfsf10 1.24 OKAY	Txnip -1.10 OKAY	Vegfa 1.50 OKAY	Wfs1 -1.18 OKAY



Supplementary figure 2: Downstream signaling genes induced by TGF-b3
(**a**) Intracellular cytokine staining of IL-17-GFP and IFN-γ from IL-17-GFP knock-in and TGF-βRII DNxIL-17-GFP KI mice naïve CD4⁺ T cells differentiated *in vitro* with TGF-β1-IL-6, TGF-β3+IL-6, or no cytokines for four days. Data is a representation of two independent experiments with similar results. (**b**) Quantitative RT-PCR analysis represented as a heat map of the TGF-β signaling PCR array from naïve CD4⁺ T cells stimulated with TGF-β1-IL-6 or TGF-β3-IL-6 for 24 hours. Raw data was uploaded to the manufacturer's website for analysis. Below the heat map is a template of the genes that correspond to the location on the heat map. Data shows the relative fold change of TGF-β3/IL-6 induced genes normalized to TGF-β1/IL-6. Data is pooled from three independent experiments. (**c**) Western blot showing smad1&5 or smad3 phosphorylation of CD4⁺ T cells stimulated with TGF-β1 or TGF-β3 for 30 minutes in serum free media.



Supplementary figure 3: Pathogenic 2D2 Tbx21$^{-/-}$ cells generated by TGF-β3-IL-6 do not express IFN-γ. Intracellular cytokine staining showing IL-17 and IFNγ from the CNS infiltrating vβ1.1^{+}CD4^{+} T cells of wt recipient mice at the peak of disease that were adoptively transferred with TGF-β3-IL-6 differentiated 2D2 or 2D2xTbx21$^{-/-}$ T_H17 cells. Data is a representation of two independent experiments.

1.5 fold cut off **Total 233 genes**

Gene Name	Fold change	Higher in TGF-β3 + IL-6
CXCL3	0.221	
GP49A;LILRB4	0.24	
IL22;ILTIFB	0.261	
TG	0.278	
LUM	0.282	
IFITM3	0.354	
IL3	0.389	
SERPINB6B	0.413	
5830443L24RIK	0.419	
TNFSF8	0.429	
CASP4	0.439	
CRABP2	0.441	
9230105E10RIK	0.444	
CDH17	0.461	
TNFSF11	0.471	
LAD1	0.476	
NEBL	0.477	
ACSL6	0.478	
GLRX	0.506	
CCL4	0.508	
5830405N20RIK	0.515	
IFIH1	0.519	
PLXNC1	0.534	
CST7	0.535	
GZMB	0.536	
AI451617;TRIM30	0.538	
AW112010	0.542	
GPR183	0.544	
NRN1	0.545	
FHOD3	0.545	
TRIM30	0.551	
TRAT1	0.552	
UPP1	0.556	
TPBG	0.559	
CCL5	0.559	
LRMP	0.559	
CASP1	0.567	
TRIM34	0.569	
CCL3	0.57	
CSF2	0.57	
EPSTI1	0.571	
GALNT3	0.571	
MS4A6B	0.571	
SPIN4	0.573	
ADH4	0.573	
CD9	0.574	
SELL	0.577	
GRAMD1B	0.579	
COBLL1	0.579	
INSL6	0.581	
6330442E10RIK	0.582	
TBX21	0.582	
CTBP2	0.583	
ICOS	0.583	
IL7R	0.585	
HIGD1C;METTL7A1;METTL7A2	0.59	
SERPINC1	0.59	
IFIT1	0.591	
GLIPR1	0.592	
ID3	0.592	
LOC100046186;RAMP3	0.593	
STAT4	0.599	
RNASEL	0.599	
RAB19	0.606	
INHBA	0.61	
ERMN	0.611	
SERPINF1	0.616	
EBF1	0.617	
GAP43	0.623	
IFI27L2A	0.624	
B3GNT5	0.628	
AKR1B8	0.628	
FAH	0.631	
CAPSL	0.635	
RFC3	0.64	
LGALS3	0.64	
ZBTB32	0.641	
AI451617	0.642	
SUPT3H	0.643	
DENND5A	0.643	
CASP6	0.643	
PTPLA	0.645	
GM10786	0.646	
LAG3	0.646	
MPA2L	0.646	
TRIB2	0.651	
ASB2	0.655	
SERPINB9	0.658	
HIST3H2BA	0.658	
PLK2	0.658	
TMEM71	0.659	
PSMB10	0.66	
FAM46A	0.66	
BB165335	0.661	
HSH2D	0.661	
CYSLTR2	0.661	
ARHGEF3	0.661	
DLG2	0.664	
DAPL1	0.665	
DTWD1	0.665	

Gene Name	Fold change	Higher in TGF-β1 + IL-6
TNIP2	1.503	
MAFF	1.503	
TMCC3	1.508	
CYP1B1	1.51	
2610024B07RIK	1.51	
FAM20A	1.51	
TEX264	1.513	
HMGA2-PS1	1.517	
2310007L24RIK	1.517	
ENAH	1.518	
IL6ST	1.526	
AHRR	1.526	
4930539E08RIK	1.53	
LOC433762	1.532	
RNF149	1.532	
CASP7	1.538	
GMFB	1.538	
LOC100046560;MAGED2	1.539	
B230120H23RIK	1.539	
SIAH1A	1.54	
BCL2L15	1.542	
PRPS1	1.547	
5730508B09RIK	1.548	
RGS1	1.554	
SPSB1	1.557	
FSCN1	1.567	
CRISPLD2	1.568	
IGFBP4	1.568	
CD40	1.579	
ILDR1	1.582	
RRAGD	1.59	
KLF10	1.603	
GOLM1	1.606	
FNBP1L	1.61	
LTB4R1	1.611	
ME2	1.613	
ANTXR2	1.633	
EAF2	1.633	
RASA3	1.635	
FRMD6	1.64	
BMPR2	1.641	
SKIL	1.643	
SEMA7A	1.645	
SLC39A8	1.648	
D18ERTD653E	1.648	
F2R	1.657	
CALHM2	1.661	
MAGED1	1.662	
TGFBR1	1.663	
MTMR7	1.663	
PELI2	1.665	
MYO5A	1.67	
TMEFF1	1.679	
B4GALT1	1.682	
APLP2	1.683	
CXCR4	1.683	
TEC	1.685	
TNK2	1.693	
ID1	1.701	
SLC44A1	1.706	
CYP2S1	1.707	
GPSM2	1.719	
CNDP2	1.736	
SBK1	1.738	
F3	1.739	
PIK3R1	1.745	
CCL20	1.748	
POU2AF1	1.749	
ASS1	1.753	
NEURL3	1.754	
KCNK6	1.772	
PYGL	1.791	
TIMP1	1.793	
PDPN	1.801	
B3GNT8	1.801	
EPB4.1L2	1.816	
CLDND1	1.818	
FAM129B	1.824	
IL1RN	1.833	
CPD	1.836	
TNFRSF13B	1.837	
CLDN4	1.841	
ANXA3	1.896	
GPR15	1.904	
PRNP	1.904	
ZFP238	1.911	
ENDOD1	1.917	
CYP11A1	1.921	
DST	1.936	
PLOD2	1.94	
4930486L24RIK	1.954	
KLRC1	1.963	
ALPK2	1.964	
IKZF3	1.966	
CTSF	1.969	
N4BP1	1.988	
PMEPA1	1.997	
GATM	2	
GM10883;GM1420;GM7202;IGK;IGK-C;IGK-V28	2.012	
ABP1	2.058	
APOL9A;APOL9B	2.101	
5430411K18RIK	2.107	
PLEKHF2	2.109	
GFPT2	2.19	
PDLIM5	2.221	
INTS4	2.321	
2010205A11RIK;GM10883;GM1420;GM7202;IGK	2.328	
9030418K01RIK	2.361	
CD86	2.439	
HAVCR1	2.449	
MAF	2.463	
LBP	2.579	
ACSBG1	2.585	
EMP1	2.588	
GCNT2	2.654	
AHR	2.761	
NT5E	2.765	
CTLA2A	3.008	
AA467197	3.02	
PROCR	3.108	
CD38	3.224	
CTLA2A;CTLA2B	3.323	
TMEM176B	3.388	
KLRD1	3.469	
AQP3	3.532	
KCTD14	3.549	
TMEM176A	3.558	
SGK1	3.93	
IL9	3.959	
CTSW	4.157	
IL10	4.249	
NIPAL1	4.32	
CTLA2B	4.673	

Supplementary table 1| Naive CD4$^+$ T cells were sorted and stimulated with the indicated cytokines. Total RNA was collected for microarray analysis. Comparison of the gene expression profiles between TGF-β3/IL-6 and TGF-β1/IL-6 T_H17 cells that showed at least a 1.5 fold change between these two conditions. Total of 233 genes were selected and ordered according to the relative fold change. The gradient triangle denotes the degree of fold change within each condition.