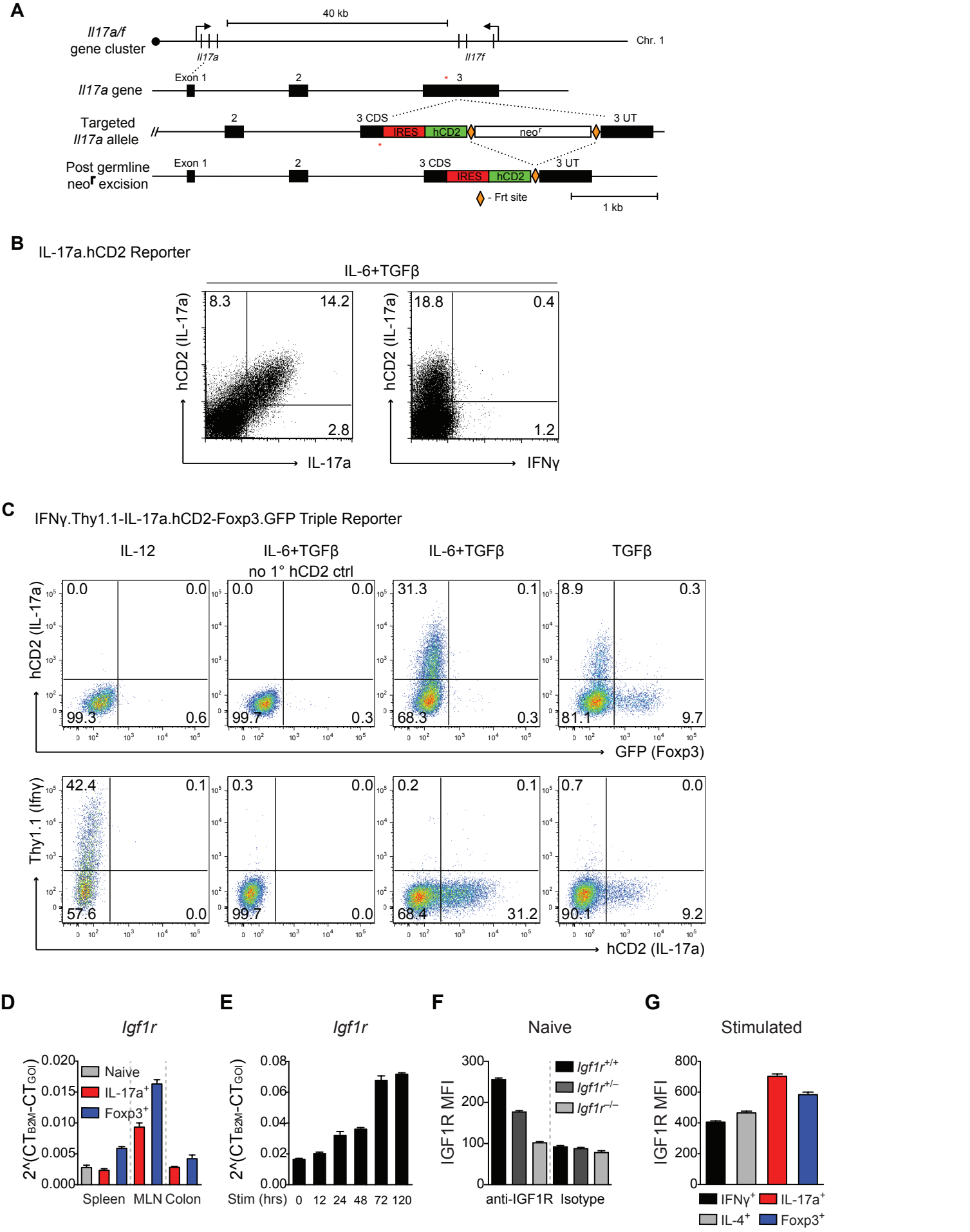
A
Il17a/f gene cluster
40 kb
Chr. 1
Il17a
Il17f
Exon 1
2
3
Il17a gene
Targeted Il17a allele
3 CDS
IRES
hCD2
neo^r
3 UT
Post germline neo^r excision
- Frt site
1 kb
B
IL-17a.hCD2 Reporter
IL-6+TGFβ
hCD2 (IL-17a)
8.3
14.2
2.8
IL-17a
18.8
0.4
1.2
IFNγ
C
IFNγ.Thy1.1-IL-17a.hCD2-Foxp3.GFP Triple Reporter
IL-12
IL-6+TGFβ
no 1° hCD2 ctrl
IL-6+TGFβ
TGFβ
hCD2 (IL-17a)
0.0
0.0
99.3
0.6
0.0
0.0
99.7
0.3
31.3
0.1
68.3
0.3
8.9
0.3
81.1
9.7
GFP (Foxp3)
Thy1.1 (Ifnγ)
42.4
0.1
57.6
0.0
0.3
0.0
99.7
0.0
0.2
0.1
68.4
31.2
0.7
0.0
90.1
9.2
hCD2 (IL-17a)
D
Igf1r
2^(CT_B2M-CT_GOI)
Naive
IL-17a+
Foxp3+
Spleen
MLN
Colon
E
Igf1r
Stim (hrs)
0
12
24
48
72
120
F
Naive
IGF1R MFI
Igf1r+/+
Igf1r+/–
Igf1r–/–
anti-IGF1R
Isotype
G
Stimulated
IGF1R MFI
IFNγ+
IL-17a+
IL-4+
Foxp3+

Figure S1

Figure S1. IL-17a reporter design and validation.

Related to Main Figure 1.

(**A**) Expression cassette and targeting strategy used to generate IL-17a.hCD2 reporter mice. The Frt-flanked neomycin resistance cassette was removed following germ line transmission by crossing founders to EIIa.Flp transgenic C57BL/6 mates. (**B**) Sorted naïve IL-17a.hCD2 $CD4^+$ T cells were stimulated in Th17 conditions for 5 days. Flow cytometric analysis of IL-17a, hCD2, and IFNγ shown. (**C**) Sorted naïve IFNγ.Thy1.1-IL-17a.hCD2-Foxp3.GFP $CD4^+$ T cells were stimulated under Th1, Th17 and Treg conditions for 5 days and analyzed by flow cytometry. (**D**) IL-17a.hCD2-Foxp3.GFP mice were orally gavaged with 1-2x10^9 CFU *Citrobacter rodentium* strain DBS100 (ATCC 51459). 8 days later, RNA was isolated from splenic naïve $CD4^+$ T cells, and IL-17a.hCD2$^+$ and Foxp3.GFP$^+$ $CD4^+$ T cells harvested from the spleen, mesenteric lymph nodes (MLN) and colon. qPCR for *Igf1r* is shown. (**E**) Sorted naïve WT $CD4^+$ T cells were cultured in Th17 conditions for the indicated times. mRNA was analyzed by qPCR for expression of *Igf1r*. (**F**) Flow cytometric analysis of IGF1R on splenic naïve $CD4^+$ T cells isolated from *Cd4.Cre Igf1r* $^{+/+}$, $^{fl/+}$, and $^{fl/fl}$ mice. (**G**) Sorted WT naïve $CD4^+$ T cells were stimulated in Th1, Th2, Th17 and Treg conditions for 5 days. Surface IGF1R on cytokine-positive cells was analyzed by flow cytometry.

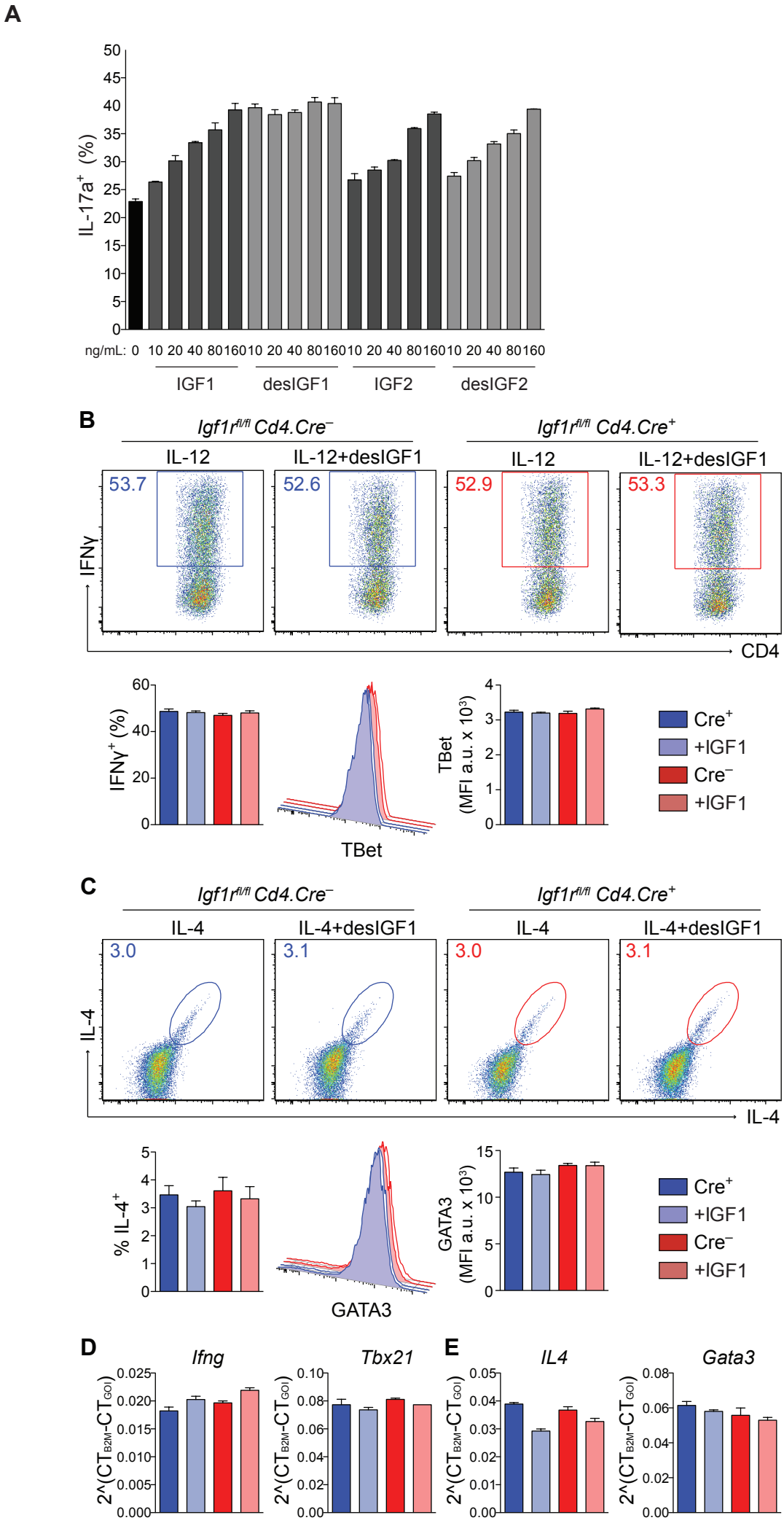
A
IL-17a+ (%)
ng/mL: 0 10 20 40 80160 10 20 40 80160 10 20 40 80160 10 20 40 80160
IGF1
desIGF1
IGF2
desIGF2
B
Igf1rfl/fl Cd4.Cre−
Igf1rfl/fl Cd4.Cre+
IL-12
IL-12+desIGF1
53.7
52.6
52.9
53.3
IFNγ
CD4
IFNγ+ (%)
TBet
TBet (MFI a.u. x 10³)
Cre+
+IGF1
Cre−
+IGF1
C
IL-4
IL-4+desIGF1
3.0
3.1
3.0
3.1
IL-4
% IL-4+
GATA3
GATA3 (MFI a.u. x 10³)
D
Ifng
Tbx21
2^(CTB2M-CTGOI)
E
IL4
Gata3

Figure S2

Figure S2. Signaling through IGF1R does not influence Th1 and Th2 differentiation in-vitro.
Related to Main Figure 1.

(**A**) Sorted naive CD4$^+$ T cells were activated in Th17 (**A**) or Treg (not shown) conditions for 5 days in the indicated concentrations of IGF1, desIGF1, IGF2 and desIGF2 then assessed for expression of IL17a and Foxp3 by flow cytometry. (**B**) Sorted naive CD4$^+$ T cells were stimulated in-vitro for 5 days in Th17 (upper panels) or Treg (lower panels) cell conditions with or without desIGF1 and qPCR preformed for expression of *Il17a*, *Rorc* and *Foxp3* mRNA isolated from sorted total cells. 1 of 3 similar experiments shown. (**C-E**) Sorted naive *Igf1r*$^{fl/fl}$ Cre$^-$ and Cre$^+$ CD4$^+$ T cells were cultured in-vitro for 5 days in Th1 (**C,E**) or Th2 (**D,F**) conditions. Data are representative of 3-5 independent experiments. (**C**) Flow cytometric analysis of IFNγ and Tbet expression in Th1 conditions. (**D**) Flow cytometric analysis of IL-4 and Gata3 expression in Th2 conditions. (**E**) RNA isolated from total live CD4$^+$ T cells cultured in Th1 conditions was analyzed by qPCR for expression of *Ifng* and *Tbx21*. (**F**) RNA isolated from total live CD4$^+$ T cells cultured in Th2 conditions was analyzed by qPCR for expression of *Il4* and *Gata3*.

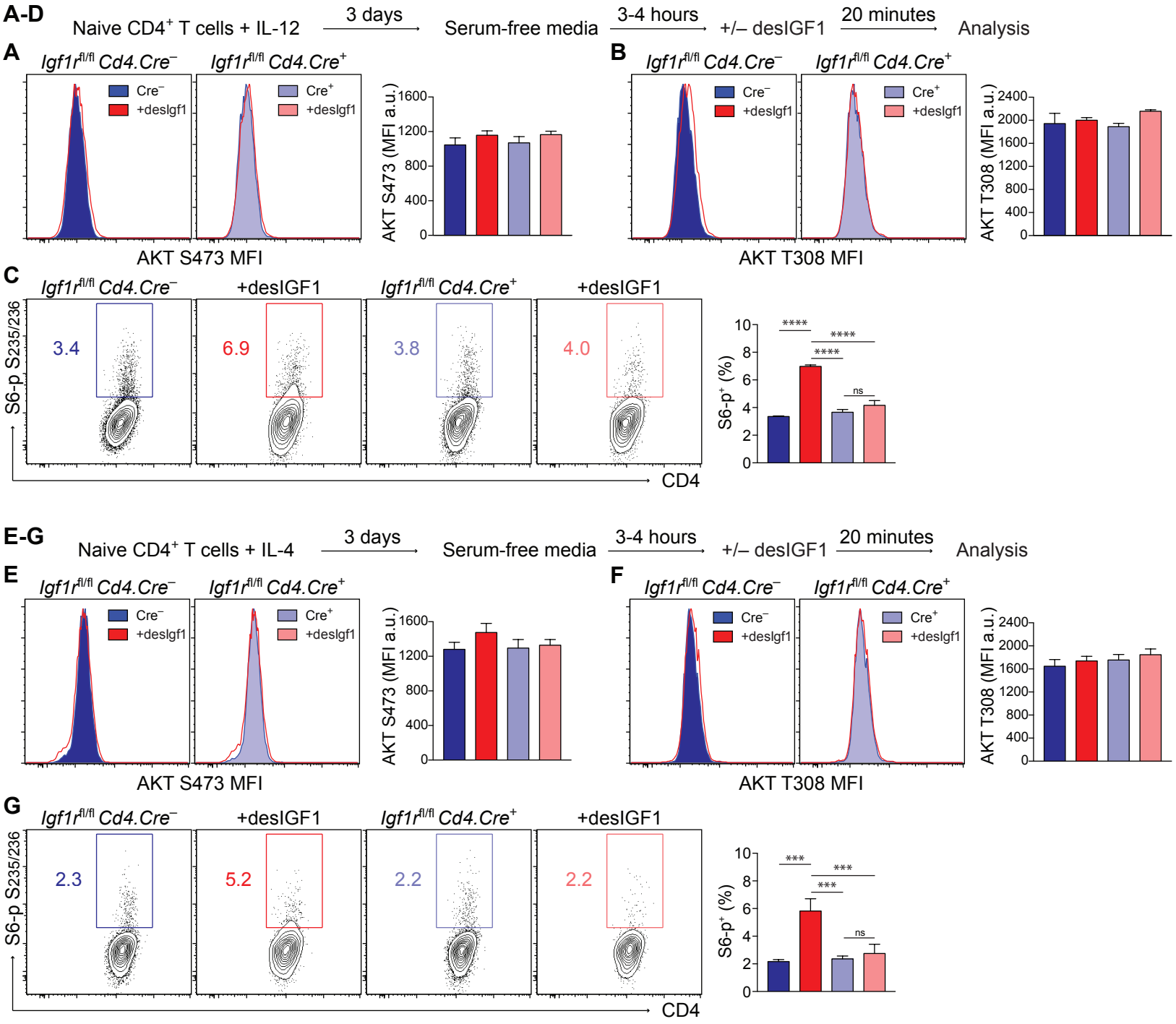
A-D
Naive CD4+ T cells + IL-12
3 days
Serum-free media
3-4 hours
+/– desIGF1
20 minutes
Analysis
A
Igf1rfl/fl Cd4.Cre–
Igf1rfl/fl Cd4.Cre+
Cre–
+deslgf1
Cre+
+deslgf1
AKT S473 MFI
AKT S473 (MFI a.u.)
B
AKT T308 MFI
AKT T308 (MFI a.u.)
C
+desIGF1
3.4
6.9
3.8
4.0
S6-p S235/236
CD4
S6-p+ (%)
****
ns
E-G
Naive CD4+ T cells + IL-4
E
F
G
2.3
5.2
2.2
2.2
***

Figure S3

Figure S3. IGF1R signaling does not activate AKT/mTOR in Th1 and Th2 CD4$^+$ T cells.
Related to Main Figure 2.
(**A-C**) Sorted naive CD4$^+$ T cells were activated for 3 days in-vitro in Th1 conditions, washed and serum-starved for 4-6 hours, then re-stimulated with desIGF1 for 15 minutes. Flow cytometric analysis of phosphorylation of AKT on serine 473 (**A**) and threonine 308 (**B**) and phosphorylation of S6 ribosomal protein (**C**) is shown. Data for A-C are representative of 3 separate experiments. (**D-F**) Sorted naive CD4$^+$ T cells were activated for 3 days in-vitro in Treg conditions, washed and serum-starved for 4-6 hours, then re-stimulated with desIGF1 for 15 minutes. Flow cytometric analysis of phosphorylation of AKT on serine 473 (**D**) and threonine 308 (**E**) and phosphorylation of S6 ribosomal protein (**F**) is shown. Data for D-F are representative of 3 separate experiments. Data in A-F were analyzed using two-way ANOVAs with Tukey's post-hoc multiple comparisons test.

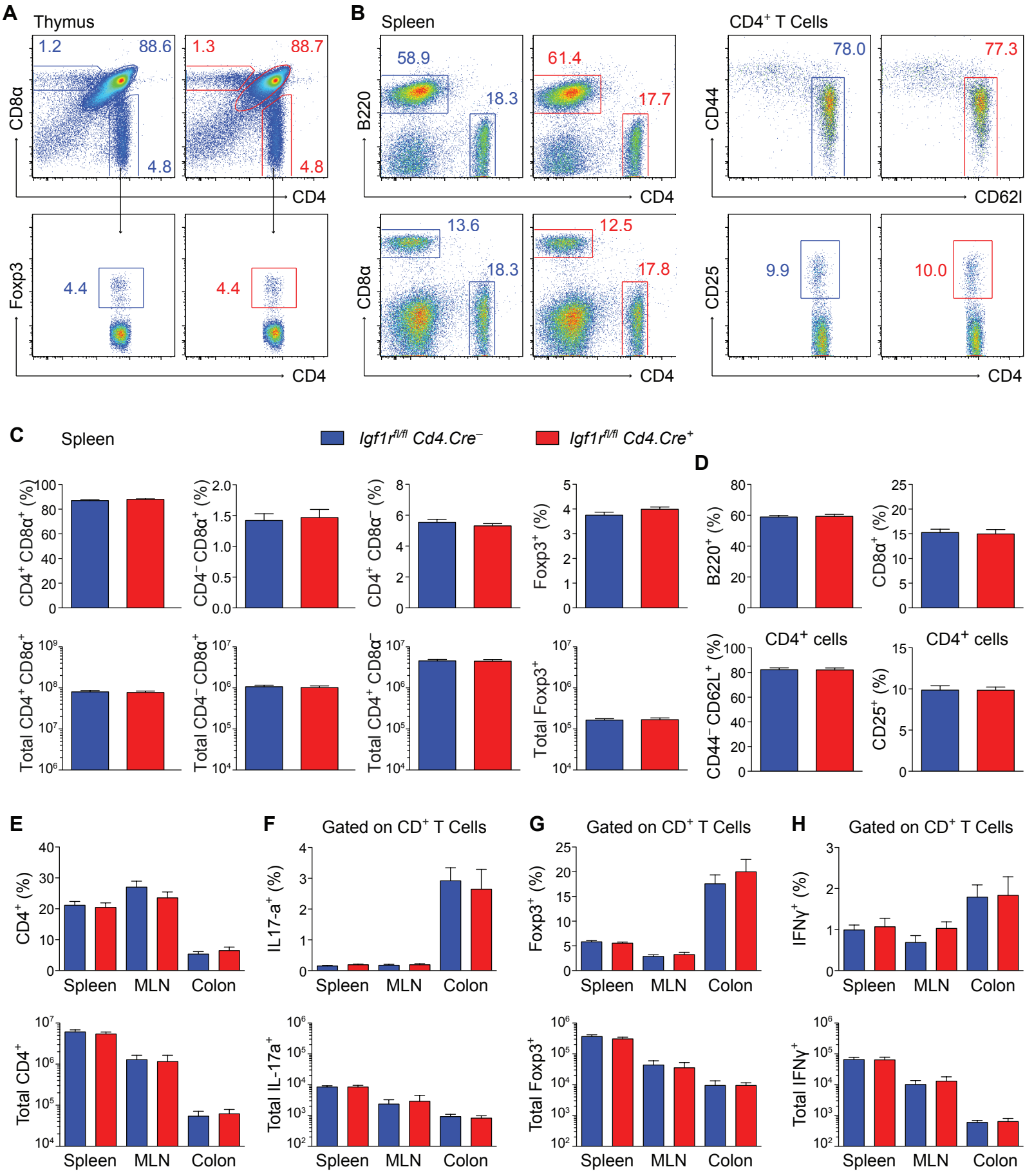
A
Thymus
1.2
88.6
1.3
88.7
CD8α
CD4
4.8
4.8
Foxp3
4.4
4.4
CD4
B
Spleen
58.9
18.3
61.4
17.7
B220
CD4
13.6
18.3
12.5
17.8
CD8α
CD4
CD4+ T Cells
78.0
77.3
CD44
CD62l
9.9
10.0
CD25
CD4
C
Spleen
Igf1rfl/fl Cd4.Cre−
Igf1rfl/fl Cd4.Cre+
CD4+ CD8α+ (%)
CD4− CD8α+ (%)
CD4+ CD8α− (%)
Foxp3+ (%)
Total CD4+ CD8α+
Total CD4− CD8α+
Total CD4+ CD8α−
Total Foxp3+
D
B220+ (%)
CD8α+ (%)
CD44− CD62L+ (%)
CD4+ cells
CD25+ (%)
CD4+ cells
E
CD4+ (%)
Total CD4+
F
Gated on CD+ T Cells
IL17-a+ (%)
Total IL-17a+
G
Gated on CD+ T Cells
Foxp3+ (%)
Total Foxp3+
H
Gated on CD+ T Cells
IFNγ+ (%)
Total IFNγ+
Spleen
MLN
Colon

Figure S4

Figure S4. Deletion of IGF1R in cells expressing CD4 does not alter endogenous lymphocyte populations.

Related to Main Figure 3.

Gender-matched litter-mate controls were used in all studies of endogenous lymphocyte populations. In all panels, data from IGF1R floxed CD4-Cre– mice are depicted in blue, while data from CD4-Cre^{+} mice are depicted in red. (**A**) Flow cytometric analysis of thymocyte expression of CD8a, CD4 and Foxp3. Data are representative of 2 separate experiments (WT n=15, KO n=12). (**B**) Flow cytometric analysis of CD4, CD8a, B220, CD44, CD25 and CD62l expression on splenic lymphocytes. Data are representative of 2 separate experiments (WT n=12, KO n=12). (**C**) Quantification of data depicted in A. (**D**) Quantification of data depicted in B. (**E-H**) Percent and total number of live (**G**), IL-17a^{+} (**F**), Foxp3^{+} (**G**), and IFNγ^{+} (**H**) $^{+}$T cells recovered from the spleen, MLN and colon. Data shown are pooled from 2 separate experiments (WT n=16, KO n=13).

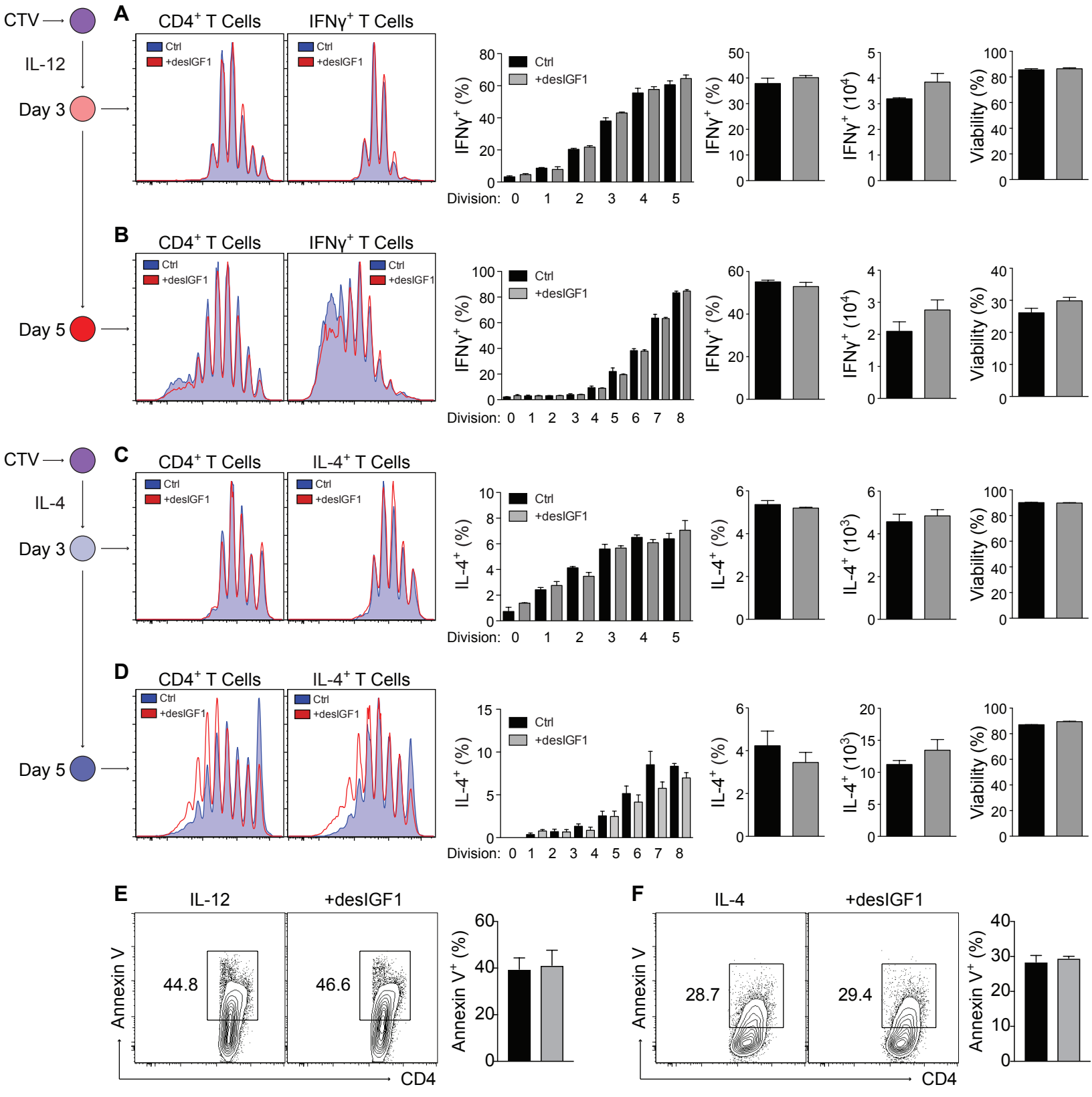
CTV
IL-12
Day 3
Day 5
A
B
C
D
E
F
CD4+ T Cells
IFNγ+ T Cells
IL-4+ T Cells
Ctrl
+desIGF1
IFNγ+ (%)
IFNγ+ (10^4)
IL-4+ (%)
IL-4+ (10^3)
Viability (%)
Division: 0 1 2 3 4 5
Division: 0 1 2 3 4 5 6 7 8
IL-12
IL-4
+desIGF1
Annexin V
CD4
44.8
46.6
28.7
29.4
Annexin V+ (%)

Figure S5

Figure S5. Insulin-like growth factors do not alter proliferation or apoptosis under Th1 or Th2 conditions.

Related to Main Figure 4.

Sorted naive CD4$^+$ T cells were labeled with CellTrace Violet and stimulated in Th1 (**A, B**) or Th2 (**C, D**) conditions with or without 20ng/mL desIGF1 for 3 (**A, C**) or 5 (**B**, **D**) days then assessed for expression of IFNγ and IL-4 by flow cytometry. Flow plots are gated on total live (left) or cytokine-positive (right) cells. The left-most bar graphs indicate the percent IFNγ$^+$ (**A, B**) or IL-4$^+$ (**C, D**) within each Cell Trace Violet peak. Bar graphs to the right indicate the percent and total number of IFNγ$^+$ or IL-4$^+$ cells as well as the percent of cells staining negative for a dead-cell marker (far right). (**E**, **F**) Sorted naive CD4$^+$ T cells were stimulated in Th1 (**E**) or Th2 (**F**) conditions for 5 days. Viability and surface levels of Annexin V were assessed by flow cytometry. Flow plots are gated on total live CD4$^+$ cells and depict cell-number controlled concatenated data of three replicates from one representative experiment. All experiments were performed 2-3 times each. Data from one representative are experiment shown. Student's t-tests were used to assess significance.

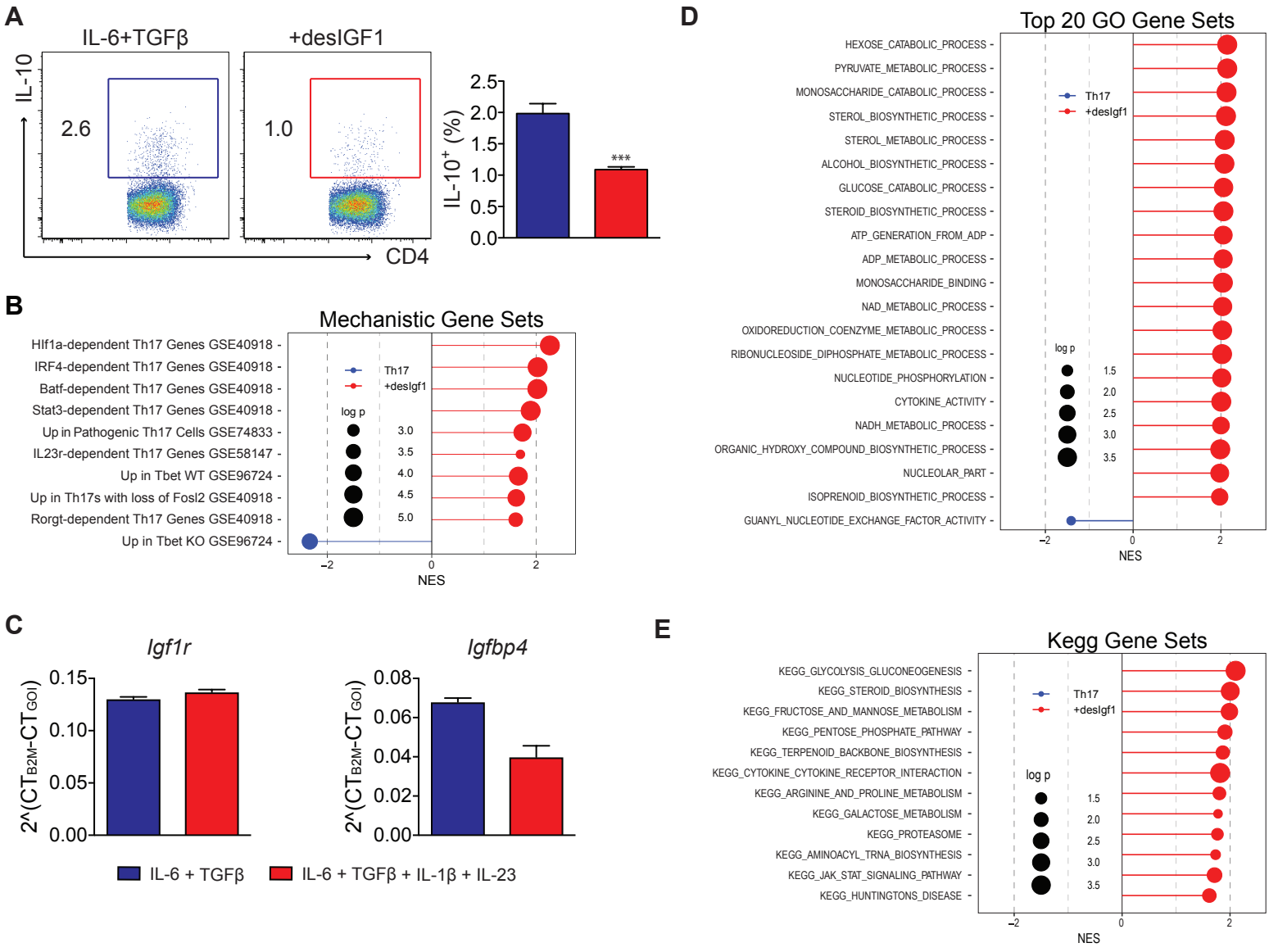
A
IL-6+TGFβ
+desIGF1
IL-10
2.6
1.0
CD4
IL-10+ (%)
***
B
Mechanistic Gene Sets
HIf1a-dependent Th17 Genes GSE40918
IRF4-dependent Th17 Genes GSE40918
Batf-dependent Th17 Genes GSE40918
Stat3-dependent Th17 Genes GSE40918
Up in Pathogenic Th17 Cells GSE74833
IL23r-dependent Th17 Genes GSE58147
Up in Tbet WT GSE96724
Up in Th17s with loss of Fosl2 GSE40918
Rorgt-dependent Th17 Genes GSE40918
Up in Tbet KO GSE96724
Th17
+desIgf1
log p
NES
C
Igf1r
Igfbp4
2^(CTB2M-CTGOI)
IL-6 + TGFβ
IL-6 + TGFβ + IL-1β + IL-23
D
Top 20 GO Gene Sets
HEXOSE_CATABOLIC_PROCESS
PYRUVATE_METABOLIC_PROCESS
MONOSACCHARIDE_CATABOLIC_PROCESS
STEROL_BIOSYNTHETIC_PROCESS
STEROL_METABOLIC_PROCESS
ALCOHOL_BIOSYNTHETIC_PROCESS
GLUCOSE_CATABOLIC_PROCESS
STEROID_BIOSYNTHETIC_PROCESS
ATP_GENERATION_FROM_ADP
ADP_METABOLIC_PROCESS
MONOSACCHARIDE_BINDING
NAD_METABOLIC_PROCESS
OXIDOREDUCTION_COENZYME_METABOLIC_PROCESS
RIBONUCLEOSIDE_DIPHOSPHATE_METABOLIC_PROCESS
NUCLEOTIDE_PHOSPHORYLATION
CYTOKINE_ACTIVITY
NADH_METABOLIC_PROCESS
ORGANIC_HYDROXY_COMPOUND_BIOSYNTHETIC_PROCESS
NUCLEOLAR_PART
ISOPRENOID_BIOSYNTHETIC_PROCESS
GUANYL_NUCLEOTIDE_EXCHANGE_FACTOR_ACTIVITY
Th17
+desIgf1
log p
NES
E
Kegg Gene Sets
KEGG_GLYCOLYSIS_GLUCONEOGENESIS
KEGG_STEROID_BIOSYNTHESIS
KEGG_FRUCTOSE_AND_MANNOSE_METABOLISM
KEGG_PENTOSE_PHOSPHATE_PATHWAY
KEGG_TERPENOID_BACKBONE_BIOSYNTHESIS
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION
KEGG_ARGININE_AND_PROLINE_METABOLISM
KEGG_GALACTOSE_METABOLISM
KEGG_PROTEASOME
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS
KEGG_JAK_STAT_SIGNALING_PATHWAY
KEGG_HUNTINGTONS_DISEASE
Th17
+desIgf1
log p
NES
F
ECAR (mpH/min)
Ctrl
+desIGF1
Rotenone
Antimycin-A
2-deoxyglucsoe
G
OCR (mpH/min)
Ctrl
+desIGF1
O
F
AA
2-DG
Minutes
H
OCR (mpH/min)
Ctrl
+desIGF1
Basal
ATP
H+ Leak
Maximal
Reserve
Non-mito

Figure S6

Figure S6. Insulin-like growth factors alter the transcriptional, translational and metabolic identity of Th17 cells.

Related to Main Figure 6.

(**A**) Sorted naïve CD4$^+$ T cells were stimulated in Th17 conditions with (red) and without (blue) desIGF1 for 5 days. Expression of IL-10 was assessed by flow cytometric intracellular staining. Data from one of five similar experiments shown. (**B**) Gene Set Enrichment Analysis (GSEA) was performed on RNA-seq data shown in Figure 7 using gene sets generated from publicly available transcriptomic datasets describing CD4$^+$ T cells lacking the indicates genes. (**C**) qPCR for *Igf1R* and *Igfbp4* was performed on RNA isolated from sorted naïve CD4$^+$ T cells stimulated in the indicated conditions for 5 days. (**D+E**) Pathway analysis was performed using all publicly available Gene Ontology gene sets (**D**) and Kyoto Encyclopedia of Genes and Genomes (**E**). A positive Normalized Enrichment Score (NES) indicates the gene set is enriched in desIGF1-treated cells (red), while a negative score indicates enrichment in control cells (blue). Adjusted p-values are indicated by diameter (see key, and extended methods for details). (**F**) Quantification of glycolysis rate assay shown in Figure 6F. (**G**) Oxygen consumption rate (OCR) trace of mitochondrial stress test (MST) performed on in-vitro generated Th17 cells cultured with (red) and without (blue) desIGF1. Measurements were performed prior to (basal) and following sequential addition of oligomycin (O; 1 μg/ml), FCCP (F; 1 μM), antimycin-A (AA; 10 μM) and 2-deoxyglucose (2-DG; 50 mM). (**H**) Quantification of glycolysis rate assay shown in Figure 6F.