Figure S1

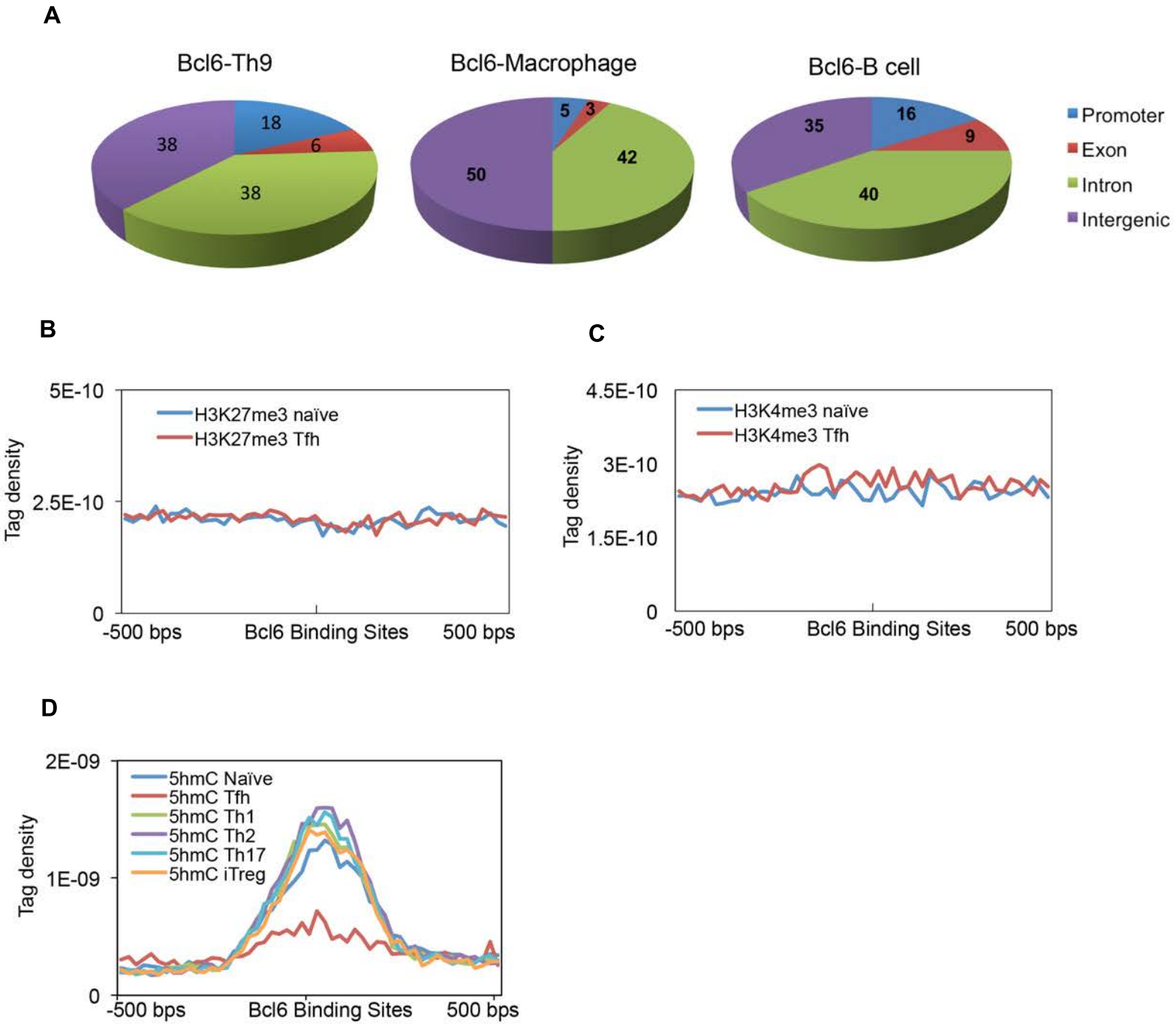

A
Bcl6-Th9
18
6
38
38
Bcl6-Macrophage
5
3
42
50
Bcl6-B cell
16
9
40
35
Promoter
Exon
Intron
Intergenic
B
5E-10
2.5E-10
0
Tag density
H3K27me3 naïve
H3K27me3 Tfh
-500 bps
Bcl6 Binding Sites
500 bps
C
4.5E-10
3E-10
1.5E-10
0
Tag density
H3K4me3 naïve
H3K4me3 Tfh
-500 bps
Bcl6 Binding Sites
500 bps
D
2E-09
1E-09
0
Tag density
5hmC Naïve
5hmC Tfh
5hmC Th1
5hmC Th2
5hmC Th17
5hmC iTreg
-500 bps
Bcl6 Binding Sites
500 bps

Figure S2

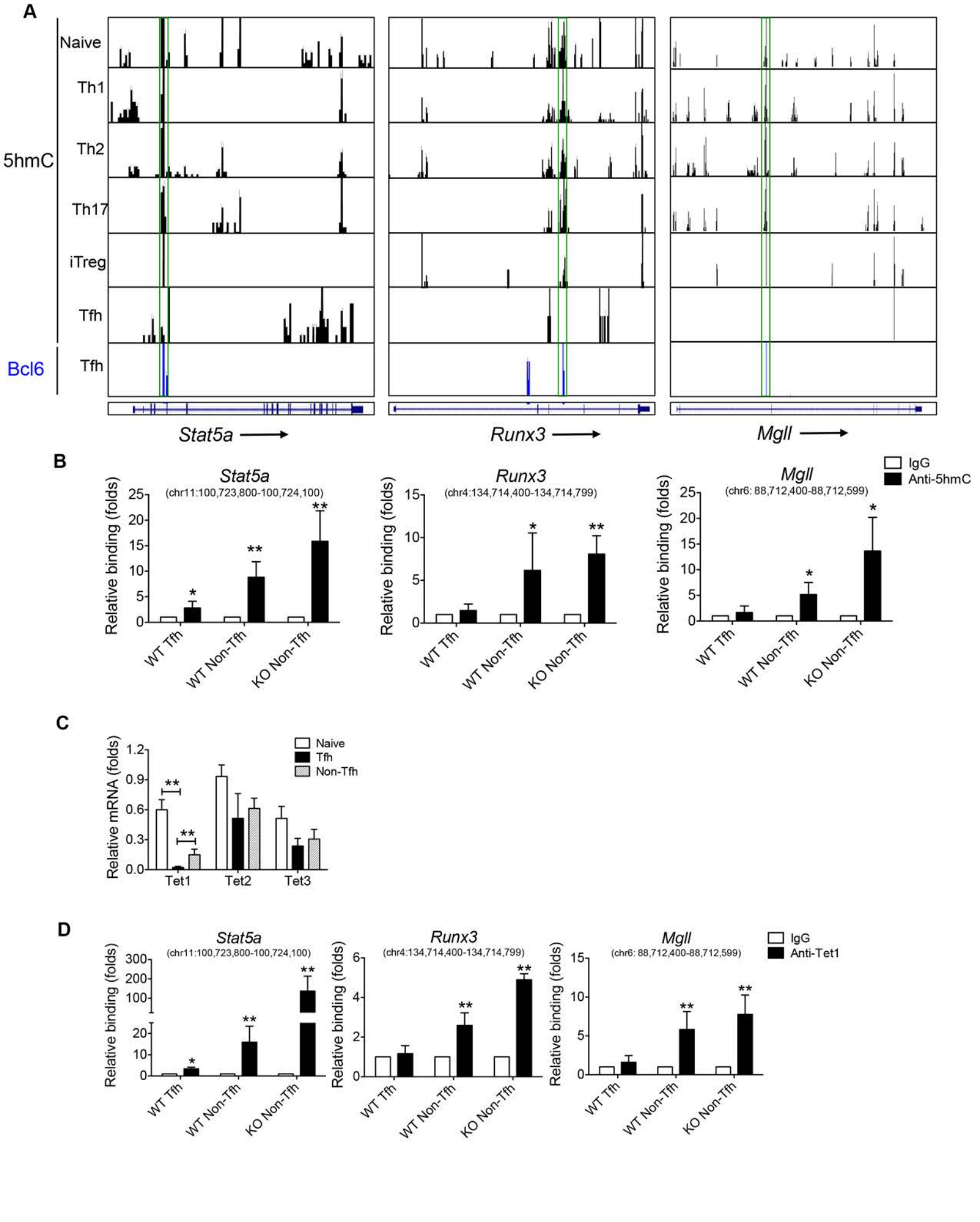

A
Naive
Th1
Th2
Th17
iTreg
Tfh
5hmC
Tfh
Bcl6
Stat5a
Runx3
Mgll
B
Stat5a
(chr11:100,723,800-100,724,100)
Runx3
(chr4:134,714,400-134,714,799)
Mgll
(chr6: 88,712,400-88,712,599)
IgG
Anti-5hmC
Relative binding (folds)
WT Tfh
WT Non-Tfh
KO Non-Tfh
C
Naive
Tfh
Non-Tfh
Relative mRNA (folds)
Tet1
Tet2
Tet3
D
Stat5a
(chr11:100,723,800-100,724,100)
Runx3
(chr4:134,714,400-134,714,799)
Mgll
(chr6: 88,712,400-88,712,599)
IgG
Anti-Tet1
Relative binding (folds)
WT Tfh
WT Non-Tfh
KO Non-Tfh

Figure S3

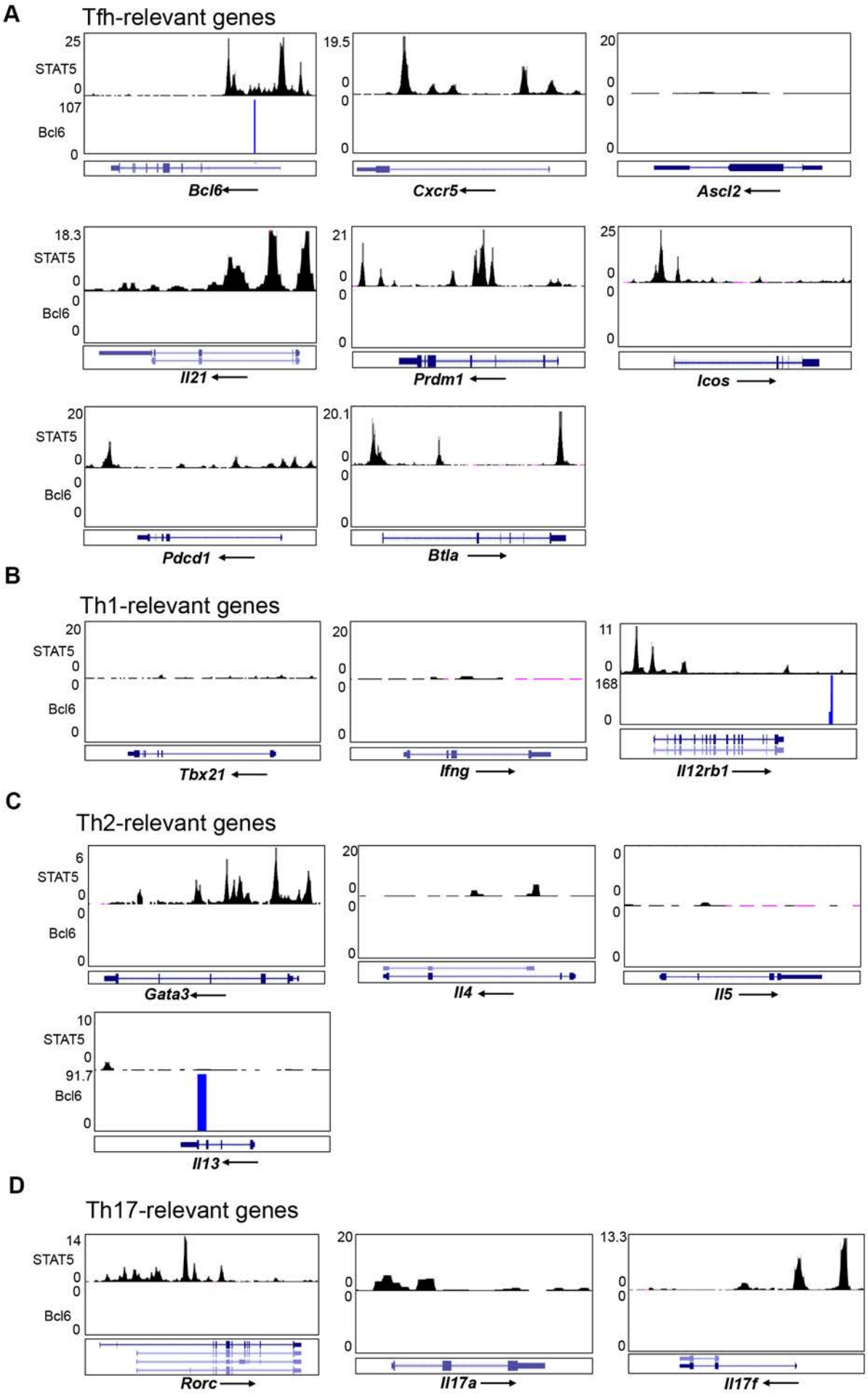

A
Tfh-relevant genes
STAT5
Bcl6
Bcl6
Cxcr5
Ascl2
Il21
Prdm1
Icos
Pdcd1
Btla
B
Th1-relevant genes
Tbx21
Ifng
Il12rb1
C
Th2-relevant genes
Gata3
Il4
Il5
Il13
D
Th17-relevant genes
Rorc
Il17a
Il17f

Figure S4

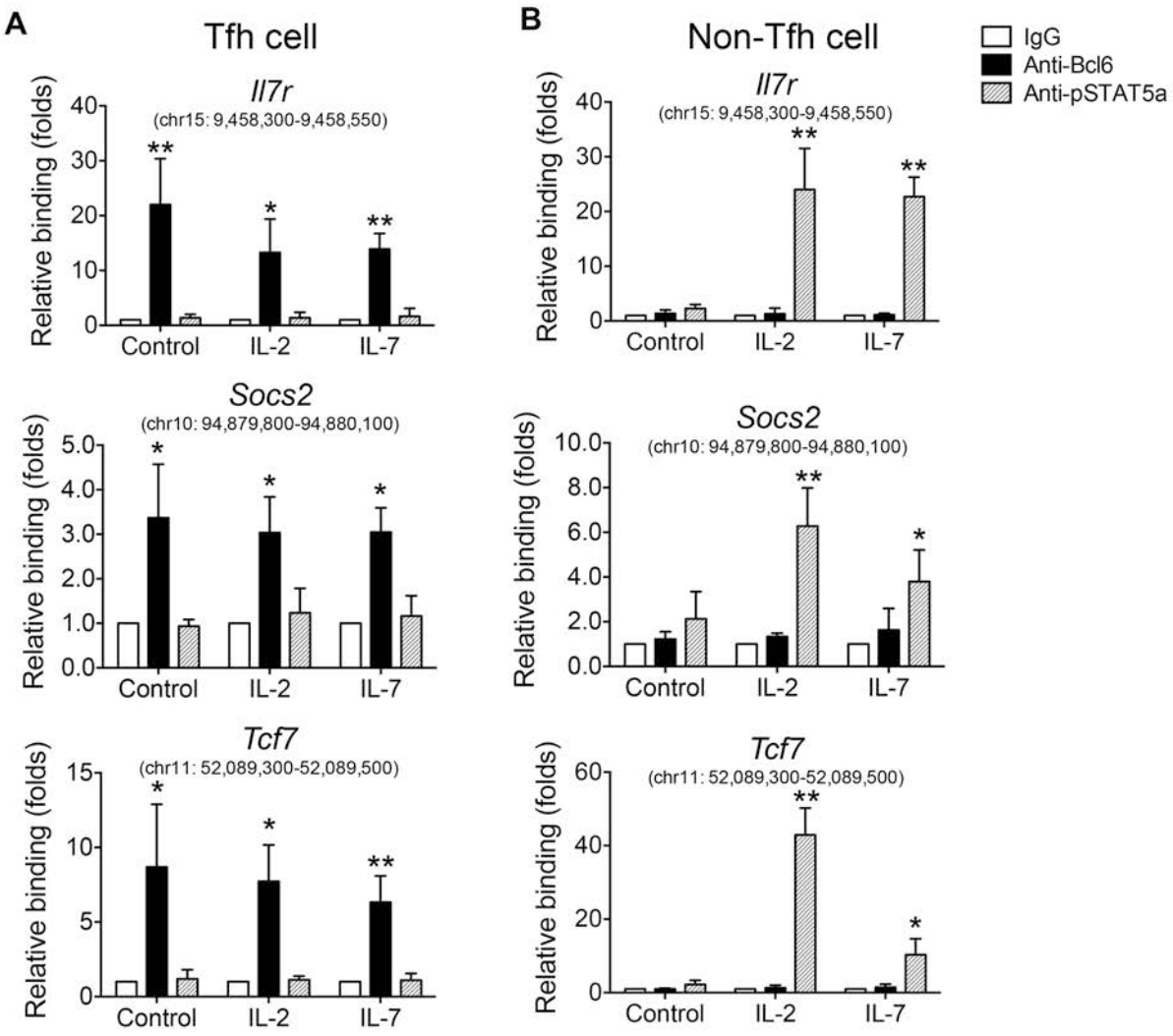
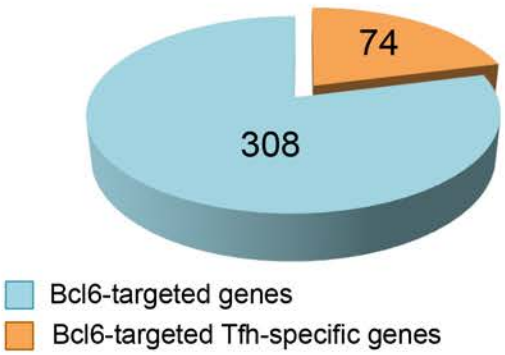

A
Tfh cell
Il7r
(chr15: 9,458,300-9,458,550)
Relative binding (folds)
Control
IL-2
IL-7
Socs2
(chr10: 94,879,800-94,880,100)
Tcf7
(chr11: 52,089,300-52,089,500)
B
Non-Tfh cell
IgG
Anti-Bcl6
Anti-pSTAT5a

Figure S5

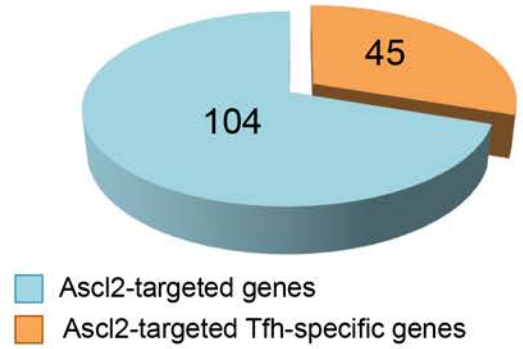
A



B

Bio function Categories	P-value	Molecules
Cell Cycle	1.60E-06	AHR, INSR, LIF
Cell Death and Survival	7.42E-06	BCL6, F2R, IL13, IL7R, INSR, LIF, NTRK3, OSM, PPID, SHMT2, SOCS2, TNFRSF18, TNFRSF
Cellular Growth and Proliferation	8.68E-06	F2R, IL13, IL7R, LIF, OSM, SOCS2
Connective Tissue Development	9.20E-06	AHR, EBF1, F2R, FOSL2, INSR, LIF, RASSF2,Tcf7
Immunological Disease	1.07E-05	AHR, BACH2, BCL6, DCK, IL7R, RASSF2, Tcf7

C



D

Bio function Categories	P-value	Molecules
Cellular Function and Maintenance,	6.13E-09	IL2, IL2RA, S1PR1, TBX21, TNFSF10
Cellular Development,	1.80E-08	CXCR4, CXCR5, GPR68, IFNG, IL2, IL2RA, LIF, S1PR1, TBX21, Tcf7, TNFSF10
Cell Death and Survival	5.55E-08	CD7, IFNG, IL2, IL2RA, SERPINB9, TBX21, TNFSF10
T Cell Differentiation	1.05E-07	IFNG, IL2, IL2RA, LIF, TBX21, Tcf7

Figure S6

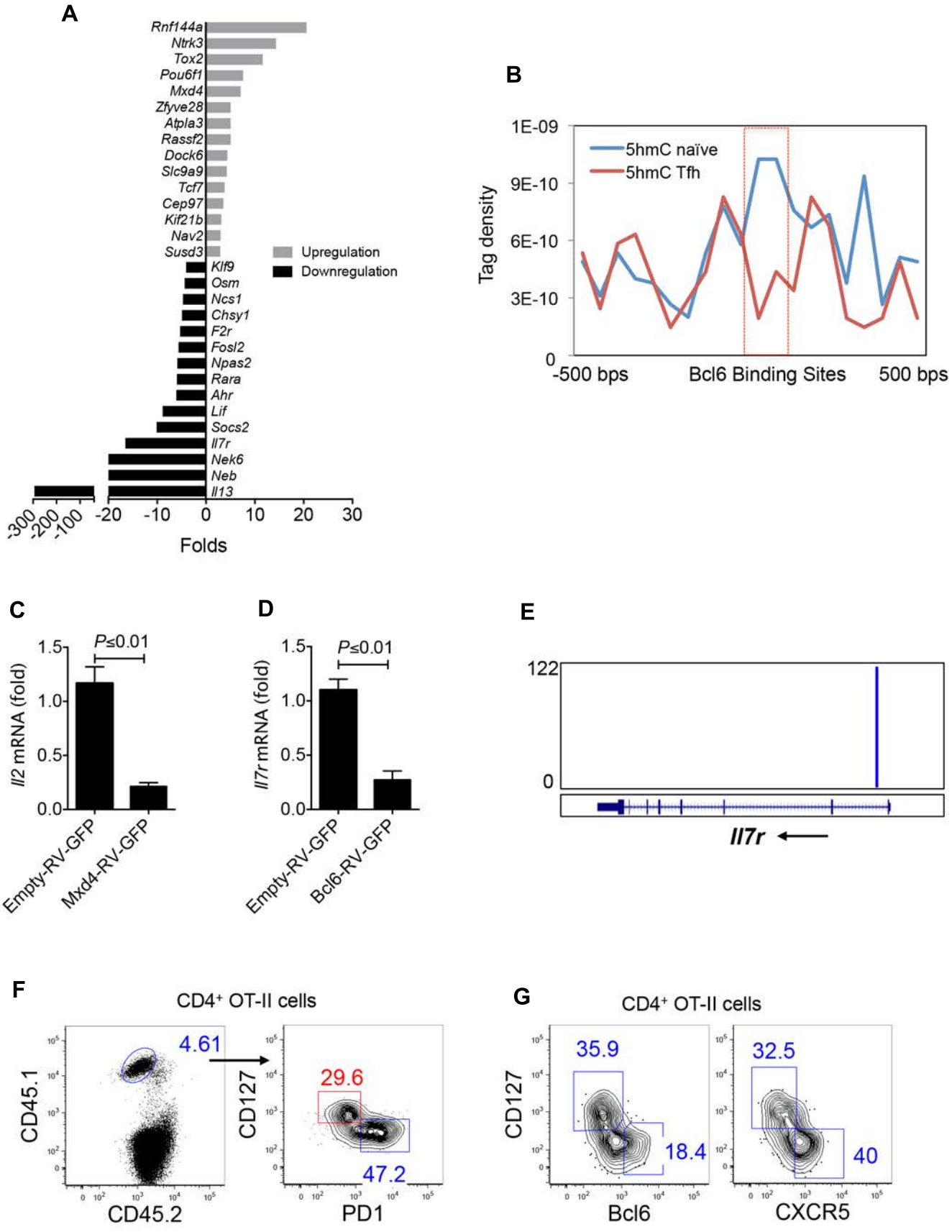
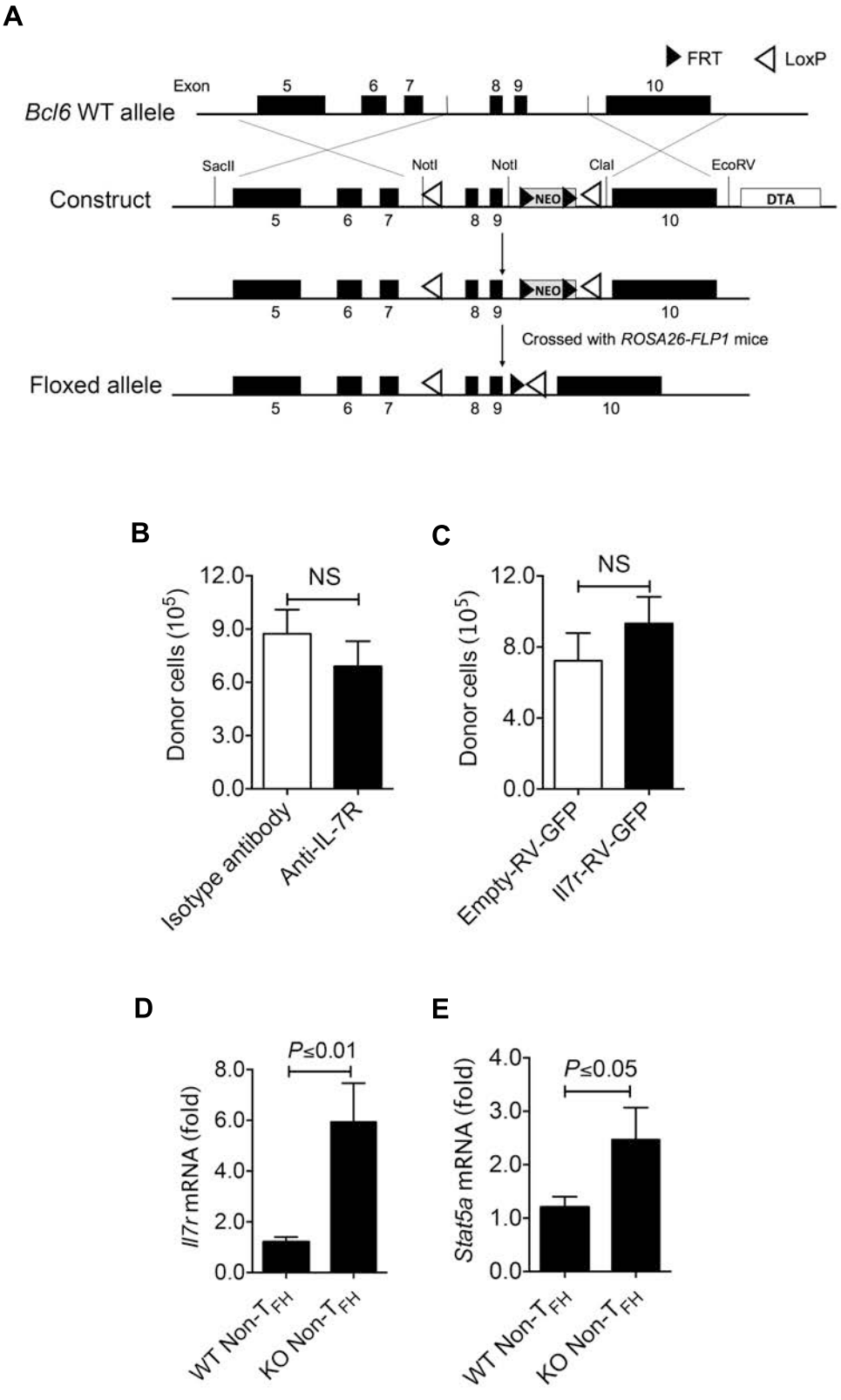

A
Rnf144a
Ntrk3
Tox2
Pou6f1
Mxd4
Zfyve28
Atpla3
Rassf2
Dock6
Slc9a9
Tcf7
Cep97
Kif21b
Nav2
Susd3
Klf9
Osm
Ncs1
Chsy1
F2r
Fosl2
Npas2
Rara
Ahr
Lif
Socs2
Il7r
Nek6
Neb
Il13
Upregulation
Downregulation
-300
-200
-100
-20
-10
0
10
20
30
Folds
B
1E-09
9E-10
6E-10
3E-10
0
5hmC naïve
5hmC Tfh
Tag density
-500 bps
Bcl6 Binding Sites
500 bps
C
Il2 mRNA (fold)
P≤0.01
1.5
1.0
0.5
0.0
Empty-RV-GFP
Mxd4-RV-GFP
D
Il7r mRNA (fold)
P≤0.01
1.5
1.0
0.5
0.0
Empty-RV-GFP
Bcl6-RV-GFP
E
122
0
Il7r
F
CD4+ OT-II cells
4.61
CD45.1
CD45.2
29.6
47.2
CD127
PD1
G
CD4+ OT-II cells
35.9
18.4
CD127
Bcl6
32.5
40
CXCR5

Figure S7


A
FRT
LoxP
Exon
5
6
7
8
9
10
Bcl6 WT allele
SacII
NotI
NotI
ClaI
EcoRV
Construct
NEO
DTA
Crossed with ROSA26-FLP1 mice
Floxed allele
B
NS
Donor cells (10^5)
12.0
9.0
6.0
3.0
0.0
Isotype antibody
Anti-IL-7R
C
NS
Donor cells (10^5)
12.0
8.0
4.0
0.0
Empty-RV-GFP
Il7r-RV-GFP
D
P≤0.01
Il7r mRNA (fold)
8.0
6.0
4.0
2.0
0.0
WT Non-TFH
KO Non-TFH
E
P≤0.05
Stat5a mRNA (fold)
4.0
3.0
2.0
1.0
0.0
WT Non-TFH
KO Non-TFH

Supplemental Figure legends

Figure S1, related to Figure 1 and 2: Distribution of Bcl6 binding peaks in Th9, macrophage and B cells; Bcl6 binding was not associated with H3K4me3 or H3K27me3 modification.
A-C. ChIP-Seq data for Bcl6 binding in Th9 cells were derived from GSE41317 (Liao et al., 2014), Bcl6 binding in macrophage was derived from GSE16723 (Barish et al., 2010), and Bcl6 binding in B cells was from GSE43350 (Huang et al., 2013).
A. At Bcl6-bound regions, H3K4me3 enrichments were compared between Naïve T and Tfh cells.
B. At Bcl6-bound regions, H3K27me3 enrichments were compared between Naïve T and Tfh cells.
C. At Bcl6-bound regions, 5-hydroxymethylcytosine (5hmC) enrichments were compared in naïve T, Tfh, Th1, Th2, Th17, and iTreg cells.
D. ChIP-seq data for H3K4me3 and H3K27me3 enrichments in Naïve T cells were derived from GenBank under accession GSE14254 (Wei et al., 2009), and those in Tfh cells were derived from GSE52840 (Liu et al., 2014). hMeDIP-Seq binding tracks for 5-hmC in Th1, Th2, Th17, and iTreg cells were derived from GSE66944 (Ichiyama et al., 2015).

Figure S2, related to Figure 2: Bcl6 binding at target gene loci reduces 5hmC levels in Tfh cells through blocking Tet1 recruitment.
A. At Bcl6-bound gene loci including *Stat5a*, *Runx3*, and *Mgll* in Tfh cell, 5hmC enrichments were compared in naïve T, Th1, Th2, Th17, iTreg, and Tfh. hMeDIP-Seq binding tracks for 5hmC in Th1, Th2, Th17, and iTreg cells were derived from GSE66944 (Ichiyama et al., 2015).
B. $CD4^+$ $CD44^{hi}$ $CXCR5^{hi}$ $PD1^{hi}$ Tfh and $CD4^+$ $CD44^{hi}$ $CXCR5^{lo}$ $PD1^{lo}$ non-Tfh cells were purified from *Bcl6*$^{fl/fl}$/*CD4-Cre* and control mice immunized with KLH/CFA for seven days. 5hmC presence to *Stat5a*, *Runx3*, and *Mgll* loci was analyzed by hMeDIP-PCR assays.
C. Quantative RT-PCT measurement of Tet1, Tet2, and Tet3 transcriptional expression in $CD4^+$ $CD25^{lo}CD44^{lo}$ $CD62L^{hi}$ naïve, $CD4^+$ $CD44^{hi}$ $CXCR5^{hi}$ $PD1^{hi}$ Tfh, and $CD4^+$ $CD44^{hi}$ $CXCR5^{lo}$ $PD1^{lo}$ non-Tfh cells.
D. $CD4^+$ $CD44^{hi}$ $CXCR5^{hi}$ $PD1^{hi}$ Tfh and $CD4^+$ $CD44^{hi}$ $CXCR5^{lo}$ $PD1^{lo}$ non-Tfh cells were purified from *Bcl6*$^{fl/fl}$/*CD4-Cre* and control mice immunized with KLH/CFA for seven days. Tet1 recruitment to *Stat5a*, *Runx3*, and *Mgll* loci was analyzed by ChIP-PCR assays.
Data are a representative of two independent experiments. Bar graph displayed as mean ± SD.

Figure S3, related to Figure 3: Distribution of ChIP-Seq peaks by Bcl6 and STAT5 on Th-relevant gene loci.
A. Bcl6 and STAT5 bindings on Tfh-relevant gene loci including *Bcl6, Cxcr5, Ascl2, Il21, Prdm1, Icos, Pdcd1,* and *Btla*.
B. Bcl6 and STAT5 bindings on Th1-relevant gene loci including *Tbx21, Ifng,* and *Il12rb1*.
C. Bcl6 and STAT5 bindings on Th2-relevant gene loci including *Gata3, Il4, Il5,* and *Il13*.
D. Bcl6 and STAT5 bindings on Th17-relevant gene loci including *Rorc, Il17a,* and *Il17f.*

Figure S4, related to Figure 3: ChIP analysis of Bcl6 and STAT5 enrichments at *Il7r*, *Socs2*, and *Tcf7* loci in Tfh and Non-Tfh cells.
$CD4^+$ $CD44^{hi}$ $CXCR5^{hi}$ $PD1^{hi}$ Tfh and $CD4^+$ $CD44^{hi}$ $CXCR5^{lo}$ $PD1^{lo}$ non-Tfh cells were sorted from WT mice immunized with KLH/CFA for seven days, and subject to IL-2, IL-7 or mock treatment for 40 minutes and ChIP-Seq assessment. Data are a representative of two independent experiments. Bcl6-and STAT5- bound DNA enrichment was relative to IgG control. Bar graph displayed as mean ± SD, n = 3 per group.
A. Bcl6 and STAT5 recruitment to *Il7r*, *Socs2*, and *Tcf7* loci in Tfh cells was analyzed by ChIP-PCR assays.
B. Bcl6 and STAT5 recruitment to *Il7r*, *Socs2*, and *Tcf7* loci in non-Tfh cells was analyzed by ChIP-PCR assays.

Figure S5, related to Figure 4: Comparison of Bcl6- and Ascl2-controlled modules in Tfh cells.
A. Bcl6 target genes and Bcl6 target Tfh-specific genes
B. IPA analysis for Bcl6-targeted genes in Tfh cells. P≤ 0.05.
C. Ascl2 target genes and Ascl2 target Tfh-specific genes
D. IPA analysis for Ascl2-targeted genes in Tfh cells. P≤ 0.05.

Figure S6, related to Figure 4-6: Among the top list of Bcl6-targeted Tfh-relevant genes, IL-7R expression is inhibited as a function of Tfh cell differentiation.
A. Top 15 of Bcl6 directly upregulated and downregulated Tfh-relevant genes.
B. Comparison of 5hmC levels on the Bcl6 binding sites located within the 74 Bcl6 target Tfh-specific genes between naïve T and Tfh cells. Red frame represents 50bp window of Bcl6 and 5hmC overlapping sites.
C. Naïve T cells were activated and transduced with Mxd4-RV-GFP or control vector under Th0 condition. After 4 days, GFP^+ T cells were sorted and subject to quantitative RT-PCR analysis of *Il2* mRNA expression. Data are a representative of two independent experiments. Bar graph displayed as mean ± SD. n = 3 per group.
D. Naïve T cells were activated and transduced with Bcl6-RV-GFP or control vector under Th0 condition. After 4 days, GFP^+ T cells were sorted and subject to quantitative RT-PCR analysis of *Il7r* mRNA expression. Data are a representative of two independent experiments. Bar graph displayed as mean ± SD. n = 4 per group.
E. Bcl6 binding peak on *Il7r* gene locus.
F-G Naïve OT-II $CD4^+$ T cells were transferred into naïve congenic mice, which were subsequently immunized with OVA/CFA subcutaneously. Data are a representative of two independent experiments.
F. At day7 post immunization, flow cytometry analysis of CD127 and PD1 expression in donor-derived OT-II cell.
G. Flow cytometry analysis of CD127, CXCR5 and Bcl6 expression in donor-derived OT-II cell.

Figure S7, related to Figure 6 and 7: *Bcl6* conditional mouse represents as a useful tool to study Tfh polarization; although manipulation of IL-7R signaling *in vivo* did not affect donor cell proliferation, *Il7r* and *Stat5a* expression was elevated in Bcl6-deficient non-Tfh cells.
A. A schematic representation of WT *Bcl6* locus, targeting construct, and recombinant allele after removal of *Neo*.
B. Naïve OT-II cells were adoptively transferred into naïve congenic mice, followed with subcutaneous OVA/CFA immunization. At day3 after immunization, anti-IL-7R or isotype control antibodies (200μg/mouse) were injected subcutaneously into mice, respectively. At day8 post immunization, flows cytometry analysis of donor-derived cells in dLNs. Data are a representative of two independent experiments. n = 4 per group. Bar graph displayed as mean ± SD. n = 4 per group.
C. Il7r-RV-GFP- or control vector- transduced OT-II cells were adoptively transferred into naïve congenic mice, respectively, followed with subcutaneous OVA/CFA immunization for seven days. Flow cytometry analysis of donor-derived cells from dLNs. Data are a representative of two independent experiments. Bar graph displayed as mean ± SD. n = 4 per group.
D-E. $Bcl6^{+/+}$/CD4-Cre and $Bcl6^{fl/fl}$/CD4-Cre mice were subcutaneously immunized with KLH/CFA. At day 8, $CD4^+$ $CD44^{hi}$ $CXCR5^{lo}$ $PD1^{lo}$ Non-Tfh cells from both $Bcl6^{+/+}$/CD4-Cre and $Bcl6^{fl/fl}$/CD4-Cre mice were sorted, re-stimulated with plate-coated anti-CD3 Data and subject to measurement of gene expression. Data are a representative of two independent experiments, Bar graph displayed as mean ± SD. n = 4 per group.
D. Quantitative RT-PCR measurement of *Il7r* mRNA expression in WT and Bcl6 KO Non-Tfh cells.
E. Quantitative RT-PCR measurement of *Stat5a* mRNA expression in WT and Bcl6 KO Non-Tfh cells.

Table S1, related to Figure 1 and 2: Summary of ChIP-Seq data.

Table S2, related to Figure 1: Bcl6 target genes (230) in Tfh/Th9/B cell /macrophage cell. (Excel file: Table S2)

Table S3, related to Figure 2: Genome-wide 5hmC reduction at Bcl6 binding sites in Tfh cells (696). (Excel file: Table S3)

Table S4, related to Figure 3: Occupancy of Bcl6 with BATF, IRF4, C-Maf, and STAT3.

Table S5, related to Figure 3: Bcl6/STAT5 Co-localized genes (424). (Excel file: Table S5)

Table S6, related to Figure 4: Bcl6-targeted Tfh-relevant genes.

Table S7, related to Figure 4: Bcl6 taget genes (382). (Excel file: Table S7)

Table S1, related to Figure 1 and 2: Summary of ChIP-Seq data

	Total Unique Tag Count	Total Number of Peaks	Promoter Peak	Exon Peak	Intron Peak	Intergenic Peak
Bcl6 Tfh	7,502,629	5191	366 (7%)	333 (6%)	2154 (41%)	2338 (46%)
5mC Naive	3,297,050	48133	1487 (3%)	4703 (10%)	20223 (42%)	21720 (46%)
5mC Tfh	4,385,983	33063	887 (3%)	5466 (17%)	15457 (47%)	11253 (34%)
5hmC Naive	6,707,746	130008	5583 (4%)	2018 (2%)	59562 (46%)	62845 (48%)
5hmC Tfh	3,169,203	35211	1203 (3%)	959 (3%)	17164 (49%)	15885 (45%)
H3K4me3 non-Tfh	15,674,354	16916	9026 (53%)	258 (2%)	2684 (16%)	4948 (29%)
H3K4me3 Tfh	19,662,955	29131	9448 (32%)	510 (2%)	7193 (25%)	11980 (41%)
H3K27me3 non-Tfh	22,913,948	38390	12202 (10%)	698 (2%)	11504 (30%)	22259 (58%)
H3K27me3 Tfh	23,827,360	30388	9901 (9%)	558 (2%)	9343 (31%)	17844 (59%)

Table S4, related to Figure 3: Occupancy of Bcl6 with BATF, IRF4, C-Maf, and STAT3

Overlays (gene number)	Genes
Bcl6/BATF (42)	4931428F04Rik, Acot7, Adpgk, Akap6, Bach2, Bcl6, Cdh23, Clic4, Dusp10, Evl, F3, Fam69a, Fam72a, Fancm, Fosl2, Gna13, Il2rb, Il7r, Lrrc56, Lrrc6, Msh5, Napepld, Nav2, Nphp4, Osm, Pde4b, Plcg2, Prr5l, Ptprs, Ptprv, Pusl1, Rab11fip4, Rassf2, Runx3, Slc2a9, Socs2, Tacc3, Tnfrsf1b, Tox, Ubash3b, Vegfa, Zmiz1
Bcl6/IRF4 (42)	1700001G17Rik, 4931428F04Rik, Adpgk, Atg9b, Bach2, Bcl6, Cdh23, Dck, Dusp10, Evl, F3, Fam69a, Fam72a, Flnb, Fosl2, Gnl3, Hk2, Id2, Il2rb, Il7r, Insr, Lrrc56, Lrrc6, Napepld, Nfkb1, Nphp4, Osm, Pde4b, Prr5l, Ptprs, Pusl1, Rab11fip4, Rassf2, Runx3, Sgsh, Socs2, Tacc3, Tnfrsf1b, Tox, Ubash3b, Vegfa, Zmiz1
Bcl6/C-Maf (32)	4931428F04Rik, Acot7, Adpgk, Bach2, Bcl6, Dck, Dusp10, Evl, F3, Fam69a, Fam72a, Fosl2, Gnl3, Id2, Il2rb, Il7r, Insr, Mgll, Mllt11, Nfkb1, Nphp4, Osm, Pde4b, Prr5l, Ptprs, Pusl1, Rab11fip4, Rassf2, Runx3, Sgsh, Tcf7, Vegfa
Bcl6/STAT3 (29)	4931428F04Rik, Adpgk, Atg9b, Bcl6, Clic4, Dck, Evl, F3, Fancm, Fosl2, Gna13, Gnl3, Hk2, Id2, Il2rb, Il7r, Lrrc56, Napepld, Nfkb1, Osm, Pde4b, Prr5l, Ptprs, Rassf2, Runx3, Sgsh, Socs2, Tacc3, Tnfrsf1b

Table S6, related to Figure 4: Bcl6-targeted Tfh-relevant genes

Up/down-regulation (gene number)	Genes
Up-regulated genes (26)	Ldlrap1, Crot, Dck, Insr, Sgsh, Slc4a8, Ankrd12, Fbxl20, Stard10, Hmha1, Hsdl1, Susd3, Nav2, Kif21b, Cep97, Tcf7, Slc9a9, Dock6, Rassf2, Atp1a3, Zfyve28, Mxd4, Pou6f1, Tox2, Ntrk3, Rnf144a
Down-regulated genes (48)	Il13, Neb, Nek6, Il7r, Socs2, Lif, Ahr, Rara, Npas2, Fosl2, F2r, Chsy1, Ncs1, Osm, Klf9, Slc7a5, Kdm6b, Myo19, Ube2l6, Chd7, Bcat1, Dot1l, Alcam, Thop1, Clic4, Bach2, Pfas, Larp1, Noc4l, Hk2, Ppid, Pusl1, Cad, Slamf7, Adpgk, Tnfrsf9, Slc29a3, Shmt2, Ubash3b, Tnfrsf18, Qtrtd1, Utp20, Dnajc11, Gnl3, Zfp593, Ppan, Naa25, Gem