Index to the Supplement

Figures

Tables

Figure S1

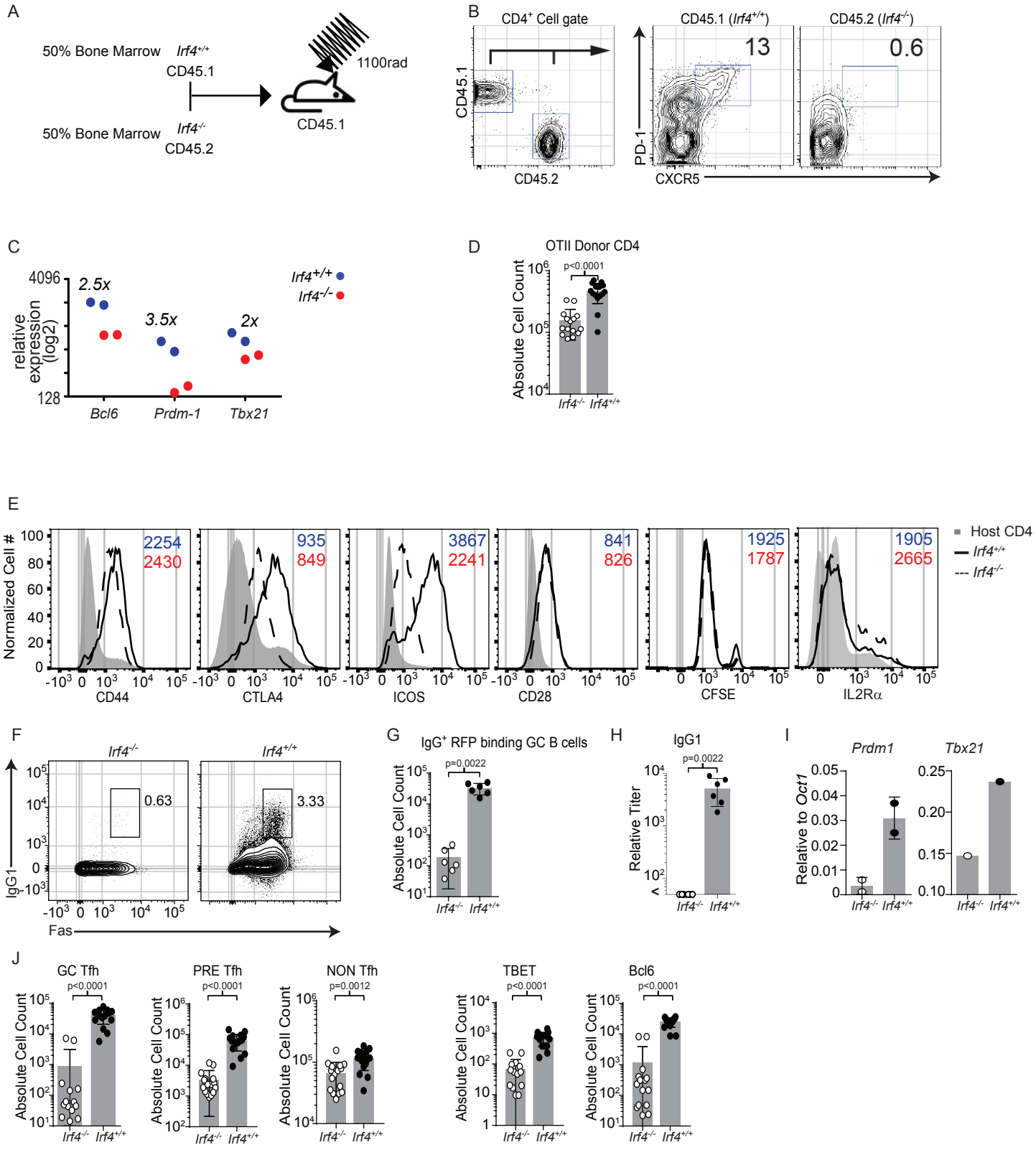
A
50% Bone Marrow
Irf4+/+
CD45.1
50% Bone Marrow
Irf4-/-
CD45.2
1100rad
CD45.1
B
CD4+ Cell gate
CD45.1
CD45.2
CD45.1 (Irf4+/+)
CD45.2 (Irf4-/-)
13
0.6
PD-1
CXCR5
C
4096
128
relative expression (log2)
2.5x
3.5x
2x
Irf4+/+
Irf4-/-
Bcl6
Prdm-1
Tbx21
D
OTII Donor CD4
Absolute Cell Count
p<0.0001
Irf4-/-
Irf4+/+
E
Normalized Cell #
2254
2430
935
849
3867
2241
841
826
1925
1787
1905
2665
CD44
CTLA4
ICOS
CD28
CFSE
IL2Rα
Host CD4
Irf4+/+
Irf4-/-
F
Irf4-/-
Irf4+/+
0.63
3.33
IgG1
Fas
G
IgG+ RFP binding GC B cells
p=0.0022
Absolute Cell Count
H
IgG1
p=0.0022
Relative Titer
I
Prdm1
Tbx21
Relative to Oct1
J
GC Tfh
p<0.0001
PRE Tfh
p<0.0001
NON Tfh
p=0.0012
TBET
p<0.0001
Bcl6
p<0.0001
Absolute Cell Count

Figure S1. Irf4 is required for both Tfh and Teff differentiation. (Accompanies Fig. 1)

A) Schematic: 1:1 mixed bone marrow chimeras were generated such that the CD45.1 expressing compartment was reconstituted with *Irf4*$^{+/+}$ hematopoietic progenitors and the CD45.2 expressing compartments was reconstituted with *Irf4*$^{-/-}$ hematopoietic progenitors.

B) Reconstituted mice from (A) were immunized with SRBC, and CD4+ T cells were analyzed on Day 7 for their expression of PD-1 and CXCR5 after gating on CD45 polymorphic alleles and CD4. Contour plots are representative of 5 mice per group of two independent experiments performed.

C) Seven days post immunization, *Irf4*$^{+/+}$ CD45.1 CD4^{+} T cells and *Irf4*$^{-/-}$ CD45.2 CD4^{+} T cells were sorted from the same mice and processed for qRT-PCR. Relative expression of *Bcl6*, *Prdm1* and *Tbx21* transcripts are depicted normalized to *Oct1*. Each point (2) represents a single mouse from one experiment.

D) Quantitation (mean±SD) of absolute numbers of donor cells from Fig 1B. Numbers of CD45.1^{-}B220^{-}MHCII^{-}CD45.2^{+}CD4^{+} donor cells within individual mice are plotted, each point (15) represents a single mouse from four experiments.

E) 5x10^{5} *Irf4*$^{+/+}$ or *Irf4*$^{-/-}$ OT-II TCR Tg cells (CD45.2+ and *Rag1*$^{-/-}$) were adoptively transferred into congenic CD45.1^{+} hosts and immunized with 50μg RFP-OVA emulsified in CFA the next day. Three days post immunization, CD45.1^{-}B220^{-}MHCII^{-}CD45.2^{+}CD4^{+} donor cells were assessed by flow cytometry for the indicated proteins. Mean fluorescent intensities are denoted in each overlay for *Irf4*$^{-/-}$ (red) and *Irf4*$^{+/+}$ (blue). Histograms represent concatenated data of four mice per group and are representative of two experiments performed.

F) RFP-binding B cells from (Fig. 1G) were analyzed for IgG1 class switch recombination. Contour plots represent concatenated data of three mice per group and are representative of two experiments performed.

G) Frequencies (mean±SD) of IgG1 class switched cells from (F). Each point (6) represents a single mouse from two experiments.

H) Serum from mice in (Fig. 1G) was analyzed for RFP-specific IgG1 by ELISA. Each point (6) represents a single mouse from two experiments. "<" indicates below detection.

I) Donor cells from Fig. 1B were sorted for CD45.1^{-}B220^{-}MHCII^{-}CD45.2^{+}CD4^{+} and processed for qRT-PCR. Relative expression of *Prdm1* and *Tbx21* transcripts normalized to *Oct1* transcripts are depicted. Each point represents the measurements from a single mouse per group, 2 for *Prdm1* and one for *Tbx21*.

J) Quantitation (mean±SD) of absolute numbers of gated cells from Figs. 1B and D. Numbers of GC Tfh, Pre Tfh, Non Tfh, Tbet+, and Bcl6+ cells within individual mice are plotted, each point (15) represents a single mouse from four experiments.

Figure S2

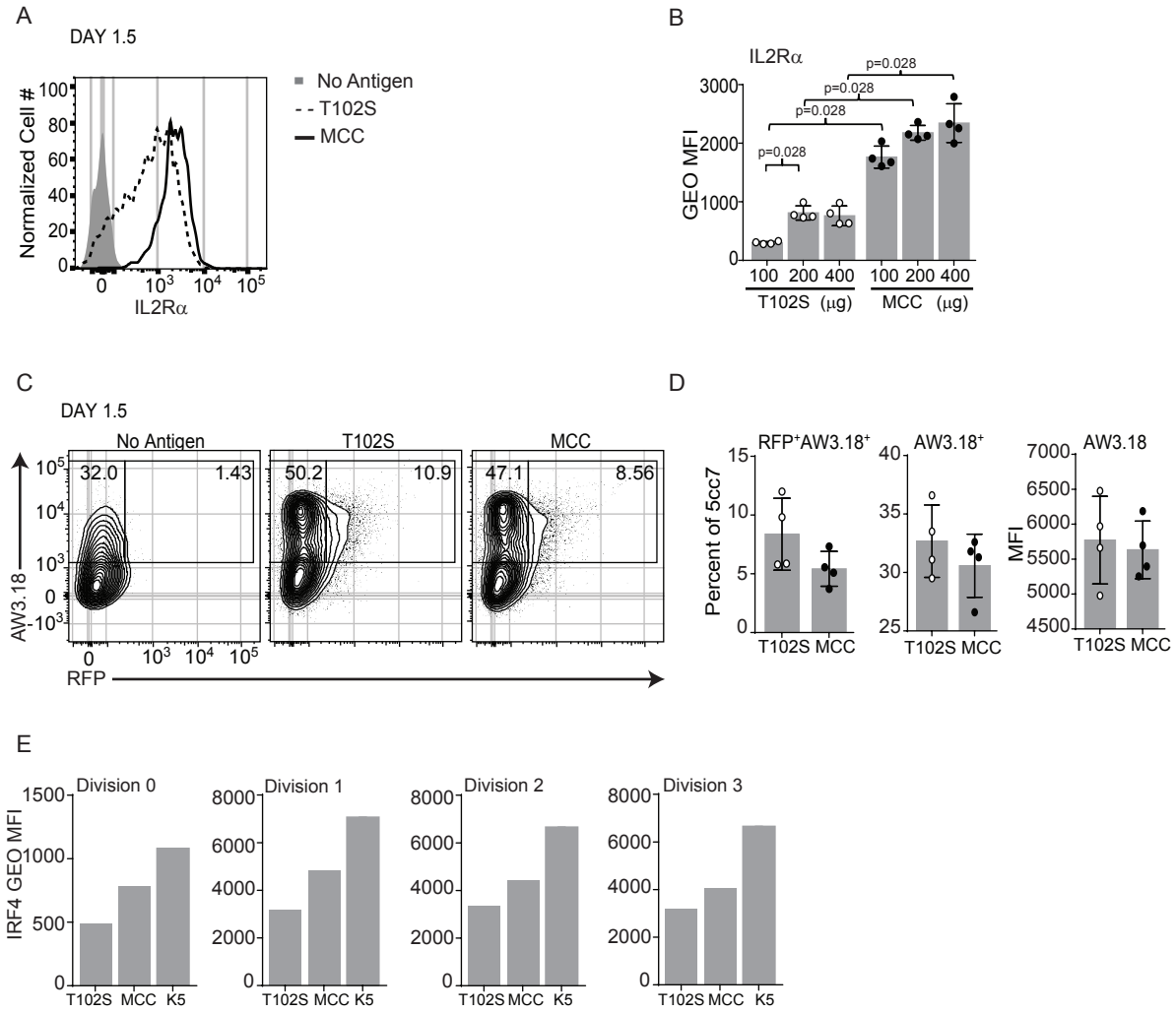
A
DAY 1.5
Normalized Cell #
IL2Rα
No Antigen
T102S
MCC
B
IL2Rα
GEO MFI
p=0.028
T102S (μg)
MCC (μg)
C
DAY 1.5
No Antigen
T102S
MCC
AW3.18
RFP
32.0
1.43
50.2
10.9
47.1
8.56
D
RFP+AW3.18+
AW3.18+
AW3.18
Percent of 5cc7
MFI
T102S MCC
E
Division 0
Division 1
Division 2
Division 3
IRF4 GEO MFI
T102S MCC K5

Figure S2. Irf4 amounts scale proportionally with increased TCR signal strength. (Accompanies Fig. 2)

A) $5x10^5$ 5c.c7 TCR Tg cells ($CD45.2^+$ and *Rag1*$^{-/-}$) were adoptively transferred into congenic $CD45.1^+$ hosts and immunized with 100, 200 or 400μg of either RFP-T102S or RFP-MCC emulsified in CFA the next day. One and a half days post immunization, $CD45.1^-B220^-MHCII^-CD45.2^+CD4^+$ donor cells were assessed by flow cytometry for the expression of CD25 protein. Shown are a representative result for 200ug immunization. Histograms represent concatenated data of four mice in each group and is representative of two independent experiments.

B) Mean fluorescent intensity (mean±SD) of populations from (A) across the dose titration. Each point (4) represents a single mouse from one representative experiment of two independent experiments.

C) $5x10^5$ 5c.c7 TCR Tg cells ($CD45.2^+$ and *Rag1*$^{-/-}$) were adoptively transferred into congenic $CD45.1^+$ hosts and immunized with 200μg of either RFP-MCC or RFP-T102S emulsified in CFA the next day. One and a half days later, the amounts of HEL_{48-62}/I-A^k pMHCII complexes were quantified on dendritic cells using the Aw3.18 mAb. Dendritc cells were identified by CD11c and CD11b coexpression. Dendritic cell red fluorescence, due to endocytosed RFP was also quantitated. Contour plots represent concatenated data of four mice in each group and is representative of two experiments.

D) Frequencies (mean±SD) of populations in (C) as well as mean fluorescence intensities of Aw3.18+ positive cells. Each point (4) represents a single mouse from one representative experiment of two performed.

E) Mean fluorescent intensity of Irf4 expression gated on cells within an indicated division from Fig. 2B at the 0.5nM dose of peptide.

Figure S3

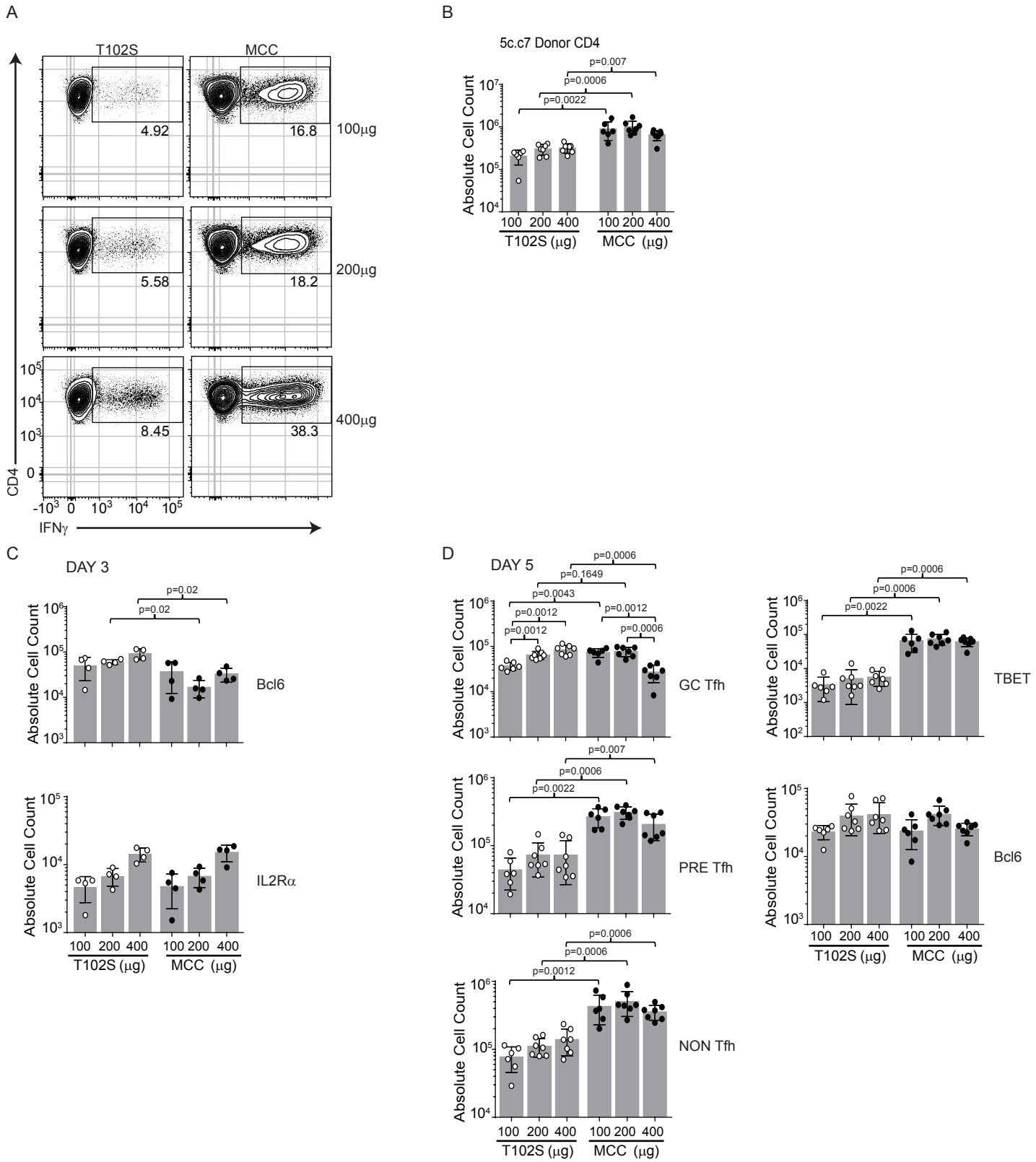
A
T102S
MCC
4.92
16.8
100μg
5.58
18.2
200μg
8.45
38.3
400μg
CD4
IFNγ
B
5c.c7 Donor CD4
Absolute Cell Count
p=0.007
p=0.0006
p=0.0022
100 200 400
T102S (μg)
MCC (μg)
C
DAY 3
p=0.02
p=0.02
Bcl6
IL2Rα
D
DAY 5
p=0.0006
p=0.1649
p=0.0043
p=0.0012
p=0.0012
p=0.0012
p=0.0006
GC Tfh
p=0.0006
p=0.0006
p=0.0022
TBET
p=0.007
p=0.0006
p=0.0022
PRE Tfh
Bcl6
p=0.0006
p=0.0006
p=0.0012
NON Tfh

Figure S3. TCR regulated Irf4 concentrations control alternate T helper cell fate decisions. (Accompanies Fig. 3)

A) $5x10^5$ 5c.c7 TCR Tg cells ($CD45.2^+$ and *Rag1*$^{-/-}$) were adoptively transferred into congenic $CD45.1^+$ hosts and immunized with 100, 200 or 400μg of either RFP-MCC or RFP-T102S emulsified in CFA the next day. Five days post-immunization, lymph node cells were stimulated with PMA/Ionomycin for six hours in the presence of monensin and brefeldinA. Six hours later, $CD45.1^-B220^-MHCII^-CD45.2^+CD4^+$ donor cells were assessed by flow cytometry for IFNγ expression. Contour plots represent concatenated data from four mice per group and is representative of two independent experiments.

B) Quantitation (mean±SD) of absolute numbers of cells analyzed from Fig. 3B. Numbers of $CD45.1^-B220^-MHCII^-CD45.2^+CD4^+$ donor cells from individual mice are plotted. Each point (7) represents a single mouse from two experiments performed.

C) Quantitation (mean±SD) of absolute numbers of gated cells from Fig. 3B. Numbers of Bcl6+ and IL2rα+ cells within individual mice are plotted, each point (4) represents a single mouse from two experiments performed.

D) Quantitation (mean±SD) of absolute numbers of gated cells from Figs. 3D and F. Numbers of GC Tfh, Pre Tfh, Non Tfh, Tbet+, and Bcl6+ cells within individual mice are plotted, each point (7) represents a single mouse from two experiments performed.

Figure S4

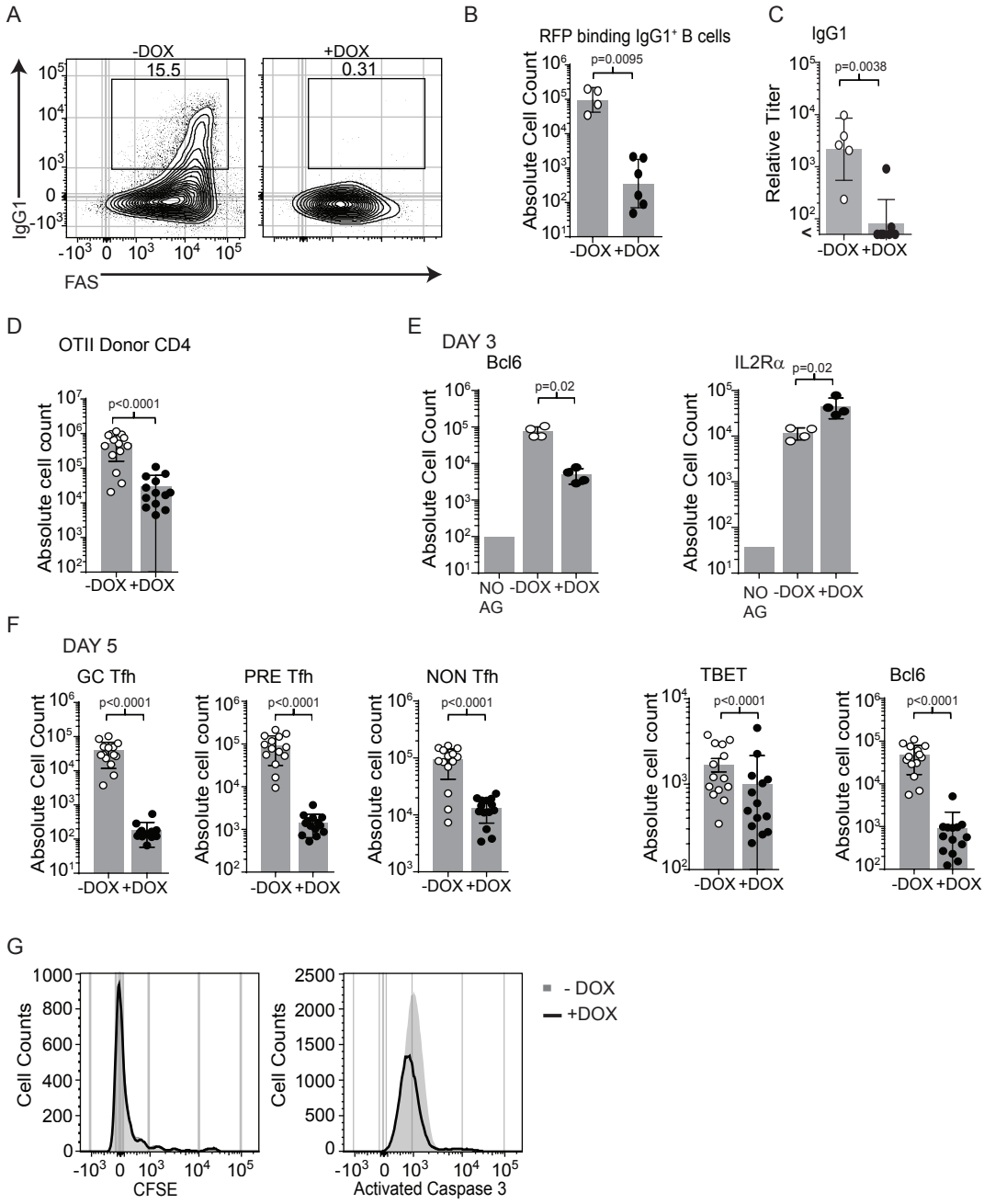
A
-DOX
15.5
+DOX
0.31
IgG1
FAS
B
RFP binding IgG1+ B cells
p=0.0095
Absolute Cell Count
-DOX +DOX
C
IgG1
p=0.0038
Relative Titer
-DOX +DOX
D
OTII Donor CD4
p<0.0001
Absolute cell count
-DOX +DOX
E
DAY 3
Bcl6
p=0.02
Absolute Cell Count
NO AG
-DOX +DOX
IL2Rα
p=0.02
Absolute Cell Count
NO AG
-DOX +DOX
F
DAY 5
GC Tfh
PRE Tfh
NON Tfh
TBET
Bcl6
p<0.0001
Absolute Cell Count
Absolute cell count
-DOX +DOX
G
Cell Counts
CFSE
Activated Caspase 3
- DOX
+DOX

Figure S4. Cell concentrations of Irf4 direct Tfh versus Teff cell fates. (Accompanies Fig. 4)

A) RFP-binding B cells from (Fig. 4I) were analyzed for IgG1 class switch recombination. Contour plots represent concatenated data of three mice per group and is representative of two independent experiments.

B) Frequency (mean±SD) of IgG1 class switched cells from (A). Each point (6) represents a single mouse from two experiments. *p value*s were calculated using parametric unpaired *t*-test.

C) Serum from mice in (Fig. 4I) were analyzed for RFP-specific IgG1 by ELISA. *p value*s were calculated using parametric unpaired *t*-test. "<" indicates below detection.

D) Quantitation (mean±SD) of absolute numbers of cells analyzed from Figs. 4F. Numbers of $CD45.1^{-}B220^{-}MHCII^{-}CD45.2^{+}CD4^{+}$ donor cells from individual mice are plotted.

E) Quantitation (mean±SD) of absolute numbers of gated cells from Fig. 4D. Numbers of Bcl6+ and IL2rα+ cells within individual mice are plotted, each point (4) represents a single mouse from one experiment performed.

F) Quantitation (mean±SD) of absolute numbers of gated cells from Fig. 4F and H. Numbers of GC Tfh, Pre Tfh, Non Tfh, Tbet+, and Bcl6+ cells within individual mice are plotted, each point (14) represents a single mouse from four experiments performed.

G) 10^5 *Irf4*-inducible (*Irf4*$^{+/+}$) OT-II TCR Tg $CD4^+$ T cells (CD45.2+ and *Rag1*$^{-/-}$) were CFSE labeled and then adoptively transferred into congenic $CD45.1^+$ hosts and then immunized with 50μg of RFP-OVA emulsified in CFA the next day. Adoptively transferred mice were administered water lacking (–DOX) or containing DOX (+DOX) for the first two days of immunization only. Three days after immunization $CD45.1^{-}B220^{-}MHCII^{-}CD45.2^{+}CD4^{+}$ donor cells were analyzed for CFSE dilution and activated Caspase3 protein. Histograms represent concatenated data from three mice per group and the experiment is representative of two independent experiments.

Figure S5

A

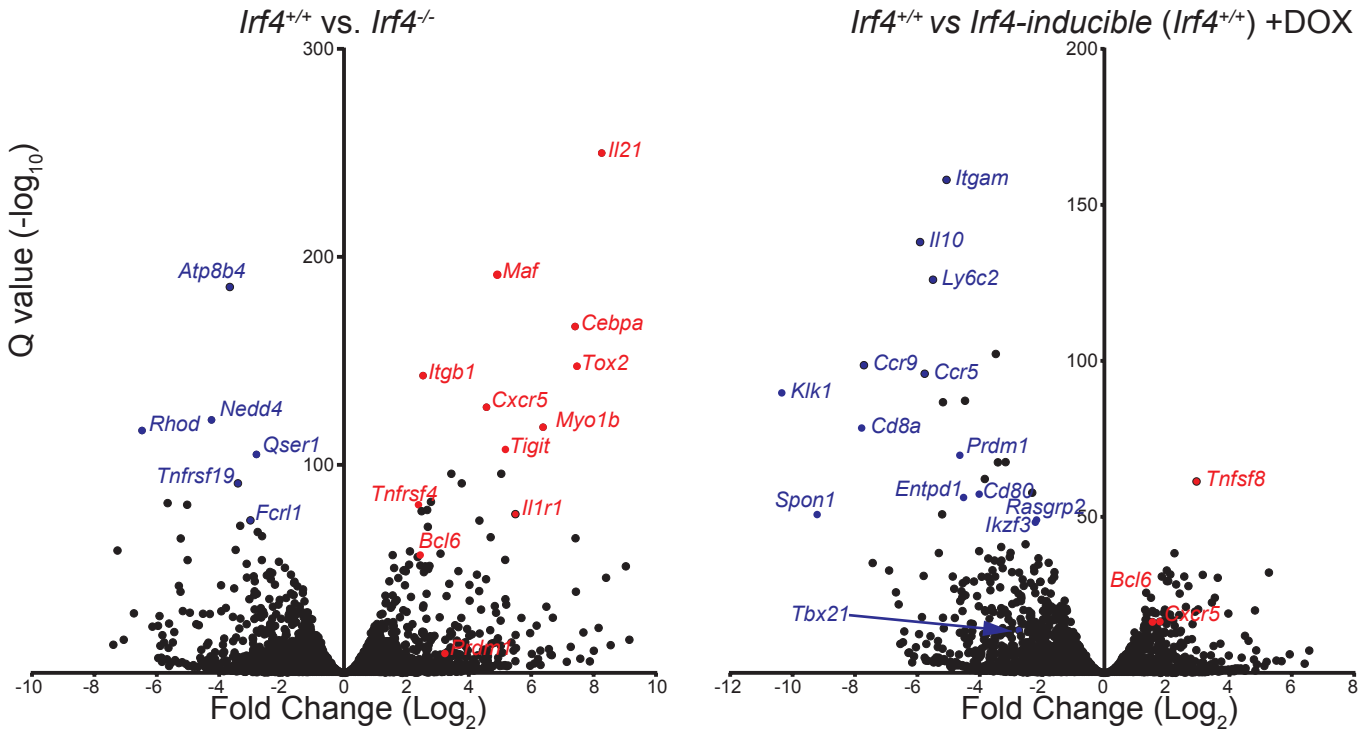
Irf4+/+ vs. Irf4-/-
Q value (-log10)
Fold Change (Log2)
Il21
Atp8b4
Maf
Cebpa
Itgb1
Tox2
Cxcr5
Rhod
Nedd4
Myo1b
Qser1
Tigit
Tnfrsf19
Tnfrsf4
Il1r1
Fcrl1
Bcl6
Prdm1
Irf4+/+ vs Irf4-inducible (Irf4+/+) +DOX
Fold Change (Log2)
Itgam
Il10
Ly6c2
Ccr9
Ccr5
Klk1
Cd8a
Prdm1
Tnfsf8
Spon1
Entpd1
Cd80
Rasgrp2
Ikzf3
Bcl6
Tbx21
Cxcr5

Figure S5. Low and high Irf4 expression levels control distinct T helper cell gene programs. (Accompanies Fig. 5)

A) Volcano plots representing fold change (Log2) on the x-axis vs. significance (Q-value; log10) on the y-axis. Each dot represents a gene and genes of interested are highlighted. The left panel represents the fold change comparison of *Irf4*$^{+/+}$ vs. *Irf4*$^{-/-}$ and the right panel represents the fold change comparison of *Irf4*$^{+/+}$ vs. *Irf4*-inducible (*Irf4*$^{+/+}$) + DOX.

Figure S6

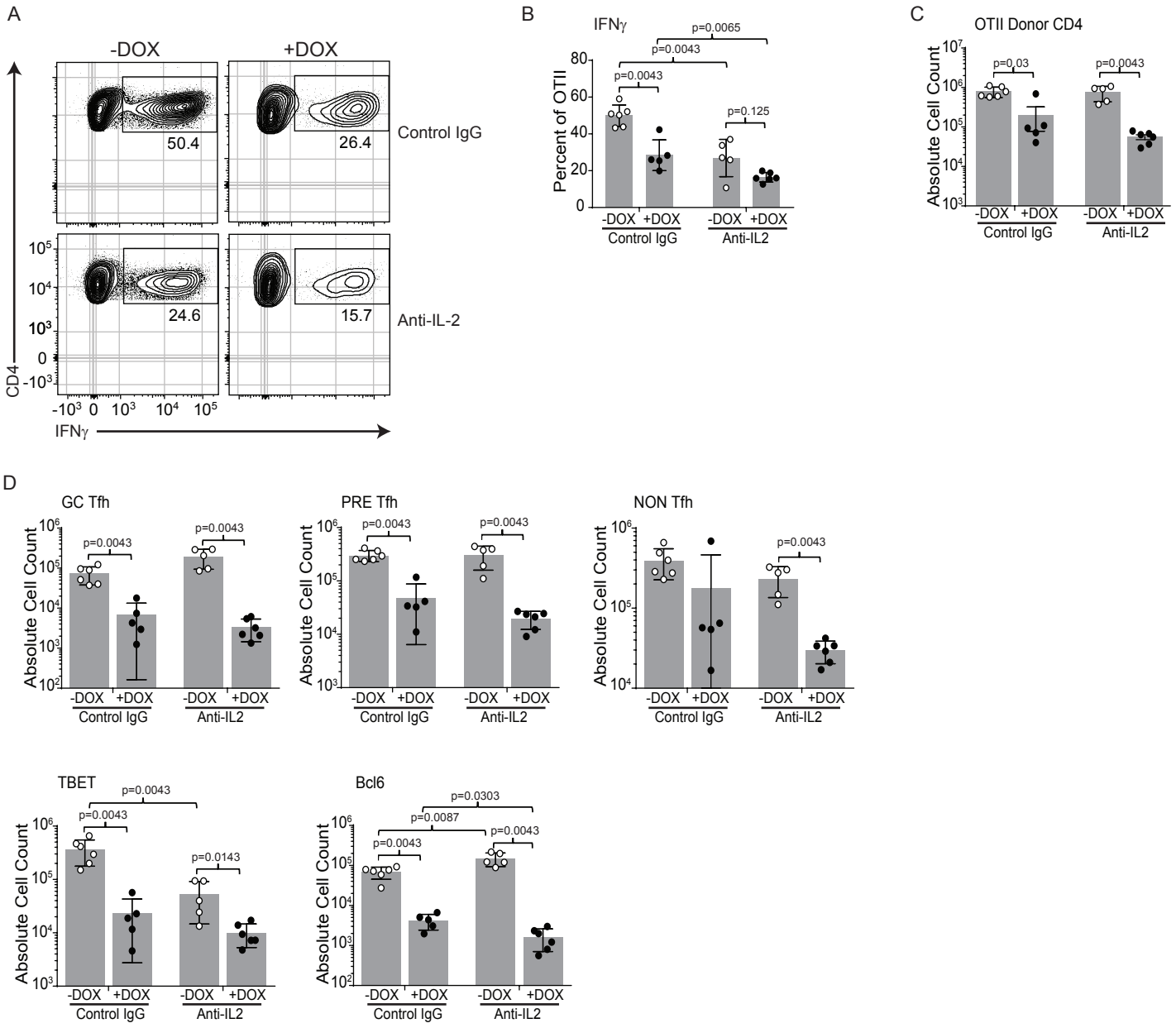
A
-DOX
+DOX
50.4
26.4
Control IgG
24.6
15.7
Anti-IL-2
CD4
IFNγ
B
IFNγ
Percent of OTII
p=0.0065
p=0.0043
p=0.0043
p=0.125
-DOX +DOX -DOX +DOX
Control IgG
Anti-IL2
C
OTII Donor CD4
Absolute Cell Count
p=0.03
p=0.0043
-DOX +DOX -DOX +DOX
Control IgG
Anti-IL2
D
GC Tfh
PRE Tfh
NON Tfh
TBET
Bcl6
Absolute Cell Count
p=0.0043
p=0.0143
p=0.0303
p=0.0087
-DOX +DOX -DOX +DOX
Control IgG
Anti-IL2

Figure S6. The effect of Irf4 concentrations on T helper cell fate is independent of IL-2 responsiveness. (Accompanies Fig. 6)

A) 10^5 *Irf4*-inducible (*Irf4*$^{+/+}$) OT-II TCR Tg CD4$^+$ T cells (CD45.2$^+$ and *Rag1*$^{-/-}$) were adoptively transferred into congenic CD45.1$^+$ hosts and then immunized with 50μg of either RFP-OVA emulsified in CFA the next day. Mice were administered control or anti-IL-2 neutralizing mAbs daily for 5 days of the immunization as well as administered water lacking DOX (–DOX) or containing DOX (+DOX) for the first two days of immunization only. Five days post-immunization, lymph node cells were stimulated with PMA/Ionomycin in the presence of monensin and brefeldinA. Six hours later, CD45.1$^-$B220$^-$MHCII$^-$ CD45.2$^+$CD4$^+$ donor cells were assessed by flow cytometry for IFNγ. Contour plots represent concatenated data of three mice per group and is representative of two experiments.

B) Frequencies (mean±SD) of indicated populations from (A). Each point (6) represents a single mouse from two experiments. *p value*s were calculated using parametric unpaired *t*-test.

C) Quantitation (mean±SD) of absolute numbers of cells analyzed from Fig 6B. Numbers of CD45.1$^-$B220$^-$MHCII$^-$CD45.2$^+$CD4$^+$ donor cells from individual mice are plotted; 6 mice from two experiments. *p value*s were calculated using parametric unpaired *t*-test.

Figure S7

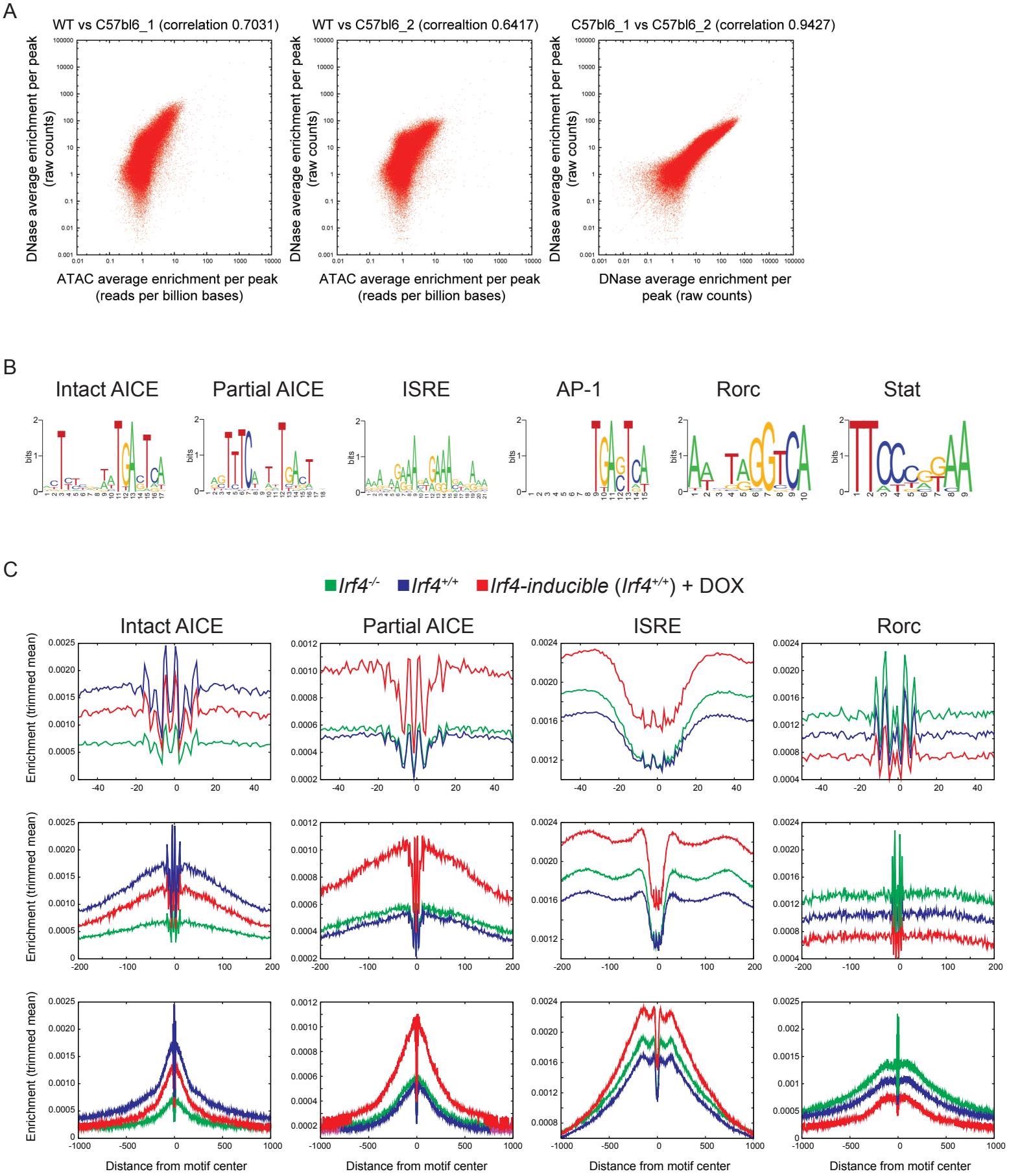
A
WT vs C57bl6_1 (correlation 0.7031)
WT vs C57bl6_2 (correaltion 0.6417)
C57bl6_1 vs C57bl6_2 (correlation 0.9427)
DNase average enrichment per peak (raw counts)
ATAC average enrichment per peak (reads per billion bases)
DNase average enrichment per peak (reads per billion bases)
DNase average enrichment per peak (raw counts)
B
Intact AICE
Partial AICE
ISRE
AP-1
Rorc
Stat
bits
C
Irf4-/- Irf4+/+ Irf4-inducible (Irf4+/+) + DOX
Intact AICE
Partial AICE
ISRE
Rorc
Enrichment (trimmed mean)
Distance from motif center

Figure S7. Irf4 targets distinct DNA motifs to control Tfh and Teff gene programs. (Accompanies Fig. 7)

A) *Irf4*$^{-/-}$, *Irf4*$^{+/+}$, or *Irf4*-inducible (*Irf4*$^{+/+}$) +DOX OT-II TCR Tg CD4^{+} T cells (CD45.2^{+} and *Rag1*$^{-/-}$) were adoptively transferred into congenic CD45.1^{+} hosts and then immunized with 50μg of RFP-OVA emulsified in CFA the next day. Three days after immunization, donor cells (CD45.1^{-}B220^{-}MHCII^{-}CD45.2^{+}CD4^{+}) were sorted and processed for ATAC-seq. Peaks from this analysis were compared to DNaseI-seq ENCODE data of 48 hour in vitro activated CD4+ cells (GEO: GSE37074; ID 5895 and 5896).

B) Logo-based depictions of Irf4 binding sequences constructed from position weight matrices in Table S7.

C) ATAC-seq peaks from *Irf4*$^{-/-}$, *Irf4*$^{+/+}$, or *Irf4*-inducible (*Irf4*$^{+/+}$) +DOX OT-II TCR Tg CD4^{+} T cells were analyzed for the presence of intact AICE, partial AICE, and ISRE-specific footprints at three different base pair intervals.

Tables S1-7; Tables S1-7 are Excel spreadsheets for download.

Table S1. Irf4 dose dependent changes in gene expression (all) and (clusters); for download

Differential expression statistics for RNA-seq. “Condition PValue” tests whether a gene is differentially expressed in any of the *Irf4*$^{+/+}$, *Irf4*$^{-/-}$, or *Irf4*-inducible conditions + DOX, using generalized linear models in edgeR; “Condition QValue” is the FDR-corrected p-value for this test. Other columns give statistics for each of three pairwise comparisons between conditions: KO/Dox (*Irf4*$^{-/-}$ vs Irf4-inducible + DOX), WT/Dox (*Irf4*$^{+/+}$ vs Irf4-inducible + DOX), and WT/KO (*Irf4*$^{+/+}$ vs *Irf4*$^{-/-}$). Each pairwise comparisons has four columns: logFC (log2 fold-change), direction indicated by the title line (WT/KO means WT over KO, so up-regulation is higher in WT); logCPM (log2 counts per million), average log-scaled normalized expression over both conditions; PValue, p-value from the exact pairwise test in edgeR; and QValue, FDR-corrected p-value. Tabs include lists of genes associated with each RNA cluster. All genes were assigned to one and only one cluster.

Table S2. ATAC batch designations

ATAC-seq data sets and the genotype for each. Batches observed in PCA clustering are shown in the third column. Corresponding batch effects were estimated and controlled for during differential enrichment analysis of ATAC-seq data sets.

Table S3. Irf4 dose dependent changes in chromatin accessibility (all) and (clusters); for download

Differential expression statistics for ATAC-seq. “Condition PValue” tests whether a gene is differentially expressed in any of the *Irf4*$^{+/+}$, *Irf4*$^{-/-}$, or *Irf4*-inducible conditions + DOX, using generalized linear models in edgeR; “Condition QValue” is the FDR-corrected p-value for this test. Other columns give statistics for each of three pairwise comparisons between conditions: Induced/KO (*Irf4*-inducible + DOX vs *Irf4*$^{-/-}$), Induced/WT (*Irf4*-inducible + DOX vs *Irf4*$^{+/+}$), and WT/KO (*Irf4*$^{+/+}$ vs *Irf4*$^{-/-}$). Each pairwise comparisons has four columns: logFC (log2 fold-change), direction indicated by the title line (WT/KO means WT over KO, so up-regulation is higher in WT); logCPM (log2 counts per million), average log-scaled normalized expression over both conditions; PValue, p-value from the exact pairwise test in edgeR; and QValue, FDR-corrected p-value. Tabs include list of peaks and their genomic coordinates (mm9) associated with each ATAC cluster. All ATAC peaks were assigned to one and only one cluster.

Table S4. Irf4 motif-containing accessible regions – correlation with Th17 IRF4 ChIP-seq; for download

Comparison of co-location of Irf4 motifs and Irf4 ChIP-seq data sets across all ATAC-seq peaks. For each motif we report, in order of the columns, (1) ATAC peaks with both a motif and a ChIP-seq peak, (2) ATAC peaks with a motif but no ChIP-seq peak, (3) ATAC peaks with a ChIP-seq peak but no motif, (4) ATAC peaks with neither a motif nor a ChIP-seq peak, (5) fraction of all ATAC peaks containing a ChIP-seq peak, (6) fraction of motif-containing ATAC peaks that also contain a ChIP-seq peak, (7) log2 enrichment ratio (log2 (6)/(5)), and (8) p-value for the co-enrichment, from Fisher's Exact Test.

Table S5. Irf4 binding motif PWM and ATAC-seq peak and motif annotation; for download

Position-weight matrices of motifs used. Motif names are indicated on lines beginning with ">", followed by the nucleotides one row at a time from 5' to 3'. Columns from left-to-right indicate the relative weight of nucleotides A, C, G, and T, respectively.

Tabs include all ATAC-seq peaks and their motif hits. The genomic coordinates and name for each peak are given on each line, along with information for each motif instance observed in that peak; each motif is in its own column, indicated by the column title line. Counts and scores for each motif are given as [count]:[score1];[score2];…, and scores are –log10 p-values from FIMO. For example, "2:4.360;4.053" indicates that this peak had 2 motif hits with scores of 4.360 and 4.053. "NA" means no motif was found.

Table S6. Motif enrichment & co-enrichment in open chromatin calculation; for download

(TAB1) Motif enrichment in each ATAC peak cluster. For each motif we report, in order of the columns, (1) count of all ATAC peaks with a motif; (2) count of all ATAC peaks without a motif; (3) count of cluster-specific ATAC peaks with a motif; (4) count of cluster-specific ATAC peaks without a motif; (5) log2 enrichment ratio; (6) enrichment p-value from Fisher's Exact Test; and (7) FDR-corrected p-value (q-value).

(TAB2) Motif co-enrichment in each ATAC peak cluster. Peaks within each cluster were examined for the co-occurrence of motifs. For each pair of motifs we report, in order of the columns, (1) count of ATAC peaks with both motifs; (2) count of ATAC peaks with only the first motif; (3) count of ATAC peaks with only the second motif; (4) count of ATAC peaks with neither motif; (5) log2 enrichment ratio; (6) enrichment p-value from Fisher's Exact Test; and (7) FDR-corrected p-value (q-value).

Table S7. Open chromatin – gene associations; for download

Associations of ATAC peak clusters with RNA gene clusters. Each ATAC cluster is listed for each associative distance range down the rows, noted in the first two columns ("Query set" is the ATAC

cluster). “Overall p-value” is from a Chi-squared test comparing the distribution of RNA clusters for the genes associated with an ATAC cluster to the distribution of RNA clusters genome-wide (i.e., the fraction of genes in each cluster). Remaining columns delineate comparisons of the ATAC cluster to each RNA cluster individually, comparing the number of genes in that RNA cluster vs not in that RNA cluster: log2 enrichment ratio is the log2-scaled fraction of ATAC cluster-associated genes in that RNA cluster, over the genome-wide fraction of genes in that RNA cluster, and p-value is from Fisher’s Exact Test.