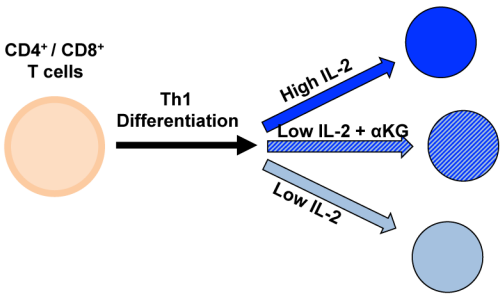
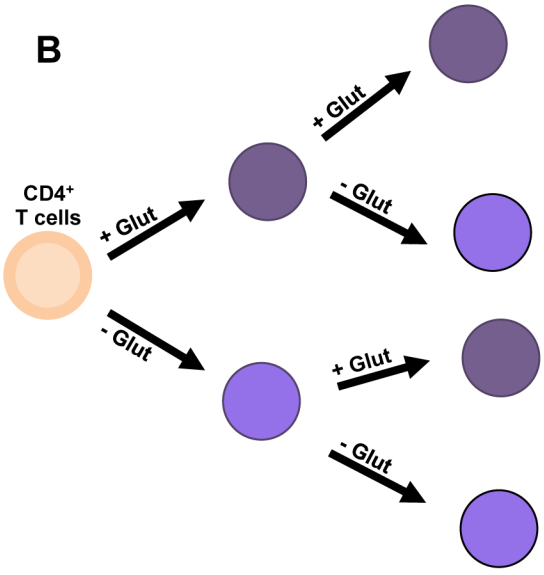
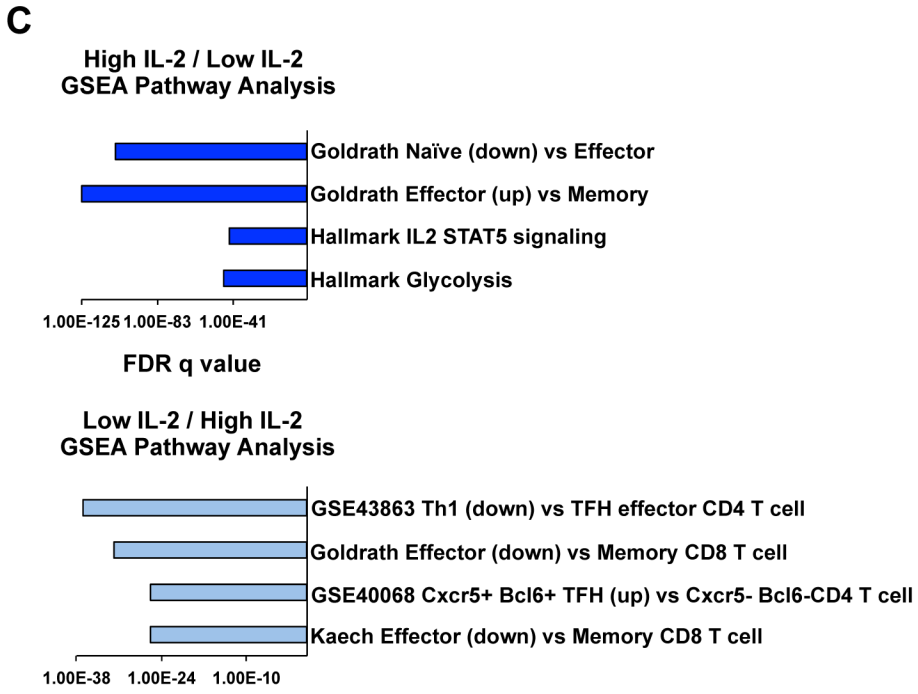
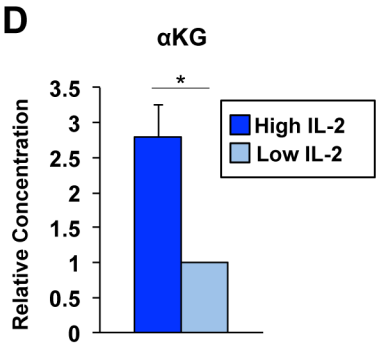
Figure S1


A
CD4+ / CD8+
T cells
Th1
Differentiation
High IL-2
Low IL-2 + αKG
Low IL-2


B
CD4+
T cells
+ Glut
- Glut
+ Glut
- Glut
+ Glut
- Glut


C
High IL-2 / Low IL-2
GSEA Pathway Analysis
Goldrath Naïve (down) vs Effector
Goldrath Effector (up) vs Memory
Hallmark IL2 STAT5 signaling
Hallmark Glycolysis
1.00E-125 1.00E-83 1.00E-41
FDR q value
Low IL-2 / High IL-2
GSEA Pathway Analysis
GSE43863 Th1 (down) vs TFH effector CD4 T cell
Goldrath Effector (down) vs Memory CD8 T cell
GSE40068 Cxcr5+ Bcl6+ TFH (up) vs Cxcr5- Bcl6-CD4 T cell
Kaech Effector (down) vs Memory CD8 T cell
1.00E-38 1.00E-24 1.00E-10
FDR q value


D
αKG
Relative Concentration
High IL-2
Low IL-2

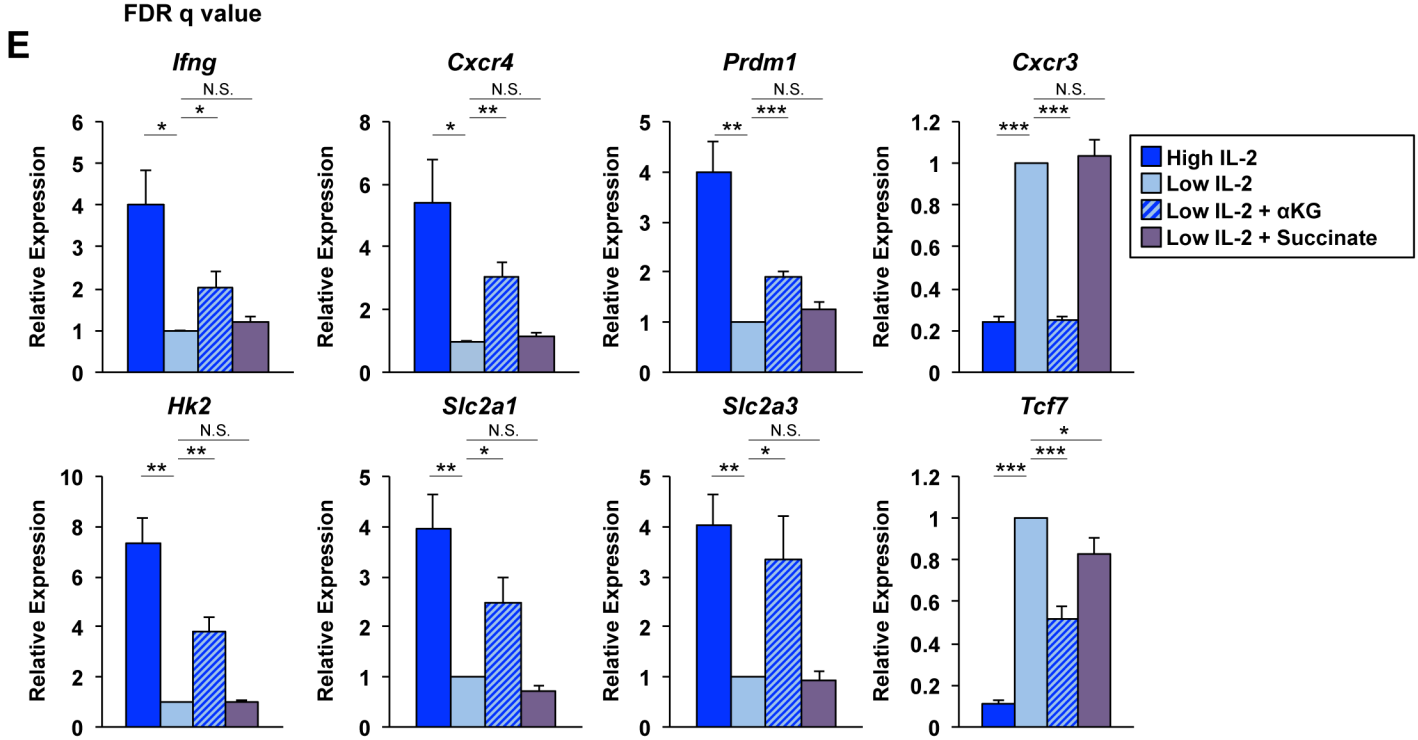

E
Ifng
Cxcr4
Prdm1
Cxcr3
Hk2
Slc2a1
Slc2a3
Tcf7
Relative Expression
N.S.
High IL-2
Low IL-2
Low IL-2 + αKG
Low IL-2 + Succinate

Figure S1. Schematic representation of experimental systems approach. Related to Figures 1, 2. **(A)** Schematic depicting the *in vitro* primary $CD4^+$ and $CD8^+$ T cell differentiation culture system. Primary $CD4^+$ or $CD8^+$ T cells isolated from wild-type (WT) C57BL/6 mice were polarized in type 1 (IL-12 and anti-IL-4) conditions and then exposed to either high environmental IL-2 (100units/mL), low environmental IL-2 (2.5units/mL) or low IL-2 with αKG conditions for two days. **(B)** Schematic depicting *in vitro* primary $CD4^+$ T cell differentiation examining glutamine sensitivity. Primary $CD4^+$ T cells were isolated from WT C57BL/6 mice and were stimulated with αCD3 and αCD28 in Th1 polarizing conditions either with glutamine (dark purple) or without glutamine (light purple). After 3 days, the cells were split and maintained in Th1 polarizing conditions either with glutamine (dark purple) or without glutamine (light purple) and either high IL-2 or low IL-2 conditions for two days. **(C)** FDR q values from a gene set enrichment analysis (GSEA) on genes that were induced in $CD4^+$ Th1 cells in high environmental IL-2 relative to low IL-2 conditions (dark blue) or genes that were more highly expressed in low IL-2 relative to high IL-2 conditions (light blue) in RNA-seq experiments. Differential gene expression was determined by a Cuffdiff analysis and genes analyzed by GSEA had at least a 2-fold statistically significant difference between conditions and an FPKM value of 1 or greater for the expression of the gene in at least one of the comparison conditions. The n= three independent biological replicates for the RNA-seq. **(D)** WT $CD4^+$ Th1 cells were cultured in either high IL-2 (250units/mL) or low IL-2 (10units/mL) conditions and αKG levels were monitored. The graph represents the relative comparison of the αKG levels found in the low IL-2 relative to the high IL-2 condition from n= three independent experiments. **(E)** Primary WT $CD4^+$ T cells polarized in Th1 conditions were exposed to high IL-2 (dark blue), low IL-2 (light blue), low IL-2 with cell permeable αKG (blue hatched) or low IL-2 with cell permeable succinate (purple) and analyzed by qRT-PCR. Each sample was first normalized to the *Rps18* control and then compared relative to the low IL-2 condition for each independent experiment. The n= at least three independent experiments for each gene in the analysis. Error bars represent SEM and an unpaired student t-test was performed. P values are indicated above each comparison by asterisks (NS not significant, * ≤0.05, **≤0.01, and ***≤0.001).

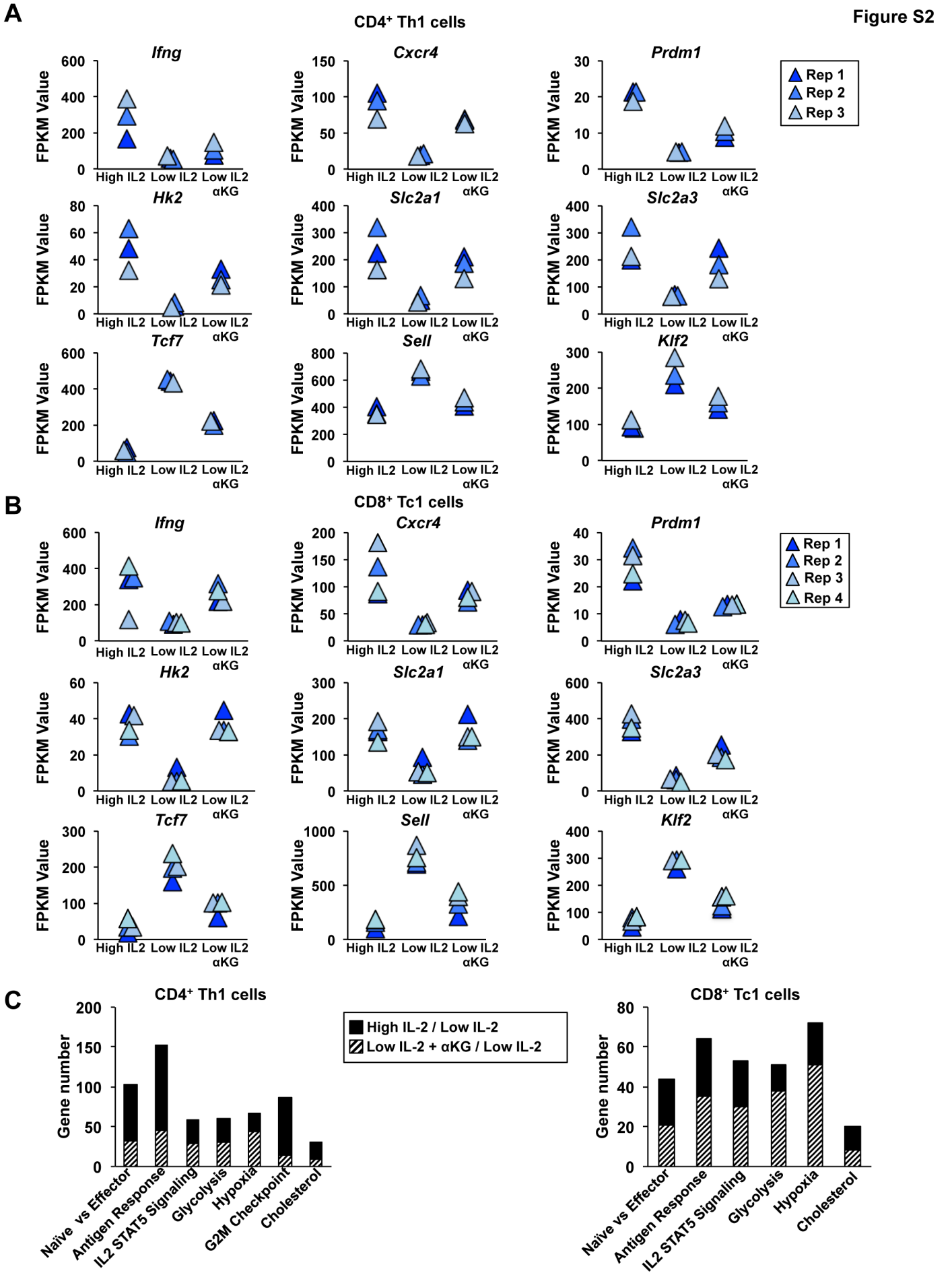

A
CD4+ Th1 cells
Ifng
Cxcr4
Prdm1
Hk2
Slc2a1
Slc2a3
Tcf7
Sell
Klf2
FPKM Value
High IL2
Low IL2
Low IL2 αKG
Rep 1
Rep 2
Rep 3
B
CD8+ Tc1 cells
Rep 4
C
CD4+ Th1 cells
CD8+ Tc1 cells
Gene number
High IL-2 / Low IL-2
Low IL-2 + αKG / Low IL-2
Naïve vs Effector
Antigen Response
IL2 STAT5 Signaling
Glycolysis
Hypoxia
G2M Checkpoint
Cholesterol

Figure S2. IL-2- and αKG-sensitive gene expression in $CD4^+$ Th1 and $CD8^+$ Tc1 cells from RNA-seq. Related to Figures 1, 2. (A, B) Primary (**A**) $CD4^+$ or (**B**) $CD8^+$ T cells polarized in type 1 conditions were exposed to high IL-2, low IL-2, or low IL-2 with αKG and RNA-seq analyses were performed on (**A**) three or (**B**) four biological replicates. Graphs represent the FPKM values for the indicated genes for each of the conditions. (**C**) GSEA was performed as described in Figure 2C and 2D. Graphs representing the number of genes in the pathways enriched by high IL-2 in comparison to low IL-2 (black bars) and low IL-2 with αKG in comparison to low IL-2 (black hatched bars) in $CD4^+$ T cells (left) or $CD8^+$ T cells (right).

Figure S3

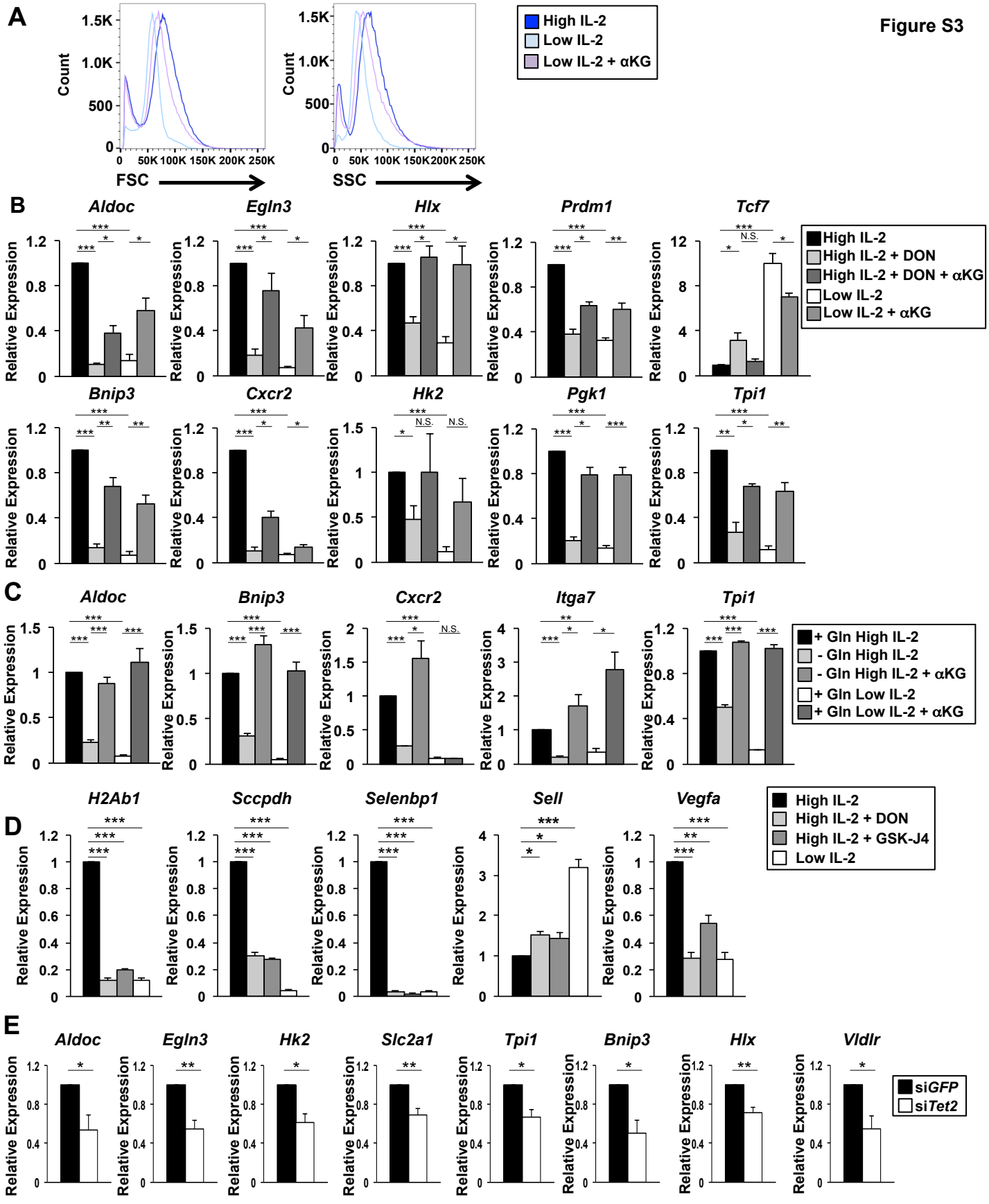

A
Count
FSC
SSC
High IL-2
Low IL-2
Low IL-2 + αKG
B
Aldoc
Egln3
Hlx
Prdm1
Tcf7
Bnip3
Cxcr2
Hk2
Pgk1
Tpi1
Relative Expression
High IL-2
High IL-2 + DON
High IL-2 + DON + αKG
Low IL-2
Low IL-2 + αKG
C
Aldoc
Bnip3
Cxcr2
Itga7
Tpi1
+ Gln High IL-2
- Gln High IL-2
- Gln High IL-2 + αKG
+ Gln Low IL-2
+ Gln Low IL-2 + αKG
D
H2Ab1
Sccpdh
Selenbp1
Sell
Vegfa
High IL-2
High IL-2 + DON
High IL-2 + GSK-J4
Low IL-2
E
Aldoc
Egln3
Hk2
Slc2a1
Tpi1
Bnip3
Hlx
Vldlr
siGFP
siTet2

Figure S3. Gln- and αKG-sensitive events contribute to IL-2-sensitive gene expression in CD4$^+$ Th1 cells. Related to Figures 1-3. (A) Flow cytometry analysis of forward scatter and side scatter for primary CD4$^+$ T cells polarized in Th1 conditions and exposed to high IL-2 (dark blue), low IL-2 (light blue), or low IL-2 with αKG (purple) from the same samples described in Figure 1C. Data are representative of three independent biological replicate experiments. **(B)** Primary CD4$^+$ T cells polarized in Th1 conditions were maintained in high IL-2 or low IL-2 conditions. Th1 cells maintained in high IL-2 conditions were left untreated (black bar), or were treated with DON (light grey) or DON with αKG (dark grey bar). Th1 cells maintained in low IL-2 were left either untreated (white bar) or treated with αKG (medium grey bar). Transcripts were monitored by qRT-PCR and samples were first normalized to the *Rps18* control and then compared relative to the high IL-2 condition for each independent experiment. **(C)** Experiment with primary CD4$^+$ T cells as described in Figure 2F. **(D)** Experiment with primary CD4$^+$ T cells as described in Figure 3C. **(E)** Primary CD4$^+$ T cells polarized in Th1 conditions were transfected with either a control siRNA to *GFP* (black bars) or an siRNA to *Tet2* (white bars). Transcripts were monitored by qRT-PCR and samples were first normalized to the *Rps18* control and then compared relative to the si*GFP* control for each independent experiment. The control siRNA sample is the same as the one used for *Ctcf* siRNA experiments described in Figure 4D and S4B. (**B-E**) Error bars represent SEM and an unpaired student t-test was performed. (**B-D**) P values are indicated above each comparison by asterisks (* ≤0.05, **≤0.001, and ***≤0.0001). (**E**) P values are indicated above each comparison by asterisks (* ≤0.05 and **≤0.01). The n= at least (**B**) 4 except DON with αKG is at least 3 (**D**) 4 except high IL-2 with DON n= 3 or (**C, E**) 3 independent biological replicates for each gene analyzed.

Figure S4

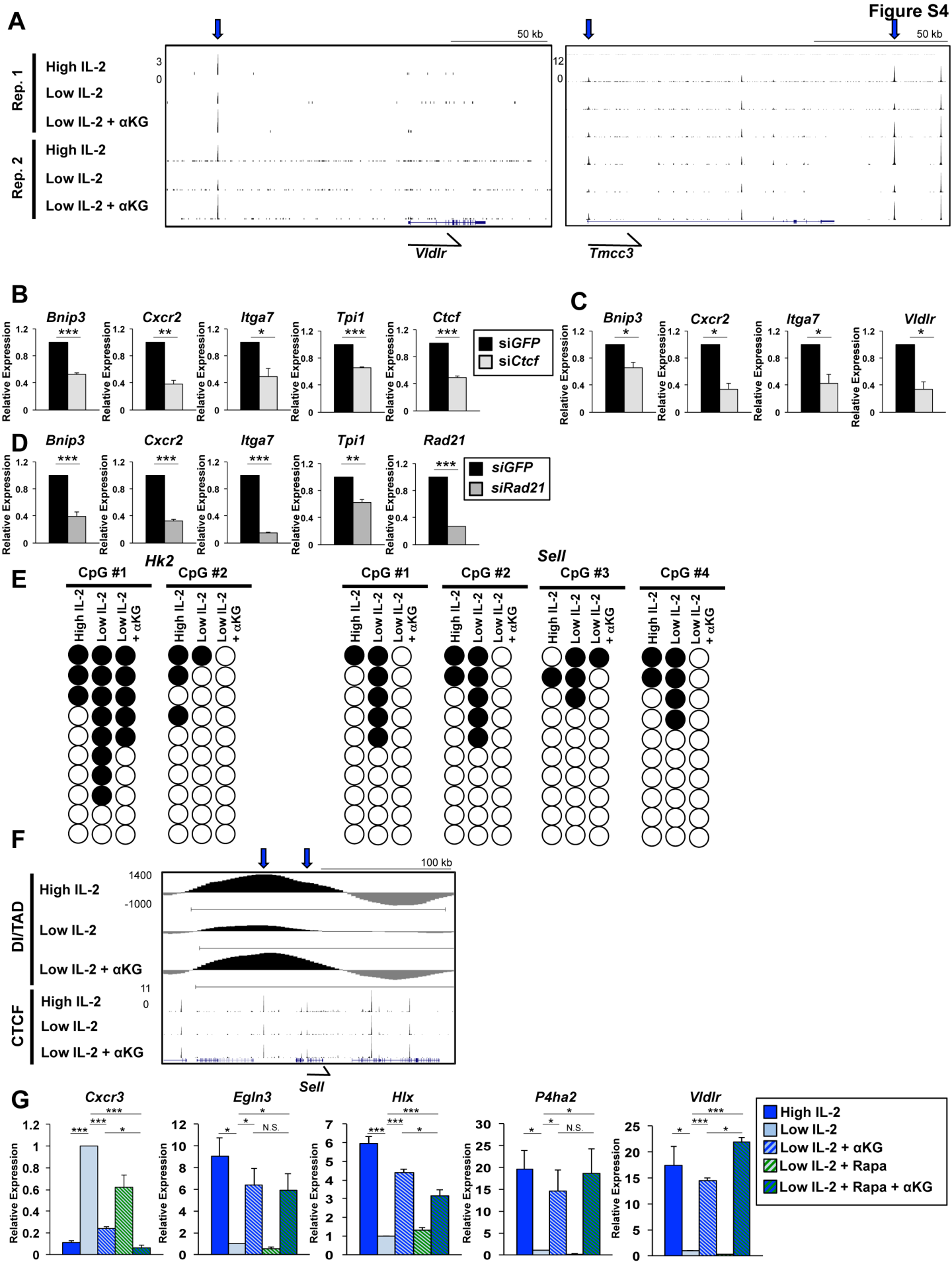

A
50 kb
Rep. 1
High IL-2
Low IL-2
Low IL-2 + αKG
Rep. 2
High IL-2
Low IL-2
Low IL-2 + αKG
Vldlr
Tmcc3
B
Bnip3
Cxcr2
Itga7
Tpi1
Ctcf
Relative Expression
siGFP
siCtcf
C
Bnip3
Cxcr2
Itga7
Vldlr
D
Bnip3
Cxcr2
Itga7
Tpi1
Rad21
siGFP
siRad21
E
Hk2
Sell
CpG #1
CpG #2
CpG #3
CpG #4
High IL-2
Low IL-2
Low IL-2 + αKG
F
100 kb
DI/TAD
CTCF
High IL-2
Low IL-2
Low IL-2 + αKG
Sell
G
Cxcr3
Egln3
Hlx
P4ha2
Vldlr
N.S.
High IL-2
Low IL-2
Low IL-2 + αKG
Low IL-2 + Rapa
Low IL-2 + Rapa + αKG

Figure S4: IL-2- and αKG-sensitive CTCF association regulates a portion of the IL-2-sensitive program. Related to Figures 4, 6. (A) UCSC genome browser tracks displaying two independent CTCF ChIP-seq replicates for primary $CD4^+$ T cells polarized in Th1 cell conditions and maintained in high IL-2, low IL-2, or low IL-2 with αKG as described in Figure 4. **(B)** Experiments were performed as described in Figure 4D. The control siRNA sample is the same as the one used for *Tet2* siRNA experiments described in Figure S3E. The *Ctcf* or *Rad21* knockdown was monitored in cells transfected with siRNA at the time of initial TCR stimulation and at the split day. **(C)** Primary $CD8^+$ T cells were polarized under type 1 conditions and cells were exposed to either an siRNA to *Ctcf* or an siRNA to *GFP* as a control. Experiments were analyzed as described in Figure 4D. **(D)** Experiments and analyses were performed as described in Figure 4E. **(E)** Independent biological replicates for bisulfite sequencing analysis of *Hk2* and *Sell* as described in Figure 6B-C. **(F)** UCSC genome browser track for *Sell* locus displaying CTCF ChIP-seq peaks and in situ Hi-C analysis of the directionality index from experiments in $CD4^+$ Th1 cells exposed to high IL-2, low IL-2, or low IL-2 with αKG displayed as in Figure 4C and S5B. (**G**) Primary Th1 cells were exposed to high IL-2 (dark blue bars), low IL-2 (light blue bars), low IL-2 with αKG (dark blue/light blue hatched bars), low IL-2 with rapamycin (green/light blue hatched bars), or low IL-2 with αKG and rapamycin (green/dark blue hatched bars). (**B-D, G**) Error bars represent SEM and an unpaired student t-test was performed. P values are indicated above each comparison by asterisks (* ≤0.05, **≤0.001, and ***≤0.0001). The n= at least (**B, G**) 3 or (**C**) 2 independent experiments for each gene analyzed. For (**D**), the n= at least 3 independent biological replicates for all genes except *Rad21* was 2 independent biological replicates.

Figure S5

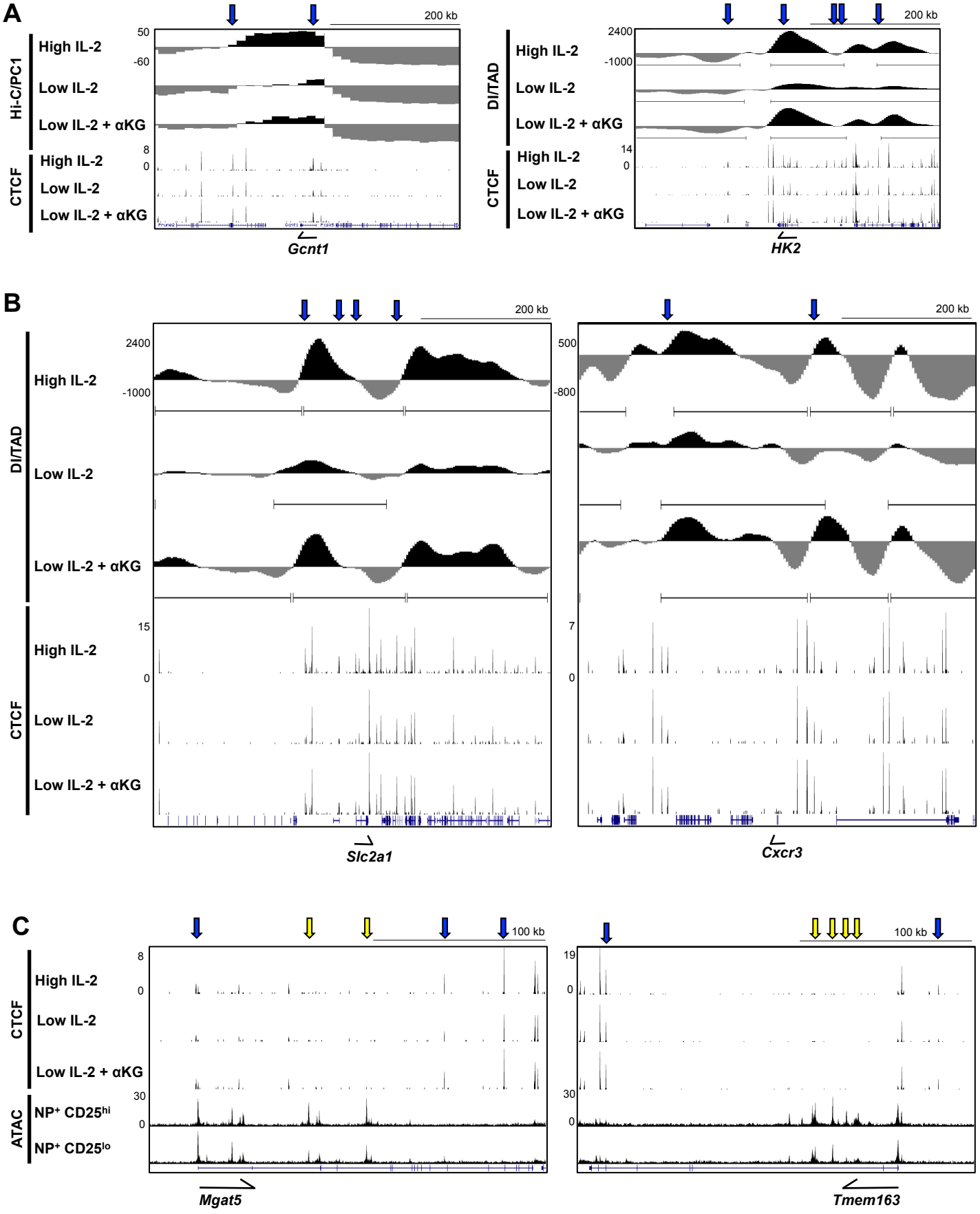

A
Hi-C/PC1
High IL-2
Low IL-2
Low IL-2 + αKG
CTCF
High IL-2
Low IL-2
Low IL-2 + αKG
200 kb
50
-60
8
0
Gcnt1
DI/TAD
High IL-2
Low IL-2
Low IL-2 + αKG
CTCF
High IL-2
Low IL-2
Low IL-2 + αKG
200 kb
2400
-1000
14
0
HK2
B
DI/TAD
High IL-2
Low IL-2
Low IL-2 + αKG
CTCF
High IL-2
Low IL-2
Low IL-2 + αKG
200 kb
2400
-1000
15
0
Slc2a1
200 kb
500
-800
7
0
Cxcr3
C
CTCF
High IL-2
Low IL-2
Low IL-2 + αKG
ATAC
NP+ CD25hi
NP+ CD25lo
100 kb
8
0
30
0
Mgat5
100 kb
19
0
30
0
Tmem163

Figure S5. IL-2- and αKG-sensitive CTCF association and genome topology in CD4$^+$ Th1 cells. Related to Figures 4, 5. (A, B) UCSC genome browser tracks displaying Hi-C PC1 analysis, directionality index, TAD boundary analysis, and CTCF ChIP-seq analysis for CD4$^+$ T cells polarized in Th1 conditions and maintained in either high IL-2, low IL-2, or low IL-2 with αKG. **(C)** ATAC-seq analysis from NP antigen specific CD8$^+$ T cells isolated 9 days after influenza infection. NP antigen specific cells were sorted into CD25^{hi} or CD25^{lo} populations for ATAC-seq analysis. Genomic regions displayed were found to have a higher probability of interaction in high IL-2 or low IL-2 with αKG compared to low IL-2 in the circos analysis in Figure 5D.

Figure S6

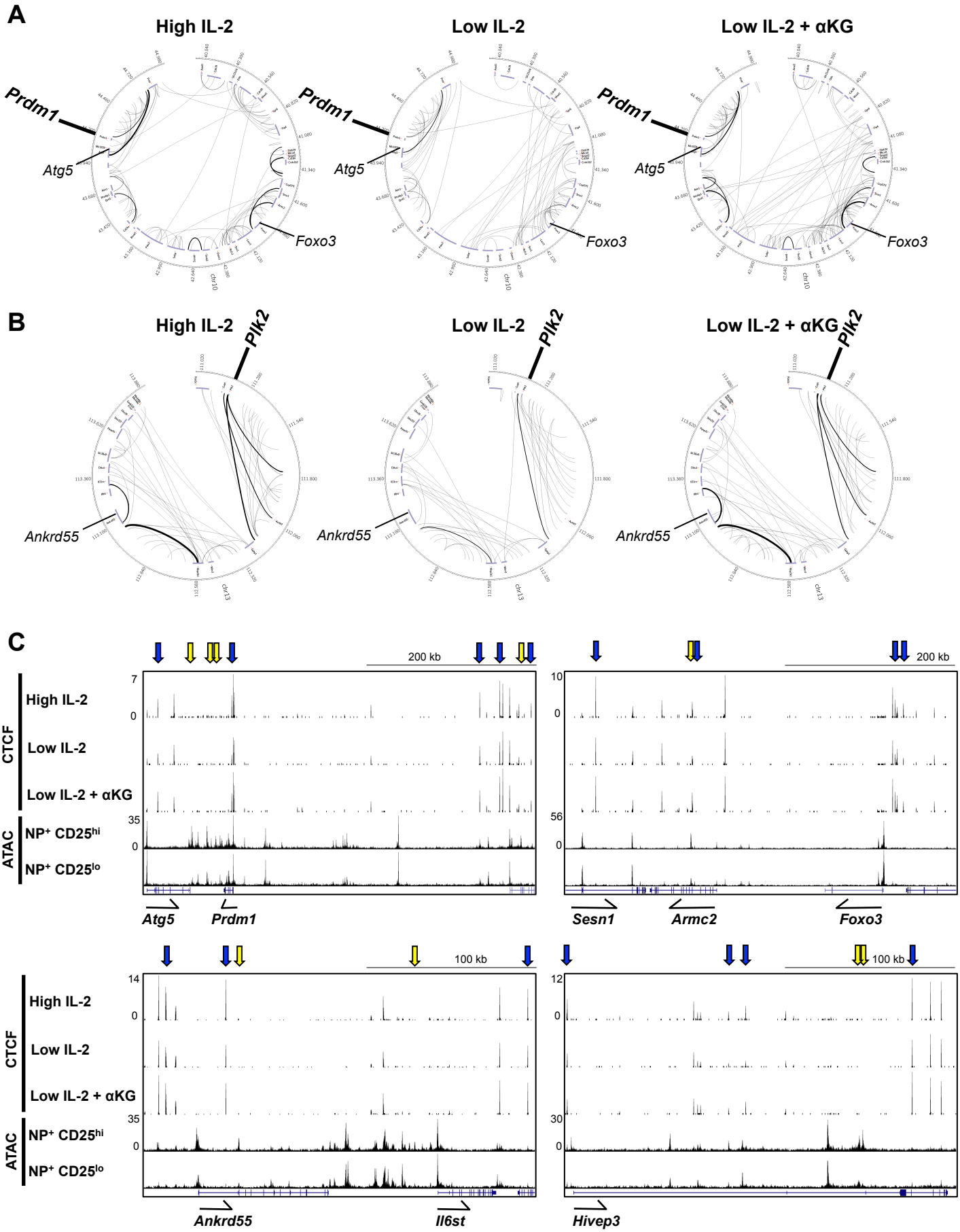
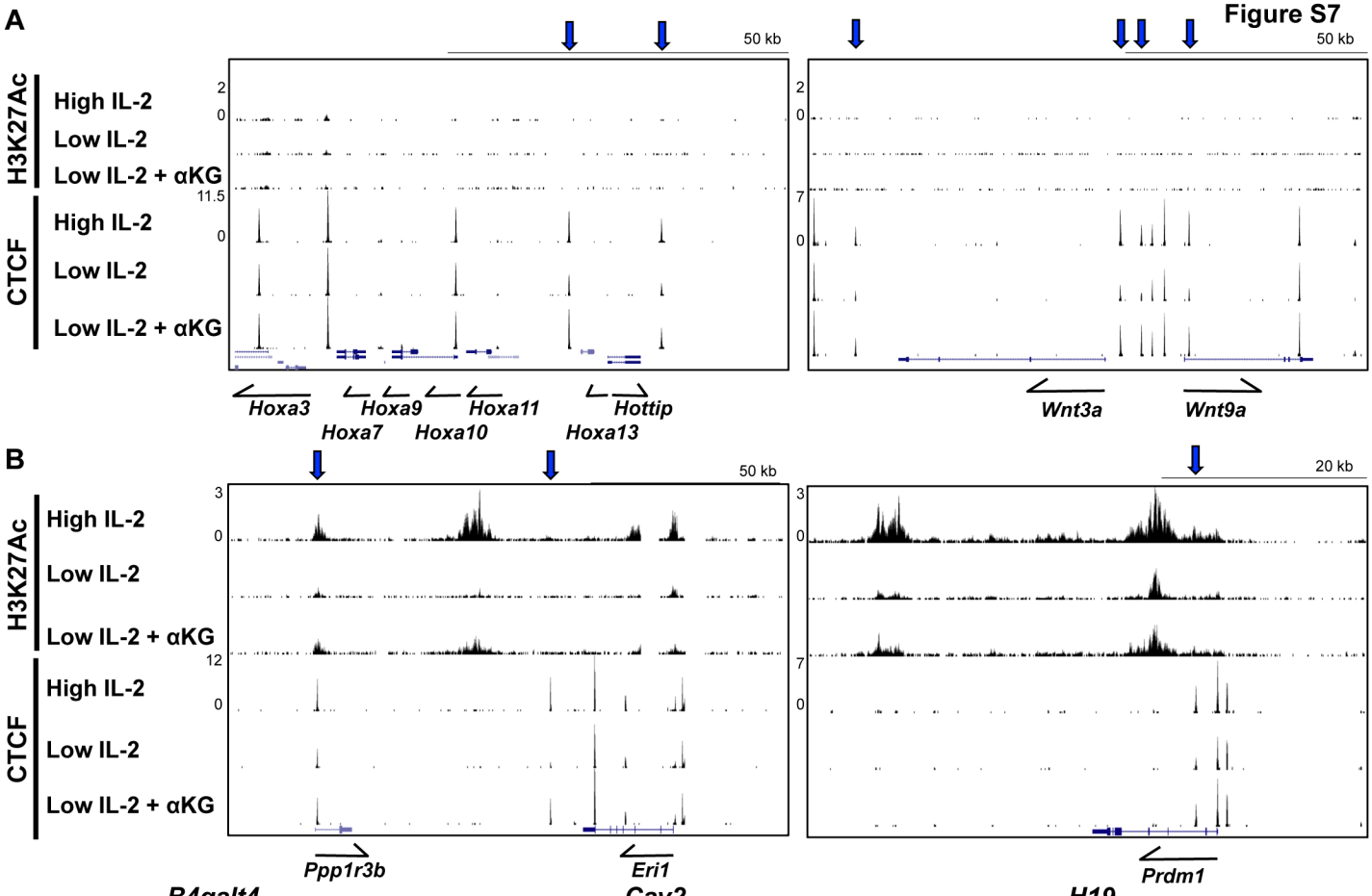

A
High IL-2
Low IL-2
Low IL-2 + αKG
Prdm1
Atg5
Foxo3
chr10
B
High IL-2
Low IL-2
Low IL-2 + αKG
Plk2
Ankrd55
chr13
C
CTCF
ATAC
High IL-2
Low IL-2
Low IL-2 + αKG
NP+ CD25hi
NP+ CD25lo
200 kb
100 kb
Atg5
Prdm1
Sesn1
Armc2
Foxo3
Ankrd55
Il6st
Hivep3

Figure S6. IL-2- and αKG-sensitive genomic interactions in CD4$^+$ T cells. Related to Figure 5. (A-C) (A, B) Circos plots displaying Hi-C interaction data for genomic regions surrounding *Prdm1* (Chr10:40,000,000-45,000,000) or *Plk2* (Chr13:111,020,000-113,880,000) or (**C**) UCSC genome browser tracks displaying CTCF ChIP-seq analysis from primary CD4$^+$ T cells polarized in Th1 conditions and maintained in either high IL-2, low IL-2, or low IL-2 with αKG. (**A**, **B**) Analysis was performed as stated in Figure 5C, 5D. (**C**) ATAC-seq analysis from NP antigen specific CD8$^+$ T cells as described in S5C. Genomic regions displayed were found to have a higher probability of interaction in high IL-2 or low IL-2 with αKG compared to low IL-2 in circos analyses.

Figure S7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H3K27Ac
High IL-2
Low IL-2
Low IL-2 + αKG
CTCF
High IL-2
Low IL-2
Low IL-2 + αKG
Hoxa3
Hoxa7
Hoxa9
Hoxa10
Hoxa11
Hoxa13
Hottip
Wnt3a
Wnt9a
B
50 kb
20 kb
Ppp1r3b
Eri1
Prdm1

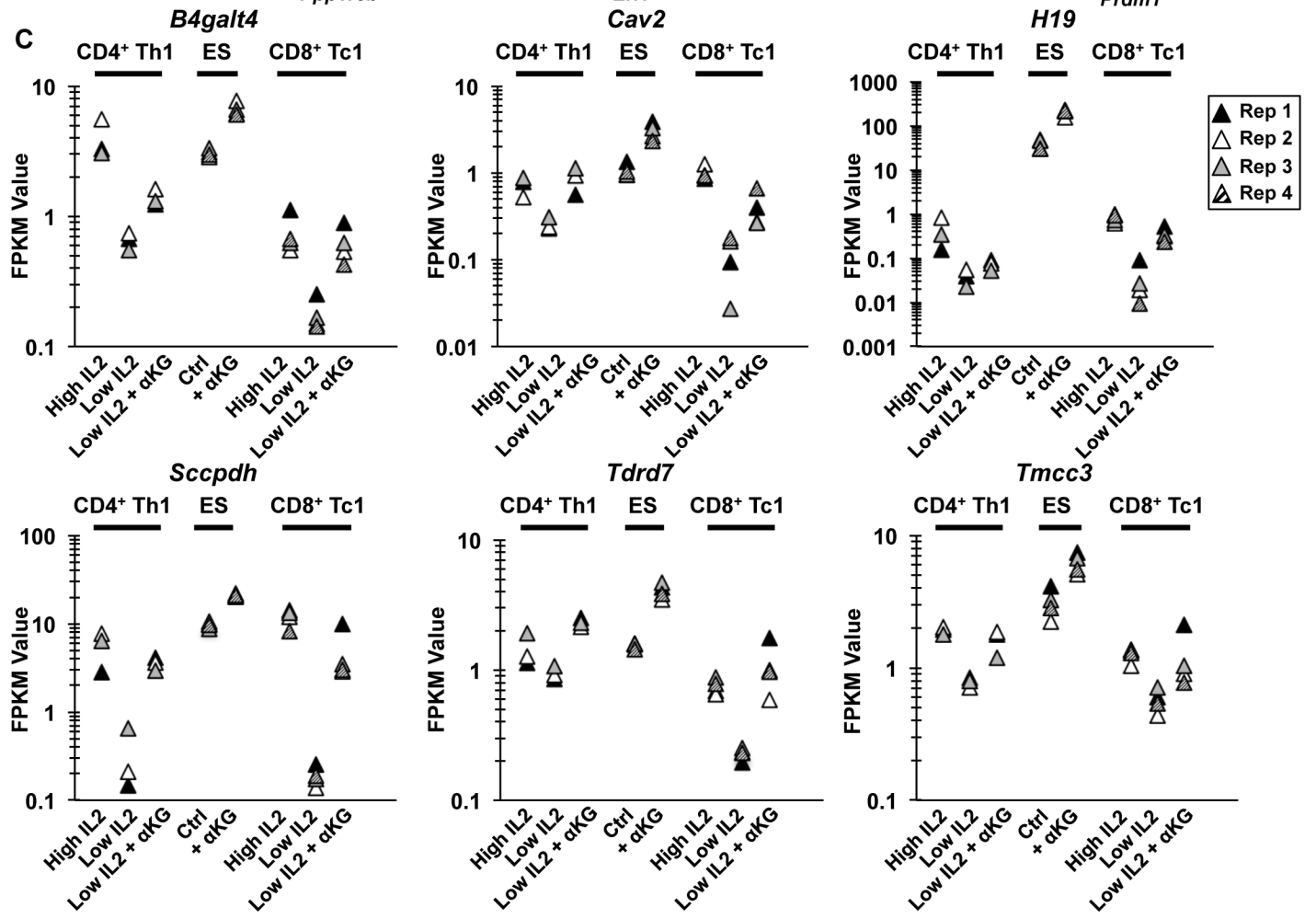
C
B4galt4
Cav2
H19
Sccpdh
Tdrd7
Tmcc3
CD4⁺ Th1
ES
CD8⁺ Tc1
FPKM Value
Rep 1
Rep 2
Rep 3
Rep 4
High IL2
Low IL2
Low IL2 + αKG
Ctrl + αKG

Figure S7. CTCF and H3K27Ac ChIP-seq tracks surrounding genes that are expressed in either Th1 or ES cells. Related to Figure 7. (A, B) UCSC genome browser tracks displaying CTCF and H3K27Ac ChIP-seq peaks from primary $CD4^+$ T cells polarized in Th1 conditions and exposed to high IL-2, low IL-2, or low IL-2 with αKG. The locations of genes in each track are shown at the bottom of each panel. **(C)** Graphs display the FPKM values from the RNA-seq analyses performed with $CD4^+$ Th1 cells (as described in Figure S2 and Figure 7C), E14Tg2a cells (as described in Figure 7C), and $CD8^+$ Tc1 cells (as described in Figure S2 and Figure 7C).

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Egln3-forward	Eurofins	TGGTCAGACCGCAGGAATCCAC
Egln3-reverse	Eurofins	GCGGATTGCACACCACAGTCAG
Tcf7-forward	Eurofins	GATGCTGGGATCTGGTGTAC
Tcf7-reverse	Eurofins	CTCCTTGAGTGTGCACTCAG
Cxcr3-forward	Eurofins	CAGCCAAGCCATGTACCTTG
Cxcr3-reverse	Eurofins	CATAATCGTAGGGAGAGGTG
Klf2-forward	Eurofins	GCAAGACCTACACCAAGAGC
Klf2-reverse	Eurofins	CTGTGACCTGTGTGCTTTCG
Hlx-forward	Eurofins	GCCTGGAGAAGAGGTTTGAG
Hlx-reverse	Eurofins	CACTTCATCCTCCGGTTCTG
Bnip3-forward	Eurofins	CGAAGCGCACAGCTACTCTCAGC
Bnip3-reverse	Eurofins	GCTAGTGGAAGTTGTCAGACGCC
P4ha2-forward	Eurofins	TGACTTCTCAAGGAGCGATGAGC
P4ha2-reverse	Eurofins	TGGCACCACCAGCTTCGACATC
Ndrg1-forward	Eurofins	CAACGTGGAGGTAGTGCACAC
Ndrg1-reverse	Eurofins	CTCCAACTACCAGCAGAGC

Vldlr-forward	Eurofins	GTGACCACAGCAG TATCAGAAG
Vldlr-reverse	Eurofins	CCACCTACTGCTG CCATCAC
Sell-forward	Eurofins	GGCATTTCTCATTT GGCTGG
Sell-reverse	Eurofins	ATGTGGGAGATGC CTGCGTG
Pgk1-forward	Eurofins	CCTTGCCTGTTGA CTTTGTC
Pgk1-reverse	Eurofins	GACCATTCCAAAC AATCTGC
Slc2a3-forward	Eurofins	TGGCTGGCTGTTG TAACTGGAC
Slc2a3-reverse	Eurofins	GAAGATGAGGAAG GCAGCGAAG
Aldoc-forward	Eurofins	GCTATGCCAGCAT CTGCCAG
Aldoc-reverse	Eurofins	GAGCAGAGTCCCT TCGAGG
Cxcr2-forward	Eurofins	GCTCACAAACAGC GTCGTAG
Cxcr2-reverse	Eurofins	GCATCTGGCAGAA TAGAGGG
Tpi1-forward	Eurofins	AAGCCGGCATCAC CGAGAAG
Tpi1-reverse	Eurofins	CACACAGGTTCAT AGGCCAGGAC
Itga7-forward	Eurofins	CTGGAACAGCACC TTTCTG
Itga7-reverse	Eurofins	GTCCAAGTACACC ATCACTG
Sccpdh-forward	Eurofins	CGAATCTGCACTC AAGTGAAG
Sccpdh-reverse	Eurofins	GGAGAAAGCTGCT CCAGGTG

Selenbp1-forward	Eurofins	CTCAGATGATCCAGCTCAGC
Selenbp1-reverse	Eurofins	CAAAGTCCACCAGAAAGTTGG
Vegfa-forward	Eurofins	GGCTGCTGTAACGATGAAGC
Vegfa-reverse	Eurofins	CACATCTGCTGTGCTGTAGG
H2Ab1-forward	Eurofins	GAGAGGTCTACACCTGTCAC
H2Ab1-reverse	Eurofins	CGAGGAAGATCACCCCAAGC
Ctcf-forward	Eurofins	CACAATGGCAAGACATGCAG
Ctcf-reverse	Eurofins	CTCCTCATTGTCATCGAGATC
Rad21-forward	Eurofins	GGAGGATGAAGATGCTTCAG
Rad21-reverse	Eurofins	CGGTTTGTGTTTCGACACAG
Rps18-forward	Eurofins	GGAGAACTCACGGAGGATGAG
Rps18-reverse	Eurofins	CGCAGCTTGTTGTCTAGACCG
Sell-forward (Bisulfite)	Eurofins	TTTTTTAAGTTAGAGTTTTGGGATTTTATTTAGG
Sell-reverse (Bisulfite)	Eurofins	CGTCCCTTCCCTTCCCTACCCTTCC
Hk2-forward (Bisulfite)	Eurofins	TAGAGTTTTTTAGAATTTTTGTTTGTTTTAGGAG
Hk2-reverse (Bisulfite)	Eurofins	AAACTTATTAAAAAACATTCAAAACCCACACACAAC

Table S1. Primers used in study. Related to Key Resource Table.