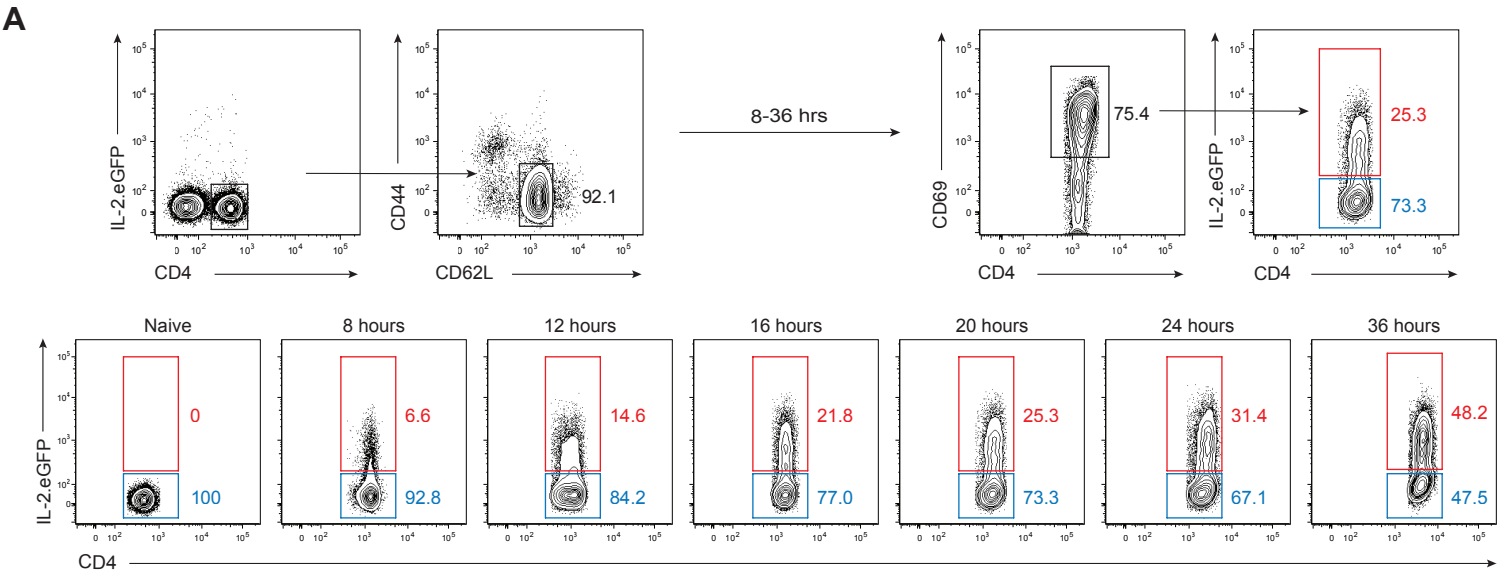
A
IL-2.eGFP
CD4
CD44
CD62L
92.1
8-36 hrs
CD69
75.4
25.3
73.3
Naive
8 hours
12 hours
16 hours
20 hours
24 hours
36 hours
0
100
6.6
92.8
14.6
84.2
21.8
77.0
25.3
73.3
31.4
67.1
48.2
47.5

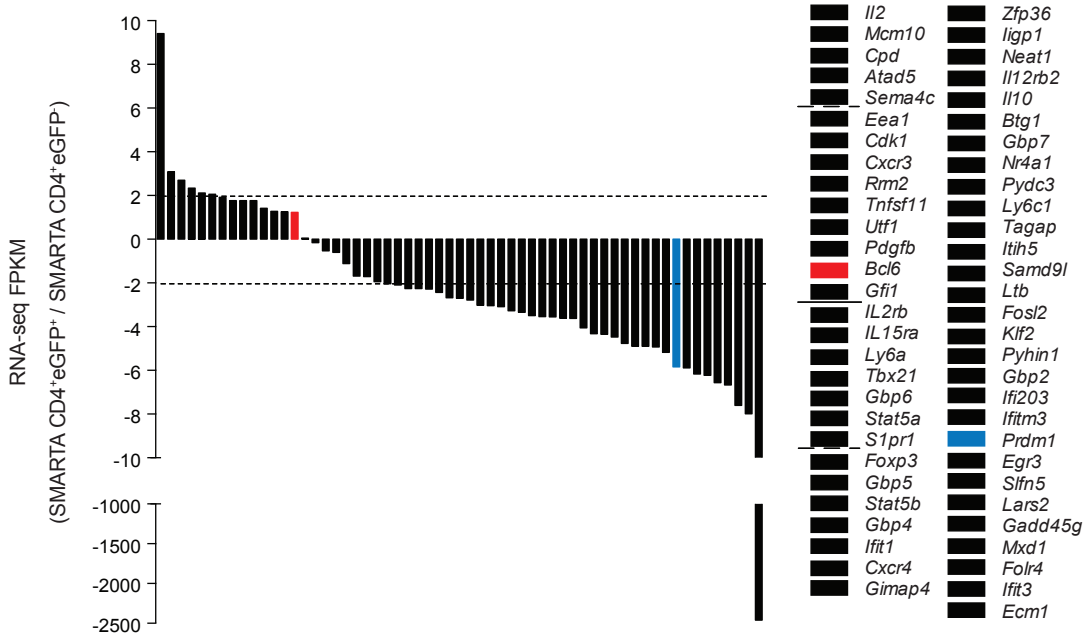
B
RNA-seq FPKM
(SMARTA CD4+eGFP+ / SMARTA CD4+eGFP-)
10
8
6
4
2
0
-2
-4
-6
-8
-10
-1000
-1500
-2000
-2500
Il2
Mcm10
Cpd
Atad5
Sema4c
Eea1
Cdk1
Cxcr3
Rrm2
Tnfsf11
Utf1
Pdgfb
Bcl6
Gfi1
IL2rb
IL15ra
Ly6a
Tbx21
Gbp6
Stat5a
S1pr1
Foxp3
Gbp5
Stat5b
Gbp4
Ifit1
Cxcr4
Gimap4
Zfp36
Iigp1
Neat1
Il12rb2
Il10
Btg1
Gbp7
Nr4a1
Pydc3
Ly6c1
Tagap
Itih5
Samd9l
Ltb
Fosl2
Klf2
Pyhin1
Gbp2
Ifi203
Ifitm3
Prdm1
Egr3
Slfn5
Lars2
Gadd45g
Mxd1
Folr4
Ifit3
Ecm1

Figure S1

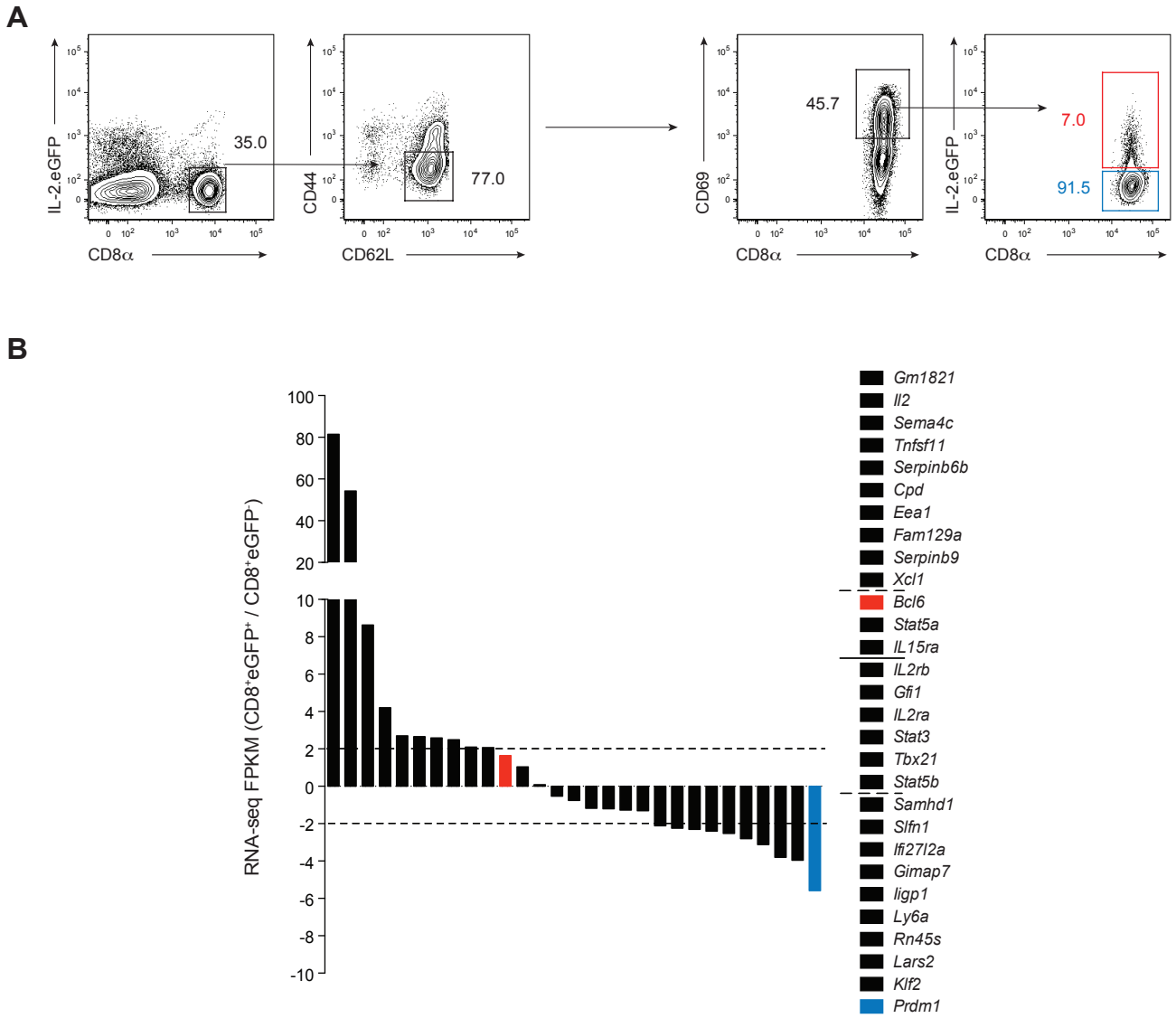
A
IL-2.eGFP
CD8α
35.0
CD44
CD62L
77.0
CD69
45.7
7.0
91.5
B
RNA-seq FPKM (CD8+eGFP+ / CD8+eGFP-)
Gm1821
Il2
Sema4c
Tnfsf11
Serpinb6b
Cpd
Eea1
Fam129a
Serpinb9
Xcl1
Bcl6
Stat5a
IL15ra
IL2rb
Gfi1
IL2ra
Stat3
Tbx21
Stat5b
Samhd1
Slfn1
Ifi27l2a
Gimap7
Iigp1
Ly6a
Rn45s
Lars2
Klf2
Prdm1

Figure S2

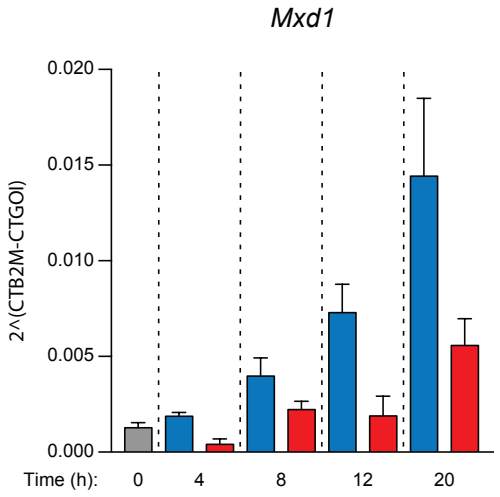


Figure S3

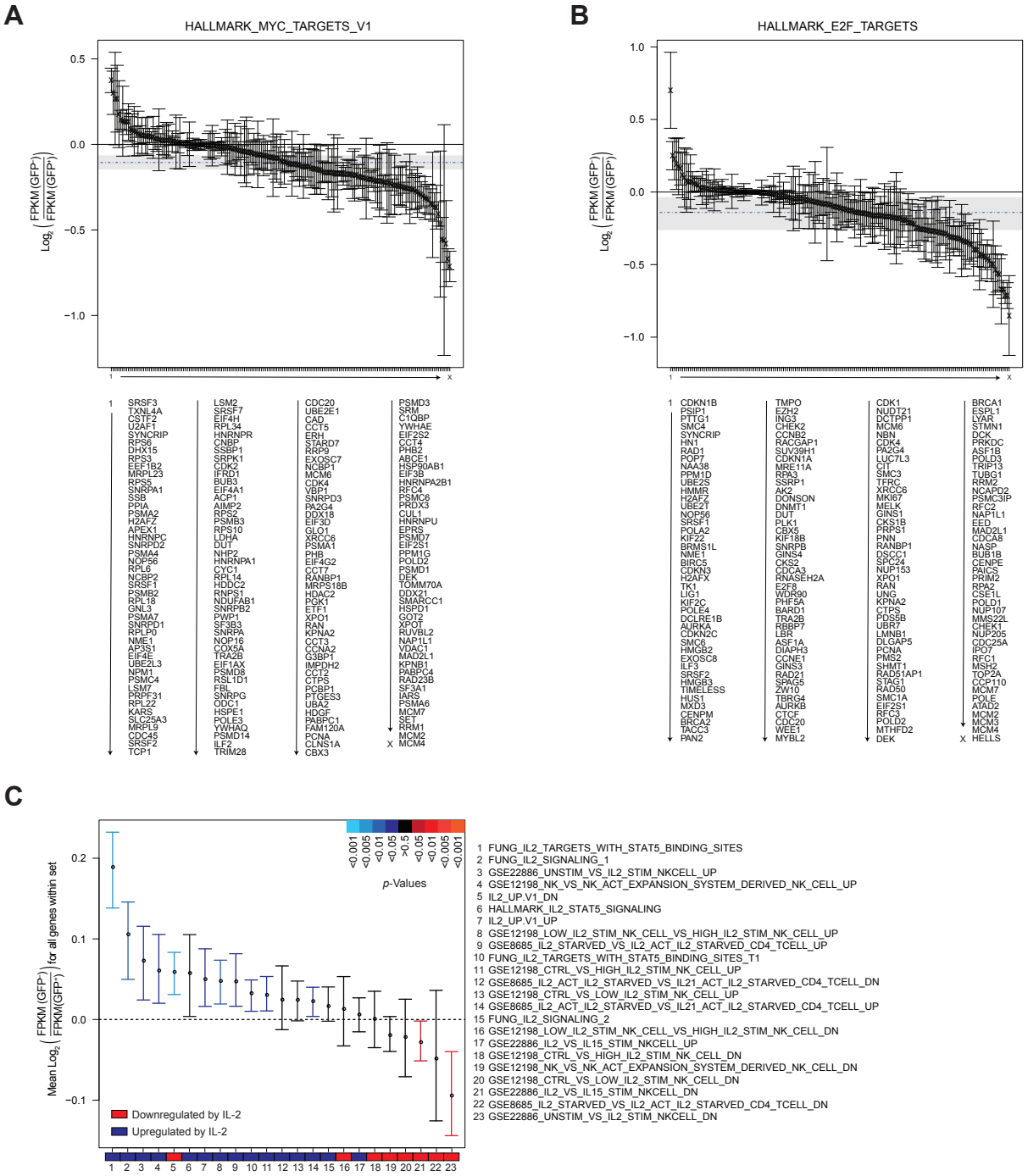
A
HALLMARK_MYC_TARGETS_V1
Log2 (FPKM (GFP+) / FPKM (GFP-))
1 SRSF3 TXNL4A CSTF2 U2AF1 SYNCRIP RPS6 DHX15 RPS3 EEF1B2 MRPL23 RPS5 SNRPA1 SSB PPIA PSMA2 HZAF2 APEX1 HNRNPC SNRPD2 PSMA4 NOP56 RPL6 NCBP2 SRSF1 PSMB2 RPL18 GNL3 PSMA7 SNRPD1 RPLP0 NME1 AP3S1 EIF4E UBE2L3 NPM1 PSMC4 LSM7 PRPF31 RPL22 KARS SLC25A3 MRPL9 CDC45 SRSF2 TCP1
LSM2 SRSF7 EIF4H RPL34 HNRNPR CNBP SSBP1 SRPK1 CDK2 IFRD1 BUB3 EIF4A1 ACP1 AIMP2 RPS2 PSMB3 RPS10 LDHA DUT NHP2 HNRNPA1 CYC1 RPL14 HDDC2 RNPS1 NDUFAB1 SNRPB2 PWP1 SF3B3 SNRPA NOP16 COX5A TRA2B EIF1AX PSMD8 RSL1D1 FBL SNRPG ODC1 HSPE1 POLE3 YWHAQ PSMD14 ILF2 TRIM28
CDC20 UBE2E1 CAD CCT5 ERH STARD7 RRP9 EXOSC7 NCBP1 MCM6 CDK4 VBP1 SNRPD3 PA2G4 DDX18 EIF3D GLO1 XRCC6 PSMA1 PHB EIF4G2 CCT7 RANBP1 MRPS18B HDAC2 PGK1 ETF1 XPO1 RAN KPNA2 CCT3 CCNA2 G3BP1 IMPDH2 CCT2 CTPS PCBP1 PTGES3 UBA2 HDGF PABPC1 FAM120A PCNA CLNS1A CBX3
PSMD3 SRM C1QBP YWHAE EIF2S2 CCT4 PHB2 ABCE1 HSP90AB1 EIF3B HNRNPA2B1 RFC4 PSMC6 PRDX3 CUL1 HNRNPU EPRS PSMD7 EIF2S1 PPM1G POLD2 PSMD1 DEK TOMM70A DDX21 SMARCC1 HSPD1 GOT2 XPOT RUVBL2 NAP1L1 VDAC1 MAD2L1 KPNB1 PABPC4 RAD23B SF3A1 IARS PSMA6 MCM7 SET RRM1 MCM2 X MCM4
B
HALLMARK_E2F_TARGETS
Log2 (FPKM (GFP+) / FPKM (GFP-))
1 CDKN1B PSIP1 PTTG1 SMC4 SYNCRIP HN1 RAD1 POP7 NAA38 PPM1D UBE2S HMMR H2AFZ UBE2T NOP56 SRSF1 POLA2 KIF22 BRMS1L NME1 BIRC5 CDKN3 H2AFX TK1 LIG1 KIF2C POLE4 DCLRE1B AURKA CDKN2C SMC6 HMGB2 EXOSC8 ILF3 SRSF2 HMGB3 TIMELESS HUS1 MXD3 CENPM BRCA2 TACC3 PAN2
TMPO EZH2 ING3 CHEK2 CCNB2 RACGAP1 SUV39H1 CDKN1A MRE11A RPA3 SSRP1 AK2 DONSON DNMT1 DUT PLK1 CBX5 KIF18B SNRPB GINS4 CKS2 CDCA3 RNASEH2A E2F8 WDR90 PHF5A BARD1 TRA2B RBBP7 LBR ASF1A DIAPH3 CCNE1 GINS3 RAD21 SPAG5 ZW10 TBRG4 AURKB CTCF CDC20 WEE1 MYBL2
CDK1 NUDT21 DCTPP1 MCM6 NBN CDK4 PA2G4 LUC7L3 CIT SMC3 TFRC XRCC6 MKI67 MELK GINS1 CKS1B PRPS1 PNN RANBP1 DSCC1 SPC24 NUP153 XPO1 RAN UNG KPNA2 CTPS PDSS5B UBR7 LMNB1 DLGAP5 PCNA PMS2 SHMT1 RAD51AP1 STAG1 RAD50 SMC1A EIF2S1 RFC3 POLD2 MTHFD2 DEK
BRCA1 ESPL1 LYAR STMN1 DCK PRKDC ASF1B POLD3 TRIP13 TUBG1 RRM2 NCAPD2 PSMC3IP RFC2 NAP1L1 EED MAD2L1 CDCA8 NASP BUB1B CENPE PAICS PRIM2 RPA2 CSE1L POLD1 NUP107 MMS22L CHEK1 NUP205 CDC25A IPO7 RFC1 MSH2 TOP2A CCP110 MCM7 POLE ATAD2 MCM2 MCM3 MCM4 X HELLS
C
Mean Log2 (FPKM (GFP+) / FPKM(GFP-)) for all genes within set
<0.001 <0.005 <0.01 <0.05 >0.5 <0.05 <0.01 <0.005 <0.001
p-Values
Downregulated by IL-2
Upregulated by IL-2
1 FUNG_IL2_TARGETS_WITH_STAT5_BINDING_SITES
2 FUNG_IL2_SIGNALING_1
3 GSE22886_UNSTIM_VS_IL2_STIM_NKCELL_UP
4 GSE12198_NK_VS_NK_ACT_EXPANSION_SYSTEM_DERIVED_NK_CELL_UP
5 IL2_UP.V1_DN
6 HALLMARK_IL2_STAT5_SIGNALING
7 IL2_UP.V1_UP
8 GSE12198_LOW_IL2_STIM_NK_CELL_VS_HIGH_IL2_STIM_NK_CELL_UP
9 GSE8685_IL2_STARVED_VS_IL2_ACT_IL2_STARVED_CD4_TCELL_UP
10 FUNG_IL2_TARGETS_WITH_STAT5_BINDING_SITES_T1
11 GSE12198_CTRL_VS_HIGH_IL2_STIM_NK_CELL_UP
12 GSE8685_IL2_ACT_IL2_STARVED_VS_IL21_ACT_IL2_STARVED_CD4_TCELL_DN
13 GSE12198_CTRL_VS_LOW_IL2_STIM_NK_CELL_UP
14 GSE8685_IL2_ACT_IL2_STARVED_VS_IL21_ACT_IL2_STARVED_CD4_TCELL_UP
15 FUNG_IL2_SIGNALING_2
16 GSE12198_LOW_IL2_STIM_NK_CELL_VS_HIGH_IL2_STIM_NK_CELL_DN
17 GSE22886_IL2_VS_IL15_STIM_NKCELL_UP
18 GSE12198_CTRL_VS_HIGH_IL2_STIM_NK_CELL_DN
19 GSE12198_NK_VS_NK_ACT_EXPANSION_SYSTEM_DERIVED_NK_CELL_DN
20 GSE12198_CTRL_VS_LOW_IL2_STIM_NK_CELL_DN
21 GSE22886_IL2_VS_IL15_STIM_NKCELL_DN
22 GSE8685_IL2_STARVED_VS_IL2_ACT_IL2_STARVED_CD4_TCELL_DN
23 GSE22886_UNSTIM_VS_IL2_STIM_NKCELL_DN
D
FUNG_IL2_TARGETS_WITH_STAT5_BINDING_SITES
Log2 (FPKM (GFP+) / FPKM (GFP-))
LTA IL2RA CCND2 LIF SOCS2 NOP16 DKC1
E
FUNG_IL2_SIGNALING_1
Log2 (FPKM (GFP+) / FPKM (GFP-))
LTA IL2RA CCND2 LIF NOP56 SOCS2 NOP16 MAT2A DKC1 BOP1

Figure S4

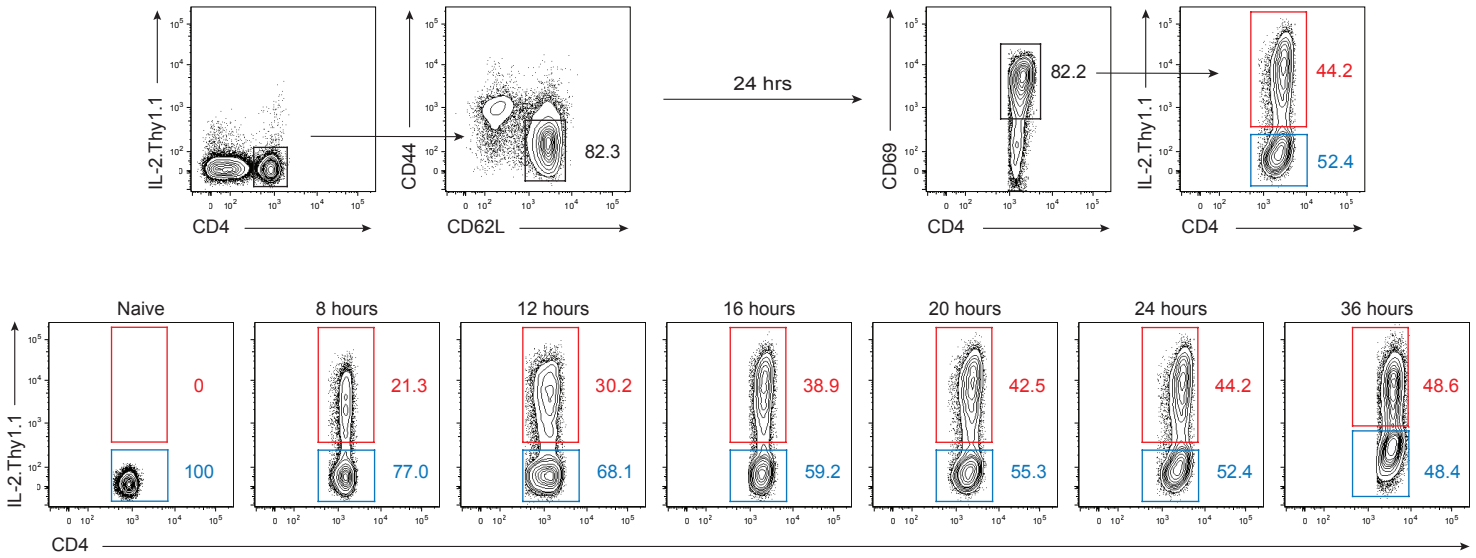
IL-2.Thy1.1
CD4
CD44
CD62L
82.3
24 hrs
CD69
82.2
44.2
52.4
Naive
8 hours
12 hours
16 hours
20 hours
24 hours
36 hours
0
100
21.3
77.0
30.2
68.1
38.9
59.2
42.5
55.3
44.2
52.4
48.6
48.4

Figure S5

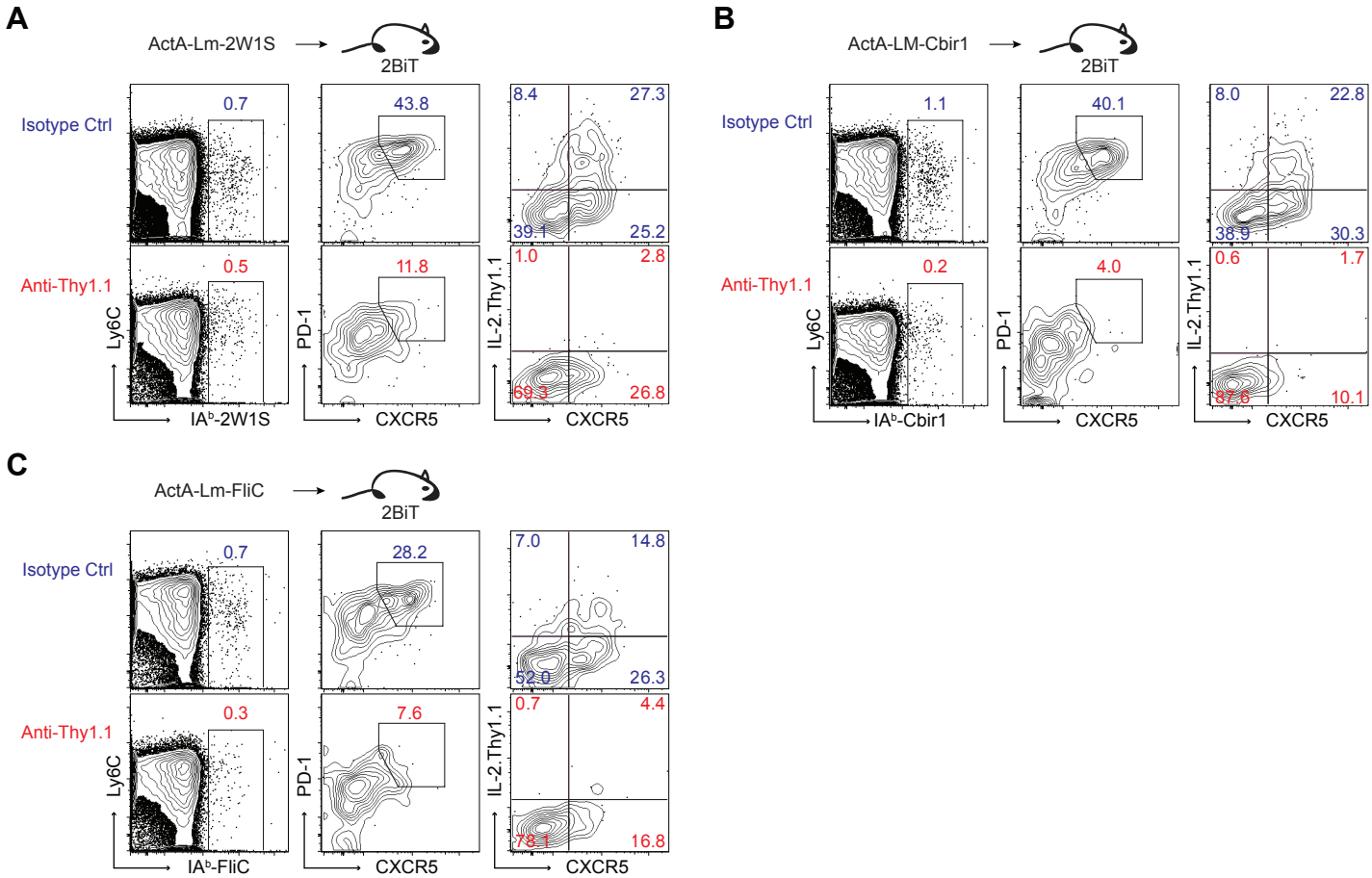
A
ActA-Lm-2W1S
2BiT
Isotype Ctrl
Anti-Thy1.1
0.7
43.8
8.4
27.3
39.1
25.2
0.5
11.8
1.0
2.8
69.3
26.8
Ly6C
IAᵇ-2W1S
PD-1
CXCR5
IL-2.Thy1.1
CXCR5
B
ActA-LM-Cbir1
2BiT
Isotype Ctrl
Anti-Thy1.1
1.1
40.1
8.0
22.8
38.9
30.3
0.2
4.0
0.6
1.7
87.6
10.1
Ly6C
IAᵇ-Cbir1
PD-1
CXCR5
IL-2.Thy1.1
CXCR5
C
ActA-Lm-FliC
2BiT
Isotype Ctrl
Anti-Thy1.1
0.7
28.2
7.0
14.8
52.0
26.3
0.3
7.6
0.7
4.4
78.1
16.8
Ly6C
IAᵇ-FliC
PD-1
CXCR5
IL-2.Thy1.1
CXCR5

Figure S6

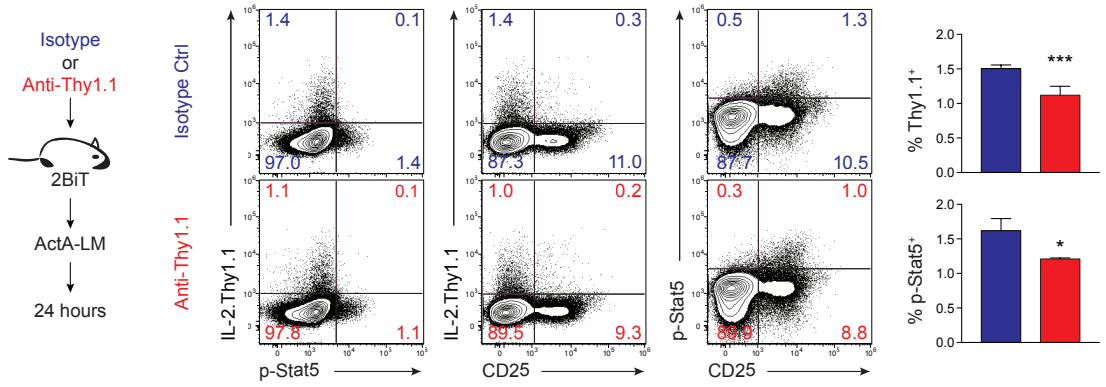
Isotype
or
Anti-Thy1.1
2BiT
ActA-LM
24 hours
Isotype Ctrl
Anti-Thy1.1
IL-2.Thy1.1
p-Stat5
CD25
p-Stat5
CD25
% Thy1.1+
% p-Stat5+
***
*

Figure S7

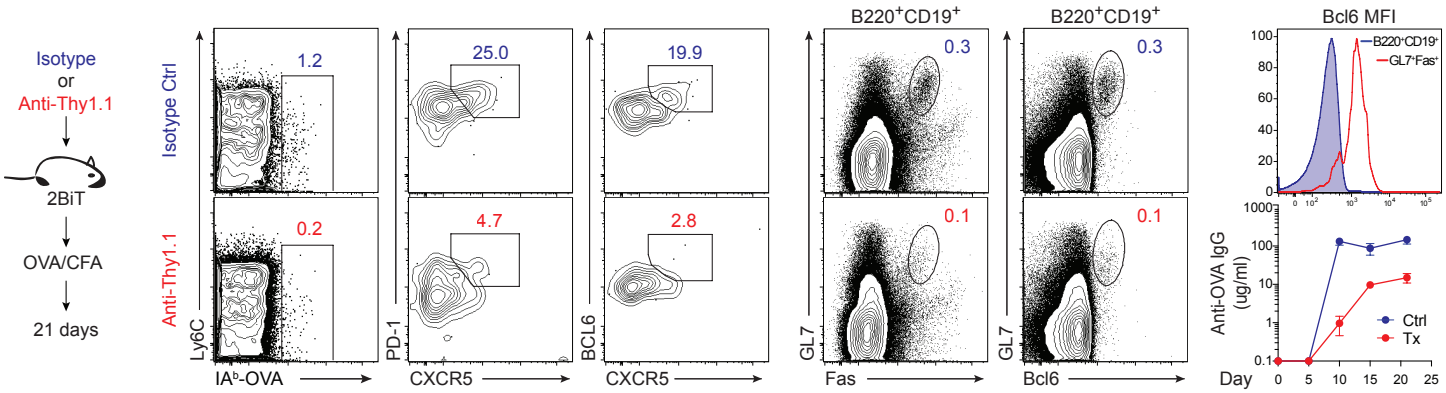
Isotype
or
Anti-Thy1.1
2BiT
OVA/CFA
21 days
Isotype Ctrl
Anti-Thy1.1
1.2
0.2
25.0
4.7
19.9
2.8
Ly6C
IAᵇ-OVA
PD-1
CXCR5
BCL6
CXCR5
B220⁺CD19⁺
0.3
0.1
GL7
Fas
B220⁺CD19⁺
0.3
0.1
GL7
Bcl6
Bcl6 MFI
B220⁺CD19⁺
GL7⁺Fas⁺
Anti-OVA IgG
(ug/ml)
Ctrl
Tx
Day

Figure S8

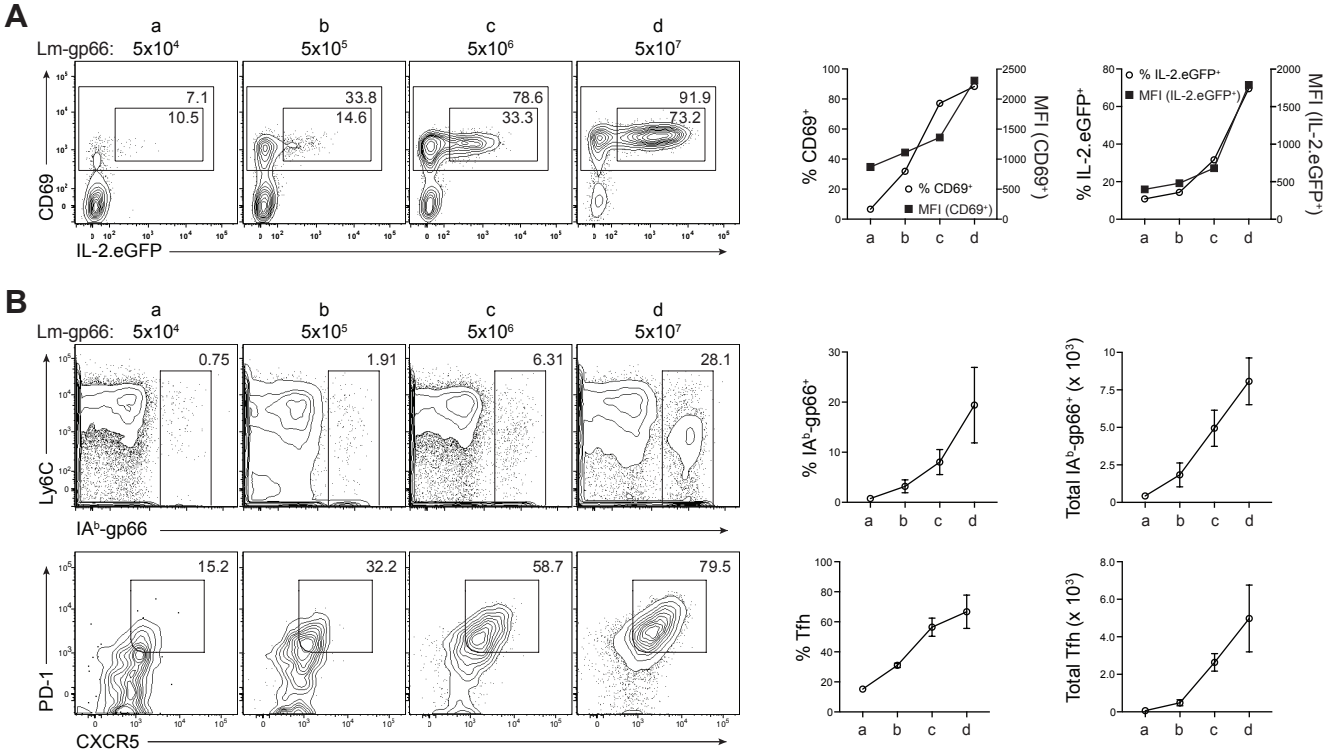
A
a
b
c
d
Lm-gp66:
5x10⁴
5x10⁵
5x10⁶
5x10⁷
7.1
10.5
33.8
14.6
78.6
33.3
91.9
73.2
CD69
IL-2.eGFP
% CD69⁺
MFI (CD69⁺)
% IL-2.eGFP⁺
MFI (IL-2.eGFP⁺)
B
0.75
1.91
6.31
28.1
Ly6C
IAᵇ-gp66
% IAᵇ-gp66⁺
Total IAᵇ-gp66⁺ (x 10³)
15.2
32.2
58.7
79.5
PD-1
CXCR5
% Tfh
Total Tfh (x 10³)

Figure S9

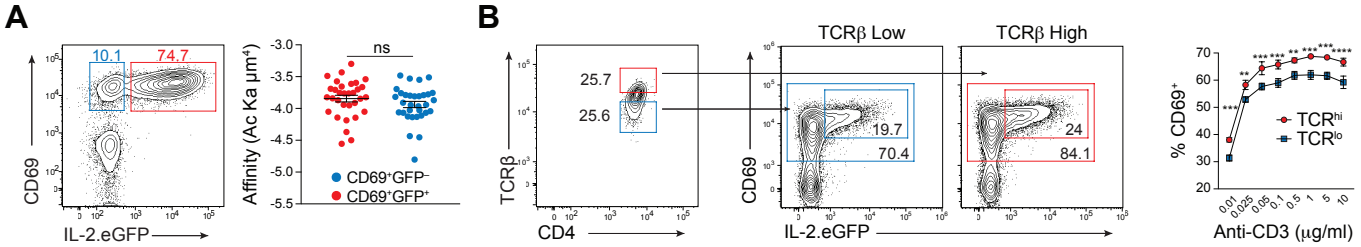
A
10.1
74.7
CD69
IL-2.eGFP
Affinity (Ac Ka μm⁴)
ns
CD69⁺GFP⁻
CD69⁺GFP⁺
B
25.7
25.6
TCRβ
CD4
TCRβ Low
TCRβ High
19.7
70.4
24
84.1
CD69
IL-2.eGFP
% CD69⁺
TCRhi
TCRlo
Anti-CD3 (μg/ml)

Figure S10

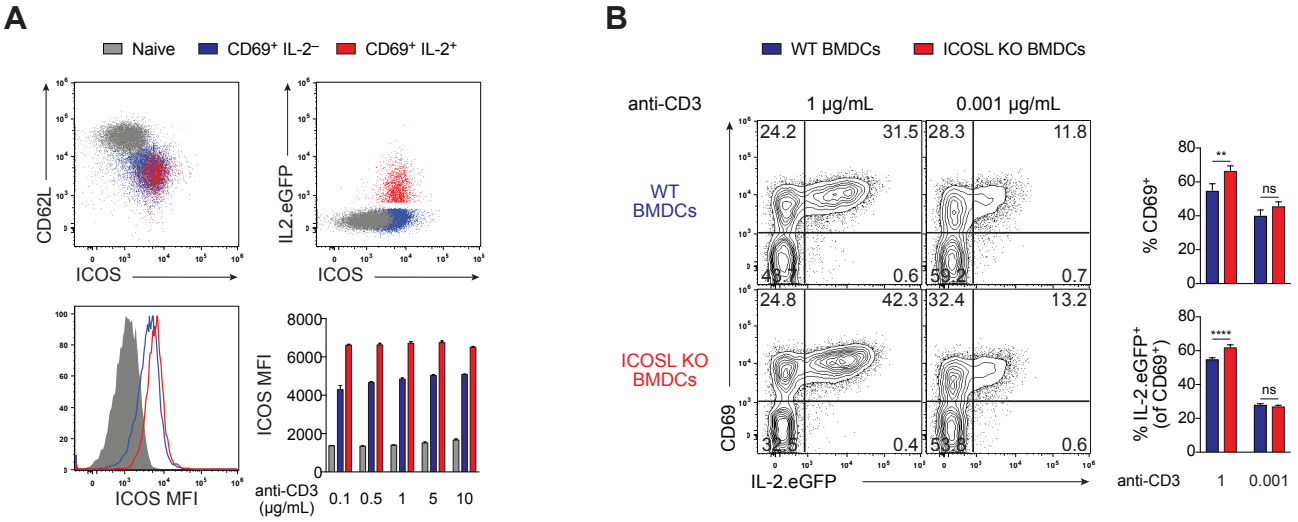
A
Naive
CD69+ IL-2−
CD69+ IL-2+
CD62L
ICOS
IL2.eGFP
ICOS
ICOS MFI
ICOS MFI
anti-CD3 (μg/mL)
0.1 0.5 1 5 10
B
WT BMDCs
ICOSL KO BMDCs
anti-CD3
1 μg/mL
0.001 μg/mL
WT BMDCs
ICOSL KO BMDCs
24.2
31.5
43.7
0.6
28.3
11.8
59.2
0.7
24.8
42.3
32.5
0.4
32.4
13.2
53.6
0.6
CD69
IL-2.eGFP
% CD69+
% IL-2.eGFP+ (of CD69+)
**
ns
****
ns
anti-CD3
1
0.001

Figure S11

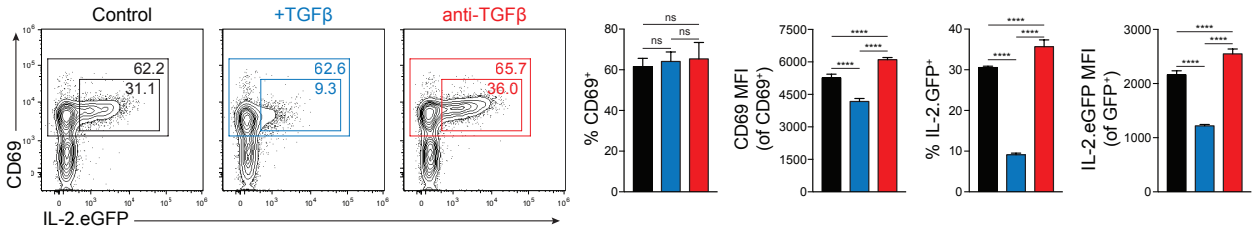
Control
+TGFβ
anti-TGFβ
62.2
31.1
62.6
9.3
65.7
36.0
CD69
IL-2.eGFP
% CD69+
CD69 MFI (of CD69+)
% IL-2.GFP+
IL-2.eGFP MFI (of GFP+)
ns
****

Figure S12

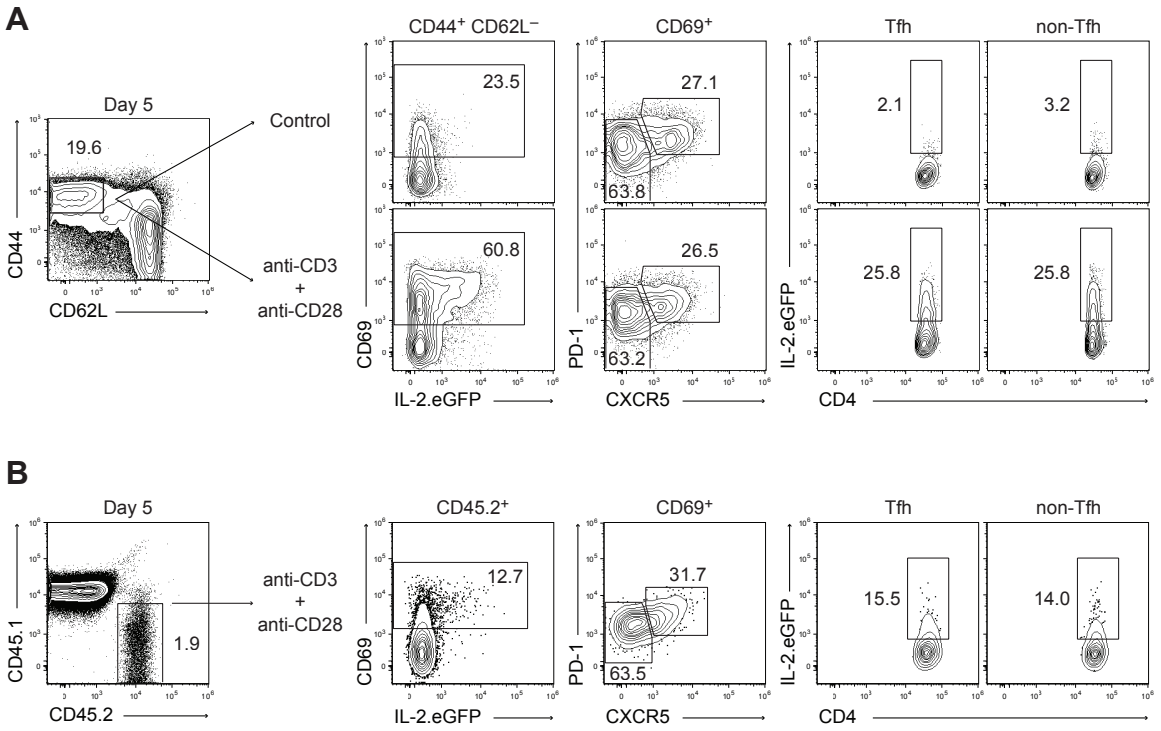
A
Day 5
19.6
CD44
CD62L
Control
anti-CD3
+
anti-CD28
CD44+ CD62L−
23.5
60.8
CD69
IL-2.eGFP
CD69+
27.1
63.8
26.5
63.2
PD-1
CXCR5
Tfh
non-Tfh
2.1
3.2
25.8
25.8
IL-2.eGFP
CD4
B
Day 5
CD45.1
CD45.2
1.9
anti-CD3
+
anti-CD28
CD45.2+
12.7
CD69
IL-2.eGFP
CD69+
31.7
63.5
PD-1
CXCR5
Tfh
non-Tfh
15.5
14.0
IL-2.eGFP
CD4

Figure S13