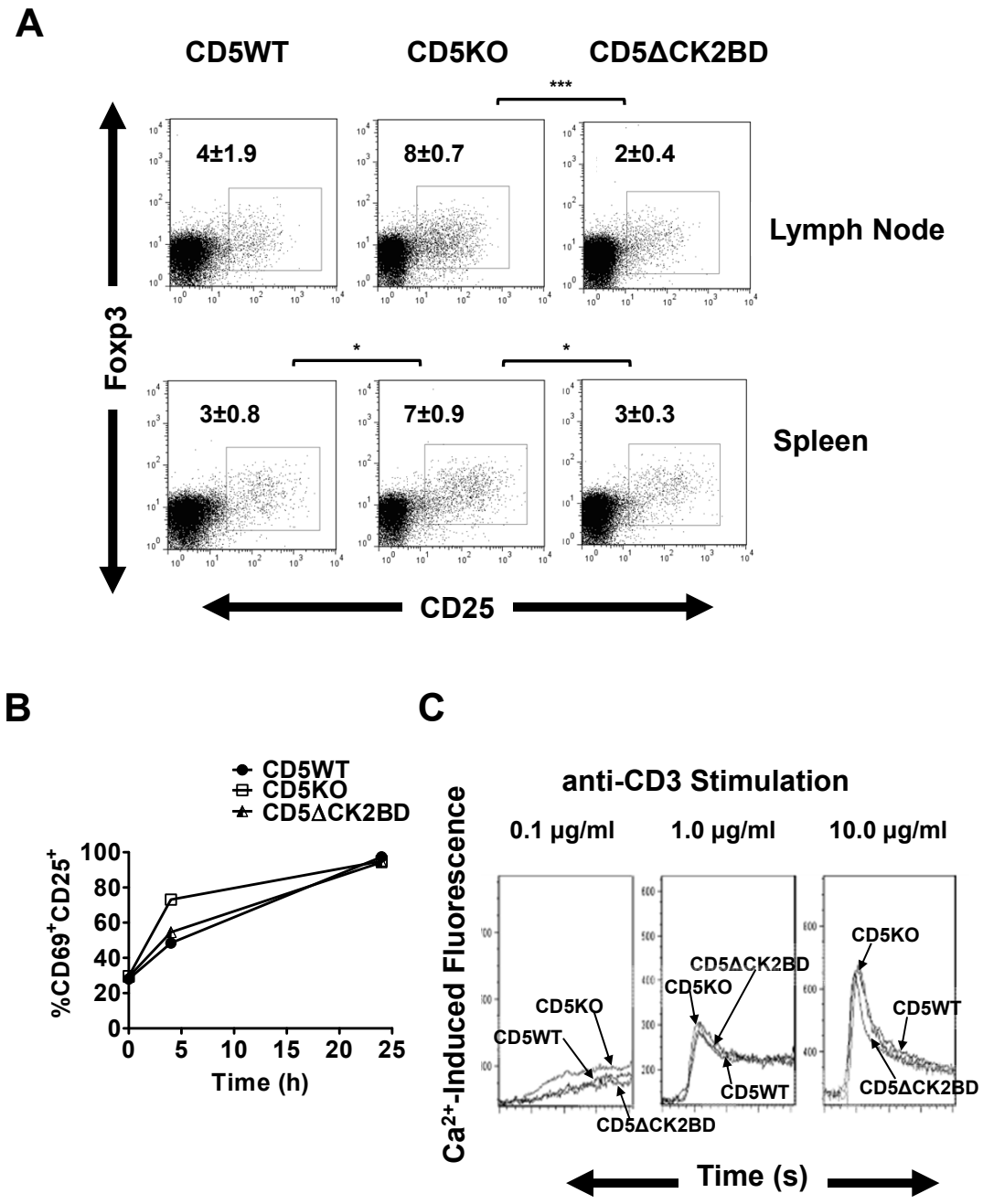
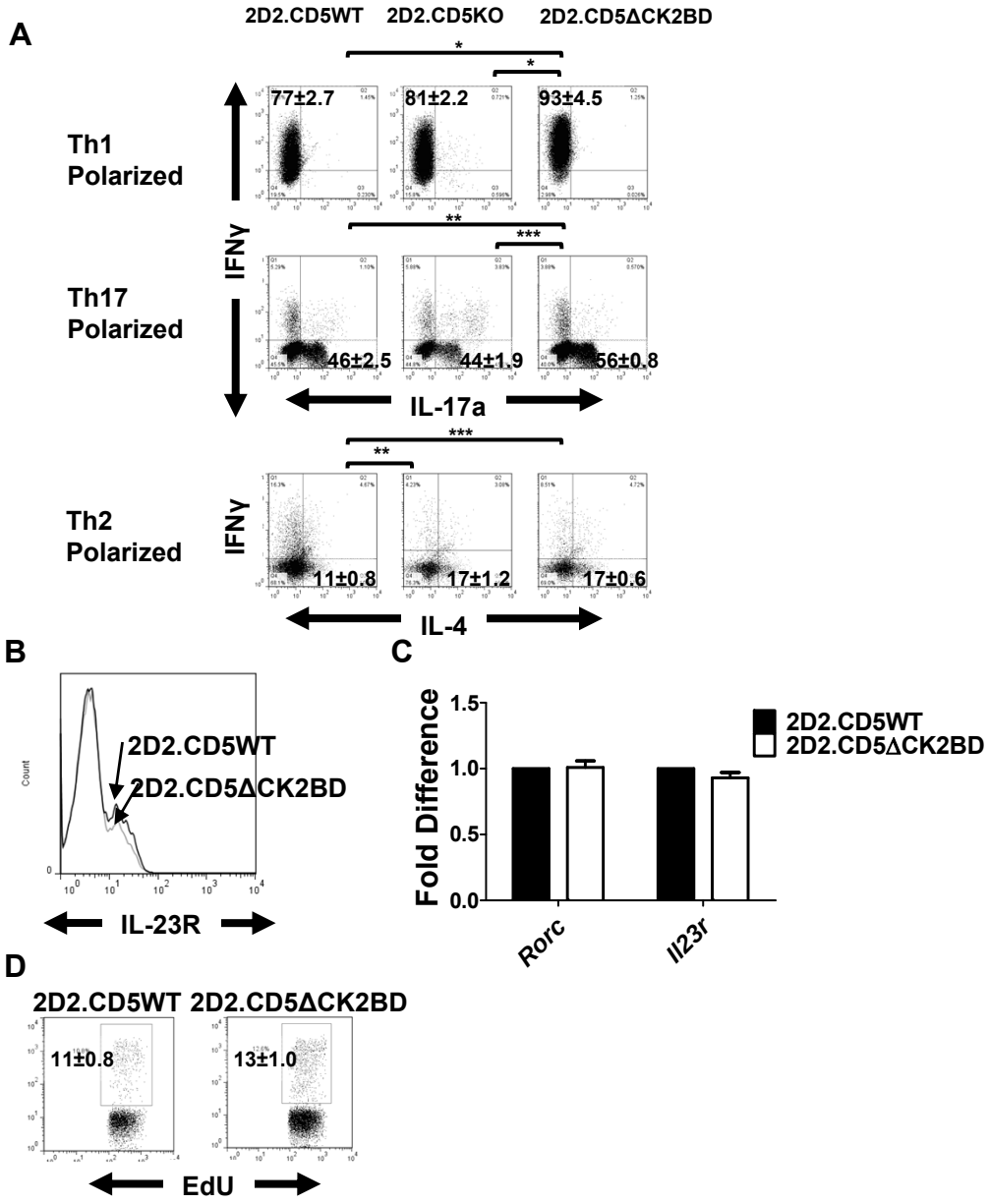
SUPPLEMENTAL FIGURE 1. *A.* Treg populations in lymph nodes and spleens of CD5WT, CD5KO or CD5ΔCK2BD mice. Data±SEM represent the averages of 3-5 mice/group**.** **p*<0.05, ****p*<0.001 with Student *t* test. *B, C*, CD5-CK2 signaling does not affect parameters of early T-cell activation. *B,* Measurement of CD25 and CD69 upregulation on $CD4^+$ T-cell development following 0-24h stimulation with anti-CD3. Data are from one mouse per group, *n*=2 experiments with 2-3 mice per experiment. *C*, Calcium mobilization in $CD4^+$ T-cells following stimulation with anti-CD3. Data are from one representative mouse per group from a total of 2-3 mice/experiment/group, *n*= 2 experiments.

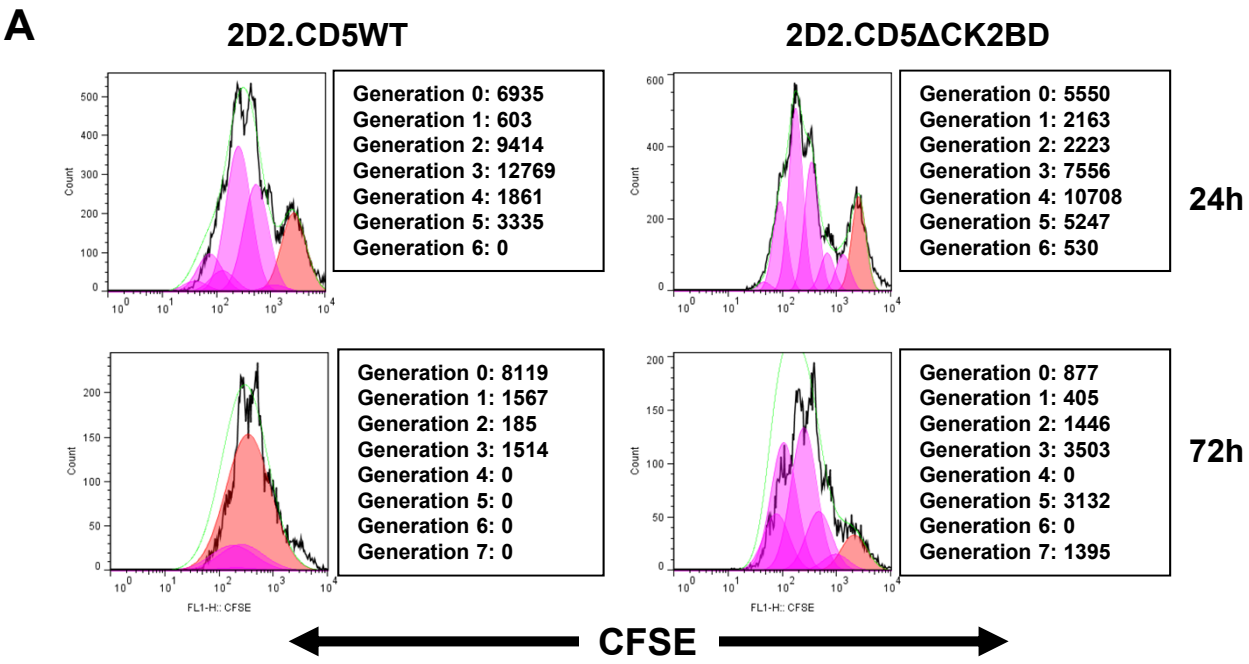
SUPPLEMENTAL FIGURE 2. *A*, Th1, Th17 and Th2 cell differentiation from naïve $CD4^+$ T-cells obtained from 2D2.CD5WT, 2D2.CD5KO or 2D2.CD5ΔCK2BD mice. Sorted $CD4^+$ T cells were co-cultured with irradiated APCs and stimulated with anti-CD3 under Th1-, Th17-, or Th2- polarizing conditions for 5 days (*A)*. Numbers in each quadrant represent the average frequency of $CD4^+$ T-cells expressing IFNγ, IL-17a and/or IL-4±SEM from four independent experiments performed with at least three mice per experiment. *B*, IL-23R expression on $CD4^+$ T-cells on day 5 after stimulation with 10 μg/ml MOG_{35-55} peptide under Th17 polarizing conditions. *C*, *Rorc* and *Il23r* RNA expression in 2D2.CD5WT and 2D2.CD5ΔCK2BD $CD4^+$ T-cells measured on day 5 of stimulation with MOG_{35-55} peptide under Th17 polarizing conditions. The fold difference was determining by normalizing to the expression of *Gapdh*. *D,* EdU incorporation determined on day 5 in Th17 polarizing cultures. Data are representative of three independent experiments performed with at least two mice per experiment. *p<0.05, **p<0.01, ***p<0.001 with Student *t* test.

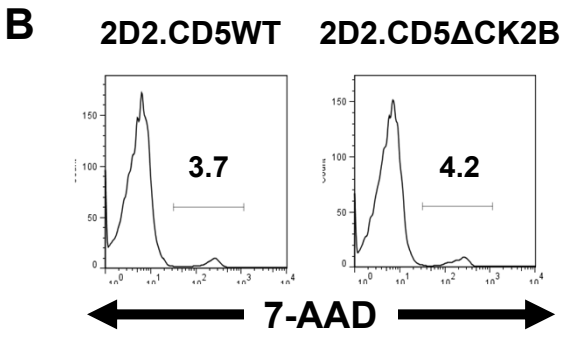
SUPPLEMENTAL FIGURE 3. T-cell proliferation and cell death following restimulation in co-cultures of previously stimulated CD4+ T-cells from 2D2.CD5WT and 2D2.ΔCK2BD mice. *A*, 2D2.CD5WT $CD4^+$ T-cells and 2D2.CD4ΔCK2 $CD4^+$ T-cells were stimulated for 24h with MOG_{35-55} peptide and rested for 3d separately. On day 3, dead cells were removed by Ficoll-Paque density gradient centrifugation and co-cultured in the presence of 10 μg/ml MOG_{35-55} after labeling one of the populations with CFSE. The dilution of CFSE was determined by flow cytometry at the indicated times and analyzed with FlowJo software *B*, 7-AAD incorporation in cells from above following 24h restimulation. *C.* 7-AAD incorporation in T-cells primed in vivo and restimulated in vitro with MOG_{35-55} peptide. Data are from one representative mouse per group from a total of 2-3 mice/experiment/group, *n*= 2 experiments.

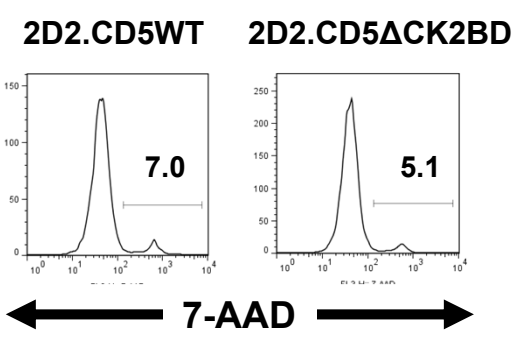
SUPPLEMENTAL FIGURE 4. The CD5-CK2 signaling pathway regulates cognate peptide or anti-CD3 mAb stimulated cytokine production from $CD4^+$ T-cells. *A-E,* Naïve 2D2.CD5WT, 2D2.CD5KO, and 2D2.CD5ΔCK2BD $CD4^+$ T-cells were co-cultured with irradiated APCs and stimulated with 10 μg/ml MOG_{35-55} peptide (*vs.* 100 μg/ml MOG_{35-55} in Figure 6) for 1-5 d. *F-J,* Naïve CD5WT, CD5KO, and CD5ΔCK2BD $CD4^+$ T-cells were co-cultured with irradiated APCs and stimulated with 1 μg/ml anti-CD3 for 1-5 d. Supernates were collected on days 1, 2, 3, and 5 to quantify secreted levels of IFNγ (*A* and *F*), IL-17a (*B* and *G*), IL-6 (*C* and *H*), IL-2 (*D* and *I*), and IL-10 (*E* and *J*) by ELISA. Data ± SEM represent values 2-3 independent mice/group/ experiment, *n*=4 experiments.

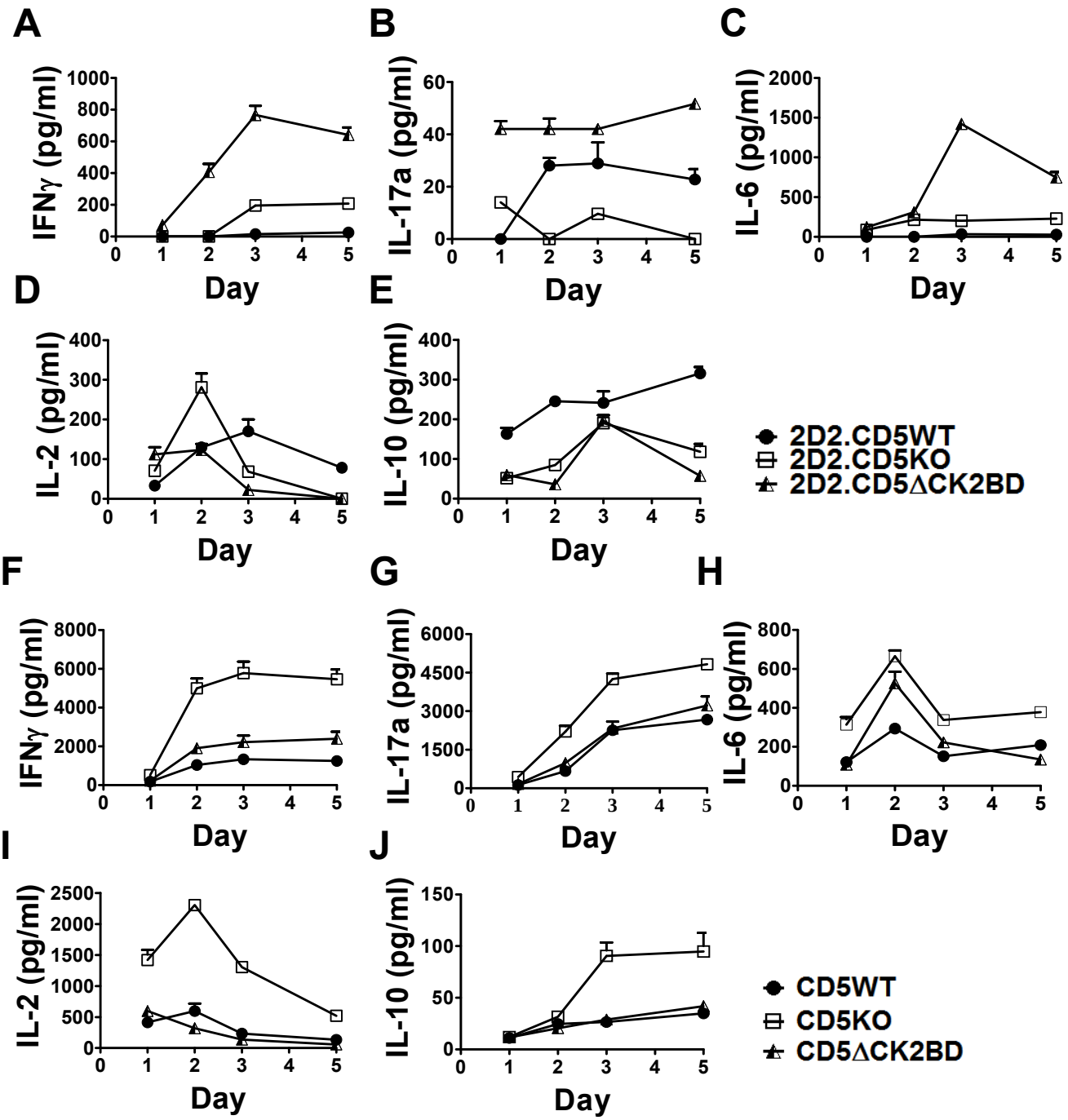

A
CD5WT
CD5KO
CD5ΔCK2BD
***
4±1.9
8±0.7
2±0.4
Lymph Node
*
*
3±0.8
7±0.9
3±0.3
Spleen
Foxp3
CD25
B
CD5WT
CD5KO
CD5ΔCK2BD
%CD69+CD25+
Time (h)
C
anti-CD3 Stimulation
0.1 μg/ml
1.0 μg/ml
10.0 μg/ml
Ca2+-Induced Fluorescence
CD5KO
CD5WT
CD5ΔCK2BD
Time (s)


A
2D2.CD5WT
2D2.CD5KO
2D2.CD5ΔCK2BD
Th1 Polarized
77±2.7
81±2.2
93±4.5
IFNγ
Th17 Polarized
46±2.5
44±1.9
56±0.8
IL-17a
Th2 Polarized
11±0.8
17±1.2
17±0.6
IL-4
B
2D2.CD5WT
2D2.CD5ΔCK2BD
IL-23R
C
Fold Difference
1.5
1.0
0.5
0.0
Rorc
Il23r
D
2D2.CD5WT
2D2.CD5ΔCK2BD
11±0.8
13±1.0
EdU


A
2D2.CD5WT
2D2.CD5ΔCK2BD
Generation 0: 6935
Generation 1: 603
Generation 2: 9414
Generation 3: 12769
Generation 4: 1861
Generation 5: 3335
Generation 6: 0
Generation 0: 5550
Generation 1: 2163
Generation 2: 2223
Generation 3: 7556
Generation 4: 10708
Generation 5: 5247
Generation 6: 530
24h
Generation 0: 8119
Generation 1: 1567
Generation 2: 185
Generation 3: 1514
Generation 4: 0
Generation 5: 0
Generation 6: 0
Generation 7: 0
Generation 0: 877
Generation 1: 405
Generation 2: 1446
Generation 3: 3503
Generation 4: 0
Generation 5: 3132
Generation 6: 0
Generation 7: 1395
72h
Count
FL1-H:: CFSE
CFSE


B
2D2.CD5WT
2D2.CD5ΔCK2BD
3.7
4.2
7-AAD


C
2D2.CD5WT
2D2.CD5ΔCK2BD
7.0
5.1
7-AAD


A
IFNγ (pg/ml)
1000
800
600
400
200
0
0 1 2 3 4 5
Day
B
IL-17a (pg/ml)
60
40
20
0
0 1 2 3 4 5
Day
C
IL-6 (pg/ml)
2000
1500
1000
500
0
0 1 2 3 4 5
Day
D
IL-2 (pg/ml)
400
300
200
100
0
0 1 2 3 4 5
Day
E
IL-10 (pg/ml)
400
300
200
100
0
0 1 2 3 4 5
Day
2D2.CD5WT
2D2.CD5KO
2D2.CD5ΔCK2BD
F
IFNγ (pg/ml)
8000
6000
4000
2000
0
0 1 2 3 4 5
Day
G
IL-17a (pg/ml)
6000
4500
3000
1500
0
0 1 2 3 4 5
Day
H
IL-6 (pg/ml)
800
600
400
200
0
0 1 2 3 4 5
Day
I
IL-2 (pg/ml)
2500
2000
1500
1000
500
0
0 1 2 3 4 5
Day
J
IL-10 (pg/ml)
150
100
50
0
0 1 2 3 4 5
Day
CD5WT
CD5KO
CD5ΔCK2BD