Online supplemental material

S1 Figure Legends

FIGURE S1. GSK3 inhibitors did not affect survival and proliferation of $CD4^+$ T cells. *A-B*, $CD4^+$ T cells were polarized toward Th17 cells for 5 days cultured on irradiated lymph node cells and splenocytes in medium supplemented with 20 ng/mL IL-6 and 5 ng/mL TGFβ, with the indicated GSK3 inhibitors 10 mM LiCl, 1 μM CHIR99021, 1 μM TDZD-8, or 10 μM SB415286. Cells were washed and stimulated for 4 hr with PMA/ionomycin and $CD4^+$ T cells were analyzed for live-dead staining by flow cytometry. *C*, $CD4^+$ T cells were polarized toward Th17 cells for 5 days cultured on anti-CD3/CD28 beads in medium supplemented with 20 ng/mL IL-6 and 5 ng/mL TGFβ, with the indicated GSK3 inhibitors 10 mM LiCl, 1 μM CHIR99021, 1 μM TDZD-8, or 10 μM SB415286. Cells were washed and stimulated for 4 hr with PMA/ionomycin and $CD4^+$ T cells were analyzed by TUNNEL staining by flow cytometry. *D*, $CD4^+$ T cells were cultured on anti-CD3/CD28 beads for 5 days with the indicated GSK3 inhibitors 10 mM LiCl, 1 μM CHIR99021, 1 μM TDZD-8, or 10 μM SB415286. Cells were washed and stimulated for 4 hr with PMA/ionomycin and $CD4^+$ T cells were analyzed by TUNNEL staining by flow cytometry. *E*, $CD4^+$ T cells were polarized toward Th17 cells for 5 days cultured on irradiated lymph node cells and splenocytes in medium supplemented with 20 ng/mL IL-6 and 5 ng/mL TGFβ, with the indicated GSK3 inhibitors 10 mM LiCl, 1 μM CHIR99021, 1 μM TDZD-8, or 10 μM SB415286. Cells were washed and stimulated for 4 hr with PMA/ionomycin and $CD4^+$ T cells were analyzed for CFSE staining by flow cytometry gated on IL-$17A^+$ $CD4^+$ T cells. *F*, $CD4^+$ T cells were cultured for 5 days on irradiated lymph node cells and splenocytes with the indicated GSK3 inhibitors 10 mM LiCl, 1 μM CHIR99021, 1 μM TDZD-8, or 10 μM SB415286. Cells were washed and stimulated for 4 hr with PMA/ionomycin and $CD4^+$ T cells were analyzed for CFSE staining by flow cytometry. *G*, GSK3α and GSK3β were knocked down by siRNA with a double-stranded

RNA oligomer labeled with Alexa-Fluor-555 in $CD4^+$ T cells for 48 h, cells were differentiated on feeder cells for 5 days, and cells were restimulated with ionomycin/PMA for 4 h and analyzed by flow cytometry to evaluate the percentage of siRNA-transfected cells.

FIGURE S2. GSK3 inhibitors reduce IL-17A-producing $CD4^+$ T cells. *A-B*, $CD4^+$ T cells were polarized toward Th17 cells for 5 days (*A*) with varying concentrations of CHIR99021, or (*B*) with 10 mM lithium added on different days of culture. T cells were recovered and stimulated with PMA/ionomycin for 4 h and IL-17A in the media was measured by ELISA. n=3, *$p<0.05$. *C*, $CD4^+$ T cells were polarized towards Th17 for 5 days, cells were restimulated with ionomycin/PMA/BFA with or without 10 mM LiCl or 1 μM CHIR99021 for 4 h, and IL-17A-producing $CD4^+$ T cells were analyzed by flow cytometry (n=2). *D*, $CD4^+$ T cells were polarized towards Th17 cells during culture with anti-CD3/anti-CD28 beads for 5 days with GSK3 inhibitors, followed by measurement of IL-17A-producing $CD4^+$ T cells by flow cytometry (n=3). *E*, mRNA levels of GSK3β and IL-17A were measured by qRT-PCR after 4 days of polarization towards Th17, with or without 1 μM CHIR99021. F, $CD4^+$ T cells from wild-type and $ROR\gamma T^{+/-}$ mice were polarized toward Th17 cells for 5 days and analyzed on different days of culture for their content in $IL\text{-}17A^+$ $CD4^+$ T cells. T cells were recovered and stimulated with PMA/ionomycin for 4 h and $IL\text{-}17A^+$ $CD4^+$ T cells were analyzed by flow cytometry (n=3), Insert: RORγT was immunoblotted in $CD4^+$ T cells from wild-type (WT) and $ROR\gamma T^{+/-}$ mice after 3 days of polarization, or not, towards Th17 cells. Blots were reblotted for the loading control β-actin. *G*, $CD4^+$ T cells isolated from GSK3 knockin mice were polarized toward Th17 cells for 5 days cultured on irradiated lymph node cells and splenocytes in medium supplemented with 20 ng/mL IL-6 and 5 ng/mL TGFβ with or without 10 μM SB415286. Cells were washed and stimulated for 4 hr with PMA/ionomycin/BFA and IL-17A-producing $CD4^+$ T cells were analyzed by flow cytometry.

FIGURE S3. GSK3 inhibitors reduce responses to IL-6, IL-23, and IL-21. *A*, Chromatin immunoprecipitation of STAT3 on the promoter of *IL-17A* in response to 30 min treatment with 20 ng/mL IL-6 with or without 10 mM LiCl. Q-PCR was applied to quantify the ChIP and input DNA. Values were normalized to input values (*$p<0.05$, n=3). *B-C*, $CD4^+$ T cells were polarized towards Th17 cells for 5 days cultured on irradiated lymph node cells and splenocytes in medium supplemented with (*B*) 10 ng/mL IL-23, 20 ng/mL IL-6 and 0.25 ng/mL TGFβ or (*C*) 75 ng/mL IL-21 and 5 ng/mL TGFβ with the GSK3 inhibitors 1 μM CHIR99021 or 1 μM TDZD-8 (n=5). IL-17A-producing $CD4^+$ T cells were analyzed after 4 h restimulation with PMA/ionomycin/BFA by flow cytometry (n=2-3). *D-E*, $CD4^+$ T cells were pretreated for 2 h with 10 mM LiCl (Li), 1 μM CHIR99021 (CH), or 1 μM TDZD-8 (TDZD) and then stimulated for 30 min with (*D*) 10 ng/mL IL-23 or (*E*) 75 ng/mL IL-21, and cell lysates were immunoblotted for phospho-Tyr705-STAT3, STAT3 and β-actin.

FIGURE S4. Effects of lithium treatment after Ft LVS or EAE. *A*, The percents of $CD4^+$ T cells, $CD8^+$ T cells, $CD11c^+$ dendritic cells and activated macrophages ($CD45^{high}$ $F4/80^+$) were determined in spleen cell preparations from lithium-treated or non-treated mice, 6 days after infection with Ft LVS. *B*, The percents of spleen IL-17A-producing, IFNγ-producing or FoxP3-expressing $CD4^+$ T cells were analyzed by flow cytometry after restimulation with PMA/ionomycin/BFA for 4 h or no restimulation (FoxP3). *C,* Spinal cord lysates were immunoblotted for phospho-Tyr705-STAT3, phospho-Tyr701-STAT1, total STAT3, STAT1, and phospho-Ser9-GSK3β (n=2).

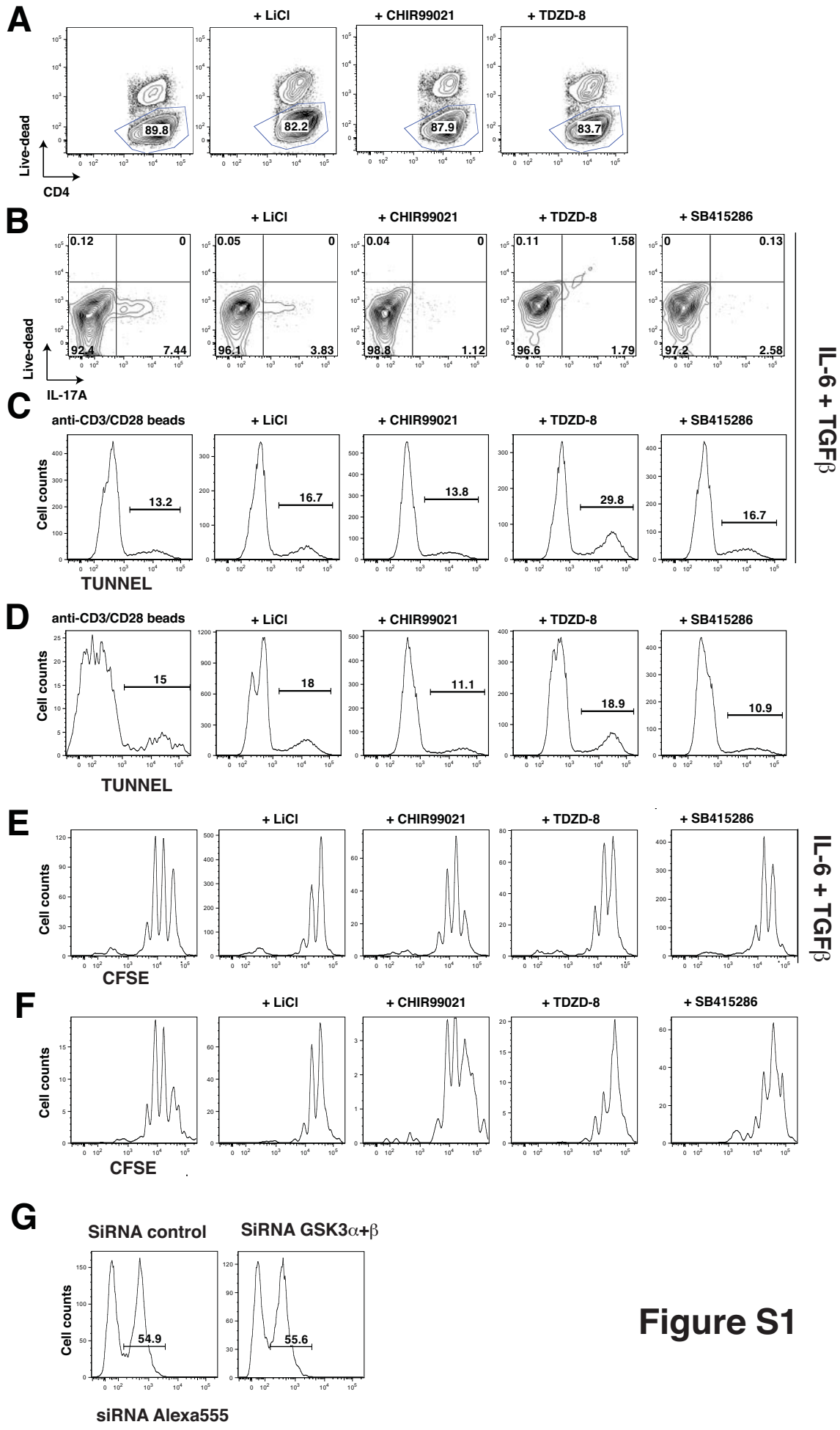

A
+ LiCl
+ CHIR99021
+ TDZD-8
Live-dead
CD4
89.8
82.2
87.9
83.7
B
+ LiCl
+ CHIR99021
+ TDZD-8
+ SB415286
Live-dead
IL-17A
0.12
0
92.4
7.44
0.05
0
96.1
3.83
0.04
0
98.8
1.12
0.11
1.58
96.6
1.79
0
0.13
97.2
2.58
IL-6 + TGFβ
C
anti-CD3/CD28 beads
+ LiCl
+ CHIR99021
+ TDZD-8
+ SB415286
Cell counts
13.2
16.7
13.8
29.8
16.7
TUNNEL
D
anti-CD3/CD28 beads
+ LiCl
+ CHIR99021
+ TDZD-8
+ SB415286
Cell counts
15
18
11.1
18.9
10.9
TUNNEL
E
+ LiCl
+ CHIR99021
+ TDZD-8
+ SB415286
Cell counts
CFSE
IL-6 + TGFβ
F
+ LiCl
+ CHIR99021
+ TDZD-8
+ SB415286
Cell counts
CFSE
G
SiRNA control
SiRNA GSK3α+β
Cell counts
54.9
55.6
siRNA Alexa555

Figure S1

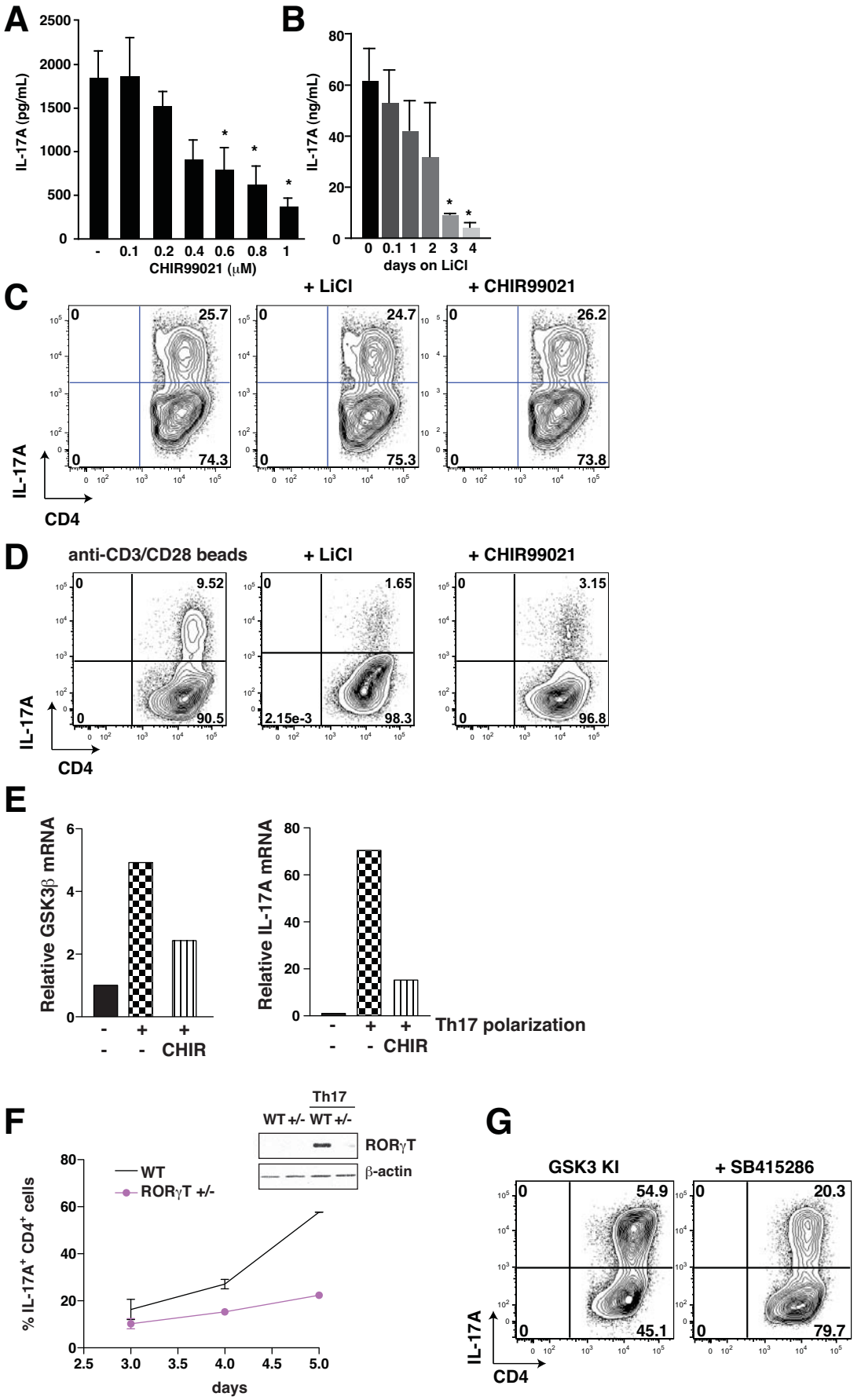

A
IL-17A (pg/mL)
2500
2000
1500
1000
500
0
-
0.1
0.2
0.4
0.6
0.8
1
CHIR99021 (μM)
B
IL-17A (ng/mL)
80
60
40
20
0
0
0.1
1
2
3
4
days on LiCl
C
+ LiCl
+ CHIR99021
25.7
74.3
24.7
75.3
26.2
73.8
IL-17A
CD4
D
anti-CD3/CD28 beads
+ LiCl
+ CHIR99021
9.52
90.5
1.65
2.15e-3
98.3
3.15
96.8
IL-17A
CD4
E
Relative GSK3β mRNA
Relative IL-17A mRNA
CHIR
Th17 polarization
F
Th17
WT +/- WT +/-
RORγT
β-actin
WT
RORγT +/-
% IL-17A+ CD4+ cells
days
G
GSK3 KI
+ SB415286
54.9
45.1
20.3
79.7
IL-17A
CD4

Figure S2

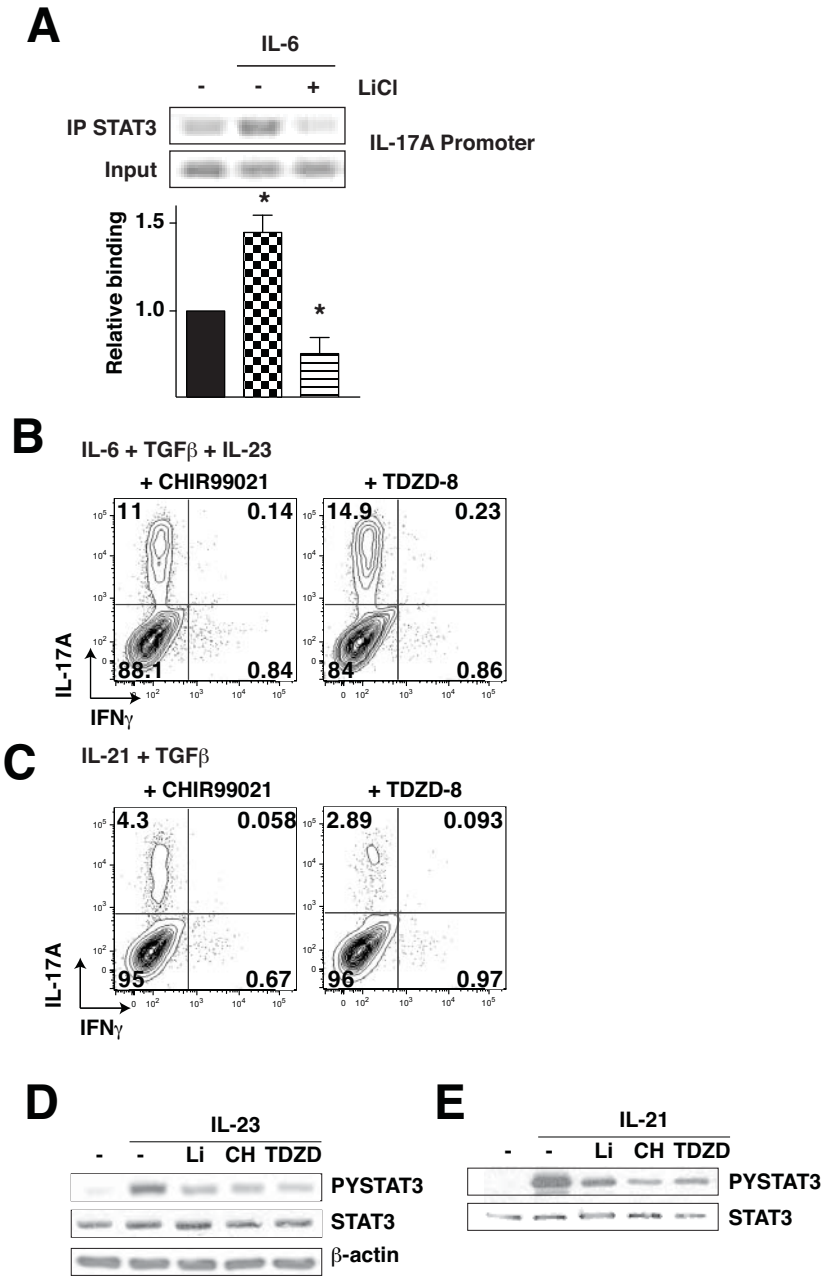
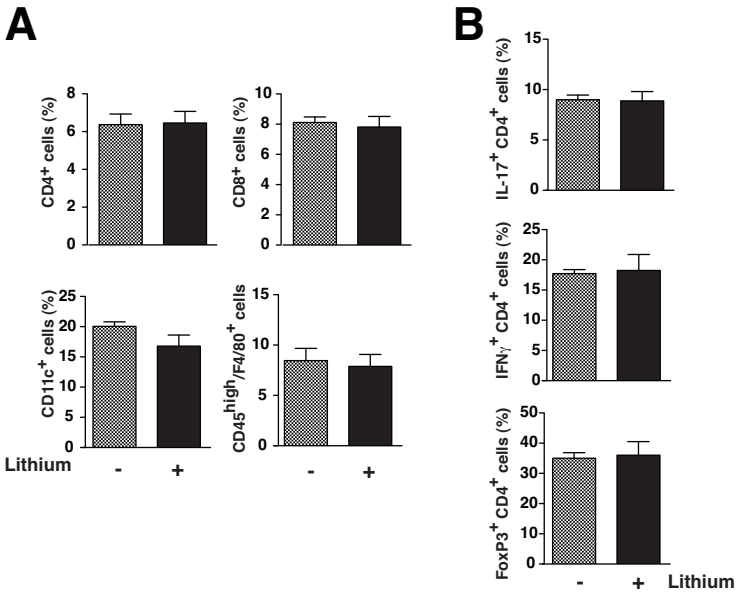

A
IL-6
- - + LiCl
IP STAT3
Input
IL-17A Promoter
Relative binding
1.5
1.0
*
*
B
IL-6 + TGFβ + IL-23
+ CHIR99021
+ TDZD-8
11
0.14
88.1
0.84
14.9
0.23
84
0.86
IL-17A
IFNγ
C
IL-21 + TGFβ
+ CHIR99021
+ TDZD-8
4.3
0.058
95
0.67
2.89
0.093
96
0.97
IL-17A
IFNγ
D
IL-23
- - Li CH TDZD
PYSTAT3
STAT3
β-actin
E
IL-21
- - Li CH TDZD
PYSTAT3
STAT3

Figure S3


A
CD4+ cells (%)
CD8+ cells (%)
CD11c+ cells (%)
CD45high/F4/80+ cells
Lithium
-
+
B
IL-17+ CD4+ cells (%)
IFNγ+ CD4+ cells (%)
FoxP3+ CD4+ cells (%)
Lithium

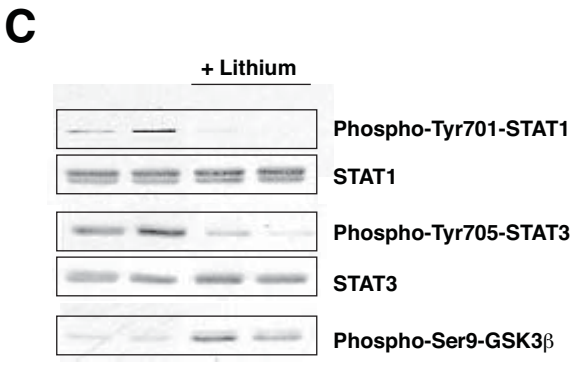

C
+ Lithium
Phospho-Tyr701-STAT1
STAT1
Phospho-Tyr705-STAT3
STAT3
Phospho-Ser9-GSK3β

Figure S4