Expanded View Figures

Figure EV1. Brd4 promotes Th2 cell differentiation.

A Flow cytometry analysis of mouse Th2 cells treated with JQ1 as indicated. Mouse Th2 cells were differentiated from mouse primary naïve $CD4^+$ T cells for 6 days before analysis.

B Flow cytometric (*left*) and statistical analysis (*right*) of IL-4 and IL-13 in Th2 cells derived from mouse primary naïve $CD4^+$ T cells treated with or without JQ1 (500 nM).

C qPCR analysis of *Il4*, *Il5*, and *Il13* in mouse Th2 cells treated with or without JQ1.

D ELISA analysis of IL-4 secretion into the supernatant of mouse Th2 cells treated with or without JQ1.

E Western blotting (*left*) and densitometry analysis (*right*) of Brd2, Brd3, and Brd4 in mouse naïve $CD4^+$ T cells, Th0, Th1, Th2, Th17, and Treg cells.

F Flow cytometric (*upper*) and statistical analysis (*lower*) of IL-4 in mouse Th2 cells infected with sh-Ctrl, sh-Brd2, sh-Brd3, or sh-Brd4 lentivirus.

Data information: Mouse naïve $CD4^+$ T cells were cultured in Th2 polarization condition and treated with or without inhibitors on Day 0 and were differentiated for 6 days before analysis, unless otherwise specified. All data represent mean ± SD and average of three independent experiments. Data are analyzed by Paired *t* test. $*P < 0.05$; $**P < 0.01$; and $***P < 0.001$.
Source data are available online for this figure.

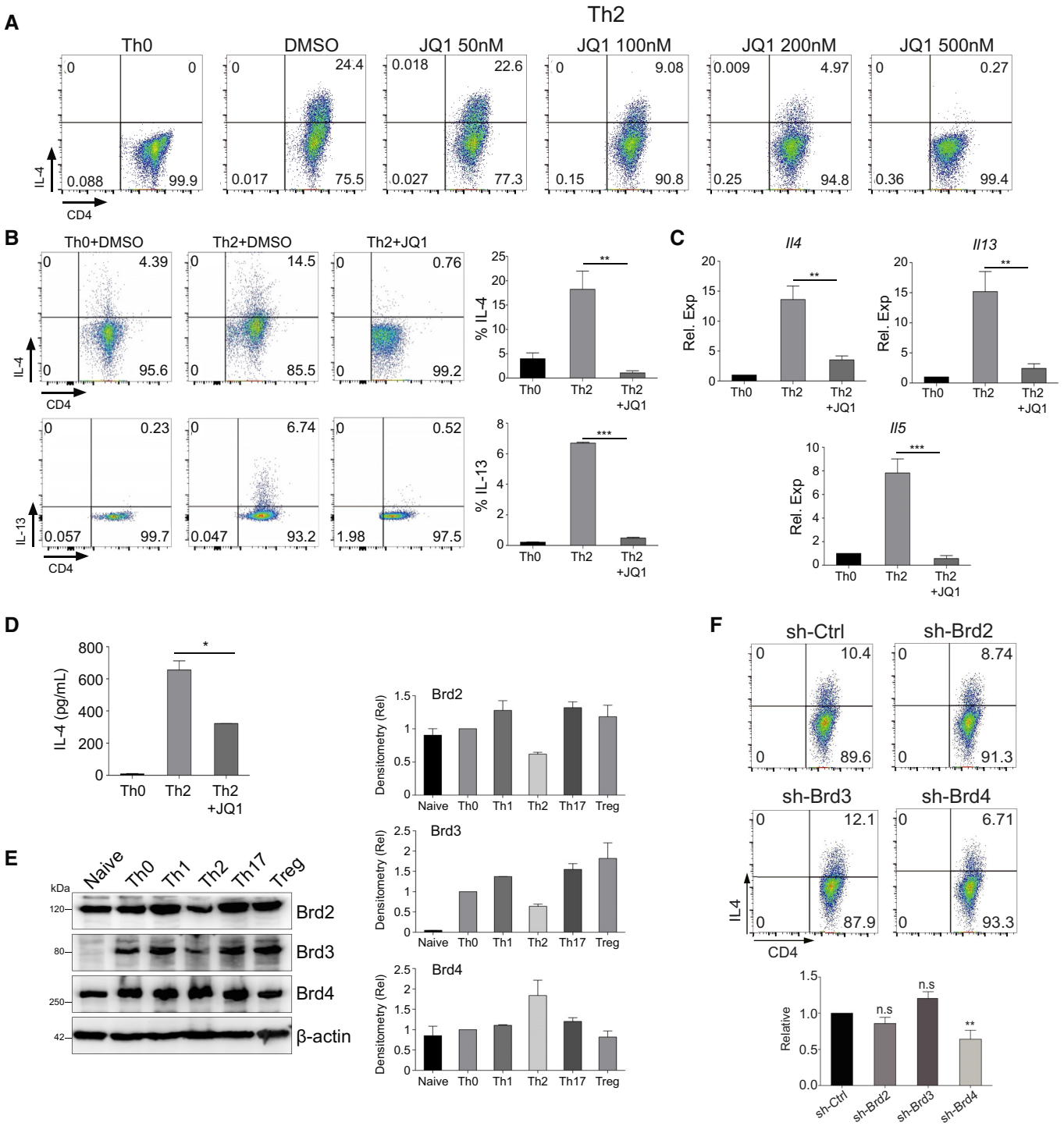


Figure EV1.

Figure EV2. Brd4 promotes murine airway inflammation.

A Representative gating strategies using side-scatter, live-dead, followed by assessment of IL-4-expressing Th2 cells, treated with control shRNA or shRNA targeting BRD4.
B Representative PI/Annexin V staining of Th2 cells, treated with control shRNA or shRNA targeting BRD4. Live cells are circled.
C Representative lung H&E staining showing infiltration of inflammatory cells indicated by arrows.
D Quik-Tiff staining of BALF (*upper*) and quantification of inflammatory cells (*lower*) in the BALF of mice transferred with mouse Th2 cells and Brd4-knockdown Th2 cells.
E Representative PAS staining showing proliferation of mucin producing cells in the airway epithelia in $Rag1^{-/-}$ hosts adoptively transferred with mouse Th2 cells and Brd4-knockdown Th2 cells.
F Quantitation of IgE in BALF with ELISA.

Data information: All data are representative of two experiments with $n = 4$. Data are analyzed by Paired t test. *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$.

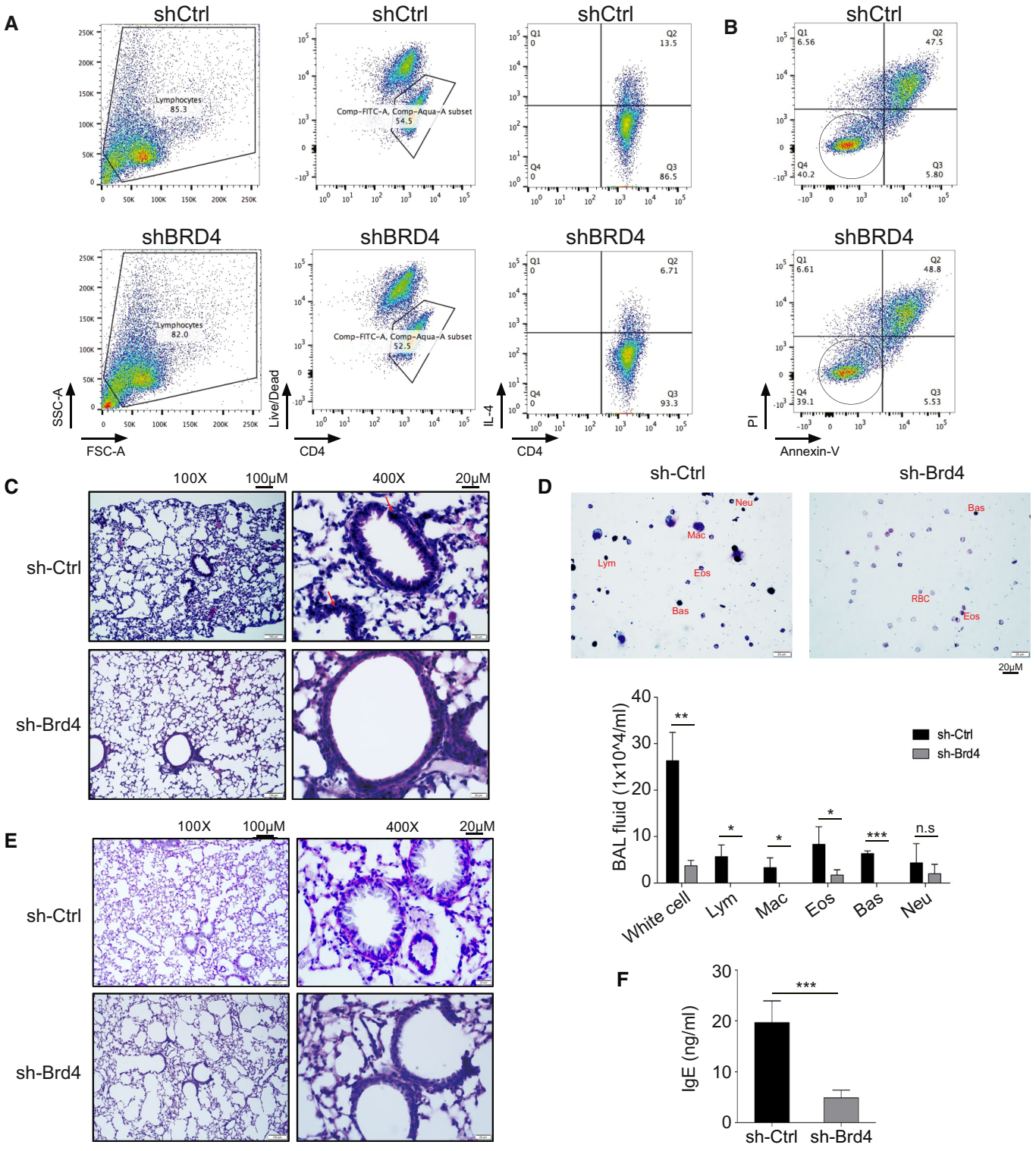


Figure EV2.

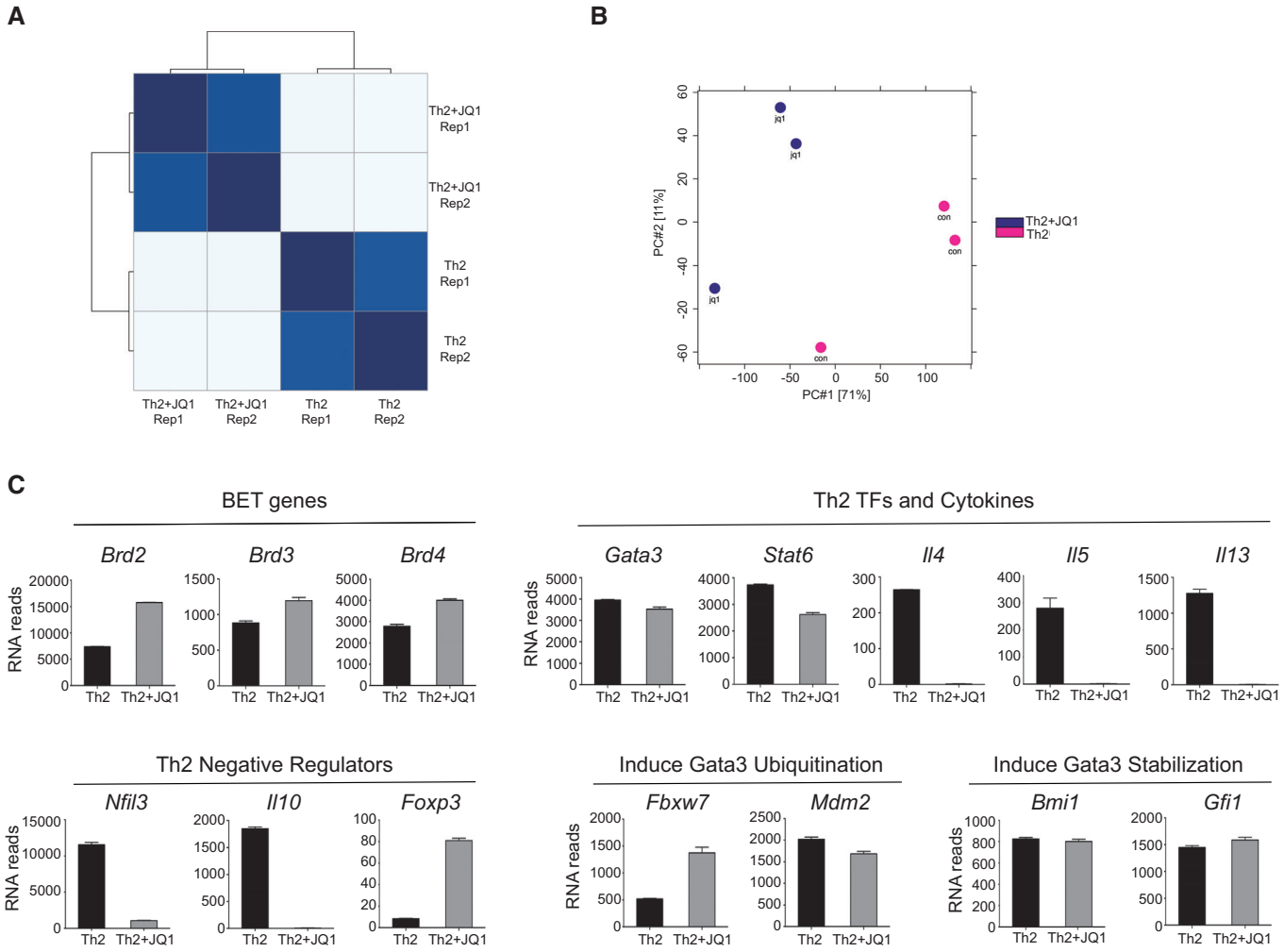


Figure EV3. RNA-seq and ChIP-seq analysis of Brd4 in Th2 cells.

A Heatmap of sample-to-sample distance of RNA-seq analysis in mouse Th2 cells treated with or without JQ1.

B Principal component analysis of ChIP-seq peaks in mouse Th2 cells treated with or without JQ1.

C RNA-seq data analysis showing expression of key genes in mouse Th2 cells treated with or without JQ1 (250 nM).

Data information: Mouse naïve $CD4^+$ T cells were cultured in Th2 polarization condition and treated with or without inhibitors on Day 0 and were differentiated for 6 days before analysis, unless otherwise specified. Data are representative of two to three biological replicates.

Figure EV4. Brd4 Inhibits *Foxp3* transcription and enhances Gata3 stability by repressing *Fbxw7* transcription to facilitate Th2 cell differentiation.

A Flow cytometric analysis of IL-4 and Foxp3 protein in mouse Th2 cells treated with or without JQ1 (500 nM).
B Statistical analysis of Foxp3 protein in mouse Th2 cells treated with or without JQ1 (500 nM).
C mRNA expression of *Foxp3* in mouse Th2 cells treated with or without JQ1 (500 nM).
D ChIP-qPCR analysis of the binding of Brd2 and Brd4 on the gene loci of *Foxp3* in mouse Th2 cells treated with or without JQ1 (250 nM).
E Dual-luciferase reporter assay of HEK293T cells transfected with Fbxw7-promoter, Brd4 and Brd4-mutant (N140A/N433A).
F Th2 cells were differentiated for 3 days and then treated with DMSO and JQ1 (250 nM) for 6, 24, and 48 h, followed by qPCR analysis of *Nr4a2*, *Foxp3*, and *Fbwx7*.
G Th2 cells were differentiated for 3 days and then treated with DMSO and MZ1 (1 μM) for 6, 24, and 48 h, followed by qPCR analysis of *Nr4a2*, *Foxp3*, and *Fbwx7*.
H qPCR analysis of *Il4*, *Il5*, *Il13*, *Gata3*, and *Foxp3* in mouse Th2 cells overexpressed with empty vector or Foxp3.
I Flow cytometric (*left*) and statistical analysis (*right*) of Foxp3 and IL-4 in Th2 cells overexpressed with empty vector or Foxp3.
J Western blotting (*upper left*) and densitometry statistical analysis (*upper right*) of Foxp3 in Th2 cells infected with sh-Ctrl and sh-Foxp3 lentivirus. Flow cytometric (*lower left*) and statistical analysis (*lower right*) of IL-4 in Th2 cells infected with sh-Ctrl and sh-Foxp3 lentivirus.
K Western blotting of Fbxw7 and Gata3 in mouse Th2 cells infected with sh-ctrl and sh-Fbxw7.
L Western blotting of Gata3 in mouse naïve $CD4^+$ T cells cultured in Th2 polarization were treated with JQ1 (500 nM) at Day 0, and then treated with DMSO or MG132 (20 μM) for 6 h before harvest.

Data information: Mouse naïve $CD4^+$ T cells were cultured in Th2 polarization condition and treated with or without inhibitors on Day 0 and were differentiated for 6 days before analysis, unless otherwise specified. All Western blotting data are representative of three independent experiments. All data represent mean ± SD and average of three independent experiments. Data are analyzed by Paired *t* test. *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$.
Source data are available online for this figure.

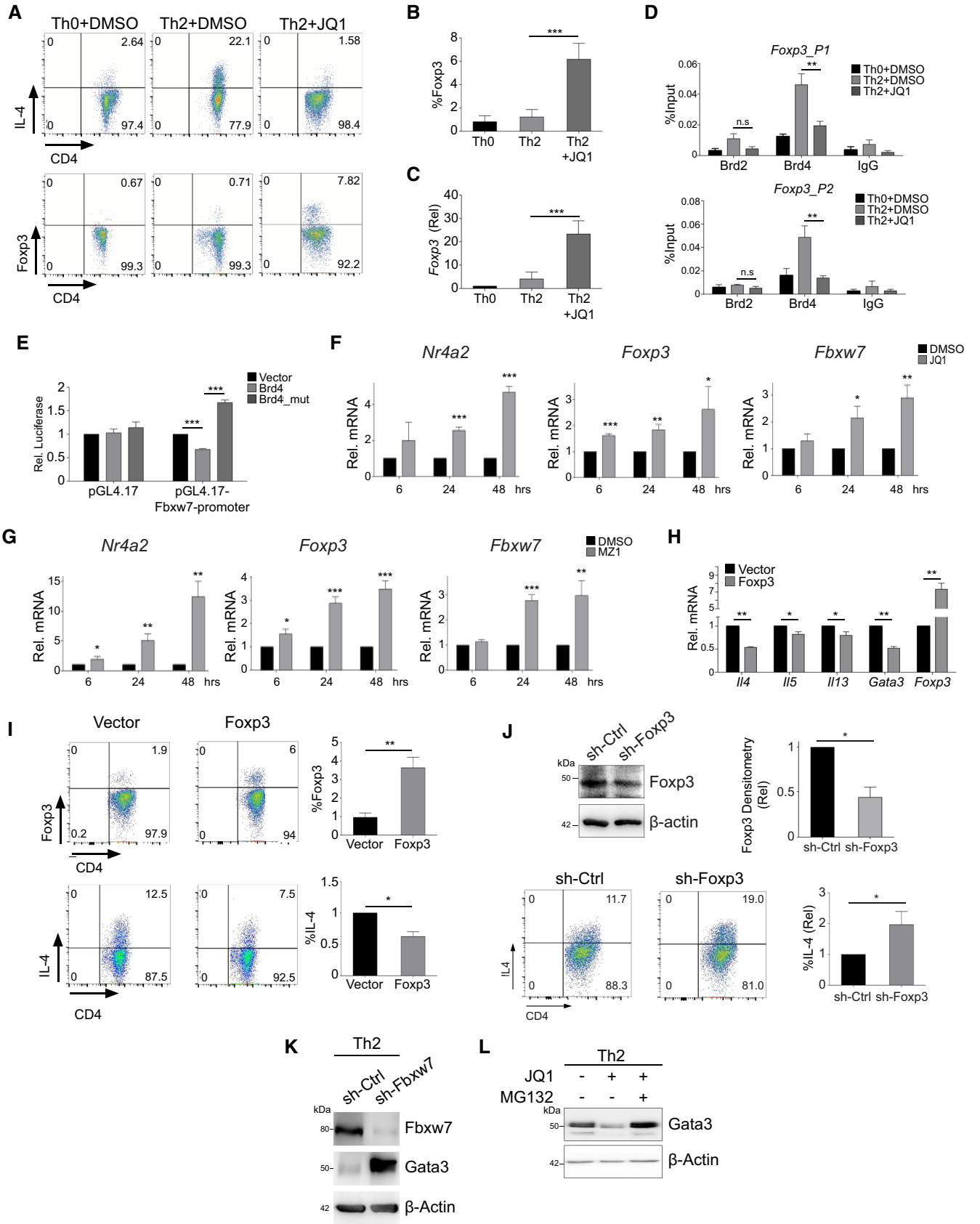
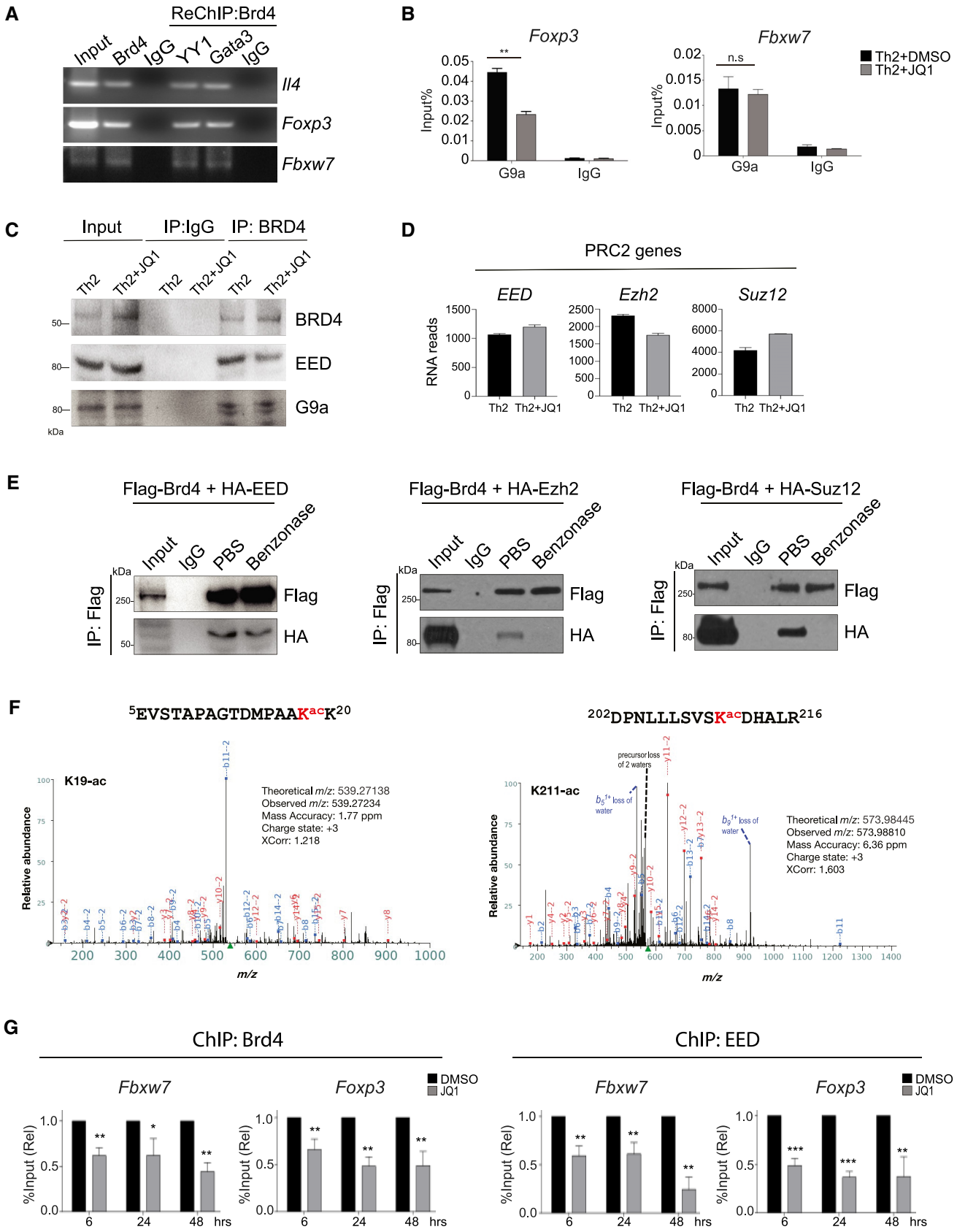

A
Th0+DMSO
Th2+DMSO
Th2+JQ1
IL-4
CD4
Foxp3
B
%Foxp3
Th0
Th2
Th2 +JQ1
C
Foxp3 (Rel)
D
Foxp3_P1
Foxp3_P2
%Input
Th0+DMSO
Th2+DMSO
Th2+JQ1
n.s
Brd2
Brd4
IgG
E
Rel. Luciferase
Vector
Brd4
Brd4_mut
pGL4.17
pGL4.17-Fbxw7-promoter
F
Nr4a2
Foxp3
Fbxw7
Rel. mRNA
DMSO
JQ1
6
24
48 hrs
G
DMSO
MZ1
H
Vector
Foxp3
Il4
Il5
Il13
Gata3
Foxp3
I
Vector
Foxp3
%Foxp3
%IL-4
J
sh-Ctrl
sh-Foxp3
kDa
Foxp3
β-actin
Foxp3 Densitometry (Rel)
%IL-4 (Rel)
K
Th2
sh-Ctrl
sh-Fbxw7
Fbxw7
Gata3
β-Actin
L
JQ1
MG132
Gata3
β-Actin

Figure EV4.

Figure EV5. Brd4-BD2 binds to acetylated-EED to repress *Foxp3* and *Fbxw7* in Th2 cells.

A ChIP-PCR of the binding of Brd4 and Re-ChIP-PCR of YY1 and Gata3 on the gene loci of *Il4*, *Foxp3*, and *Fbxw7* in mouse Th2 cells.
B ChIP-qPCR analysis of the binding of G9a on the gene loci of *Foxp3* and *Fbxw7* in mouse Th2 cells treat with or without JQ1 (250 nM).
C Th2 cells were differentiated for 6 days, treated with JQ1 (250 nM) on Day 0. Lysates are immunoprecipitated with BRD4 followed by Western blotting analysis of BRD4, EED, and G9a.
D RNA-seq data analysis showing expression of PRC2 key genes in mouse Th2 cells treated with or without JQ1 (250 nM). Data are representative of two biological replicates.
E HEK293T cells are transfected with Flag-Brd4 and HA-EED (*left*), HA-Ezh2 (*middle*), or HA-Suz12 (*right*) plasmids. Lysates are immunoprecipitated with Flag-tagged Brd4, treated *in vitro* with PBS and Benzonase, followed by Western blotting of HA to detect HA-EED, HA-Ezh2, and HA-Suz12.
F HEK293T cells are transfected with Flag-Brd4 and HA-EED plasmids. Lysates are immunoprecipitated with HA, followed by liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis. MS/MS spectrum for the identified acetylated peptides containing Lysine acetylation at position K19 and K211. Peak heights show the relative abundance of the corresponding fragmentation ions. The peptide sequences are shown at the top of the corresponding MS/MS spectrum with acetylated lysine residues highlighted in red. The identified fragmentation y (red color) and b (blue color) ions are indicated.
G Th2 cells were differentiated for 3 days and then treated with DMSO and JQ1 (250 nM) for 6, 24 and 48 h, followed by ChIP-qPCR analysis of binding of Brd4 and EED on *Foxp3* and *Fbwx7*.

Data information: Mouse naïve $CD4^+$ T cells were cultured in Th2 polarization condition and treated with or without inhibitors on Day 0 and were differentiated for 6 days before analysis, unless otherwise specified. All Western blotting data are representative of three independent experiments. All ChIP-qPCR data represent mean ± SD and are representative of three independent experiments. Data are analyzed by Paired *t* test. *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$.
Source data are available online for this figure.


A
ReChIP:Brd4
Input
Brd4
IgG
YY1
Gata3
IgG
Il4
Foxp3
Fbxw7
B
Foxp3
Fbxw7
Input%
**
n.s
Th2+DMSO
Th2+JQ1
G9a
IgG
C
Input
IP:IgG
IP: BRD4
Th2
Th2+JQ1
BRD4
EED
G9a
kDa
D
PRC2 genes
EED
Ezh2
Suz12
RNA reads
Th2
Th2+JQ1
E
Flag-Brd4 + HA-EED
Flag-Brd4 + HA-Ezh2
Flag-Brd4 + HA-Suz12
Input
IgG
PBS
Benzonase
IP: Flag
Flag
HA
F
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202DPNLLLSVSKacDHALR216
K19-ac
Theoretical m/z: 539.27138
Observed m/z: 539.27234
Mass Accuracy: 1.77 ppm
Charge state: +3
XCorr: 1.218
K211-ac
precursor loss of 2 waters
Theoretical m/z: 573.98445
Observed m/z: 573.98810
Mass Accuracy: 6.36 ppm
Charge state: +3
XCorr: 1.603
Relative abundance
m/z
G
ChIP: Brd4
ChIP: EED
Fbxw7
Foxp3
DMSO
JQ1
%Input (Rel)
6
24
48
hrs

Figure EV5.

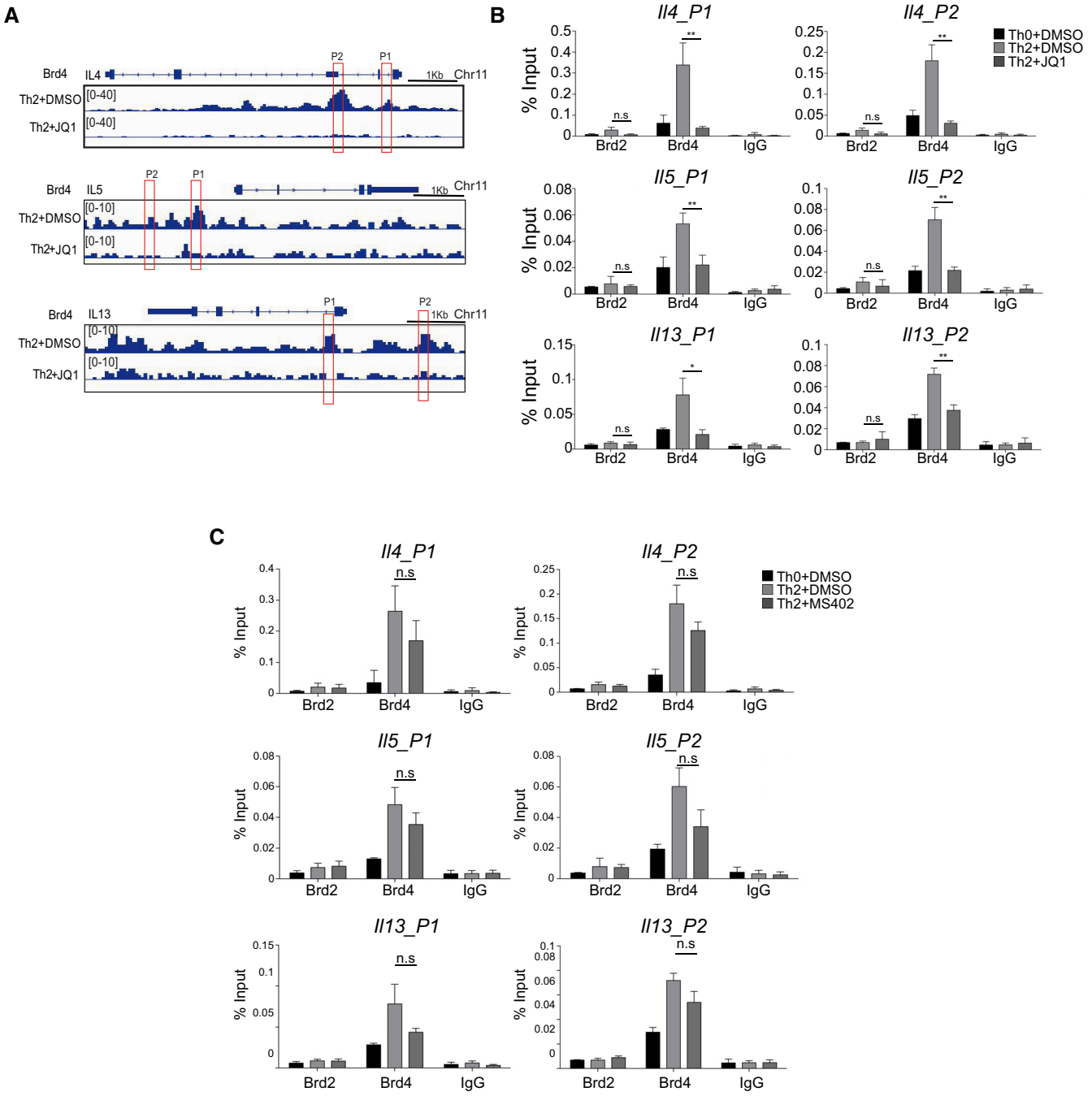

Figure EV6. ChIP-qPCR analysis of Brd2 and Brd4 on *Il4/Il5/Il13* promoter treated with or without JQ1 and MS402.

A ChIP-seq tracks of Brd4 occupancy at the promoters of *Il4*, *Il5*, and *Il13* in mouse Th2 cells treated with or without JQ1 (250 nM).

B ChIP-qPCR analysis of Brd2 and Brd4 on *Il4/Il5/Il13* promoter treated with or without JQ1 (250 nM).

C ChIP-qPCR analysis of Brd2 and Brd4 on *Il4/Il5/Il13* promoter treated with or without MS402 (3 μM).

Data information: Mouse naïve $CD4^+$ T cells were cultured in Th2 polarization condition and treated with or without inhibitors on Day 0 and were differentiated for 6 days before analysis, unless otherwise specified. All data represent mean ± SD and are representative of two independent experiments. *$P < 0.05$; and **$P < 0.01$.