SUPPLEMENTARY MATERIAL

BRG1-Mediated Chromatin Remodeling Regulates Differentiation And Gene Expression Of T Helper Cells

Wurster and Pazin

SUPPLEMENTAL METHODS:

Proliferation Assay. Nucleofected naïve Th cells were plated at $1x10^5$ cells/well in triplicate in 96 well plates coated with indicated concentrations of anti-CD3 and 2 μg/ml anti-CD28. Proliferation was assessed after 72 hours of culture using CyQuant Cell Proliferation Kit (Molecular Probes). Similar results were seen either in unskewed or Th2 skewed conditions.

Cell Surface Staining. Naïve Thp cells were transduced with control and BRG1 RNAi reagents, differentiated for 48 hours under Th2 skewing conditions, and then directly stained with FITC-conjugated antibodies from Pharmingen against CD3 (01084D), CD4 (01064D), CD69 (01504A) and Rat IgG1 isotype control (554684). Cell-surface expression was assessed by flow cytometry using a FACSCalibur with CellQuest Pro software (BD Biosciences), and analyzed with FloJo software (Tree Star, Inc.).

ChIP Antibodies. BRM, Abcam ab15597; BAF250, gift from Weidong Wang (Nie et al., 2000).

Immunoblotting antibodies. GATA3: Santa Cruz (sc-268); c-Maf: Novus Biologicals (NB600-267), phospho-Stat6: Cell Signaling Technology (9361S), Stat6: Santa Cruz (sc-981); HDAC2: Abcam (ab7029-50).

ELISA. Culture supernatants from Th2 effector cells stimulated with anti-CD3 for 48 hours were analyzed for IL-4 and IFNg using BD OptE1A (BD Biosciences).

BAF250 RNAi reagent. Dharmacon Arid1a SmartPool M-040694-00.

Sequential IP method. Modified our method based on (Metivier et al., 2003).

MNase Digestion. Control and BRG siRNA transfected Th2 cells were rested or stimulated for 1.5 hours with PMA/Ionomycin. Cells were washed once with PBS, twice with MNase Wash Buffer (10 mM Tris pH 7.5 containing 10 mM NaCl, 3 mM MgCl2, 0.5 % NP40, 1 mM PMSF) and resuspended in 100µl MNase Buffer (10 mM Tris pH 7.5 containing 10 mM NaCl, 3 mM MgCl2, 1 mM CaCl2, 4% NP40). MNase (0.03U/ml) was added and reactions were incubated for 3 minutes at room temperature. The digestion was stopped by the addition of 10mM EDTA, 1mM EGTA, 1%SDS and 0.5 mg/ml proteinase K. Samples were incubated overnight at 37 degrees and DNA was purified using DNeasy Blood and Tissue Kit (Qiagen).

Intracellular Cytokine Staining. Th2 effector cells were stimulated with platebound anti-CD3 for 5 hours in the presence of 3µM monensin and subsequently stained for

cytokine expression using Cytofix/Cytoperm (BD Biosciences) per manufacturer's instructions. Intracellular cytokine staining was performed using PE-conjugated anti-IL-4 (554435 BD Biosciences) and FITC-conjugated anti-IFNg (554411 BD Biosciences).

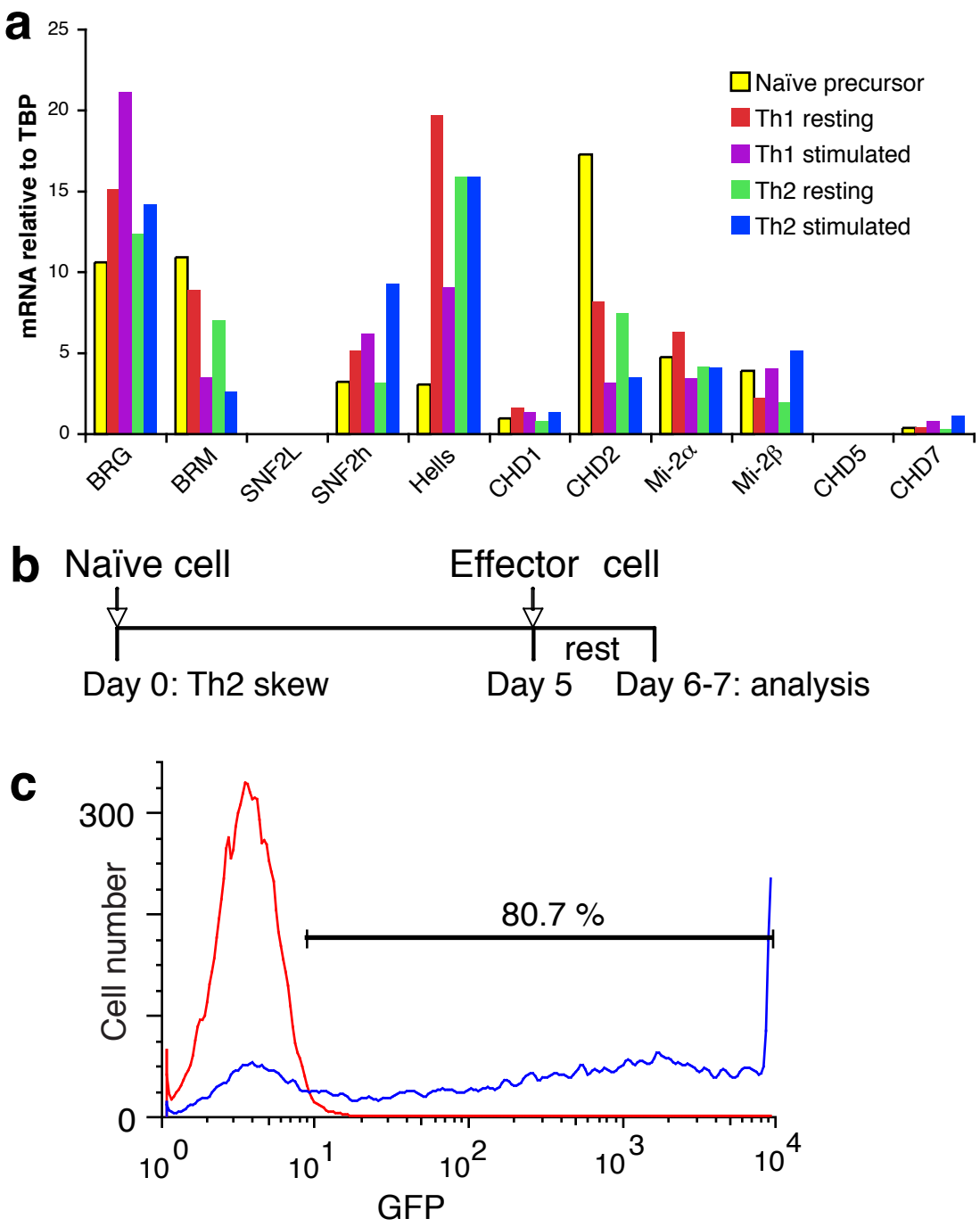

Figure S1 Remodeling ATPase expression and Th2 cell transduction efficiency.
A) ATPase mRNA expression was measured by real-time RT-PCR and normalized to TBP expression in CD4+/CD62L+ naive cells and resting and activated Th1 and Th2 cells differentiated from them.
B) Scheme for differentiation and electroporation. Arrows indicate time of transduction during (Naive) and after (Effector) differentiation.
C) Transduction efficiency after Th2 differentiation measured by GFP expression.
Red, Control plasmid. Blue, GFPmax plasmid.

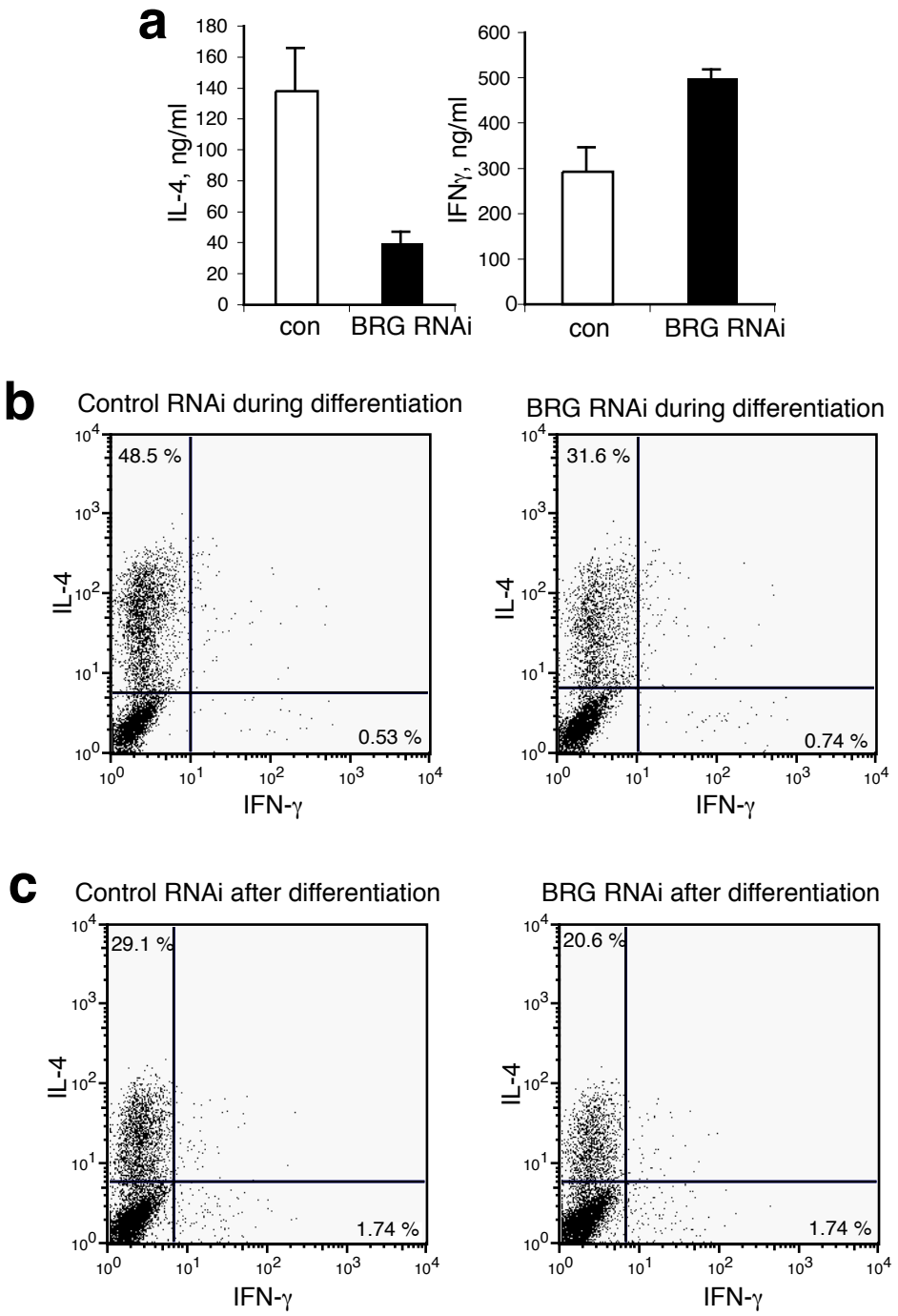

Figure S2 BRG1 RNAi impairs expression of Th2 cytokine proteins.
A) Analysis of IL-4 and IFN-γ proteins from cell culture supernatants using ELISA. Similar results were obtained using either CD4+/CD62L+ or CD4+ lymph node cells as starting material. Control and BRG1 RNAi were performed *during* Th2 differentiation.
B) Intracellular cytokine staining of IL-4 and IFN-γ proteins in restimulated Th2 cells. Control and BRG1 RNAi were performed *during* Th2 differentiation.
C) Intracellular cytokine staining of IL-4 and IFN-γ proteins in restimulated Th2 cells. Control and BRG1 RNAi were performed *after* Th2 differentiation was complete.

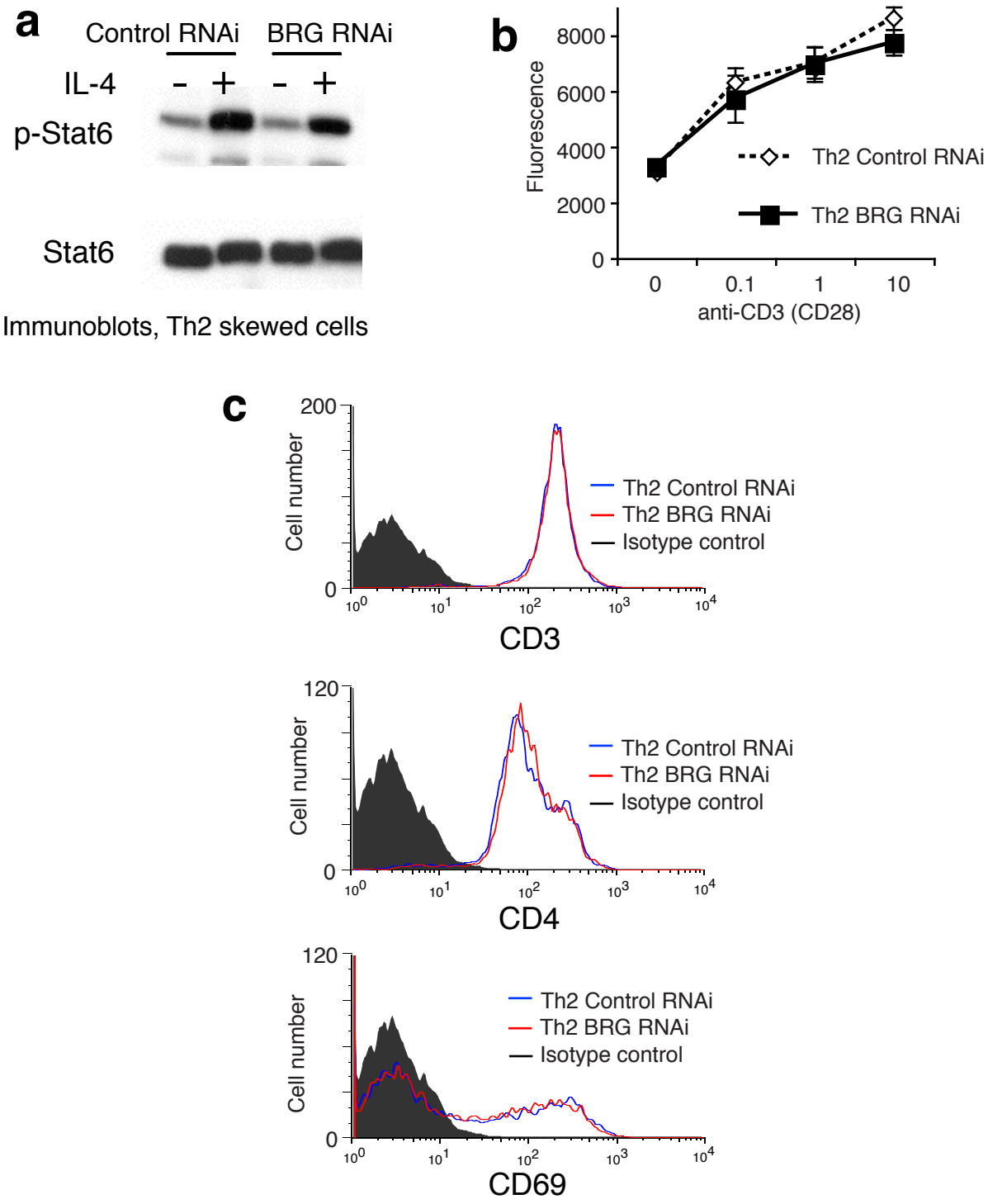

Figure S3. BRG1 RNAi does not alter viability, proliferation, STAT6 phosphorylation, or cell surface markers.

A) STAT6 tyrosine phosphorylation and protein expression are not strongly altered by BRG1 RNAi during Th2 differentiation.

B) Proliferation in response to antigen receptor stimulation during differentiation is unchanged by BRG1 RNAi during differentiation.

C) Cell surface staining for T cell markers. Intact cells were directly stained 48 hours after culture in Th2 conditions with FITC-conjugated antibody specific for the indicated protein or isotype control. The overlay histogram plots show BRG1 RNAi cells with red lines, control knockdown with blue lines, and isotype control with black.

Supplemental Figure 3 Wurster and Pazin

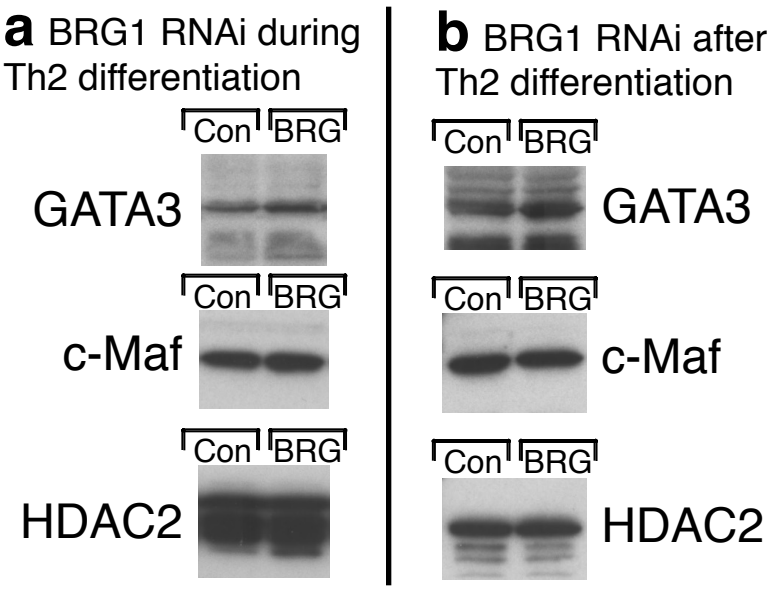

Figure S4. Protein expression of the Th2 transcription factors is unchanged after BRG1 RNAi. Restimulated Th2 effector cells were lysed with RIPA buffere, clarified lysates were analyzed by SDS-PAGE, transferred to PVDF membranes, and the indicated proteins were detected by immunoblotting. Antibodies are listed in the supplemental methods.HDAC2 is a loading control. A) BRG1 RNAi (BRG) or control RNAi (Con) during Th2 differentiation. B) BRG1 RNAi (BRG) or control RNAi (Con) after Th2 differentiation.

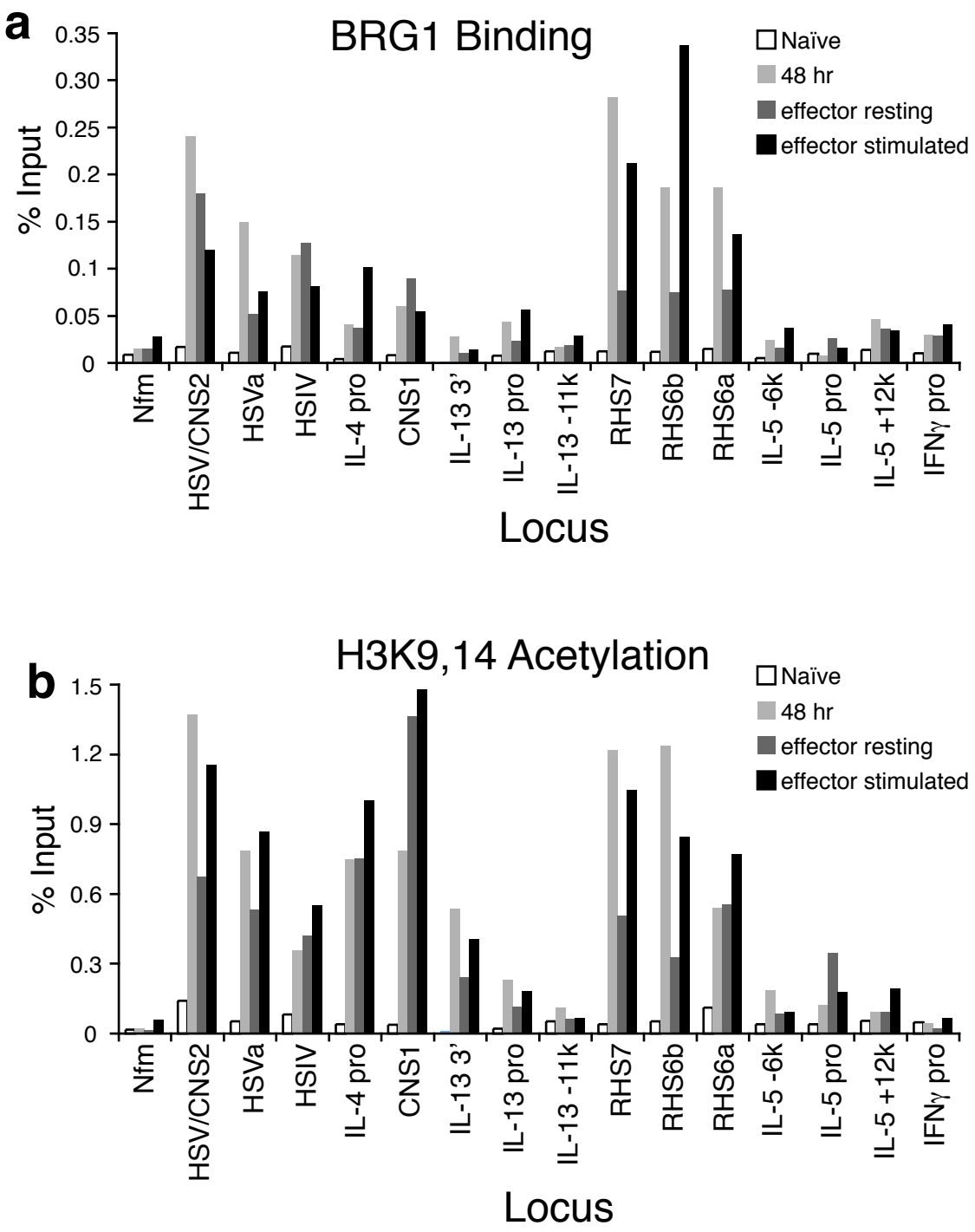

Figure S5. Replicate experiment showing BRG1 binds to Th2 cytokine loci in differentiated, but not undifferentiated (CD4+ lymph node) cells. All of the Th2 cytokine locus samples are from one experiment, using one batch of cells.
These results are typical of at least 3 independent experiments. This is a completely independent experiment from Figure 3 (Different mice, cells made on different days, ChIP and PCR done on different days).

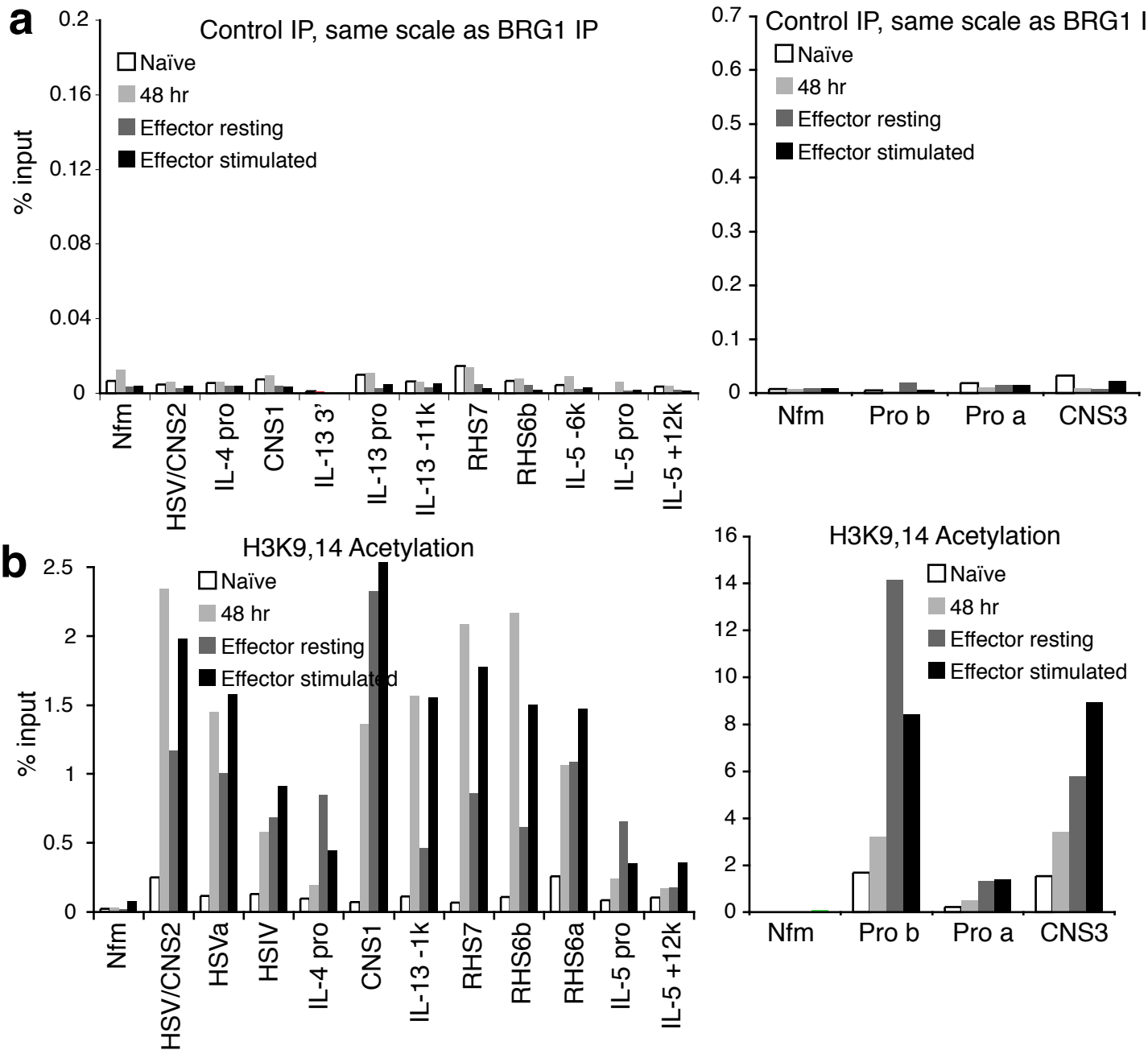

Figure S6. H3K9,14 acetylation in Th2 cells parallels BRG1 binding at the Th2 locus.
A) IgG control IP, from same chromatin used in experiments in Figure 3, performed at the same time; these panels are plotted at the same scales as Figure 3 for direct comparison of background.
B) H3 acetylation, from same chromatin used in experiments in Figure 3, performed at the same time.

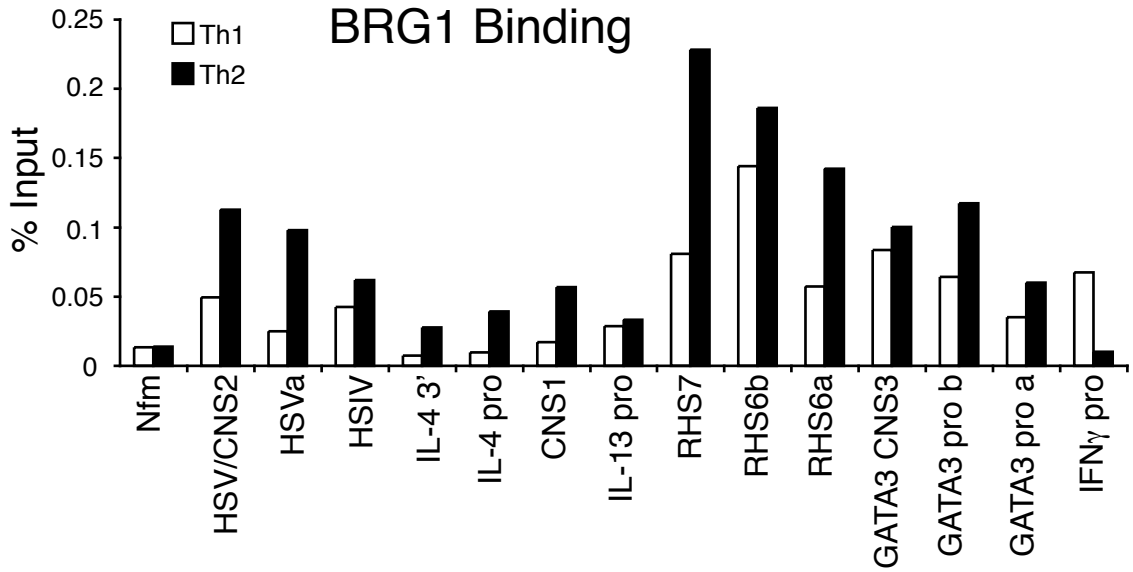

Figure S7. Replicate experiment showing more BRG1 is bound to Th2 loci in Th2 cells than in Th1 cells. Similar results were obtained with either CD4+/CD62L+ or CD4+ lymph node cells as starting material.
This experiment is completely independent from Figure 4A (Different mice, cells made on diffferent days, ChIP and PCR done on different days).

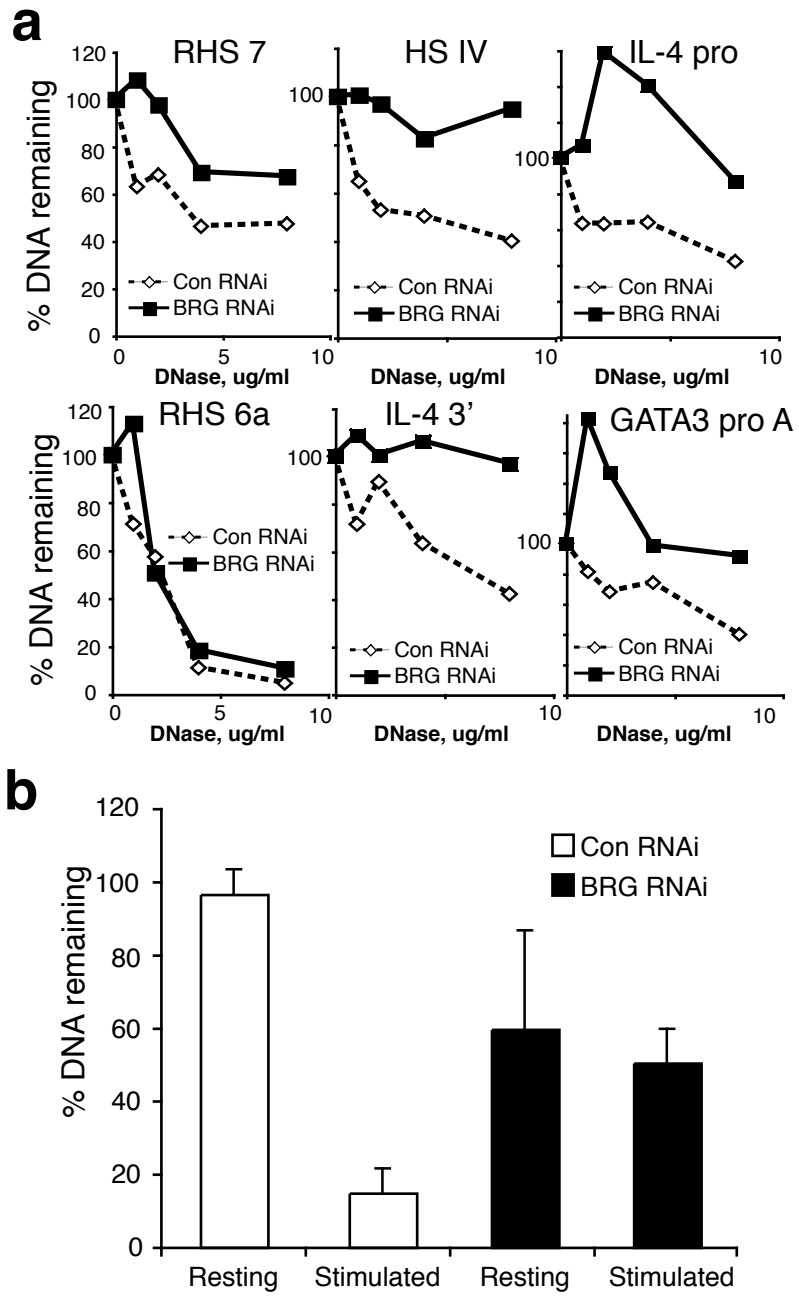

Figure S8. BRG1 is required for chromatin structure after Th2 differentiation is complete.
CD4+ lymph node cells were differentiated into Th2 cells in culture; BRG1 or control RNAi was performed after Th2 differentiation was complete.
A) Replicate experiment showing BRG1 is required for the DNase I HS at RHS7, HSIV, IL-4 promoter, IL-4 3' end, and GATA3 promoter a. This is a completely independent experiment from Figure 5A (Different mice, cells made on different days, DNaseI digests and PCR done on different days).
B) BRG1 is required for MNase sensitivity at RHS7. MNase was used as an alternative probe of chromatin structure. Averages and standard deviations of two completely independent experiments are shown.

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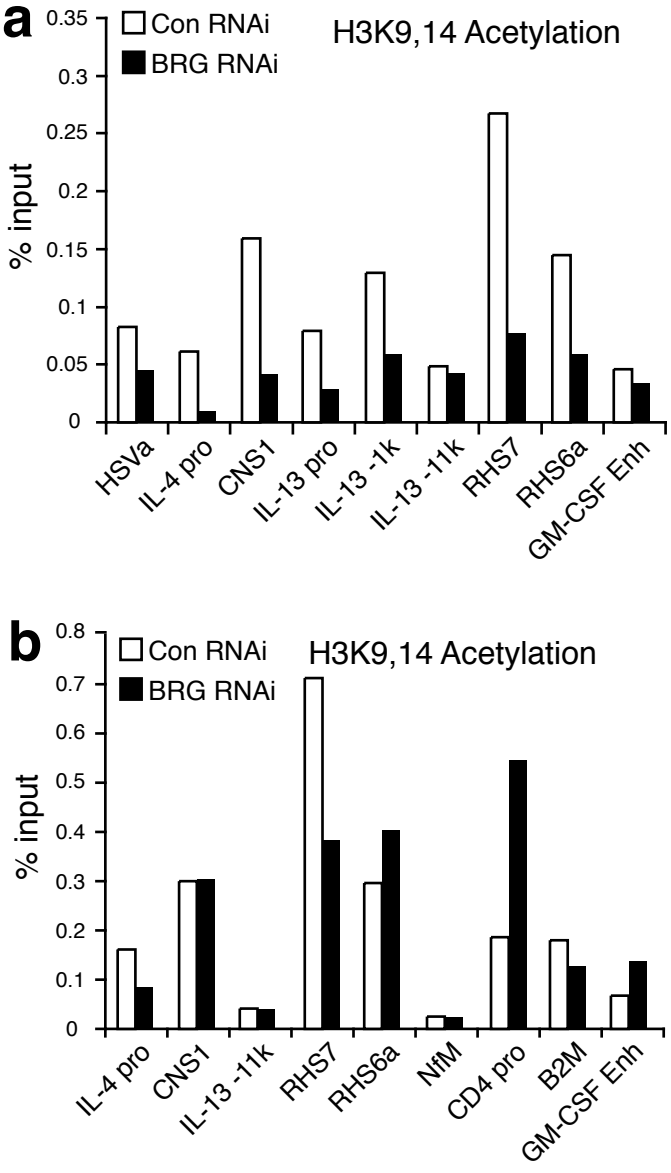

Figure S9. Replicate experiments showing BRG1 is required for histone acetylation in the Th2 locus. CD4+ lymph node cells were differentiated into Th2 cells in culture; BRG1 or control RNAi was performed after Th2 differentiation was complete. Histone acetylation was measured using C-ChIP.
A and B are completely independent experiments from each other and Figure 5B (Different mice, cells made on different days, ChIP and PCR done on different days).

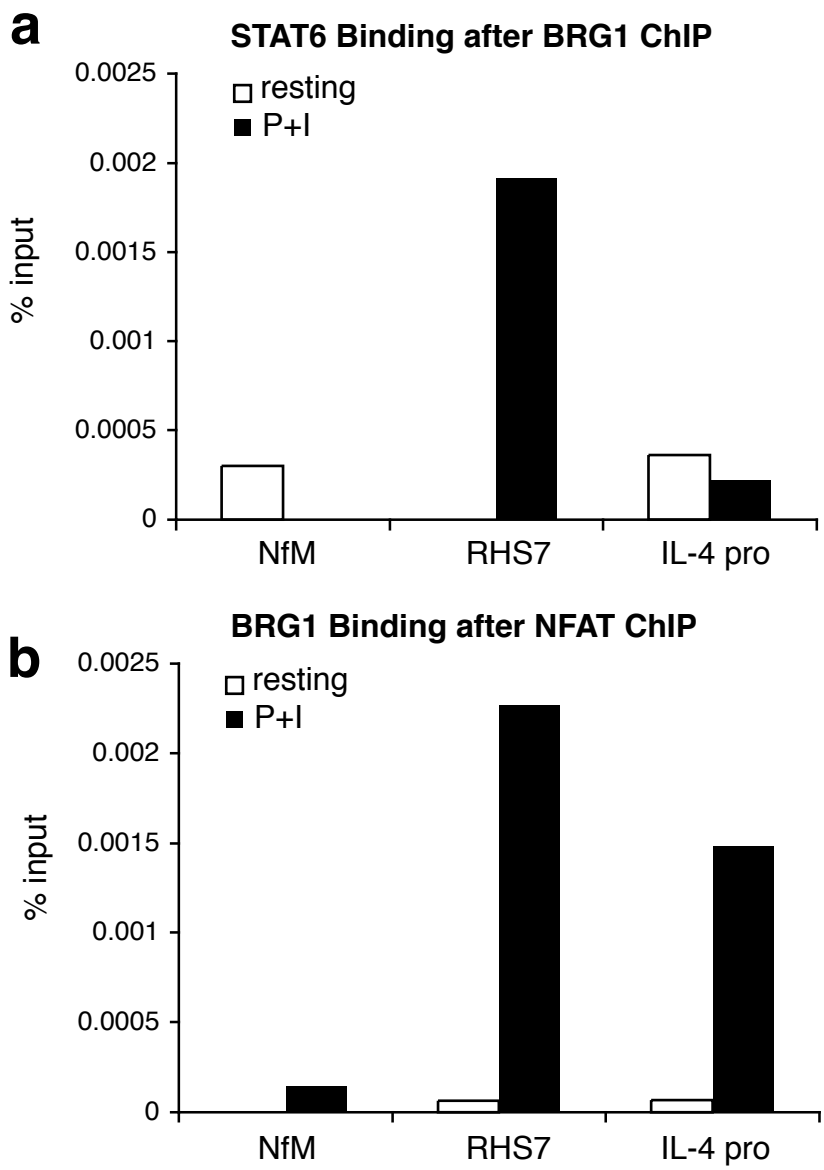

Figure S10. BRG1 and transcription factors occupy the same alleles in Th2 effector cells.
Th2 cells were generated in culture from CD4+/CD62L+ cells obtained from lymph nodes.
After differentiation was complete, remodeling enzyme and transcription factor binding was measured by sequential ChIP, using resting and restimulated cells.
A) After ChIP using BRG1 antisera, chromatin was eluted with DTT, diluted, and immunoprecipitated again with STAT6 antibodies.
B) After ChIP using NFAT antibodies, chromatin was eluted with DTT, diluted, and immunoprecipitated with BRG1 antisera.

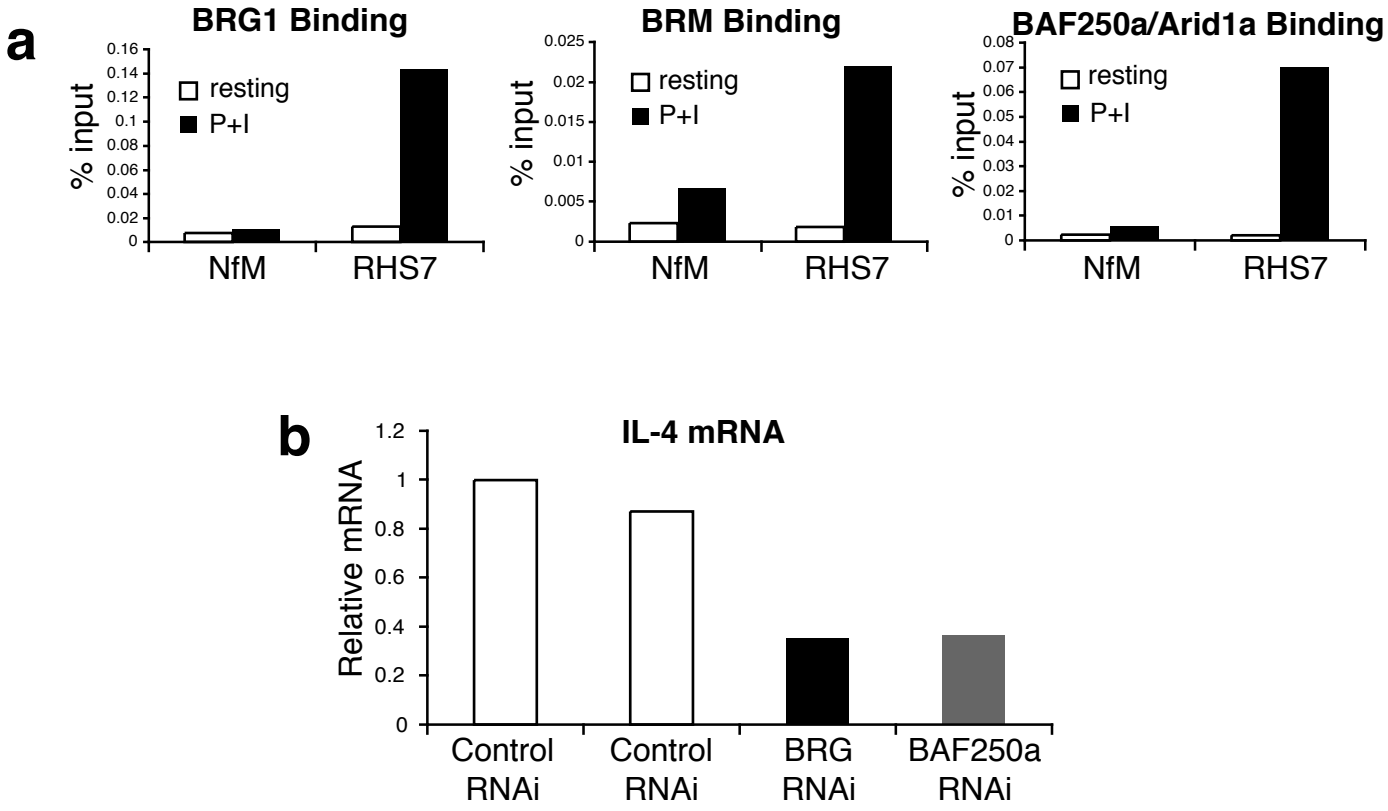

Figure S11. Components of the BAF form of the SWI/SNF complex are directly involved in regulating Th2 gene expression in Th2 effector cells.
A) After Th2 differentiation to effector cells was complete, remodeling enzyme binding was measured in resting and restimulated cells, by performing ChIP with antibodies to the indicated proteins.
B) Cytokine mRNA amounts were measured in restimulated Th2 effector cells. RNAi was performed *after* Th2 differentiation was complete, using reagents for the indicated targets.

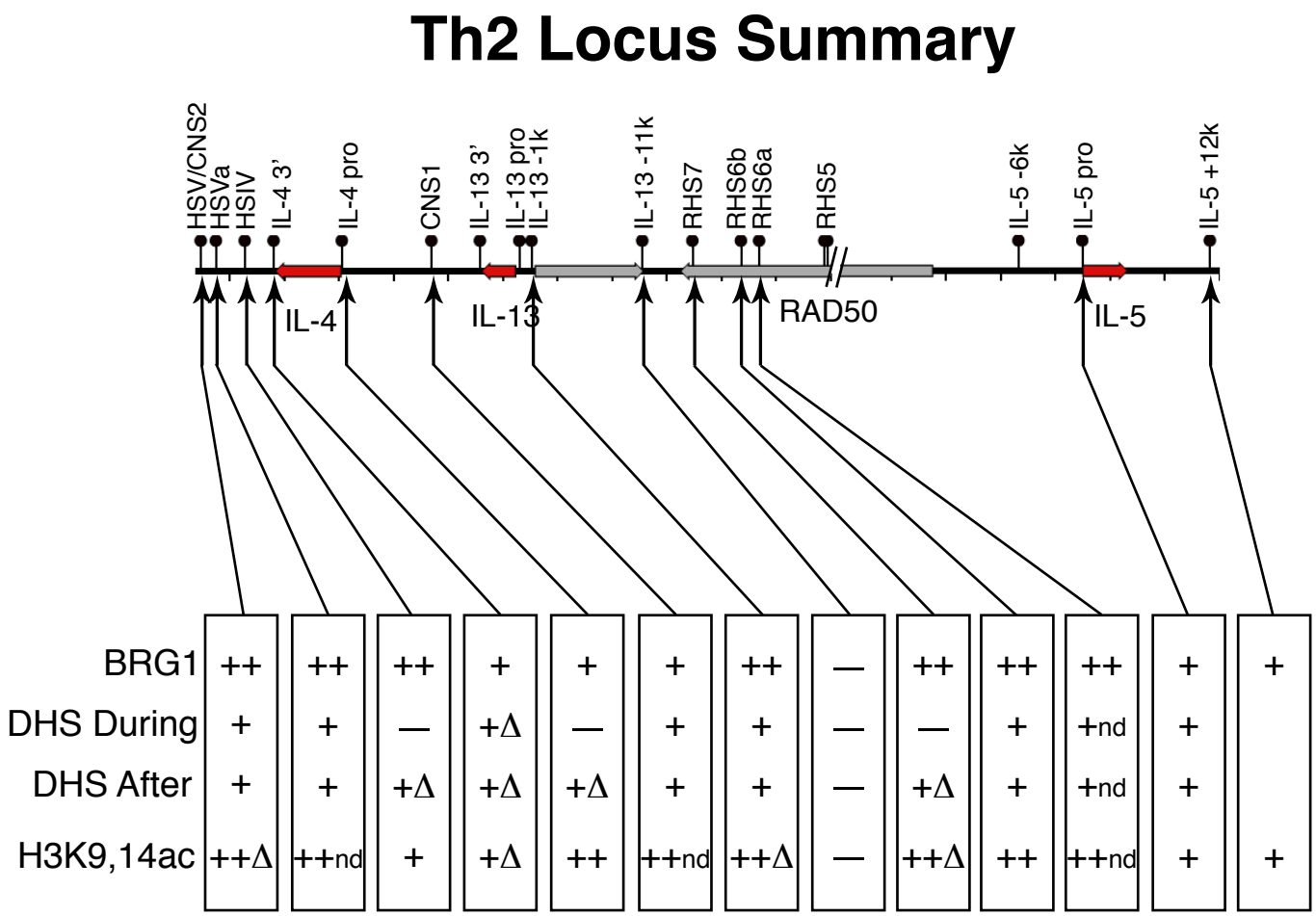

Figure S12. Th2 cytokine locus summary. On the scale physical map at the top of the figure, Th2 genes are indicated as red horizontal arrows on the sequence pointing in the direction of transcription, the Rad50 housekeeping gene is indicated with a green horizontal arrow, black circles above the sequence indicate named regulatory regions, and marks below the sequence are spaced 10 kb apart. For BRG1 binding, - indicates no binding, + indicates binding, ++ indicates strong binding, eg. Fig 3a. For DNase I HS, - indicates no hypersensitivity, + indicates hypersensitive. "DHS during" row reports chromatin analyzed 72 hours after Th2 differentiation begun; Δ in this row indicates BRG1 knockdown during differentiation alters the typical chromatin structure, nd indicates no difference with knockdown; data not shown. "DHS after" row reports chromatin analyzed in restimulated Th2 effector cells, eg. Fig 5a; Δ in this row indicates BRG1 knockdown after differentiation changed the chromatin structure, nd indicates no difference with knockdown. For histone acetylation, - indicates weak or no signal, + indicates acetylation, ++ indicates strong acetylation, and Δ in this row indicates BRG1 RNAi after Th2 differentiation alters the acetylation pattern, nd indicates no difference with knockdown, eg Fig. 5b. Vertical arrows connect experimental results to the physical map location of features.

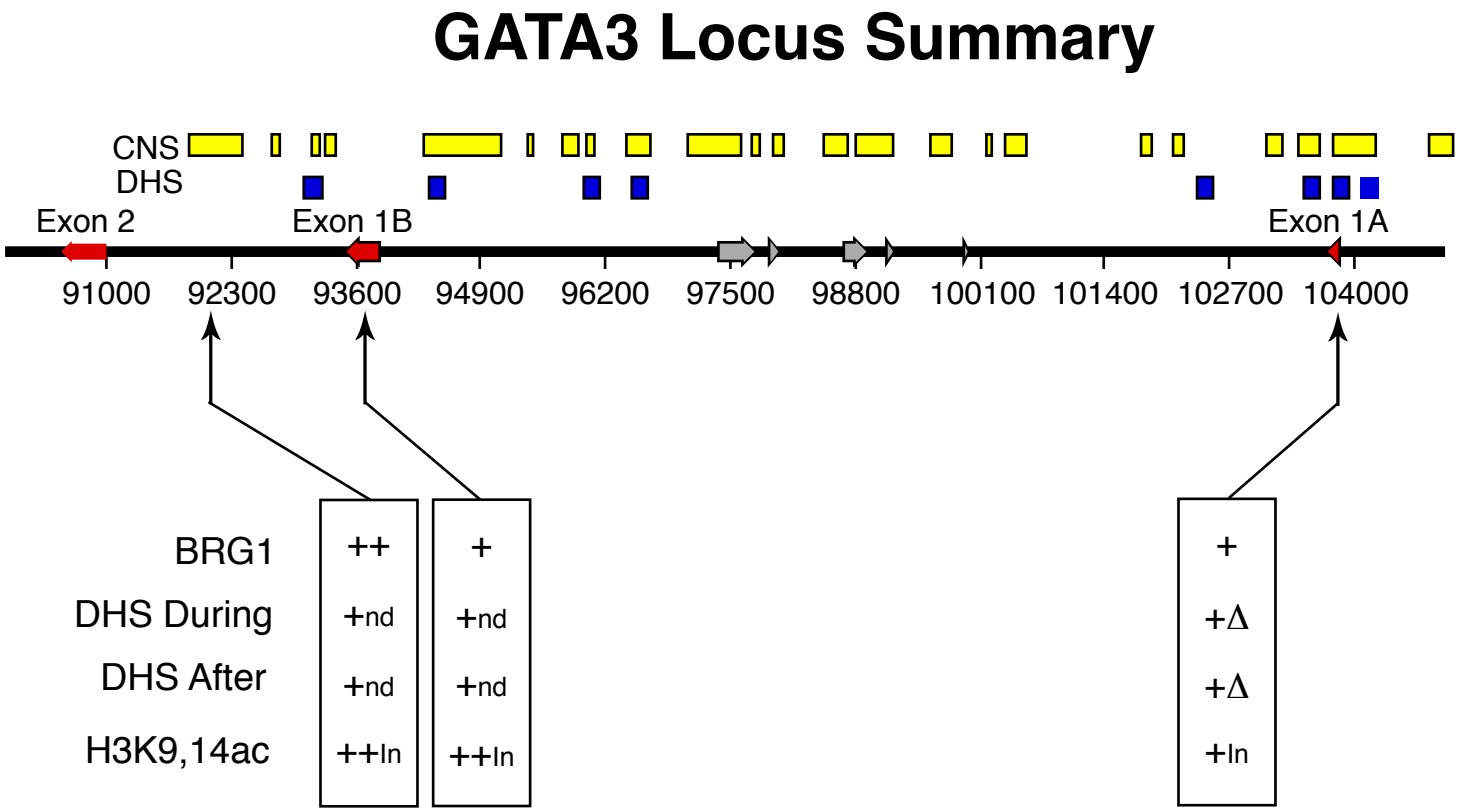

Figure S13. GATA3 locus summary. On the scale physical map, selected GATA3 exons are indicated on the sequence as red horizontal arrows pointing in the direction of transcription, exons of a putative transcript (Riken cDNA 4390412013Rik) are indicated with grey arrows, blue boxes above the sequence (DHS row) indicate DNase I HS sites (Lieuw et al., 1997), yellow boxes above the sequence (CNS row) indicate conserved non-coding sequences predicted using VISTA (Frazer et al., 2004), by comparing mouse and human, numbers below the sequence are spaced 1.3 kb apart, and indicate position (in base pairs) relative to an arbitrary location. For BRG1 binding, - indicates no binding, + indicates binding, ++ indicates strong binding eg. Fig 3b. For DNase I HS, - indicates no hypersensitivity, + indicates hypersensitive. "DHS during" row reports chromatin analyzed 72 hours after Th2 differentiation begun; Δ in this row indicates BRG1 knockdown during differentiation alters the typical chromatin structure, nd indicates no difference with knockdown; data not shown. "DHS after" row reports chromatin analyzed in restimulated Th2 effector cells, eg. Fig 5a; Δ in this row indicates BRG1 knockdown after differentiation changed the chromatin structure, nd indicates no difference with knockdown. For histone acetylation, - indicates weak or no signal, + indicates acetylation, ++ indicates strong acetylation and In indicates Inducible acetylation during differentiation, eg. Supp Fig 3b. Vertical arrows connect experimental results to the physical map location of features.

Supplementary Figure 14

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Primers for steady-state RNA

Gene	Primer name	Primer 1	Primer 2
IL-4	MP 588	ACAGGAGAAGGGACGCCAT	GAAGCCCTACAGACGAGCTCA
IL-5	MP 779	AGCACAGTGGTGAAAGAGACCTT	TCCAATGCATAGCTGGTGATTT
IL-13	MP 590	AGACCAGACTCCCCTGTGCA	TGGGTCCTGTAGATGGCATTG
IL-10	MP 1016	GGCGCTGTCATCGATTTCTC	GCTCCACTGCCTTGCTCTTATTT
IFNγ	MP 1015	TGGCATAGATGTGGAAGAAAAGAG	TGCAGGATTTTCATGTCACCAT
IL-2	MP 672	GAAACTCCCCAGGATGCTCA	GTGGCCTGCTTGGGCA
TNFα	MP 594	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC
GMCSF	MP 773	GCCATCAAAGAAGCCCTGAA	GCGGGTCTGCACACATGTTA
IL-3	MP 771	GGAAGCTCCCAGAACCTGAAC	TCAGAGAGGGTCCTTCATCATCA
GATA3	MP 604	AGAACCGGCCCCTTATCAA	AGTTCGCGCAGGATGTCC
c-maf	MP 1028	CCGAATTTTTCATGTGAGTGTGA	GACCCCCACGGAGCATTT
T-bet	MP 602	CAACAACCCCTTTGCCAAAG	TCCCCCAAGCAGTTGACAGT
BRG	MP 670	TCAACGGGCCTTTCCTCA	CCCAGTTTGACAGTGTCGAGAG
BRM	MP 777	CGCCATCATTGATACTGTGATAAAC	TGGAAGGTAACTGAATGAAGACTTCA
SNF2L	MP 749	CAGACTGGAATCCACAGGTTGA	CGTACTGGCTTCTTCTGACCAA
SNF2H	MP 620	TGTATTACAGGAAAACTATTGGGTATAAGGTA	TTAGGTAGATCAGGACTCCGAGGTA
CHD1	MP 1107	TCGCCCCTGCCTTCAGA	GAGCATCGGACACTACAGACTTTTT
CHD2	MP 1108	TCCTGGCCGAGTACCTGACTAT	CATCCAGGCGCTGGAAAG
CHD3/Mi-2α	MP 1109	GGAGGACTACCACACACTTACCAATT	GACATCGGGATCTTAGGATTCTTC
CHD4/Mi-2β	MP 1110	CTCCTTTGAAGACAATGCCATTC	ATGGTGATCAACTCATAGGATGTCA
CHD5	MP 1049	GCCCGCGCTGTACATGTC	CAGTGTAGGATCCGCTGAACTTT
CHD7	MP 1050	CAGAGATTGAGGATGACCTTTTTAATC	GTGCTACGTGCAAAGTCCATTATC
Hells	MP 634	GAATGCTGCCCGAACTTAAAA	CATTTGTGAAAACACCAGAACCTT
TBP	MP 935	CTTCGTGCAAGAAATGCTGAATAT	TGTCCGTGGCTCTCTTATTCTCA
actin	MP 598	AGAGGGAAATCGTGCGTGAC	CAATAGTGATGACCTGGCCGT

Primers for ChIP and DNase I

Locus	Primer name	Primer 1	Primer 2
Nfm	MP 855	CCACGGCGCTGAAGGA	CTGGTGCATGTTCTGGTCTGA
Th2 HSV/CNS2	MP 825	ATCACGTCGTCTTACCCAAACA	TGTGGGAGAGCGTCTGATCTG
Th2 HSVa	MP 823	CCCTCCATACAGTTCCTGCAGTA	ACCAGGGCACTTAAACATTGCT
Th2 HSIV	MP 821	CTGCATACCTTCCCTGATTGG	CCCTGCCCACATGAAATACC
Th2 IL-4 3'	MP 813	AAGGGTGGGAACTCCTGGAA	TTTGGCCATAAGGTTTCTACTGTTAG
Th2 IL-4 pro	MP 1132	GCAGGATGACAACTAGCTGGG	ACGGCACAGAGCTATTGATGG
Th2 CNS1	MP 827	CACACACTGGTCCACTGTGATG	GACGCAGGCACCAAAATTAAA
Th2 IL-13 3'	MP 817	ACTGGGCTCCTCAGTTTGGA	GCCTGCTTTGGCTGTCTTTT
Th2 IL-13 pro	MP 1111	ACCCAGAACCTGGAAACCCT	GTGGCCGCTAAAGGAAAGAGT
Th2 IL-13 -1k	MP 835	GCCAGATTCCATCTTCTCACAAA	GGGAGCTGGGCAGGACTAG
Th2 IL-13 -11k	MP 819	GGCAAGCCCCACACTGTT	ATGAGACGCACCAGCACAGA
Th2 RHS7	MP 998	TGCCTGCCCTGCAAACA	TGTGGGAGGAGATAGGACTCTTACC
Th2 RHS6b	MP 831	CACCTTTGTCACTTAAACACATCGT	GCAGCCAGTGCTCCTAAGGA
Th2 RHS6a	MP 829	TGCAGGCTGCTGAAACAGTAA	CAGAGATGAGCGTGGGTTAGAA
Th2 IL-5 -6k	MP 837	TGGCCAGGTATTGTCTTTCCA	CAAACCATAAATTCTGCCTAACCA
Th2 IL-5 pro	MP 815	TTTCCTCAGAGAGAGAATAAATTGCTT	GCTGGCCTTCAGCAAAGG
Th2 IL-5 +12k	MP 839	AAGGTGAAAGACTTGAATCAGCACTA	GGTGTGCATCTCTTCCTGTGTTT
GATA3 CNS3	MP 887	TGTTAAGGCGGTCATGAGTTGT	TGCGCTCCGAGTTTAAAGGT
GATA3 pro b	MP 934	CCCTGACGGAGTTTCCGTAGT	GGTGGACGTACTTTTTAACATCGA
GATA3 pro a	MP 885	CGGGTGGGCAAGAAACATT	GACAGTGGGCTGGCTTTGTAG
GM-CSF enh	MP 1006	CACGGGCAGGCATTCC	CAGCAAGGCTGTCTGATGCTAT
CD4 pro	MP 1313	TAGTCTGGCCTTGAGCTTGTG	ACCCCCAGTTGTTGGGGAAG
β2M pro	MP 1314	AGGCTGAACGACCAGATACAC	AGGTTACAAAGGGACTTTCCC
IFNγ pro	MP 807	TCCCGAGGAGCCTTCGA	CAGCCGATGGCAGCTATAGC

Primers for nascent (unspliced) RNA

Gene	Primer name	Primer 1	Primer 2
IL-4	MP 1079	TGTGCCAGGTCACTTGCAA	GCTTTTCGATGCCTGGATTC
IL-5	MP 1080	GCCAACCTGACAGTCTGTTCTTT	ACTCTTGCAGGTAATCCAGGAACT
IL-13	MP 1081	TGGGCTACTTCGATTTTGGTATC	CCCTCTAATGGATGGGCTTCT
IL-10	MP 1082	CCAATGGGTACTAACCAGATGCT	AATTCATTCATGGCCTTGTAGACA

siRNA Sequences, sense strands

Brg-1 siRNA (m), sc-29830, pool of three, Santa Cruz Biotechnology
CAACCCCAUUUAACCAGAAtt
GAACCCAGGGUAUGAAGUAtt
GGUAGAGUAUGUCAUCAAAtt
Brg-1 siRNA (m2), sc-44289, pool of three, Santa Cruz Biotechnology
CUGUGGAGAUCCUACAAGAtt
CAGGGAGUAUCACAGAUCAtt
GGUAGAGUAUGUCAUCAAAtt
siGENOME SMARTpool M-041135-00-0005, pool of 4, Dharmacon
GGUCAACGGUGUCCUCAAAUU
GAUAAUGGCCUACAAGAUGUU
GAGCGAAUGCGGAGGCUUAUU
CAACGGGCCUUUCCUCAUCUU

Supplementary references:

Frazer, K.A., Pachter, L., Poliakov, A., Rubin, E.M., and Dubchak, I. (2004). VISTA: computational tools for comparative genomics. Nucleic Acids Res *32*, W273-279.

Lieuw, K.H., Li, G., Zhou, Y., Grosveld, F., and Engel, J.D. (1997). Temporal and spatial control of murine GATA-3 transcription by promoter-proximal regulatory elements. Dev Biol *188*, 1-16.

Metivier, R., Penot, G., Hubner, M.R., Reid, G., Brand, H., Kos, M., and Gannon, F. (2003). Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. Cell *115*, 751-763.

Nie, Z., Xue, Y., Yang, D., Zhou, S., Deroo, B.J., Archer, T.K., and Wang, W. (2000). A specificity and targeting subunit of a human SWI/SNF family-related chromatin-remodeling complex. Mol Cell Biol *20*, 8879-8888.