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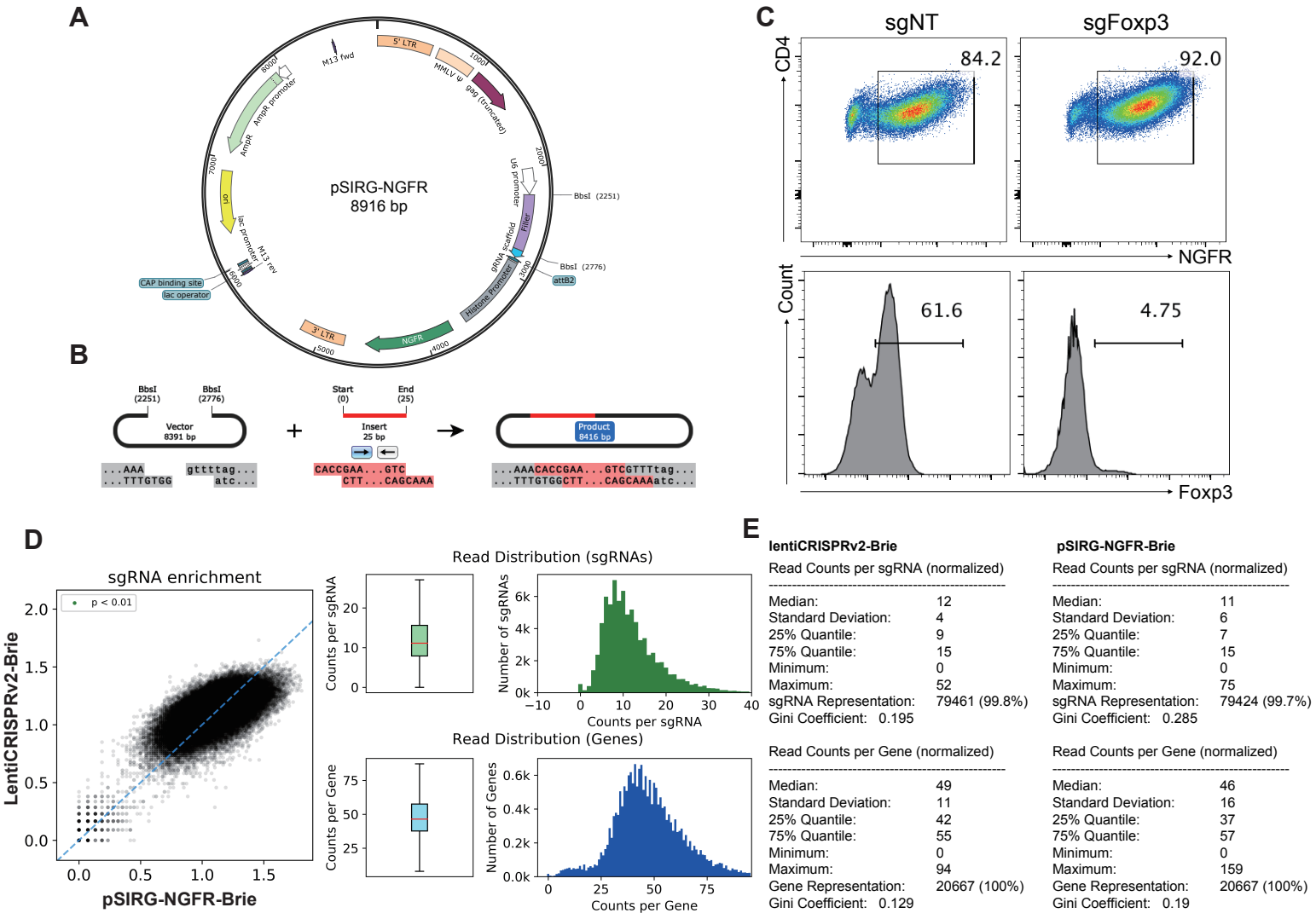
Supplemental Information

A Genome-wide CRISPR Screen Reveals a Role for the Non-canonical Nucleosome-Remodeling BAF Complex in Foxp3 Expression and Regulatory T Cell Function

Chin-San Loo, Jovylyn Gatchalian, Yuqiong Liang, Mathias Leblanc, Mingjun Xie, Josephine Ho, Bhargav Venkatraghavan, Diana C. Hargreaves, and Ye Zheng

Figure S1



E

lentiCRISPRv2-Brie	
Read Counts per sgRNA (normalized)	
Median:	12
Standard Deviation:	4
25% Quantile:	9
75% Quantile:	15
Minimum:	0
Maximum:	52
sgRNA Representation:	79461 (99.8%)
Gini Coefficient:	0.195
Read Counts per Gene (normalized)	
Median:	49
Standard Deviation:	11
25% Quantile:	42
75% Quantile:	55
Minimum:	0
Maximum:	94
Gene Representation:	20667 (100%)
Gini Coefficient:	0.129

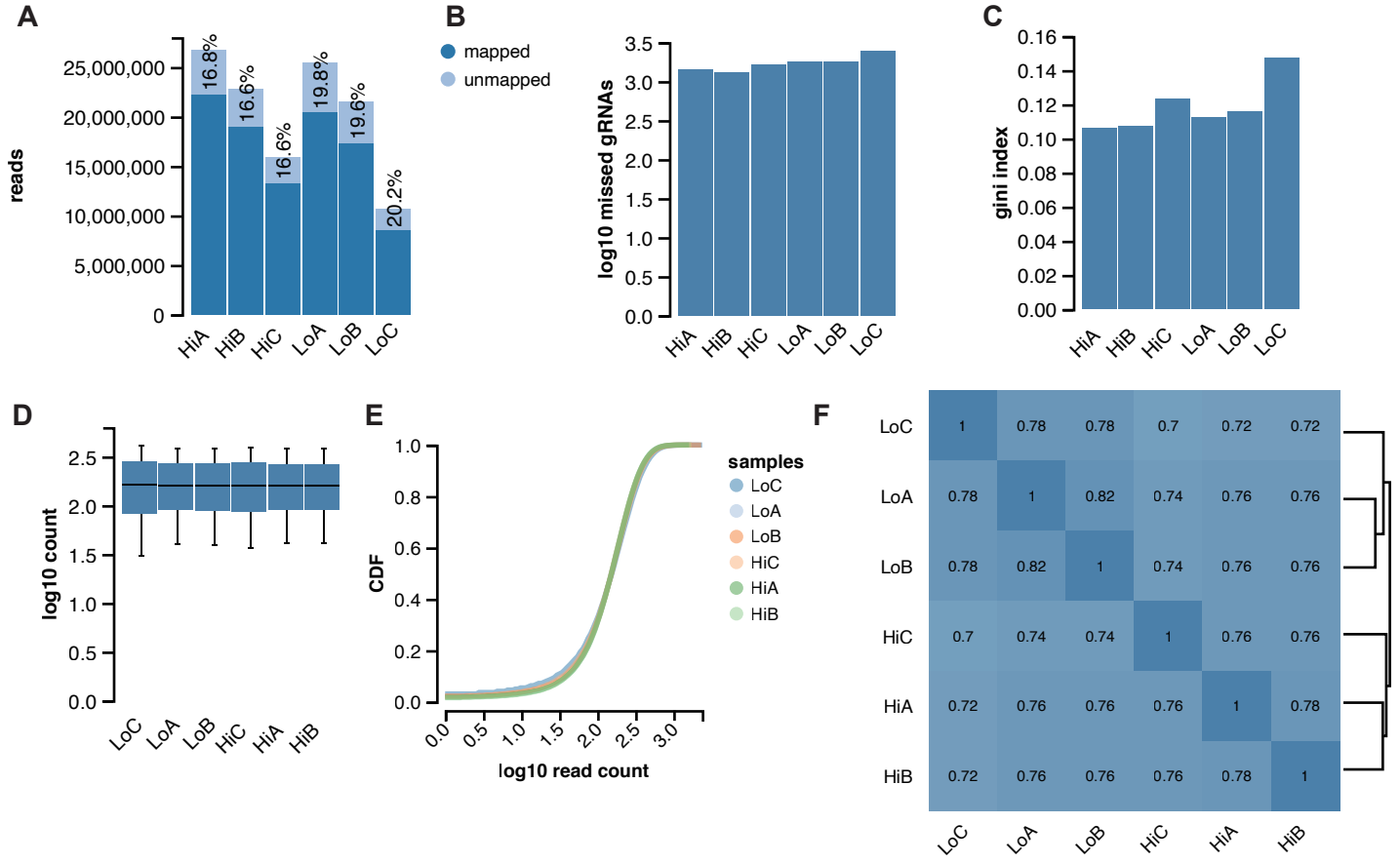
pSIRG-NGFR-Brie	
Read Counts per sgRNA (normalized)	
Median:	11
Standard Deviation:	6
25% Quantile:	7
75% Quantile:	15
Minimum:	0
Maximum:	75
sgRNA Representation:	79424 (99.7%)
Gini Coefficient:	0.285
Read Counts per Gene (normalized)	
Median:	46
Standard Deviation:	16
25% Quantile:	37
75% Quantile:	57
Minimum:	0
Maximum:	159
Gene Representation:	20667 (100%)
Gini Coefficient:	0.19

Figure S1. Construction of a retroviral sgRNA CRISPR library (pSIRG-NGFR-Brie), Related to Figure 1.

A, The map of pSIRG-NGFR. A self-inactivating retroviral vector containing a sgRNA expressing cassette and a truncated human NGFR surface marker. **B,** Overview of the process to clone a sgRNA into pSIRG-NGFR. A pair of annealed sgRNA oligomers can be directly cloned into BbsI-digested pSIRG-NGFR by T4 ligation. **C,** Validation of the transduction and knockout efficiency of pSIRG-NGFR. Cas9-expressing naïve CD4 T cells were transduced with either non-targeting control virus (sgNT) or Foxp3 targeting virus (sgFoxp3) in the presence of TGF-β and IL-2 for Foxp3 induction. NGFR and Foxp3 expression were measured by FACS 3 days post-infection. **D,** Correlation of sgRNA representation comparing lentiCRISPRv2-Brie library to pSIRG-NGFR-Brie library (left). Read distribution of sgRNAs and genes in pSIRG-NGFR-Brie (right). **E,** Statistics of sgRNAs and genes represented in lentiCRISPRv2-Brie and pSIRG-NGFR-Brie. Quantification of sgRNAs and genes was computed by PinAPL-Py program.

Figure S2

Foxp3^{Lo} Vs. Foxp3^{Hi}



Day6 Vs. Day3

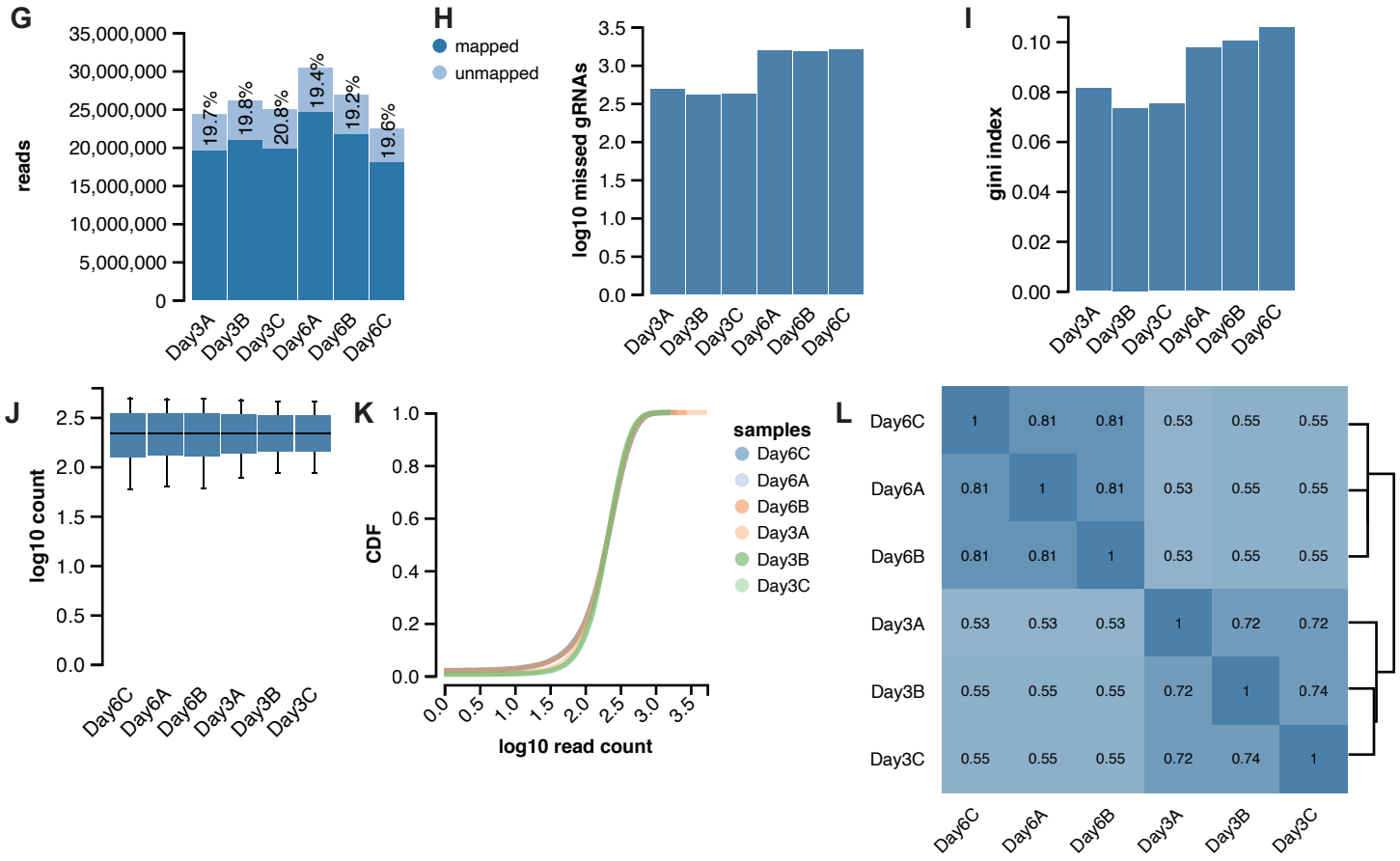


Figure S2. Quality control analysis of samples generated from the screen in Treg cells, Related to Figure 1.

Quality control analysis of samples comparing between Foxp3^{Lo} and Foxp3^{Hi} populations (**A-F**) or between Day 6 and Day 3 NGFR+ transduced populations (**G-L**). **A**, **G**, Mapped (dark blue) and unmapped (light blue) reads for each sample. Percentage of unmapped reads is labeled on each bar. **B**, **H**, Number of missed gRNAs with zero mapped reads. **C**, **I**, Gini Index for each sample measuring inequality between read counts. **D**, **J**, Distribution of normalized read counts for each sample. **E**, **K**, Cumulative distribution function of normalized read counts for each sample. **F**, **L**, Correlation between normalized log10 read counts of samples.

Figure S3

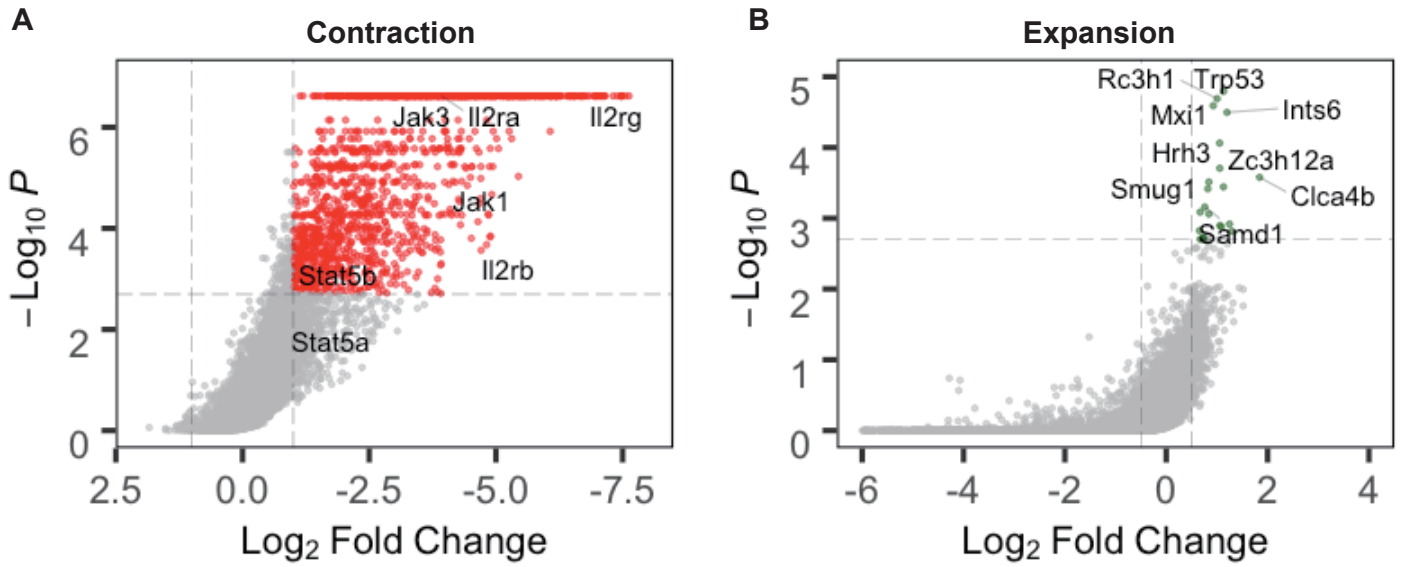


Figure S3. Identification of genes that regulate cell proliferation and survival from the screen in Treg cells, Related to Figure 2.

A,B Scatter plots showing genes enriched in the cell contraction pool (**A**) or cell expansion pool (**B**) by comparing NGFR+ transduced cells on day 6 to NGFR+ transduced cells on day 3, from the screen in Treg cells. Cutoff was set for contraction is P-value <0.002 and LFC>1 (Red dots), whereas cutoff for expansion was set P value <0.002 and LFC >0.5 (Green dots).

Figure S4

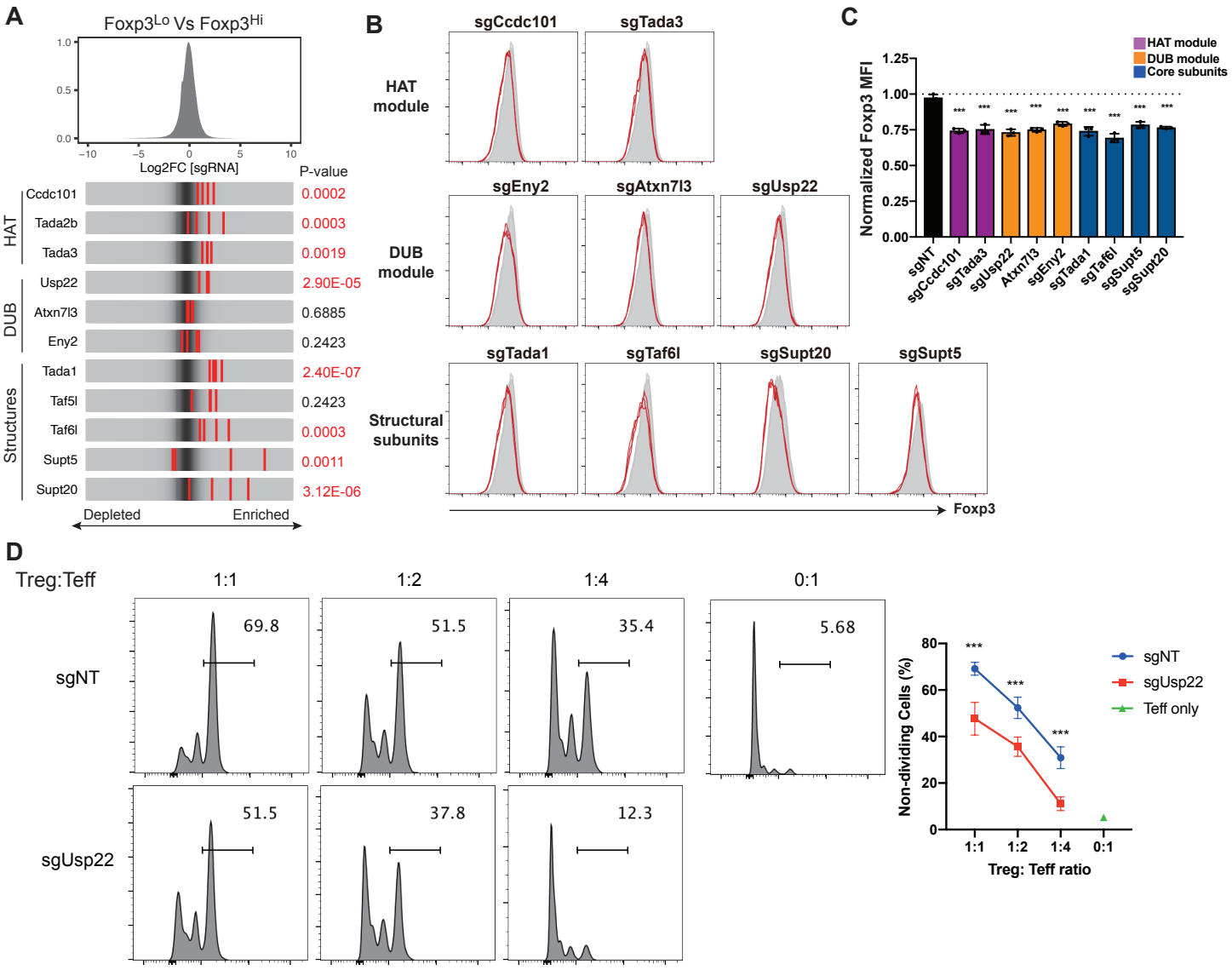


Figure S4. The SAGA complex regulates Foxp3 expression and Treg suppressor activity, Related to Figure 2.

A, Distribution of sgRNA Log2FC comparing Foxp3^{Lo} to Foxp3^{Hi}. Red stripes represent sgRNAs from positive Foxp3 regulators. Genes with a P-value of less than 0.01 are shown in red. **B**, FACS plot of Foxp3 expression in Treg cells transduced with sgRNAs against *Ccdc101*, *Tada3*, (HAT module), *Eny2*, *Atxn7l3* and *Usp22* (DUB module), and *Tada1*, *Taf6l*, *Supt20*, *Supt5* (structural subunits) of SAGA complex (n=3 per group.). **C**, Mean fluorescent intensity (MFI) of Foxp3 in Treg cells transduced with sgRNAs against SAGA subunits. **D**, In vitro suppression assay of Treg cells transduced with sgUsp22. sgNT is non-targeting control. n=3 per group. Data represent mean ± s.d. Statistical analyses were performed using unpaired two-tailed Student's t test (***p<0.001).

Figure S5

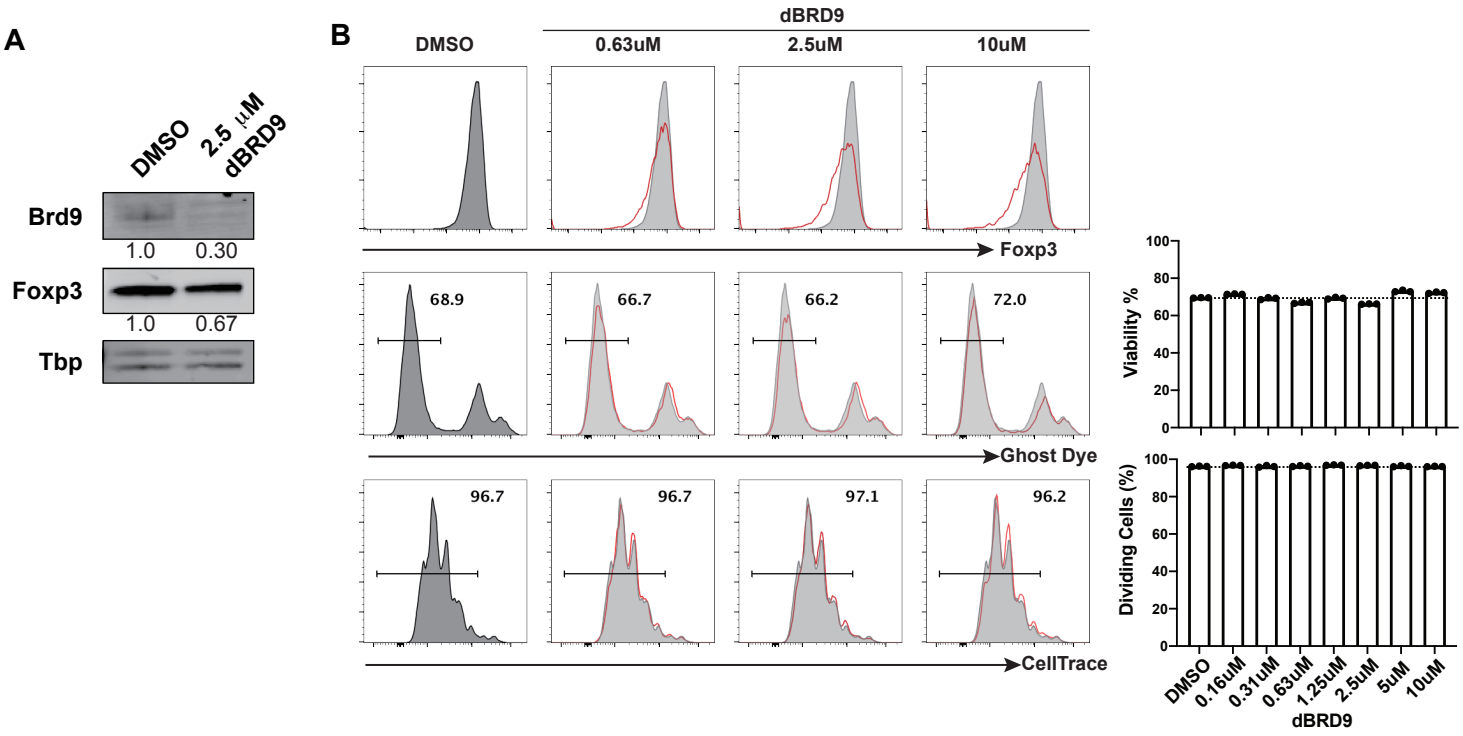


Figure S5. Brd9 degrader dBRD9 reduces Foxp3 expression without affecting cell viability and proliferation, Related to Figure 3

A, Immunoblotting analysis of Brd9, Foxp3, and TATA-binding protein (Tbp) in nuclear lysates from Treg cells treated with either DMSO or 2.5 μM dBRD9 for four days. Normalized protein levels are indicated. **B,** Foxp3 expression, cell viability labeled by Ghost Dye, and cell division determined by CellTrace dilution in Treg cells after treatment of dBRD9 in increasing concentrations for 4 days (n=3 per group). Grey shade: DMSO. Red line: dBRD9. See also Figure 3E. Data represents mean ± sd. Statistical analyses were performed using unpaired two-tailed Student's t-test. (*p<0.05, **p<0.01, ***p<0.001).

Figure S6

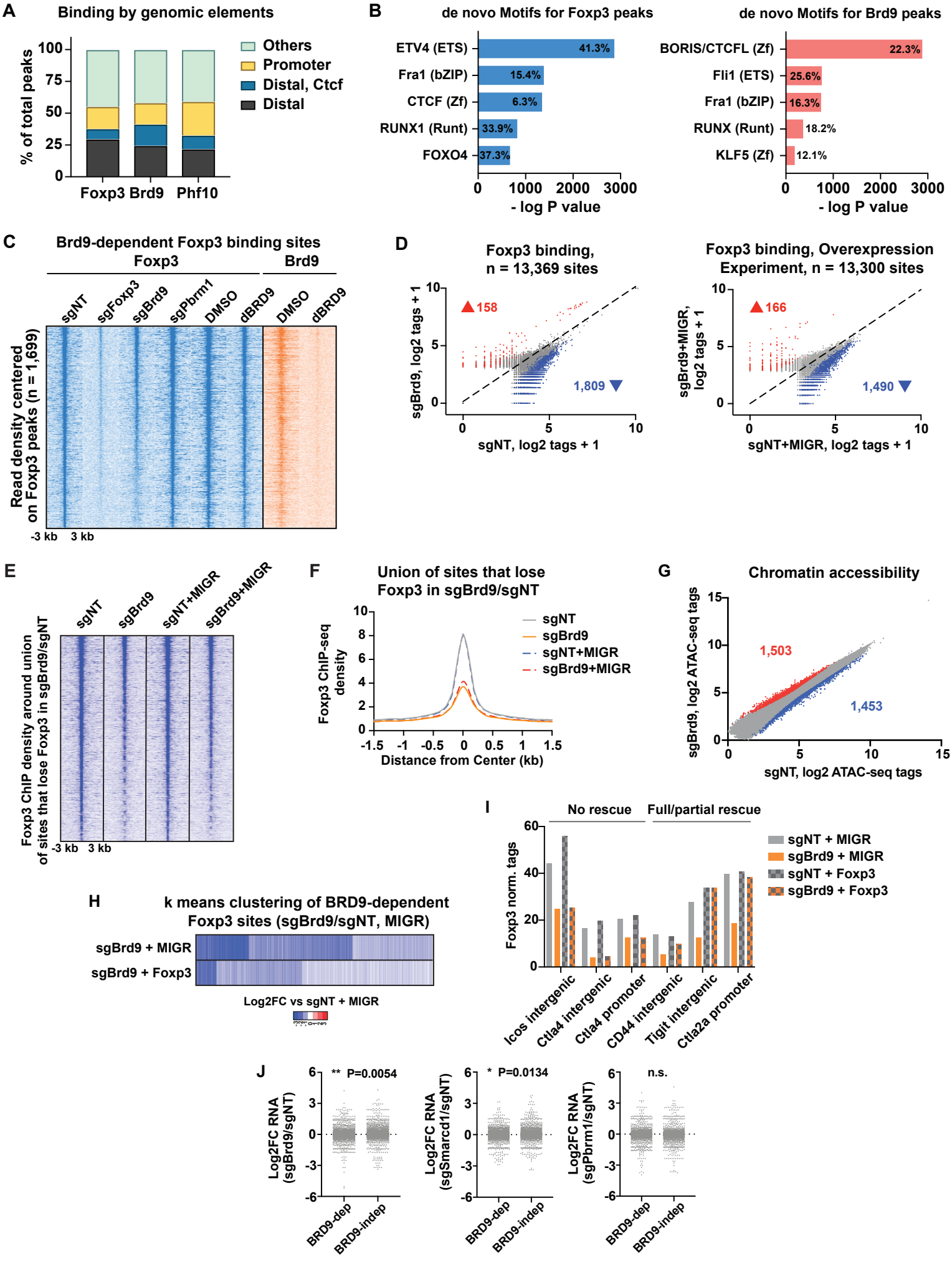

A
Binding by genomic elements
% of total peaks
100
75
50
25
0
Foxp3 Brd9 Phf10
Others
Promoter
Distal, Ctcf
Distal
B
de novo Motifs for Foxp3 peaks
ETV4 (ETS)
Fra1 (bZIP)
CTCF (Zf)
RUNX1 (Runt)
FOXO4
41.3%
15.4%
6.3%
33.9%
37.3%
0 1000 2000 3000
- log P value
de novo Motifs for Brd9 peaks
BORIS/CTCFL (Zf)
Fli1 (ETS)
Fra1 (bZIP)
RUNX (Runt)
KLF5 (Zf)
22.3%
25.6%
16.3%
18.2%
12.1%
0 1000 2000 3000
- log P value
C
Brd9-dependent Foxp3 binding sites
Foxp3
Brd9
sgNT
sgFoxp3
sgBrd9
sgPbrm1
DMSO
dBRD9
DMSO
dBRD9
Read density centered on Foxp3 peaks (n = 1,699)
-3 kb 3 kb
D
Foxp3 binding, n = 13,369 sites
158
1,809
sgBrd9, log2 tags + 1
sgNT, log2 tags + 1
Foxp3 binding, Overexpression Experiment, n = 13,300 sites
166
1,490
sgBrd9+MIGR, log2 tags + 1
sgNT+MIGR, log2 tags + 1
E
sgNT
sgBrd9
sgNT+MIGR
sgBrd9+MIGR
Foxp3 ChIP density around union of sites that lose Foxp3 in sgBrd9/sgNT
-3 kb 3 kb
F
Union of sites that lose Foxp3 in sgBrd9/sgNT
Foxp3 ChIP-seq density
sgNT
sgBrd9
sgNT+MIGR
sgBrd9+MIGR
Distance from Center (kb)
G
Chromatin accessibility
1,503
1,453
sgBrd9, log2 ATAC-seq tags
sgNT, log2 ATAC-seq tags
H
k means clustering of BRD9-dependent Foxp3 sites (sgBrd9/sgNT, MIGR)
sgBrd9 + MIGR
sgBrd9 + Foxp3
Log2FC vs sgNT + MIGR
I
No rescue
Full/partial rescue
Foxp3 norm. tags
sgNT + MIGR
sgBrd9 + MIGR
sgNT + Foxp3
sgBrd9 + Foxp3
Icos intergenic
Ctla4 intergenic
Ctla4 promoter
CD44 intergenic
Tigit intergenic
Ctla2a promoter
J
** P=0.0054
Log2FC RNA (sgBrd9/sgNT)
* P=0.0134
Log2FC RNA (sgSmarcd1/sgNT)
n.s.
Log2FC RNA (sgPbrm1/sgNT)
BRD9-dep
BRD9-indep

Figure S6. Brd9 and Foxp3 co-localize on chromatin; Brd9 regulates Foxp3 binding to a subset of Foxp3 binding sites, Related to Figure 4 and 5.

A, Stacked bar graph of sites bound by Foxp3, Brd9, and Phf10 that localize to the indicated genomic elements. **B**, Bar graph showing the top five *de novo* motifs enriched at Foxp3 (left) and Brd9 (right) ChIP-seq peaks, the percentage of sites that contain the motif, and the negative log of P value (Binomial distribution against random genomic background). **C**, Heatmap of Foxp3 ChIP-seq signal in sgNT, sgFoxp3, sgBrd9, and sgPbrm1 transduced Treg cells and DMSO- and dBRD9-treated Treg cells at sites that significanly lose Foxp3 binding in sgBrd9/sgNT and sgFoxp3/sgNT (FC 1.5, Poisson p value < 0.0001). BRD9 ChIP-seq signal is also shown in DMSO- and dBRD9-treated Treg cells. Signal is plotted ± 3 kb centered on Foxp3 peaks. **D**, Scatterplot of Foxp3 ChIP-seq tags in sgNT and sgBrd9 (left) and sgNT+MIGR and sgBrd9+MIGR (right) at all Foxp3-bound sites. Sites that are significantly up and down by 1.5-fold (Benjamin Hochberg FDR < 0.05) in sgBrd9 vs sgNT are colored red and blue, respectively. Black dashed line represents y = x. **E**, Heatmap of Foxp3 ChIP-seq density at the union of sites that significantly lose Foxp3 in sgBrd9 vs sgNT in the two experiments shown in D. **F**, Metaplot of Foxp3 ChIP read density surrounding the peak center of sites in E. **G**, Scatterplot of Log2 ATAC-seq mean tags of duplicates in sgNT versus sgBrd9 Treg cells. **H**, Heatmap of k-means clusters based on Log2FC Foxp3 ChIP-seq signal in sgBrd9+MIGR vs sgNT+MIGR and sgBrd9+Foxp3 vs sgNT+MIGR at sites that significantly lose Foxp3 binding in sgBrd9+MIGR vs sgNT+MIGR. **I**, Bar graph showing Foxp3 ChIP-seq signal at select genomic regions. **J**, Log2FC RNA in sgBrd9/sgNT, sgSmarcd1/sgNT, and sgPbrm1/sgNT of genes that are annotated to sites that are most and least affected by Brd9-dependent Foxp3 change in binding. See Methods section for details of analysis. Unpaired two-tailed Student's t test.

Figure S7

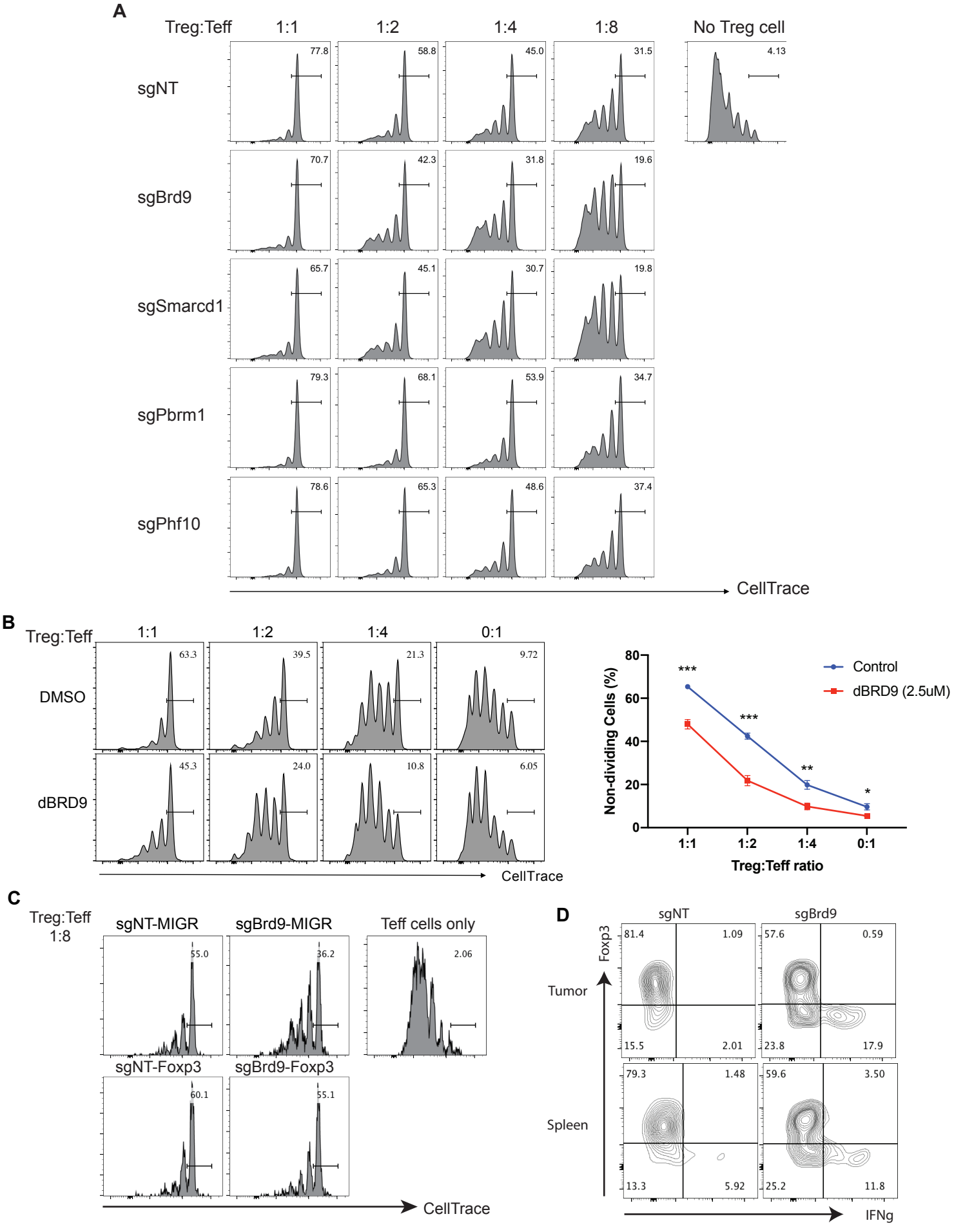

A
Treg:Teff
1:1
1:2
1:4
1:8
No Treg cell
sgNT
77.8
58.8
45.0
31.5
4.13
sgBrd9
70.7
42.3
31.8
19.6
sgSmarcd1
65.7
45.1
30.7
19.8
sgPbrm1
79.3
68.1
53.9
34.7
sgPhf10
78.6
65.3
48.6
37.4
CellTrace
B
Treg:Teff
1:1
1:2
1:4
0:1
DMSO
63.3
39.5
21.3
9.72
dBRD9
45.3
24.0
10.8
6.05
CellTrace
Non-dividing Cells (%)
Control
dBRD9 (2.5uM)
***
***
**
*
Treg:Teff ratio
C
Treg:Teff
1:8
sgNT-MIGR
sgBrd9-MIGR
Teff cells only
55.0
36.2
2.06
sgNT-Foxp3
sgBrd9-Foxp3
60.1
55.1
CellTrace
D
sgNT
sgBrd9
Foxp3
Tumor
81.4
1.09
15.5
2.01
57.6
0.59
23.8
17.9
Spleen
79.3
1.48
13.3
5.92
59.6
3.50
25.2
11.8
IFNg

Figure S7. sgRNA targeting of ncBAF or PBAF subunits or chemical degradation Brd9 alters Treg lineage stability and suppressor function. Related to Figure 6 and 7.

A, *In vitro* suppression assay of Treg cells transduced with sgBrd9, sgSmarcd1, sgPbrm1, and sgPhf10. sgNT was used as non-targeting control. See also Figure 6A. **B,** *In vitro* suppression assay using Treg cells treated with dBRD9 or vehicle DMSO. Representative histograms of effector T cell divisions in different Treg:Teff ratios. **C,** *In vitro* supression assay of Treg cells transduced with sgNT or sgBrd9, with ectopic expression of Foxp3 or empty vector MIGR. Representative histogram of effector T cells divisions in Treg:Teff mixed in 1:8 ratio. See also Figure 6B. (n=3 per group, data represent mean ± s.d.). Statistical analyses were performed using unpaired two-tailed Student's t test (ns: $p >= 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). **D,** FACS analysis of Foxp3 and IFN-γ expression in donor Treg cell population (CD4+ GFP+) in MC38 tumor and spleen at the end point. See also Figure 7J and 7K.