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Supplemental Information

Transcriptional Repressor HIC1 Contributes to Suppressive Function of Human Induced Regulatory T Cells

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Supplemental Experimental Procedures:

Isolation of Mouse $CD4^+$ Cells and Their Differentiation to iTreg

Single cell suspensions were prepared from mouse spleen using glass homogenizer. $CD4^+CD62L^+$ naïve cells were then isolated using Miltenyi kits following manufacturer's instructions (Miltenyi biotec 130-106-643). Cells were then activated with plate bound CD3 (325 ng/well; BD553238) and soluble CD28 (500 ng/ml; BD553294) either in absence of cytokines (Th0) or in the presence of IL-2 (12ng/ml), TGF-β (10ng/ml), and ATRA (10nM) (iTreg). Cells were seeded at the density of approximately 2 million cells per well in 1 ml IMDM media supplemented with 10% FCS, 500 µl penicillin/streptomycin, L-glutamine, 0.1% β-mercaptoethanol, 1X non-essential amino acid solution (NEAA).

siRNA-Mediated Gene Knockdown

For HIC1 knockdown experiments, freshly isolated $CD4^+$ $CD25^-$ cells were suspended in Optimem I (Invitrogen) and transfected with small interfering RNA (siRNA) oligonucleotides (Sigma) using the nucleofection technique (Lonza). Four million cells were transfected with 300 pmol of siRNA (HIC1-siRNA1: 5´-AGUUCGCACAGCAACGCAACCUCAU-3´, HIC1-siRNA2: 5´-CCUAGUCUCCUCUAUCGCUGGAUGA-3´, HIC1-siRNA3: 5´-CAUCGACCGUUUCUCUCCCACCUAG-3´ or non-targeting (NT) siRNA: AAUUCUCCGAACGUGUCACGU). The transfected cells were rested for 24 h in RPMI 1640 medium (Sigma-Aldrich) supplemented with pen/strep, 2 mM L-glutamine and 10% FCS at 37^0C ($2x10^6$ cells/ml) and subsequently activated and cultured as above.

Antibodies for Flow Cytometry

The following antibodies were used: anti-human FOXP3-PE (eBioscience, Cat. No. 12-4776-42); rat IgG2a isotype control (eBioscience, Cat. No. 72-4321-77A); anti-human HIC1 (H6) (Santa Cruz Biotechnology, Heidelberg, Germany, sc-271499) combined with Alexa Fluor 647-goat anti-mouse IgG (H+L) secondary antibody (ThermoFisher Scientific, A21235); and anti-human IFN-γ-FITC (Invitrogen, MHCIFG01). All samples were acquired by FACS LSRII (BD Biosciences, Franklin Lakes, NJ) and analyzed either with FlowJo (FLOWJO, LLC) or with Flowing Software (CBT, Turku, Finland).

Western Blotting

Samples were lysed in Triton-X sample buffer (50 mM Tris-HCl, pH 7.5; 150 mM NaCl; 0.5% Triton-X-100; 5% glycerol; 1% SDS), containing proteinase (Roche, Espoo, Finland) and phosphate inhibitors (Roche) and sonicated (Bioruptor UCD-200; Diagenode, Seraing, Belgium). Sonicated samples were centrifuged at maximum speed for 20 min at 4°C, and supernatants were collected. Samples were quantified (DC Protein Assay; Bio-Rad) and boiled with 6× loading dye (330 mM Tris-HCl, pH 6.8; 330 mM SDS; 6% β-ME; 170 µM bromophenol blue; 30% glycerol). Samples were loaded on 10% or 12%

Mini-PROTEAN TGX Precast Protein Gels (BioRad Laboraotries, Helsinki, Finland) and transferred to PVDF membranes (Trans-Blot Turbo Transfer Packs, BioRad Laboratories). The following antibodies were used: 1:1000 anti-human HIC1 (H-123) rabbit polyclonal (Santa Cruz, sc-28703), 1:1000 anti-human HIC1 (H6) mouse monoclonal (Santa Cruz**,** sc-271499), 1:1000 anti-human FOXP3 (PCH101) monoclonal (eBioscience, Ref 14-4776-82), 1:200 TBET (Santa Cruz, sc-21749), 1:1000 GATA3 (BD Pharmingen, 558686), 1:10000 anti-GAPDH (HyTest, *5G4*. *MAbs*: *6C5)*, 1:10000 anti-Tubulin monoclonal (Sigma, T6074) and LSD1 (Diagenode, Cat# 15410067). Horseradish peroxidase-conjugated anti-mouse IgG (Santa Cruz, sc-2005) and anti-rabbit IgG (BD Pharmingen, 554021) were used as secondary antibodies.

Cell Fractionation

For the cellular localization of HIC1 protein, cytoplasmic and nuclear extracts from Th0 and iTreg cells were fractionated at 72 and 120 h post-activation and polarization using the NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (ThermoFisher Scientific, 78833) and analyzed by western blotting on 4–20% SDS-PAGE Mini-PROTEAN® TGX™ Precast Protein Gels (BioRad Laboratories 4561094). The localization of HIC1 expression in cytoplasm and nuclear fractions was detected using a primary antibody specific for HIC1 (1:1,000) (Santa Cruz Biotechnology, sc-271499). Primary antibodies for α-tubulin (Sigma, T6074) and LSD1 (Digenode, C15410067) were used as loading control for cytoplasmic and nuclear fractions, respectively.

RNA Isolation, RNA-Seq Sample Preparation and Data Analysis

RNA was isolated (RNeasy Mini Kit; QIAGEN, Hilden, Germany) and treated in-column with DNase (RNase-Free DNase Set; QIAGEN) for 15 min. The removal of genomic DNA was ascertained by an additional treatment of the samples with DNase I (Invitrogen). After RNA quantification (using Nanodrop 2000) and quality control (using BioRad Experion and Agilent Bioanalyzer), libraries for RNA-Seq were prepared using Illumina TruSeq® Stranded mRNA Sample Preparation Guide (part #15031047). The high quality of the libraries was confirmed with Advanced Analytical Fragment Analyzer (Advanced Analytical Technologies, Heidelberg, Germany) or with Agilent Bioanalyzer, and the concentrations of the libraries were quantified with Qubit® Fluorometric Quantitation (Life Technologies, ThermoFisher). Sequencing was performed at the Finnish Functional Genomics Centre (FFGC) using HiSeq2500 or Hiseq3000 Next-Generation Sequencing platforms.

*Mapping***:** Paired-end reads were aligned with Tophat (Kim et al., 2013) (version 2.0.8b for iTreg/Th0 data and version 2.1.1 for HIC1 data) and Bowtie2 (version 2.1.0 or iTreg/Th0 data and version 2.2.5 for HIC1 data) to the human reference genome (hg19) and Gencode transcriptome (v19). Based on the calculation for one million reads mapped to the reference

genome, we set the mean insert size and standard deviation for each sample separately. Read counts were determined using HTSeq-count (version 0.6.1) (Anders et al., 2015) with parameters "–stranded no –order name."

DE calling: Bioconductor package edgeR (Robinson et al., 2009) was used for differential expression calling. Genes with Reads Per Kilobase of transcript per Million mapped reads (RPKM) <3 in at least two samples in both conditions were filtered out before differential expression calling. Differential expression calling was done for each time separately and taking into account the paired design in the experiments. The dispersion was estimated as gene-wise dispersion. Finally, DE genes were detected with cut offs FDR<0.05 for HIC1 KD data and FDR<0.05 and |log2 FC|>1 for iTreg/Th0 time series data.

Chromatin Immunoprecipitation Assay (ChIP) and ChIP-Seq

$CD4^+$ T cells were cultured in iTreg polarizing condition for 72 h. ChIP was performed as described (Hawkins et al., 2013) with slight modifications. The cells were subjected to sonication using Bioruptor® Pico sonication device (Diagenode) to obtain 100–500-bp chromatin fragments. A total of 250 μg of sonicated chromatin fragments were incubated with 10 μg of HIC1 antibody (Santa Cruz**,** sc-271499) and incubated for crosslinking with magnetic beads (no. 11201D, Dynabeads® M-280 sheep anti-mouse IgG, Dynal Biotech, Invitrogen). The crosslink samples were reversed at 65°C overnight, and precipitated DNA was treated with Rnase A and proteinase K and purified with QIAquick PCR purification kit (QIAGEN). DNA libraries were prepared as per the guidelines from Illumina by Fasteris Life Sciences (Plan-les-Ouates, Switzerland). Input DNA was sequenced and used as a control. DNA libraries were sequenced on Illumina HiSeq2500 producing 25–35 million reads per sample. The 50-nucleotide reads were aligned to the hg19 build of human reference genome by bowtie2 (version 2.2.9) (Langmead and Salzberg, 2012). Uniquely mapped reads were retained (~20–25 million reads per sample) for further analysis. HIC1 binding sites relative to input DNA were identified using MACS2 (version 2.1.1.20160309) (Zhang et al., 2008) with the default parameter settings. *De novo* motifs from HIC1 ChIP-Seq peaks were discovered using Homer (version 4.8) (Heinz et al., 2010). The binding sites were annotated in terms of genomic annotations using the "annotatePeaks.pl" script supplied as part of Homer. HIC1 motif locations in ChIP-Seq peaks were scanned using Homer with default parameters (with the addition of –local argument).

SNP Analysis

For the RNA-Seq data, SNPs from NHGRI GWAS catalog (Welter et al., 2014) were extracted, and genes that were located ±100 kb of SNPs were associated with the corresponding diseases. For time series data, genes that were DE at any time point were considered for the analysis. For HIC1 knock-down data, genes that were regulated by HIC1 in at least one time were

considered. The diseases with more than one associated gene were considered for the analysis. Disease enrichment was calculated using hypergeometric distribution, and Benjamini-Hochberg method (Benjamini and Hochberg, 1995) was used for multiple testing corrections of p-values.

For ChIP-Seq data, the SNPs of 11 autoimmune diseases in NHGRI catalog were extracted. SNPs from studies with meta-analysis of more than one disease and from population other than Caucasian were excluded from further analysis. Using SNAP server (Johnson et al., 2008), proxies within a distance of 100 kb and r2 > 0.8 were determined in CEU population from 1000 genomes data. Proxies and lead SNPs were overlaid with HIC1 ChIP-Seq peaks.

Analysis for enrichment of HIC1 sites was carried out separately for each trait. SNPs from the HLA region were excluded, and correlated SNPs were clumped (distance = 1000 kb and LD r2 = 0.8). Random sampling of 1000 SNP sets from EUR population, with the same size as the original disease set, was done using SNPsnap (Pers et al., 2015) with default parameters (except distance = 1000 kb, LD buddies ±20%, r2 = 0.8). Proxies for both the disease associated SNPs and randomly generated SNPs within a window of 1000 kb and r2 = 0.8, were generated using plink (version 1.9) (Chang et al., 2015) from 1000 genomes EUR population. The proxy SNPs together with the lead SNPs were then overlaid with HIC1 ChIP-Seq peaks and p-values were determined based on the empirical background distribution. The Benjamini-Hochberg method (Benjamini and Hochberg, 1995) was used for multiple testing correction.

Network Analysis

The HIC1-TF network was constructed and visualized in Cytoscape (Shannon et al., 2003). The connections (edges) include the ChIP-Seq and RNA-Seq data as well as protein-DNA interaction and protein-protein interaction of TFs from IPA (www.ingenuity.com).

DNA Affinity Precipitation Assay

Biotinylated sense and non-biotinylated antisense bait oligonucleotides were purchased from Sigma (UK). HIC1-specific sequences were used as a positive control. Oligonucleotide probes containing HIC1 DNA binding motif, with or without SNP mutation were designed as shown in Table S5. Mutations introduced to the oligonucleotides are highlighted in red and bold. Annealing of all oligonucleotides was performed by incubating them at 95°C for 5 min, followed by gradual cool down at room temperature (RT). Neutravidin beads (Ultralink immobilized neutravidin protein, Pierce) were washed 4x with buffer A (10 mM HEPES pH 7.9, 60 mM KCl, 2 mM EDTA, 1 mM EGTA, 0.1% Triton X-100, 1 mM DTT, and protease and phosphatase inhibitors from Roche). Annealed oligonucleotides were incubated with 25–30 µl of beads in 250 µl buffer A for 90 min at +4°C with rotation at 360° rotator, followed by 4x wash with buffer A. Nuclear fractions prepared from iTreg

cultures for 72 h and further diluted with 60 mM KCl using buffer 2 (10 mM HEPES, pH 7.9, 2 mM EDTA, 1 mM EGTA, 0.1% Triton X-100, 1 mM DTT, and protease and phosphatase inhibitors from Roche) to dilute any KCl salt. Pre-clearing was performed with unconjugated beads by incubating for 90 min in a 360° rotator at +4°C. Pre-cleared lysates were subjected for binding reactions by incubating with bead-conjugated oligonucleotides for 3 h at +4°C, followed by washing four times with buffer A. Protein pull-down precipitates were eluted by incubating beads at 95°C for 5 min in 100 µl of 2xSDS buffer (125 mM Tris-HCl, pH 6.8, 4% w/v SDS, 20% glycerol, 100 mM DTT). HIC1 protein was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and western blotting was performed using the mouse monoclonal HIC1 antibody from Santa Cruz (H-6).

Analysis of Transcription Factor Binding Sites (TFBS)

Overrepresentation of TFBS on the promoters of DE genes was performed using the commercial version of an FMatch tool at the TRANSFAC database (Release 2017.2). For respective time points, a separate analysis was performed for up- and downregulated genes. The -10 kb to +1 kb sequence was taken as a promoter except for 72 h DE genes because it exceeded the limit that can be analyzed by the tool. Therefore, for 72 h DE genes, -8kb to +800 bp was used instead. A randomly generated gene set of approximately the same size was taken as a background for calculating overrepresentation. A custom profile was generated where we only took matrices corresponding to the TFs DE in Th17 or iTreg cells (Table S2). For 311 DE TFs, TRANSFAC database had 332 high quality motifs corresponding to 184 TFs. We tested if the promoters in test set had enrichment of these motifs as compared to a set of background genes of similar size. The enrichment was calculated using binomial test. The p-value was corrected by Benjamini & Hochberg method in r (version 3.3.1). FDR<0.05 was considered significant.

Gene Set Enrichment Analysis

The gene set for the analysis was “iTreg signature genes”, which were defined as those upregulated more than fourfold in iTreg conditions at 48 h in the time series data. The enrichment in HIC1-deficient RNA-Seq data of 48 h was calculated using GSEA tool (Subramanian et al., 2005). The parameters were as follows. Number of permutations: 1000; permutation type: phenotype; enrichment statistics: weighted; metric for ranking: Signal2Noise; gene list sorting: real. Signal2Noise metric uses the “difference of mean scaled by standard deviation”. Signal to noise ratio is defined as $(\mu_A-\mu_B)/(\sigma_A+\sigma_B)$ where μ is mean and σ is standard deviation of samples A and B. The p-value cutoff used was 0.05.

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Figure S1

Supplemental Figures and Legends

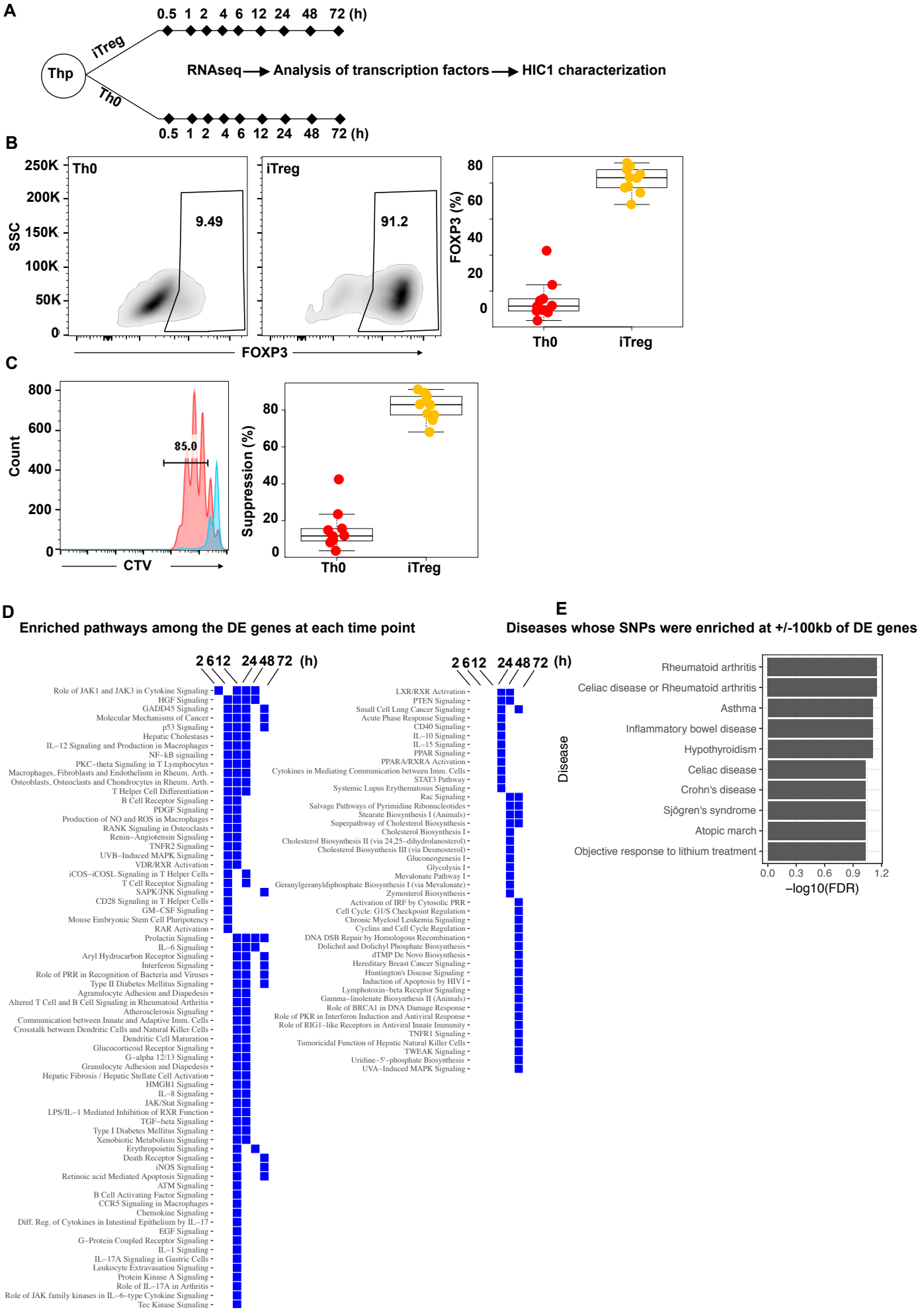


Figure S1. Immune Related Pathways and Autoimmune Disease Associated SNPs are Enriched in and Around iTreg DE Genes. Related to Figure 1.

(A) Overall design of the study. Naive $CD4^+$ T cells were isolated from cord blood and differentiated to iTreg. Cells that received only TCR stimulations served as control (Th0). RNAseq analysis on three biological replicates was performed at the indicated time points (h).

(B) FOXP3 expression was measured in Th0 and iTreg cells at 72 h in one representative experiment (left) and in 10 biological replicates (right).

(C) Suppression of responder cell proliferation by Th0 (red) and iTreg (blue) cells was measured after 4 days. The left panel shows suppression in a representative experiment, and the right panel shows the data from 10 biological replicates.

(D) IPA pathways enriched among the DE genes in iTreg cells at different time points (indicated on top) are shown. Only pathways with FDR0.01 are shown in the figure. All enriched pathways can be found in Table S2. Each filled square represents enrichment. Names of some pathways are shortened for clarity. Left panel shows pathways that are enriched at 2-72 h while right panel shows pathways that are enriched only at later time points (24-72 h).

(E) NHGRI diseases/traits whose SNPs were enriched near (±100 kb) genes DE at one or more time points during differentiation are shown. Disease with FDR <0.1 are shown in the figure. Complete list of diseases with their FDR can be found in Table S3: Sheet 1.

Supplemental Figures and Legends

Figure S2

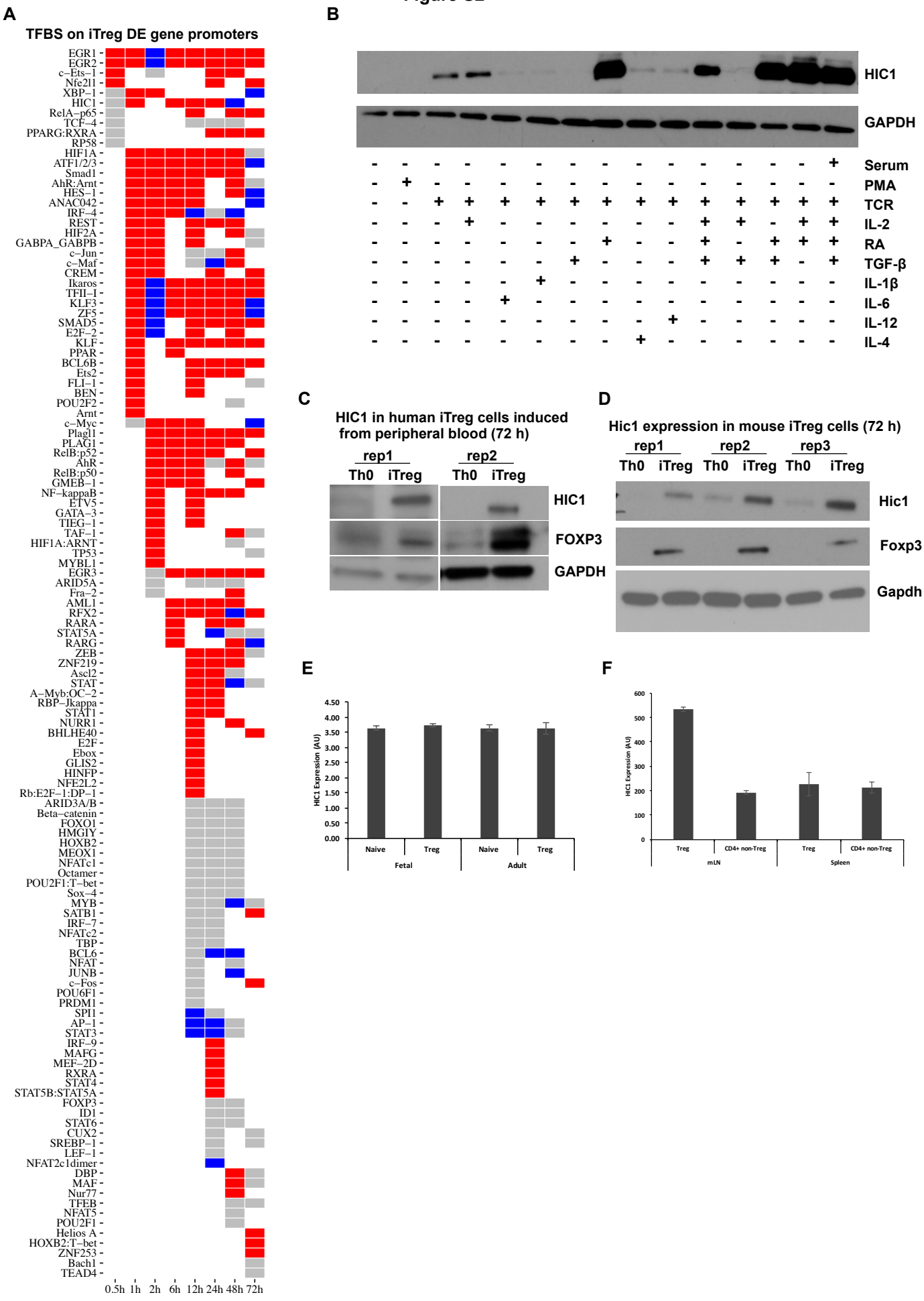


Figure S2. Binding sites for RA Induced TF HIC1 are enriched on the promoters of iTreg DE genes. Related to Figure 1 and 2.

(A) Tile plot showing enrichment of TFBS on the promoters of up- (red) and downregulated (grey) genes. Blue indicates the enrichment of the TF both on up as well as downregulated gene promoters. The time points are indicated at the bottom.

(B) The cells were activated in presence (+) or absence (-) of indicated factors for 24 h followed by measurement of HIC1 expression by WB. A representative of three biological replicates is shown.

(C) The blots are showing the expression of HIC1 in human iTreg cells differentiated from $CD4^+$ T cells isolated from peripheral blood (72 h).

(D) The blots are showing the expression of Hic1 in mouse iTreg and Th0 cells cultured for 72 h.

(E) The bar chart is showing the expression of HIC1 in naive T cells and Treg cells isolated from blood. The data is taken from GSE25087.

(F) The bar chart is showing the expression of Treg and effector T cells from mLN and spleen of mice. The data is taken from GSE41229.

Figure S3

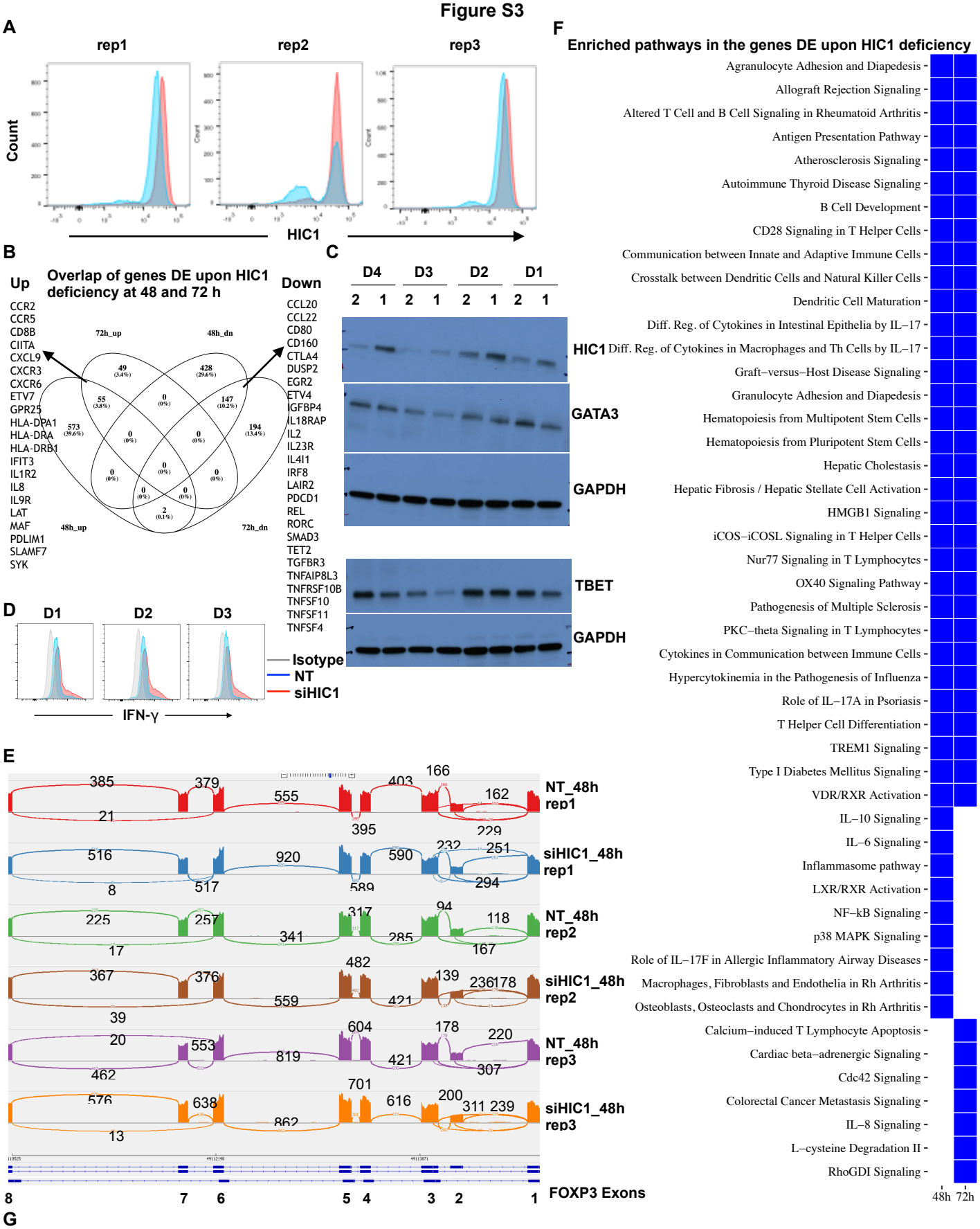

A
rep1
rep2
rep3
Count
HIC1
B
Up
Overlap of genes DE upon HIC1 deficiency at 48 and 72 h
Down
CCR2
CCR5
CD8B
CIITA
CXCL9
CXCR3
CXCR6
ETV7
GPR25
HLA-DPA1
HLA-DRA
HLA-DRB1
IFIT3
IL1R2
IL8
IL9R
LAT
MAF
PDLIM1
SLAMF7
SYK
72h_up
48h_dn
48h_up
72h_dn
49 (3.4%)
428 (29.6%)
55 (3.8%)
0 (0%)
147 (10.2%)
573 (39.6%)
194 (13.4%)
2 (0.1%)
CCL20
CCL22
CD80
CD160
CTLA4
DUSP2
EGR2
ETV4
IGFBP4
IL18RAP
IL2
IL23R
IL4I1
IRF8
LAIR2
PDCD1
REL
RORC
SMAD3
TET2
TGFBR3
TNFAIP8L3
TNFRSF10B
TNFSF10
TNFSF11
TNFSF4
C
D4
D3
D2
D1
2 1 2 1 2 1 2 1
HIC1
GATA3
GAPDH
TBET
GAPDH
D
D1
D2
D3
Isotype
NT
siHIC1
IFN-γ
E
NT_48h rep1
siHIC1_48h rep1
NT_48h rep2
siHIC1_48h rep2
NT_48h rep3
siHIC1_48h rep3
FOXP3 Exons
8 7 6 5 4 3 2 1
F
Enriched pathways in the genes DE upon HIC1 deficiency
Agranulocyte Adhesion and Diapedesis
Allograft Rejection Signaling
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis
Antigen Presentation Pathway
Atherosclerosis Signaling
Autoimmune Thyroid Disease Signaling
B Cell Development
CD28 Signaling in T Helper Cells
Communication between Innate and Adaptive Immune Cells
Crosstalk between Dendritic Cells and Natural Killer Cells
Dendritic Cell Maturation
Diff. Reg. of Cytokines in Intestinal Epithelia by IL−17
Diff. Reg. of Cytokines in Macrophages and Th Cells by IL−17
Graft−versus−Host Disease Signaling
Granulocyte Adhesion and Diapedesis
Hematopoiesis from Multipotent Stem Cells
Hematopoiesis from Pluripotent Stem Cells
Hepatic Cholestasis
Hepatic Fibrosis / Hepatic Stellate Cell Activation
HMGB1 Signaling
iCOS−iCOSL Signaling in T Helper Cells
Nur77 Signaling in T Lymphocytes
OX40 Signaling Pathway
Pathogenesis of Multiple Sclerosis
PKC−theta Signaling in T Lymphocytes
Cytokines in Communication between Immune Cells
Hypercytokinemia in the Pathogenesis of Influenza
Role of IL−17A in Psoriasis
T Helper Cell Differentiation
TREM1 Signaling
Type I Diabetes Mellitus Signaling
VDR/RXR Activation
IL−10 Signaling
IL−6 Signaling
Inflammasome pathway
LXR/RXR Activation
NF−kB Signaling
p38 MAPK Signaling
Role of IL−17F in Allergic Inflammatory Airway Diseases
Macrophages, Fibroblasts and Endothelia in Rh Arthritis
Osteoblasts, Osteoclasts and Chondrocytes in Rh Arthritis
Calcium−induced T Lymphocyte Apoptosis
Cardiac beta−adrenergic Signaling
Cdc42 Signaling
Colorectal Cancer Metastasis Signaling
IL−8 Signaling
L−cysteine Degradation II
RhoGDI Signaling
48h 72h
G
TFs whose binding sites are enriched on the promoters of the genes DE upon HIC1 deficiency
72h
48h
Egr−2
MYB
SPI1
SREBP−1
HOXB2;T−bet
IRF−9
NFIX
PLAG1
PPARGAMMA
Smad1
Ebox
HIF1A:ARNT
RelA−p65
TP53
ZEB
AhR
Bcl−6
c−MAF
IRF−4
STAT3
A−Myb:FIGLA
AhR:Arnt
AML1
ANACO42
Arnt
Ascl2
ATF−1
ATF−1
BEN
c−Ets−1
c−Rel
CREM
EGR
ETV7
GMEB1
Hbp1
HES−1
HIF−1alpha
HIF2A
Ikaros
JunB
KLF7
NF−kappaB
NFAT5
NFE2L2
NFKAPPAB
NUR81
RARA
Rb:E2F−1:DP−1
RBP−Jkappa
RelB:p52 (NF−kappaB)
REST
TFEB
TFII−I
TIEG−1
ZFP202
KLF
ZF5
EGR−1
BCL6B
E2F−2
EGR3
KLF5
Plag1
GRHL1
MAF
SMAD5
XBP−1
AP−1
ARID5A
Beta−catenin
c−Jun
CUX2
HMGIY
HOXB2
ID1
IRF−7
LEF−1
MEOX1
NFAT2:dimer
NFATc1
NFATc2
Octamer
POU2F1:T−bet
SATB1
Sox−4
STAT5A
STAT6
TBP
TCF−4
FOXO1

Figure S3. HIC1 Deficient iTreg Cells Have Effector Like Gene Expression Signature. Related to Figure 3.

(A) Overlay histograms plots are showing HIC1 expression in NT- (red) and siHIC1- (blue) treated iTreg cells at 48 h in the three replicates of RNAseq samples.

(B) Venn diagram is showing the overlap of genes DE (FDR <0.05) upon HIC1 deficiency at 48 and 72 h. A list of both up and downregulated interesting genes are shown on the sides.

(C) NT- (1) or siHIC1- (2) treated cells from four biological replicates (D1-4) were cultured in iTreg conditions for 72 h. WB analysis is showing regulation of TBET and GATA3 in HIC1-deficient cells. GAPDH was used as loading control.

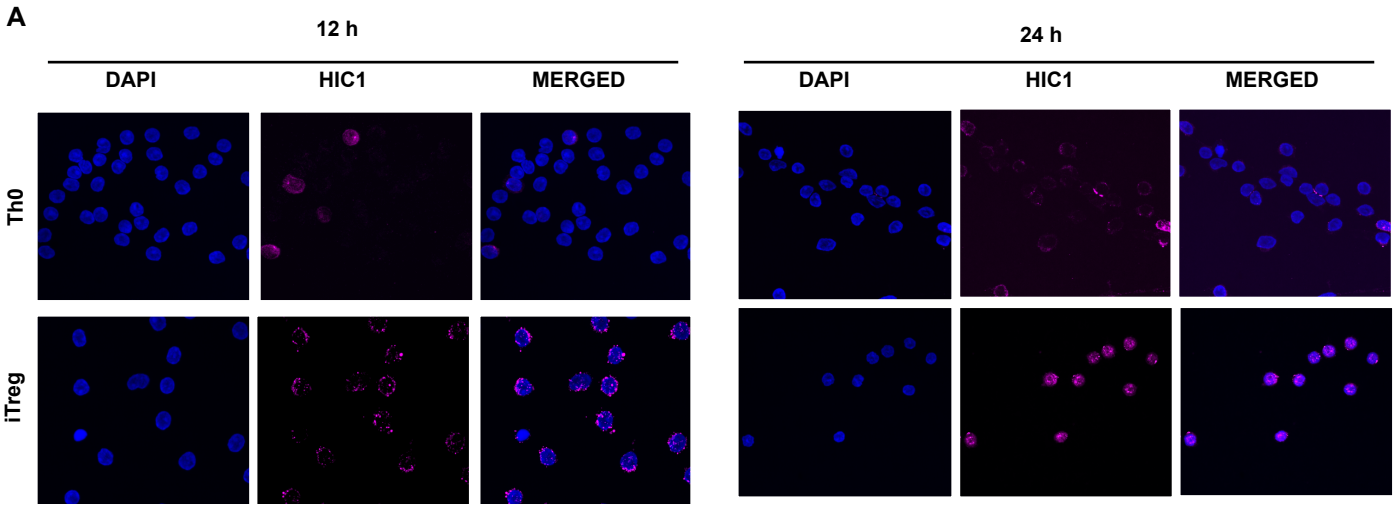
(D) Histogram plots are showing the intracellular IFN-γ expression by NT or siHIC1 treated iTreg cells upon re-stimulation. The data are from three donors (D1-3).

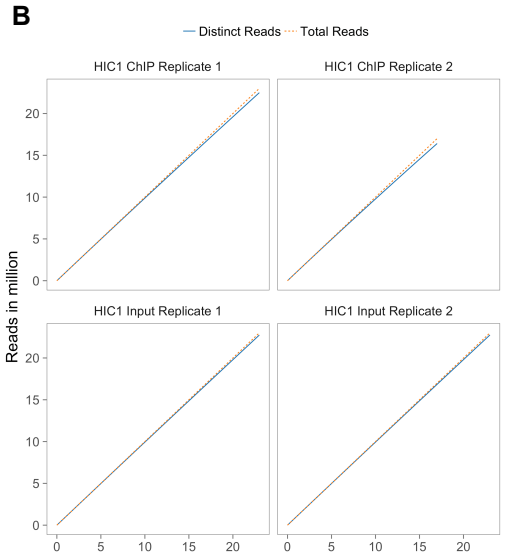
(E) Sashimi plots of 48 h HIC1 KD RNAseq data showing the number of sequencing reads connecting the FOXP3 exons in the three replicates. Samples (NT/siHIC1) are labeled on the right.

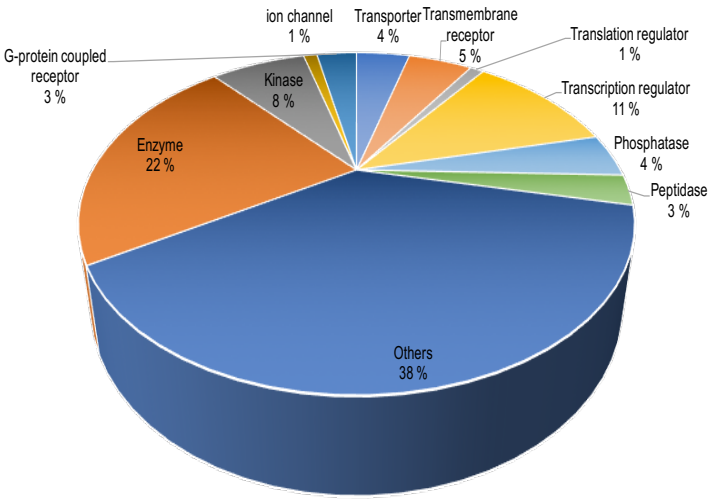
(F) The tile plot is showing the IPA pathways that were enriched among the genes DE upon HIC1 silencing at the indicated time points (bottom).

(G) Tile plot is showing enrichment of TFBS on the promoters of genes DE upon HIC1 silencing. The enrichment on the promoters of up- (red) and downregulated (grey) genes are shown. Blue indicates the enrichment of the TF both in up as well as downregulated gene promoters. The time points are indicated on the right.

Figure S4


A
12 h
24 h
DAPI
HIC1
MERGED
DAPI
HIC1
MERGED
Th0
iTreg


B
Distinct Reads
Total Reads
HIC1 ChIP Replicate 1
HIC1 ChIP Replicate 2
HIC1 Input Replicate 1
HIC1 Input Replicate 2
Reads in million


C
ion channel 1 %
Transporter 4 %
Transmembrane receptor 5 %
Translation regulator 1 %
Transcription regulator 11 %
Phosphatase 4 %
Peptidase 3 %
Others 38 %
Enzyme 22 %
Kinase 8 %
G-protein coupled receptor 3 %

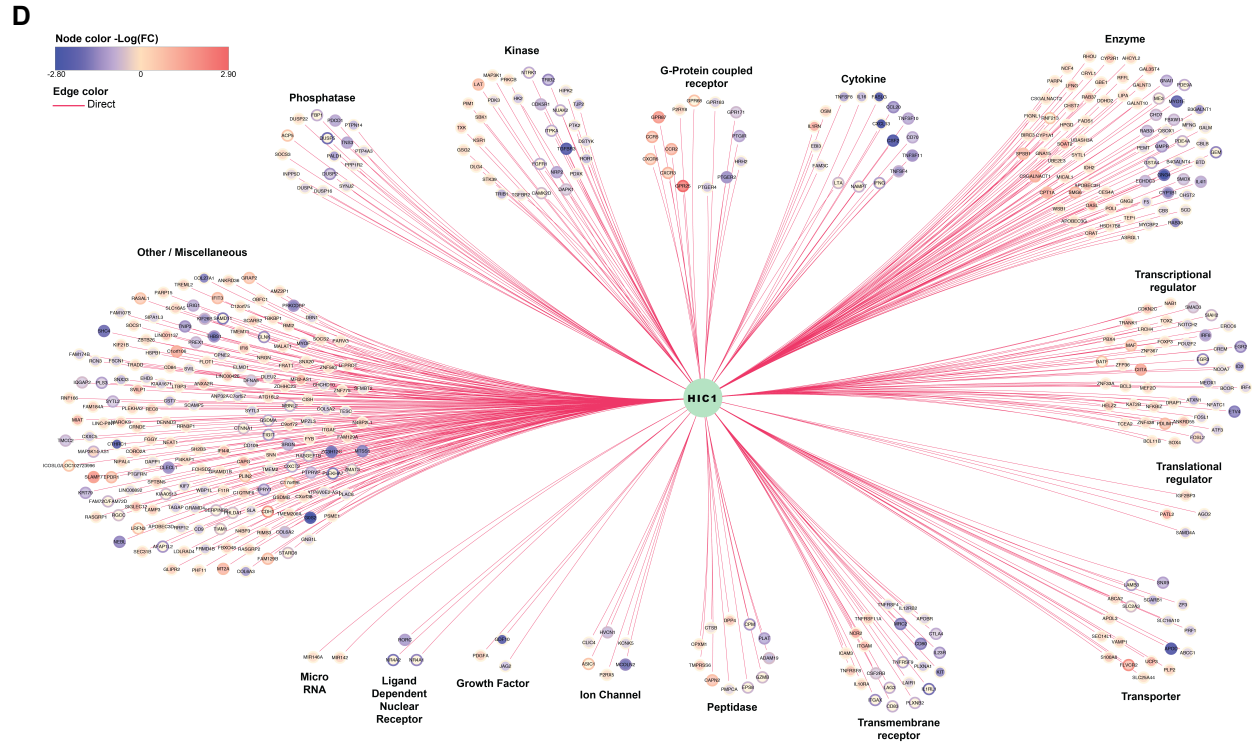

D
Node color -Log(FC)
-2.80
0
2.90
Edge color
Direct
HIC1
Kinase
G-Protein coupled receptor
Cytokine
Enzyme
Phosphatase
Other / Miscellaneous
Transcriptional regulator
Translational regulator
Transporter
Transmembrane receptor
Peptidase
Ion Channel
Growth Factor
Ligand Dependent Nuclear Receptor
Micro RNA

Figure S4. HIC1 Translocates to the Nucleus after 24 h of iTreg Differentiation and binds to its targets. Related to Fig. 4.

(A) Cells were activated under iTreg condition for the indicated times and stained with DAPI (blue) or HIC1 antibody (red) on a fluorescent microscope.

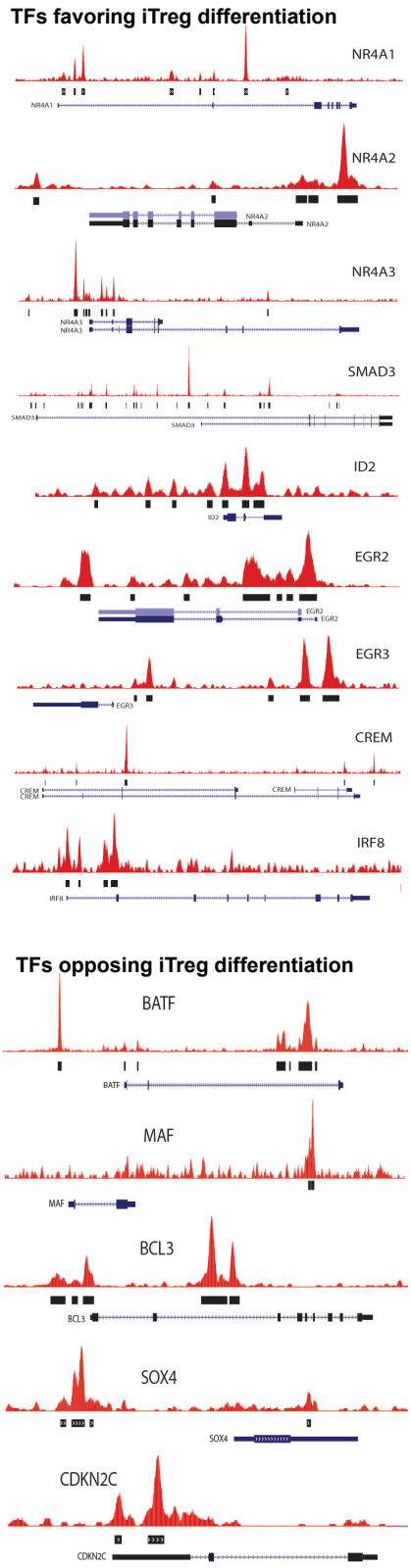
(B) The figure showing the estimation of library complexity for two independent ChIPseq replicates.

(C) The figure shows the molecular function of the identified HIC1 direct target genes, according to IPA software.

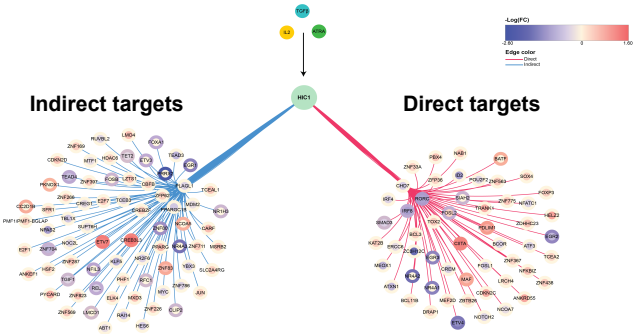
(D) The network is showing the functional classes of genes directly regulated by HIC1 during iTreg cell differentiation.

Figure S5

A



B



C

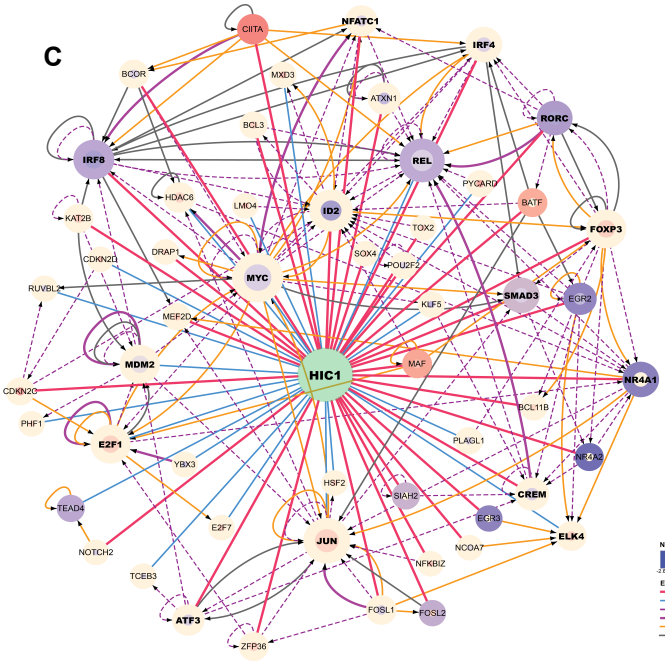


Figure S5. HIC1 binds to TFs supporting as well as opposing iTreg differentiation. Related to Fig. 4.

(A) UCSC genome browser shots are showing the HIC1 binding near the TFs downregulated or upregulated upon HIC1 silencing.

(B) Network is showing the TFs (nodes) that were the direct (red edges) or indirect (blue edges) targets of HIC1. The expression data are plotted as node color where downregulation (blue) or upregulation (orange) of both the time points 48 (inner circle) and 72 h (outer circle) are shown. The node color white indicates that the gene was not DE at that time point.

(C) Network is showing the interactions between TFs that are direct (red edges) and indirect (blue edges) targets of HIC1. Annotations for interactions between TFs were obtained from IPA, and the network was visualized using Cytoscape. Expression data are plotted as node color where downregulation (blue) or upregulation (orange) of both the time points 48 (inner circle) and 72 h (outer circle) are shown. The color scale is shown in the figure. The edges in yellow connect a pair involved in protein DNA interaction; magenta edges connect pairs involved in transcription; dashed magenta edges connect pairs involved in expression; and black edges connect pairs involved in protein-protein interaction.

Supplementary Tables

Table S1. iTreg DE genes and pathways. Related to Fig. 1

Table S2. TFs among iTreg DE genes and TFBS on their promoters. Related to Fig. 1

Table S3. HIC1 Knock down DE genes and the TFBS on their promoters. Related to Fig. 3

Table S4. HIC1 ChIP-Seq data. Related to Fig. 4

Table S5. DAPA oligonucleotides. Related to Fig. 5