SUPPLEMENTAL EXPERIMENTAL PROCEDURES

ATAC-Seq. Samples prepared as described in experimental procedures were purified using MinElute spin columns (Qiagen). Nextera index adapters (i7 and i5) were used to amplify transposed DNA fragments as per manufacturer's protocol (Illumina). Libraries were purified using AMPure XP beads (Beckman-Coulter), pooled (2-3 samples) and subjected to PE50 sequencing on an Illumina HiSeq2500.

Human cell cultures. Human Th1 or 17 cells sorted from blood were expanded for 20 days on irradiated allogeneic feeders, PHA (1 µg/ml; HA16 Remel) and human IL-2 (20ng/ml; Peprotech).

Ultra-low Input ChIP Assays. Aliquots of 30,000 cells were resuspended in 20 µl EZ nuclei isolation lysis buffer (Sigma-Aldrich). Chromatin was digested by adding 20 µl MNase master mix (MNase buffer, MNase, 3mM DTT, dH_20) and incubating for 5 minutes at 37°C. The addition of EDTA, Triton and DOC was used to halt the reaction. Digested chromatin was diluted in immunoprecipitation buffer (20mM Tris-HCl pH 8.0, 2mM EDDTA, 150 mM NaCl, 0.1% Triton X-100, 5mM sodium butyrate) with EDTA-free protease inhibitors (Roche, France) and pre-cleared with Dynabeads (Invitrogen) coated with Protein-A for an hour at 4°C. Pre-cleared chromatin was subjected to immunoprecipitation overnight at 4°C with each antibody conjugated to Dynabeads coated with Protein-A (H3K27ac, Abcam; H3K4me3, Abcam; or isotype control, Santa Cruz Biotechnologies). Samples were washed as described (Brind'Amour et al, 2015) with the following buffers: low-salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris [pH 8.0], 150 mM NaCl, dH_2O), high-salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris [pH 8.0], 500 mM NaCl, dH_2O), and eluted with an SDS buffer (1% SDS, 100mM $NaHCO_3$, dH_2O) for 1 hour at 65°C. DNA was purified with phenol:chloroform:isoamylalcohol (25:24:1, pH 8) using Maxtract tubes (Qiagen), then precipitated overnight. The DNA was subjected to a final clean-up with Ampure XP beads. ChIP or input DNA was used for indexed library preparation, pooled (4-6 samples), and subjected to 50 bp single-end sequencing per manufacturer's protocol (Illumina HiSeq2500).

Epigenome Analysis Pipeline. Sequencing tags from ATAC- and ChIP-Seq were aligned to the reference genome (build GRCh37/hg19) with Novoalign (Hatem et al., 2013). MACS (Feng et al., 2012) was used to identify H3K27ac or H3K4me3 peaks as described (Koues et al., 2015). ATAC samples were processed with MACS using the following changes to default settings, --nomodel --shiftsize=50. Overlapping peaks for all chromatin marks were merged to generate a consolidated list of putative regulatory elements as described (Koues et al., 2015). Sequencing tags in 200 bp bins spanning these merged regions were pulled from wig files and reads per million (RPM) aligned reads were calculated, quantile normalized, and subjected to pairwise comparisons for identification of peaks with differential activity as described (Koues et al., 2015). Distal regulatory elements ($ATAC^+K27ac^+$) were connected to genes within 250 kb that exhibited concordant changes in H3K4me3 at their transcription start sites (TSS) as well as concordant expression changes.

Analysis of Active, Poised and Unique enhancers. To analyze active, unique and poised enhancers, we examined H3K27Ac and ATAC peak files called by MACS using at least two biological replicates for each cell type, which were concatenated and merged. We pruned CTCF (GEO acquisition: GSM935611) and RefSeq annotated TSS sites from ATAC and K27Ac peak files using BEDTools (Quinlan and Hall, 2010). After intersecting files to create a list of all ATAC+ regions, we used cell type-matched H3K27ac data to categorize enhancers as follows: active (intersecting H3K27Ac–ATAC peaks), poised (ATAC peak but no H3K27AC peak) or off (no H3K27ac or ATAC peak). To assay expression proximal to enhancers, we assigned enhancer peak files to genes within 30 kb using the BEDTools closest algorithm, followed by removing any gene over 30 kb from an annotated TSS. Expression values of genes across biological replicates (microarray data) were represented as either fold change (Fig 2, S2A) or as absolute probe intensities (Fig S2C). Significance levels of fold change values were determined by one-way anova analysis of gene sets between cell types (* $p < 0.001$).

SE Identification. We ranked $H3K27ac^+$ regions using the HOMER (Heinz et al., 2010) findPeaks algorithm, set for processing histone modifications (program options findPeaks.pl <H3K27Ac Tag Directory> -superslope 1000 -k 0). Peaks within 12.5 kb were stitched together, ranked according to H3K27ac density, and identified as SEs as described (Hnisz et al., 2013). To compare SEs across different cell types, SE files were first intersected using

BEDTools (Quinlan and Hall, 2010) multiintersect and Ryan Layers's clustering algorithm. Lastly, assignment of genes to the combined SE files (< 30 kb from SE borders), was performed with the BEDTools closest algorithm with the following change to the default settings: -t first, followed by removal of any SE:Gene combination >30 kb from an annotated TSS.

TF Motif Analyses. Cell type-restricted enhancers were defined by the following criteria from our epigenetic analysis pipeline: p value > 0.05, ATAC+, K27AC 1.5 fold enriched either between ILCs or Ths (Fig 4A) or enriched uniquely for individual cell types (Fig 4B). ATAC peak centers at enriched enhancers were extracted and analyzed by HOMER motif pipeline (Heinz et al., 2010) using default parameters and all identified enhancer peaks (Hg19) as background. To normalize for enhancer counts, we used equal numbers of randomly chosen enhancers for each analysis. Relative enrichment of motifs was scored as changes in p-values between samples (Fig 3A) or represented as enrichment p-values for motifs corresponding to differentially expressed TFs (Fig 3B).

SNP Annotation within SEs. Disease associated SNPs were downloaded from NCBI's dbSNP database (http://www.ncbi.nlm.nih.gov/projects/gapplusprev/sgap_plus.htm) in July 2015. Redundancies were pruned from the list by removing SNPs lacking a dbSNP ID or SNP records that contained multiple SNPs for a given trait. SNP enrichment was determined by counting SNPs in observed SEs and p-values were calculated using permutation analysis to count disease-associated SNPs in random genomic regions, excluding unmappable regions, of sizes proportional to SEs over 10^6 permutations (BEDTools shuffle). To assess potential effects on gene expression, the SE-SNPs were analyzed by HaploReg (www.broadinstitute.org/mammals/haploreg/), for with known eQTLs or TF motif disruption (Ward et al., 2010).

Primers and Probes.

For 3C assays on the IL22 locus

IL22-97	ccggataaatatcagcgccaatg
IL22-198	ccatcttcacaggccgaattc
IL22-112	ttccctaaagcctccatgagac
SE22-211	tggtccgtgtaacaaacagc
YWHAZ L	aactaagcatcaataggtgtcag
YWHAZ R	gttaggatagcaacctctgtcttac
YWHAZ Probe	gatggacgtccaaggtgagt
IL22 L (anchor)	aagccctctttaagagcagcag
IL22 probe	tgcccctgatggaccaagca

For cloning enhancers into luciferase vectors:

IL22 -104 kb fwd2_Asp	ggtacccttttctgtactaagcatttatc
IL22 -104 kb rc2_Xho	ctcgagaatccactttcagaaagaatgct
IL22 -112 kb fwd2_Asp	ggtaccgtgctttctttttttcctttgag
IL22 -112 kb rc2_Xho	ctcgagtgtgtgcaatgctatctagt

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