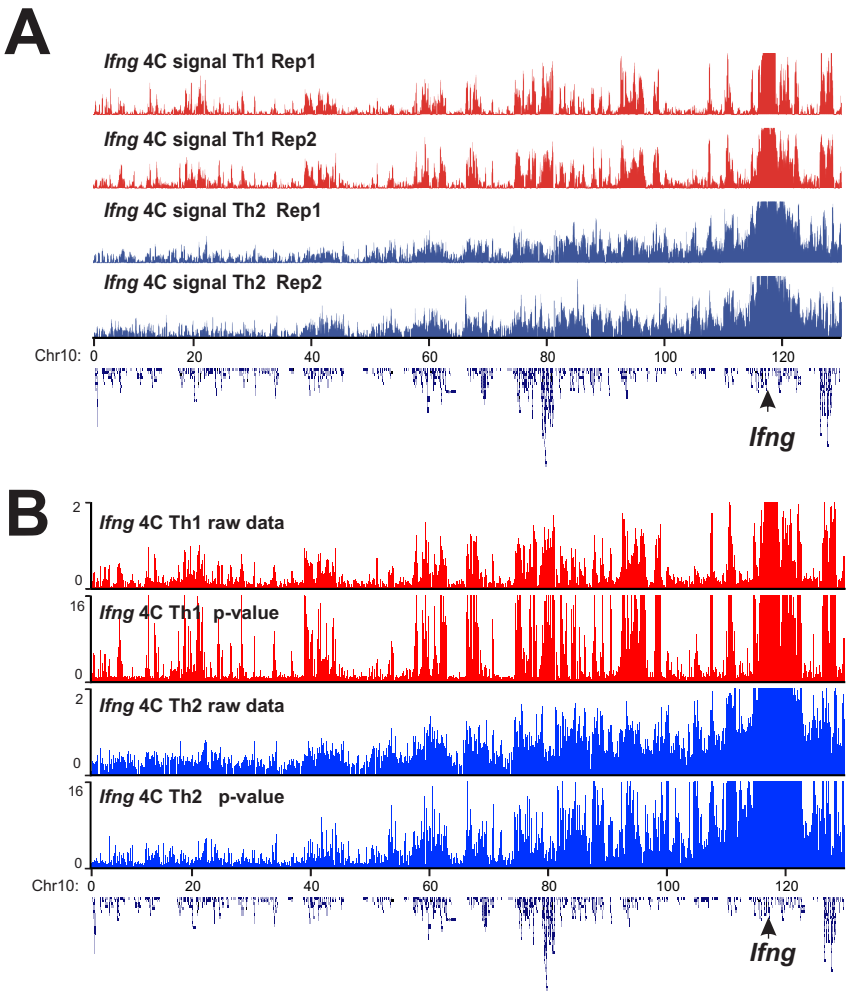
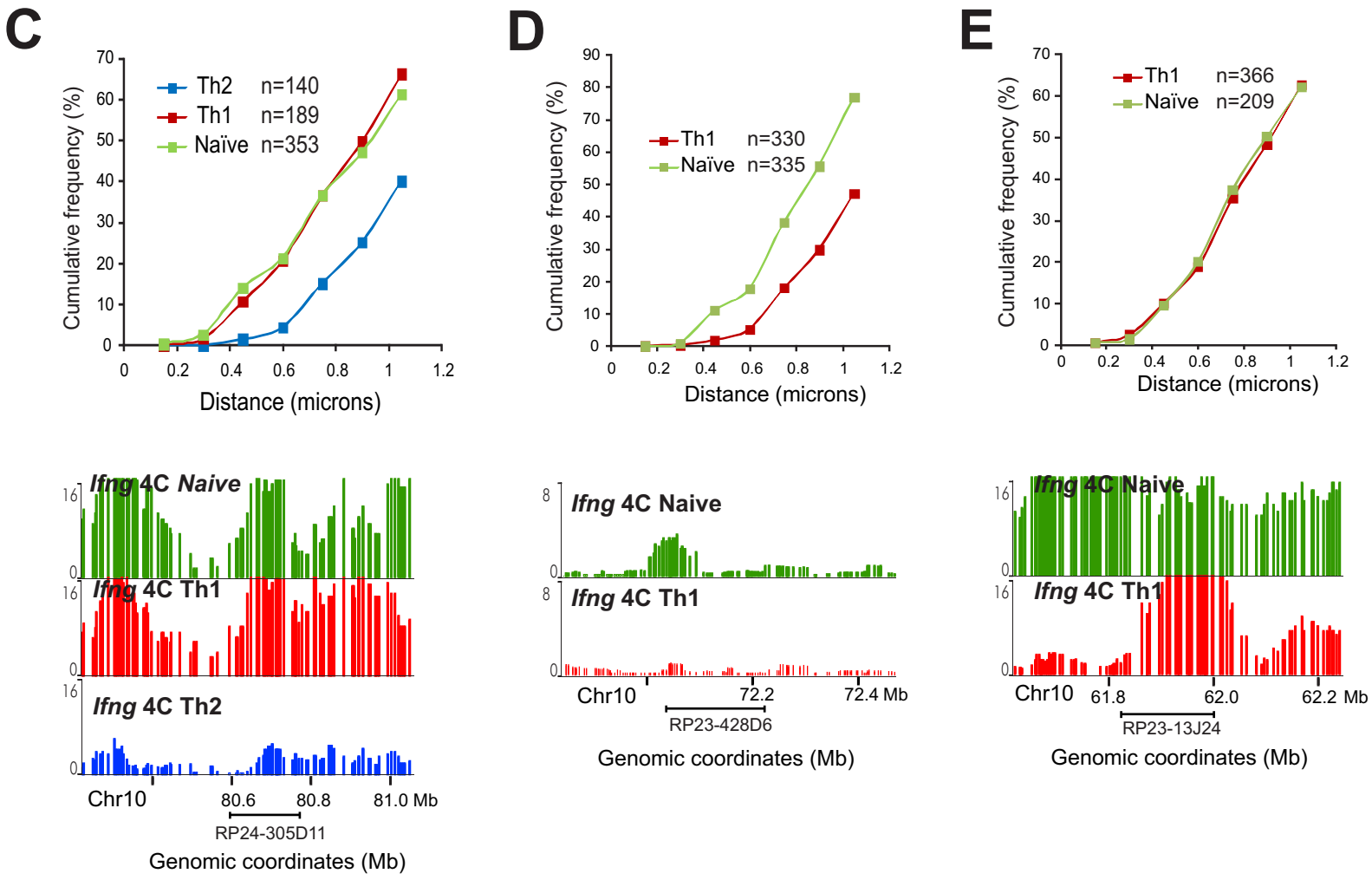
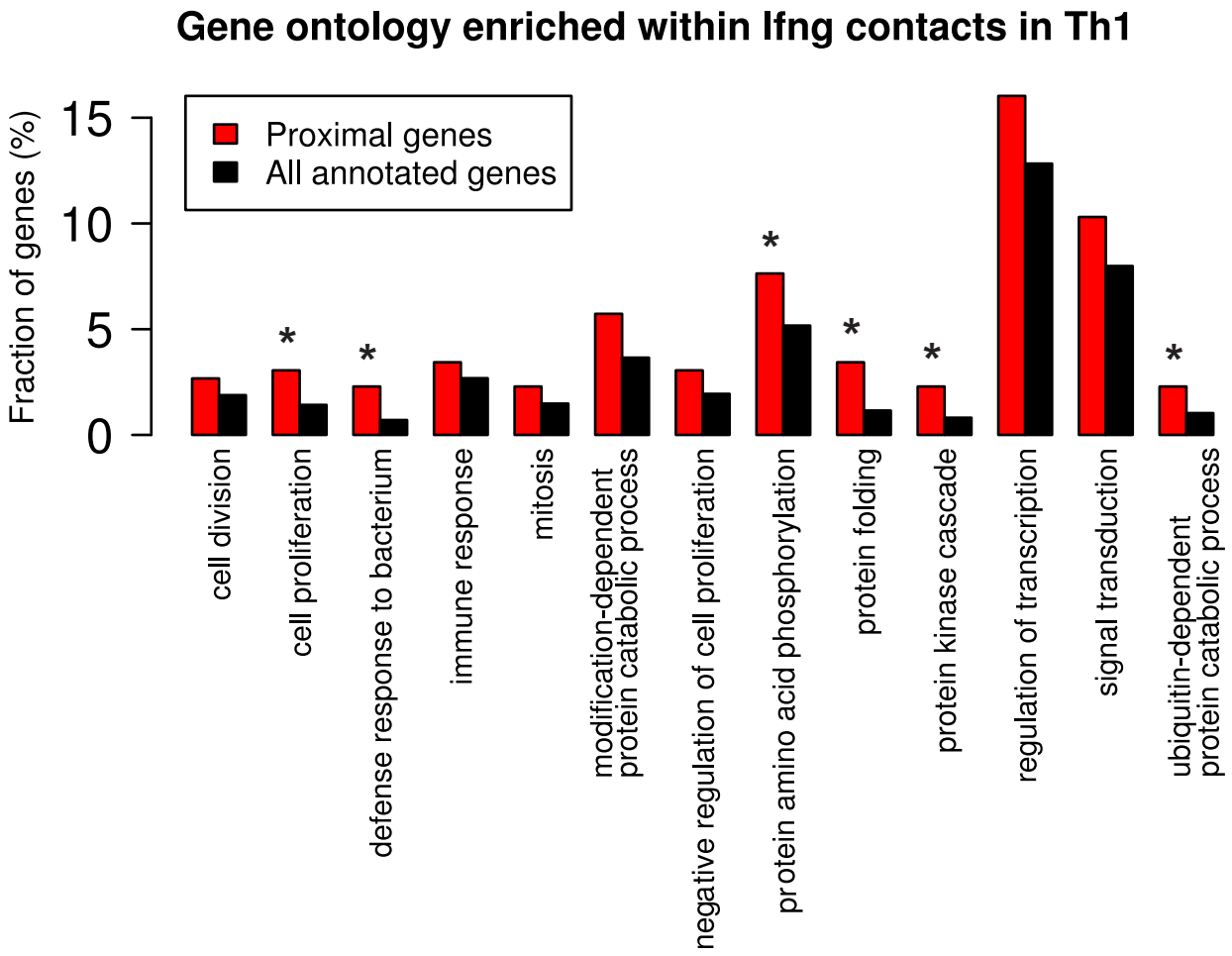


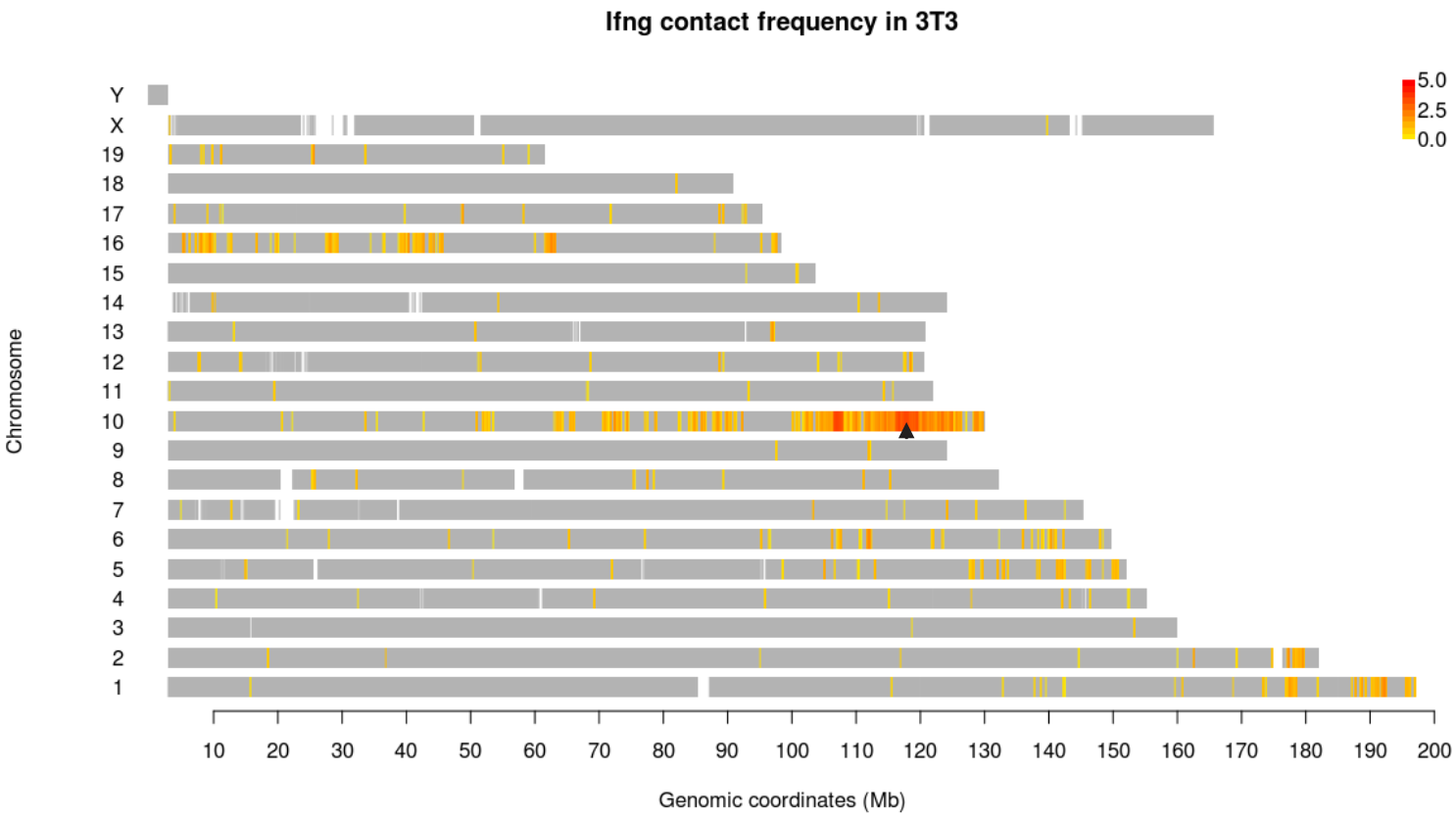
Supplemental Figure S1. *Ifng* 4C data analysis and correlation with 3D DNA FISH contact frequency

(A) Reproducibility of *Ifng* 4C in biological replicates. Raw data are shown as running mean of NimbleGen probe logratios using 100 kb windows. (B) Detection of significant interactions by statistical test of enrichment. Two replicates average 4C microarray logratios (4C DNA/ genomic input, "raw data" panels) and p score enrichment of 4C signal ("p-value" panels) are shown for Th1 and Th2. (C, D, E) Agreement between 4C and 3D DNA FISH. Cumulative frequency of the 3D DNA FISH distances between *Ifng* and one of three BAC loci tested (up), together with *Ifng* 4C contact profile (p score) at the BAC region (underlined with BAC clone number). Naïve (green), Th1 (red), and Th2 (blue). Genomic coordinates (mm8) in Mb.

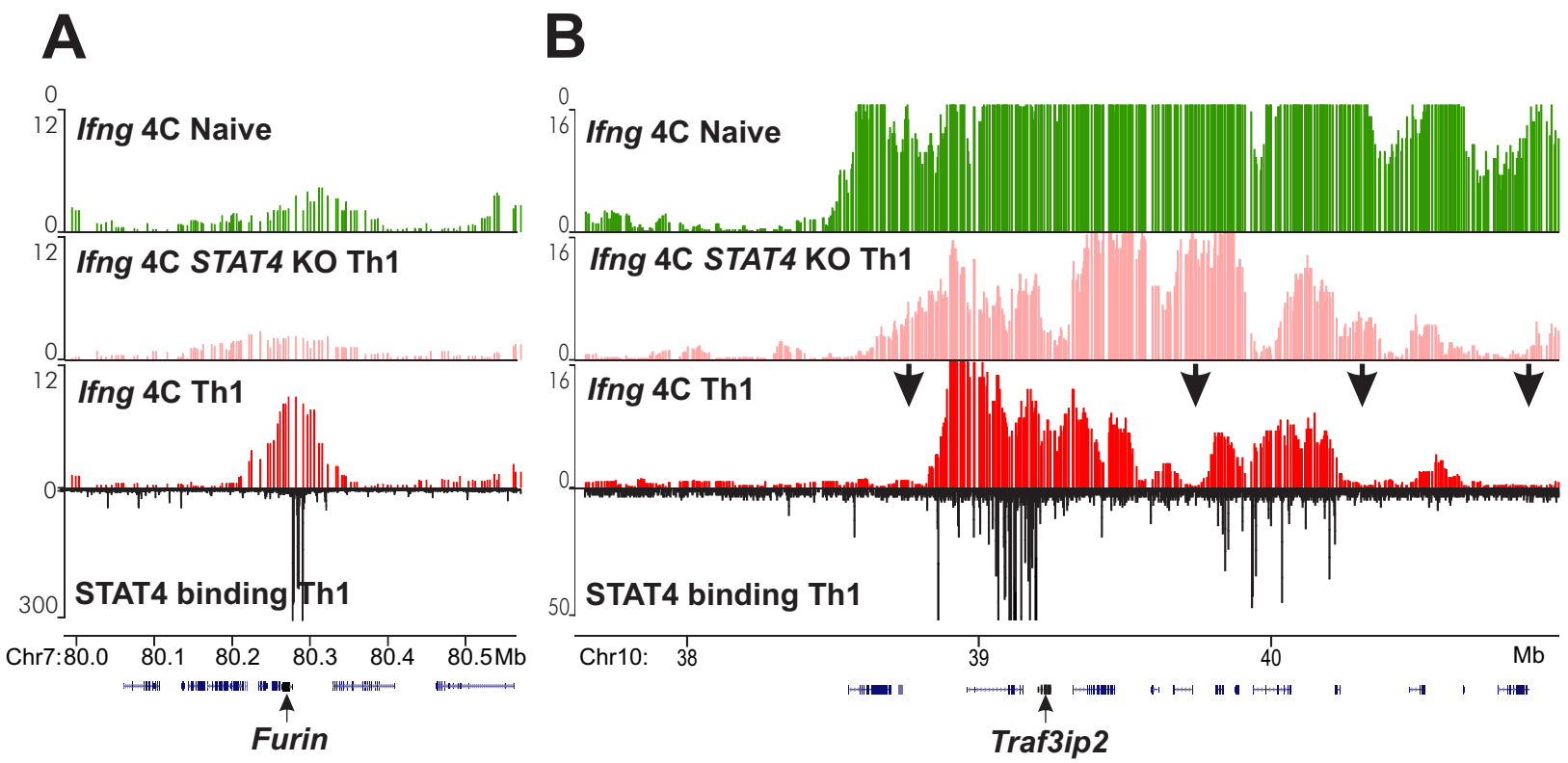




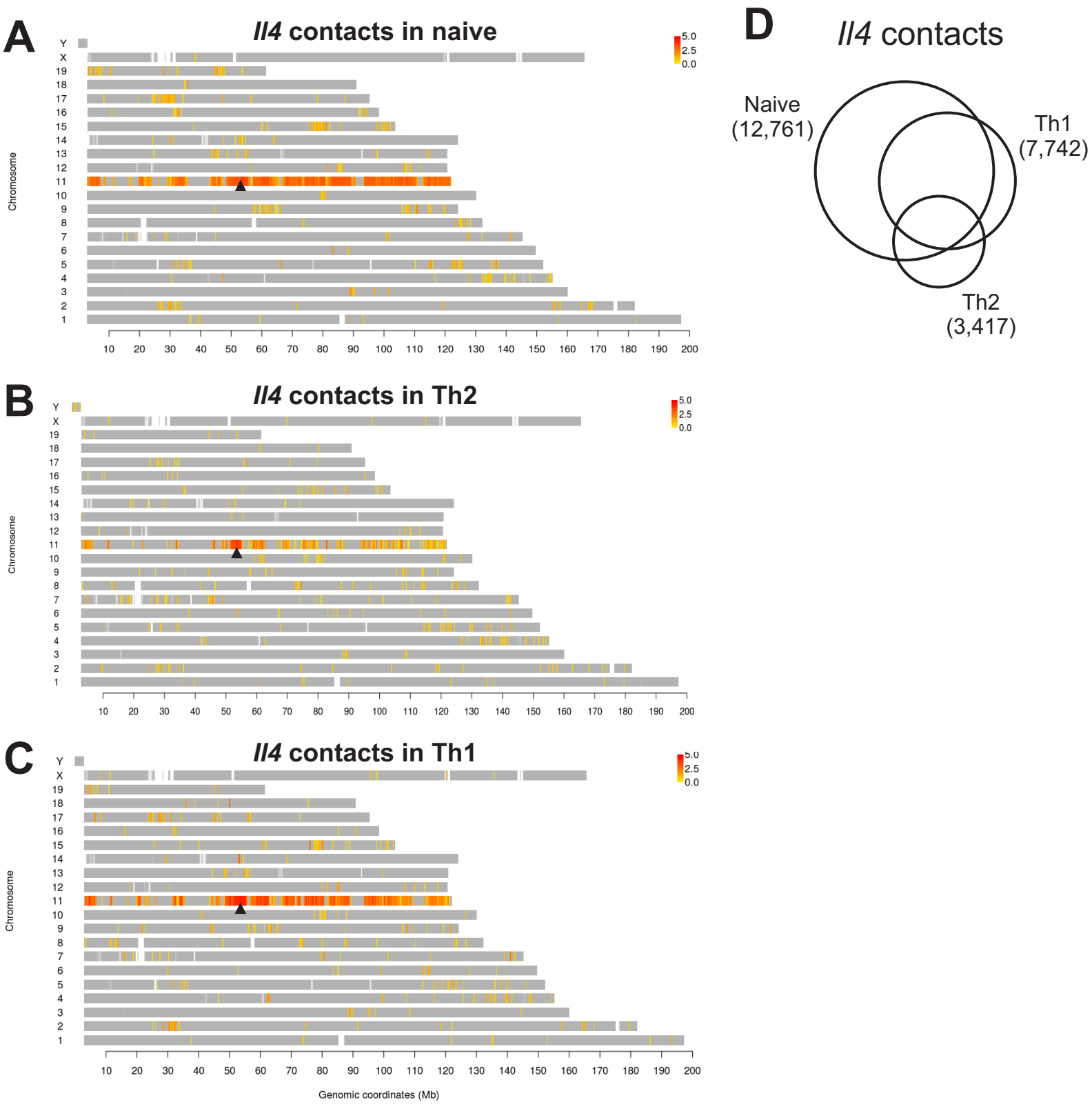
Supplemental Figure S2. Gene ontology of the genes on *Ifng* contact loci in Th1
Ontology categories that are over-represented in the genes from 4C-positive regions in Th1 (red). The baseline distribution of the ontology groups from the whole genome is shown in black bars. * indicates enrichment beyond p <= 0.05 (Fisher exact test).



Supplemental Figure S3. Genomic map of *Ifng* contacts in 3T3-L1 mouse fibroblasts
Positive 4C probes (p score > 4; see Methods), marked in a yellow-to-red color scale according to the probe signal (log2 of 4C/input DNA) reflecting contact frequency. Locations of all other probes are shown in gray.



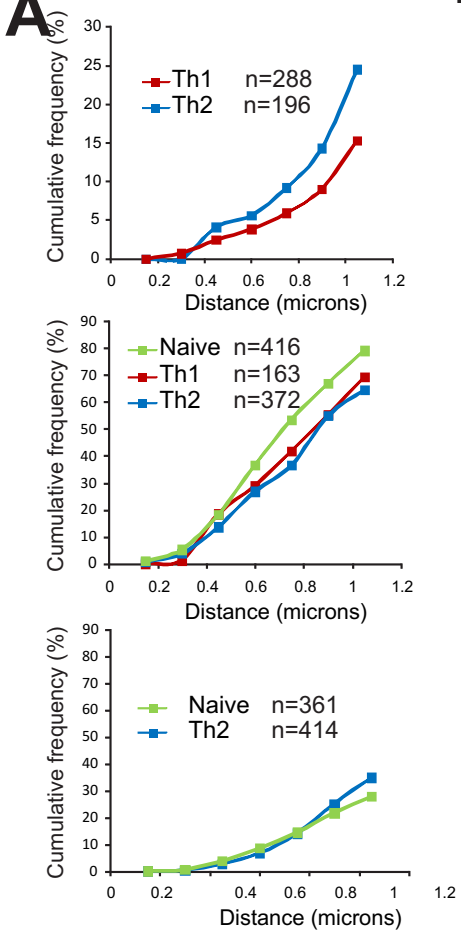
Supplemental Figure S4. STAT4 binding at *Ifng* Th1-specific contacts
(A, B) Examples of STAT4 binding (black track), *Ifng* contacts in naïve (green), *Stat4* knock-out (pink), Th1 (red) cells. (A) Inter-chromosomal contact created *de novo* at STAT4 binding cluster. (B) Contacts are lost during differentiation at regions lacking STAT4 binding (arrows), while the surrounding contacts at STAT4 binding loci remain in Th1. p score enrichment of 4C signal is presented, genomic coordinates (mm8) in Mb.

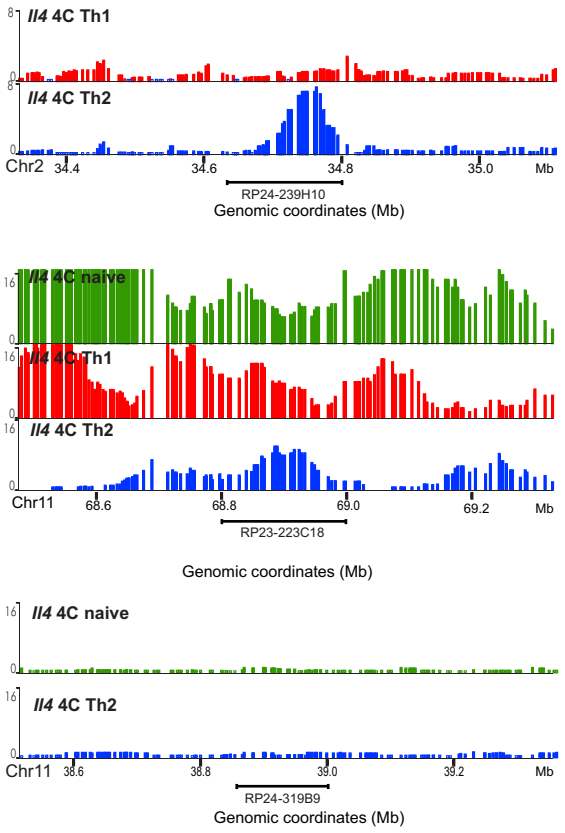


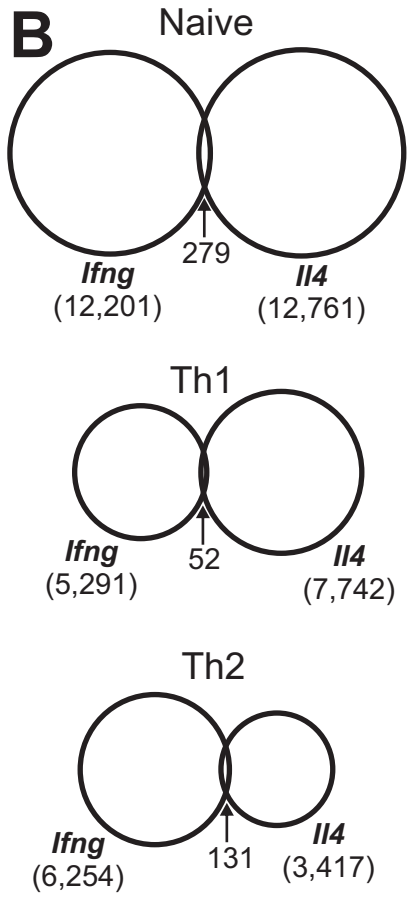
Supplemental Figure S5. Reciprocal re-organization of the *Il4* interactome in the Th2 helper lineage where *Il4* functions as the signature cytokine.

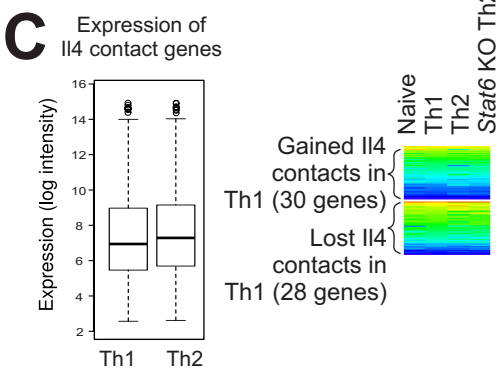
(**A, B, C**) The genomic map of *Il4* contacts in the naive (**A**), Th2 (**B**), Th1 (**C**) lymphocytes shows the locations of positive 4C probes (p score > 4; see Methods), marked in a yellow-to-red color scale according to the probe signal (log2 ratio of 4C/input DNA) reflecting contact frequency. Locations of all other probes are shown in gray. The location of the *Il4* gene is marked with an arrowhead. (**D**) The Venn diagram shows the number of positive 4C probes (*Il4* contact regions) in each of the T cell types.

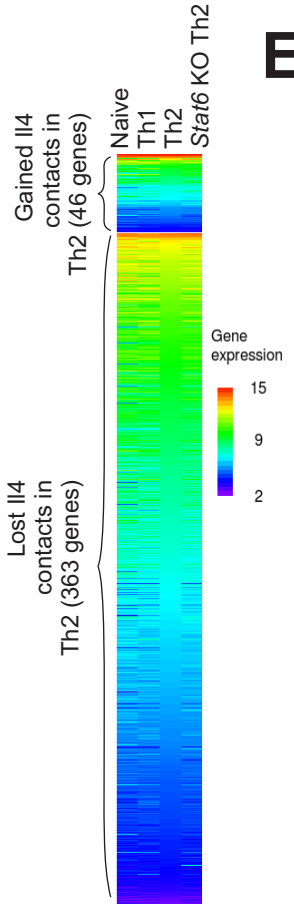
Hakim Supplemental Figure S6

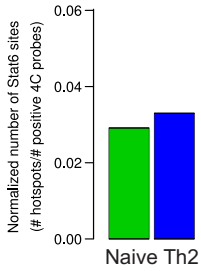


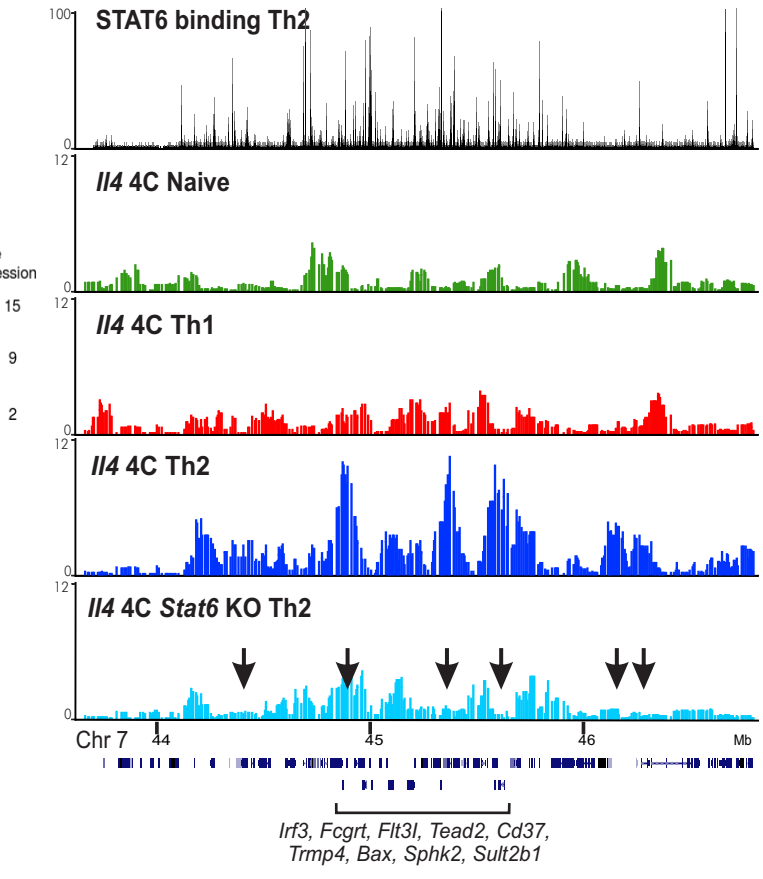


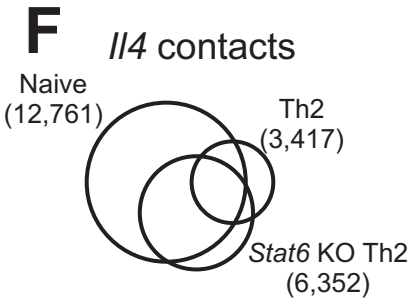












Supplemental Figure S6. Lineage-specific re-organization of the *Il4* interactome
(A) Cumulative frequency of the 3D DNA FISH distances between *Il4* and one of three BAC loci tested (left), together with *Il4* 4C contact profile (p score) at the BAC region (underlined with BAC clone number) in the naive (green), Th1 (red), and Th2 (blue) lymphocytes, validating the 4C contact profile. Genomic coordinates (mm8) in Mb. (B) The *Ifng* and *Il4* interactomes are disjoint. The Venn diagrams represent the overlaps between positive probes (contacts) from *Ifng* and *Il4* 4C analysis in each cell type. (C) The expression of *Il4* contact genes in Th1 and Th2 is similar (box plot). Genes that gain or lose interactions with *Il4* (heat maps) have similar expression levels across all the cell types (see Methods). The heat maps include expression data from the other cell types, and are sorted by the expression values in Th1 (left panel) and Th2 (right panel) within each category of genes. (D) The Il4 contact loci have a slight enrichment of STAT6 binding sites in Th2 cells. The plot shows the numbers of Th2-derived STAT6 ChIP-seq sites per positive 4C probe for each 4C data. (E) An example of STAT6 binding (black track), *Il4* contacts in naïve (green), Th1 (red), Th2 (blue), and Th2-induced *Stat6* knock-out (light blue) T cells. Immune-related genes are indicated at the bottom. Arrows indicate contact loci that are disrupted in KO versus WT Th2. p score enrichment of 4C signal is presented, genomic coordinates (mm8) in Mb. (F) The Venn diagram represents the overlaps between positive 4C probes (contacts) from the naive, Th2, and Th2-induced *Stat6* KO.