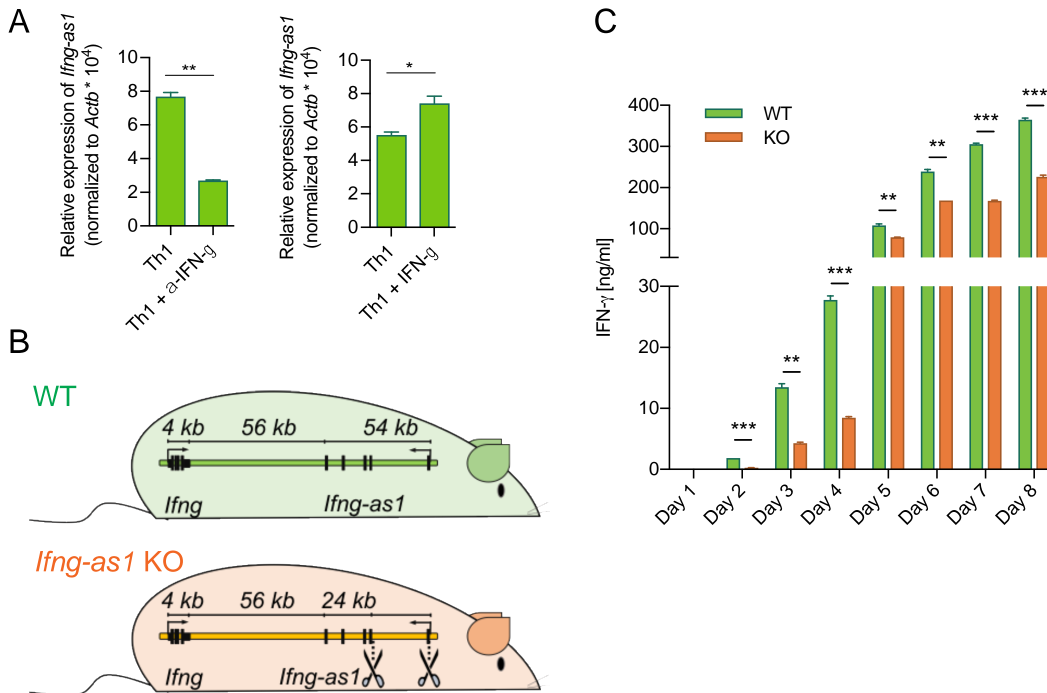
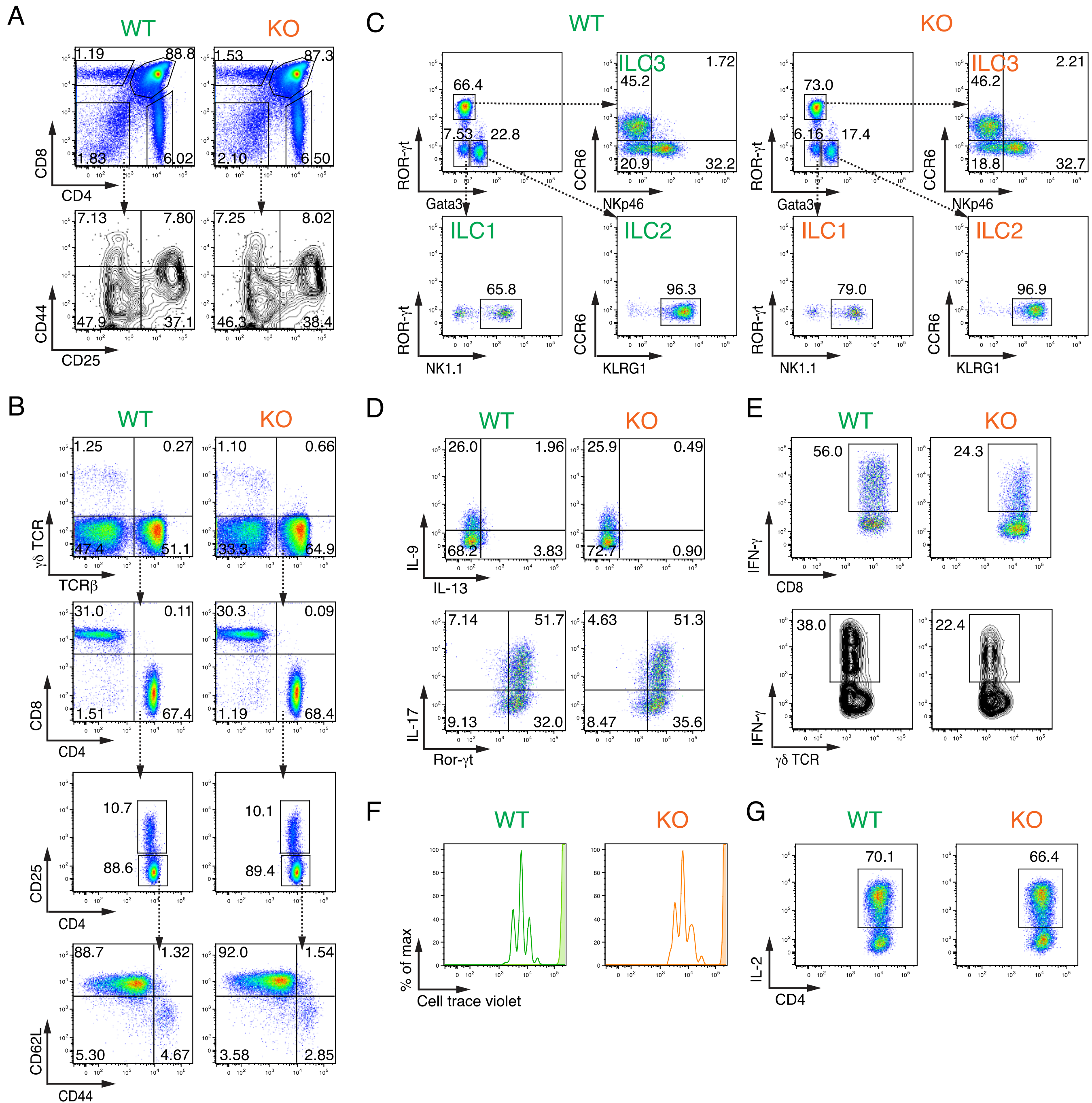
Supplementary Figure 1



Supplementary Figure 1. *Ifng* expression is enhanced by IFN-γ and *Ifng-as1*. Related to Figure 1.

(A) Naïve T cells from WT mice were isolated by FACS and cultured under Th1 polarization conditions for 6 days with or without the addition of αIFN-γ or IFN-γ. *Ifng-as1* expression was analyzed by RT-qPCR and normalized to the expression of *β-actin*. Shown are representative graphs from n=3 independent experiments. Graphs show mean ± sem. *$p<0.05$ **$p<0.01$. (B) Exon 1 and intron 1 (~ 30 kb) of the *Tmevpg1* gene which include the TSS were replaced with an EGFP and an FRT-flanked positive selection cassette. The targeting vector has been generated using BAC clones from the C57BL/6J RPCIB-731 BAC library and was transfected into the TaconicArtemis C57BL/6N Tac ES cell line. Homologous recombinant clones were isolated using positive (Puro resistance) and negative (Thymidine kinase) selections. The constitutive knock in allele was obtained after Flp-mediated removal of the selection marker. (C) IFN-γ concentration in cell culture supernatants was measured by ELISA.

Supplementary Figure 2

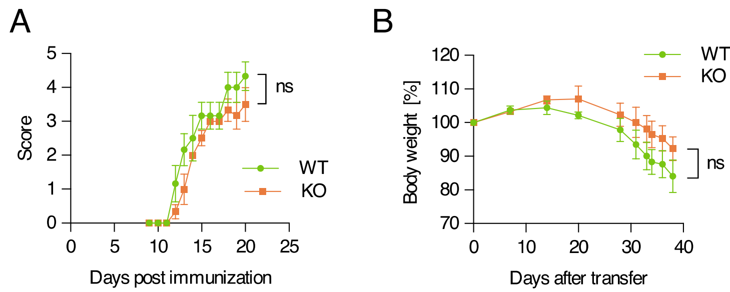


Supplementary Figure 2. Fractions of lymphocyte subsets and autoimmune disease susceptibility are unaltered in *Ifng-as1* KO mice. Related to Figure 1.

(A)(B) Flow cytometric analysis of cells isolated from thymi (A) or from lymph nodes (B) from WT or *Ifng-as1* KO mice. (C) Flow cytometric analysis of innate lymphoid cells isolated from small intestines from WT or *Ifng-as1* KO mice. (D) Naïve T cells from WT mice or *Ifng-as1* KO mice were isolated by FACS and cultured under Th9 (top panel) or Th17 (bottom panel) polarization conditions for 3 days. (E) $CD3^+$ T cells from WT mice or *Ifng-as1* KO mice were isolated by MACS sorting and cultured with IL-12 for 3 days. IFN-γ expression in $CD8^+$ cells and γδ TCR^+ cells was assessed by flow cytometry. (A)-(E) Shown are representative flow cytometry plots from n=3 independent experiments. (F)(G) Naïve T cells from WT mice or *Ifng-as1* KO mice were isolated by FACS sorting and cultured under Th1 polarization conditions for up

to 6 days. (F) Flow cytometric analysis of cell proliferation on day 3 of culture. (G) Flow cytometric analysis of IL-2 production on day 6 of culture. Shown are representative flow cytometry plots from n=2 independent experiments.

Supplementary Figure 3

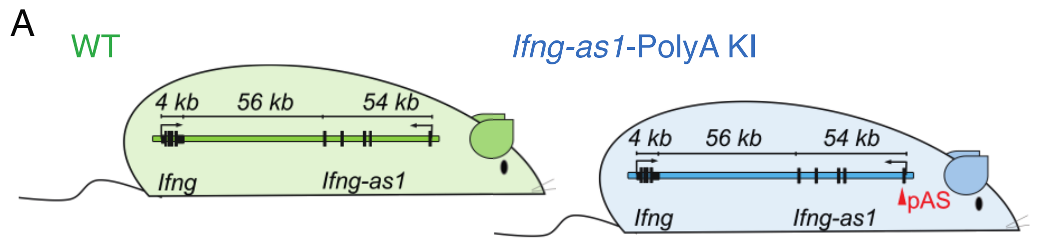


Supplementary Figure 3. EAE and colitis disease severity are unchanged between WT and *Ifng-as1* KO mice. Related to Figure 2.

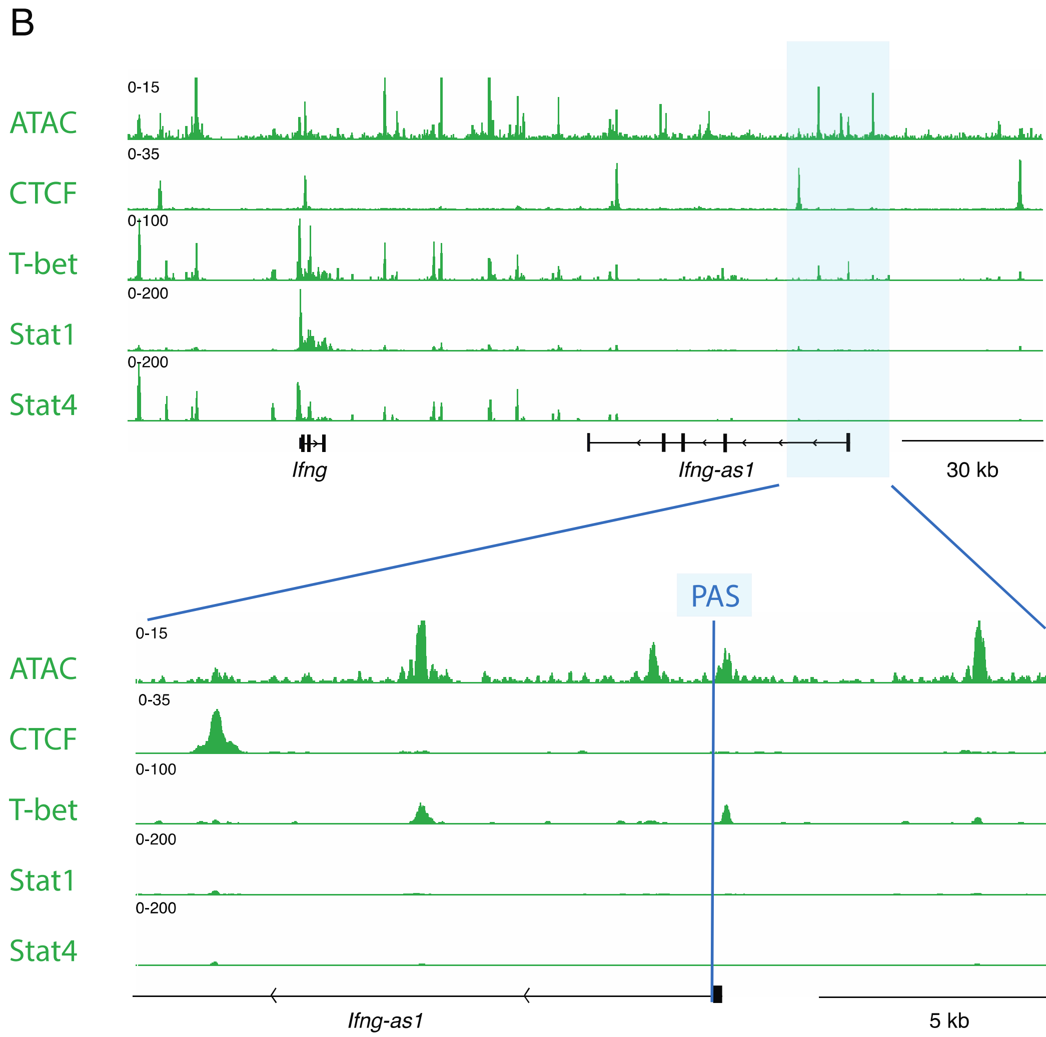
(A) EAE disease score of WT and *Ifng-as1* KO mice in which EAE was induced by subcutaneous injection of $MOG_{35\text{-}55}$ peptide emulsified in complete Freund's adjuvant and i.v. injection of Pertussis toxin. (B) Body weight of *Rag2* KO mice in which 0.5 x 10^6 naïve ($CD4^+CD25^-CD44^-CD62L^+$) WT or *Ifng-as1* KO cells were adoptively transferred. Shown are representative data from n=2 independent experiments.

Supplementary Figure 4

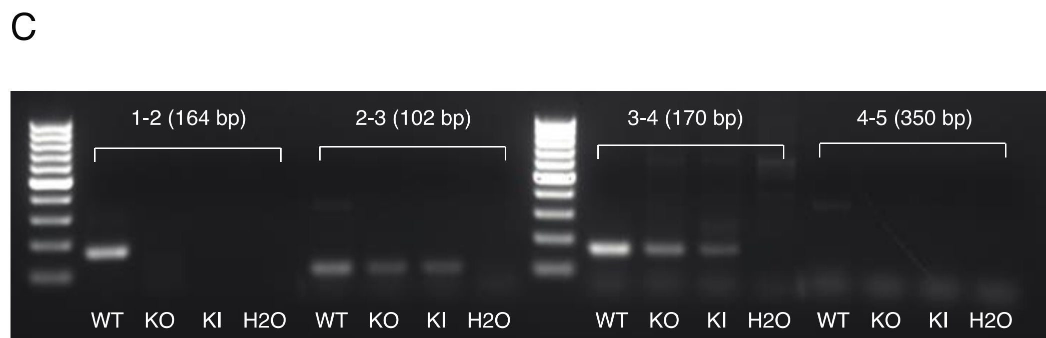
A



B



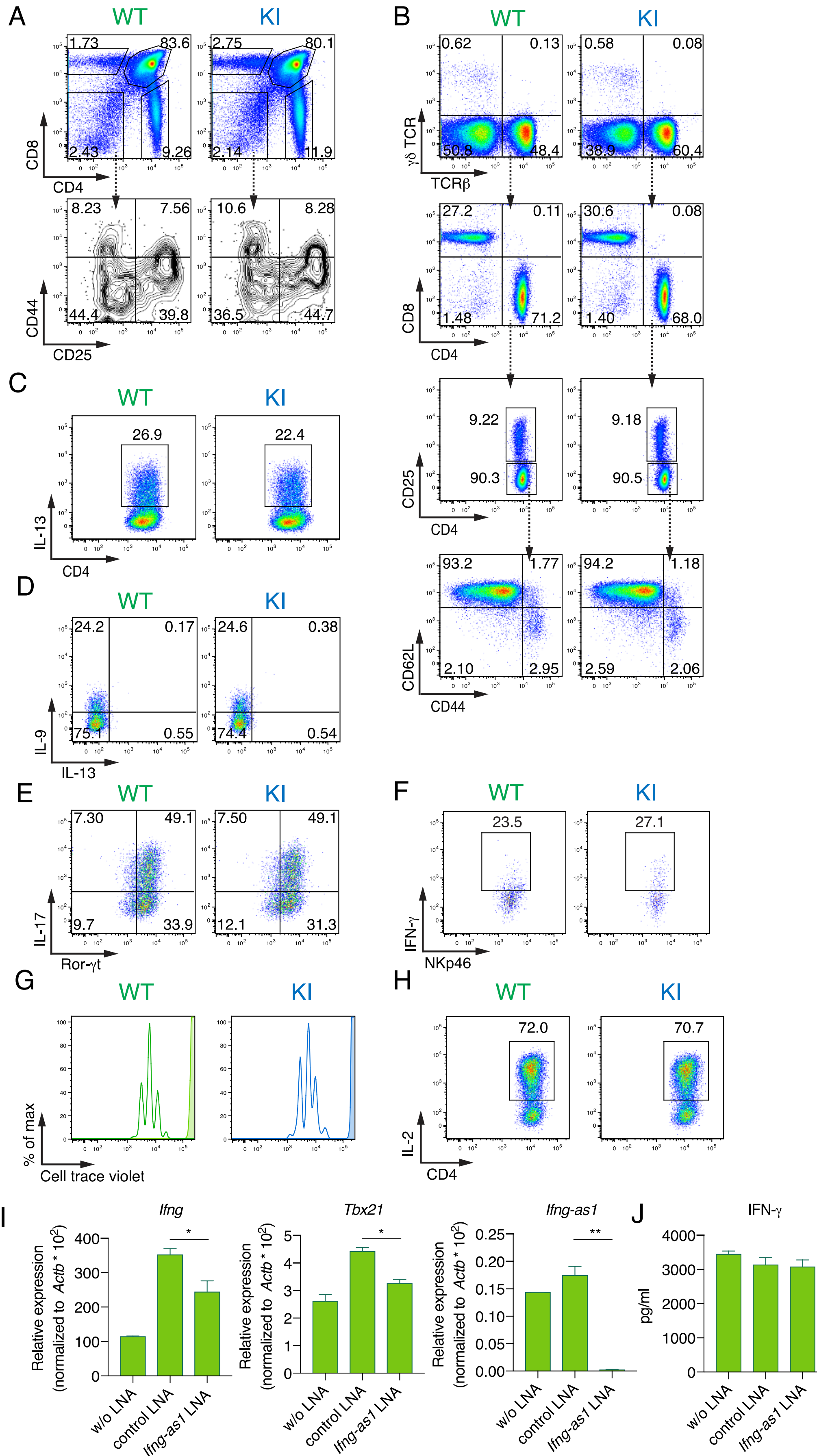
C



Supplementary Figure 4. Fractions of lymphocyte subsets are unaltered in *Ifng-as1*-PolyA KI mice. Related to Figure 3.

(A) Generation of *Ifng-as1*-PolyA KI mouse using CRISPR/Cas9 mediated gene editing. The BGH polyadenylation sequence was inserted into the region immediately after the *Ifng-as1* TSS. (B) Location of PAS insertion. A BGH polyadenylation sequence was inserted 70 base pairs after *Ifng-as1*'s TSS. A site as close as possible to the TSS was chosen based on available data concerning locus accessibility (ATAC), CTCF, Tbet, Stat1 and Stat4 binding in WT Th1 cells. (C) On day 6 of Th1 differentiation, RNA was isolated and *Ifng-as1* expression was measured by PCR in wild type (WT), *Ifng-as1* KO (KO), and *Ifng-as1*-PolyA KI (KI) cells using primer pairs spanning exons 1-2, 2-3, 3-4 and 4-5, respectively (from left to right).

Supplementary Figure 5

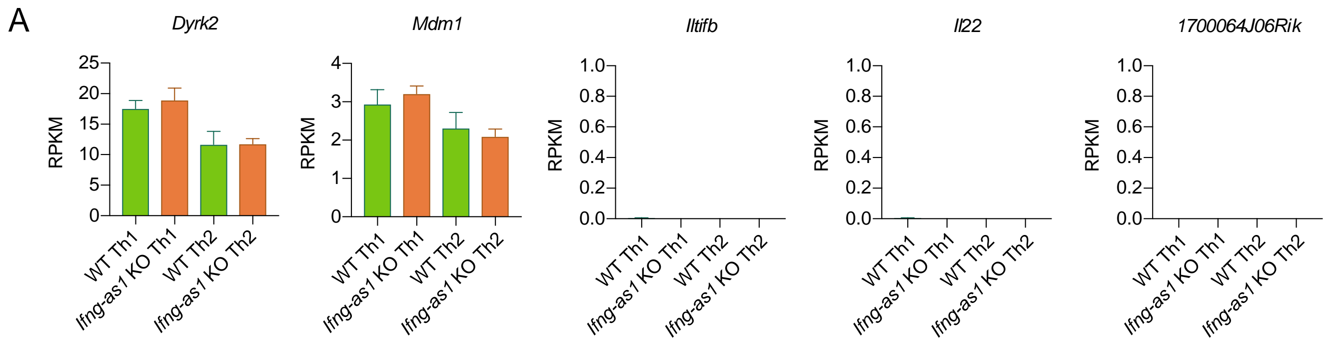


Supplementary Figure 5. Fractions of lymphocyte subsets are unaltered in *Ifng-as1*-PolyA KI mice. Related to Figure 3.

(A)(B) Flow cytometric analysis of cells isolated from thymi (A) or from lymph nodes (B) from WT or *Ifng-as1*-PolyA KI mice. (C)-(E) Naïve T cells from WT mice or *Ifng-as1*-PolyA KI mice were isolated by FACS and cultured under Th2 (C), Th9 (D) or Th17 (E) polarization conditions for 3 days. (F) Splenocytes from WT mice or *Ifng-as1*-PolyA KI mice were isolated and cultured in the presence of IL-12 and IL-2 for 6 hours. IFN-γ expression in NKp46$^+$ NK cells was measured by flow cytometry. (A)-(F) Shown are representative flow cytometry plots from n=3 independent experiments. (G)(H) Naïve T cells from WT mice or *Ifng-as1*-PolyA KI mice were isolated by FACS and cultured under Th1 polarization conditions for 3 days. (G) Flow cytometric analysis of cell proliferation on day 3 of culture. (H) Flow cytometric analysis of IL-2 production on day 6 of culture. Shown are representative flow cytometry plots from n=2 independent experiments. (I)(J) On day 3 of Th1 differentiation control LNAs or LNAs targeting *Ifng-as1* were added to the culture. Cell were stimulated with PMA/Ionomycin on day 6 of culture, RNA was isolated and *Ifng, Ifng-as1 and Tbx21* expression was measured by qPCR (I) or ELISA using day 6 supernatants (J).

Supplementary Figure 6

A



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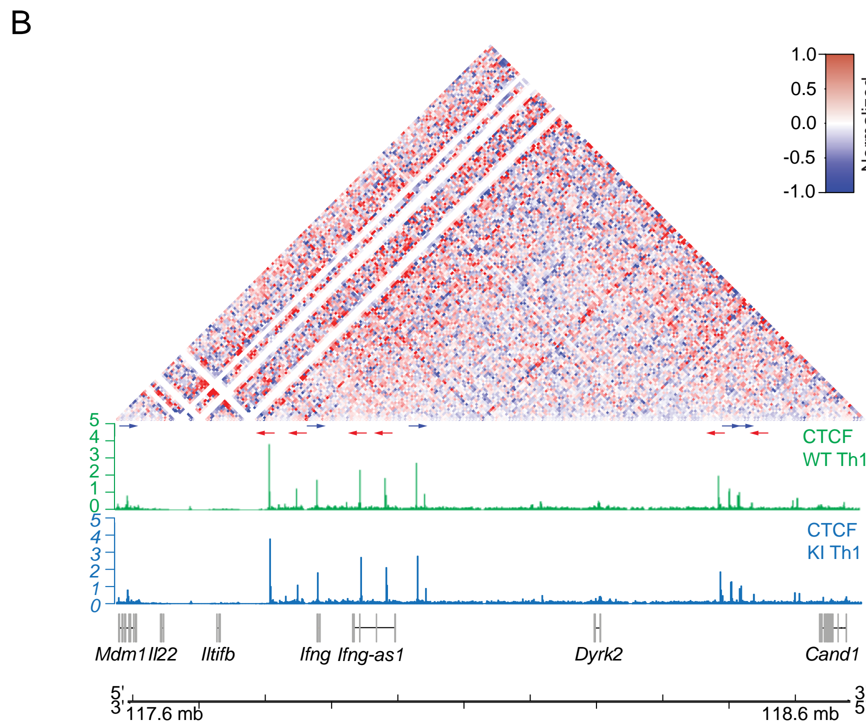
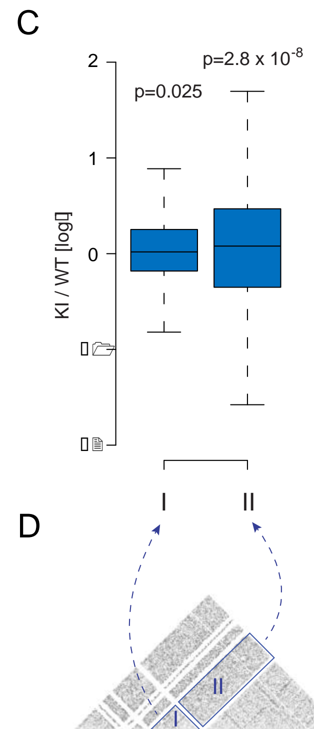


C

D



E

Genotype	IFN-γ response				Chromatin architecture		RNA	Regulatory elements in deleted 30 kb	
	IFN-γ ICS in-vitro Th1	*Ifng* qPCR in-vitro Th1	*T.gondii* Acute survival day 7	*T.gondii* Cyst burden day 60	3C *Ifng* - *Ifng-as1*	HiC Aberrant interaction region II	*Ifng-as1* full-length RNA	ATAC	CTCF ChIP
WT *Ifng-as1* DNA *Ifng-as1* RNA	100%	100%	90%	<500	+	-	+	5	1
KI *Ifng-as1* DNA ~~*Ifng-as1* RNA~~	90% (ns)	40%	50%	<500	N/A	-	-	5	1
KO ~~*Ifng-as1* DNA~~ ~~*Ifng-as1* RNA~~	70%	20%	50%	>1500	-	+	-	0	0

Supplementary Figure 6. Expression of genes adjacent to *Ifng* is unaltered in *Ifng-as1* KO Th1 cells. Related to Figure 4.

(A) Expression of genes adjacent to *Ifng* or *Ifng-as1* was measured by RNA sequencing. (B)-(D) In vitro polarized Th1 cells (day 6 of culture) from WT mice and *Ifng-as1*-PolyA KI mice were subjected to local Hi-C targeting an approximately 1 Mb large sequence containing the *Ifng* and *Ifng-as1* loci. Shown are pooled data from n=4 independent experiments. (B) Shown are contact matrices and CTCF binding tracks. Arrows indicate CTCF binding site orientation. (B)-(D) The interactions in the domain surrounding *Ifng* ("I") and in the one situated upstream (telomeric) of *Ifng-as1* ("II") were quantified by local Hi-C. For both regions, the per-bin log2 ratios were computed. The significance of the deviation of the above derived ratios from 0 was evaluated with a two-sided t-test. (E) Table summarizes *in-vitro* and *in-vivo* IFN-γ response, chromatin architecture, *Ifng-as1* full-length transcript levels, accessibility (ATAC-seq peaks), and CTCF recruitment (binding sites) in *Ifng-as1* KO and *Ifng-as1*-PolyA KI mice compared to WT mice.

Supplementary Table 1

PRIMER	SEQUENCE (5'-3')
Ifng (+66).HindIII.anchor	GACACTAGCTGGGCGTCAA
Ifng (+66).HindIIII.probe	CCCACCAAAGGTGGTAACTAAACCTGAGC
Ifng (+2).HindIII.primer	AGATCTCACCAGAGGCAAAG
Ifng (+66).HindIII.F	CCACCTTTGGTGGGTAGATT
Ifng (+95).HindIII.F	GTGCTTTCTGATCCACTCAC
Ifng (+105).HindIII.F	CAGACAGCATGCTTGAGAAC
Ifng (+146).HindIII.F	CCCGTCCGAATCTCACTTAG
Ifng (-29).HindIII.F	CGTTTAGACTGCTTCTCTGG
HindIII.digest.F	ATAGCGCTGCGTCTTACTGG
HindIII.digest.R	GTCAAAGGCTCCCACACATC
HindIII.undigest.F	TCCAGAGATTCGAGGTTTGG
HindIII.undigest.R	CCAGTAAGACGCAGCGCTAT
Gapdh_HindIII.anchor	AGGGAGGAAGGCTGGAATAGG
Gapdh_HindIII.probe	CTGTCCTTGGGAGTCGCCTGTAGGTGTG
Gapdh_proximal.F	CGCTCCTGGAAGATGGTGATG
Gapdh_distal.F	GCGGCAAGTAGGAGACTGTG

Supplementary Table 1: 3C Primers. Related to Figure 5.