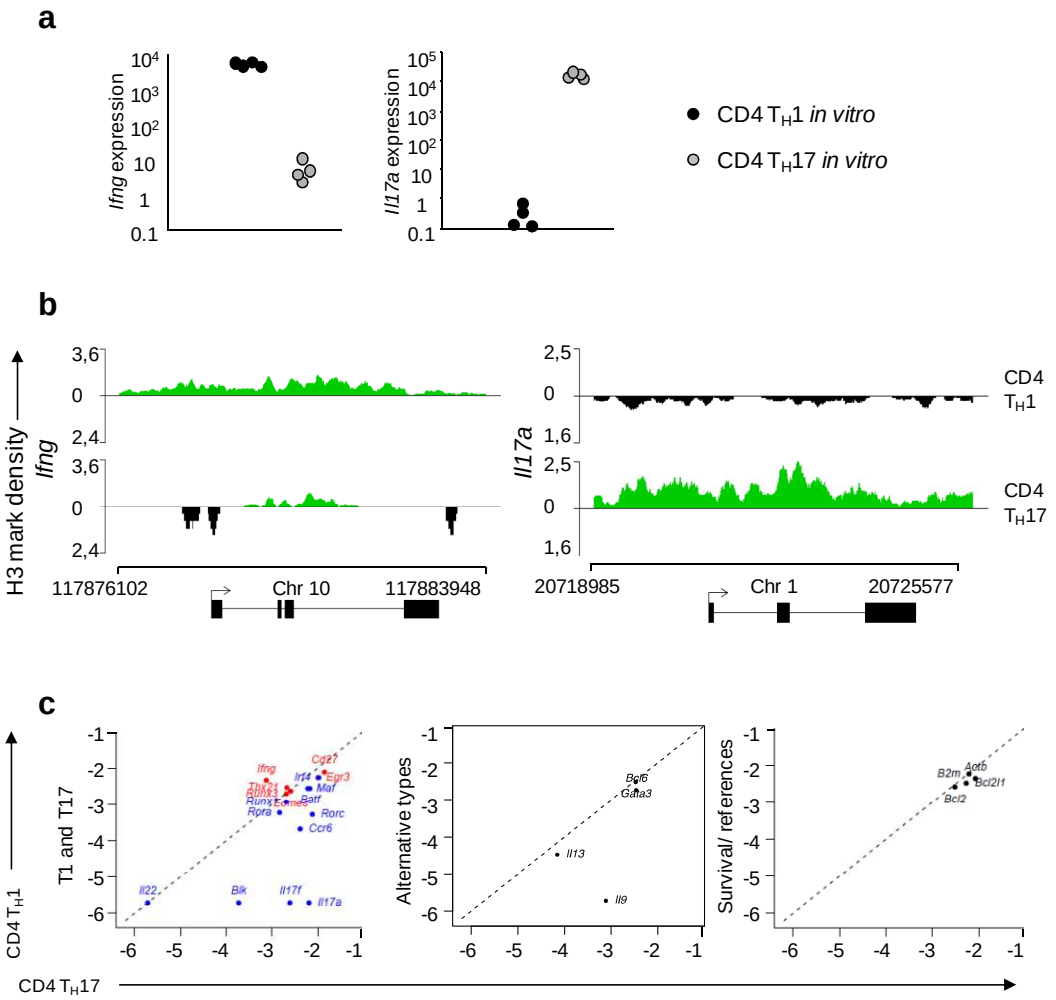
SUPPLEMENTARY FIGURES AND TABLES

Epigenetic and transcriptional signatures of stable versus plastic differentiation of proinflammatory γδT cell subsets

Schmolka N, Serre K, Grosso A, Rei M, Pennington DJ, Gomes AQ, and Silva-Santos B



Supplementary Fig. 1. Histone modifications and transcription of *Ifng* and *Il17a* in $CD4^+$ T_H1 and T_H17 cell subsets. **(a)** RT-qPCR data for *Ifng* and *Il17a* expression (relative to *Actb*) on *in vitro*-generated (as described in Methods) $CD4^+$ T_H1 or T_H17 cells. **(b)** ChIP-seq plots for H3K4me2 (green) and H3K27me3 (black) modifications on *Ifng* and *Il17a* loci in $CD4^+$ T_H1 or T_H17 cells. **(c)** Pair-plots comparing quantitative levels (log_{10}-transformed) of gene-specific H3K4me2 modifications between $CD4^+$ T_H1 and T_H17 cells. Genes were grouped as: type 1 (red) or type 17 (blue) factors; alternative effector cell types; and housekeeping reference or survival genes.

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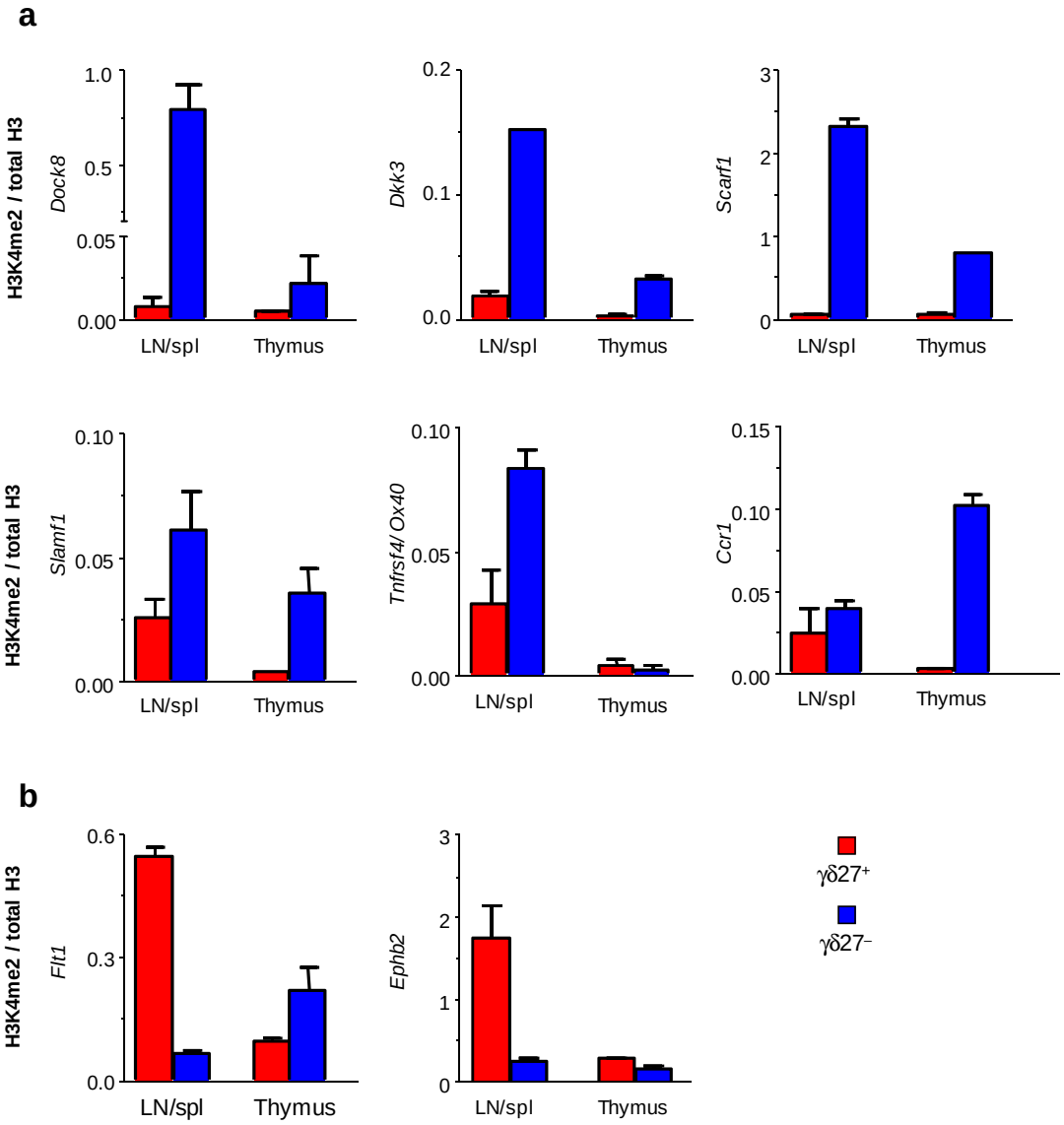

Supplementary Fig. 2. Permissive histone H3K4me2 modifications in candidate genes on thymic and peripheral γδ T cell subsets. Candidate genes (from Table 1) enriched for H3K4me2 modifications in CD27^{-} (γδ^{27-}) **(a)** or CD27^{+} (γδ^{27+}) **(b)** γδ T cells were analyzed by ChIP-qPCR on populations FACS-sorted from pooled lymph nodes and spleen (LN/spl) or from the thymus. Data are normalized against total H3 (mean ± SD).

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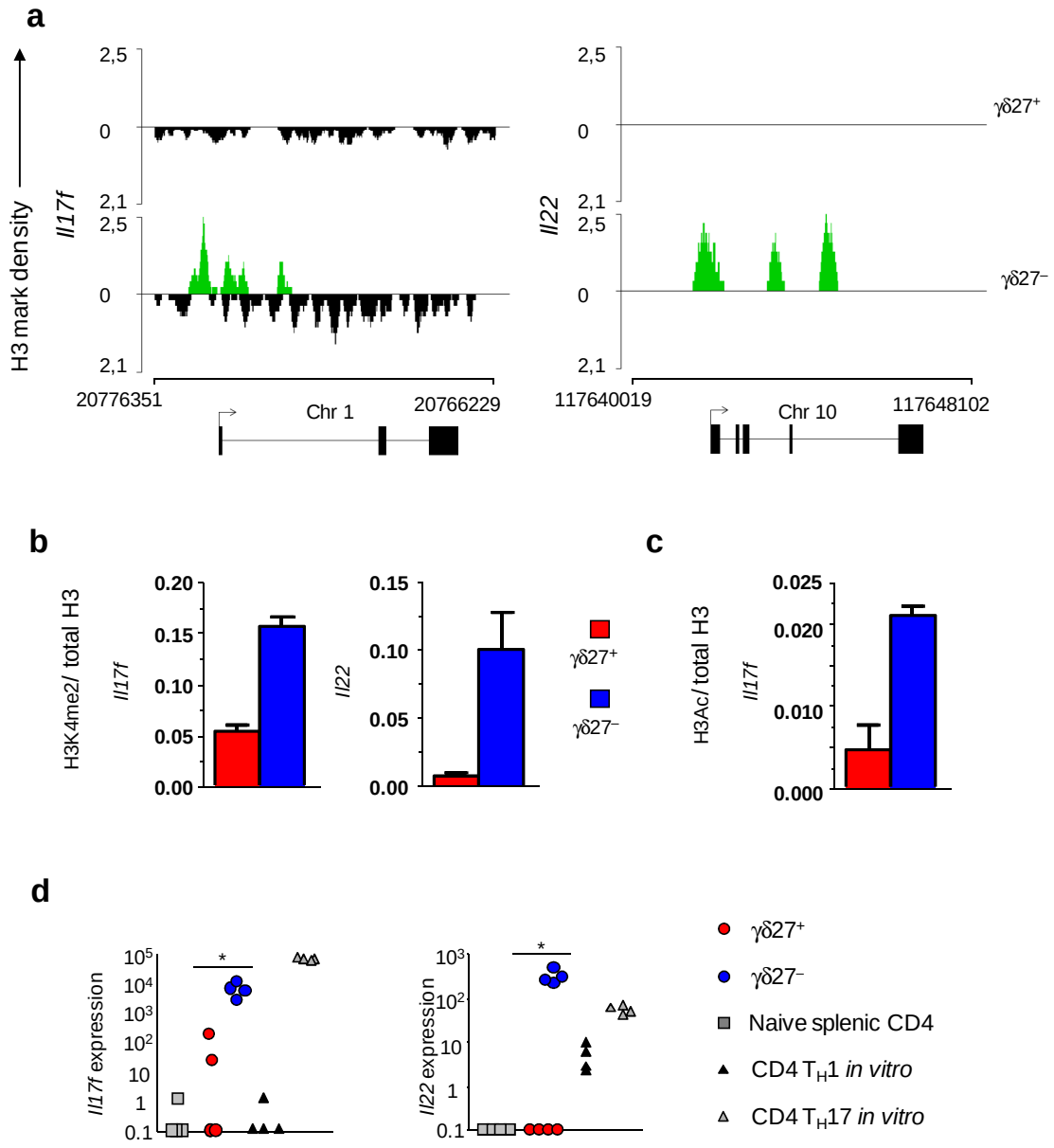

Supplementary Fig. 3. Histone modifications and transcription of *Il17f* and *Il22* in γδ T cell subsets. **(a)** ChIP-seq plots for H3K4me2 (green) and H3K27me3 (black) modifications on *Il17f* and *Il22* loci in peripheral CD27^{+} (γδ^{27+}) and CCR6^{+} CD27^{-} (γδ^{27-}) γδ T cells. **(b)** ChIP-qPCR validation of H3K4me2 modifications on *Il17f* and *Il22* in peripheral γδ^{27+} and γδ^{27-} T cells (mean ± SD). **(c)** ChIP-qPCR for H3Ac modifications on the *Il17f* promoter in peripheral γδ^{27+} and γδ^{27-} T cells (mean ± SD). **(d)** RT-qPCR data for *Il17f* and *Il22* expression (relative to *Actb*) on populations derived from peripheral T cells: *ex vivo* CD4^{+}, CD27^{+} (γδ^{27+}) and CCR6^{+} CD27^{-} (γδ^{27-}) γδ cells; and *in vitro*-generated CD4^{+} T_H1 and T_H17 cells.

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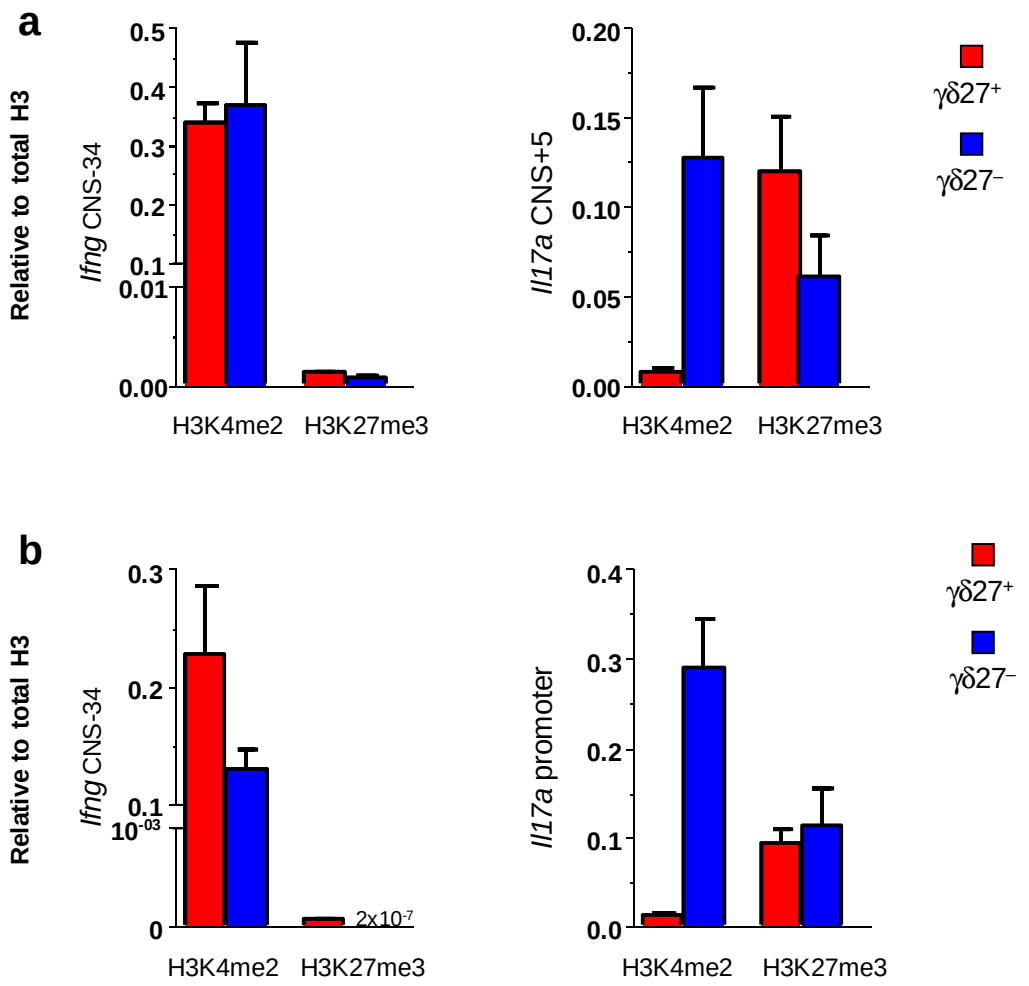

Supplementary Fig. 4. ChIP-qPCR validation of ChIP-seq data for H3K4me2 or H3K27me3 modifications on *Ifng* and *Il17a* loci in γδ T cell subsets. Peripheral **(a)** or thymic **(b)** CD27^{+} (γδ^{27+}) and CCR6^{+} CD27^{-} (γδ^{27-}) γδ T cells were analyzed by ChIP-qPCR for H3K4me2 and H3K27me3 modifications on *Ifng* (CNS-34 region) and *Il17a* (promoter or CNS+5 regions). Data are normalized against total H3 (mean ± SD).

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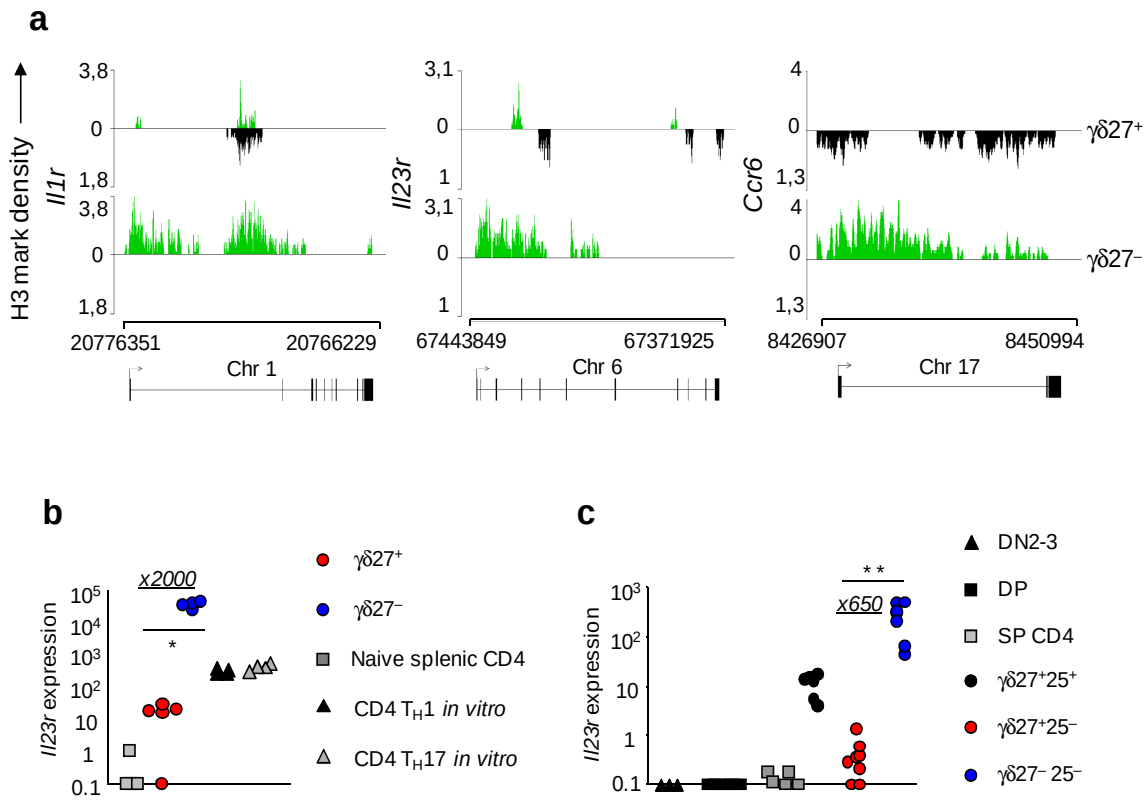

Supplementary Fig. 5. Histone H3 methylation patterns for *Il1r1*, *Il23r* and *Ccr6* in γδ T cell subsets. **(a)** ChIP-seq plots for H3K4me2 (green) and H3K27me3 (black) modifications on *Il1r1*, *Il23r and Ccr6* loci in peripheral CD27$^+$ (γδ^{27+}) or CCR6$^+$ CD27$^-$ (γδ^{27-}) γδ T cells. **(b)** RT-qPCR data for *Il23r* expression on populations derived from peripheral T cells: *ex vivo* CD4$^+$, CD27$^+$ (γδ^{27+}) and CCR6$^+$ CD27$^-$ (γδ^{27-}) γδ cells; and *in vitro*-generated CD4$^+$ T_H1 and T_H17 cells. **(c)** RT-qPCR data for *Il23r* expression on thymocyte subsets: CD4$^-$ CD8$^-$ CD25$^+$, double negative stages 2 and 3 (DN2-3) common progenitors; CD4$^+$ CD8$^+$ double positive (DP) and CD4$^+$ single positive (SP) cells of the αβ T cell lineage; and CD25$^+$ CD27$^+$ (γδ^{25+}), CD25$^-$ CD27$^+$ (γδ^{27+}) and CD25$^-$ CD27$^-$ (γδ^{27-}) thymocytes of the γδ T cell lineage.

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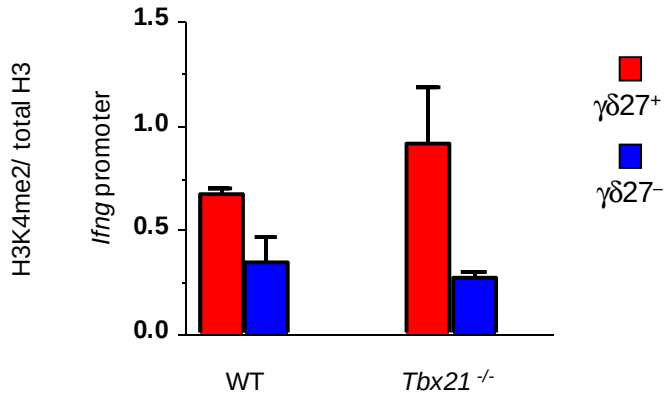

Supplementary Fig. 6. Histone H3K4me2 modifications on the *Ifng* promoter of *Tbx21*$^{-/-}$ γδ T cell subsets. Peripheral CD27^{+} (γδ^{27+}) or CD27$^{–}$ (γδ^{27-}) γδ T cells from wild-type (WT) or *Tbx21*-deficient (*Tbx21*$^{-/-}$) mice were analyzed by ChIP-qPCR for H3K4me2 modifications on the *Ifng* promoter. Data are normalized against total H3 (mean ± SD).

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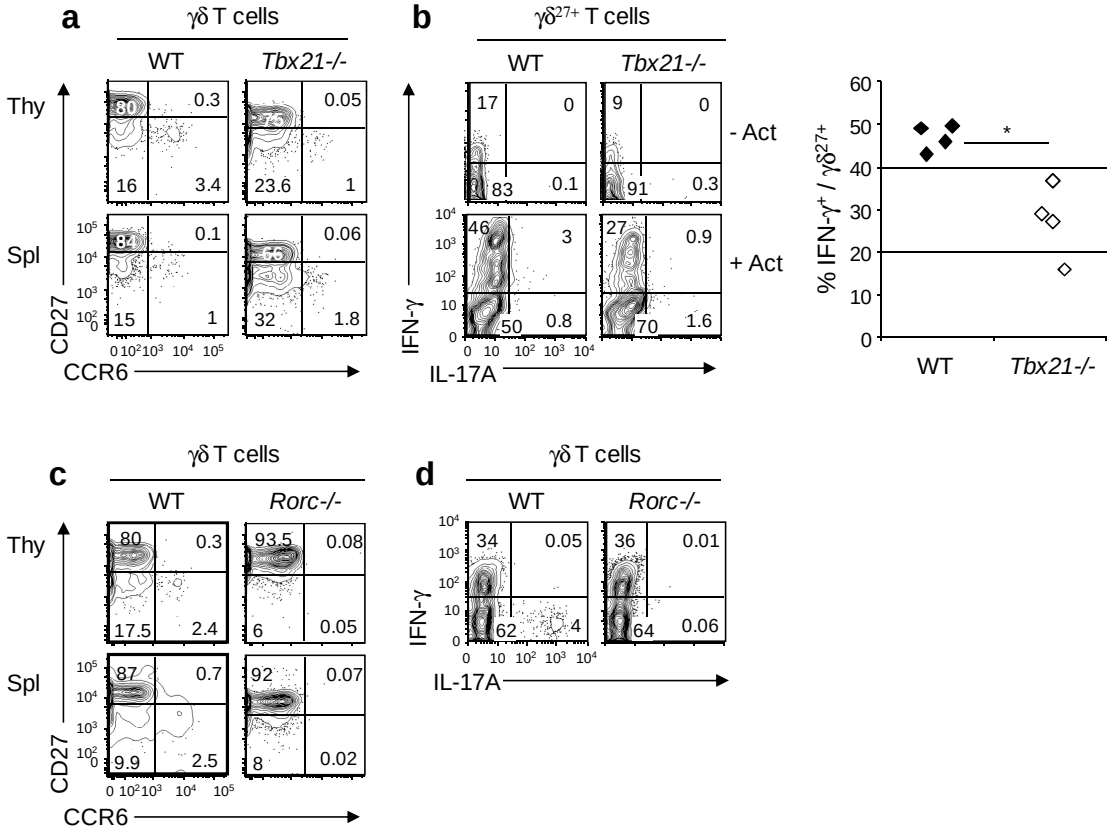

Supplementary Fig. 7. Analysis of effector γδ T cell populations in *Tbx21*$^{-/-}$ and *Rorc*$^{-/-}$ mice. Flow cytometry data for surface CD27 and CCR6 expression **(a,c)**; and intracellular IFN-γ and IL-17A cytokine production **(b,d)** in total γδ T cells **(a,c,d)** or CD27^{+} γδ T cells **(b)**.

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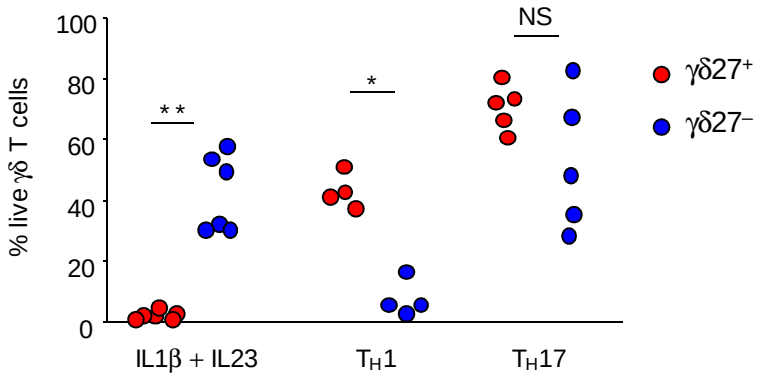

Supplementary Fig. 8. Maintenance of isolated $\gamma\delta^{27+}$ and $\gamma\delta^{27-}$ T cells in cytokine-defined culture media. $\gamma\delta^{27+}$ and $\gamma\delta^{27-}$ T cells were FACS-sorted from pooled spleen and lymph nodes from groups of 5 mice and stimulated for 48 hr in the presence of IL-1β plus IL-23, or standard T_H1 or T_H17 conditions (as described in Methods). Graph indicates percentages of live cells after 48 hr of culture, as assessed by forward/ side scatter on flow cytometry analyses. Each dot represents an independent culture. Mann-Whitney two-tailed statistical differences are indicated as NS, non significant; *$p<0.05$; **$p<0.01$.

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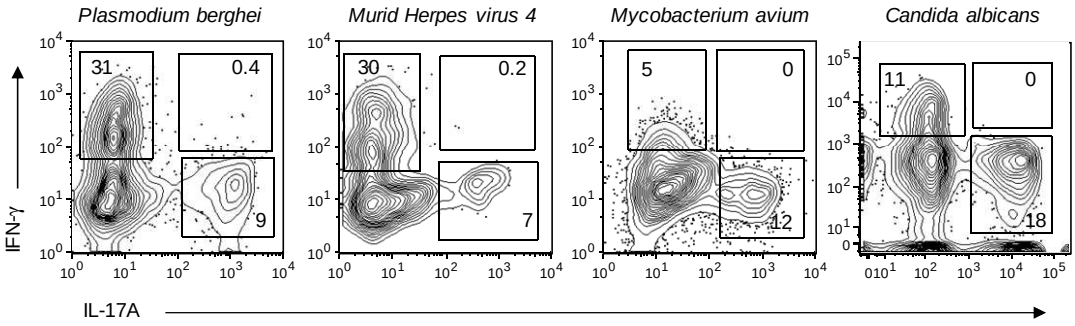

Supplementary Fig. 9. Cytokine production by peripheral lymphoid γδ T cells in systemic acute responses to infection. Flow cytometry analysis of intracellular IFN-γ and IL-17A protein expression in total γδ T cells isolated from the spleen and lymph nodes of C57BL/6 mice infected with the noted microorganisms (as described in Methods). Cells were stimulated for 4 hr with PMA and ionomycin before intracellular staining. Numbers adjacent to outlined areas indicate percentages of IFN-γ^+, IFN-γ^+ IL-17^+ or IL-17^+ γδ T cells.

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SUPPLEMENTARY TABLE 1

Genes differentially modified by H3K4me2 or H3K27me3 marks between $\gamma\delta^{27+}$ and $\gamma\delta^{27-}$ T cells, or between $CD4^+$ T_H1 and T_H17 cell subsets.

The annexed Excel file (**Supplementary Table 1.xls**) lists all genes with differential H3K4me2 and H3K27me3 marks between *ex vivo*-isolated $\gamma\delta^{27+}$ (gdCD27+) *versus* $\gamma\delta^{27-}$ (gdCD27-) T cells; or between *in vitro*-generated T_H1 *versus* T_H17 $CD4^+$ populations. The ChIP-seq profiles of histone modifications were quantitatively analyzed using the bioinformatics tools (described in Methods). Only gene containing regions with a density fold-enrichment of 1.5 or above (for each comparison) are listed. The table contains gene symbol, transcript ID, chromosome start and end coordinates, gene region; gene ontology categories; and fold enrichment in the indicated population (relative to either gdCD27+ *versus* gdCD27- or T_H1 *versus* T_H17 comparisons).

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SUPPLEMENTARY TABLE 2

Genes highly modified by H3K4me2 or H3K27me3 marks in γδ T cell subsets

Histone Mark	Gene Symbol	Fold Change	γδ Subset	Histone Mark	Fold Change	CD4 Subset
	Top 25 miscellaneous					
K4	*Gstcd*	67.74	CD27-			
K4	*Acyp2*	61.71	CD27-			
K4	*Dock8*	42.15	CD27-	K27	13.57	Th17
K4	*Pdzd2*	42.15	CD27-			
K4	*Wwox*	37.63	CD27-	K27	10.89	Th17
				K27	10.87	Th1
				K4	9.72	Th17
K4	*Pla2g2d*	37.46	CD27-			
K4	*Clip1*	37.20	CD27+			
K4	*Dkk3*	34.62	CD27-			
K4	*Kcnq5*	33.12	CD27-	K27	9.97	Th17
K4	*Ppp1r14c*	33.12	CD27-	K27	16.39	Th17
				K4	13.97	Th17
K4	*Sgip1*	30.10	CD27-			
K4	*Zp1*	30.10	CD27-			
K4	*Hivep1*	28.60	CD27-			
K4	*Slc17a4*	28.60	CD27-			
K4	*Dcp1b*	27.90	CD27+			
K4	*Crmp1*	27.09	CD27-			
K4	*Psme4*	27.09	CD27-			
K4	*Ptk2*	27.09	CD27-			
K4	*Reps2*	27.09	CD27-			
K4	*Sntb2*	27.09	CD27-	K27	15.08	Th17
K4	*Zranb3*	27.09	CD27-			
K4	*Eif4g3*	26.34	CD27-			
K4	*Myom1*	25.59	CD27-			
K4	*Zfp408*	25.25	CD27+			
K4	*Cdon*	25.09	CD27-			
	Receptor Activity					
K4	*Trpm6*	45.16	CD27-	K27	16.97	Th17
K4	*Nrp2*	37.20	CD27+			
K4	*Il1r1*	33.12	CD27-	K4	10.69	Th17
K4	*Il17rd*	28.60	CD27-	K27	15.08	Th17
K4	*Ntrk2*	24.91	CD27+	K27	16.97	Th17
K4	*Fbn1*	21.92	CD27+	K4	23.36	Th1
K4	*Pvrl1*	20.57	CD27-	K27	16.02	Th17
K4	*Ptprn2*	19.57	CD27-			
K4	*Lrrn2*	17.23	CD27-			
K4	*Gpr160*	16.86	CD27-			
K4	*Grik1*	16.18	CD27-	K27	16.34	Th17
K4	*Gpr174*	15.81	CD27-			
K4	*Itpr1*	15.81	CD27-	K27	25.14	Th17
K4	*Scarf1*	14.30	CD27-	K27	11.85	Th17
K4	*Ramp1*	13.80	CD27-			
K4	*Ccr6*	13.76	CD27-			
K4	*Xpr1*	13.55	CD27-			
K4	*Ccr1*	12.26	CD27-			
K4	*Ryk*	11.44	CD27-			
K4	*Ptprm*	10.63	CD27+	K27	14.61	Th17
K4	*Tnfrsf4*	10.35	CD27-			
K4	*Vipr2*	10.11	CD27-	K4	9.93	Th17
K4	*Grik2*	9.78	CD27-	K27	12.57	Th17
K4	*Sorl1*	9.78	CD27-			
K4	*Ncoa7*	9.53	CD27-			
K4	*Stab1*	9.53	CD27-			
K4	*Igf1r*	9.46	CD27-			
K4	*Flt1*	9.43	CD27+			
K4	*Cd44*	9.41	CD27-			
K4	*Gpr98*	9.41	CD27-			
K4	*Slamf1*	9.28	CD27-			
K27	*Dner*	9.11	CD27+	K27	10.10	Th17
K4	*Ptpra*	9.03	CD27-			
K27	*Grm7*	8.98	CD27+	K27	33.94	Th17
K4	*Ptpn5*	8.92	CD27+			
K4	*Il1r2*	8.73	CD27-	K27	10.77	Th17
K4	*Sema6a*	8.60	CD27-			
K4	*Ephb2*	8.35	CD27+			
K4	*Mtus1*	8.28	CD27-	K27	11.92	Th17
K4	*Il23r*	8.03	CD27-			

Histone Mark	Gene Symbol	Fold Change	γδ Subset	Histone Mark	Fold Change	CD4 Subset
	Cytokines					
K4	*Il5*	20.17	CD27-			
K4	*Il21*	16.81	CD27-			
K4	*Il17a*	16.06	CD27-	K4	11.89	Th17
K4	*Il17f*	13.25	CD27-	K4	8.08	Th17
K4	*Il22*	10.54	CD27-			
	Signal Transduction					
K4	*Tnk2*	24.33	CD27-			
K4	*Brca1*	20.07	CD27-			
K27	*Pde3b*	16.75	CD27-	K27	30.16	Th17
K4	*Prkca*	15.05	CD27-	K4	46.33	Th1
K4	*Tiam1*	14.62	CD27-	K27	15.08	Th17
K4	*Psd*	13.55	CD27-			
K4	*Wisp1*	13.55	CD27-			
K4	*Optn*	13.17	CD27-	K27	8.30	Th17
K4	*Txnl1*	12.04	CD27-			
K4	*Baiap2l1*	11.83	CD27-	K27	12.57	Th17
K4	*Dapk1*	11.21	CD27+			
K4	*Fgd3*	11.14	CD27-	K4	8.76	Th17
K4	*Gpsm2*	11.04	CD27-			
K4	*Irak3*	11.04	CD27-			
K4	*Sh3gl3*	11.04	CD27-			
K27	*Gna15*	11.02	CD27+	K27	8.01	Th17
K27	*Pde8b*	10.61	CD27+			
K4	*Mapk10*	10.24	CD27-	K4	9.45	Th17
K4	*Cradd*	10.16	CD27-	K27	18.85	Th17
K4	*Bcar3*	10.03	CD27-	K27	11.31	Th17
K27	*Brc*	9.79	CD27+			
K4	*Rasgrp3*	9.78	CD27-			
K4	*Camk4*	9.03	CD27-			
K4	*Nedd9*	9.03	CD27-			
K4	*Camk2g*	8.66	CD27-	K27	10.44	Th17
K4	*Rhot1*	8.53	CD27-			
K4	*Pik3ca*	8.43	CD27-			
K4	*Elmo1*	8.11	CD27-			
	Transcription Factor Activity					
K4	*Zbtb38*	31.61	CD27-			
K4	*Tshz2*	21.26	CD27+	K27	20.74	Th17
K4	*Stat5b*	18.06	CD27-			
K27	*Satb1*	14.70	CD27-			
K4	*Arnt2*	14.30	CD27-			
K27	*Mef2c*	13.47	CD27+			
K4	*Mllt10*	12.54	CD27-			
K4	*Dlx3*	12.29	CD27+			
K4	*Gabpb2*	11.67	CD27-			
K4	*Vdr*	11.18	CD27-			
K4	*Atf6*	11.04	CD27-			
K4	*Runx2*	10.54	CD27-			
K4	*Tcf7l1*	10.54	CD27-			
K4	*Pbx1*	10.16	CD27-	K27	25.77	Th17
K4	*Pbx3*	10.16	CD27-	K27	25.77	Th17
K27	*Clock*	9.38	CD27+			
K4	*Lcor*	9.03	CD27-			
K4	*Rora*	9.03	CD27-	K27	20.16	Th1
				K27	15.08	Th17
				K4	11.70	Th17
K4	*Erg*	8.80	CD27+			
K4	*Foxn2*	8.36	CD27-			
K4	*Arntl*	8.28	CD27-			
K4	*Nfatc2*	8.28	CD27-			

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Selection of 120 genes highly differentially modified by H3K4me2 (K4) or H3K27me3 (K27) marks between $CD27^+$ and $CD27^-$ γδ T cell subsets. From the full list of differentially modified gene regions (**Supplementary Table 1**), a cut-off of 8-fold difference between $CD27^+$ and $CD27^-$ γδ T cells was used to select various candidates: the top 25 (miscellaneous) and genes belonging to particular functional categories of interest: receptor activity, cytokines, signal transduction and transcription factor activity. Differences above the 8-fold cut-off are also indicated for $CD4^+$ T_H1 and T_H17 cells. Details about coordinates of enriched regions within each gene are provided in Supplementary Table 1.

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SUPPLEMENTARY TABLE 3

List of primers used for ChIP-qPCR analyses

Primer name	**FWD primer (5'-3')**	**REV primer (5´-3´)**
Ifng Promoter	CGAGGAGCCTTCGATCAGGT	GGTCAGCCGATGGCAGCTA
Ifng CNS-34	TGCTTTCTCCCCTGTCTCAATTAT	ACACACACACACCCTTTCTTCATT
Il17a Promoter	GAACTTCTGCCCTTCCCATCT	CAGCACAGAACCACCCCTTT
Il17a CNS+5	AGGCCCACAATGTAGGTCAG	CAGGCTGGGAAGTCTCTCTG
Il17f Promoter	ACTGCATGACCCGAAAGCA	TTTAATTCCCCCACAAAGCAA
Rorc	TCCAATACCTTGGCCAAAAC	CTTTGCCTCGTTCTGGACTATAC
Eomes	TGGAGATATTCTGTCCACTTCG	TCAGGGTTTTTCCTTAAGTGTG
Tbx21	GGGAACCGCTTATATGTCCA	GAGCTTTAGCTTCCCAAATGAA
Il22	TCCCTTATGGGGACTTTGG	GGAAGTTGGACACCTCAAGC
Dock 8	CCTCTACCAATGGCATTTTCC	ACACAAGGCTCTGTTGTAGCC
Dkk3	CAGAGCGAAACTCAAAACAGC	GCCCAGAATAACCTCAAACTTGT
Scarf1	GCTTTTCCCCATTGTGAGAC	GGTTATTTATTGCACTGGTCACCT
Ephb2	CGCAGCAGTGGTCTCTCC	TGAGAAACATTTGGGCTGAA
Slamf1	ACTGGACCCTTATATTGTTTGAACTT	GTCATGTTCTTACAACTTCATCTCATT
Flt1	CAGCGCGTAAGGCAAGAC	GCCAAGCAGAAGCAGGAG
Tnfrsf4	TCTCCAGGGCTATCTGACCA	CCTCCTGGCCTCTCCTCTAA
Ccr1	CGATAACAAATTCCTCAATATCACTG	TTGGGAAATGATGACAATGC

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