Supplementary Figures

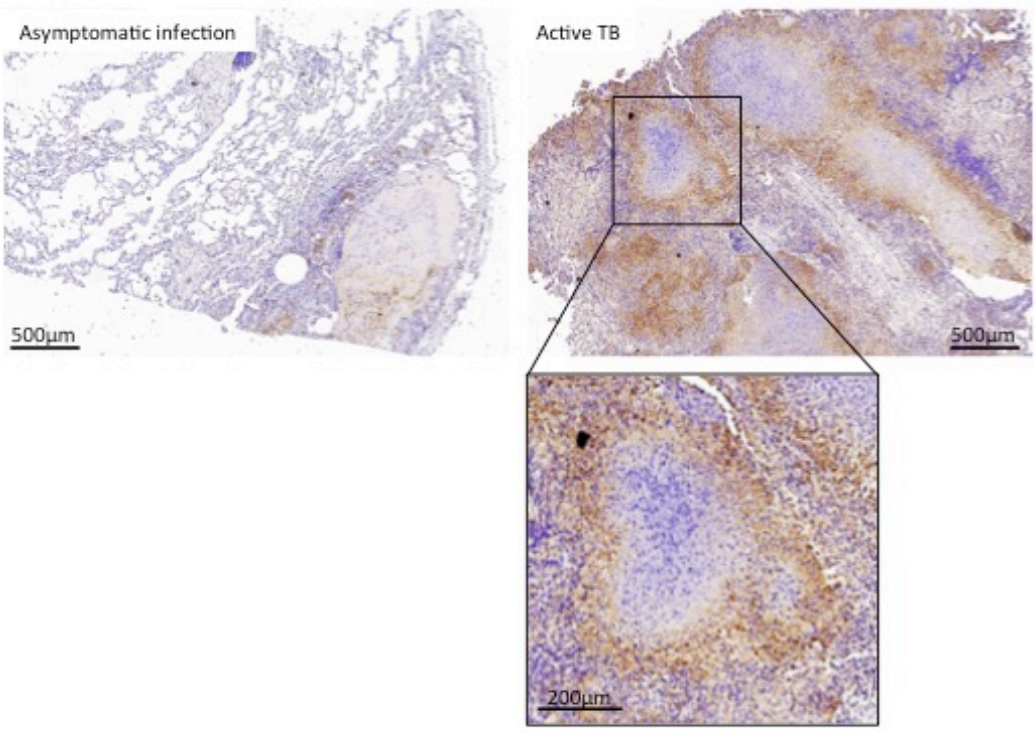


Fig. S1 Immuno-histological analysis of DCIR expression in the lungs of *M. tuberculosis*-infected non-human primates (macaques) during asymptomatic infection (left panel) or active TB disease (right panels).

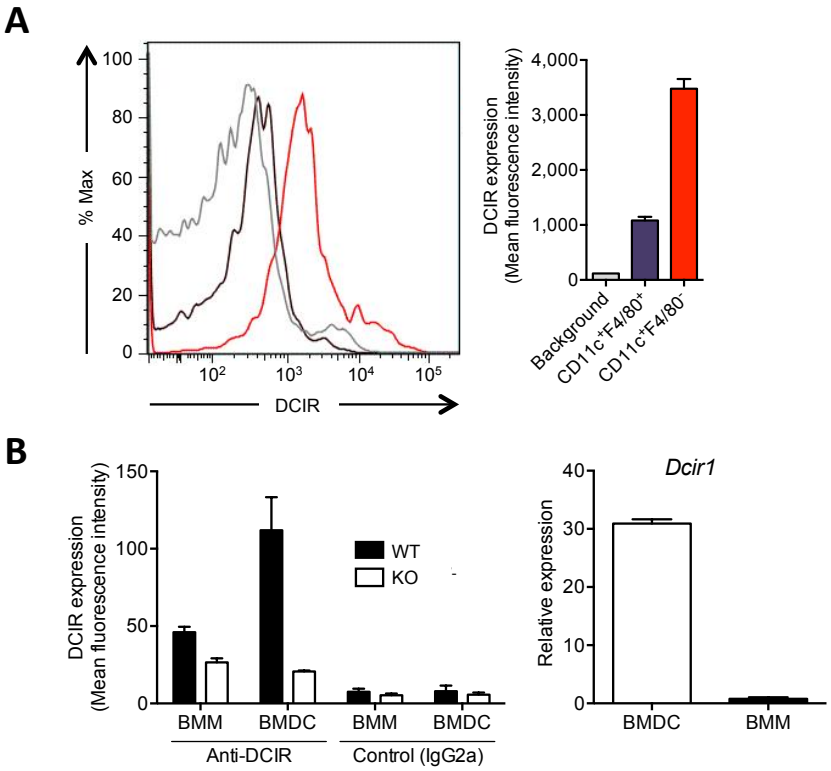
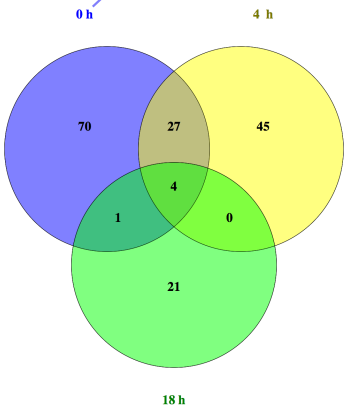


Fig. S2 DCIR (Dcir1/Clec4a2) expression in mouse macrophages and DCs, and phenotypic characterization of DCIR-KO and WT DCs. (*A*) FACS analysis of DCIR expression in DC11c^{+}F4/80^{+} macrophages and CD11c^{+}F4/80^{-} DCs in the lungs of naïve mice. Left panel shows a representative FACS analysis (grey line, control isotype; dark line, macrophages; red line, DCs). Right panel shows DCIR mean fluorescence intensity as mean±s.e.m. of 4 biological replicates. (*B*) DCIR expression in mouse bone marrow-derived macrophages (BMM) or DCs (BMDC). Left panel shows DCIR mean fluorescence intensity in cells from WT or KO mice as mean±s.e.m. of 3 biological replicates. Right panel shows RT-qPCR quantification of the DCIR mRNA in BMDC and BMM as mean±s.e.m. of 3 biological replicates and is representative of 2 independent experiments. Statistical analysis was performed using Student's *t*-test; **P*<0.05.

A

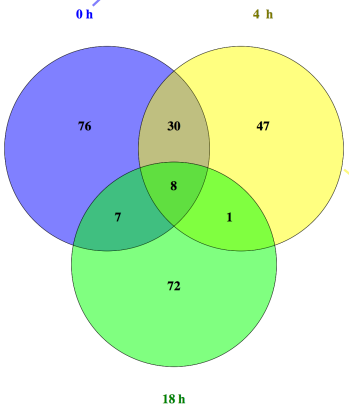
GO biological process complete	Reference list	Experiment	Expected	Fold enrichment	P-value
positive regulation of phosphorylation (GO:0042327)	899	15	3.26	4.60	6.12E-03
positive regulation of protein phosphorylation (GO:0001934)	857	14	3.11	4.50	1.92E-02
positive regulation of phosphorus metabolic process (GO:0010562)	1017	15	3.69	4.06	2.78E-02
positive regulation of phosphate metabolic process (GO:0045937)	1017	15	3.69	4.06	2.78E-02
locomotion (GO:0040011)	1027	15	3.73	4.02	3.13E-02
regulation of multicellular organismal process (GO:0051239)	2621	25	9.51	2.63	2.93E-02
positive regulation of biological process (GO:0048518)	5093	37	18.48	2.00	3.81E-02



UP-regulated in DCIR-KO as compared to WT

B

GO biological process complete	Reference list	Experiment	Expected	Fold enrichment	P-value
response to interferon-alpha (GO:0035455)	20	5	.08	63.41	1.68E-04
negative regulation of viral genome replication (GO:0045071)	41	7	.16	43.30	3.10E-06
response to interferon-beta (GO:0035456)	36	6	.14	42.27	6.72E-05
defense response to virus (GO:0051607)	130	14	.51	27.31	1.75E-12
regulation of viral genome replication (GO:0045069)	66	7	.26	26.90	8.03E-05
negative regulation of viral process (GO:0048525)	81	8	.32	25.05	1.18E-05
negative regulation of viral life cycle (GO:1903901)	77	7	.30	23.06	2.28E-04
response to virus (GO:0009615)	173	15	.68	21.99	3.22E-12
negative regulation of multi-organism process (GO:0043901)	159	9	.63	14.36	1.28E-04
regulation of viral process (GO:0050792)	146	8	.58	13.90	1.07E-03
regulation of viral life cycle (GO:1903900)	135	7	.53	13.15	9.67E-03
regulation of symbiosis, encompassing mutualism through parasitism (GO:0043903)	177	8	.70	11.46	4.51E-03
innate immune response (GO:0045087)	458	20	1.81	11.08	1.11E-11
immune effector process (GO:0002252)	388	15	1.53	9.81	3.03E-07
defense response to other organism (GO:0098542)	487	15	1.92	7.81	6.73E-06
regulation of multi-organism process (GO:0043900)	419	11	1.65	6.66	6.82E-03
response to other organism (GO:0051707)	737	17	2.91	5.85	3.39E-05
response to external biotic stimulus (GO:0043207)	737	17	2.91	5.85	3.39E-05
immune response (GO:0006955)	873	20	3.44	5.81	1.30E-06
response to biotic stimulus (GO:0009607)	765	17	3.02	5.64	5.87E-05
defense response (GO:0006952)	1060	20	4.18	4.79	3.60E-05
immune system process (GO:0002376)	1674	27	6.60	4.09	1.17E-06
response to external stimulus (GO:0009605)	1559	22	6.15	3.58	9.51E-04
multi-organism process (GO:0051704)	1641	22	6.47	3.40	2.30E-03



DOWN-regulated in DCIR-KO as compared to WT

GO biological process complete	Reference list	Experiment	Expected	Fold enrichment	P-value
defense response to virus (GO:0051607)	130	6	.35	17.17	1.18E-02
response to virus (GO:0009615)	173	7	.47	15.05	3.57E-03
immune system process (GO:0002376)	1674	17	4.50	3.78	9.93E-03

Fig. S3 Venn diagram of genes up (*A*)- or down (*B*)-modulated in DCIR-deficient DCs, compared to in WT cells, at 0, 4 or 18 h after *M. tuberculosis* infection. The boxes show functional gene set enrichment analysis using Gene Ontology.

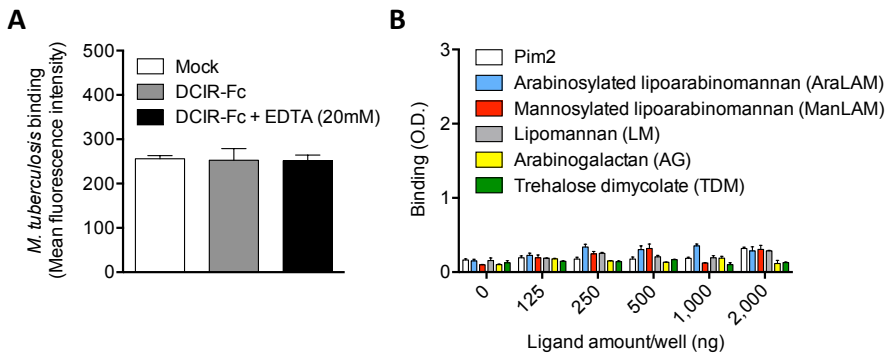


Fig. S4 DCIR does not recognize *M. tuberculosis* or mycobacterial surface glycosylated ligands. (*A*) *M. tuberculosis* bacteria were incubated for 1 h at 37°C with DCIR-Fc (10µg/ml) in the presence or absence of EDTA, or in Lectin buffer (Mock). DCIR-Fc was immune-detected with a PE-coupled anti-human IgG antibody and binding was analyzed by FACS. (*B*) Different constituents of the mycomembrane, PIM_2, AraLAM, ManLAM, LM, AG, and TDM were coated onto 96-well plates at different concentrations. The plates were then incubated with 10 µg/ml of chimeric DCIR-hFc at room temperature during 2 h. Binding was detected using a biotinylated polyclonal anti-human IgG and streptavidin-HRP. Colorimetry analysis was analyzed at 450 -570 nm using a spectrophotometer. Data are presented as mean±s.e.m. of at least 3 replicates, and are representative of at least 2 independent experiments.

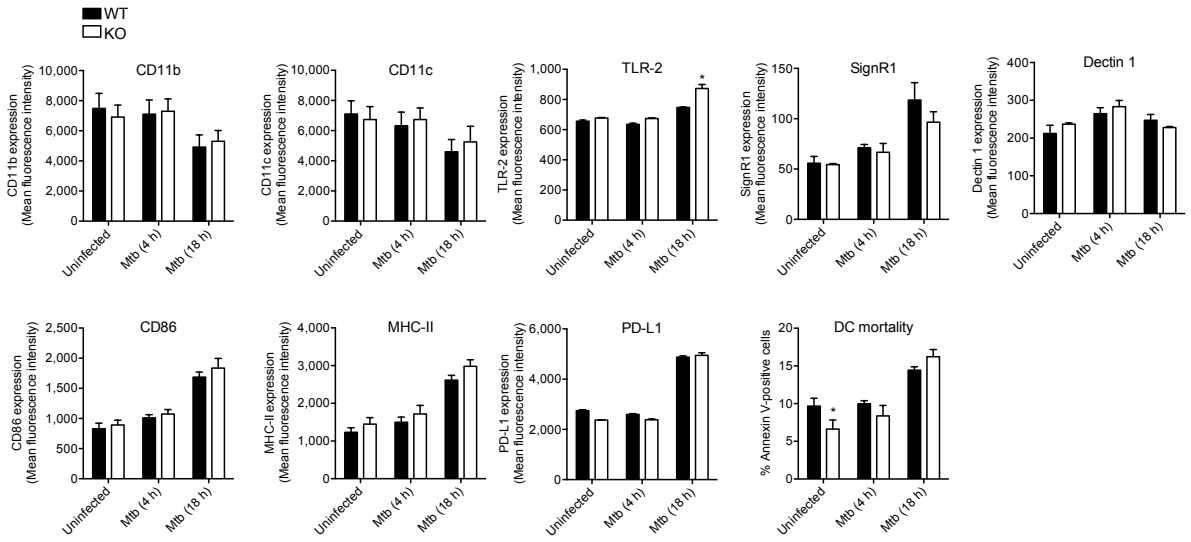


Fig. S5 FACS quantification of receptors (CD11b, CD11c, TLR-2, SignR1, Dectin 1), molecules involved in T cell stimulation or inhibition (CD86, MHC-II, PDL-1), and mortality in BMDC during *M. tuberculosis* infection. Data are presented as mean±s.e.m. of 3 independent experiments. Statistical analysis was performed using Student's *t*-test; **P*<0.05.

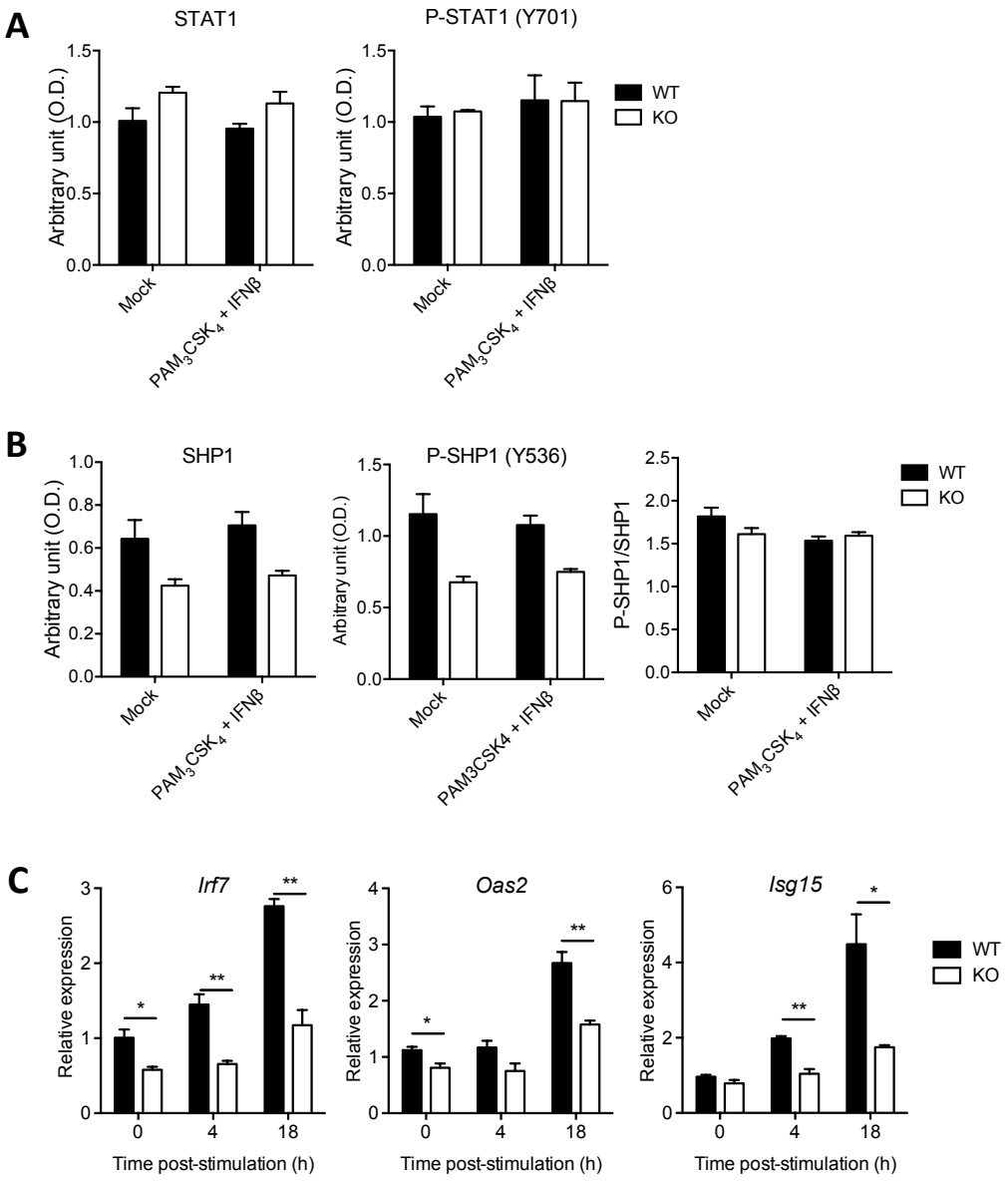
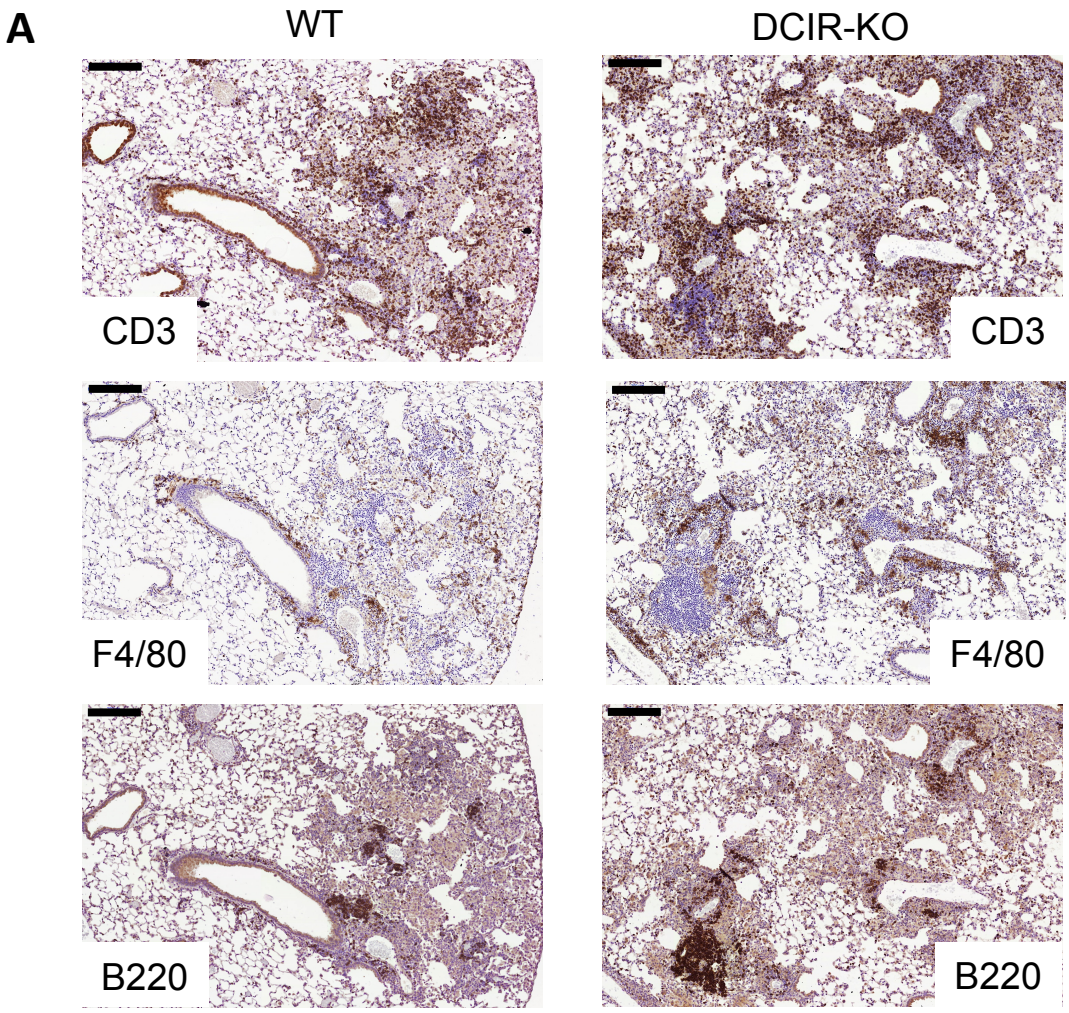


Fig. S6 Analysis of the phosphorylation status of STAT1 (Tyr701) and SHP1 (Tyr536) and of type II IFN signaling in WT vs. KO cells. (*A,B*) WT or KO DCs were left unstimulated (Mock), or co-stimulated with the TLR2 agonist lipopeptide Pam_3CSK_4 and IFNβ for 4 h, as indicated. STAT1 (*A*) and SHP1 (*B*), and their phosphorylated forms were quantified in cell lysates by ELISA. (*C*) WT or KO DCs were stimulated with 20 ng/ml IFNγ for the indicated periods of time. Relative expression of the ISGs *Irf7*, *Oas2*, and *Isg15* was quantified by RT-qPCR. In all panels, data are presented as mean±s.e.m. of at least 3 biological replicates. Statistical analysis was performed using Student's *t*-test; **P*<0.05, ***P*<0.01.


A
WT
DCIR-KO
CD3
CD3
F4/80
F4/80
B220
B220

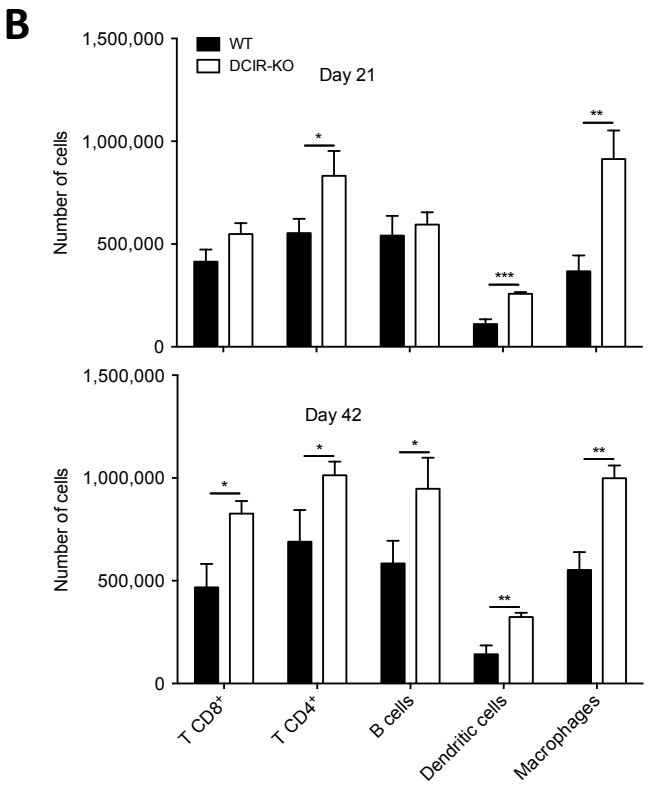

B
WT
DCIR-KO
Day 21
Day 42
Number of cells
1,500,000
1,000,000
500,000
0
T CD8+
T CD4+
B cells
Dendritic cells
Macrophages

Fig. S7 (*A*) Immuno-histological analysis of CD3-, B220- and F4/80-expressing T lymphocytes, B lymphocytes and macrophages, respectively, in the lungs of *M. tuberculosis*-infected WT or KO mice 42 days after infection. Bars correspond to 200 μm. (*B*) Flow cytometry analysis of $CD4^+$ and $CD8^+$ T cells, B cells ($CD19^+$), DCs ($CD11b^+CD11c^+F4/80^-$) and macrophages ($CD11b^+$ $F4/80^+$) in the lungs of *M. tuberculosis*-infected WT or KO mice 21 (top panel) or 42 (bottom panel) days after infection. Data show mean±s.e.m. of 5 biological replicates. Statistical analysis was performed using Student's *t*-test; **P*<0.05, ***P*<0.01, ****P*<0.001.

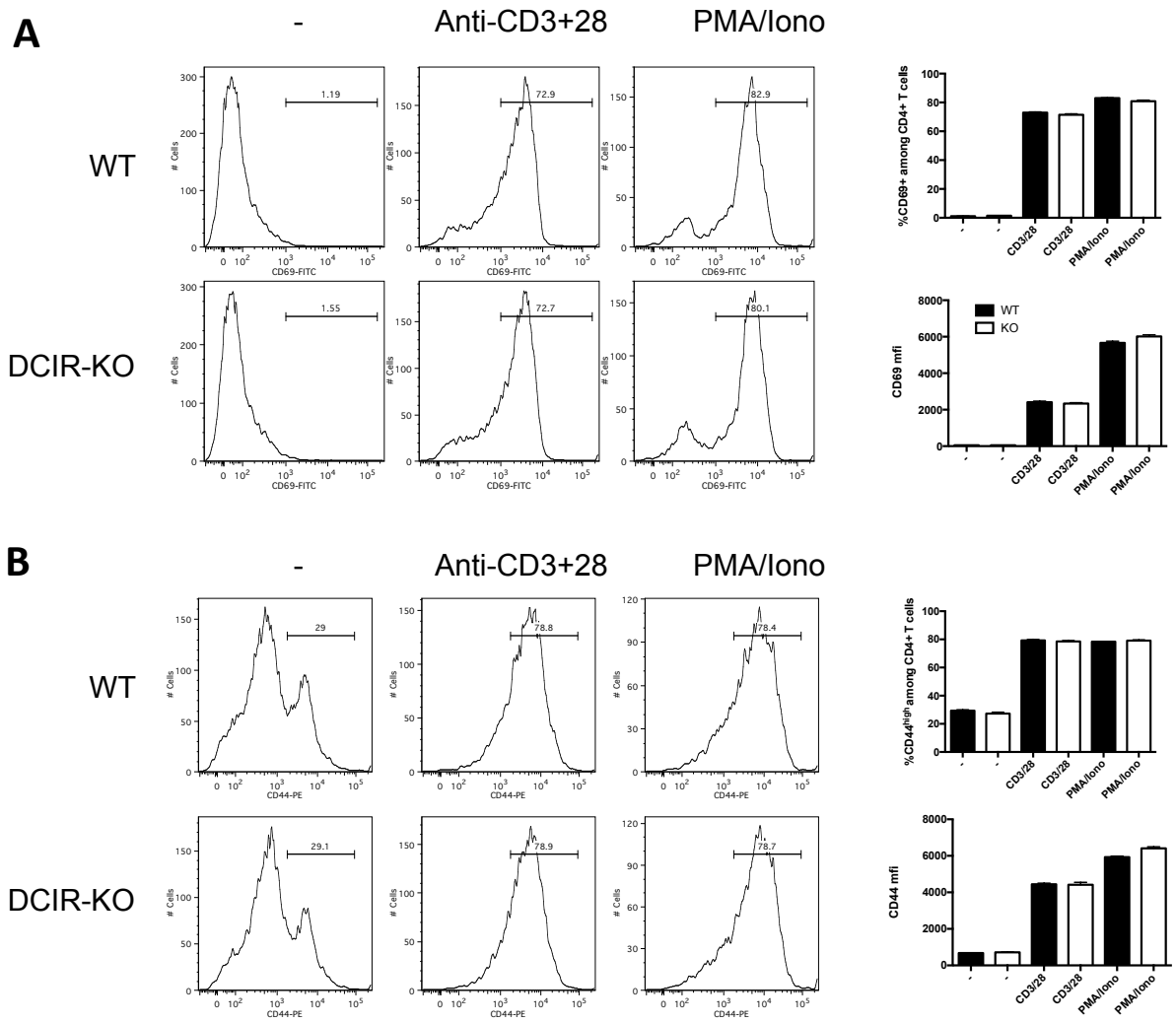


Fig. S8 No intrinsic differences in activation of WT or DCIR KO T cells. (A) CD4+ T cells purified from the spleen of WT or KO mice were left unstimulated (-) or stimulated in vitro using anti-CD3+28 mAb or PMA/ionomycine then anlyzed by flow cytometry for the upregulation of the CD69 activation marker. Flow cytometry histograms are representative examples of CD69 staining in the indicated conditions for WT (top panels) or KO (lower panels) CD4+ T cells. Right histograms represent the quantification of the % CD69 cells among total CD4+ T cells (top panel) or CD69 mfi (lower panel) on CD4+ T cells for WT (black bars) or KO (white bars) T cells. B) As in A) excet that CD44 expression was analyzed. animal. Data are representative of 2 independent experiments performed in triplicates.

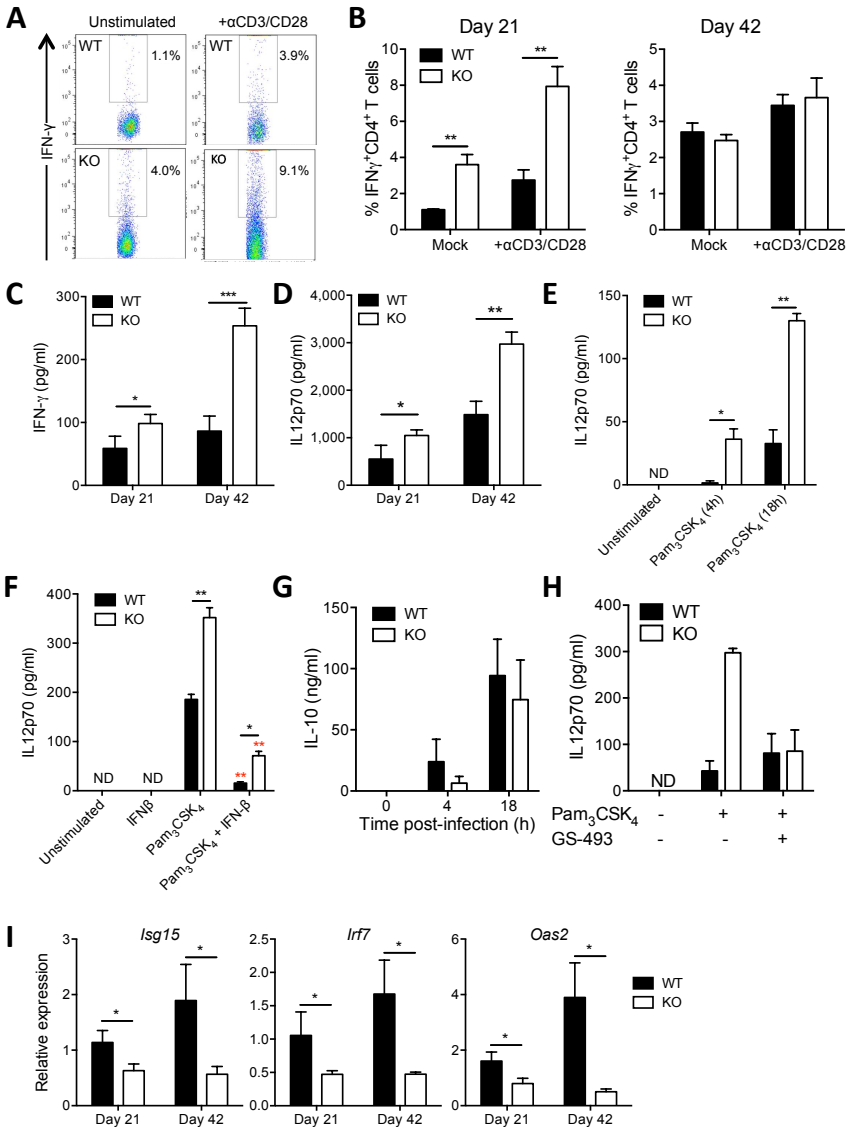


Fig. S9 DCIR impairs anti-*M. tuberculosis* Th1 immunity in the LNs. Mice were infected as in Figure 3. (*A,B*) IFNγ-producing $CD4^+$ Th1 cells were quantified by intracellular cytokine FACS staining after either stimulation with anti-CD3 and -CD28 antibodies or in the absence of restimulation (Mock), as indicated. A representative experiment at day 21 is depicted in (A). (B) represents analysis of 6 biological replicates. (*C,D*) ELISA quantification of total IFNγ (C) or IL-12p70 (D) in LN lysates of infected mice. (*E,F*) ELISA quantification of IL-12p70 in bone marrow-derived DCs from WT or KO mice stimulated with the TLR-2 agonist Pam_3CSK_4 in the absence (E) or presence (F) of IFNβ. In (F) cells were stimulated for 18 h. (*G*) ELISA quantification of IL-10 in *M. tuberculosis*-infected DCs after various periods of time. (*H*) ELISA quantification of IL-12p70 in bone marrow-derived DCs from WT or KO mice stimulated for 4 h with the TLR-2 agonist Pam_3CSK_4 in the absence or presence

of the SHP2 inhibitor GS-493. (*I*) RT-qPCR quantification of gene expression of the ISGs *Isg15*, *Irf7* and *Oas2* in LN lysates of *M. tuberculosis*-infected WT or KO mice. In (C-I), data are presented as mean±s.e.m. of at least 3 biological replicates, and are representative of at least 2 independent experiments. Statistical analysis was performed using Student's *t*-test; **P*<0.05, ***P*<0.01.

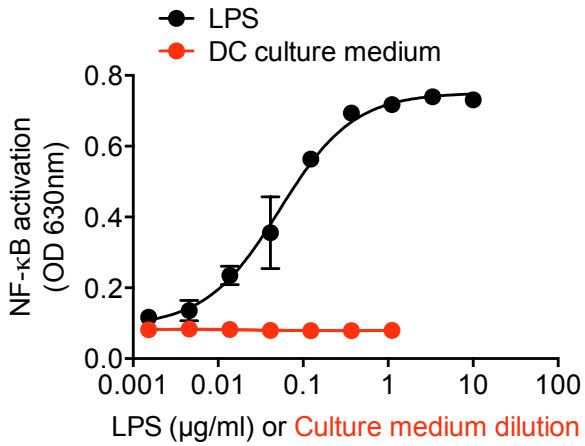


Fig. S10 NF-κB activation in HEK-Blue™ TLR4 cells by various stimuli. Cells (5 × 104) were plated in 96-wells plates and stimulated at 37°C for 2h by the indicated doses of LPS or the indicated dilutions of DC culture medium (1 means pure culture medium). NF-κB activity was determined by reading OD at 630 nm.

Supplementary Tables

Table S1. List of IFN-stimulated genes (ISG) and –inhibited genes (IIG) genesets used for generating the Bubblemaps in Fig. 1B

ISG_geneset	IIG_geneset
Ly6a	Scd2
Irf7	Emb
Usp18	Serpinb1a
Ifit2	Elovl6
Ctse	Gde1
Ifi204	Gbp2b
Csprs	Enc1
Ifi44	Tle1
Ifi27l2a	Brix1
Eif2ak2	1600029D21Rik
Cxcr4	Sgms1
Cmpk2	Snx24
Slfn8	Gcsh
Slfn5	Fcgr2b
Ai607873	Ado
Xaf1	Hepacam2
Rtp4	Lyz1

Oas1c	Setp03
Isg20	Xpot
Rsad2	Polr3g
Oas1a	Bst1
Oas2	Ppargc1b
Loc667373	Ctsl
Ddx58	Acsl3
Pydc4	Pgm2L1
Pydc3	Ppp1R3d
I830012O16Rik	Sod2
Ifit1	Cpd
Spon1	Znrf2
Mx1	Ccbl2
Asprv1	Ptpla
Oasl2	Btg2
Apod	5430435G22Rik
Parp9	Ppfia4
Fap	1300007C21Rik
Il18	Zfp236
Il15	Fcgr3
Nat5	Loc622147

Slfn1	
Sct	
Ms4a6b	
Ms4a4d	
Daxx	
Asb13	
Stk39	
Pttg1	
Sgcb	
Mx2	
St8Sia1	
Abcb1a	
Etnk1	
Tktl1	
Ms4a4c	
Lgals9	
Car13	
Trim34b	
Ly6c2	
Slfn3	
Stat2	

Tor3a	
Oprd1	
Sp100-rs	
H2-T24	
Loc270335	
Gjb2	
Ildr1	
Rpl39l	
Ms4a4b	
Oasl1	
1600014C10Rik	
Tmem219	
Loc432823	
Pgap2	
Wdfy1	
Trim34a	
Oas1b	
Oas3	
Trim25	
Mitd1	
Ifih1	

Tdrd7	
Parp12	
Znfx1	
Slfn4	
Dopey2	
Zc3hav1	
Pvrl4	
Dck	
Pik3ap1	
9030607L20Rik	
Hmgn3	
Herc6	
Parp14	
Mthfr	
Frmd4a	
Sp100	
Mxd1	
Pyhin1	
Helz2	
C130026I21Rik	
Trim30d	

Bc147527	
Aw011738	
9530028C05	
Cdc42bpg	
5230400M03Rik	
B430306N03Rik	
Fam46a	
Phf11d	
Adar	
Chic1	
Synj1	
Loc240327	
G530011O06Rik	
Lrp8	
Ppm1k	
Hsh2d	
Serinc4	
2010109K09Rik	
D130084N16Rik	
Samhd1	
Pnpt1	

Loc665317	
Usp25	
Atp10a	
Rilpl1	
Pml	
Ifi203	
Kdr	
Ehd4	
H2-T10	
Ms4a6c	
Xdh	
Nt5C3	
Rab7l1	
Ddx60	
Tcstv3	
Trim30a	
Ankle2	
Rnf213	
Whsc1l1	
Fndc3a	
Pou3f1	

Samd9l	

Table S2. Primers used for RT-qPCR experiments

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Hprt	GTTCTTTGCTGACCTGCTGGAT	CCCCGTTGACTGATCATTACAG
Irf7	ACTTCAGCACTTTCTTCCGAGAAC	AGGTAGATGGTGTAGTGTGGTGAC
Isg15	AGCAATGGCCTGGGACCTAAAG	AGTCACGGACACCAGGAAATCG
Ifit1	AGCAGAGAGTCAAGGCAGGTTTC	TGGTCACCATCAGCATTCTCTCC
Oas2	AAAGTCCTGAAGACCGTCAAGGG	ACAACAATGTCAGCATCTGATCCC
Cxcl9	AGCAGTGTGGAGTTCGAGGAAC	AGGGATTTGTAGTGGATCGTGC
Tnfsf10	GCTGTGTCTGTGGCTGTGACTTAC	TCATCCGTCTTTGAGAAGCAAGC
Ccl2	CCACTCACCTGCTGCTACTCATT	TTCCTTCTTGGGGTCAGCACAGA
Ifnβ	GCACTGGGTGGAATGAGACT	AGTGGAGAGCAGTTGAGGACA
Ifnα	TCTGATGCAGCAGGTGGG	AGGGCTCTCCAGACTTCTGCTCTG
Tnfα	CAAAATTCGAGTGACAAGCCTGT	CCACTTGGTGGTTTGCTACGA
Nos2	TCCTCACGCTTGGGTCTTGTTC	TCCAACGTTCTCCGTTCTCTTGC
Mrc1	AATGCCAAAAATTATTGATCGTG	ACGGTGACCACTCCTGCTG
Ym1	TACCCTATGCCTATCAGGGTAAT	CCTTGAGCCACTGAGCCTTC
Arg1	AATGAAGAGCTGGCCTGGTGTGGT	ATGCTTCCAACTGCCAGACTGTG

Table S3. Histopathological scoring of TB lesions in NHPs

	Type of lesions	Granuloma Size	Type of granuloma	Distribution pattern	Cellular composition	Notes
Asymptomatic infection **#46**	Granulomatous	Small	Non-necrotizing	Focal	- Lymphocytic cuff (1) - Epithelioid Mφ - Fibrosis (+++)	1: with few plasma cells
Active TB **#55**	Granulomatous	Medium	Caseous (admixed with some PMN) Non-necrotizing (Mixed)	Multifocal coalescing	- Lymphocytic cuff (1) - Epithelioid & foamy Mφ (2) - MGC	1: with plasma cells 2: presence of foamy Mφ in the alveoli in the vicinity of granulomas

Abbreviations: Mφ: Macrophages; MGC: Multinucleated giant cells, PMN: Polymorphonuclear neutrophils.

Table S4. Antibodies used for histological analysis

Antigen	Clone / Number	Supplier	Dilution	Incubation time	Antigen retrieval
DCIR	Rabbit polyclonal, HPA007842	Sigma-Aldrich	1/200	1 h	HIER/Citrate pH6/MO*
CD45R/B220	RA3-6B2 Rat mAb	Bio-Rad	1/400	1 h	HIER/ Citrate ph6/MO*
CD3	SP7 Rabbit mAb	Spring Bioscience	1/100	1 h	HIER/ Tris-EDTA ph9/WB
F4/80	CI:A3-1 Rat mAb	Bio-Rad	1/100	1 h	Proteinase K proteolytic digestion
T-bet	4B10, Mouse mAb	BioLegend	1/100	1 h	HIER/Tris-EDTA ph8/MO**

Abbreviations: mAb : monoclonal antibody; Ab: antibody; HIER: Heat-induced epitope retrieval.; MO : Boiling in microwave oven twice 10 minutes each/MO* or 7 minutes/MO**; WB: Heating in water-bath at 95°C for 40 minutes.