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Supporting Information

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Enhanced protection to *Mycobacterium tuberculosis* infection in IL-10-deficient mice is accompanied by early and enhanced Th1 responses in the lung

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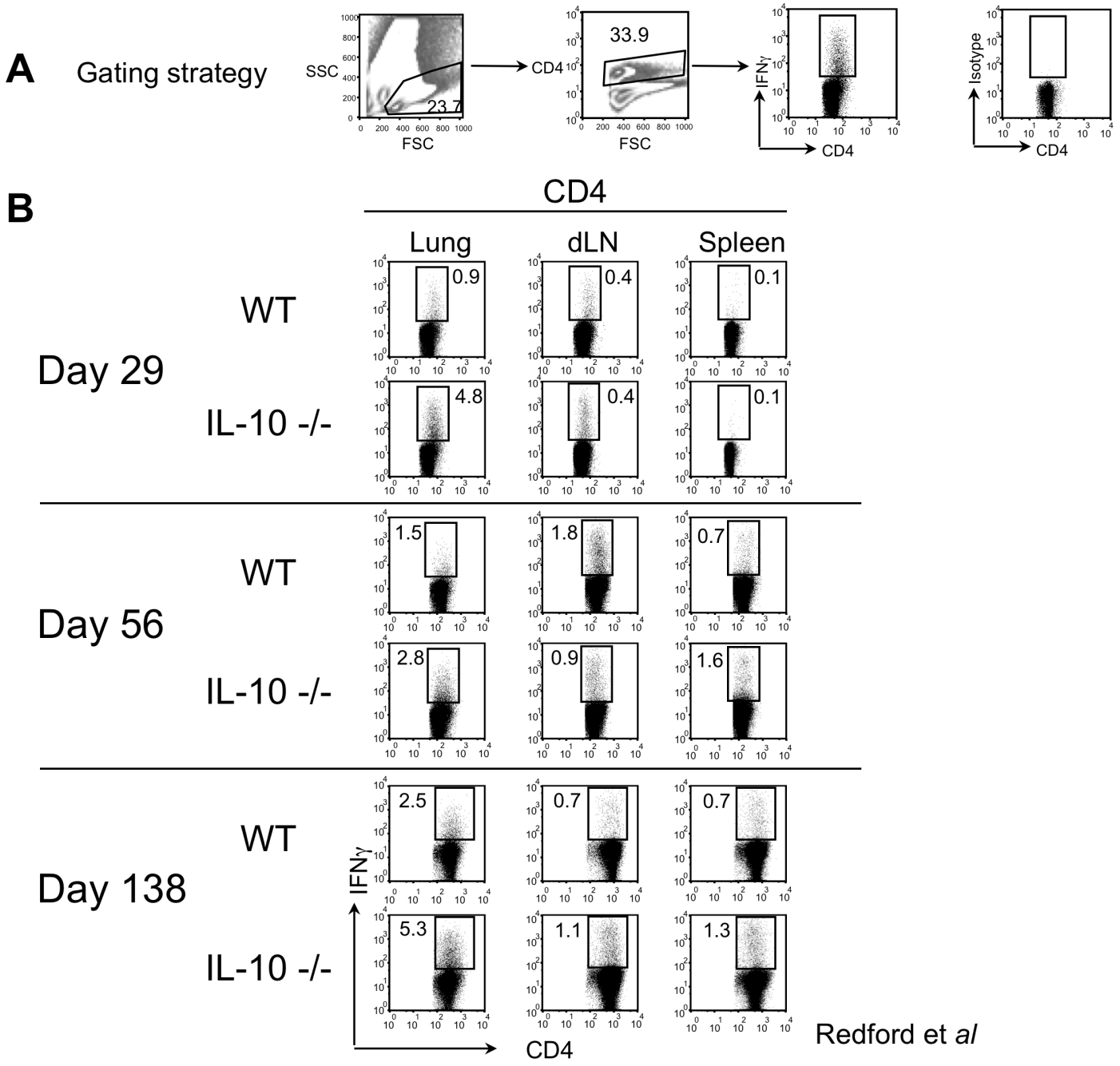
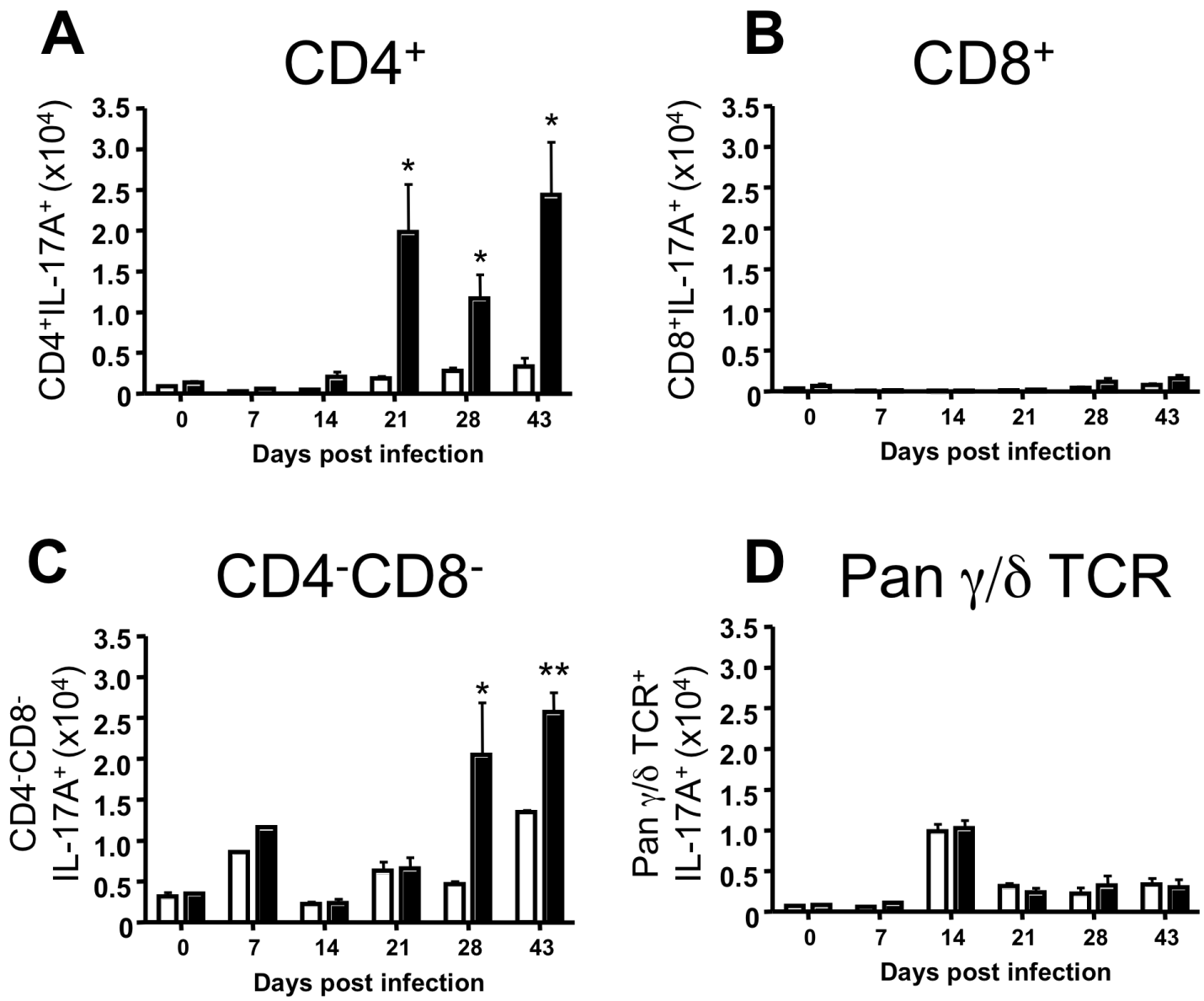


Supporting information Figure 1: IL-10-/- mice have early enhanced percentages of IFN-γ⁺ CD4+ cells in the lungs.

Whole organ homogenates from *M. tuberculosis* infected wild type BALB/c and BALB/c IL-10-/- mice were stimulated *ex vivo* with PPD for 48 hours before cells were stained for flow cytometric analysis. (A) The gating strategy to determine the percentage production of IFN-γ from $CD4^+$ cells along with an appropriately labelled isotype control antibody for IFN-γ. (B) Percent $CD4^+IFN\text{-}\gamma^+$ cells present in the lungs, dLN and spleen at the indicated time-points post *M. tuberculosis* infection. FACS plots shown are concatenations of 3-4 individual mice per group per time-point and are representative of at least 2 independent experiments.



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Supporting information Figure 2: The proportion of IL-17A+ cells is enhanced early in MTb infected IL-10-/- mice.

Wild type BALB/c (open bars) and BALB/c IL-10-/- (closed bars) mice were infected with *M. tuberculosis* as described in Fig. 1, and whole lung homogenates were restimulated with PMA and Ionomycin as described in Materials and Methods. IL-17A production from (A) $CD4^+$; (B) $CD8^+$; (C) $CD4^-CD8^-$ (D) g/d T cells was analysed by flow cytometry as described according to the gating strategy in Fig. S1 A. Total numbers of IL-17A-producing cells of each subset are shown from at least 4 individual mice per group per time-point. The effect attributed to the absence of IL-10 was tested statistically using an unpaired Students *t* test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Supporting information Table 1: Preliminary results from an initial screen of Lung supernatants by Multiplex analysis from uninfected and *Mtb* infected (day 28-29) wild type and IL-10-/- mice (BALB/c background).

Analyte:	Independent Expts.	WT non-infected	IL-10-/- non-infected	WT *Mtb* day 28	IL-10-/- *Mtb* day 28
IL-1α	Expt. 1	3.523 ± 1.361	10.53 ± 2.770	29.58 ± 6.124	127.6 ± 30.28
IL-1α	Expt. 2	12.47 ± 5.694	11.63 ± 1.807	90.03 ± 4.319	127.2 ± 5.053
IL-1β	Expt. 1	1.028 ± 0.6507	3.030 ± 0.3233	13.70 ± 1.485	44.53 ± 9.032
IL-1β	Expt. 2	4.822 ± 2.009	4.647 ± 0.8207	13.12 ± 2.515	16.77 ± 1.839
IL-2	Expt. 1	<<	<<	6.550 ± 1.728	37.90 ± 16.59
IL-2	Expt. 2	<<	<<	8.090 ± 0.3749	50.25 ± 15.96
IL-4	Expt. 1	<<	<<	1.650 ± 0.02887	3.525 ± 0.6860
IL-5	Expt. 1	8.985 ± 2.620	6.400 ± 0.3559	36.50 ± 12.50	21.18 ± 3.886
IL-7	Expt. 1	1.018 ± 1.018	3.125 ± 3.125	8.643 ± 2.613	11.90 ± 2.041
IL-9	Expt. 1	<<	<<	17.28 ± 6.212	35.70 ± 5.431
IL-9	Expt. 2	1.475 ± 1.475	1.573 ± 1.573	<<	1.907 ± 1.436
IL-12(p70)	Expt. 1	<<	<<	4.238 ± 1.780	9.350 ± 1.883
IL-13	Expt. 1	4.135 ± 0.3893	5.750 ± 0.9526	13.60 ± 2.305	23.08 ± 3.175
IL-15	Expt. 1	<<	<<	<<	<<
CCL2 (MCP1)	Expt. 1	842.8 ± 185.7	505.5 ± 61.26	454.8 ± 59.41	239.3 ± 26.94
CCL2 (MCP1)	Expt. 2	317.8 ± 39.68	211.2 ± 35.20	530.7 ± 60.66	149.0 ± 13.48
CCL3 (MIP1α)	Expt. 1	174.2 ± 9.283	263.3 ± 14.43	719.3 ± 137.5	1139 ± 246.9
CCL3 (MIP1α)	Expt. 2	116.7 ± 13.47	210.3 ± 24.94	841.8 ± 111.8	841.7 ± 184.8
CCL5 (RANTES)	Expt. 1	4.525 ± 0.3010	3.175 ± 0.2394	41.30 ± 7.208	79.15 ± 17.83
CXCL1 (KC/GRO)	Expt. 1	1838 ± 283.5	2176 ± 52.38	2534 ± 268.9	4079 ± 638.2

a) All Means shown are ± S.E.M. (as outlined in Figure 3).
b) << = below detection (<1pg/ml).
c) Expt.1 = preliminary screen; Expt.2 = repeat of selected analytes to confirm initial findings.

Supporting information Table 2: Preliminary results from an initial screen of serum samples by Multiplex analysis from uninfected and *Mtb* infected (day 28) wild type and IL-10-/- mice (BALB/c background).

Analyte:	WT non-infected	IL-10-/- non-infected	WT *Mtb* day 28	IL-10-/- *Mtb* day 28
IL-1α	28.9 ± 2.9	25.9 ± 6.65	41.8 ± 10.3	36 ± 9.22
IL-1β	<<	9.4 ± 0.37	<<	9.4 ± 5.79
IL-2	<<	<<	<<	<<
IL-4	<<	<<	<<	<<
IL-5	4.9 ± 1.3	2.8 ± 0.4	6.3 ± 1.5	8.5 ± 5.3
IL-7	9.4 ± 8.2	5.1 ± 3.3	12.1 ± 10.6	2.4 ± 1.7
IL-9	<<	11.7 ± 3	50.8 ± 26	<<
IL-10	<<	<<	<<	<<
IL-12(p70)	33.5 ± 1.5	25 ± 6.2	6.3 ± 1.8	18.7 ± 5.02
IL-13	12.0 ± 1.8	11.9 ± 1.9	13 ± 1.7	11.3 ± 1.7
IL-15	98.7 ± 38.6	186.3 ± 51.6	179.8 ± 39	51.9 ± 8.8
GM-CSF	44.7 ± 14.5	23.7 ± 16.76	10.9 ± 6.4	24.4 ± 3.7
CCL2 (MCP1)	18.5 ± 6.2	27.9 ± 7	13.4 ± 2.7	74.1 ± 19.1
CCL3 (MIP1α)	22.5 ± 3.8	20.2 ± 2.6	15.7 ± 3.8	21.3 ± 4.6
CCL5 (RANTES)	7.3 ± 1.6	9.8 ± 2.1	6.4 ± 0.9	13.7 ± 7.5
CXCL1 (KC/GRO)	31.5 ± 16.4	47.8 ± 27.6	17.4 ± 6.6	56.4 ± 22.4

a) All Means shown are ± S.E.M. (as outlined in Figure 4)
b) << = below detection (<1pg/ml)