

Innate myeloid cell TNFR1 mediates first line defence against primary *Mycobacterium tuberculosis* infection.

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Running title: TNFR1 from myeloid cells in mycobacterial infections

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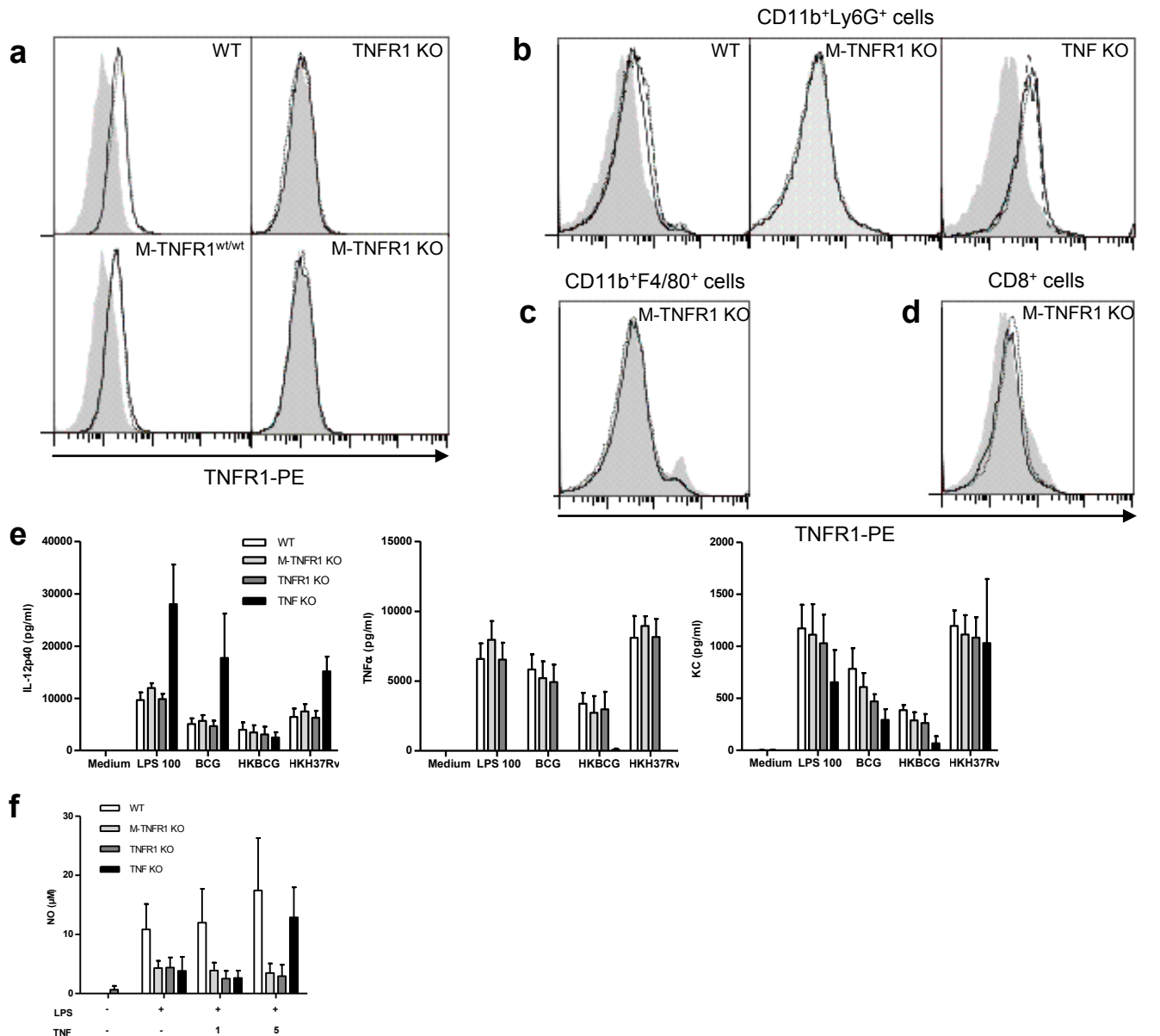


Figure S1. Macrophages from M-TNFR1 KO mice do not express functional TNFR1 but respond to mycobacterial stimuli.

(a) Bone marrow derived macrophages (BMDM) from C57BL/6 (WT), TNFR1 KO, *Tnfrsf1α^{fl/fl} LysM^{wt/wt}* (M-TNFR1^{wt/wt}) or *Tnfrsf1α^{fl/fl} LysM^{cre/wt}* (M-TNFR1 KO) mice were incubated with hamster α-mouse TNFR1/CD120a and revealed with biotinylated α-hamster IgG plus PE-streptavidine (n=2, dark line and dotted line), as compared to isotype controls (grey). **(b-d)** Spleen cells from WT, M-TNFR1 KO or TNF KO mice were isolated and stained for cytometry analysis 41 days after *M. bovis* BCG infection. TNFR1/CD120a expression was measured in CD11b⁺Ly6G⁺ cells **(b)**, CD11b⁺F4/80⁺ cells **(c)** and CD8⁺ cells **(d)**. **(e)** BMDM from WT, M-TNFR1-, TNFR1- or TNF KO mice were stimulated with LPS (100ng/ml), or live or heat-killed mycobacteria (MOI 2) for 20h. IL-12p40, TNF and KC were measured in supernatants. Results are mean +/- SEM of n=6 mice per group, from 3 independent experiments **(f)** BMDMs were primed with TNF at 5ng/ml for 16h prior to LPS stimulation (at 1ng/ml) for 18h and NO measured in supernatants. Results are mean +/- SEM of n=4 mice per group from 2 independent experiments.

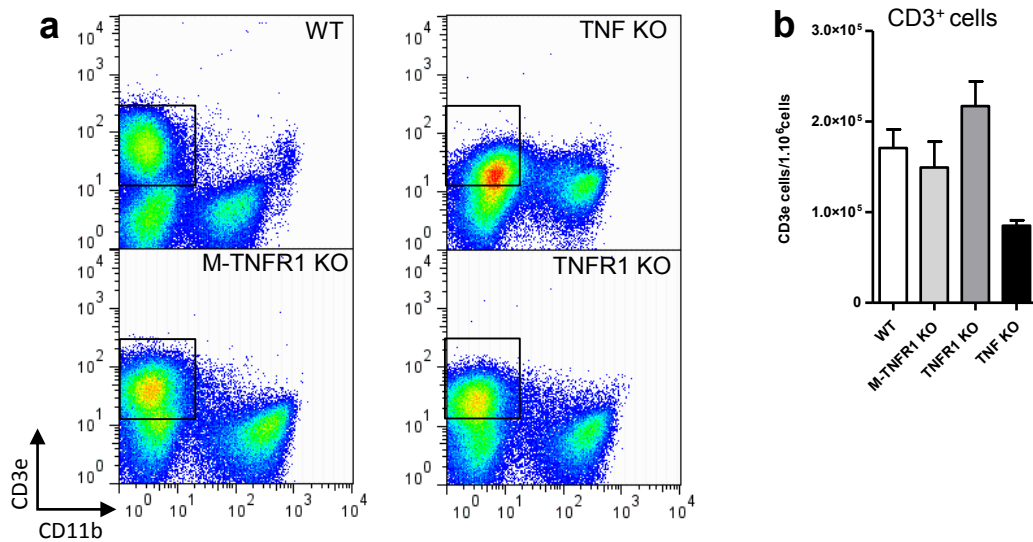


Figure S2. Pulmonary T-cells recruitment after *M. tuberculosis* infection independent of TNFR1 signalling

Lungs from M-TNFR1-, TNFR1- or TNF α -deficient mice and wild-type controls were harvested on day 28 after *M. tuberculosis* H37Rv infection (1000 CFU/mouse i.n.) for flow cytometry analysis. **(a)** Representative dot plots of CD11b⁺CD3 ϵ ⁺ cells. **(b)** Bargraph of CD3 ϵ ⁺ cells in the lungs of M-TNFR1-, TNFR1-, or TNF α -deficient mice and wild-type controls. Results are expressed as mean \pm SEM of n=2-5 mice per group from two independent experiments. *, p<0.05; **, p<0.01; ***, p<0.001.

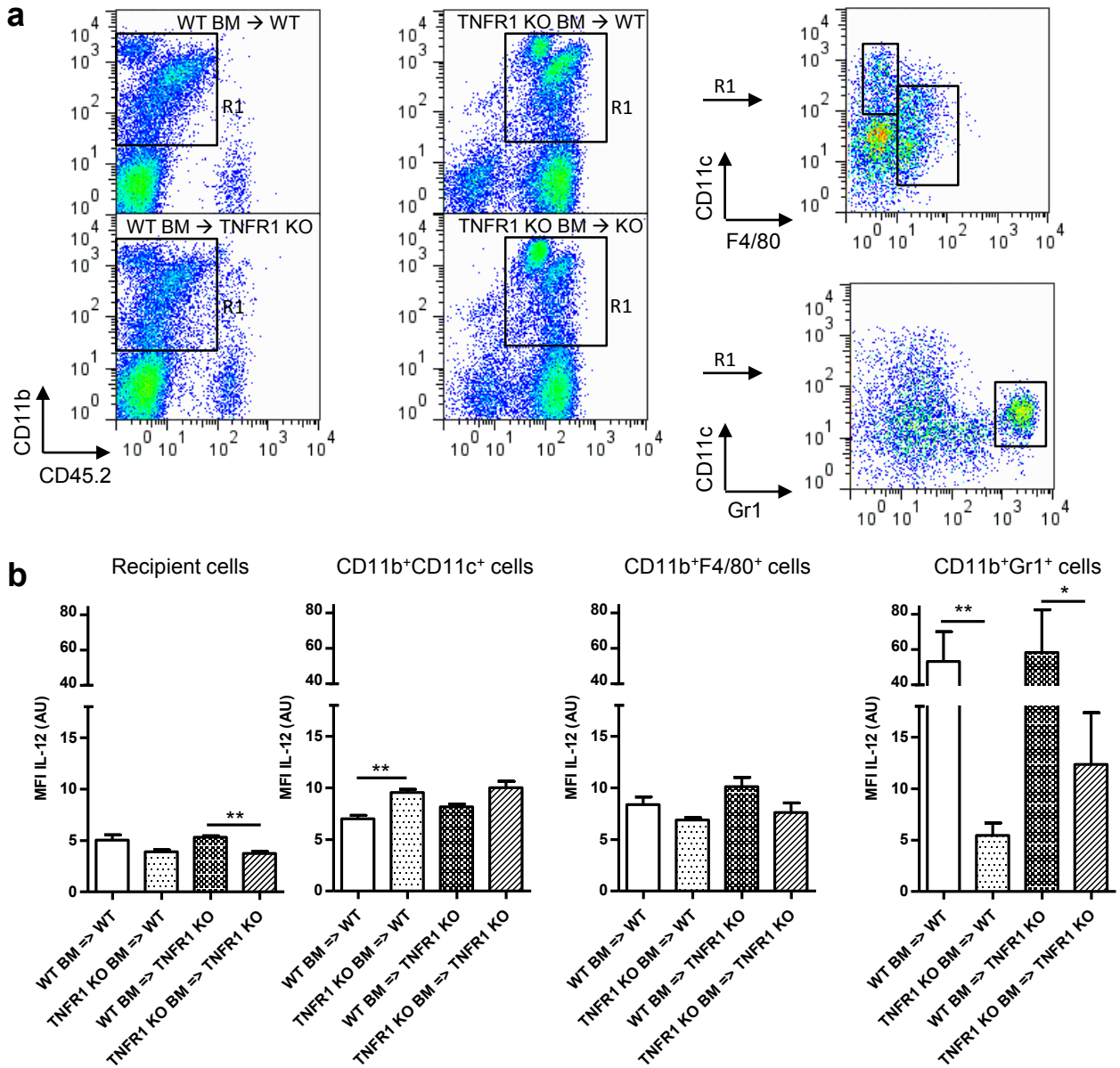


Figure S3. Hematopoietic cells are the major source of IL-12p40 in the lungs after *M. tuberculosis* infection.

TNFR1 deficient mice and WT mice were lethally irradiated and reconstituted with bone marrow from either WT mice or TNFR1 KO mice before *M.tuberculosis* intranasal infection. Lungs were harvested 30 days post-infection and stained for CD45.2, CD11b, CD11c, F4/80, Gr1 and IL-12p40. (a) Gating strategy used for IL-12p40 MFI determination is shown as CD45.2/CD11b, F4/80/CD11c and Gr1/CD11c dot plots. (b) Bargraphs of IL-12p40 mean fluorescence intensity in each cell population, including cells of recipient origin, and donor CD11b⁺CD11c⁺, CD11b⁺F4/80⁺ and CD11b⁺Gr1⁺ cells. Results are expressed as mean +/- SEM (n=6-7 mice per group). *, p<0.05; **, p<0.01; ***, p<0.001.

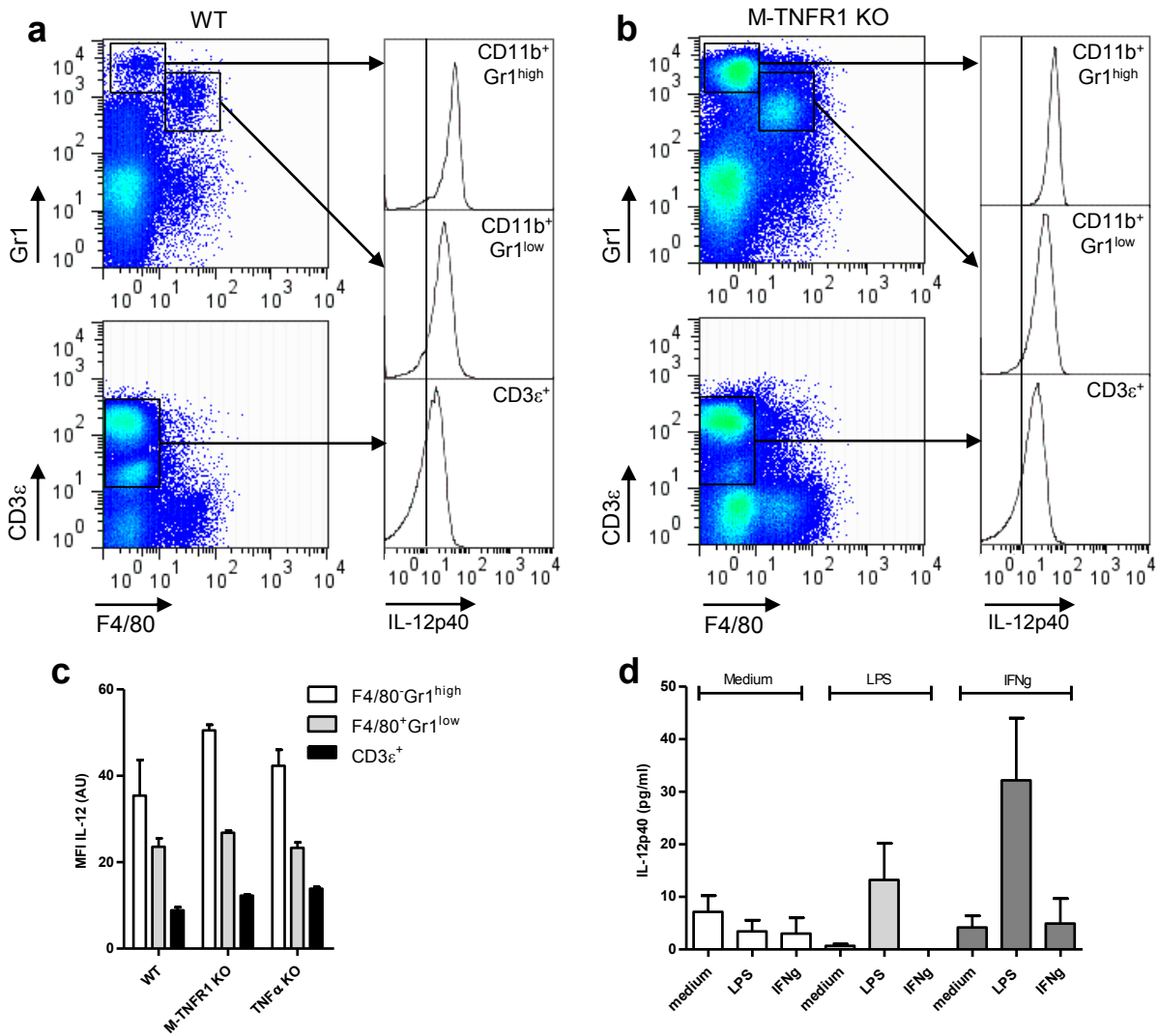


Figure S4. Splenic neutrophils express IL-12p40 after *M.bovis* BCG infection

(a-c) Intracytoplasmic IL-12p40 staining was measured in splenic CD11b⁺Gr1^{high}, CD11b⁺Gr1^{low} or in CD3ε⁺ cells from WT (a) and M-TNFR1 KO mice (b). IL-12p40 mean fluorescence intensity (MFI) is represented as bar graph in WT, M-TNFR1 KO and TNF KO mice (c). (d) Neutrophils isolated from C57BL/6 mice bone marrow were primed with LPS or IFNγ for 12 hours prior to stimulation with LPS or IFNγ for 12 hours. Supernatants were harvested for IL-12p40 determination by ELISA. Results are expressed as mean +/- SEM of n=3-6 pooled from 2 independent experiments. *, p<0.05; **, p<0.01; ***, p<0.001.

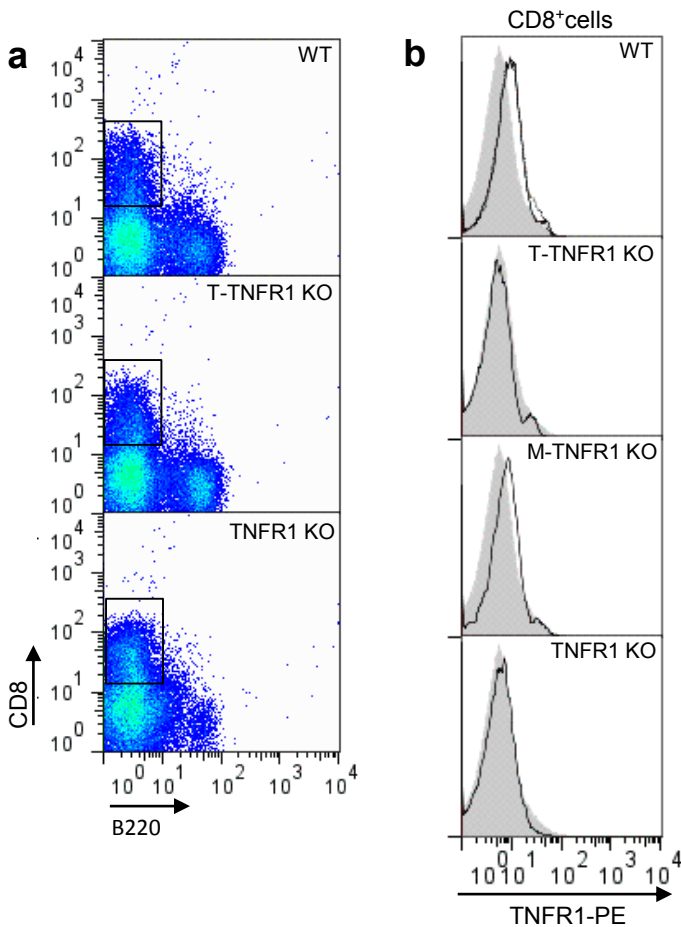


Figure S5. Lymphocytes from T-TNFR1 KO mice do not express TNFR1 ex-vivo

Lung cells from WT and T-TNFR1⁻, M-TNFR1⁻, and TNFR1-deficient mice infected with *M. tuberculosis* H37Rv (1000 CFU/mouse i.n.) were isolated on day 28 and stained for CD8 and B220. (a) Representative dot plots for CD8⁺ B220⁺ cells are shown for each experimental group. (b) TNFR1 expression was measured as mean fluorescence intensity in CD8⁺ cells as compared to isotype control (in grey).

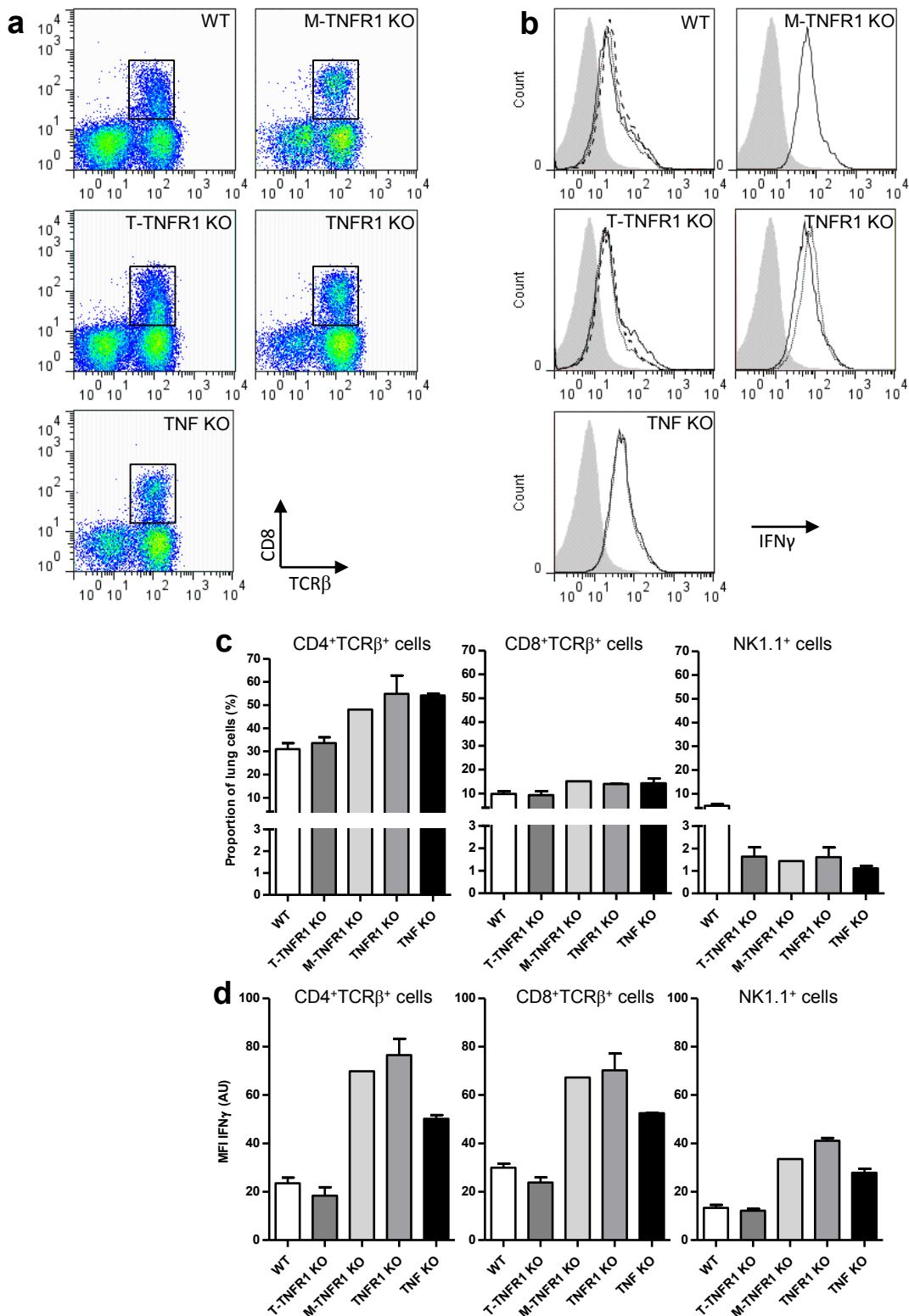


Figure S6. High IFN γ secretion is correlated with susceptibility to *M. tuberculosis* infection but does not require TNF/TNFR1 pathway.

Lung cells from WT and TNF⁻, TNFR1⁻, M-TNFR1-deficient mice infected with *M. tuberculosis* H37Rv (1000 CFU/mouse i.n.) were harvested on day 28 and stained for CD3 ϵ , CD4, CD8, TCR β , NK1.1 and IFN γ using a secretion kit. **(a)** Representative dot plots for CD3 ϵ ⁺ TCR $\alpha\beta$ CD8⁺ cells are shown for each experimental group. **(b)** Secreted IFN γ intensity in TCR $\alpha\beta$ CD8⁺ cells was plotted for each group (n=2-3, dark, dotted and dashed lines) as compared to isotype control (in grey). **(c-d)** The percentages of CD3 ϵ ⁺ CD4⁺TCR $\alpha\beta$, CD3 ϵ ⁺ CD8⁺TCR $\alpha\beta$ cells and CD3 ϵ ⁻NK1.1⁺ cell populations **(c)** and IFN γ MFI **(d)** are represented as bar graphs. Results are expressed as mean \pm SEM of n=2-3 mice.

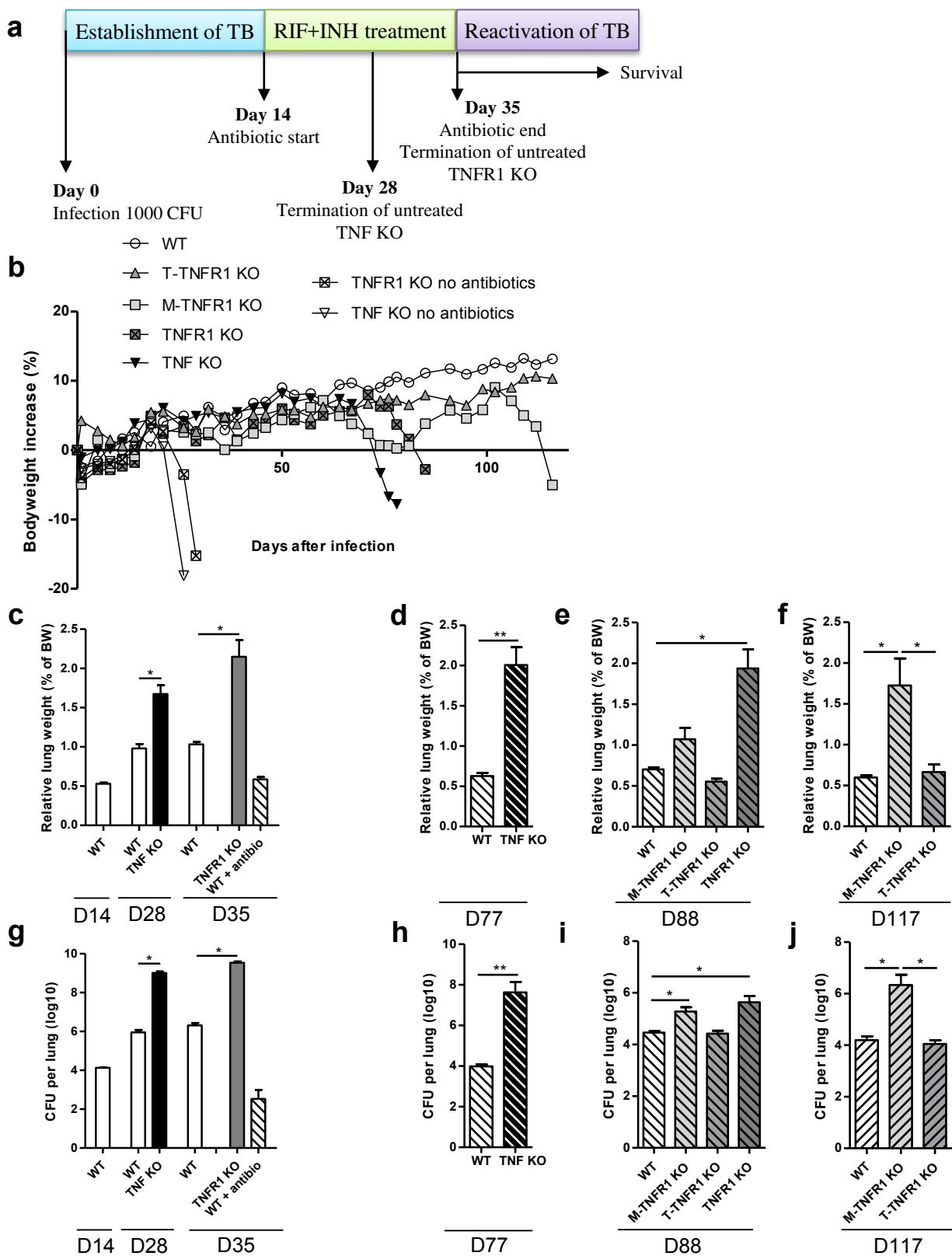


Figure S7. Lack of TNFR1 signalling in myeloid cells leads to a reactivation of *M. tuberculosis* infection

M-TNFR1-, T-TNFR1-, TNFR1 and TNF-deficient mice and wild-type controls were exposed to *M. tuberculosis* H37Rv and treated with isoniazid (INH) and rifampicin (RIF) antibiotics (antibio; each 0.1g/L in drinking water) from 14 days to 35 days post-infection (a). Mice were then monitored for bodyweight (b). Relative lung weight (c, d, e, f) and bacterial load (g, h, i, j) were assessed at the indicated days. Results are expressed as mean +/- SEM from n=4-6 mice per group. *, p<0.05; **, p<0.01; ***, p<0.001.

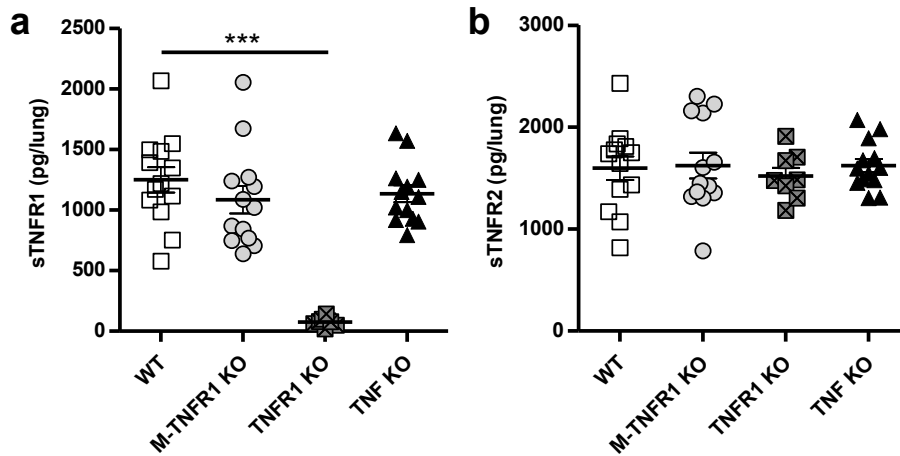


Figure S8. Soluble TNFR1 and TNFR2 levels in the absence of TNFR1 on myeloid cells.

Soluble TNFR1 (**a**) and soluble TNFR2 (**b**) concentrations were determined in lung homogenates of mice deficient for TNF, TNFR1, or myeloid-TNFR1 and wild-type mice 28 days after *M. tuberculosis* infection. Results are expressed as single points and mean +/- SEM of n=8-13 from 3 experiments. *, p<0.05; **, p<0.01; ***, p<0.001.

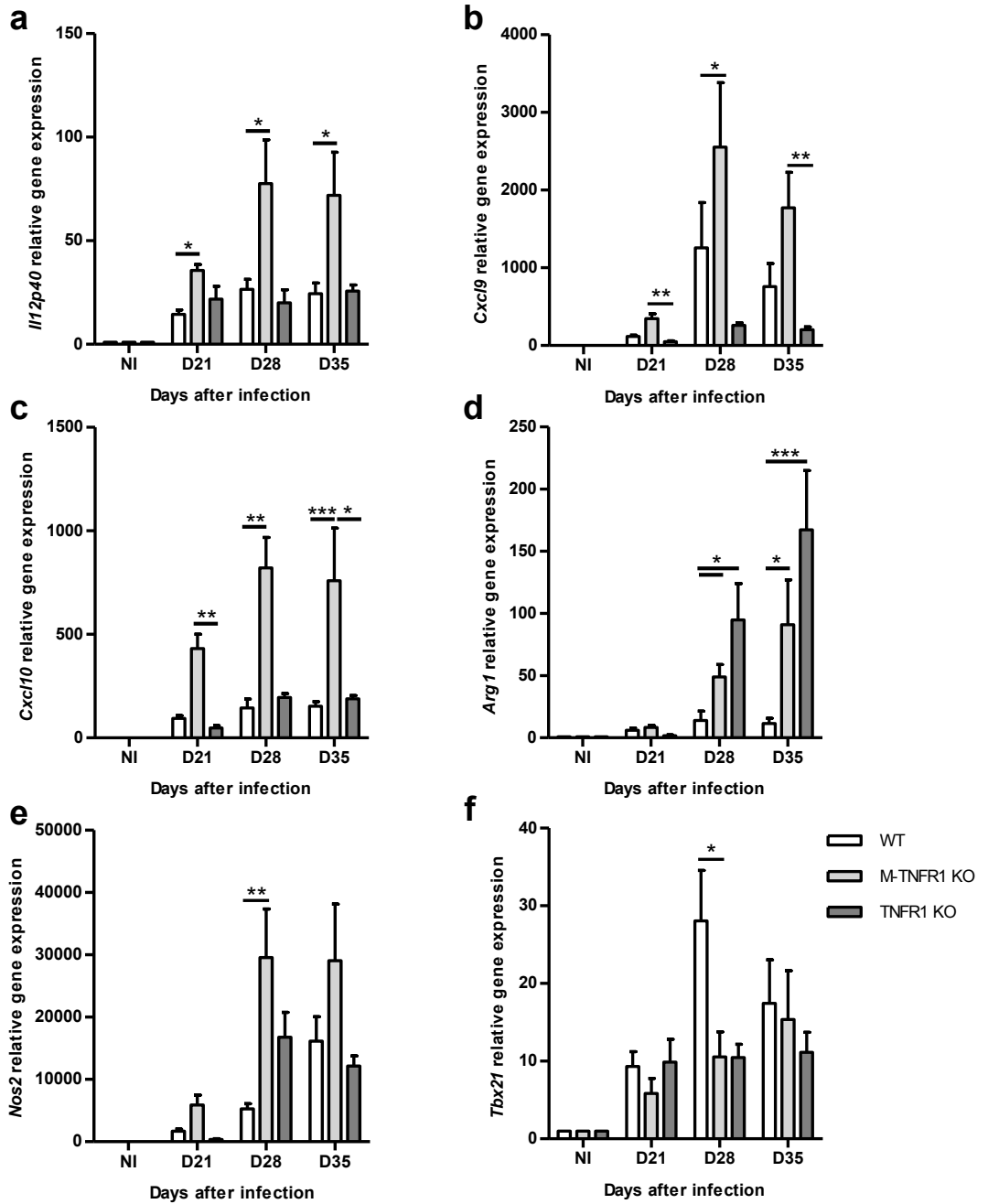


Figure S9. T-cell adaptive response-related gene expression in the absence of TNFR1 pathway. Relative gene expression of *I12p40* (a), *Cxcl9* (b), *Cxcl10* (c), *Arg1* (d) *Nos2* (e) and *Tbx21* (f) were determined in the lung of TNF, TNFR1, or myeloid-TNFR1 deficient mice and wild-type mice 21, 28 and 35 days after *M. tuberculosis* infection. Results are expressed as mean \pm SEM of n=4-12 from 2 independent experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Supporting information

Macrophage cultures.

Murine bone marrow cells isolated from femurs were differentiated into macrophages after culturing at 10^6 cells/ml for 7 days in DMEM (Sigma) supplemented with 10 mM L-glutamine, 25mM HEPES, 100U/mL Penicillin and 100U/ml Streptomycin, plus 20% horse serum and 30% L929 cell-conditioned medium as a source of M-CSF¹. Three days after washing and re-culturing in fresh medium, the cell preparation contained a homogenous population of macrophages.

Macrophages were plated in 96 well microculture plates (at 10^5 cells/well in supplemented DMEM as above), and stimulated with LPS (*Escherichia coli*, serotype O111:B4, Sigma, St Louis, MO, at 100 ng/ml), heat-killed *M. tuberculosis* H37Rv (heat-killed 90 min at 80°C), live or heat-killed *M. bovis* BCG (HKBCG; from Pasteur Institute, Paris), at a MOI of 2 bacteria per cell. Cell supernatants were harvested after 24 h of stimulation for IL-12p40, CXCL1/KC and TNF quantification by ELISA (R&D Duoset). For assessing functional TNFR1 response, cells were plated as described and incubated with TNF for 16h before LPS/IFN γ stimulation for 20h². Supernatants were harvested for nitric oxide determination by Griess reaction as described³.

Reactivation model.

In a pharmacological model of *M. tuberculosis* infection reactivation adapted from the original Cornell model⁴, mice were infected with *M. tuberculosis* H37Rv (1000 CFU i.n.) for 2 weeks, and treated with isoniazid (INH) and rifampicin (RIF) (each 0.1g/L in drinking water) for 3 weeks to control the infection, as described⁴ (Figure S7). Under this protocol, the bacterial burden is drastically reduced, and the infection may reactivate thereafter in the presence of a primed anti *M. tuberculosis* immune response.

References

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