SUPPLEMENTAL INFORMATION

ESAT-6-dependent cytosolic pattern recognition drives noncognate tuberculosis control *in vivo*

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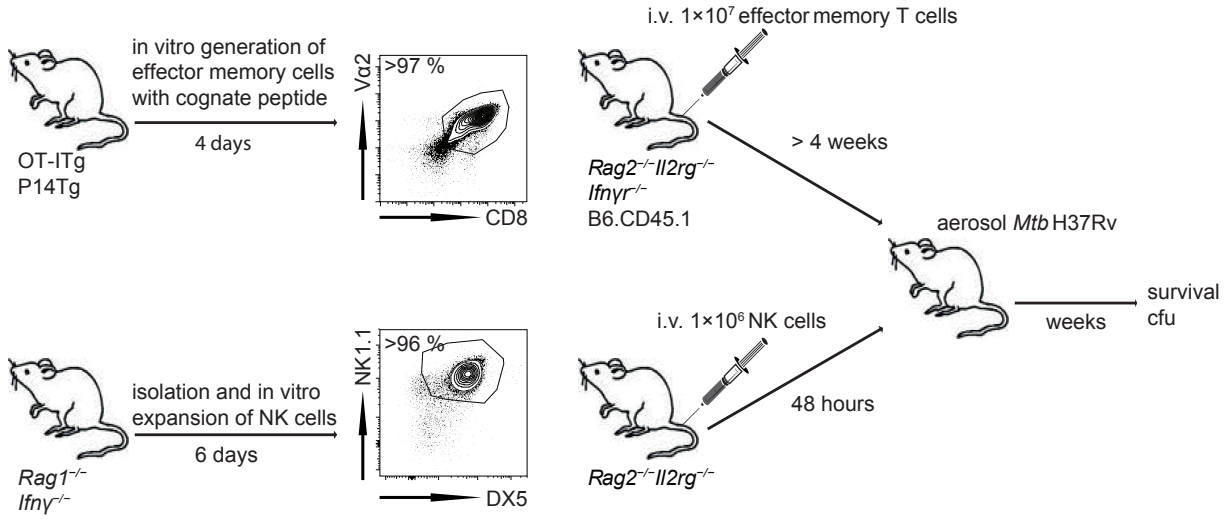
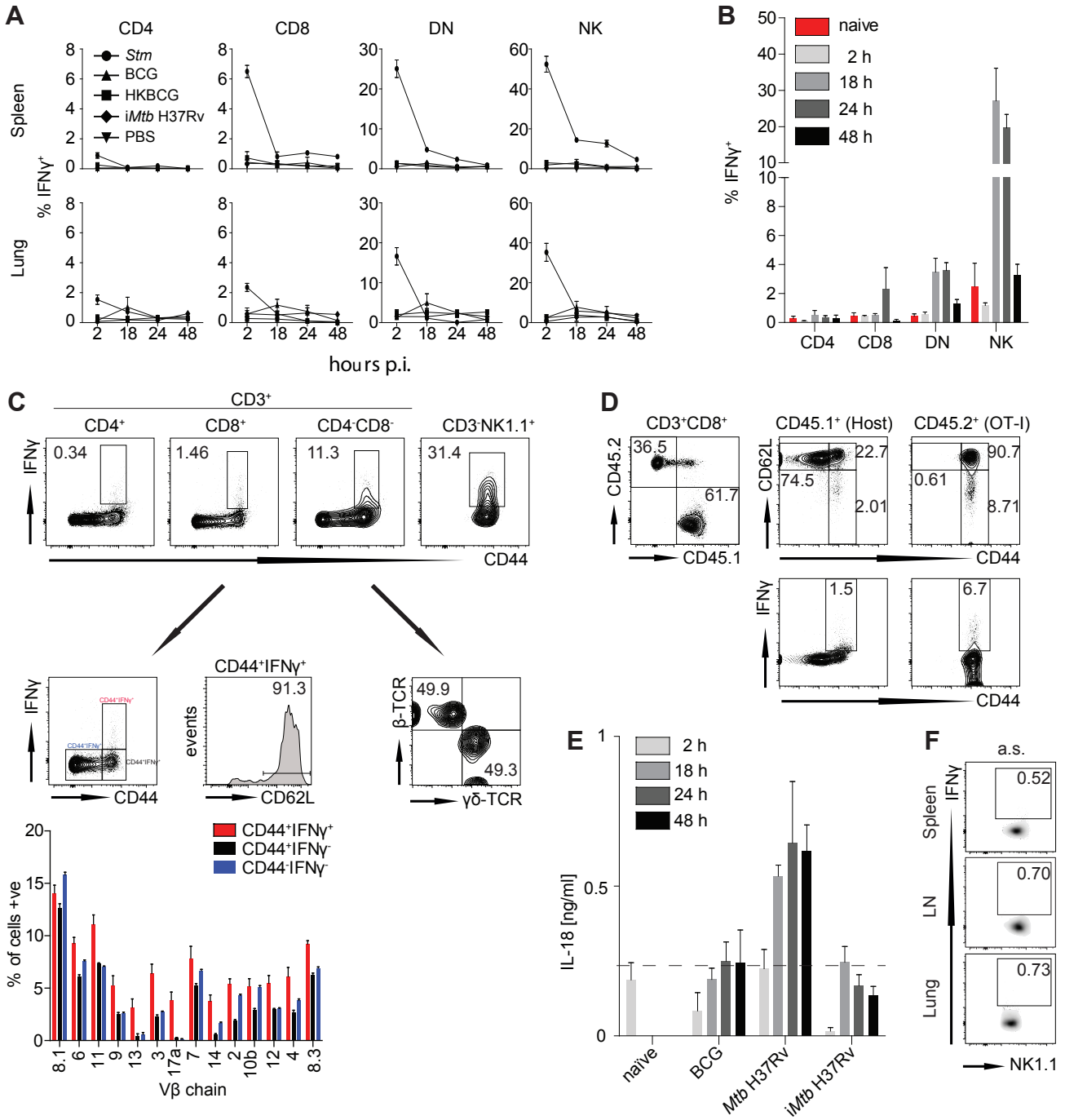
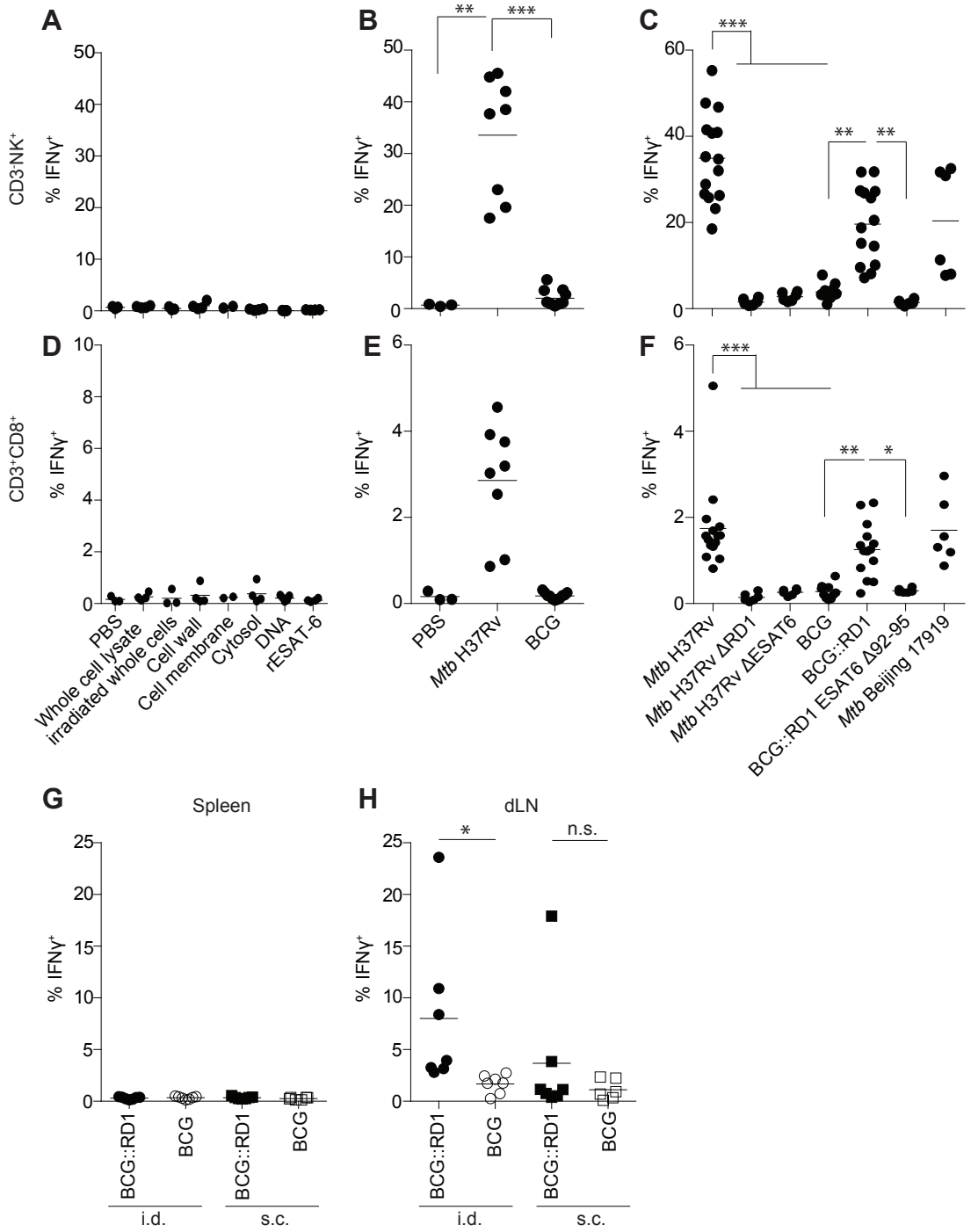


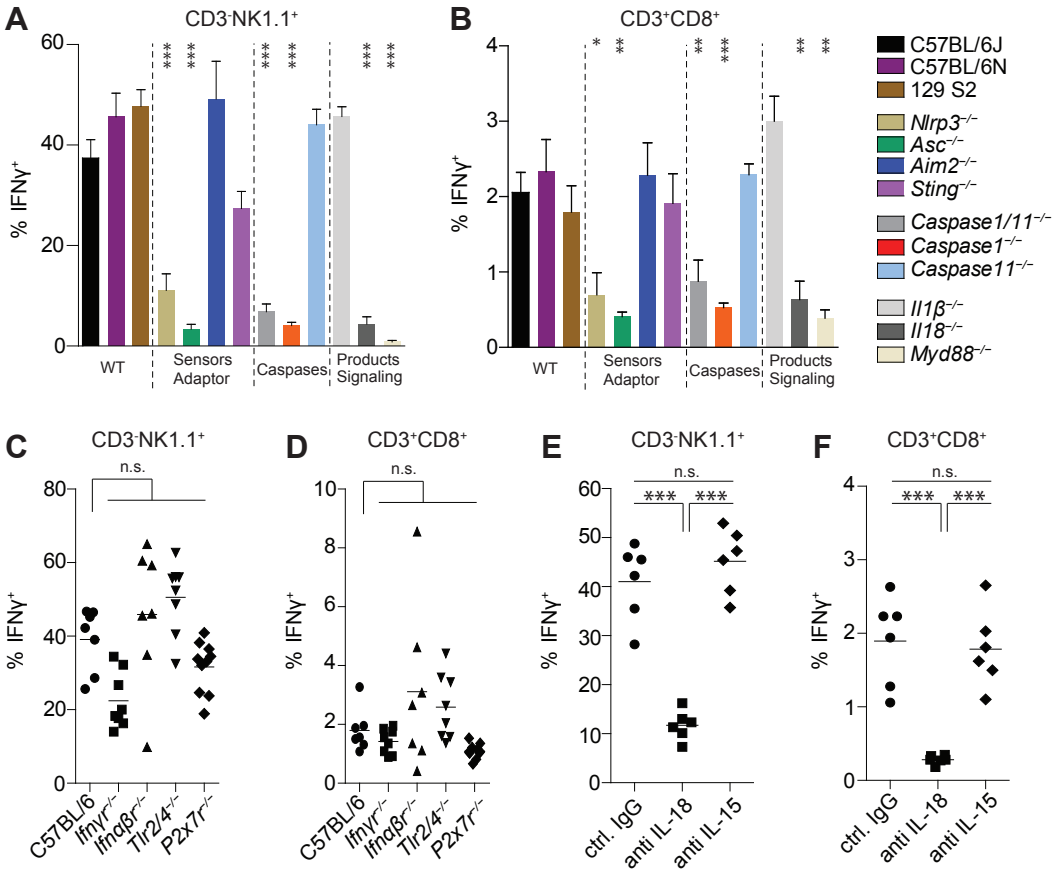
Supplemental Figure 1: Generation and expansion of memory CD8$^+$ T cells and NK cells (Related to Figure 1). TCR transgenic effector memory OT-ITg and P14Tg CD8$^+$ T cells were generated through in vitro activation of splenocytes with the cognate peptides SIINFEKL or KAVYNFATC, respectively. After 4 days cells were purified and 1×10^7 Tg cells were transferred i.v. into naïve *Rag2$^{-/-}$Il2rg$^{-/-}$, Ifnγr$^{-/-}$* or B6 (CD45.1$^+$) mice. Mice were left untouched for at least 4 weeks to ensure that transferred Tg cells converted into central memory CD8$^+$ T cells. For NK cell transfers, 1×10^6 purified and in vitro expanded NK cells from *Rag1$^{-/-}$* or *Ifnγ$^{-/-}$* donors were injected i.v. into naïve *Rag2$^{-/-}$Il2rg$^{-/-}$* mice on day 5 and 6 after in vitro culture. Mice were infected 48 hours after NK cell transfer. Representative FACS plots are shown.



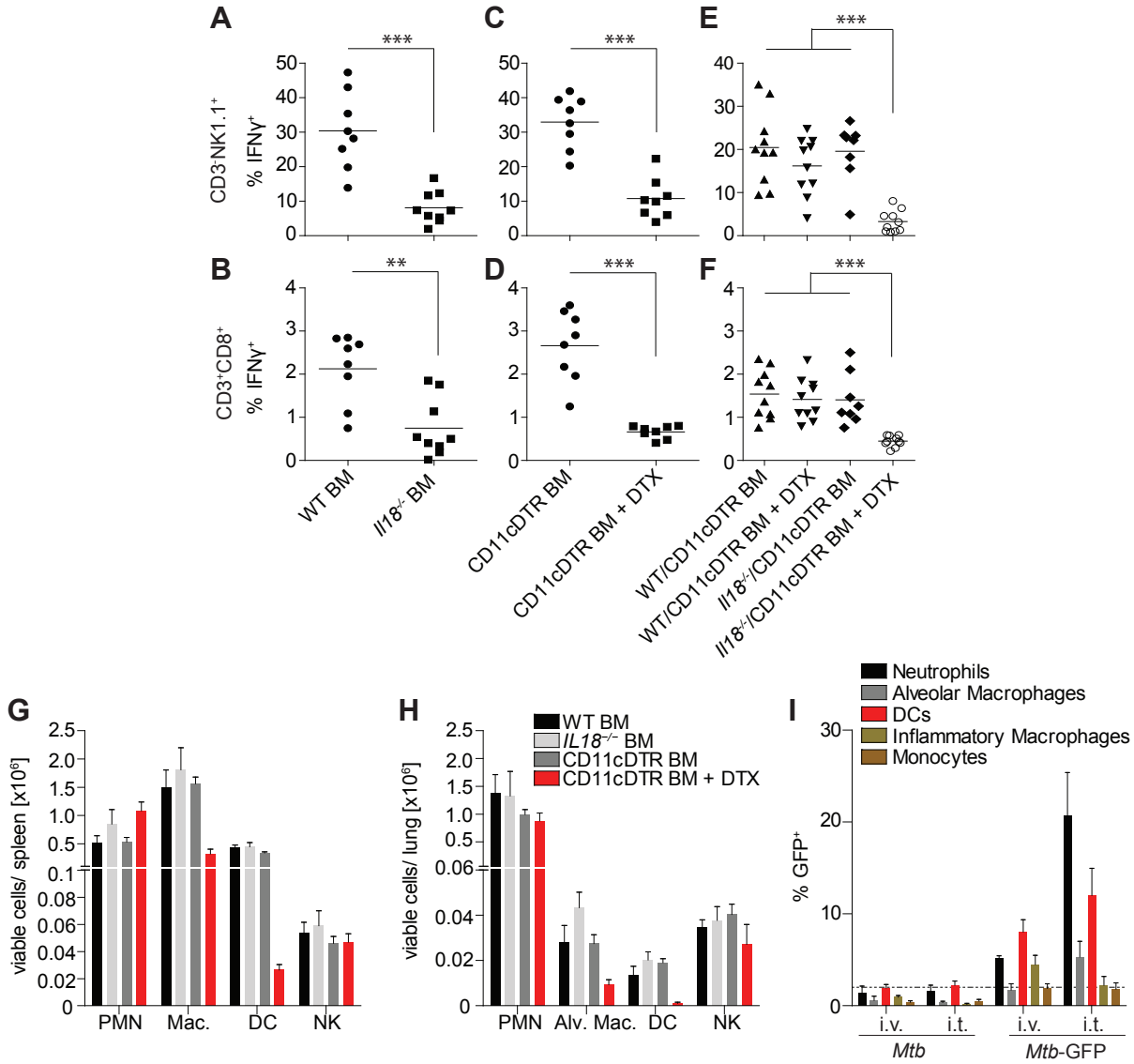
Supplemental Figure 2: Temporal and spatial characterization of innate IFN-γ secretion in response to *Mtb* (Related to Figure 2). (A) Percent of IFN-γ$^+$ cells amongst total viable splenic and lung CD3$^+$CD8$^+$, CD3$^+$CD4$^+$, CD3$^+$CD4$^-$CD8$^-$ T cells and CD3$^-$NK1.1$^+$ cells at different time-points after injection of B6 mice with 1×10^8 cfu of *S.* Typhimurium (*Stm*), BCG, heat-killed BCG (HKBCG), irradiated *Mtb* H37Rv (i*Mtb*) or PBS. (B) Percent of IFN-γ$^+$ cells amongst total viable CD3$^+$CD8$^+$, CD3$^+$CD4$^+$, CD3$^+$CD4$^-$CD8$^-$ T cells and CD3$^-$K1.1$^+$ cells in the lung at different time-points after injection of B6 mice with 1×10^8 cfu *Mtb* H37Rv. (C) Representative FACS plots and histograms of splenic CD3$^+$CD8$^+$, CD3$^+$CD4$^+$, CD3$^+$CD4$^-$CD8$^-$ T cells and CD3$^-$NK1.1$^+$ cells at 24 hours after B6 mice were injected with 1×10^8 cfu *Mtb* H37Rv. (D) Representative FACS plots of endogenous (CD45.1$^+$) and exogenous (CD45.2$^+$, OT-ITg) splenic CD3$^+$CD8$^+$ cells at 24 hours after B6 mice were injected with 1×10^8 cfu *Mtb* H37Rv. (E) Serum IL-18 concentrations at different time-points after i.t. delivery of B6 mice with 1×10^8 cfu BCG, *Mtb* H37Rv, irradiated *Mtb* H37Rv or PBS. (F) Percent of IFN-γ$^+$ CD3$^-$NK1.1$^+$ cells from either spleen, lung or draining lymph nodes 24 hours after infection with 1×10^3 cfu *Mtb* H37Rv via aerosol (a.s.) route. Results are presented as pooled data means ± SEM (A – D) and representative FACS plots (C, E) of 4–9 (A), 5–10 (B), 4 (C), 3 (D), 5–7 (E) and 10 (F) mice per group from at least 2 pooled independent experiments.



Supplemental Figure 3: RD1-dependence is not organ specific (Related to Figure 3). (A–C) Percent of viable IFN-γ^+ cells amongst total viable splenic CD3$^-$NK1.1$^+$ (A–C) and CD3$^+$CD8$^+$ (D–F) cells 24 hours after i.v. injection of purified mycobacterial ligands (A, D) or 1×10^8 cfu wildtype or recombinant *Mtb* or BCG strains (B, C, E, F). (G, H) Percent of viable IFN-γ^+ cells amongst total viable CD3$^-$NK1.1$^+$ cells from spleen (G) or draining lymph node (H) 24 hours after i.d. or s.c. injection of 1×10^8 cfu BCG or BCG::RD1. Results are presented as individual data points of 3-15 mice per group from at least 2 pooled independent experiments. Statistical analyses: One-way ANOVA; significant differences are indicated by asterisks: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; n.s. not significant.



Supplemental Figure 4: Rapid IFN-γ production by NK cells and *Mtb*-unrelated memory CD8⁺ T cells requires NLRP3-dependent IL-18 secretion (Related to Figure 4). (A–D) Percent of viable IFN-γ⁺ cells amongst total viable splenic CD3⁻NK1.1⁺ (A, C) and CD3⁺CD8⁺ (B, D) cells 24 h after i.v. injection of 1×10^8 cfu *Mtb* H37Rv into different mouse strains lacking key components of the cytosolic PRR pathways (A, B), key surface receptors involved in *Mtb* recognition and innate immune responses (C, D) or B6 mice treated with different antibodies (E, F). Results are presented as individual data points (C - F) or pooled data means plus standard error of the mean (A, B) of 5–16 (A, B), 7–10 (C, D) or 6 (E, F) mice per group from at least two pooled independent experiments. Statistical analyses: One-way ANOVA; significant differences relative to B6 controls (A, D) are indicated by asterisks: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; n.s. not significant.



Supplemental Figure 5: CD11c$^+$ cells are involved in early uptake of *Mtb* (Related to Figure 5). (A–F) Percent of IFN-γ$^+$ cells amongst total splenic CD3$^-$NK1.1$^+$ (A, C, E) and CD3$^+$CD8$^+$ (B, D, F) cells of straight WT or *Il18*$^{-/-}$ (A, B) and CD11cDTR bone marrow chimeras (C, D) as well as DTX- and PBS-treated CD11cDTR mixed bone marrow chimeras (E, F) 24 hours after injection of 1×10^8 cfu *Mtb* H37Rv. (G, H) Number of viable PMNs, macrophages, DCs and NK cells per spleen (G) and lung (H) 24 hours after i.v. injection with 1×10^8 cfu *Mtb* H37Rv of WT, *Il18*$^{-/-}$ and DTX- or PBS-treated CD11cDTR bone marrow chimeras 6 weeks after bone marrow transplantation. (I) Uptake of *Mtb* H37Rv-GFP by individual lung myeloid cell populations 24 hours after i.v or i.t. delivery of 1×10^8 cfu. Results are presented as individual data points (A–F) or pooled data means plus standard error of the mean (G–I) of 8-10 (A–H) or 6 (I) mice per group from 2 (I) or 3 (A–H) pooled independent experiments. Statistical analyses: One-way ANOVA; significant differences are indicated by asterisks: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.