Supplementary data

TREM-2 promotes Th1 responses by interacting with CD3ζ/ZAP70 following Mycobacterium tuberculosis infection

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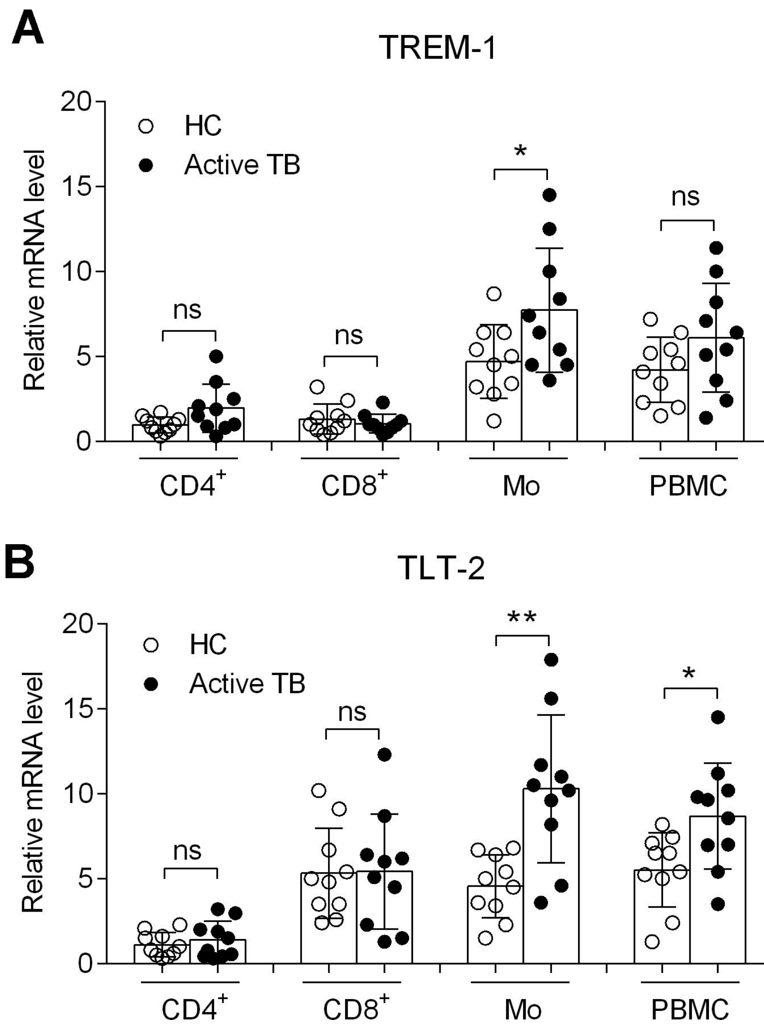

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13 **Figure S1. The expression of TREM-1 and TLT2 in HC and active TB patients.**

14 (**A, B**) The expression levels of TREM-1(**A**) and TLT-2 (**B**) in $CD4^+$ T cells, $CD8^+$ T

15 cells, $CD14^+$ monocytes (Mo) or PBMCs from healthy donors (HC) or active

16 tuberculosis (TB) patients were analyzed by quantitative real-time PCR. Data

17 represent mean ± SD. Unpaired Student's t-test was performed in **A-B**. ns, no

18 significant difference. $*P < 0.05$, $**P < 0.01$.

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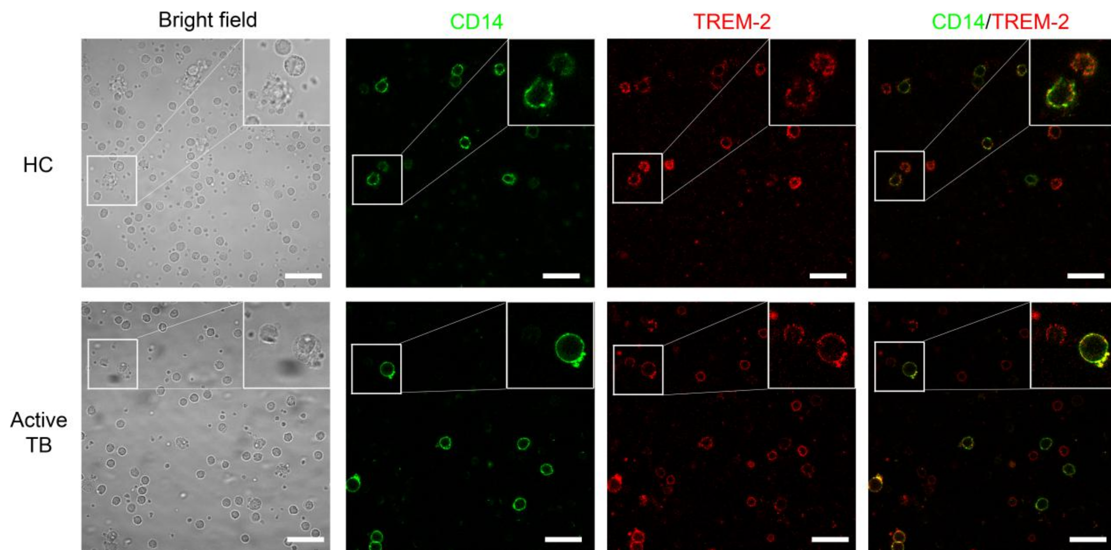

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23 **Figure S2. TREM-2 expression on peripheral CD14^{+} monocytes by confocal**

24 **microscopy.** PBMCs from HC or active TB patients were double stained with anti-

25 CD14 (Green) and anti-TREM-2 (Red) Abs, and then observed by confocal

26 microscopy. Scale bars, 50μm.

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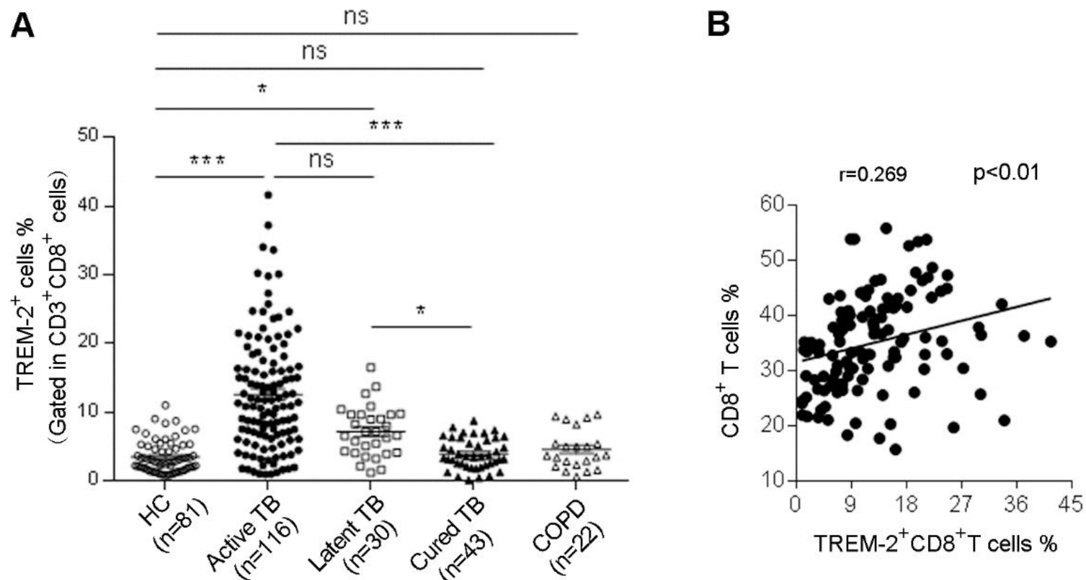

Figure S3. The expression of TREM-2 on CD8$^+$ T cells from clinical patients. (**A**) The frequency of TREM-2$^+$CD8$^+$ T cells (Gated in live CD8$^+$ T cells) were analyzed in healthy donors (HC, n=81) and patients with active TB (n=116), latent TB (n=30), cured TB (n=43) or COPD (n=22). (**B**) Correlation between the frequency of CD8$^+$ T cells and TREM-2$^+$ surface expression was analyzed in active TB patients (n=116) by SPSS Software. r, correlation coefficient. Data represent mean ± SD. One way ANOVA test was performed in **A**. Spearman correlation analysis was performed to analyze the correlations in **B**. ns, no significant difference.**P* < 0.05. ****P* < 0.001.

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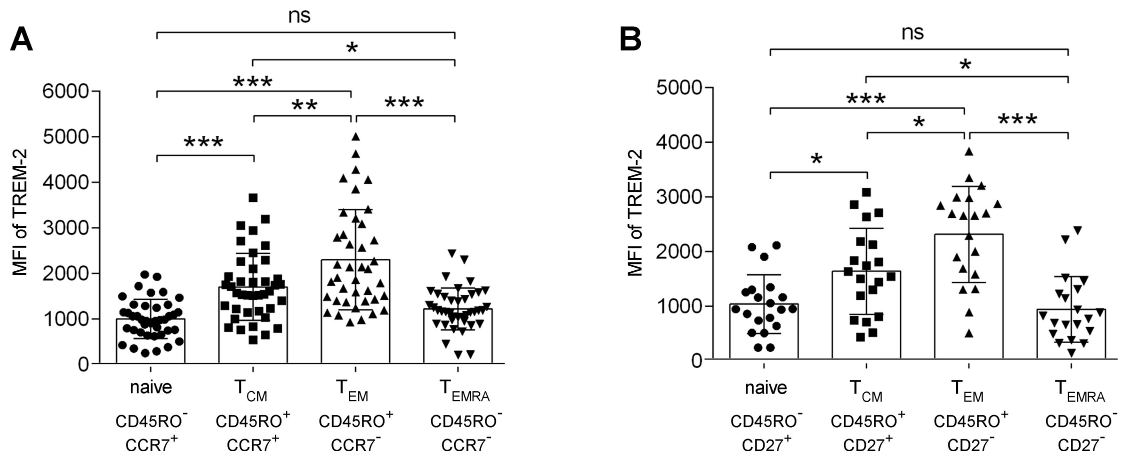

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43 **Figure S4. TREM-2 is highly expressed in** effector and memory T cells**.** (**A, B**) The
44 mean fluorescence intensity (MFI) of TREM-2 in naive T cells, T_{EM}, T_{CM}, T_{EMRA} were
45 analyzed by flow cytometry. Data represent mean ± SD. One-way ANOVA test was
46 performed in **A-B**. ns, no significant difference. **P* < 0.05. ***P* < 0.01. ****P* < 0.001.

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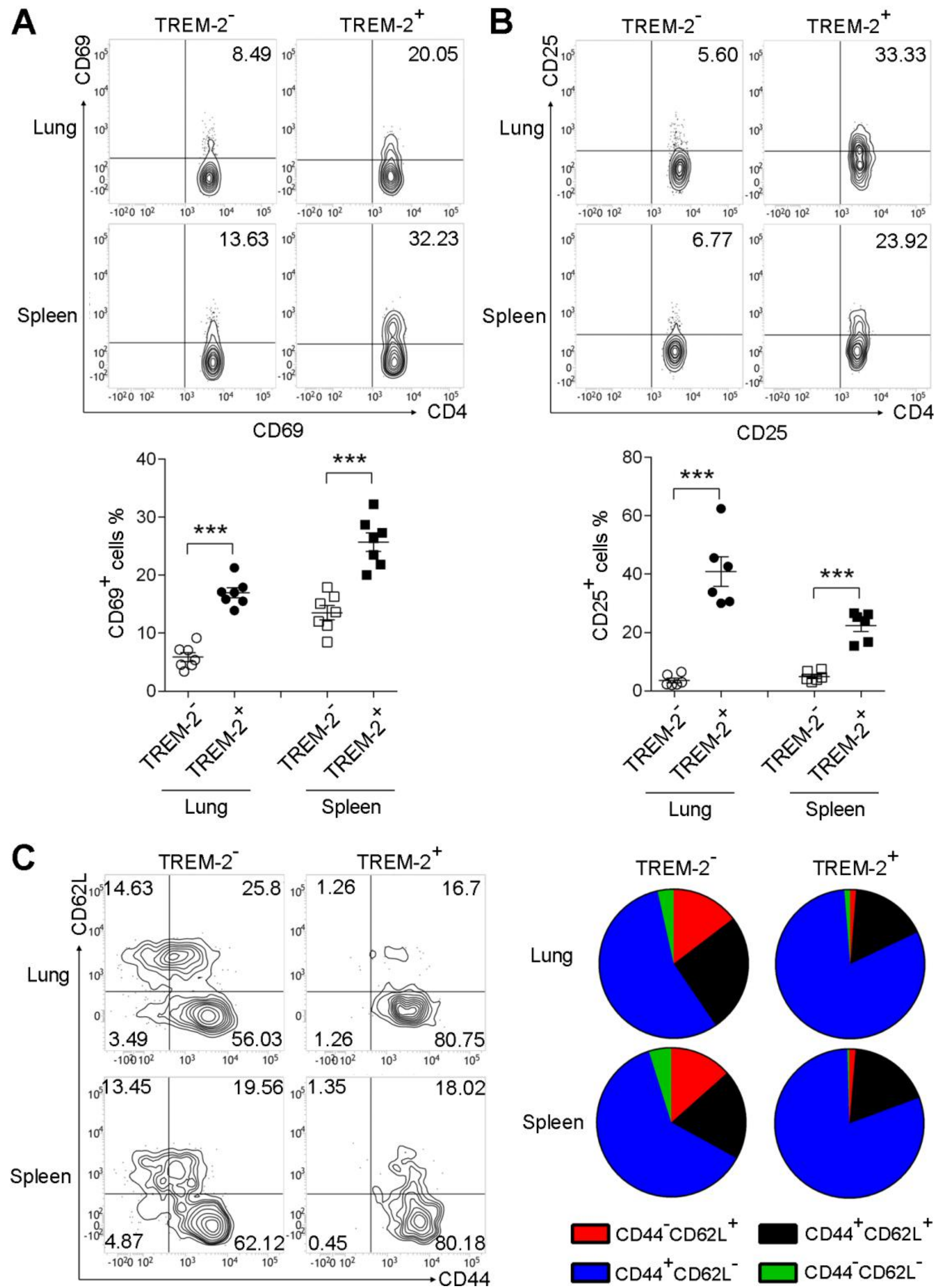

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49 **Figure S5. TREM-2⁺CD4⁺ T cells displayed activation and effector memory**
50 **phenotype in *Mtb*-infected mice.** C57BL/6 mice were injected i.p. with $1x10^6$ CFU
51 of H37Rv. (**A-B**) Expressions of CD69 and CD25 in TREM-2^+ or TREM-2^-CD4^+ T
52 cells from lungs and spleens were determined by flow cytometry. The percentages of
53 CD69 or CD25-positive cells were compared in TREM-2^+ vs TREM-2^-CD4^+ T cells.
54 (**C**) CD4^+ T cells were defined by flow cytometry with CD44 and CD62L staining.

55 The percentages of naive T cells ($CD44^{-}CD62L^{+}$), T_{EM} ($CD44^{+}CD62L^{-}$), T_{CM}

56 ($CD44^{+}CD62L^{+}$) and T_{EMRA} ($CD44^{-}CD62L^{-}$) were shown as piechart in TREM-2^{+} or

57 TREM-$2^{-}$$CD4^{+}$ T cells in the infected lungs or spleens. Data represent mean ± SD

58 from at least three independent experiments. Unpaired Student's t-test was performed

59 in **A-B**. ****P* < 0.001.

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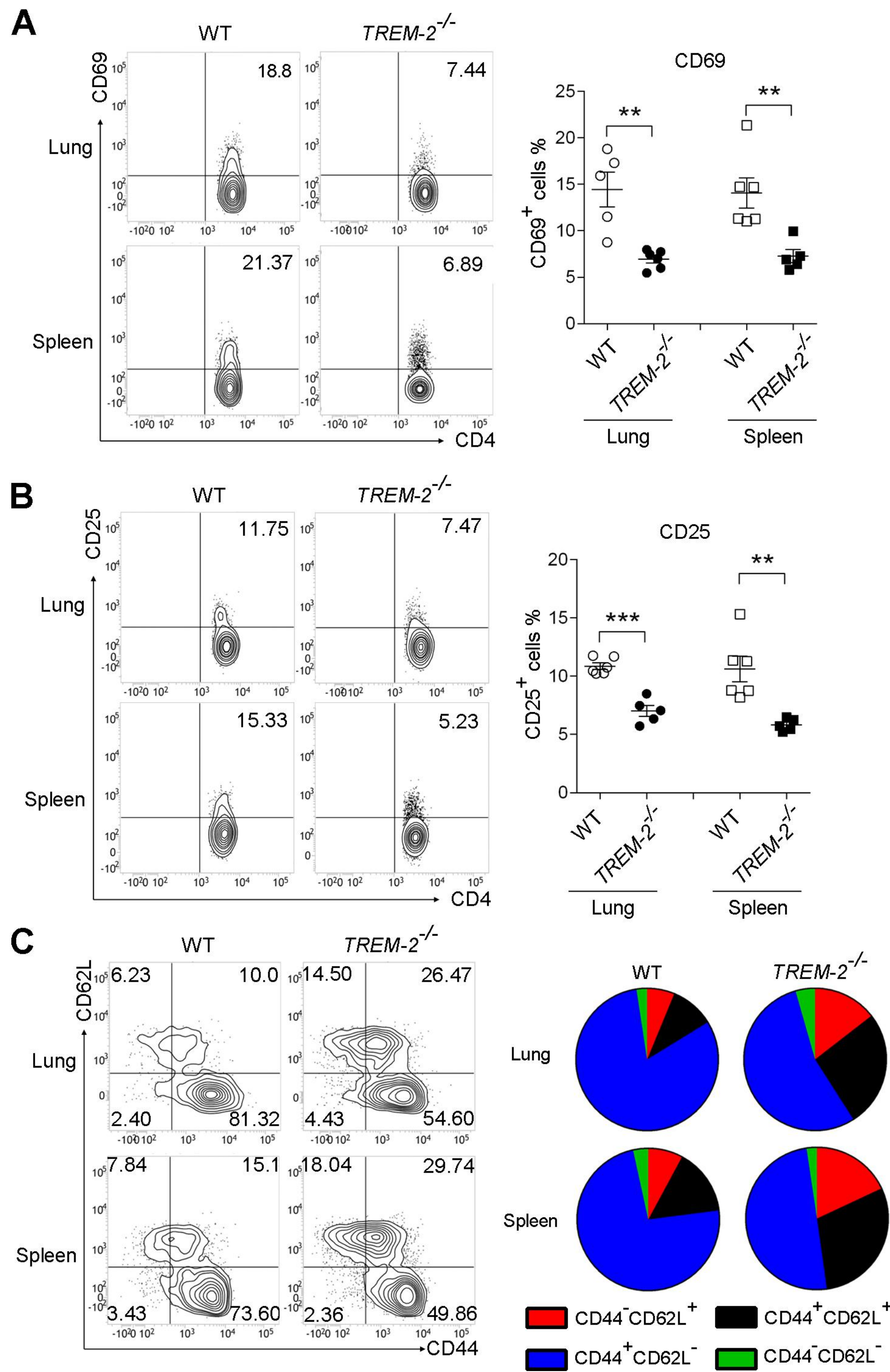

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62 **Figure S6. TREM-2 deficiency suppressed CD4^{+}T cell activation and effector**

63 **memory differentiation in vivo.**

64 C57BL/6 and *TREM-2$^{-/-}$* mice were injected i.p. with 1x10^{6} CFU of H37Rv. (**A, B**)

65 Expression of CD69 and CD25 in WT or *TREM-2$^{-/-}$* CD4^{+} T cells from lungs and

66 spleens were detected by flow cytometry. The percentages of CD69 **(A)** or CD25 (**B**)

67 positive cells were compared in TREM-2^{+} vs TREM-2^{-}CD4^{+} T cells. (**C**) CD4^{+} T cells
68 were defined by flow cytometry with CD44 and CD62L staining. The percentages of
69 naive T cells (CD44^{-}CD62L^{+}), T_{EM} (CD44^{+}CD62L^{-}), T_{CM} (CD44^{+}CD62L^{+}) and T_{EMRA}
70 (CD44^{-}CD62L^{-}) were shown as piechart in WT or *TREM-2$^{-/-}$* CD4^{+} T cells. Data
71 represent mean ± SD from at least three independent experiments. Unpaired Student's
72 t-test was performed in **A-B**. **$P < 0.01$. ***$P < 0.001$.
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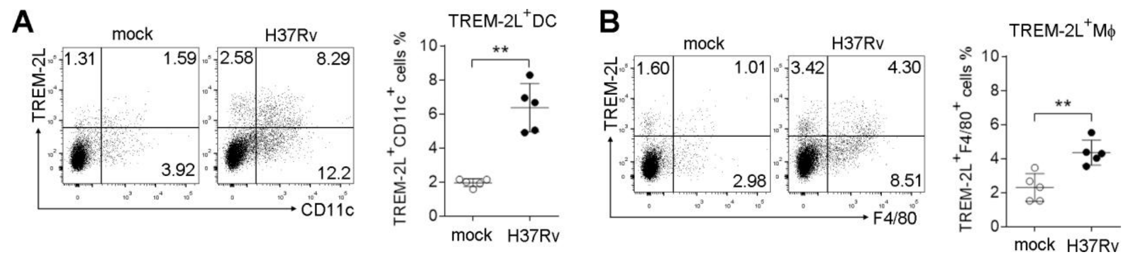

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77 **Figure S7. TREM-2L expression in Mϕ and DC was increased after *Mtb* infection.**

78 (**A, B**) The frequency of TREM-2L on splenic F4/80$^+$ Mϕ and CD11c$^+$ DC in

79 uninfected and infected mice was detected by flow cytometry. Data represent mean ±

80 SD from at least three independent experiments. Unpaired Student's t-test was

81 performed in **A-B**. ***P* < 0.01.

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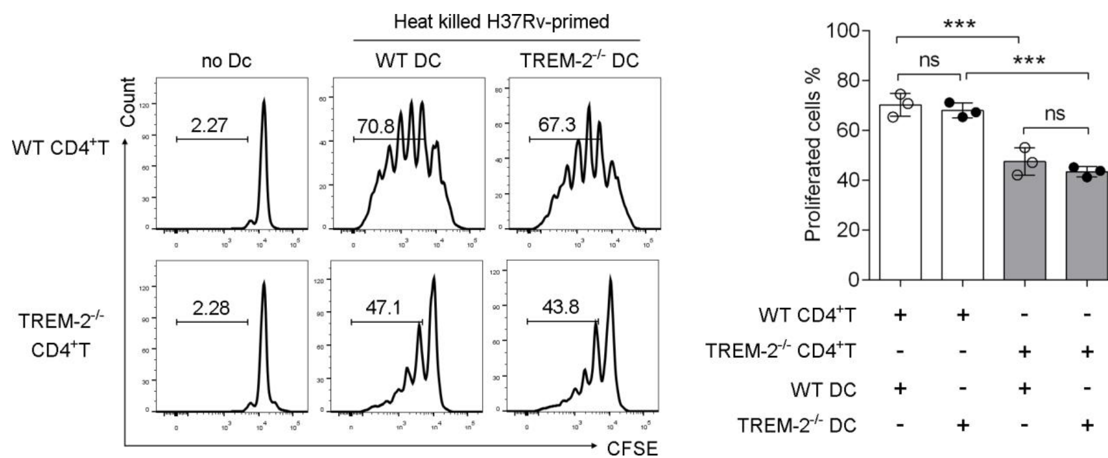

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87 **Figure S8. TREM-2 deficiency in DCs did not affect CD4+ T cell proliferation in**

88 **vitro.** Splenic T cells sorted from WT or *TREM-2-/-* mice (n=3) were labeled with

89 CFSE, and then co-cultured with heat-killed H37Rv-primed WT or *TREM-2-/-* DCs at

90 a ratio of 5:1 for 5 days, respectively. Cell proliferations of WT or *TREM-2-/-* CD4+ T

91 cells were examined by flow cytometry. Data represent mean ± SD from at least three

92 independent experiments. One way ANOVA test was performed. ns, no significant

93 difference. ****P* < 0.001.

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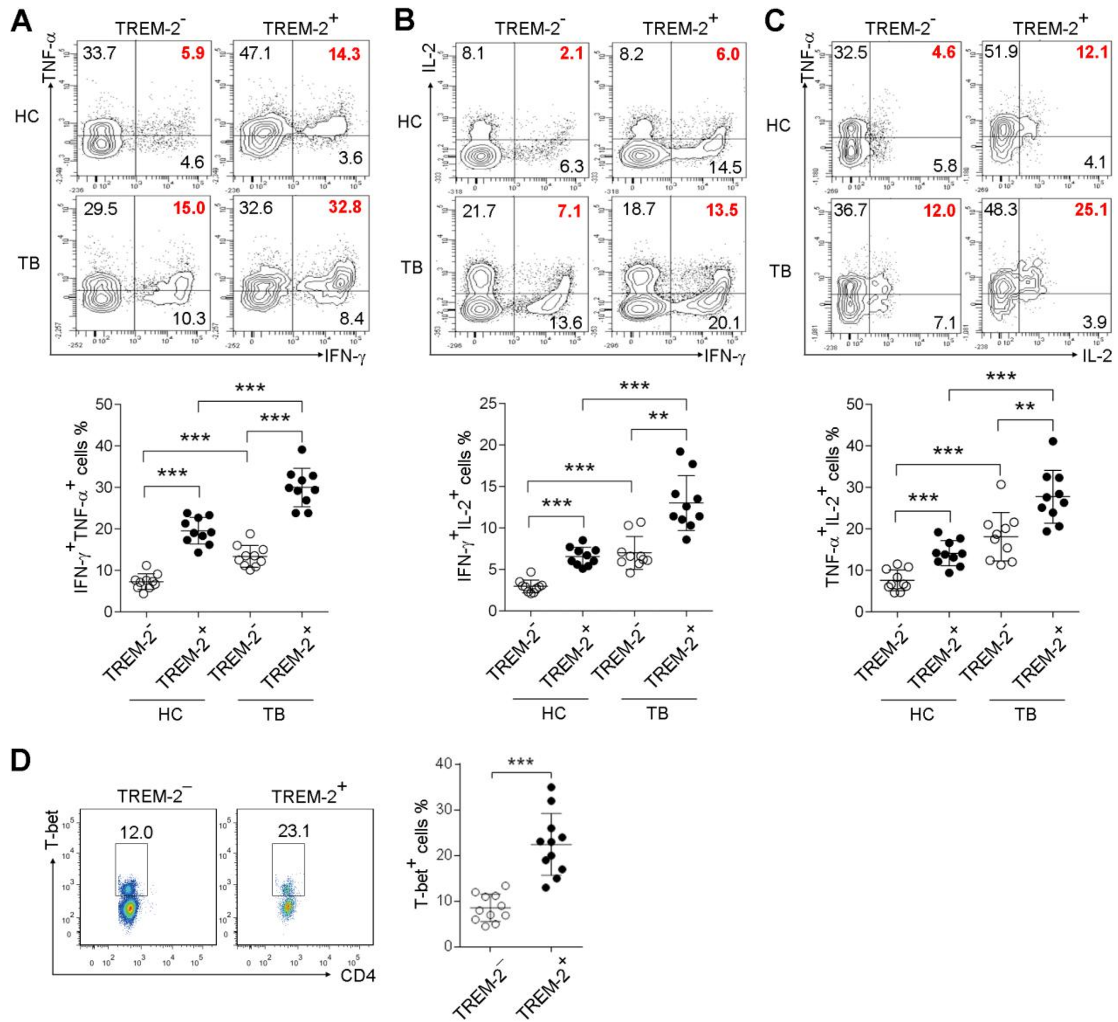

Figure S9. TREM-2 promoted Th1 cytokine production in peripheral CD4⁺ T cells of active TB patients. PBMCs from HC (n=9) and active TB patients (n=10) were stimulated with anti-CD3/CD28 (1μg/ml) and BFA (10μg/ml) for 12 hours. The percentages of IFN-γ$^+$TNF$^+$ (**A**), IFN-γ$^+$IL-2$^+$ (**B**) and TNF$^+$IL-2$^+$ (**C**) cells were analyzed by flow cytometry. (**D**) PBMCs from active TB subjects (n=12) were stained with specific Abs against CD3, CD4, TREM-2 or T-bet, followed by flow cytometry analysis. The percentages of T-bet positive cells were shown in TREM-2$^+$ vs TREM-2$^-$ CD4$^+$ T cells. Data represent mean ± SD from at least three independent experiments. One way ANOVA test was performed in **A-C**. Unpaired Student's t-test was performed in **D**. ***P* < 0.01. ****P* < 0.001.

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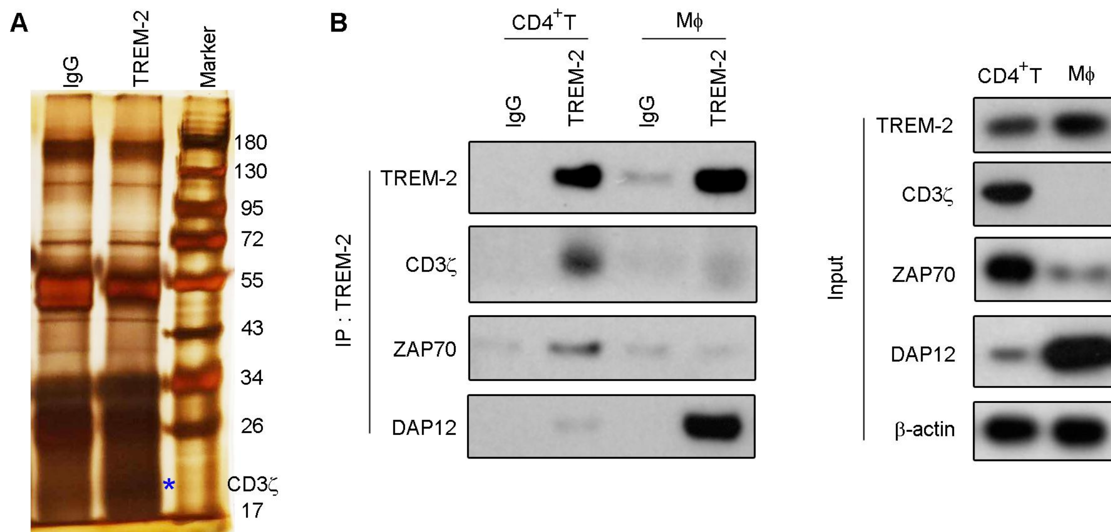

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109 **Figure S10. Identified proteins interacted with TREM-2 in CD4$^+$ T cells.** (**A**)

110 Primary CD4$^+$ T cells were sorted from C57BL/6 mice and then cell lysate protein

111 was respectively immunoprecipitated with anti-TREM-2 Ab or isotype-matched IgG.

112 Immunoprecipitates were analyzed by silver staining and the band as pointed with

113 asterisk (17-26kDa) was cut and examined by liquid chromatography- mass

114 spectrometry (LC-MS). The results indicated CD3ζ as the most potential protein. (**B**)

115 Sorted CD4$^+$ T cells or F4/80$^+$ macrophages were treated with anti-CD3 mAb (1μg/ml)

116 or LPS (1μg/ml) for 30 min respectively. Cell lysates (input) and anti-TREM-2

117 immunoprecipitates were analyzed for TREM-2, CD3ζ, DAP12 and ZAP70.

118 Immunoprecipitates performed with isotype-matched control IgG were used as

119 negative control.

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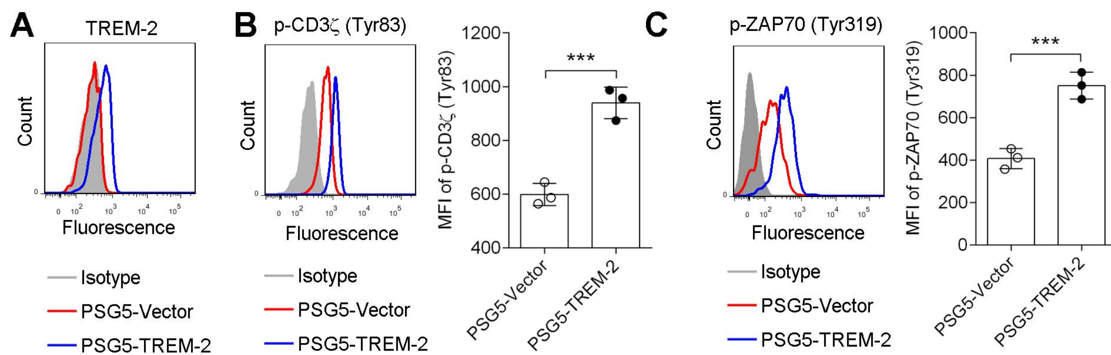

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123 **Figure S11. TREM-2 promoted phosphorylation of CD3ζ and ZAP70 in Jurkat**
124 **cells.** (**A**) 293T cells were transfected with HA-tagged TREM-2 plasmids. Surface
125 TREM-2 expression was determined by flow cytometry. (**B, C**) Jurkat cells (n=3)
126 were transfected with or PSG5 vector vs human TREM-2-expressing plasmids for 24
127 hours, and then treated with anti-CD3 mAb (1μg/ml). TREM-2, phosphorylated CD3ζ
128 and ZAP70 were analyzed by flow cytometry. Data represent mean ± SD from at least
129 three independent experiments. Unpaired Student's t-test was performed in **D**.***$P <$
130 0.001.

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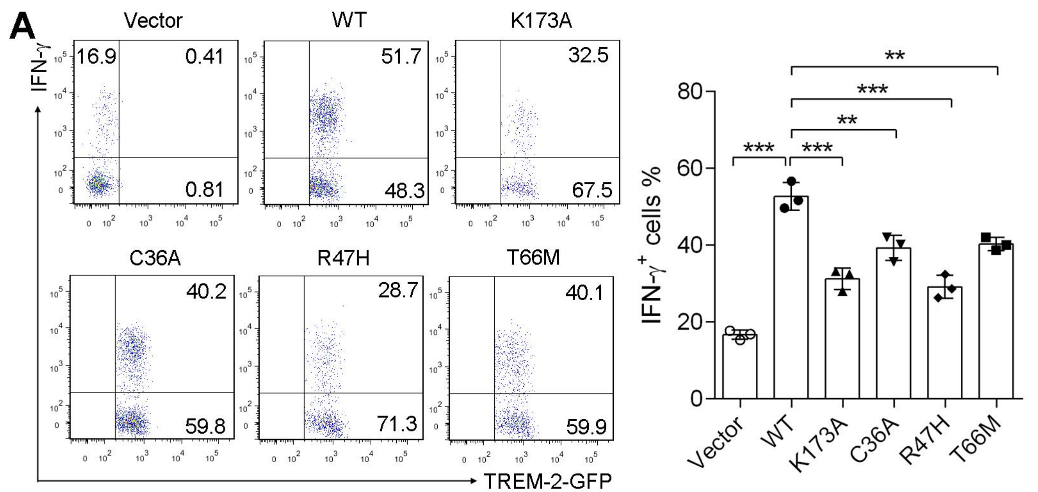

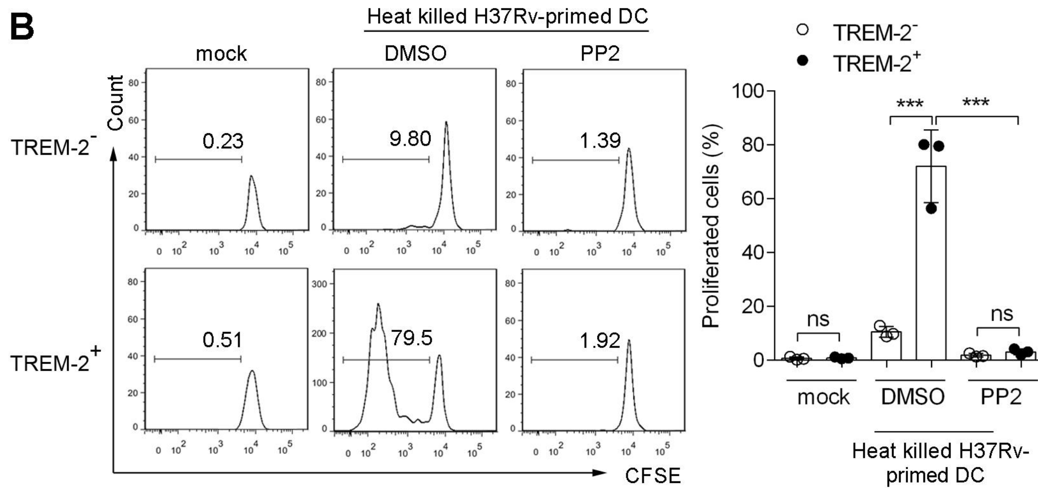

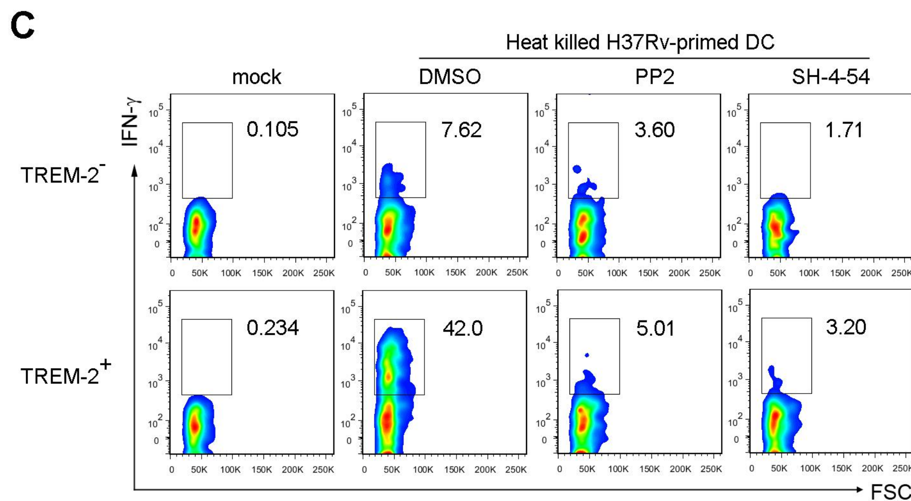

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134 **Figure S12. TREM-2/ZAP70/STATs signaling was required in $CD4^+$ T cell**

135 **proliferation and IFN-γ production.** (**A**) Sorted $CD4^+$ T cells (n=3) were transfected

136 with mutant TREM-2-GFP plasmid, and then stimulated with anti-CD3/CD28
137 (1μg/ml), IFN-γ (10ng/ml) and IL-12 (10ng/ml). IFN-γ production and GFP
138 expression was determined by flow cytometry. Bar chart showed the frequency of
139 IFN-γ positive cells (gated in $GFP^{+}CD4^{+}$ T cells). (**B, C**) TREM-2^{+} or TREM-$2^{-}CD4^{+}$
140 T cells sorted from human PBMCs (n=3) were pretreated with PP2 (1μM) or SH-4-54
141 (1μM), and then coculture with heat killed H37Rv-primed DCs. Cell proliferation (**B**)
142 and IFN-γ production (**C**) was determined by flow cytometry. Data represent mean ±
143 SD from at least three independent experiments. One way ANOVA test was performed
144 in **A-B**. ns, no significant; $*P < 0.05$, $***P < 0.001$.

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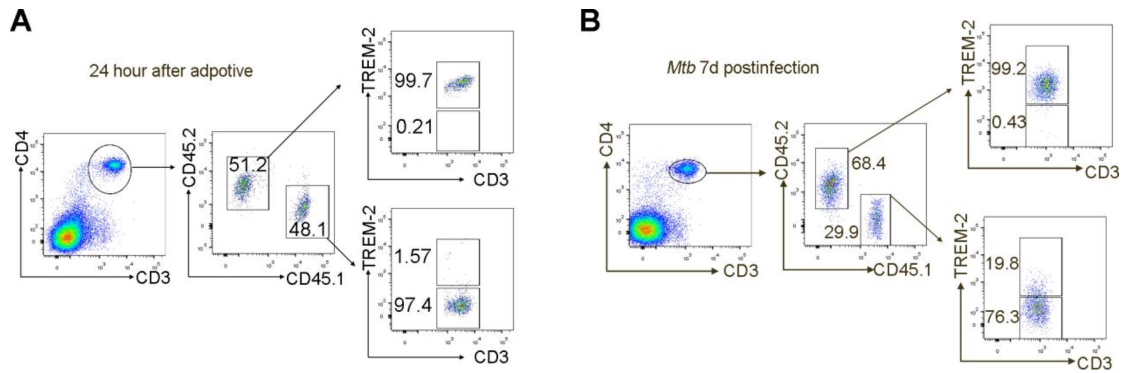

147 **Figure S13. TREM-2 expression stability in $CD4^+$ T cells after transfer to *Rag2*$^{-/-}$**

148 **mice.** TREM-2^- or TREM-$2^+$$CD4^+$ T cells were sorted from spleen cells from CD45.1

149 or CD45.2 transgenic mice. TREM-2^-CD45.1^+ and TREM-2^+CD45.2^+ $CD4^+$ T cells

150 were co-transferred into *Rag2*$^{-/-}$ mice at 1:1 ratio. Recipient *Rag2*$^{-/-}$ mice were infected

151 with *Mtb* at 24 hours after T cell transfer. TREM-2 expression was determined in

152 transferred CD45.1^+ and CD45.$2^+$$CD4^+$ T cells at 24 hours after adoptive transfer (**A**)

153 and Day 7 p.i. (**B**).

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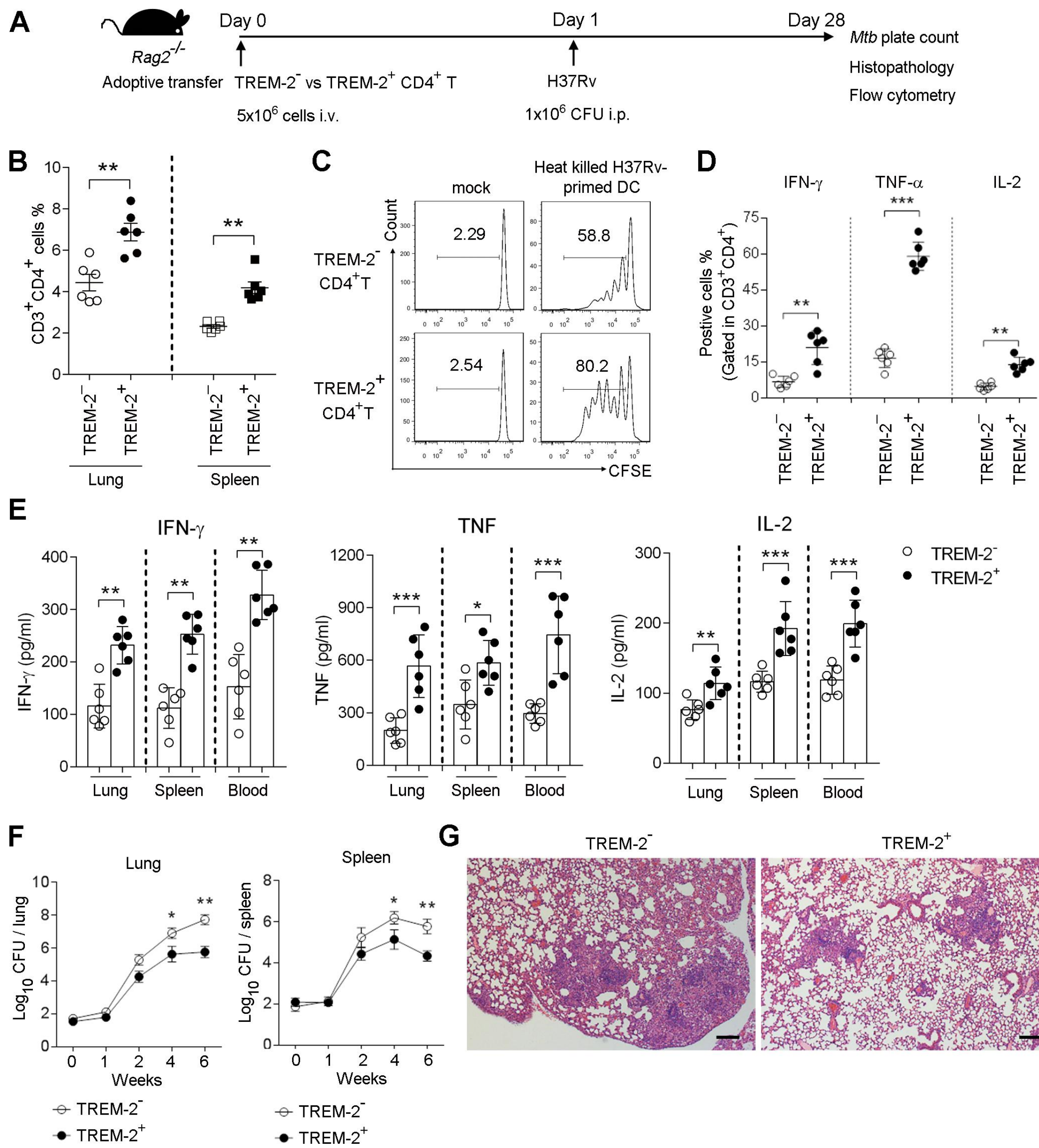

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157 **Figure S14. TREM-2 enhanced pro-inflammatory Th1 responses against *Mtb***

158 **infection in vivo.** (**A**) *Rag2*$^{-/-}$ mice (n=6) were injected i.v. with 5 x 10^6 with TREM-

159 2^- vs TREM-2^+CD4$^+$ T cells, following by injection i.p. with 1x10^6 CFU of H37Rv. At

160 28 days p.i., the lungs and spleens were collected and analyzed for the following tests.

161 (**B**) The frequency of pulmonary and splenic CD4$^+$ T cells was determined by flow

162 cytometry. (**C**) CFSE-labeled TREM-2^+ or TREM-2^-CD4$^+$ T cells from active TB

163 patients were stimulated with heat killed H37Rv-primed DC, anti-CD3/CD28 (1μg/ml)

164 for 3 days, and then cell proliferation was examined by flow cytometry. (**D**)
165 Splenocytes were stimulated with PMA (50nM), ionomycin (1μg/ml) and BFA
166 (1μg/ml) for 6 hours. Percentages of IFN-γ , TNF and IL-2-producing cells in TREM-
167 2^{-} vs TREM-2^{+} $CD4^{+}$ T cells were analyzed by flow cytometry. (**E**) Concentrations of
168 IFN-γ, TNF and IL-2 in the lungs, spleens and peripheral blood were determined by
169 ELISA. (**F**) Bacteria burden in the lungs was determined by plate count and
170 calculated as colony-forming units (CFU) per lung or spleen. (**G**) Lung sections were
171 stained with hematoxylin-eosin (H&E) and checked for histopathology under
172 microscope. Data represent mean ± SD from at least three independent experiments.
173 Unpaired Student's t-test was performed in **B-F**.**$P < 0.01$. ***$P < 0.001$.
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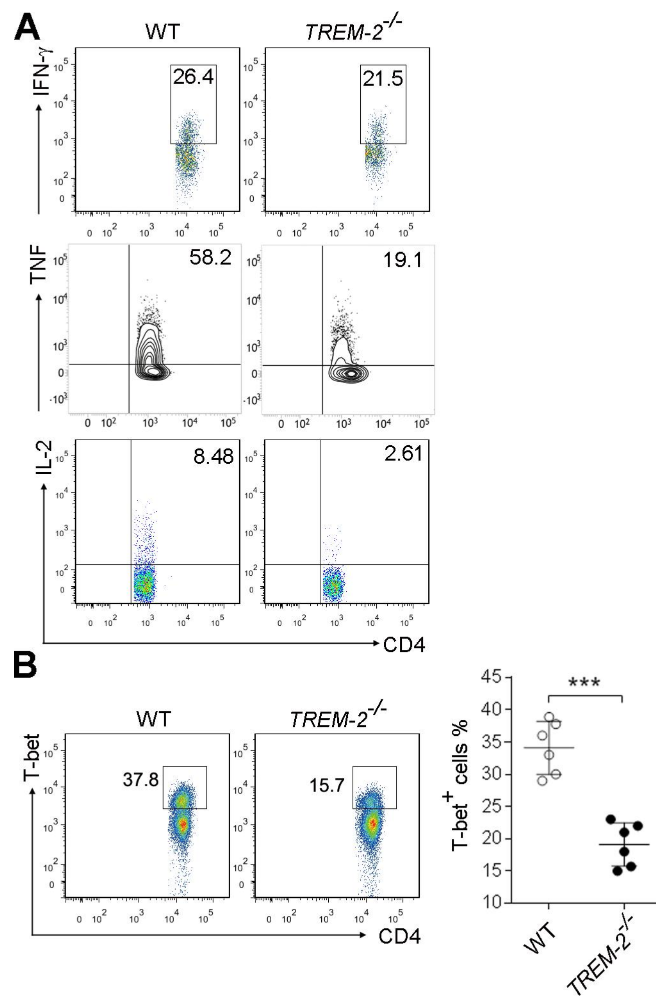

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177 **Figure S15. TREM-2 enhanced Th1 cytokine and T-bet expression in $CD4^+$ T cell**

178 **in vivo.** (**A**) Splenocytes were stimulated with PMA (50nM), ionomycin (1μg/ml) and

179 BFA (1μg/ml) for 6 hours. Percentages of IFN-γ , TNF and IL-2-producing cells in

180 WT vs *TREM-2*$^{-/-}$ were analyzed by flow cytometry. (**B**) Percentages of T-bet$^+$ cells in

181 WT vs *TREM-2*$^{-/-}$ $CD4^+$ T cells (n=6) were detected by flow cytometry. Data represent

182 mean ± SD from at least three independent experiments. Unpaired Student's t - test

183 was performed in **B**.****P* < 0.001.

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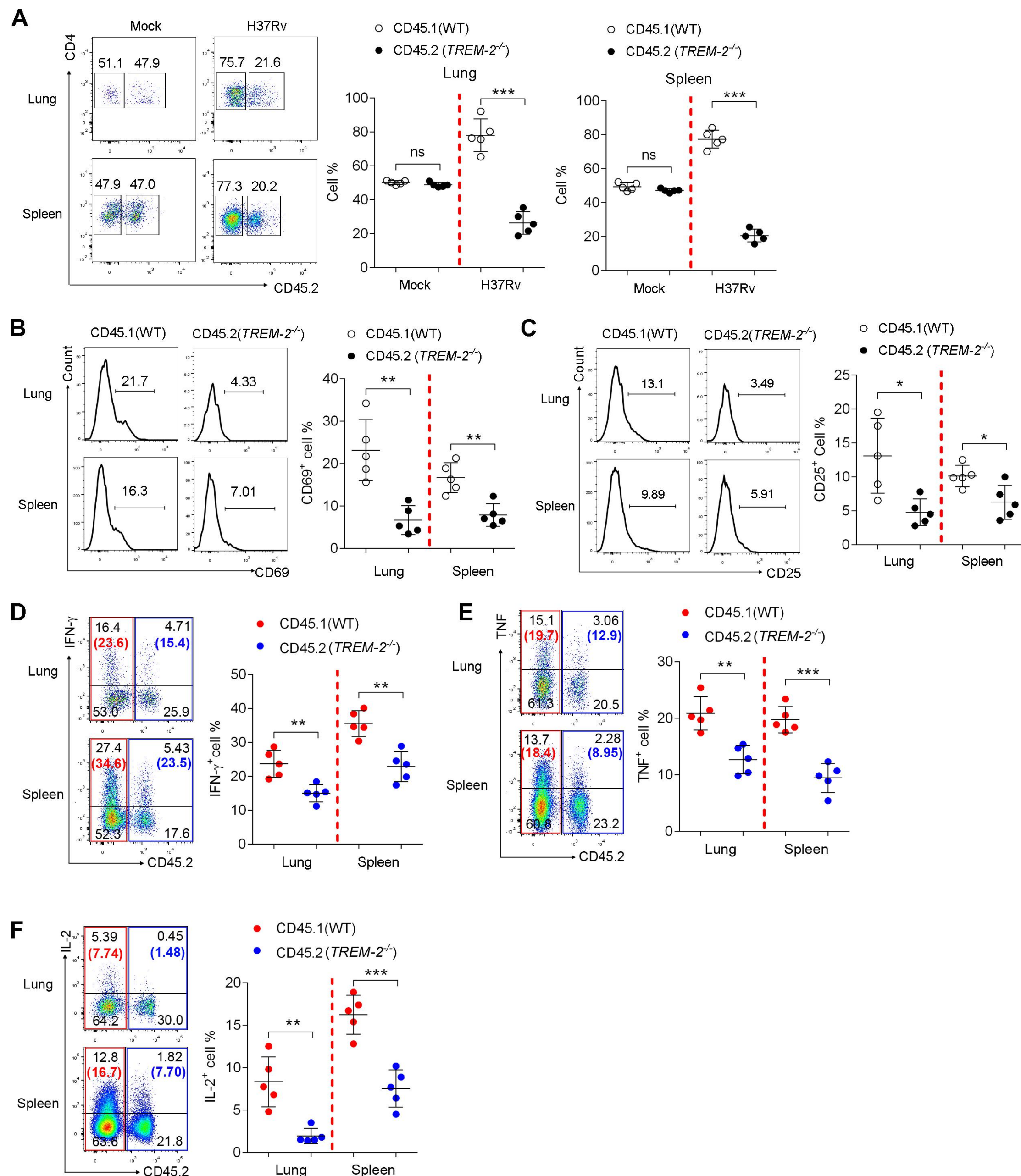

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186 **Figure S16. TREM-2 knockout reduced Th1 responses in bone marrow chimeric**

187 **mice during *Mtb* infection.** Irradiated *Rag2*$^{-/-}$ mice (n=5) were reconstituted with

188 CD45.1^{+} WT and CD45.2^{+} *TREM-2*$^{-/-}$ BM cells at ratio of 1:1. Reconstituted mice

189 were infected i.v. with H37Rv, and lung and spleen cells were analyzed at Day 28 p.i..

190 (**A**) The frequencies of CD45.1^{+} and CD45.2^{+} T cells (gated in CD3^{+}CD4^{+} T cells)

191 were determined by flow cytometry. (**B, C**) Expressions of CD69 **(B)** and CD25 **(C)**

192 were determined by flow cytometry in CD45.1^{+} or CD45.2^{+}CD4^{+} T cells. (**D-F**) Cells

193 were stimulated with PMA, ionomycin and BFA for 6 hours. Percentages of IFN-γ,

194 TNF and IL-2-producing cells in $CD45.1^+$ or $CD45.2^+CD4^+$ T cells were analyzed by

195 flow cytometry. Data represent mean ± SD from at least three independent

196 experiments. Unpaired Student's t-test was performed in **A-F**. ns, no significant

197 difference. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

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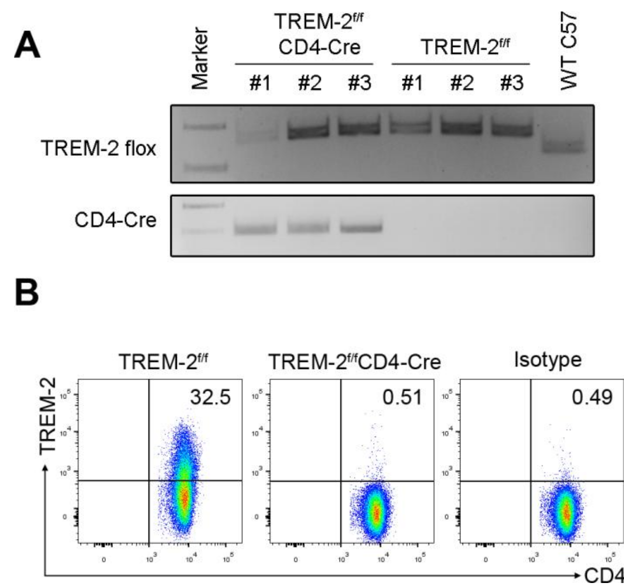

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201 **Figure S17. Generation of CD4 specific TREM-2 knockout mice.**

202 Mice with loxP-flanked alleles of TREM-2 exon 2/3 (TREM-$2^{f/f}$) were crossed with

203 mice expressing Cre recombinase under the control of a CD4 promoter (CD4-Cre) to

204 achieve CD4-specific deletion of TREM-2 (TREM-$2^{f/f}$CD4-Cre). (**A**) PCR analysis of

205 TREM-2 with loxP-flanked alleles and CD4-Cre recombinase from extracted DNA.

206 (**B**) Flow cytometry analysis of TREM-2 protein expression in spleen $CD4^+$T cell

207 from TREM-$2^{f/f}$ or TREM-$2^{f/f}$CD4-Cre mice.

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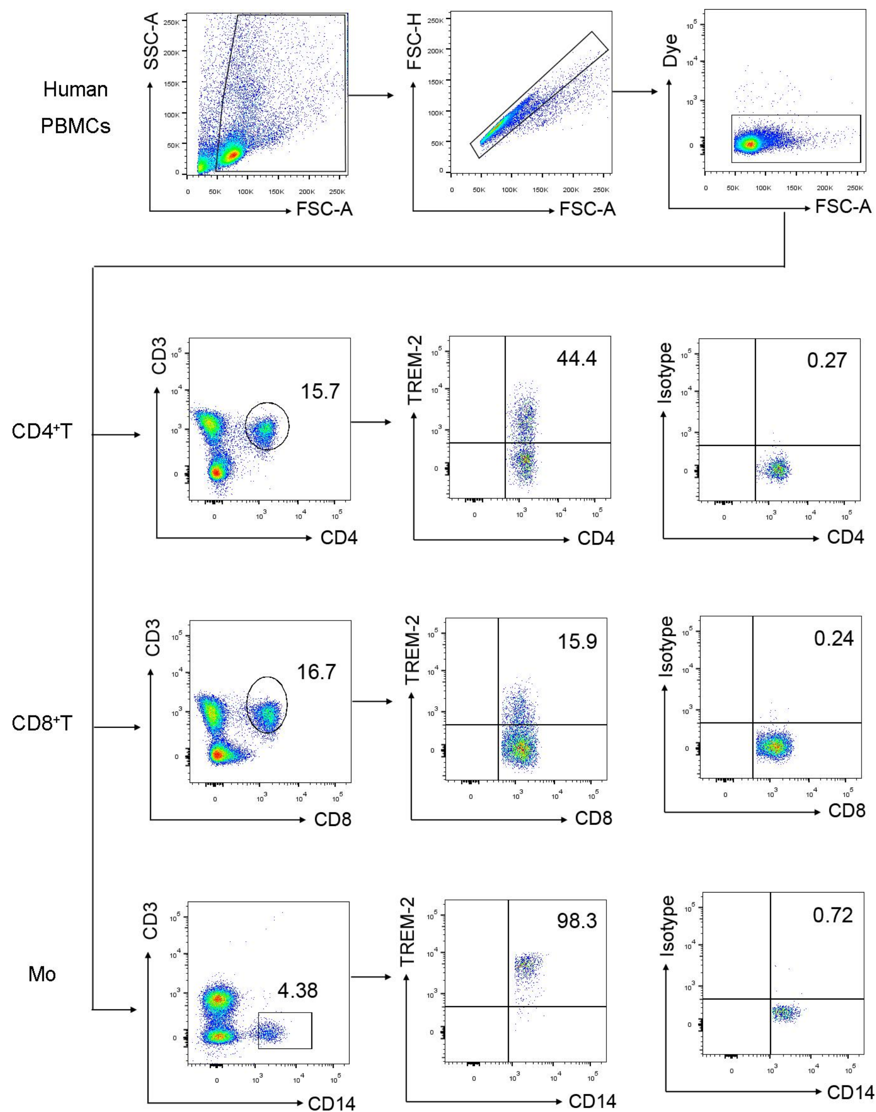

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210 **Figure S18. Gating strategy used to determine TREM-2 expression on**
211 **populations in human PBMCs.** Cells were gated as single, live (Viability dye$^-$) cells.
212 CD4$^+$ T cells, CD8$^+$T cells and monocytes (Mo) were gated as CD3$^+$CD4$^+$, CD3$^+$CD8$^+$
213 and CD3$^-$CD14$^+$ cells, respectively. TREM-2 Ab and IgG isotype were used to set the
214 cutoff value of TREM-2$^+$ cells and TREM-2$^-$ cells.

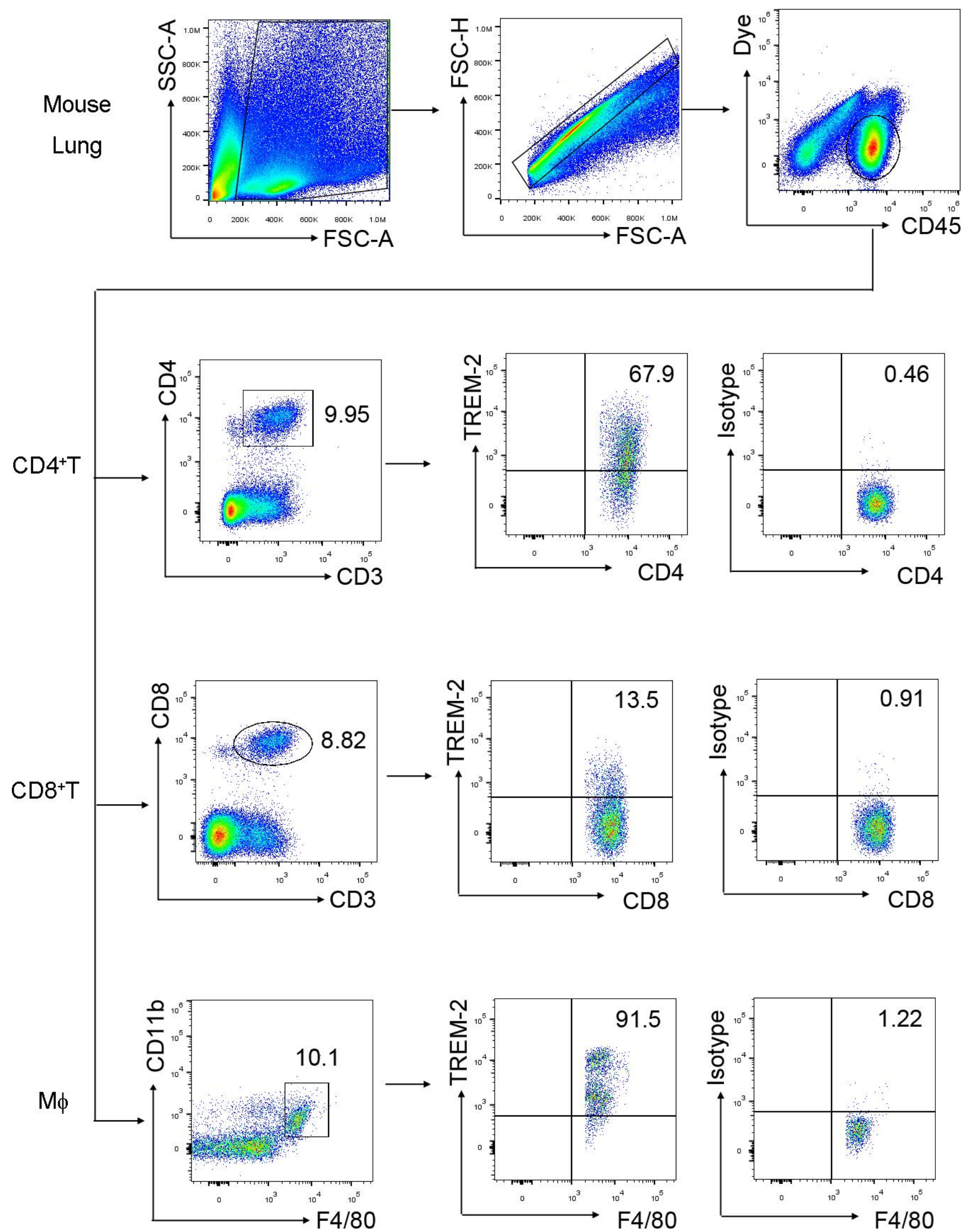
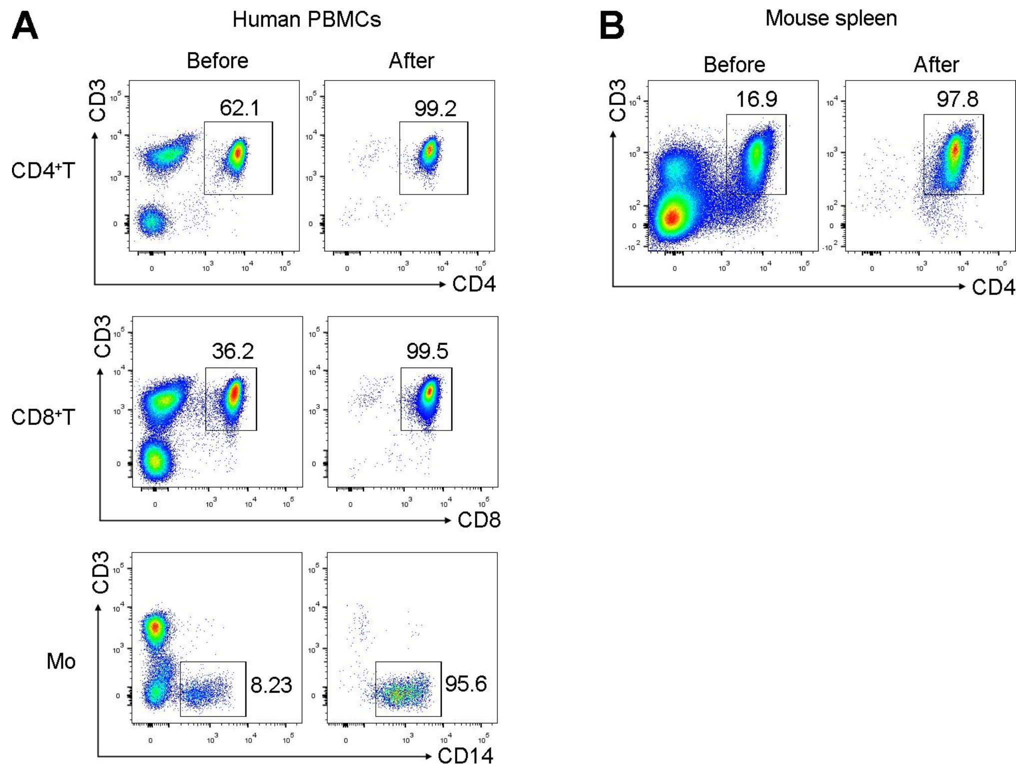


Figure S19. Gating strategy used to determine TREM-2 expression on populations in mouse lung sample. Cells were gated as single, live (viability dye$^-$) cells. CD4$^+$ T cells, CD8$^+$ T cells and macrophage (Mϕ) were gated as CD3$^+$CD4$^+$, CD3$^+$CD8$^+$ and CD11b$^+$F4/80$^+$ cells, respectively. TREM-2 Ab and IgG isotype were used to set the cutoff value of TREM-2$^+$ cells and TREM-2$^-$ cells.



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225 **Figure S20. The purity of sorted cells.** (**A**) Human CD4$^+$ T cells, CD8$^+$ T cells and
226 monocytes (Mo) were sorted from human PBMCs by positive selection using the
227 magnetic cell sorting system. The purity of the above subpopulations was analyzed by
228 gating on CD3$^+$CD4$^+$, CD3$^+$CD8$^+$ or CD3$^-$CD14$^+$ before and after sorting. (**B**) Mouse
229 CD4$^+$ T cells were sorted from mouse spleen by positive selection using the magnetic
230 cell sorting system. The purity of mouse CD4$^+$ T cells was analyzed by gating on
231 CD3$^+$CD4$^+$ before and after sorting.

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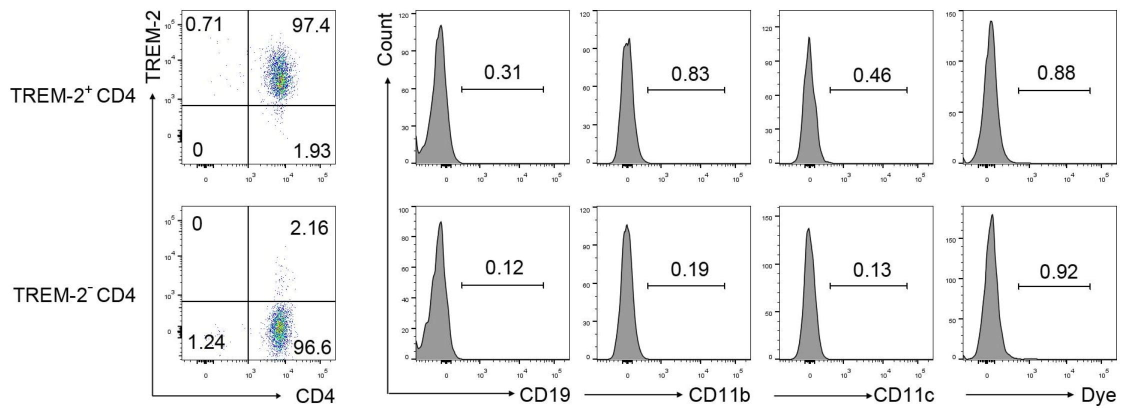

234 **Figure S21. The purity of TREM-2- and TREM-2+ CD4+T cells.** The sorted

235 TREM-2+ or TREM-2-CD4+ T cells were analyzed using flow cytometry by staining

236 with T cell marker CD4, B cell marker CD19, myeloid cell marker CD11b, dendritic

237 cell marker CD11c and viability dye.

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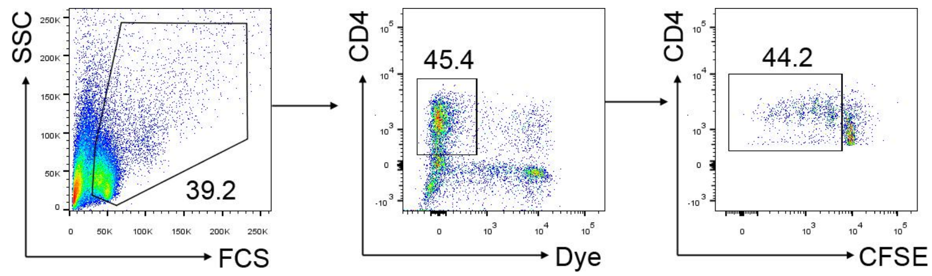

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243 **Figure S22. Gating strategy of CFSE proliferation array.** Cells from T cell/DC co-

244 culture system were collected and examined by flow cytometry. Proliferated T cells

245 were gated as live (viability dye^{-}) CD4^{+}cells. The proliferation of divided CD4^{+}T cells

246 was analyzed by CFSE.

247

248

249 **Supplemental Table 1. Summary of clinical features and laboratory results of**

250 **patients**

	HC	Active TB	Latent TB	Cured TB	COPD
Number	81	116	30	43	22
Age (years)	38.1(±2.38)	40.4(±5.16)	36.2(±3.36)	37.7(±4.43)	45.6(±5.23)
Sex (M/F)	30/51	45/71	12/18	18/25	12/10
Symptom					
Fever	NA	88(75.9%)	NA	NA	0(0%)
Cough	NA	77(66.4%)	NA	NA	22(100%)
Weakness	NA	34(29.3%)	NA	NA	20(90.9%)
Rhinorrhea	NA	19(16.4%)	NA	NA	17(77.3%)
Chest pain	NA	13(11.2%)	NA	NA	18(81.8%)
Sputum smear	NA	116(100%)	0(0%)	0(0%)	0(0%)
Sputum culture	NA	116(100%)	NA	NA	NA
TST	0(0%)	116(100%)	30(100%)	30(100%)	0(0%)
T-SPOT.TB	0(0%)	116(100%)	30(100%)	30(100%)	0(0%)
FEV1	NA	NA	NA	NA	51.8(±4.0)
FEV1/FVC	NA	NA	NA	NA	46.2(±2.5)

251 F, Female; M, male; NA, not applicable; TST, tuberculin skin testing; T-SPOT.TB
252 Tuberculosis antigen T-cell enzyme-linked immunospot assay; FEV1, forced expiratory
253 volume in one second; FVC, forced vital capacity. Data were shown by mean ± SEM.

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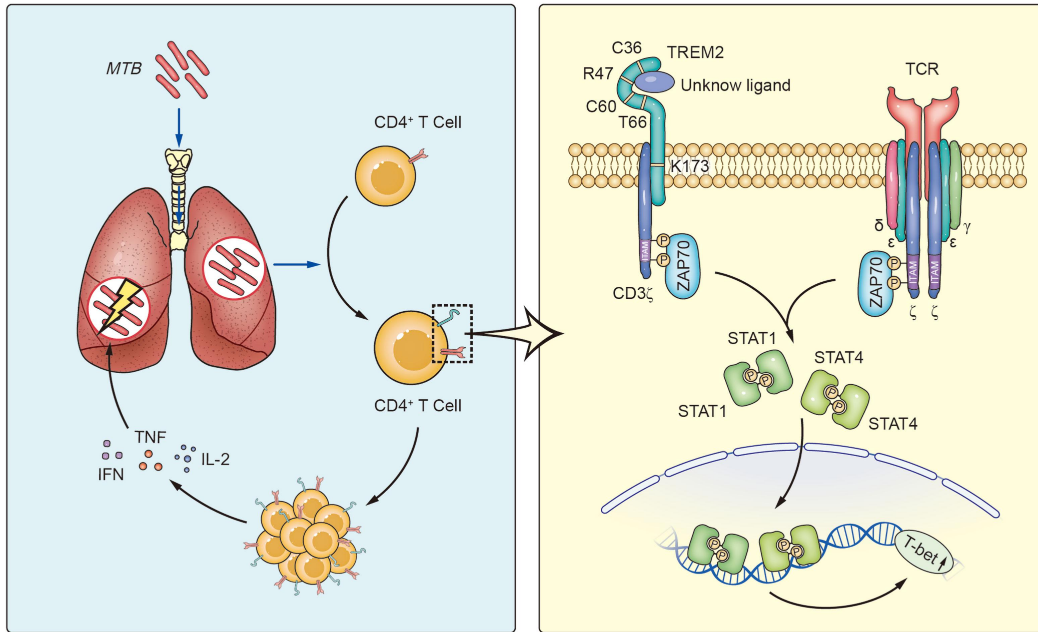

256 **Graphical abstract.** *Mtb* infection induces TREM-2 expression on the surface of

257 $CD4^+$ T cells. After the ligation with unknown ligand, TREM-2 interacts with

258 CD3ζ/ZAP70 complex by its transmembrane domain via K173 site. TREM-2 signals

259 through CD3ζ/ZAP70 complex to induce downstream STAT1/4 activation and T-bet

260 transcription, which is largely dependent on the amino acid sites C36, R47, C60, T66

261 and K173 located in the extracellular ligand-binding domain and transmembrane

262 domain respectively. Activation of TREM-2/CD3ζ/ZAP70 signaling subsequently

263 evokes $CD4^+$ T cell proliferation and Th1 differentiation. Activated Th1 cells are

264 recruited to the infected lungs and secrete pro-inflammatory cytokines like IFN-γ,

265 TNF and IL-2, triggering host cellular immunity and inflammatory responses to

266 eliminate the intracellular *Mtb*.

267