

1 **SUPPLEMENTARY INFORMATION**

2 **Glutamine metabolism inhibition has dual immunomodulatory and antibacterial activities**
3 **against *Mycobacterium tuberculosis***

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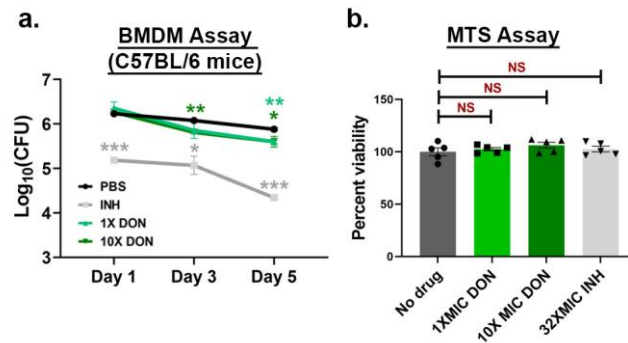
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Fig S1.



27

28 **Fig S1. The effect of JHU083 on the intracellular survival of *Mtb* and on bone-marrow**
 29 **derived macrophage (BMDM) viability.** BMDMs were harvested from the femurs of 8-12
 30 weeks old C57BL6 mice (n=10/experiment), activated using IFN γ . (a) IFN γ -activated BMDMs
 31 were infected with *Mtb* H37Rv at an MOI of 2 (n=3 wells/group). They were then treated with
 32 1X and 10X MIC concentrations of DON assuming a MIC value of 1 mg/ml. Isoniazid (INH)
 33 was used as the positive control. The cells were lysed at indicated time points and plated on
 34 7H11 selection plates. (b) BMDM viability as assessed by an MTS assay was performed after 5
 35 days of daily drug treatment at the indicated concentration (n=5 wells/group). The experiment
 36 was performed in triplicate. Statistical significance was calculated using a two-tailed student t-
 37 test considering unequal distribution. The exact p-values are provided in the Source Data file.
 38 *<0.05, **<0.01, ***<0.001. CFU stands for colony-forming units. All the experiments were
 39 performed in triplicates. MTS stands for 3-(4,5-Dimethylthiazol-2-yl)-5-(3-
 40 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt. CFU stands for colony-forming
 41 units. NS stands for non-significant change, p-value was >0.05.

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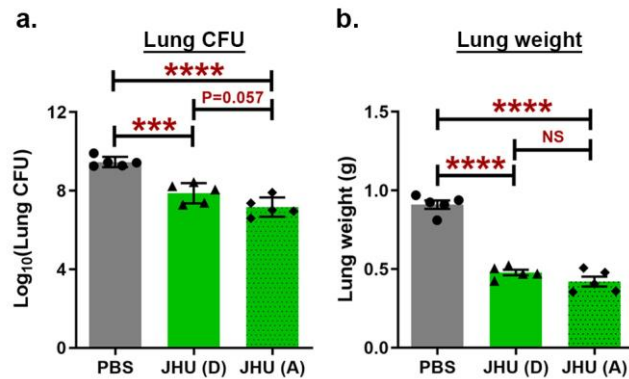
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Fig S2.



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49 **Fig S2. Both daily and alternate JHU083 dosing regimen are effective in vivo.** 129S2 mice
 50 (n=5/group) were aerosol infected with ~200-300 CFU of *Mtb* H37Rv. Two different dosing
 51 regimens; (1) Daily (JHU-D; 1 mg/kg dose per day for the first week, followed by 0.3 mg/kg
 52 daily 5/7 dosing M-F) and (2) Alternate (JHU-A; 1 mg/kg dose per day for the first week,
 53 followed by 3/7 dosing with 1 mg/kg on Mon, Wed and Fri) were administered. The mice were
 54 sacrificed at day 0 and week 5 post-infection/treatment. The lungs were harvested,
 55 homogenized, serially diluted, and plated on 7H11 selection plates. After 21-25 days, colonies
 56 were counted, and counts were transformed into log_{10} values and plotted. Graphs depicting the
 57 effect of both dosing regimen upon (a) lung bacillary burden and (b) lung weight at 5 weeks post
 58 infection/treatment. Data is plotted as mean \pm SEM. Statistical significance was calculated
 59 using a two-tailed student t-test considering unequal distribution. The exact p-values are
 60 provided in the Source Data file. * <0.05 . CFU stands for colony-forming units. CFU stands for
 61 colony-forming units. NS stands for non-significant change, p-value was >0.05 . The experiment
 62 was performed twice.

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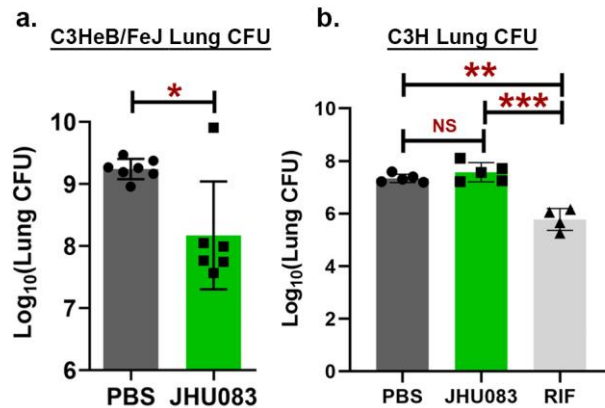
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Fig S3.



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69 **Fig S3. Impact of JHU083 treatment on lung bacillary burden in *Mtb*-infected C3HeB/FeJ**
70 **and C3H mice.** C3HeB/FeJ (n=7/group) and C3H mice (n=5/group) were aerosol-infected with
71 150-200 CFU of *Mtb* H37Rv. The mice were then treated with JHU083 via oral gavage starting
72 one day after infection. 1 mg/kg JHU083 was given daily for the first 5 days and then the dose
73 was reduced to 0.3 mg/kg daily (5/7, M-F). The mice were sacrificed on day 0 and week 5 post-
74 infection/treatment. The lungs were harvested, homogenized, serially diluted, and plated on
75 7H11 selection plates. After 21-25 days, colonies were counted, and counts were transformed
76 into log_{10} values and plotted. Lung bacillary burden in **(a)** C3HeB/FeJ mice at 4.5 weeks post
77 infection/treatment and **(b)** C3H mice at 5 weeks post infection/treatment. Data is plotted as
78 mean \pm SEM. Statistical significance was calculated using a two-tailed student t-test considering
79 unequal distribution. The exact p-values are provided in the Source Data file. * <0.05 . CFU
80 stands for colony-forming units. CFU stands for colony-forming units. NS stands for non-
81 significant change, , p-value was >0.05 . One mouse each from JHU083-treated C3HeB/Fej and
82 RIF-treated C3H groups died prematurely. The experiment was performed twice.

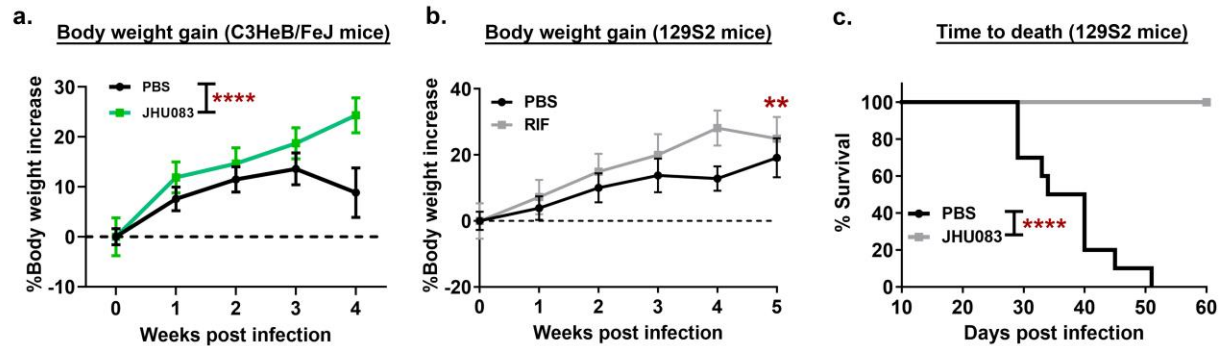
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Fig S4.



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88 **Fig S4. JHU083 and RIF administration improved body weight and survival of *Mtb*-**

89 **infected mice.** 129S2 or C3HeB/FeJ mice were aerosol-infected with 200-300 CFU of *Mtb*

90 *H37Rv*. Next day of the infection, 1 mg/kg of JHU083 was given daily for the first five days,

91 and then the dose was reduced to 1 mg/kg on alternate days (3/7, M-F). 12.5 mg/kg RIF was

92 given daily orally for 5-weeks. The mice were then treated with JHU083 12.5 mg/kg RIF daily

93 via oral gavage starting one day after infection. The mice were sacrificed at day 0 and week 5

94 post-infection/treatment. Graphs depict (a) body weight gain in C3HeB/FeJ mice (n=10/group)

95 treated with PBS or JHU083 and, (b) body weight gain in 129S2 mice (n=5/group) treated with

96 PBS and RIF. (c) Survival of 129S2 mice (n=10/group) treated with RIF or PBS. Data is plotted

97 as mean \pm SEM. For body weight, a two-way Anova test was used for calculating statistical

98 significance. For survival curve, statistical significance was calculated using both Log-rank

99 (Mantel-Cox) and Gehan-Breslow-Wilcoxon tests. The exact p-values are provided in the

100 Source Data file. **<0.01, ****<0.0001. The experiment was performed twice.

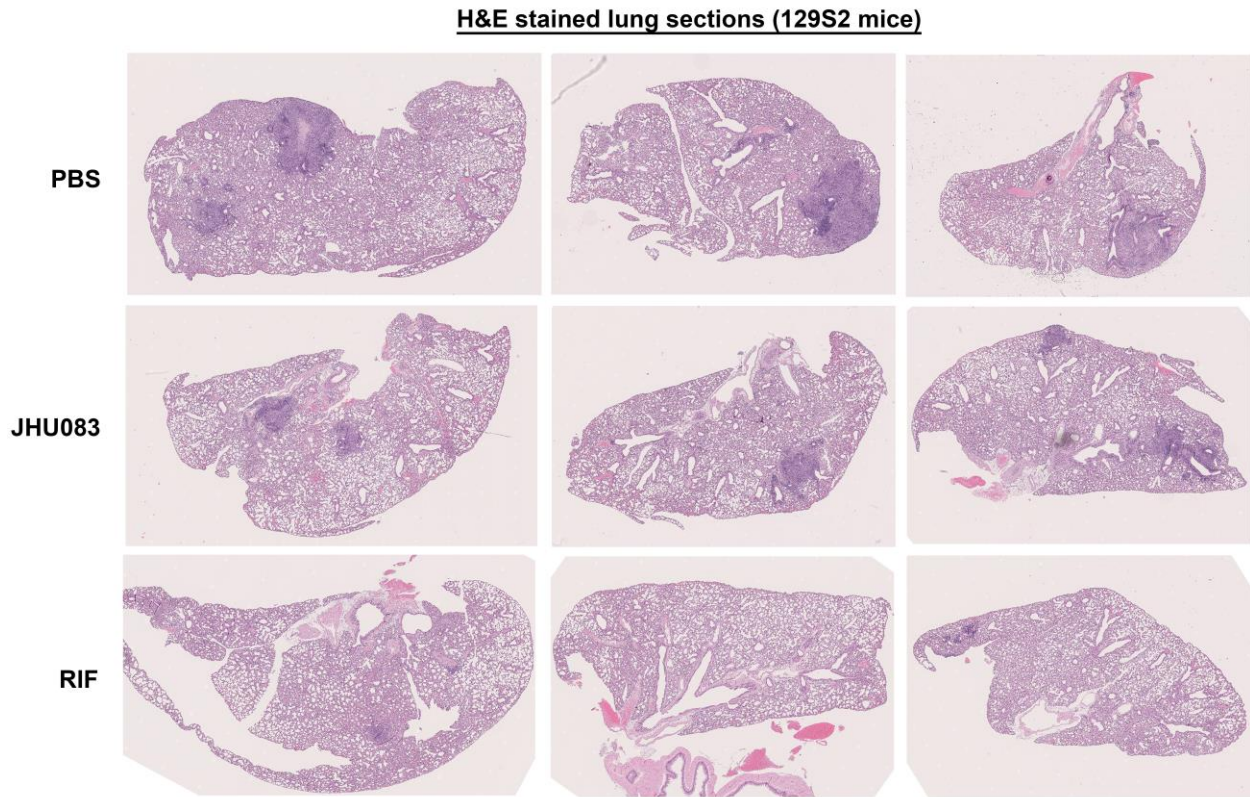
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Fig S5



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106 **Fig S5. The histopathology of the lungs isolated from 129S2 mice infected with *Mtb* H37Rv**
107 **at week 5 post-infection/treatment.** 129S2 or C3HeB/FeJ mice (n=3/group) were aerosol
108 infected with ~200-300 CFU of *Mtb* H37Rv. The mice were treated with JHU083 or RIF via oral
109 route one day after infection. 1 mg/kg JHU083 was given daily for the first five days, and then
110 the dose was reduced to 0.3 mg/kg daily (5/7, M-F). The lungs were formalin fixed, sectioned,
111 and stained with hematoxylin and eosin. All the experiments were repeated at least twice.

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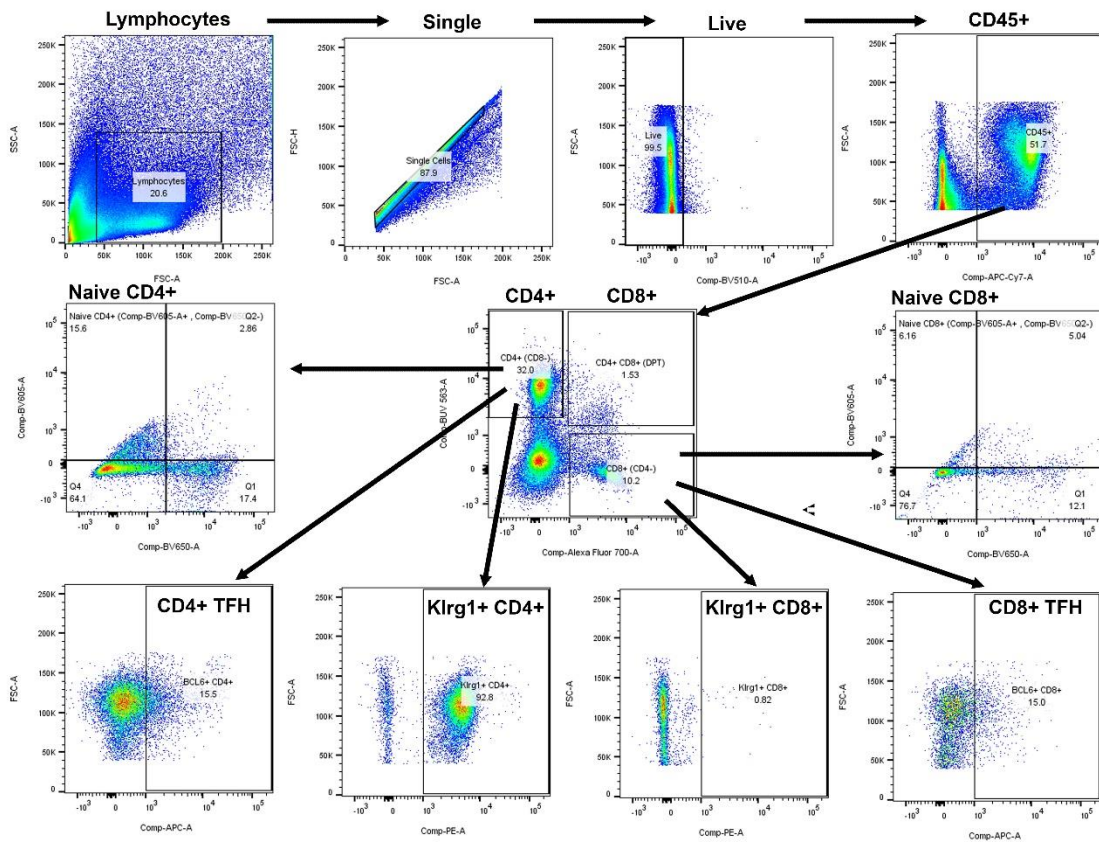
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Fig S6.

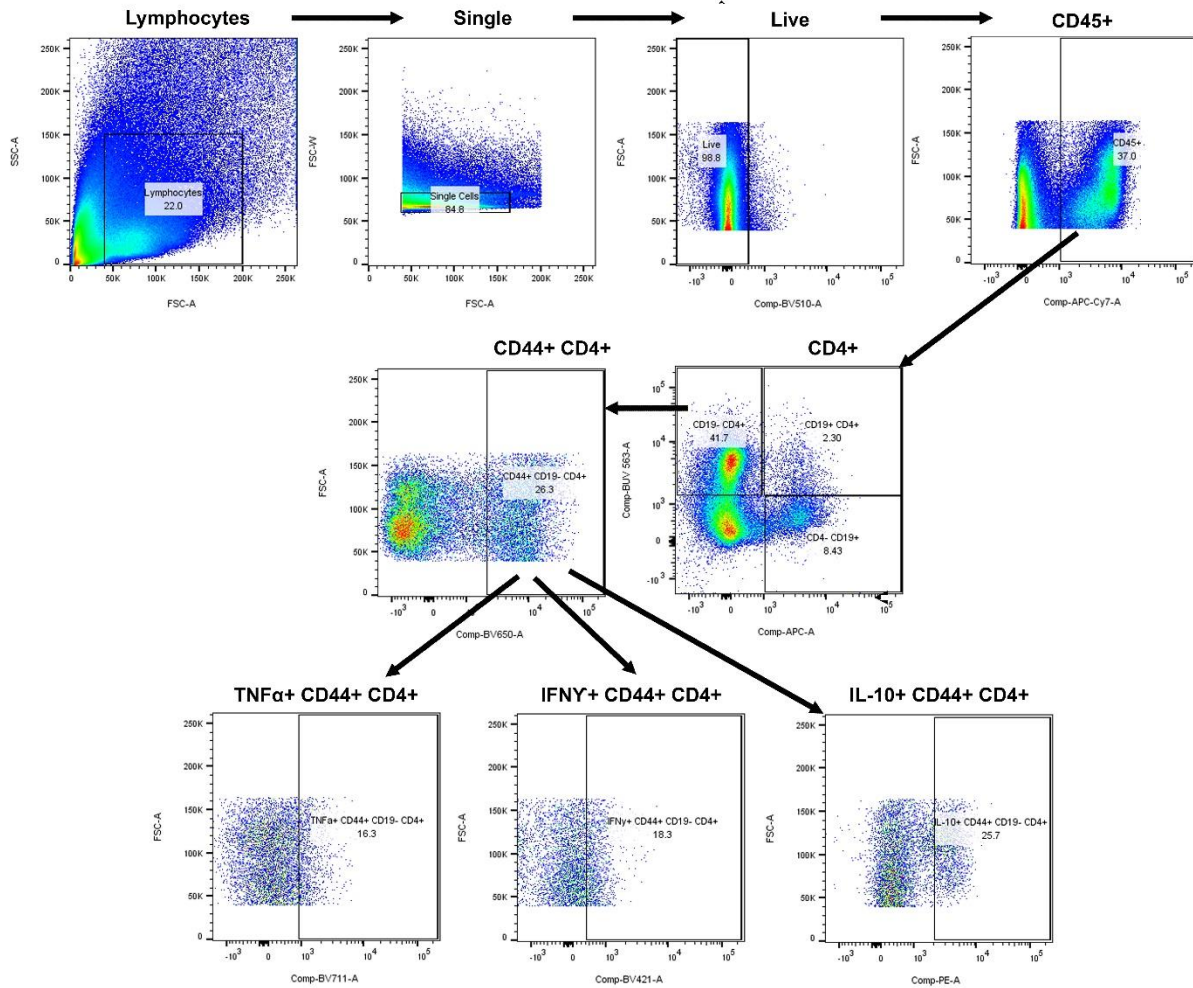


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118 **Fig S6. Two-dimensional gating strategies for flow cytometrical identification of T-cell**
 119 **subsets.** We excluded doublets and debris and gated on single live CD45⁺ cells. We identified
 120 CD4⁺ T-cells (CD45⁺ CD8⁻ CD4⁺), CD8⁺ T-cells (CD45⁺ CD4⁻ CD8⁺), Naïve CD4⁺ T-cells
 121 (CD45⁺ CD8⁻ CD4⁺ CD44⁻ CD26L⁺), Naïve CD8⁺ T-cells (CD45⁺ CD8⁺ CD4⁻ CD44⁻ CD26L⁺),
 122 Follicular helper CD4⁺ T-cells (CD45⁺ CD8⁻ CD4⁺ BCL6⁺), Follicular helper CD8⁺ T-cells
 123 (CD45⁺ CD8⁺ CD4⁻ BCL6⁺), Klrp1⁺ CD4⁺ T-cells (CD45⁺ CD8⁻ CD4⁺ Klrp1⁺), Klrp1⁺ CD8⁺ T-
 124 cells (CD45⁺ CD8⁺ CD4⁻ Klrp1⁺). All flow antibodies were titrated to identify the concentration
 125 with maximum specificity coupled with minimum possible spillover. The gating strategy was
 126 defined using single-stain and FMO controls (for low-expression markers).

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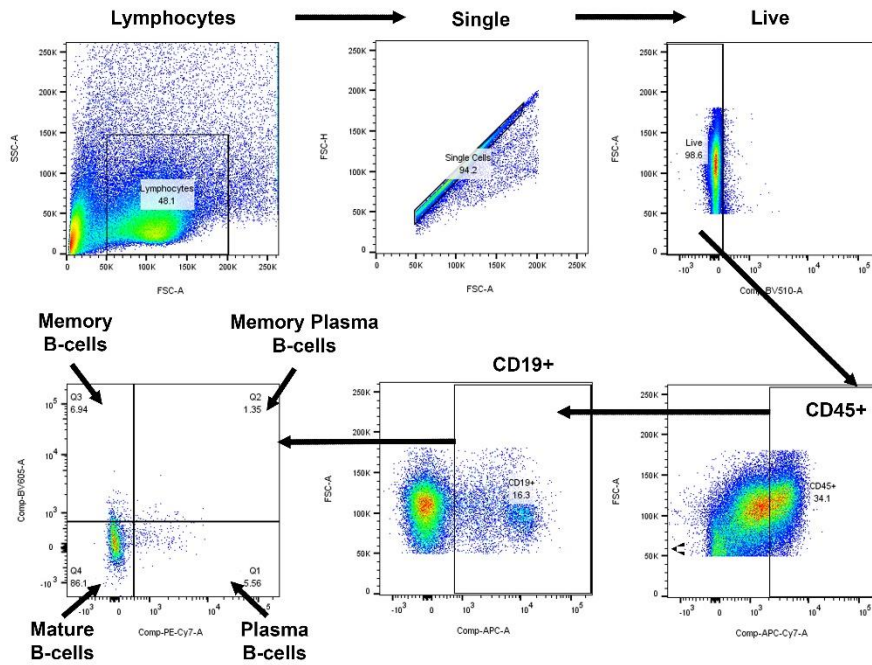
Fig S7.



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129 **Fig S7. Two-dimensional gating strategies for flow cytometrical identification of cytokine-**
130 **producing T-cell subsets.** We excluded doublets and debris and gated on single live CD45+
131 cells. We identified CD4⁺ T-cells (CD45⁺ CD19⁻ CD4⁺), activated CD4⁺ T-cells (CD45⁺ CD19⁻
132 CD44⁺ CD4⁺), TNFα⁺ activated CD4⁺ T-cells (CD45⁺ CD19⁻ CD44⁺ CD4⁺ TNFα⁺), IFNγ⁺
133 activated CD4⁺ T-cells (CD45⁺ CD19⁻ CD44⁺ CD4⁺ IFNγ⁺) and IL-10⁺ activated CD4⁺ T-cells
134 (CD45⁺ CD19⁻ CD44⁺ CD4⁺ IL10⁺). All flow antibodies were titrated to identify the concentration
135 with maximum specificity coupled with minimum possible spillover. The gating strategy was
136 defined using single-stain and FMO controls (for low-expression markers).

Fig S8.



137

138 **Fig S8. Two-dimensional gating strategies for flow cytometrical identification of B cell**
139 **subsets.** We excluded doublets and debris and gated on single live CD45⁺ cells. We identified
140 total T-cells (CD45⁺ CD19⁺), mature B-cells (CD45⁺ CD19⁺ CD27⁻ CD138⁻), memory B-cells
141 (CD45⁺ CD19⁺ CD27⁺ CD138⁻), plasma cells (CD45⁺ CD19⁺ CD27⁻ CD138⁺), plasma memory
142 B-cells (CD45⁺ CD19⁺ CD27⁺ CD138⁺). All flow antibodies were titrated to identify the
143 concentration with maximum specificity coupled with minimum possible spillover. The gating
144 strategy was defined using single-stain and FMO controls (for low-expression markers).

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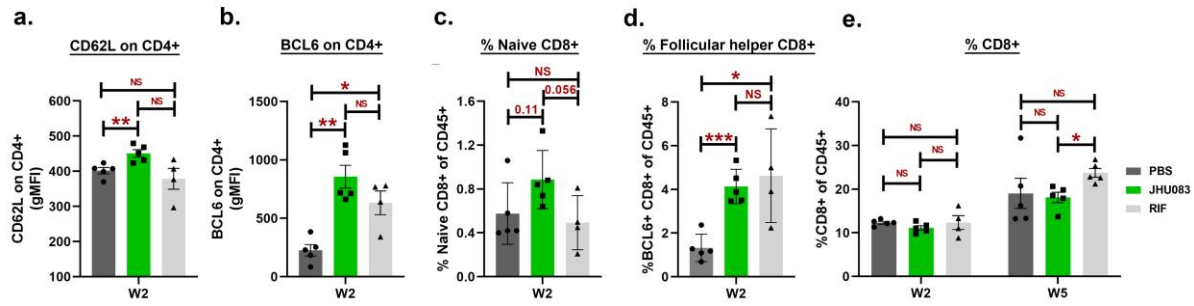
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Fig S9.



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154 **Fig S9. Effect of JHU083 administration upon T-cells in the lungs at weeks 2 and 5.** As
155 described in Fig 2a, *Mtb*-infected 129S2 mice were treated with JHU083 and RIF every day
156 starting day 1 post-infection. The mice were sacrificed at week 2 and week 5, and the lungs were
157 harvested. Single cell suspensions of the lungs from all three groups were stained with
158 appropriate antibodies and analyzed using multicolor-flow cytometry (n=5/group except RIF-
159 treated group in graph panels a-d where n=4). We found differences in the (a) CD62L
160 expression upon CD4⁺ T-cells, (b) BCL6 expression upon CD4⁺ T-cells, (c) frequency of naïve
161 CD8⁺ T-cells, (d) frequency of follicular helper CD8⁺ T-cells and, (e) total CD8⁺ T-cells The X-
162 axis shows the timepoint at which the lungs were harvested for the flow cytometry analysis.
163 Data were plotted as Mean ± SEM and are shown as the frequency of CD45⁺ population. gMFI
164 stands for geometric mean fluorescence intensity and was used to define the expression of the
165 individual markers upon the indicated cell types. Statistical significance was calculated using a
166 two-tailed student t-test considering unequal distribution. The exact p-values are provided in the
167 Source Data file. *<0.05, **<0.01, ***<0.001. CFU stands for colony-forming units. NS stands
168 for non-significant change, p-value was >0.05. The experiment was repeated two times.

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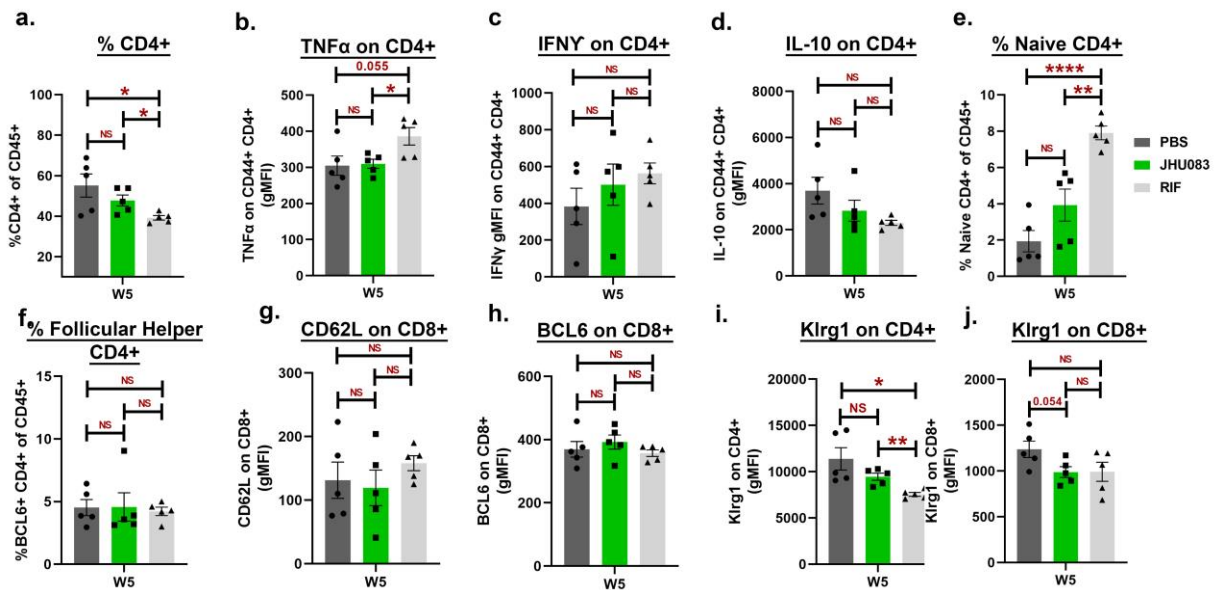
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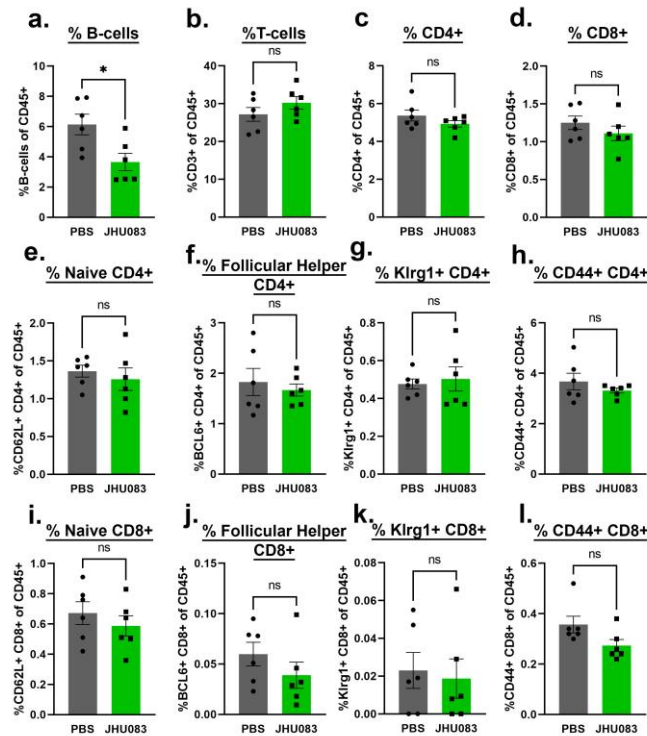
Fig S10.



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175 **Fig S10. Effect of JHU083 administration upon T-cells in the lungs at week 5.** As described
176 in Fig 2a, *Mtb*-infected 129S2 mice (n=5/group) were treated with JHU083 and RIF every day
177 starting day 1 post-infection. The mice were sacrificed at week 2 and week 5, and the lungs were
178 harvested. Single cell suspensions of the lungs from all three groups were stained with
179 appropriate antibodies and analyzed using multicolor-flow cytometry (n=4-5). We found no
180 difference in the (a) CD4⁺ T-cell frequency, (b) TNF α expression upon activated CD4⁺ T-cells,
181 (c) IFN γ expression upon activated CD4⁺ T-cells, (d) IL-10 expression upon activated CD4⁺ T-
182 cells, (e) Naive CD4⁺ T-cells, (f) Follicular helper T-cells, (g) CD62L expression on CD8⁺ T-
183 cells, (h) BCL6 expression on CD8⁺ T-cells, (i) Klrg1 expression on CD4⁺ T-cells, (j) Klrg1
184 expression on CD8⁺ T-cells. The X-axis shows the timepoint at which the lungs were harvested
185 for flow cytometry analysis. Data were plotted as Mean \pm SEM and are shown as the frequency
186 of CD45⁺ population. gMFI stands for geometric mean fluorescence intensity and was used to
187 define the expression of the individual markers upon the indicated cell types. gMFI was mostly
188 used for low abundance cell surface markers and transcription factors. Statistical significance
189 was calculated using a two-tailed student t-test considering unequal distribution. The exact p-
190 values are provided in the Source Data file. *<0.05, **<0.01, ***<0.001, ****<0.0001. CFU
191 stands for colony-forming units. NS stands for non-significant change, p-value was >0.05. The
192 experiment was repeated twice.

Fig S11.



194

195 **Fig S11. Effect of JHU083 administration upon T-cell subsets in uninfected 129S2 mice**

196 **lungs at week 2.** Uninfected six to ten weeks old female 129S2 mice (n=6/group) were treated
 197 with JHU083 every day. The mice were sacrificed at week 2, and the lungs were harvested.

198 Single cell suspensions of the lungs from all three groups were stained with appropriate
 199 antibodies and analyzed using multicolor-flow cytometry. We found (a) reduced frequency of
 200 B-cells. While there was no difference in the frequency of (b) CD3⁺ T-cells, (c) CD4⁺ T-cells,
 201 (d) CD8⁺ T-cells, (e) Naïve CD4⁺ T-cells, (f) Follicular helper CD4⁺ T-cells, (g) proliferating
 202 Ki67⁺ CD4⁺ T-cells, (h) activated CD44⁺ CD4⁺ T-cells, (i) Naïve CD8⁺ T-cells, (j) Follicular
 203 helper CD8⁺ T-cells, (k) proliferating Ki67⁺ CD8⁺ T-cells and, (l) activated CD44⁺ CD8⁺ T-cells.

204 Data were plotted as Mean ± SEM and are shown as the frequency of CD45⁺ population.

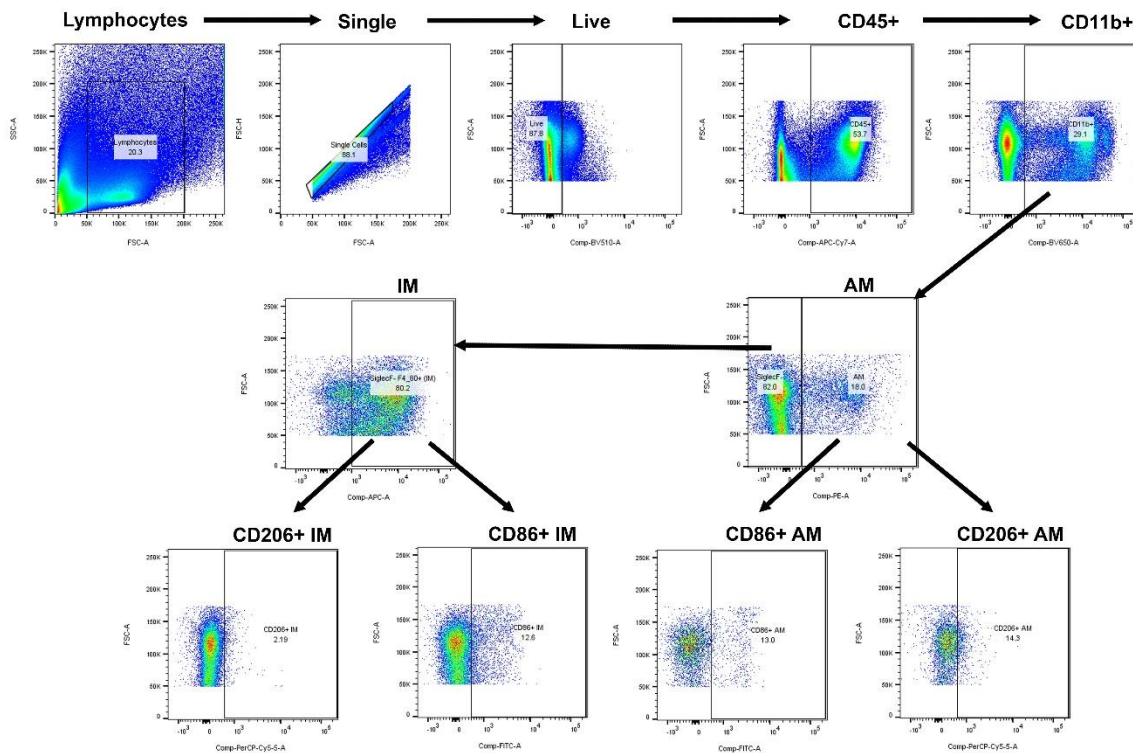
205 Statistical significance was calculated using a two-tailed student t-test considering unequal

206 distribution. The exact p-values are provided in the Source Data file. *<0.05. CFU stands for

207 colony-forming units. NS stands for non-significant change, p-value was >0.05. The experiment

208 was performed twice.

Fig S12.

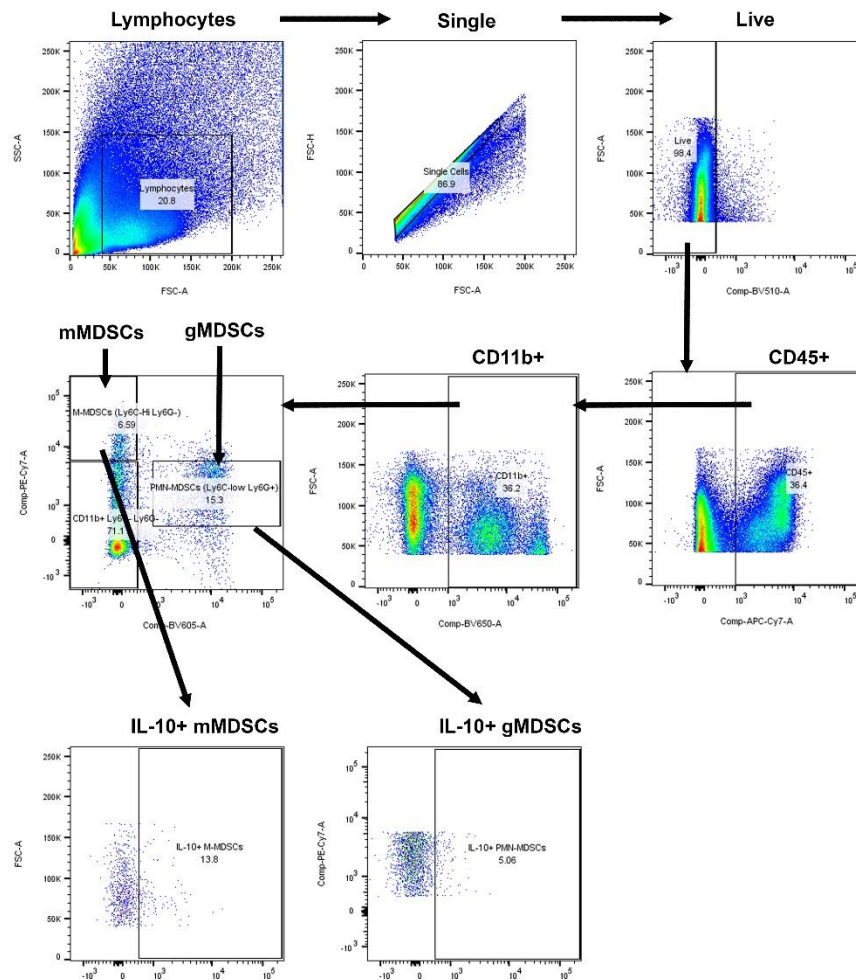


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211 **Fig S12. Two-dimensional gating strategies for flow cytometrical identification of myeloid**
 212 **cell subsets.** We excluded doublets and debris and gated on single live CD45⁺ cells. We
 213 identified myeloid cells (CD45⁺ CD11b⁺), alveolar macrophages (AM; CD45⁺ CD11b⁺
 214 SiglecF⁺), interstitial macrophages (IM; CD45⁺ CD11b⁺ SiglecF⁻ F4/80⁺), CD86⁺ alveolar
 215 macrophages (AM; CD45⁺ CD11b⁺ SiglecF⁺ CD86⁺), CD206⁺ alveolar macrophages (AM;
 216 CD45⁺ CD11b⁺ SiglecF⁺ CD206⁺), CD86⁺ interstitial macrophages (IM; CD45⁺ CD11b⁺
 217 SiglecF⁻ F4/80⁺ CD86⁺), CD206⁺ interstitial macrophages (IM; CD45⁺ CD11b⁺ SiglecF⁻ F4/80⁺
 218 CD206⁺). All flow antibodies were titrated to identify the concentration with maximum specificity
 219 coupled with minimum possible spillover. The gating strategy was defined using single-stain and
 220 FMO controls (for low-expression markers).

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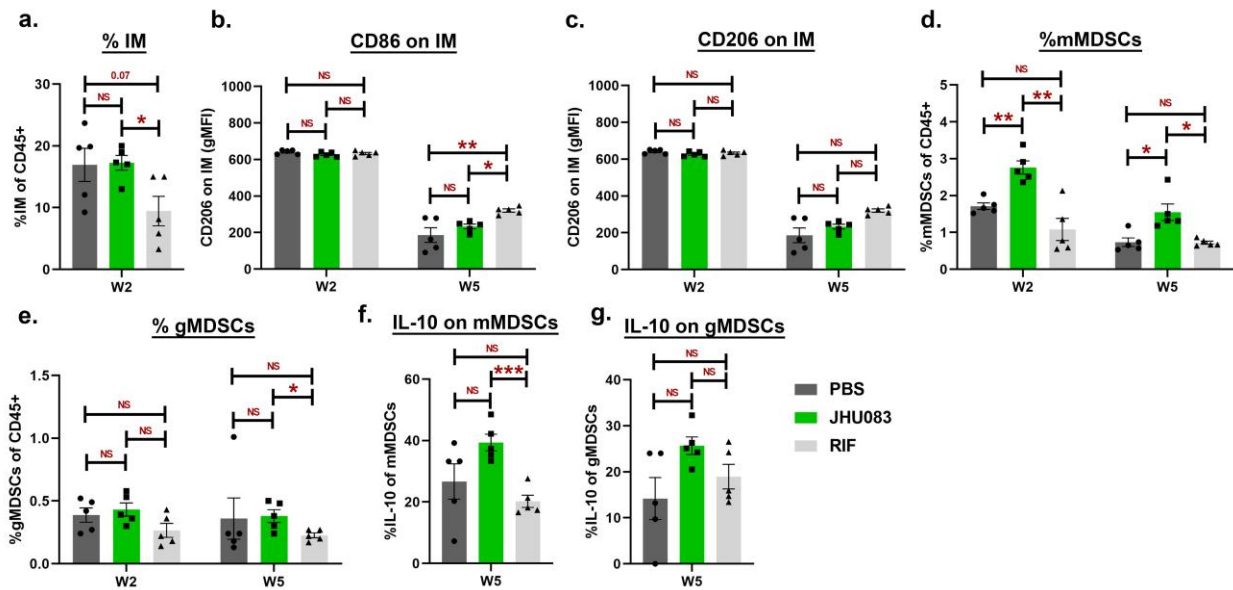
Fig S13.



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223 **Fig S13. Two-dimensional gating strategies for flow cytometrical identification of MDSC**
224 **subsets.** We excluded doublets and debris and gated on single live CD45⁺ cells. We identified
225 myeloid cells (CD45⁺ CD11b⁺), monocytic MDSCs (CD45⁺ CD11b⁺ Ly6G⁻ Ly6C^{High}), IL-10⁺
226 monocytic MDSCs (CD45⁺ CD11b⁺ Ly6G⁻ Ly6C^{High}, IL-10⁺), granulocytic MDSCs (CD45⁺
227 CD11b⁺ Ly6G⁺ Ly6C^{low}), granulocytic MDSCs (CD45⁺ CD11b⁺ Ly6G⁺ Ly6C^{low}, IL-10⁺). All
228 flow antibodies were titrated to identify the concentration with maximum specificity coupled with
229 minimum possible spillover. The gating strategy was defined using single-stain and FMO controls
230 (for low-expression markers).

Fig S14.

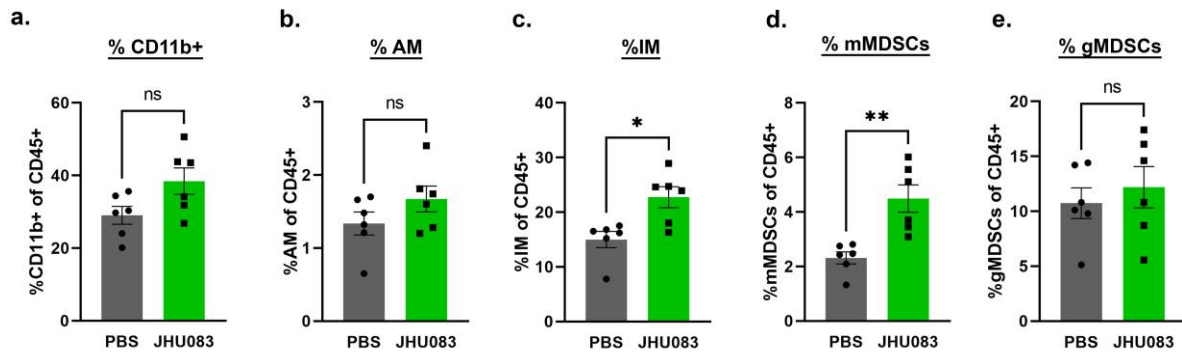


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232 **Fig S14. Effect of JHU083 treatment upon myeloid cell subsets.** As described in Fig 2a,
 233 *Mtb*-infected female 129S2 mice (n=5/group) were treated with JHU083 and RIF every day
 234 starting day 1 post-infection. The mice were sacrificed at week 2 and week 5, and the lungs were
 235 harvested. Single cell suspension of the lungs from all three groups were stained with
 236 appropriate antibodies and analyzed using multicolor-flow cytometry (n=5). Details are
 237 provided in “Methods” section. We found no difference in the (a) interstitial macrophages (IM),
 238 (b) CD86 expression on IM, (c) CD206 expression on IM, (d) monocytic myeloid-derived
 239 suppressor cells (mMDSCs), (e) granulocytic myeloid-derived suppressor cells (gMDSCs), (f)
 240 IL-10 expression upon mMDSCs, (g) IL-10 expression upon gMDSCs. The X-axis shows the
 241 timepoint at which the lungs were harvested for flow cytometry analysis. Data were plotted as
 242 Mean ± SEM and are shown as the frequency of CD45⁺ population. gMFI stands for geometric
 243 mean fluorescence intensity and was used to define the expression of the individual markers
 244 upon the indicated cell types. gMFI was mostly used for low abundance cell surface markers
 245 and transcription factors. Statistical significance was calculated using a two-tailed student t-test
 246 considering unequal distribution. The exact p-values are provided in the Source Data file.
 247 *<0.05, **<0.01, ***<0.001, ****<0.0001. CFU stands for colony-forming units. NS stands for
 248 non-significant change, p-value was >0.05. The experiment was repeated two times.

249

Fig S15.



250

251 **Fig S15. Effect of JHU083 administration upon myeloid cell subsets in uninfected 129S2**
252 **mice lungs at week 2.** Uninfected 129S2 mice (n=6/group) were treated with JHU083 every
253 day. The mice were sacrificed at week 2, and the lungs were harvested. Single cell suspensions
254 of the lungs from all three groups were stained with appropriate antibodies and analyzed using
255 multicolor-flow cytometry. We found no difference in the (a) CD11b+ myeloid cells and, (b)
256 alveolar macrophages (AM). There was increased frequency of (c) interstitial macrophages (IM)
257 and, (d) monocytic MDSC (mMDSCs) while (e) frequency of granulocytic MDSC (gMDSCs)
258 remained unaltered. Data were plotted as Mean \pm SEM and are shown as the frequency of
259 CD45⁺ population. Statistical significance was calculated using a two-tailed student t-test
260 considering unequal distribution. The exact p-values are provided in the Source Data file.
261 *<0.05. CFU stands for colony-forming units. NS stands for non-significant change, p-value
262 was >0.05. The experiment was performed twice.

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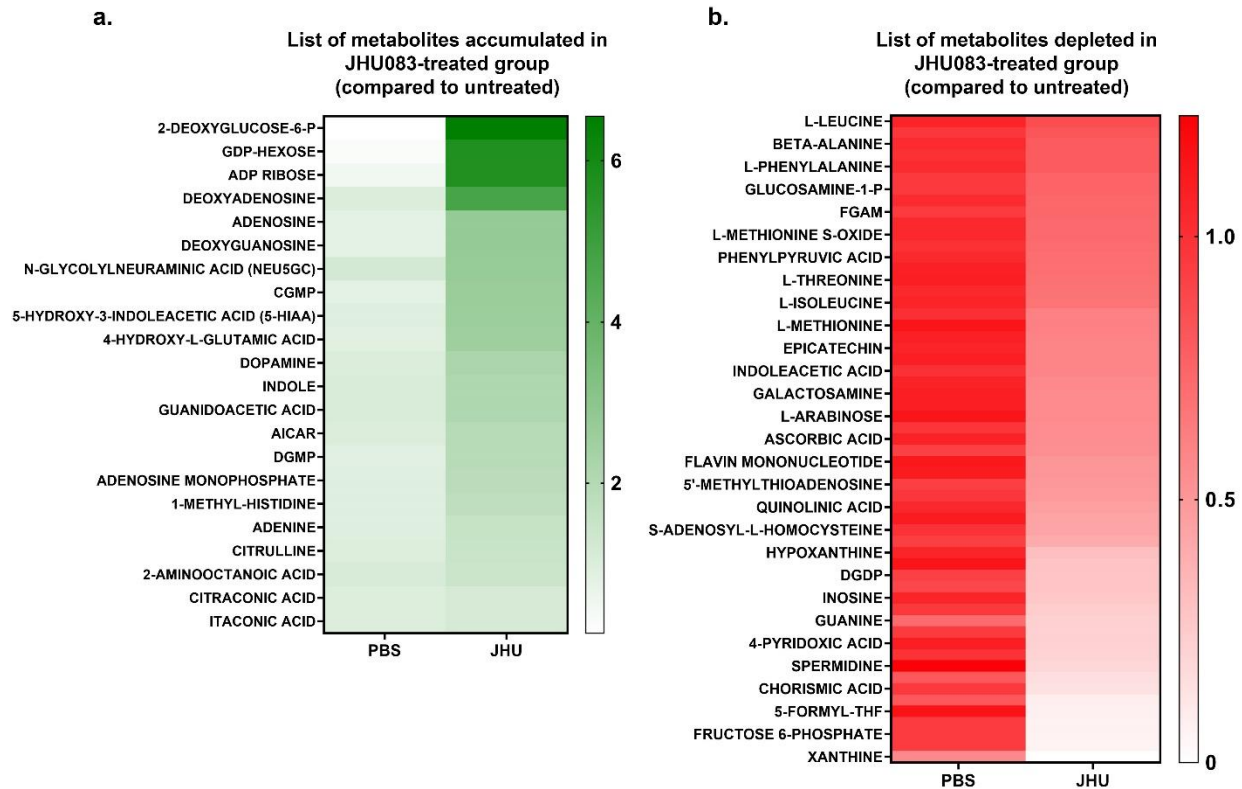
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Fig S16.



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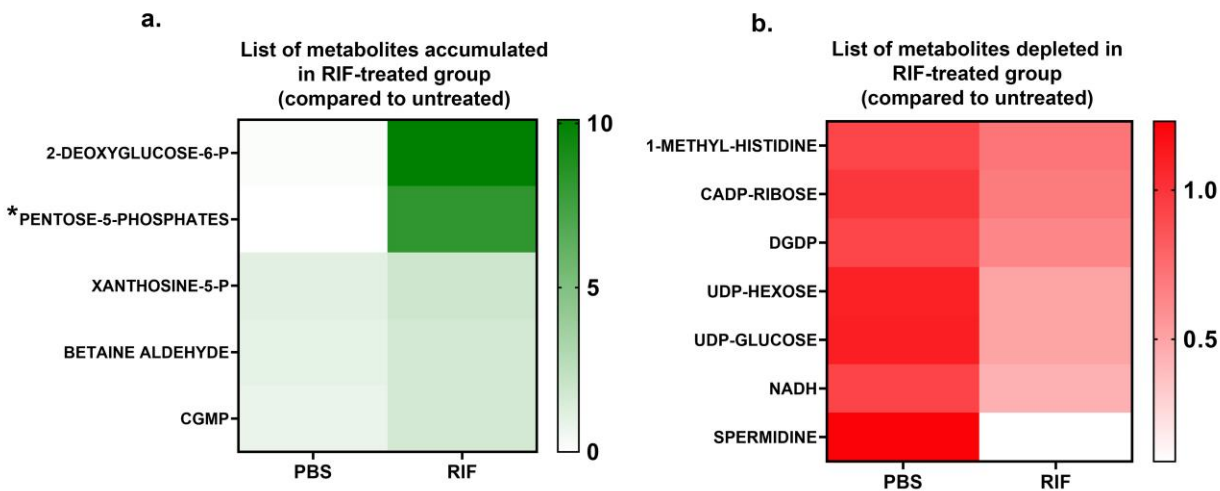
272 **Fig S16. Heatmap representing the top metabolites that were either (a) enriched or (b)**
 273 **depleted in the lungs harvested from the animals treated with JHU083 (right-lane)**
 274 **compared to untreated group (left lane).** The metabolites were methanol-extracted from the
 275 total lungs harvested at week 2 post infection and treatment (n=5/group). The metabolite
 276 abundance was normalized to the total lung tissue used for the extraction and untreated control.
 277 Statistical significance was calculated using a two-tailed student t-test considering unequal
 278 distribution. The exact p-values are provided in the Source Data file. The experiment was
 279 performed twice.

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281

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Fig S17.



283

284 **Fig S17. Heatmap representing the top metabolites that were either (a) enriched or (b)**
285 **depleted in the lungs harvested from the animals treated with RIF (right-lane) compared to**
286 **untreated group (left lane).** The metabolites were methanol-extracted from the total lungs
287 harvested at week 2 post infection and treatment (n=5/group). The metabolite abundance was
288 normalized to the total lung tissue used for the extraction and untreated control. The exact p-
289 values are provided in the Source Data file. Statistical significance was calculated using a two-
290 tailed student t-test considering unequal distribution. *Pentose-5-phosphates include arabinose-5-
291 phosphate, ribose-5-phosphate, and xylulose-5-phosphate. These three molecules have identical
292 MW and RT and hence, are indistinguishable based on mass-spectrometry. The experiment was
293 performed twice.

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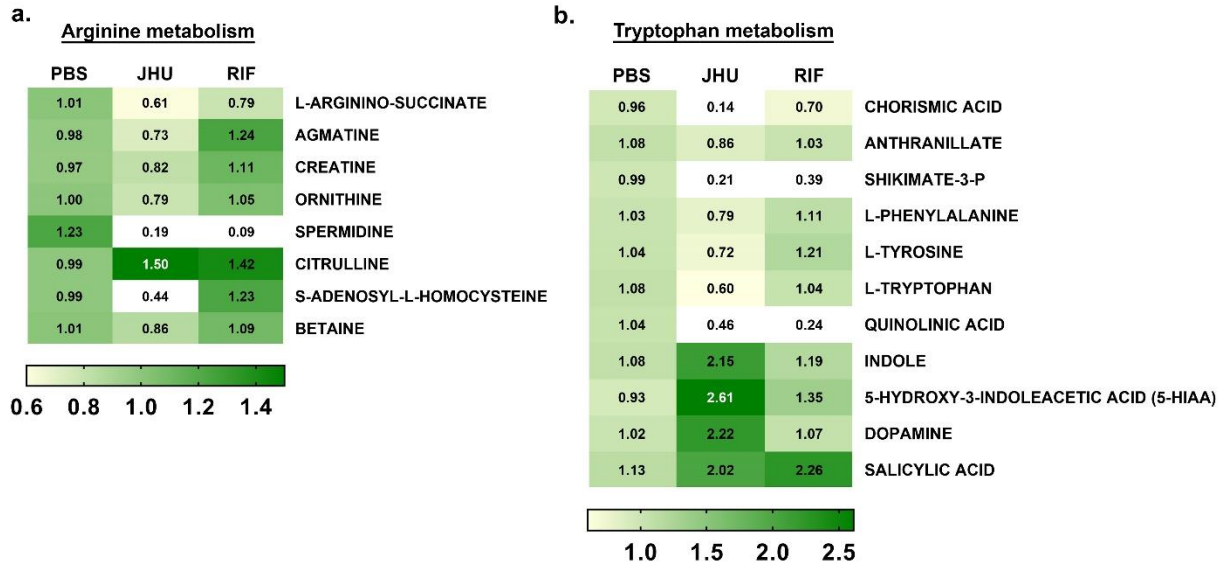
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Fig S18.



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302 **Fig S18. Heatmaps listing the metabolites that are affected by JHU083-treatment.** As
 303 described in Fig 2a, *Mtb*-infected 129S2 mice were treated with JHU083 and RIF every day
 304 starting day 1 post-infection (n=5/group). Mice were sacrificed at week 2, the lungs were
 305 harvested, and total metabolites were methanol extracted as described in “Methods”. The total
 306 metabolites were normalized to the tissue weight and then to the untreated controls. We detected
 307 changes in the level of metabolites belonging to both **(a)** arginine and, **(b)** tryptophan
 308 metabolism pathways. Statistical significance was calculated using a two-tailed student t-test
 309 considering unequal distribution. The values in the cells correspond to the median value of the
 310 dataset. The experiment was performed once. The metabolomics data is provided as a separate
 311 excel sheet with normalized values along with the p-value calculations. The exact p-values are
 312 provided in the Source Data file. The experiment was performed twice.

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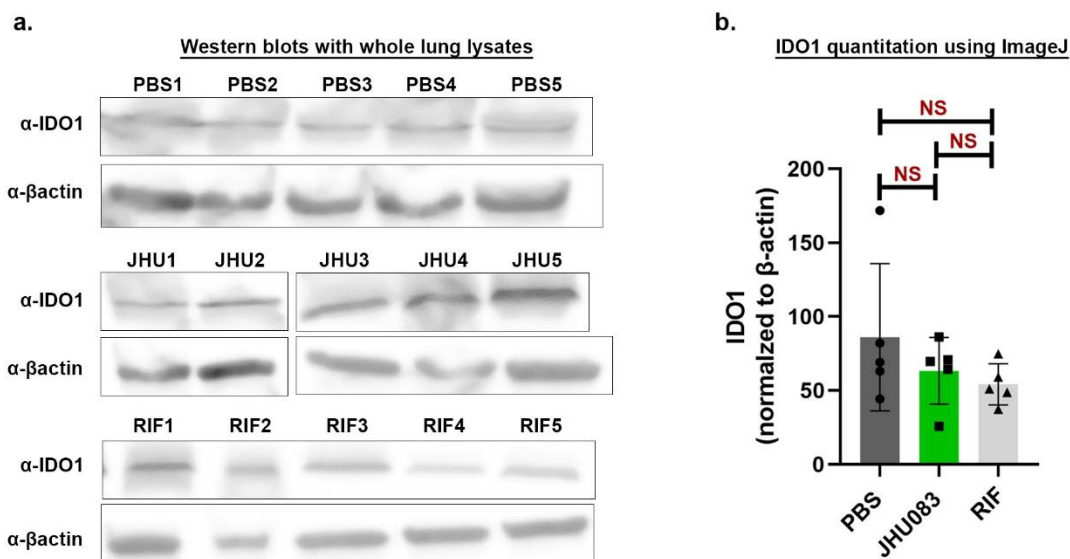
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Fig S19.



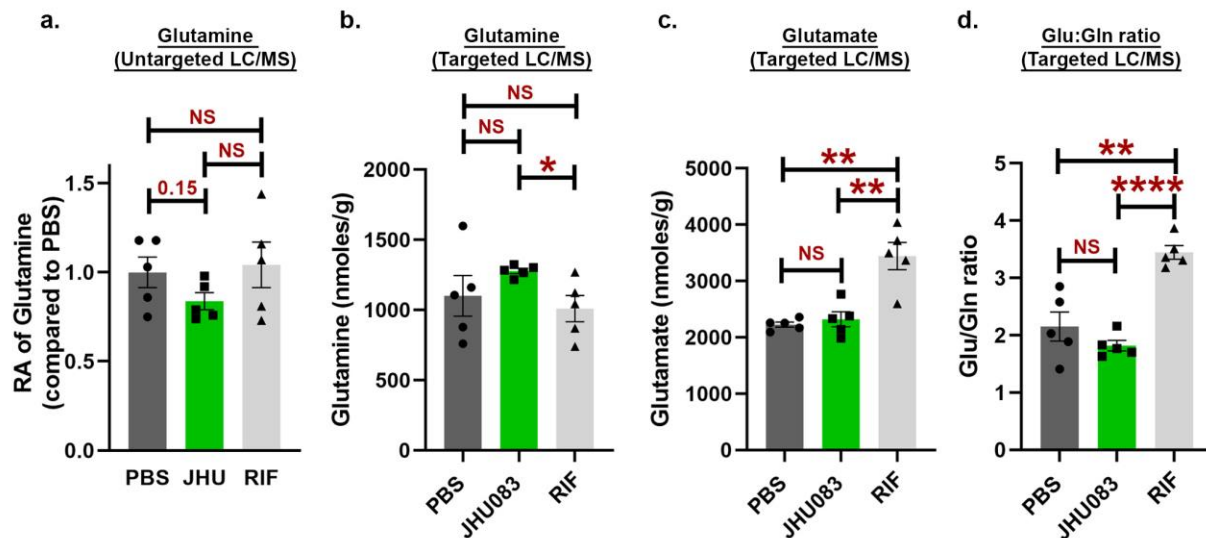
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319 **Fig S19. JHU083 administration does not alter the IDO1 levels in *Mtb*-infected lungs. As**
320 **described in Fig 2a**, six to ten weeks old 129S2 female mice (n=5/group) were aerosol infected
321 with ~200-300 CFU of *Mtb H37Rv*. The mice were treated with JHU083 or RIF via oral route
322 one day after infection. 1 mg/Kg JHU083 was given daily for the first five days, and then the
323 dose was reduced to 0.3 mg/Kg daily (M-F). The mice were sacrificed at week 2 post-
324 infection/treatment. The lungs were harvested and homogenized to prepare whole lung lysate.
325 Lung lysate corresponding to 10 μ g protein was loaded per lane, electrophoresed, transferred to
326 PVDF membrane. The level of IDO1 and β -actin was determined using specific primary
327 antibody specified in the materials and methods section. (a) Western blot showing the IDO1 and
328 β -actin levels in whole lung lysate from all three treatment groups. (b) Quantitation of IDO1
329 levels in the whole lung lysate using ImageJ based densitometry. Data were plotted as Mean \pm
330 SEM. Statistical significance was calculated using a two-tailed student t-test considering
331 unequal distribution. The exact p-values are provided in the Source Data file. CFU stands for
332 colony-forming units. NS stands for non-significant change, p-value was >0.05. The experiment
333 was repeated twice.

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Fig S20.



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338 **Fig S20. JHU083 treatment does not alter glutamine level in the *Mtb*-infected lungs.** As
 339 described in Fig 2a, *Mtb*-infected 129S2 mice (n=5/group) were treated with JHU083 and RIF
 340 every day starting day 1 post-infection. Mice were sacrificed at week 2, the lungs were
 341 harvested, and total metabolites were methanol extracted as described in “Methods”. The total
 342 metabolites were normalized to the tissue weight and then to the untreated controls. We detected
 343 no change in the level of (a) glutamine as per the untargeted metabolomics. We also observed
 344 no change in the levels of (b) glutamine, (c) glutamate and (d) glutamate and glutamine ratio,
 345 quantified as per targeted metabolomics. Data were plotted as Mean \pm SEM. Statistical
 346 significance was calculated using a two-tailed student t-test considering unequal distribution.
 347 The exact p-values are provided in the Source Data file. * <0.05 , ** <0.01 , *** <0.001 ,
 348 **** <0.0001 . CFU stands for colony-forming units. NS stands for non-significant change, p-
 349 value was >0.05 . The experiment was repeated twice.

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354 **LEGEND FOR TABLE**

355 **Supplementary Data 1:** As described in Fig 2a, *Mtb*-infected 129S2 mice (n=5/group) were
356 treated with JHU083 and RIF daily starting day 1 post-infection. Mice were sacrificed at weeks
357 2 and 5, the lungs were harvested, and total metabolites were extracted with methanol as
358 described in “Methods.” Sheet 1, labeled “Sample details,” includes the description of all the
359 samples that were used for the experiment. Sheet 2, labeled “Week 2_Normalized data” lists all
360 the metabolites that were identified in the *Mtb*-infected lungs week 2 post-infection/treatment.
361 The metabolite abundances were normalized to the tissue weight and then to the untreated
362 controls. Data were plotted as Mean \pm SEM. Statistical significance was calculated using a two-
363 tailed student t-test considering unequal distribution. The exact p-values are provided in the
364 table. Sheet 3, labeled “Week 5_Normalized data” lists all the metabolites that were identified in
365 the *Mtb*-infected lungs, week 5 post-infection/treatment. The metabolite abundances were
366 normalized to the tissue weight and then to the untreated controls. Data were plotted as Mean \pm
367 SEM. Statistical significance was calculated using a two-tailed student t-test considering
368 unequal distribution. The exact p-values are provided in the table. Sheet 4, labeled “All
369 metabolites” lists the metabolites that were expected to give a peak on the MS spectra. The
370 values represent the area under the curve for individual metabolite peaks, present in *Mtb*-infected
371 lungs weeks 2 and 5 post-infection/treatment. Zero indicates that we could not detect the
372 specified metabolite peak in the corresponding sample. Sheet 5, labeled “Metabolites with signal
373 peak” lists all the metabolites that were detected in the *Mtb*-infected lungs weeks 2 and 5 post-
374 infection/treatment. The values represent the area under the curve for individual metabolite
375 peaks, present in *Mtb*-infected lungs weeks 2 and 5 post-infection/treatment. on the
376 corresponding mass spectra. Sheet 6, labeled “Metabolites with no signal peak” lists all the
377 metabolites that could not be detected in the *Mtb*-infected lungs, weeks 2 and 5 post-
378 infection/treatment. The values represent the area under the curve for individual metabolite peaks
379 observed on the corresponding mass spectra.

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