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Supporting Information

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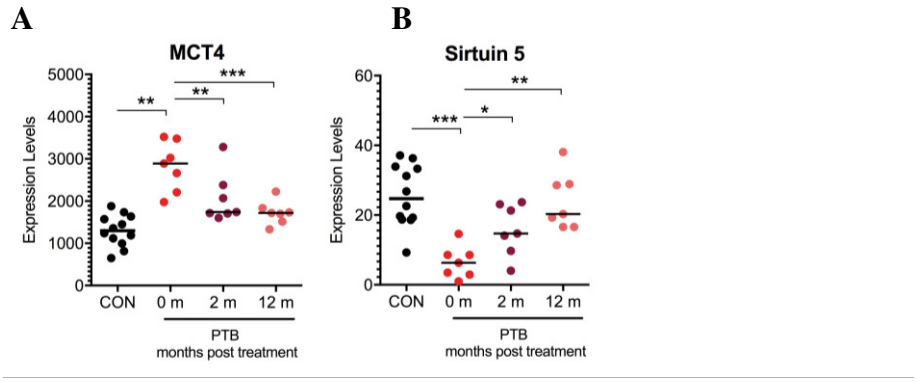
Rewiring cellular metabolism via the AKT/mTOR pathway contributes to host defence against *Mycobacterium tuberculosis* in human and murine cells

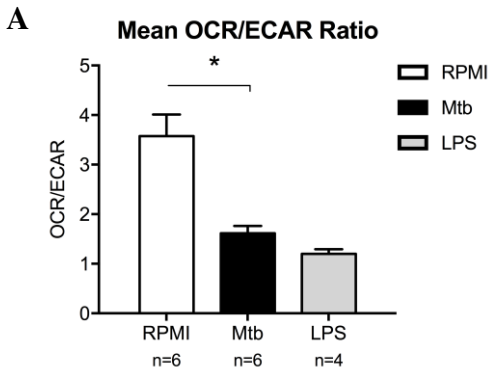
Supporting Information Figure 1



Supporting Information Figure 1. Transcriptional Regulation of Glucose Metabolism

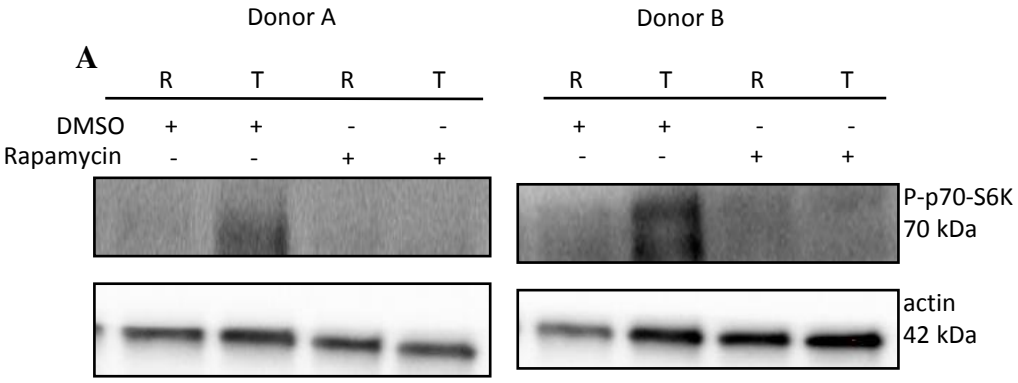
(A-B) Data are represented as mean scatter plots for gene expression of MCT4 and Sirtuin 5 in LTBI and PTB individuals in the South African validation cohort of Berry et al. [13] and in controls and PTB patients on 0 m, 2 m or 12 m of treatment with anti-tubercular drugs. Symbols represent individual samples and data are shown as means. Means were compared using the Mann-Whitney U test. *p<0.05, **p<0.01, ***p<0.001.

Supporting Information Figure 2.



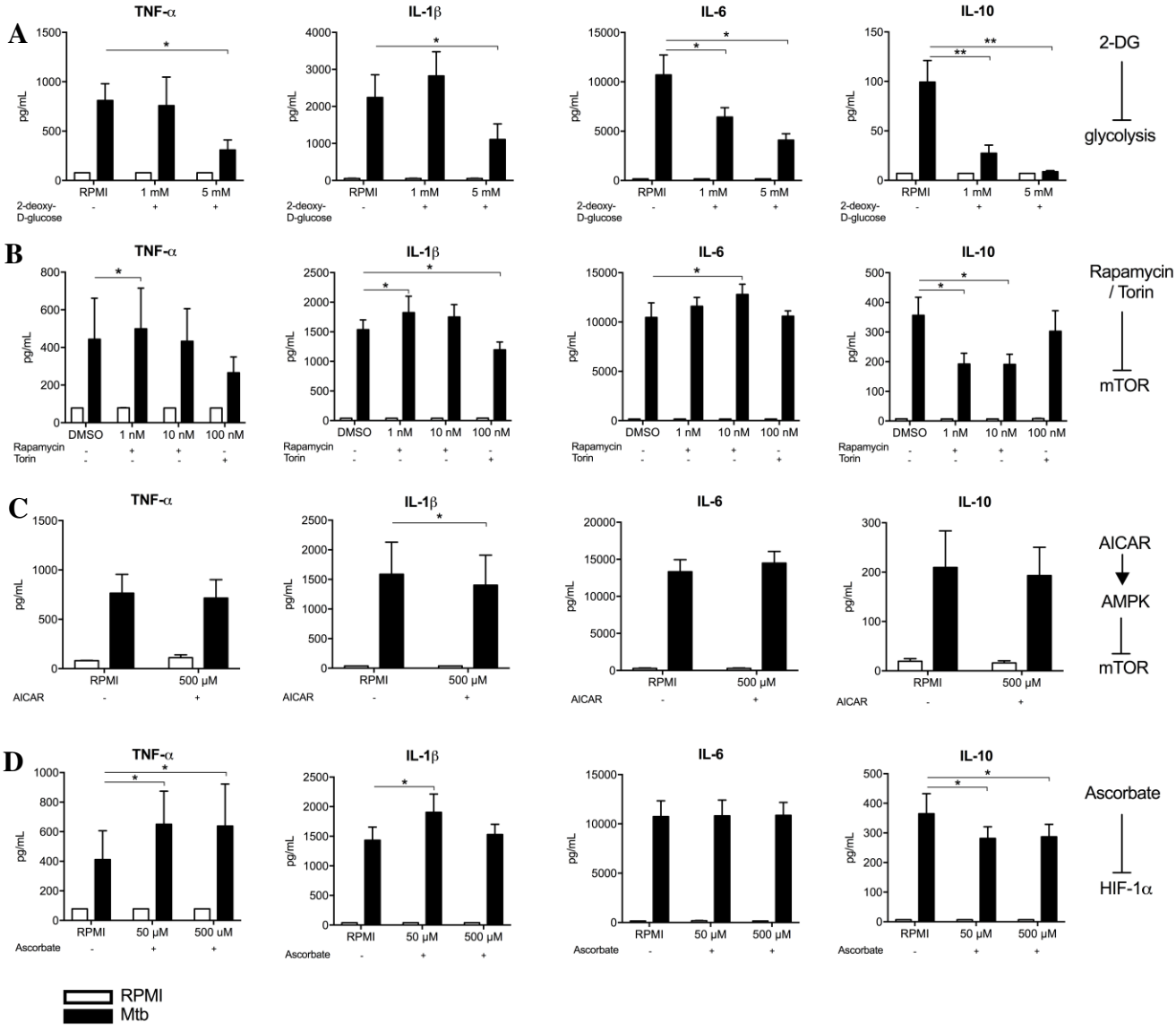
Supporting Information Figure 2. Physiology of the Metabolic Response to Mtb Stimulation
(A) CD14+ monocytes were stimulated for 24 h with Mtb lysate (n=6) and LPS (n=4) and the resulting ECAR and OCR rates were determined using the Seahorse metabolic analyser. Three baseline measurements were determined. Data are shown as means ± SEM of n = 4 to 6 from a single experiment. Means were compared using the Wilcoxon signed-rank test (*$p<0.05$).

Supporting Information Figure 3



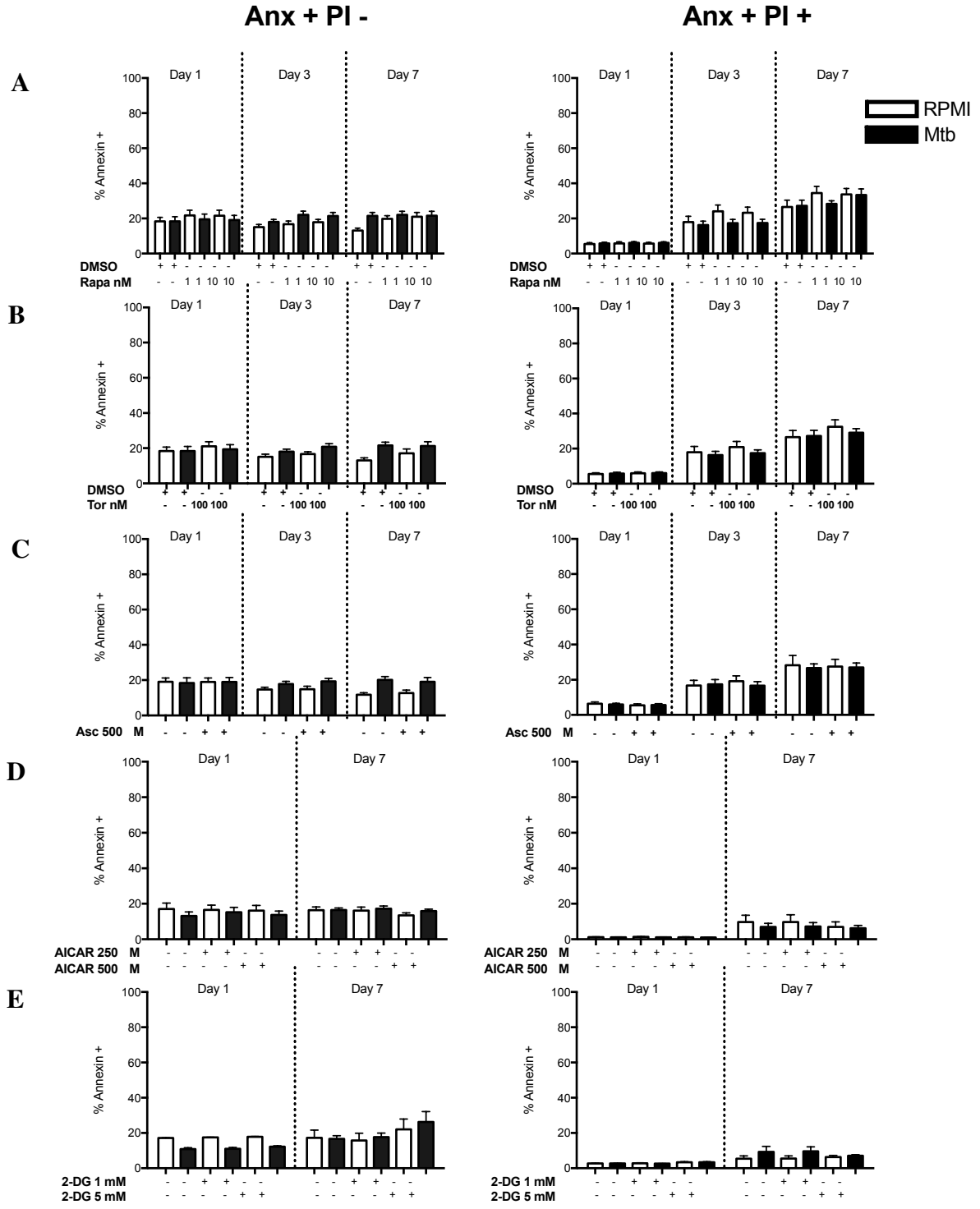
Supporting Information Figure 3. Induction of Glycolysis is Mediated by the AKT-mTOR Pathway
(A) CD14+ monocytes pre-incubated with DMSO (vehicle control) or rapamycin (mTOR inhibitor) were stimulated with RPMI (R) or Mtb lysate (T) for 2 h. Cell lysates were analysed by Western blot for phosphorylation of p70-S6K and actin levels using specific antibodies. Blots from two donors are shown.

Supporting Information Figure 4.



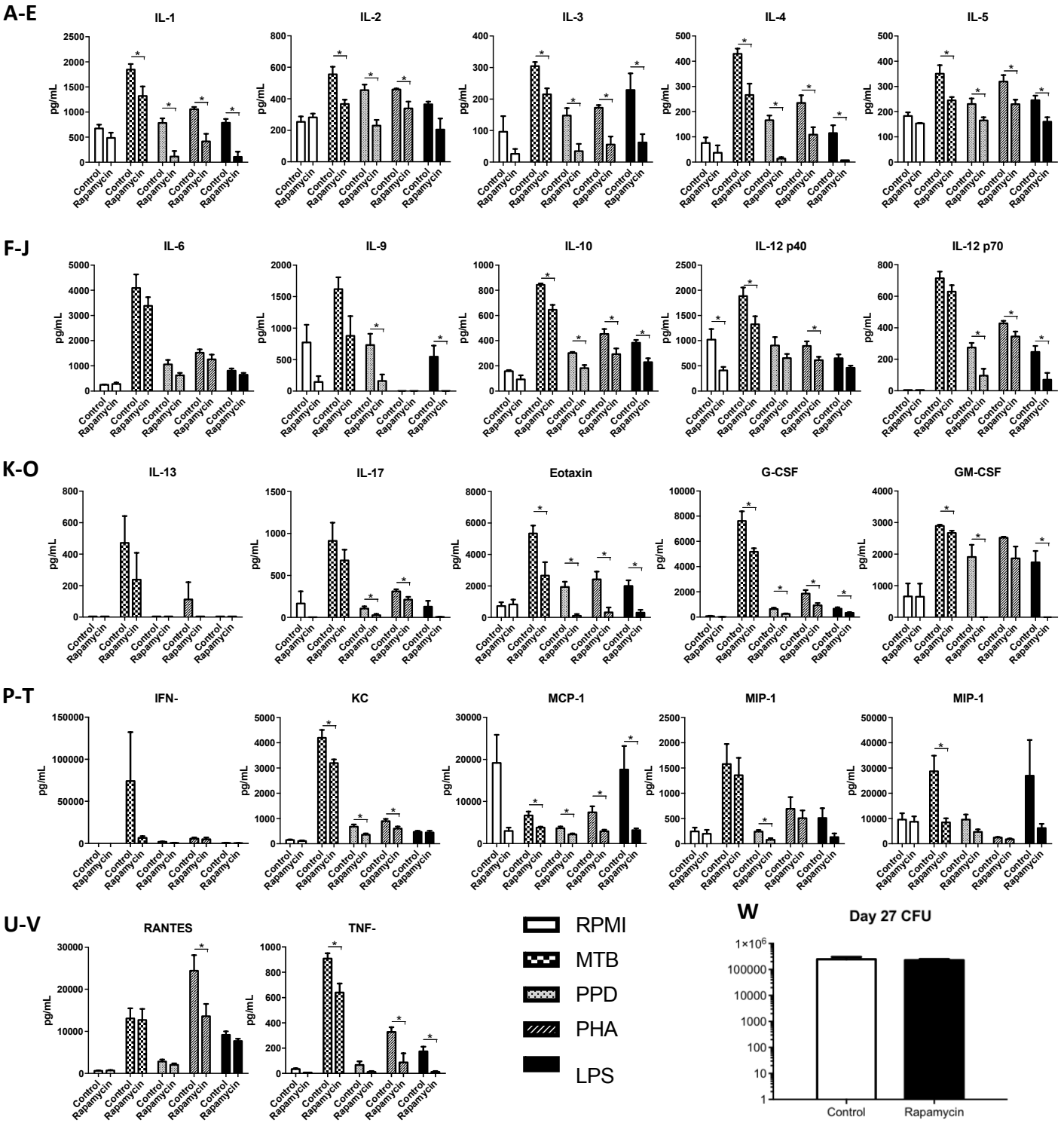
Supporting Information Figure 4. Regulation of Monocyte-Derived Cytokines by Aerobic Glycolysis (A-D) PBMCs were pre-incubated with DMSO (vehicle control) or (A) 1 mM or 5 mM 2DG, (B) 1 nM or 10 nM rapamycin, 100 nM torin, (C) 500 µM AICAR or (D) 50 µM or 500 µM ascorbate or for 1 h prior to stimulation with Mtb lysate. TNF-α, IL-1β, IL-6 and IL-10 levels were measured from culture supernatants by ELISA. Data are shown as means ± SEM of n = 6 to 9 pooled from three independent experiments. Means were compared using the Wilcoxon signed-rank test (*p<0.05, **p<0.01).

Supporting Information Figure 5.



Supporting Information Figure 5. Effects of Metabolic Pathway Inhibitors on Cell Death
(A–E) PBMCs were pre-incubated with DMSO (vehicle control) or (A) 1 mM or 5 mM 2DG, (B) 1 nM or 10 nM rapamycin, 100 nM torin, (C) 500 µM AICAR or (D) 50 µM or 500 µM ascorbate or for 1 h prior to stimulation with Mtb lysate for 1, 3 or 7 d after which the cells were harvested and stained with an Annexin-V / PI stain to determine levels of cell death. Data are shown as means ± SEM of n = 4 to 6 pooled from three independent experiments. Means were compared using the Wilcoxon signed-rank test.

Supporting Information Figure 6.



Supporting Information Figure 6. In vivo Effects of Rapamycin on Ex vivo Splenocyte Restimulation (A–W) C57/BL6 mice were treated with rapamycin or vehicle (mixed PBS/100% EtOH) from 1 day prior to aerosol infection until 28 d post-infection. Mice were euthanized. Splenocytes were harvested and re-stimulated with Mtb lysate, PPD, PHA or LPS for 6 d after which a bead-based immunoassay was performed (A-U). Lung homogenates were harvested and CFUs plated and counted after three weeks (W). Data are shown as means ± SEM of n =6 samples from a single experiment. Means were compared using the Mann–Whitney U test (*$p<0.05$).