Supplementary Material

Humanized mouse model mimicking pathology of human tuberculosis for *in vivo* evaluation of drug regimens

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**Supplementary Figure 1. Human immune cell reconstitution in peripheral blood and lungs.** (A) Representative dot plots showing flow cytometric gating strategy applied to identify human leukocyte populations (FSC/SSC leukocytes → human CD45+ leukocytes → human CD3+ T cells/human CD19+ B cells → human CD4+ T cells and human CD8+ T cells). (B) Percentages of immune cells in peripheral blood of HIS-NSG mice 10 weeks post-transplantation (*n* = 23) reconstituted from the same donor are shown. Each symbol represents an individual mouse. Frequencies of CD3+ T cells and CD19+ B cells within human CD45+; and frequencies of CD4+ and CD8+ T cells amongst CD3+ cells are indicated. Horizontal bars represent means. (C, D) Representative tissue sections of HIS-NSG mice lungs 10 weeks post-transplantation. (C) Hematoxylin and Eosin (400X, scale bar = 50 µm). (D) Immunostaining with human CD68 (oval indicates parenchymal macrophages, box indicates alveolar macrophages), human CD15, human CD3 and human CD20. Black arrows indicate positive nuclear staining of CD15+ neutrophils and CD20+ B cells (400X, scale bar = 50 µm).

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**Supplementary Figure 2. HIS-NSG mice are susceptible to aerosolized Mtb.** (A)Bacterial burden in spleen, liver, kidney and bone marrow at day 35 p.i. Data were pooled from 2 independent experiments, mean ± SEM, Mann-Whitney U test, ns = no significance, \* *P* < 0.05. (B) Images from macroscopic (photographs) analysis of lung lesions in HIS-NSG, PBS-NSG and BL/6 lungs at day 35 p.i.. Data are representative of two independent experiments.



**Supplementary Figure 3. HIS-NSG mice develop splenic lesions upon Mtb infection.** (A) Macroscopic (photographs) and (B) microscopic analysis (Hematoxylin and Eosin 50X, scale bar = 500 µm) in representative HIS-NSG, PBS-NSG and BL/6 spleens at day 35 p.i. was carried out in addition to (C) mycobacterial identification (Ziehl-Neelsen 100X, scale bar = 200 µm). Data are representative of two independent experiments.

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**Supplementary Figure 4. Flow cytometric gating strategy of human innate and adaptive immune cells in HIS-NSG lungs.** Representative dot plots showing flow cytometric gating strategy applied to identify human innate and adaptive immune cells (FSC/SSC leukocytes→ human CD45+ granulocytes → human CD33+CD66+a/c/e/b neutrophils; FSC/SSC leukocytes→ human CD45+ monocytes→ human CD33+CD14+ monocytes/macrophages; FSC/SSC leukocytes→ human CD45+ monocytes→ human CD33+CD11c+ dendritic cells; FSC/SSC leukocytes→ human CD45+ lymphocytes → human CD3+ T cells and CD19+ B cells; human CD3+ T cells→ CD4+ T cells and CD8+ T cells; human CD19+ B cells → human CD20+ B cells).

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**Supplementary Figure 5. Human biomolecule expression in Mtb infected HIS-NSG lungs.** Human biomolecules were determined in lung homogenates of HIS-NSG mice. Bar graphs showing protein levels at 14, 21 and 28 days p.i. *n* = 4 - 5 mice per time point, Mean ± SEM, Kruskal-Wallis/Dunn’s multiple comparisons test. n.d., not detected/below detection limit, dotted line: detection limit; \*\* *P* < 0.01, \* *P* < 0.05.



**Supplementary Figure 6. Changes in HIS-NSG lung architecture as TB disease progresses.** Entire left lung lobes collected from representative HIS-NSG mice at 14, 21 and 28 days post Mtb infection show increased loss of spongy architecture as TB progressed. Hematoxylin and Eosin stain of tissue slide scans; Hematoxylin and Eosin stain 100X, scale bar = 200 µm.