BCG vaccination reduces the rate of Mycobacterium tuberculosis dissemination between murine lungs

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Supplemental Information

ID model assuming intermediate tissue is blood

In the ID model, if blood is treated as an intermediate tissue, Mtb neither replicates nor dies in this compartment. Instead, blood will serve solely as a transport medium, carrying Mtb from Lung 1 to Lung 2. Thus, the ID model equations will be modified as mentioned below:

$$\begin{aligned}
\frac{dL_1(t)}{dt} &= (1-\epsilon_r)r_L(t)L_1(t) - \frac{\delta(t)}{1-\epsilon_\delta}L_1(t) - (1-\epsilon_m)m_1L_1(t), && \text{(S.1)}\\
\frac{dT(t)}{dt} &= (1-\epsilon_{m_1})m_1L_1(t) - m_2(1-\epsilon_m)T(t), && \text{(S.2)}\\
\frac{dL_2(t)}{dt} &= \theta(L_2-1)\Big[(1-\epsilon_r)r_L(t)L_2(t) - \frac{\delta(t)}{1-\epsilon_\delta}L_2(t)\Big] + m_2(1-\epsilon_m)T(t), && \text{(S.3)}
\end{aligned}$$

Modeling Mtb dynamics in the whole lung

To evaluate how BCG vaccination impacts early Mtb dynamics in mice infected with an ULD or conventional dose (**CD**) of Mtb, we modified our previously proposed mathematical model[60] to include dynamics of immune response E and suppression of Mtb replication by immunity[61]:

$$\frac{\mathrm{d}B}{\mathrm{d}t} = \frac{(r+\delta)B}{1+h^nE(t)^n} - \delta B, \tag{S.4}$$

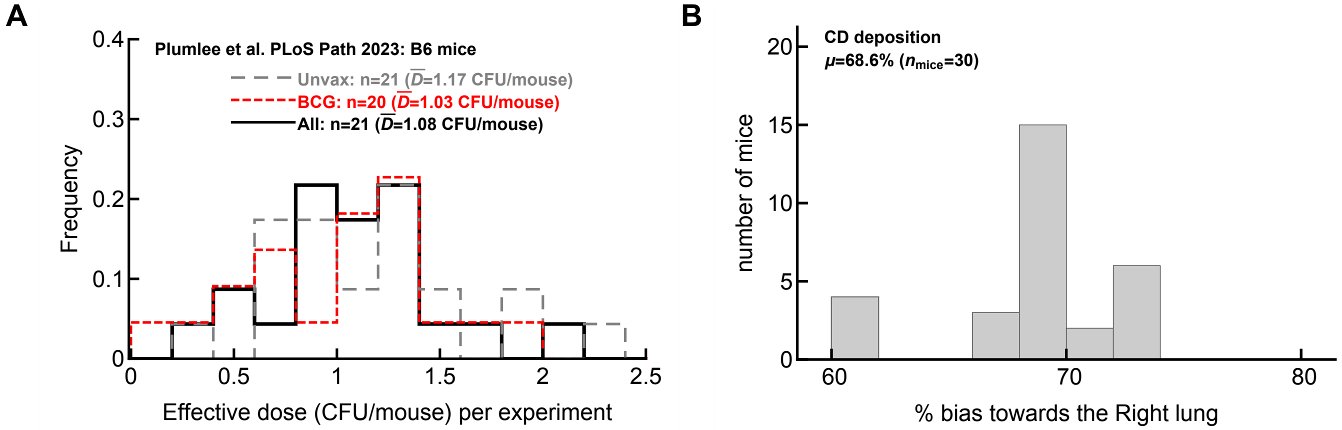
where B is the number of bacteria in the lung, r and δ are the rates of Mtb replication and death, respectively, h is the level of immunity at which immune-mediated suppression is half of its maximum, and $E(t) = \mathrm{e}^{\rho t}$ where ρ is the expansion of the immune response. We characterize kinetics of the immune response by time $T_E = -\ln(h)/\rho$ that is the time when immunity reaches 50% of its suppressive capacity. We estimated $\rho \approx 0.4-0.6/\text{day}$ for Mtb-specific CD4 T cell response (Batabyal et al. (in prep)) but specific value of ρ is typically not very critical for the model behavior as long as it is sufficiently large[61]. In the model, infection starts with B_0 bacteria.

Calculation of effective dose in ULD-infected mice

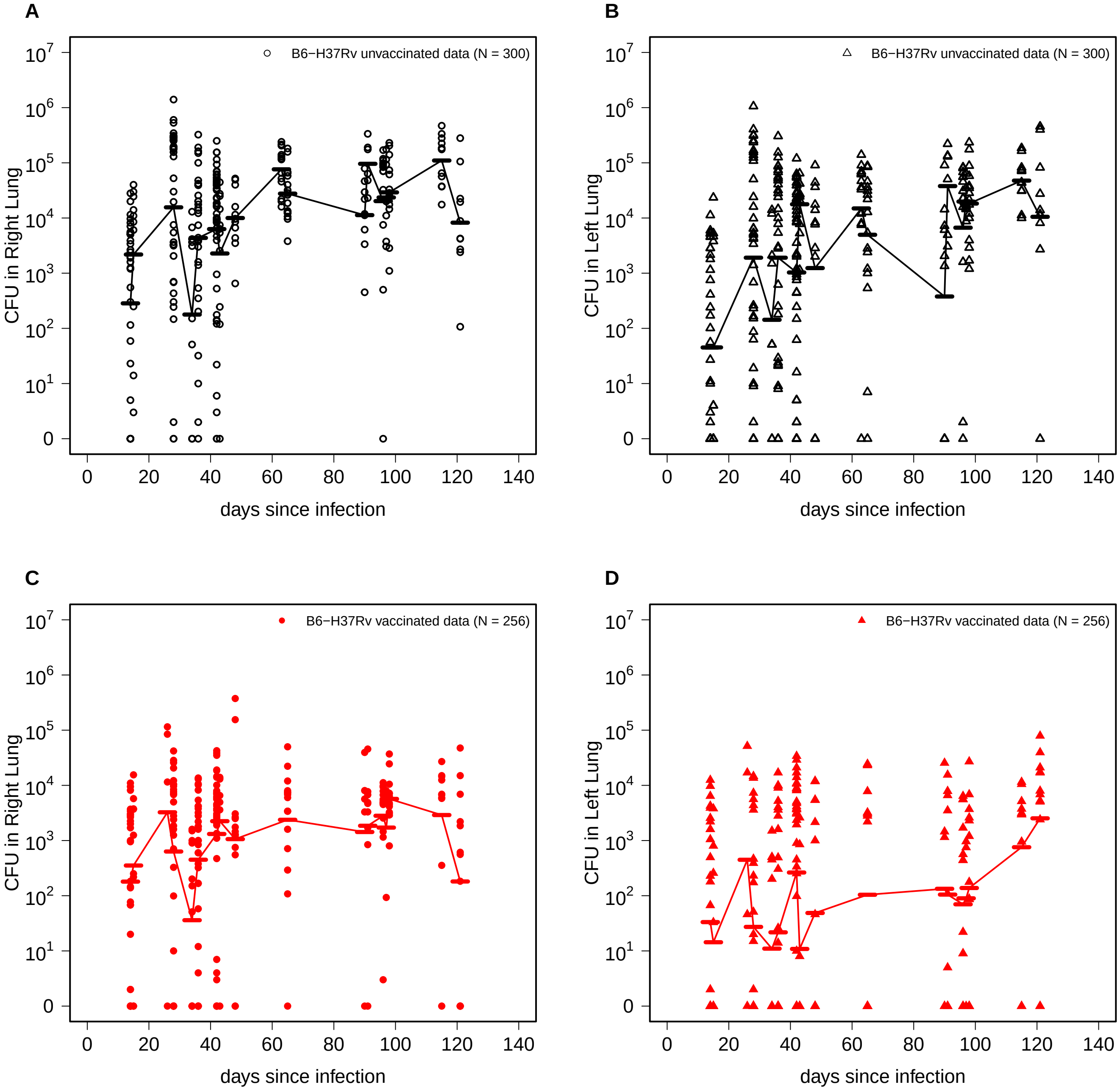
Let f_0 be the fraction of mice that remain uninfected, and D be the average dose of Mtb infection. Since Mtb infection is random, the number of bacteria inhaled by individual mice will follow a Poisson distribution[41,62]. Then, the probability that a mouse receives k bacteria is,

$$P(k) = \frac{D^k e(-D)}{k!}, \tag{S.5}$$

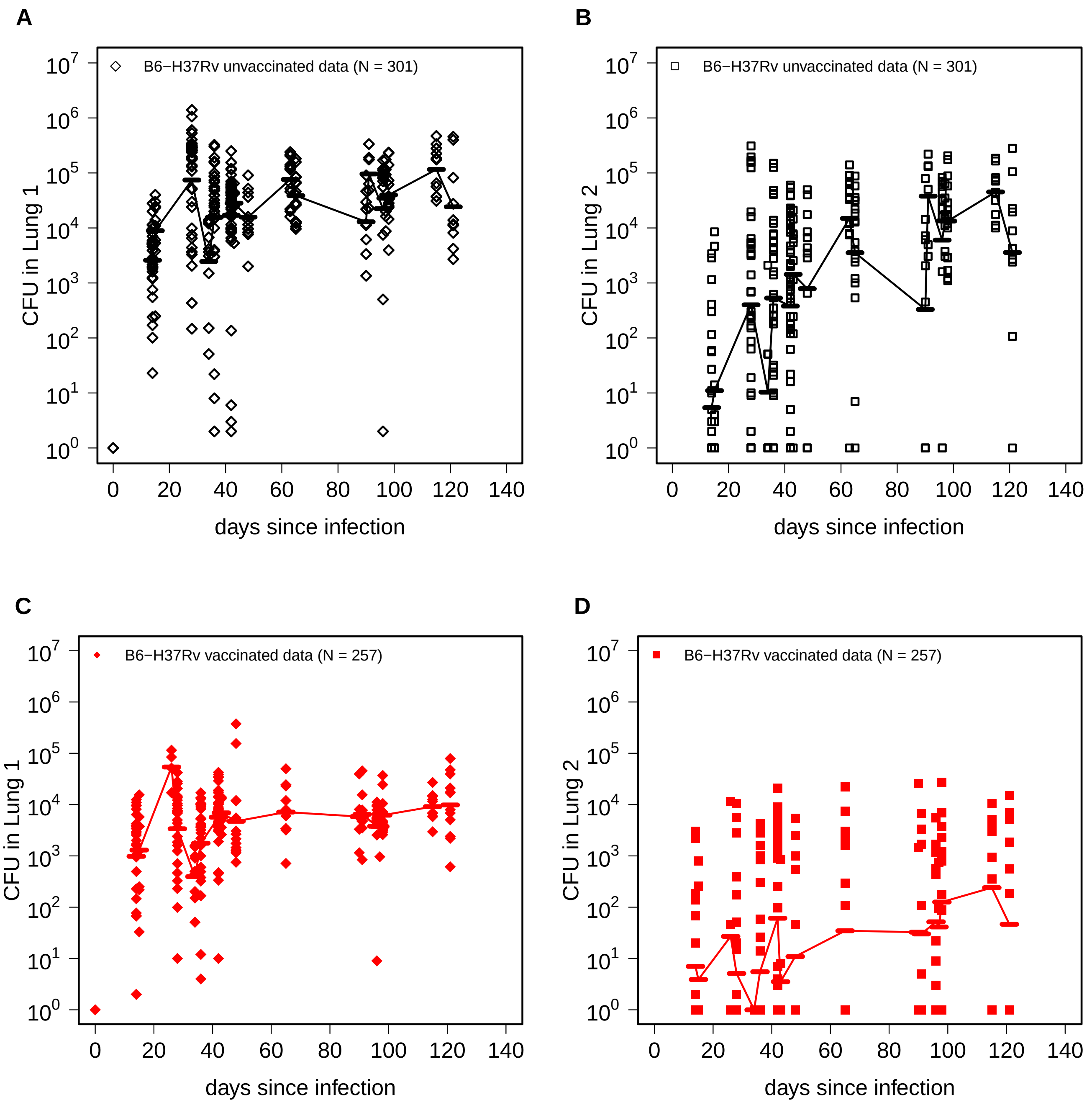
where the probability of no infection is: $P(0) = e^{-D} = f_0$. Thus, the effective dose $D = -\ln(f_0)$. Note that the actual dose that mice are exposed to may be higher because of additional death rate of Mtb during the infection (**eqn. (3)**).



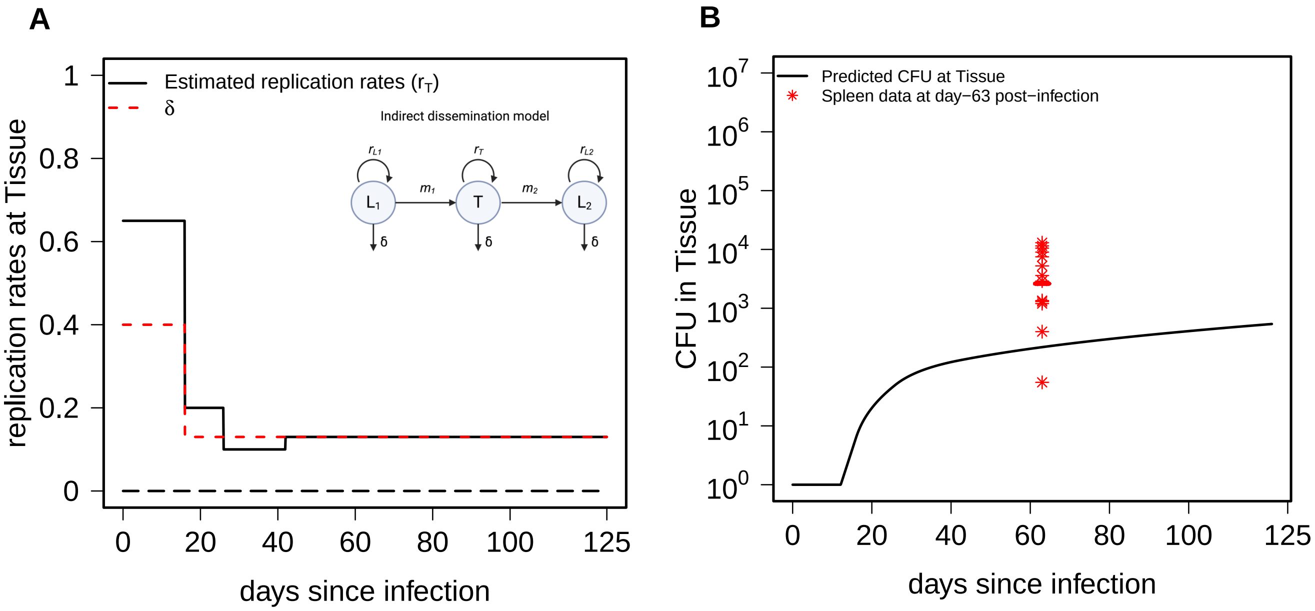
Supplemental Figure S1: Distribution of estimated dose in mice exposed to ultra-low dose of Mtb and bias of initial infection towards the right lung of mice. **A**: For every experiment of $n = 21$ experiments of Plumlee *et al.*[39] we calculated the proportion of uninfected mice for unvaccinated group, BCG-vaccinated group, or all mice in the experiment (f_0) and then calculated the average effective deposition dose using a Poisson distribution $D = -\ln(f_0)$ (ref[41]). In cases when all mice were infected, the dose could not be calculated, and such an experiment was excluded from the calculations. (Note a different number of experiments n used for calculation of the dose.) **B**: We calculated the bias in the CFUs recovered from right ($\mathrm{CFU_{RL}}$) or left ($\mathrm{CFU_{LL}}$) lung 1 day after Mtb infection of B6 mice with a conventional dose[41]; the bias was calculated for each mouse as $b = \mathrm{CFU_{RL}}/(\mathrm{CFU_{RL}} + \mathrm{CFU_{LL}})$.



Supplemental Figure S2: Bacterial burden at right lung and left lung of ultra-low dose infected mice Plumlee *et al.*[39]. Mice were exposed to an ultra-low dose of Mtb H37Rv and bacterial burden at right lung (RL) and left lung (LL) was measured at different days post-infection after sacrificing the mice. Plumlee *et al.*[39] conducted multiple experiments, categorizing the mice into two cohorts: unvaccinated and BCG-vaccinated, comprising a total of (300 + 256) mice (excluding entries marked as ‘NA’ and the mice with no detectable infection i.e., mice with zero CFUs in both lungs or mice in which CFU was only measured in the whole lung without subdivision into right and left lungs). (A) and (B) present the CFU counts in the RL and LL of unvaccinated mice across all experiments, while (C) and (D) show the corresponding CFU data for BCG-vaccinated mice.



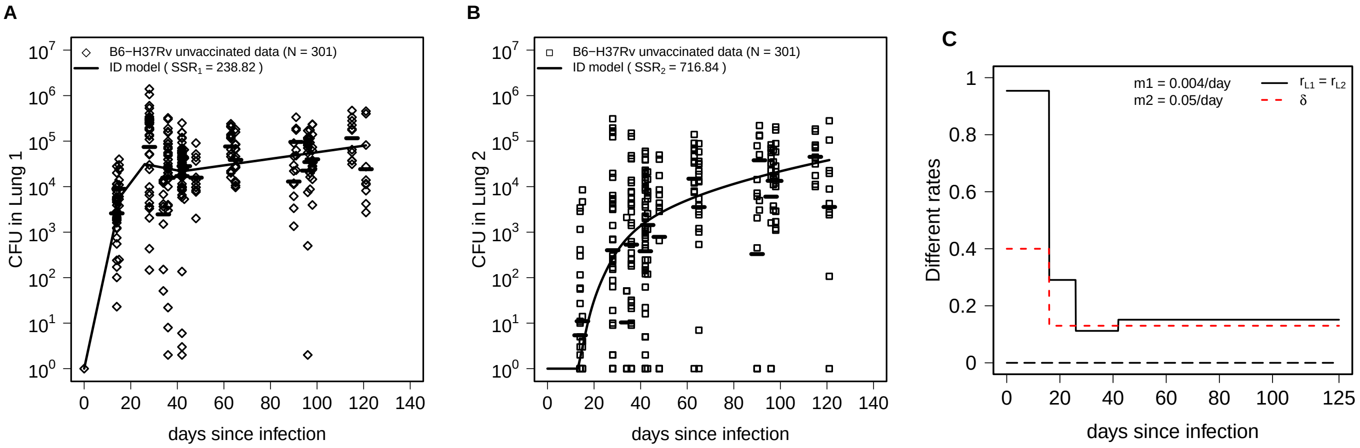
Supplemental Figure S3: Reclassification of right lung and left Lung to Lung 1 and Lung 2. We have redefined the CFU data of right lung and left lung to Lung 1 and Lung 2 such that Lung 1 always has higher CFU than Lung 2. This reclassification reflects our assumption that the initial infection starts at Lung 1 (Lung 1 = 1 CFU at day 0), and then it could go to Lung 2 or other tissues as prescribed in the mathematical model section. Thus, due to the addition of an initial CFU data point, the number of mice in the dataset used for our analyses increases to 301 for unvaccinated mice (A, B) and to 257 for vaccinated mice (C, D).



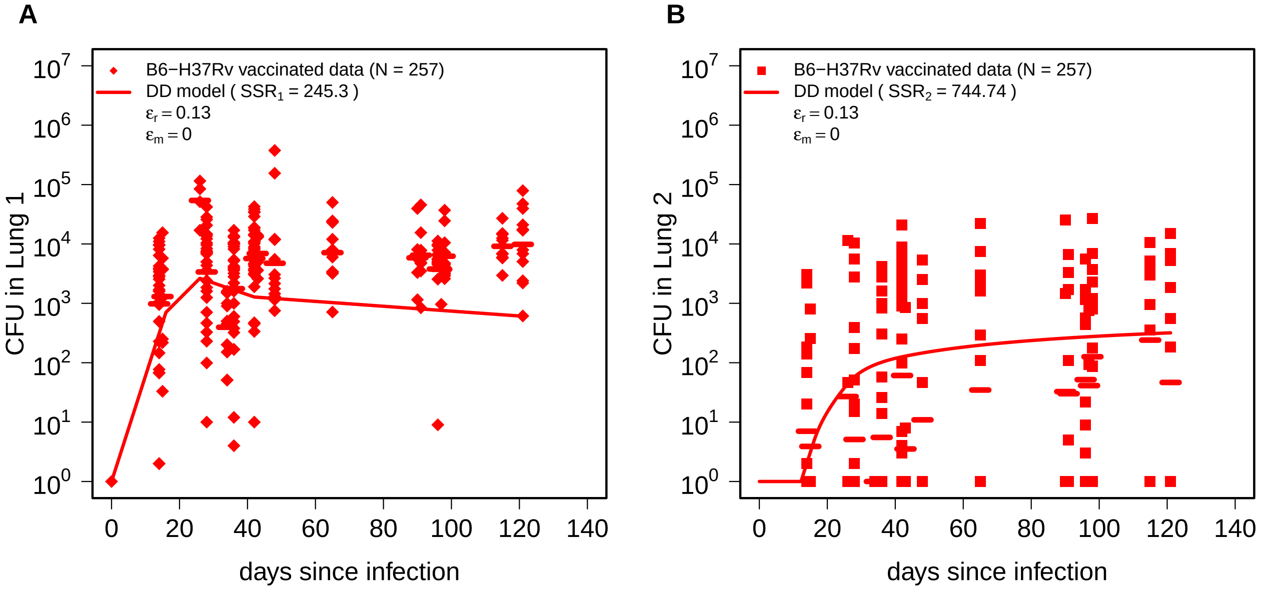
Supplemental Figure S4: Estimated replication rates and predicted bacterial burden at an intermediate tissue predicted by the best fit of the ID model. **A**: We plot time-varying replication rates of Mtb in the intermediate tissue as estimated from fitting the indirect dissemination model to the data. **B**: We plot prediction of the model on the bacterial numbers in an intermediate tissue (line) and experimentally measured CFU in the spleen of ULD-infected mice (markers).

Model	r_1	r_2	r_3	r_4	r_{T_1}	r_{T_2}	r_{T_3}	r_{T_4}	m	m_1	m_2
DD model	0.94 (0.89–0.98)	0.32 (0.28–0.33)	0.10 (0.09–0.11)	0.14 (0.13–0.16)	-	-	-	-	0.0027	-	-
ID model	0.97	0.23	0.11	0.15	0.65	0.20	0.10	0.13	-	0.004	0.042

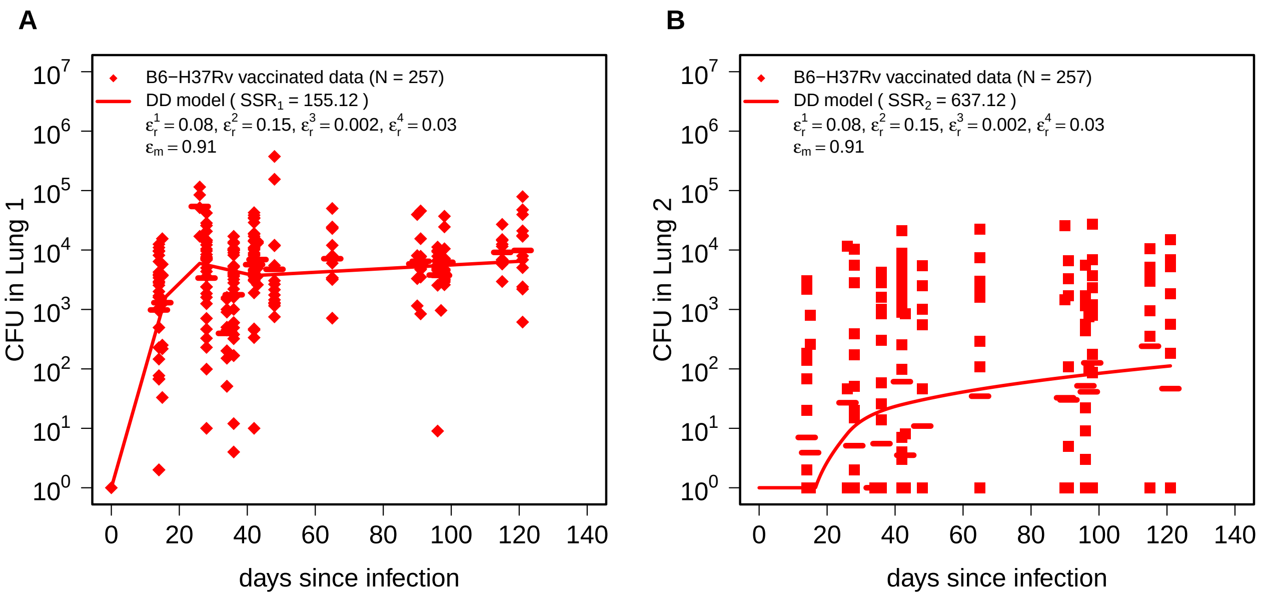
Supplemental Table S1: Estimates of replication rates of Mtb in the lung (r_i) or lung (r_i) and intermediate tissue (r_{T_i}), providing the best fit of the DD or ID model, respectively (model fits are shown in **Fig. 2**). The rates are time-dependent as defined in **eqn. (4)**. All rates are given in per day units, and $-$ denotes not applicable. The 95% confidence intervals (CIs) for the parameter estimates were generated using `modFit`. We only report CIs for the DD model because `modFit` failed to provide CI estimates for the ID model due to the larger number of parameters.



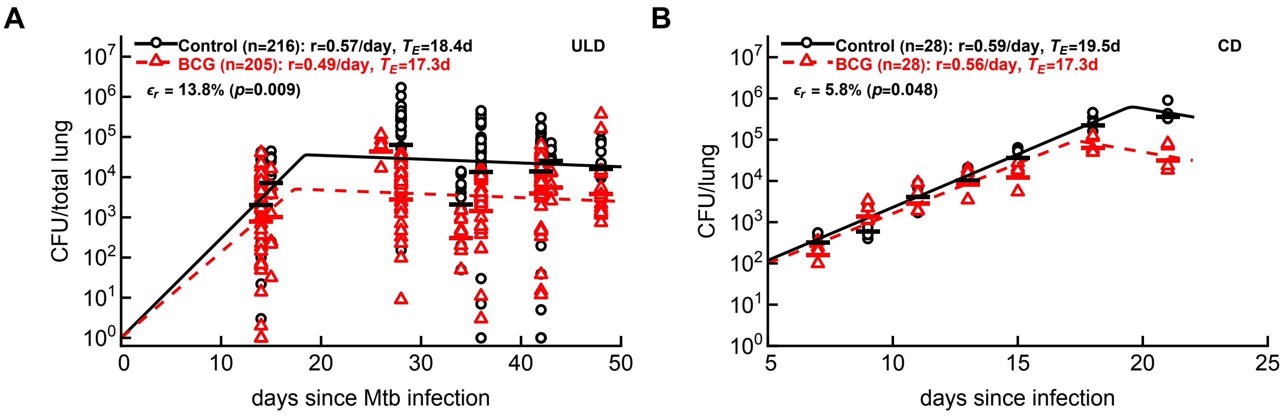
Supplemental Figure S5: Indirect dissemination model explains the data well even if the intermediate tissue is assumed to be blood. We assume that in the ID model the intermediate tissue is blood, and there is no replication and death in the this intermediate tissue. Then, we fit the model to the data and the best-fitted model predicts the CFU burden at Lung 1 and Lung 2 (A, B). The best-fitted model estimate of the dissemination rate from blood to Lung 2 was $m = 0.05/\text{day}$, and the time-dependent Mtb replication rate in the lung behaves similarly to that in **Fig. 2**C.



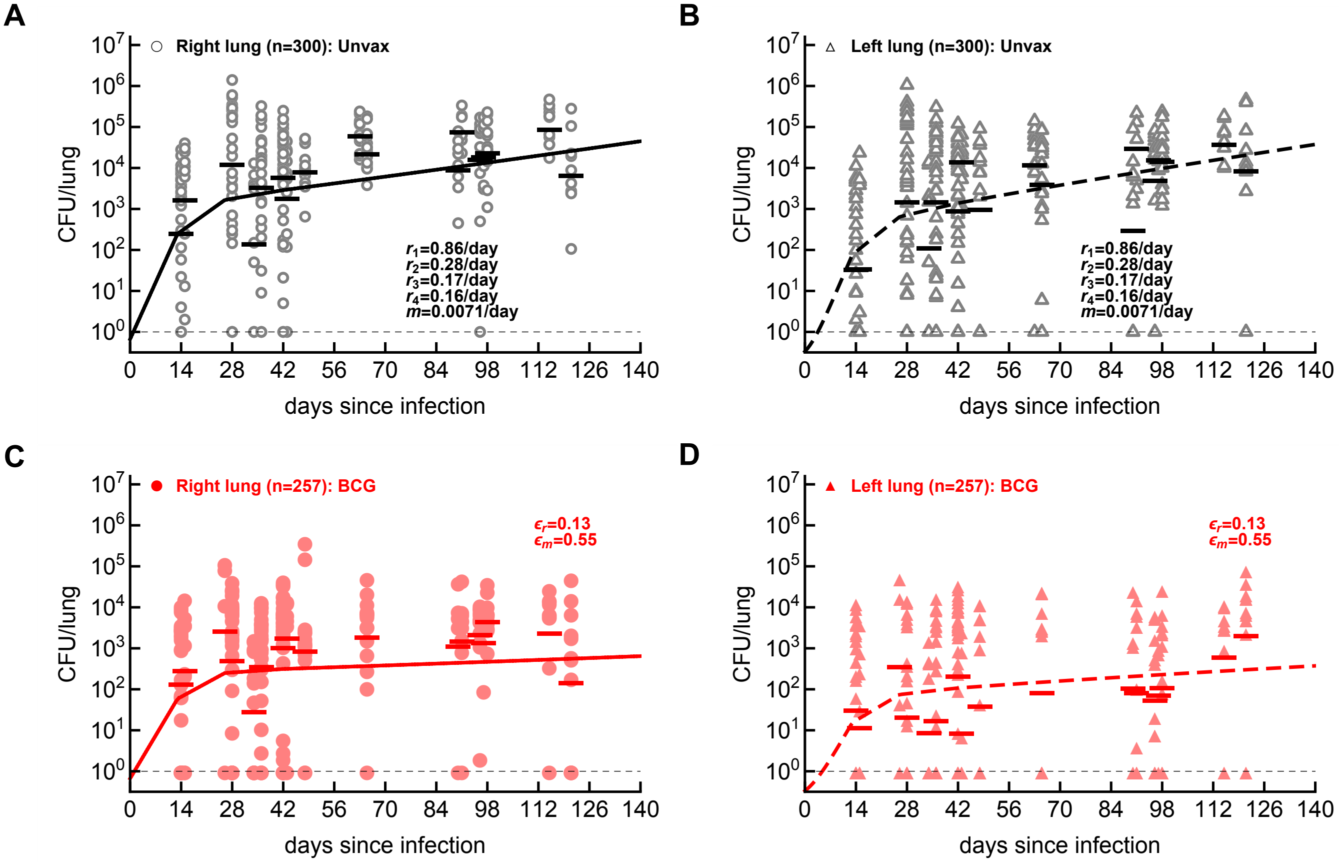
Supplemental Figure S6: The DD model assuming that BCG only reduces the rate of Mtb replication does not accurately fit the data. We fit the DD model (**eqns. (1)–(2)**) to the BCG-vaccinated data by assuming that BCG vaccination only impacts the Mtb replication rate (ϵ_r) but not the Mtb death rate and dissemination rate ($\epsilon_\delta = \epsilon_m = 0$); estimated $\epsilon_r = 0.13$. Other notations are similar to those in **Fig. 3**A-B. SSR values of the fit are shown on individual panels and AIC $= 338.95$ (see also **Suppl. Table. S2**).



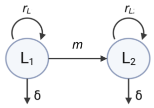
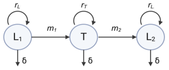
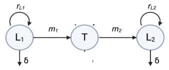
Supplemental Figure S7: Efficacy of BCG vaccine declines with time since infection. We fit the DD model (**eqns. (1)–(2)**) to the data from BCG-vaccinated mice by assuming that BCG vaccine efficacy in reducing the rate of Mtb replication ϵ_r depends on time since infection in time intervals defined in **eqn. (3)**). The fit had much lower SSR (see **Suppl. Table. S2**) and slightly lower AIC = 232.5.



Supplemental Figure S8: BCG vaccination reduces net Mtb replication rate early during ULD and CD infection of B6 mice. We fit our novel model that predicts Mtb dynamics in the presence of exponentially expanding immune response (**eqn. (S.4)**) to the data on Mtb numbers in lungs of B6 mice infected with an ultra-low dose (**A**, **Suppl. Fig. S2**) or conventional dose (**B**, ref[63]) of H37Rv strain of mice. In panel A we only take the data the first 50 days of the total lung CFU of ULD-infected mice (**Suppl. Fig. S2**). We fit the model to data from both unvaccinated or BCG-vaccinated mice simultaneously only allowing for the parameters r and h (or $T_E = -\ln(h)/\rho$) to vary between the datasets; fixing the rate of Mtb replication between the datasets resulted in fits of poorer quality (A: $F_{1,416} = 6.84$, $p = 0.009$; B: $F_{1,49} = 4.1$, $p = 0.048$). Estimated efficacy of BCG vaccination was calculated as $\epsilon_r = (r_{unvax} - r_{BCG})/r_{unvax}$. Other estimated parameters are as follows. Panel A: $B_0 = 1$ (assumed), $\delta = 0.22$/day, $h_{unvax} = 1.62 \times 10^{-5}$, $h_{BCG} = 3.02 \times 10^{-5}$, $r_{unvax} = 0.57$/day, $r_{BCG} = 0.49$/day; panel B: $B_0 = 6.12$, $\delta = 0.24$/day, $h_{unvax} = 8.22 \times 10^{-6}$, $h_{BCG} = 3.1 \times 10^{-5}$, $r_{unvax} = 0.59$/day, $r_{BCG} = 0.56$/day. Parameters $\rho = 0.6$/day and $n = 20$ (**eqn. (S.4)**) were fixed in model fits to data.



Supplemental Figure S9: Deterministic version of realistic simulations of the DD model also predicts that BCG vaccination reduces Mtb replication and dissemination rates. We fit the DD model in which we consider random deposition of inhaled Mtb bacilli into right or left lungs, growth in the lungs, and dissemination between the lungs to the data from unvaccinated (panels **A-B**) or BCG-vaccinated (**C-D**) mice together assuming that BCG vaccine reduces the rate of Mtb replication (by ϵ_r) and the rate of Mtb dissemination (by ϵ_m). We plot experimental data (by markers) and model predictions (by lines); horizontal dashed line denotes the limit of detection of 1 CFU. Best fit parameters are shown on the panels and are $r_1 = 0.86$/day, $r_2 = 0.28$/day, $r_3 = 0.17$/day, $r_4 = 0.16$/day, $m = 0.0071$/day, $\epsilon_r = 0.13$, and $\epsilon_m = 0.55$; the death rates are assumed as in **eqn. (3)**. The best model fit resulted in SSR = 3494.14. To generate model predictions and fit the model to data, we solved the DD model (**eqns. (1)–(2)**) for a collection of initial conditions with an infection initiated by n bacteria ($n = 1, 2 \ldots n_{\max}$) that distributed between right and left lung (e.g., $(1, 0)$, $(0, 1)$, $(2, 0)$, $(1, 1)$, $(0, 2) \ldots (i, j)$ for $(\mathrm{RL}, \mathrm{LL})$, respectively). For each of these initial conditions we calculated the probability $p_{(i,j)}$ of such initial condition (i, j) happening using 0-truncated Poisson distribution and 2:1 bias in infection of the right:left lung. After solving the model for all initial conditions (e.g., assuming $n_{\max} = 3$) we generated the average model predictions by weighting the solutions with a given initial conditions by their respective probabilities. To calculate the SSR, we use paired model predictions of the CFU number in the right and left lung and those observed experimentally; the residuals for a model prediction with initial condition (i, j) were squared and weighed by the probability $p_{(i,j)}$ and summed over to generate SSR.

Fitting Models to:	**Type of model**	**SSR**	**AIC**	**Akaike weights**
Unvaccinated	 Direct dissemination (DD) model	939.1	**277.79**	**0.91**
	 Indirect dissemination (ID) model	930.52	282.53	0.08
	 ID model (blood as a carrier)	955	290.35	0.00
BCG vaccinated	DD model fitted with ϵ_r	990.04	338.95	0.0
	DD model with ϵ_δ	1081.22	384.23	0.0
	DD model with ϵ_r, ϵ_m	804.66	**234.4**	**0.82**
	DD model with $\epsilon_\delta, \epsilon_m$	835.91	244.58	0.005
	DD model with $\epsilon_r, \epsilon_m, \epsilon_\delta$	805.25	237.41	0.18
	DD model with varying ϵ_r,	792.24	232.5	Not counting

Supplemental Table S2: Comparison of model fits (SSR and AIC) for unvaccinated and BCG-vaccinated data. We show statistics of fits of alternative direct and indirect dissemination models, fitted to data from unvaccinated or BCG-vaccinated mice infected with ULD of H37Rv.

Category	**Efficacy Value**	**Model predicted sample size per group**
Vaccination efficacy on replication (ϵ_r)	0.2	500
	0.3	177
	0.4	69
	0.5	36
	0.6	<20
	0.7	<20
	0.8	<20
	0.9	<20
Vaccination efficacy on dissemination (ϵ_m)	0.85	165
	0.90	95
	0.95	48
	0.99	22

Supplemental Table S3: Calculation of number of mice needed to detect the efficacy of a hypothetical vaccine if the initial dose is $\lambda = 2$. We compared the sample size estimates required to achieve 80% power for different levels of vaccine efficacy, controlling replication (ϵ_r) or dissemination (ϵ_m). The power analysis is implemented by using realistic stochastic simulation to predict the sample size per group, as described in the Materials and methods section (and see **Table. 3**).