Supporting Information

Reed *et al*. 10.1073/pnas.0712077106

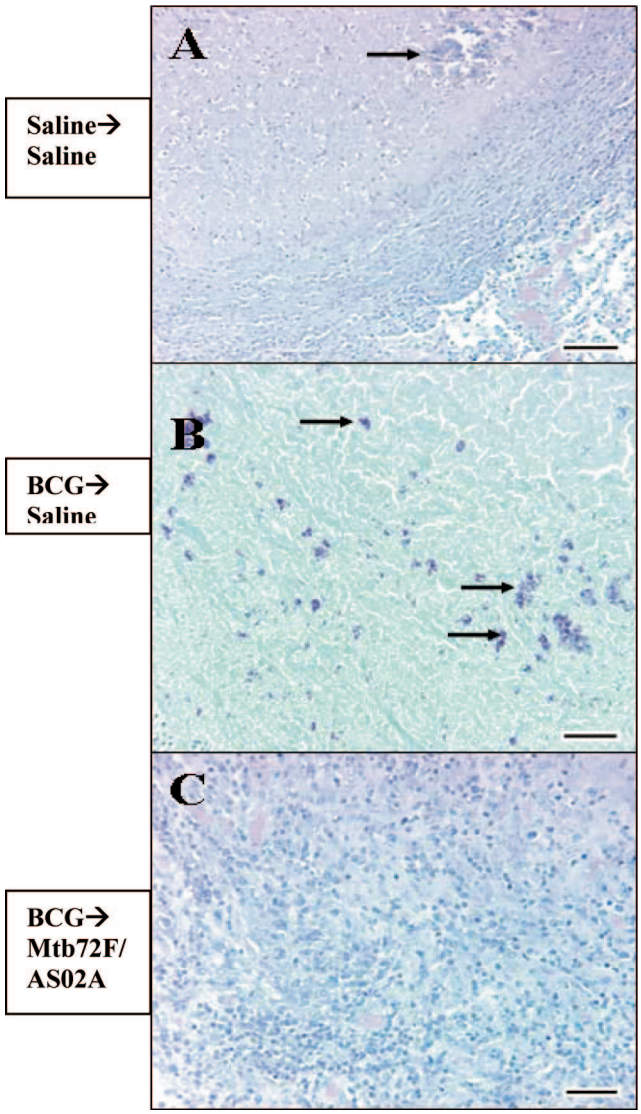

Fig. S1. Acid Fast Staining of lung tissues harvested from monkeys primed with bacillus Calmette–Guérin and immunized with Mtb72F-containing vaccines. Cynomolgus monkeys were injected with saline alone (*A*–Right Lung), bacillus Calmette–Guérin followed by saline (*B*–Right Lung), bacillus Calmette–Guérin followed by Mtb72F/AS02A (*C*–Right lung), 3 times 4 weeks apart and then infected 4 weeks later with *Mycobacterium tuberculosis*. Ziehl Neelsen and Fite's stain were used to stain for acid fast bacilli. Bar = 100 μm.

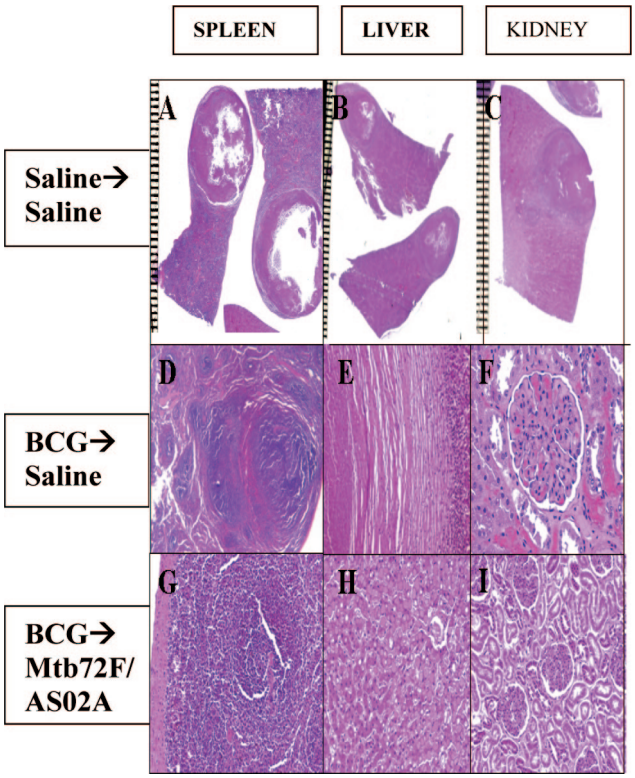

Fig. S2. Histologic appearance of spleen, liver, and kidney tissues harvested from monkeys primed with bacillus Calmette–Guérin and boosted withMtb72F vaccines. Cynomolgus monkeys were injected with saline or were primed with bacillus Calmette–Guérin followed 32 weeks later by boosting with Mtb72F /AS02A or were injected with saline 3 times 4 weeks apart. All monkeys were infected with *Mycobacterium tuberculosis* 4 weeks after the last injection. Spleens (*A*, *D*, *G*), livers (*B*, *E*, *H*), and kidneys (*C*, *F*, *I*) were removed from monkeys and stained with hemtoxylin and eosin, and von Kossa for histopathological evaluation.

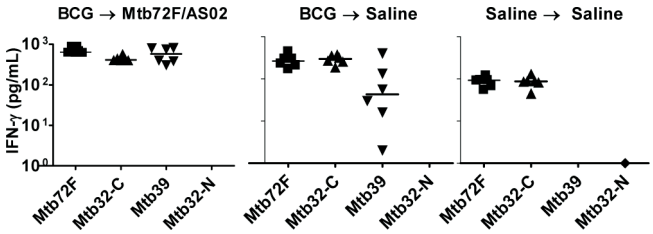

Fig. S3. Cellular responses to mycobacterial antigens after injection with saline, or immunization with bacillus Calmette–Guérin followed by 3 boost immunizations with Mtb72F/AS02A or saline. Monkeys (6 per group) were injected with saline or were primed with bacillus Calmette–Guérin followed 32 weeks later by injections of Mtb72F/AS02A or saline alone administered 3 times 4 weeks apart on weeks 32, 36, and 40. In vitro IFN-γ release (pg/ml) by whole blood in response to mycobacterial antigens (Mtb72F, Mtb32-C, Mtb39, Mtb32-N; each at a concentration of 10 μg/ml) were measured by ELISA at three weeks after the last immunization (week 43).

Table S1. Experimental plan for Mtb72F vaccine development in nonhuman primates

Experimental groups	Conditions	Immunization schedule	Baseline readouts	Monthly/Exams/Immunizations	Postinfection monthly exams
1	Mtb39 + AS02A Mtb39 + Mtb32n + AS02A BCG AS02A	Injections were administered on weeks 0, 4, and 8	Physical exam, weight, temperature, CBC with differential, ESR, serum chemistry profile, direct fecal exam, rectal culture, CXR, WBA, and PPD-ST	Mtb72F-containing vaccines were administered 3 times, 4 weeks apart. BCG prime injections were followed 16 or 32 weeks later by Mtb72F-containing vaccines. Monthly exams included measurements of weights, temperature, hematocrit, and ESR. Injection site were monitored at 30 minutes, and 2, 24, and 48 hours post each injection.	Weight, temperature, ESR, hematology, CBC, CXR, PPD-ST, WBA, collection of plasma
2	Adjuvant Saline Mtb39 + Mtb32c/ AS02A	Injections were administered on weeks 0, 4, and 8			
3	AS02A BCG Mtb72F/AS02A	Injections were administered on weeks 0, 4, and 8			
4	AS02A BCG Mtb32c + Mtb39 + Mtb32n/AS02A Mtb72F/AS02A	Injections were administered on weeks 0, 4, and 8			
5	Saline BCG Mtb72F/AS02A	Injections were administered on weeks 0, 4, and 8			
6 BCG Prime-Vaccine Boost	AS02A BCG → AS02A BCG → Mtb32c + Mtb39 + Mtb32n/ AS02A BCG → Mtb72F/AS02A	NHP were primed with BCG on week 0 and boosted with Mtb72F/AS02A on week 16, 20, and 24			
7 BCG Prime-Vaccine Boost	Saline → Saline BCG → Saline BCG → Mtb72F/AS02A	NHP were primed with BCG on week 0 and boosted with Mtb72F/AS02A on weeks 32, 36, and 40			

Seven experiments were performed in monkeys (5 or 6 per group) that were injected with AS02A adjuvant system, saline, Mtb72F/AS02A, or its component antigens (Mtb39, Mtb32n, Mtb32c) delivered in a mixture or singly with AS02A, or monkeys were primed with BCG followed 16 or 32 weeks later by injections of AS02A, saline, or Mtb72F/AS02A administered 3 times 4 weeks apart. In each experiment, monkeys were challenged by intratracheal instillation of 500 CFU *Mtb* Erdman strain 4 weeks after the last immunization. Survival was monitored for 75 to100 weeks post infection. Baseline examinations included physical exams, weights, temperature, CBC with differential, ESR, serum chemistry profile, direct fecal exam, rectal culture, CXR, WBA, and PPD-ST.