Peptide #	Rv #	Residue #	Sequence
1	Rv0640	12-26	KLQIVAGQANPAPPV
2	Rv0640	67-81	SFTFTLKTPPAAKLL
3	Rv0651	68-82	GLDELFVGPTAIAFV
4	Rv0651	146-160	KAAGLFNAPASQLAR
5	Rv0652	30-44	ETFEVTAAAPVAVAA
6	Rv0670	53-67	IYVHAPYLINLASAN
7	Rv0670	221-235	AAVKAAGAPVICETA
8	Rv0672	11-25	VPPLENYNPASSPVL
9	Rv0675	23-37	SRNAVNGPTAAALCA
10	Rv0675	64-78	ADLKAFGTPEANSVH
11	Rv0676c	919-933	LIVRSFMTPSIAALL
12	Rv0688	387-401	VRGKIAAGAPIAEVL
13	Rv0691c	48-62	RTLFRYYASKNAIPW
14	Rv0696	223-237	PHSTVSYVPSAAIVC
15	Rv0696	293-307	RKAFYGGSAAPLAVR
16	Rv0701	25-39	PVTVVKAGPNVVTRI
17	Rv0706	45-59	ILRWAPQAASGPVAK
18	Rv0706	57-71	VAKVIASAAANAQNN
19	Rv0719	104-118	LEFALGYSHPVVIEA
20	Rv0725c	112-126	DARAYRLPWPAGTVV

Table SI. Sequences and locations of peptides included in Pool 12. The Mtb (strain H37Rv) gene of origin, residue numbers and sequences are shown for each of the peptides within Pool 12 of the IKEPLUS peptide library (**Fig. 1D**).



IKEPLUS culture (washed bacteria)

EmulsiFlex Homogenizer – 15,000 psi x2 in lysis buffer (70 mM KCl, 10 mM MgCl₂, 10 mM Tris-HCl [pH 7.4])

Clarify (30,000xg, 45min at 4°C), filter (0.22um)

Apply 1mL (3mg/mL) to QA monolithic disk column

HPLC (In lysis buffer, with 35%, 47%, and 100% 1M NaCl)









Figure S1. Characterization of enriched ribosomes from IKEPLUS.

(A) Schematic of the ribosome enrichment procedure used.

(B) Ribosomes were enriched from IKEPLUS using a standard HPLC ion exchange and size separation method. Isolations were performed at a flow rate of 2 mL/min, using lysis buffer (Buffer A) and lysis buffer containing 1M NaCl (Buffer B). Sequential elutions with 35% (fraction QA1), 47% (QA2), and 100% (QA3) buffer B were collected and further analyzed.

(**C**) Fractions QA2 and QA3, as well as IKEPLUS lysate, were also analyzed by SDS-PAGE and visualized with Coomassie blue staining.

(**D**) IL-2 ELISA was also used to examine fractions QA1, QA2, and QA3 for ribosomes by culturing the fractions in the presence of APCs and an $rplJ_{TB146-160}$ -sepcific TCH (clone RplJ.03). Data are mean values and standard errors for duplicate samples.



Figure S2. Quantification of CFU in immunized mice following Mtb challenge. Mice (C57BL/6) were immunized with 5 × 10⁷ CFU IKEPLUS i.v., 10⁷ CFU BCG-Danish s.c., or sham-immunized with saline s.c. (naïve). Ten weeks later, immunized and naïve mice were challenged with low-dose aerosol Mtb strain H37Rv (~100 CFU). Six weeks after challenge, lungs, spleens, and livers were harvested, processed, and quantified as previously described (60). ***p < 0.001, and ****p < 0.0001 (ANOVA).