Table S1. Analysis of random clones after treatment of primary phages (P01) with various concentrations of trypsin.

Trypsin concentration	I. Fold reduction in titre	II. Aligning to *M. tb* genome	III. ORFs in-frame with *PelBss* and *gIIIp* *	IV. Aligning to the *M. tb* proteome †
A. MTBLIB25 Library (100-300 bp)				
Without trypsin	-	100 (32/32)	-	-
10 µg/ml	1 x 10^4	100 (32/32)	93.7 (30/32)	40.0 (12/30)
100 µg/ml	4 x 10^4	100 (32/32)	96.8 (31/32)	32.25 (10/31)
200 µg/ml	10^6	100 (32/32)	93.7 (30/32)	33.33 (10/30)
B. MTBLIB27 Library (300-800 bp)				
Without trypsin	-	100 (47/47)	-	-
10 µg/ml	1.3 x 10^4	100 (44/44)	100 (44/44)	65.9 (29/44)
100 µg/ml	1.7 x 10^4	100 (44/44)	93.2 (41/44)	53.6 (22/41)
200 µg/ml	10^5	97.7 (43/44)	95.4 (42/44)	57.1 (24/42)

Primary phages (10^{11}) from the (A) MTBLIB25 library (100-300 bp) and (B) MTBLIB27 library (300-800 bp) were incubated with four different concentrations of trypsin (0, 10, 100 and 200 µg/ml) and then used to infect *E. coli* TOP10F' cells to determine the reduction in phage titer. Randomly selected clones were analyzed by PCR and DNA sequencing to estimate the percentage of ORF (genic and non-genic ORF) and non-ORF clones.

* Percentage of clones in-frame with the *PelB* signal sequence and *gIIIp* in the phagemid.

† Percentage of total in-frame clones from Stage III that aligned with the *M. tuberculosis* (*M. tb*) proteome (genic clones).

Number of positive clones/total clones analyzed is given in brackets.

Table S2. Size distribution of randomly sequenced clones at various stages of library construction.

A. MTBLIB25 Library (100-300 bp)				
Size	1. MTBLIB25C01 (95)	2. MTBLIB25P01 (47)	3. MTBLIB25C02 (48)	4. MTBLIB25P02 (48)
< 100 bp	2	2	3	2
100 - 200 bp	42	25	24	29
200 - 300 bp	47	20	20	17
> 300 bp	1	-	1	-
B. MTBLIB27 Library (300-800 bp)				
Size	1. MTBLIB27C01 (95)	2. MTBLIB27P01 (47)	3. MTBLIB27C02 (48)	4. MTBLIB27P02 (48)
< 300 bp	-	-	-	-
300 - 400 bp	21	11	15	20
400 - 500 bp	36	10	16	10
500 - 600 bp	20	8	9	9
600 - 700 bp	10	7	4	4
700 - 800 bp	4	4	2	3
> 800 bp	1	4	-	-

The total number of clones analyzed at each stage are indicated in brackets. 1, Transformants obtained after large-scale electroporation of the ligation sample; 2, transductants obtained after infection of TOP10F' cells with the primary phage library; 3, transductants obtained after infection of TOP10F' cells with the trypsin-treated ORF phages; 4, transductants obtained after infection of TOP10F' cells with the secondary phage library.

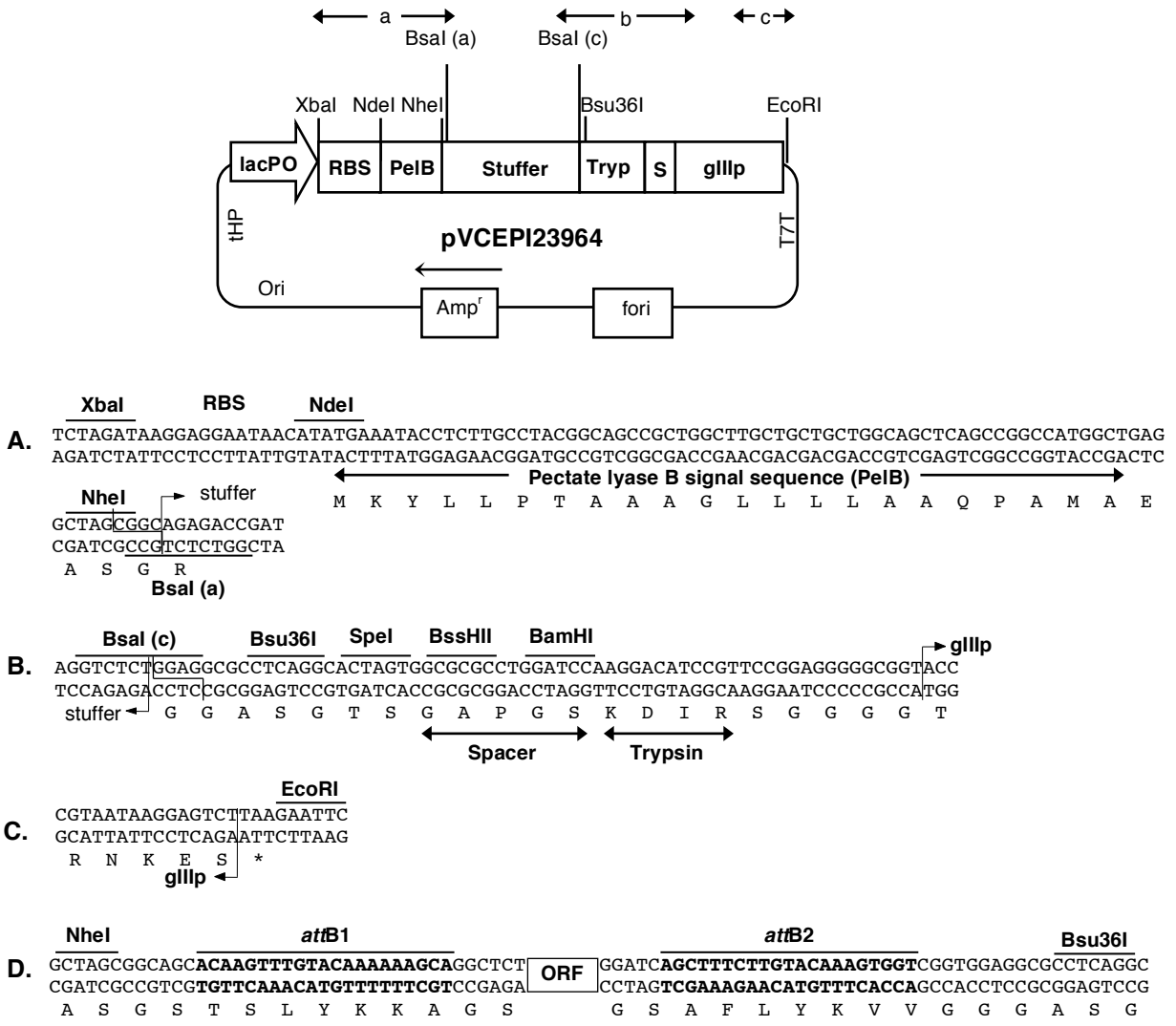

Fig. S1: Schematic representation of the vector pVCEPI23964.
Only the relevant genes and restriction sites are shown. The map is not to scale. lacPO, lac promoter-operator; RBS, ribosome-binding site; PelB, pectate lyase signal sequence; Stuffer, 1.8 Kbp nucleotide sequence flanked by *Bsa*I sites (a) and (c); Tryp, trypsin protease cleavage site; S, spacer; gIIIp, segment encoding amino acid residues 2 - 405 of the gene III of filamentous phage; fori, origin of replication of filamentous phage; Amp^r, β-lactamase gene; Ori, ColE1 origin of replication; tHP, transcriptional terminator. Details of regions marked a, b and c by double-headed arrows are shown in A, B and C, respectively. 'D' shows the sequence flanking the ORF after cloning into the phagemid vector harboring *att*B1 and *att*B2 site-specific recombination sites (in bold) based on Gateway Technology. Amino acids are shown in single-letter code below the nucleotide sequence. Restriction enzyme sites are shown above the nucleotide.

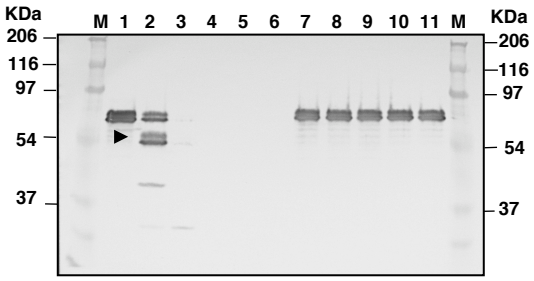

Fig. S2: Western blot analysis of helper phages.
Trypsin-untreated and -treated VCSM13 and AGM13 phages (1.5 x 10^{10}) were separated by 10% SDS-PAGE under reducing conditions, transferred onto 0.45 µ PVDF membranes and probed with anti-gIIIp MAb 30421, which targets an epitope located in the N2 domain of gIIIp from the bacteriophage M13. M, Prestained marker; Lane 1-5, 1.5 x 10^{10} VCSM13 phages treated with 0, 0.1, 1, 10 and 100 µg/ml trypsin, respectively; Lane 6, empty; Lane 7-11, 1.5 x 10^{10} AGM13 phages treated with 0, 0.1, 1, 10 and 100 µg/ml trypsin, respectively.