## Supplementry Figure Legends

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2 Supplementary Figure S1. Maps of single cos (pYUB1551) and double cos (pYUB1552) 3 vectors expressing mVenus from the p<sub>left</sub> promoter of phage L5. Plasmid pYUB1551 is 4 the name allocated to the sequenced plasmid pYUB1391(6). Unique restriction sites are 5 indicated except for the Pacl recognition sequence, which is unique in pYUB1551 but 6 appears twice in pYUB1552. 7 8 Supplementary Figure S2. Construction of the DS6A shuttle phasmid. The indicated 9 DNA samples were evaluated by electrophoresis on agarose gels. (A) High quality 10 genomic DNA isolated from DS6A. (i) Genomic DNA isolated from D29 phage amplified 11 on M. smegmatis using the standard phage amplification protocol. (ii) Genomic DNA 12 isolated from DS6A phage amplified on Mtb using the standard phage amplification protocol. (iii) Genomic DNA isolated from DS6A phage amplified with the modified 13 14 amplification protocol resulting in high quality phage DNA suitable for shuttle phasmid 15 construction. (B) Restriction digestion confirming shuttle phasmids from DS6A phage. A 16 DS6A deletion library was electroporated into *Mtb.* Genomic DNA from the recombinant 17 phages obtained was isolated, and the presence of plasmid was confirmed by Pacl 18 digestion. A band of the correct size for pYUB328 plasmid (~3.9 kb) was released from 19 seven of the eight DS6A recombinant phages (indicated by the lower arrow). 20 21 Supplementary Figure S3. Phylogenetic trees of selected DS6A proteins analyzed 22 against the mycobacteriophage database. Phylogenetic trees of DS6A structural 23 proteins (i) capsid, (ii) major tail subunit, (iii) tape measure protein, functional proteins 24 (iv) Lysin A and (v) Lysin B, and DNA modifying proteins (vi) DNA polymerase III, (vii)

- integrase, (viii) MazG, and (ix) Primase were assembled via maximum parsimony
- analysis. The colored shape next to the phage name indicates its cluster assignment.

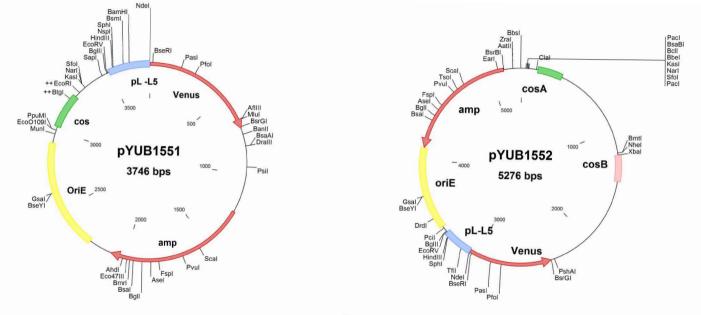


Fig. S1

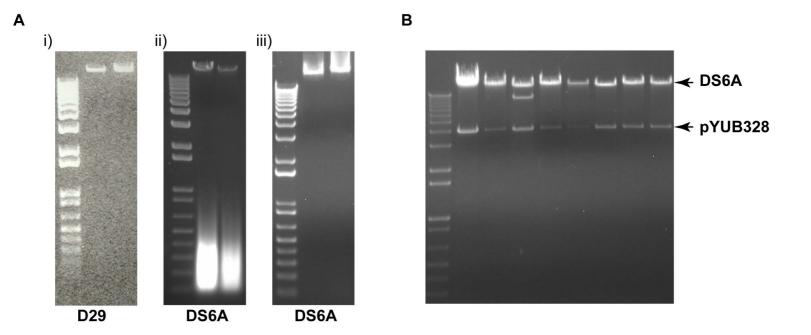


Fig. S2

## Cluster assignment legend

Cluster A		Cluster CU	▲ Cluster R
Cluster AK		Cluster CV	Cluster S
Cluster AR		Cluster DB	▲ Cluster T
Cluster AS		Cluster DE	▲ Cluster V ▲ Cluster W
Cluster B	<b>•</b>	Cluster F	
Cluster BE		Cluster G	
Cluster BH		Cluster H	Cluster X
Cluster BU		Cluster I	Cluster Y
Cluster BV		Cluster J	Singleton
Cluster BW	×	Cluster K	Unclassified
Cluster C	Ă	Cluster L	▲ DS6A
Cluster CB			
Cluster CC		Cluster M	

Cluster N

Cluster O

▲ Cluster Q

Cluster CQ

Cluster CR

Cluster CT

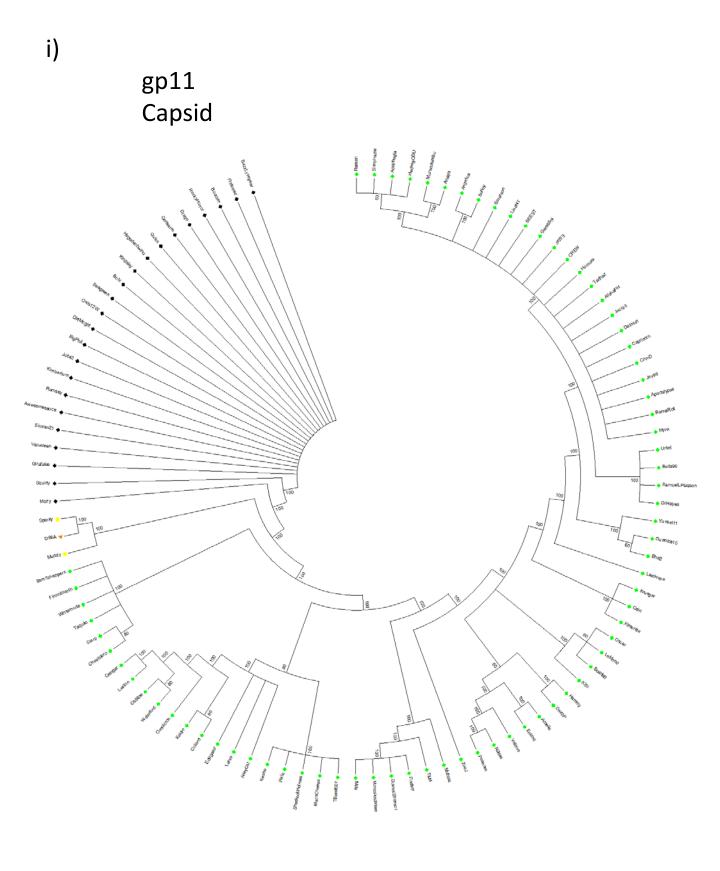


Fig. S3

ii)

gp16 Major tail subunit

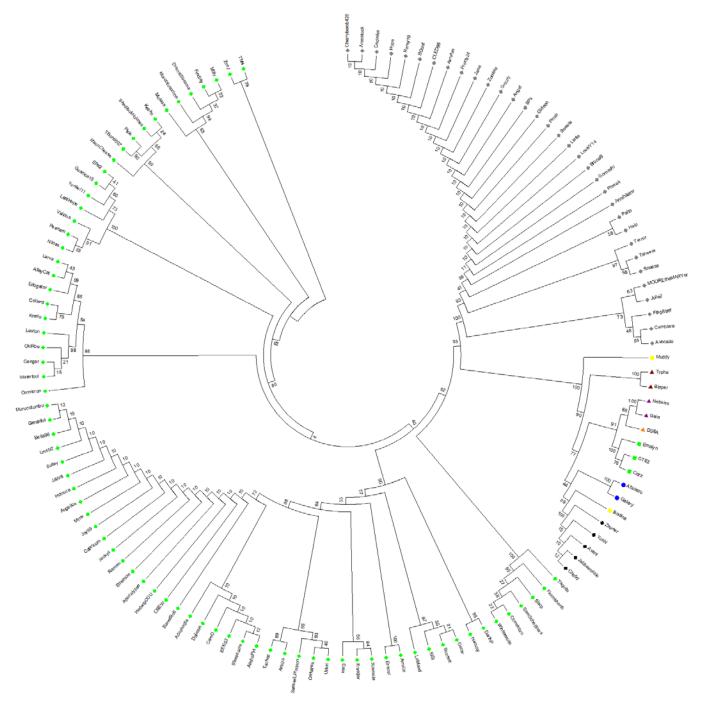


Fig. S3

iii)

gp19 Tape measure protein

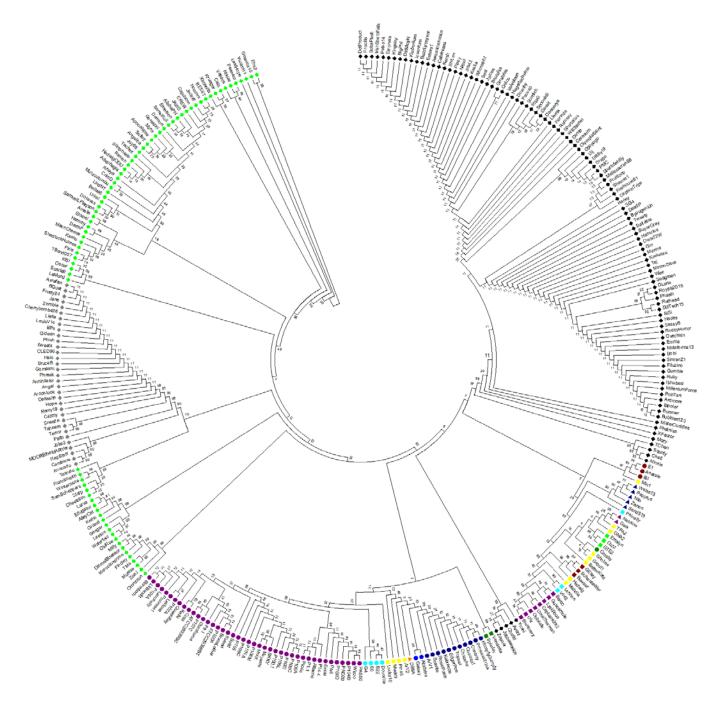


Fig. S3

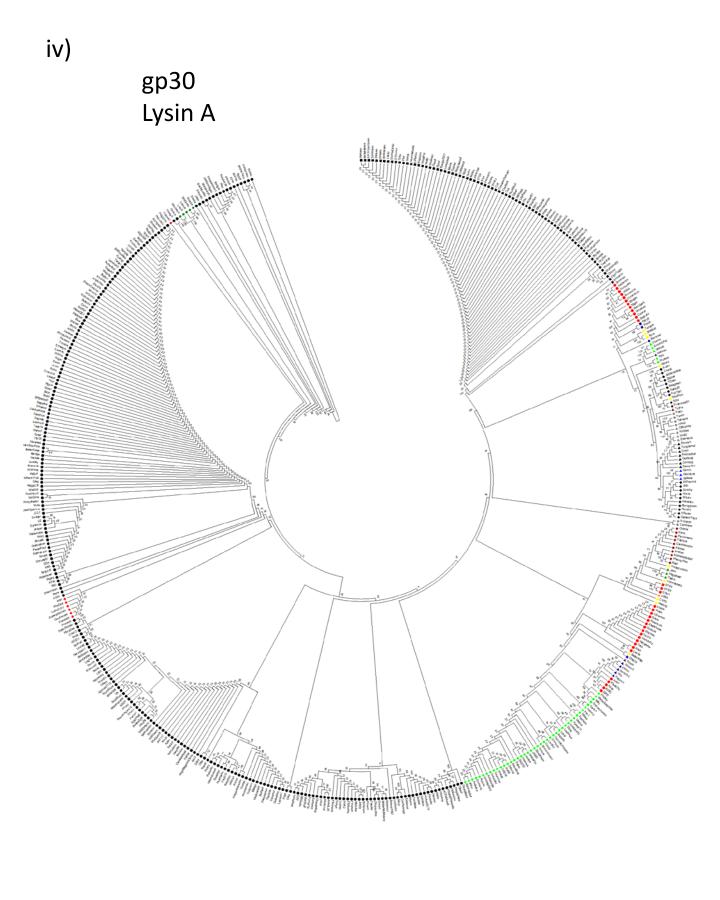


Fig. S3

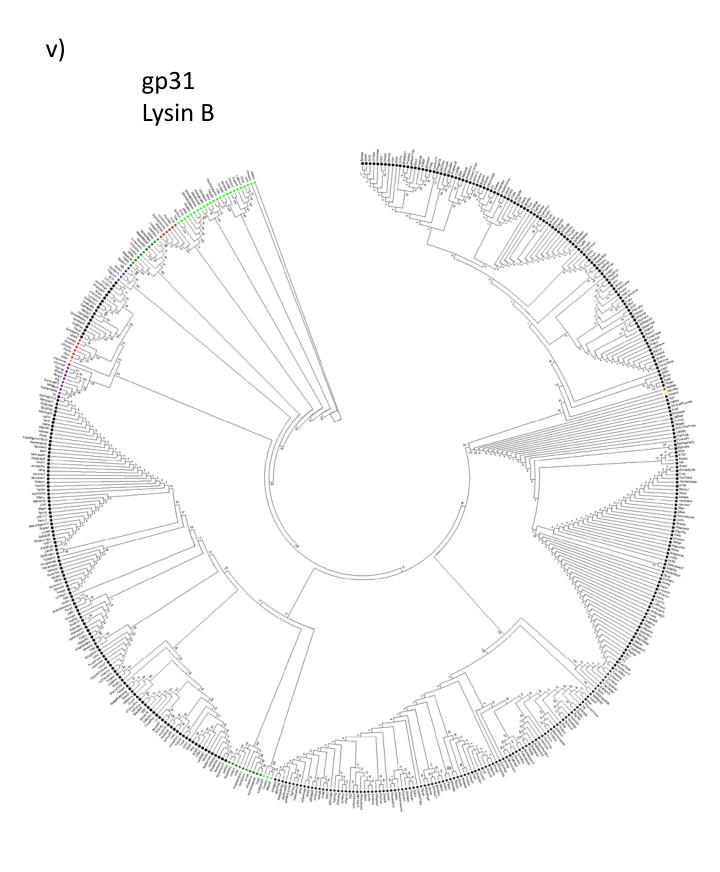


Fig. S3

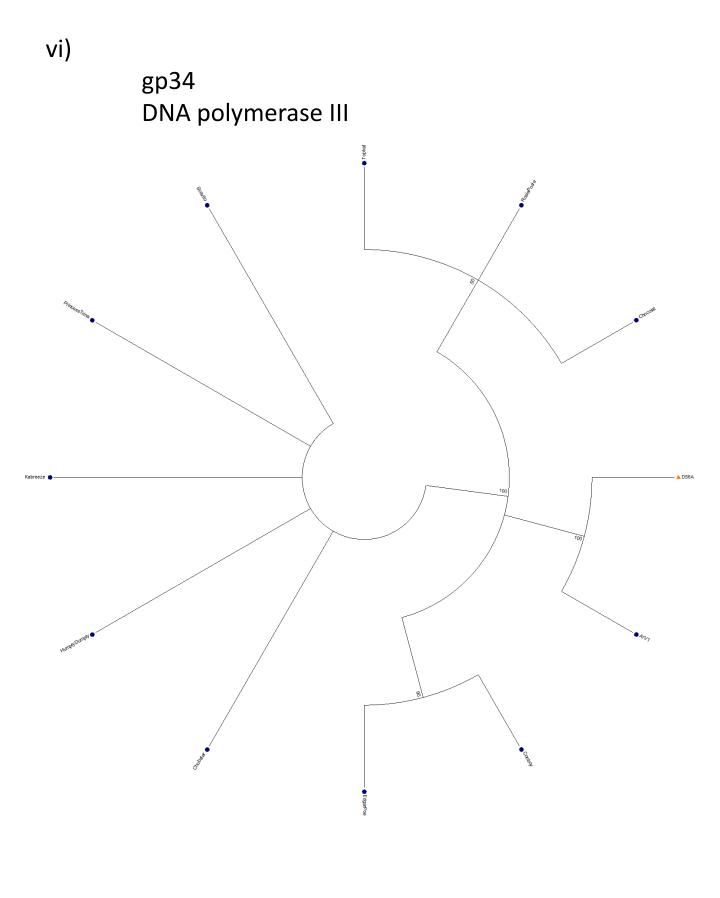


Fig. S3

vii) gp56 Integrase

Fig. S3

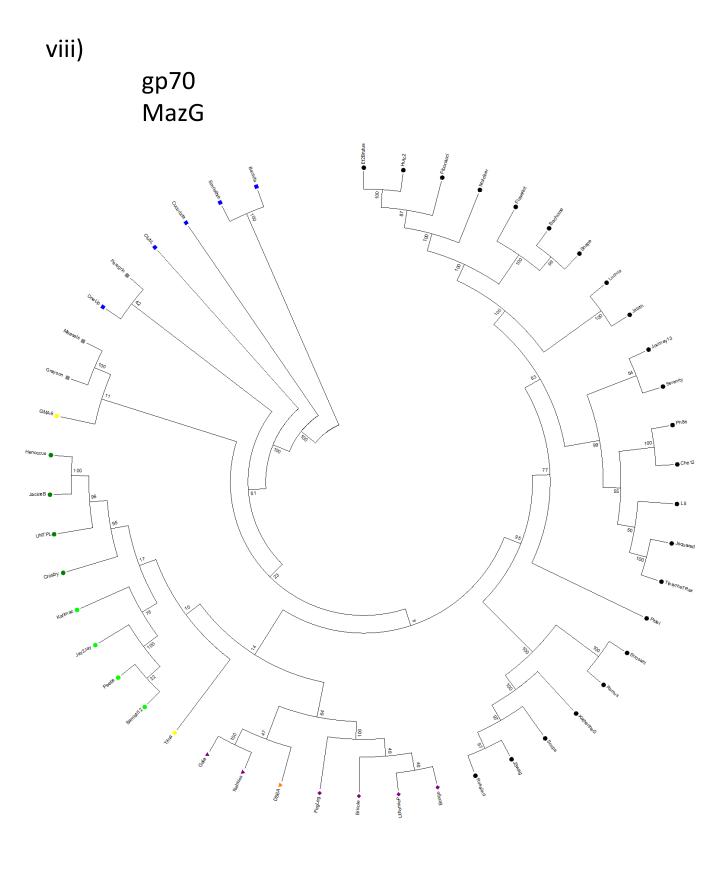


Fig. S3

ix) gp77 Primase

Fig. S3