Figure S1. Genome maps of the three mycobacteriophages HelDan (JF957058), BTCU-1 (KC172839) and Rockstar (JF704111). The shading with colors from the violet towards the red end of the spectrum reflects a reduced level of similarity defined by BLASTN between the genomes. No shading means that there is no similarity with a BLASTN score of $10^{-4}$ or better. Key annotations of the gene products encoded by BTCU-1 are shown. The putative lysis cassette of BTCU-1 composed of *lysA* and *lysB* is highlighted by a red bar. The boundary between the two major gene clusters located on different strands is indicated by a blue line.
**Figure S2.** Lytic activity of BTCU-1 against two *M. smegmatis* strains and seven *M. tuberculosis* isolates was detected by the formation of clear plaques. The mixture of BTCU-1 with different *Mycobacterium* strains ((A,B) *M. smegmatis*; (C–I) *M. tuberculosis* clinical isolates) was added to agar and the plates were incubated. Some of the clear plaques formed are highlighted by white arrows. The lysis of the host bacteria *M. tuberculosis* TCGH 58339 (D) and 45545 (H) was very effective, producing clear plates.

In this result, BTCU-1 presented a broad lytic host range, affecting nearly all the tested *Mycobacterium* strains except *M. tuberculosis* TCGH59490 (F).