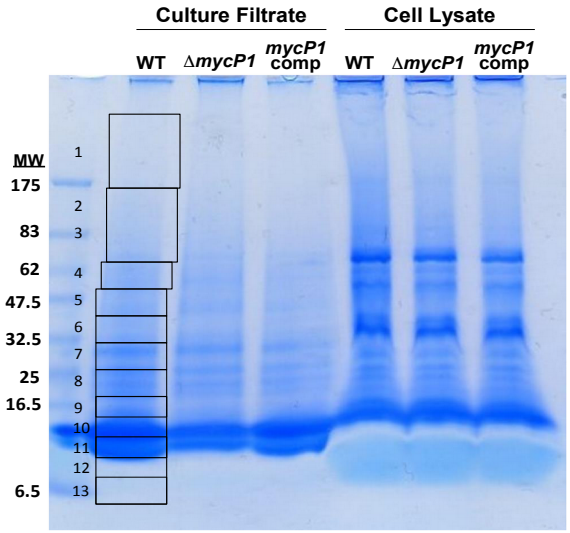
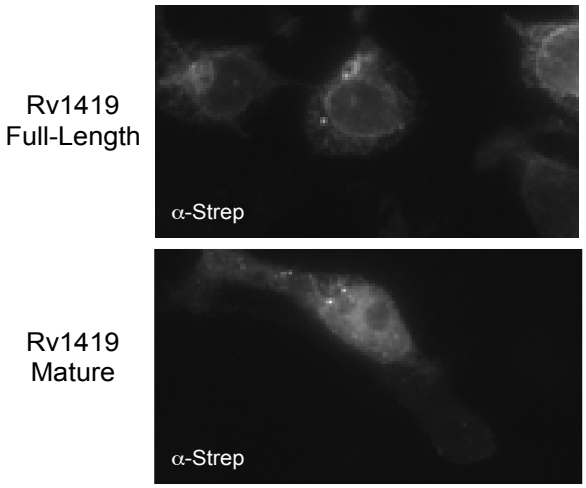
Figure S1

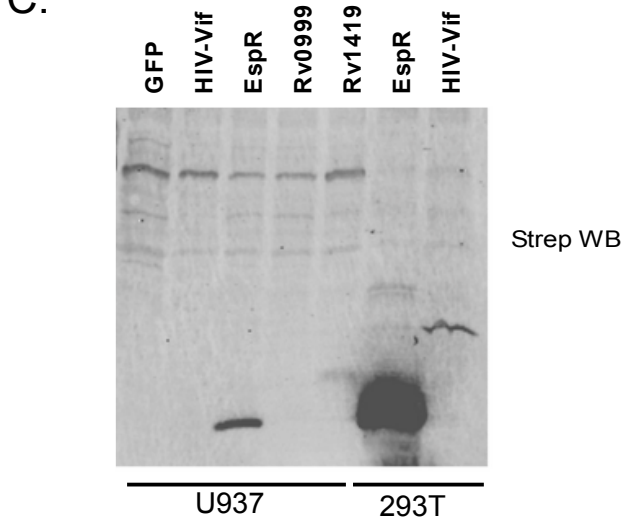
A.



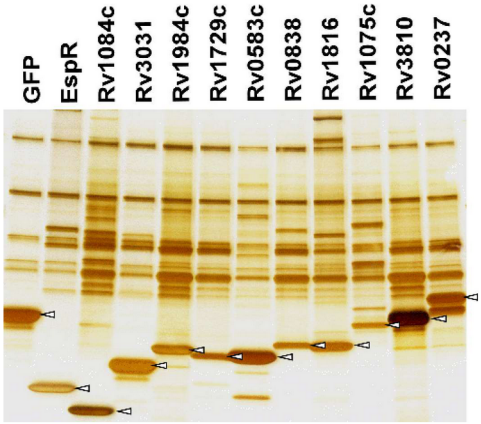
B.



C.



D.



E.

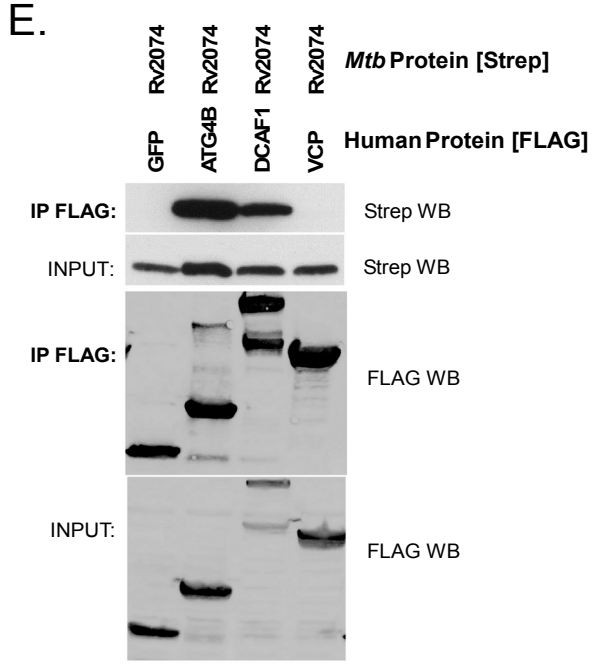


Figure S2

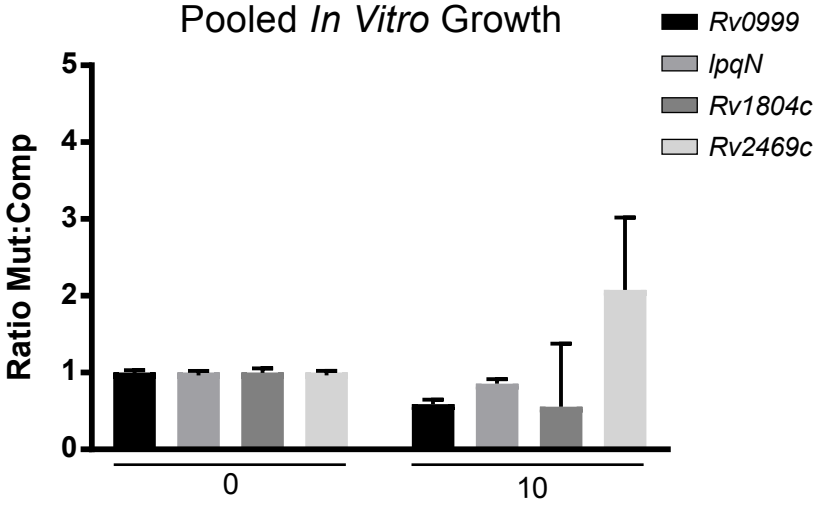

Pooled *In Vitro* Growth
Rv0999
lpqN
Rv1804c
Rv2469c
Ratio Mut:Comp
5
4
3
2
1
0
0
10

Figure S3

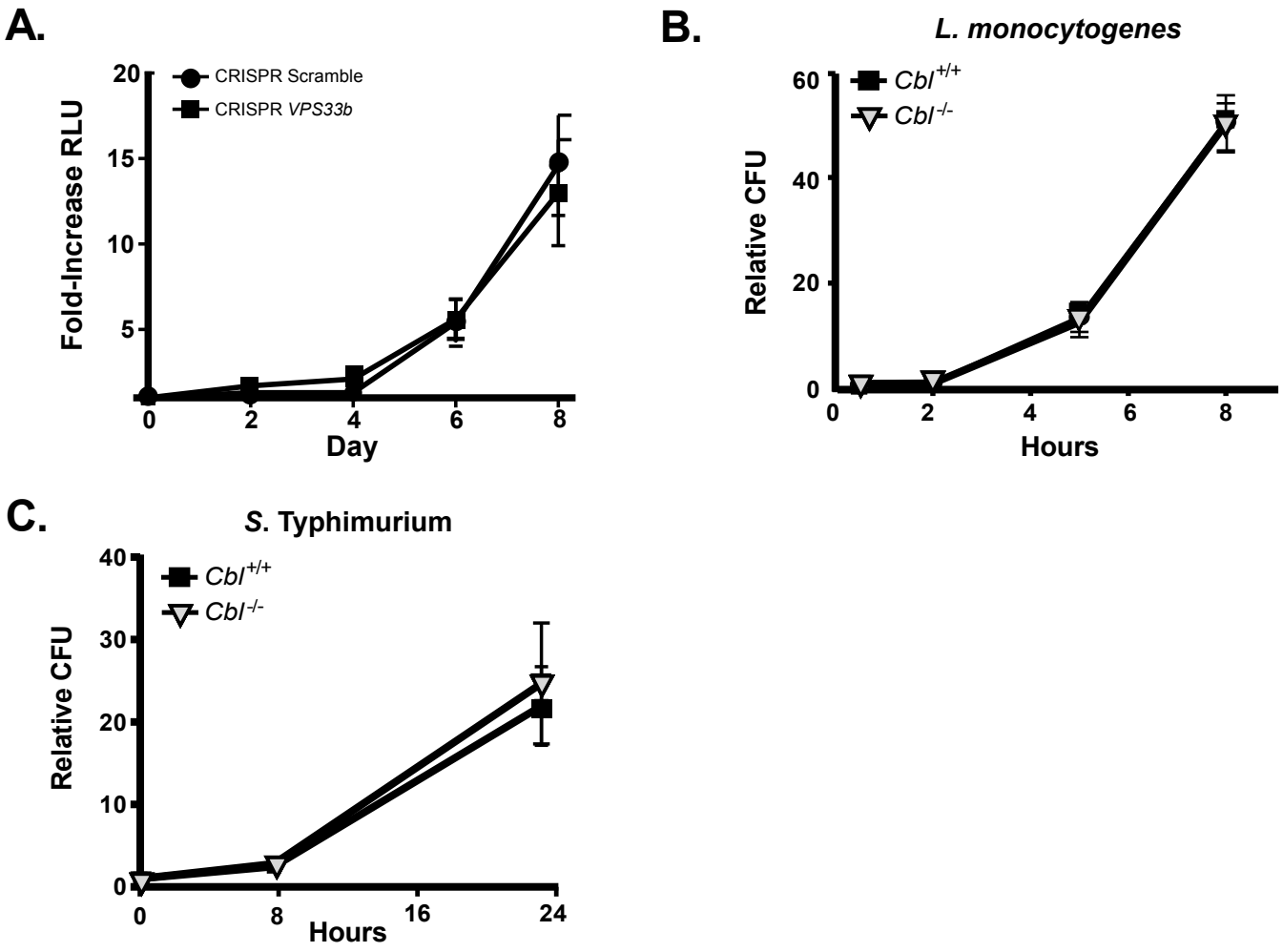
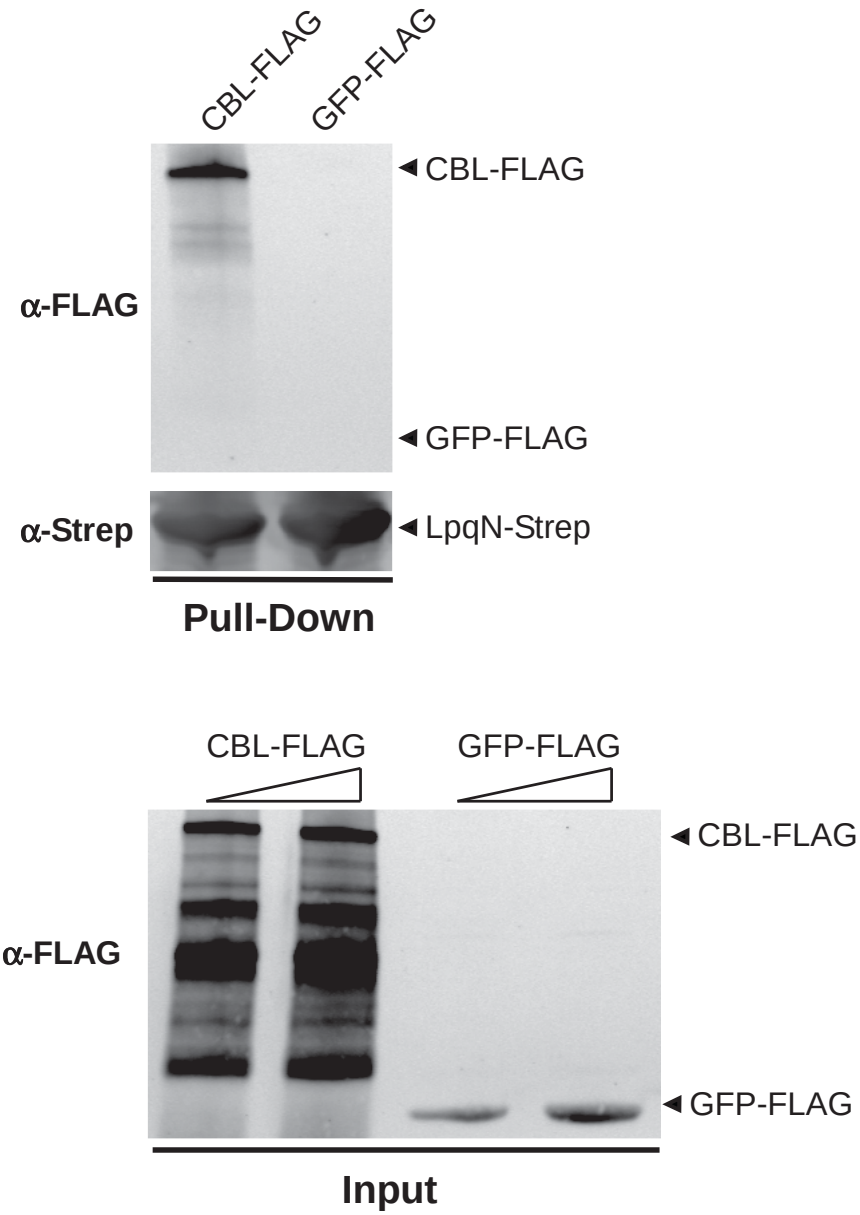

A.
CRISPR Scramble
CRISPR VPS33b
Fold-Increase RLU
Day
B.
L. monocytogenes
Cbl+/+
Cbl-/-
Relative CFU
Hours
C.
S. Typhimurium
Cbl+/+
Cbl-/-
Relative CFU
Hours

Figure S4


CBL-FLAG
GFP-FLAG
α-FLAG
◄ CBL-FLAG
◄ GFP-FLAG
α-Strep
◄ LpqN-Strep
Pull-Down

CBL-FLAG
GFP-FLAG
α-FLAG
◄ CBL-FLAG
◄ GFP-FLAG
Input

Figure S1. Related to Figure 1. Establishing conditions for proteomic analysis of secreted *Mtb* proteins in human cells.

(A) Culture filtrates were prepared from wild-type cultures, separated by SDS-PAGE, and proteins identified by mass spectrometry. The gel was cut into sequential regions as indicated, and each slice was individually subjected to in-gel trypsin digestion and LC-MS/MS (Table S1). Supernatants from ESX-1 mutants (Δ*mycP1*) indicate that most of the secreted proteins are not ESX-1 substrates, and cell lysates were added as controls for lysis.

(B) Immunofluorescence microscopy of 293T cells expressing either the full-length uncleaved *Mtb* Rv1419 protein, or the form predicted to be released after signal peptidase cleavage.

(C) Western blot analysis of U937 macrophages and 293T cells transfected with plasmids encoding the indicated Strep-tagged proteins, showing much higher levels of expression in 293T cells. The majority of proteins were undetectable from U937 cells, but were expressed to sufficient levels in 293T cells to purify and detect host interactors.

(D) Affinity purifications from lysates derived as described in Figure 1 were separated by SDS-PAGE and visualized by silver staining, demonstrating the efficient expression and purification of bacterial proteins. Arrowheads denote the tagged bacterial proteins.

(E) Example of reciprocal co-immunoprecipitation validation studies. 293T cells were co-transfected with plasmids expressing Strep-tagged *Mtb* Rv2074 and with FLAG-tagged versions of GFP (negative control), or each of three putative interactors identified in the PPI map, VPRBP, ATG4B, and VCP. The host proteins were immunoprecipitated with anti-FLAG antibody and Rv2074 was detected by Western blot using anti-Strep antibodies.

Figure S2. Related to Figure 4. The LpqN mutant competes normally in mixed cultures grown in liquid media.

An aliquot of the pool of eight strains used to infect mice for the *in vivo* competition assay (Figure 4) was grown in liquid 7H9 media for 10 days and the relative proportion of each strain was quantified by qPCR of the genomic sequence tag. Mean ± SEM is displayed.

Figure S3. Related to Figure 4. Genetic interactions between Host *Cbl* and *Mtb lpqN* in RAW264.7 cells.

(A) The gene encoding VPS33b, a putative LpqN interactor, was mutated by CRISPR/Cas9 in RAW264.7 cells. These cells were infected with the *Mtb lpqN* mutant strain expressing the *luxBCADE* operon from *Vibrio harveyi,* and bacterial growth was assessed by measuring luminescence. The assay was performed twice with three independent mutant clones and two independent wild-type control clones. Each condition was measured in quadruplicate; mean ± SEM from representative clones are displayed.

(B) The *Cbl*$^{-/-}$ CRISPR/Cas9 clones of RAW264.7 described in Figure 4E were infected with *L. monocytogenes* and CFU enumerated at the indicated times. Mean change in CFU relative to t=0 is shown ± SEM. Representative results from two independent experiments are shown.

(C) Infection as in (B) with *S.* Typhimurium.

Figure S4. Related to Figure 4. Direct interaction between LpqN and CBL *in vitro.*

LpqN-Strep was expressed in *E. coli* BL21DE3 cells, and whole cell lysates were mixed with

similar lysates from cells expressing either CBL-FLAG or GFP-FLAG for 3h at 4˚C to allow for binding. LpqN-Strep was affinity purified using Strep-Tactin resin, and co-purification of the host fusion proteins were detected by western blotting using anti-FLAG antibodies.