

Screening strategy

Grow J774 macrophages
in 96-well plates

Grow 2660 *Mtb* mutants and controls
in 96-well plates in duplicate

Measure OD600 of mutants

Transfer 2660 mutants and controls
in quadruplicates to macrophage plates

2406 analyzed by imaging phenotype (discarded 254 mutants with $OD_{600} < 0.05$)

361 mutants with intracellular growth defect

Perform multiplex cytokine
Luminex analysis on
361 mutants and WT

**113 mutants selected based
on altered cytokine secretion
from WT infected cells**

Confirm CFU of 71
mutant hits

47 mutant hits confirmed by CFU
24 mutant hits did not confirm by CFU
15 mutant hits literature confirmed

Perform multiparametric clustering analysis based on cytokine and imaging profiles