Redelman-Sidi et al

Supplementary Methods

Pharmacologic inhibitors

The pharmacologic inhibitors used in this study are listed in the table below:

Name	Obtained from	Molecular Target	Reference
Wortmannin	Sigma-Aldrich	PI3K	(1)
Ethyl-isopropyl amiloride (EIPA)	Sigma-Aldrich	Na+/H+ pump	(2)
Rapamycin	Sigma-Aldrich	mTOR	(3)
Cytochalasin D	Sigma-Aldrich	Actin polymerization inhibitor	(4)
Genistein	Sigma-Aldrich	Tyrosine Kinases	(5)
Staurosporine	Sigma-Aldrich	Protein Kinases	(6)
Blebbistatin	Sigma-Aldrich	Non-muscle myosin	(7)
IPA-3	Sigma-Aldrich	Pak1	(8)
Gö-6983	Sigma-Aldrich	Protein Kinase C	(9)
Akt inhibitor XIII	Calbiochem	Akt	(10)
Y-27632	Gift from Dr. Michael Overholtzer	RhoA	(11)
BKM120	Selleck Chemicals	PI3K	(12)

Plasmids and transfections

The shRNA sequences used in this study were as follows:

PTEN shRNA: 5’-CCGGCCACAGCTAGAACTTATCAAACTCGAGTTTGATAAGTTCTAGCTGT-3’

Pak1 shRNA#1: 5’-CCGGGCATTCGAACCAGGTCATTCACTCGAGTGAATGACCTGGTTCGAATGCTTTTTTG-3’

Pak1 shRNA#2: 5’-CCGGGAGCTGCTACAGCATCAATTCCTCGAGGAATTGATGCTGTAGCAGCTCTTTTTTG-3’

Pak2 shRNA#1: 5’-CCGGCAGACCTCCAATATCACCAAACTCGAGTTTGGTGATATTGGAGGTCTGTTTTT-3’

Pak2 shRNA#2: 5’-CCGGGTCTCTGGGTATCATGGCTATCTCGAGATAGCCATGATACCCAGAGACTTTTT-3’

PDK1 shRNA#1: 5’-CCGGCCAGGGTGTGATTGAATACAACTCGAGTTGTATTCAATCACACCCTGGTTTTT-3’

PDK1 shRNA#2: 5’-CCGGGAAGTAGAAGTCTACCATATTCTCGAGAATATGGTAGACTTCTACTTCTTTTTTG-3’

CHC shRNA#1: 5’-CCGGCGTGTTCTTGTAACCTTTATTCTCGAGAATAAAGGTTACAAGAACACGTTTTT-3’

CHC shRNA#2: 5’-CCGGGCCCAAATGTTAGTTCAAGATCTCGAGATCTTGAACTAACATTTGGGCTTTTT-3’

CHC shRNA#3: 5’-CCGGCCTGTGTAGATGGGAAAGAATCTCGAGATTCTTTCCCATCTACACAGGTTTTT-3’

The lentiviral constructs pQCXIP-Rac1 (T17N) (13), pQCXIP-Cdc42 (T17N) (14), pQCXIP-Rac1 (Q61L), and pQCXIP-Cdc42 (Q61L) were a kind gift from Dr. Alan Hall. pcDNA3.1-PTEN (wild type) and PTEN (C124S) (15) were provided by Dr. Xuejun Jiang. PTEN cDNA was amplified from these constructs and cloned into pQCXIP-IRES-puro using the BamHI and EcoRI restriction sites. The polyadenylation site AATAAA in both inserts was mutated synonymously to AACAAG. PCMV6-Pak1 (WT), Pak1 (T423E), Pak1 (L107F) and Pak1 (K299R) (16) were a generous gift from Dr. Jonathan Chernoff. The constructs were cut with the restriction enzymes BamHI and EcoRI, and the Pak1 cDNA fragment was cloned into pQCXIP-IRES-puro using the BamHI and EcoRI restriction sites. RCAS-K-ras (G12D) (17) and PWZL-H-ras (G12V) (18) were kindly given by Dr. Eric Holland. K-ras and H-ras cDNA was amplified from these constructs and cloned into pQCXIP-IRES-puro using the BamHI and EcoRI restriction sites. All amplified inserts were sequenced prior to cloning to confirm that no mutations arose during amplification. The empty lentivirus pQCXIP-IRES-puro was used as control for overexpression constructs. The dynamin constructs pEGFP-dynamin2aa (WT) and pEGFP-dynamin2aa (K44A) (19) were a kind gift from Dr. Mark McNiven. Cells were transiently transfected with the dynamin constructs in 6-well plates, using X-treme Gene HP DNA transfection reagent (Roche) as per the manufacturer’s instructions. Infection with BCG was carried out

24 hours after transfection. As these constructs express GFP, BCG-mCherry was used in these experiments.

References:

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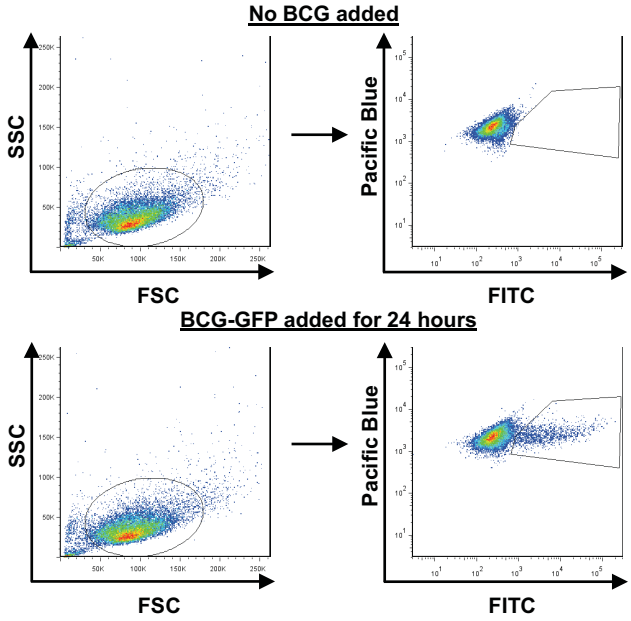

Figure S1: Gating strategy for flow cytometry
Representative flow-cytometry analysis showing the gating strategy to determine the percent of BCG-GFP infected cells. Cells were pre-gated in an FSC/SSC scattergram. Because of auto-fluorescence in the cell lines used, an empty channel (Pacific-blue) was used to facilitate discrimination of GFP-positive events. In each experiment uninfected cells were used as a control to optimize gating.

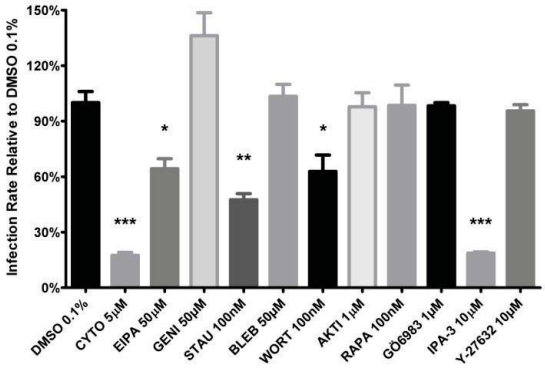

Figure S2: Effect of small-molecule inhibitors on the uptake of fixed BCG

UM-UC-3 was pretreated for one hour with the specified small molecule inhibitors at the stated concentration. BCG-GFP was fixed in 4% PFA, washed twice, and added to the cells for 4 hours in the presence of the inhibitors. At the end of the incubation period the cells were washed, and BCG uptake measured by flow-cytometry. For each inhibitor, the percent of cells infected by BCG-GFP is shown as compared with percent of infected cells in the presence of DMSO (vehicle control). Killing of the BCG by the fixative was confirmed by plating the fixed BCG on 7H10 plates and observing no colonies.

DMSO: Dimethyl sulfoxide. CYTO: Cytochalasin D. EIPA: 5-(N-Ethyl-N-isopropyl) amiloride. GENI: Genistein. STAU: Staurosporine. BLEB: Blebbistatin. WORT: Wortmannin. AKTI: Akt inhibitor XIII. RAPA: Rapamycin

*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$ compared with DMSO

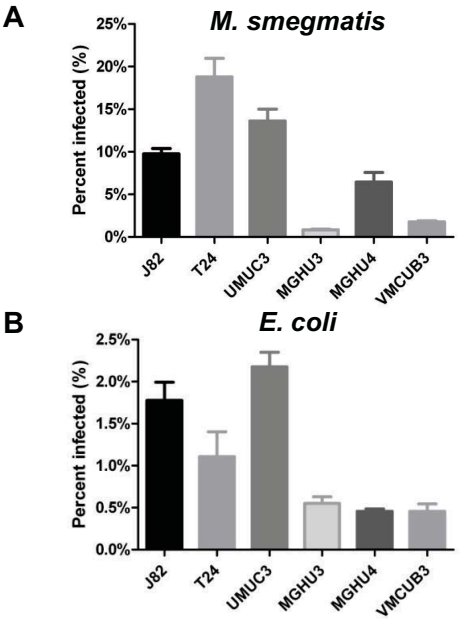

Figure S3: Uptake of organisms other than BCG

The cell lines J82, T24, UM-UC-3, MGH-U3, MGH-U4, and VMCUB-3 were incubated with either: A. GFP-expressing *M. smegmatis* (MOI 10:1) for 4 hrs; or B. GFP-expressing *E. coli* (MOI 10:1) for 24 hrs. At the end of the incubation period uptake of fluorescent bacteria was measured by flow-cytometry. The data corresponds to the mean of three independent experiments ± SEM.

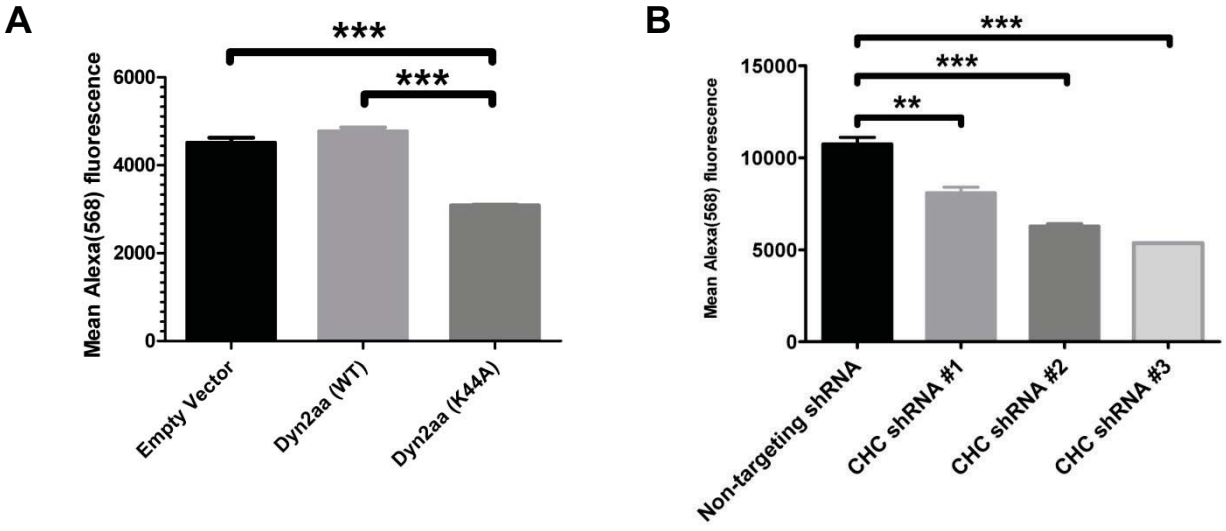

Figure S4: Effect of dynamin constructs and clathrin shRNA on uptake of fluorescent transferrin

A. T24 was transiently transfected with empty vector or with GFP-tagged dynamin 2 (aa) wild type or GFP-tagged dynamin 2 (aa) (K44A). 24 hours after transfection the cells were incubated with fluorescent transferrin for 15 minutes and uptake was measured by flow-cytometry. Shown is the mean Alexa 568 fluorescence for each sample. The data corresponds to the mean of three independent experiments ± SEM.

B. T24 was stably transduced with lentiviruses bearing non-targeting or three shRNAs targeting the clathrin heavy chain. Cells were incubated with fluorescent transferrin for 15 minutes and uptake was measured by flow-cytometry. Shown is the mean Alexa 568 fluorescence for each sample. The data corresponds to the mean of three independent experiments ± SEM.

***, $P < 0.0005$. CHC: clathrin heavy chain

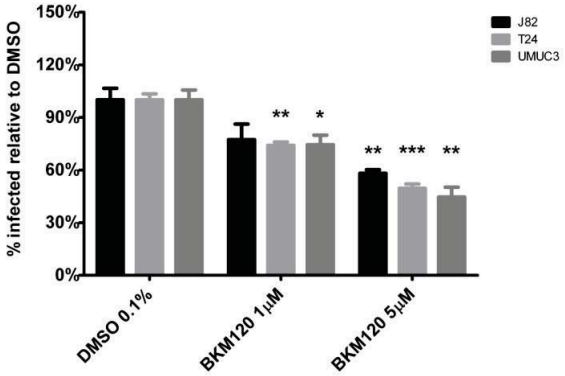

Figure S5: Effect of BKM120 on BCG uptake

The cell lines J82, T24, and UM-UC-3 were pretreated with the PI3K inhibitor BKM-120 at the stated concentrations. After 1 hour BCG-GFP was added, and incubated with the cells for 4 hours in the presence of the inhibitor. At the end of the incubation period the cells were washed, and BCG uptake was measured by flow-cytometry. The percent of cells infected by BCG-GFP is shown as compared with percent of infected cells in the presence of DMSO (vehicle control). The data corresponds to the mean of three independent experiments ± SEM. *, $P < 0.05$; **, $P < 0.005$ compared with DMSO.

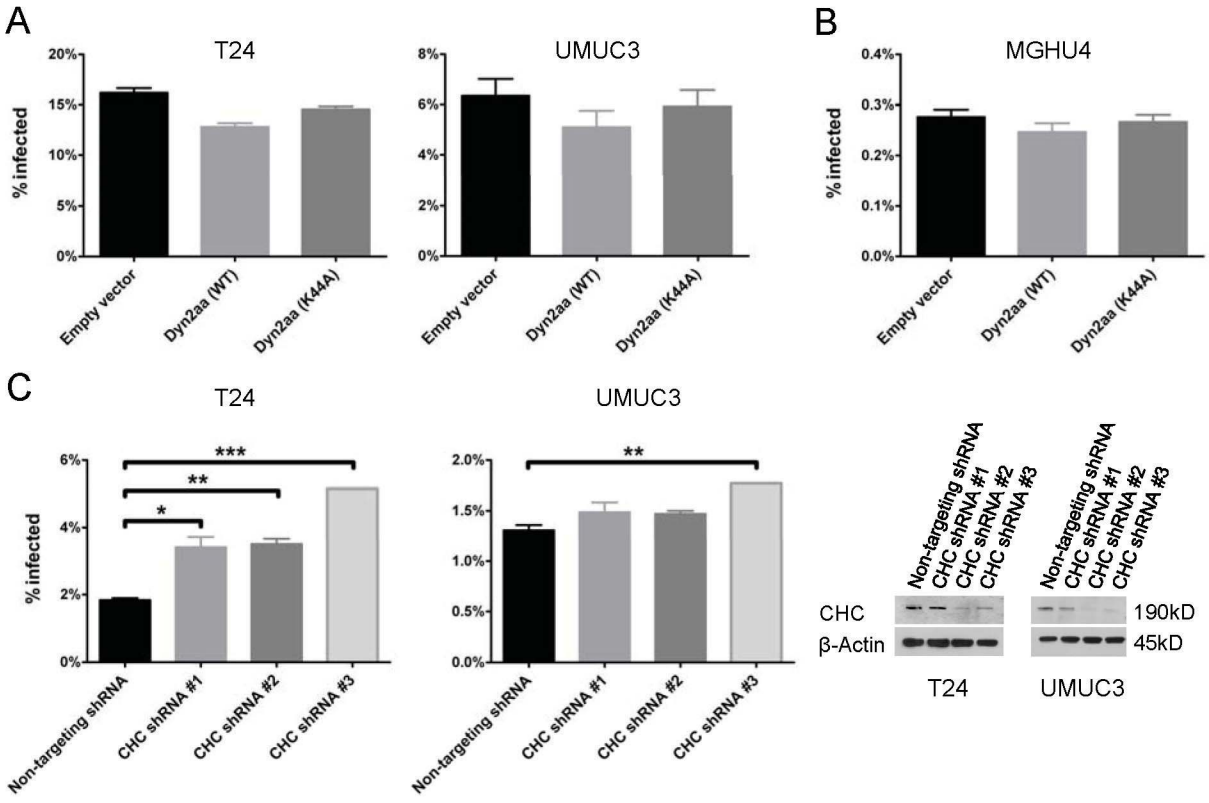

Figure S6: BCG uptake is independent of dynamin and clathrin

A. T24 and UM-UC-3 were transiently transfected with empty vector or with GFP-tagged dynamin2 or GFP-tagged dynamin2 (K44A) (a DN form of dynamin). 24 hours after transfection the cells were washed and infected with BCG-mCherry at an MOI of 10:1. Uptake of BCG by cells expressing the GFP-tagged protein was measured after 24 hours using flow-cytometry. The data corresponds to the mean of three independent experiments ± SEM.

B. MGH-U4 was transiently transfected with empty vector or with GFP-tagged dynamin2 wild type or GFP-tagged dynamin2 (K44A). 24 hours after transfection the cells were washed and infected with BCG-mCherry at an MOI of 10:1. Uptake of BCG by cells containing the GFP-tagged protein was measured after 24 hours using flow-cytometry. The graphed data is the mean of three independent experiments and error bars are ± SEM.

C. T24 and UM-UC-3 were stably transduced with lentiviruses bearing non-targeting or three shRNAs targeting the clathrin heavy chain (CHC). Cells were incubated with BCG-GFP for 4 hours, and uptake of BCG was measured by flow-cytometry. Knock-down of CHC was demonstrated by Western blotting. The graphed data is the mean of three independent experiments and error bars are ± SEM.

* $P < 0.05$; ** $P < 0.005$; *** $P < 0.0005$. CHC: clathrin heavy chain