

Drug discovery for Tuberculosis

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Institut Pasteur Korea

We are Pasteurians fighting disease in Korea for all mankind

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Introduction

Part 1.

TUBERCULOSIS AND DISEASE PROGRESSION

Facts on Tuberculosis

Facts about TB (2017)

140,000 Children

1.6 million deaths

1st killer of HIV patients

400,000 HIV+

1 million children

10.4 million infections

1.1 million HIV+

58% in SE Asia

50% success rate

480,000 MDR-TB

3x increase since 2009

10% XDR

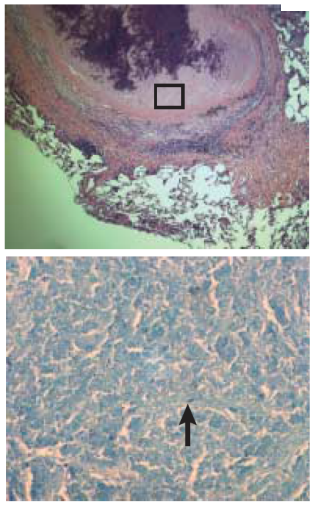
STR (1946). Resistance: 1947
BDQ (2013). Resistance: 2015

Disease progression

Primo infection

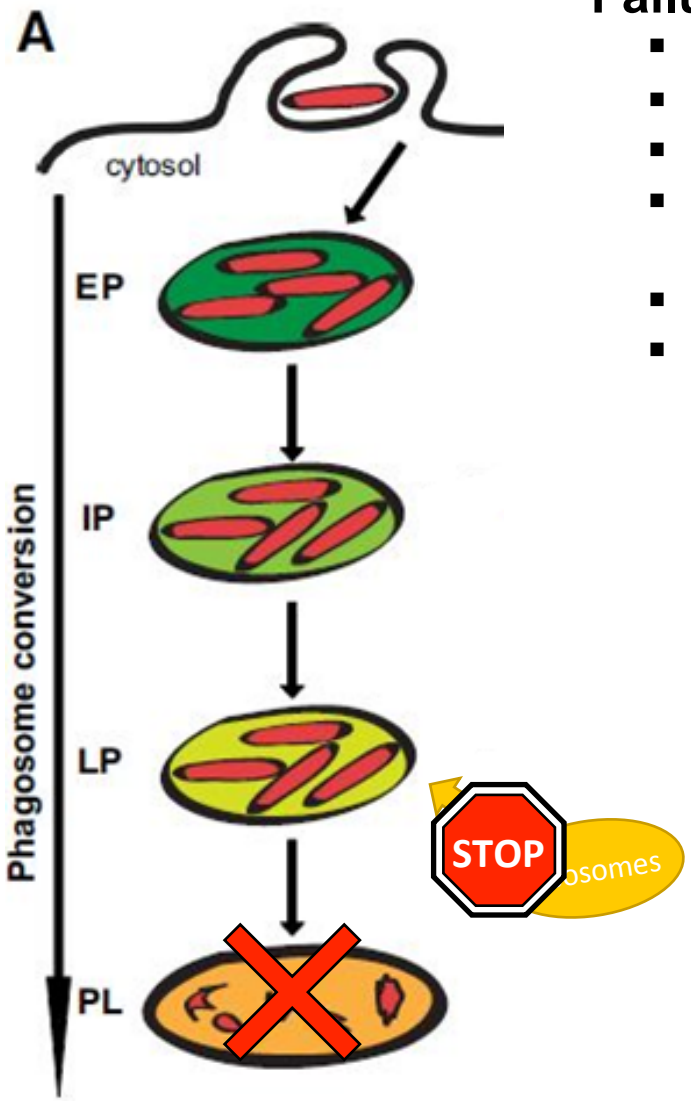
1/3 of the global population!

1.6 million deaths



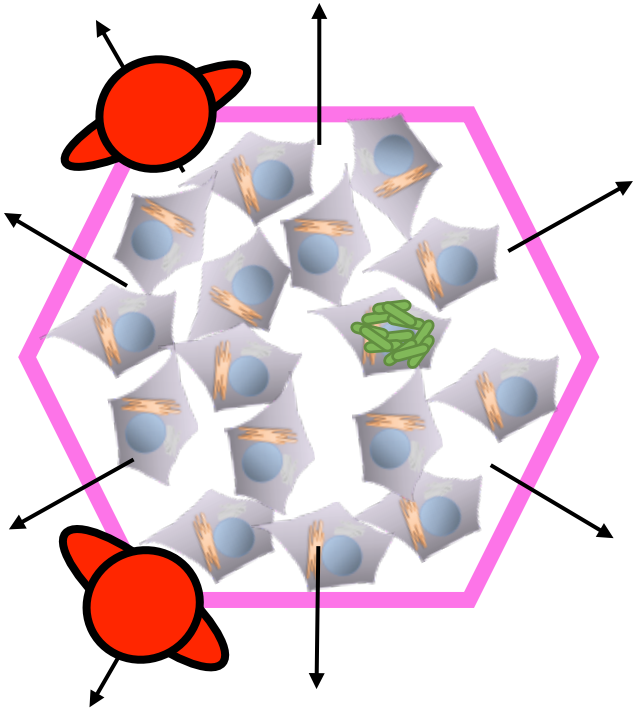
Boshoff *et al.* **2005** Nat. Rev. Microbio. 3: 70-80

Phagosomal escape



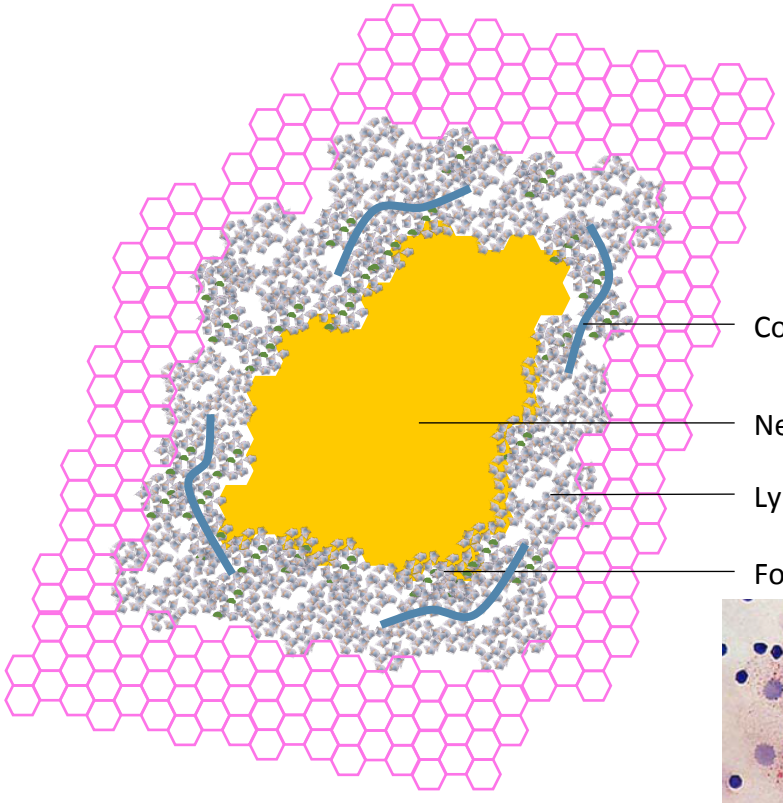
Failure of bacterial killing by macrophages

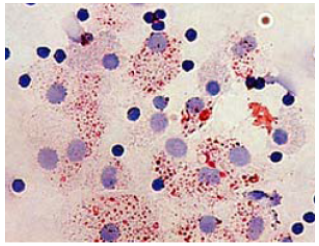
- Bacterial replication
- Cell death
- Inflammation
- Large influx of cells
  - Mostly macrophages and neutrophils
- High Local pressure on epithelium and capillary
- Collapsing of capillary and alveolus



Granuloma

Granuloma with a necrotic core
Necrotic core surrounded by foamy macrophages

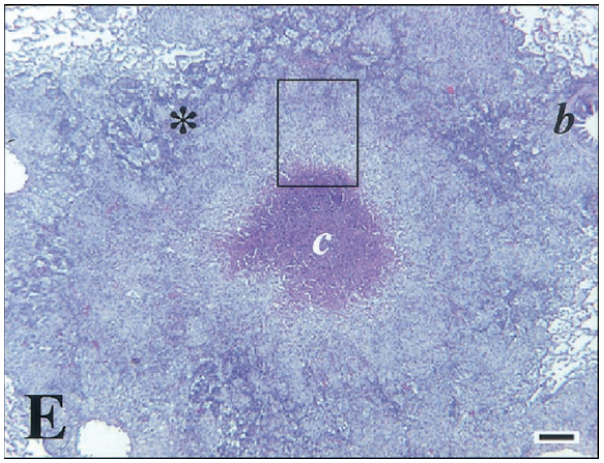




Peyron *et al.* **2008** PLoS Pathog 4(11): e1000204

Stage III
31 days in guinea pig model
Large amount of lymphocytes in the edge
Bacterial propagation stopped

Turner *et al.* **2003** Infect Immun (71) 864-871



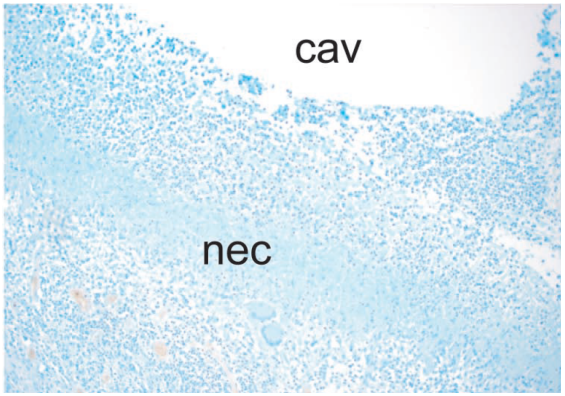
c, necrotic core
b, bronchiole
* Lymphocytes

100 µm

10% of TB cases: cavitation and propagation

Case courtesy of Dr Frank Gaillard, Radiopaedia.org

Boshoff *et al.* **2005** Nat Rev Microbio (3) 70-80

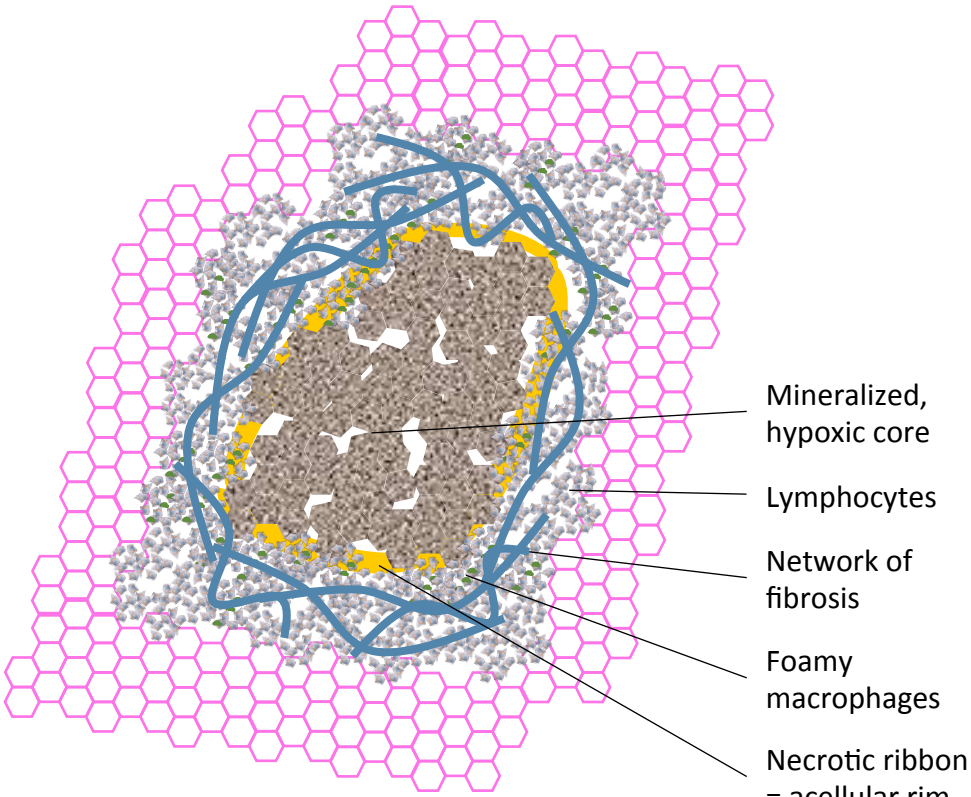


cav, cavity
nec, necrotic area

Kaplan *et al.* **2003** Infec Immun (71) 7099–7108

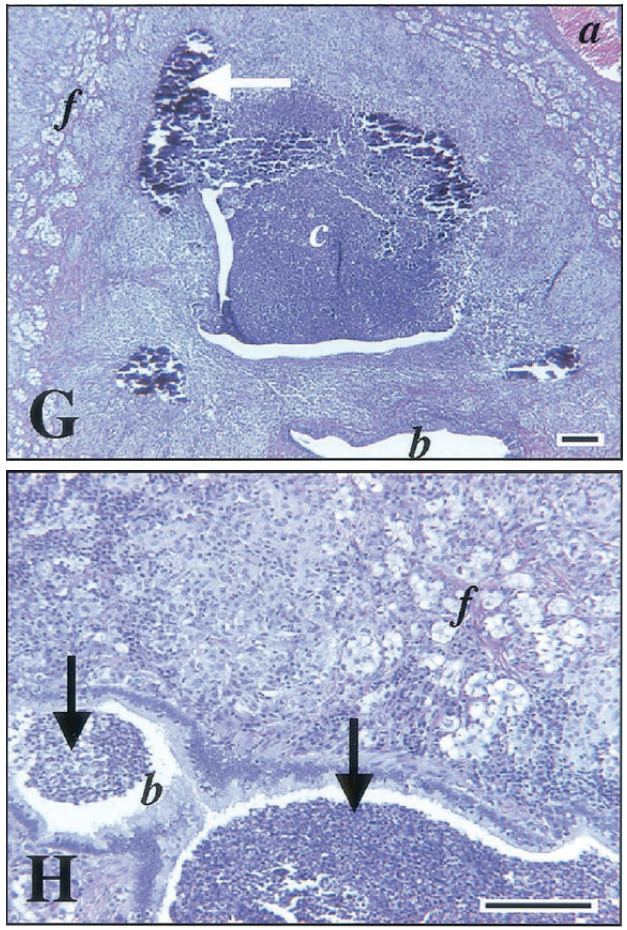
90% of cases: Granuloma calcification

Mature granuloma with a mineralized core
Surrounded by foamy macrophages and lymphocytes



Stage IV
93 days in guinea pig model
Fibrosis and mineralization, healing process by calcification

Turner *et al.* **2003** Infect Immun (71) 864-871



c, mineralized core
f, fibrosis
a, artery
b, bronchiole

100 µm

b, bronchiole
f, fibrosis

100 µm

Arrows: Necrotic debris

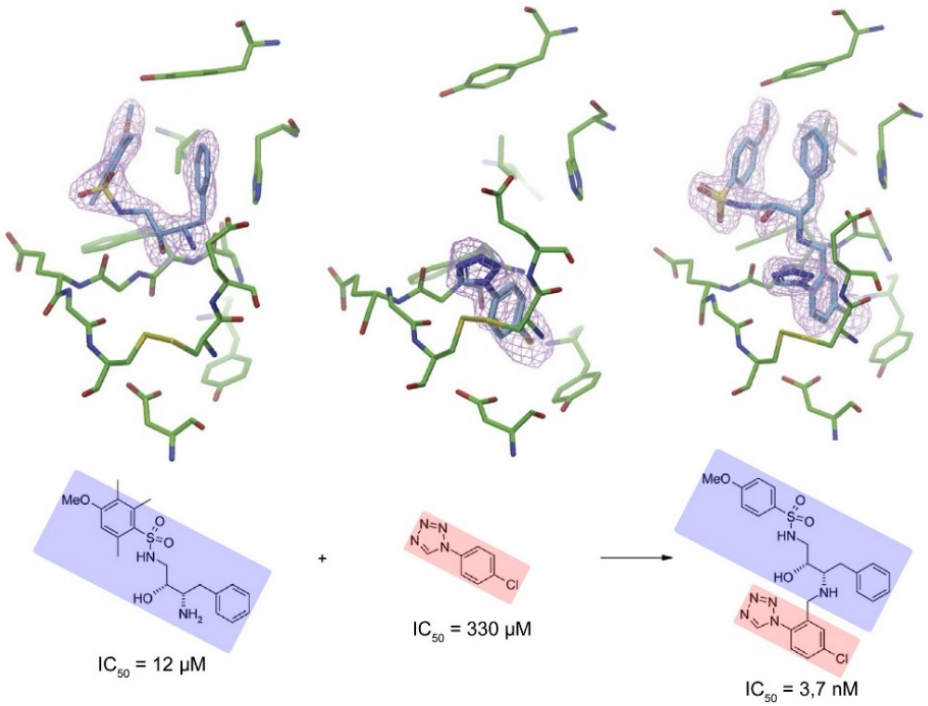
Part 1.

DRUG DISCOVERY IN TB FIELD

Screening strategies

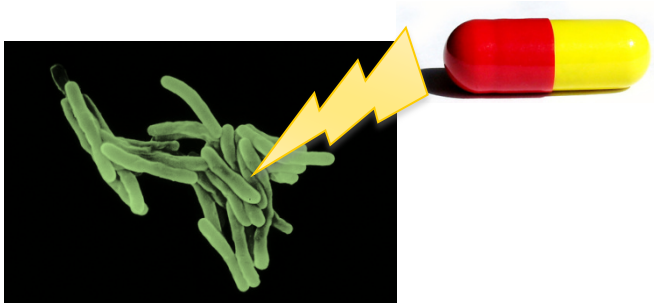
Target-based screening

- Enzymatic assays
- Fragment / *in silico* approaches
- Need a protein/crystal/model!

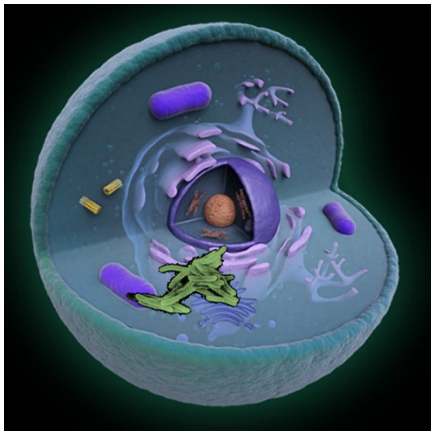


Target-free / Phenotypic screening

- Bacterial replication / survival assays

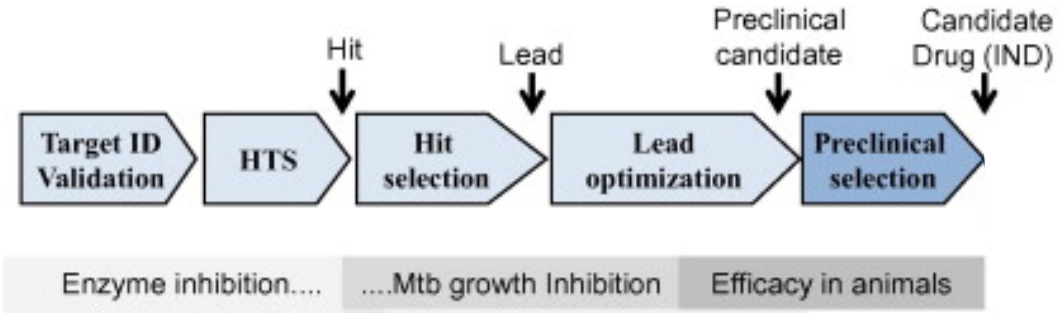


- Infected cells



Drug discovery pipeline

Target-based screening



Targe

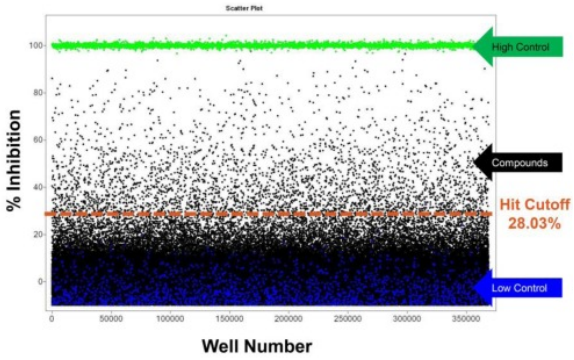
Hit selection

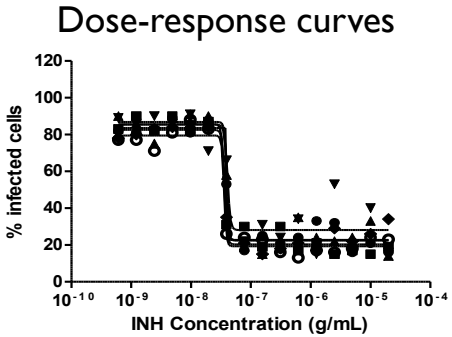
Primary screening

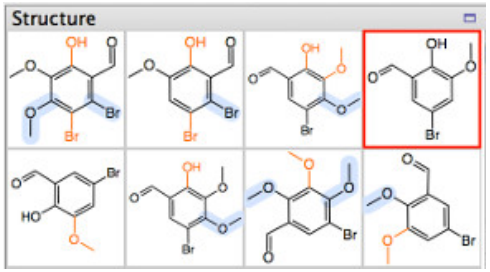
- Single dose
- Select compounds with activity > Threshold

Secondary screening

- Cherry-picking & validation by dose-response curve (DRC)
- Repurchase & Resynthesize, validate again by DRC



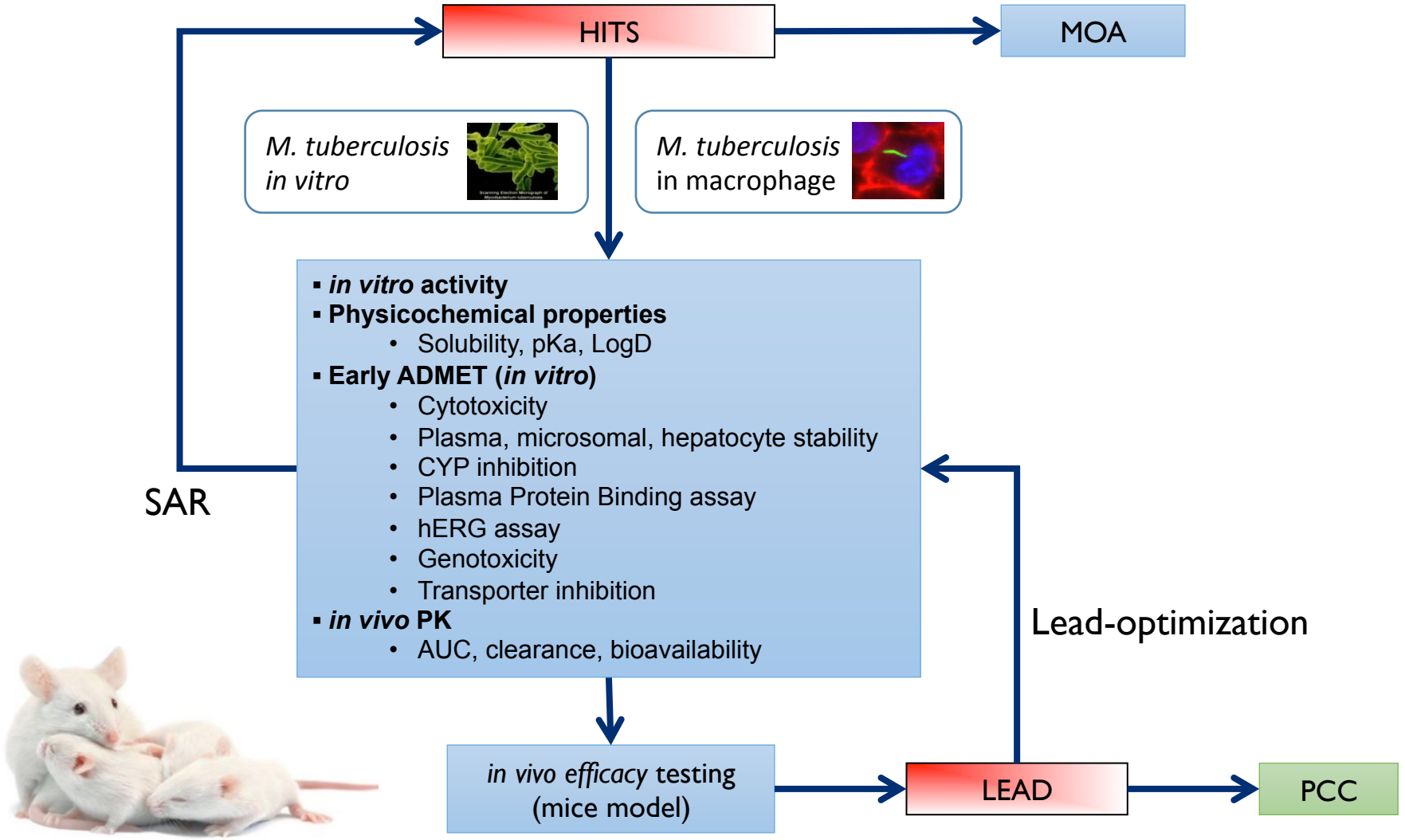




Hits

- Structure and activity confirmed
- Clustering & prioritization

Hit-to-lead

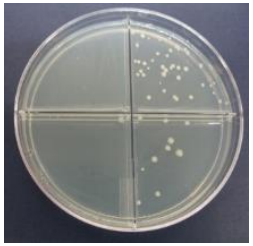

HITS
MOA
M. tuberculosis in vitro
M. tuberculosis in macrophage
▪ in vitro activity
▪ Physicochemical properties
• Solubility, pKa, LogD
▪ Early ADMET (in vitro)
• Cytotoxicity
• Plasma, microsomal, hepatocyte stability
• CYP inhibition
• Plasma Protein Binding assay
• hERG assay
• Genotoxicity
• Transporter inhibition
▪ in vivo PK
• AUC, clearance, bioavailability
SAR
Lead-optimization
in vivo efficacy testing (mice model)
LEAD
PCC

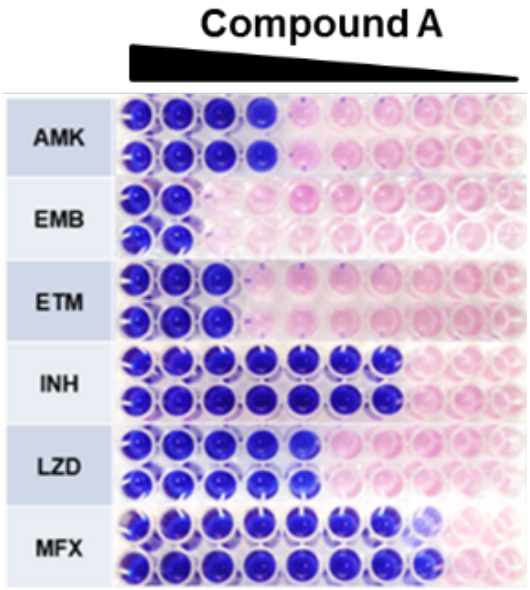
Phenotypic screening assays for Tuberculosis research

Phenotypic assays – Quantification?

Wild-Type bacteria

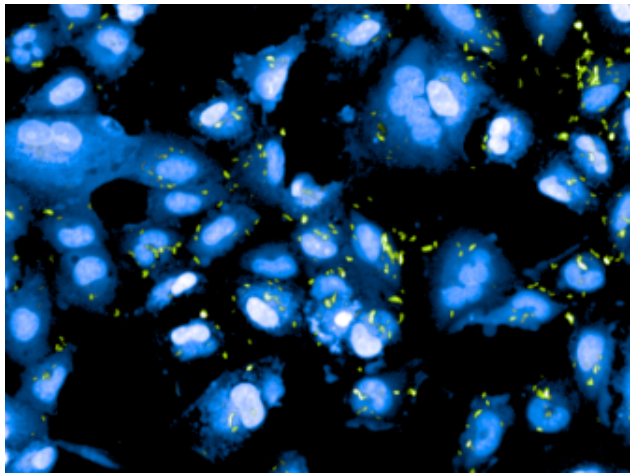
- **CFU counting**
  - Agar
  - Bactec MGIT
- **OD reading**
- **Resazurin**





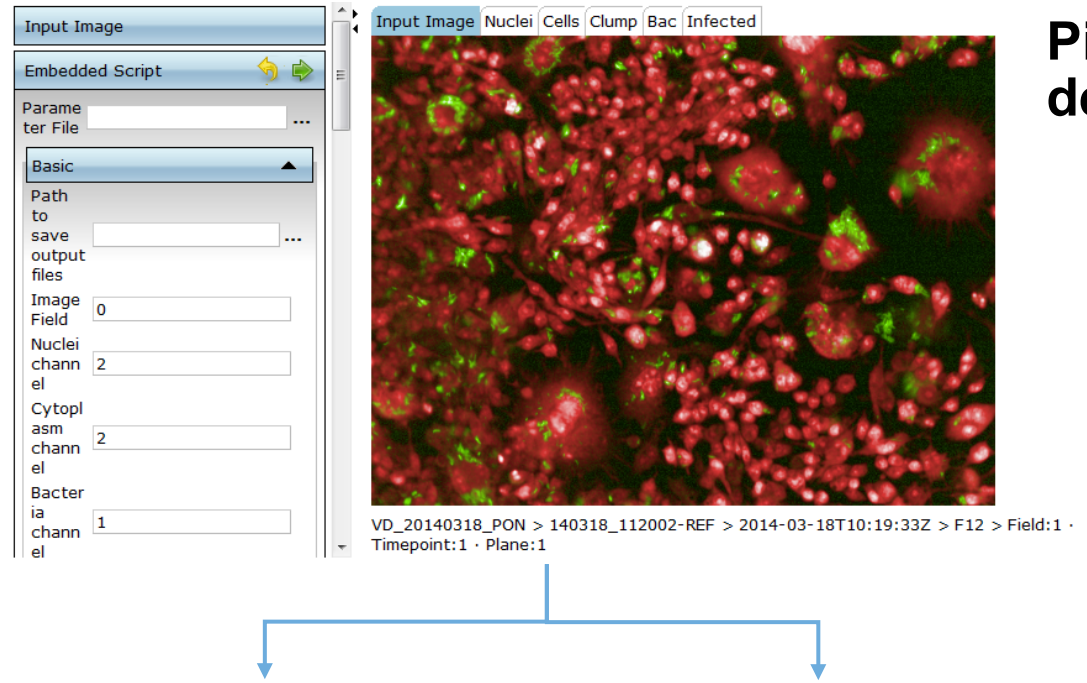
Reporter strains

- **Luminescence, fluorescence (GFP, RFP…)**
- **Constitutive vs stimuli-driven expression**



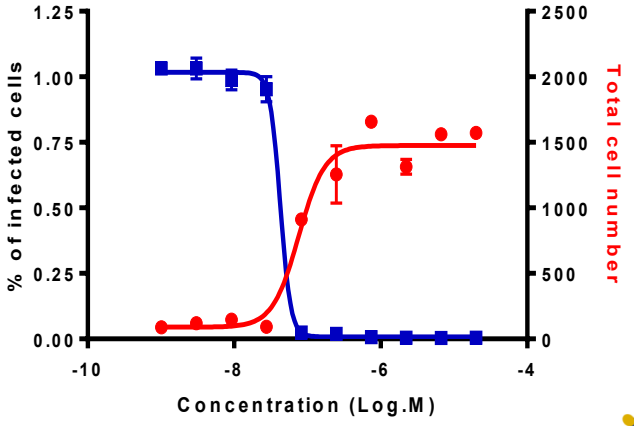
Dyes or antibodies

Multiparametric image analysis

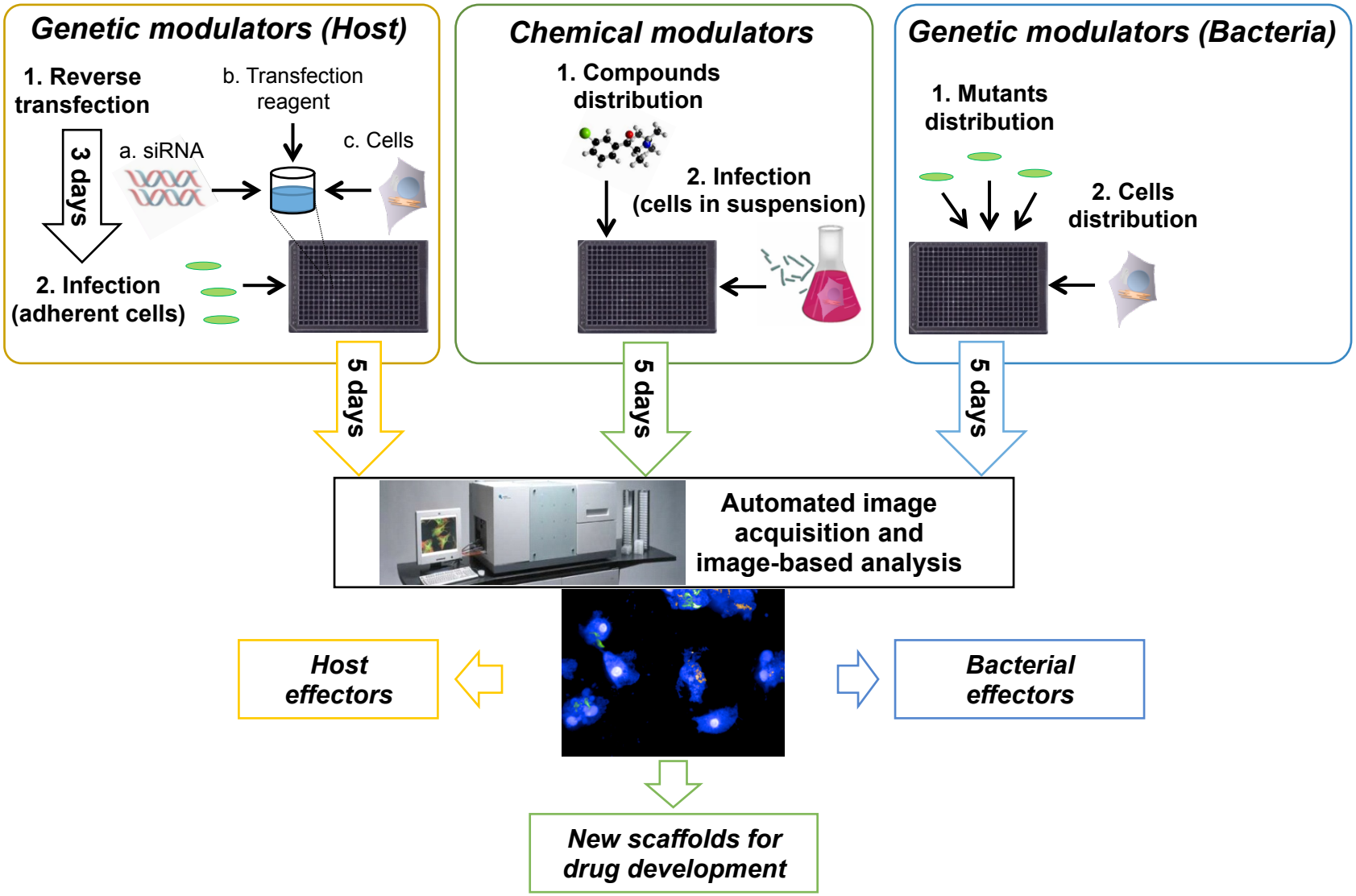


Pictures analyzed by dedicated scripts

- Definition of two Populations: cells & bacteria
- Several parameters used to quantify compound efficiency:
  - Number of cells
  - Total area of bacteria (px)
  - Average area of bacteria per infected cells (px)
  - Ratio of infected cells



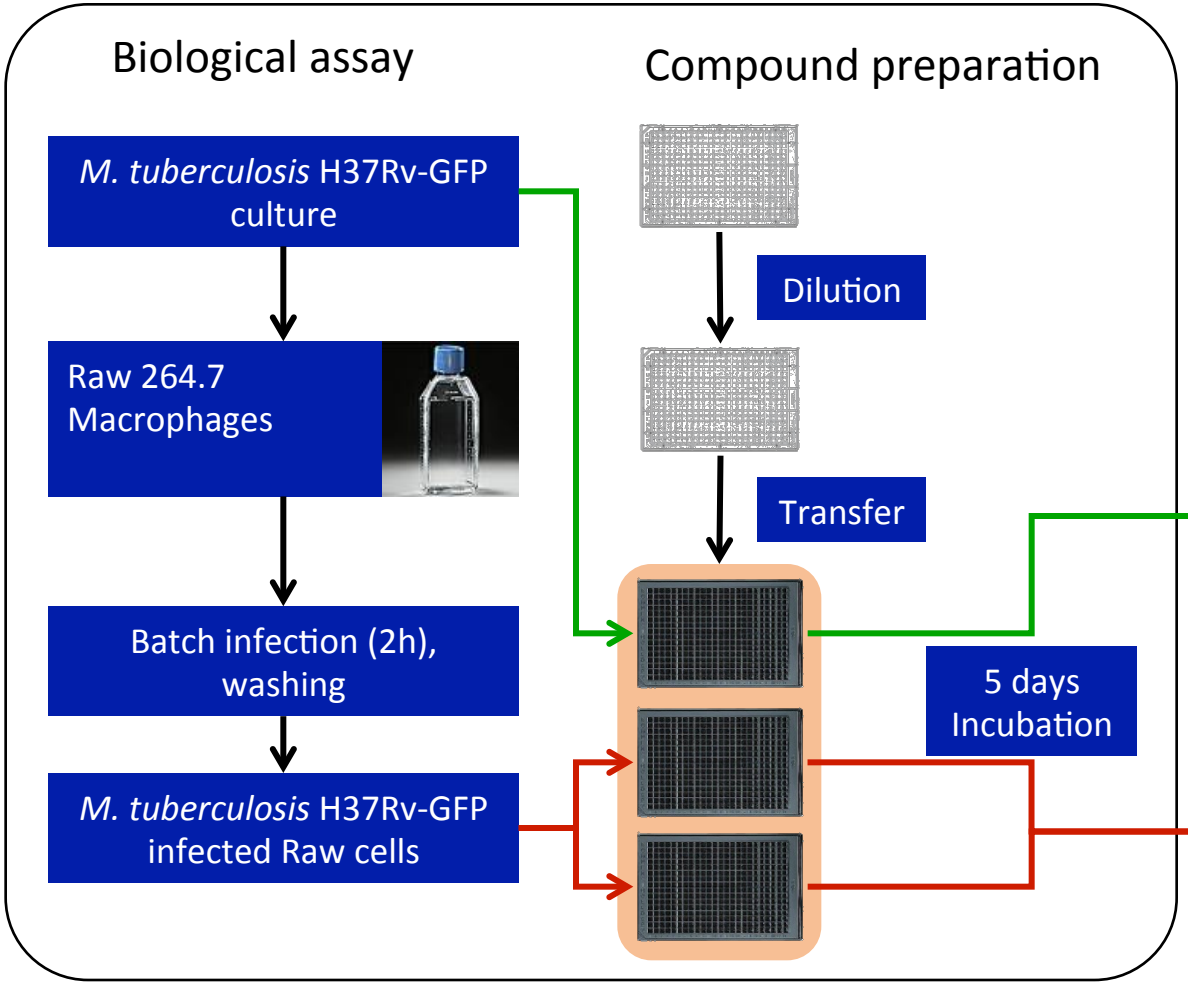
Phenotypic screening assays - overview

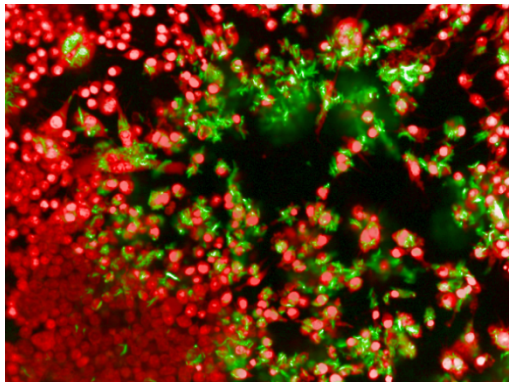


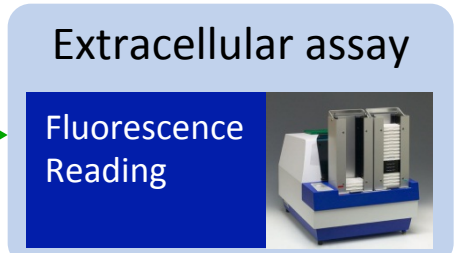
Part 1.

CHEMICAL MODULATORS

Chemical modulators - Screening process





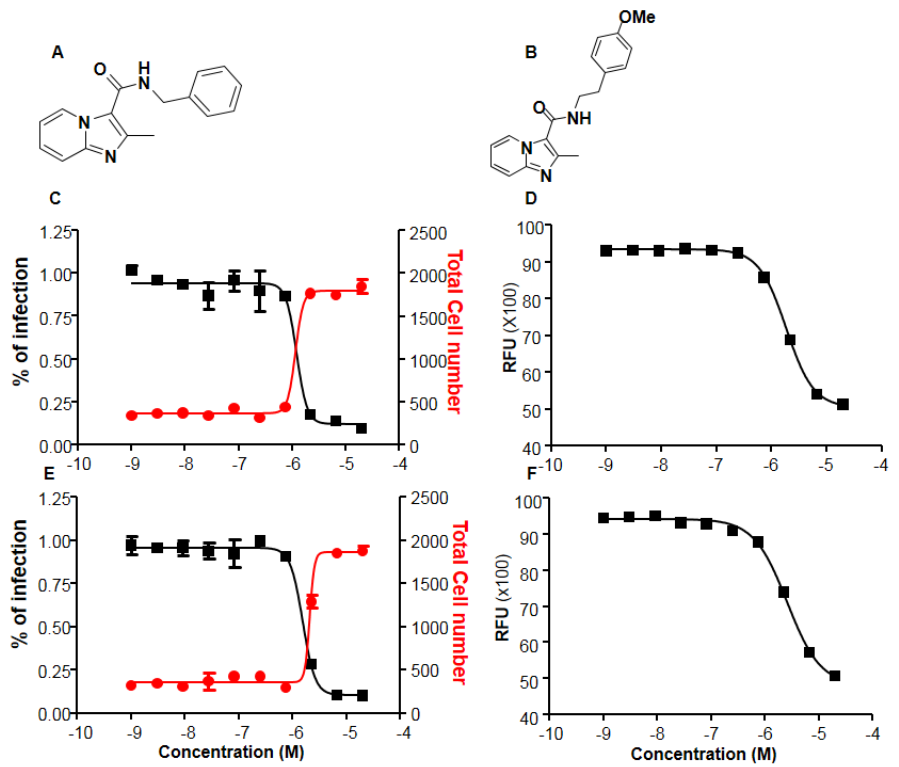


Christophe *et al.* **2010** Future Med. Chem. 2: 1283-1293

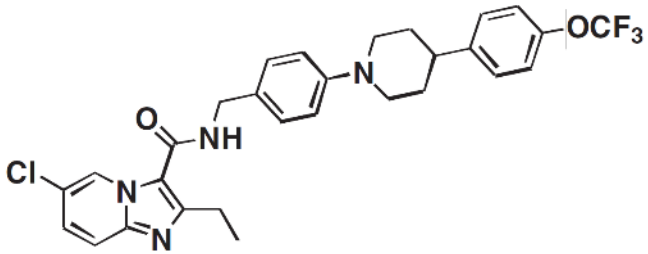
Chemical modulators: Q203

Q203

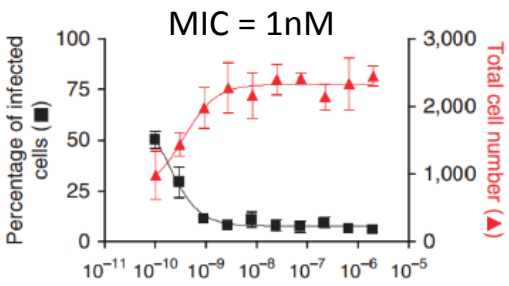
- Imidazo[1,2-a]pyridine amide (IPA) Family
- From a screening of 120,000 compounds
- Identified by infected macrophage assay (QIM)
- Hit compounds were active in the µM range

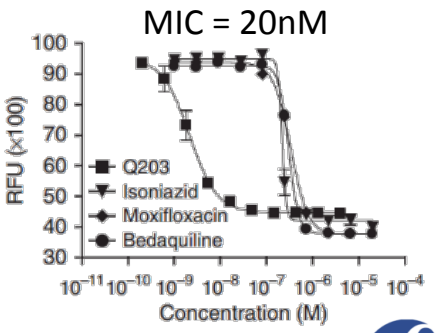


Optimizations gave preclinical candidate



Pethe *et al.* **2013** Nat. Med

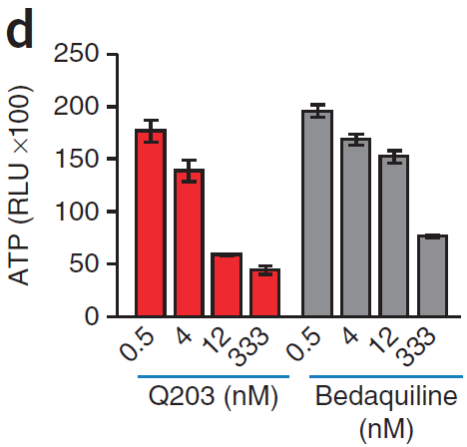




Q203 - Overview

Triggers ATP depletion

- More potent than BDQ
- Active against MDR-XDR

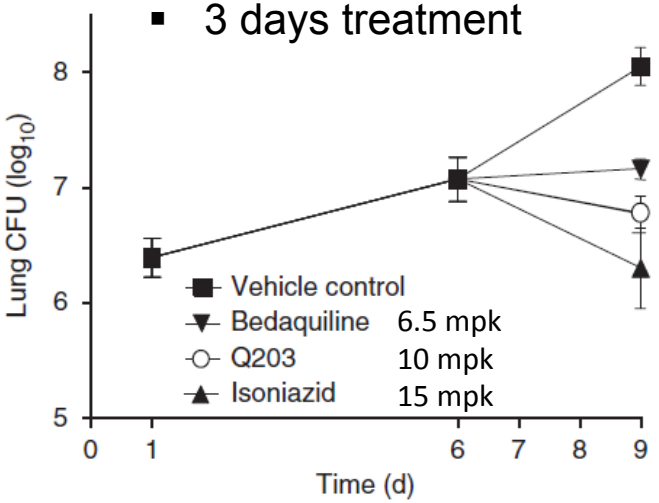


Type	#	Family	INH	Rif	Strept	Oflox	MIC_{90} (nM)
MDR	4	Beijing	R	R	R	S	0.43
	5	Beijing	R	R	S	R	<0.43
	6	Beijing	R	R	S	R	<0.43
	7	Beijing	R	R	S	R	<0.43
	8	-	R	R	S	R	<0.43
	9	Beijing	R	R	R	S	<0.43
	10	Beijing	R	R	S	S	<0.43
	11	Beijing	R	R	S	R	<0.43
	12	Beijing	R	R	S	R	0.98
	13	Beijing	R	R	S	S	<0.43
	14	Beijing	R	R	S	S	0.88
	15	Beijing	R	R	S	R	3.51
	16	Beijing	R	R	R	S	<0.43
	17	Beijing	R	R	R	R	0.43
	18	Beijing	R	R	R	R	<0.43
	19	-	R	R	R	R	<0.43
	20	-	R	R	R	R	<0.43
	21	Beijing	R	R	R	R	<0.43
	22		R	R	R	R	<0.43
	23		R	R	R	R	<0.43
XDR	24	Beijing	R	R	R	R	<0.43
	25	Beijing	R	R	R	R	<0.43
	26	-	R	R	R	R	<0.43
	27	Bei	R	R	R	R	7.02
	28		R	R	R	R	<0.43
			R			R	7.02
		Bei	R			R	<0.43
		Bei	R			R	28

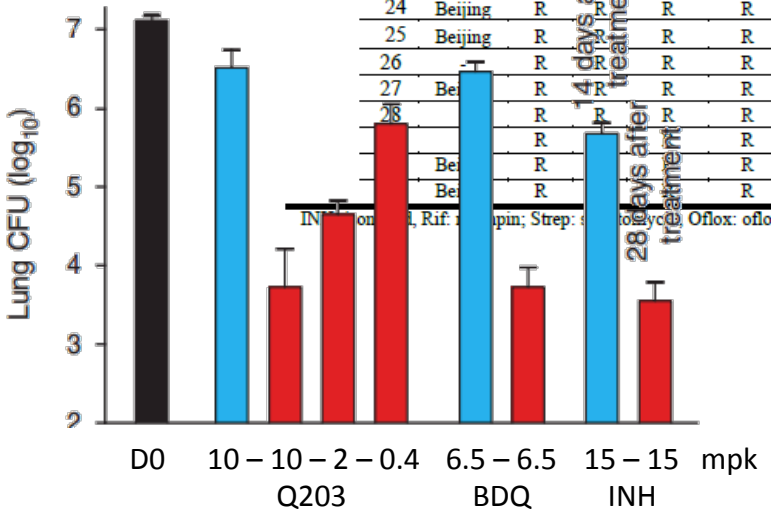
INH: [illegible]onid, Rif: [illegible]pin; Strep: [illegible]tomycin, Oflox: ofloxacin

In vivo efficacy in mice

- Acute model (GSK)
  - 3 days treatment



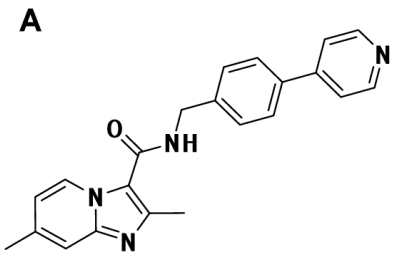
- Chronic model
  - Gavage, 5x / weeks



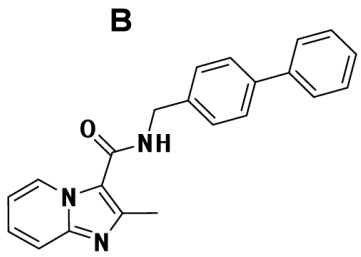
Q203 & QcrB

Target identification

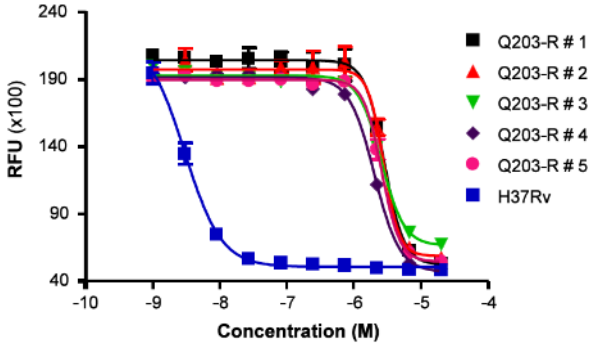
- Generation of spontaneous resistant mutants



IC_{50} = 10 nM



IC_{50} = 5 nM

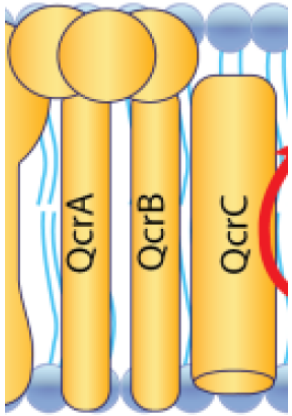


Mutation freq.
2.4×10^{-8}

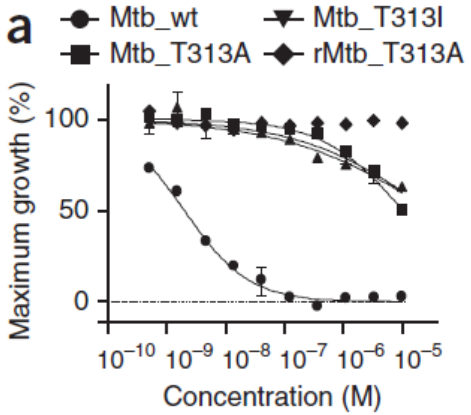
- Whole genome sequencing: qcrB

H37Rv	301	SAGSQPDFYMMWTEGLARI	319
CDC1551	301	SAGSQPDFYMMWTEGLARI	319
W4	301	SAGSQPDFYMMWTEGLARI	319
XDR #27	301	SAGSQPDFYMMWTEGLARI	319
XDR #29	301	SAGSQPDFYMMWTEGLARI	319
XDR #31	301	SAGSQPDFYMMWTEGLARI	319
Q203-R #1	301	SAGSQPDFYMMWAEGLARI	319
Q203-R #2	301	SAGSQPDFYMMWAEGLARI	319
Q203-R #3	301	SAGSQPDFYMMWAEGLARI	319
Q203-R #4	301	SAGSQPDFYMMWAEGLARI	319
Q203-R #5	301	SAGSQPDFYMMWAEGLARI	319

Pethe *et al.* **2013** Nat. Med



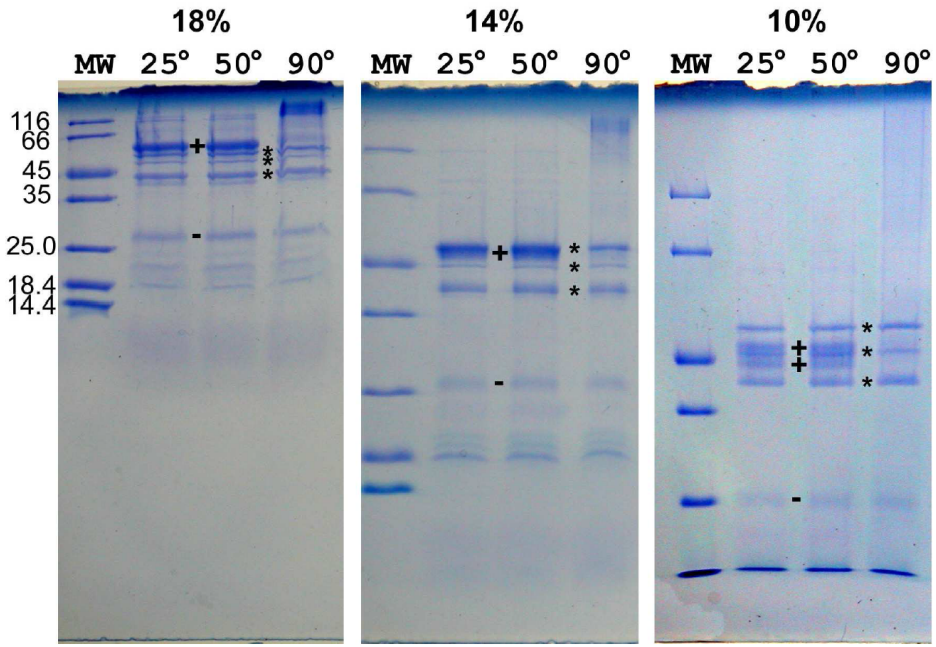
Respiratory complex III
(Cytochrome *bc* oxidase)

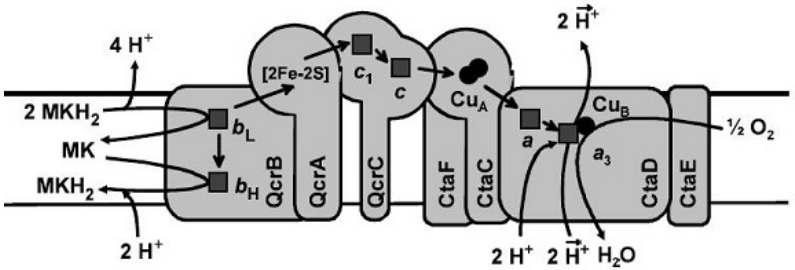


Q203 & QcrB

Target purification

- Collaboration with Dr. E. Berry (Upstate Univ)
- Hybrid supercomplex
  - *M. smegmatis* cytochrome aa_3 + *M. tuberculosis* QcrCAB (bcc)





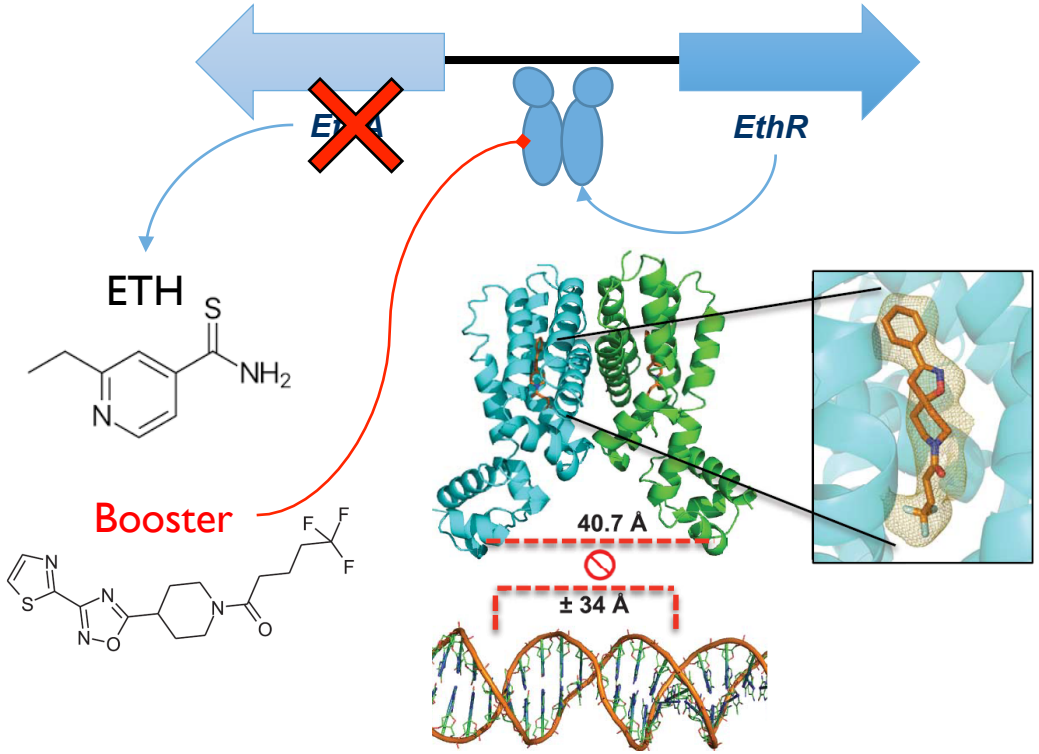
Kim *et al.* **2015** J Biol Chem

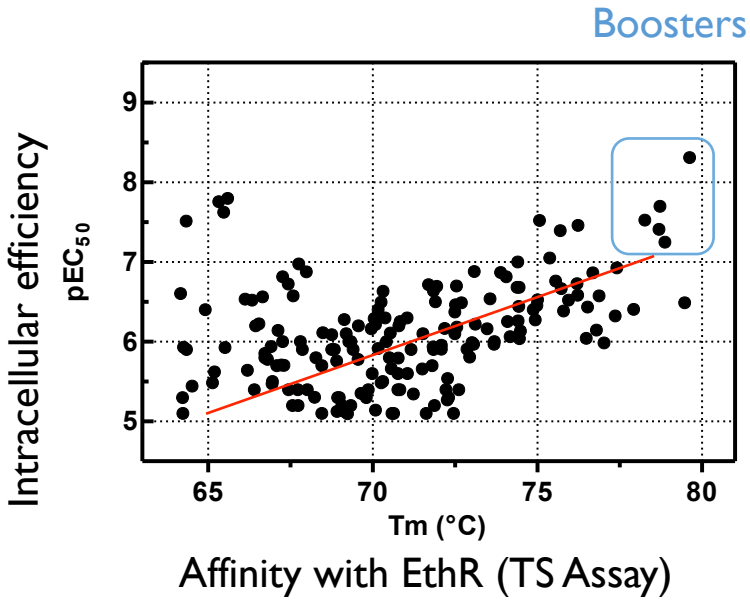
+, QcrB, Cox1; -, QcrC; *, QcrA, Cox2, Rieske

Chemical modulators - optimization

Adding value by combining screening strategies

- Finding compounds active in multiple models



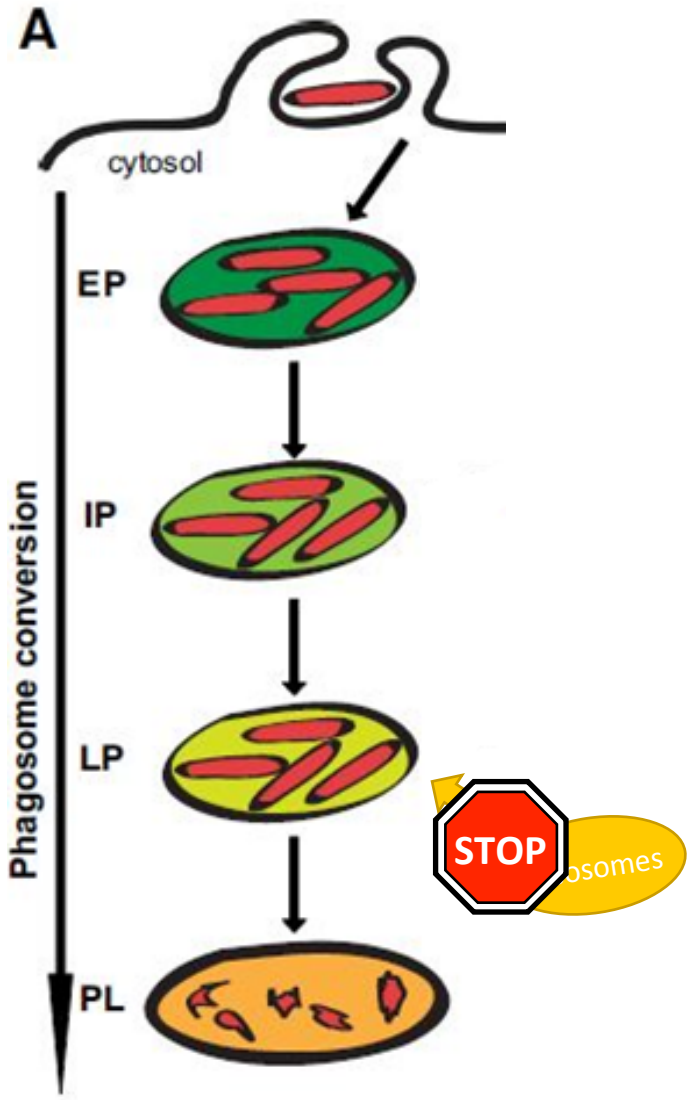


Blondiaux *et al.* **2017** Science

Part 3.

GENETIC MODULATORS (BACTERIA SIDE)

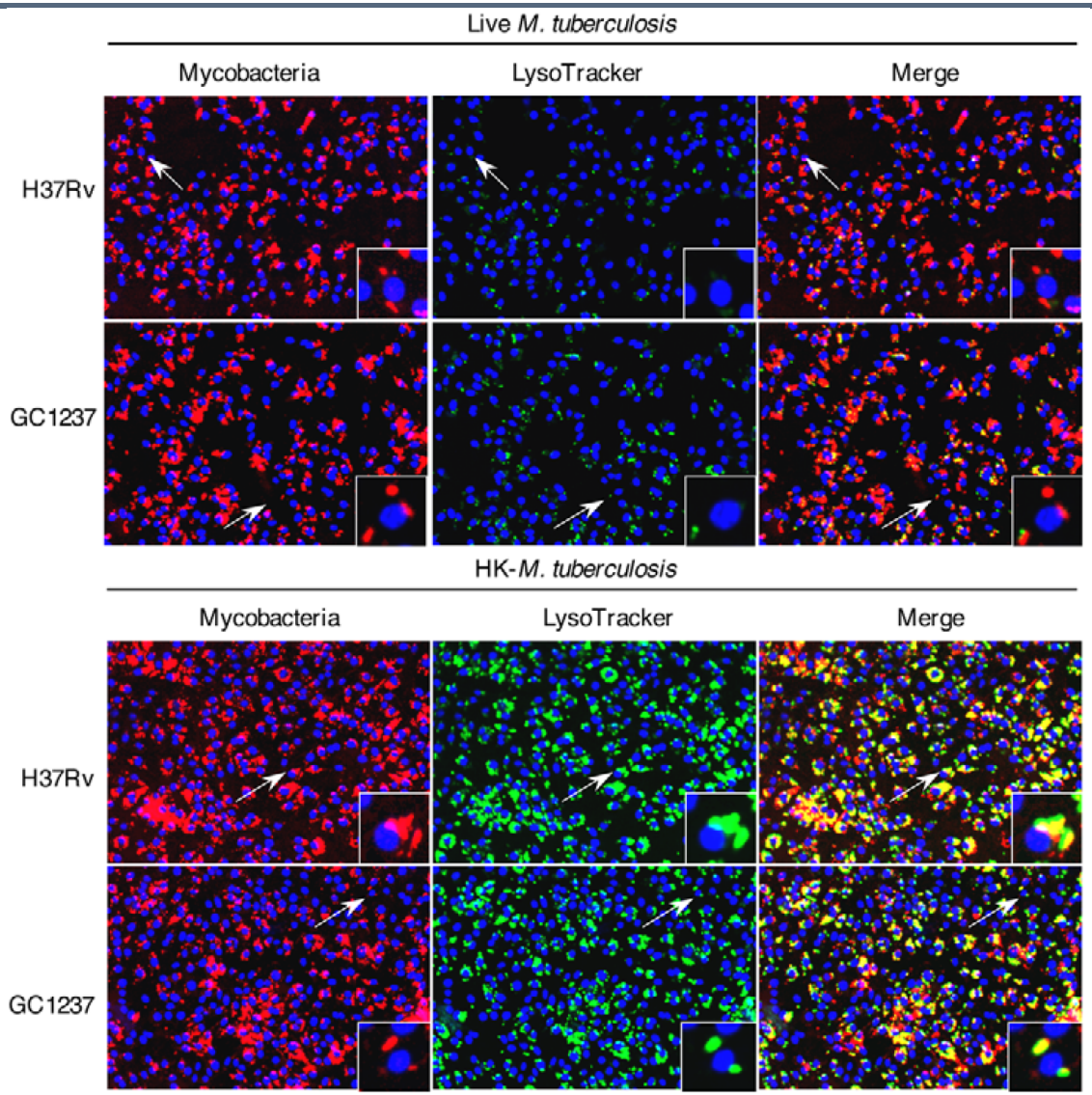
Genetic modulators – Bacteria side



Failure of lysosomal fusion with *M. tuberculosis* containing phagosomes

- Which bacterial effectors are implied?
- Use of a library of ~11,000 transposon mutants build in *M. tuberculosis* Beijing GC1237 strain
- Follow lysosomal fusion using the pH-sensitive Lysotracker probe
- Read-out: number of bacteria in acidified compartments

Genetic modulators - Screening principle



Validation using H37Rv and Beijing GC1237 fluorescent (RFP) strains

- Live and heat killed (HK) bacteria
- Increase in the number of acidified compartment with the HK strains

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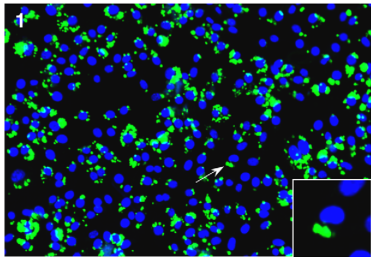
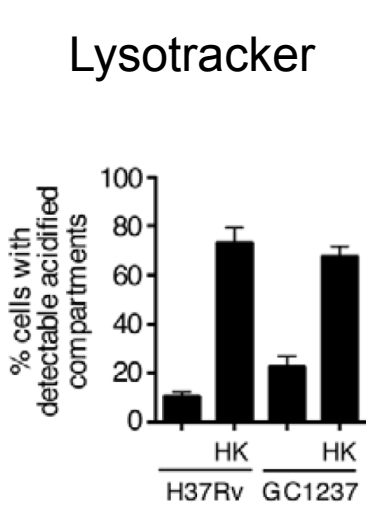
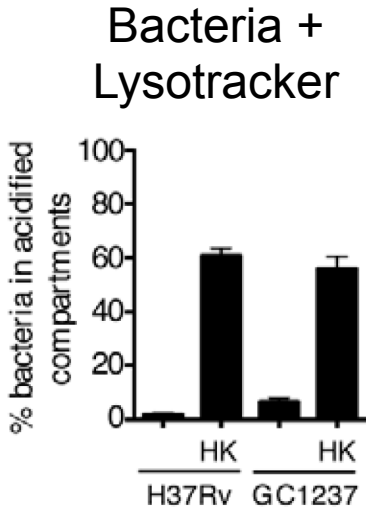
Genetic modulators - Quantification

Image analysis

- Detection of cell nuclei
- Detection of acidified compartment
  - Detection of bacteria is optional,
  - Quantification gave similar results







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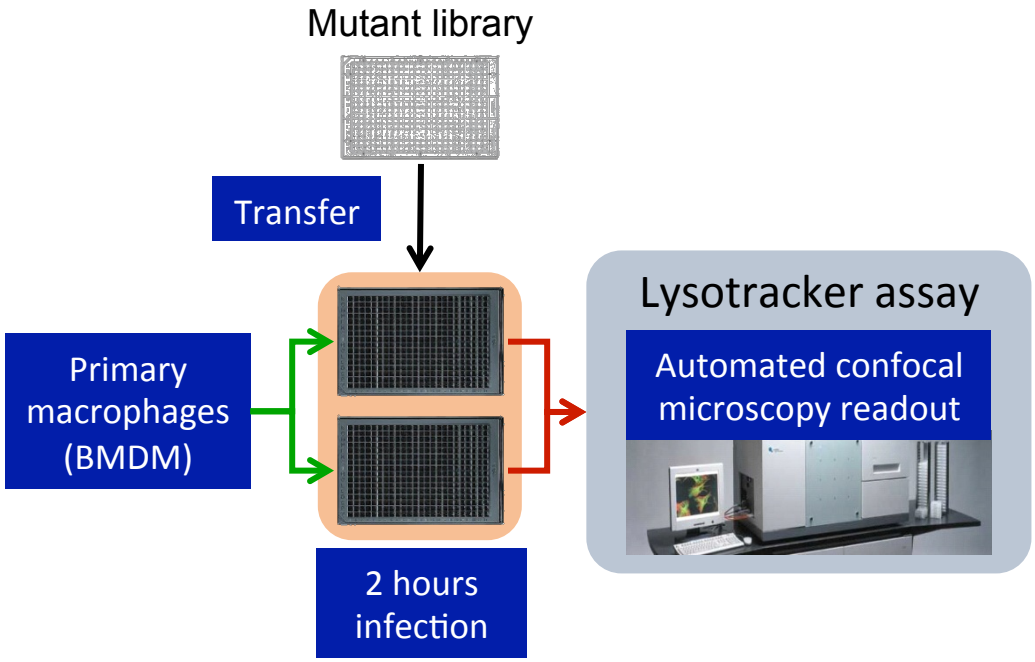
Genetic modulators - Process and screening

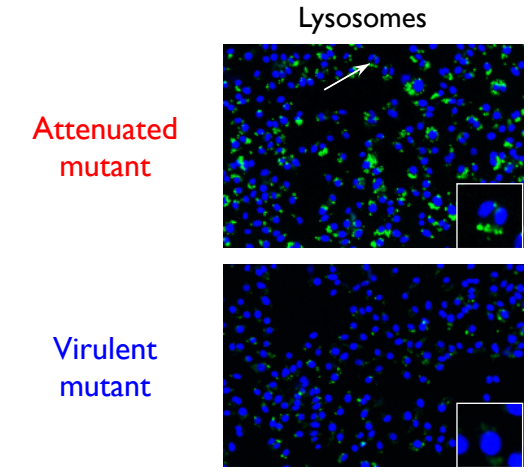
Process:

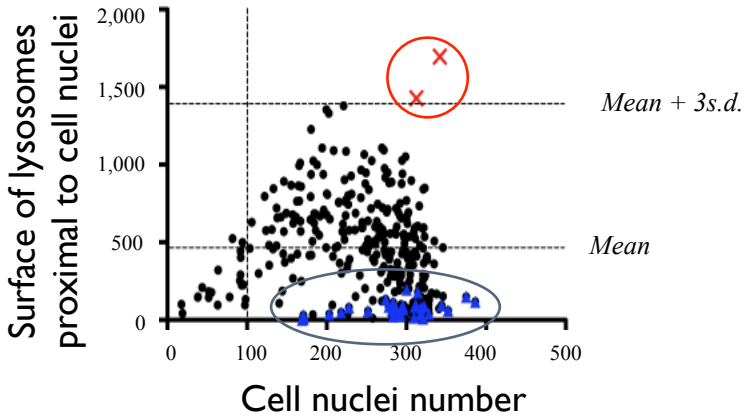
- Mutant distribution
- Cell distribution
- 2h incubation
- Staining, fixation, reading



Results:

- Two extreme phenotypes:





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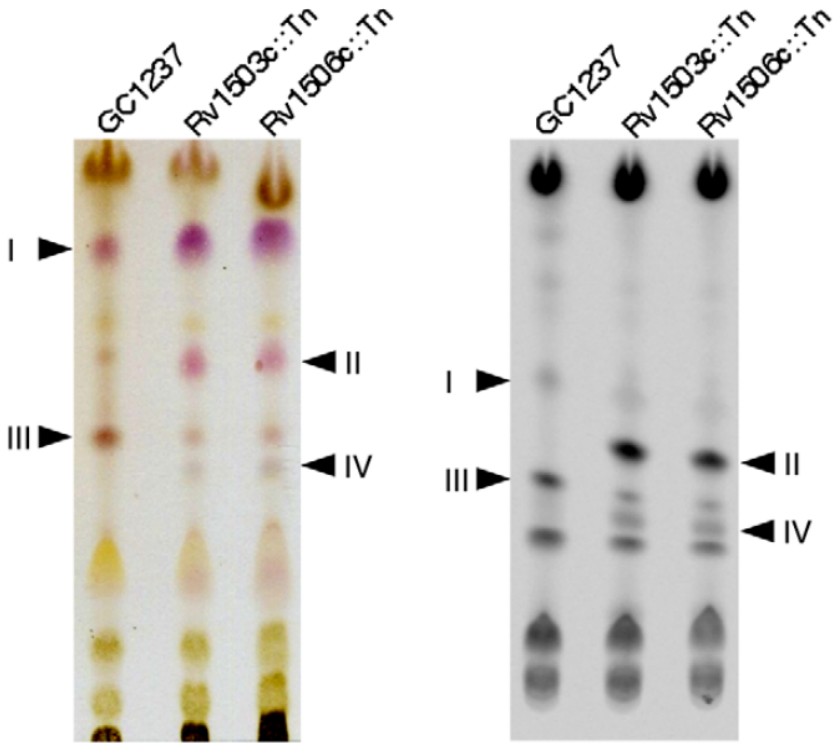
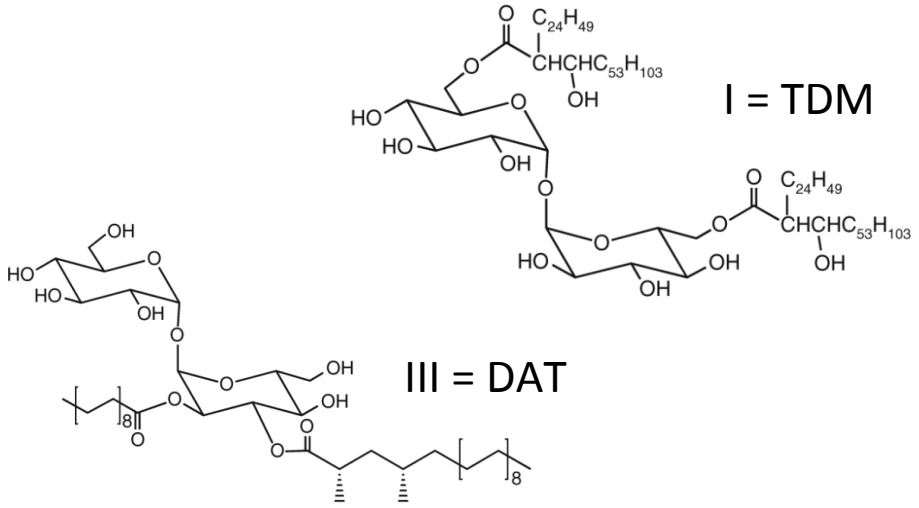
Genetic modulators – Results

Set of 10 mutants with defect in escaping lysosomal fusion



Mutant id	Gene (nt[a])	Putative function
P69D07	*pstS3* (482)	Phosphate transport
P117C08	Rv1503c (186)	TDP-4-oxo-6-deoxy-D-glucose transaminase (glycosyl aminotransferase)
P2E07	Rv1506c (211)	Methyltransferase
P65B12	*lppM* (272)	Lipoprotein of unknown function
P1E07	Rv2295 (-35)	Unknown
P58C04	*fadD28* (1252)	DIM biosynthesis
P32E07	*moaC1* (507)	Molybdopterin biosynthesis
P36D07	*moaD1* (49)	Molybdopterin biosynthesis
P55C04	*ppe54* (1126)	Unknown
P39E07	Rv3880c (269)	Unknown



Defect in DAT production

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Part 3.

GENETIC MODULATORS (HOST SIDE)

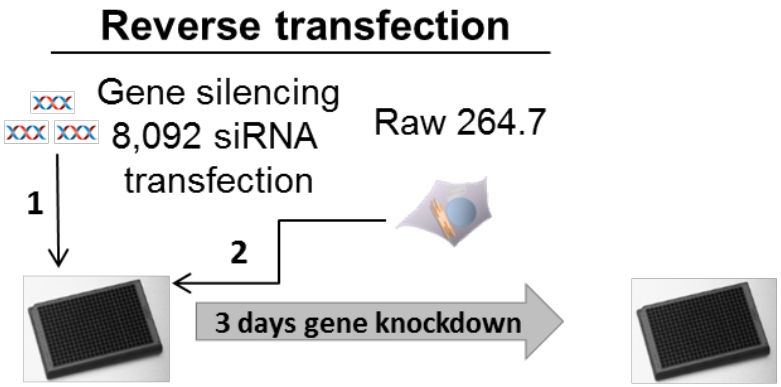
Genetic modulators – host side

Objectives:

- To identify essential pathways manipulated by the bacteria

Method:

- Bacterial replication assay in siRNA transfected cells

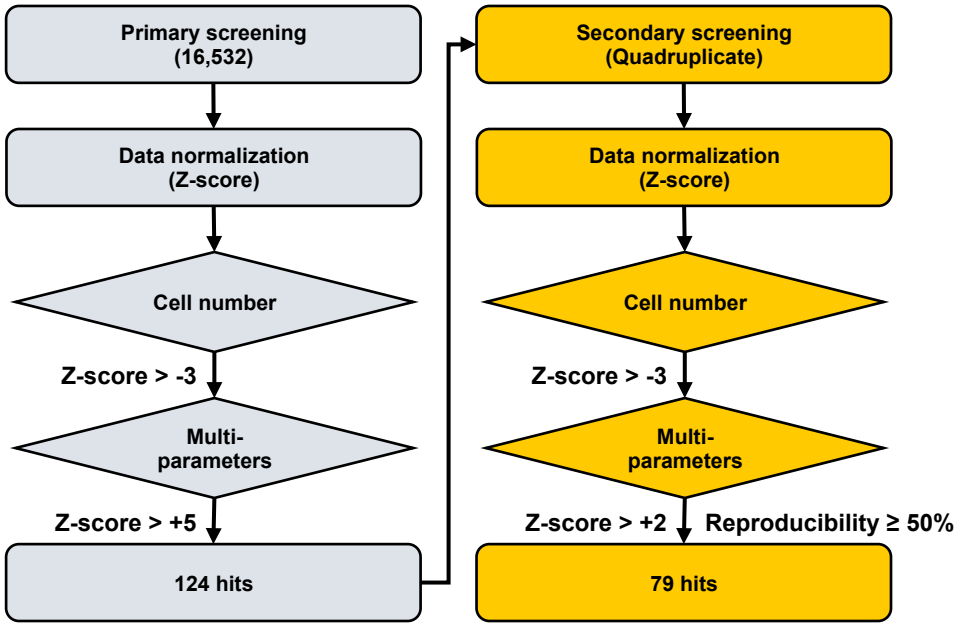


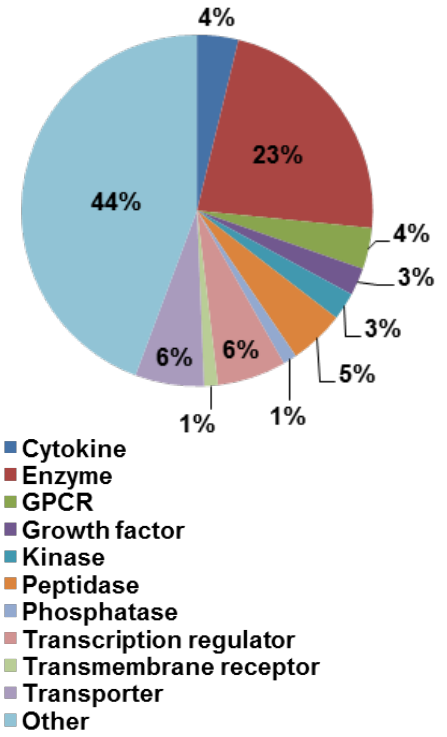
Genetic modulators – Host side

Strategy can be applied to different cell types

- **Macrophages**
- **Epithelial cells**

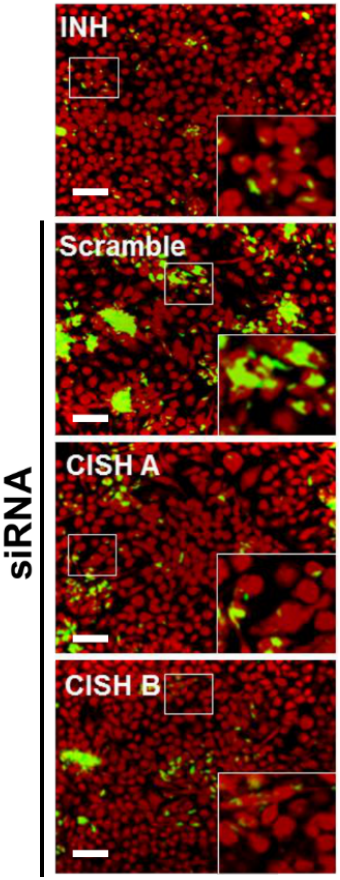
Validation process:





Identification of CISH

CISH is required for efficient *Mtb* replication in macrophages



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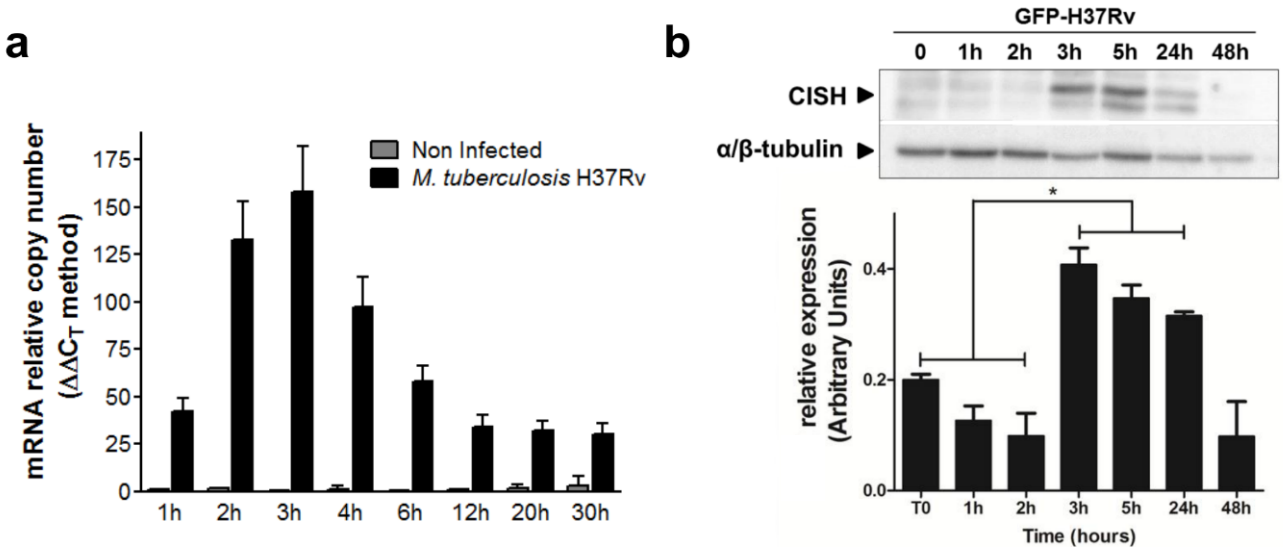
CISH expression pattern



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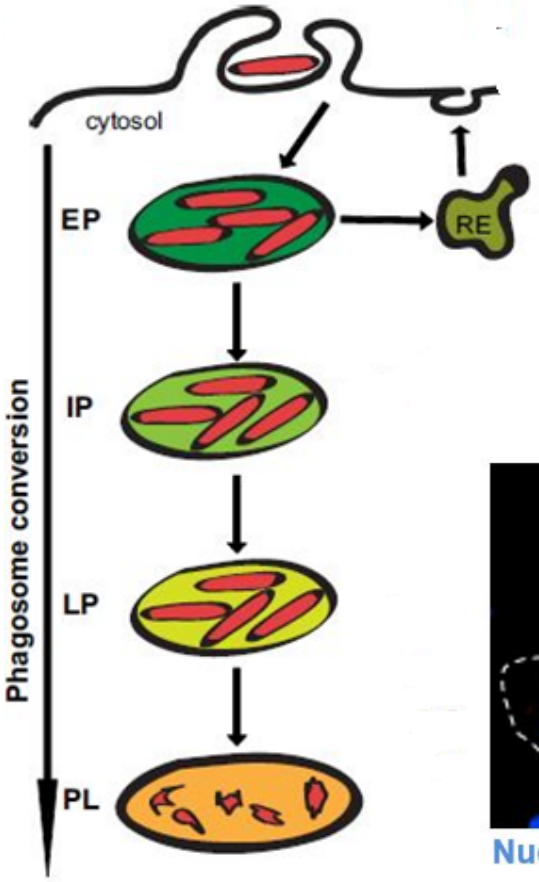
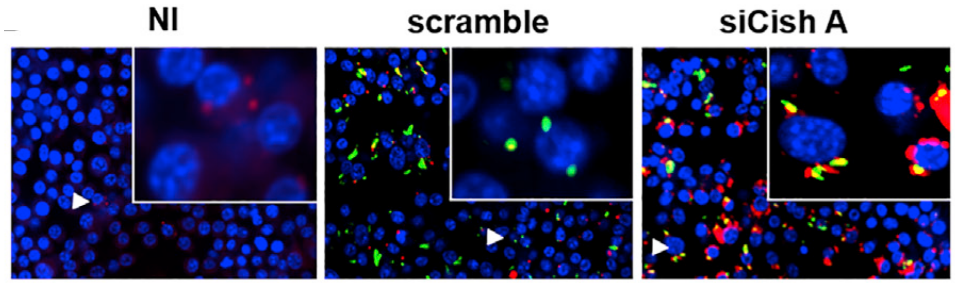
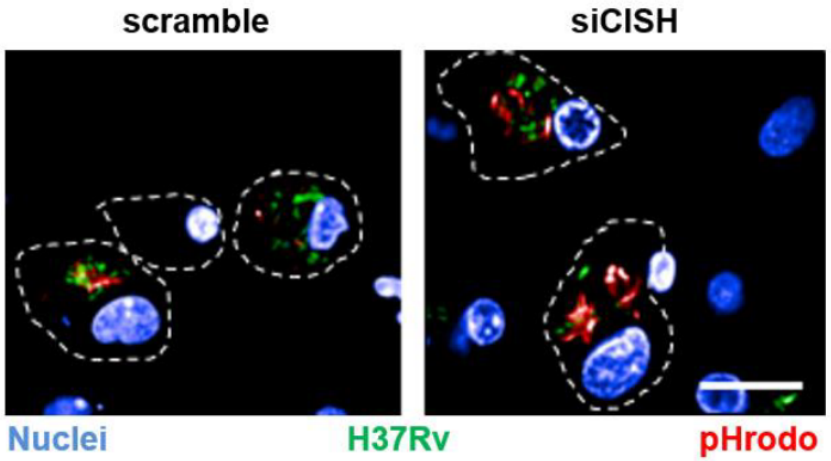
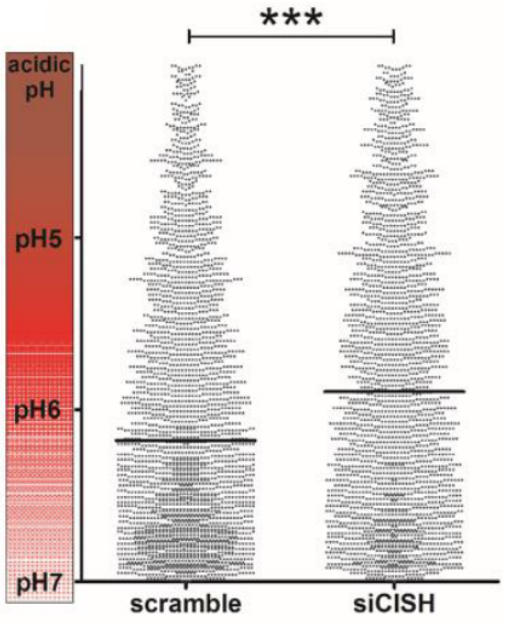
CISH interferes with phagosome acidification









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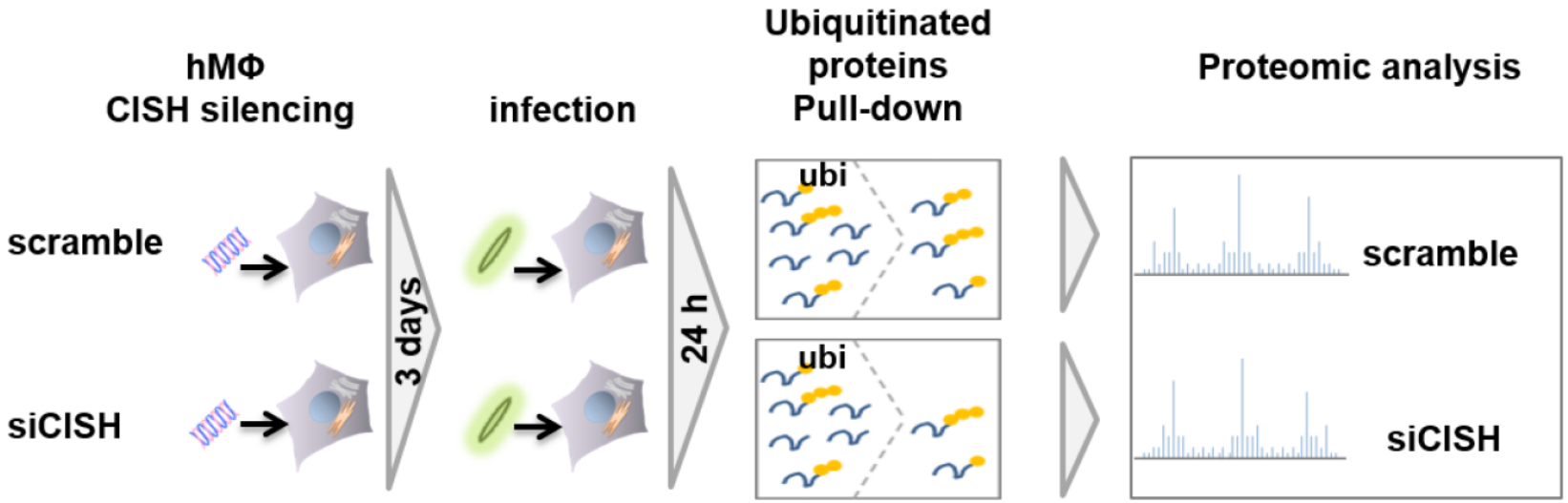
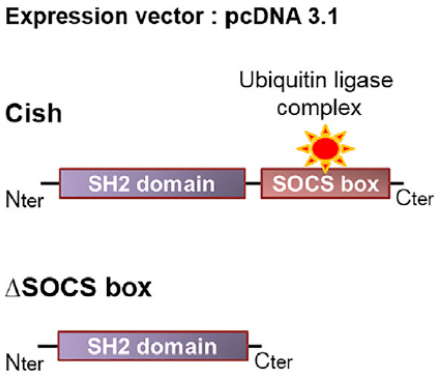
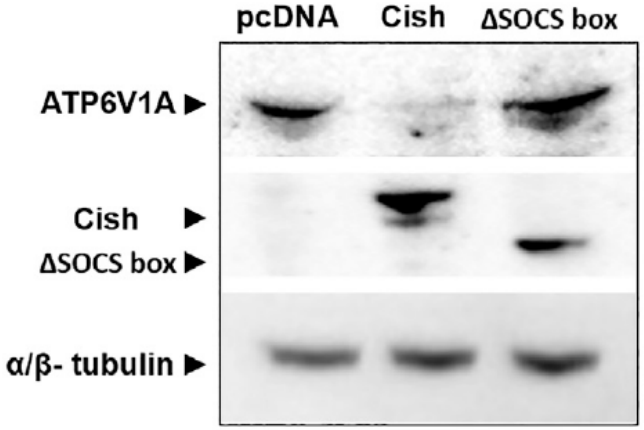
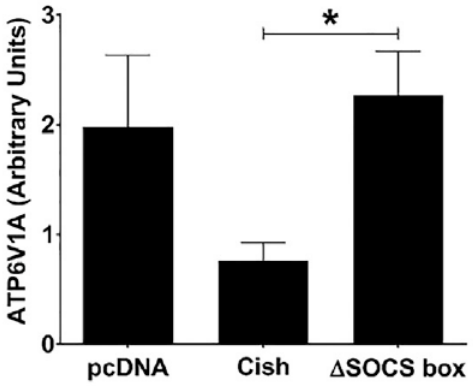
CISH targets V-ATPase









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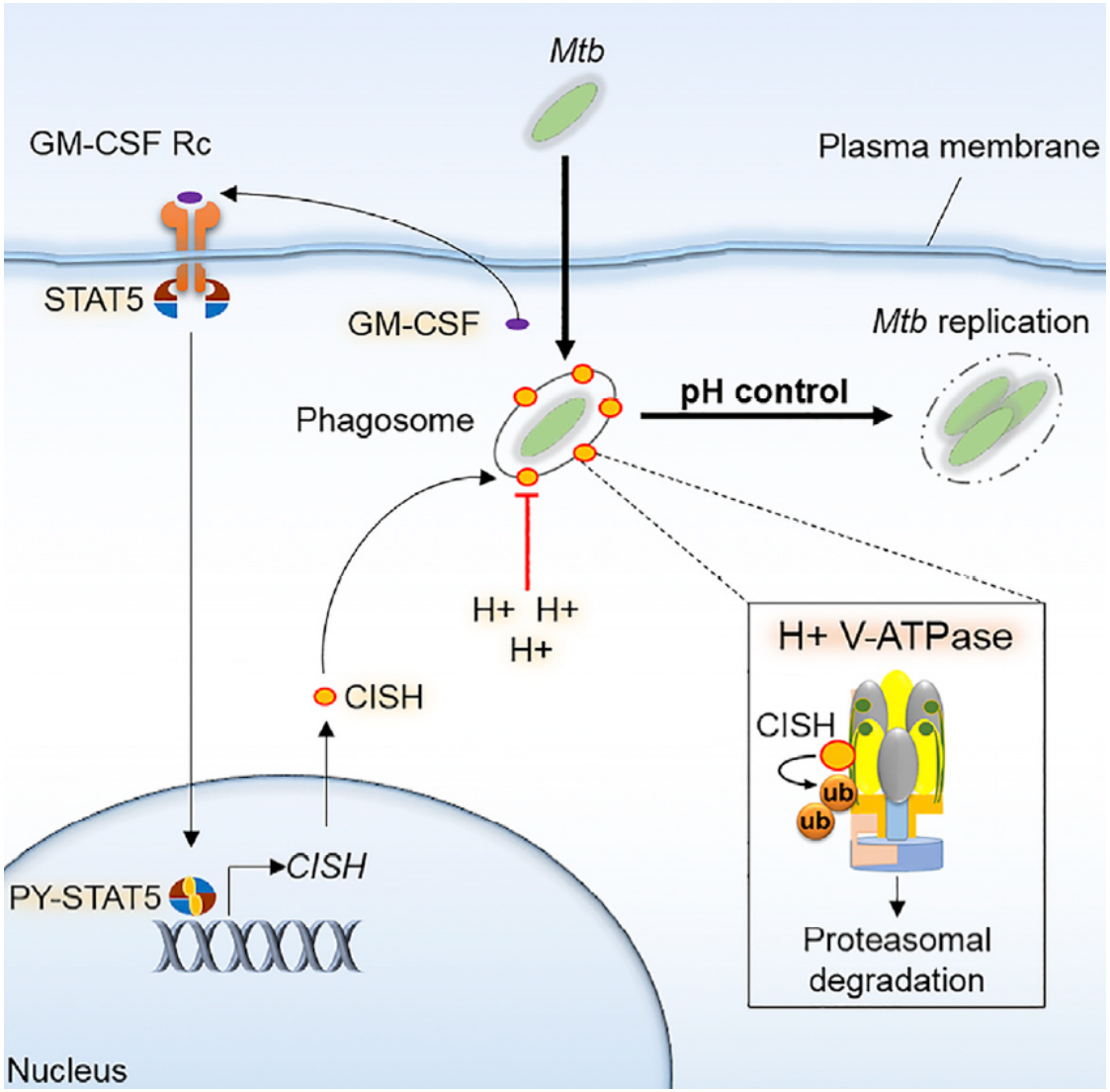
CISH – A summary



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Thank you!

Q & A

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