

Mycobacterial rifampicin tolerance: all roads lead to Rome?

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Medicine
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for
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Apologia

Apologia #1

- I don't know any Physics – or Maths!

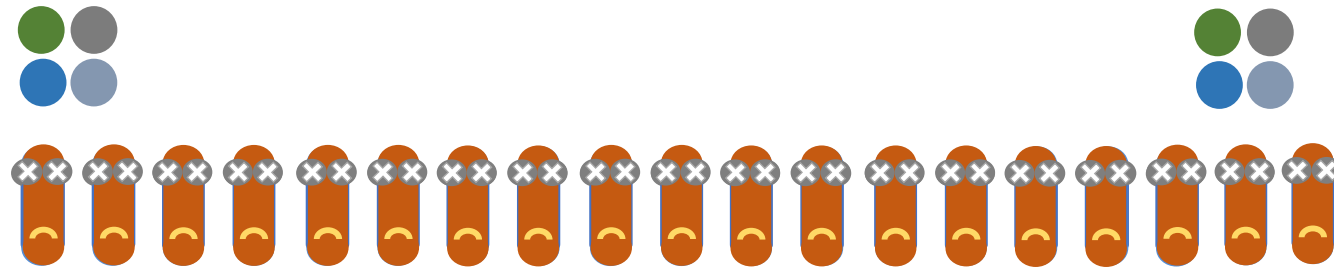
Apologia #2

- I mostly study phenotypes in growing mycobacteria – specifically excluding the ones that don't grow!

No one gets the right treatment for TB!

- The standard “short course” for drug-susceptible TB is 6 months

Mtb grows slowly...perhaps it also dies really slowly?



1 months to eradicate **>99%** of all bacteria

≥5 months to kill the rest **1%**

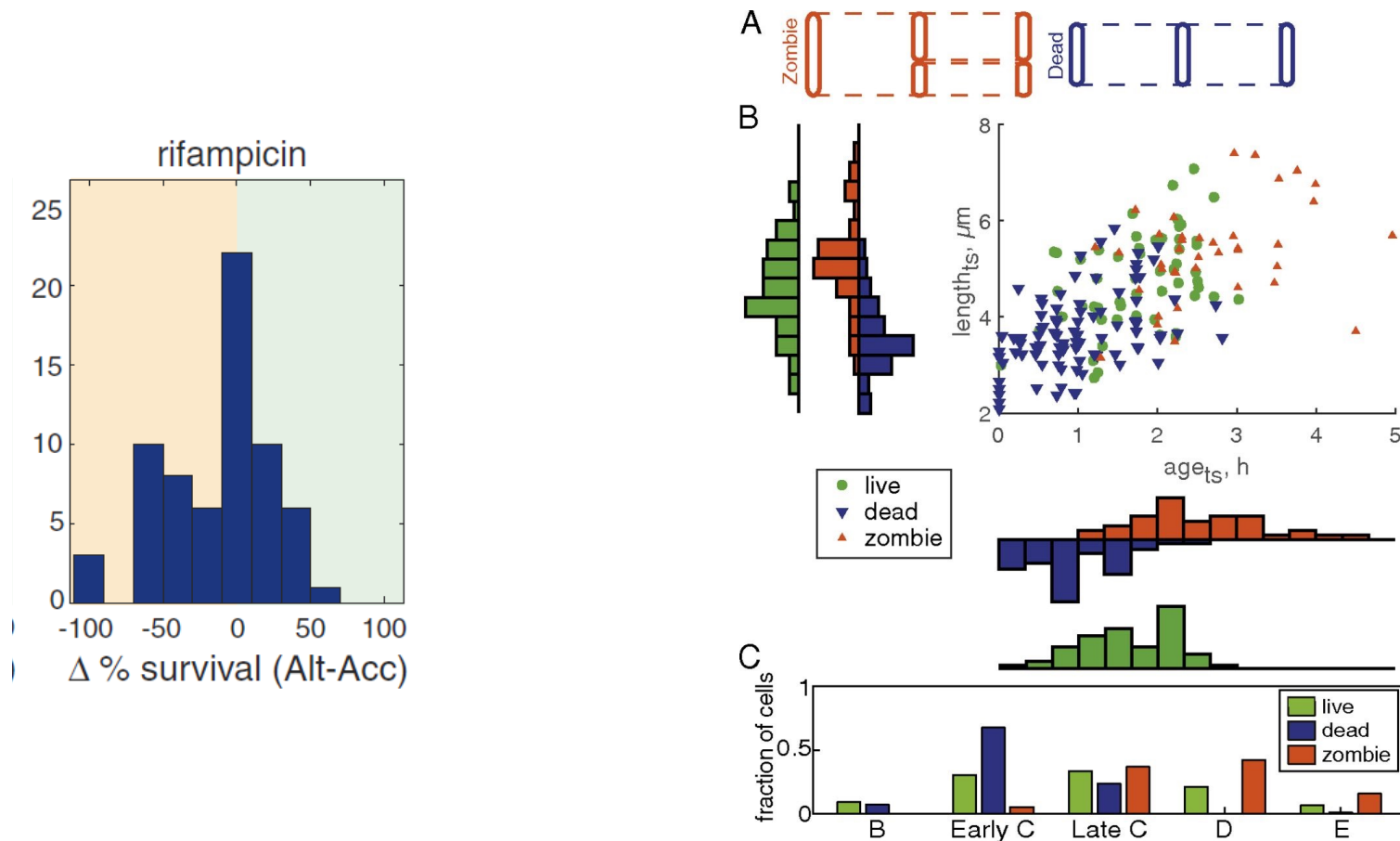
No one gets the right treatment for TB!

- The standard “short course” for drug-susceptible TB is 6 months
- 5% relapse/ fail even with 6 months therapy
- 80% of patients are cured in 3-4 months

We cannot predict which 80%

- Understanding bacterial heterogeneity to antibiotic therapy may allow specific targeting of those needing shorter or longer treatment times

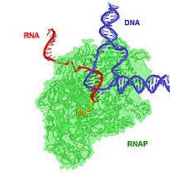
Rifampicin tolerance has *many* causes...



A little bit of an aside on the central dogma..

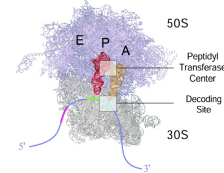
DNA

CCCGCCTGCATGGCAAAC



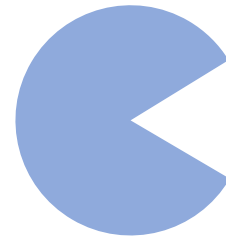
RNA

CCCGCCUGCAUGGCAAAC



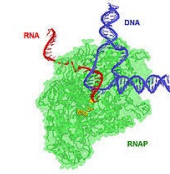
Protein

PACMAN



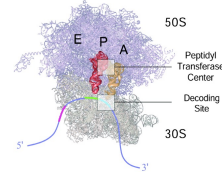
DNA

CACGCCTGCATGGCAAAC



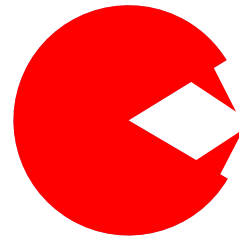
RNA

CACGCCUGCAUGGCAAAC



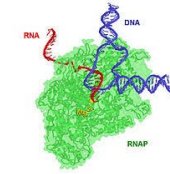
Protein

HACMAN



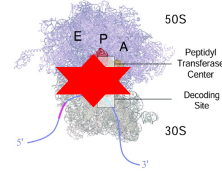
DNA

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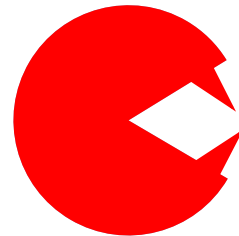
RNA

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Protein

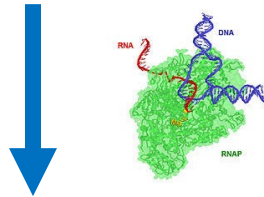
HACMAN



Too many errors → error catastrophe

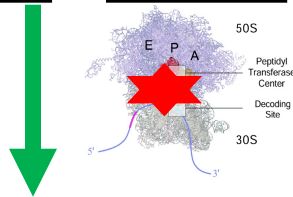
DNA

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RNA

CCCGCCUGCAAUGGGCAAAC



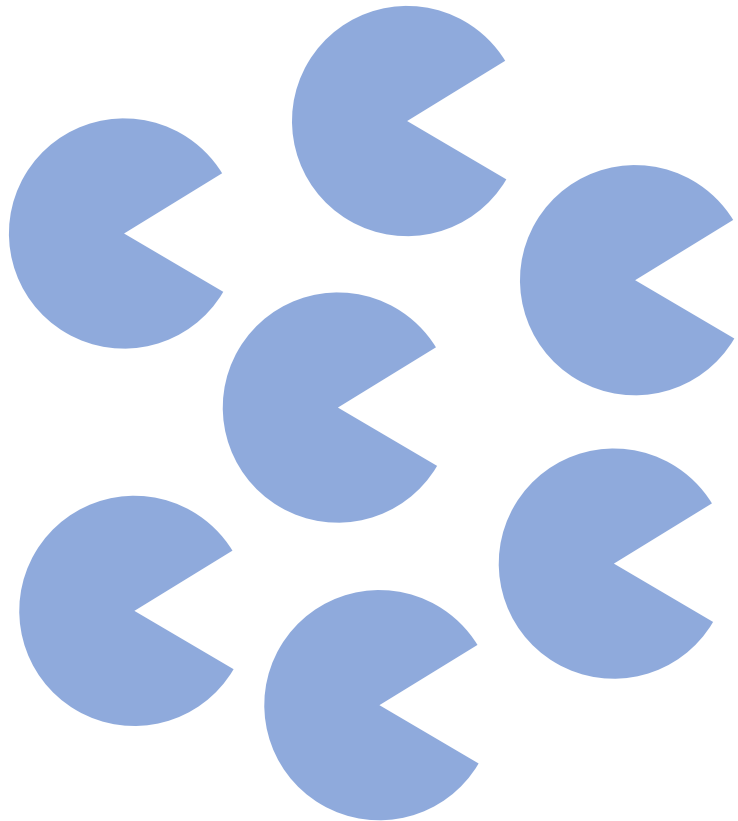
Protein

HAMBUN

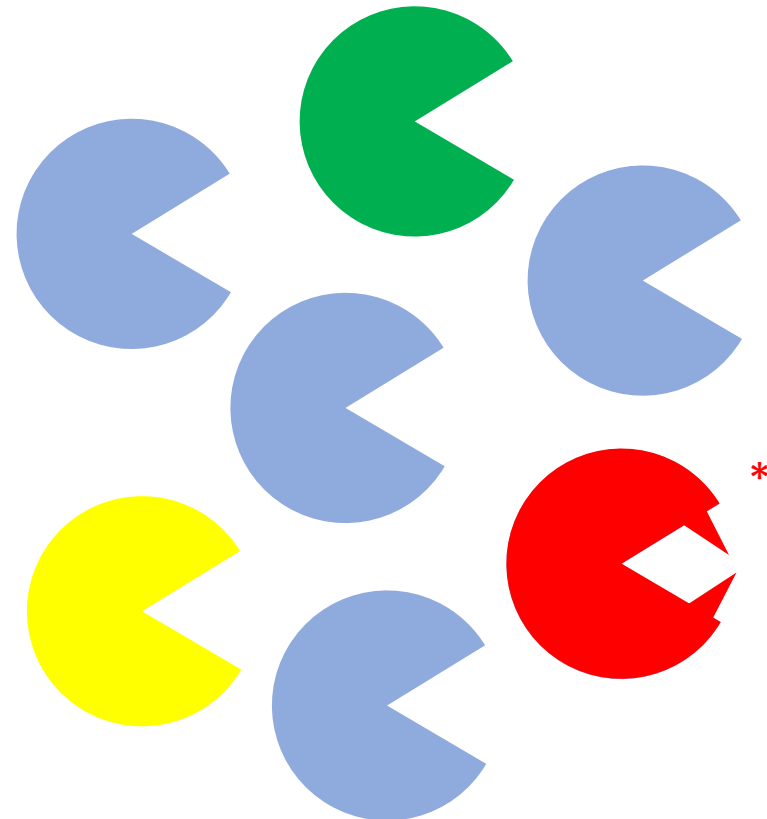


Mistranslation as a diversity generator

IDEAL TRANSLATION FIDELITY



INCREASED MISTRANSLATION



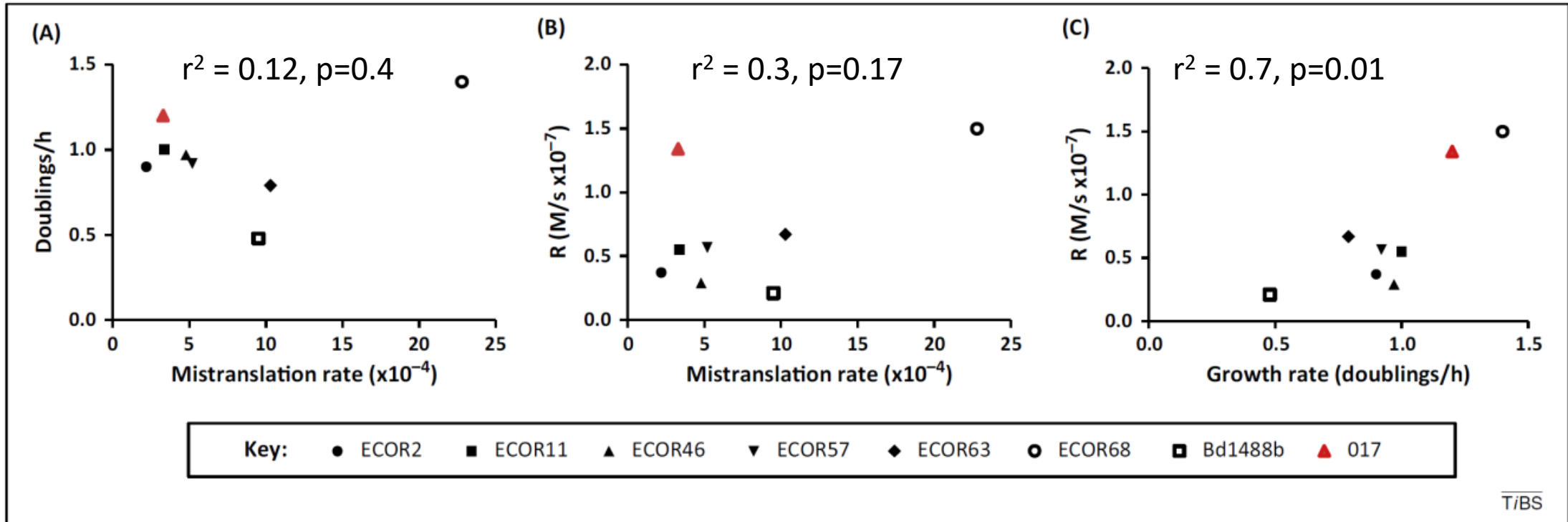
* gain of function phenotype

Are translational errors the 'cost of doing business'?

Are translational errors the 'cost of doing business'?

- Mikkola and Kurland (1992):
- 7 natural isolates of *E. coli* and one lab strain (O17)
- Measured growth rate, protein synthesis rate and translational error rate (Leu misincorporation from polyU) before/after 300 generation passage in a chemostat

Are translational errors the 'cost of doing business'?



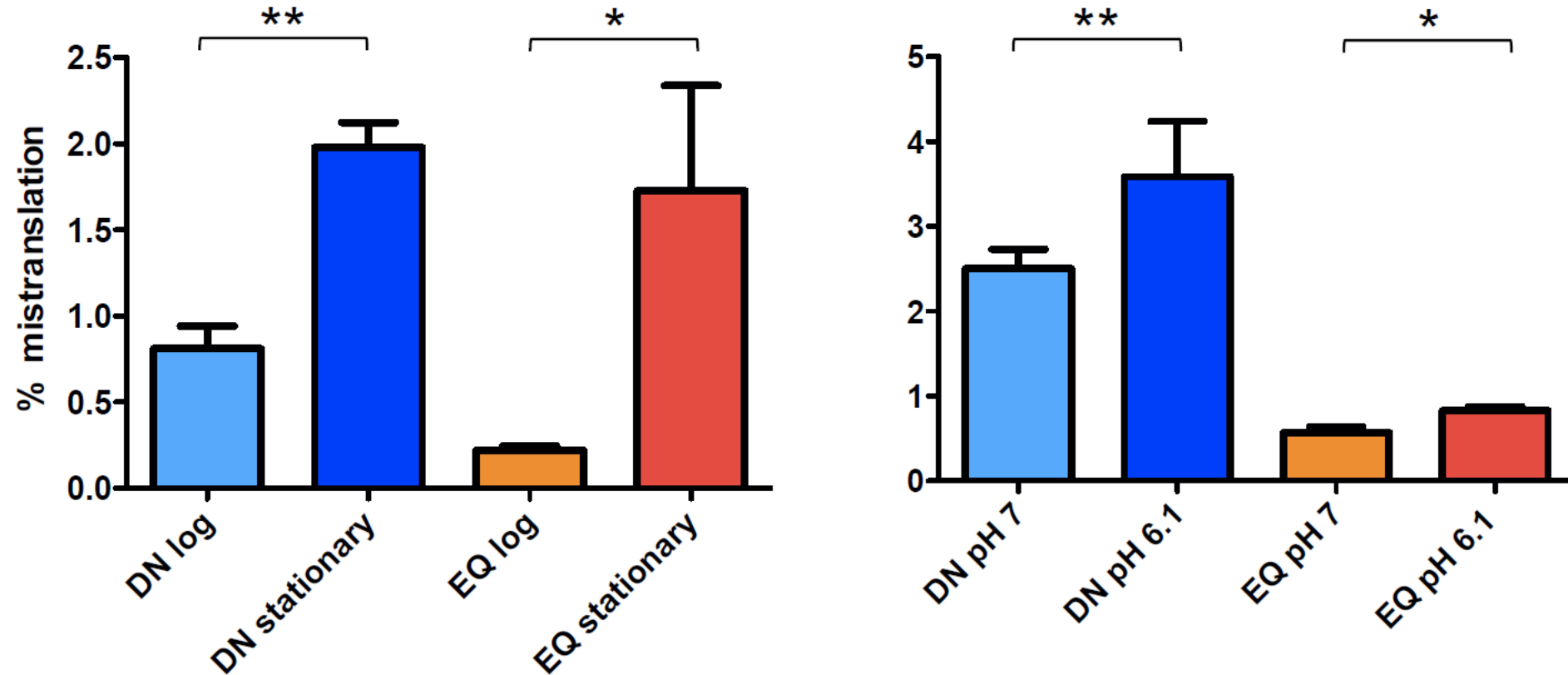
Are translational errors the 'cost of doing business'?

- Mikkola and Kurland (1992):
- 7 natural isolates of *E. coli* and one lab strain (O17)
- Measured growth rate, protein synthesis rate and translational error rate (Leu misincorporation from polyU) before/after 300 generation passage in a chemostat
- **AFTER PASSAGE**: all strains 'reverted' to O17-like phenotype: fast growth, efficient protein synthesis and ~low error rate.

Model organisms we study in my lab

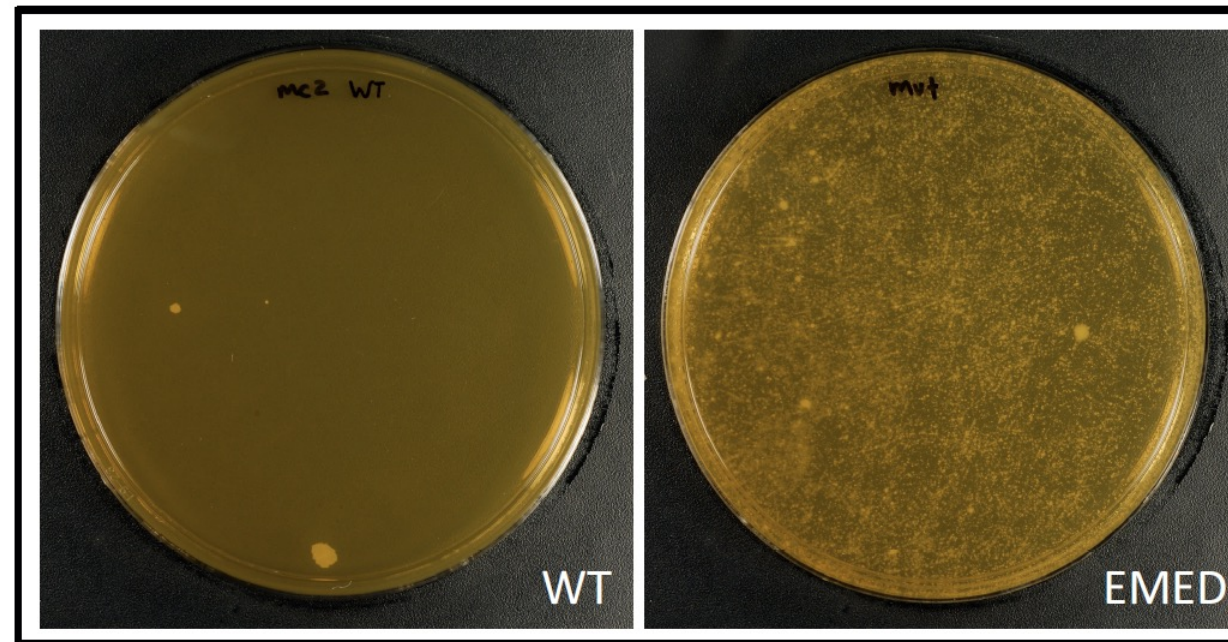
- *M. smegmatis* -- non-pathogenic, average doubling time in rich axenic culture ~3hrs
- *M. tuberculosis* – BSL-3 containment, average doubling time in rich axenic culture (AND early animal infection) ~ 20 hrs

Mycobacteria have high and variable translation error rates

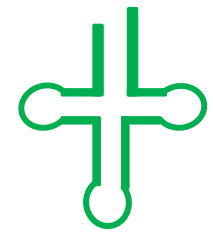


M. smegmatis

Mistranslation is sufficient for Rif-Specific Phenotypic Resistance



RIFAMPICIN



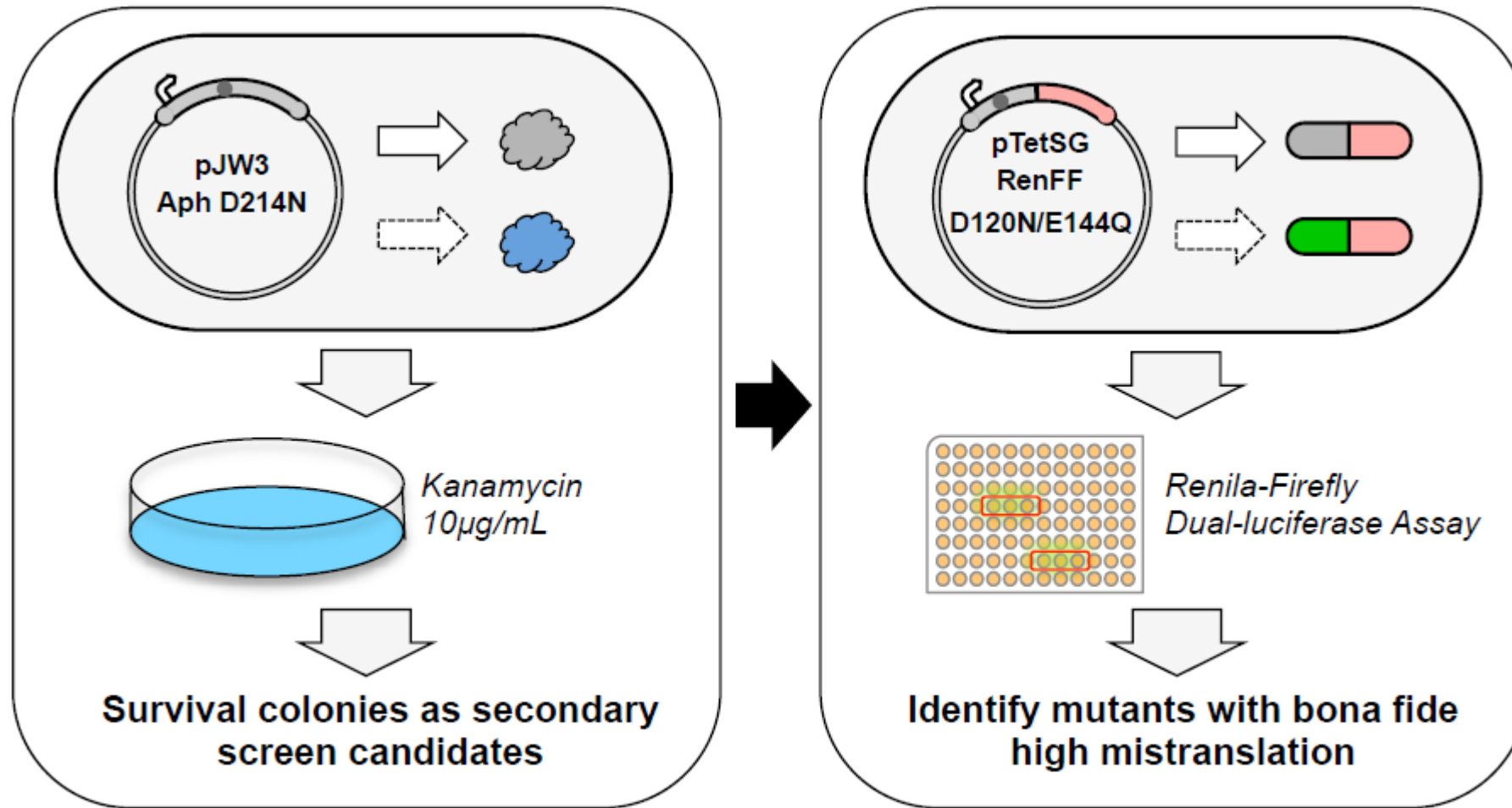
Express
mutated tRNAs
in trans

EMED: “excess mistranslation E+D”: Gln → Glu, Asn → Asp

M. smegmatis

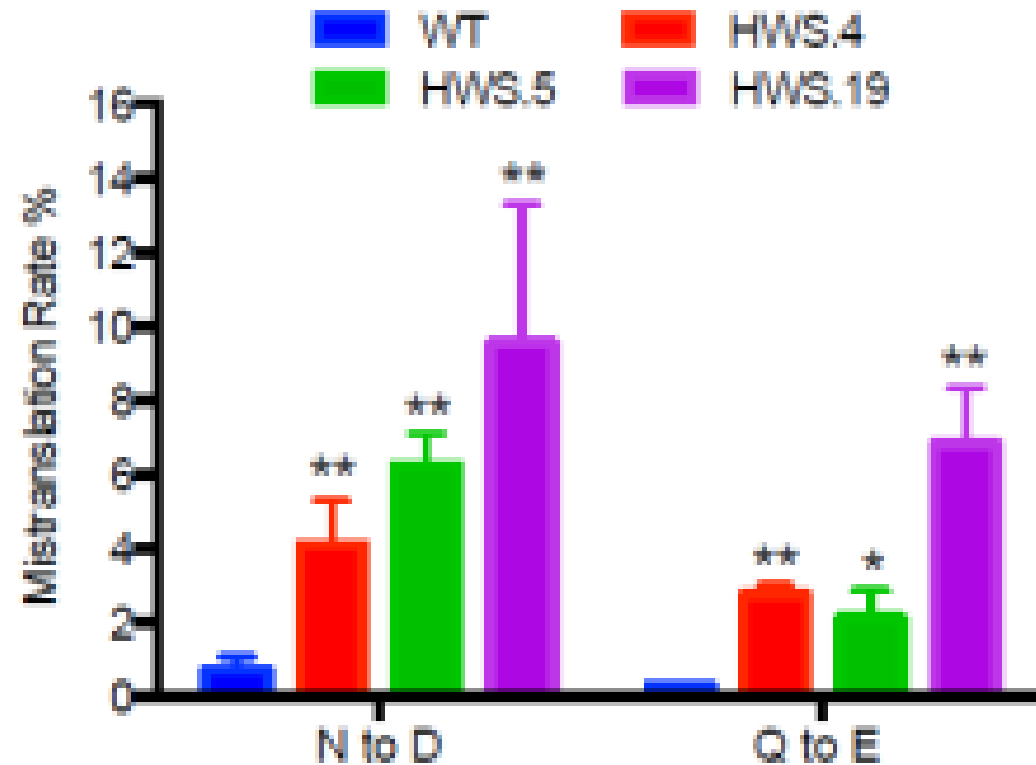
These are NOT RIF-RESISTANT BACTERIA!

A selection/ screen strategy to identify high mistranslator mutants



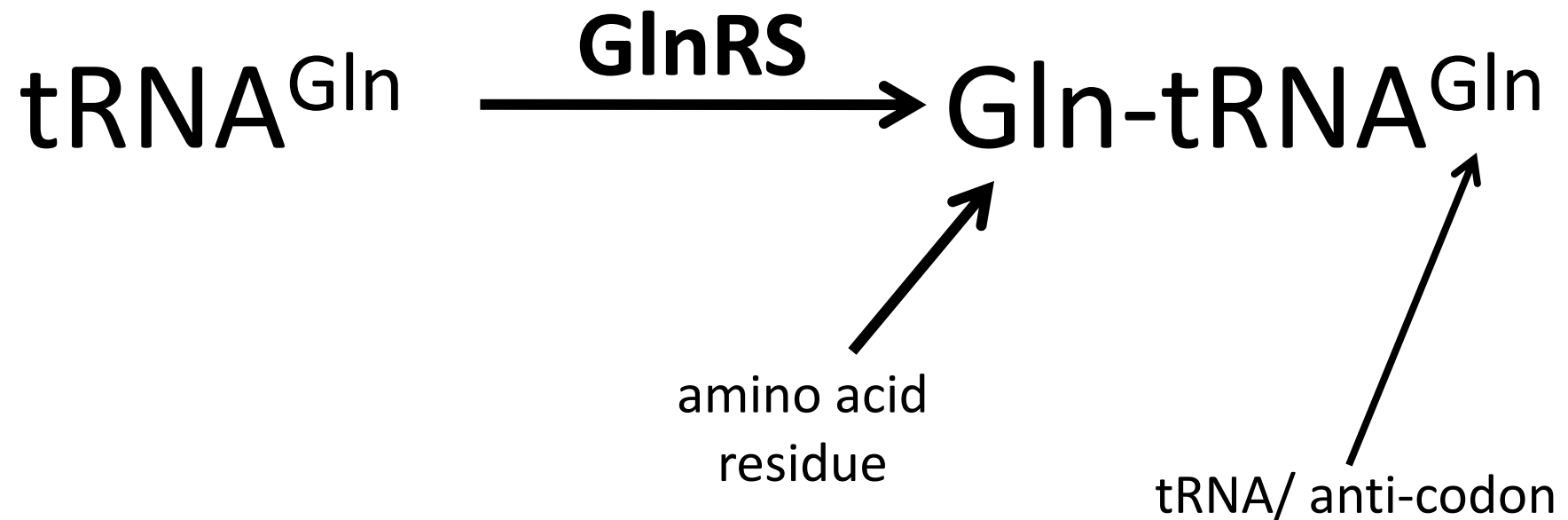
Hongwei Su

gatA mutations cause high mistranslation rates



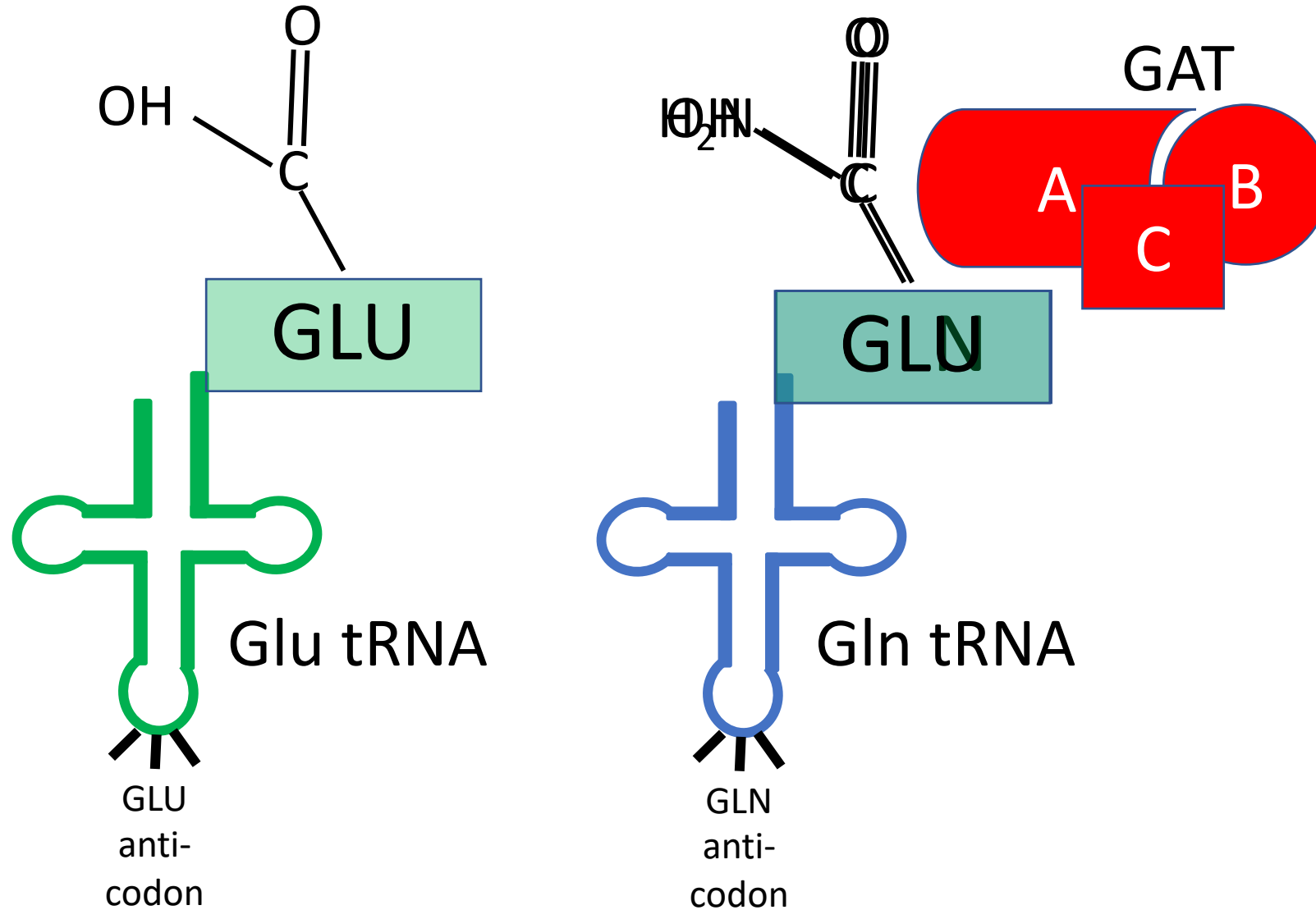
M. smegmatis

Direct and indirect Gln-tRNA^{Gln} synthesis

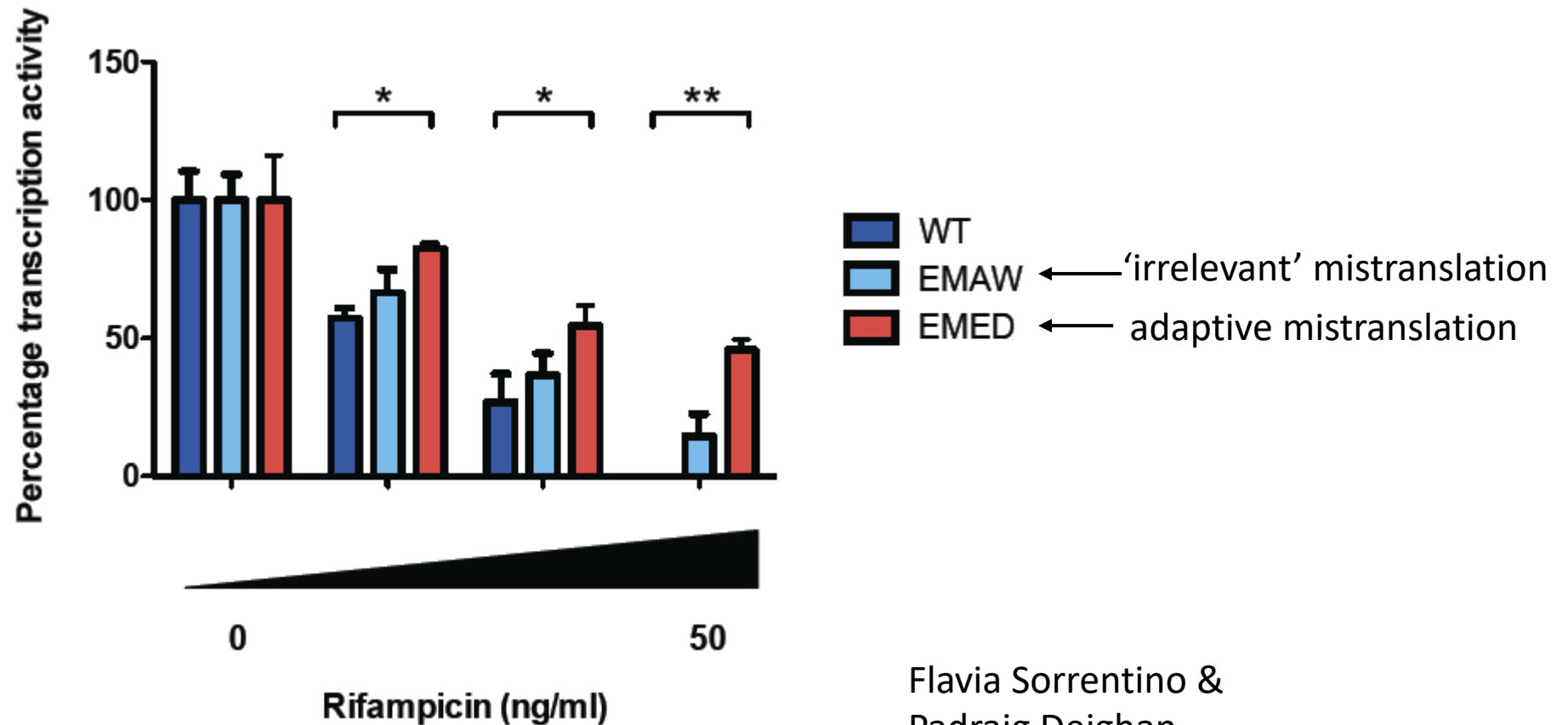


DIRECT tRNA amino-acylation pathway

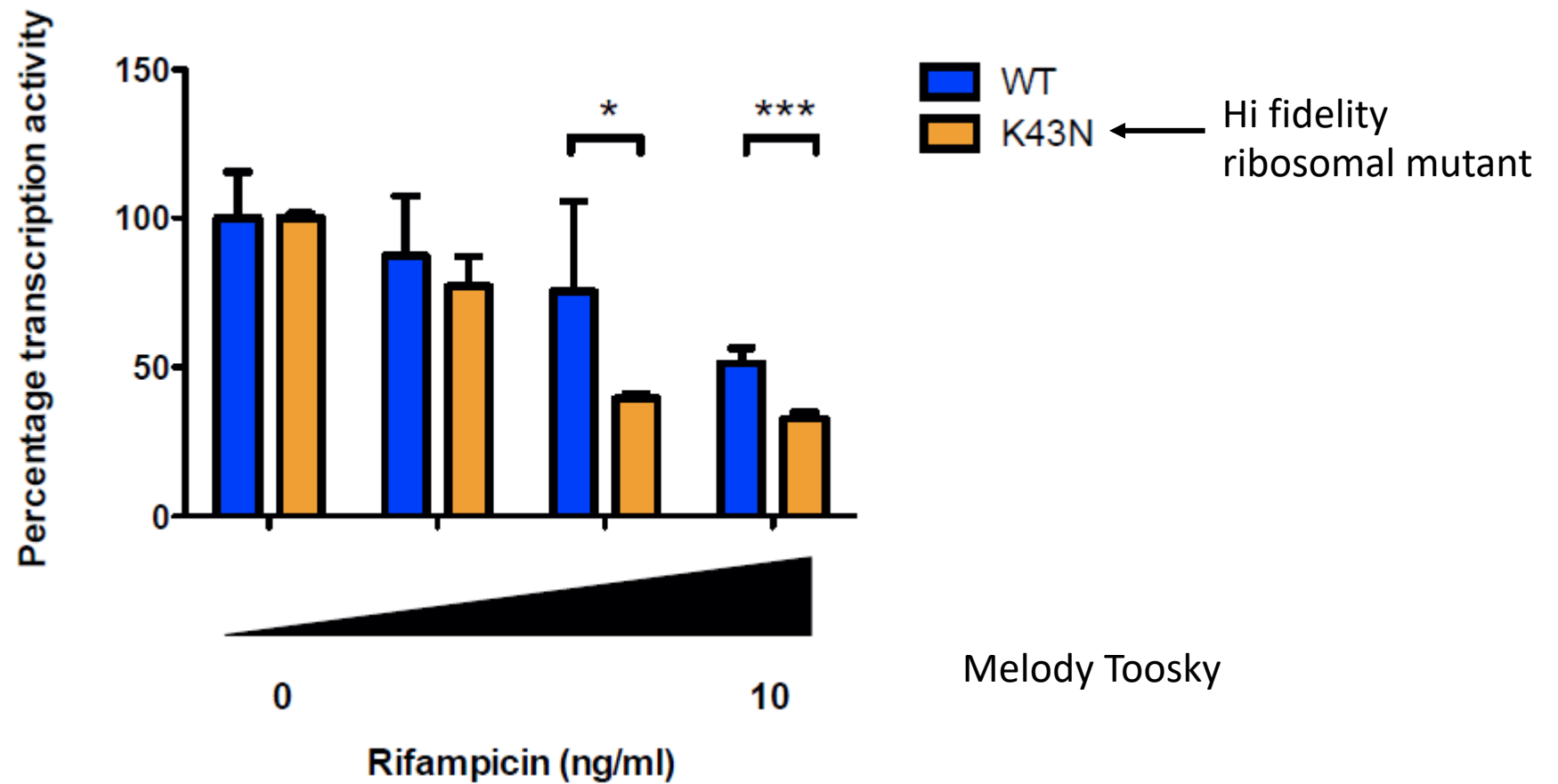
The indirect pathway utilises GatCAB



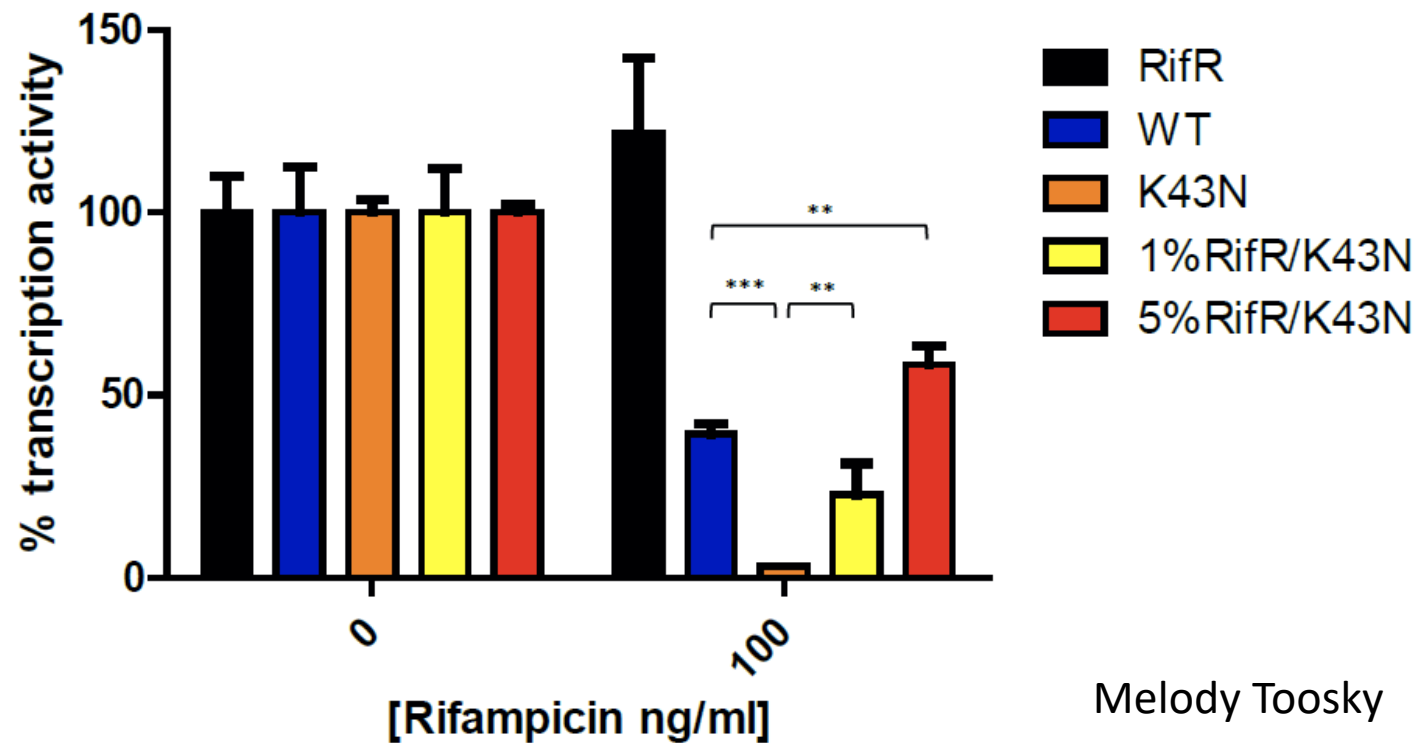
Mistranslation results in fitness at the protein level for RNAP



Improving translational fidelity reverses protein-level adaptation

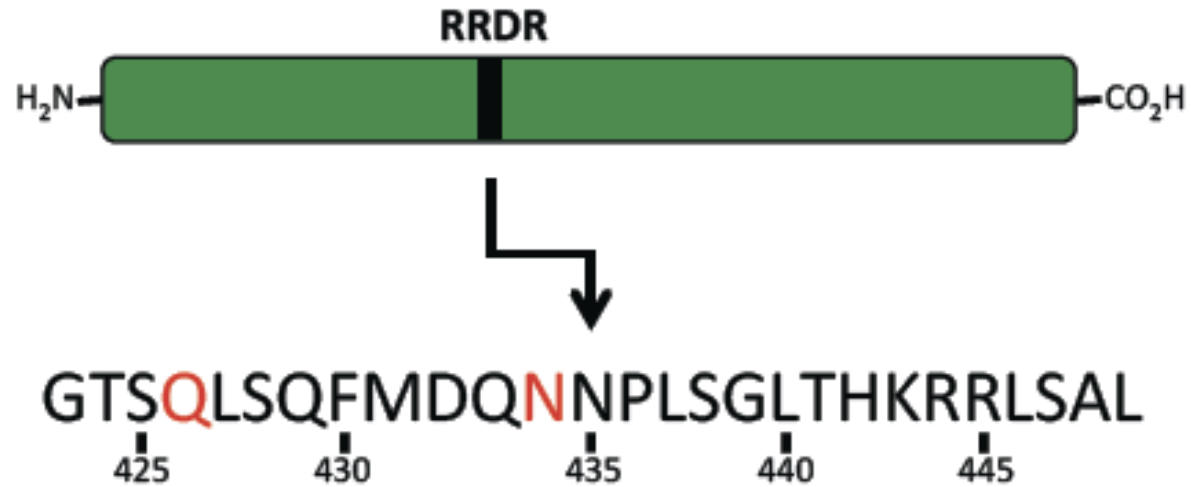


Low levels of 'resistant' protein are sufficient to phenocopy mistranslation

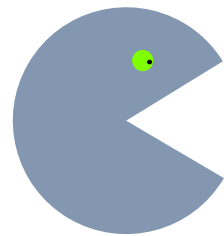
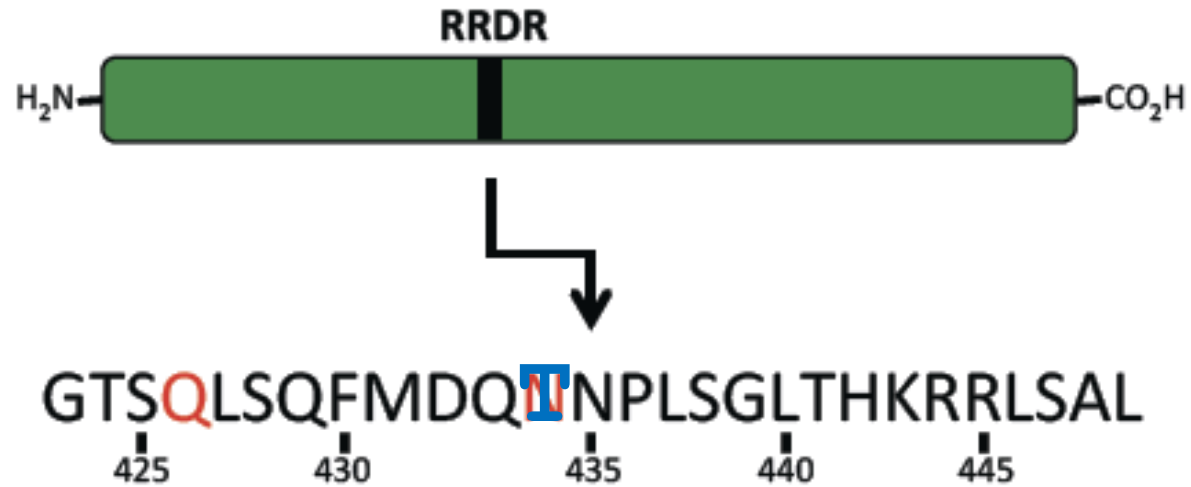


Melody Toosky

Making a 'high fidelity' RpoB



Making a 'high fidelity' RpoB



N

Mistranslation



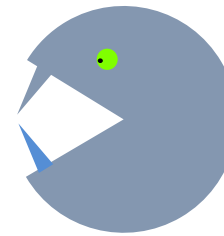
D

T

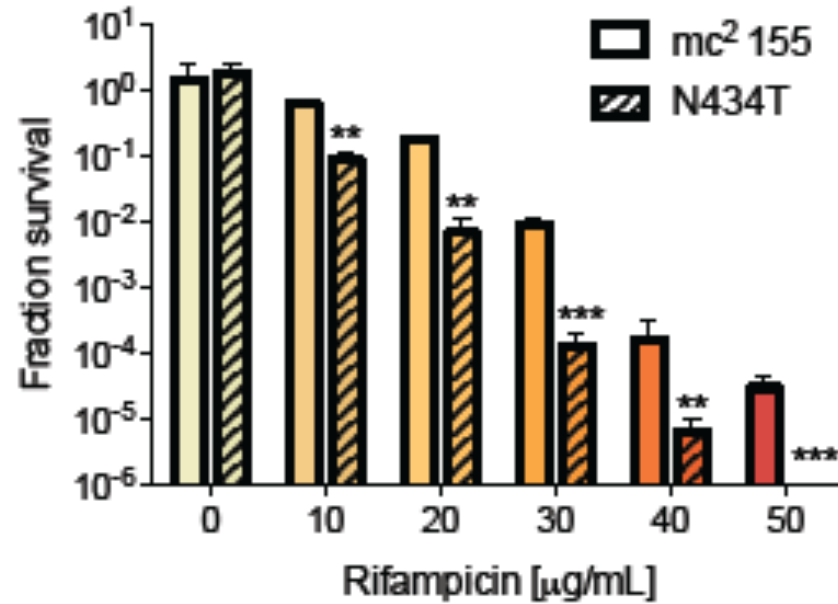
Mistranslation



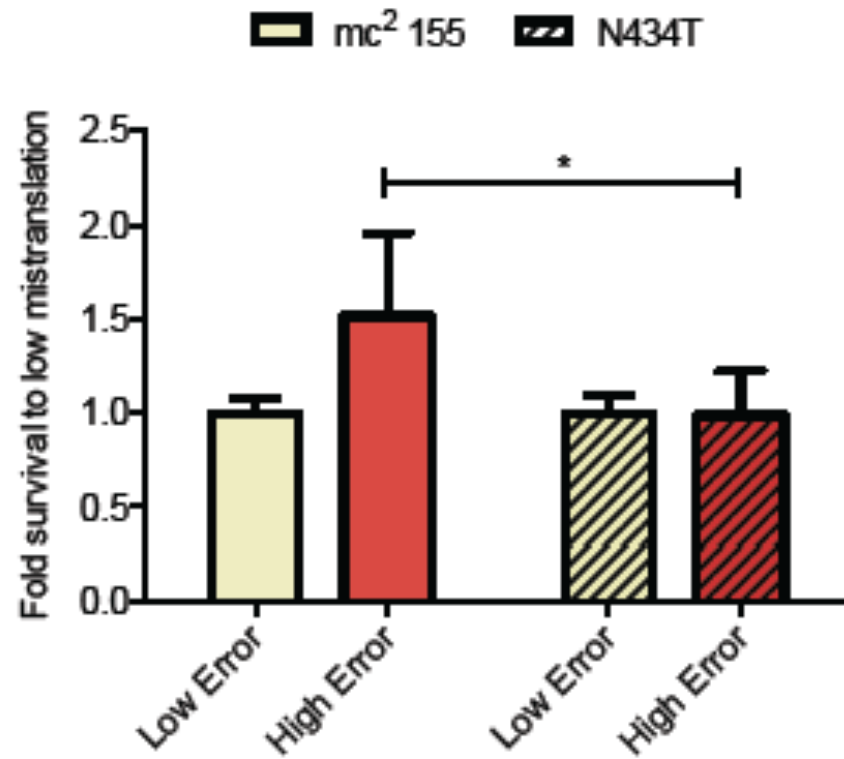
D



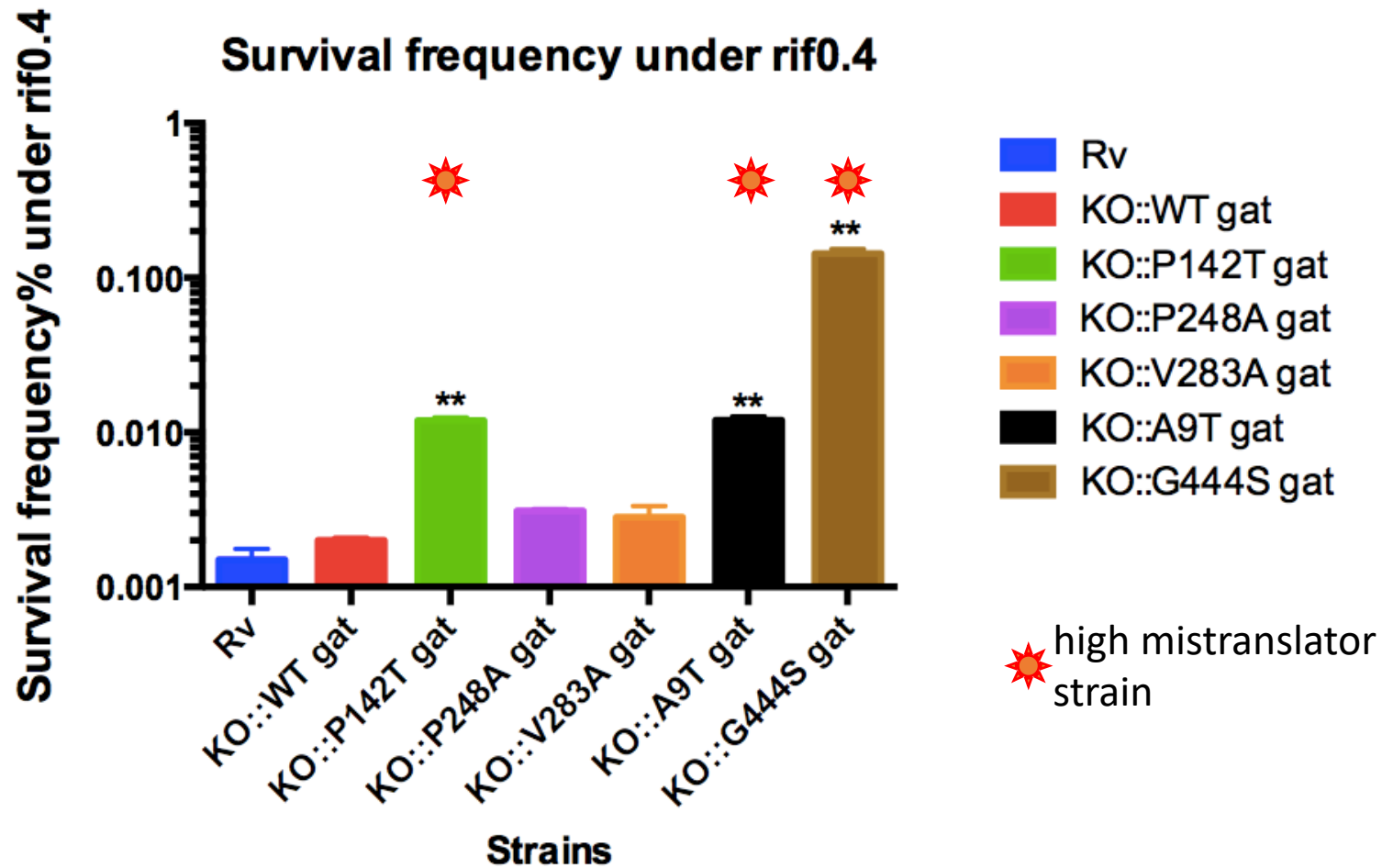
A strain with 'hi-fi' RpoB has slightly higher MIC to rifampicin than the parent but is less phenotypically resistant



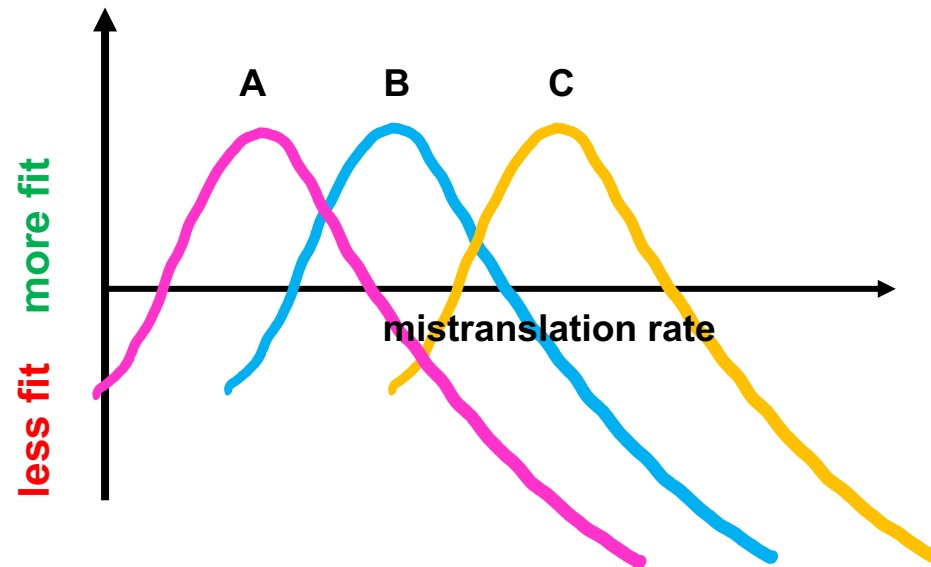
'Hi-fi' RpoB is now mistranslation insensitive for rifampicin phenotypic resistance



Some but not all TB *gatA* mutants have high rifampicin phenotypic resistance



Optimal mistranslation rates are (likely)
context specific

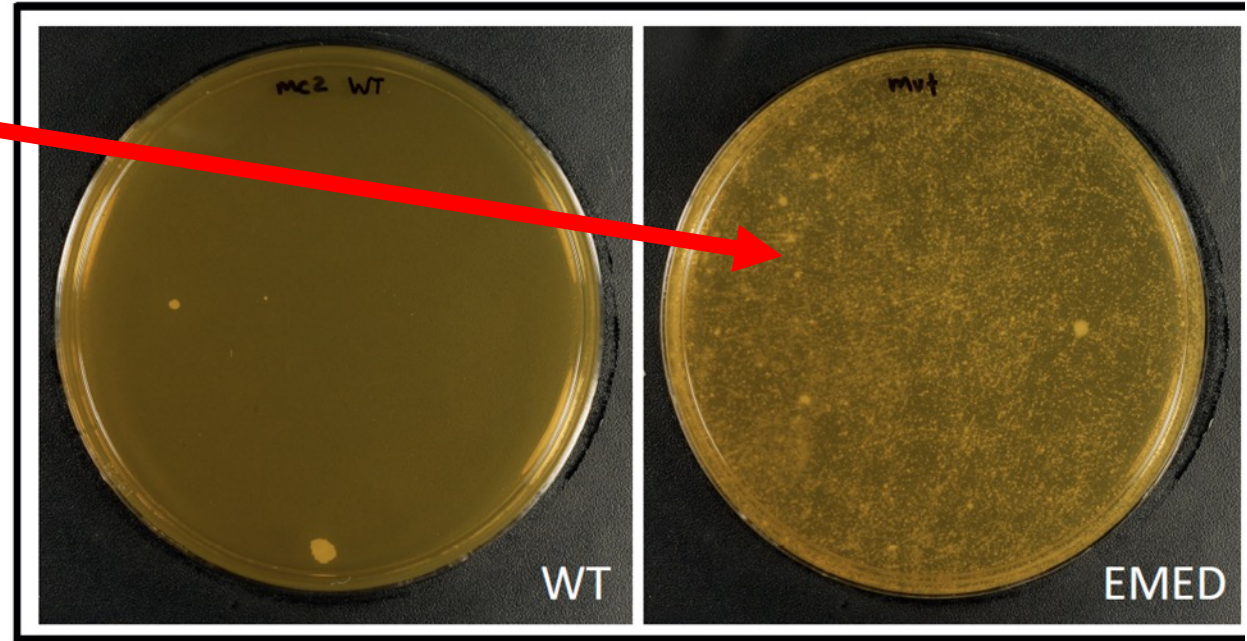


A: ? Axenic culture?
B: ? Animal infection?
C: ? RIF tolerance?

The joys of having an insubordinate student

Me to JH: study these guys

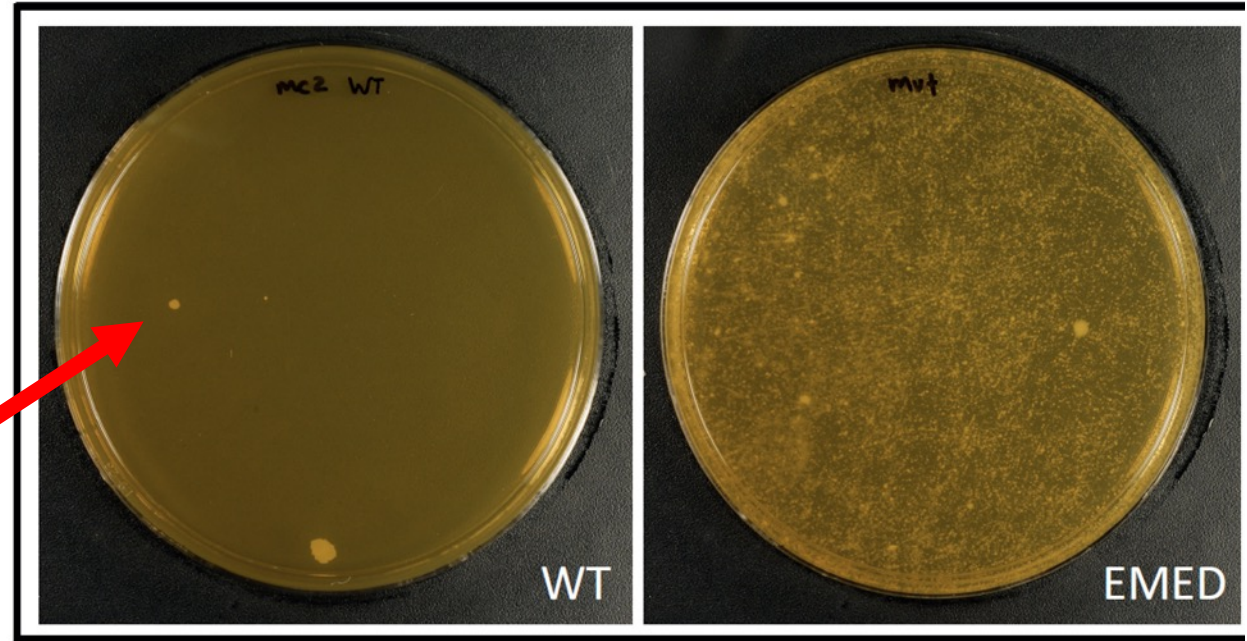
Junhao Zhu



RIFAMPICIN

The joys of having an insubordinate student

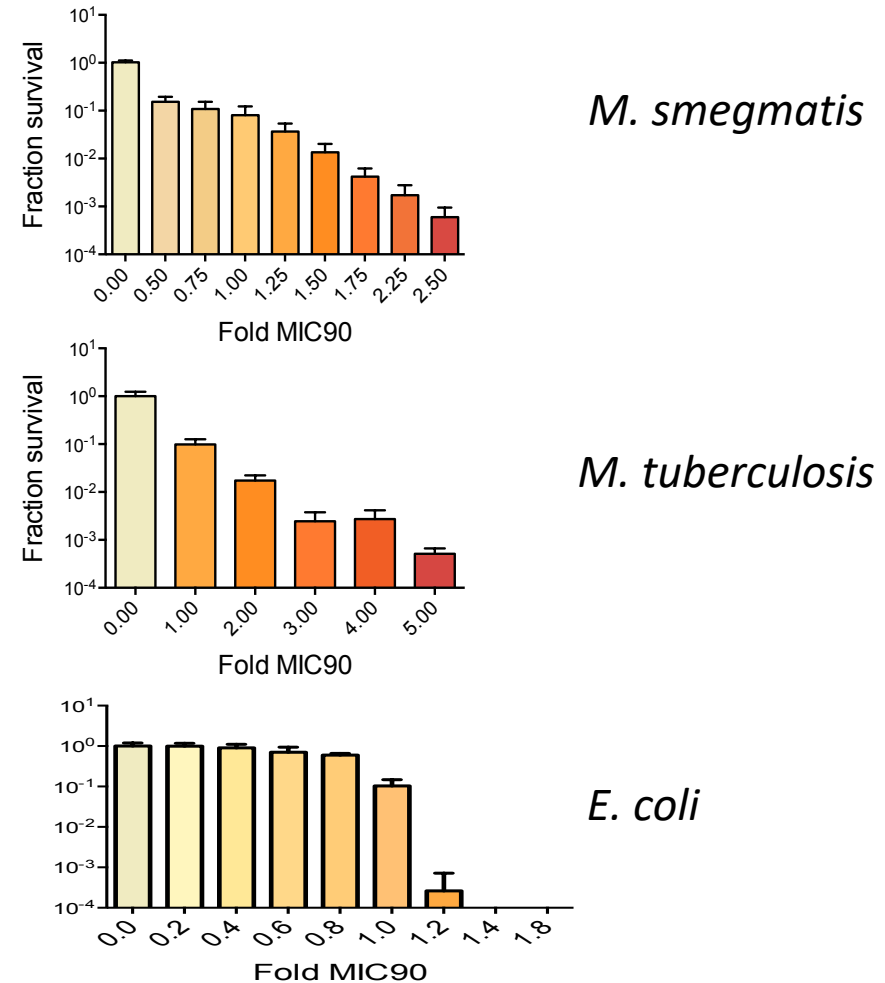
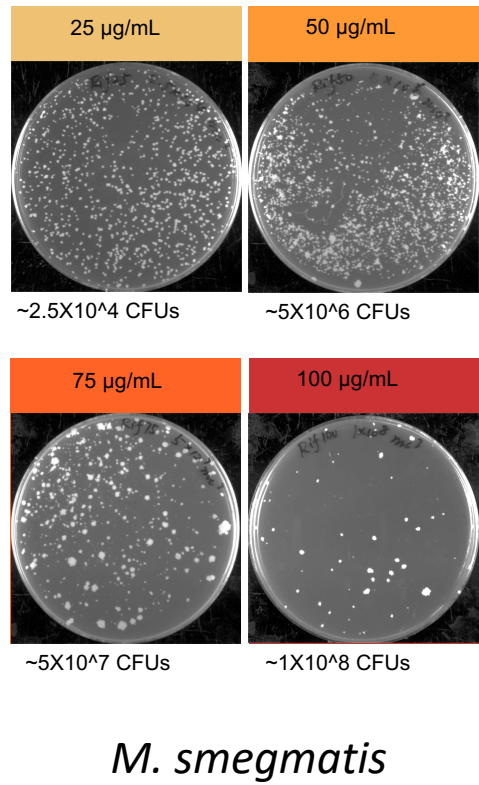
Junhao Zhu



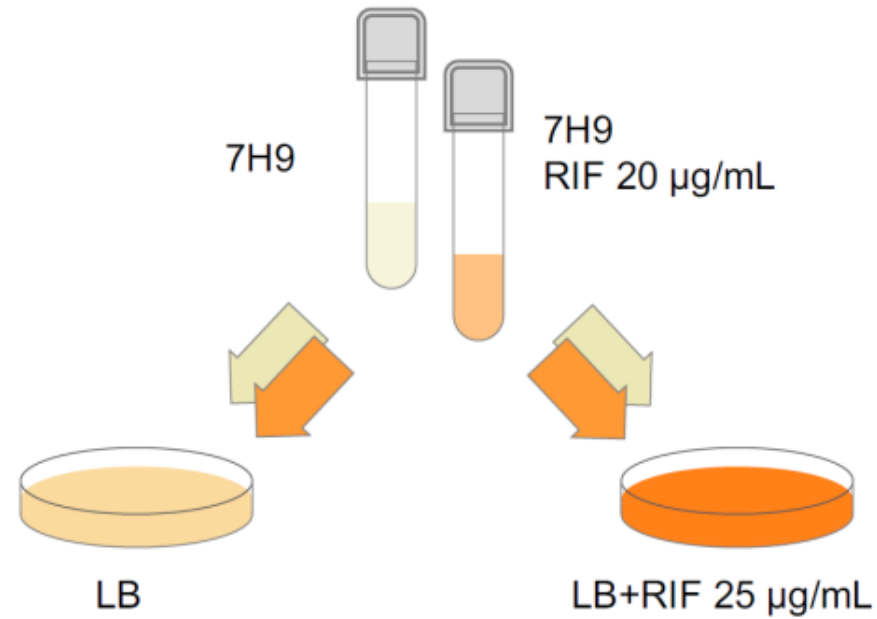
JH: I want to study these guys!

RIFAMPICIN

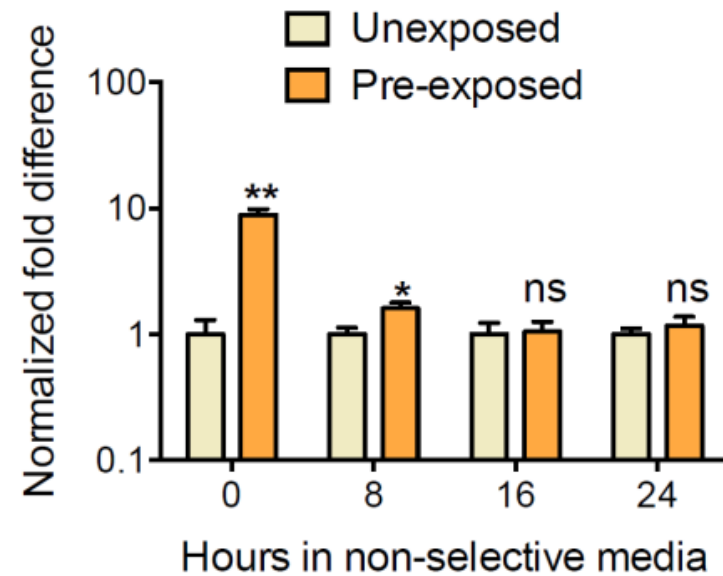
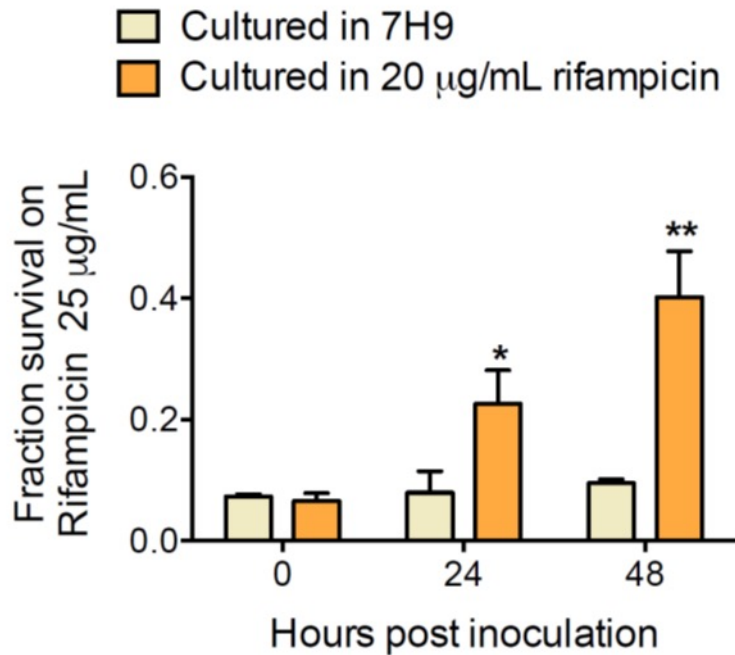
Rifampicin-specific phenotypic resistance is a specific form of antibiotic tolerance



Rifampicin exposure leads to drug-specific hypertolerance

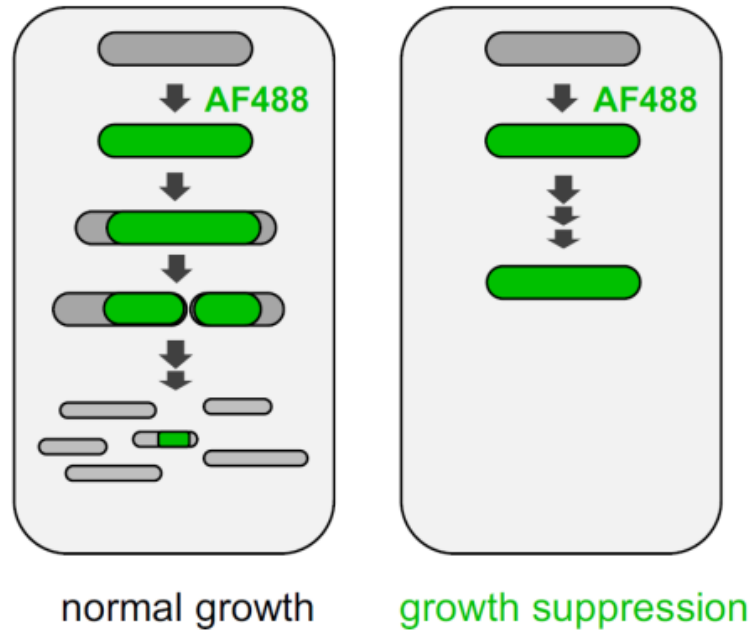


Rifampicin exposure leads to semi-heritable hypertolerance

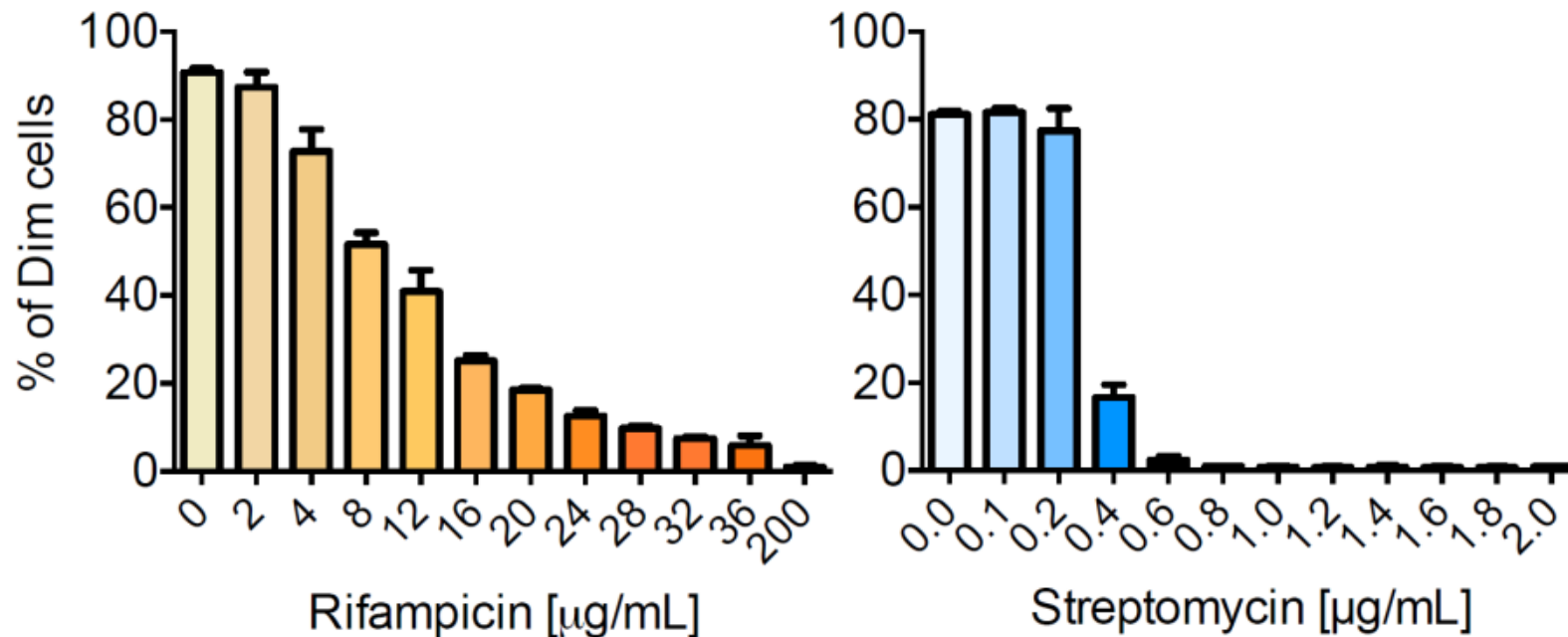
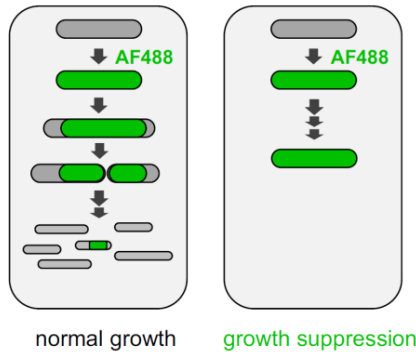


M. smegmatis

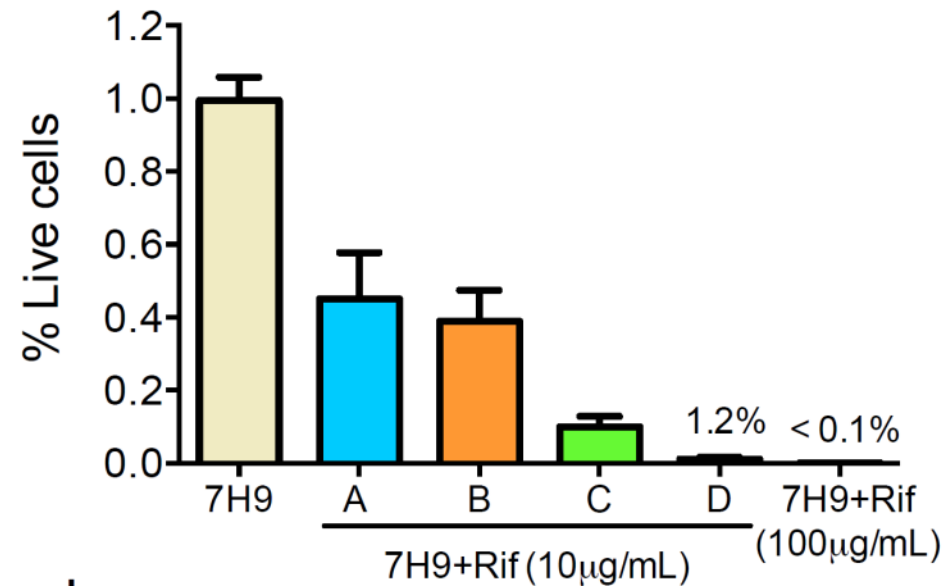
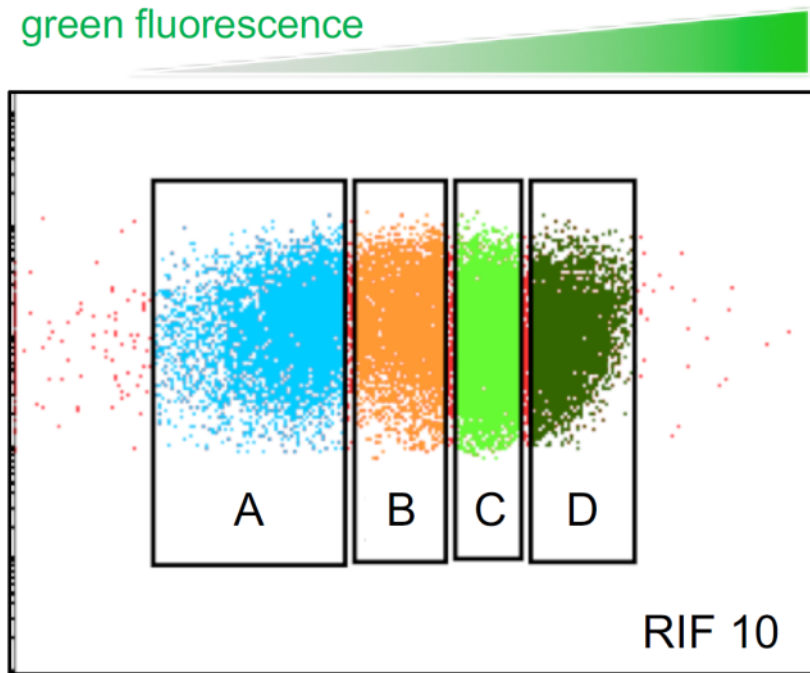
A fluorescence dilution assay allows identification of phenotypic resistors



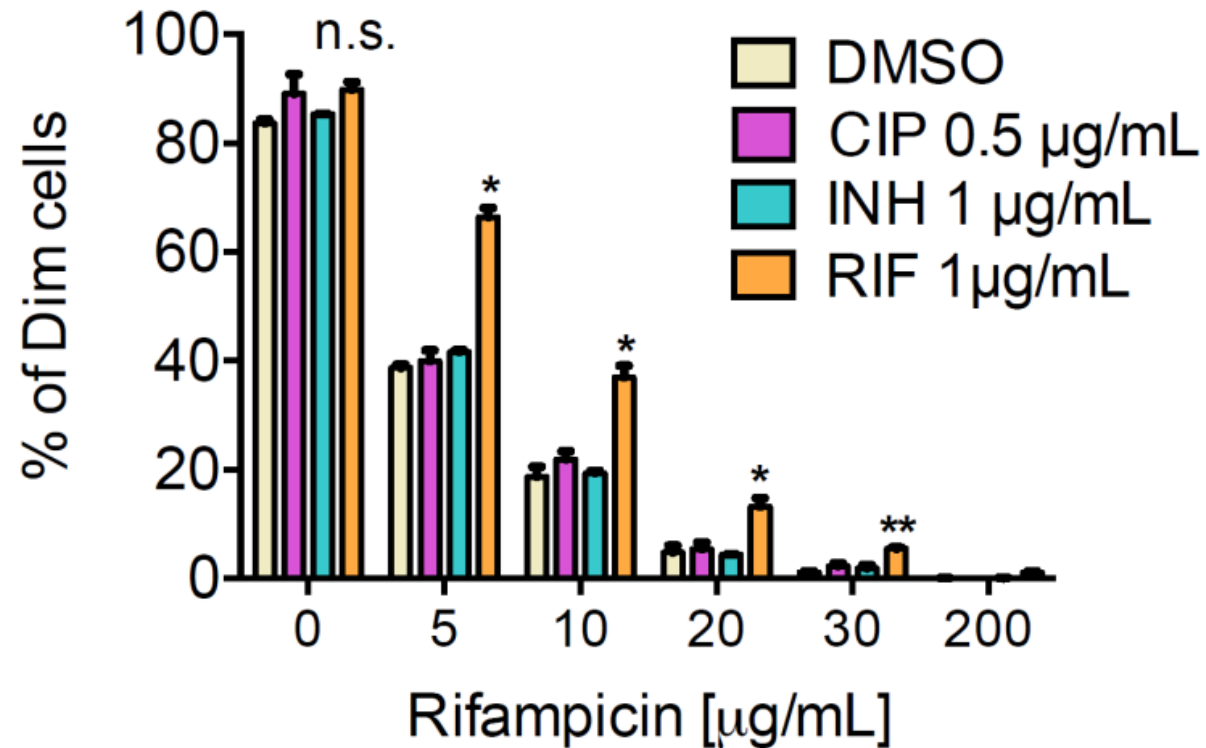
A fluorescence dilution assay allows identification of phenotypic resistors



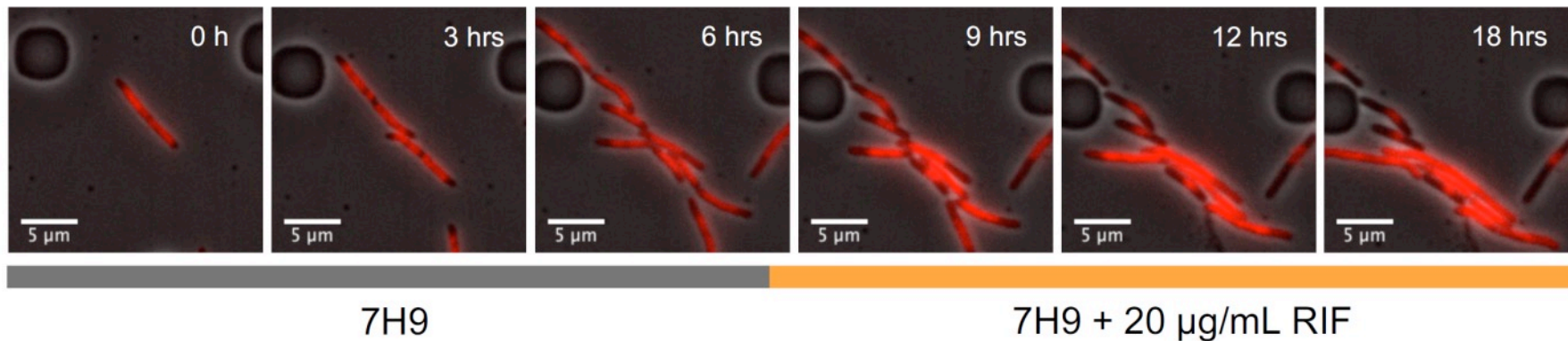
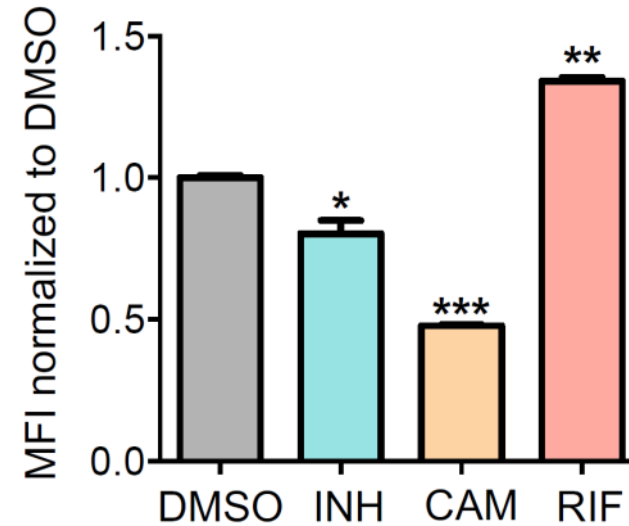
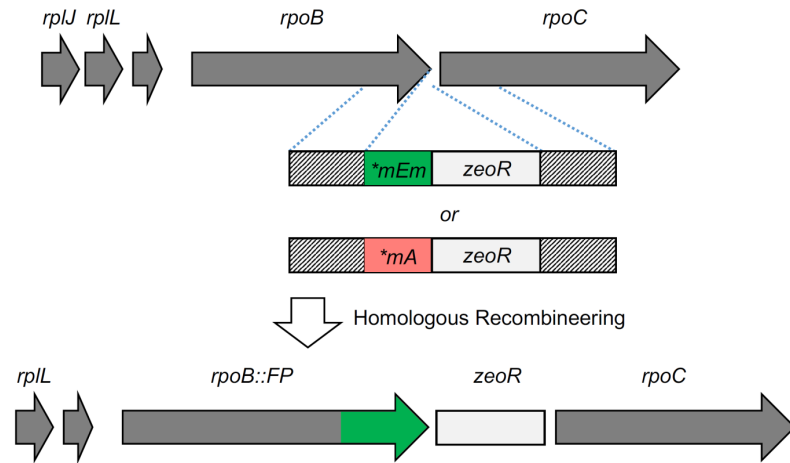
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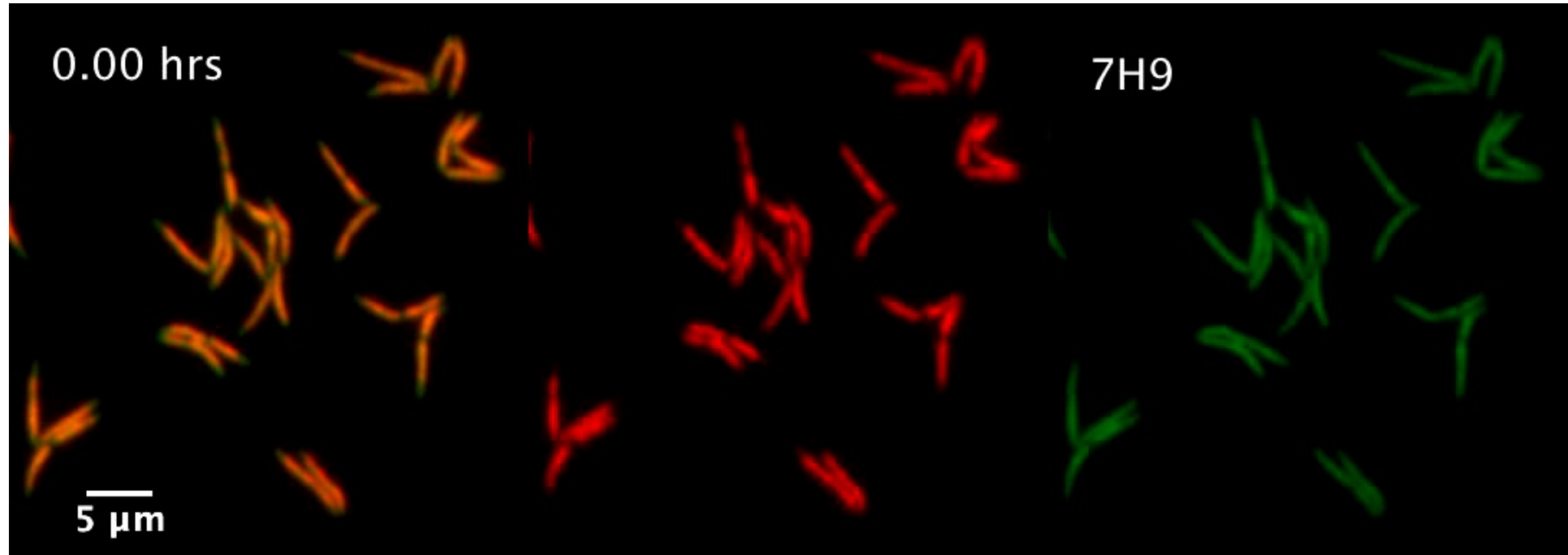
Rifampicin exposure increases rifampin-specific tolerance



RpoB expression increases after rifampicin exposure



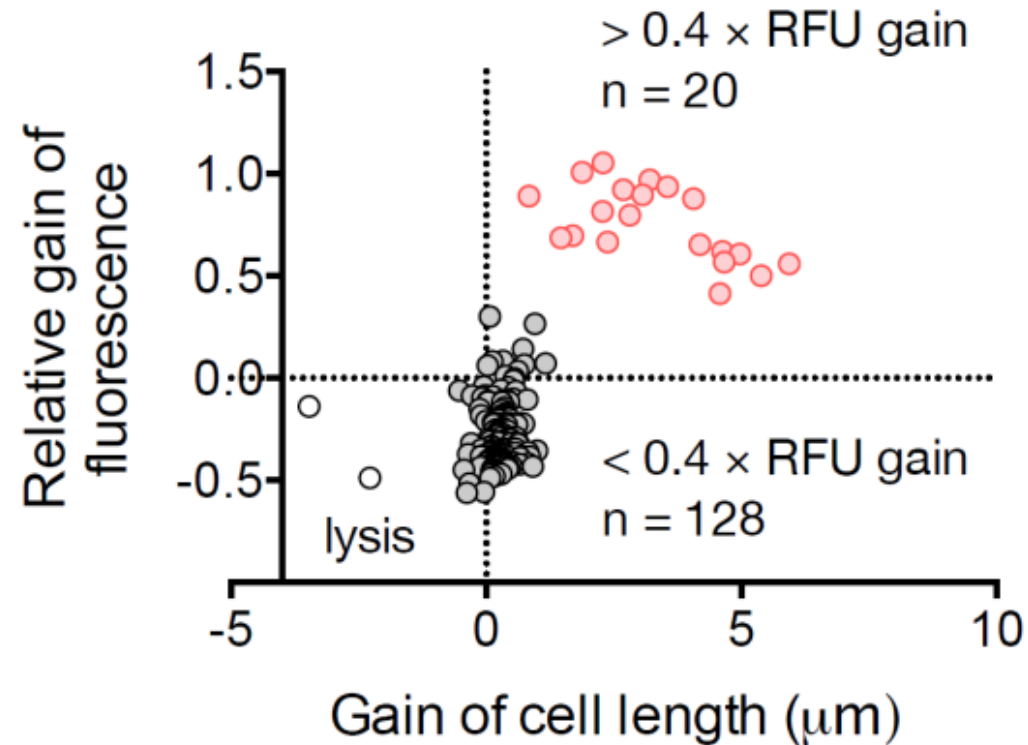
Rifampicin induces its own tolerance via upregulation of its cellular target



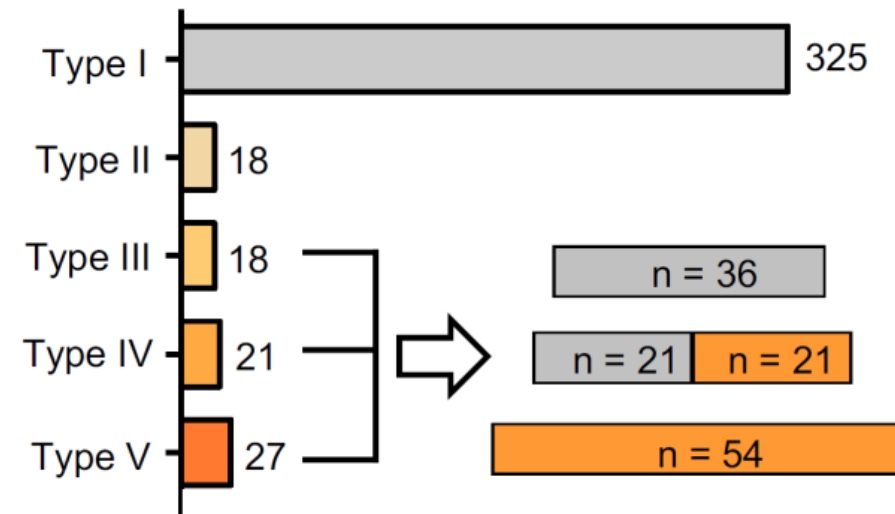
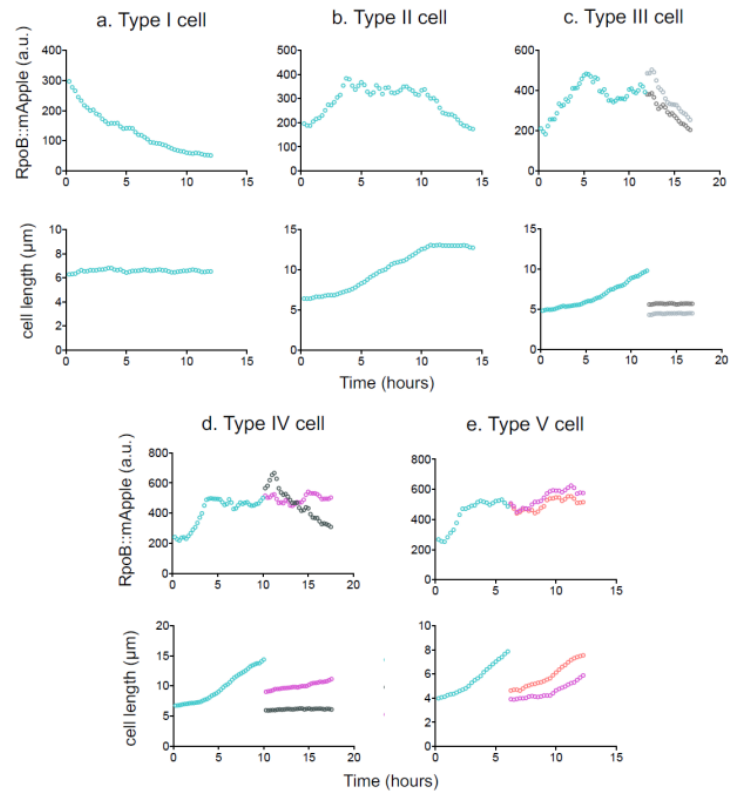
M. smegmatis::*rpoB*-mApple

M. smegmatis::*PrpoB*-mEm

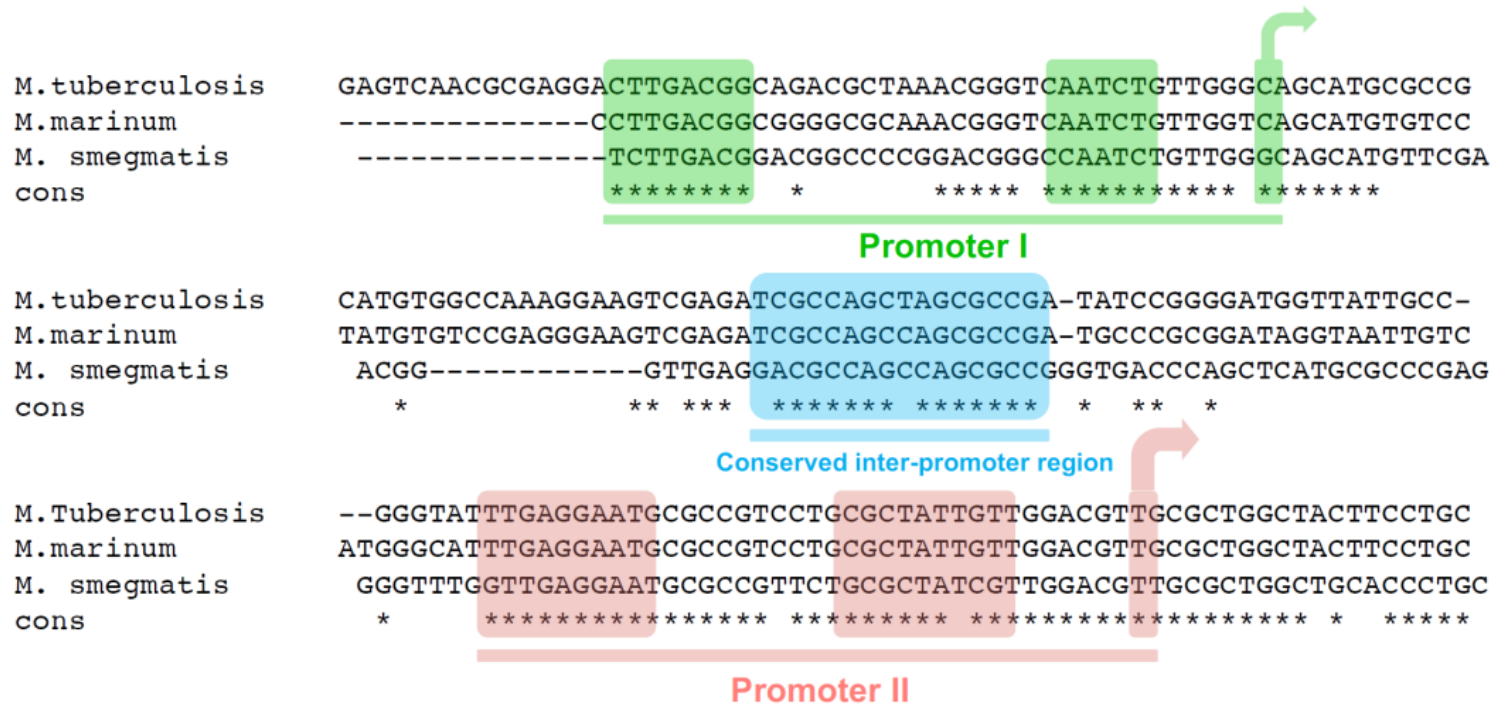
Rifampicin treatment bisects the mycobacterial population response



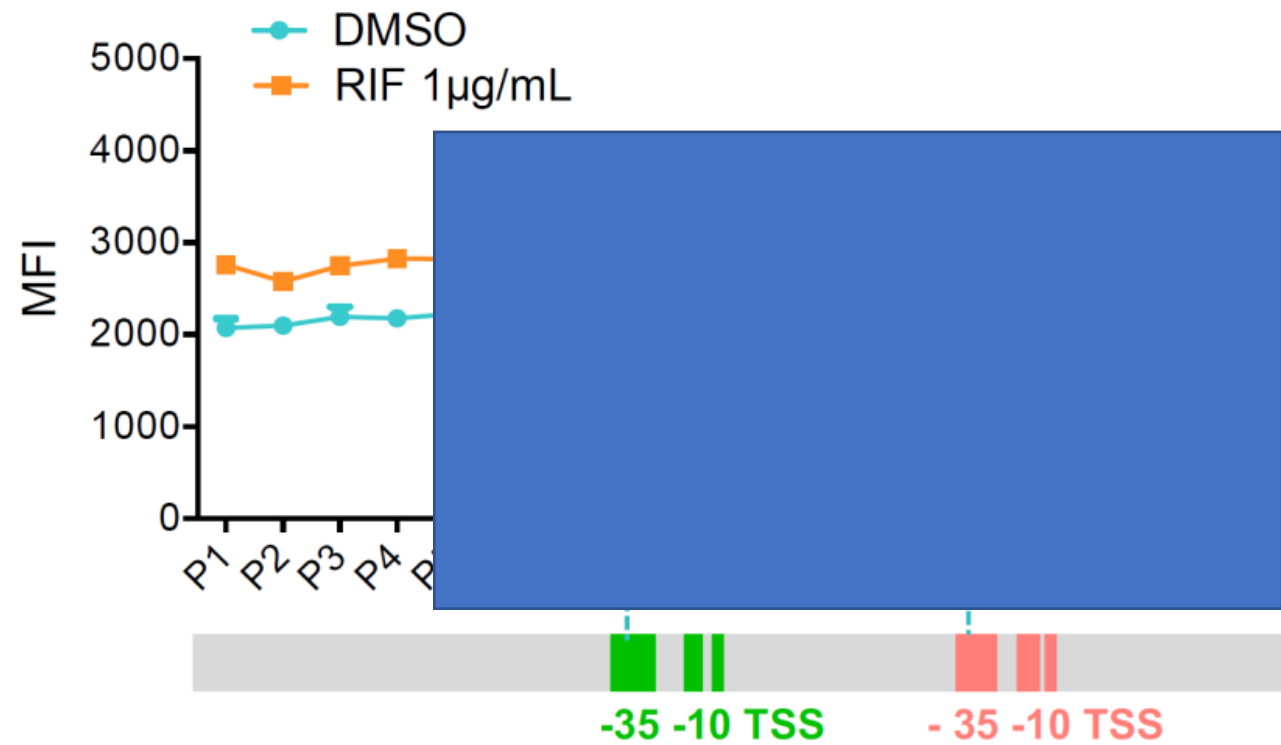
'Growers' are more likely to have grower daughters



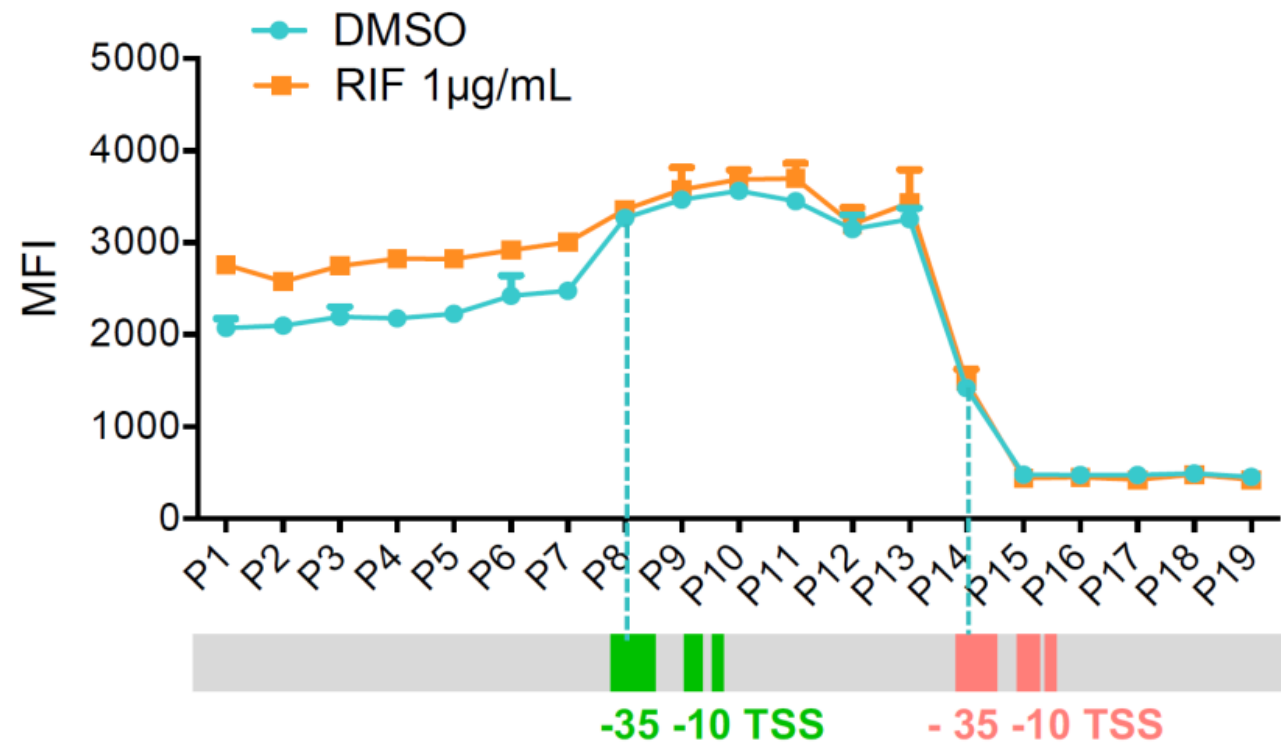
Mycobacterial *rpoB* has two highly conserved promoters



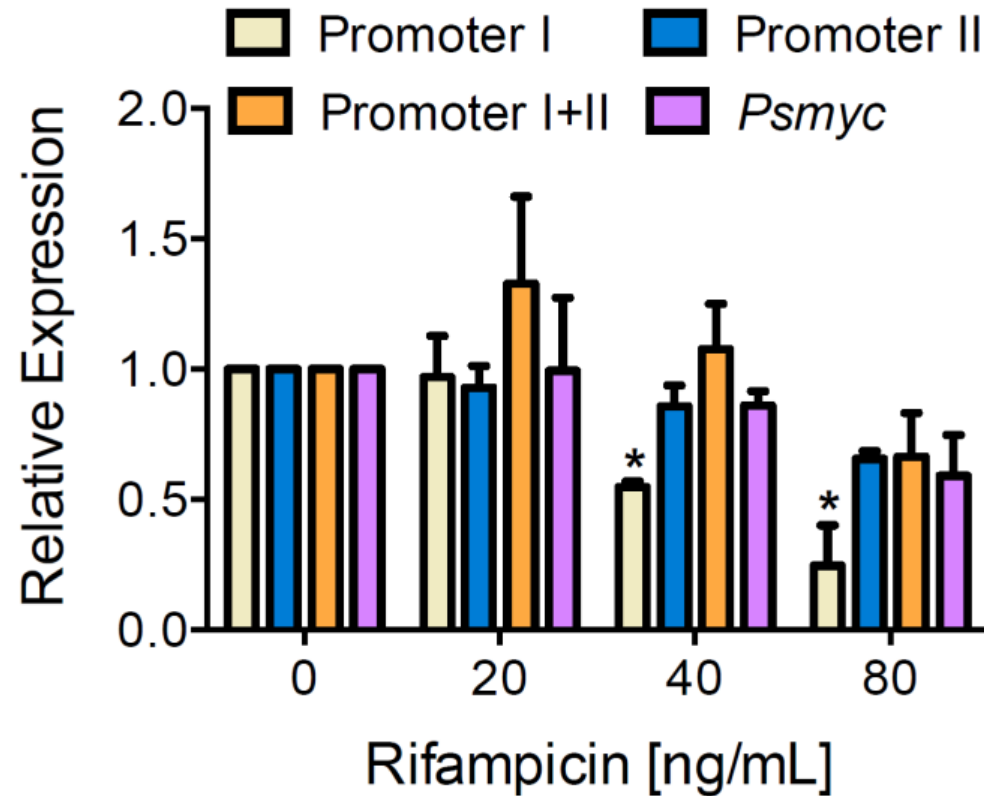
rpoB promoter I negatively regulates promoter II expression and is inhibited by RIF



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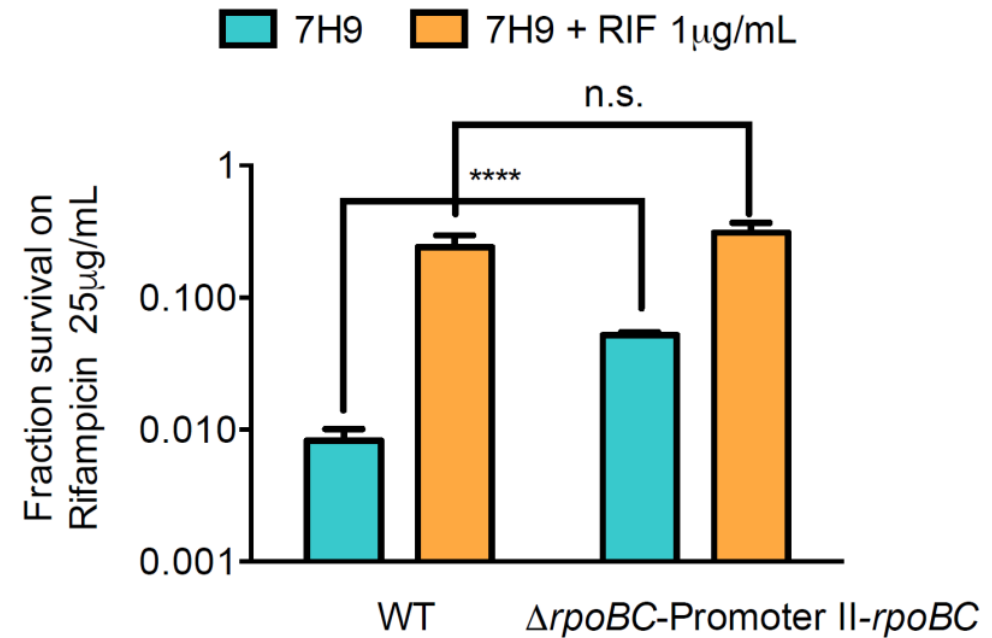
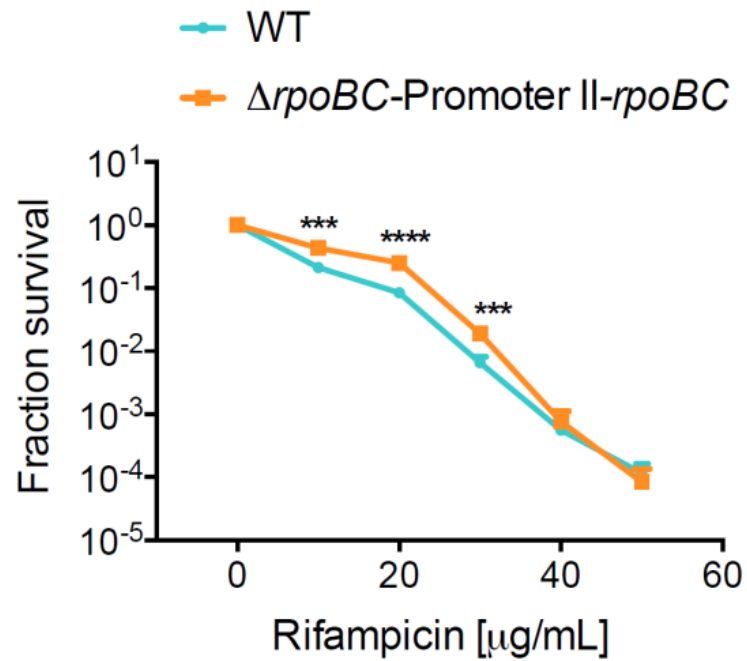


rpoB promoter I negatively regulates promoter II expression and is *sensitively* inhibited by RIF

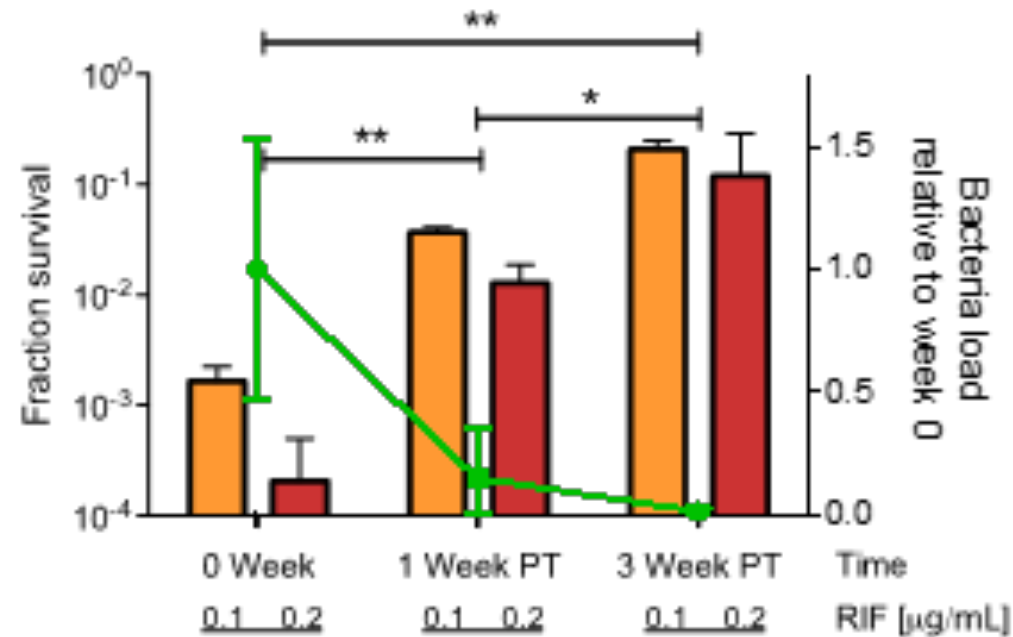


in vitro RNAP transcription

Disrupting *rpoB* promoter structure alters rif-specific tolerance



RSPR 'growers' are enriched upon treatment of drug-sensitive TB patients



Perspectives

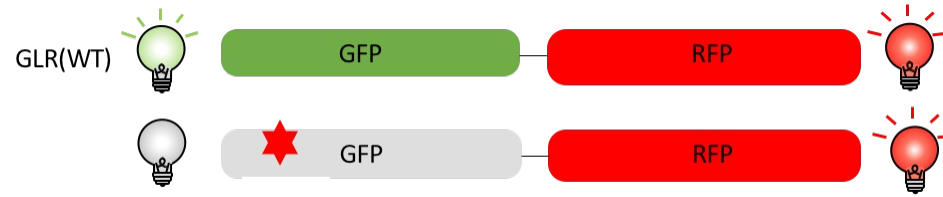
- Mtb uses mistranslation to adapt to hostile environments – antibiotics and the host
- There are *many* routes to rifampicin tolerance in mycobacteria: in both growing and non-growing populations: which ones are most important in the clinical response to antibiotics or give rise to *bona fide* drug resistance is not known
- Studying mycobacteria informs not only pathogenesis/ drug tolerance but uncovers entirely novel biology

If there's still 5 minutes time...

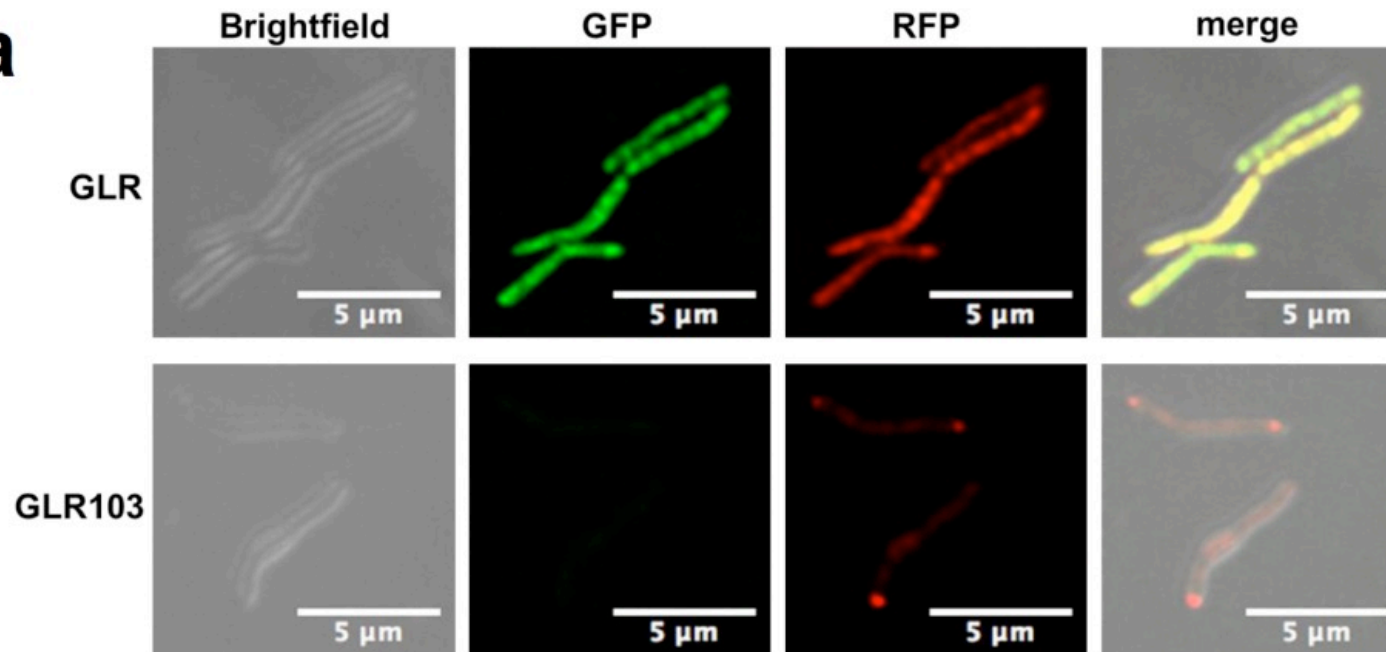
HspX is a protein pro-aggregase and sortase

- HspX is a small heat shock protein (~16 kDa)
- Highly upregulated in response to prolonged microaerophilic conditions (the “hypoxic dormancy regulon”)
- One of the most upregulated proteins in Mtb in infection and a leading vaccine antigen candidate
- Biochemically characterized as a misfolded protein ‘holdase’
- Yet, its cellular function unknown

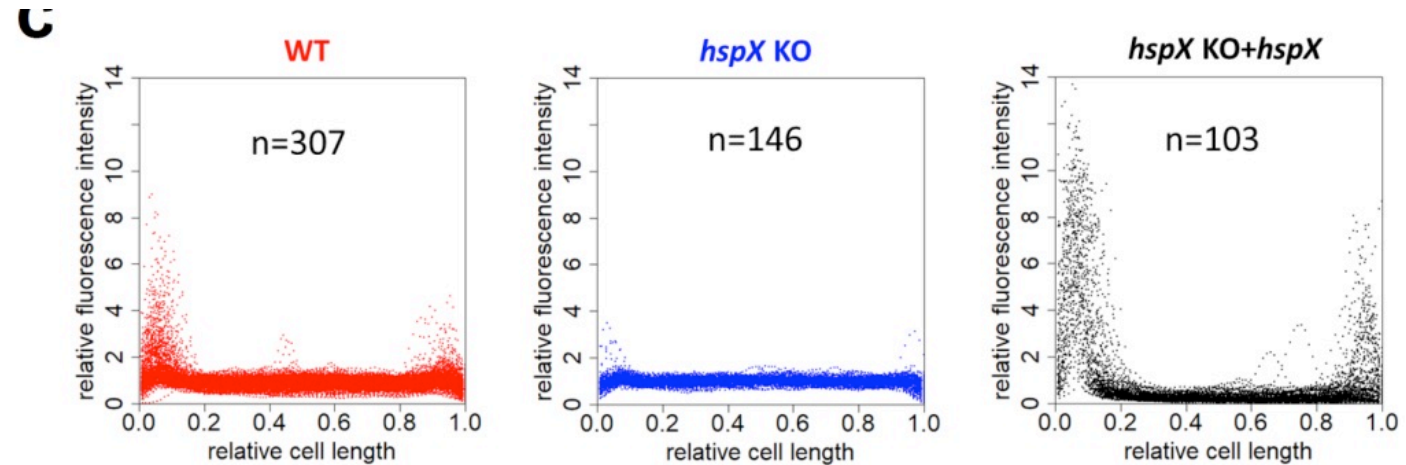
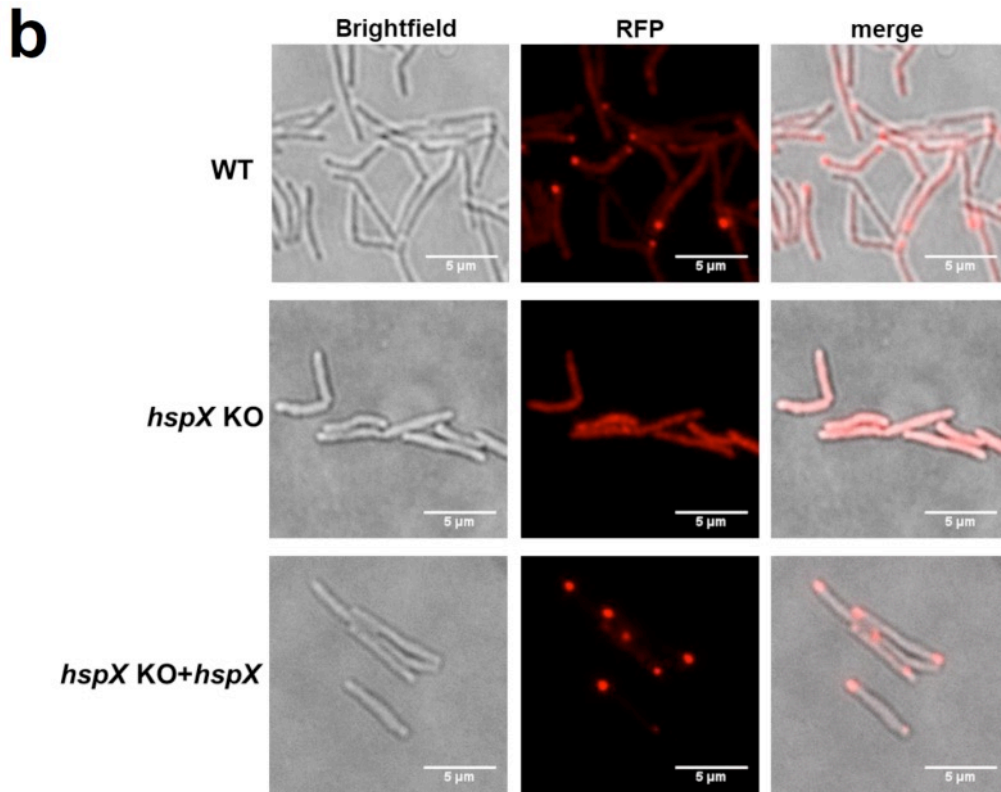
A fluorescent 'misfolding' reporter



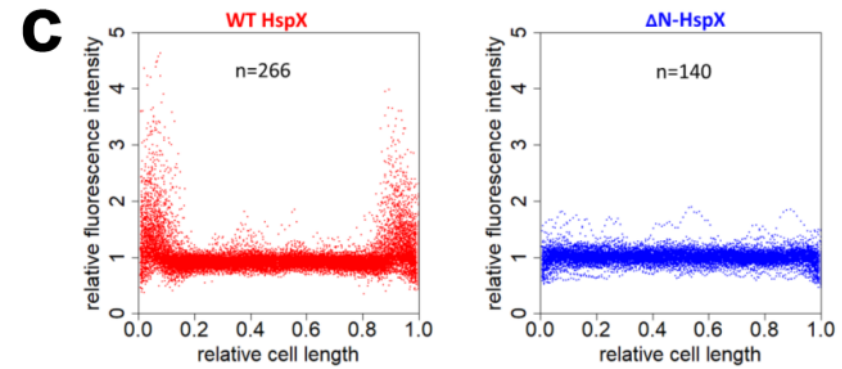
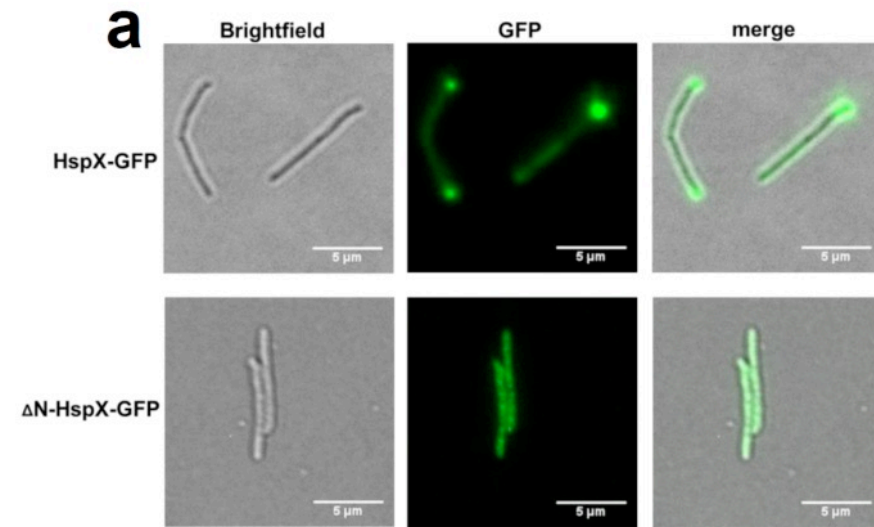
a



Misfolded proteins traffic to the pole in an HspX-dependent manner

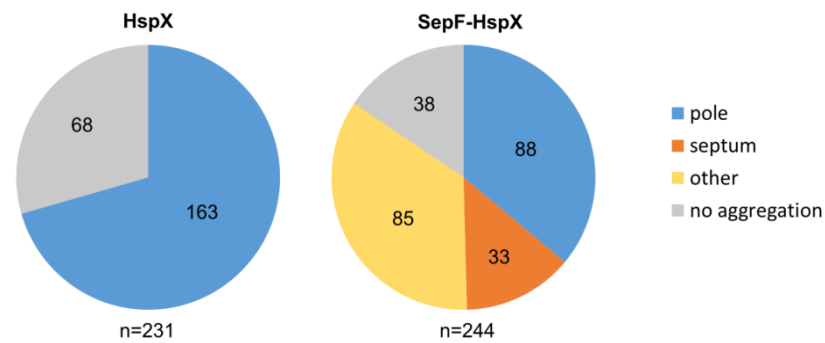


HspX itself localizes to the pole

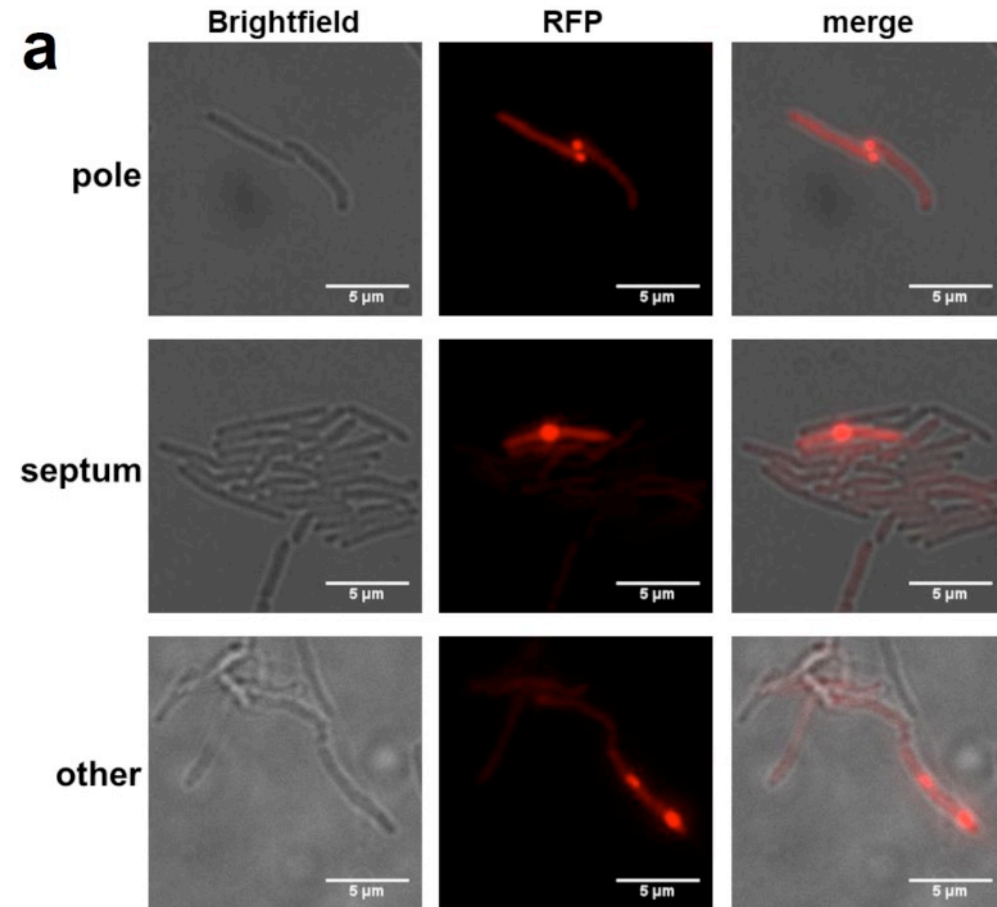


Re-directing HspX alters misfolded protein localization

b

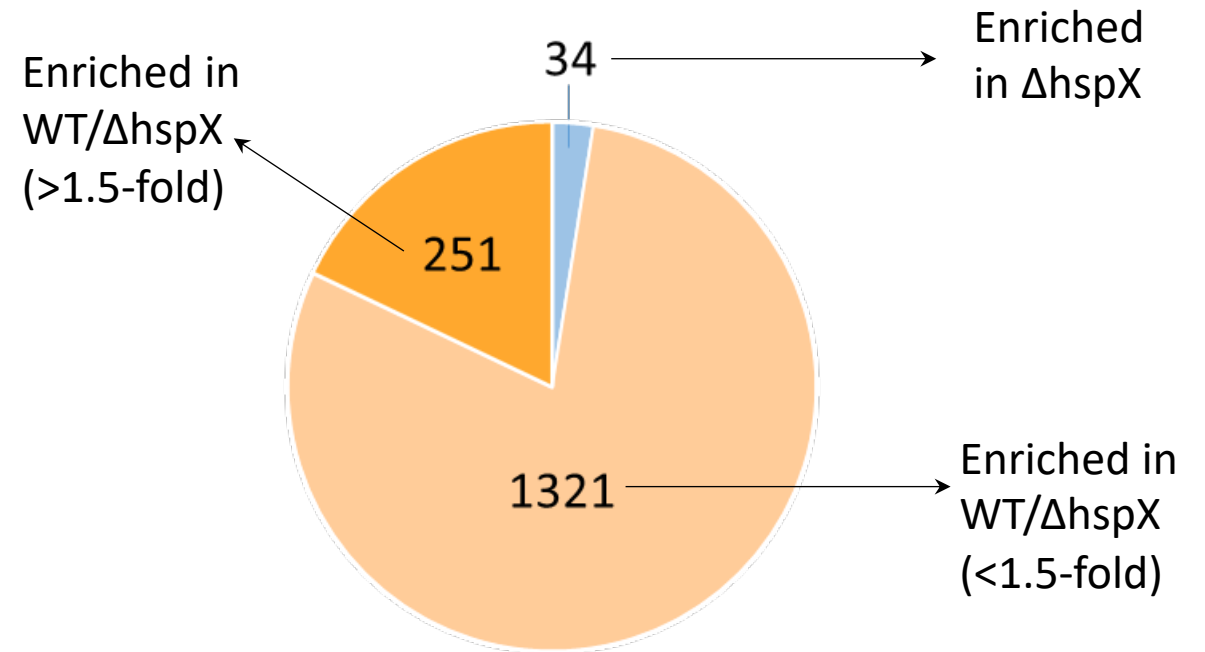


a



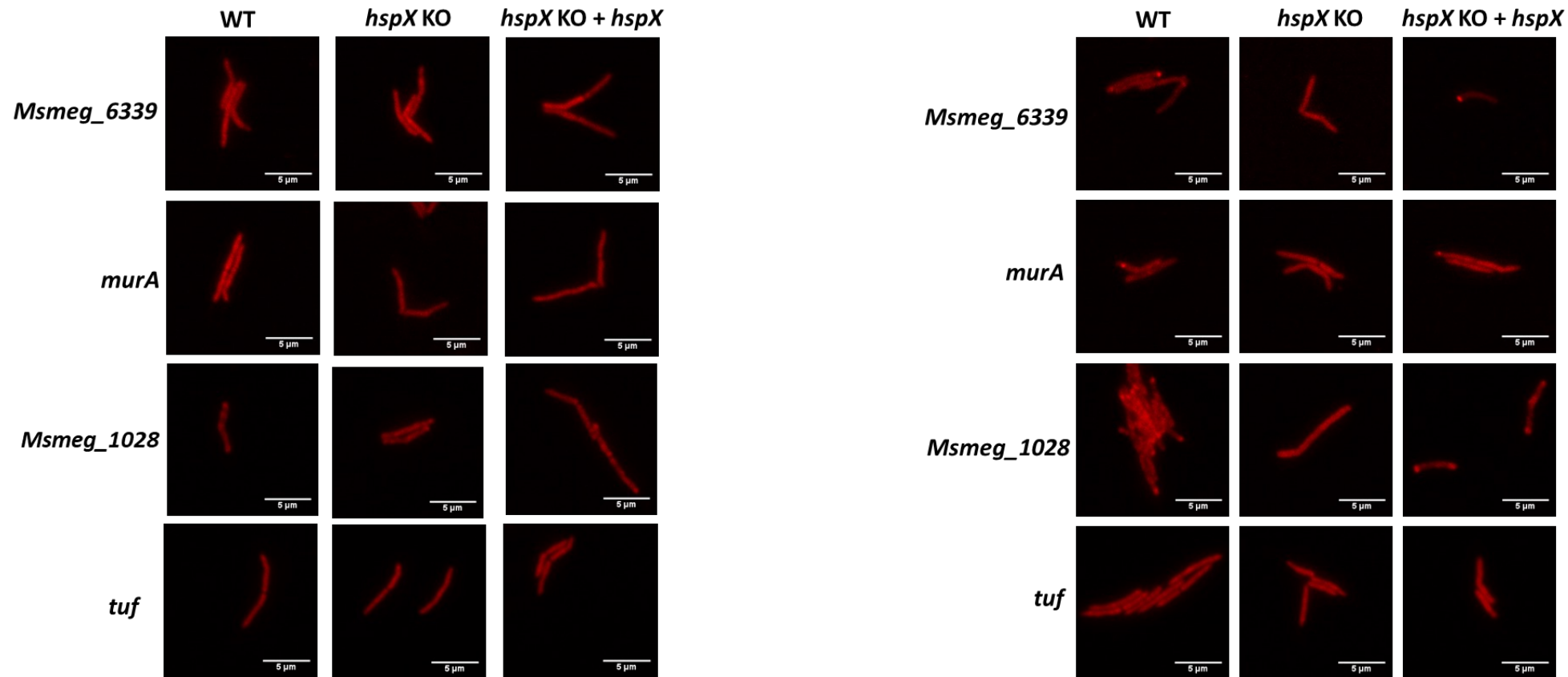
Are there native HspX client proteins?

- Isolate inclusion bodies in WT, $\Delta hspX$, *hspX-OE* strains
- Semi-quant MS with TMT tags



1355: proteins in total

Native HspX-clients misfold in an HspX-dependent manner under stress



Axenic culture

Heat shock

Acknowledgements

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Junhao Zhu*

Hongwei Su*

Rongjun Cai*

Swarnava Chaudhuri*

Biwei Wang*

Yiwei Zhang*

Ernst Lab (UCSF)

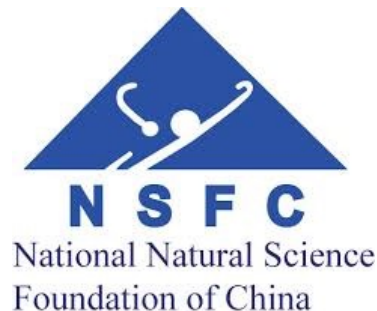
Thuong Lab (OUCRU)

Bavesh Kana (Witts)

Hesper Rego (Yale)



BILL & MELINDA
GATES foundation



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Swarnava Chaudhuri*

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