

**A protein complex from human milk enhances the activity of antibiotics  
and drugs against *Mycobacterium tuberculosis***

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## SUPPLEMENT

### Supplementary Tables

Name	Sequence (5' to 3')
NdeI-his-mutLA	TATTAACATATGCACCACCACCACCACCACCACCACAAACAGTTCACAAAATC TGAATTG
HindIII-LA rev	CTAGATAAGCTTTACAGTTTCTCGCTA

**Table S1: Oligonucleotides used in this work.**

Strain	Parent strain and relevant genotype	Reference
<i>E. coli</i> DH5 $\alpha$	recA1; endA1; gyrA96; thi; relA1; hsdR17(rK-;mK+); supE44; $\Phi$ 80 $\Delta$ lacZ $\Delta$ M15 $\Delta$ lacZYA-argF; UE169	(1)
<i>E. coli</i> BL21(DE3)	<i>E. coli</i> B derivative; F <sup>-</sup> <i>ompT gal dcm lon hsdS<sub>B</sub>(r<sub>B</sub><sup>-</sup> m<sub>B</sub><sup>-</sup>)</i> $\lambda$ (DE3 [ <i>lacI lacUV5-T7 gene 1 ind1 sam7 nin5</i> ])	(2)
<i>M. tuberculosis</i> H37Rv	wild-type	ATCC 25618
<i>M. tuberculosis</i> mc <sup>2</sup> 6206	H37Rv derivative, $\Delta$ <i>leuCD</i> , $\Delta$ <i>panCD</i>	(3)
<i>S. pneumoniae</i> R36A	Non-encapsulated derivative of the wild-type strain D39; obtained from Dr. D. E. Briles	(4)

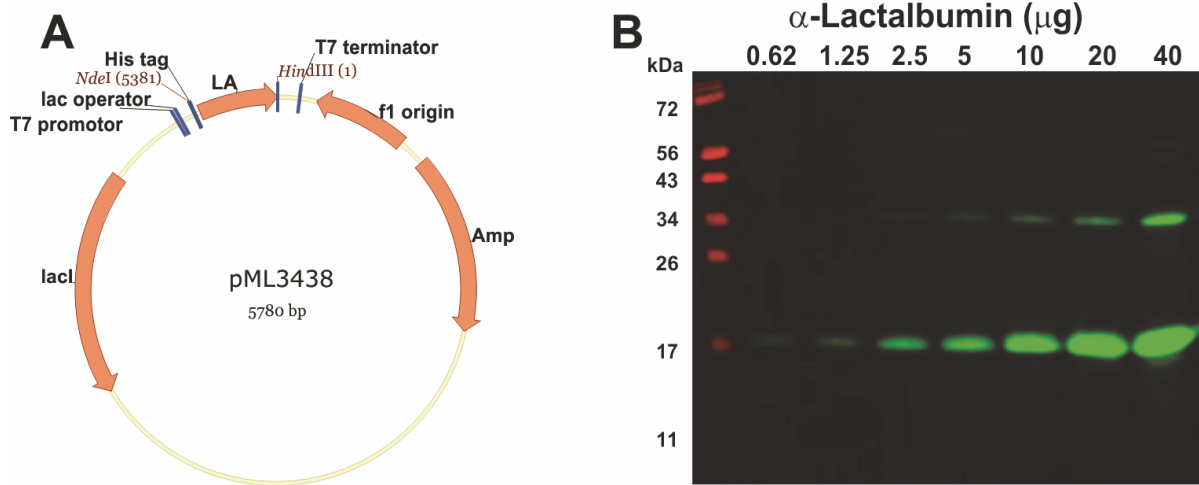
**Table S2. Strains used in this work.**

Plasmid	Components and properties	Reference
pET-21(b)+	f1 origin of replication, amp <sup>R</sup> , T7lac	Novagen
pML3438	pET-21(b)+ - derivative; $\alpha$ -lactalbumin expression plasmid; ColE1 origin; amp <sup>R</sup> ; 5780 bp	(this study)

**Table S3. Plasmids used in this work.**

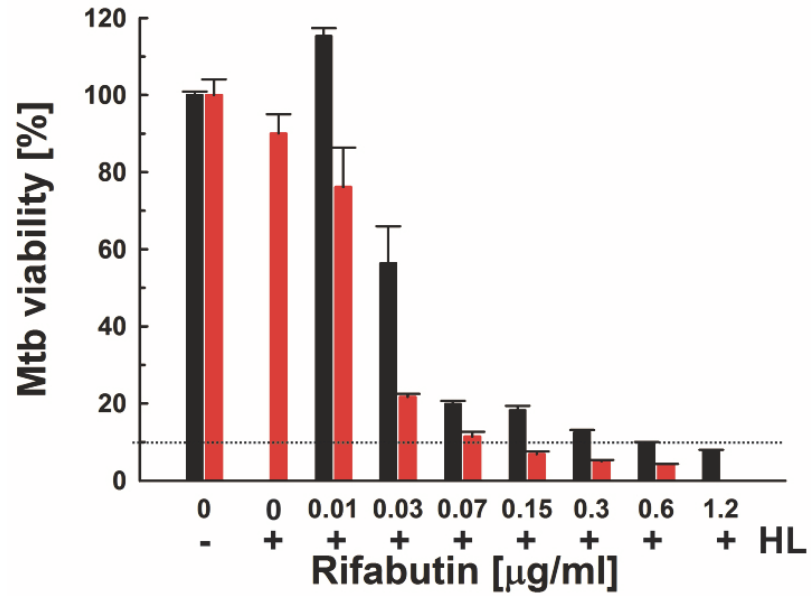
The annotation amp<sup>R</sup> indicates that the plasmid confers resistance to ampicillin. "Origin" denotes the origin of replication.

## Supplementary Figures



**Fig. S1. Expression vector and production of recombinant  $\alpha$ -lactalbumin from *E. coli*.**

**A.** Map of the  $\alpha$ -lactalbumin expression plasmid pML3438. The recombinant  $\alpha$ -lactalbumin gene (*hALA*) encodes a mutated human  $\alpha$ -lactalbumin (C6S, C28S, C61S, C73S, C77S, D87A, C91S, C111S, C120S) with an N-terminal histidine tag comprising eight histidines. Transcription of the *hALA* gene is initiated from the bacteriophage T7 promoter. The *bla* gene confers resistance to ampicillin. **B.** The recombinant  $\alpha$ -lactalbumin protein was purified by Ni(II) affinity chromatography. Different amounts of  $\alpha$ -lactalbumin (LA) were loaded on a protein gel and detected by a monoclonal antibody in a western blot. The theoretical molecular mass of the recombinant  $\alpha$ -lactalbumin is 15 kDa. The band with an apparent molecular mass of app. 34 kDa is probably dimeric  $\alpha$ -lactalbumin.



**Fig. S2. HAMLET potentiates the efficacy of rifabutin against *M. tuberculosis***

*M. tuberculosis* H37Rv was incubated with increasing concentrations of rifabutin in the absence (black bars) or presence (red bars) of 90 µg/ml HAMLET (75% of the MIC<sub>90</sub>). The viability of *M. tuberculosis* H37Rv was determined by the microplate Alamar Blue assay. Error bars represent standard errors of the mean values of biological triplicates.

## Supplementary References

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2. **Studier, F. W., and B. A. Moffatt.** 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. **189**:113-130.
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