

## Supplementary Information

### **RecA-NT homology motif in ImuB is essential for mycobacterial ImuA'-ImuB protein interaction and mutasome function**

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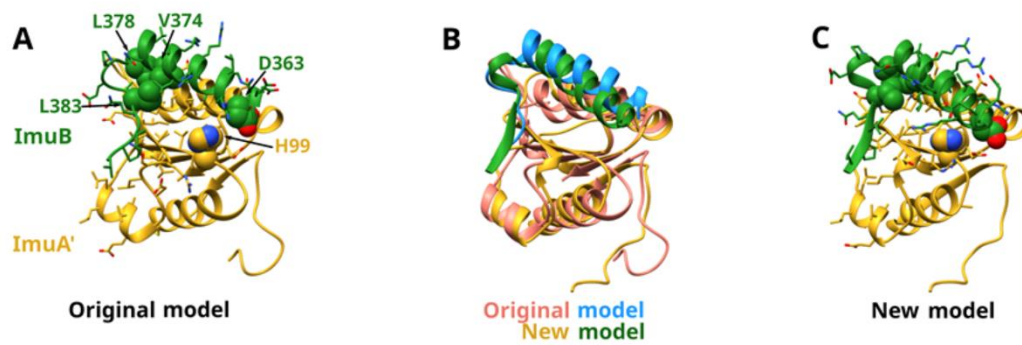
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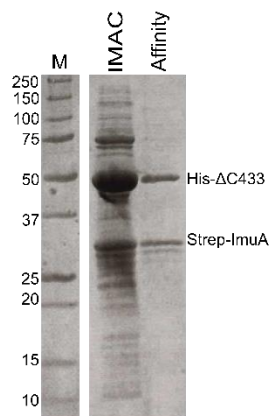
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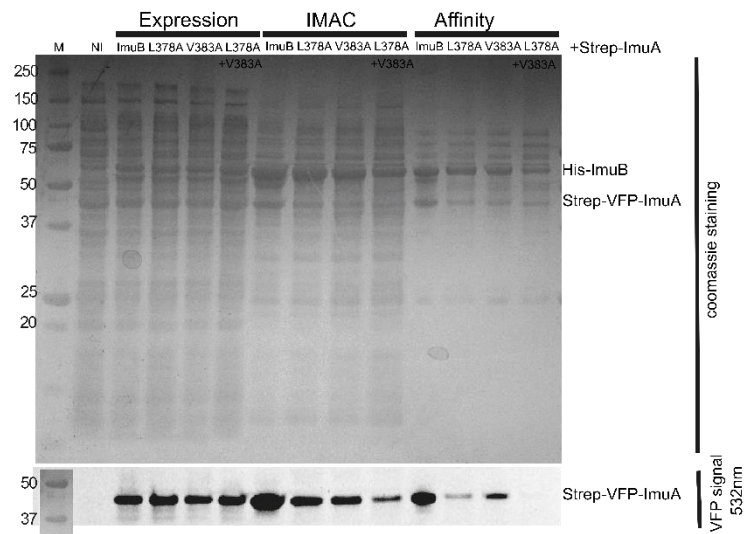
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**Fig. S1. Comparison of original Modeller model A. to new AlphaFold model C. with B. showing both models superimposed (original model – salmon/blue, new model - gold/green). Only structurally corresponding parts to the original model are shown for the new model: 63-178 of ImuA' and 360-387 of ImuB. The original model includes 67-194 of ImuA', but the C-terminal beta-strand is modeled incorrectly according to the AlphaFold prediction.**



**Fig. S2 - His-ImuBΔC433, in which the full RecA-NT is preserved, forms a stable complex with ImuA'.** SDS-PAGE analysis of the purification of His-ImuBΔ433 with Strep-ImuA' after two sequential pull-downs with HisTag and StrepTag, respectively.



**Fig. S3. Biochemical confirmation of disruption of ImuA'-ImuB interaction in L378A+V383A (double mutant, DM) using a fluorescent tagged version of ImuA' (Strep-VFP-ImuA').** SDS-PAGE analysis of the co-expression of His-ImuB mutants with Strep-ImuA' and the two subsequent pull-downs, followed by size-exclusion chromatography. Expression samples correspond to the total expression after growth for 3h following induction with IPTG. Above is the SDS-PAGE gel stained with Coomassie and below is the same gel imaged at 532nm for detection of the VFP fluorescent tag in ImuA'.

**Table S1 Interface surface areas between ImuB constructs and full length ImuA'.** Calculated with VoroContacts from the ImuA'-ImuB AlphaFold model.

ImuB construct	Interface area with ImuA', Å <sup>2</sup>
ImuB, full	1804
ImuB, 360-388 (RecA-NT motif)	694
ImuB, 422-525 (ImuB-C)	960
ImuB, 1-359 (C-terminal region removed)	0