

Reduced beta-lactam susceptibility in group A streptococcus

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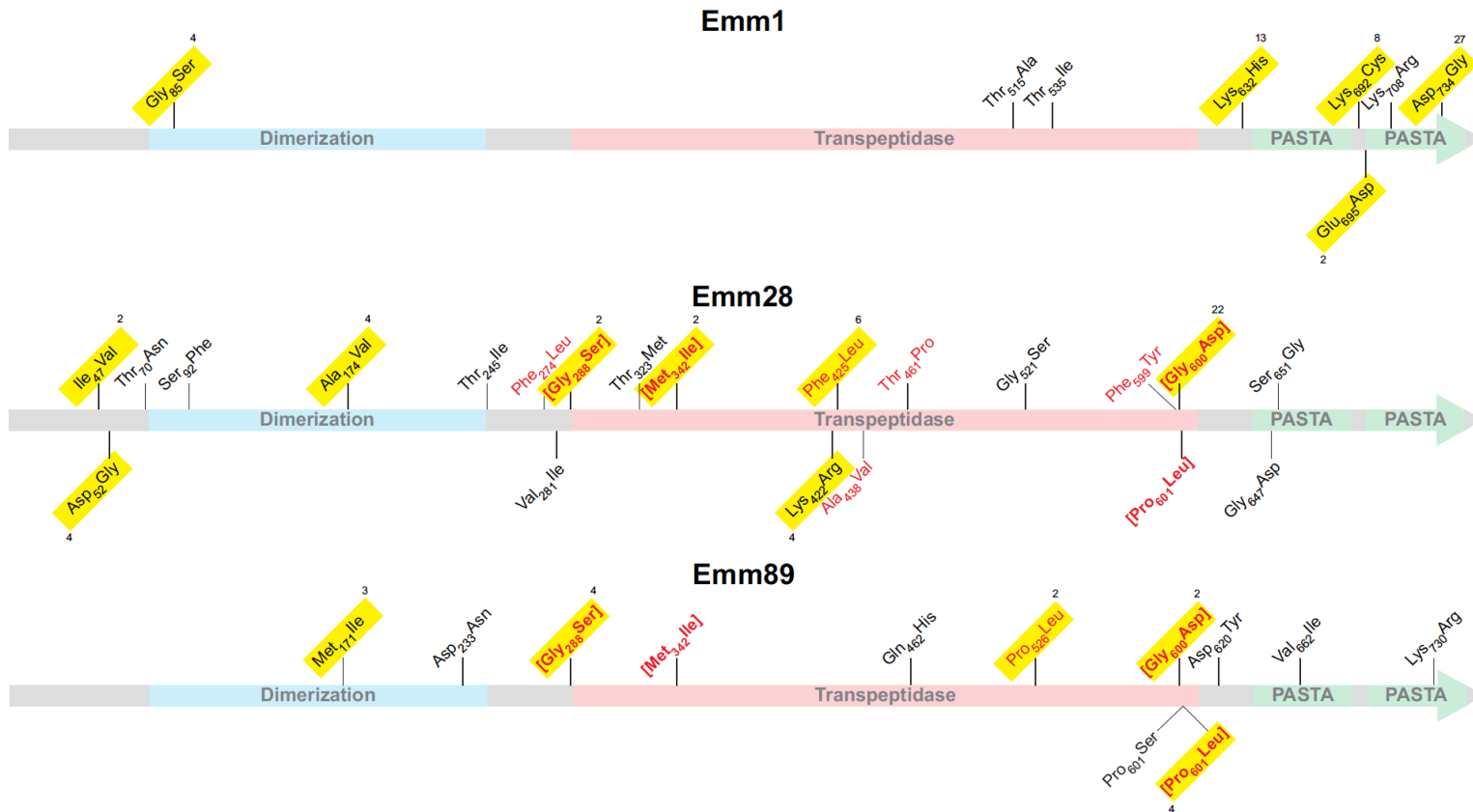
Group A Streptococcus is universally susceptible to beta-lactam antibiotics

- For generations, microbiologists, physicians, and infectious disease experts have been taught that *Streptococcus pyogenes* (Group A Streptococcus; GAS) is universally susceptible to beta-lactam antibiotics
- Clinical laboratories do not routinely perform antimicrobial susceptibility testing on GAS isolates
- GAS strains may be accumulating mutations in genes such as Penicillin Binding Protein 2X (PBP2X) and evolving towards a beta-lactam resistance phenotype

- Our laboratory and others have recently reported GAS strains with nonsynonymous (amino acid altering) mutations in PBP2X
- We hypothesized that PBP2X mutations in GAS are geographically widespread, alter beta-lactam susceptibility *in vitro*, and increase fitness *in vivo*
- To test this hypothesis, we
 - interrogated our population genomic data of 7,025 international serotype M1, M28 and M89 GAS strains
 - performed susceptibility testing
 - performed virulence studies

Identification of PBP2X amino acid replacements

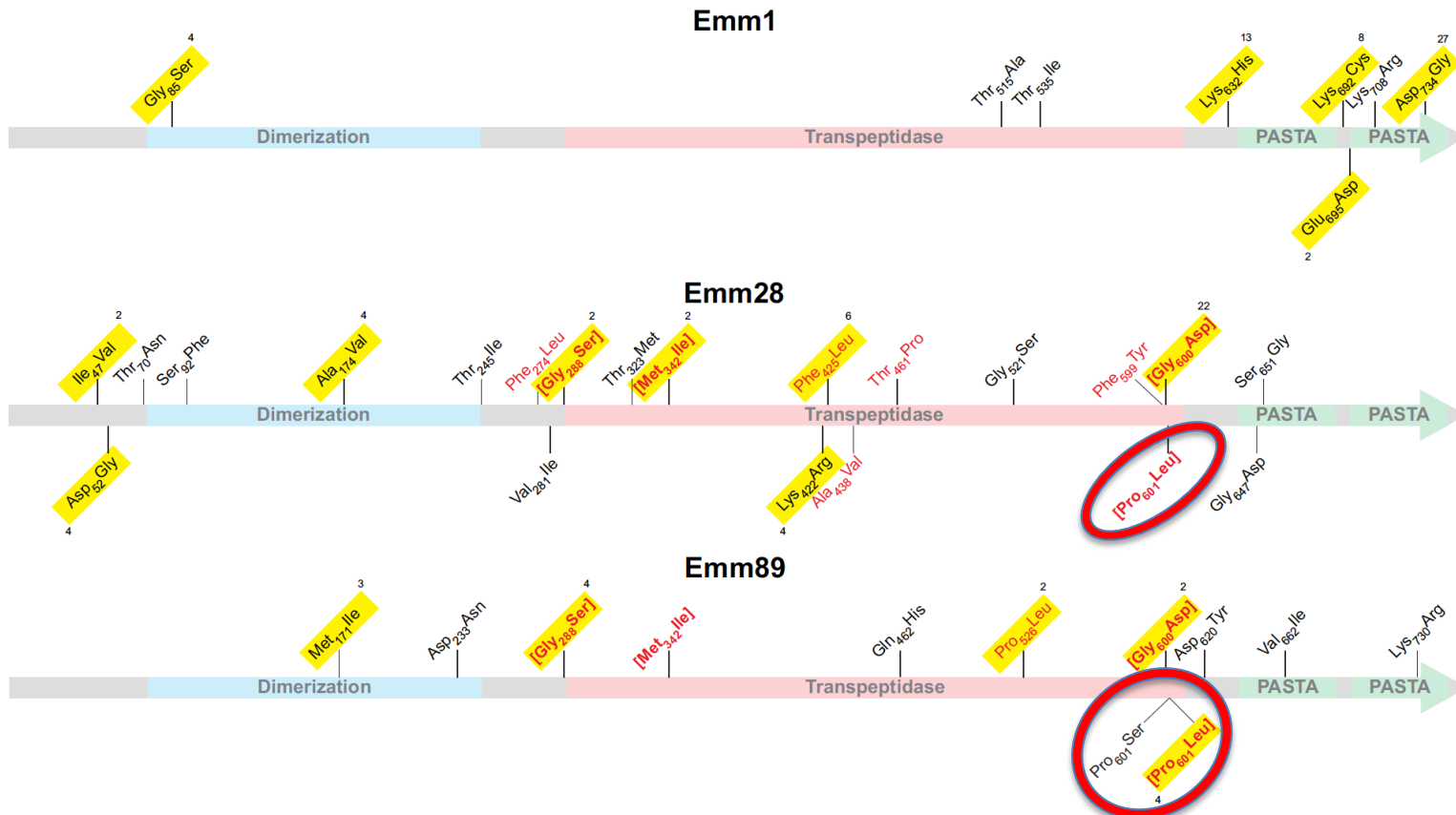
Among 7,025 whole genome sequences examined, we identified 137 strains with 37 different nonsynonymous mutations in PBP2X, which is significantly greater than would be expected by random chance



Amino acid replacements identified in multiple isolates are highlighted in yellow

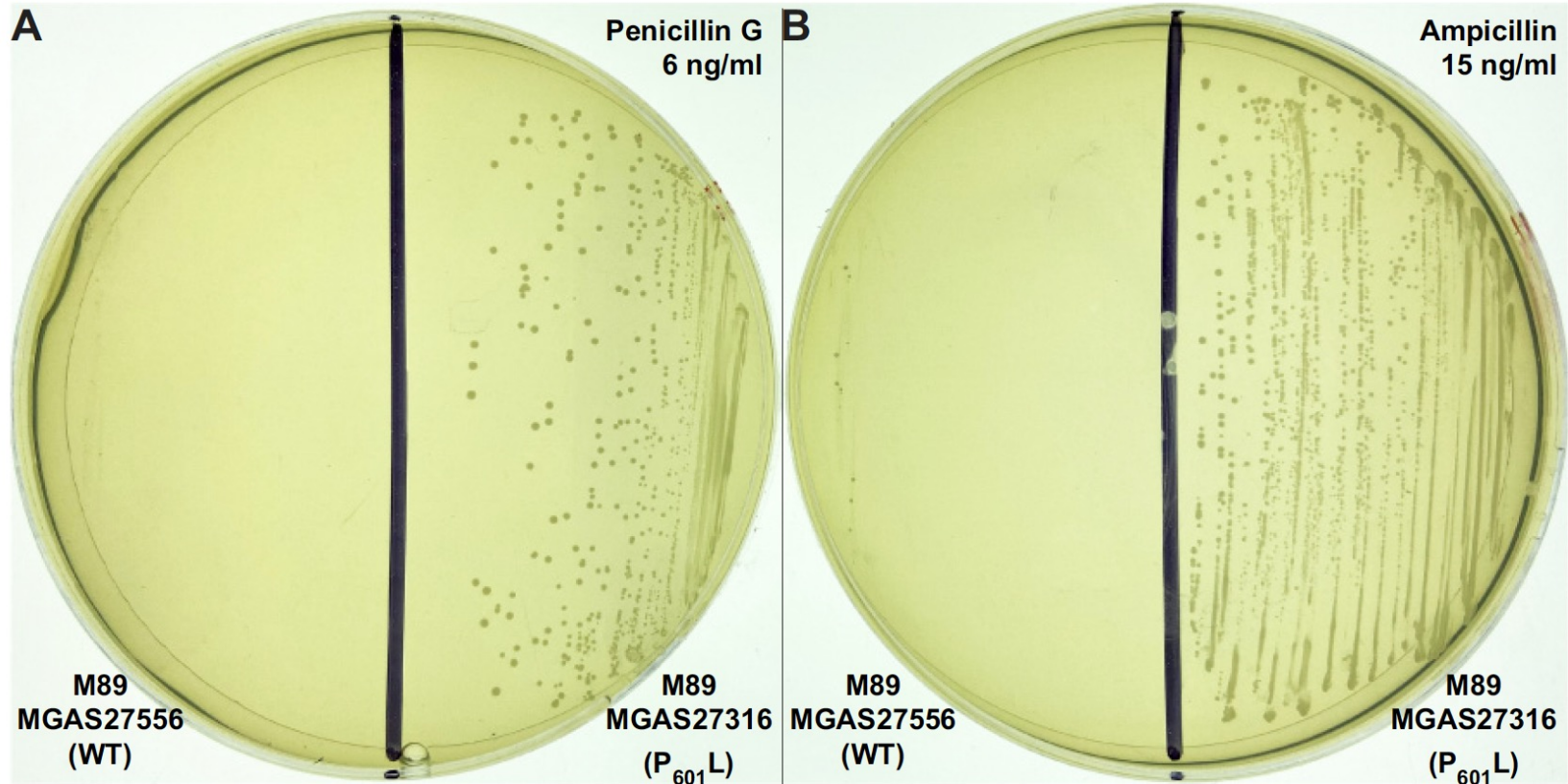
Identification of PBP2X amino acid replacements

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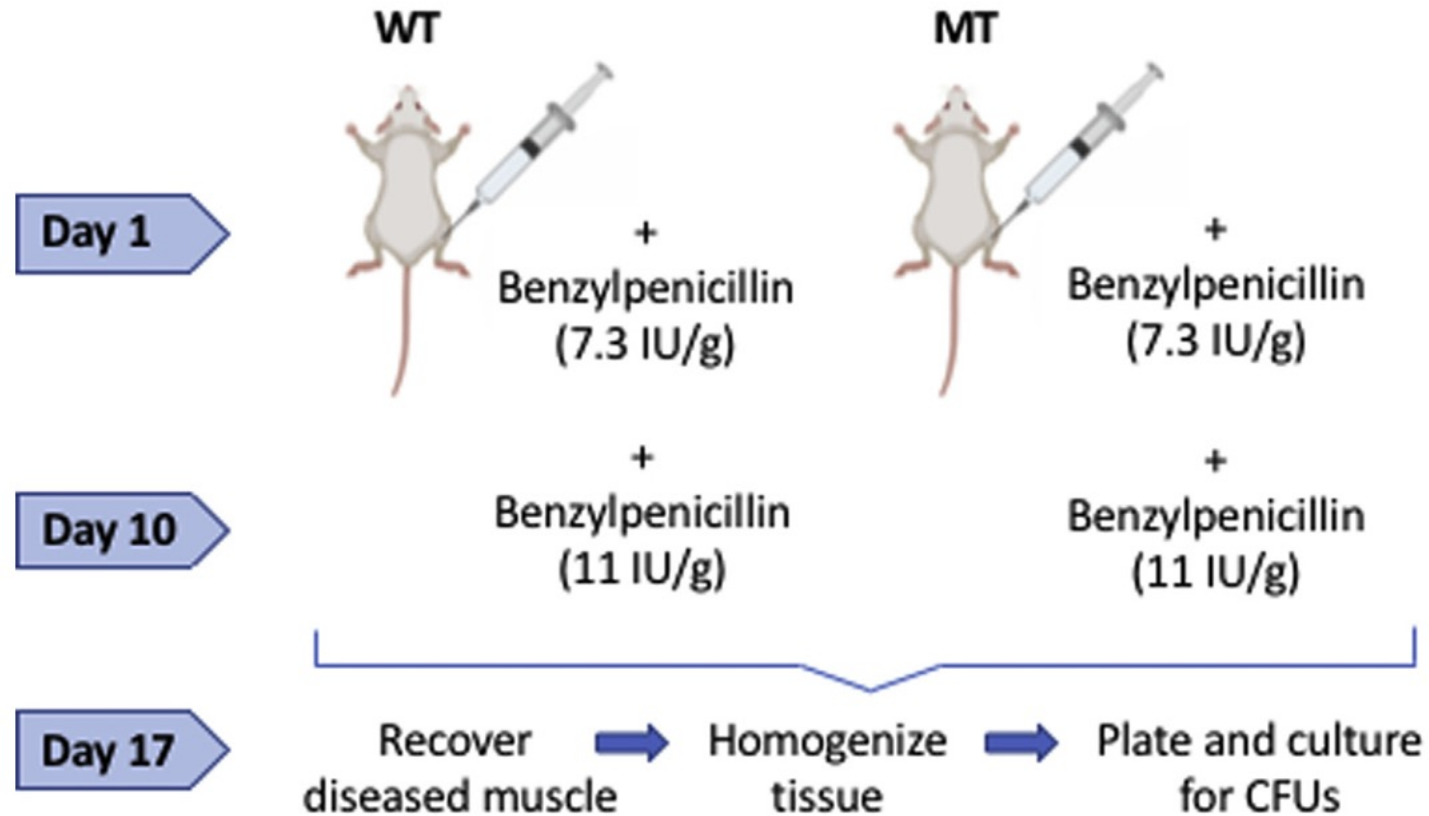
Amino acid replacements identified in multiple isolates are highlighted in yellow

Hypothesis: Amino acid replacements in PBP2X, such as P601L, alter susceptibility to beta-lactam antibiotics *in vitro*



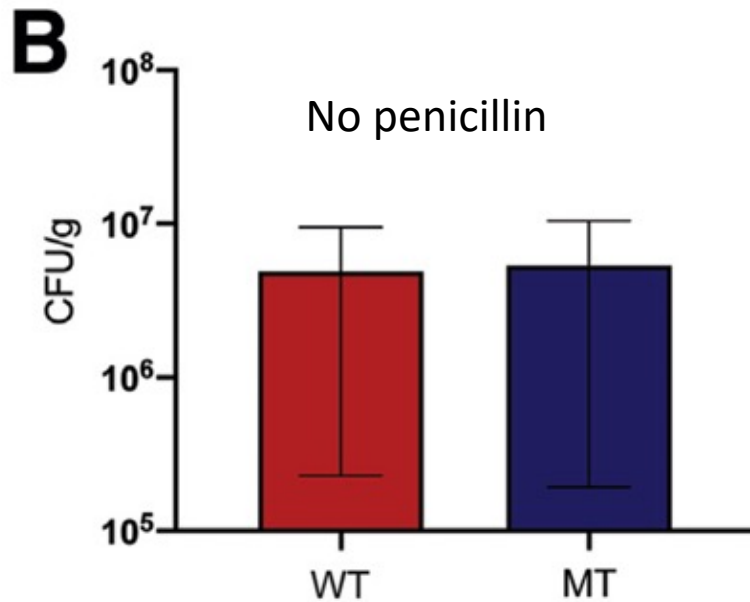
Compared to the PBP2X wild-type strain, the P601L mutant strain grows in the presence of low concentrations of Penicillin and Ampicillin

Hypothesis: Amino acid replacements in PBP2X, such as P601L, increase GAS fitness *in vivo*

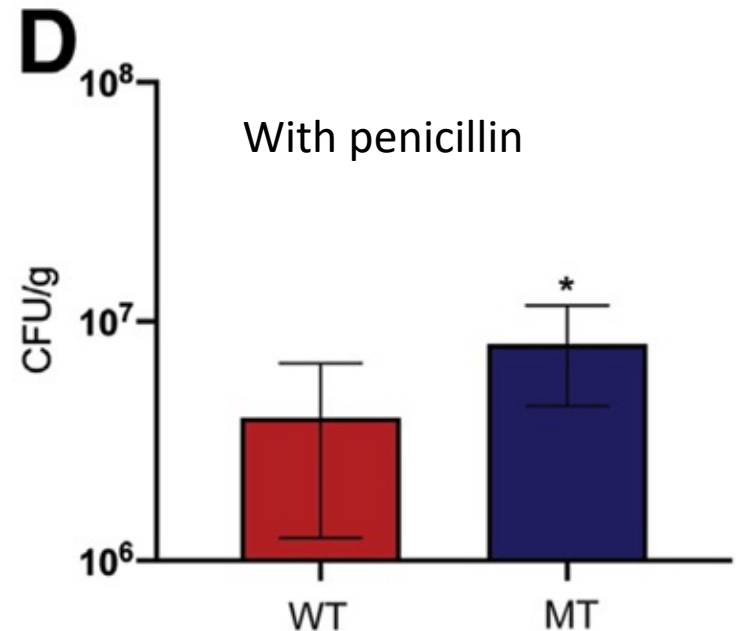


A mouse model of necrotizing myositis in the presence or absence of intermittent subtherapeutic penicillin treatment was used to compare virulence of wild-type and P601L isogenic mutant strains

Significantly more CFUs were recovered from mice infected with the PBP2X P601L strain



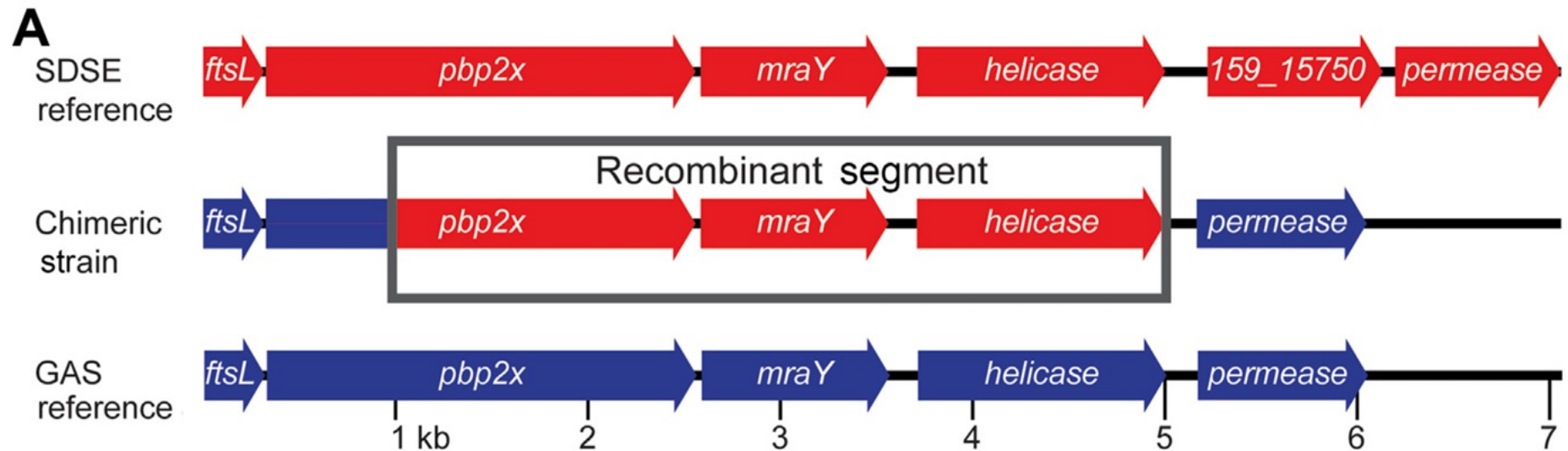
In the absence of penicillin, the number of CFUs recovered from mice infected with the wild-type or P601L isogenic mutant strains did not significantly differ



In the presence of intermittent subtherapeutic penicillin treatment, significantly more CFUs were recovered from mice infected with the P601L strain

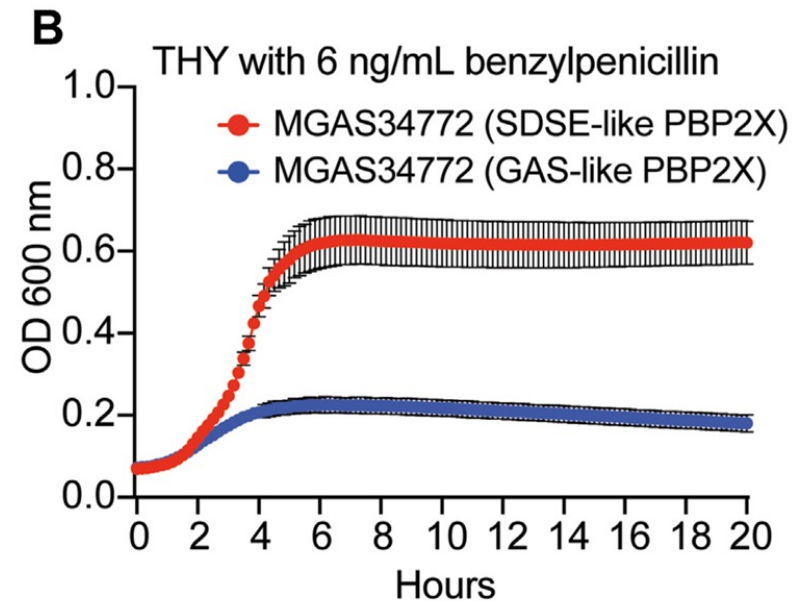
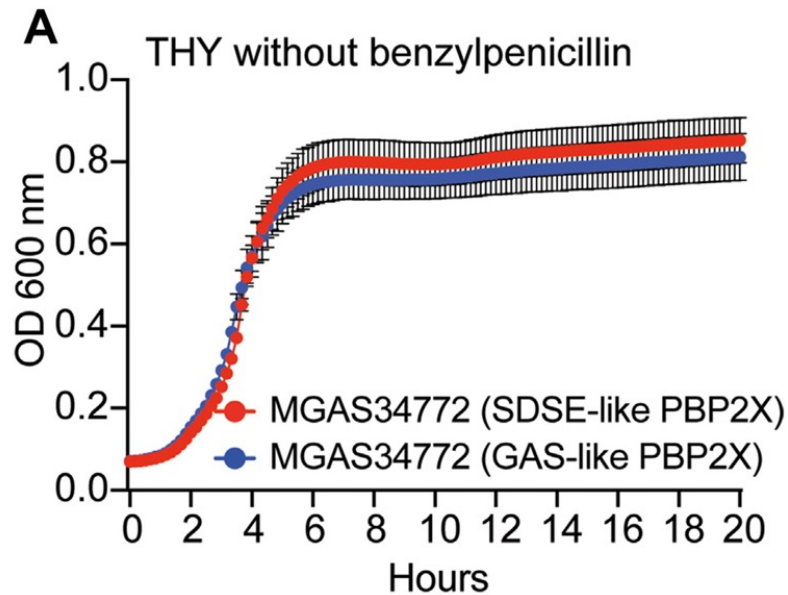
The first chimeric PBP2X in GAS

During the whole genome sequence analysis, we identified a GAS strain with a chimeric PBP2X containing a recombinant segment from *Streptococcus dysgalactiae subspecies equisimilis* (SDSE)



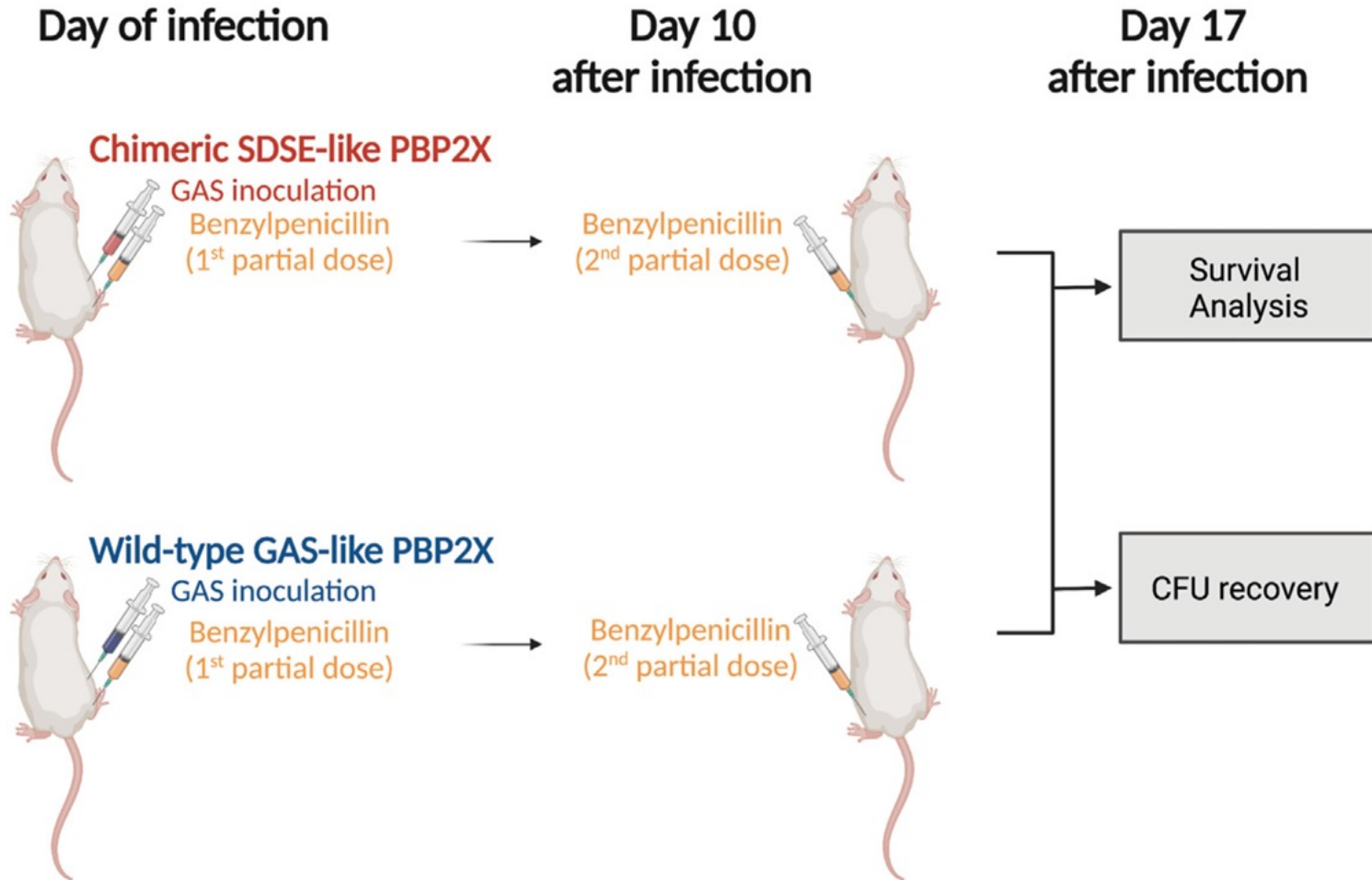
To investigate the effect of a chimeric PBP2X on beta-lactam susceptibility and fitness, isogenic strains with a WT (GAS-like) and mutant (SDSE-like) PBP2X were generated

Hypothesis: The chimeric PBP2X alters susceptibility to beta-lactam antibiotics

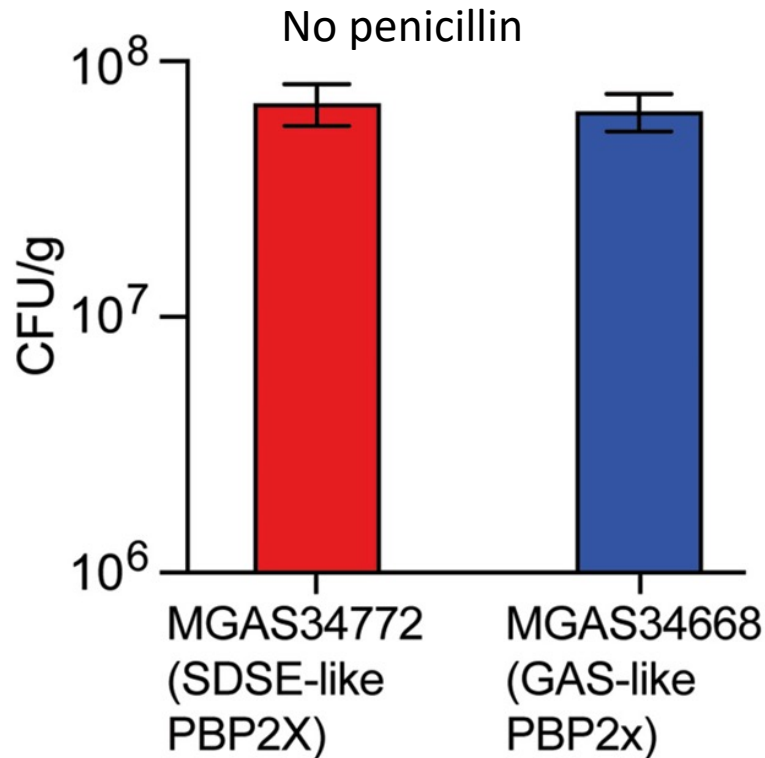


Compared to the isogenic wild-type strain, the chimeric SDSE-like PBP2X strain grows to a higher OD600 *in vitro* in the presence of low concentrations of penicillin

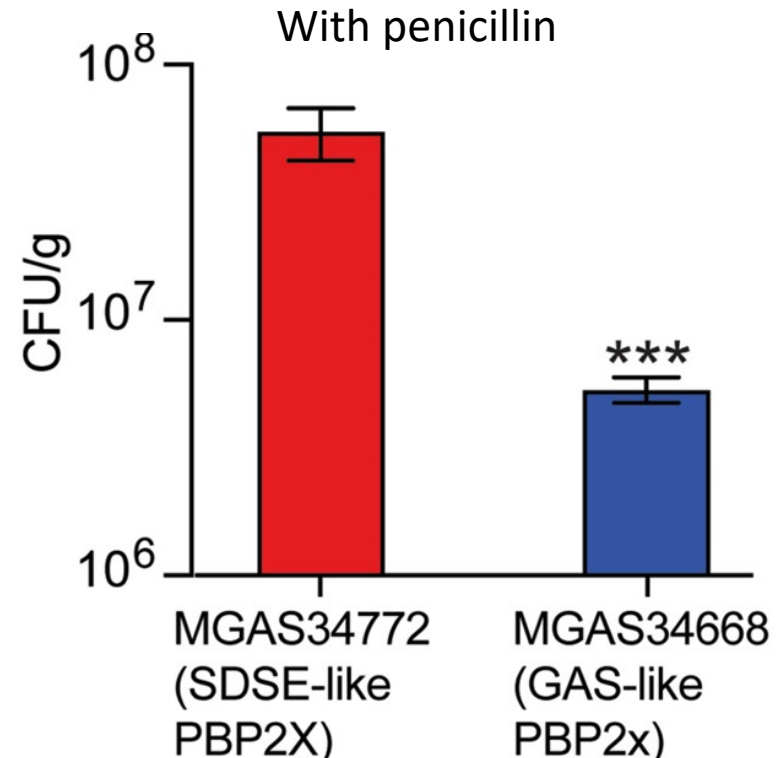
Hypothesis: The chimeric PBP2X increases GAS fitness *in vivo*



Significantly more CFUs were recovered from mice infected with the chimeric PBP2X strain



In the absence of penicillin, the number of CFUs recovered from mice infected with the wild-type or chimeric SDSE-like PBP2X strains did not significantly differ



In the presence of intermittent subtherapeutic penicillin treatment, significantly more CFUs were recovered from mice infected with the chimeric SDSE-like PBP2X strain

PBP2X amino acid replacements and recombination in GAS

- **Geographically widespread**
- **Reduced susceptibility to beta-lactam antibiotics *in vitro***
- **Increased fitness *in vivo***
- **Cause of concern for evolution to a resistant phenotype**

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