## **Supporting Information**

# Tracking of Engineered Bacteria *In Vivo* Using Non-Standard Amino Acid Incorporation

#### **Authors:**

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\*,†These authors contributed equally.

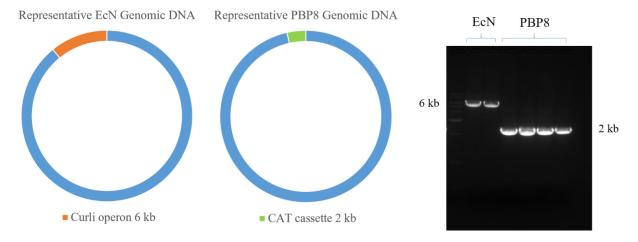
\*\*Corresponding author and lead contact

#### **Affiliations:**

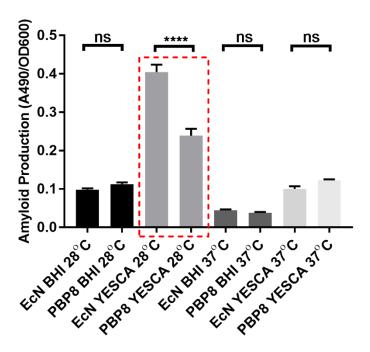
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wtCsgA mutCsgA	1 1	MKLLKVAAIAAIVFSGSALAGVVPQYGGGGNHGGGGNNSGPNSELNIYQYGGGNSALALQ MKLLKVAAIAAIVFSGSALAGVVPQYGGGGNHGGGGNNSGPNSELNIYQYGGGNSALALQ
wtCsgA	61	${\tt TDARNSDLTITQHGGGNGADVGQGSDDSSIDLTQRGFGNSATLDQWNGKNSEMTVKQFGG}$
mutCsgA	61	${ t TDARNSDLTITQHGGGNGADVGQGSDDS}{-1DLTQRGFGNSATLDQWNGKNSEMTVKQFGG}$
wtCsgA	121	GNGAAVDQTASNSSVNVTQVGFGNNATAHQYGSGGSGGSGGSGHHHHHH
mutCsgA	120	GNGAAVDQTASNSSVNVTQVGFGNNATAHQYGSGGSGGSGGSGHHHHHH

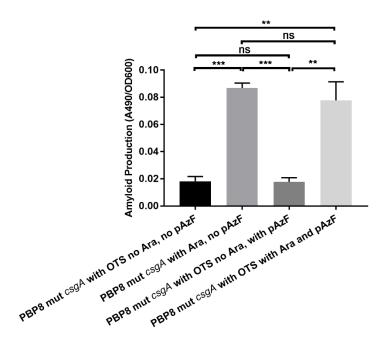
**Supplementary figure 1.** Protein sequences of wild type CsgA and mutant CsgA, which contains an amber stop codon (UAG) for the incorporation of non-standard amino acid.



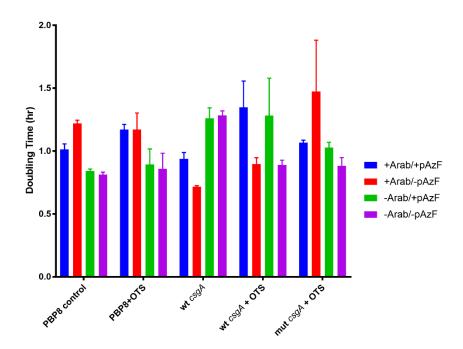
**Supplementary figure 2.** Curli operon deletion of *E. coli* Nissle 1917 (left panel) representative genomic DNA of EcN with curli operon labeled in orange (middle panel) representative genomic DNA of PBP8 with chloramphenical acetyl transferase (CAT) cassette labeled in green replacing curli operon (right panel) DNA gel shift assay verifying the change of curli operon into CAT cassette by PCR amplification of the sequence adjacent to the curli operon in EcN and PBP8 genomic DNA



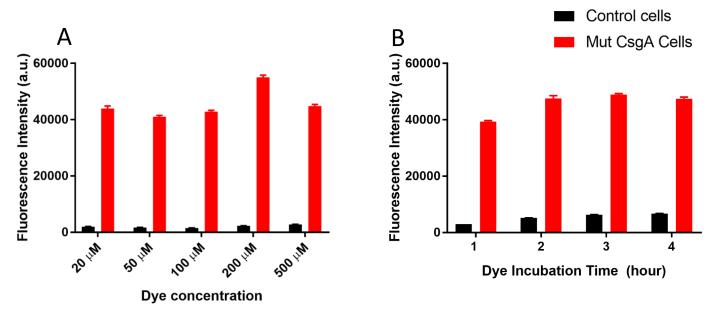
**Supplementary figure 3.** Confirmation of curli production deficiency in PBP8 cells. EcN and PBP8 cells were grown using brain heart infusion (BHI) media (high osmolarity) and YESCA media (low osmolarity) at 28°C (low temperature) and 37°C. It is known that EcN produces curli fibers when grown in low osmolarity media at low temperature. Hence, we observe the high production at YESCA 28°C. However, with the PBP8 strain, the curli operon is deleted. Therefore, we observe significant reduction in the amyloid production under this condition.



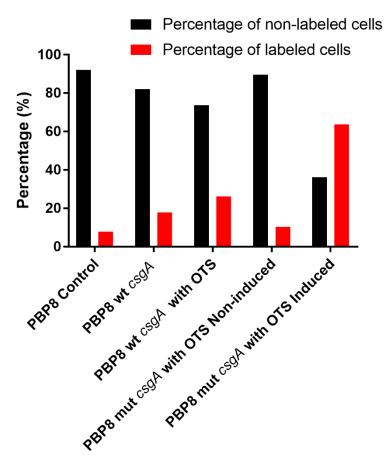
**Supplementary figure 4.** Amyloid production assay for PBP8 mut csgA strain with various induction conditions (Ara = L-arabinose, pAzF = p-azido-L-phenylalanine)



**Supplementary figure 5.** Doubling time comparison of PBP8 variants across different types of induction conditions.



**Supplementary figure 6.** (A) Fluorescence intensity after labeling of PBP8 cells (grey) and mut CsgA cells (red) as a function of Cy5-DBCO concentration (B) Fluorescence intensity after labeling of PBP8 cells (grey) and mut CsgA cells (red) as a function of incubation time with Cy5-DBCO.



**Supplementary figure 7.** Percentage of non-labeled and labeled PBP8 cells as interpreted by flow cytometry data.

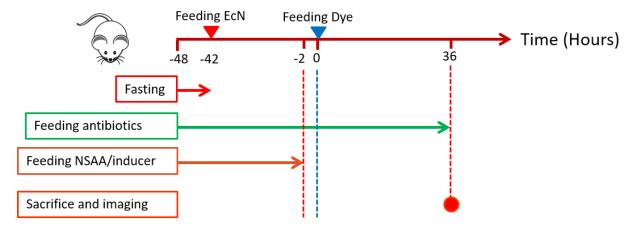
Time after dye gavage -1 0 6 12 24 36

wt csgA induced

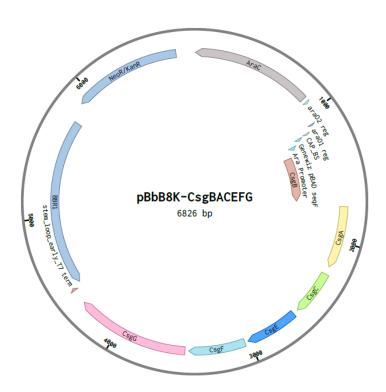
mut csgA + OTS not induced

mut csgA + OTS induced

**Supplementary figure 8.** Auto-fluorescence of the *in vivo* mouse imaging.

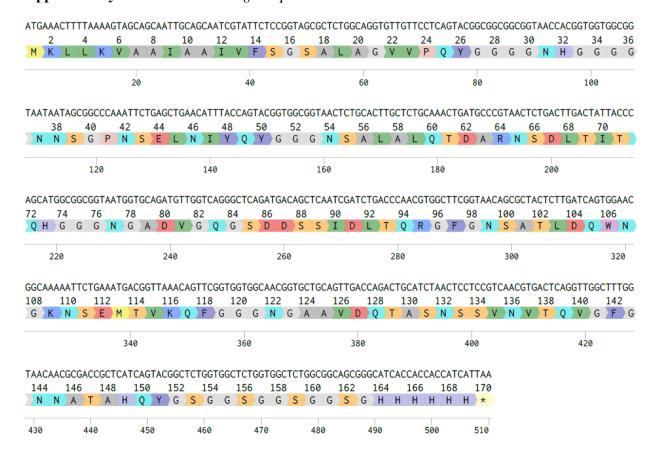


Supplementary figure 9. Mouse experiment timing illustration.



**Supplementary figure 10.** pBbB8k-wt *csgA* plasmid map.

### Supplementary information 1. wt csgA sequences



#### Supplementary information 2. mut csgA sequences

