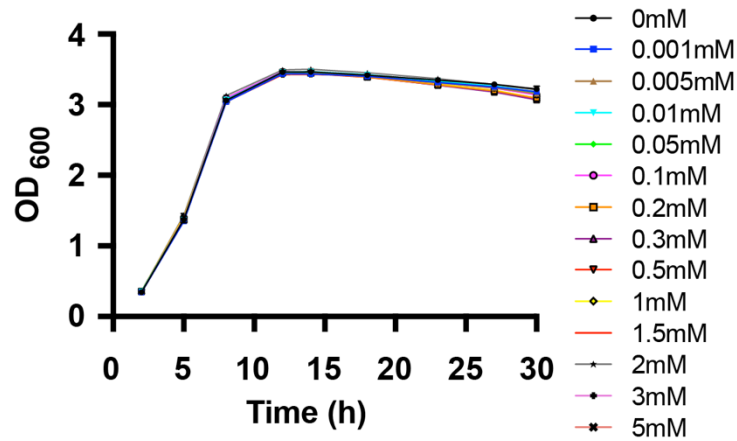
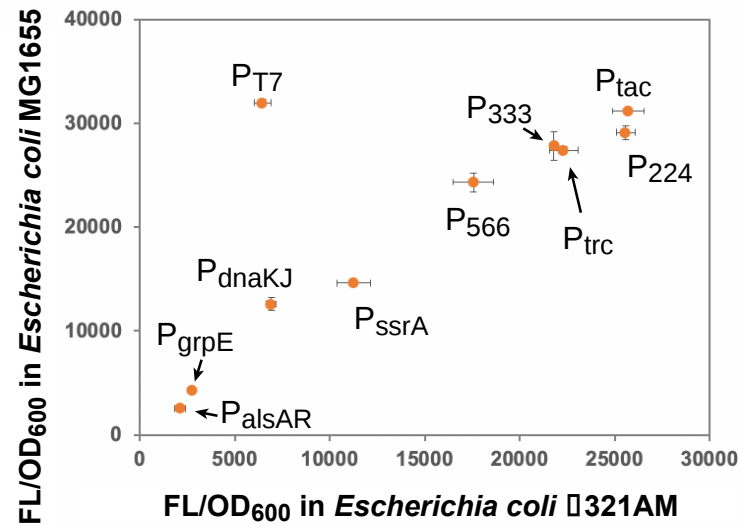


**Titration bacterial growth and chemical biosynthesis for  
efficient *N*-acetylglucosamine and *N*-acetylneuraminic acid  
bioproduction**

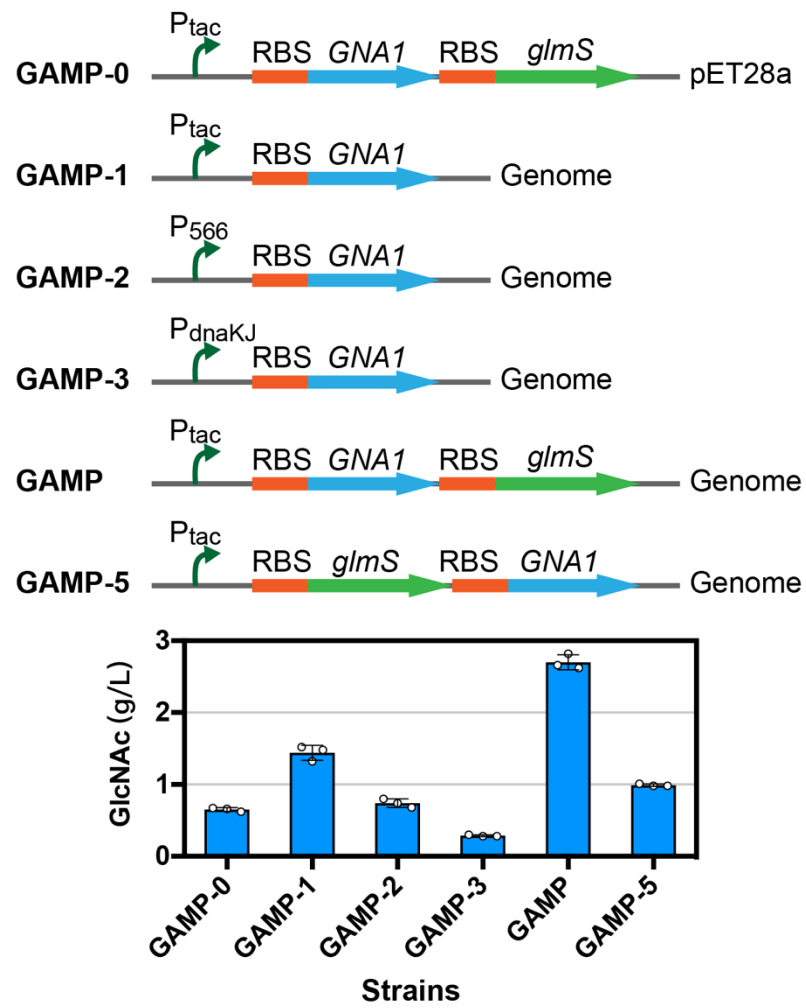
Tian *et al.*



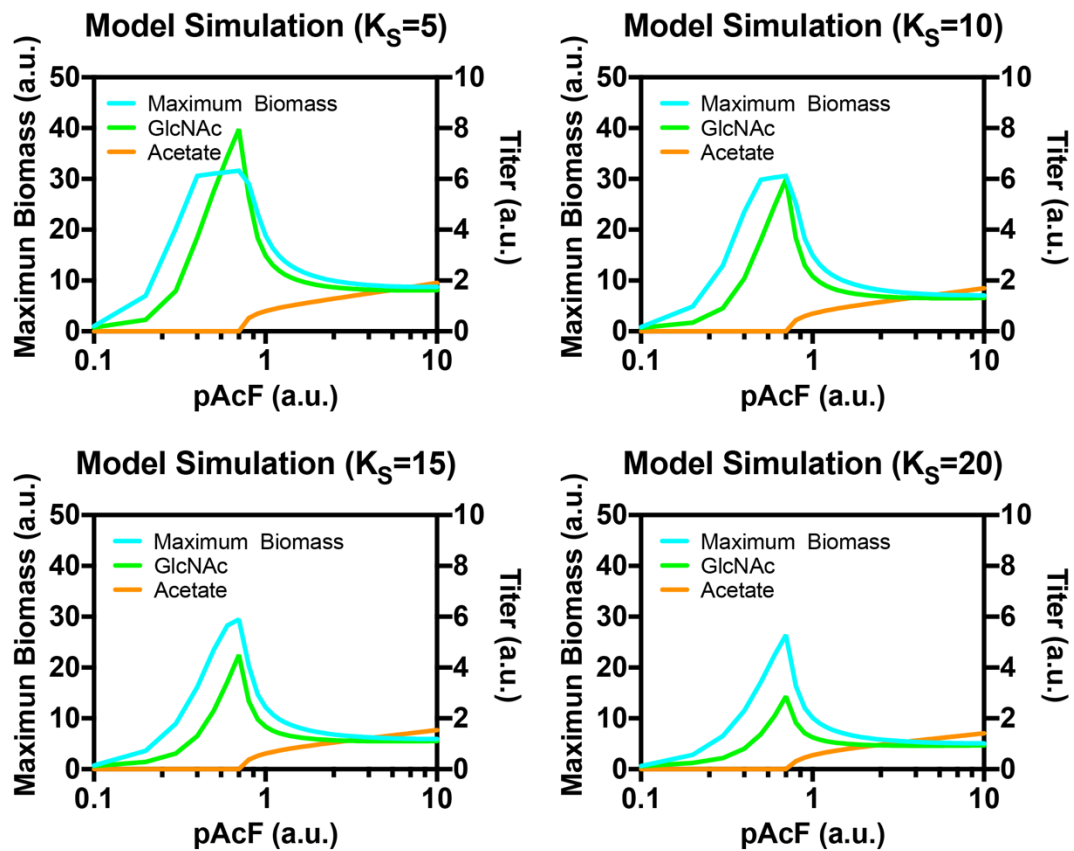
**Supplementary Figure 1. Growth curve of *Escherichia coli* Δ321AM-pEVOL in LB medium with different pAcF concentrations.** Optical density at 600 nm (OD<sub>600</sub>) was used to characterize biomass and quintuplicate experiments were carried out for characterizing OD<sub>600</sub> of *E. coli* Δ321AM-pEVOL at different times. The data are expressed as the mean ± SD from five (n = 5) biologically independent replicates. Source data are provided as a Source Data file.



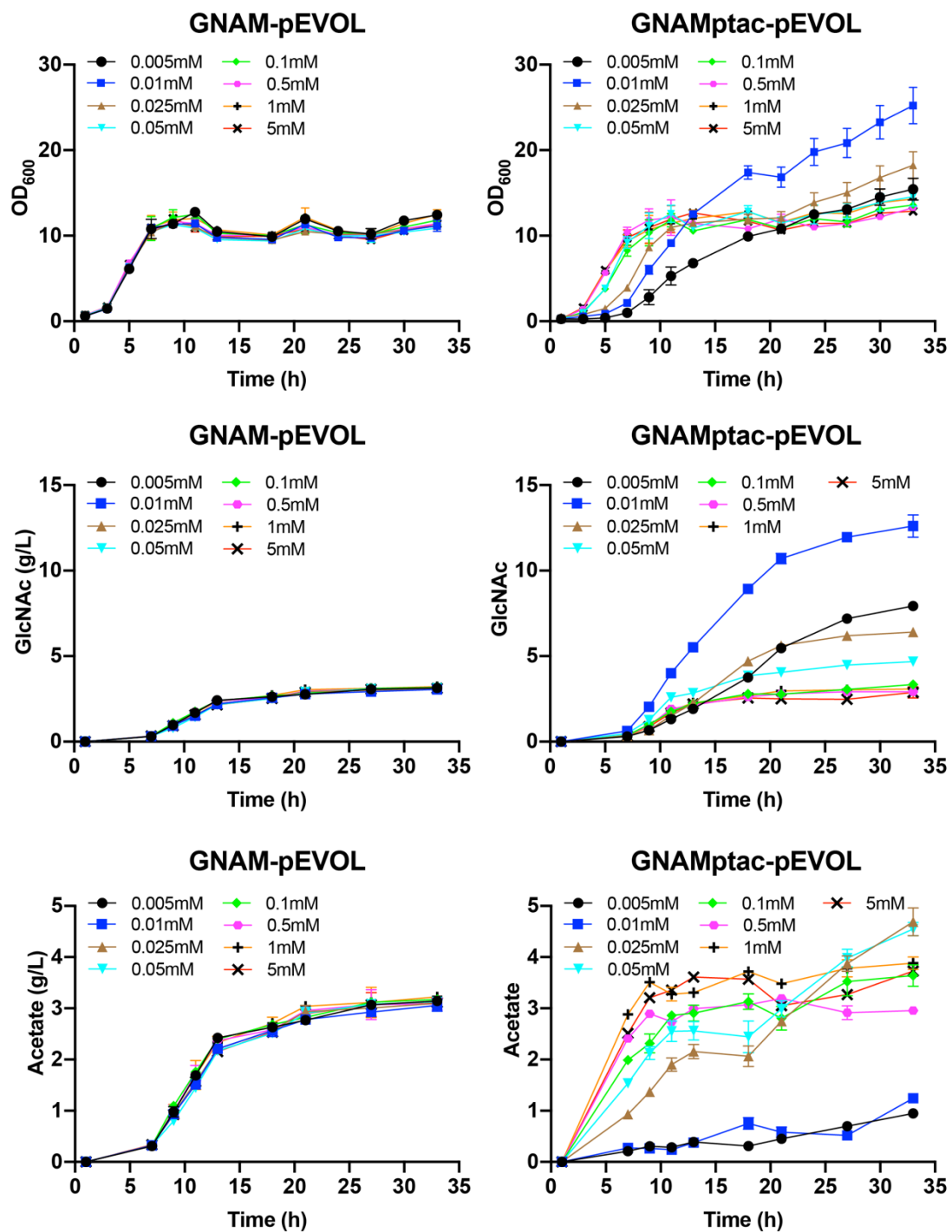
**Supplementary Figure 2. A series of tested promoters and their relative strengths in *E. coli* MG1655 and *E. coli* 321.** Green fluorescent protein (GFP) gene was used as a reporter gene. The data are expressed as the mean  $\pm$  SD from five (n = 5) biologically independent replicates. Source data are provided as a Source Data file.



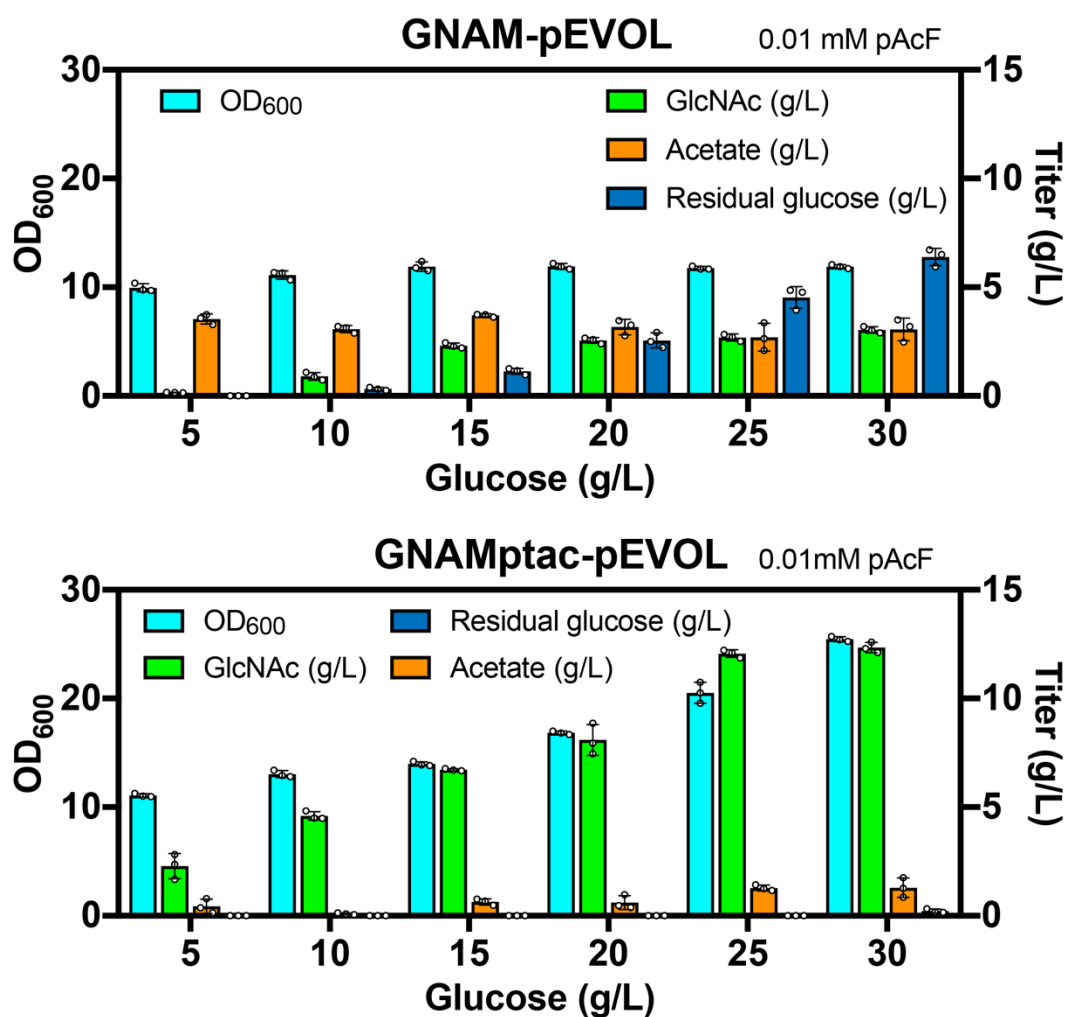
**Supplementary Figure 3. Fermentation results of five engineered GlcNAc-producing strains using TMM medium.** The data are expressed as the mean  $\pm$  SD from three ( $n = 3$ ) biologically independent replicates. Source data are provided as a Source Data file.



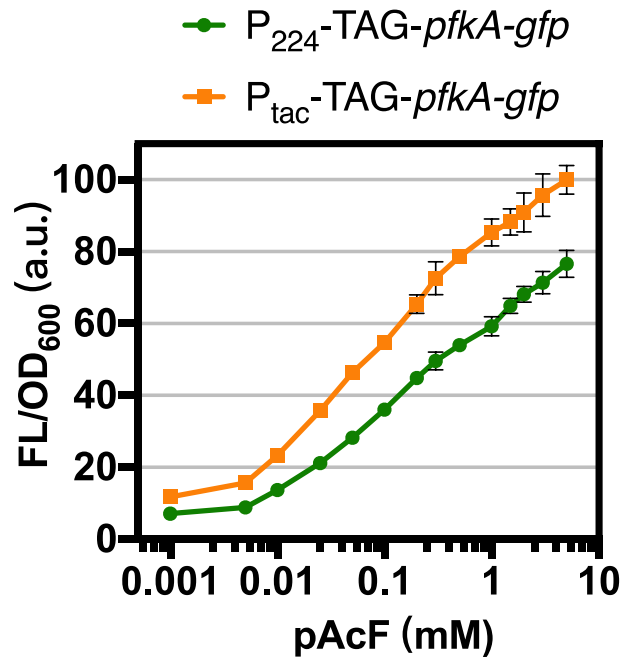
**Supplementary Figure 4. The model simulated fermentation results.** Source data are provided as a Source Data file.



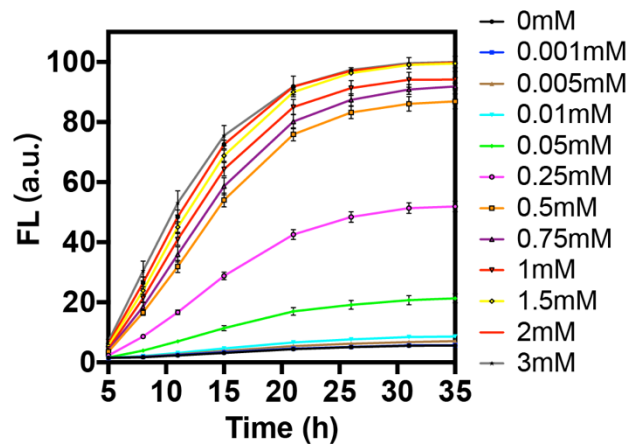
**Supplementary Figure 5. 24-well plate fermentations of GNAM-pEVOL and GNAMptac-pEVOL using TMM medium with different pAcF concentrations.** The data are expressed as the mean  $\pm$  SD from three ( $n = 3$ ) biologically independent replicates. Source data are provided as a Source Data file.



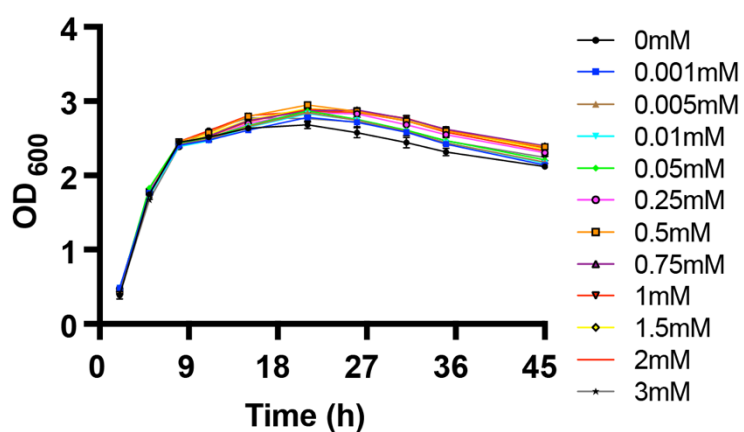
**Supplementary Figure 6. Fermentation of GNAM-pEVOL and GNAMptac-pEVOL using TMM medium with different initial glucose concentrations.** The data are expressed as the mean  $\pm$  SD from three ( $n = 3$ ) biologically independent replicates. Source data are provided as a Source Data file.



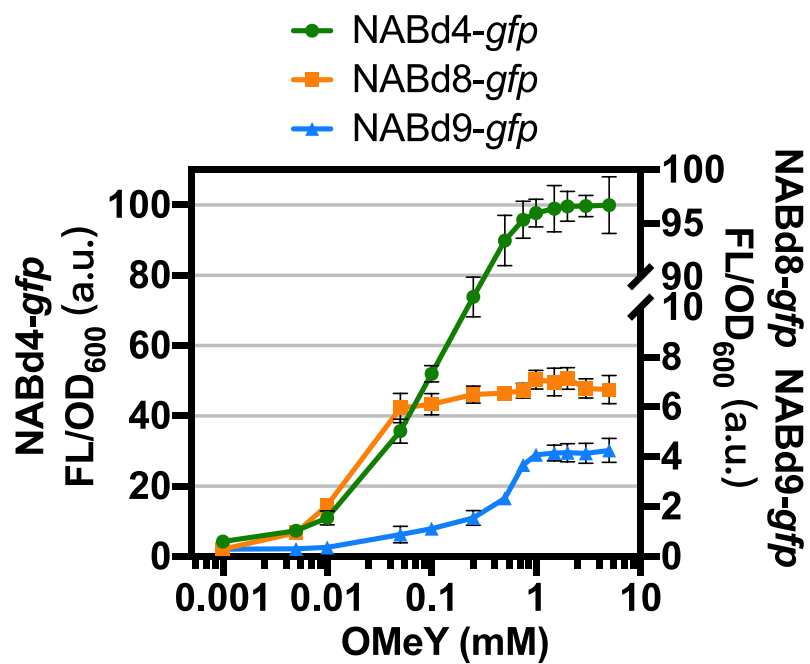
**Supplementary Figure 7. *pfkA* expression levels at different concentrations of pAcF using GFP as a reporter.** The data are expressed as the mean  $\pm$  SD from three ( $n = 3$ ) biologically independent replicates. Source data are provided as a Source Data file.



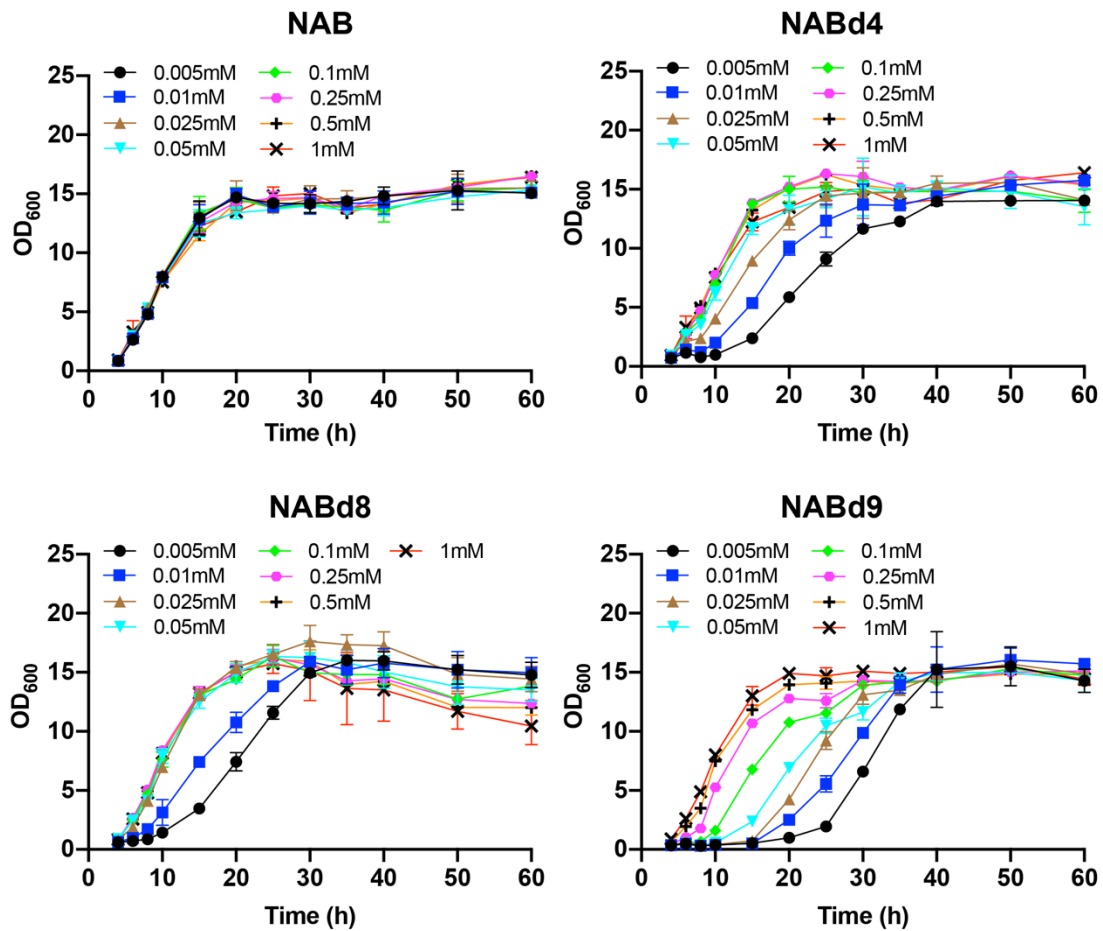
**Supplementary Figure 8. Relative fluorescence intensity curve of BSU168-pBUA in LB medium with different OMeY concentrations.** The data are expressed as the mean  $\pm$  SD from five ( $n = 5$ ) biologically independent replicates. Source data are provided as a Source Data file.



**Supplementary Figure 9. Growth curve of BSU168-pBUA in LB medium with different OMeY concentrations.** Optical density at 600 nm (OD<sub>600</sub>) was used to characterize biomass. The data are expressed as the mean  $\pm$  SD from five ( $n = 5$ ) biologically independent replicates. Source data are provided as a Source Data file.

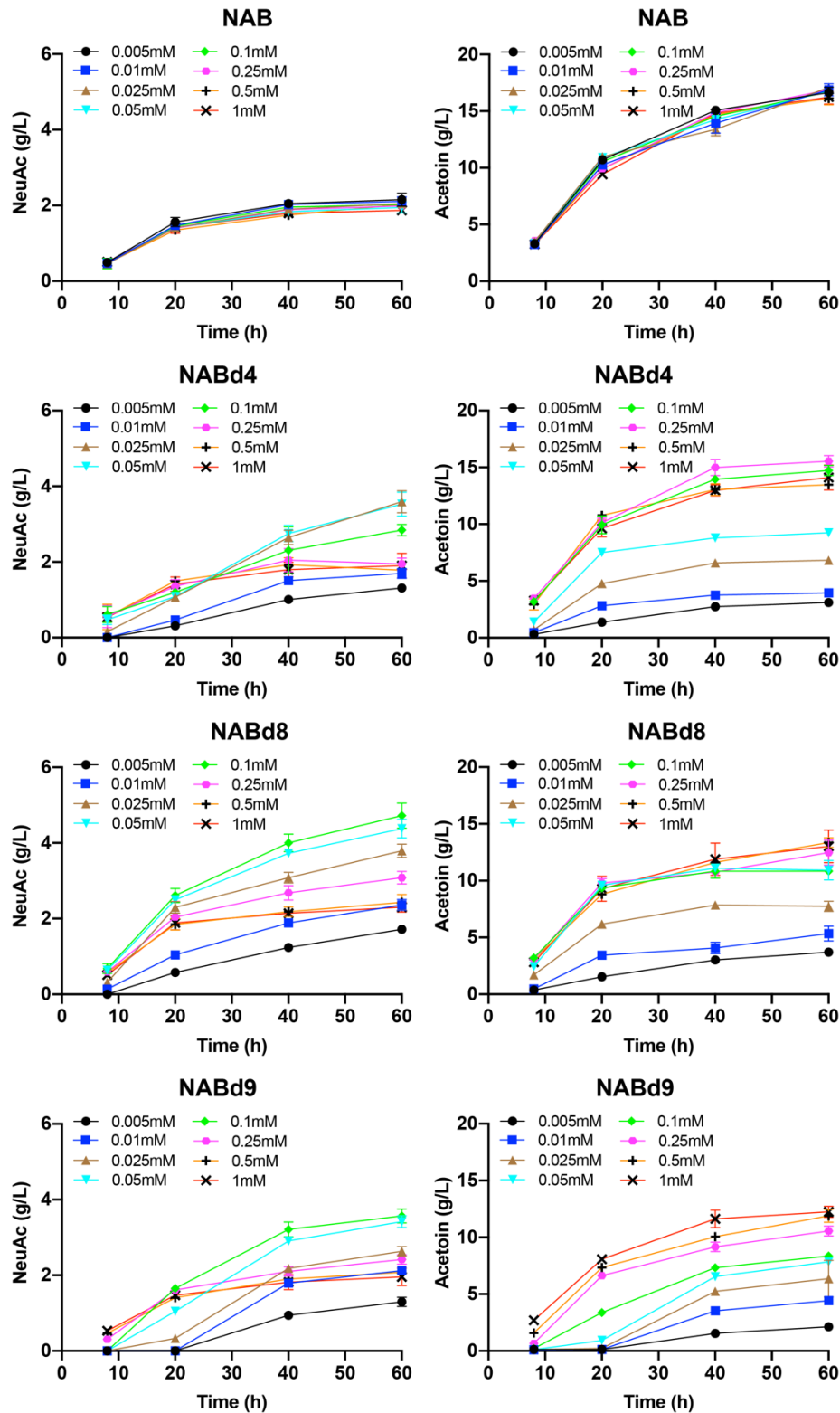


**Supplementary Figure 10. Expression levels of three essential genes (*murB*, *walR*, *cdsA*) at different concentrations of OMeY using GFP as a reporter.** The data are expressed as the mean  $\pm$  SD from three ( $n = 3$ ) biologically independent replicates. Source data are provided as a Source Data file.



**Supplementary Figure 11. Growth curve of four engineered *B. subtilis* fermentation experiments using BFM medium with different OMeY concentrations in 24-well plates.**

The data are expressed as the mean  $\pm$  SD from three ( $n = 3$ ) biologically independent replicates. Source data are provided as a Source Data file.



**Supplementary Figure 12. 24-well plate fermentations of four engineered *B. subtilis* using BFM medium with different OMeY concentrations.** The data are expressed as the mean  $\pm$  SD from three ( $n = 3$ ) biologically independent replicates. Source data are provided as a Source Data file.

**Supplementary Table 1. Cost per cubic of culture for ncAAs used in this study.**

| Name of ncAA                       | CAS#      | MW Cat# for<br>1g | Price of<br>1g (\$) | Optimal conc.<br>(mM) | Cost per<br>cubic of<br>culture (\$) |
|------------------------------------|-----------|-------------------|---------------------|-----------------------|--------------------------------------|
| 4-Acetyl-L-phenylalanine<br>(pAcF) | 2018-61-3 | 207.23            | 6.8                 | 0.01-0.025            | 14 -35                               |
| 4-Methoxy-L-tyrosine<br>(OMeY)     | 6230-11-1 | 195.22            | 1.4                 | 0.1-0.5               | 27-75                                |