

**Supplementary figure 1.** Nine sgRNAs targeting *PFK1* (A) or *PYK1* (B) were tested for GlcNAc titer. 2627EE strains expressing dCas9 targeting different location of either *PFK1* or *PYK1* were cultivated in defined minimal medium with 20 g/L glucose. After 5 days of cultivation, final GlcNAc titer was measured and compared with control strain 2627EE to select a best sgRNA for each gene. PFK1-4 and PYK1-4 were selected and used in this study.