



S3 Figure. Uxt1- and Uxt2-mediated UDP-Galp uptake into proteoliposomes.

(A) LC-MS/MS analysis of UDP-Galp prepared from UDP-Galp utilizing *E. coli* UDP-galactopyranose mutase (GLF). (B - D) Proteoliposomes prepared from *S. cerevisiae* expressing vector alone (B), Uxt1 (C), or Uxt2 (D) were preloaded with 30 mM UMP, and analyzed by LC-MS/MS after a 10 min incubation with 700 μ M UDP-Galp and 10 μ g purified GLF. Based on mass and retention time, the minor peak between UDP-Galp and UDP-Galp is likely UDP-Glc, presumably present in the reaction starting material. (E and F) Quantification of nucleotide sugar uptake into proteoliposomes preloaded with 30 mM UMP (E) or 30 mM GMP (F). Amounts were calculated using a UDP-Galp standard

and normalized to the total protein content of the proteoliposome preparations, and the mean \pm SD of four assays are plotted. All assays were performed at 37 °C.