Supplementary data

Extracellular *Toxoplasma gondii* tachyzoites metabolize and incorporate unnatural sugars into cellular proteins

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Running title: Protein glycosylation in *Toxoplasma*

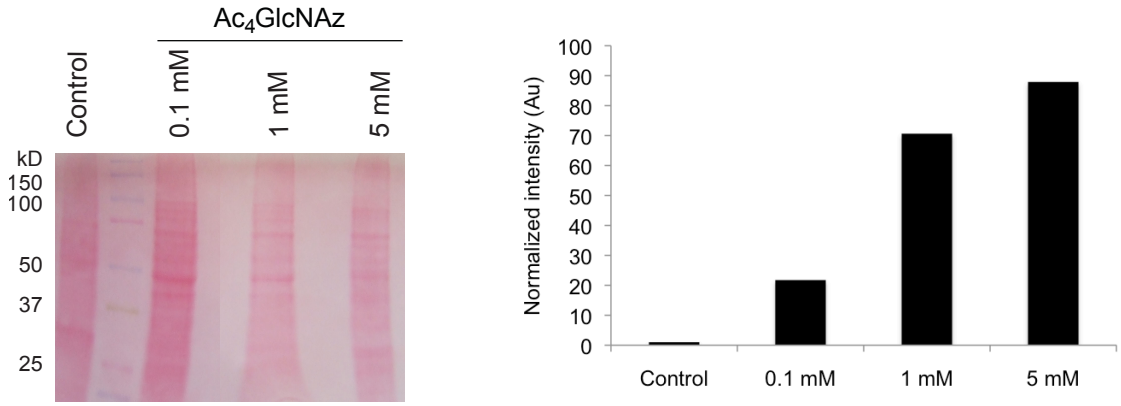


Figure S1. Equivalent protein loading for the blot pictured in Figure 1B was confirmed via staining with Ponceau S (left). Densitometry of Figure 1B (right).

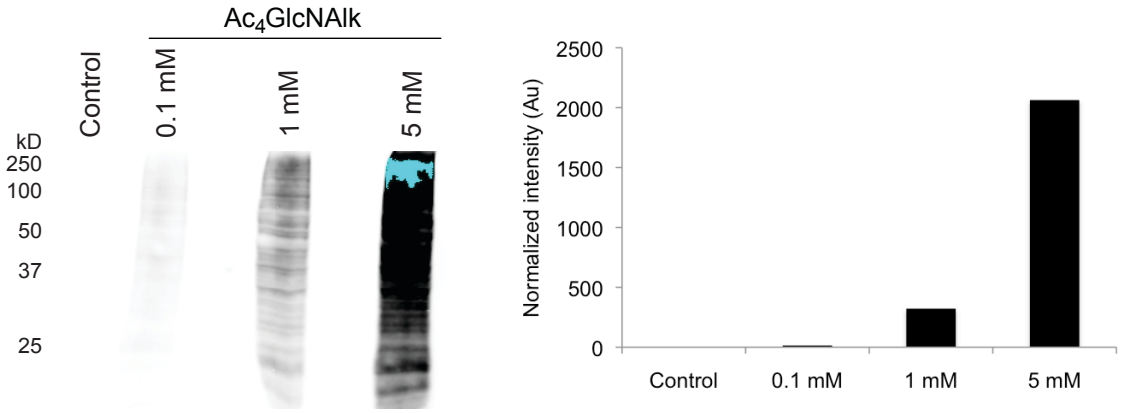


Figure S2. *Toxoplasma* tachyzoites incorporate Ac_4GlcNAlk in a dose-dependent manner. Parasites were incubated with unnatural sugar (0.1-5 mM) or the control sugar Ac_4GlcNAc for 8 hours, lysed, and reacted with a biotin-az tag via "click" chemistry. The labeled proteins were separated via gel electrophoresis and detected via immunoblot with streptavidin (left). Densitometry analysis of the blot (right).

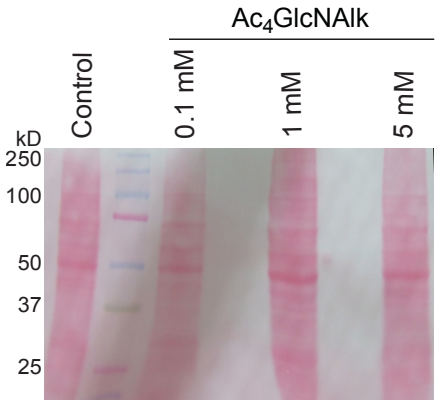


Figure S3. Equivalent protein loading for the above blot was confirmed via staining with Ponceau S.

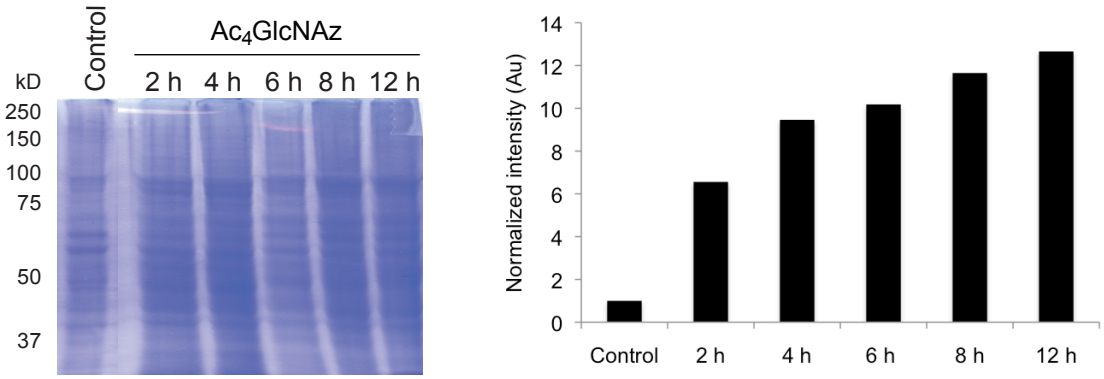


Figure S4. Equivalent protein loading for the blot pictured in Figure 1C was confirmed via staining with Ponceau S (left). Densitometry of Figure 1C (right).

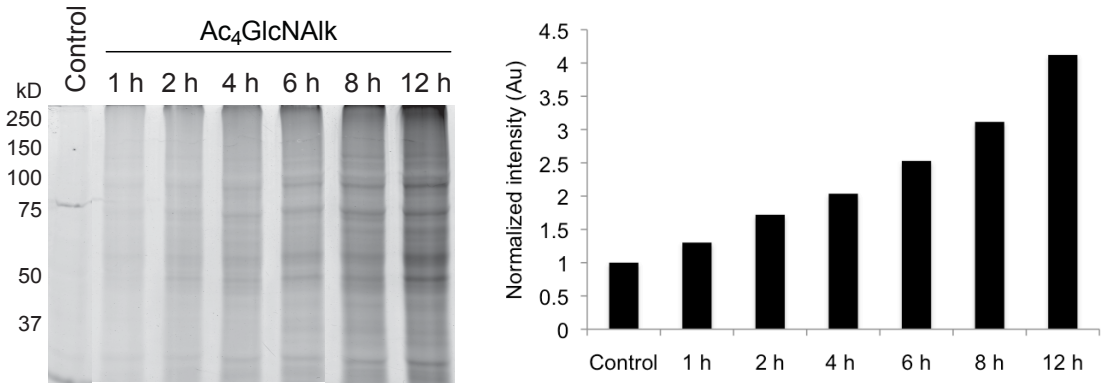


Figure S5. *Toxoplasma* tachyzoites incorporate Ac_4GlcNAlk in a time-dependent manner. Parasites were incubated with Ac_4GlcNAlk (1 mM) or the control sugar Ac_4GlcNAc (1 mM) for 1-12 h hours, lysed, and reacted with a rhodamine-azide probe. Labeled proteins were separated via gel electrophoresis and analyzed via in-gel fluorescence (left). Densitometry analysis of the blot (right).

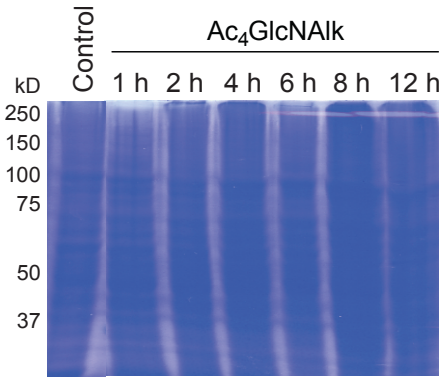


Figure S6. Equivalent protein loading for the above blot was confirmed via staining with Ponceau S.

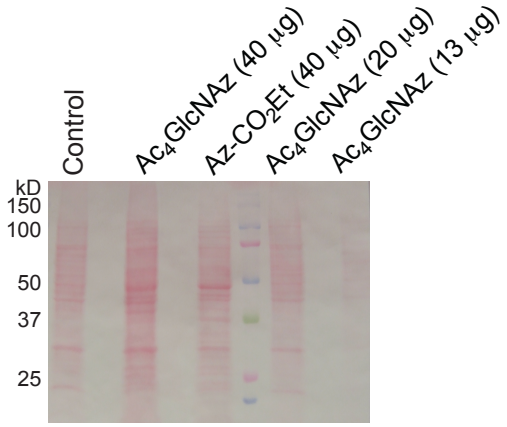


Figure S7. Equivalent protein loading for the blot pictured in Figure 1D was confirmed via staining with Ponceau S.

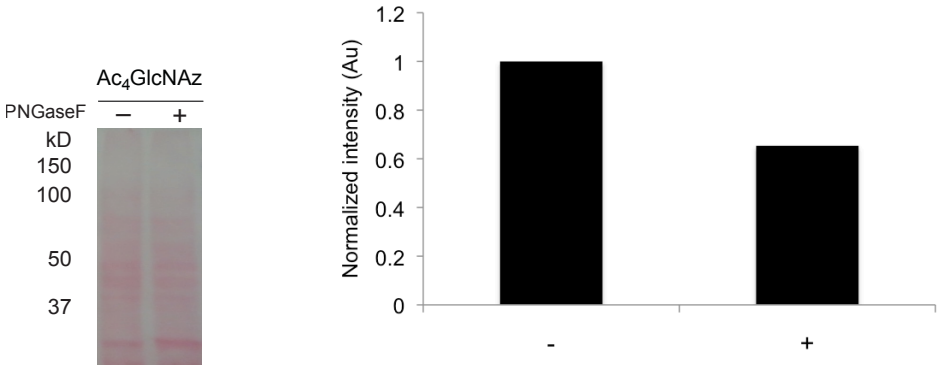


Figure S8. Equivalent protein loading for the blot pictured in Figure 3A was confirmed via staining with Ponceau S (left). Densitometry of Figure 3A (right).

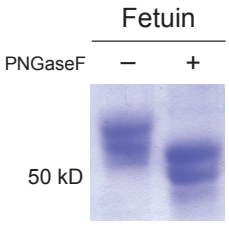


Figure S9. Fetuin protein was treated with PNGase F. The samples were analyzed via gel electrophoresis and stained with Coomassie Brilliant Blue.

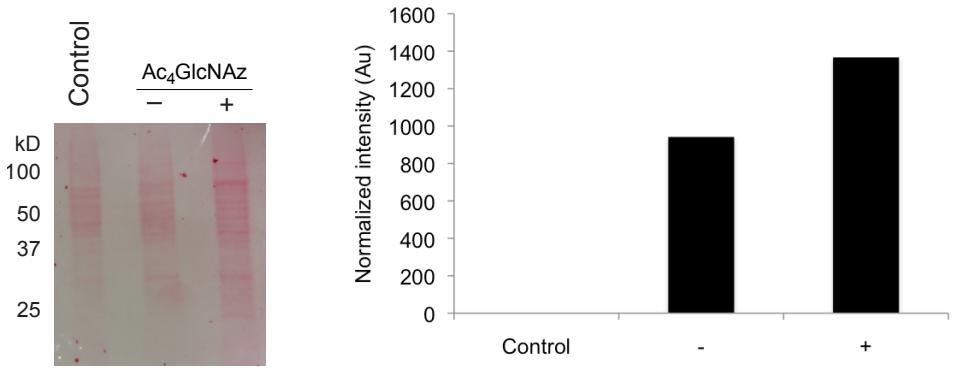


Figure S10. Equivalent protein loading for the blot pictured in Figure 3B was confirmed via staining with Ponceau S (left). Densitometry of Figure 3B (right).

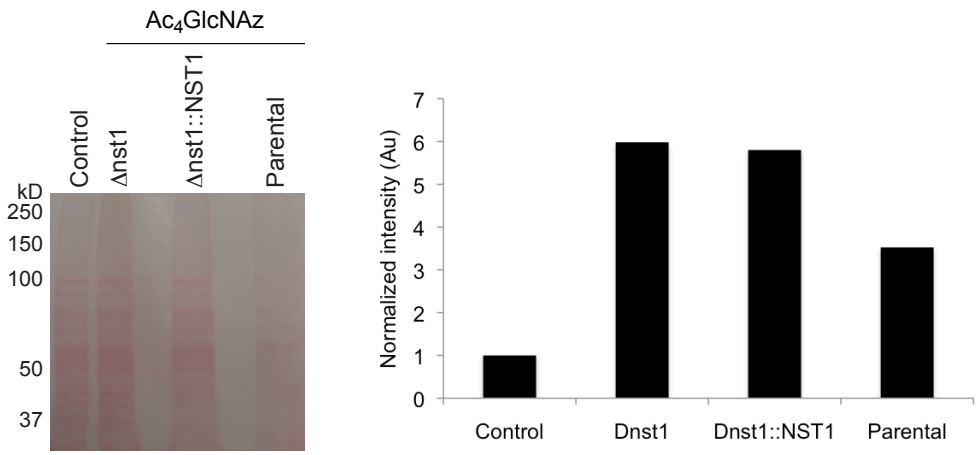


Figure S11. Equivalent protein loading for the blot pictured in Figure 3C was confirmed via staining with Ponceau S (left). Densitometry of Figure 3C (right).

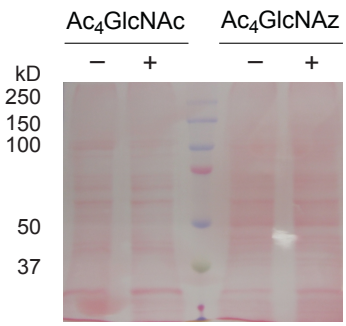
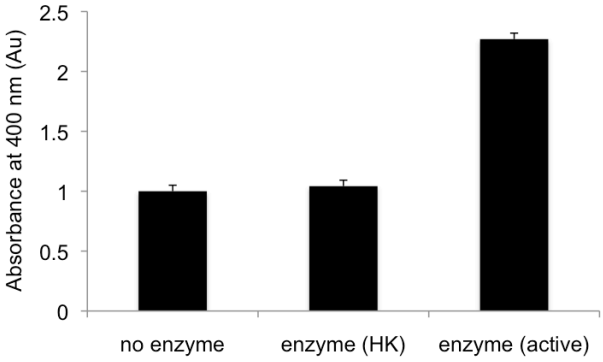


Figure S12. A. Equivalent protein loading for the blot pictured in Figure 3D was confirmed via staining with Ponceau S.



B. As a control, 45 μL of 0.1 mg/mL 4-Nitrophenyl-N-acetyl-β-D-glucosaminide (Sigma) was incubated with 5 μL of G2 reaction buffer and 1 μL of hexosaminidase*f* (active or heat-killed) or 1 μL of water for 2 h at RT. The amount of released 4-Nitrophenol was measured by reading absorbance at 400 nm.

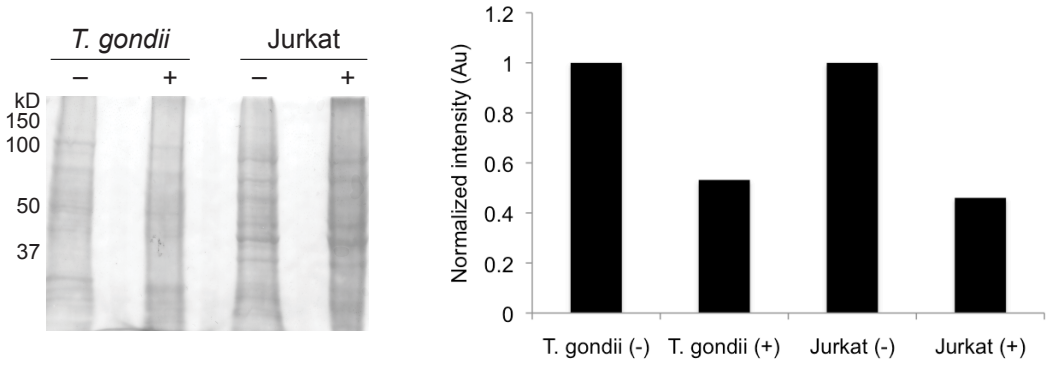


Figure S13. Equivalent protein loading for the blot pictured in Figure 3E was confirmed via staining with Ponceau S (left). Densitometry of Figure 3E (right).

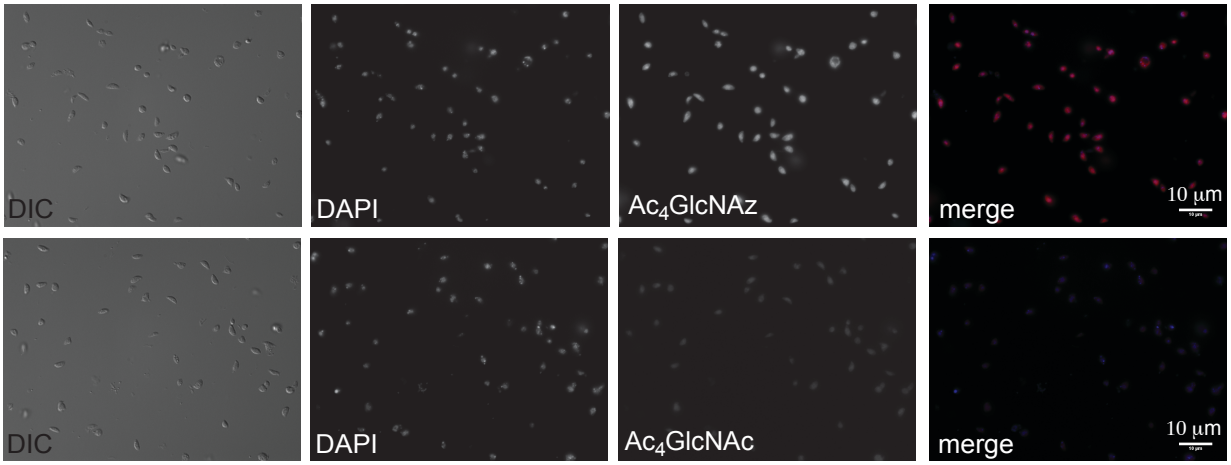


Figure S14. Labeling of *Toxoplasma* tachyzoites with unnatural sugars in DMEM media via fluorescence microscopy. Parasites were incubated with Ac_4GlcNAz (1 mM) or Ac_4GlcNAc (1 mM) for 8 h, prior to fixation with 4% paraformaldehyde in PBS for 15 min at rt Subsequent reaction with biotin-alk and incubation with streptavidin-AlexaFluor594 enabled fluorescence detection of modified glycoconjugates.

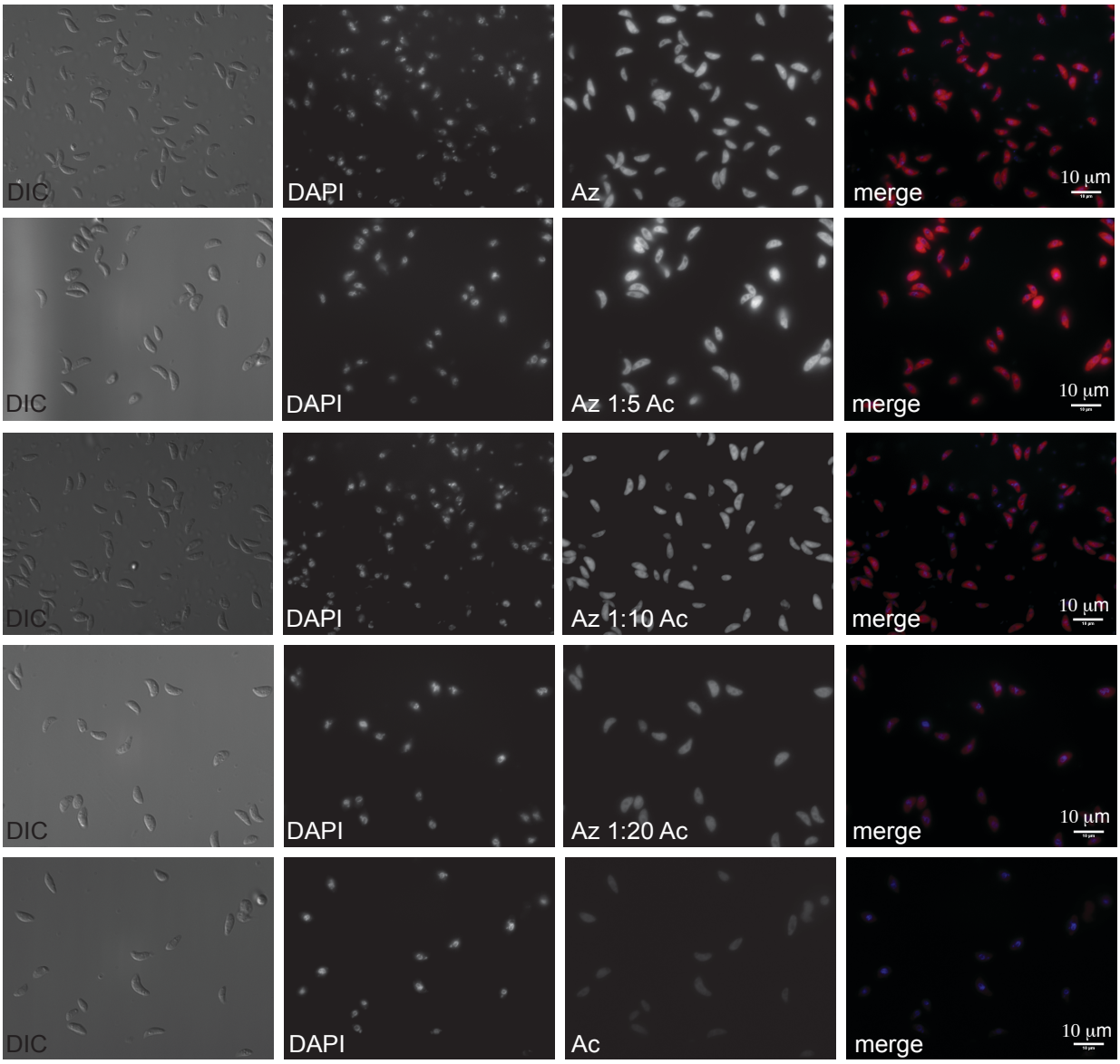


Figure S15. Intensity of Ac_4GlcNAz signal can be decreased by the addition of Ac_4GlcNAc. Parasites were incubated with Ac_4GlcNAz (1 mM) or a mixture of Ac_4GlcNAz (Az) and Ac_4GlcNAc (Ac) (from 1:5 to 1:20 ratio) or Ac_4GlcNAc (1 mM) for 8 h, prior to fixation with 4% paraformaldehyde in PBS for 15 min at rt Subsequent reaction with biotin-alk and incubation with streptavidin-AlexaFluor594 enabled fluorescence detection of modified glycoconjugates.