Supporting Information for

Recognition of diazirine-modified O-GlcNAc by human O-GlcNAcase

Andrea C. Rodriguez and Jennifer J. Kohler*

Department of Biochemistry, University of Texas Southwestern Medical Center

*corresponding author: jennifer.kohler@utsouthwestern.edu

Page Contents

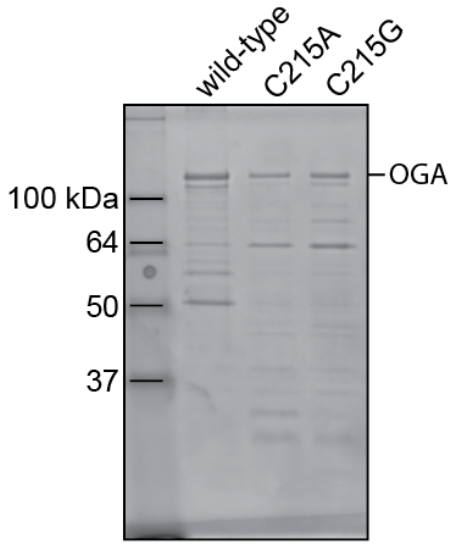

Fig. S1. Purified recombinant proteins. Recombinant OGA and OGA mutant proteins were expressed in *E. coli* and purified by IMAC chromatography. Eluted protein (7 μg) was analyzed by SDS-PAGE with detection by Coomassie stain.

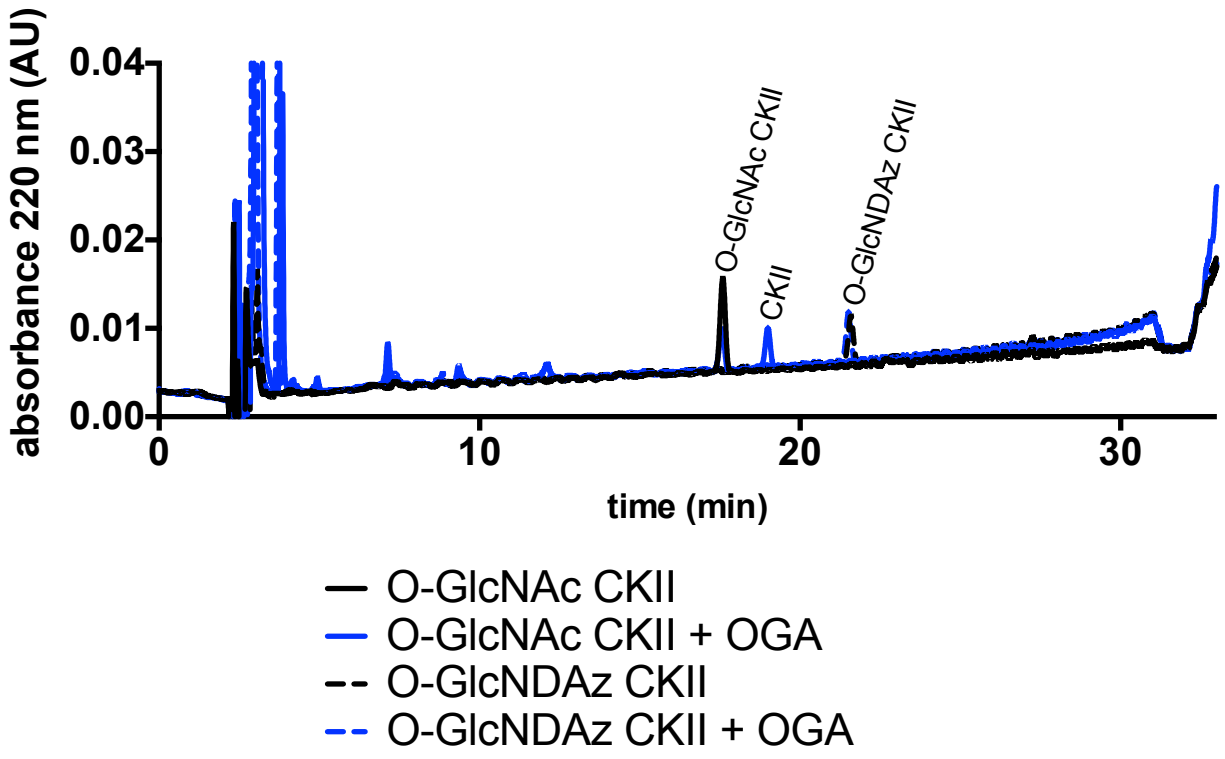

Fig. S2. Complete HPLC traces of peptide deglycosylation by wild-type OGA. CKII peptide was enzymatically modified with O-GlcNAc or O-GlcNDAz, as described in the methods section. Glycosylated peptides were incubated with or without wild-type OGA in 50 mM sodium cacodylate at pH 6.5, then analyzed by HPLC.

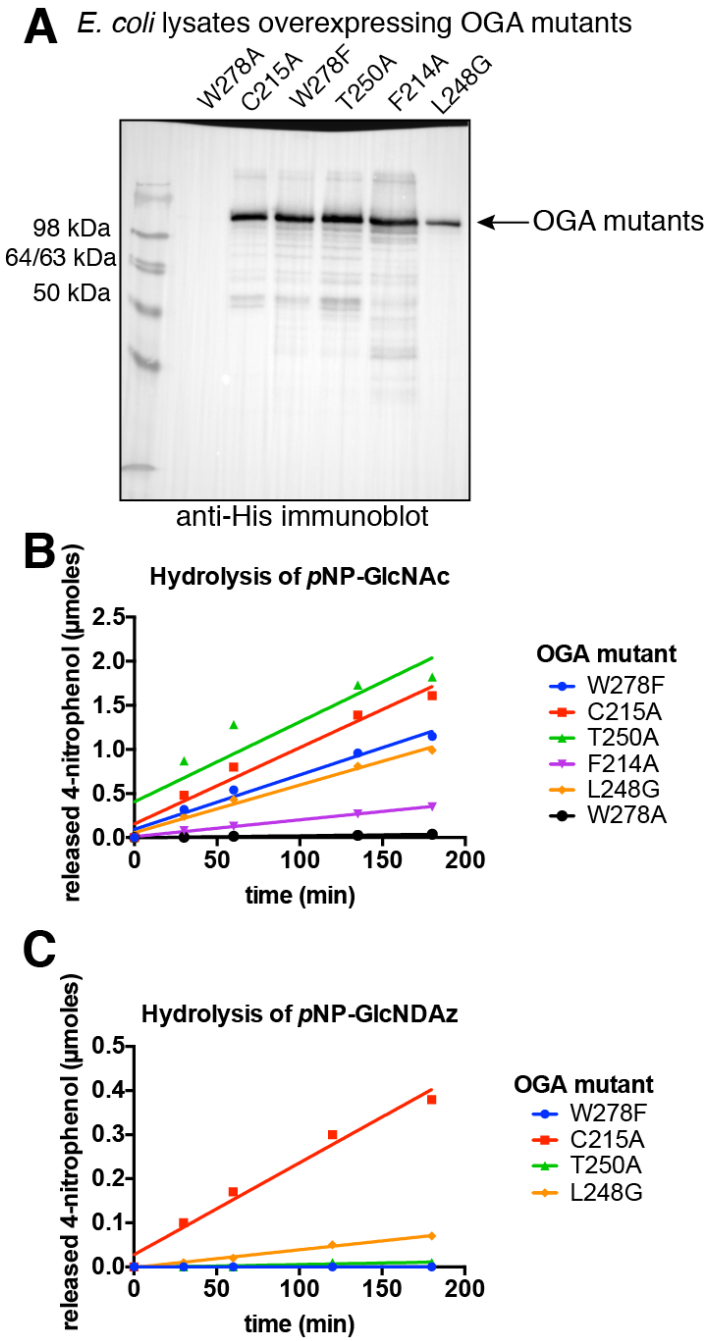

Fig. S3. Hydrolysis activity of bacterial lysates overexpressing OGA mutants. (**A**) OGA mutant expression in bacterial lysates was detected by immunoblot using an anti-His antibody. Lysates from bacteria overexpressing OGA mutants were incubated with *p*NP-GlcNAc (**B**) or *p*NP-GlcNDAz (**C**). Hydrolysis resulting in the release of 4-nitrophenol was monitored by measuring absorbance at 405 nm.

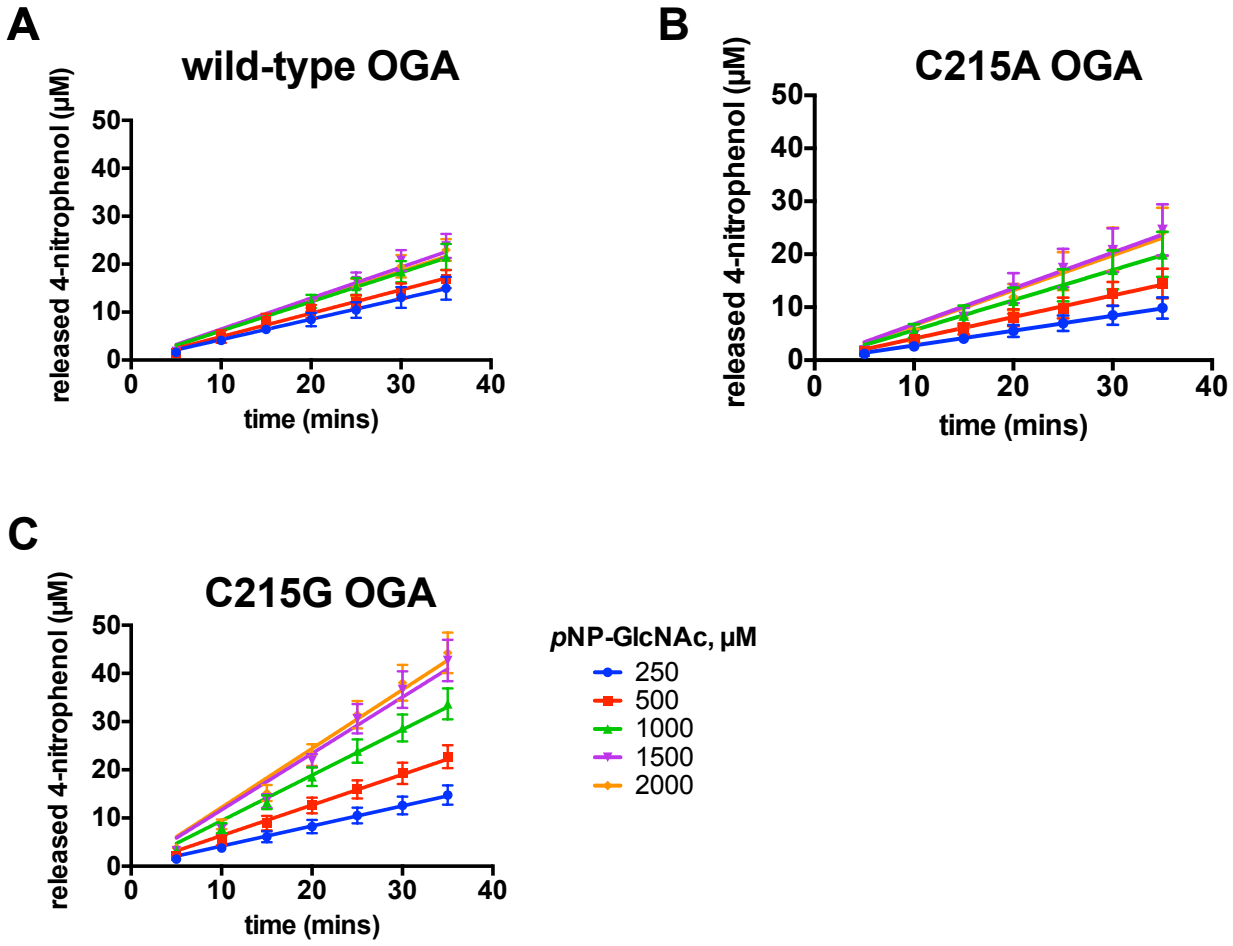

Fig. S4. Initial rates of OGA and OGA mutant activity toward *p*NP-GlcNAc. Recombinant wild-type OGA (**A**), OGA(C215A) (**B**), or OGA(C215G) (**C**) was incubated with *p*NP-GlcNAc. Concentrations of *p*NP-GlcNAc varied from 250 to 2000 µM. Absorbance at 405 nm was measured in continuous assay format. The amount of released *p*NP was calculated by comparison to absorbance of a 4-nitrophenol standard measured in the same buffer.

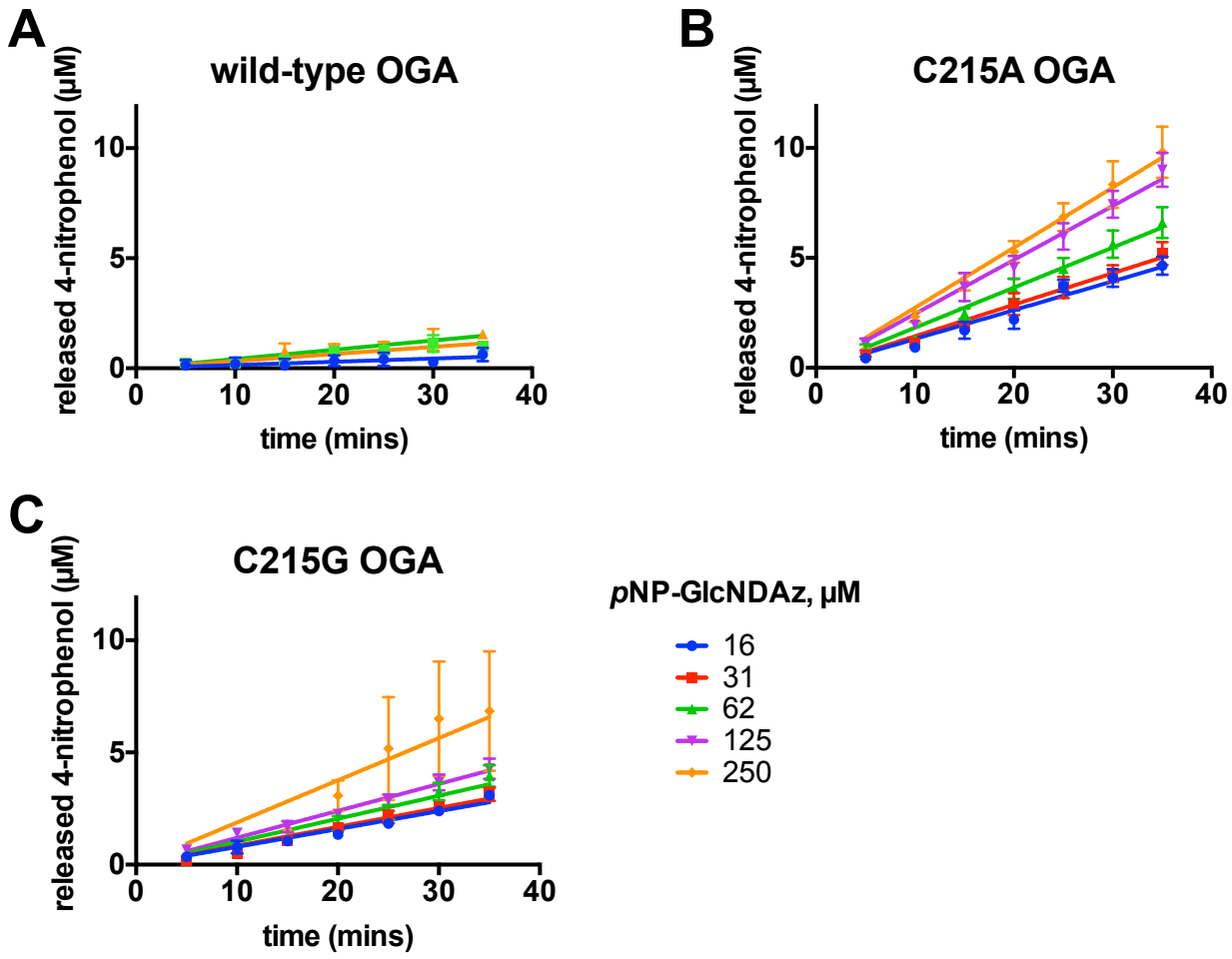

Fig. S5. Initial rates of OGA and OGA mutant activity toward *p*NP-GlcNDAz. Recombinant wild-type OGA (**A**), OGA(C215A) (**B**), or OGA(C215G) (**C**) was incubated with *p*NP-GlcNDAz. Concentrations of *p*NP-GlcNDAz varied from 16 to 250 μM. Absorbance at 405 nm was measured in continuous assay format. The amount of released 4-nitrophenol was calculated by comparison to absorbance of a 4-nitrophenol standard measured in the same buffer.

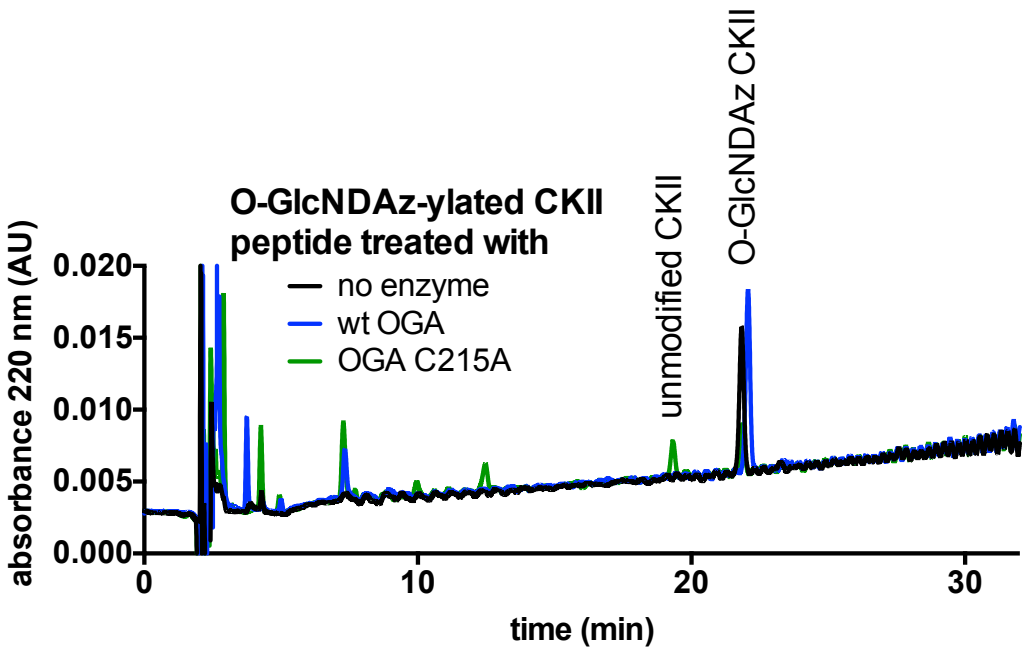

Fig. S6. Complete HPLC traces of O-GlcNDAz peptide deglycosylation. Purified CKII peptide was modified with O-GlcNDAz, as described in the methods section. O-GlcNDAz-ylated CKII was incubated in 50 mM sodium cacodylate at pH 6.5 with no enzyme, wild-type OGA, or OGA(C215A). Reaction products were analyzed by HPLC.