Supporting Information for

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

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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 pNP-GlcNAc (B) or pNP-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 pNP-GlcNAc.** Recombinant wild-type OGA (A), OGA(C215A) (B), or OGA(C215G) (C) was incubated with pNP-GlcNAc. Concentrations of pNP-GlcNAc varied from 250 to 2000 µM. Absorbance at 405 nm was measured in continuous assay format. The amount of released pNP 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 pNP-GlcNDAz. Recombinant wild-type OGA (A), OGA(C215A) (B), or OGA(C215G) (C) was incubated with pNP-GlcNDAz. Concentrations of pNP-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.