Supplementary Material

Identification of Asp^{174} and Asp^{175} as the Key Catalytic Residues of Human *O*-GlcNAcase by Functional Analysis of Site-Directed Mutants.

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Table S1. Full details of the assays used for analyzing the kinetic parameters of the WT *O*-GlcNAcase and the D174A and D175A mutants toward a series of aryl 2-acetamido-2-deoxy-β-D-glucopyranosides.

Substrate	pK_a[a]	Enzyme	λ (nm)	ε (M^{-1} cm^{-1})	[Enzyme] (μM)
3,4-DNP-GlcNAc	5.42	WT	400	12900	0.044
		D174A (pH 7.4)	400	12900	10.0
		D174A (pH 5.0)	400	400	10.0
		D175A	400	12900	0.050
3FpNPGlcNAc	6.42	WT	388	17600	0.014
		D174A (pH 7.4)	388	17600	10.0
		D174A (pH 5.0)	388	700	10.0
		D175A	388	17600	0.011
pNP-*O*-GlcNAc	7.18	WT	400	13200	0.044
		D174A (pH 7.4)	400	13200	10.0
		D174A (pH 5.0)	360	2000	10.0
		D175A	400	13200	0.380
4-MU-GlcNAc	7.50	WT	360	8600	0.22
		D174A (pH 7.4)	360	8600	19.2
		D175A	360	2000	1.1
mNP-GlcNAc	8.39	WT	330	650	2.2
		D175A	330	650	3.8
pClP-GlcNAc	9.47	WT	280	720	0.59
pNHAcP-GlcNAc	9.5	WT	280	400	0.67
P-*O*-GlcNAc	9.99	WT	269	970	0.98
		D175A	269	970	25.1

Figure S1. 10% SDS-PAGE of the WT and mutant full length recombinant *O*-GlcNAcase from *E. coli* and purified as described in the methods and materials.

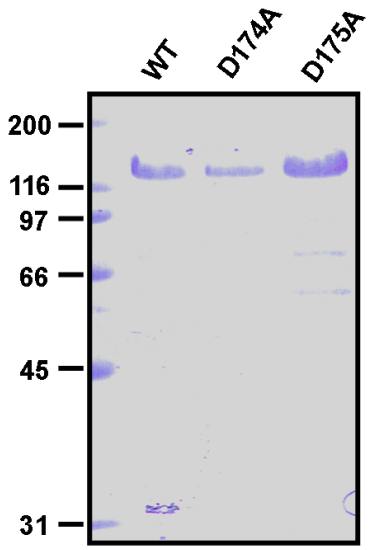

Figure S2: Thin layer chromatography (TLC) analysis of the D175A catalyzed hydrolysis of 3,4-DNP-GlcNAc in the presence and absence of NaN_3. The reaction was carried out at 37 ºC in pH 7.4 PBS containing 5 mM 3,4-DNP-GlcNAc in the presence or absence of 400 mM NaN_3. For the NaN_3 containing buffer the pH was adjusted to pH 7.4 prior to the addition of enzyme. The final volume of the reactions was 100 µL and the final concentration of enzyme in the assay mixtures was 0.5 mg/mL. The upper panel shows a TLC plate under UV light. The middle panel shows a TLC plate developed using sulfuric acid stain (10% H_2SO_4 in ethanol) and heat. The bottom panel shows a TLC plate developed using ninhydrin and heat. Lane 1 (Rxn-Az) shows the enzyme reaction in the absence of azide, lane 2 (Rxn+Az) shows the enzyme reaction in the presence of azide, lane 3 (GlcNAc-Az) shows a synthetic standard of 2-acetamido-2-deoxy-β-D-glucopyranosyl azide, lane 4 (Az) shows a standard of NaN_3, lane 5 (GlcNAc) shows a standard of GlcNAc, lane 6 (3,4-DNP-GlcNAc) shows a standard of 3,4-dinitrophenyl-β-D-2-acetamido-2-deoxy-β-D-glucopyranoside, lane 7 (3,4-DNP) shows a standard of 3,4-dinitrophenol. As can be seen from the TLC analyses in the absence of NaN_3 only the two anomers of GlcNAc and 3,4-DNP are observed in the reaction after along with a small amount of residual substrate (3,4-DNP-GlcNAc) that appears faintly on the plate charred by H_2SO_4. In the reaction containing azide the majority of product formed is 2-acetamido-2-deoxy-β-D-glucopyranosyl azide (GlcNAc-Az) and 3,4-DNP. Only a small amount of GlcNAc is formed in the reaction and a small amount of residual substrate is seen from examination of the TLC plate under UV detection and by charring with H_2SO_4. TLC plates were developed using 3:1 Ethyl Acetate:Methanol.