SUPPLEMENTARY EXPERIMENTAL PROCEDURES

GlcNAc-competition Immunoblots – Immunoblots prepared as in "Experimental Procedures" were blocked in Tris-buffered saline with 0.1 % (v/v) Tween-20 with 3 % (w/v) bovine serum albumin and 1 M N-acetyl-D-glucosamine (GlcNAc; Sigma). The blocked membranes were then incubated overnight at 4 °C with primary antibody against O-GlcNAc (CTD110.6) with 1 M GlcNAc. The blots were then washed, incubated with secondary antibody, developed using ECL (GE Healthcare), and exposed to Hyperfilm ECL (GE Healthcare).

Proteasome Inhibition – The glucose deprivation protocol outlined in "Experimental Procedures" was modified as follows. Thirty minutes prior to the start of glucose deprivation, DMSO (Sigma) or 10 μM MG132 proteasome inhibitor ((1,2); Sigma) were added to the cells. After 30 min, the cells were washed twice with glucose-free media containing DMSO or MG132 and then incubated in the same media for the indicated times. Samples were then subjected to immunoblotting for O-GlcNAc (CTD110.6) or actin (Sigma) as described in "Experimental Procedures."

SUPPLEMENTARY REFERENCES

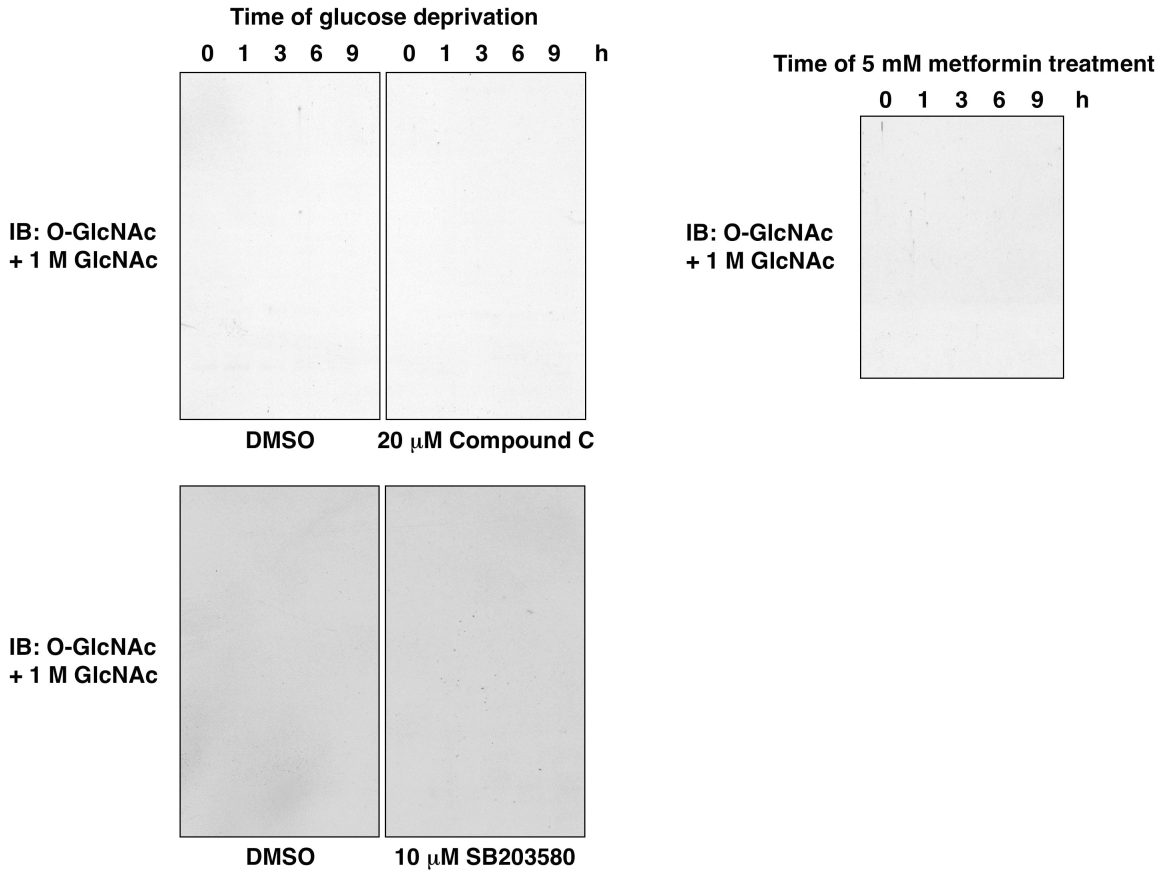
1. Palombella, V. J., Rando, O. J., Goldberg, A. L., and Maniatis, T. (1994) *Cell* **78**(5), 773-785
2. Wang, C. Y., Mayo, M. W., and Baldwin, A. S., Jr. (1996) *Science* **274**(5288), 784-787

SUPPLEMENTARY FIGURE LEGENDS

Supp. Fig. 1. Protein O-GlcNAcylation immunoblots are specific for O-GlcNAc. Lysates from Neuro-2a cells glucose deprived or treated with 5 mM metformin for the indicated times (following 30 min pre-treatment with DMSO, 20 μM compound C, or 10 μM SB203580) were immunoblotted for O-GlcNAc in the presence of 1 M GlcNAc.

Supp. Fig. 2. Glucose deprived-induced protein O-GlcNAcylation is independent of protein degradation. Lysates from Neuro-2a cells glucose deprived for the indicated times (following 30 min pre-treatment with DMSO or 10 μM MG132 proteasome inhibitor) were immunoblotted for O-GlcNAc and actin.

Supplementary Figure 1



Supplementary Figure 2