Data supplements

Supplement Figure 1: After 20 min zero-flow ischemia, NAG-thiazoline treatment during reperfusion significantly improved the recoveries of left ventricular developed pressure (LVDP) and maximum rate of left ventricular pressure change (max dP/dt) in a dose- and drug-dependent manner. Time course of changes in **A:** max dP/dt; and **B:** LVDP during 60 min of reperfusion in untreated hearts (Control; n=7) and hearts subjected to treatments in NBt50 (n=7), NBt100 (n=5) and NAe (n=3) groups. * $P<0.05$ vs. Control; † $P<0.05$ vs. NBt50; ‡ $P<0.05$ vs. NBt100;

Supplement Figure 2: Representative anti-O-GlcNAc immunoblots (CTD110.6) and densitometric results demonstrate the effect of ischemia-reperfusion and NAG-thiazoline treatment on cardiac O-GlcNAc levels. **A:** At the end of reperfusion, cardiac O-GlcNAc levels significantly decreased in untreated, ischemia-reperfusion hearts (I/R, Control; n=5) compared to the time-control, normoxic hearts (Norm; n=5). Treatments at the time of reperfusion with **B:** 50 μM NAG-Bt (NBt50; n=5), and **C:** 100 μM NAG-Bt (NBt100; n=5) significantly increased cardiac O-GlcNAc levels compared to the untreated, ischemia-reperfusion hearts (I/R, Control; n=5). **B and C:** Addition of 50 μM NAG-Ae (NAe; n=3) during reperfusion resulted in the highest increase in cardiac O-GlcNAc levels compared to both the untreated ischemia-reperfusion group (Control) and the treated NBt50, NBt100 groups. The entire lane mean intensities are normalized to calsequestrin levels shown as protein loading control and are relative to the Control group. § $P<0.001$ vs. Norm; * $P<0.001$ vs. Control; † $P<0.001$ vs. NBt50; ‡ $P<0.05$ vs. NBt100;

Supplement Figure 3: O-GlcNAc is associated with the Z-line proteins desmin (**A**) and vinculin (**B**) in normoxic hearts. Fluorescent images were taken simultaneously and merged. The same region of interest was chosen on both red (desmin or vinculin) and green (O-GlcNAc) images

and the plot profiles of longitudinal sections (perpendicular to the Z-lines) were analyzed using ImageJ (National Institutes of Health). The plot profiles clearly show overlap of O-GlcNAc with both desmin and vinculin signals.

Supplement Figure 4: Original anti-OGT immunoblot demonstrates specific changes in OGT protein content in the nuclear and cytosolic compartments in response to ischemia-reperfusion and NAG-thiazoline treatment in hearts at the end of time-control normoxic perfusions in the presence (Norm+NBt) and absence of 50 μM NAG-Bt (Norm), and hearts after ischemia-reperfusion in the untreated control group (I/R) and the treated NBt50, NBt100 and NAe groups. OGT appears as a single band at ~110 kDa in the nuclear fraction as well as in the cytosolic fraction from the normoxic time-control group, where there were no evidence of immunoreactive bands between 150-250 kDa either with short (A) or longer (B) exposition times. However, intense high molecular weight immunoreactive bands were observed following ischemia-reperfusion (I/R), which were decreased with OGA inhibitor treatment in parallel with the increase in the 110 kDa band (NBt50, NBt100, NAe), suggesting that these high molecular weight OGT multimers rather than non-specific antibody binding.

Supplement Figure 5: Immunoblot analyses of **A:** desmin, **B:** total vinculin, and **C:** phospho-Tyr(822) vinculin levels in cardiac whole tissue lysates (n=3-4 hearts/group) of time-control, normoxic hearts (Norm), and hearts after ischemia-reperfusion in the untreated group (Control) and NAG-thiazoline-treated groups (NBt50, NBt100, NAe). Results are normalized to calsequestrin levels and are expressed as % of Control group. * $P<0.05$ vs. Control; † $P<0.05$ vs. NBt50; § $P<0.05$ vs. Norm;