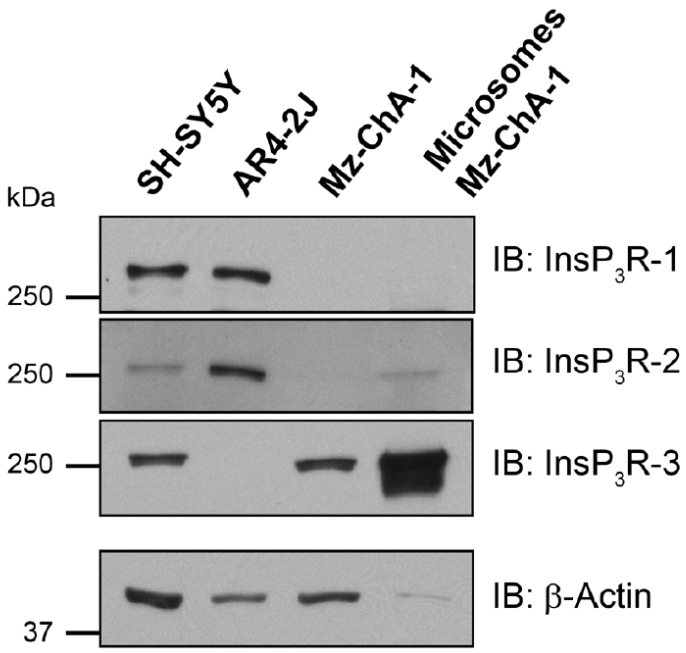
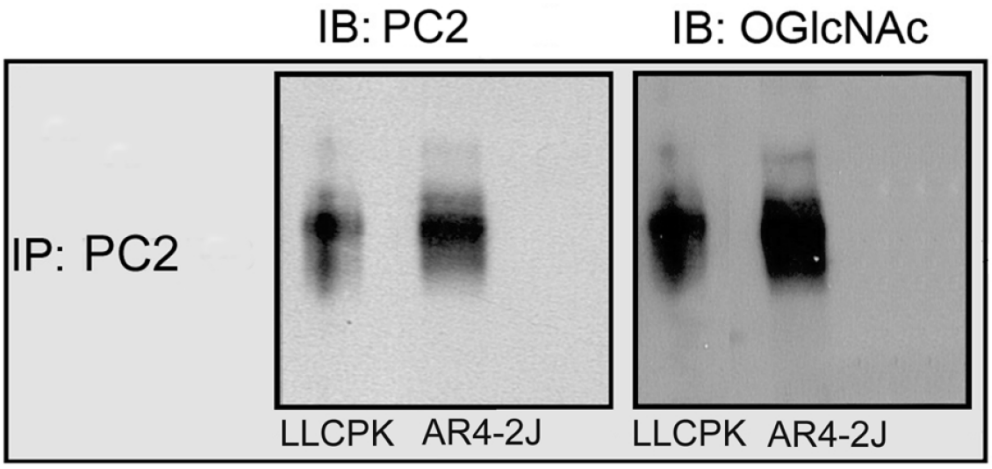
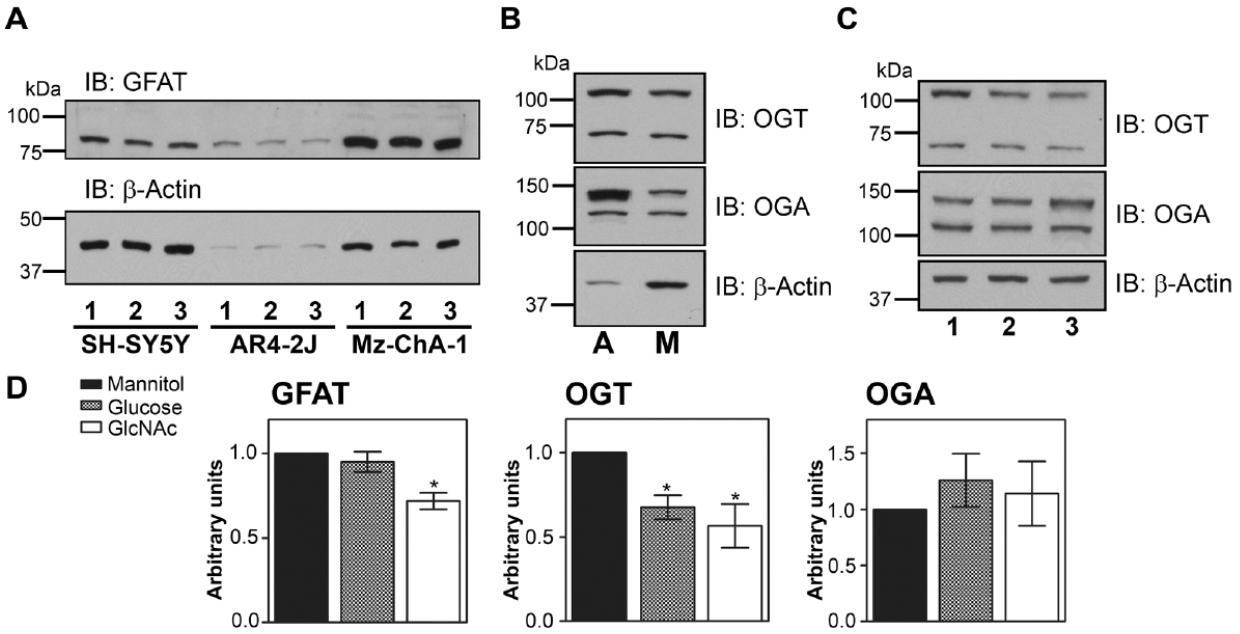
SUPPLEMENTAL MATERIAL



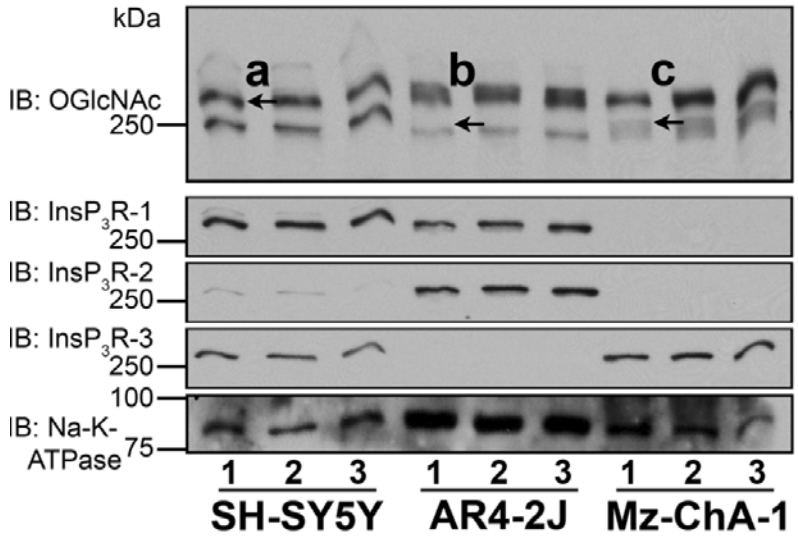
Supplemental Figure S1. $InsP_3R$-subtype expression in different cell lines. Western blot analysis of whole cell lysates of SH-SY5Y, AR4-2J and Mz-ChA-1 cells and Mz-ChA-1 microsomal preparation. Membranes were probed with anti-$InsP_3R$-1, anti-$InsP_3R$-2, anti-$InsP_3R$-3 and anti-β-actin antibody respectively. Representative blots are shown. Order of probing did not change results.



Supplemental Figure S2. PC2 is modified by O-GlcNAcylation. Western blot analysis of endogenous PC2 immunoprecipitated from LLCPK (left lane) and AR4-2J (right lane) cell lysates using anti-PC2 antibody (left panel) or anti-O-GlcNAc antibody (RL2; right panel). Lanes were removed from a larger gel for presentation. The PC2 antibody was kindly provided by Prof. S. Somlo, Yale University, New Haven, CT. LLCPK cells were cultured M199 media (Invitrogen) supplemented with 3% fetal calf serum (FCS).



Supplemental Figure S3. Enzymes involved in O-GlcNAc glycosylation are regulated. Western blot analysis of the level of OGT (two subunits at approximately 110 kDa and respectively 78 kDa (68)), OGA (at approximately 130 kDa, a second band may be nonspecific binding or a caspase cleavage product of the enzyme (69)) or GFAT (at approximately 77 kDa (70)) in each of the cell types used for this study *A.* SH-SY5Y, AR4-2J and Mz-ChA-1 cells were grown for 72 hours in (1) 20 mM mannitol, (2) 20 mM glucose, (3) 8 mM GlcNAc. Equal protein amounts (25 µg) were analyzed through gel electrophoresis and western blot analysis was performed. Membranes were probed for GFAT and β-actin. *B.* Analysis of enzyme levels of whole cell lysate of AR4-2J (A) and Mz-ChA-1 (M) cells. 25 µg of protein were analyzed and membranes were probed with anti-OGT, anti-O-GlcNAcase or anti-β-actin antibody respectively. *C.* Mz-ChA-1 cells were grown for 72 hours in (1) 20 mM mannitol, (2) 20 mM glucose, (3) 8 mM GlcNAc and equal protein amounts (25 µg) were analyzed through gel electrophoresis and western blot analysis was performed. Membranes were probed for OGT, O-GlcNAcase and β-actin respectively. *D.* Analysis of GFAT, OGT and OGA protein expression levels in Mz-ChA-1 lysate grown in different media. Representative blots are shown in panel A and panel C. Protein levels are shown as arbitrary units of densitometry values of GFAT, OGT and OGA bands in relation to corresponding densitometry data of β-actin bands. Ratios were normalized internally per blot to mannitol data and results were averaged, n=4 for each enzyme and condition**.** * represents $p<0.05$



Supplemental Figure S4. O-GlcNAc glycosylation of different $InsP_3R$ subtypes. Western blot analysis of whole cell lysates of SH-SY5Y, AR4-2J and Mz-ChA-1 cells. Cells were grown for 72 hours in (1) 20 mM mannitol, (2) 20 mM glucose and (3) 8 mM GlcNAc. Samples were lysed, and analyzed through western blot analysis. Membranes were probed for O-GlcNAc, $InsP_3R$-1, $InsP_3R$-2, $InsP_3R$-3 and Na-K-ATPase as a loading control. Arrows on the O-GlcNAc blot are showing the apparent molecular mass of a) $InsP_3R$-1, b) $InsP_3R$-2 and c) $InsP_3R$-3. For $InsP_3R$-1 and $InsP_3R$-3 a corresponding band could be detected with RL2 whereas no band could be detected for $InsP_3R$-2.