β_1-adrenergic receptor O-glycosylation regulates N-terminal cleavage and signaling responses in cardiomyocytes

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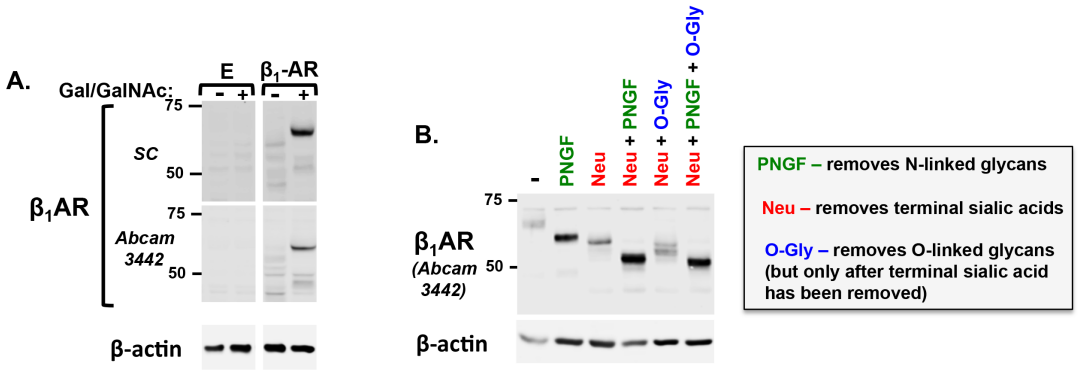

Figure S1. Glycosylation profile of an untagged β_1AR. *Panel A:* An untagged β_1AR was heterologously overexpressed in *ldlD* cells cultured without or with Gal (20 μM) and GalNAc (200 μM) as indicated. Immunoblot analysis with two different β_1AR antibodies shows that a ~69-kDa β_1AR species (that corresponds to the full-length fully glycosylated β_1AR) and lesser amounts of smaller β_1AR species accumulate only when ldlD cells are cultured with Gal/GalNAc. *Panel B*: The β_1AR species that accumulate in ldlD cells cultured with Gal/GalNAc were subjected to deglycosylation protocols. The deglycosylation experiment establishes that that the ~69-kDa band contains N-linked glycans (since its mobility increases in response to treatment with PNGF) as well as sialylated O-linked glycans (since neurominidase and O-glycosidase produce further increases in β_1AR electrophoretic mobility - over than produced by PNGF treatment alone). These results indicate that epitope tags on the N- and C-terminus do not grossly influence maturational processing (glycosylation) of the β_1AR.

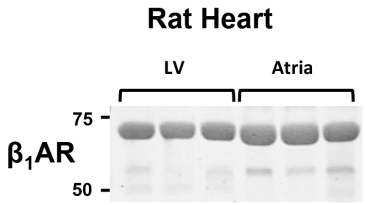

Figure S2. Lighter exposure of Figure 5B.