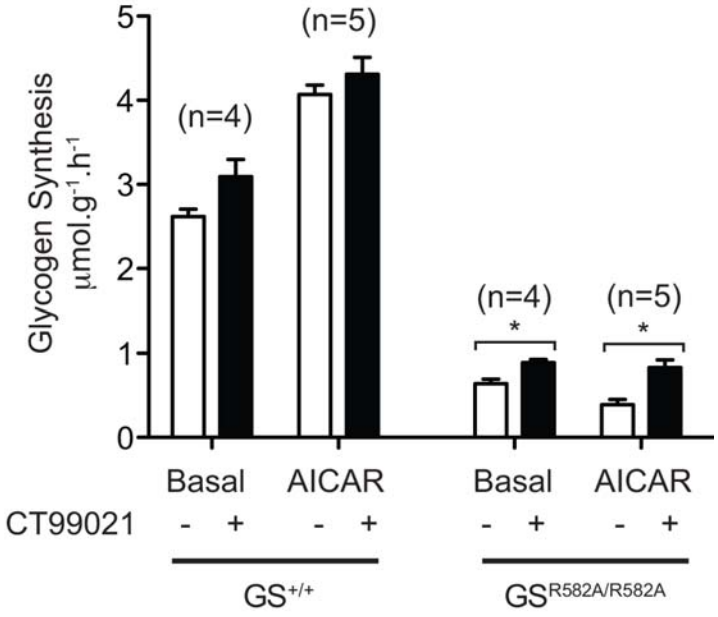
SUPPLEMENTARY DATA

Supplementary Figure 1. GSK3 inhibition modestly improves glycogen synthesis in AICAR-stimulated GS$^{R582A/R582A}$ muscles. EDL muscles from the indicated genotypes were incubated with either vehicle (0.2 % DMSO) or 3 μM CT99021 (GSK3 selective inhibitor) in the presence of buffer (resting) or 2 mM AICAR in KRB containing D-[U-^{14}C]glucose (5.5 mM) for 40 min. Glycogen synthesis was assayed as described in the Research Design and Methods.



SUPPLEMENTARY DATA

Supplementary Figure 2. G6P does not significantly influence AMPK-mediated phosphorylation of Ser8 GS. Baculovirus-expressed muscle glycogen synthase (GS):GST-glycogenin-1 complex was dephosphorylated *in vitro* with recombinant PP1γ in 50 mM HEPES pH 7.4, 1 mM $MnCl_2$, 0.1 mM EGTA and 1 mM DTT for 30 min at 30° C. PP1 was blocked using 1 μM microcystin-LR and the GS preparation was phosphorylated *in vitro* with recombinant AMPKα2β2γ1 complex produced in E.coli activated with CaMKKβ (active AMPKα2β2γ1 complex was donated by D. Grahame Hardie, University of Dundee). Briefly, 100 ng GS was incubated with 50 mM HEPES pH 7.4, 0.1 % (w/v) glycogen, 0.1 mM AMP, 10 mM $MgCl_2$, 5 mU AMPK (assayed against AMARA peptide) and varying concentrations of G6P for 5 min at 30 °C. 5 mU recombinant protein kinase A (PKA) catalytic subunit (Promega) was used as a positive control. The reaction was started by the addition of 0.2 mM ATP, incubated for a further 20 min at 30° C and stopped by the addition of SDS sample buffer. Reactions were separated by SDS-PAGE and blotted for pSer8 and total GS. Results are representative of two independent experiments.