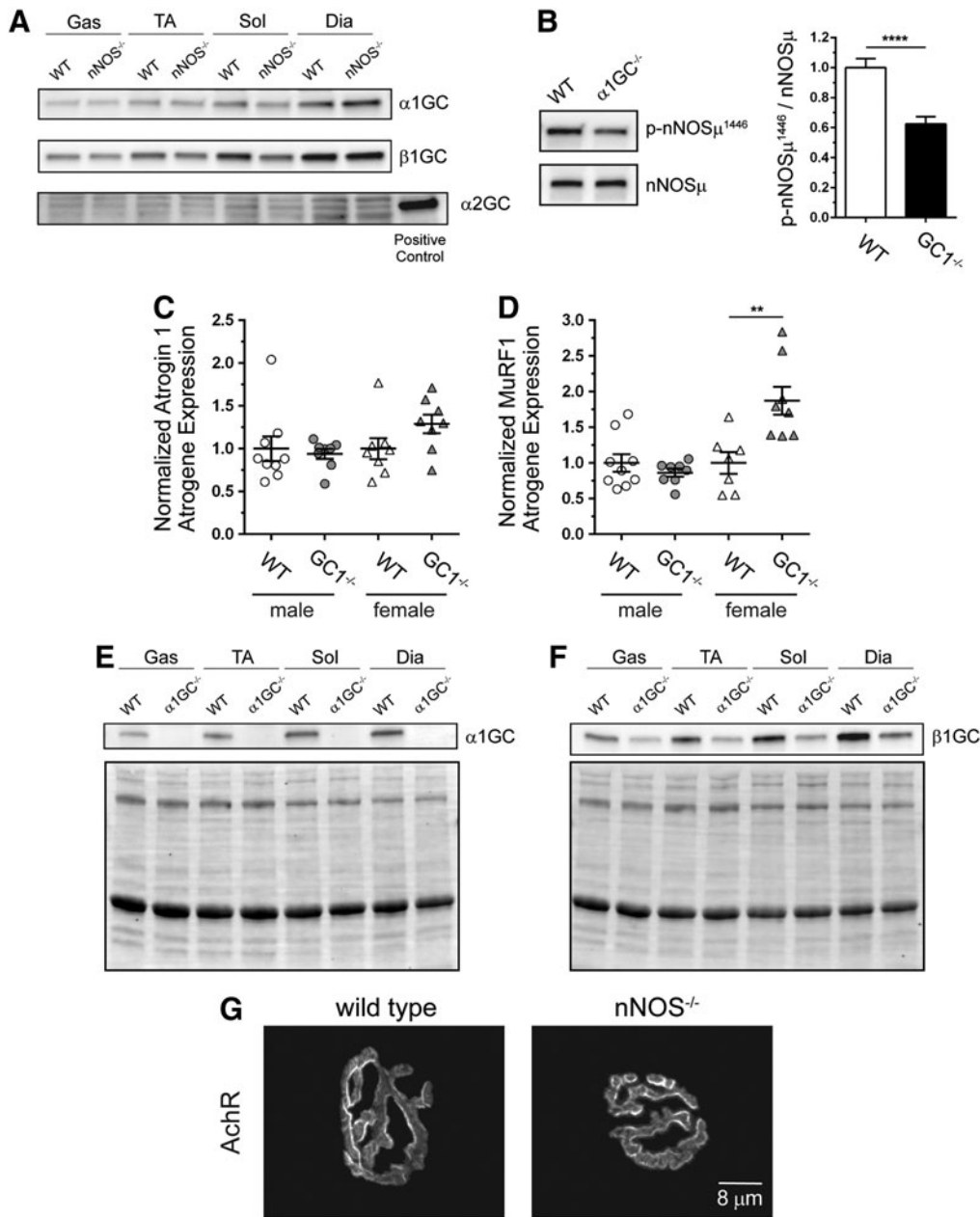


Supplementary Data



SUPPLEMENTARY FIG. S1. GC enzyme expression in nNOS^{-/-} muscles and the fraction of active nNOS_μ and atrogene mRNA expression in GC1^{-/-} muscles. (A) Representative Western blots of α1GC, β1GC, and α2GC subunit expression in gas, TA, sol, and dia muscles from WT and nNOS^{-/-} mice. *n* = 8 for all groups. The positive control for α2GC is striatum. nNOS deficiency does not impact α1GC, β1GC, and α2GC expression. (B) Representative Western blots and densitometric quantitation of nNOS_μ and serine 1446 phosphorylated nNOS_μ expression in TA muscles from WT and GC1^{-/-} mice. Loss of GC1 decreases the fraction of active ser1446 phosphorylated nNOS_μ by ~40%, suggesting a feedback loop between GC1 and nNOS_μ. *n* = 24–27 for each group. *****p* < 0.0001 by Student's unpaired *t*-test comparison of WT GC1^{-/-} groups. (C) Quantitation of Atrogin 1 transcript expression by qPCR in TA muscles from male and female WT and GC1^{-/-} mice. Elevated Atrogin 1 mRNA expression marks depressed protein synthesis. Atrogin 1 expression was not significantly affected by loss of GC1. (D) Quantitation of MuRF1 transcript expression by qPCR in TA muscles from male and female WT and GC1^{-/-} mice. MuRF1 targets contractile proteins for breakdown and its expression is increased by muscle disuse. MuRF1 mRNA expression was unaffected in male GC1^{-/-} muscle, but significantly increased in female GC1^{-/-} muscle consistent with the poorer fatigue resistance and suggesting greater muscle disuse in female GC1^{-/-} mice. For (C), *n* = 9 for the WT male group and *n* = 8 for all other groups. For (D), *n* = 9 and 7 for WT male and female groups, respectively. *n* = 8 for male and female GC1^{-/-} groups. These data argue against induction of atrophy in GC1^{-/-} TA and are supported by similar TA muscle cell sizes in control and GC1^{-/-} mice (Fig. 4H, I). (E) Representative α1GC Western blot from Figure 1F (top panel) with loading control (lower panel). ***p* < 0.01 (F) Representative β1GC Western blot from Figure 1F (top panel) with loading control (lower panel). (G) Representative high-magnification confocal micrographs showing an *en face* view of the neuromuscular junction labeled with AchR (left panel) from WT and nNOS^{-/-} gastrocnemius myofibers. AchR distribution is unaffected by nNOS deficiency. GC, soluble guanylate cyclase; Dia, diaphragm; Gas, gastrocnemius; nNOS, neuronal nitric oxide synthase; Sol, soleus; TA, tibialis anterior; WT, wild-type.