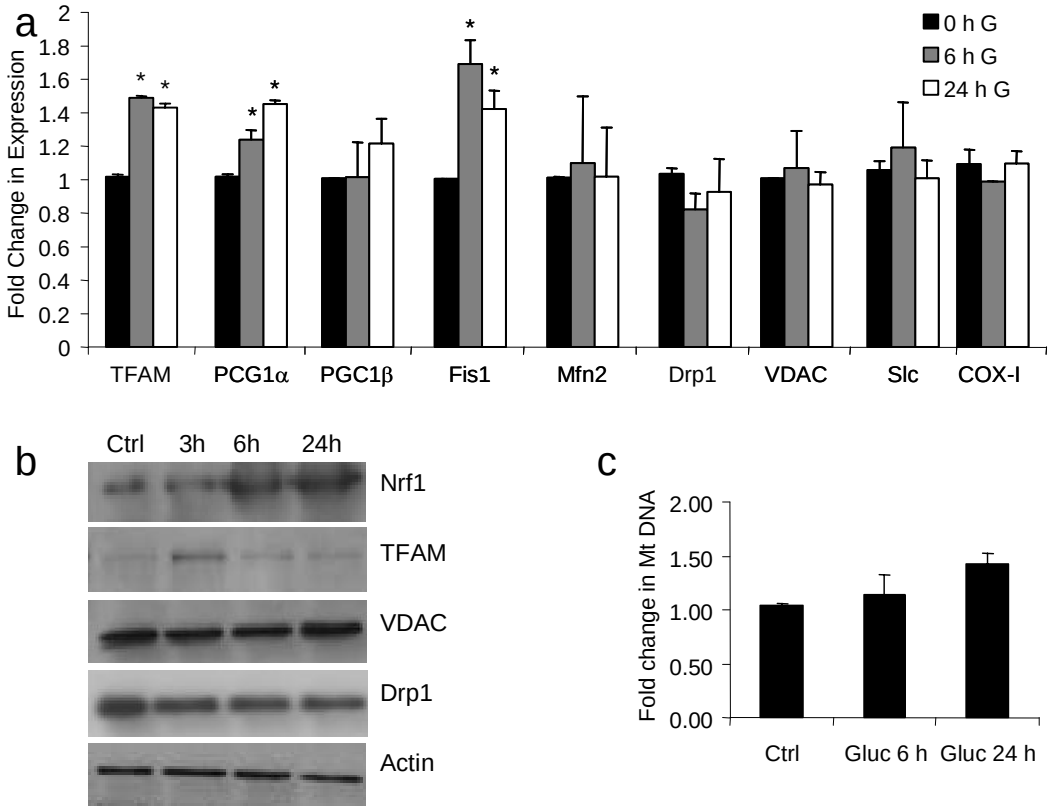
Supplementary Fig. 1

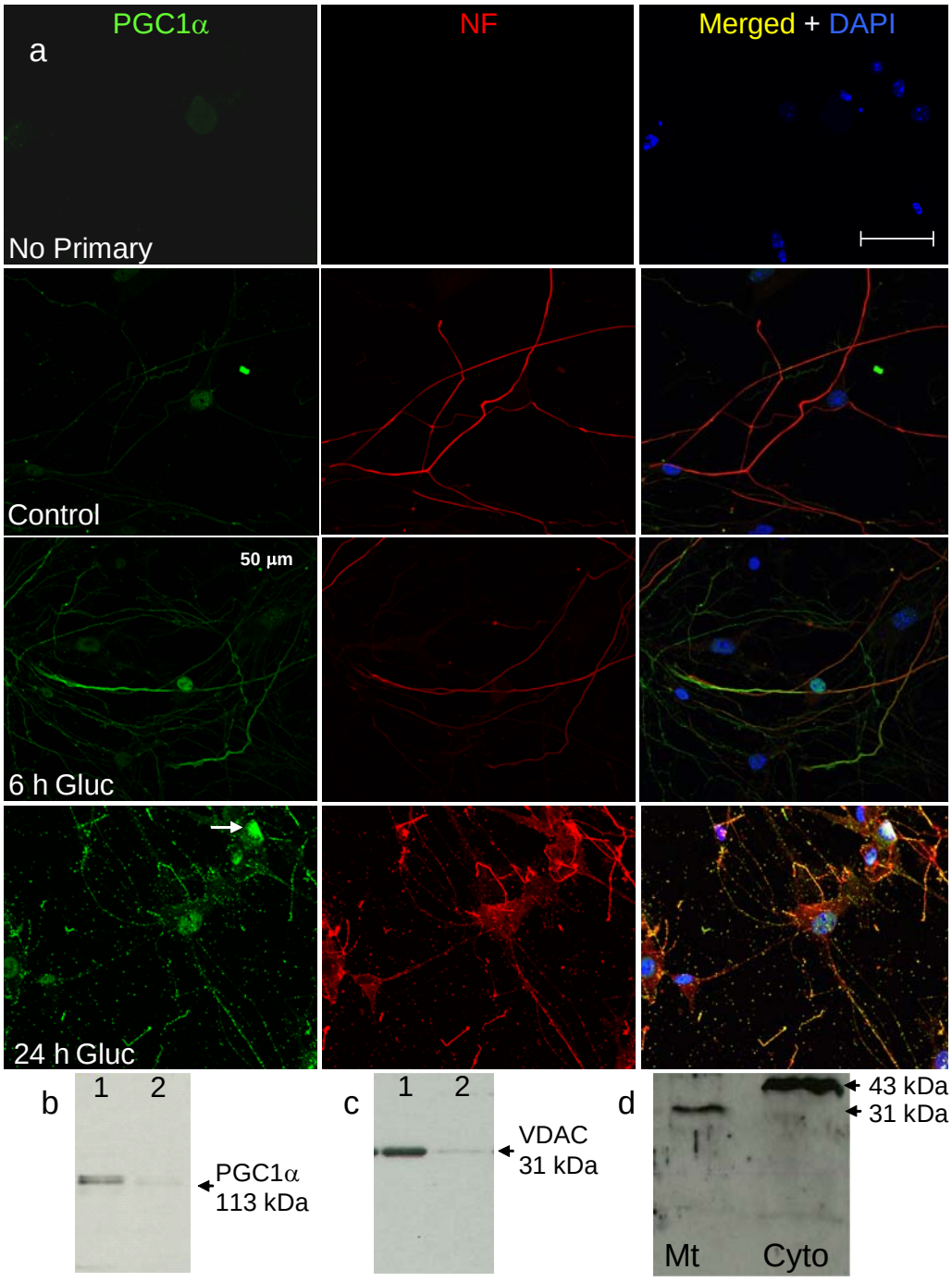
. Mitochondrial Biogenesis in DRG Neurons in Response to Hyperglycemia. Since there are increased numbers of mitochondria in the neurites, a proportion of which may be accounted for by mitochondrial biogenesis (evidenced by mitochondrial DNA synthesis), we assessed mitochondrial transcription in the DRG neurons plus or minus glucose treatment.



DRG neurons from C57/Bl6 mice were exposed to 25.7 mM glucose for 0, 6, or 24 h, then mRNA, mitochondrial DNA, and protein were isolated. In (a), mRNA was subjected to quantitative RT-PCR for mitochondrial transcription factors, fission and fusion proteins, and mitochondrial functional proteins. *TFAM, PGC1α, and Fis1 were significantly increased after 6-24 h hyperglycemia, $p<0.01$. In (b), protein levels of selected factors were also analyzed by Western blotting. The transcription factors Nrf1 and TFAM were increased. In (c), mitochondrial DNA was normalized to the levels of nuclear DNA. *After 24 h hyperglycemia, there was a significant increase in mitochondrial DNA relative to nuclear DNA, $p<0.01$. .

Supplementary Fig. 2.

The Subcellular Localization of PGC1α in DRG in Response to Hyperglycemia. In addition to regulation at the level of expression, transcription factors also function only at specific cellular locations. The Nrf1 signal was weak and did not appear to re-localize in high glucose (not shown). In contrast, we observed a re-localization of PGC1α from primarily nuclear localization in basal glucose to increasing throughout the cell body and neurites in high glucose. By 24 h, there is frequently a significant accumulation of PGC1α label in the perinuclear region (arrow).



In (a), DRG neurons from C57/Bl6 mice were exposed to 25.7 mM glucose for 0, 6, or 24 h, then fixed and immunolabeled for PGC1α (green) and VDAC (red). Merged images (right) show co-localization in yellow. Bar =50 µm. In (b-d), the quality of the antibodies is explored by Western blotting. PGC1α (b) and VDAC (c) antibodies were tested on DRG neuron lysates. In (b-c), lane 1 contains untreated lysate and lane 2 contains lysate pre-incubated with 1 mg/mL control peptide supplied by the manufacturer. In (d), we further confirm that the VDAC

antibody immunolabels mitochondria in fractionated DRG neurons. The cultured cells are fractionated by ultracentrifugation per our published protocol [1] and the mitochondria (Mt) and cytoplasm/membrane fragments (Cyto) are run separately on the Western blot. The 31 kDa VDAC band appears only in the Mt fraction, while 43 kDa actin is only in the Cyto fraction.

Reference

[1] Vincent AM, McLean LL, Backus C, et al. (2005) Short-term hyperglycemia produces oxidative damage and apoptosis in neurons. FASEB J 19:638-640