SUPPLEMENTARY MATERIALS AND METHODS

Chemicals

2-deoxy-D-Glucose (2DG) was purchased from Cayman Europe OU, Estonia. Metformin hydrochloride and sodium azide (NaN_3) were purchased from Toronto Research Chemicals, Canada.

Drug treatment

After 3 days in vitro, culture medium was changed and treatments were initiated. Oxidative ATP production was blocked with 100 µ M NaN_3 for 24 hr in the presence of 2.5 mM glucose. 10 mM 2DG was applied to neurons for 24 hr in growth medium in the presence of a reduced amount of glucose (1mM).

Mitochondrial fusion rate and motility analysis

Cortical neuronal cultures were transfected with mito-KikGR1 plasmid and plasmids of interest as described earlier (Cagalinec et al., 2013). A laser scanning confocal microscope (LSM 510 Duo, Carl Zeiss Microscopy GmbH) equipped with a LCI Plan-Neofluar 63×/1.3 water immersion DIC M27 objective was used. The temperature was maintained at 37°C using a climate chamber. For fusion analysis, mito-KikGR1 was illuminated with a 488 nm argon laser line to visualize the intense green mitochondrial staining. Selected mitochondria were then photo-converted to red using a 405 nm diode laser and illuminated using a 561 nm DPSS laser. The images were taken at 10s intervals for 10 min, and the fate of all activated mitochondria was followed throughout the time-lapse and the fusion and fission events recorded. The number of fusions and fissions of photo-activated mitochondria was summarised per dish and then averaged over 12 dishes. To compare mitochondrial velocities, 10–20 mitochondria per neurite (including non-activated mitochondria) were tracked and the fraction of time moving and their movement velocity were calculated.

ATP Levels

Cortical neurons were transfected with plasmids expressing firefly luciferase, *Renilla* luciferase, and the plasmids of interest. To measure firefly luciferase activity, cells were incubated for 10 min with 25µM 1-(4,5-dimethoxy-2-nitrophenyl)ethyl (DMNPE) caged luciferin at 37 °C and the luminescence was measured by MicroBeta® TriLux. To measure *Renilla* luciferase activity, the neurons were lysed and the luminescence was measured using Dual-Glo Luciferase Assay reagent. Firefly luciferase activity from living cells normalized to *Renilla* luciferase activity from lysed cells was used to estimate ATP levels in transfected cells. Sixteen independent samples were analysed per group.

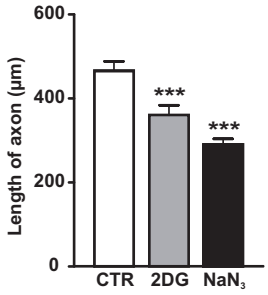

Fig. S1. Chemical inhibition of glycolysis or oxidative phosphorylation supresses axonal growth. On DIV3, oxidative ATP production was blocked with 100 µM NaN_3 for 24 hr in the presence of 2.5mM glucose. Glycolytic ATP production was inhibited by applying 10 mM 2DG to neurons for 24 hr in growth medium in the presence of a reduced amount of glucose (1mM). Both treatments significantly reduced axonal growth. ***$p<0.001$ compared with the control group (n=40 axons per group).

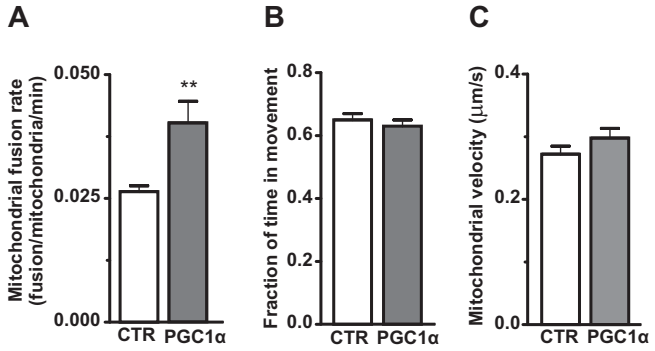

Fig. S2. Effect of PGC-1α overexpression on mitochondrial fusion and movement. Cortical neuronal cultures were co-transfected with mito-KikGR1 and PGC-1α-overexpressing plasmids and mitochondrial fusion rate and motility parameters were analysed as described in Supplementary Materials and Methods. (A) Mitochondrial fusion rate was significantly increased in PGC-1α-overexpressing neurons. **p<0.001 compared with the control group, n=12 dishes. (B and C) The fraction of time spent moving by mitochondria and the average velocity of mitochondria were similar between groups (n=656 and 786 mitochondria in the control and PGC-1α-overexpressing group respectively, p = 0.33 for fraction of time spent moving and p = 0.66 for mitochondrial velocity.

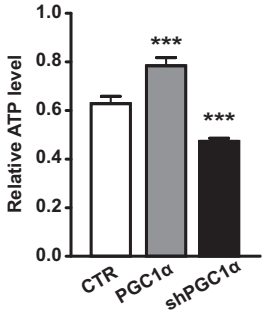

Fig. S3. The effect of PGC-1α overexpression or silencing by PGC-1α shRNA on neuronal ATP levels. Cortical neurons were co-transfected with firefly- and *Renilla* luciferase-expressing plasmids. Firefly luciferase activity was measured 72 h later in living cells using DMNPE-caged luciferin as a substrate. The results were normalized to Renilla luciferase activity measured after cell lysis. ***$p<0.001$ compared with the control group (16 independent samples were analysed per group).

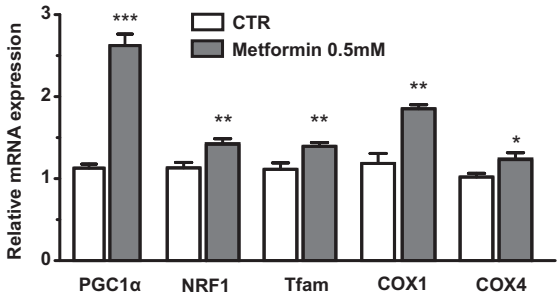

Fig. S4. Quantitative PCR analysis of mitochondrial biogenesis and energy production-related genes in primary cortical neurons following Metformin treatment. The mRNA levels of PGC-1α, NRF1, Tfam, Cox1 and Cox4 (cytochrome c oxidase subunit 1 and 4; encoded in mitochondrial and nuclear DNA, respectively) were normalised to HPRT mRNA levels and expressed relative to control groups (n=4 dishes per group). *p<0.05, **p<0.01, and ***p<0.001 compared with respective control groups.