O-GlcNAcylation of Ogg1 Impairs Oxidative Mitochondrial DNA Lesions Repair in Diabetic Hearts

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Running Title: O-GlcNAc inhibits Ogg1 mtDNA repair activity

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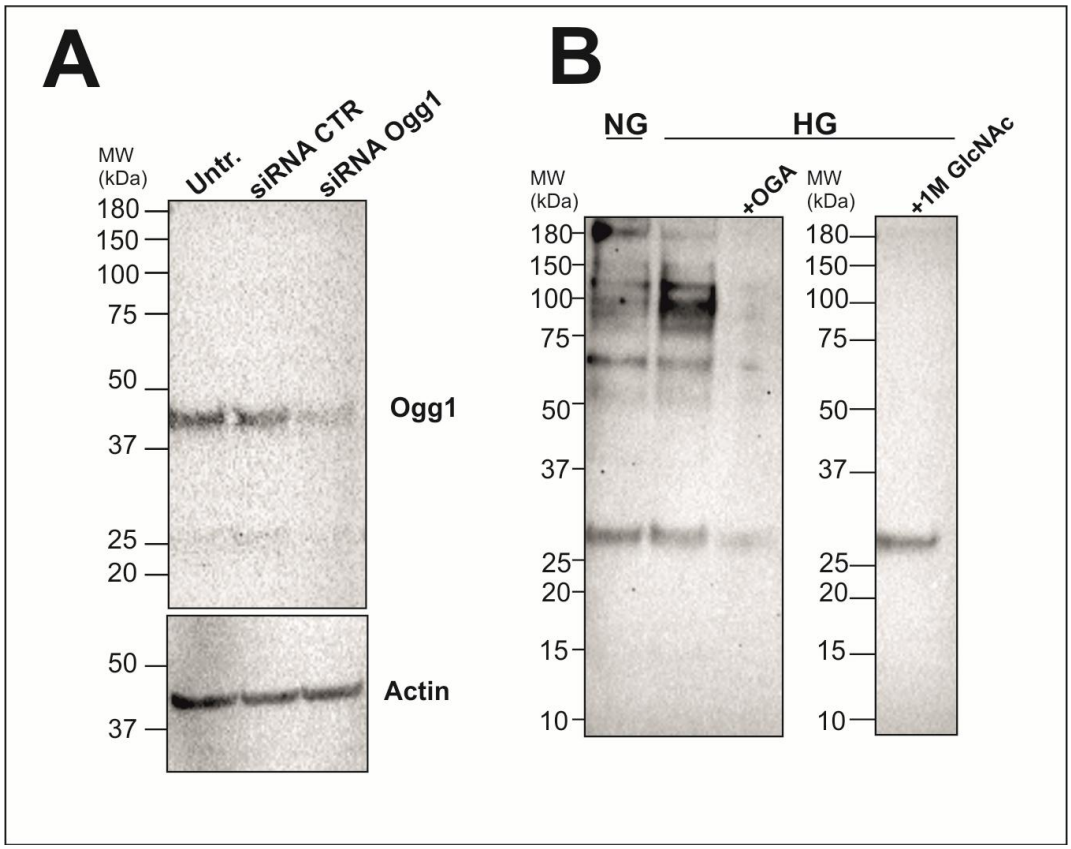

Figure S1 Anti-Ogg1 and anti-O-GlcNAc (RL2) antibody validation. A) For anti-Ogg1 validation NCM cells were exposed to 25 mM glucose and after 24 h cells were transfected with 100 pmol Ogg1-specific siRNA (siRNA Ogg1) or control siRNA (siRNA CTR) for 48 h using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. Western blots of Ogg1 (45 kDa) levels are shown. Actin was used as loading control. B) For RL2 validation cells were exposed to either normal (NG) or high glucose (HG) for 72 h, harvested and 50 µg lysate was analyzed for total O-GlcNAcylation by Western blot. For biological RL2 antibody validation 50 µg lysate from HG cells was incubated for 2 h at 37°C with approximately 200 µg/ml recombinant OGA (+OGA). For technical RL2 validation 50 µg lysate from HG cells was separated by SDS PAGE and after transfer to PVDF the membrane was cut and incubated with RL2 antibody diluted in 5% milk/TBS 0.05% Tween + 1M GlcNAc (+1M GlcNAc).

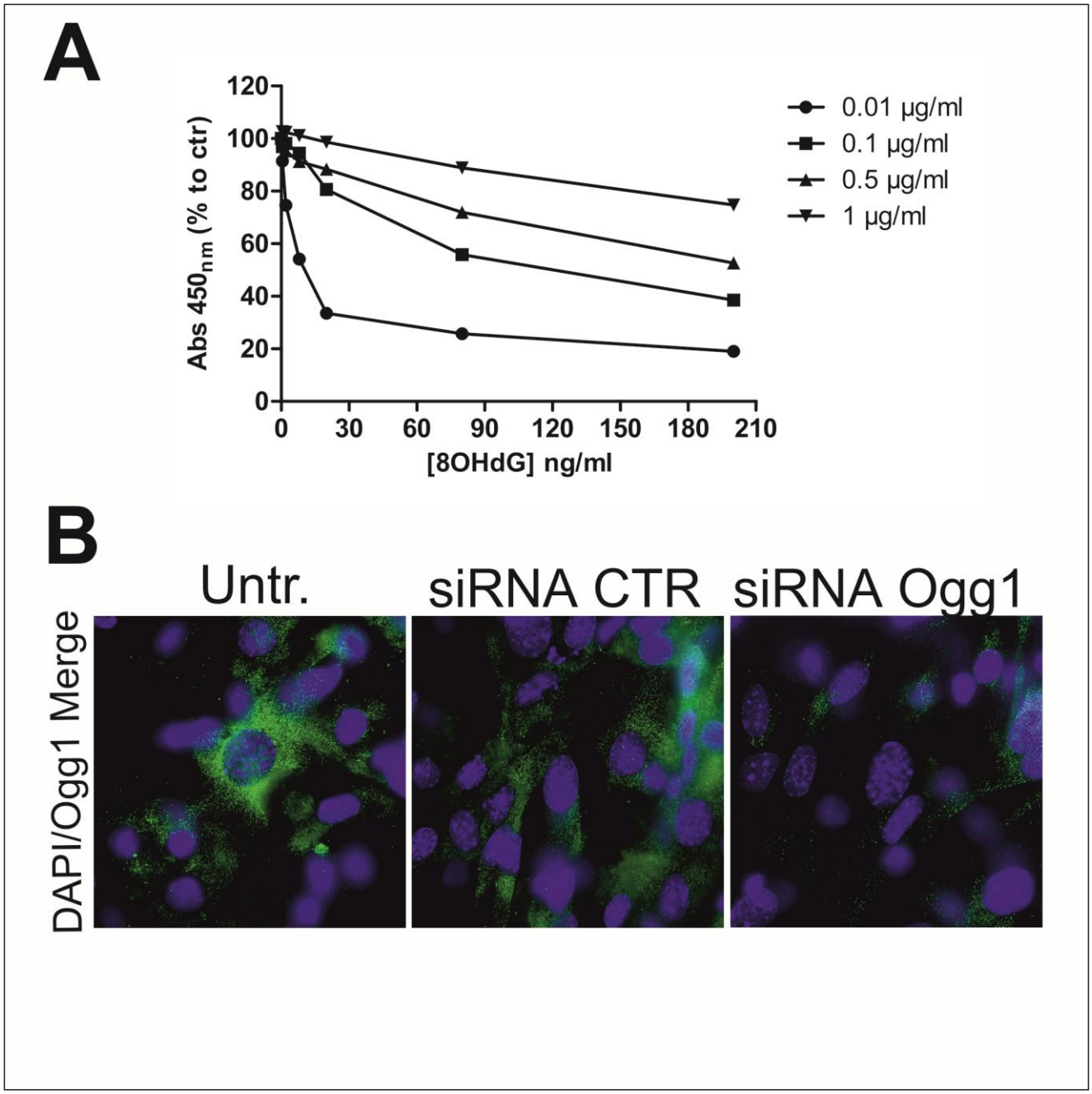

Figure S2 Anti-8-OHdG and IF anti-Ogg1 antibody validation. A) The anti-8-OHdG antibody was validated using a competitive enzyme-linked immunosorbent assay (ELISA) for quantitative measurement of 8-OHdG (BIOXYTECH® 8-OHdG-EIA™ Kit). The anti-8-OHdG antibody used in our studies (Genetex: GTX41980) was used in place of the antibody provided by the kit. Four different anti-8-OHdG antibody concentrations ranging from 0.01 to 1 µg/ml were tested. B) NCM cells were treated as they were for anti-Ogg1 Western blot (See supplemental Fig. 1) and Ogg1 immunofluorescence staining was performed according to procedures described in materials and methods.

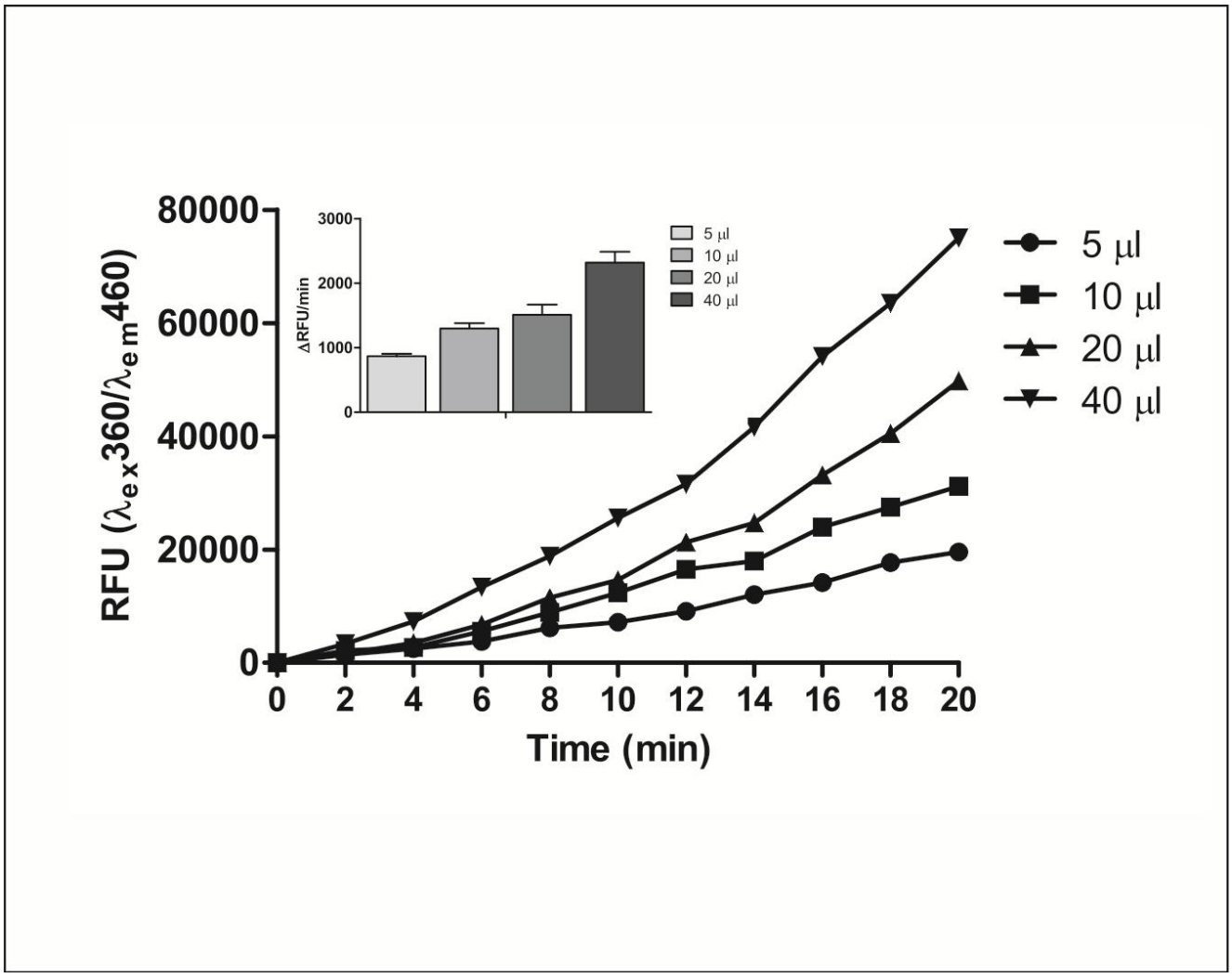

Figure S3 Recombinant His-tagged OGA *in vitro* activity assay. After purification and prior to every application requiring it, OGA activity was assessed in 200 µl total reaction volume in the presence of 50 mM sodium cacodylate pH 6.4, 3% BSA, 1 µM 4-methylumbellifery 2-deoxy-2-N-acetyl-β-D-glucopyranoside (MU-GlcNAc). Activity was followed on a Biotek® Synergy 2 Multi-Mode Reader. Samples were excited at 360/40 nm (λ_{ex}) and fluorescence emission readings at 460/40 nm (λ_{em}) were taken every 2 minutes. RFU and ΔRFU/min (inset) for the different enzyme volumes tested are reported.