SUPPLEMENTARY INFORMATION

OPA1 gene therapy prevents retinal ganglion cell loss in a Dominant Optic Atrophy mouse model.

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SUPPLEMENTARY FIGURES

Figure S1

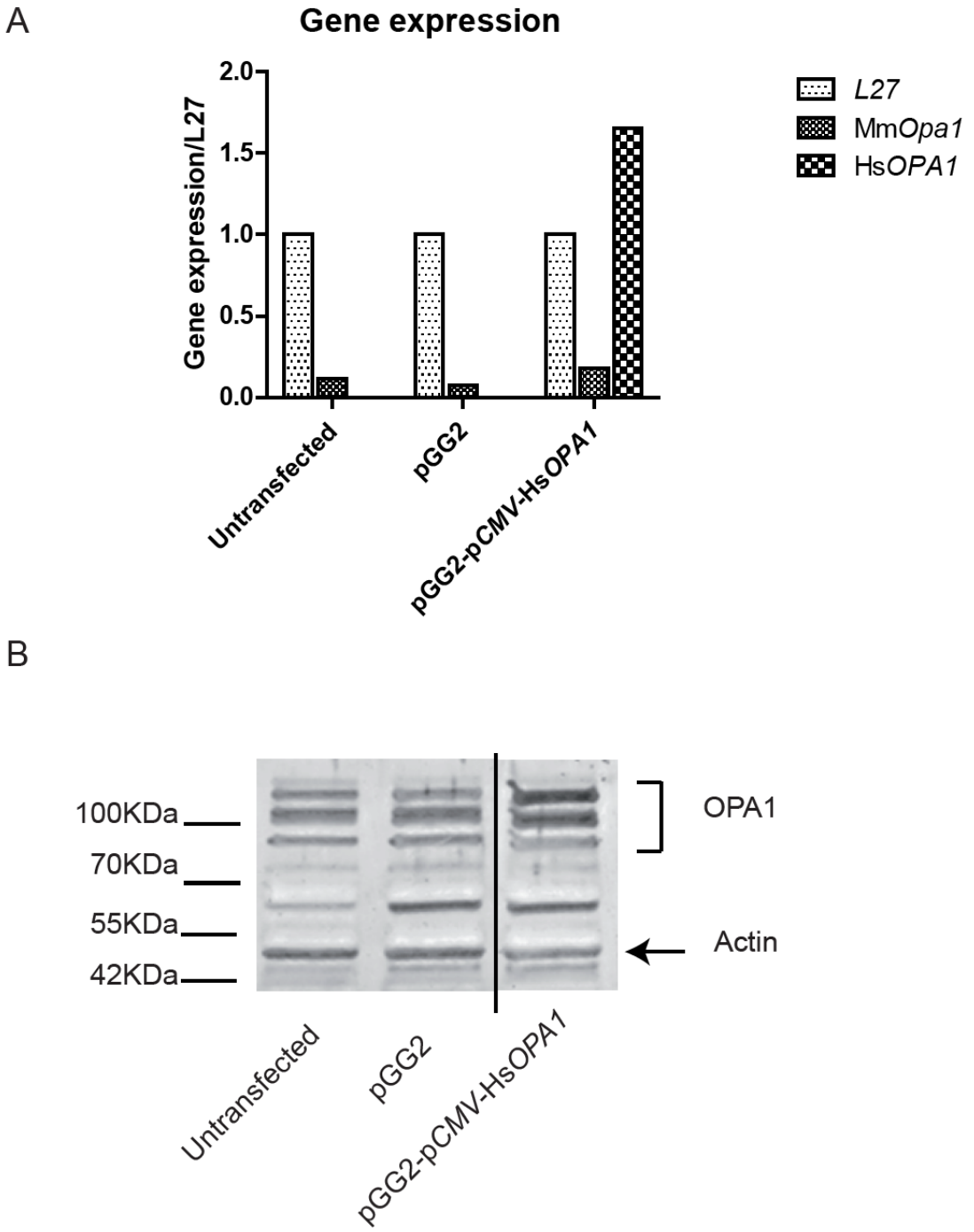

Figure S1: Control of the Hs*OPA1* gene and protein expression in the NIH3T3 mouse strain. (A) real-time quantitative PCR of murine *Opa1* and Hs*OPA*1 transcripts reported to the L27 gene expression (*n*=3). (B) Western blot using a home-made-OPA1 and actin antibodies.

Figure S2

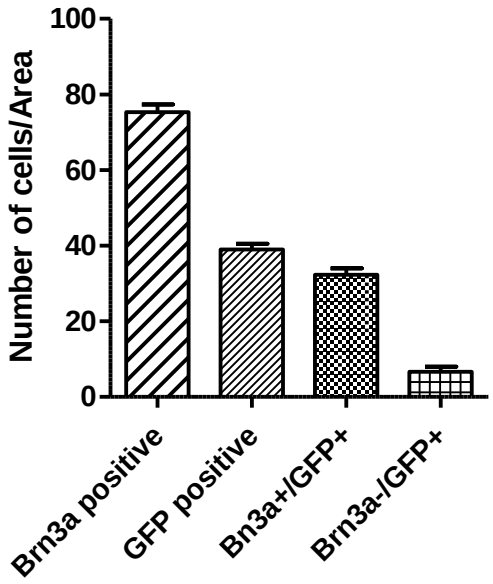

Figure S2: Quantification of GFP and Brn3a staining on whole-mount retina. Data were expressed as the mean ± SEM.

SUPPLEMENTARY MATERIAL AND METHOD

NIH3T3 transfections and control of the HsOPA1 protein expression

NIH3T3 cells were cultured in complete culture medium (Dulbecco's modified Eagle's medium, supplemented with 10% fetal calf serum and 100 units/ml penicillin/streptomycin, Invitrogen) and maintained at 37 °C in a humidified 5% CO2 atmosphere. Cells were seeded at a concentration of a 2×10^5 into 6-well plates. Transient transfections with the pGG2-p*CMV*-Hs*OPA1* construction were performed using Lipofectamine 2 000 in Optimem (Invitrogen) according to the manufacturer's protocol. Empty pGG2 vector was used as control. Twenty-four hours after transfection, cells were harvested and protein were extracted in RIPA buffer (50 mM Tris–HCl, pH8.0, 150 mM sodium chloride, 1% NP-40, 0.1% SDS, and 0.5% sodium

deoxycholate). Proteins (40 μg) were separated by 7.5% SDS-PAGE and transferred onto a nitrocellulose membrane (Bio-Rad, Hertfordshire, UK). Primary antibodies against OPA1 protein (home-made antibody) and Actin (1:5000, Sigma-Aldrich) were visualized using horseradish peroxidase-conjugated secondary antibody.