Supplemental Information

NMNAT2 is downregulated in glaucomatous

RGCs, and RGC-specific gene therapy rescues

neurodegeneration and visual function

Fang Fang, Pei Zhuang, Xue Feng, Pingting Liu, Dong Liu, Haoliang Huang, Liang Li, Wei Chen, Liang Liu, Yang Sun, Haowen Jiang, Jiangbin Ye, and Yang Hu
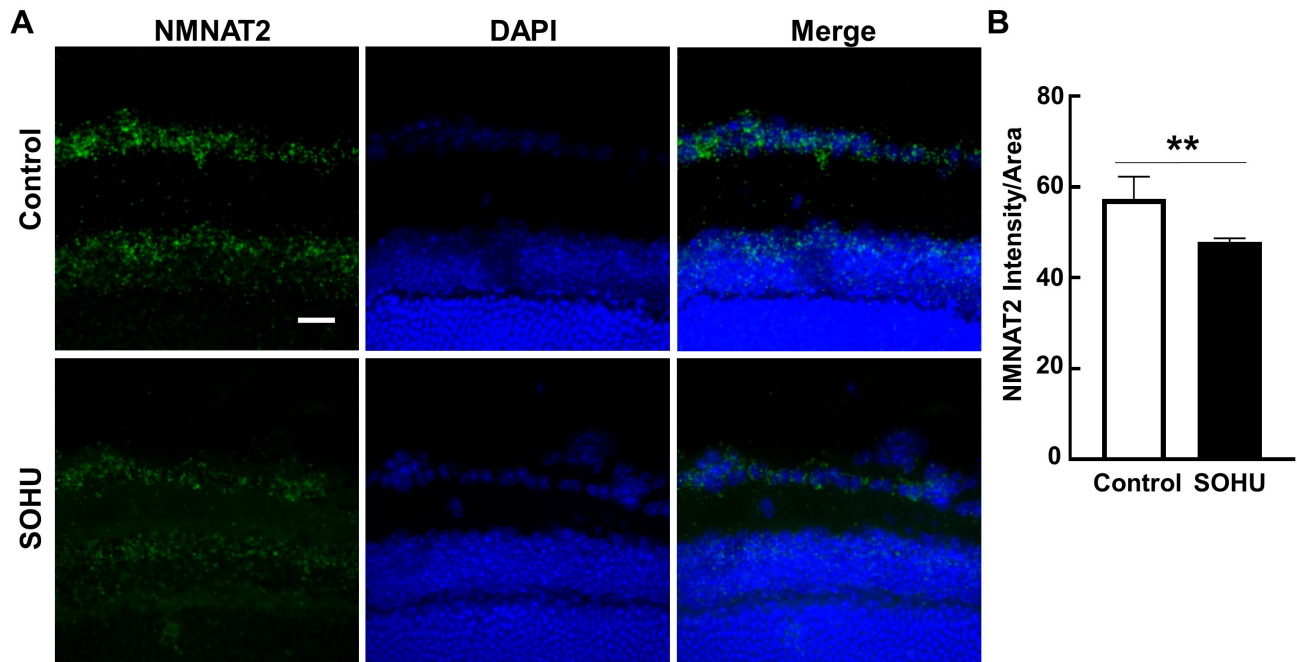
A

NMNAT2 | DAPI | Merge

Control

SOHU

B

Fig. S1

Figure S1. NMNAT2 mRNA level is decreased in glaucomatous RGCs. (A) Representative

confocal images of retina cross-sections showing NMNAT2 mRNA levels in GCL by ISH, one week

after SO injection. GCL: ganglion cell layer. Scale bar, 20 μm. **(B)** Quantification of mean

fluorescence intensity of NMNAT2, n = 5. Data are presented as means ± SD, **: $p<0.01$, two-tailed

unpaired t test.
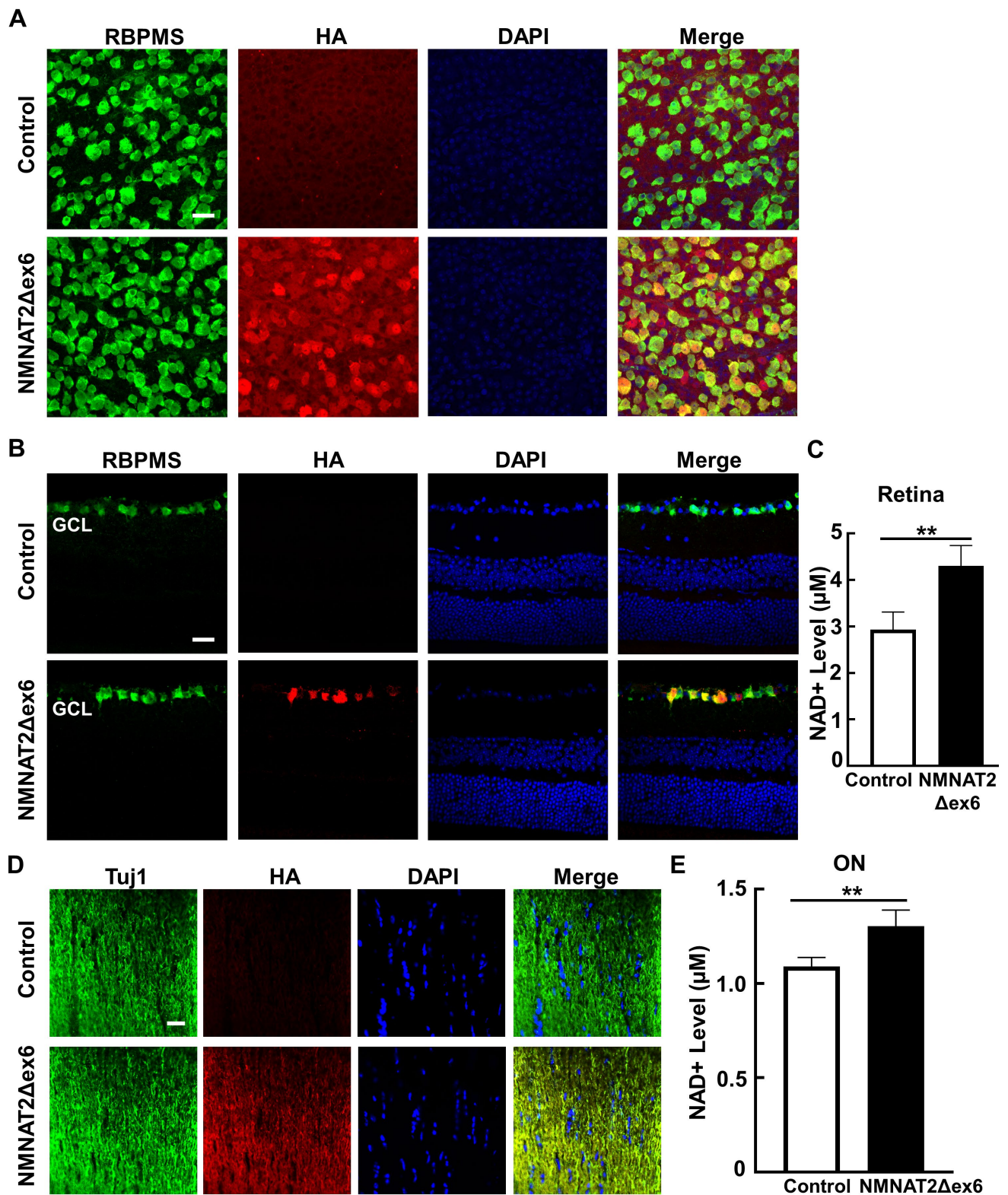
Figure S2. AAV2-mSncg-mediated RGC-specific expression of NMNAT2Δex6 after intravitreal

injection. (A) Representative confocal images of retina wholemounts showing RBPMS positive

10   (green) RGCs and HA-tagged NMNAT2 (red) overexpression in mice 2 weeks after intravitreal

11   injection of AAV2-mSncg-3HA-NMNAT2Δex6, but not in mice injected with control AAV2. Scale

12   bar, 20 μm. **(B)** Representative confocal images of retina cross-sections showing HA-tagged NMNAT2

13   (red) expression in RBPMS positive (green) RGCs but not in other layers of retina, 2 weeks after

14   intravitreal injection. GCL: ganglion cell layer. Scale bar, 20 μm. **(C, E)** The NAD^+ level in whole

15   retina lysates or ON lysates measured by NAD+ biochemistry assay kit, 2 weeks after AAV2-mSncg-

16   NMNAT2Δex6 intravitreal injection. Data are presented as means $\pm$ SD, $n = 4$ each group; **: p<0.01,

17   two-tailed unpaired t test. **(D)** Representative confocal images of ON longitudinal sections

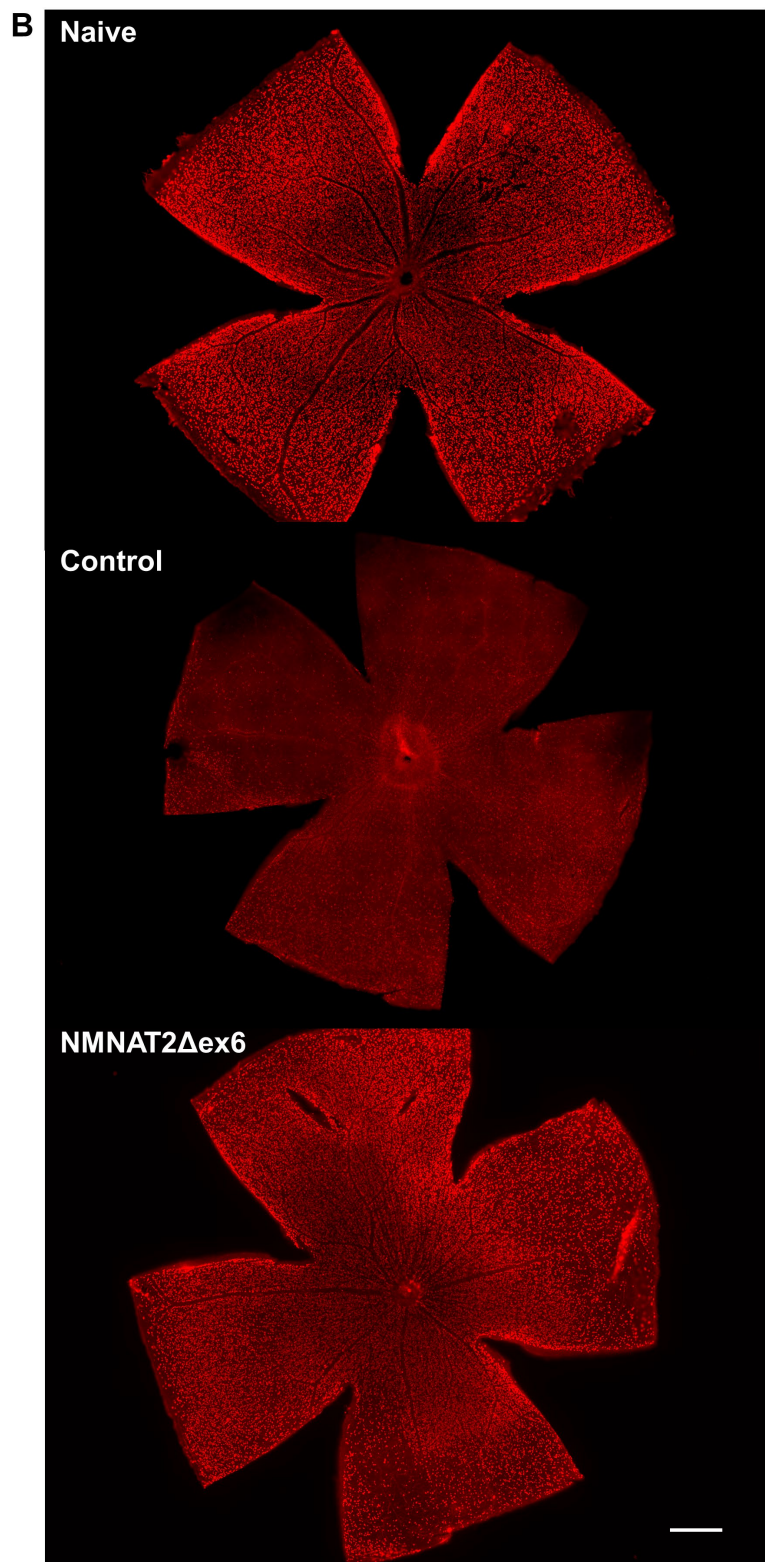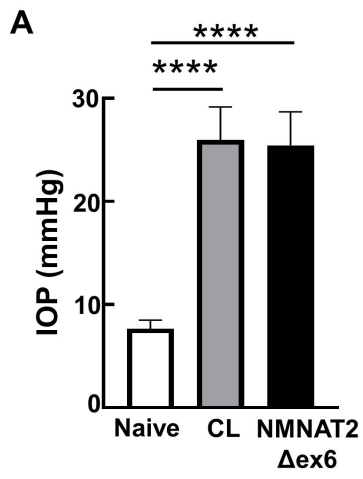18   immunostained for Tuj1 and HA in mice 2 weeks after intravitreal injection. Scale bar, 20 μm.

19

A

B

Naive

Control

NMNAT2Δex6

Fig. S3

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4

21 **Figure S3. NMNAT2 overexpression does not affect IOP elevation but protects RGCs**

22 **significantly in glaucomatous mice. (A)** IOP measurements at 3wpi. Naïve, n = 20, all groups. Data

23 are presented as means ± SD, ****: p<0.0001, one-way ANOVA with Tukey's multiple comparisons

24 test. **(B)** Representative fluorescence microscope images of the whole flat-mounted retinas showing

25 surviving RBPMS positive (red) RGCs at 3wpi. Scale bar, 500 µm.

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