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Supplemental Information

Ad- and AAV8-mediated *ABCA1* gene therapy in a murine model with retinal ischemia/reperfusion injuries

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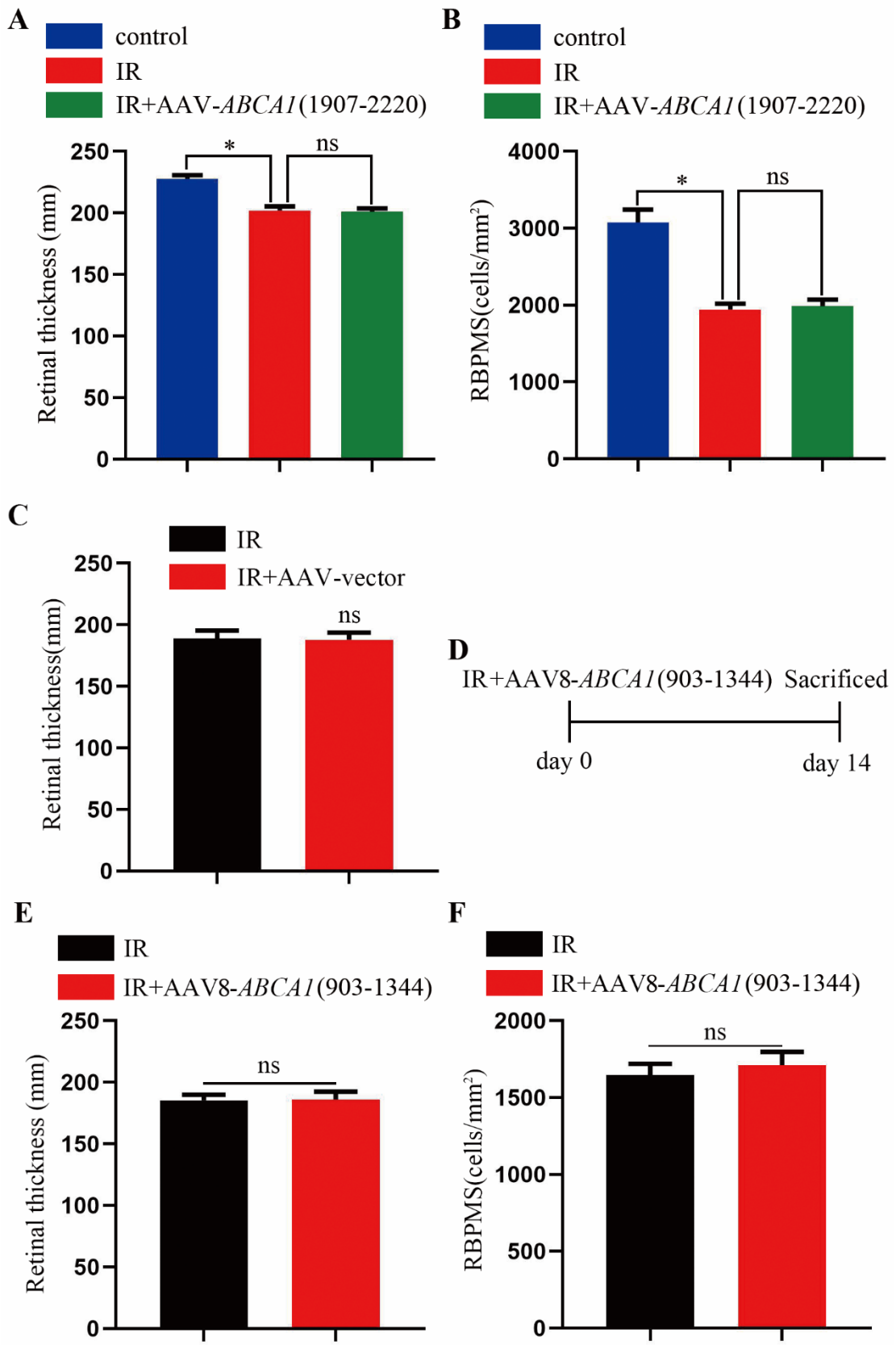
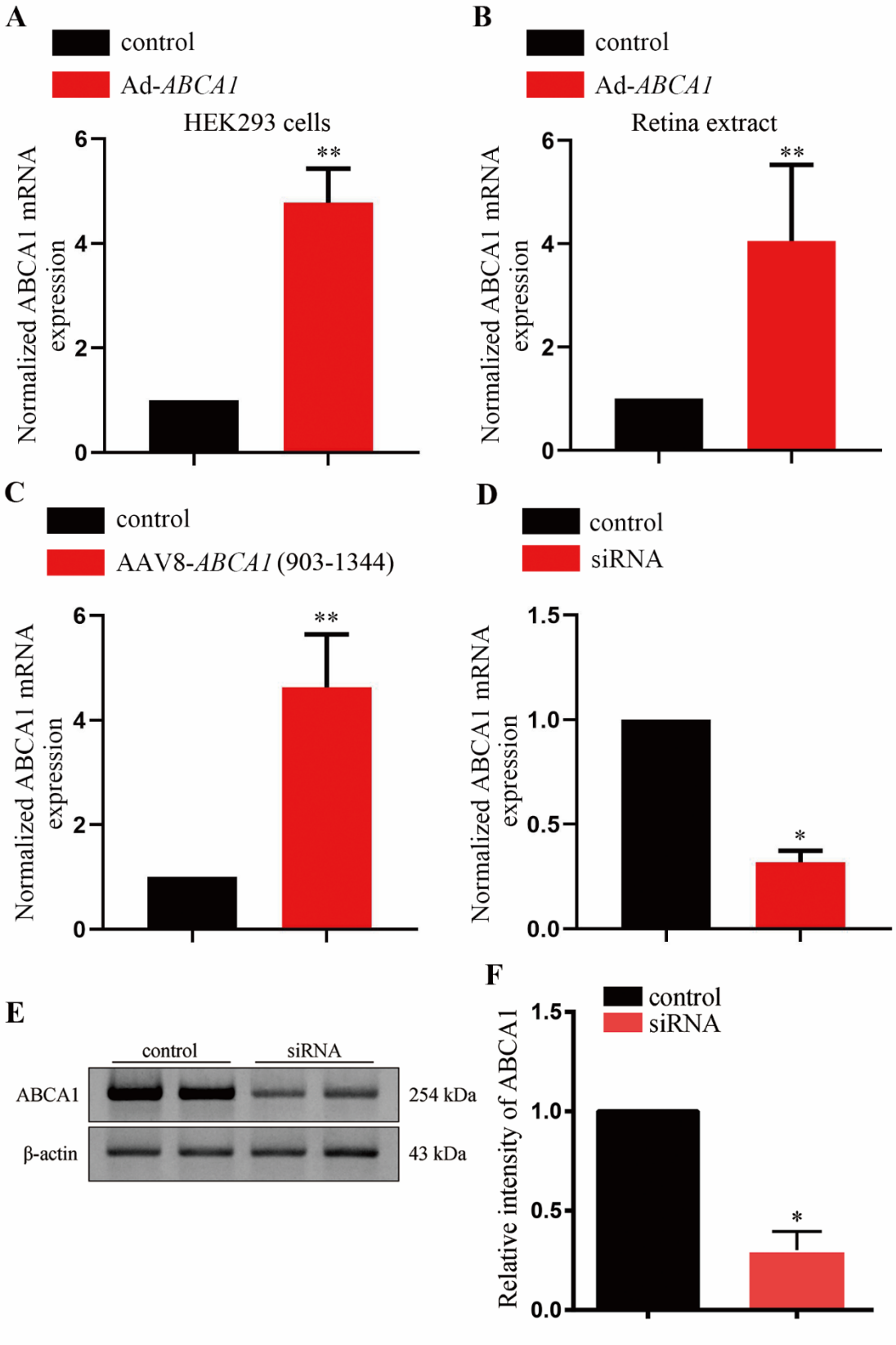
Supplementary figure 1



Supplementary figure 1 The effect of AAV8-ABCA1 on retina degeneration and RGC loss after IR. (A) Statistical analysis of SD-OCT results for mice retinal thickness.

The data are expressed as mean ±S.E.M. (n = 8 retinas per group). *P < 0.05 compared with control retina, one-way ANOVA followed by Tukey's post hoc test. (B) Statistical analysis of RGC number. The data are expressed as mean ±S.E.M. (n = 8 retinas per group). *P < 0.05 compared with control retina, one-way ANOVA followed by Tukey's post hoc test. (C) Statistical analysis of SD-OCT results for mice retinal thickness. The data are expressed as mean ±S.E.M. (n = 8 retinas per group), unpaired Student's t-test vs IR group. (D) Experimental scheme of AAV8-ABCA1 administration and mice IR model. (E) Statistical analysis of SD-OCT results for mice retinal thickness. The data are expressed as mean ±S.E.M. (n = 8 retinas per group), unpaired Student's t-test vs IR group. (F) Statistical analysis of RGC number. The data are expressed as mean ±S.E.M. (n = 8 retinas per group), unpaired Student's t-test vs IR group.

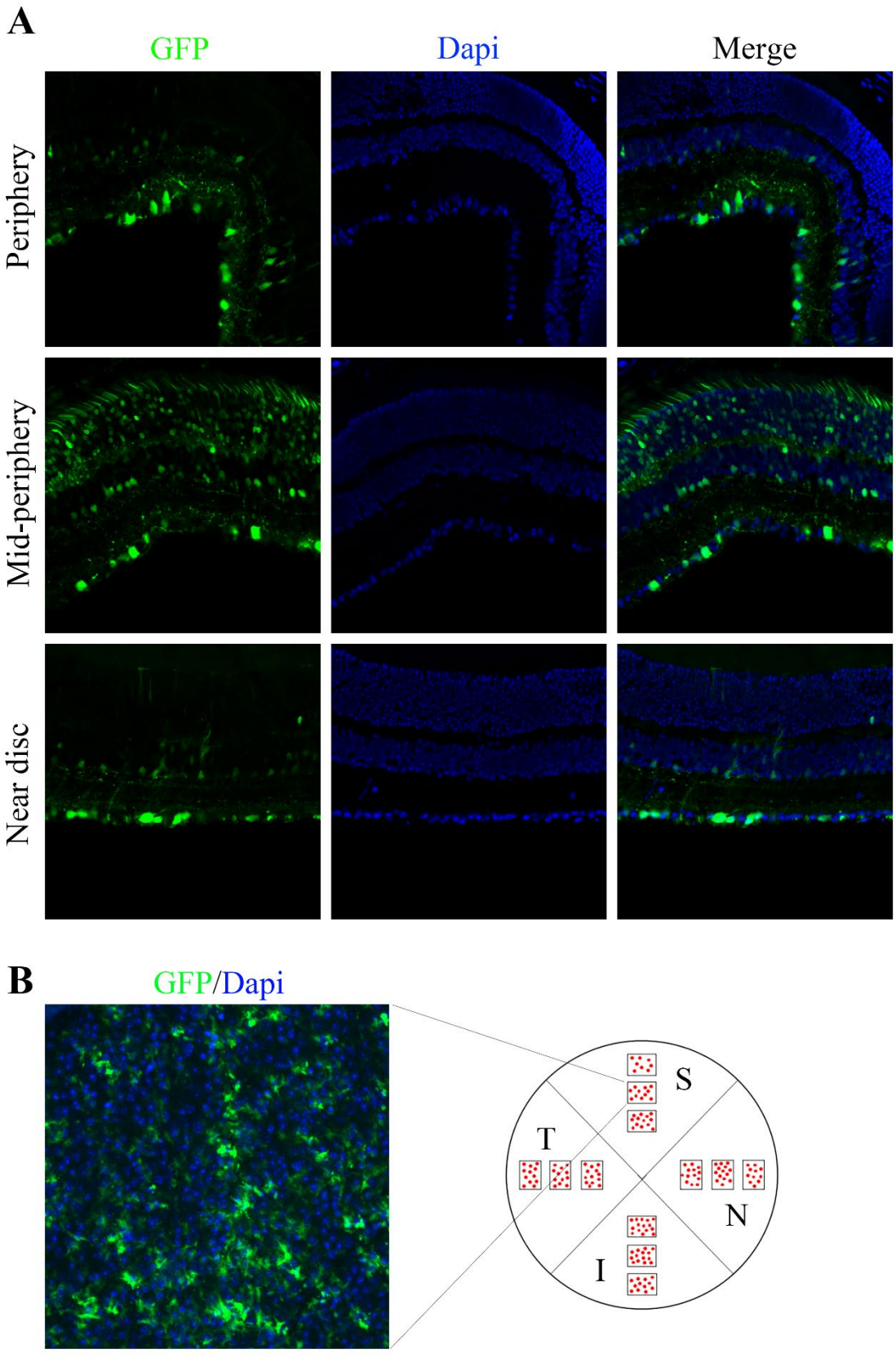
Supplementary figure 2



Supplementary figure 2 ABCA1 semiquantitative analysis. (A) qPCR results for ABCA1 mRNA expression from HEK293 cells after Ad-ABCA1 infection. The data of

three independent experiments expressed as the means ± S.D. **P < 0.01, unpaired Student's t-test vs control. (B) qPCR results for ABCA1 mRNA expression from retina extract after Ad-ABCA1 injection. The data of three independent experiments expressed as the means ± S.D. **P < 0.01, unpaired Student's t-test vs control. (C) qPCR results for ABCA1 mRNA expression from retina extract after AAV8-ABCA1 (903-1344) injection. The data of three independent experiments expressed as the means ± S.D. **P < 0.01, unpaired Student's t-test vs control. (D) Statistical analysis of ABCA1 mRNA expression in HEK293 cells. The data of three independent experiments expressed as the means ± S.D. *P < 0.05, unpaired Student's t-test vs control. (E) Western blot analysis showing the expression of ABCA1 after ABCA1 siRNA treatment. (F) Statistical analysis of (E), *P < 0.05, compared with control, unpaired Student's t-test vs control.

Supplementary figure 3



Supplementary figure 3 The representative images for Ad-ABCA1 infection. (A)

Representative immunofluorescence images of GFP expression on c-section retina slice.

(B) Representative immunofluorescence images of GFP expression on flat mount of retina.

Supplemental Methods

Quantitative RT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen), and 1 μg RNA was reverse transcribed. qRT-PCR was performed on an ABI StepOne Plus using SYBR Green ® Premix Ex Taq (Takara, Tokyo, Japan). The qPCR data were shown as relative mRNA expression versus control group. The results showed the fold-change (2-delta /delta Ct) of relative expression versus control group (-delta /delta Ct = -{sample (CT gene-CT actin)- con(CT gene-CT actin)}). For mRNA, the gene expression was normalization to β-actin.

Sequences of primers for qPCR:

Human Actin forward: TGGCACCCAGCACAATGAA

Human Actin reverse: CTAAGTCATAGTCCGCCTAGAAGCA

Human ABCA1 forward: GCTGGTGTGGACCCTTACTC

Human ABCA1 reverse: GCAGCTTCATATGGCAGCAC

Human ABCA1 (fragment 903-1344) forward: GCTGGTGTGGACCCTTACTC

Human ABCA1 (fragment 903-1344) reverse: GCAGCTTCATATGGCAGCAC

SD-OCT analysis

The OCT scanner has optical axial resolution of 7 μm, digital resolution of 3.5 μm, scan depth of 1.8 mm, and scan rate of 40 kHz. All animals were measured rapidly following anesthesia and pupillary dilation. We applied lubricating eye drops over the mouse eyes and covered them with custom-made contact lenses to prevent ocular surface issues. For imaging, animals were placed on an adjustable platform, and the camera was aligned perpendicular to the animal directly in front of and very close to the eye using a three-dimensional micromanipulator. Once the optic disc was centered and in focus using infrared imaging, we performed the circular scan (scan angle 12°) using the enhanced depth imaging (EDI) and high-resolution mode, with each B-scan consisting of 1536 A-scans centered around the optic disc. We averaged 16 frames per B-scan. We also performed posterior pole scans (scan angle 30°×25°) using EDI in high-speed mode, with each two-dimensional B scan consisting of 768

A-scans, average 9 frames per B-scan; and 25 line scans (scan angle 25°×15°) in high resolution mode, average 16 frames per B-scan. Only images with adequate signal strength index were saved and used for analysis. All OCT scans were performed by one investigator to maximize consistency, and the best image from each eye was selected for segmentation.

Viral vectors transduction in vitro

The HEK293 cells were used to transfected with Ad-ABCA1. The MOI for Ad vector was 50 genome copies (GC)/cell, incubated the virus at 37°C for 4 h, then replaced the medium with virus-free medium, and analyzed the results 48 h post-infection with ABCA1 qPCR detection. Human ABCA1 siRNA obtained from Thermo Fisher (Assay ID 107728, Catalog #AM16708, Shanghai, China). The siRNA transfection protocol was according to transfection reagent manufacturer's instructions. Briefly, in the 24-well plate, 10000-15000 HEK293 cells per well were cultured in 0.5 ml of complete growth medium 24 h prior to transfection. 40 ul of serum free medium, 4.5 ul of transfection reagent and 30 nM of siRNA were used as transfection complexes. Then we incubated the HEK293 cells with transfection complexes at 37°C for 48 h and analyzed the results with ABCA1 qPCR detection.