

# PNAS

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Supplementary Information for

Chronic Dicer1 deficiency promotes atrophic and neovascular outer retinal pathologies in mice

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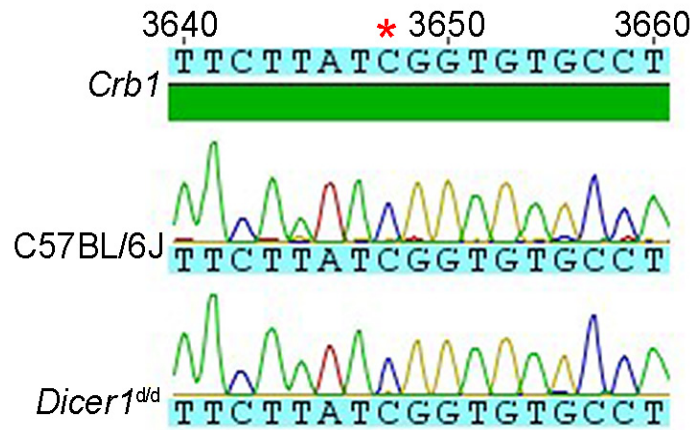
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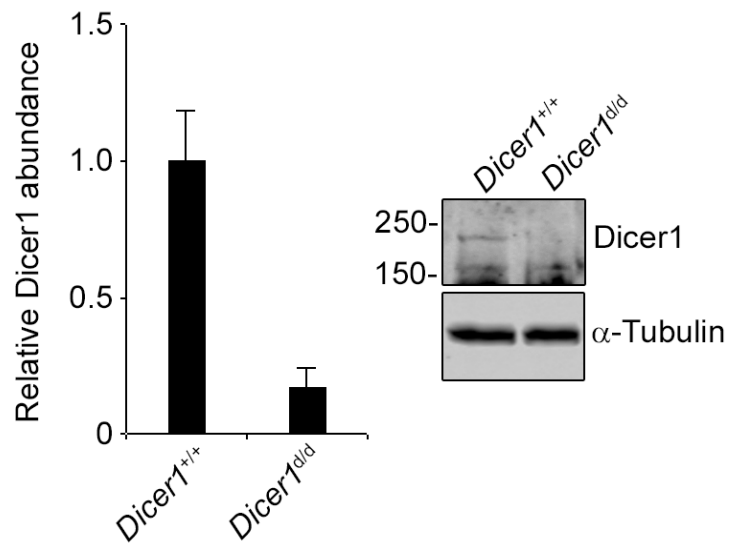
Figures S1 to S9

Fig. S1



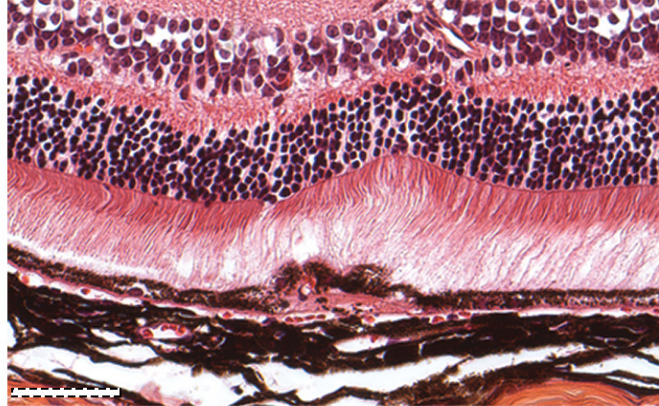
**Fig. S1.** Sequencing that of *Crb1* in *Dicer1<sup>dd</sup>* confirming the absence of rd8 mutation. rd8 arises due to a deletion of cytosine 3647 (asterisk).

Fig. S2

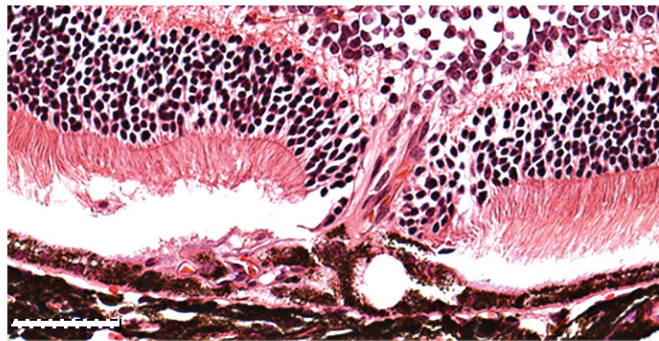


**Fig. S2.** Quantitation of Dicer1 by quantitative densitometry (left) and representative immunoblot of protein (right) from retina of littermate wild type and *Dicer1*<sup>dl/d</sup> mice.

Fig. S3



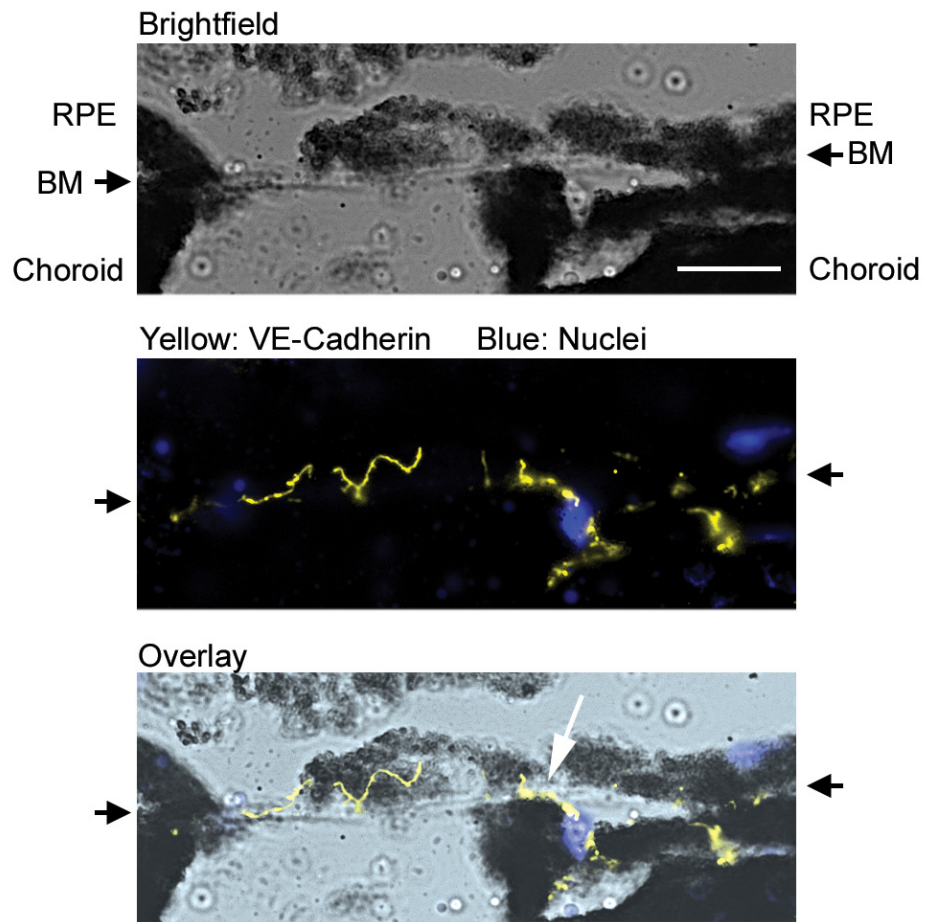
Type 1 sub-RPE lesion



Type 3 choroidal-retinal anastomosis

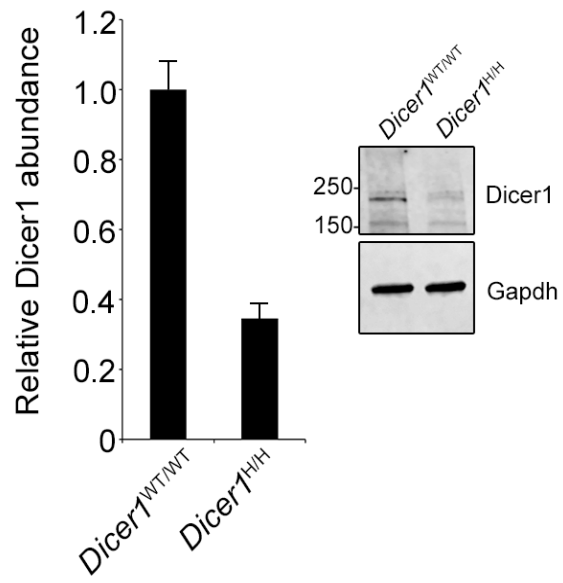
**Fig. S3.** High-resolution micrographs of hematoxylin and eosin stained retina from *Dicer1<sup>d/d</sup>* mice showing sub-RPE choroidal neovascularization (top) and chorioretinal anastomosis (bottom). Scale bar = 50  $\mu$ m.

Fig. S4



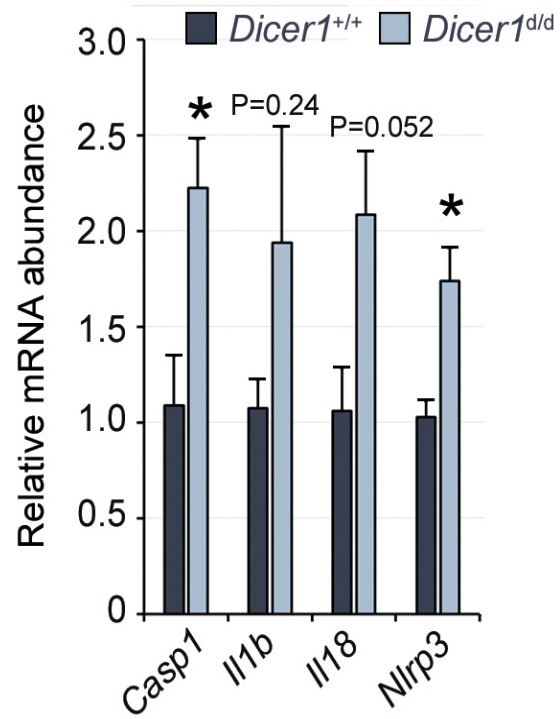
**Fig. S4.** High-resolution bright field and fluorescent micrographs of choroidal vessels traversing Bruch's membrane (BM) in *Dicer1<sup>dd</sup>* mice showing sub-RPE choroidal neovascularization (top) and chorioretinal anastomosis (bottom). White arrow denotes VE-Cadherin-positive endothelial cell crossing BM. Nuclei were labeled with DAPI.

Fig. S5



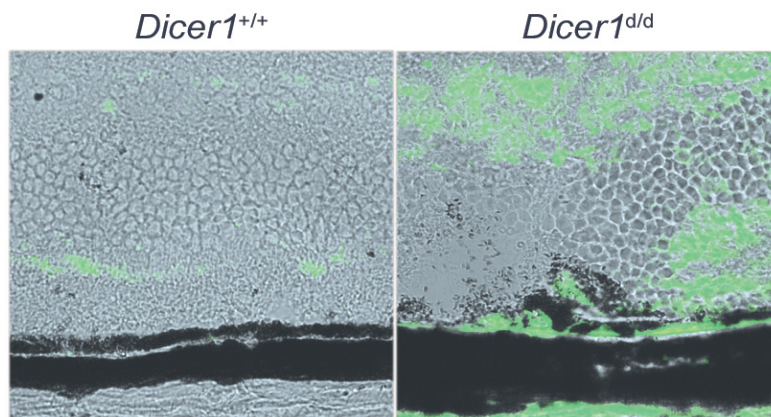
**Fig. S5.** Representative immunoblot and densitometry quantification of Dicer1 abundance in retina from *Dicer1*<sup>H/H</sup> relative to wild type littermate control mice.

Fig. S6



**Fig. S6.** Quantitative RT-PCR of cDNA from whole retinas of 15-month old *Dicer1*<sup>d/d</sup> and littermate control. N=3-4, \*P<0.05.

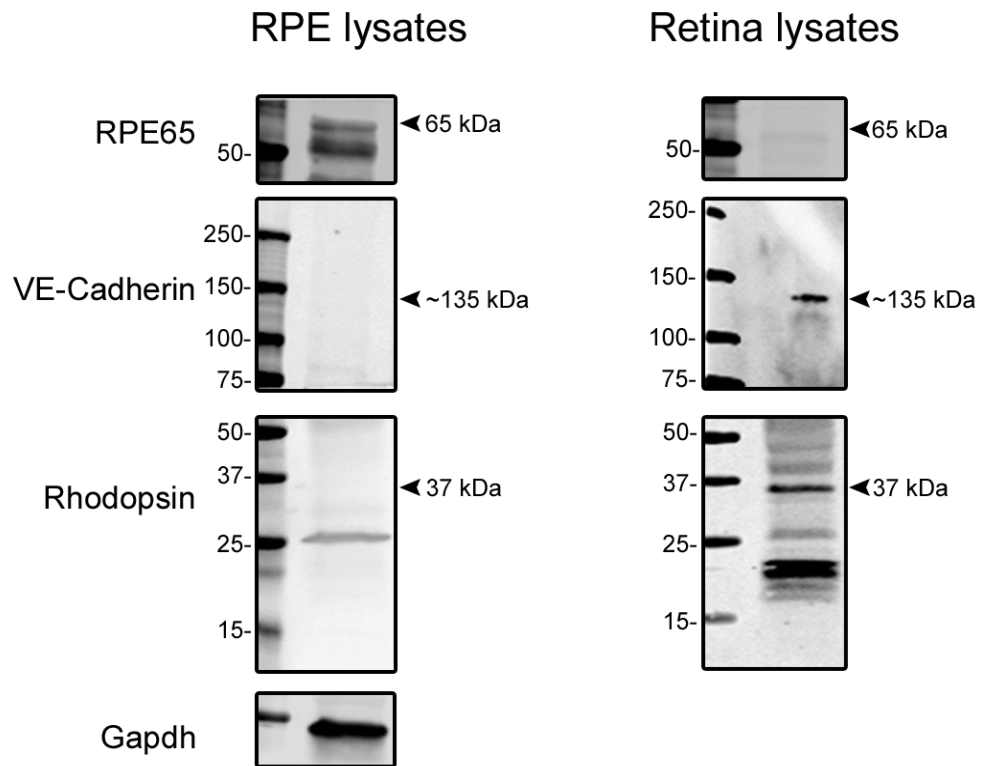
Fig. S7



**Fig. S7.** *In situ* fluorescent labeling caspase-1 activity in unfixed retinal cryo-sections of 10-month-old wild type and *Dicer1*<sup>d/d</sup> mice. Green fluorescent signal arises from a caspase-1 peptide substrate that becomes fluorescent upon cleavage. Signal was observed in the neovascular lesions.

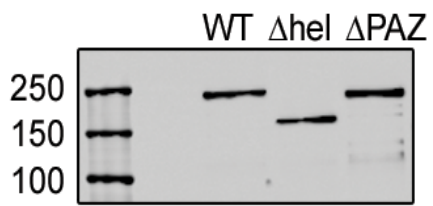


Fig. S8



**Fig. S8.** Immunoblotting to assess purity of RPE and retina lysates. RPE lysates isolated from wild-type mice are enriched for RPE65, and lack detectable rhodopsin and VE-Cadherin compared to retinal lysates.

Fig. S9



**Fig. S9.** Immunoblotting of purified DICER1 constructs expressed in HEK293T cells after transient transfection.