Supporting Information

Meira *et al.* 10.1073/pnas.0807030106

SI Text

AP Site Determination. Six- to 8-week-old mice were injected with MMS (75 mg/kg), and their retinas were dissected at numerous time points after treatment (0, 3, 6, 18, and 24 h) in PBS containing deaminase inhibitors (5 μg/mL coformycin, 0.66 μg/mL desferrioxamine, and 50 μg/mL tetrahydrouridine). The retinas were lysed overnight at 4 °C with DNA lysis buffer (Qiagen). DNA was extracted with 2 phenol/chloroform/isoamyl alcohol extractions (Invitrogen, 1 chloroform/isoamyl alcohol extraction, and 1 ethanol precipitation. AP sites were determined by using the DNA Damage Quantification Kit (Dojindo) and performed according to the manufacturer's instructions. The AP sites were normalized to the concentration of ARP-DNA as quantitated by spectrophotometric measurement of the absorbance at A260.

TUNEL Assay. One-week-old wild-type and $Aag^{-/-}$ neonates were injected i.p. with MMS (75 mg/kg). Forty-eight hours later, the eyes were harvested and fixed overnight in Bouin's fixative. Five-micrometer unstained sections were placed on slides, which were deparaffinized and rehydrated as described in *Methods*. The TUNEL assay was performed with a APO-BrdU TUNEL assay kit (Molecular Probes/Invitrogen), following the manufacturer's instructions. All staining was performed in humidified chambers. As an alternative to staining with the propidium iodide provided in the kit, the slides were counterstained with DAPI (1 μg/mL). Slides were examined as described in *Methods*.

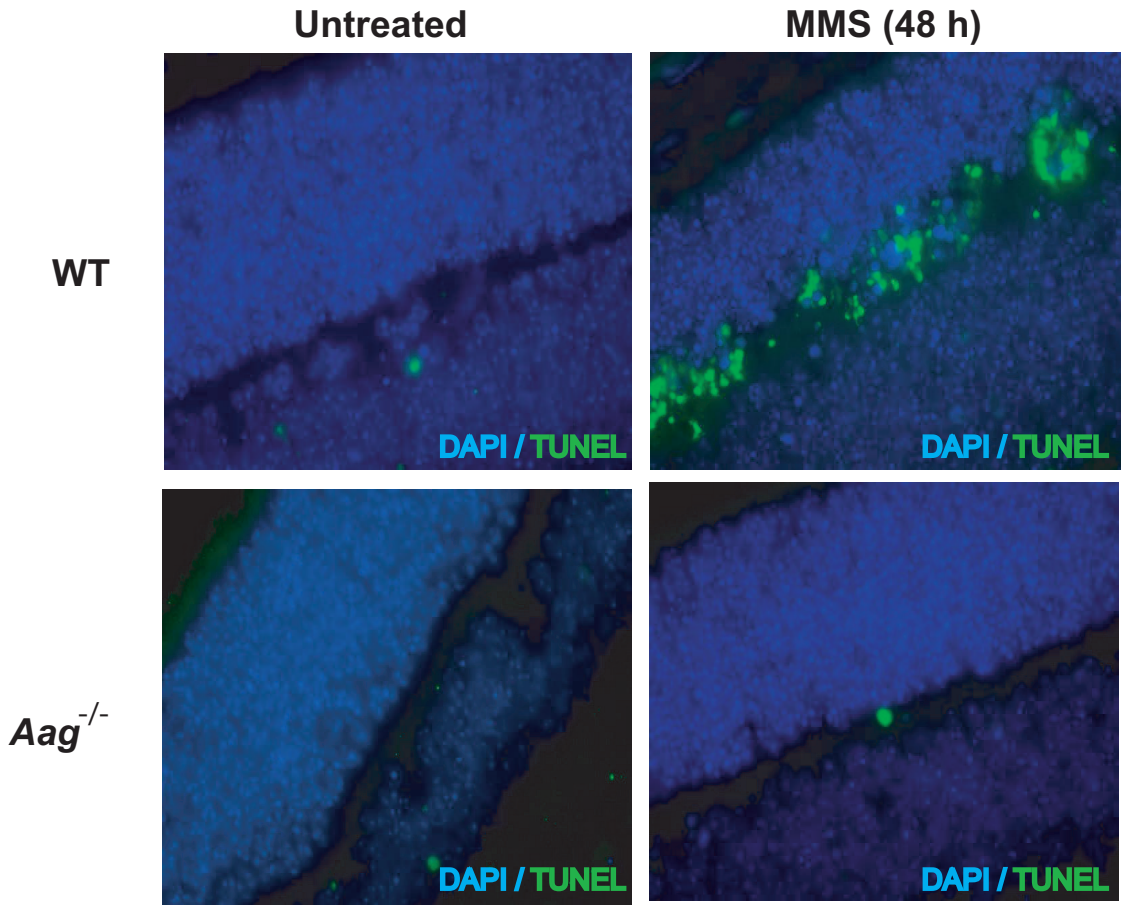

Fig. S1. Wild-type mice exhibit positive TUNEL staining in the outer nuclear layer of retina after MMS treatment. Seven-day-old wild-type (*Upper*) and *Aag*$^{-/-}$ (*Lower*) mice were either untreated (*Left*) or injected with 75 mg/kg MMS or PBS (*Right*), and their eyes were harvested 48 h after treatment. TUNEL staining and DAPI counterstaining were performed, and immunofluorescence images were captured. (Magnification: 600×).

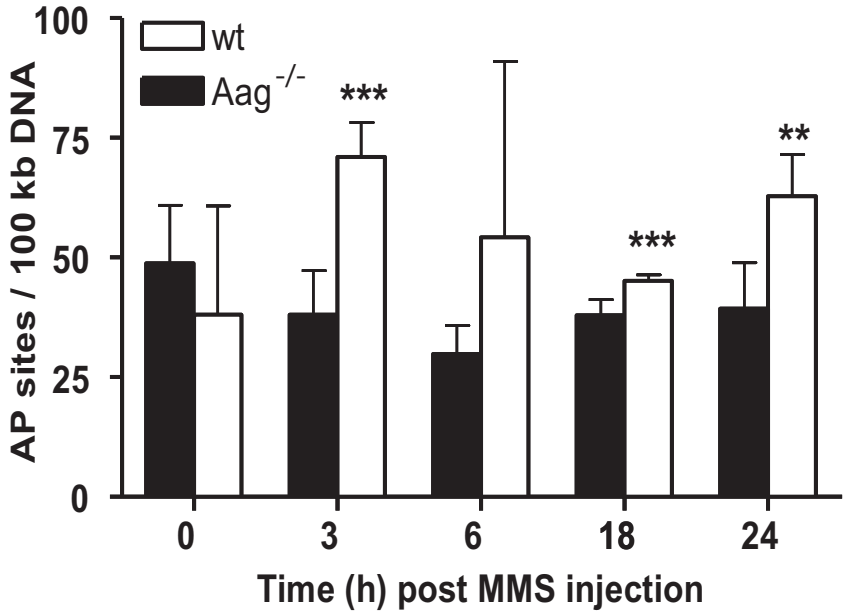

Fig. S2. Wild-type mice show significantly more AP sites after MMS treatment. Six- to 8-week-old mice ($n = 2$ per genotype) were treated with 75 mg/kg MMS, and retinal DNA was isolated at the indicated time points. AP sites were measured and normalized to total ARP DNA concentrations. Data are presented as mean ± SD. **, $P < 0.01$; ***, $P < 0.001$.

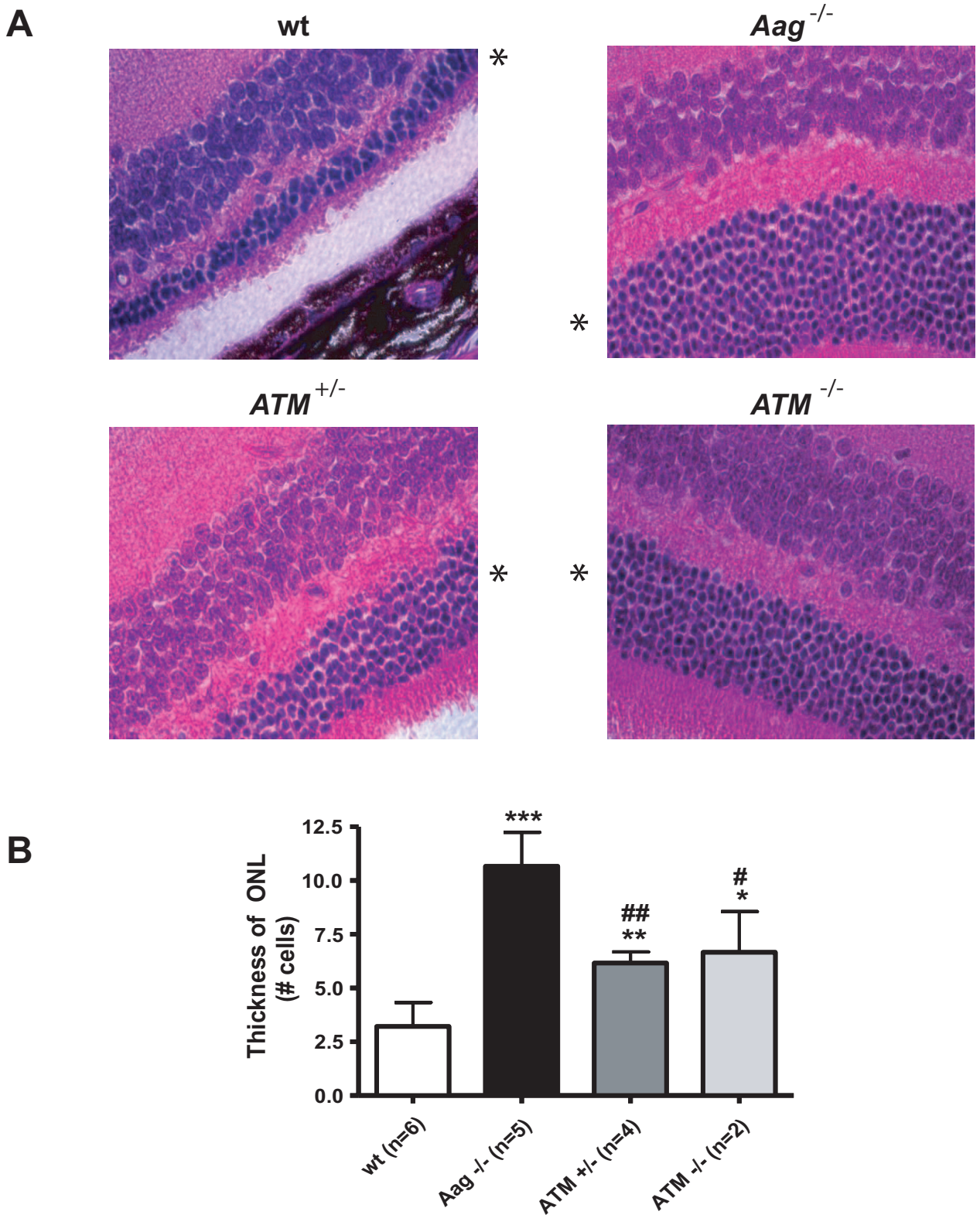

Fig. S3. ATM-deficient animals demonstrate partial rescue against MMS-induced retinal degeneration. (*A*) Six- to 8-week-old mice were injected with 75 mg/kg MMS, and their eyes were harvested 7 days later. Asterisks indicate the outer nuclear layer. (*B*) Determination of the thickness of the outer nuclear layer was performed by counting 3 representative areas of the retinas of treated wild-type ($n = 6$), $Aag^{-/-}$ ($n = 5$), $Atm^{+/-}$ ($n = 4$), and $Atm^{-/-}$ ($n = 2$) mice. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, #, $P < 0.05$; ##, $P < 0.01$, where *'s indicate significance compared with wild type, and #'s indicate significance compared with $Aag^{-/-}$ mice. Data are presented as mean ± SD.