Myeloid HDAC3 deletion ameliorates neurovascular degeneration in a mouse model of retinal ischemia-reperfusion injury

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Background and Rationale

► Retinal ischemia injury is involved in many potentially blinding pathological diseases such as diabetic retinopathy, retinal artery or vein occlusion, and retinopathy of prematurity.

► To date, there is no clinically effective treatment that can preserve the retinal neurons and vasculature.

► Retinal neurovascular injury involves myeloid cell (microglia / macrophages (MΦ)) activation/infiltration.

► Histone deacetylases (HDACs) are a class of enzymes that regulate gene expression and protein function. The isoform HDAC3 is a class I histone deacetylase that play a central role in macrophage inflammatory response.

► The role of myeloid HDAC3 in retinal ischemia and macrophage efferocytic (phagocytic) function remains unknown.

► The main aims of this study are:

1) Determine the role of myeloid HDAC3 in neurovascular degeneration and immune cell response after retinal IR injury.

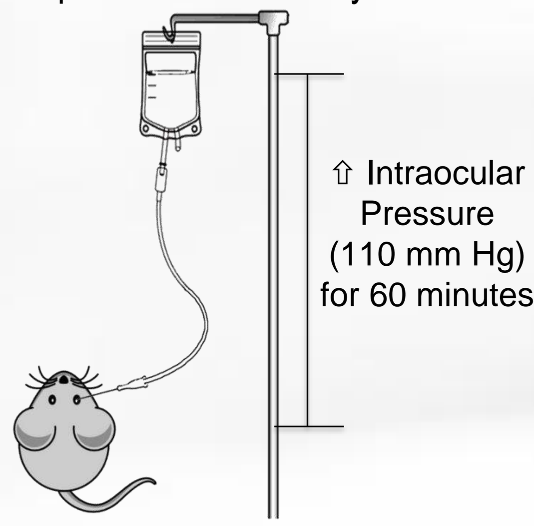
2) Test the previously unexplored role of HDAC3 in efferocytosis, a process by which myeloid cells engulf and clear dead cells and hence facilitate tissue repair.

3) Exploring the underling mechanism by which HDAC3 modulate efferocytosis in macrophages following injury.

METHODS

We generated a myeloid specific HDAC3 KO mouse under the LysM cre promoter to investigate the potential role of myeloid HDAC3 in retinal IR injury.

► Retinal IR injury model is achieved under anesthesia through raising the eye intraocular pressure (IOP) to 110 mm Hg for 1 h via cannulating the anterior chamber with a needle attached to a line infusing sterile saline.

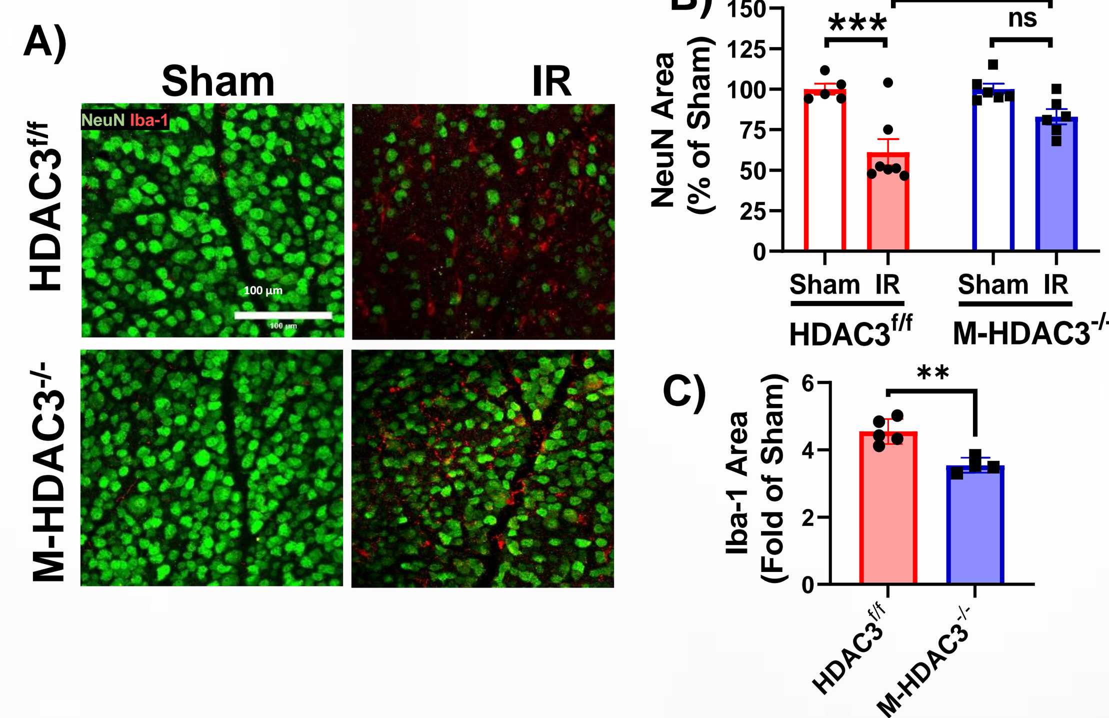


► Following IR injury, we analyzed the retinal neurodegeneration, morphology, inflammation, efferocytosis and microvascular degeneration in floxed control and myeloid HDAC3 KO mice using TUNEL assay, immunohistochemistry, optical coherence tomography (OCT), and flow cytometer.

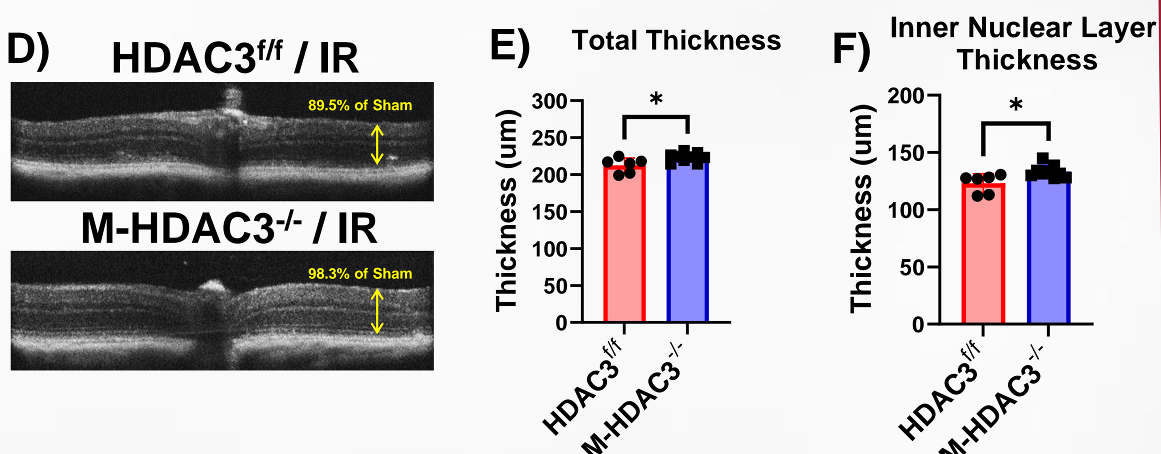
► In vitro efferocytosis assays: Isolated macrophages from HDAC3 KO, Arginase 1 (A1) KO and control mice were treated with or without the A1 inhibitor (ABH). The macrophages were incubated with labeled or unlabeled apoptotic or non-apoptotic cells for 40 minutes then we performed Western blot, RT-PCR and confocal imaging.

RESULTS

1) Myeloid HDAC3 deletion is neurovascular protective in retinal IR injury.

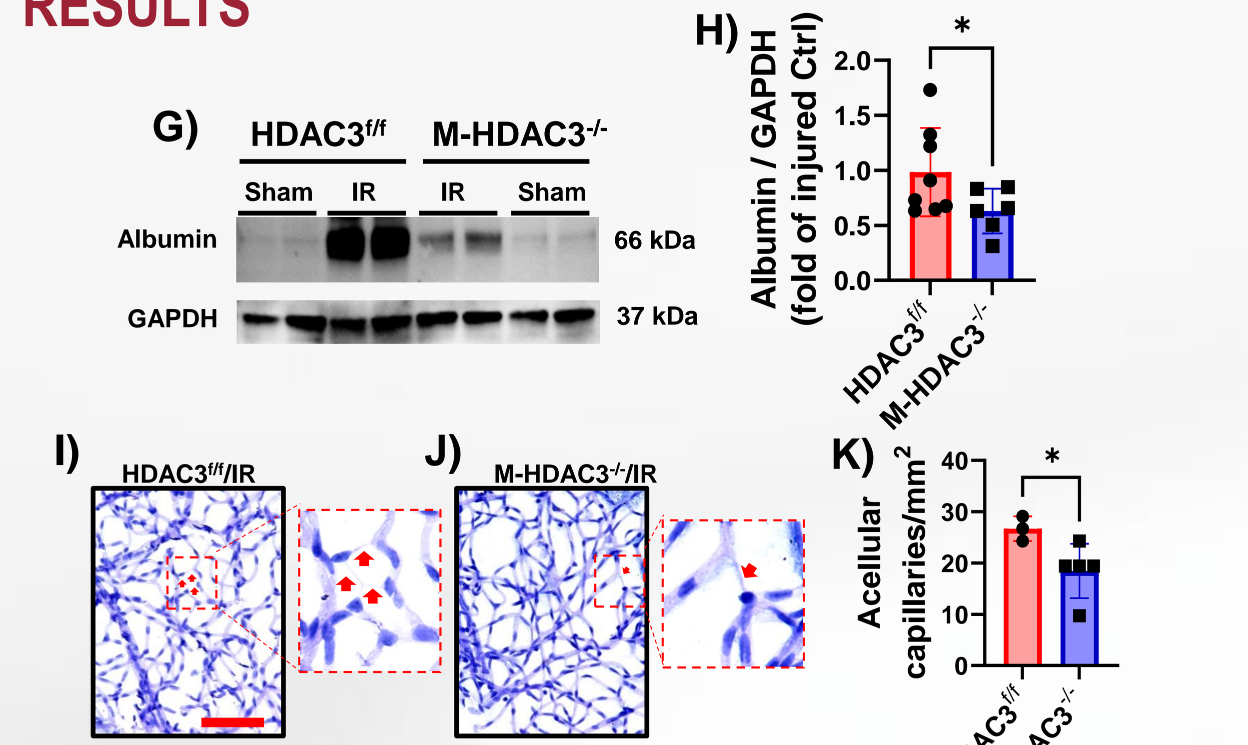


A-C) Immunolabeling at 7 days after injury and quantification showed reduced neurodegeneration (marked by the neuronal marker, NeuN) and microglia/MΦ numbers (marked by Iba1) in the M-HDAC3 KO retinas as compared to WT. *p<0.05; **p<0.01; ***p<0.005



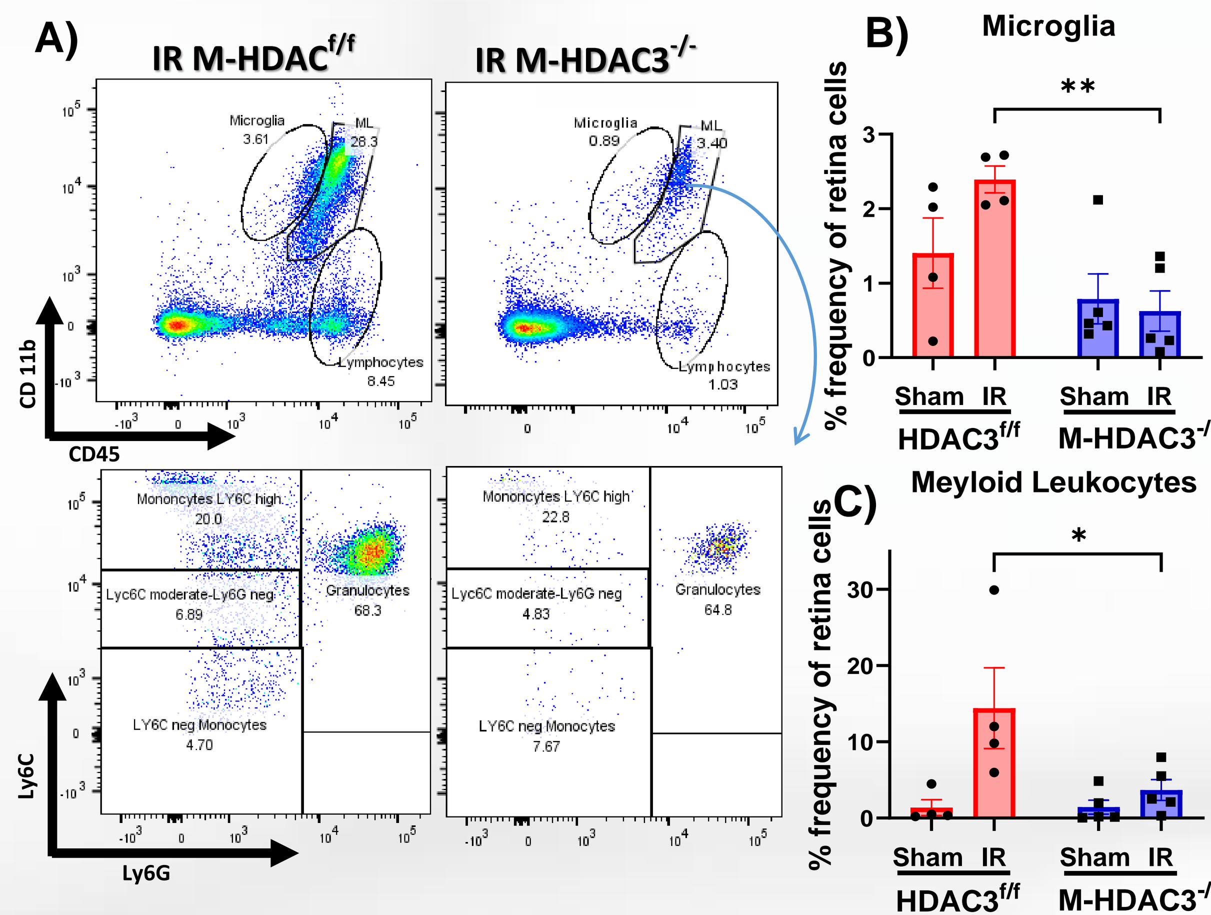
D-F) Optical coherence tomography (OCT) in live mice at 7 days after IR show M-HDAC3 deletion preserves the internal retinal layer (INL) and total thickness. *p<0.05

RESULTS



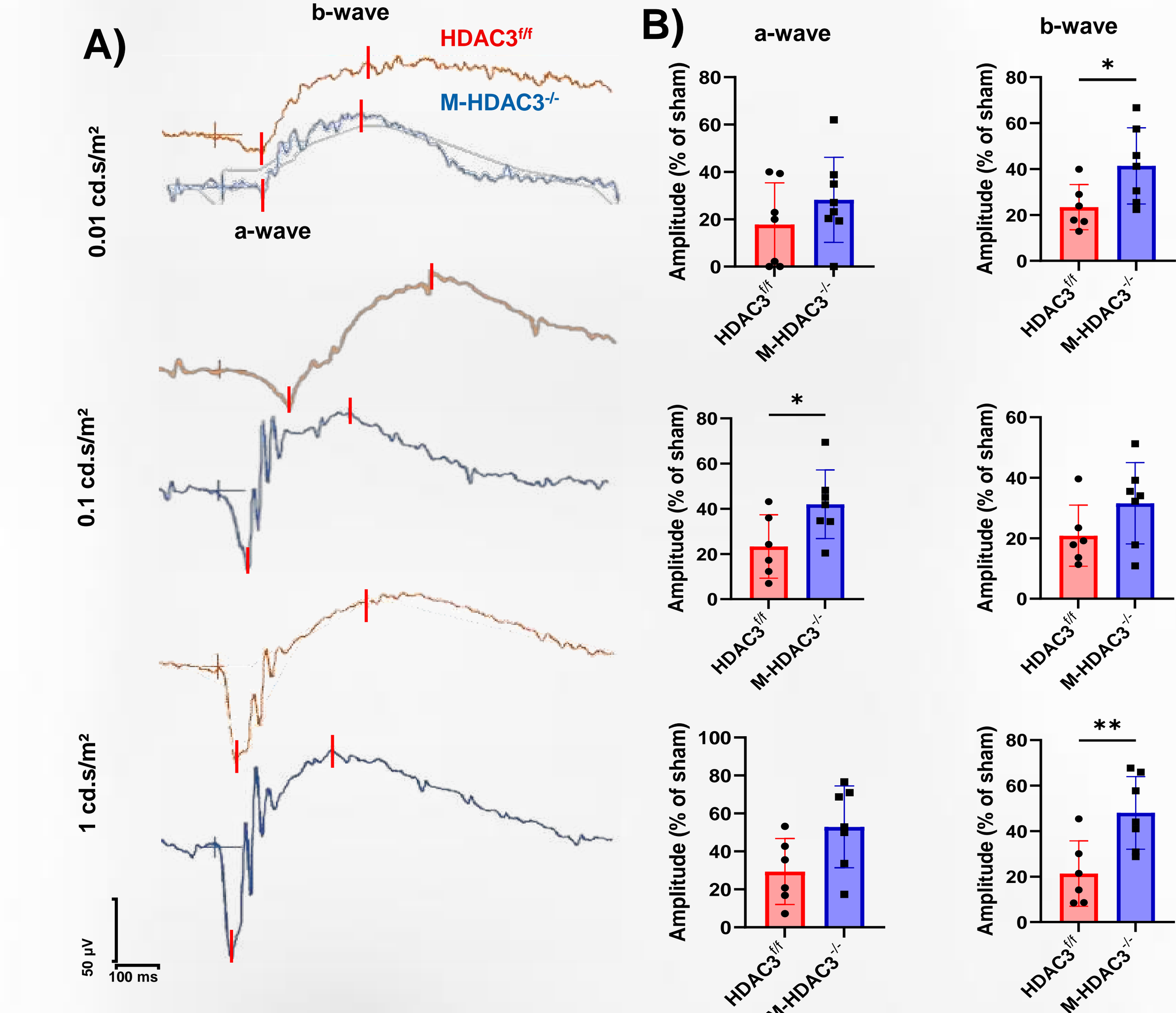
G, H) albumin extravasation at day 2 post-IR was measured in perfused retinas. I-K) Representative images from vascular digests at 14 days post-IR and quantification of the acellular capillaries (enlarged inset, red arrows) *p<0.05

2) M-HDAC3 deletion mitigates the IR-induced microglia proliferation and myeloid cell infiltration into the retina.



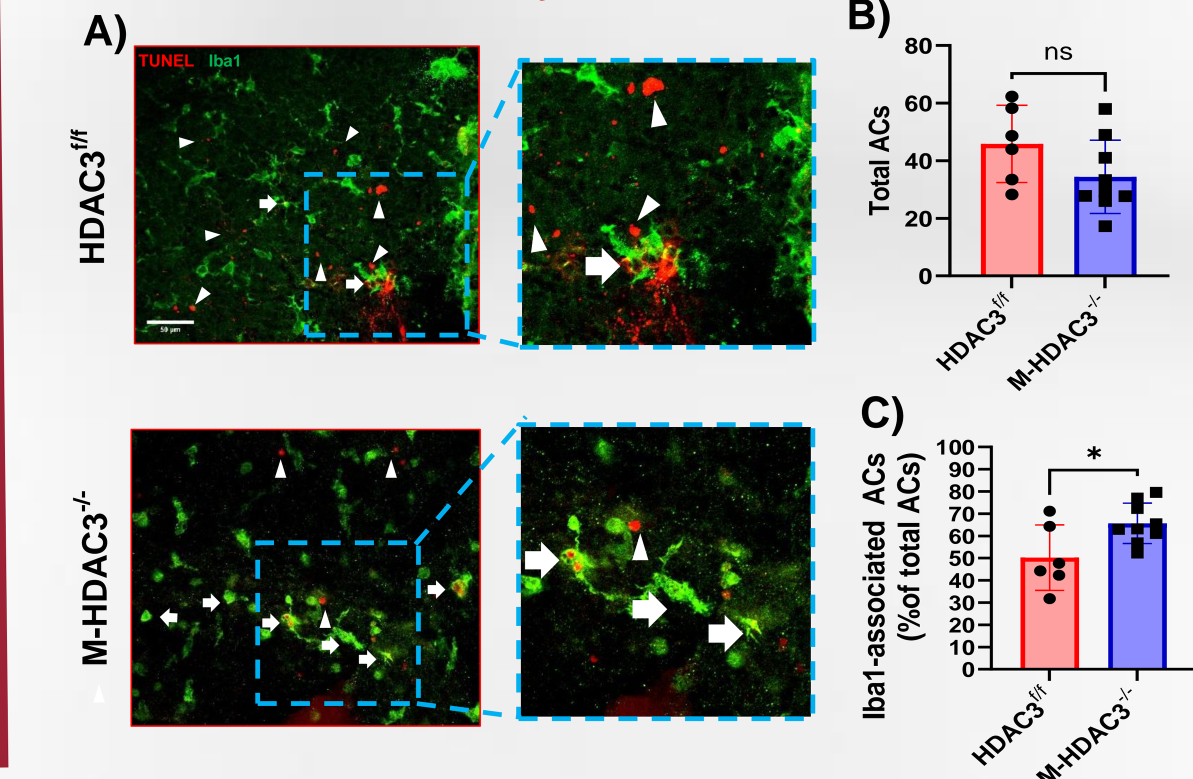
A) Representative scatter-graphs showing the flow-cytometric analysis used to quantify immune cell populations in the retina at day 2 post-IR injury. B) CD11b⁺/CD45^{low} (principally microglia), CD11b⁺/CD45^{hi} myeloid leukocytes (ML), and CD11b^{neg}/CD45^{hi} lymphocytes. C) Myeloid leukocytes were gated into Ly6C^{hi}/Ly6G^{neg} monocytes, Ly6C^{moderate}/Ly6G^{neg} Monocytes, Ly6C^{neg}/Ly6G^{neg} monocytes, and Ly6C⁺/Ly6G⁺ granulocytes. . *p<0.05; **p<0.01

3) Myeloid HDAC3 deletion improves retinal ERG after IR



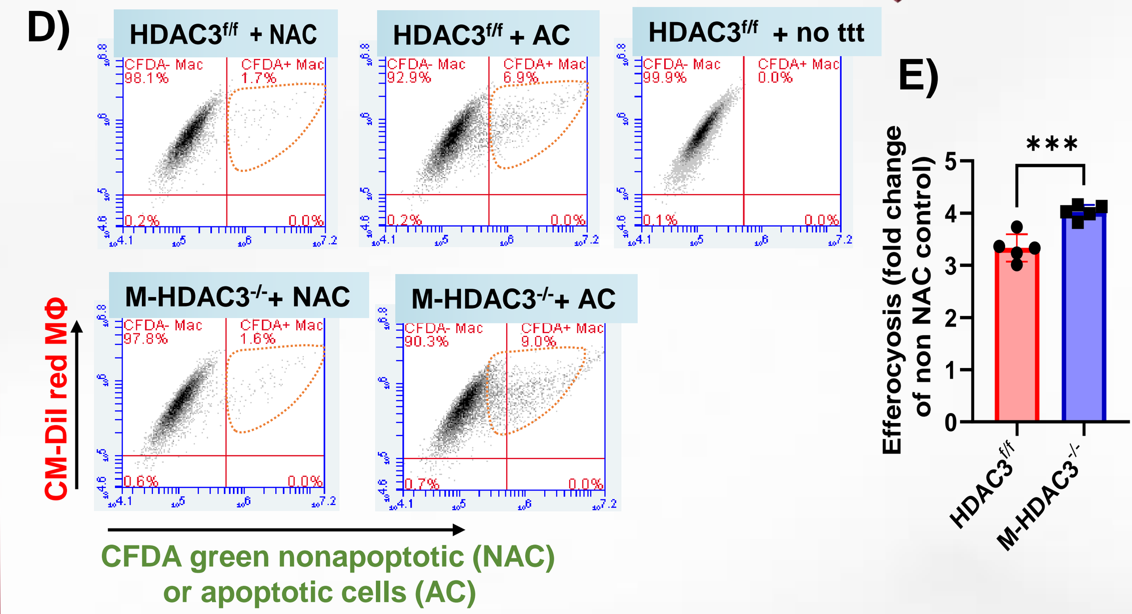
A) Representative waveforms of electroretinography (ERG) at day 14 after IR at various light intestines. B) Quantification and comparison of amplitudes show improved b-wave response in M-HDAC3⁻/⁻ retinas as compared to HDAC3$^{f/f}$ at 0.01 and 1 cd.s/m². *p<0.05; **p<0.01

4) M-HDAC3 deletion improves the macrophages' apoptotic cell clearance and efferocytosis



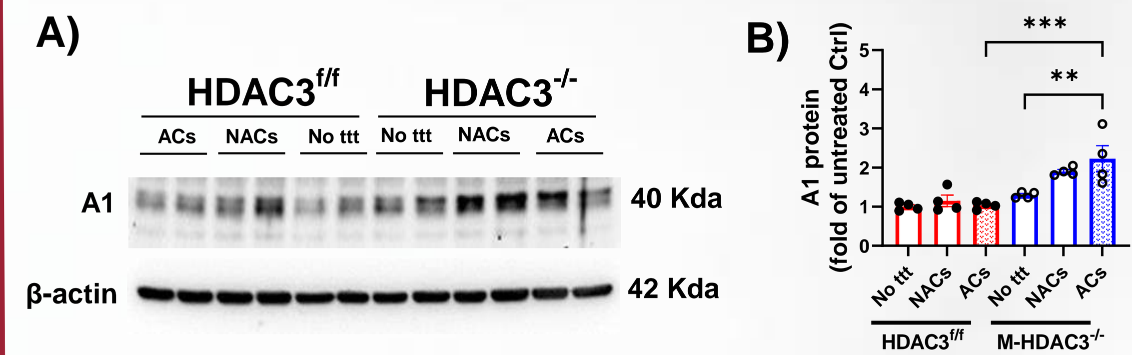
A) Representative confocal images of TUNEL (red) and Iba-1 (green) immunostaining 2d post IR injury. Arrows and arrowheads depict macrophage (MΦ)-associated and free TUNEL+ cells, respectively. B) Quantification of total apoptotic cells (ACs)TUNEL+ cells).

RESULTS

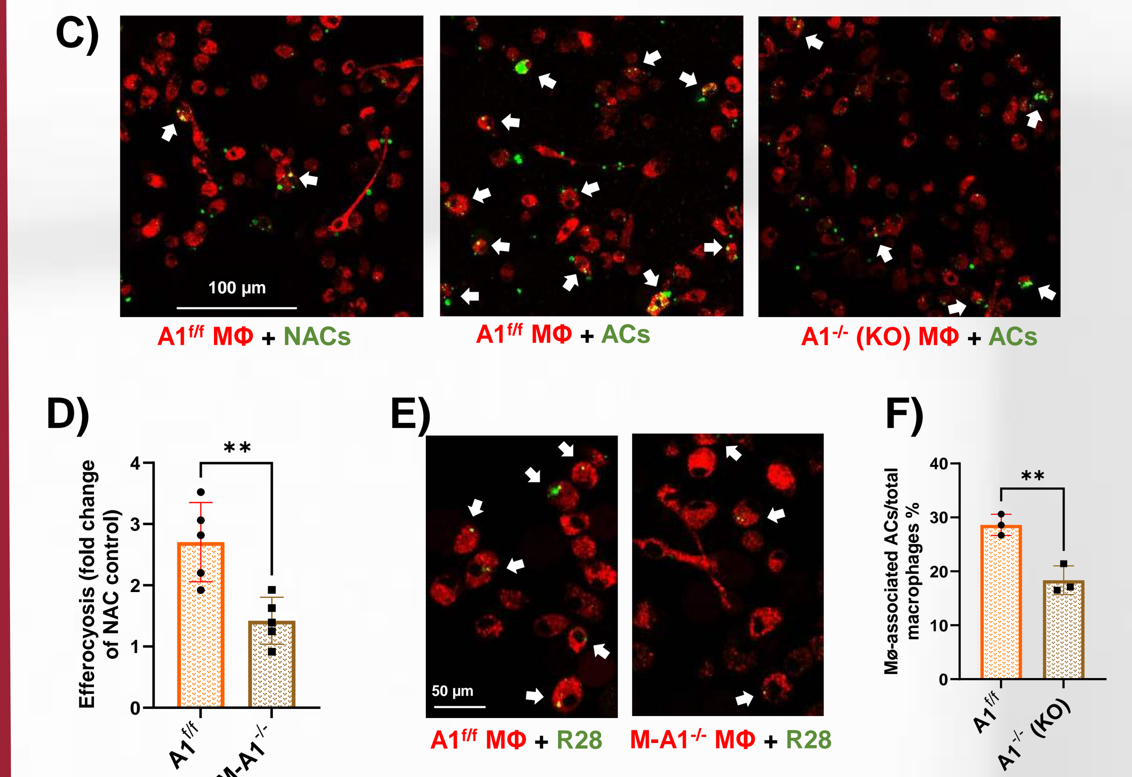


D-E) Representative fluorescence-activated cell sorting (FACS) plots of efferocytosis coculture experiments after non-apoptotic (NAC) or apoptotic (AC) CFDA green-labeled K-562 cells were added to Dil red-labeled MΦ. E) Quantification of MΦ engaged in efferocytosis of apoptotic AP (Dil+/CFDA+) detected by FACS .*p<0.05, .**p<0.01, .***p<0.005

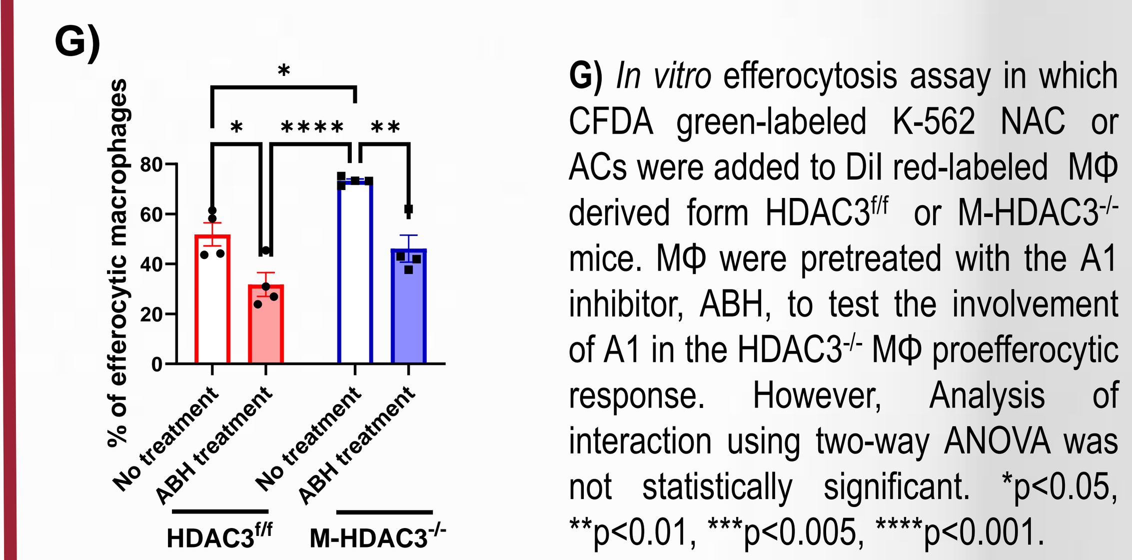
5) Upregulation of arginase 1 (A1) in HDAC3⁻/⁻ MΦ and its deletion inhibits efferocytosis in vitro.



A) Western blot and (B) analysis of arginase 1 (A1) expression in HDAC3$^{f/f}$ and M-HDAC3⁻/⁻ macrophages (MΦ) after incubation without (no ttt) or with K-562 non-apoptotic (NACs), or apoptotic cells (ACs).



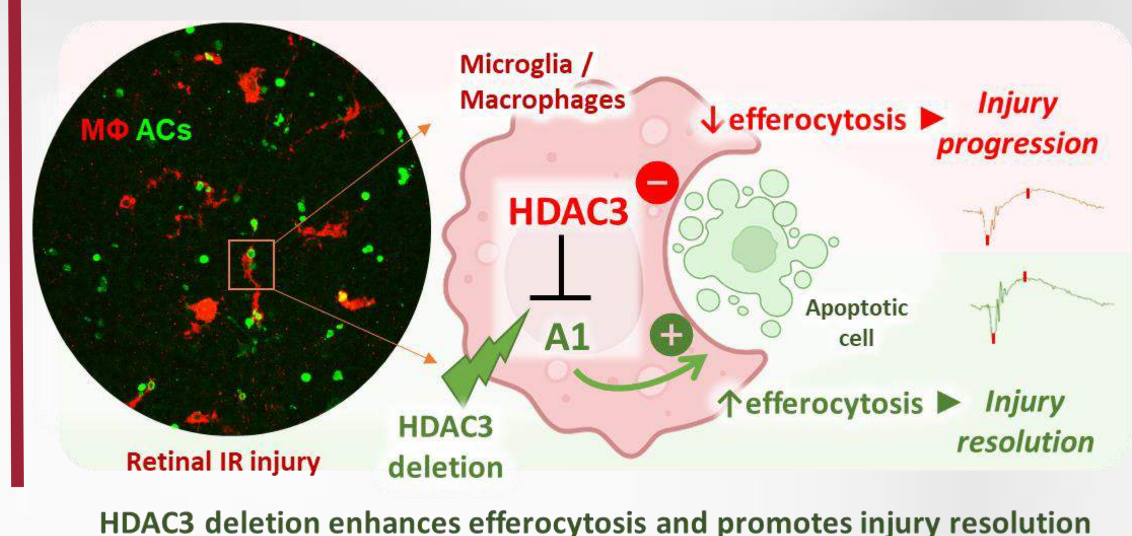
C) Representative images of in vitro efferocytosis assay where CFDA green-labeled K-562 NAC or ACs were added to CM-Dil red-labeled macrophages (MΦ) derived from A1f/f or A1-/- mice. D) Quantification of MΦ astrocytic capacity of the ACs presented as fold of NAC efferocytosis showed a reduction in efferocytosis due to A1 deletion. Similarly, the deletion of A1 reduced the efferocytosis of CFDA green-labeled-R28 (retinal neural cell line) ACs by MΦ (E, F). *p<0.05, **p<0.01.



G) *In vitro* efferocytosis assay in which CFDA green-labeled K-562 NAC or ACs were added to Dil red-labeled MΦ derived form HDAC3$^{f/f}$ or M-HDAC3⁻/⁻ mice. MΦ were pretreated with the A1 inhibitor, ABH, to test the involvement of A1 in the HDAC3⁻/⁻ MΦ proefferocytic response. However, Analysis of interaction using two-way ANOVA was not statistically significant. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001.

CONCLUSIONS

- Myeloid-specific deletion of HDAC3 protects against IR-induced neurodegeneration, microglia/MΦ infiltration, microvascular degeneration and blood retina barrier breakdown.
- Myeloid HDAC3 deletion improves retina function after IR as evidenced by retinal ERG.
- Myeloid-specific deletion of HDAC3 improves macrophages efferocytosis function in vivo and in vitro.
- HDAC3 deletion upregulates A1 expression, however, A1 does not appear to mediate the efferocytic response of MΦ lacking HDAC3.
- Inhibition of myeloid HDAC3 might serves as a new therapeutic strategy to limit retinal IR injury.



HDAC3 deletion enhances efferocytosis and promotes injury resolution

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