Review of: "Melanosomes degrade lipofuscin and precursors that are derived from photoreceptor membrane turnover in the retinal pigment epithelium—an explanation for the origin of the melanolipofuscin granule"

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The manuscript by Y. Lyu et al., entitled “Melanosomes degrade lipofuscin and precursors that are derived from photoreceptor membrane turnover in the retinal pigment epithelium – an explanation for the origin of melanolipofuscin granules”, describes an interesting study seemingly providing experimental evidence for in vivo removal of RPE lipofuscin in Abca4$^{-/-}$ mice. Considering that the accumulation of lipofuscin in human RPE is one of the most remarkable changes observed in association with AMD and Stargardt disease, the novel results presented in the reviewed manuscript are very important, if they are reproduced by independent study.

Although Lyu et al, proposed a hypothetical explanation of the observed phenomena, several issues need to be resolved and convincing physicochemical evidence must be obtained to validate any proposed mechanism of action of the drugs that release nitric oxide. The most relevant are the following:

1. According to the manuscript, removal of RPE lipofuscin requires melanin pigmentation of the cells. Did the authors only observe reduction of the lipofuscin component of melanolipofuscin granules or also reduction in the number of single lipofuscin granules?
2. If the key process leading to lipofuscin degradation requires melanin oxidation and formation of a reactive product by melanin interaction with NO and superoxide, the procedure used by the authors to deliver sin-1 or horseradish peroxidase into the ocular tissue, is likely be extremely inefficient. It is difficult to explain how sin-1 and even more so the enzyme will be able to diffuse from the subretinal area into RPE. Unfortunately, the formed by sin-1 peroxynitrite is not stable enough to justify its slow diffusion though the ocular tissues.
3. The authors suggest that superoxide anion was probably involved in the key chemical processes leading to oxidation of melanin. Although lipofuscin and melanin are known to photogenerate superoxide anion, their ability to generate this reactive oxygen species in the dark (no light experiments has been described in the manuscript), is extremely low.

The authors stated that superoxide anion alone was able to degrade A2E-bisretionoid, referring to a paper by Ueda et al., PNAS 2016 (Discussion). However, the cited paper demonstrated photooxidation of A2E, but no evidence was provided for the role of superoxide anion.