SI Materials and Methods

Human specimens

Eyes with histories of AMD (85.5 ± 8.17 years old; mean ± SD), age-matched controls with no history of AMD (79.5 ± 4.81), and young donors (43.7 ± 9.54) were obtained from National Disease Research Interchange (NDRI, Philadelphia, PA) (n = 6 eyes, each group). Ages of AMD and age-matched control donors were not significantly different (Student t-test). AMD donor eyes used in this study excluded neovascular AMD and geographic atrophy AMD. AMD eyes were confirmed to have signs of drusen in histopathological autopsy reports provided by NDRI. Similarly, age-matched control eyes were confirmed not to have drusen. Specimens were formalin fixed within 6 h postmortem. Each eye globe was placed in 120 ml of 10% buffered formalin for 24 to 48 h. The eyes were dissected and processed for paraffin infiltration using standard histology protocols. This involved subjecting the tissues fixed in formalin to graded ethanol, then graded xylenes and finally paraffin wax. All sections were placed on electrostatic slides suitable for immunohistochemistry and stored in the dark at 21°C. Procedures including initial handling eye globes and tissue sectioning were performed by NDRI and immunochemistry was performed in our laboratory. Procedures using human eye specimens were conducted in accordance with the Declaration of Helsinki. The use of human tissues in this study was granted exemption from the institutional review board of Massachusetts Eye and Ear Infirmary.

Animals

All procedures using mice were performed in strict accordance with the guidelines for Association for Research in Vision and Ophthalmology (ARVO). The experimental protocols were approved by the Animal Care Committee of Massachusetts Eye and Ear Infirmary. Adult male *Lamp2* KO mice (*Lamp2*$^{y/-}$), their WT littermates (*Lamp2*$^{y/+}$), or C57BL6J mice (Stock No. 000664, Jackson laboratory, Bar Harbor, ME) were used at the age of 2, 6, 12 months in this study. *Lamp2* KO mice, originally

generated by Prof. Saftig's lab, were provided from Shiga University for this study. *Lamp2* KO mice used in this study were backcrossed to C57BL6J mice for at least five generations in our laboratory. We confirmed that strains of *Lamp2* KO mice and their WT littermates do not carry the rd8 mutation by DNA sequencing. All tissue collections were performed at the same time of day (1:00 pm) due to the presence of circadian changes (1).

Fundus photography and autofluorescence in mice

For *in vivo* imaging, mice were anesthetized with an intraperitoneal injection of 50 mg/kg ketamine and 10mg/kg xylazine and subjected to pupillary dilation with topical 1% tropicamide and 2.5% phenylephrine hydrochloride. Fundus photography was performed with a commercial fundus camera and imaging system (TRC 50 IX and IMAGEnet, Topcon, Tokyo, Japan). Fundus autofluorescence (FAF) images (55° angle lens and a 488 nm excitation wavelength) were obtained by a confocal scanning laser ophthalmoscopy (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany).

Electroretinography (ERG)

The animals were dark adapted overnight and further handling was performed under deep red illumination. Mice were anesthetized by peritoneal injection of 50 mg/kg ketamine and 10 mg/kg xylazine. The pupils were dilated with topical 1% tropicamide and 2.5% phenylephrine hydrochloride. Body temperature was maintained at 37°C with a heating pad. ERG was recorded from both eyes using active contact lens electrodes. A gold wire loop placed in the mouth was used as reference and a ground electrode was on the tail. Dark-adapted ERG (dim or intense flash; 0.01 or 30.0 cd・s/m^2, respectively) and light-adapted ERG (3.0 cd・s/m^2) were recorded. Stimulation and data recording were controlled using the PuRec device system (Mayo Corporation, Inazawa, Japan).

Primary antibodies

Following primary antibodies were used in this study (see Table 1): LC3 (#4108, Cell Signaling Technology, Beverly, MA), SQSTM1 (#5114, Cell Signaling Technology), anti-human LAMP2 (clone H4B4, sc-18822, Santa Cruz Biotechnology, Santa Cruz, CA), anti-mouse LAMP2 (clone ABL-93, ab25339, Abcam, Cambridge, MA), anti-human LAMP1 (H4A3, 555798, BD Pharmingen, San Diego, CA), RPE65 (mice; ab78036, human; ab231782, Abcam), APOE (EPR19378, ab183596, Abcam), clusterin (AF2747, R&D), vitronectin (MAB38751, R&D), GAPDH (clone 14C10, #2118, Cell Signaling Technology), beta-Actin (13E5, #4970, Cell Signaling Technology), TJP1 (61-7300, Invitrogen), Iba-1 (019-19741**,** Wako, Tokyo, Japan), collagen IV (2150-1470, Bio-Rad, Hercules, CA), laminin (ab11575, Abcam), fibronectin (ab23750, Abcam), APOA1 (ab20453, Abcam), APOB (ab20737, Abcam), MMP-2 (ab92536, Abcam), and isotype IgGs (sc-2025, Santa Cruz Biotechnology).

Immunohistochemistry

For paraffin sections of formalin fixed human eyes, sections were deparaffinzed with xylene, rinsed with pure ethanol, and rehydrated. For melanin bleaching, sections were treated with 10% hydrogen peroxide in PBS at 60°C for 30 minutes. Then sections were blocked with blocking buffer (Blocking One, 03953-95, Nacalai tesque), incubated with anti-human LAMP2 antibody (clone H4B4, Santa Cruz Biotechnology) at 4°C overnight and HRP-polymer-labeled secondary antibodies (Dako, Carpinteria, CA) at room temperature for 30 minutes, and followed by 3-amino-9-ethylcarbazole (AEC; SK-4200, Vector laboratories, Burlingame, CA) staining. Regions within 6 mm from the foveal center were photographed in AMD eyes, age-matched controls, and young donors by masked photographers. To assess regional variability, RPE areas were divided into two groups; central (0-3 mm) or peripheral (3-6 mm) from the foveal center. Immunohistochemistry of human eyes were performed at the same time under the same experimental and microscopy conditions. Color images were obtained by means of an Axio observer (Carl Zeiss, Oberkochen, Germany). Images were white balanced by Zen software (Carl Zeiss) in advance and

RGB channels were split. Green channels were subtracted from red channel to subtract background using Image J software. The areas of RPE were defined with the original images and integrated densities per RPE area were calculated.

Immunofluorescence

Mice eyes were fixed with 4% paraformaldehyde at 4°C overnight and paraffin-embedded or frozen for cryosections. Sections were blocked and incubated with primary antibodies at 4 °C overnight and secondary antibodies conjugated to Alexa Fluor dyes (Invitrogen, Carlsbad, CA) at room temperature for 1 h. Autofluorescent images of mouse paraffin sections were obtained by 450/490 and 500/550 filter sets for excitation and emission, respectively. TUNEL assay was performed according to the manufacturer's protocol (ApoTag Fluorescein In Situ Apoptosis Detection Kit, S7110; Millipore). Nuclei were stained with DAPI (Thermo Fischer Scientific). Mouse RPE flatmount specimens were obtained from WT and *Lamp2* KO mice as follows. For Iba-1 immunofluorescence in RPE flatmount, enucleated mice eyes were fixed in 4 % paraformaldehyde for 30 min and further fixed for another 30 min after removal of corneas. Then lenses and retinas were removed to isolate eyecups including RPE. For autofluorescence in RPE flatmount, corneas, lenses, and retinas were immediately removed after enucleation. Then RPE flatmount specimens were fixed in 4 % paraformaldehyde. Fluorescent images were obtained using a microscope Axio imager M2 or LSM 800 laser confocal microscopy (Carl Zeiss, Oberkochen, Germany). Immunofluorescence at the basal side of RPE were quantified in 4 defined regions with 0.5 mm interval from optic nerve head per eye (n = 6 mice). Similar immunofluorescent detections were performed on ex vivo cultures of primary mouse RPE on transwell membranes. Immunofluorescence observed under the RPE was quantified at 6 regions per membrane (n = 4 membranes per group).

Filipin staining

For quantification of unesterified cholesterol (UC), cryosections were stained with 0.05 mg/ml filipin III (F4767, Sigma) for 1 hour, washed with PBS, and the fluorescent images were photographed using a UV filter set. For esterified cholesterol (EC), native UC was extracted from cryosections by two 5 min rinses in 70% ethanol, native EC was hydrolysed with cholesterol esterase (228180, Sigma-Aldrich; enzyme category 3.1.1.13) at a concentration of 1.65 units/mL in 0.1 M potassium phosphate buffer (pH 7.4) for 3 hr at 37°C, and UC newly released by EC hydrolysis was stained with filipin as described above.

***ex vivo* culture of mouse primary RPE monolayer**

Collections of RPE monolayers from 2 month old WT or Lamp2 KO mice were performed at the same time of day (1:00 pm) due to the presence of circadian changes. Culture medium for primary RPE cells were prepared by adding 5 ml of N1 Supplement (Sigma-Aldrich), 5 ml of glutamate (Invitrogen), 5 ml of nonessential amino acids (Gibco; Carlsbad, CA), 125 mg of taurine (Sigma-Aldrich), 10 mg of hydrocortisone (Sigma-Aldrich) and 0.0065 mg of triiodothyronin (Sigma-Aldrich), penicillin-streptomycin, and 10% heat inactivated FBS to 500 ml of MEM–Alpha (Sigma-Aldrich). To isolate the monolayer of primary RPE cells, eyes were enucleated and the corneas were removed. The eyecups were incubated with Dulbecco's PBS without Ca^{2+} and Mg^{2+} (Gibco) containing 1% Dispase II (Wako Pure Chemical) for 30 min at 37°C. Enzymatic reaction was terminated by transferring eyecups into cold RPE culture medium. An incision was made at ora serata to remove the limbus, and the retinas with RPE were separated from the choroid. The retina-RPE complex was incubated with new culture medium for 60 minutes at 37°C. RPE monolayers were peeled from the retina using fine forceps. Quadrants of an isolated RPE sheet were transferred into transwells (Corning #3460, 0.4 μm pore polyester membrane insert). Basolateral side of RPE was allowed to viscoelastically attach to the membrane inserts using 10mg/ml sodium hyaluronate (Sigma-Aldrich) in the syringe with 26G needle. The RPE were incubated with humidified 5% CO2 at 37 °C. Culture medium were changed 3 h after the attachment to the

membrane and then every 3-4 days. For cryosections of primary RPE sheet cultures, RPE was similarly isolated and cultured on polytetrafluoroethylene transwell membrane inserts (CLS3494, Sigma). The transwell membrane inserts were pre-coated with 166 μl of Matrigel (growth factor reduced, phenol red-free, 356231, BD biosciences) diluted 1:20 in cold water and air-dried overnight to create a matrigel film. The primary RPE sheets were pre-cultured for a week and then cultured with/without repetitive POS administration (10 particles/cell of POS incubation for 6 h every 3 days for 2 weeks). Cultured primary RPE cells have shown hexagonal morphology and well preserved polarized characteristics (SI Appendix, Fig. S9).

Porcine POS isolation

POS were isolated from porcine eyes (Visiontech, Mesquite, TX) for in vitro phagocytosis assays as previously described (2). Briefly, ten porcine retinas were isolated from the eye cup after removing cornea lens and the limbus with scalpel blade and scissor. Collected retinas were kept in homogenization solution (20% sucrose, 20 mM tris acetate pH 7.2, 2 mM $MgCl^2$, 10 mM glucose and 5 mM taurine) on ice. The suspensions were shaken vigorously by hand for 2 min in order to break POS off the rest of photoreceptors. After allowing large visible tissue fragments to drop down, the crude retinal homogenates were gently laid on fresh chilled continuous 25-60% sucrose gradient in ultracentrifuge tubes and centrifuged at 50,000 g for 100 min at 4 °C. Most of the solution above the orange-pink band in the upper third of gradient was removed by aspiration to collect the orange-pink band. The collected band including POS was diluted with ice-cold 20 mM tris acetate pH 7.2 with 5 mM taurine and centrifuged at 3,000 x g for 10 min at 4 °C. The supernatants were carefully discarded. Pellets were resuspended in 10% sucrose, 20 mM tris acetate pH 7.2 and 5 mM taurine and spun down at 3,000 g for 10 min. Pellets were resuspended in 10% sucrose, 20 mM sodium phosphate pH 7.2 and 5 mM taurine and spun down at 3,000 x g for 10 min. Collected POS pellets were resuspended in DMEM with 2.5% sucrose and aliquots were frozen at -80 °C until use.

Transmission electron microscopy (TEM)

The posterior segments of human or mice eyes and primary RPE cultures were fixed with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer with 0.08 M $CaCl_2$ at 4°C overnight, post-fixed for 1.5 h in 2% aqueous OsO_4, dehydrated in ethanol and water, and embedded in epoxy resin. For retinal structure analysis, 1 μm semi-thin sections were cut from the resin block along the vertical meridian and stained with Azure II. ONL thickness was measured at 4 defined regions starting from the optic nerve head and extending along the vertical meridian. For TEM imaging, ultrathin sections were cut from the resin blocks, mounted on copper grids, and observed with transmission electron microscopes (H-7770; Hitachi, Tokyo, Japan or Tecnai G2 Spirit; FEI, OR, USA). The maximum thickness of sub-RPE deposits or Bruch's membrane was measured in six regions defined with 200 um intervals from optic nerve head. The numbers of phagosomes that contained the identifiable multilayered membrane structure of POS (disk) or autophagic vacuoles that did not contain such identifiable disk structures were counted and quantified in the RPE as per area (mm^2) using sections including the optic nerve for similar defined areas (n = 6 mice per group). For primary RPE cultures, 6 polyester transwell membrane inserts per group were imaged by TEM. The maximum thickness of basolateral extracellular material was measured at 6 regions per membrane (n = 4 membranes per group).

Western Blot Analysis

The retinas and RPE/choroid were isolated by removing cornea, lens, and iris. For isolation of RPE monolayers, the eye cups were incubated with 1 % dispase for 30 minutes similar to the procedure for mouse primary RPE cultures. Collected mouse tissues or ARPE-19 cells were lysed in Mammalian Protein Extraction Reagent (M-PER; Thermo Scientific, Waltham, MA) containing protease inhibitors (cOmplete Mini, Roche, Indianapolis, IN), followed by Bradford protein concentration assay. Lysates were denatured with NuPAGE LDS Sample Buffer (Invitrogen) containing 4% beta-mercaptoethanol,

electrophoresed on 4-12% Bis-Tris gel (NuPAGE; Invitrogen), and transferred onto polyvinylidene difluoride membranes (Millipore). The blotted membranes were blocked and incubated with primary antibodies at 4°C overnight followed by labeling with HRP-conjugated secondary antibodies at room temperature for 30 minutes. Immunoreactive bands were visualized by ECL and detected by a commercial imaging system (ChemiDoc MP; Hercules, CA) and analyzed by Image Lab software (Bio-Rad Labs, Richmond, CA).

siRNA transfection and beta-hexosaminidase assay

ARPE-19 cells, a spontaneously arising human RPE cell line, were purchased from American Type Culture Collection and cultured in 12-well plates for beta-hexosaminidase assay. Cells at passages 3 to 5 were cultured with DMEM/F-12 medium containing 10% FBS, 100 U/mL penicillin, and 100 μg/ml streptomycin in humidified 5% CO2 at 37°C. ARPE-19 cells were transfected with scrambled negative control siRNA duplex or 3 unique 27mer siRNA duplexese targeting human *LAMP2* gene (Trisilencer-27, SR302652, Origene) using RNAi MAX (Invitrogen) for 48 hours according to manufacture's protocol. Culture medium was changed at 48 hours and cells were further incubated with DMEM/F-12 plus 10% FBS and antibiotics for 3 days prior to experiments. For beta-hexosaminidase activity assay, siRNA treated ARPE-19 cells were washed with HBSS (calcium+ magnesium+) and incubated with HBSS (calcium+ magnesium+) in the presence or absence of 10 μM ionomycin (I0634, Sigma) for 10 min. Cell culture supernatants were collected after centrifuge at x800g for 4 min. Lysates were collected with 0.5 % Triton X in PBS, centrifuged at x800g for 4 min, and pellets were discarded. Then, 25 μL of fluorescent substrate, 4-methyl-umbellyferyl-N-acetyl-beta-D-glucosaminide (M2133, Sigma) 4 mM in 20mM Na citrate-phosphate buffer, pH4.5 were added to 175 μL of collected supernatants or lysates. beta-hexosaminidase activities were determined by means of a microplate reader Gemini XPS (Molecular Devices, Orleans, CA). The supernatant/lysate ratios of beta-hexosaminidase activity were calculated for 6 wells per group.

Statistical Analysis

Statistical differences between the two groups were analyzed by means of Student *t*-test. Multiple group comparison was performed by One-way ANOVA with post-hoc Tukey HSD test. Differences were considered significant at $P < 0.01$ (*), $P < 0.001$ (**), and $P < 0.0001$ (***) for Student *t*-test and $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***) for Tukey HSD test. All values were expressed as the means ± SDs.

References

1. Yao J, et al. (2014) Circadian and noncircadian modulation of autophagy in photoreceptors and retinal pigment epithelium. Invest Ophthalmol Vis Sci 55(5):3237-3246.

2. Parinot C, Rieu Q, Chatagnon J, Finnemann SC, & Nandrot EF (2014) Large-scale purification of porcine or bovine photoreceptor outer segments for phagocytosis assays on retinal pigment epithelial cells. in J Vis Exp.

Figure S1

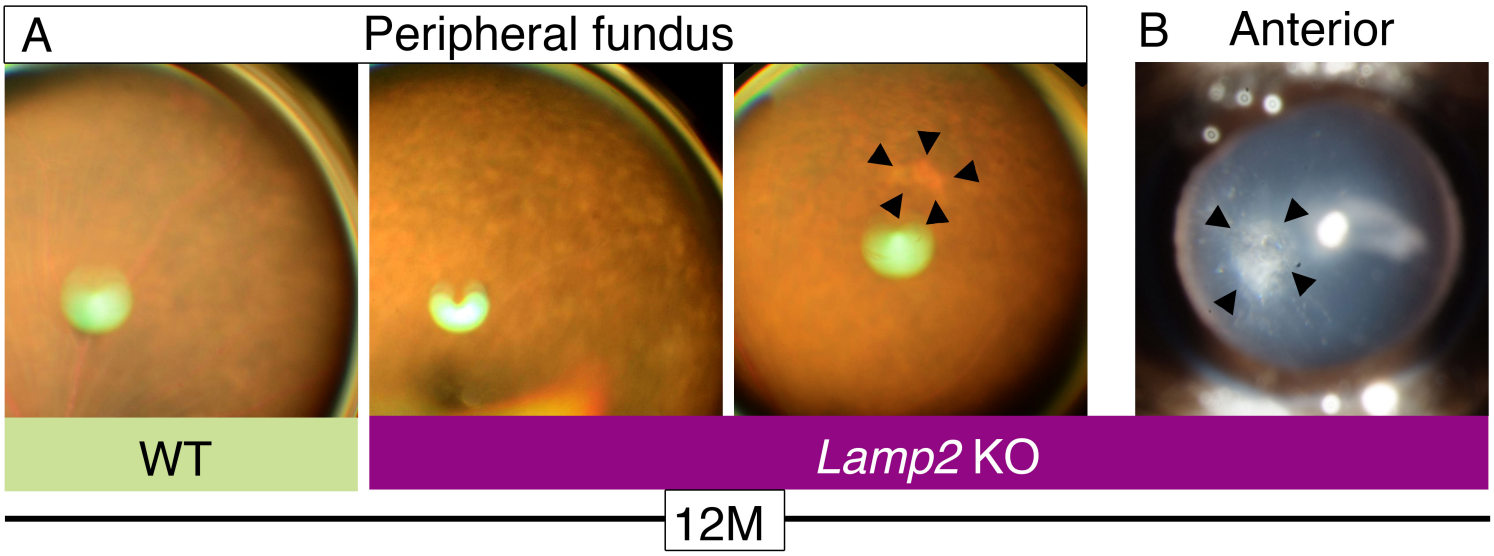


Figure S2

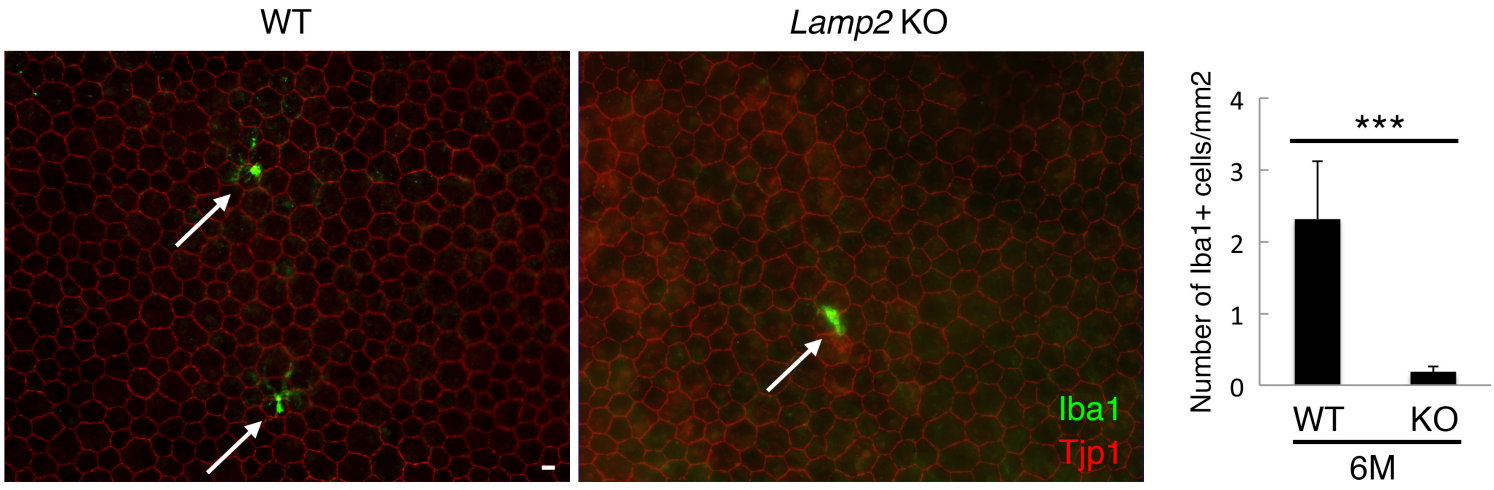
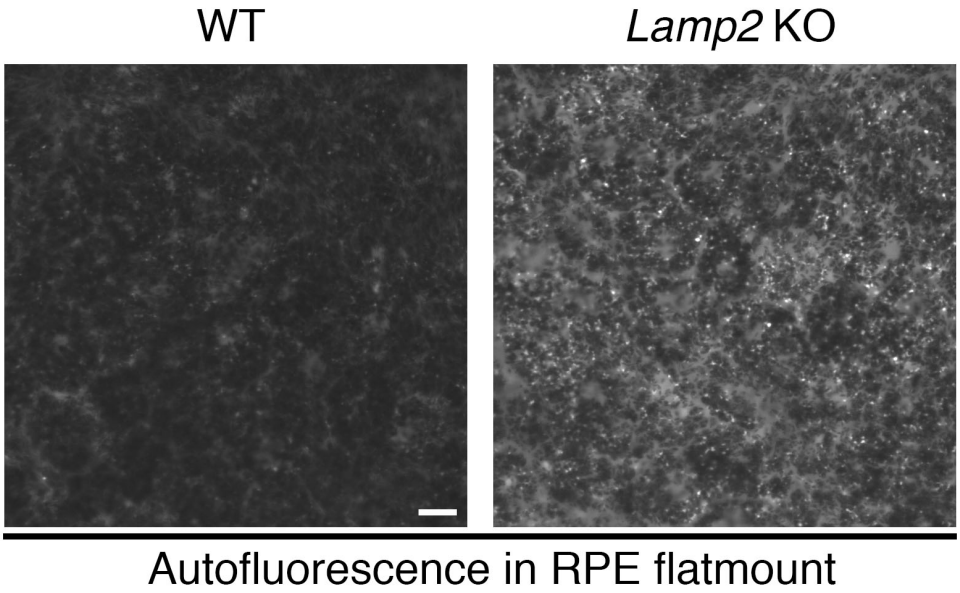


Figure S3



Autofluorescence in RPE flatmount

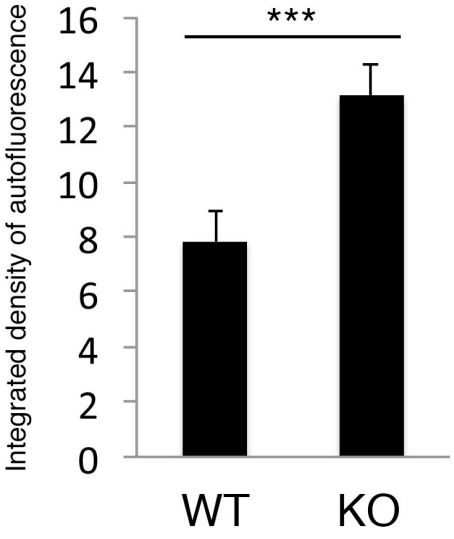
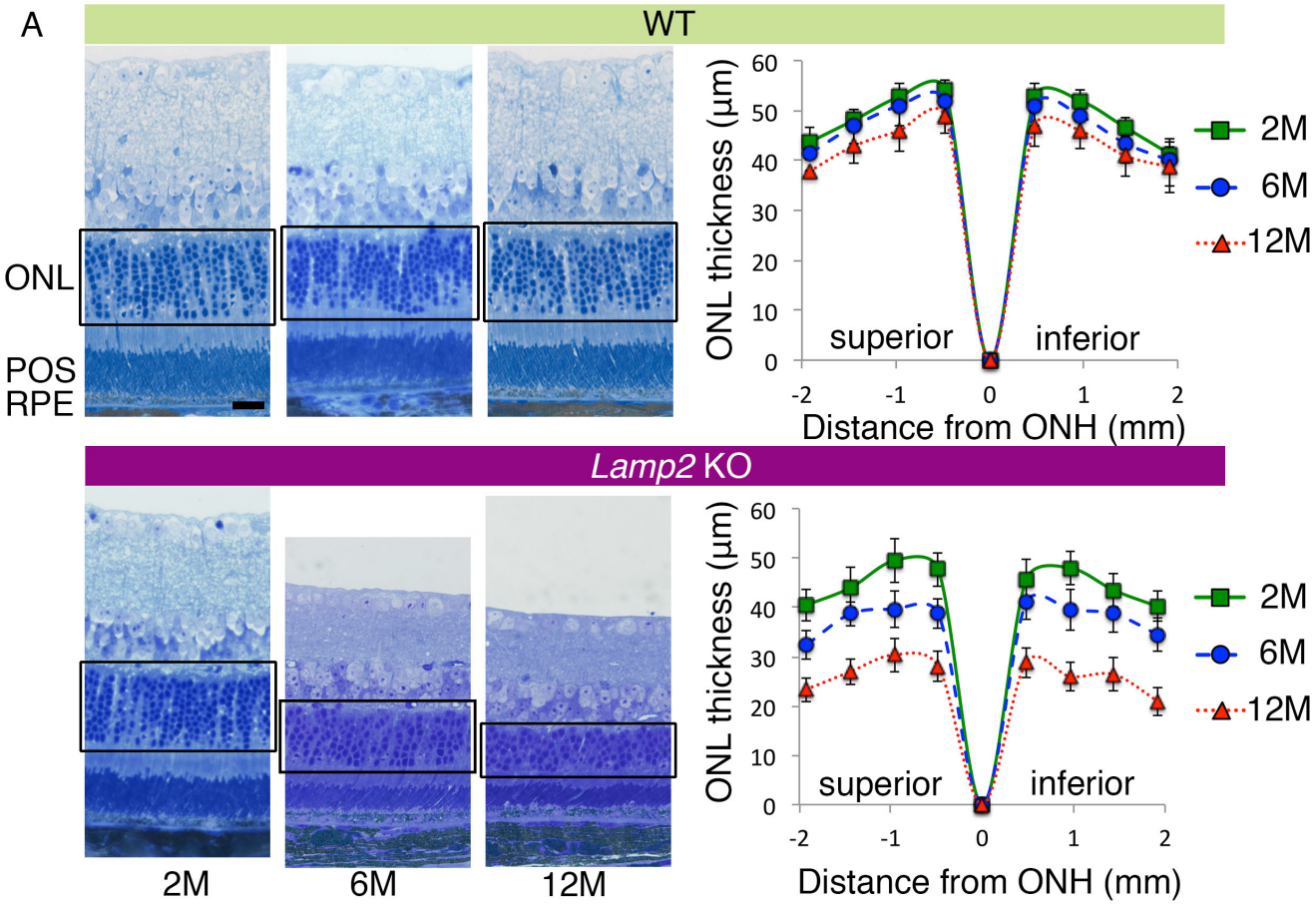


Figure S4


A
WT
ONL
POS
RPE
ONL thickness (μm)
superior
inferior
Distance from ONH (mm)
2M
6M
12M
Lamp2 KO
2M
6M
12M

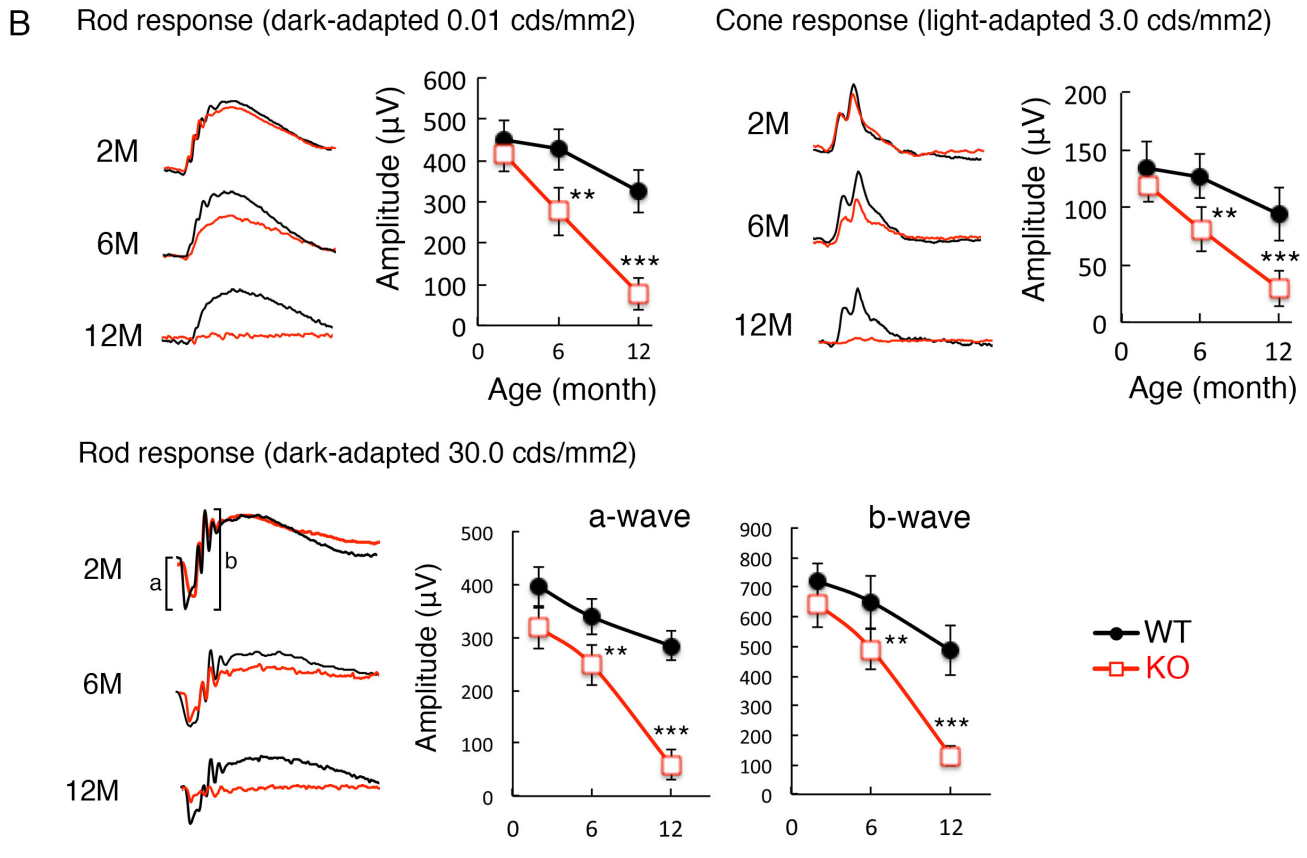

B
Rod response (dark-adapted 0.01 cds/mm2)
Cone response (light-adapted 3.0 cds/mm2)
Amplitude (μV)
Age (month)
Rod response (dark-adapted 30.0 cds/mm2)
a-wave
b-wave
WT
KO

Figure S5

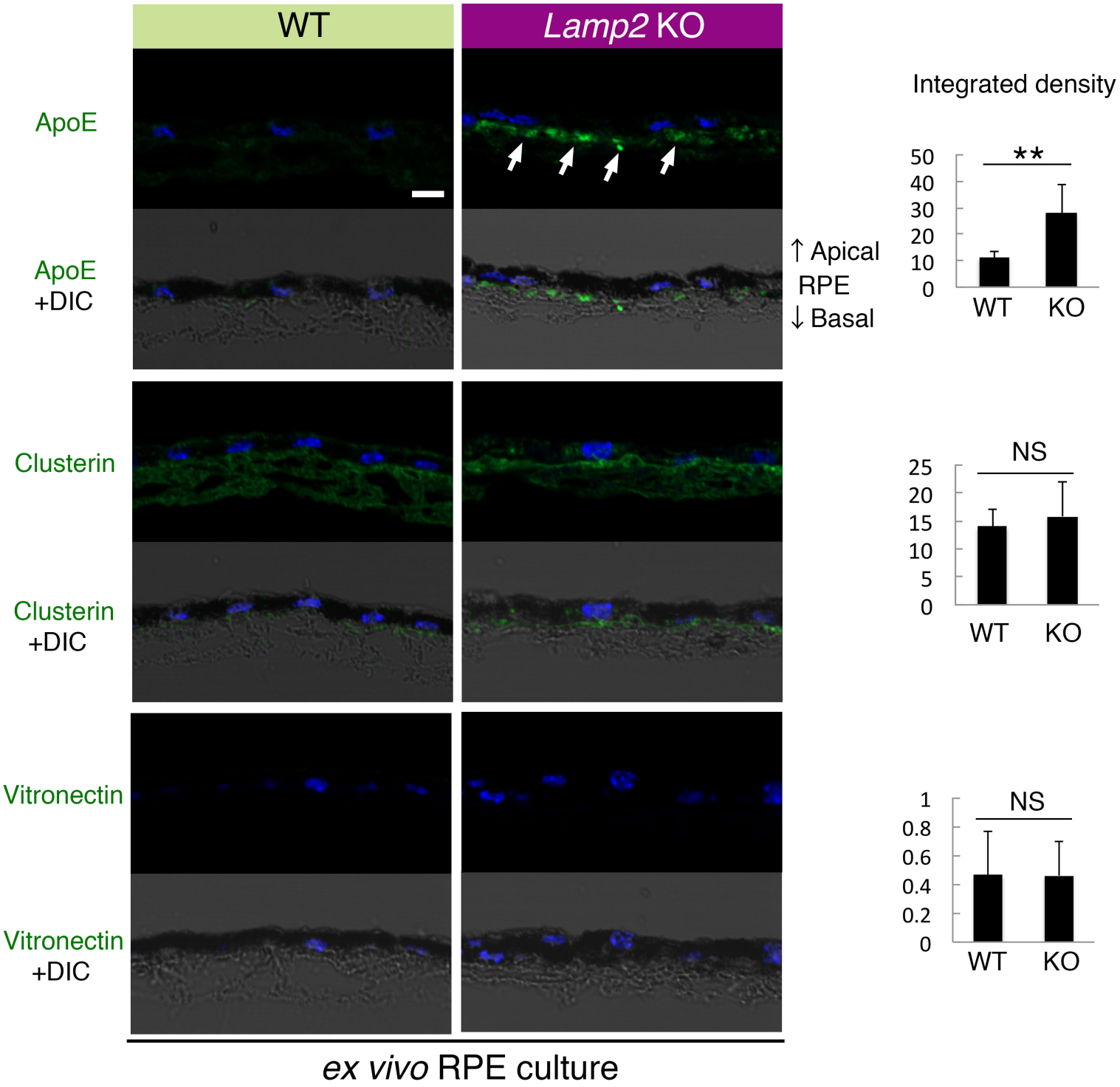

WT
Lamp2 KO
ApoE
ApoE
+DIC
↑ Apical
RPE
↓ Basal
Integrated density
**
50
40
30
20
10
0
WT
KO
Clusterin
Clusterin
+DIC
NS
25
20
15
10
5
0
WT
KO
Vitronectin
Vitronectin
+DIC
NS
1
0.8
0.6
0.4
0.2
0
WT
KO
ex vivo RPE culture

Figure S6

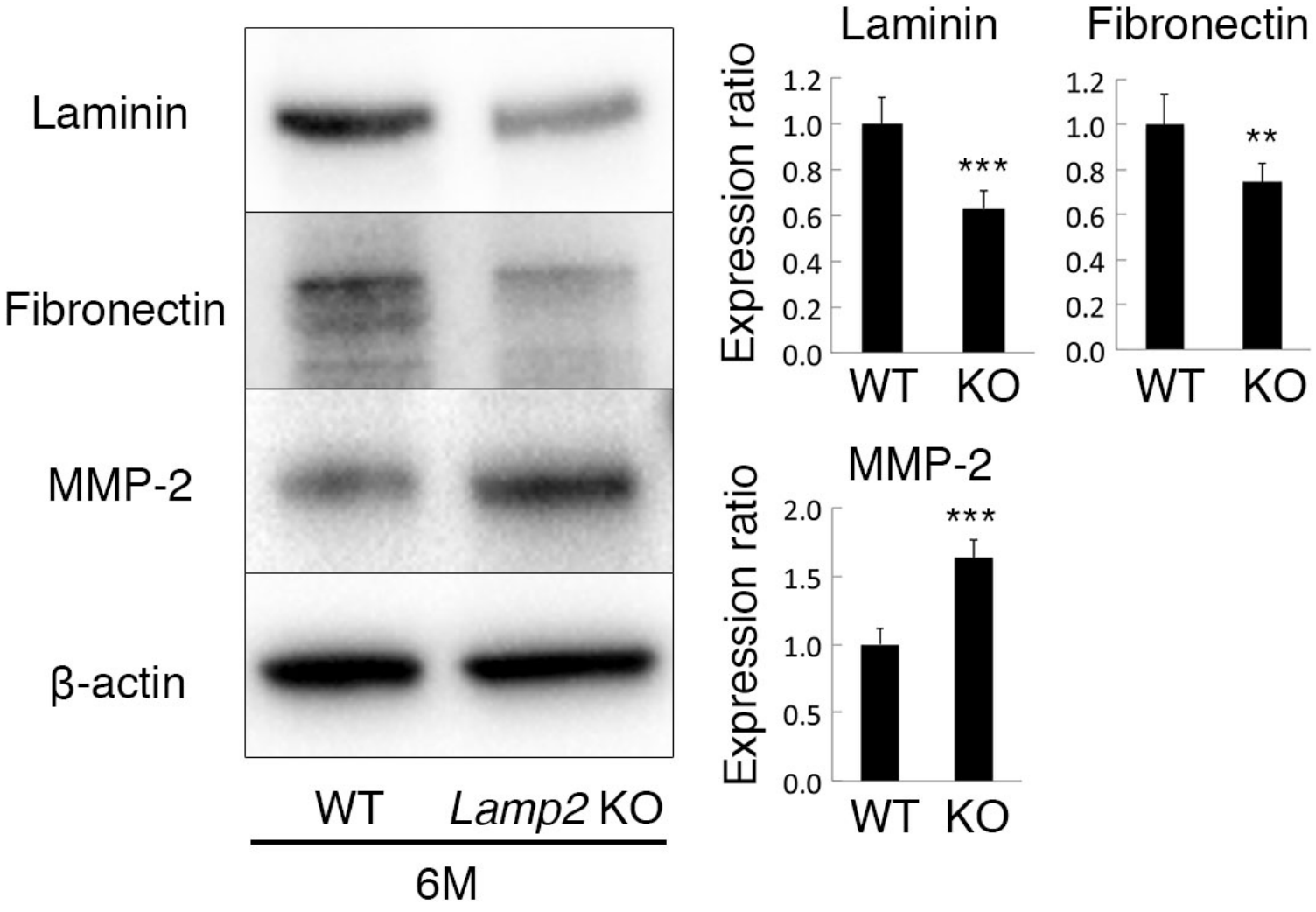
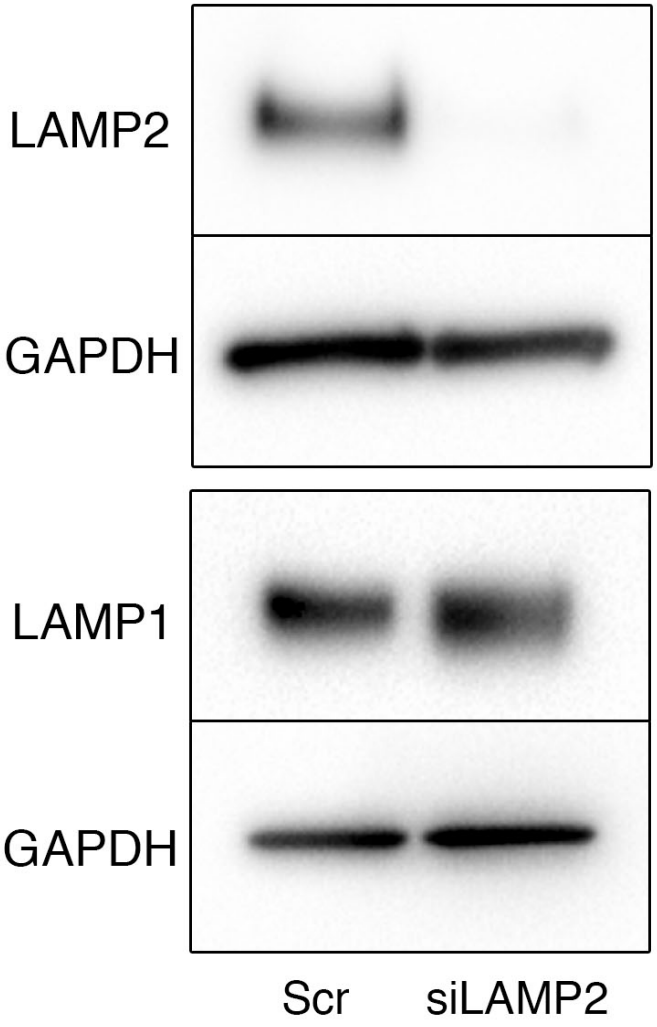

Laminin
Fibronectin
MMP-2
β-actin
WT
Lamp2 KO
6M
Laminin
Fibronectin
Expression ratio
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
**
WT
KO
MMP-2
2.0
1.5
1.0
0.5
0.0
***
WT
KO

Figure S7


LAMP2
GAPDH
LAMP1
GAPDH
Scr
siLAMP2

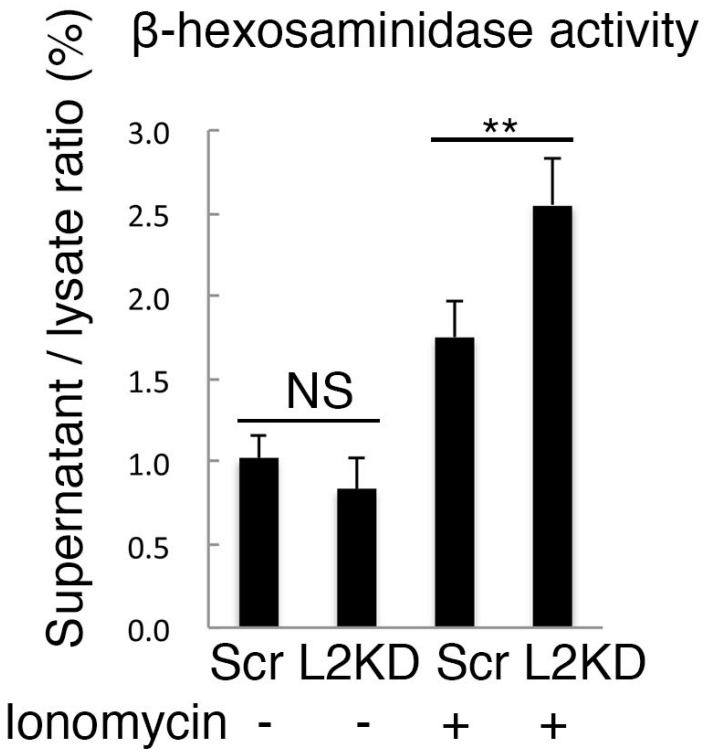

β-hexosaminidase activity
Supernatant / lysate ratio (%)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
NS
Scr
L2KD
Scr
L2KD
Ionomycin - - + +

Figure S8

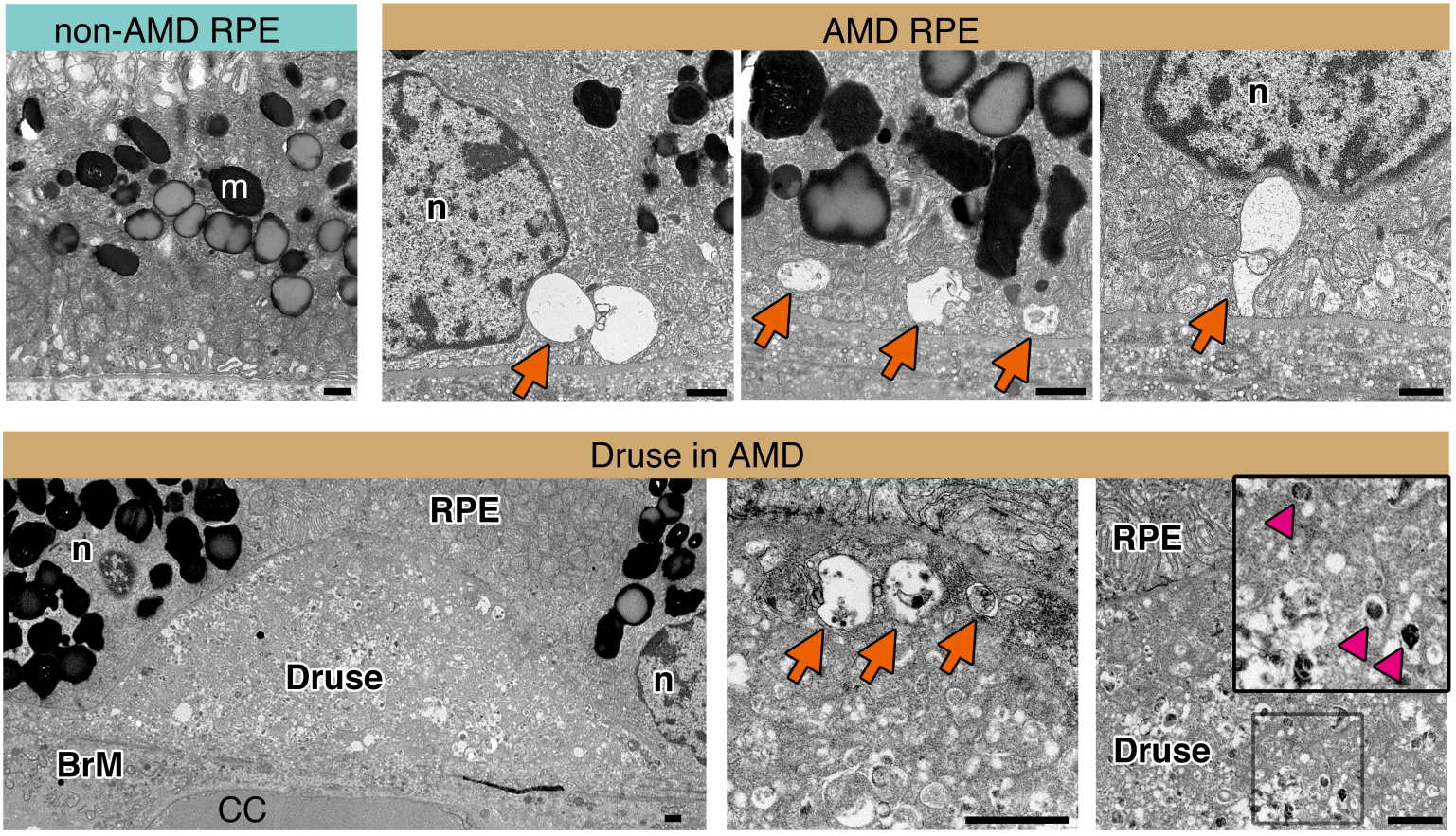
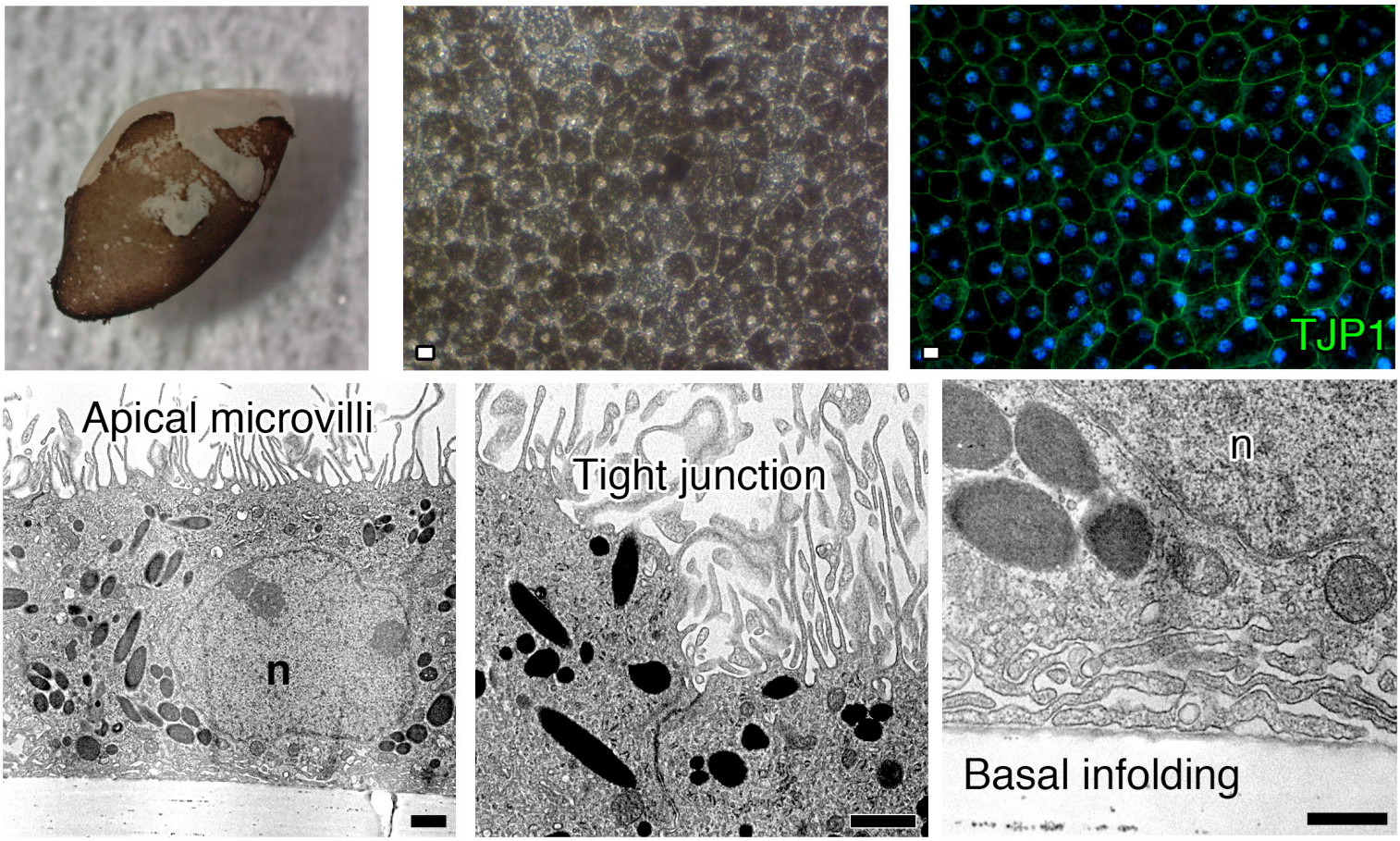

non-AMD RPE
m
AMD RPE
n
n
Druse in AMD
RPE
n
Druse
n
BrM
CC
RPE
Druse

Figure S9

ex vivo culture of mouse primary RPE



Antigen	clone name	product number	company	tested speicie:	dilution (IF)	dilution (WB)
LC3		4108	CST	mice		1/1000
SQSTM1		5114	CST	mice		1/1000
LAMP2	H4B4	sc-18822	Santa Cruz	human		1/1000
LAMP1	H4A3	555798	BD	human		1/1000
LAMP2	ABL93	ab25339	Abcam	mice		1/1000
LAMP1	1D4B	sc-19992	Santa Cruz	mice		1/1000
NPC1	EPR5209	ab134113	Abcam	mice		1/1000
LIMP2		ab16522	Abcam	mice		1/1000
ABCA1	AB.H10	MAB10005	Millipore	mice		1/1000
Cathepsin D	EPR3057Y	ab75852	Abcam	mice		1/1000
Cathepsin B	D1C7Y	31718	CST	mice		1/1000
CI-MPR	EPR6599	ab124767	Abcam	mice		1/1000
Rab7	D95F2	9367	CST	mice		1/1000
ABCB9		ab86222	Abcam	mice		1/1000
β-Actin	13E5	4970	CST	mice		1/1000
TJP1		61-7300	Invitrogen	mice	1/400	
RPE65		ab78036	Abcam	mice		1/1000
APOE	EPR19378	ab183596	Abcam	mice	1/400	1/1000
clusterin		AF2747	R&D	mice	1/400	1/1000
vitronectin		MAB38751	R&D	mice	1/400	1/1000
GAPDH	14C10	2118	CST	mice, human		1/2000
Iba-1		019-19741	Wako	mice	1/400	
Collagen-4		2150-1470	Bio-Rad	mice	1/400	
Laminin		ab11575	Abcam	mice	1/400	
Fibronectin		ab23750	Abcam	mice	1/400	
APOA1		ab20453	Abcam	mice	1/400	
APOB		ab20737	Abcam	mice	1/400	
RPE65	EPR22579-44	ab231782	Abcam	human	1/400	
MMP-2	EPR1184	ab92536	Abcam	mice		1/1000
Isotype IgG		sc-2025	Santa Cruz	human	1/400	

Legends to supplemental figures

Figure S1.

A: The patchy fundus abnormalities extended to the peripheral region of *Lamp2* KO mice. A large patchy pigment atrophy was occasionally observed (arrowheads). **B**: Formation of posterior subcapsular cataracts in aged *Lamp2* KO mice (arrowheads).

Figure S2.

Immunofluorescence for Iba-1 and TJP1 in the RPE flatmount from WT and LAMP2 KO flatmount specimens. Note that Iba-1 positive cells found in *Lamp2* KO mice showed amoeboid morphology. Total numbers of Iba-1 positive cells per eye were counted and expressed as cells per area. n = 6 mice per group. ***$P < 0.0001$. Student t-test. Values were expressed as the means ± SDs.

Figure S3.

Autofluorescence observed in the RPE flatmounts from WT and LAMP2 KO mice. Integrated density of autofluorescent images obtained by 450/490 and 500/550 filter sets for excitation and emission, respectively, were quantified in 6 regions per eye. n = 6 mice per group. ***$P < 0.0001$. Student t-test. Values were expressed as the means ± SDs.

Figure S4.

Age-dependent retinal degeneration and loss of light-electrical response in *Lamp2* KO mice.

A: Histology of WT mice and *Lamp2* KO mice and the quantified ONL thickness (Azure II stain). ONL thickness was measured at 4 defined regions per eye (see Material and Methods). **B**: ERG measurements of dark-adapted rod responses (dim or intense flash; 0.01 or 30.0 cd･s/m^2, respectively) and light-adapted cone responses (3.0 cd･s/m^2) in *Lamp2* KO or WT mice. n = 6 mice per group. **$P < 0.001$, ***$P <$ 0.0001. Student t-test. Values were expressed as the means ± SDs. Scale bar: 20 μm.

Figure S5.

Immunofluorescence for APOE, clusterin, and vitronectin were performed on *ex vivo* cultures of primary RPE cells from 2 month-old WT or *Lamp2* KO mice. RPE cells were cultured for seven days on the plastic membranes. Arrows indicates punctate accumulations of APOE under the cultured RPE from *Lamp2* KO mice. Immunofluorescence observed under RPE was quantified at 6 regions per membrane (n = 4 membranes per group). Scale bars: 10 μm. $^{**}P < 0.001$. Student t-test. Values were expressed as the means ± SDs.

Figure S6.

Immunoblot analyses of the expression of laminin, fibronectin, and MMP-2 in the RPE/choroid. Lysates of RPE/choroid were collected from 6 eyes per group and ratiometric analyses were performed on at least three times repetitive blotting. $^{***}P < 0.001$, $^{**}P < 0.001$. Student t-test. Values were expressed as the means ± SDs.

Figure S7.

Lysosomal exocytosis increased by LAMP2 deficiency in ARPE-19 cells. ARPE-19 cells were treated with scramble or LAMP2 siRNA for 48 hours and further cultured for 3 days after medium change at 48 hours. Expression of LAMP2 but not LAMP1 was depleted by *LAMP2* siRNA. Beta-hexosaminidase activity ratios (supernatant/lysate) in the presence or absence of 10 μM ionomycin treatment for 5 min after the siRNA treatments (n = 6 wells per group). $^{**}P < 0.01$. One-way ANOVA with post-hoc Tukey HSD test. Values were expressed as the means ± SDs.

Figure S8.

Representative TEM images of RPE in non-AMD or AMD eyes. The structure of normal basal infolding was preserved in the RPE of control subjects while largely dilated autophagic or phagocytic vacuoles were observed around the basolateral plasma membrane of RPE from AMD eyes (arrows). Note TEM images of an accumulation of granular material within the dome-shaped druse in AMD (arrowheads). Dr: druse, BrM: Bruch's membrane, CC: choriocapillaris, m: melanin, n: nucleus. Scale bars: 1 μm.

Figure S9.

A representative photograph of the isolated RPE from the choroid in mice (top left panel). Bright light field (top middle) and immunofluorescence for tight junction protein 1 (TJP1; top right in green) of *ex vivo* cultures of primary RPE cells from WT mice. Scale bars: 10 μm. TEM showing epithelial polarities such as apical microvilli, tight junction, and basal infolding of ex vivo cultures of primary RPE cells (bottom panels). Scale bars: 1 μm. n: nuclei.