Supporting Information

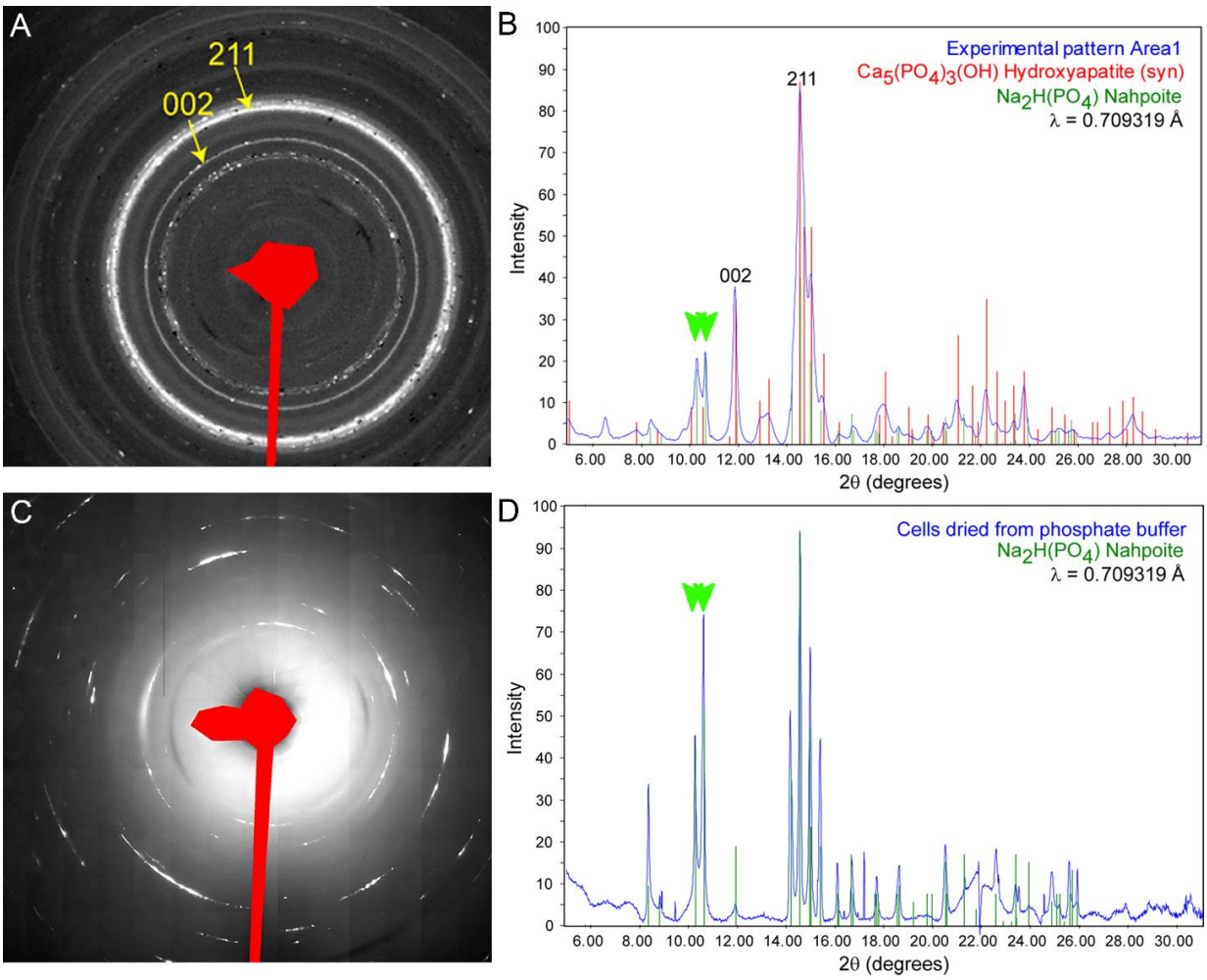
Pilgrim, M., Lengyel, I., Lanzirotti, A., Newville, M., Fearn, S., Emri, E., Knowles, J. C., Messinger, J. D., Read, R. W., Guidry, C., Curcio, C. A. Sub-retinal pigment epithelial deposition of hydroxyapatite and other drusen components in primary cell culture model

Corresponding Address:
Christine A. Curcio, PhD; Department of Ophthalmology; EyeSight Foundation of Alabama Vision Research Laboratories; 1670 University Boulevard Room 360; University of Alabama School of Medicine; Birmingham AL 35294-0019; Email curcio@uab.edu

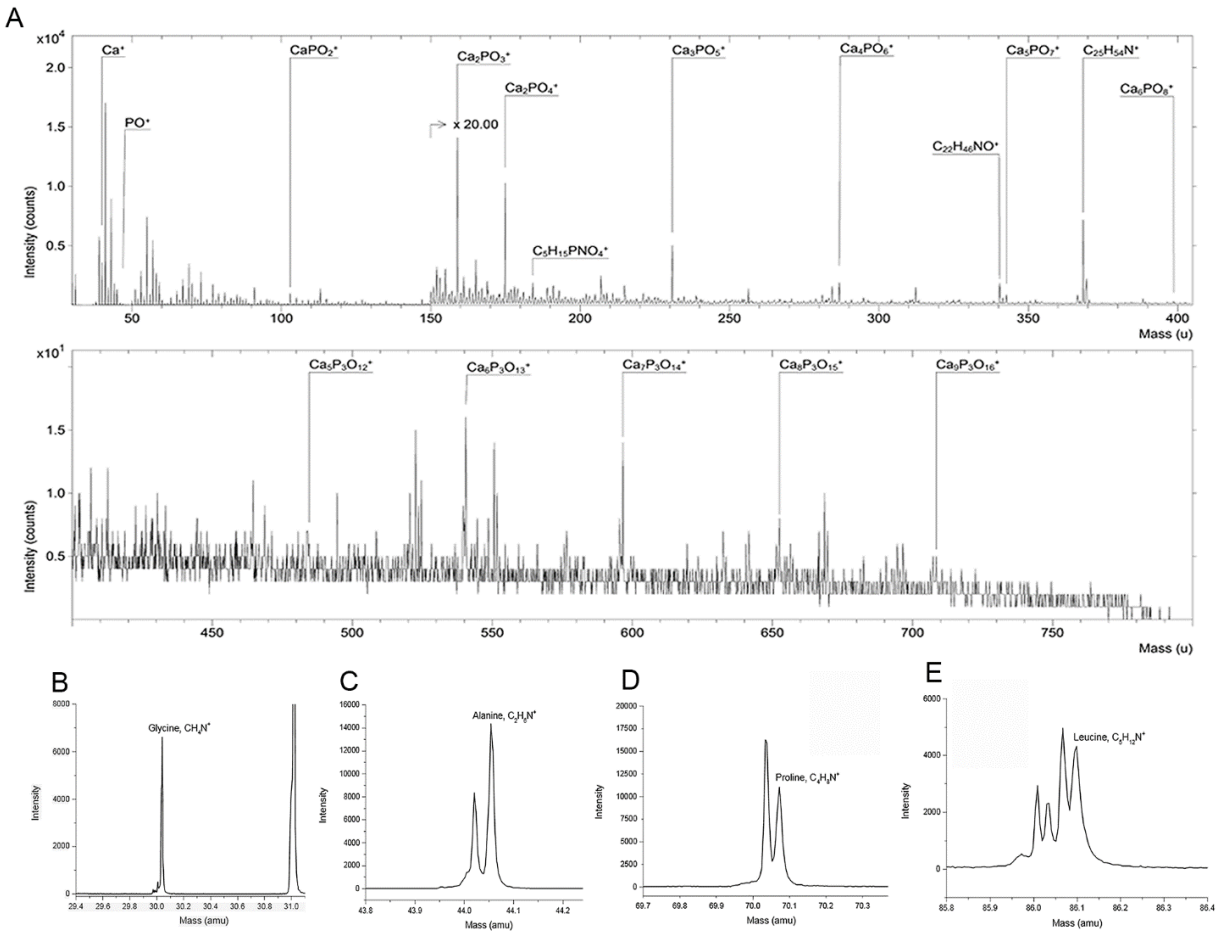
Supporting Information Figure Legends

Supporting Information Figure S1. Sample preparation affects XRD pattern. In the presence of PBS in the storage buffer we could identify Nahpoite ($Na_2H(PO_4)$) deposition in our samples after drying (B and D, green bars). The double peaks labeled with green arrowheads (B and D) are missing from a sample dried from a PBS free buffer (Fig. 4G). The XRD pattern suggest that the microcrystalline material appearing on (A) is derived from Nahpoite (C) which is absent from Fig. 4H, indicating that sample preparation requires appropriate buffers to avoid artifacts.

Supporting Information Figure S2. The presence of HAP and proteins within sub-RPE deposits was confirmed by secondary ion mass spectrometry.
A positive ion mode mass spectrum obtained from a focal deposit exhibited molecular ion peaks representative of HAP **(A)**. Molecular ion peaks were separated by 55.9 *m/z* **(A)**. This is characteristic of CaO, a building block of the hydroxyapatite lattice**.** Molecular ion peaks representative of the amino acids glycine (30.0347 m/z) **(B)**, alanine (44.0512 m/z) **(C),** proline (70.0671 m/z) **(D)** and leucine (86.1005 m/z) **(E)** confirmed the presence of proteins within sub-RPE deposits produced by cell cultures.



Supporting Information Figure S1



Supporting Information Figure S2

Supplementary Table 1

Supplementary Table: Extracellular lesions of AMD (from inner to outer) compared with sub-RPE deposits in primary porcine RPE culture			
Lesion, location	**Evidence for lipid**	**Evidence for calcium phosphate**	**Present in culture**
Subretinal drusenoid deposit (between RPE and photoreceptors)	Unesterified cholesterol, undetectable esterified cholesterol (3, 4)	No refractile material, *Fig. 9B*	No
BLamD (between RPE plasma membrane and basement membrane)	Tracks of lipoprotein particles (5)	No refractile material, *Fig. 9A-B*	No
Basal mounds (soft druse material within BLamD)	(6); *Fig. 9B*	Refractile material, *Fig. 9B*	No
BLinD/ soft drusen (lipoprotein-derived debris between RPE basal lamina and inner collagenous layer of Bruch's membrane (7))	(5); *Fig. 9A*	*Fig. 9A*	Yes: *Fig. 2*
Hard drusen (lipoproteins, proteins, trace metals between RPE basal lamina and inner collagenous layer of Bruch's membrane)	(8-10); *Fig. 9D,E*	(11-13); *Fig.9E*	Yes: *Fig. 2*