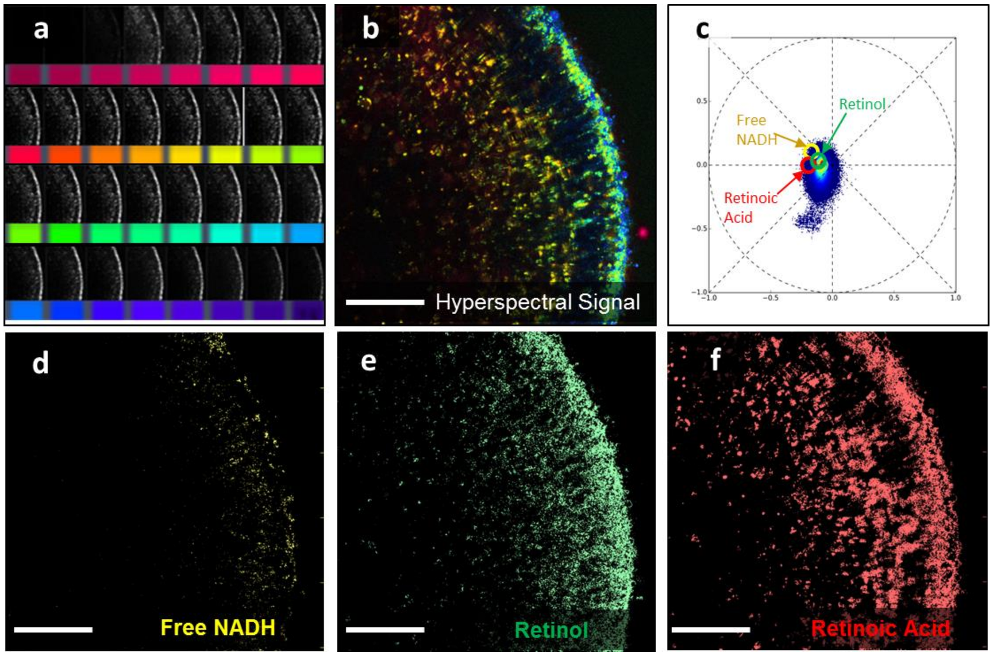
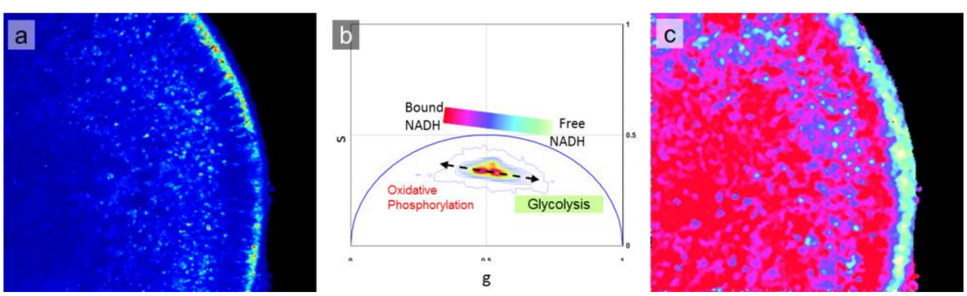
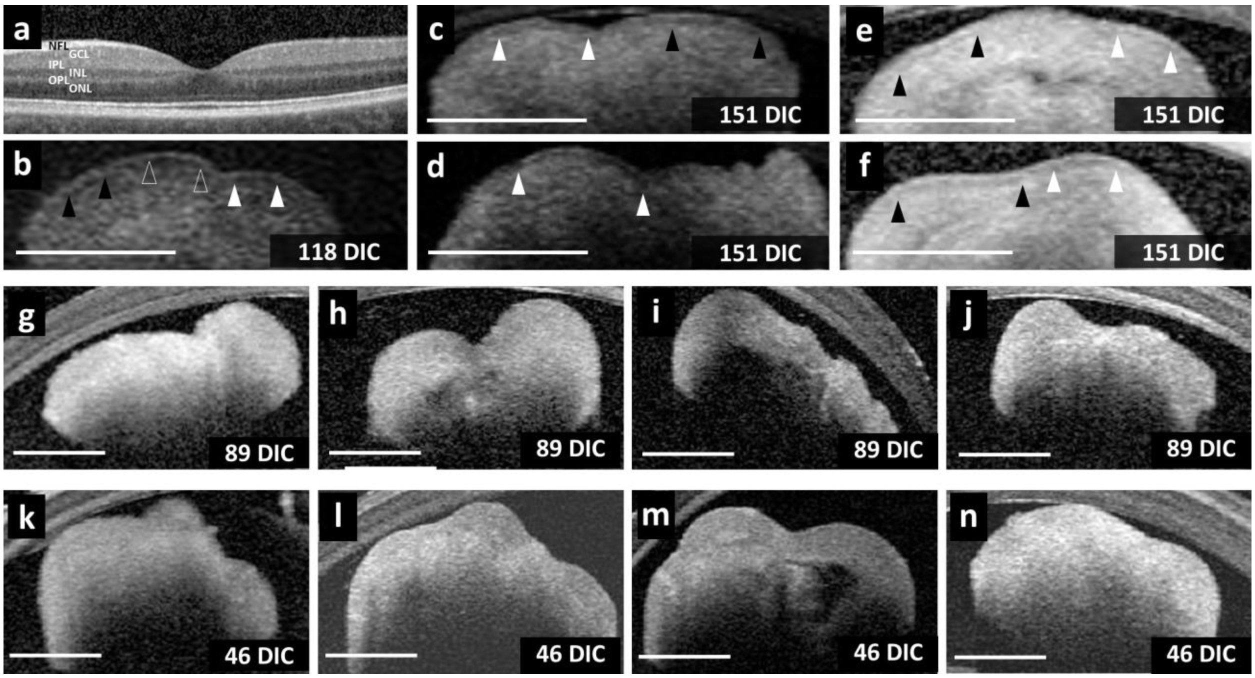


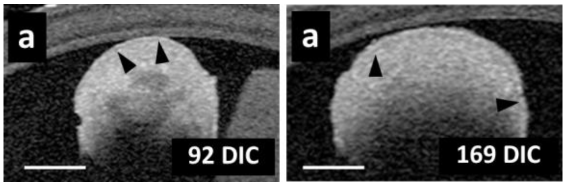
Supplementary Figure S1: Hyperspectral imaging of 151 DIC hESC-derived retinal organoid. 2-photon stimulation produces a broad spectral autofluorescence (a) and each autofluorescent wavelength can be merged with the bulk autofluorescent image (b) where each pixel is color coded for the detected autofluorescent emission wavelength from that pixel. Phasor analysis (c) of autofluorescent spectral signature from each pixel is correlated with previously characterized autofluorescent species such as free NADH (d), retinol (e) and retinoic acid (f) [16]. Scale bars are 50 μm.



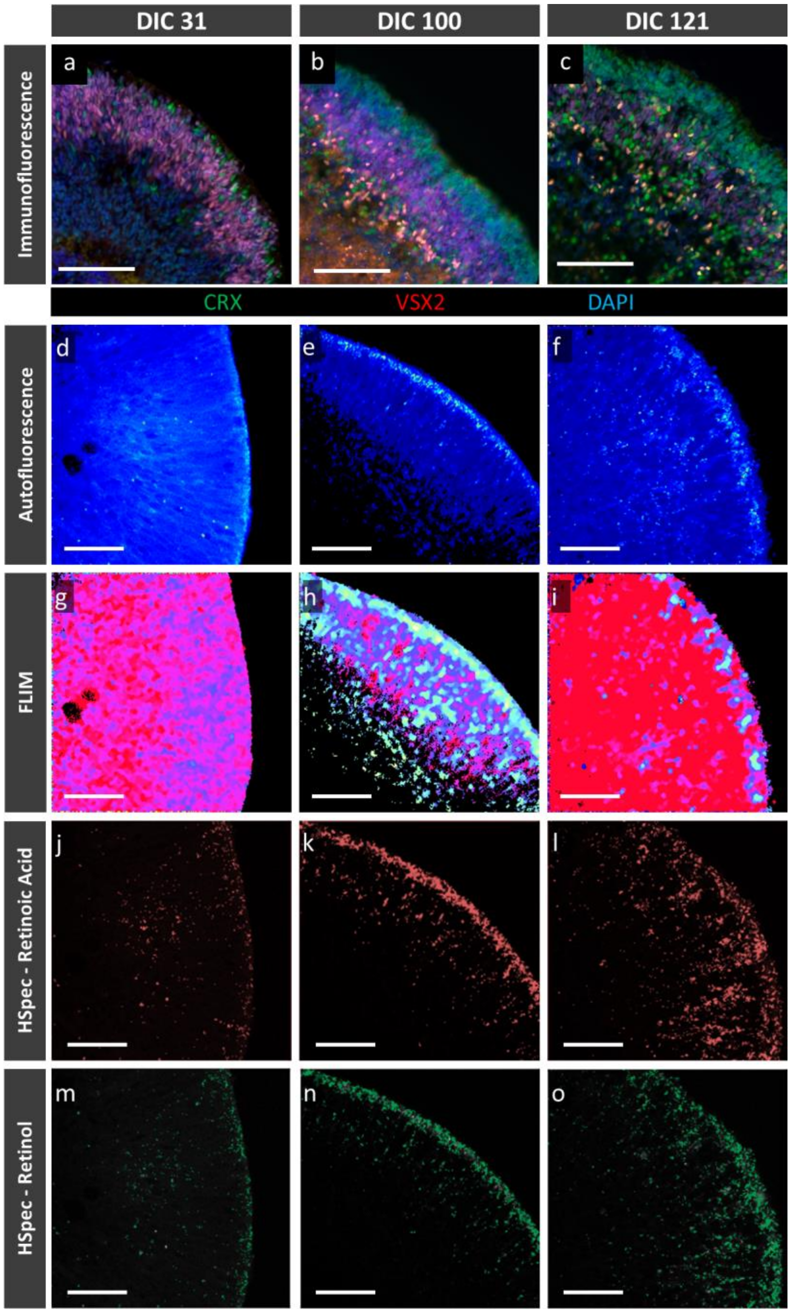
Supplementary Figure S2: Fluorescence lifetime imaging microscopy of 151 DIC old hESC-derived retinal organoid analyzed using a FLIM metabolic trajectory.[23] The total autofluorescent signal (a) is analyzed by decomposing each pixel into a light signal with decay profile modeled as a phasor histogram (b). The phasor model allows assignment of a metabolic signature to each pixel along a color-coded metabolic trajectory. The metabolic signature is used to color-code regions of the autofluorescent image to indicate metabolic states of different regions (c).[10, 11]



Supplementary Figure S3: Spectral domain OCT (SDOCT) imaging adult human retina and hESC-derived retinal organoids. **a,** Normal SDOCT of adult human macula. Retinal nuclear layers are hypointense (dark), whereas, axonal and dendritic connections in the inner and outer plexiform layers are hyperreflective (bright). **b-f**, Heidelberg spectralis images of 118 (b) and four 151 DIC (c-f) retinal organoids in tissue culture media showing a hyporeflective band at the organoid surface demarcated by complementary arrowheads. OCT of less mature organoids (g-n) demonstrate an absence of a superficial hyporeflective band. Scale bars, 0.5 mm. ONL, outer nuclear layer with Henle's fiber layer, OPL, outer plexiform layer, INL, inner nuclear layer, IPL, inner plexiform layer, GCL, ganglion cell layer, and NFL, nerve fiber layer.



Supplementary Figure **4**: iPSC-derived retinal organoids imaged with OCT demonstrate a hyporeflective surface band as early as 92 DIC. Scale bars, 1 mm



Supplementary Figure **5**: Human iPSC-derived retinal organoids of ages 31 DIC, 100 DIC and 121 DIC. (a-c) Immunofluorescence co-stained for retinal progenitor cell marker VSX2 (CHX10), photoreceptor marker CRX, and DAPI. (d-f) Total autofluorescence under 2-photon excitation at 740 nm and detection at 420-500 nm. (g-i) Fluorescence lifetime images. Hyperspectral signal with phasor analysis for retinoic acid (j-l) and retinol (m-o). Scale bars were 100 μm for immunofluorescence and 50 μm for 2-photon techniques.

Resolution Mode	High Resolution
Scan Focus	34.00 D
Camera Objective	Anterior Segment
Internal Target	None
External Target	ON
Examination Time	14:21:09 (UTC)
Examined Structure	Cornea
Application	Cornea
IR Image	
Scan Angle	20°
Size X	1024 pixel (11.1 mm)
Size Y	1024 pixel (11.1 mm)
Scaling	10.84 µm/pixel
ART Mode	ON (9 images averaged)
ART Normalization	ON
Sensitivity (DC/DC)	75%
Total Sensitivity	40
IR Laser Power	25%
Filter State	FA Filter
Lookup Table	Linear
ERG Mode	OFF
Auto-Brightness State	OFF
Grey Value Offset	0.887034
OCT Image	
Scan Angle	15°
Size X	768 pixel (8.3 mm)
Size Z	496 pixel (1.9 mm)
Scaling X	10.84 µm/pixel
Scaling Z	3.87 µm/pixel
ART Mode	ON (15 images averaged)
Eye Length	Unknown (1210)
Quality	22 dB
EDI Mode	OFF
OCT Scan Pattern	
Number of B-Scans	25
Pattern Size	15° x 3° (8.3 x 1.7 mm)
Distance between B-Scans	69 µm
Device	
Camera Model	Spectralis HRA+OCT (S3610-CIFP)
Camera S/N	006975
Power Supply S/N	006803
Touch Panel S/N	004840
HRA Camera FW Version	2.4.3.0
Power Supply FW Version	1.5.4.0
Touch Panel FW Version	1.6.0.0
OCT Camera FW Version	1.51.0.0
OCT Controller FW Version	1.3.2.0
OCT Camera FPGA Version	1.41.0.0
Acquisition Software Version	5.7.5.0

Supplementary Figure **6**: OCT imaging parameters used for organoids.