phenocell

— stem cells for better drugs —

Focus on AMD

Products and services to support drug discovery and research for
Age-related Macular Degeneration (AMD)

FOCUS on AMD

Age-related Macular Degeneration (AMD) is the **leading cause of blindness** in the elderly population, currently affecting around **200 million people** worldwide[1]. And with an aging population, rates of AMD are predicted to **increase by 44%** by 2040[1].

AMD is characterized by the **progressive loss of central vision** and can be split into two broad types: **"wet" AMD** (~10% of cases) and **"dry" AMD**, which accounts for around **90%** of all AMD cases. Despite the dominant prevalence of dry AMD, there is currently **no treatment**[2,3].

To address this pressing need for dry AMD therapies, scientists are turning to *in vitro* models that use **human iPSCs** from healthy and AMD patient donors. The cells generated from these iPSCs **retain the donor characteristics**, enabling researchers to generate iPSC-derived cells that can fuel **advanced *in vitro* AMD models** for research and to screen potential therapies on a more human-relevant platform.

**1** Wong WL et al. doi: 10.1016/S2214-109X(13)70145-1. Epub 2014 Jan 3. PMID: 25104651.
2 Ambati J, Fowler BJ. doi: 10.1016/j.neuron.2012.06.018. PMID: 22794258; PMCID: PMC3404137.
3 Fleckenstein M et al. doi: 10.1038/s41572-021-00265-2. PMID: 33958600.

Human iPSC-derived retinal pigment epithelium cells

The human retina is in direct contact with a monolayer of pigmented cells called the **retinal pigment epithelium** (RPE). The RPE plays an essential role in the **maintenance and survival** of photoreceptors, including the **phagocytosis of cellular debris** produced daily by photoreceptors.

Over time, there is accumulation of a molecule called **N-retinylidene-N-retinylethanolamine (A2E)** which undergoes photoexcitation to produce **reactive oxygen species** and **cellular damage**. This damage can ultimately cause the death of RPE cells and, therefore, of the photoreceptors that depend on them. Over time, the progressive atrophy and death of photoreceptors in the macula of the retina can lead to the development of **AMD**.
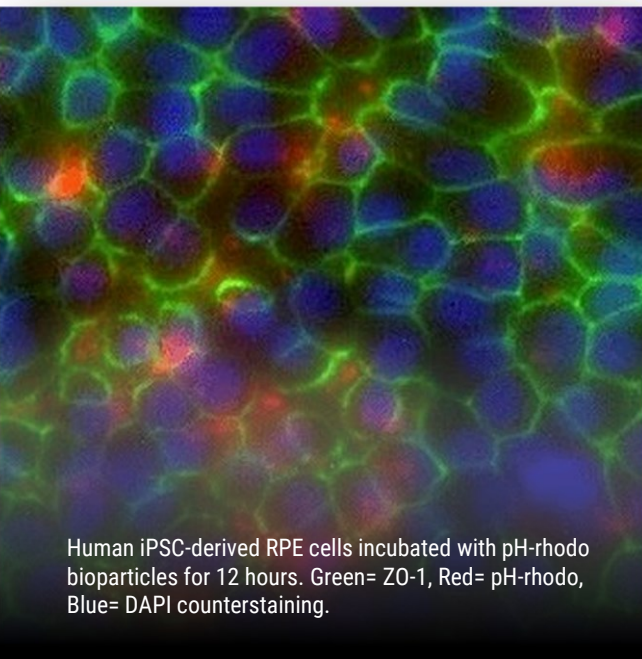
Human iPSC-derived RPE cells incubated with pH-rhodo bioparticles for 12 hours. Green= ZO-1, Red= pH-rhodo, Blue= DAPI counterstaining.

Key highlights of our iPSC-derived RPE cells include:

- Exhibit typical **pigmented** and **cobblestone** morphology
- Display the key **phenotypic and functional characteristics** of primary human RPE cells including **key marker expression** (MITF, ZO-1, PMEL17) and **phagocytosis**
- Display a **normal karyotype** with **high genomic stability**
- Available in **bulk quantities** with excellent batch-to-batch and inter-batch **reproducibility**

Fuel your *in vitro* dry AMD models with our iPSC-derived RPE cells to build a **robust and relevant tool** for your drug screening campaigns and Hit/Lead validation.

Developing faithful *in vitro* dry AMD models

Characterization of iPSC-derived RPE cells

We have **extensively characterized** our iPSC-derived RPE cells for phenotypic and functional relevance.

Key highlights include:
- Expression of **key markers** (MITF, ZO-1, PMEL17) by immunostaining (Fig 1A.)
- Typical **pigmented** and **cobblestone** morphology (Fig 1B.)
- High **purity**, with >95% PMEL 17 expression on flow cytometry (Fig 1C.)
- Outer retinal barrier resistance measured via volt/ohm meter
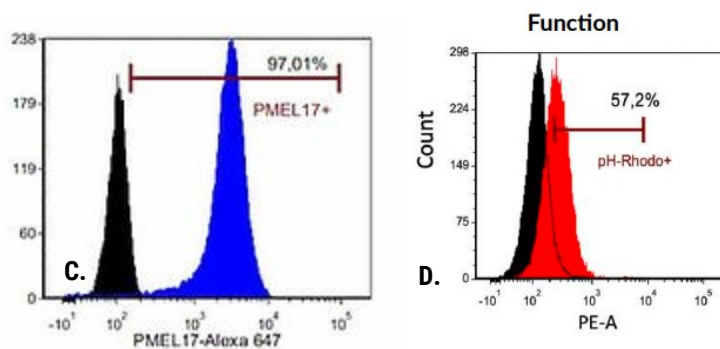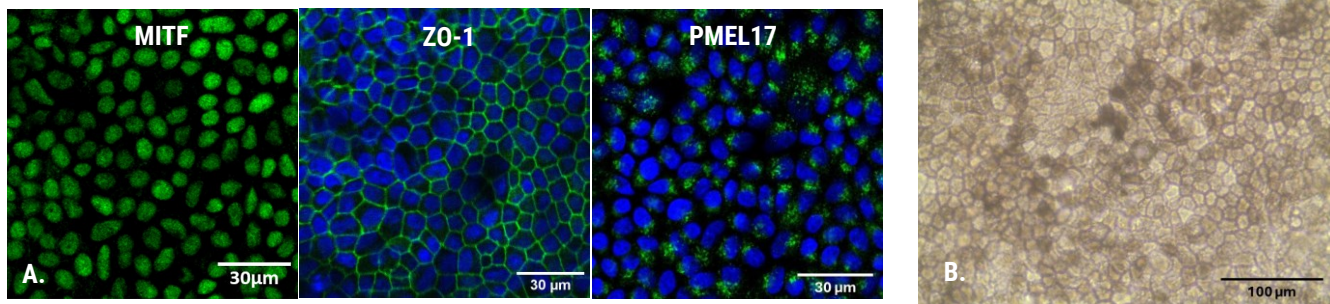- Functional relevance in phagocytosis assay (Fig 1D.)

Figure 1. Characterization of iPSC-derived RPE cells.
1A: ICC images demonstrating expression of key markers MITF, ZO-1 and PMEL17.
1B: Microscopy image demonstrating typical cobblestone morphology and pigmentation of iPSC-derived RPE cells.
1C: Flow cytometry demonstrating >95% PMEL 17 expression (blue) against isotype control (black).
1D: The phagocytotic potential of iPSC-derived RPE cells was assessed using a pH-Rhodo bioparticle assay (Thermo Fisher), demonstrating phagocytotic activity in 57.2% of the RPE cells, which is within the range described in the literature.

Recapitulating AMD pathophysiology

We utilize **chronic N-retinylidene-N-retinylethanolamine (A2E)** treatment combined with **blue light irradiation** to induce apoptosis, oxidative stress and inflammation. This helps to better reproduce pathological AMD conditions to produce more human-relevant AMD models.
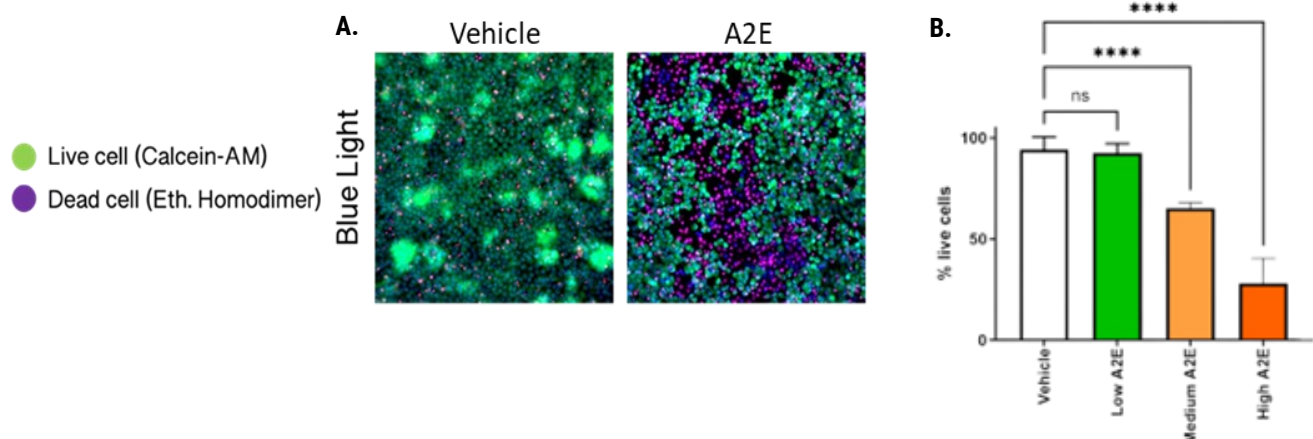

Figure 2. A2E treatment combined with blue light can reproduce AMD conditions. **2A**: Images demonstrating enhanced cell death with combination treatment (A2E and blue light) versus vehicle control and blue light. **2B**: Increasing A2E concentrations produce a significant increase in cell death. This assay was used to guide the most appropriate concentration of A2E for chronic treatment. Ns: $P > 0.05$, **** $P \leq 0.0001$.

Demonstrating dry AMD disease phenotype

We have performed extensive characterization to validate our dry AMD model against key hallmarks of the disease, including pro-inflammatory cytokine release (Fig 3A.), AMD-associated drusen protein expression (Fig 3B.), oxidative stress (Fig 3C.) and complement immunostaining (Fig 3D).
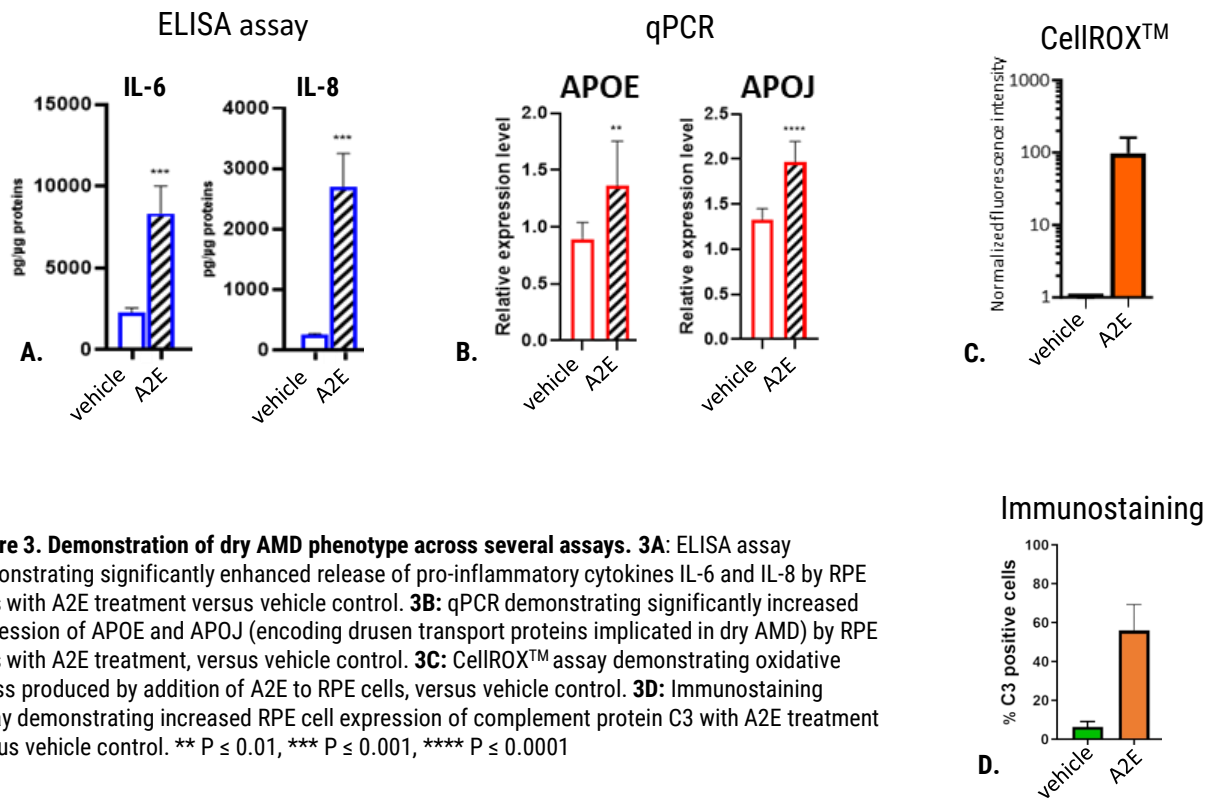

Figure 3. Demonstration of dry AMD phenotype across several assays. 3A: ELISA assay demonstrating significantly enhanced release of pro-inflammatory cytokines IL-6 and IL-8 by RPE cells with A2E treatment versus vehicle control. **3B:** qPCR demonstrating significantly increased expression of APOE and APOJ (encoding drusen transport proteins implicated in dry AMD) by RPE cells with A2E treatment, versus vehicle control. **3C:** CellROX™ assay demonstrating oxidative stress produced by addition of A2E to RPE cells, versus vehicle control. **3D:** Immunostaining assay demonstrating increased RPE cell expression of complement protein C3 with A2E treatment versus vehicle control. ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$

iPSC products for dry AMD research

Human iPSC-RPE lines

We can support your dry AMD model with human iPSC lines differentiated from **healthy control donors.**

If you would like more information on our dry AMD iPSC lines, please **get in contact**.

Optimized culture medium

PhenoCULT©-RPE has been optimized for post-thaw recovery and amplification of iPSC-derived RPE cells.

It is suitable for use on human iPSC-derived RPE cells and primary RPE cells and is available in 100ml and 500ml formats.

RPE kit

Our all-in-one RPE kit includes cryopreserved iPSC-derived RPE cells with optimized media and supplements. This kit offers a complete, ready-to-use solution for dry AMD research and drug discovery.

Kit contents:
- 1 vial of PCi-RPE cells (2 M cells/0.5 mL)
- 1 bottle of PhenoCULT®-RPE culture medium (100 mL)

High-throughput screening services for dry AMD research & testing

We're applying our extensive technical expertise and operational excellence to offer **a range of outsourced services** for dry AMD research. Let us do the **scientific "heavy lifting",** freeing you up to focus on the science.

Below you can find our main **high-throughput bioassays** for iPSC-derived retinal pigment epithelial cells. If you have a custom request, please contact us for more information.

Area	Name	Method	Endpoint	Reference
Retinal disorders	VEGF (growth factor) secretion	OD reading	VEGF concentration	RPE-VEGF
Retinal disorders	PEDF (growth factor) secretion	OD reading	PEDF concentration	RPE-PEDF
Retinal disorders	Outer retinal barrier resistance	Volt/Ohm meter	Resistance in Ohm/cm²	RPE-ORB
Retinal disorders	Phagocytosis assay	Bioparticles labeling	Normalized fluorescence	RPE-PHAG
Retinal disorders	mRNA expression of characterized markers	RT-qPCR	Relative expression level	RPE-RNA
Pigmentation	TYRP1 expression	Immunolabeling	Normalized fluorescence	RPE-TYRP1
Pigmentation	Melanin content	OD reading	Melanin concentration	RPE-MEL
Pigmentation	PMEL17 expression	Immunolabeling	Normalized fluorescence	RPE-PMEL17
Oxidative stress	Cellular oxidative stress	CellROX™ Staining	Normalized fluorescence	RPE-CRX
Oxidative stress	Mitochondrial Oxidative Stress	Immunolabeling	Normalized fluorescence	RPE-MSX
Identification	ZO1-expression	Immunolabeling	Cell population purity	RPE-ZO1
Identification	MITF expression	Immunolabeling	Cell population purity	RPE-MITF
Cell viability	Cell viability / survival	Calcein AM - EthD1	Living cells / dead cells	RPE-LCC
Apoptosis	RPE apoptosis	TUNEL staining	Normalized fluorescence	RPE-TUN

What does a typical service project look like?

While every project is unique, we utilize **established workflows** to ensure projects are **conducted efficiently** with a kick-off meeting, open communication and full data sharing.

Management of your R&D Project

Preliminary study
NDA
Project framework
Feasibility study
Quotation
Service agreement order

Experiment
Compounds reception
RPE culture
Testing & Bioassays

Data management
Quality control
Data analysis
Data mining

Report
Report writing
Report meeting