hTERT-immortalized Primary Epidermal Cells: Key Components in Complex Toxicological Models

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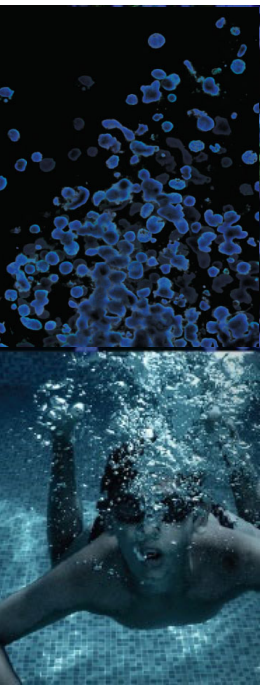
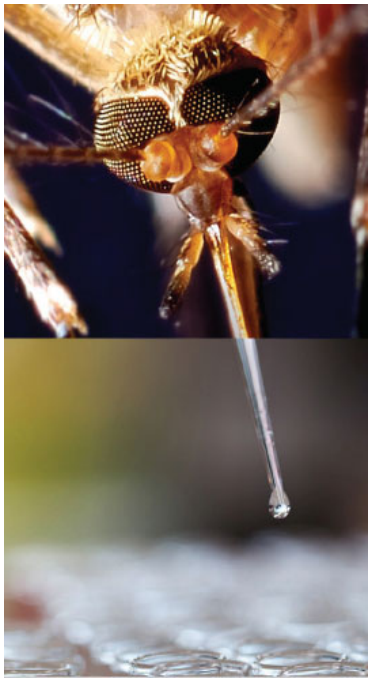
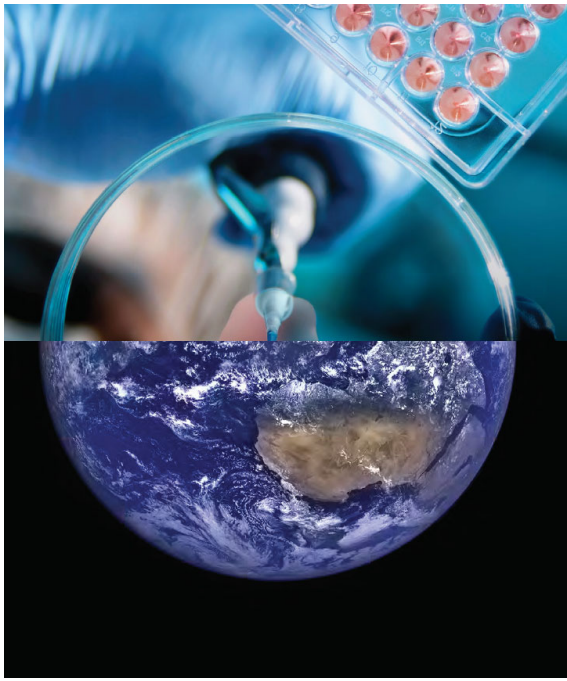
Credible Leads to Incredible™







About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"
- Innovative R&D company featuring gene editing, differentiated stem cells, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 550+ employees, over one-third with advanced degrees

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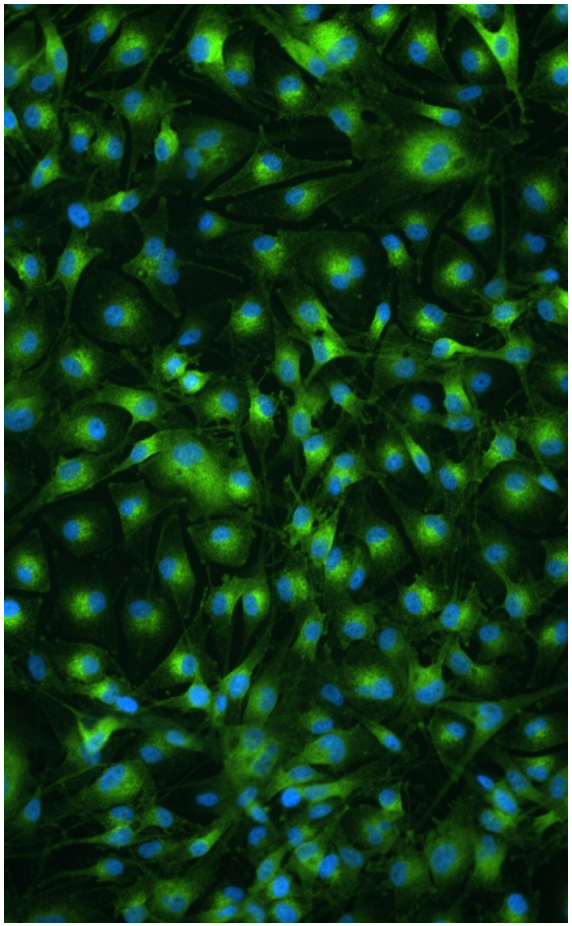
Agenda

- Epidermal biology background
- Applications for dermal cell models
- Comparison of various cell models (primary and immortal)
- Immortalized cell models – key characteristics
- hTERT-immortalized keratinocytes – data
- hTERT-immortalized melanocytes – data
- hTERT-immortalized fibroblasts – data



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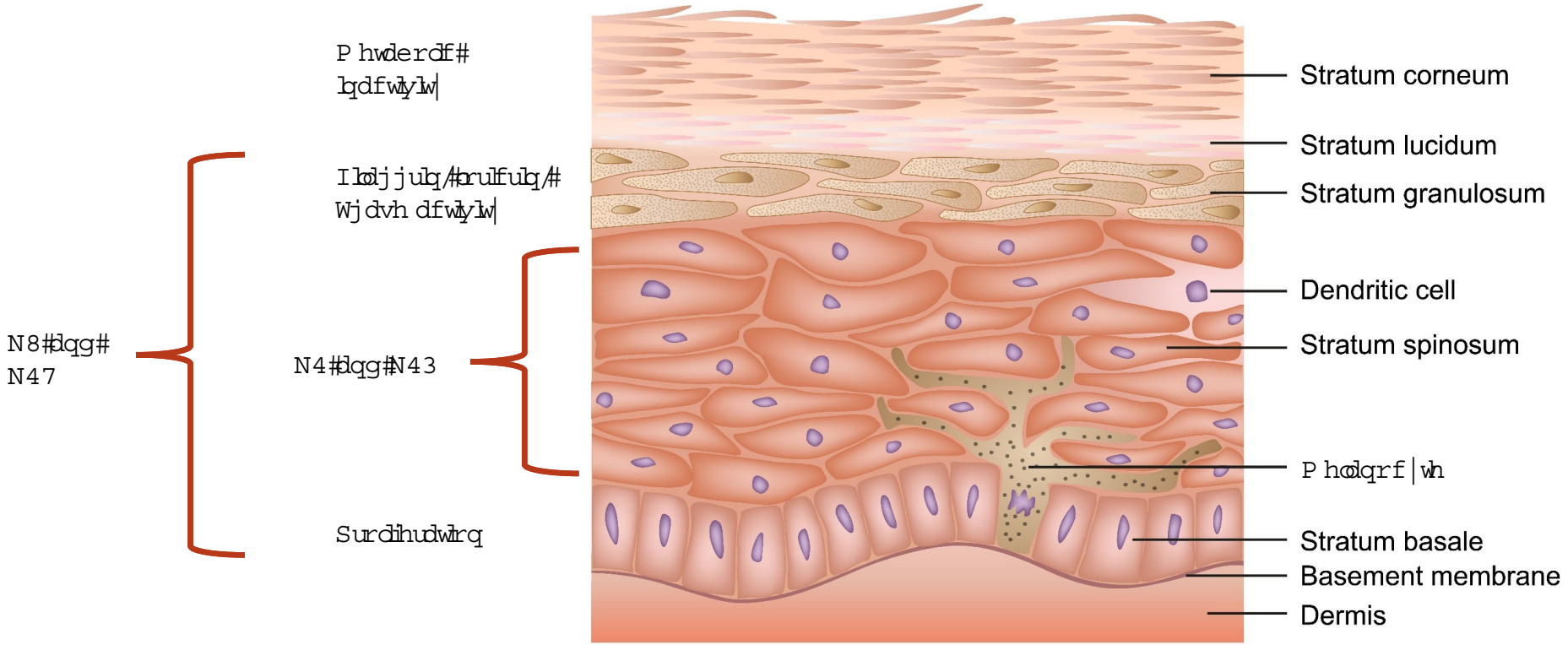
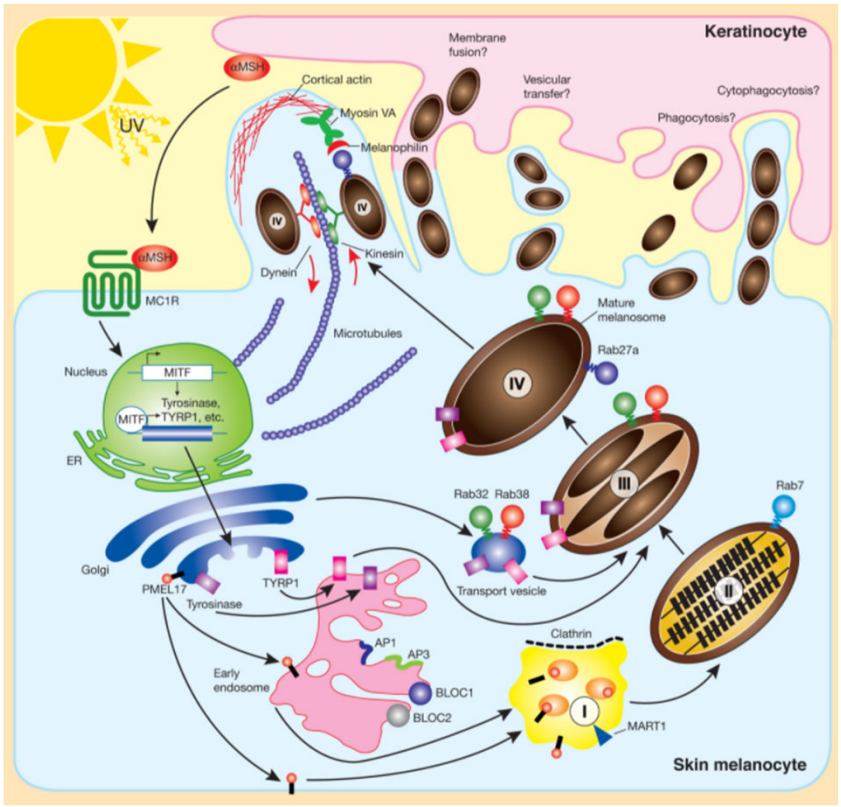
Epidermal Stratification, Keratinization, and Cornealization

A multi-step process resulting in barrier formation



Skin Pigmentation Background – Step 1

First main step – complex cellular and biochemical process to produce and package melanosomes



Melanosome biogenesis – 4 distinct phases:

I. Non-pigmented, pre-melanosome vacuole
II. Acquire striations
III. Striations receive pigment deposits
IV. Transported to membrane for exocytosis

Journal of Cell Science 2008 121: 3995-3999

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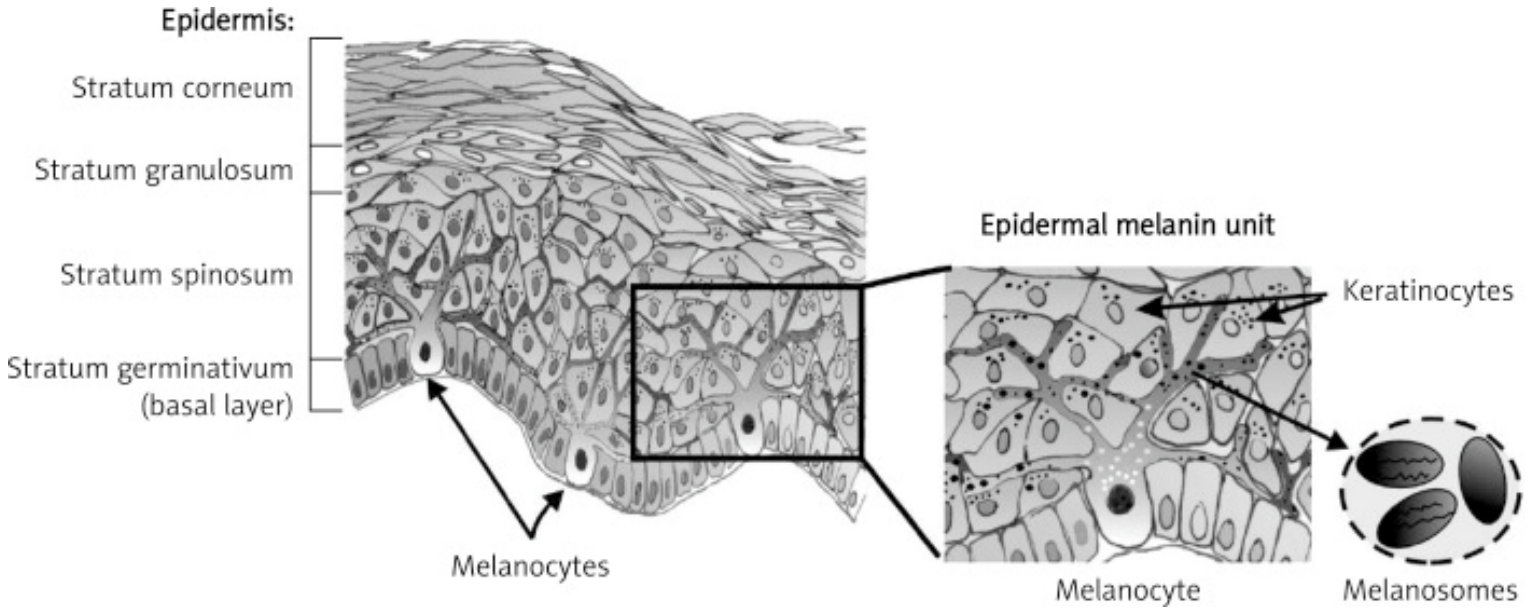
Skin Pigmentation Background – Step 2

Second main step: stored in neighboring keratinocytes – protects underlying tissue



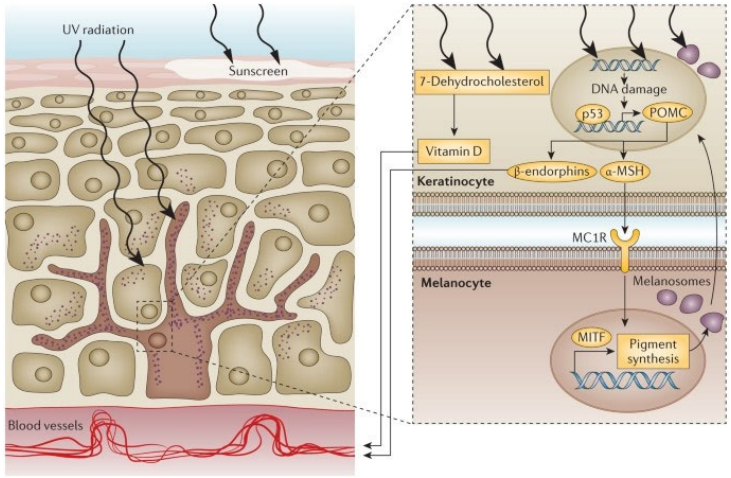
Melanosomes are exocytosed (by melanocytes) then endocytosed by adjacent keratinocytes

Postepy Dermatol Alergol. 2013 Feb; 30(1): 30–41.

Applications of Dermal Cell Models – Toxicology

1. Reliable reagent for traditional toxicology (LD_{50}): Skin corrosion testing, and cosmetics testing
2. Advanced Toxicology: Understand the complex interplay of genetic background and environmental agents
3. Toxicology of chemotherapeutic agents: Melanoma, basal cell carcinoma, and squamous cell carcinoma
4. Develop treatments: Skin conditions such as hypopigmentation/hyperpigmentation and psoriasis

https://www.chemicalindustryjournal.co.uk/your-guide-to-in-vitro-skin-corrosion-testing



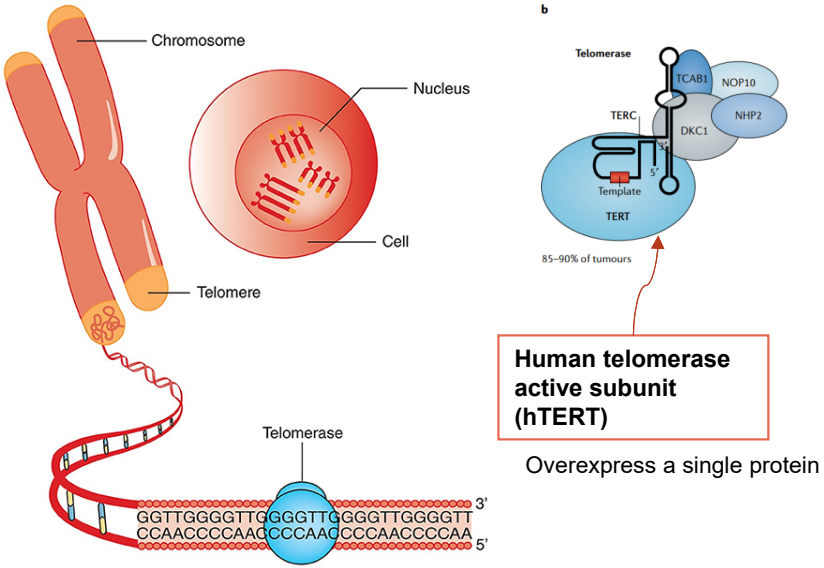
**Nature Reviews Disease Primers volume 1, Article number: 15003 (2015)*

https://www.chemistryworld.com/news/fluorinated-compounds-in-cosmetic-products/3009868.article

Cell Immortalization Processes

Quick note about process

Telomerase – prevents cell aging



Human telomerase active subunit (hTERT)

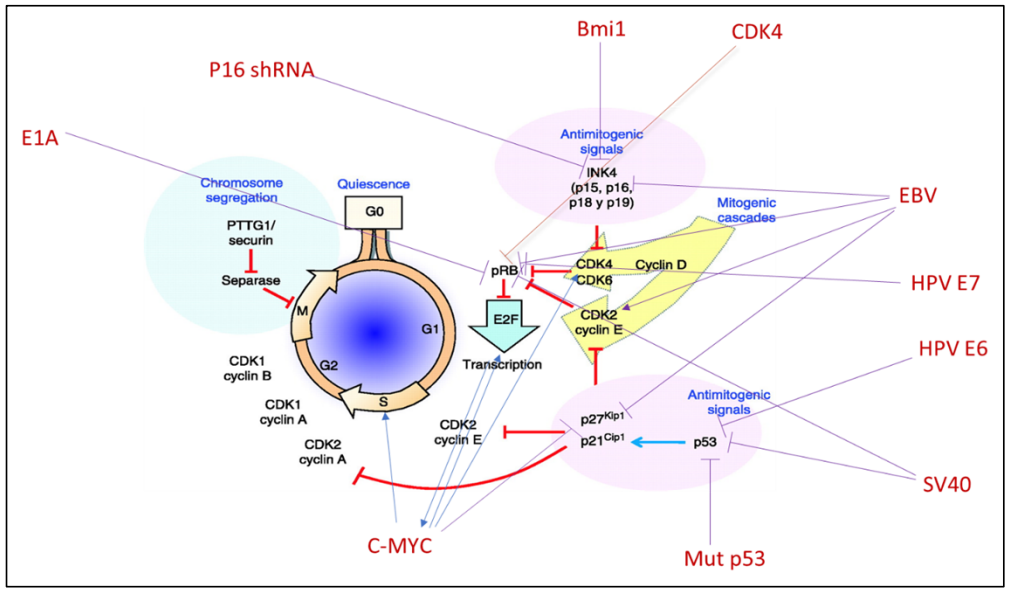
Overexpress a single protein

hTERT: human telomerase reverse transcriptase

Front. Genet., 21 January 2021 | https://doi.org/10.3389/fgene.2020.630186

Nat Rev Genet 20, 299–309 (2019).

Cell cycle – removes stops or otherwise encourages the cell cycle



Immortalization using telomerase differs from methods where cell cycle proteins are inhibited or overexpressed.

ATCC has expertise in several methods

Characteristics of various cell models

	Continuous (cancer) cell lines	Primary cells	hTERT-immortalized primary cells
Mimic *in vivo* characteristics	+	++++	+++
Proliferative capacity	+++	+	+++
Experimental reproducibility	+++	+	+++
Predictability in toxicological studies	+	+++	+++
Genomic stability	Aneuploid	Diploid	Diploid/near diploid
Supply	+++	+	+++
Cost	+++	+	++
Ease of use	+++	+	++

Primary: Ideal when donor diversity is needed
Immortalized: Ideal for screening or when a consistent source is needed

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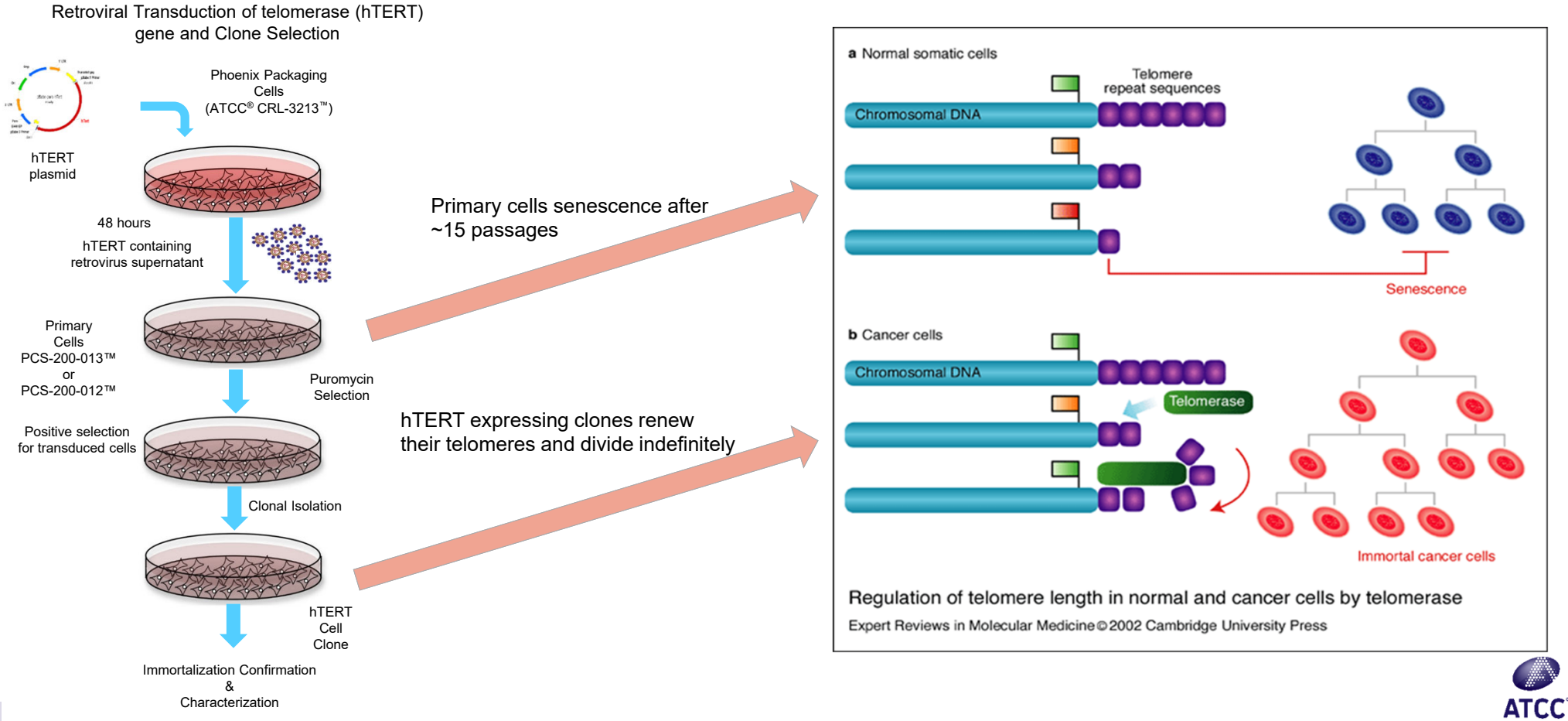
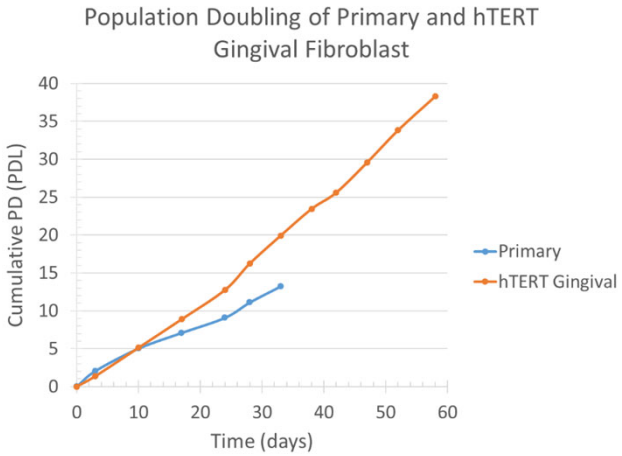
Cell Immortalization Process

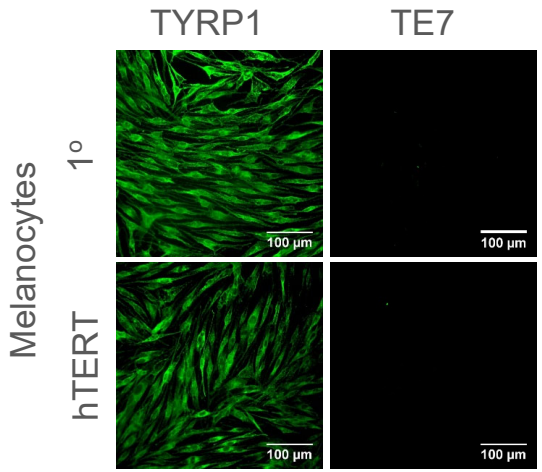
Melanocytes have been immortalized by expression of human telomerase gene

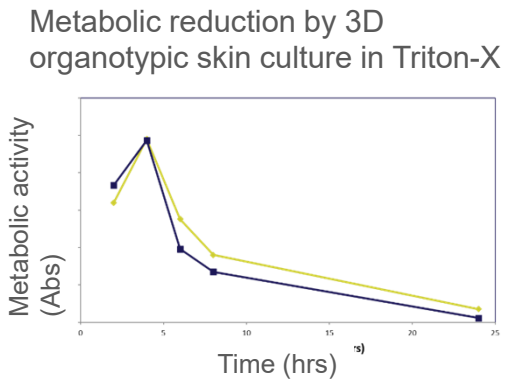


hTERT-immortalized Cells – Key Characteristics

- Growth:
  - Cells retain replicative capacity ("immortalized")
- Morphology and marker expression:
  - Similar to primary cells
    - Do epithelial cells still express epithelial markers?
    - Are they still negative for fibroblast markers?
- Toxicology responses:
  - Within expected range, similar to primary cells

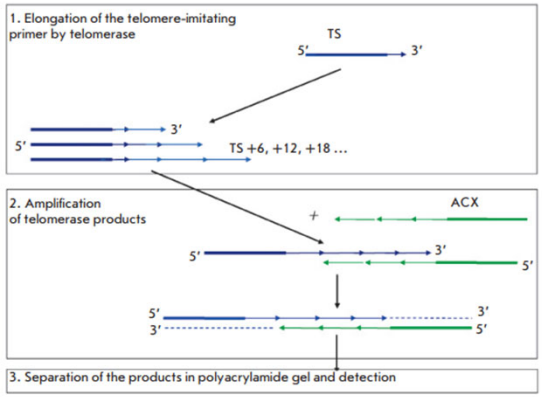


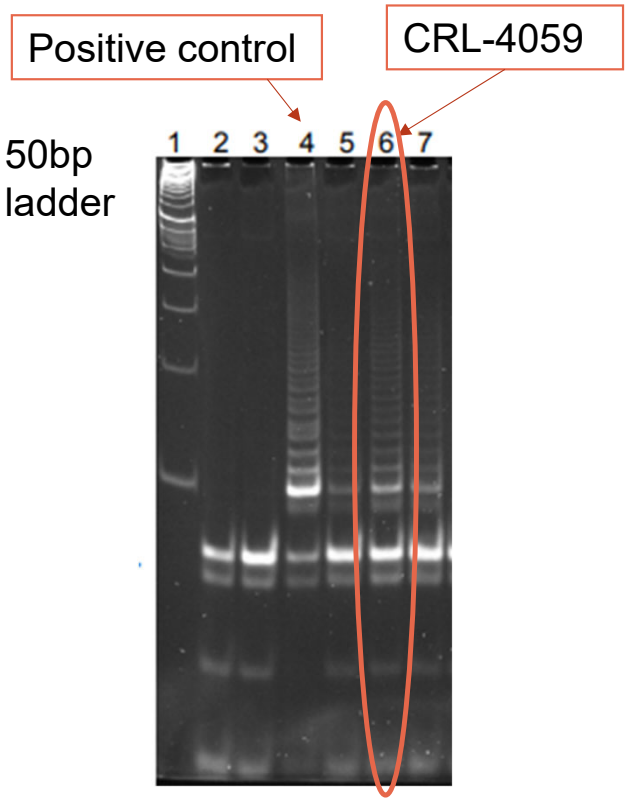


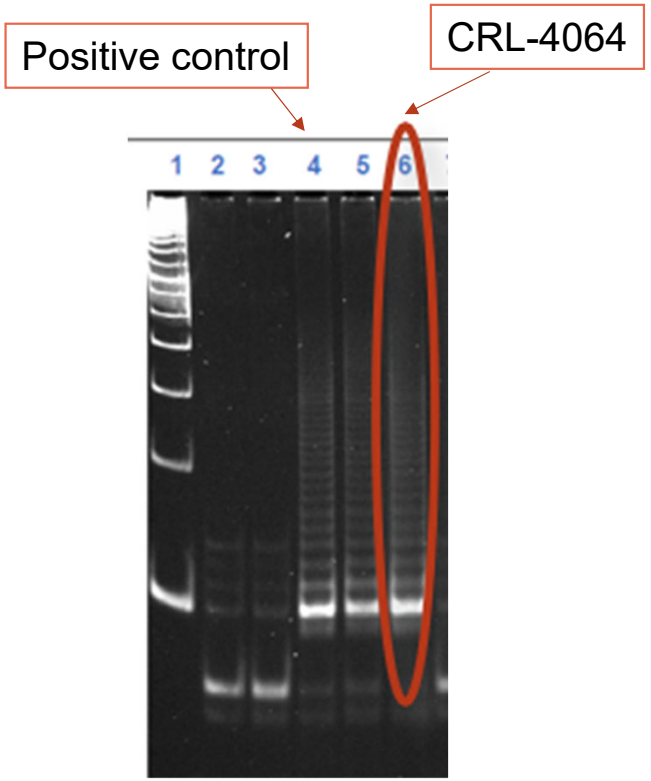


Confirmation of hTERT Expression by TRAP Assay

Telomerase Reverse Transcriptase Amplification Protocol (TRAP)







Assays for detection of telomerase activity. Acta Naturae. 2011 Jan;3(1):48-68. PMID: 22649673

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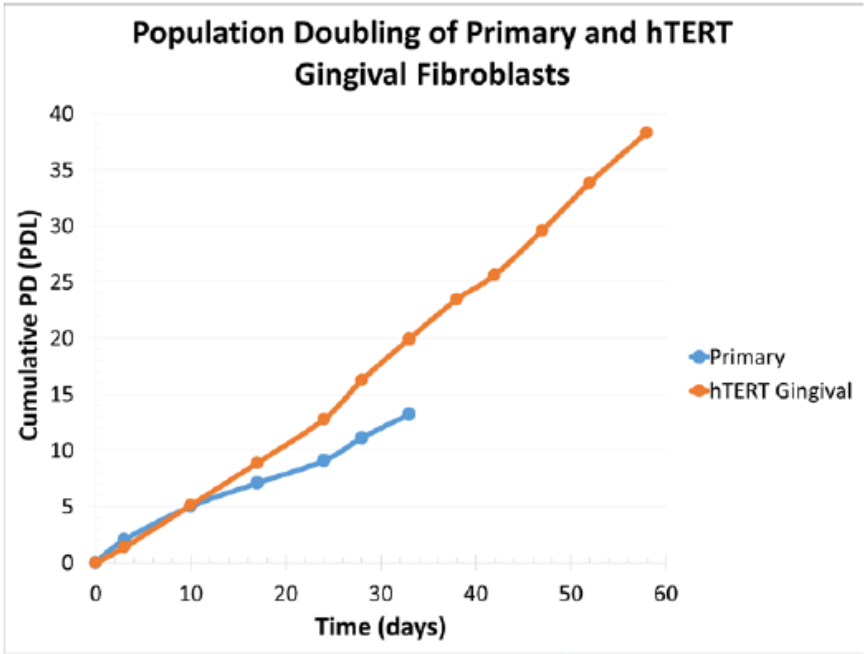
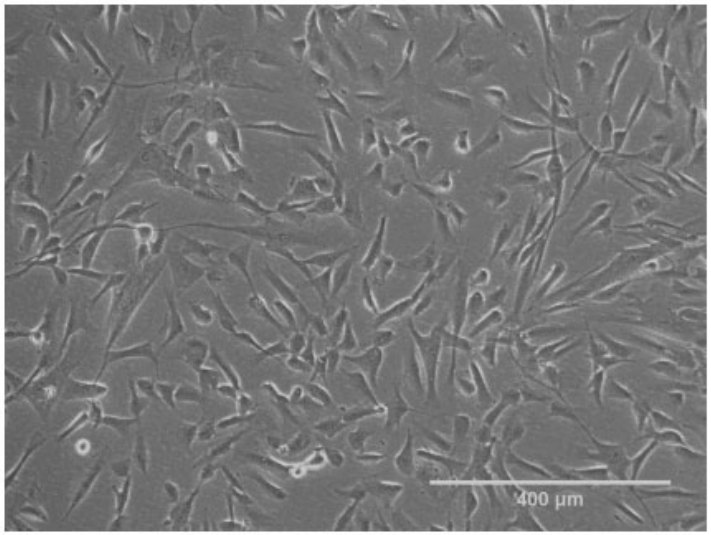
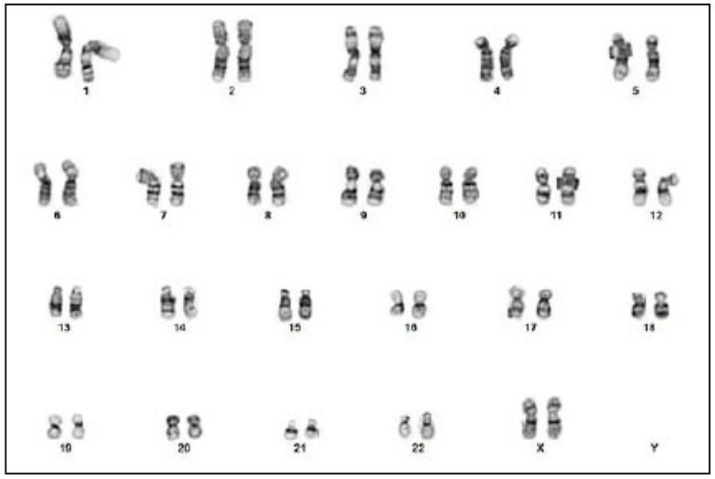
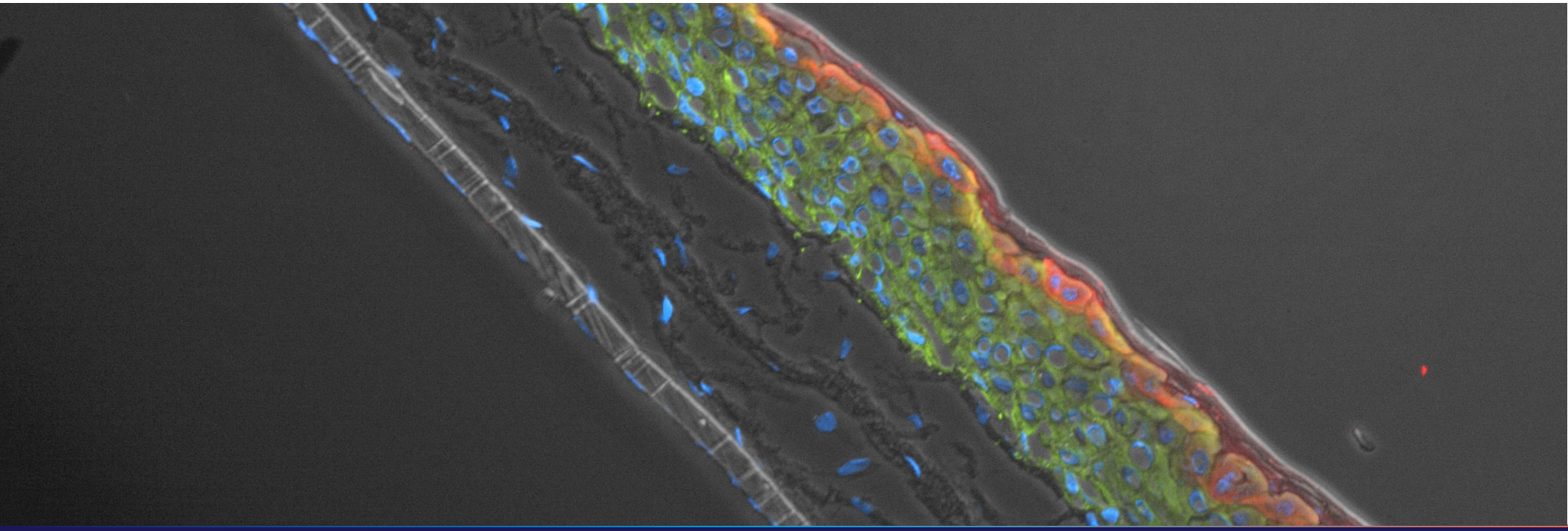
Cell Immortalization, Morphology, and Karyotype of hTERT-immortalized Fibroblasts Cell Lines





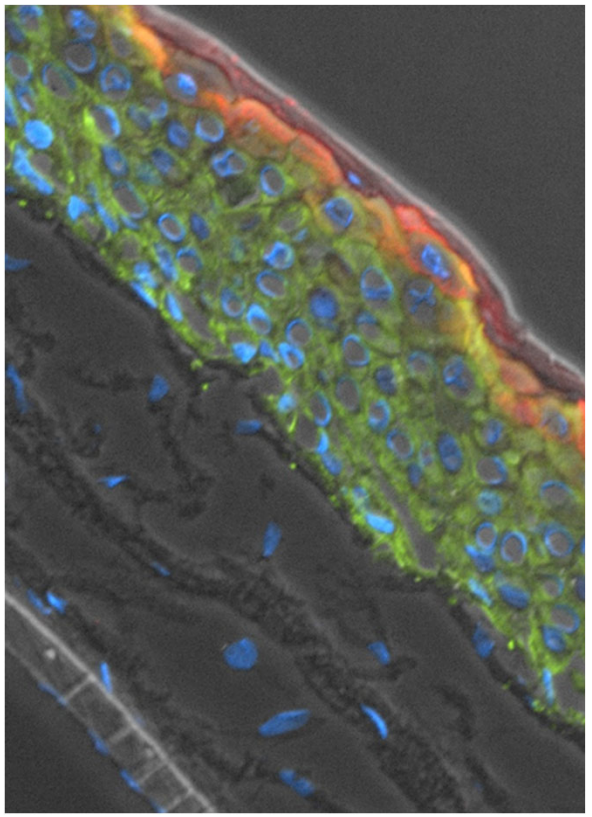




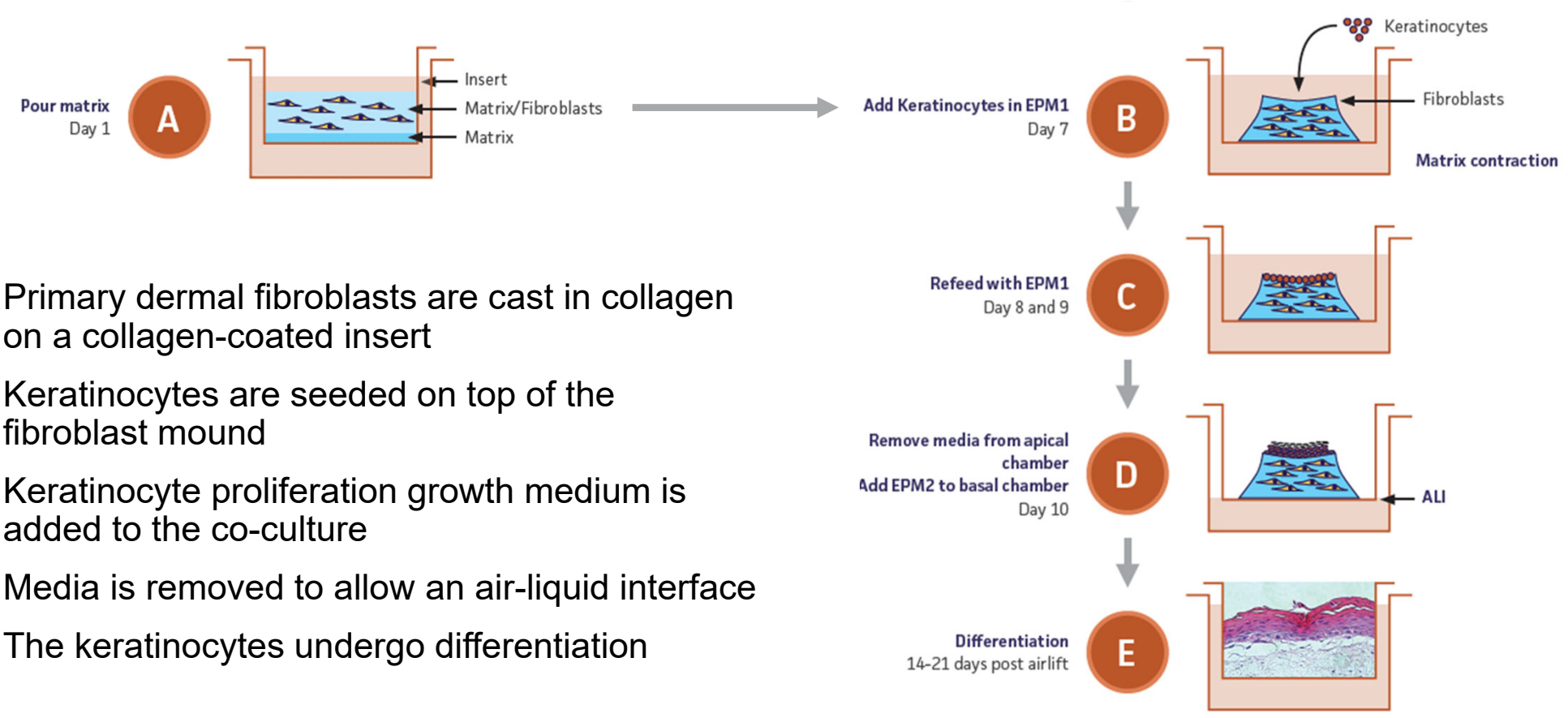
hTERT Immortalized Dermal Keratinocytes - Data

ATCC Epidermal Keratinocyte Models

- ATCC provides several keratinocyte cell lines to support research and development efforts
- From basic research through discovery and development to product testing
  - Primary cells
    - Primary Epidermal Keratinocytes, Adult (ATCC® PCS-200-011™)
    - Primary Epidermal Keratinocytes, Neonatal (ATCC® PCS-200-010™)
  - hTERT-immortalized primary cells
    - Ker-CT, Adult (ATCC® CRL-4048™)
- Portfolio features
  - Reliability
  - Fully characterized cells
  - Optimized growth protocols
  - Scalable to research needs
  - Biological relevancy



Overview of Co-culture of Keratinocytes and Fibroblasts



A. Primary dermal fibroblasts are cast in collagen on a collagen-coated insert

B. Keratinocytes are seeded on top of the fibroblast mound

C. Keratinocyte proliferation growth medium is added to the co-culture

D. Media is removed to allow an air-liquid interface

E. The keratinocytes undergo differentiation

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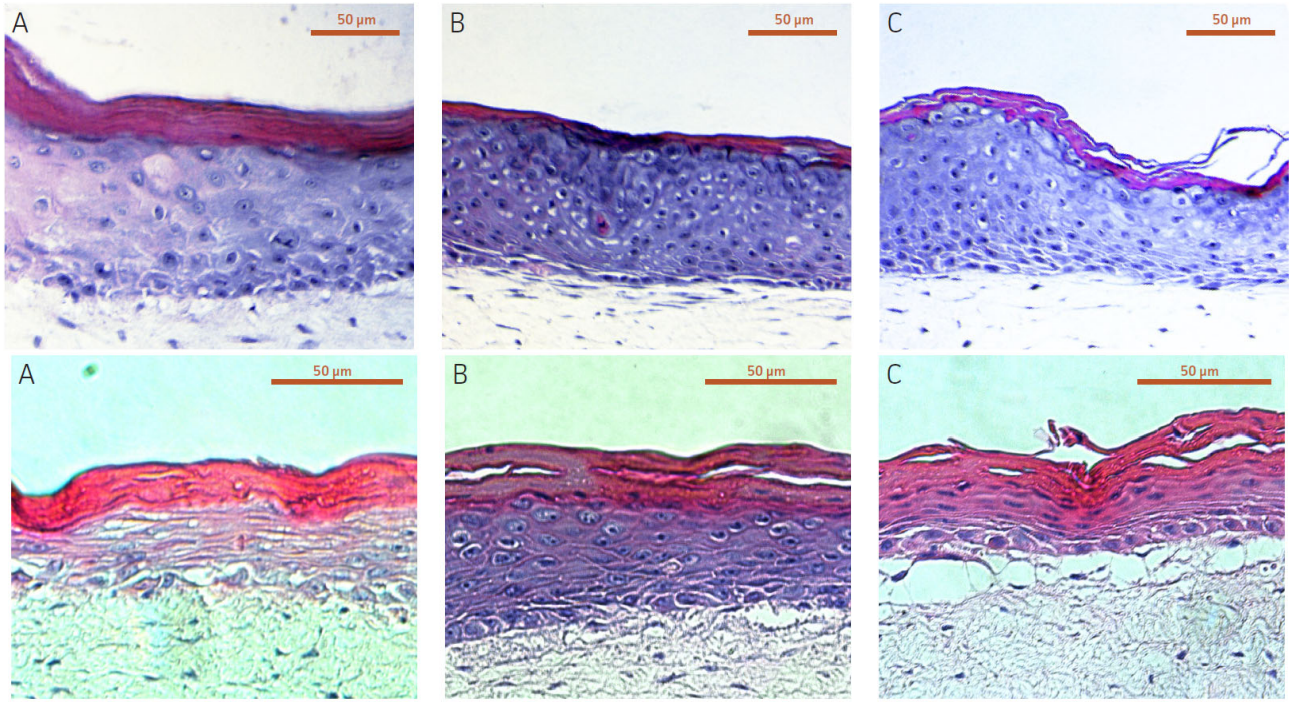
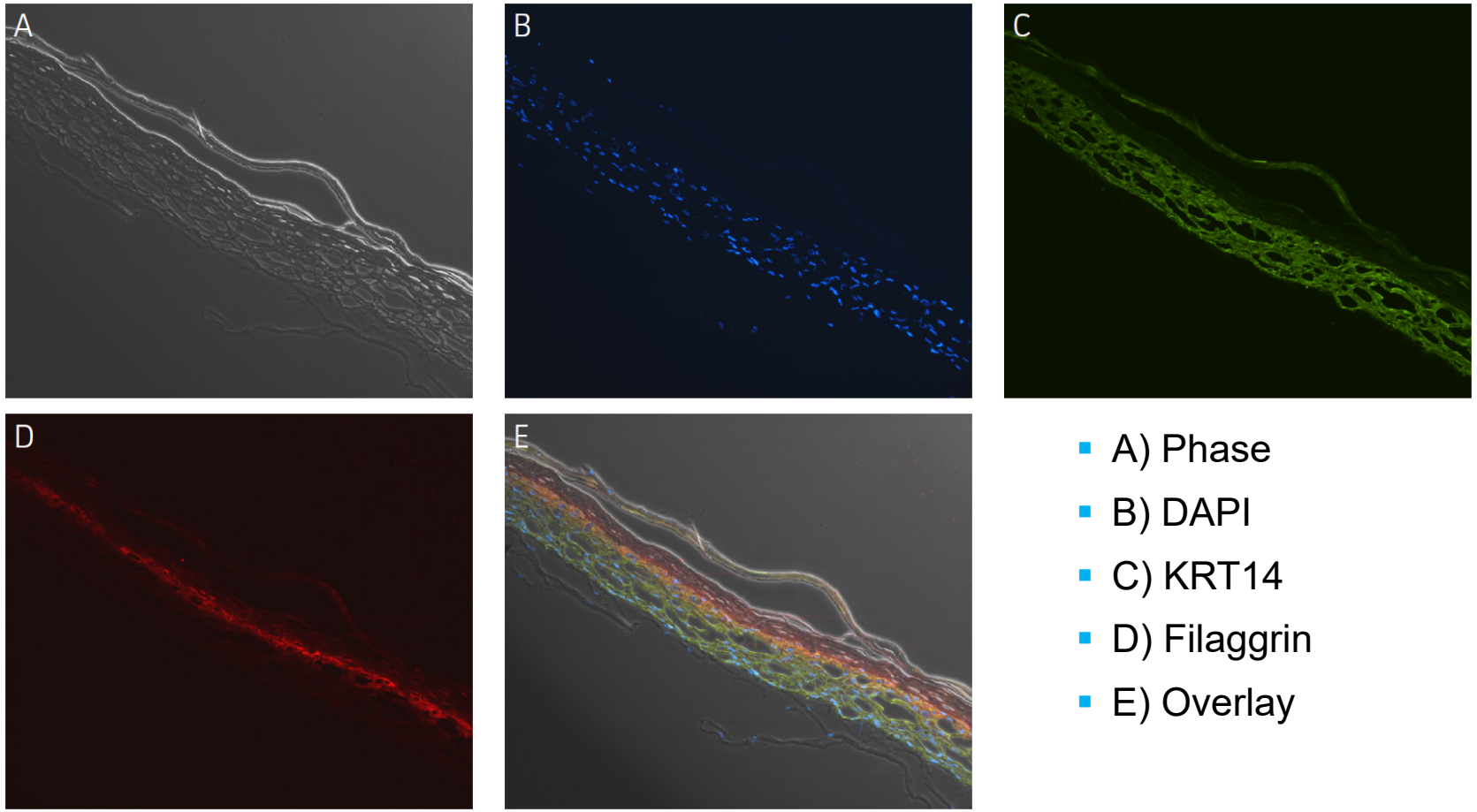
Micrograph of primary foreskin keratinocytes and Ker-CT at low and high passage

- A) primary foreskin keratinocytes at passage 2
- B) Ker-CT at passage 6
- C) Ker-CT at passage 15
- Top panels 11 days post ALI culture
- Bottom panels 21 days post ALI culture

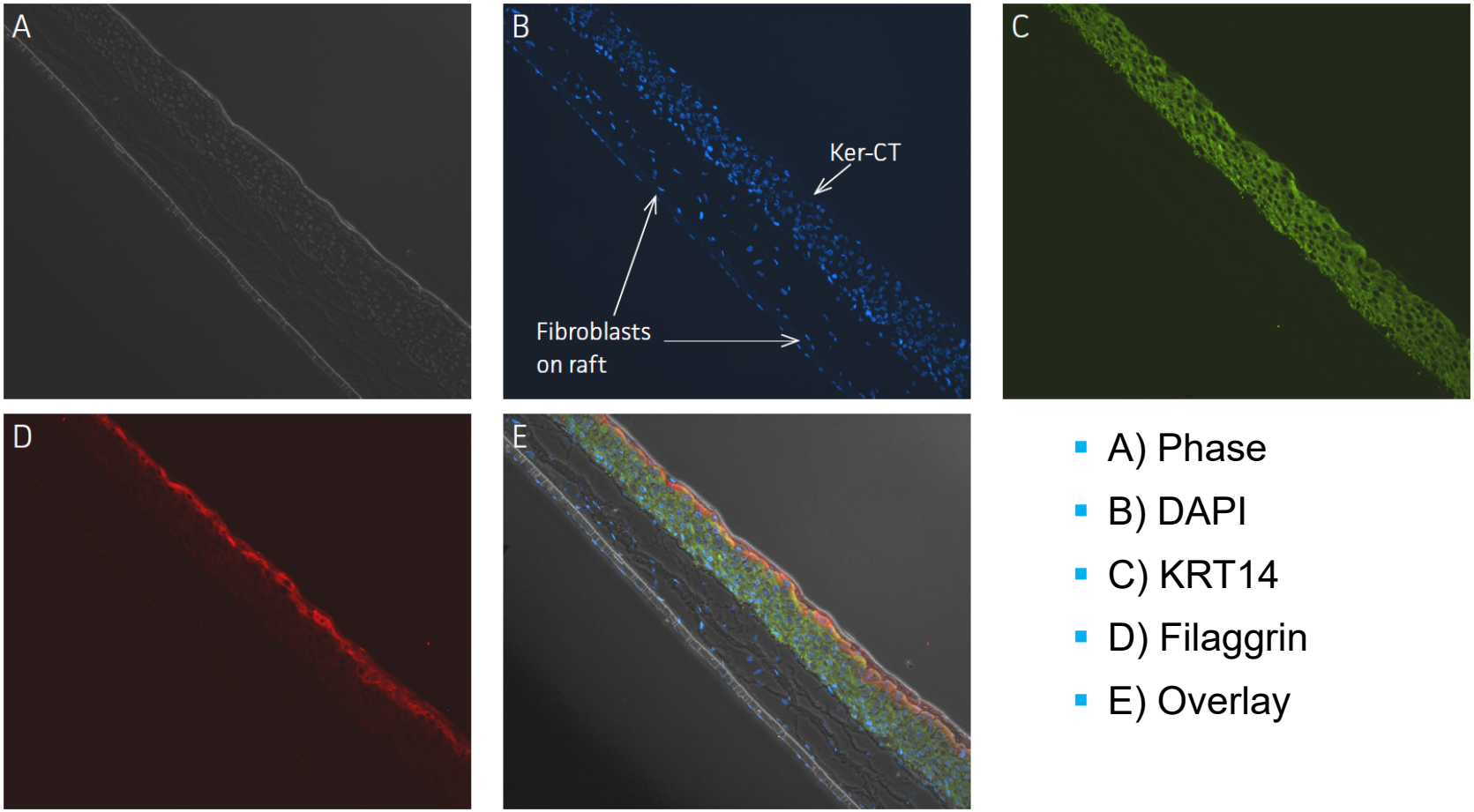


Primary keratinocytes cultured at ALI display similar architecture to skin in vivo

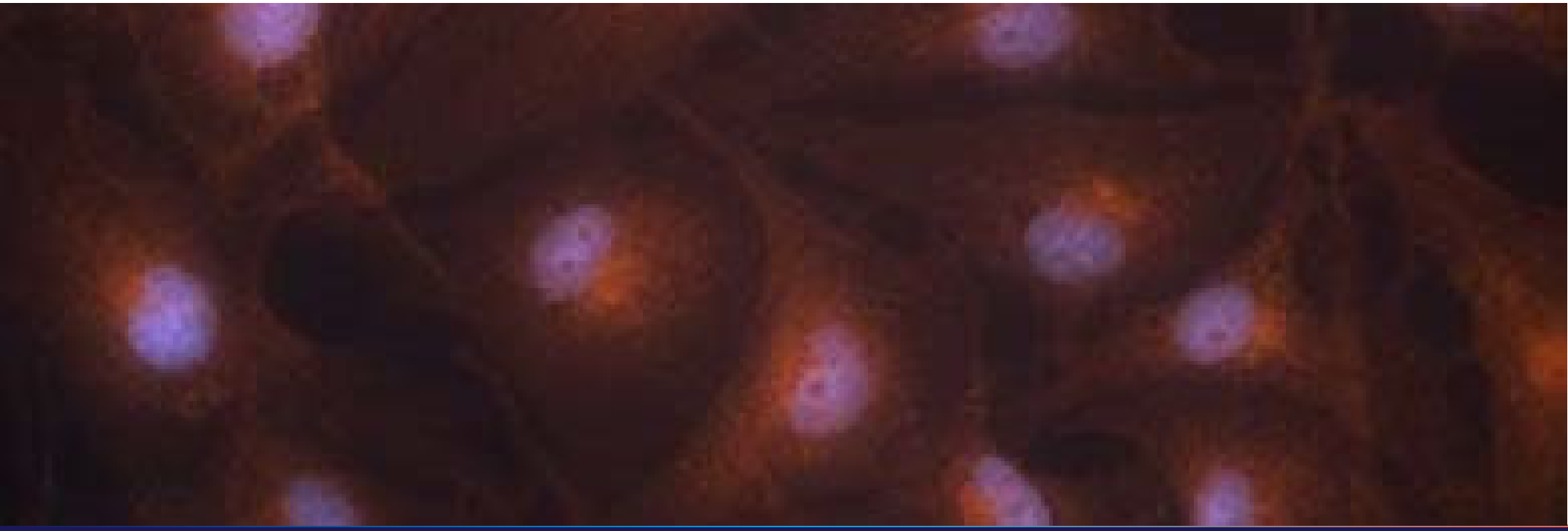


- A) Phase
- B) DAPI
- C) KRT14
- D) Filaggrin
- E) Overlay

Ker-CT cultured at ALI display similar architecture to skin in vivo



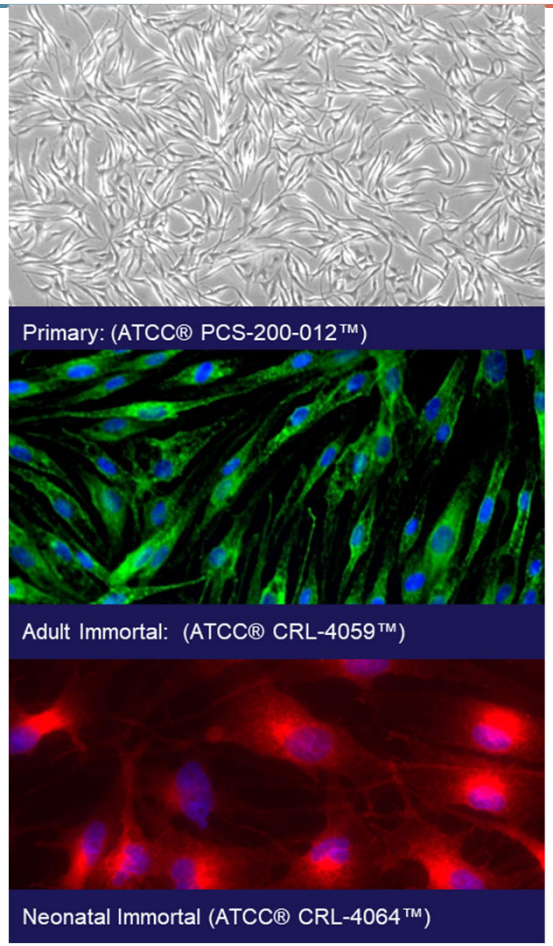
- A) Phase
- B) DAPI
- C) KRT14
- D) Filaggrin
- E) Overlay



hTERT Immortalized Dermal Melanocytes - Data

ATCC Melanocyte Models

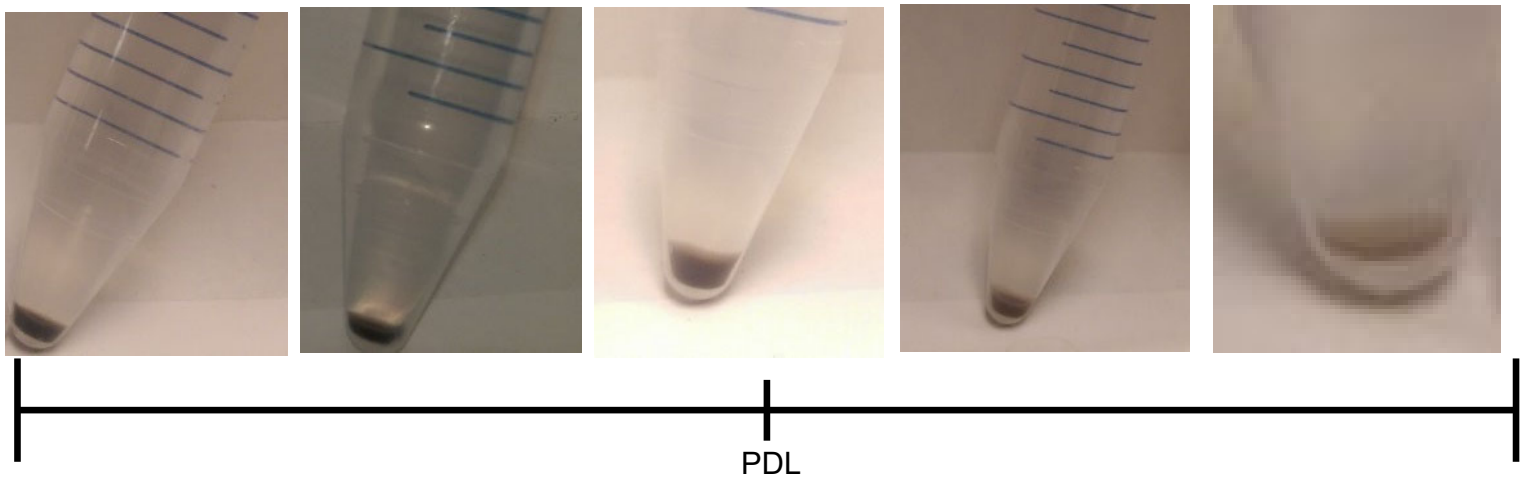
- ATCC provides several melanocyte cell lines to support research and development efforts
- From basic research through discovery and development to product testing
  - Primary cells
    - Adult and Neonatal
  - hTERT-immortalized primary cells
    - Adult Female Caucasian Donor
    - Neonatal Male Asian Donor
- Portfolio features
  - Reliability
  - Fully characterized cells
  - Optimized growth protocols
  - Scalable to research needs
  - Biological relevancy



Melanin Expressed and Maintained Throughout Many Passages

Cell pellets in centrifuge tube

hTERT melanocytes



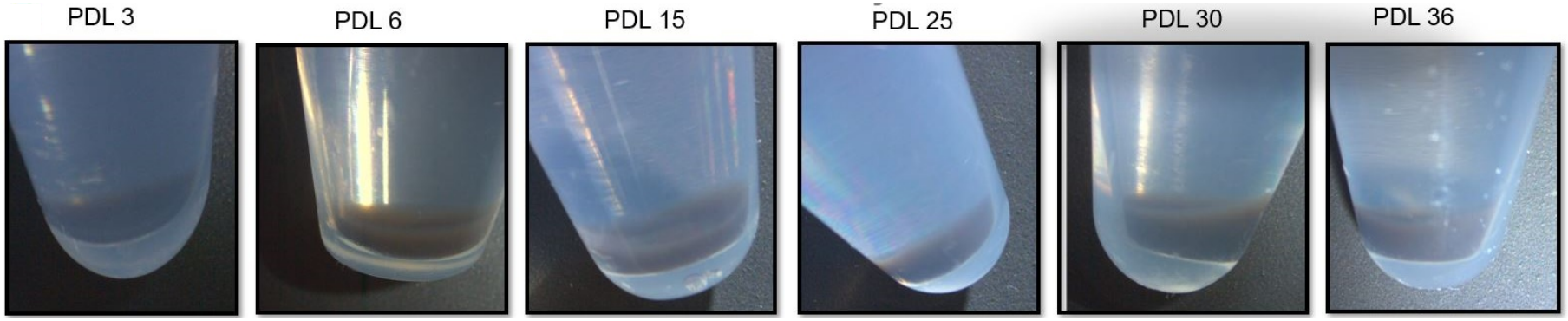
Primary adult melanocytes

negative control (adipose) cell pellet

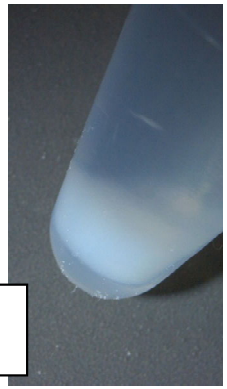
Melanin Expressed and Maintained Throughout Passaging

Cell pellets in centrifuge tube

hTERT Immortalized Neonatal Melanocyte Cell Pellet

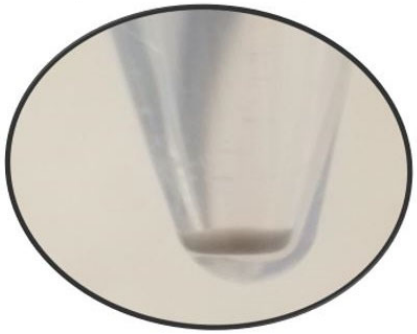


- hTERT Immortalized Neonatal Melanocyte Cells were detached from flask using trypsin and pelleted in centrifuge tube
- Images are taken at given time points throughout several months of continuous passaging



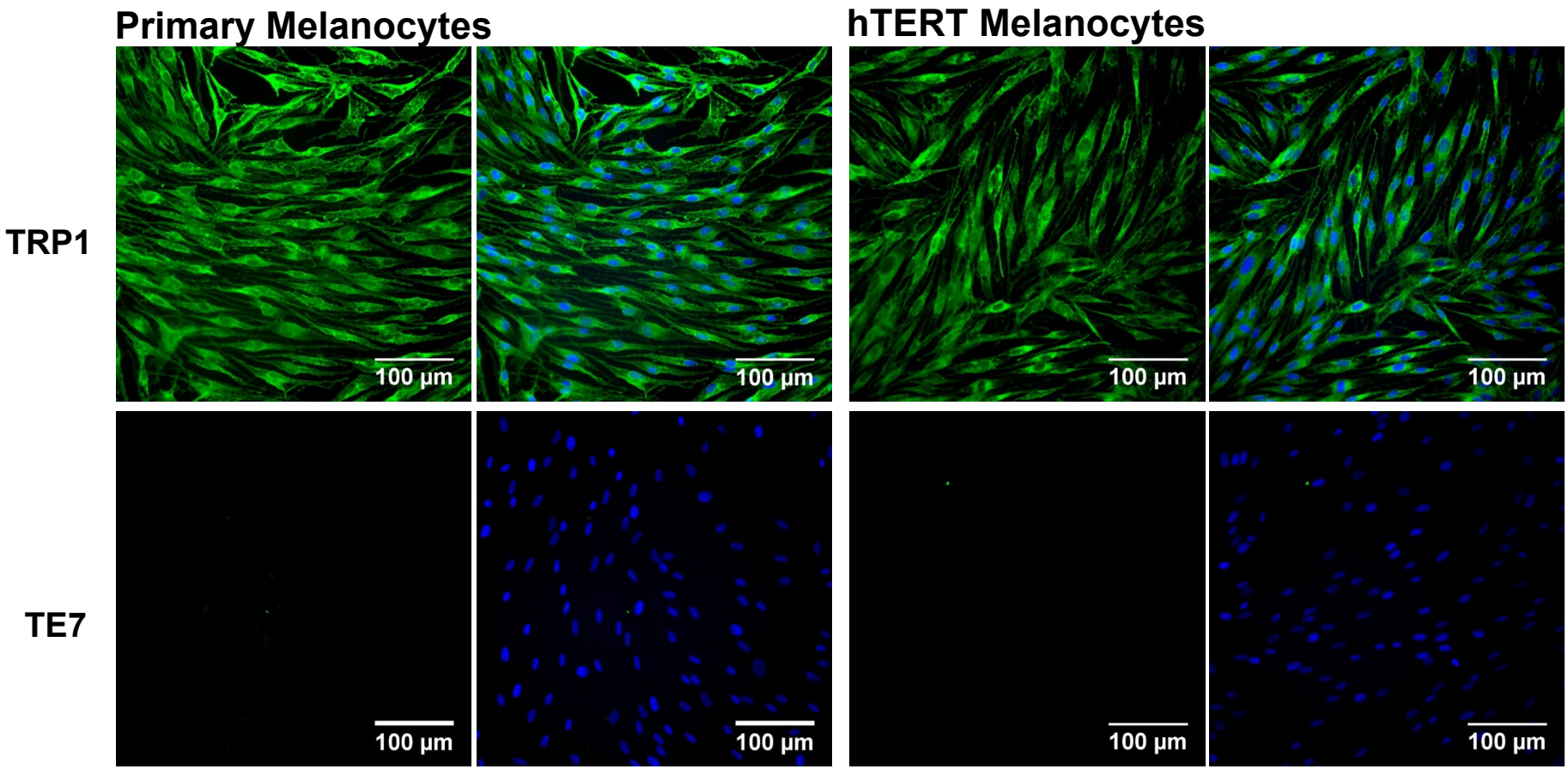
Negative control (adipose) cell pellet

Primary neonatal melanocyte cell pellet



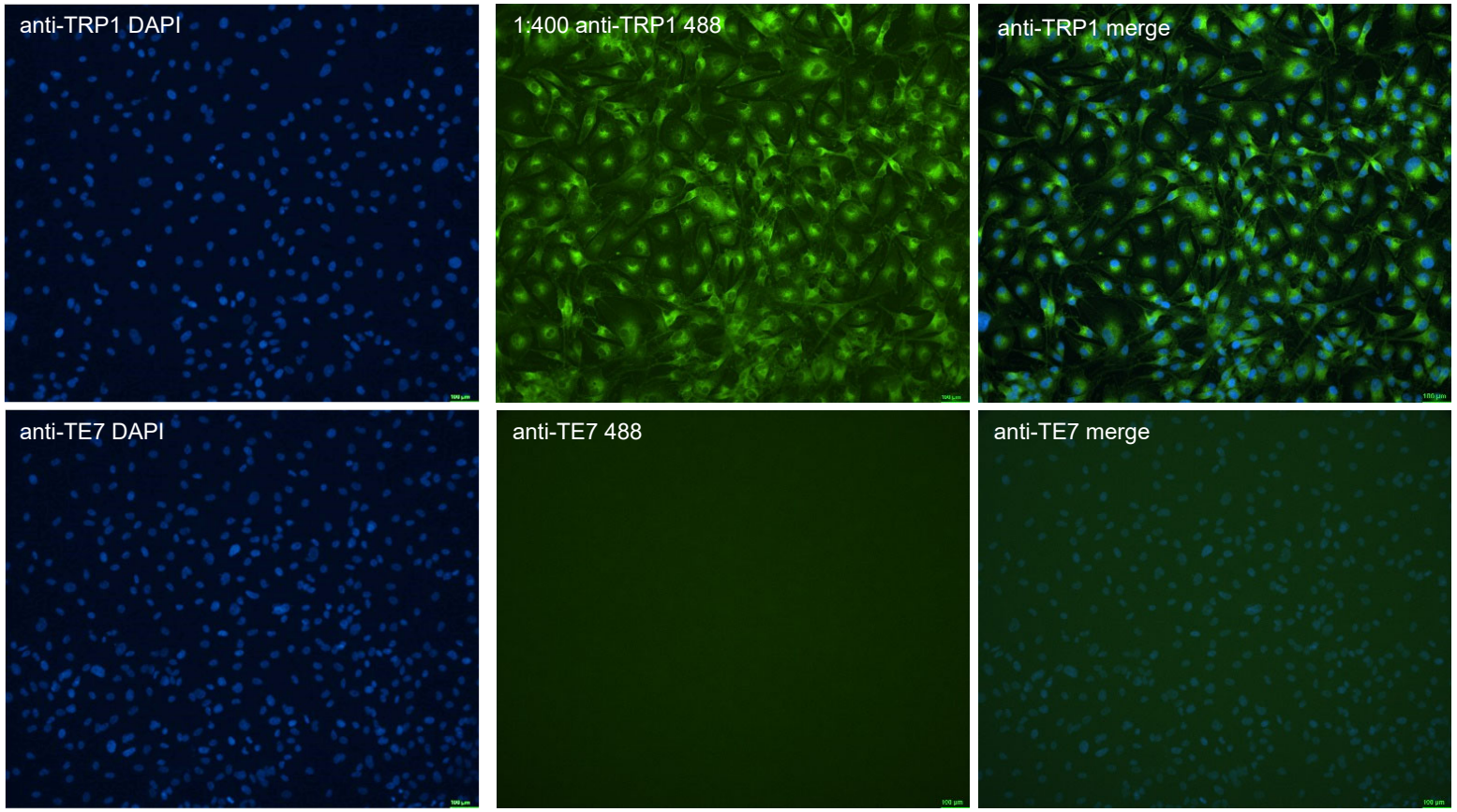
Adult Melanocyte Characteristics: Molecular Markers

Immunocytochemistry – Molecular marker staining of adult melanocytes



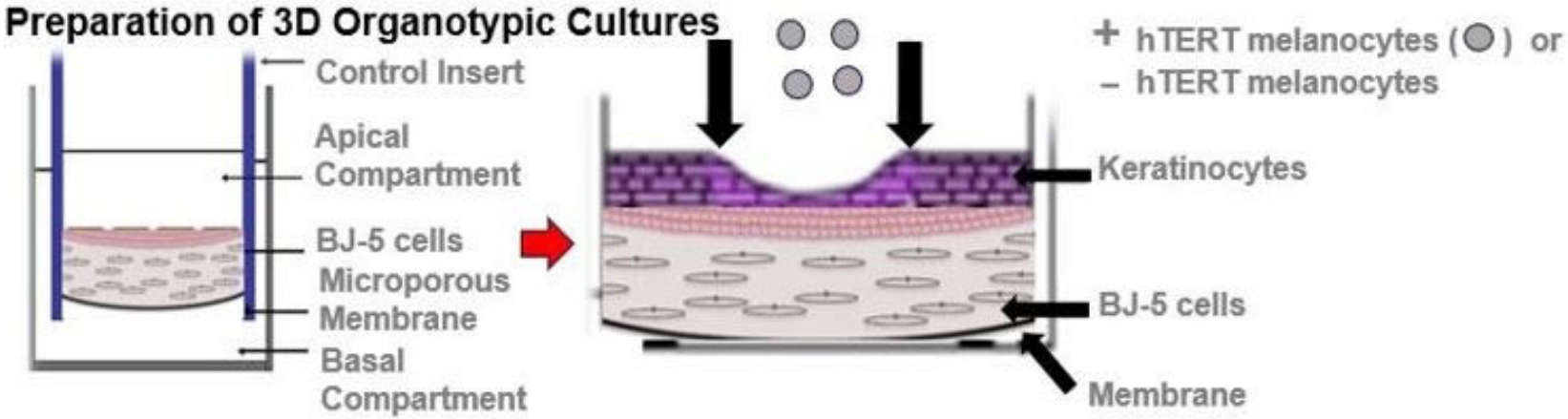
Neonatal Melanocyte Characteristics: Molecular Markers

Immunocytochemistry – Molecular marker staining of neonatal melanocytes



Scale Bar (in green) = 100 µm

Melanocyte 3D Organotypic Culture – Method



Embed BJ-5 cells into a collagen matrix contained in a single deep well with a control insert

Create conditions with only fibroblasts and keratinocytes or with all three cells fibroblasts, melanocytes, and keratinocytes

Grow for 14 days → fix and stain (Fontana Masson)

hTERT Immortalized Fibroblasts: CRL-4001™
hTERT Immortalized Keratinocytes: CRL-4048™

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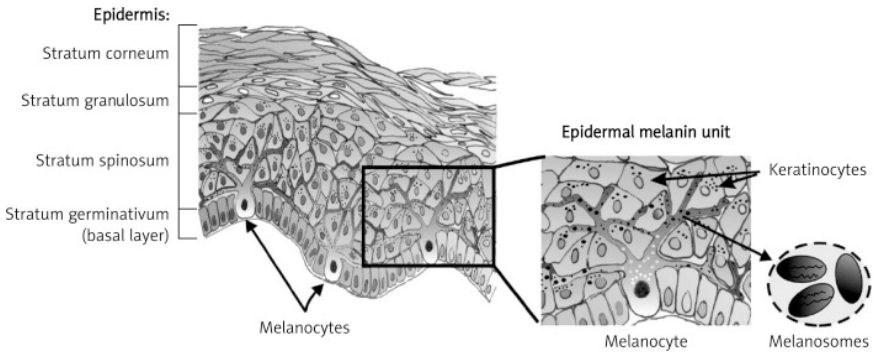
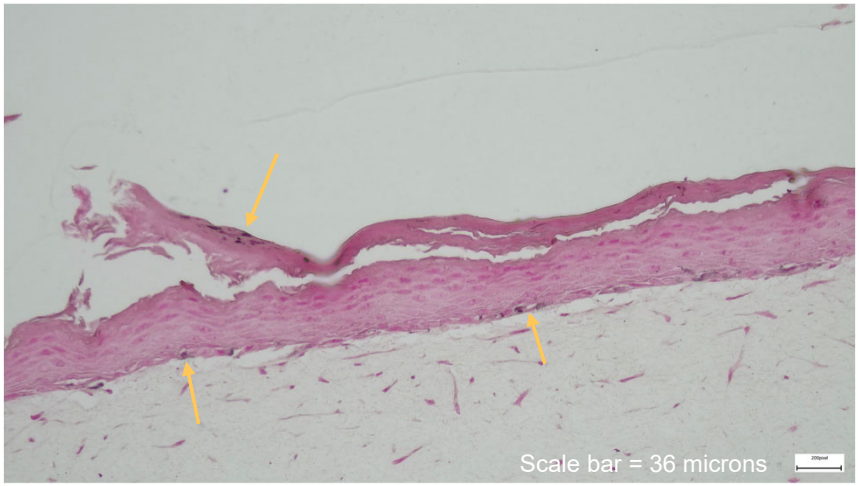
Adult Melanocyte 3D Organotypic Culture



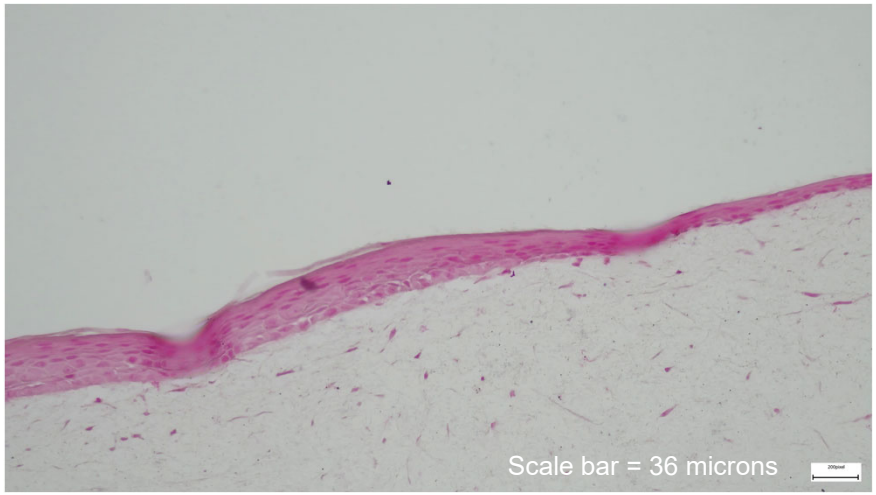
Postepy Dermatol Alergol. 2013 Feb; 30(1): 30–41.

- Brightfield images of fixed paraffin embedded sections
- Fontana Masson stain
- Brightness adjusted +20%
- Yellow arrows indicate melanin deposits
- Cultures with melanocytes develop more fully

Fibroblast/Keratinocytes + hTERT Melanocytes

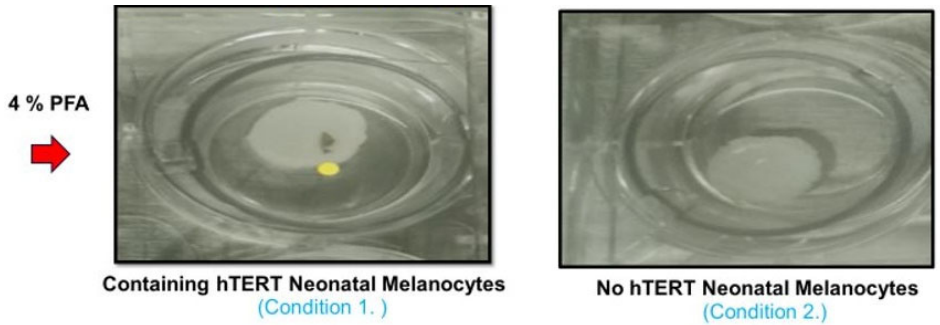


Fibroblast/Keratinocytes



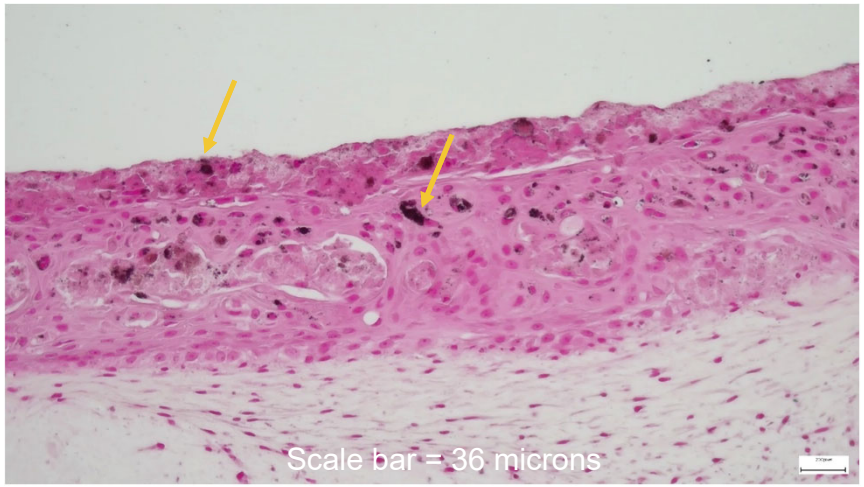
Neonatal Melanocyte 3D Organotypic Culture

Melanin deposits visible in 3D organotypic co-culture

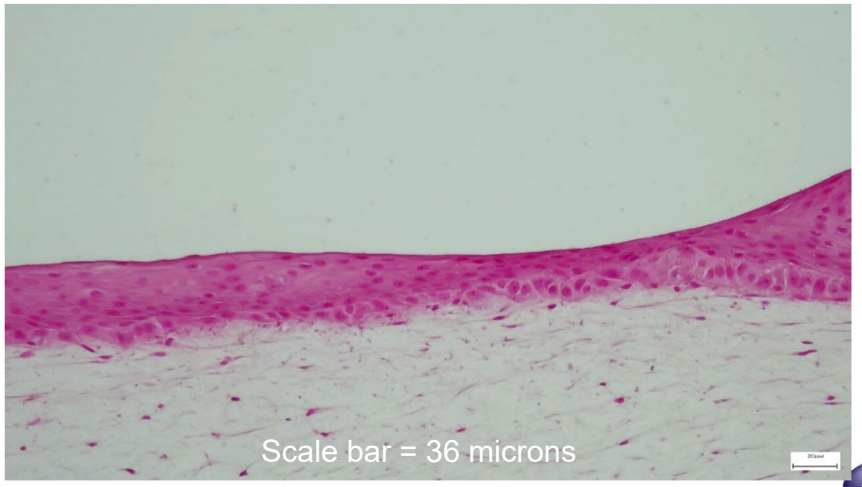


- Melanin visible in macroscopic & microscopic images of 3d cultures.
- Generally, less tissue development is observed in cultures without melanocytes.

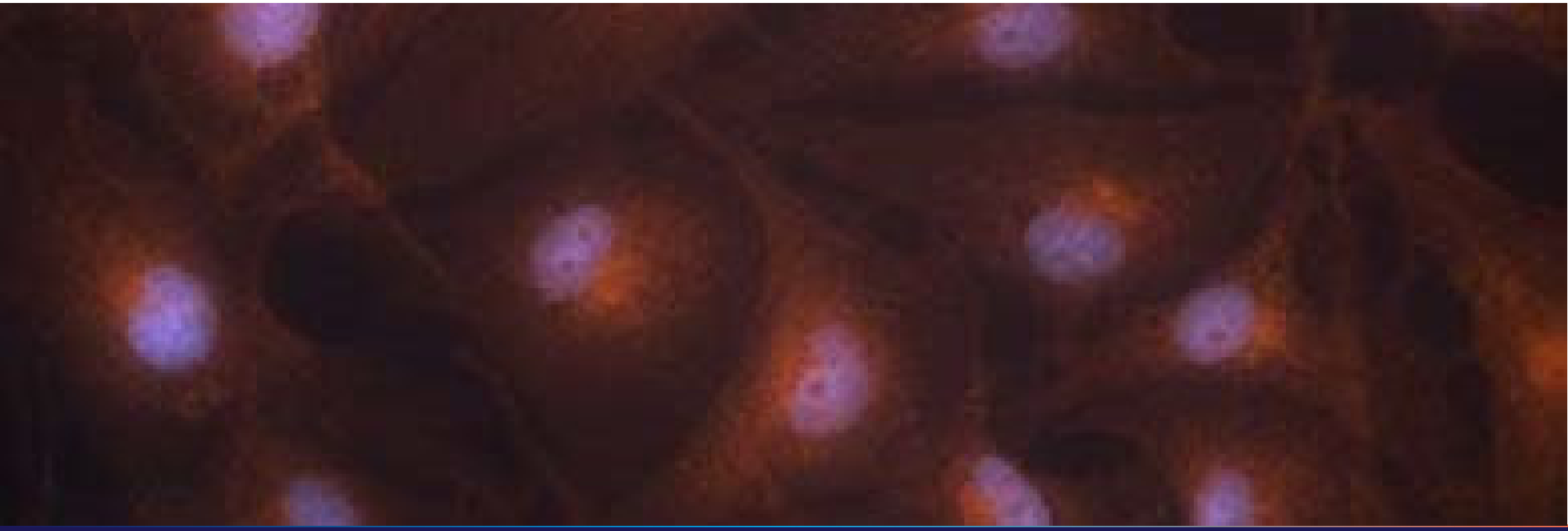
Fibroblast/Keratinocytes + hTERT Neo Melanocytes



Fibroblast/Keratinocytes



Fontana Masson Stain, 20x Brightfield, Brightness +20%



Melanin Synthesis - Data

Melanin Synthesis Pathway

Inhibit/stimulate melanogenesis with specific compounds

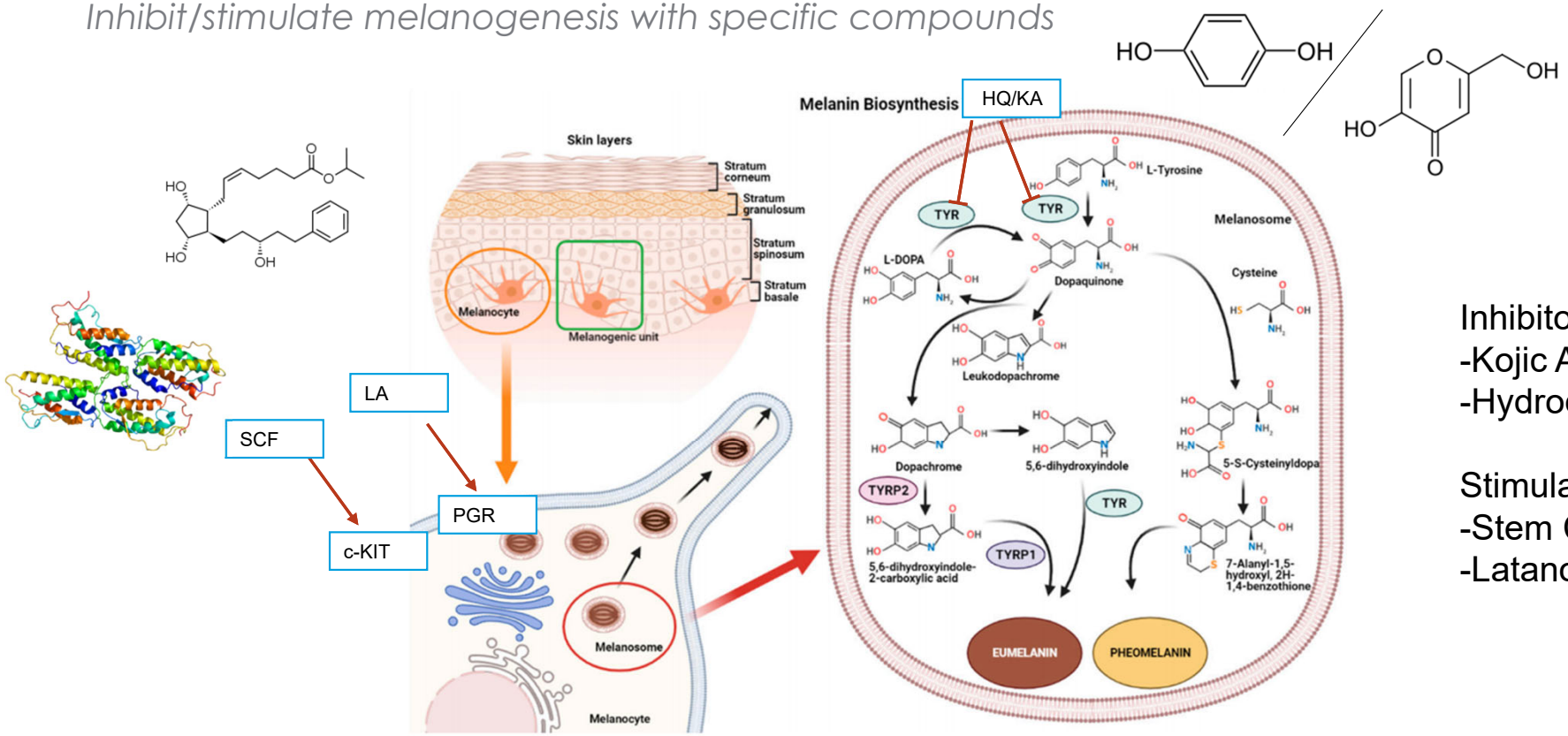


Figure 1. Representation of the melanogenic unit and melanin synthesis in melanosomes (**left**). Schematic representation of eumelanin and pheomelanin biosynthetic pathways (**right**).

Hushcha Y, Blo I, Oton-Gonzalez L, et al. microRNAs in the Regulation of Melanogenesis. *Int J Mol Sci*. 2021;22(11):6104. Published 2021 Jun 5. doi:10.3390/ijms22116104

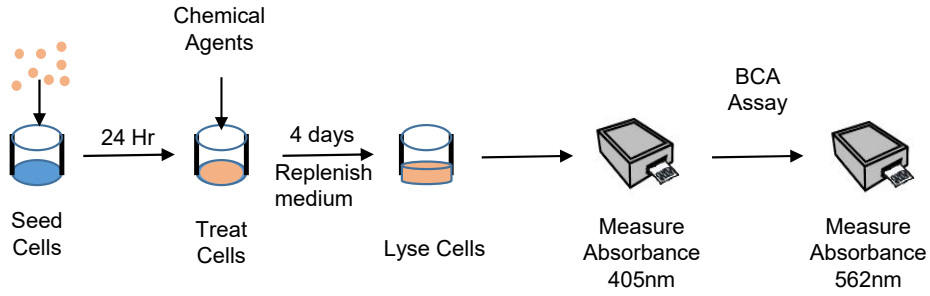
Inhibitors:
-Kojic Acid
-Hydroquinone

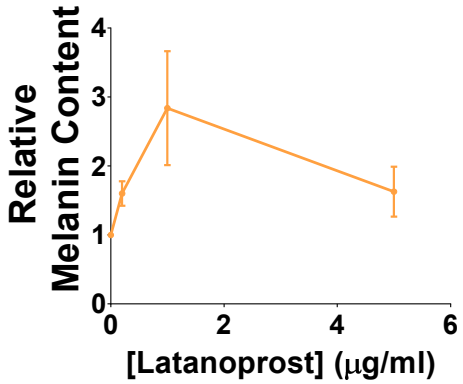
Stimulators:
-Stem Cell Factor
-Latanoprost

ATCC

Adult Melanocyte Stimulation and Inhibition Study

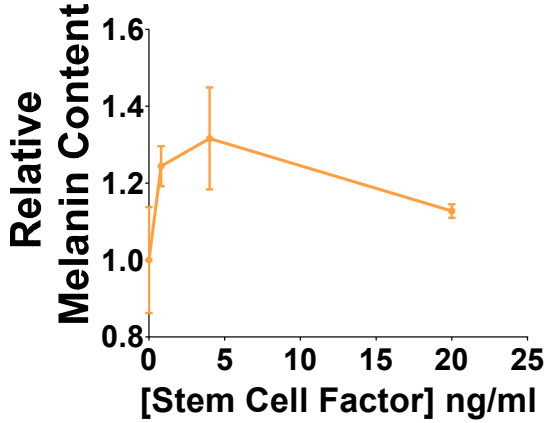
Testing responsiveness to stimulators and inhibitors of melanogenesis

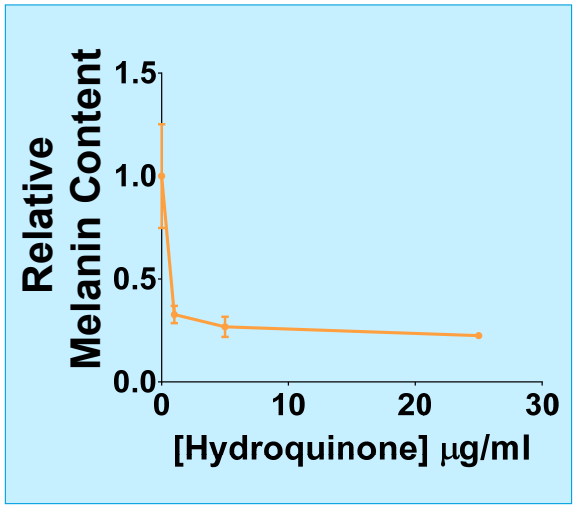




- Total protein determined by BCA assay and fitting to standard curve of 8 concentrations
- Melanin content adjusted relative to total protein and untreated control

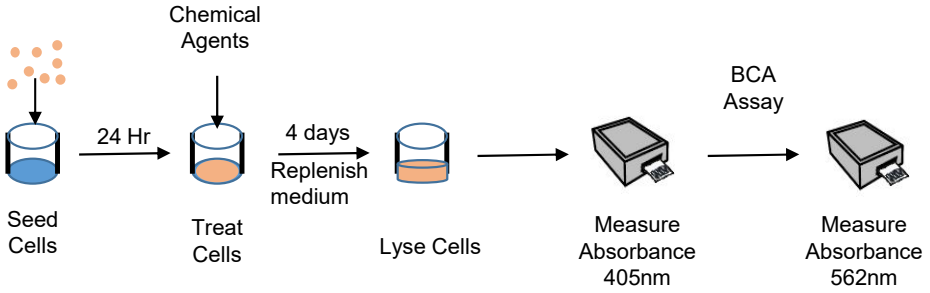
$$Rel.Mel.cont_i = \frac{\frac{A405_i}{Total\ Protein_i}}{\frac{A405_{untreated}}{Total\ Protein_{untreated}}}$$

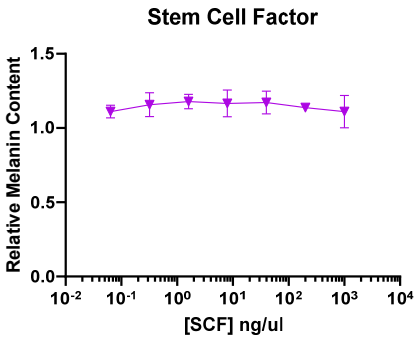


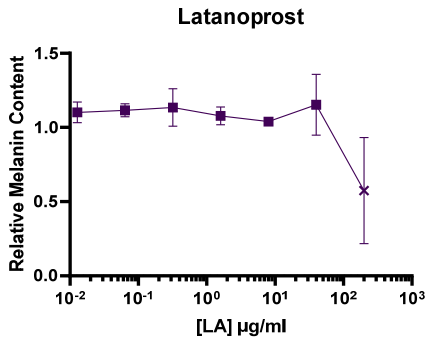


Neonatal Melanocyte Stimulation and Inhibition Study

Testing responsiveness to stimulators and inhibitors of melanogenesis

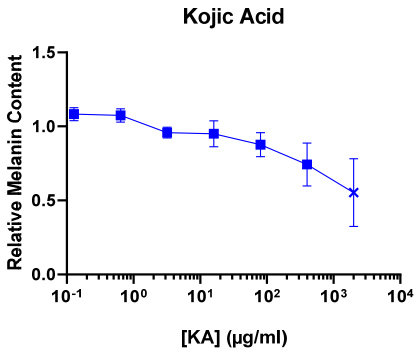


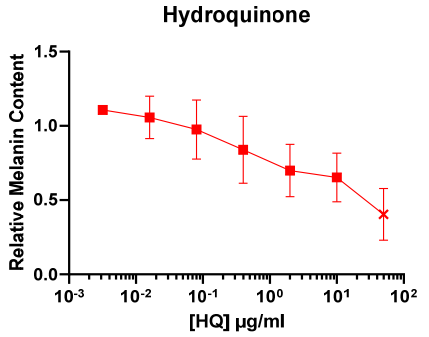


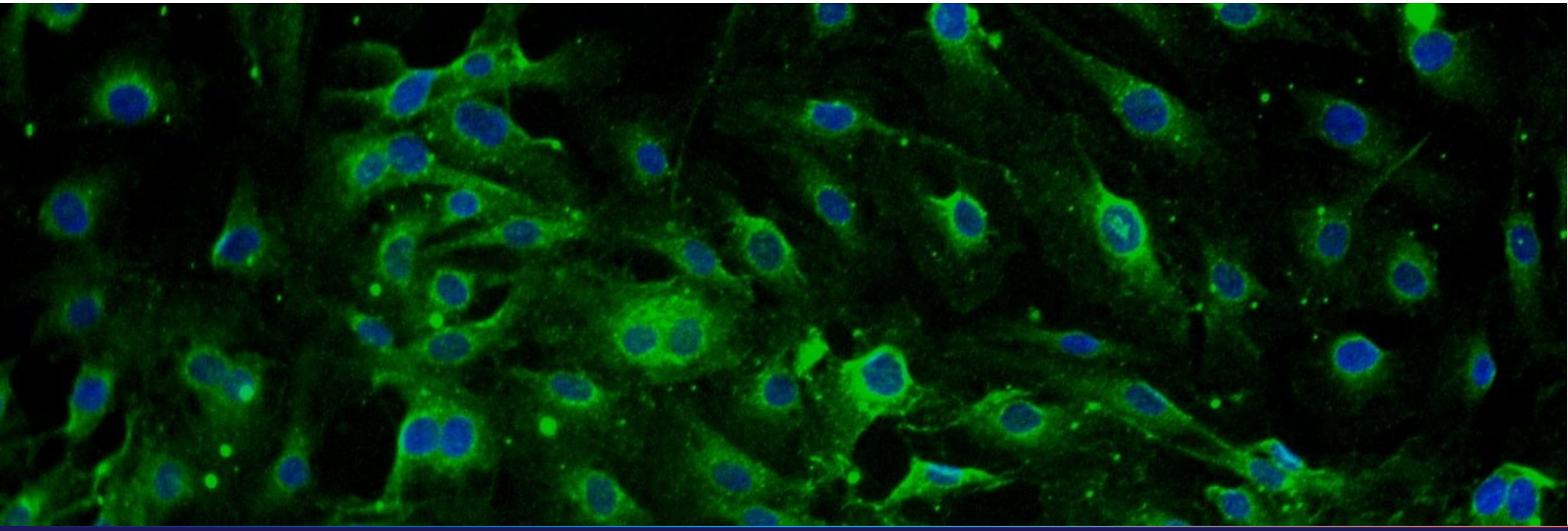


Neonatal cells show minimal response to stimulators and respond to inhibitors in a dose-dependent manner

Adult and neonatal primary cells also tested using the same conditions (data not shown) and showed similar responses



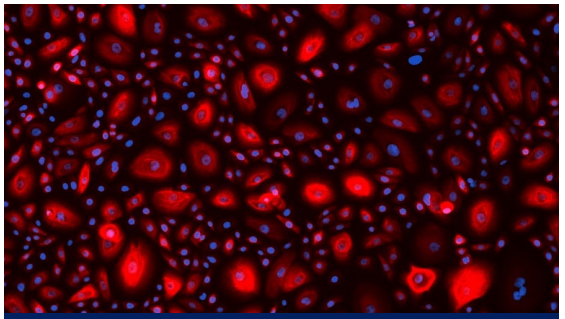




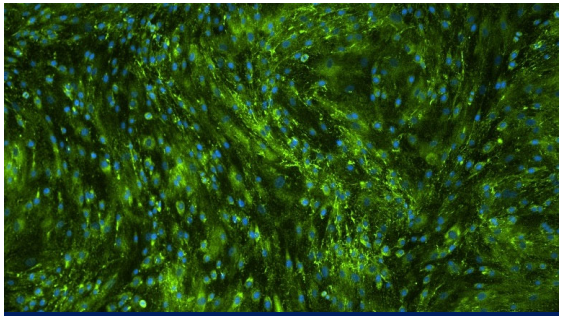
hTERT Immortalized Gingival Fibroblasts and Keratinocytes- Data

ATCC Gingival Fibroblast and Keratinocyte Models

- ATCC provides several gingival cell lines to support research and development efforts
- From basic research through discovery and development to product testing
  - Primary cells
    - Primary Gingival Keratinocytes (ATCC® PCS-200-014™)
    - Primary Gingival Fibroblasts (ATCC® PCS-201-018™)
  - hTERT-immortalized primary cells
    - hTERT TIGKs (ATCC® CRL-3397™)
    - hTERT Gingival Fibroblasts (ATCC® CRL-4061™)
- Portfolio features
  - Reliability
  - Fully characterized cells
  - Optimized growth protocols
  - Scalable to research needs
  - Biological relevancy

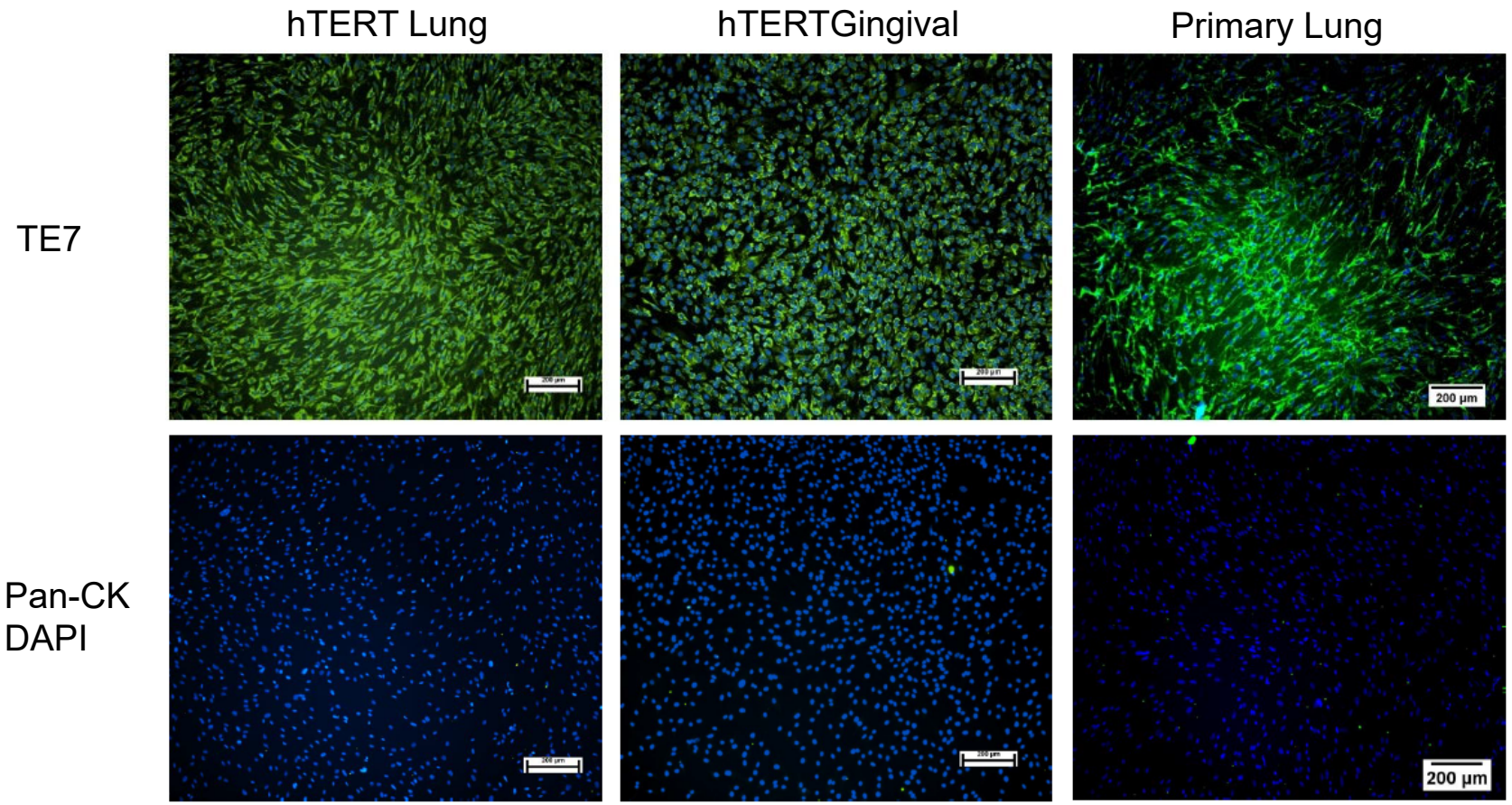


Primary Gingival Keratinocytes



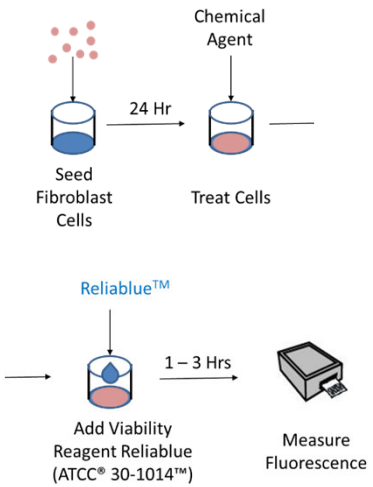
Primary Gingival Fibroblasts

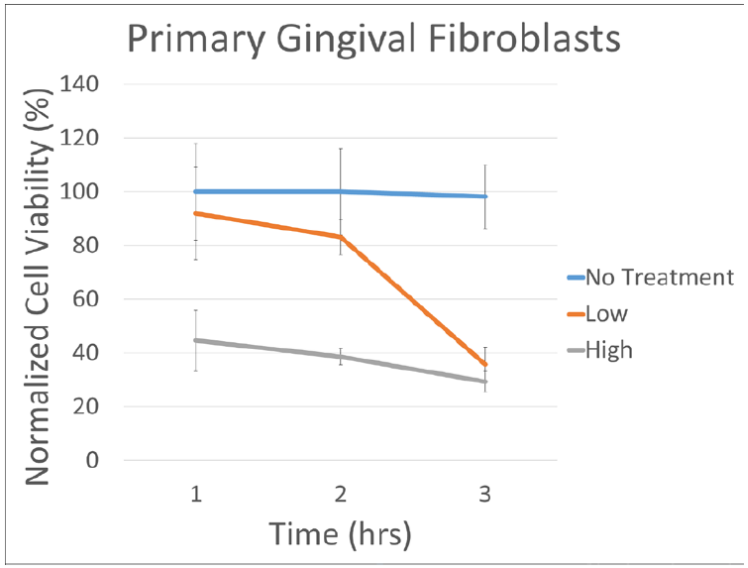
Immortalized Fibroblasts Maintain a Fibroblast-specific Markers

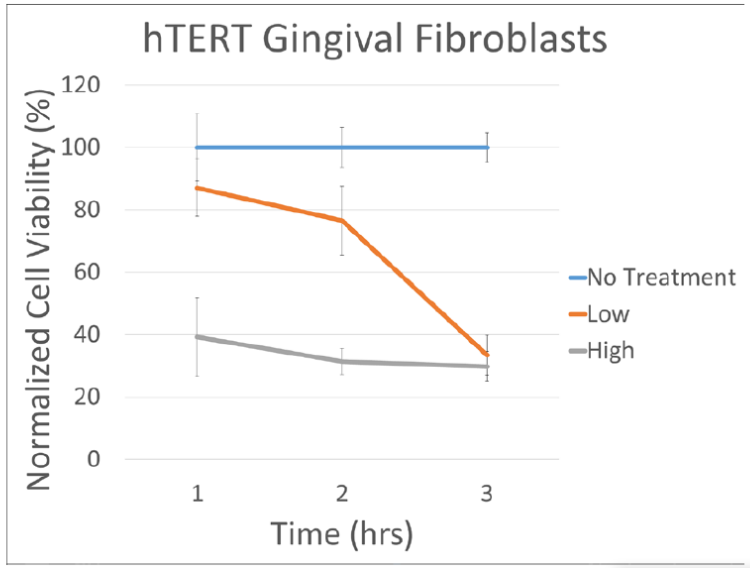


hTERT gingival fibroblasts respond to chlorhexidine

Cellular cytotoxicity of lung fibroblasts by chlorhexidine is dose-dependent

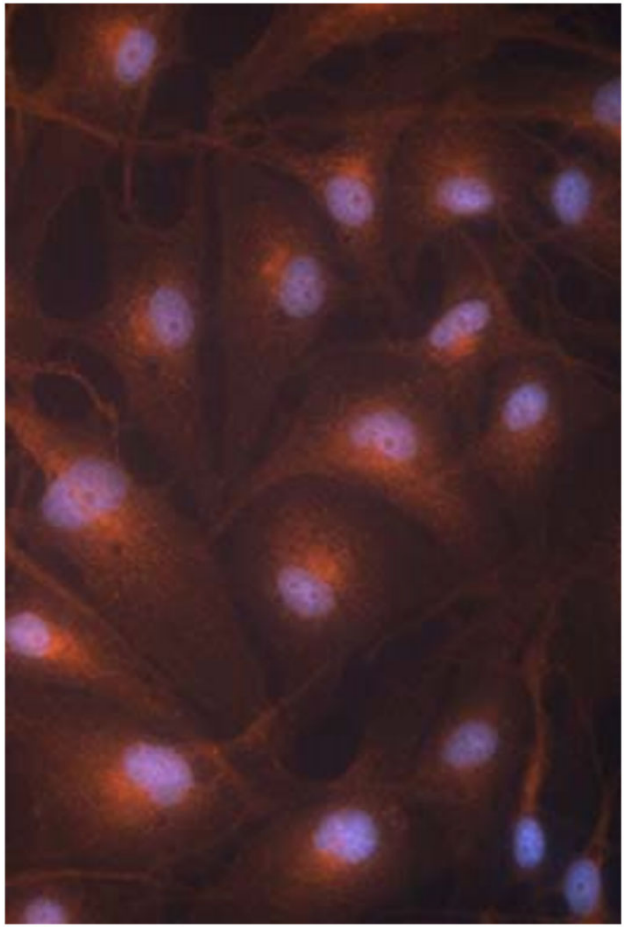






Summary and Conclusions

- Immortalized keratinocytes, melanocytes, and fibroblasts are available
- hTERT immortalized cell lines show key physiological characteristics:
  - Multi-dendritic morphology, expression of key molecular markers, keratin and melanin production
  - Form epidermal structures in a 3D organotypic co-culture system
  - Show responsiveness to stimulators and inhibitors of melanogenesis
- ATCC hTERT-immortalized primary melanocytes
  - Replicate primary cell characteristics
  - Provide greatly increased longevity
  - Complement ATCC's current primary melanocyte offerings

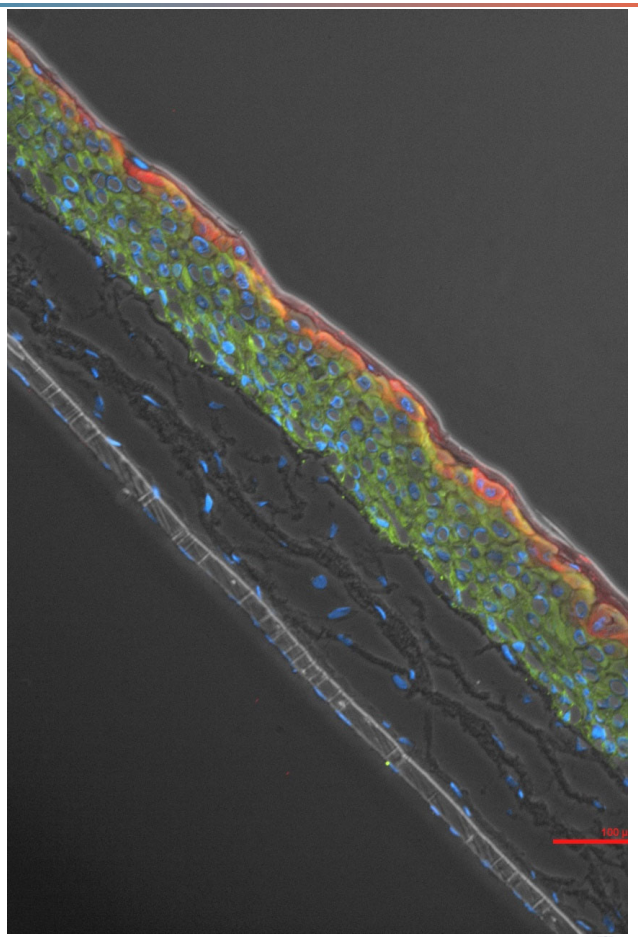


ATCC

Summary and resources

- ATCC provides a portfolio of over 50 hTERT-immortalized primary cells to the life science research community
- ATCC R&D actively develops new immortalized cell lines
  - Custom immortalization service is available
  - A variety of technologies are available
- hTERT-immortalized primary cells provide primary cell functionality with increased longevity
- hTERT cells are a user-friendly solution for building reliable cell models for a variety of research needs
- Multiple primary cell and hTERT-immortalized primary cell resources are available at

www.atcc.org/TOX



ATCC

Thank you and questions

Coming attractions:

Skin Microbiome: Considerations, Applications, and Future Directions
August 11 at 12:00 PM EST
Tasha Santiago-Rodriguez, PhD

For more information about ATCC toxicological models:

www.atcc.org/TOX