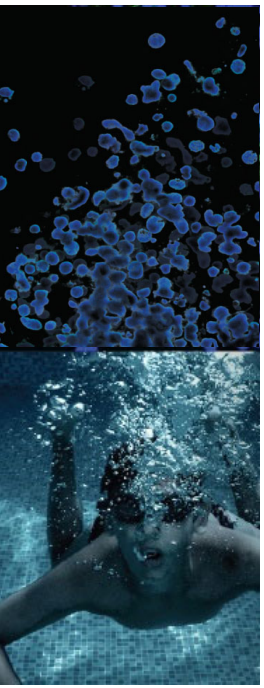
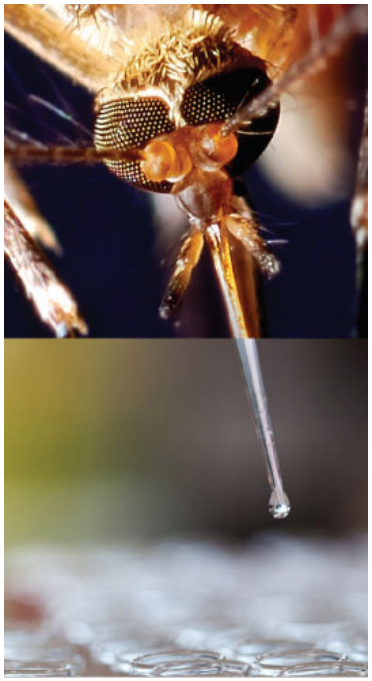
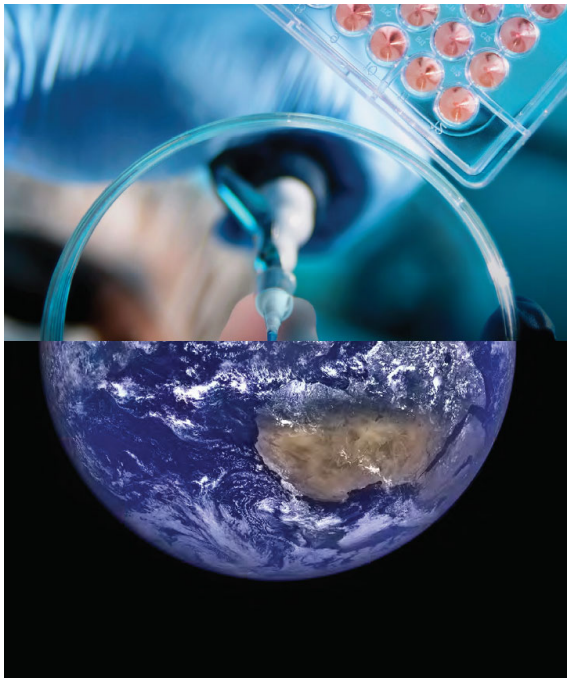
hTERT Immortalized Melanocytes - Advanced Models for Your Dermal Toxicology Studies

Michael Maddox, BS
Biologist, ATCC







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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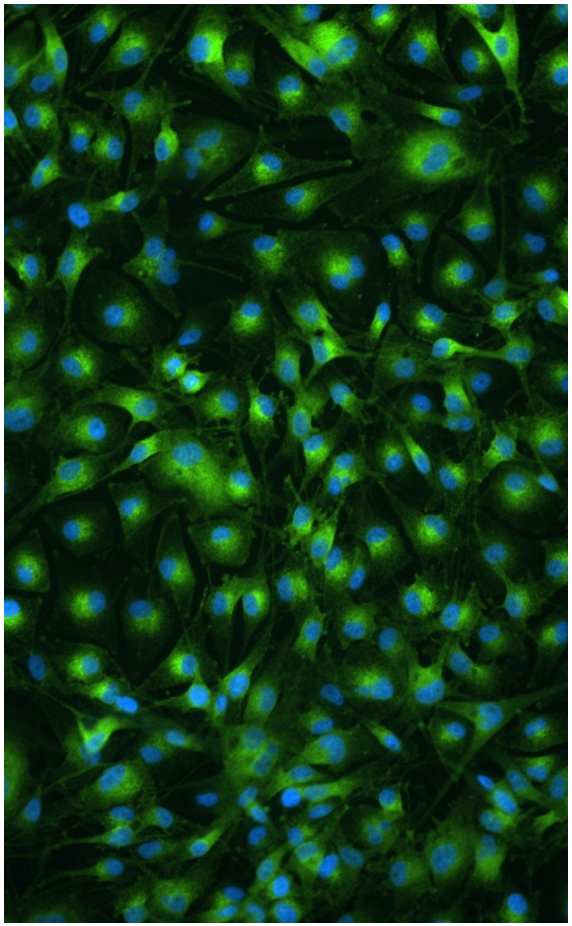
Agenda

- Skin pigmentation background
- Applications for melanocyte cell models
- Comparison of various cell models (primary and immortal)
- Immortalized cell models – key characteristics
- hTERT-immortalized Adult Melanocyte cell culture model - data
- hTERT-immortalized Neonatal Melanocyte cell culture model - data



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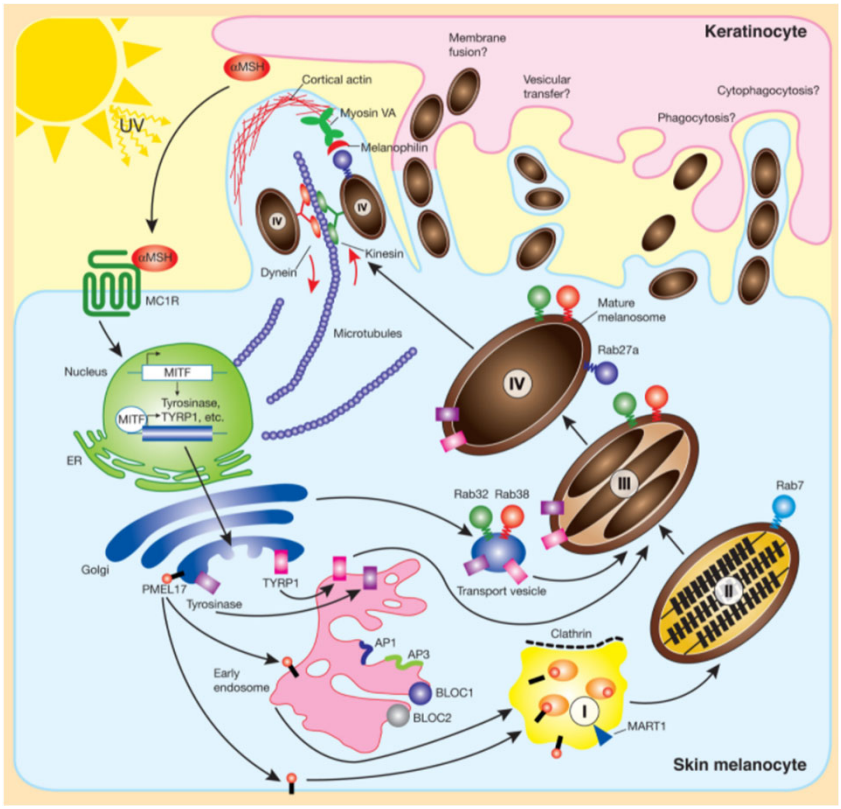
Skin Pigmentation Background – Step 1

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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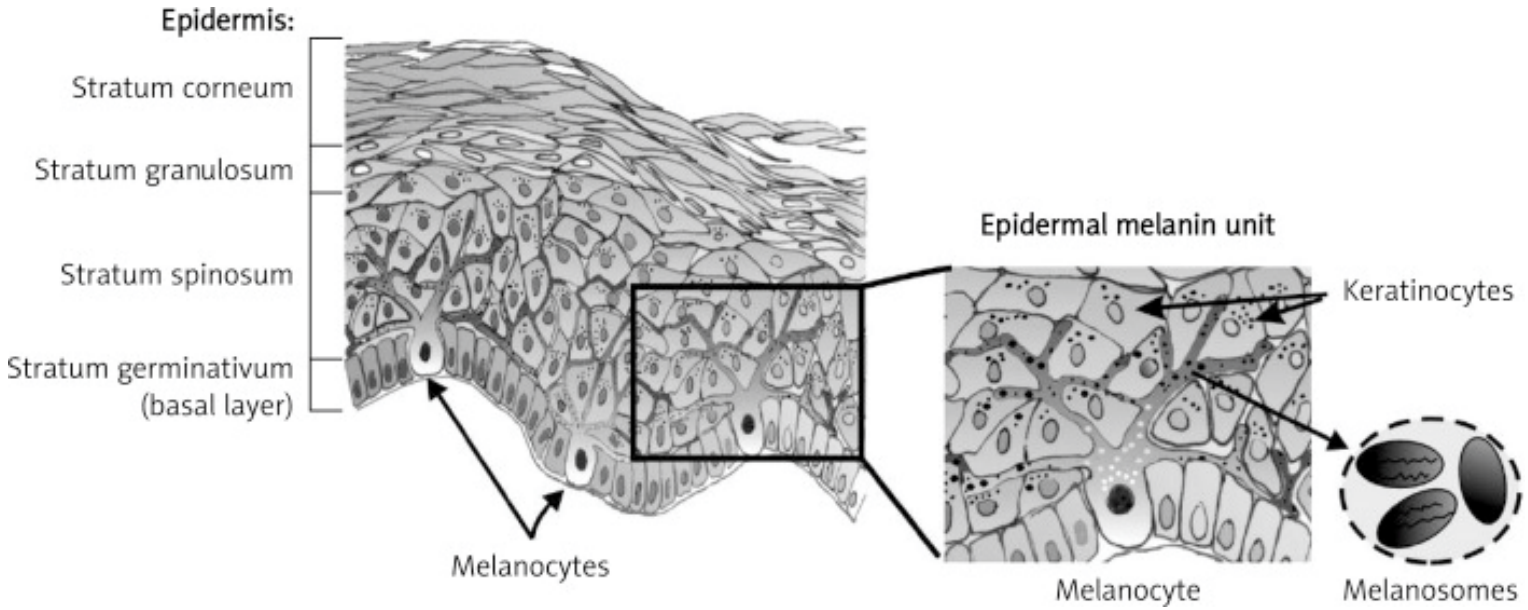
Skin Pigmentation Background – Step 2

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

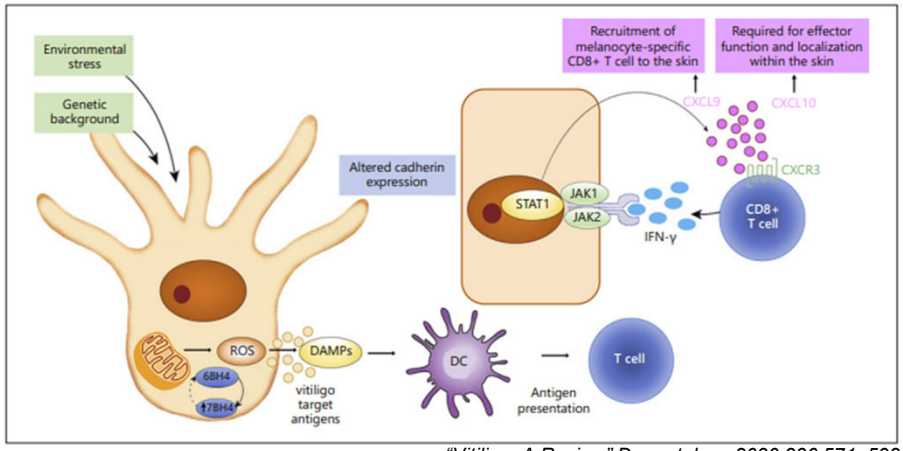


Melanosomes are exocytosed (by melanocytes) then endocytosed by adjacent keratinocytes

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

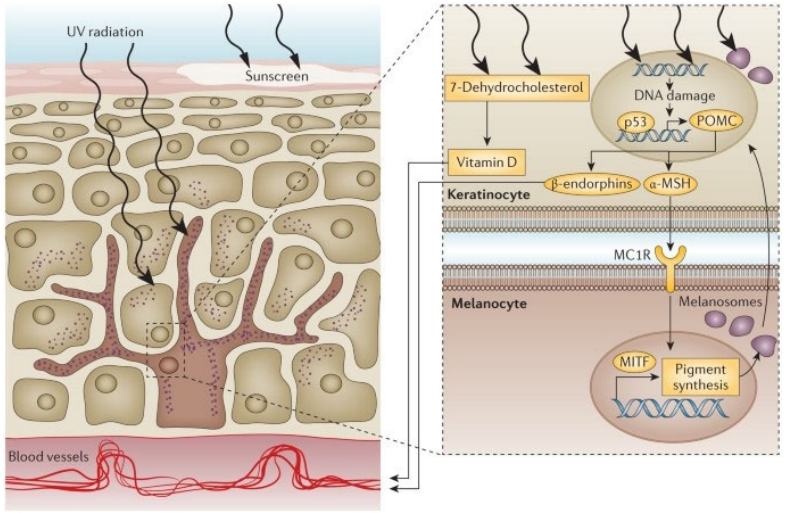
Applications of Melanocyte Cell Models – Toxicology

1. Reliable reagent for traditional toxicology (LD_{50})
2. Advanced Toxicology: Understand the complex interplay of genetic background and environmental agents that can stress melanocytes
3. Toxicology and chemotherapeutic agents: Melanoma, a common cancer in the western world with an increasing incidence* begins in melanocytes
4. Develop treatments: Skin conditions such as hypopigmentation, hyperpigmentation, or combined disorders with hypo-/hyperpigmentation



"Vitiligo: A Review" Dermatology 2020;236:571–592

http://www.MedlinePlus/ hyperpigmentationdisorders



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

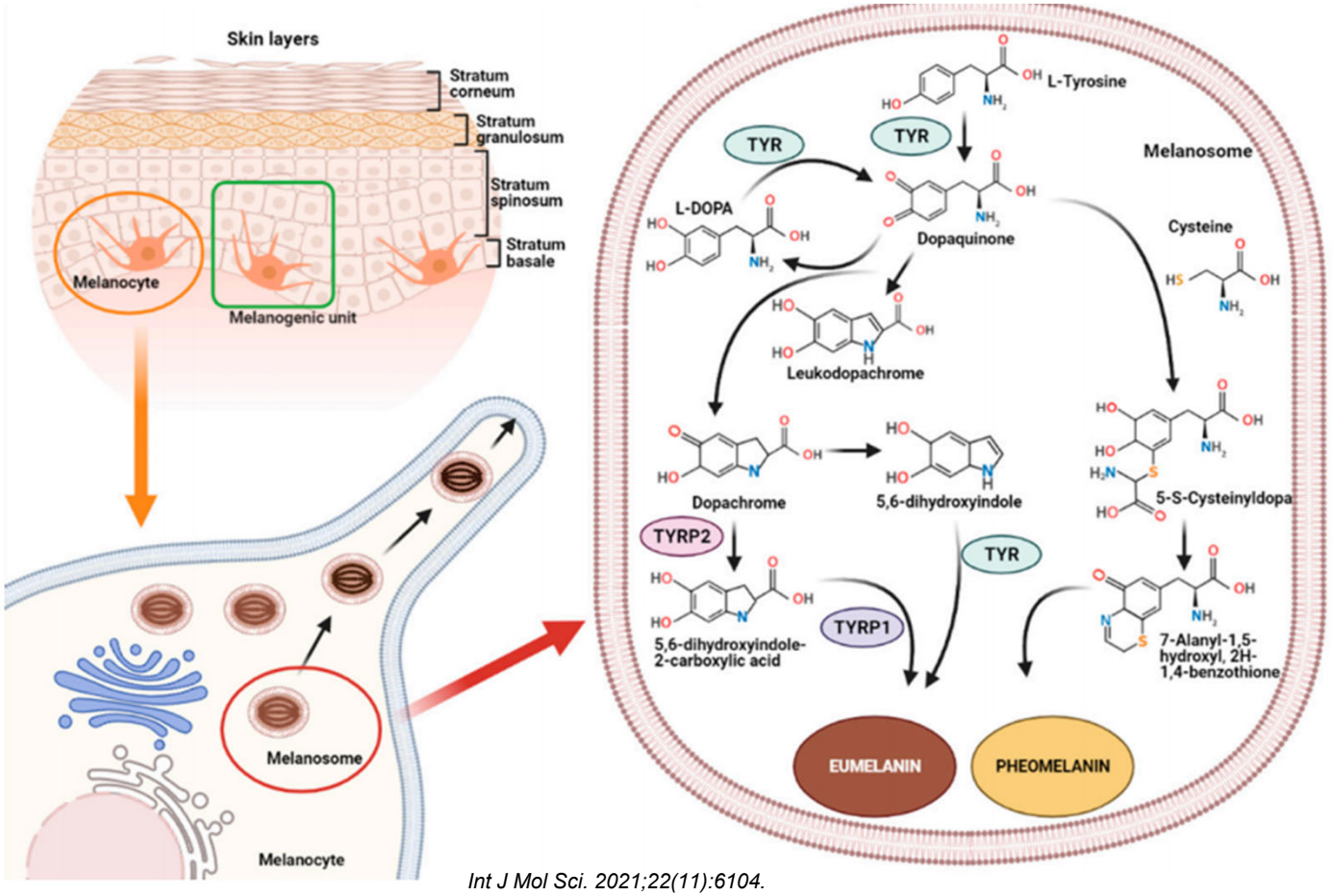
Accurate models needed to study the complex system

Applications of Melanocyte Cell Models – Studying Biochemical Processes

Melanocytes provide a model to study cell metabolic processes

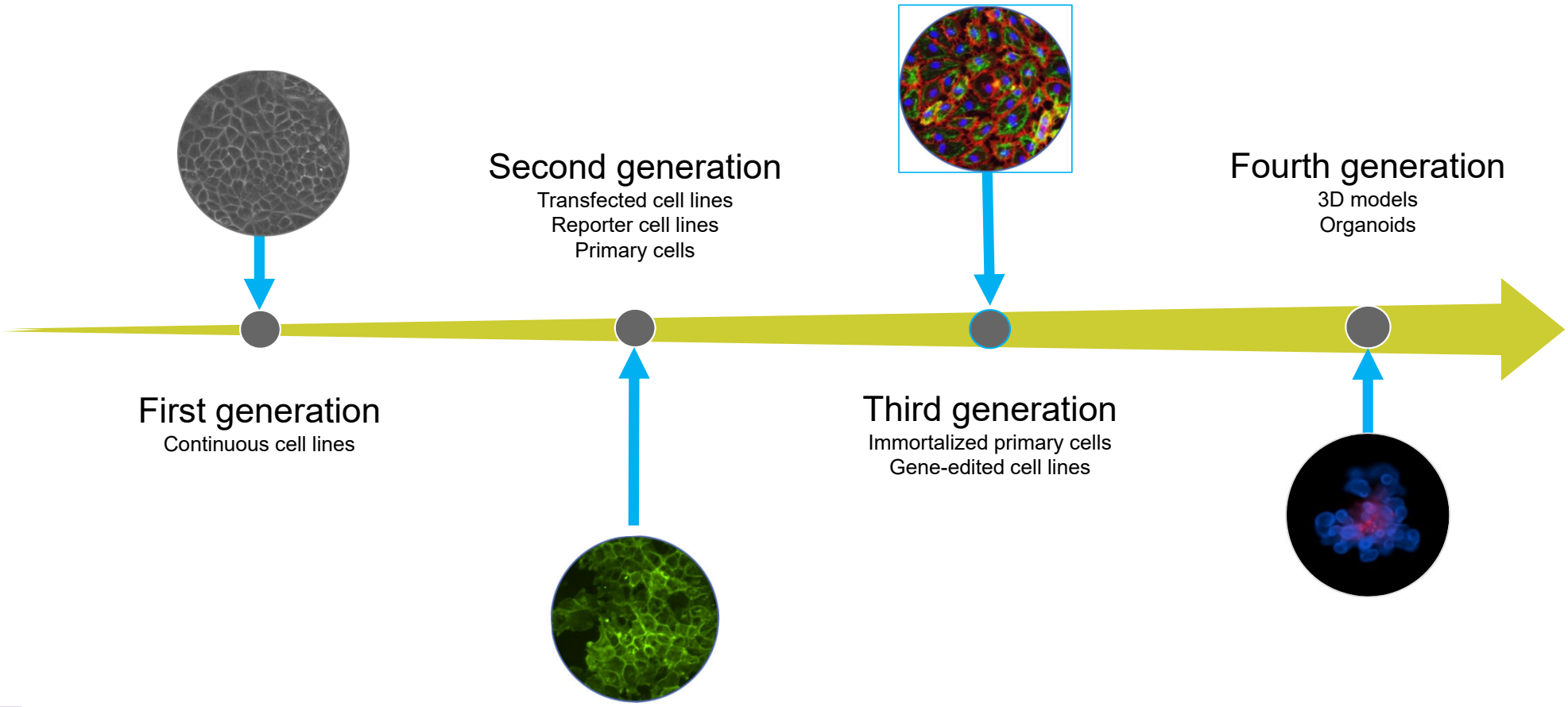
1. In-depth studies of melanin biosynthesis and metabolism (Cosmetics)

2. Melanin pigments are relatively simple to detect and measure making melanogenesis an ideal model system for general studies of cell metabolism



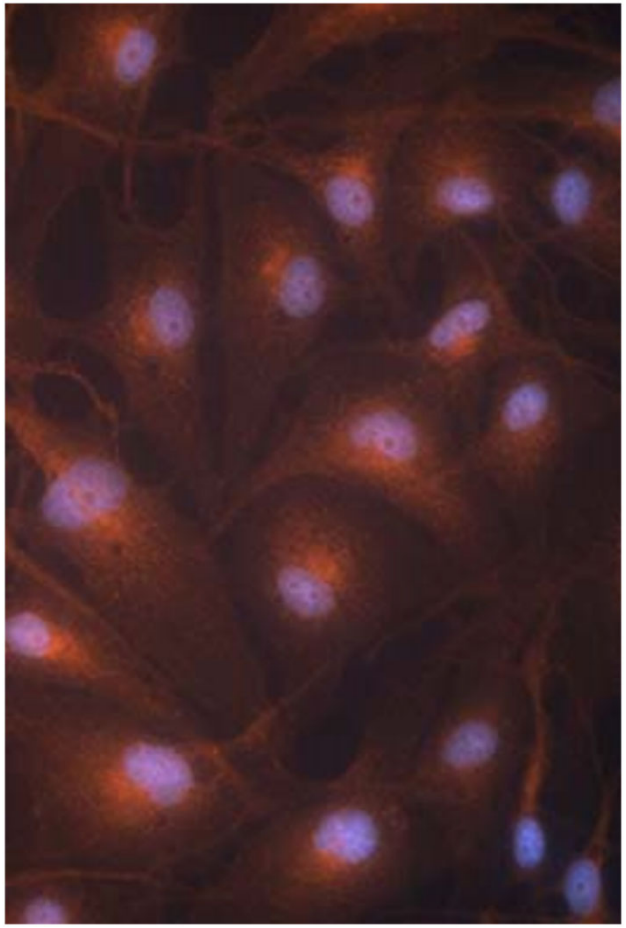
Int J Mol Sci. 2021;22(11):6104.

Immortalized Cells – Advanced In Vitro Model



Characteristics of Two Physiologically Relevant Cell Models

	Primary cell Melanocytes	hTERT-immortalized primary melanocytes
Mimic *in vivo* characteristics	++++	+++
Proliferative capacity	+	+++
Experimental reproducibility	+	+++
Predictability in toxicological studies	+++	+++
Genomic stability	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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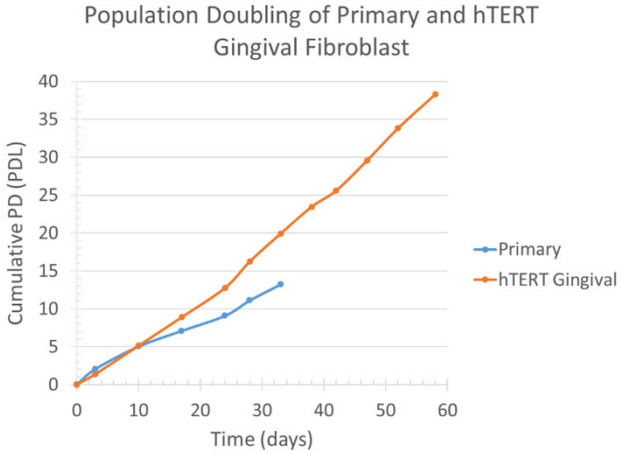
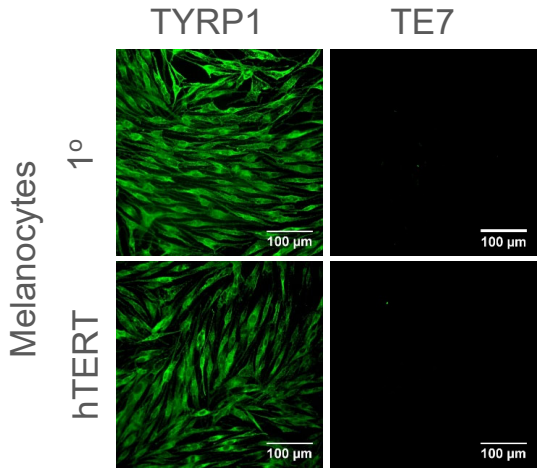
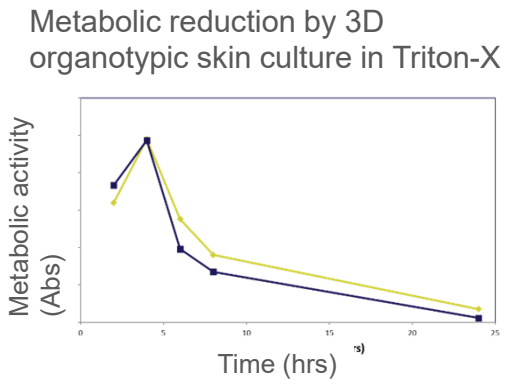
hTERT-immortalized Cells – Key Characteristics

- 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

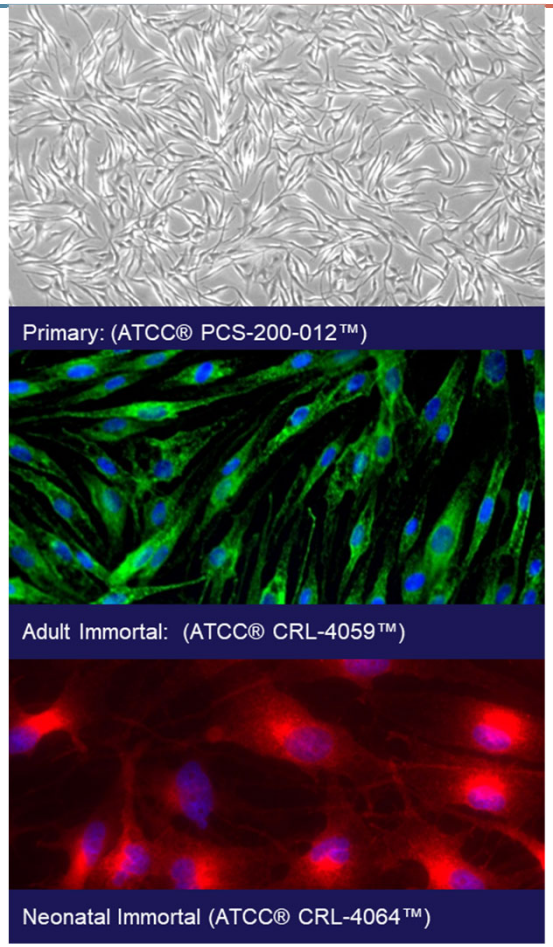


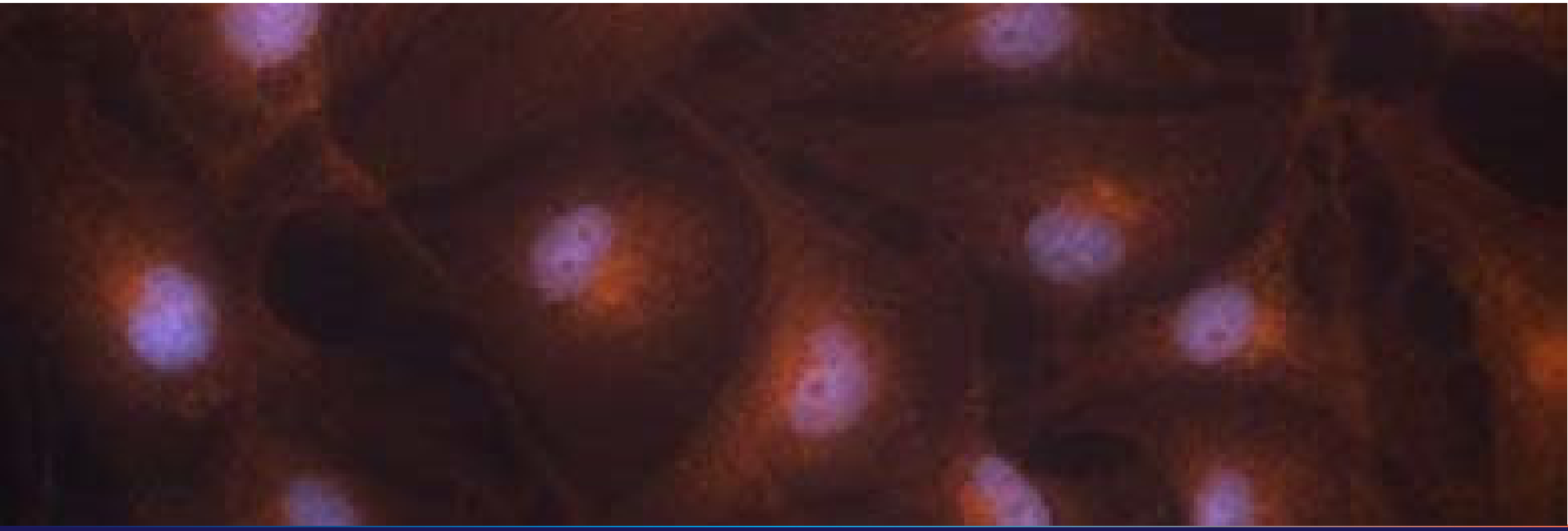




ATCC Melanocyte Models

- 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



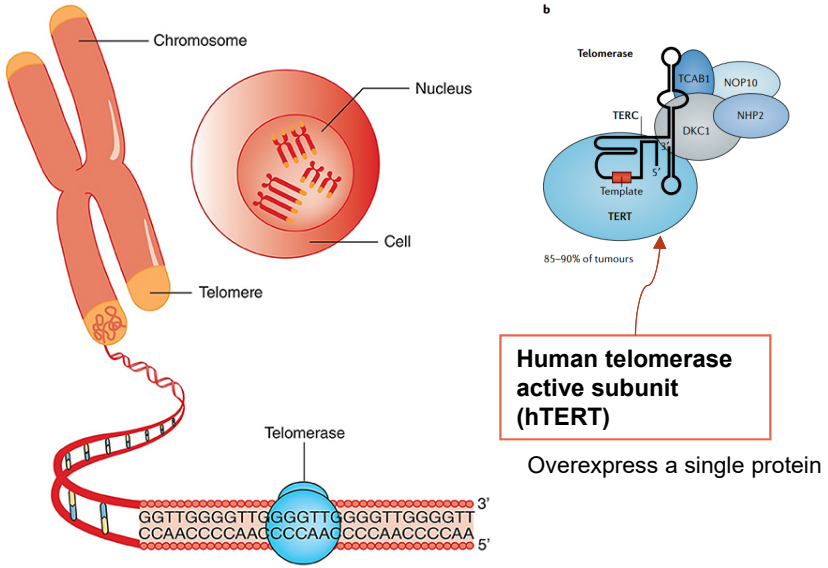


hTERT Immortalized Dermal Melanocytes - Data

Cell Immortalization Processes

Quick note about process

Telomerase – prevents cell aging



Human telomerase active subunit (hTERT)

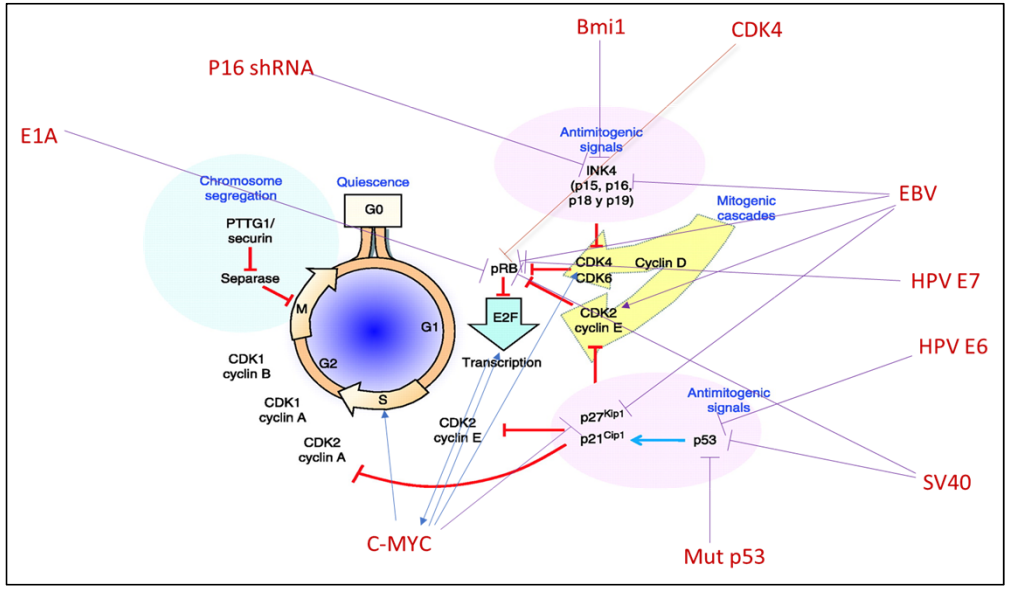
Overexpress a single protein

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

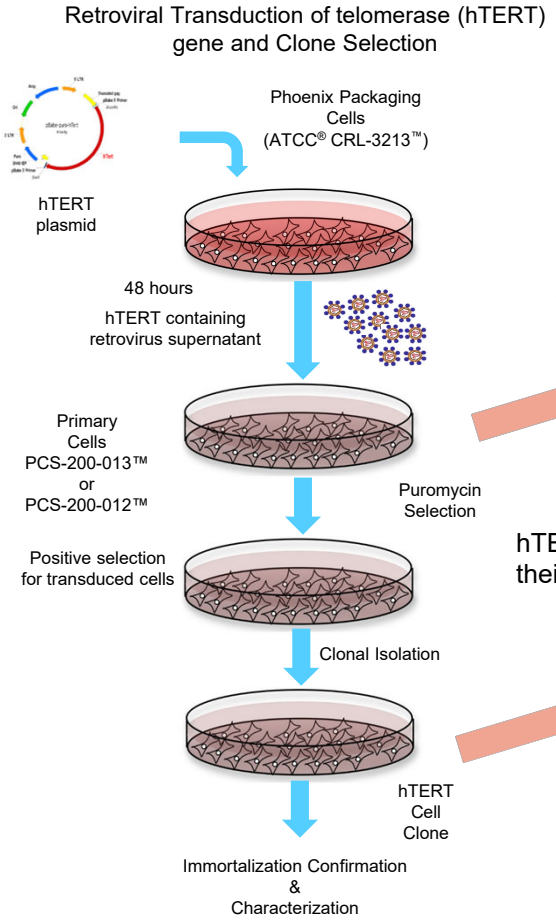


Immortalization using telomerase differs from methods where cell cycle proteins are inhibited or overexpressed.

ATCC has expertise in several methods

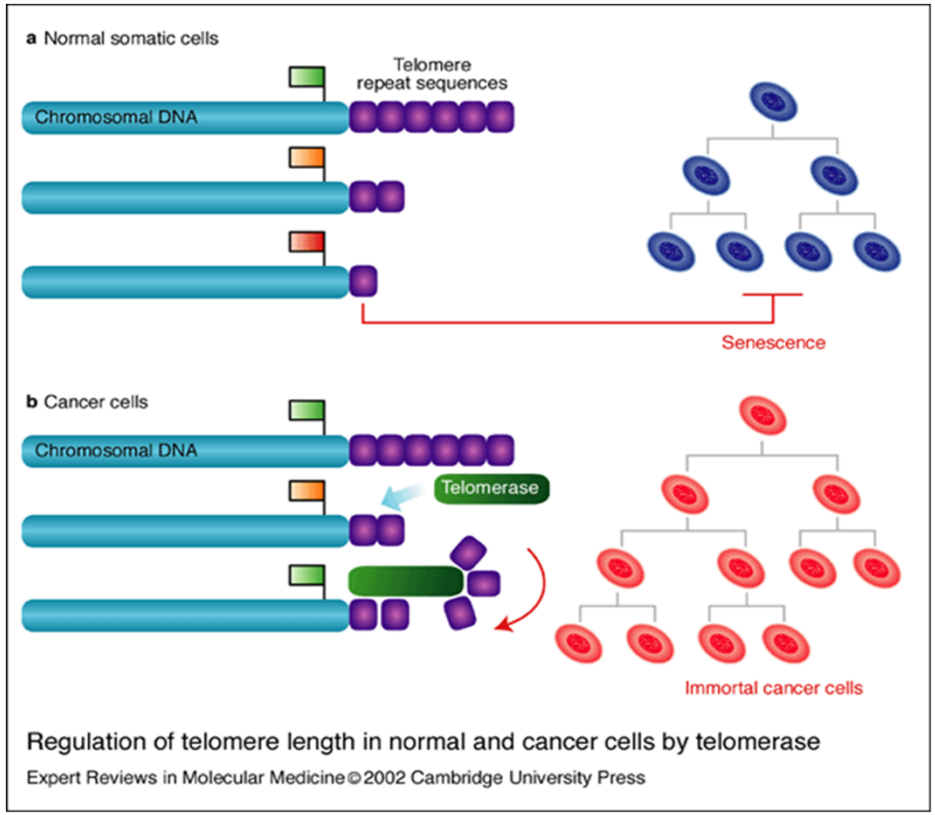
Cell Immortalization Process - hTERT Alone

Melanocytes have been immortalized by expression of human telomerase gene



Primary cells senescence after ~15 passages

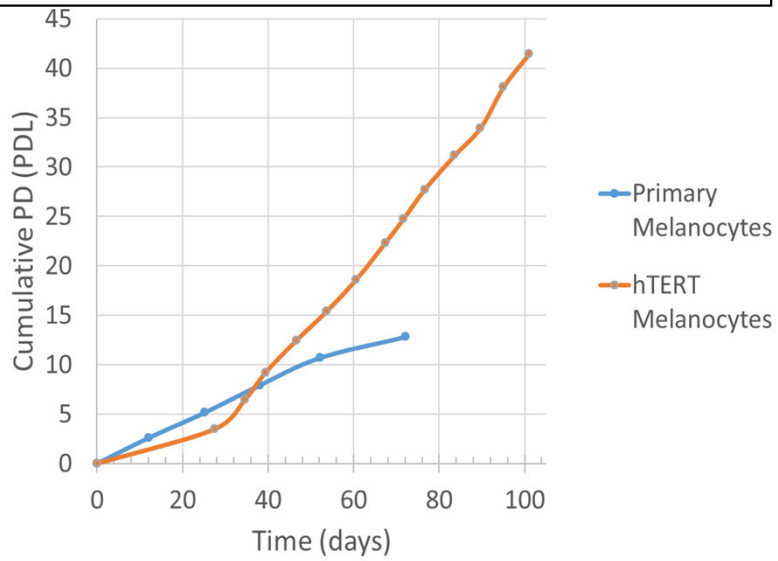
hTERT expressing clones renew their telomeres and divide indefinitely



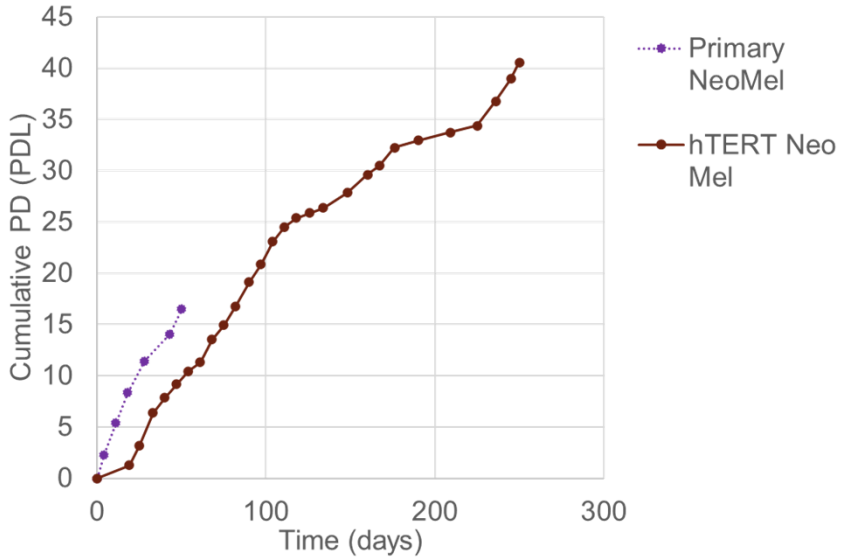
Growth of Immortalized Melanocyte Models

Consistent growth up to 40 population doublings (PD)

Growth of ATCC® CRL-4059™ Adult Dermal Melanocytes



Growth of ATCC® CRL-4064™ Neonatal Dermal Melanocytes

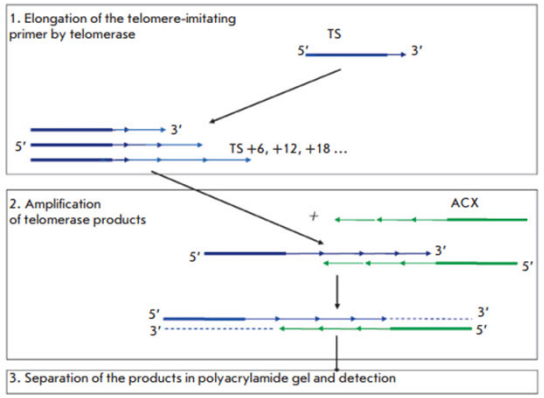


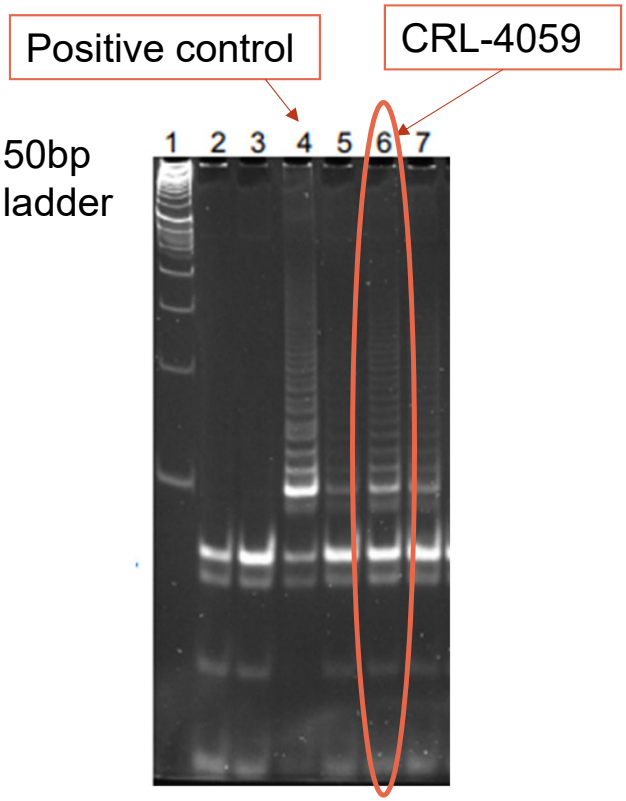
hTERT-immortalized Dermal Melanocytes Growth Media:

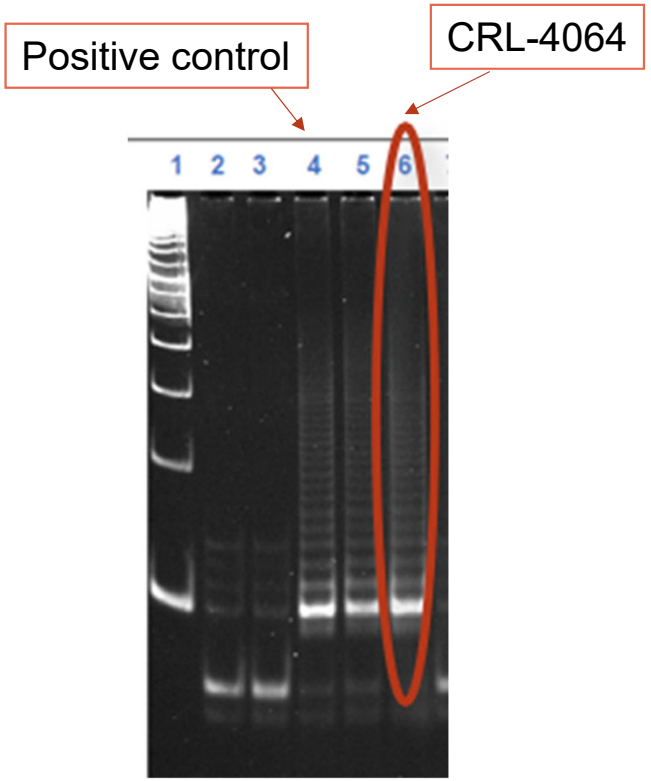
- Dermal Cell Basal Medium (ATCC® PCS-200-030™)
- Adult Melanocyte Growth Kit (ATCC® PCS-200-042™)
- 0.5 μg/mL puromycin

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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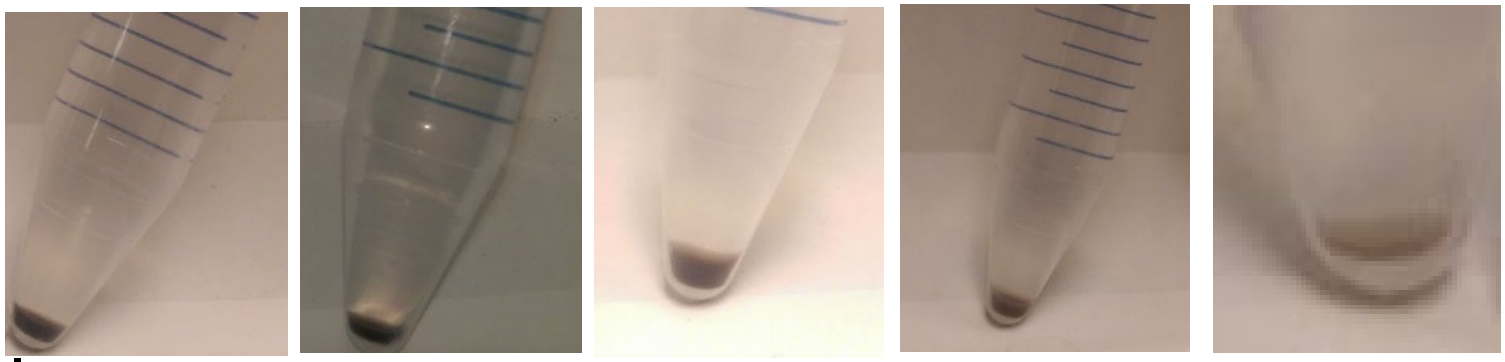
Melanin Expressed and Maintained Throughout Many Passages

Cell pellets in centrifuge tube

hTERT melanocytes



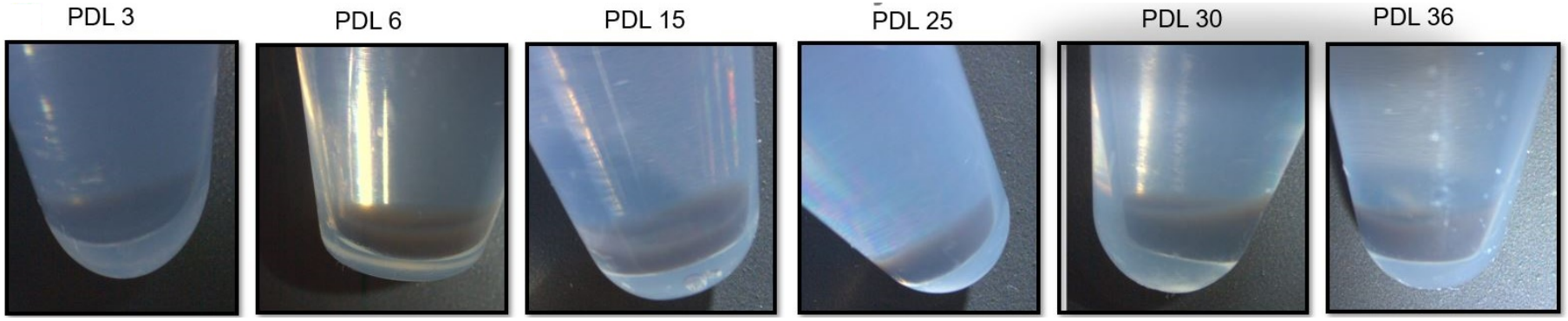
Primary adult melanocytes

negative control (adipose) cell pellet

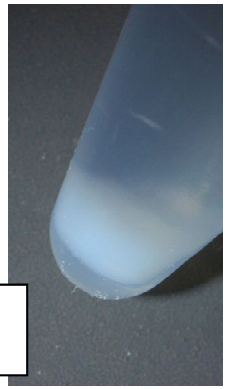
Melanin Expressed and Maintained Throughout Passaging

Cell pellets in centrifuge tube

hTERT Immortalized Neonatal Melanocyte Cell Pellet

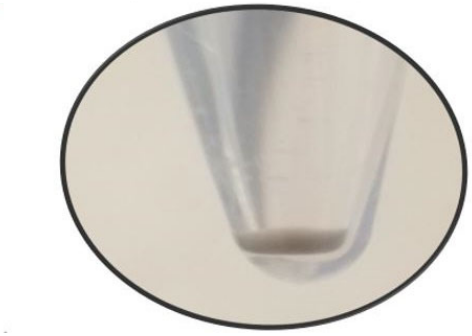


- hTERT Immortalized Neonatal Melanocyte Cells were detached from flask using trypsin and pelleted in centrifuge tube
- Images are taken at given time points throughout several months of continuous passaging



Negative control (adipose) cell pellet

Primary neonatal melanocyte cell pellet

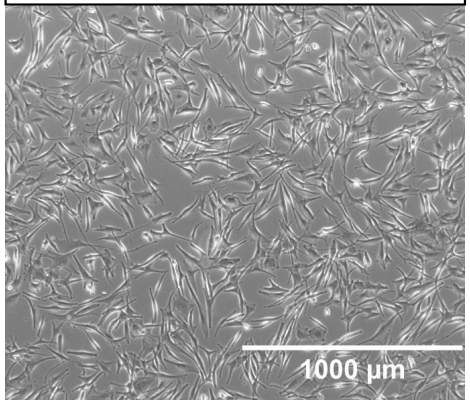


Immortalized Adult Melanocyte Morphology

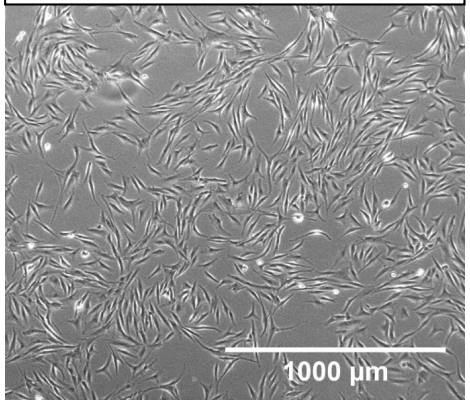
Morphology closely resembles primary cell

hTERT immortalized cells display the multi-dendritic morphology characteristic of melanocytes

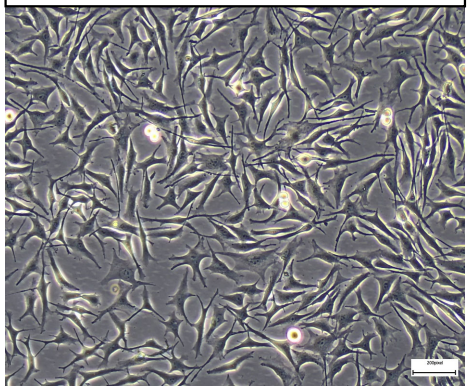
Primary Adult Melanocytes PCS-200-013™



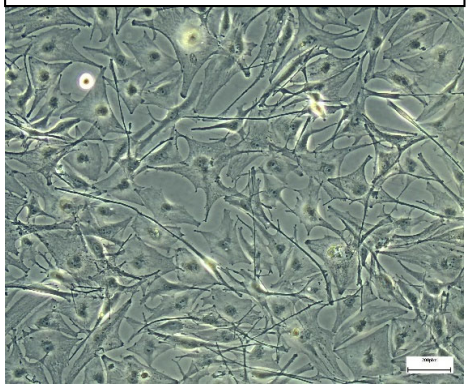
hTERT Adult Melanocytes CRL-4059™



Primary Neonatal Dermal Melanocytes PCS-200-012™



hTERT Neonatal Dermal Melanocytes CRL-4064™



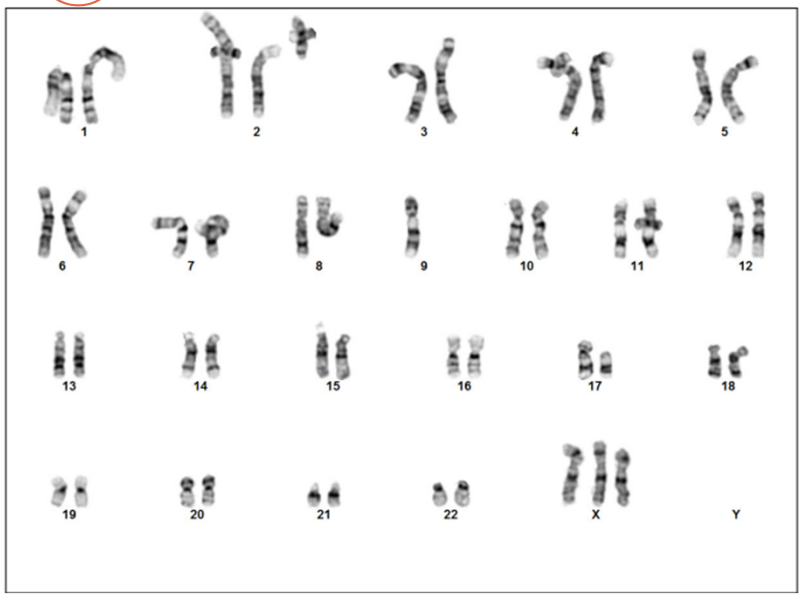
Scale bar for neonatal micrographs = 82 µm

Immortalized Adult Melanocyte Karyotype

Compared to typically polyploid cancer cell lines hTERT melanocytes have a relatively stable karyotype

hTERT Adult Melanocytes

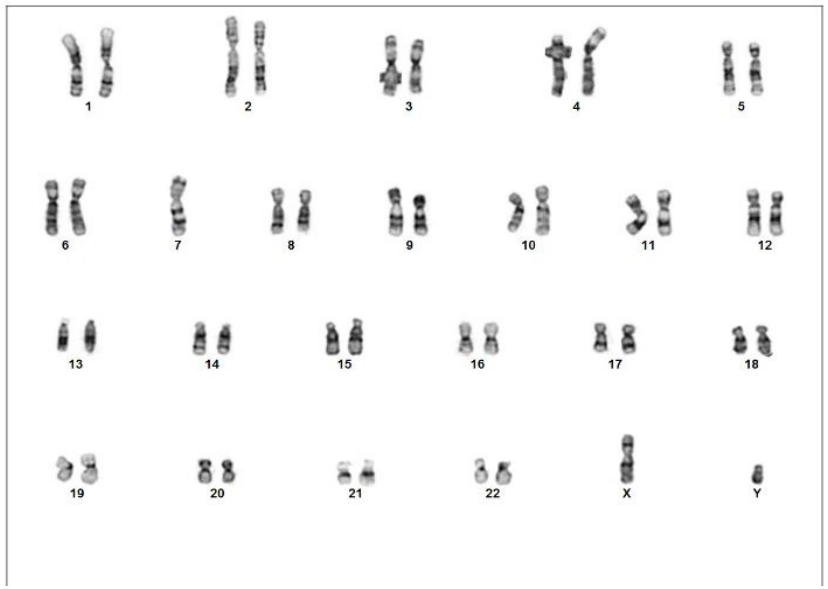
Result: 47,XX,+X,del(2)(q10),+der(2;17)(q10;p10),der(7)t(7;9)(p22;q22),-9,del(17)(p10)



near diploid female karyotype

hTERT Neonatal Dermal Melanocytes

Result: 45,XY,der(4)t(4;17)(p16;q11.2),-7

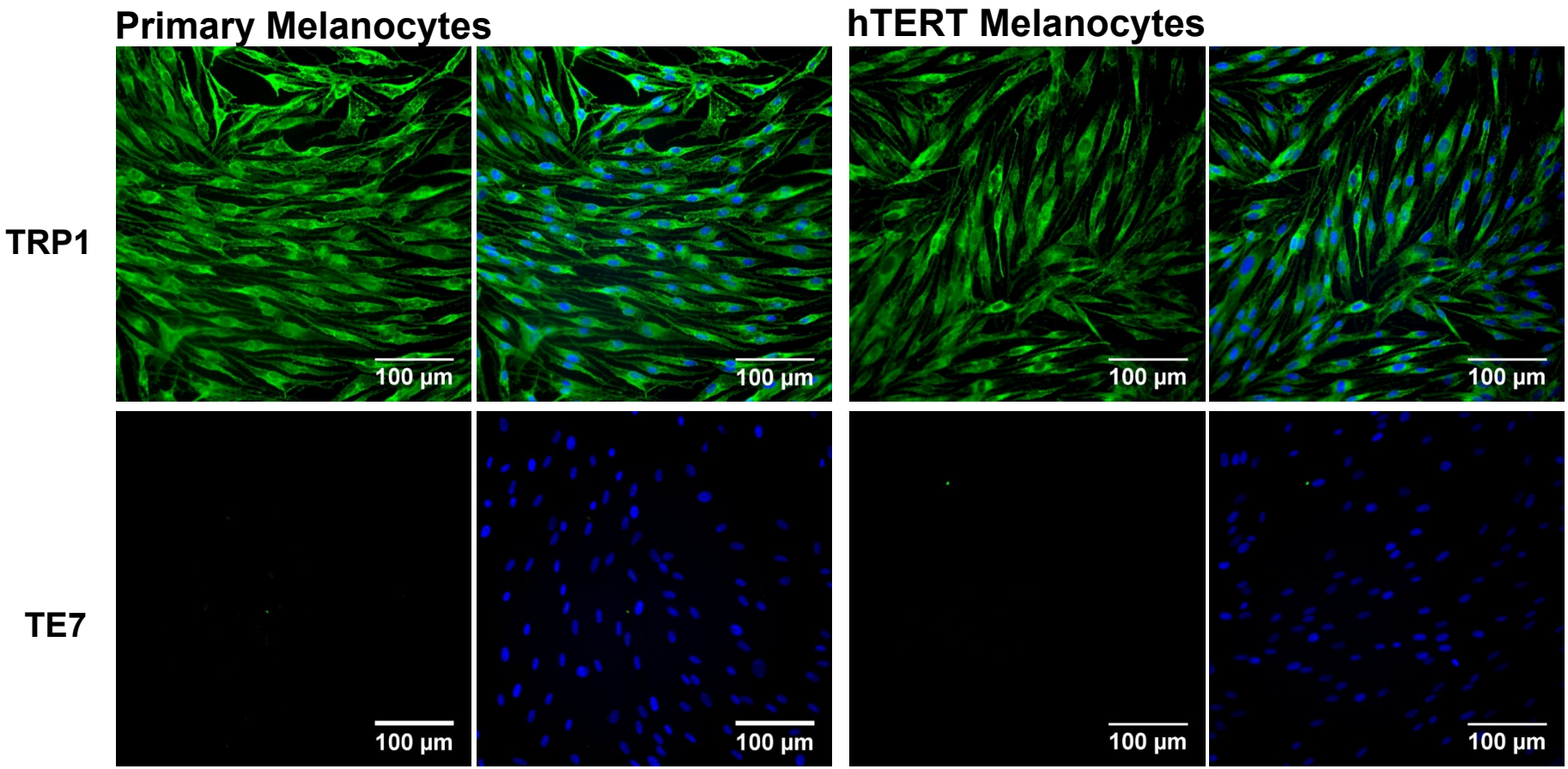


near diploid male karyotype

Karyotype performed at high passage number

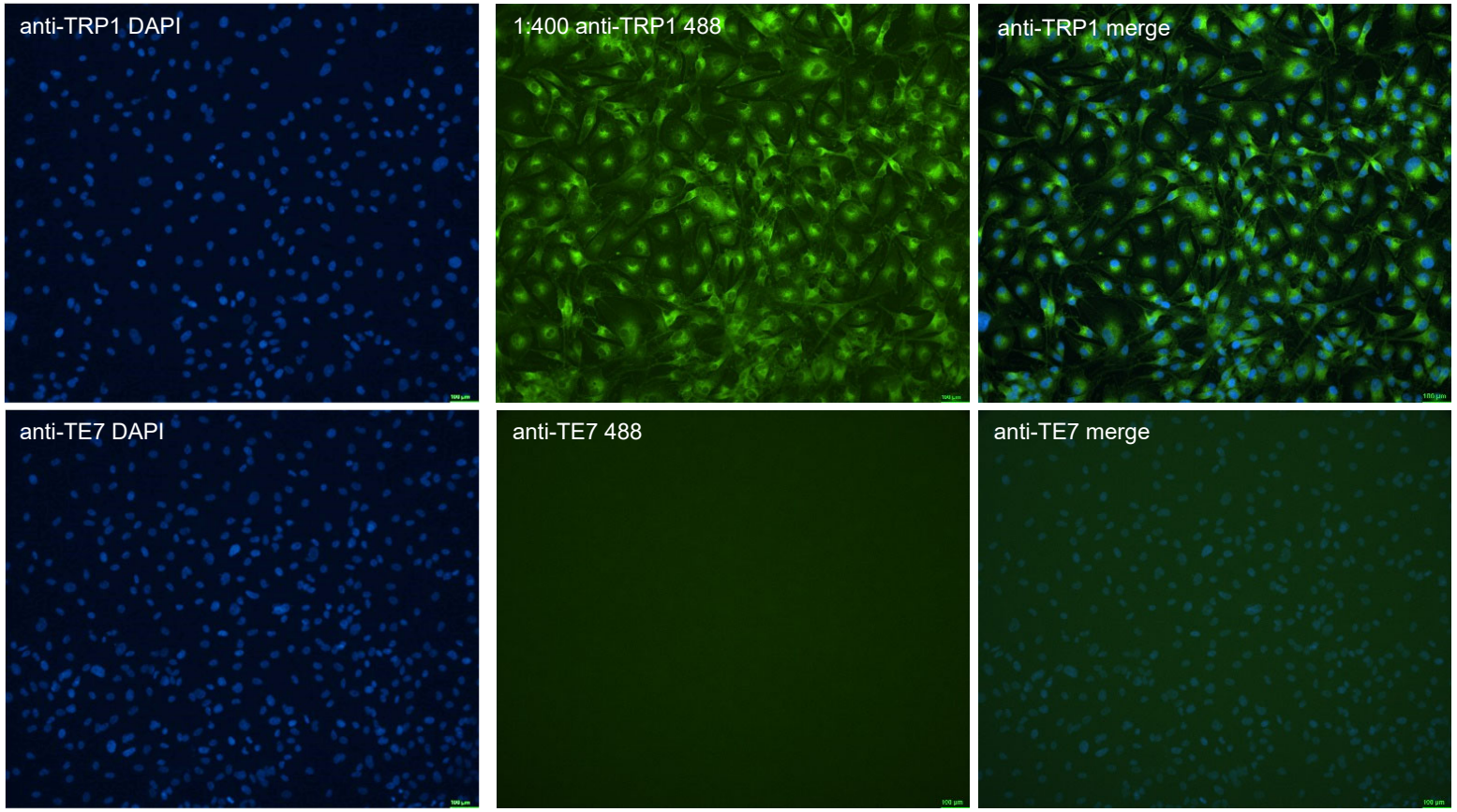
Adult Melanocyte Characteristics: Molecular Markers

Immunocytochemistry – Molecular marker staining of adult melanocytes



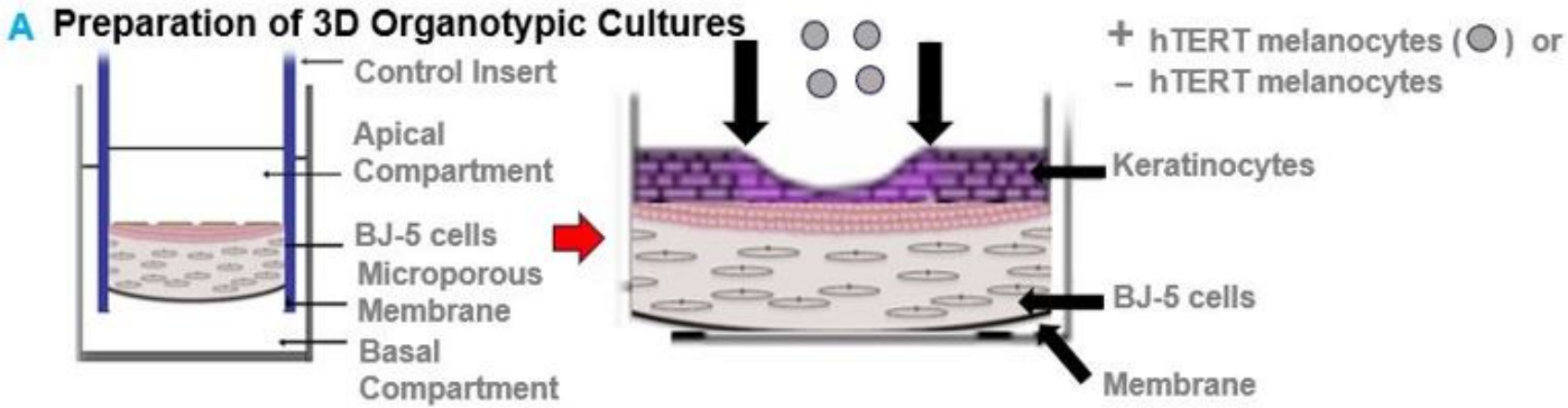
Neonatal Melanocyte Characteristics: Molecular Markers

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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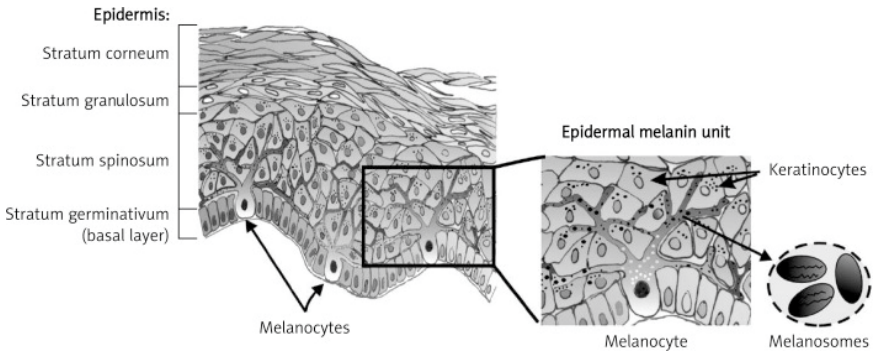
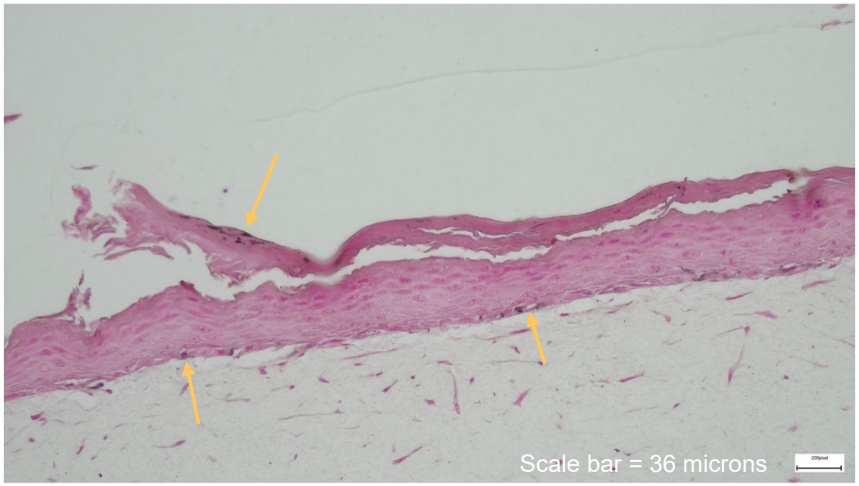
Adult Melanocyte 3D Organotypic Culture



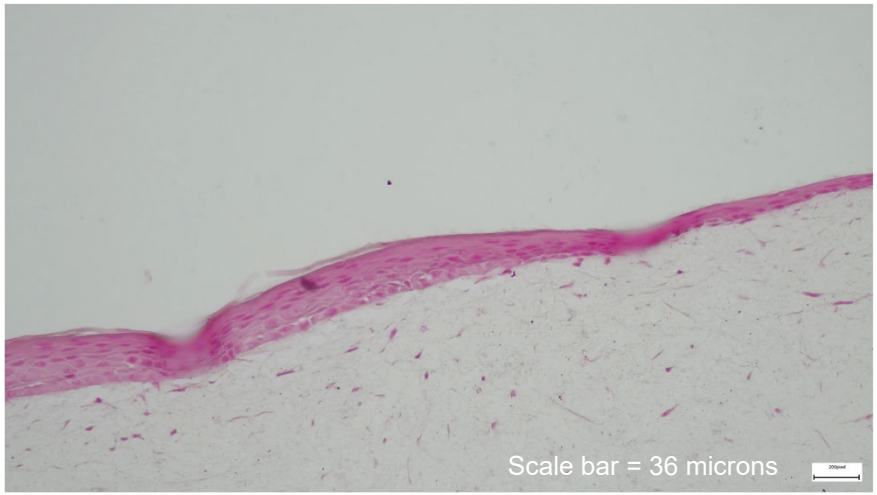
Postepy Dermatol Alergol. 2013 Feb; 30(1): 30–41.

- 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

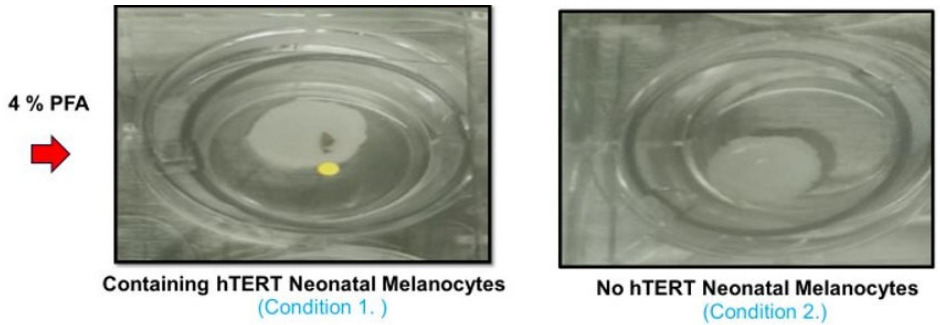


Fibroblast/Keratinocytes



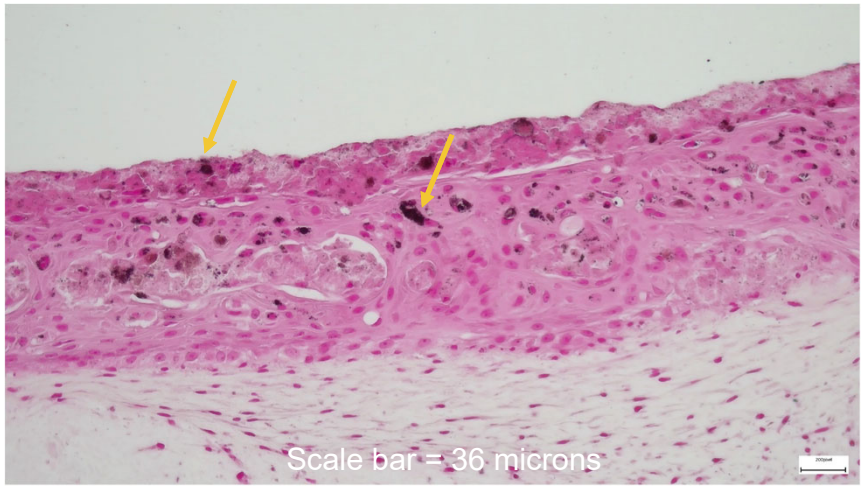
Neonatal Melanocyte 3D Organotypic Culture

Melanin deposits visible in 3D organotypic co-culture

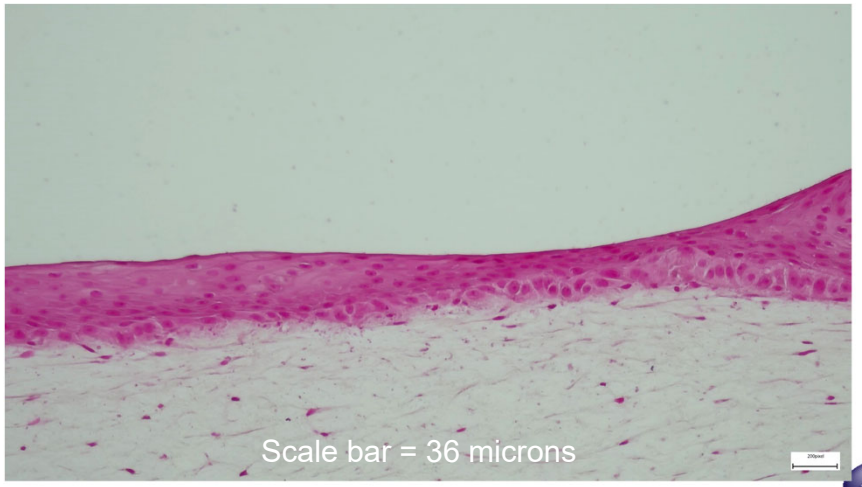


- Melanin visible in macroscopic & microscopic images of 3d cultures.
- Generally, less tissue development is observed in cultures without melanocytes.

Fibroblast/Keratinocytes + hTERT Neo Melanocytes



Fibroblast/Keratinocytes



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

Melanin Synthesis Pathway

Inhibit/stimulate melanogenesis with specific compounds

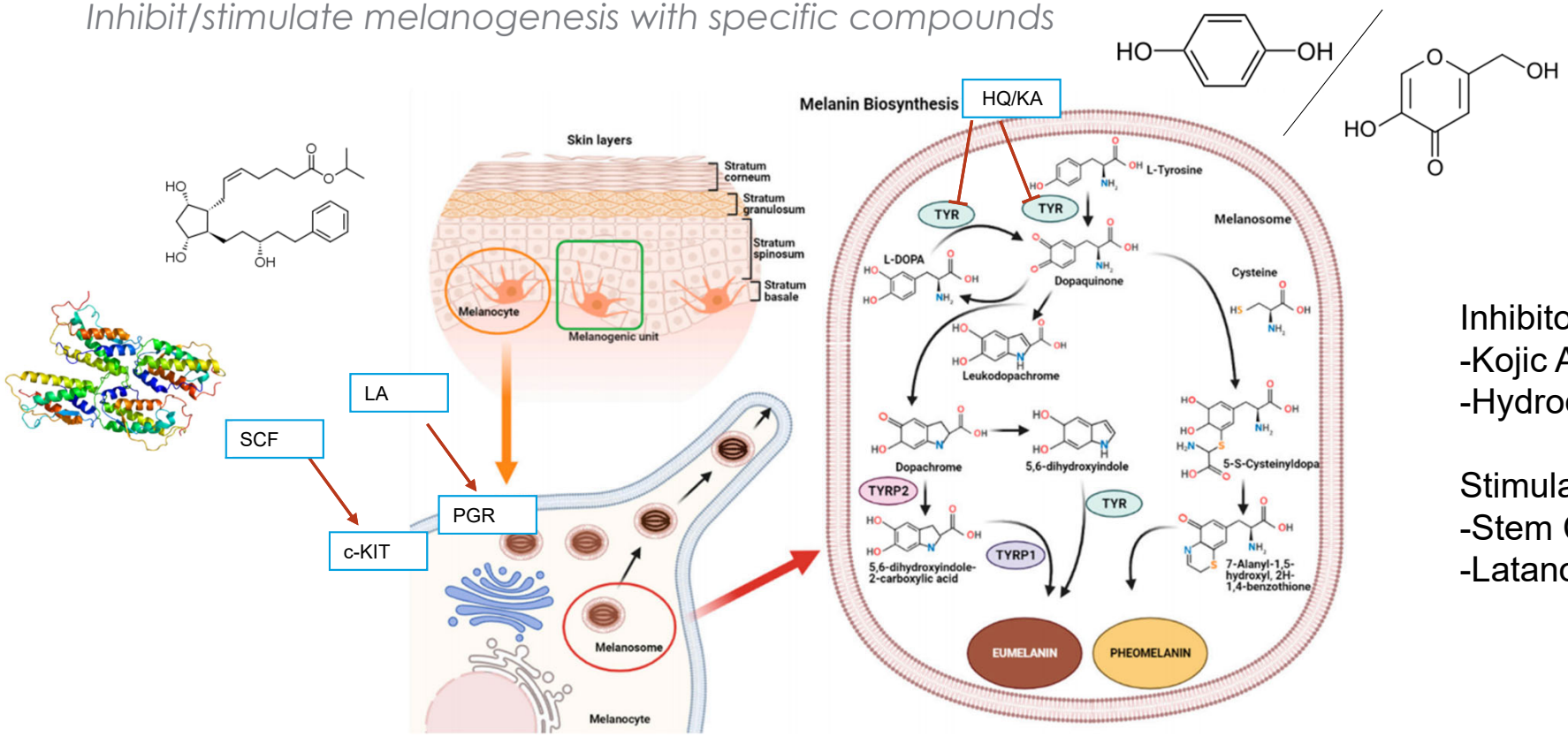


Figure 1. Representation of the melanogenic unit and melanin synthesis in melanosomes (**left**). Schematic representation of eumelanin and pheomelanin biosynthetic pathways (**right**).

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

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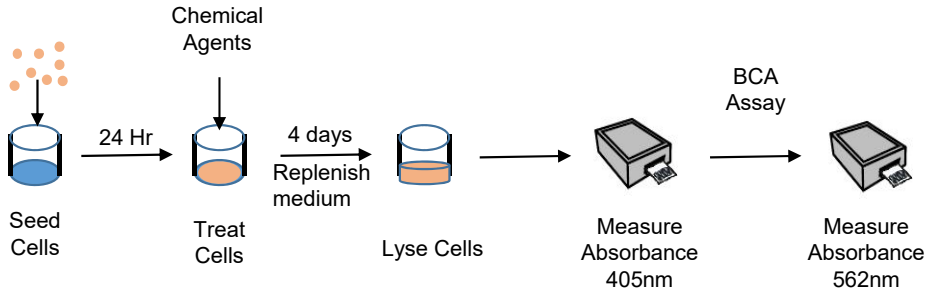
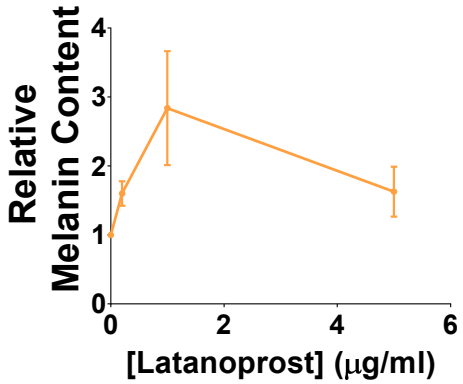
Adult Melanocyte Stimulation and Inhibition Study

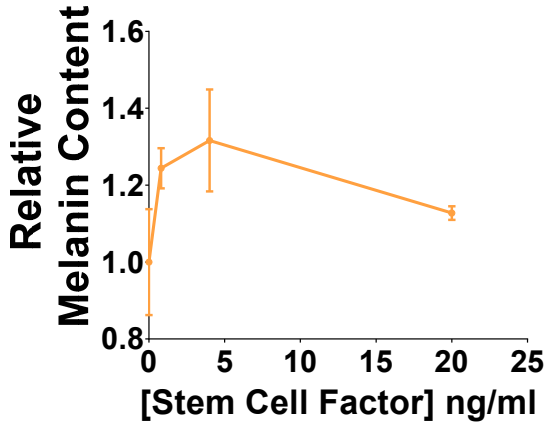
Testing responsiveness to stimulators and inhibitors of melanogenesis

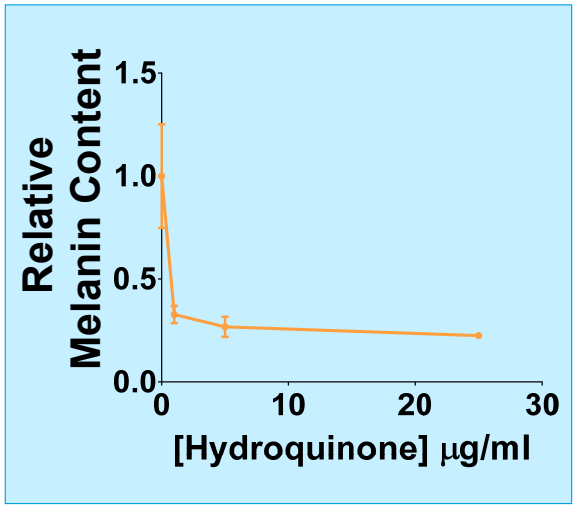




- Total protein determined by BCA assay and fitting to standard curve of 8 concentrations
- Melanin content adjusted relative to total protein and untreated control

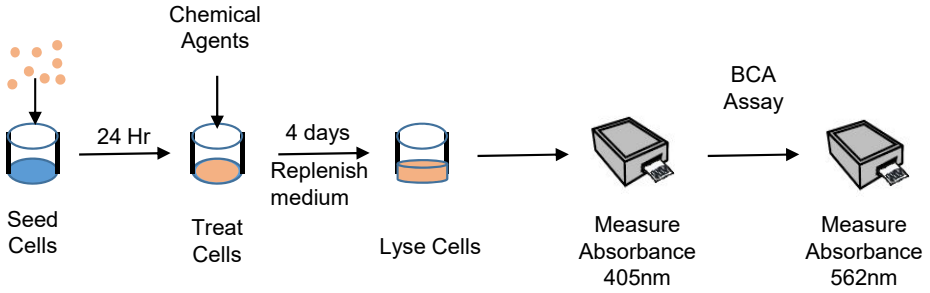
$$Rel.Mel.cont_i = \frac{\frac{A405_i}{Total\ Protein_i}}{\frac{A405_{untreated}}{Total\ Protein_{untreated}}}$$

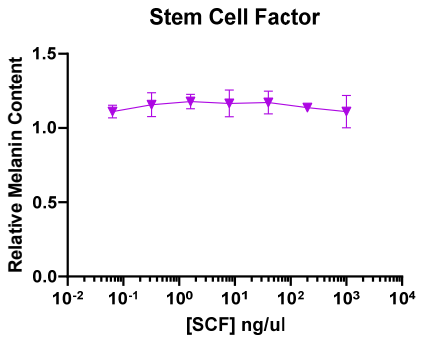


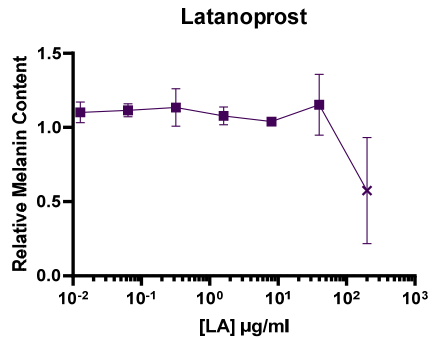


Neonatal Melanocyte Stimulation and Inhibition Study

Testing responsiveness to stimulators and inhibitors of melanogenesis

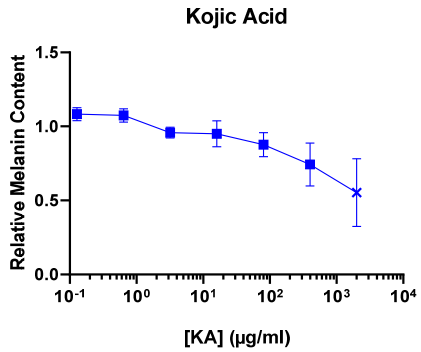


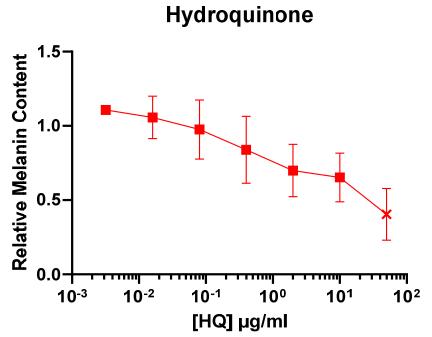




Neonatal cells show minimal response to stimulators and respond to inhibitors in a dose-dependent manner

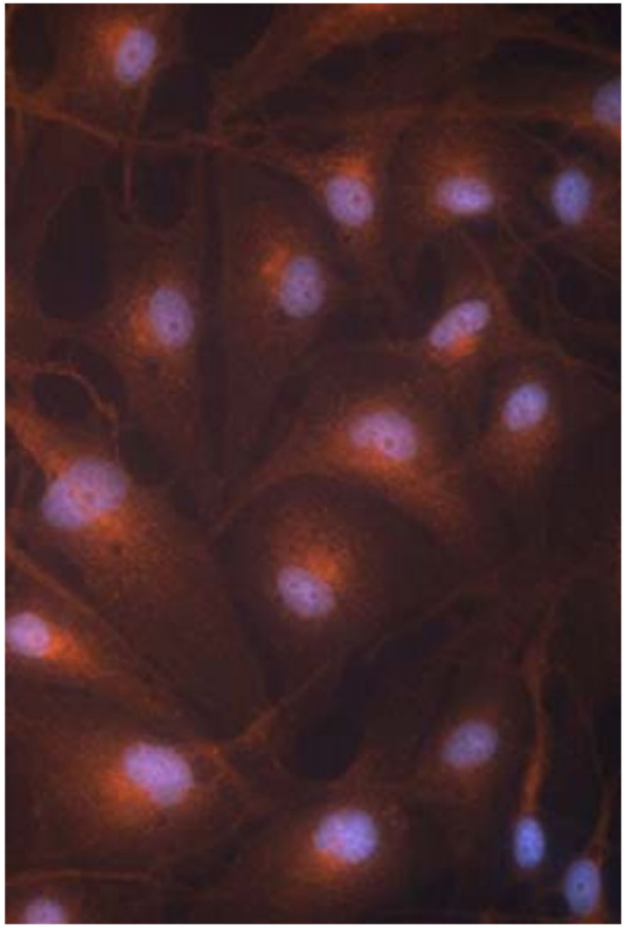
Adult and neonatal primary cells also tested using the same conditions (data not shown) and showed similar responses





Summary and Conclusions

- Immortalized melanocytes are available from adult and neonatal donors
- CRL-4059/64 hTERT immortalized cell lines show key melanocyte characteristics:
  - Multi-dendritic morphology, expression of key molecular markers, 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



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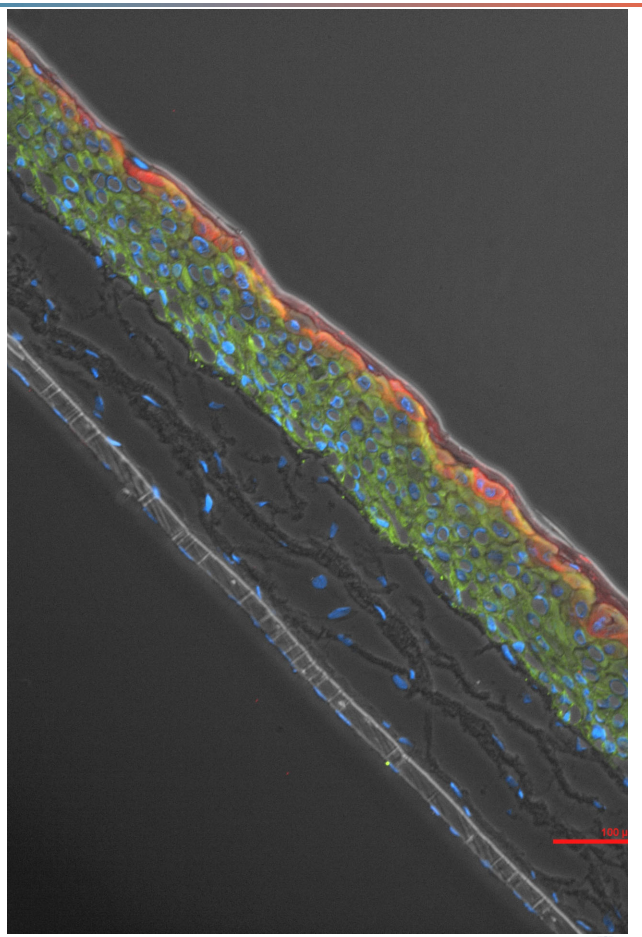
Summary and resources

- 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/hTERT



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