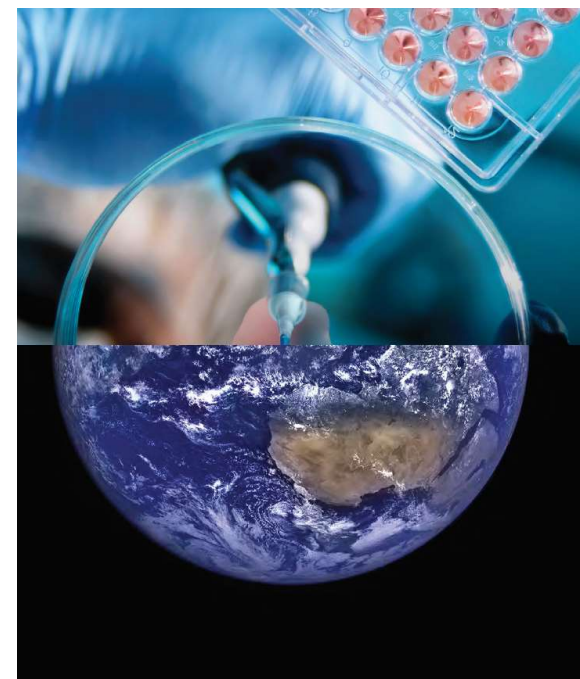
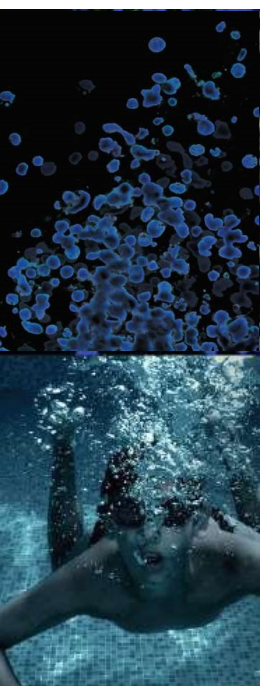




# On the edge of the bubble: Use of exosomes as reference materials in biomedical research HJ14

Siddhartha Paul, Ph.D.  
*Scientist, ATCC Cell Biology*

Credible Leads to Incredible™



## Slide 1

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**HJ14** Check consistency of font, especially on the slide headings  
Huuskonen, Jarkko, 10/22/2019

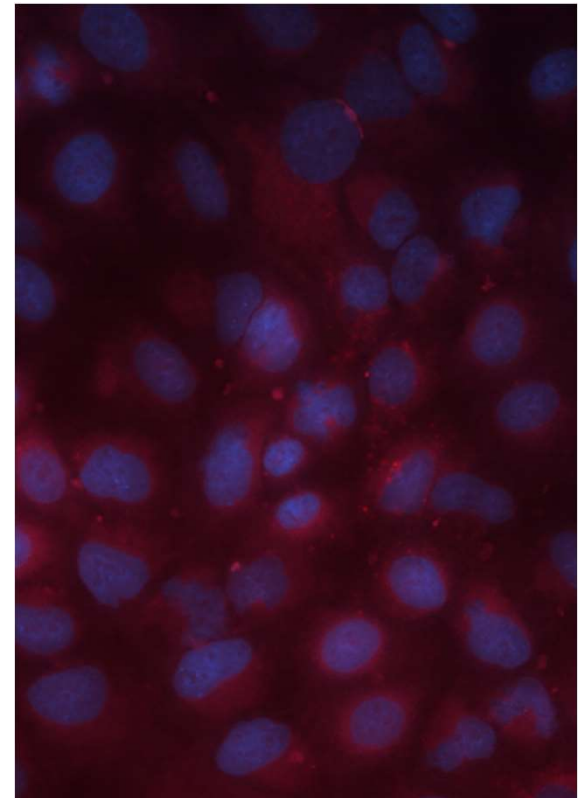
# About ATCC

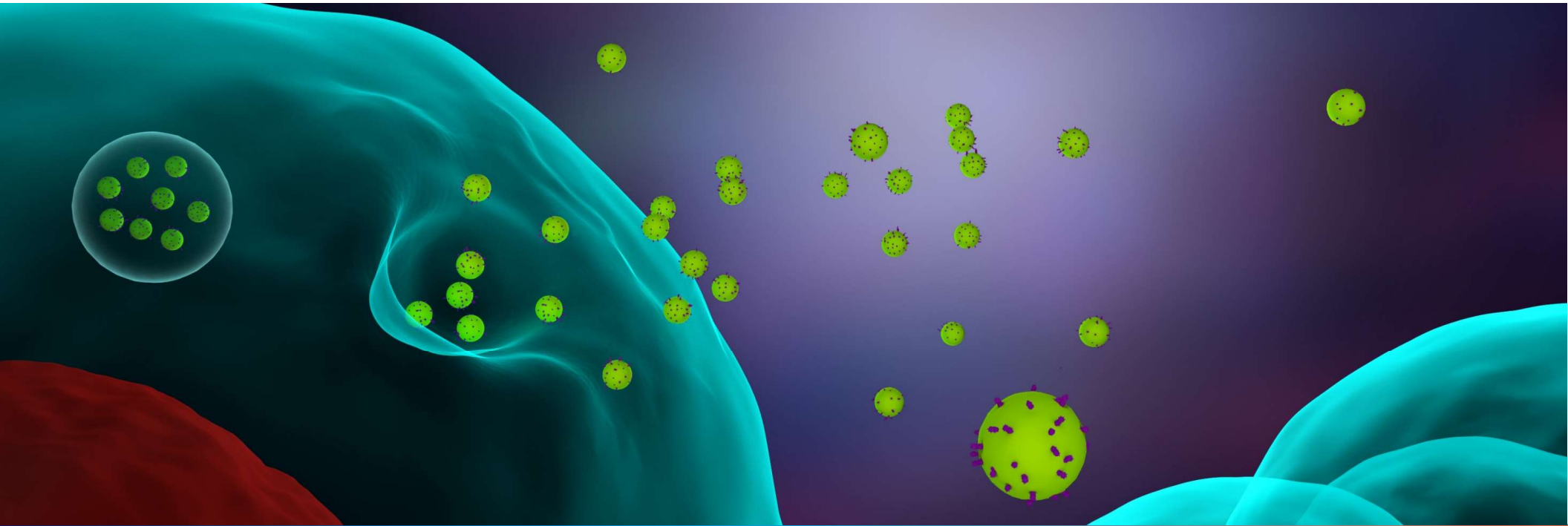
- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for microbes – the “*gold standard*”
- Innovative R&D company featuring gene editing, microbiome, NGS, 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, 18 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

# Agenda

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- I. Exosomes and extracellular vesicles
- II. Exosome reference material – ATCC quality and reproducibility
- III. Validation data to support ATCC exosome reference materials





# Exosomes and extracellular vesicles

# Extracellular vesicles and exosome biogenesis

- Cell-derived
- Present in all biological fluids
- 30-200 nm with lipid bilayer
- Serve as “cargo” for RNA and protein molecules
- Diverse functions:
  - Cellular communication
  - Waste product management

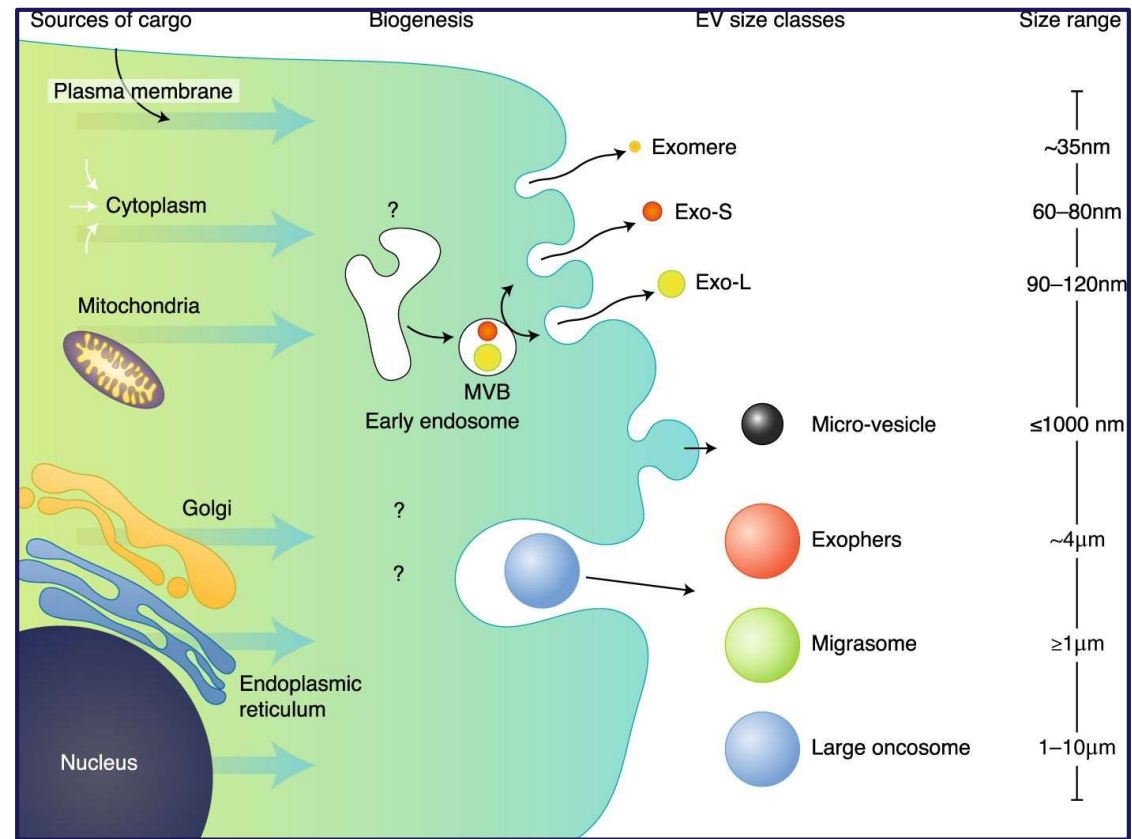
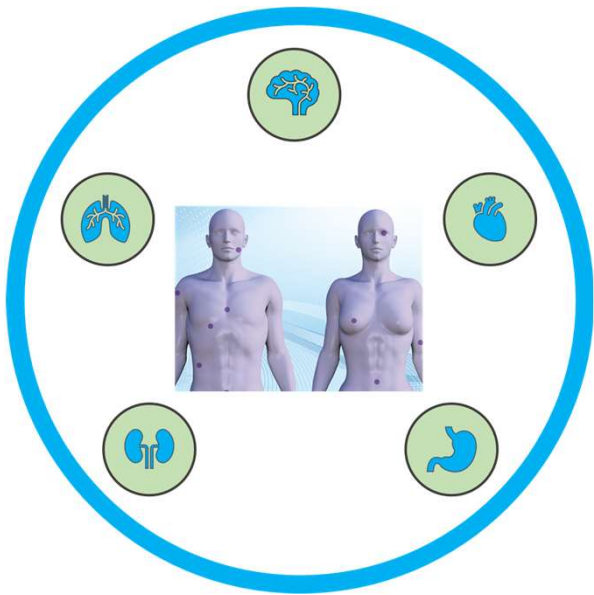
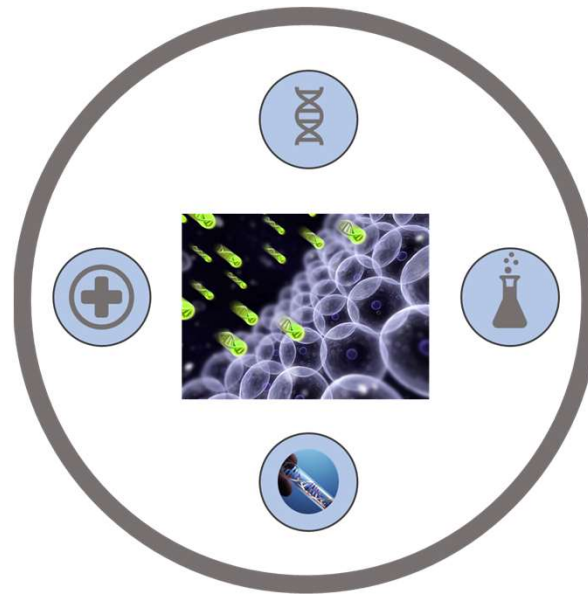


Image adapted from Zijlstra et al, Nature Cell Biology, 2018

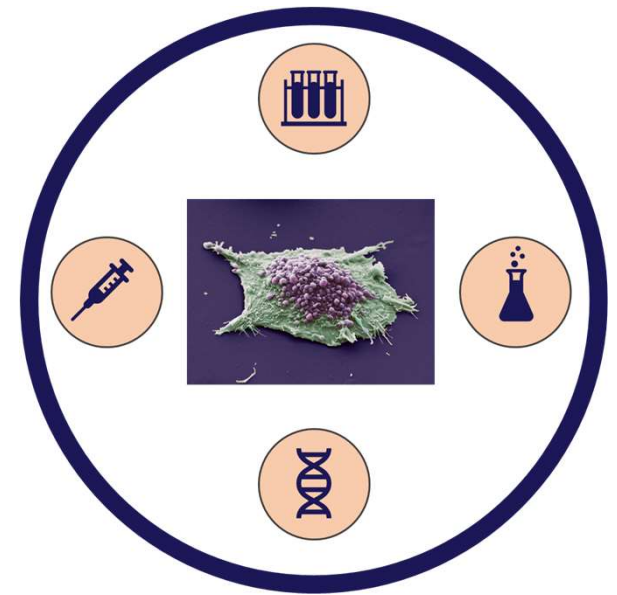
# Application of exosomes



Liquid biopsy/Diagnostics



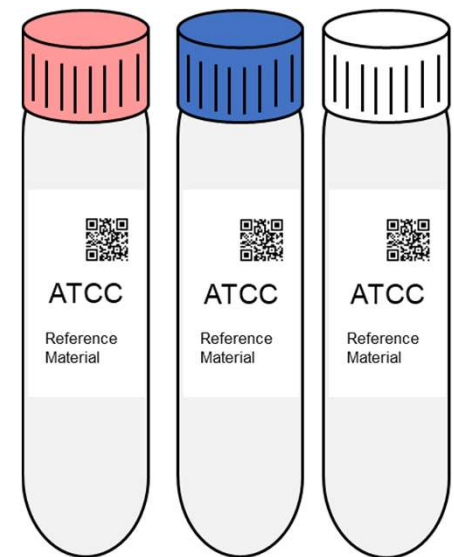
Cell therapy/Regenerative medicine



Payload/Drug delivery

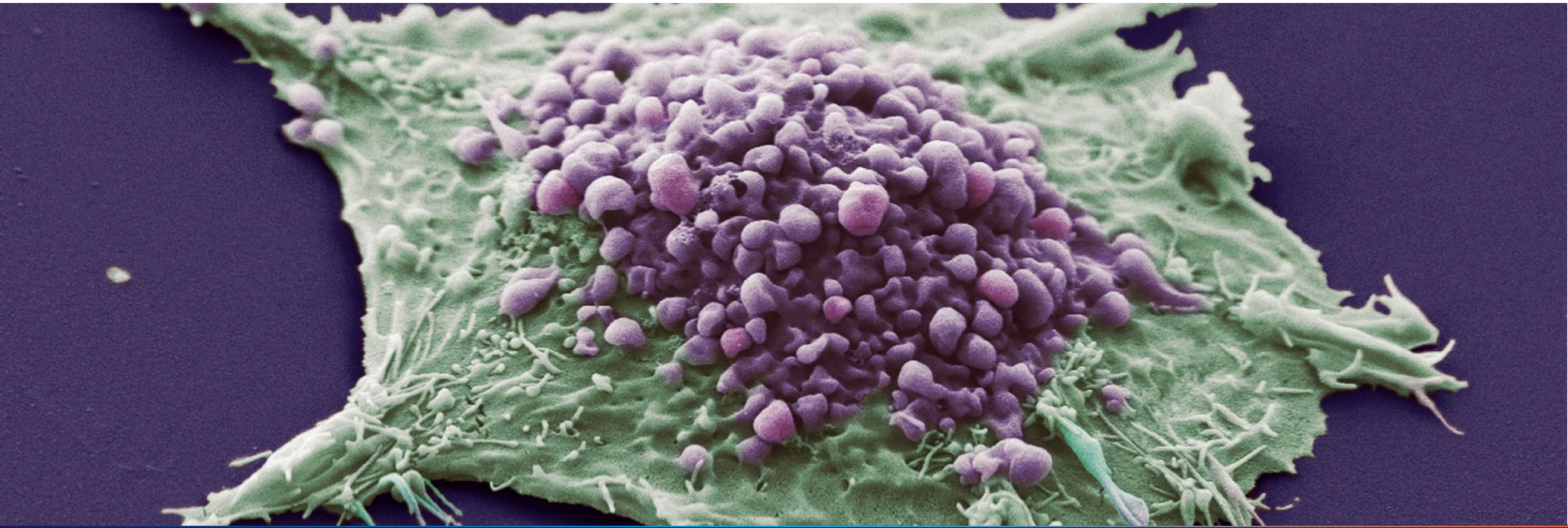
# Need for reference standards

- Reference standards reduce the time and costs of developmental work
- Reference standards increase reproducibility of the assays
- Reference materials help to regulate the quality of one's own material



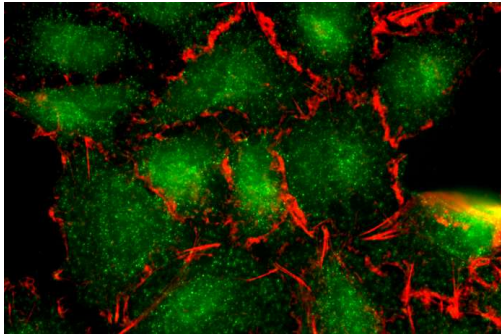
*Reference standards add value to research work or product development*





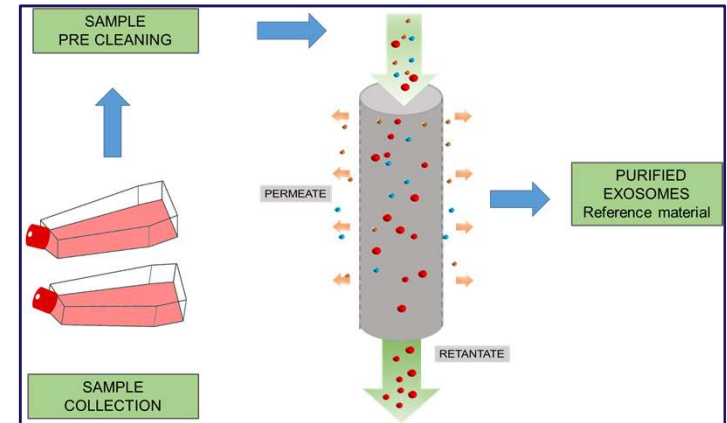
## Exosome reference materials

# A platform for exosomes isolation and quality control

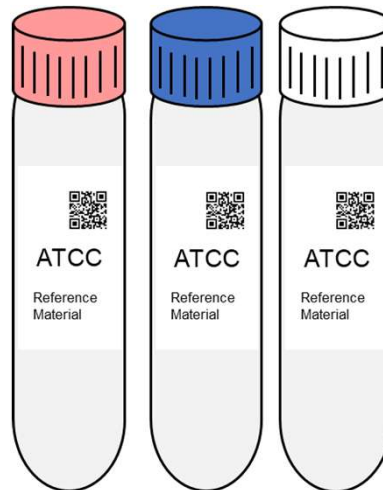


ATCC authenticated cell lines

ATCC's TFF exosome isolation strategy



Exosome reference materials



Assay development

Size standards

Control for lab developed tests

TFF: Tangential flow filtration

## ATCC exosome portfolio

ATCC® No.	Parental cell designation	Cancer type	Status
SCRC-4000-EXM™	hTERT-immortalized adipose-derived mesenchymal stem cell (MSC)	N/A	AVAILABLE
CCL-185-EXM™	A549	Carcinoma, lung	AVAILABLE
CRL-1435-EXM™	PC-3	Adenocarcinoma, prostate	COMING SOON
CCL-247-EXM™	HCT-116	Carcinoma, colorectal	COMING SOON
CRL-1740-EXM™	LnCap	Carcinoma, prostate	COMING SOON
HTB-26-EXM™	MDA-MB-231	Adenocarcinoma, breast	COMING SOON
HTB-14-EXM™	U-87 MG	Glioblastoma	COMING SOON
CCL-2-EXM™	HeLa	Adenocarcinoma, cervix	COMING SOON
HTB-22-EXM™	MCF-7	Adenocarcinoma, breast	COMING SOON

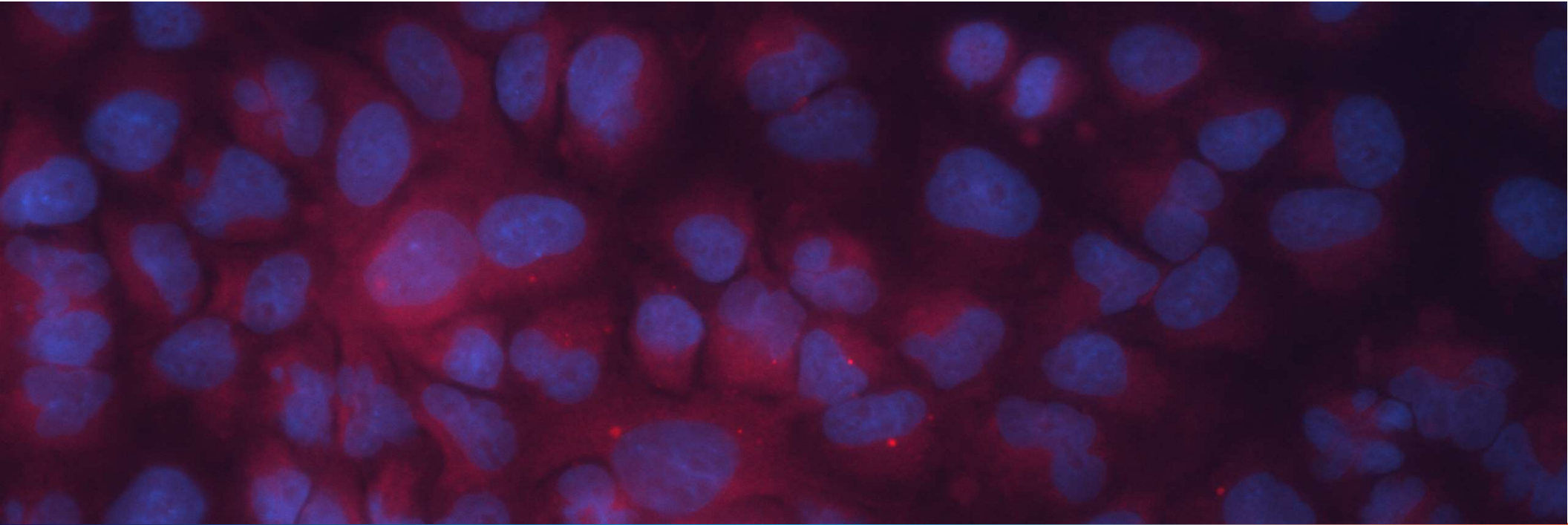
***Immortalized cell lines and continuous cell lines reduce lot to lot variability***

# ATCC quality control for each lot of production

Attributes	Test	Specification
Protein concentration/vial	BCA assay	Approximately 100 µg
Particle number /vial	NTA analysis	≥ 10 <sup>9</sup> particles
Size distribution (% particle within 50-200 nm)	NTA analysis	Cell type dependent
Protein Marker expression	Western Blotting	Transmembrane and cytosolic proteins
Sterility	Bacteria, yeast, fungi	Sterile
Mycoplasma	PCR based assay	Negative

# Comparison of ATCC and competitor exosomes

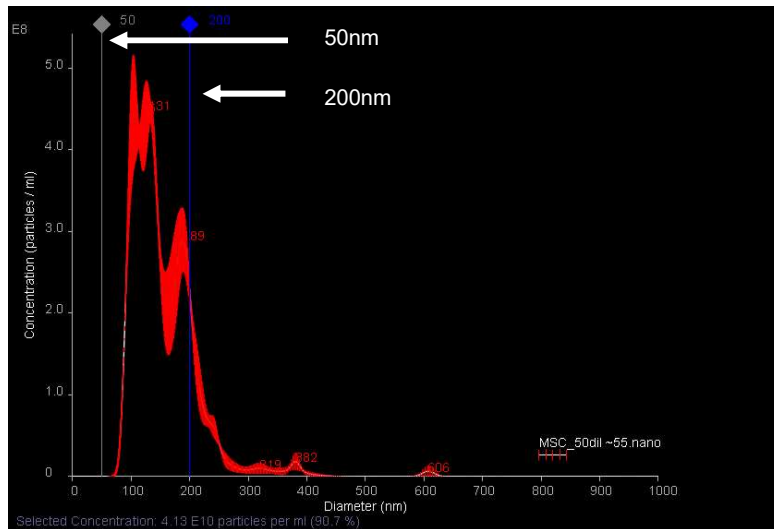
Product Attributes	ATCC	Competitor H	Competitor A	Competitor B	Competitor Z
Defined size range	✓	✗	✗	✗	✗
Marker verification	✓	✓	✗	✗	✗
Particle verification	✓	✗	✗	✗	✗
Functional data	✓	✗	✓	✓	✓
Frozen or lyophilized	Frozen	Lyophilized	Frozen	Frozen	Frozen



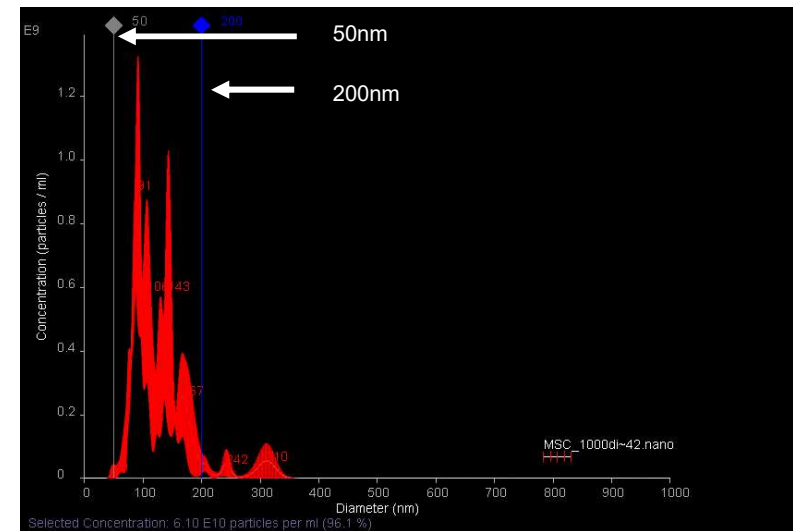
## Characterization of ATCC exosomes

# Characterization of TFF-purified exosomes: Size distribution analysis via NTA

MSC Exosomes Lot 1



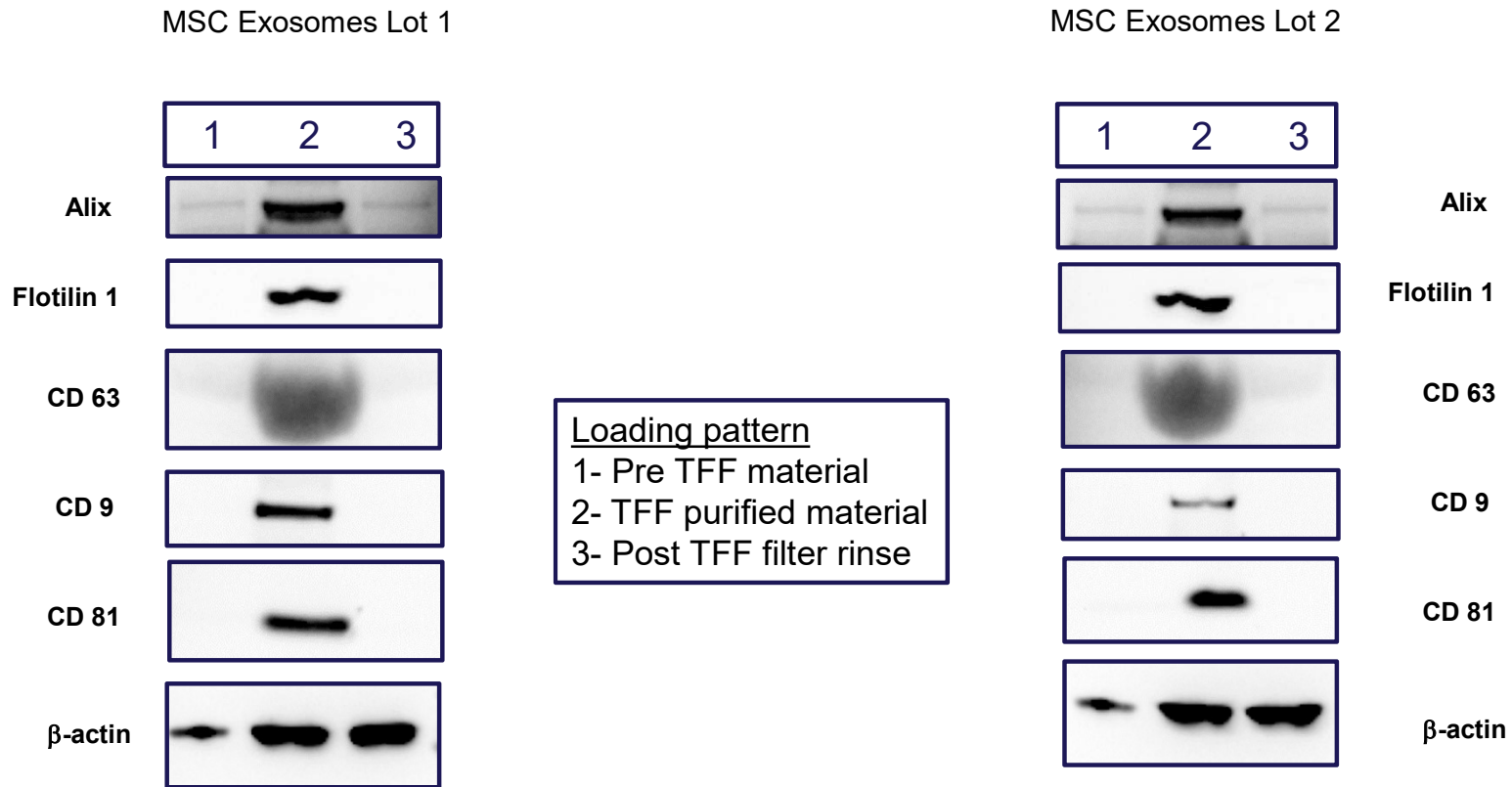
MSC Exosomes Lot 2



Cell Type	Particle number	% Particles between 50- 200nm size range
MSC exosome Lot 1	4.1×10 <sup>10</sup> particles/mL	90.7
MSC exosome Lot 2	6.1×10 <sup>10</sup> particles/mL	96.1

***Consistent and high yield of particles in exosome size range was observed in two separate production lots***

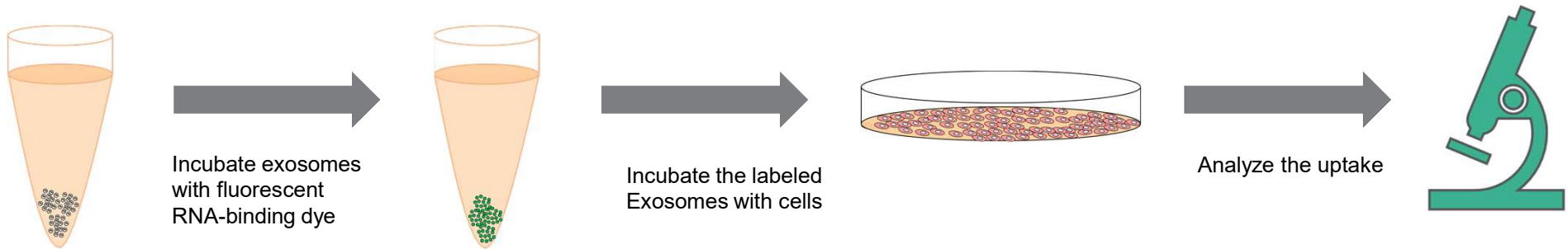
# Characterization of TFF-purified exosomes



*Consistent expression of exosomal biomarkers was observed in two separate production lots*



# Functional testing: Exosome uptake assay

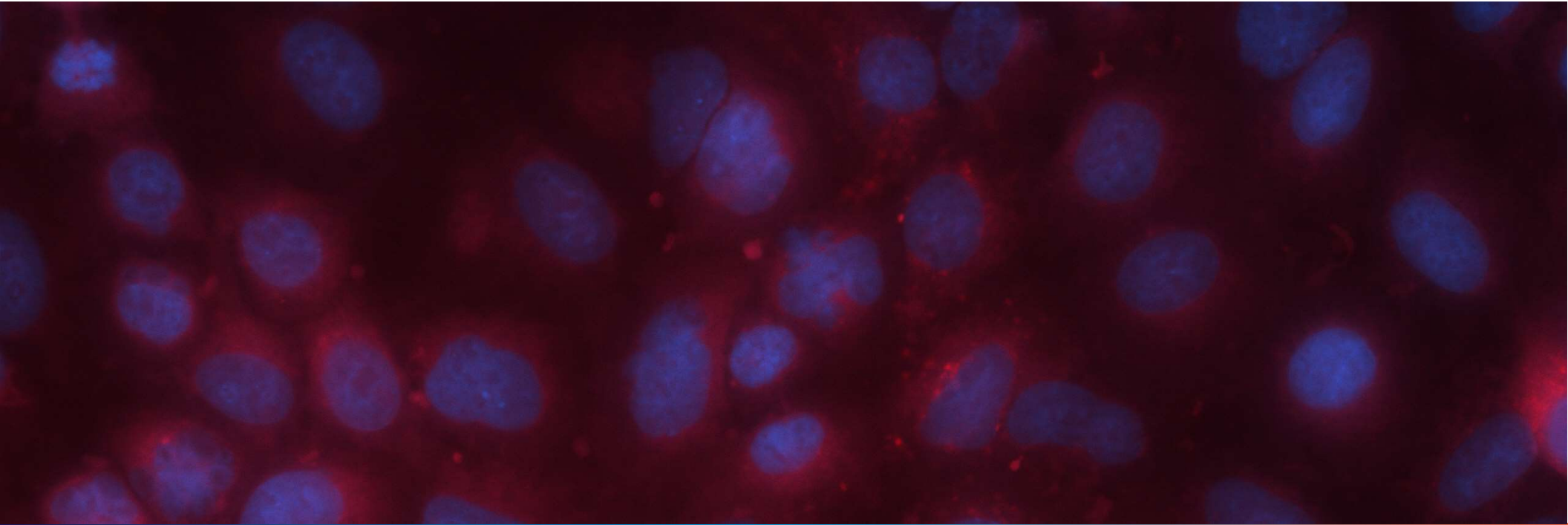


DYE-labeled exosomes

DYE only

No treatment

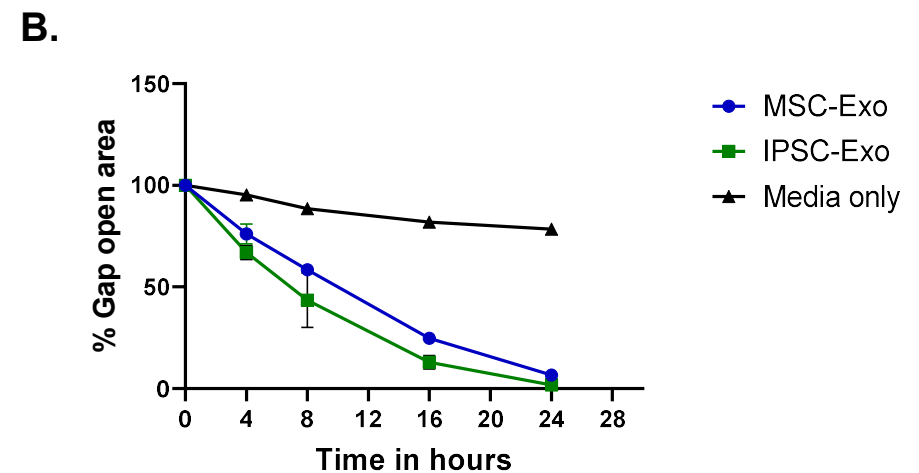
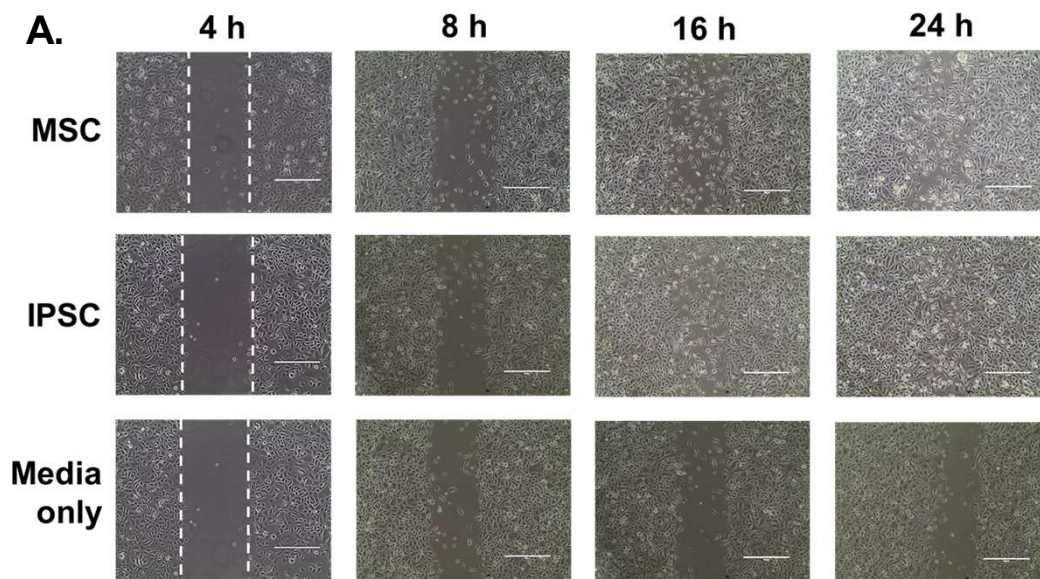
*Exosomes uptake was tested in multiple cell types*



## Functional data using ATCC exosomes

# Functional testing: Wound healing and migration

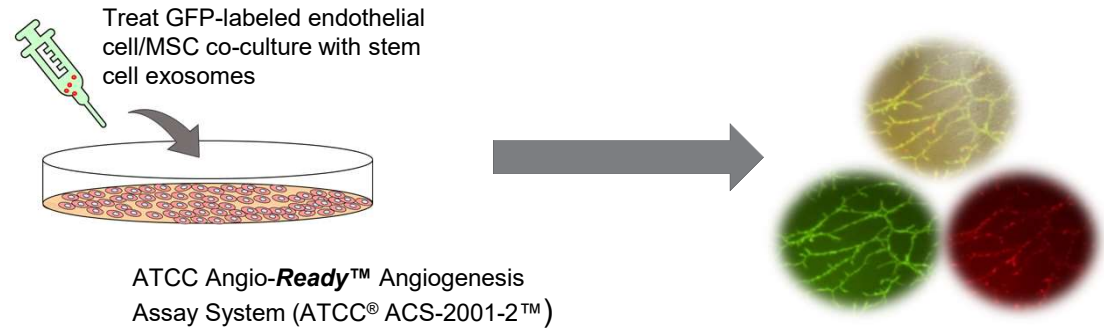
A gap was created on Primary Gingival Keratinocytes (ATCC® PCS-200-014™), followed by treatment with exosomes derived from MSCs and iPSCs



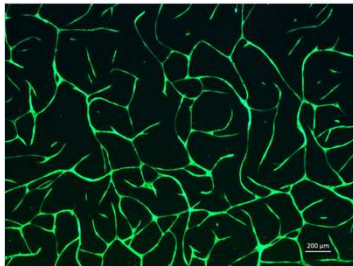
*Stem cell-derived exosomes promote cell migration*

# Functional testing: *In vitro* endothelial tubule formation

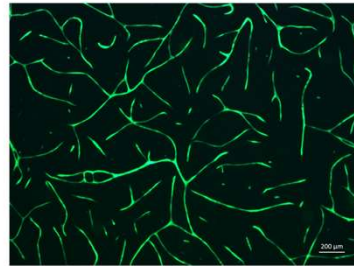
A.



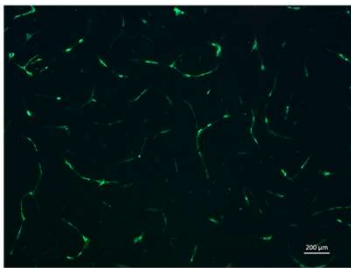
MSC exosomes



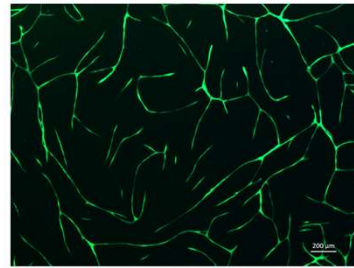
iPSC exosomes



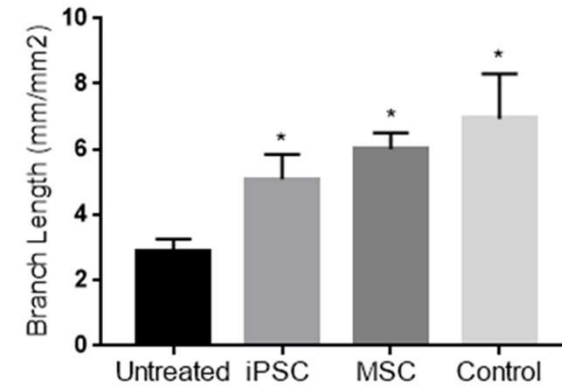
Untreated



Positive control



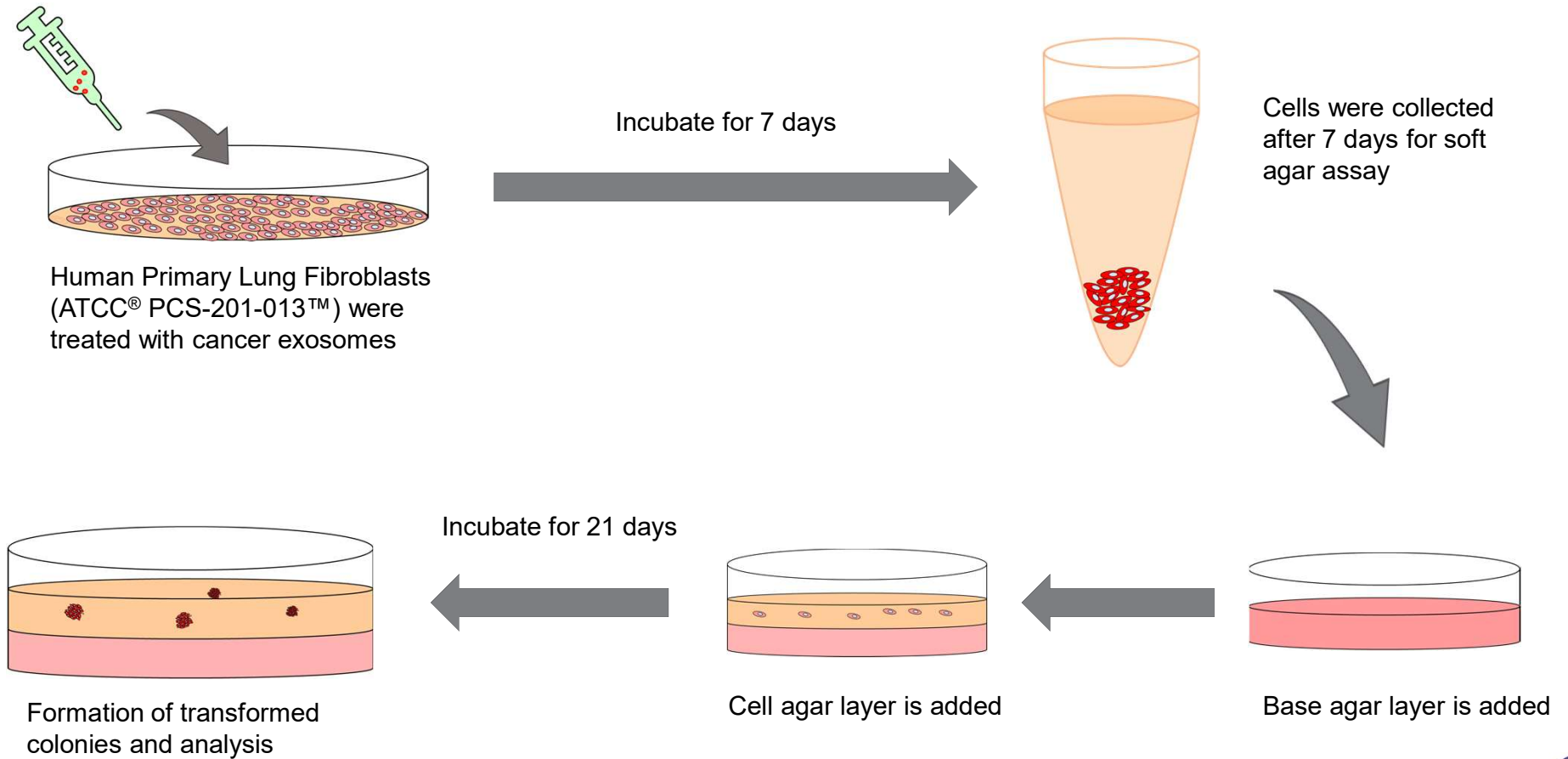
B.



\*=p < 0.05

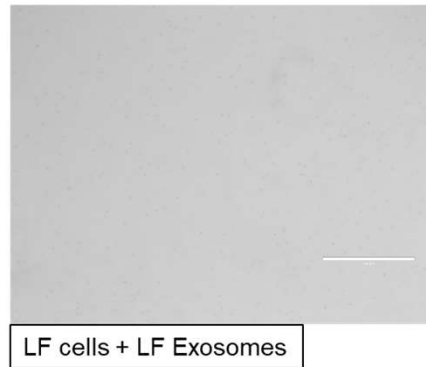
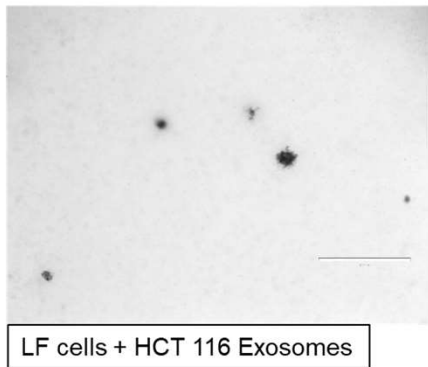
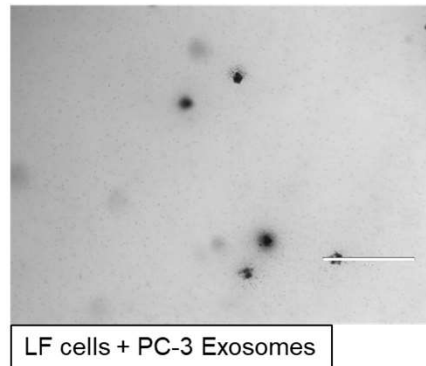
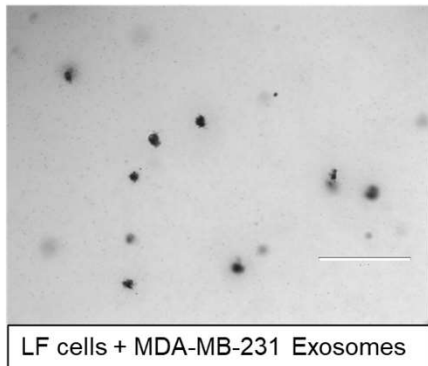
***Stem cell exosomes promote angiogenesis***

# Functional testing: Anchorage-independent growth

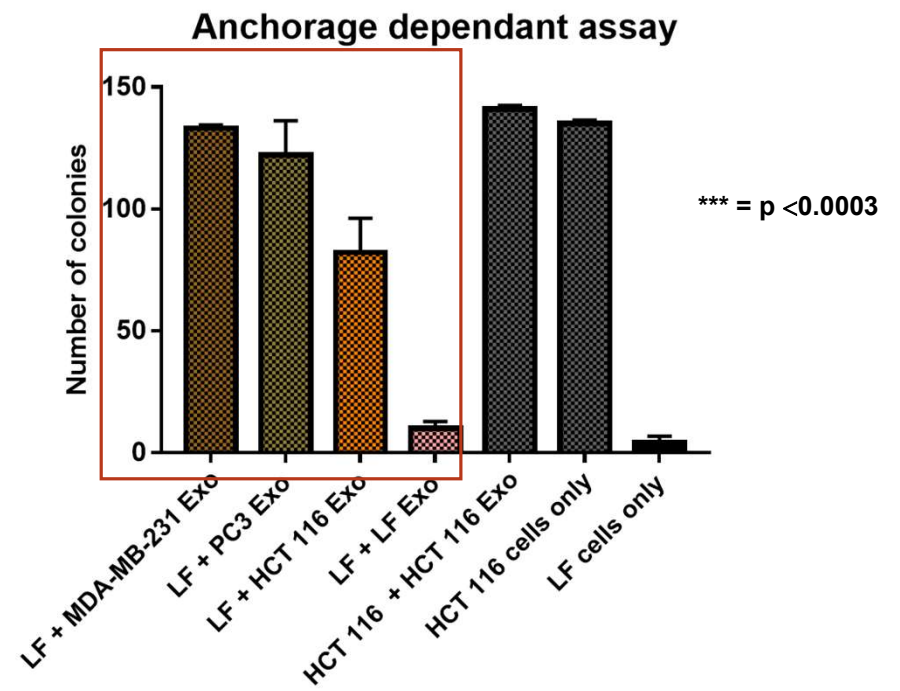


# Functional testing: Anchorage-independent growth

A.



B.

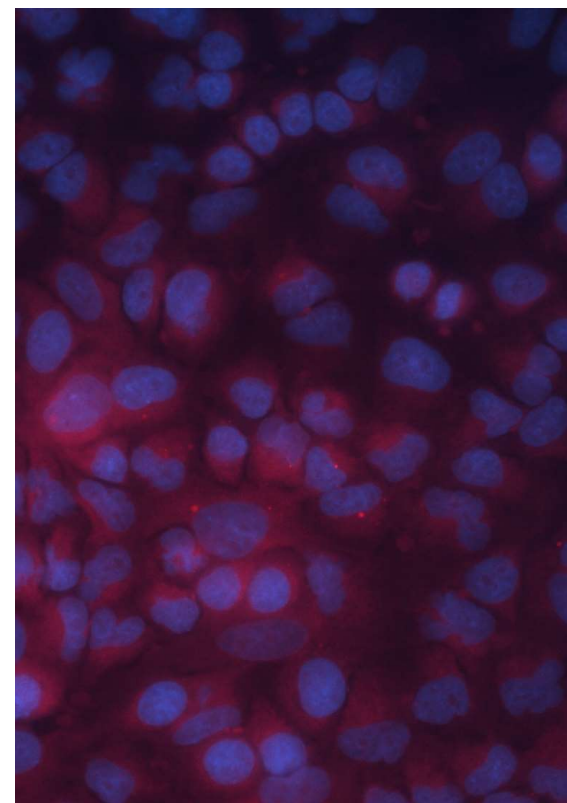


***Tumor cell-derived exosomes induce normal cells to exhibit a malignant phenotype***

# Summary

- Exosomes were derived from well-characterized and authenticated ATCC cell lines
- ATCC exosomes demonstrate the expected size distribution and expression of characteristic protein markers
- The exosomes demonstrated functionality in multiple *in vitro* assays
- Our in-house exosome isolation strategy ensures high purity, high reproducibility, and low lot-to-lot variability
- ATCC also offers custom exosome isolation services

[www.atcc.org/exosomes](http://www.atcc.org/exosomes)



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- **Prevent Analysis Variability by Using Reference-quality Microbial Genomes — Shift from Consensus to Discernible**  
November 14, 12:00 ET

[www.atcc.org/webinars](http://www.atcc.org/webinars)

