



Human Adipose Stem Cells Derived Lyophilized Exosomes at Room Temperature Retain Therapeutic Efficacy for Traumatic Brain Injury and Open Wounds



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Disclosures

- Supported by Department of Veterans Affairs
 - Merit Awards I01BX005591, I0BX005708 and I01BX003836
 - Research Career Scientist Award IK6BX005387
- US patents and the provisional patent described here are awarded to the PIs and have VA-asserted rights.

Disclaimer: This work does not reflect the views or opinions of the James A. Haley Veterans Hospital or the US Government.

Schematics made with BioRender



Collaborator (TBI)

Dr. Paula Bickford (USF and JAH VH)



Patel Lab Members



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 - Rekha Patel
 - Briana Jones, Ph.D.
 - Bangmei Wang
 - Yan Shi, PhD
 - Robert Sparks, PhD *
 - Tradd Dobbins *
 - William Lawless, PhD *
 - Houston Edmondson *
 - Brenna Osborne
 - Suhaas Singireddy
 - Alexandria Bustabad
 - Ryan Shaw
 - Anna Kharitonova
 - Ashley Lui, Ph.D.
 - Ghattas El Bassit, MD
- * **Veteran**



Collaborators (JAH VA)

Dr. Paul Albear
Dr. Risa Richardson
Dr. Stephen Mastorides
Dr. Omar Santana-Hernandez



Polytrauma in Military Service Members

Curr Phys Med Rehabil Rep (2019) 7:323–332

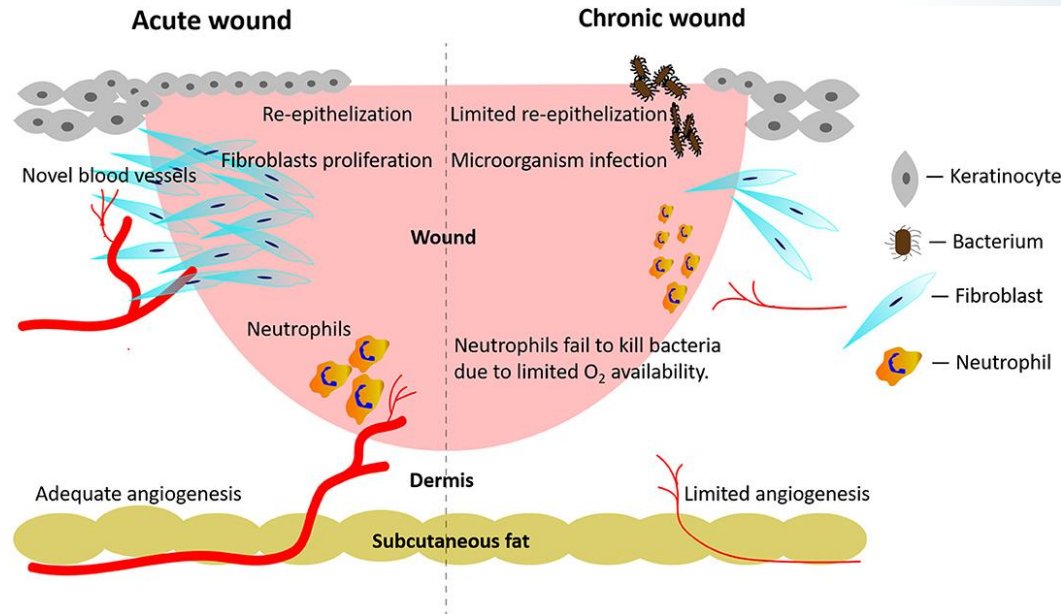


Fig. 1 Four basic mechanisms of blast injury are characterized as primary, secondary, tertiary, and quaternary injuries

US Department of Defense and the Defense and Veteran's Brain Injury Center:

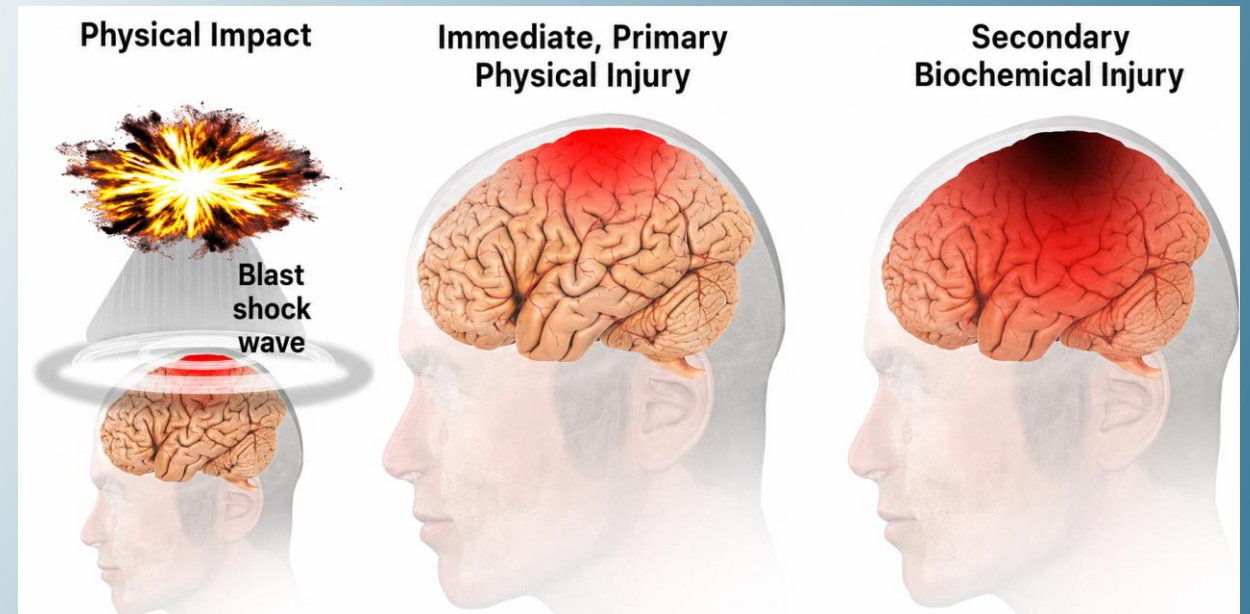
- 22% of combat casualties (Afghanistan and Iraq) vs 12% (Vietnam)
- 60-80 % of soldiers with other blast injuries may have TBI.
- Blast, MVAs, gunshots.
- Polytrauma System of Care (symptomatic)

Wounds



Slow or delayed wound healing often results in **chronic recalcitrant wounds**.

Traumatic Brain Injury



<https://vet.purdue.edu/cpr/traumatic-brain-injury.php>

Utmost need for regenerative therapy to halt the progression of TBI and mediate recovery.

Human Adipose-derived Stem cells (hASC)

We isolated and characterized hASC for use in regenerative medicine:

- Ease of isolation.
- Personalized treatment.
- Large quantities.

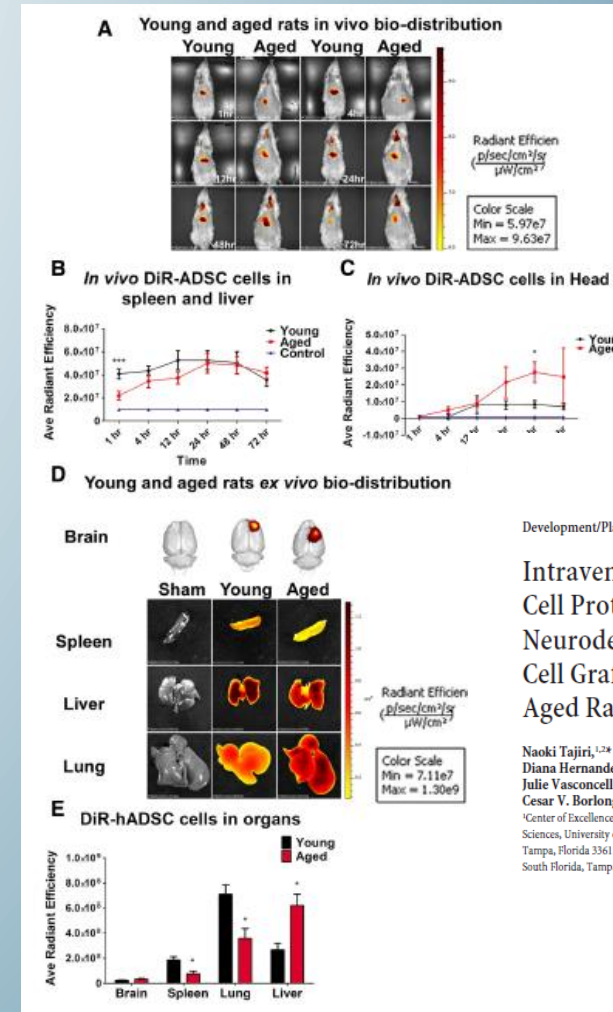


Table 1 Comparison of stem cell antigens and markers

Maker	Other names	sc-ASCn (%)
CD31	PECAM-1	1.10
CD34	L-selectin ligand	Negative
CD44	Pgp-1	89.10
CD45	LCA	0.10
CD73	eNT	88.10
CD90	Thy-1	99.40
CD105	Endoglin	18.00
CD106	VCAM-1	Negative
CD117	c-Kit	0.30

Stem Cell Investig 2016;3

Transplantation of hASC post TBI



Development/Plasticity/Repair

Intravenous Transplants of Human Adipose-Derived Stem Cell Protect the Brain from Traumatic Brain Injury-Induced Neurodegeneration and Motor and Cognitive Impairments: Cell Graft Biodistribution and Soluble Factors in Young and Aged Rats

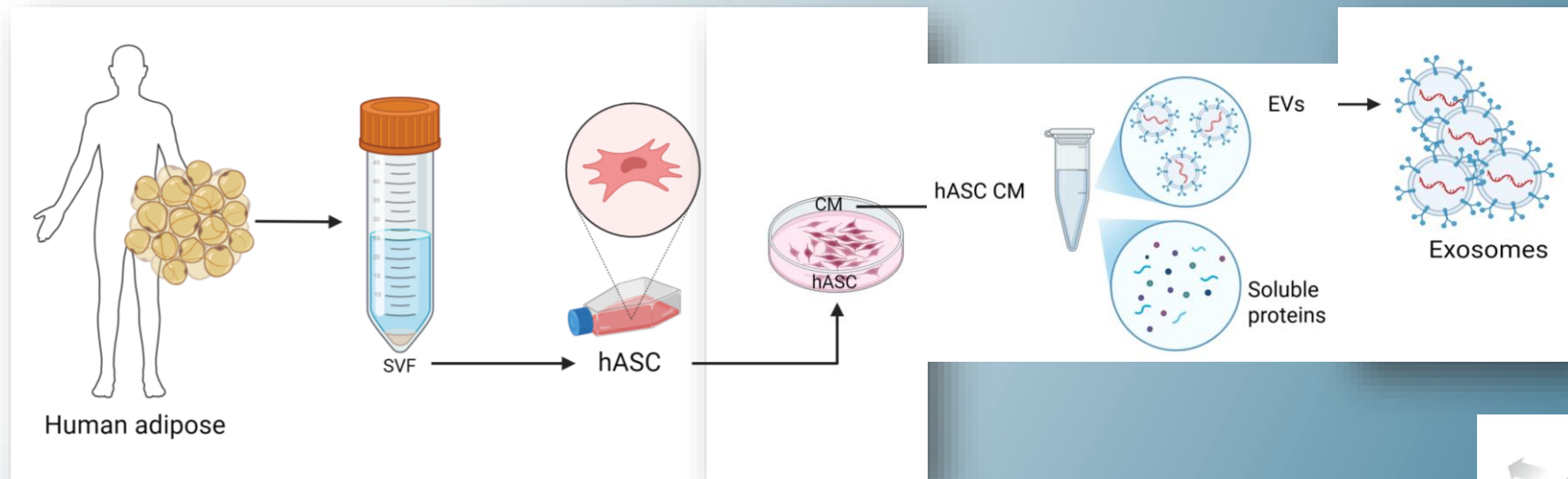
Naoki Tajiri,^{1,2*} Sandra A. Acosta,^{1*} Md Shahaduzzaman,^{1,2*} Hiroto Ishikawa,¹ Kazutaka Shinozuka,¹ Mibel Pabon,¹ Diana Hernandez-Ontiveros,^{1,2} Dae Won Kim,¹ Christopher Metcalf,¹ Meaghan Staples,¹ Travis Dailey,¹ Julie Vasconcellos,¹ Giorgio Franyuti,¹ Lisa Gould,^{1,2} Niketa Patel,^{1,2} Denise Cooper,^{1,2} Yuji Kaneko,¹ Cesar V. Borlongan,^{1,2} and Paula C. Bickford^{1,2,3,4}

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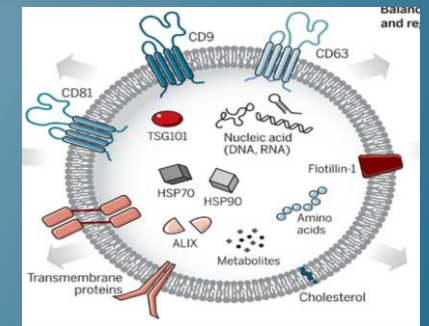
Regenerative ability of adult human adipose stem cells (hASC)

US Patent 9,822,539
US Patent 10,857,187
US Patent 12,016,884B2

Problems with infusion of stem cells: Proliferate and differentiate into several phenotypes.



Exosomes are nanovesicles (25-120 nm) derived from adult human adipose stem cells (hASC).

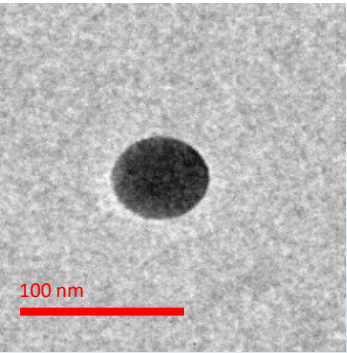
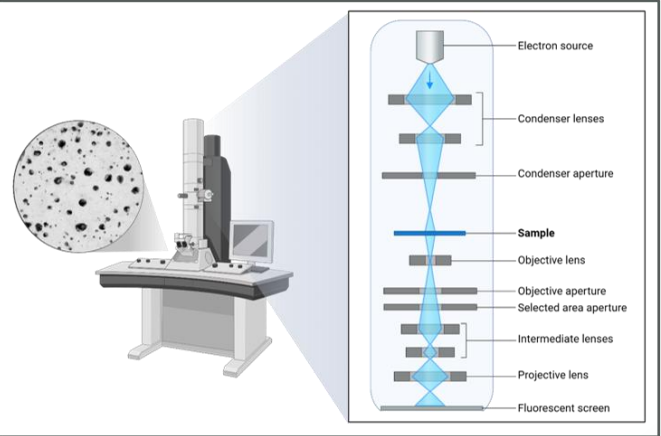


Exosomes

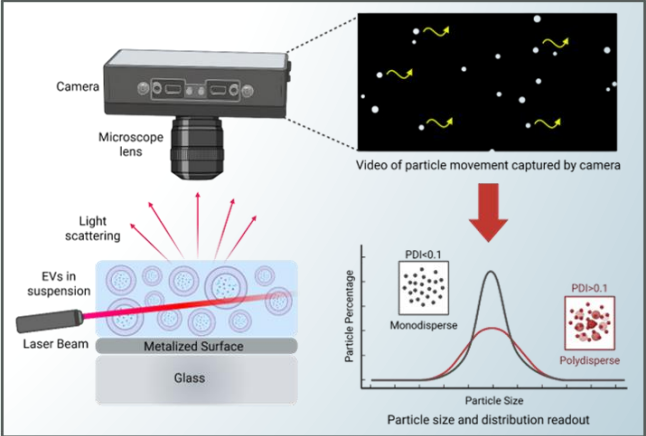
Characterization of hASC exosomes

The small extracellular vesicles (sEV) are characterized as **exosomes** using these methods

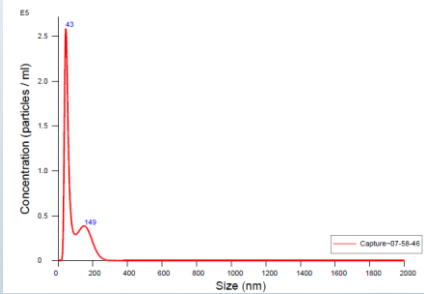
Transmission Electron Microscopy (TEM)



Nanoparticle Tracking Analysis (NTA)



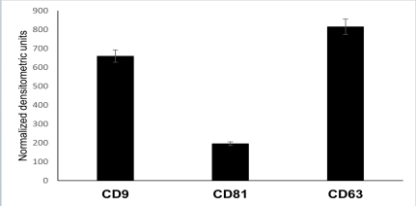
Nanoparticle tracking analysis (NanoSight v3.2.01)		
	Peak diameter (nm)	Concentration/ml (x 10 ⁸)
hASCexo	94 ± 7	1.1 ± 0.08



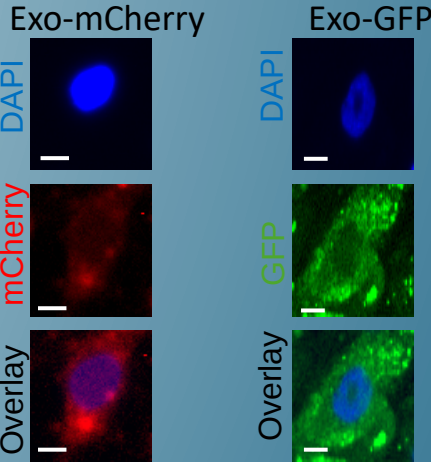
Exosomes-specific Cell surface markers

Western Blot

IB: CD 9 95 kDA
IB: CD 81 25 kDA
IB: CD 63 25 kDA

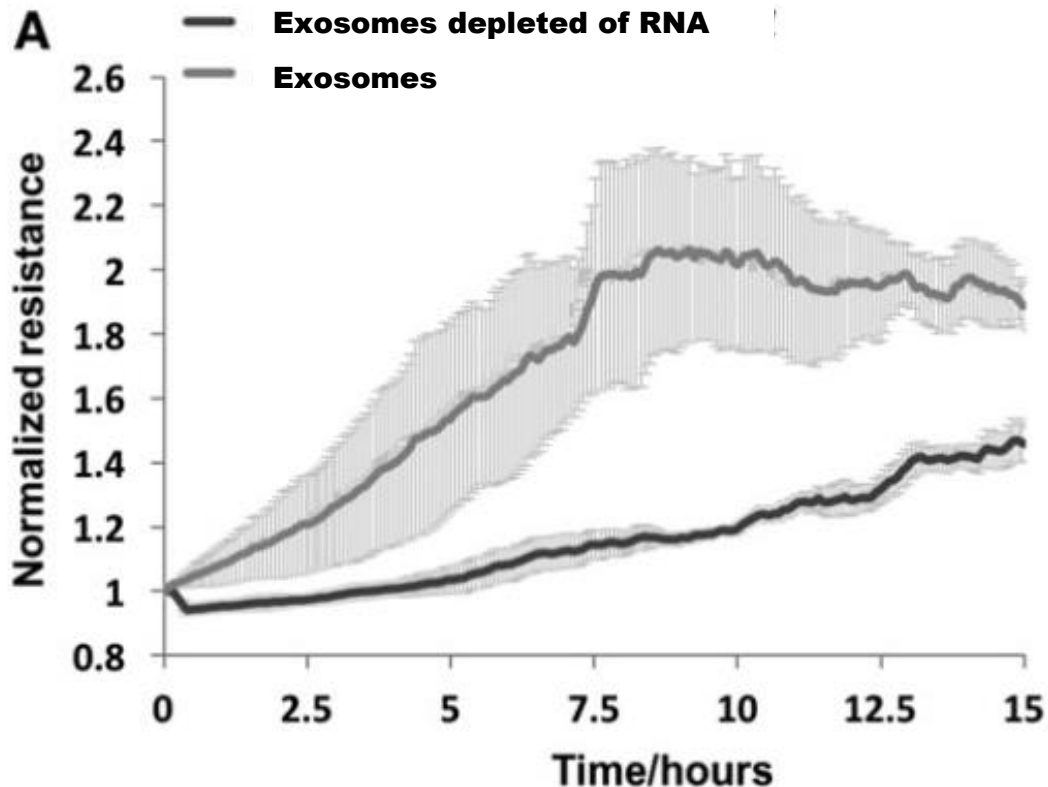


hASC_{exo} Uptake

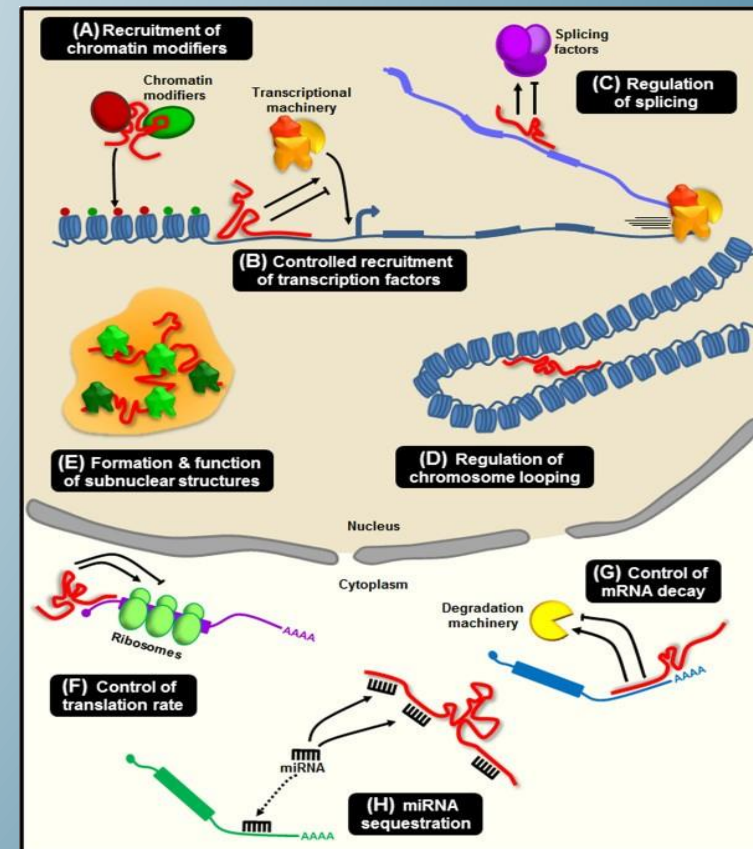


LncRNAs are packaged into hASC exosomes

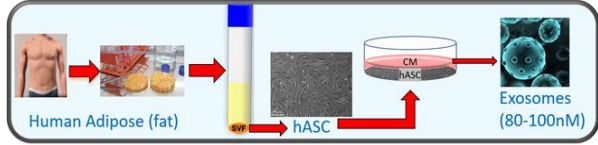
ECIS Wound Healing assay:
RNA cargo drives exosomes' repair function



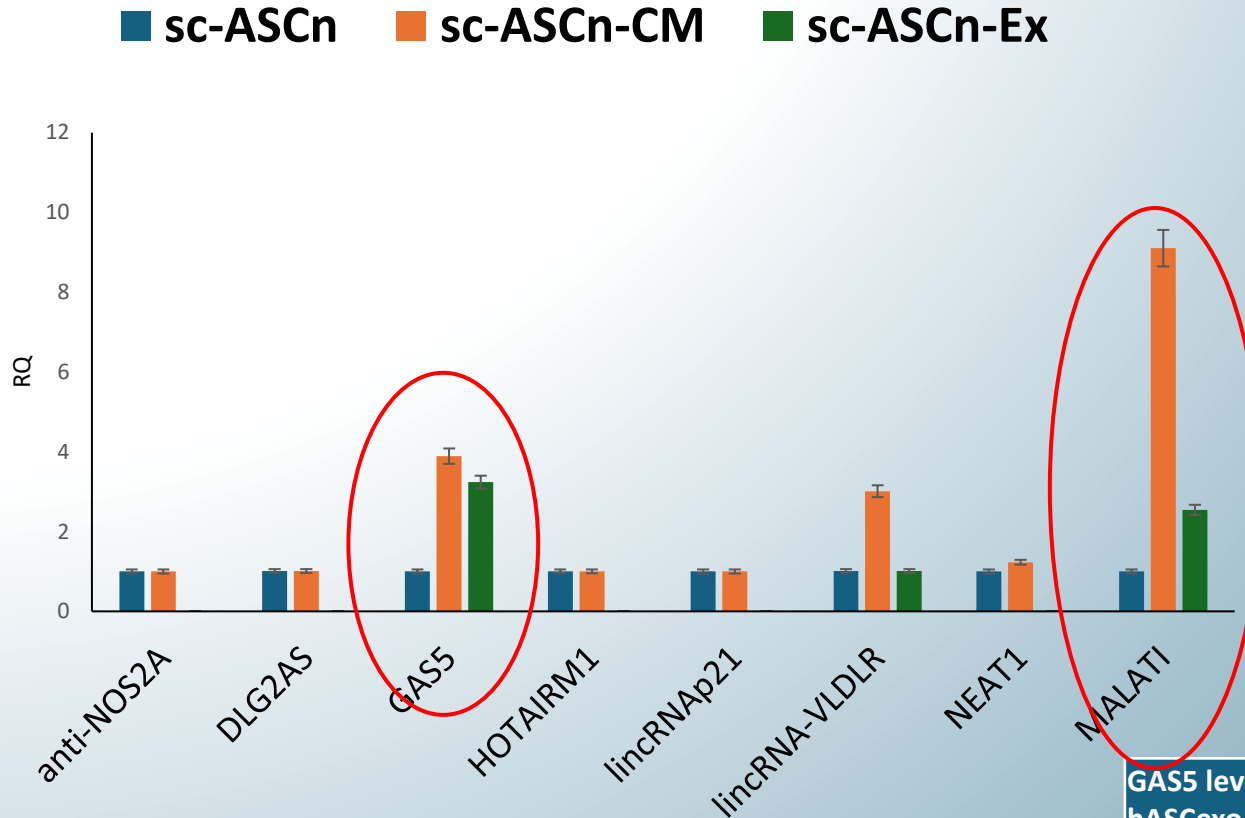
Long Noncoding RNA (lncRNA):
Master orchestrators of gene regulation



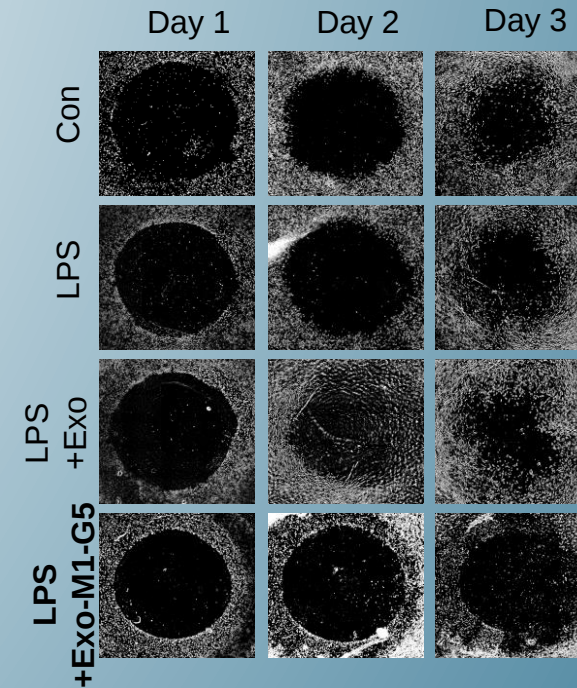
MALAT1 and GAS5 drive the repair ability of hASC exosomes



LncRNA profiles



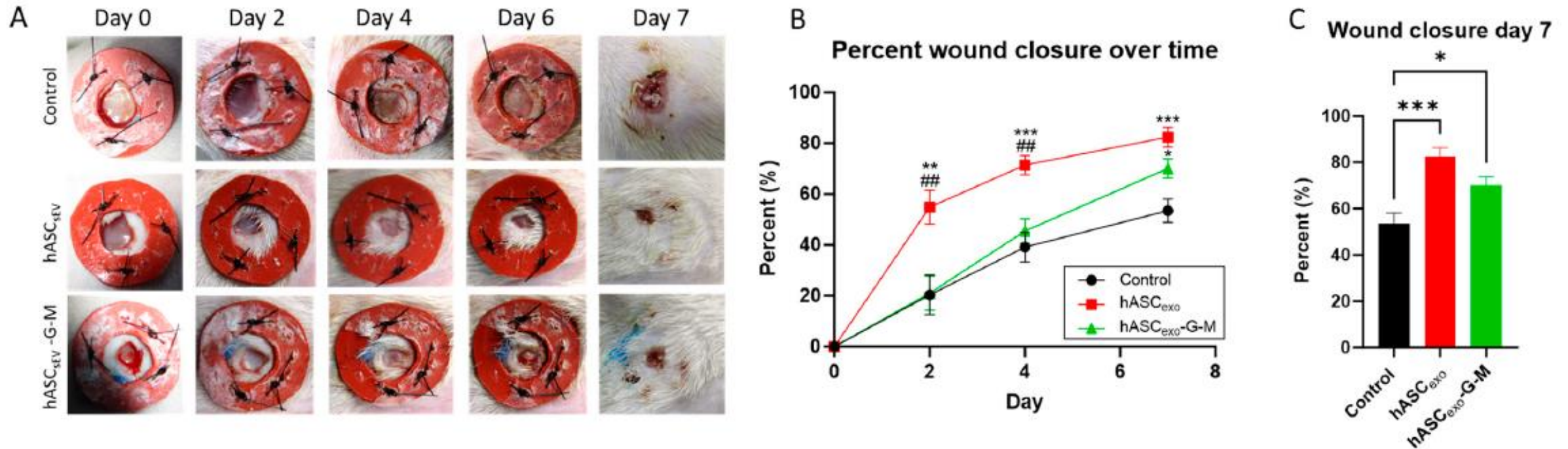
Chronic Inflammation



GAS5 levels/1μg hASCexo	MALAT1 levels/1μg hASCexo
0.24 μg ±0.03	0.15 μg ±0.02

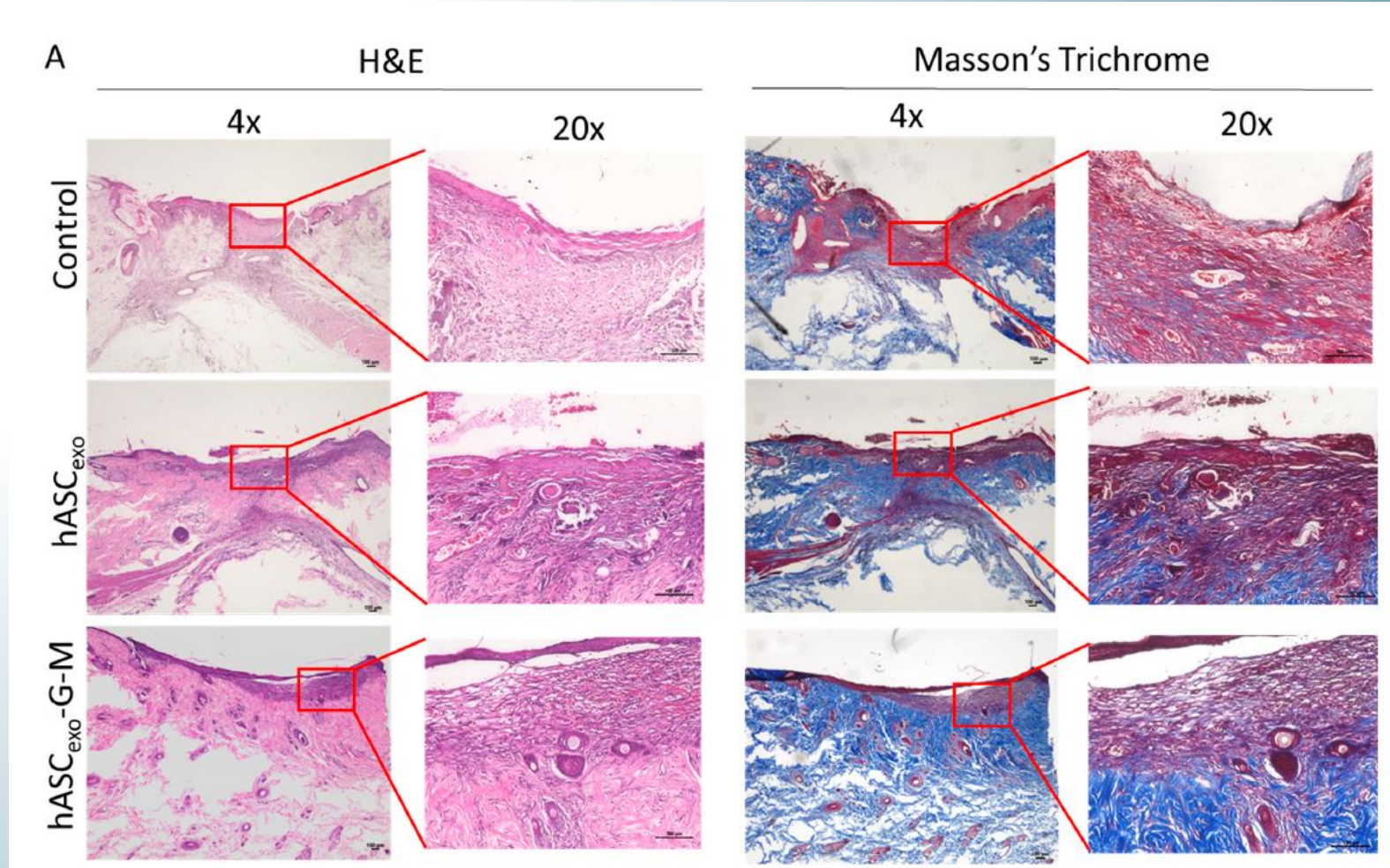
LncRNA GAS5 and MALAT1 contained in hASCexo drive regeneration and repair

Topical application



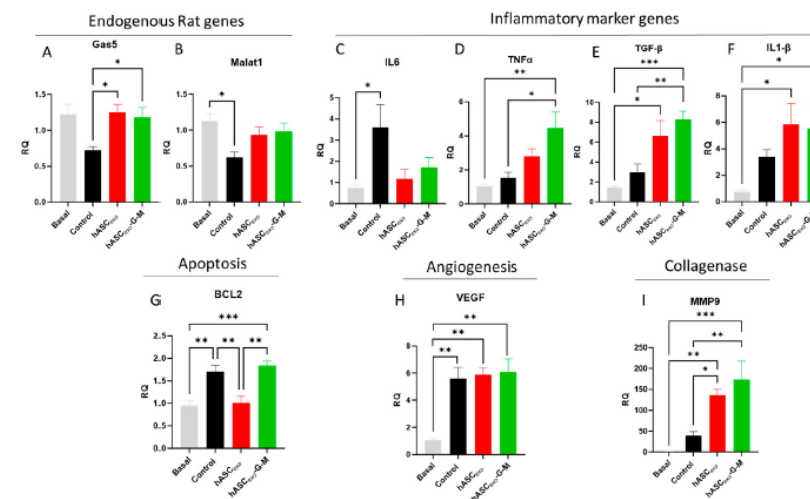
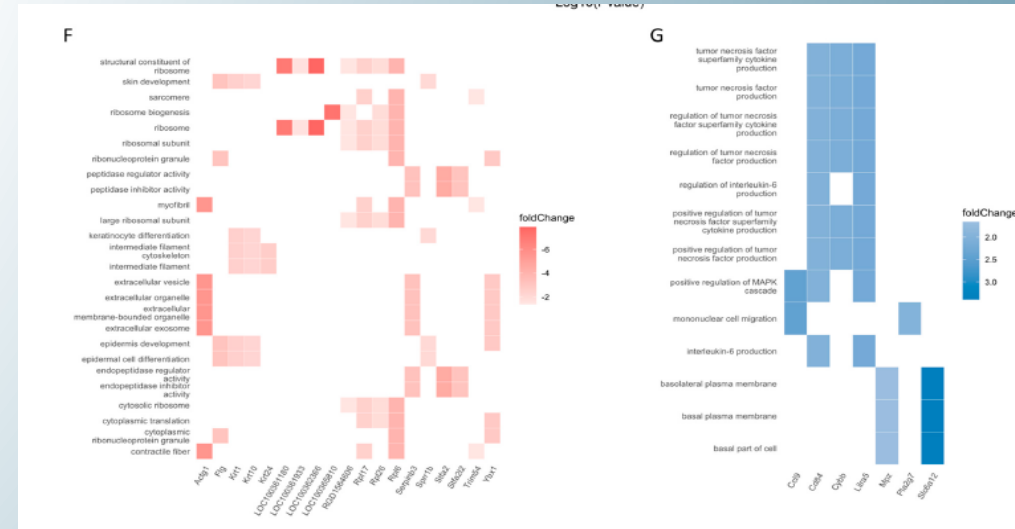
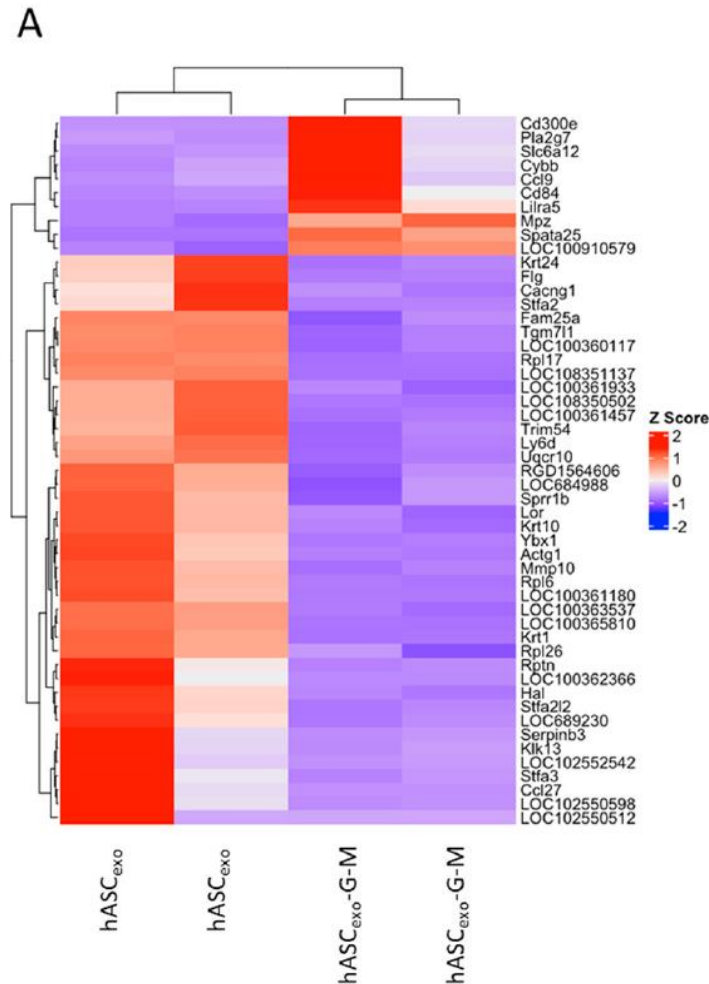
LncRNA GAS5 and MALAT1 contained in hASCexo drive regeneration and repair

Topical application



LncRNA GAS5 and MALAT1 contained in hASCexo drive regeneration and repair

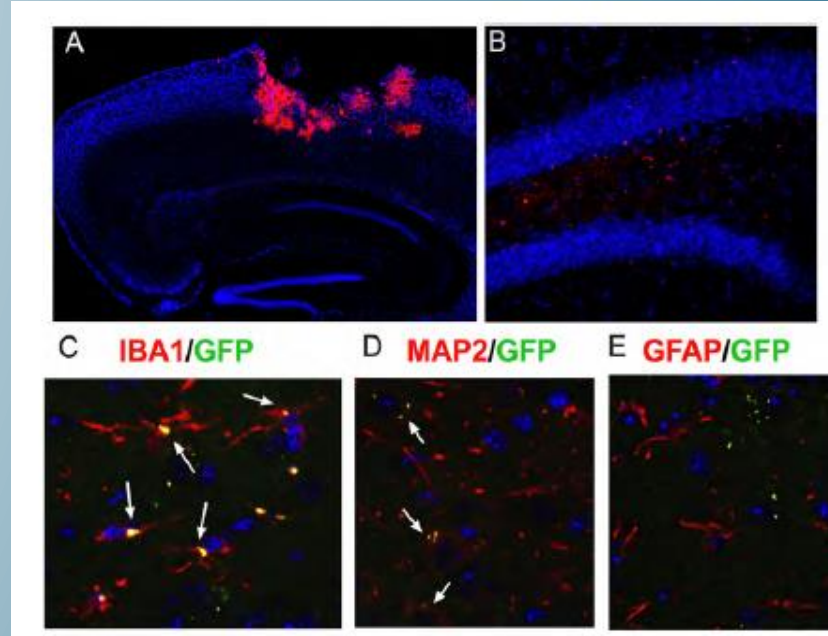
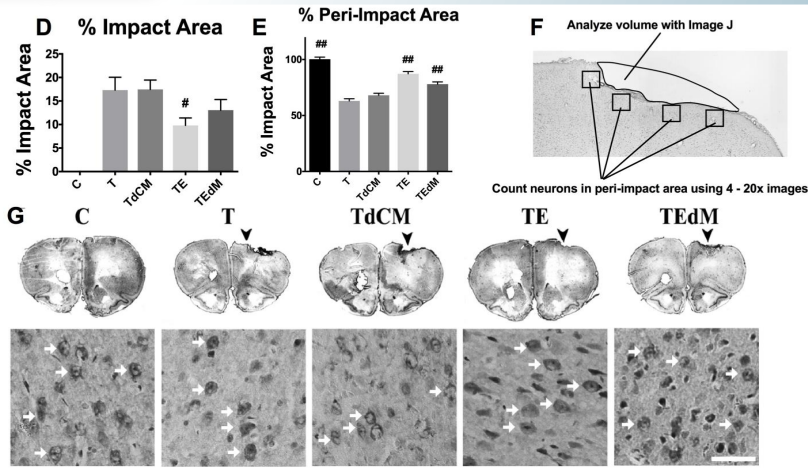
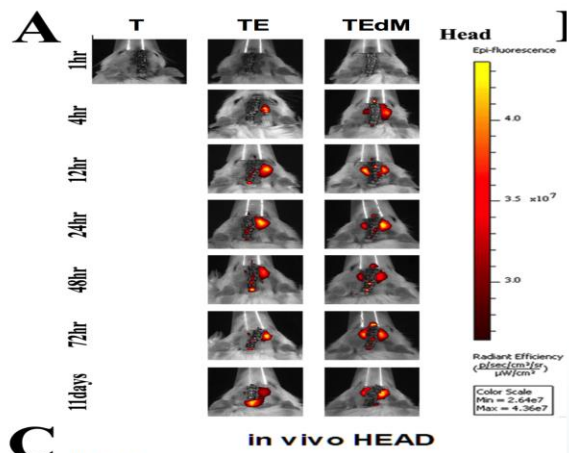
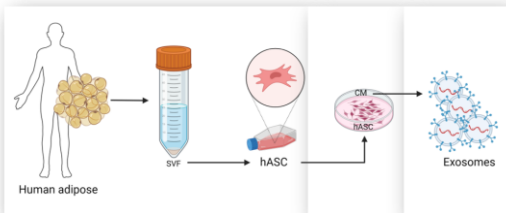
Topical application: RNAseq





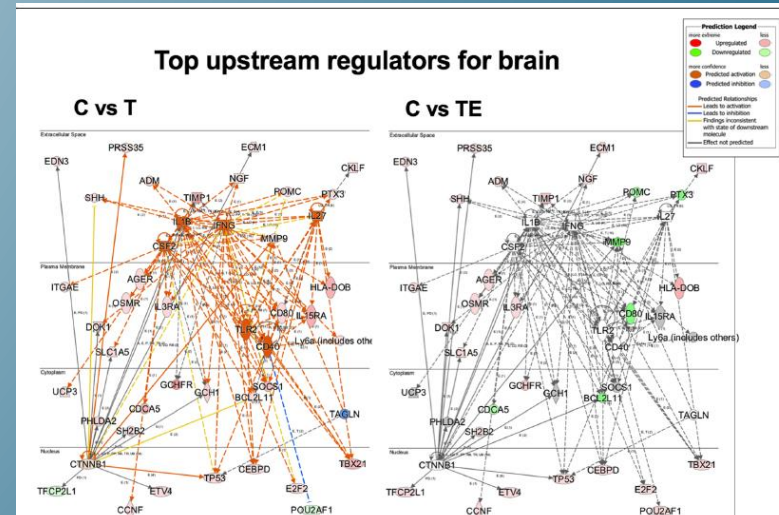
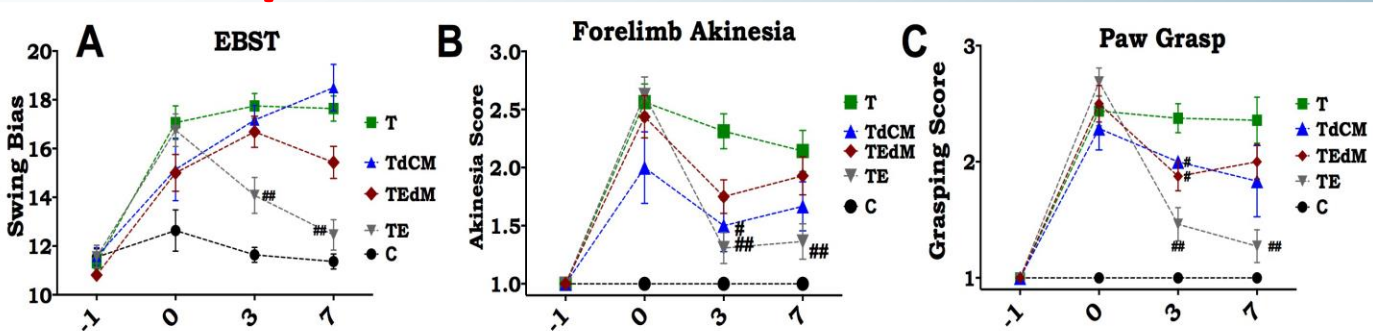
Dr. Bickford

Intranasal delivery of hASC Exosomes in a TBI model

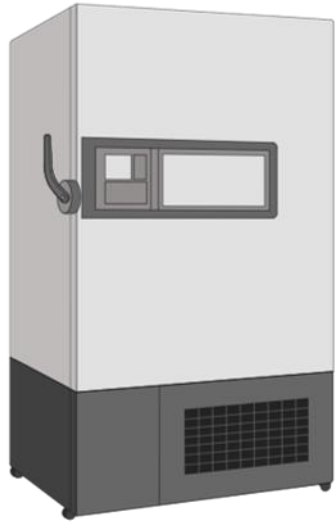


RNAseq

Behavior and motor skills significantly improved with exosomes treatment



Caveats for exosomes usage

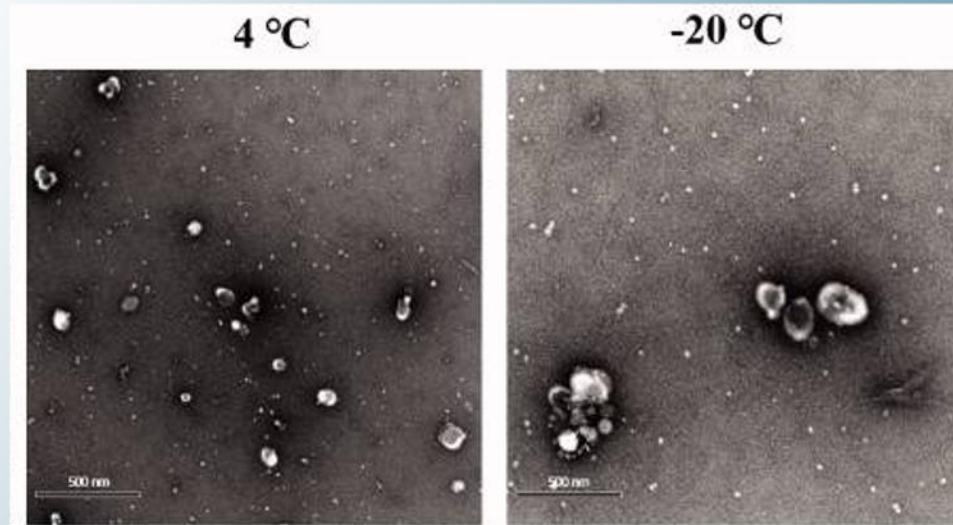


Storage temps at -80 °C,
-20 °C or 4 °C

Preserve
Exosomes

Protects integrity
of exosomes

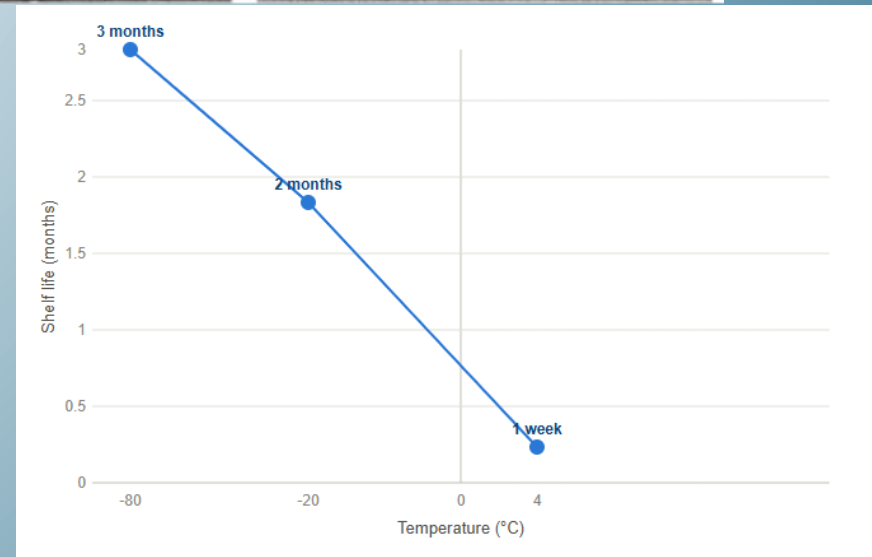
Reproducibility



Wu et al, 2021.

Storage of exosomes:

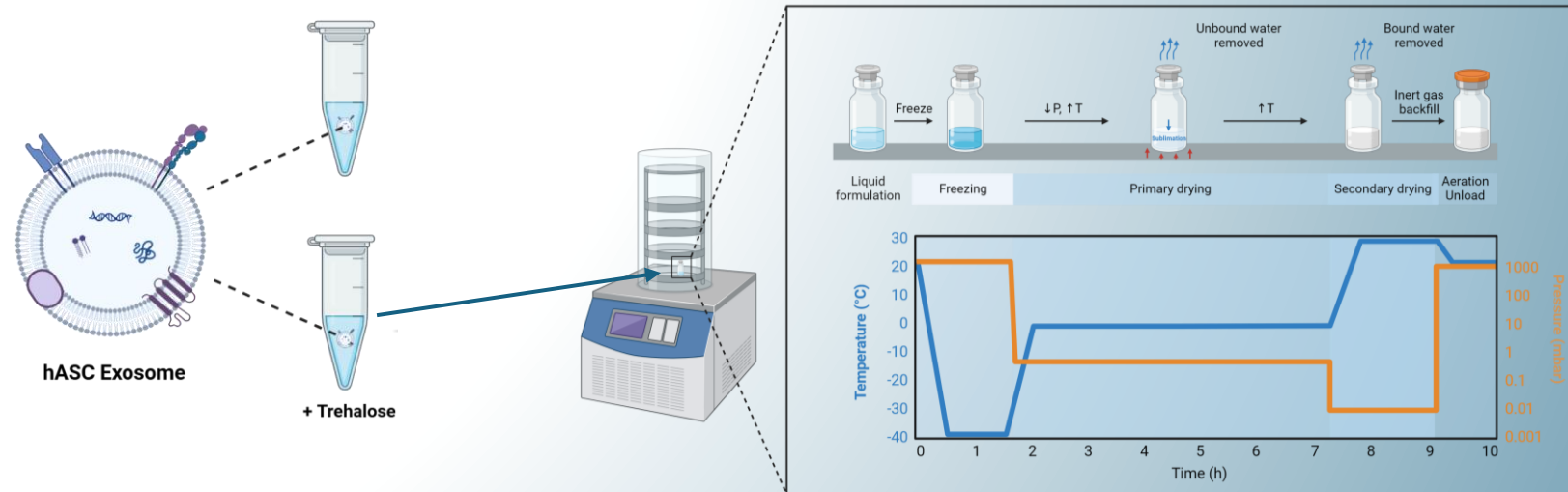
- 80°C for 3 months, but expect 10-20% degradation
- 20°C for 2 months, but expect 10% degradation
- 4°C for 1 week, but expect 5% degradation



Translating Discovery and Inventions to Innovations in healthcare



Store and carry exosomes in lyophilized form



Advantages:

- Store at room temperature
- Inclusion in first-aid kits, on battlefields
- Integrated into clinics



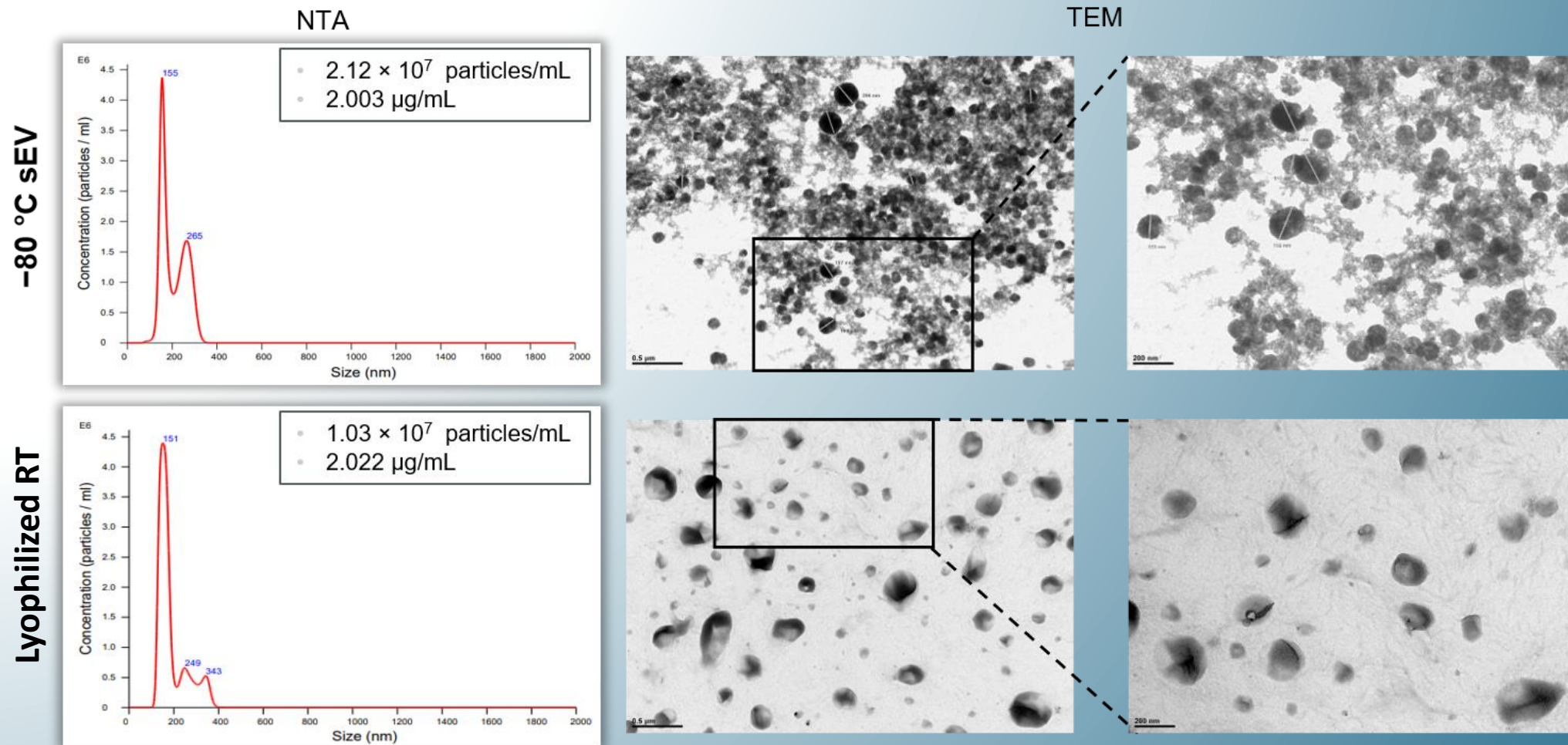
Article

Lyophilized Small Extracellular Vesicles (sEVs) Derived from Human Adipose Stem Cells Maintain Efficacy to Promote Healing in Neuronal Injuries

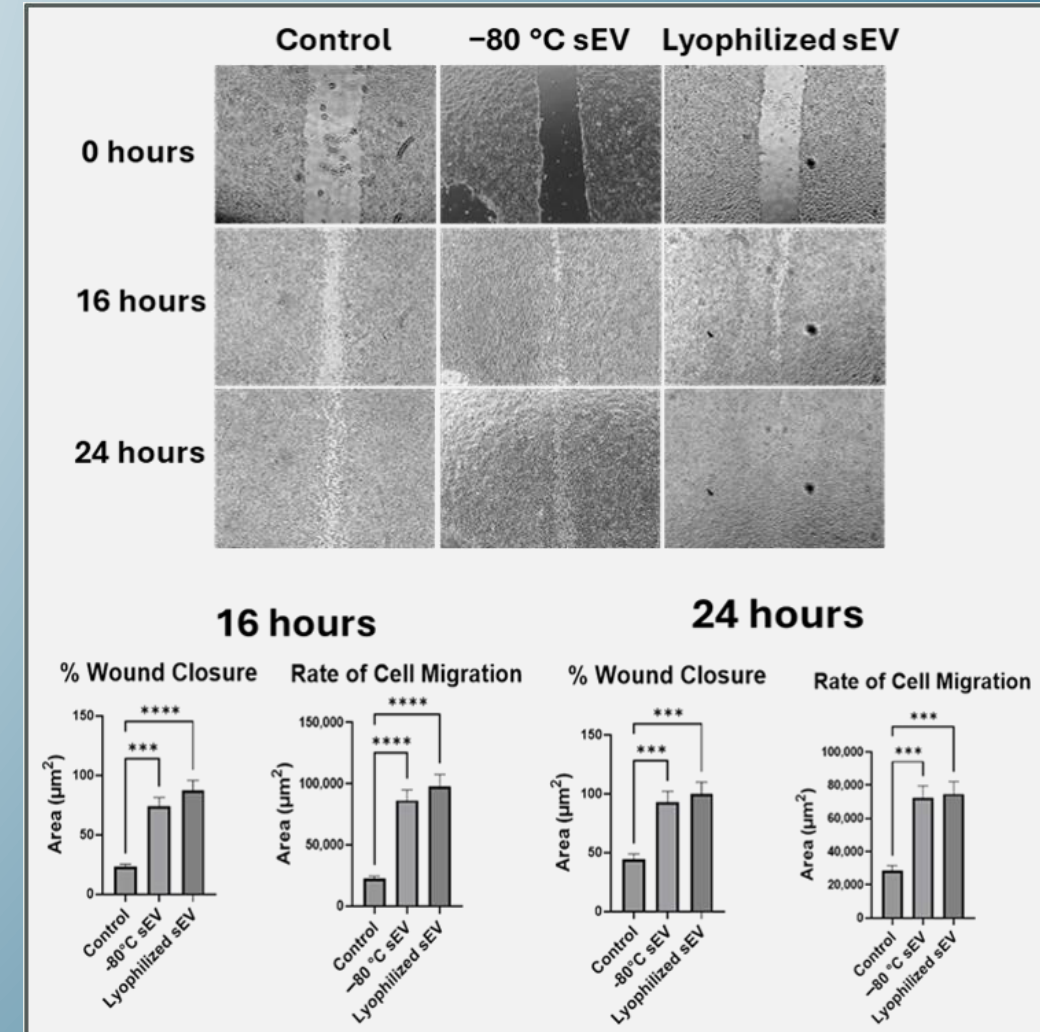
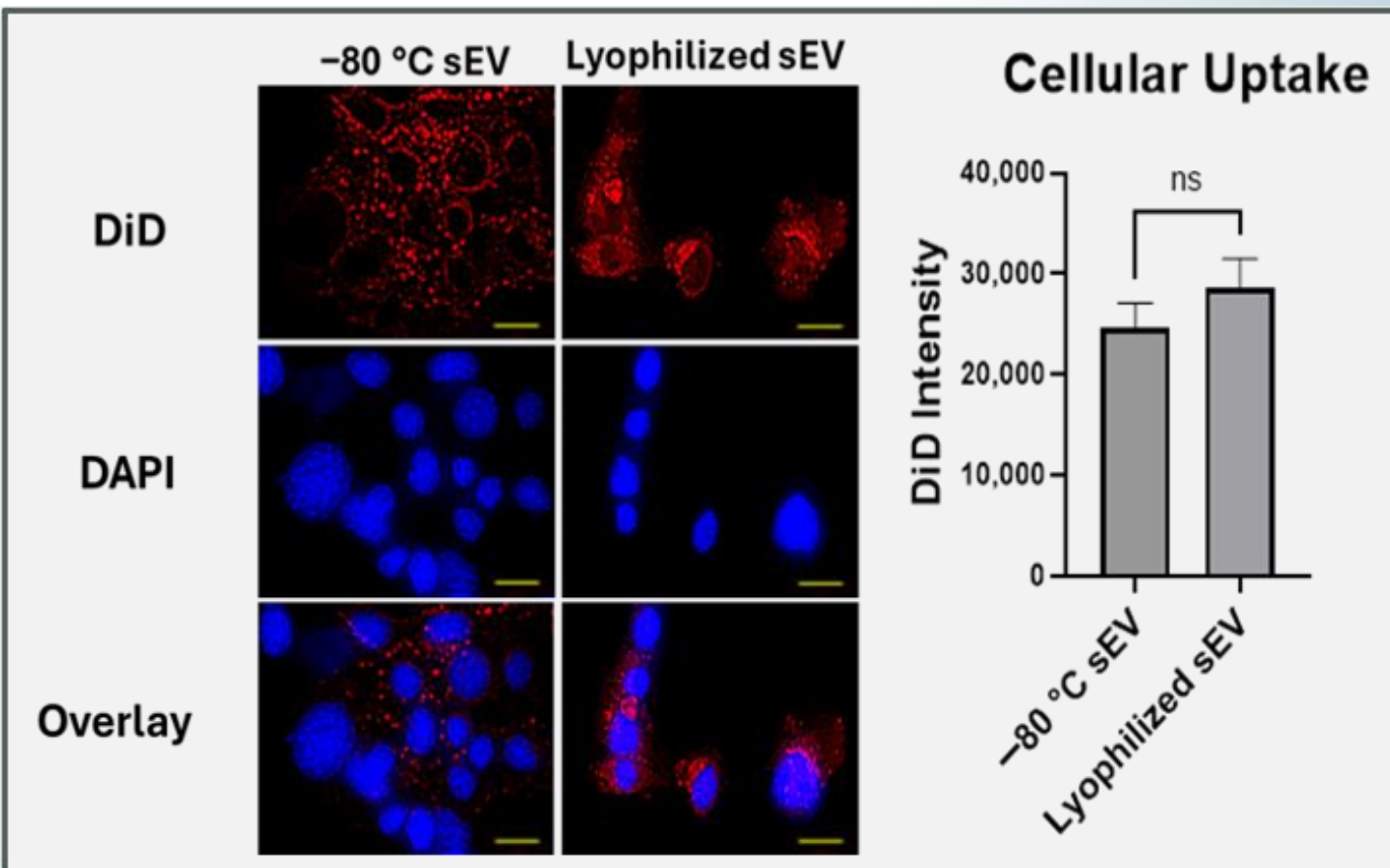
Brianna Jones ^{1,2}, Rekha Patel ¹, Bangmei Wang ¹, Theresa Evans-Nguyen ² and Niketa A. Patel ^{1,3,*}

¹ Research Service, J.A. Haley Veterans Hospital, Tampa, FL 33612, USA; brianna.jones2@va.gov (B.J.); niketa.patel@va.gov (N.A.P.); rekha.patel1@va.gov (R.P.); bangmei.wang@va.gov (B.W.)
² ³

Lyophilized exosomes stored for 2 months at room temperature maintain structural integrity



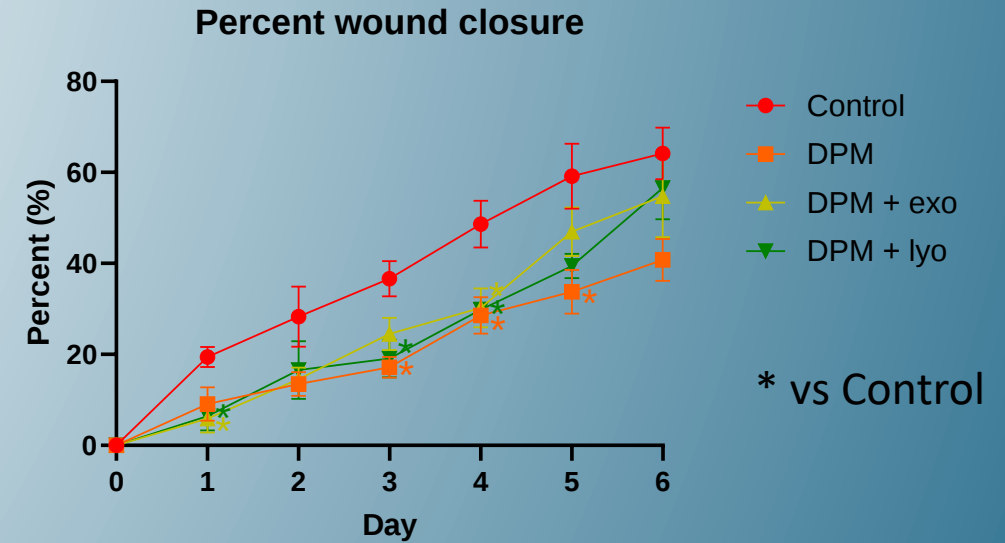
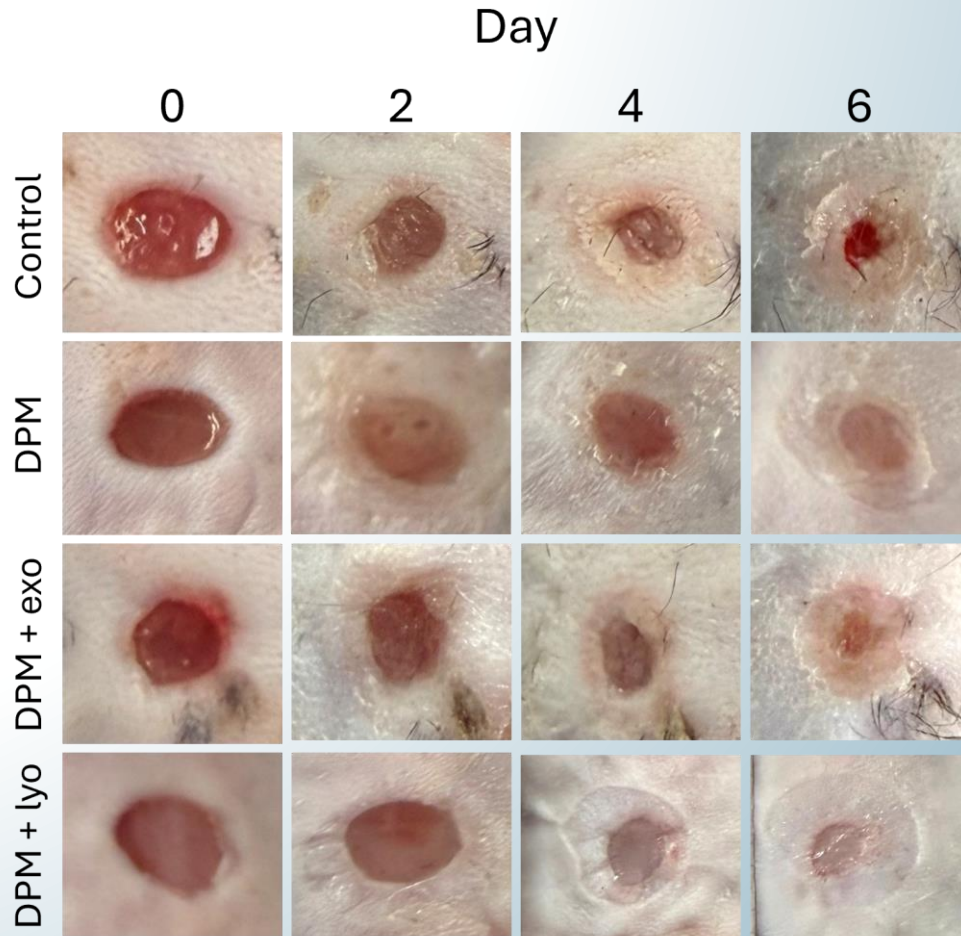
Lyophilized exosomes stored for 2 months at room temperature maintain biological activity



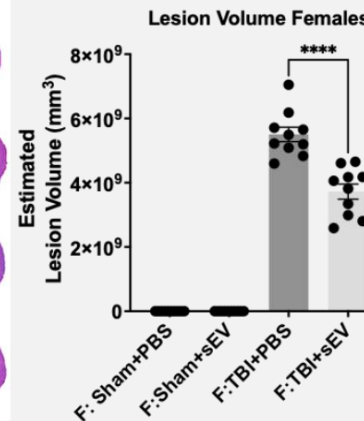
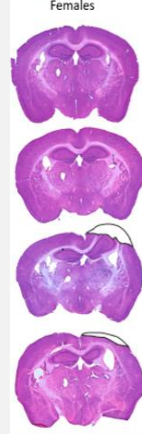
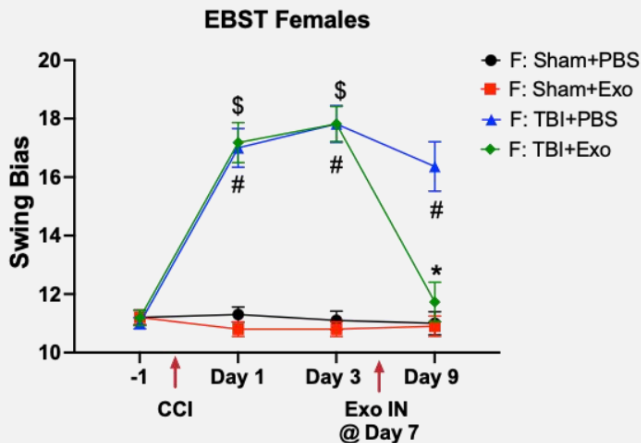
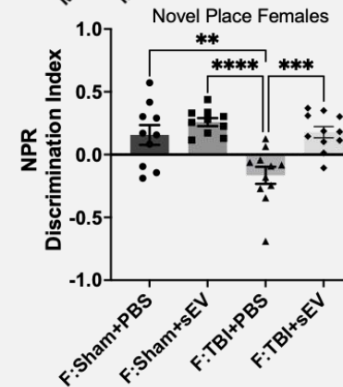
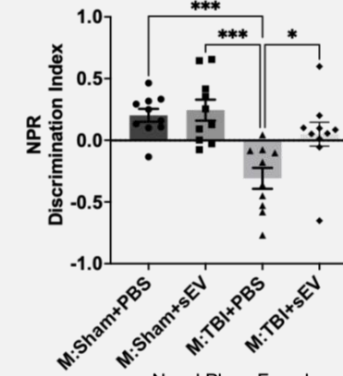
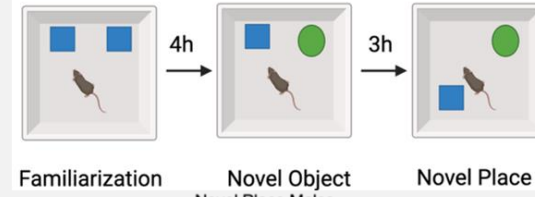
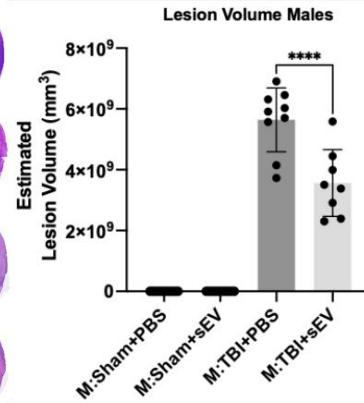
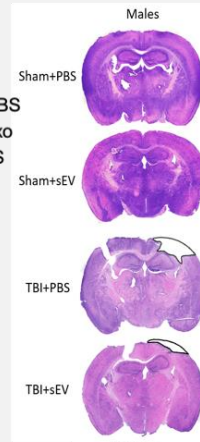
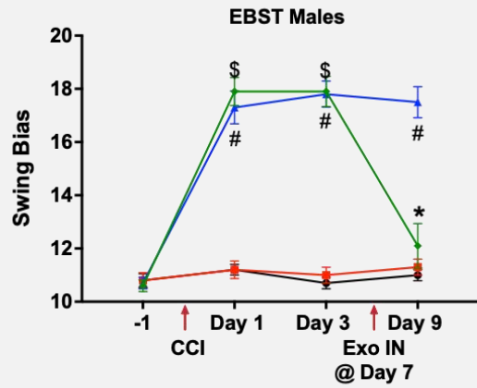
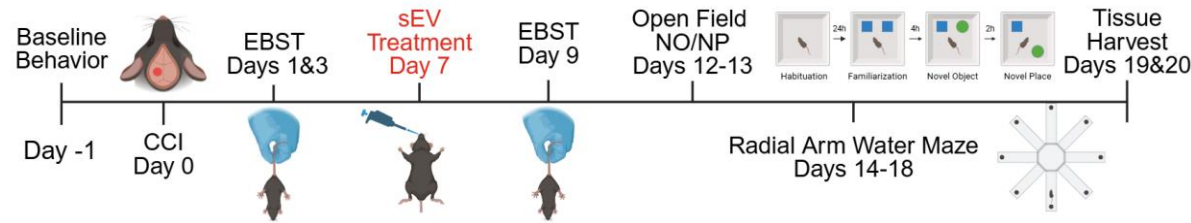
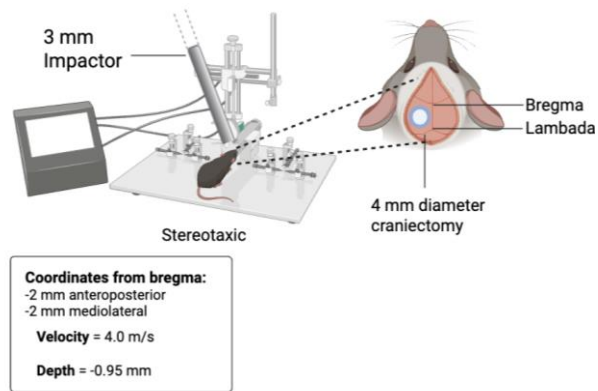
Efficacy of lyophilized exosomes stored at room temperature **in vivo**

- Open wound model
- TBI model

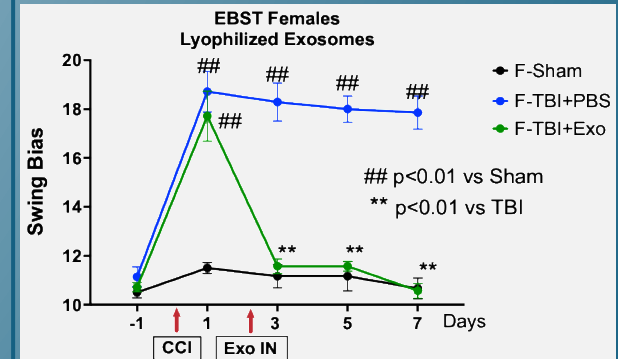
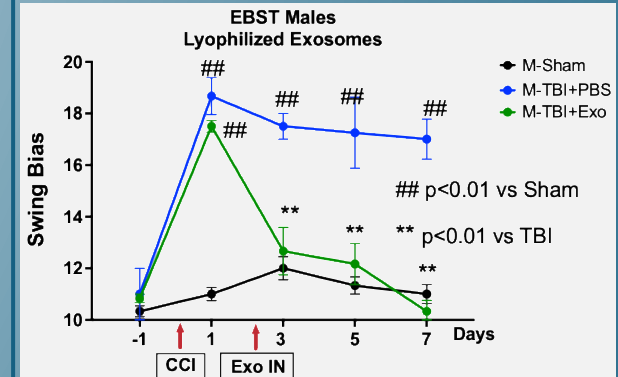
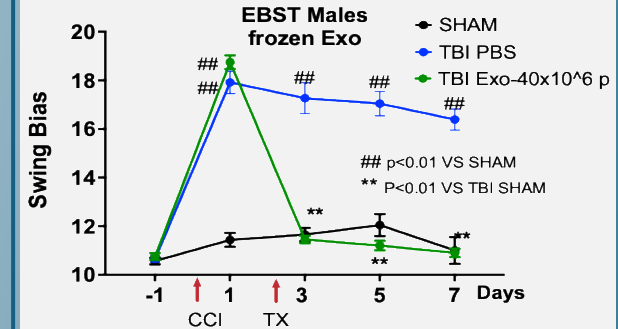
Lyophilized hASC exosomes efficacy in DPM-contaminated wounds



Lyophilized hASC exosomes efficacy in TBI



Lyophilized exosome treatment on day 2



Conclusions

- Exosomes derived from human adipose stem cells provides a cell-free regenerative approach to treat wounds and significantly improve the outcome of TBI and wound healing.
- Lyophilized hASC exosomes are viable and maintain efficacy to treat open wounds and TBI.
- The lab is a pioneer in this field of research and has innovated methods of the storage and transport of exosomes to bring the technology to clinic and in battlefields.

Thank you!