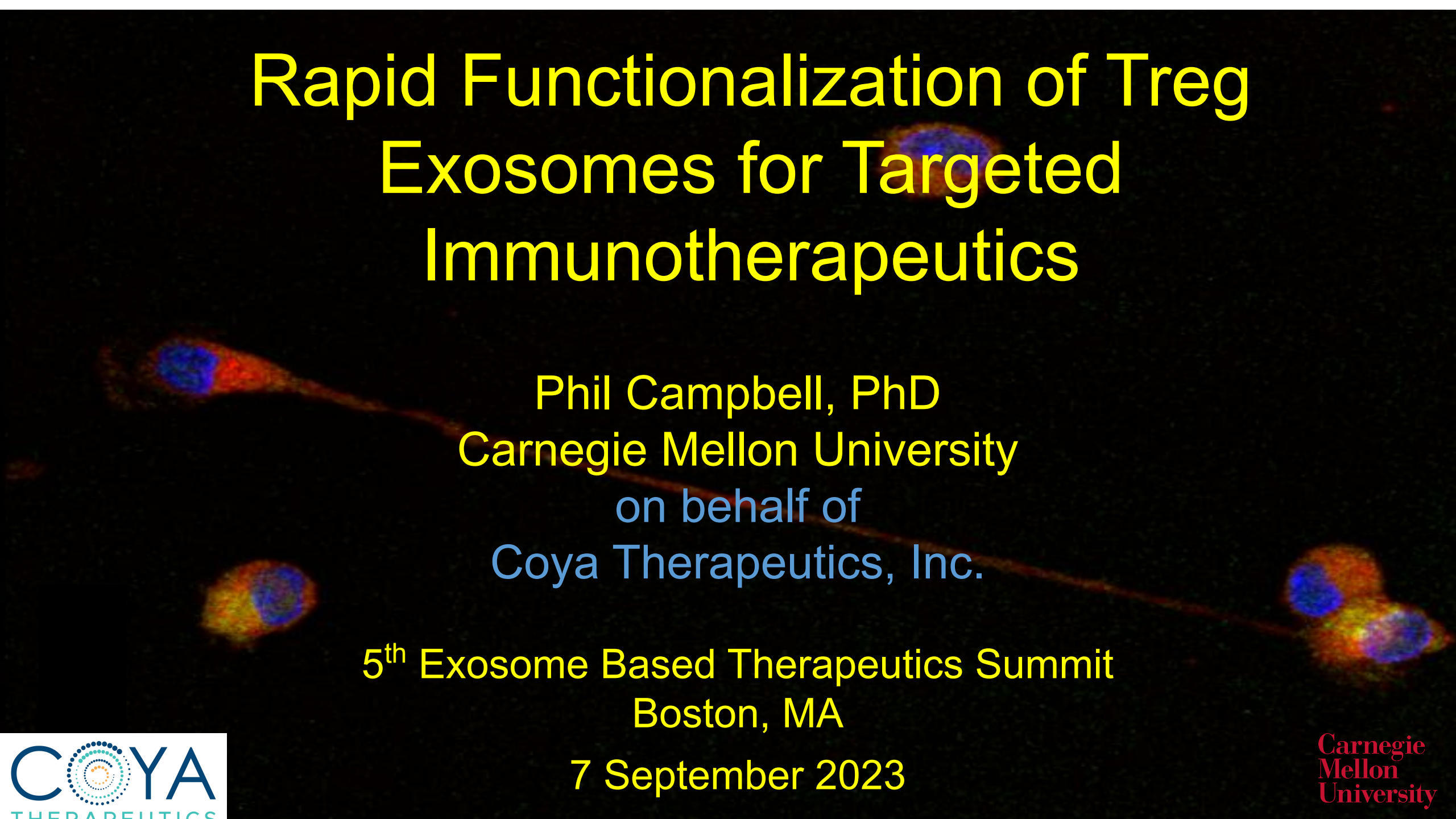
Rapid Functionalization of Treg Exosomes for Targeted Immunotherapeutics



Phil Campbell, PhD
Carnegie Mellon University
on behalf of
Coya Therapeutics, Inc.

5th Exosome Based Therapeutics Summit
Boston, MA
7 September 2023

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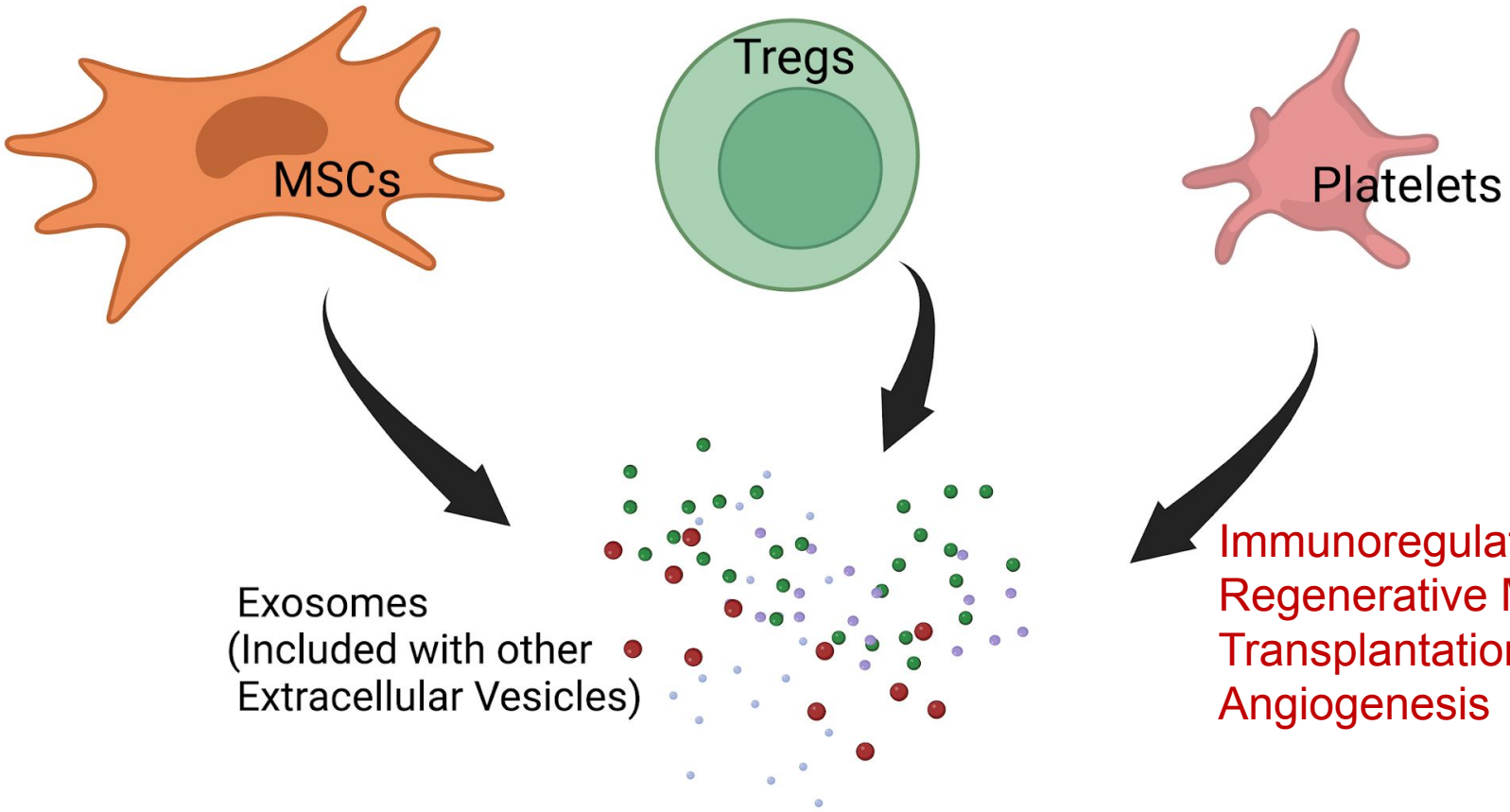
Carnegie Mellon University

Therapeutic Potential of Exosomes



The potential innate therapeutic properties of MSC, Treg and Platelet exosomes mirror those of source cells themselves.

Thus, these exosomes have the potential to simplify therapy logistics while providing source cell therapeutic effects in an alternative, cell-free manner.

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Just Like the Mythological Janus, MSC-Exosomes and Treg Exosomes have Two Sides-

- They can be Drivers of immunoregulation promoting tissue regeneration and transplant immunotolerance.

- They can be Drivers for the promotion of cancer development.

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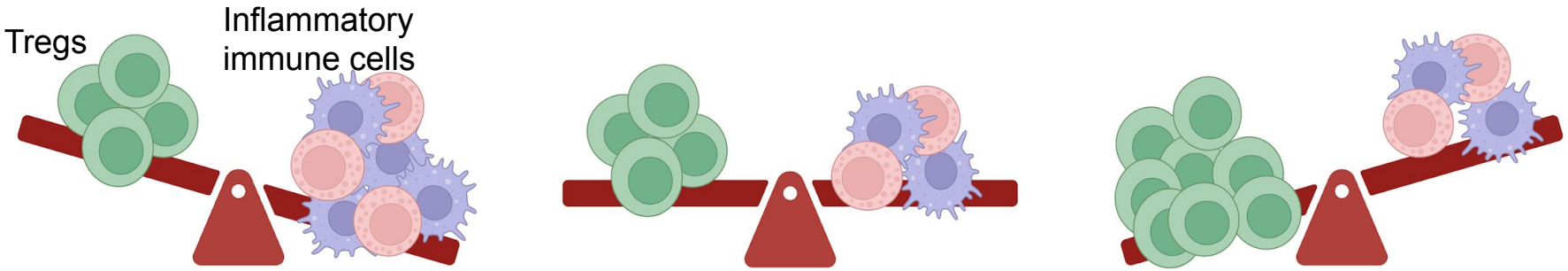
Tregs are Important Immunomodulatory Cells and are Drivers Controlling Inflammation, Enabling Tolerance, Promoting Healing and Regenerative Processes, but……



Autoimmunity

Reduction and loss of Treg population

- Loss of homeostasis and peripheral tolerance
- Loss of adequate immune response and regulation to prevent non-specific symptoms
- Promotes abnormal autoimmunity and autoimmune diseases

Healthy

Balanced Treg and inflammatory immune cell populations

- Promotes homeostasis and peripheral tolerance
- Regulates immune response to prevent non-specific symptoms
- Permits cancer immuno-surveillance

Cancer

Abnormal increase of Treg population

- Loss of cancer immuno-surveillance
- Promotes suppression of anti-tumor response
- Promotes cancer progression

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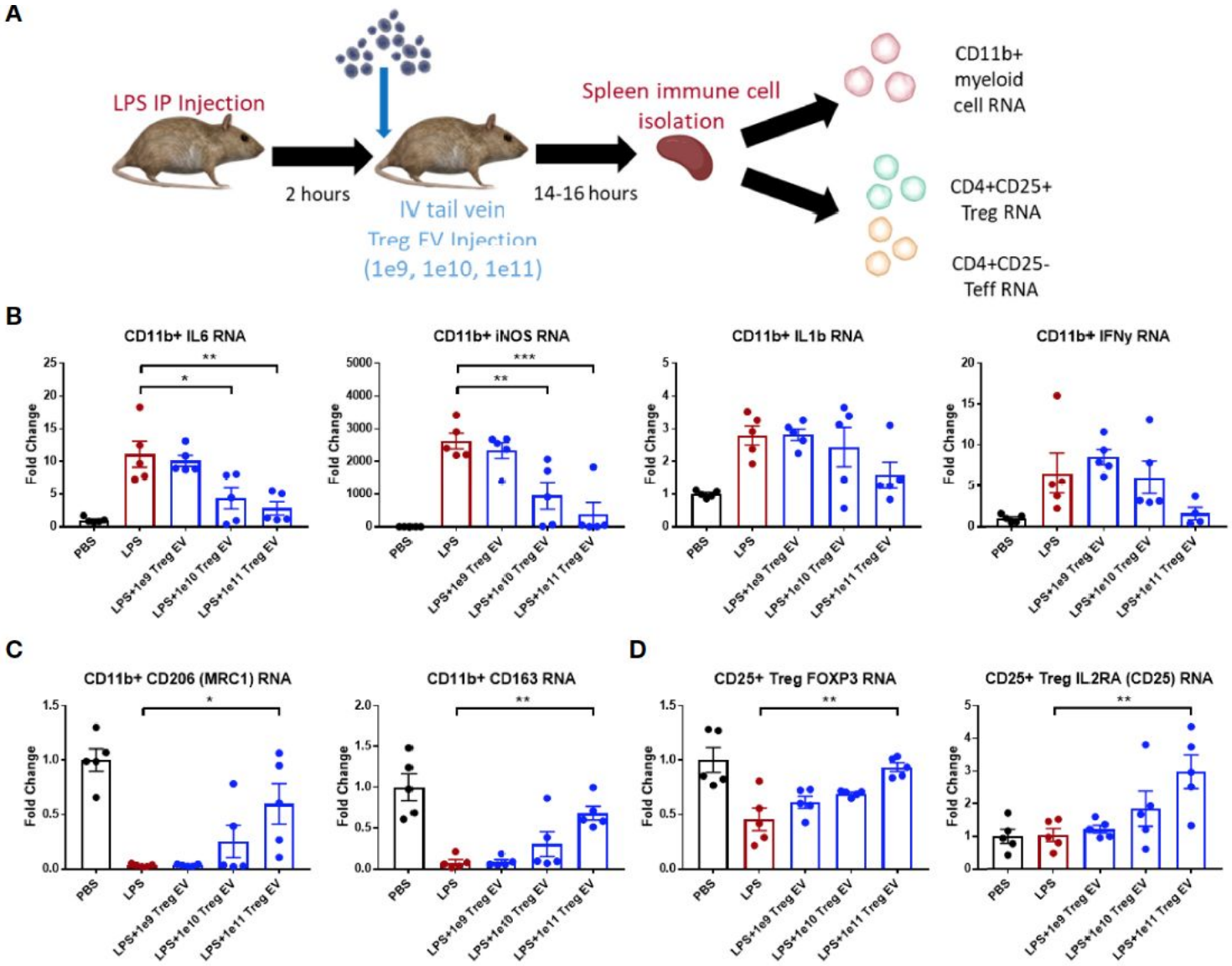
And, exosomes, as part of the Treg secretome, include many of the cell signaling aspects of their parent cells thus mediate Treg physiological and pathophysiological conditions.

Treg Exosomes Suppress Pro-Inflammatory Myeloid Cells and T Cell Proliferation



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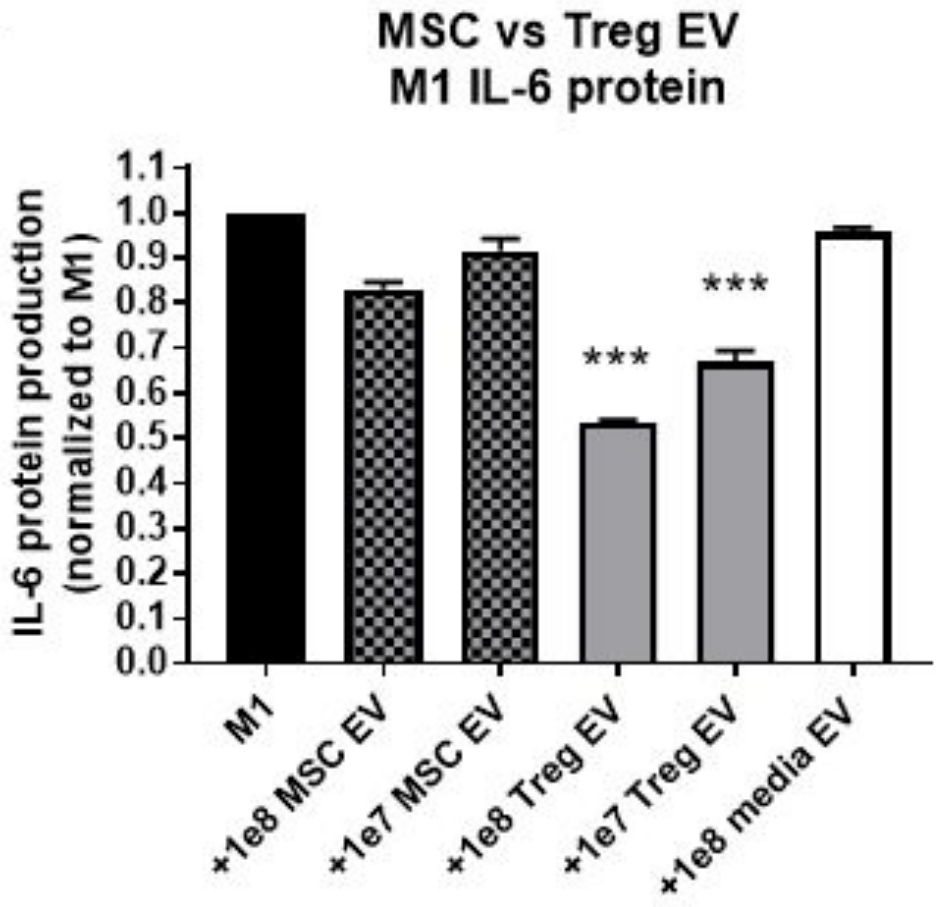
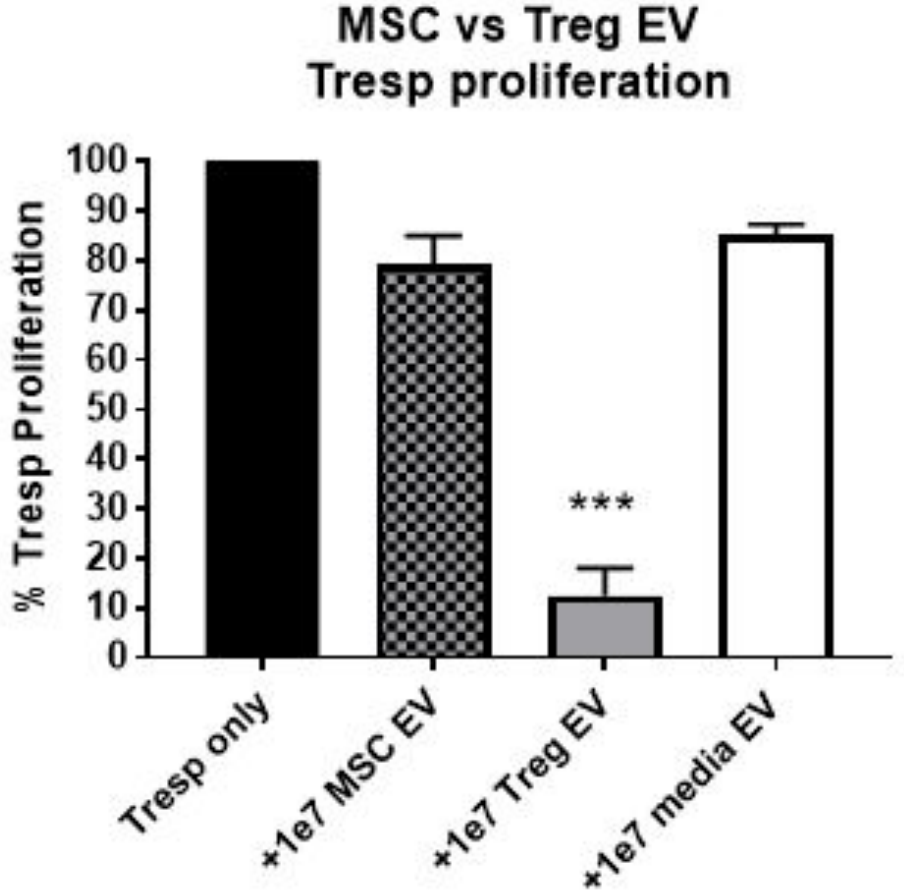
Treg Exosomes Can Have ”Better” Immunomodulation to MSC Exosomes





However, the primary cavate is that these cells are primary so hugely heterologous both within and across isolates.

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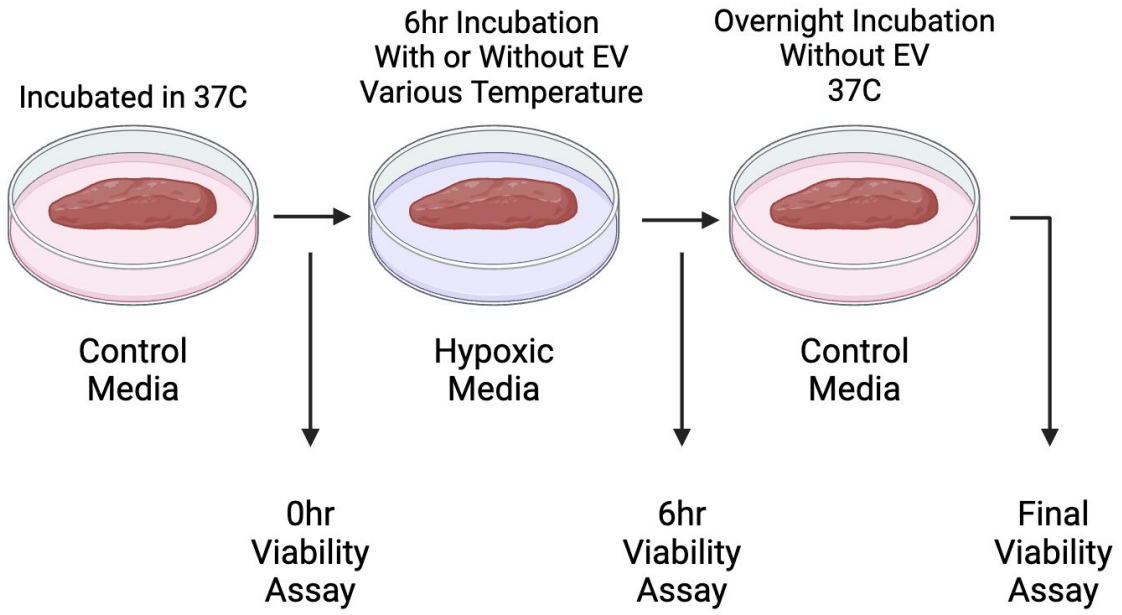
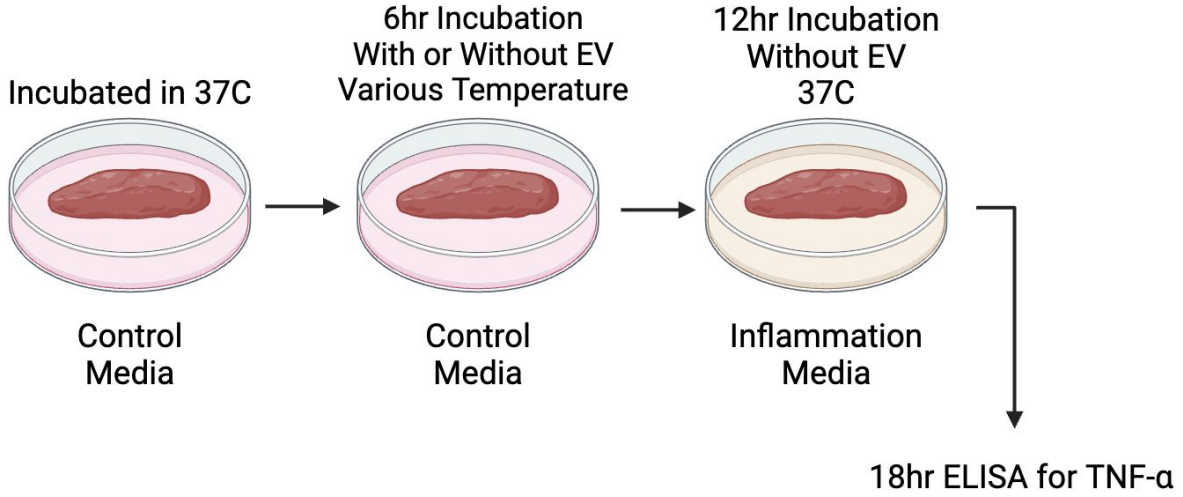
Precision Cut Tissue Slices as a Model to Study Treg Exosome Function

EV Against Ischemic Injury



EV Against Inflammation



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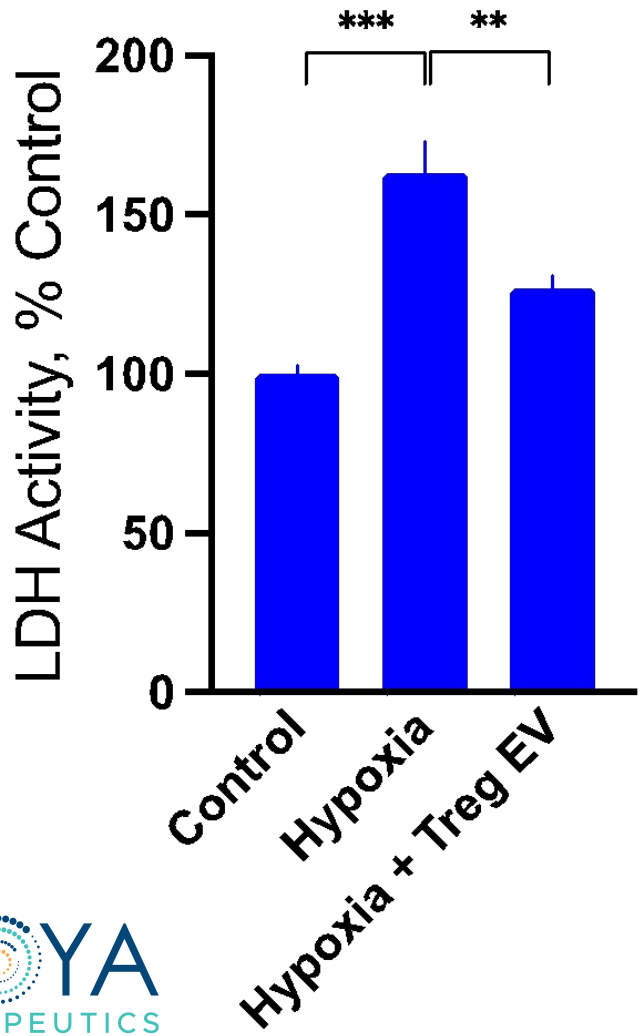
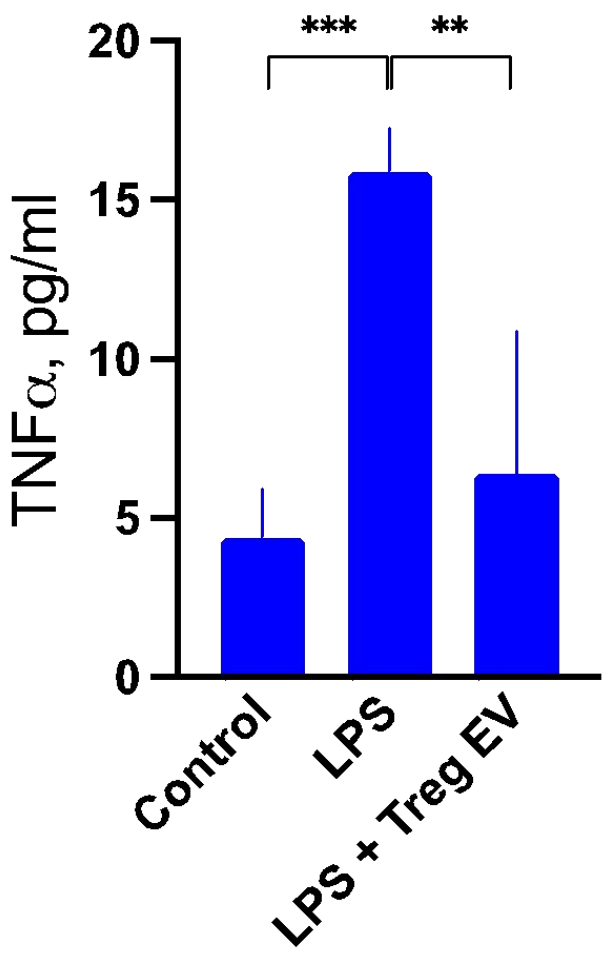
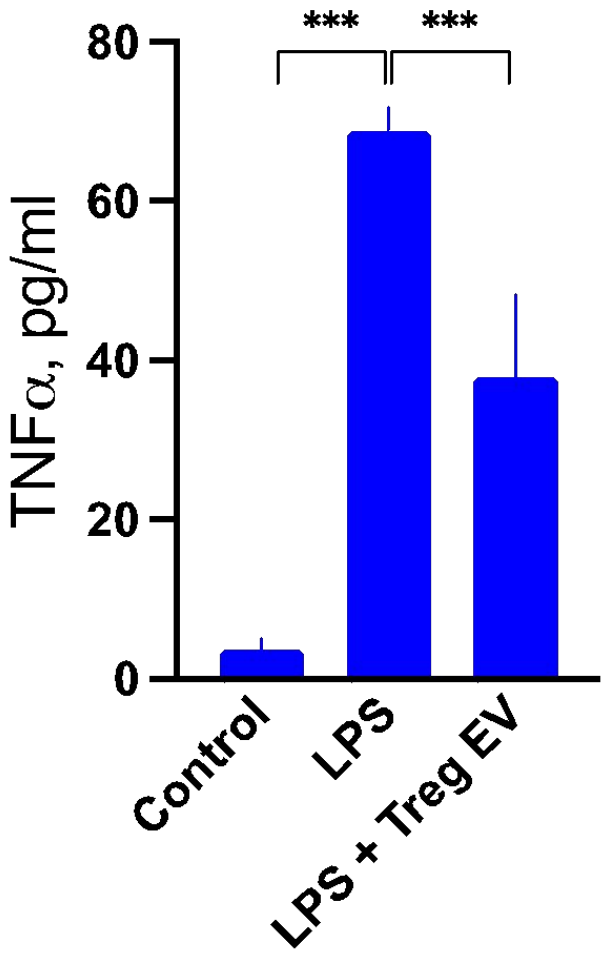
Examples of Treg EV Protection of PCTS From Either Hypoxia or Inflammation







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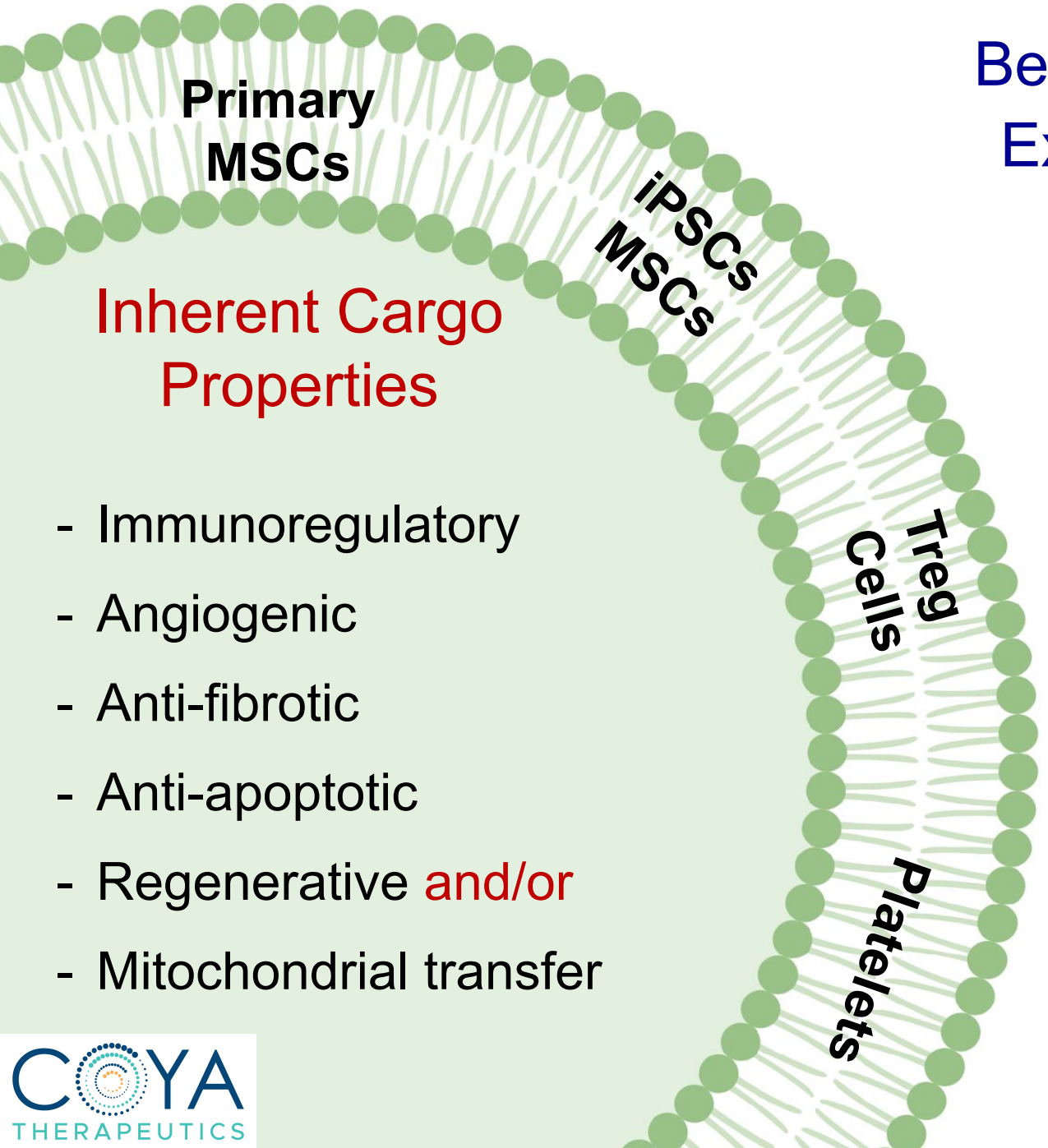

Inherent Cargo Properties

- Immunoregulatory
- Angiogenic
- Anti-fibrotic
- Anti-apoptotic
- Regenerative and/or
- Mitochondrial transfer

Beyond the Inherent Cargo Properties, Exosomes are Nature's Nanoparticle Drug Delivery Vehicle

- Designed for receiver cell uptake.
- Crosses physiological barriers that the "typical" man-made nanoparticle cannot (ie. BBB).
- Exosomes offer low toxicity, high biocompatibility and low immunogenicity.
- Inherent targeting capacity.

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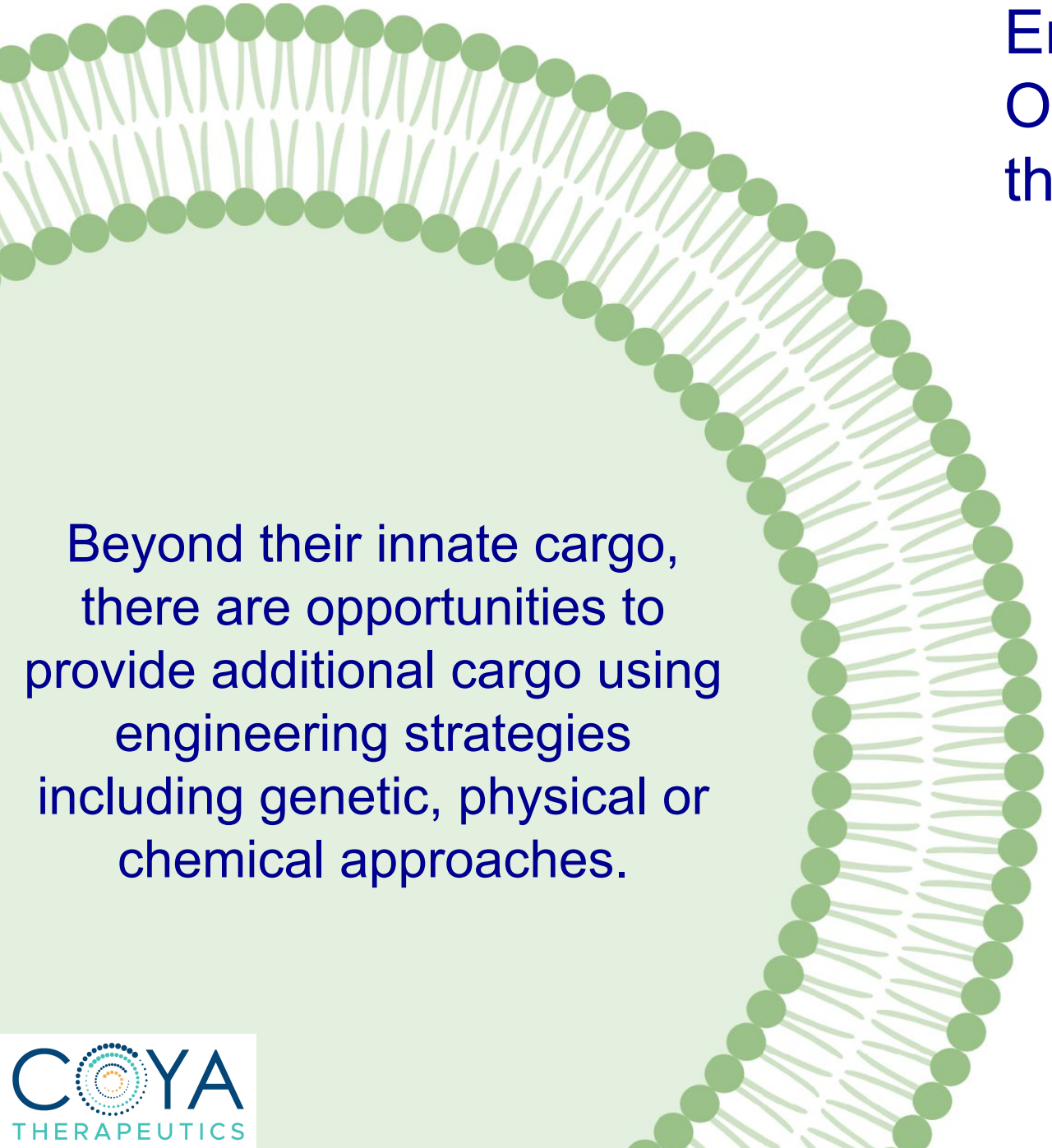

Beyond their innate cargo, there are opportunities to provide additional cargo using engineering strategies including genetic, physical or chemical approaches.

Engineering Exosome Cargo Presents Opportunities to Improve Their therapeutic Potential

- Improving stability
- Increasing plasma retention during systemic delivery
- Altering biodistribution
- Increasing residence time during local delivery
- Enhancing cell targeting with systemic delivery
- Enhancing cell targeting and uptake with local delivery
- Enhancing targeted therapeutic effects

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We at CMU are Developing Non-Genetic Engineering and Direct Approaches to Load Both Luminal and Surface Exosome Cargo

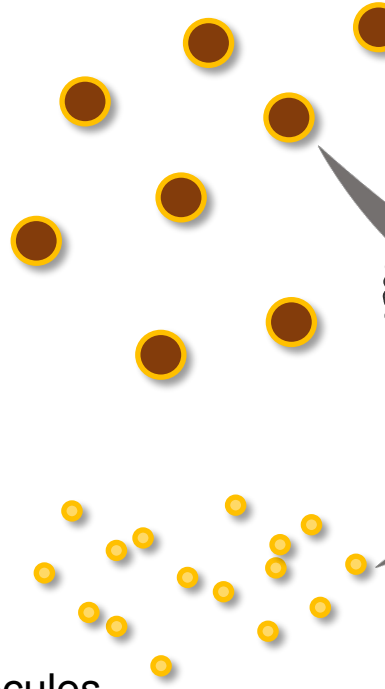
Engineering Exosome Luminal Cargo

Yerneni et al. 2021 J Extracell Vesicles 10:e12155

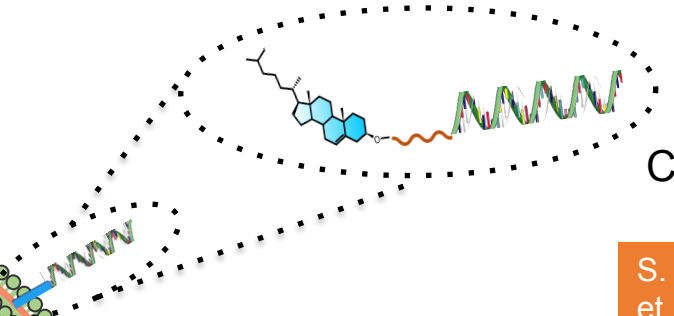
Proteins (Albumin, BMP2) Nucleotides

Yerneni et al 2022 Acta Biomaterialia 149:198

Hydrophobic Drugs and Small Molecules (Curcumin)



Engineering the Exosome Surface



Cholesterol-DNA Tethers

S. S. Yerneni*, S. Lathwal* et. al., *ACS Nano* (2019)

Small Molecules

Proteins

Polymers

S. Lathwal et. al., *PNAS* (2021)

Click Chemistry, Glycan Metabolic Labeling

Xing et al. Biomaterials 2022; 281:121357

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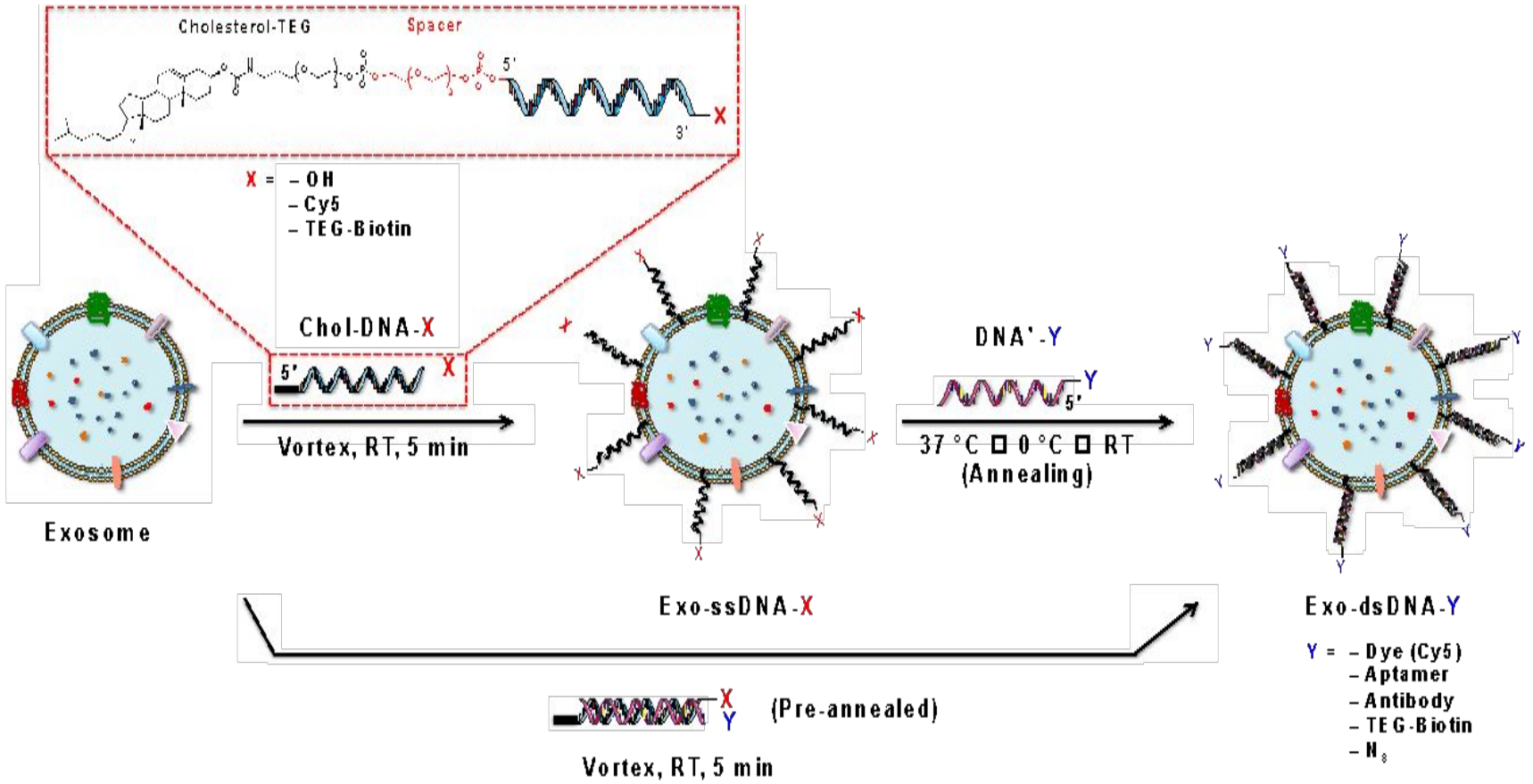
Generalized and Direct Exosome Surface Functionalization Using DNA Tethers



Sai Yerneni
Sushil Lathwal
Krzysztof Matyjaszewski
Subda Das
Julia Cuthbert

-Independent of cell source
-Multivalent functionalization
-Scalable
-Direct ssDNA cargo loading or annealing to dsDNAA using complementary DNA with cargo
-Reversible with DNase

-Control over surface loading
-2000-8000 DNA tethers/EV
-Stable tethering at 4C and 37C

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S. S. Yerneni*, S. Lathwal* et. al., *ACS Nano* (2019)

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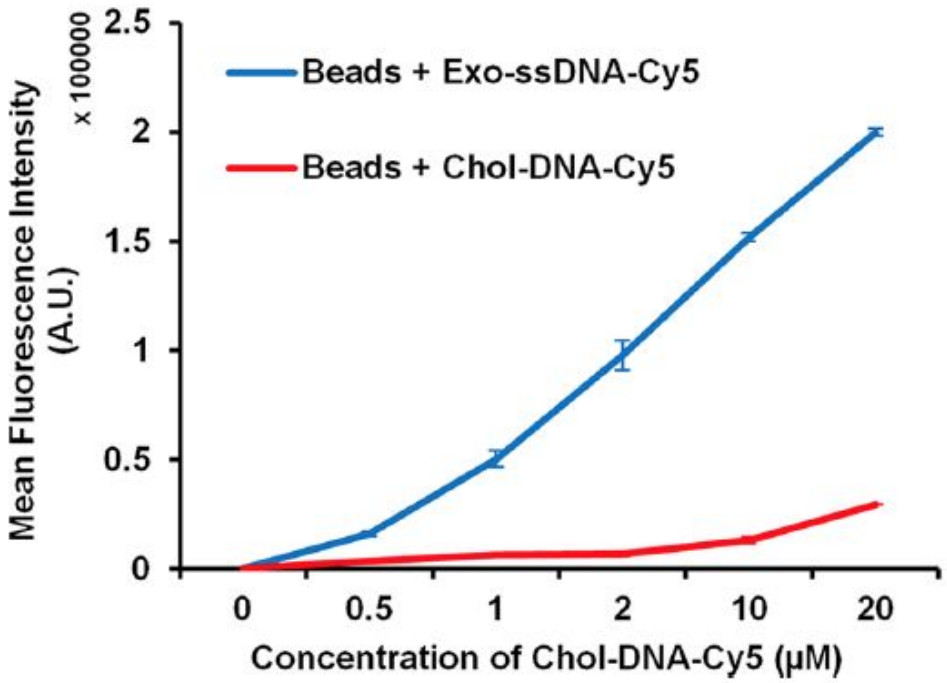
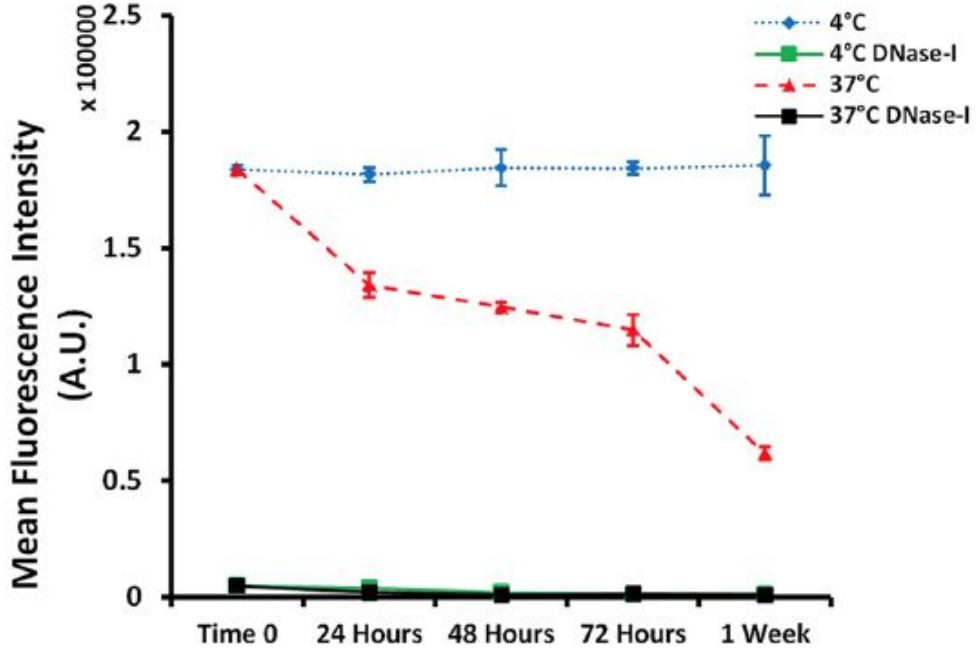
Dose Dependency of ssDNA Tethers



Stability of ssDNA Tethers on Exosomes

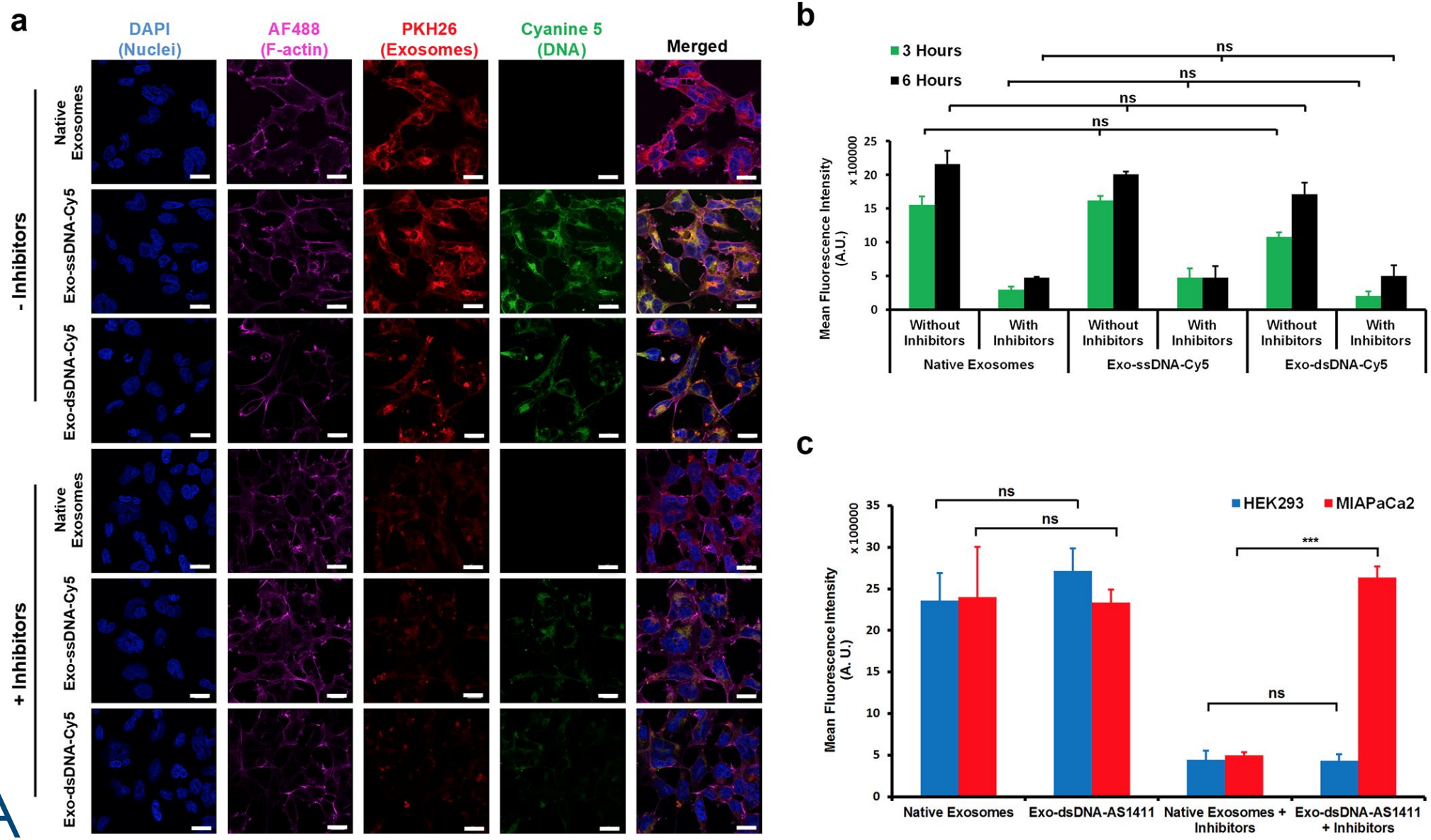


COYA THERAPEUTICS

S. S. Yerneni*, S. Lathwal* et. al., *ACS Nano* (2019)

Carnegie Mellon University

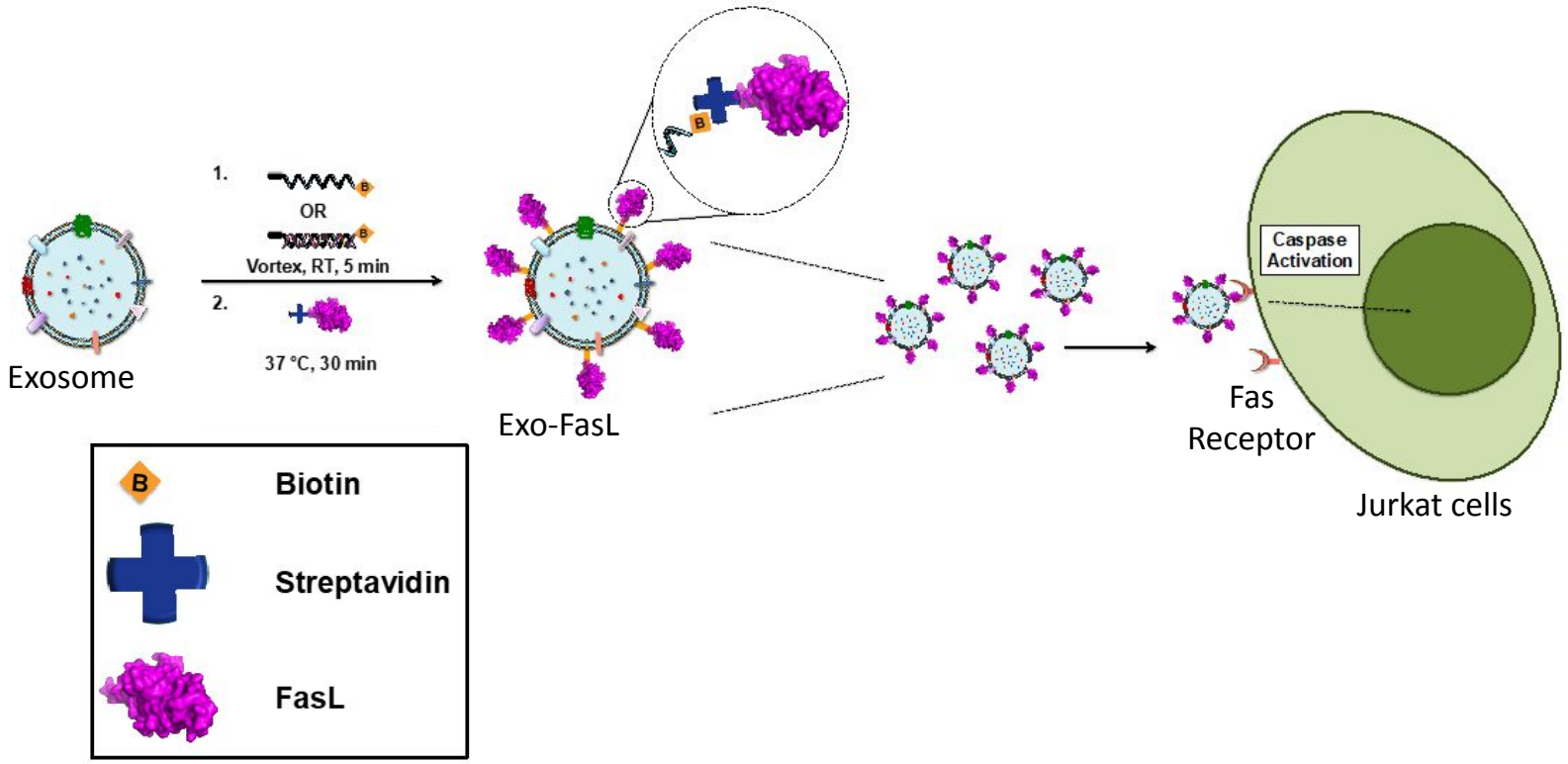
Cell Uptake of DNA-Tethered Exosomes



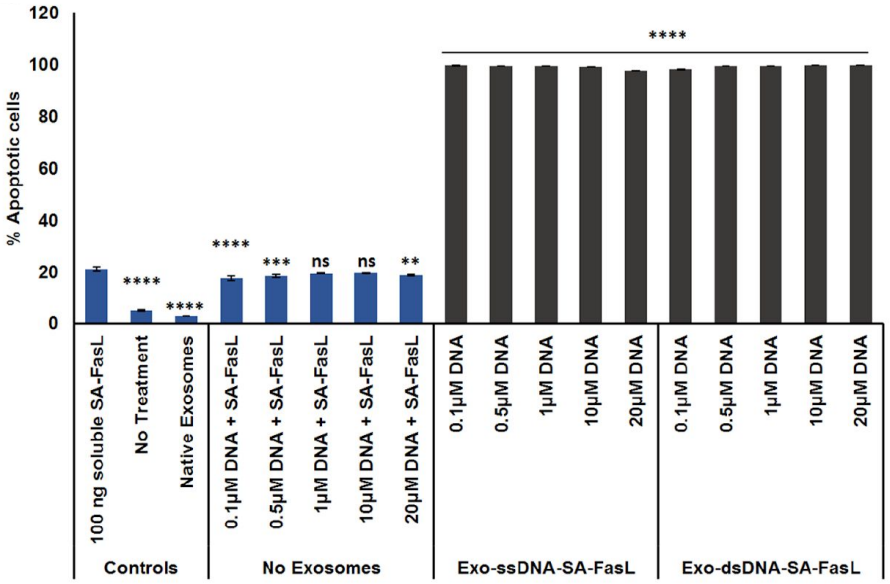
S. S. Yerneni*, S. Lathwal* et. al., *ACS Nano* (2019)

Exosomes Functionalized With FasL (Exo-FasL) are Bioactive In Vitro

FasL induces apoptosis in Fas receptor-bearing cells



Exo-FasL induce potent apoptosis in Jurkat cells



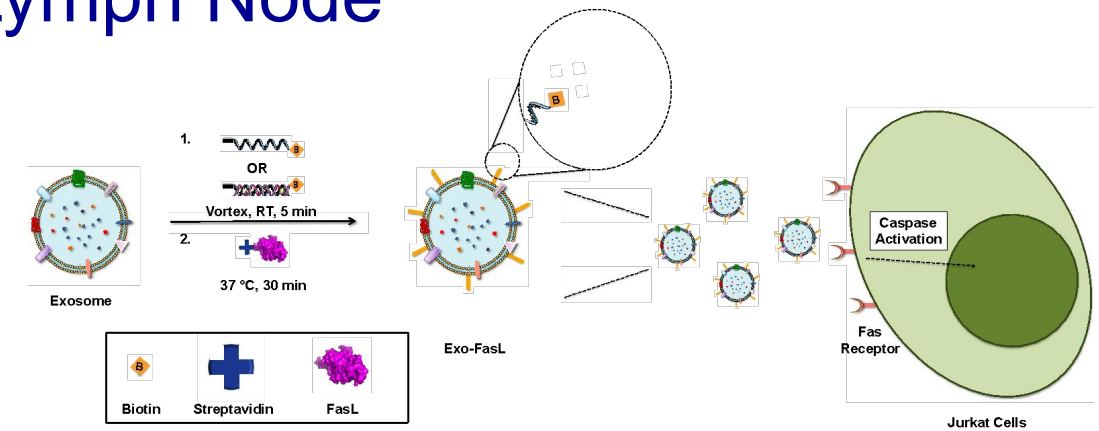
COYA THERAPEUTICS

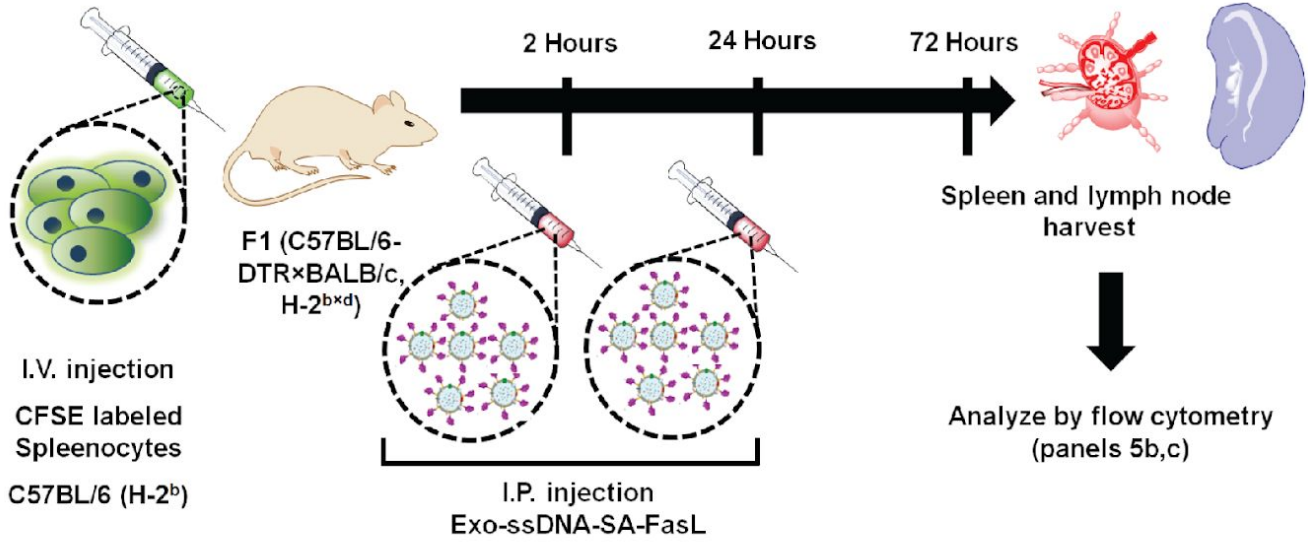
S. S. Yerneni*, S. Lathwal* et. al., *ACS Nano* (2019)

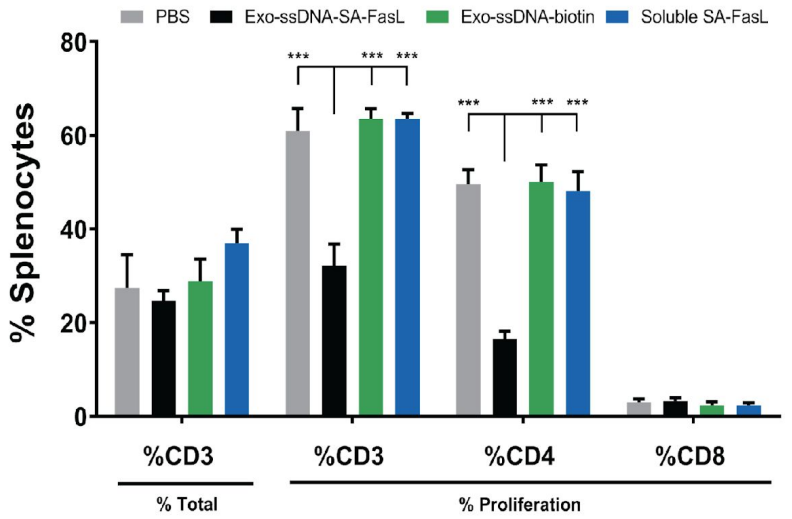
Carnegie Mellon University

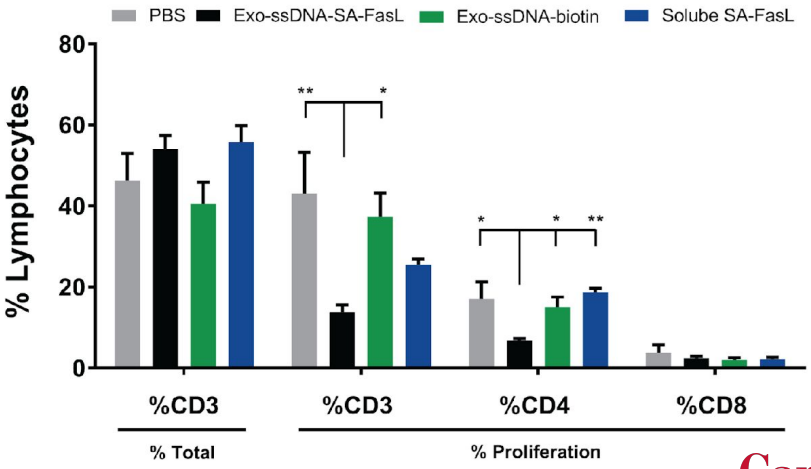
Exosomes Functionalized with FasL-Strep to EVs via DNA Tethers Alters the Immune Microenvironment of the Spleen and Lymph Node

Sai Yerneni
Suba Das
Haval Shirwan
Esma Yolcu
Pradeep Shrestha









COYA THERAPEUTICS

S. S. Yerneni*, S. Lathwal* et. al., *ACS Nano* (2019)

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We asked the question, could we engineer the surface of the Treg exosome to improve their delivery to immune cells?

We considered increasing the EV surface of CTLA4 using our DNA-cholesterol tethering approach.

We created tethers with terminal aptamers to either His or Fc tagged recombinant proteins, ie. His-CTLA4 or Fc-CTLA4

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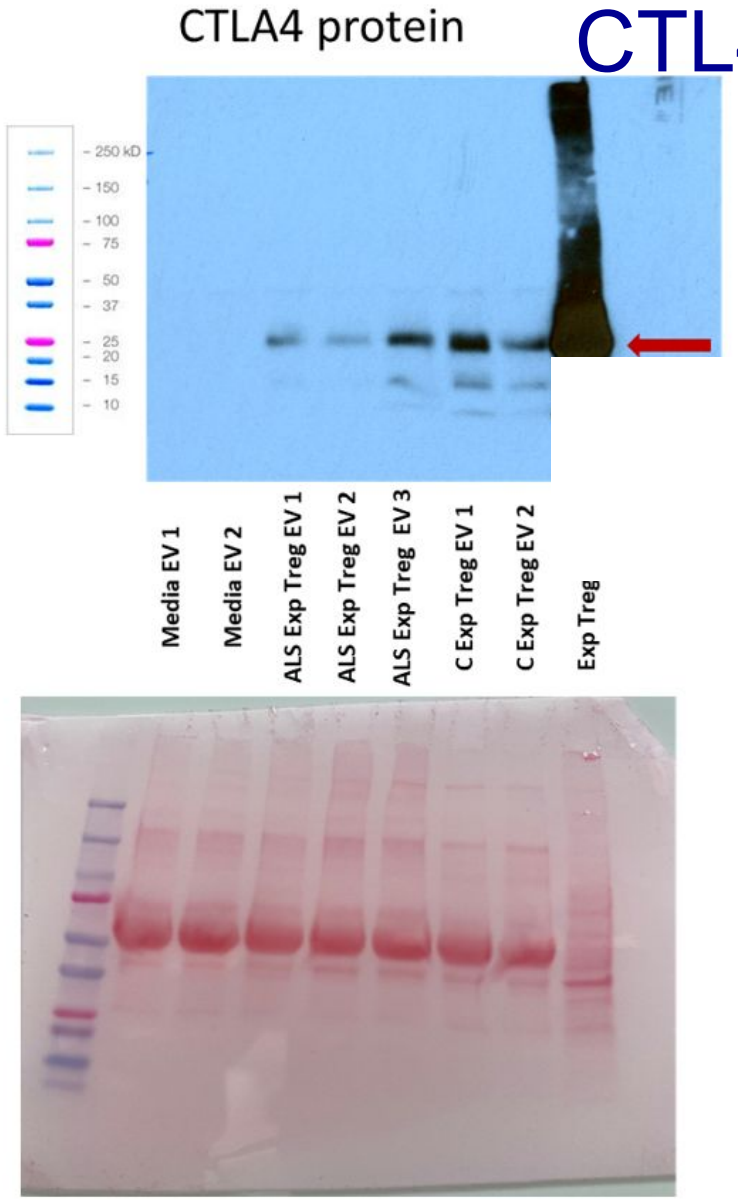
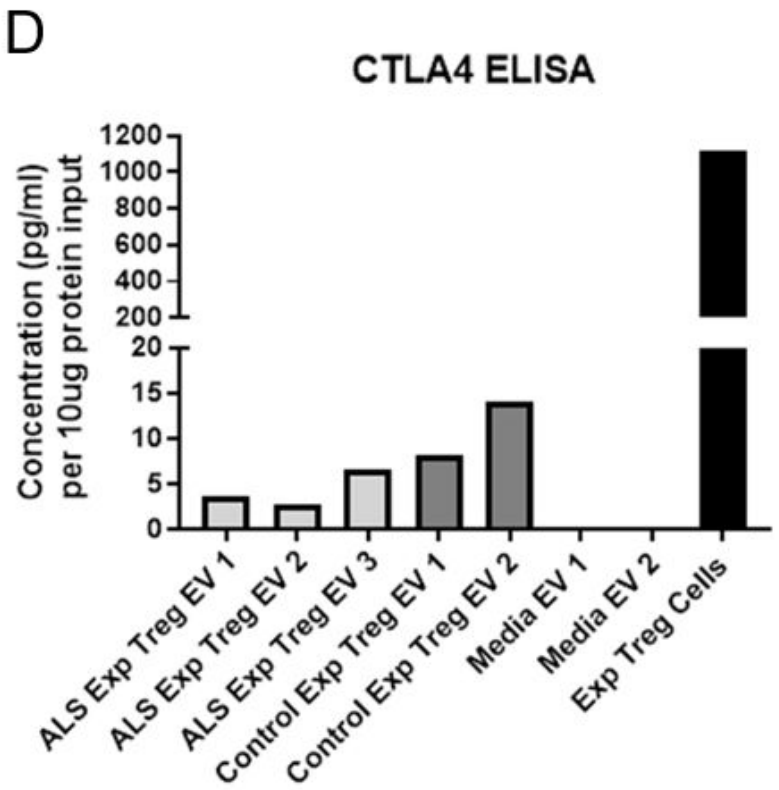
CTL4 is contained in/on Treg Exosomes





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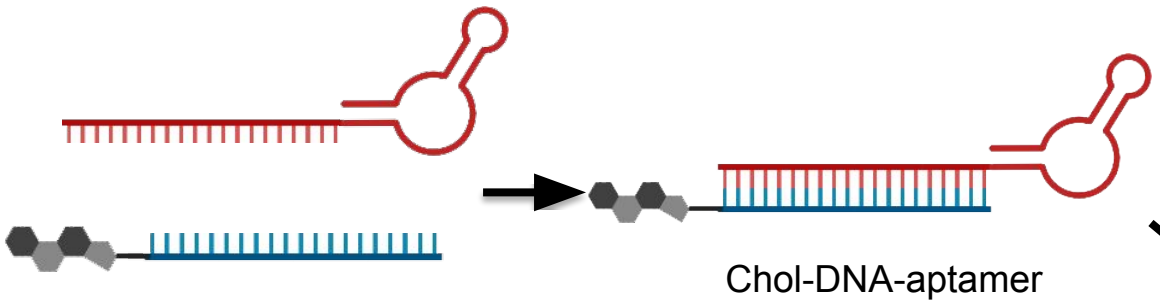
Thome 2022 Front Immunol 13:875825

Aptamer Sequences with chol-DNA Tethers for Direct Engineering of CTLA4 onto Treg Exosomes

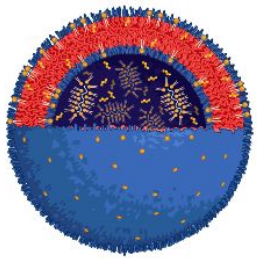


Chol-DNA-aptamer

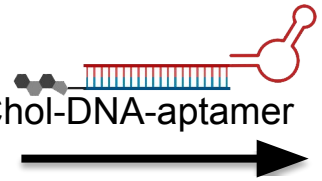
DNA'-3T-ApFc
AGCTATGGGATCCAACTGCAGTTTTGCCACATTAGTCTCACCACTACCTGCGTACCTACCGCCGC

DNA'-3T-ApHis
AGCTATGGGATCCAACTGCAGTTTTGTTTGCCGGTGGGCAGGTTTAGGGTCTGCTCGGGATTGCGGAGGAACA
TGCGTCGCAAAC

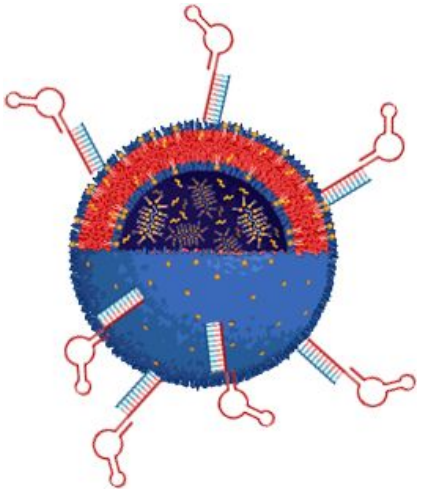
DNA'-3T-ApNCtrl
AGCTATGGGATCCAACTGCAGTTTTGCCATACACAGACTCTCCTCTCTCCCCAACTTCCCACTTT



Exosome

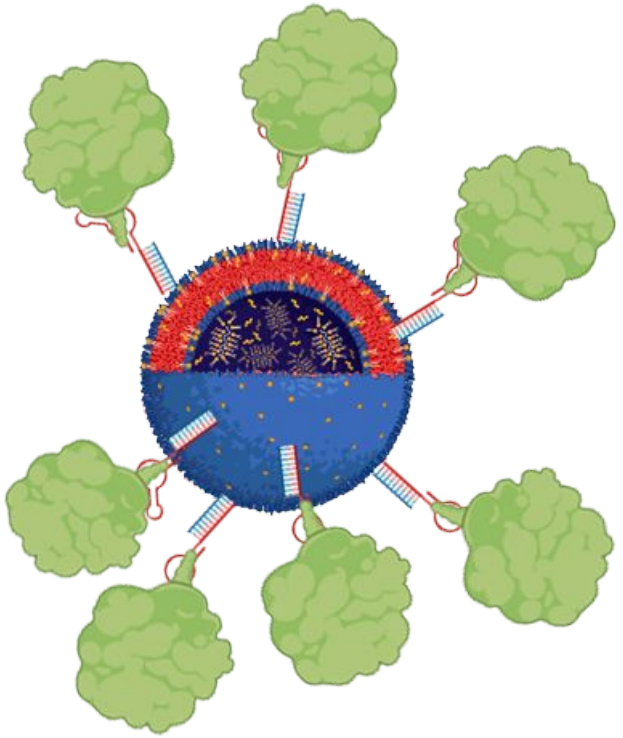


Chol-DNA-aptamer



Exosome-DNA-aptamer

[Tag]-CTLA4



Exosome-DNA-CTLA4
(via aptamer-Tag binding)

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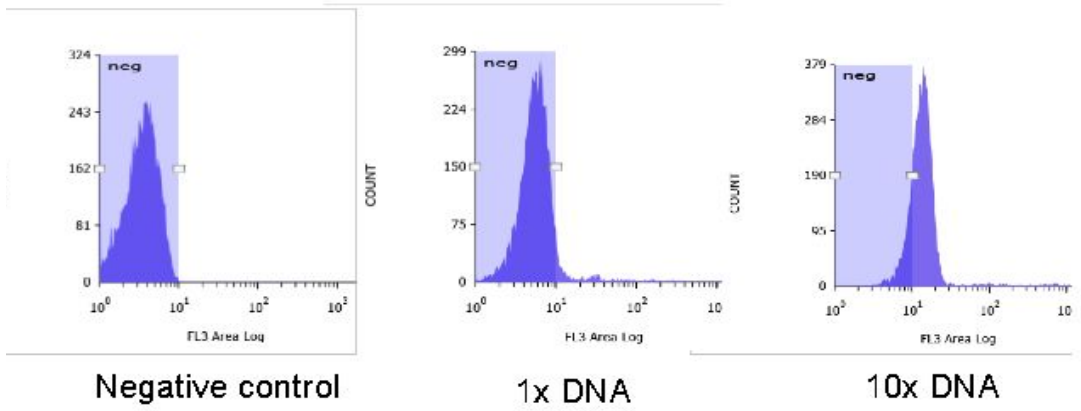
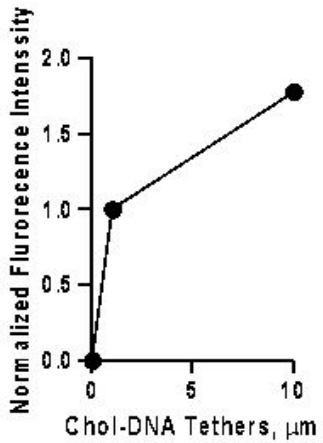
Chol-DNA Tethers can be Loaded onto Treg EVs





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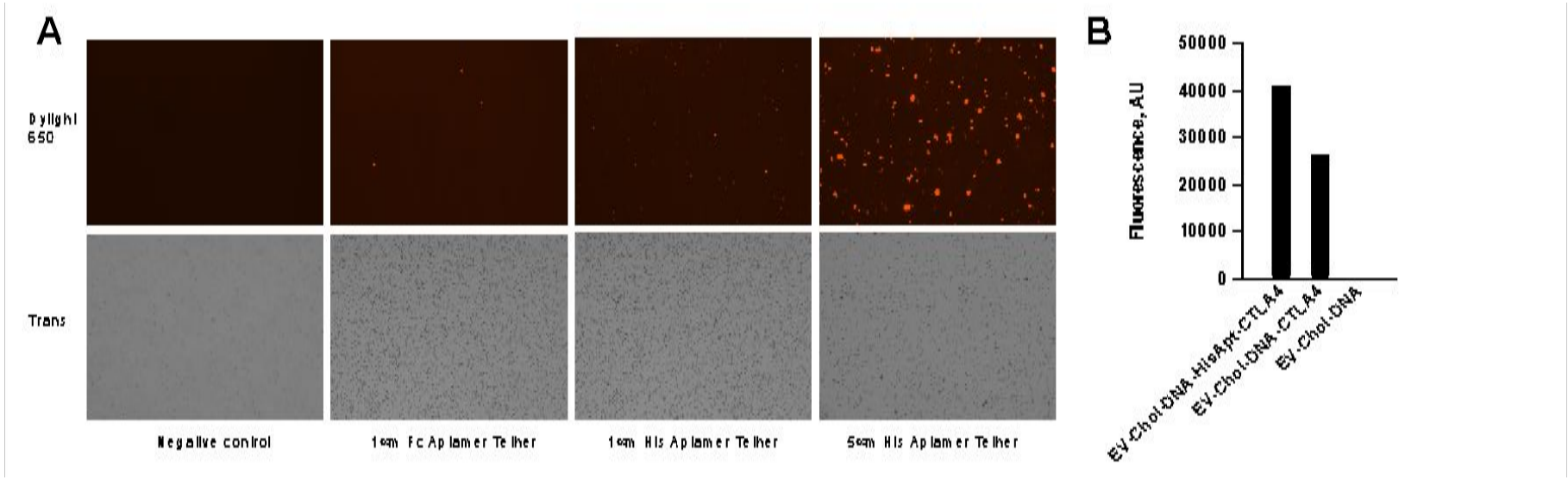
CTLA4 can be Tethered to Treg Exosomes Using Aptamers Sequences on the chol-ssDNA Tethers



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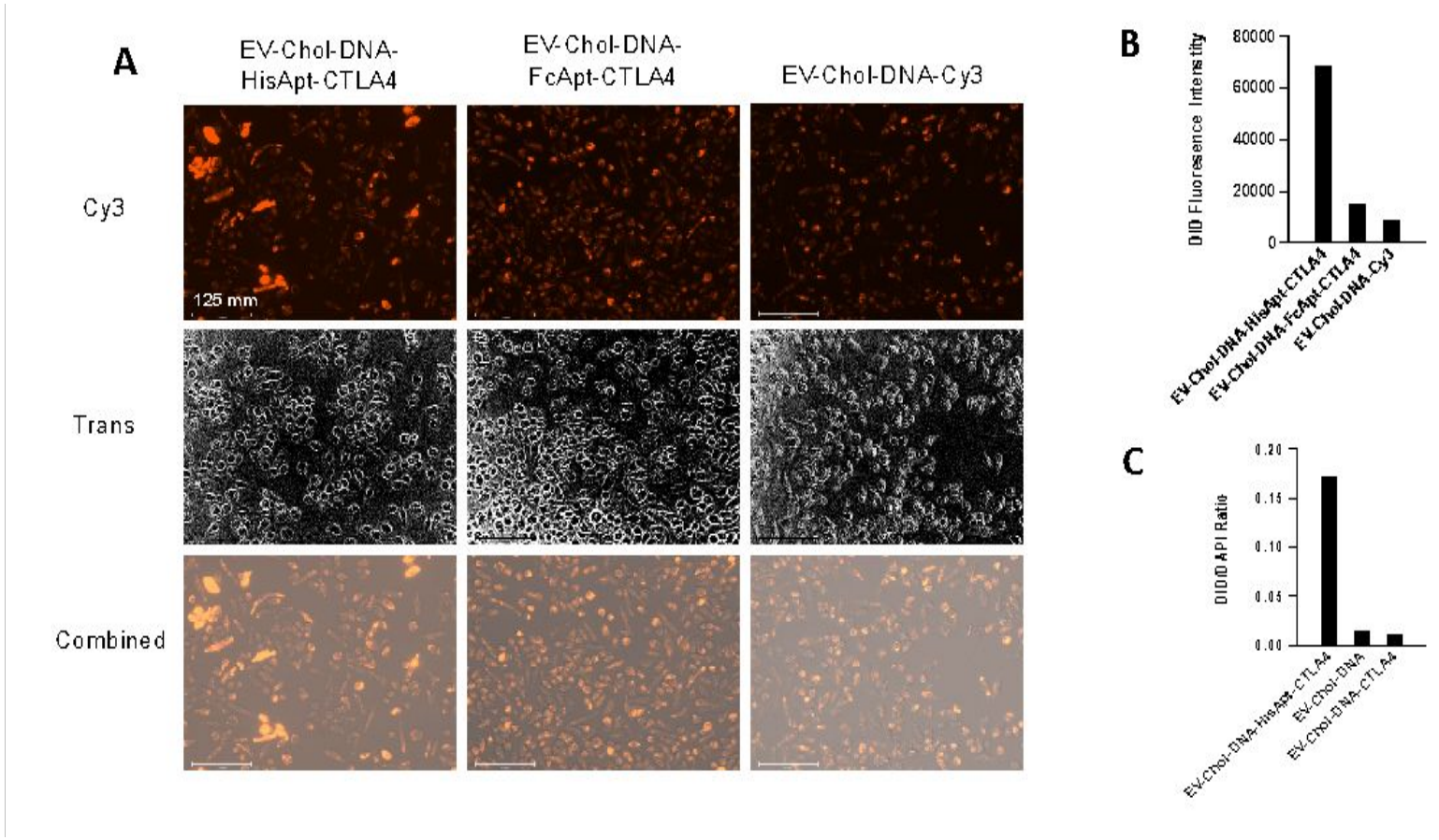
CTLA4 Increases Uptake of Treg Exosomes into Macrophages



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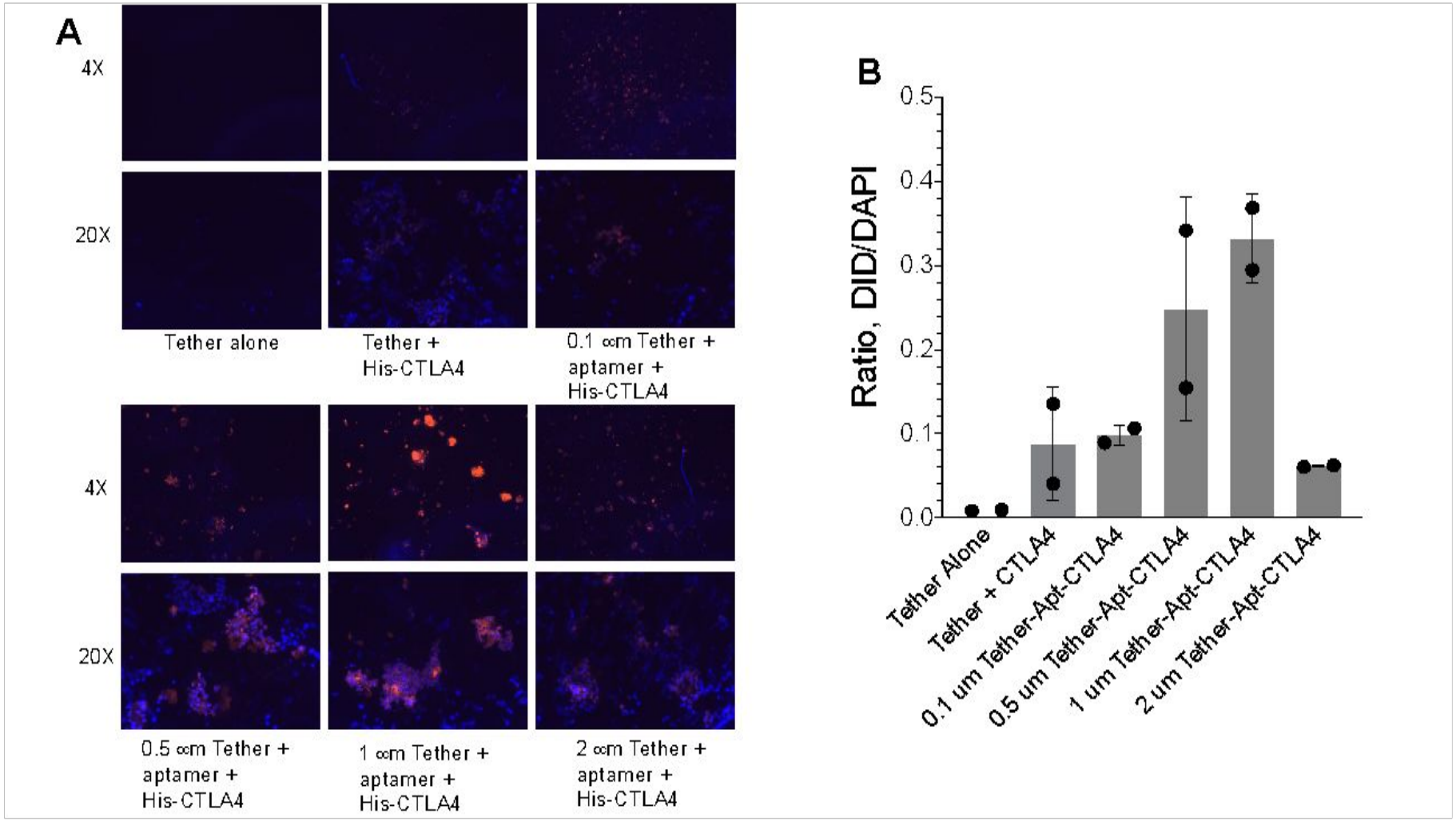
CTLA4 Increases Uptake of Treg Exosomes into T Cells.



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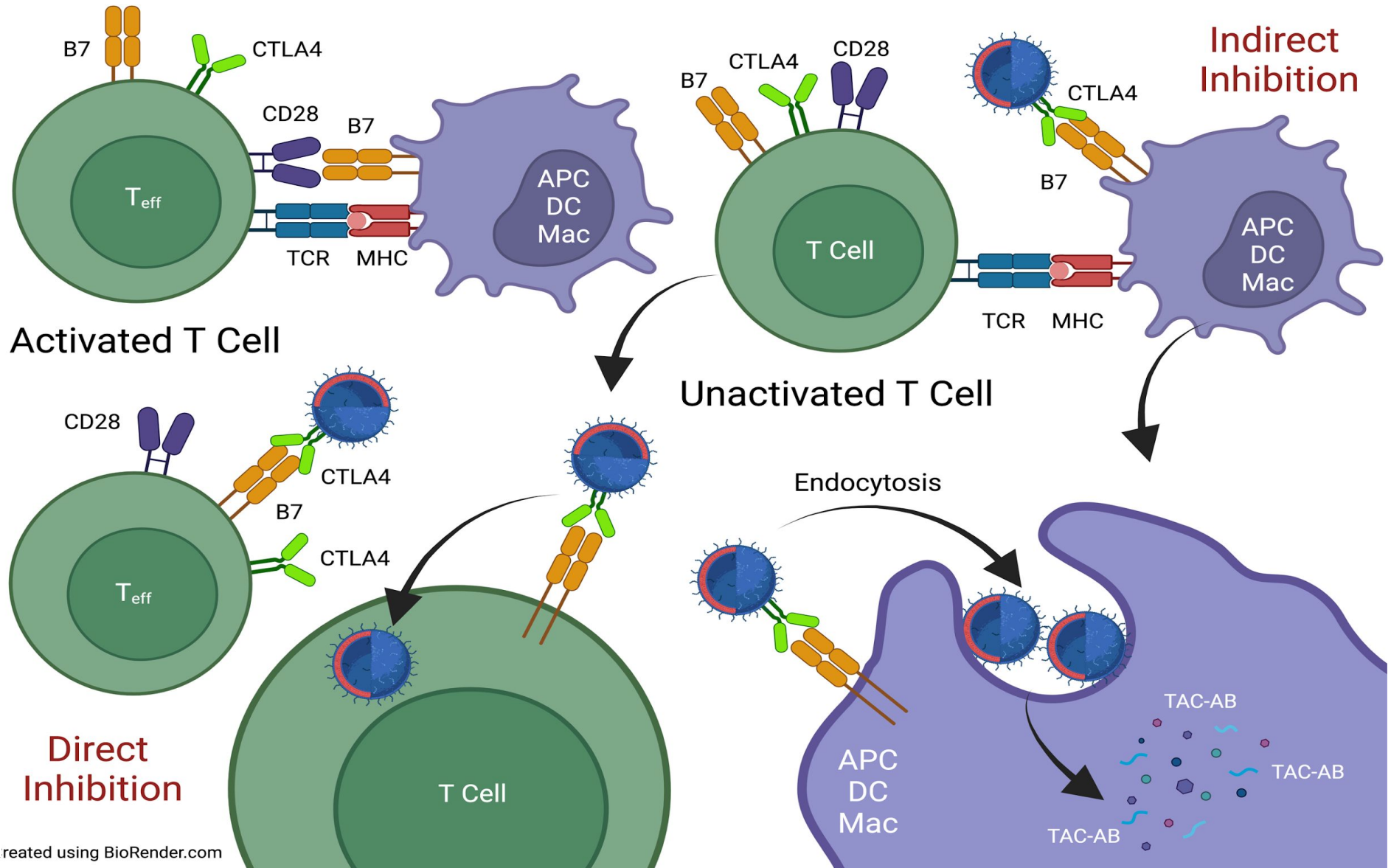
Rationale for Engineering CTLA4 onto Treg Exosomes to Increase Targeting to Immune Cells.



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Created using BioRender.com

Summary

- Engineering exogenous CTLA4 onto Treg exosomes results in their increased binding and internalization into immune cells.

- Therefore, the delivery of the Treg exosome cargo (both surface and luminal) to modify both innate and adaptive immune cells is expected to be enhanced. We will be determining this in upcoming experiments.

- We will also be delivering CTLA4-Treg exosomes in future experiments in vivo with the intent to alter immune environment to inhibit/reverse chronic inflammatory conditions, and to promote transplantation.

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