Exosomes as therapeutic agents

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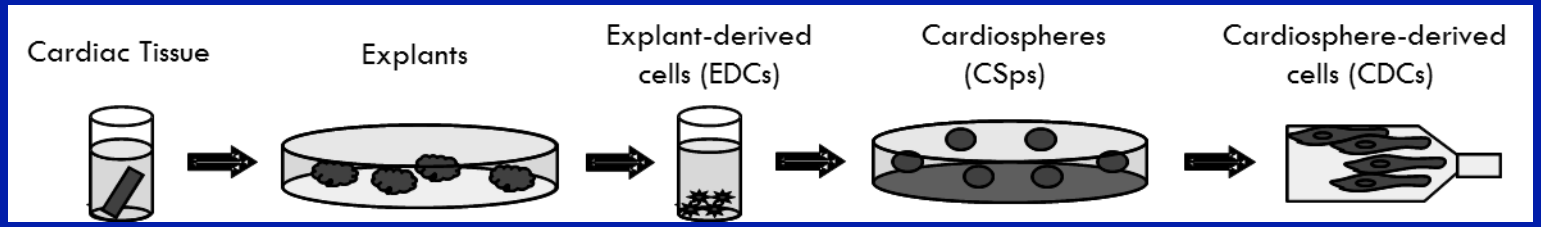
Disclosure: founder & SAB chair, Capricor Inc.

Cardiosphere-derived cells (CDCs)



First described by RR Smith et al., *Circulation* 2007; methods and bioactivity reproduced by at least 26 labs worldwide

Cell Type	Human heart stem cell/cardiac stromal cell
Characteristics	CD105+, CD45-; secreted SDF-1 and exosomes containing distinctive miRs
Clinical Trials	**CADUCEUS**-completed-autologous phase 1. Twenty-five patient study showed regeneration in CDC-treated post-MI subjects with mild HFrEF
	ALLSTAR-phase 1 (n=14) completed, now in phase 2- allogeneic CDCs post-MI with mild HFrEF
	DYNAMIC-ongoing-phase 2a/b study of allogeneic CDCs in patients with advanced HFrEF
	HOPE-Duchenne-allo CDCs for DMD cardiomyopathy, enrolling
Mechanism of action	Paracrine regenerative effects ▪ **Promote cardiomyomyogenesis** ▪ **Prevent cardiomyocyte apoptosis** ▪ **Anti-fibrotic** ▪ **Anti-inflammatory**

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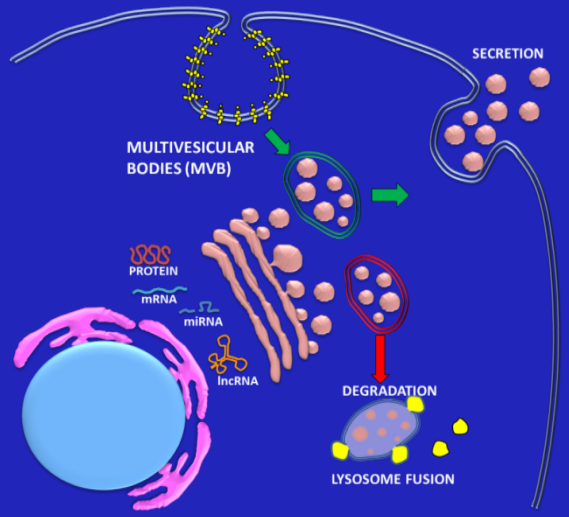
Disadvantages of cells for therapeutics

- Cells work, but fragile living material
- QA/QC, release & identity criteria complex
- Suboptimal in closed compartments
- Immune memory?

Cell-free therapeutics

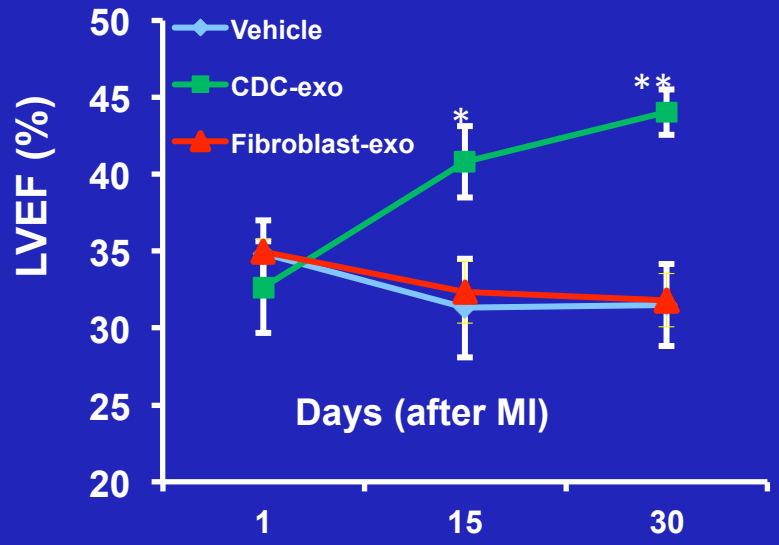
- If effects do not require permanent presence of transplanted cells, why use cells?
- Is there a single entity that can mimic all the salient benefits?

Exosomes are bioactive nanoparticles

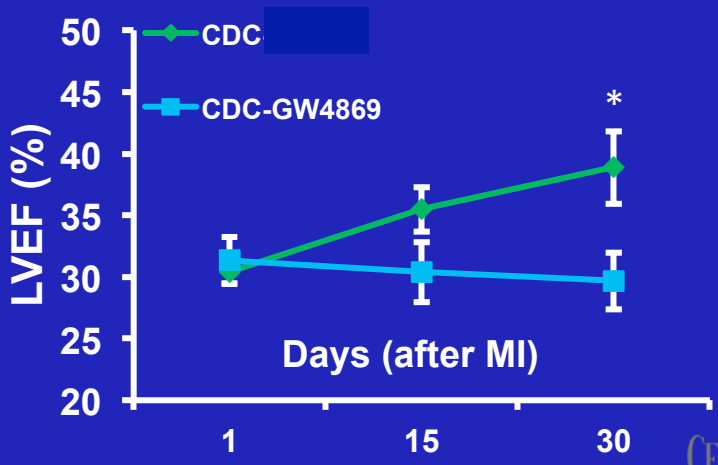


- 30-150 nm particles
- Present in all body fluids
- Released by nearly all cell types
- Loaded with miRs and other bioactive contents
- Payload very cell-specific

CDC exosomes ↑ EF



Blocking exosome biosynthesis abrogates CDC benefit



Ibrahim et al., *Stem Cell Reports* 2014

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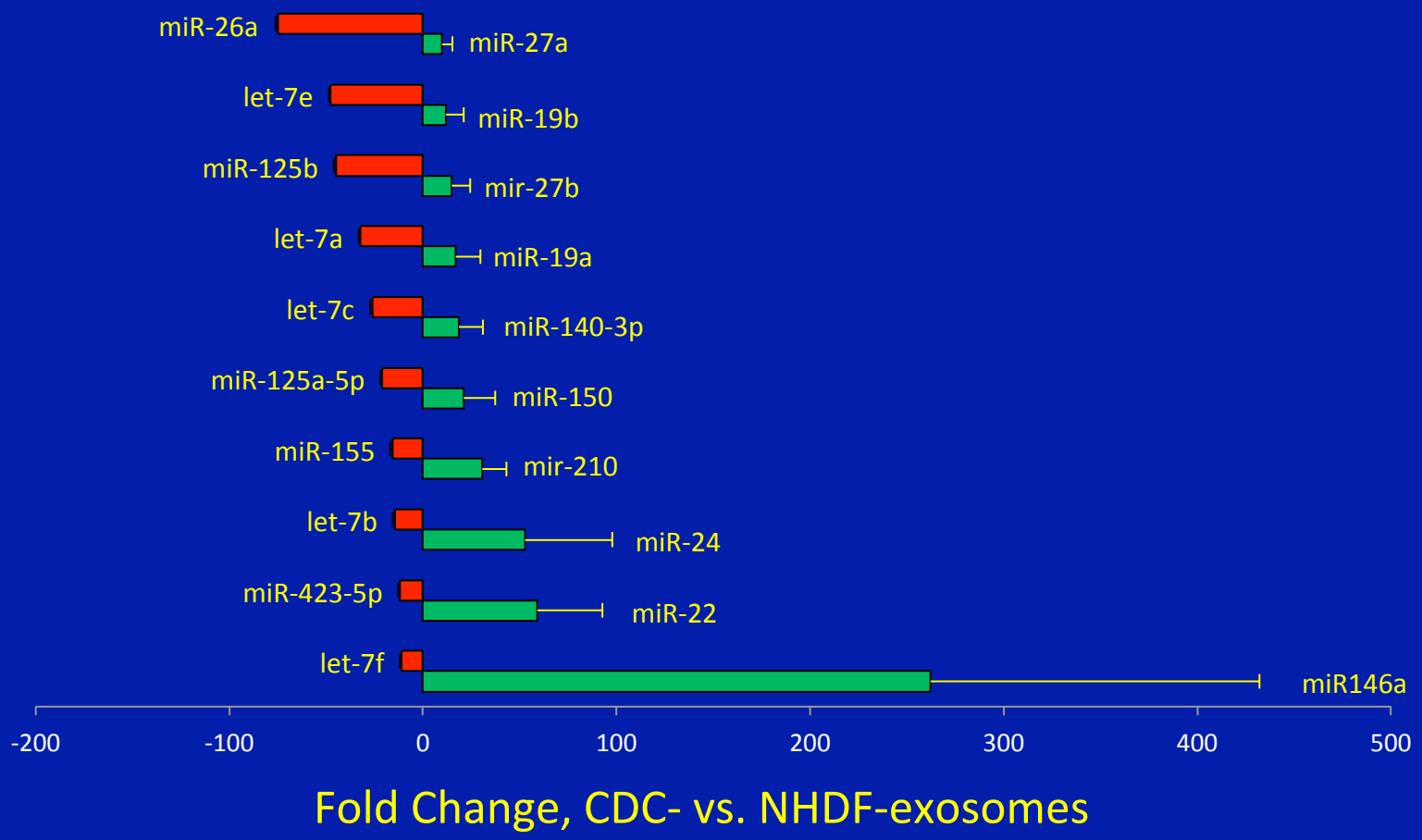
CDC-exosomes exhibit a distinctive miR profile



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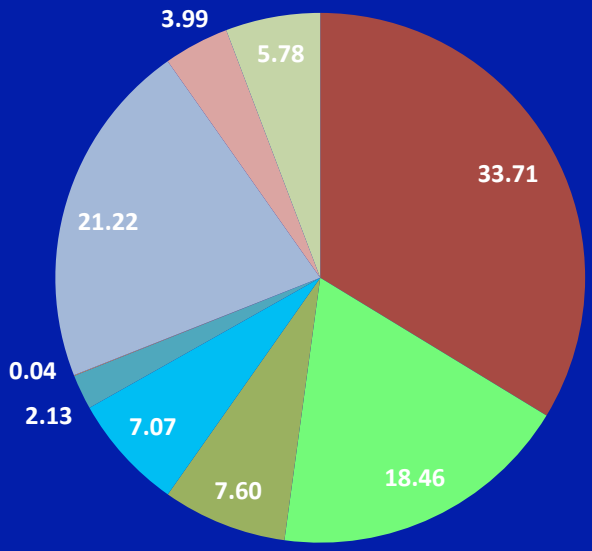
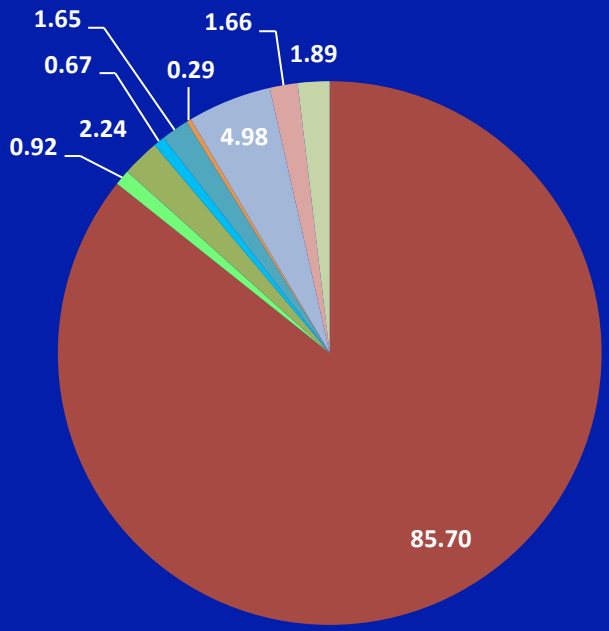
…but miRs are minority of exosomal RNA



CDC-exo

%tRNA
% Y RNA
%rRNA
%mirRNA
%linc RNA
%piRNA
%<15bp
%UnMapped (mRNA etc)
%other (snRNA, snoRNA etc)



NHDF-exo

Marbán lab, unpublished

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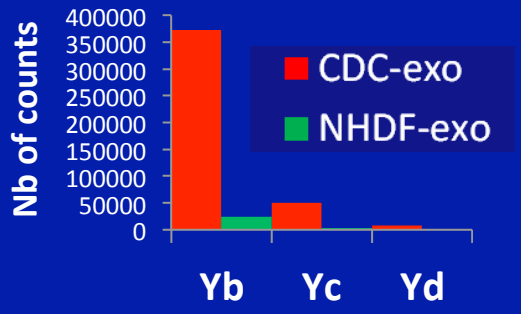
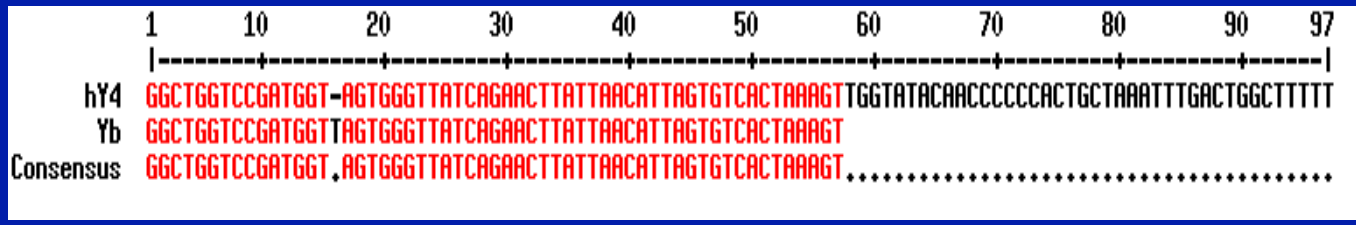
Plentiful Y RNA fragment regulates IL-10 expression

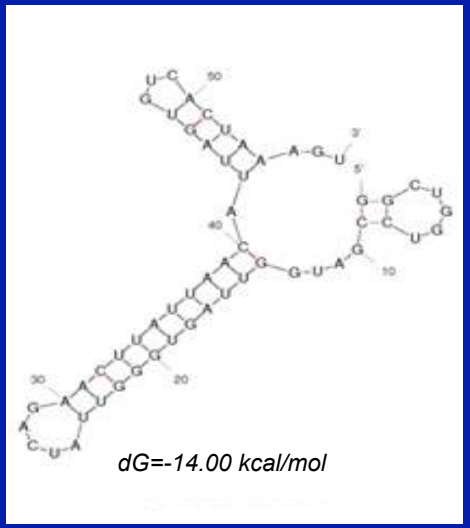
Y RNA abundance



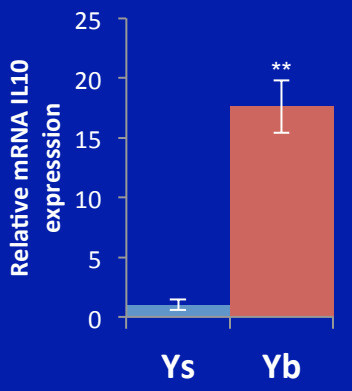
Y RNA alignment reveals Yb homology to Y4

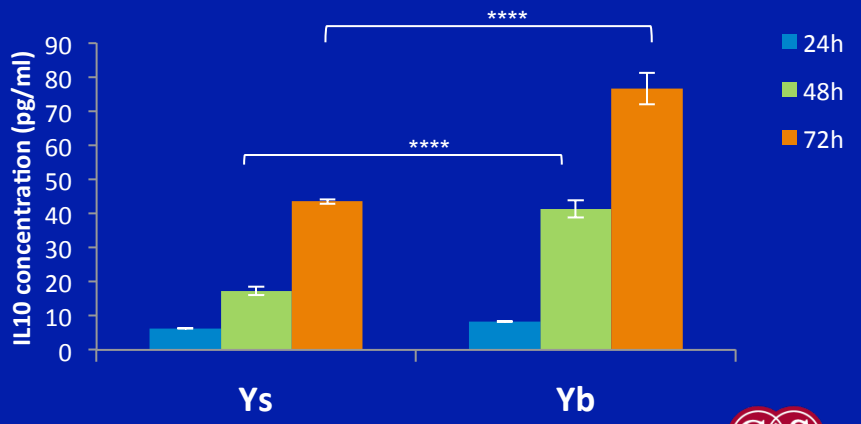


Predicted structure



IL-10 transcript (left) and secreted protein (right) in macrophages





L. Cambier & G. DeCouto, Marbán lab, unpublished

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Working hypotheses

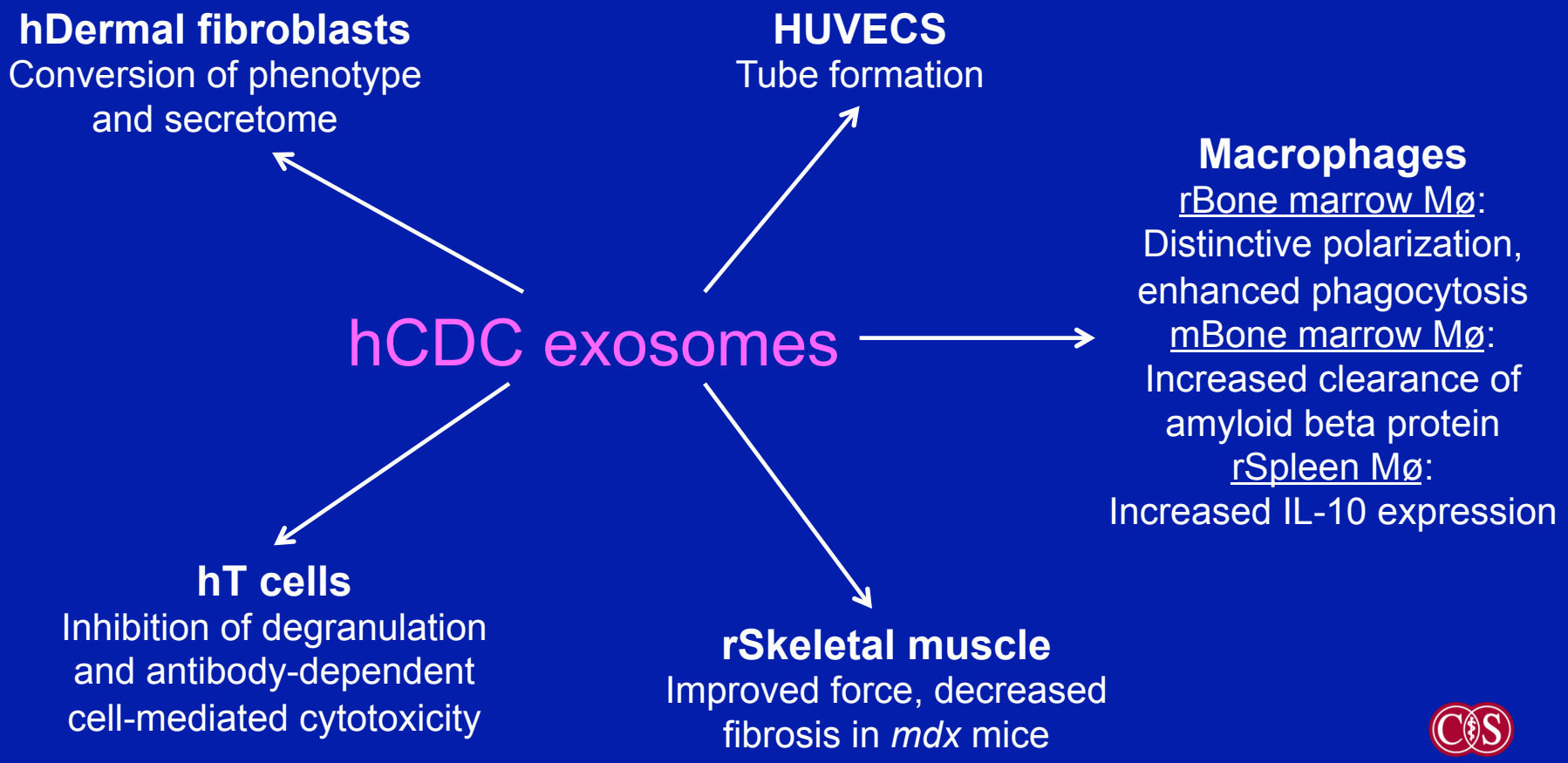
- No single RNA species can account for all the benefits of exosomes
- Individual miRs or other RNA species may prevail in any given setting
- The totality of exosomal contents required for full manifestation of bioactivity

Exosome payloads mimic CDC effects on multiple biological processes

	CDCs	CDC-XO
• Regenerative	√ 1,2	√ 11,12
• Antifibrotic	√ 1-4	√ 11,12
• Anti-apoptotic	√ 3-5	√ 11,13
• Angiogenic	√ 1,6	√ 11
• Anti-inflammatory	√ 9	√ 12,13
• Immunomodulatory	√ 9,10	√ 12

1. RR Smith et al, *Circ* 2007; 2. Makkar et al., *Lancet* 2012; 3. E. Tseliou et al., *PLoSOne* 2014; 4. E. Tseliou et al., *BRIC* 2014; 5. T-S Li et al, *JACC* 2012; 6. I. Chimenti et al, *Circ Res* 2010; 7. K. Malliaras et al, *Circ* 2012; 8. K. Malliaras et al., *EMBO Mol Med* 2013; 9. M. Aminzadeh et al., *EHJ* 2014; 10. L. Lauden et al, *Circ Res* 2013; 11. A. Ibrahim et al. *Stem Cell Reports* 2014; 12. M. Aminzadeh et al., *Circulation AHA abstracts* 2015; 13. G. DeCouto et al., *Circulation AHA abstracts* 2015

Diverse *non-cardiac* effects of CDC exosomes



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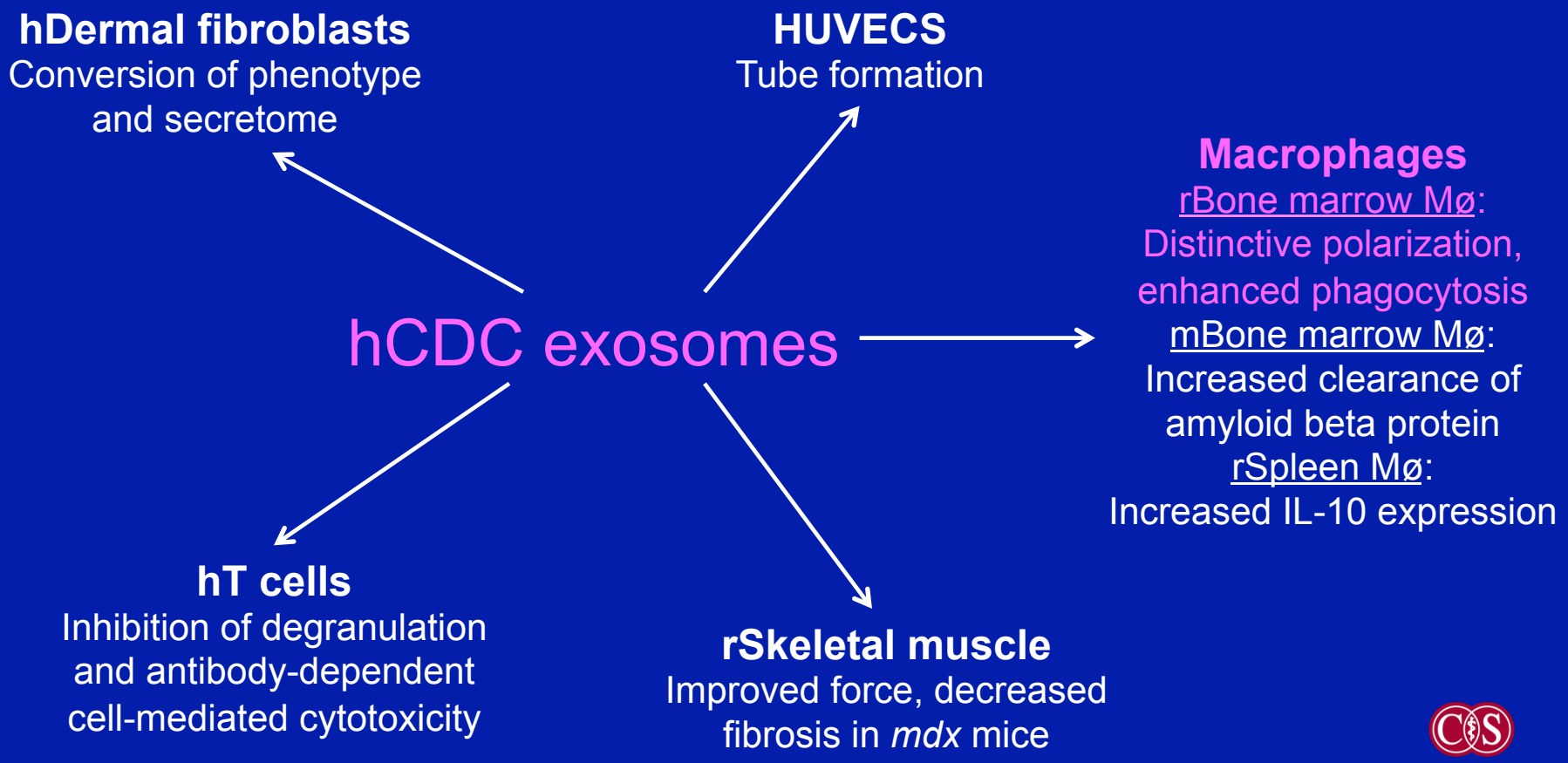
Diverse *non-cardiac* effects of CDC exosomes



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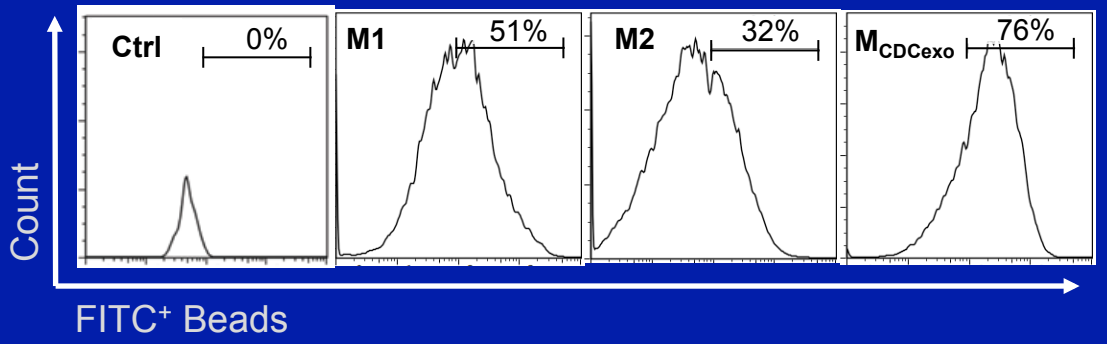
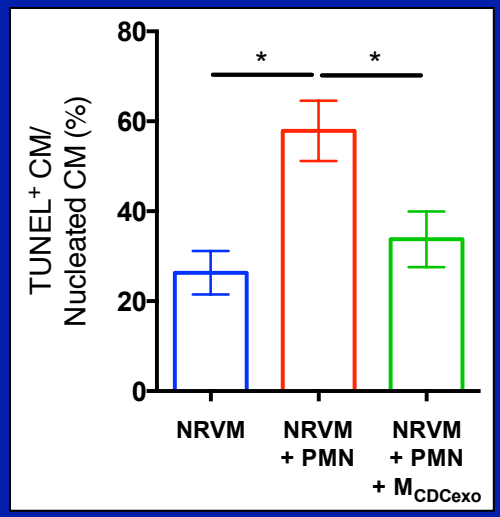
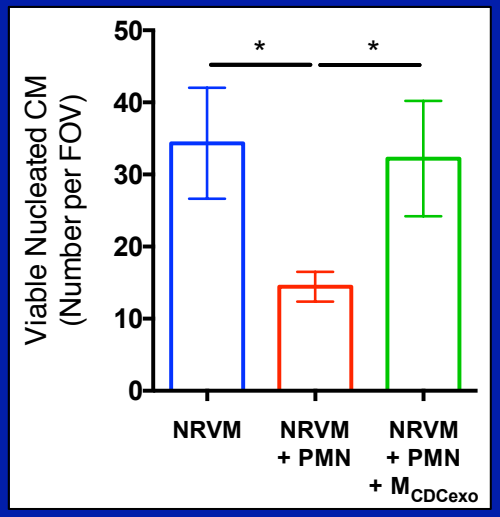
Cardioprotective effects of bone marrow-derived macrophages polarized by CDC exosomes

CDCexo enhance macrophage phagocytosis

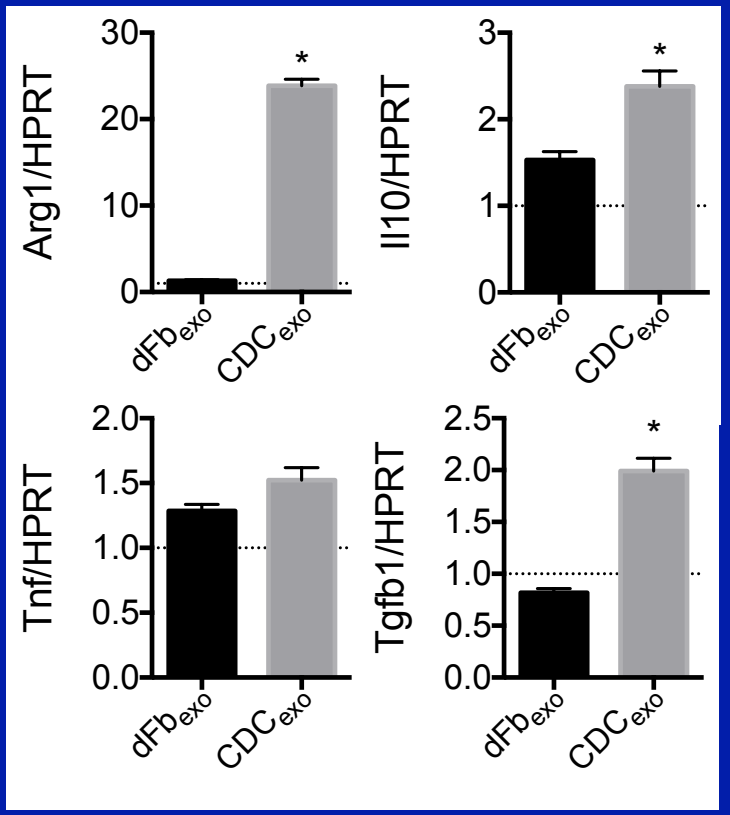


M_{CDCexo} reduce neutrophil-induced myocyte death





CDCexo elicit a distinct gene expression profile in M_{CDCexo}



G. DeCouto, Marbán lab, unpublished

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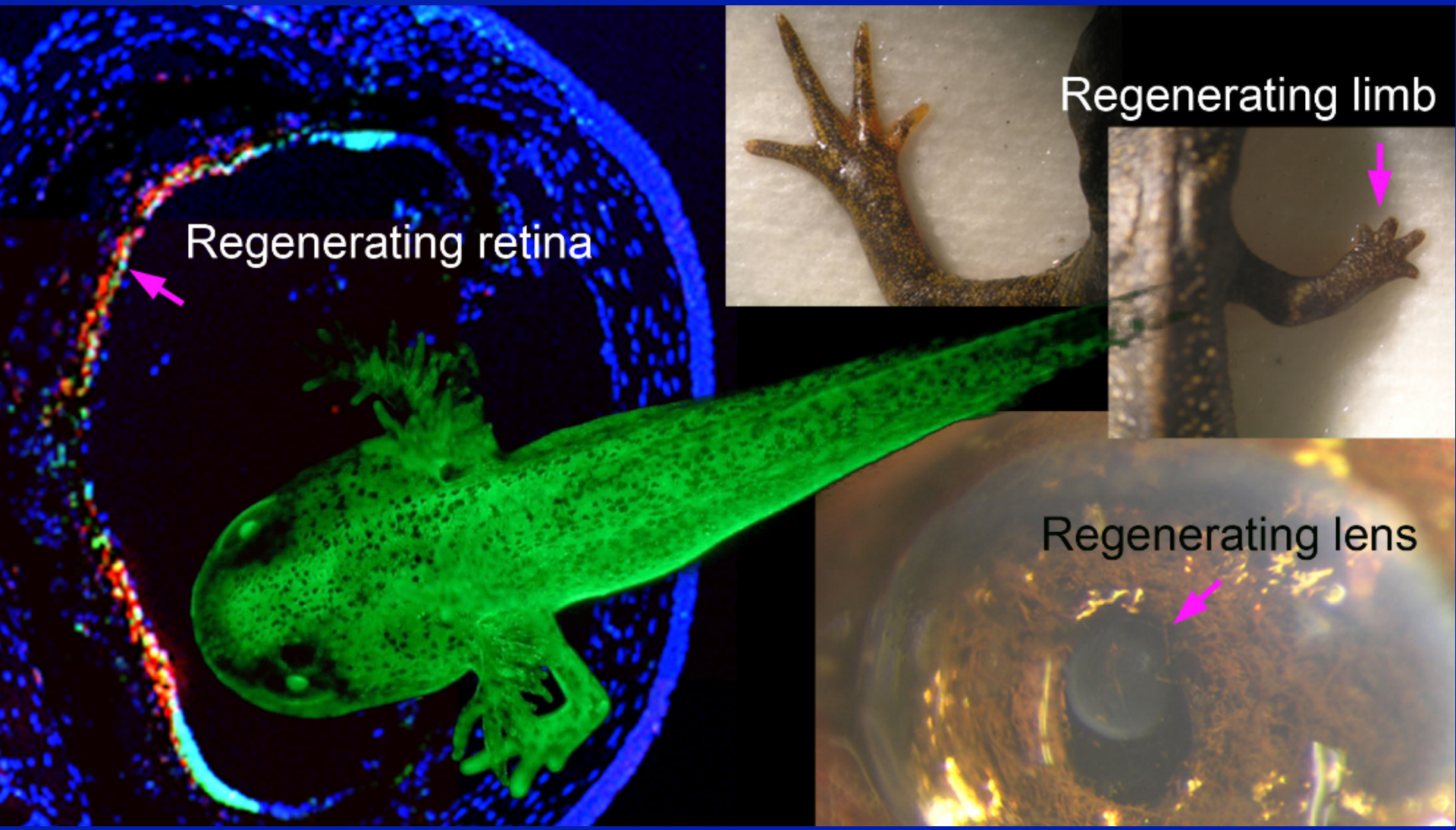
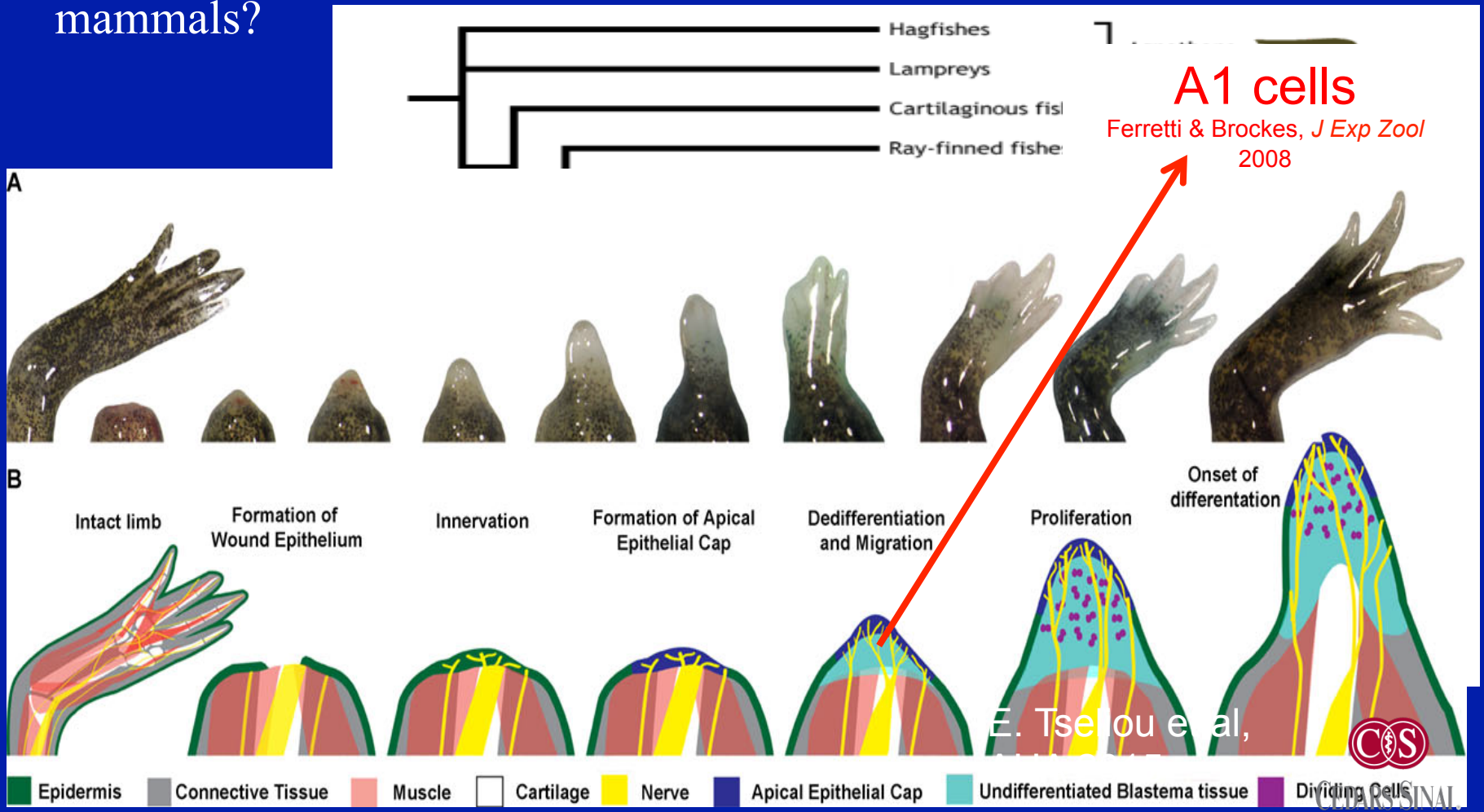
Regeneration in newts>>in mammals



Chiba lab, Tsukuba University, Japan

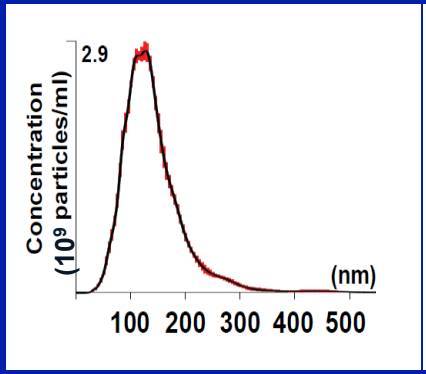
Is newt biology translatable to mammals?

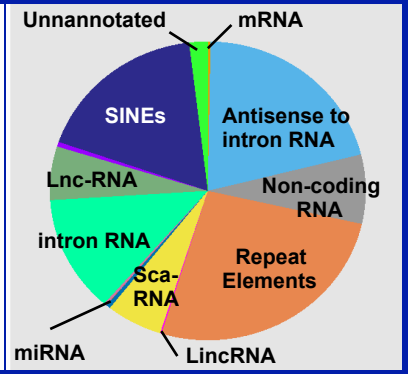
- Separated from mammalian lineage ~**300 million years ago**
- Can newt cells make exosomes? Are the exosomes biologically active in mammals?



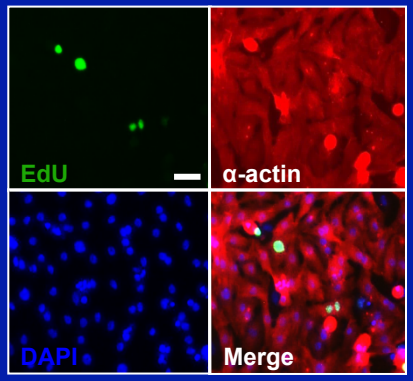
Newt A1 cells make exosomes that are regenerative in rats

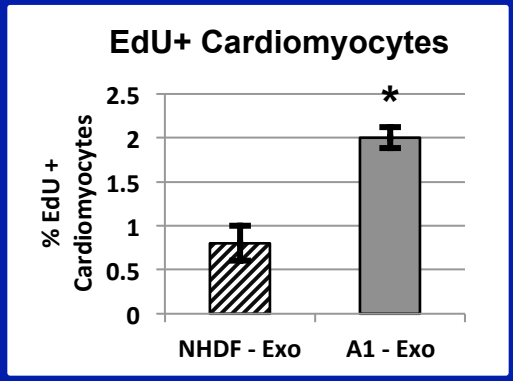
Newt exosome size & RNA cargo profile



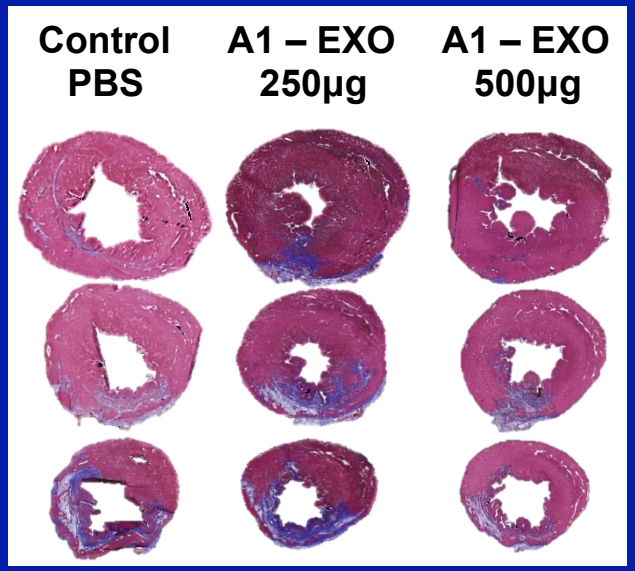


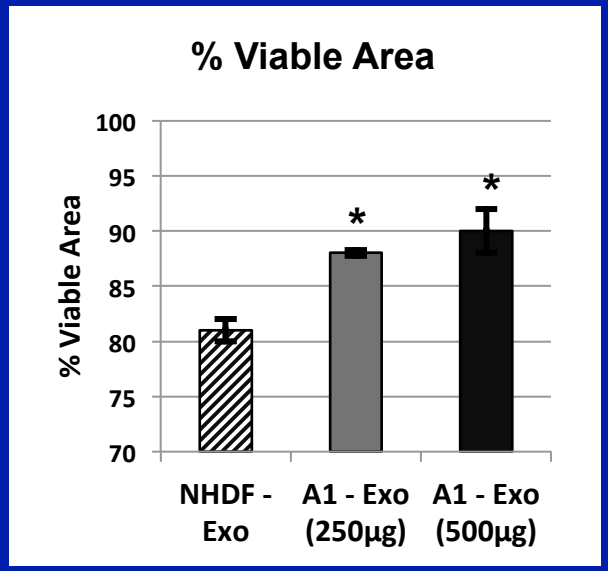
Rat cardiomyocyte proliferation *in vitro*

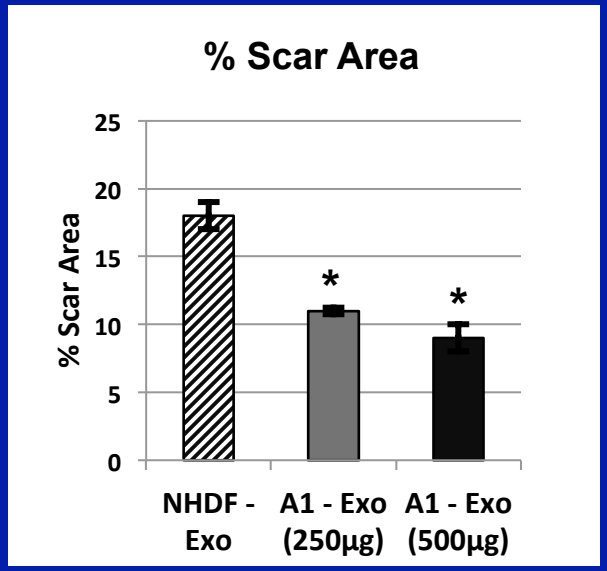




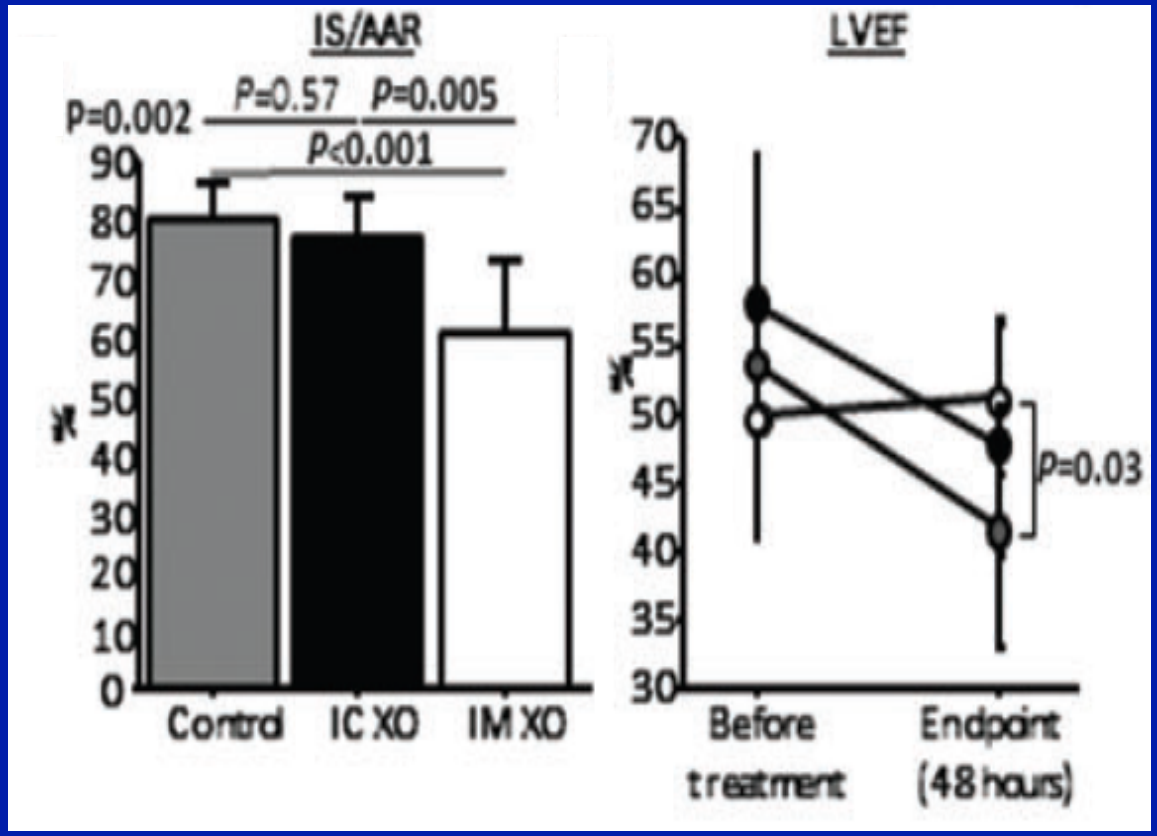
Rat heart morphology two weeks post-infarction: Newt A1- vs. NHDF-exosomes

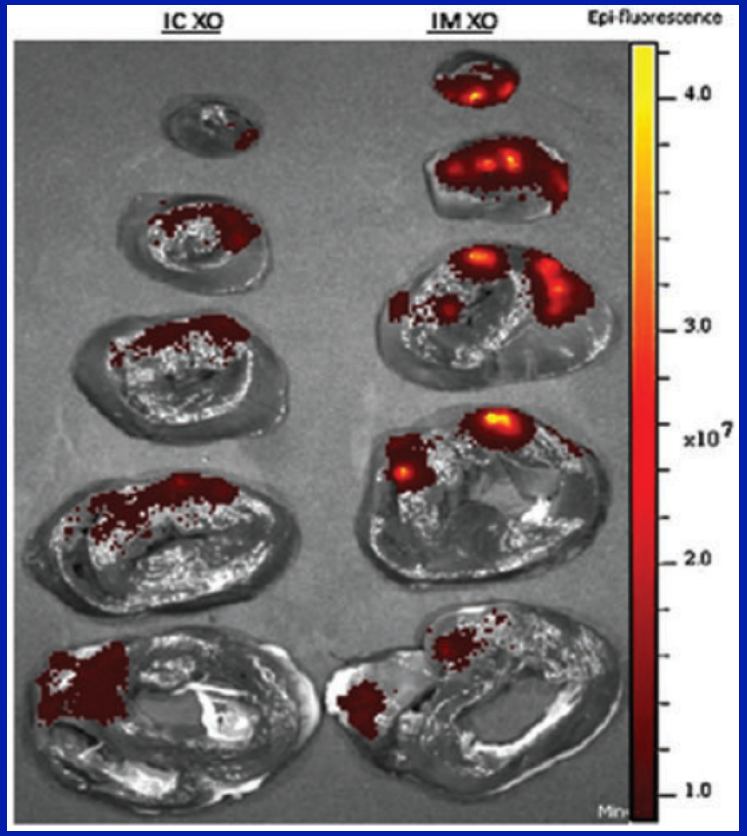






Disadvantage for cardiac applications: exosomes work IM but not IC





R. Gallet et al., Marbán lab, *EHJ* in press 2016

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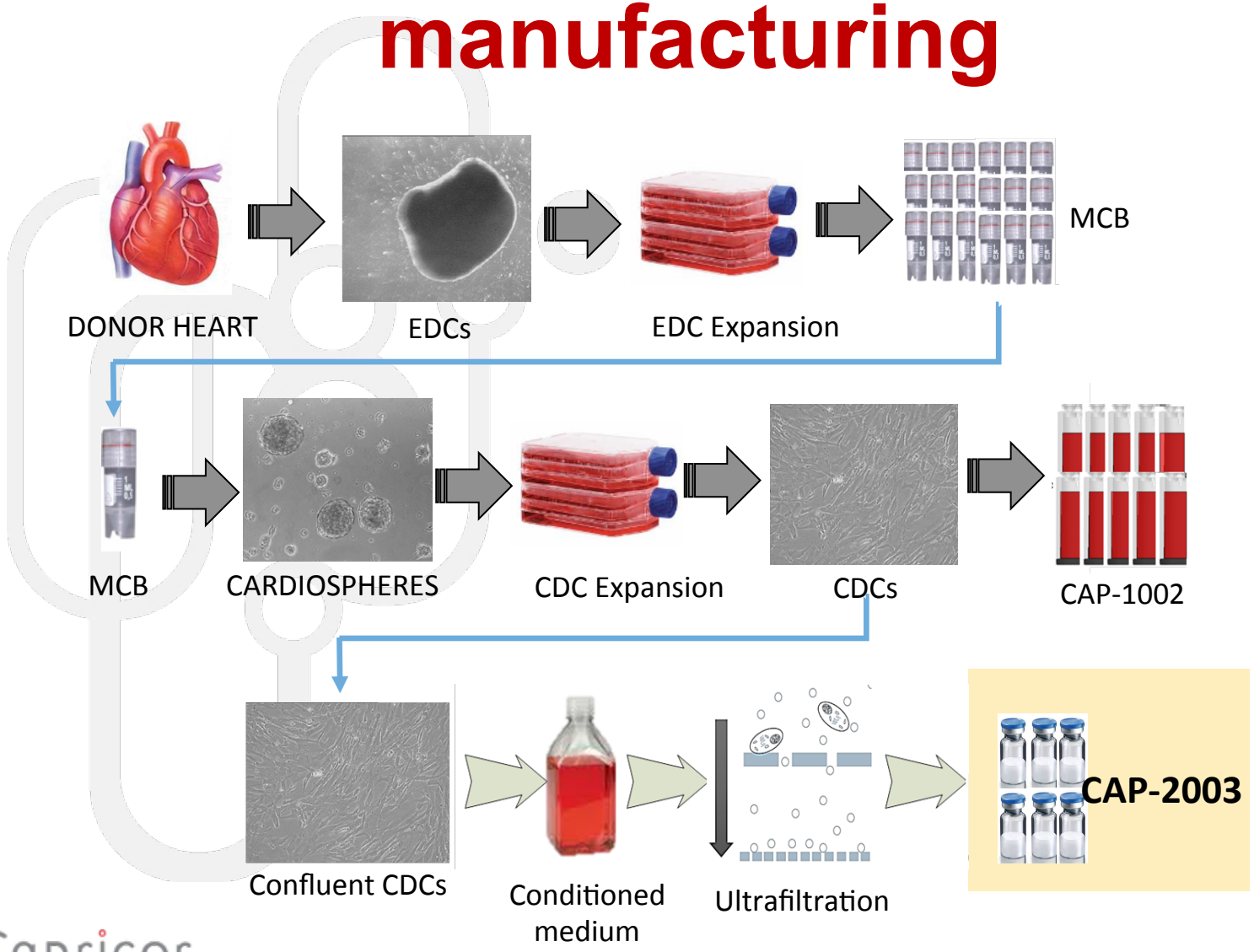
Will therapeutic exosomes ever reach the clinic?

CDC-exosome clinical manufacturing



Capricor Therapeutics™

Ongoing projects

- Mechanistic studies of exosomes and their contents
- Product development of exosomes as next-gen therapeutic candidates
- Focal cardiac applications
- Extracardiac applications (diseases with prominent fibrosis, inflammation)

Acknowledgments

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California Institute for Regenerative Medicine

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in the Advanced
Health Sciences
Pavilion

Properties of CDCs

- CD105+/CD45- cells[1,2] of intrinsic cardiac origin[3]
- Not MSCs, fibroblasts, myofibroblasts, or cardiomyocytes[1]
- Shrink scar and increase viable myocardium[1,2,4-8]
- Functionally superior to other clinically-applied cells[5]
- Multipotent & clonogenic,[4] but long-term engraftment & differentiation not necessary for benefit[6-9]
- c-kit^{+} subpopulation irrelevant[10]

1. RR Smith et al, *Circ* 2007; 2. Makkar et al., *Lancet* 2012; 3. A White et al., *EHJ* 2011; 4. D Davis *PLoS One* 2010; 5. T-S Li et al, *JACC* 2012; 6. I. Chimenti et al, *Circ Res* 2010; 7. K. Malliaras et al, *Circ* 2012; 8. K. Malliaras et al., *EMBO Mol Med* 2013; 9. P Johnston et al, *Circ* 2010; 10. K. Cheng et al., *JAHA* in press

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