

Exosomes from red blood cell units bind to monocytes and induce proinflammatory cytokines, boosting T-cell responses in vitro

blood

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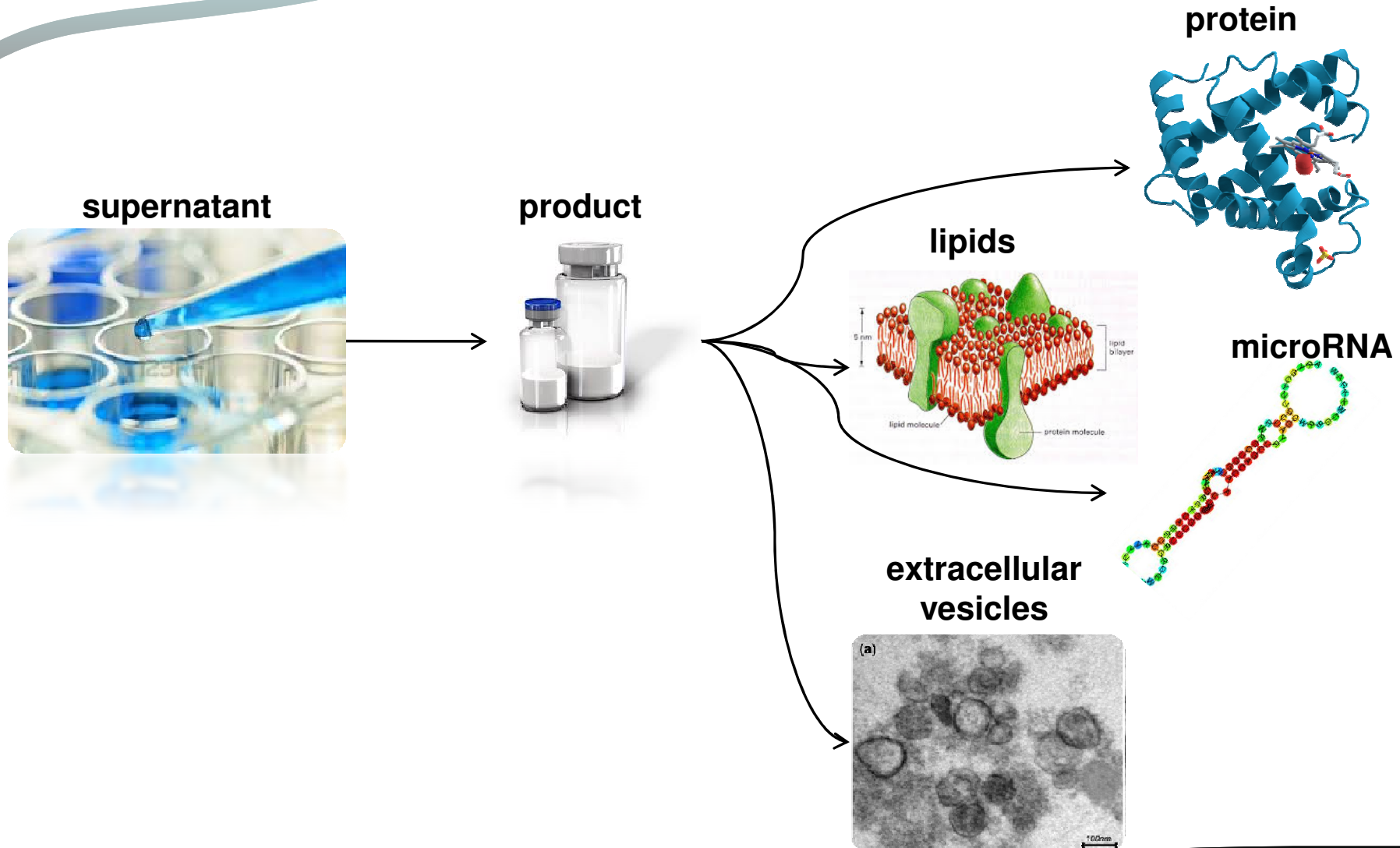
*Ali Danesh, Heather C. Inglis, Rachael P. Jackman, Shiquan Wu,
Xutao Deng, Marcus O. Muench, John W. Heitman, and Philip J.
Norris*

Blood 2014;123:687-696

Outline

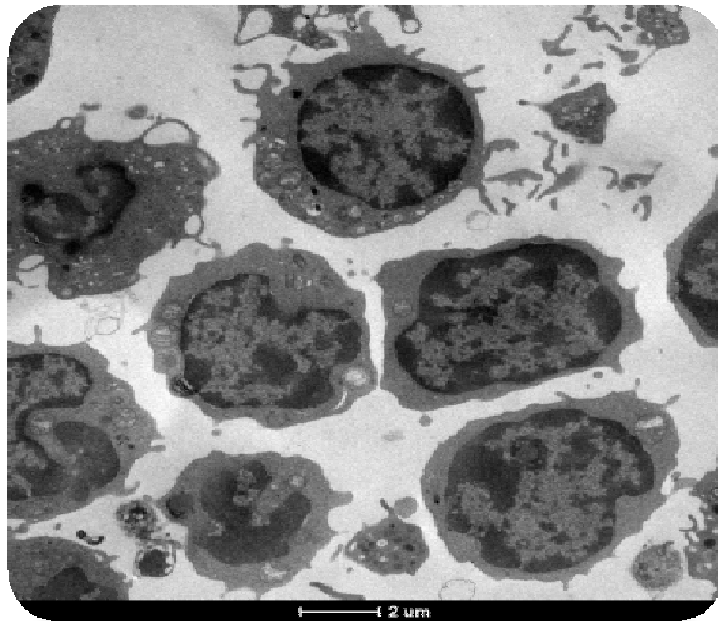
- Introduction
 - Why is this paper important for our group?
 - What are exosomes?
- Special methods used in this study
- Key results
- Discussion

Relevance

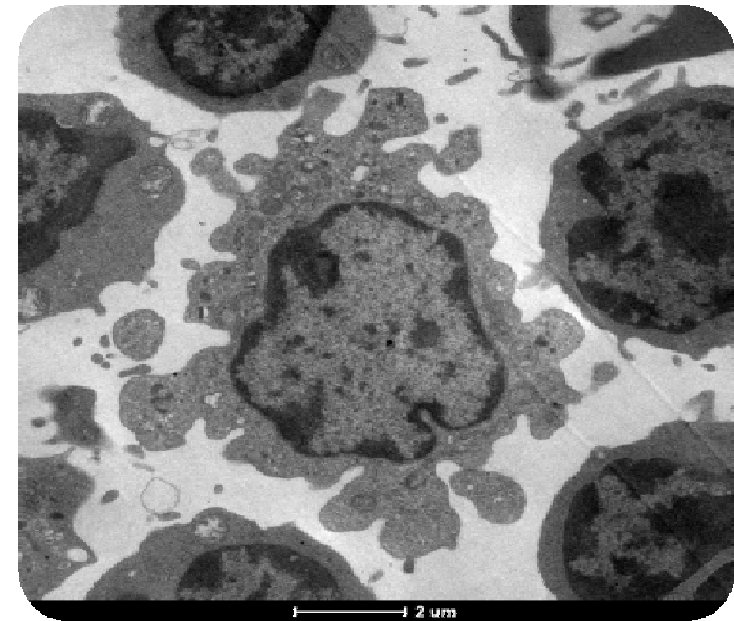


Extracellular Vesicles

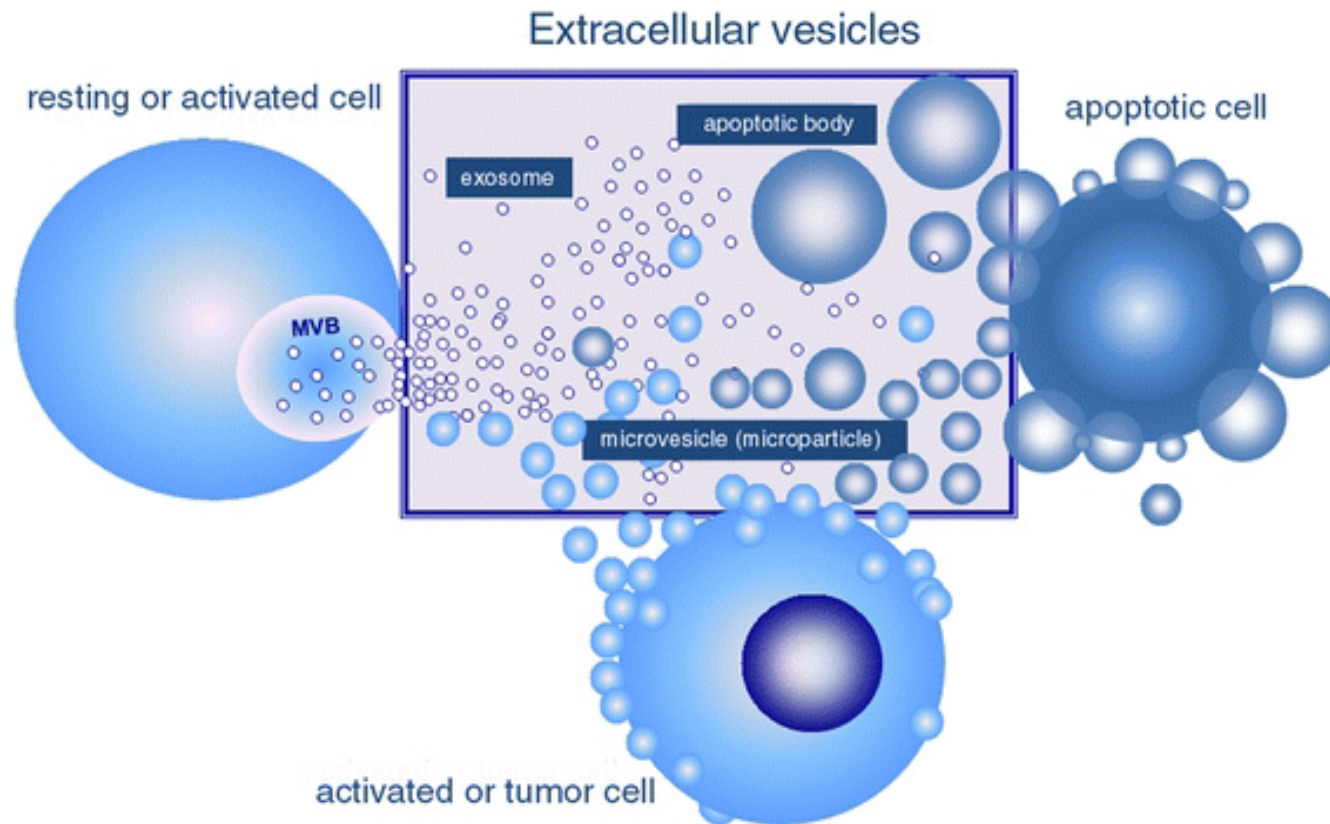
non-radiated PBMC



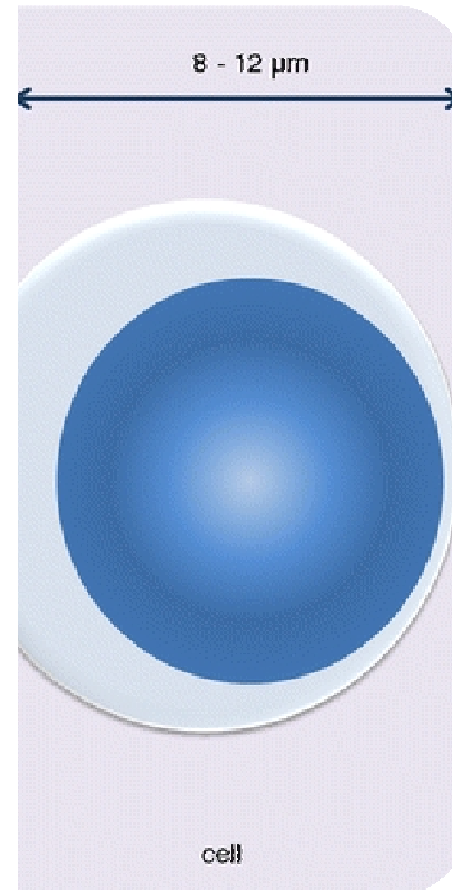
radiated PBMC



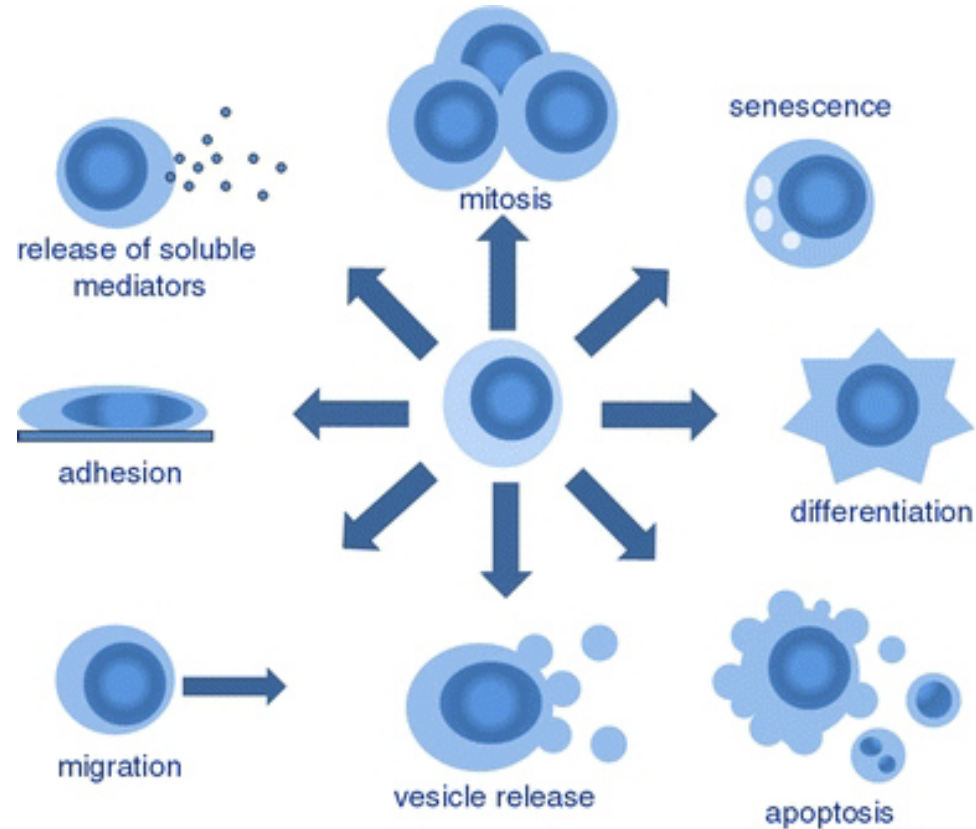
Extracellular Vesicles



Extracellular Vesicles



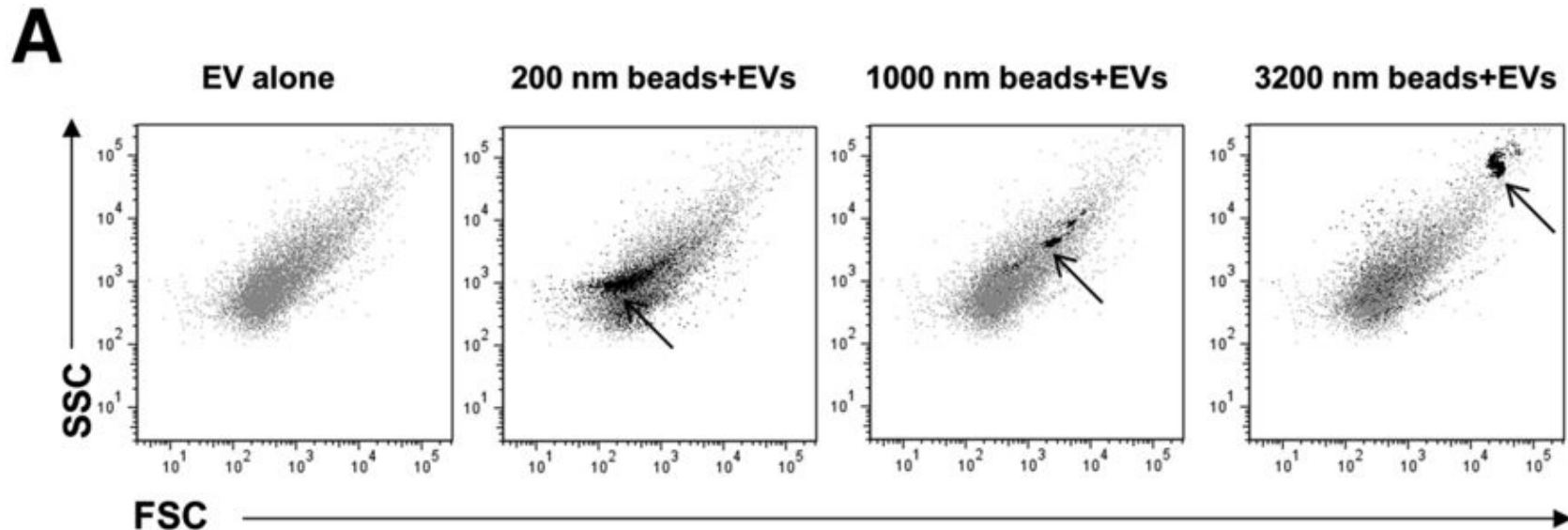
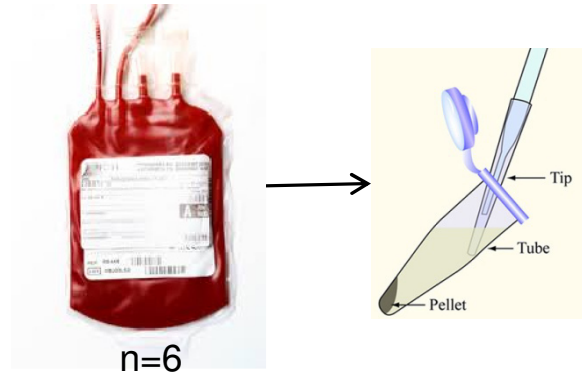
Extracellular Vesicles



Study Aim

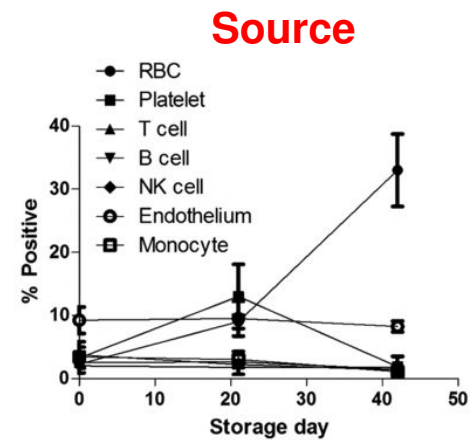
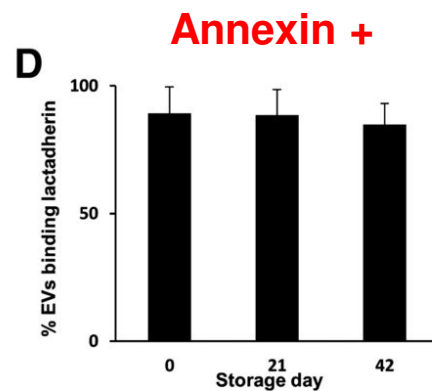
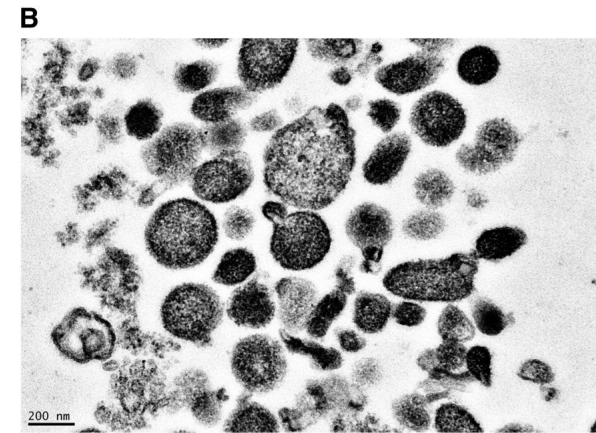
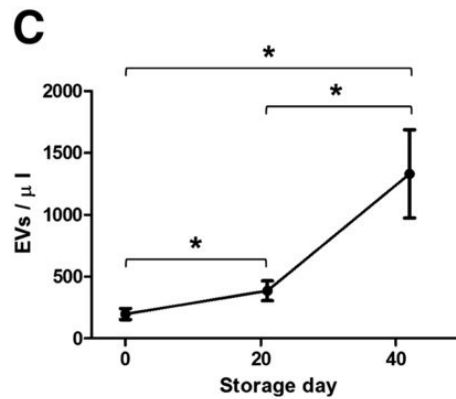
Do red blood cell units contain
extracellular vesicles?

Results

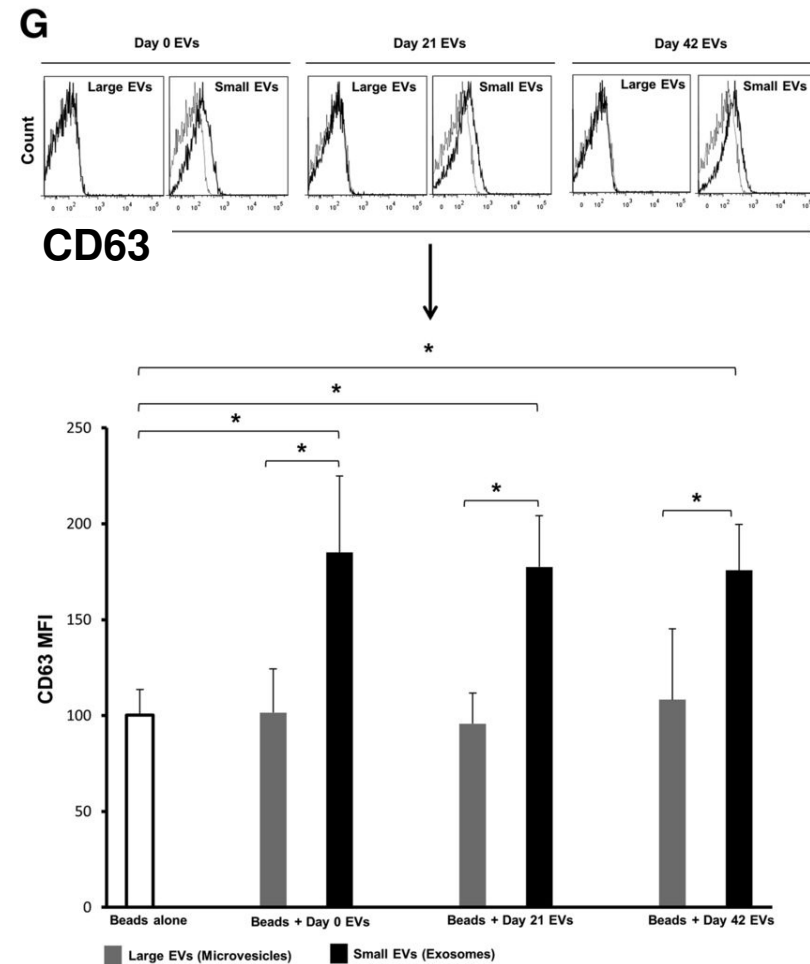


EV relative **size** stored RBC units.

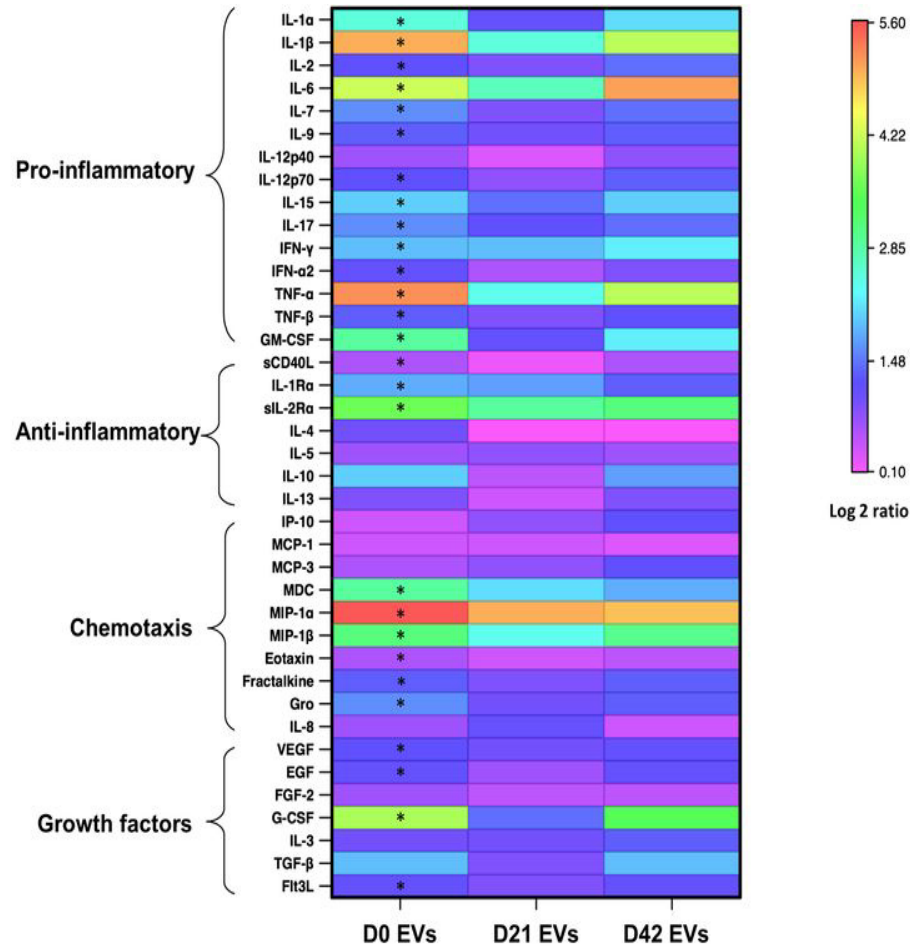
EV absolute count in stored RBC units.



Exosome vs. Microparticle



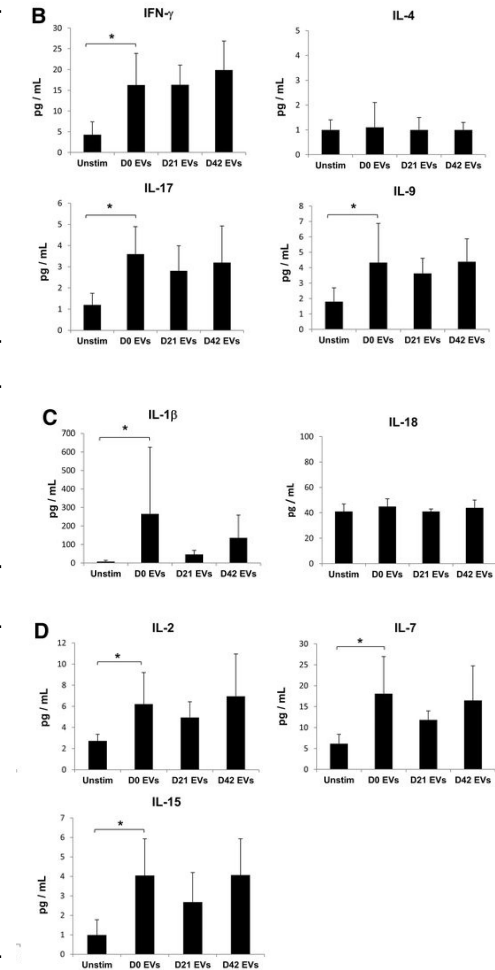
Cytokine and growth factor secretion by PBMCs stimulated with EVs.



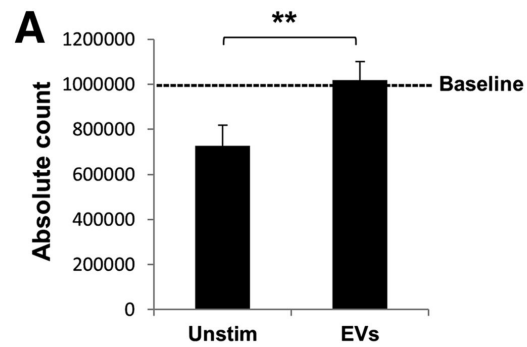
T helper cell subset

NLRP3 Inflammasome

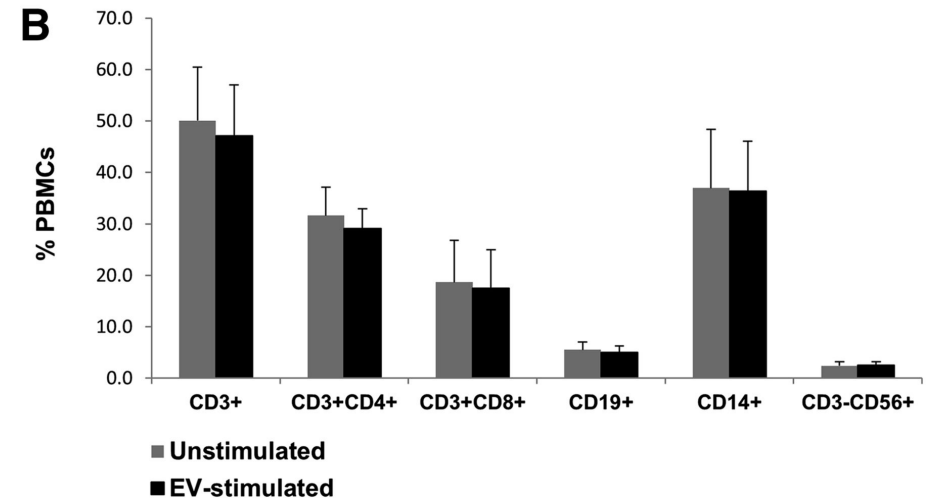
T cell growth factors



PBMC survival

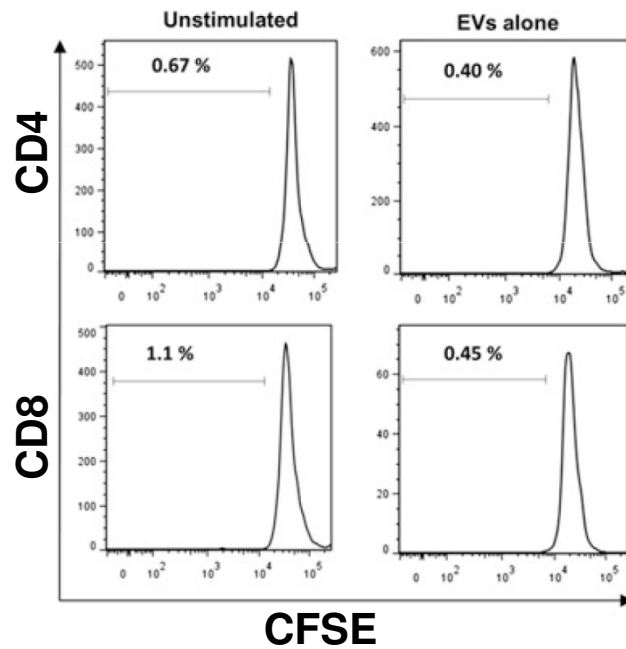


Cell population analysis

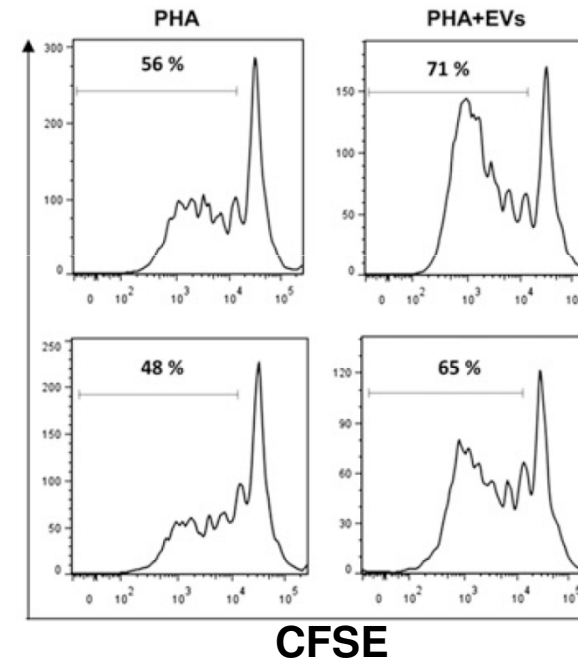


EV-induced immune augmentation of T-cell response

Proliferation: EV

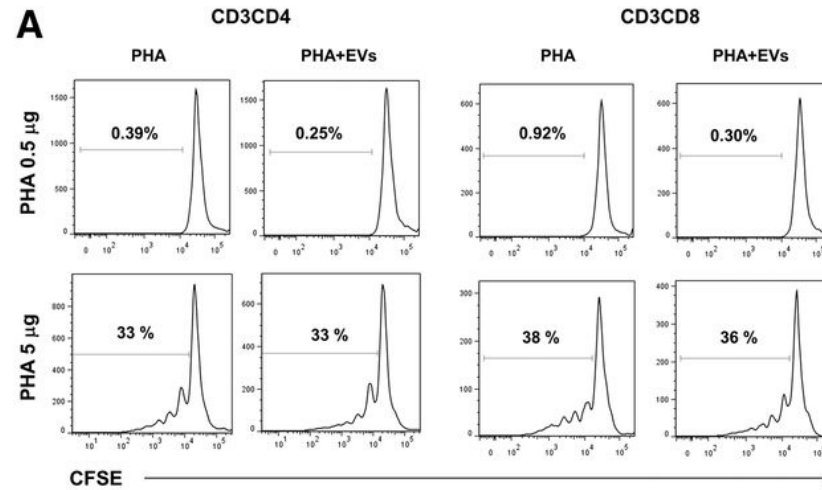


Proliferation PHA+EV

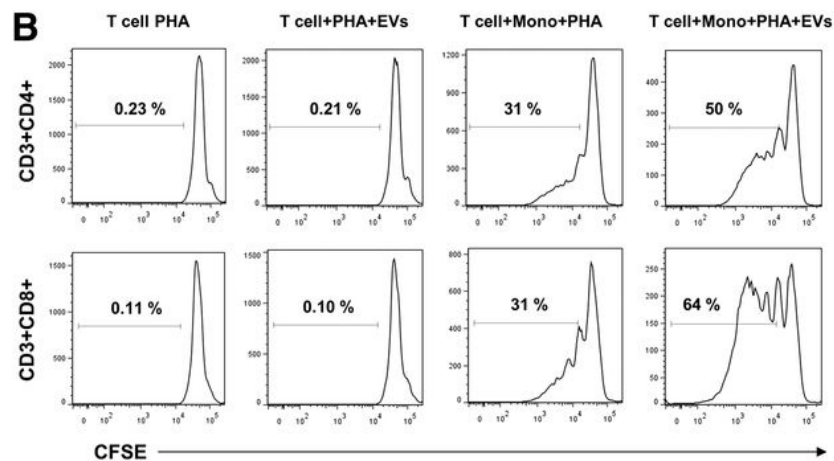


Mechanism of EV-induced immune augmentation.

PHA + EV



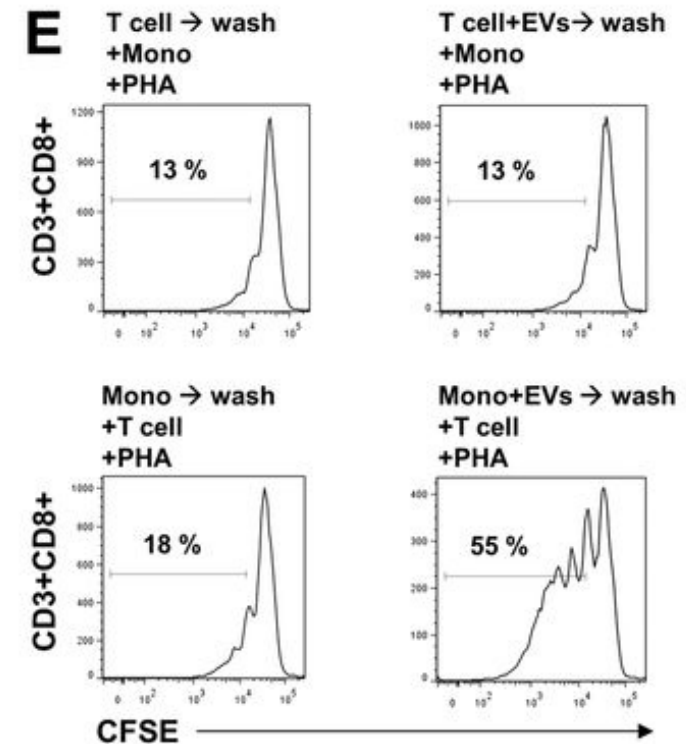
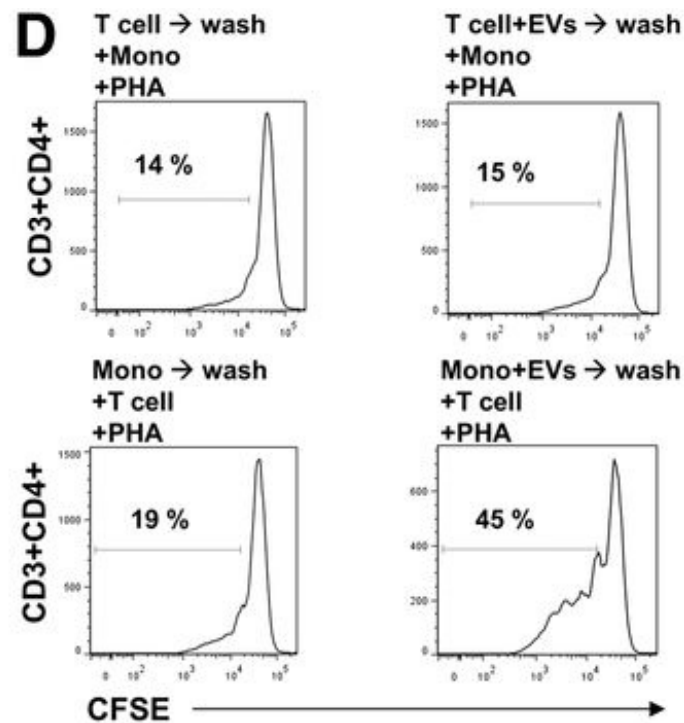
PHA + EV + Monocytes



EV act via monocyte activation

CD4+

CD8+



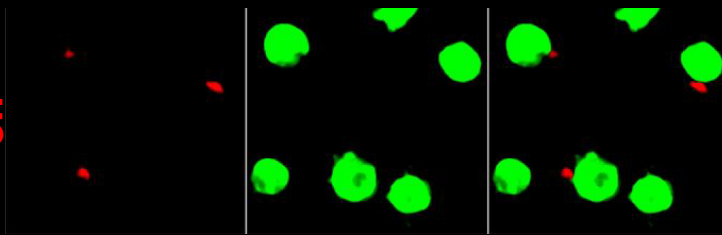
Interaction PBMC

EVs.

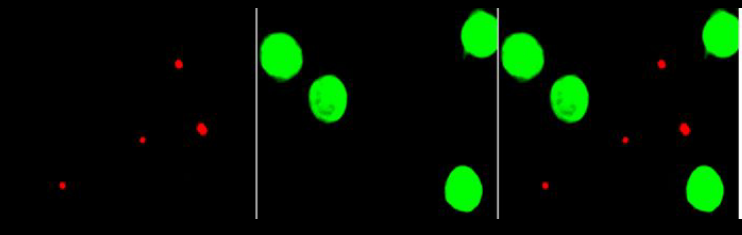
Monocytes

T-cells

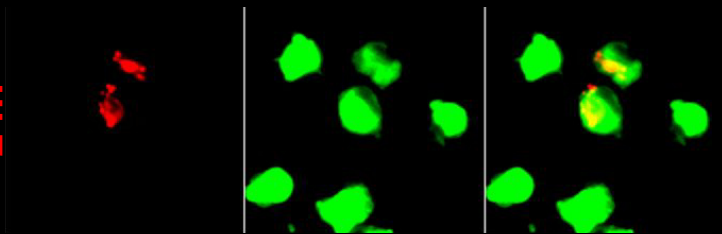
3h



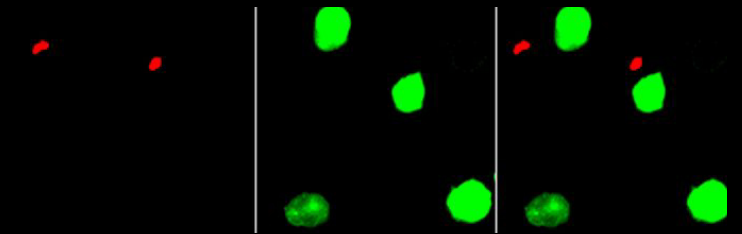
3h



24h

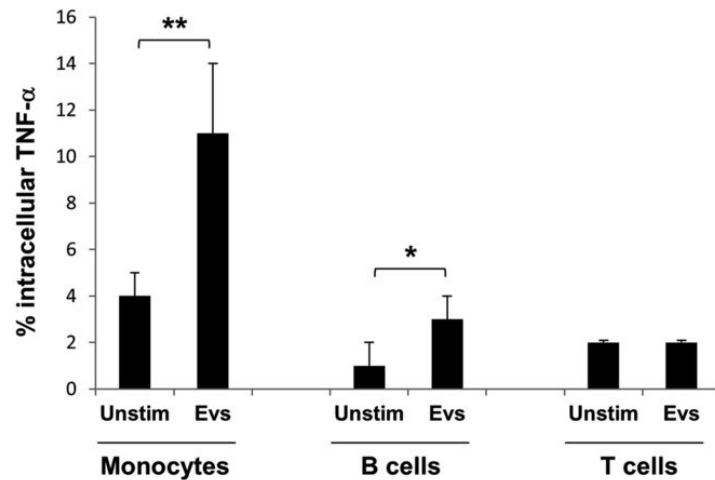


24h

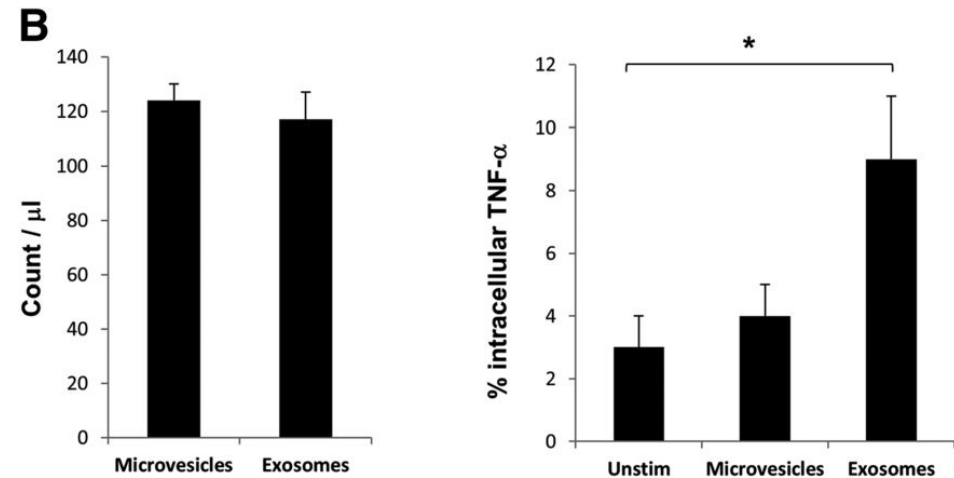


TNF- α production indicates monocytes as primary interacting cells with EVs

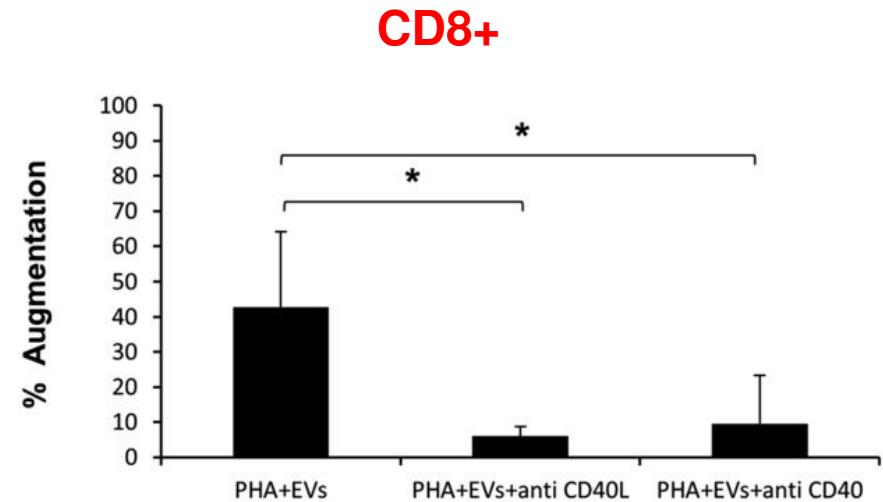
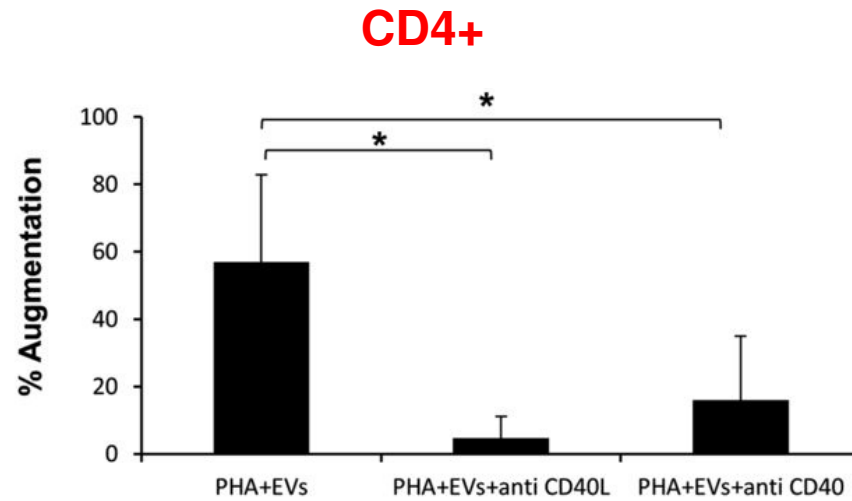
Monocyte TNF- α



Exosomes stimulate TNF- α release

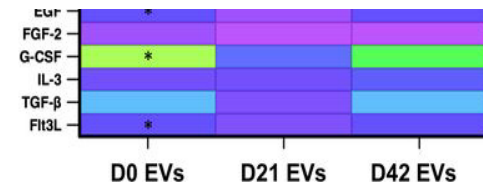
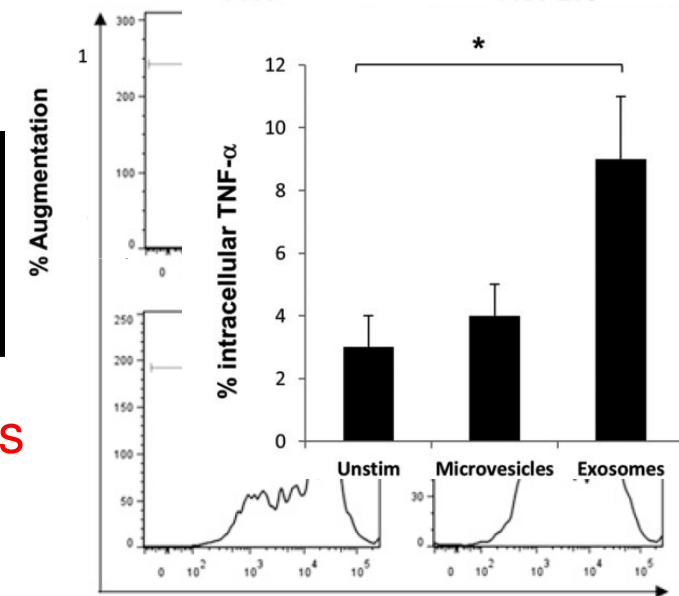
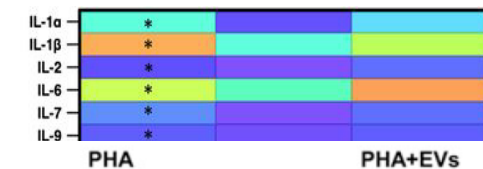


Exosome signaling via CD40 CD40L interaction



Summary

1. Packed red blood cells
 1. contain **extracellular vesicles (EV)**
 2. EV originate mainly from **red blood cells** and **platelets**
 3. Modify **cytokine** secretion of PBMCs
 4. EV effect **PBMC survival**
 5. EV augment **T-cell** proliferation
 6. EV mediate via fusion with **monocytes**
 7. blockade **CD40/CD40L** interaction attenuated EV mediated effects
 8. biological active EV are **<200 nm**



Thank you for
your attention!

Exosome

	Exosomen	Mikrovesikel	Apo-Körper
Größe	30-100 nm	100-1000 nm	1-5 µm
Sekretion	Exozytose	Budding	Blasenbildung durch Apoptose
Isolierung	Vorzentrifugation + Ultracentrifugation mit mind. <u>100,000</u> g	Zentrifugation mit ca. 20,000g	Keine definitive Isolationsmöglichkeit, meist Zellüberstand von apo. Zellen
Detektion	TEM, Western-Blotting FACS, Massenspek.	FACS	FACS
Marker	Annexin 5, CD63...	Integrine, Selektine, CD40L	Histone