

# Research status of extracellular world: Micro-vesicles, Exosomes, and RNPs.

Siqi Wang

2018.04.28

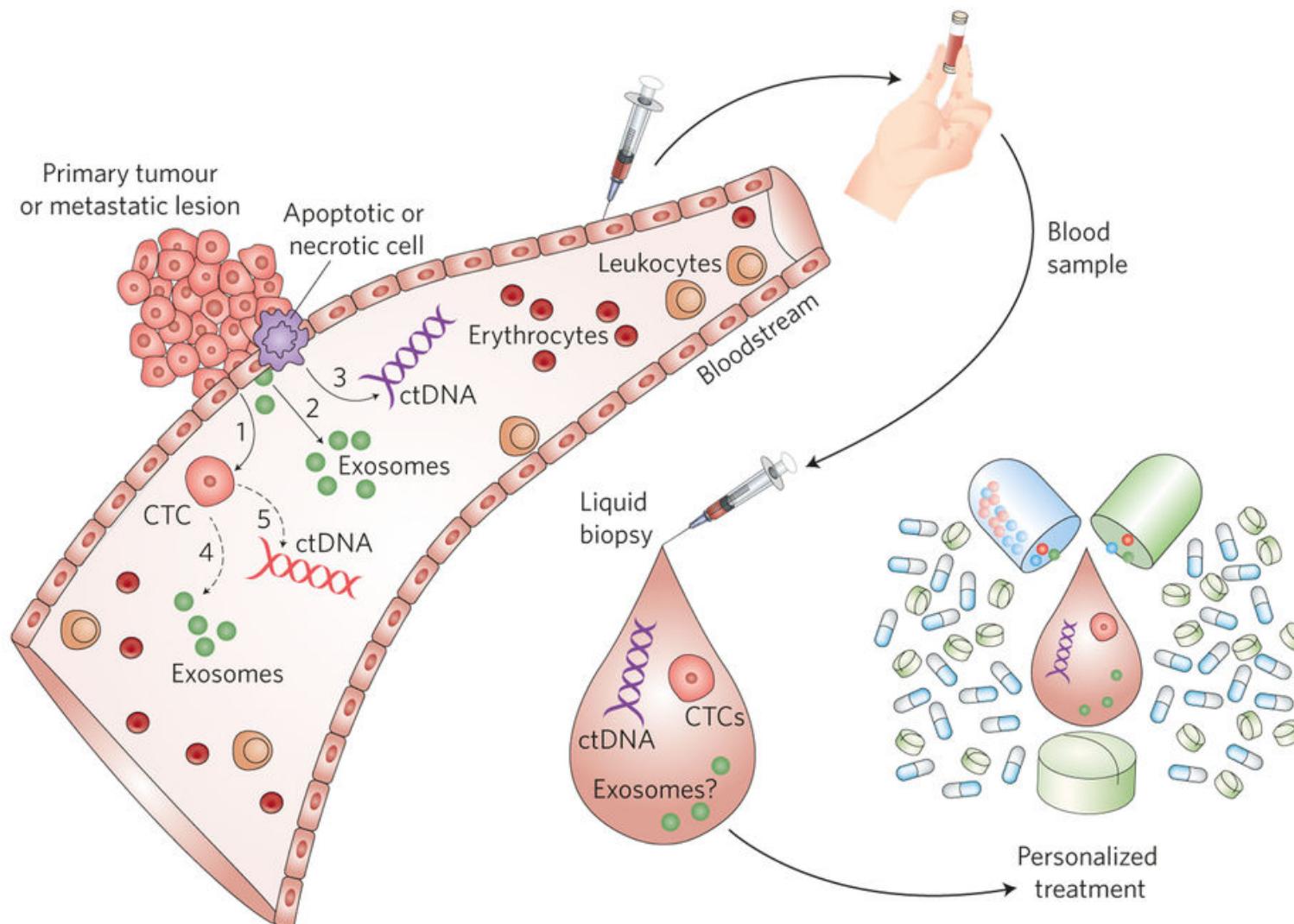
# Outlines

1. Introduction of extracellular components.
  - Microvesicles
  - Exosomes
  - RNPs
2. Paper sharing
  - Review: microvesicles vs exosomes.
  - Methods && comparison of MVs, exosomes, RNPs.
  - Small RNA sorting character into MVs, exosomes, RNPs.
3. Preliminary results of microvesicles, exosomes, RNPs.

# 1. Introduction of extracellular components.

- Microvesicles.
- Exosomes.
- RNPs.

# Liquid Biopsy → Biomarkers in body-fluids

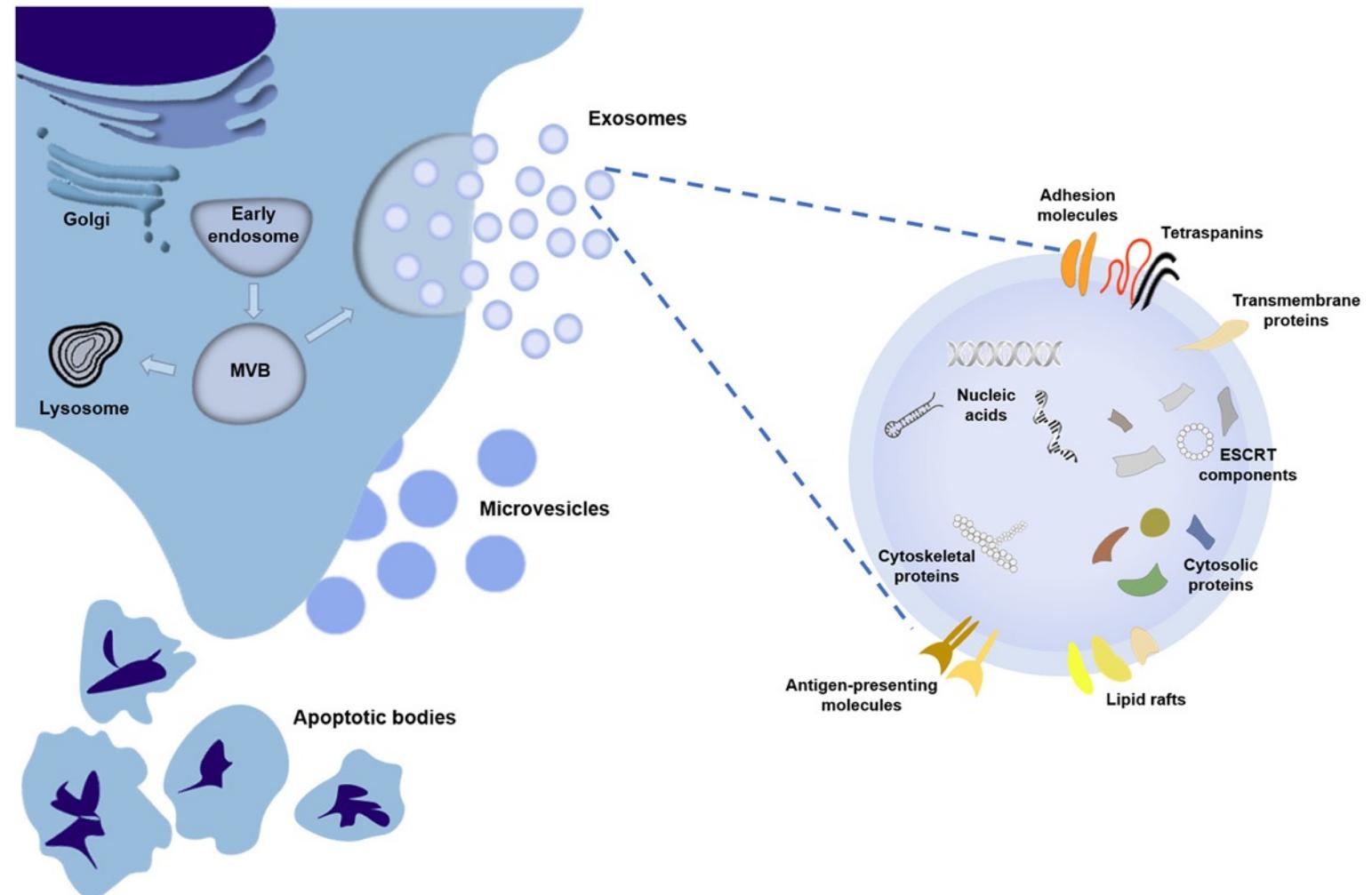


## Biomarkers

- Blood cells
  - Protein
  - CTC
  - ctDNA(methylation)
  - **exRNA**
  - **Exosomes**
- Microvesicles  
➤ RNPs

# Exosomes

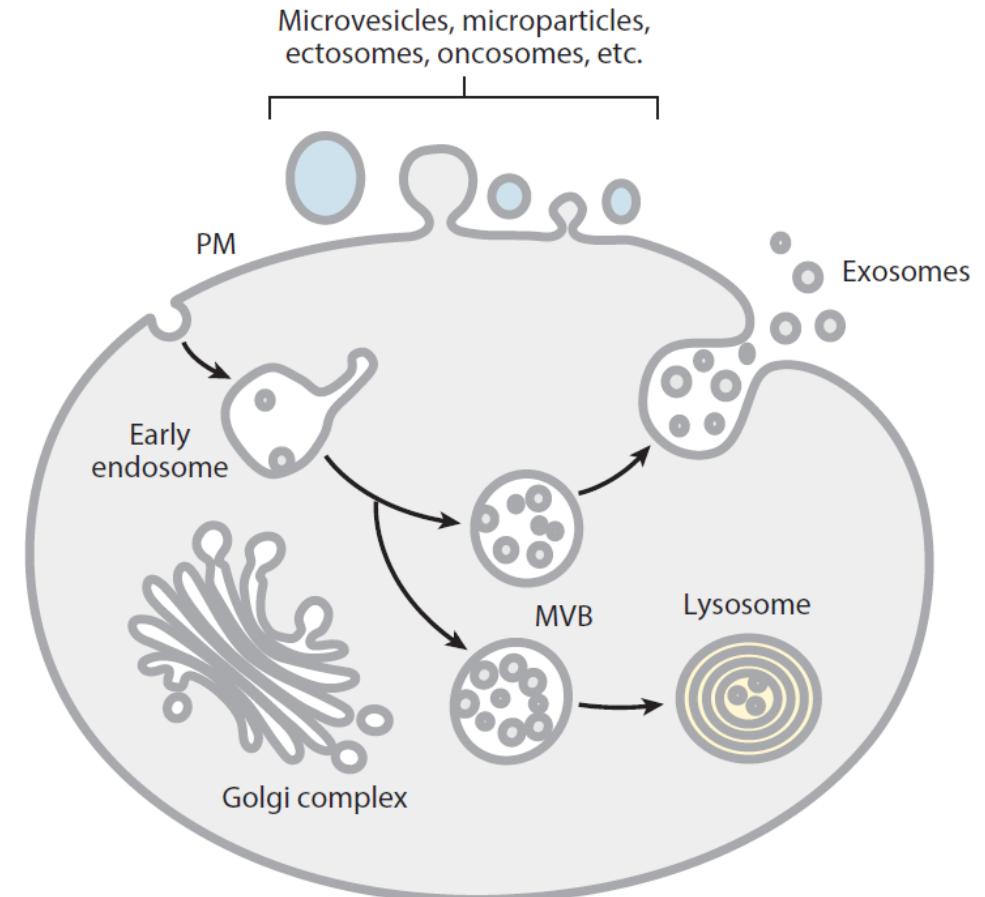
- Secreted by all of cell types.
- Composition:
  - lipid bilayer.
  - Proteins.
  - DNA(chromosomes).
  - **RNA.**



(David R. 2017, *molecular & cellular proteomics*)

# Extracellular components

- **Extracellular Vesicles**
  - **Exosomes:** 30-120 nm
  - **Microvesicles:** 50-1000 nm, more irregular in shape.
  - apoptotic bodies, oncosomes...
- **Ribonucleoproteins(RNPs)**
  - Complex of ribonucleic acid and RNA-binding protein.



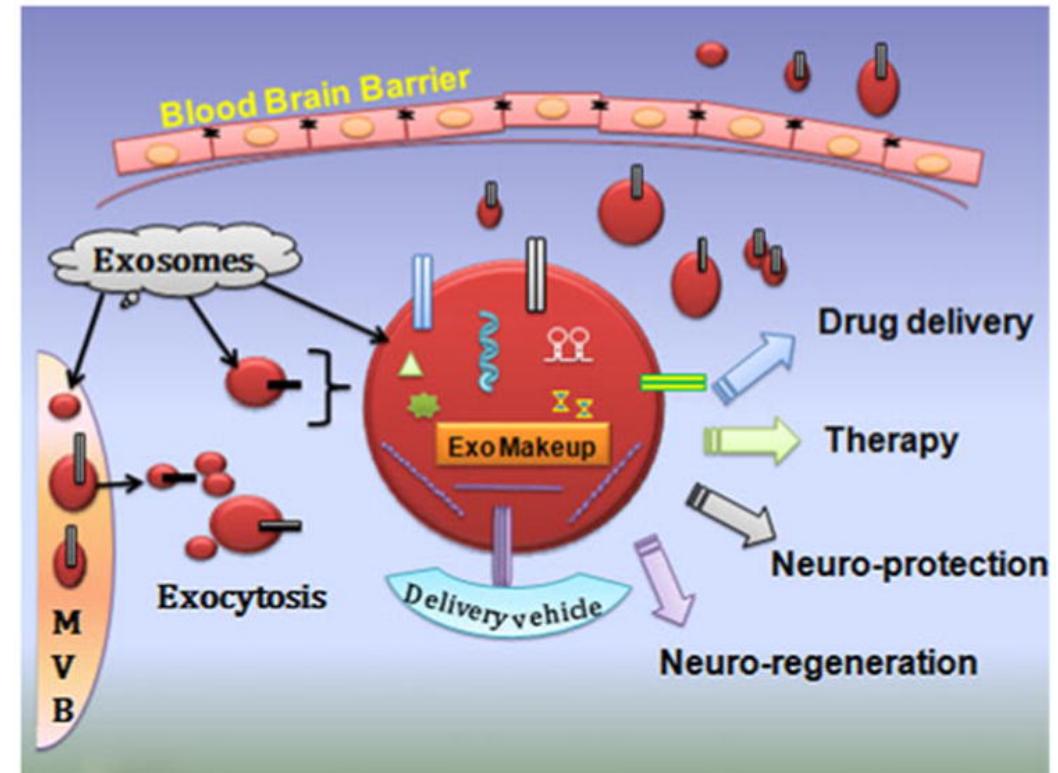
# RNPs

- **RNPs**
  - complex of ribonucleic acid and RNA-binding protein.
  - Biological functions: DNA replication, regulating gene expression, regulating RNA metabolism.
  - RNPs: ribosome, argonaute 2(ago2), heterogeneous nuclear RNP(hnRNP).
  - Currently, over 2000 RNPs can be found in the RCSB Protein Data Bank (PDB) .
- **exRNPs**
  - **~90%** of circulating miRNAs is reportedly present in ribonucleoprotein complexes.
  - Currently research: RNA bound to ago2, lipoprotein(HDL, LDL).

(Arroyo JD. 2011, *PNAS*)  
(Turchinovich A. 2011, *NAR*)  
(Wang K. 2010, *NAR*)

# Functions of Microvesicles and Exosomes

- **Protein/ DNA/ RNA cargo.**
- **Cell communication.**
- Activate signaling pathways in recipient cells.
- Activate immune response.
- Delivery siRNA/drugs.
- Facilitating tumorigenesis by regulating angiogenesis, immunity, and metastasis.
- on-going...



(Mercedes T. 2016, *Cell*)  
(Raghu Kalluri. 2016, *J Clin Invest*)

## 2. Paper sharing

- Review: microvesicles vs exosomes.
- MVs, exosomes, RNPs.
- Small RNA sorting character into MVs, exosomes, RNPs.

# Microvesicles vs Exosomes

- “Exosomes is generally refer to a **mixed population** of small EVs without demonstration of their intracellular origin.”  
– 2016 *cell.*
- “Current methodologies obtained exosomes may **not strictly discriminate** from microvesicles.”  
– 2016 *J Clin Invet.*

# Paper-1: microvesicles vs exosomes.

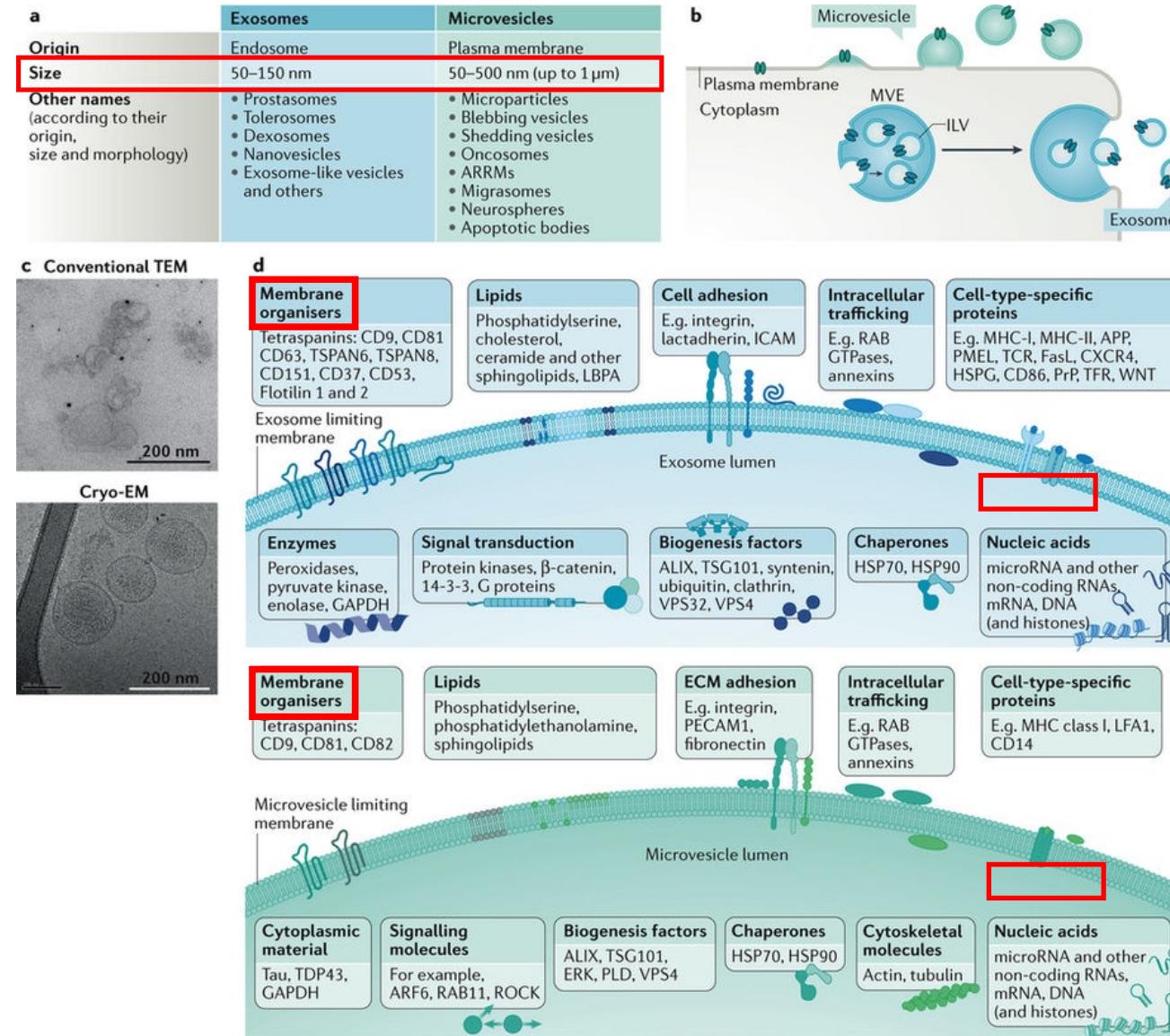
NATURE REVIEWS | MOLECULAR CELL BIOLOGY

## Shedding light on the cell biology of extracellular vesicles

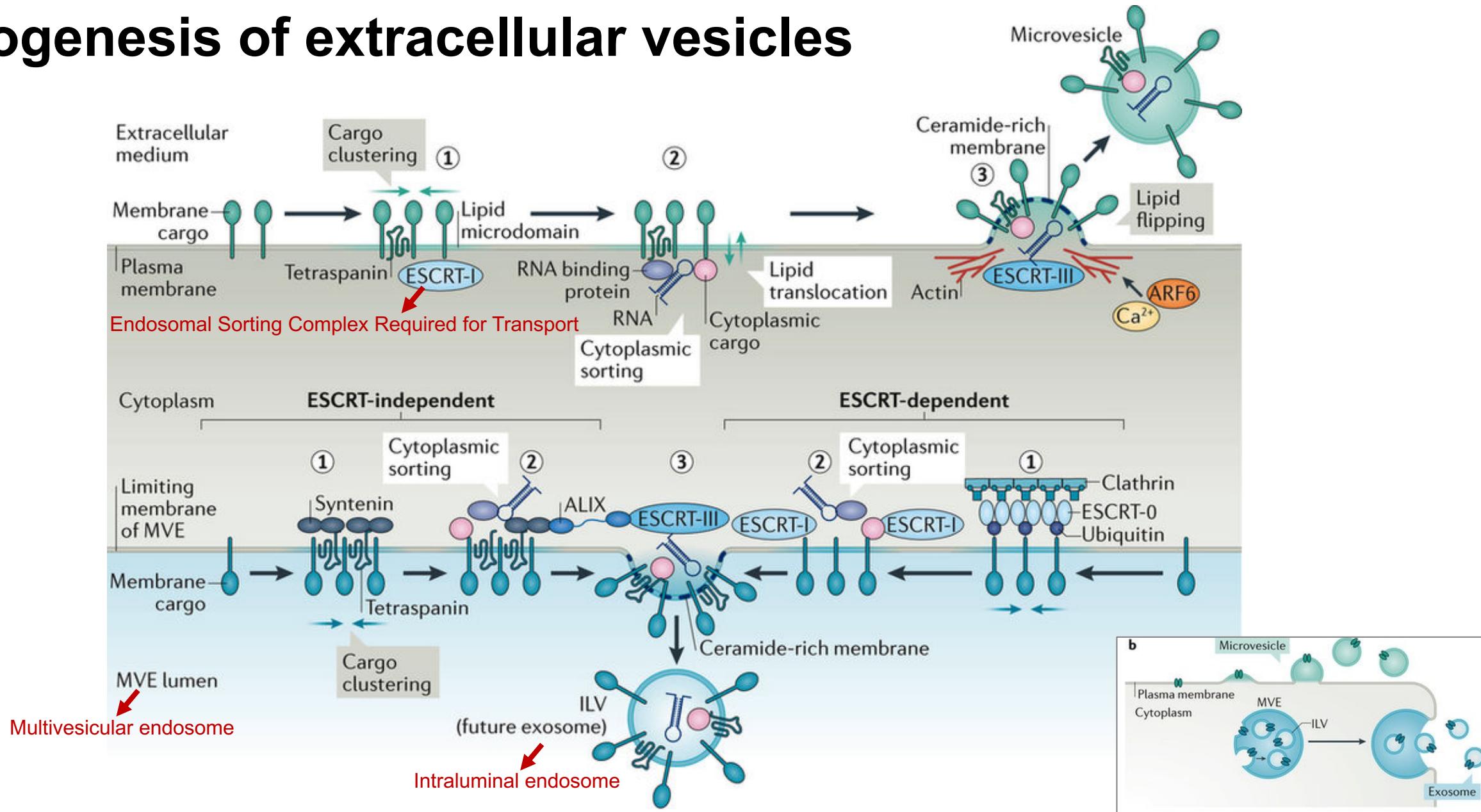
*Guillaume van Niel<sup>1</sup>, Gisela D'Angelo<sup>2</sup> and Graça Raposo<sup>2</sup>*

**Abstract** | Extracellular vesicles are a heterogeneous group of cell-derived membranous structures comprising exosomes and microvesicles, which originate from the endosomal system or which are shed from the plasma membrane, respectively. They are present in biological fluids and are involved in multiple physiological and pathological processes. Extracellular vesicles are now considered as an additional mechanism for intercellular communication, allowing cells to exchange proteins, lipids and genetic material. Knowledge of the cellular processes that govern extracellular vesicle biology is essential to shed light on the physiological and pathological functions of these vesicles as well as on clinical applications involving their use and/or analysis. However, in this expanding field, much remains unknown regarding the origin, biogenesis, secretion, targeting and fate of these vesicles.

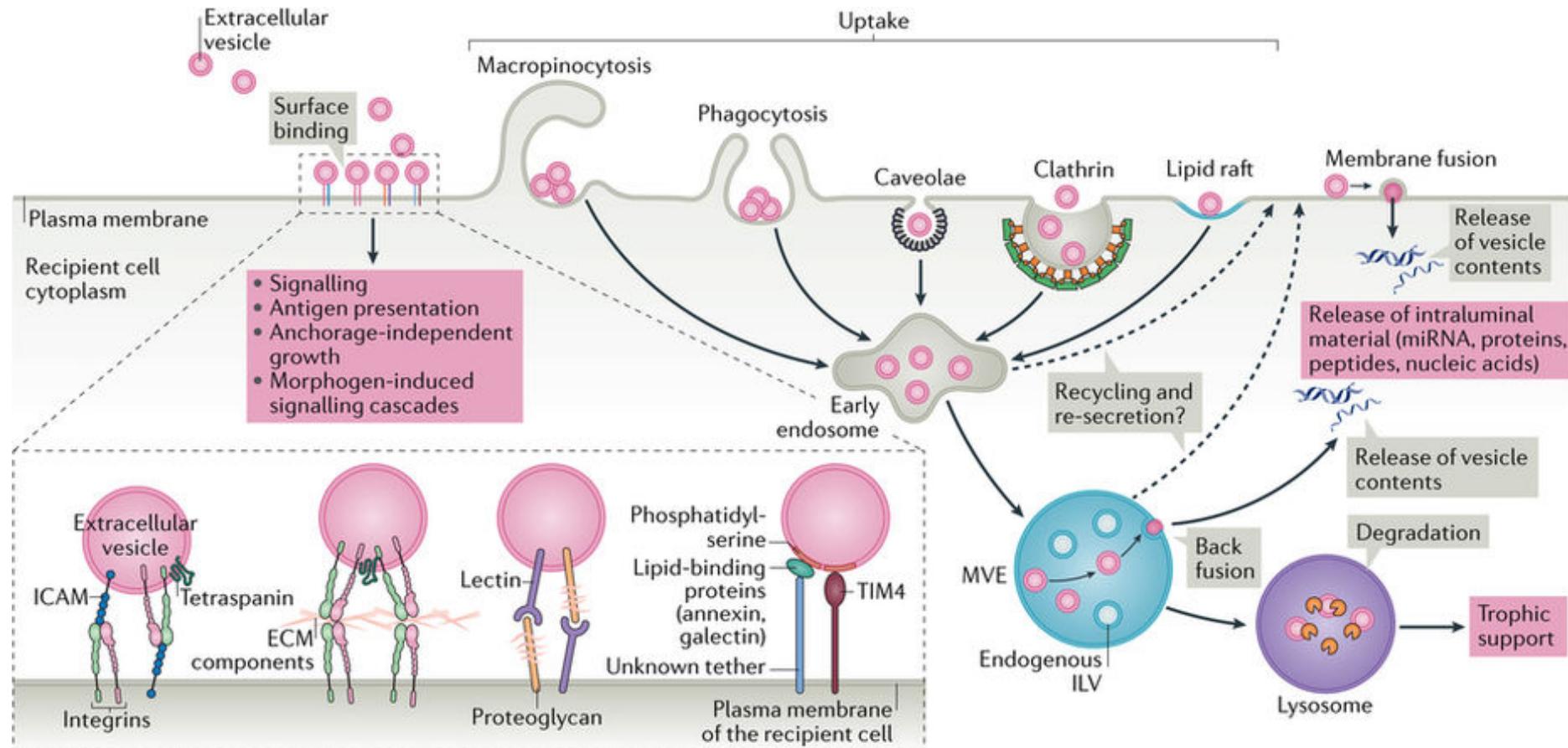
# Main features of extracellular vesicles



# Biogenesis of extracellular vesicles



# Fate of extracellular vesicles in recipient cells



# Paper-2: MVs, exosomes, RNPs.



ARTICLE

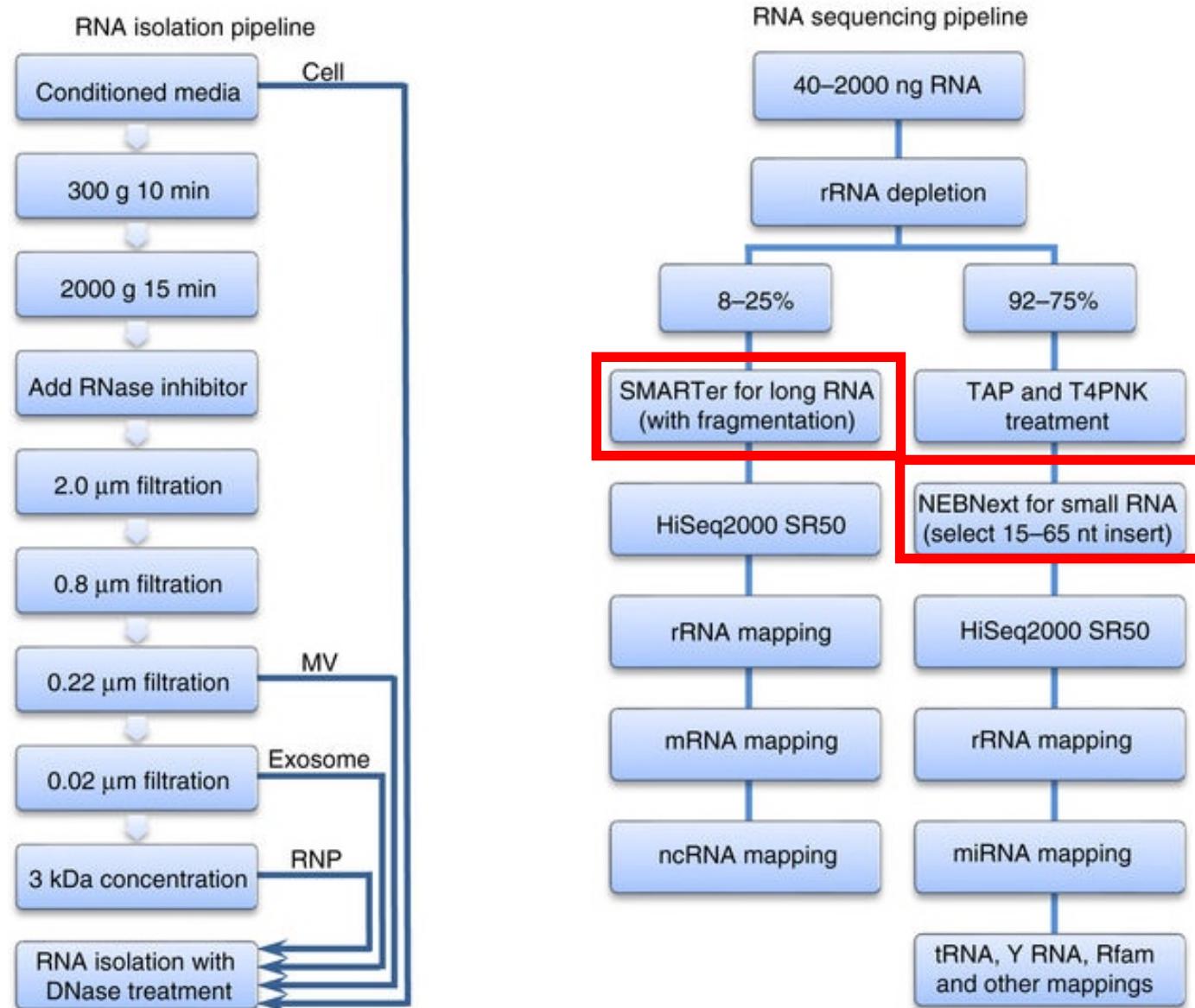
DOI: 10.1038/s41467-017-01196-x

OPEN

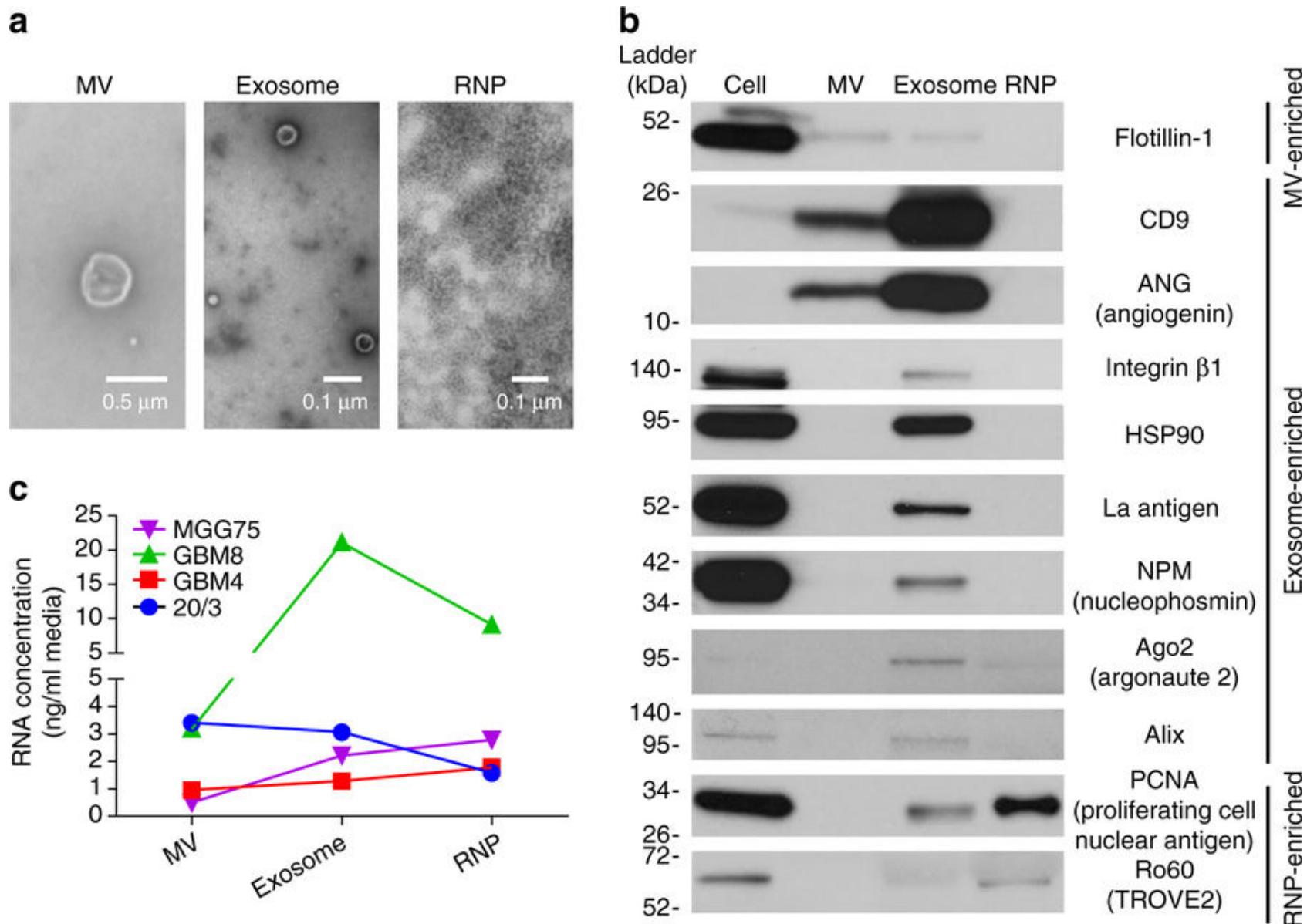
## Coding and noncoding landscape of extracellular RNA released by human glioma stem cells

Zhiyun Wei<sup>1</sup>, Arsen O. Batagov<sup>2</sup>, Sergio Schinelli<sup>3</sup>, Jintu Wang<sup>4</sup>, Yang Wang<sup>1</sup>, Rachid El Fatimy<sup>1</sup>, Rosalia Rabinovsky<sup>1</sup>, Leonora Balaj<sup>5</sup>, Clark C. Chen<sup>6</sup>, Fred Hochberg<sup>7,8</sup>, Bob Carter<sup>7</sup>, Xandra O. Breakefield<sup>5</sup> & Anna M. Krichevsky<sup>1</sup>

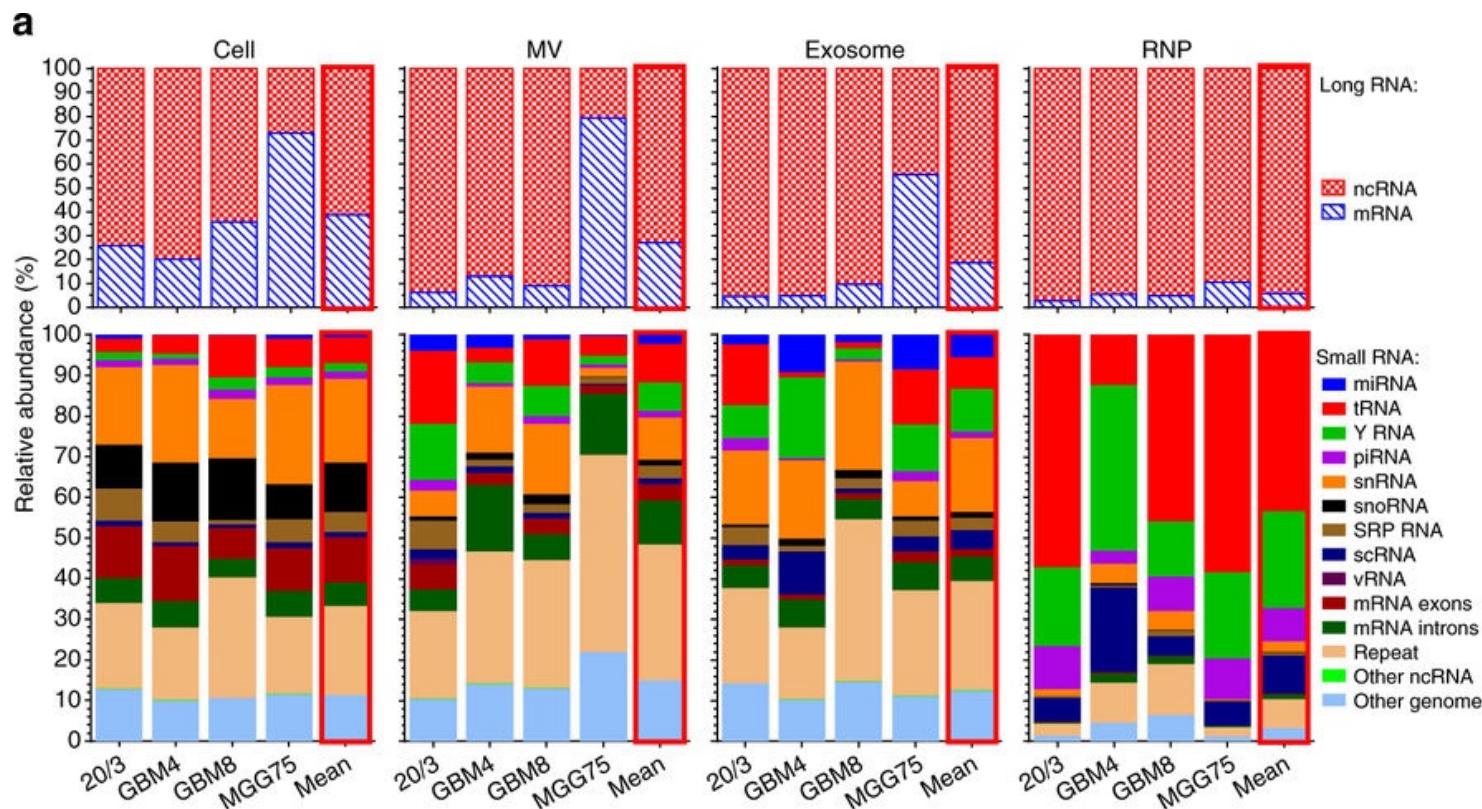
# Flowchart of the exRNA fractionation and sequencing



# Quality control of the fraction separation

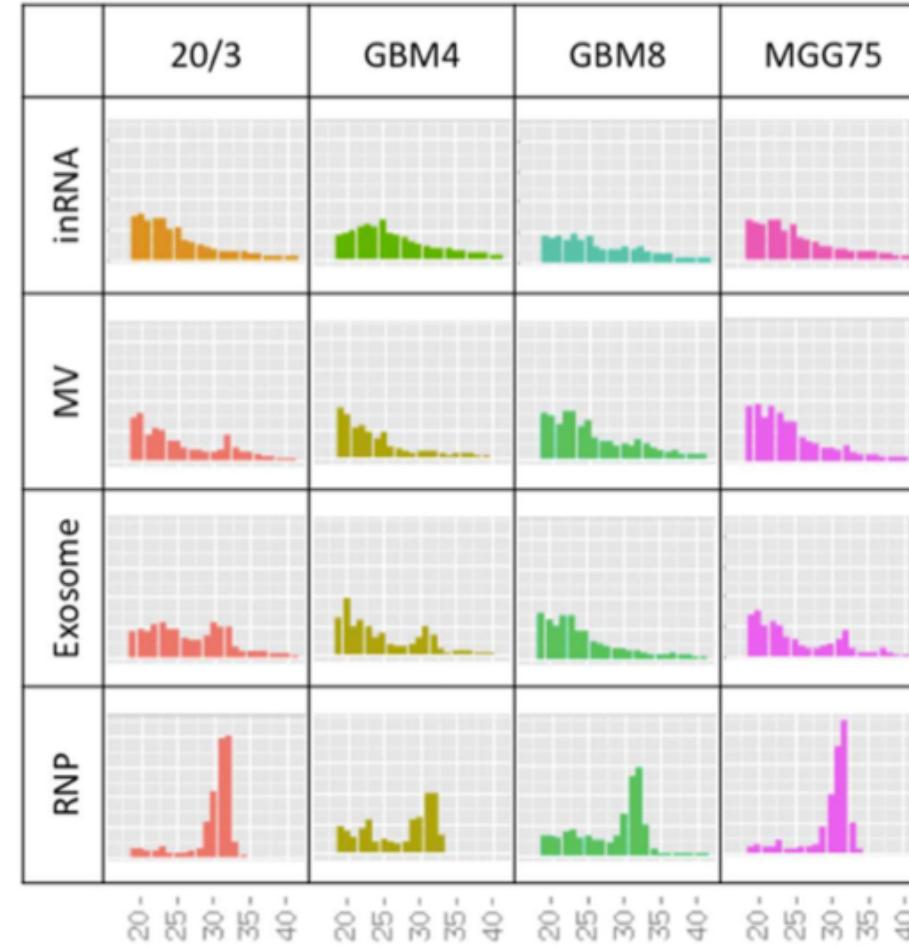


# RNA composition



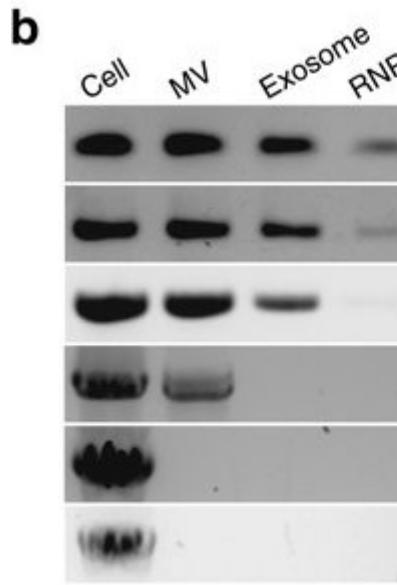
- Different extracellular fractions exhibited distinct characteristics of RNA repertoires.
- miRNA enriched in exosomes, but not in MVs or RNPs.
- RNPs are highly enriched in Y RNA, tRNA, scRNA fragments.
- “tRNA and Y RNA are commonly misidentified due to the poor quality of annotation.”

# Reads' length distribution

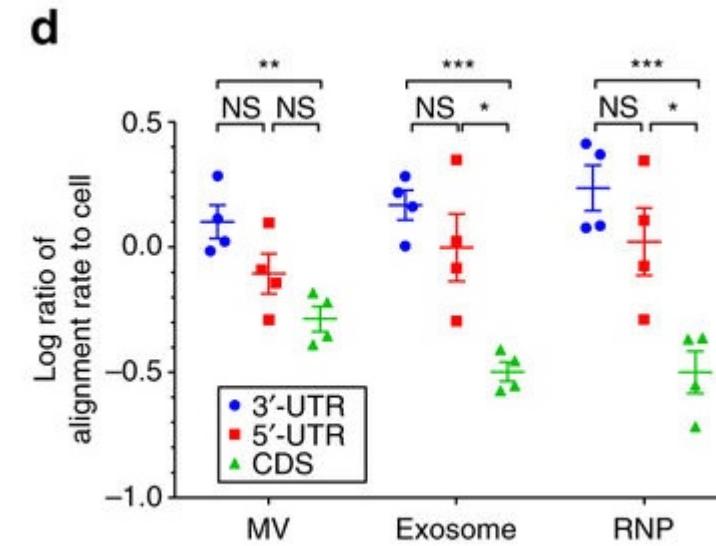


- tRNA and Y RNA fragments are predominant in the RNPs, producing the sharp ~32nt peak.

# Is full-length mRNAs exist in extracellular?



mRNA	Full length	Amplicon length
RPS27	361 nt	306 nt
RPLP2	511 nt	451 nt
RPS6	829 nt	724 nt
EEF2	3163 nt	2996 nt
YY1AP1	2705 nt	2509 nt
PROM1	3857 nt	3708 nt



- RT-qPCR analysis of selected mRNAs abundant in exRNA.
- Long mRNAs are excluded in exosomes and RNPs.
- 3' UTRs were enriched in extracellular fractions.

# Inequality and heterogeneity of the RNA repertoire

evenness factor ( $\epsilon$ ):  
 $\epsilon\%$  of RNA species =  
 $(100-\epsilon)\%$  of total abundance

**a**

Evenness factors of RNA species abundance distribution

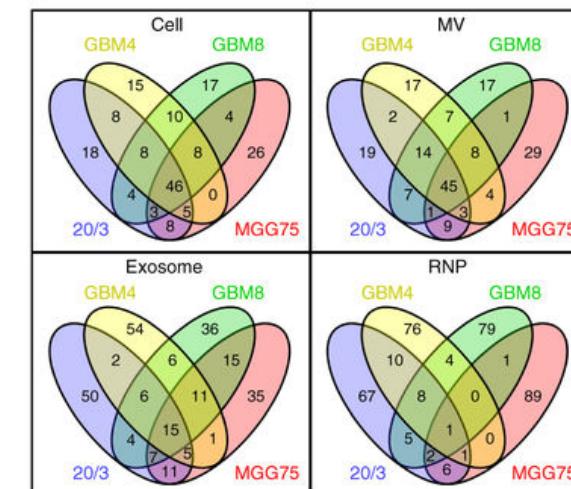
RNA category	Cell	MV	Exosome	RNP
All long RNA	6.37±1.49	4.71±1.53	4.67±1.04	4.92±0.47
--mRNA	11.70±0.89	12.12±0.36	14.27±3.85	16.30±2.45
--ncRNA	2.59±0.72	4.26±0.89	2.89±1.76	3.81±0.40
All small RNA	3.94±0.26	3.12±0.10*	2.26±0.19**	0.91±0.15***
--miRNA	6.09±0.34	5.27±0.12	5.39±0.35	4.76±0.30*
--snoRNA	16.62±1.79	17.98±0.72	16.24±1.27	9.41±2.57
--snRNA	11.63±0.57	8.10±0.69**	6.98±0.58**	5.99±0.99**
--SRP RNA	17.81±0.79	15.57±0.45*	12.32±1.05**	14.19±1.43
--tRNA	19.20±0.45	17.41±0.45*	11.61±0.89***	8.48±0.45***
--vRNA	31.25±6.25	31.25±6.25	25.00±0.00	18.75±6.25
--Y RNA	11.27±0.98	8.33±0.56*	6.54±0.27**	5.88±0.38**

**b**

Fold change of  $\chi^2$  value to estimate heterogeneity difference

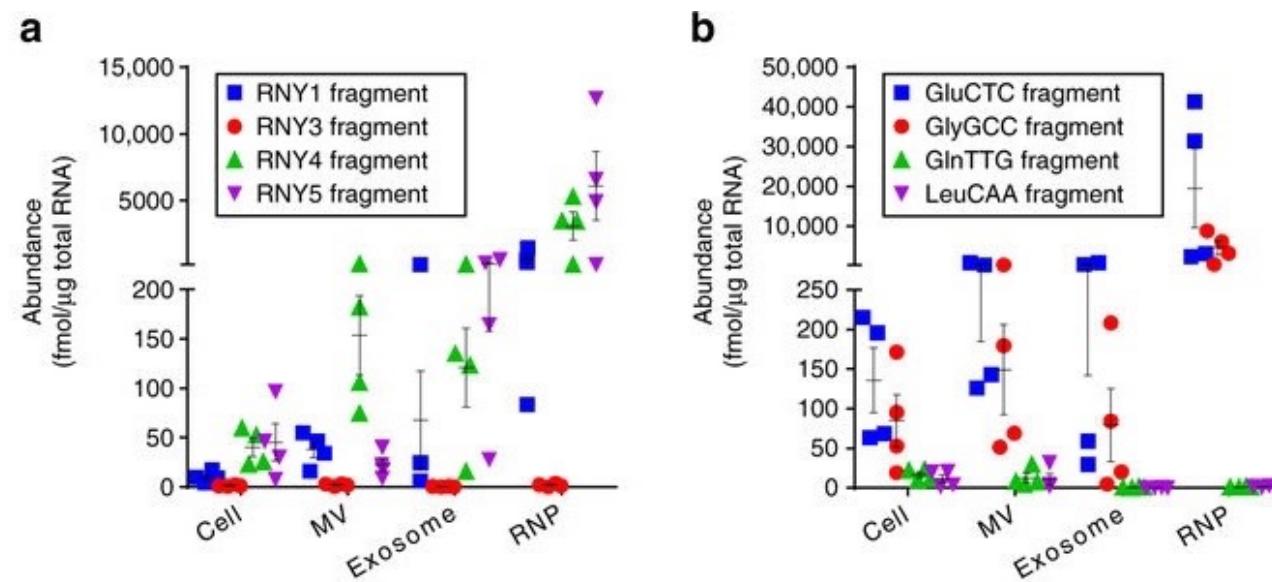
RNA category	Cell	MV	Exosome	RNP
All long RNA	1	1.340	1.720	1.660
--mRNA	1	0.967	2.746	2.905
--ncRNA	1	1.792	2.137	1.998
All small RNA	1	1.868	2.651	1.624
--miRNA	1	1.045	0.838	1.001
--snoRNA	1	2.034	1.762	2.196
--snRNA	1	2.060	1.995	4.266
--SRP RNA	1	1.073	3.520	3.069
--tRNA	1	0.423	0.916	1.889
--vRNA	1	0.692	0.068	0.958
--Y RNA	1	0.401	1.030	1.002

**c**

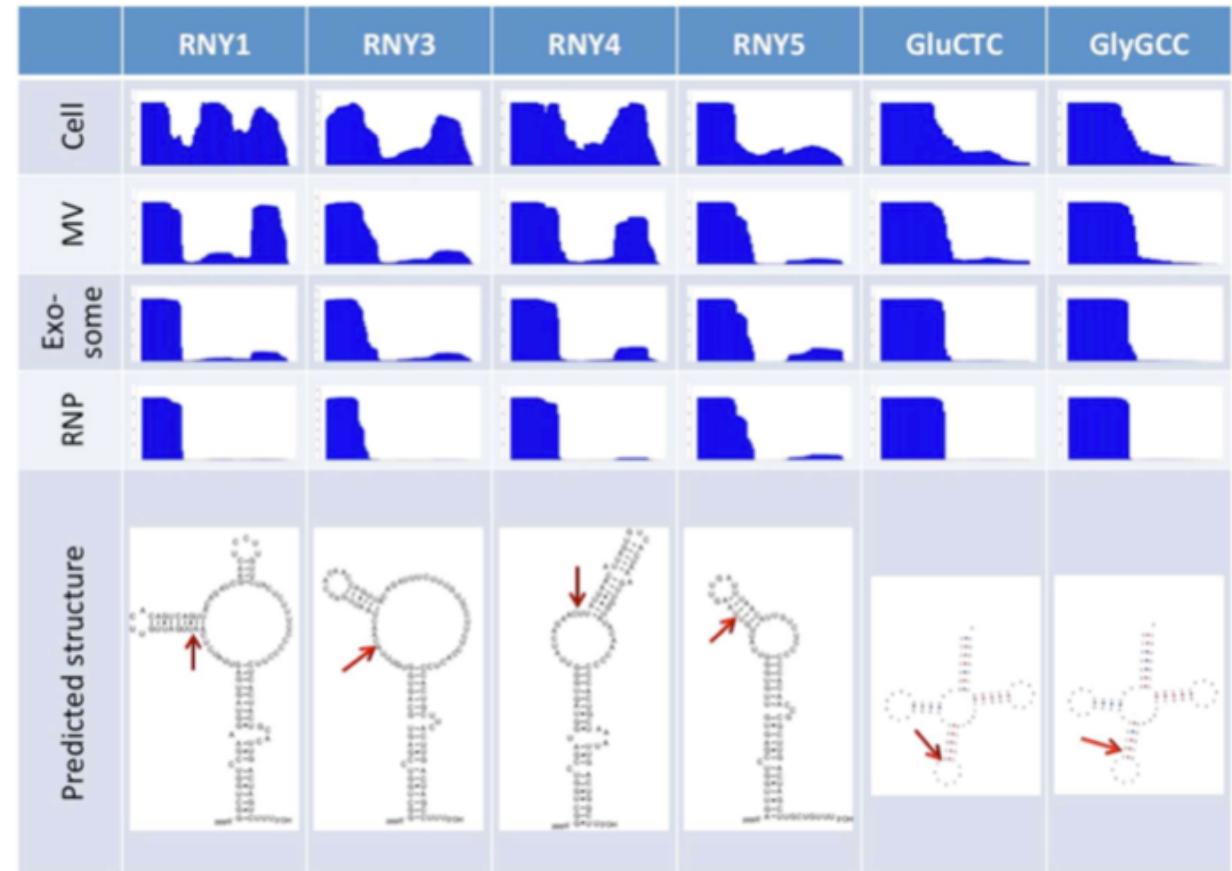
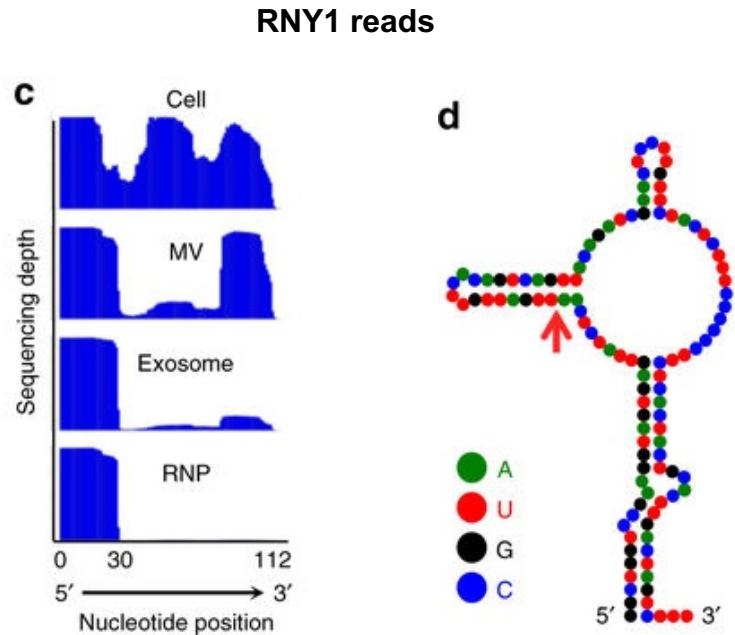


- Venn diagrams depict the number of common species among 100 top abundant mRNAs, showed less commonalities for mRNAs in exosomes and RNPs.

# YRNA and tRNA fragments are enriched in extracellular, especially in RNPs



# Character of YRNA and tRNA mapped reads

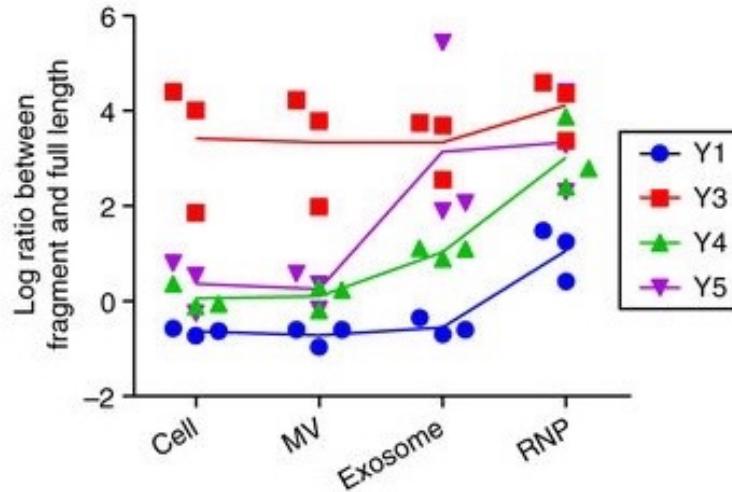


- Reads mapped to Y RNAs and tRNAs suggested the **special processed fragments**.
- located within the loop domains that are known to bind several proteins.

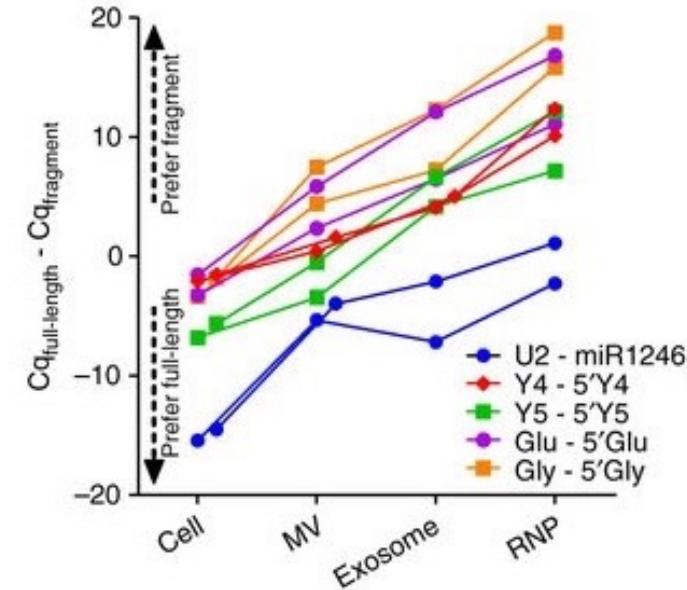
# Full-length vs Fragment

Fragment / full-length

e RNA-seq

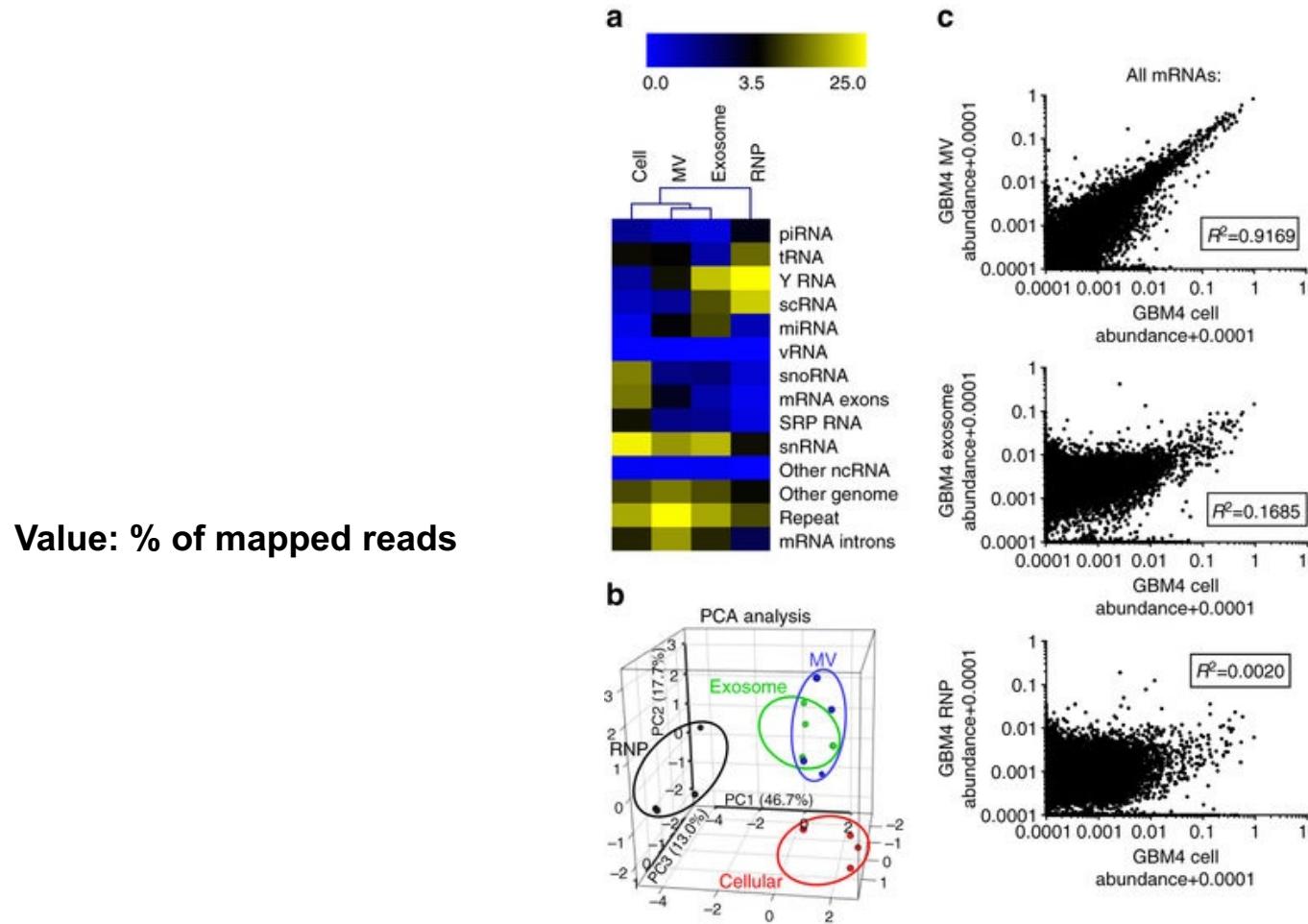


f qPCR



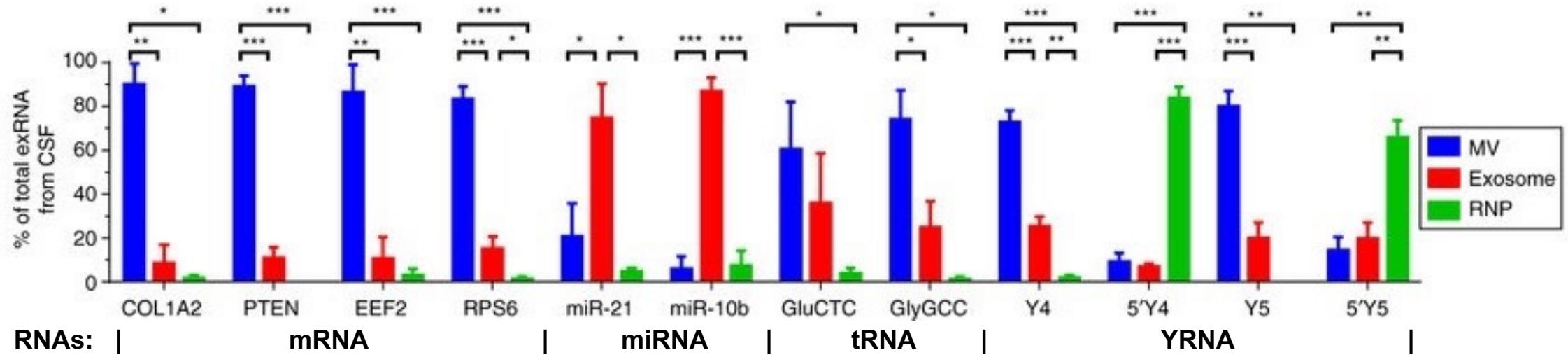
- the fragment to full-length ratio was significantly higher in extracellular vs cellular fractions for Y RNA and tRNA.

# MVs most closely reflect cellular RNA composition



- An extracellular fraction that closely mirrors the cellular transcriptome would be more valuable.
- **MVs appear to be a good source for RNA biomarker discovery.**

# MVs and exosomes should be analyzed separately



- Samples: 3ml CSF.
- mRNAs were preferentially associated with MVs, miRNAs with exosomes, tRNA with MVs, YRNA with RNPs.
- MV is suitable for mRNA biomarkers, while exosomes represent a superior source for miRNA biomarkers.

# Summary

- **Revealing the composition of extracellular components** help to understanding the function of exRNA, as well as discovery of RNA biomarkers.
- **Developing a protocol** enabling quantitative, minimally biased analysis of exRNAs in MVs, exosomes, and RNPs.
- Challenges the commonly assumed predominant role of miRNA in intercellular communication.
- Need for in-depth investigation of **other classes of exRNAs** and use as biomarkers.

# Paper-3: Small RNA sorting character into MVs, exosomes, RNPs.

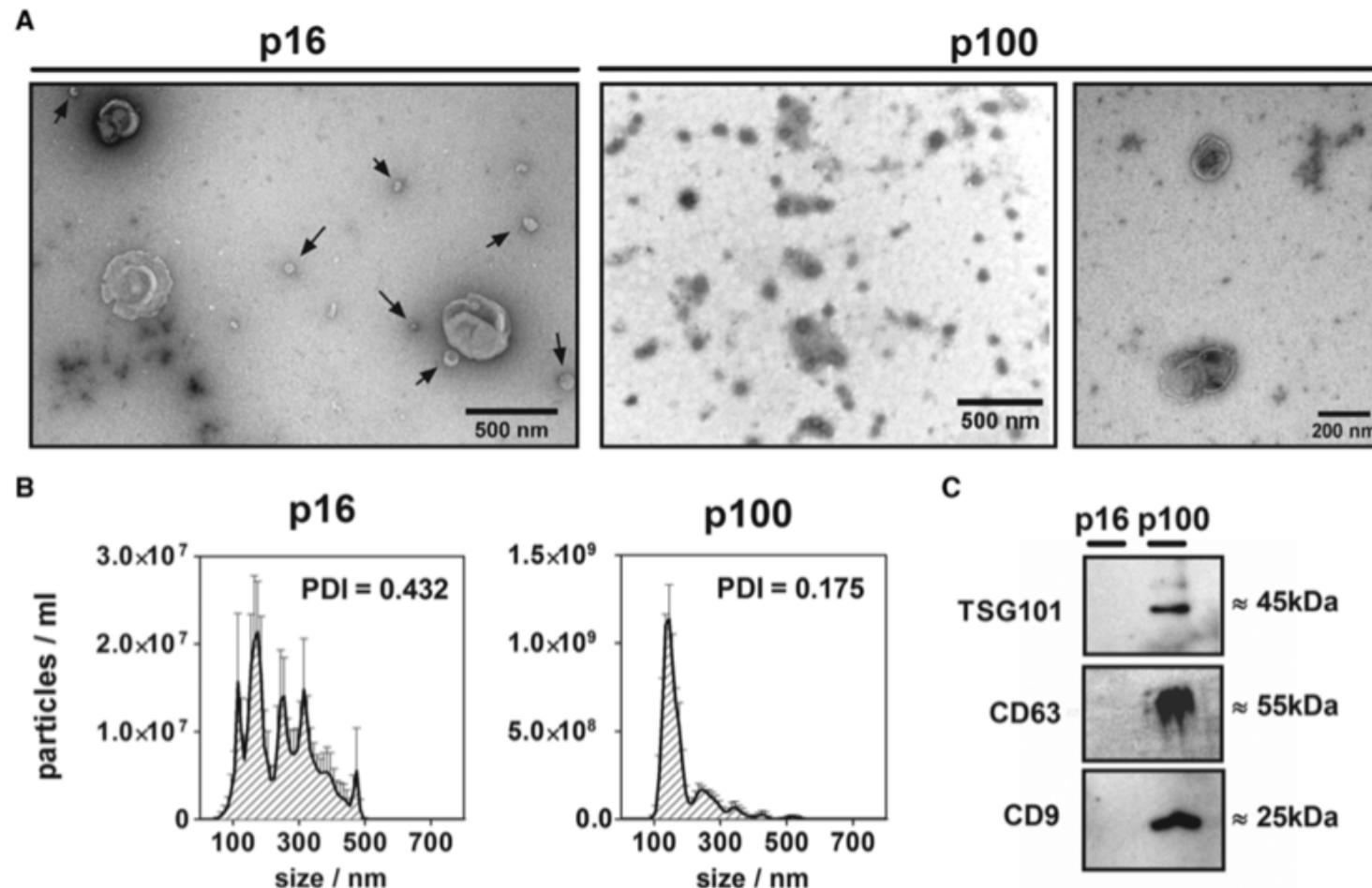
Published online 04 May 2015

*Nucleic Acids Research*, 2015, Vol. 43, No. 11 5601–5616  
doi: 10.1093/nar/gkv432

## **Assessment of small RNA sorting into different extracellular fractions revealed by high-throughput sequencing of breast cell lines**

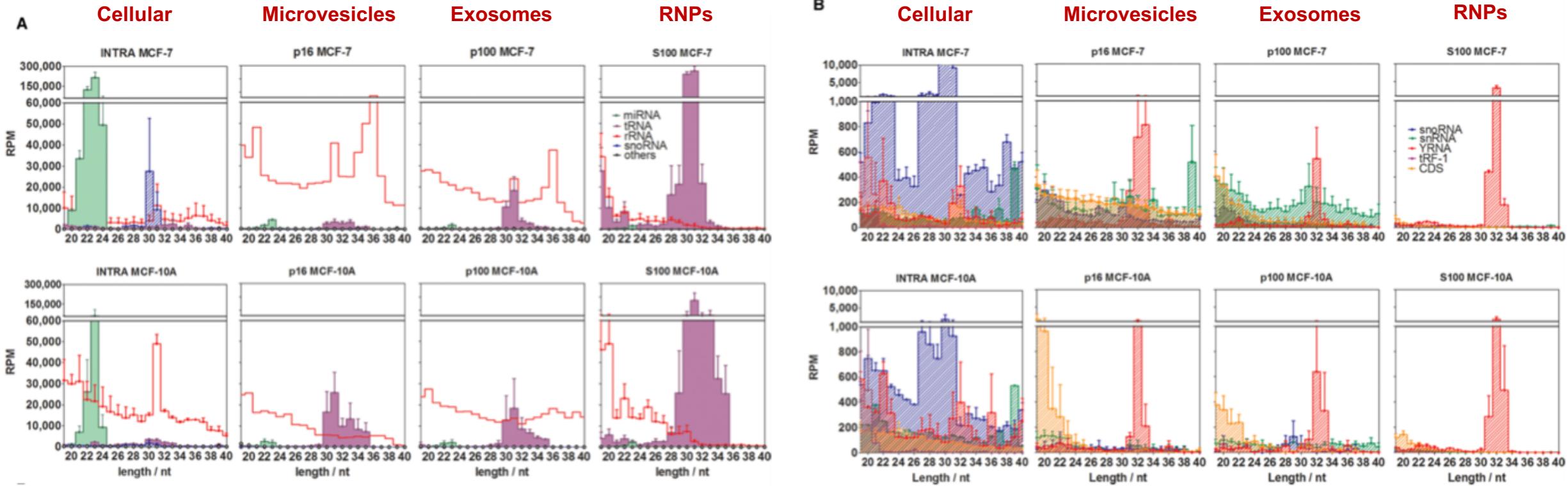
Juan Pablo Tosar<sup>1,2</sup>, Fabiana Gámbaro<sup>1</sup>, Julia Sanguinetti<sup>1</sup>, Braulio Bonilla<sup>1</sup>, Kenneth W. Witwer<sup>3</sup> and Alfonso Cayota<sup>1,4,\*</sup>

# Characterization of extracellular components



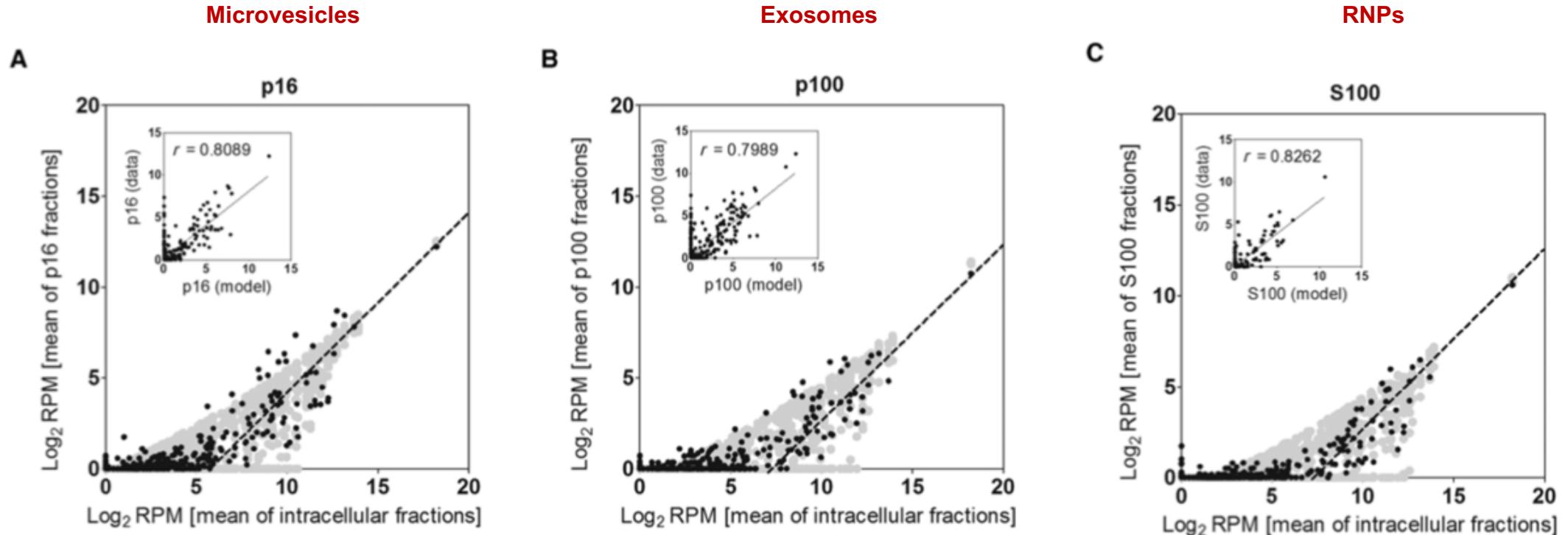
- p16: microvesicles, p100: exosomes;
- TSG101, CD63, CD9: exosomes' surface protein markers.

# Size distributions and abundances of small RNA



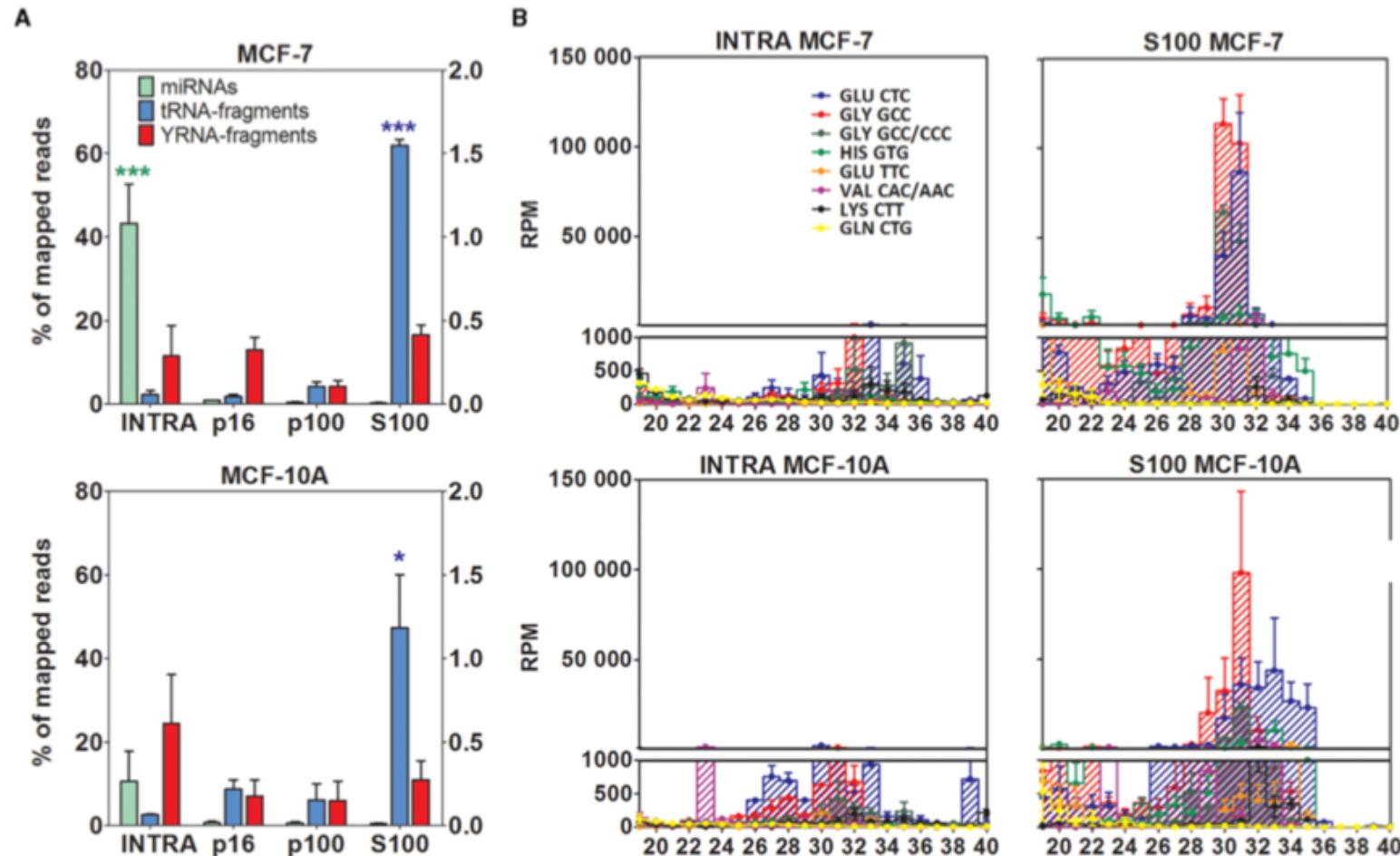
- Differences in the small RNA profiles between extracellular fractions and cell.
- tRNA, YRNA enriched in RNPs.

# miRNAs are sorted to the extracellular non-selectively



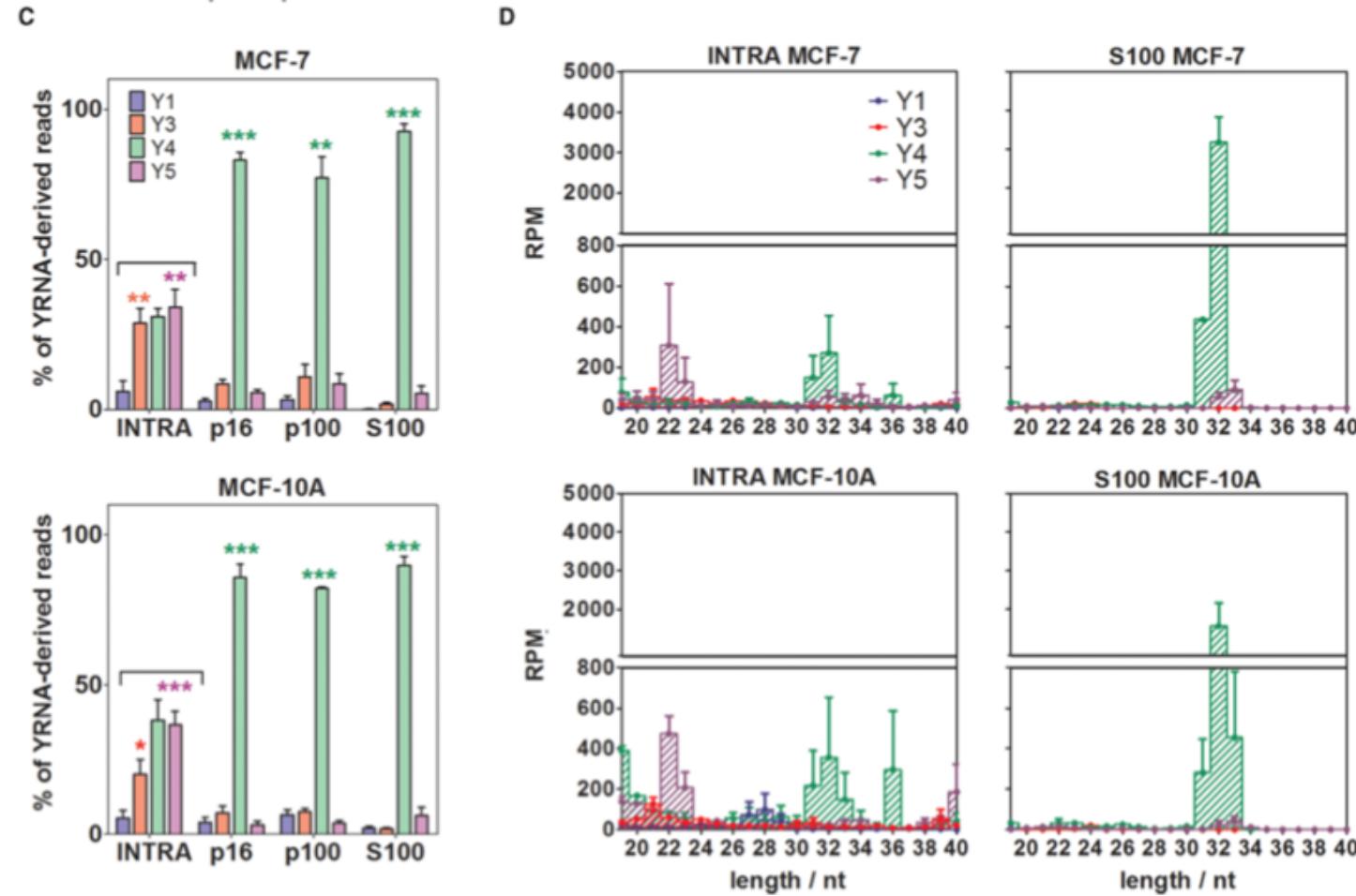
- Constructed a theoretical model(grey) to predict the extracellular abundance of each miRNA.
- Based on its corresponding intracellular expression and assuming non-selective secretion.

# 5' tRNA fragments enriched in extracellular fraction, especially in RNPs



- Size distributions and abundances of tRNA-derived sequences.

# YRNA fragments enriched in extracellular fraction, especially Y4



- Size distributions and abundances of YRNA-derived sequences.

# Summary

- To date, most attention is centered on exosomes and miRNA.
- Circulating sRNAs have been described in a variety of RNase-insensitive protein complexes, or encapsulated inside different types of EVs.
- The vast majority (above 90%) of circulating miRNAs is reportedly present in stability-conferring ribonucleoprotein complexes
- Altered miRNA profiles in cancer patients and donors are usually reported, but discordant results appear across publications.
- An alternative strategy is to focus in **extracellular fractions instead of whole plasma or serum.**

# Discussion

## Status

- Altered miRNA profiles in cancer patients and donors are usually reported, but discordant results appear across publications.
- An alternative strategy is to focus in **extracellular fractions** instead of whole plasma or serum.

## Future

- Need for **revealing the composition of extracellular components**, which could be used to discover RNA biomarkers.
- Need for **in-depth investigation of other classes of exRNAs** and use as biomarkers.
- Need for **development of novel experimental techniques and computational resources** for integrating complex data sets into comprehensive biologic networks.

### 3. Preliminary results

- microvesicles, exosomes, RNPs

# Literature research

- Methods to isolation, characterization, and results.
- 11 top level papers about MV, exosome, RNP in extracellular.
- Combined and improve to adjust for plasma samples.

## LETTER

Macrophages redirect phagocytosis by non-professional phagocytes and influence inflammation  
Claudia Z. Hart<sup>1,2</sup>, Ignacio J. Juncahita<sup>1</sup>, Jason M. Kirschner<sup>1</sup>, Monica W. Buckley<sup>1,3</sup>, Alexander L. Khokhlov<sup>1,4</sup>, Kelly Dryden<sup>2</sup>, Susa Ortega-Gomariz<sup>2</sup>, Uta Erdmann<sup>2</sup>, Stephen D. Turner<sup>1</sup>, Yun M. Shim<sup>1</sup>, Kenneth S. Tung<sup>1,2</sup> & Kodi S. Ravichandran<sup>1,2</sup>  
*Cell*. 2016 January 14; 164(0): 246–257. doi:10.1016/j.cell.2015.11.051.

Extracellular vesicles from *Trypanosoma brucei* mediate virulence factor transfer and cause host anemia  
Sonia Celli-Riv and Rep (2017) 13:226–240  
DOI 10.1007/s13010-016-0713-1

Exosome and Microvesicle-Enriched Fractions Isolated from Mesenchymal Stem Cells by Gradient Separation Showed Different Molecular Signatures and Functions on Renal Tubular Epithelial Cells  
Published online 04 May 2015  
*Nucleic Acids Research*, 2015, Vol. 43, No. 11 5603–5616  
doi:10.1093/nar/gkv452

Assessment of small RNA sorting into different extracellular fractions revealed by high-throughput sequencing of breast cell lines

Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma  
Jason D. Arvey<sup>1</sup>, John A. Chevallet<sup>1</sup>, Evan M. Kraft<sup>2</sup>, Ingrid K. Ruf<sup>2</sup>, Colin C. Pritham<sup>3</sup>, Donald F. Gibson<sup>4</sup>, Francis S. Mitchell<sup>1</sup>, Christopher F. Bennett<sup>1</sup>, Ira L. Pogosova-Agadjanyan<sup>2</sup>, Derek L. Stirewalt<sup>2</sup>, Jonathan F. Tait<sup>2</sup>, and Marlene Tewari<sup>1</sup>

<sup>1</sup>Institute of Human Genetics, Fred Hutchinson Cancer Research Center, Seattle, WA 98109 USA; <sup>2</sup>Department of Laboratory Medicine, University of Washington, Seattle, WA 98195 USA

<sup>3</sup>Department of Cell Biology, University of Washington, Seattle, WA 98195 USA; <sup>4</sup>Department of Laboratory Medicine, University of Washington, Seattle, WA 98195 USA

MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins

## ARTICLE

Glycan-1 identifies cancer exosomes and detects early pancreatic cancer

## LETTER

Microenvironment-induced PTEN loss by exosomal microRNA primes brain metastasis outgrowth

## ARTICLE

Tumour exosome integrins determine organotropic metastasis

## ARTICLE

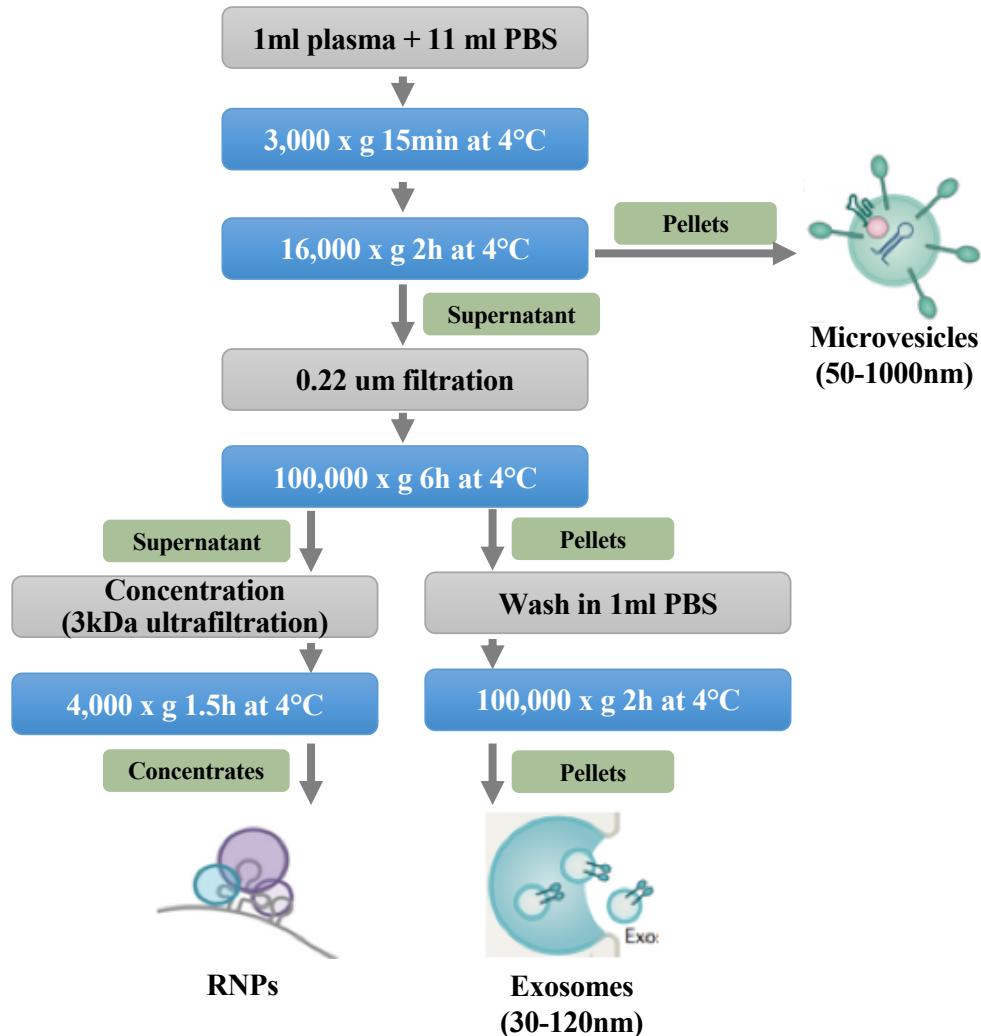
Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer



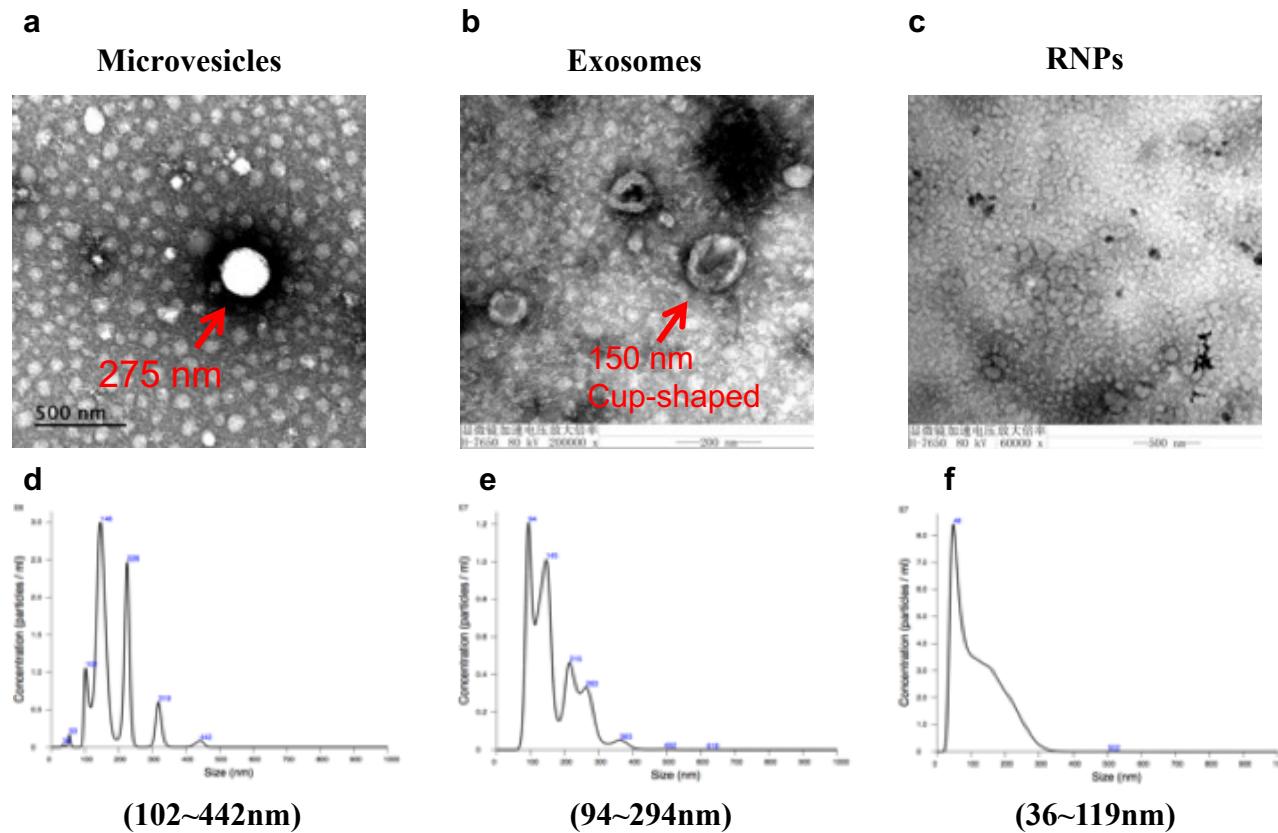
Exosomes Mediate Stromal Mobilization of Autocrine Wnt-PCP Signaling in Breast Cancer Cell Migration

# exRNA problem 1 – degradable: Fractionation of Microvesicles, Exosomes, RNPs

## □ Experimental procedure



## □ Characterization results



**a-c:** Morphology by Transmission Electronic Microscope.

**d-f:** Particle size distribution by Nanoparticles Tracing Analysis.

**Thank you !**