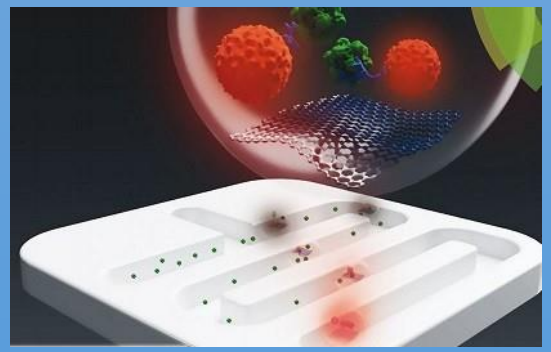

EXTRACELLULAR VESICLE GROUP
SEMMELWEIS UNIVERSITY
DRARPAD.com
WRAP UP
Annual Meeting – ISEV2017
Toronto, Canada
18-21 May 2017
www.isev.org

# NEWS IN

## FLOW CYTOMETRY

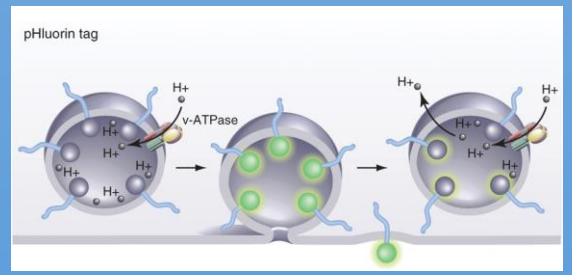
- hrFC
- hollow silica beads
- nanoFCM
- NP-40 detergent

## EV ISOLATION



- EV sorting
- Microfluidics
- ExoDisk

## EV CHARACTERIZATION




- live imaging
- pHluorin
- EV uptake
- Different MVBs
- Single vesicle analysis

## FUNCTIONAL STUDIES

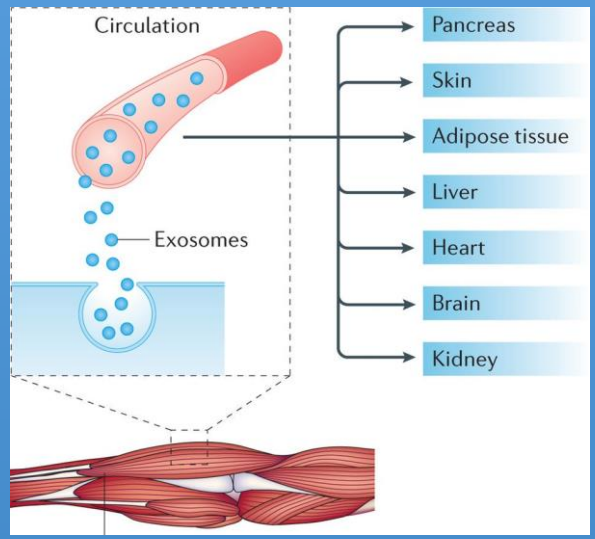
-EV uptake

-“cloud of vesicles”

-EVs and Cardiovascular system

-EVs and Pericardial fluid

-EVs and AMI



# EV BIOPHYSICS AND BIOCHEMISTRY

EV diameter ↑, then stochastic events ↑

EV molecular imaging – wireless network

EV labeling by location:
- inside
- transmembrane
  - outer membrane
  - inner membrane

EV detergent sensitivity may vary between subpopulations?

Interference of optiprep use with lowry based protein assay

Molecular heterogeneity

Circulating plasma older and newer EVs?

Selection mechanism(s)

Different MVBs → different exosomes

ARRDC1 –recruitment of MVB to PM

CD9+ EVs are independent of RABs

EV internalization is inhibited by latrunculin

Nsmase2 control secretion of miR via exosomes

Live cell imaging of exosomes:
https://pdfs.semanticscholar.org/9128/842ec2e6351feafc4ad5633ddcafe4b39756.pdf

Fusion with PM through vSNARE/ tSNARE - how are EVs are escaping EE?

# EV UPTAKE

## ENDOCYTOSIS

A. Clathrin dependent
B. Caveolin dependent
C. Lipid raft mediated (FLOT may be involved)

Stripping proteins from EVs reduces their uptake
(eg. Proteinase K treatment)

Proteins involved in uptake:

→ tetraspannins: CD81, CD9, CD63
→ integrins and immunoglobulins: CD51, CD61
→ proteoglycans: HSPGs (Christianson 2013)
→ lectins Abs again DCSIGN reduces EV uptake- DEC205

## PHAGOCYTOSIS

Through PS and other lipids

## MACROPINOCYTOSIS

Lipid rafts

**"KISS and RUN" – delivery type**

hemifusion

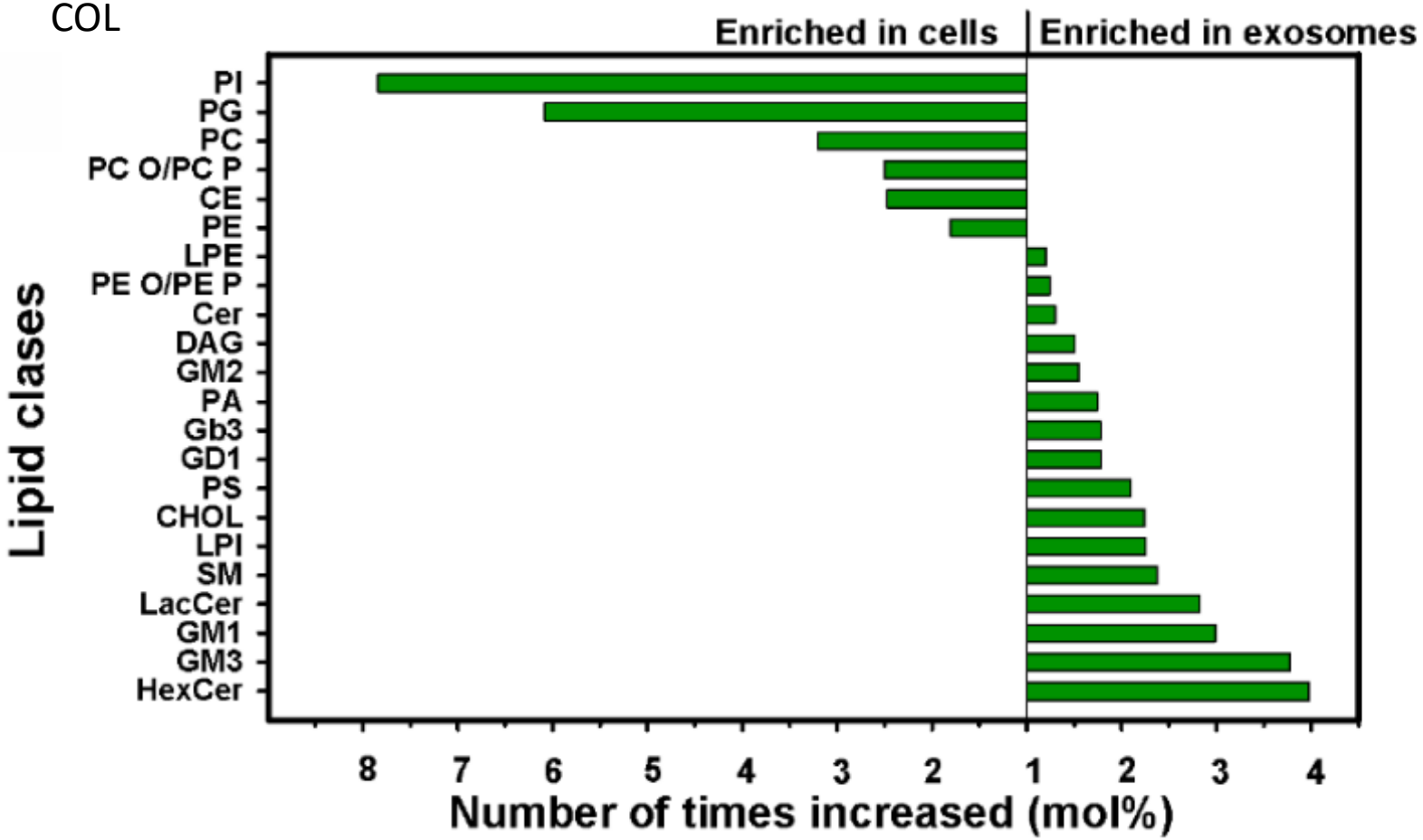
Inhibition of binding: tripsinidase, neutralizing Abs

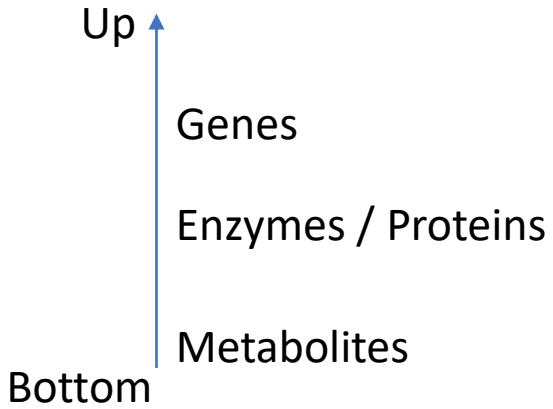
Inhibition of endocytosis: Dynasore, Pitstop + list of drugs

Zomer et al, Nat Protocols, 2016; Mulcahy et al, 2014 JEV

# Lipids in exosomes

PL
SM
GM3
COL

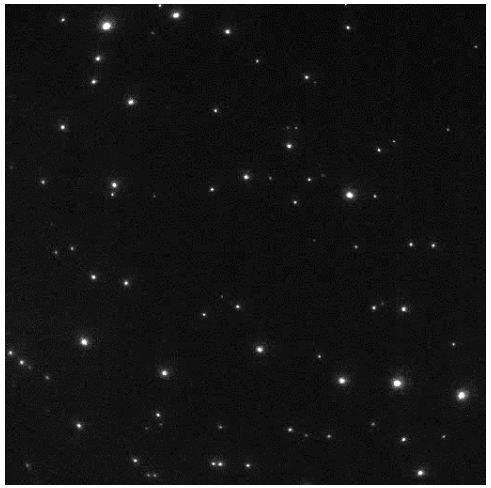



hr proteomic and lipidomic analysis Haraszti et al 2016 JEV

Skotland et al 2017 Prog Lipid Res

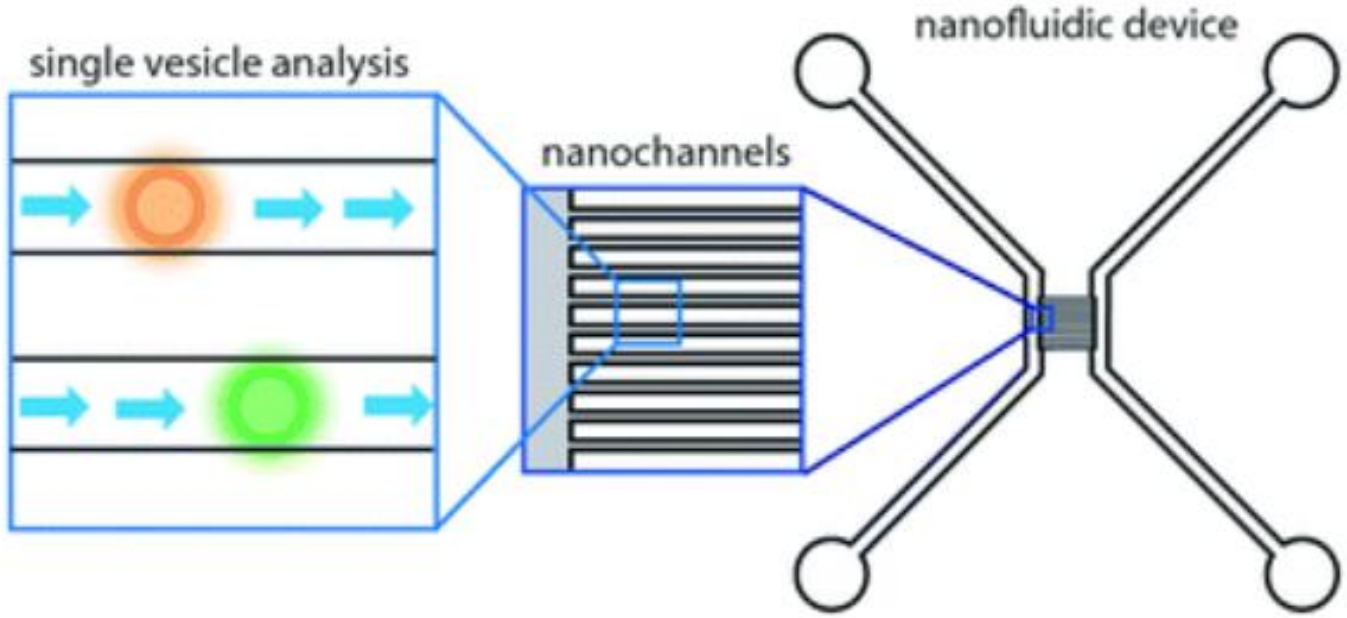
# Single vesicle analysis



**Proposed isolation method**: microfluidics

**Confocal microscopy**: mitochondria DSRED detects vesicles containing mitochondrias

EV quant vs NTA: 0.99 correlation, can detect small vesicles up to 50 nm down;
detect multiple surface markers on EVs




Friedrich et al, 2017 Lab on a Chip

# EV CHARACTERIZATION

- EV quantification: EV/ Cell/ Hour
- Saturation: reach concentration → dilution : concentration dependent curve (±co-culture experiments)
- Chris Gardiner : NTA/TRPS/DLS do not distinguish the protein aggregates from EVs
- EV best measured in isotonic aqueous medium
- To prevent loss of particles over time in diluted suspensions: dilute immediately before use
- Reduce sample error: 5-20 short measurements are better instead of 1 long
- Check regularly the instrument calibration!
- DLS can be useful when combined with SEC
- TEM: wide EM + 1-2 close view ± immunogold labeling; CryoSEM Koffman ez al, 2017
- DOT BLOT permeabilization: Tween20, inside vs outside of EVS: Sung et al, 2017 Cell Adh Migr
- Surface biothinylation followed by MS

# EV ISOLATION

**C. Thery**: no pressure based filtration isolation: could break large EVs and create artificial small EVs
tagging proteins may alter the effects of EVs

**Membrane filtration**

-low purity
-it can process large volumes
-combing filters→ reduces yield

**Acoustic separation**

-no mechanical or chemical stress on EVs
-SAW (surface acustic wave)
Lee et al, ACS NANO 2015

**Immuno-magnetic separation**

-bead quoted with Ab → highly specific
! selection of Abs;
mixture of Abs (CD9, CD63, CD81)
-low yield
Jeoung et al, ACS NANO 2016

**Affinity separation**

-up to 12 marker in one sample
-Ab based
-ELISA and nPLEX

**ODG**

EV + HDL together

Remnant OPTIPREP can be successfully removed by SEC

**ExoDISK**

lab-on-a-disc with 2 nanofilters
20-600 nm range
Wo et al, ACS Nano 2017


**Sucrose gradient density centrifugation**: EVs may shrink + ↑ density was measured of the EV fractions → use **Optiprep** instead

# FLOW CYTOMETRY (1)

evflowcytometry.org

High frequency of small signals are buried in noise

**Hollow silica beads**: RI:1,46; do not sediment, membrane thickness: 5 nm

MIEV Flow Cyt Checklist

nanoFCM: detection 1-10 epitope vs. Astrios nanoFACS detection 30-200 epitopes (vs. conventional FACSCalibur +200 epitope)

Plasma EV epitope profile with hrFC biolegend +300Ab

Synthetic MP: Smith et al Circulation 2017

EVs from cardiomyocytes can be sorted and isolated (GFP+EVs) Das et al

non fluorescent particles affect fluorescent labeled EVs: High concentration of unstained particles negatively impact the fluorescent thresholding

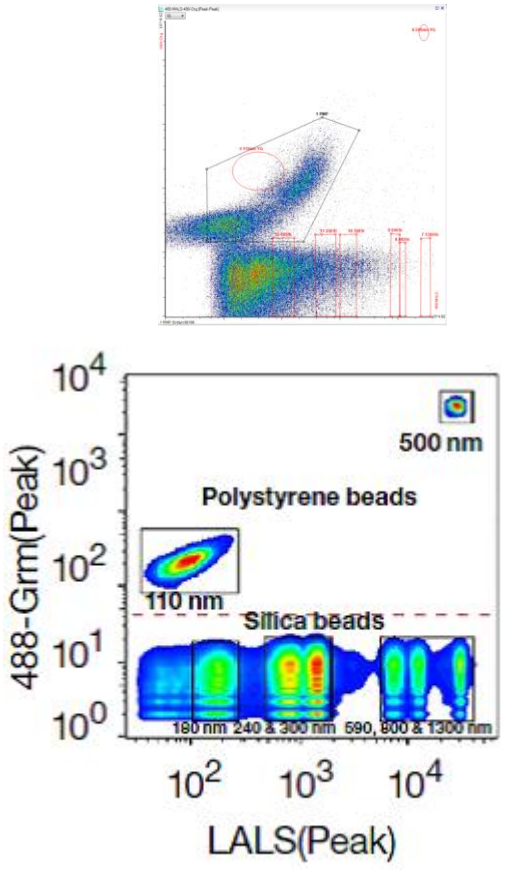
NanoFACS: sorted EVs are biologically viable and can be used for RNAseq Zabeo et al 2017 JEV

Kormelink et al, 2016; Stomer et al, 2016

# hr FLOW CYTOMETRY (2)

## *Apogee A50+*

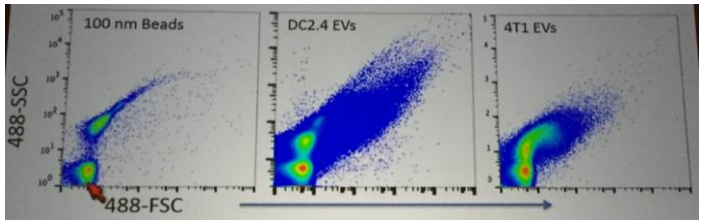
- 80 nm Fluorescent particle detection

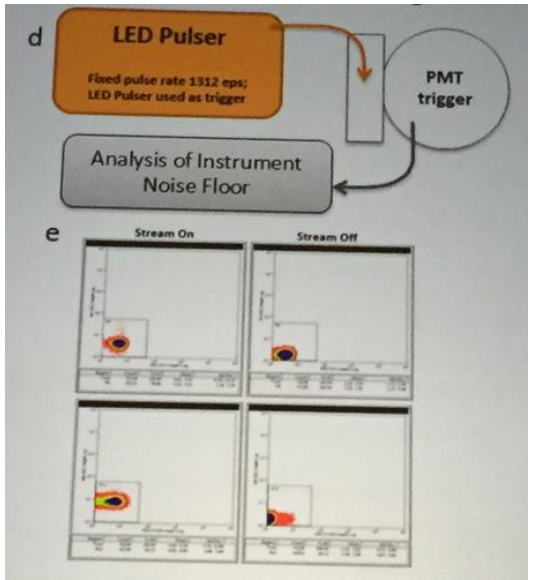



http://apogeeflow.com/

## *Astrios-EQ nanoFACS*

- High speed detection
- EV sorting

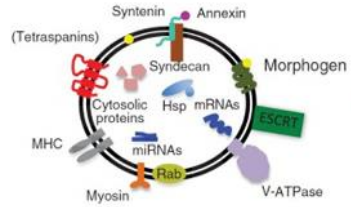


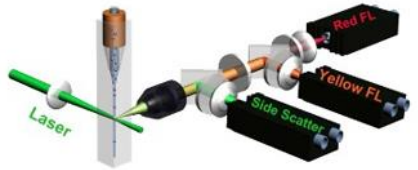




Jones et al 2016

## *nanoFCM – prototype May 2017*

- Highly dedicated setup
- Limited dynamic range
- Best detection limit




| Parameter | Fluorescent Probe | Applications |
|---|---|---|
| Size | Label-free | Rapid, high-resolution size measurement of EVs |
| Concentration | Label-free | Determine the abundance of EVs in body fluids |
| Protein | Fluorescent Immunolabeling | Determination of subcellular origin, and biochemical properties |
| RNA | Nucleic Acid Staining | Origin and biological function analysis |

www.nanofcm.com

# EV RNA (1)

exosome-rna.com

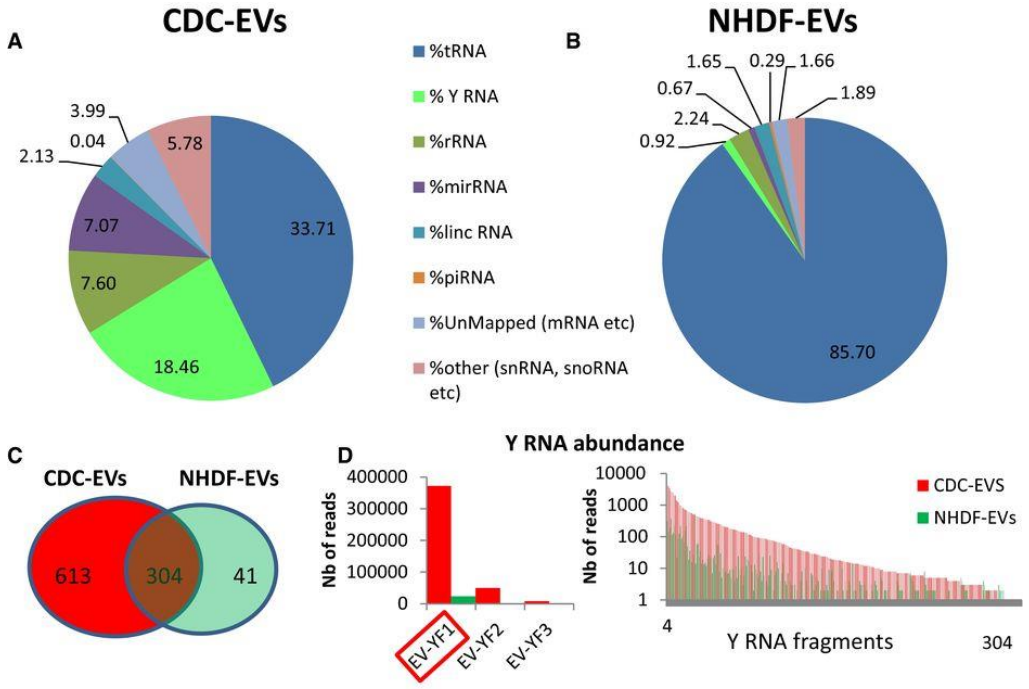
2 different exRNA signatures released by a single cell type

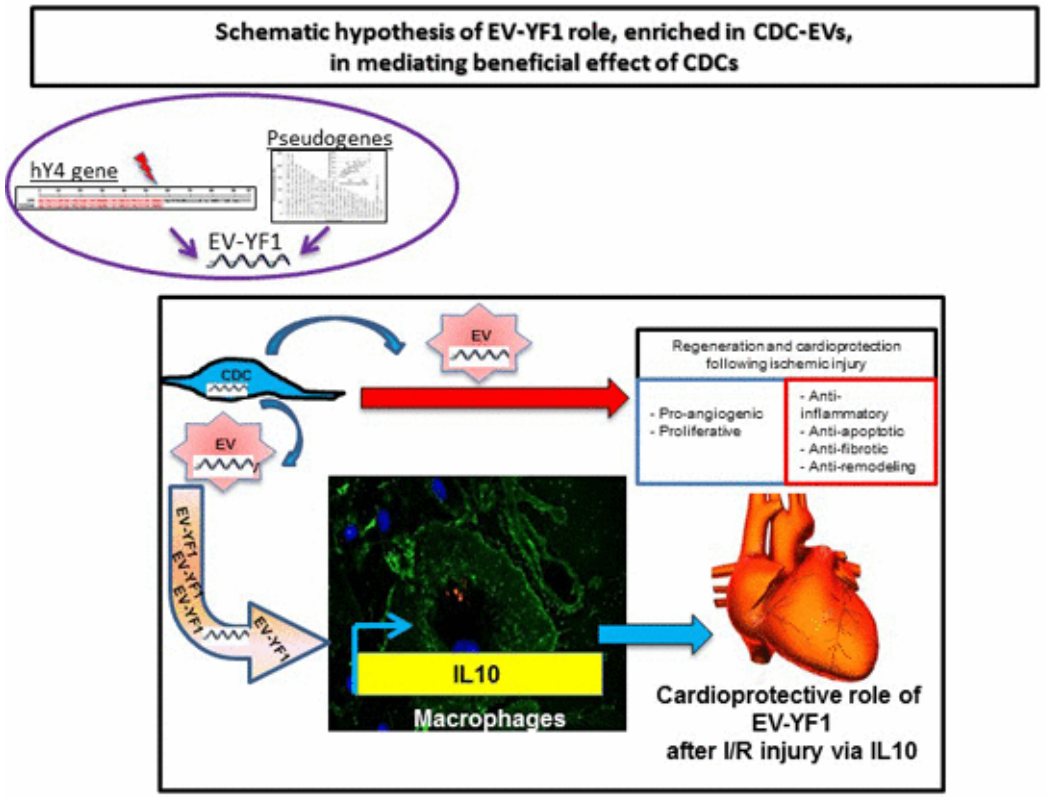
There is not one miRNA, that is constantly secreted in exosome

Y RNAs are present in EVs and they are altered in DC activation

Treating EVs with UV for 220 sec: successfully decreases RNA without damaging proteins

RNA- protein complexes co-pellet with vesicles




Cambier et al, 2017

Lässer et al, 2017

# EV RNA (2)

How does bovine serum-derived RNA affect EV-RNA analysis?

Observation of miRNA species in EV pellets of cell-conditioned medium that could not be detected in parent cell
Eg. Liver hsa-miR-122 in glioblastoma cell supernatant



1. Depletion of (EV) RNA from bovine serum prior to cell culture may not be efficient
2. Bovine RNAs can map to murine or human genome (up to 10% alignment for both miR, mRNA, snoRNA)

Wei et al, 2017 Shelke et al, 2014

# APOPTOTIC BODIES

IAV induces apoptosis and apoptotic bodies production

Epithelial cell line A549 treated with AB derived from IAV infected THP-1 cells induced infection in vitro

THP-1 cell derived AB could induce the same infection in vivo in mice

AB from infected cell can elicit inflm response and IAV specific T cell response

Can we impair viral infection by influencing the AB production?

Inhibiting AB disassembly with drug x→ partial restorement of cell disassembly

Athkin Smith et al, Sci Rep 2016

# EVs and the CARDIOVASCULAR SYSTEM

tRNAs are increased in primary myocardiocytes derived EVs

Diabetes promotes release of miR503 enriched MP from endothelium

CD34+ exosomes: are pro-angiogenic and have an increased has-miR-130a, 126a and 92a
In hypoxia CD34+ exosome conc is increased

CD34+ exosomes induce angiogenesis in vivo (mice)
loss of miR-126a reduced the angiogenic potential of Cd34+ exosomes.
Uptake of CD34+ exosomes injected in the heart: by endothelial (via SIRPA)
and cardiomyocyte cells, and no uptake by fibroblast cells.
These exosomes may induce the cell cycle in endothelial cells.

hsa-miR-21 activates cardiac fibroblasts + antagomir miR-21 as general anti-fibrotic drugs (Phase II clinical trial)

CVD alter neuronal targets of circulating EVs

CD151 and mucin 16 is increased with age in EVs

Hepatocyte's derived EVs: Royo et al, 2017

# EVs and ACUTE MYOCARDIAL INFARCTION

After AMI 2-3 days: inflammatory monocytes M1
After AMI 4 days : M2 type monocytes

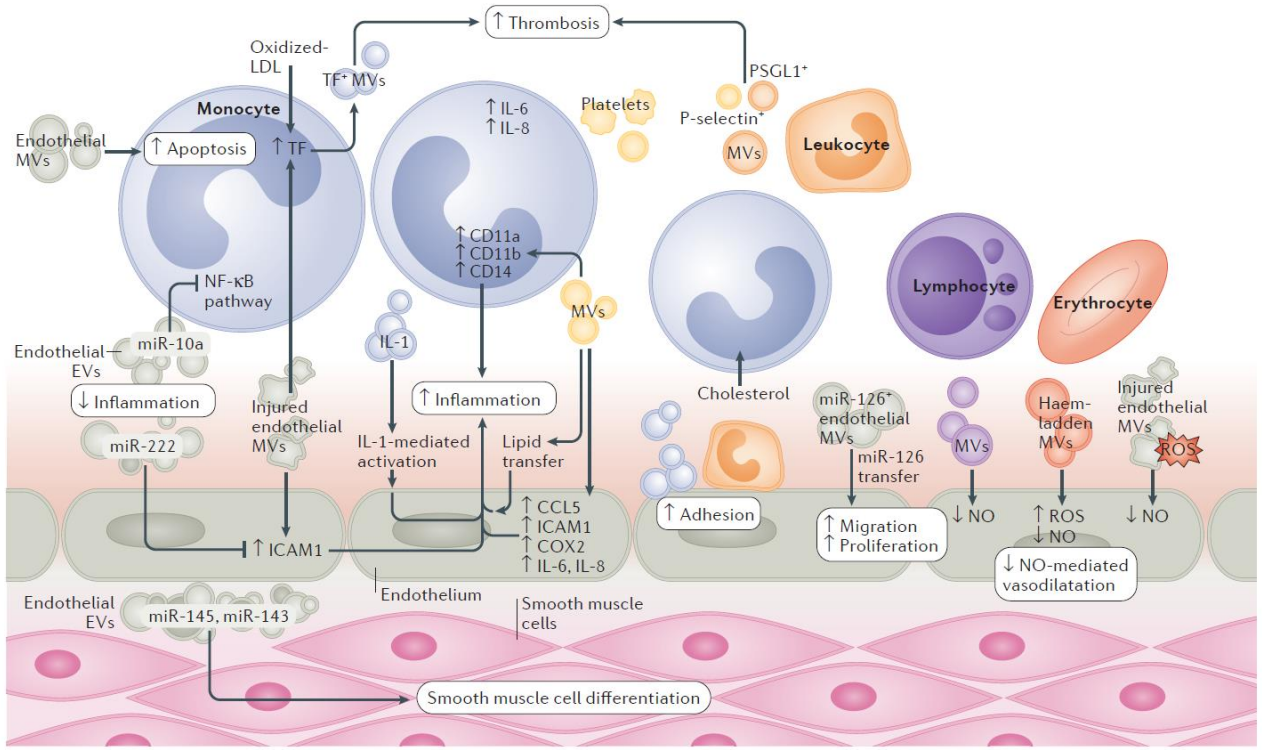
↑VCAM1+ EVs  Anti-VCAM1 reduces the EC migration (Boyden chamber in vitro)
↑↑TNF+ EVs

AMI modulates EV miRNA:
↑ miR:126-3p, 126-5p, 26b-5p, 1472, 23a-3p, 151a-3p, 374b-5p

EC-EVs mobilize the monocytes from spleen in vivo mice

In human: Monocytes from spleen are mobilized after AMI

EVs reduce oxidative stress under hyperglycemic conditions




Boulanger et al, 2017  Chistiakov et al 2016

# EVs and the PERICARDIAL FLUID

CABG diabetes pericardial fluid EVs (CABG non diabetes, MVR)

Exosomes: CD63, Alix, TSG101 + Beltrami et al, 2017
RNA:
→ Proangiogenic response: let-7b-5p; hsa-mir-21-5p; has-mir126-3p (relative to cel-miR-39)
→ increase apoptosis in EC
→ comparing microRNA signature with the miRNA pattern in piece of atrium and piece of aortic tissues
MS:
→↑expression of apoptotic proteins in CABG DM PF
→APAF1 protein (under control by let-7b-5p; protein presence validated by WB)
WB
CD63 APAF1 double positive EVs

Costanza Emanueli: The pericardial fluid exosomes as new cell-to-cell communicators worsening ischaemic heart disease in diabetes

Andrew I.U. Shearn: Methodological considerations for nanoparticle tracking analysis (NTA) of neat biofluids obtained from cardiac surgery

Sezin Aday: Bio-inspired synthetic exosomes carrying microRNA let-7b for post- ischemic vascular regeneration

# EVs from other species

Host- virus interaction in ocean

Algal bloom in ocean → 50% of O2 production

Emiliania huxleyi ← are killed by EhV viruses (dsDNA, 500 genes)

Viral free lysate =VFL induces vesicle production, in these vesicles RNA and lipid profile identified, no DNA

In virus infected E. huxleyi derived EVs: different lipidomic profile: TAG and SL
different small RNA profile: with protein targeting chromatin remodeling

EV: 1. provirus; 2. prohost

BODIPY stain for vesicles

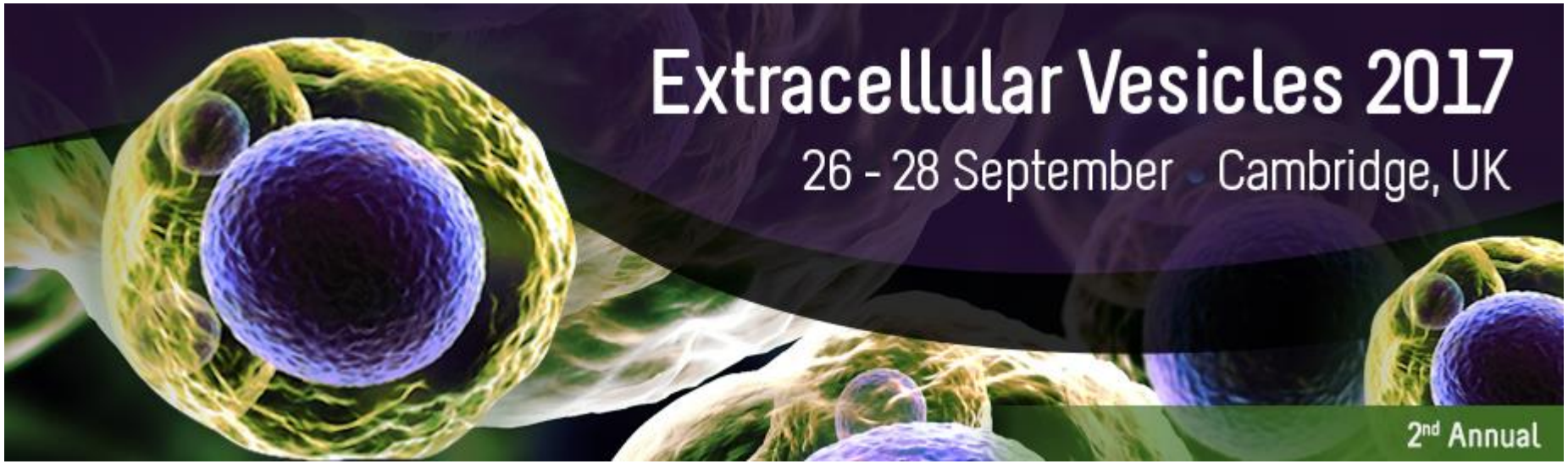
EVs lead to increased stability of the virus→ EV/EhV ratio is ↑ → EhV half life is ↑

**Vesiclomics in the ocean Project**: collection of EVs from the ocean in marine environment

Schatz et al, 2017 Nat Microbiology; Ziv et al, 2014; Rosenwarver et al, 2014

# EVs in therapy

- Injection of EVs, interspecies
- In vitro and in vivo (animal models)
- One patient studied in GVH
- Experiments started for large scale production of therapeutic EVs
- Toxicity/immunogeneity/ safety
- MSC




## Keynote Speakers

**Samir EL-Andaloussi**
Assistant Professor, Karolinska Institute

**Pascale Zimmermann**
Directeur de Recherche, Inserm

**Andrew Hill**
Professor, La Trobe University

**Esther Nolte-'t Hoen**
Assistant Professor, Utrecht University

**Sai Kiang Lim**
Research Director, Institute of Medical Biology, A*STAR

**Giovanni Camussi**
Professor, University of Torino

https://www.selectbiosciences.com/conferences/index.aspx?conf=EVE2017

# TOP 5: RBC-derived EVs role in cardiac remodeling in vivo

AMI: Mo/MF, Fibr, Endoth, RBC → infiltration into heart→ heart remodeling→ Dysfunction in Kidney, Brain and Hematopoetic System

Cre-lox system: ROSA26: kidney receives cre from the heart EVs

Cardiomyocytes EVs in plasma are 5-8%
RBC releases EVs with complement activation: possible mechanism to target EVs

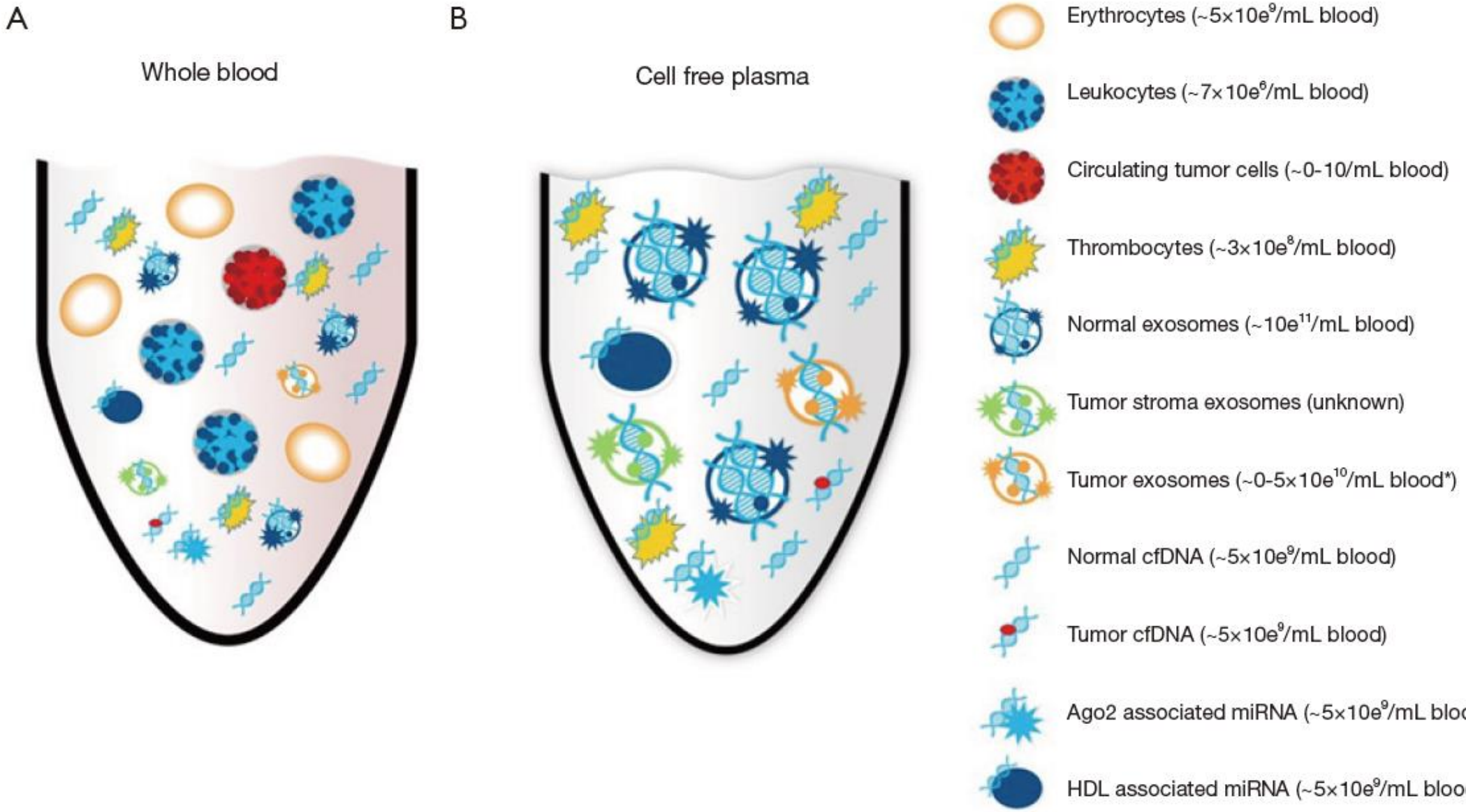
EpoR-cre-mTmG mice have GFP+ RBC derived EVs

EVs from EPO-Cre RBC can transfer functional cre

RBC EVs communicate with heart (cardiomyocytes), kidney (prox tubular cells), spleen (splenic cells), lung

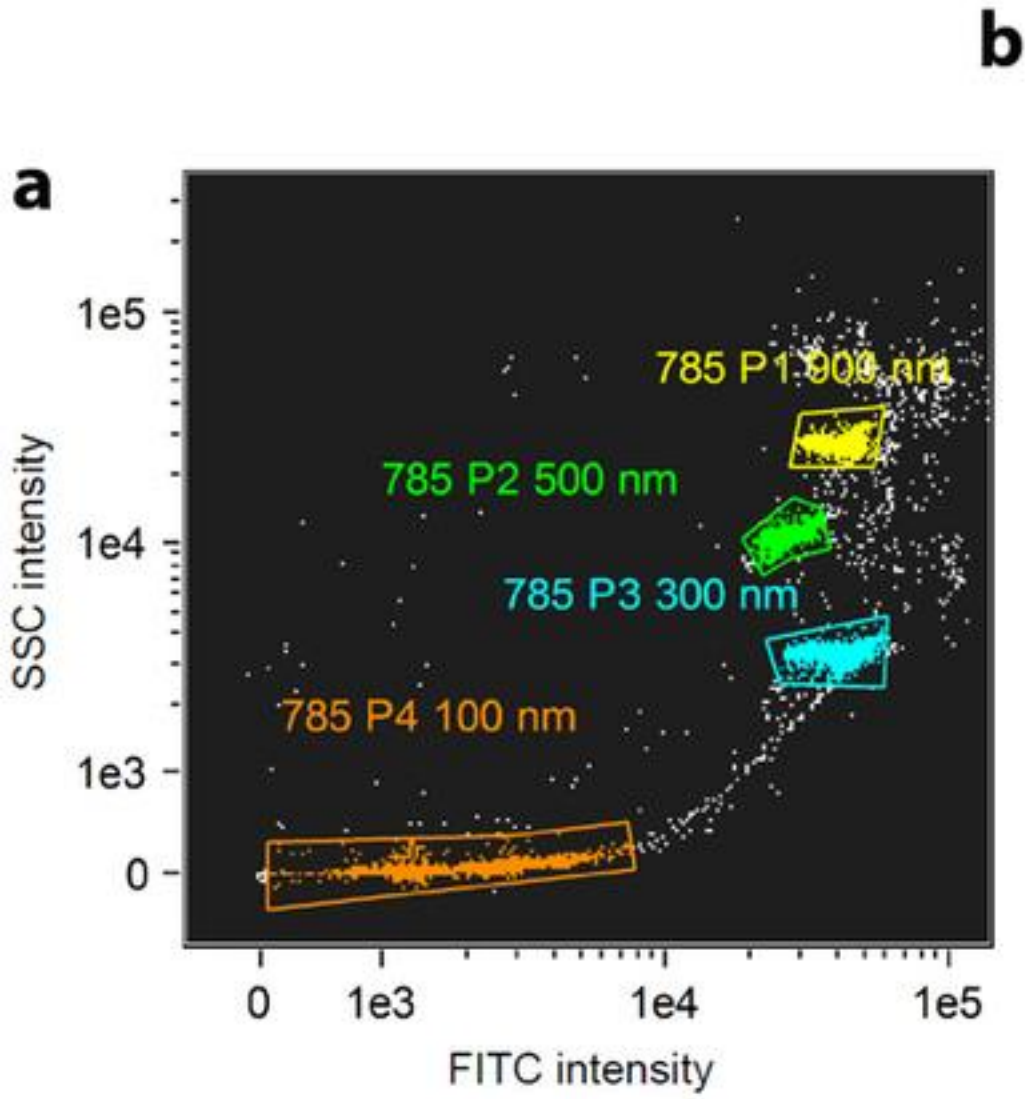
+ control experiment to exclude ectoptic expression (FC assay)

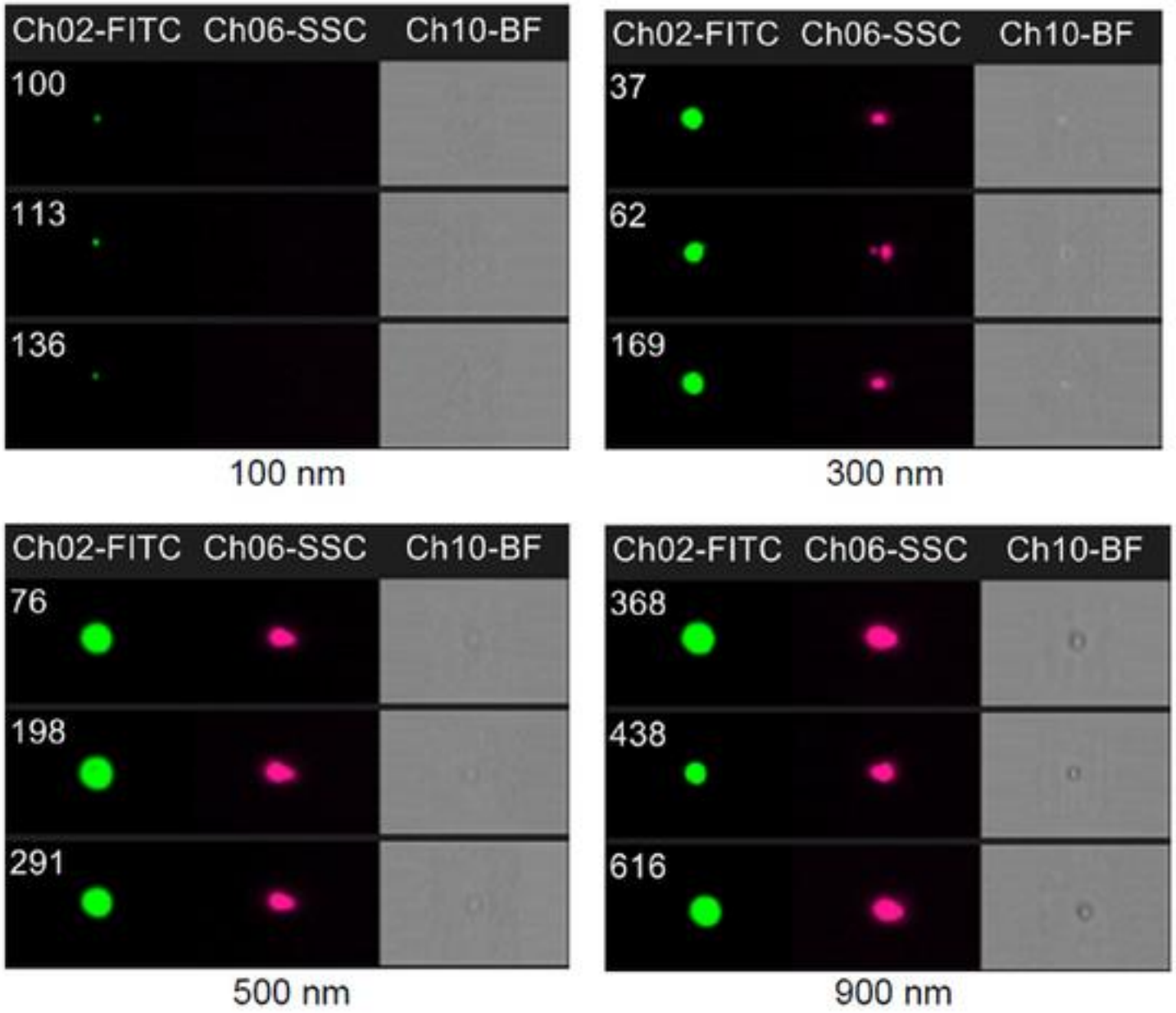
# TOP 4: Circulating cancer associated EVs



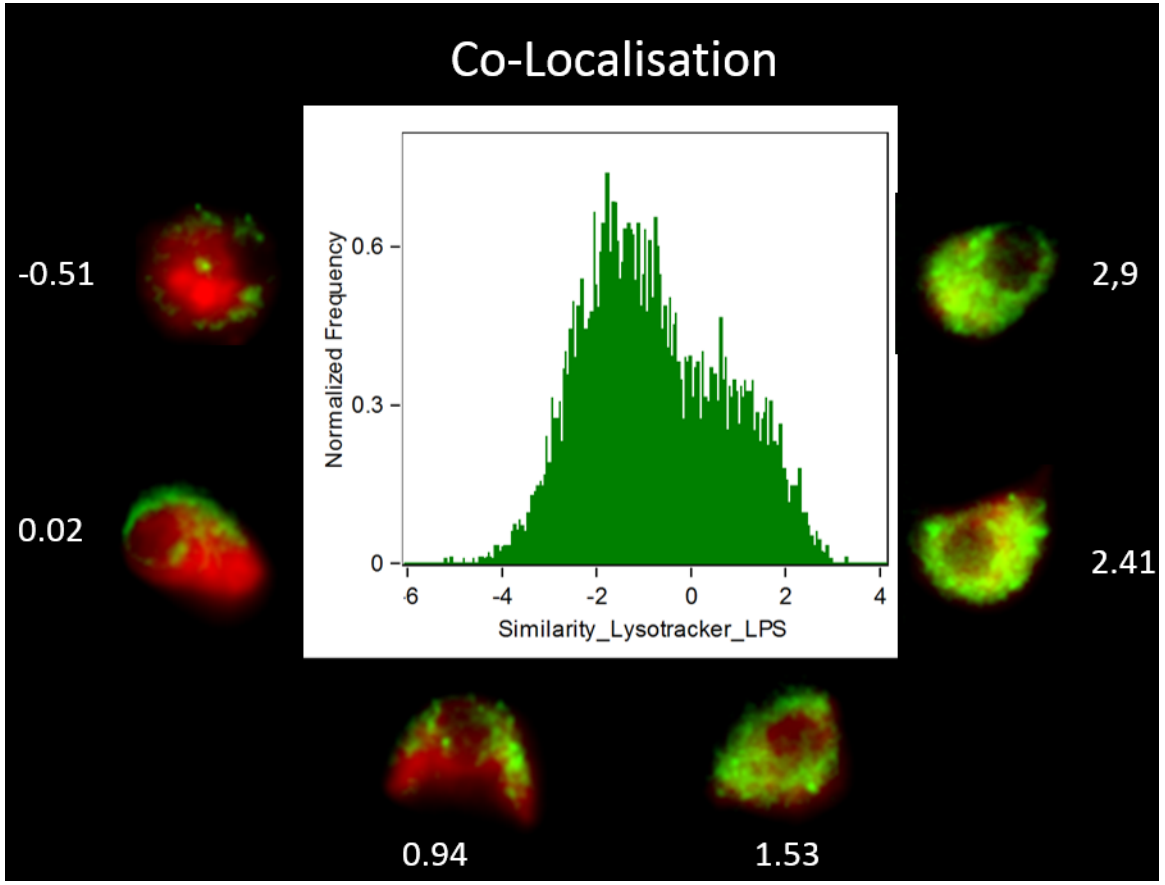

Brock et al, 2015

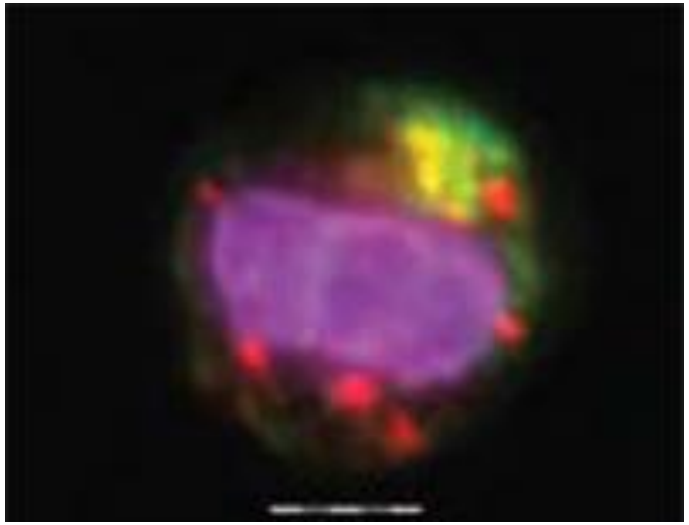
# TOP 3: Imagestream$^{x}$ hrFC



# TOP 2: Imagestream$^{x}$ Mark II



K562 cell line, DAPI (purple), the lysosomes were stained with CD107a AF647 (red), and the endosomes were stained with CD71 FITC (green)

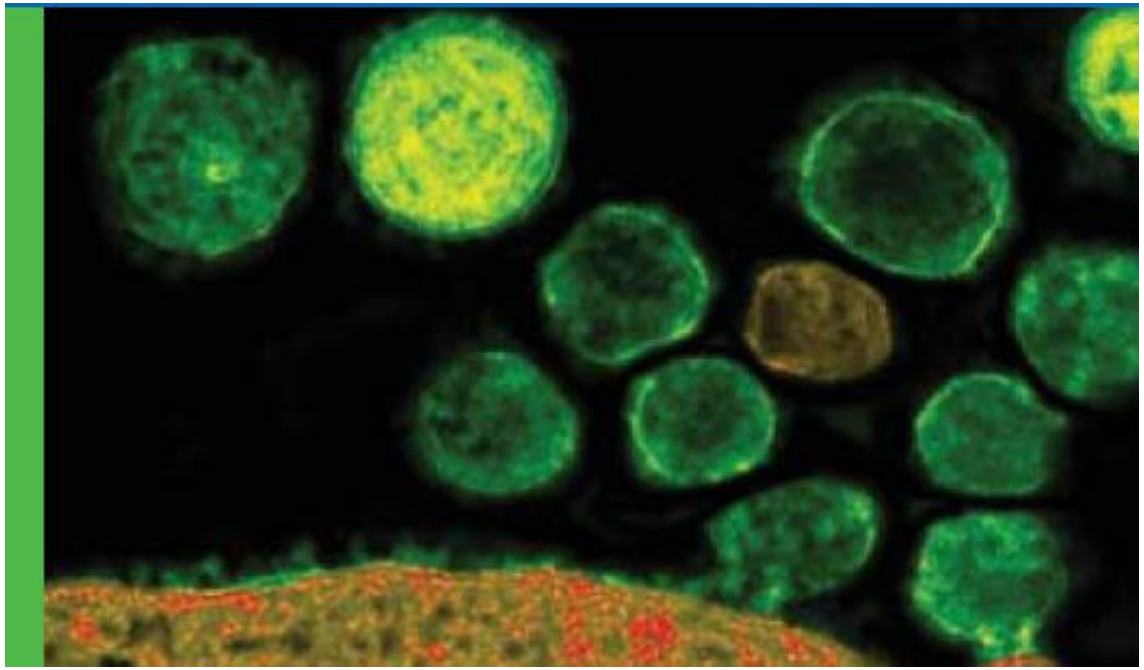
# TOP 1: In vivo tracking of exosomes

Frederik Verweij, Institut Curie, Paris

Zebrafish placenta: yolk specific expression of CD63pHluorin

Active uptake of EVs by MF: initially are not degraded/ acidified

Extravasation of EVs towards interstitial fluid: form a major repository for exosomes



# Thank you for your attention!

Extracellular
vesicles or vesicles?