# Direct detection of donor-derived extracellular vesicles in kidney transplant recipients

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# Disclosure

## In relation to this presentation, I declare that there are no conflicts of interest.

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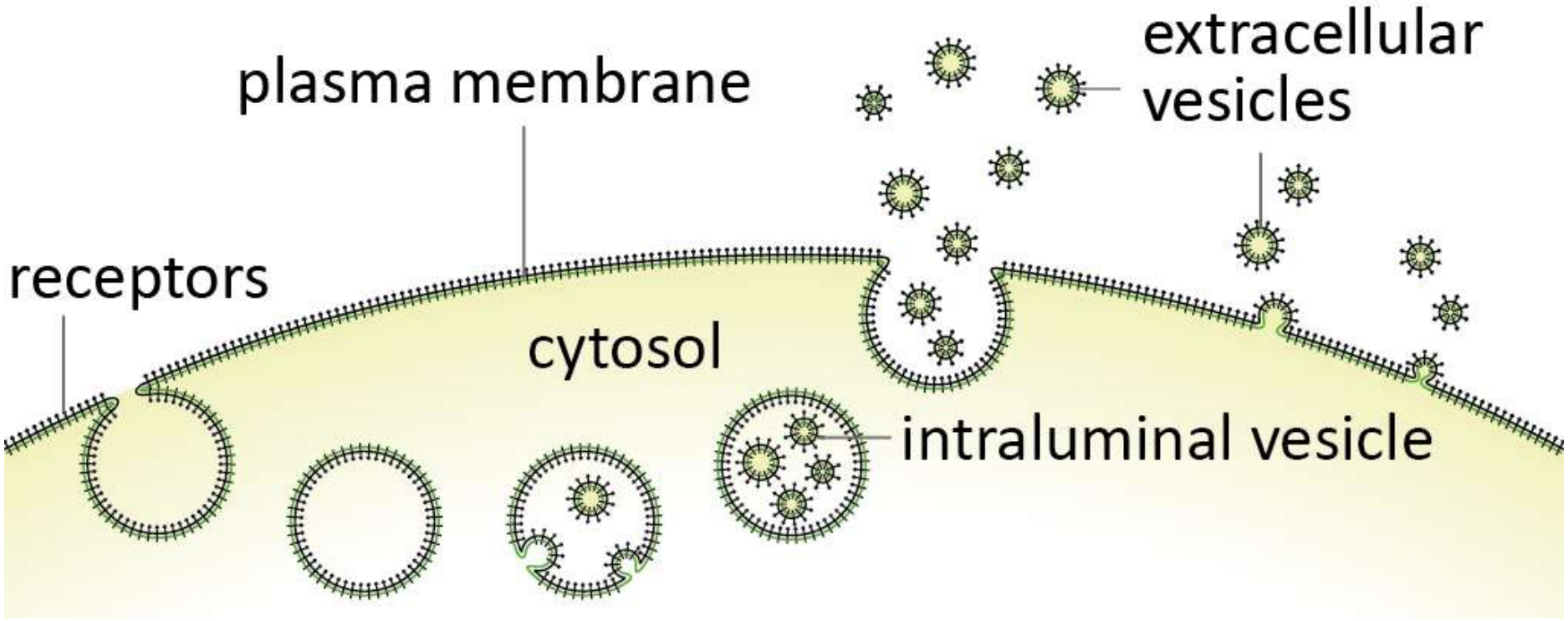
# Extracellular Vesicles (EVs)




*van der Pol, E., et al., Pharmacol Rev 2012*

- Biological nanoparticles with receptors, DNA, RNA
- Reflect the status of their cell of origin
- Clinically relevant

# EVs are challenging to measure

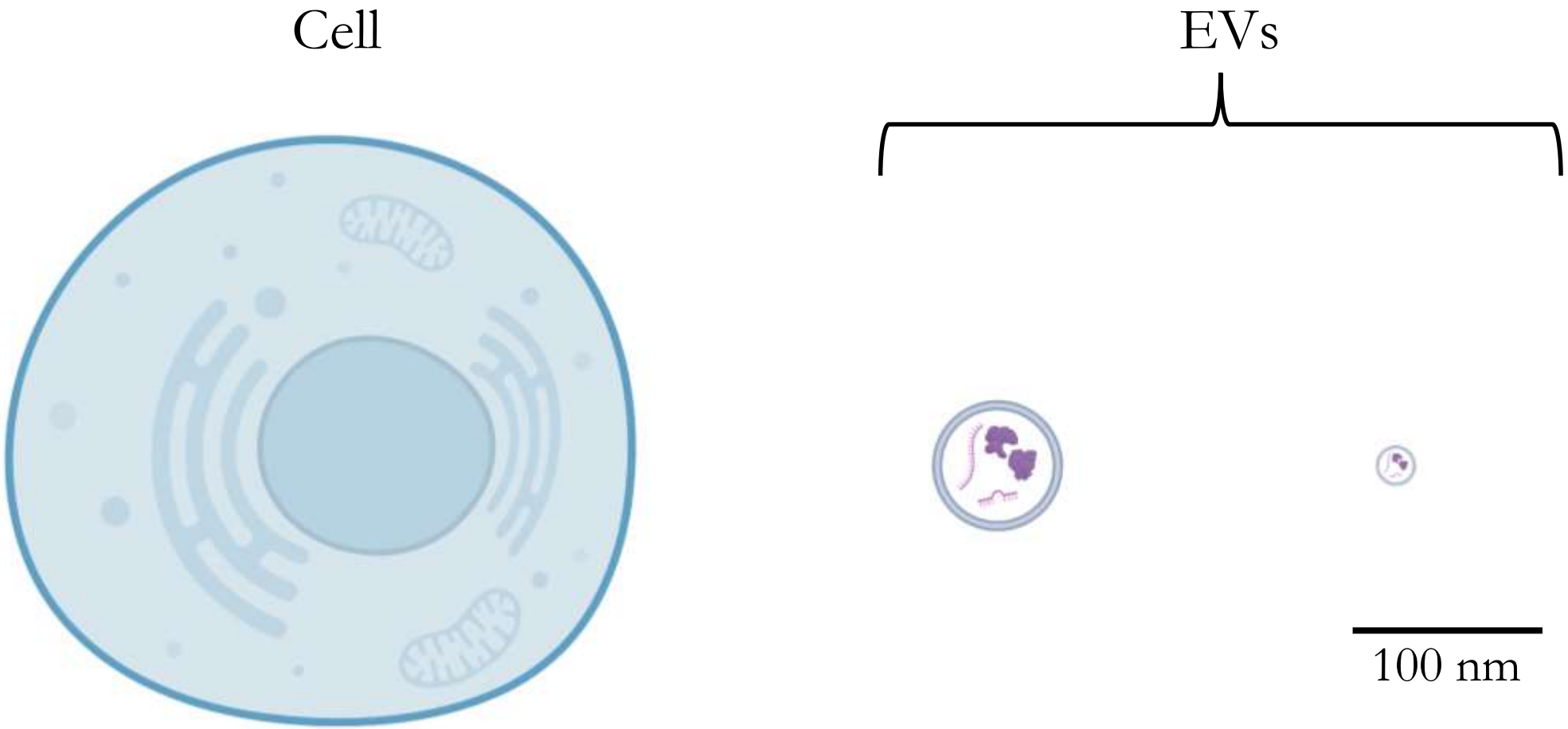
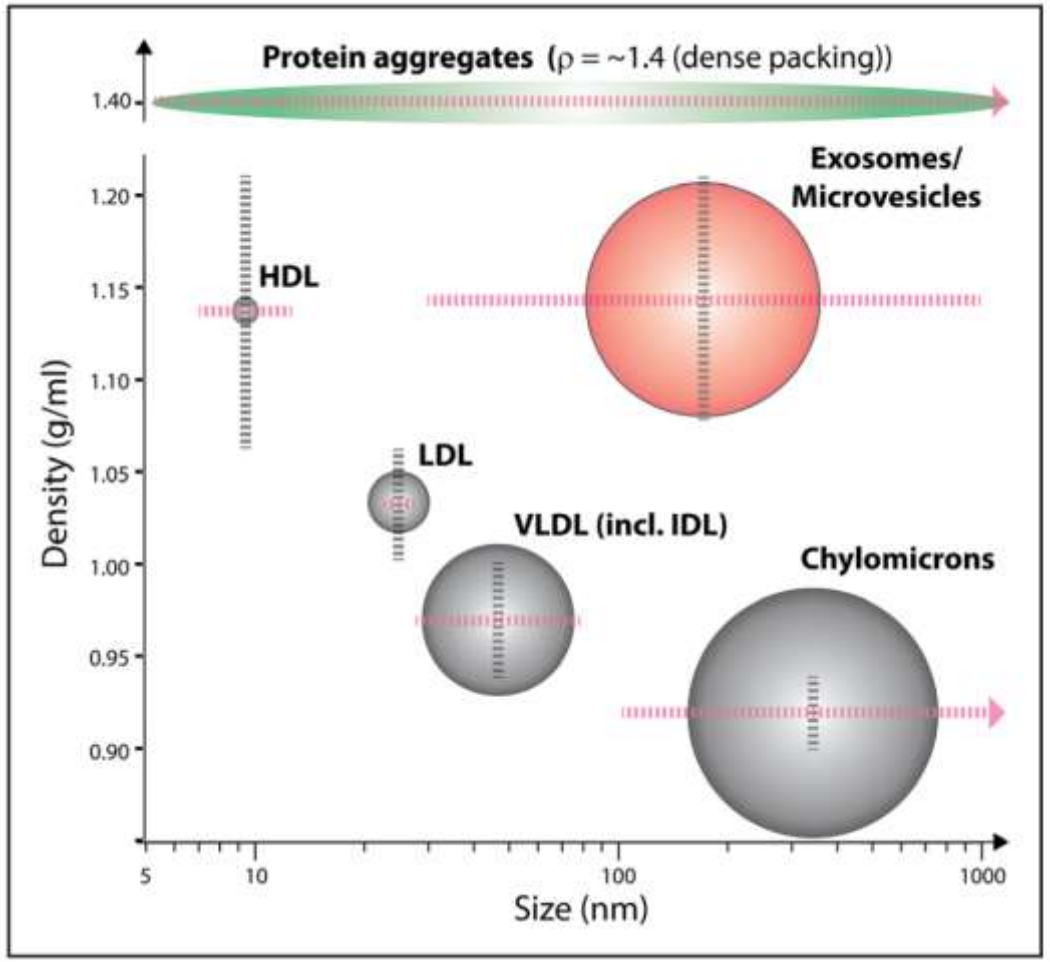



Image generated using biorender.com

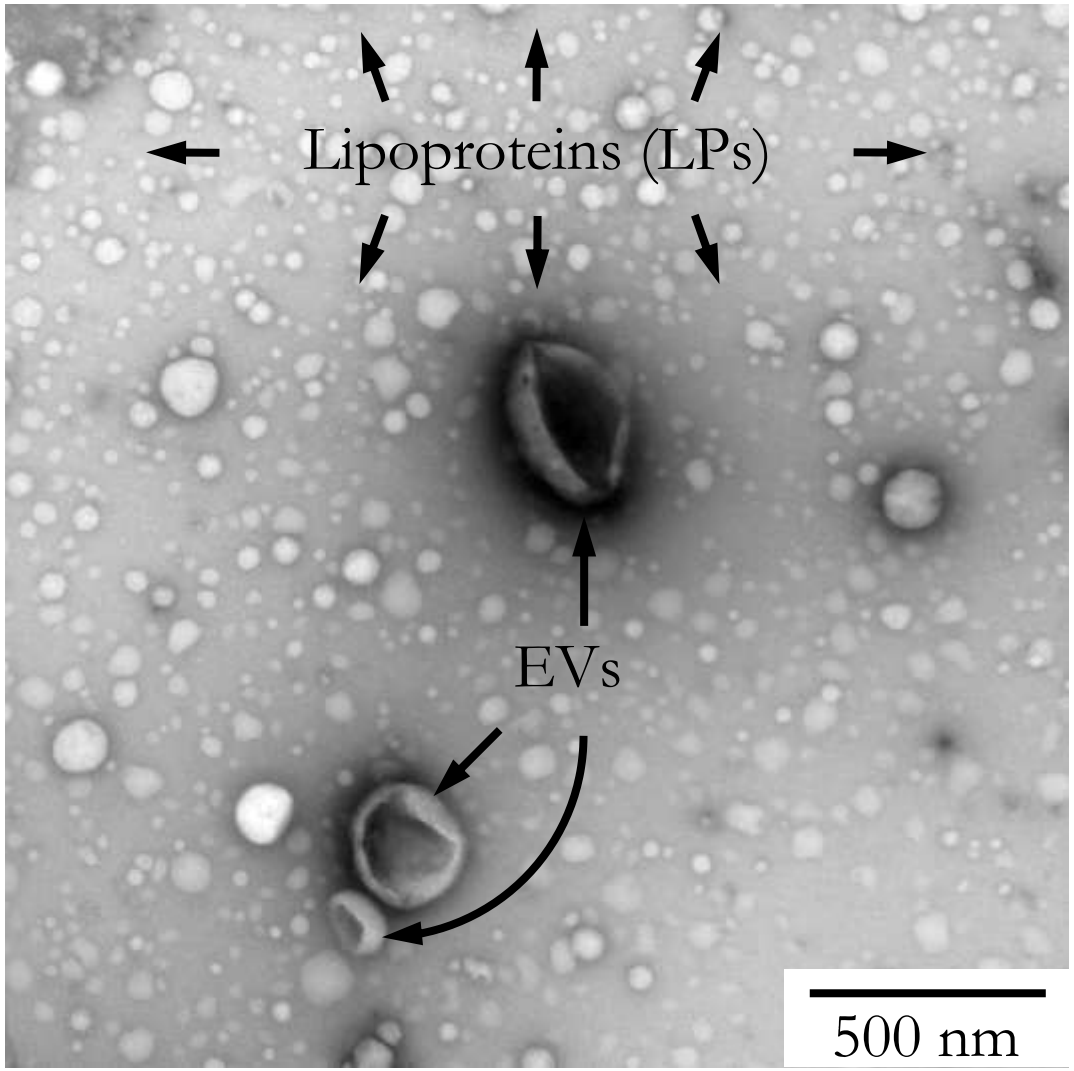
# (Human) Plasma associated challenges

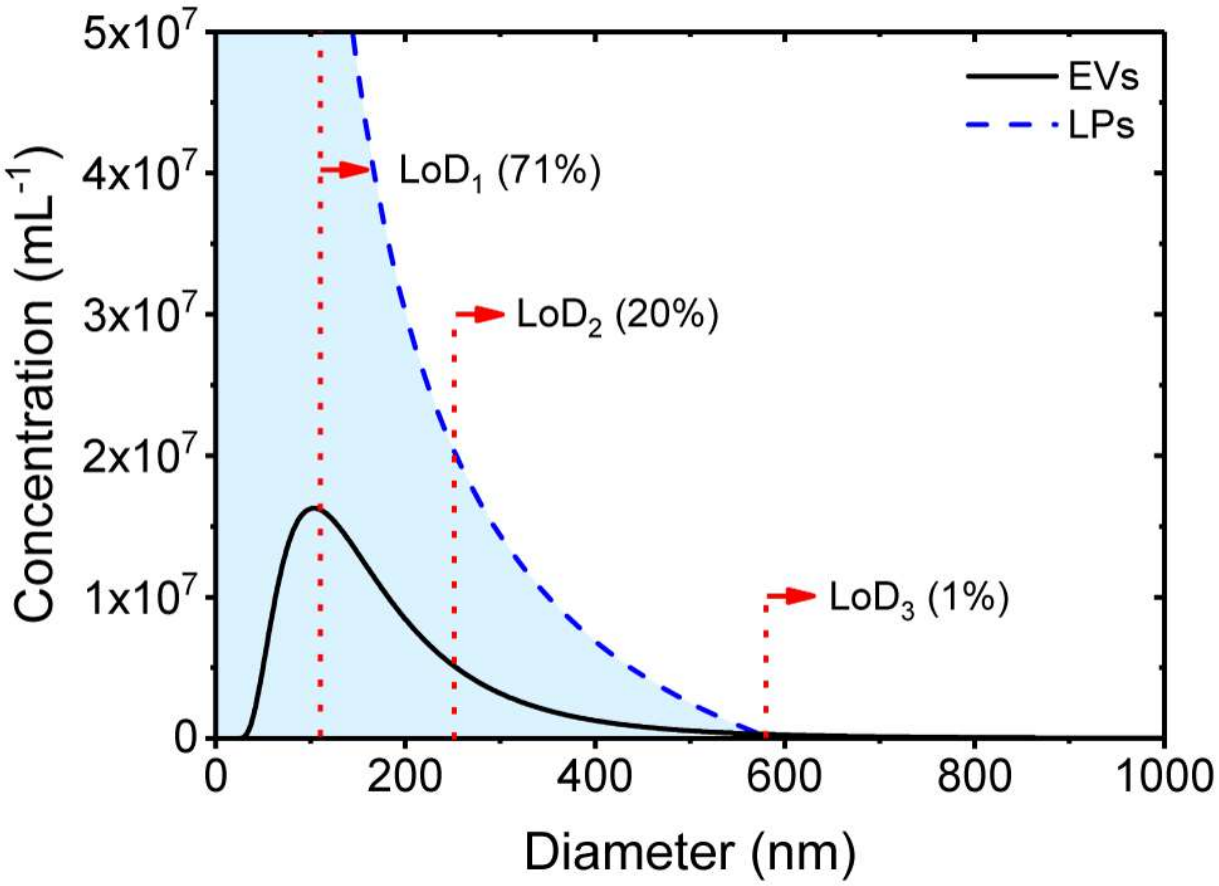



*Simonsen, J.B., Circ Res 2017.*

- Biochemical properties (size & density) of EVs overlap with **lipoproteins**
- Total plasma EV pool is a **heterogeneous mixture**

# (Human) Plasma associated challenges



# Aim

To decipher the information represented by EVs

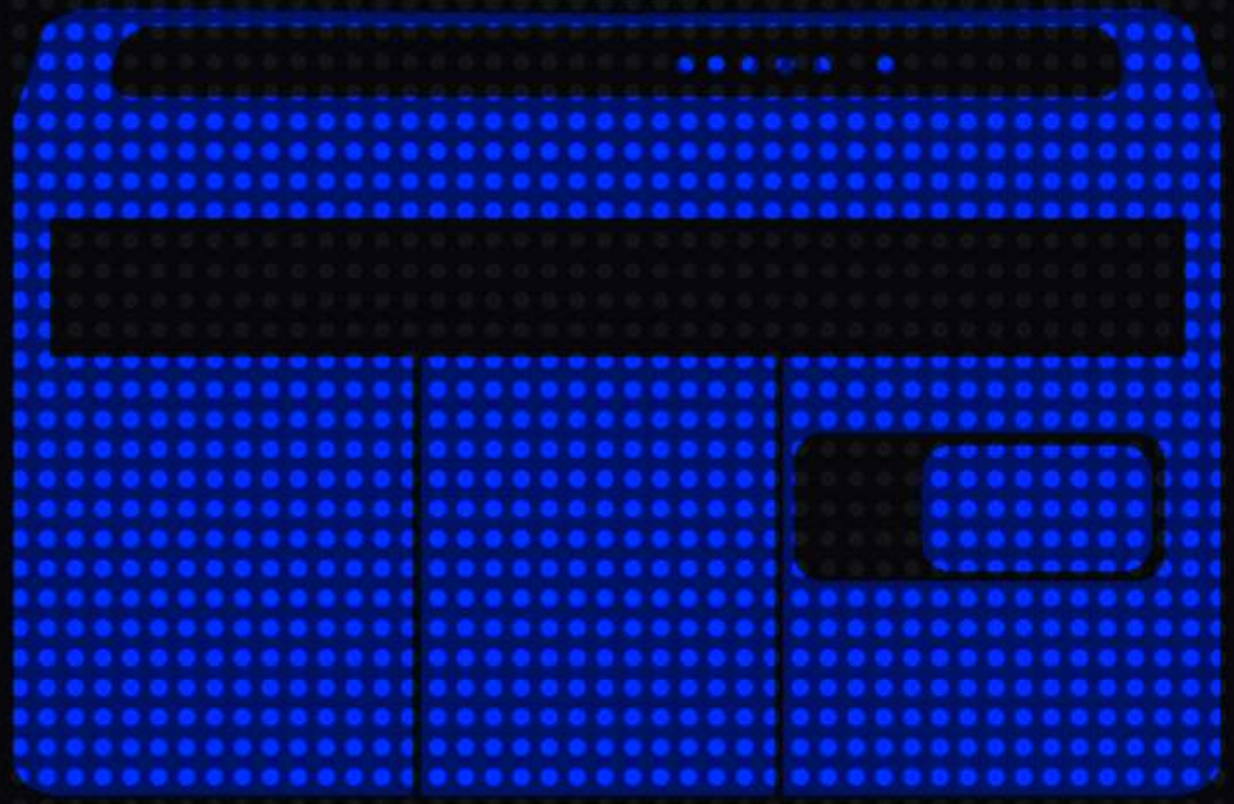
Part I:

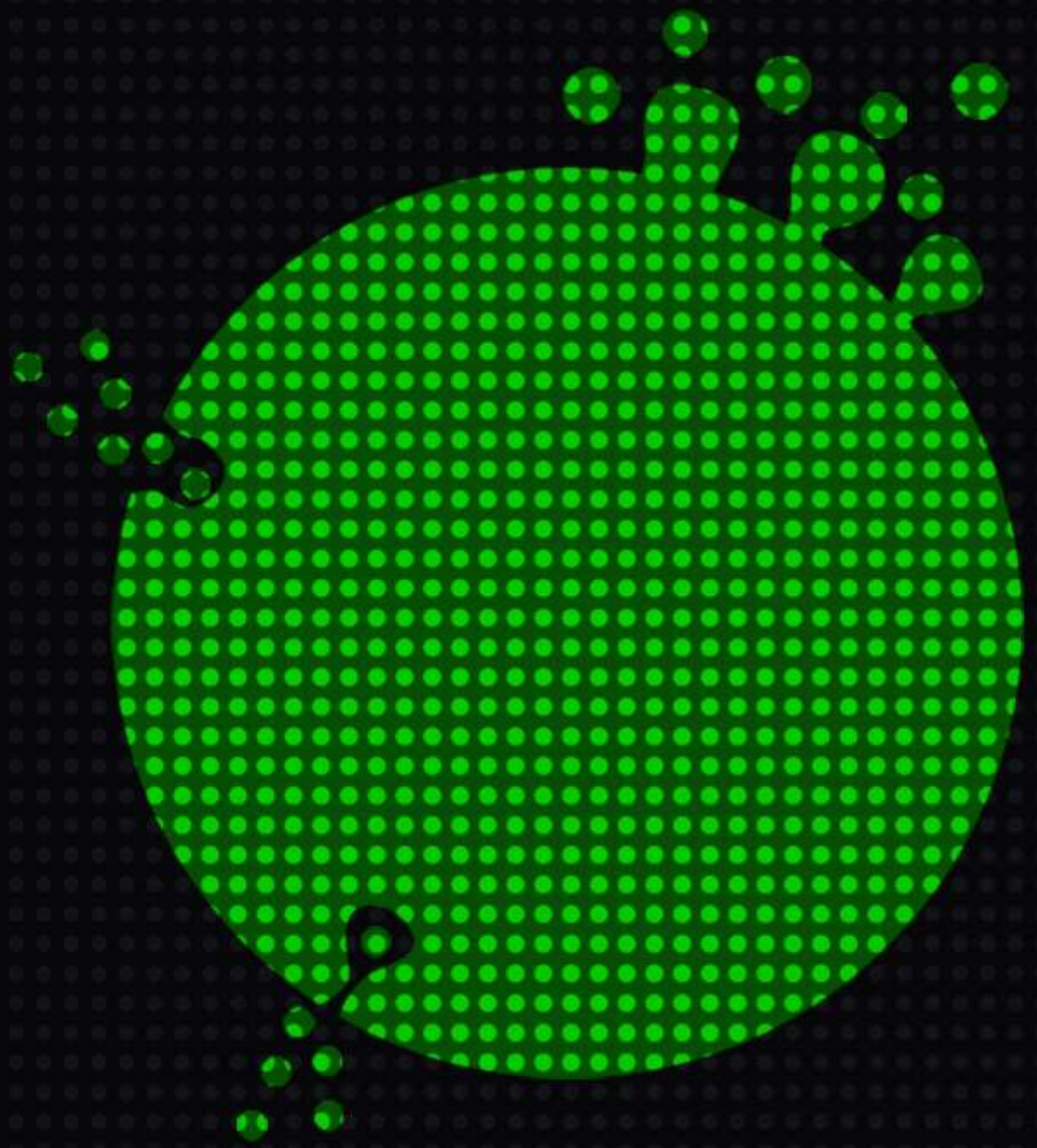
Develop a standardized assay to measure single EVs **directly** in diluted, labelled human plasma

Part II:

Apply the developed assay in the context of clinical kidney transplantation

# Part I

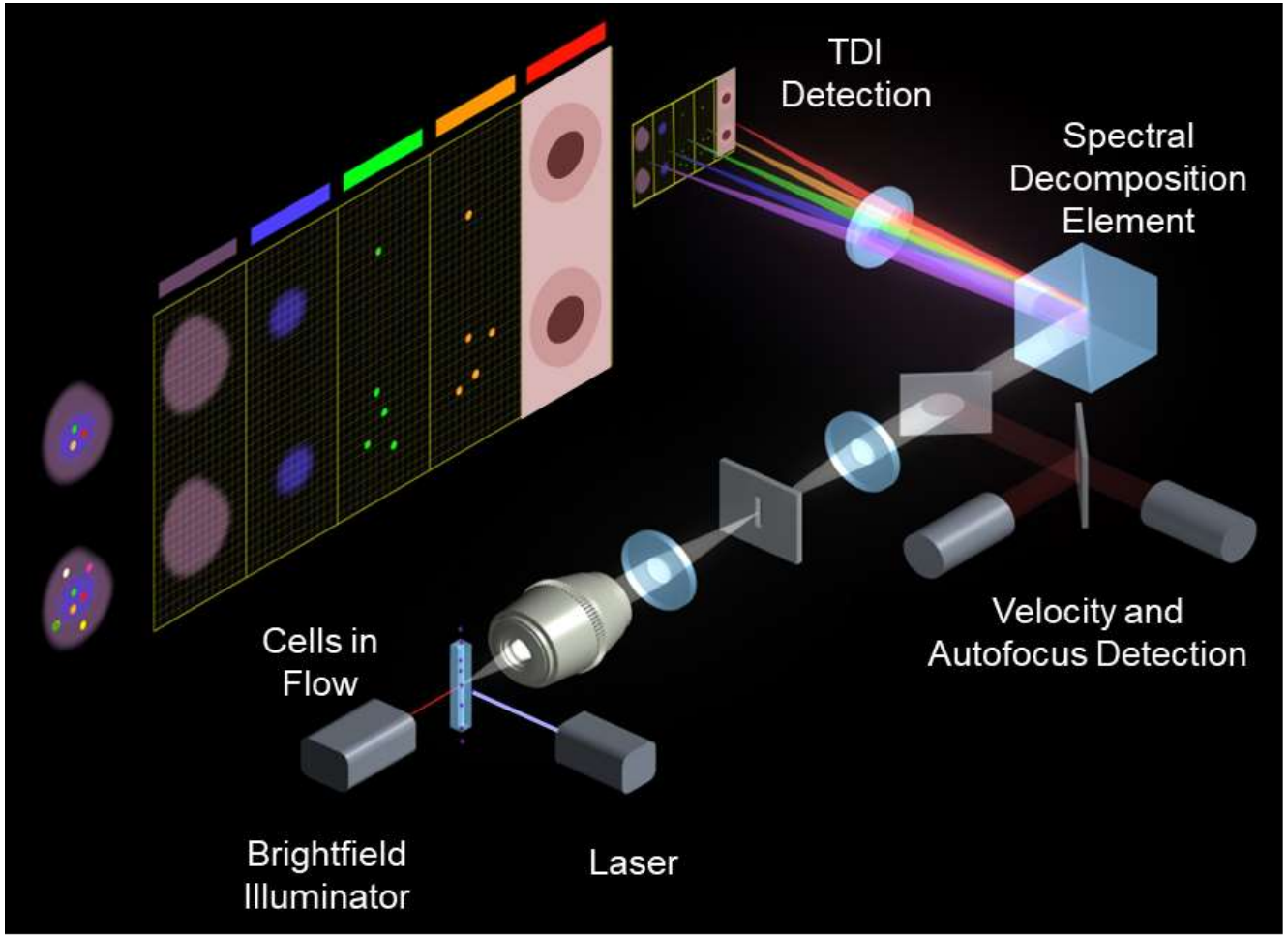




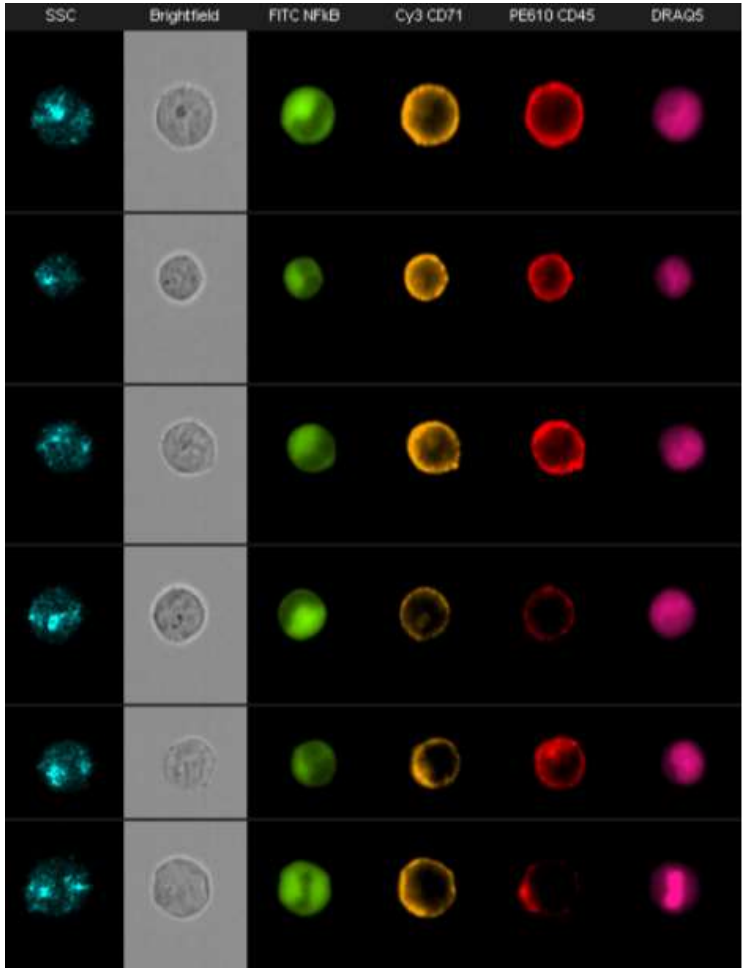
## Assay development

# Imaging Flow Cytometry

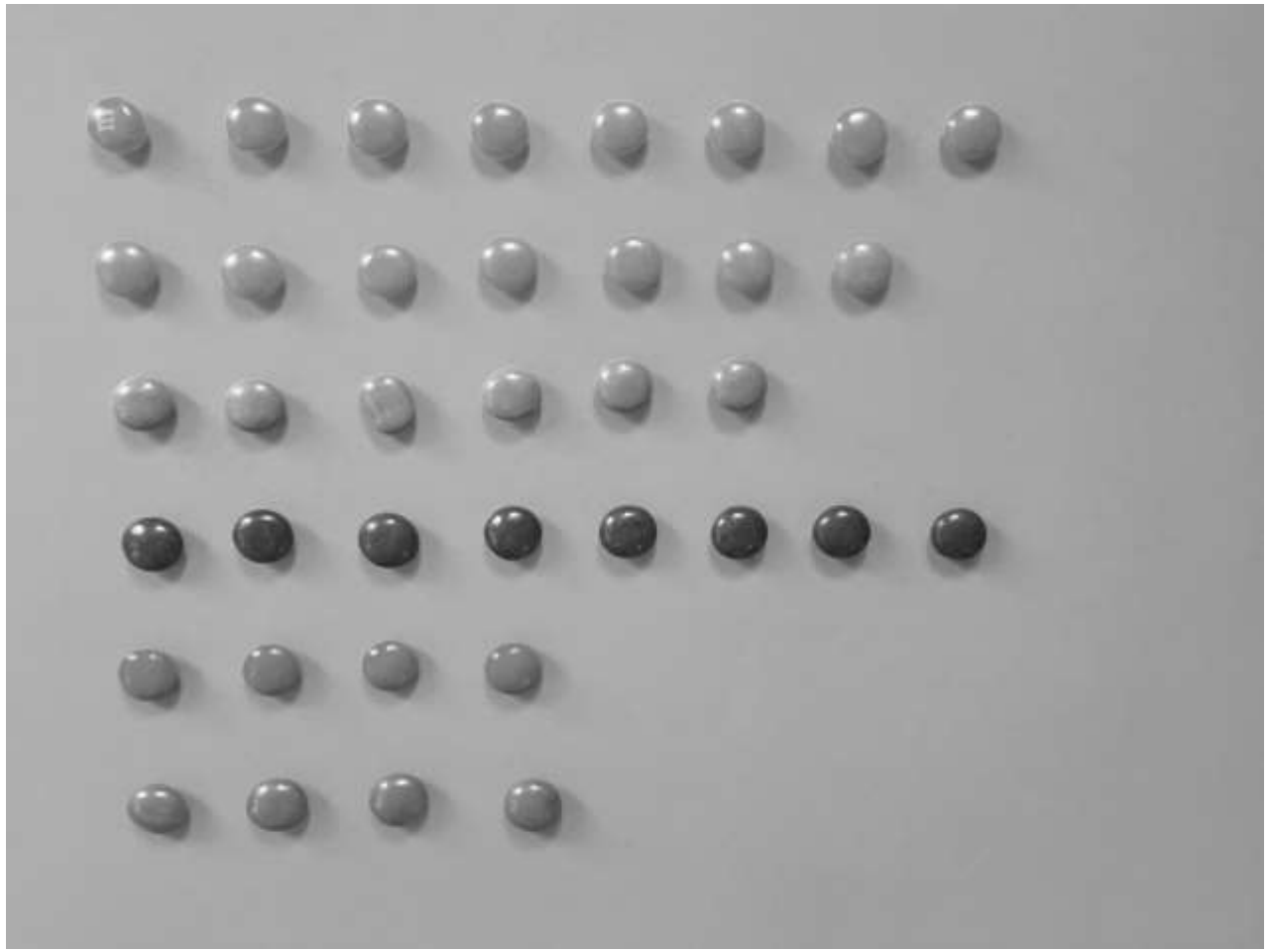
ImageStreamX MKII




Multi-Spectral Imagery

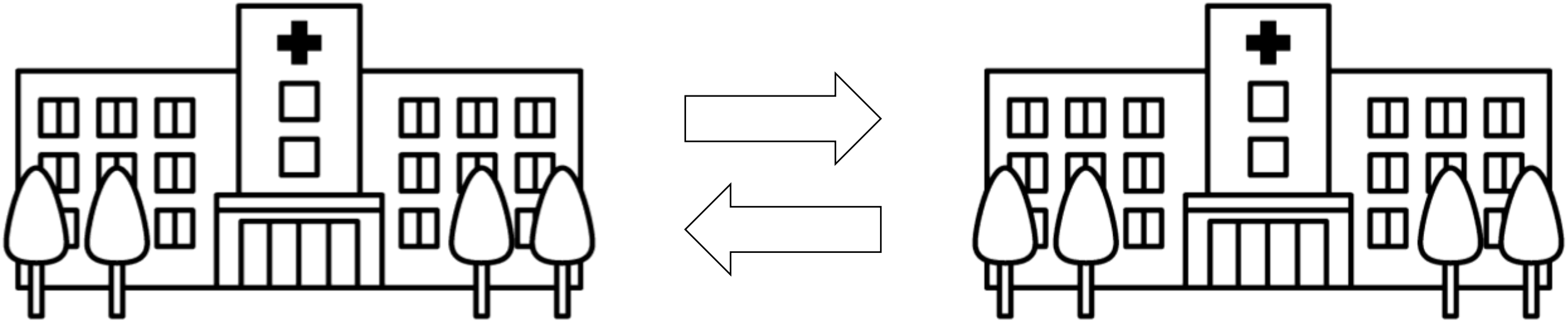


# Goal 1: Direct detection of single EVs



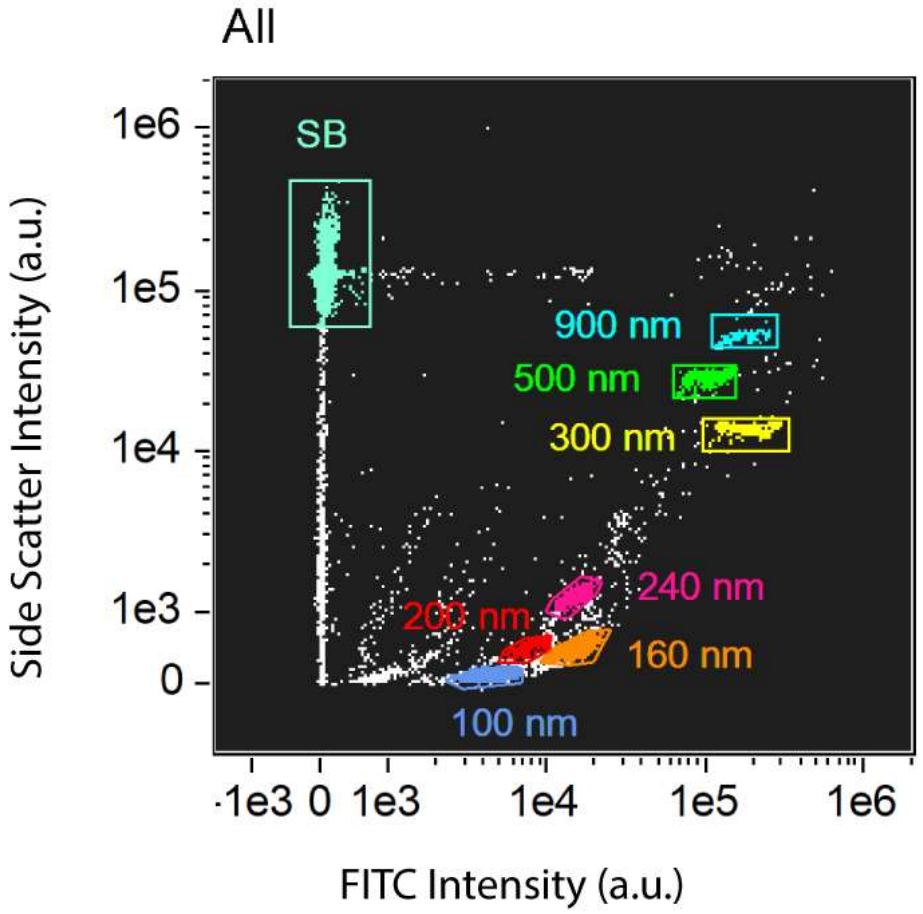
# Goal 2: Characterization of single EVs

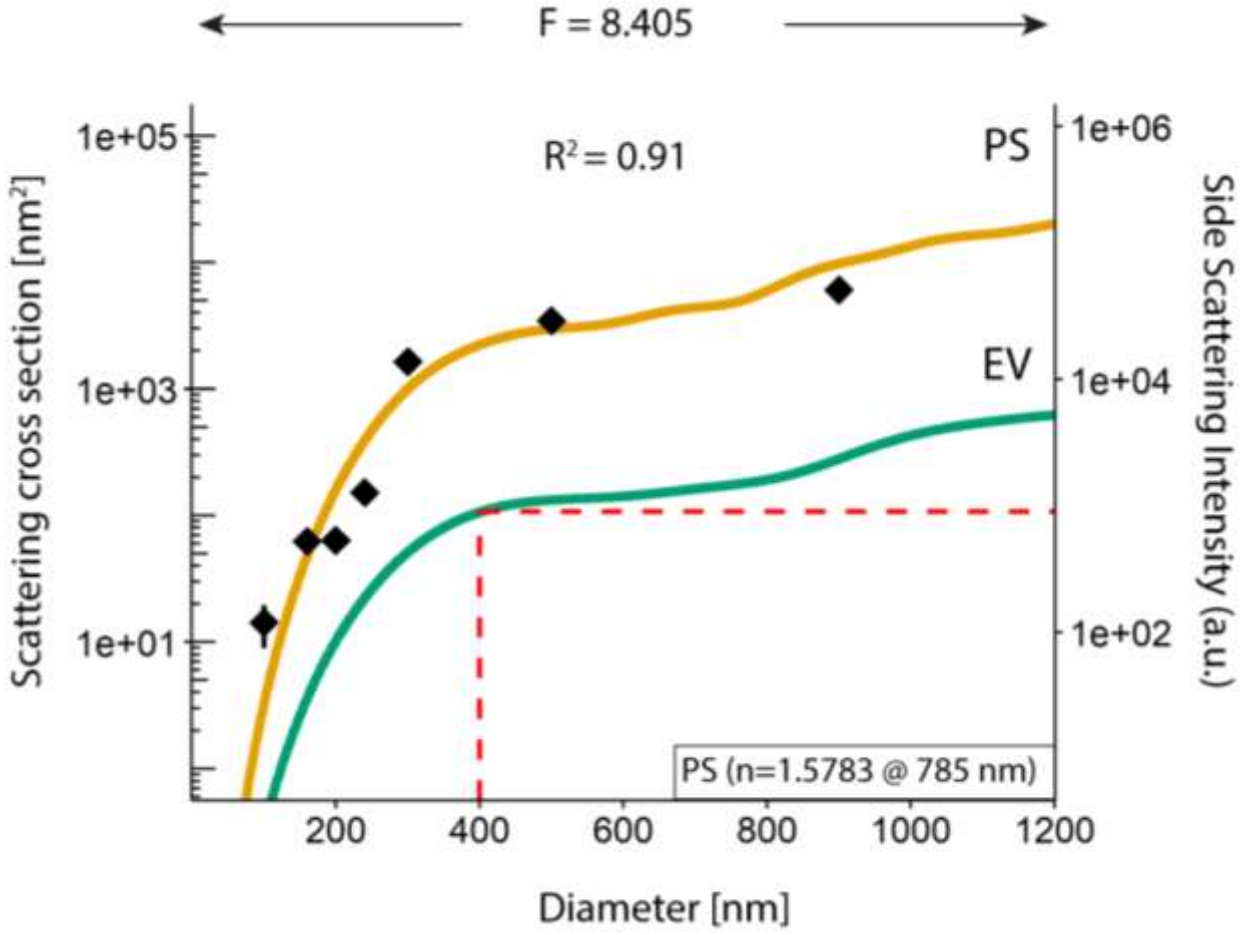
# Goal 3: Standardization of assay results



Requirement: <u>full</u> calibration of the platform

# IFCM SSC Size Calibration

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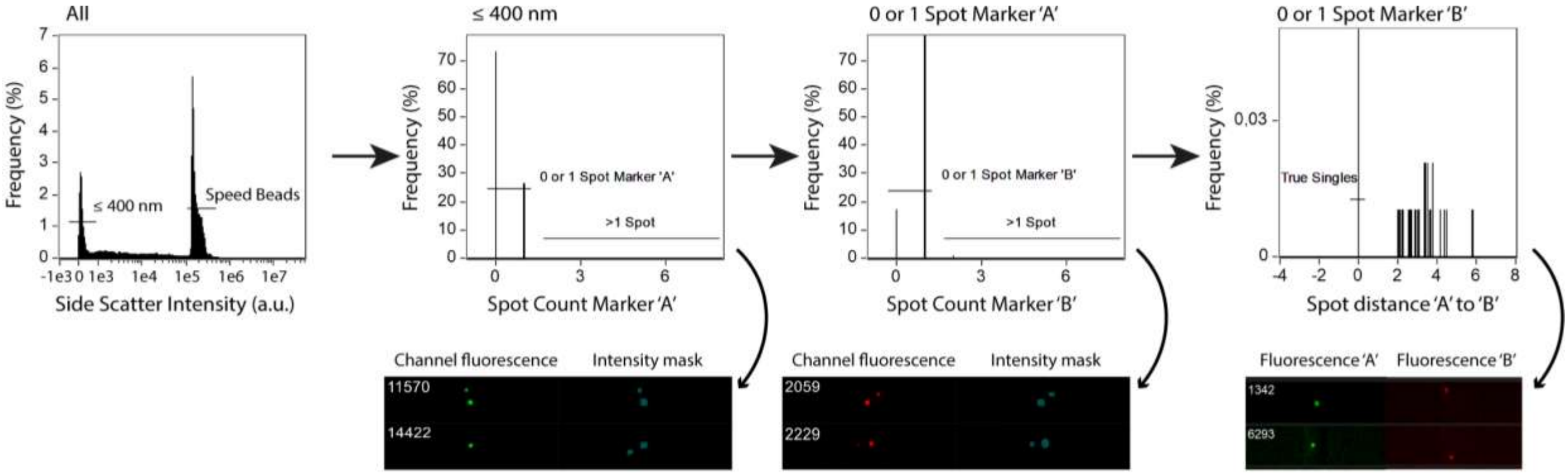
# Gating Strategy

Selection of single fluorescent EVs ≤ 400 nm in plasma:

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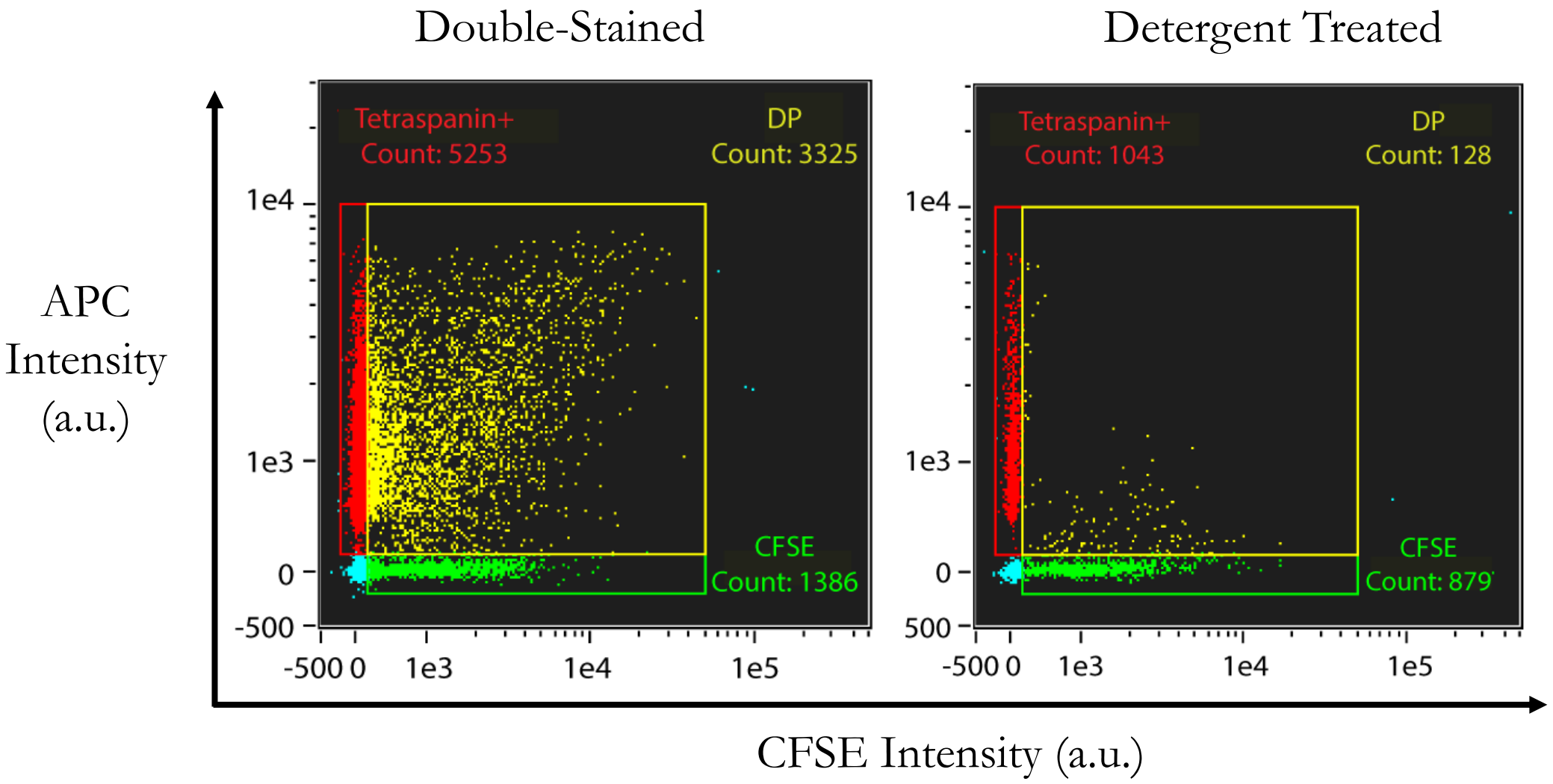
# PPP stained with α-Tetraspanin mixture & CFDA-SE

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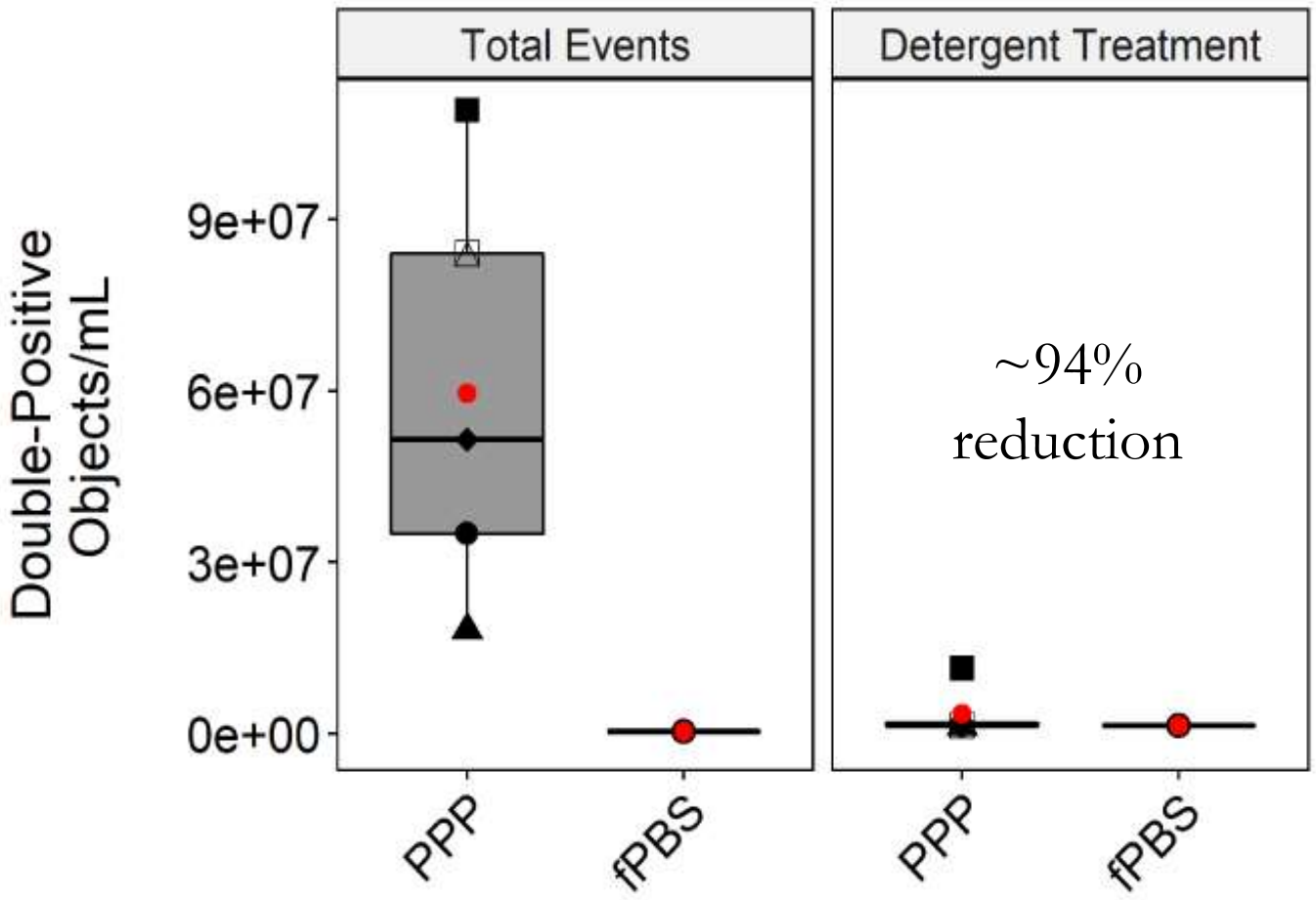
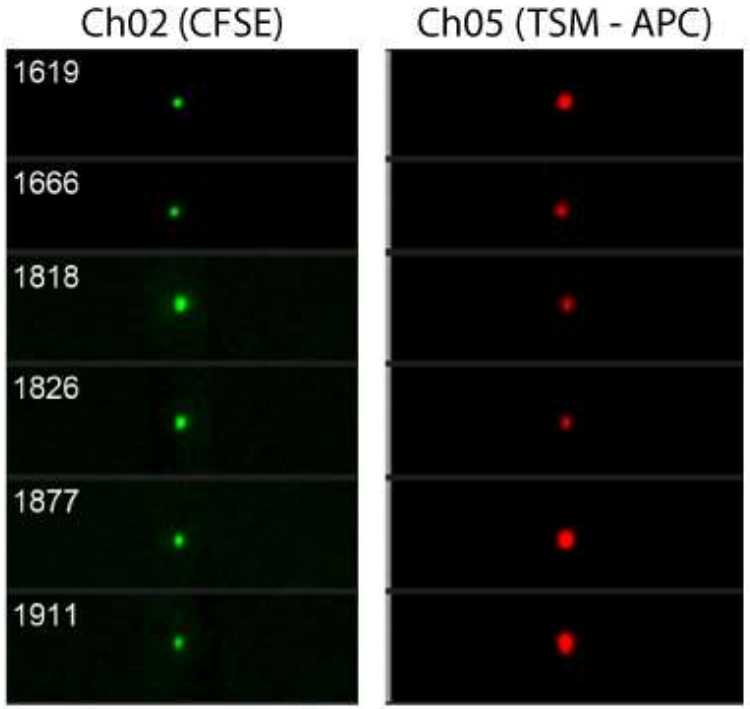
# Detergent Treatment

CFSE+Tetraspanin+ EVs




Double-positive EVs

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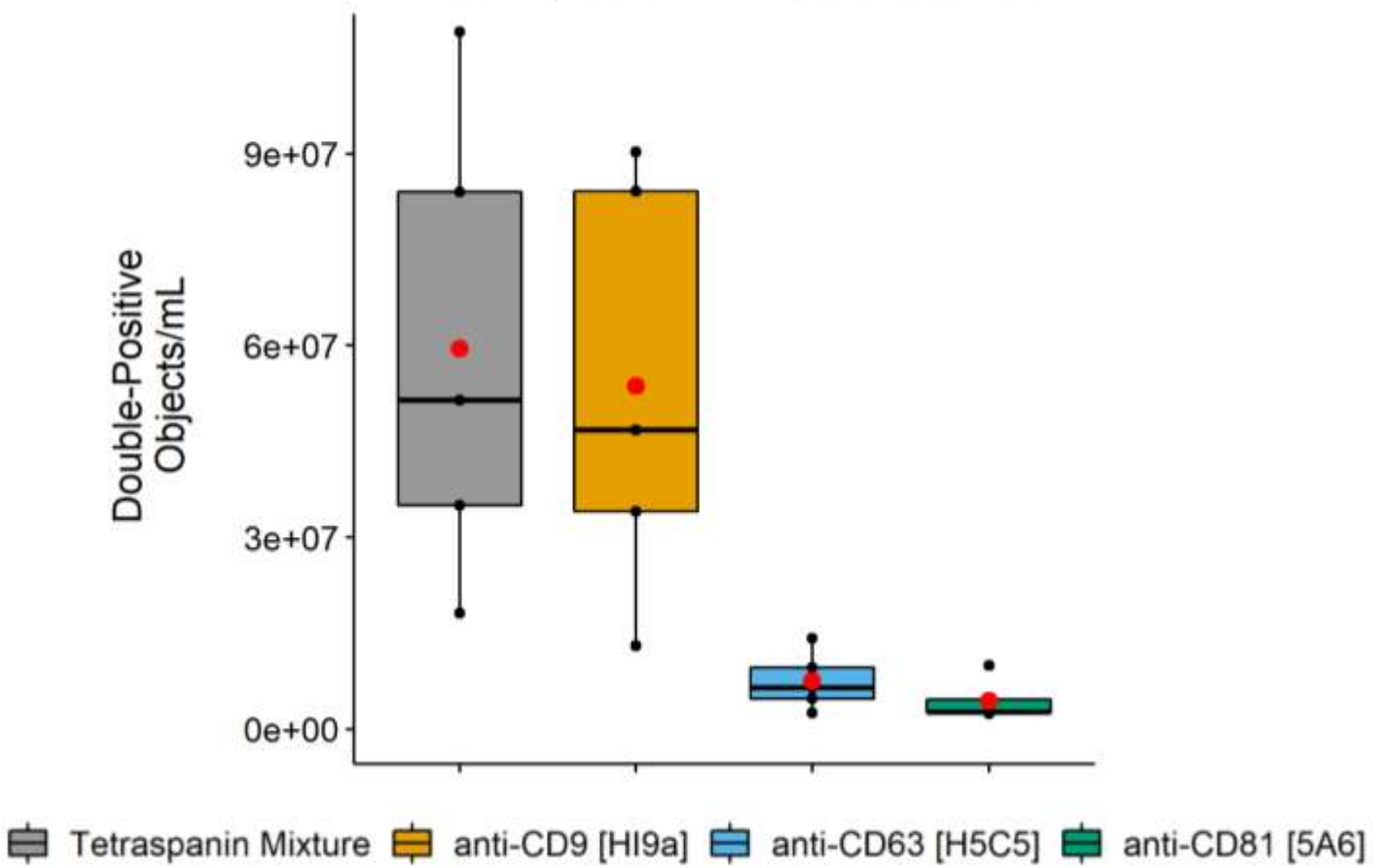
# EV Characterization - Tetraspanin Distribution

CFSE+Tetraspanin+ concentrations
per tetraspanin labelling

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communications biology

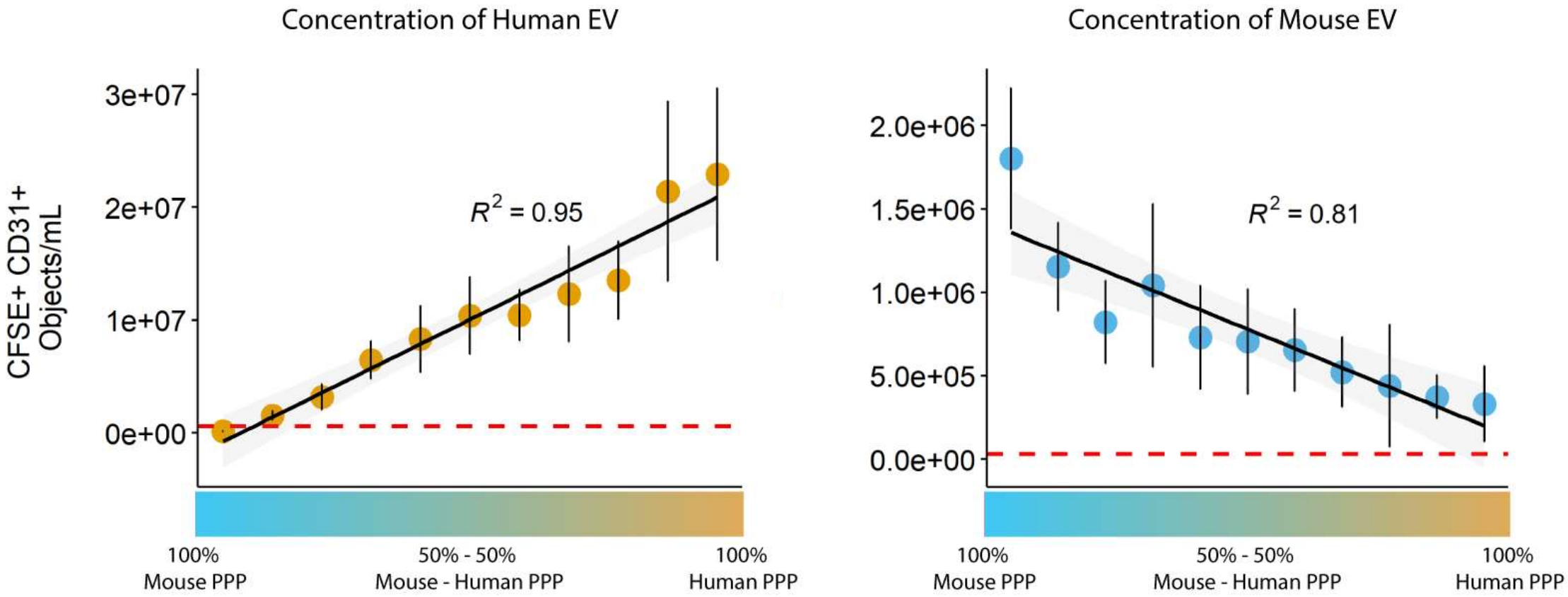
Article | Open Access | Published: 29 June 2022

# An imaging flow cytometry-based methodology for the analysis of single extracellular vesicles in unprocessed human plasma

Wouter W. Woud, Edwin van der Pol, Erik Mul, Martin J. Hoogduijn, Carla C. Baan, Karin Boer & Ana Merino

*Communications Biology* **5**, Article number: 633 (2022) | Cite this article

# Direct detection of single EV subsets ≤400 nm in complex biofluids with IFCM

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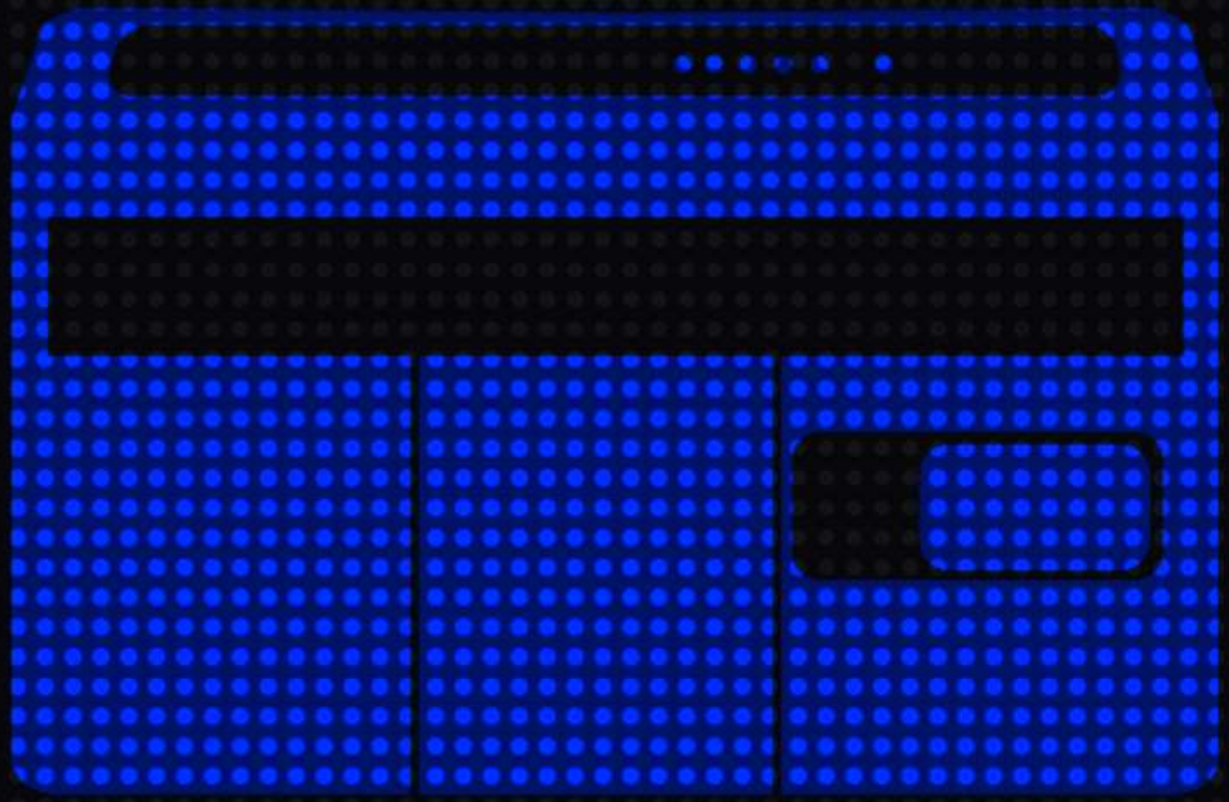
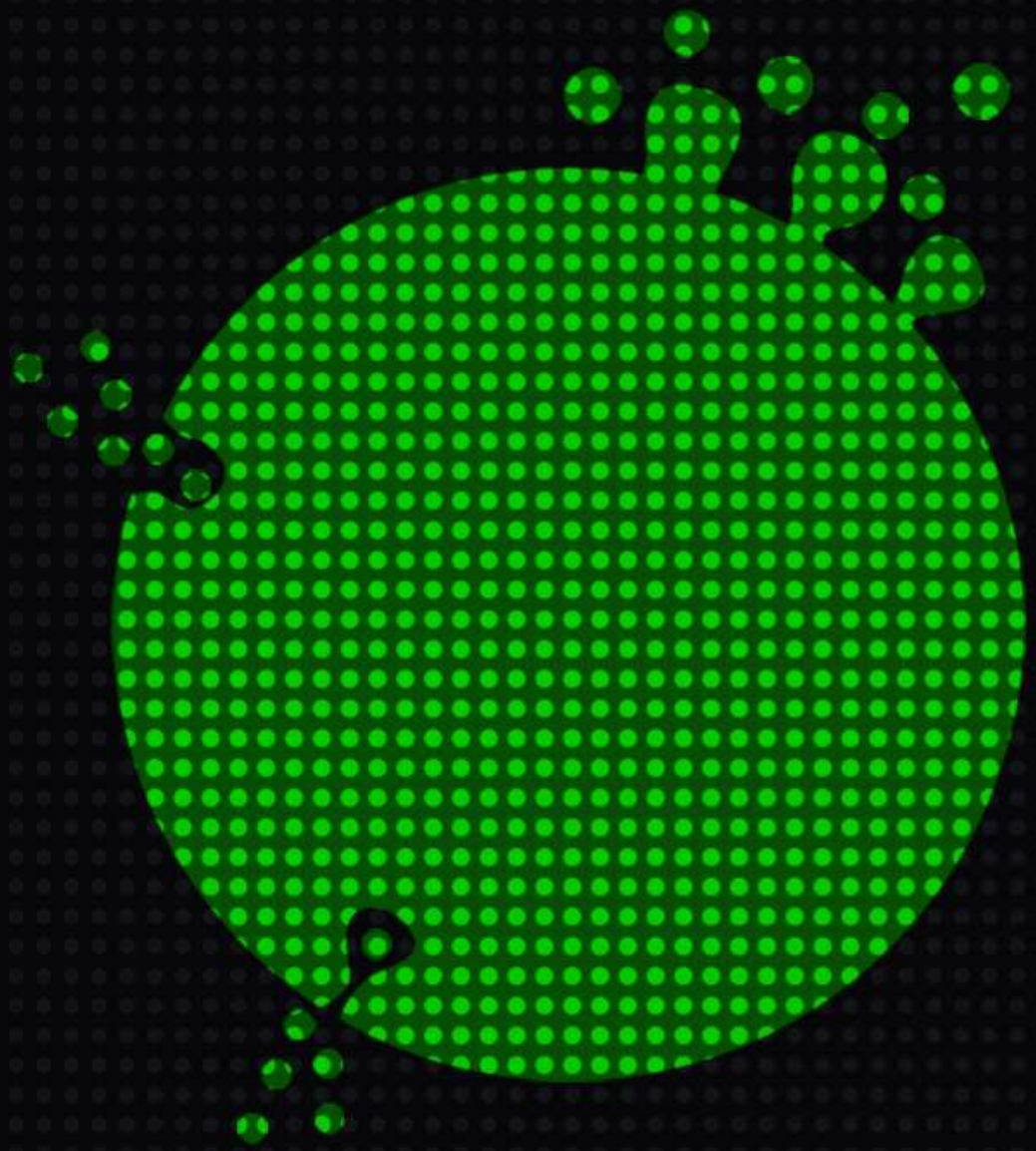
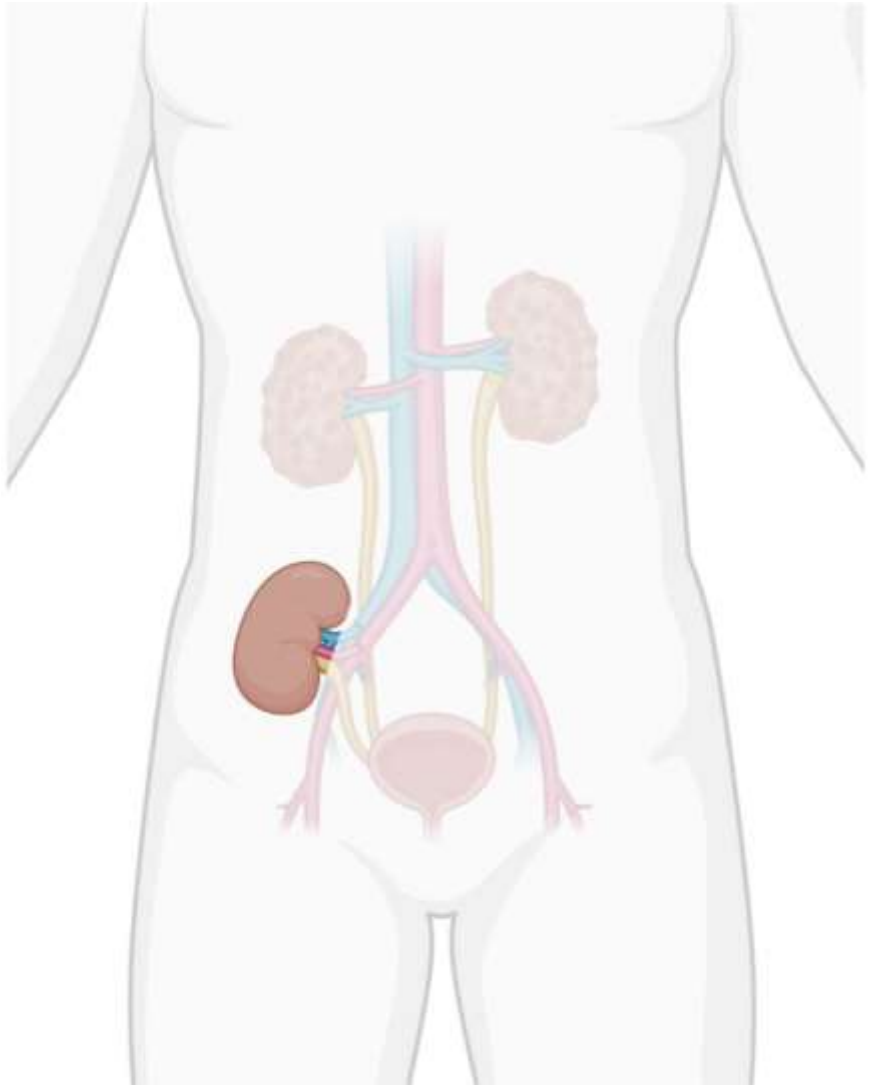
# Part II





## Clinical applications

# Kidney Transplantation (KTx)



EVs as potential indicators of:

- ❖ Kidney status <u>before</u> KTx – *Ex vivo*
- ❖ Kidney status <u>after</u> KTx – *In vivo*

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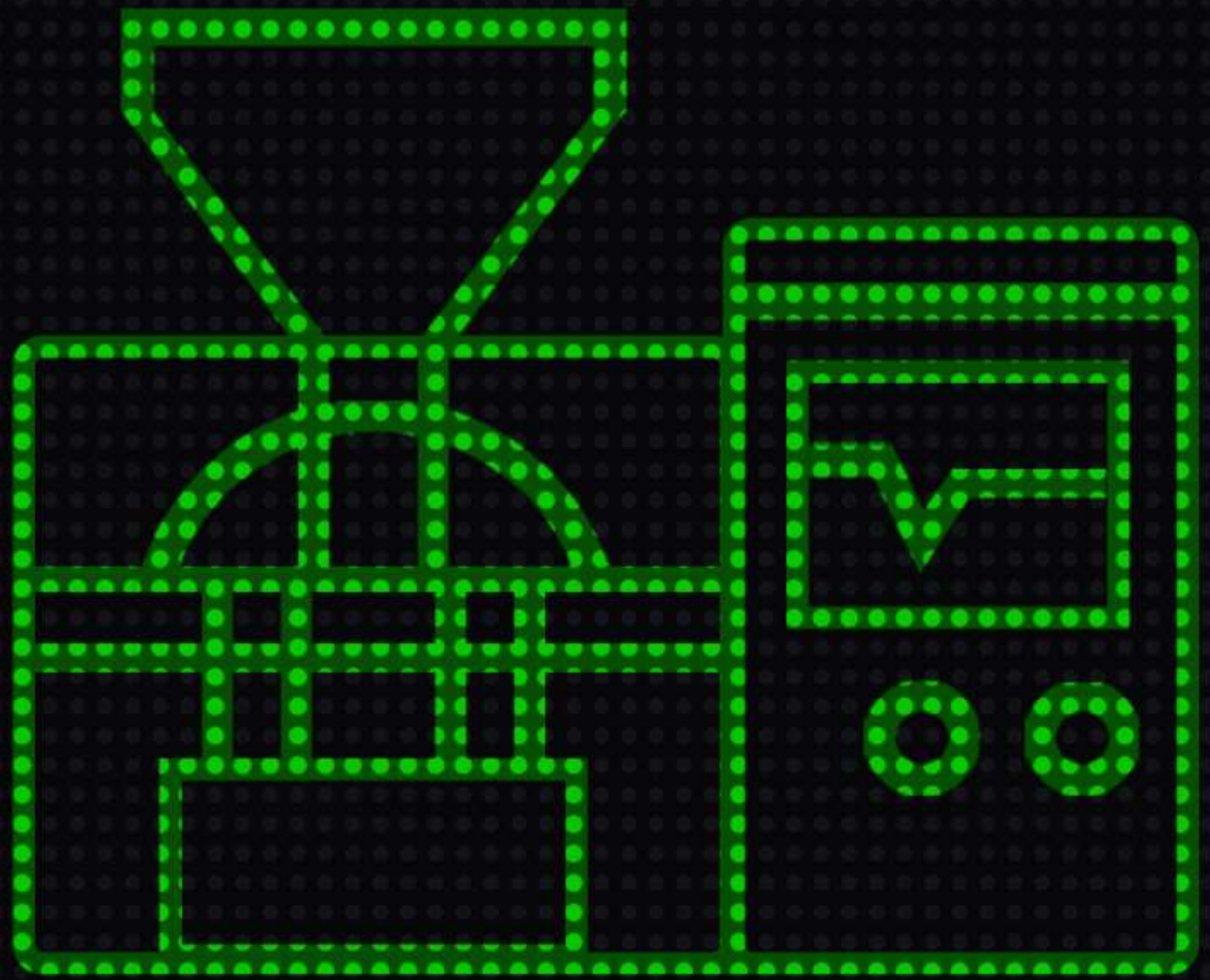
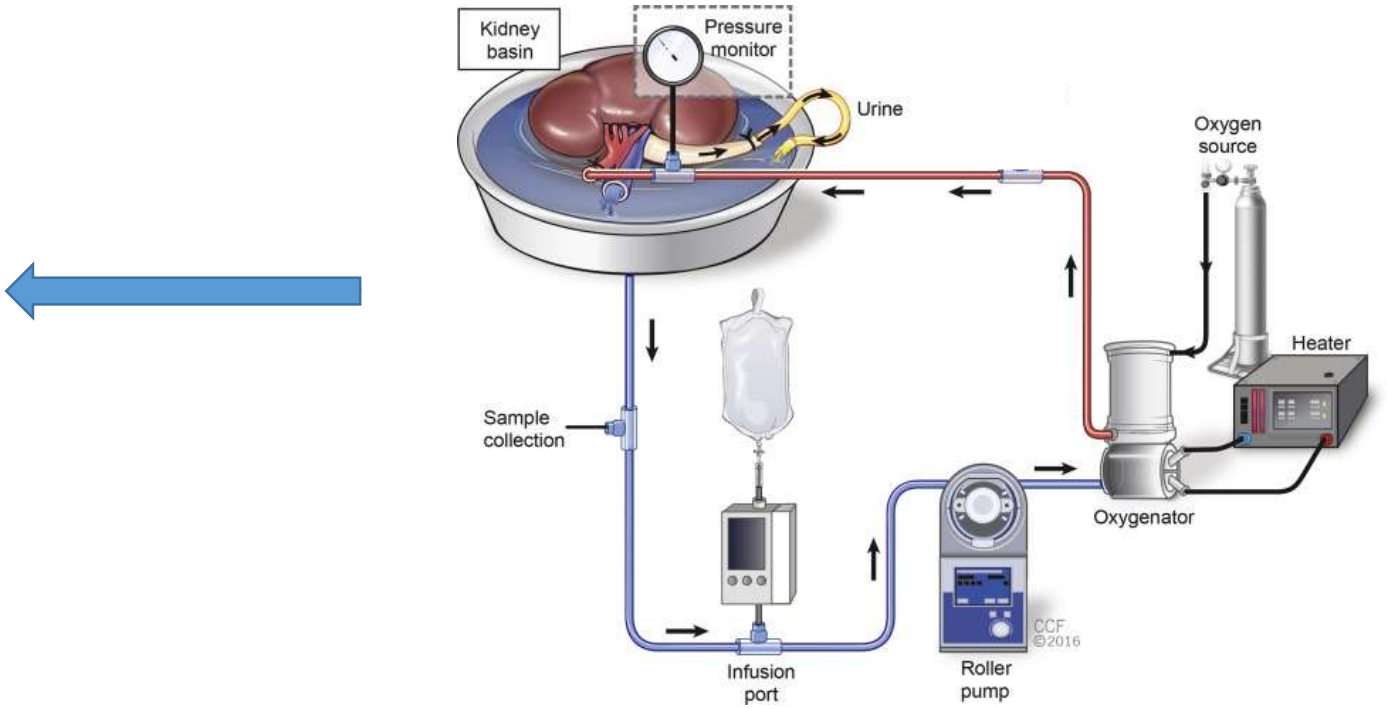
# *Ex vivo*

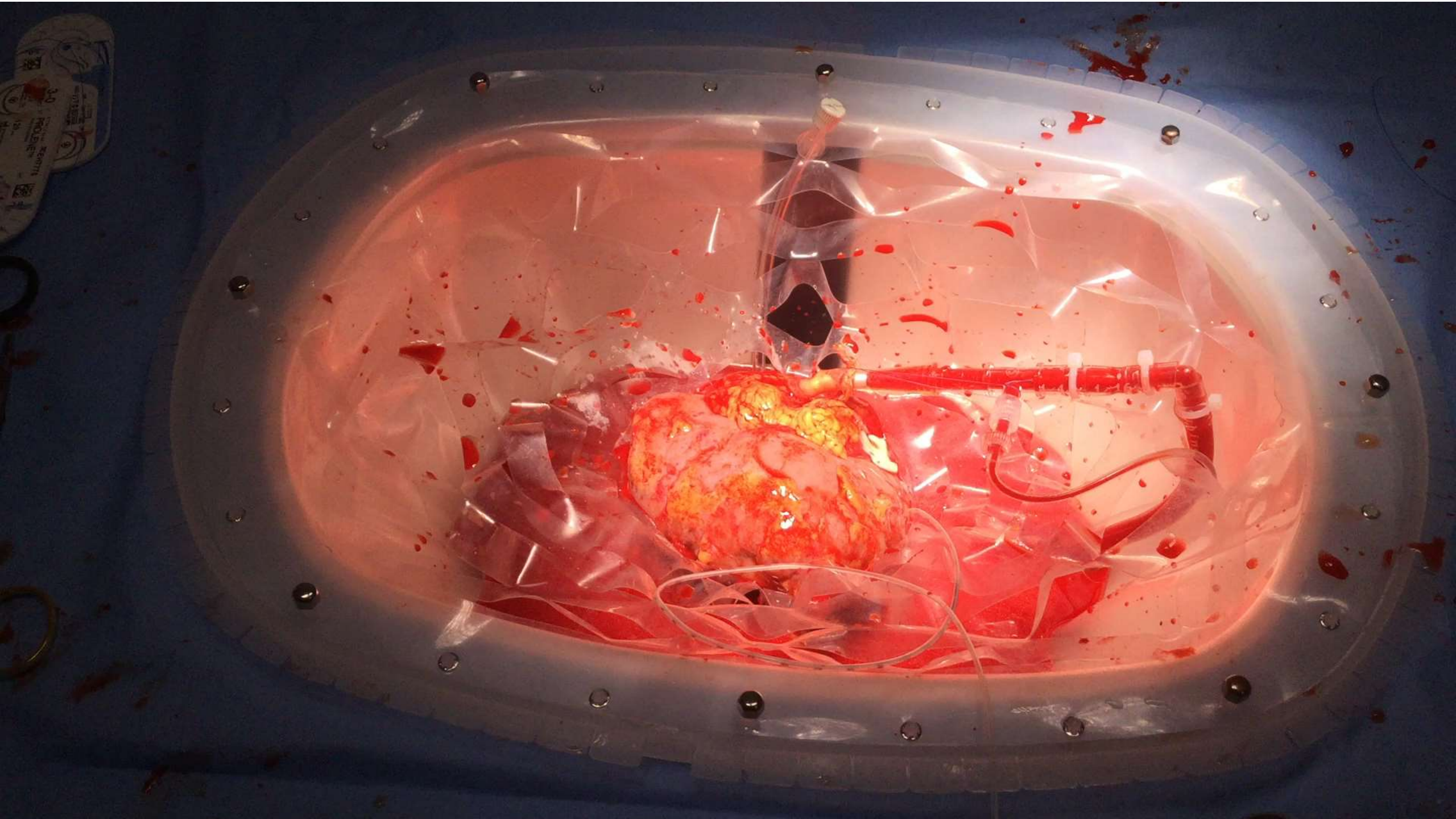


# Kidney status <u>before</u> KTx – *Ex vivo*

## Normothermic Machine Perfusion

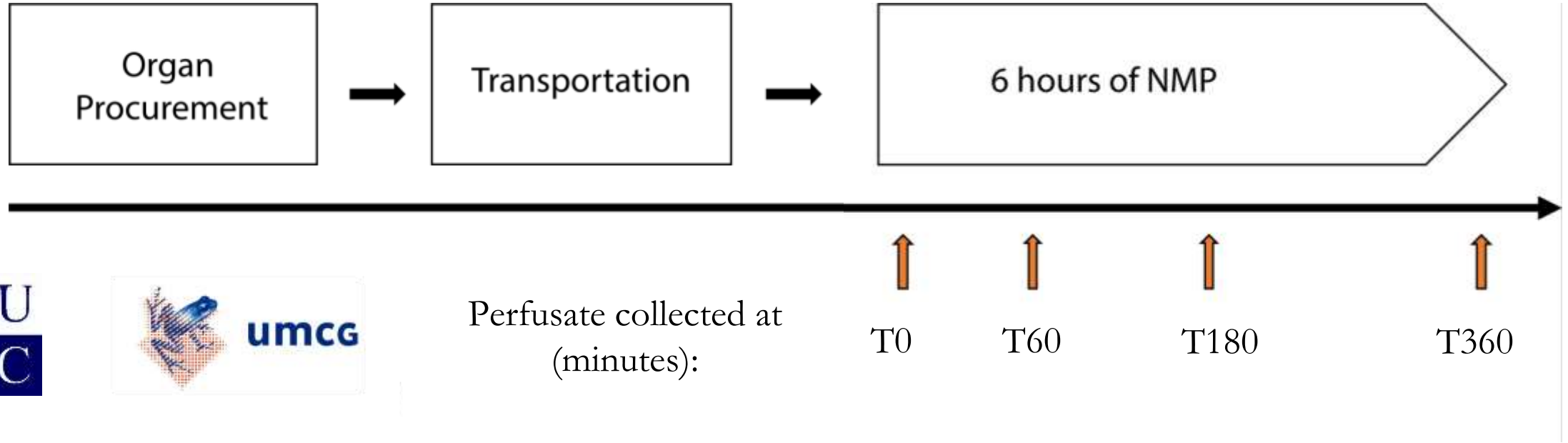
- Activated metabolism
- Assessment of graft status prior to KTx through analysis of biomarkers in perfusion fluids





# Study Design:

Quantify and characterize EV release in perfusion fluids of 8 discarded sub-marginal kidneys during 6 hours of NMP.

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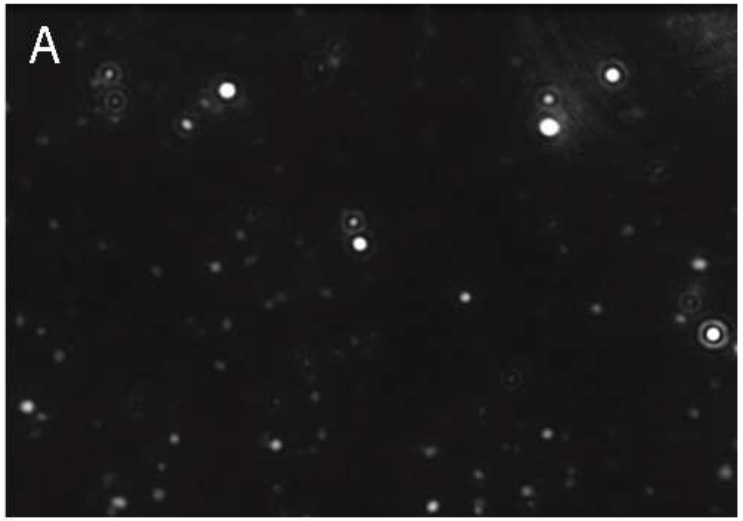
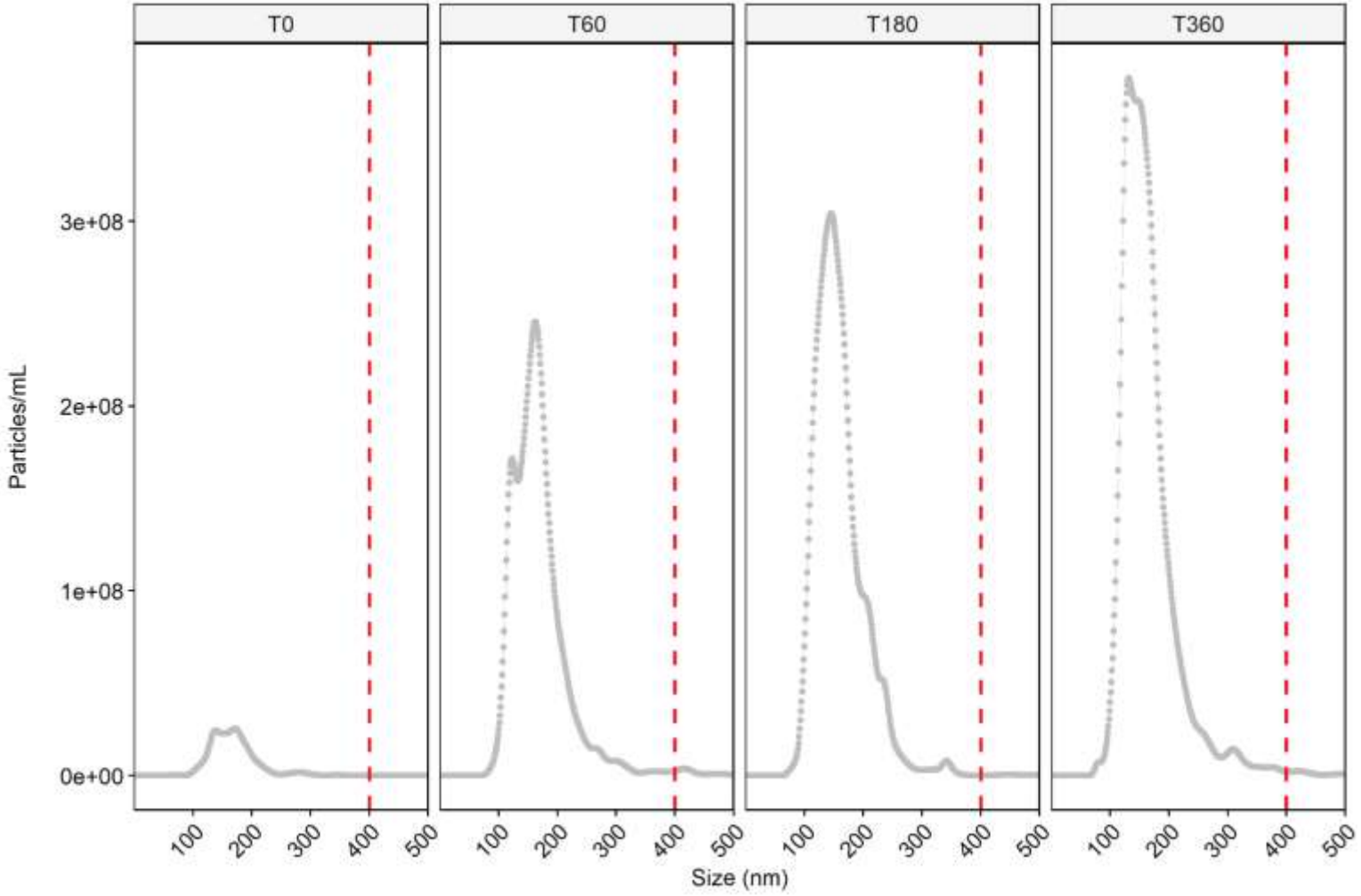
# ECD kidneys release nanoparticles during NMP

Perfusates analysed with Nanoparticle Tracking Analysis




Kidney-derived nanoparticles in suspension

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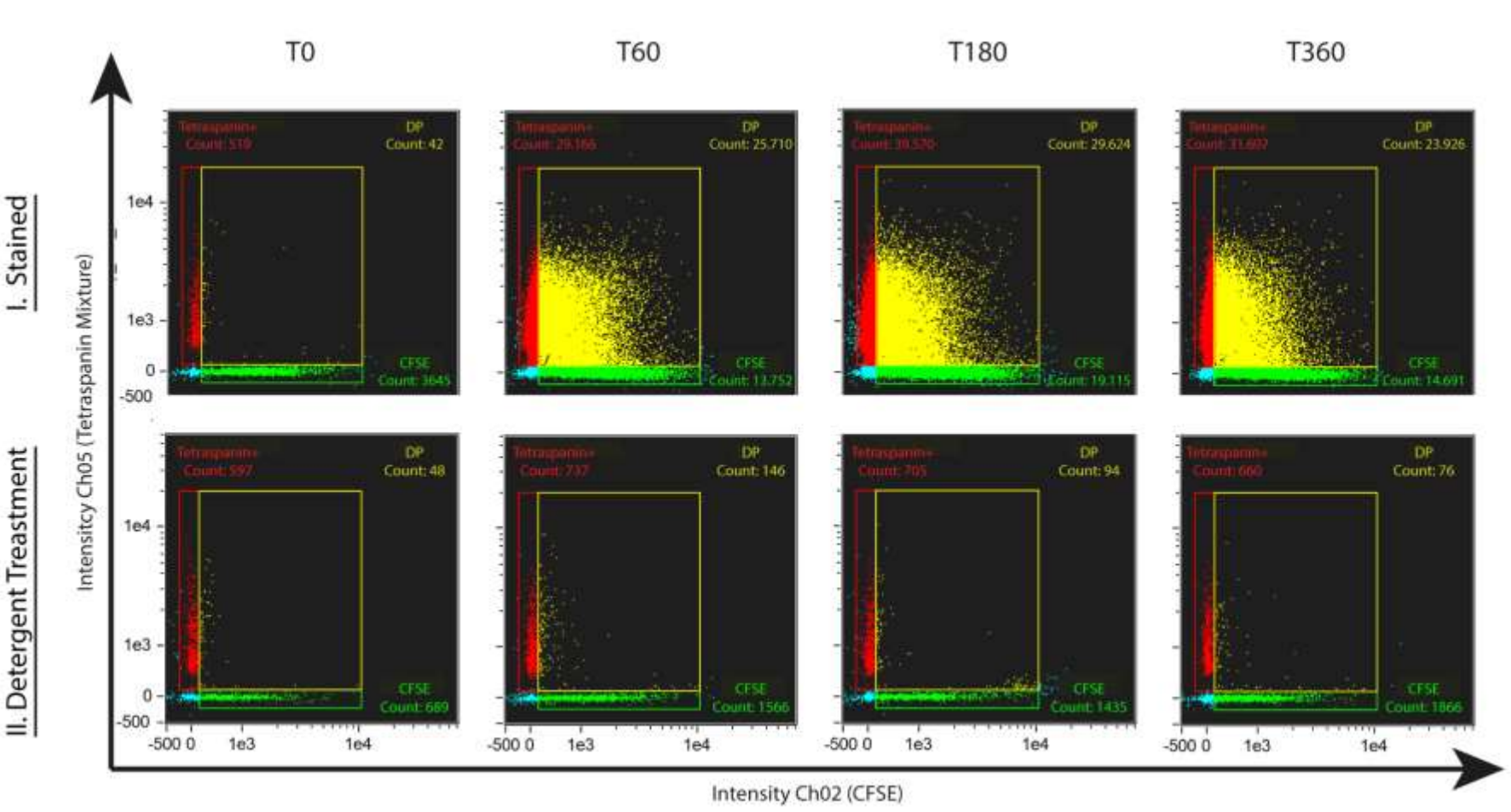
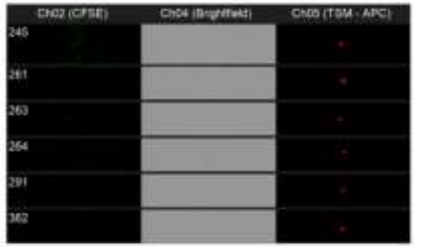
# Characterization of released Nanoparticles

CD9
CD63
CD81

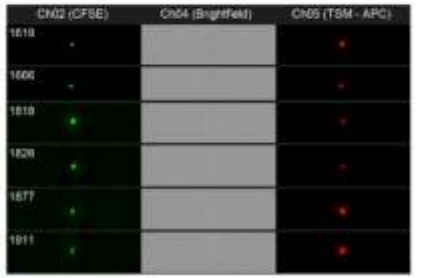



CFSE Single-Positives

Tetraspanin Single-Positives



Double-Positives



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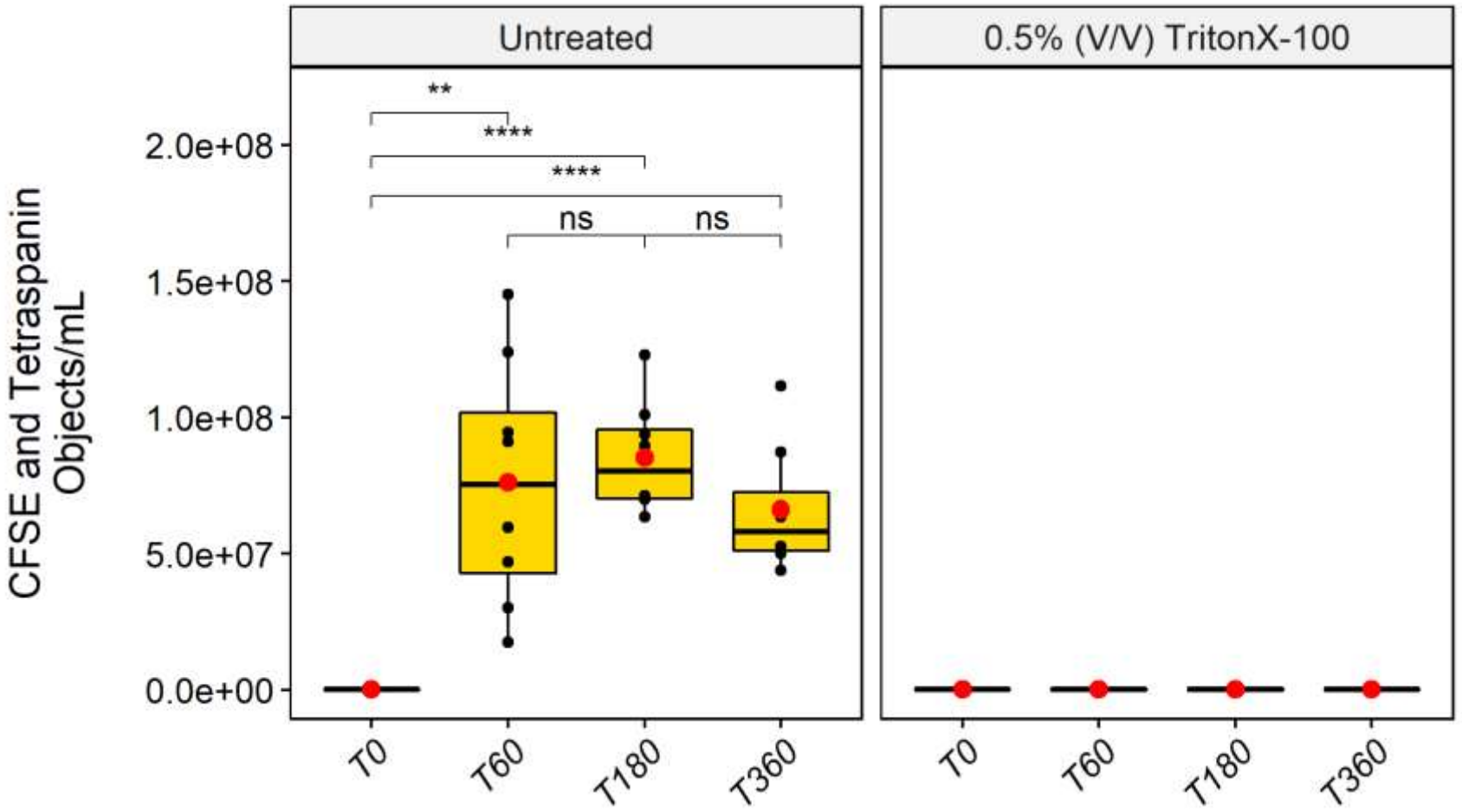
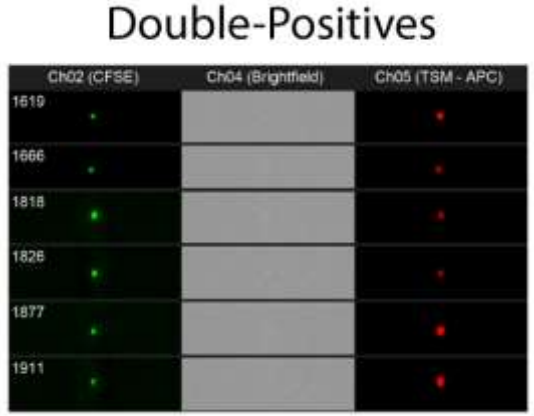
# Detergent Treatment confirms EV measurements

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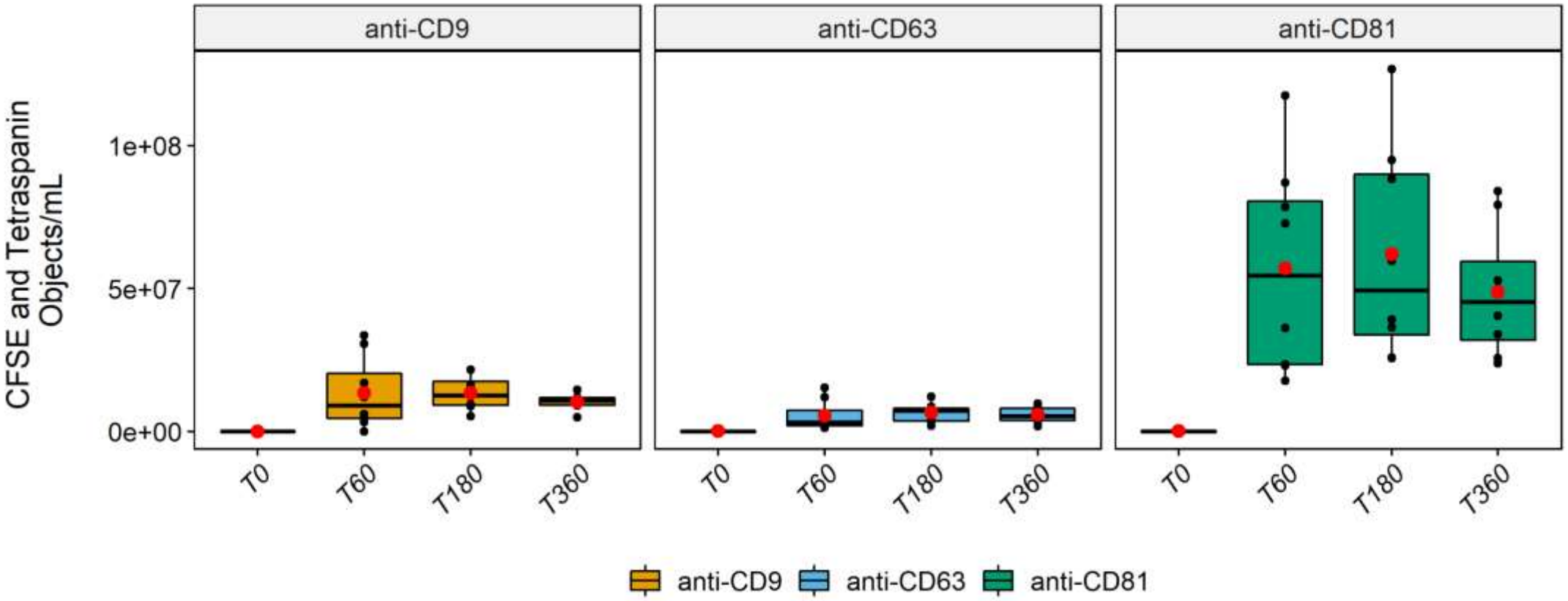
# Majority of released EVs is CD81+




Comparison of means:

~16%

~8%

~75%

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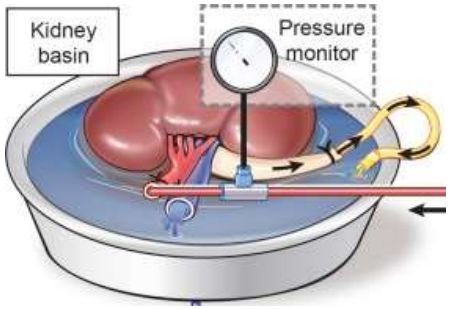
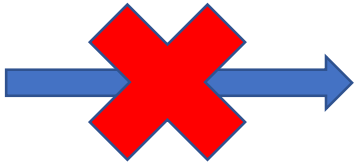
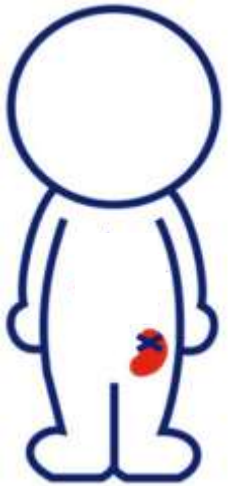
# Correlation with clinical parameters

Donor kidney characteristics

- Age
- Gender
- Donor Type
- Ischemia times
- Preservation method before NMP
- Kidney weight
- Transplantability assessment*





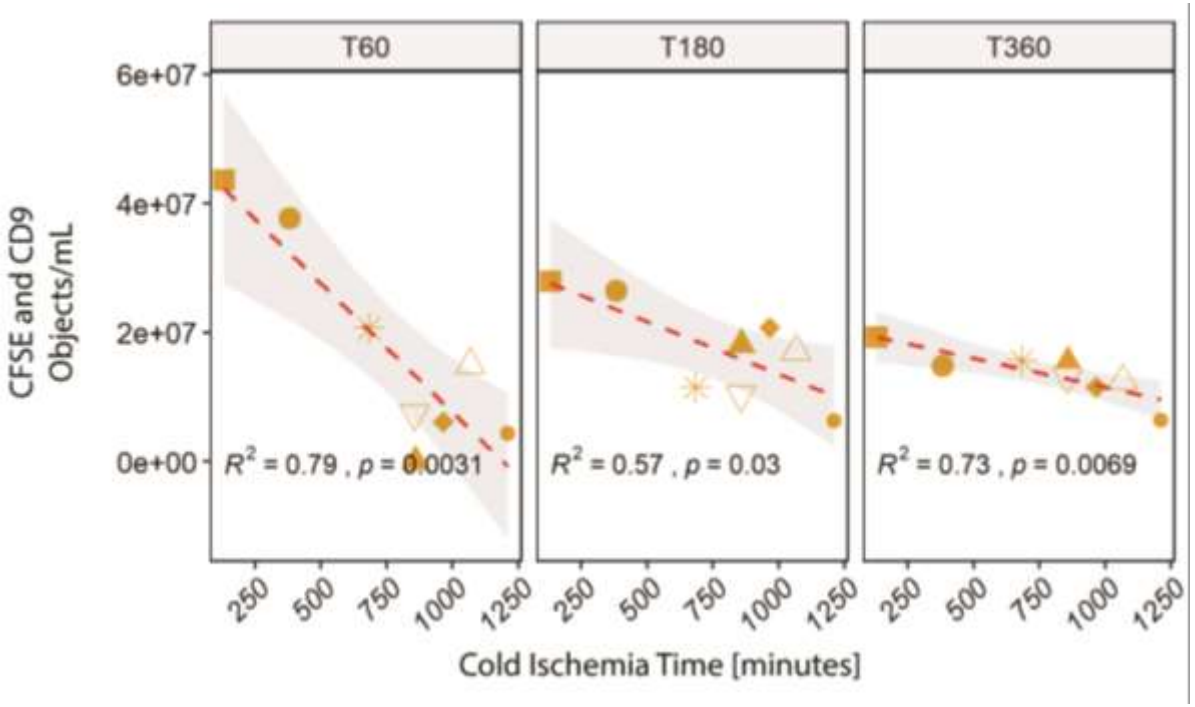
NMP viability characteristics

- Renal flow
- Renal flow resistance
- Total urine production

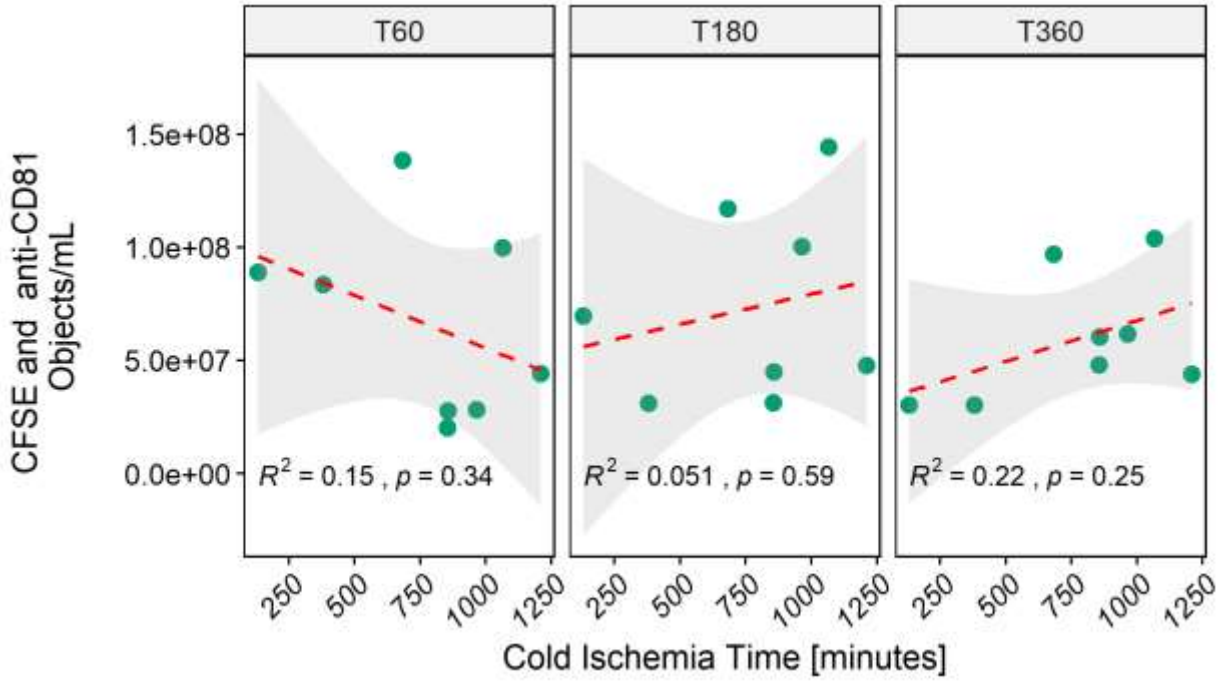
* As judged by an independant transplant surgeon and nephrologist

# Donor kidney characteristics

CFSE+CD9+ EVs are correlated with CIT




CFSE+CD81+ EVs are **not** correlated with CIT





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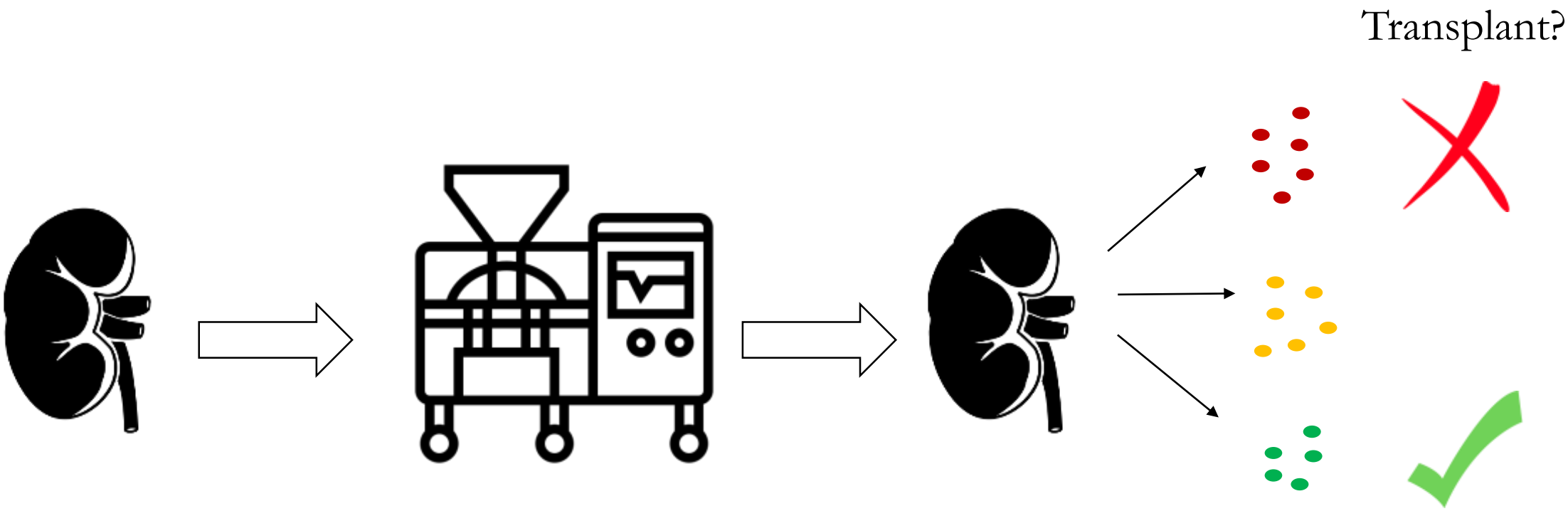
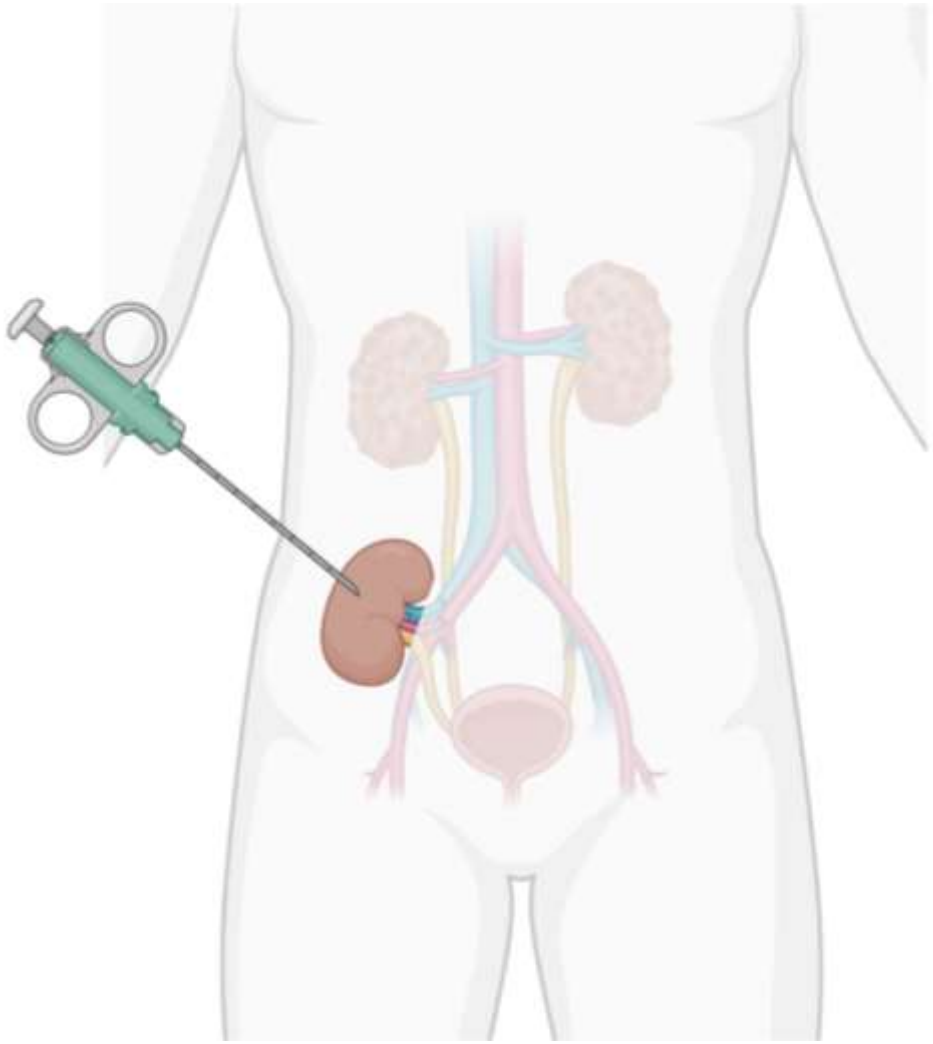
# Summary




Future !

# *In vivo*

# Kidney status <u>after</u> KTx



- Decline of kidney function post KTx → Biopsy
- Invasive
- Risk of complications
  - Bleedings
  - Infections
- Local sample

Image generated using biorender.com

# Need for minimally-invasive biomarkers

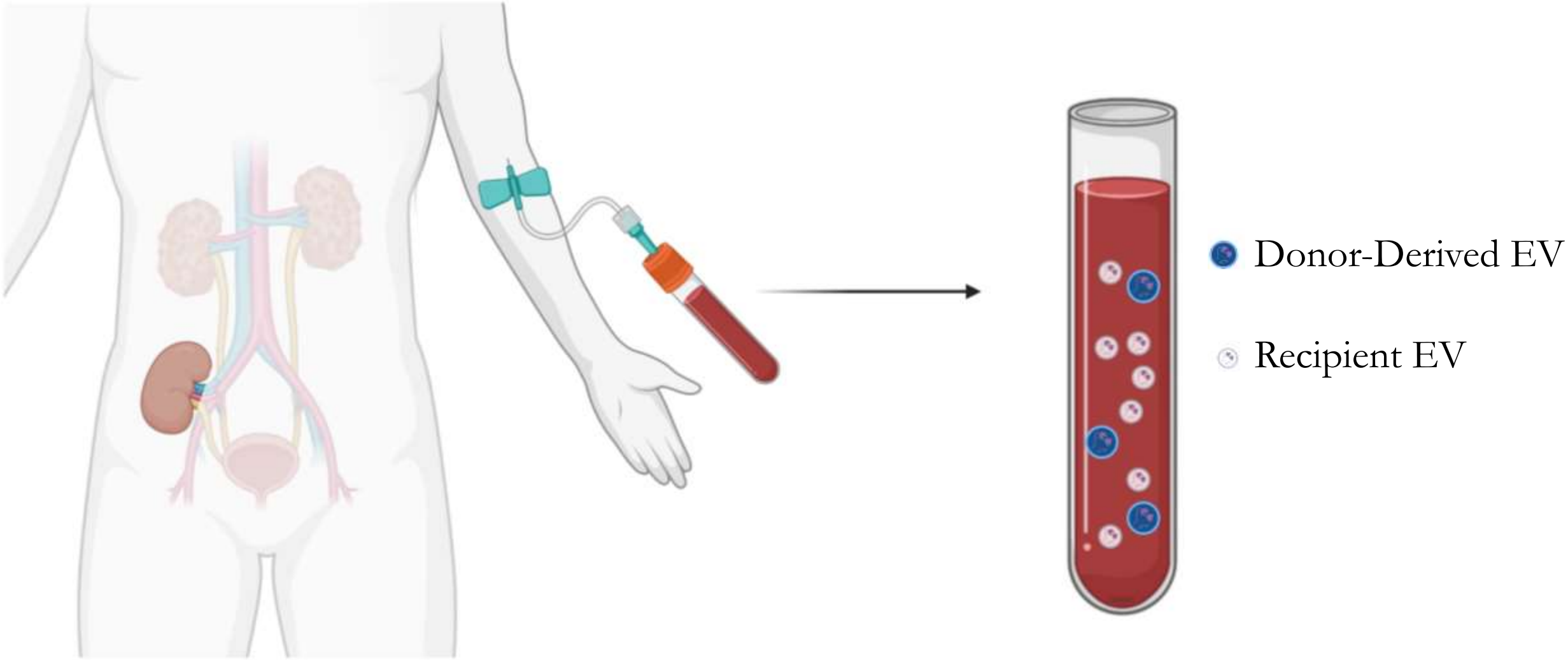
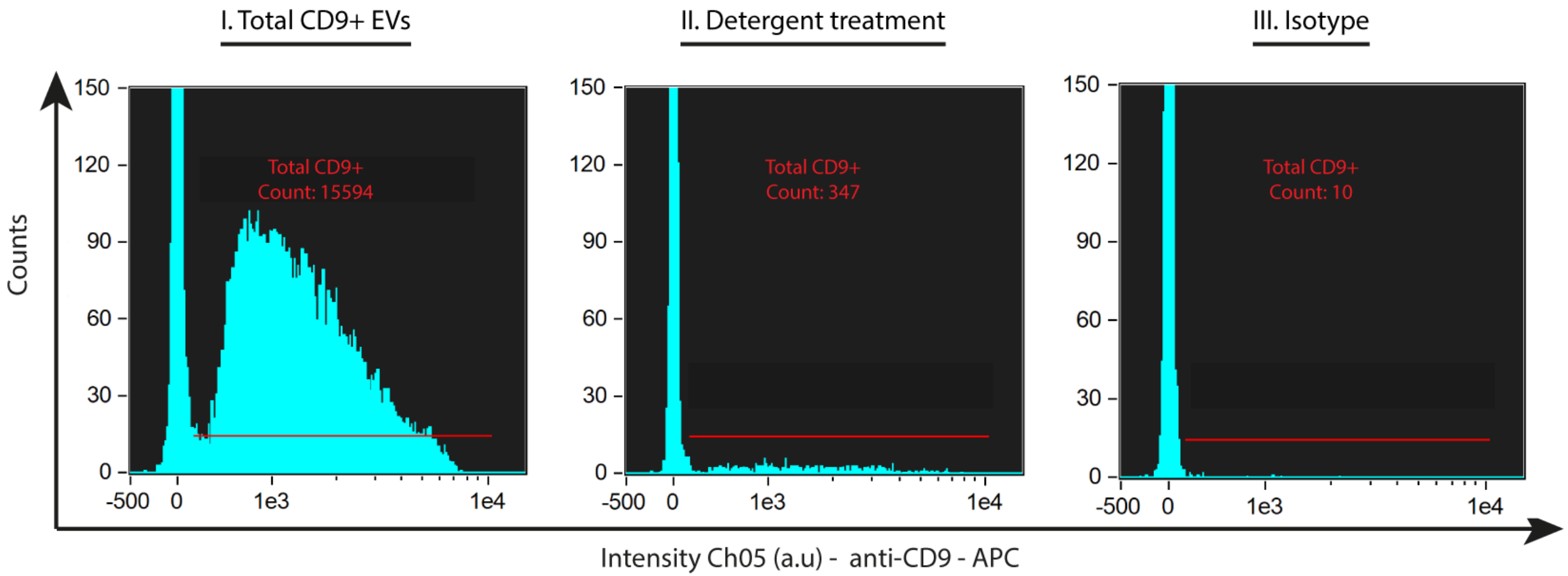



Image generated using biorender.com

# Direct detection of CD9+ EVs ≤ 400 nm

Note: High Gain Upgrade

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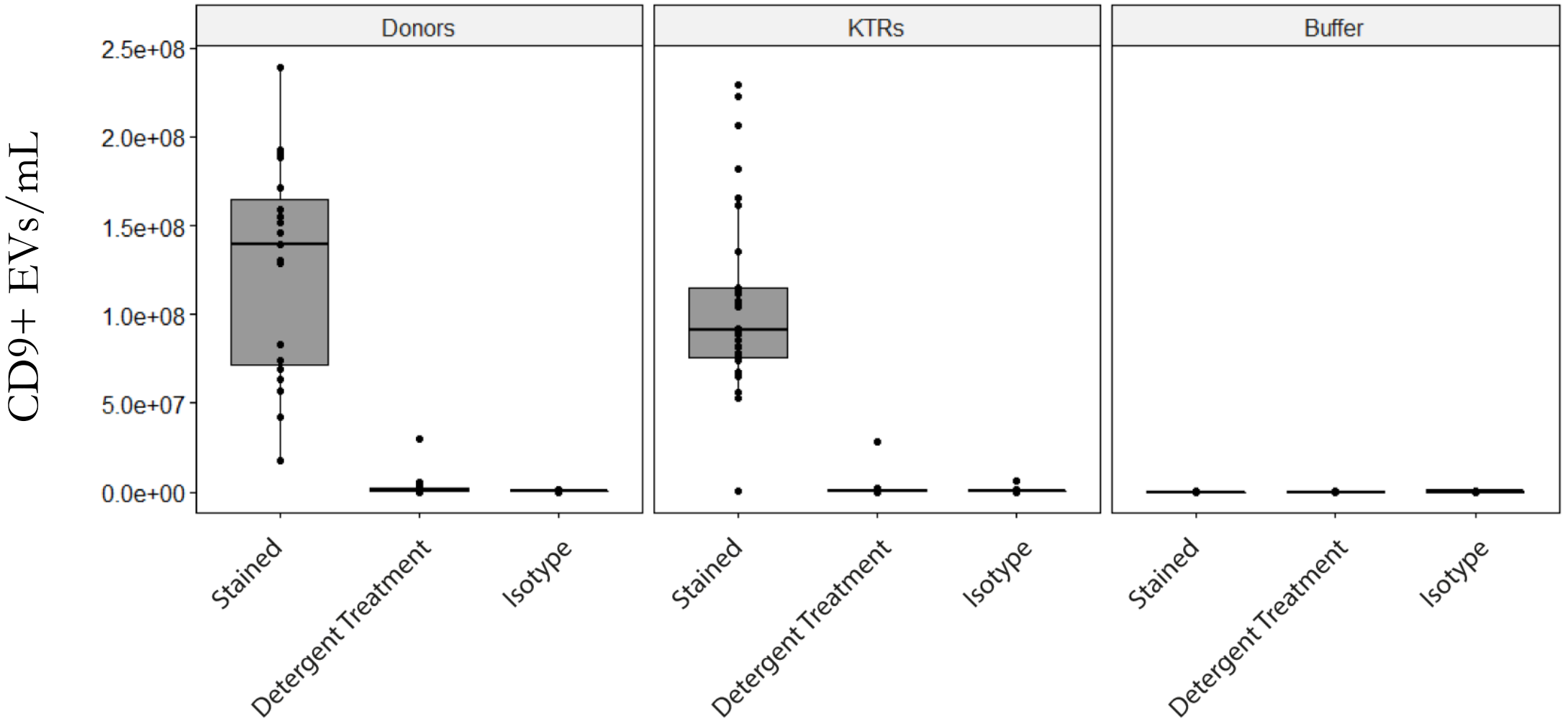
# Direct detection of CD9+ EVs ≤ 400 nm

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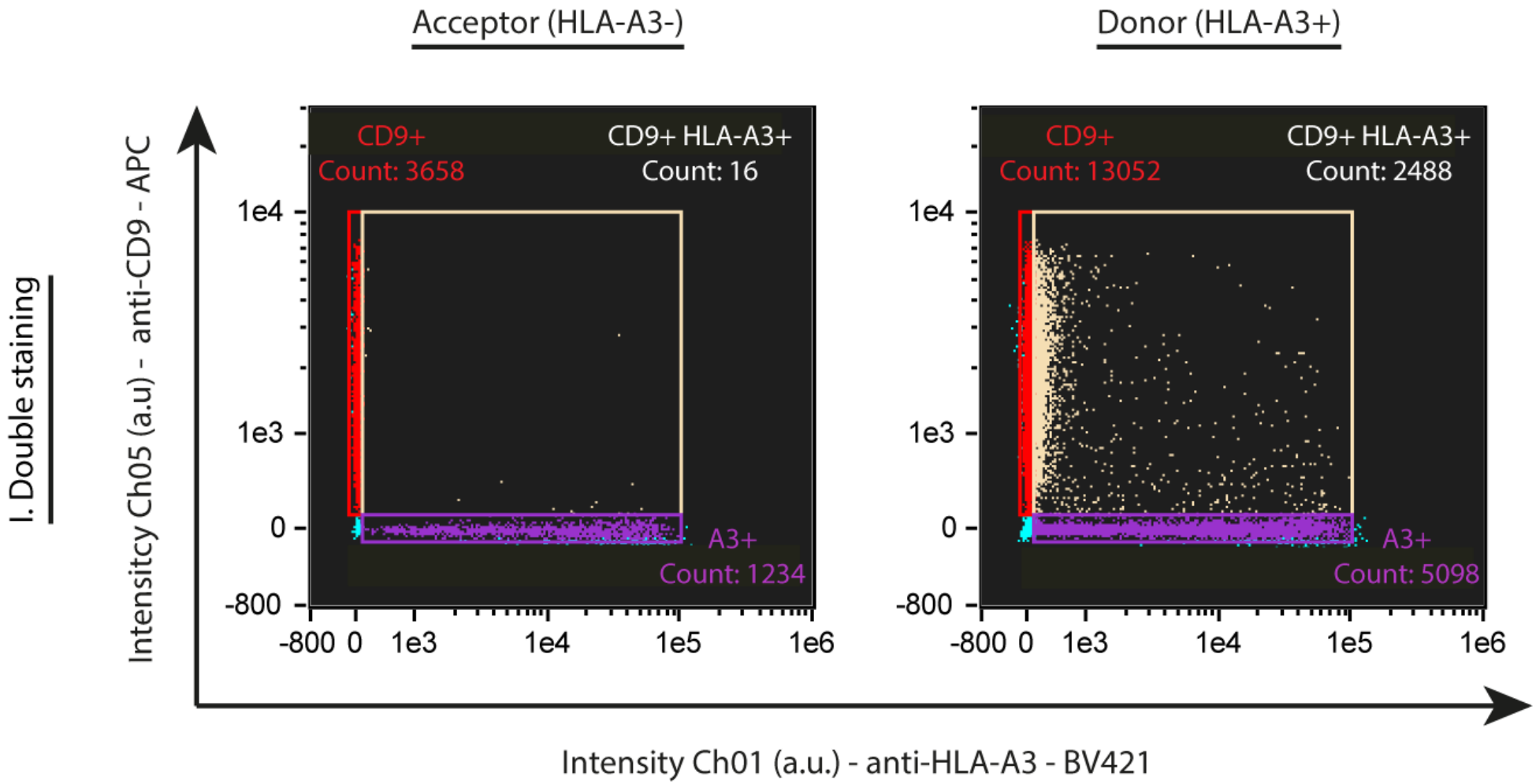
# Use HLA mismatching to identify donor EVs

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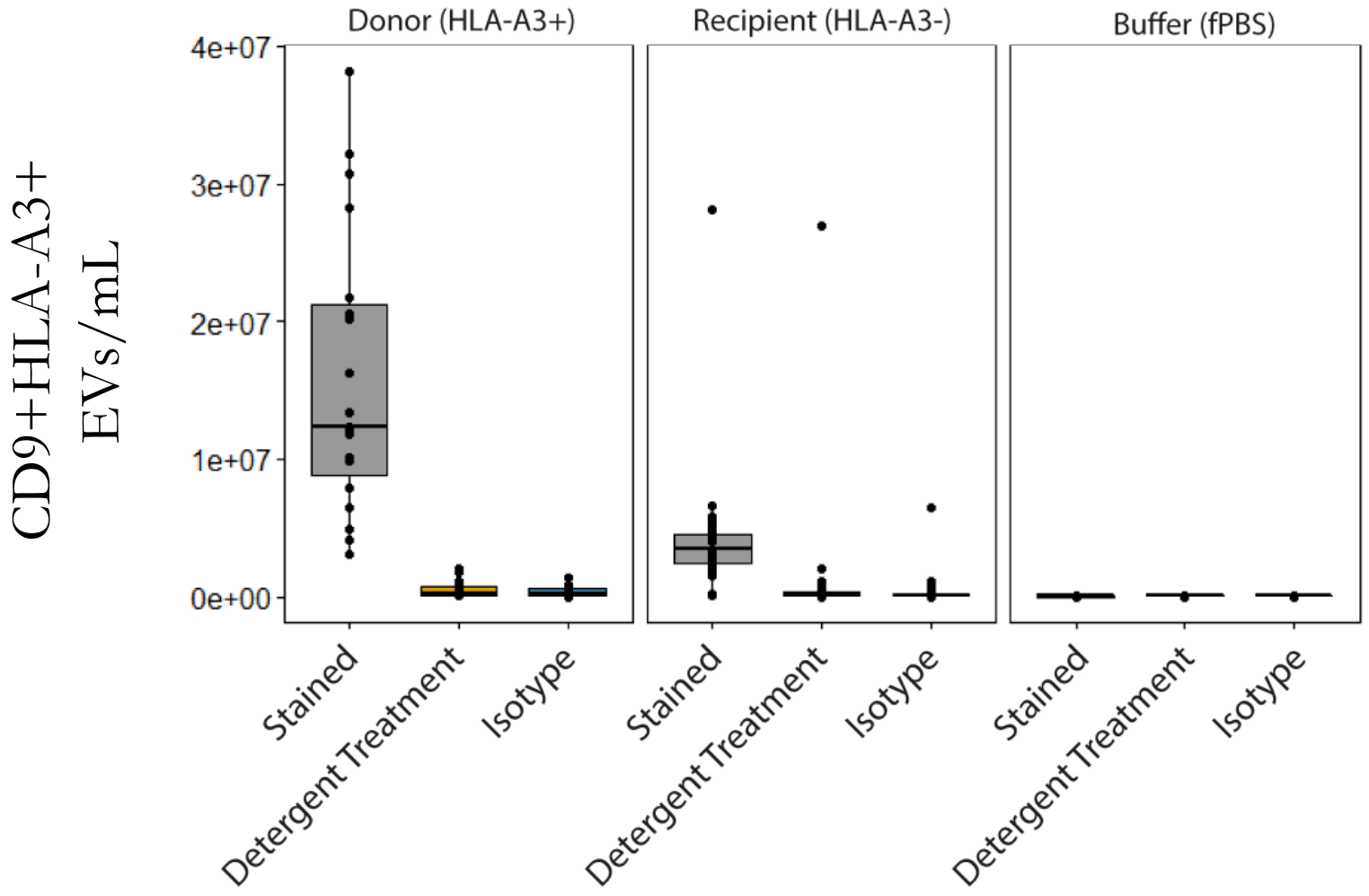
# Discriminate between donor and recipient EVs

CD9+HLA-A3+ events




Pre-KTx samples

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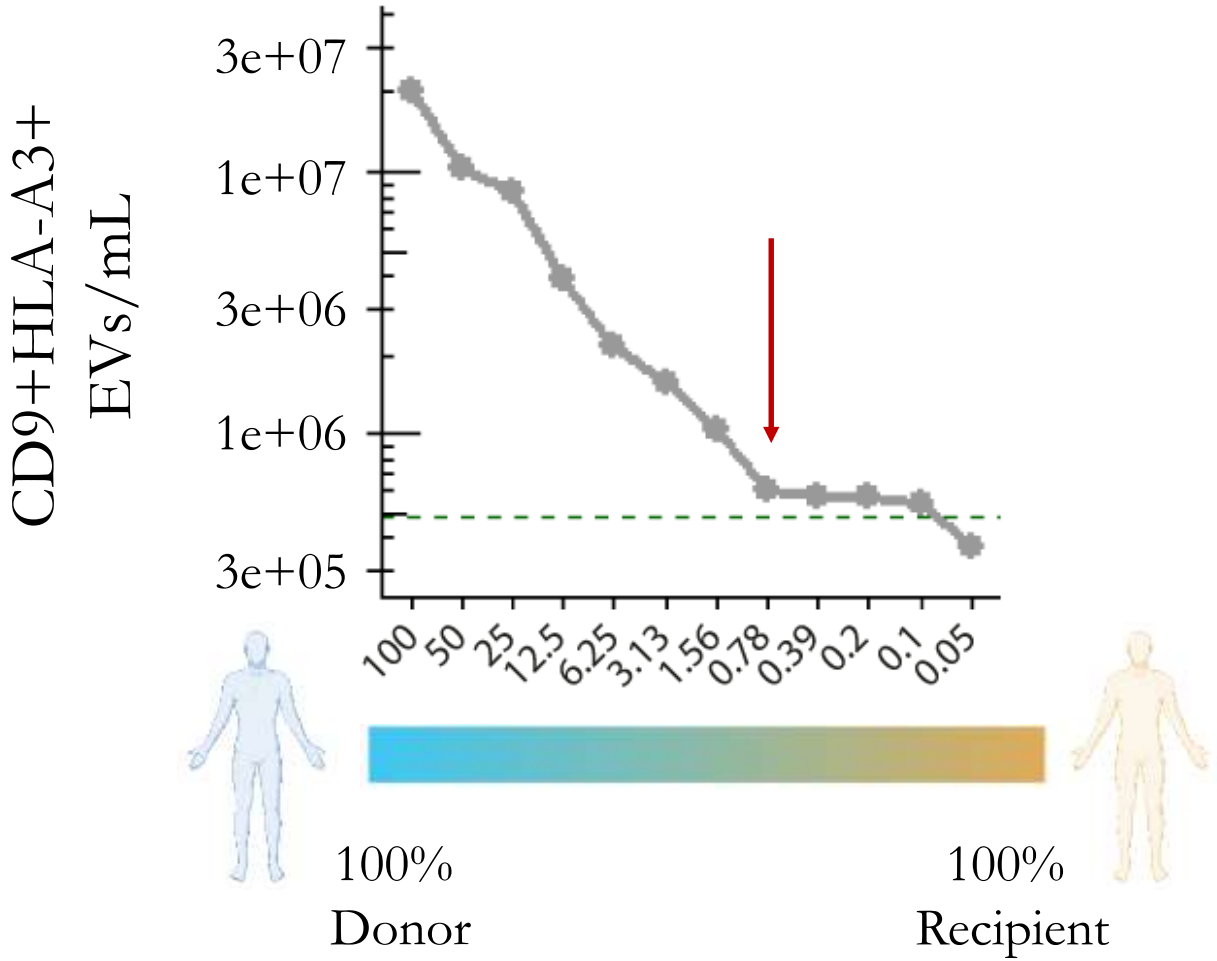
# Determine the sensitivity of the assay




Detection of donor-derived EVs down to ~1% above (recipient specific) background

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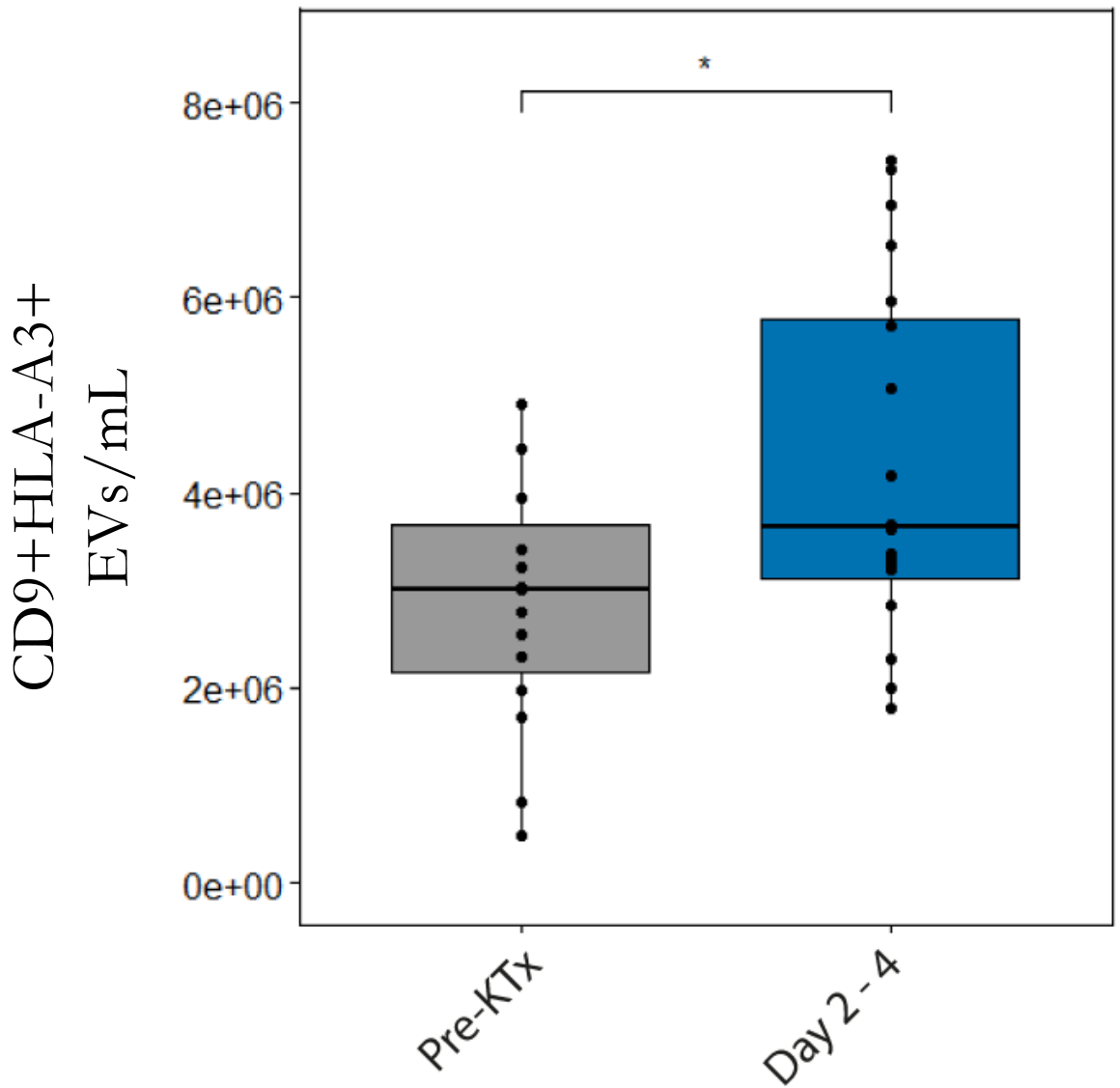
# Donor-derived EVs are detected ~3 days after KTx

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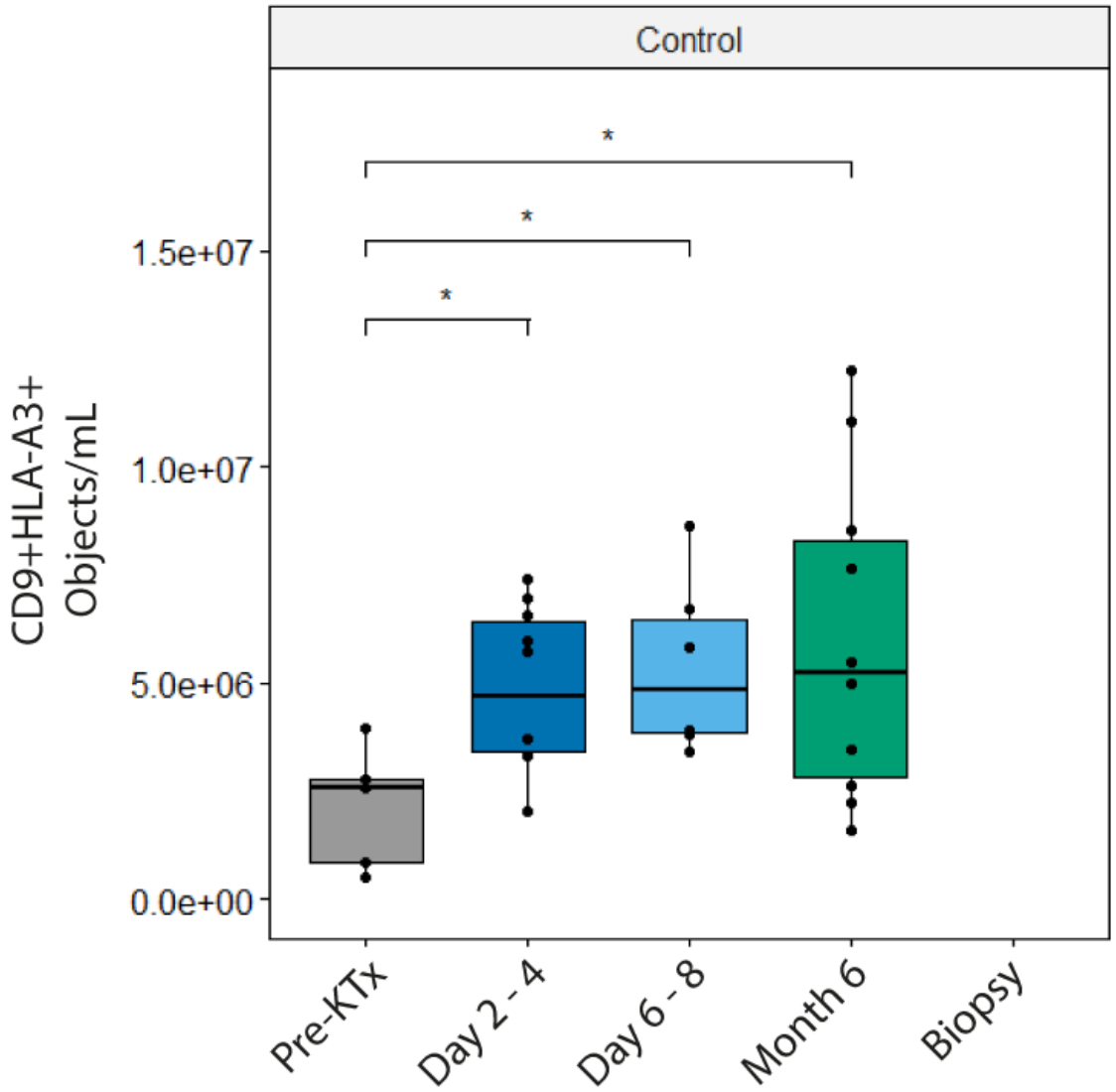
# Donor-derived EV detection in recipients




dd-EVs are detected
in KTRs with
**stable allograft function**

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# In summary

- Direct-detection and characterization of single EVs with IFCM
- Fully standardized platform
- EV subset analysis demonstrated in complex, clinically relevant (bio)fluids:
  - Platelet-Poor Plasma (*Communications Biology, 2022 & Scientific Reports, 2022)*
  - Urine (*Nanomedicine: Nanotechnology, Biology, and Medicine, 2023)*
  - Perfusion Fluids (*Transplantation, 2022)*
- Applicable in both health and disease

# Acknowledgements