



# Effect of Shear on EVs

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# Aggregation of EVs

## Experimental Parameters:

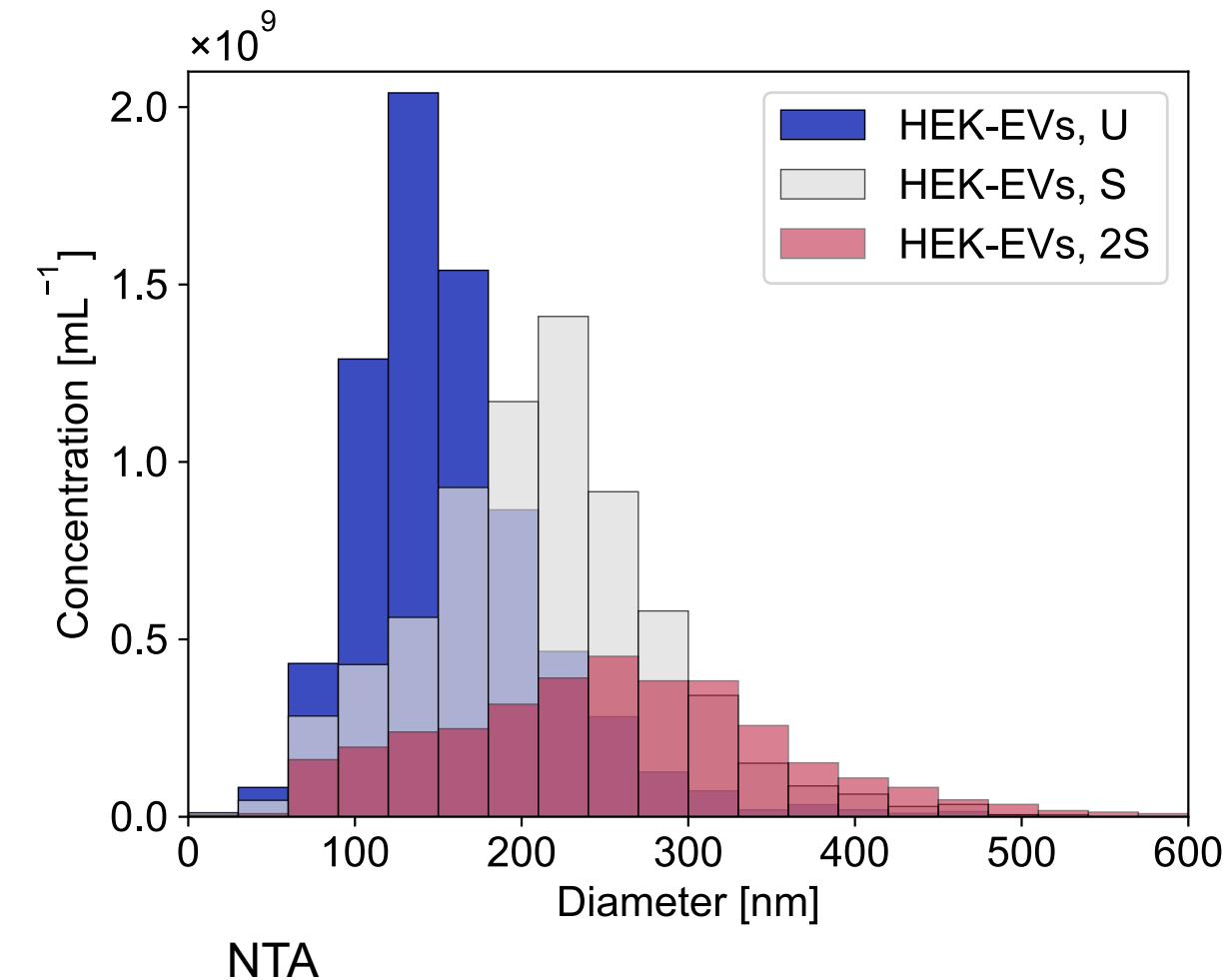
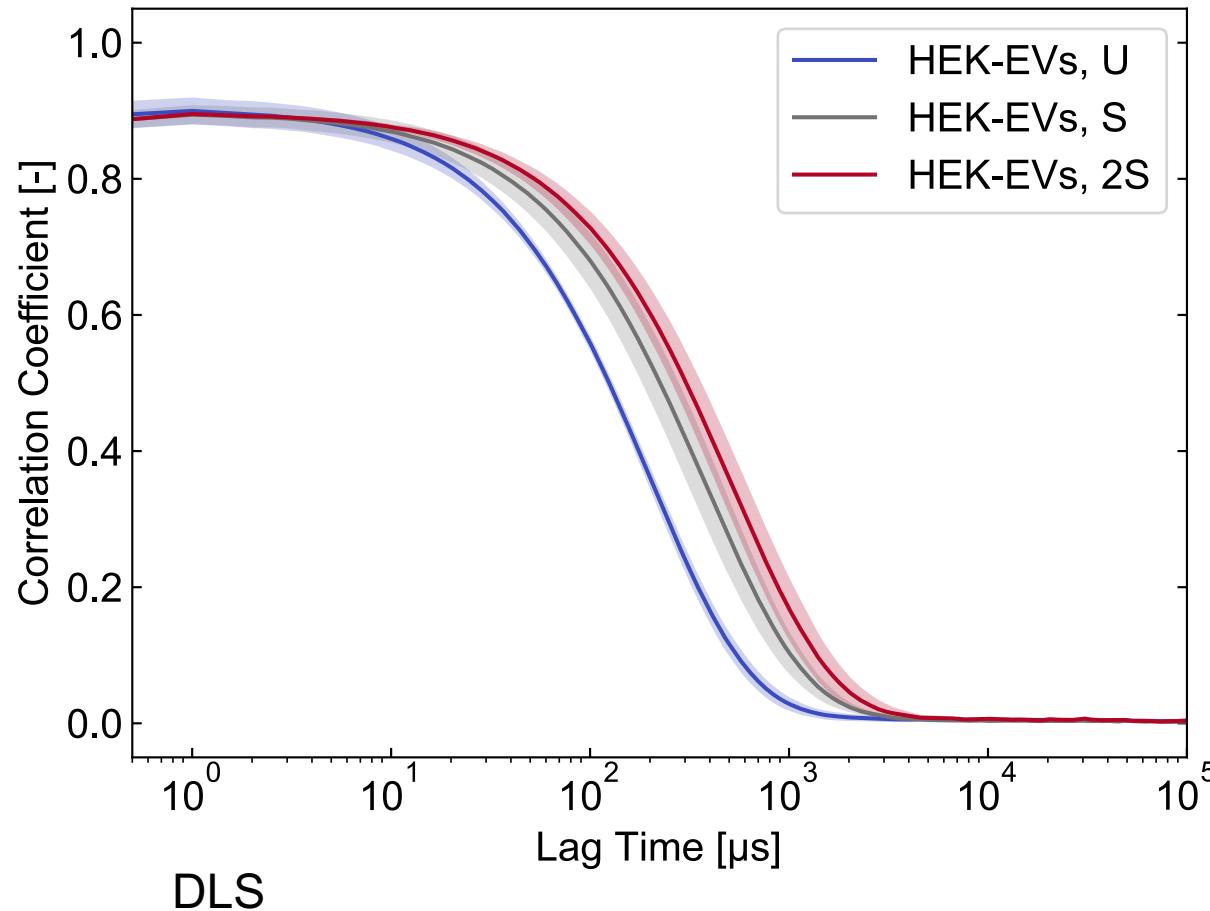
|                    |                        |  |
|--------------------|------------------------|--|
| Rotational Speed:  | 300 rpm                | → Shear Rate $\sim 1'300 \text{ s}^{-1}$ |
| Experimental Time: | 20 min                 | → Total Shear $\sim 1'500'000$           |
| Materials:         | Stainless Steel & PMMA |  |

To minimize sample consumption, the same EV sample was measured:

1. Before application of shear, i.e. unsheared (U)
2. After a single shear experiment (S)
3. After a second shear experiments (2S)

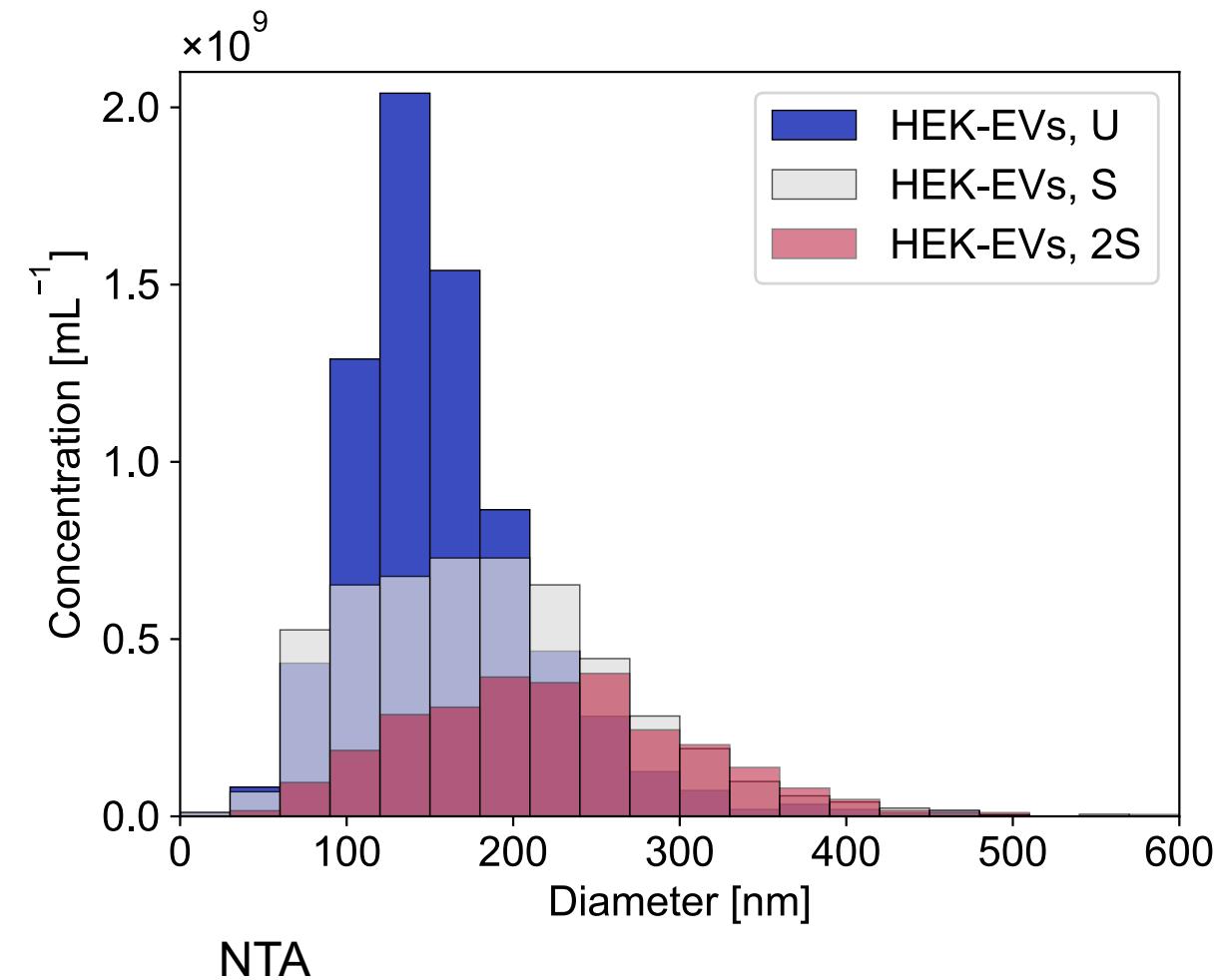
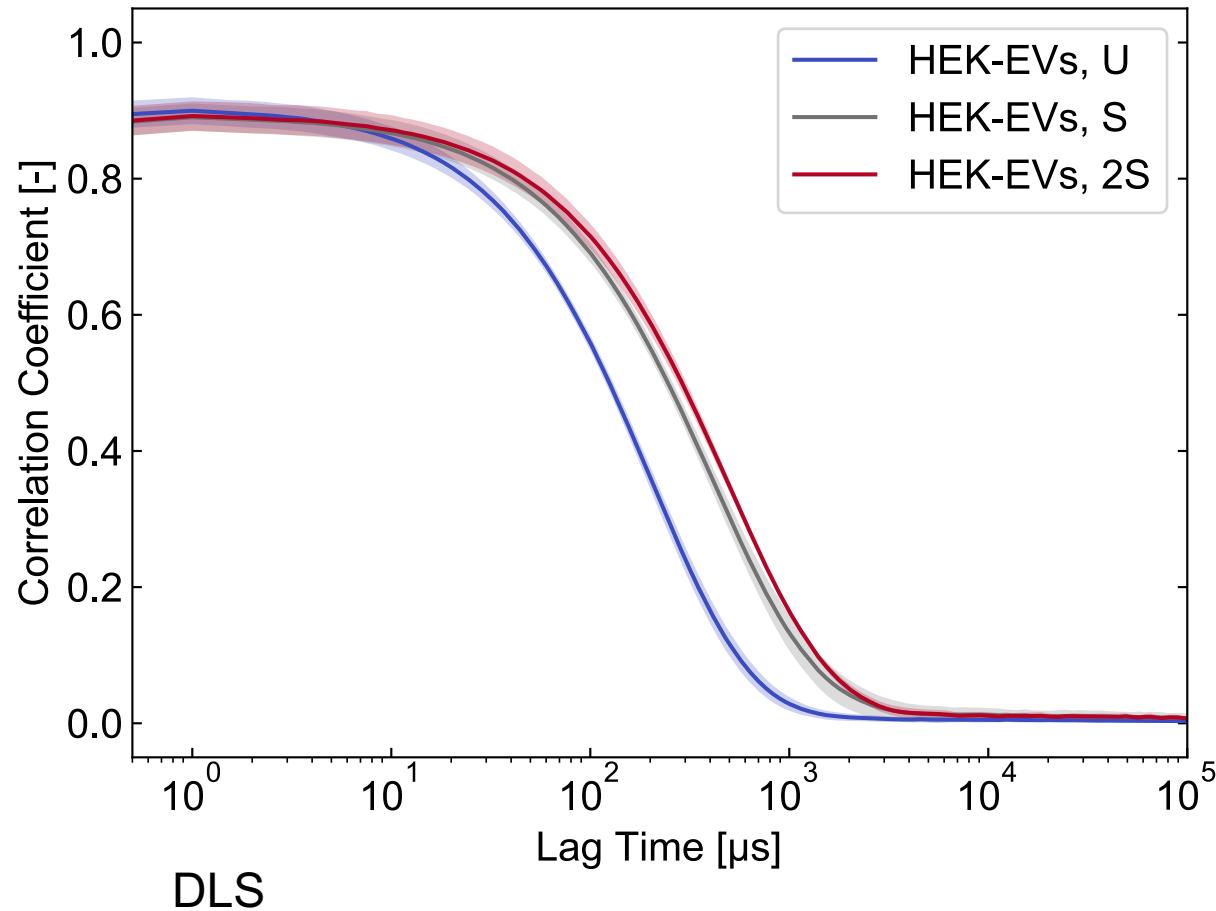
# Experimental Results

HEK-EVs, Steel:



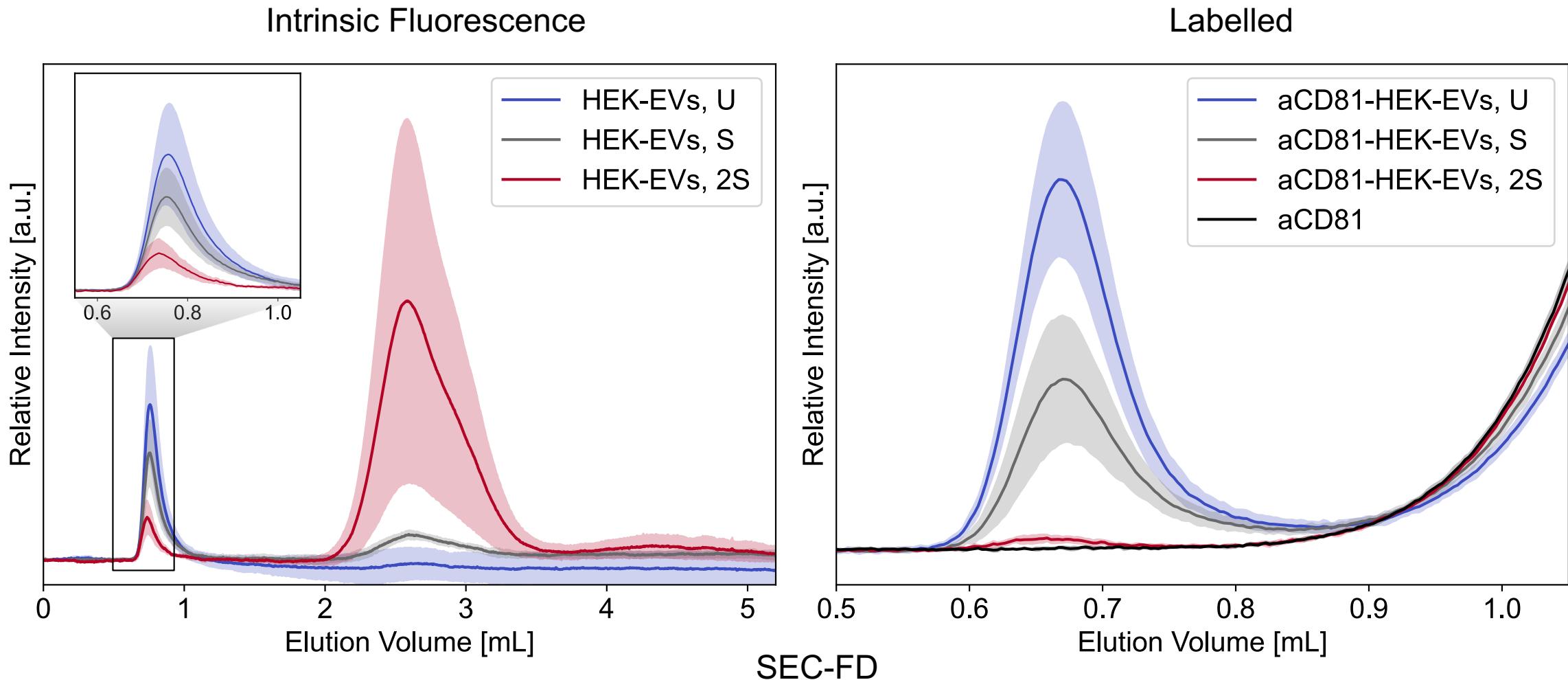
# Experimental Results

HEK-EVs, PMMA:



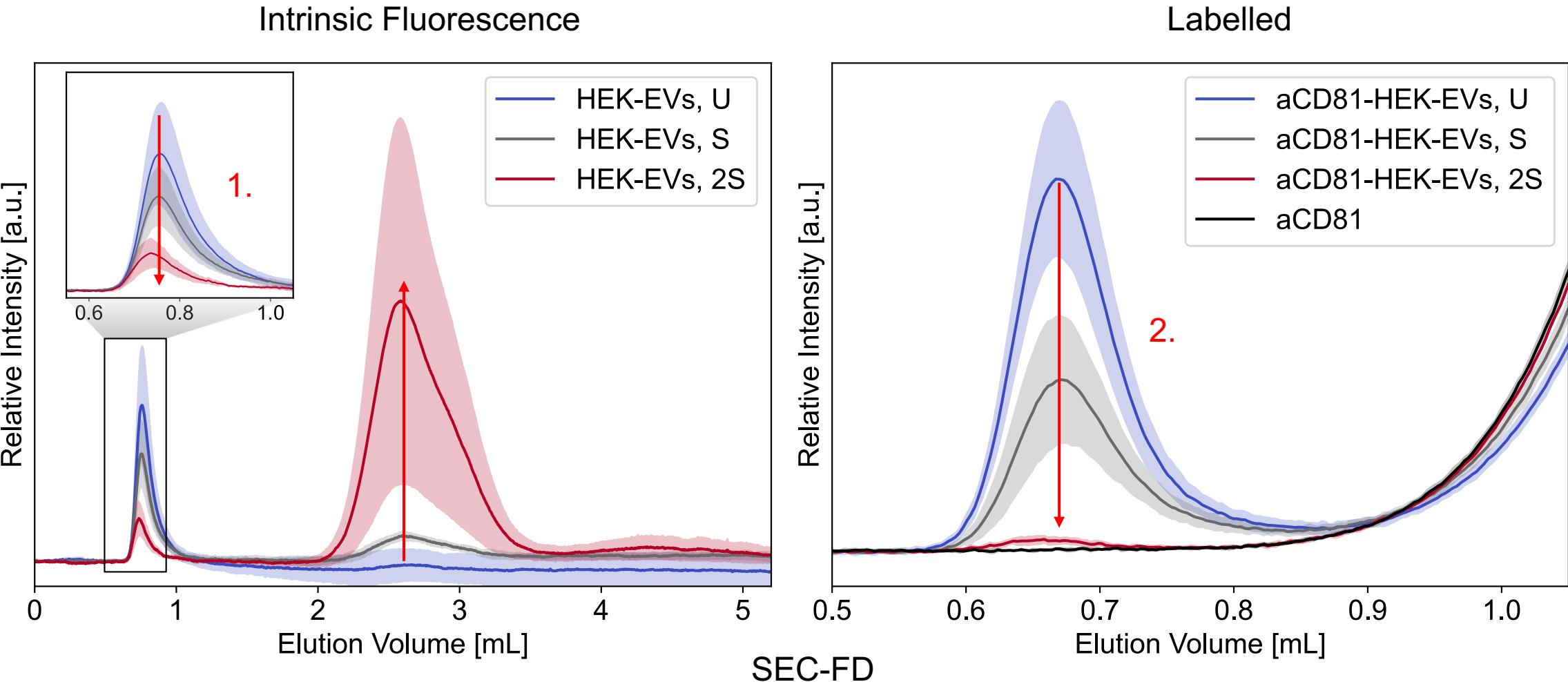
# Experimental Results

HEK-EVs, Steel:



# Experimental Results

HEK-EVs, Steel:



## Effects of Shear Stress:

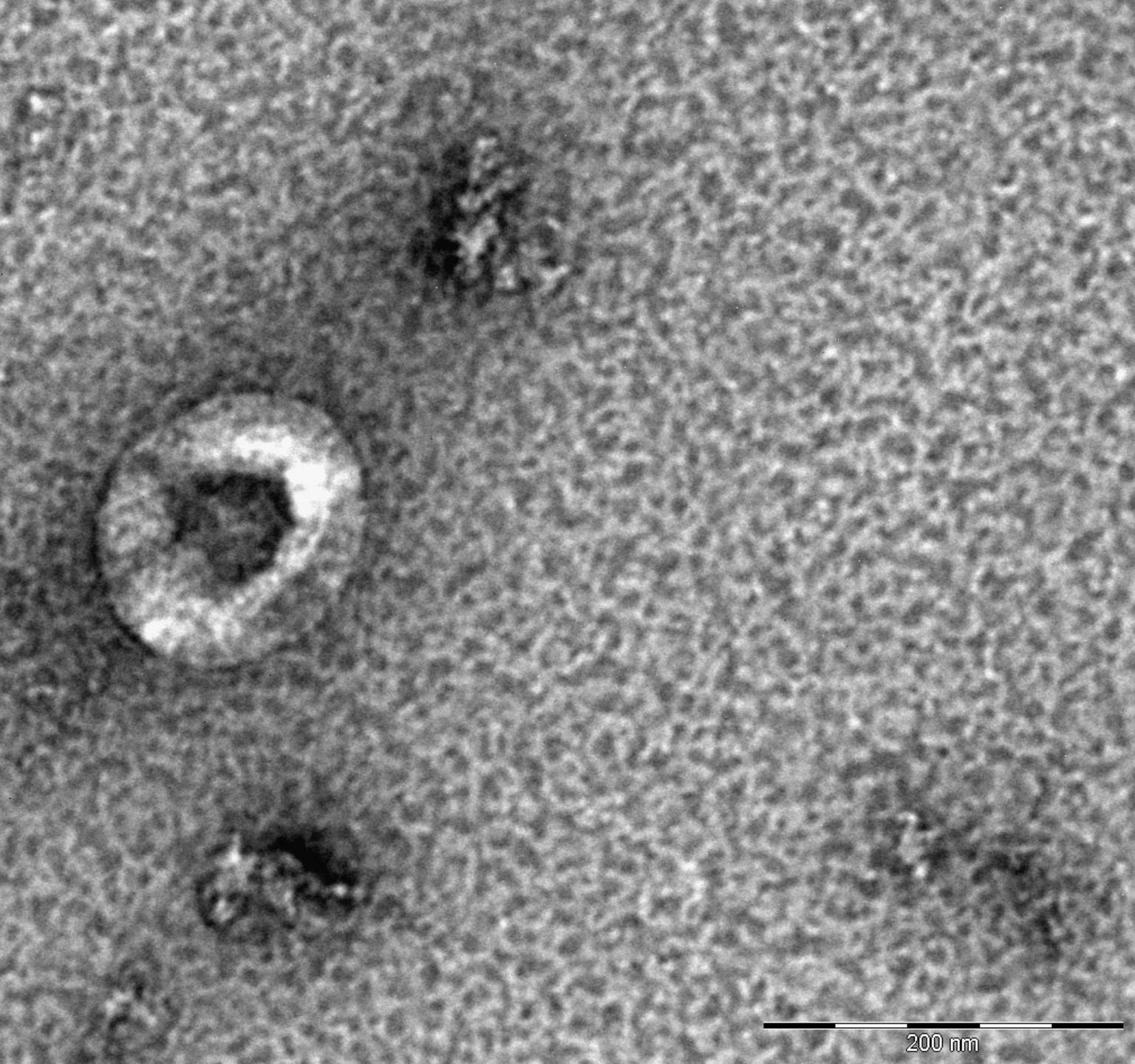
1. Decrease of EV total protein amount
2. Decrease of surface markers

# Experimental Results

HEK-EVs, Steel:

**TEM – HEK-EV, U**

Diameter ~ 150 nm

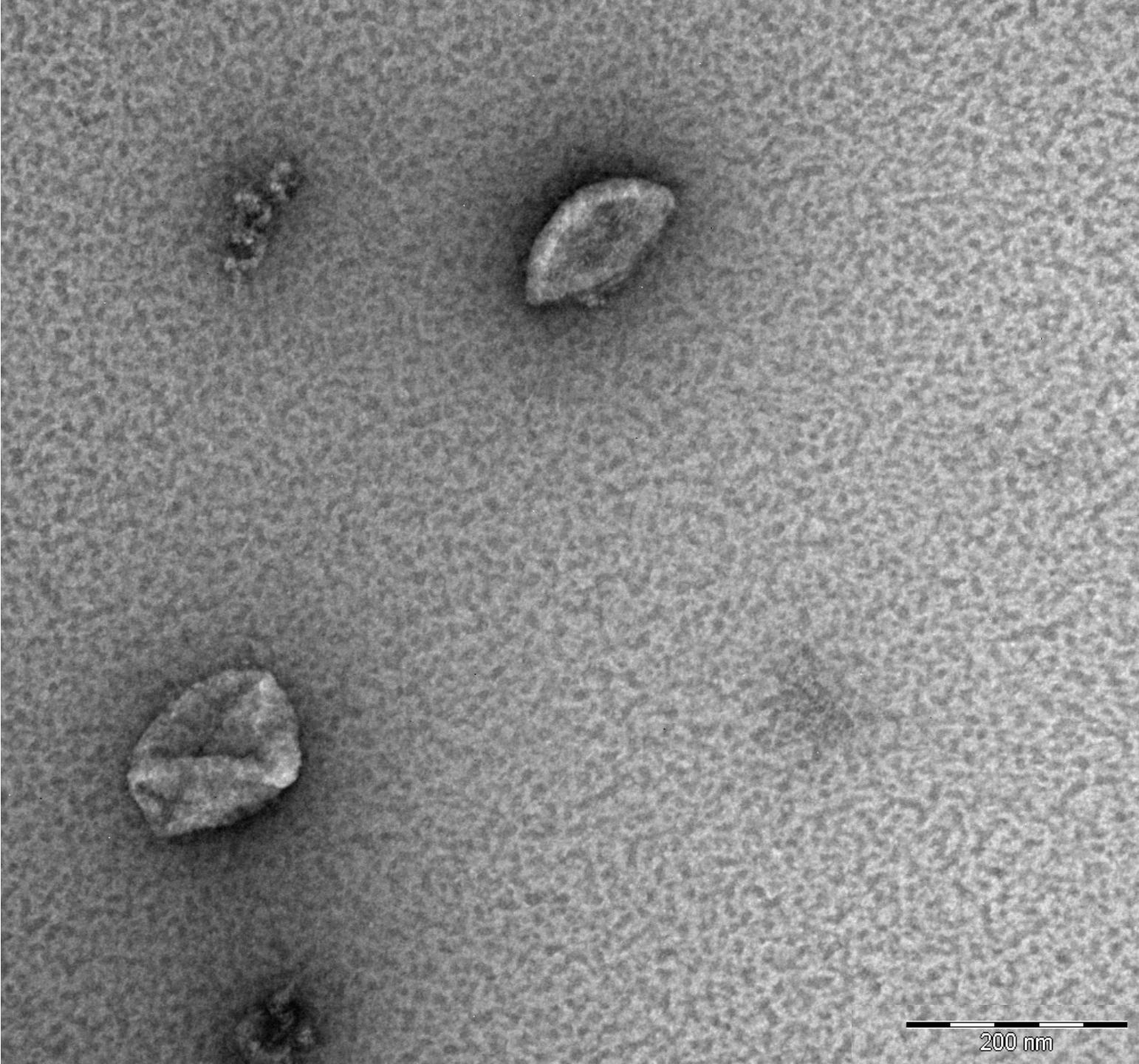


# Experimental Results

HEK-EVs, Steel:

**TEM – HEK-EV, S**

Diameter ~ 150 nm

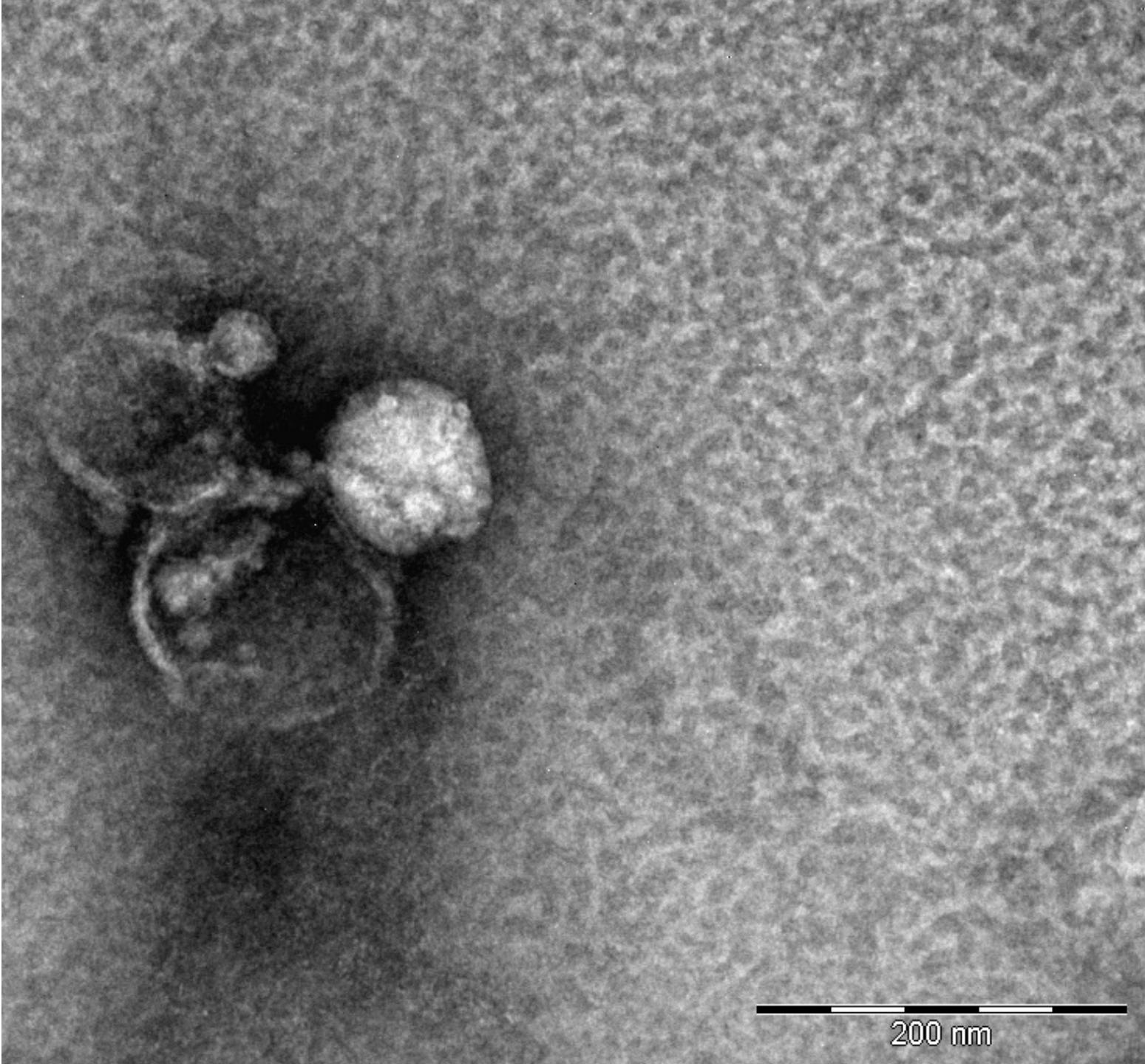


# Experimental Results

HEK-EVs, Steel:

**TEM – HEK-EV, 2S**

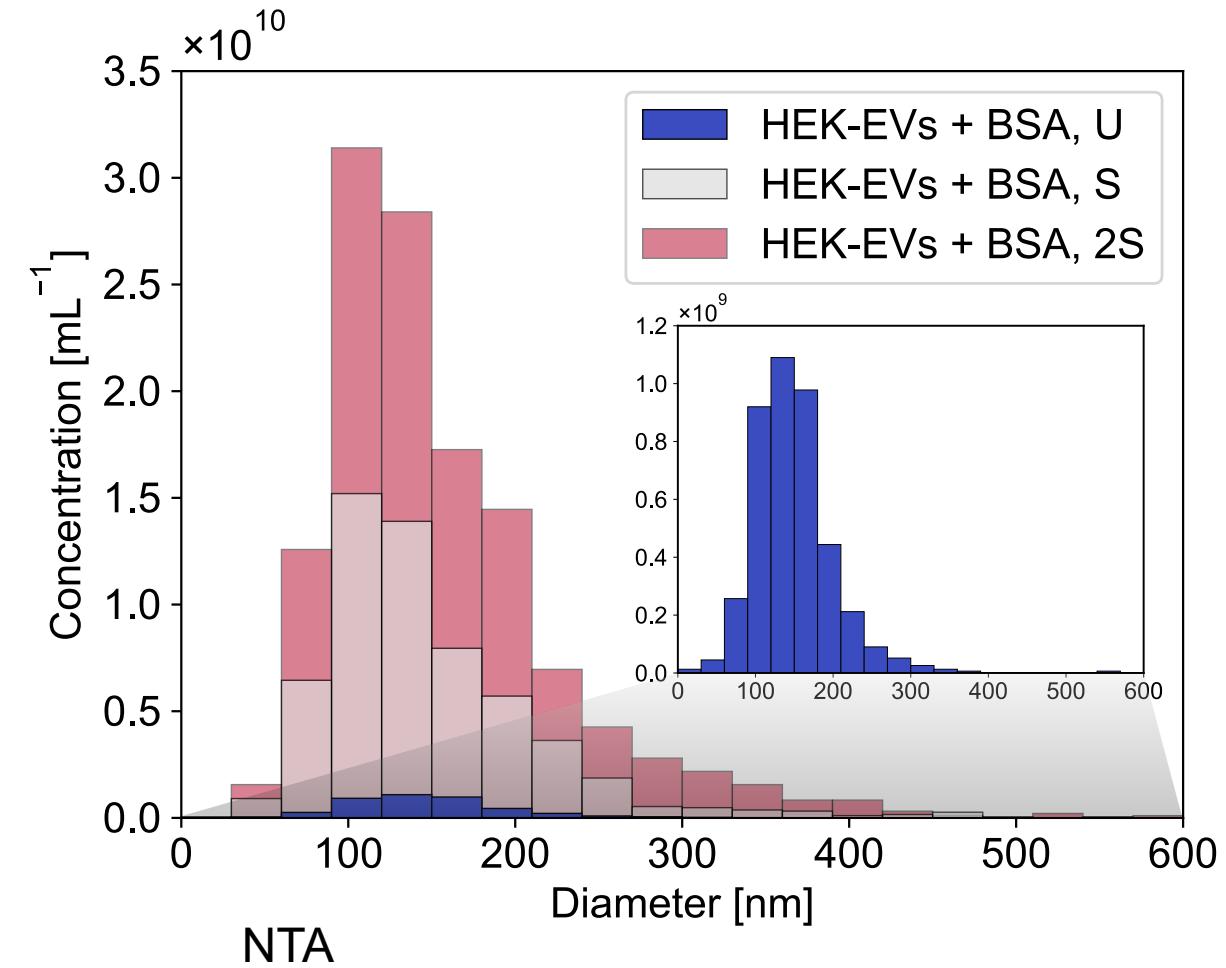
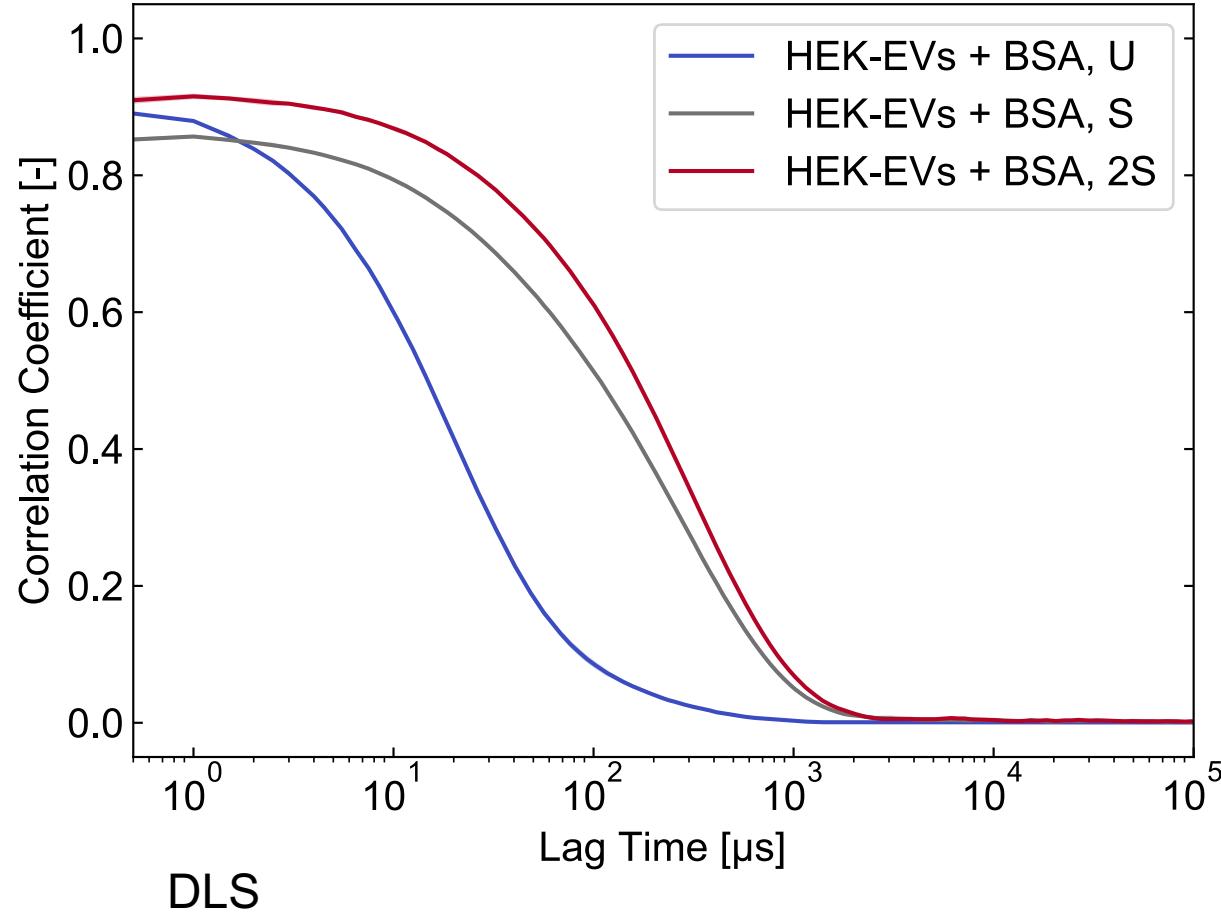
Diameter ~ 250 nm



200 nm

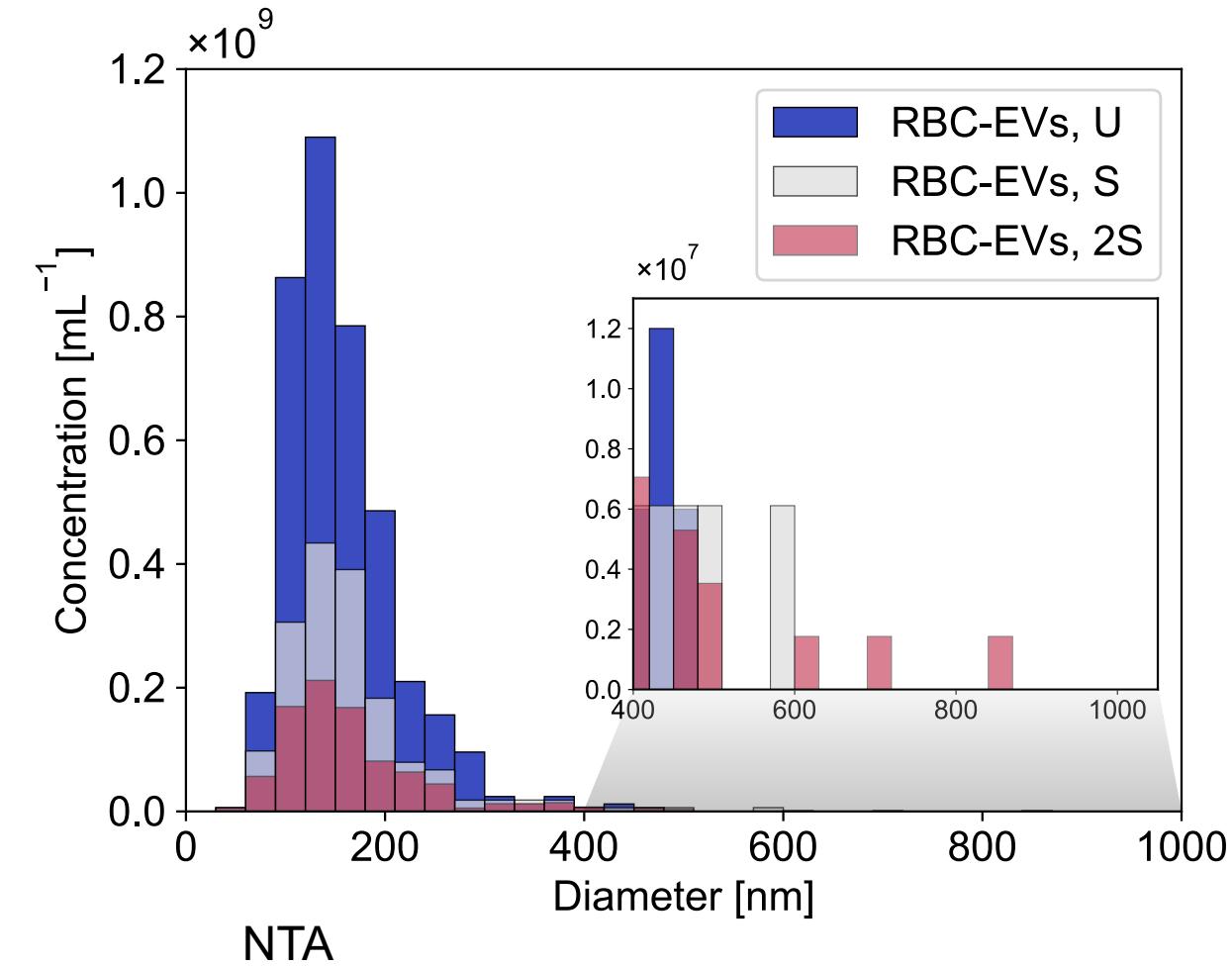
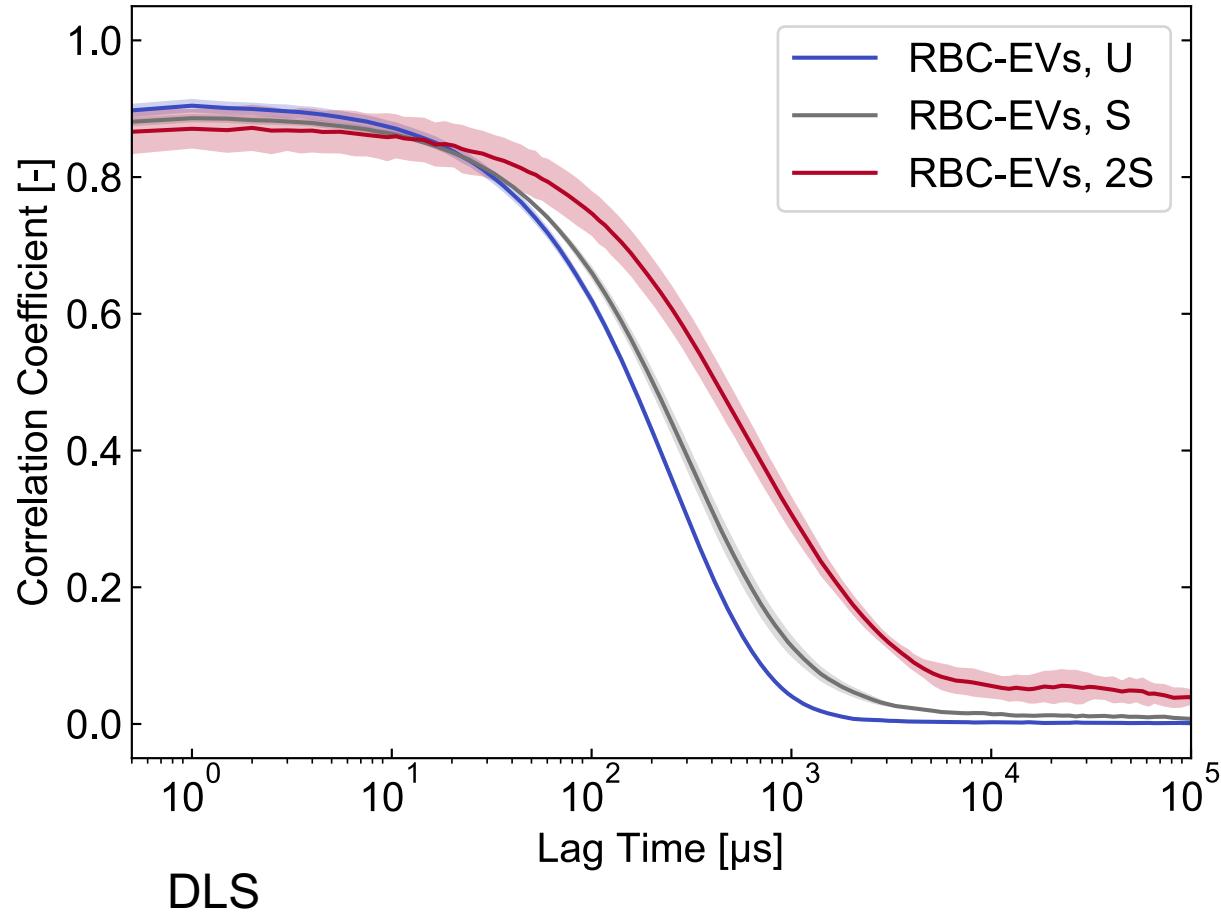
# Experimental Results

HEK-EVs + BSA, Steel:



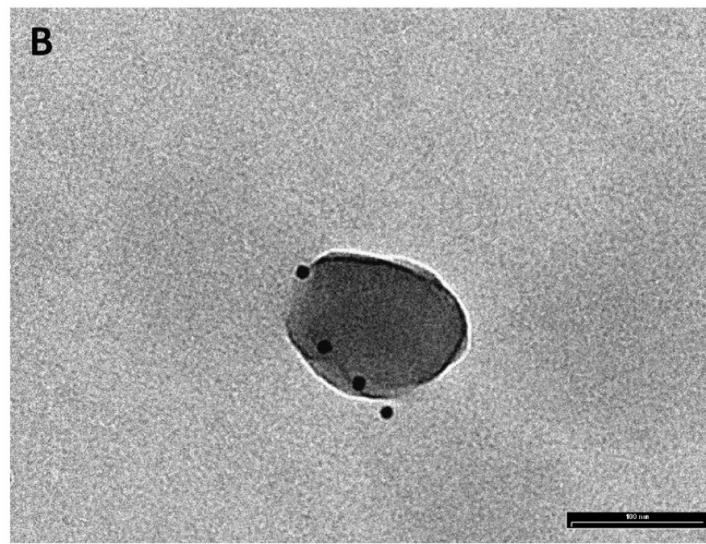
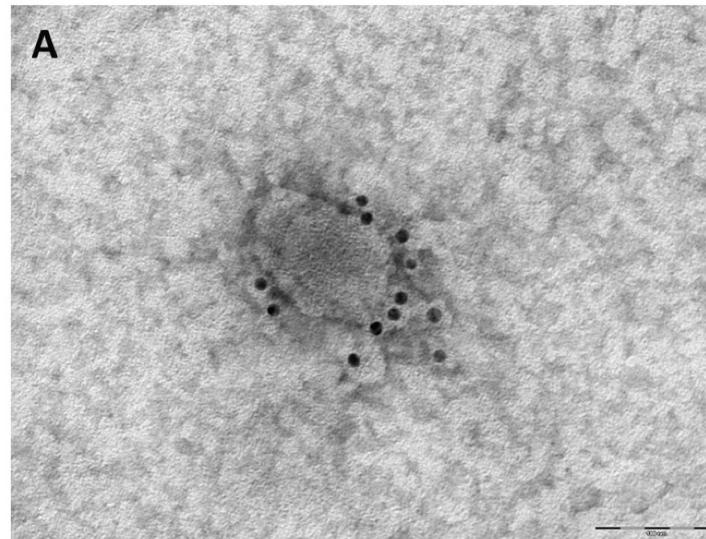
# Experimental Results

RBC-EVs, Steel:



# How to Continue...?

- Imaging of stained EVs (e.g. aCD81)?  
→ Easier to differentiate actual EVs from artifacts..
- How to continue with RBC-EVs?
- Prevention of Shear Induced Effects?



**Fig. 9.** Immuno-TEM of EVs from Mesenchymal Stromal Cell culture supernatants. A. Immuno-negative stain of CD90-positive EVs. Scale bar, 100 nm [76] (Reproduced from Capomaccio et al. 2019 under the terms of the Creative Commons Attribution License). B. Immuno-positive stain of flotillin-positive EVs. Scale bar, 100nm (Unpublished image by Pascucci L.).