ISOLATION OF ANTI-EXOSOME SINGLE-DOMAIN ANTIBODIES BY DIRECT PANNING ON EXTRA-CELLULAR VESICLE-ENRICHED FRACTIONS

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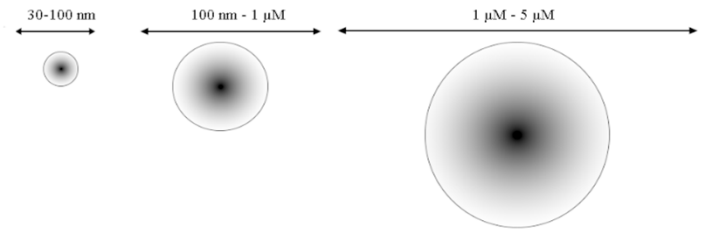
Extra-cellular vesicles (EV)

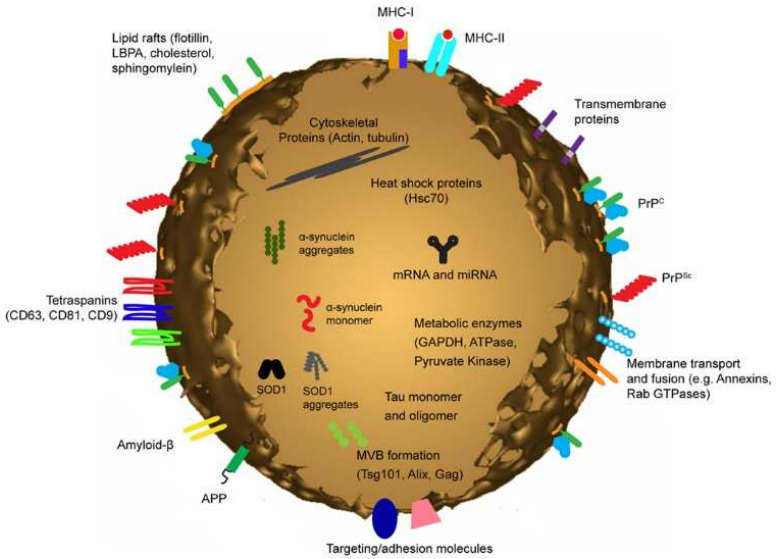


	Exosomes	Microvesicle	Apoptotic body
Origin	Endolysosomal pathway; intra-luminal budding of multivesicular bodies and fusion of multivesicular body with cell membrane	Cell surface; outward budding of cell membrane	Cell surface; outward blobbing of apoptotic cell membrane
Markers	Tetraspanins (such as TSPAN29 and TSPAN30), ESCRT components, PDCD6IP, TSG101, flotillin MFGE8	Integrins, selectins, CD40 ligand	Extensive amounts of phosphatidylserine
Content	mRNA, microRNA (miRNA) and other non-coding RNAs; cytoplasmic and membrane proteins including receptors and major histocompatibility complex (MHC) molecules	mRNA, miRNA, non-coding RNAs, cytoplasmic proteins and membrane proteins, including receptors	Nuclear fractions, cell organelles

Popović and de Marco (2017). Transl Cancer Res **7** (S209-S225)

- Most eukaryotic cells release membrane derived vesicles
- Paracrine and endocrine signaling
- Extracellular vesicles (EVs) are classified based on:
  - cellular origin
  - biological function
  - biogenesis

Exosomes

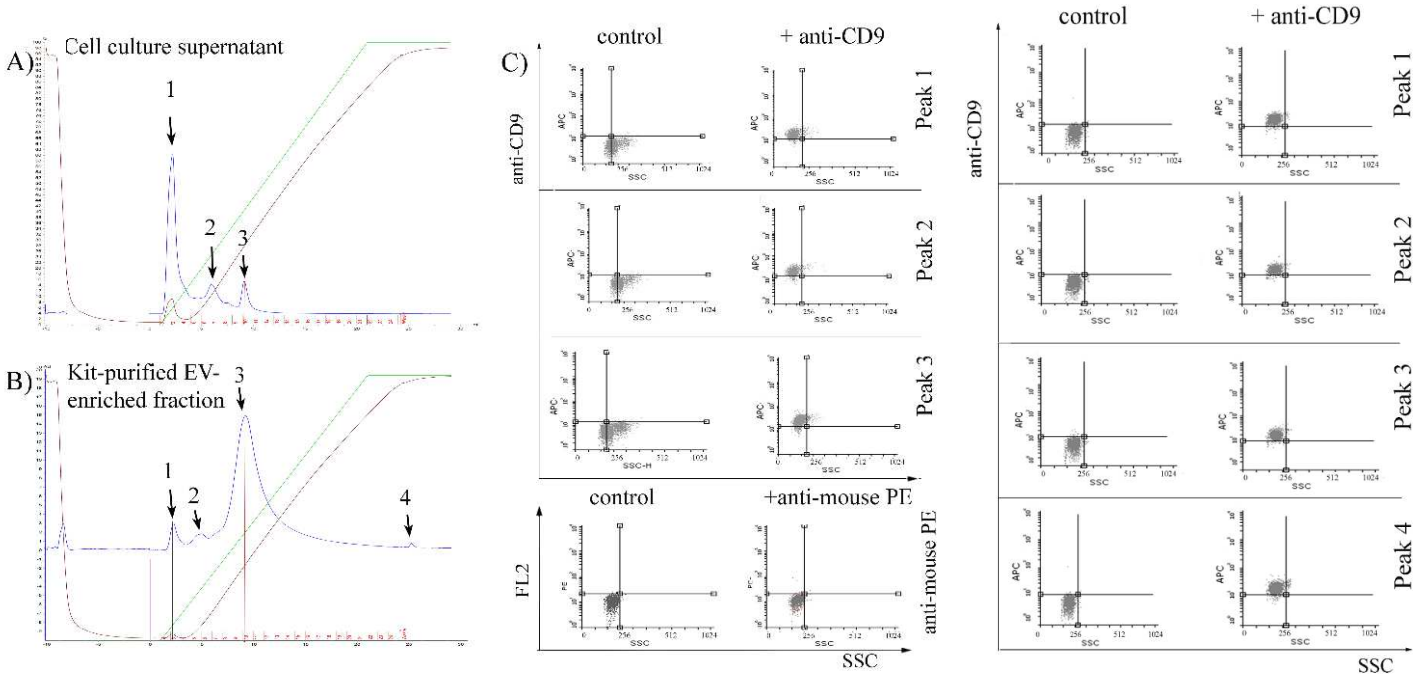


- Biological fluids (blood, urine, cerebrospinal fluid, milk, ascites)
- Physiological processes: coagulation, intercellular signaling and waste management)
- Pathological conditions: inflammation, tumor growth and metastasis.
- Biomarkers with potential to application in diagnosis and therapy.
- Identification of EV sub-groups has been challenged by purification methods (cumbersome, not reproducible, or insufficient to yield homogeneous material)

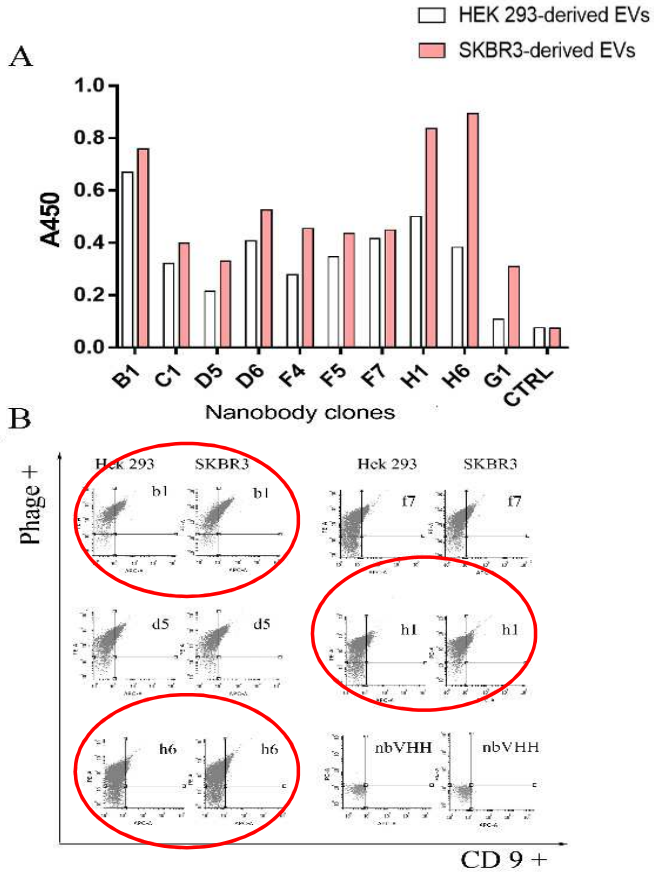
We aimed at isolating anti-exosome nanobodies to use for vesicle immunopurification by panning a pre-immune singe-chain llama antibody (VHH) phage library directly against exosomes derived from cell culture supernatant.

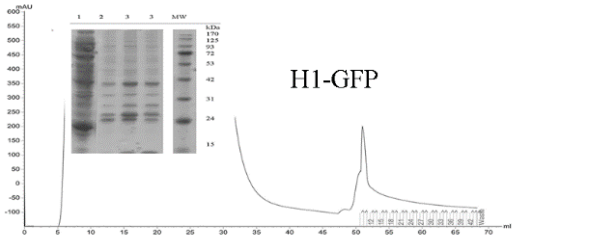
Bellingham, Guo et al. (2012). Frontiers Physiol **3**(124).
van der Pol, Boing et al. (2012). Pharmacol Rev **64**(3): 676-705.

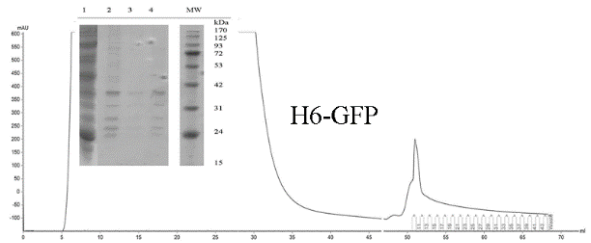
Purification of Evs from cell culture supernatant



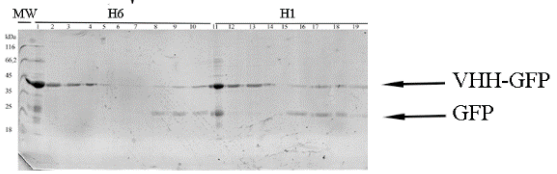
Panning on EVs and VHH production



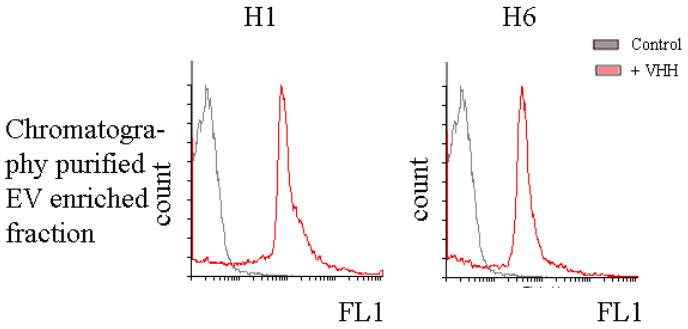


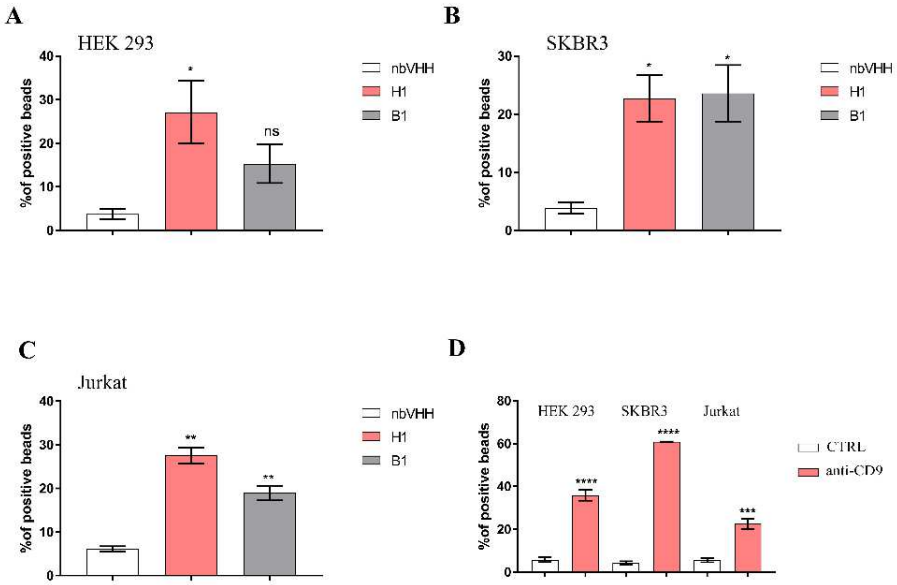


desalting + IEX

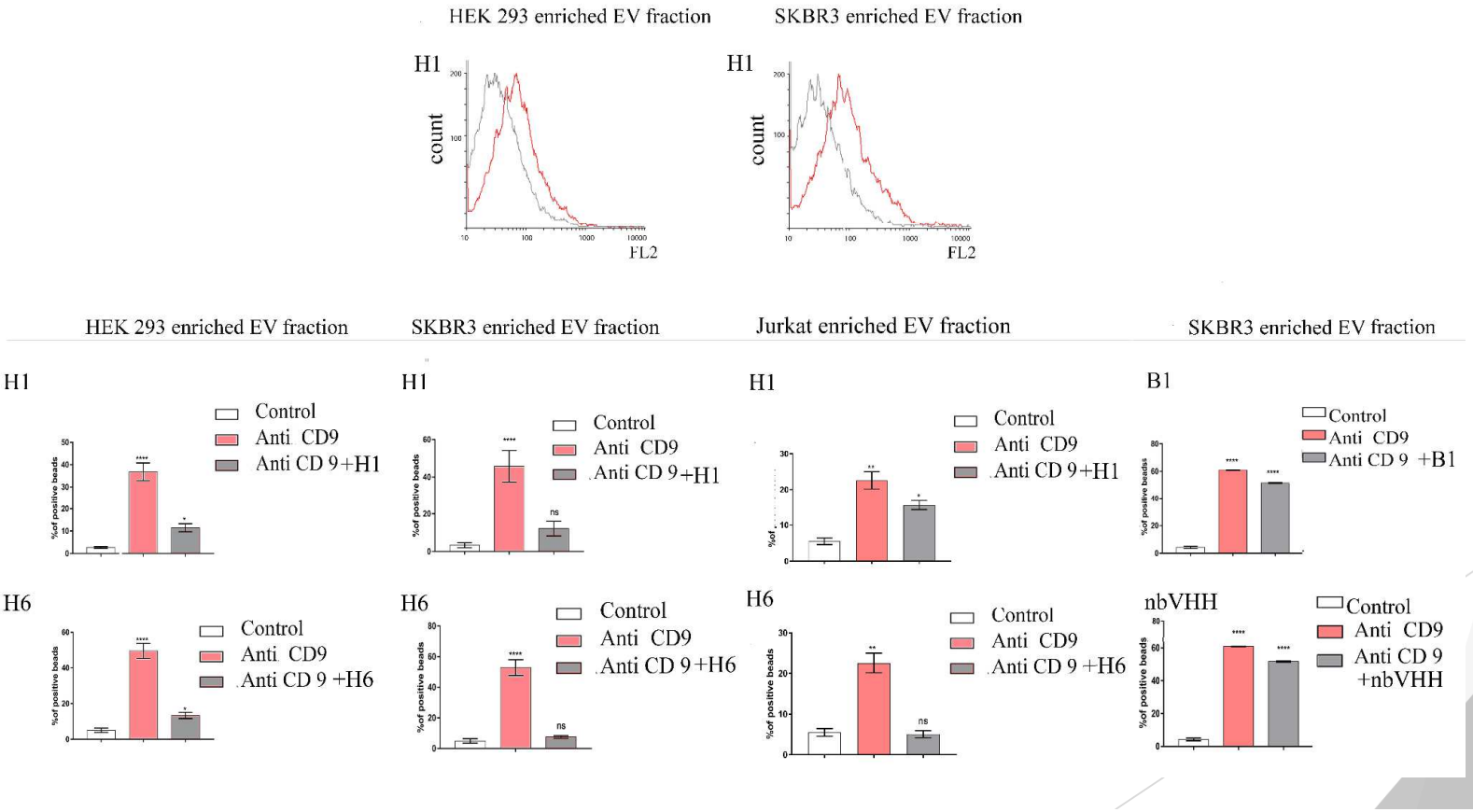


Isolated VHH bind strongly to HPLC isolated or precipitated EVs

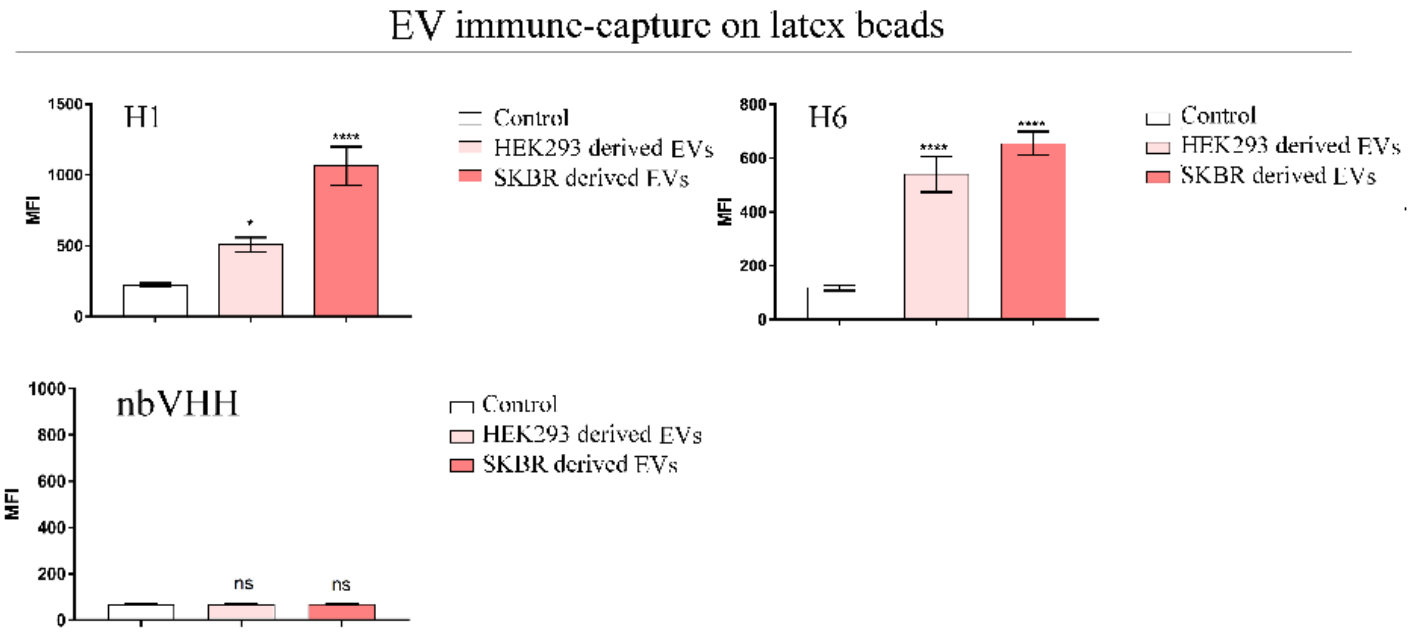




H1 and H6 VHH-GFP constructs compete with anti-CD9 antibodies



Nanobody-mediated EV capture on solid surfaces



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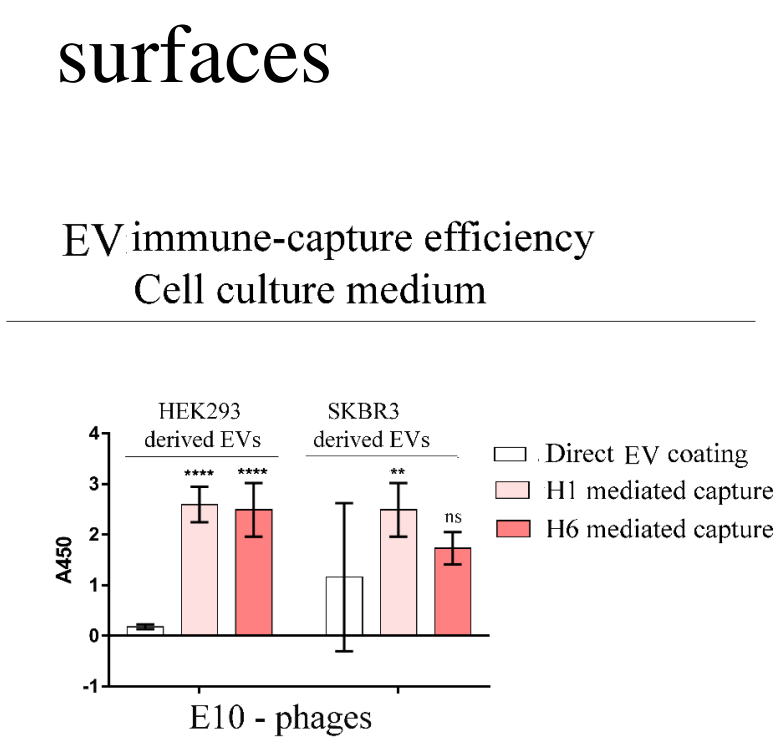

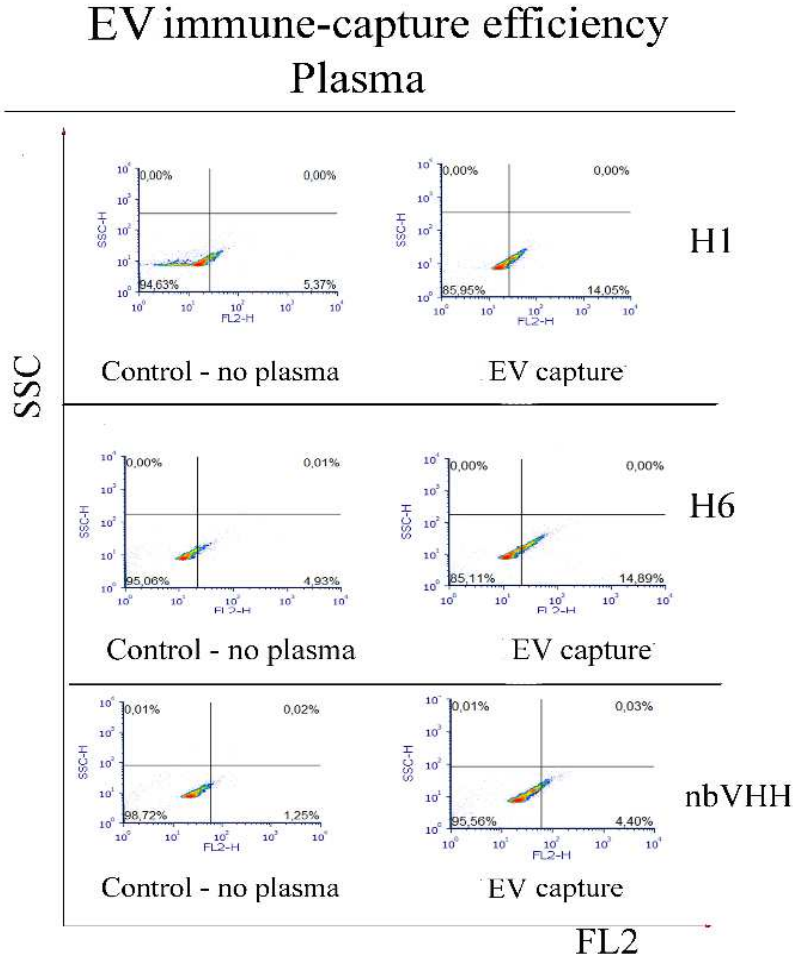

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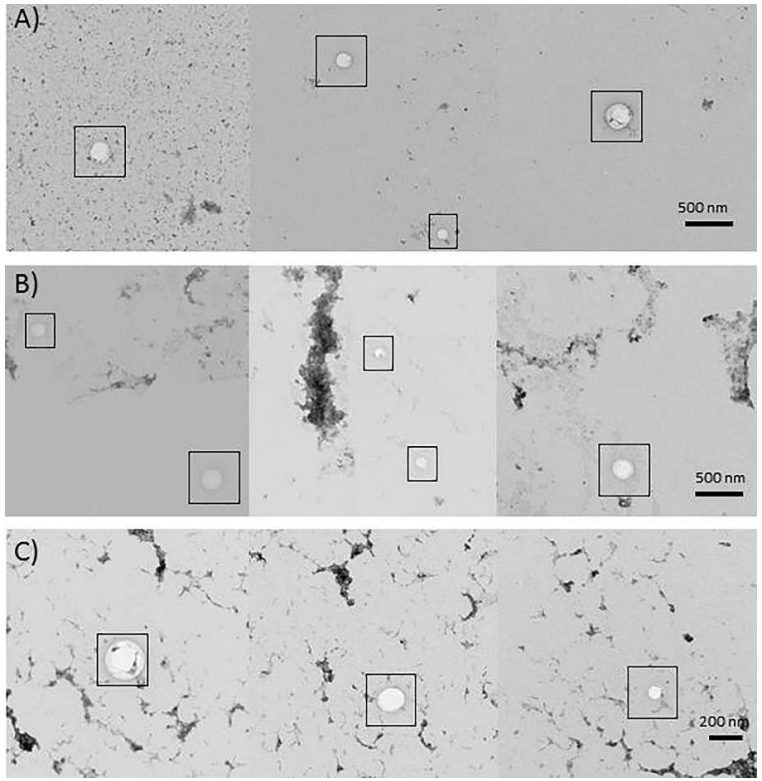

Cell culture

Unfractionated human plasma

Conclusion

- This was the first successful panning performed directly against intact EVs that yielded functional recombinant antibody fragments for exosome surface markers.
- This achievement and further optimization of the panning procedure will open the way to the discovery of VHHs able to discriminate between EV sub-populations and of selective EV surface biomarkers.
- The EV stratification made possible by such reagents could have a great clinical impact because it will enable to correlate EV subgroups to specific pathology or prognosis.

Thank you for your attention!

- University of Belgrade, Faculty of Chemistry,
  - Prof. Dr Marija Gavrovic-Jankulovic
  - Andjela Platisa
- University of Nova Gorica, Laboratory for Environmental and Life Sciences
  - Prof. Dr Ario de Marco
  - Dr Elisa Mazzega
- Integrated University Hospital of Udine, Institute of Anatomy and Pathology, Udine
  - Barbara Toffoletto
- University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences
  - Dr Rita Musetti

This work was supported by the Serbia-Slovenia bilateral program (Project “Isolation of recombinant nanobodies specific for exosome subclasses”, BI-RS/16-17-014), and by the Grant No. 172049 from Ministry of Education, Science and Technological Development of the Republic of Serbia.