



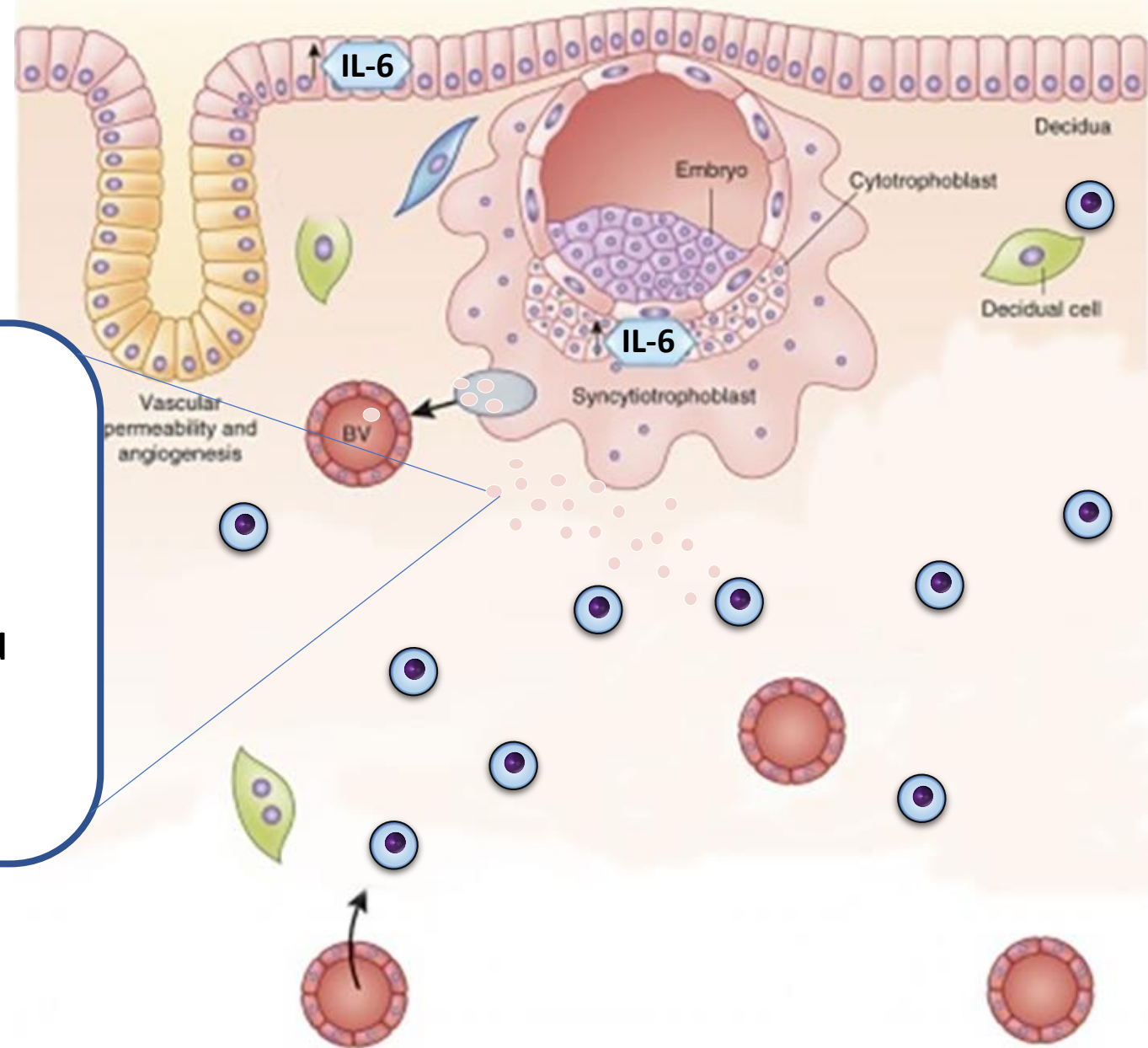
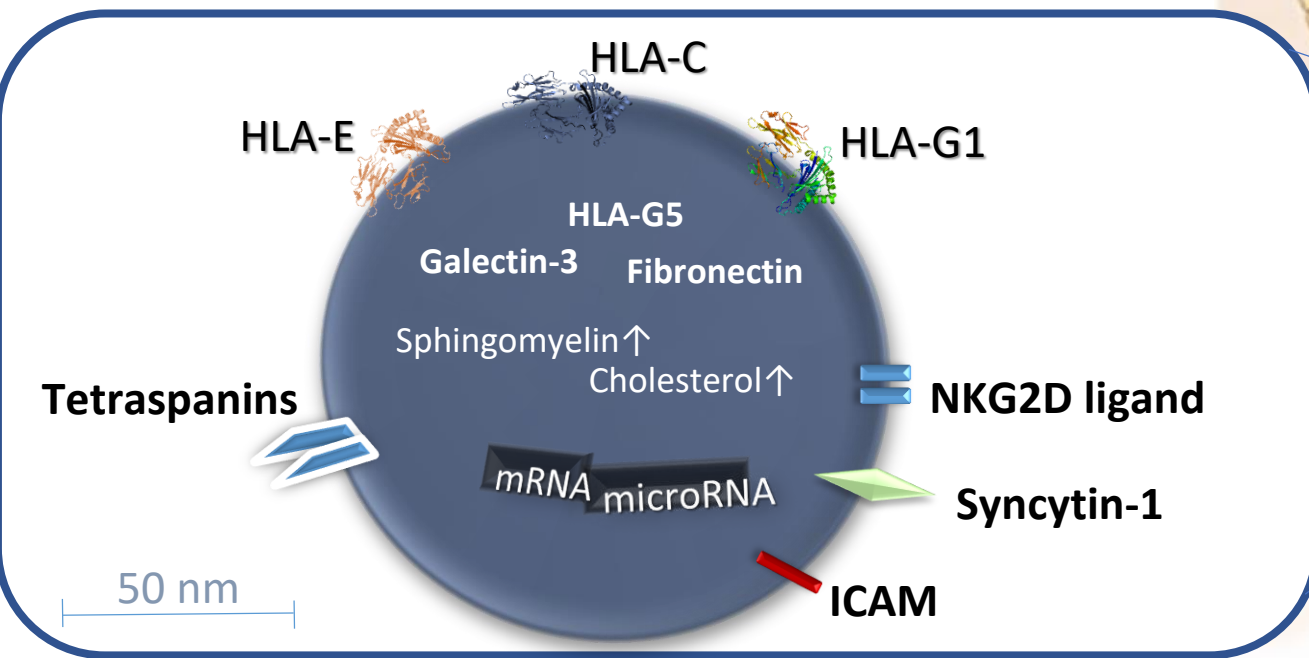
IMMUNOMODULATORY EFFECTS OF BEWO TROPHOBLASTIC CELL-DERIVED EXTRACELLULAR VESICLES ON HUMAN LYMPHOCYTES

Árpád Ferenc Kovács¹, Nóra Fekete¹, Bálint Alasztics², Gábor Joó², János Rigó², Edit Buzás¹, Éva Pállinger¹

¹Dept. of Genetics, Cell and Immunobiology, Semmelweis University ²1st Dept. of Obstetrics and Gynecology, Semmelweis University

18. 10. 2018

PERI-IMPLANTATION AND EARLY FIRST TRIMESTER



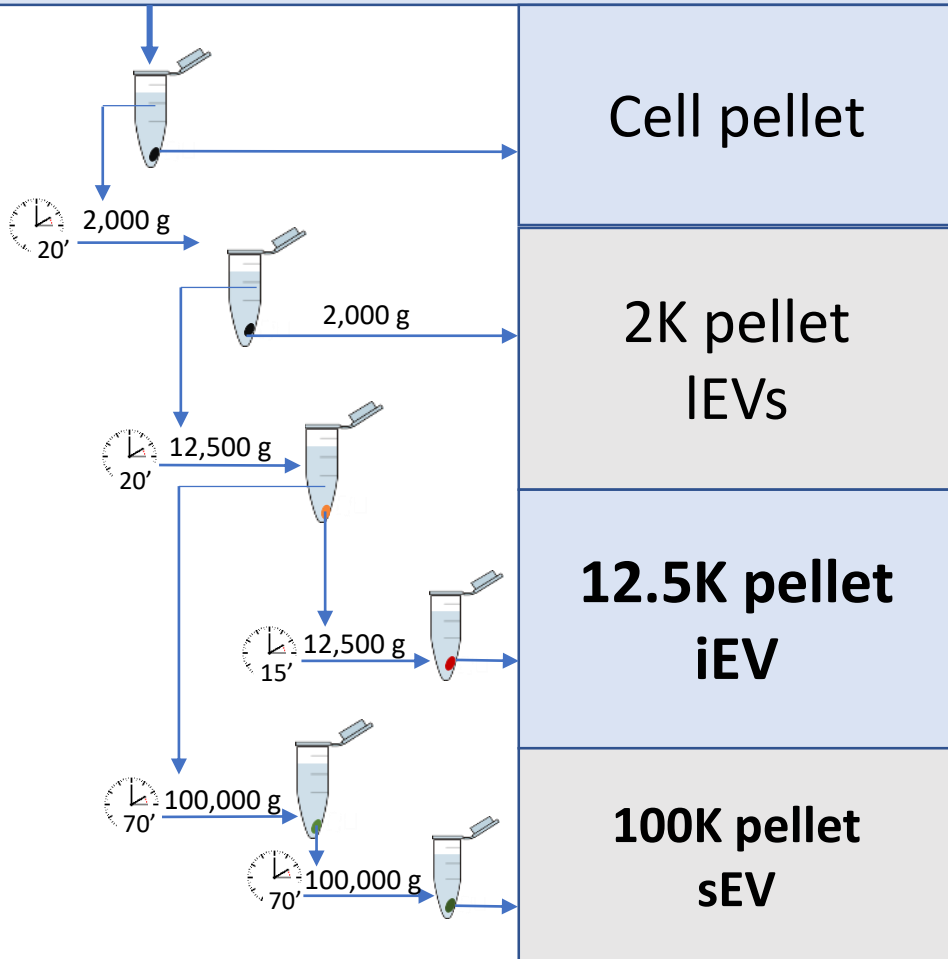
AIM

THE INVESTIGATION OF IMMUNOMODULATORY
PROTEINS FOUND IN BeWo TROPHOBLASTIC-
DERIVED iEVS

METHODS (1)

EV ISOLATION PROTOCOL

10 mL of serum free BeWo Supernatant



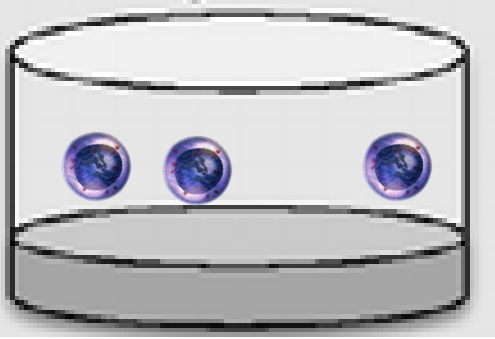
EV CHARACTERIZATION

- ✓ **VESICULAR NATURE** – FLUORESCENT MICROSCOPY, TEM
- ✓ **EV MORPHOLOGY AND SIZE** – TEM
- ✓ **EV PROTEIN content** – MICRO BCA
- ✓ **EXOFACIAL PROTEIN PATTERN** – FLOW CYTOMETRY
- ✓ **EV MARKERS** – WESTERN BLOT, IMMUNOGOLD TEM
- ✓ **EV-ASSOCIATED PROTEIN CARGO** – MASS SPECTROMETRY
- ✓ **EV miRNA content** – QUBIT
- ✓ **EV dsDNA content** - QUBIT

METHODS (2)

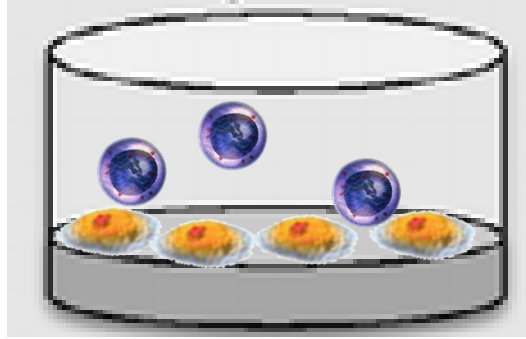
Control

1×10^6 LYMPHOCYTES



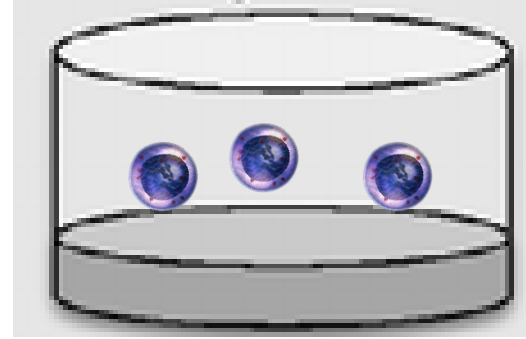
DIRECT CELL-CELL INTERACTION

1×10^6 LYMPHOCYTES
 2×10^5 BeWo cells



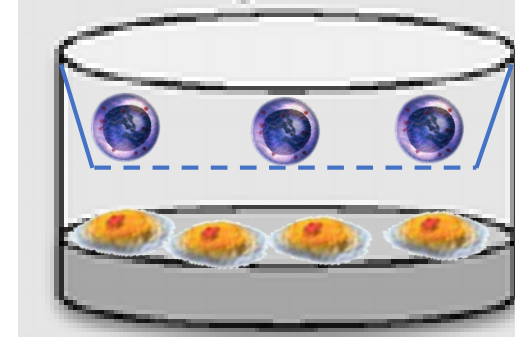
iEV – LYMPHOCYTE INTERACTION

1×10^6 LYMPHOCYTES
 3×10^6 BeWo-derived iEVs



TRANSWELL INTERACTION

1×10^6 LYMPHOCYTES
 2×10^5 BeWo cells



Culturing for 24 hours

Add 0.02 ng/mL rIL-6

Incubation for 4 hours

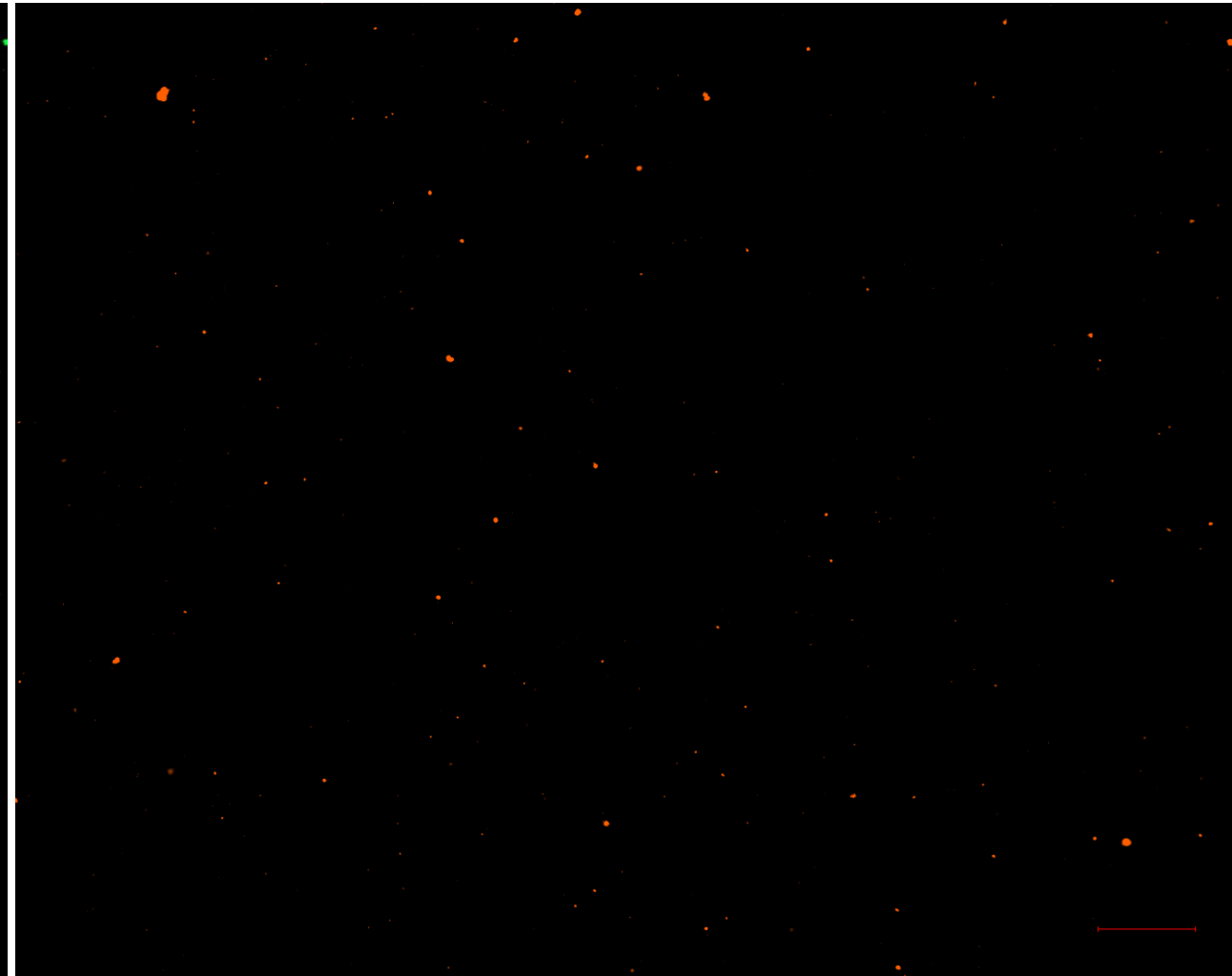
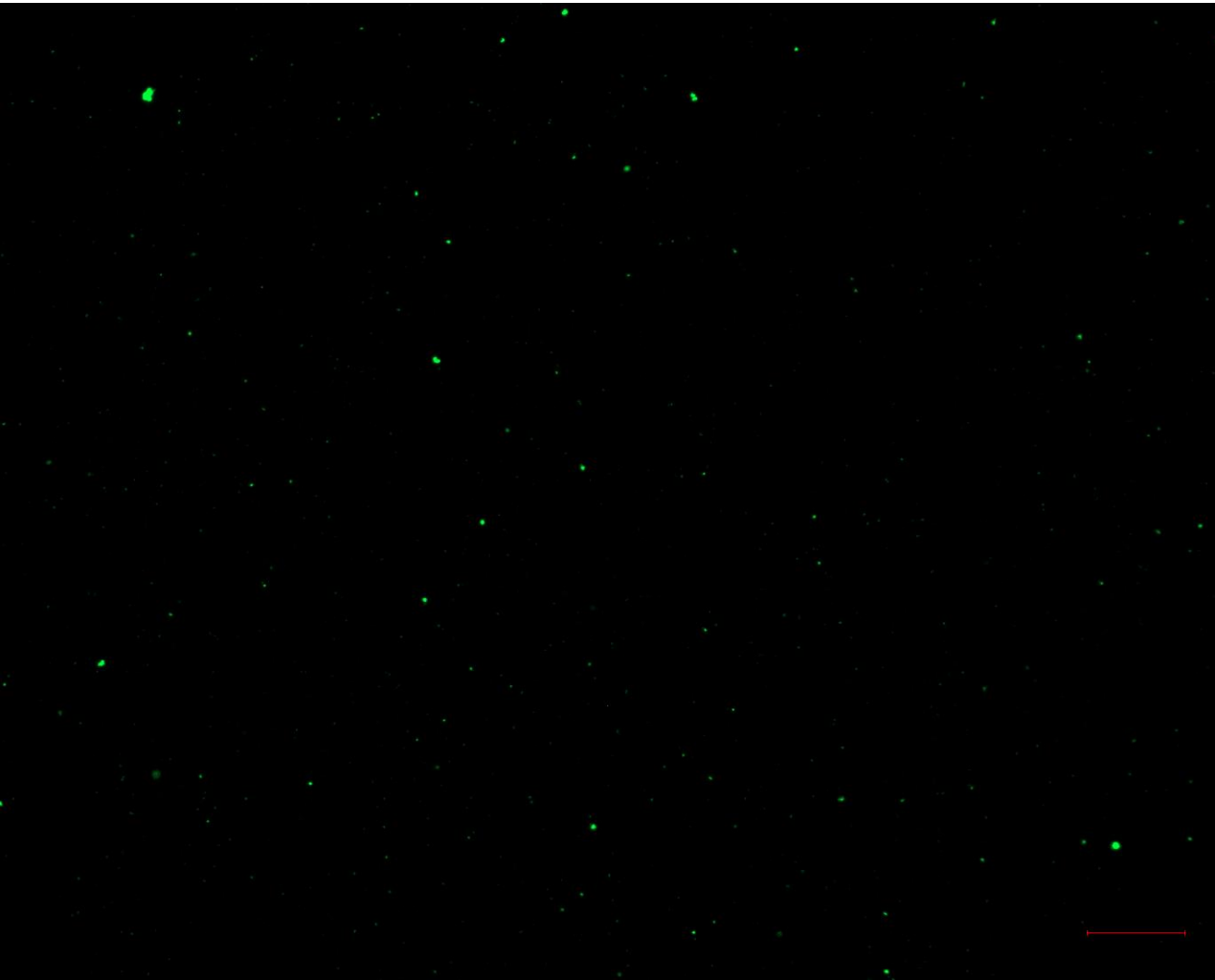
RNA ISOLATION

PROTEIN EXPRESSION

I. BeWo iEV CHARACTERIZATION

CELLDISCOVERER7 single channel view

Calcein-AM and PKH26 stained iEVs (12.5K EV enriched fraction)



GREEN – CALCEIN-AM STAINED iEVs **RED** – PKH26 STAINED iEVs **YELLOW** – CALCEIN-AM AND PKH26 DOUBLE STAINED iEVs

I. BeWo iEV CHARACTERIZATION

CELLDISCOVERER7

Calcein-AM and PKH26 stained iEVs (12.5K EV enriched fraction)



After 0.1% TRITON X-100 DETERGENT LYSIS

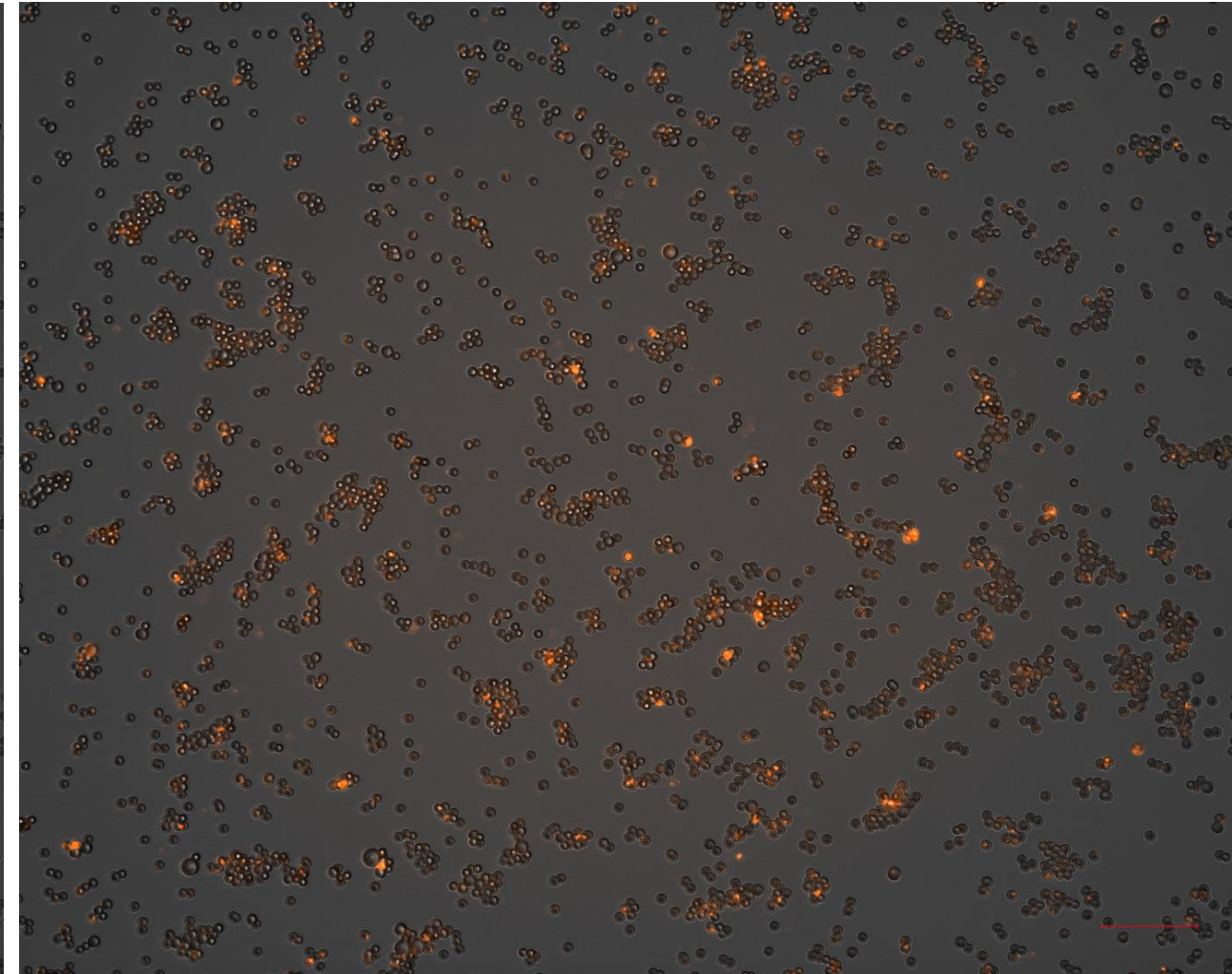
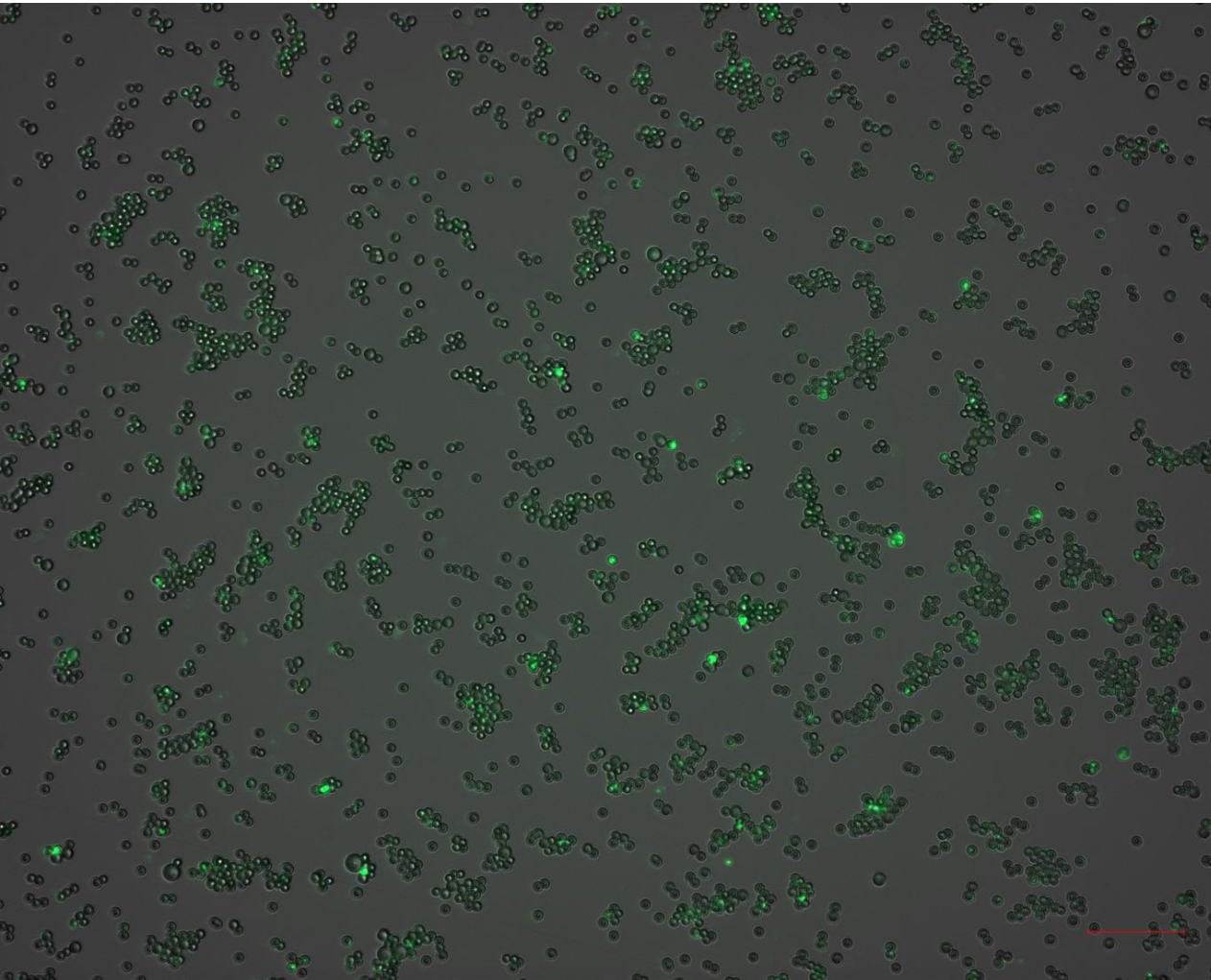


GREEN – CALCEIN-AM STAINED iEVs **RED** – PKH26 STAINED iEVs **YELLOW** – CALCEIN-AM AND PKH26 DOUBLE STAINED iEVs

I. BeWo sEV CHARACTERIZATION

CELLDISCOVERER7 – single channel view

Calcein-AM and PKH26 stained sEVs bound to 3.7 μ m latex-aldehyde beads (100K EV enriched fraction)

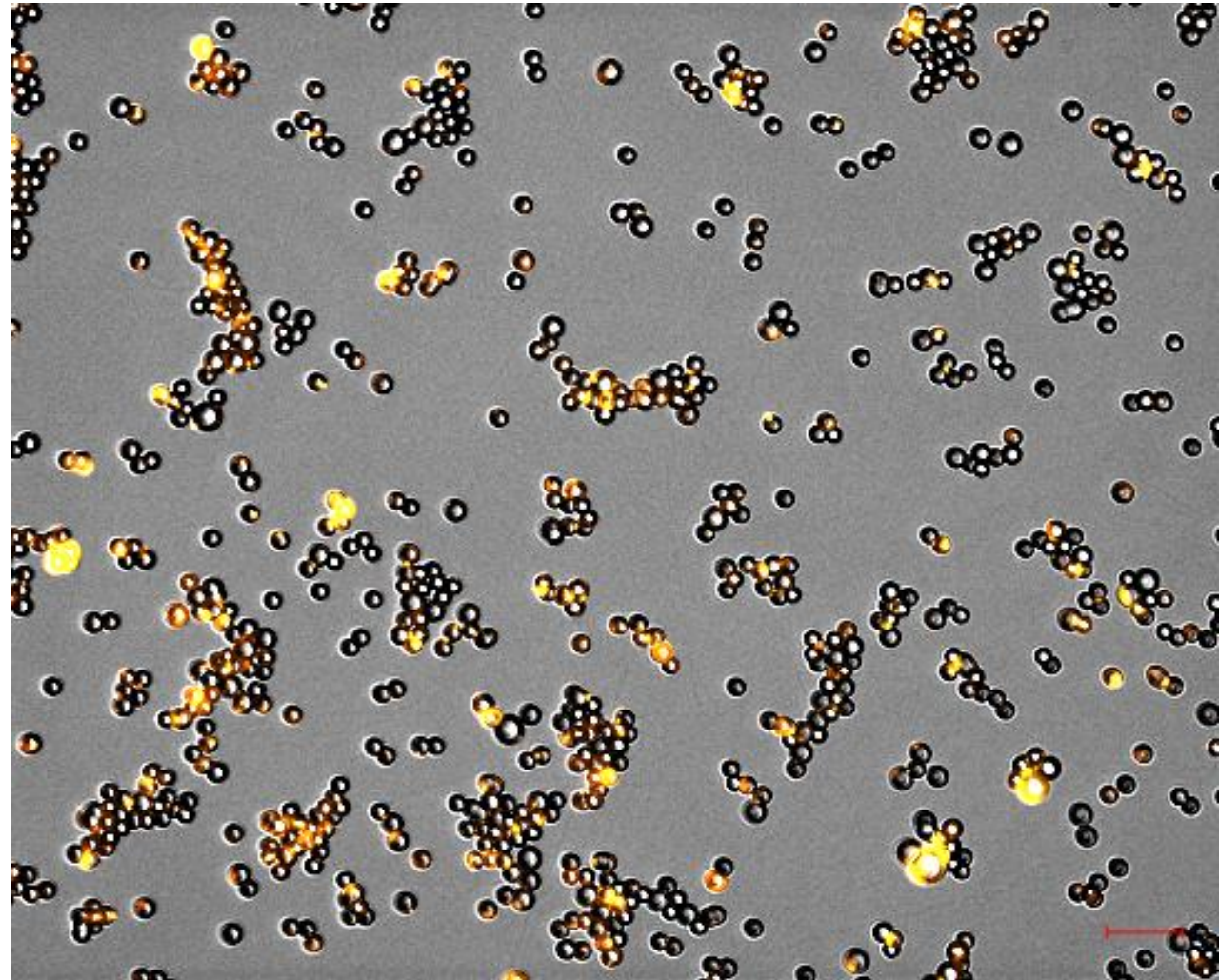
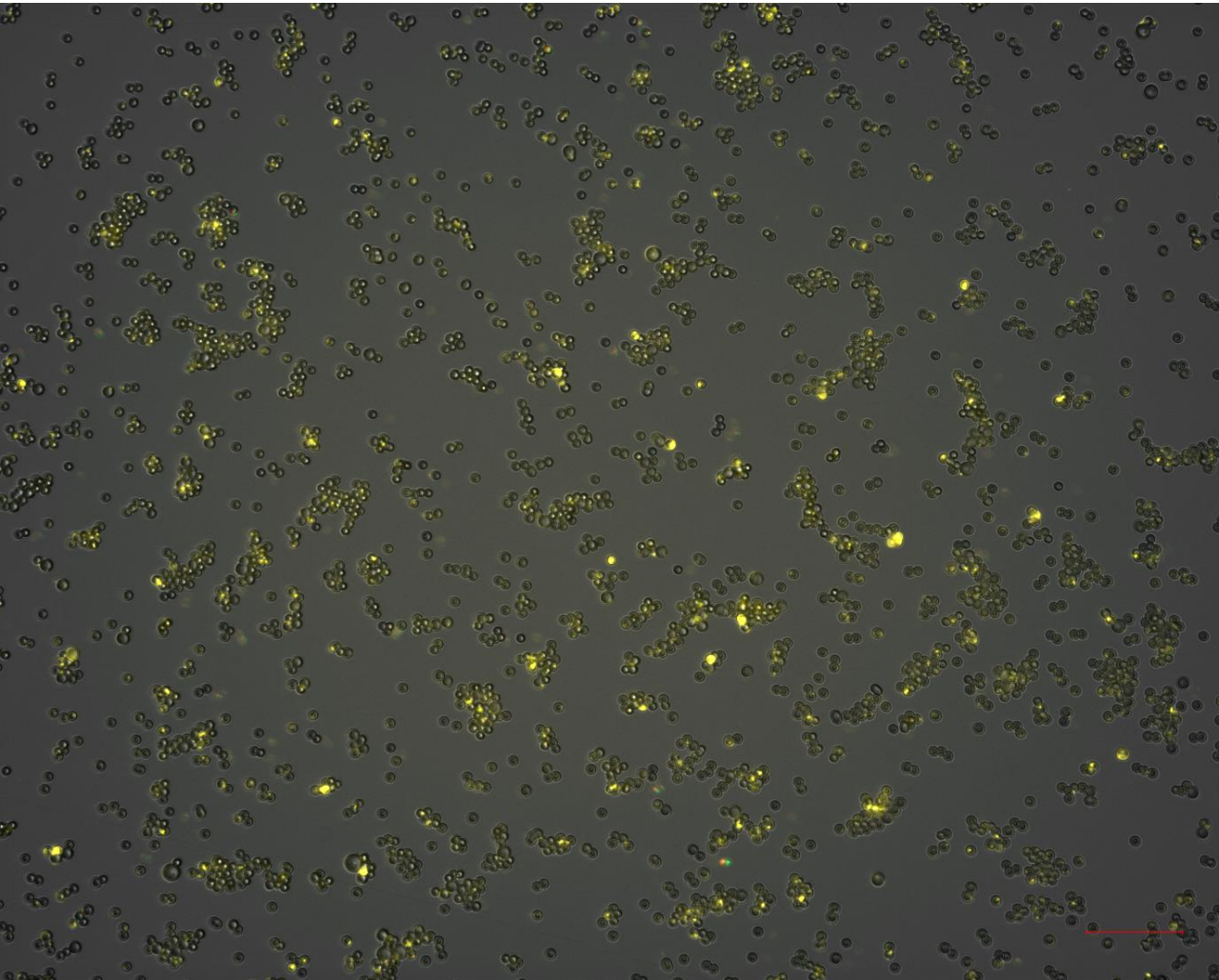


GREEN – CALCEIN STAINED iEVs **RED** – PKH26 STAINED iEVs **YELLOW** – CALCEIN-AM AND PKH26 DOUBLE STAINED iEVs

I. BeWo sEV CHARACTERIZATION

CELLDISCOVERER7

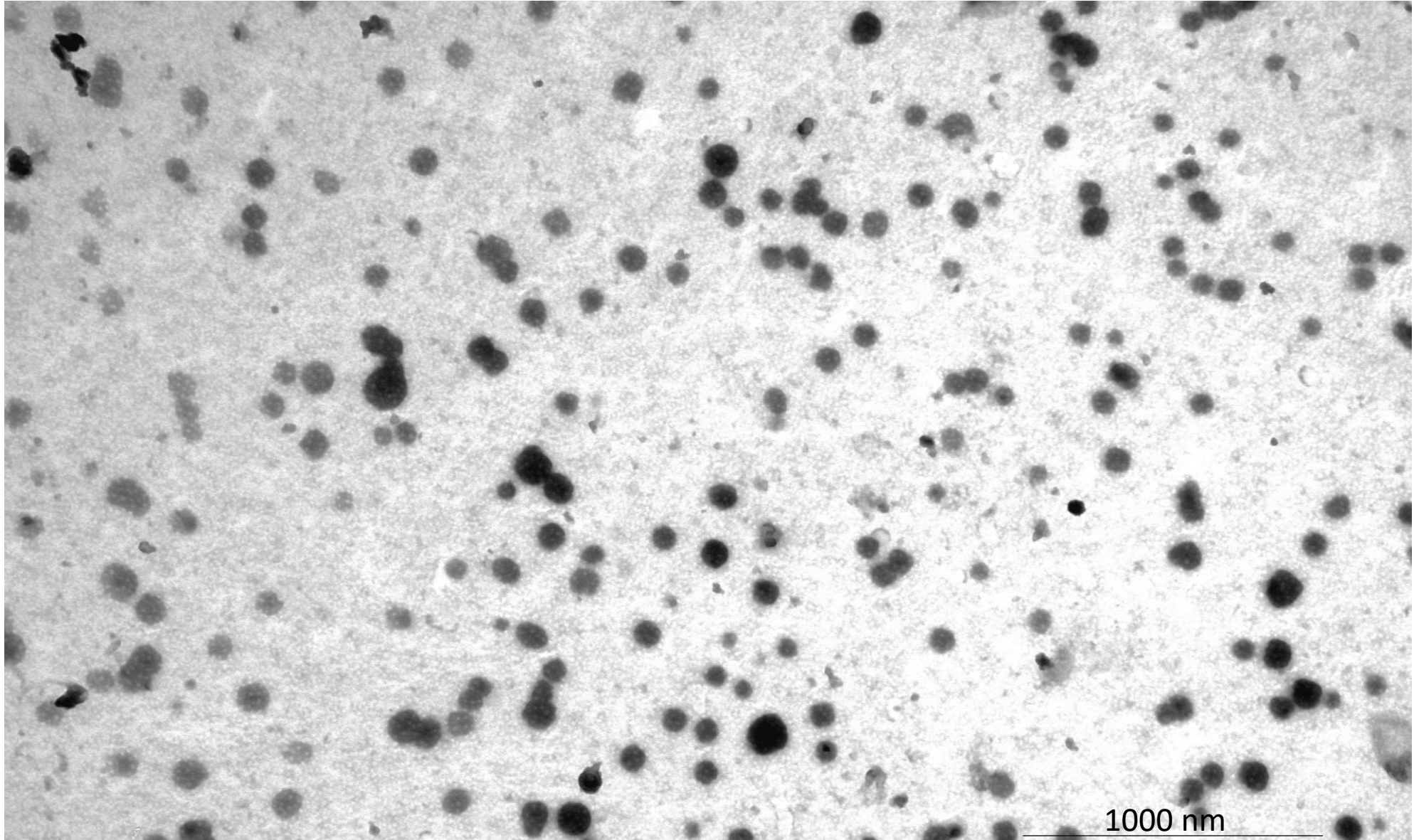
Calcein-AM and PKH26 stained sEVs bound to 3.7 μm latex-aldehyde beads (100K EV enriched fraction)



GREEN – CALCEIN STAINED iEVs **RED** – PKH26 STAINED iEVs **YELLOW** – CALCEIN-AM AND PKH26 DOUBLE STAINED iEVs

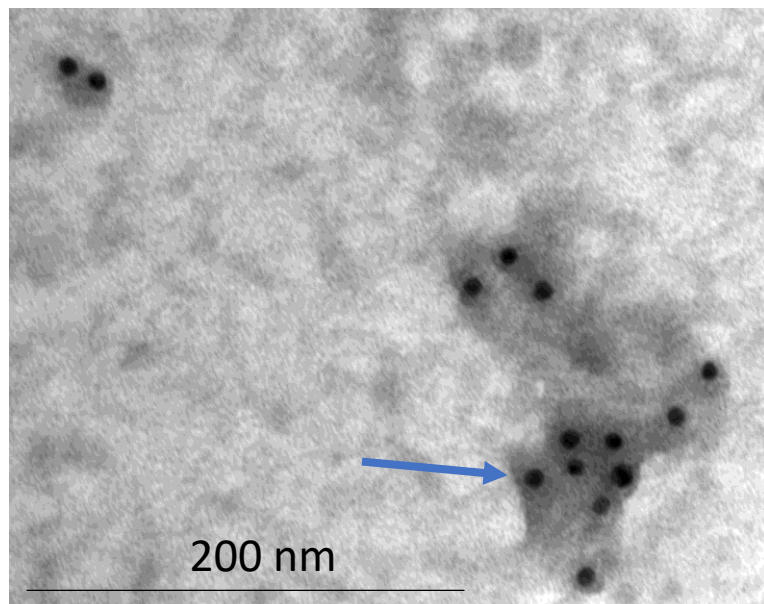
I. BeWo iEV CHARACTERIZATION

TEM negative stain



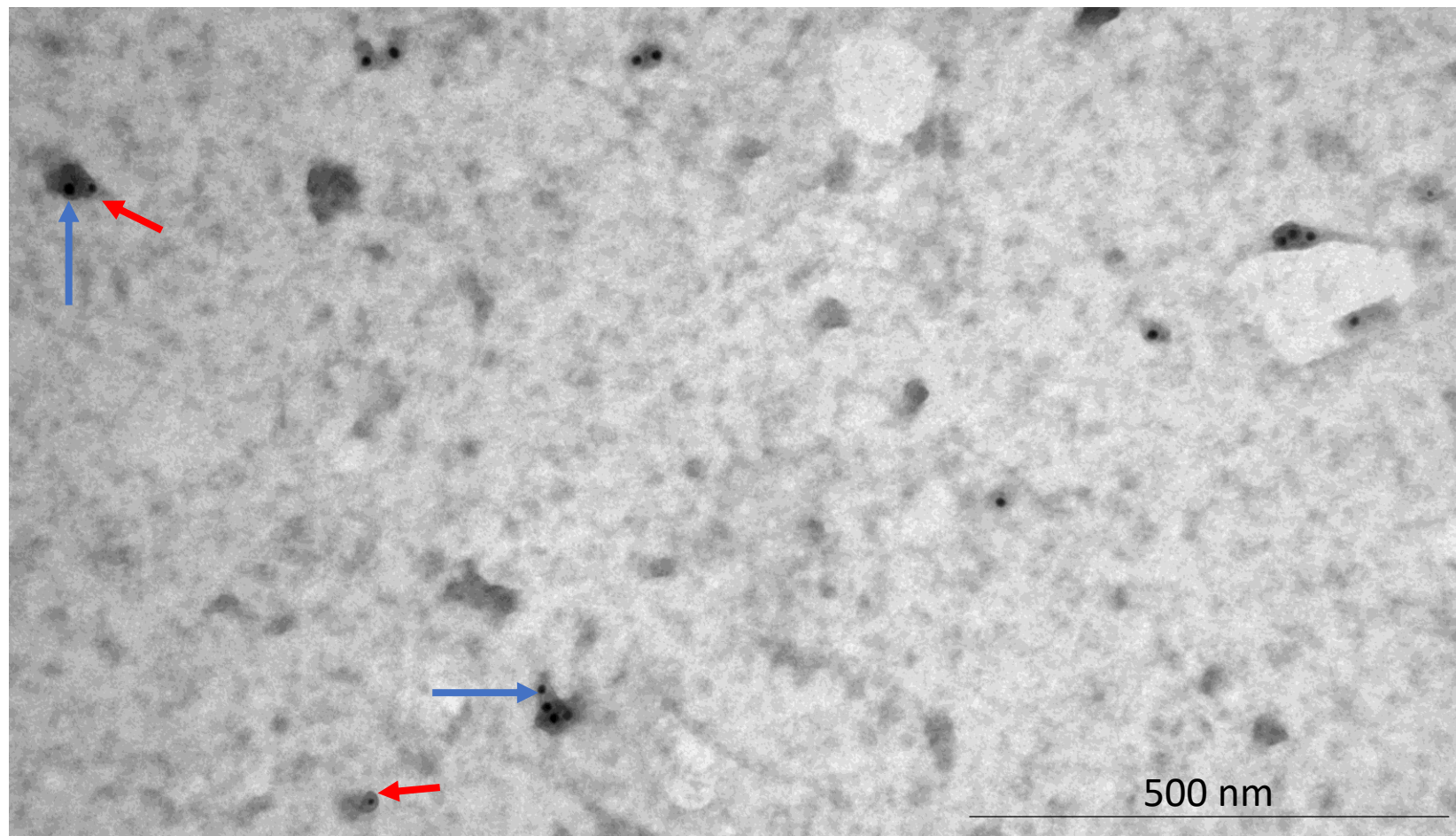
I. BeWo iEV CHARACTERIZATION

TEM – CD63 AND HLA-G IMMUNOGOLD LABELLING



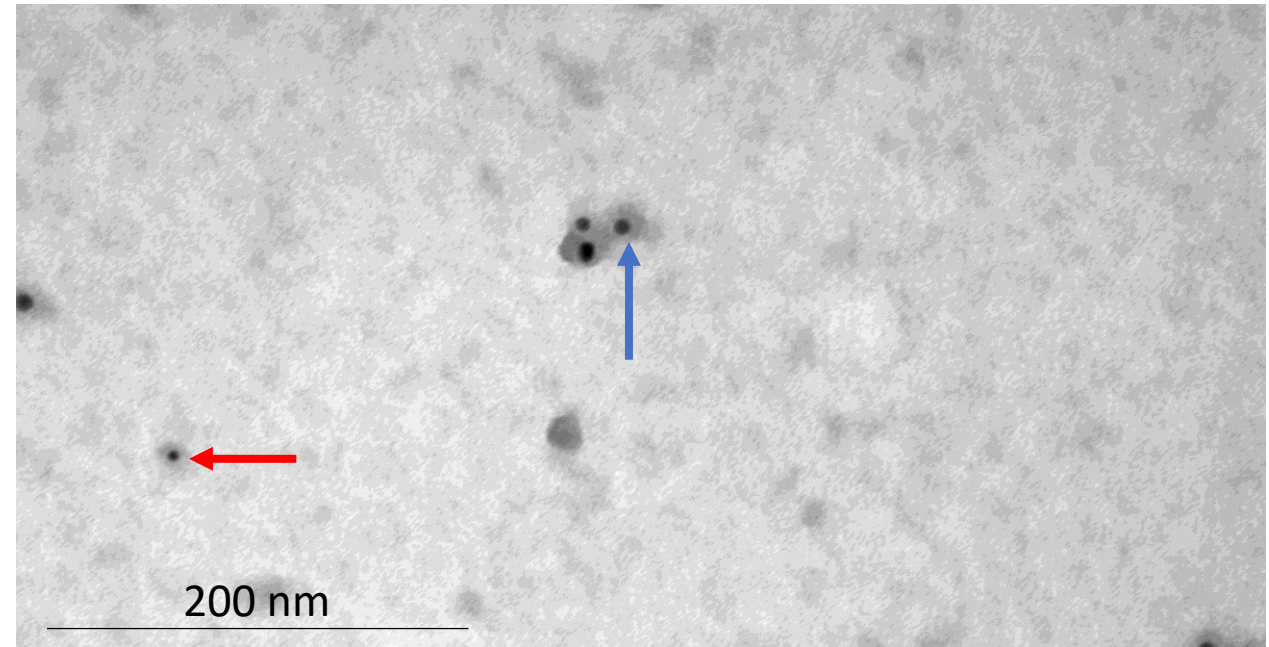
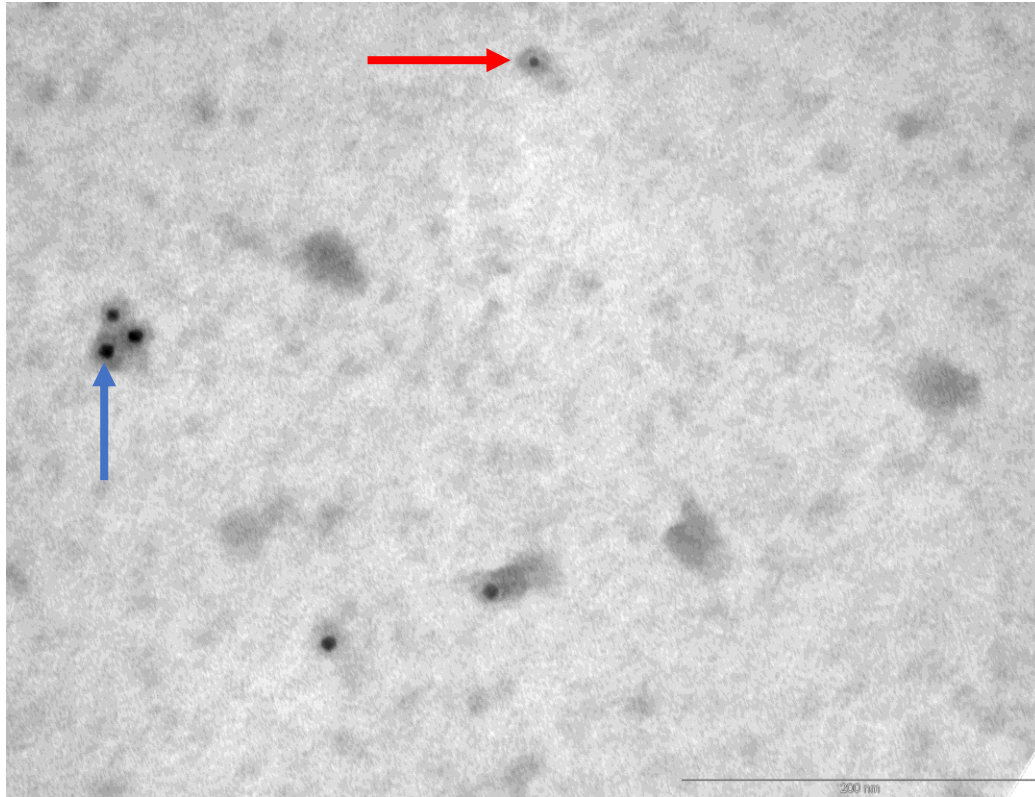
CD63

HLA-G →



I. BeWo sEV CHARACTERIZATION

TEM – CD63 AND HLA-G IMMUNOGOLD LABELLING



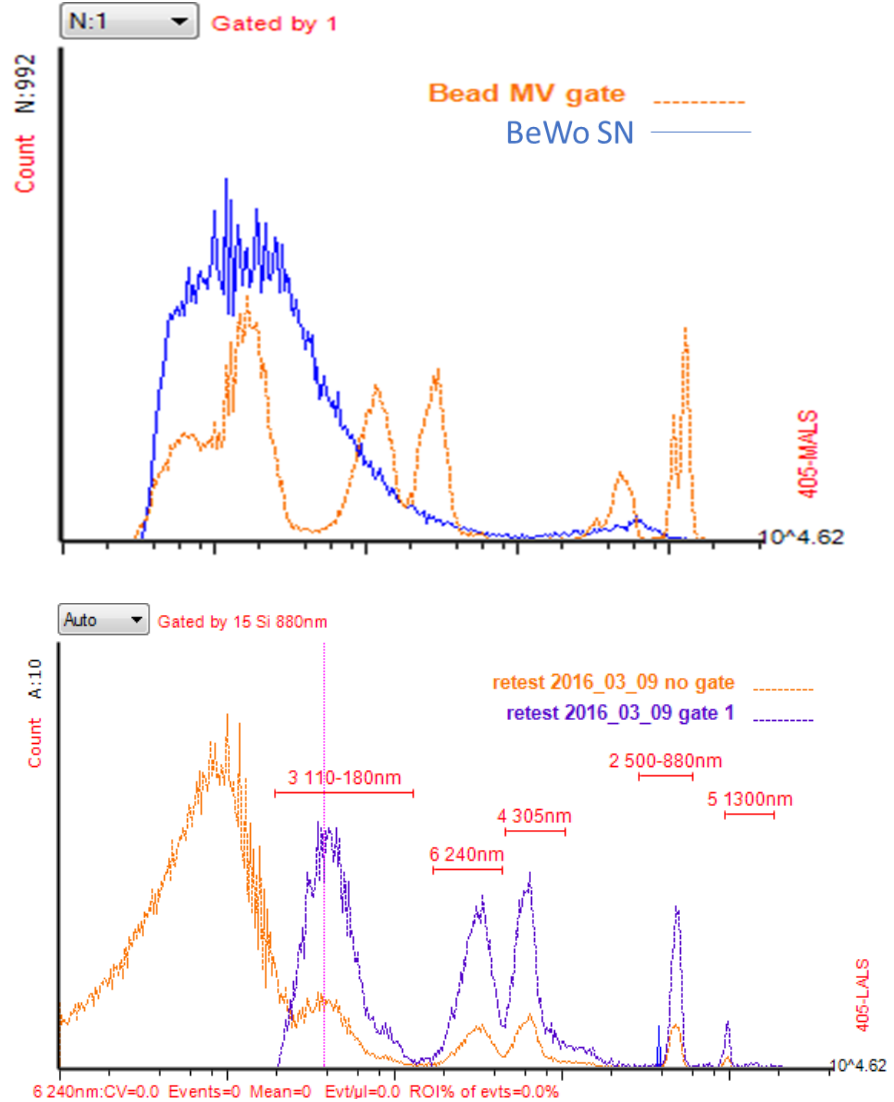
HLA-G →

CD63

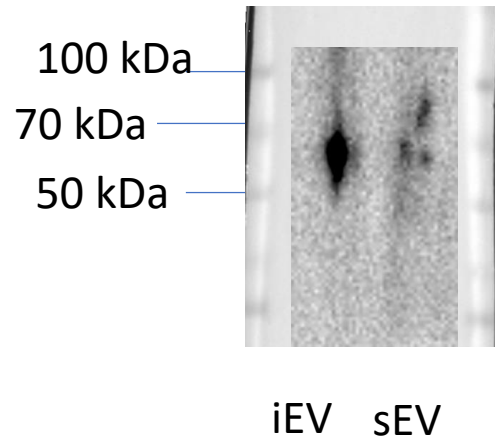
→

I. BeWo EV CHARACTERIZATION

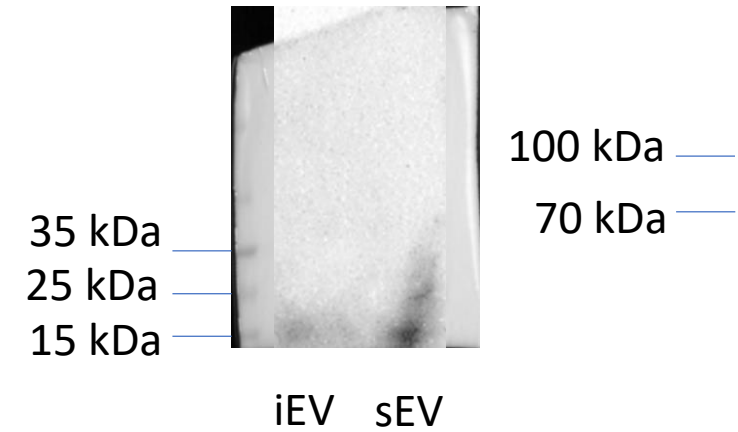
EV high resolution flow cytometry and western blot



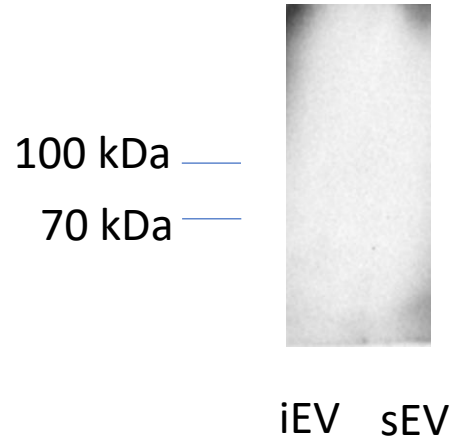
CD63



HLA-G



MPO



I. BeWo iEV and sEV CHARACTERIZATION

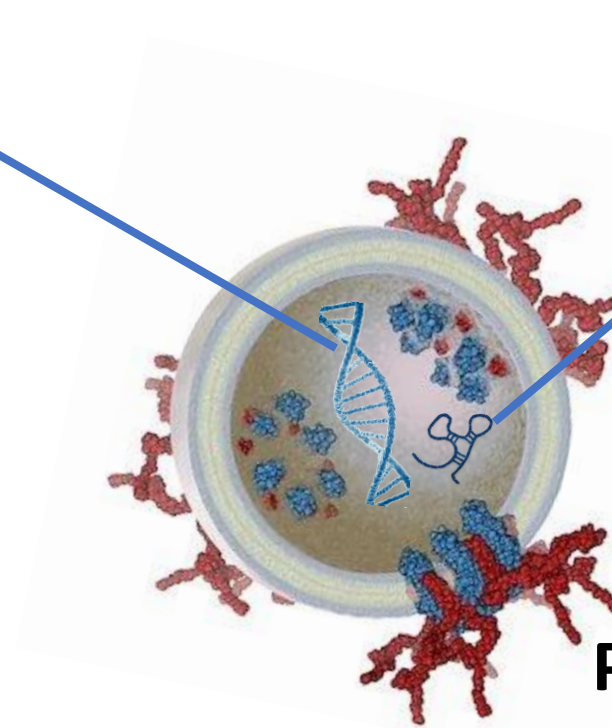
Protein and nucleic acid content of BeWo-derived EVs

dsDNA (Qubit assay)

iEV	334 ± 222 ng/mL
sEV	2716 ± 1044 ng/mL
EV poor	700 ng/mL

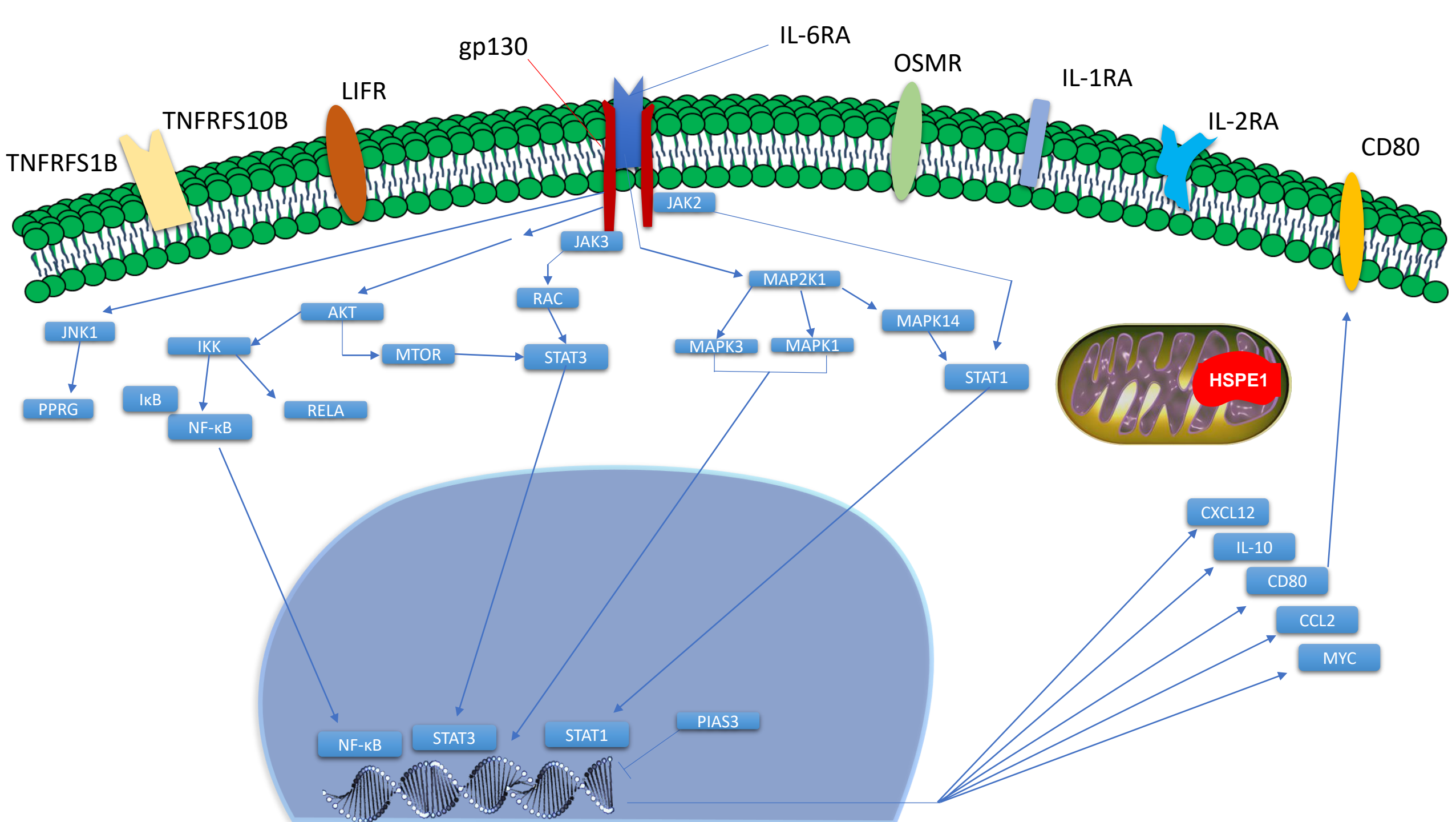
miRNA (Qubit assay)

iEV	1180 ng/mL
sEV	17387 ± 5712 ng/mL
EV poor	4340 ng/mL

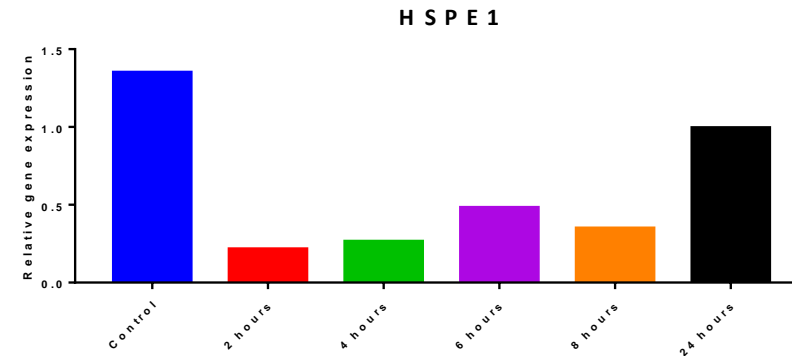
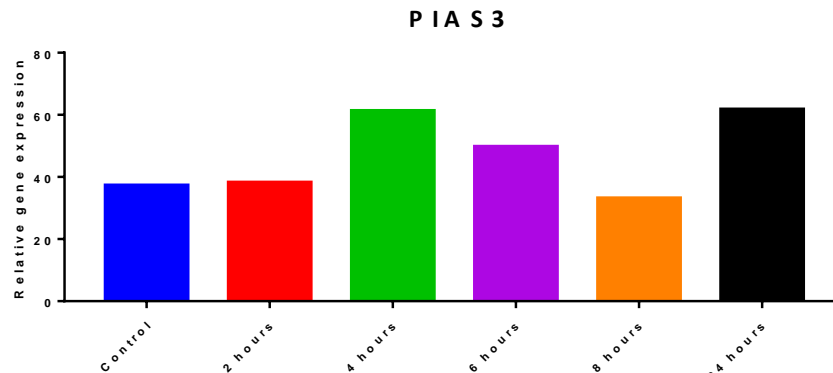
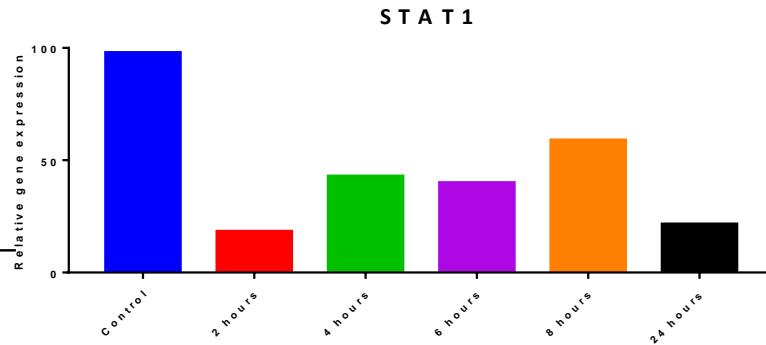
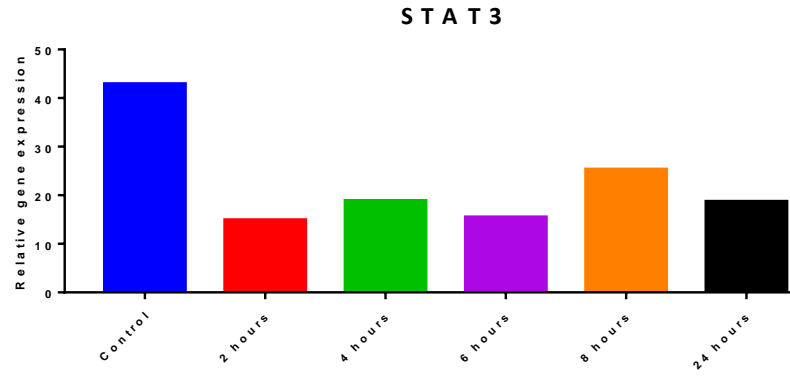
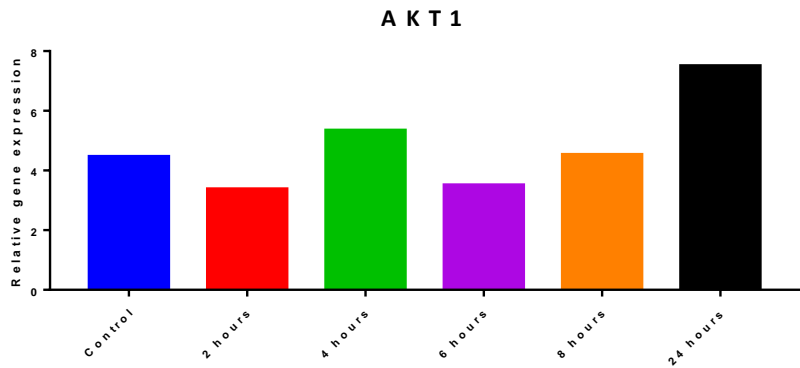
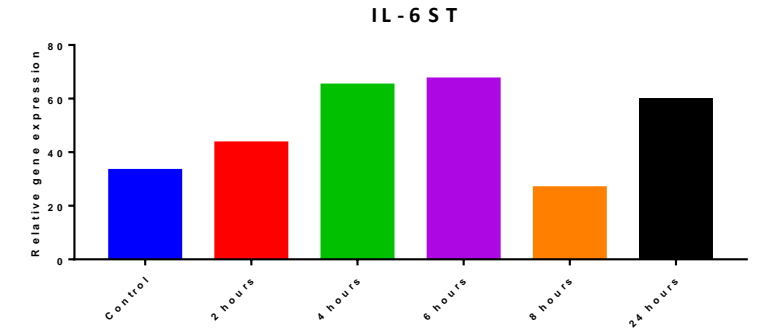
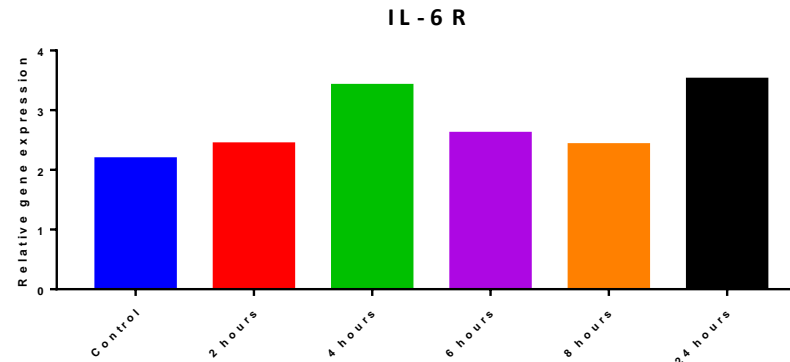
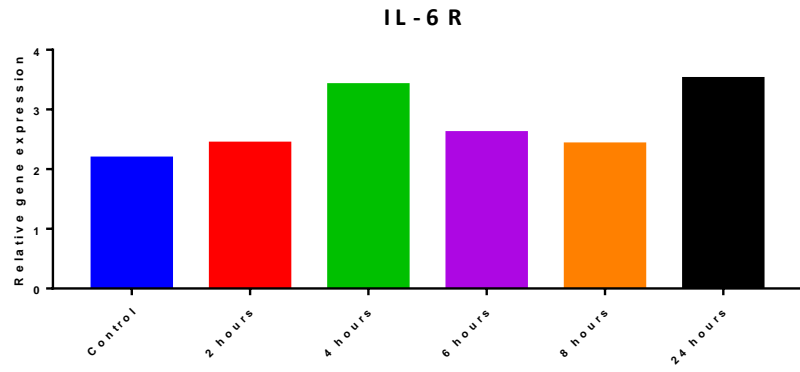


Protein (Micro BCA assay)

iEV	0.218 ± 0.08 mg/mL
sEV	0.09 ± 0.03 mg/mL
EV poor	0.127 ± 0.04 mg/mL

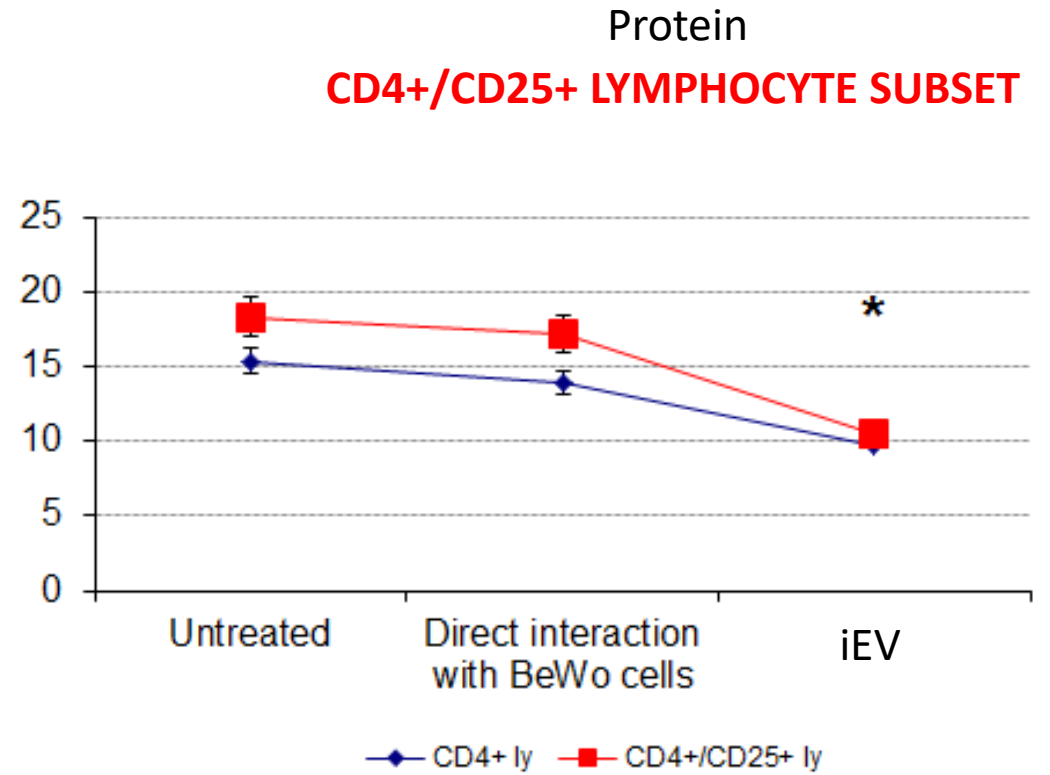
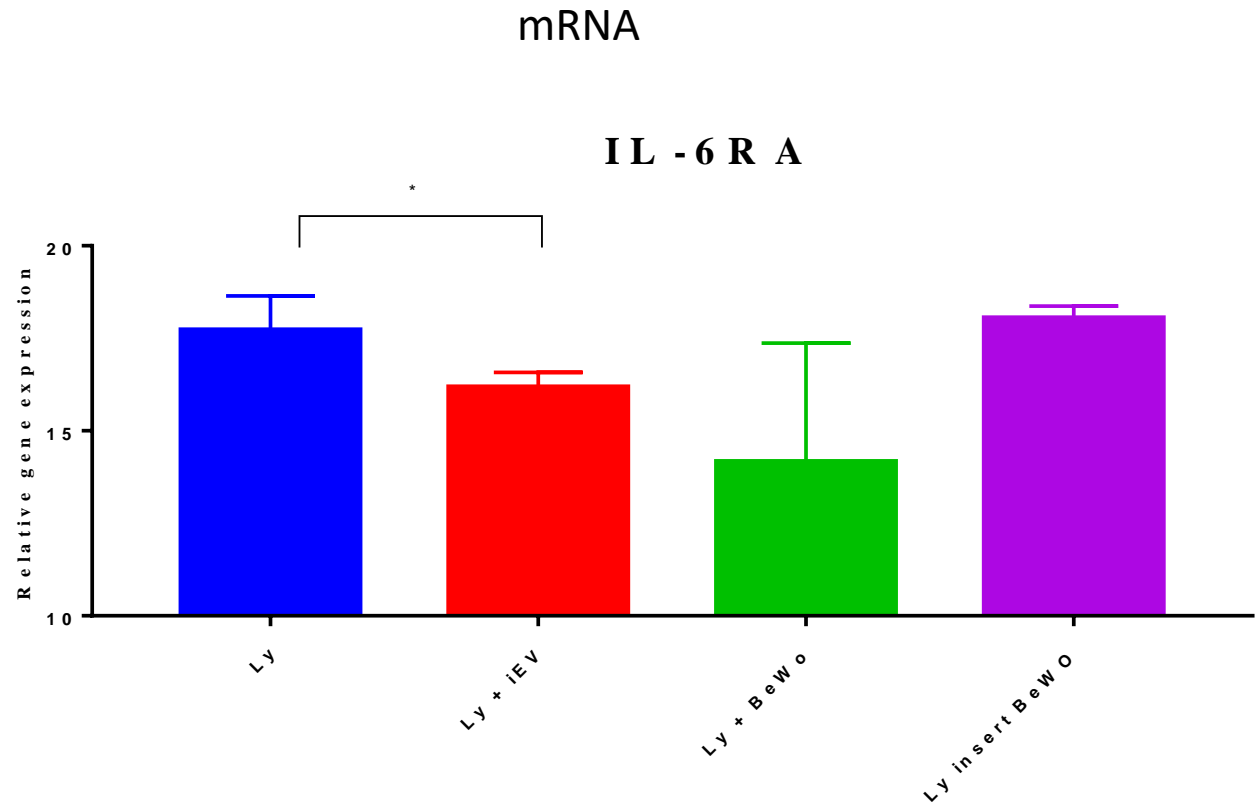


II. EFFECTS OF IL-6 ON THE LYMPHOCYTE'S GENE EXPRESSION

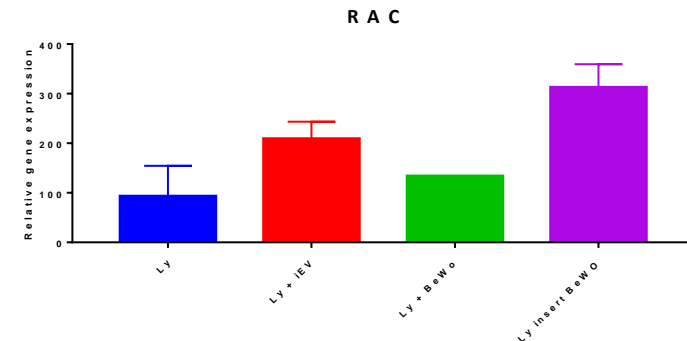
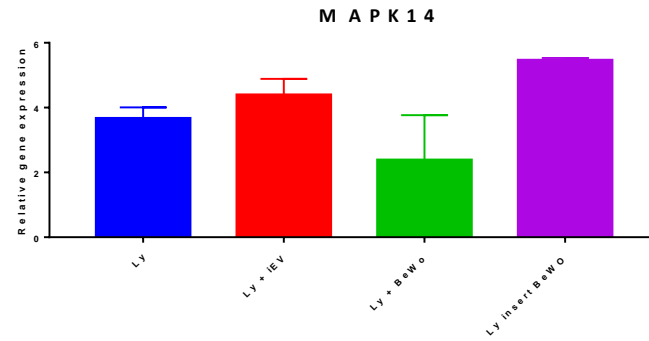
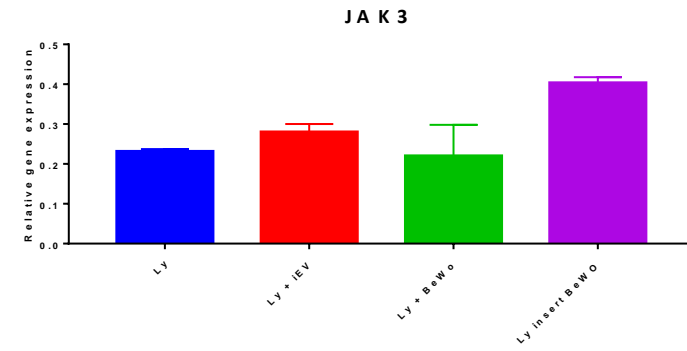
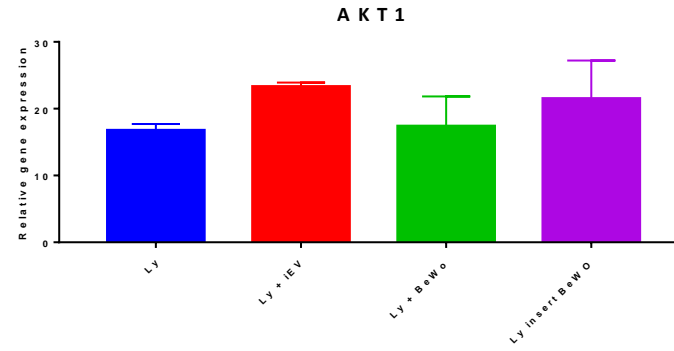
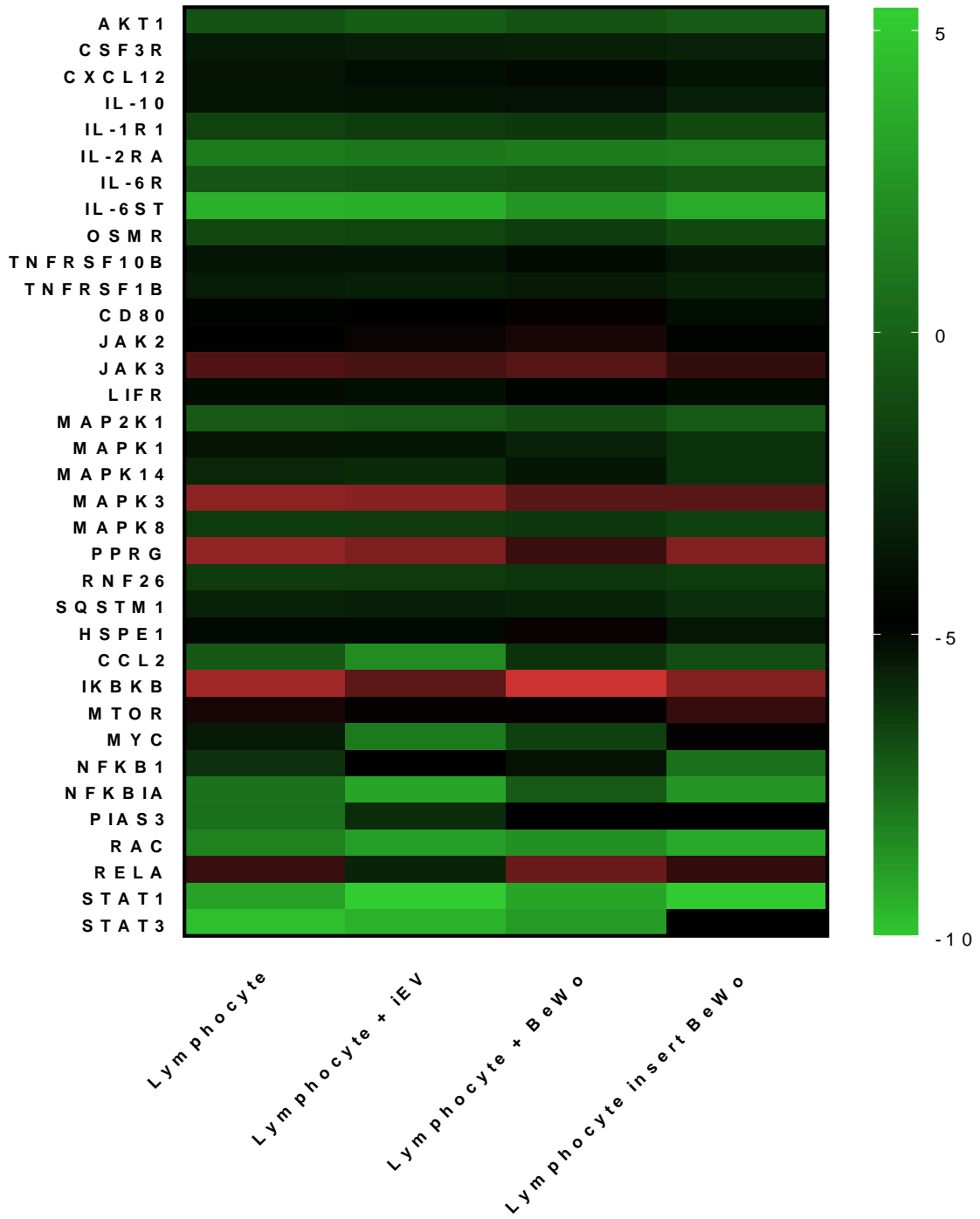


Normalized to HPRT reference gene, displayed values: $2^{-\Delta Ct}$

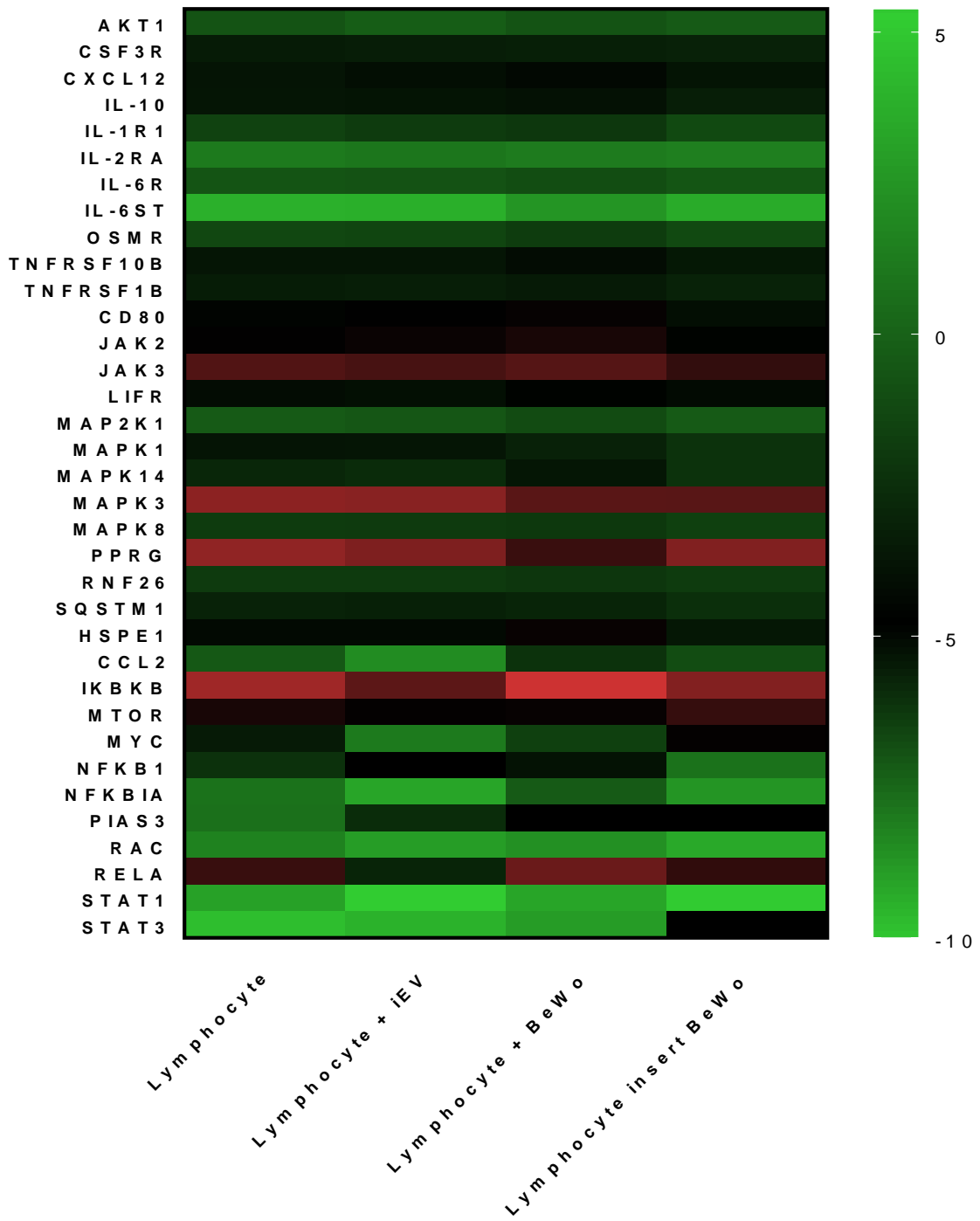
II. IL-6R DOWNREGULATION



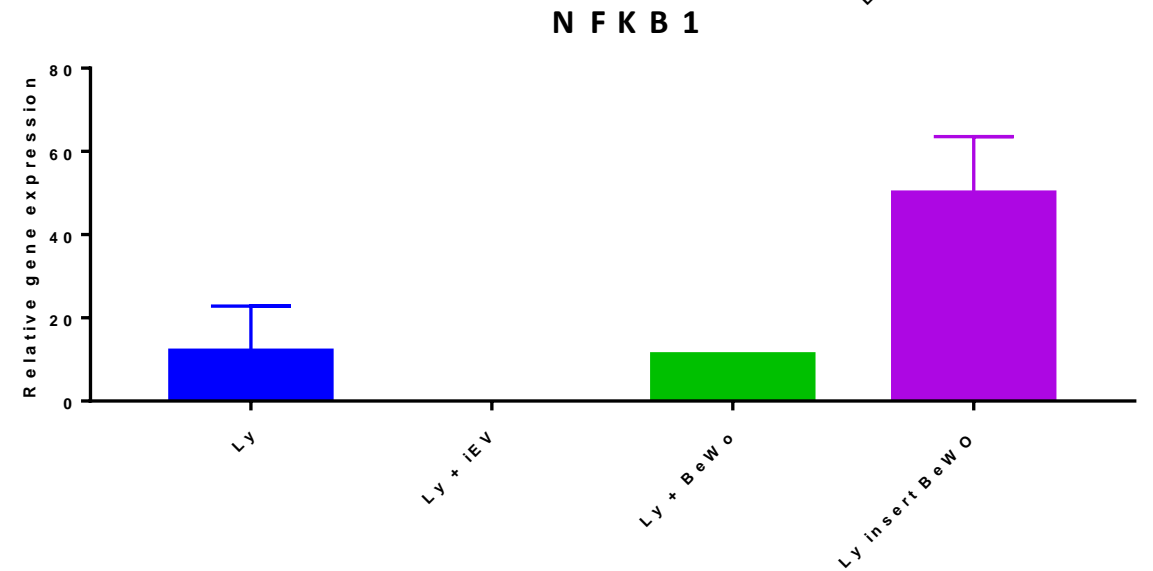
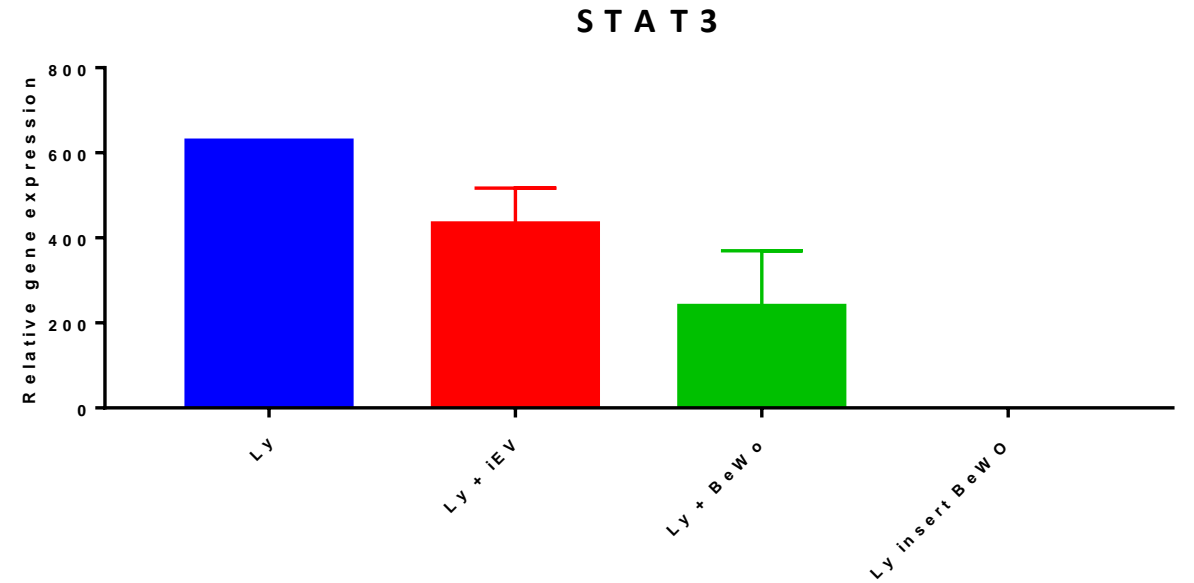
SIGNALING UPSTREAM OF STAT3

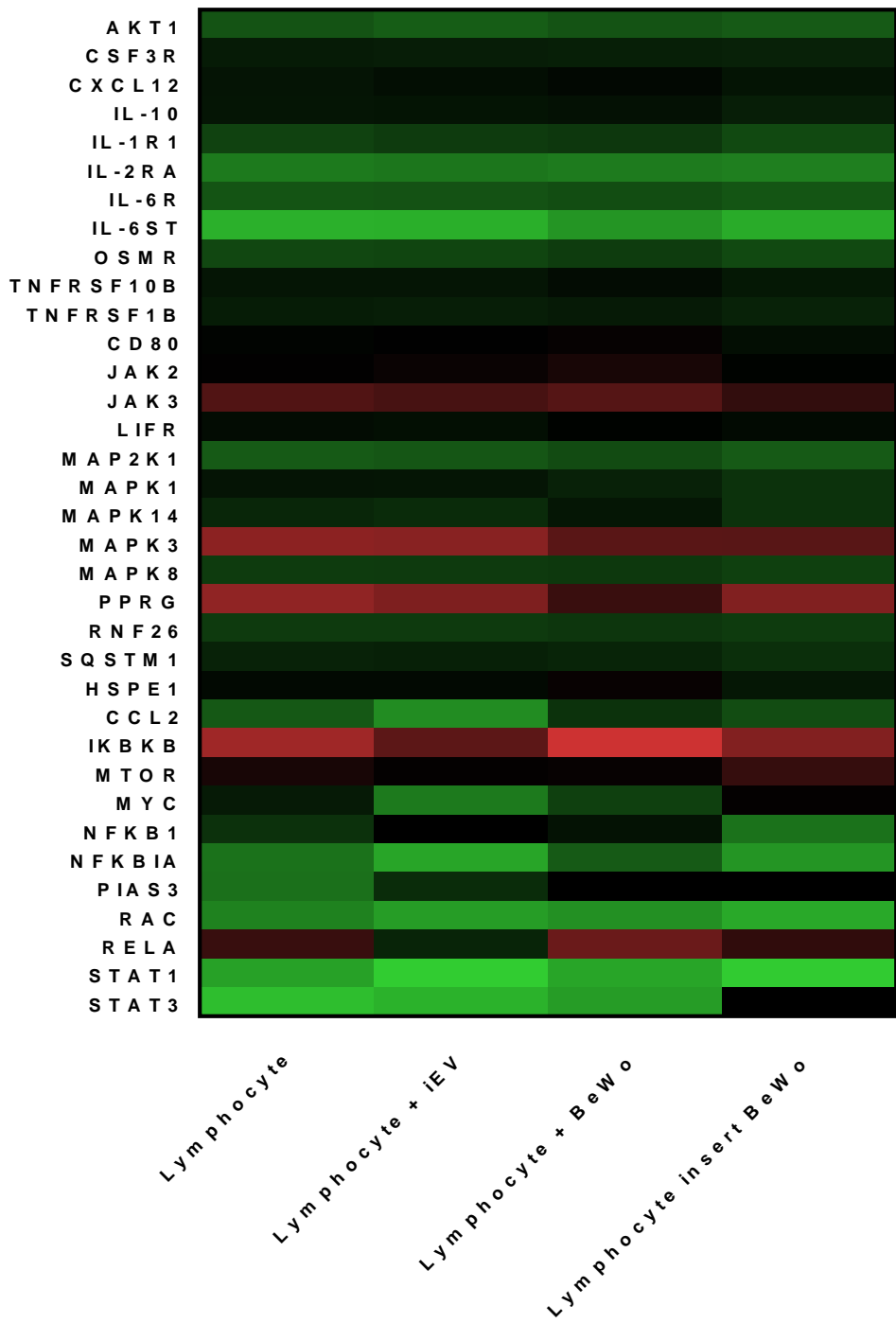


TLR4 WAS NOT EXPRESSED ON LYMPHOCYTES

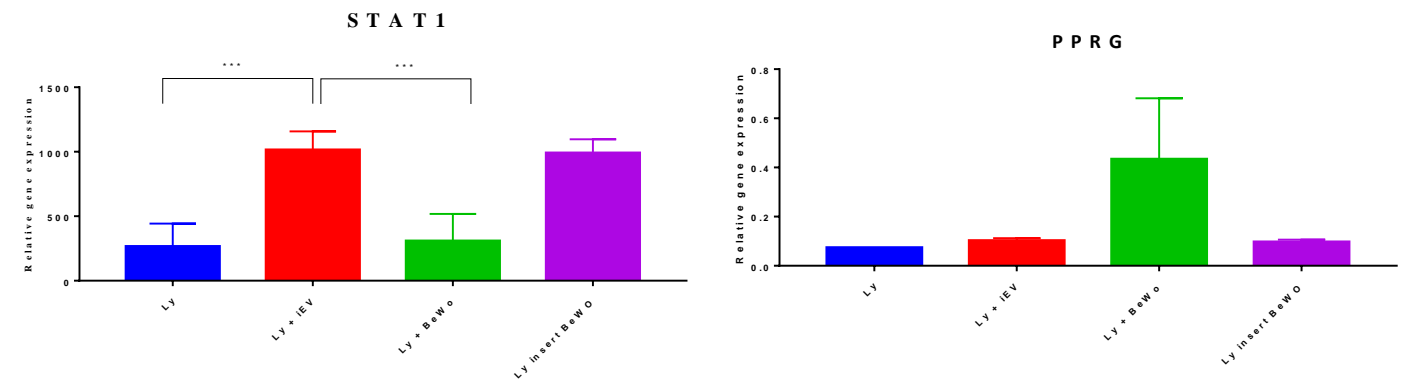


EXPRESSION OF STAT3 and NFKB1

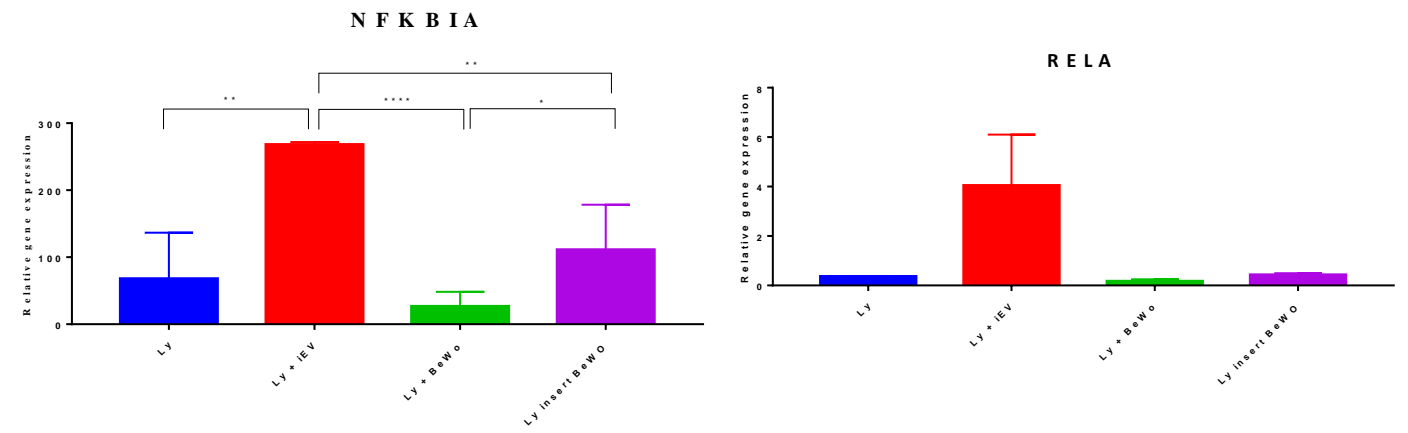




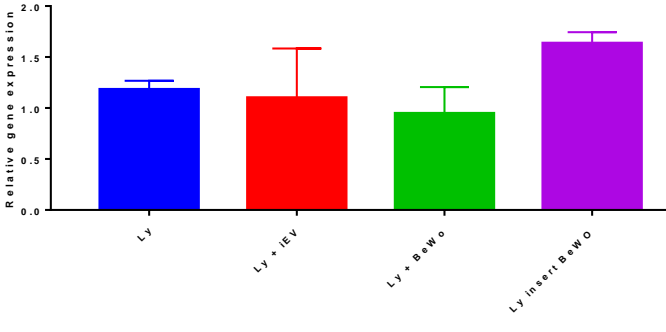
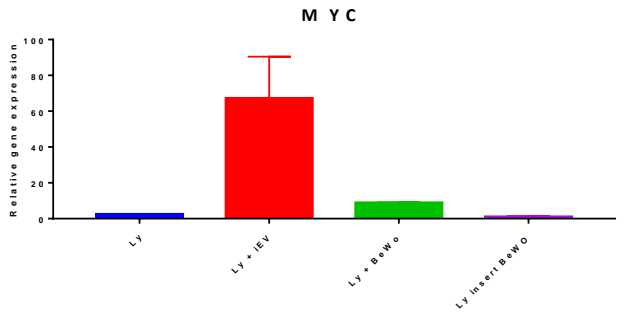
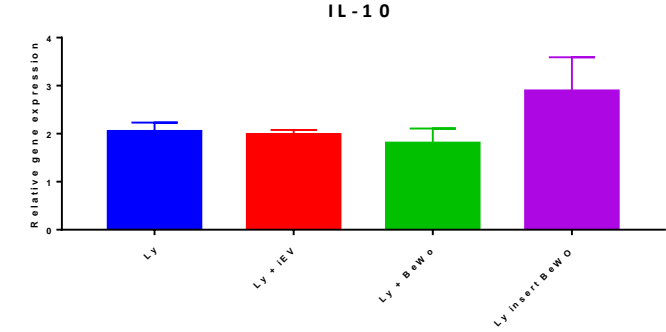
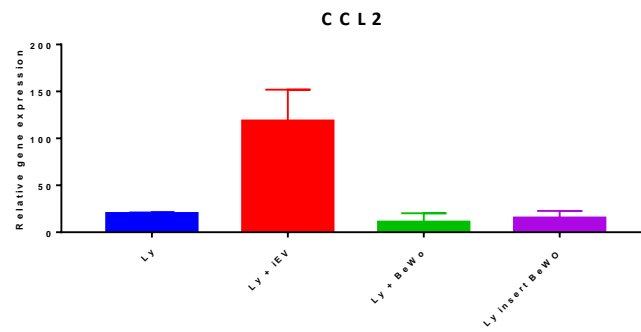
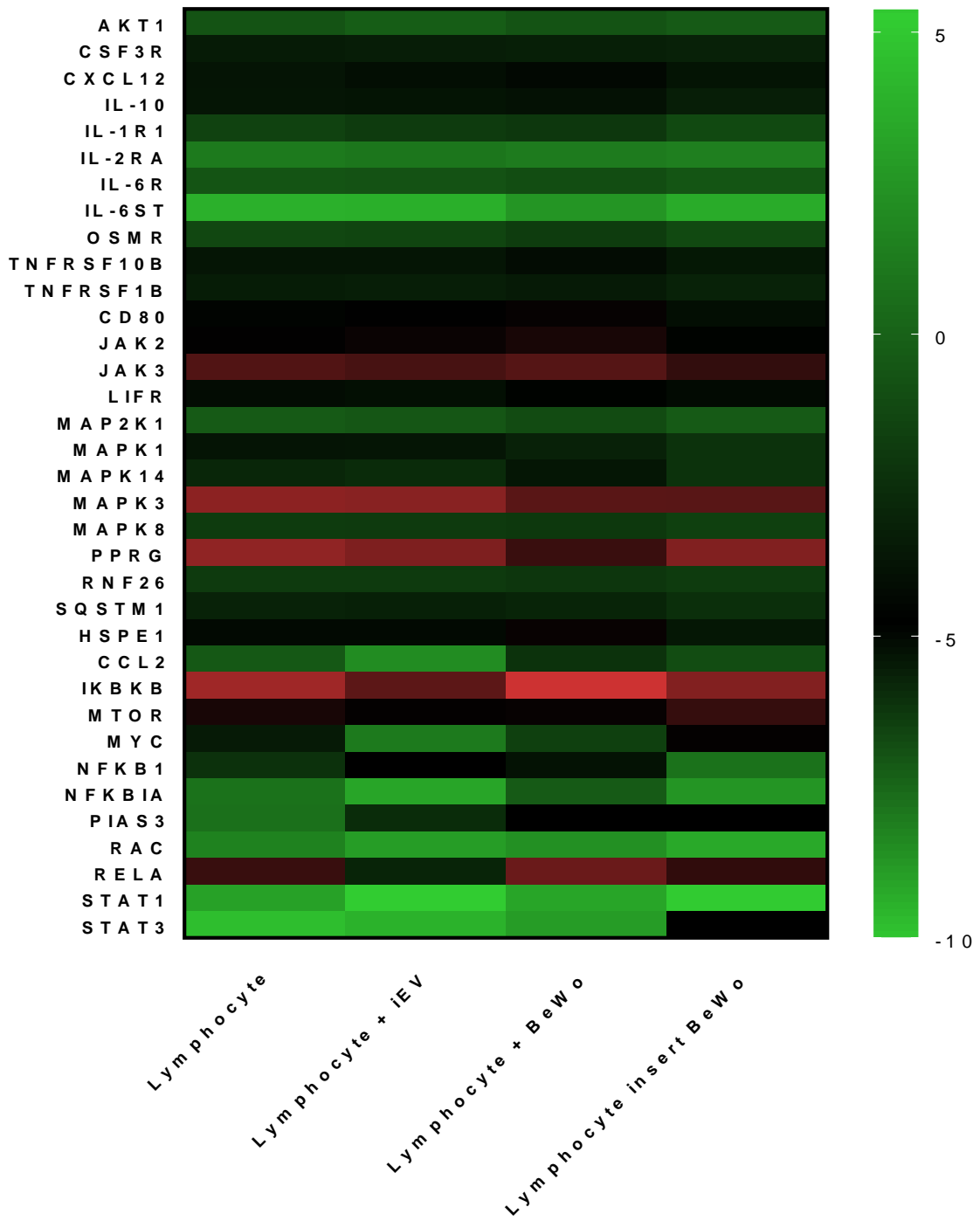
STAT1 SIGNALING

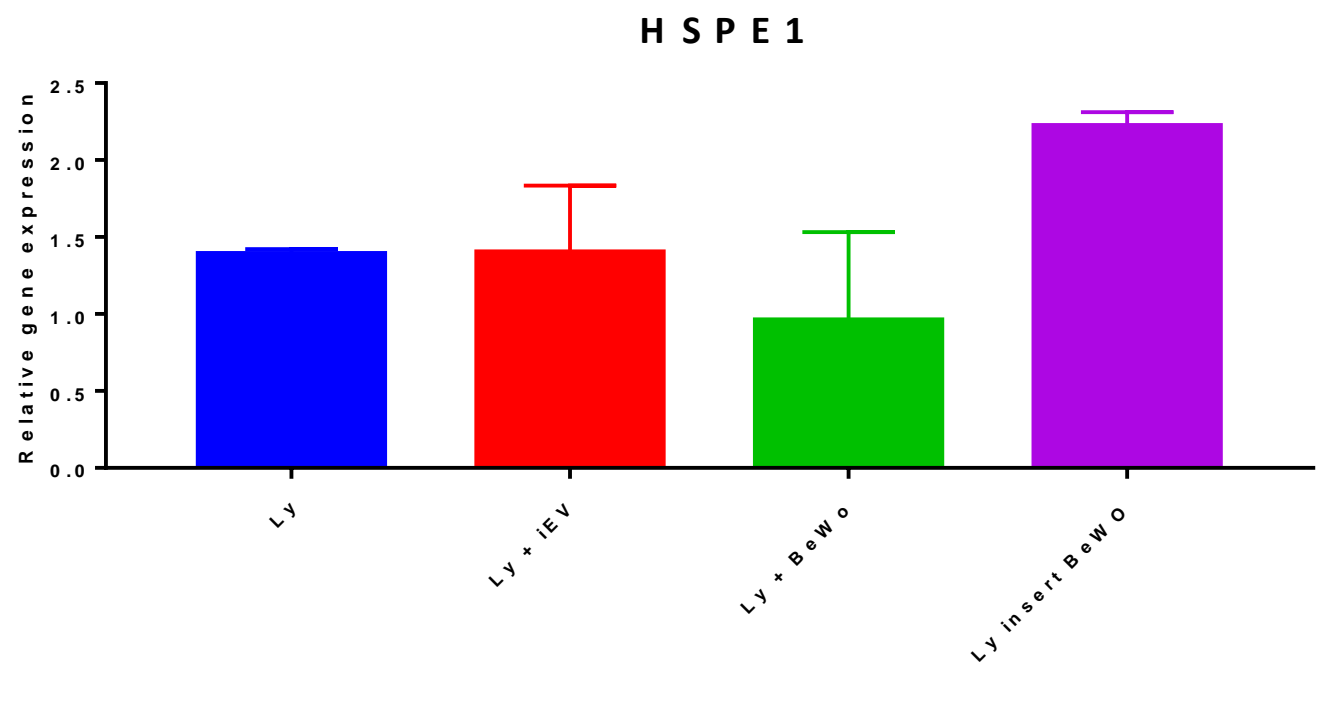
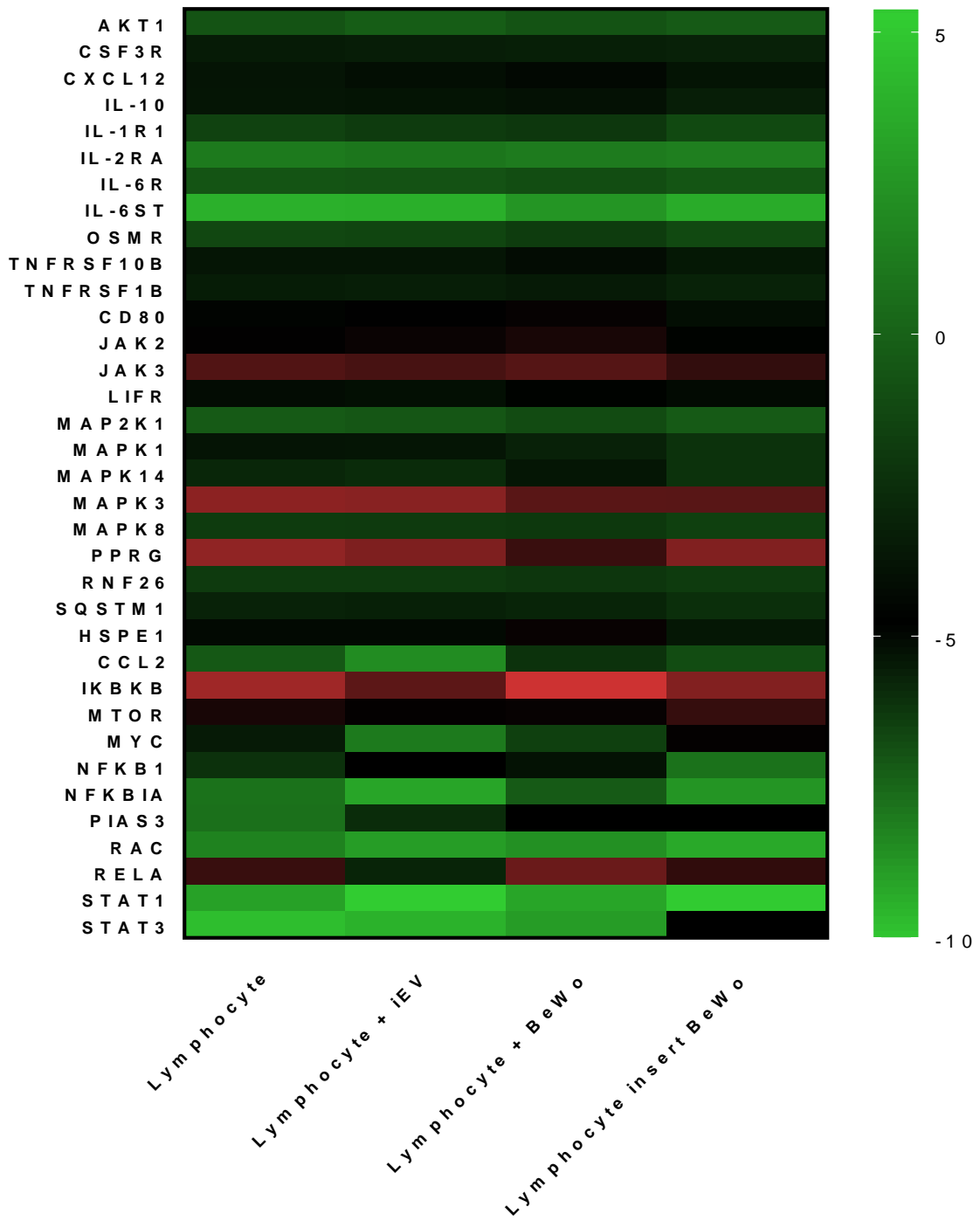


NFKB SIGNALING

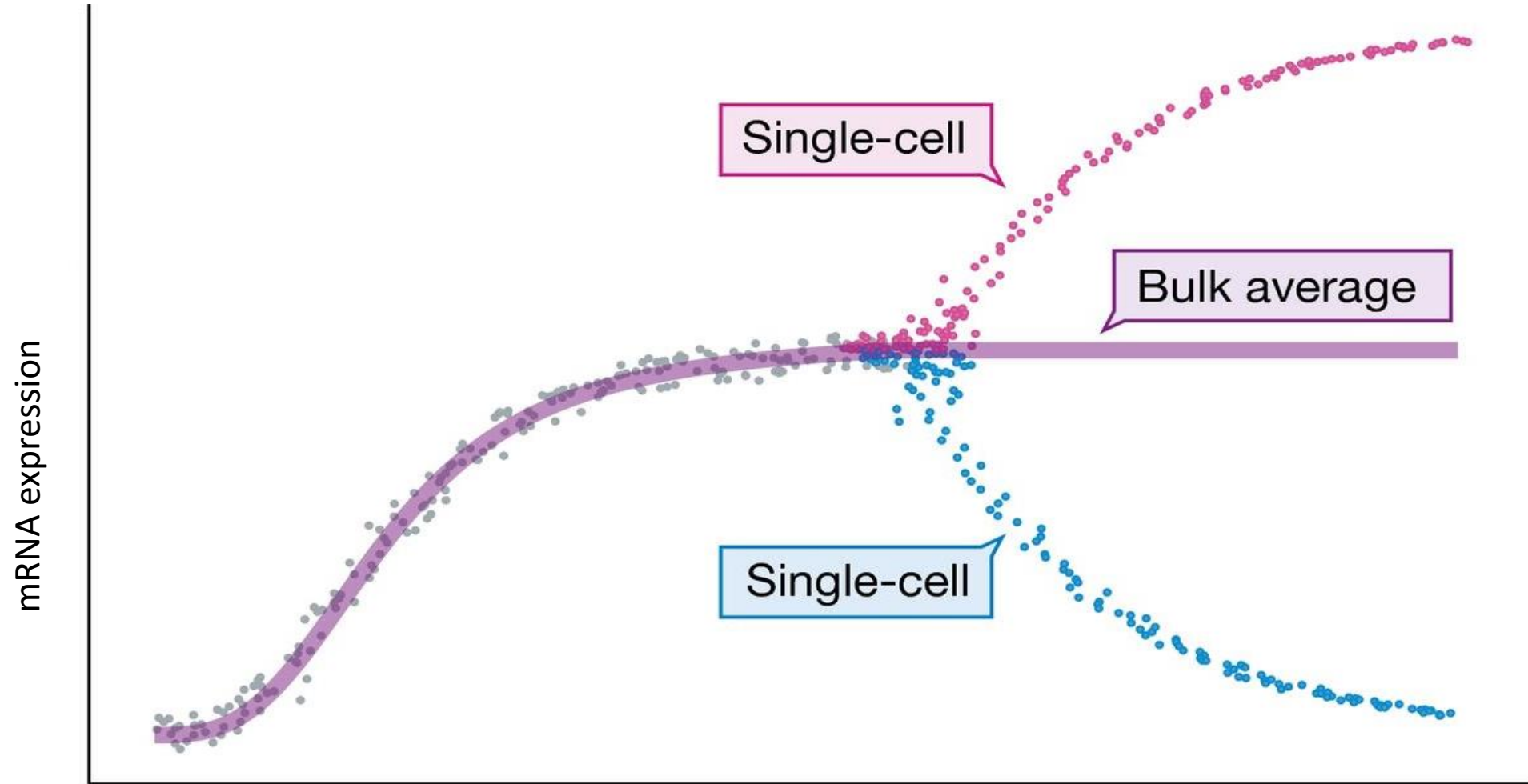


SIGNALING TARGET GENES



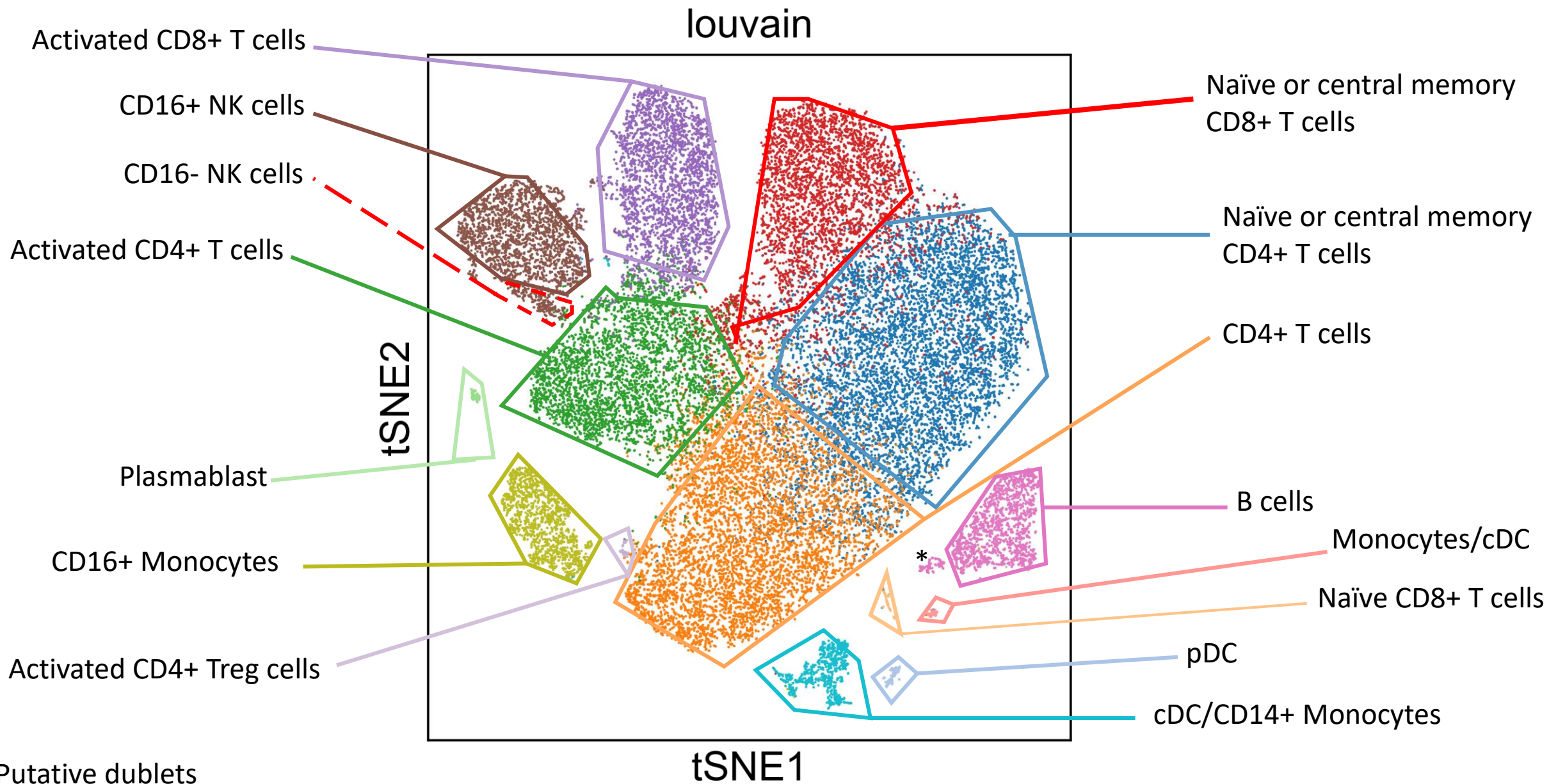


HSPE1 EXPRESSION IN HUMAN LYMPHOCYTES



HSPE1 expression after IL-6 + iEV stimulation in immune cell subsets

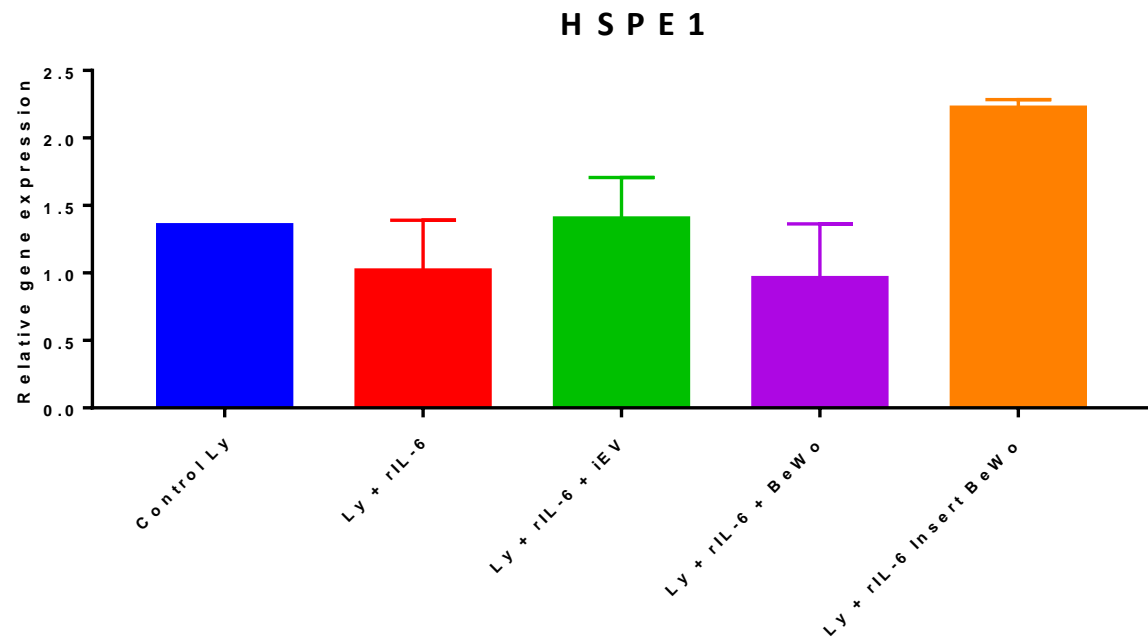
SAMPLE	PLATFORM	SOFTWARE	PROTOCOL	UMAP	tSNE	CELL NUMBER
68K PBMC	10X	SCANPY 1.2.2	SEURAT	YES	YES	68 000



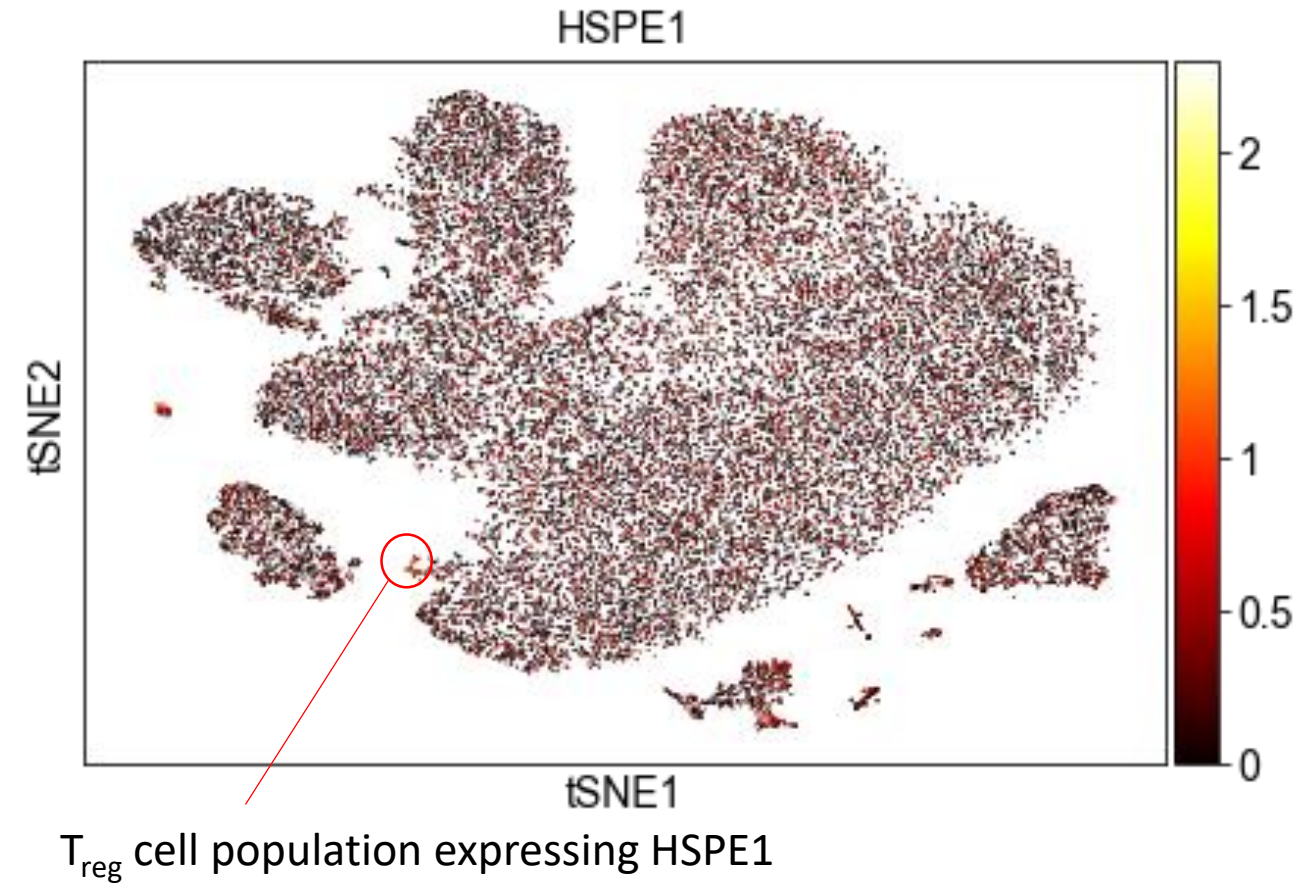
* Putative doublets

HSPE1 EXPRESSION IN HUMAN LYMPHOCYTES

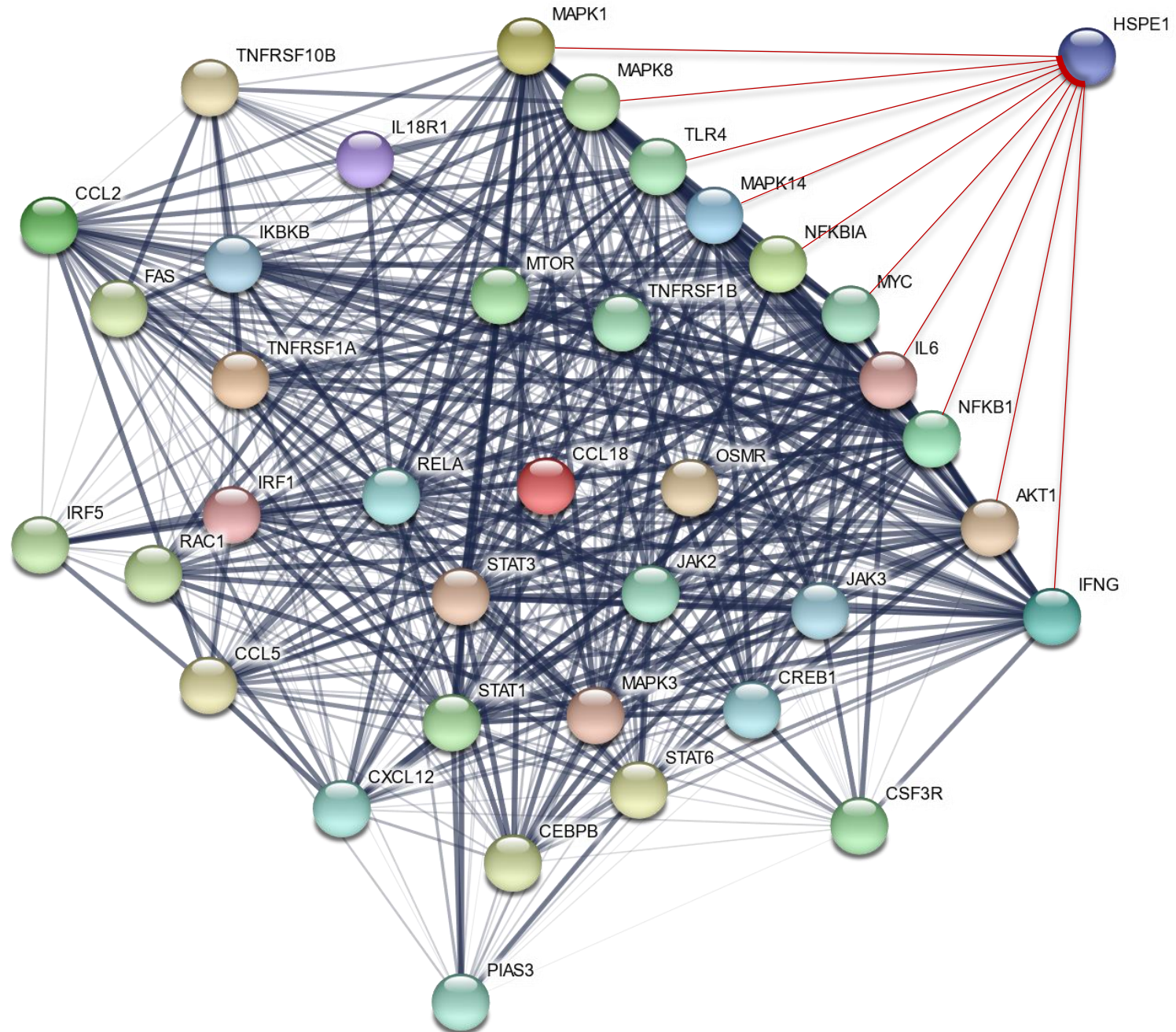
Bulk RNA HSPE1 expression

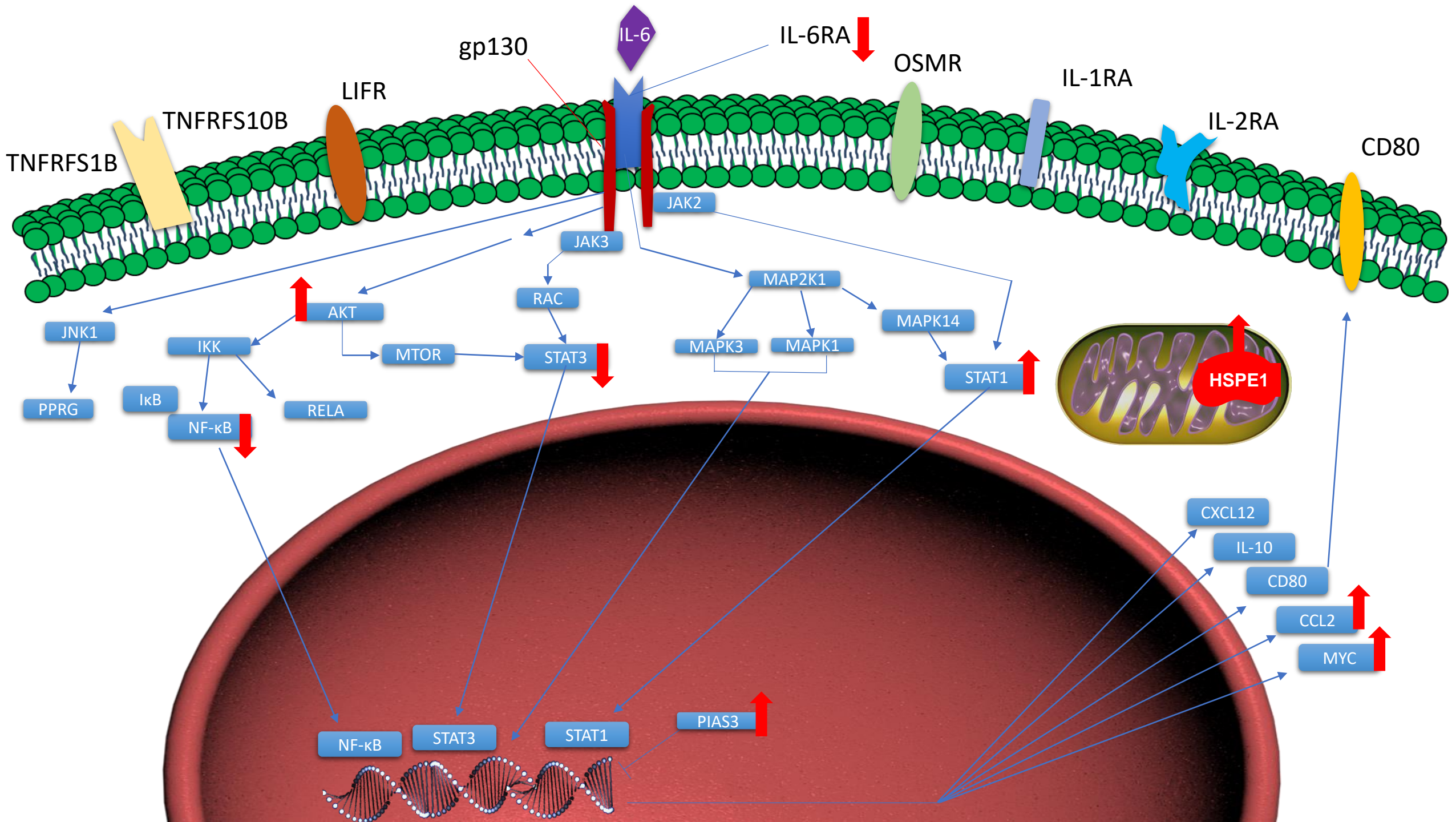


Single-cell RNA-Seq HSPE1 expression



HSPE1 INTERACTION WITH THE MEMBERS OF THE IL-6 SIGNALING PATHWAY





CONCLUSIONS

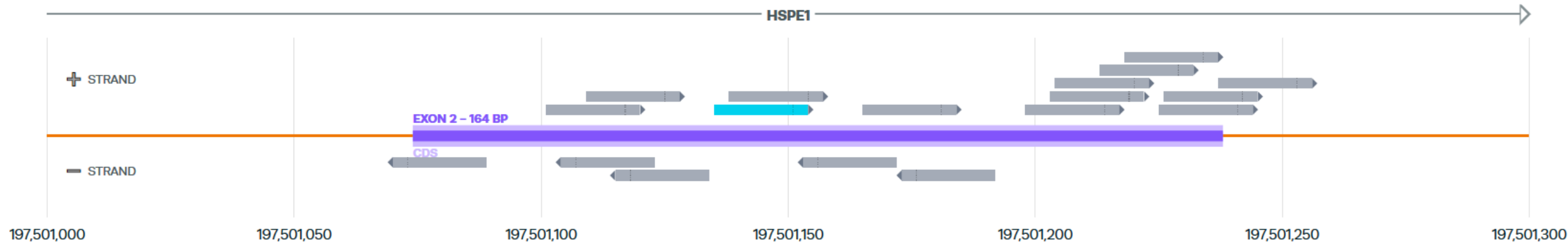
- BeWo-derived iEVs decrease the IL-6R expression in target lymphocytes both at mRNA and protein levels
- iEVs further decrease the STAT3 mRNA levels
- EVs increase the immunomodulatory HSPE1 levels

Our preliminary data suggest that BeWo-derived iEVs have an immunomodulatory protein cargo which may have an impact on the success of pregnancy.

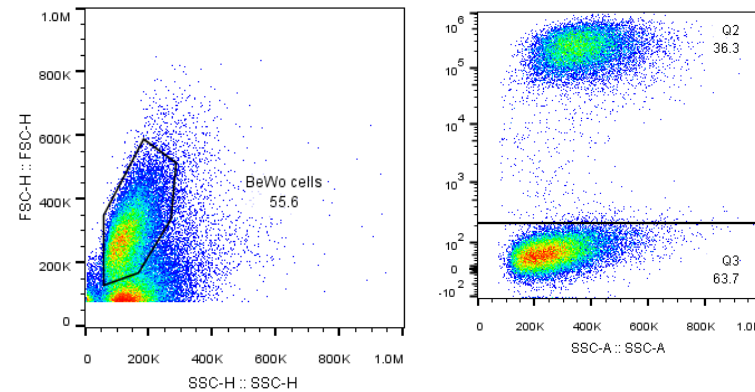
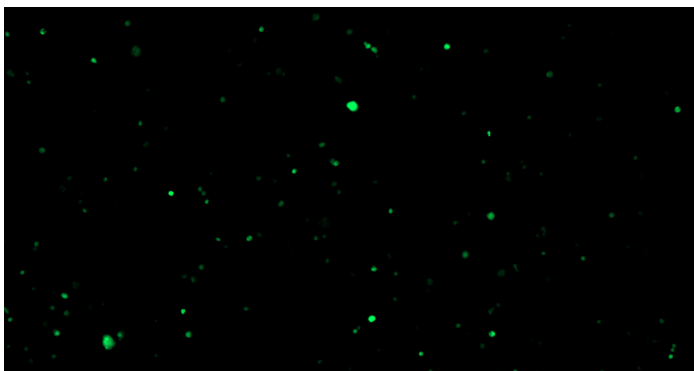
Next steps

INVESTIGATING THE MECHANISM OF ACTION OF HSPE1 IMMUNOMODULATORY PROTEIN

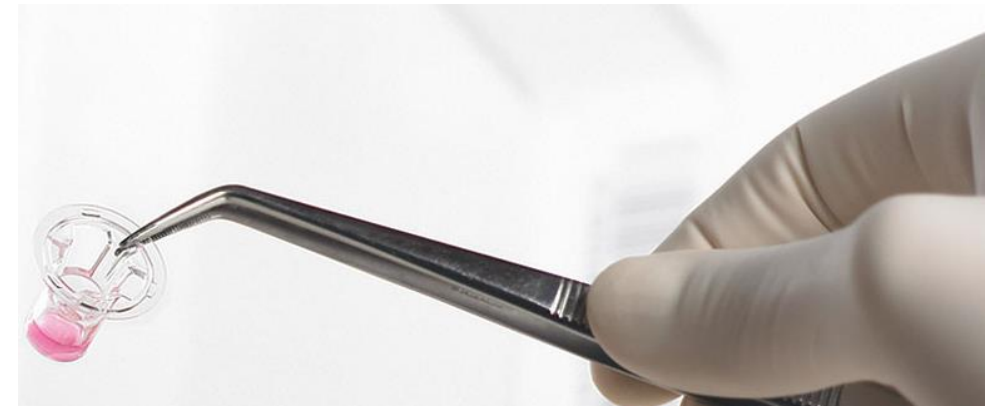
1. GENERATION OF HSPE1 KO BeWo cell line USING CRISPR-Cas9 SYSTEM BASED MODIFIED sgRNA INDUCING dsDNA breaks in exon 2 of HSPE1 gene



2. FACS based sorting of successfully transfected cells and clone selection



3. HSPE1 gene DNA sequencing and RNA expression validation



ACKNOWLEDGMENT

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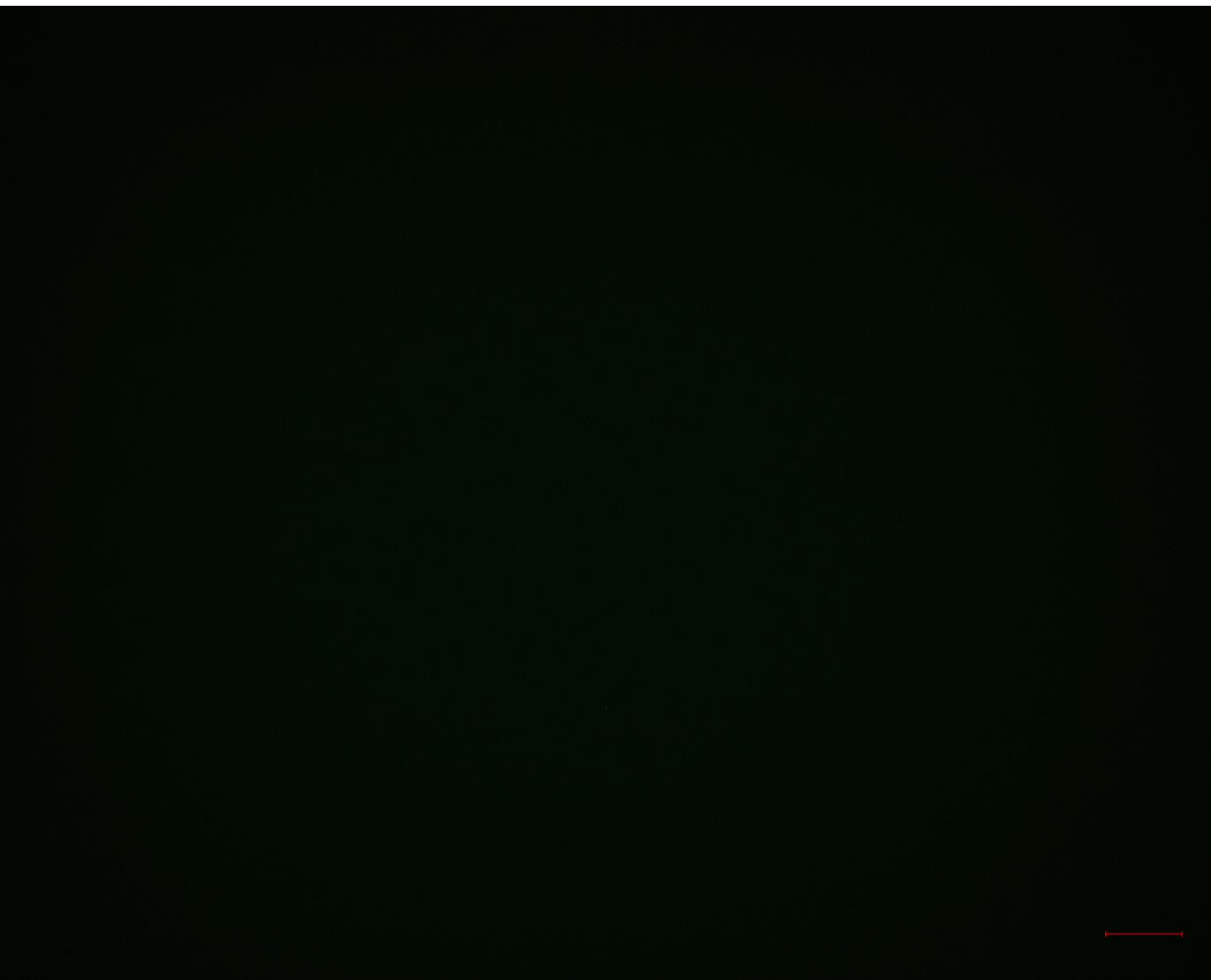
Ghaith Abdessalem



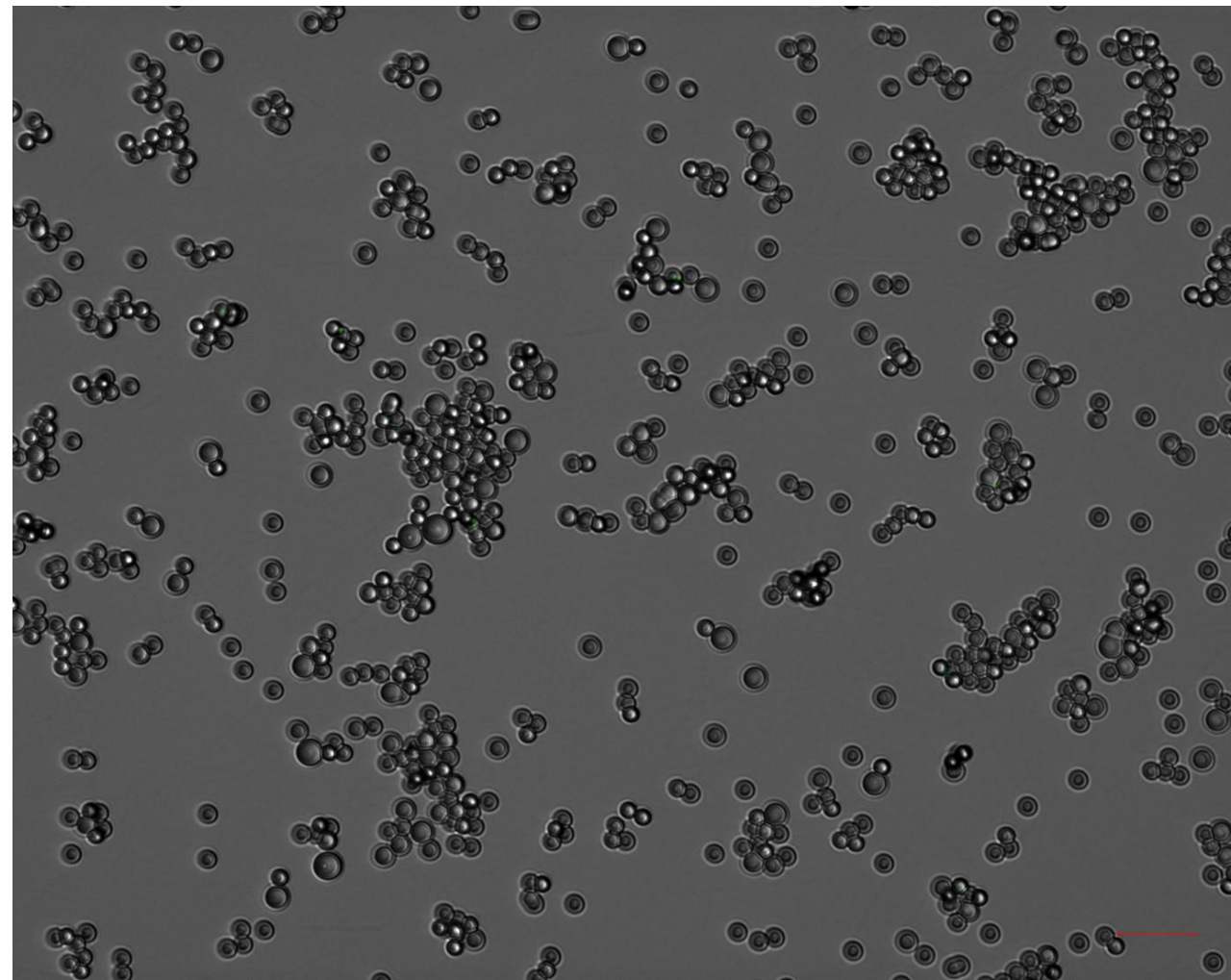
I. BeWo EV CHARACTERIZATION – MISEV2018 CHECKLIST 1

CELLDISCOVERER7

MOCK CONTROL (12.5K fraction)

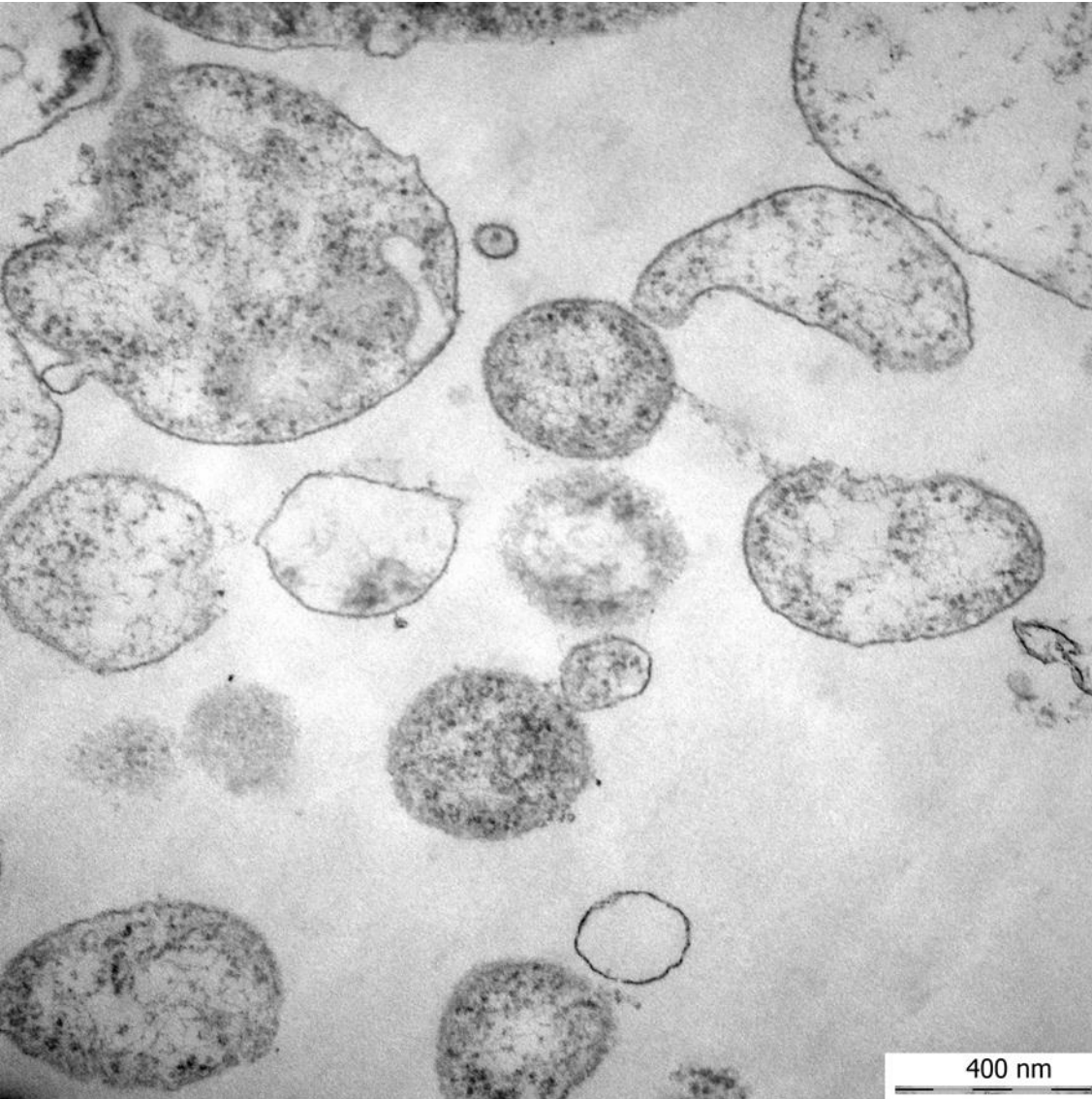


MOCK CONTROL (100K fraction)



I. BeWo iEV CHARACTERIZATION

EV vesicular nature and size distribution



After Triton X-100 detergent lysis

