

Melanoma-derived exosomes: their cargo, functions and potential role as biomarkers of disease progression

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Immunosuppressive factors and cells contributing to immune-cell dysfunction in cancer

- Inhibitory receptors/ ligands
- (e.g., PD-1/PD-L1)

- Soluble factors:

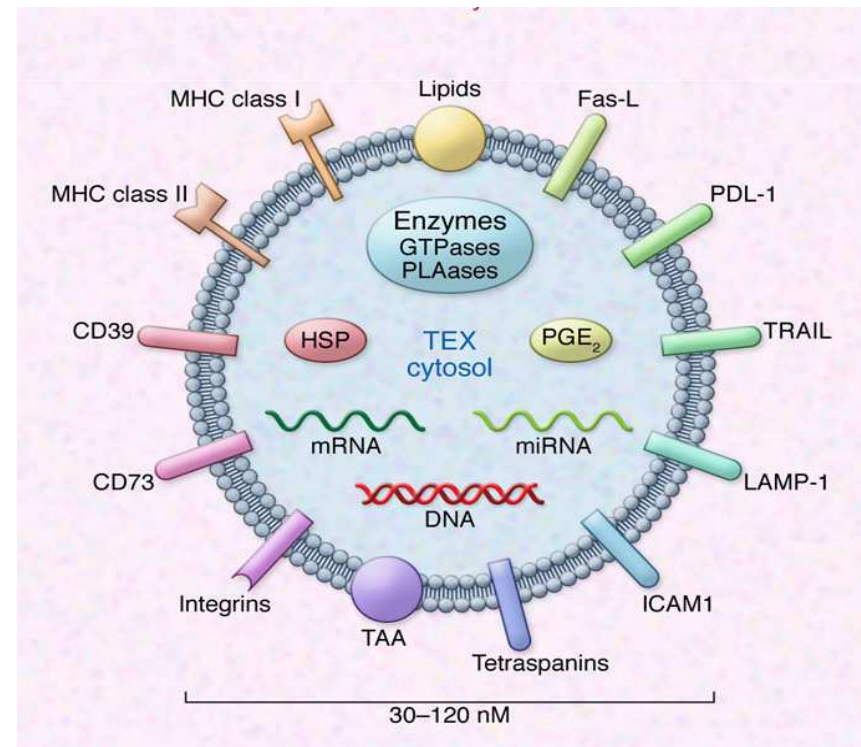
IL-10, TGF- β , IL-35
IDO, arginase
Galectin -9
Adenosine, COX-2, PGE2
Oxygen radicals

- Regulatory cells:

iTreg
MDSC

- Metabolic checkpoints
e.g., glucose deprivation

- MHC class I down-regulation/loss
 β 2-microglobulin inactivation
on tumor cells
- **Tumor-derived immunoinhibitory exosomes**

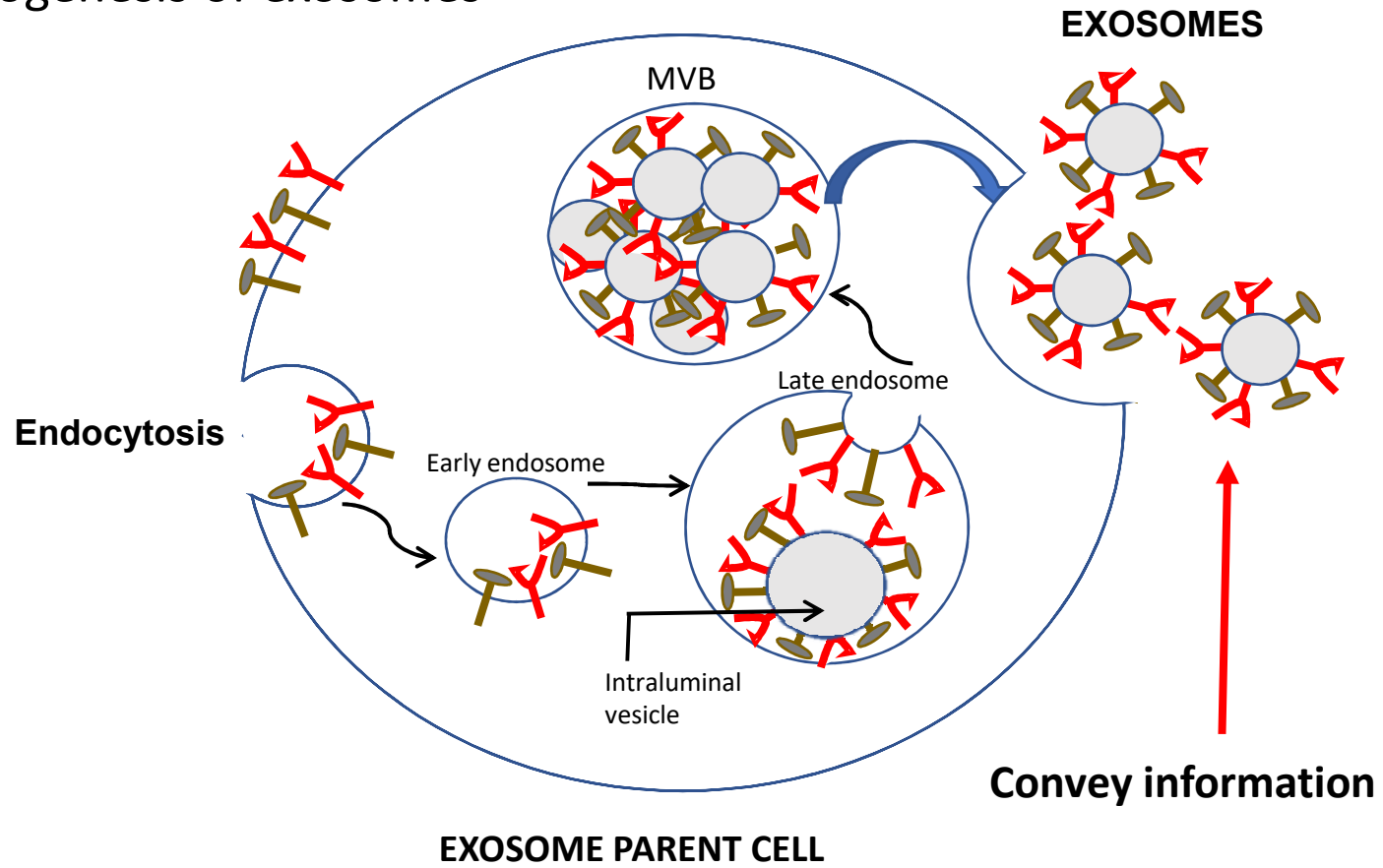


Tumors produce and release a variety of extracellular vesicles (EVs);
exosomes are the smallest EVs (30-150nm) that differ from larger MVs by the endocytic origin

Why are we especially interested in exosomes??

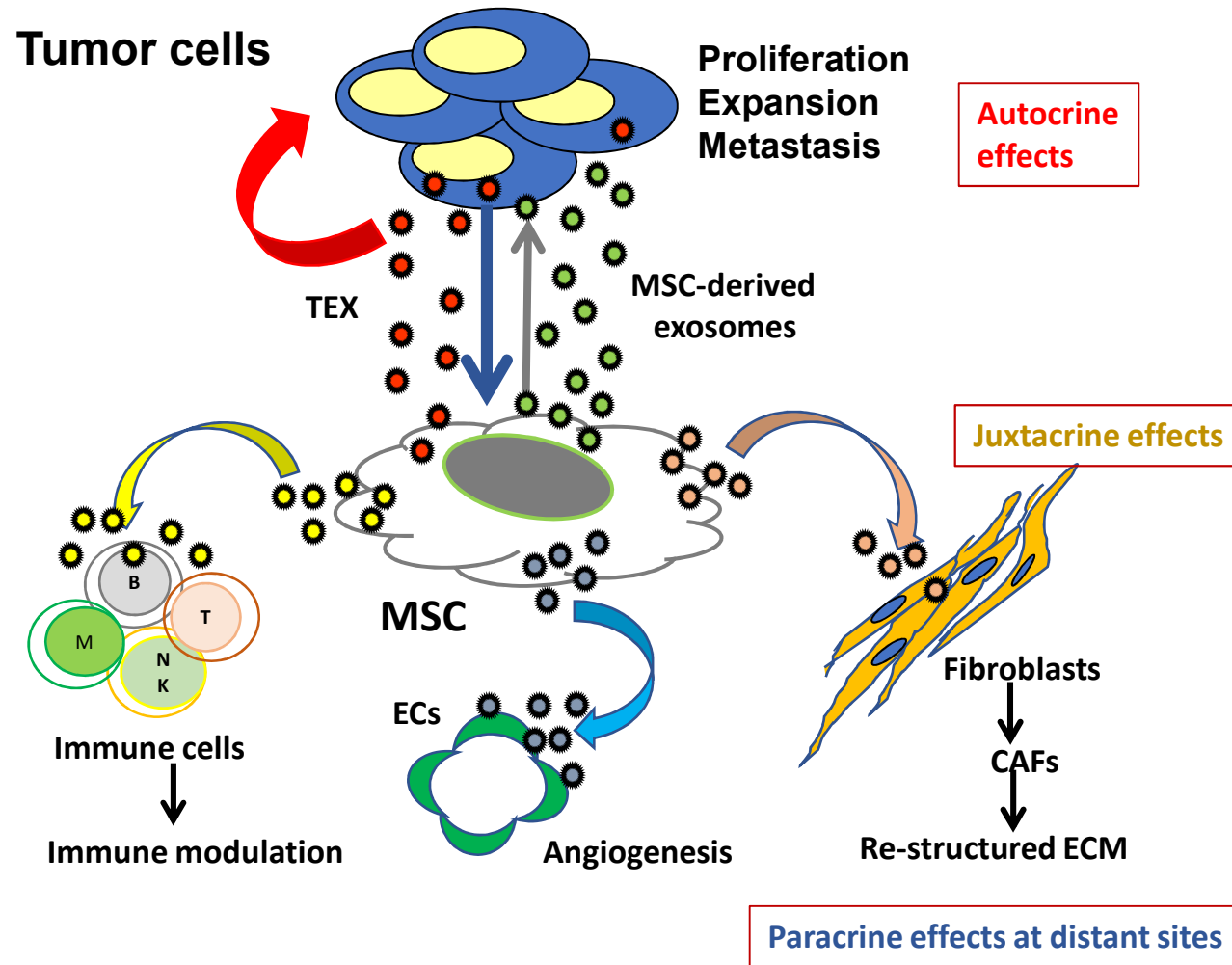
- ❑ Because of their origin and their content: **TEX** are potential biomarkers for parent tumor cells (“**liquid biopsy**” concept)
- ❑ **TEX** are a communication system operating between the tumor and other cells in the TME
- ❑ **TEX** emerge as key elements responsible for reprogramming of the TME into a tumor-promoting environment

Biogenesis of exosomes

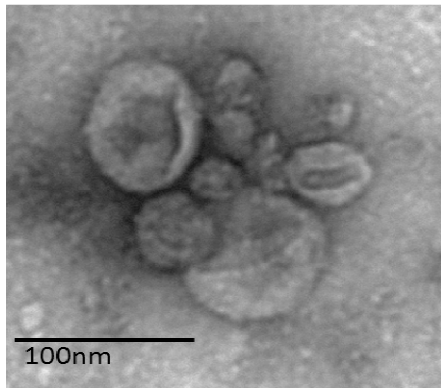


Whiteside TL. Adv Clin Chem. 2016;74:103-41

TEX-MEDIATED REPROGRAMMING OF RECIPIENT CELLS



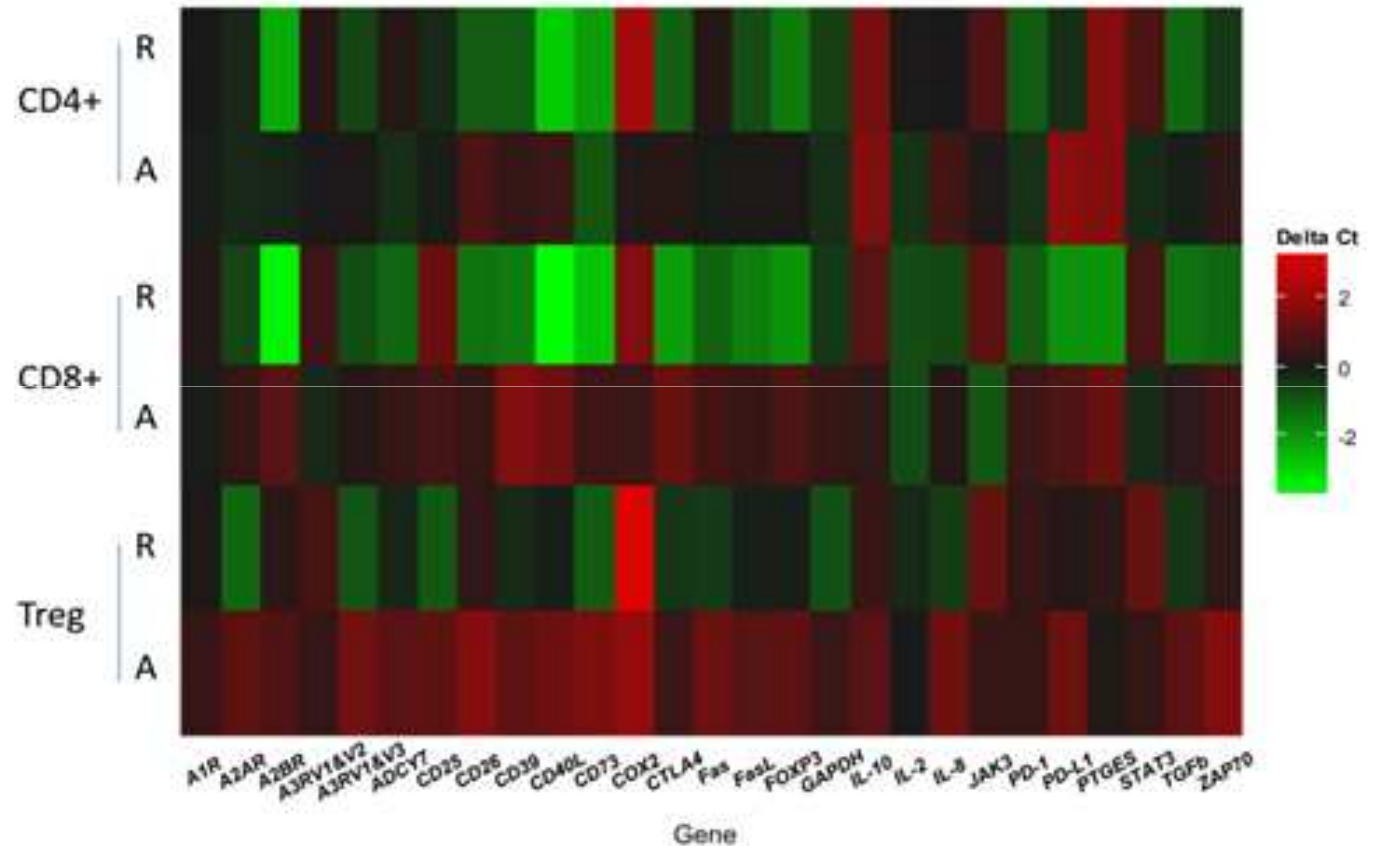
A supervised heat map for mRNA expression changes in resting or activated T cells co-incubated with TEX



T-cell subsets: isolated by immunoaffinity from PBMC of NC: resting or *in vitro* activated via the TCR



Examine mRNA/protein profiles in resting or activated T-cells + TEX relative to the baseline (no TEX) Ct values

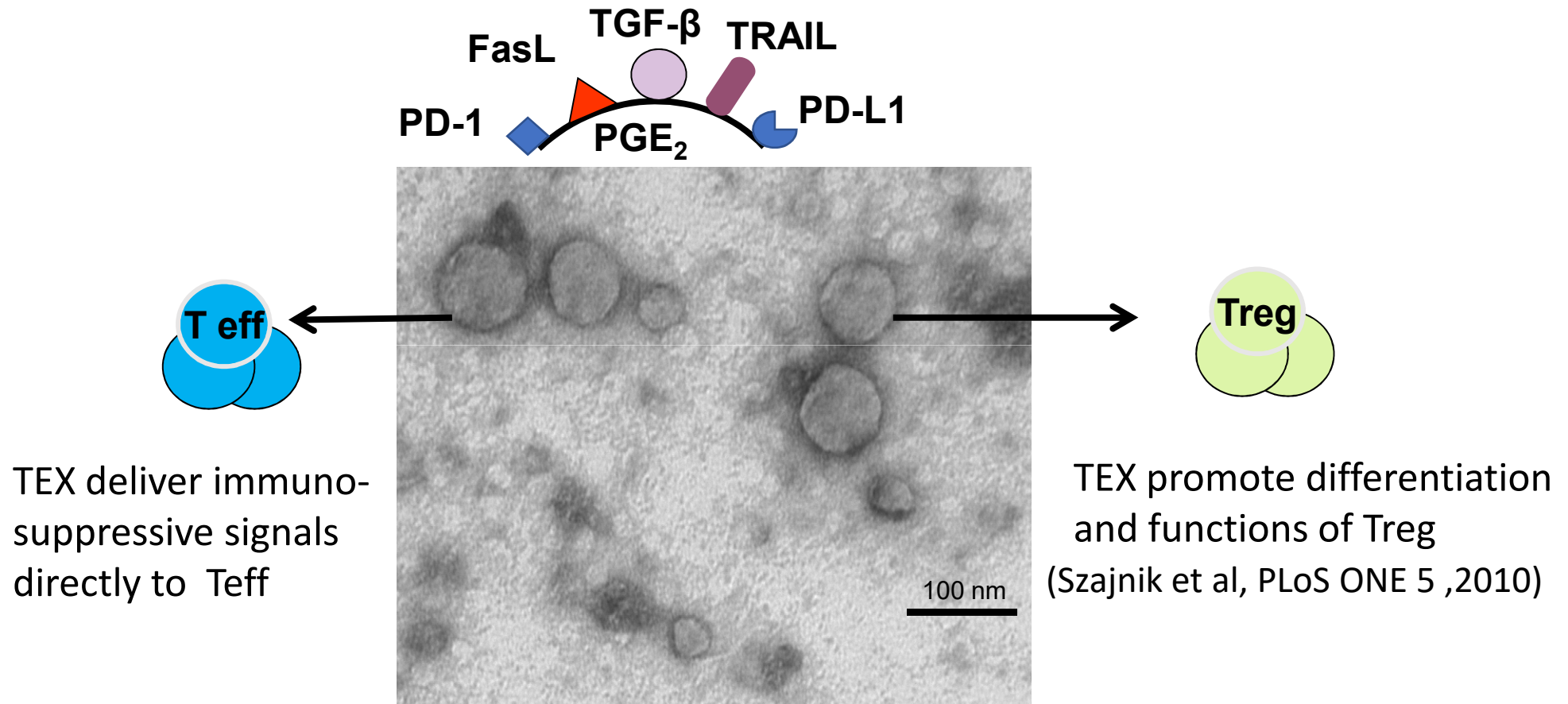


(mRNA expression levels were measured as a fold change from baseline)

Muller L et al, Sci. Reports, 2016

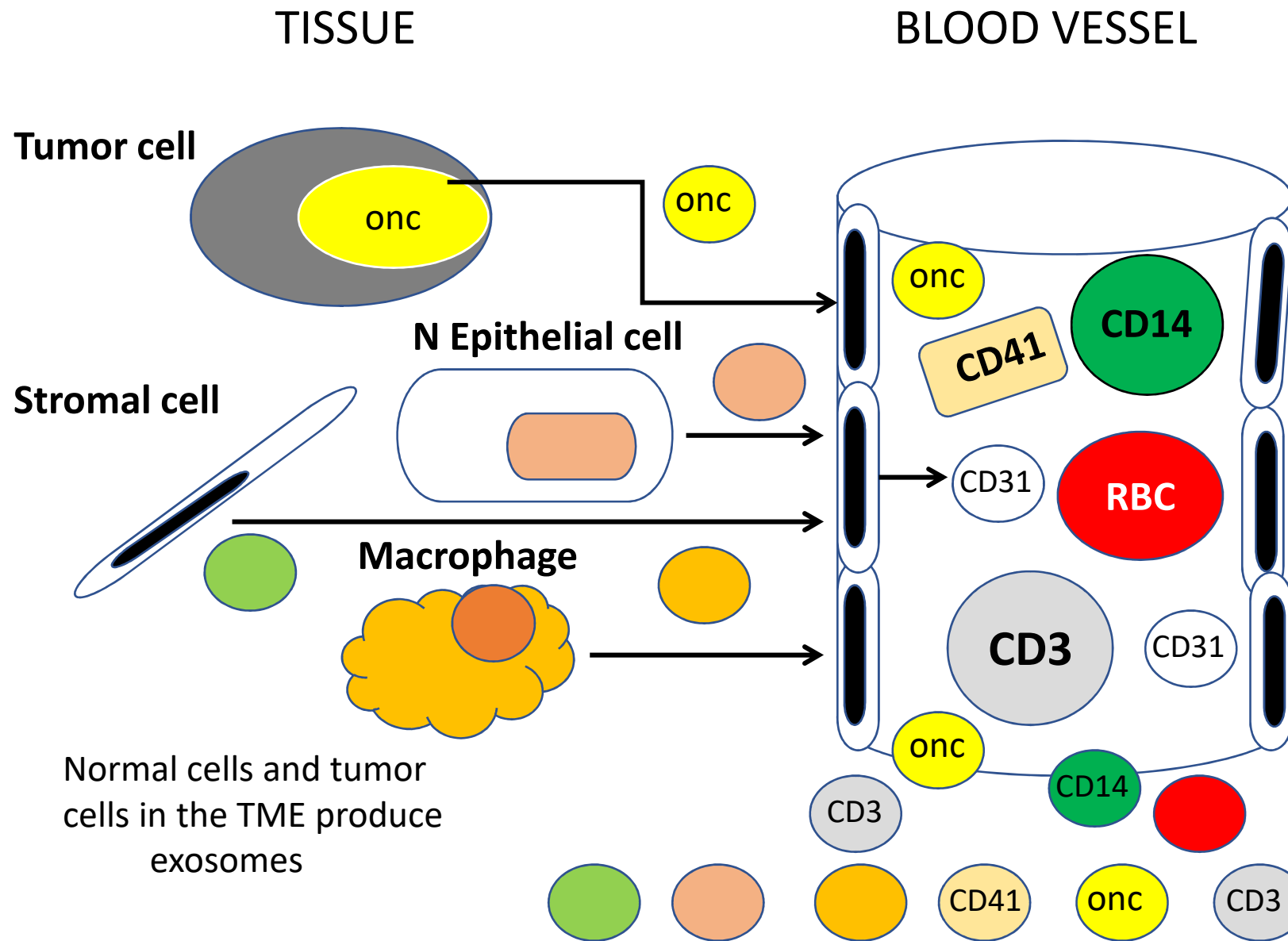
Reprogramming of recipient T cells by TEX

TEX which carry and deliver suppressive factors as a “bundle”



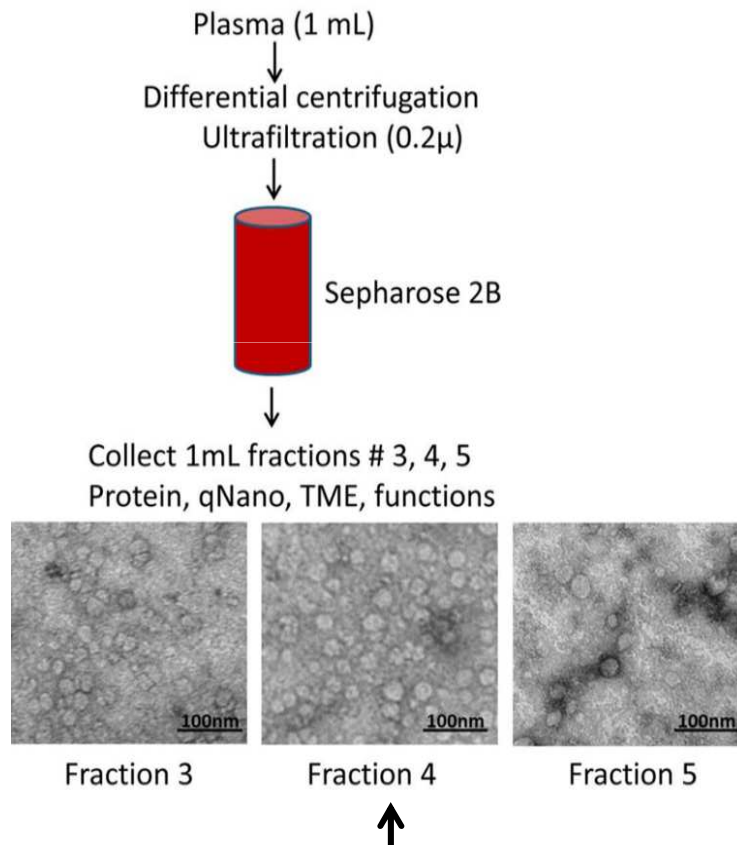
TEX modify immune-cell functions

Whiteside TL. Bochem Soc. Transactions (London), 2013

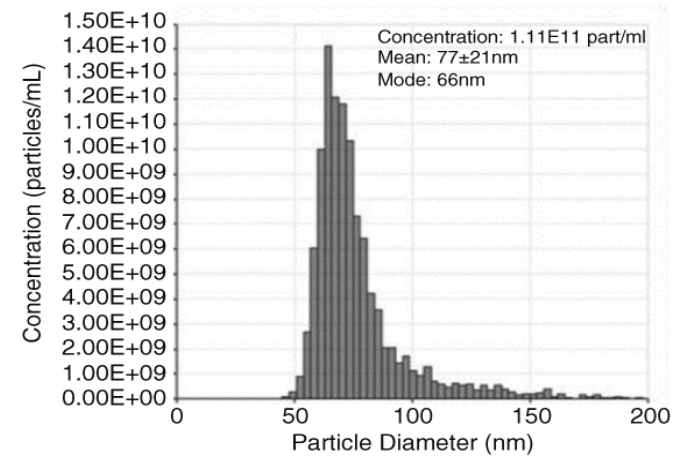
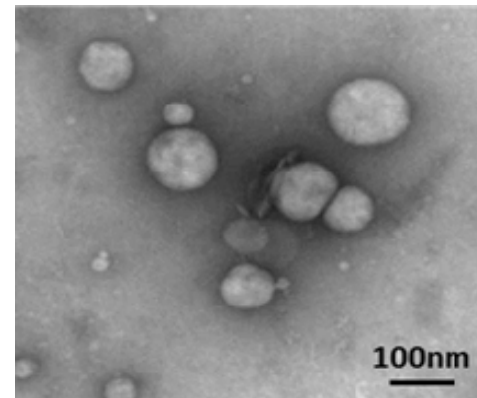


Exosomes in the circulation are mixed populations of normal cell-derived and tumor-derived vesicles

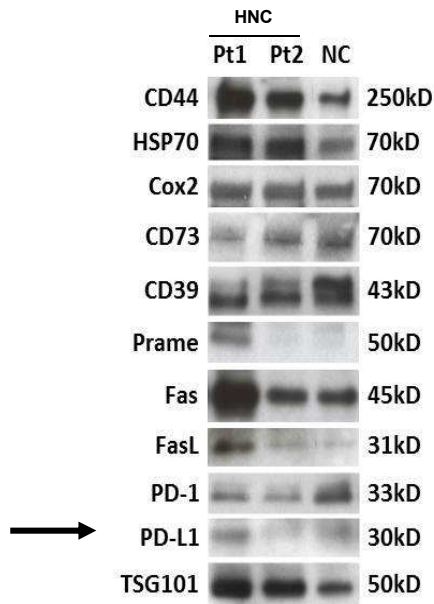
Exosome isolation from a patient's plasma or supernatants of cell lines



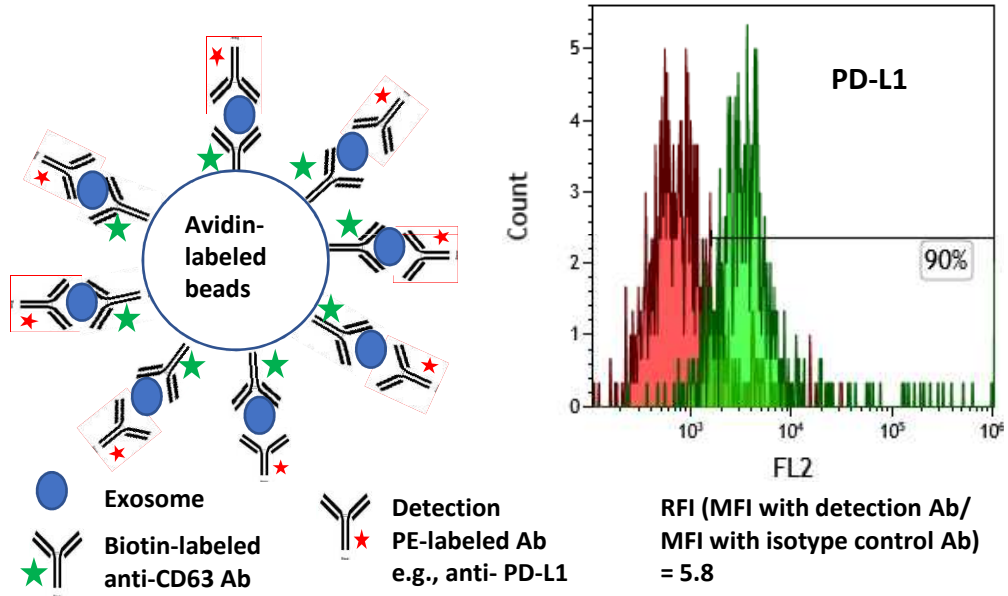
Exosome characteristics



Western blots



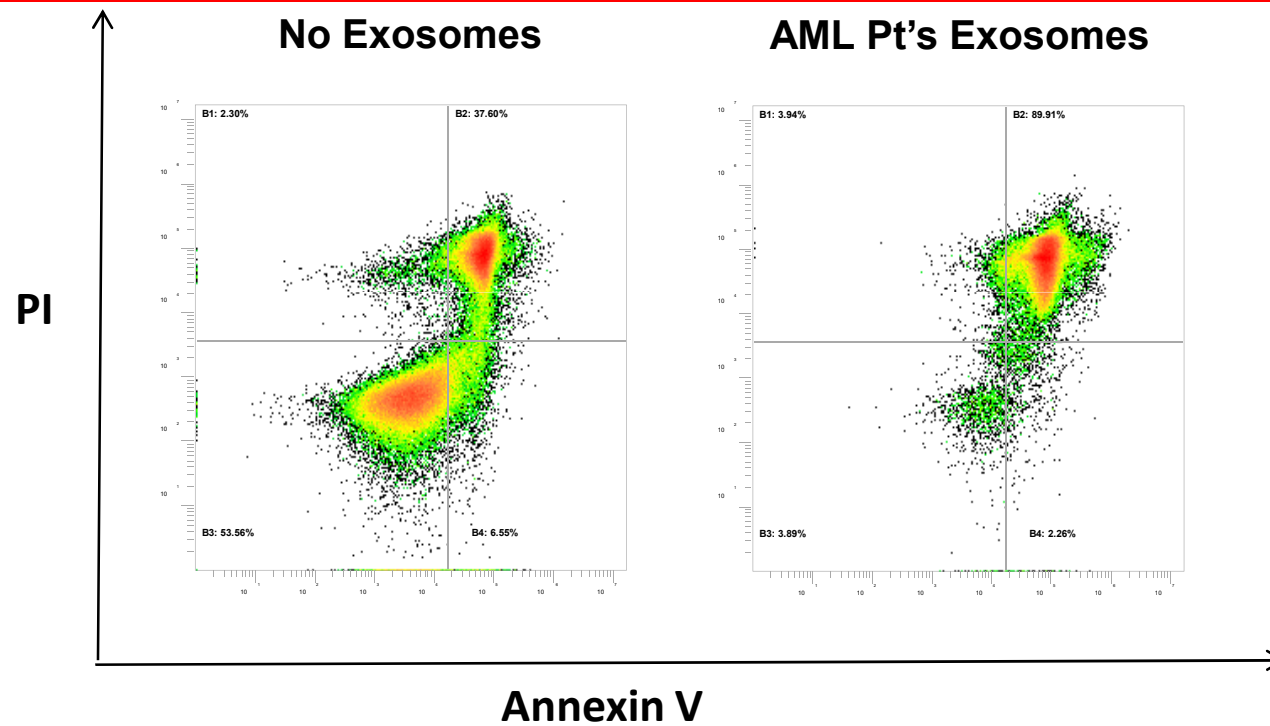
Quantitative on-bead flow cytometry for measuring exosome molecular cargos



Exosomes isolated from plasma of HNC patients carry PD-1 and PD-L1

Mass Spectrometry of exosomes (Dr. Monika Pietrowska)

Exosomes isolated from plasma of patients with cancer induce apoptosis of activated T cells



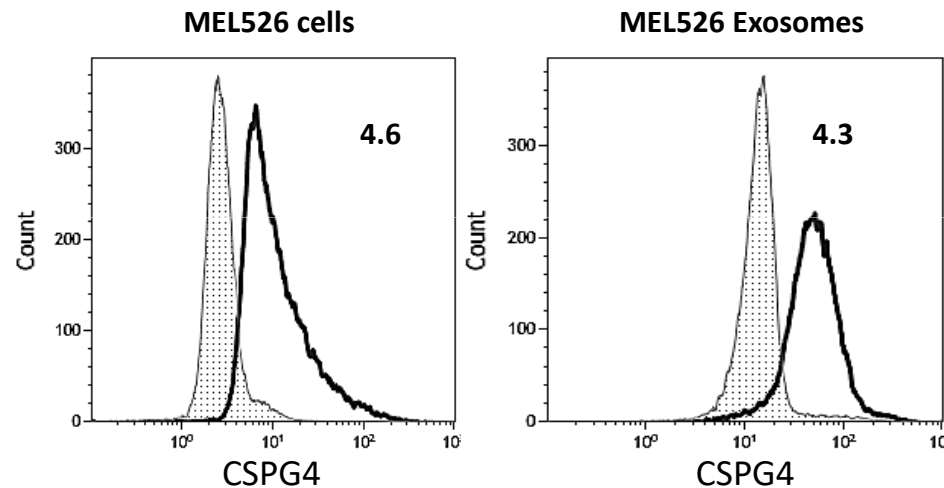
4h co-incubation of activated CD8+ T cells with exosomes (5 μ g protein)

Separation of melanoma cell-derived exosomes (**MTEX**) from exosomes produced by normal cells (**non-MTEX**) by immune capture:

- (a) **isolation** from plasma of total exosomes
- (b) **immune capture** of MTEX on beads
- (c) **detection** of molecular cargos in MTEX and non-MTEX

TEX mimic the molecular content of parent tumor cell

CSPG4 expression on MEL526 cells or exosomes from MEL526 cell line
(Chondroitin sulfate peptidoglycan 4)



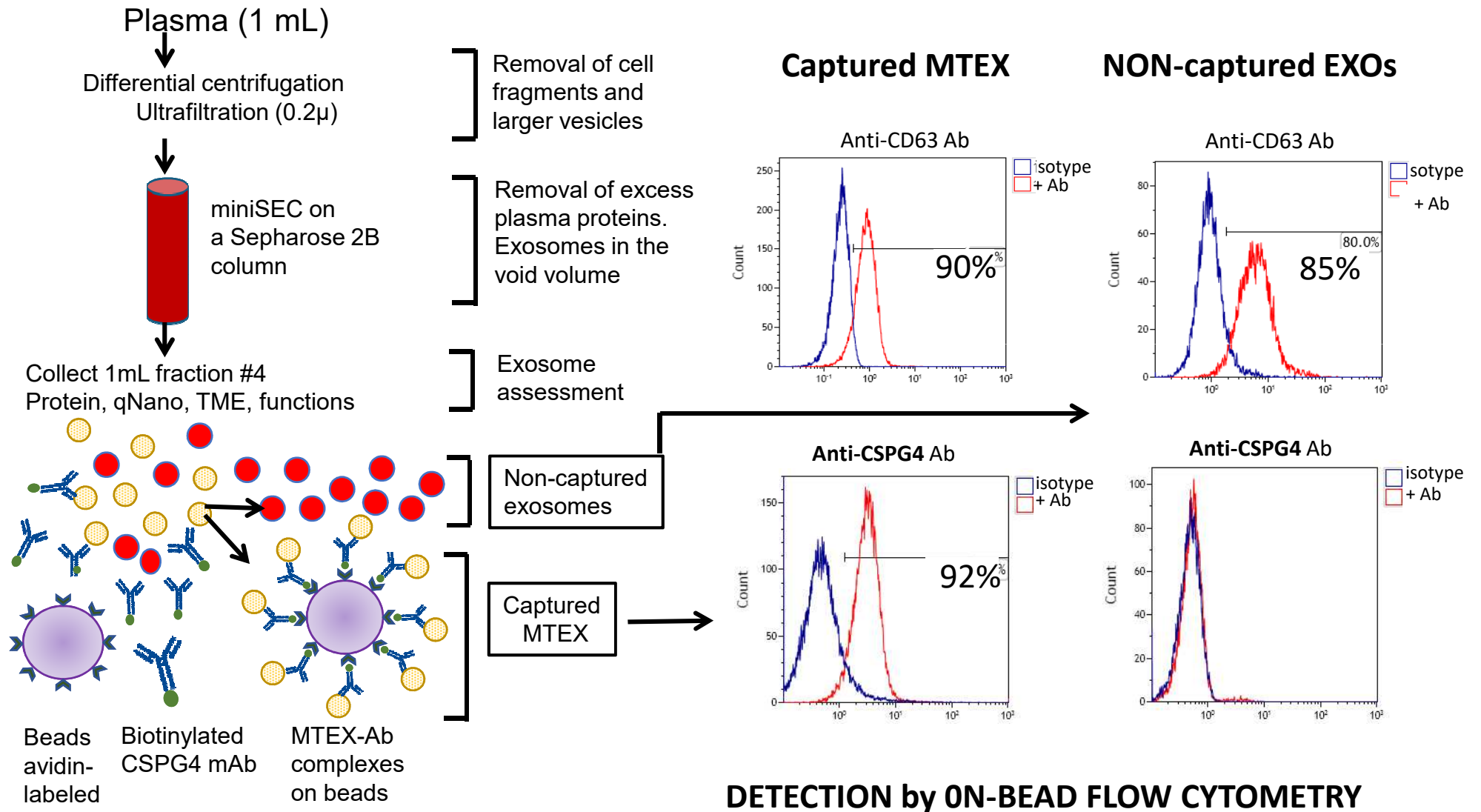
Mel526 cells express high levels of CSPG4, and the exosomes these cells produce also carry high levels of CSPG4. Relative Fluorescence Index (RFI) value for exosomes = 4.3.

Molecular profiles and functional analysis of MTEX and non-MTEX from patients' plasma

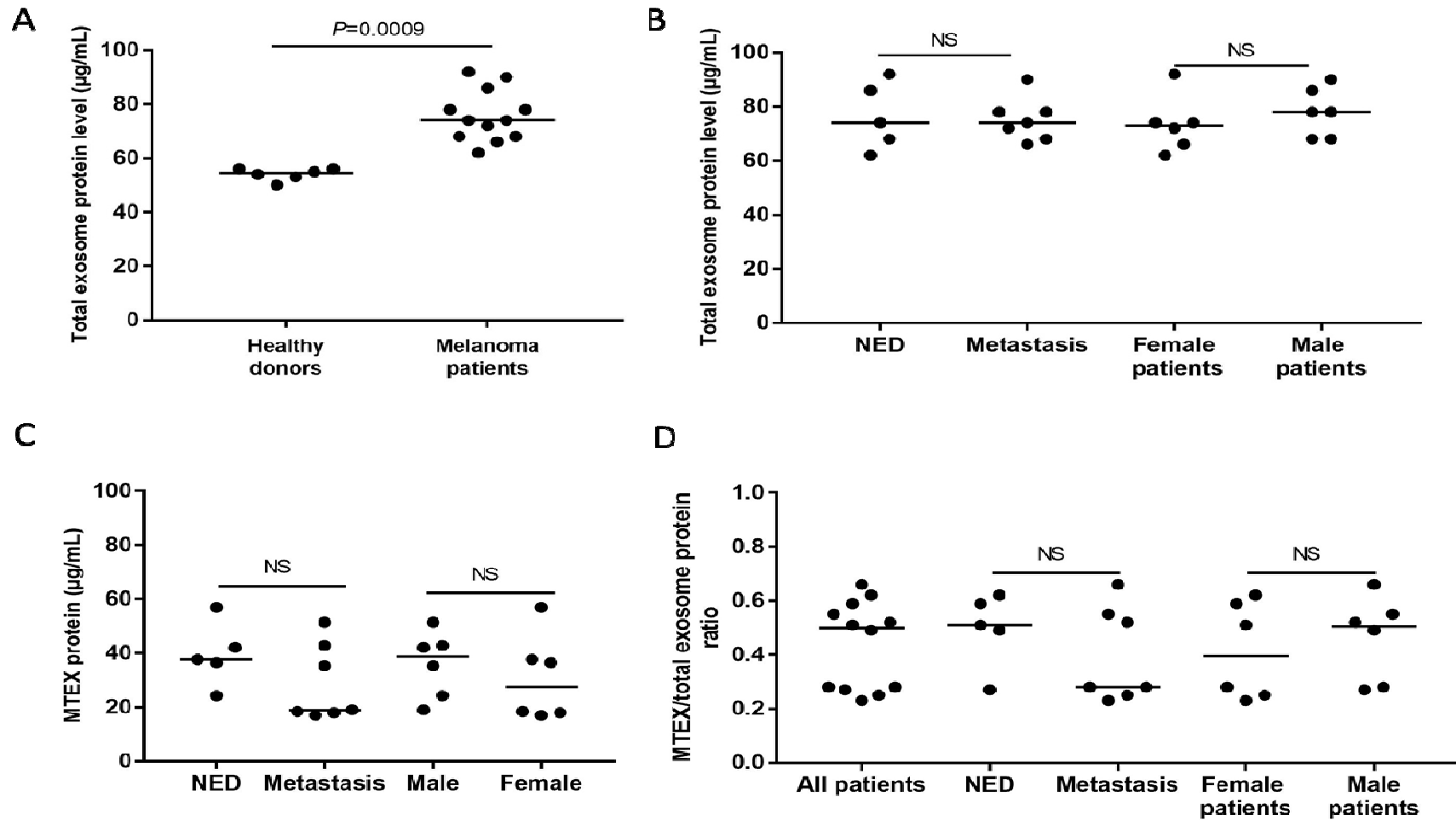
- ❑ A pilot study of exosomes isolated from plasma of 12 patients with melanoma and 6 age- and sex-matched HDs
- ❑ Annotated plasma from the Melanoma SPORE BANK established at the Hillman Cancer Center
- ❑ 5 patients were NED at blood draw and 7 had metastatic disease
- ❑ Flowcytometry-based profiling: Immunosuppressive score
Immunostimulation score
Immsupp/immstim ratio
- ❑ Functional studies: TEX + primary human immune cells
- ❑ Clinicopathological correlations

Objectives: (a) the identification of immunosuppressive exosomes in plasma
(b) preliminary indication whether TEX could serve as correlates of disease

Immune capture of melanoma-derived exosomes (MTEX) from plasma of patients with melanoma



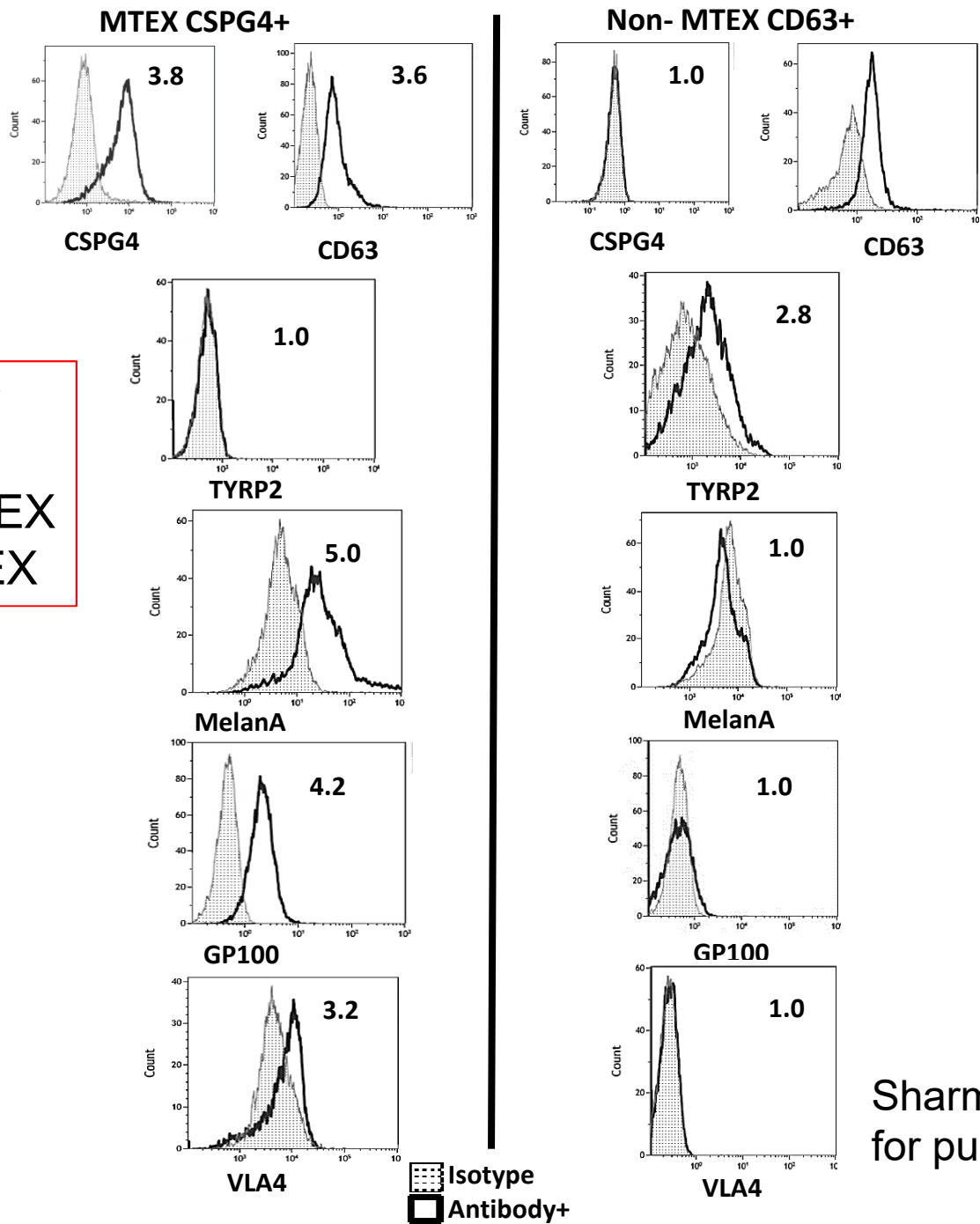
PROTEIN LEVELS of TOTAL EXOSOMES or MTEX in PLASMA



MTEX = from 23% to 66% of total plasma exosomes

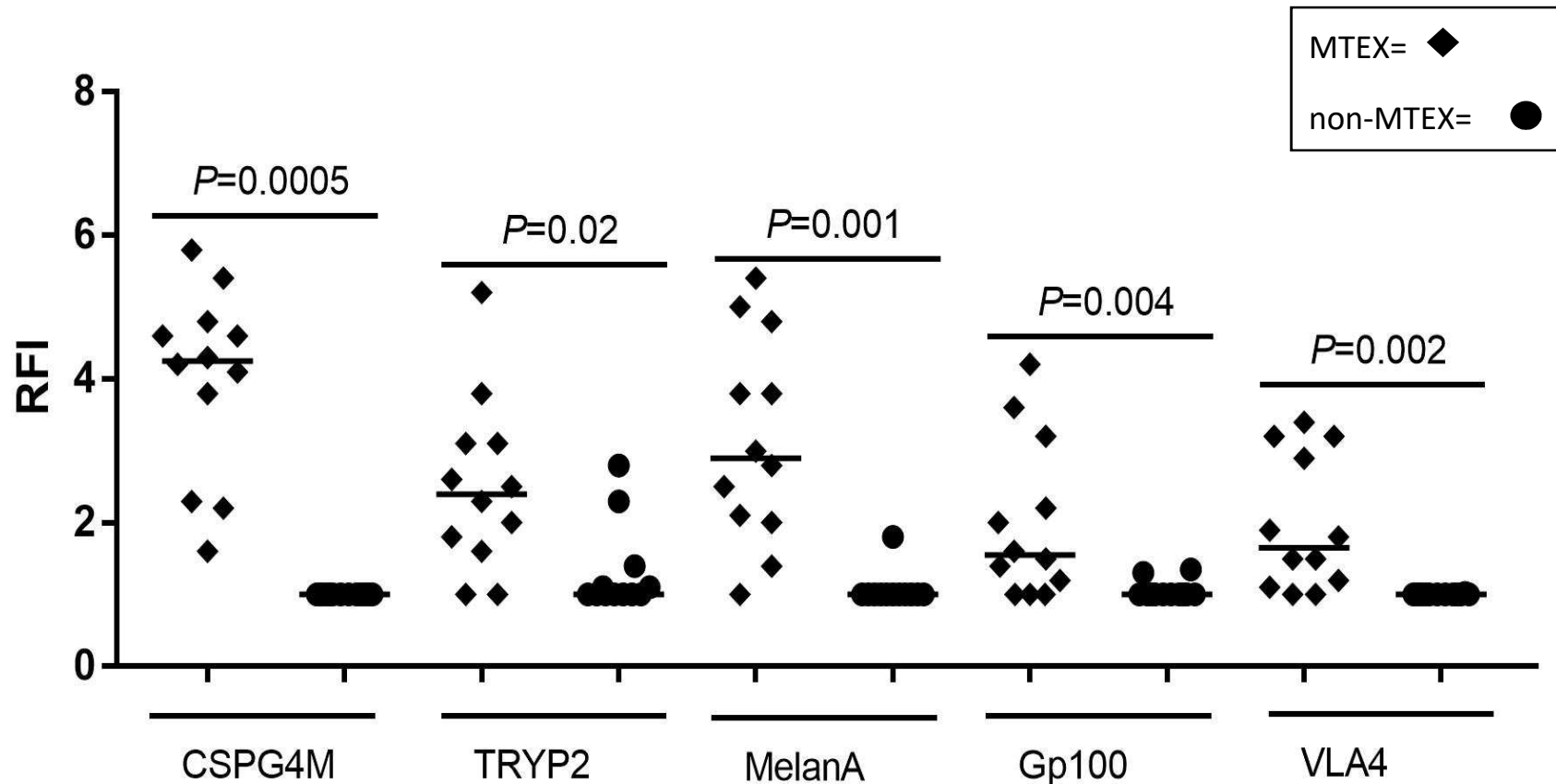
Sharma et al, submitted for publication, 2019

On-bead flow cytometry for MAAs on MTEX and non-MTEX



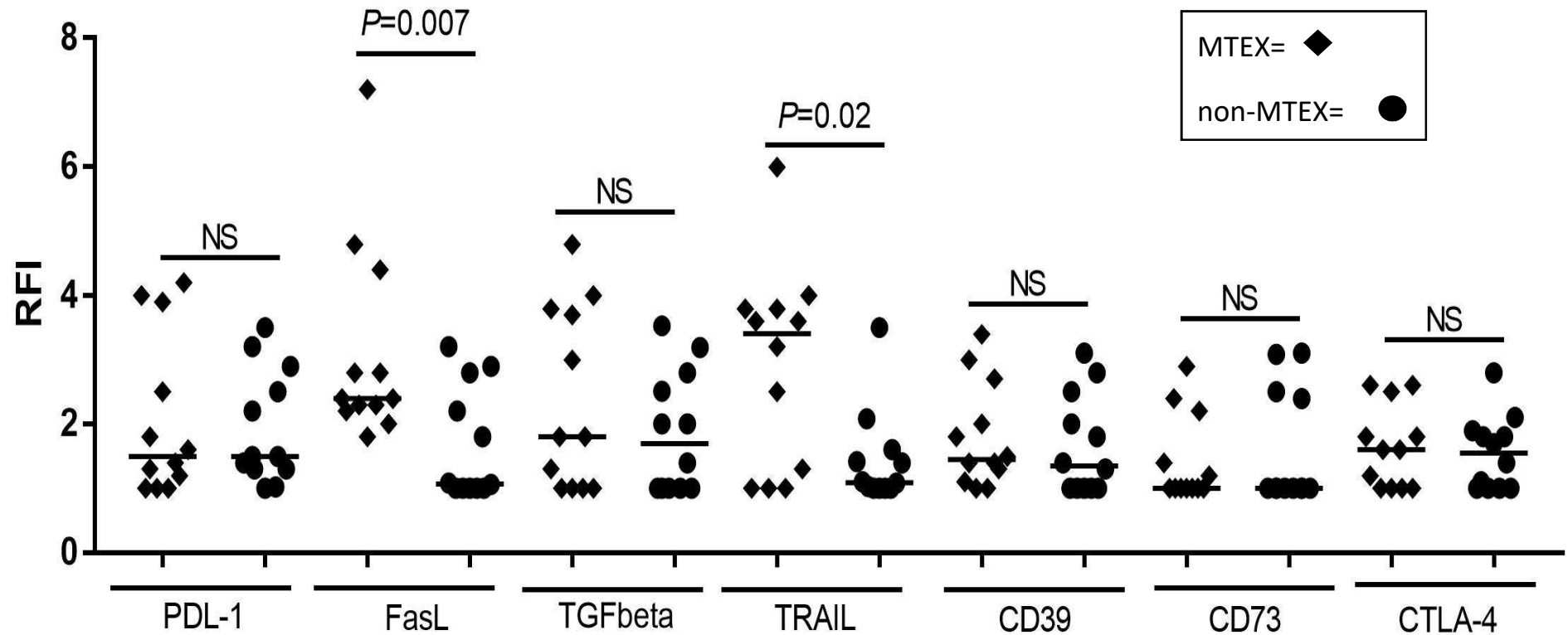
Sharma et al, submitted for publication, 2019

Melanoma associated antigens (MAAs) in MTEX and non-MTEX



MTEX score for MAAs = 13.6
Non-MTEX score for MAAs = 5.4

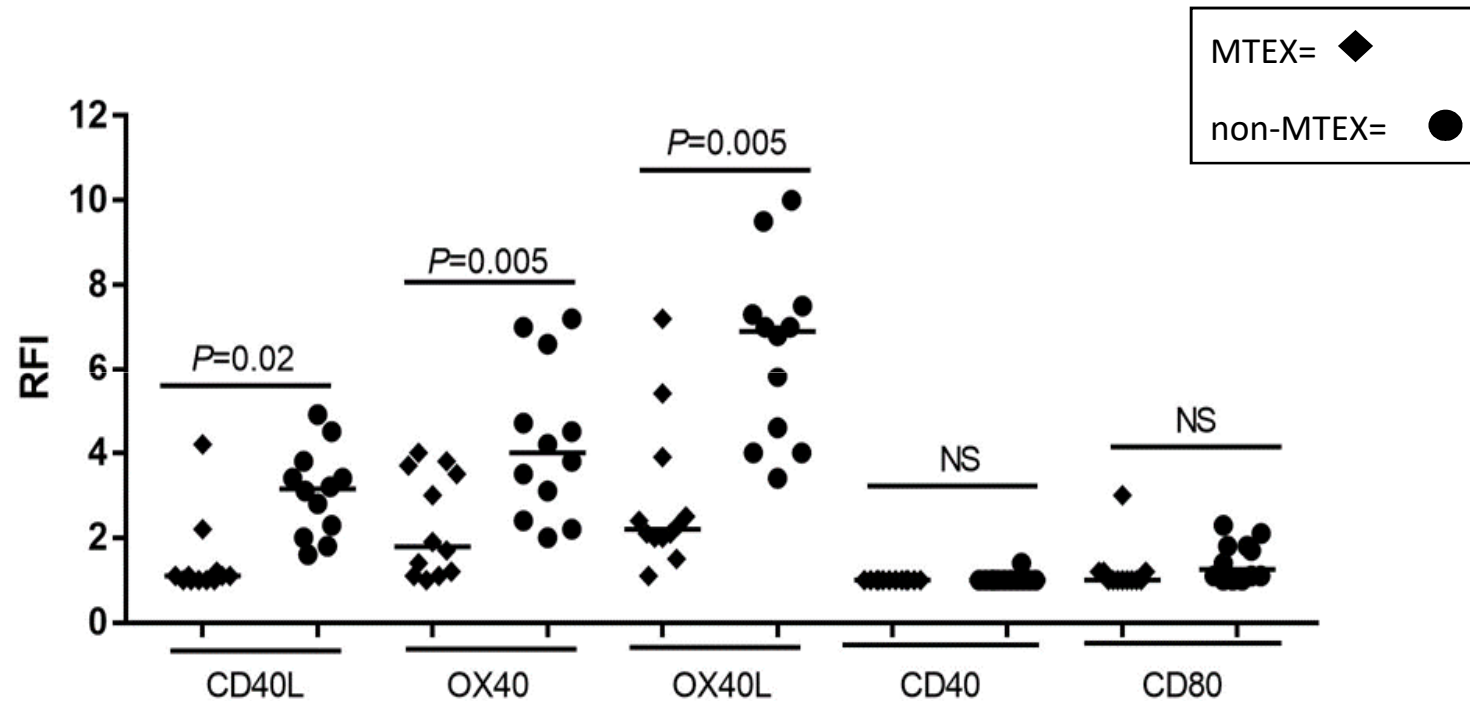
Immunoinhibitory proteins in MTEX and non-MTEX



MTEX immsupp score = 15.3

Non-MTEX immsupp score = 11.7

Immunostimulatory proteins in MTEX and non-MTEX

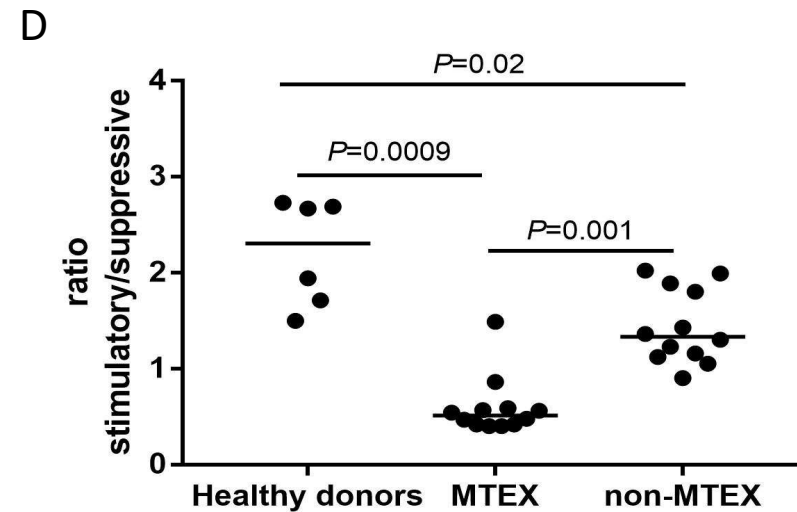
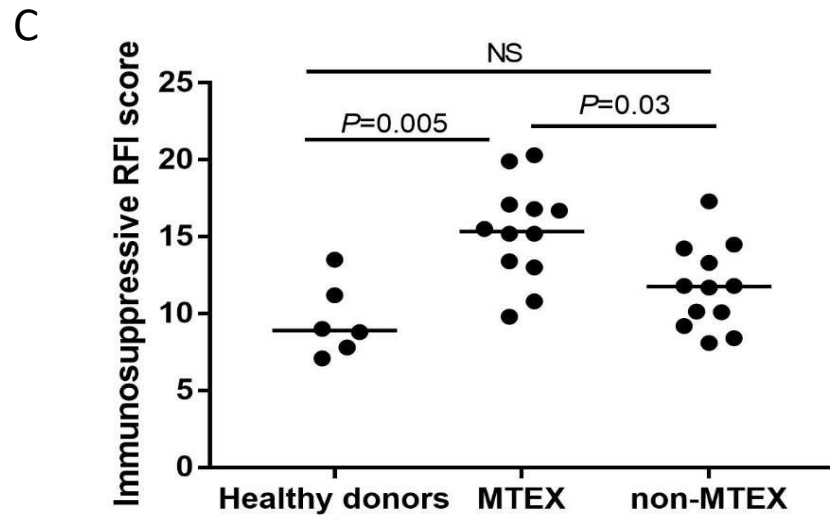
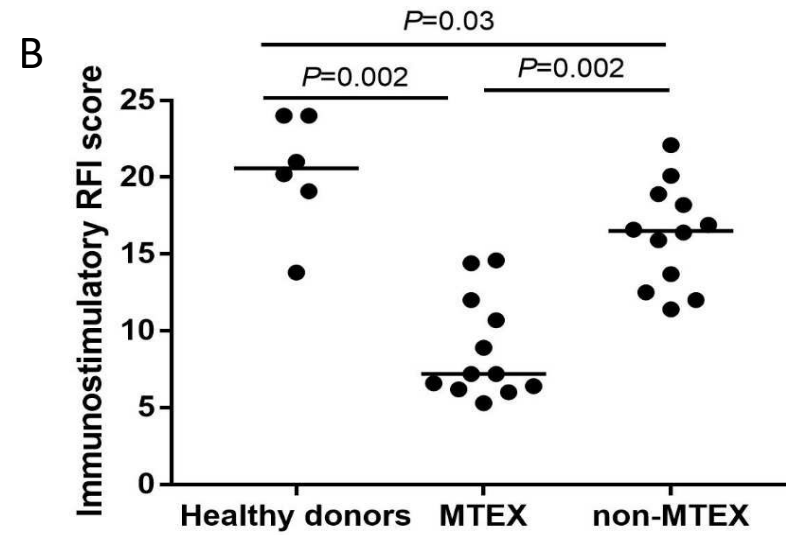
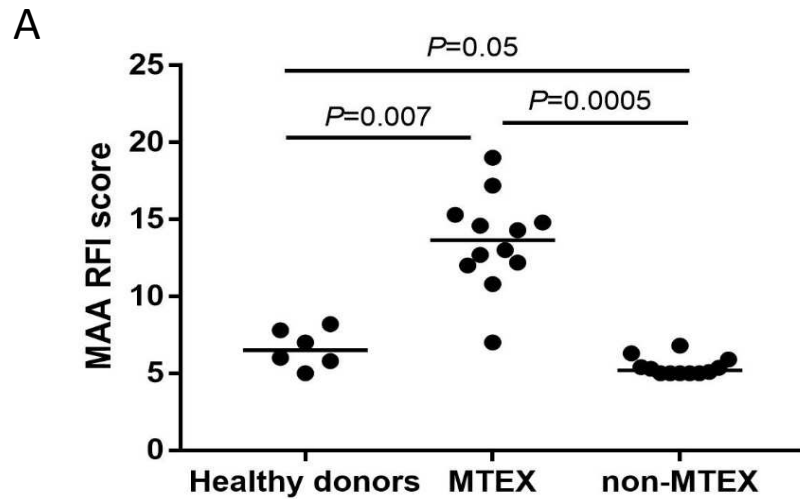


MTEX immstim score =8.8

Non-MTEX immstim score=16.2

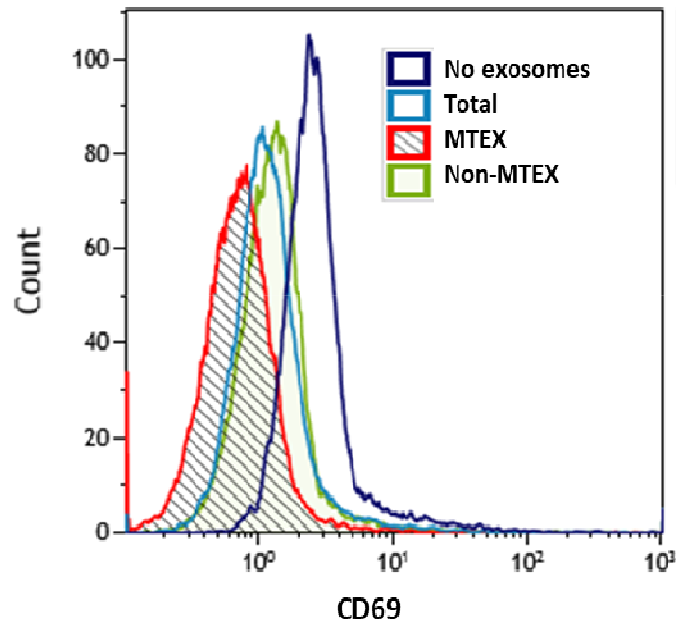
Sharma et al, submitted for publication, 2019

RFI scores and ratios for MTEX and non-MTEX

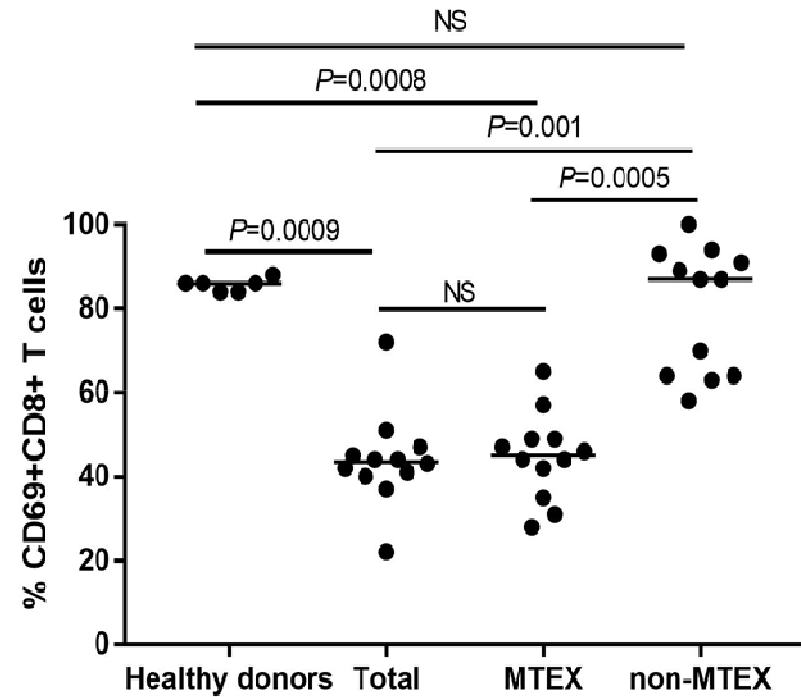


Exosome-mediated effects on CD69 expression on primary human CD8+ T cells

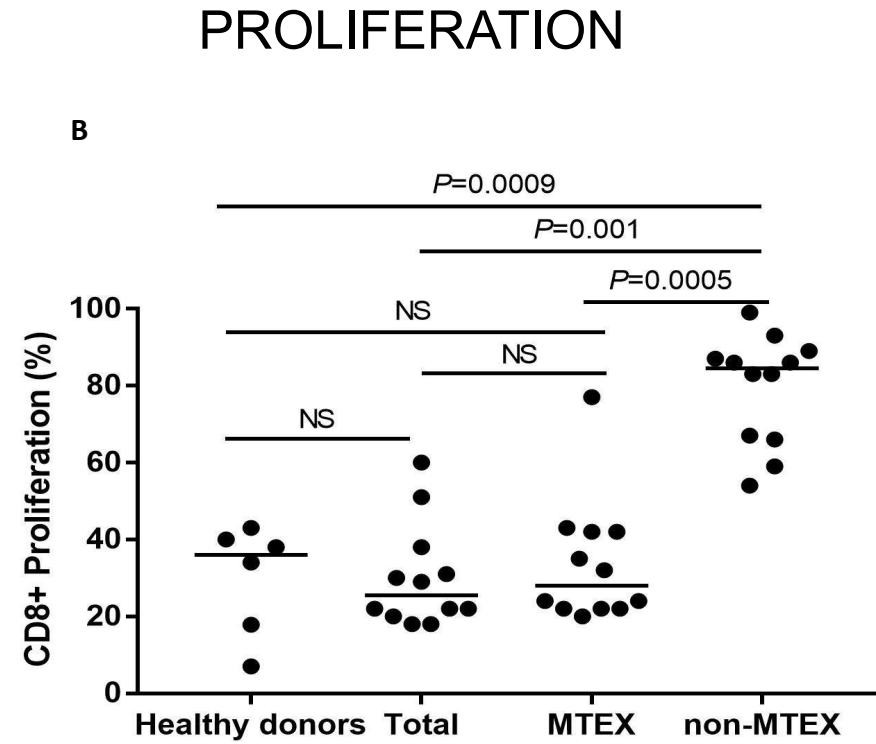
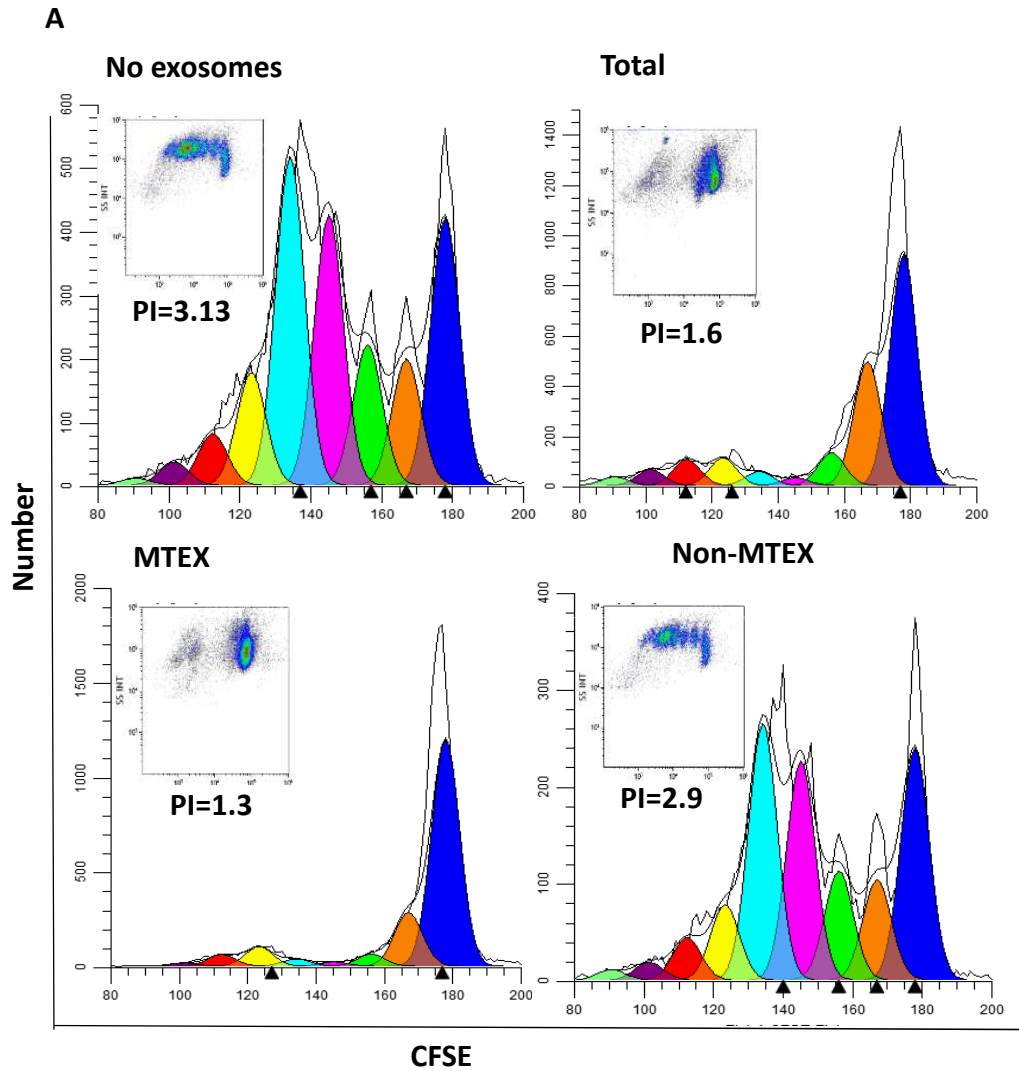
A



B

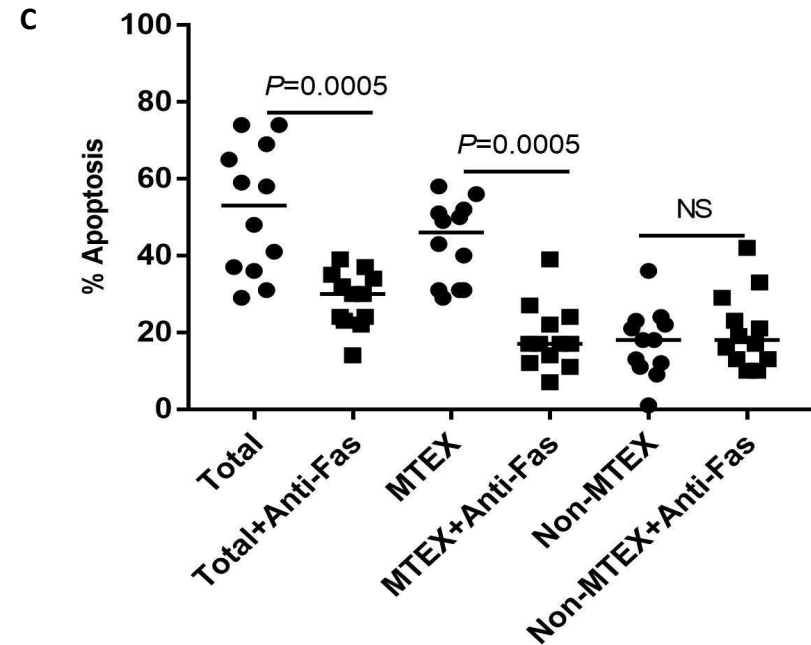
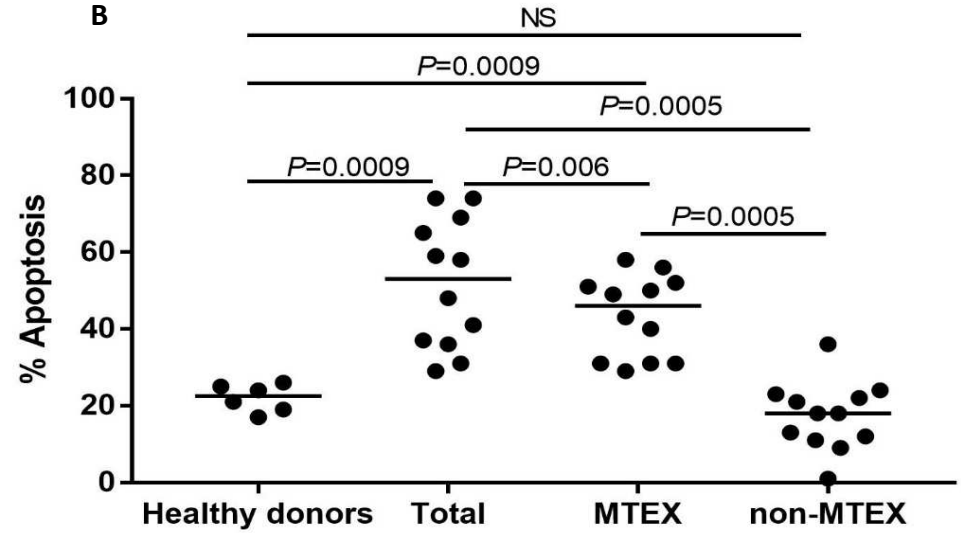
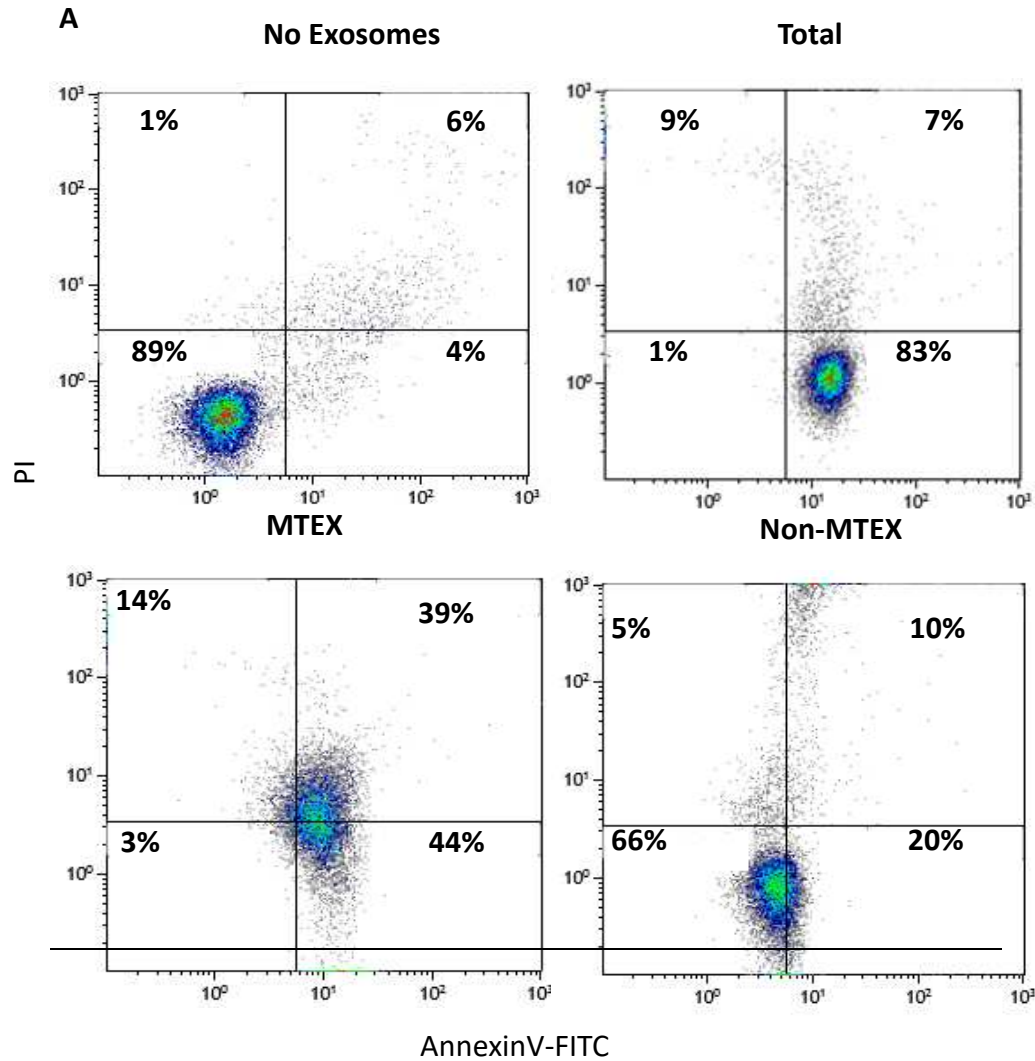


All values are normalized to NO EXO controls



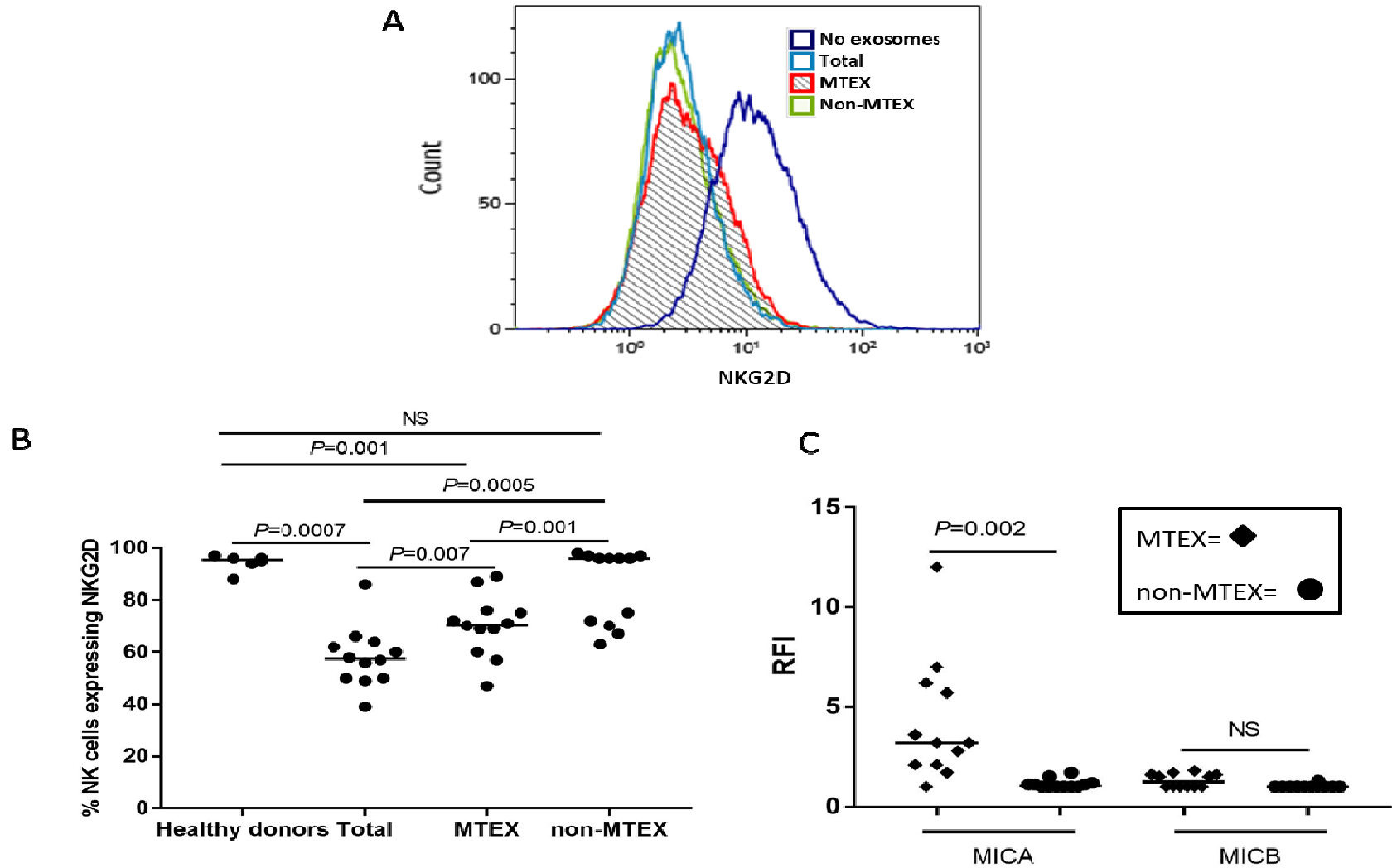
MTEX and non-MTEX co-incubated with CD8+ T cells

Apoptosis



MTEX and non-MTEX co-incubated with CD8⁺ T cells for 6h

Exosome-mediated effects on NKG2D receptor expression levels and % NKG2D+ human primary NK cells after co-incubation with exosomes



Spearman's correlation coefficients for non-MTEX

	Total protein	MTEX/Total protein ratio	Supp score	Stim score	Stim/supp ratio	CD8+ prolif	CD69	NKG2D	% Apop	Age at dx	Age at draw	Stage	Sex	Disease status
Total protein	1					-0.59 <i>P</i> =0.046								
MTEX/Total protein ratio		1						0.61 <i>P</i> =0.04						
Supp score			1					-0.64 <i>P</i> =0.03						
Stim score				1				-0.72 <i>P</i> =0.009						
Stim/supp ratio			-0.76 <i>P</i> =0.005		1			0.81 <i>P</i> =0.002	-0.73 <i>P</i> =0.007			-0.83 <i>P</i> =0.0007		
CD8+ prolif	-0.59 <i>P</i> =0.046			0.59 <i>P</i> =0.04		1								
CD69							1							
NKG2D		0.61 <i>P</i> =0.04	-0.64 <i>P</i> =0.03		0.81 <i>P</i> =0.002			1						
% Apop				-0.72 <i>P</i> =0.009	-0.73 <i>P</i> =0.007				1			0.61 <i>P</i> =0.04		
Age at dx										1	0.84 <i>P</i> =0.0006		0.63 <i>P</i> =0.03	
Age at draw										0.84 <i>P</i> =0.0006	1			
Stage						-0.83 <i>P</i> =0.0007			0.61 <i>P</i> =0.04			1		
Sex										0.63 <i>P</i> =0.03			1	
Disease status														1

Blue = positive correlatino

Yellow = inverse correlation

What have we learned?

- ❑ Total exosome protein levels higher in MTEX than HDs' plasma
- ❑ MTEX are enriched in MAAs and immunosuppressive proteins
- ❑ Non-MTEX are enriched in immunostimulatory proteins
- ❑ Quantitative differences in molecular profiles of MTEX, non-MTEX and HD's exosomes: no single, unique profile distinguishing MTEX from non-MTEX
- ❑ MTEX strongly suppress functions of human immune cells and emerge as main contributors to immune suppression in melanoma patients
- ❑ Few correlations between exosome molecular profiles and functions were evident, with the ability of MTEX and non-MTEX to mediate CD8+ T cell apoptosis emerging as the strongest functional correlate of disease
- ❑ PD-L1 was present in MTEX and non-MTEX but no evidence for its association with disease was seen in this patient cohort

Contributors

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