



Role of melanoma exosomes in pro-tumor conversion of immune cells

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**FONDAZIONE IRCCS
ISTITUTO NAZIONALE
DEI TUMORI**



SITC 2015, November 4-8 2015, National Harbor, MD

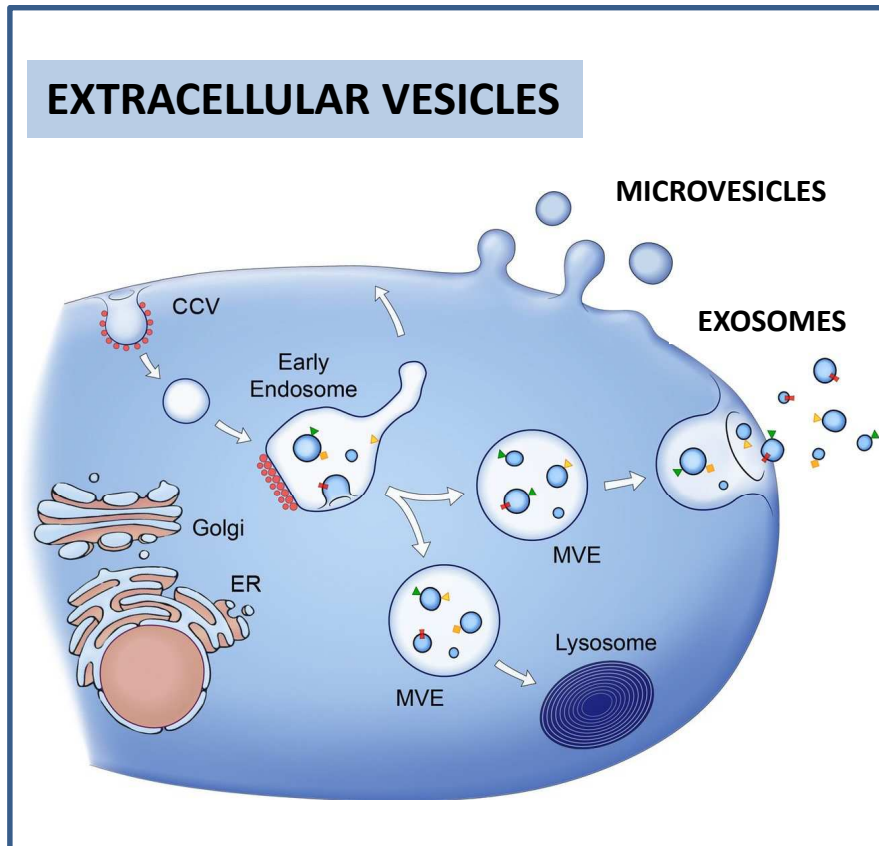
Presenter disclosure Information

CAMISASCHI CHIARA

The following relationships exist related to this presentation:

<No Relationships to Disclose>

EXTRACELLULAR VESICLES



Raposo G, JCB 2013

Cells release several types of EV that differ in size:

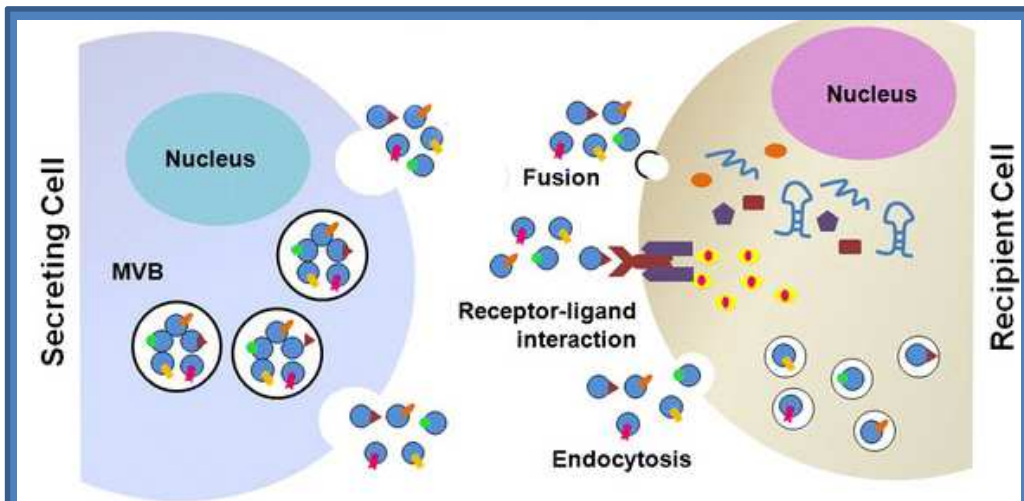
- **apoptotic bodies** (1000-5000nm)
- **microvesicles** MV (200-1000nm) formed by blebbing of cellular membrane
- **exosomes** (30-150nm)

Exosomes are produced by all type of cells and present in all body fluids

Exosomes biogenesis involves the endosomal compartment, suggesting a role in maintaining cellular homeostasis

Exosomes as nano-replica of the secreting cell, thus putative tumor biomarker

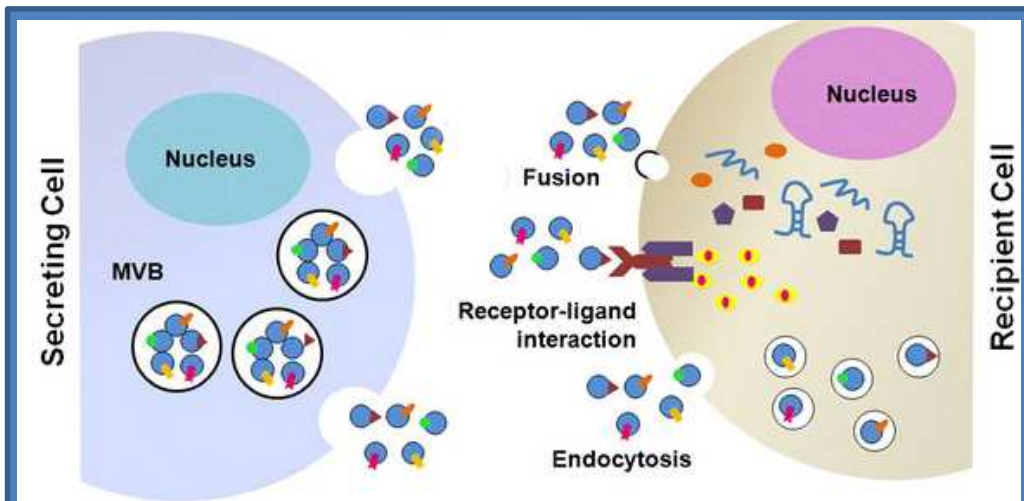
Central role in INTERcellular cross-talk



Exosomes are internalized by recipient cells by receptor mediated **endocytosis**, **pinocytosis**, **phagocytosis** or **fusion with the cell membrane**.

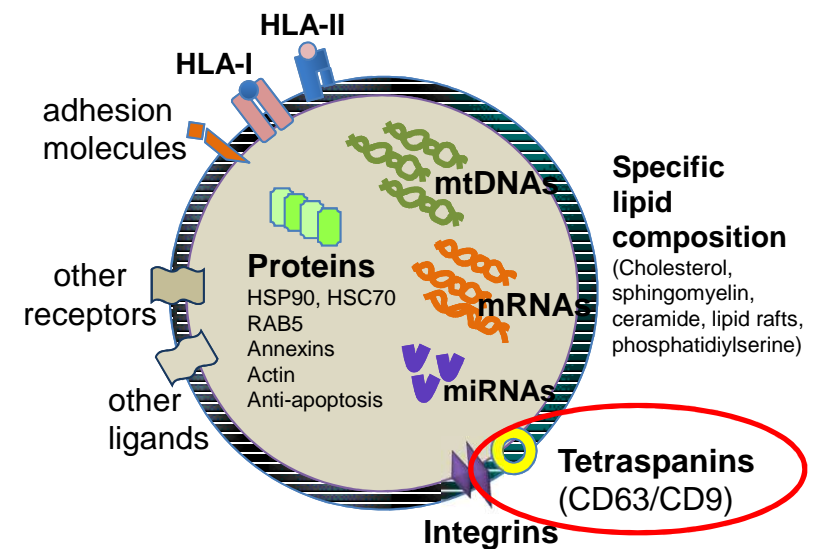
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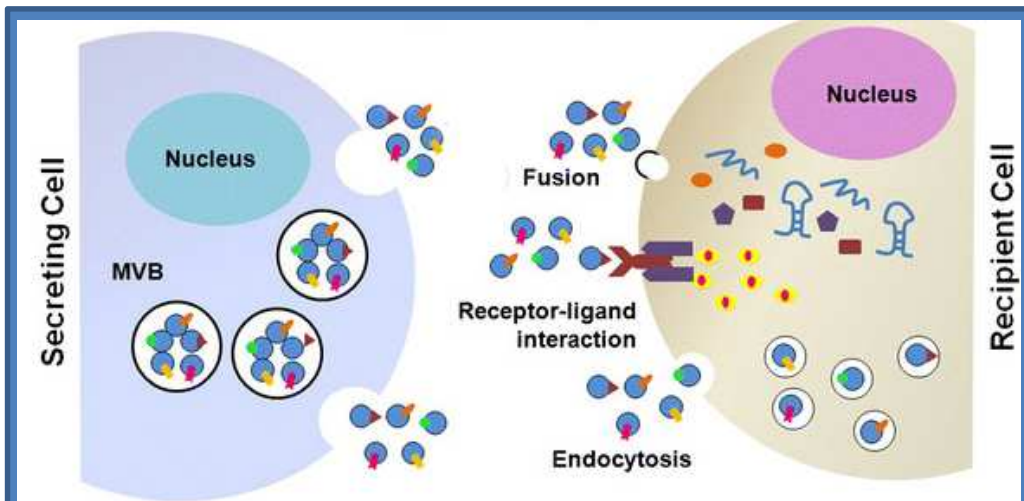
They transfer **proteins**, **lipids**, **DNA**, **mRNA** and **miRNA** in a functionally-active form, locally and systemically. They can condition **myelopoiesis** and regulate gene-expression in recipient cell determining their behavior.



Zhang et al. Journal of Hematology & Oncology 2015 8:83

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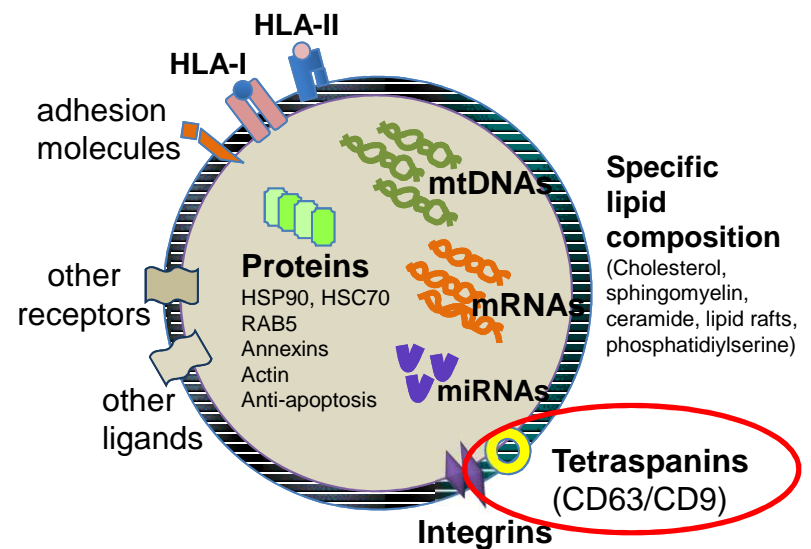


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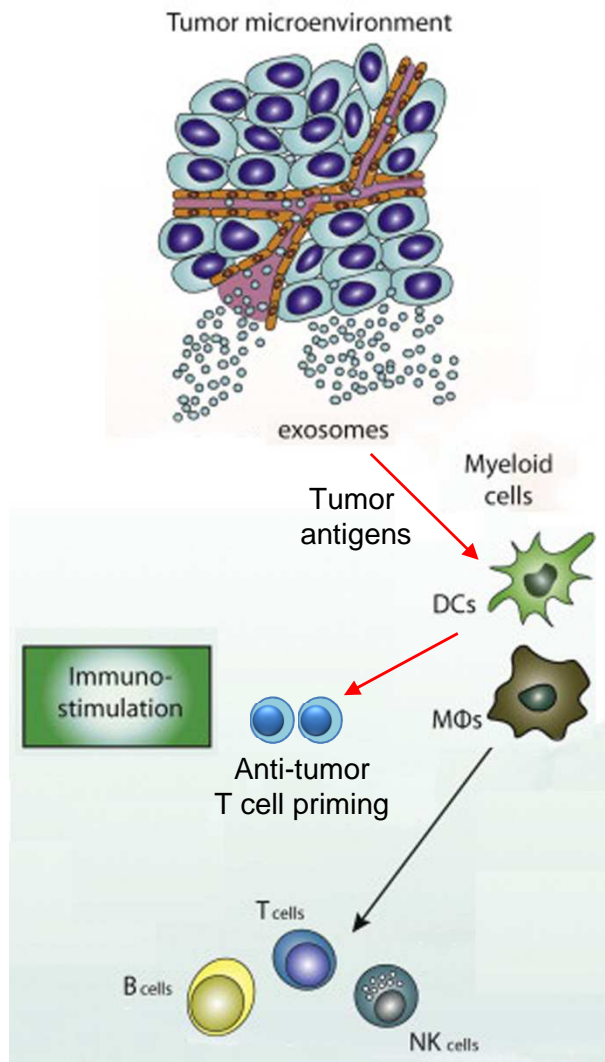
Zhang et al. Journal of Hematology & Oncology 2015 8:83

Exosomes are augmented in CANCER PATIENTS compared to HD and express higher levels of miRNA

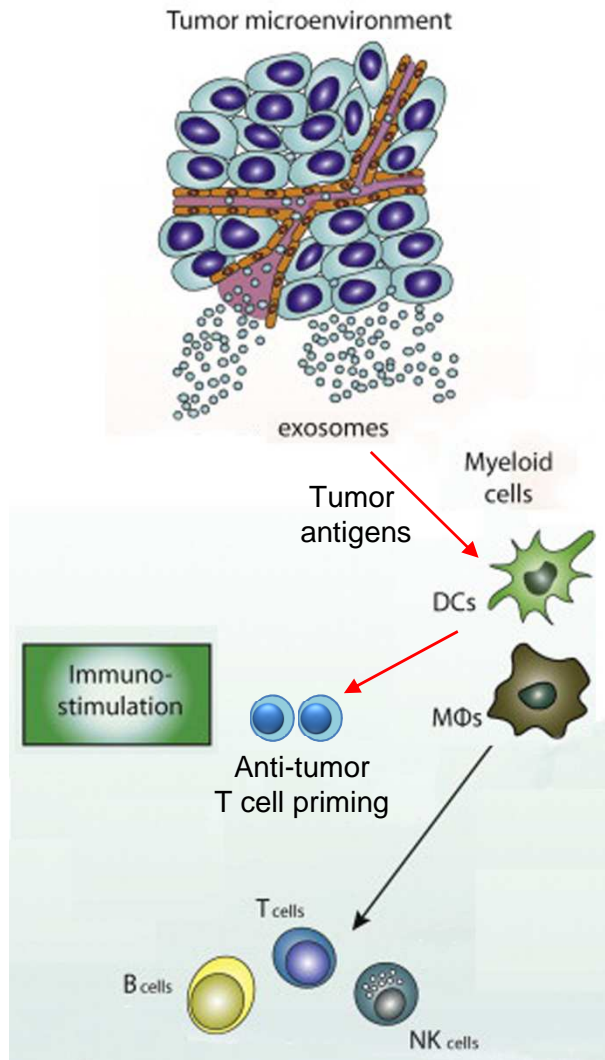
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Multiple role of tumor exosomes in cancer, immune stimulation or immune suppression



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Modulation of tumor microenvironment

Angiogenesis

Multi-drug resistance

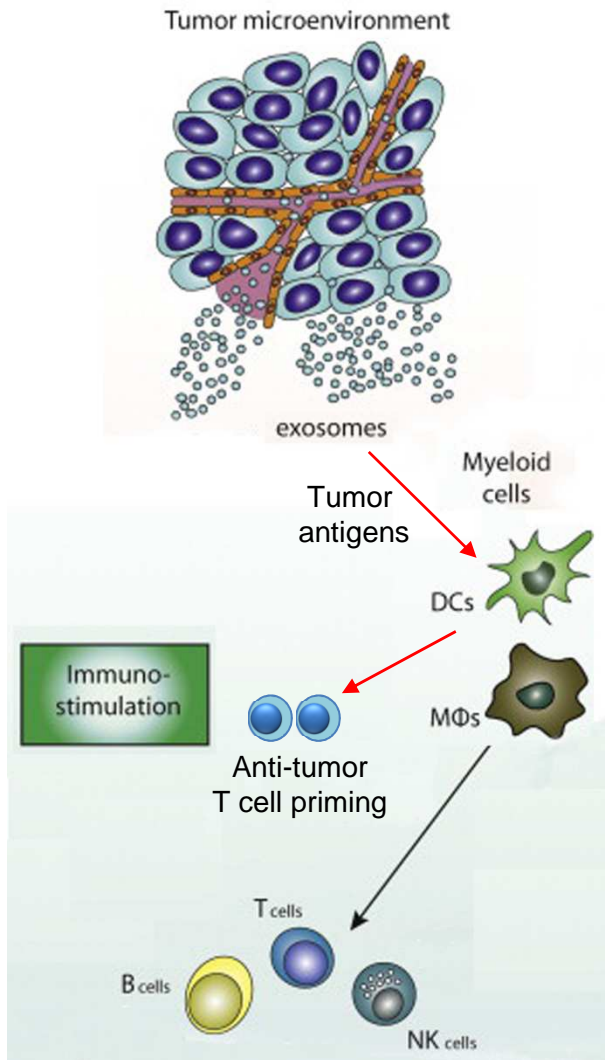
Invasion and metastasis

Acquisition of a more aggressive phenotype

Promotion of tumorigenesis
Cell-independent microRNA

Evasion of immune surveillance

Multiple role of tumor exosomes in cancer, immune stimulation or immune suppression



Modulation of tumor microenvironment

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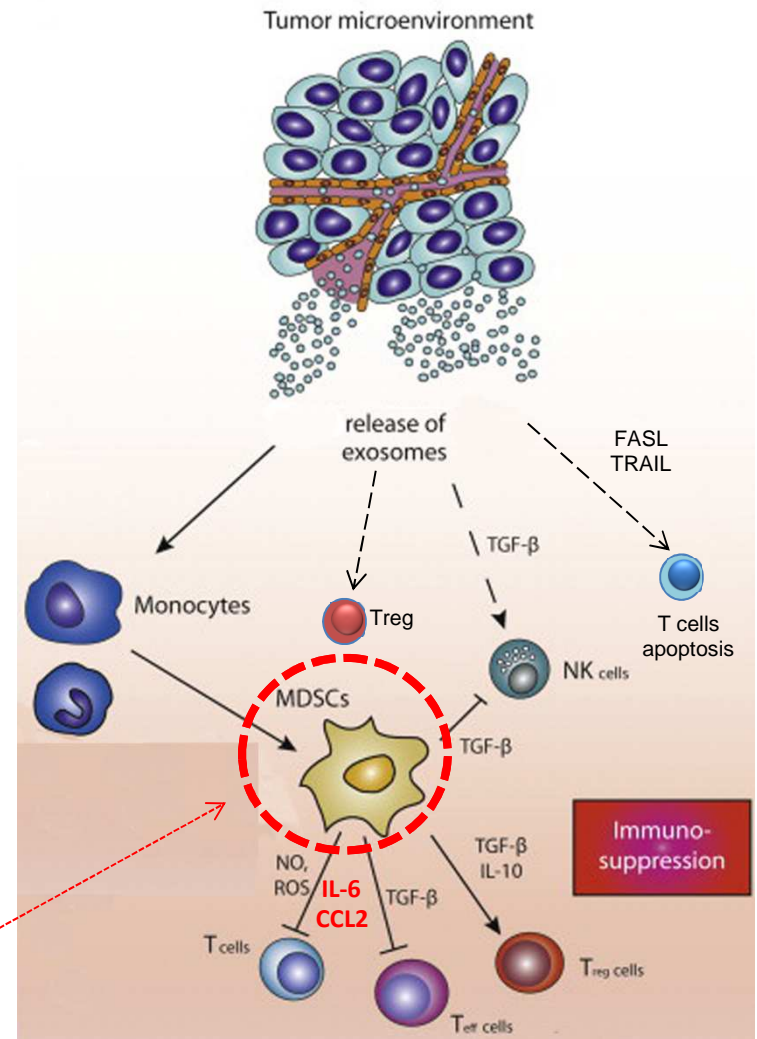
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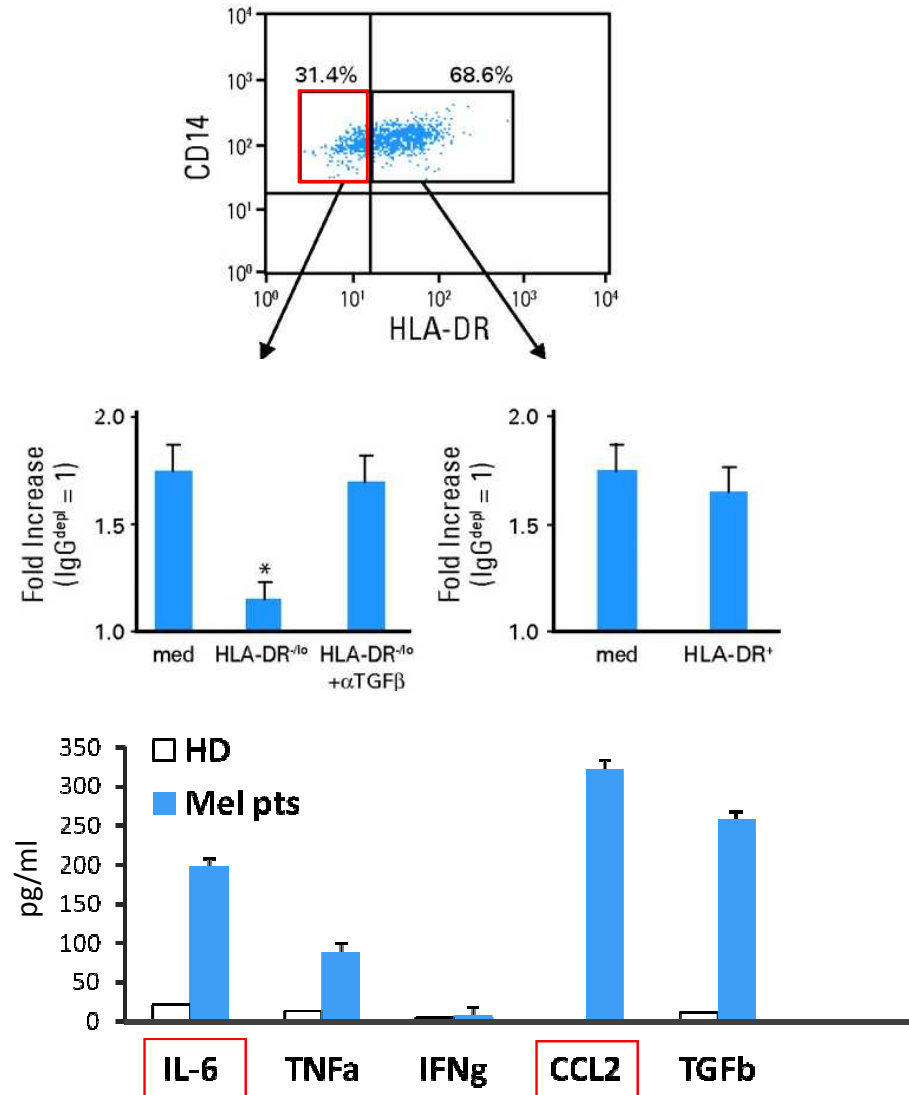
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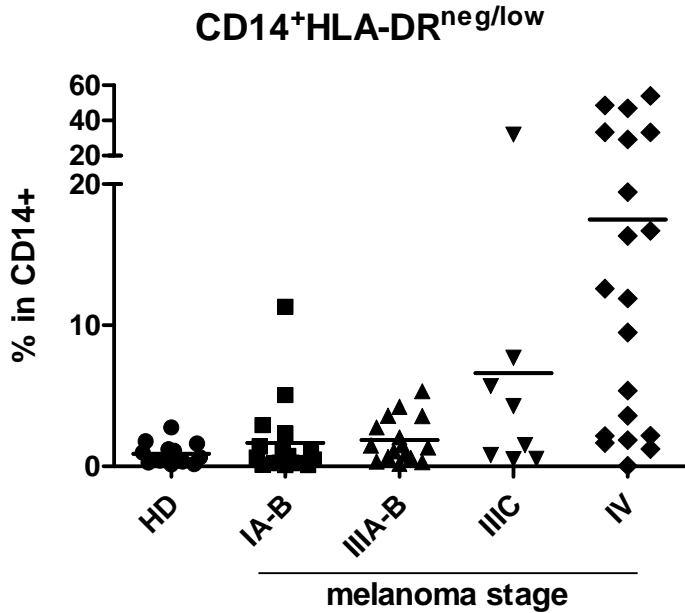
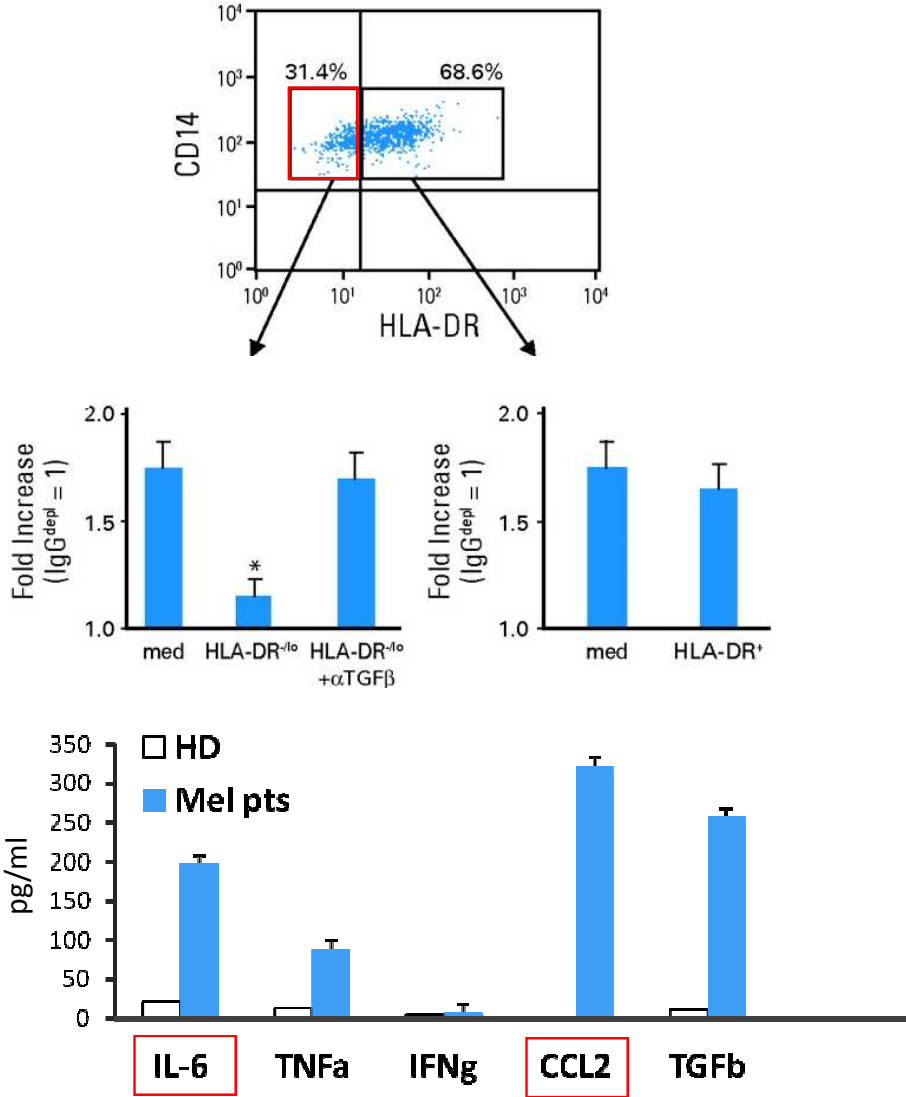
Evasion of immune surveillance



Immunosuppressive activity of monocytes from melanoma patients and MDSC accumulation

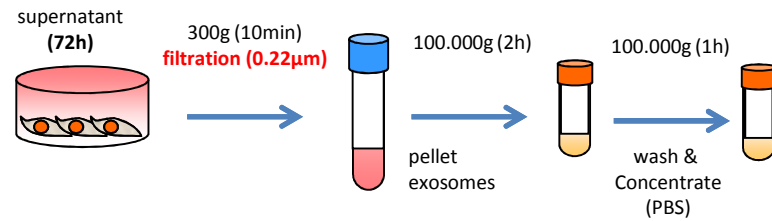


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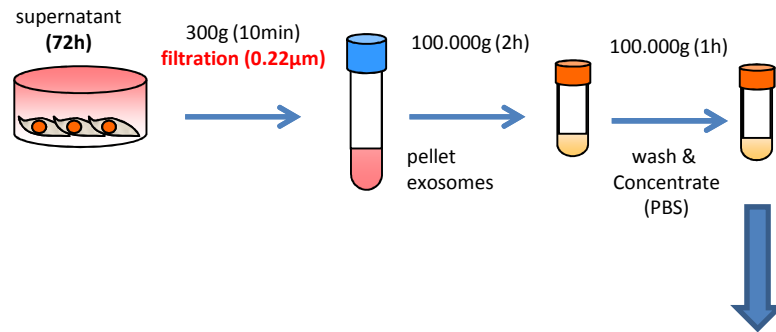
Are exosomes involved in MDSC differentiation? *in vitro* model

purification

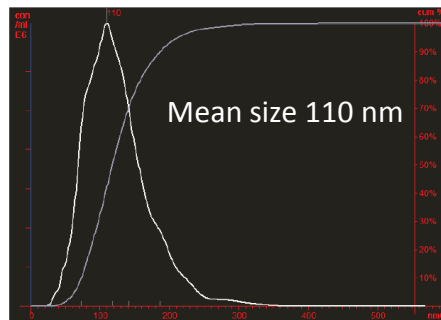


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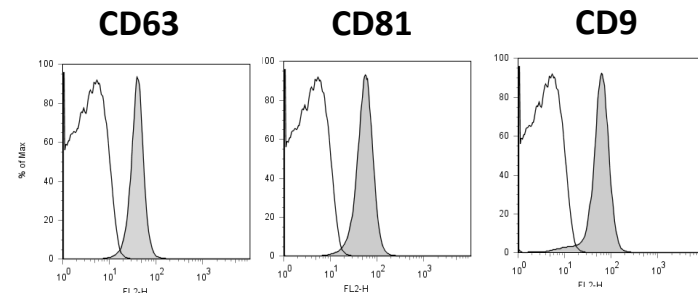
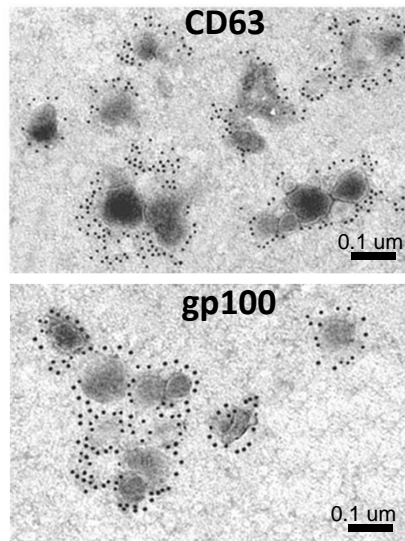
purification



Nanosight

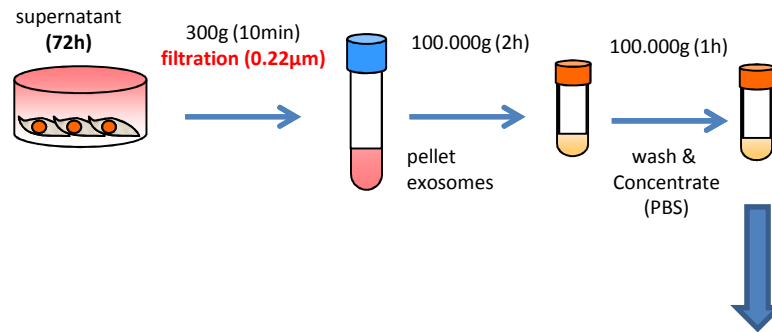


RESULTS:
Size Distribution: Mean: 125 nm, Mode: 110 nm, SD: 46 nm
Cumulative Data (nm): D10: 73, D50: 118, D90: 186, D70: 141
User Lines: 0 nm, 0 nm
Total Concentration: 47.11 particles / frame, 7.06E8 particles / ml



Are exosomes involved in MDSC differentiation? *in vitro* model

purification



Melanoma cells

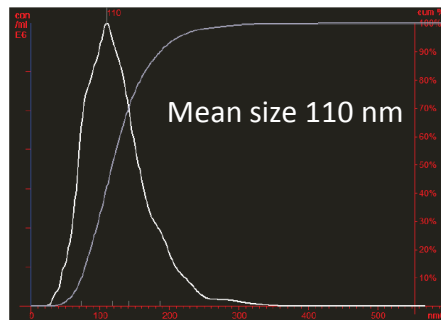
healthy donors' monocytes

Exosomes

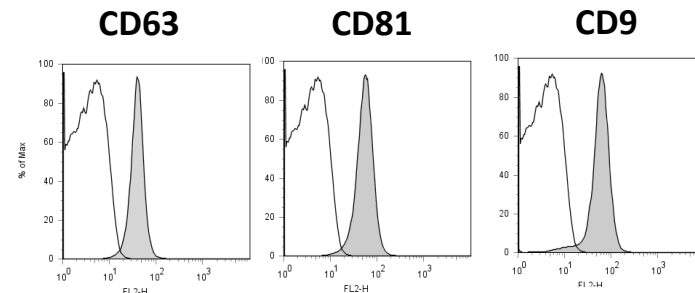
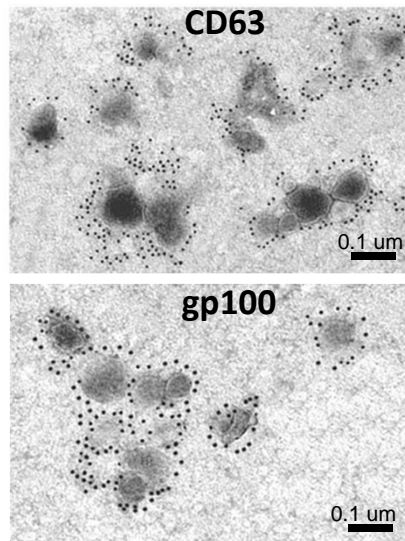
24/72h

Exo-MDSC
CD14⁺HLA-DR^{neg}
endowed with T cell
suppressive activity

Nanosight

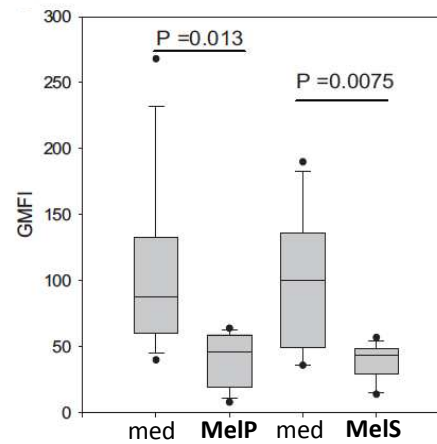
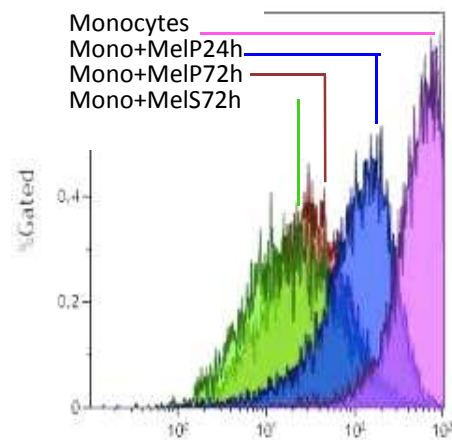


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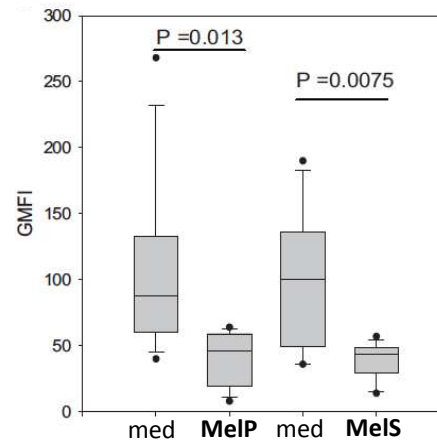
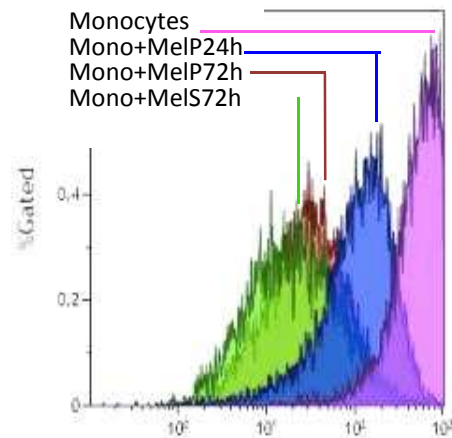
Melanoma exosomes induce MDSC *in vitro*

HLA-DR down-modulation

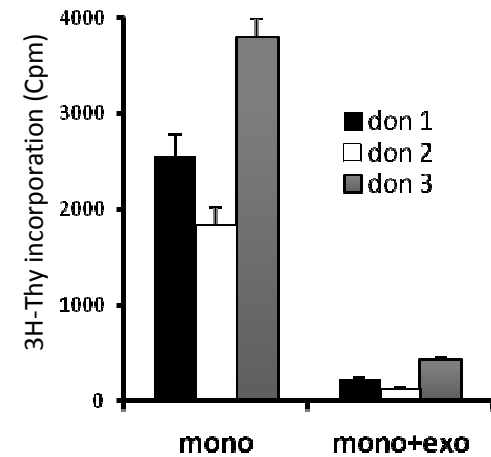


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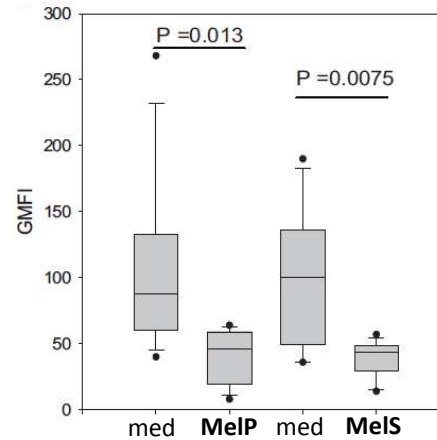
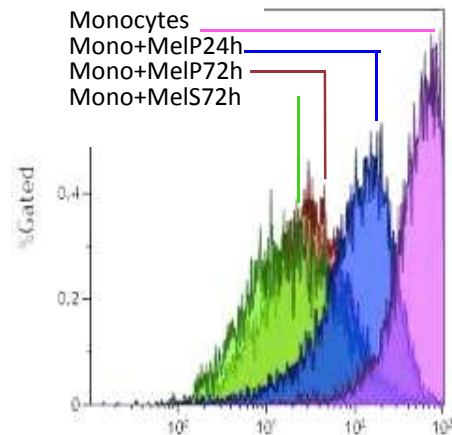


Inhibition of T cell proliferation

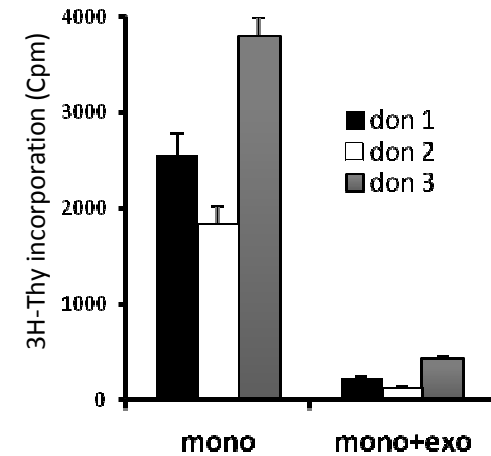


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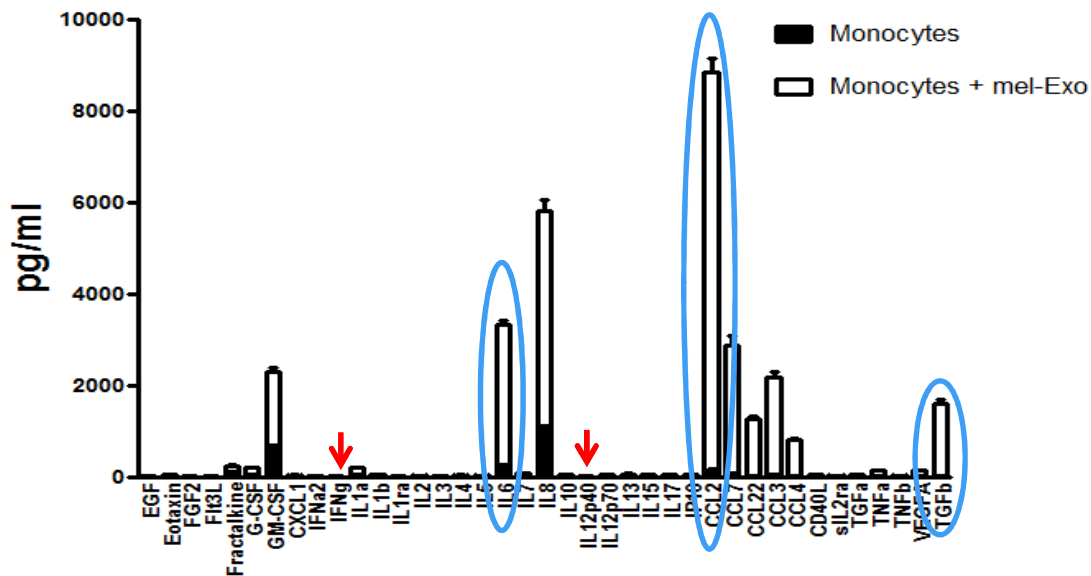
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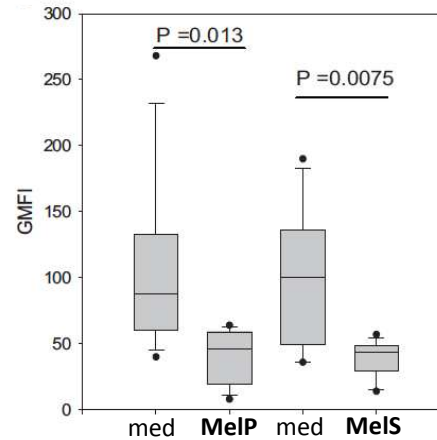
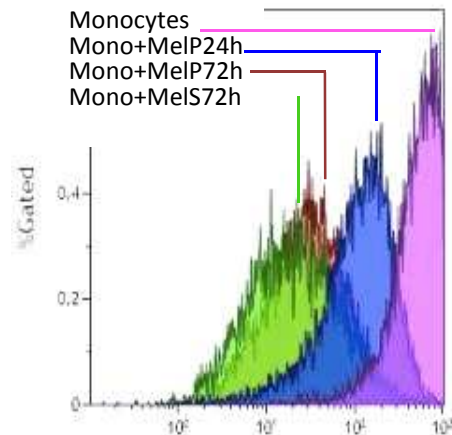


Release of immunosuppressive/proinflammatory cytokines and chemokines

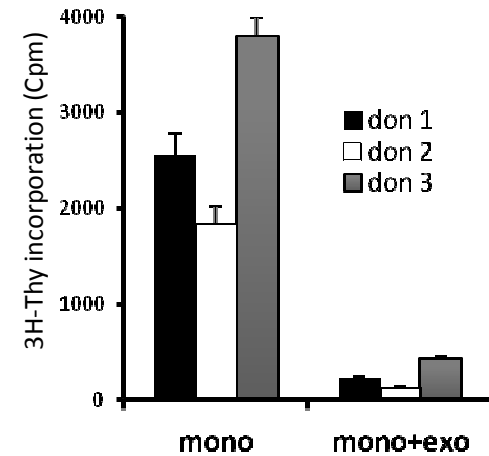


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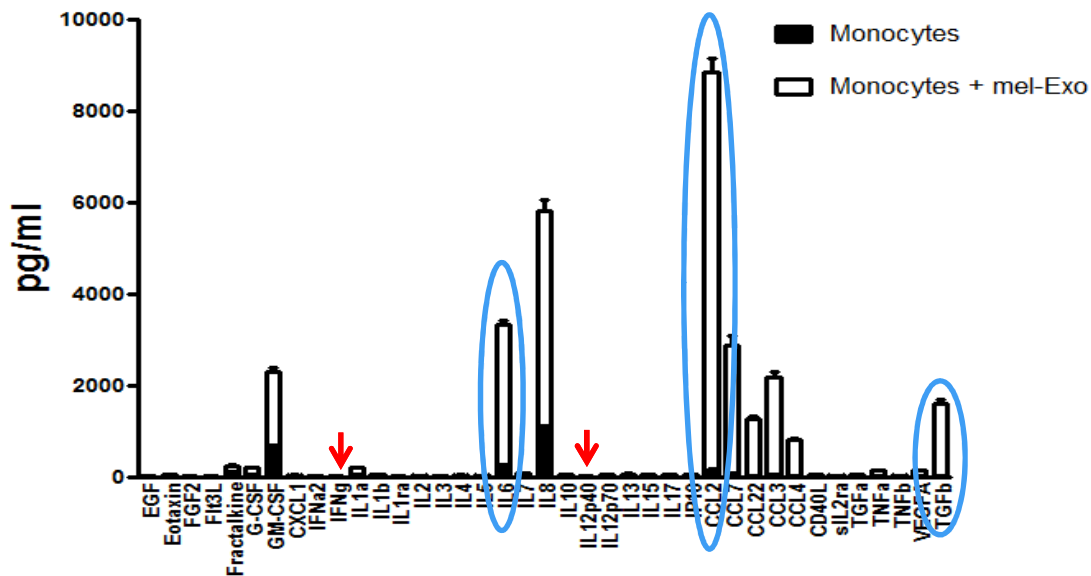
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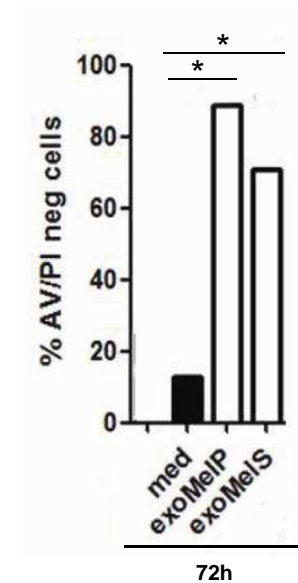
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Release of immunosuppressive/proinflammatory cytokines and chemokines



Prolonged survival





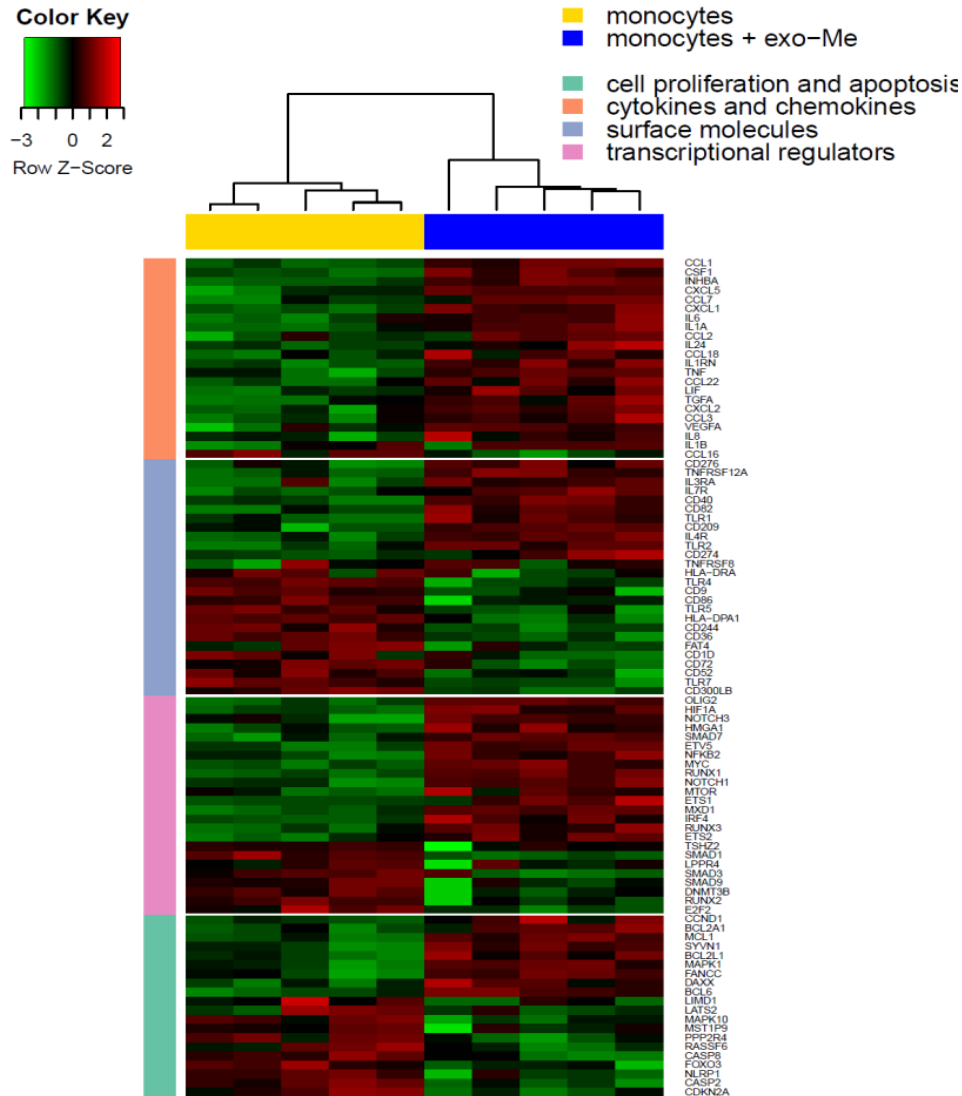
Which are the mechanisms involved in this process?

**Looking for molecules implicated in “education” of
myeloid cells and their conversion into MDSC**

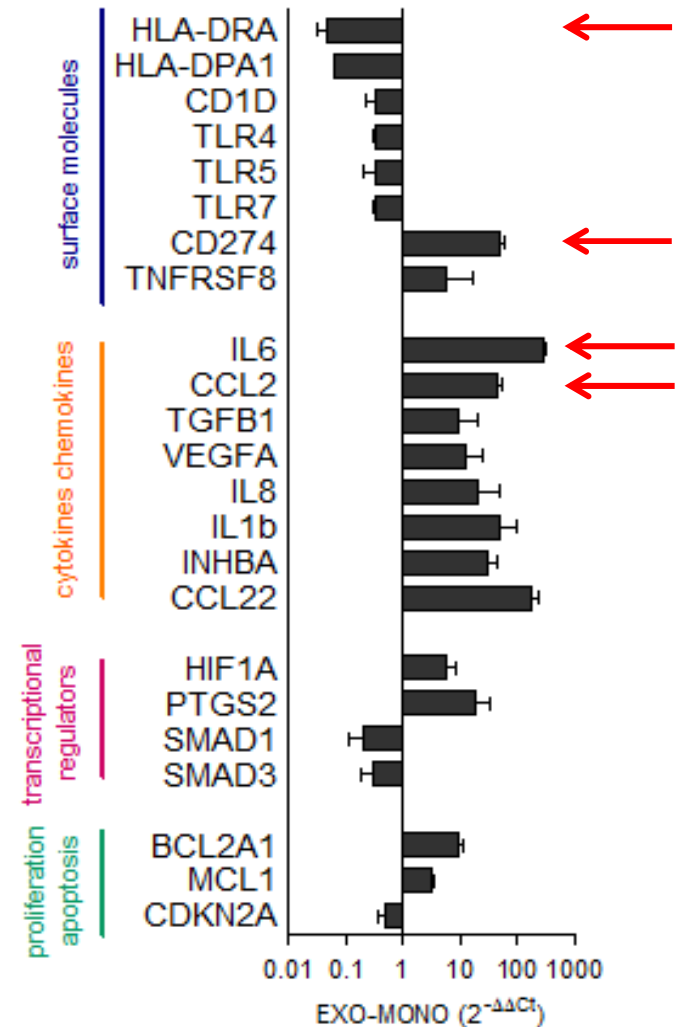


gene expression and miRNA profiling

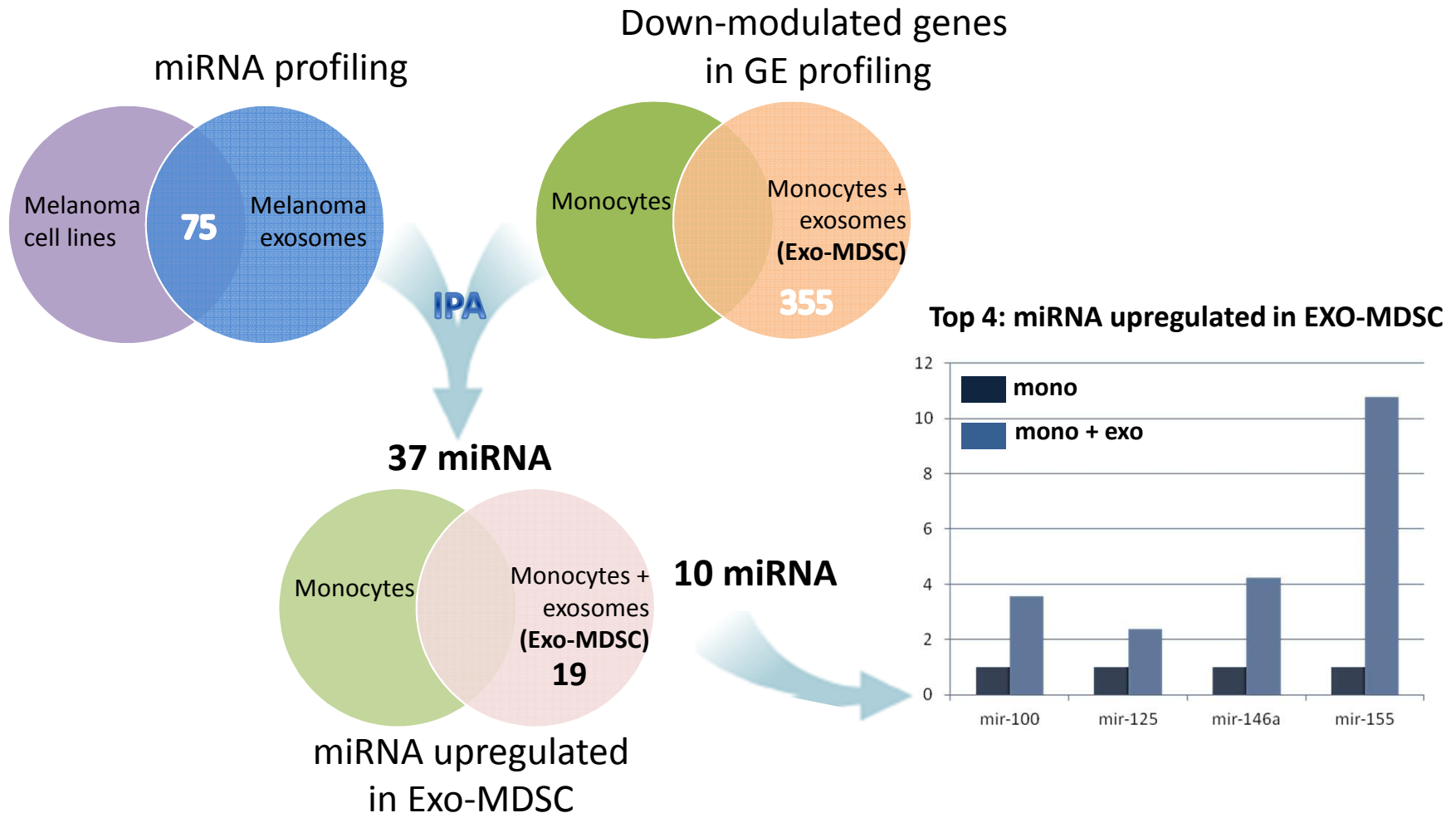
Transcriptional profile of Exo-MDSC



Heatmap of the genes resulted as differentially expressed between monocytes and monocytes treated with melanoma exosomes



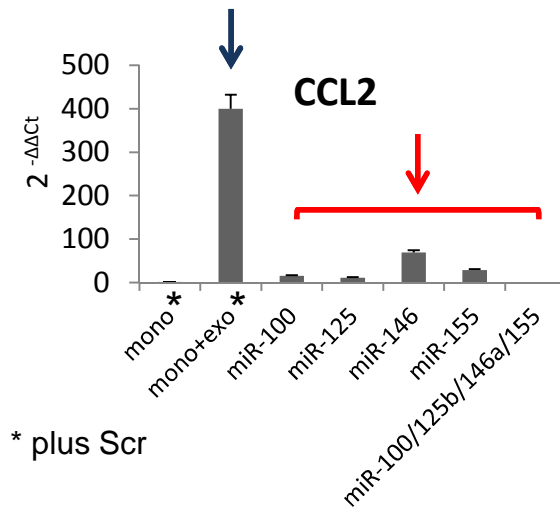
Selection of miRNA contained in tumor exosomes and involved in the generation of exo-MDSC



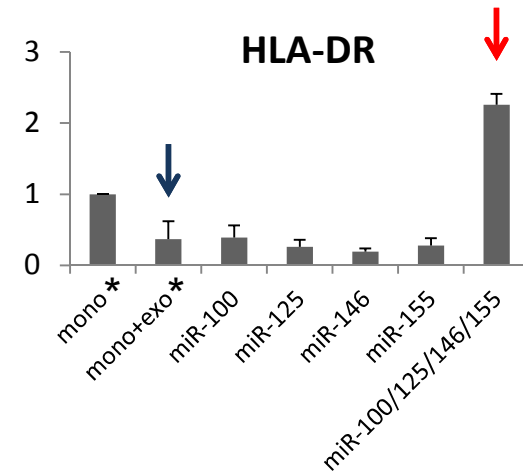
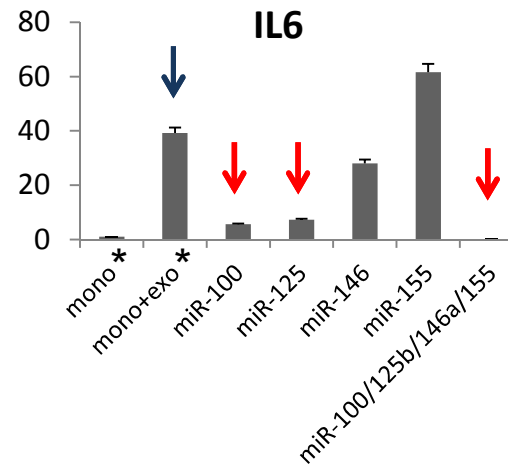
Role of selected miRNA in exosome-mediated MDSC conversion

- effect at miRNA inhibitors* -

antagomir



Transcriptional level

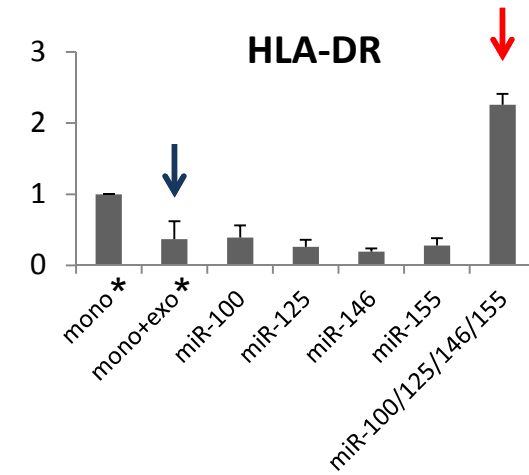
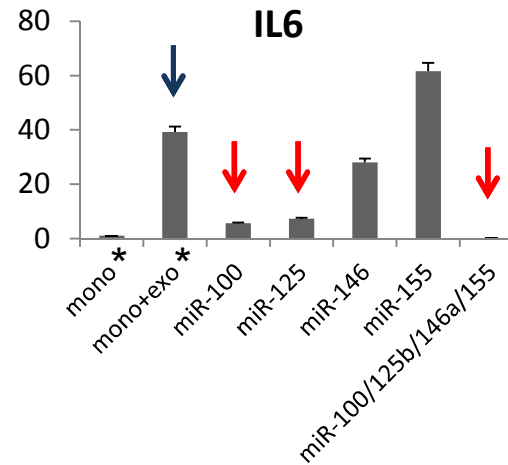
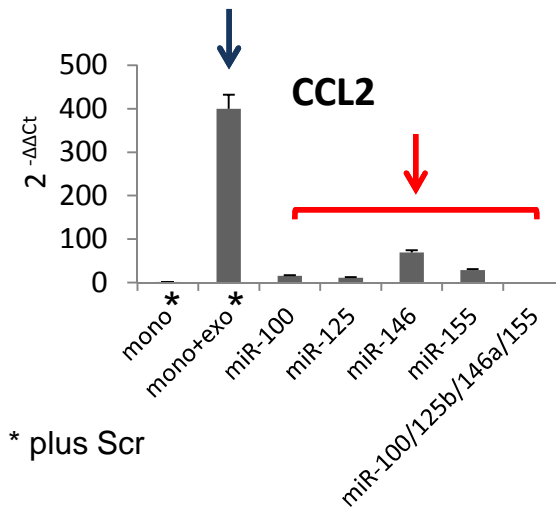


Role of selected miRNA in exosome-mediated MDSC conversion

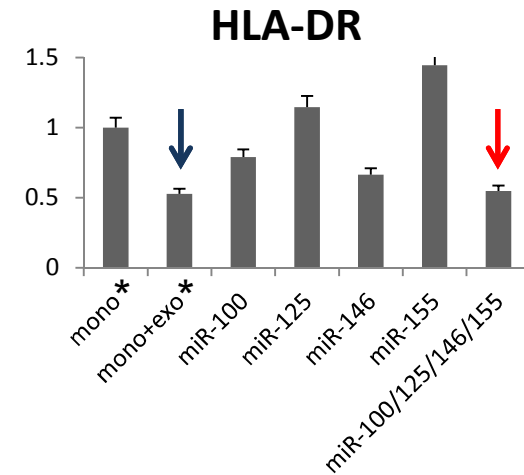
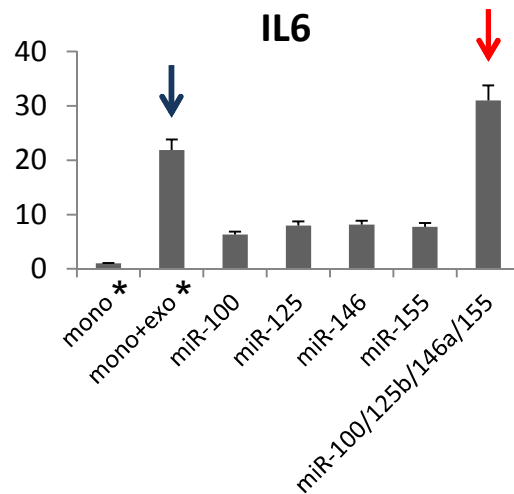
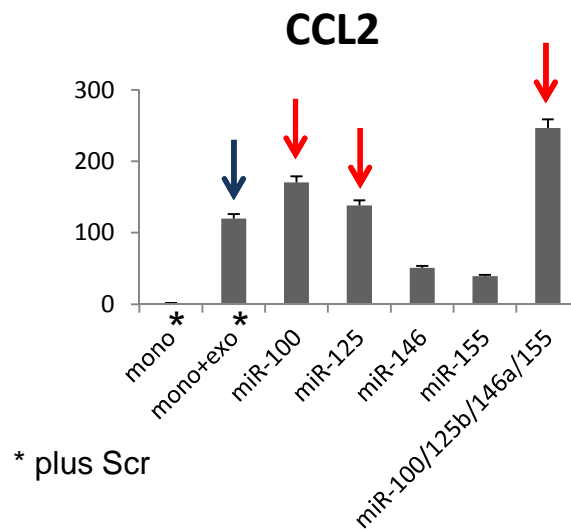
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Transcriptional level

antagomir



miRNA mimics

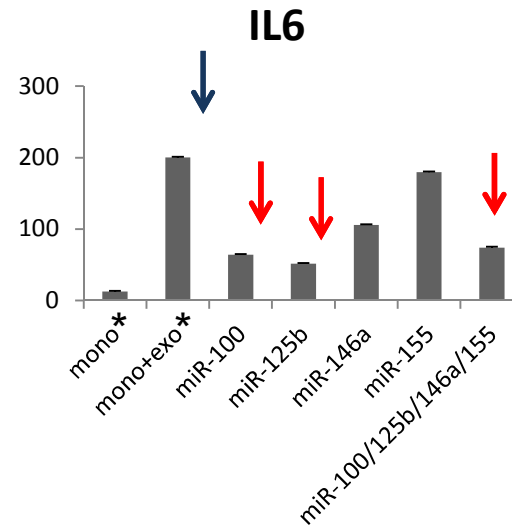
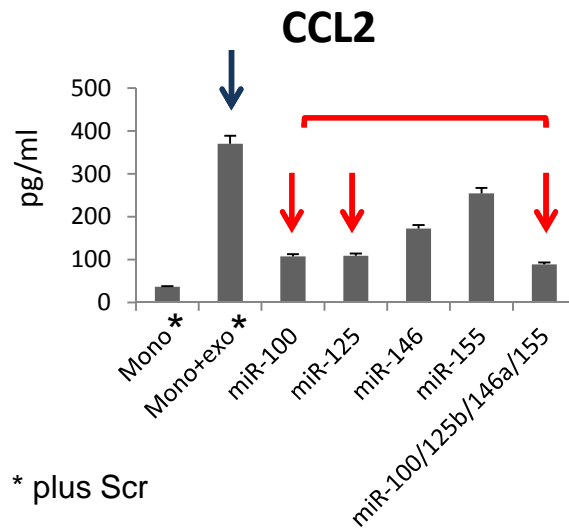


Role of selected miRNA in exosome-mediated MDSC conversion

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Protein level

CBA

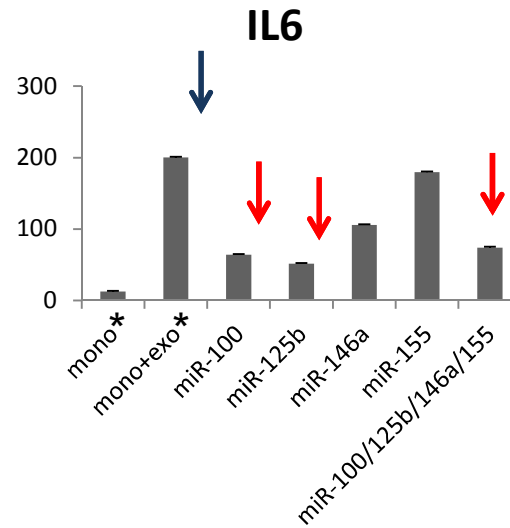
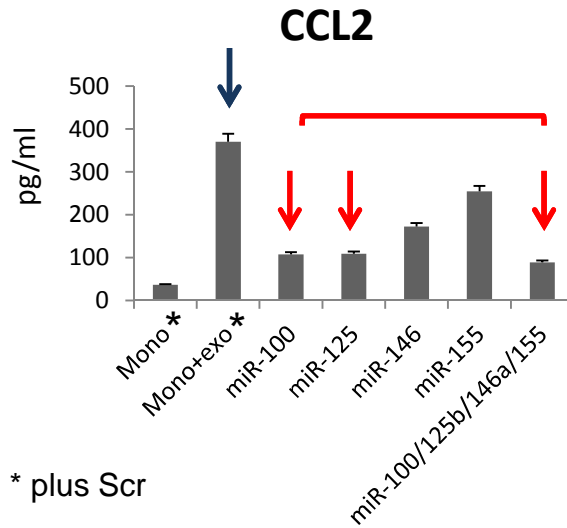


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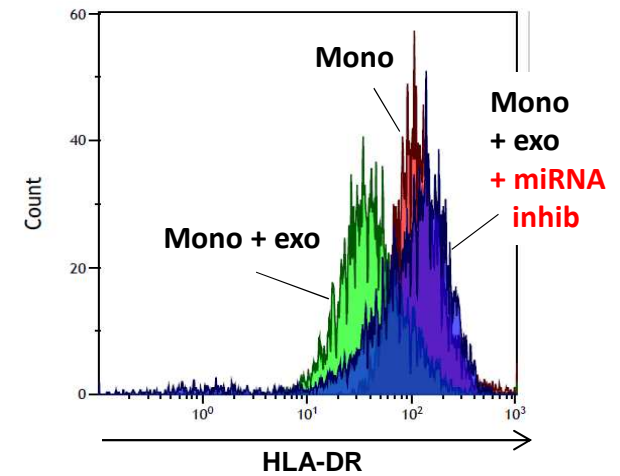
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Protein level

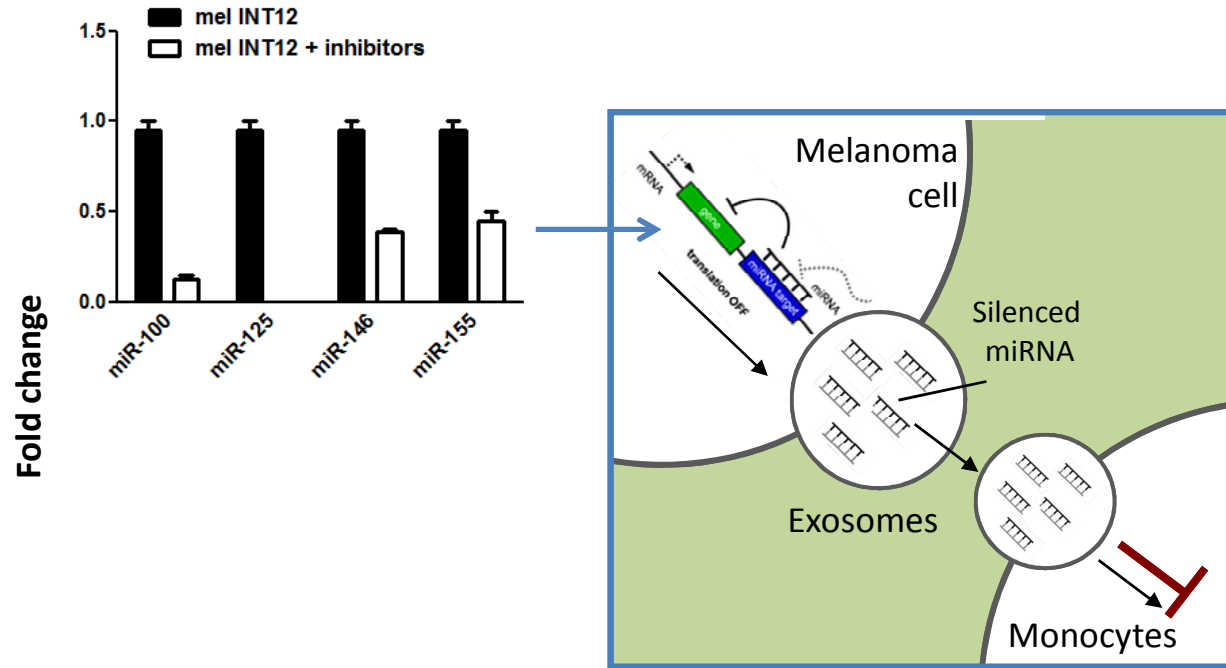
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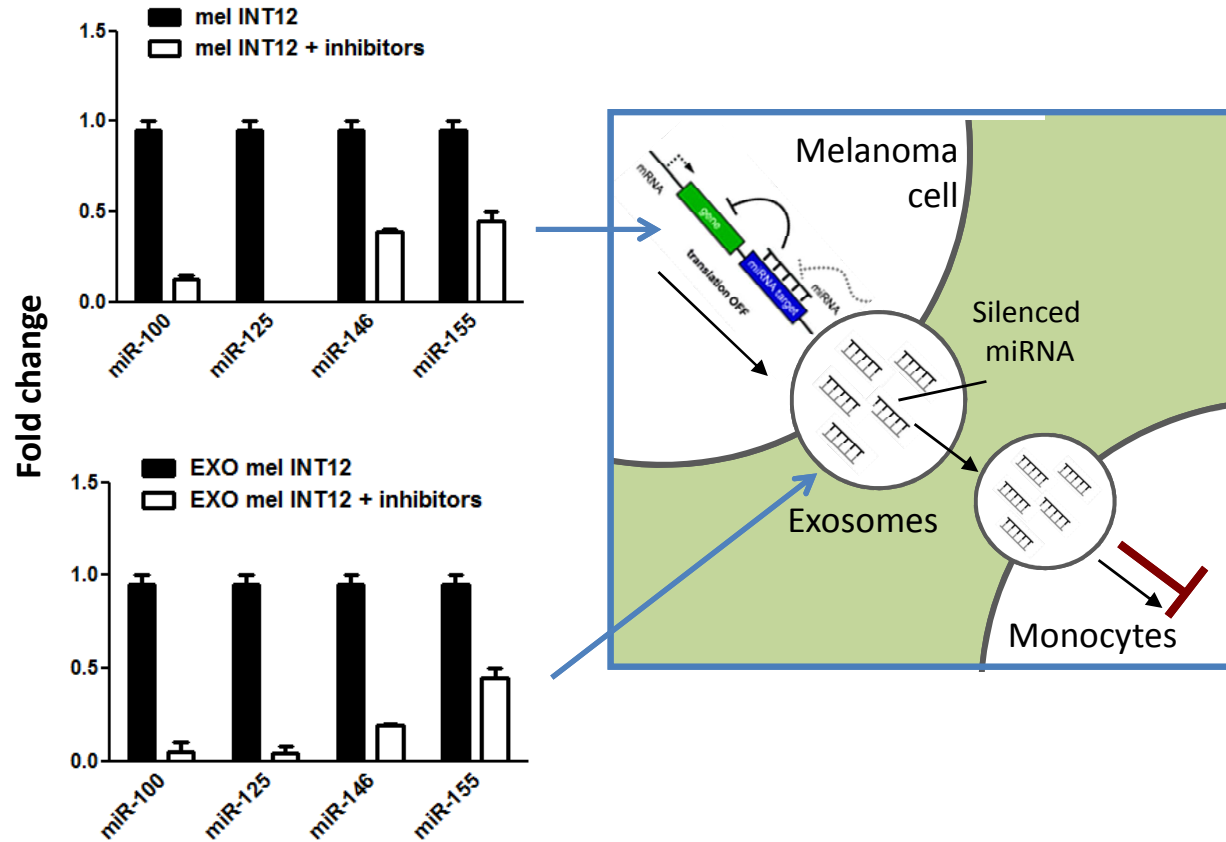
FACS



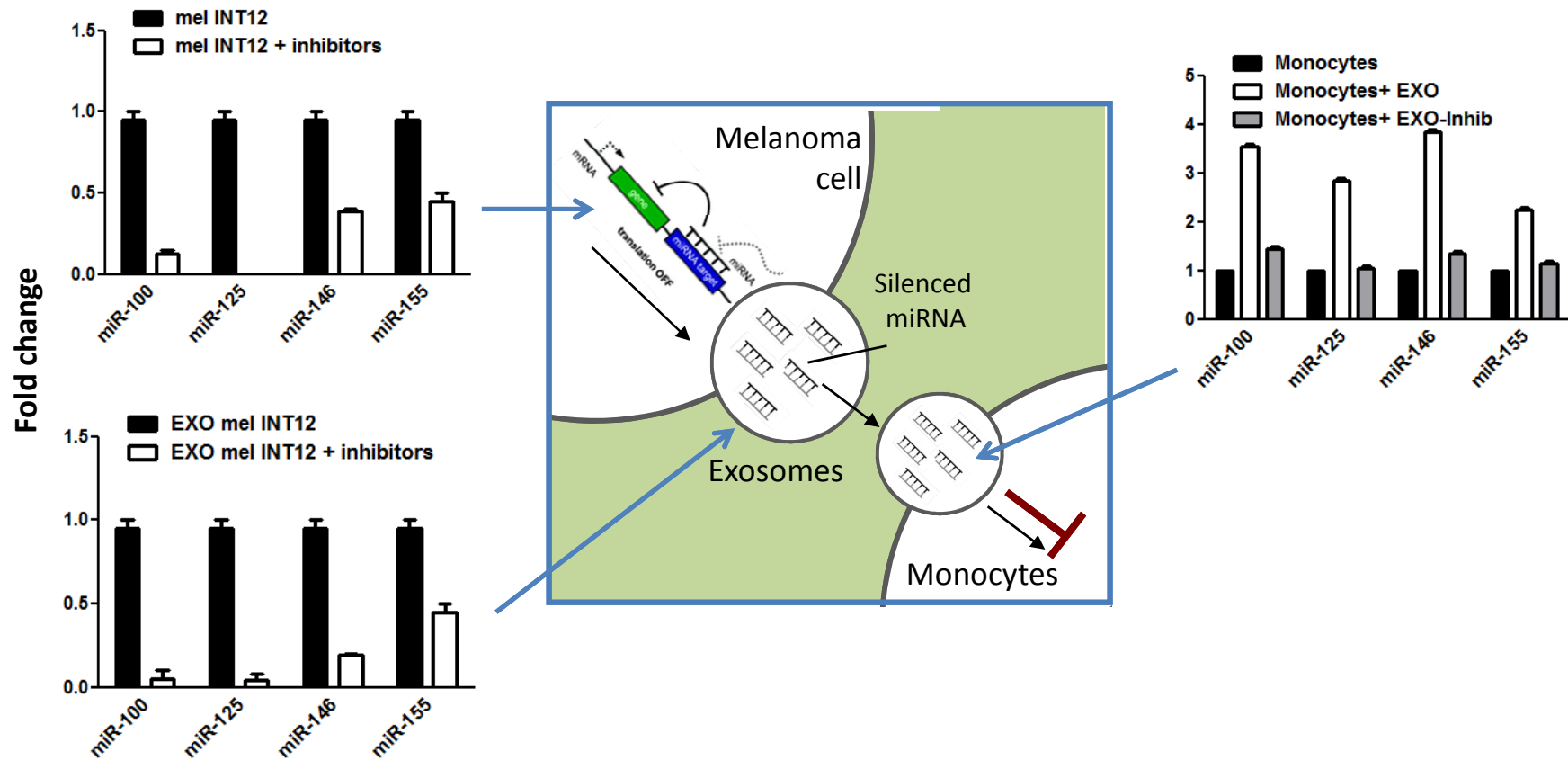
Role of miRNA transfer by exosomes in MDSC conversion



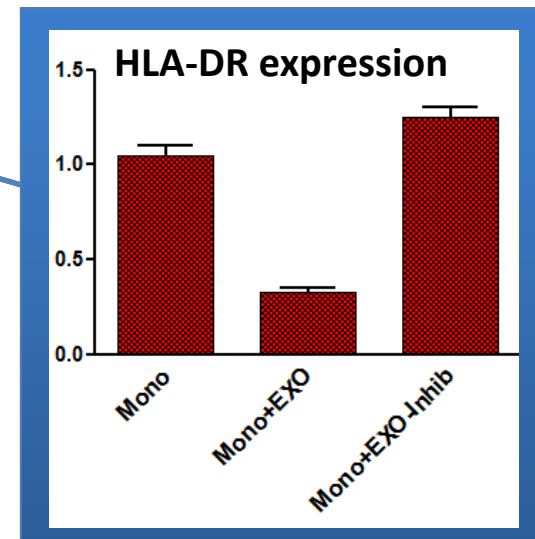
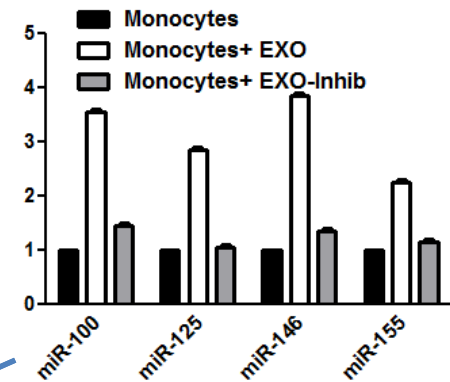
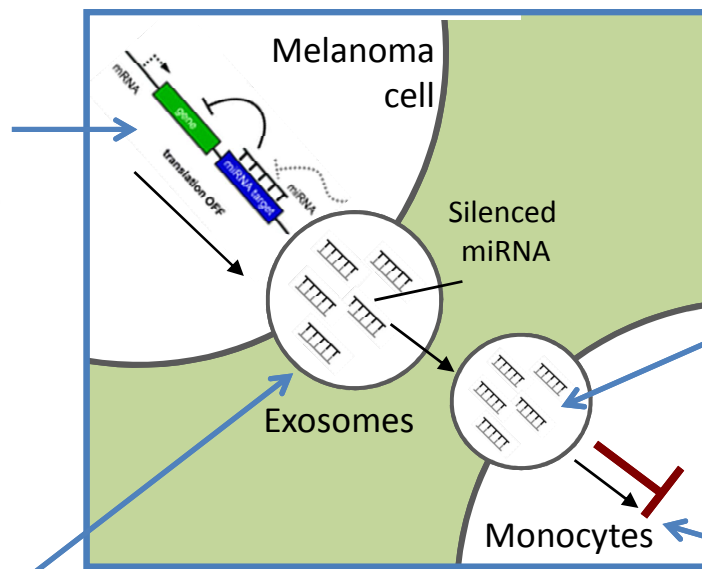
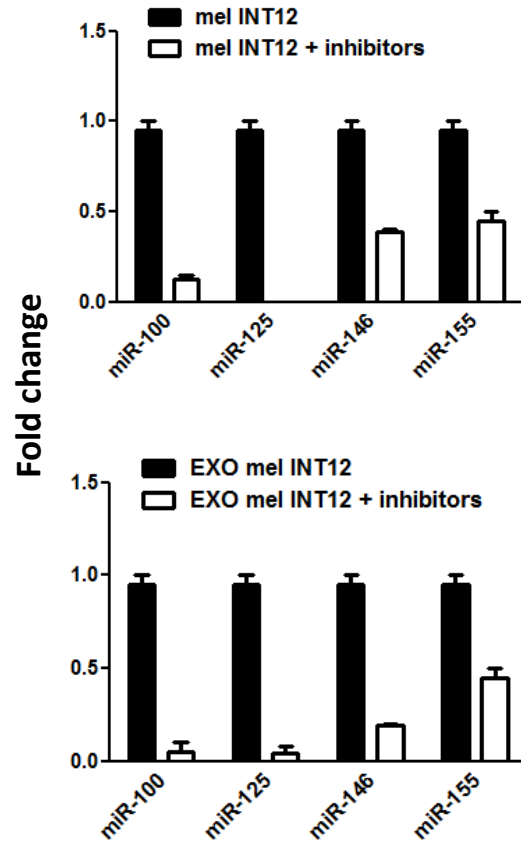
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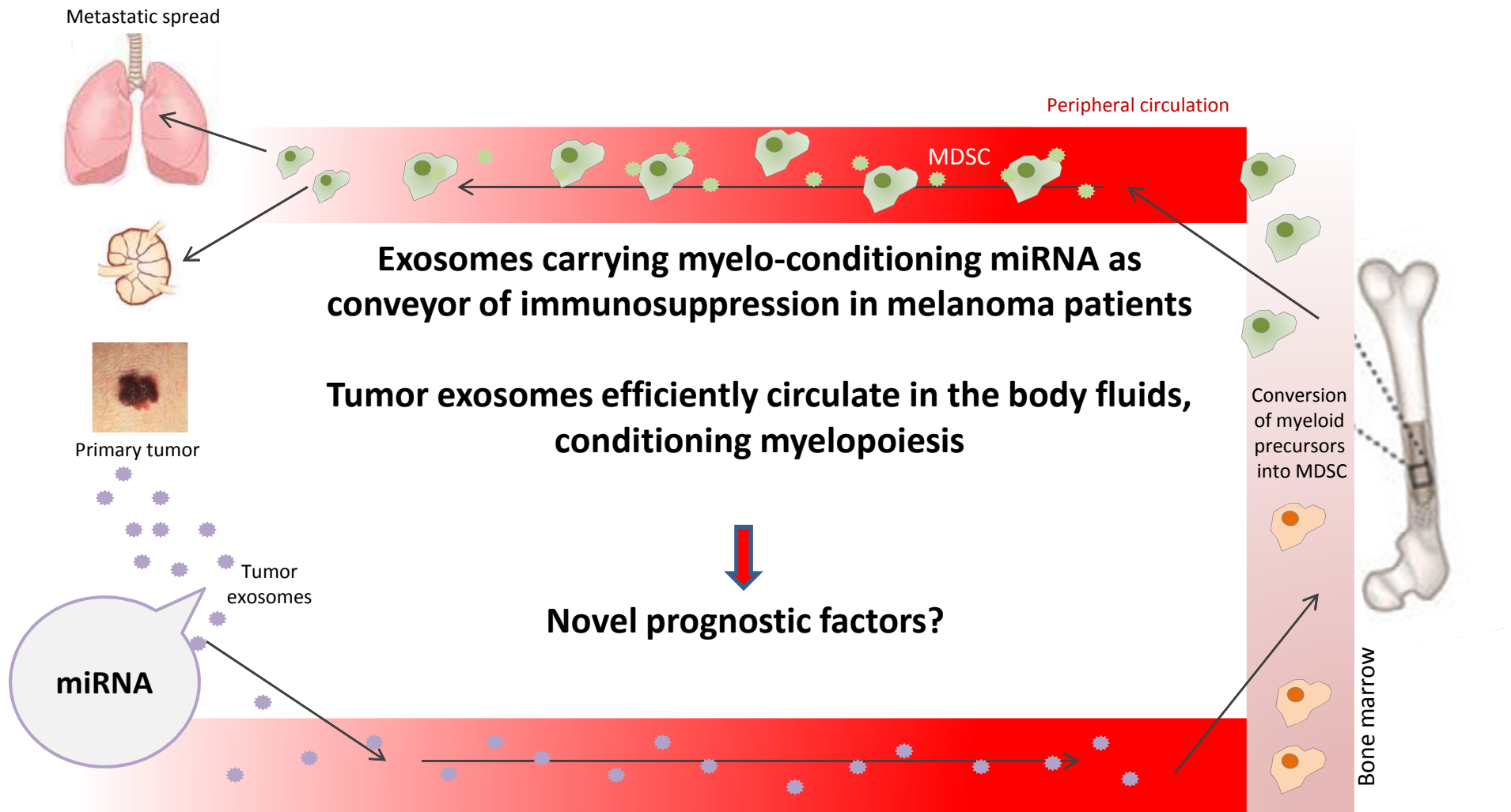
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Role of miRNA transfer by exosomes in MDSC conversion



Melanoma patients

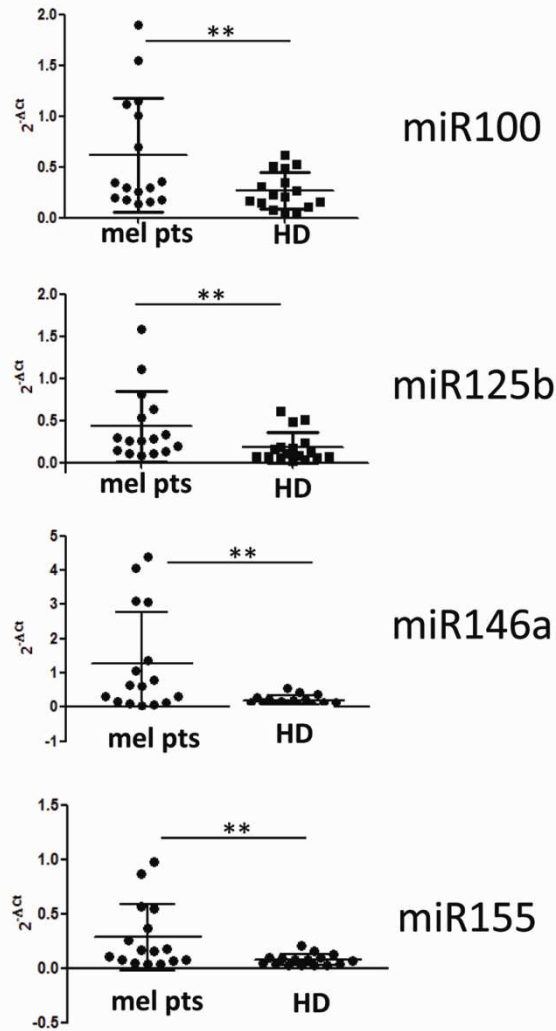


EXO-MDSC miRNA signature can be detected in peripheral blood monocytes and biopsies of melanoma patients

CD14⁺ monocytes sorted from PBMC



HD	Mel pts
16	16

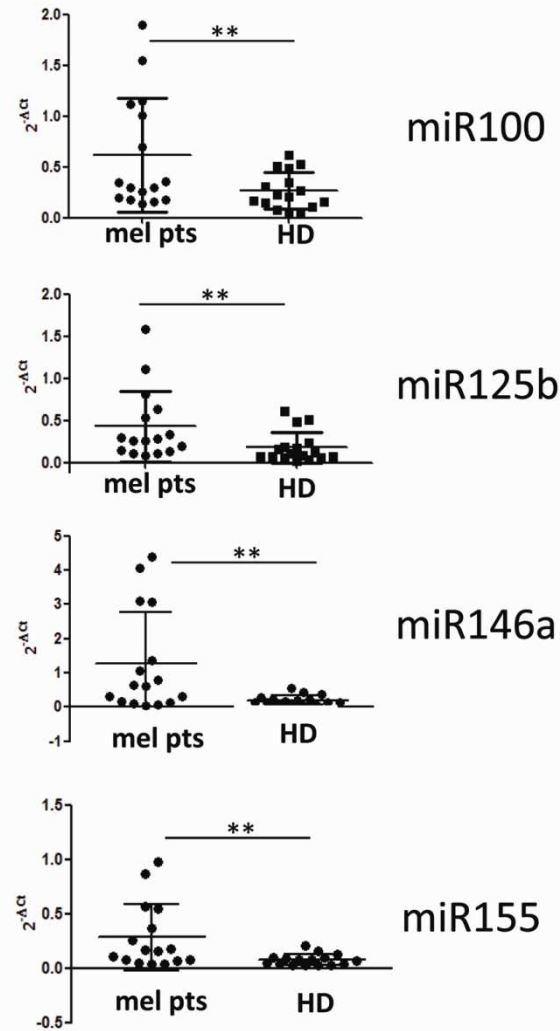


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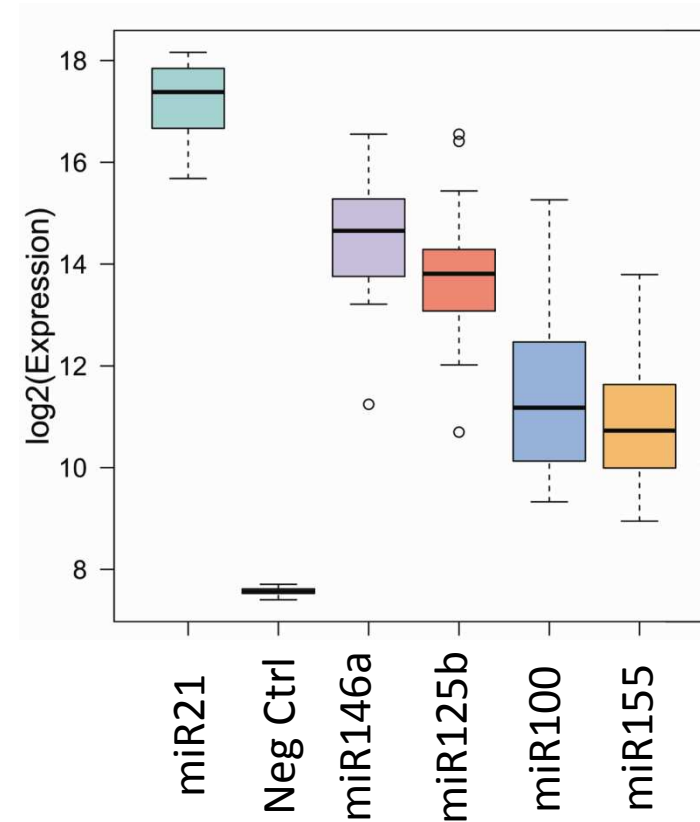
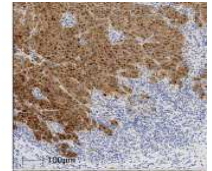
CD14+ monocytes sorted from PBMC



HD	Mel pts
16	16



Melanoma biopsies (stage IIIc-IV)



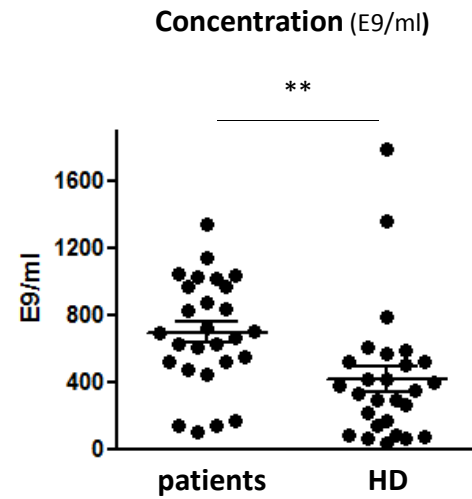
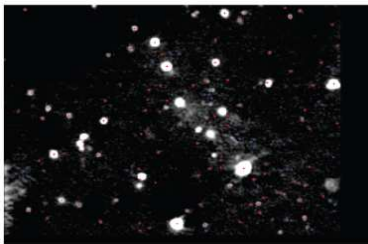
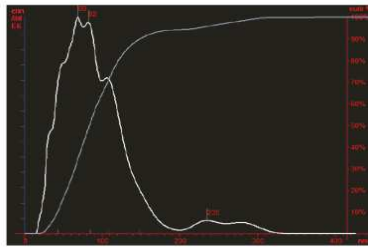
Mel pts

23

EV in whole plasma and MDSC-related miRNAs in EV of melanoma patients (stage IV)

HD	Mel pts
27	27

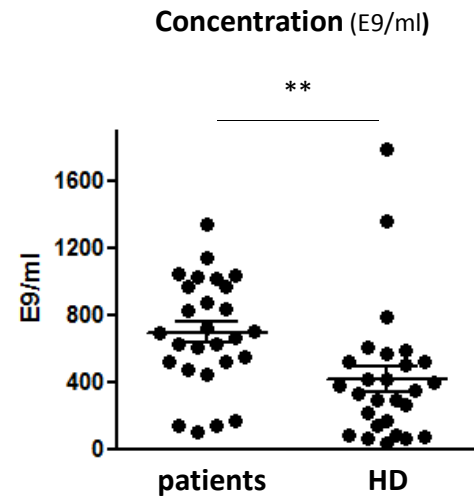
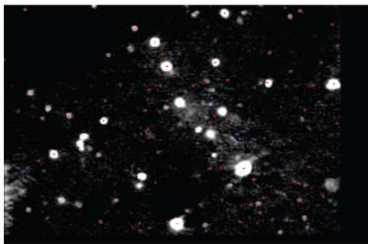
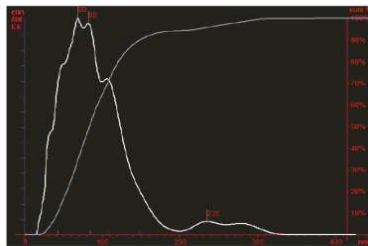
Whole PLASMA



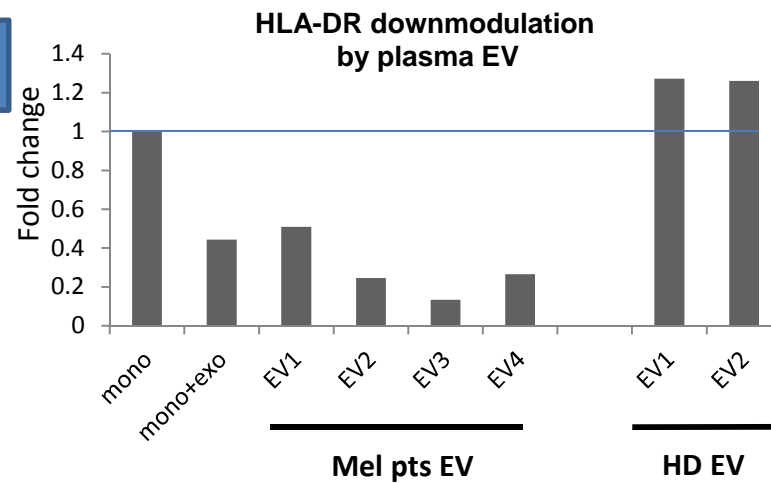
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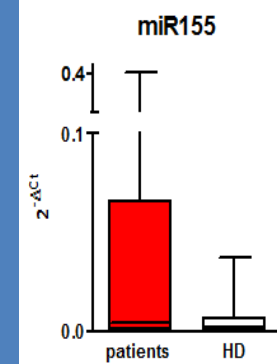
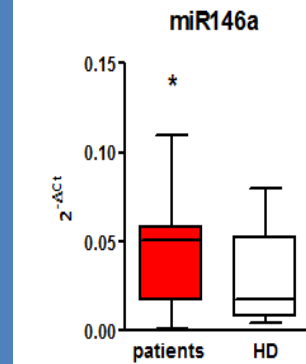
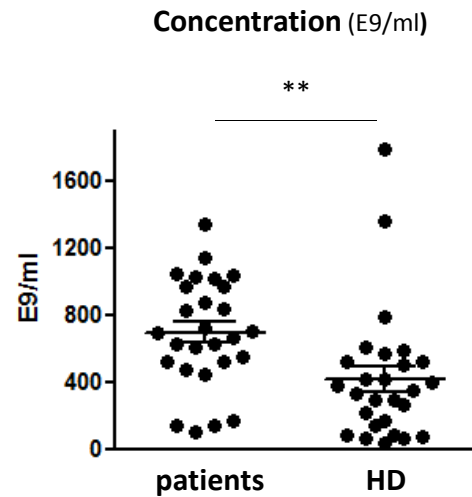
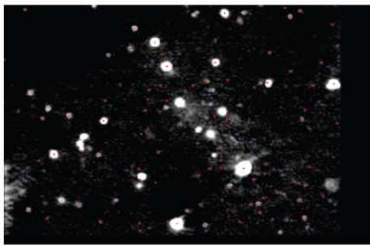
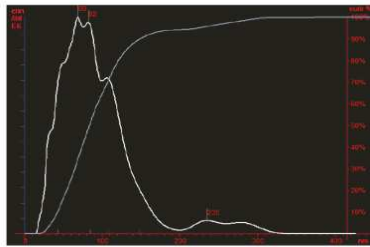
EV



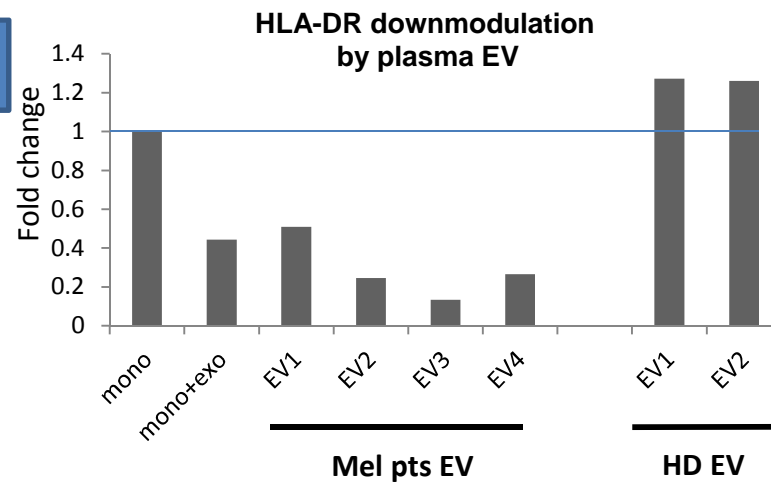
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Whole PLASMA



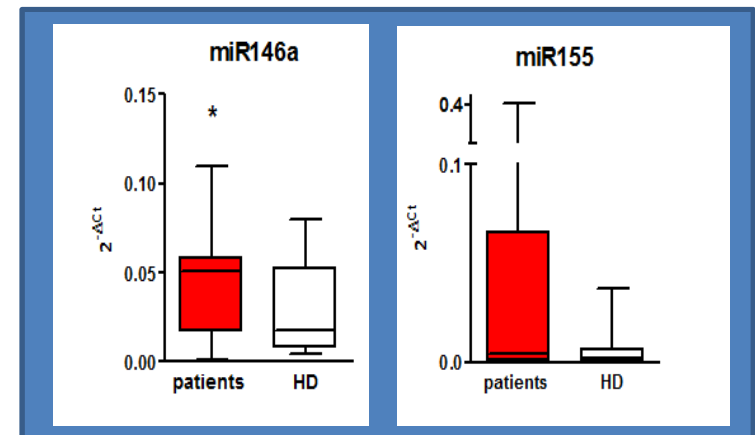
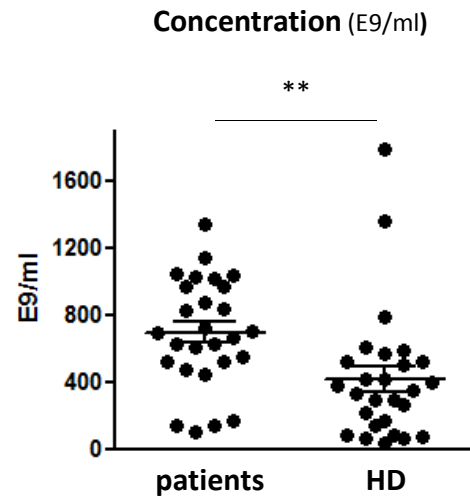
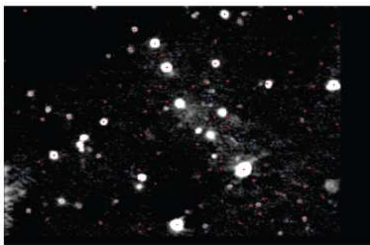
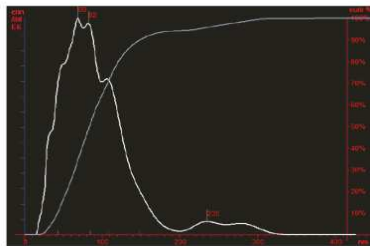
EV



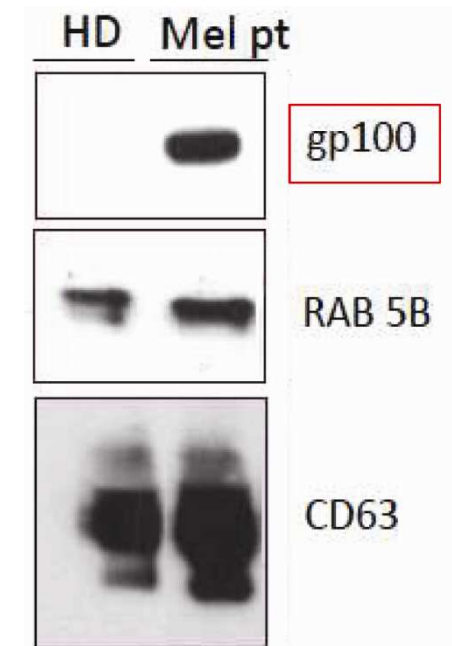
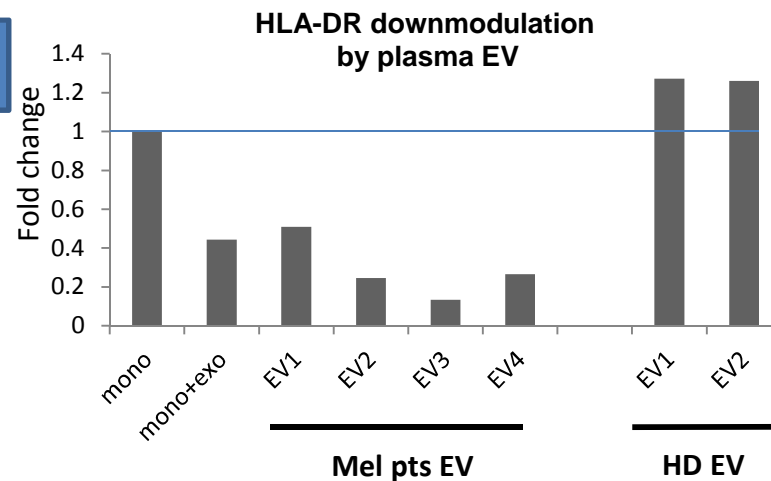
EV in whole plasma and MDSC-related miRNAs in EV of melanoma patients (stage IV)

HD	Mel pts
27	27

Whole PLASMA



EV



Lessons and Take Home Messages

Key points and lessons learned:

- Tumor exosomes induce MDSC conversion *in vitro* by transferring myeloid-conditioning miRNA to CD14+ monocytes
- miRNA 100, 125b, 146a and 155 are involved in down-modulating HLA-DR expression and inducing IL6 and CCL2 secretion, thus suggesting a role in the generation of immunosuppressive and pro-tumorigenic myeloid cells
- Clear signs of the occurrence of this pathway *in vivo* in melanoma patients are detected in blood CD14+ cells, tumor lesions and plasma

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Potential impact on the field:

Since MDSC accumulate in peripheral blood of melanoma patients in association with disease progression, and plasma contains high level of tumor exosomes, the identification of exosomes and myelo-conditioning miRNA, paves the way to the development of **novel immune-based therapeutic strategies** and **prognostic/disease course biomarkers** in cancer patients

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