

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

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**FONDAZIONE IRCCS
ISTITUTO NAZIONALE
DEI TUMORI**



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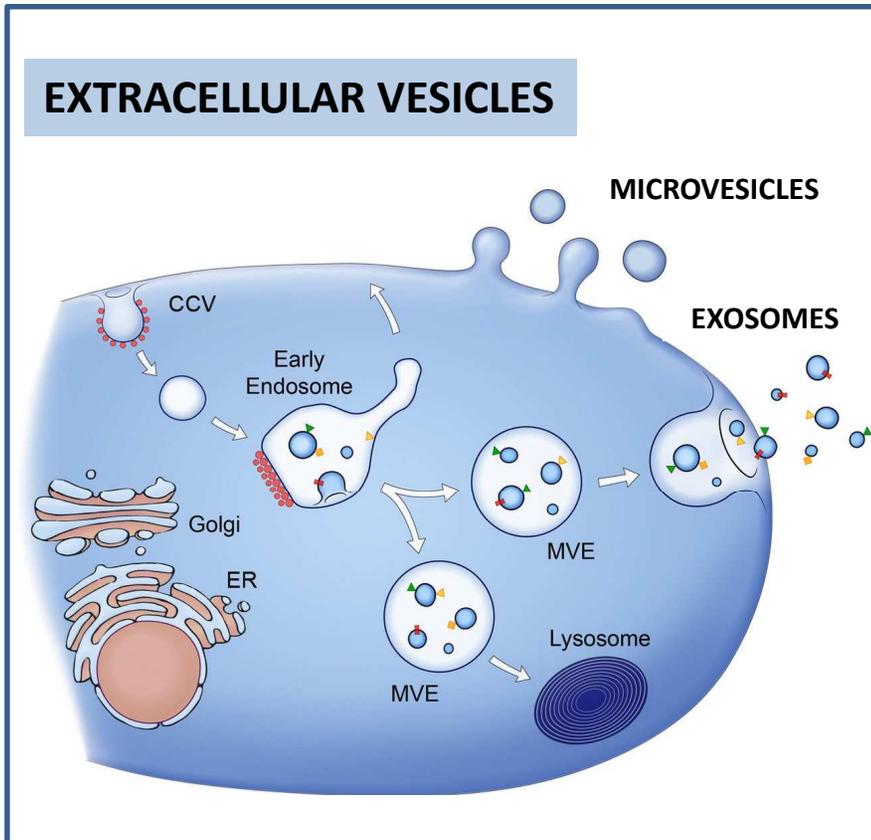
Presenter disclosure Information

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:

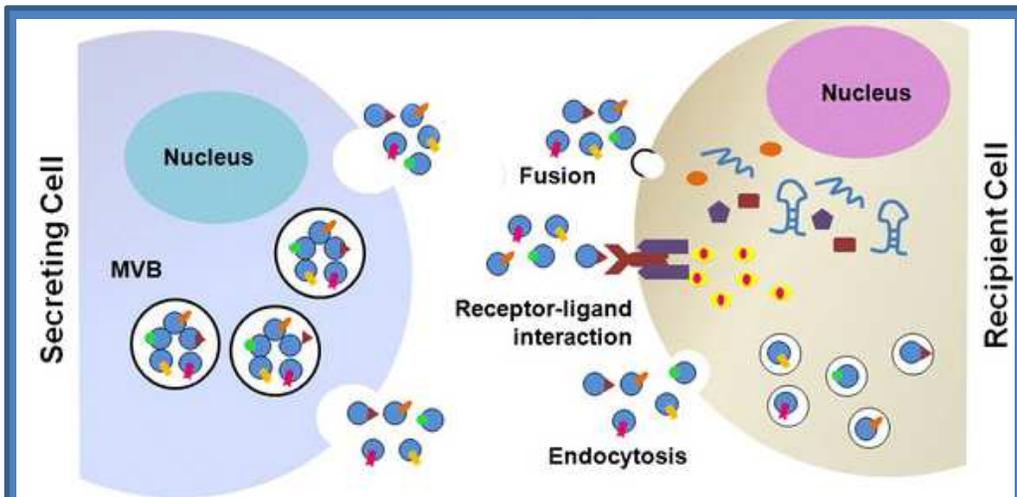
- **apoptotic bodies** (1000-5000nm)
- **microvesicles** MV (200-1000nm) formed by blebbing of cellular membrane
- **exosomes** (30-150nm)

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

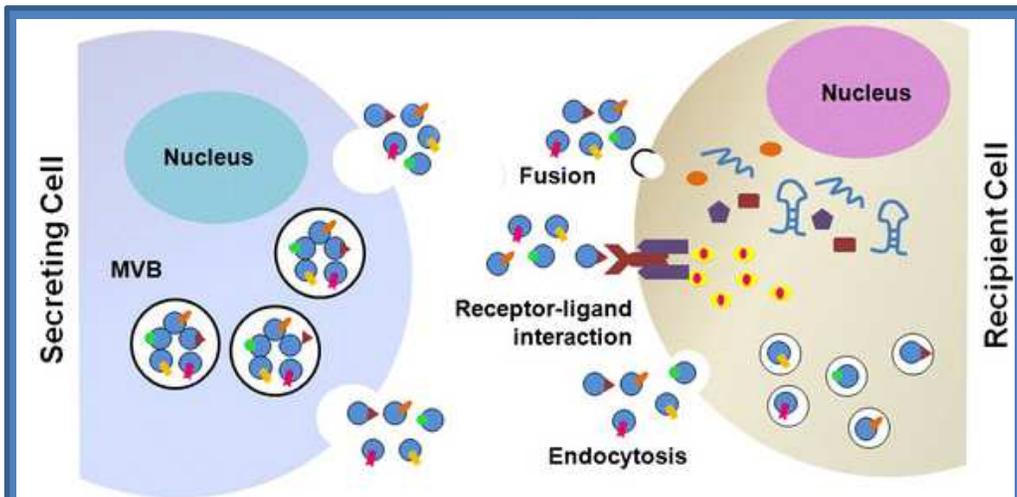
Central role in INTERcellular cross-talk



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

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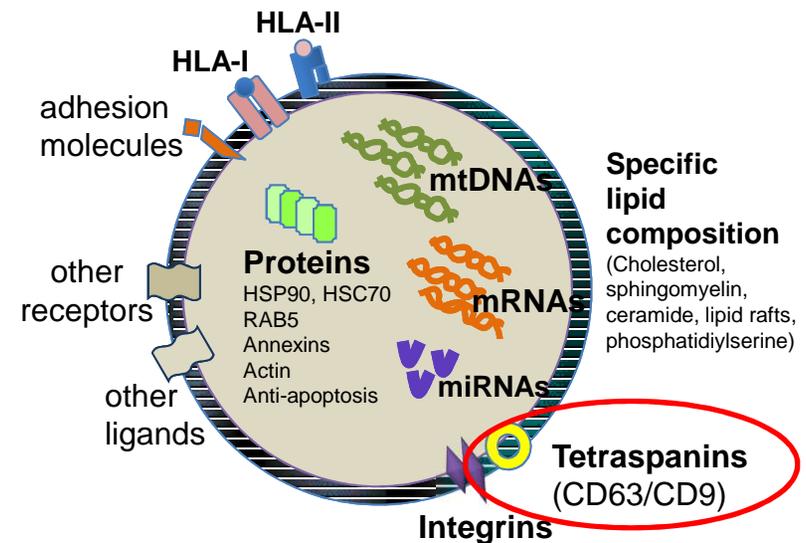
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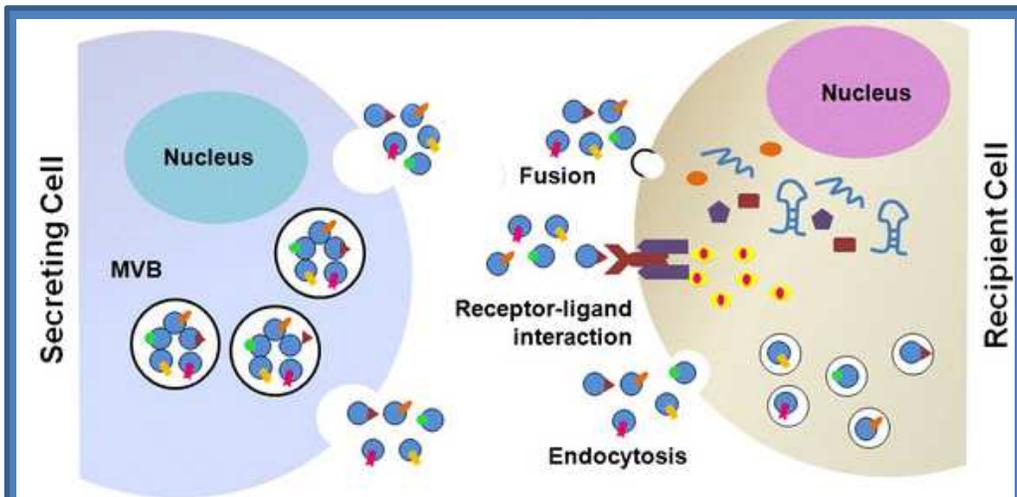
They transfer **proteins**, **lipids**, **DNA**, **mRNA** and **miRNA** in a functionally-active form, locally and systemically. They can condition **myelopoiesis** and regulate gene-expression in recipient cell determining their behavior.

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



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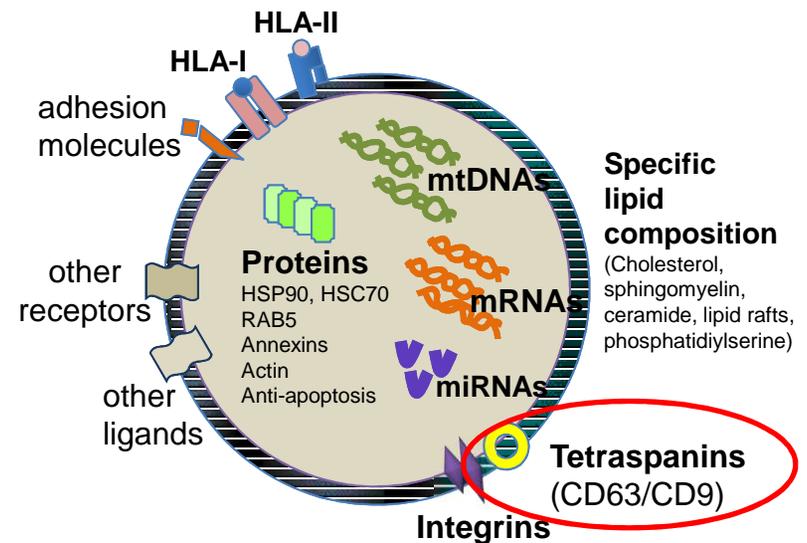


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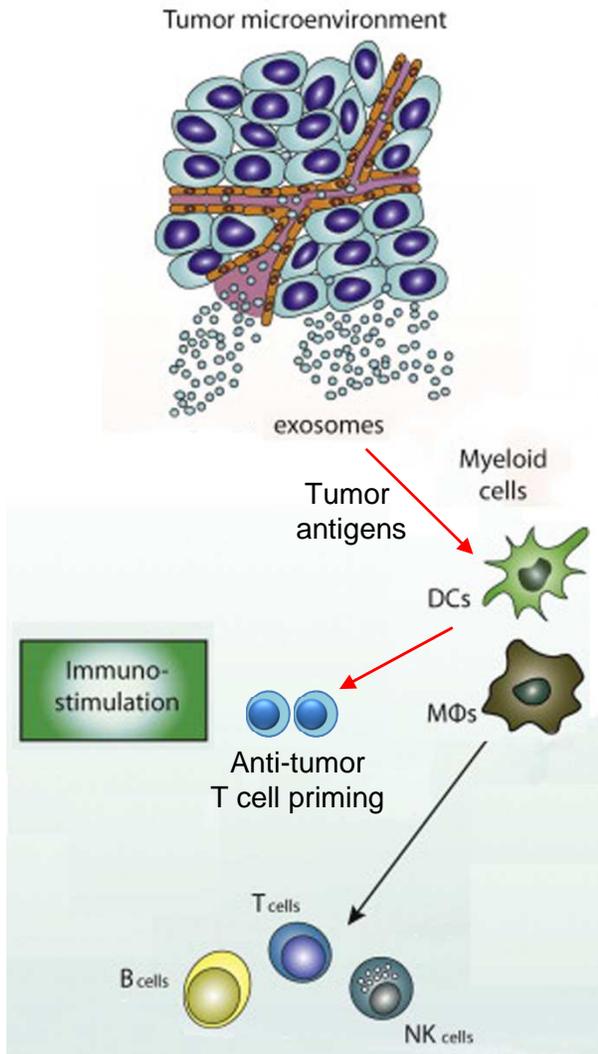
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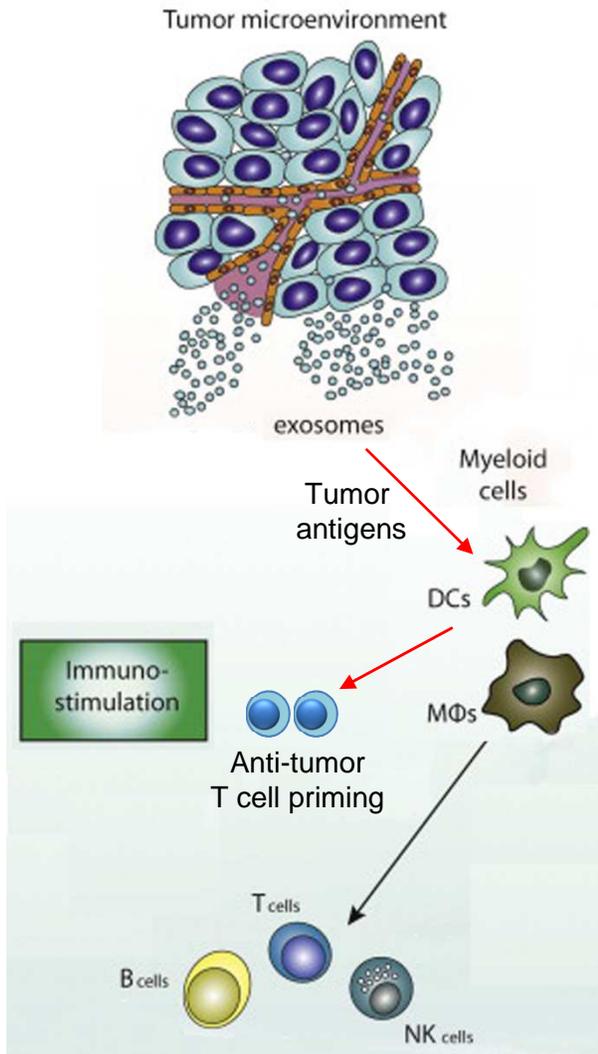
Exosomes are augmented in CANCER PATIENTS compared to HD and express higher levels of miRNA



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



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Modulation of tumor microenvironment

Angiogenesis

Multi-drug resistance

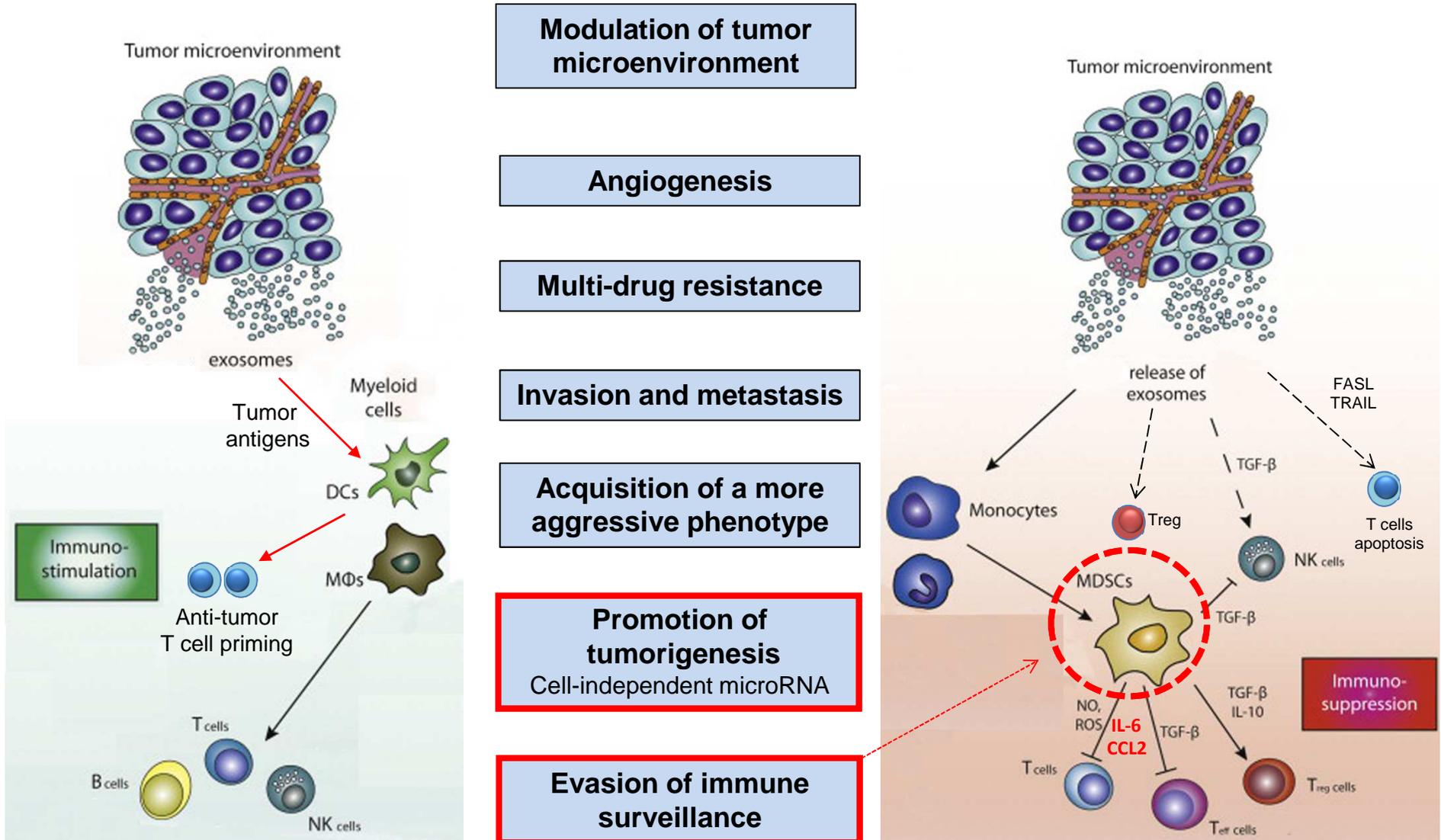
Invasion and metastasis

Acquisition of a more aggressive phenotype

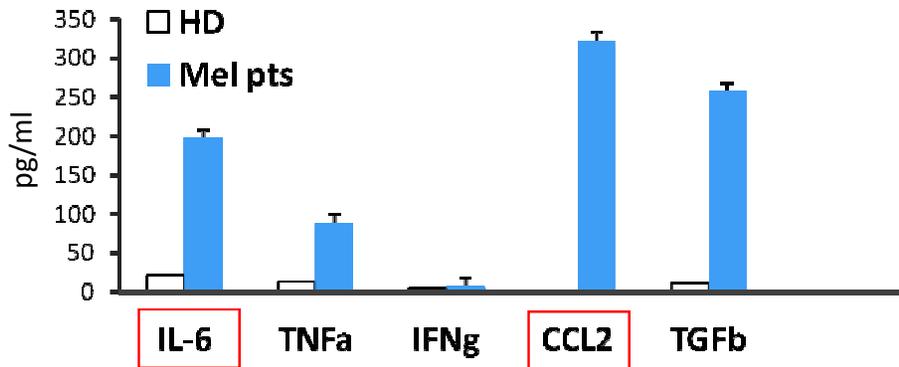
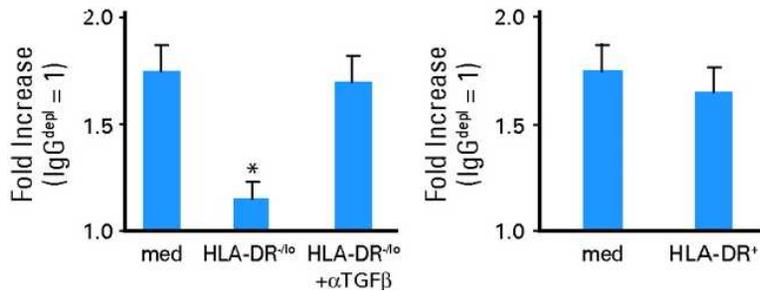
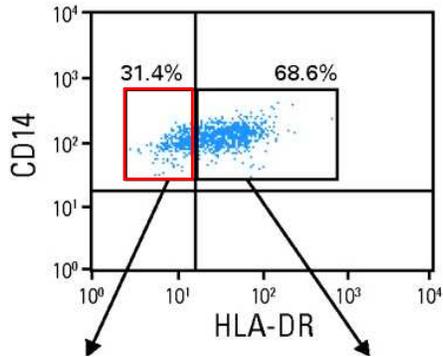
Promotion of tumorigenesis
Cell-independent microRNA

Evasion of immune surveillance

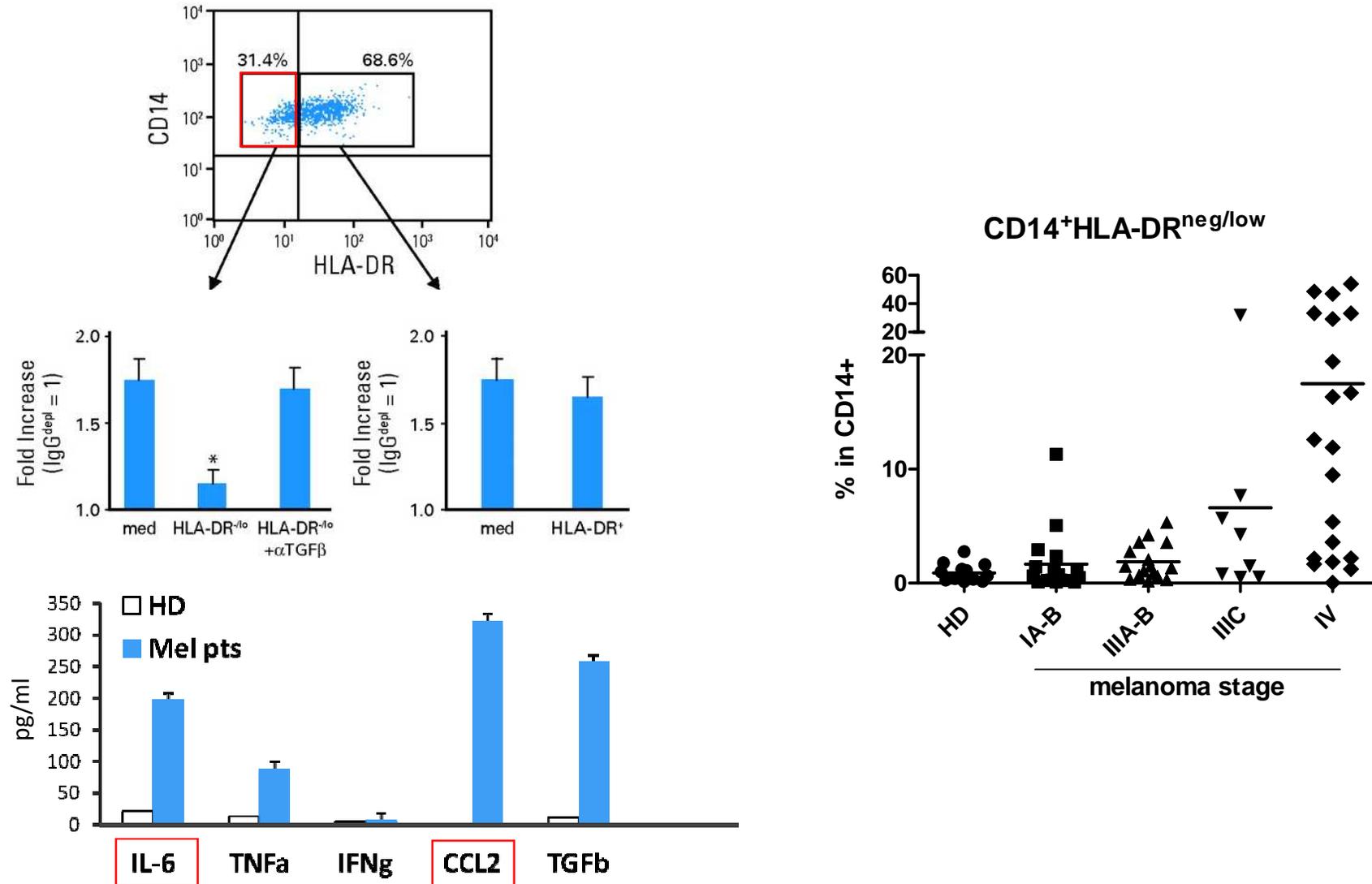
Multiple role of tumor exosomes in cancer, immune stimulation or immune suppression



Immunosuppressive activity of monocytes from melanoma patients and MDSC accumulation

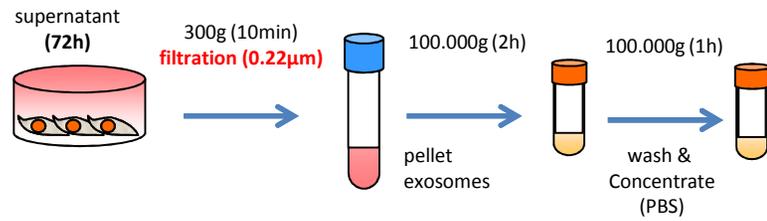


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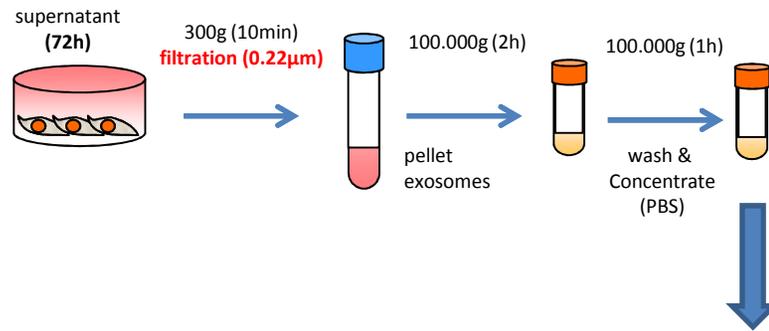
Are exosomes involved in MDSC differentiation? *in vitro* model

purification

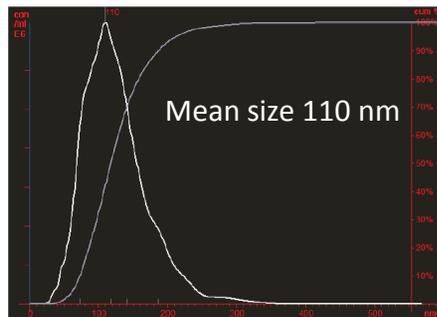


Are exosomes involved in MDSC differentiation? *in vitro* model

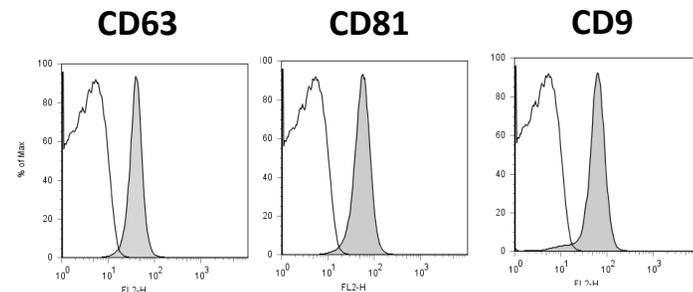
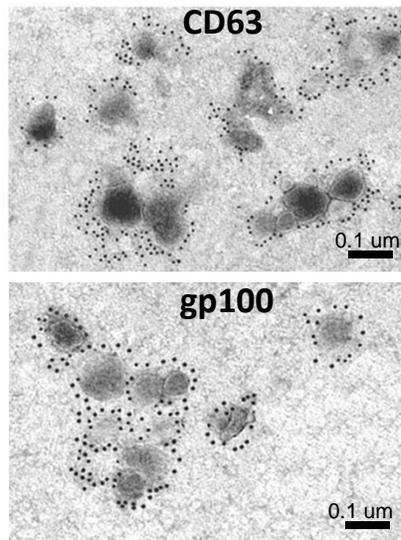
purification



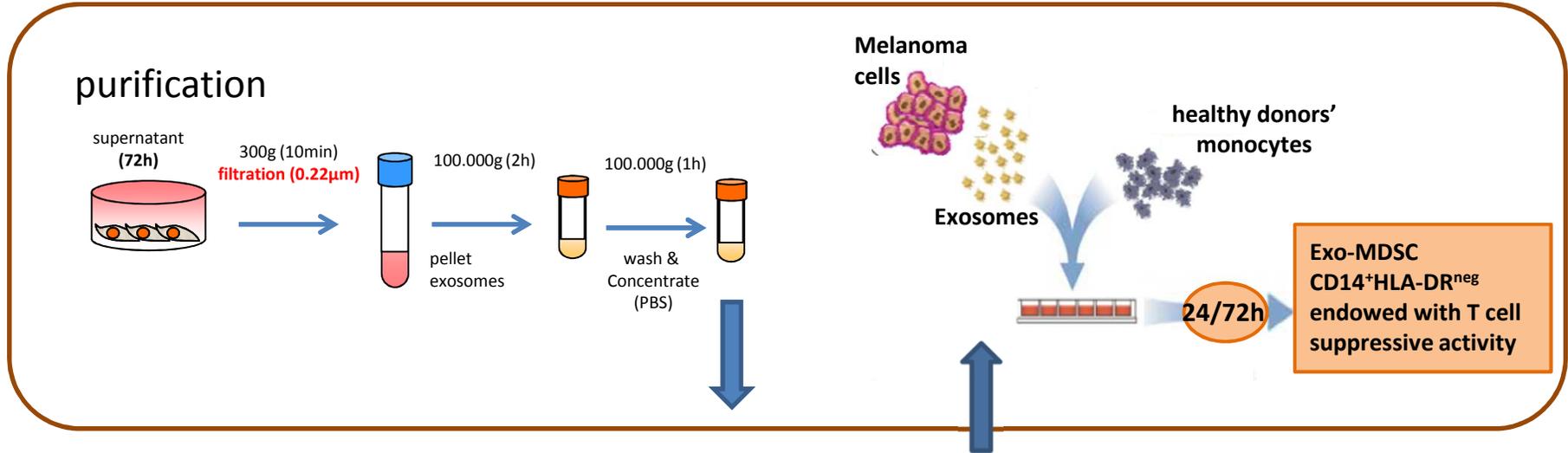
Nanosight



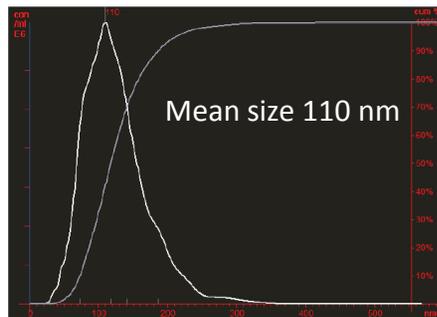
RESULTS:
Size Distribution: Mean: 125 nm, Mode: 110 nm, SD: 46 nm
Cumulative Data (nm): D10: 73, D50: 118, D90: 186, D70: 141
User Lines: 0 nm, 0 nm
Total Concentration: 47.11 particles / frame, 7.06E8 particles / ml



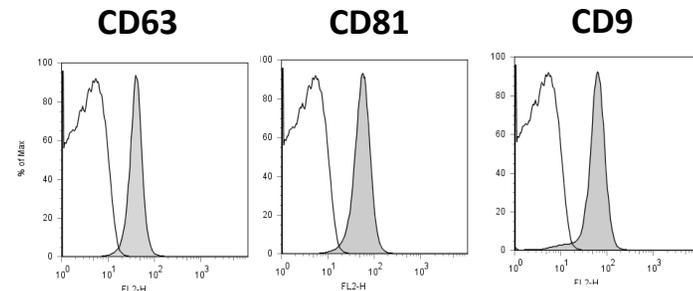
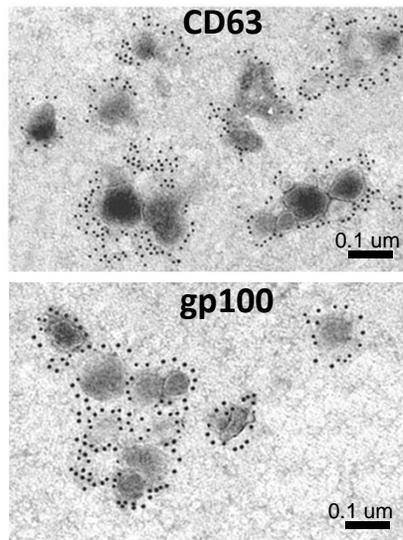
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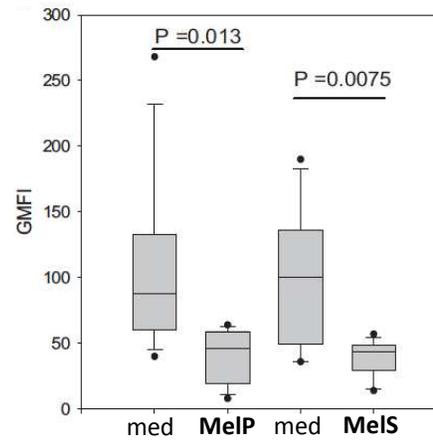
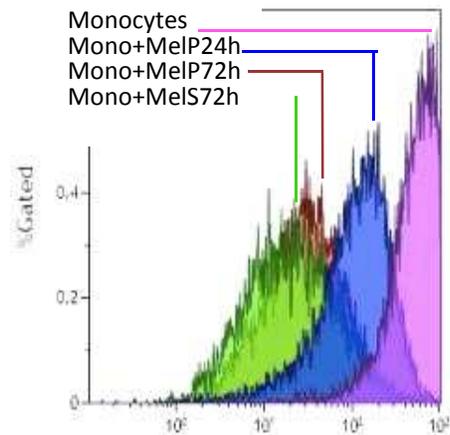


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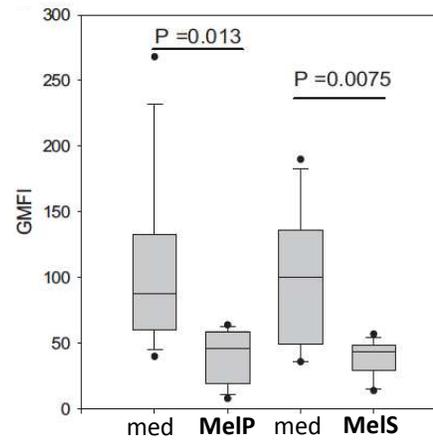
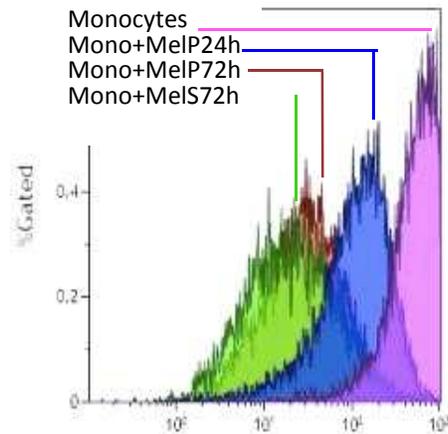
Melanoma exosomes induce MDSC *in vitro*

HLA-DR down-modulation

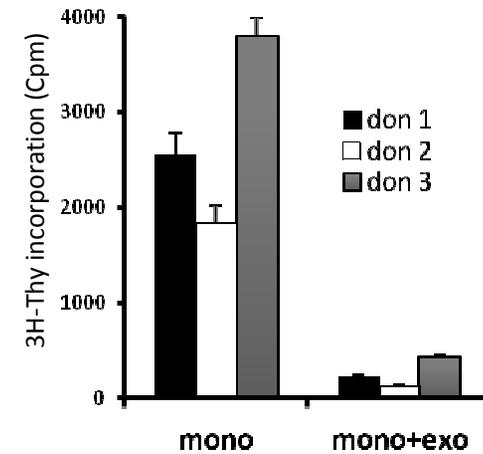


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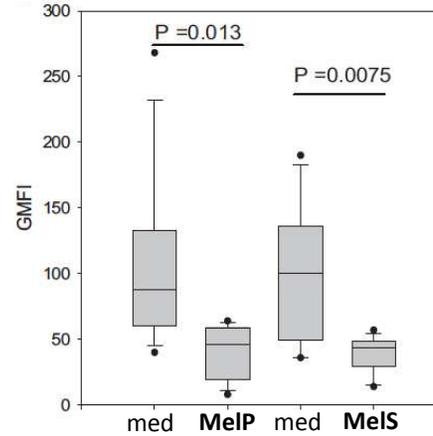
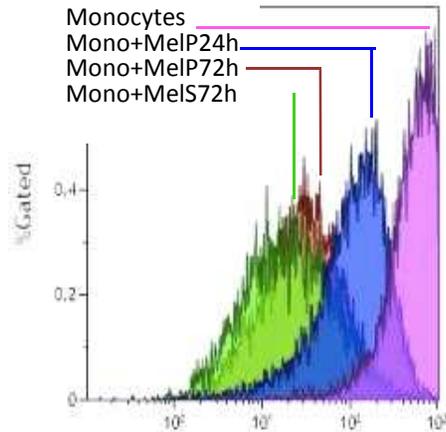


Inhibition of T cell proliferation



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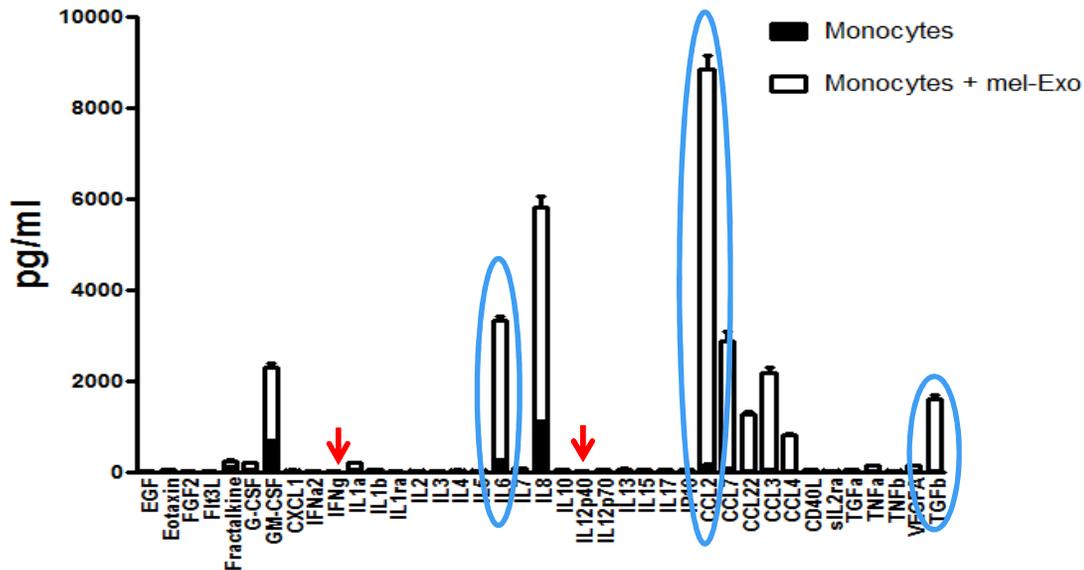
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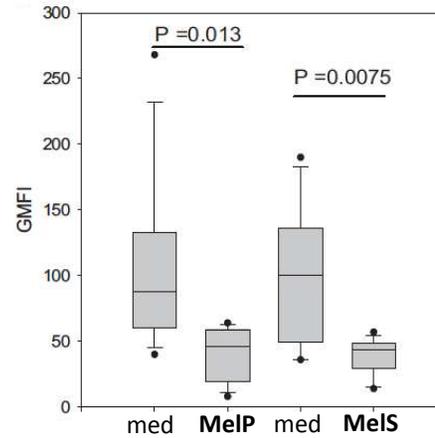
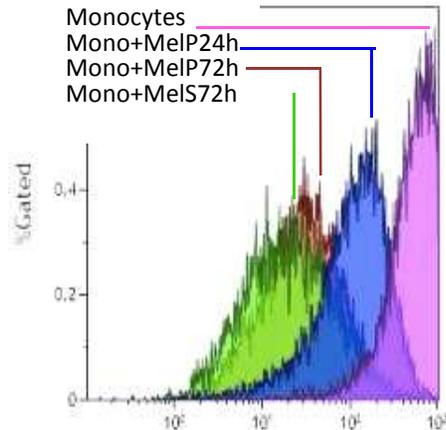


Release of immunosuppressive/proinflammatory cytokines and chemokines

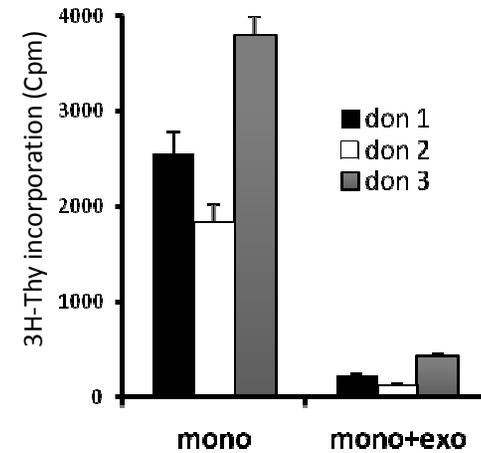


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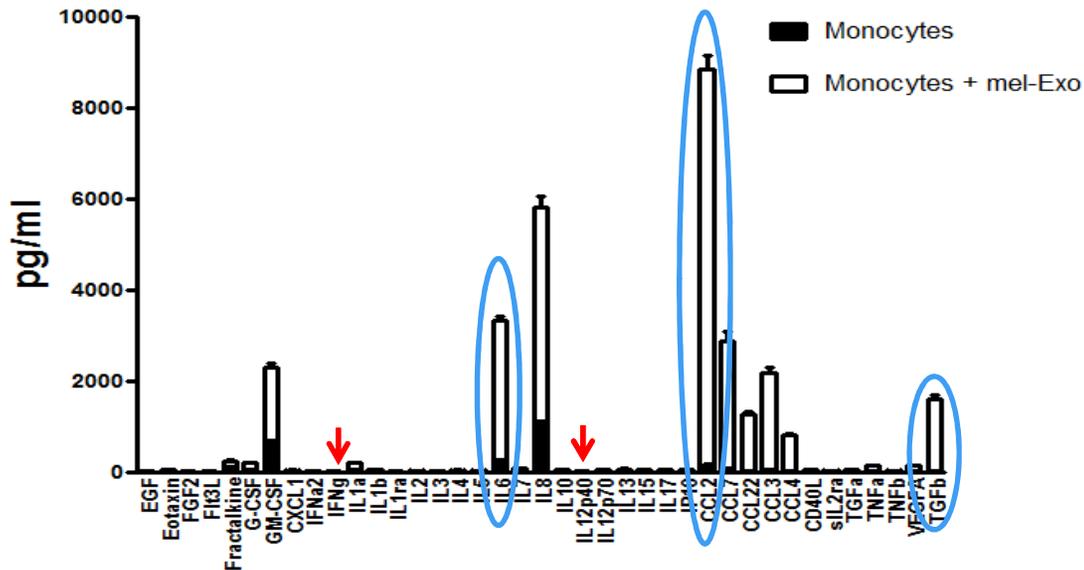
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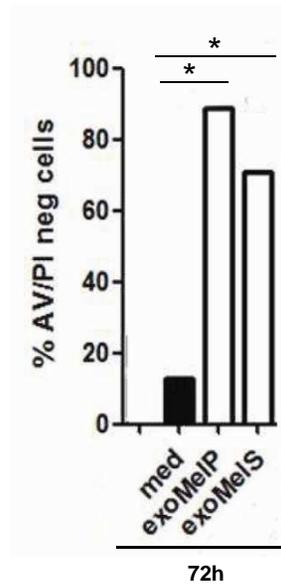
Inhibition of T cell proliferation



Release of immunosuppressive/proinflammatory cytokines and chemokines



Prolonged survival





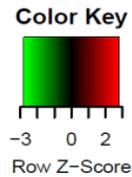
Which are the mechanisms involved in this process?

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

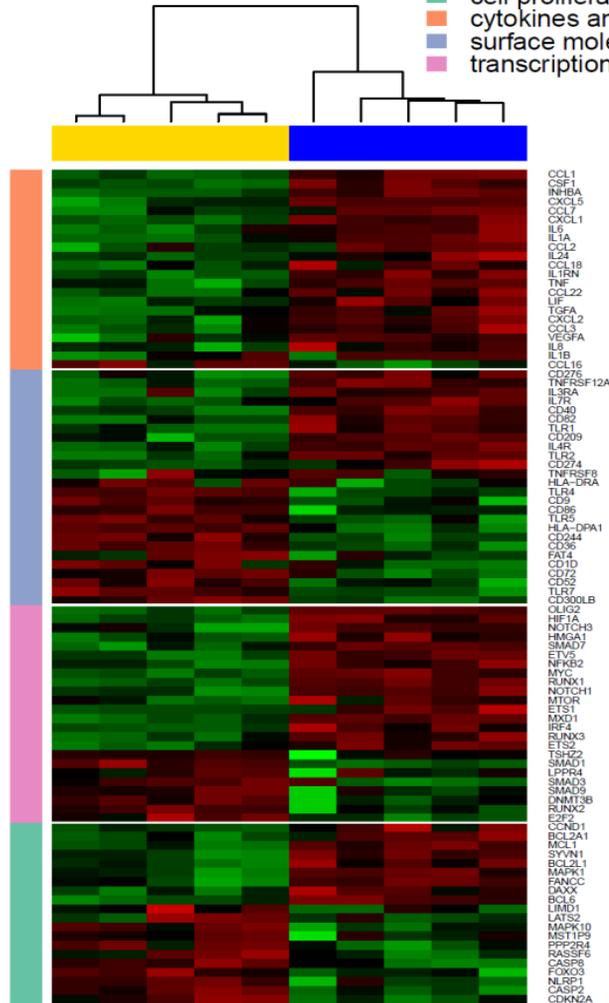


gene expression and miRNA profiling

Transcriptional profile of Exo-MDSC

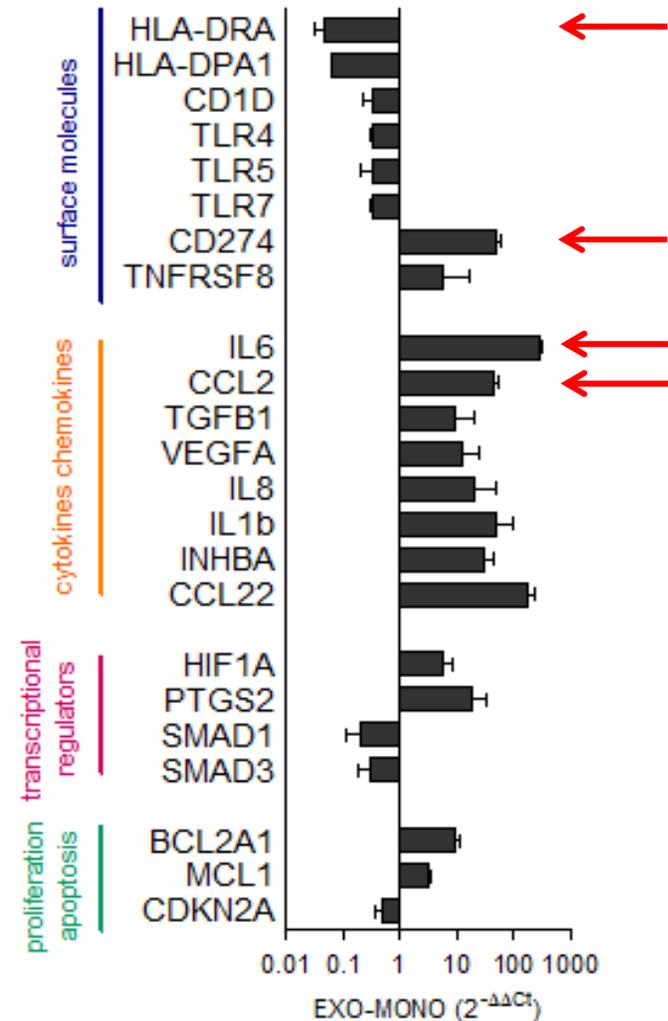


- monocytes
- monocytes + exo-Me
- cell proliferation and apoptosis
- cytokines and chemokines
- surface molecules
- transcriptional regulators

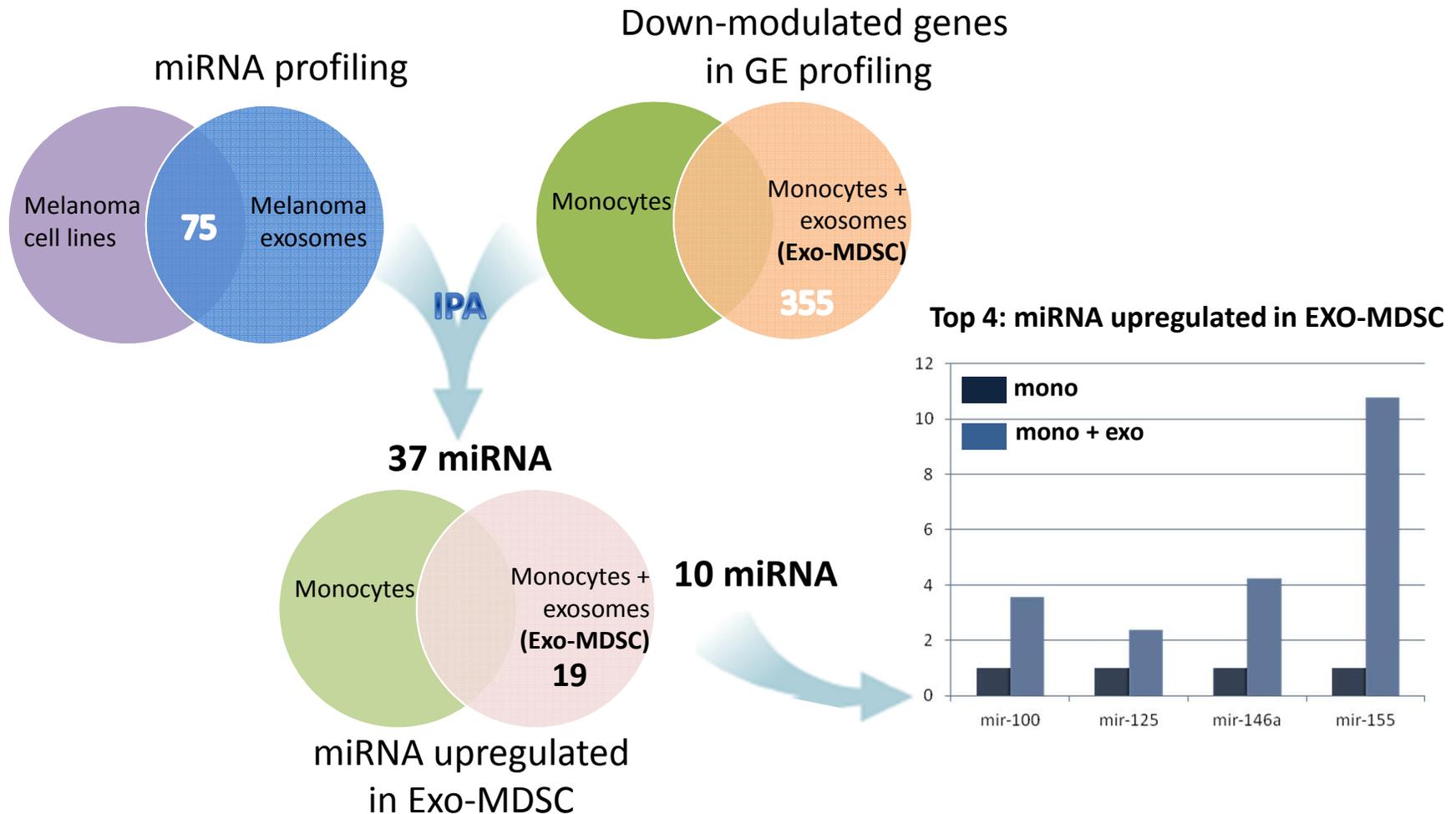


Gene expression (HumanHT-12_V4_WGDASL Illumina). N= 5 HD

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



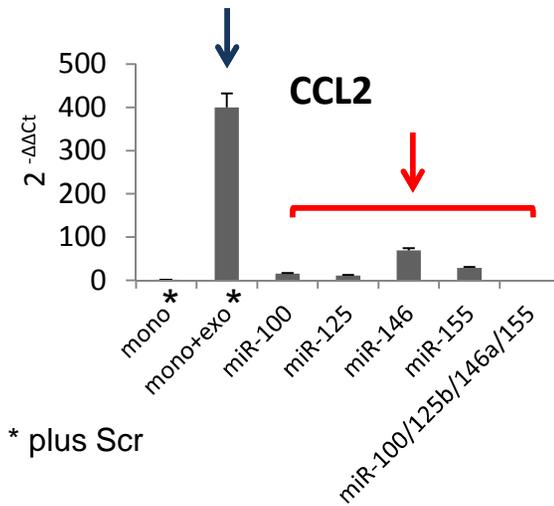
Selection of miRNA contained in tumor exosomes and involved in the generation of exo-MDSC



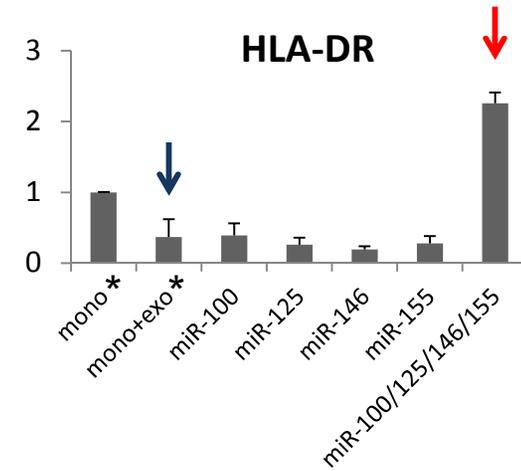
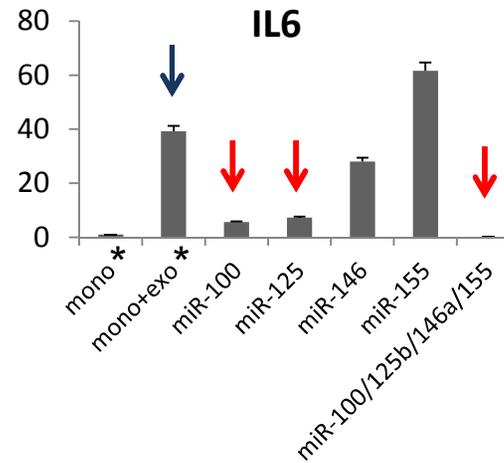
Role of selected miRNA in exosome-mediated MDSC conversion

- effect at miRNA inhibitors* -

antagomir



Transcriptional level

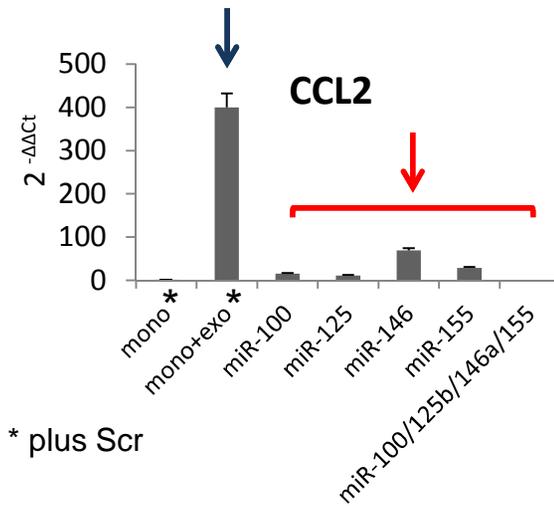


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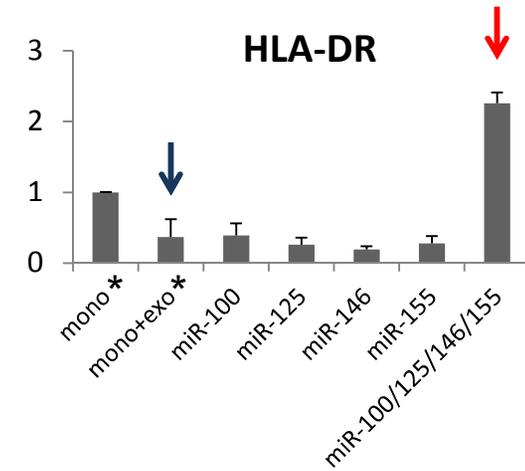
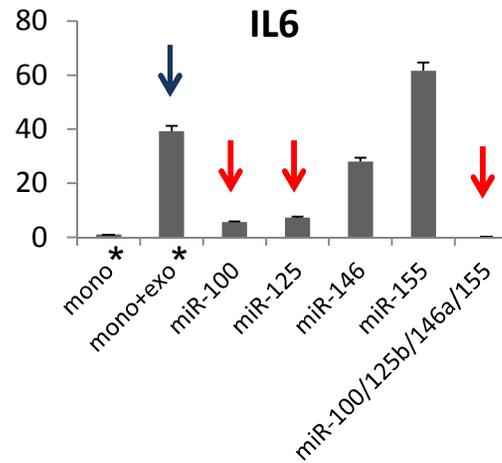
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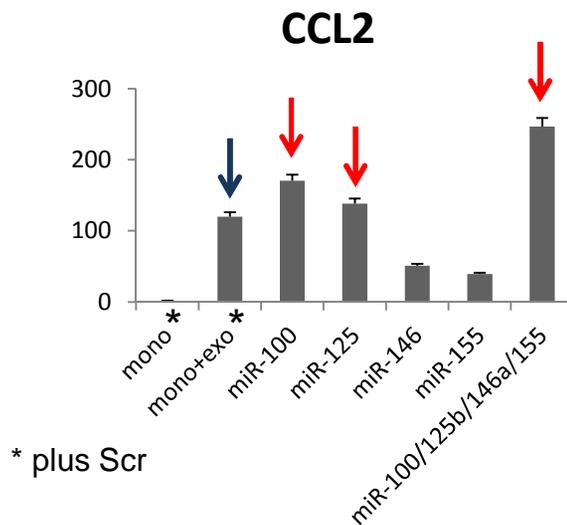
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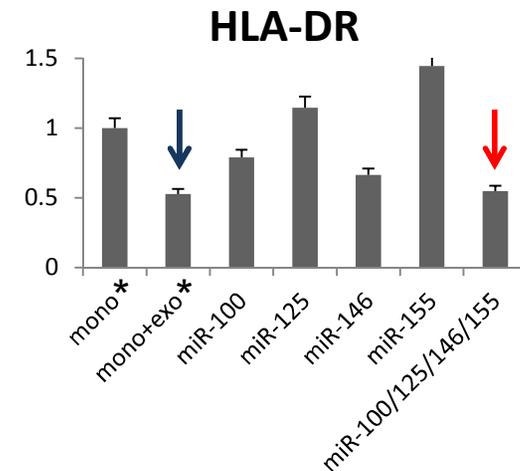
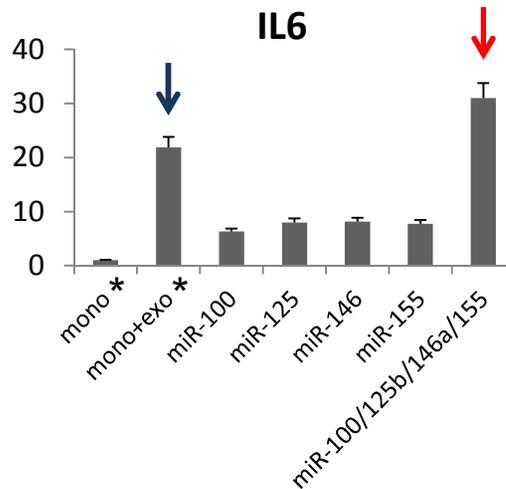
* plus Scr



miRNA mimics



* plus Scr

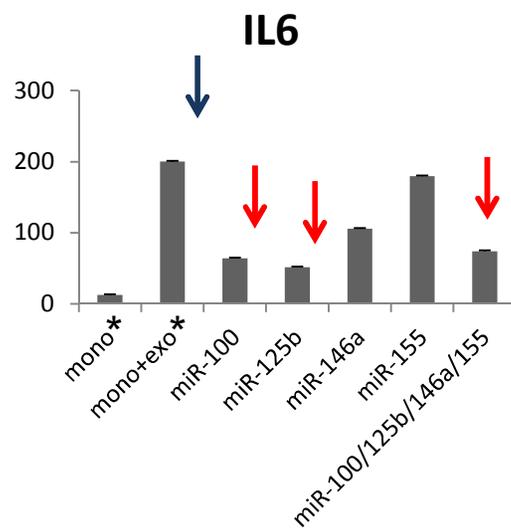
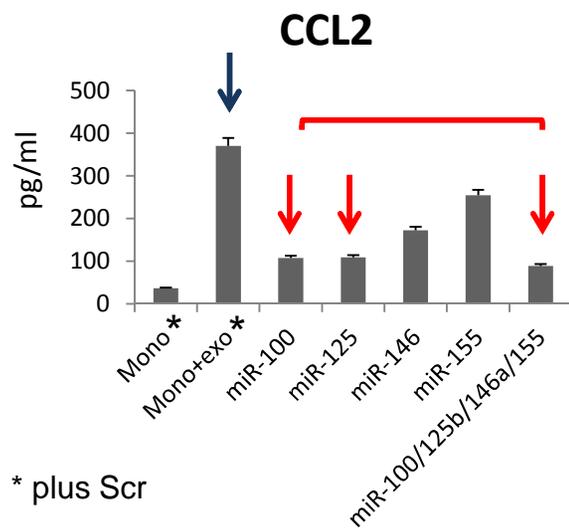


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Protein level

CBA

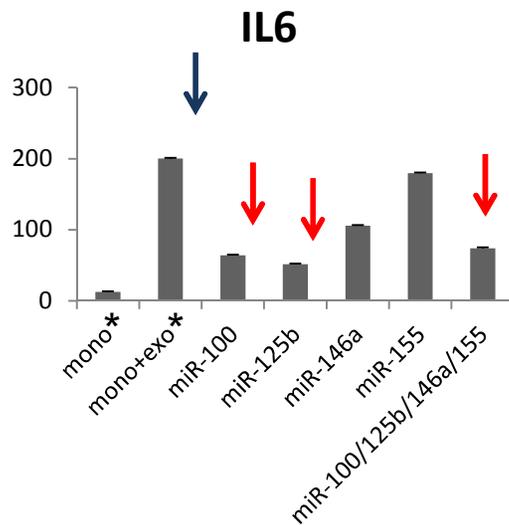
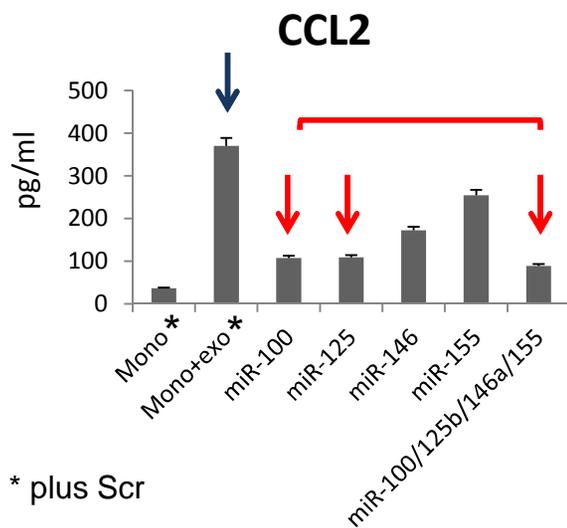


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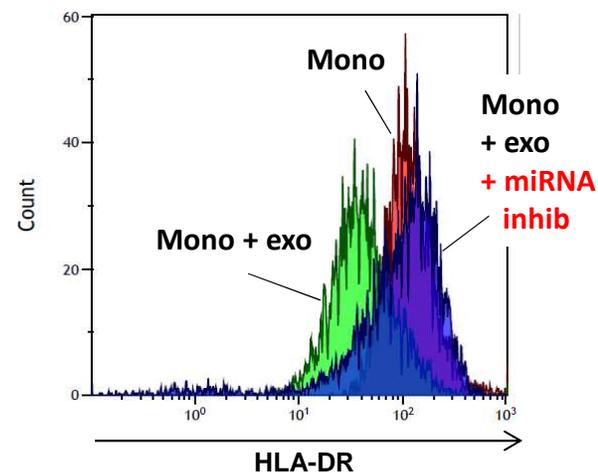
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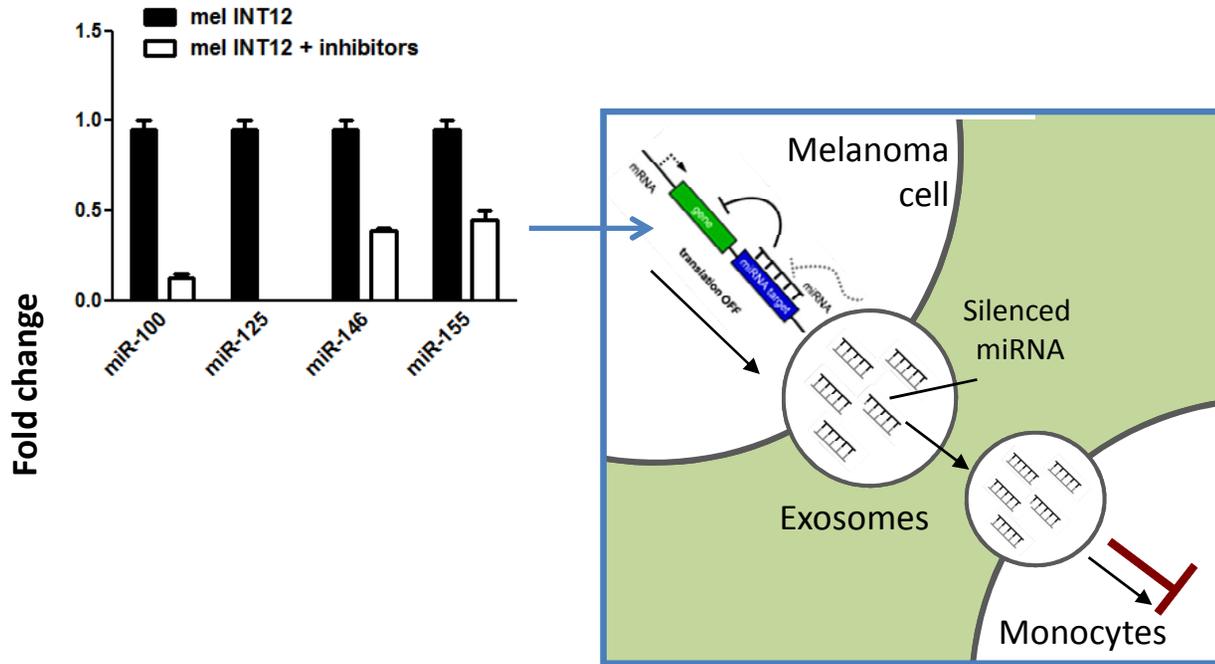
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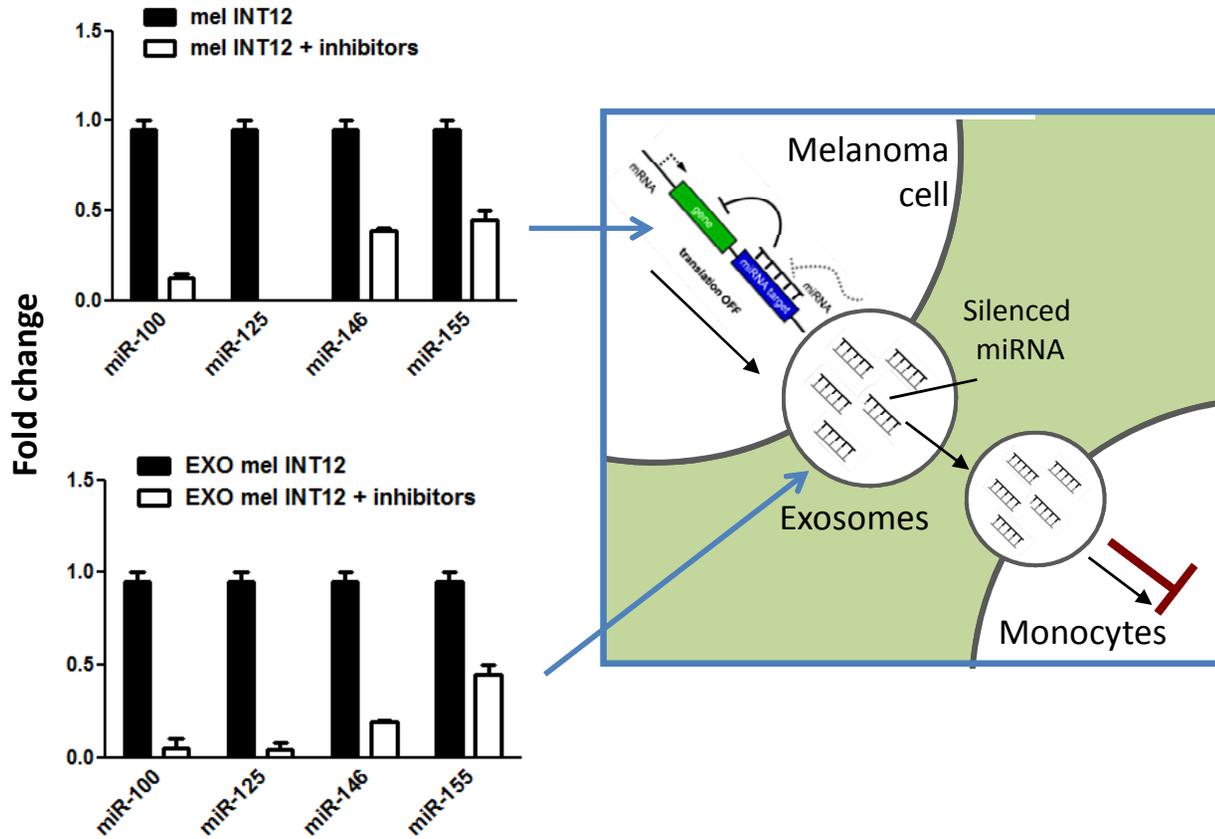
FACS



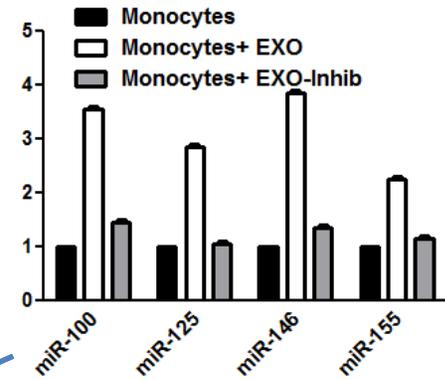
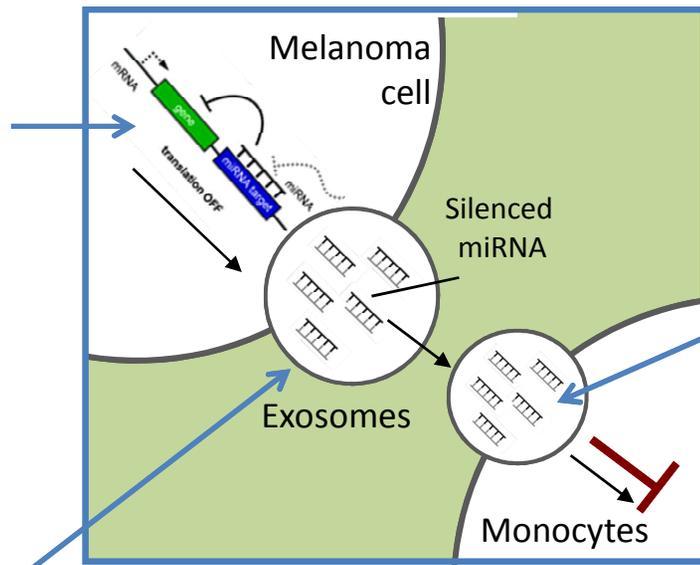
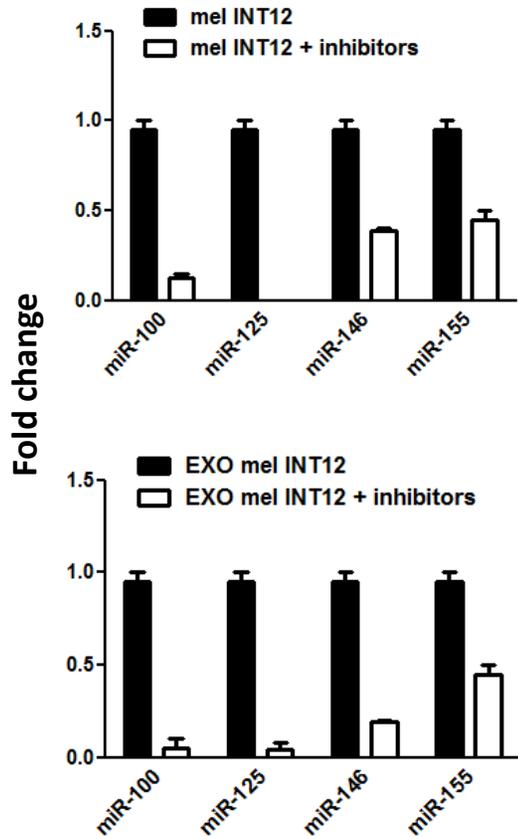
Role of miRNA transfer by exosomes in MDSC conversion



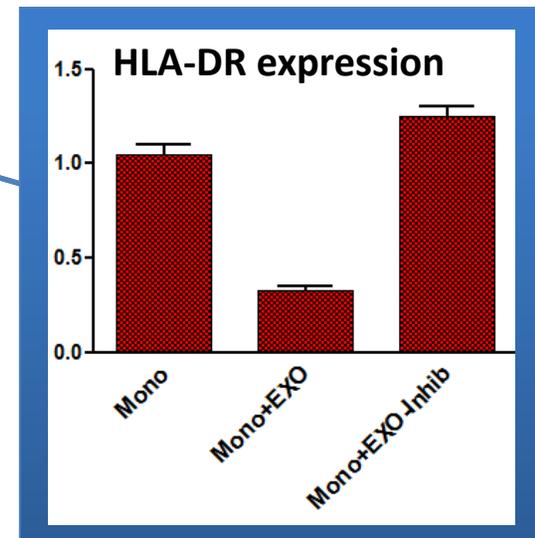
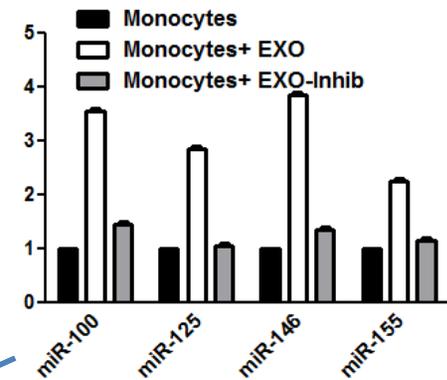
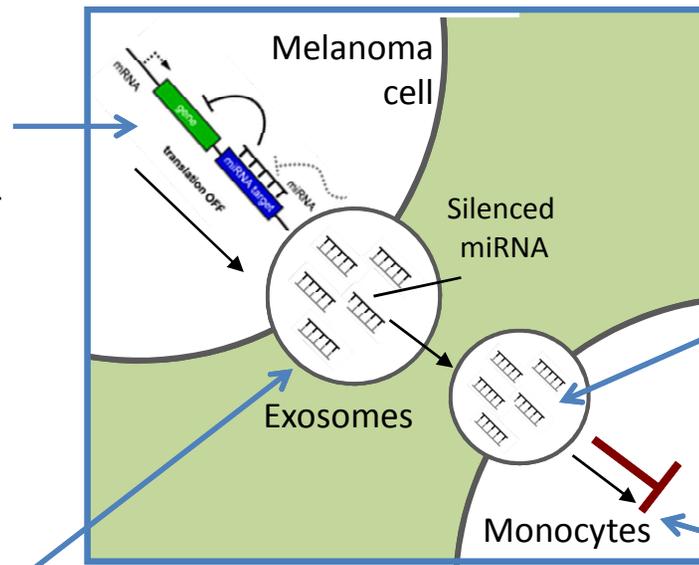
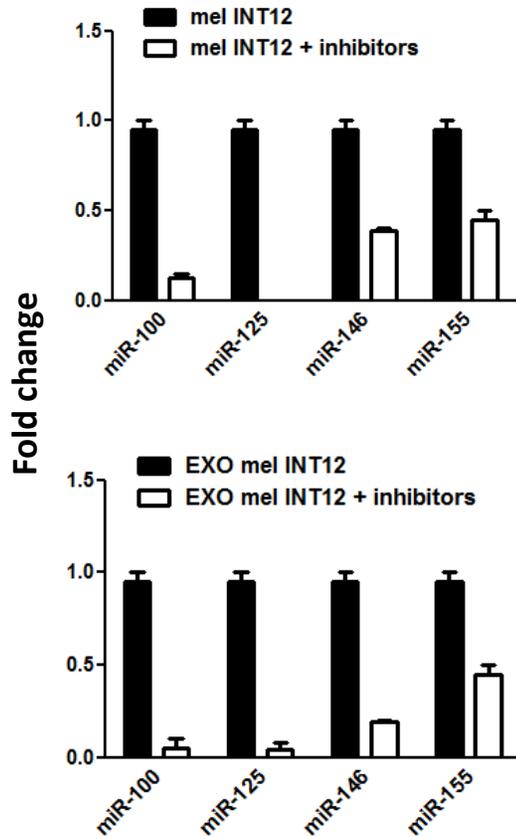
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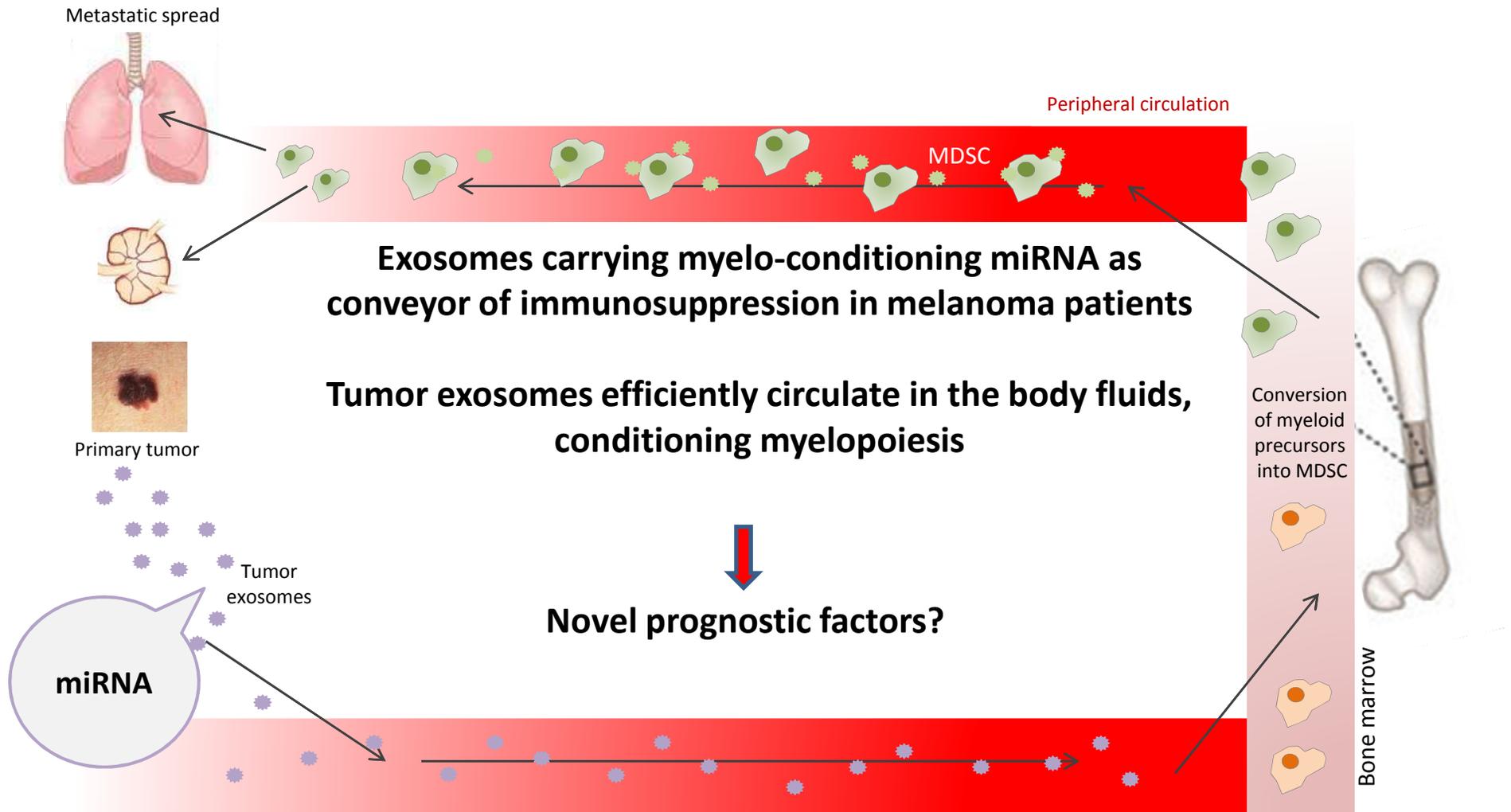
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Role of miRNA transfer by exosomes in MDSC conversion



Melanoma patients

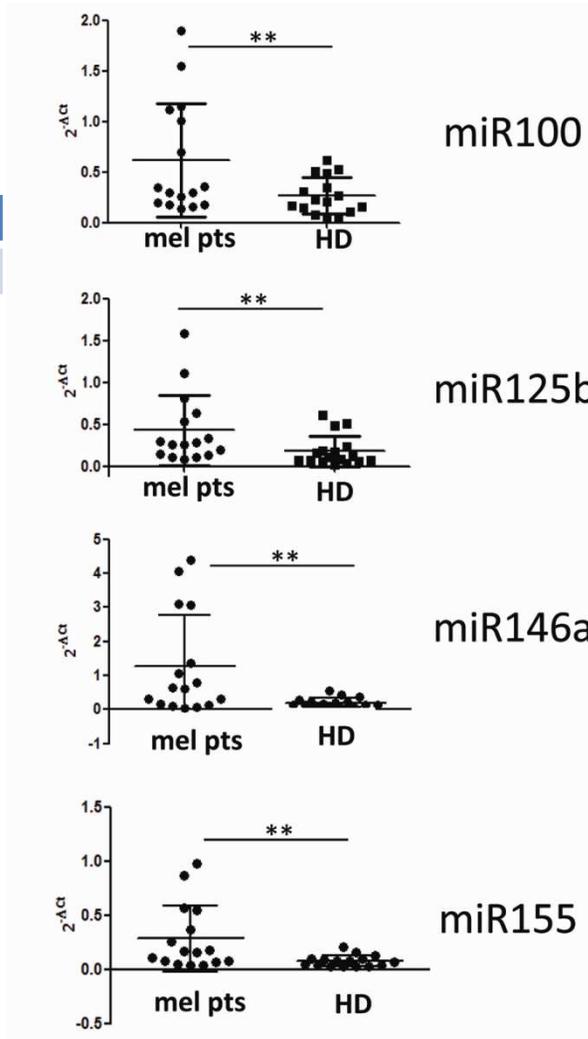


EXO-MDSC miRNA signature can be detected in peripheral blood monocytes and biopsies of melanoma patients

CD14+ monocytes sorted from PBMC



HD	Mel pts
16	16

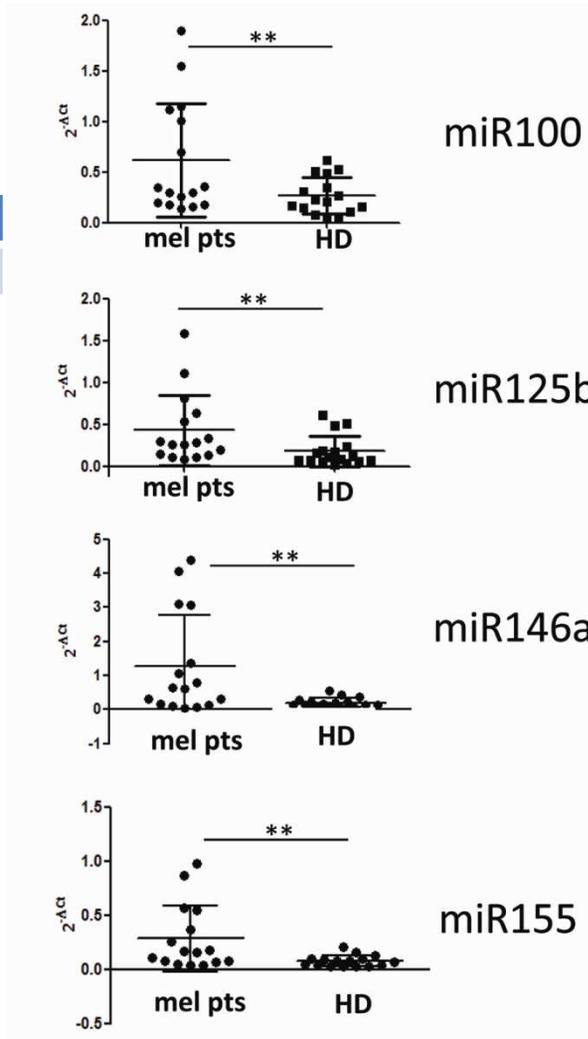


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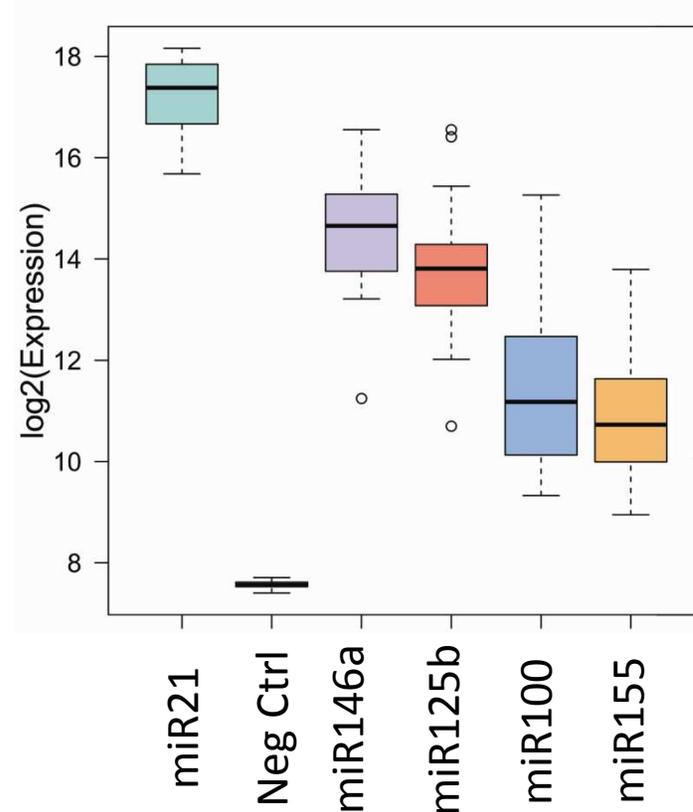
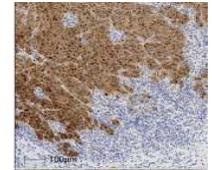
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Melanoma biopsies (stage IIIc-IV)

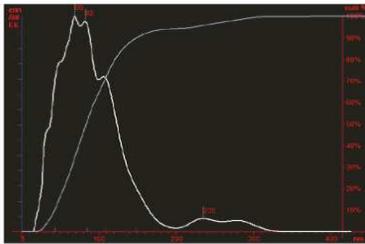


Mel pts
23

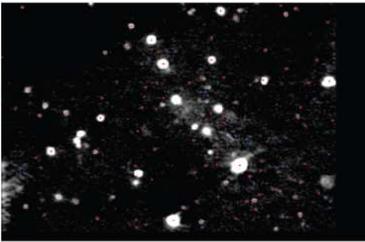
EV in whole plasma and MDSC-related miRNAs in EV of melanoma patients (stage IV)

HD	Mel pts
27	27

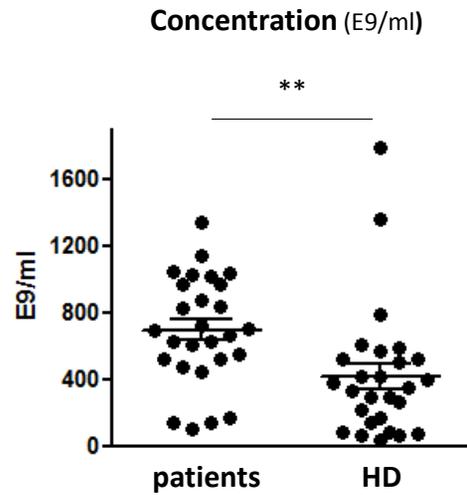
Whole PLASMA



Particle Size / Concentration



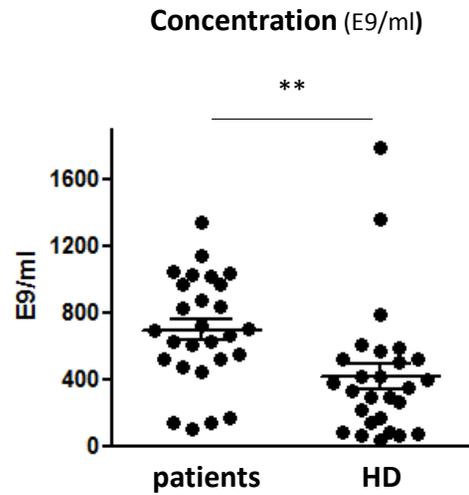
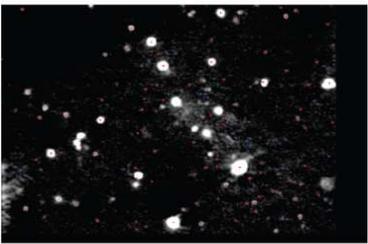
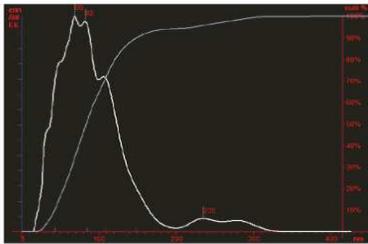
Sample Video Frame



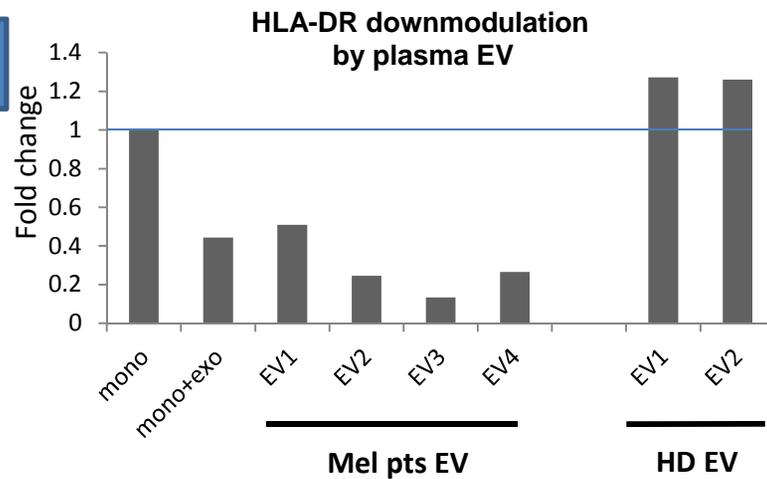
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Whole PLASMA



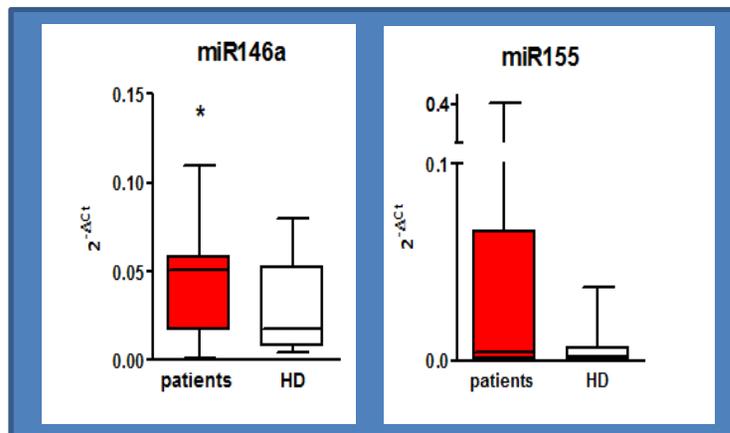
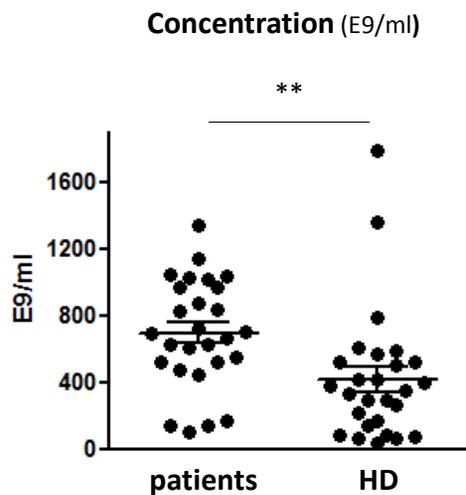
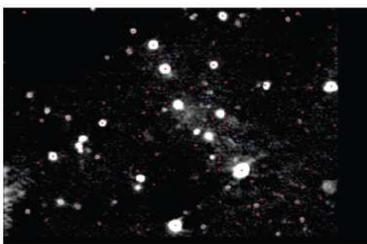
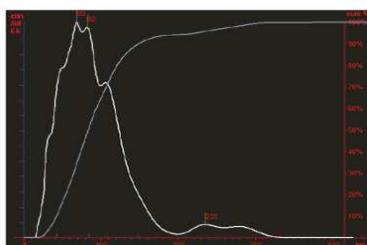
EV



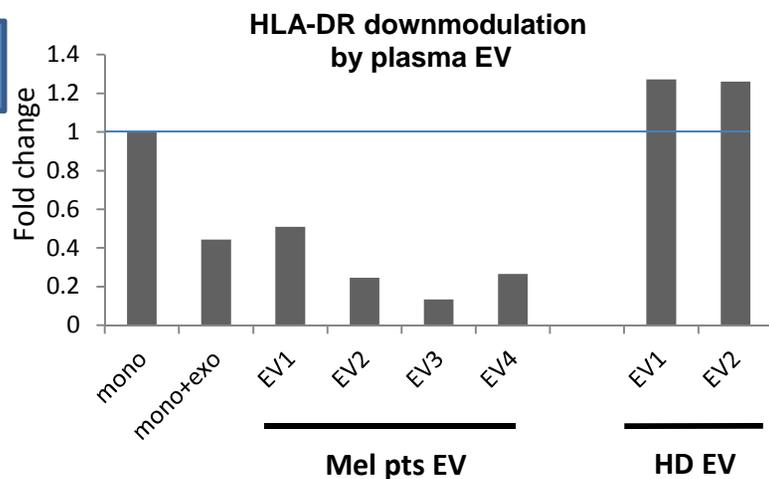
EV in whole plasma and MDSC-related miRNAs in EV of melanoma patients (stage IV)

HD	Mel pts
27	27

Whole PLASMA



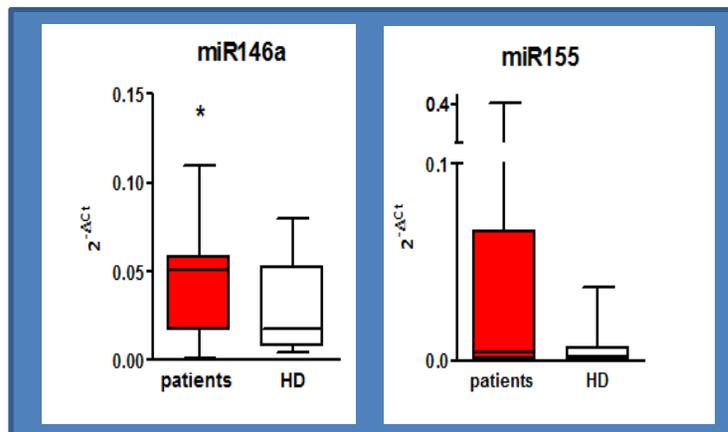
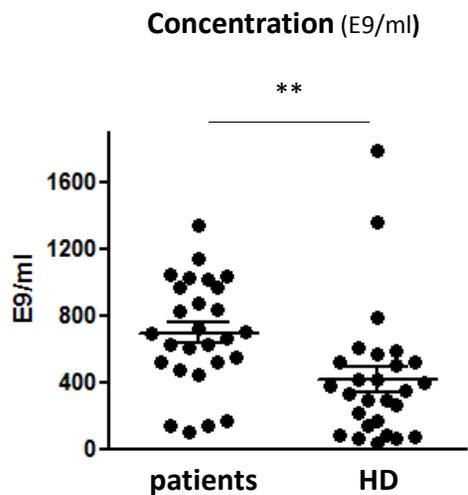
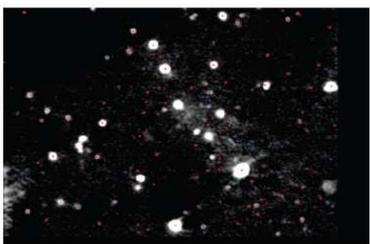
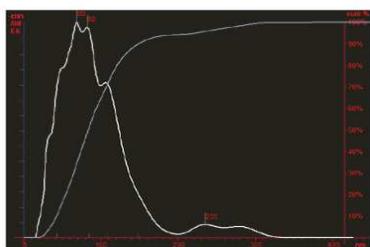
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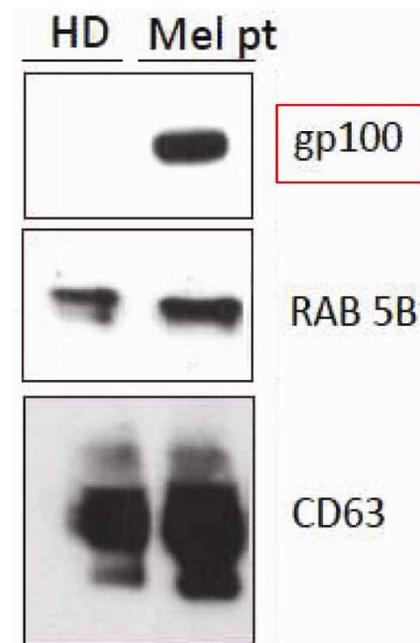
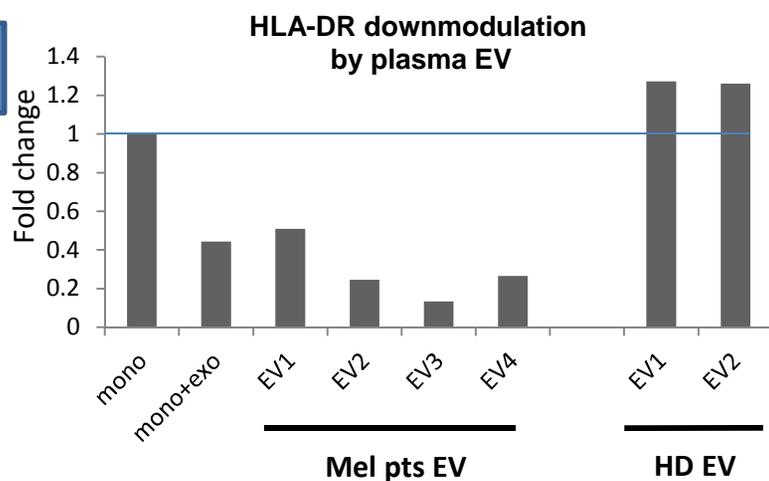
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EV



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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