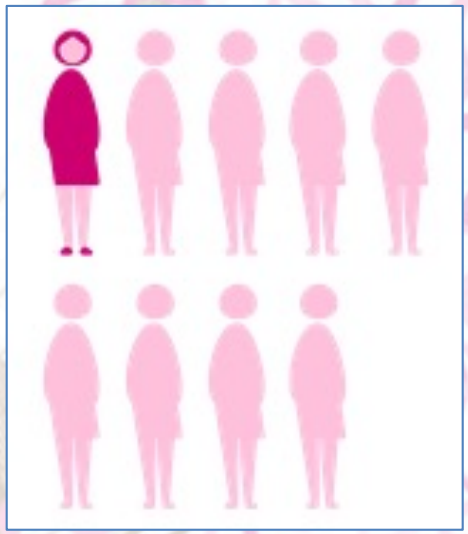
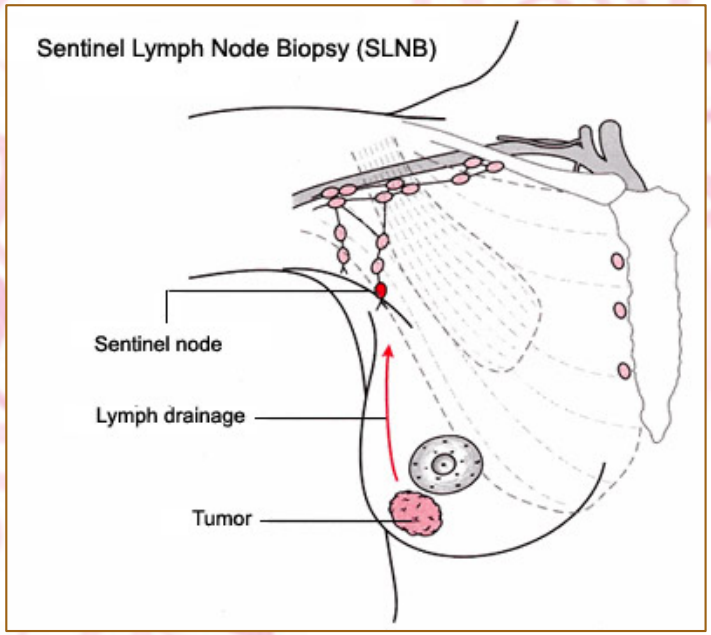
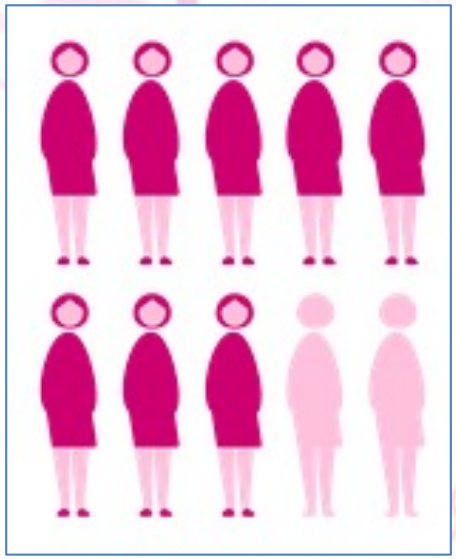
Myeloid suppressor cells in human breast cancer

KARIN LEANDERSSON, CANCER IMMUNOLOGY, LUND UNIVERSITY







Breast Cancer

- Most common type of cancer in women (1/9)
- Second leading cause of cancer deaths among women
- Spread to **lymph nodes (MLN^{+})** – *worse prognosis*
- Metastatic disease (stage IV) – 20% 5-year survival rate
- Different breast cancer subtypes – *TNBC worst*

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Pictures from Cancerfonden

Breast Cancer Subtypes

Luminal A (~40%)
ER^{+} and/or PR^{+} $Her2^{-}$ $Ki67^{low}$

Normal-like (~2-8%)
ER^{+} and/or PR^{+} $Her2^{-}$
$Ki67^{low}$

Luminal B (~20%)
ER^{+} and/or PR^{+} $Her2^{-/+}$
$Ki67^{hi}$

Her2 enriched (~10-15%)
$ER^{-}PR^{-}Her2^{+}$

Triple negative (TNBC) (~15-20%)
$ER^{-}PR^{-}Her2^{-}$

Best prognosis

Worst prognosis

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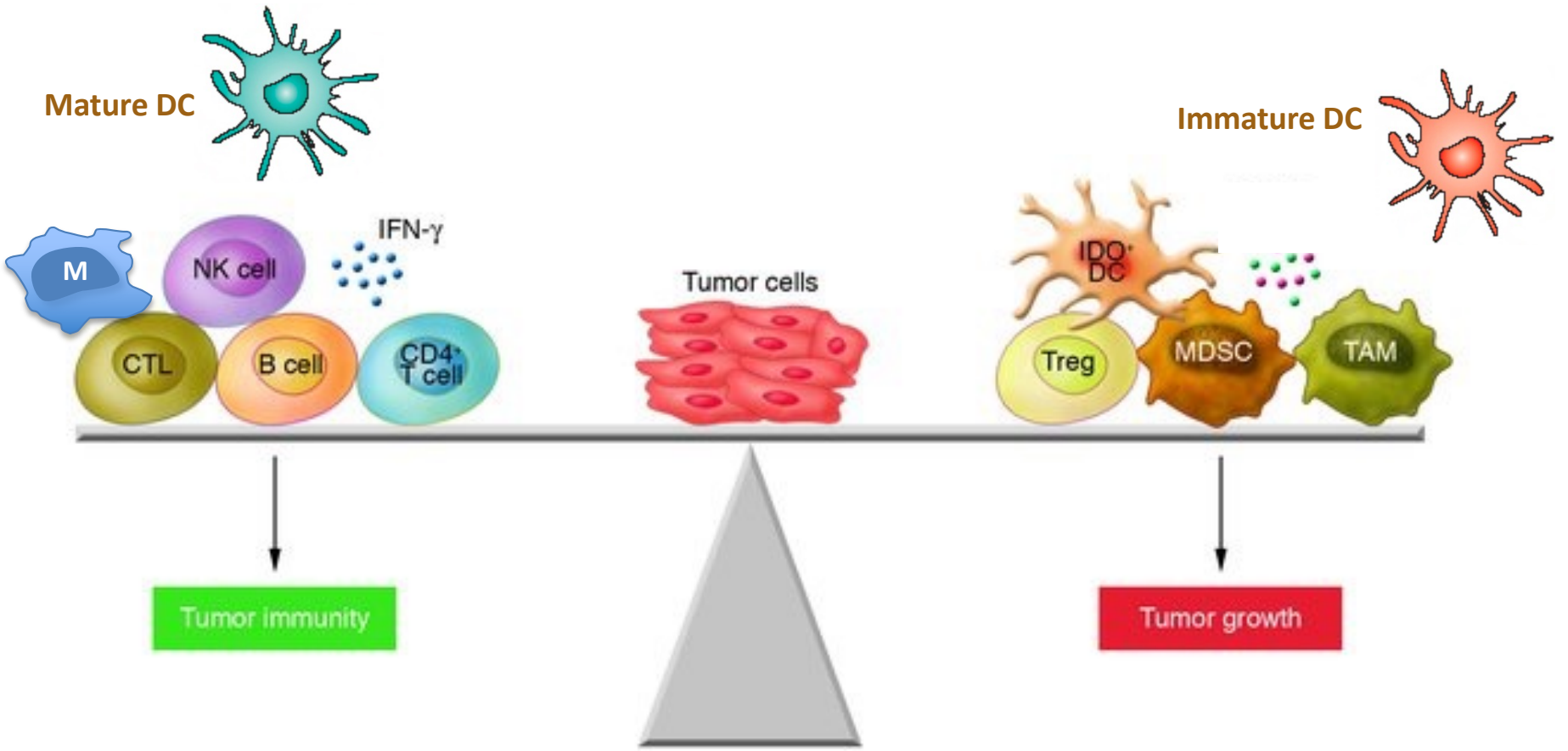

Inflammatory

Induce inflammation

Eradicate non-self

Anti-Inflammatory

Stop the inflammation

Induce wound healing mechanisms

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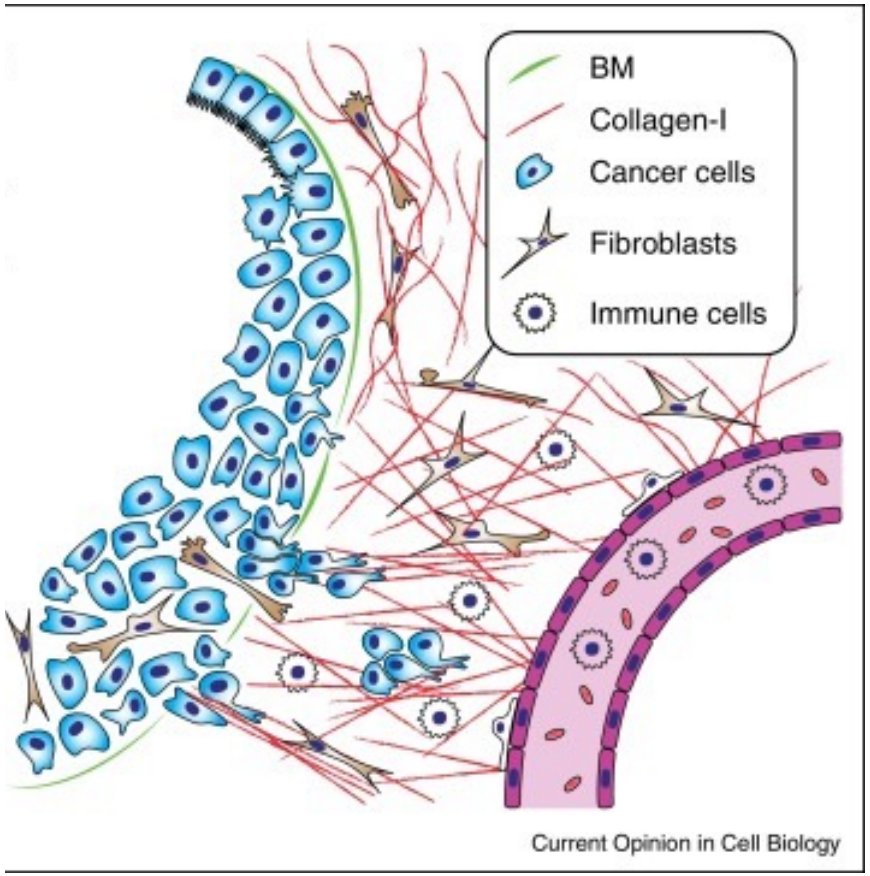
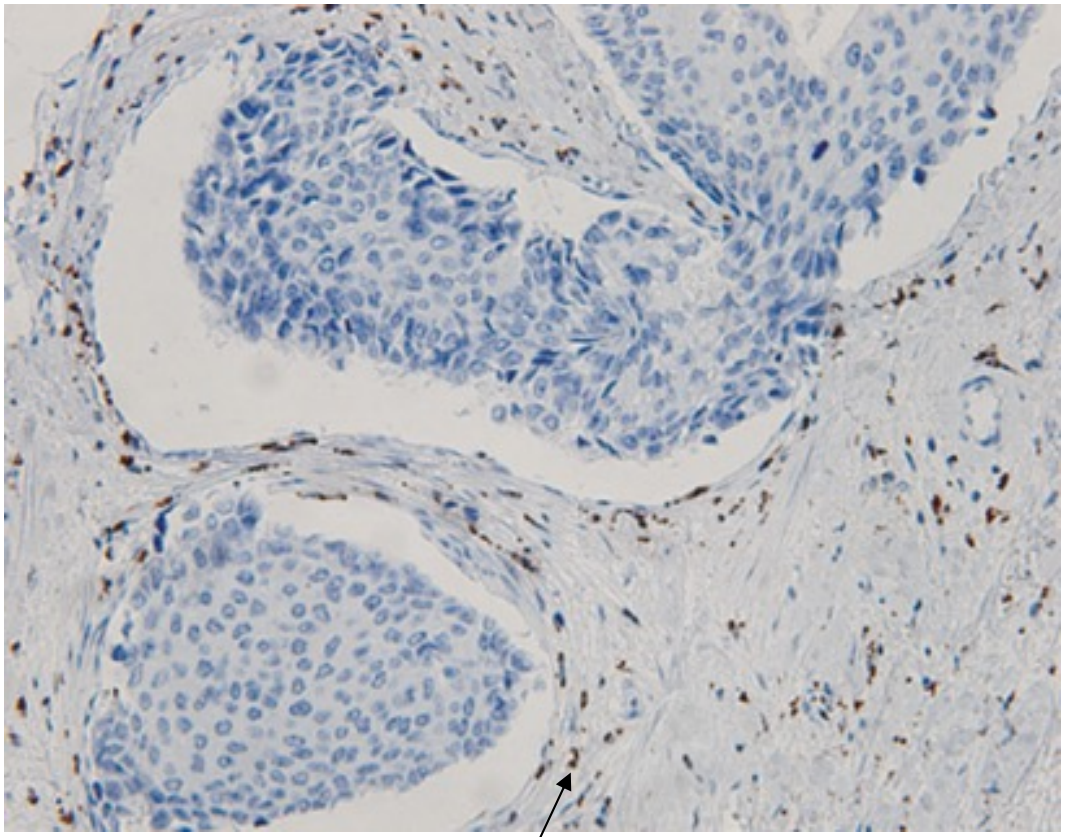
Adopted and modified from Bhardwaj JCI 2007

Tumor microenvironment





Tumor associated myeloid cells

→ worse prognosis

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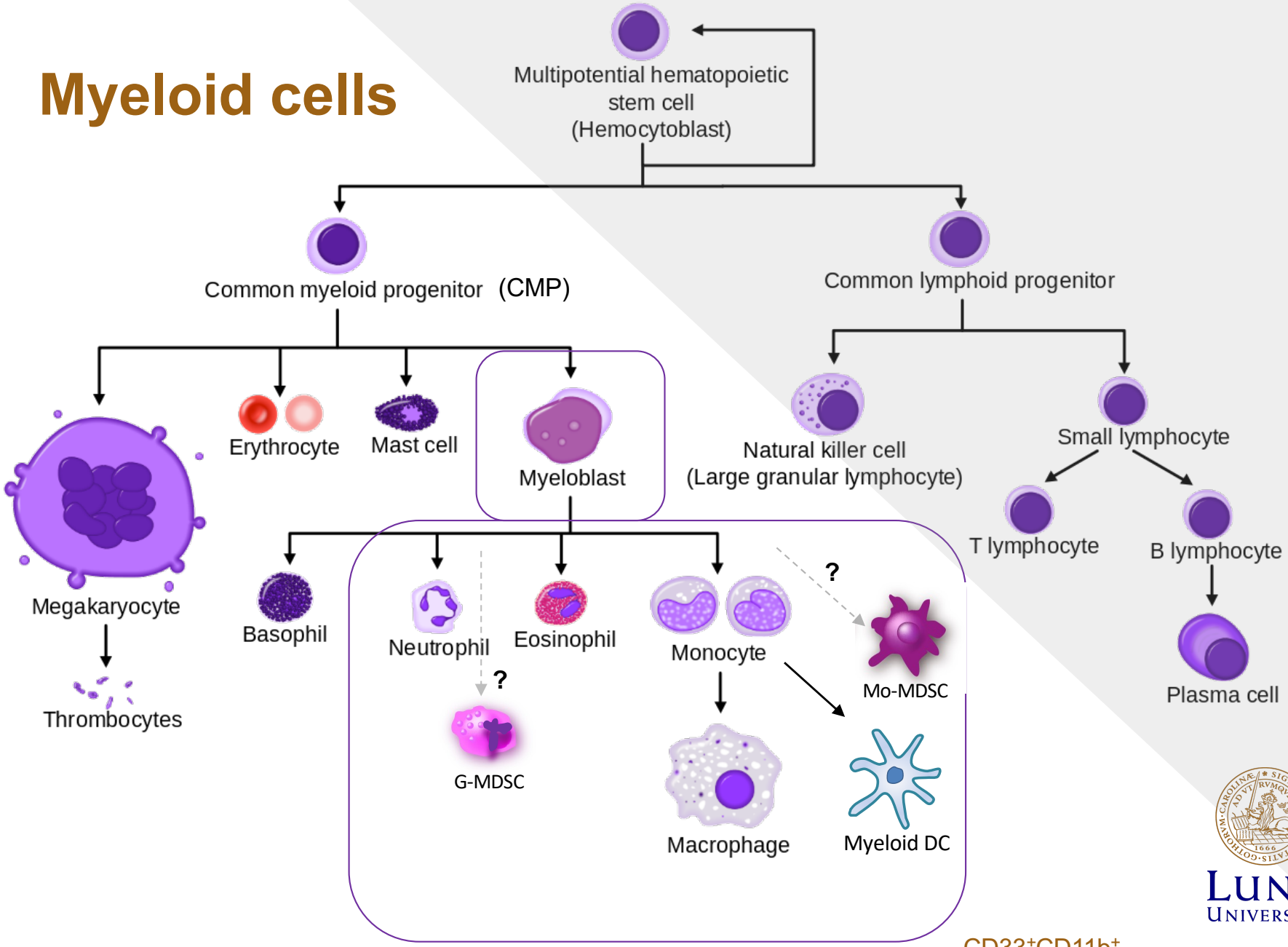
Myeloid cells



CD33⁺CD11b⁺

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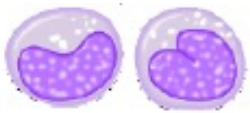
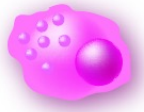
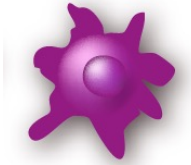
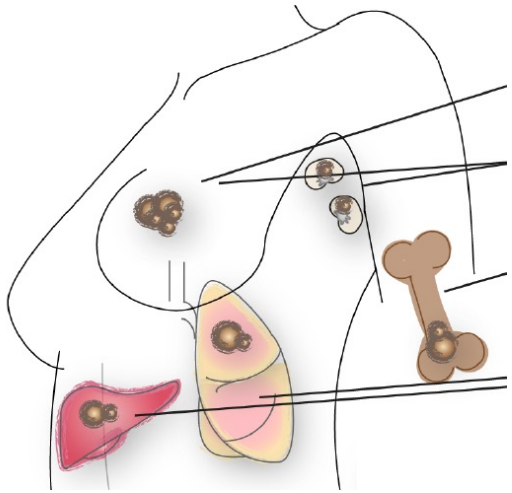






Circulating/systemic anti-inflammatory myeloid cells in cancer patients?



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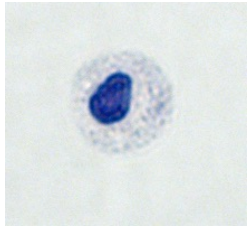
Myeloid derived suppressor cells

- Functional phenotype: Potent immunosuppression
- Cancer patients –*and infections and inflammatory conditions*
- Myeloid precursors – *Immature* – *Plasticity?*
- Based on surface phenotype

Monocytic MDSCs

(Mo-MDSCs)



Granulocytic MDSCs

(G-MDSCs)

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Myeloid derived suppressor cells

$CD11b^{+}Ly6C^{high}Ly6G^{-}$

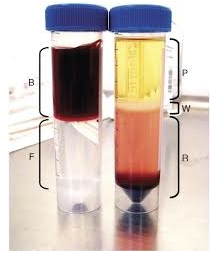
$CD11b^{+}Ly6C^{low}Ly6G^{+}$

?

$CD11b^{+}CD14^{+}HLADR^{-/low}$

$CD11b^{+}CD33^{+}\ CD15^{+}$
$Lin^{-}\ HLADR^{-/low}$

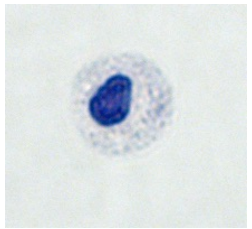
Low density



- Based on surface phenotype

Monocytic MDSCs

(Mo-MDSCs)



Granulocytic MDSCs

(G-MDSCs)

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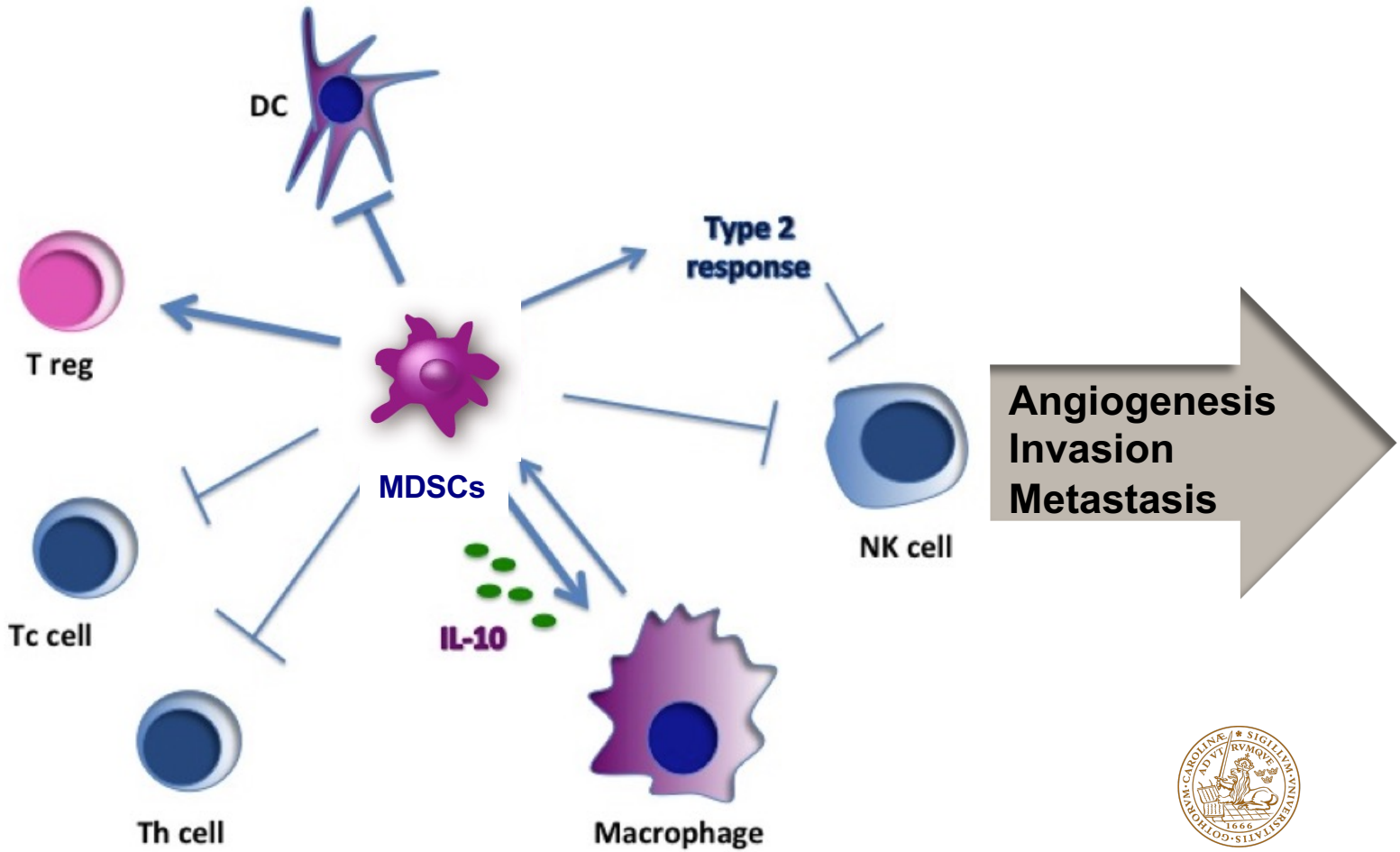
Myeloid derived suppressor cells

VEGF
HGF
EGF
MMP9
TGFβ
IL-10
Arginase
ROS
IDO
IL-6
S100A8/A9



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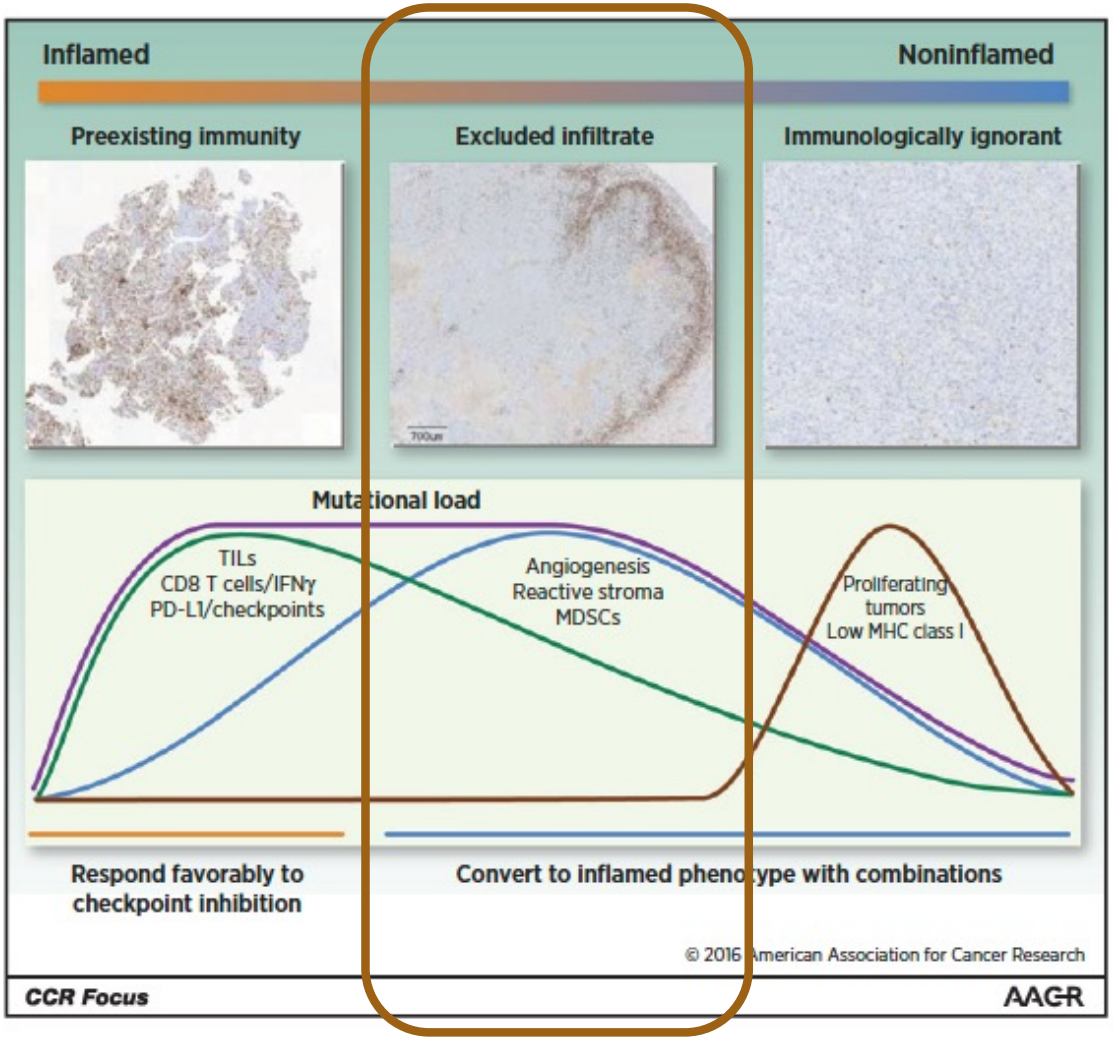
Human MDSCs involved in immune exclusion?



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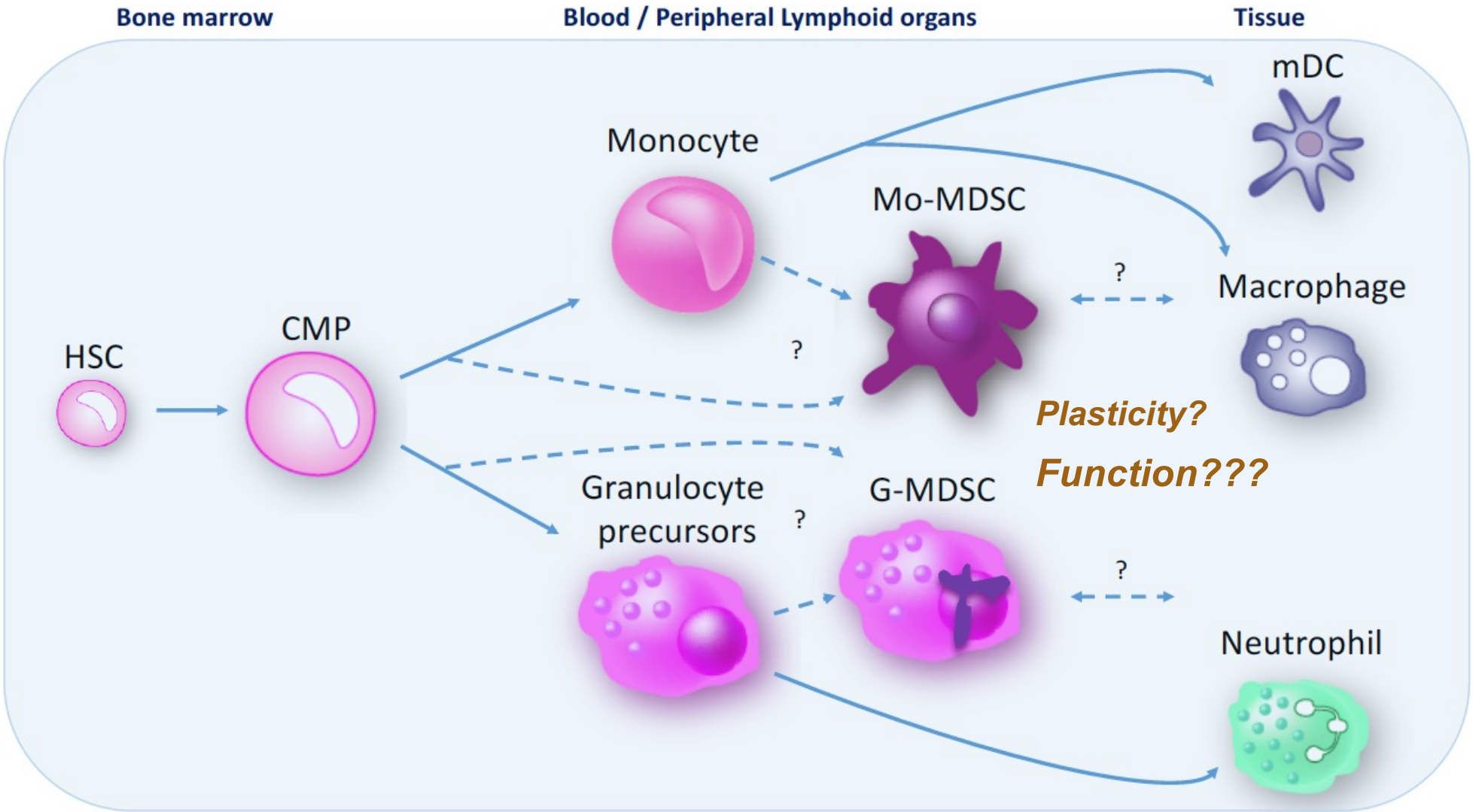
The generation of MDSCs



Systemic Anti-inflammatory myeloid cells

Generation and function of human MDSCs in breast cancer patients

What are MDSCs in humans?

Can / should we block them?

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Outline

→ Origin / mechanism of generation of human Mo-MDSCs

- Origin and function of human G-MDSCs

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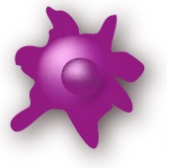
Systemic Anti-inflammatory myeloid cells

A summary of our research on human Mo-MDSCs



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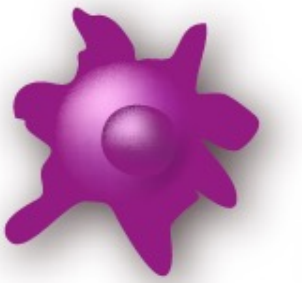
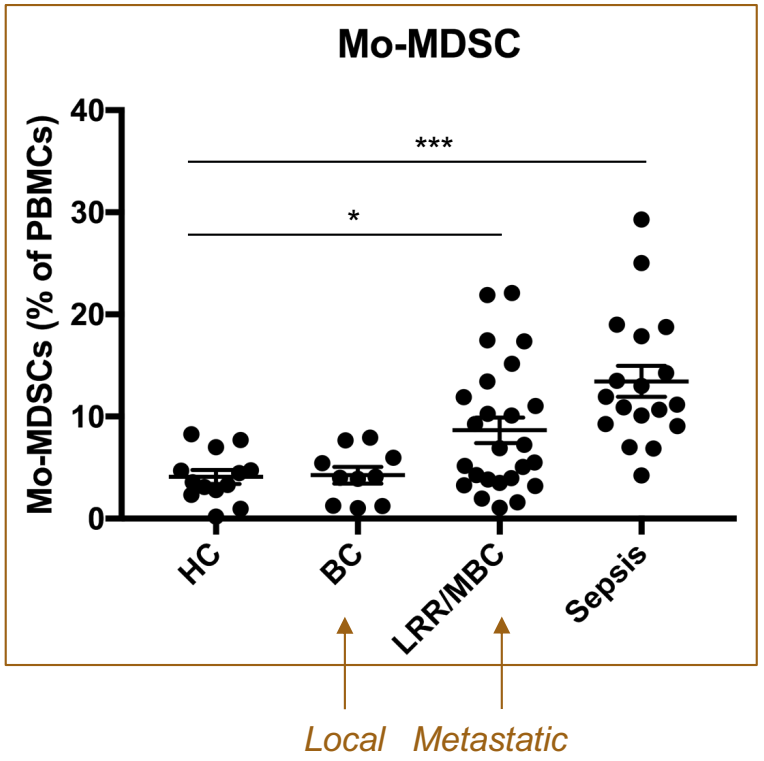
Systemic Mo-MDSCs in breast cancer correlate with disease progression

Mo-MDSCs





Bergenfelz et al Plos ONE 2015
(N=25)

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Systemic Mo-MDSCs correlate with disease progression

Table 1 Levels of Mo-MDSCs and clinicopathological variables.

Clinicopathological variables (n=22)	All patients (n=54)	**Low Mo-MDSCs** (n=28)	**High Mo-MDSCs** (n=26)	***P* value**
PT Hormone status				
ER-	12	3 (11,5)	9 (40,9)	**0,02**
ER+	36	23 (88,5)	13 (59,1)	
Unknown	6			
Metastatic sites:				
Lymph node				
Negative	31	18 (64,3)	13 (50,0)	0,29
Positive	23	10 (35,7)	13 (50,0)	
Lung				
Negative	33	16 (57,1)	17 (65,4)	0,54
Positive	21	12 (42,9)	9 (34,6)	
Liver				
Negative	38	23 (82,1)	15 (57,7)	**< 0,05**
Positive	16	5 (17,9)	11 (42,3)	
Bone				
Negative	12	10 (35,7)	2 (7,7)	**0,01**
Positive	42	18 (64,3)	24 (92,3)	
Time from PT to MBC				
De novo MBC	12	2 (7,1)	10 (38,5)	**0,006**
Distant recurrent MBC	42	26 (92,9)	16 (61,5)	

TNBC

Liver

Bone

MBC

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Bergenfelz et al 2020 (PI: MD PhD Anna-Maria Larsson, Oncology Unit, Lund University, Sweden) N=54

Systemic Anti-inflammatory myeloid cells

Mo-MDSCs are present in metastatic breast cancer patients and associate with ER- and disease progression

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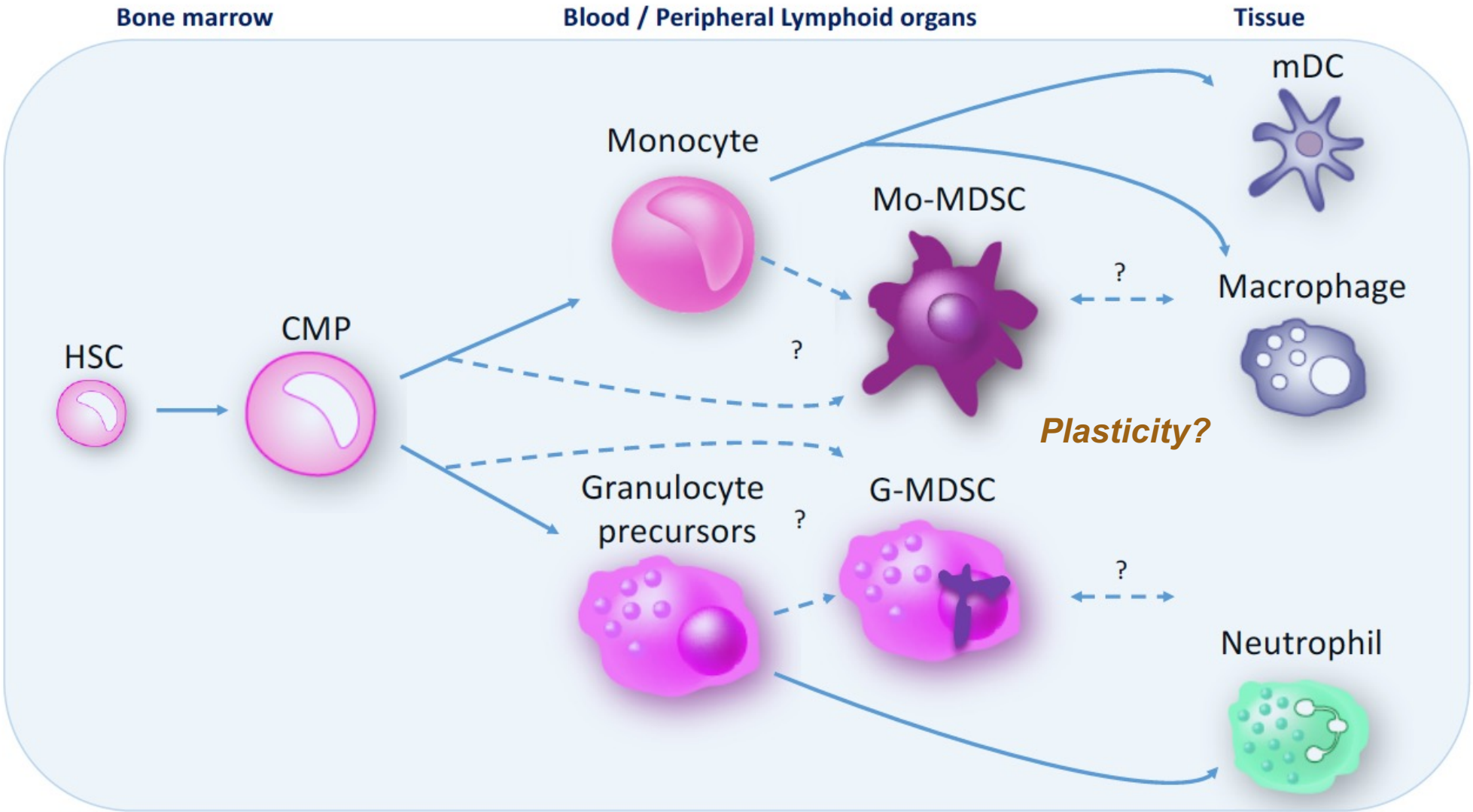
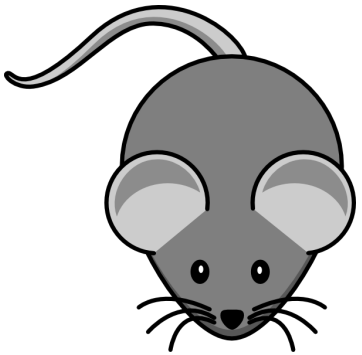
The generation of MDSCs



Myeloid derived suppressor cells



$CD11b^{+}Ly6C^{high}Ly6G^{-}$

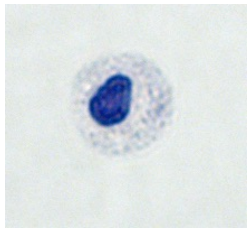
?

$CD11b^{+}CD14^{+}HLADR^{-/low}$

- Based on surface phenotype

Monocytic MDSCs

(Mo-MDSCs)



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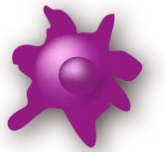
Sepsis

Blood

Monocyte

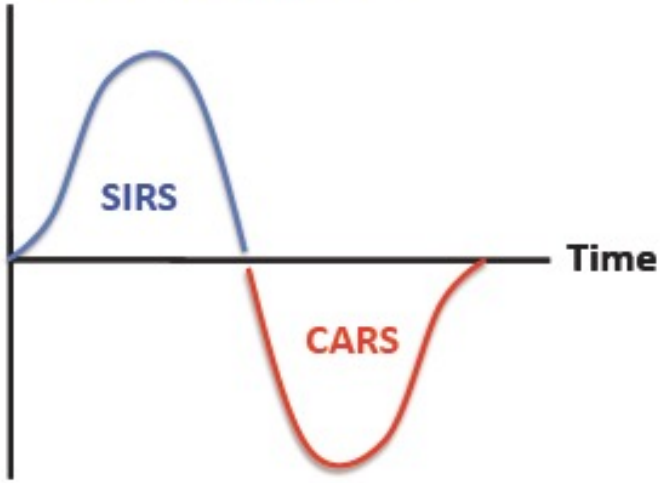
Inflammatory Anti-inflammatory

Danger dogma



Mo-MDSC?

SIRS – Systemic inflammatory response syndrome



CARS – Compensatory anti-inflammatory response syndrome

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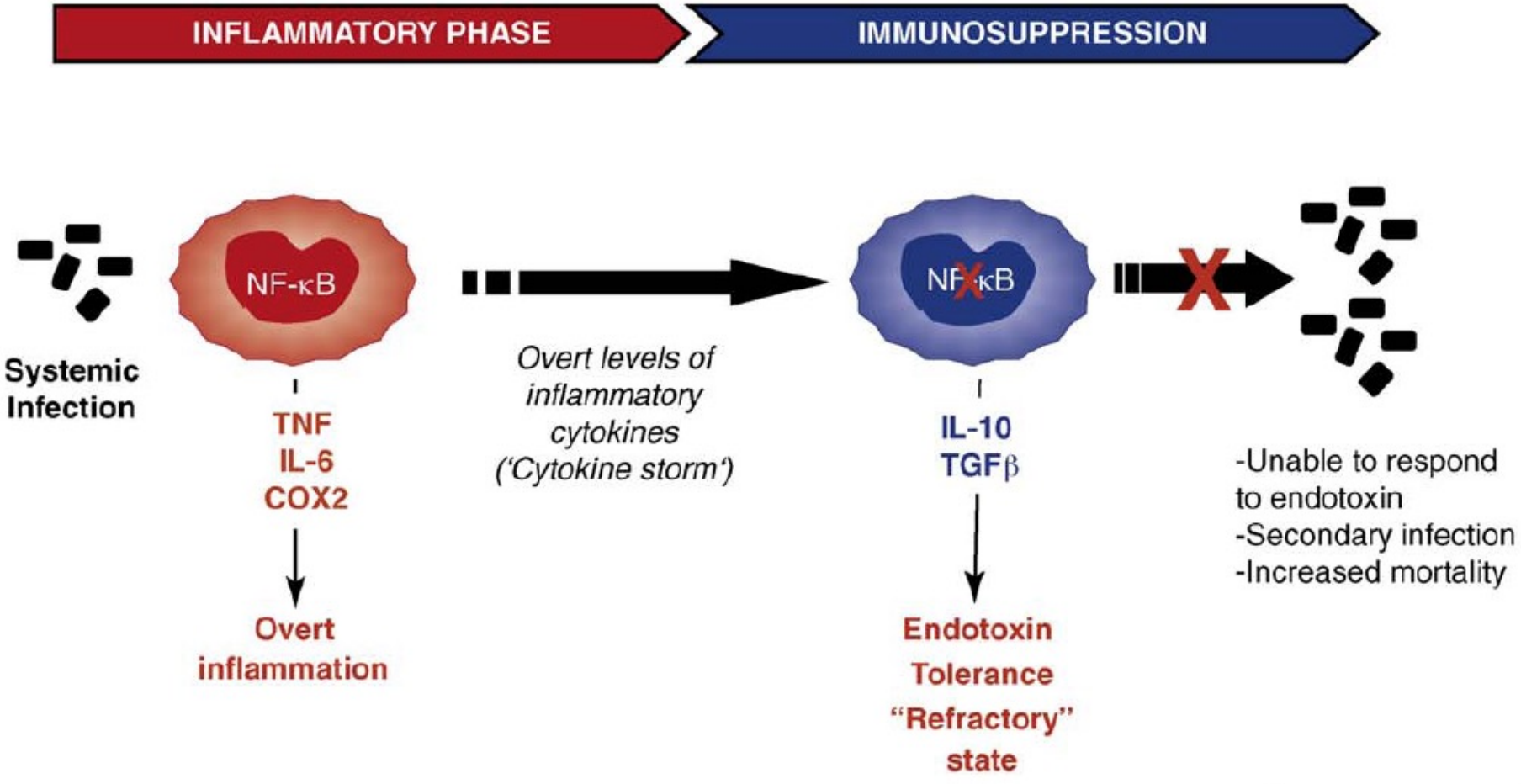
Sepsis – Monocyte reprogramming

Endotoxin tolerance (1947)



TRENDS in Immunology

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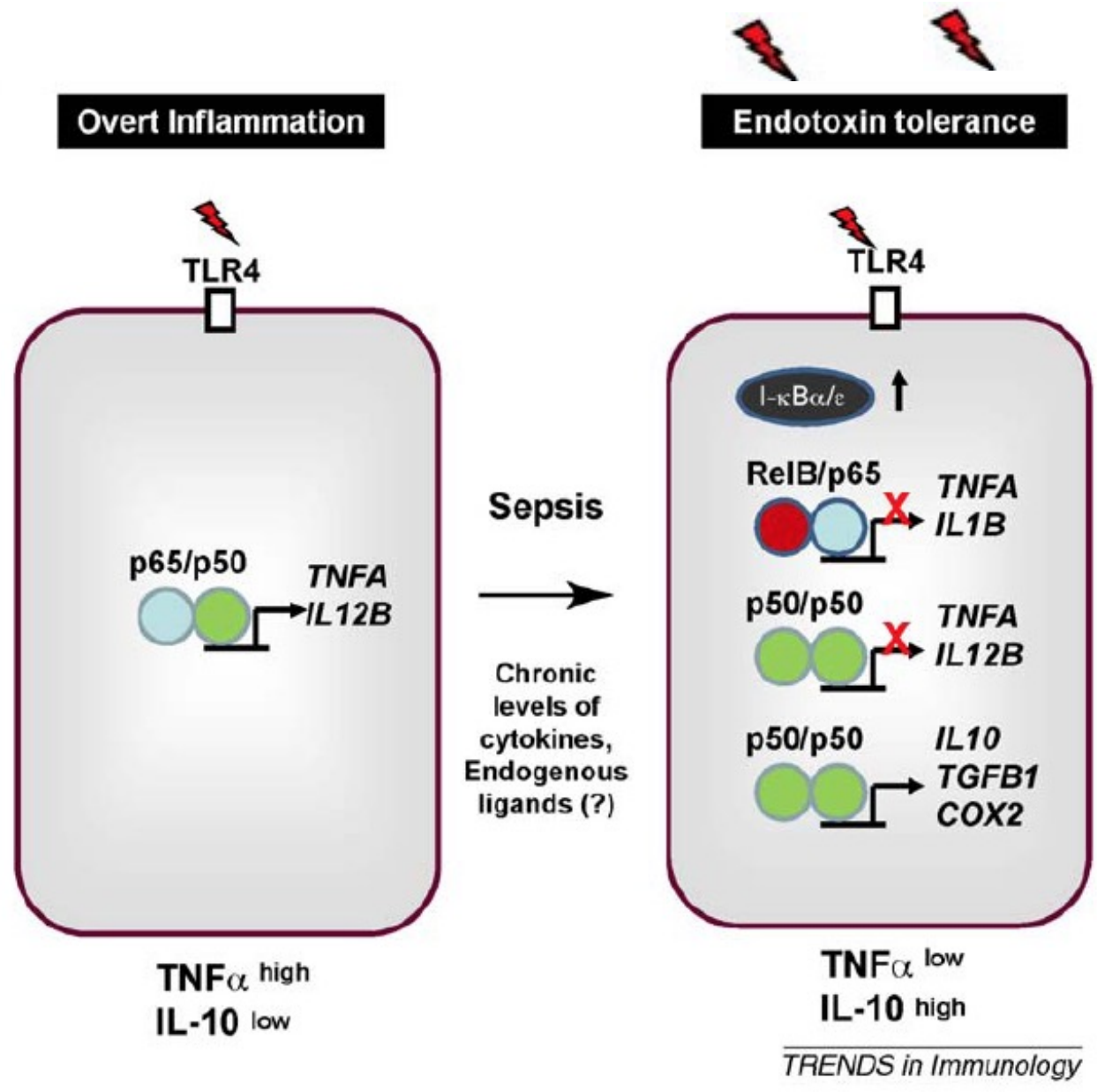
Hypothesis: Are reprogrammed monocytes in sepsis patients Mo-MDSCs?



PAMP
DAMP?

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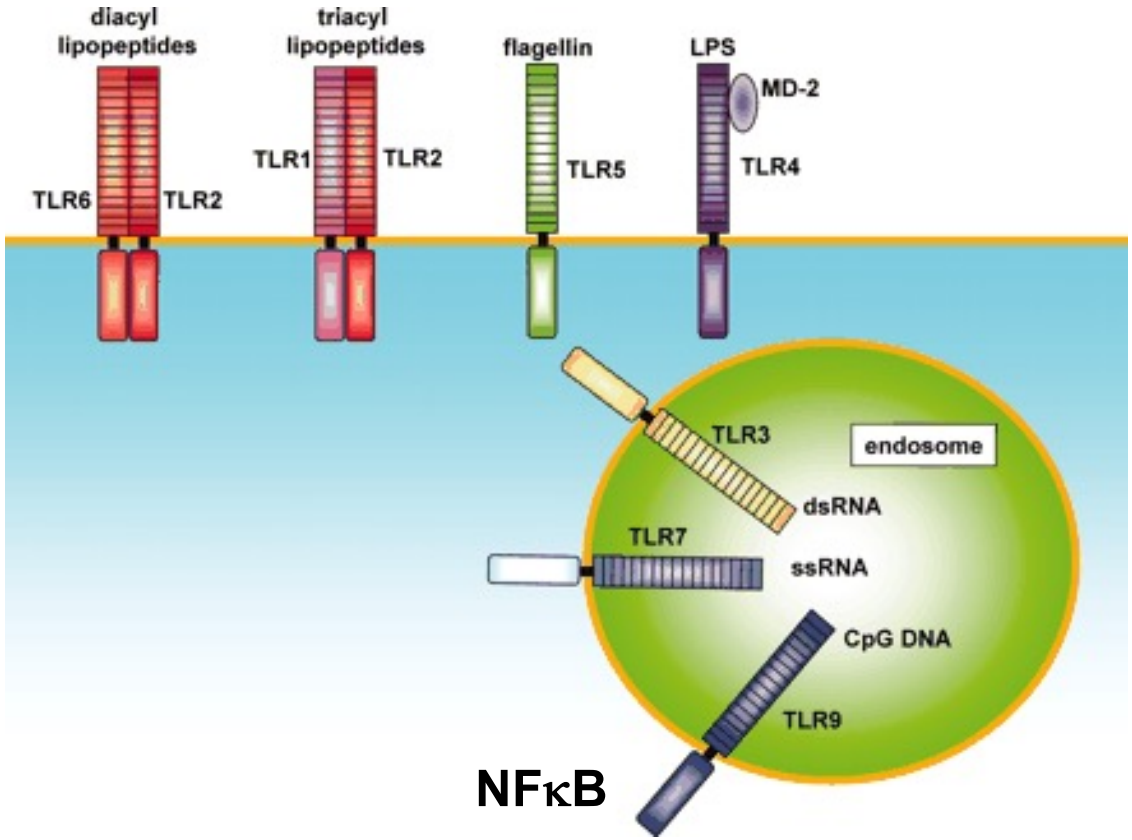
If PAMPs induce monocyte reprogramming during sepsis…

PAMPs

- Pathogen associated molecular patterns
- Bind Pattern recognition receptors (PRRs eg. TLRs)
- Activates NFκB signaling



- **LPS**
- **Flagellin**
- **Lipotheichoic Acid**
- **Peptidoglycan etc**

Figure adapted and modified; from Int Immunol, Volume 17, Issue 1, January 2005, Pages 1–14, https://doi.org/10.1093/intimm/dxh186

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Can DAMPs induce monocyte reprogramming in the sterile cancer microenvironment?

DAMPs

- Damage associated molecular patterns
- Endogenous PRR ligands
- Activates NFκB signaling

- **HMGB1**
- **S100A**
- **HSP**
- **dsRNA**

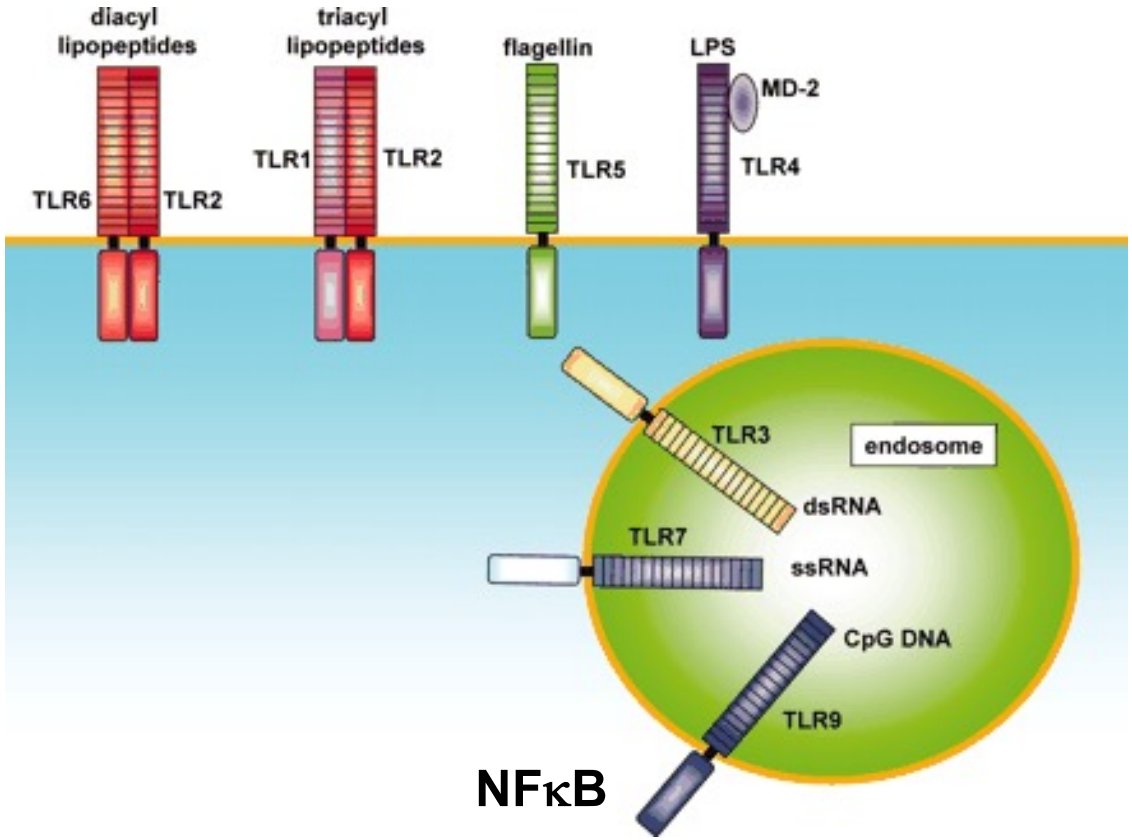

Figure adapted and modified; from Int Immunol, Volume 17, Issue 1, January 2005, Pages 1–14, https://doi.org/10.1093/intimm/dxh186

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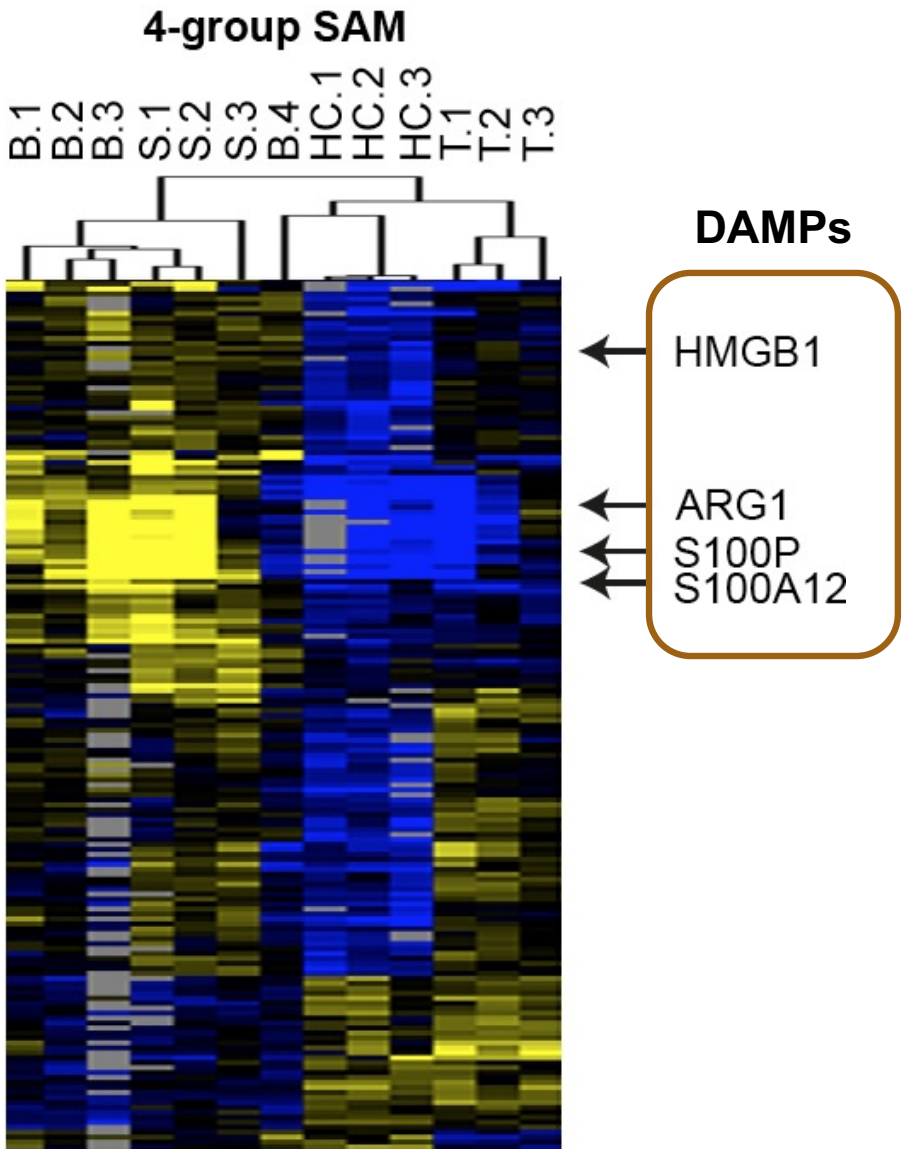
The molecular signature of Mo-MDSCs from breast cancer patients is similar to reprogrammed tolerance sepsis monocytes



PAMP
DAMP
TAMP?

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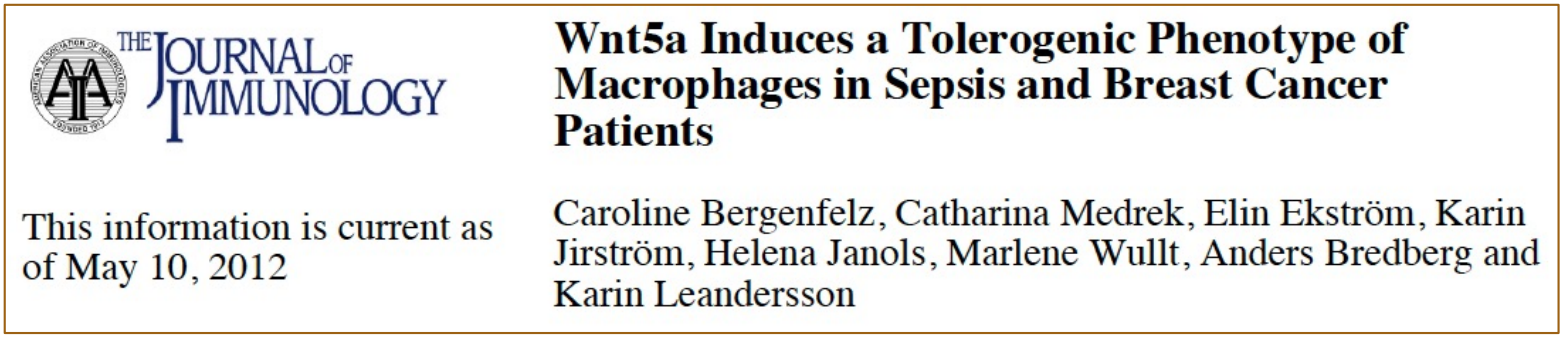
Bergenfelz et al Plos ONE 2015

Wnt5a - a tolerance associated molecular pattern "TAMP"?

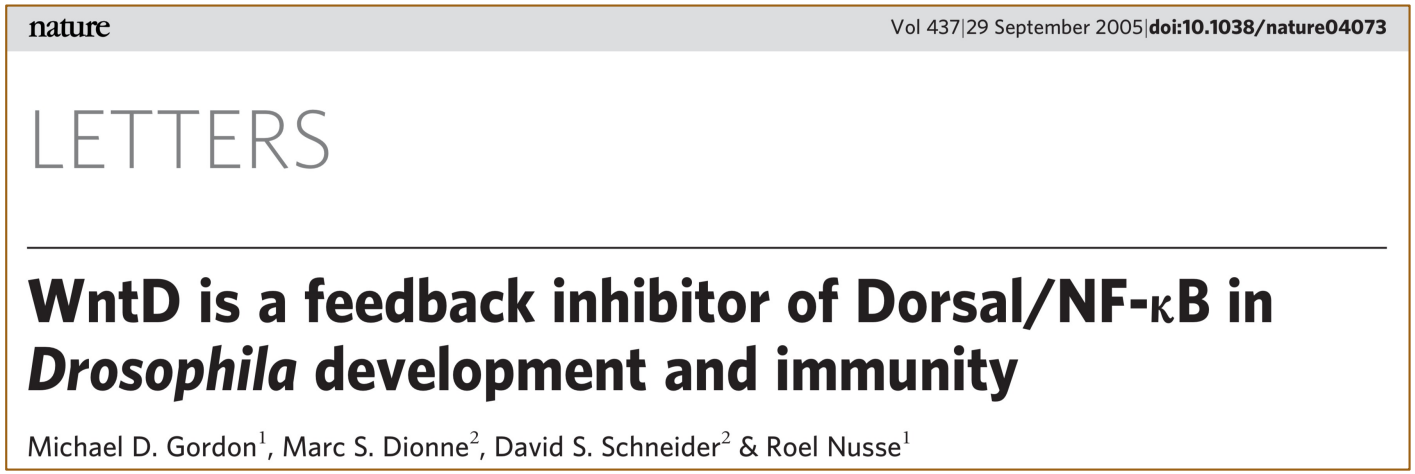


THE JOURNAL OF IMMUNOLOGY

This information is current as of May 10, 2012

Wnt5a Induces a Tolerogenic Phenotype of Macrophages in Sepsis and Breast Cancer Patients

Caroline Bergenfelz, Catharina Medrek, Elin Ekström, Karin Jirström, Helena Janols, Marlene Wullt, Anders Bredberg and Karin Leandersson



nature Vol 437|29 September 2005|doi:10.1038/nature04073

LETTERS

WntD is a feedback inhibitor of Dorsal/NF-κB in *Drosophila* development and immunity

Michael D. Gordon[1], Marc S. Dionne[2], David S. Schneider[2] & Roel Nusse[1]

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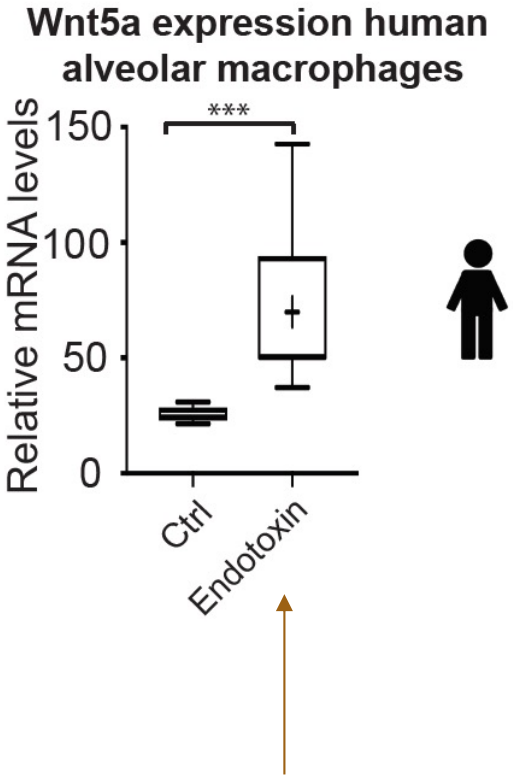

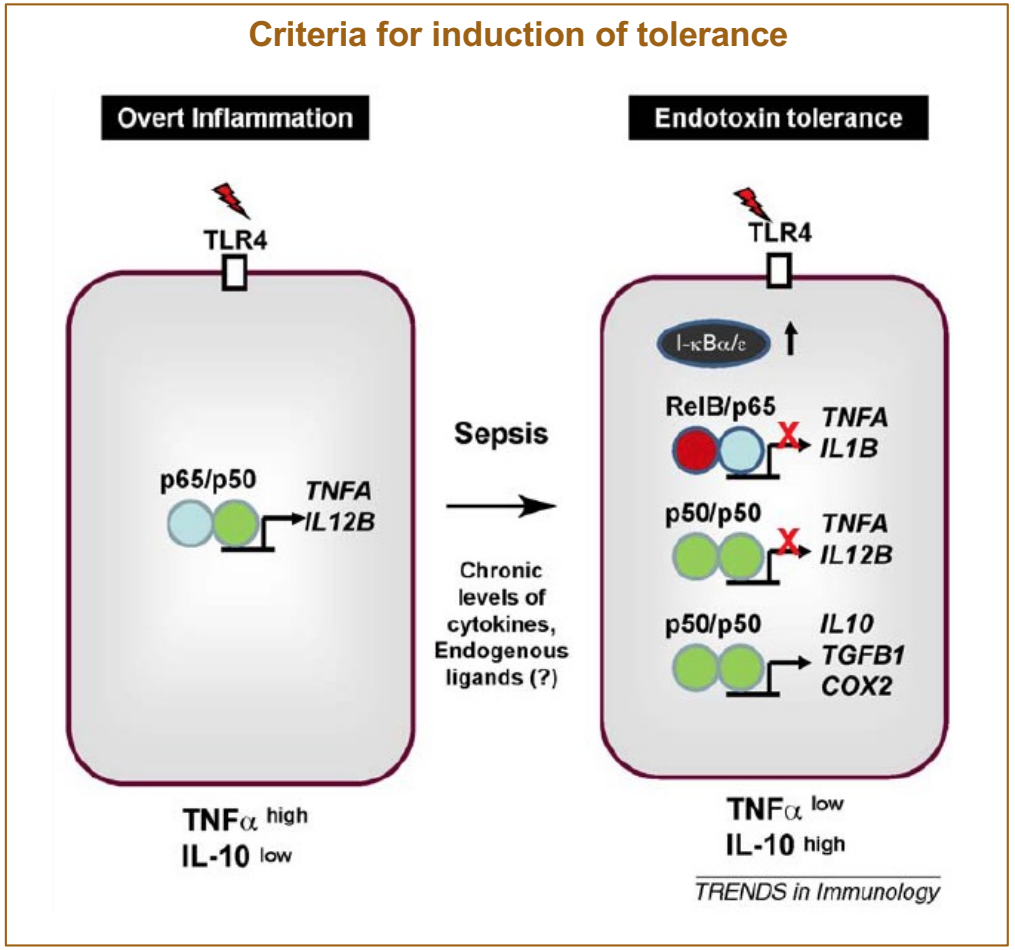

Communications Biol Mehmeti et al 2019

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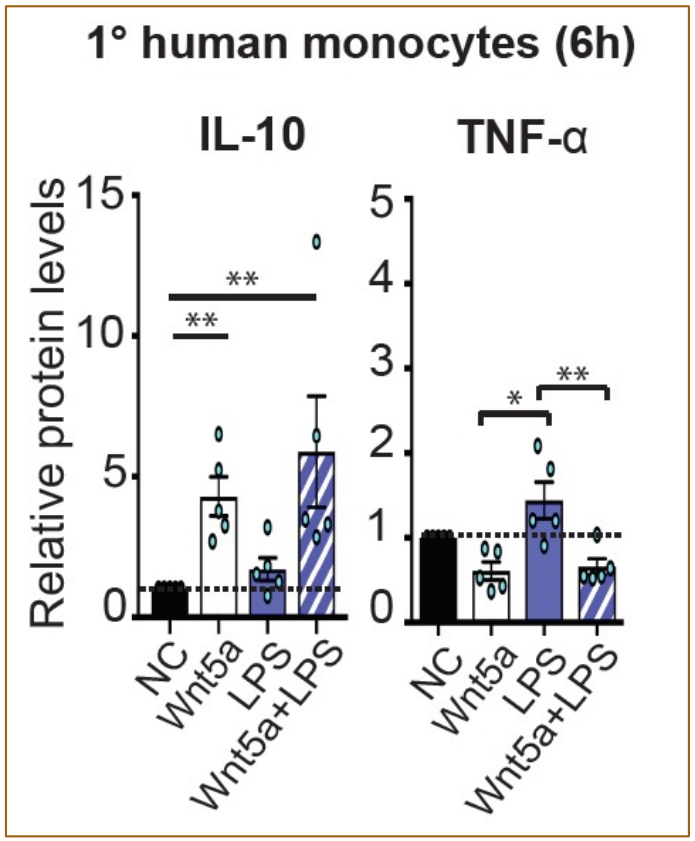
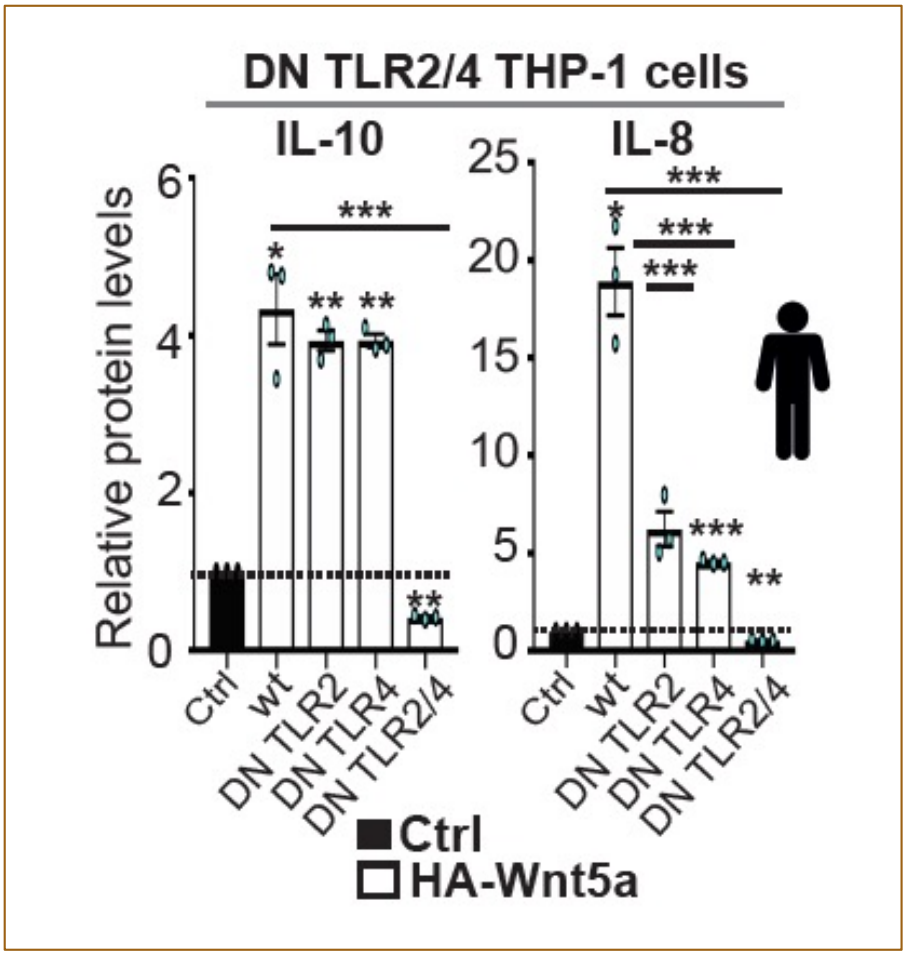
Wnt5a is a novel TLR4 ligand inducing tolerance in human myeloid cells (Mo-MDSCs)





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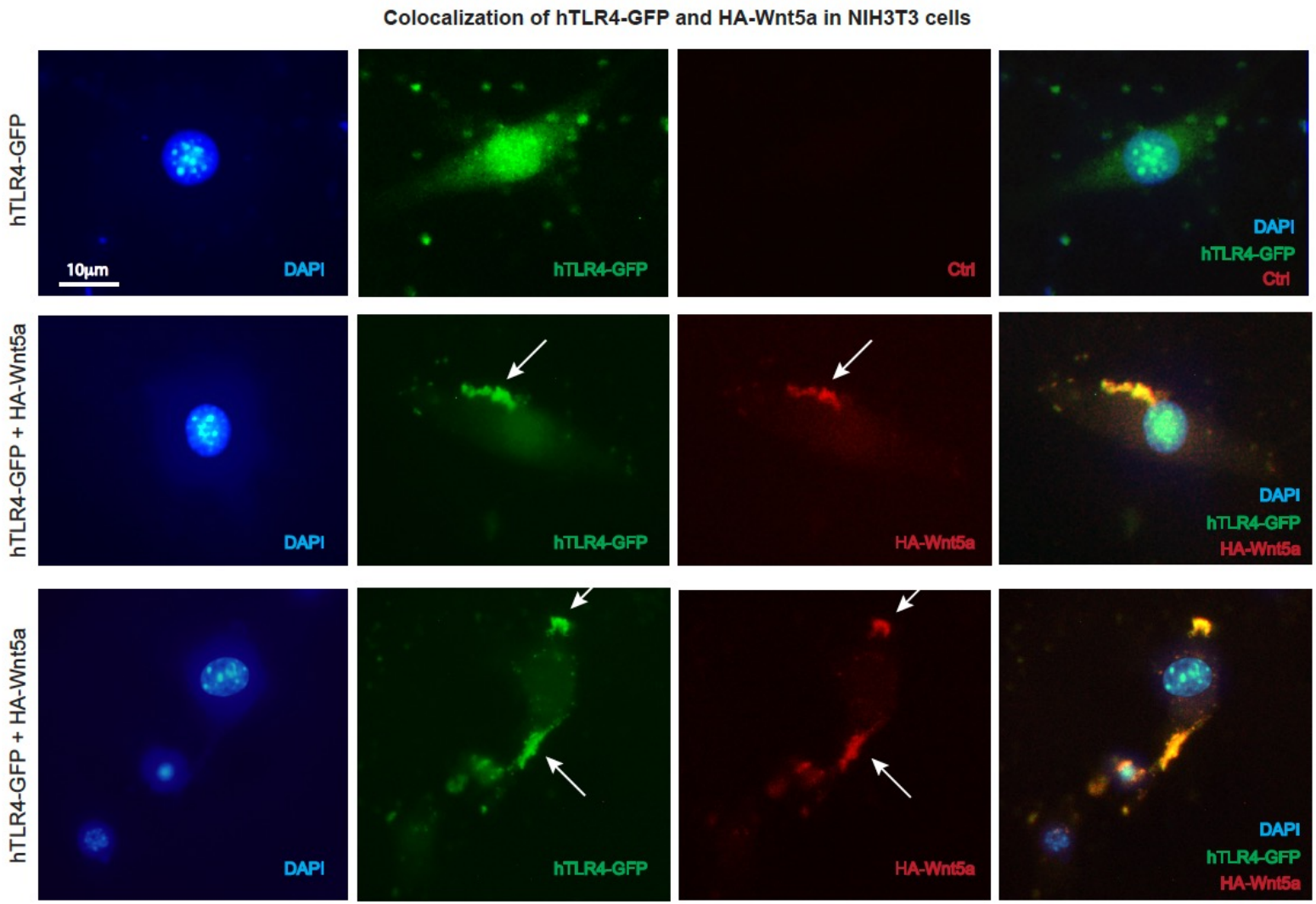

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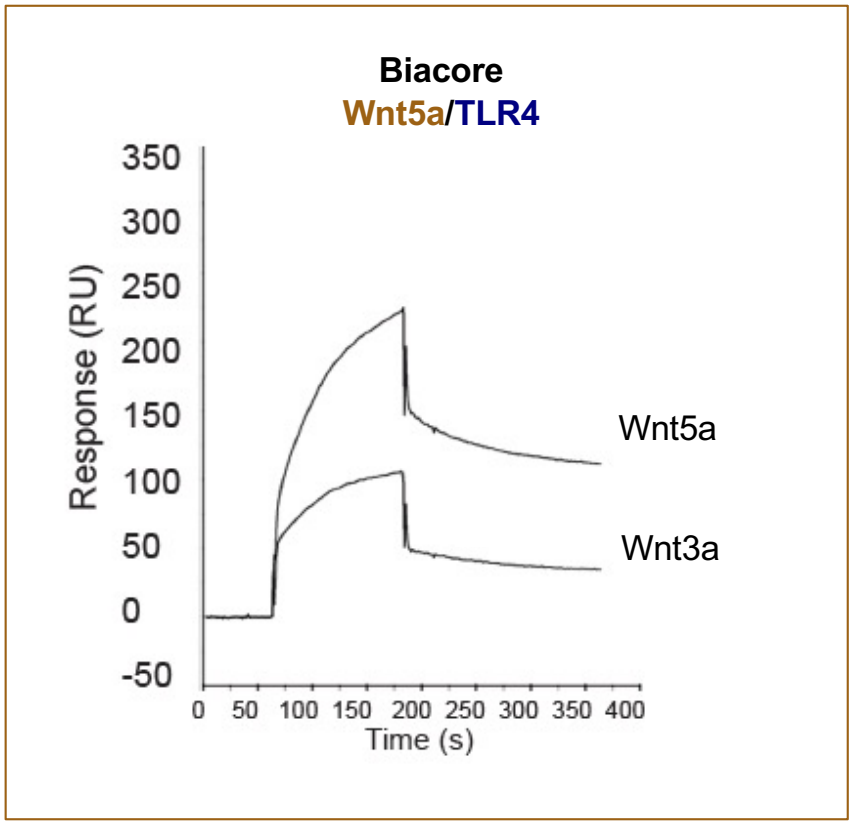

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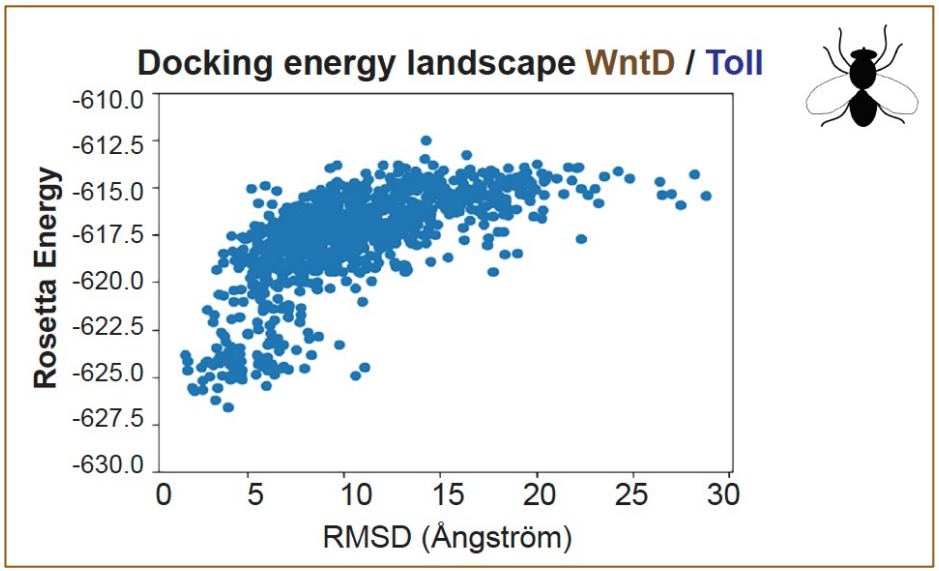

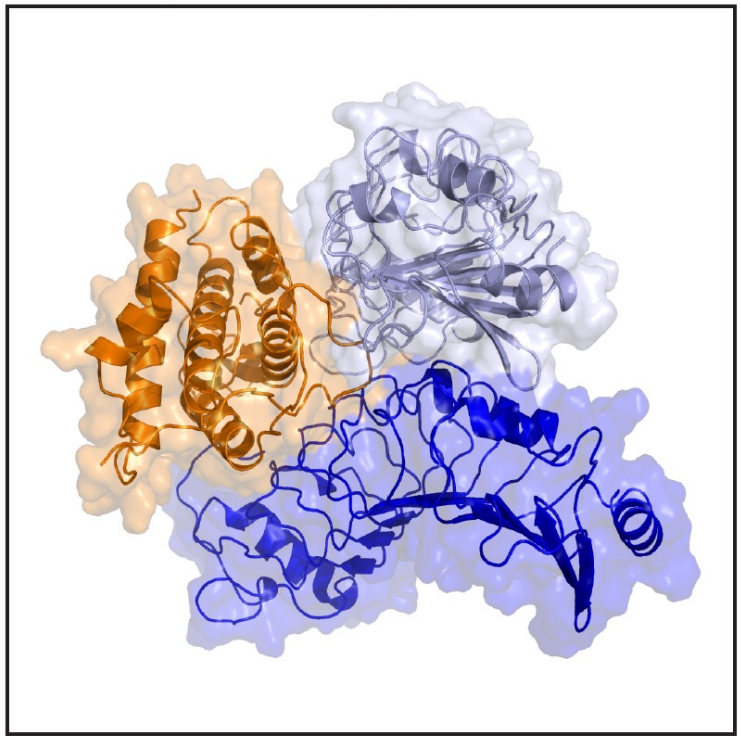

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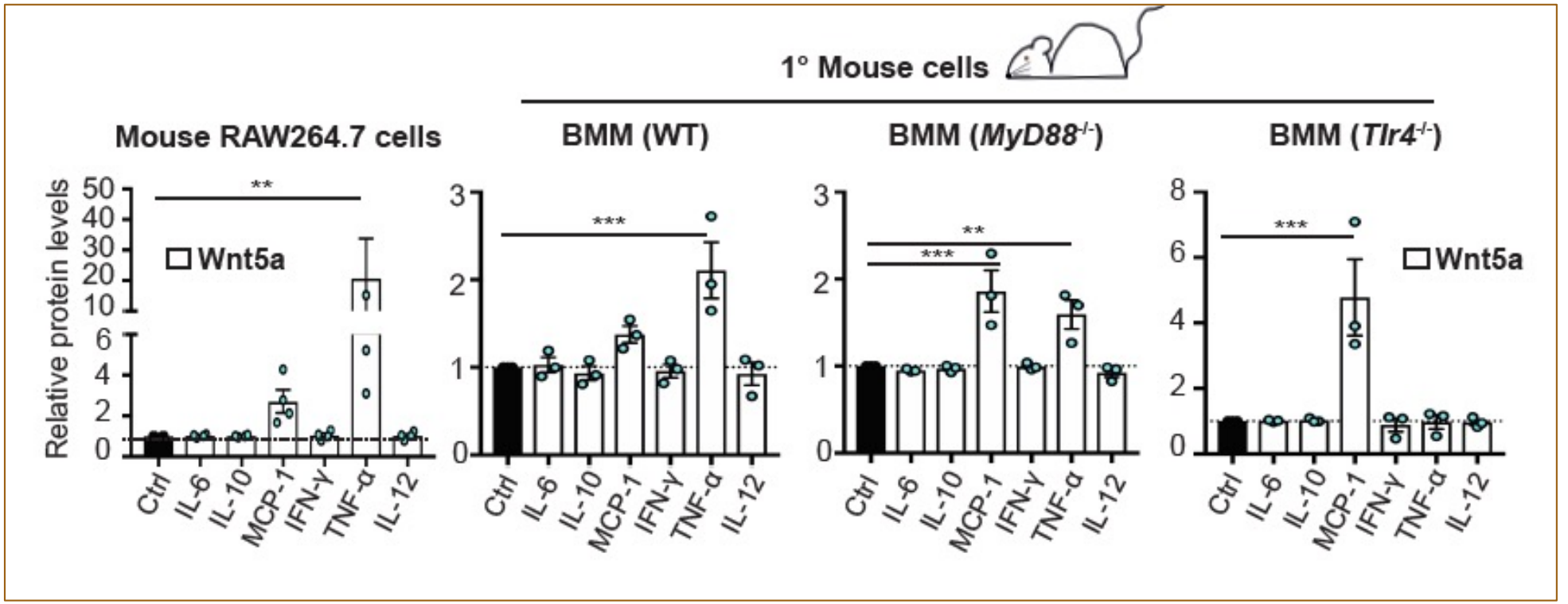

Opposite (pro-inflammatory) effect in mouse!

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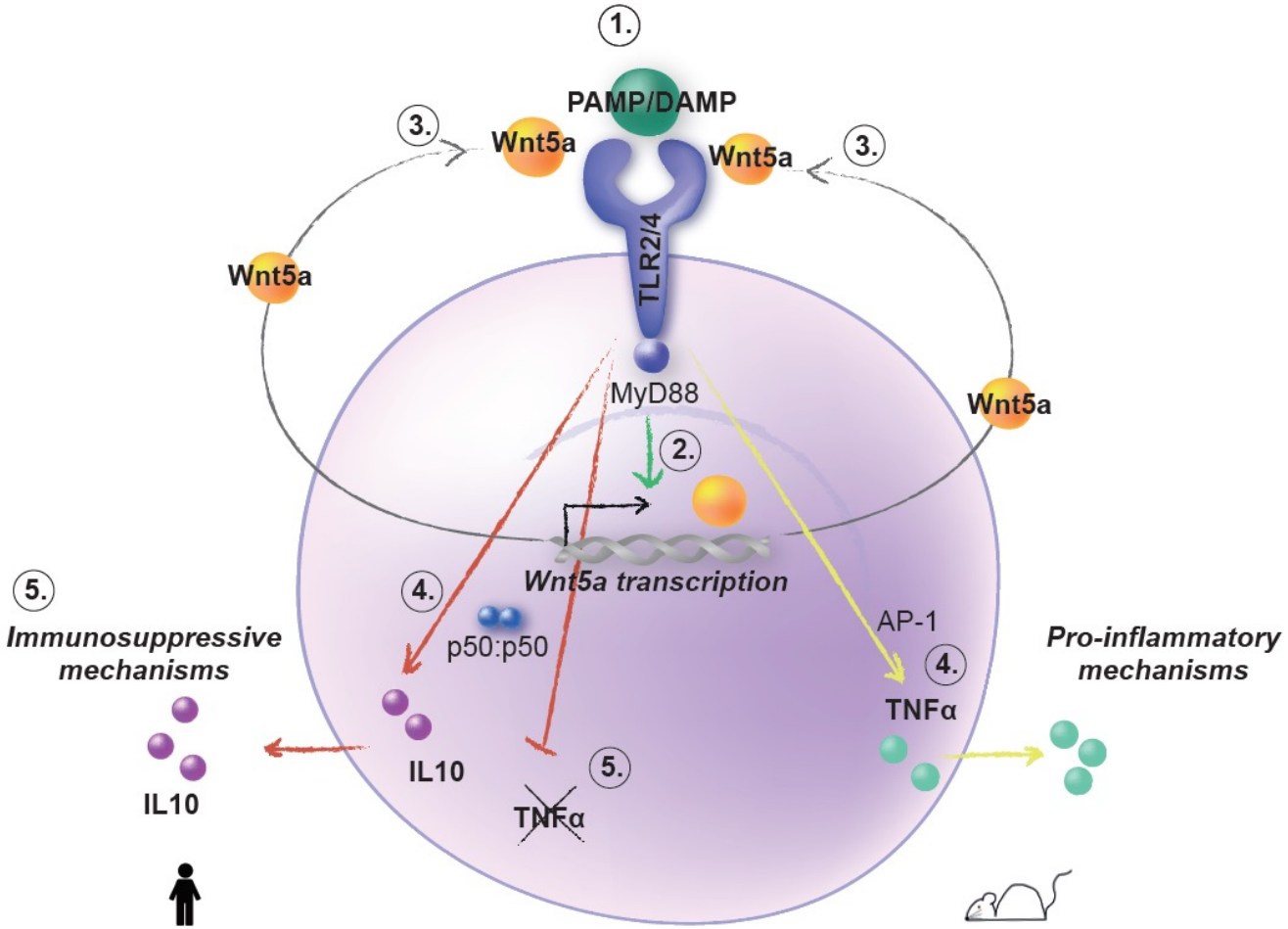
Wnt5a is a novel TLR4 ligand inducing tolerance in human myeloid cells (Mo-MDSCs)



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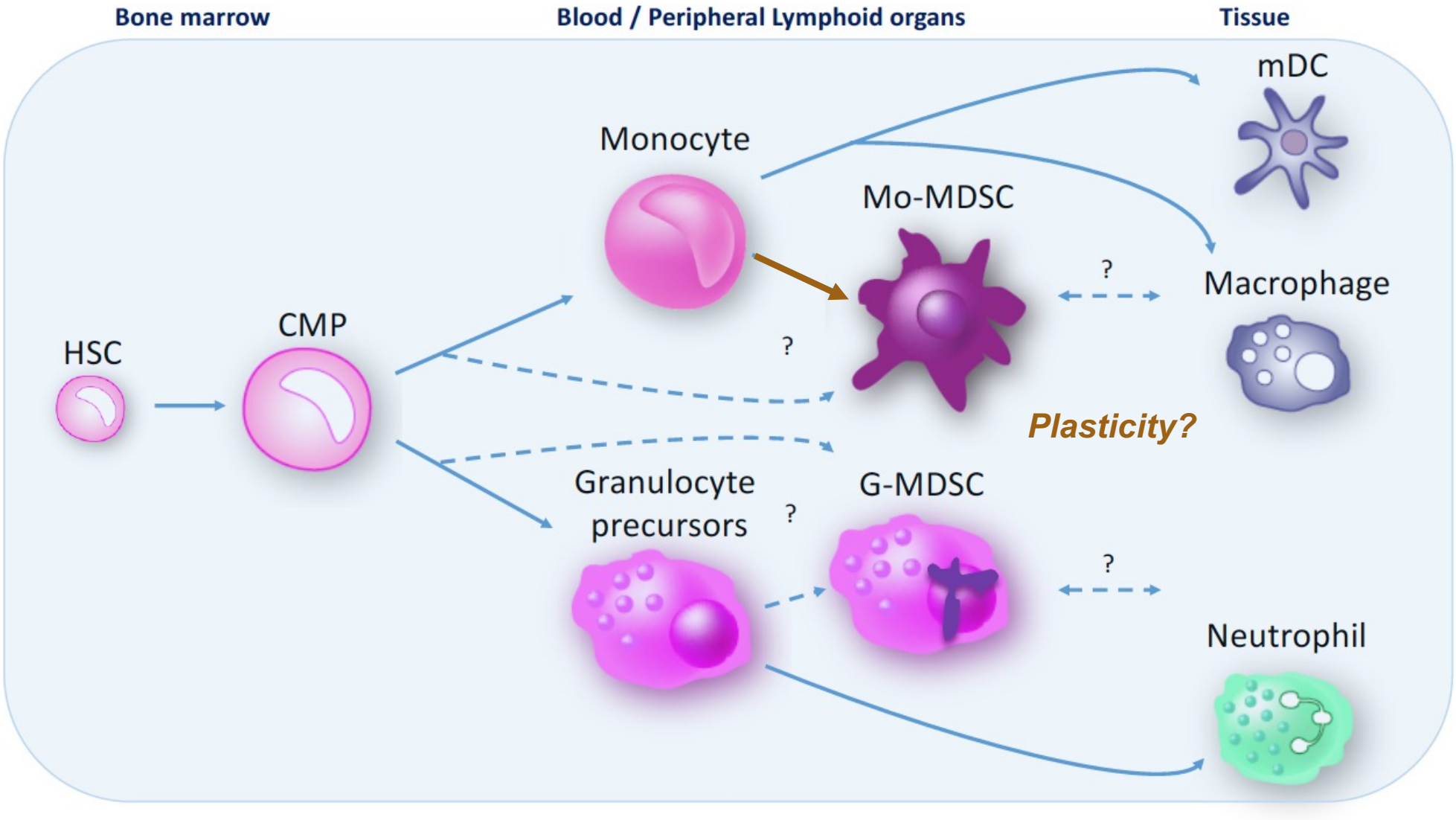
The generation of MDSCs



Systemic Anti-inflammatory myeloid cells

Mo-MDSCs are reprogrammed anti-inflammatory monocytes also in cancer patients

Functions in a tumor context?

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How does Breast tumor subtype affect monocyte differentiation and function?

- *Recruitment*
- *Survival*
- *Proliferation*
- *Differentiation*
- *Function*

1) Xenotransplants – NSG mice

Luminal A:

- MCF-7 or T47D cells

-/+ 1° Monocytes (Mo)

TNBC: - MDA-MB-231 or SUM-159 cells

-/+ 1° Monocytes (Mo)

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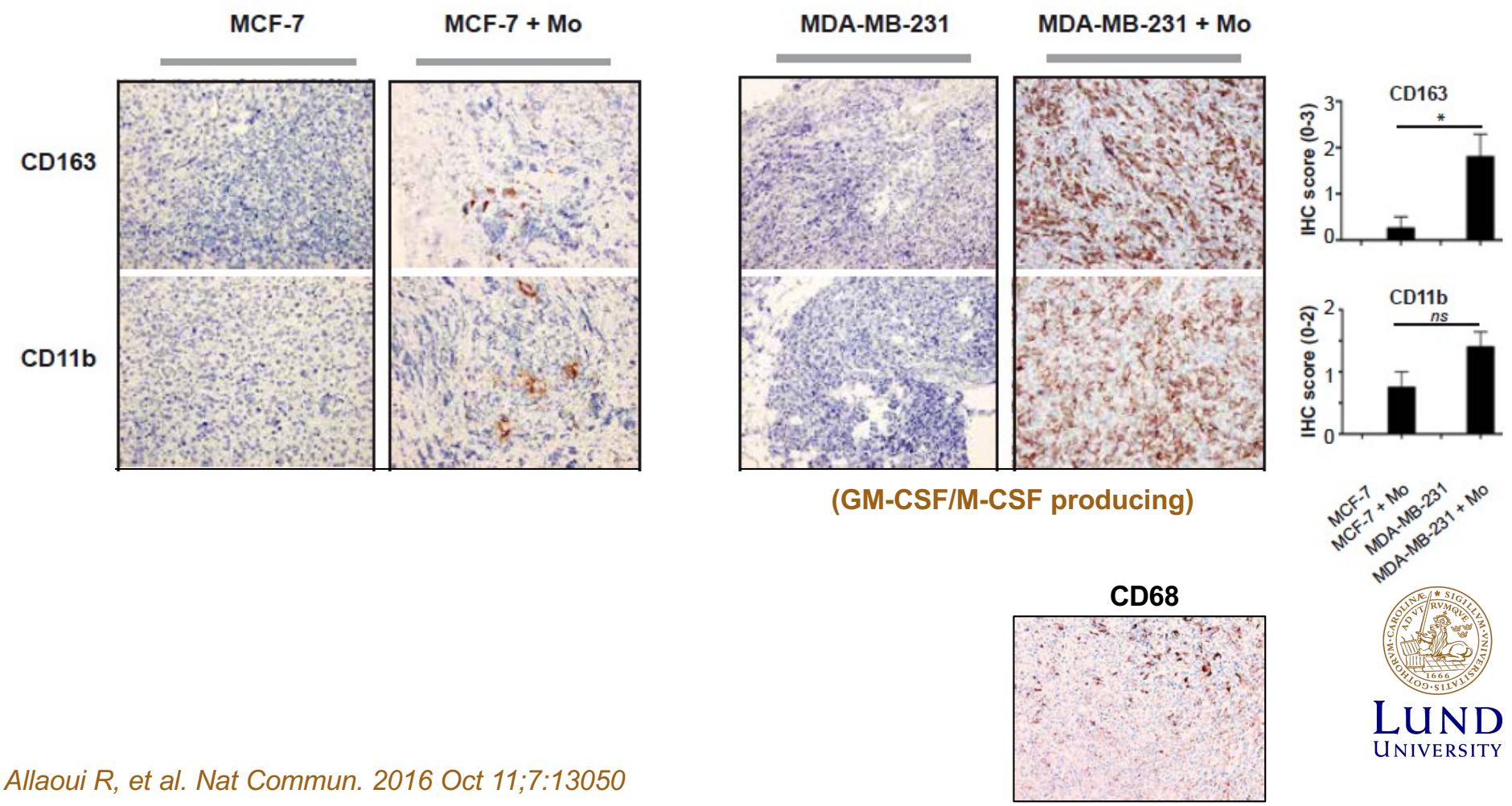
Allaoui R, et al. Nat Commun. 2016 Oct 11;7:13050

Monocytes differentiate into M2-like TAMs and Mo-MDSC like cells in a TNBC environment

Survival: Monocytes survive and expand primarily in a TNBC environment



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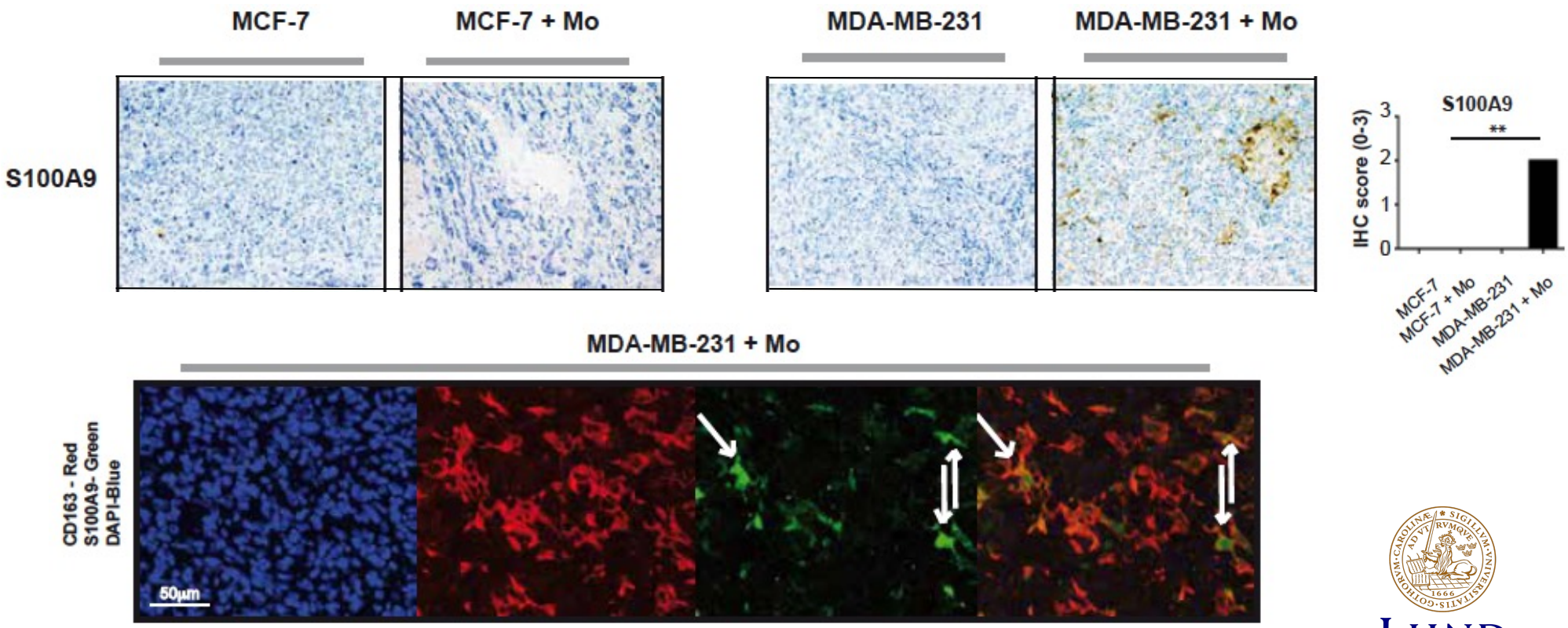
Allaoui R, et al. Nat Commun. 2016 Oct 11;7:13050

Monocytes differentiate into M2-like TAMs and Mo-MDSC like cells in a TNBC environment

Differentiation: Monocytes differentiate into M2-like TAMs and Mo-MDSC like cells in a TNBC environment

(CD163^{+}CD68$^{-/+}$S100A9^{+}) "MDSC marker"



Allaoui R, et al. Nat Commun. 2016 Oct 11;7:13050

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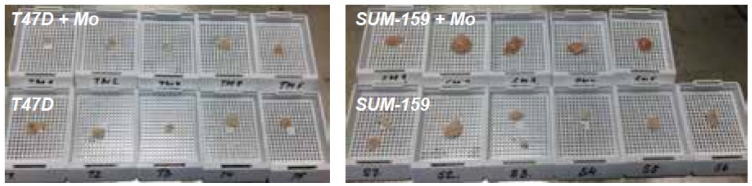
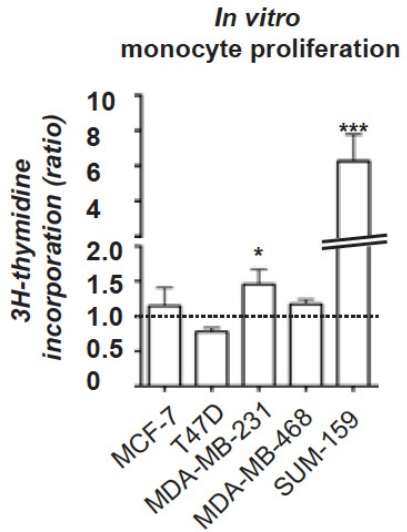
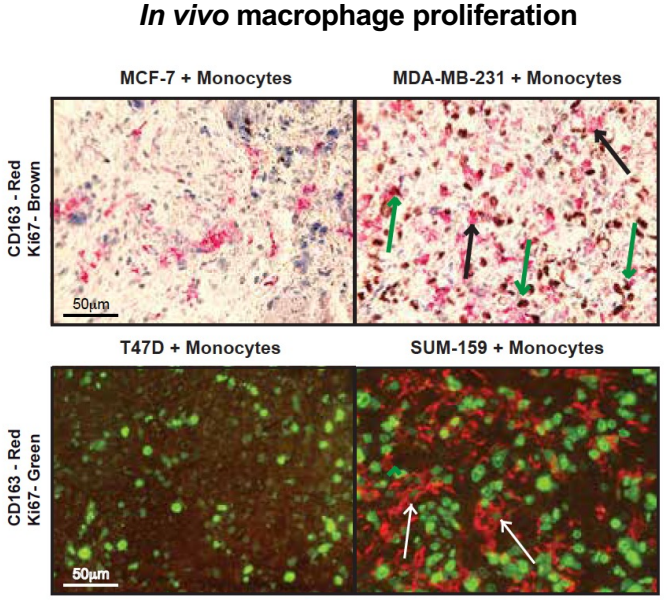
Monocytes differentiate into M2-like TAMs and Mo-MDSC like cells in a TNBC environment

Proliferation:
Monocytes and TAMs proliferate in a TNBC context







Allaoui R, et al. Nat Commun. 2016 Oct 11;7:13050

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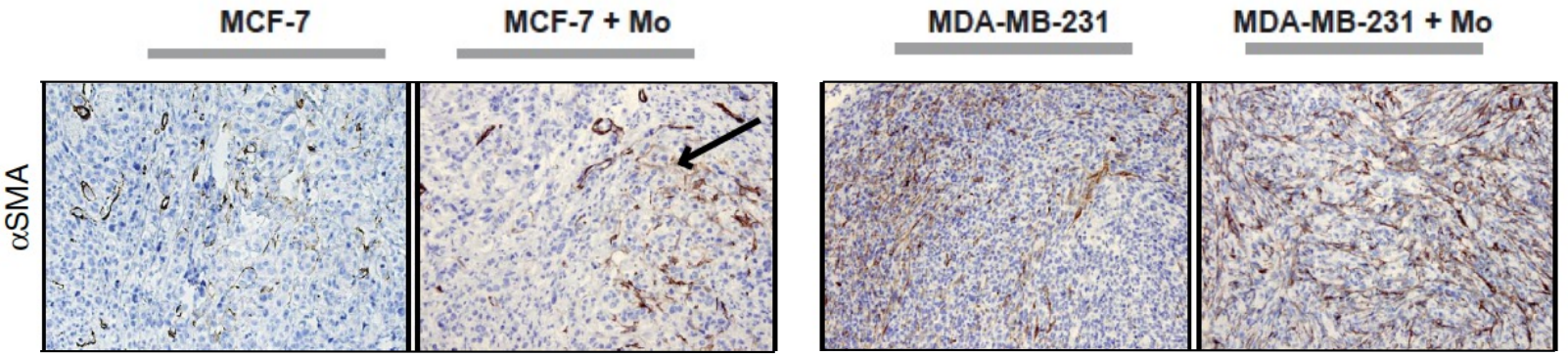
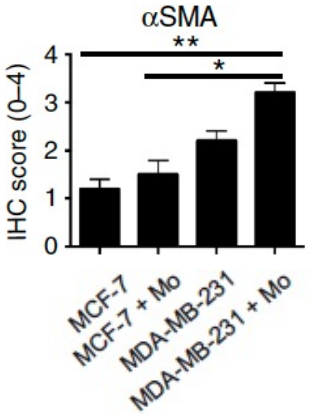
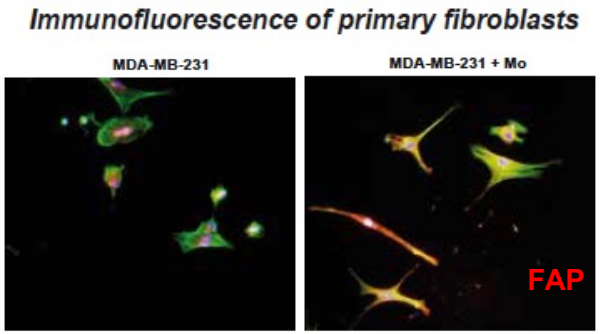
Monocytes differentiate into M2-like TAMs and Mo-MDSC like cells in a TNBC environment

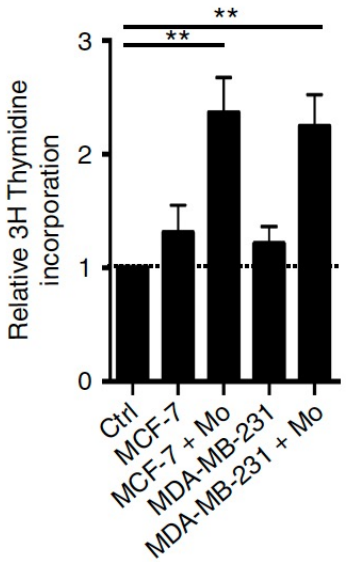
Effect on TME: Myeloid cells activate fibroblasts primarily in a TNBC environment





Primary fibroblast proliferation in co-culture supernatants





Allaoui R, et al. Nat Commun. 2016 Oct 11;7:13050

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Summary Mo-MDSCs

Human Mo-MDSCs are

”**reprogrammed**” anti-inflammatory monocytes that

associate with breast tumor type (ER^-) and disease progression.

Human Mo-MDSCs can promote tumor progression

by activating fibroblasts, tumor growth and myeloid cell proliferation primarily in a TNBC context.

- *Mehmeti et al Commun Biol May 9 2019*
- *Allaoui et al. Nat Commun. 2016 Oct 11*
- *Millrud et al Oncotarget. 2016 Sep 27*
- *Bergenfelz et al. PLoS One. 2015 May 20*
- *Janols et al. J Leukoc Biol. Nov 2014*
- *Bergenfelz et al J Immunol April 2 2012*

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Outline

- Origin / mechanism of generation of human Mo-MDSCs

→ Origin and function of human G-MDSCs

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Systemic Anti-inflammatory myeloid cells

Summary of our research on human G-MDSCs

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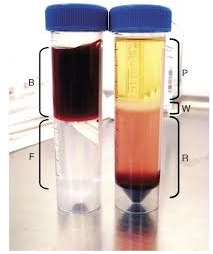
Myeloid derived suppressor cells

CD11b^{+}Ly6C^{low}Ly6G^{+}

?

CD11b^{+}CD33^{+} CD15^{+}
Lin^{-} HLADR$^{-/low}$



- Based on surface phenotype

Granulocytic MDSCs

(G-MDSCs or PMN-MDSC)

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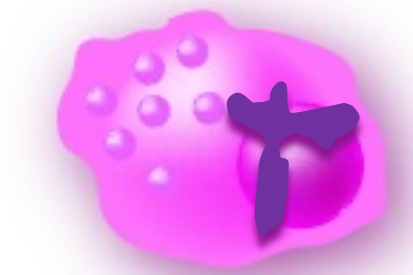
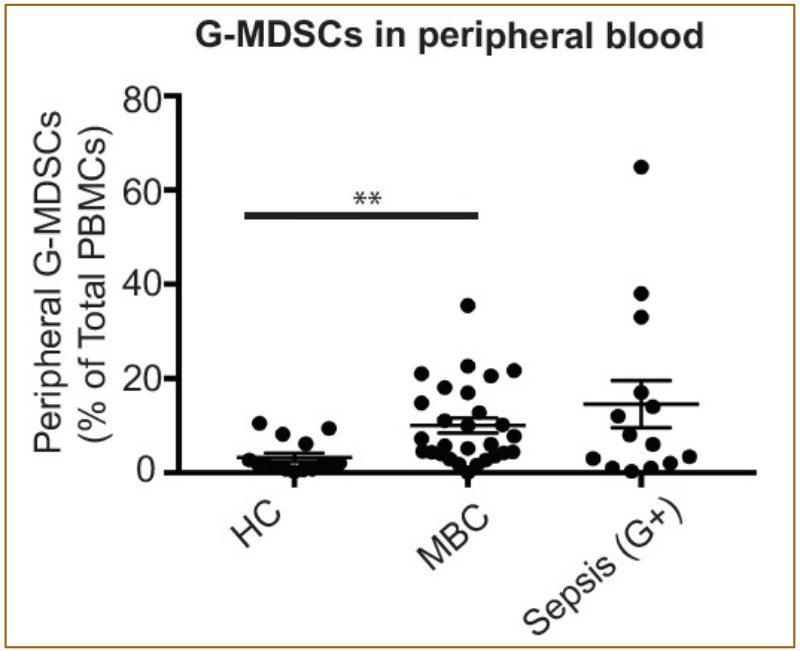
Systemic G-MDSCs in breast cancer patients

- *Increased but does not correlate with any clinical parameter*
- *or disease progression*

G-MDSCs





…Except peripheral blood neutrophil count
(P=0.05)

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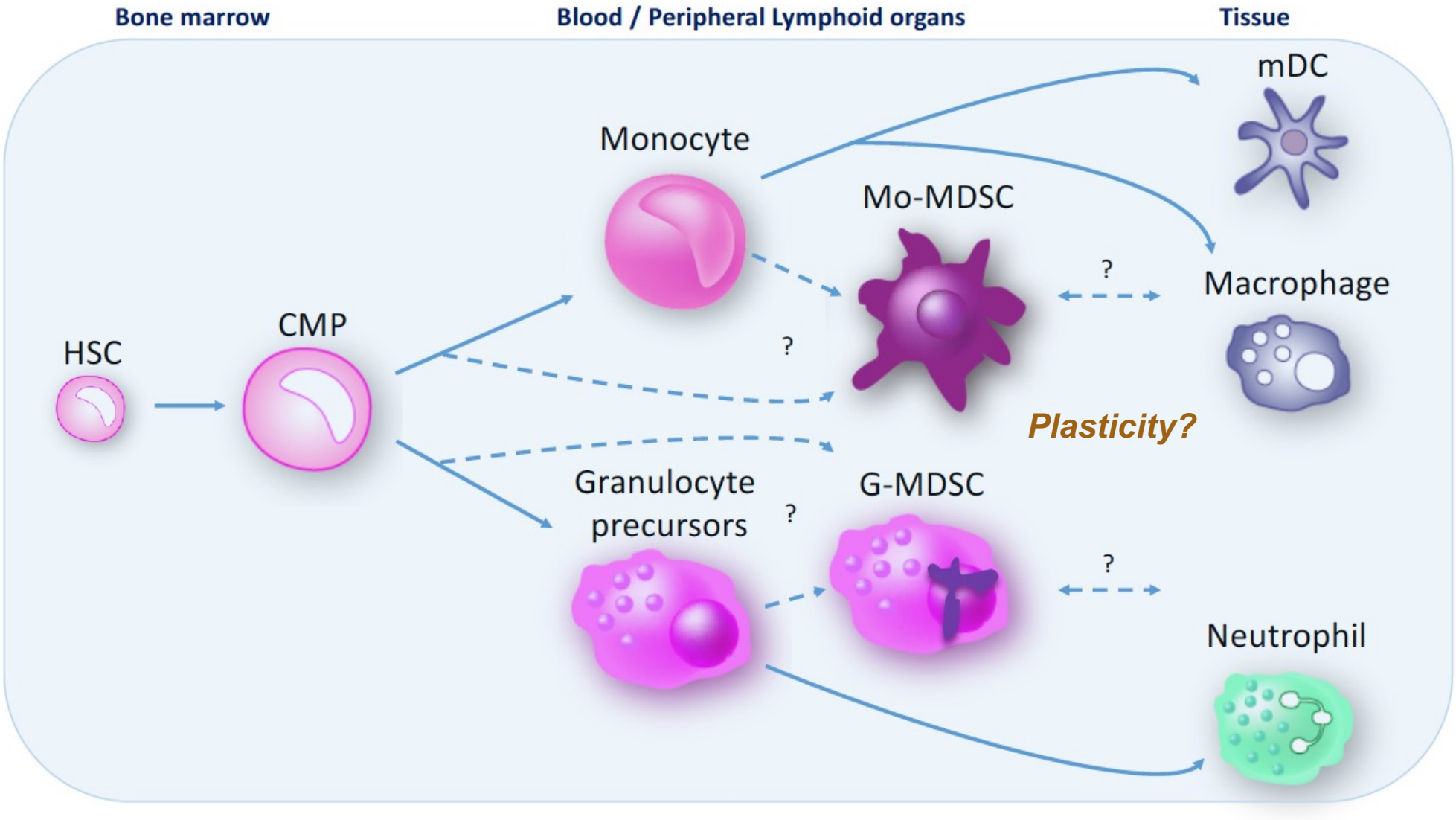
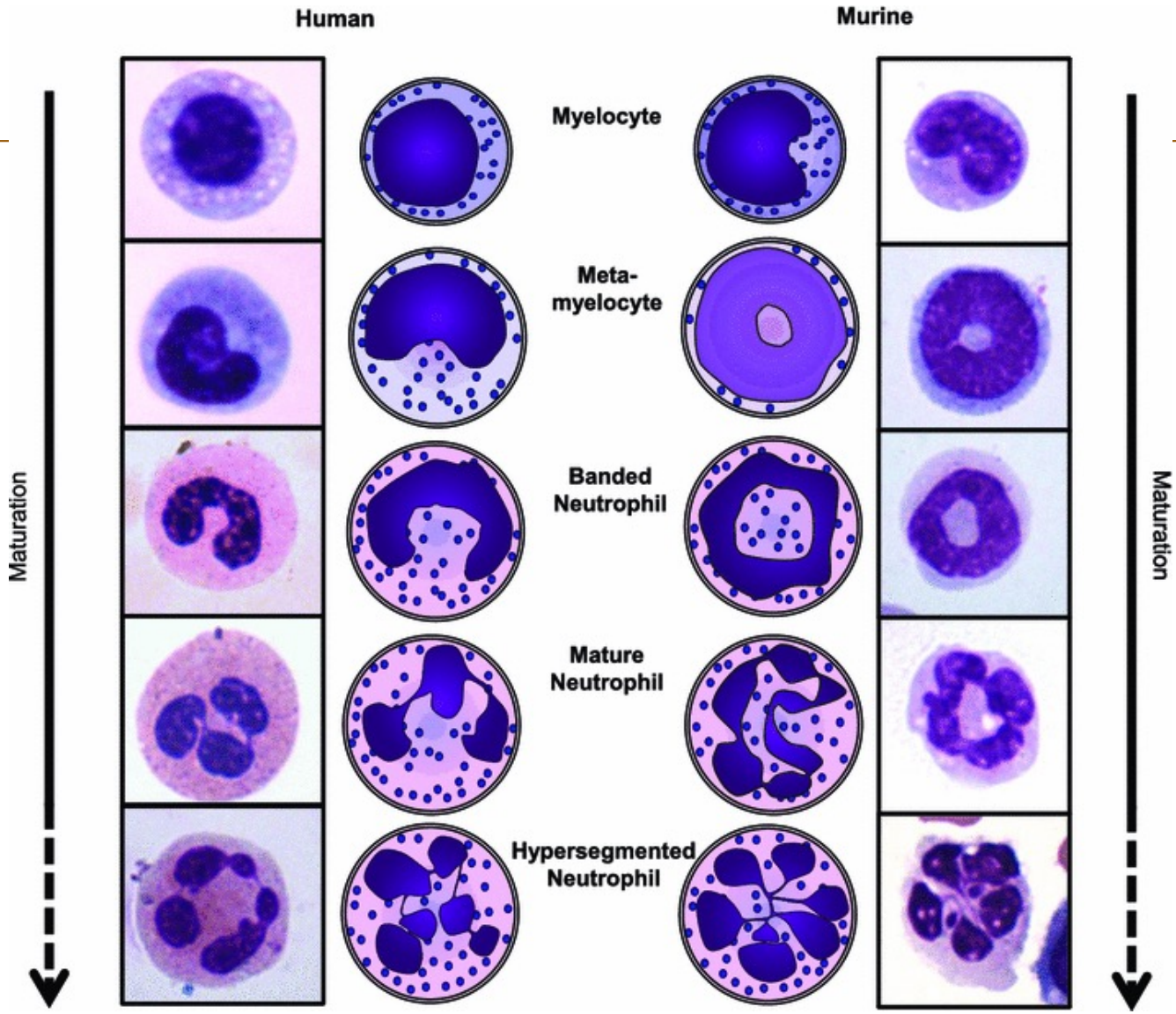
Mehmeti et al 2020 LSA

The generation of MDSCs



Neutrophil maturity



Adopted from Pillay et al Cell Mol Life Sci 2013

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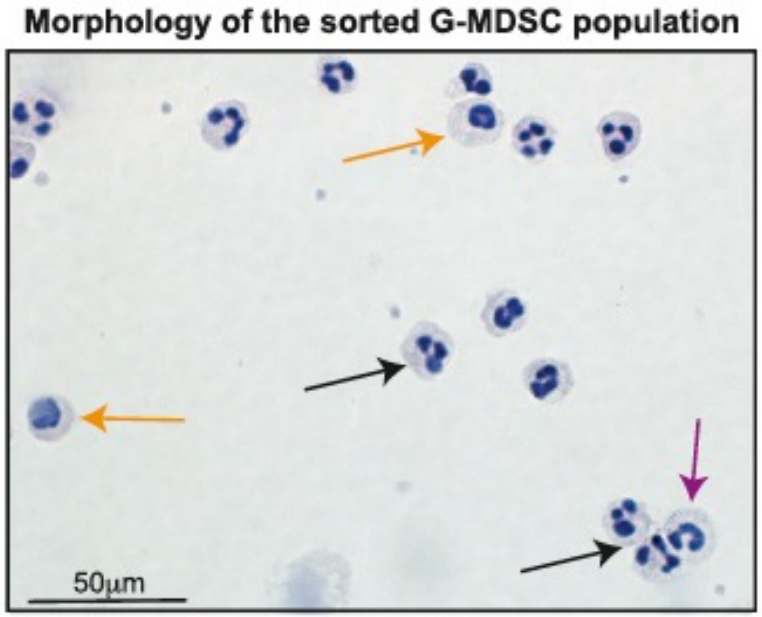
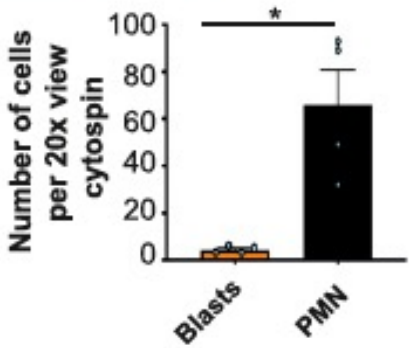
Human G-MDSCs are neutrophils at distinct maturation stages promoting tumor growth in breast cancer





Human PMN G-MDSCs → Activated neutrophils

Human blast *G-MDSCs* → *Immature neutrophils*

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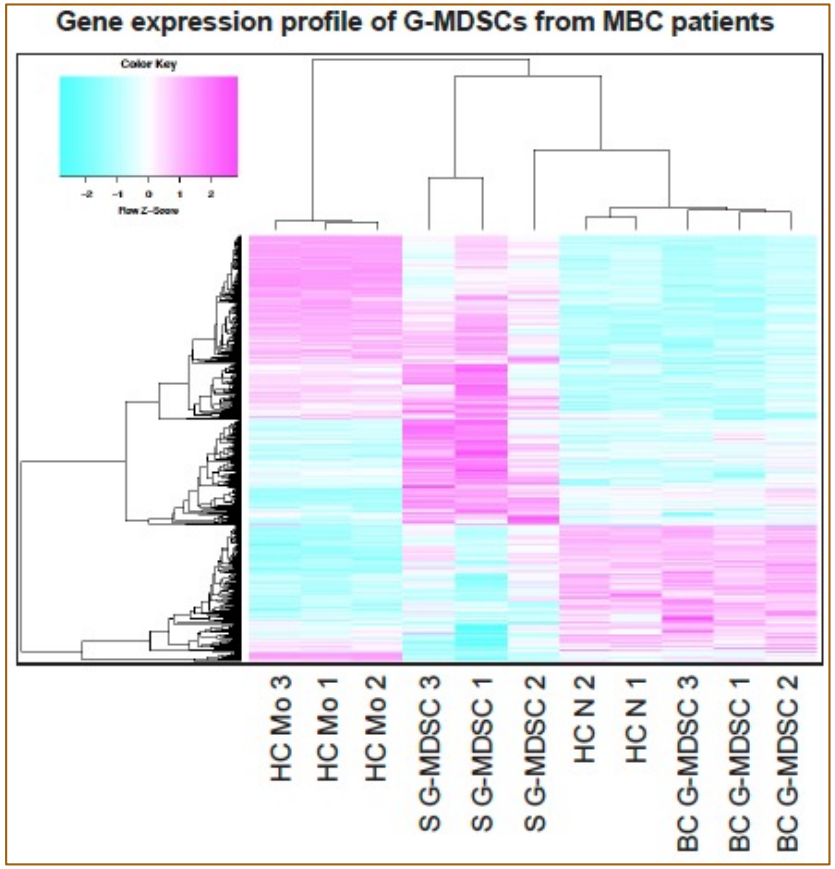
Mehmeti et al 2020 LSA

Human G-MDSCs are neutrophils at distinct maturation stages promoting tumor growth in breast cancer



HC Mo – Healthy donor monocytes (negative ctrl)
HC N- Healthy donor neutrophils
BC G-MDSC- MBC G-MDSCs
S G-MDSC- Sepsis G-MDSCs (positive ctrl)

Selected genes of interest HC N > MBC and Sepsis	
CXCL8	Chemotaxis, inflammation
Selected genes of interest MBC and HC N > Sepsis	
CSF1R (CD115)	Differentiation, Survival
CXCR2 (IL8Rβ)	Chemotaxis, MDSC marker
CXCL1	Chemotaxis
EPHB1	Angiogenesis
ppp1R16b (TIMAP)	Angiogenesis
FGF6	Tissue repair, metastasis
Selected genes of interest MBC and Sepsis > HC N	
ARG1	Immunosuppression
MMP8	Invasion, metastasis
TLR5	TLR (flagellin, HMGB1 ligands)
CD177	Transendothelial migration, Ly6-family
Selected genes of interest MBC > Sepsis and HC N	
FGF2	Angiogenesis, Immune exclusion
LYVE-1	Lymphangiogenesis
FGL2	Immunosuppression
TIAM2	Metastasis, Rho-GTPase signaling
Selected genes of interest Sepsis > MBC and HC N	
CD163	Immunosuppression
TGFBR1	Immunosuppression
CD24	Neutrophils, myelocytes

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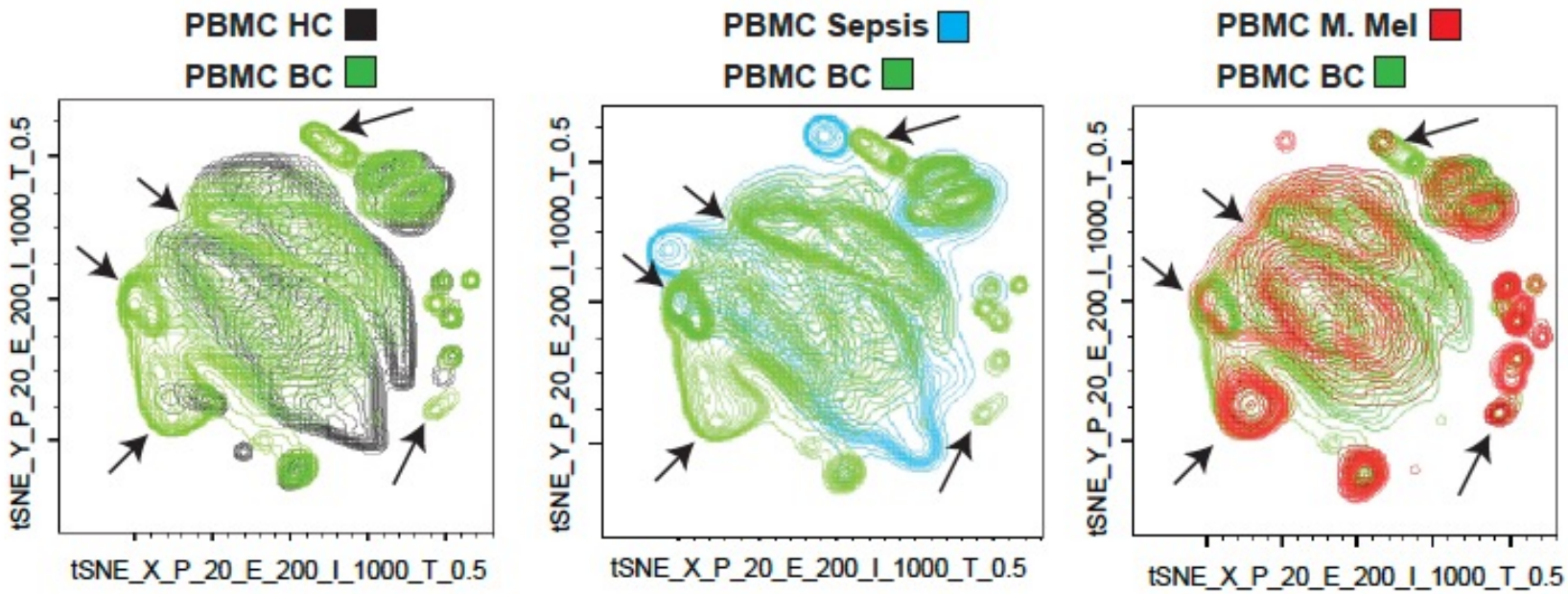
G-MDSCs from metastatic breast cancer patients:

- *- are dissimilar to sepsis induced G-MDSCs*
- *are similar to G-MDSCs from other type of cancer patients*
- *are mostly similar to mature neutrophils from healthy donors…but in cancer patients distinct maturation stages of neutrophils are represented*

Subpopulation of *immature* neutrophils in cancer patients ($CD10^{-}CD13^{-}$)



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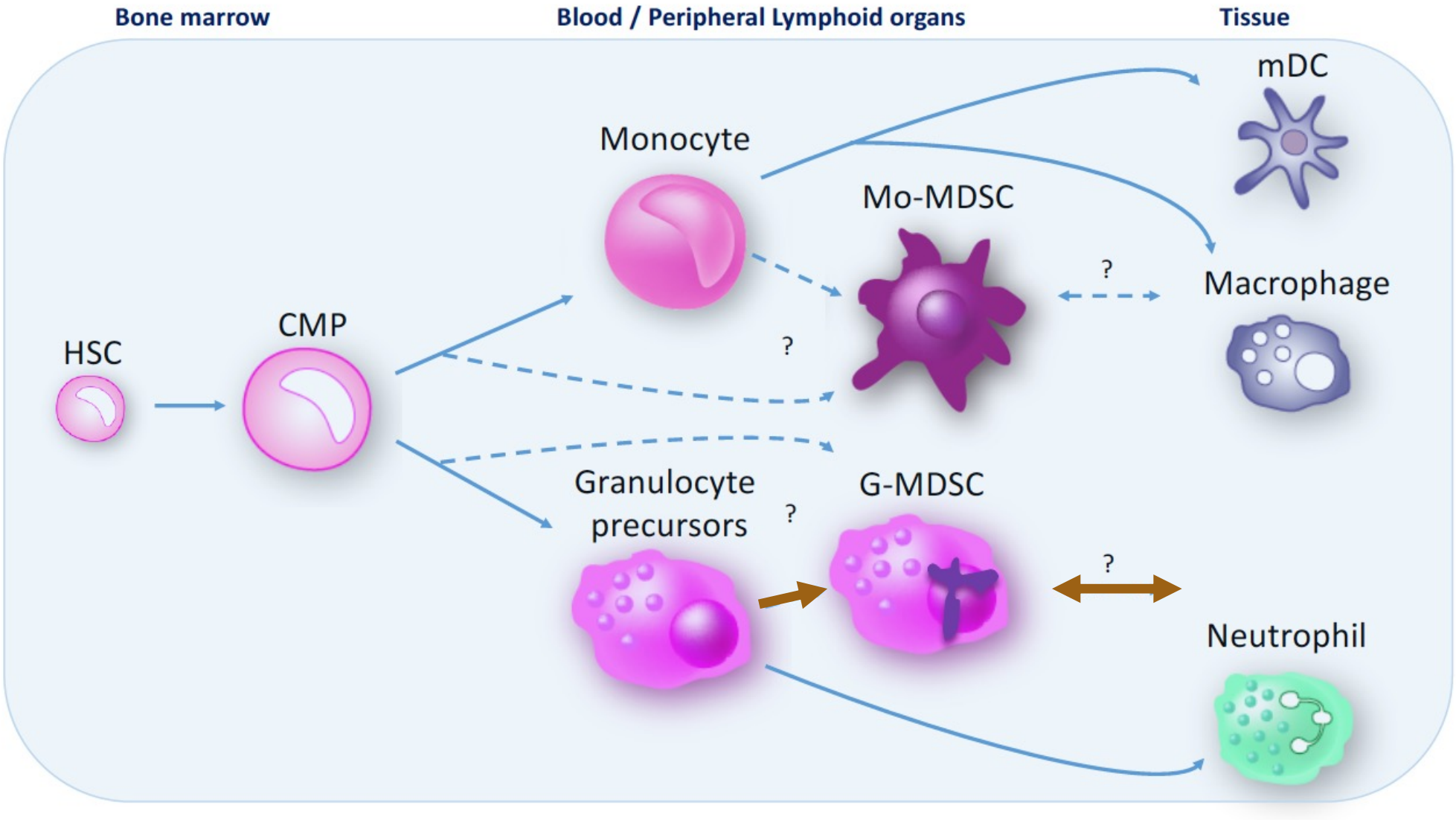
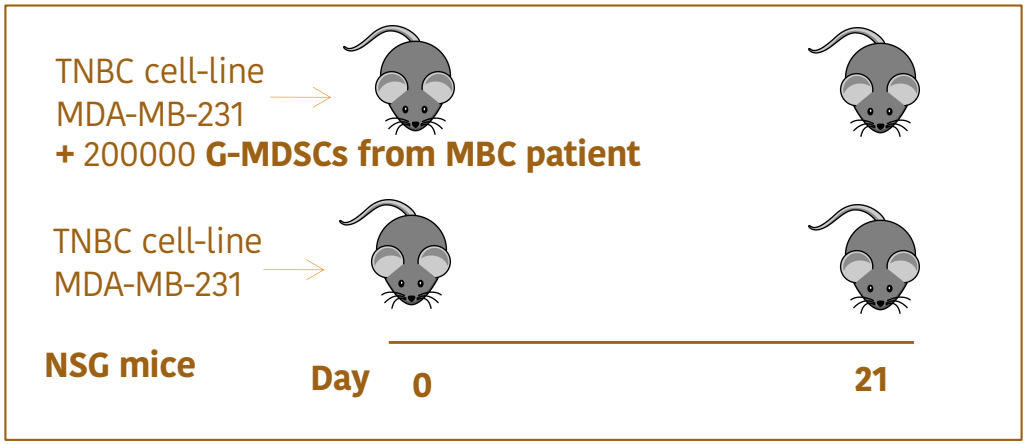
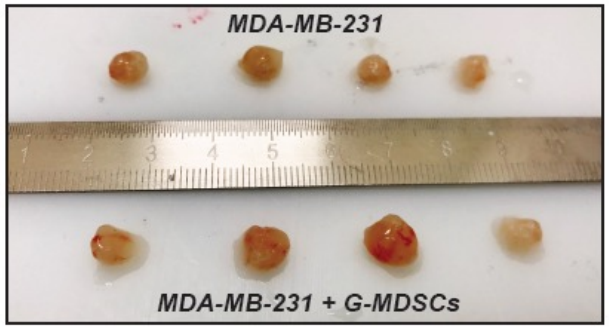
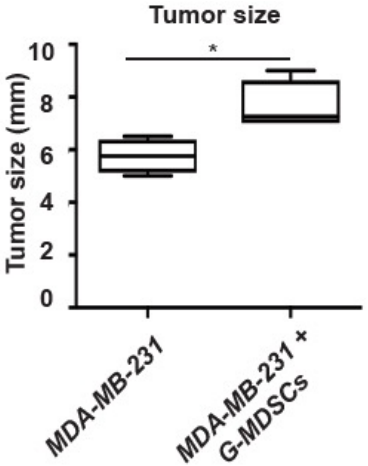
The generation of MDSCs

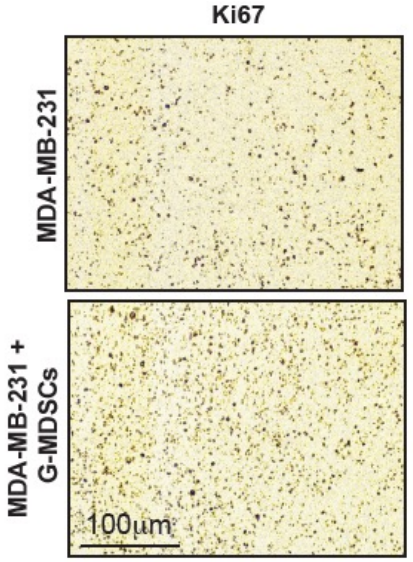


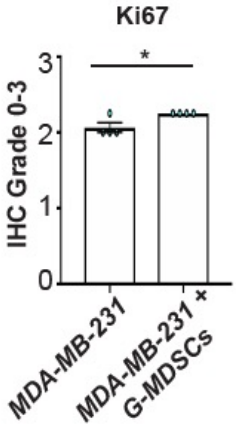
Human G-MDSCs are neutrophils at distinct maturation stages promoting tumor growth in breast cancer











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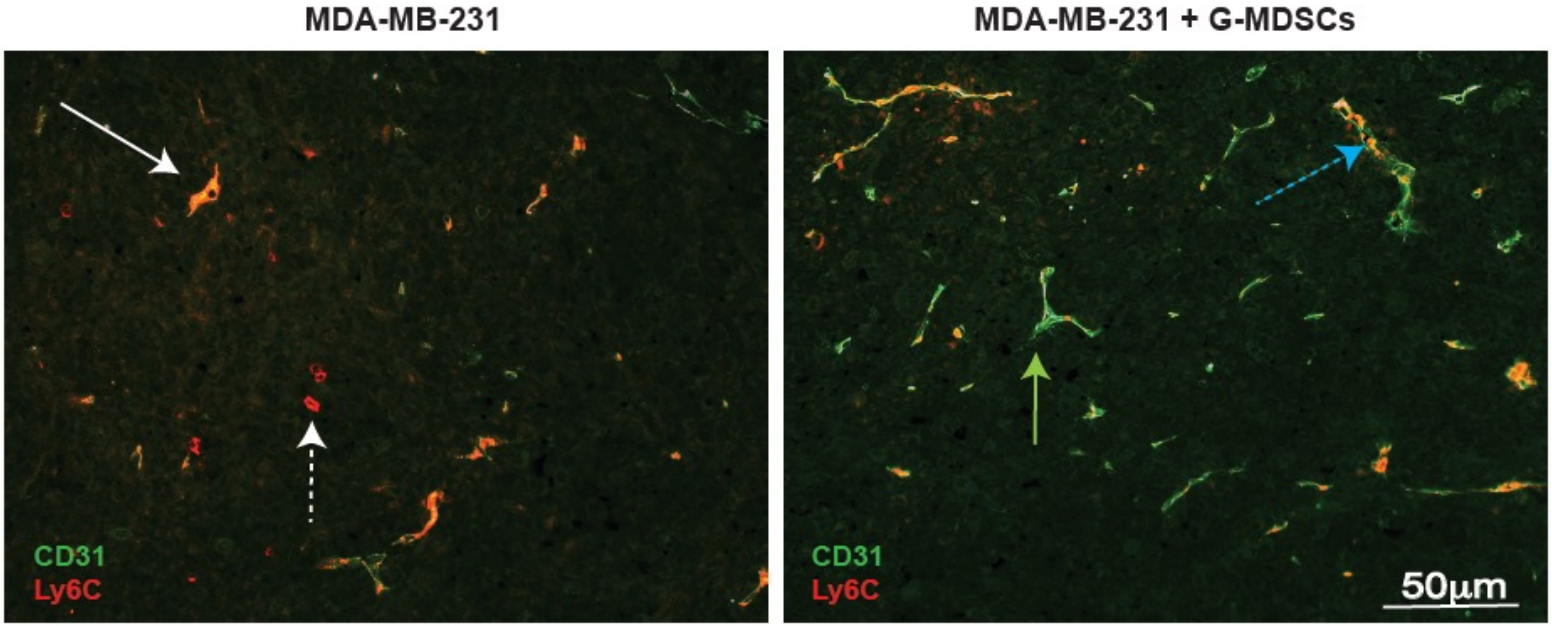
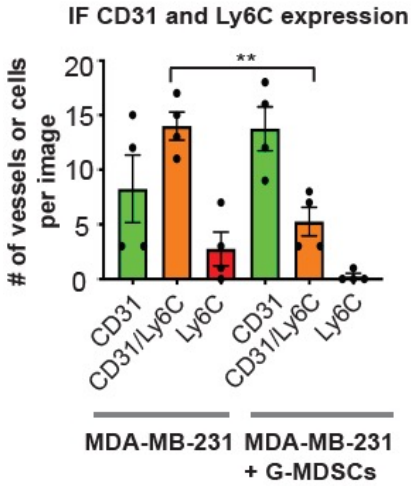
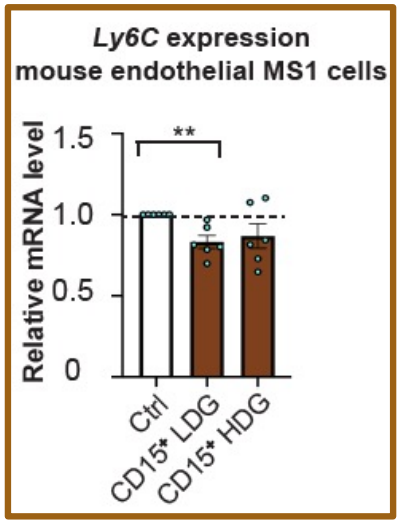
Ly6C is expressed on endothelial like structures only in tumors *without* G-MDSCs







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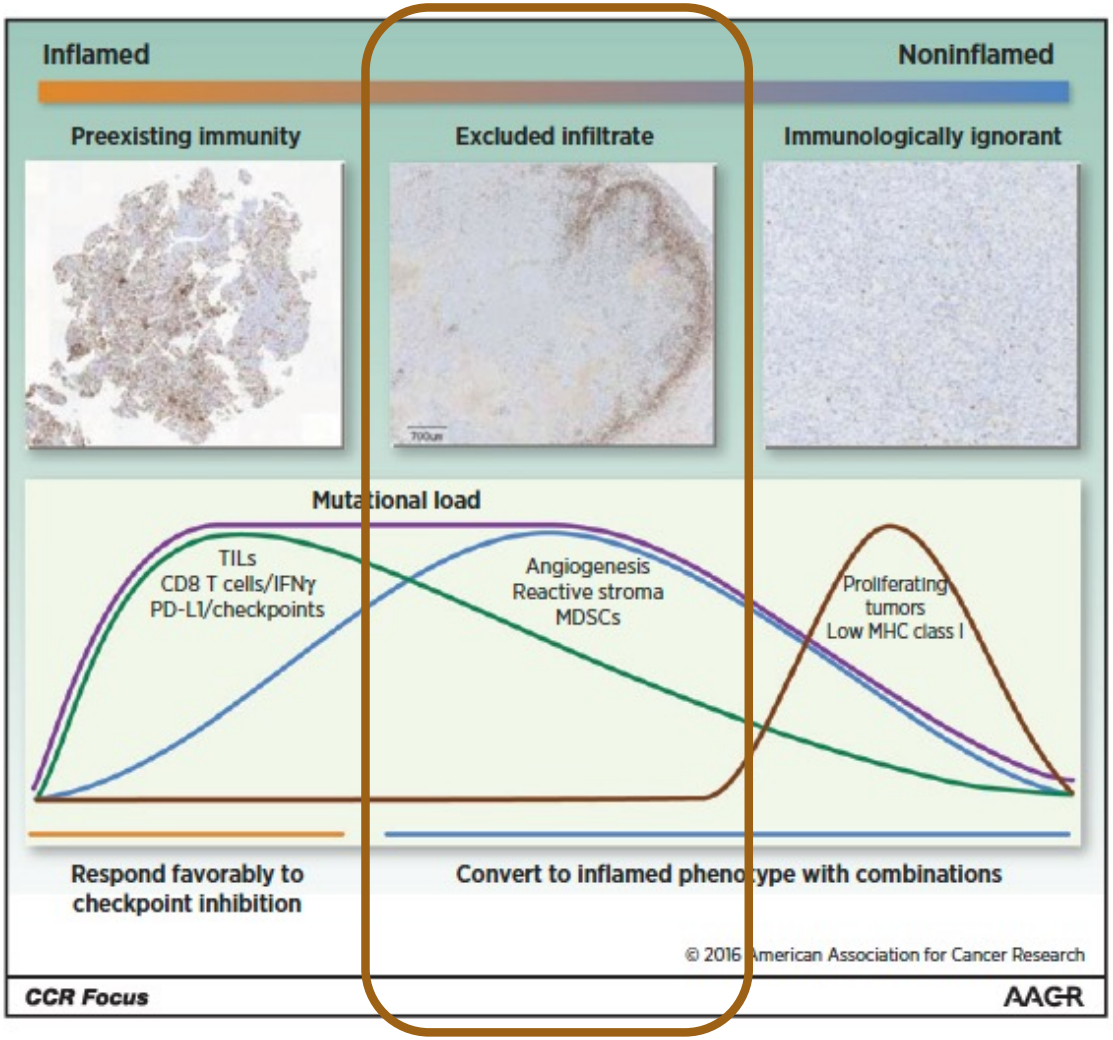
Human MDSCs involved in immune exclusion?



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Systemic Anti-inflammatory myeloid cells

Human G-MDSCs affect tumor growth and blocks myeloid immune infiltration *in vivo*

→ *Mechanism immune exclusion?*

- *Janols et al. J Leukoc Biol. Nov 2014*
- *Mehmeti et al 2020 LSA*
- *Review Bergenfelz and Leandersson Front Oncol 2020*

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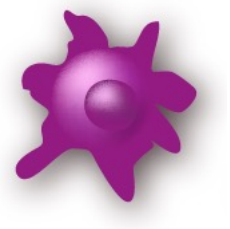
Summary



- Mo-MDSCs and G-MDSCs are enriched in breast cancer patients.
- Mo-MDSCs correlate with breast cancer type and disease progression in breast cancer patients – *Soluble Biomarker for metastatic breast cancer?*

- Human Mo-MDSCs → derive from Reprogrammed monocytes (TAMPs)
- Human G-MDSCs → Majority are activated neutrophils
  - *G-MDSC blasts – Immature neutrophils, Bona fide G-MDSCs?*

- Human Mo-MDSCs induce tumor growth & fibroblast activation *in vivo*
- Human G-MDSCs affect tumor growth *in vivo*

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- Professor **Ingemar André,** Biochemistry, Lund University
- Professors **Fredrik Ivars** and **Bengt Johansson-Lindbom,** Immunology, Lund University
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- PhD **Per Björk**, Active Biotech AB, Lund
- PhD **Robert Carlsson** and Professor **Gesine Paul-Visse** Translational Neurology, Lund University
- PhD **Jörgen Adolfsson**, **SciLife**, Linköping University, Sweden
- All co-authors

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Percy Falks stiftelse

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Cancerfonden

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