

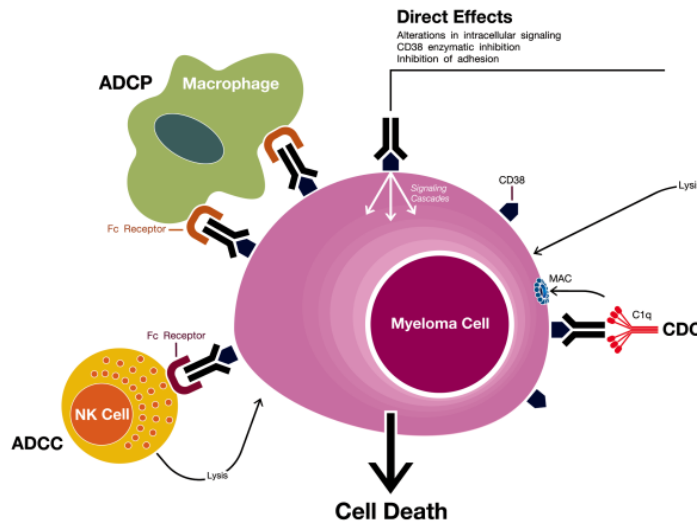
Anti-CD38 antibody therapy: basic science and combined immunotherapy strategies

F. Malavasi, Barbara Castella, F. Morandi, A. C. Faini,
Yuliya Yakymiv & A. Horenstein

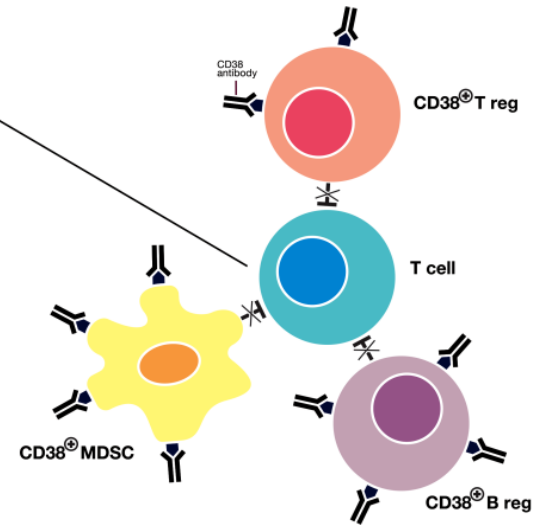
Lab of Immunogenetics
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A

Fc-dependent immune effector mechanisms and direct effects



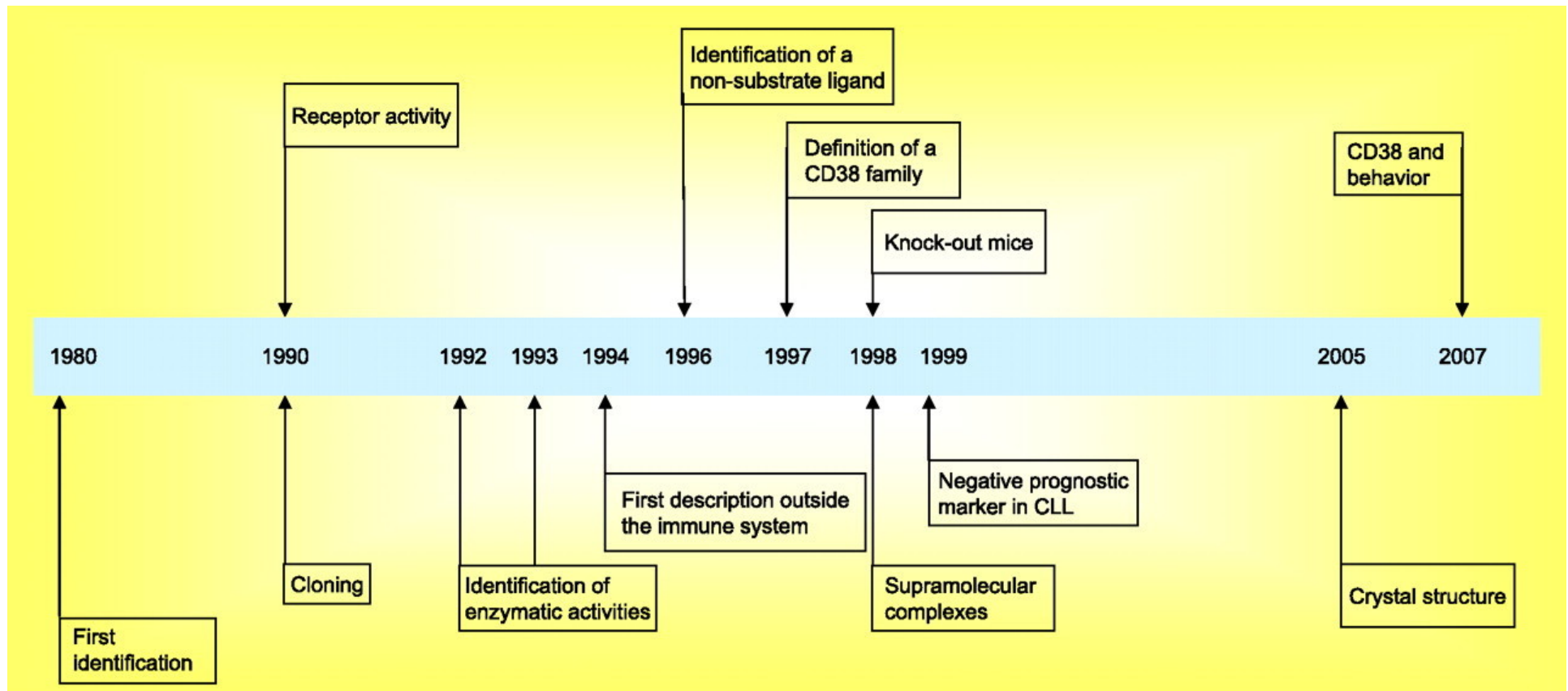
Immunomodulatory Effects



B

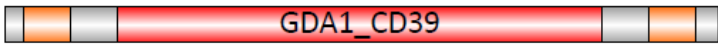
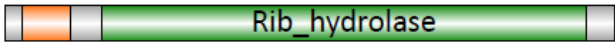




MoA	DARATUMUMAB	ISATUXIMAB	MOR202
Origin, isotype	Human IgG-kappa	Chimeric IgG1-kappa	Human IgG1-lambda
CDC	+++	+	+
ADCC	++	++	++
ADCP	+++	nd	++
PCD direct	-	++	-
PCD cross linking	+++	+++	+++
Modulation ectoenzyme function	+	+++	-

Story of the CD38 gene family before therapeutic antibodies

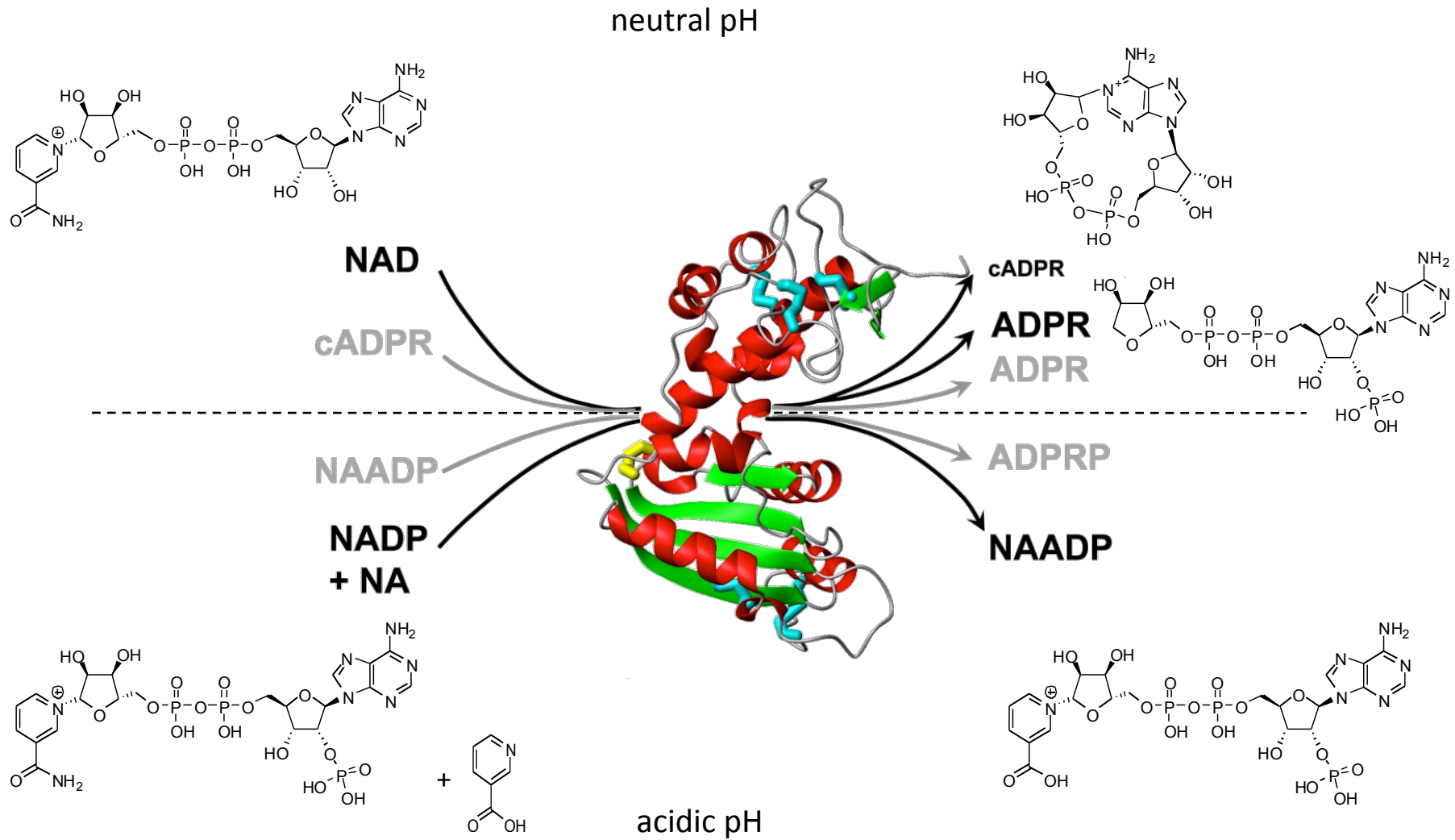


Monoclonal antibody therapy
and tumor escape:
the Daratumumab model

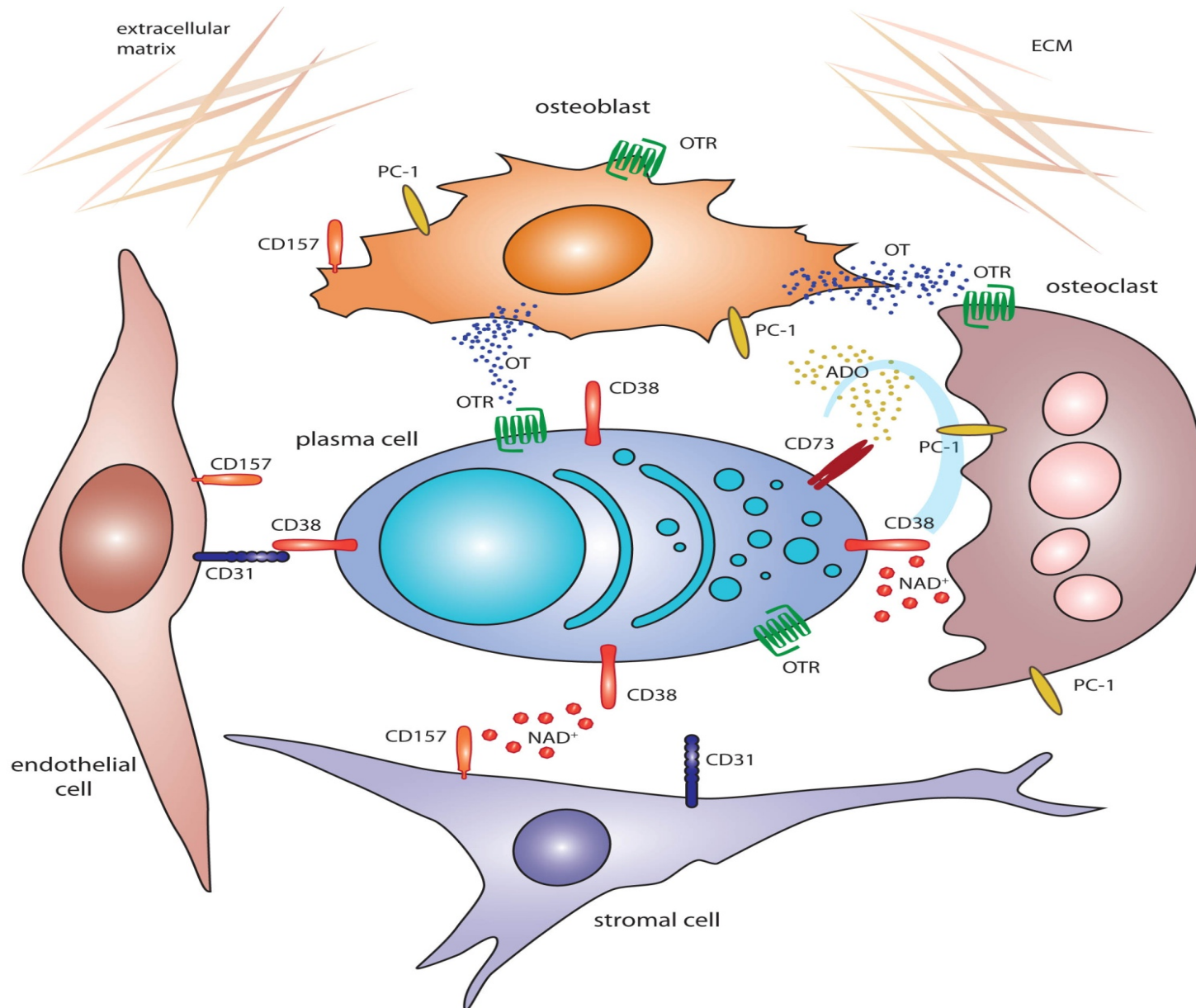
Ectoenzymes and protein structure

	Human protein domain organization		% amino acid identity with human ortholog		
			Mouse	Chicken	Xenopus
CD39		510 aa →	76%	63%	55%
CD38		300 aa →	59%	45%	42%
CD157		318 aa →	74%	52%	50%
CD73		574 aa →	86%	64%	66%
CD203a		925 aa →	79%	66%	60%
CD203c		875 aa →	81%	67%	62%

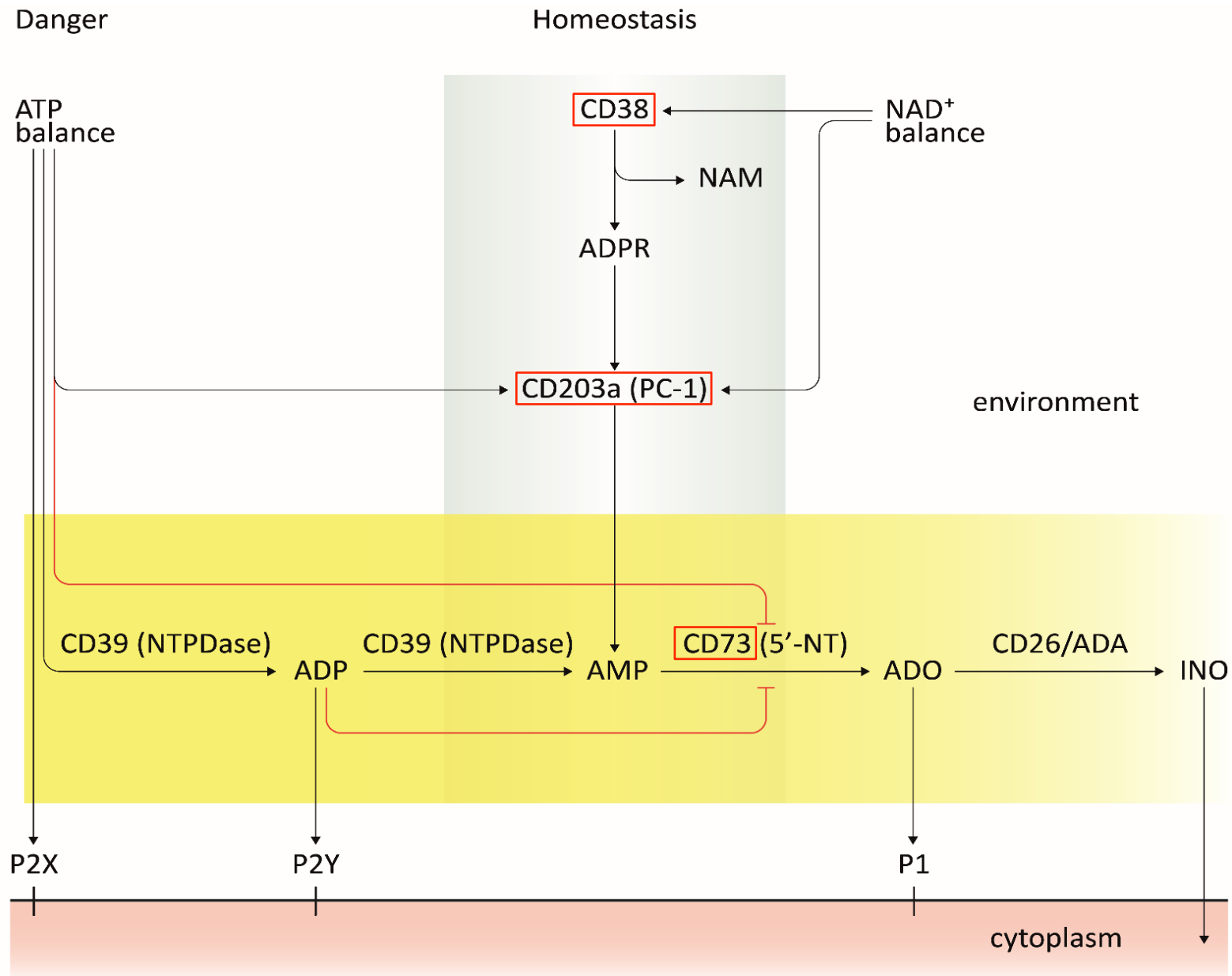
ENZYMATIC FUNCTIONS EXERTED BY CD38/CD157



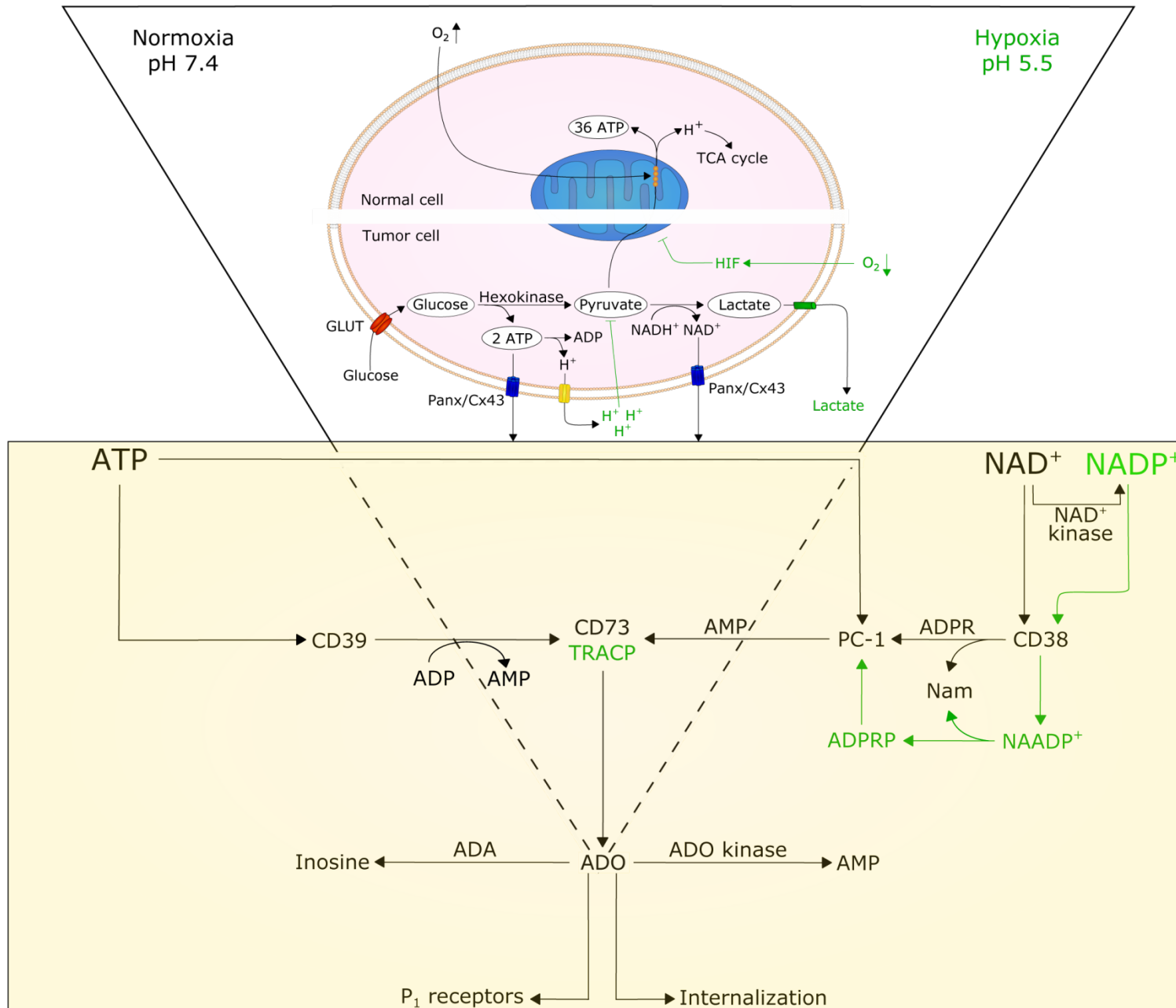
Choice of the disease: the human myeloma model



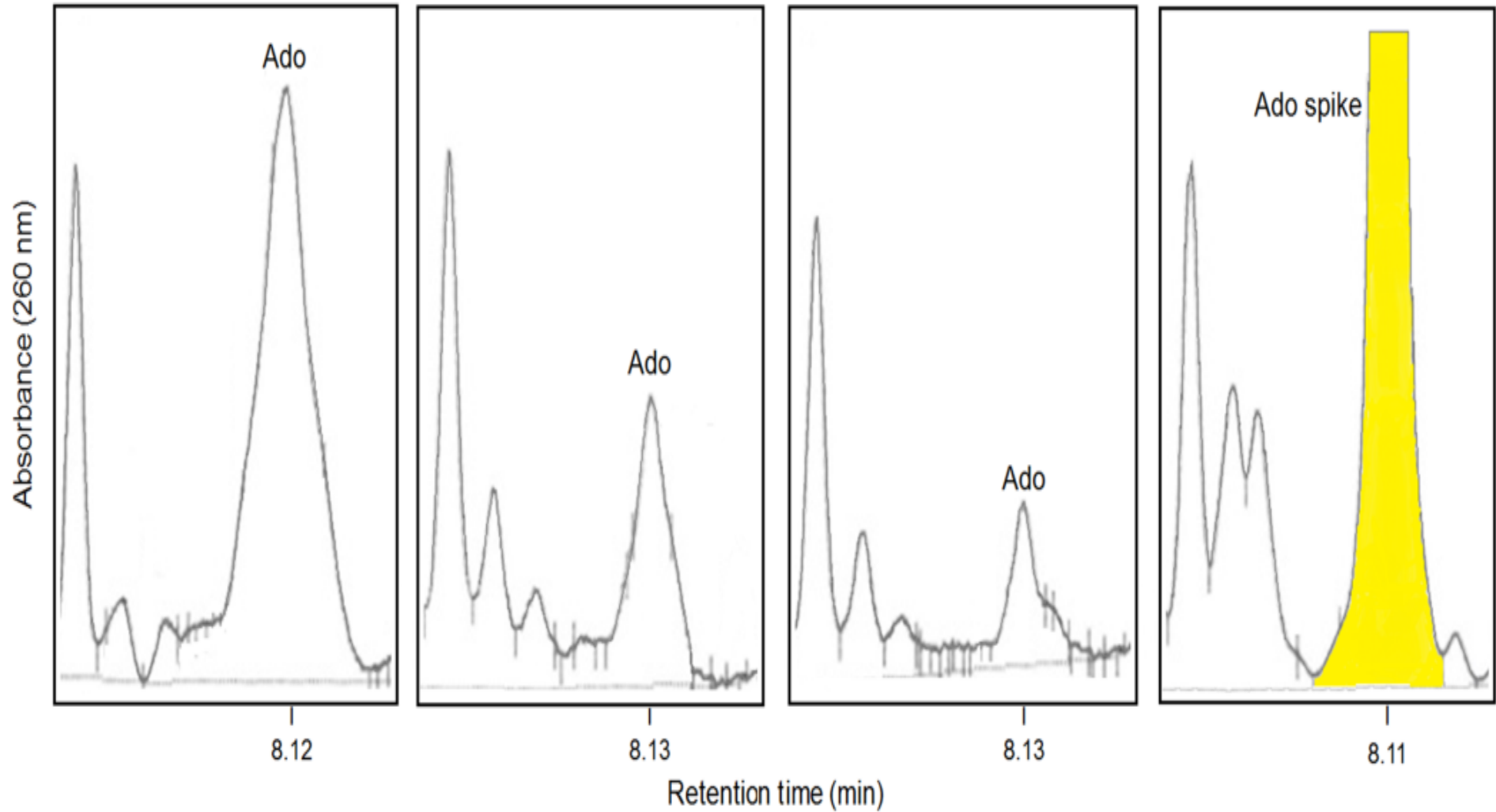
Memo: pathways of adenosine production



Interplay among ectoenzymes, their substrates and products in the BM niche

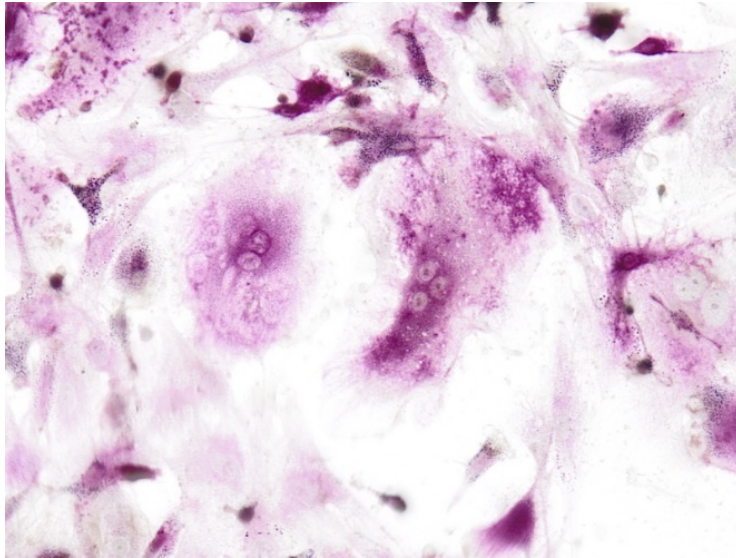


Proof-of-principle: BM plasma from MM patients contains ADO

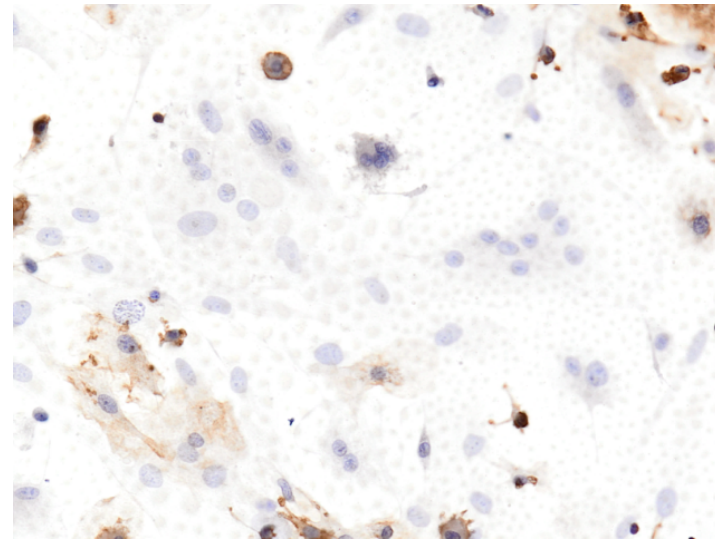


Human Osteoclasts express ADO receptors

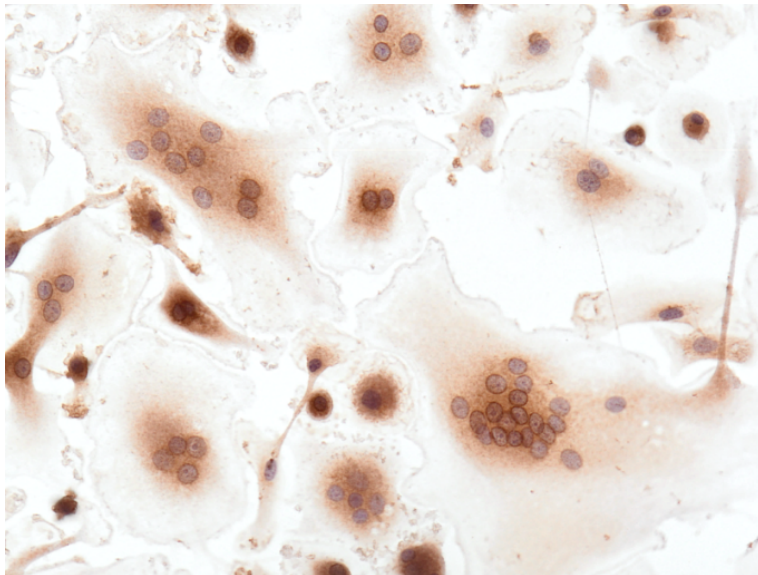
TRAP



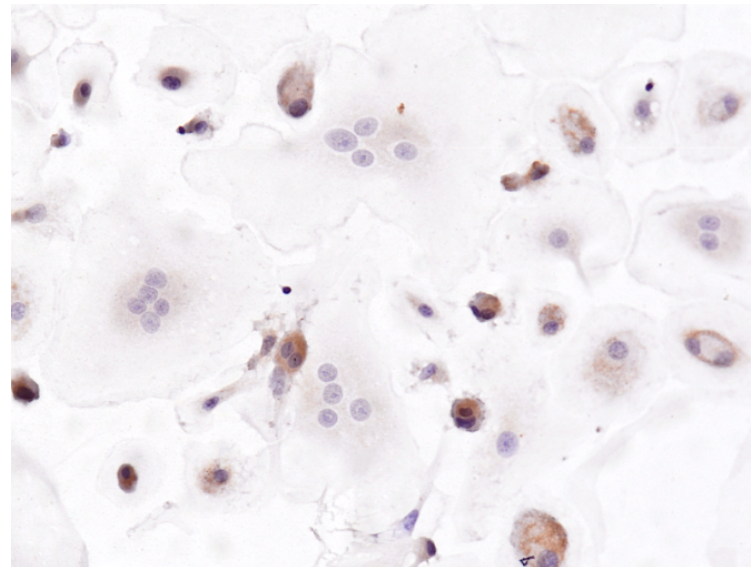
ADORA1



ADORA2a



ADORA2b

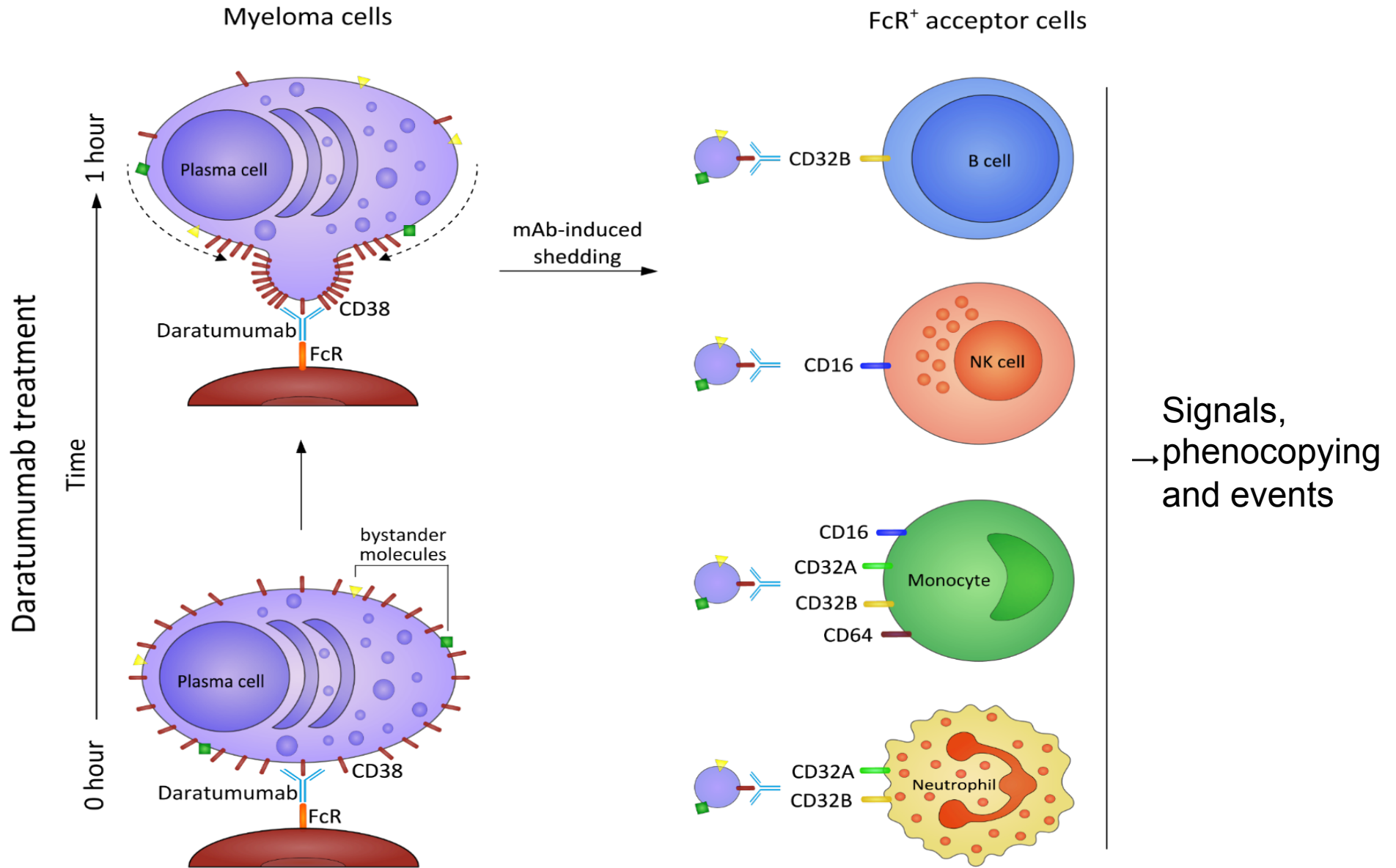


V. Quarona, (unpublished)

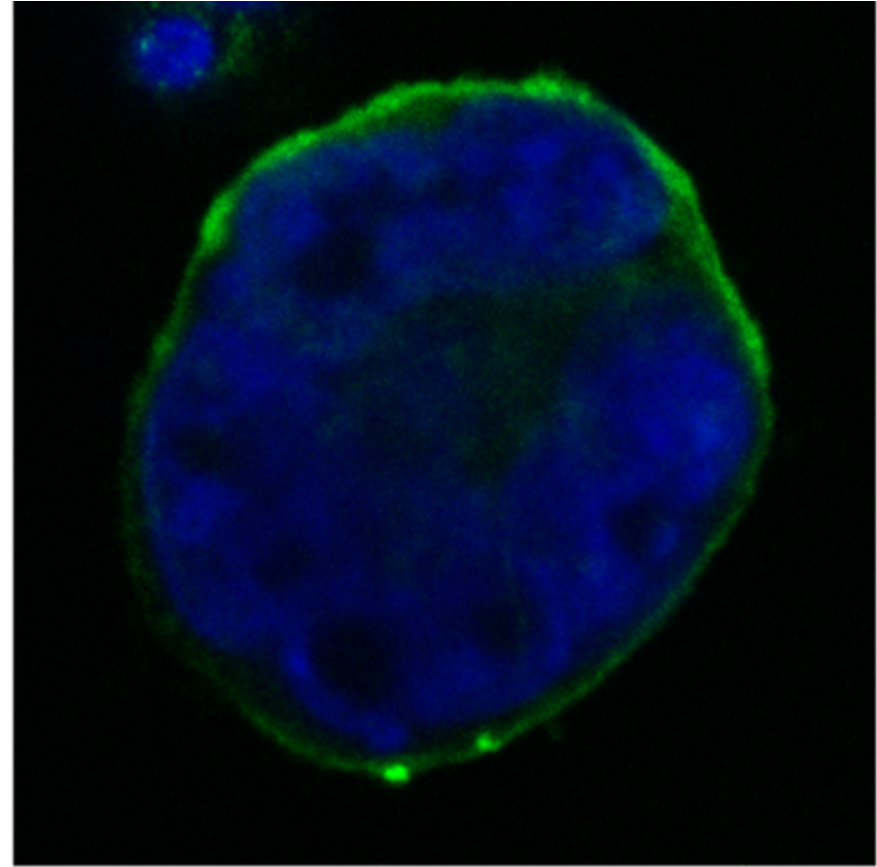
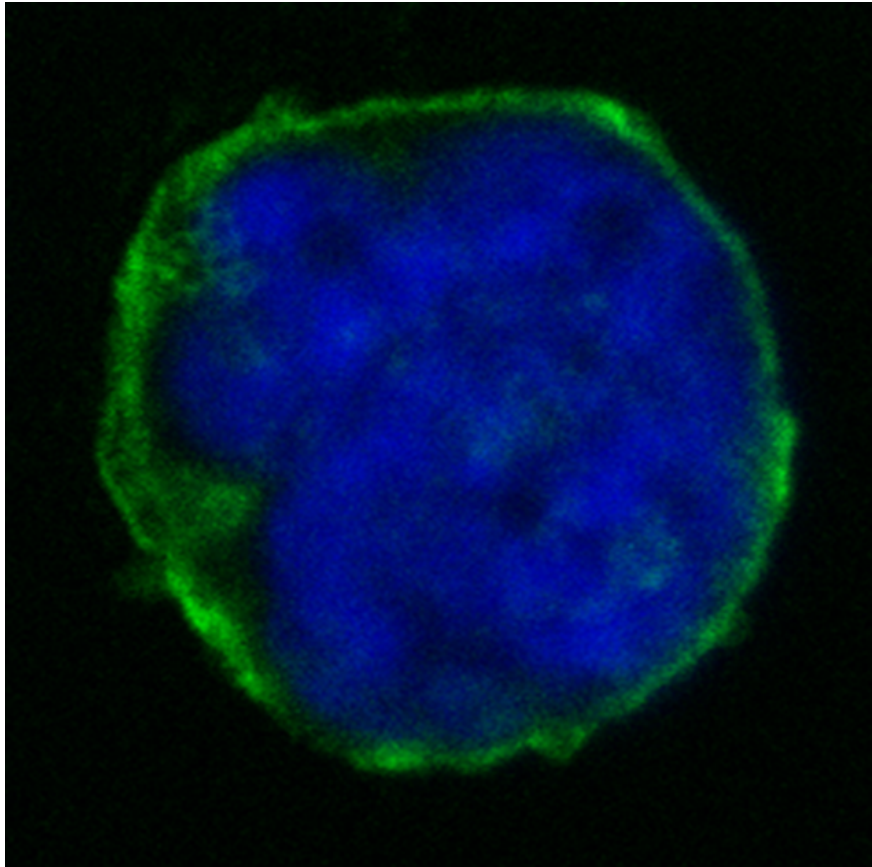
Human IgG receptor expression pattern

Name	FcγRI	FcγRIIA	FcγRIIB	FcγRIIC ^h	FcγRIIIA	FcγRIIIB	FcRn
CD	CD64	CD32A	CD32B	CD32C	CD16A	CD16B	-
B cell	-	-	+	-	-	-	-
T cell	-	-	-	-	-	-	-
NK cell	-	-	- [#]	+ ^h	+	-	-
Mono/Macro	+	+	+/-	+ ^h	+	-	+
Neutrophil	(+)	+	+/-	+ ^h	-	+	+
Dendritic Cell[§]	+	+	+	-	-	-	+
Basophil	-	+	+	-	-	+/-	-
Mast cell	(+)	+	-	-	-	-	NA
Eosinophil	-	+	-	-	-	-	-
Platelet	-	+	-	-	-	-	NA
Endothelium	-	-	-	-	-	-	+

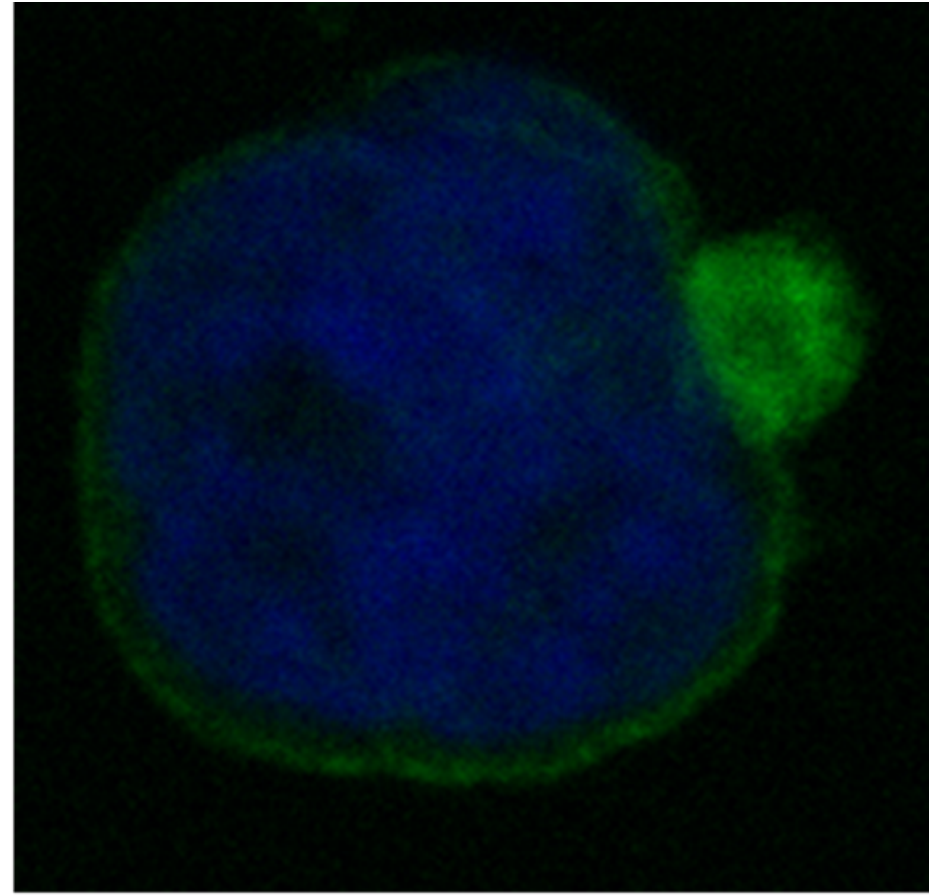
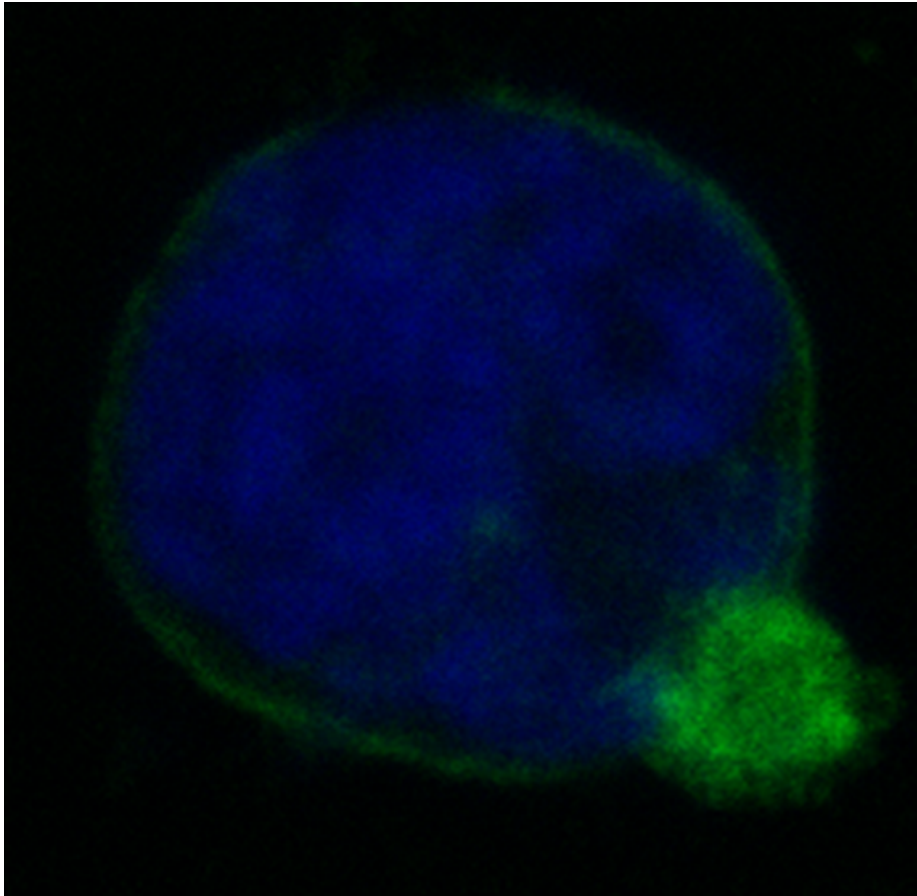
In vivo events elicited by a mAb reaching its myeloma target



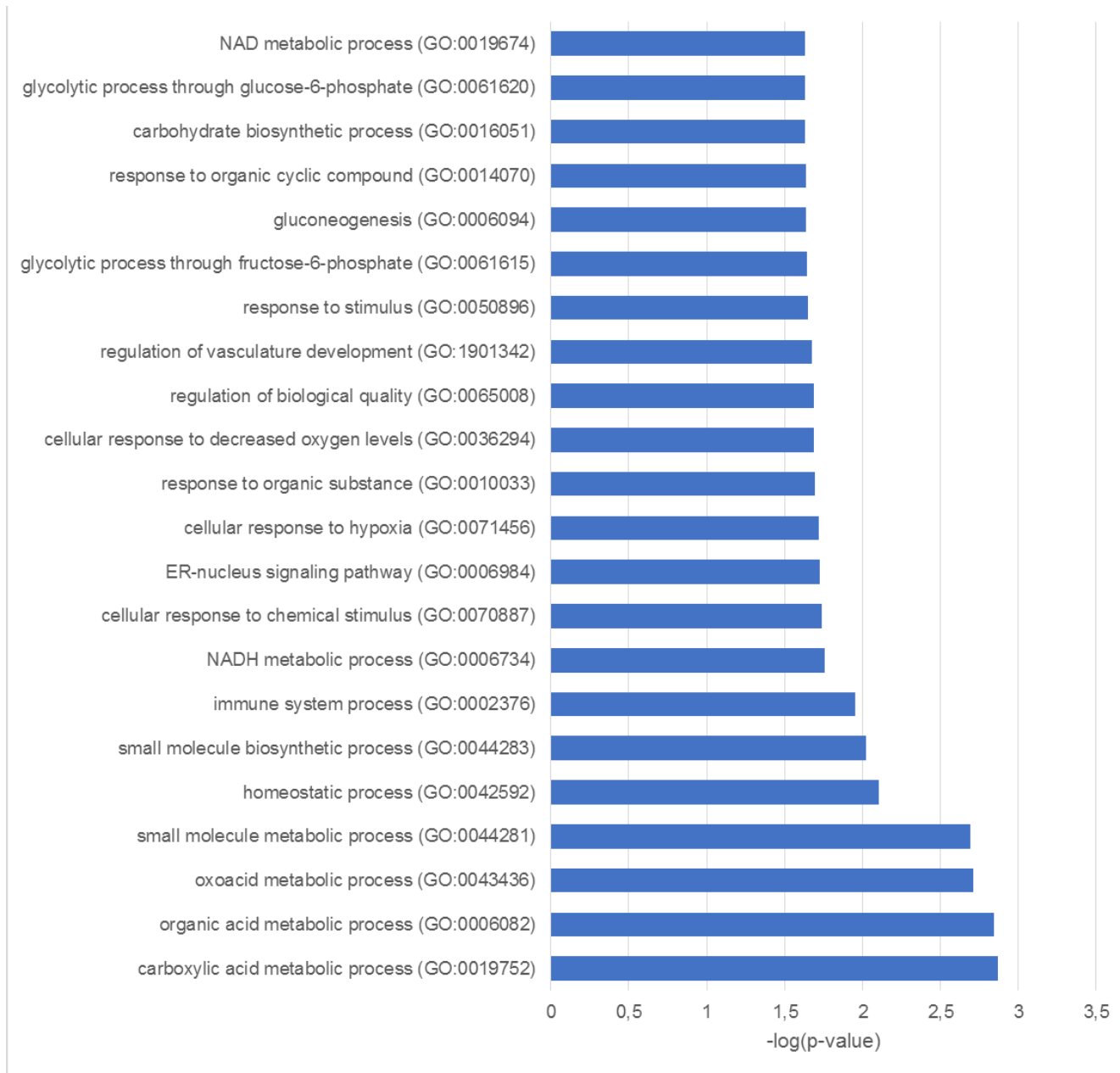
Confocal microscopy analysis of CD38/DARA interaction (4°C) on a relapsed myeloma



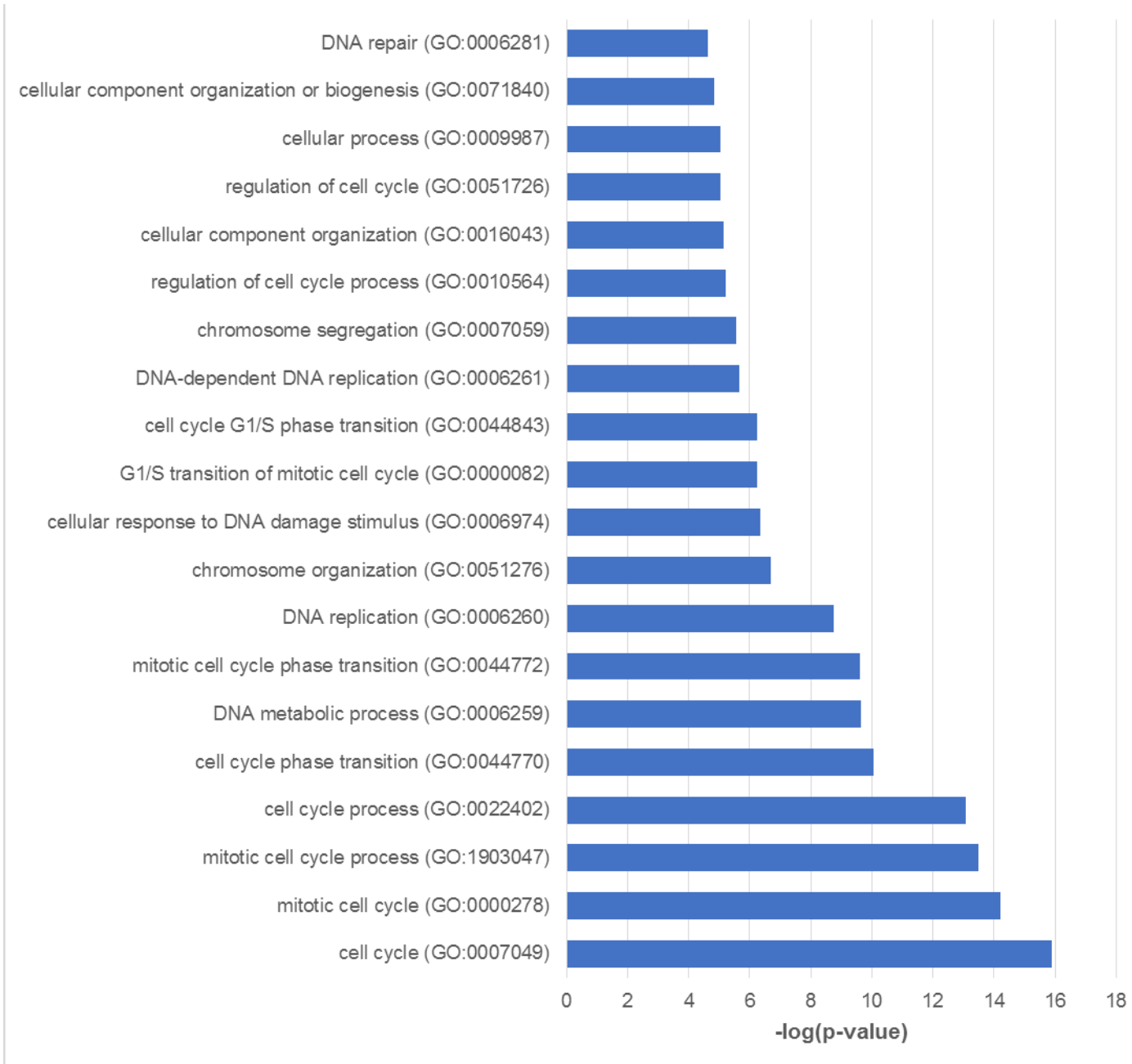
Confocal microscopy analysis of CD38/DARA interaction
(37 °C, 2 h) on a myeloma at diagnosis



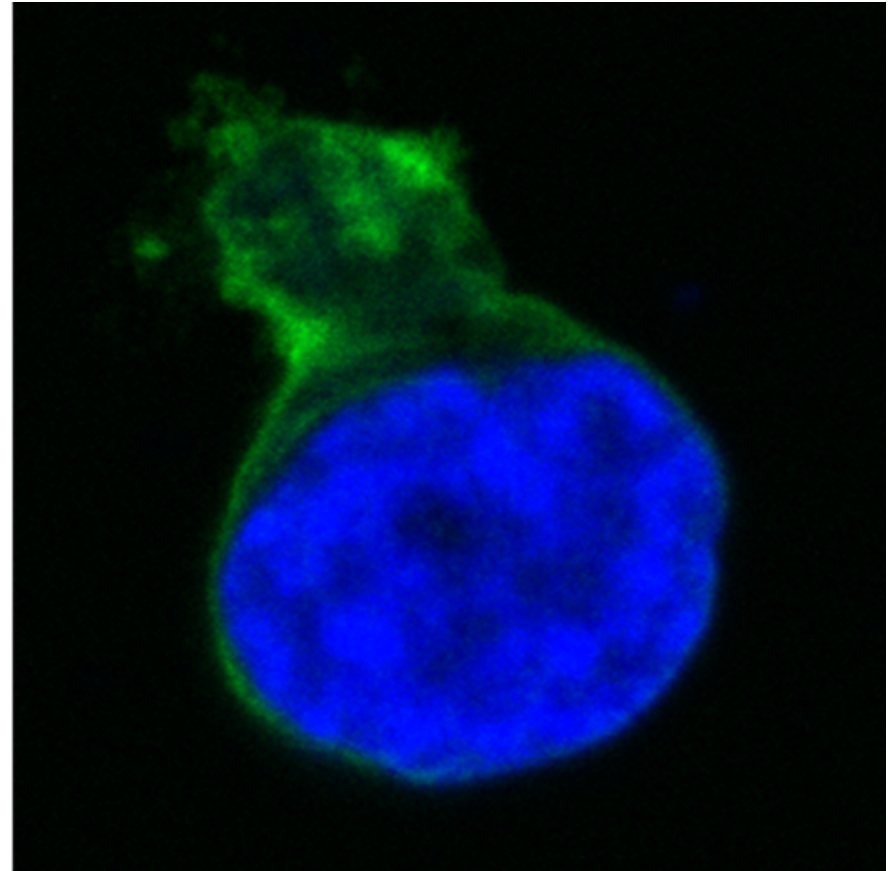
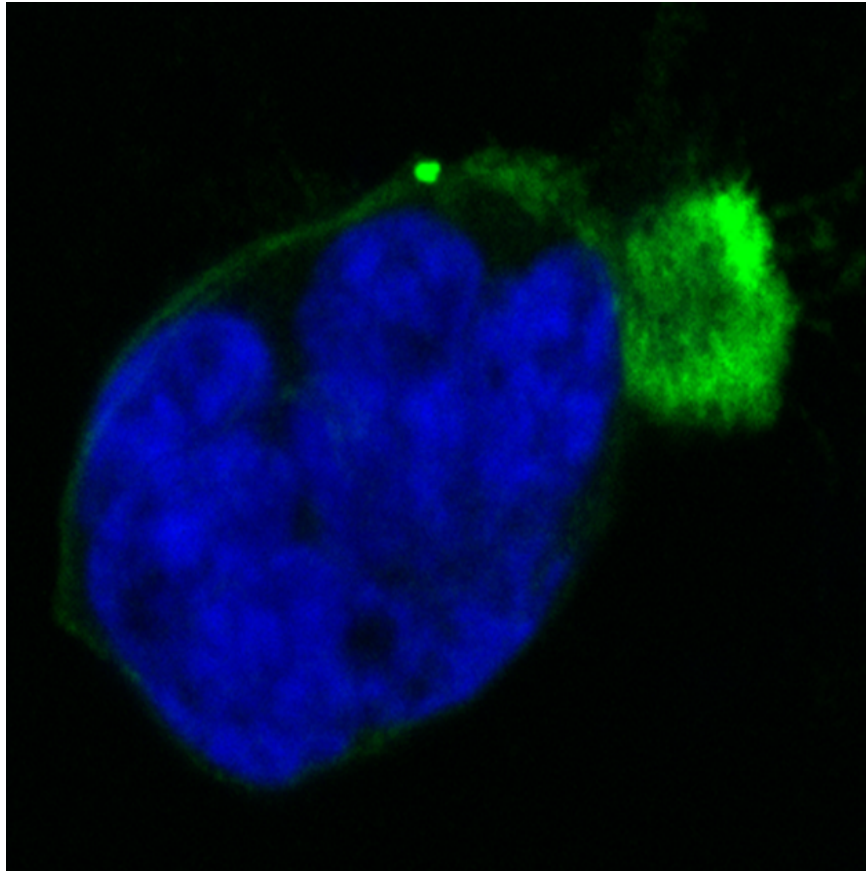
Up-regulated genes in human myeloma upon Dara ligation



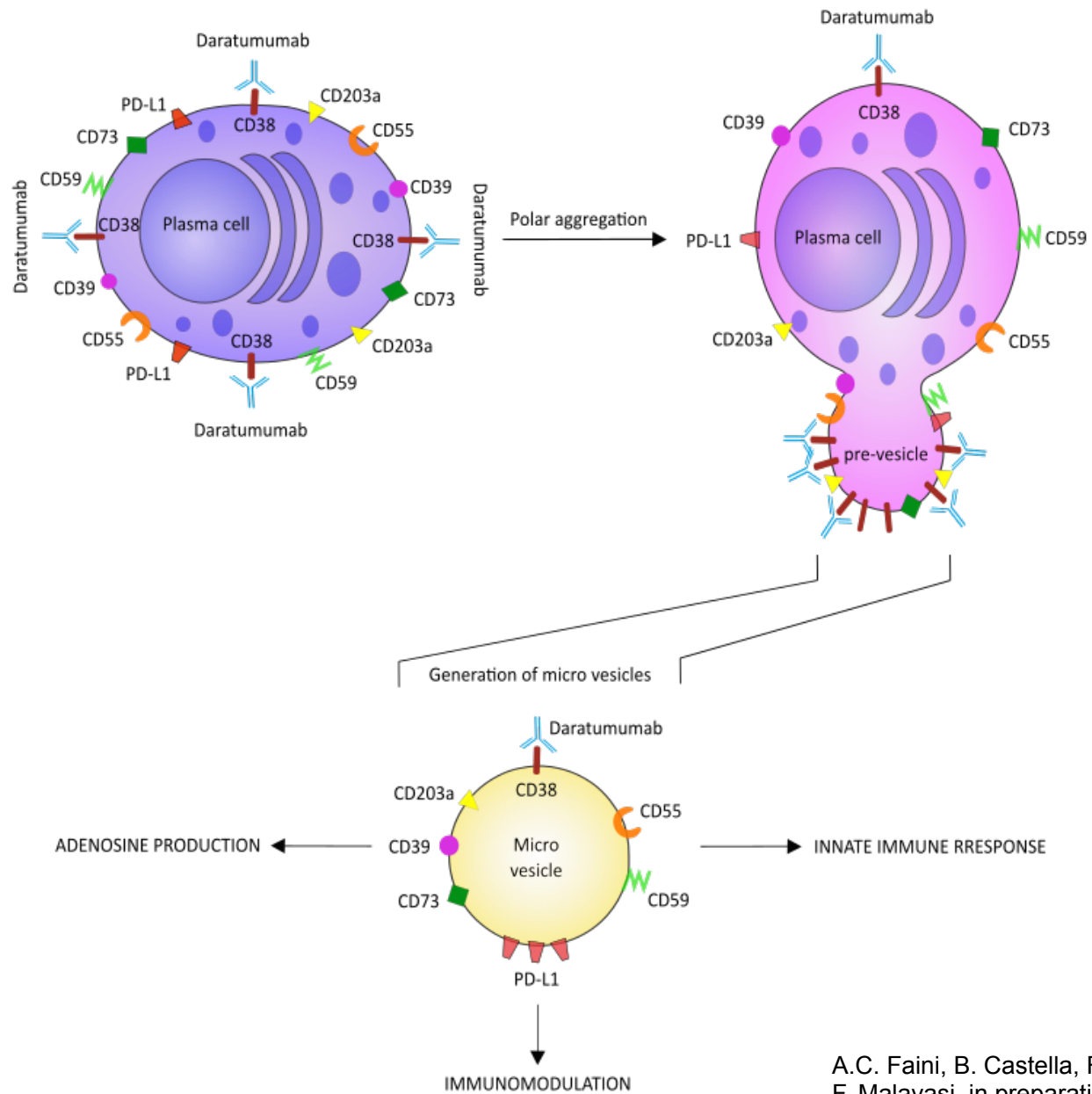
Down-regulated genes in human myeloma upon Dara ligation



Confocal microscopy analysis of CD38/DARA interaction (37 °C, 1 h) on a relapsed myeloma



Schematic view of events following DARA treatment *in vivo*

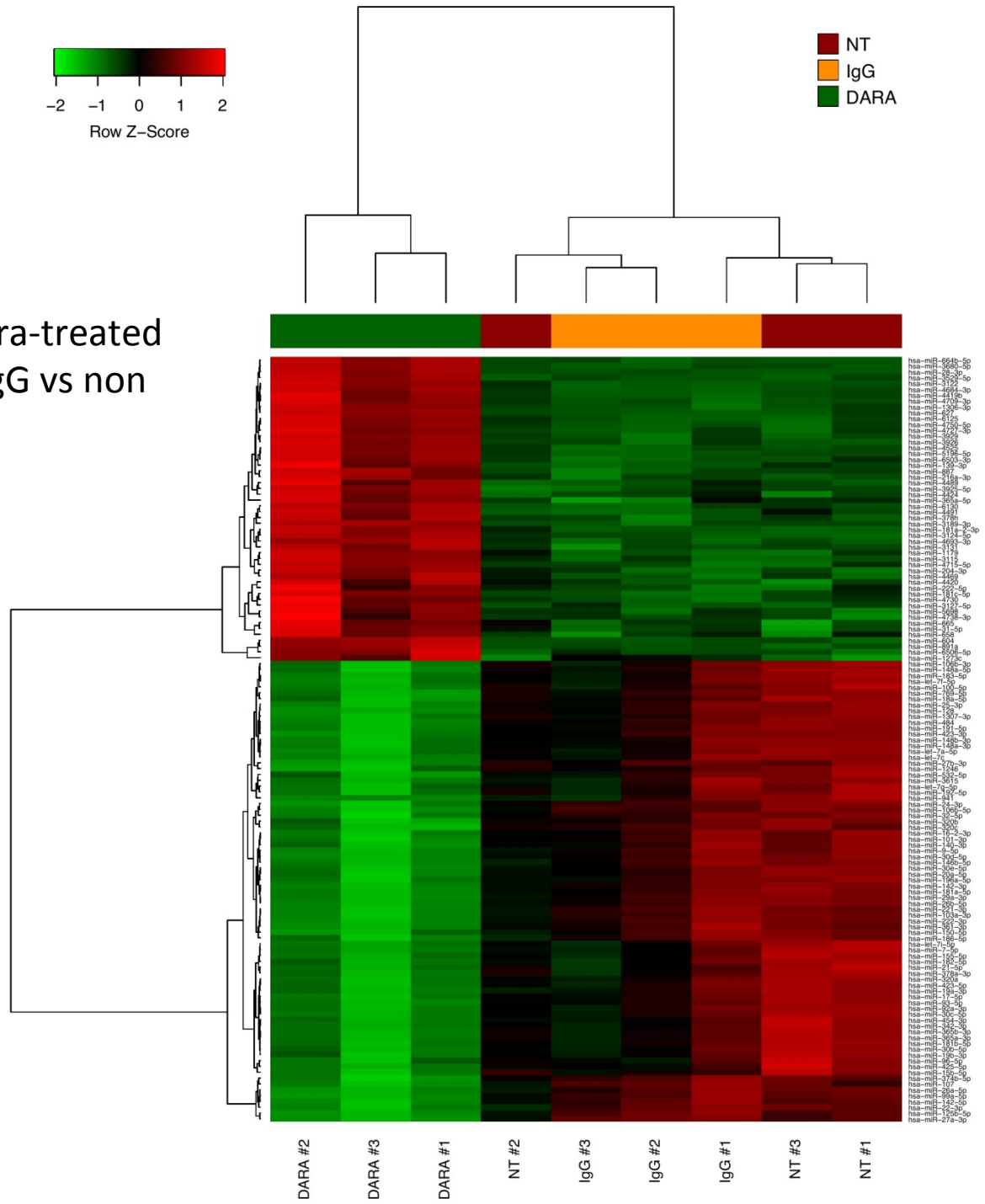


A.C. Faini, B. Castella, F. Morandi and F. Malavasi, in preparation 2018

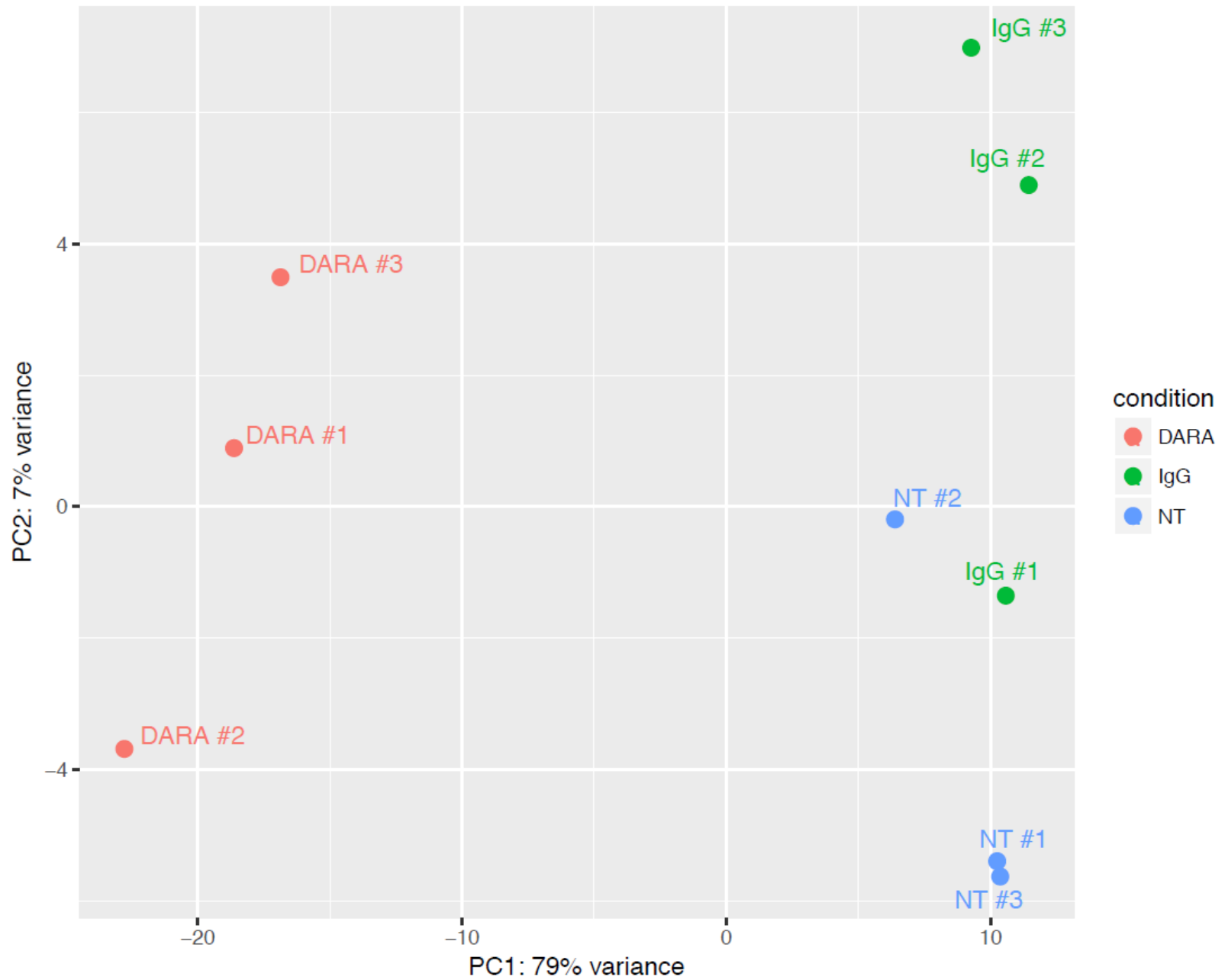
Heatmap of miRNA in Dara-treated myeloma vs irrelevant IgG vs non treated



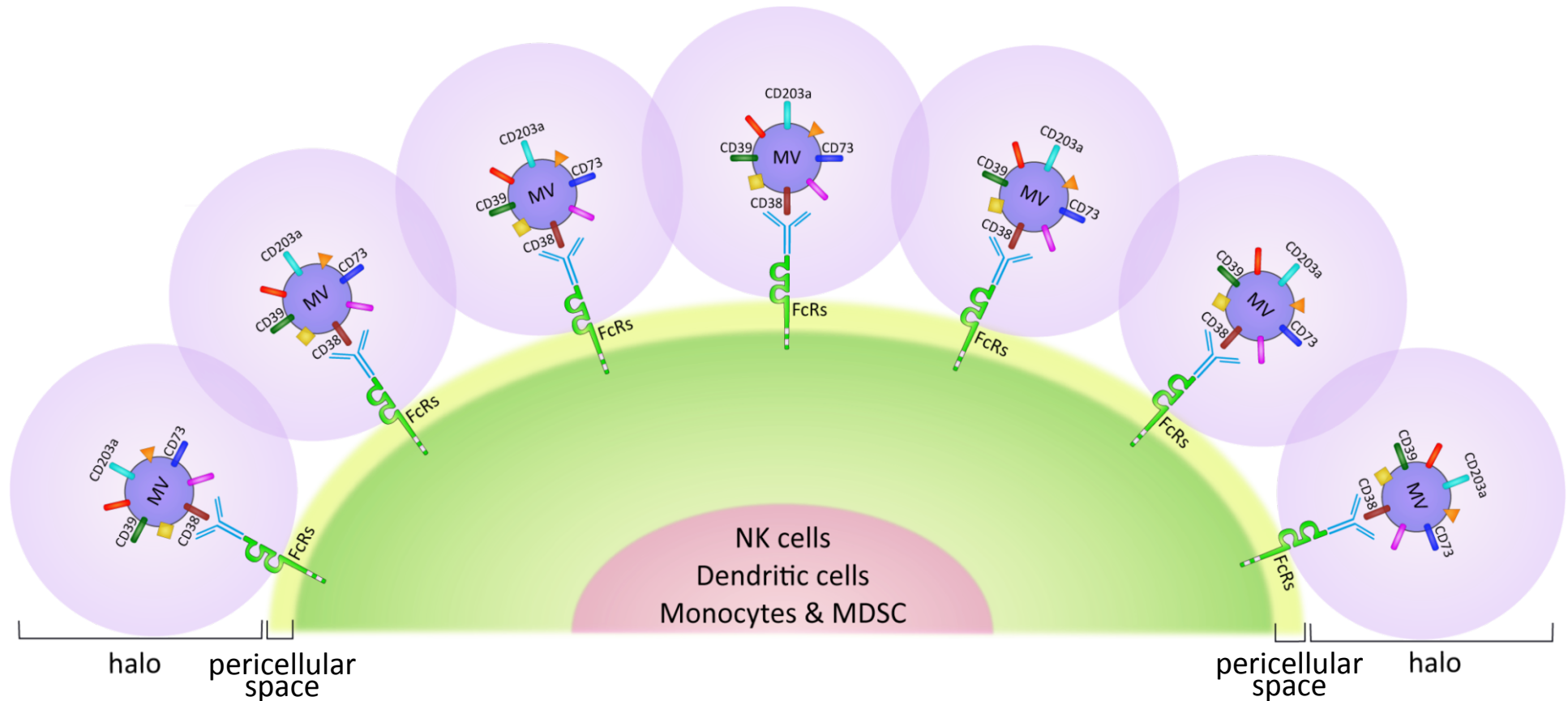
- NT
- IgG
- DARA



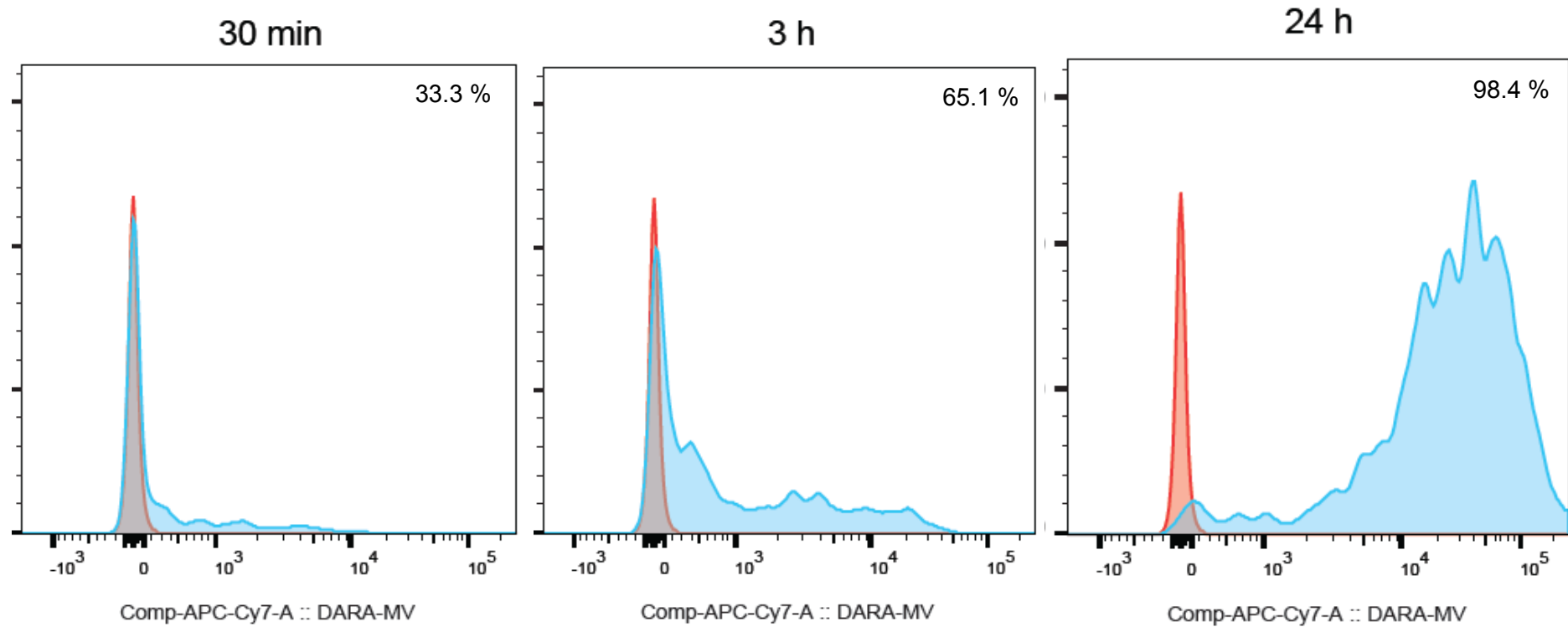
Distribution of miRNA from Dara-treated vs IgG-treated or vs non-treated



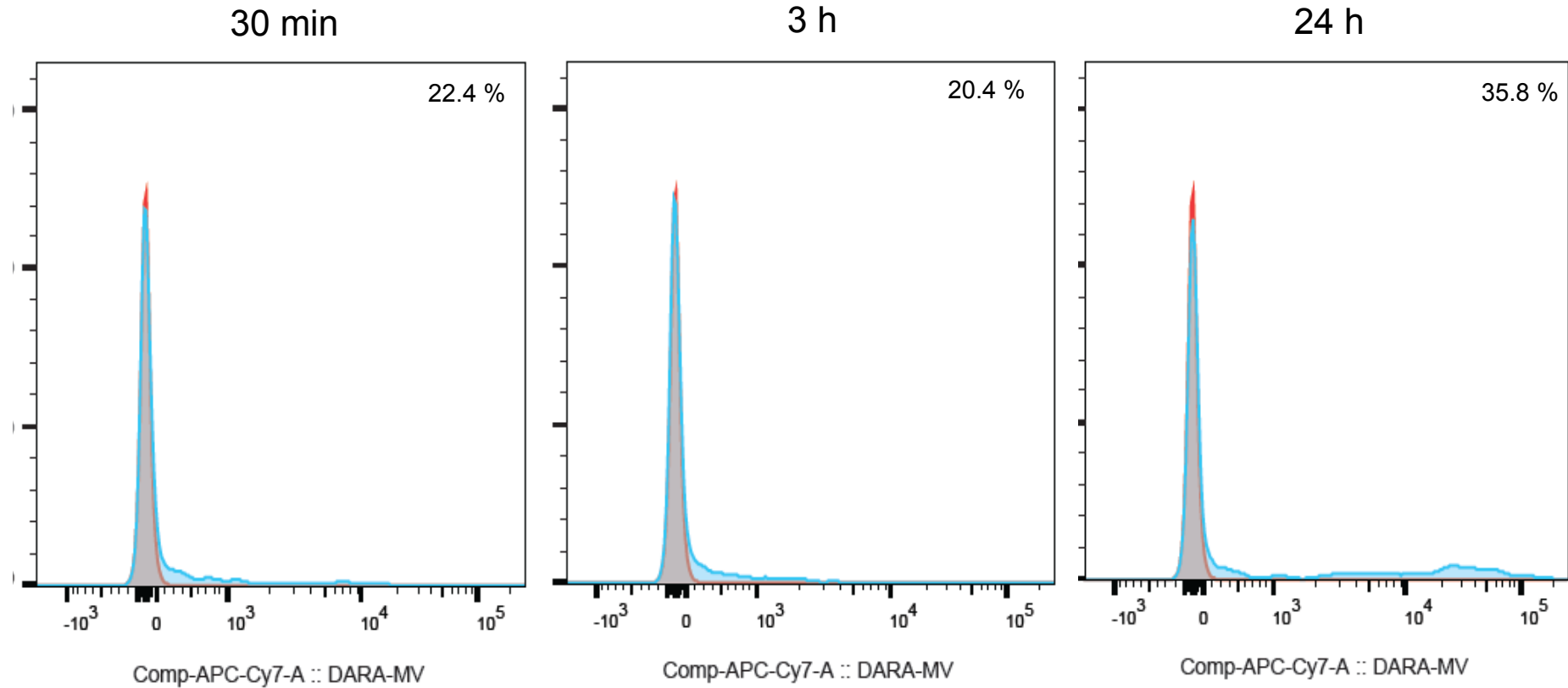
Enzymatic halo and MV define a pericellular space



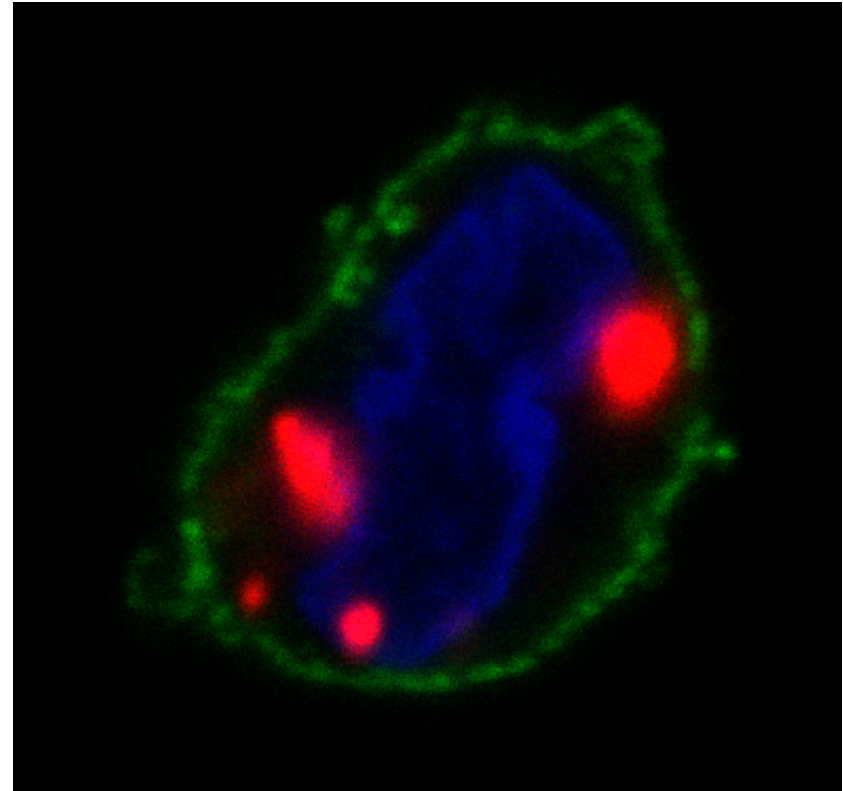
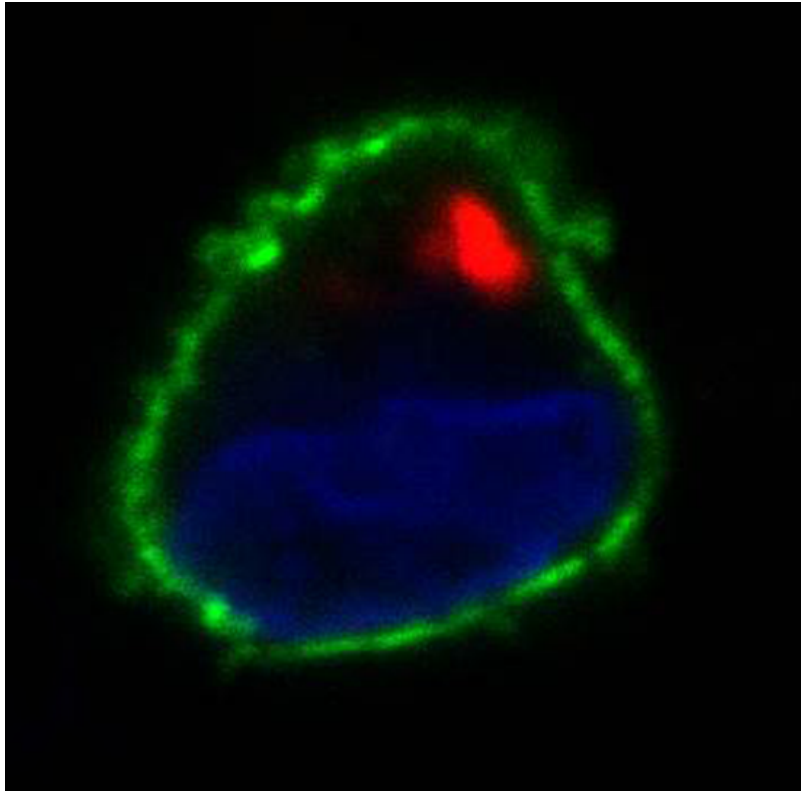
Whither MV from multiple myeloma: accumulation on monocytes (CD14⁺)



Whither MV from multiple myeloma: accumulation on NK cells (CD16⁺)

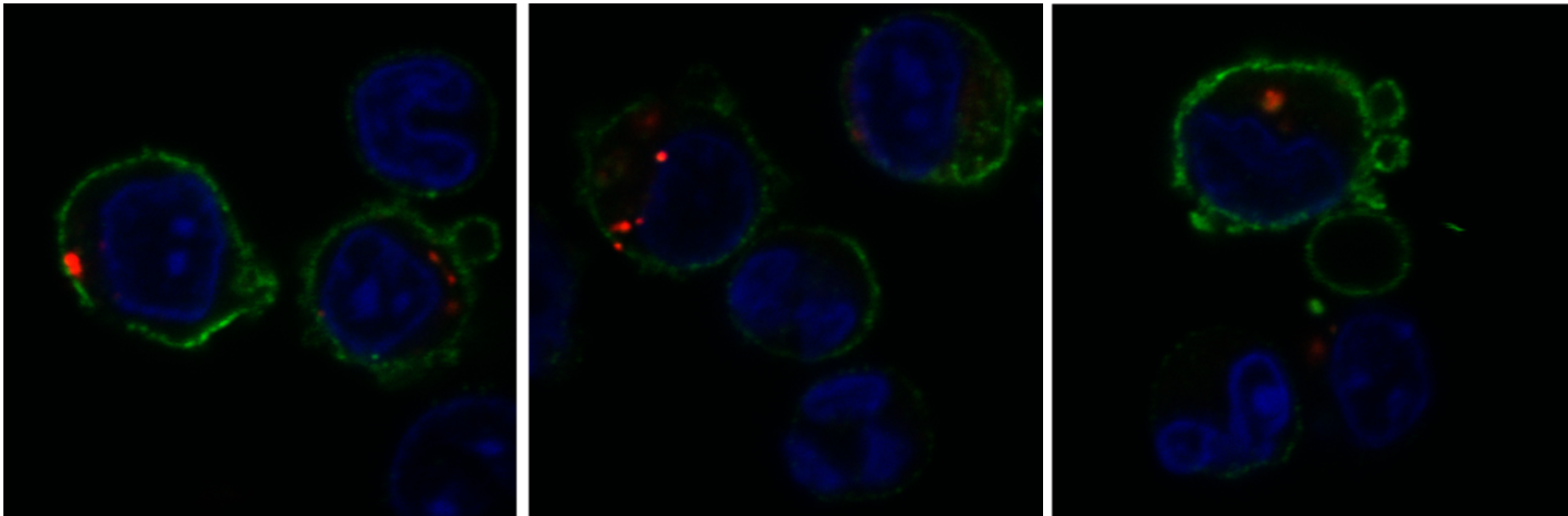


Whither MV from multiple myeloma: Entering monocytes (CD14⁺)



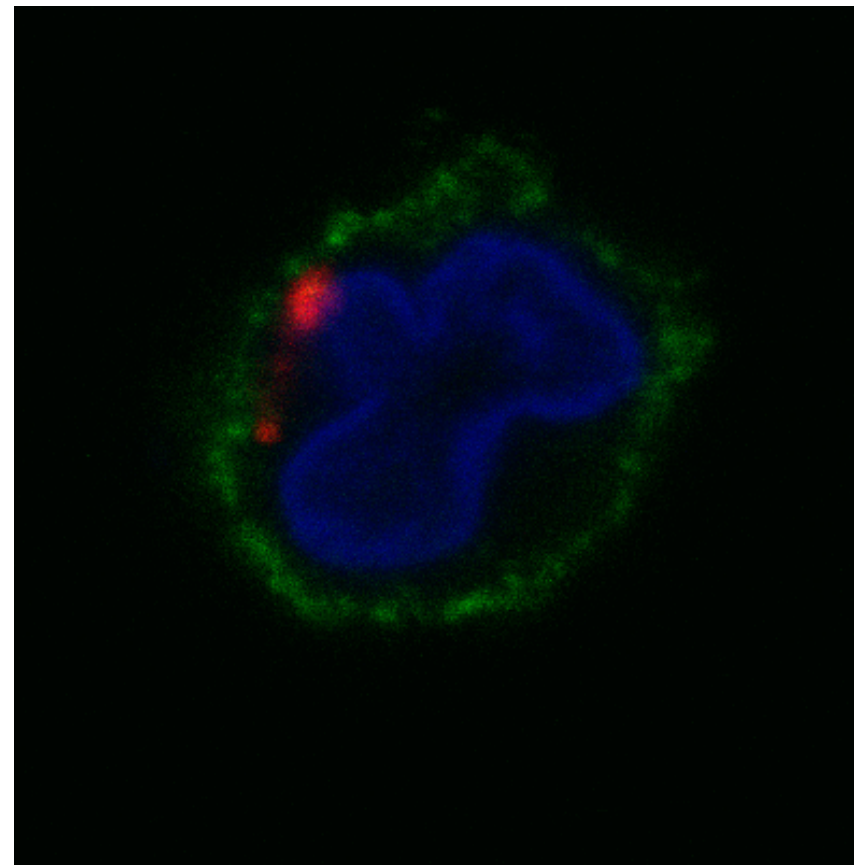
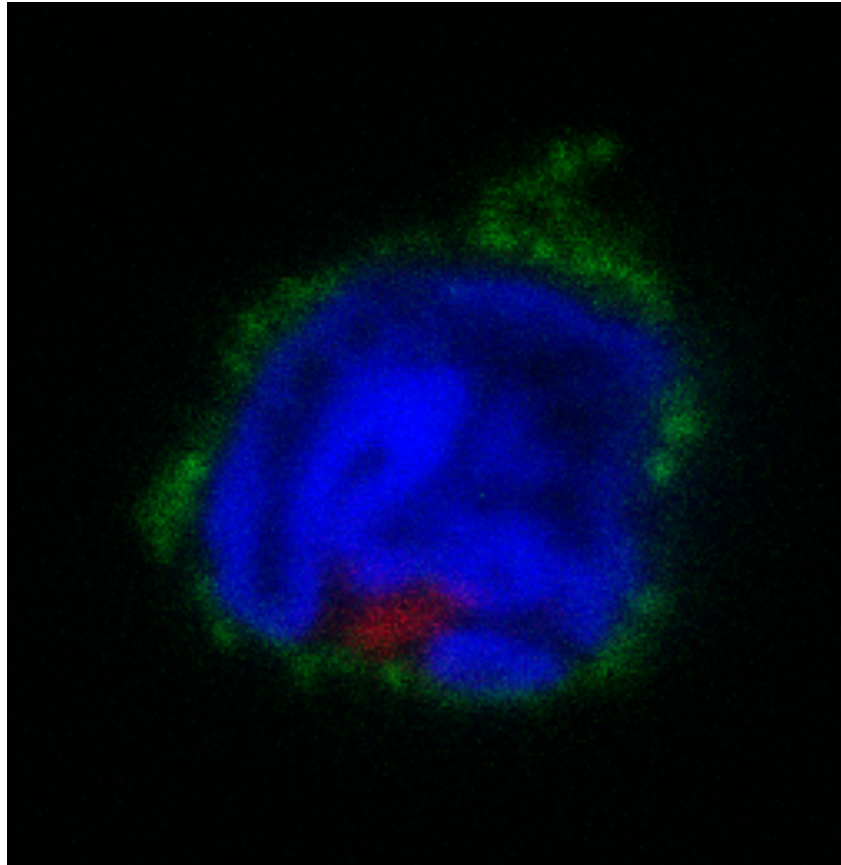
Green = anti-CD14 mAb plus anti-mouse IgG-Alexa 488
Red = MV labeled with 1,1'-Diiodo-3,3',3'-tetramethylindodicarbocyanine
4-chlorobenzenesulfonate (DiI)
Blue = 4',6-Diamidino-2-Phenylindole (DAPI)

Whither MV from multiple myeloma: Entering MDSC (CD15⁺/CD33⁺/CD11b⁺)



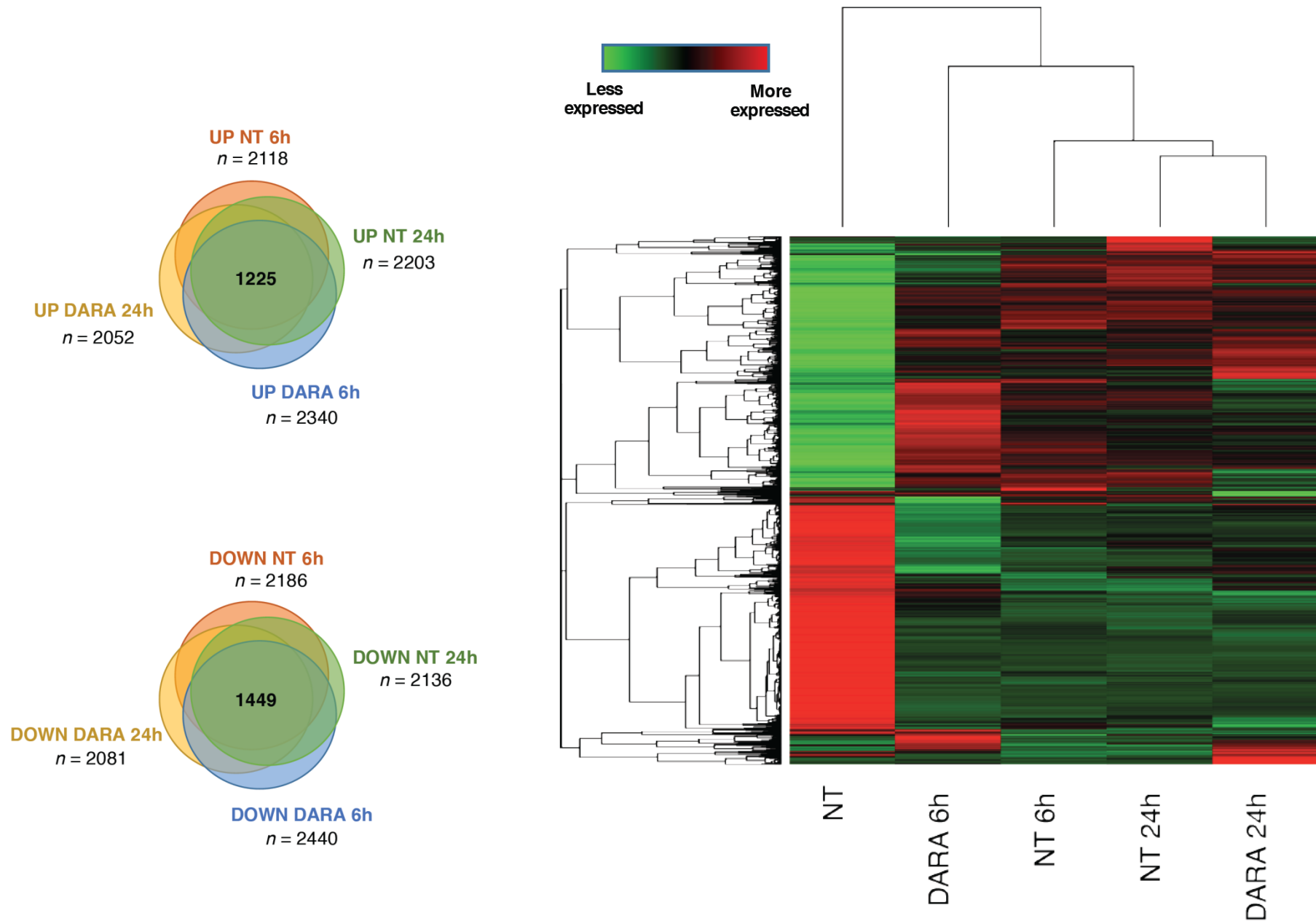
Green = anti-CD14 mAb plus anti-mouse IgG-Alexa 488
Red = MV labeled with 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine
4-chlorobenzenesulfonate (DiD)
Blue = 4',6-Diamidino-2-Phenylindole (DAPI)

Whither MV from multiple myeloma: Entering NK cells (CD16⁺)

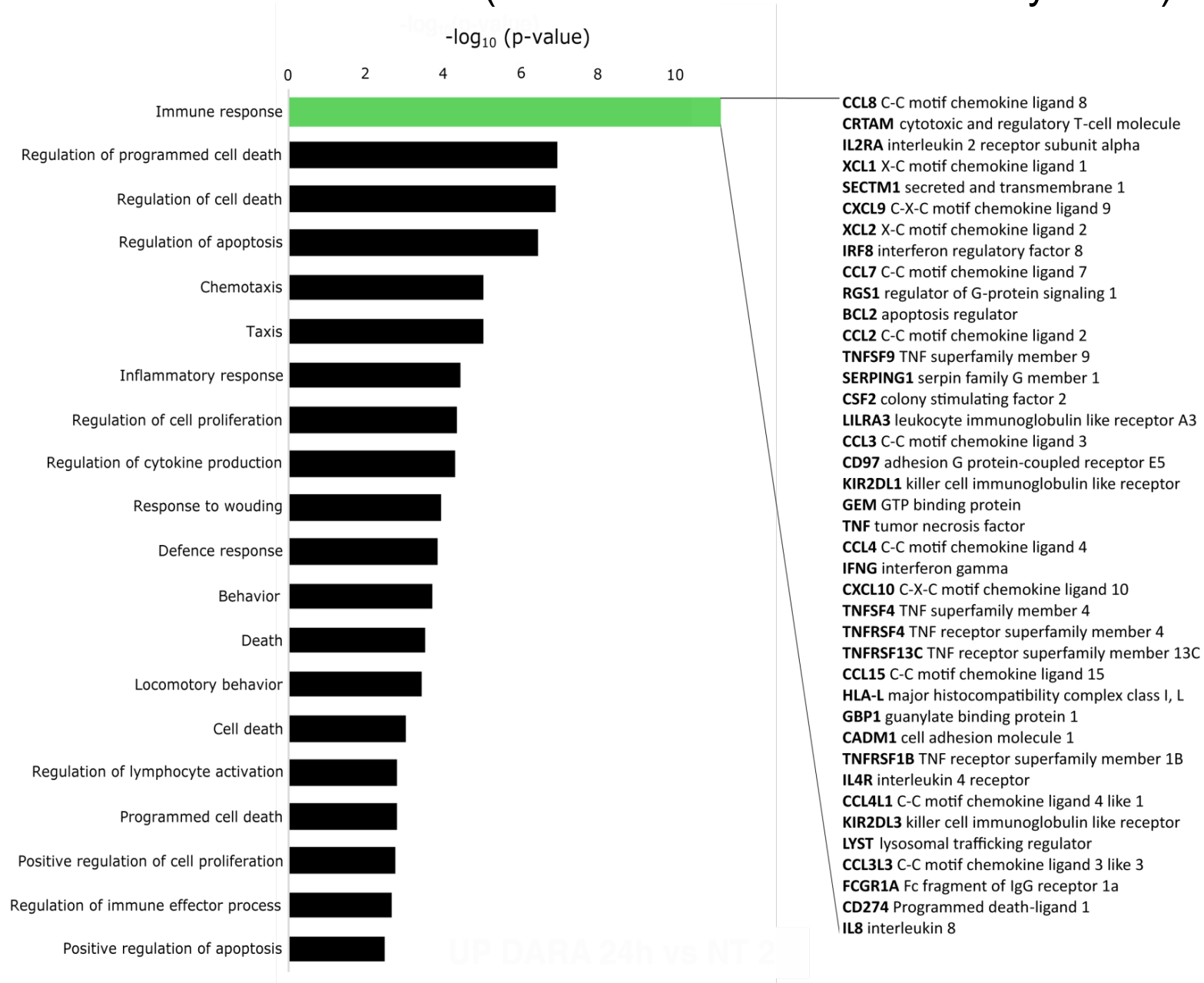


Green = anti-CD16 mAb plus anti-mouse IgG-Alexa 488
Red = MV labeled with 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine
4-chlorobenzenesulfonate (DiD)
Blue = 4',6-Diamidino-2-Phenylindole (DAPI)

Whither MV from multiple myeloma: Molecular effects observed on NK cells (CD16⁺/CD56⁺)

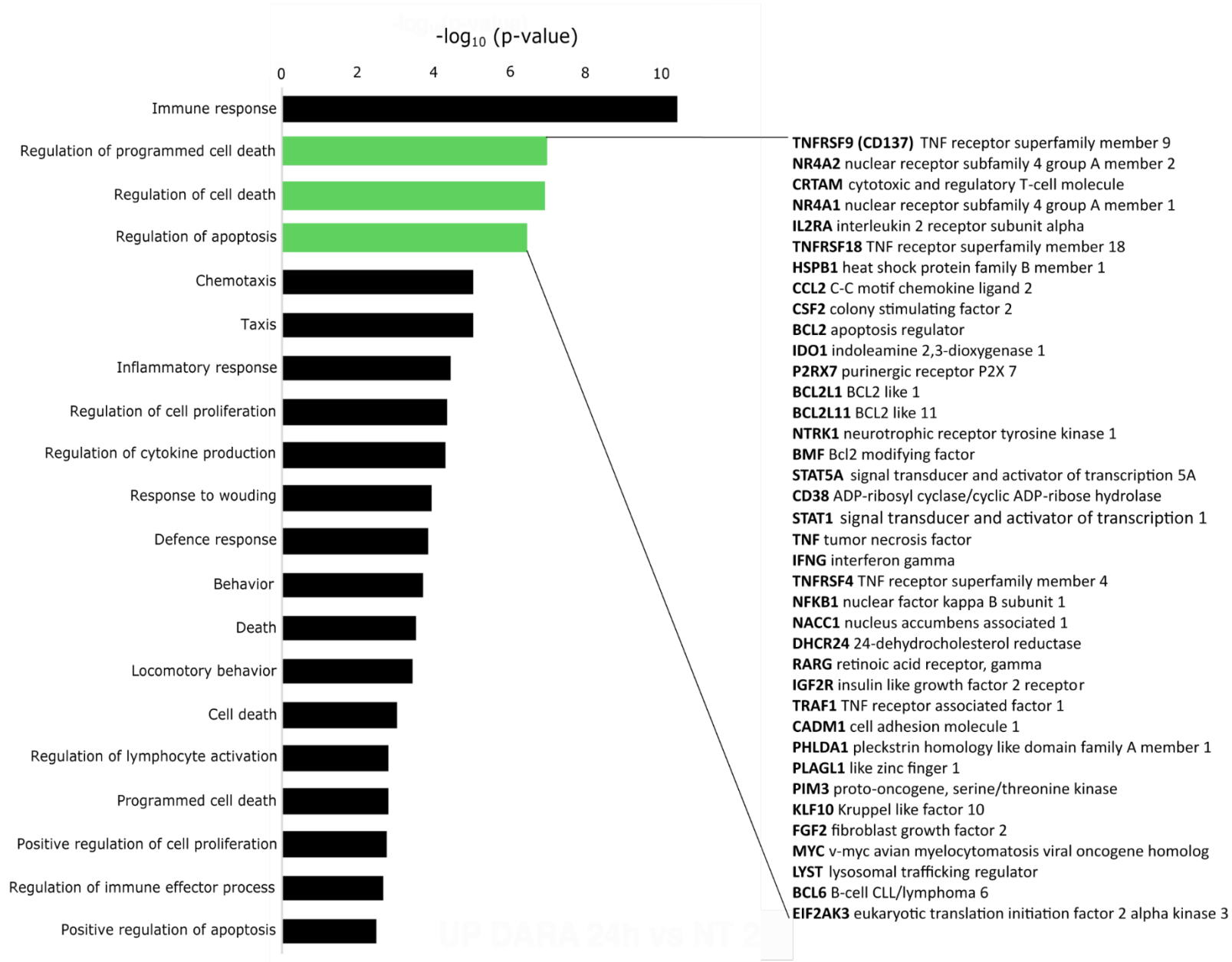


Comparative analysis of up modulated genes (RNA polyA) after exposure of NK cells to MV-DARA (control: MV from untreated myeloma)



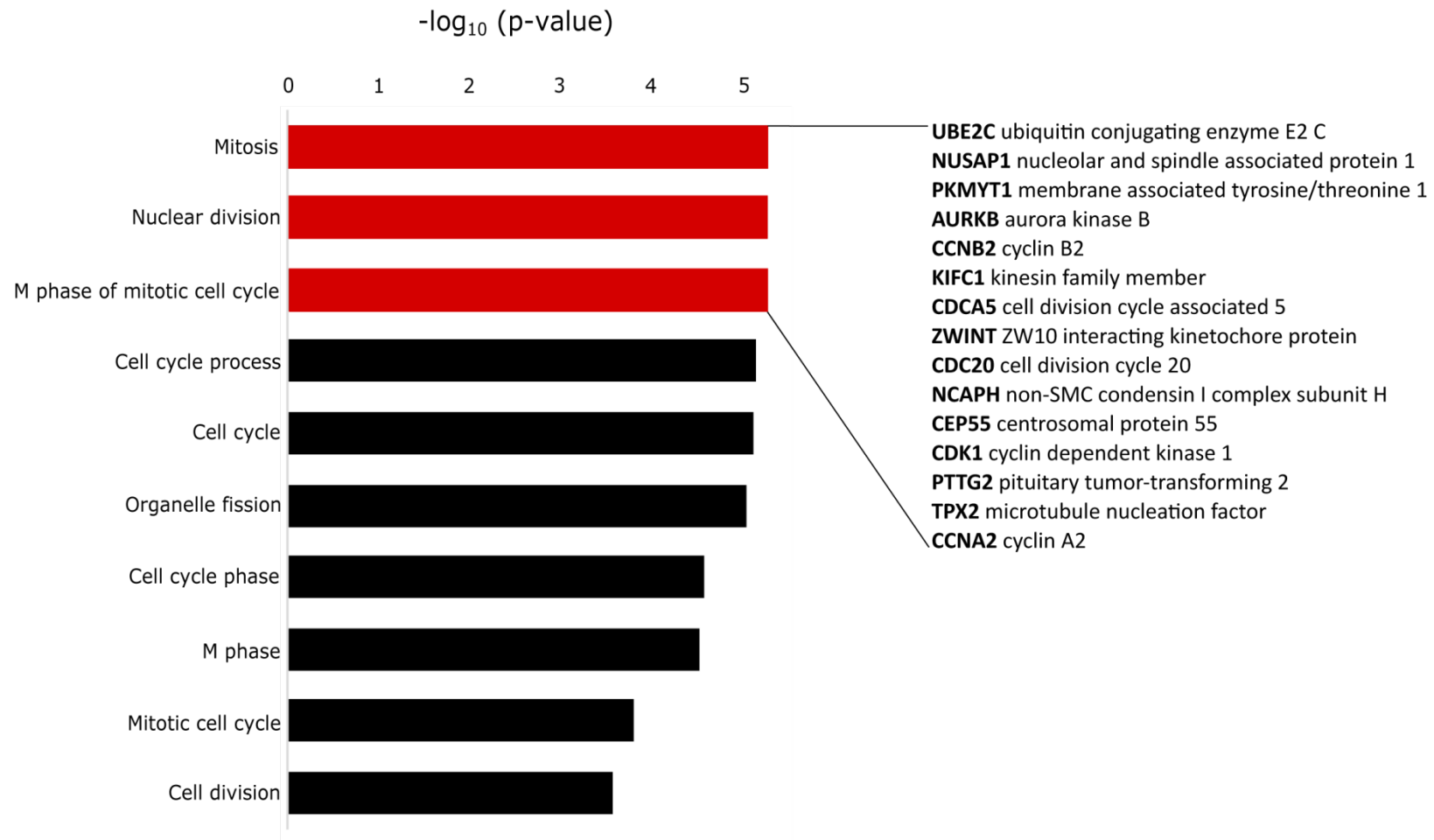
- CCL8** C-C motif chemokine ligand 8
- CRTAM** cytotoxic and regulatory T-cell molecule
- IL2RA** interleukin 2 receptor subunit alpha
- XL1** X-C motif chemokine ligand 1
- SECTM1** secreted and transmembrane 1
- CXCL9** C-X-C motif chemokine ligand 9
- XL2** X-C motif chemokine ligand 2
- IRF8** interferon regulatory factor 8
- CCL7** C-C motif chemokine ligand 7
- RGS1** regulator of G-protein signaling 1
- BCL2** apoptosis regulator
- CCL2** C-C motif chemokine ligand 2
- TNFSF9** TNF superfamily member 9
- SERPING1** serpin family G member 1
- CSF2** colony stimulating factor 2
- LILRA3** leukocyte immunoglobulin like receptor A3
- CCL3** C-C motif chemokine ligand 3
- CD97** adhesion G protein-coupled receptor E5
- KIR2DL1** killer cell immunoglobulin like receptor
- GEM** GTP binding protein
- TNF** tumor necrosis factor
- CCL4** C-C motif chemokine ligand 4
- IFNG** interferon gamma
- CXCL10** C-X-C motif chemokine ligand 10
- TNFSF4** TNF superfamily member 4
- TNFRSF4** TNF receptor superfamily member 4
- TNFRSF13C** TNF receptor superfamily member 13C
- CCL15** C-C motif chemokine ligand 15
- HLA-L** major histocompatibility complex class I, L
- GBP1** guanylate binding protein 1
- CADM1** cell adhesion molecule 1
- TNFRSF1B** TNF receptor superfamily member 1B
- IL4R** interleukin 4 receptor
- CCL4L1** C-C motif chemokine ligand 4 like 1
- KIR2DL3** killer cell immunoglobulin like receptor
- LYST** lysosomal trafficking regulator
- CCL3L3** C-C motif chemokine ligand 3 like 3
- FCGR1A** Fc fragment of IgG receptor 1a
- CD274** Programmed death-ligand 1
- IL8** interleukin 8

Comparative analysis of up modulated genes (RNA polyA) after exposure of NK cells to MV-DARA (control: MV from untreated myeloma)



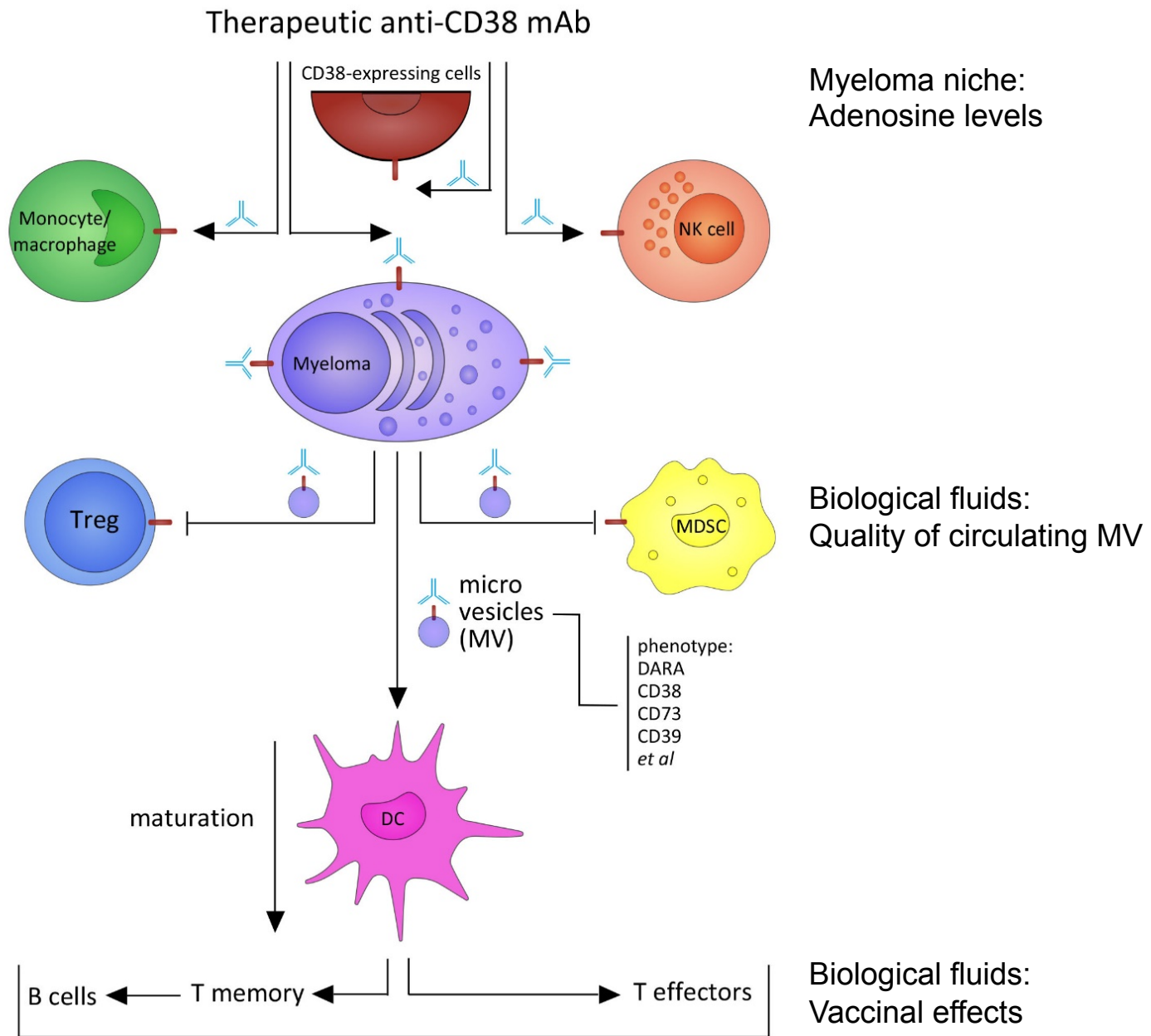
UP DARA 24h vs NT 2

Comparative analysis of down modulated genes (RNA polyA) after exposure of NK cells to MV-DARA (control: MV from untreated myeloma)

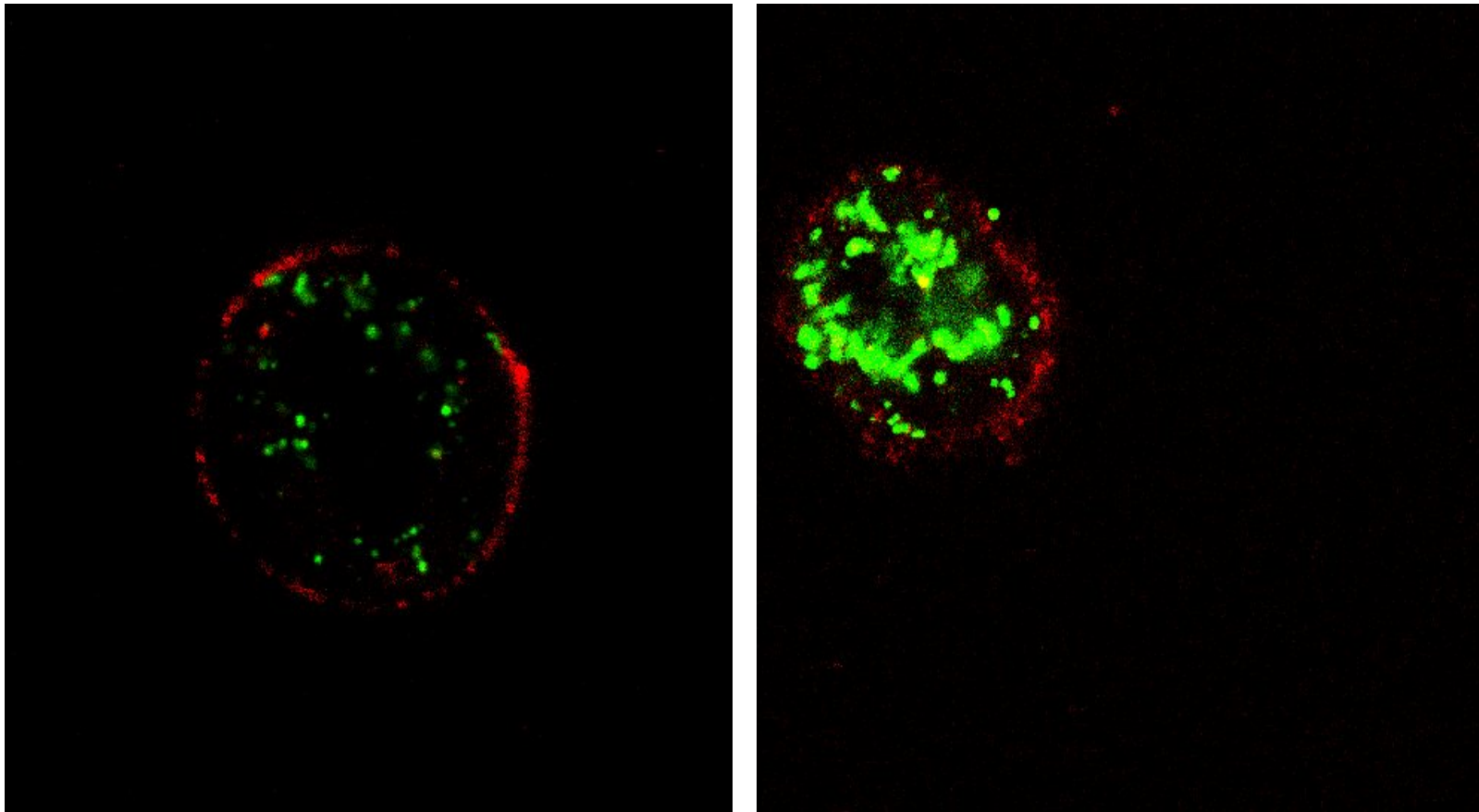


CD38 in the time of therapeutic mAbs

Proposals



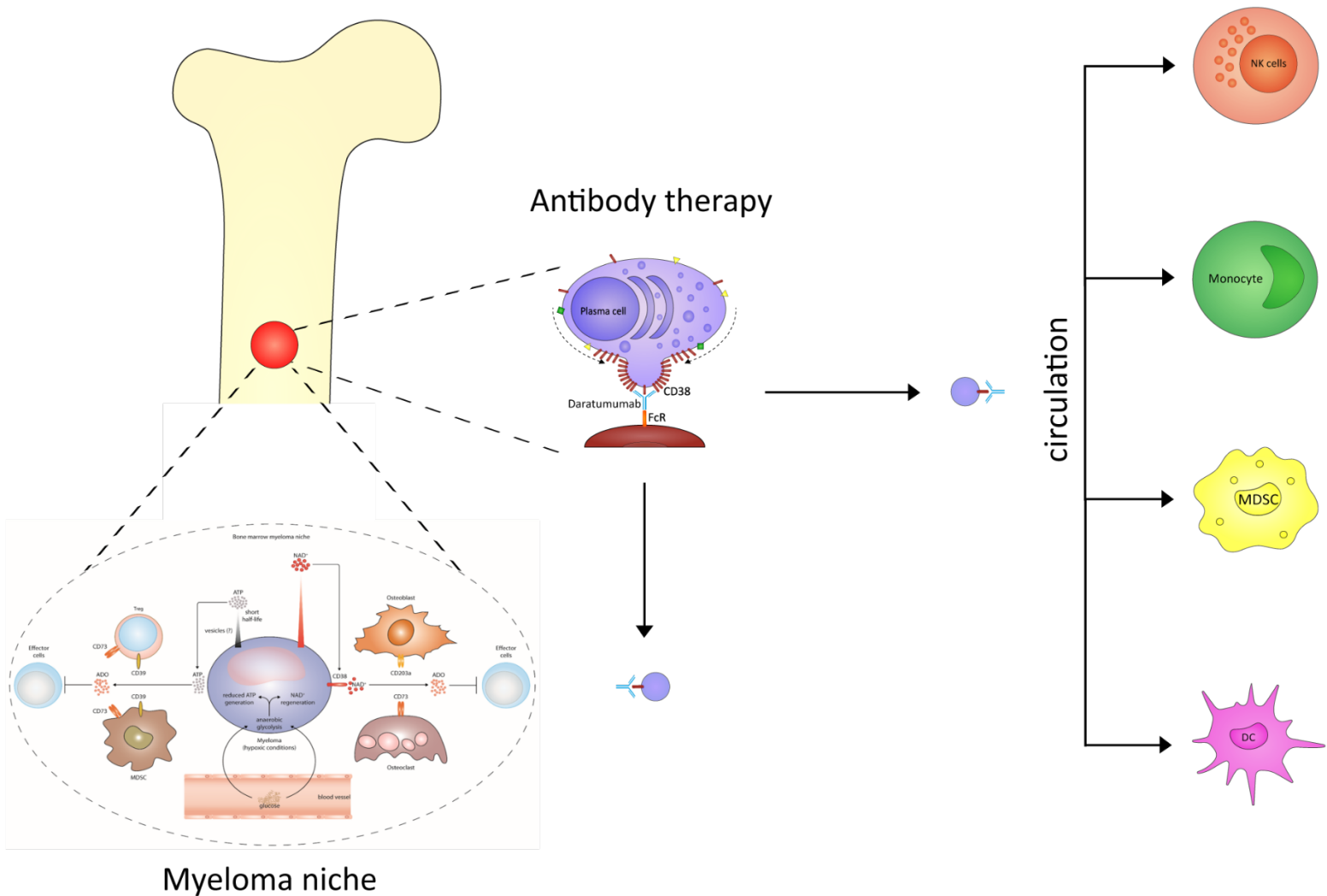
Internalization of MV from human multiple myeloma membranes into dendritic cells



RED=CD80-Alexa546
GREEN=DiO-labelled MV

Faini A.C, Castella, Y. Yakymiv, et al.,.and Malavasi f., 2018, in preparation

Soluble and particulate communications between myeloma and cells *in situ* and afar: a hypothesis



F. Malavasi, B. Castella, A.C. Faini, Y. Yakimiv *et al.*, 2018, in preparation

Questions to be answered

Can anti-CD38 mAbs be active in various phases of treatment (induction, consolidation, maintenance)?

May anti-CD38 mAbs influence escape strategies of myeloma cells?

Can anti-CD38 mAb resistance be predicted?



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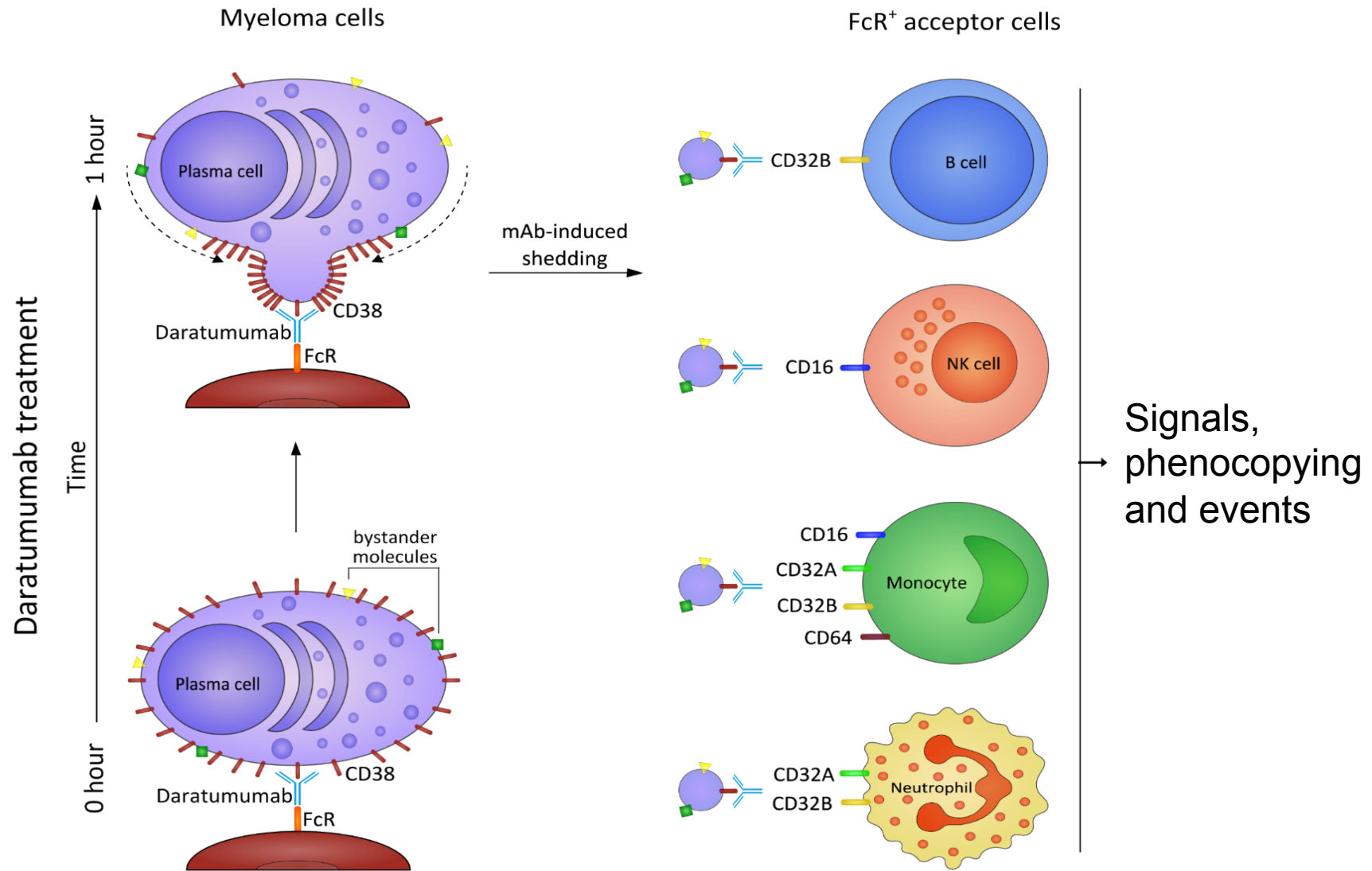
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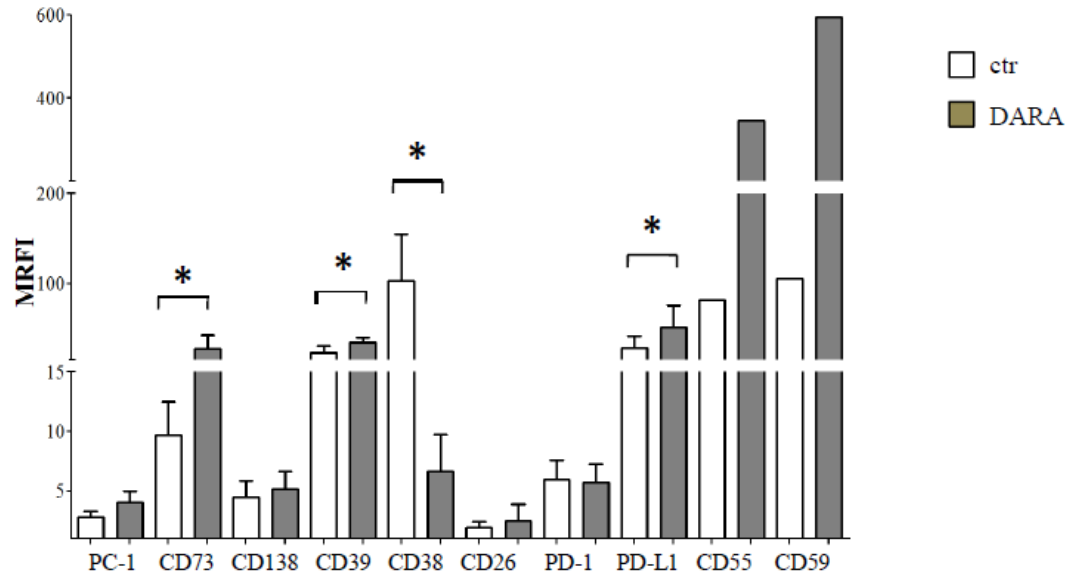
Ilaria Schiavoni
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Clara Ausiello
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In vivo events when a mAb reaches its myeloma target

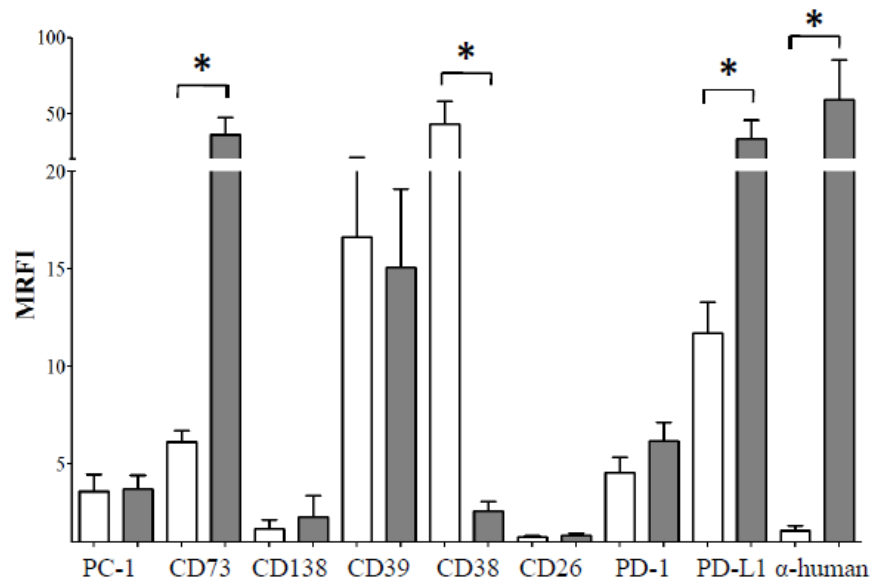


MV phenotypes from patients and myeloma line, after Daratumumab treatment

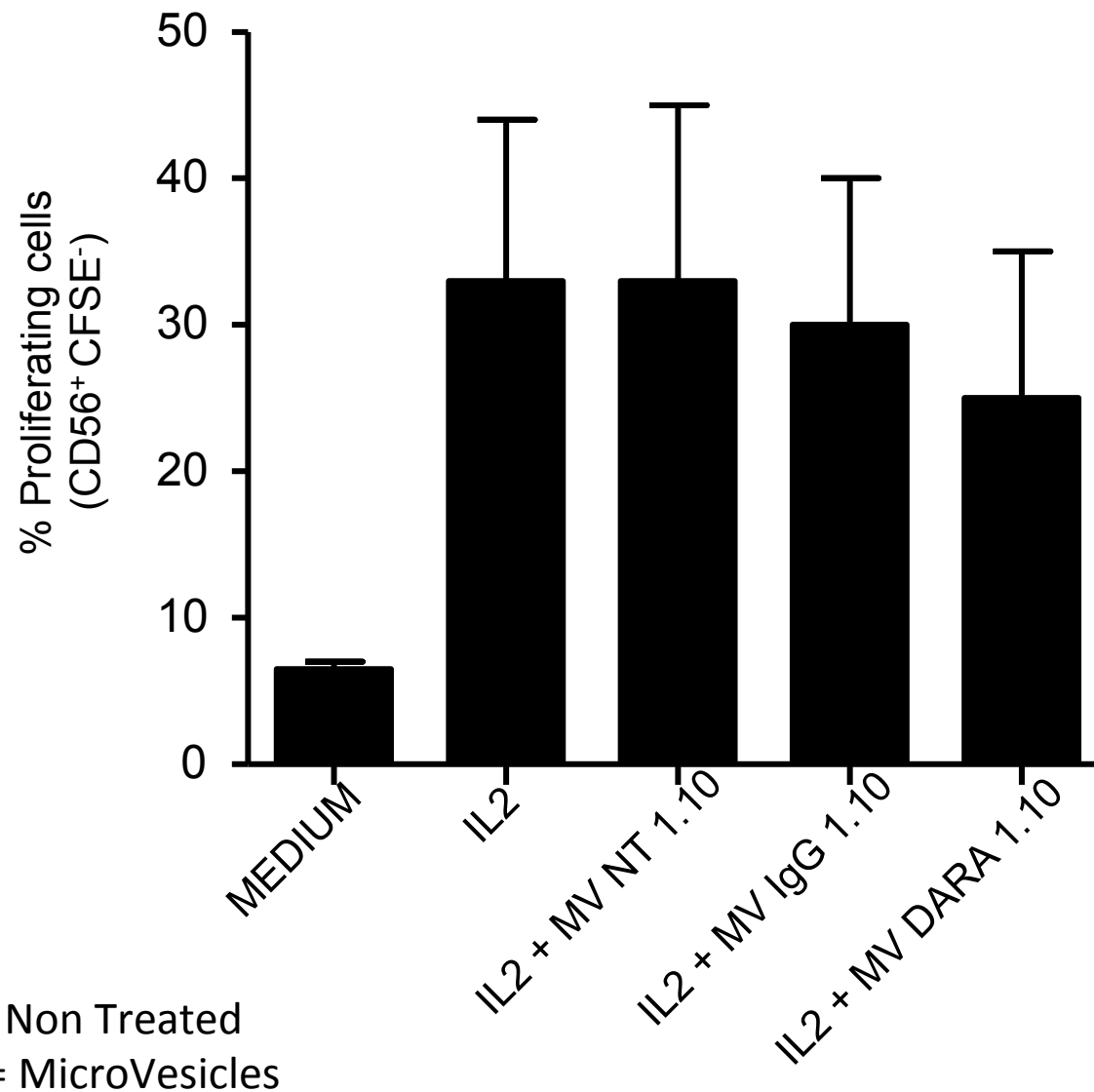
MM patients



BF01 myeloma line

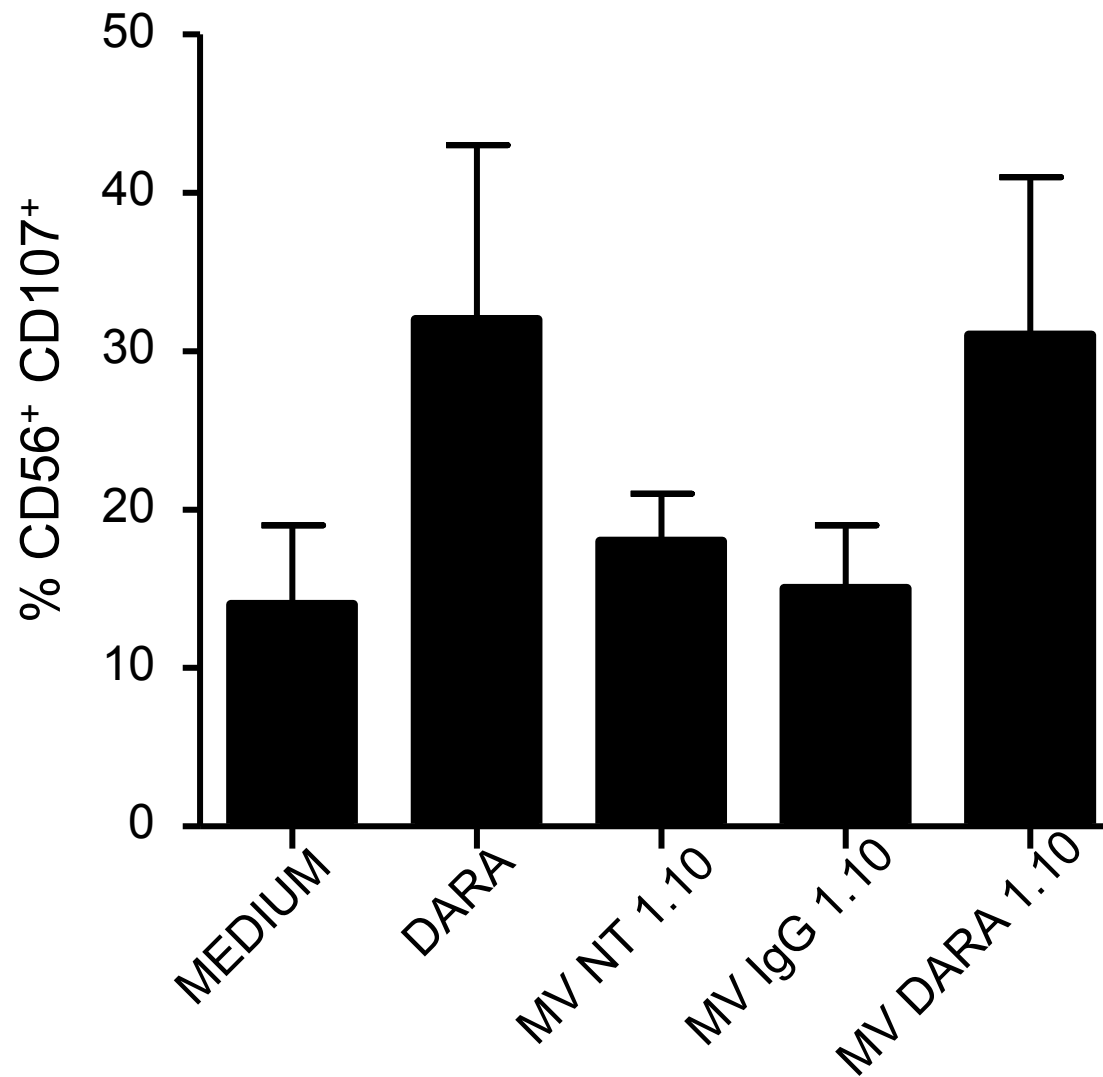


NK PROLIFERATION (PB CTRL + MV MM)

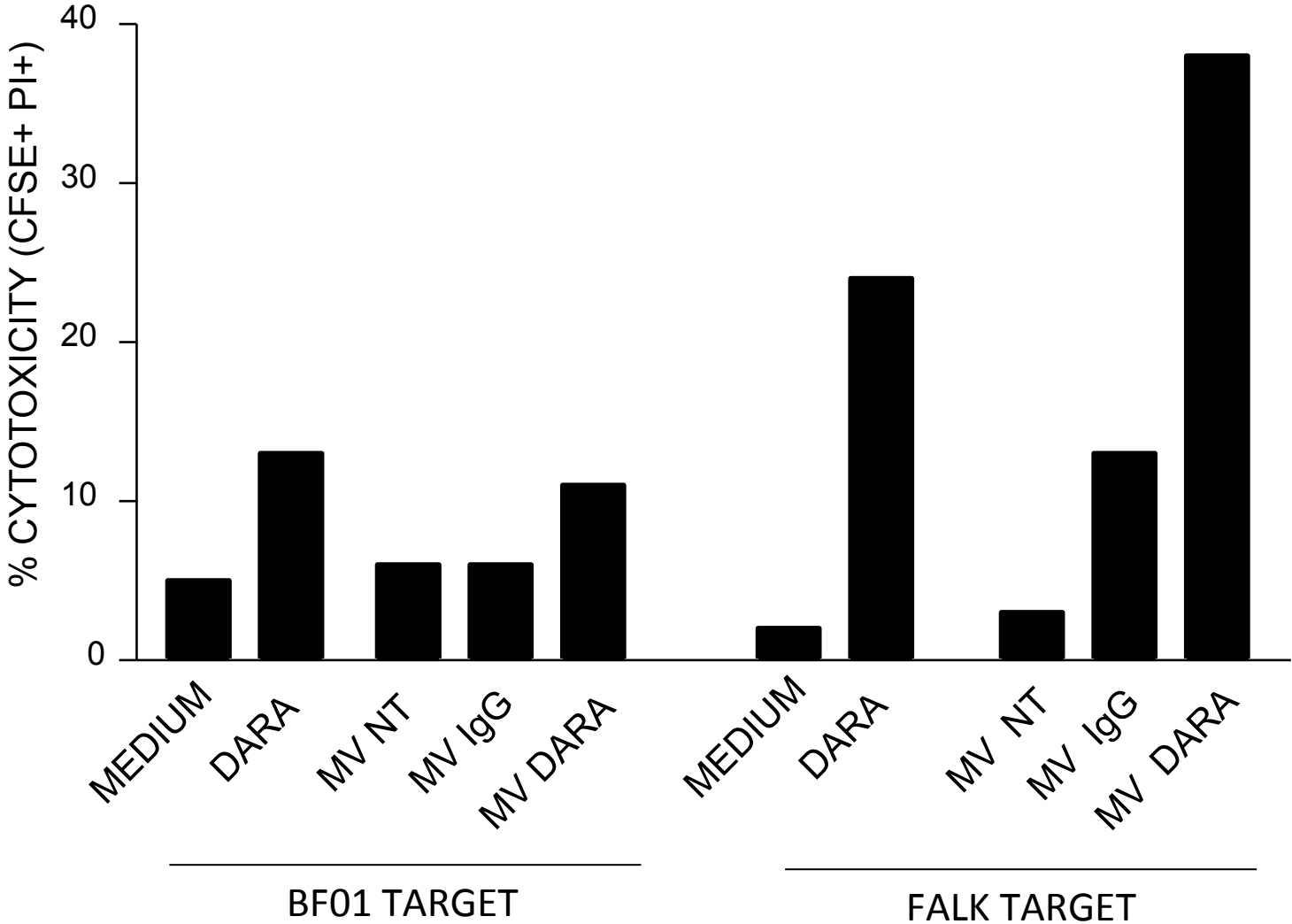


(B. Castella, 2018, in preparation)

NK DEGRANULATION (PB CTRL + MV MM)

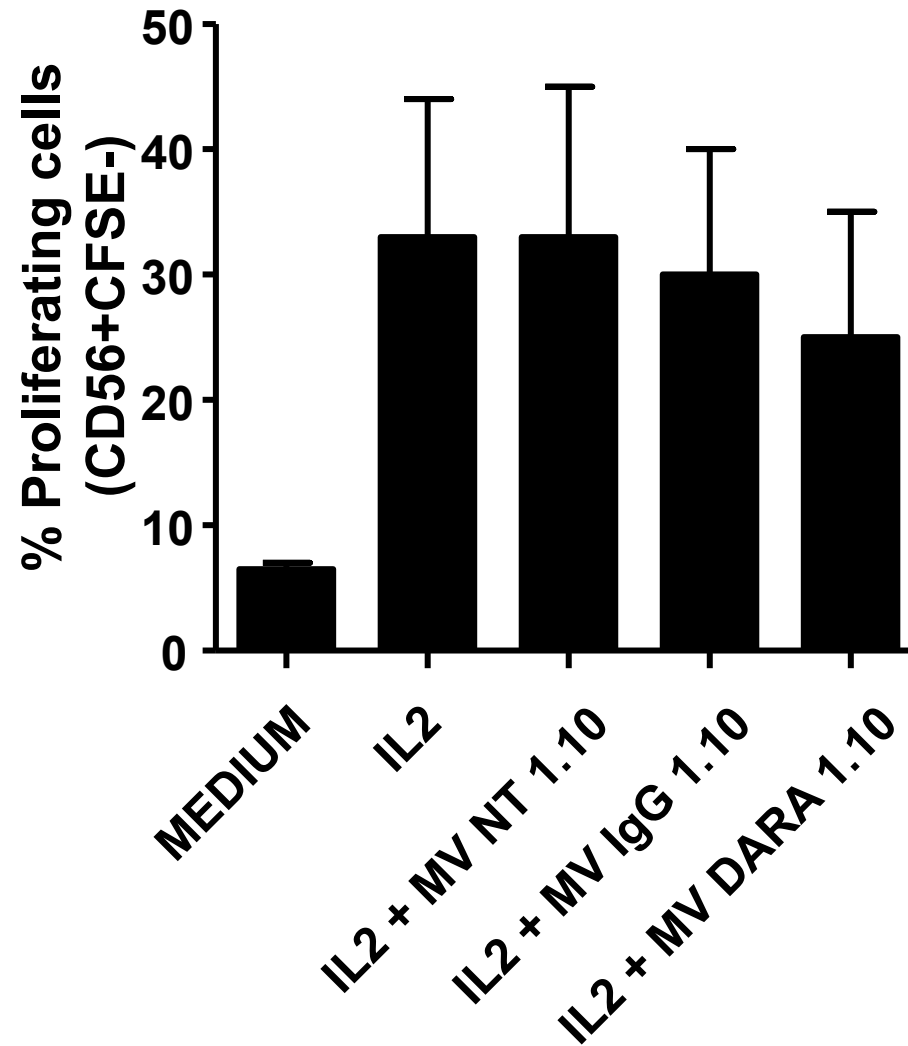


NK CYTOTOXICITY (PB CTRL + MV MM)



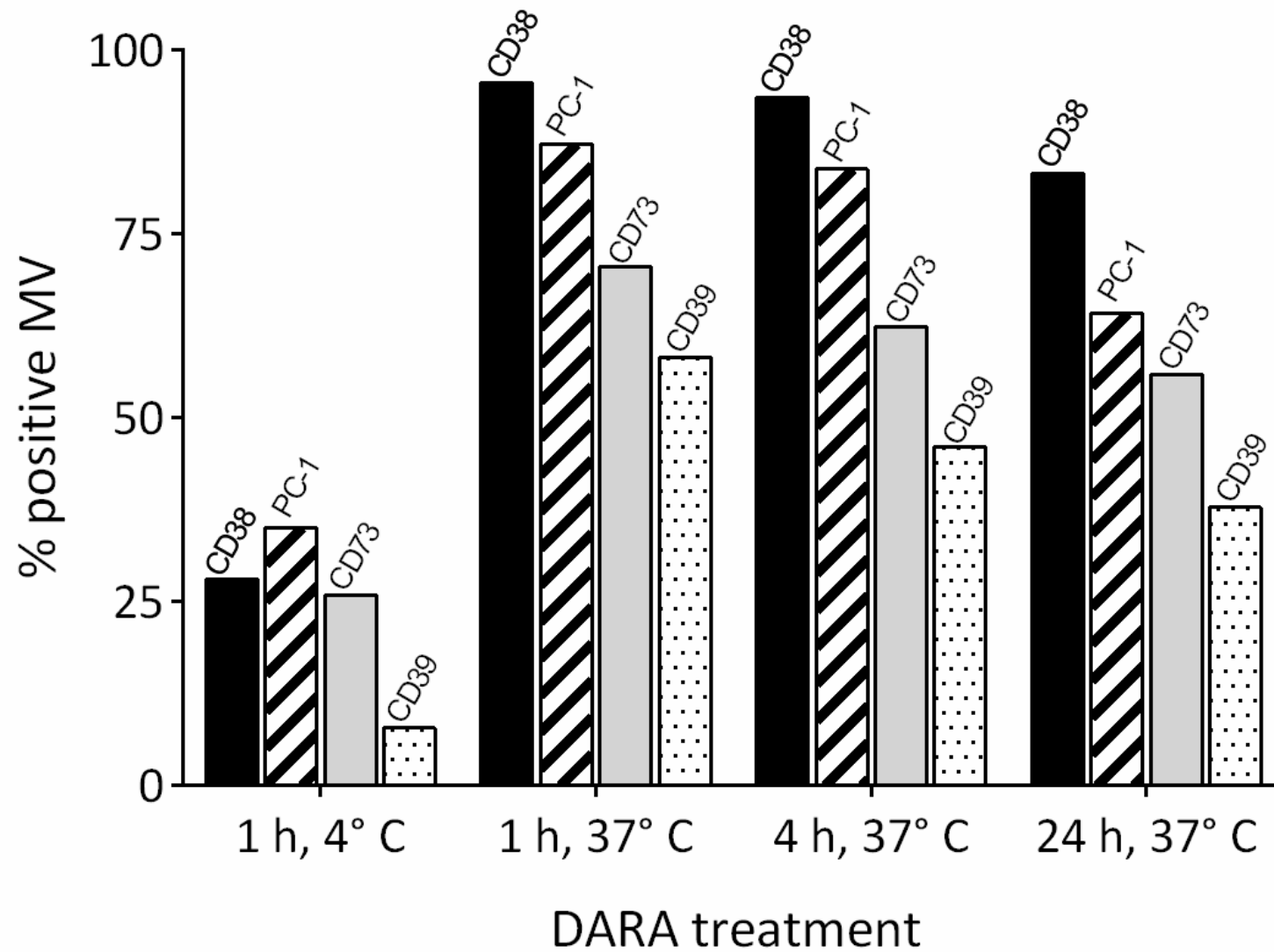
B Castella (2018, in preparation)

NK PROLIFERATION (PB CTRL + MV MM)

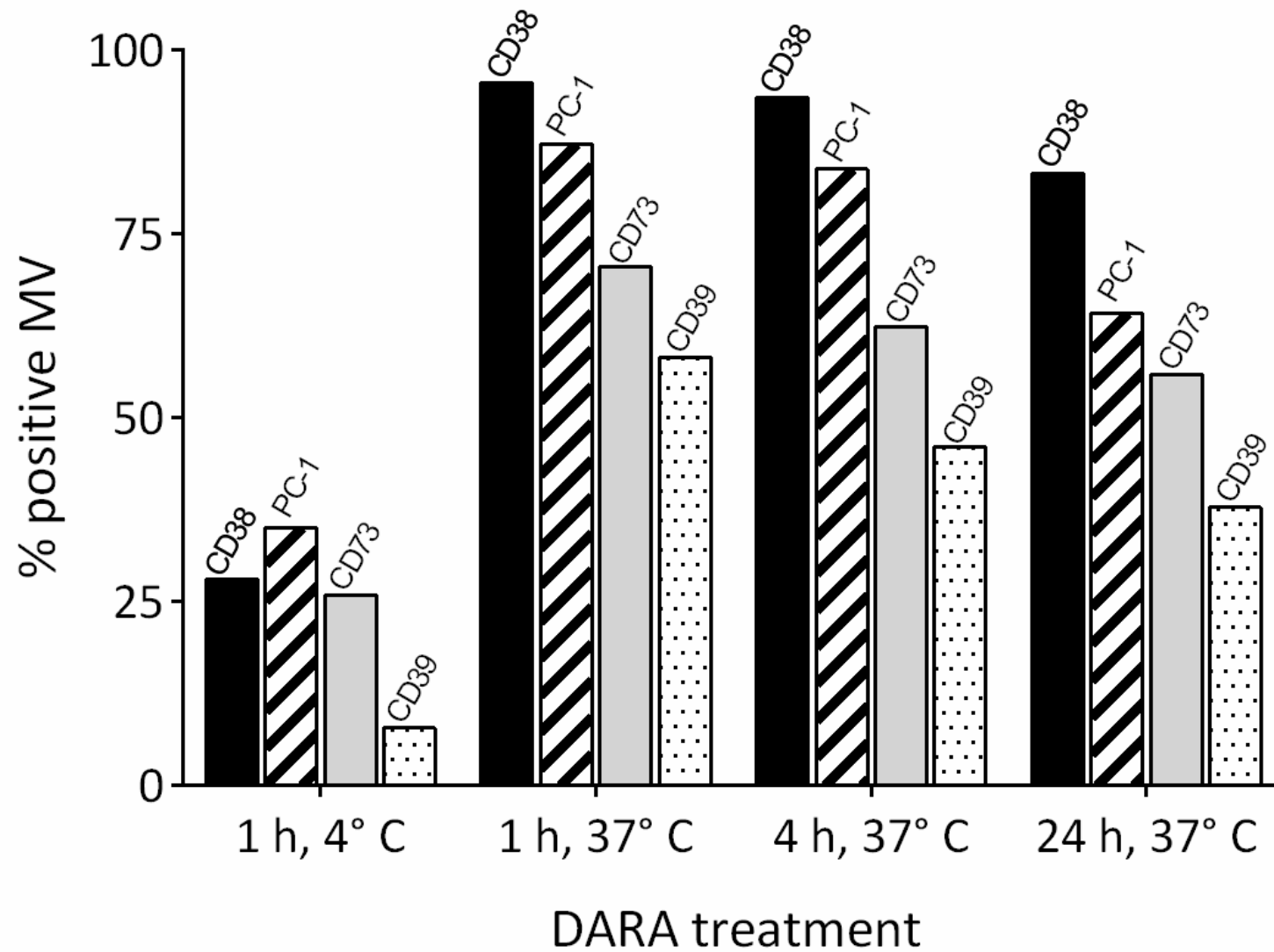


B. Castella (2108, in preparation)

MV phenotype from MM after treatment with anti-CD38 mAbs



MV phenotype from MM after treatment with anti-CD38 mAbs


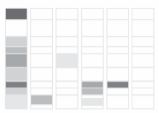





Immunomodulatory properties of antibodies

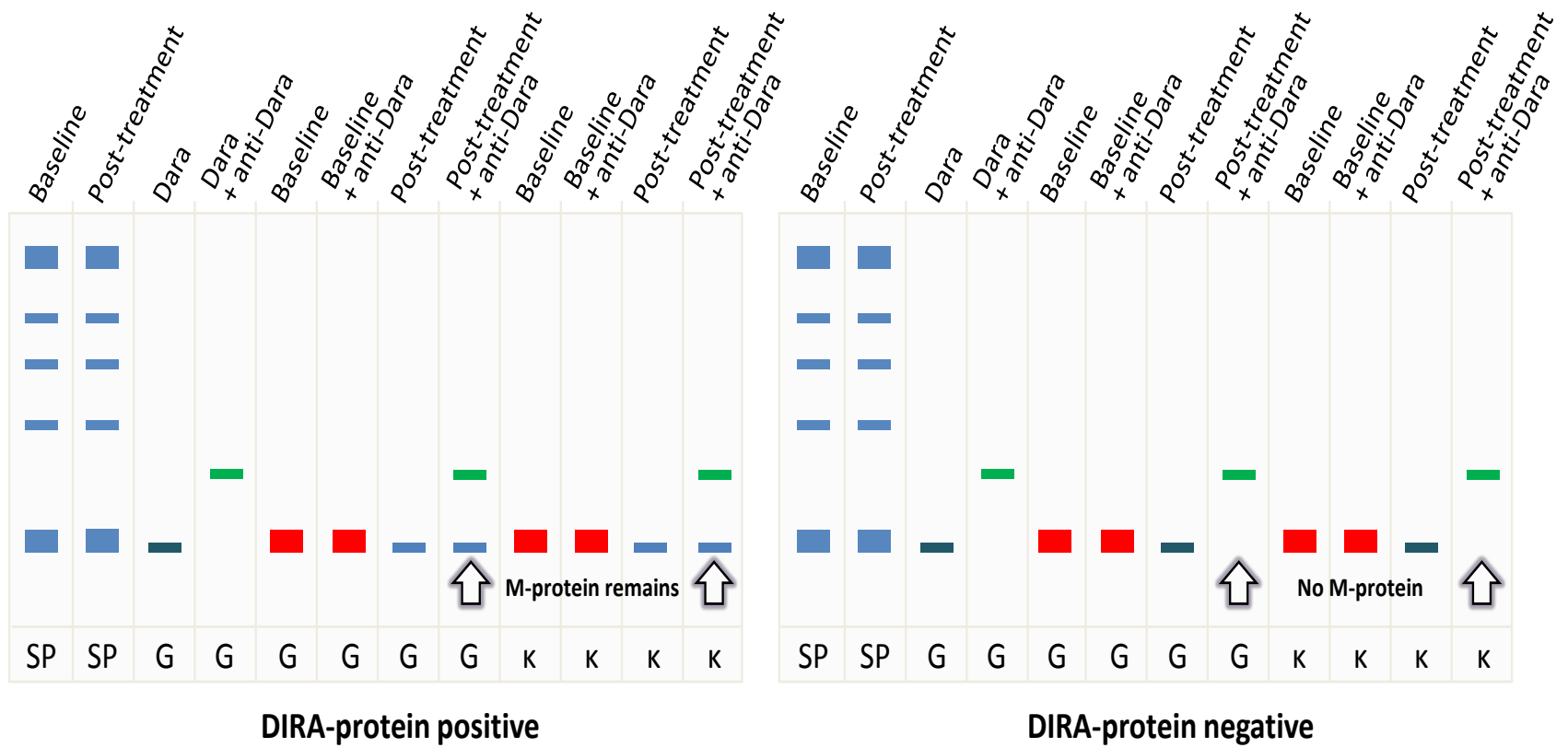
1) Tumors shield themselves from the immune system through immunosuppressive mechanisms in the tumor microenvironment. One instance is shedding of surface molecules.

2) Antibodies that target not only the tumor, but also immunoregulatory pathways mediated by cells of tumor environment or of the immune system, provided therapeutic successes.

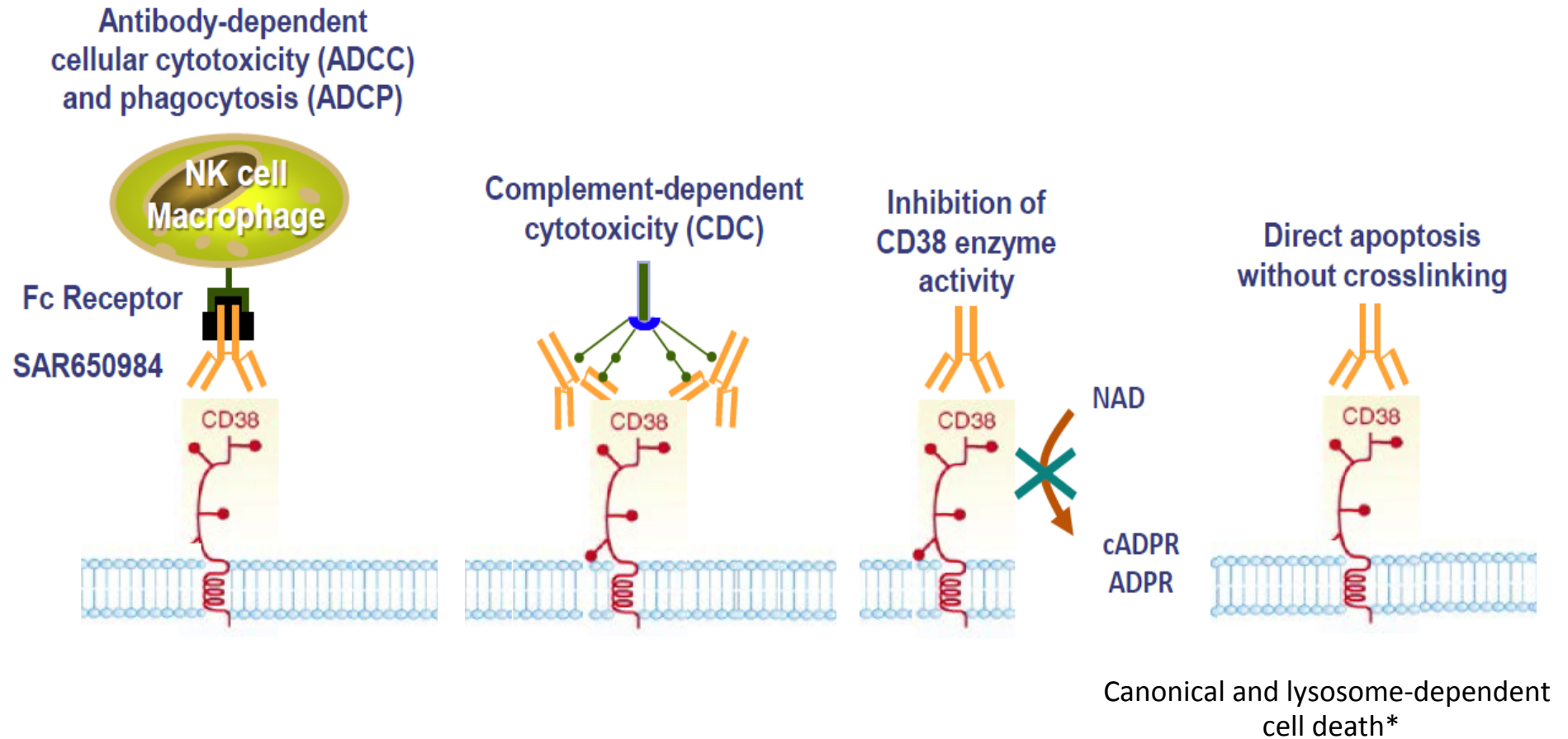
3) CD38 is both a target molecule in myeloma and at the same time an immunomodulatory receptor in immunity

	Management Aspect	Applicable to	Management and Developments
	Administration -Duration of infusion -Infusion-related reactions	All antibodies	Appropriate pre- and post-infusion medication; subcutaneous administration of daratumumab
	Interference of therapeutic antibody with SPEP/IFE assays	All antibodies but dependent on plasma concentrations	Shift assays; mass spectrometry
	Auto-immune adverse events	PD-1/PD-L1 neutralizing antibodies (not observed with CD38 antibodies)	Institution of appropriate treatment (including prompt treatment with prednisone)
	Interference with blood transfusion tests	CD38-targeting antibodies (and CD44-targeting antibodies)	DTT, anti-idiotypic, serotyping/genotyping before start therapy; provide patients with blood transfusion card
	Infections	Dependent on antibody type and other drugs in combination regimens	Herpes zoster prophylaxis is recommended for CD38 antibodies

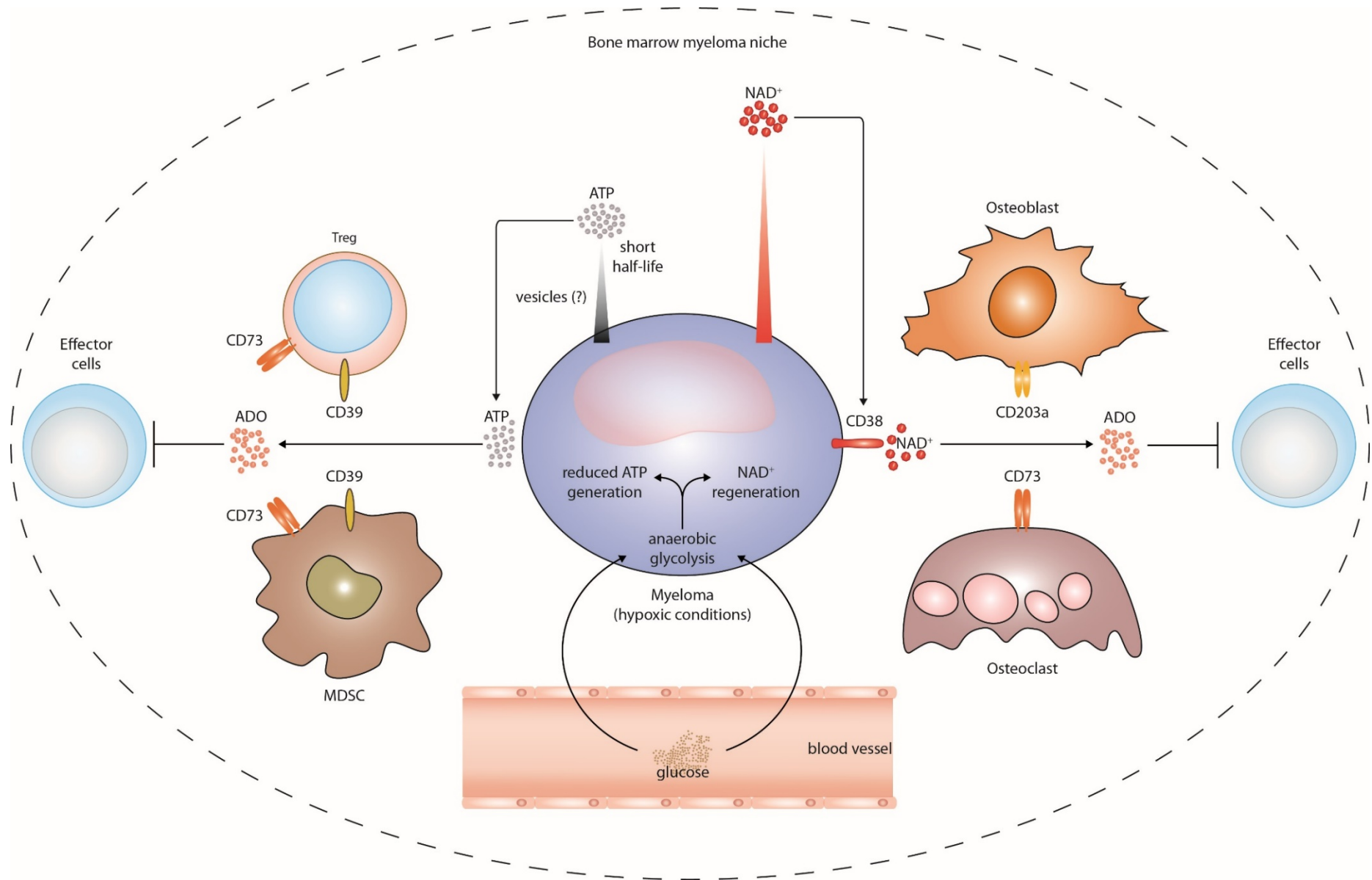
A



Isatuximab (anti-CD38) induces direct apoptosis and suppresses Tregs to mitigate immune impairment in multiple myeloma



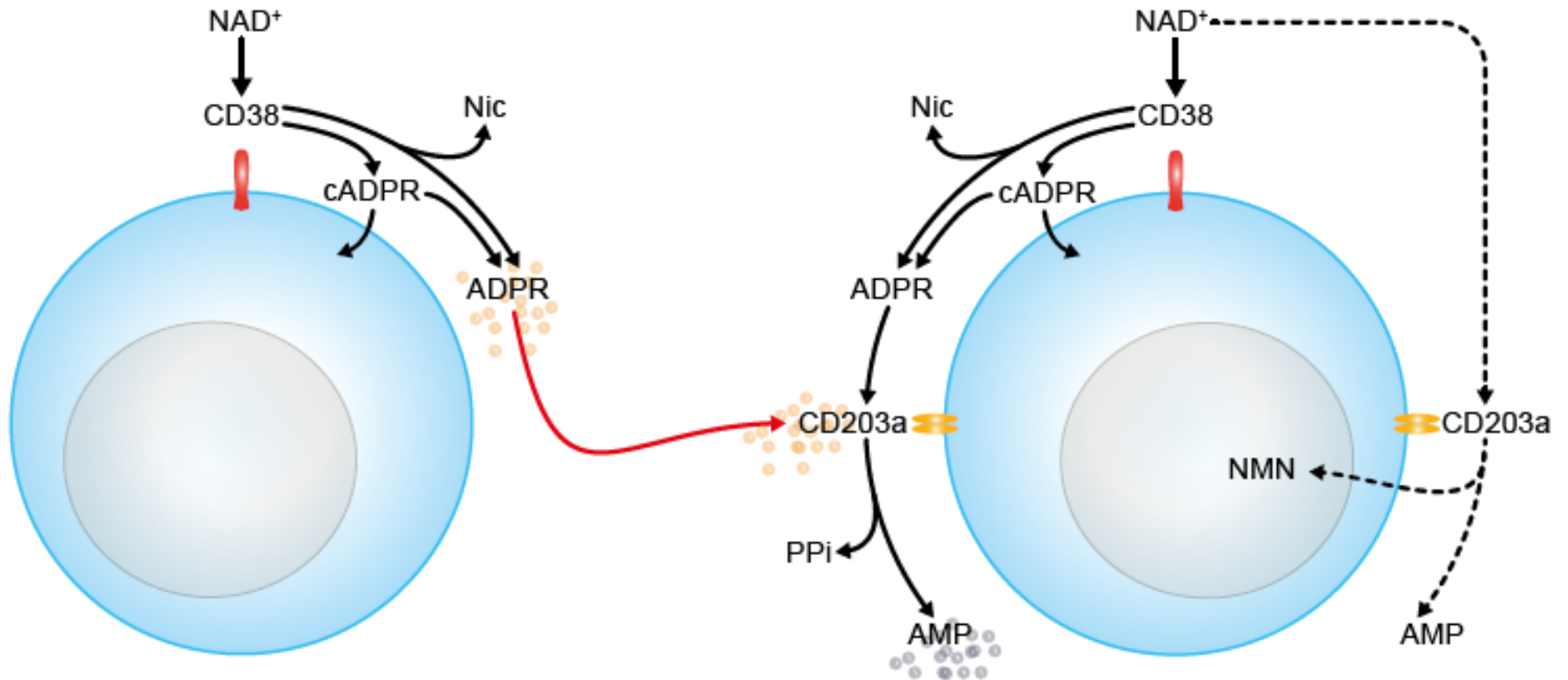
Metabolic balance between ATP and NAD⁺ in the BM niche



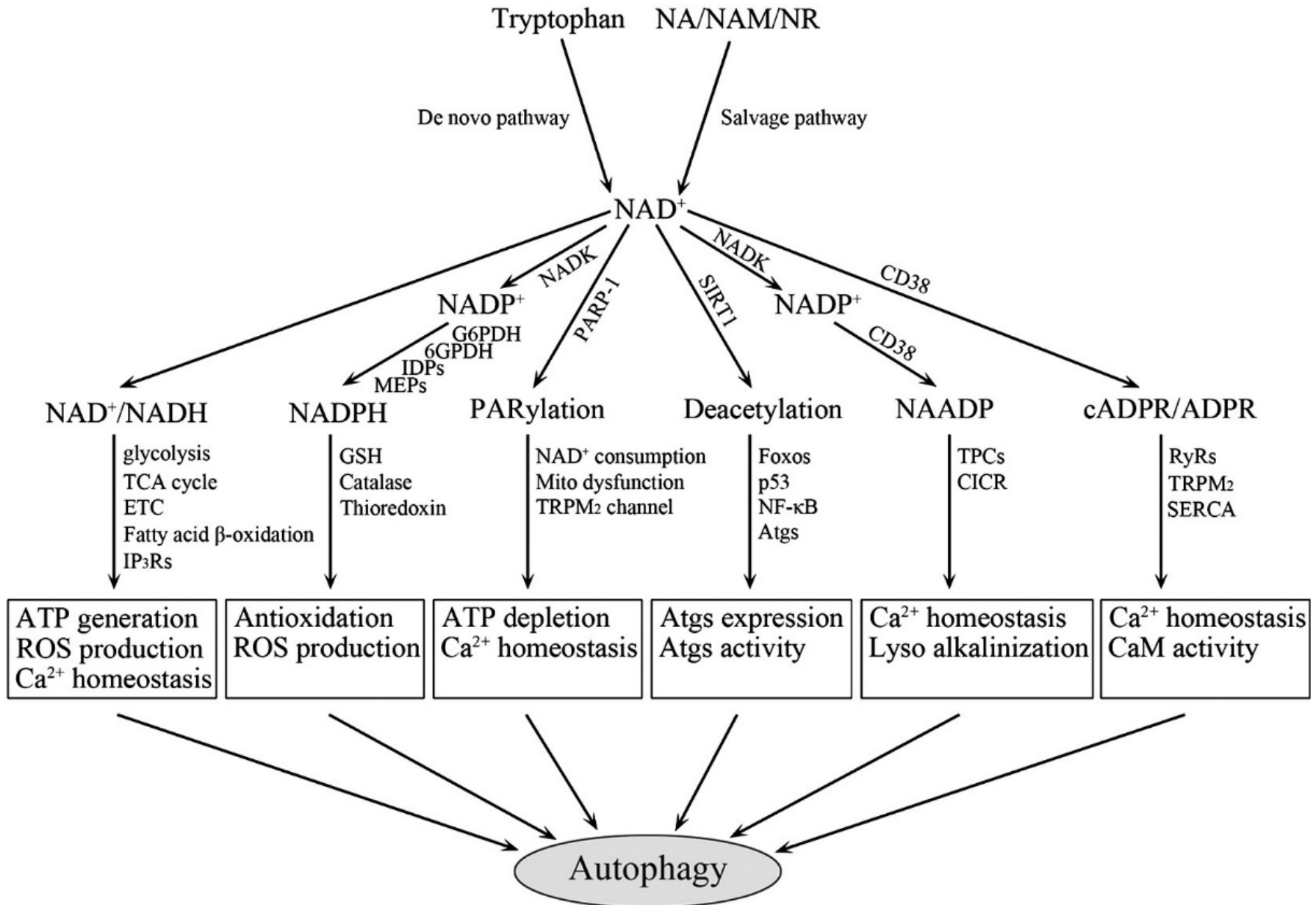
Schematic representation of the discontinuous ectoenzymatic CD38/CD203a/CD73 network

Resting T lymphocyte

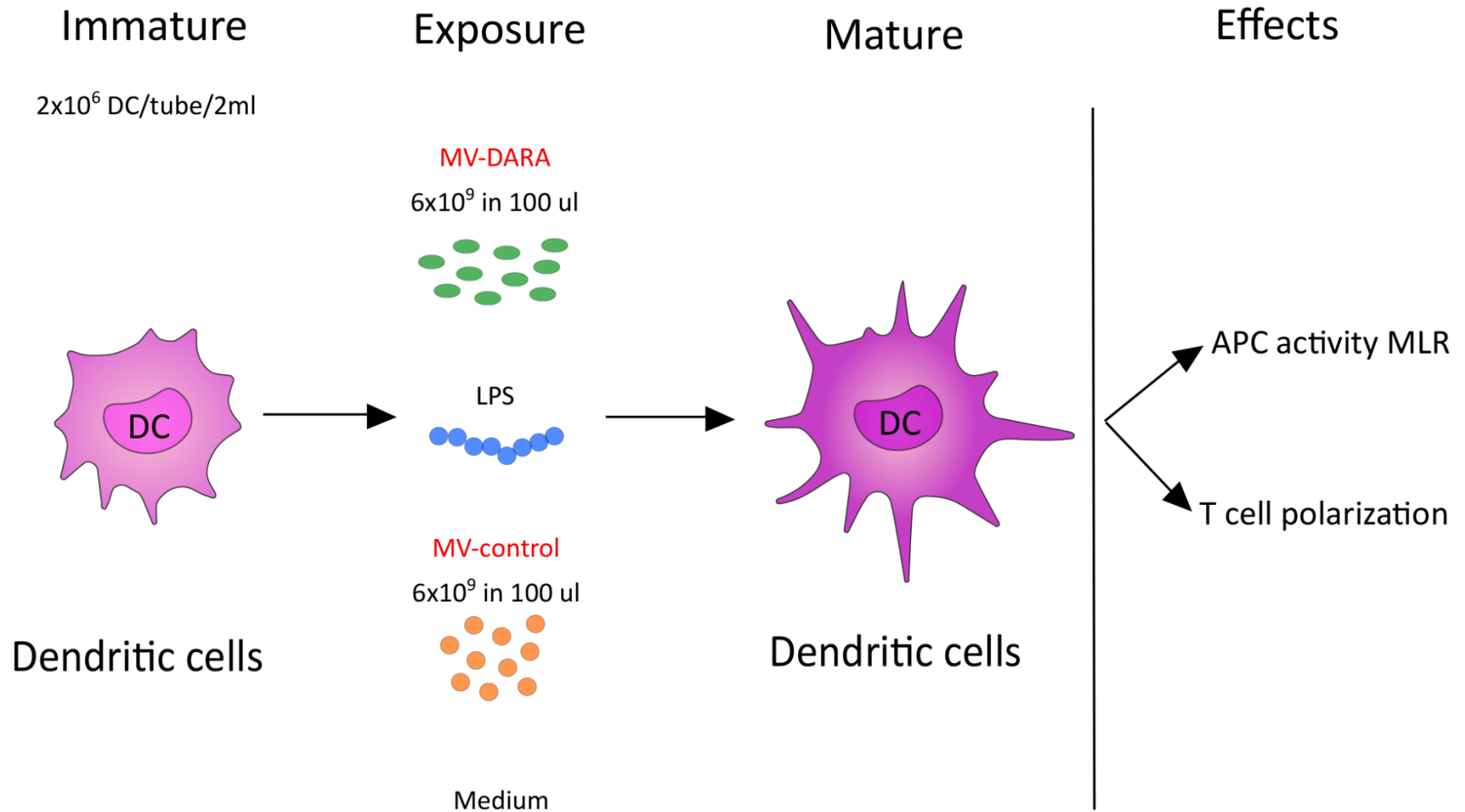
Activated T lymphocyte



Extracellular NAD⁺ can be metabolized by CD38-NADase generating Nic, cADPR and ADPR. The latter compound is transformed to AMP by the CD203a-NPP .

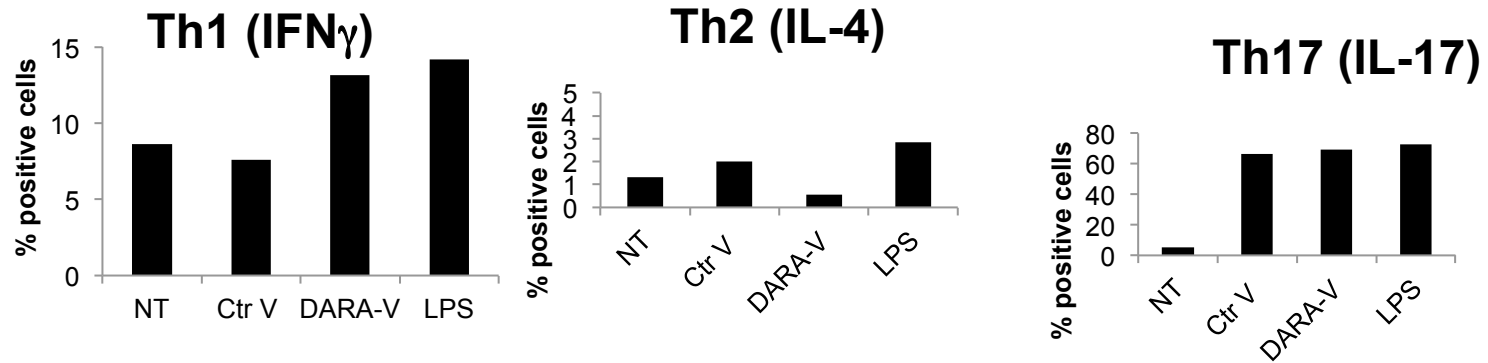


Vaccinal effects after DARA-microvesicles (MV)

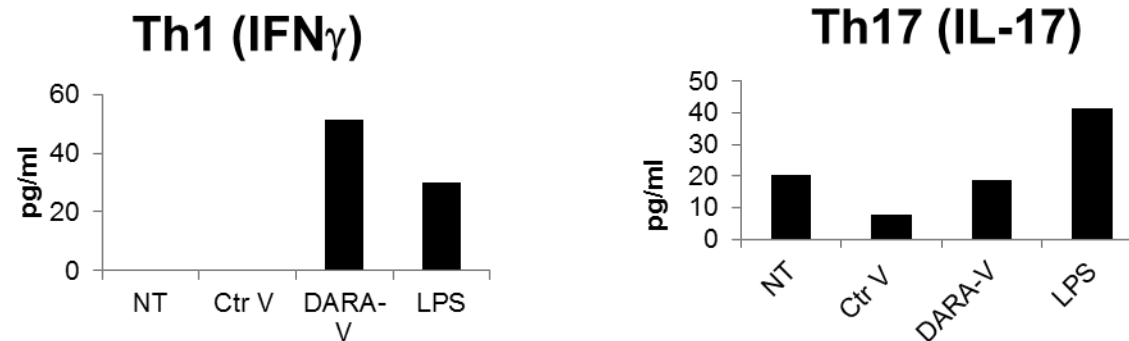


Polarization: T cell response polarized by DC treated with DARA-MV

Cytokines production (intracellular staining):



Cytokines production (Immuno-enzymatic assay):



Anti-CD38 antibody-mediated therapy in myeloma: some unbeaten paths of potential application

- 1) BM contains a panel of growth-permissive and restrictive signals from the tumor microenvironment. These signals likely co-evolve with the tumor. Can the enzymatic activities exerted by CD38 play a role in these events?
- 2) Does the enzymatic activities of CD38 collaborate with other ectoenzymes in the bone marrow niche?
- 3) Do therapeutic anti-CD38 antibodies interfere with the enzymatic activities ruled by CD38?
- 4) Do the products derived from the ectoenzymes operate outside the niche?

Immunomodulatory properties of antibodies

1) Tumors shield themselves from the immune system through immunosuppressive mechanisms in the tumor microenvironment, for example, shedding of surface molecules

2) Antibodies that target not only the tumor, but immunoregulatory pathways mediated by cells of the immune system, provided therapeutic successes

3) CD38 is both a target molecule in myeloma and at the same time an immunomodulatory receptor in immunity

Rationale for targeting CD38

Functions:

1) Receptor-mediated adhesion and signaling functions

2) Enzymatic activities

Contributes to intracellular calcium mobilization

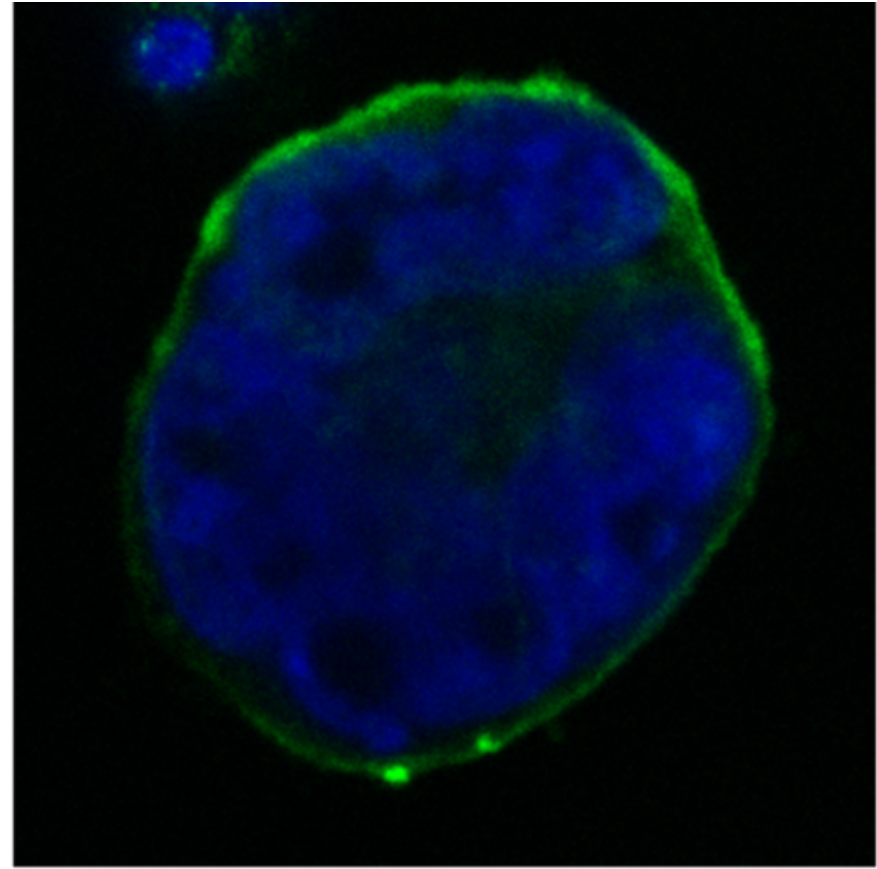
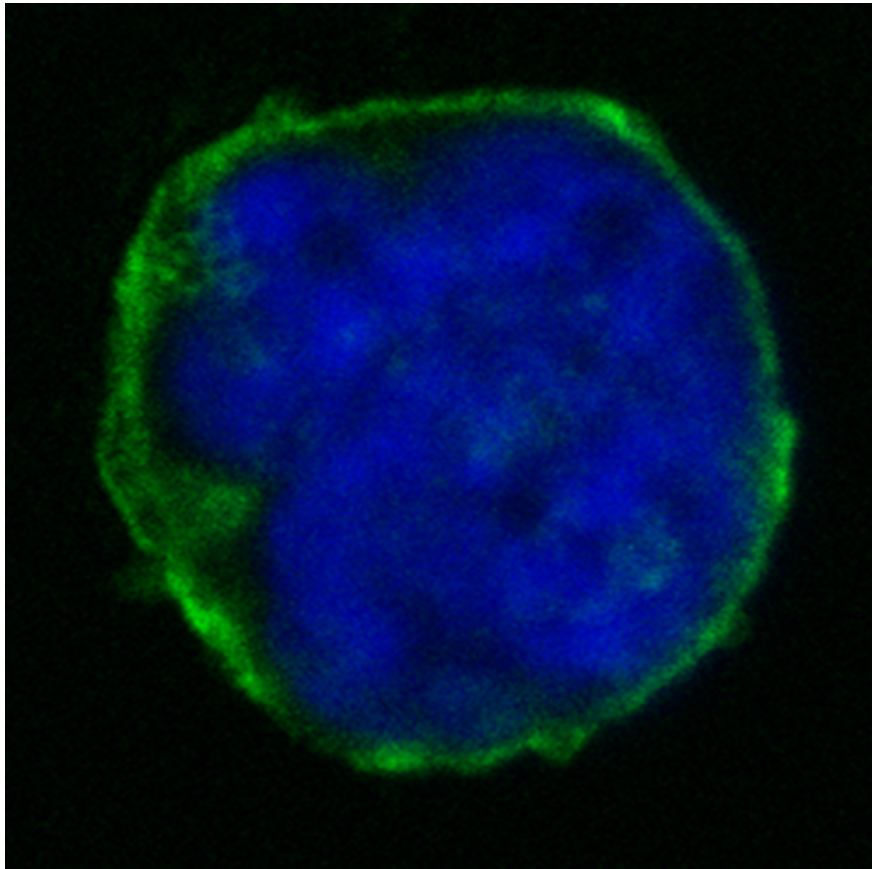
Involved in production of adenosine: important for induction of local immunological tolerance → implicated in local survival strategy of the neoplastic plasma cell in the bone marrow milieu

Expression levels:

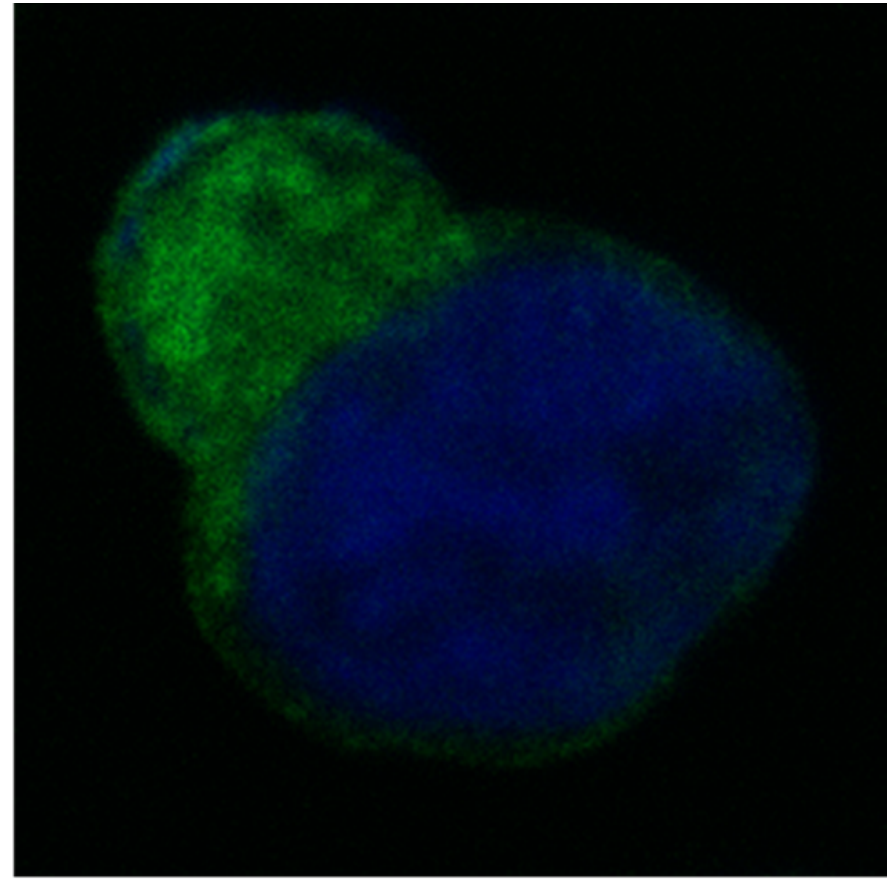
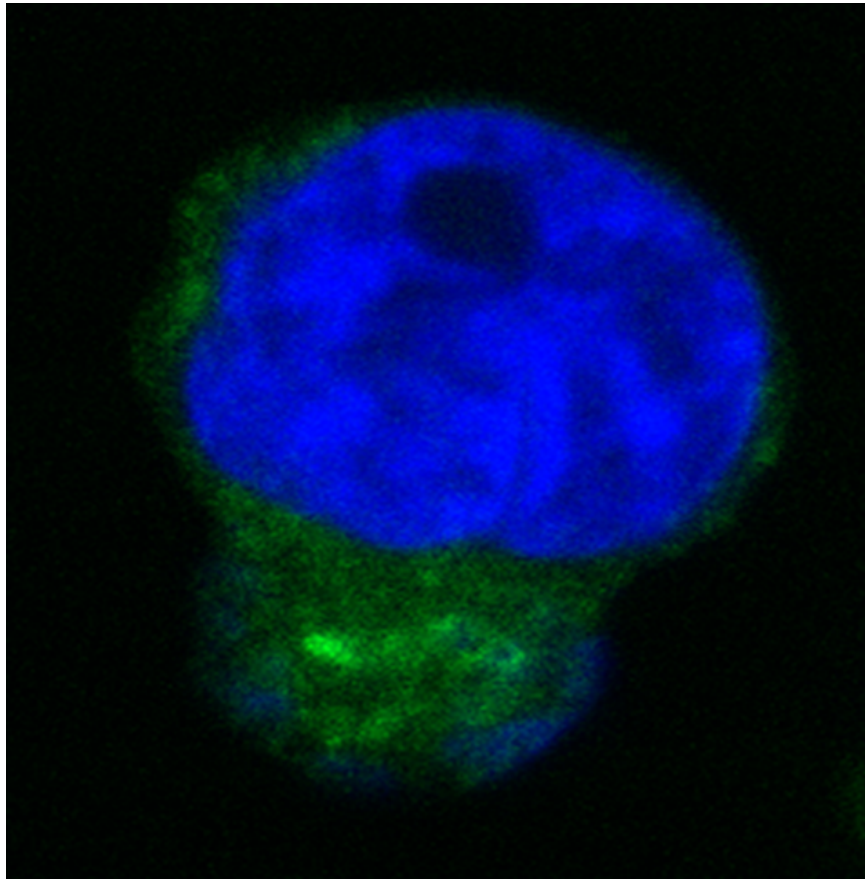
1) Low expression of CD38 on lymphoid and myeloid cells under normal conditions

2) High expression of CD38 on multiple myeloma cells

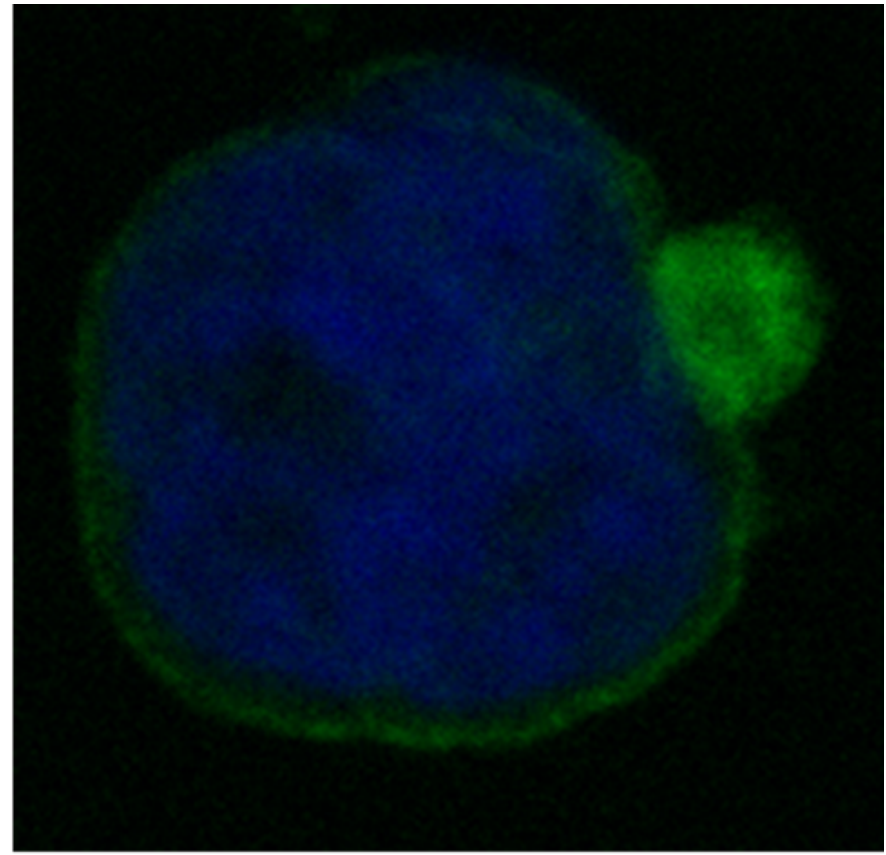
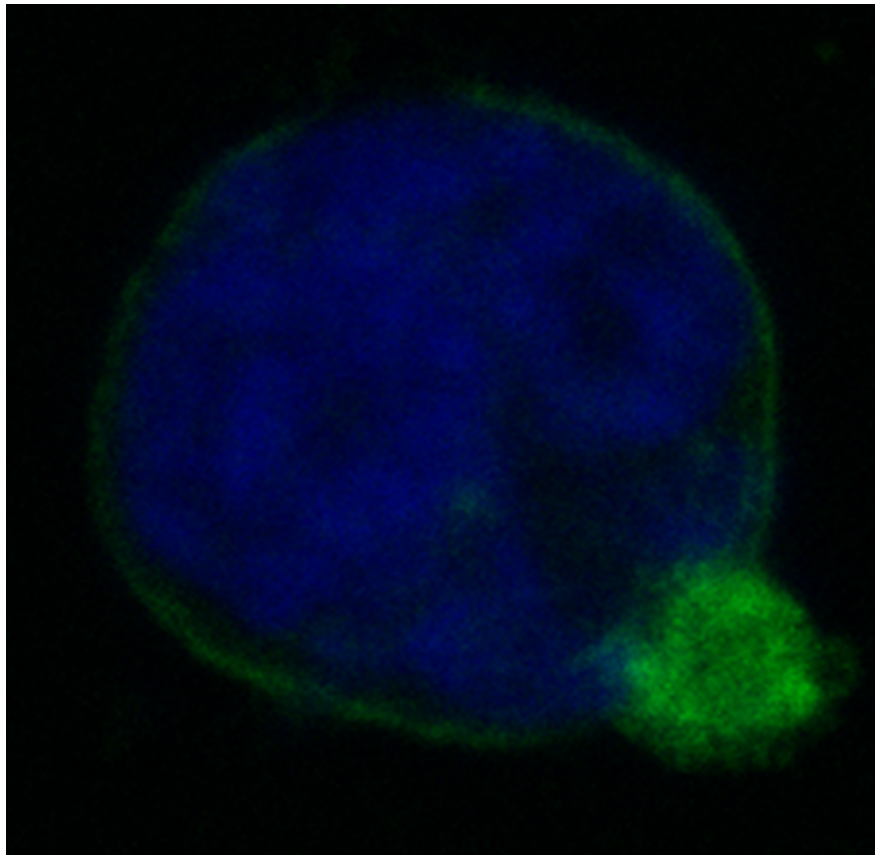
Confocal microscopy analysis of CD38/DARA interaction
(4°C) on a relapsed myeloma



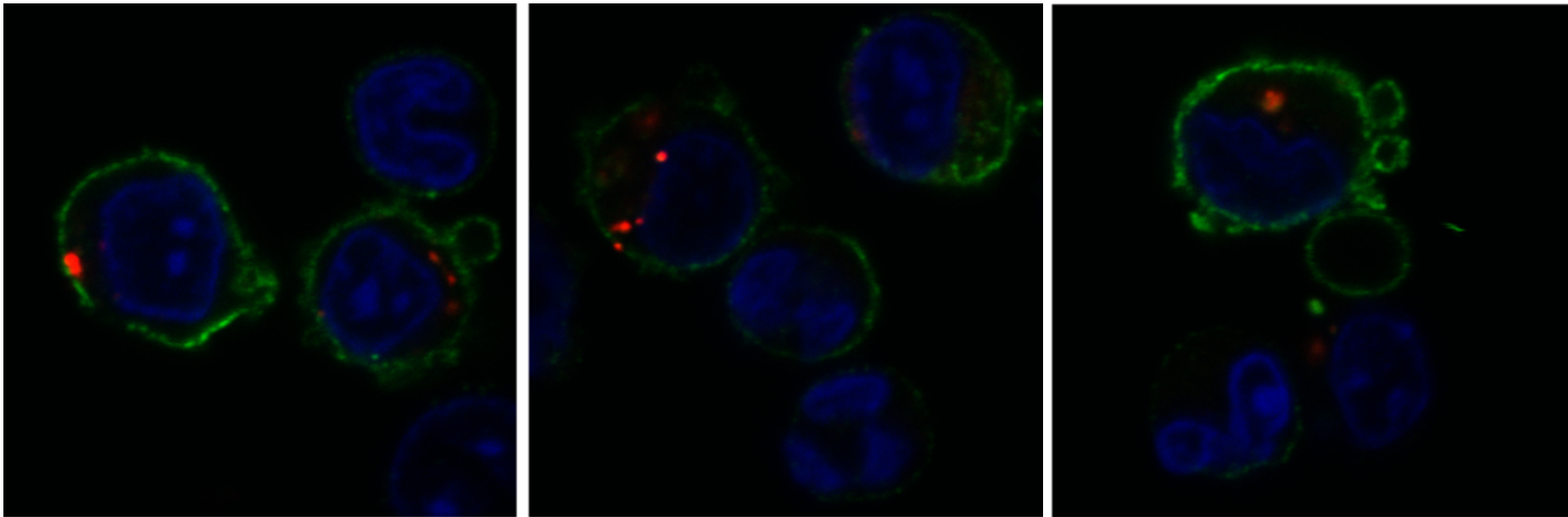
Confocal microscopy analysis of CD38/DARA interaction
(37 °C, 3 h) on a myeloma at diagnosis



Confocal microscopy analysis of CD38/DARA interaction
(37 °C, 2 h) on a myeloma at diagnosis



Whither MV from multiple myeloma: 2) Entering MDSC (CD15⁺/CD33⁺/CD11b⁺)

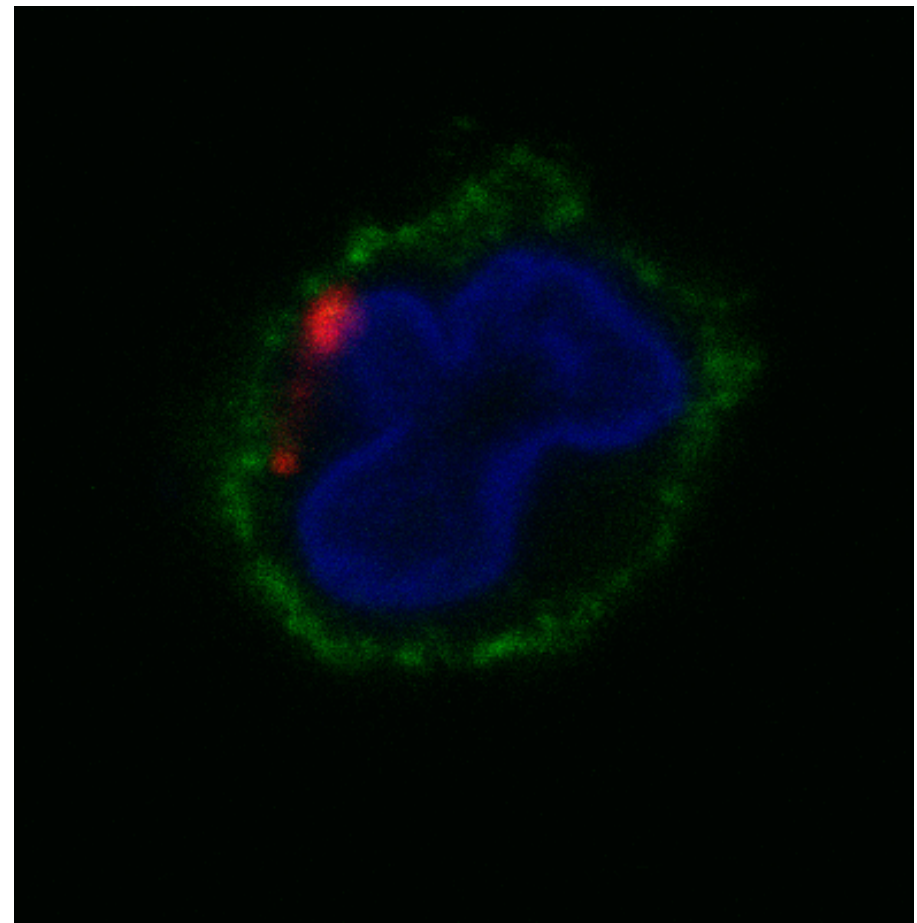
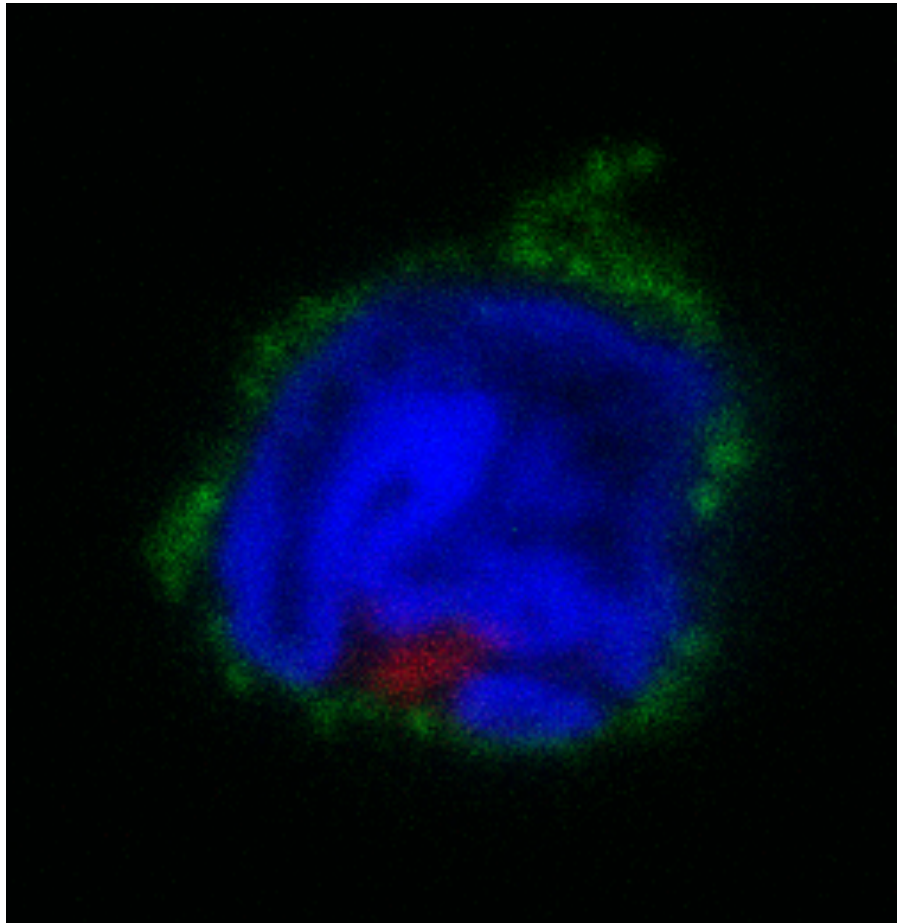


Green = anti-CD14 mAb plus anti-mouse IgG-Alexa 488

Red = MV labeled with 1,1'-Dioctadecyl-3,3',3'-tetramethylindodicarbocyanine 4-chlorobenzenesulfonate (DiD)

Blue = 4',6-Diamidino-2-Phenylindole (DAPI)

Whither MV from multiple myeloma: 3) Entering NK cells (CD16⁺)

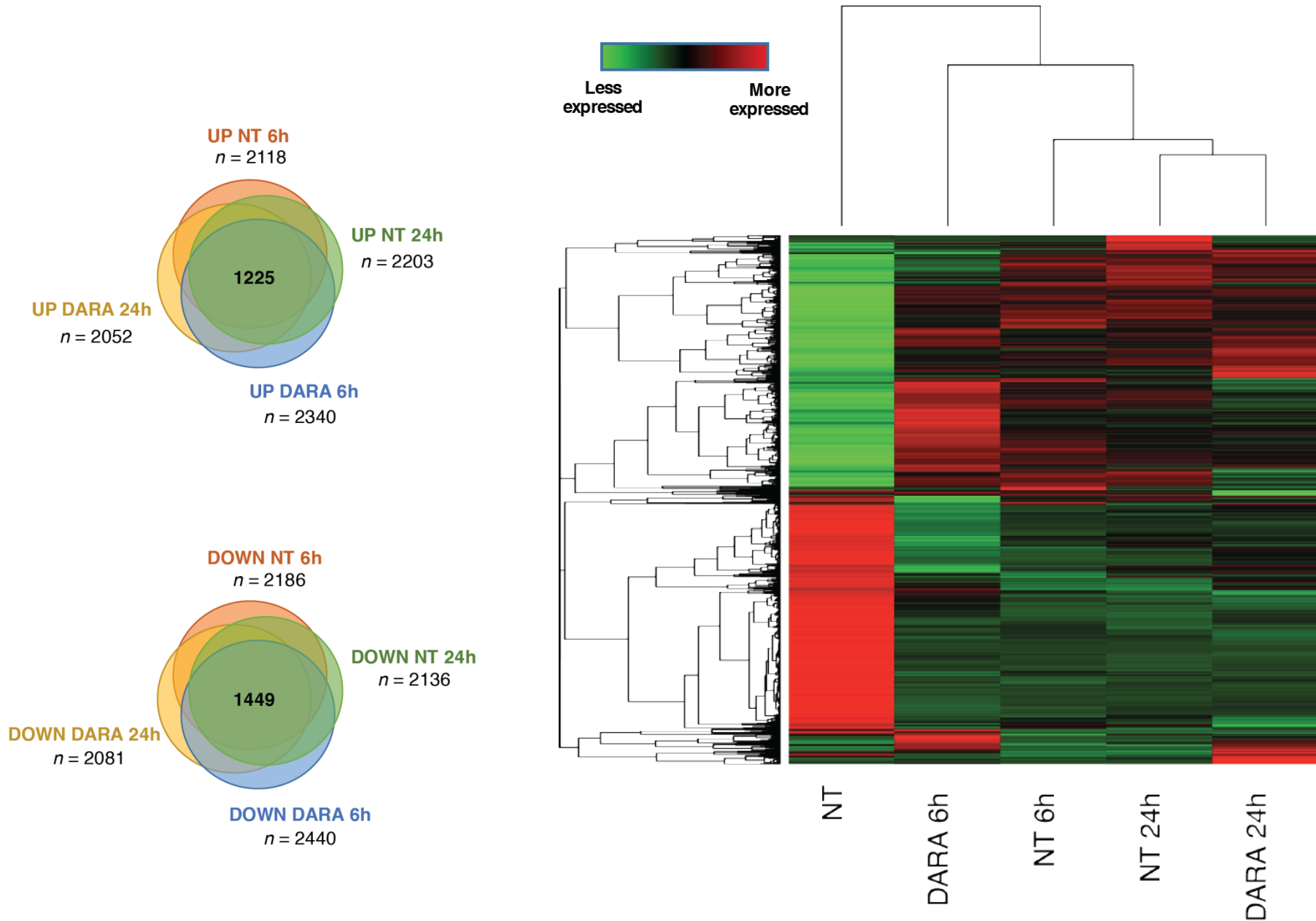


Green = anti-CD16 mAb plus anti-mouse IgG-Alexa 488

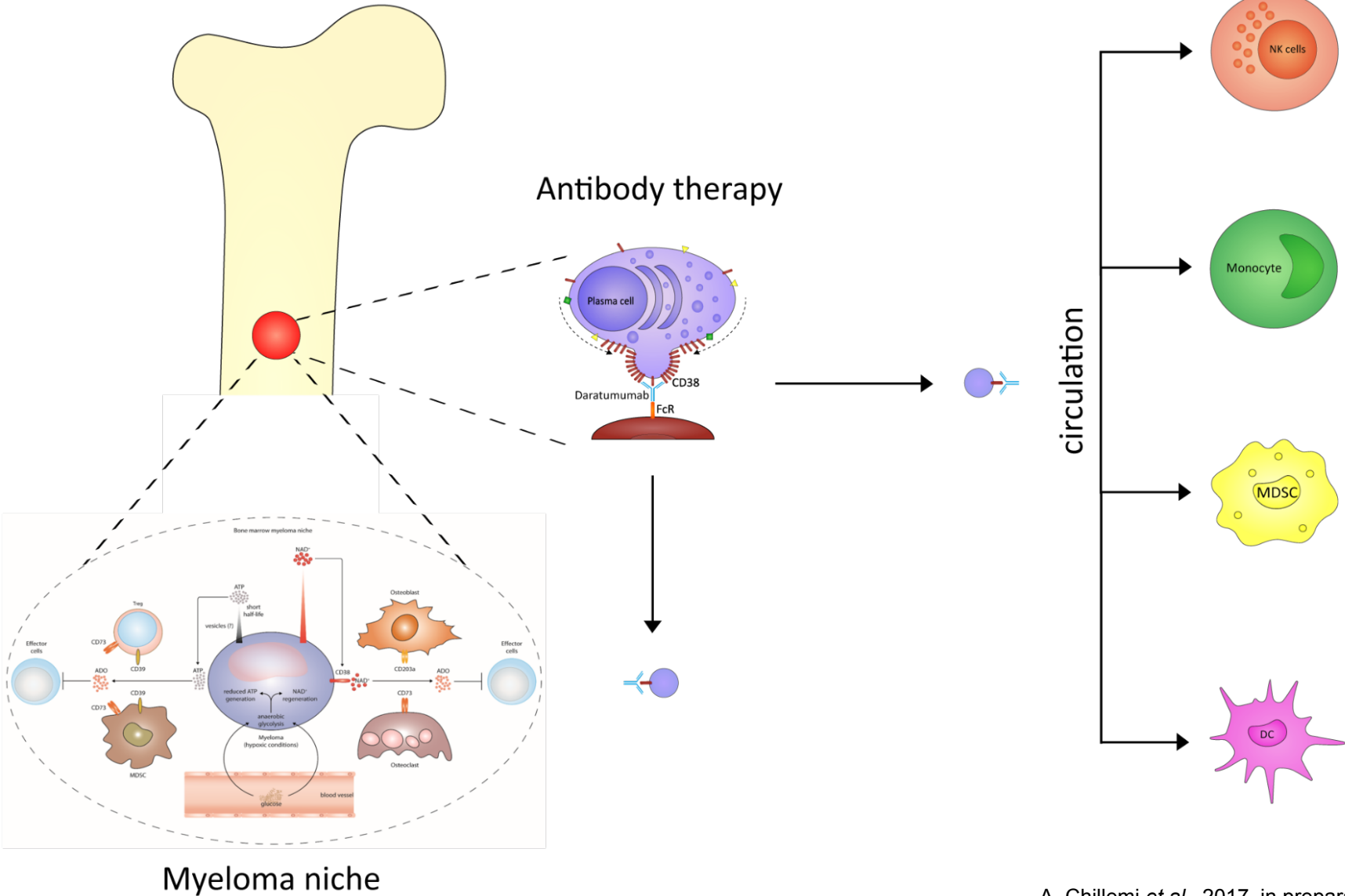
Red = MV labeled with 1,1'-Diocetyl-3,3',3'-tetramethylindodicarbocyanine 4-chlorobenzenesulfonate (DiD)

Blue = 4',6-Diamidino-2-Phenylindole (DAPI)

Whither MV from multiple myeloma: 4) Molecular effects observed on NK cells (CD16⁺/CD56⁺)



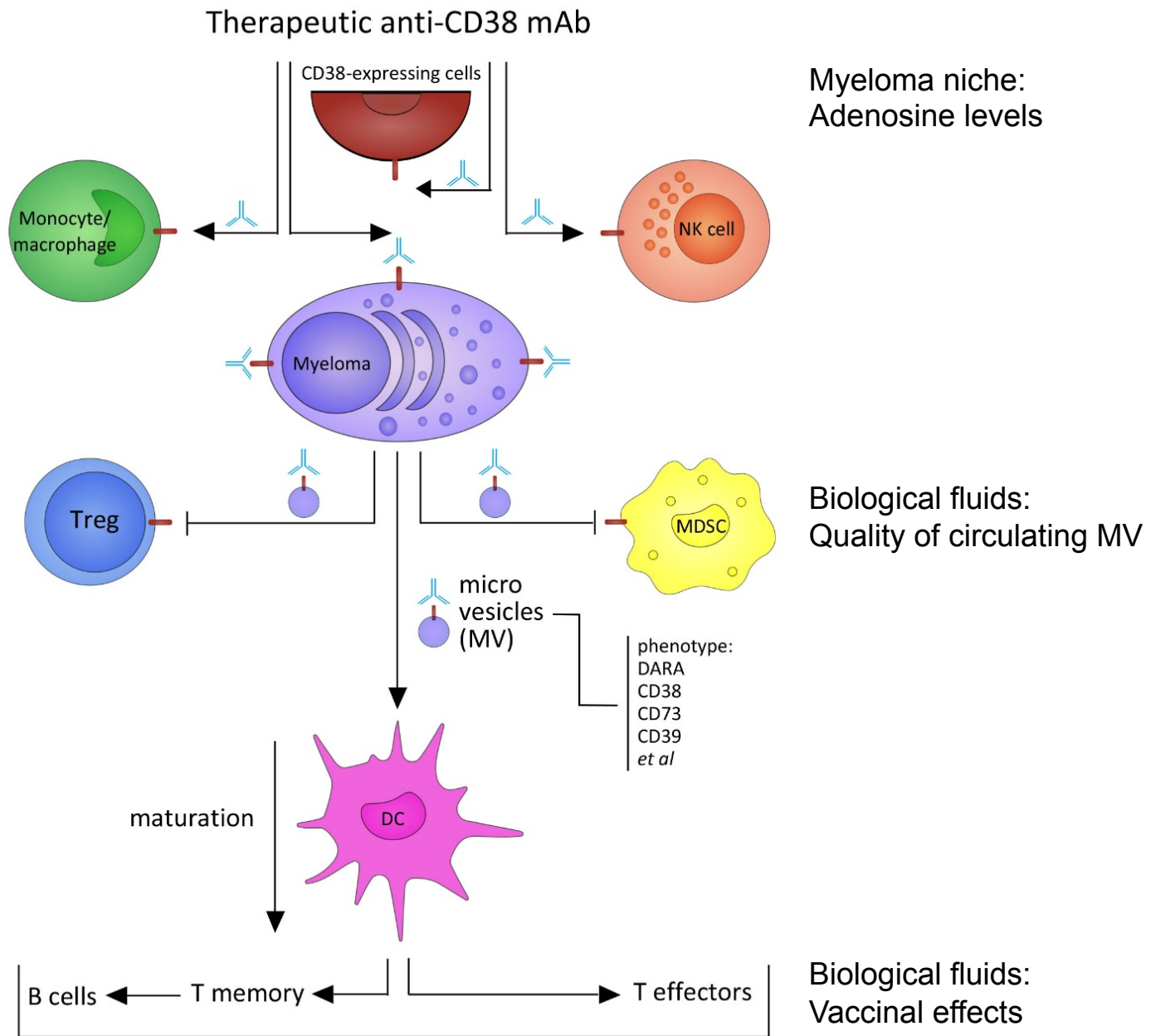
Soluble and particulate communications between myeloma and cells *in situ* and after antibody treatment: a hypothesis



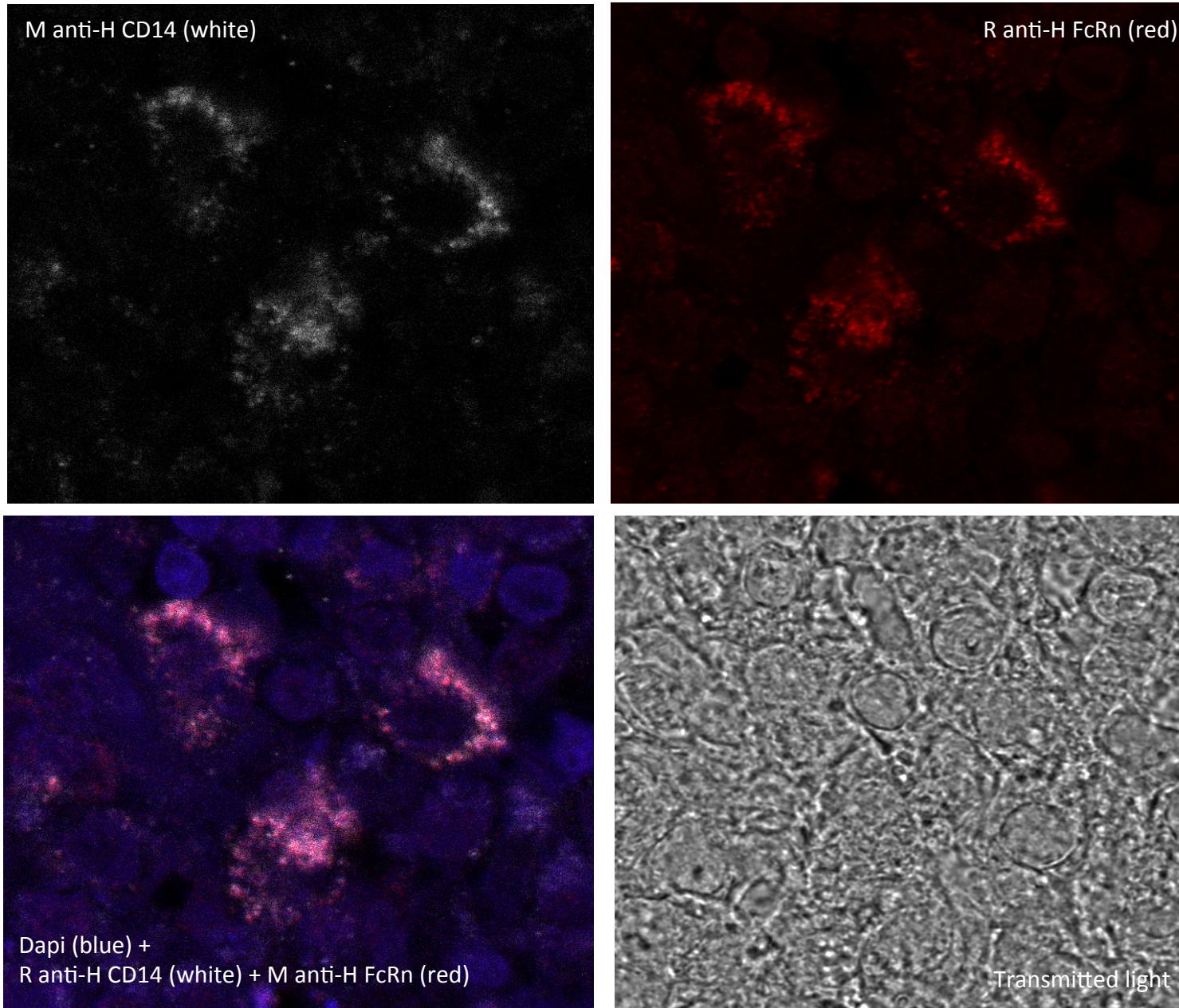
A. Chillemi *et al.*, 2017, in preparation

CD38 in the time of therapeutic mAbs

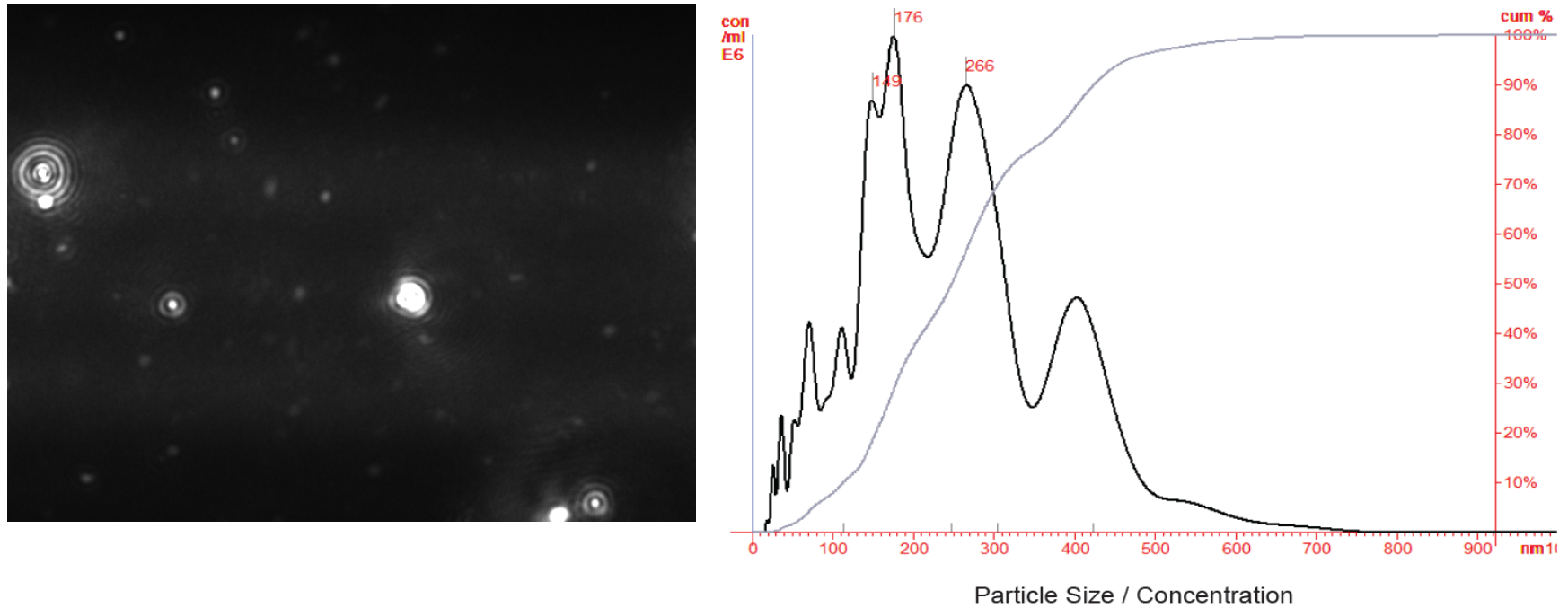
Proposals



Osteomedullary biopsy from MM patient: confocal analysis of CD14⁺/FcRn⁺cells

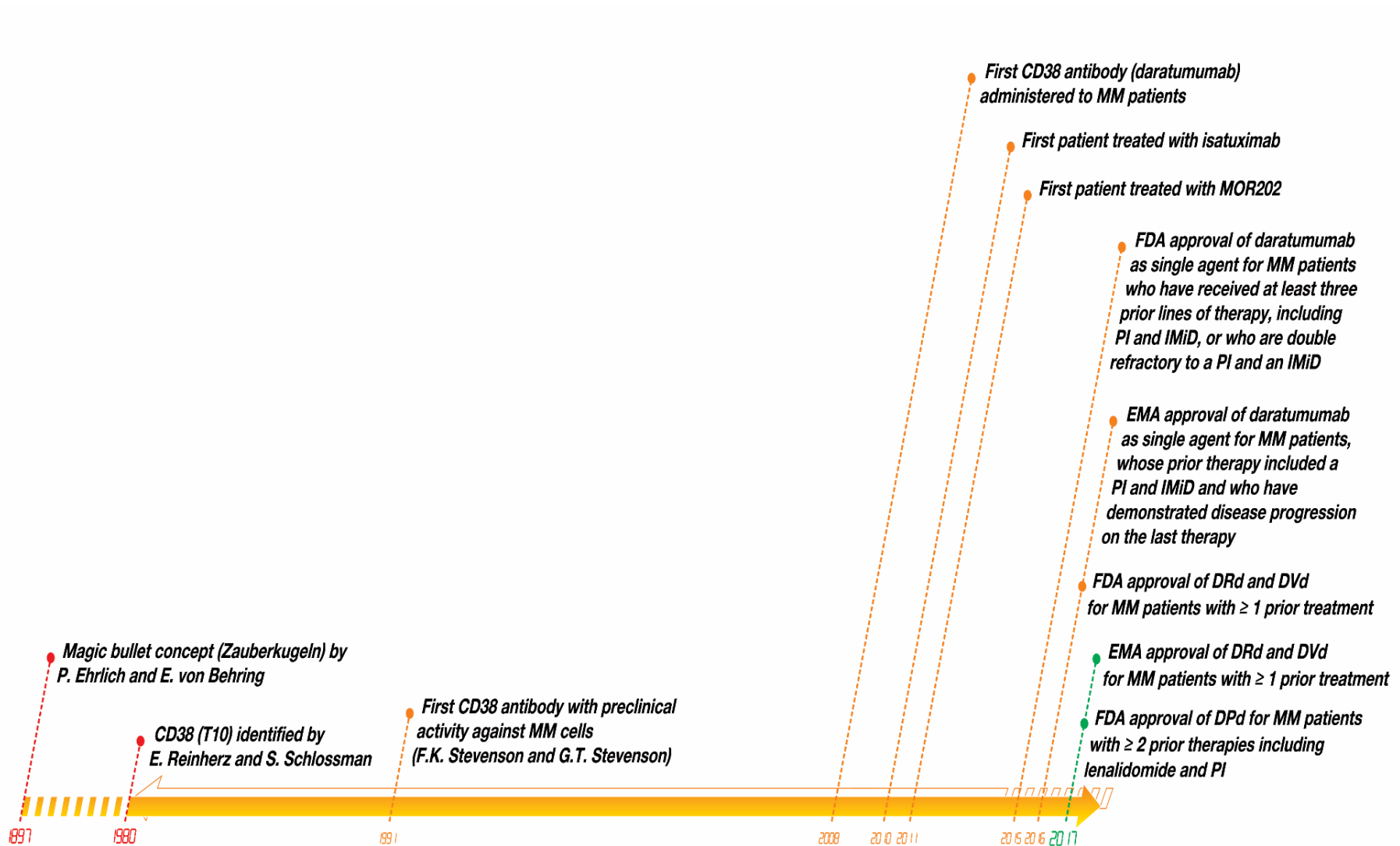


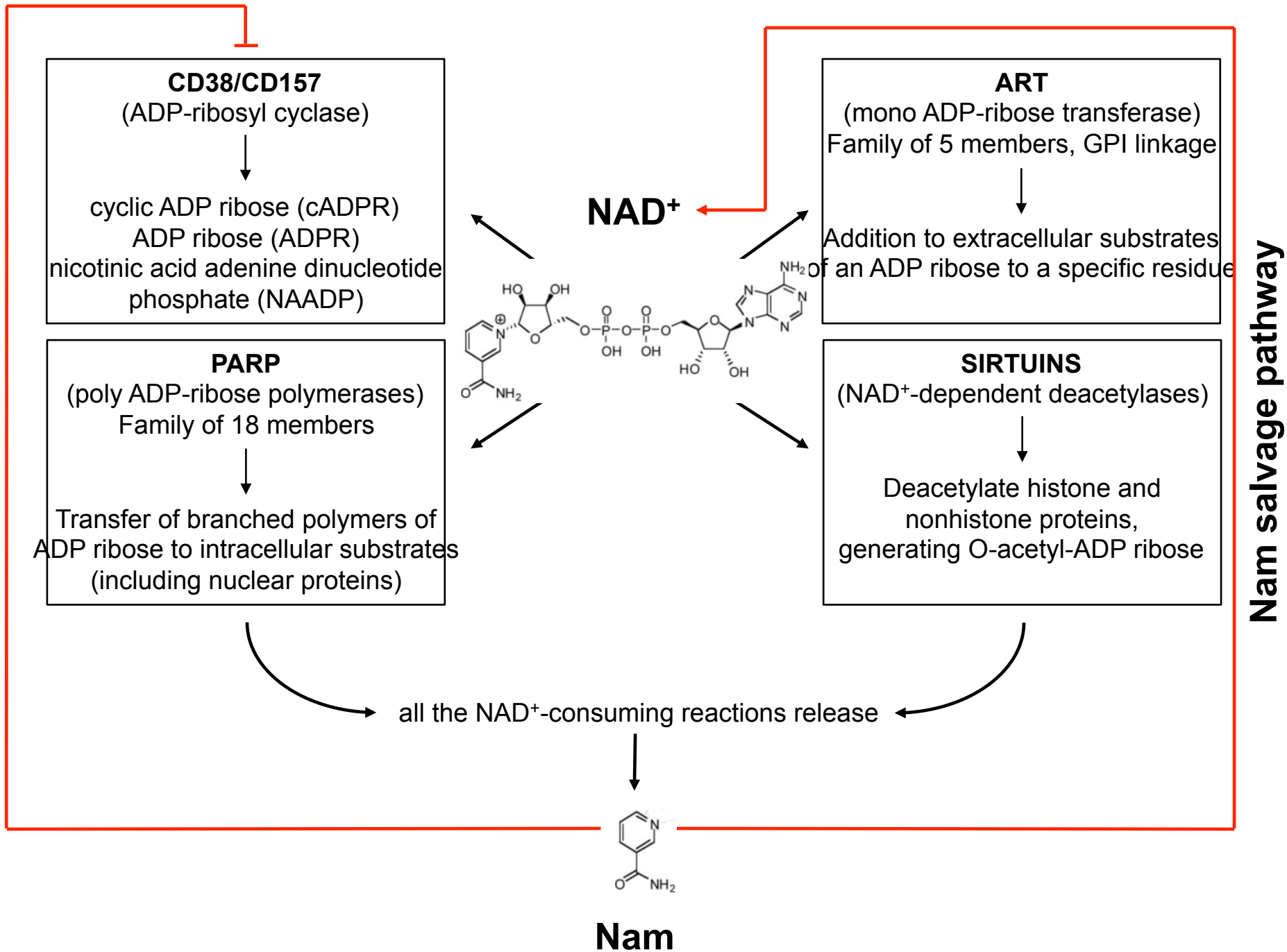
MV released after therapeutic anti-CD38 mAb treatment



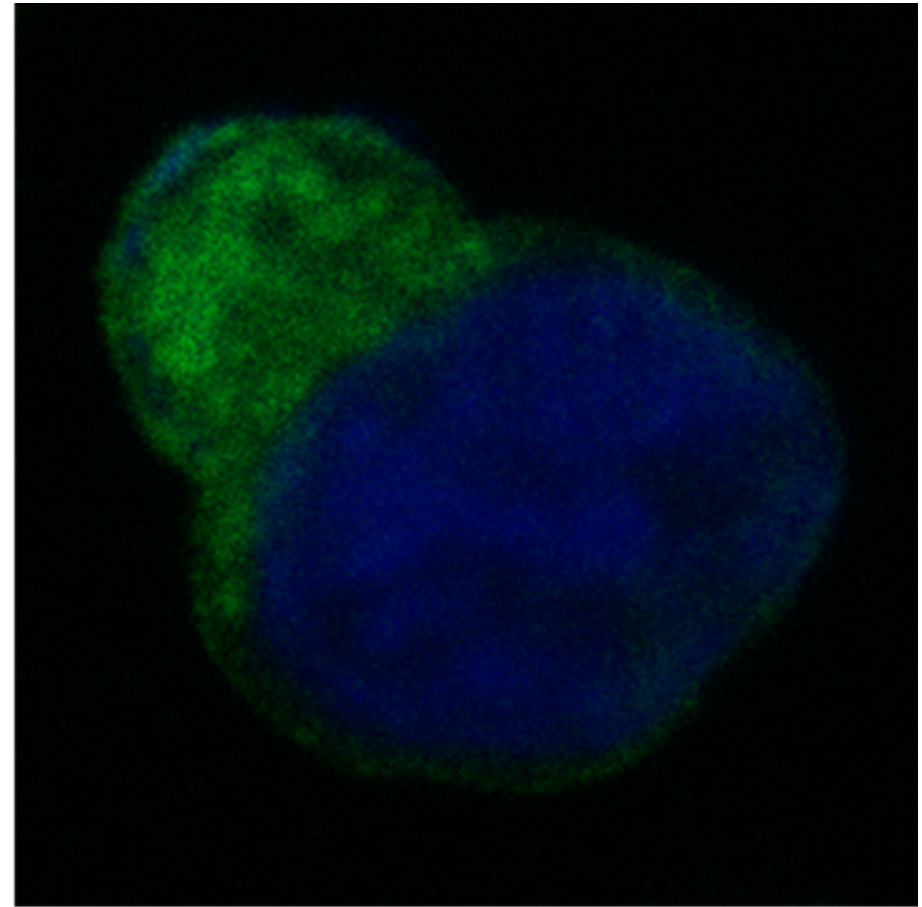
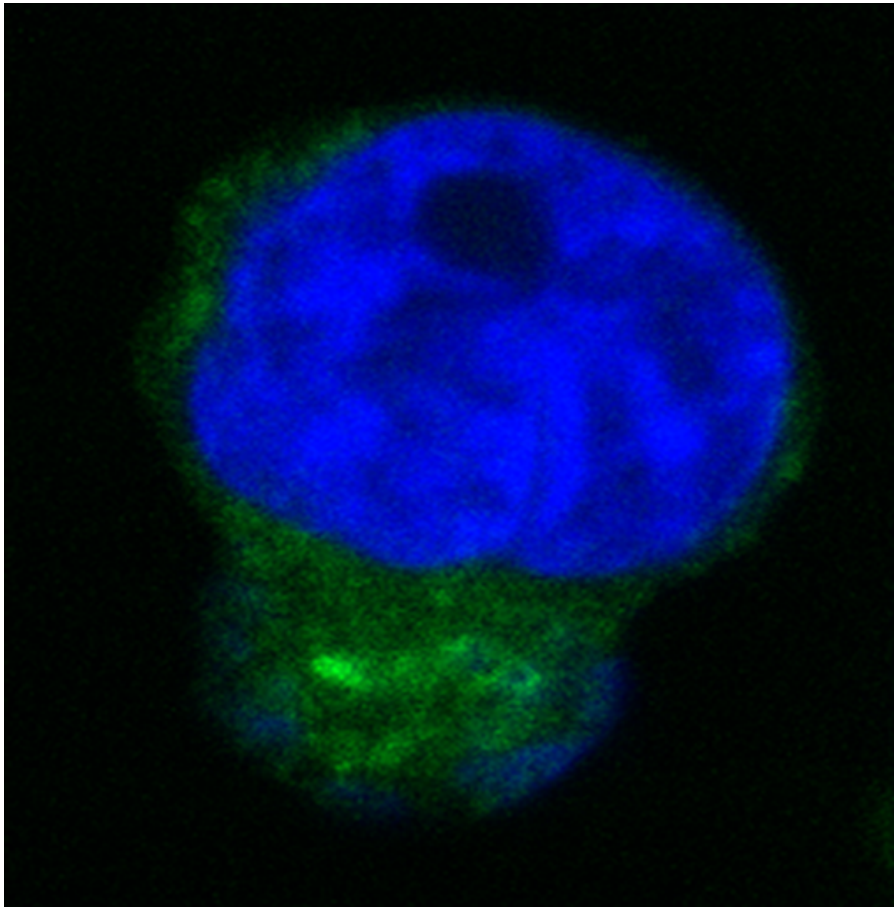
Size Distribution: Mean: 258 nm, Mode: 176 nm, SD: 120 nm
Cumulative Data (nm): D10: 113, D50: 248, D90: 424, D70: 305
User Lines: 0 nm, 0 nm
Total Concentration: 22.04 particles / frame, 3.39E8 particles / ml

Historical steps in antibody-mediated myeloma therapy

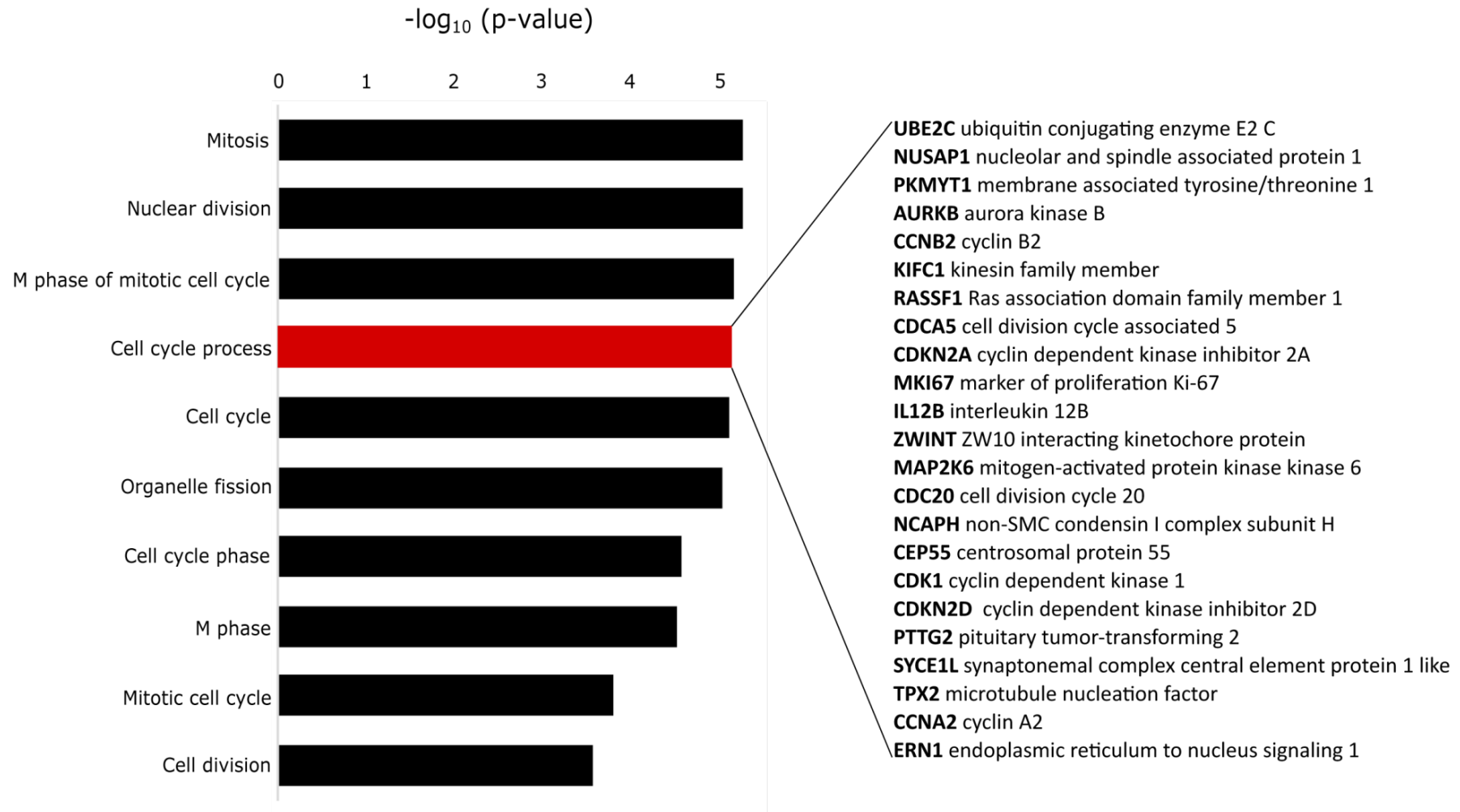




Confocal microscopy analysis of CD38/DARA interaction
(37 °C, 3 h) on a myeloma at diagnosis



Comparative analysis of down modulated genes (RNA polyA) after exposure of NK cells to MV-DARA (control: MV from untreated myeloma)



Rationale for targeting CD38

Functions:

1) Receptor-mediated adhesion and signaling functions

2) Enzymatic activities

Contributes to intracellular calcium mobilization

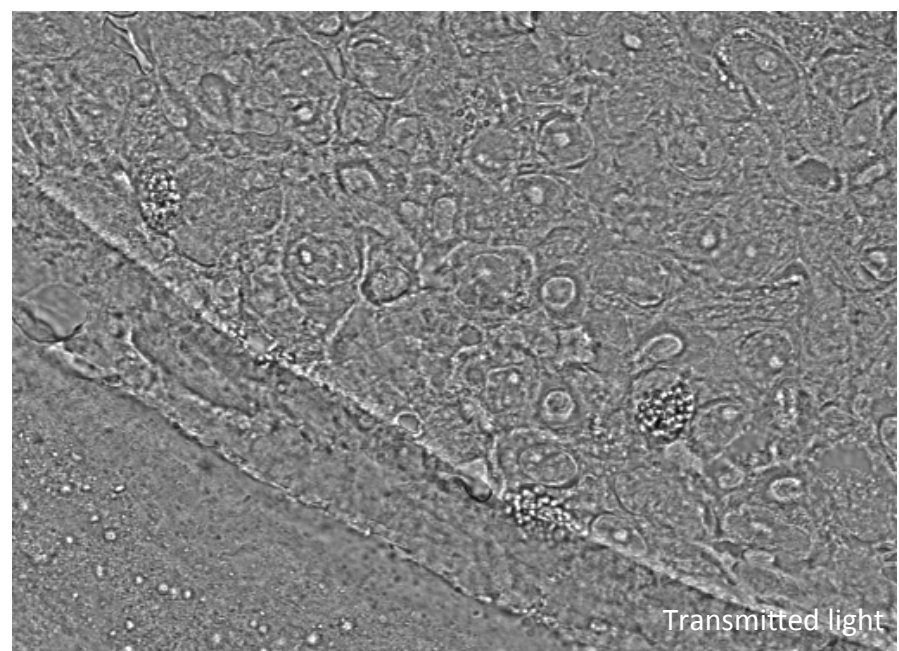
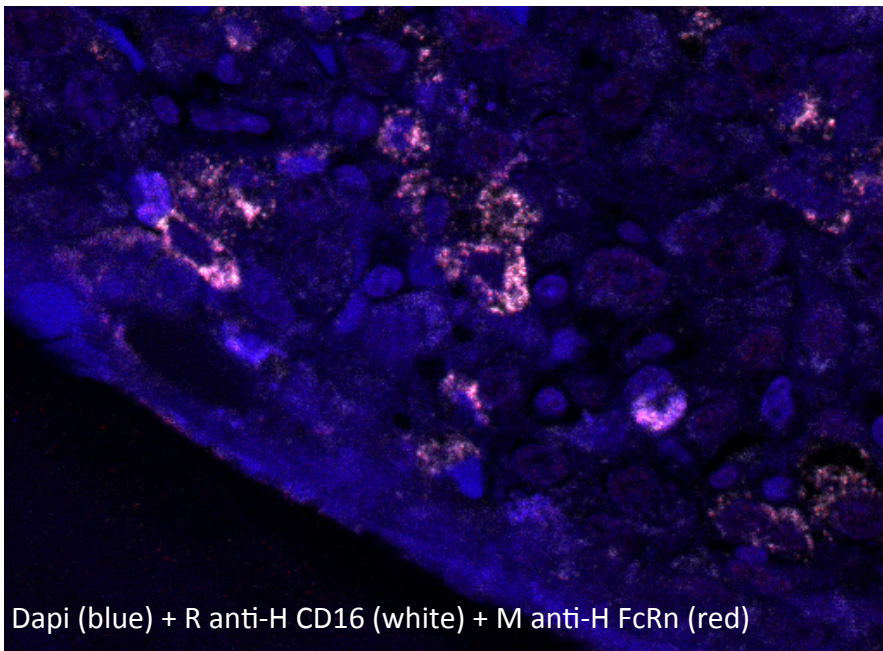
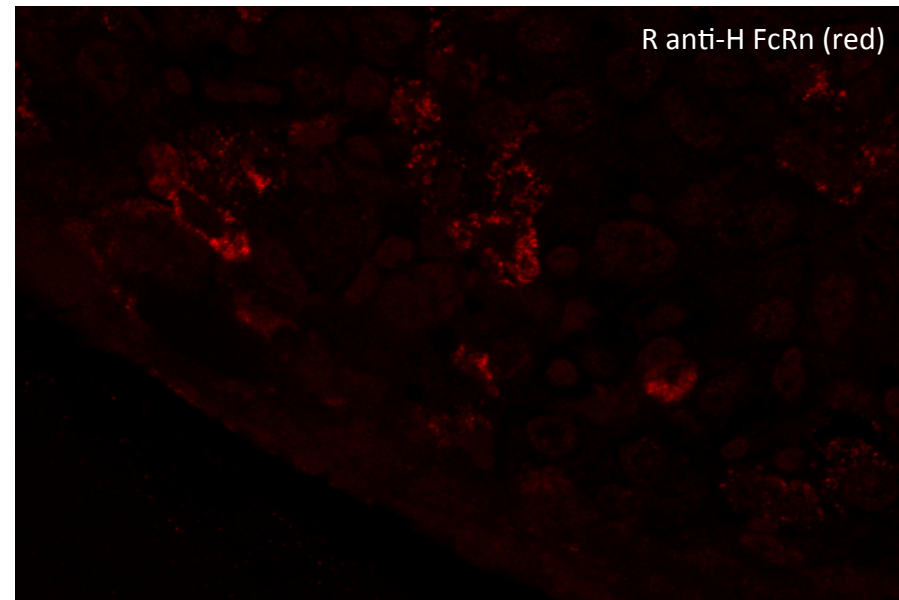
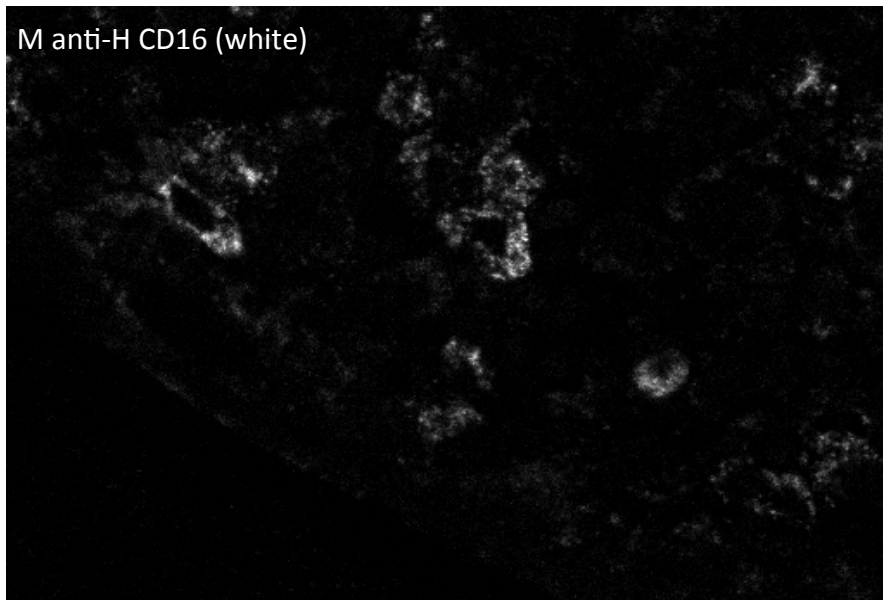
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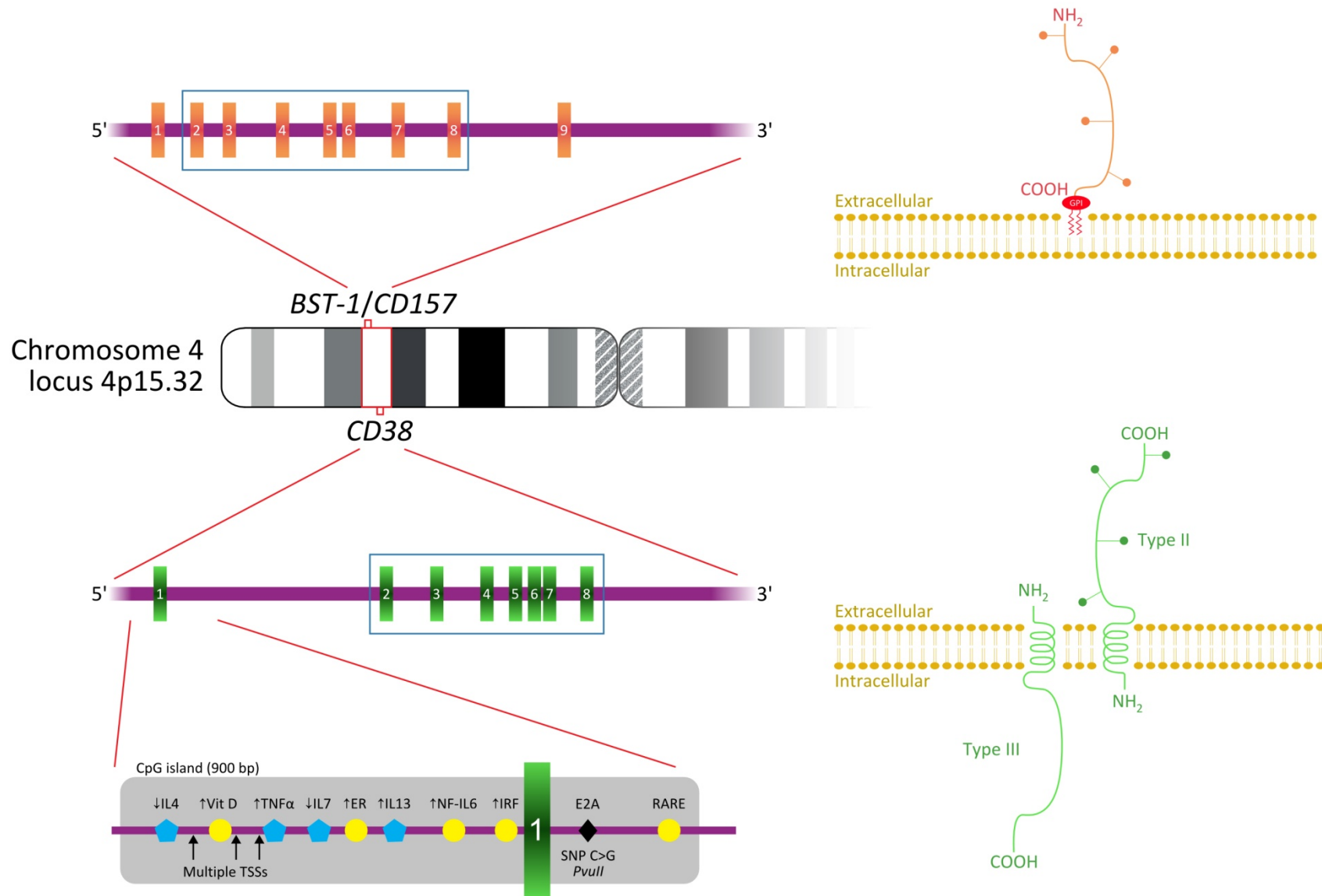
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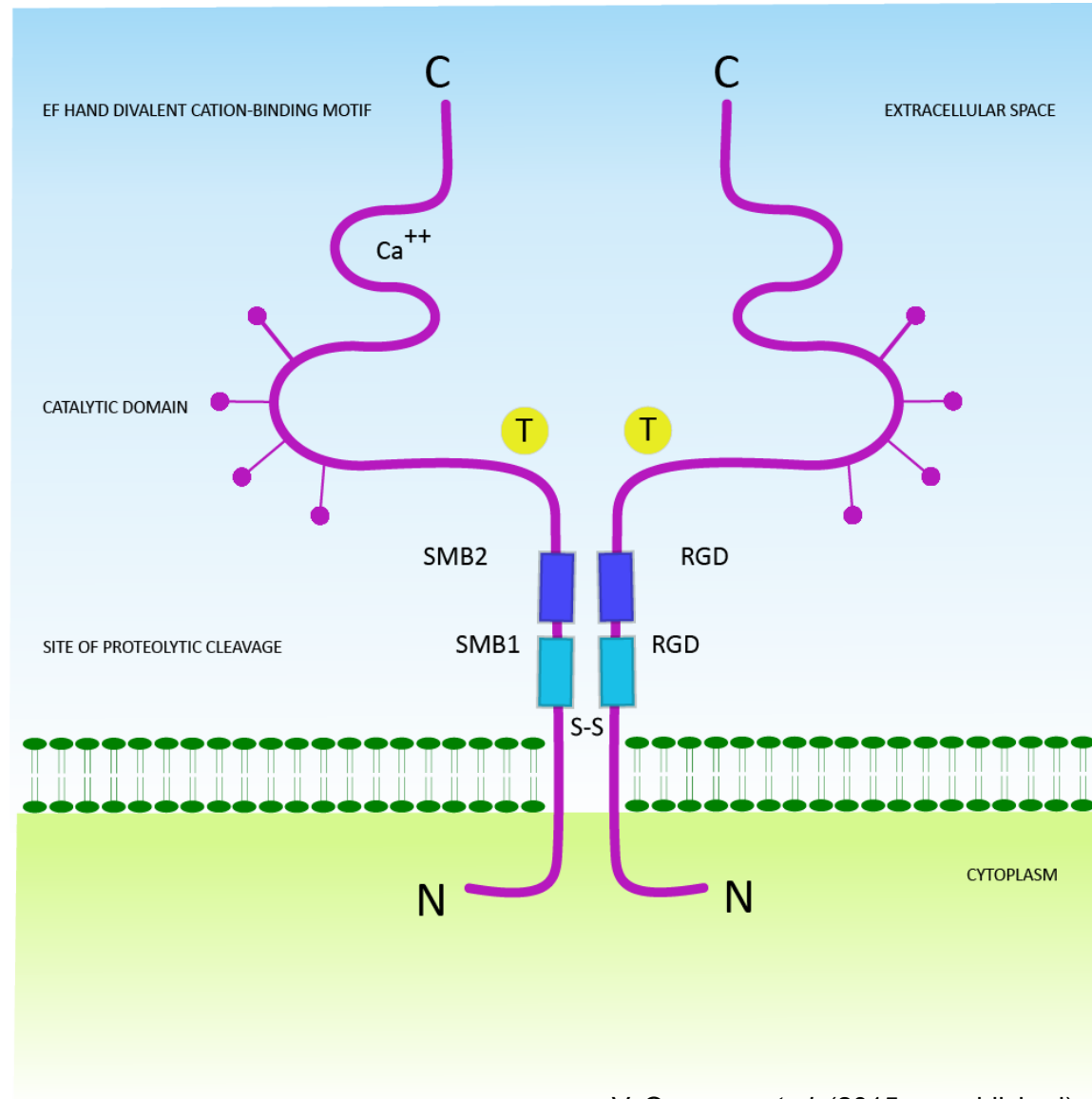
Osteomedullary biopsy from MM patient: confocal analysis of CD16⁺/FcRn⁺cells



Schematic representation of the CD38/CD157 gene family and products



Schematic representation of the Plasma Cell-1/CD203a/ENPP-1 (ectonucleotide pyrophosphatase/phosphodiesterase-1) molecule



V. Quarona *et al.* (2015, unpublished)