Inducible paracrine factors from peripheral blood mononuclear cells modulate immunological function

Doctoral Viva – Rigorosum – PhD-Programm N094

Dr. med. univ. Dragan Copic

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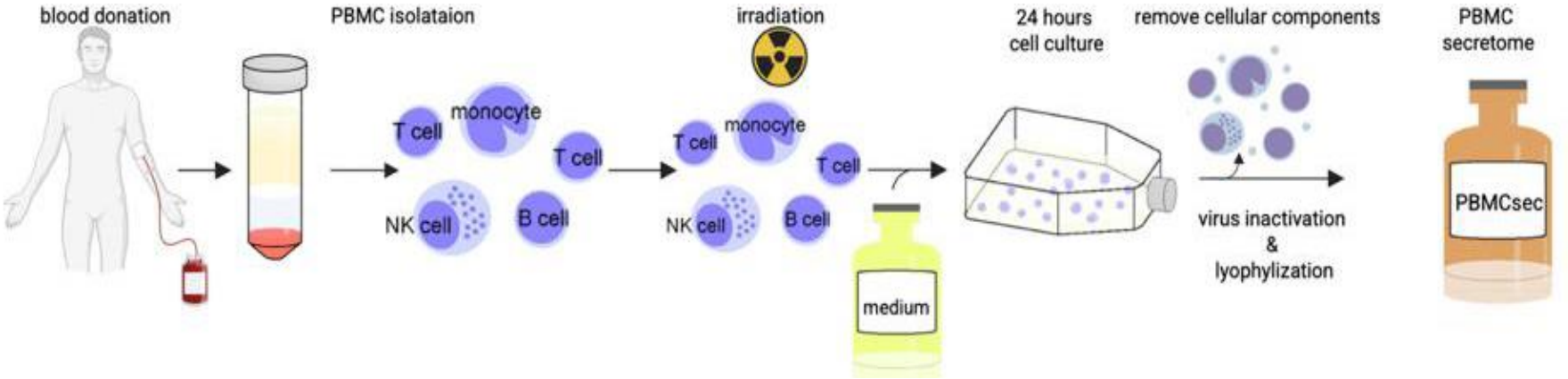
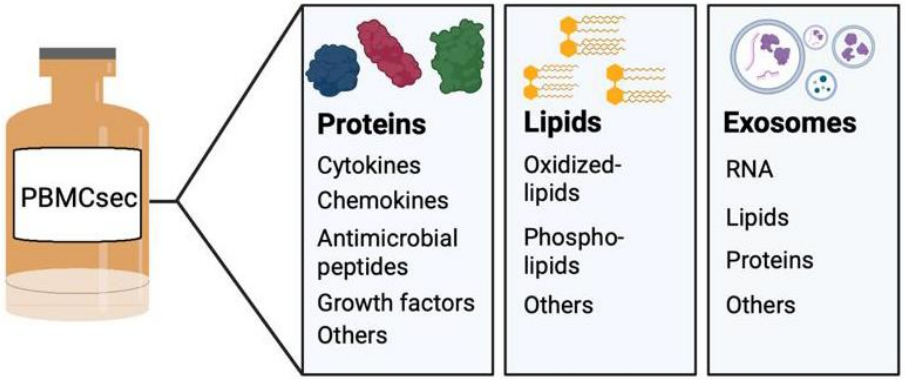
Secretome of γ-irradiated PBMCs (PBMCsec)

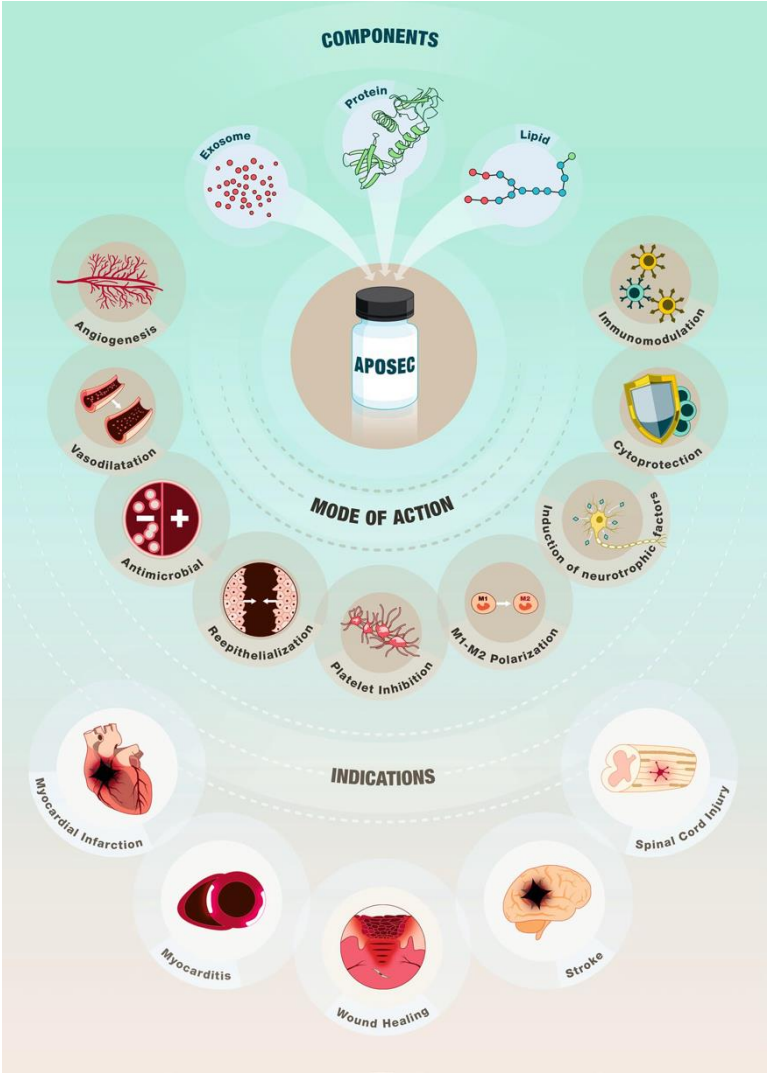




Tissue-regenerative effects mediated by PBMCsec

Experimental model

- Wound healing
- Myocardial infarction
- Autoimmune myocarditis
- Ischemic stroke
- Spinal cord injury



Beer et al. Peripheral blood mononuclear cell secretome for tissue repair. Apoptosis. 2016

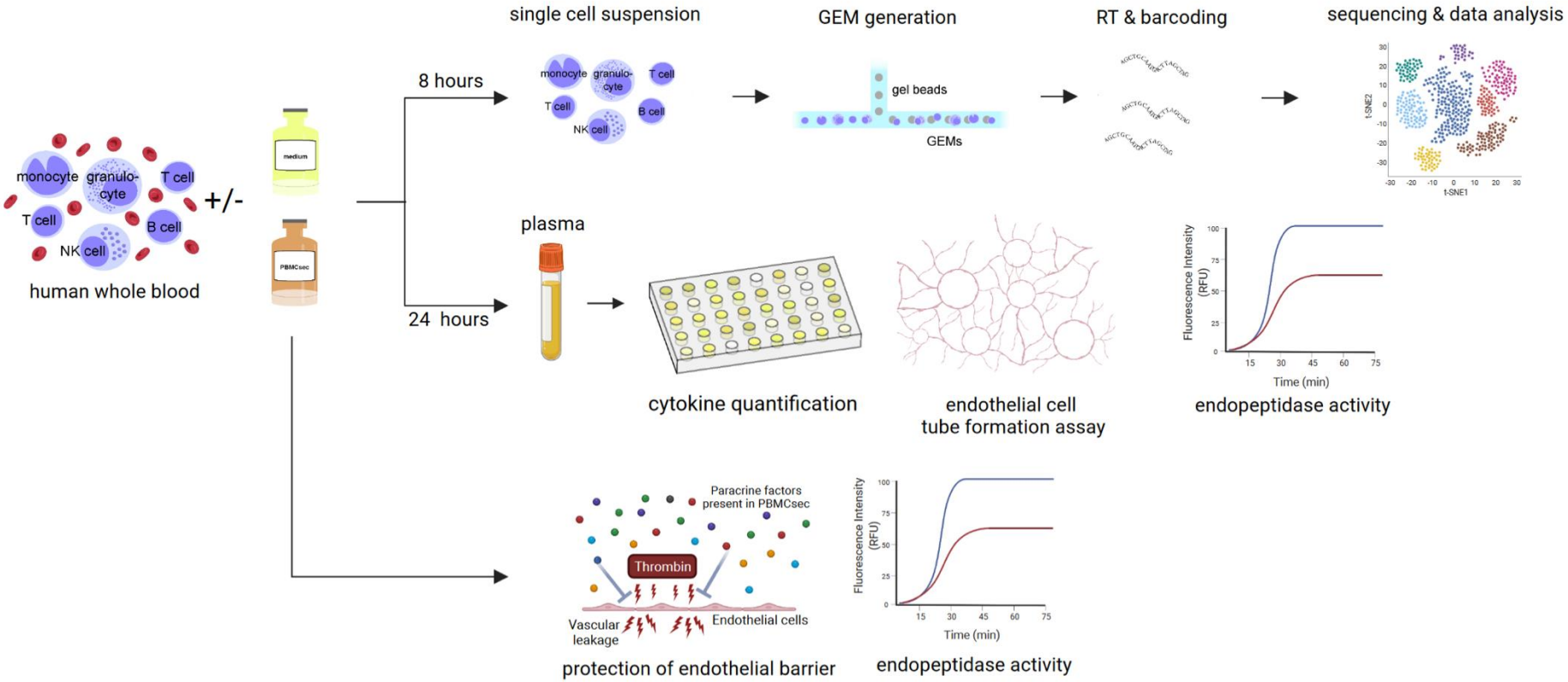
Translation of pre-clinical work to patient relevant settings

- MARSYAS I – safety and tolerability of topically administered autologous PBMCsec for the treatment of dermal wounds
- MARSYAS II – safety and efficacy of topically administered allogeneic PBMCsec for the treatment of diabetic foot ulcers
- Systemic application in future therapeutic approaches

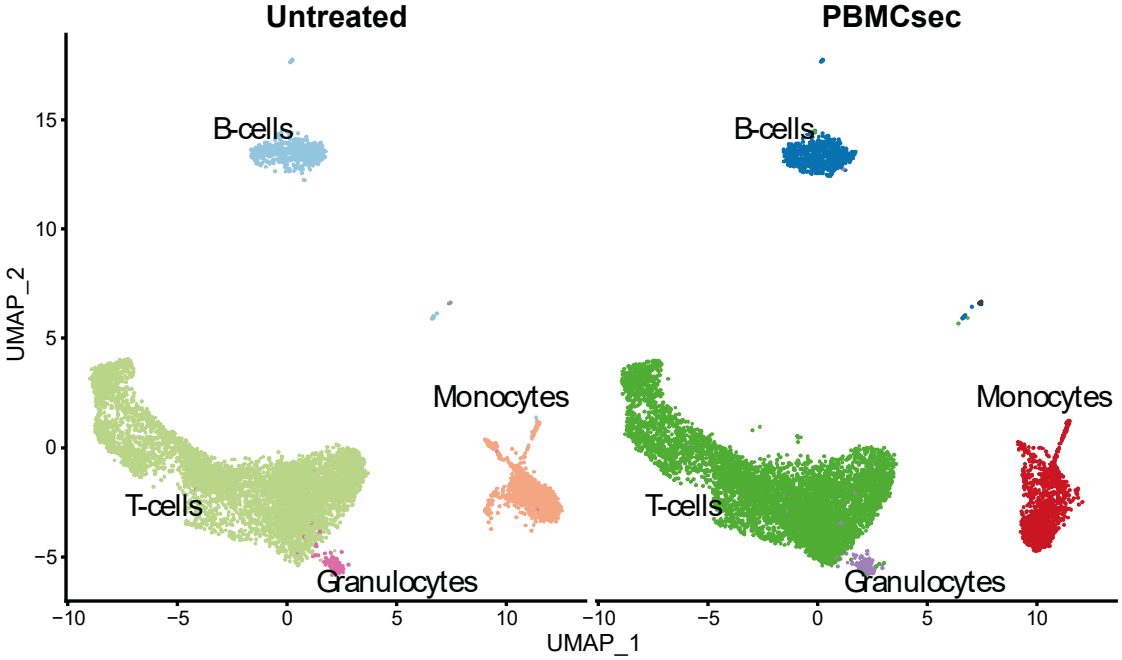
Aim

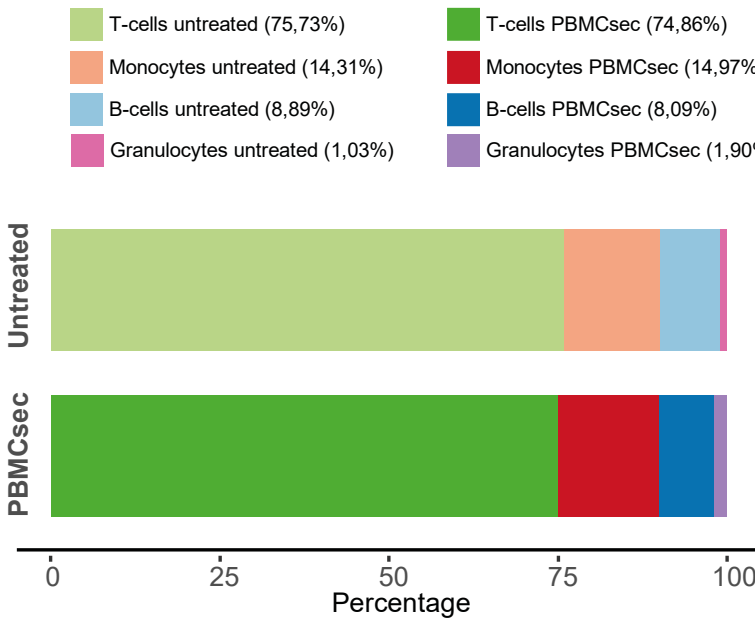
Identification and dissection of transcriptional changes induced in immune cells from human whole blood following exposure to PBMCsec and map the resulting output to biological functions translating to tissue protective effects

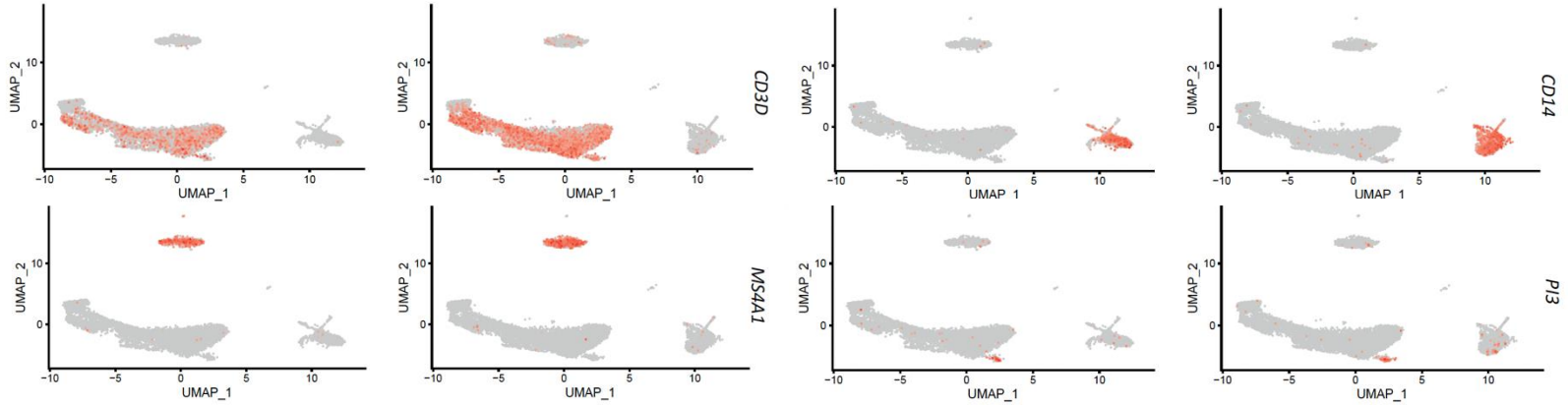
Experimental Setup



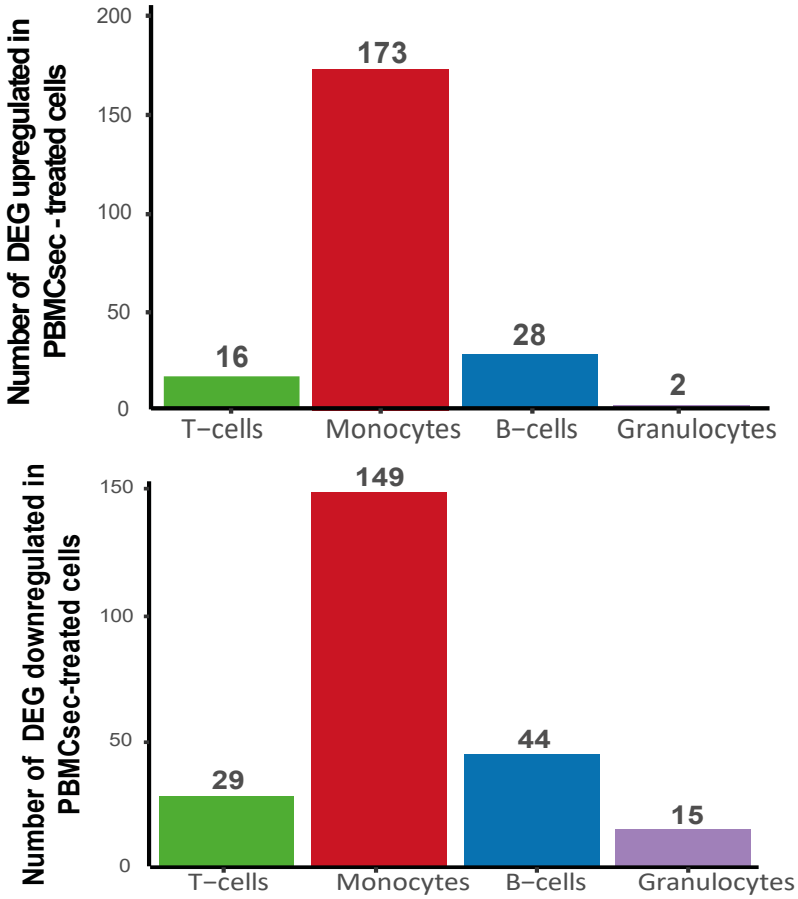
Immune cell clusters and frequencies are not altered by PBMCsec

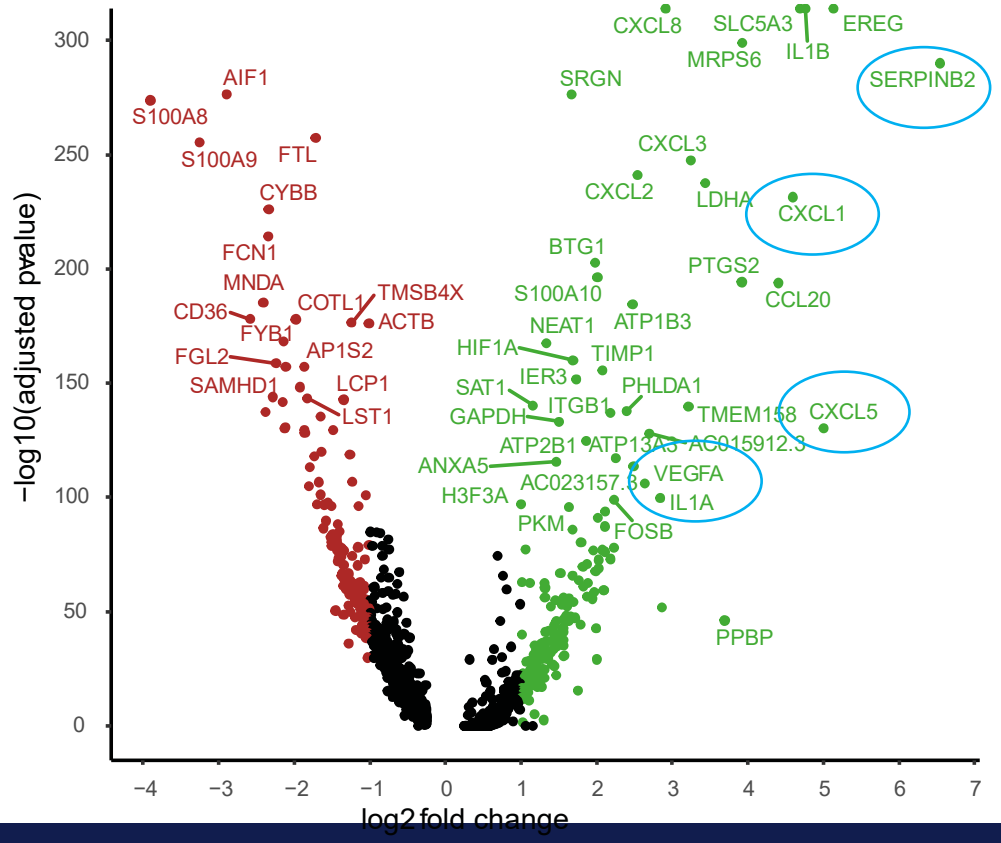






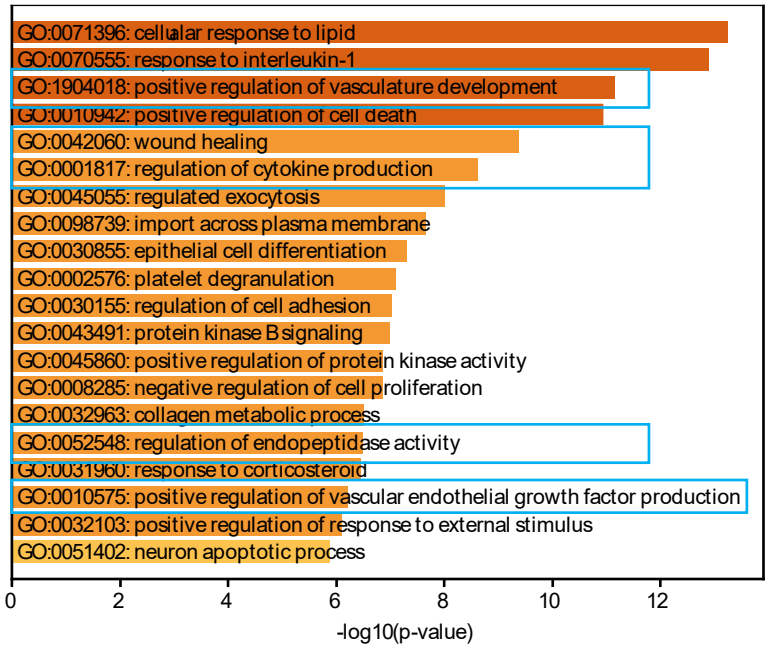
PBMCsec induced DGE is highest in Monocytes



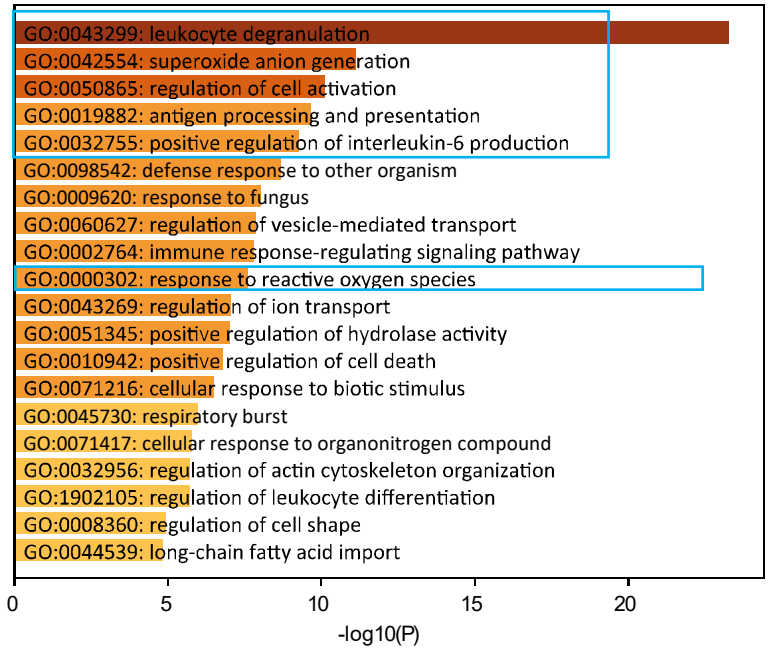


Pathways modulated by differentially up- and downregulated genes in monocytes treated with PBMCsec associate with tissue regeneration

Pathways affected by upregulated genes in Monocytes PBMCsec (Biological Function)



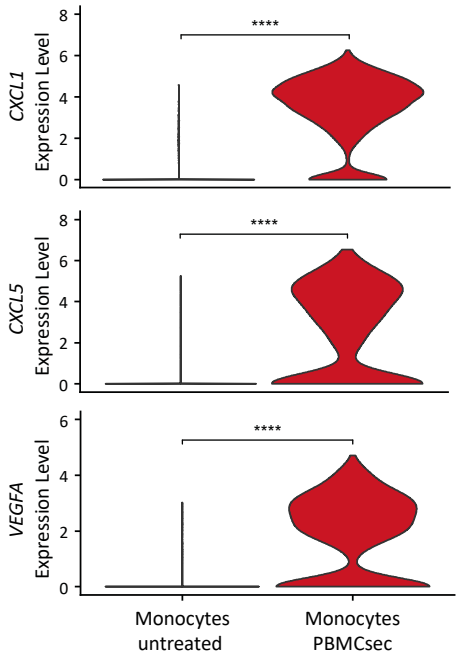
Pathways affected by downregulated genes in Monocytes PBMCsec (Biological Function)

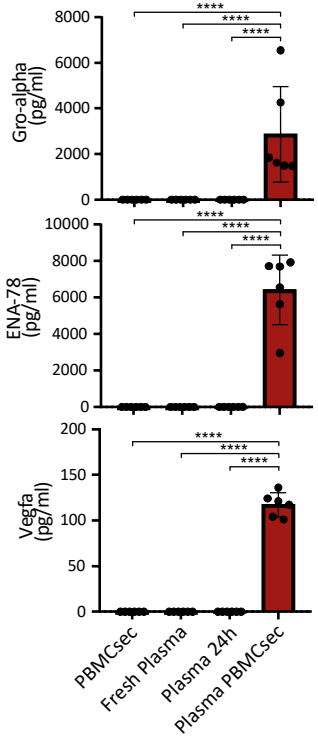


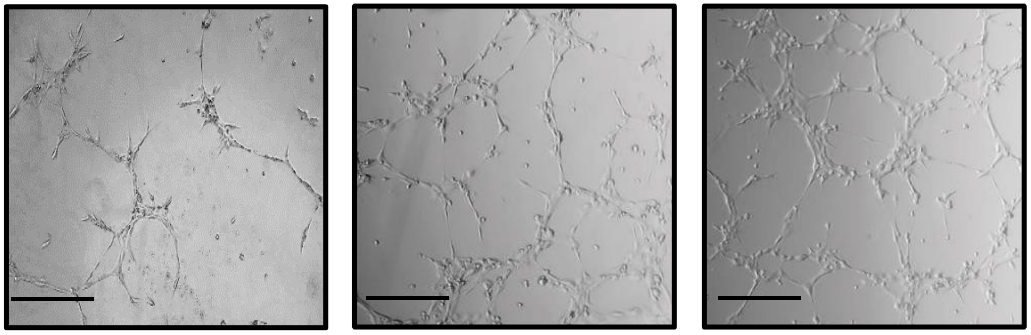
PBMCsec causes transcriptional changes in the subset of Monocytes

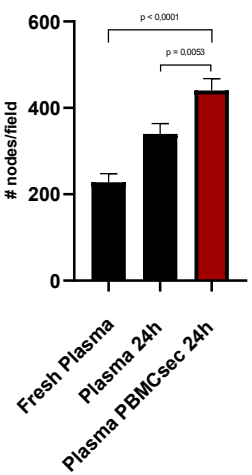
upregulated genes are involved in processes such as wound healing, cytokine production, growth factor production and endopeptidase activity

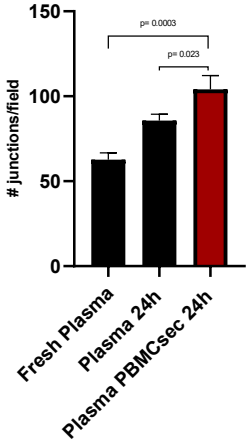
PBMCsec increases plasma levels of pro-angiogenic cytokines and promotes *in vitro* EC tube formation

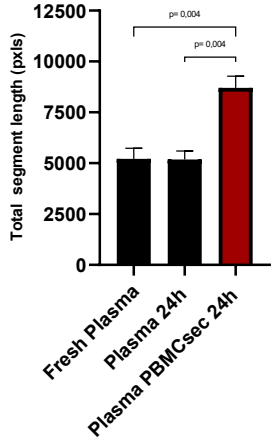








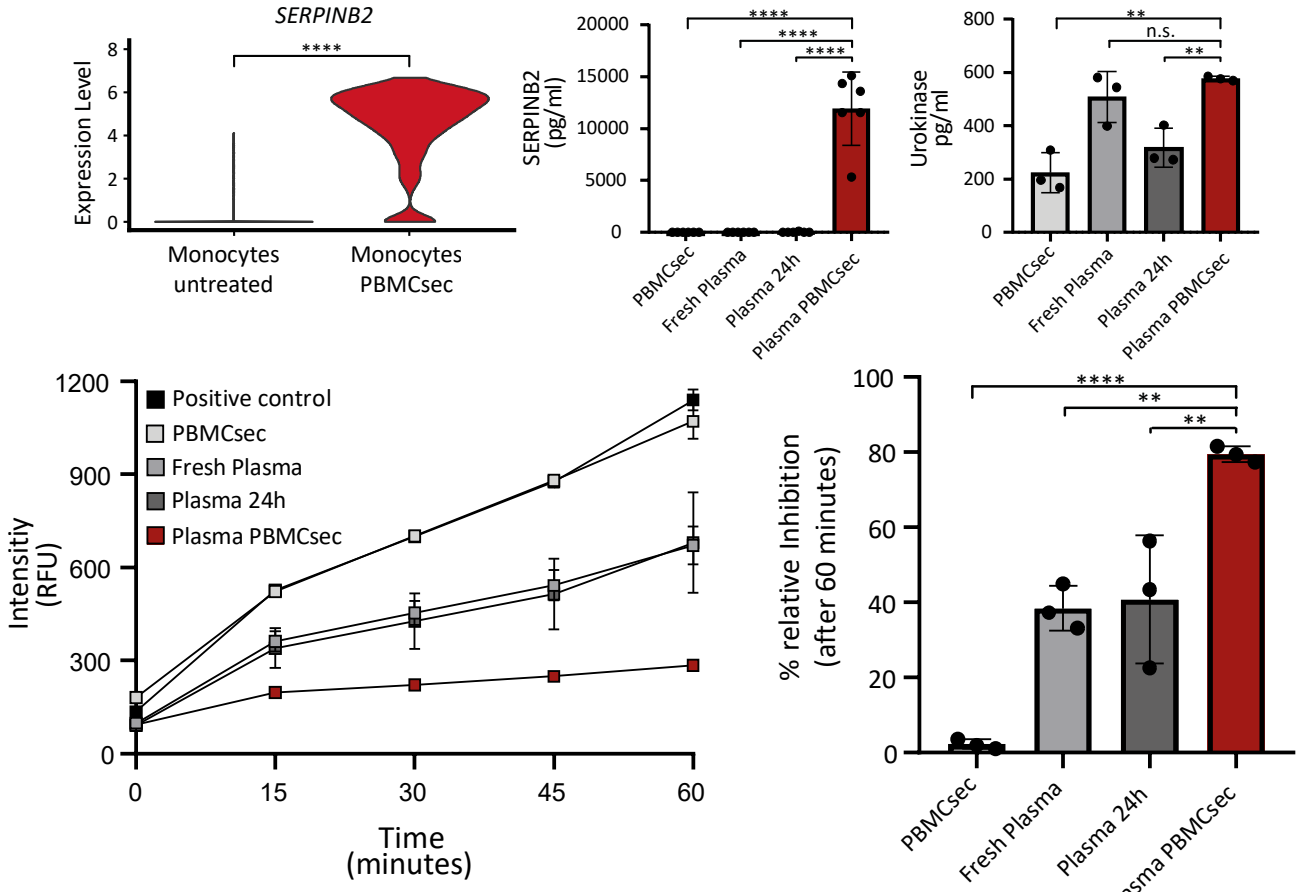




Gene expression for CXCL1, CXCL5 and VEGFA are increased in Monocytes PBMCsec

In line with elevated gene expression, plasma levels of Gro-α, ENA-78 and VEGF are significantly increased

PBMCsec induced SerpinB2 associates with inhibition of urokinase activity



Summary (1)

- Transcriptional responses to PBMCsec were strongest in the subset of monocytes
- Modulated biological processes included
  - Cytokine production
  - Vasculature development
  - Inhibition of endopeptidase activity
- Monocytes take up a pro-angiogenic, anti-proteolytic secretory phenotype and amplify tissue-protective mechanisms innate to PBMCsec

Anti-thymocyte globulin (ATG)

- Polyclonal immune globulins
- Clinical use: Induction therapy prior to
  - Stem cell transplantation
  - Solid organ transplantation
- Diverse immunosuppressive effects
  - T cell depletion (ADCC, AICD, CDCC, opsonization)
  - Masking of T-cell antigens
  - Expansion of regulatory T-cells (T_{regs})
  - Dendritic cell modulation

Mohty M (2007) Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond. *Leukemia* 21: 1387-1394

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PLOS ONE

Anti-Thymocyte Globulin Induces Neoangiogenesis and Preserves Cardiac Function after Experimental Myocardial Infarction

Michael Lichtenauer[1,2], Michael Mildner[3], Gregor Werba[2,4], Lucian Beer[2,4], Konrad Hoetzenecker[2,4], Andrea Baumgartner[5], Matthias Hasun[5], Stefanie Nickl[2,4], Andreas Mitterbauer[2,4], Matthias Zimmermann[2,4], Mariann Gyöngyösi[6], Bruno Karl Podesser[5], Walter Klepetko[4], Hendrik Jan Ankersmit[2,4]*

Table 2. Accumulation of cytokines, chemokines and growth factors in whole blood.

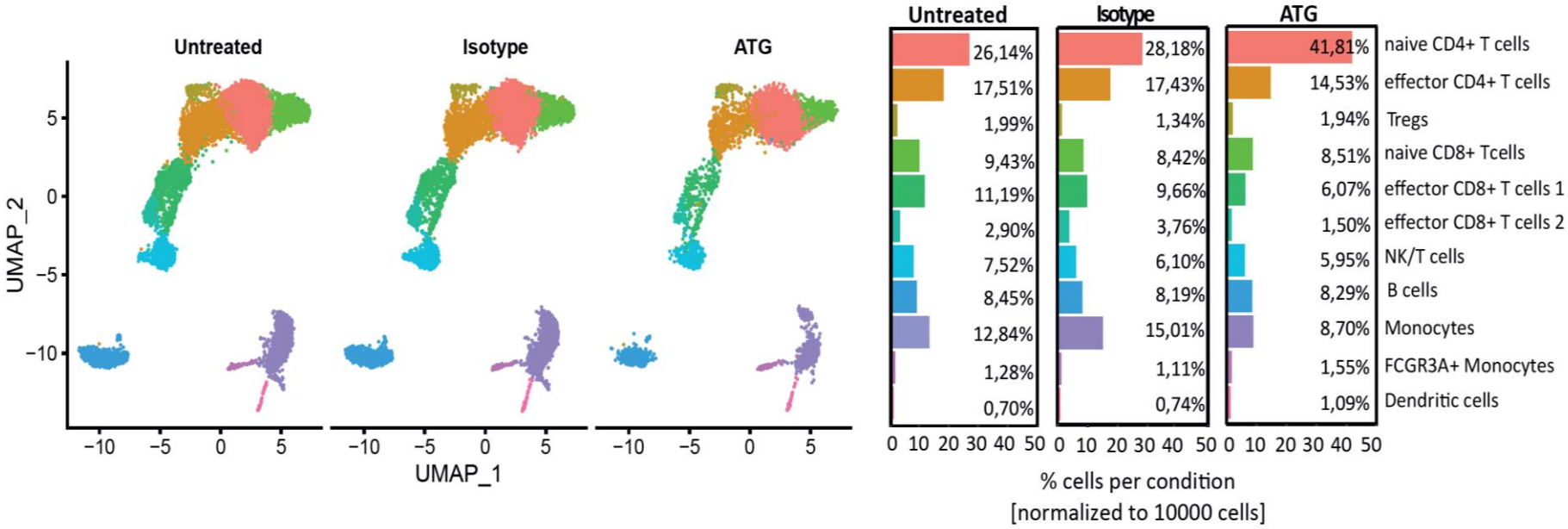
Whole Blood Incubation

	0 h	24 h	24 h +20 µg ATG	*sig.*	24 h +100 µg ATG	*sig.*	24 h +20 µg IVIG	*sig.*
TNF-alpha	0.8±0.5	11.9±9.7	69.9±15.3	*	58.8±17.6	‡	3.1±3.1	*ns*
IL-2	0.3±0.3	0.9±0.6	3.2±1.9	*ns*	36.4±8.8	‡	0.0±0.0	*ns*
IFN-gamma	8.7±8.7	39.9±22.6	715.0±84.9	‡	415.9±72.6	‡	2.6±2.6	*ns*
IL-1beta	1.4±0.6	39.5±8.7	52.8±6.6	*	171.7±27.9	‡	36.4±8.6	*ns*
IL-1ra	0.0±0.0	1030.8±204.8	20492.2±1935.6	‡	26902.0±2360.4	‡	916.3±226.1	*ns*
IL-4	21.8±5.6	25.6±7.6	25.7±6.2	*ns*	27.9±5.9	*ns*	17.7±3.2	*ns*
IL-10	1.3±0.7	1.7±0.6	17.2±4.4	‡	32.1±6.2	‡	4.8±1.9	*ns*
IL-8	202.7±7.3	2427.1±304.3	4801.2±1108.2	†	6478.3±1051.5	‡	2713.5±243.7	*ns*
GRO-alpha	5.8±1.5	130.7±31.0	126.3±32.4	*ns*	501.4±91.8	†	98.9±29.9	*ns*
ENA-78	59.6±34.2	1236.1±147.1	549.8±129.9	‡	1955.0±306.3	*ns*	1486.5±297.1	*ns*
MCP-1	0.0±0.0	57.2±27.3	1107.0±114.7	‡	1846.9±91.5	‡	98.4±35.7	*ns*
VEGF	137.6±26.2	126.9±23.6	88.1±16.0	†	74.3±14.9	†	62.6±19.6	*ns*
HGF	17.9±13.0	140.5±31.3	677.1±63.0	‡	567.4±63.8	‡	175.2±40.4	*ns*
TGF-beta	0.0±0.0	0.0±0.0	0.0±0.0	*ns*	0.0±0.0	*ns*	0.0±0.0	*ns*

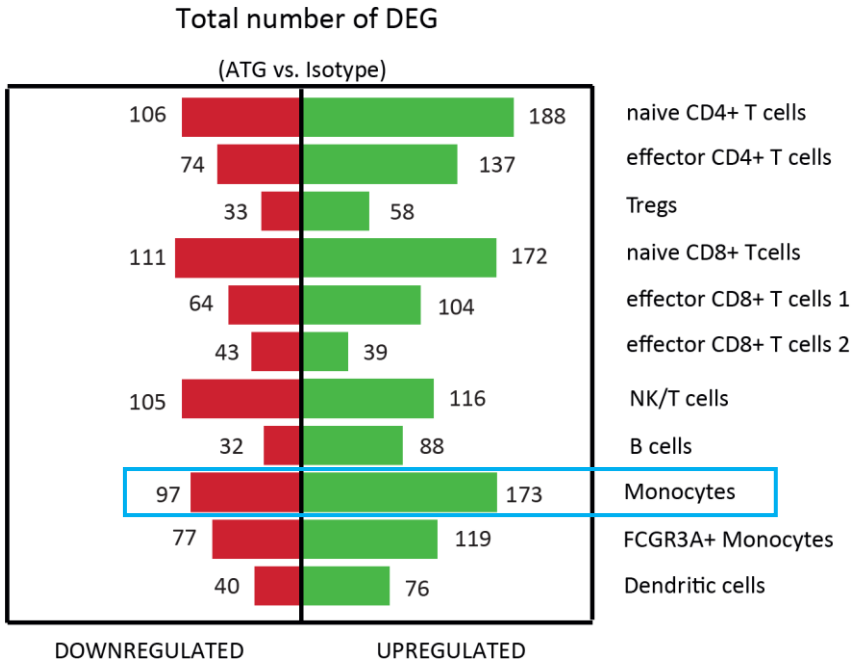
Aim

Capture transcriptional changes in immune cells from healthy human whole blood after *ex vivo* stimulation with ATG on a single cellular level to identify novel mechanisms by which ATG regulates T cell effector functions

ScRNA-seq of whole blood cells following ex vivo treatment with ATG

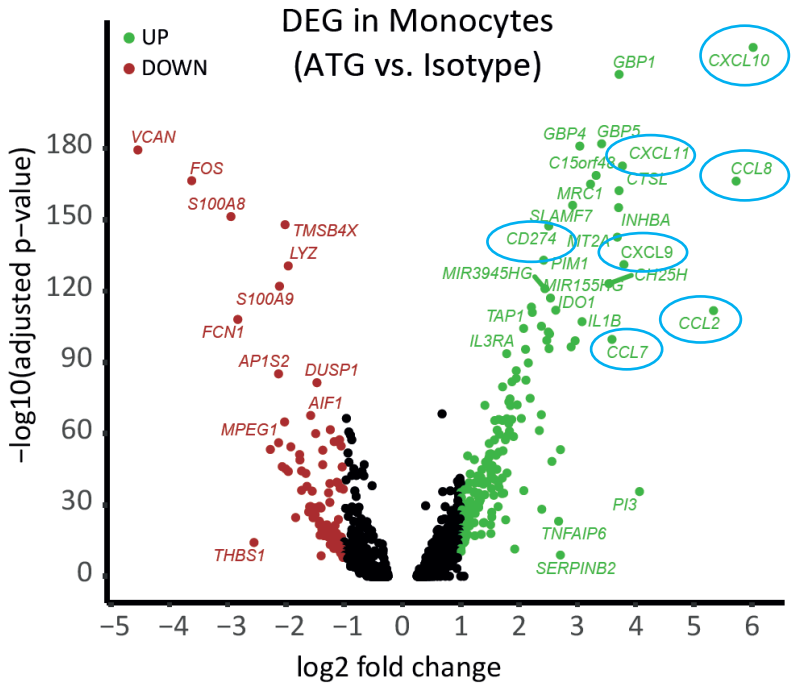


ATG alters gene expression in all identified cell clusters



Bar plot displays total number of DEGs in ATG treated compared to Isotype control for every cluster

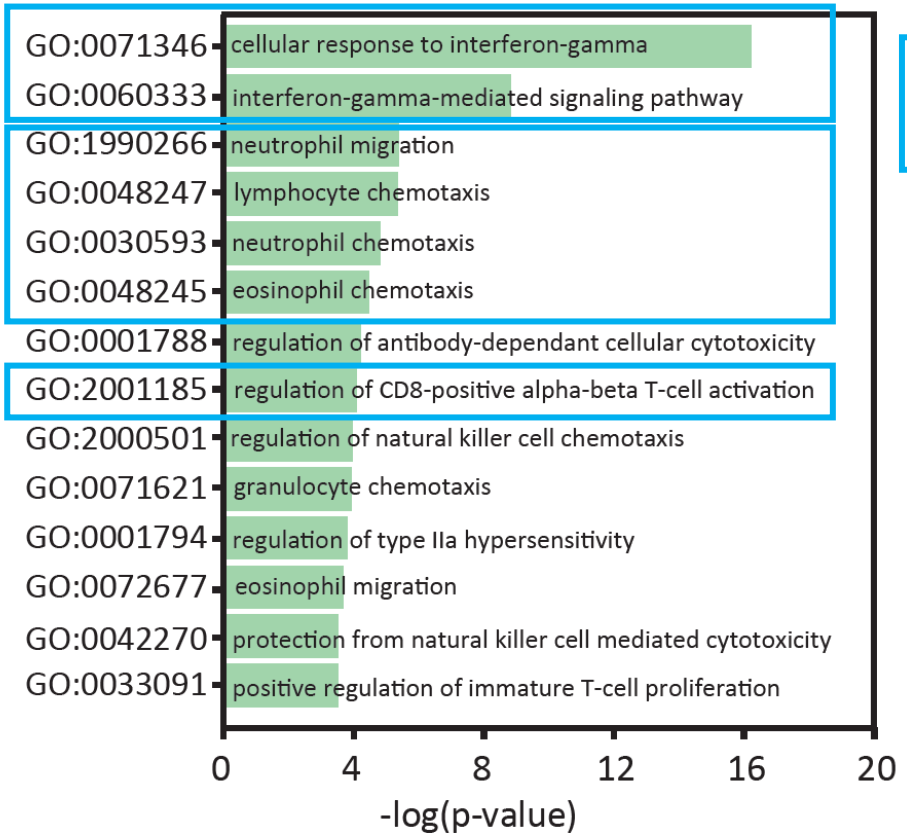
Criteria for inclusion: adj.pvalue < 0.05 & log_2Foldchange ≥ 1 or ≤-1

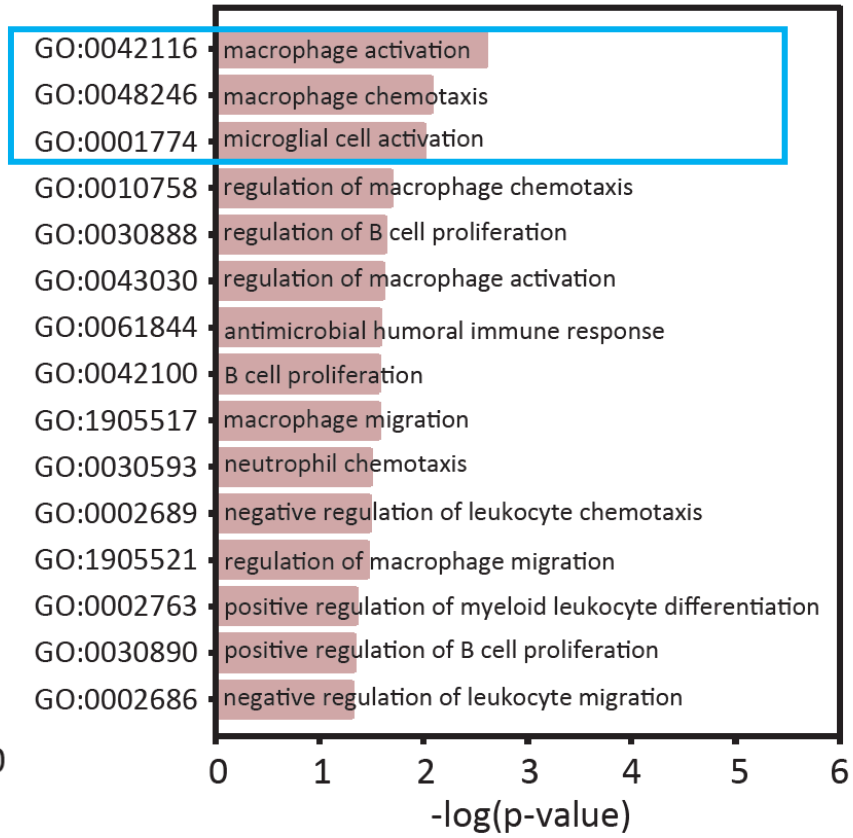


Volcano plot of DEGs in monocytes from ATG-treated whole blood compared to Isotype

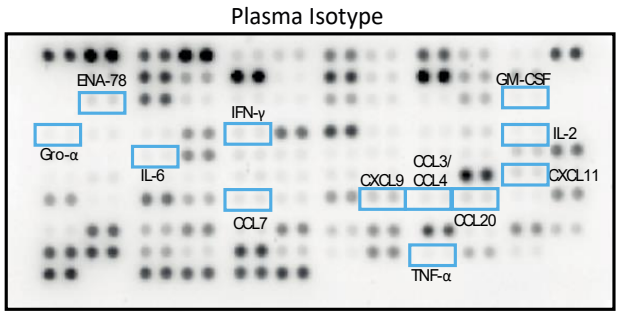
Upregulation of various *CXCLs*; *CCLs* and *CD274* (PD-L1)

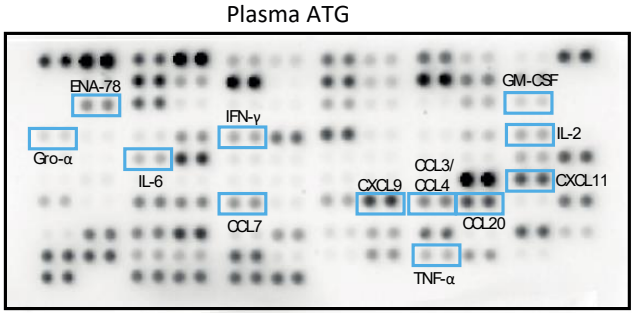
Immune System processes associated with the up- and downregulated genes in Monocytes of ATG-treated whole blood

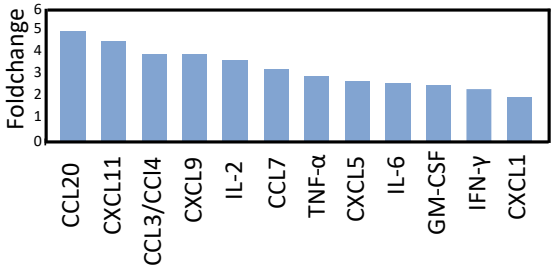




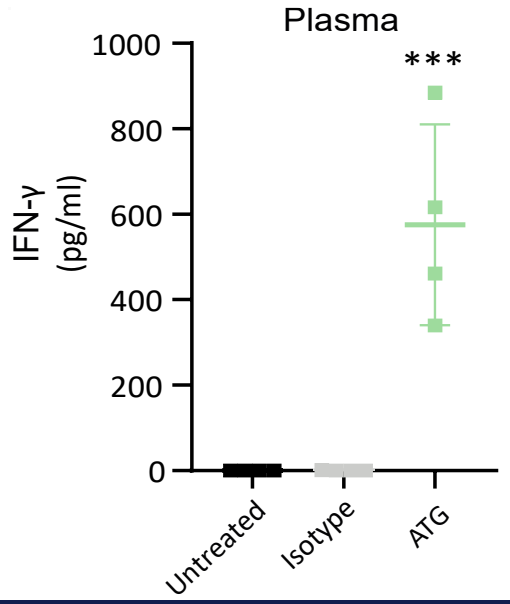
Composition of paracrine factors is altered in plasma of ATG-treated whole blood



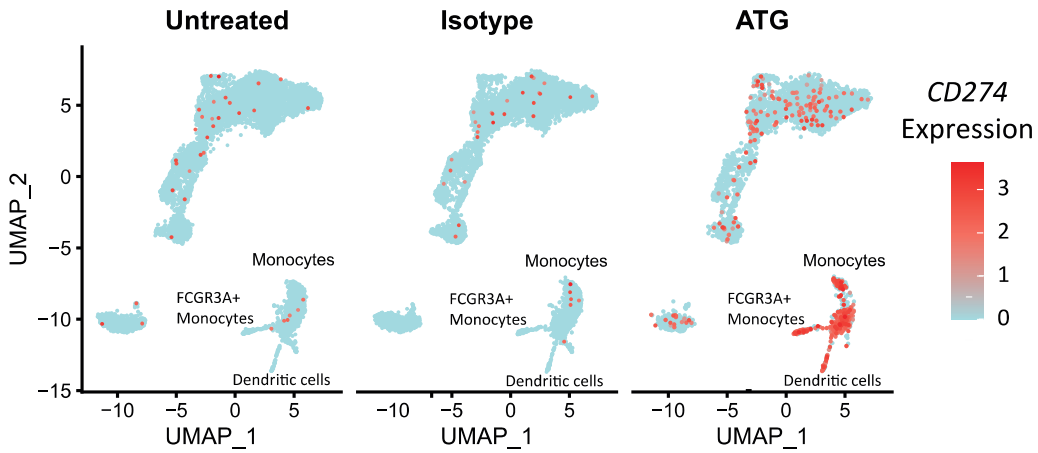


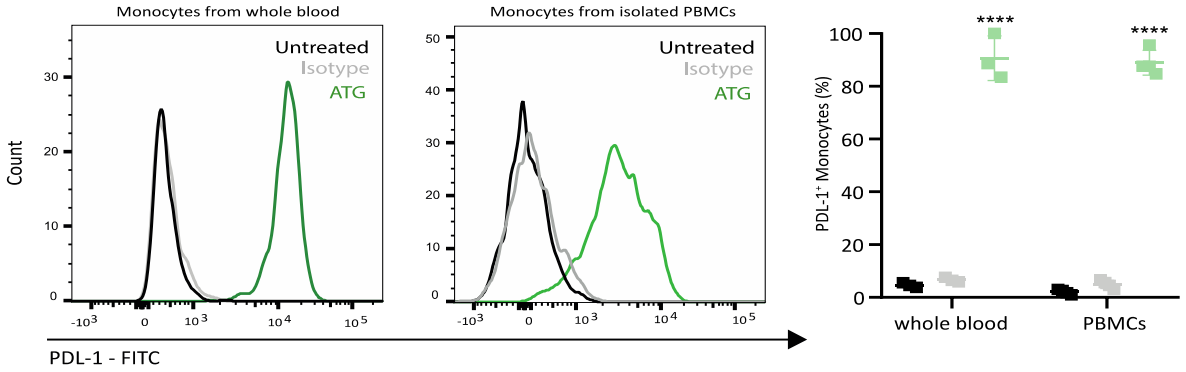


Increased levels of IFN-γ and its response cytokines CXCL9 and CXCL11 were detectable in Plasma ATG



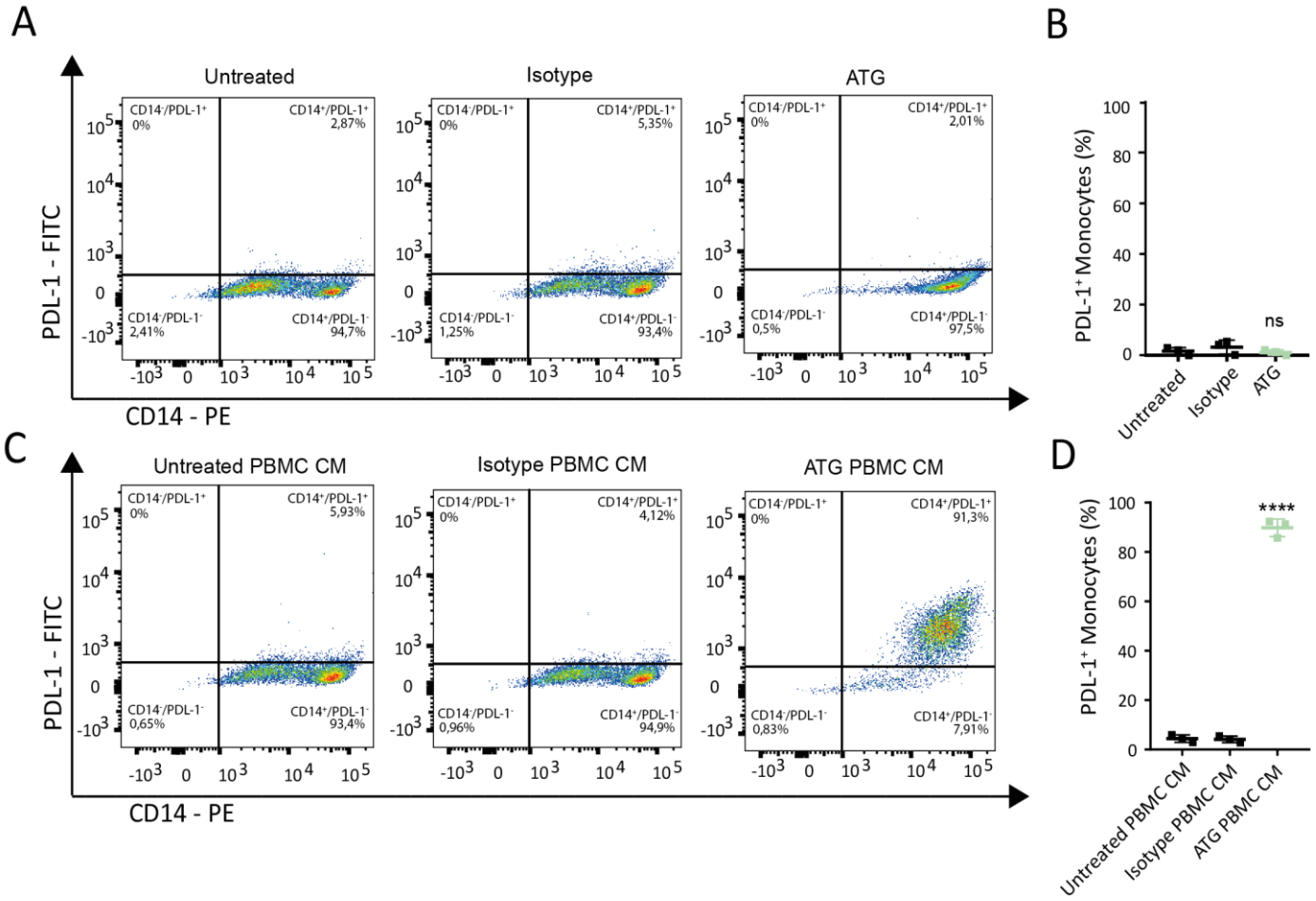
ATG increases surface expression of PD-L1 on monocytes from human whole blood





Percentage of PD-L1^{+} Monocytes increased significantly after treatment with ATG

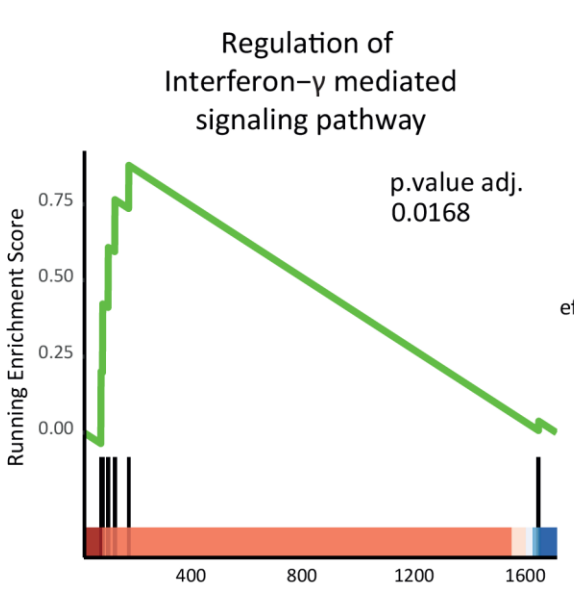
PD-L1 expression on monocytes is modulated by paracrine factors

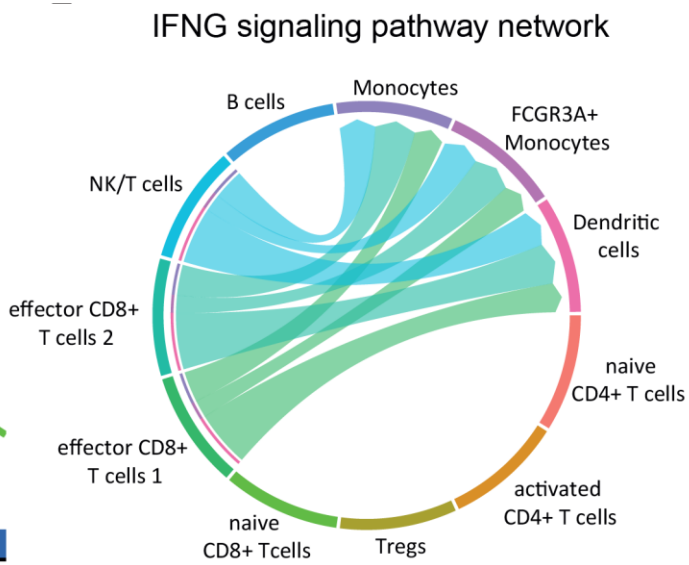


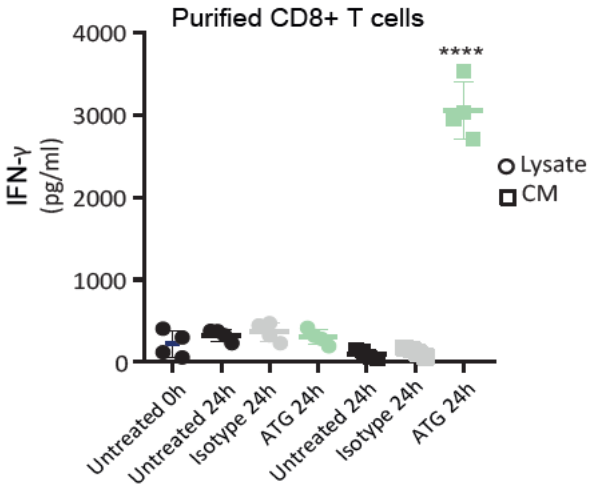
Addition of ATG to purified Monocytes did not alter expression of PD-L1

Treatment of purified Monocytes with CM of ATG-treated PBMCs increased surface expression of PD-L1 on purified monocytes

Expression of PD-L1 is regulated by Interferon-γ signalling (1)



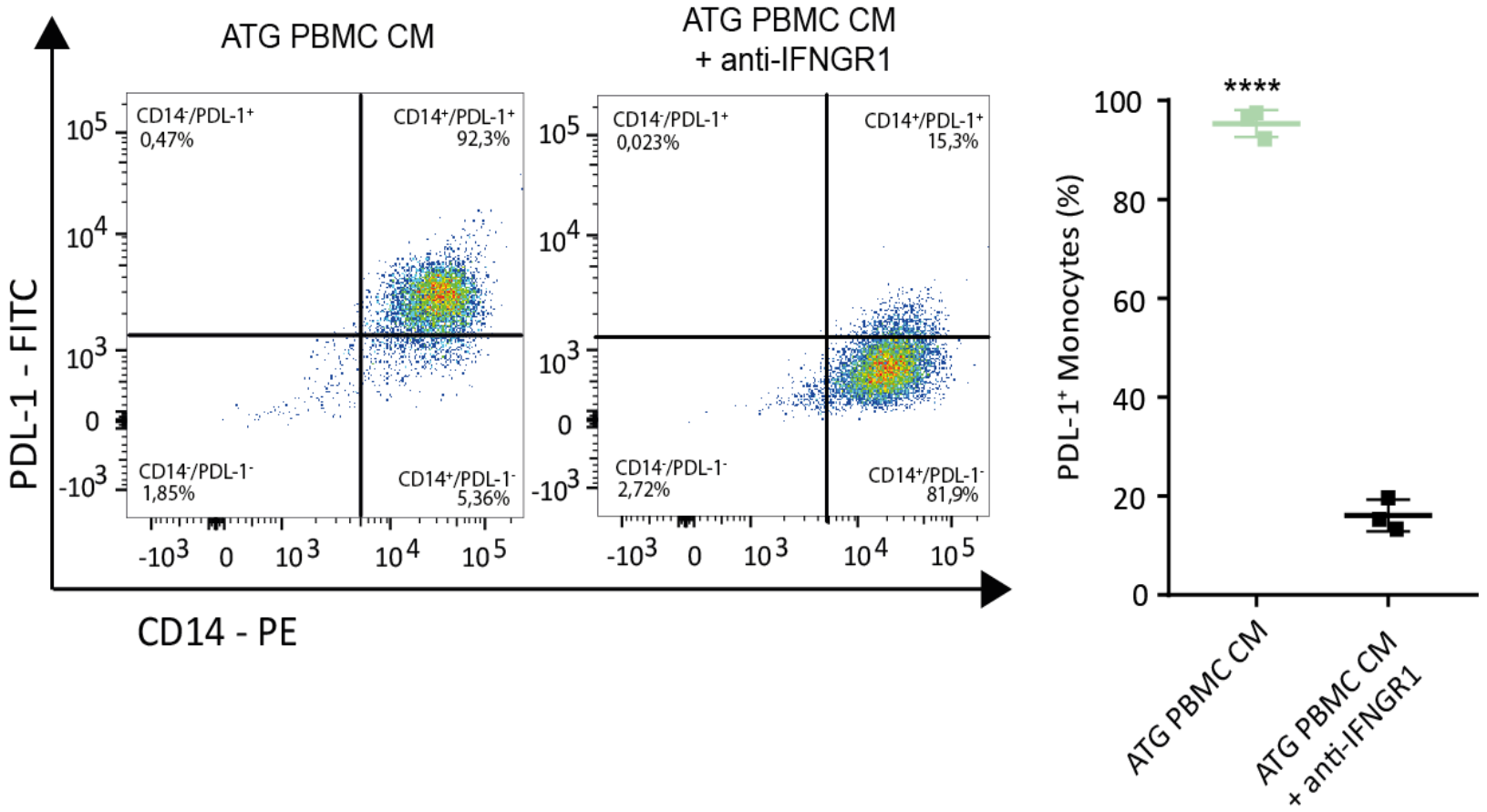




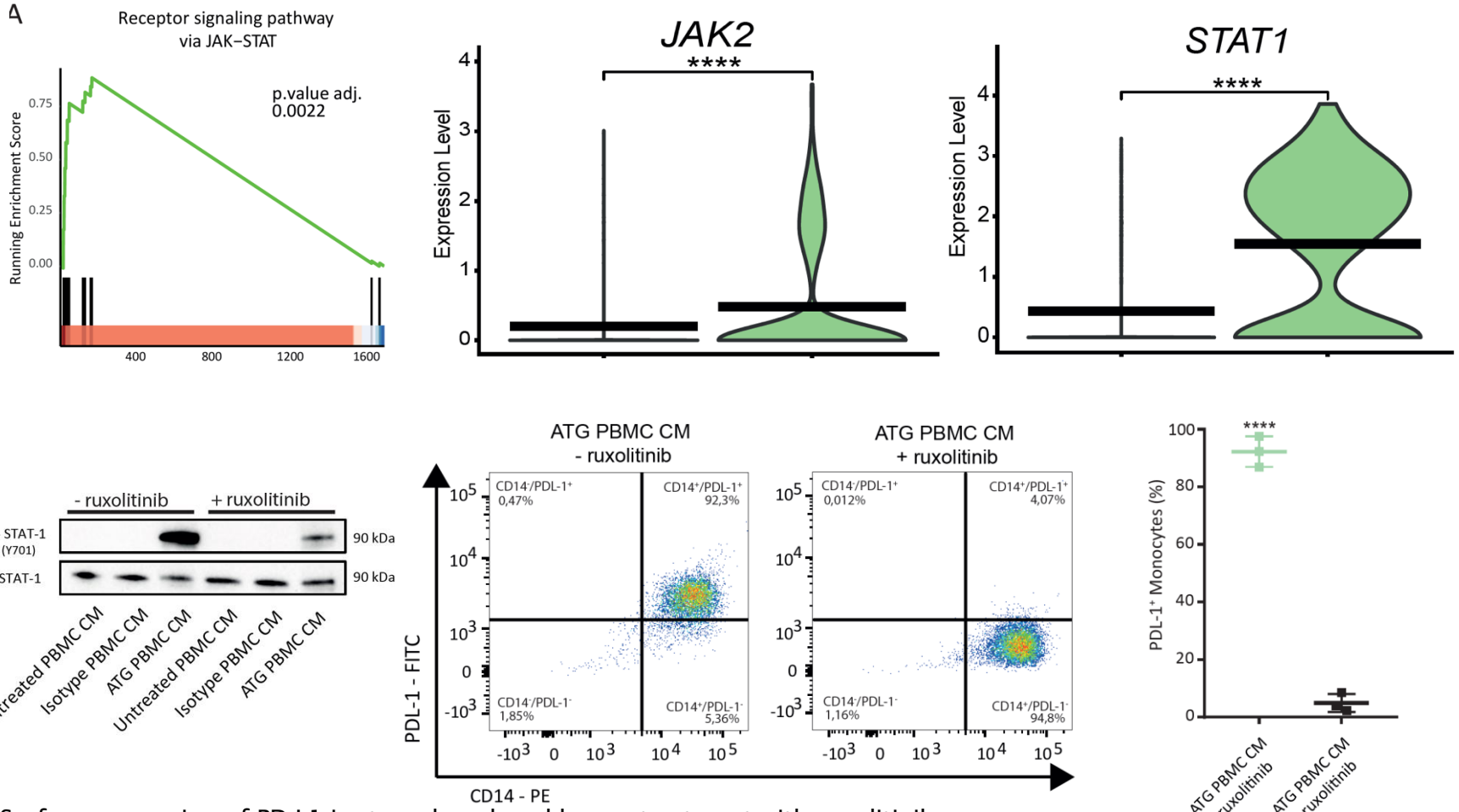
Enrichment for IFN-γ mediated signalling pathway cell-cell interaction

ATG modulates IFN-γ in effector subsets of $CD8^+$ T cells

ATG mediated expression of PD-L1 is regulated by Interferon-γ signalling (2)

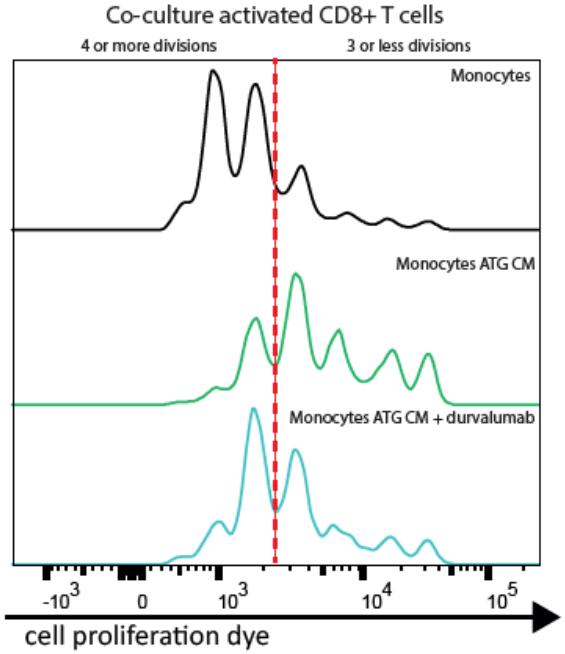


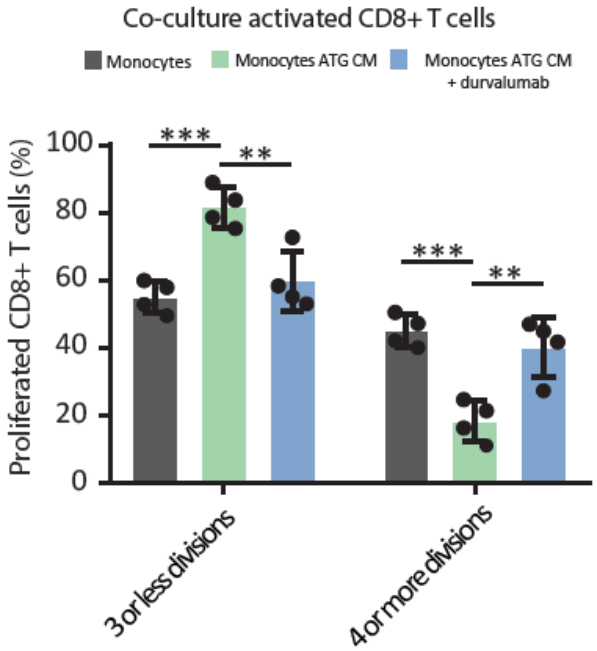
Paracrine induction of PD-L1 relies on JAK/STAT signalling

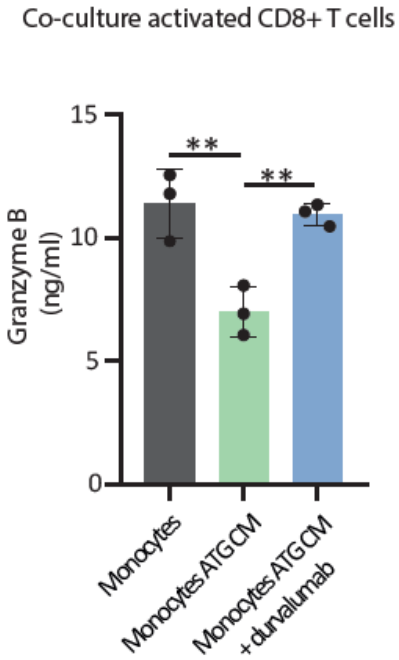


Surface expression of PD-L1 is strongly reduced by pre-treatment with ruxolitinib

PD-L1^{+} monocytes inhibit T-cell effector functions *in vitro*







T cells were activated with αCD3/αCD28 antibodies and co-cultured with monocytes

Proliferation was assesed on day 5 of co-culture

Summary (2)

- ATG changes the transcriptional profile of monocytes from human whole blood
- Effects of ATG on monocytes are in part mediated by CM and play an important role in the induction of immunoregulatory molecules (PD-L1)
- JAK/STAT- signaling is modulated in monocytes after stimulation of whole blood with ATG and leads to increased surface expression of PD-L1
- PD-L1^{+} monocytes inhibit proliferation and production of granzyme B in activated CD8^{+} T cells *in vitro*

Conclusion

PBMCsec and ATG extend their mechanistic repertoire by inducing endogenous secondary mediators in host immune cells

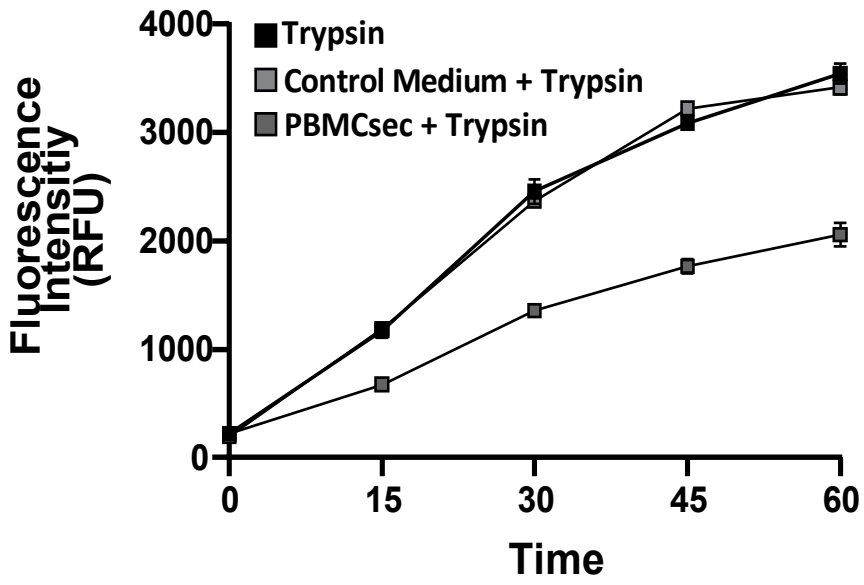
The induced mediators act as amplifiers of innate functions and introduce novel modes of actions

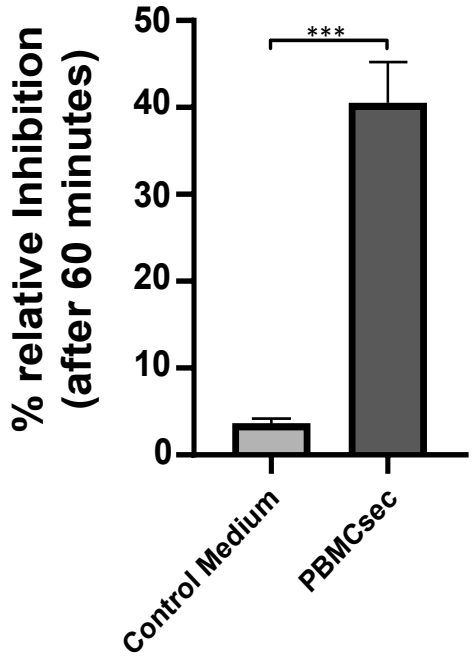
Aposcience AG
Jan Ankersmit
Katharina Klas
Martin Direder
Daniel Bormann

Department of Dermatology
Research Division of Biology and Pathobiology of the Skin
Michael Mildner

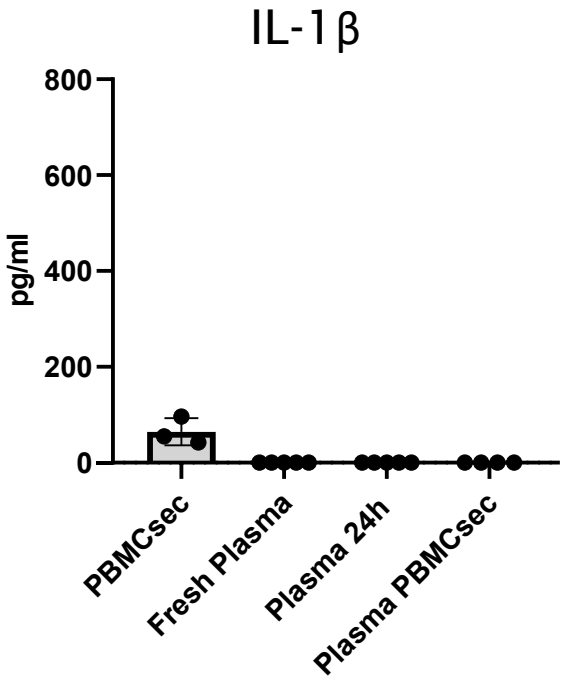
Thank you for your attention!

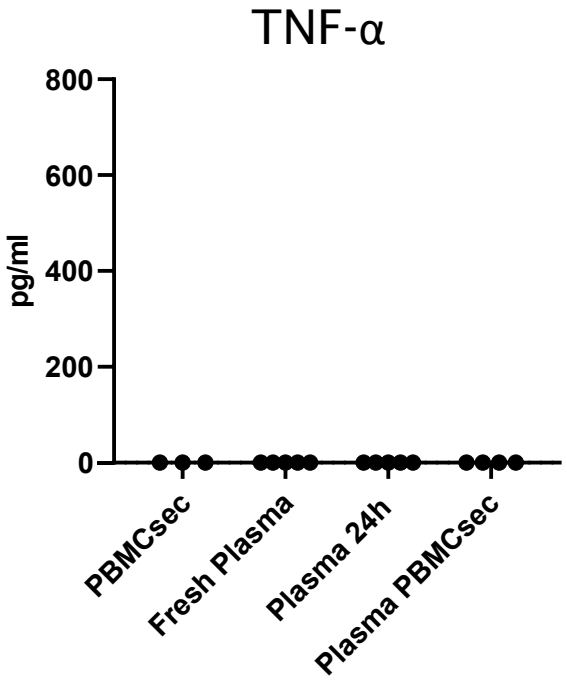
PBMCsec inhibits enzymatic activity of the unselective serine-protease Trypsin

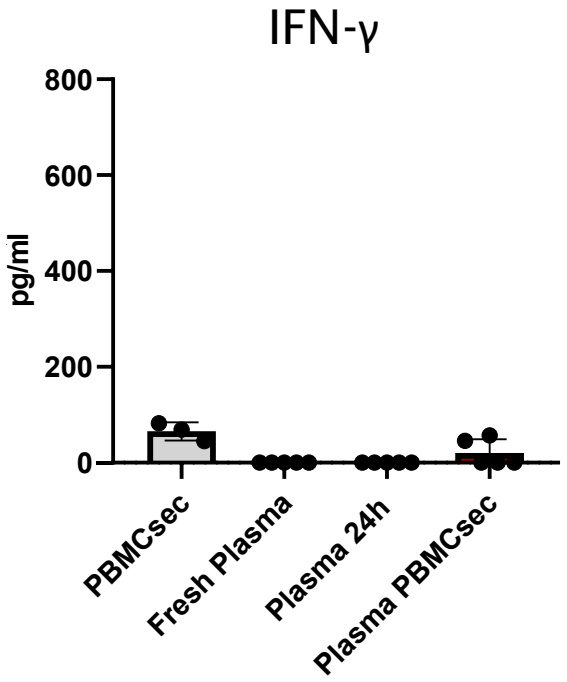




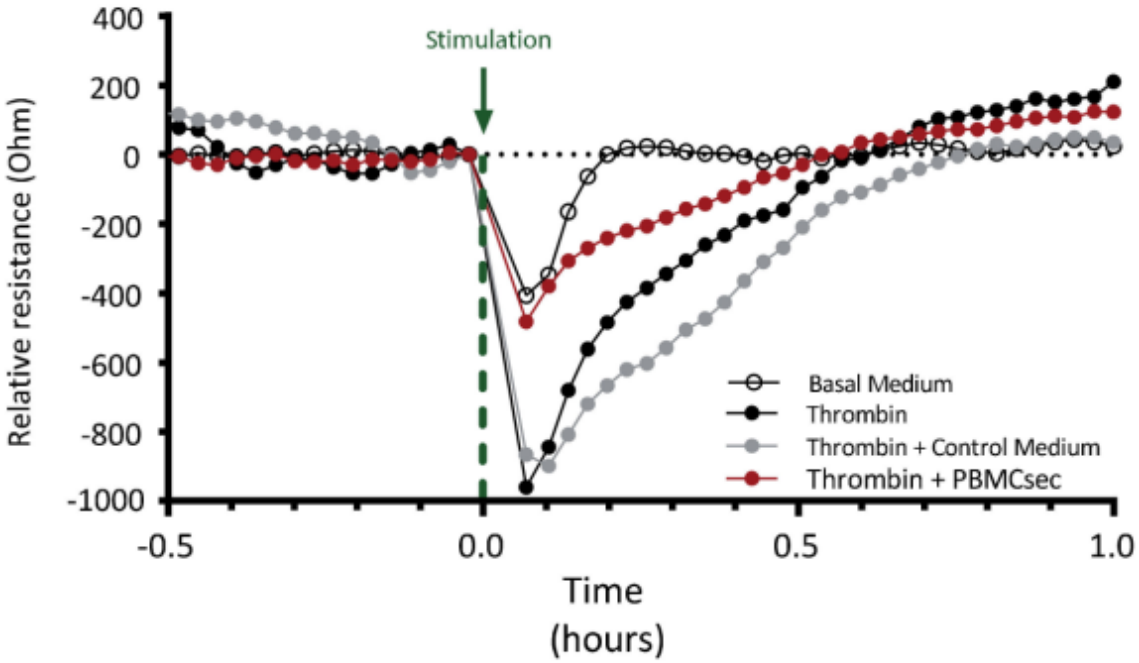
No induction of pro-inflammatory cytokines IL-1β , TNF-α and IFN-γ

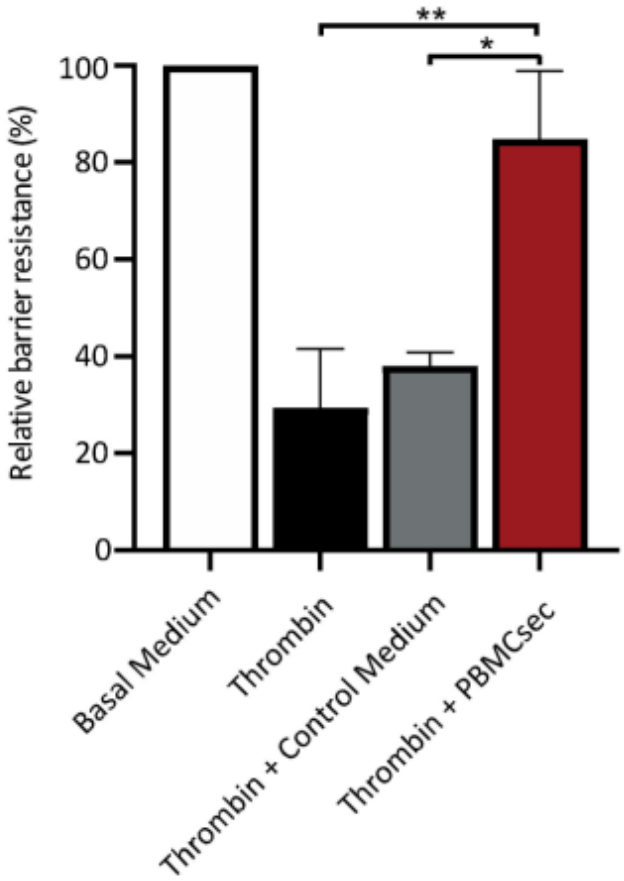






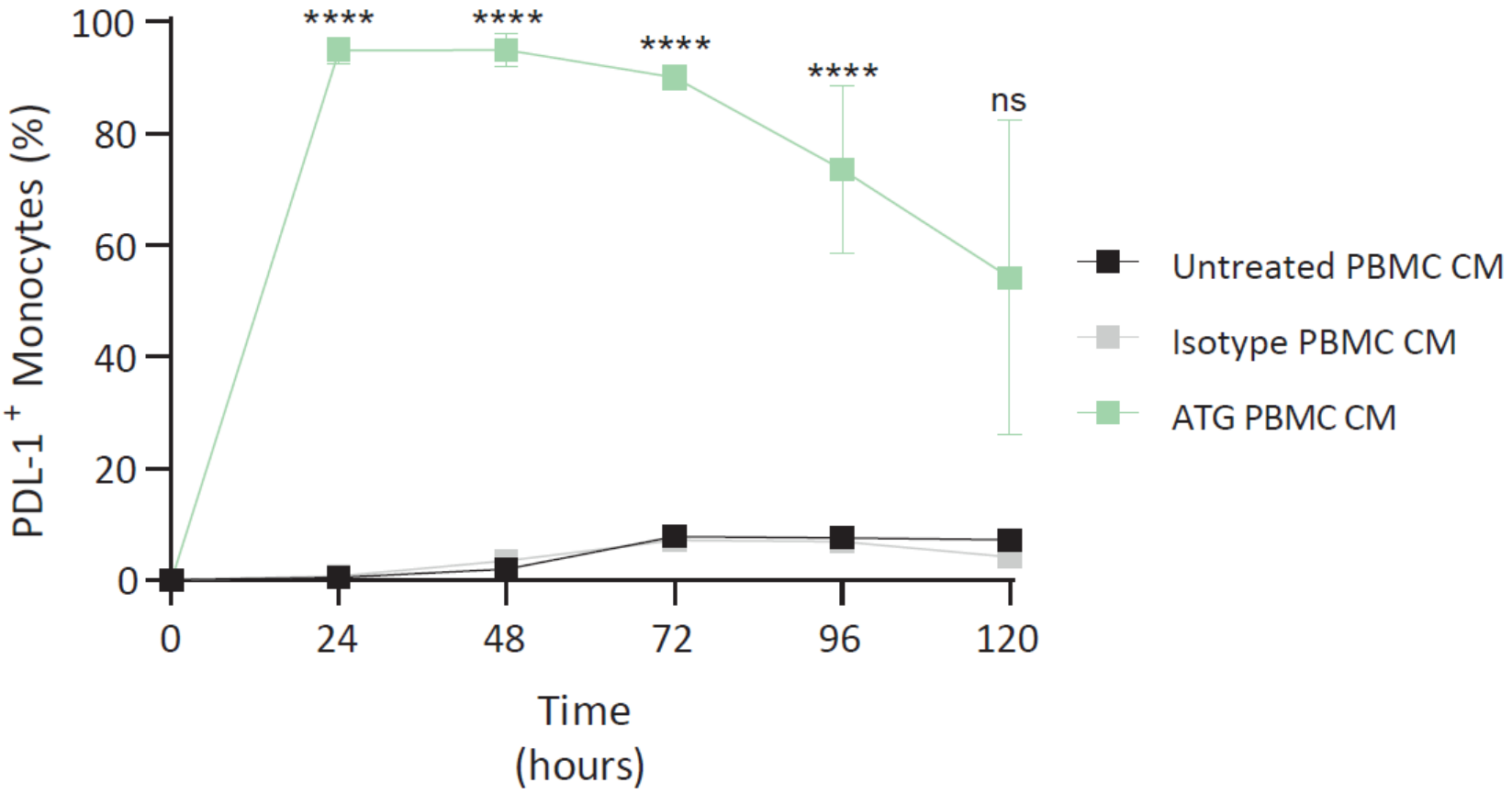
PBMCsec ameliorates thrombin-induced decrease in endothelial barrier function





Thrombin causes a transient drop in barrier resistance
This drop significantly reduced by PBMCsec

Expression of PDL-1 on monocytes over time



Combined treatment of ATG and hydrocortisone does not abolish induction of PD-L1