Supplemental Figures and Tables

Mixed-polarization phenotype of ascites-associated macrophages in human ovarian carcinoma: correlation of CD163 expression, cytokine levels and early relapse

Silke Reinartz[1], Tim Schumann[2], Florian Finkernagel[2], Annika Wortmann[2], Julia M. Jansen[1], Wolfgang Meissner[2], Michael Krause[2], Anne Schwörer[1,2], Uwe Wagner[1], Sabine Müller-Brüsselbach[2#] and Rolf Müller[2#]

[1]Clinic for Gynecology, Gynecological Oncology and Gynecological Endocrinology
Philipps University, Baldingerstraße
35033 Marburg, Germany

[2]Institute of Molecular Biology and Tumor Research (IMT)
Philipps University, Emil-Mannkopff-Strasse 2
35032 Marburg, Germany

[#]SMB and RM: shared senior authorship
[#]Email: smb@imt.uni-marburg.de, rmueller@imt.uni-marburg.de

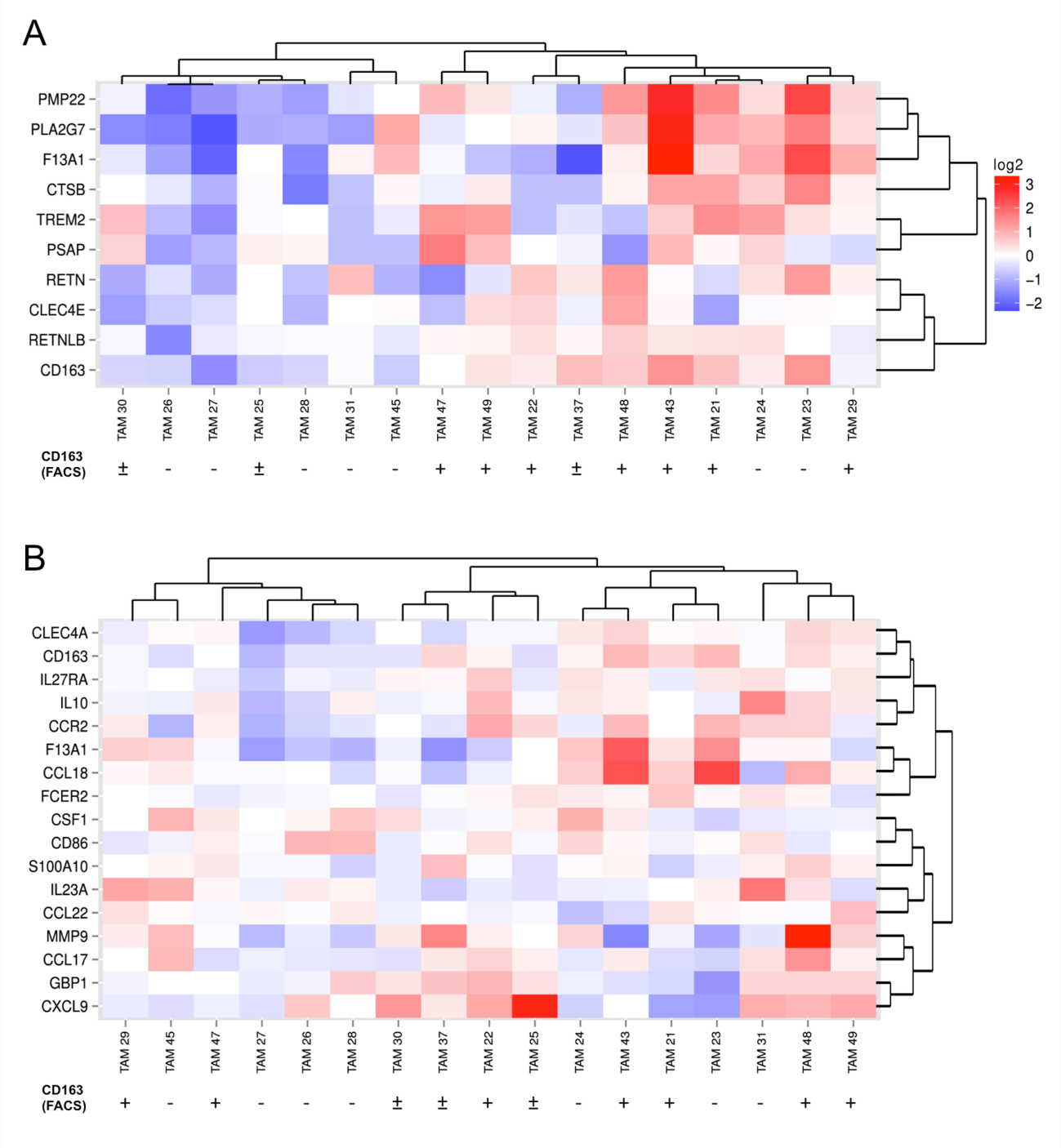

Figure S1. CD163 surface expression is unrelated to M2 gene signatures. Hierarchal clustering of TAM samples according to their similarity with (A) the Ghassabeh signature, a gene set common to type II cytokine-associated murine myeloid cells[15], and (B) the "Beyer signature", discriminating M1- and M2-polarized human MDMs[18]. The corresponding surface expression of CD163 is shown below the heat maps (+, more than 5% above median; ±, median ± 5%; -, less than 5% below median).

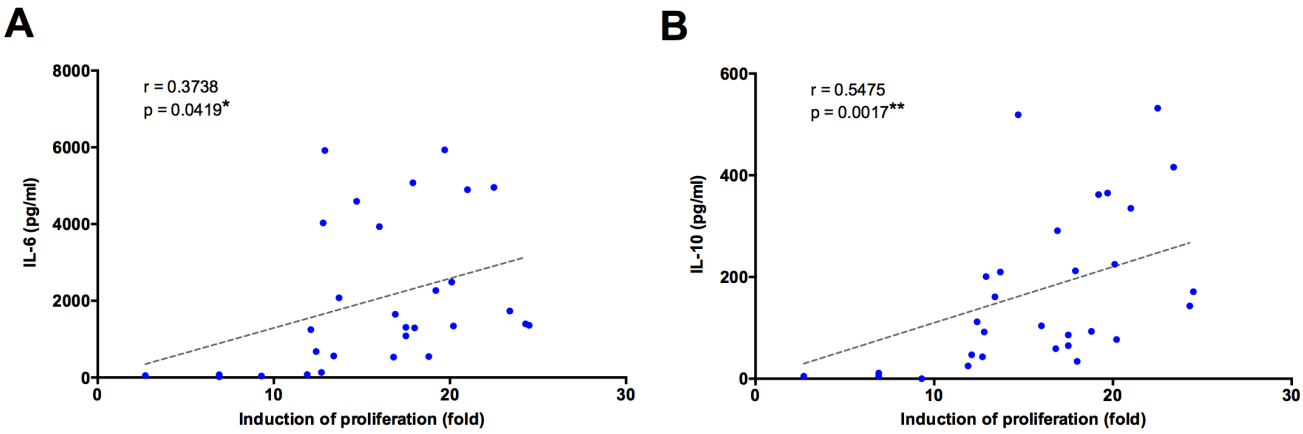

Figure S2. Stimulation of ovarian carcinoma cell proliferation by cytokines from ascites. SKOV-3 cells were serum-deprived and stimulated with 10% ascites from ovarian cancer patients for 6 days. Data were plotted against the levels of IL-6 (A) and IL-10 (B) in the ascites samples. *, $p<0.05$; **$p<0.01$ by Pearson's r correlation.

Methods: The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay (Sigma-Aldrich) was used to assess the influence of ascites or defined cytokines on the proliferation of the human ovarian cancer cell line SKOV-3 (ATCC, HTB-77). SKOV-3 cells were seeded in triplicates on 96-well plates ($1x10^3$ per well) in serum-deprived RPMI1640 medium (PAA) with or without 10% cell-free ascites from ovarian cancer patients. After six days, 10 µl MTT (5 mg/ml) was added for 4 h at 37°C. MTT formazan crystals were dissolved with 10 % SDS + 0.01 M HCl (o/n at 37°C) and the color development was measured at A560 (reference A650). The proliferation index was calculated by calculating for each time point the corresponding ratio of $A560^{ascites}$ /$A560^{control}$. In a single experiment, the MTT assay results were confirmed by a BrdU (5-bromo-2'-deoxyuridine)-based proliferation assay (Roche Applied Science, Mannheim, Germany).

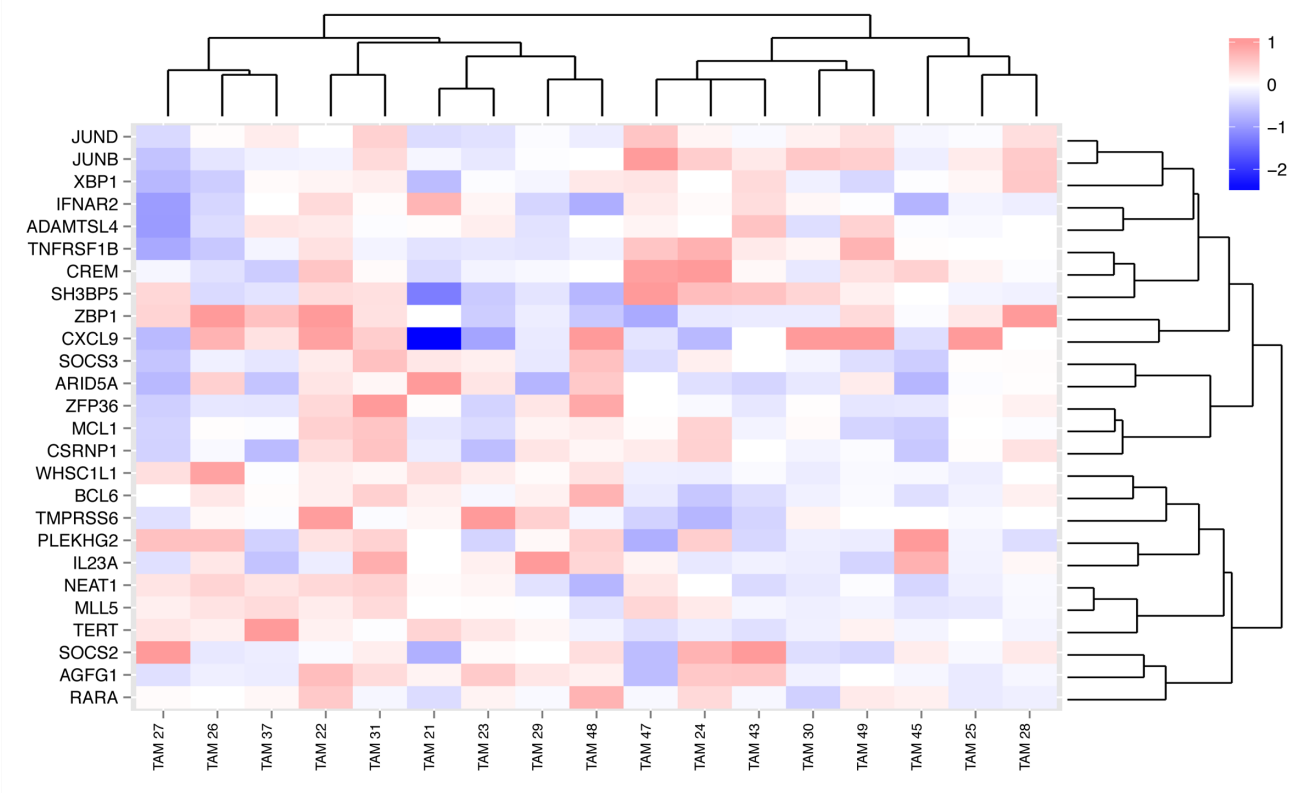

Figure S3. Hierarchal clustering of TAM samples biased on their similarity with a 65-gene STAT signature [24]. The corresponding surface expression of CD163 is shown below the heat maps (+, more than 5% above median; ±, median ± 5%; -, less than 5% below median).

Table S1: Patients included in the present study.

Patient ID	age (y)	surgery date	Grade	Lymph nodes	Stage (FIGO)	Site
TAM15	69	18.04.11	G3	pos	III	Ovary
TAM17	66	04.05.11	G2	pos	III	Ovary
TAM18	65	17.05.11	G2	neg	III	Ovary
TAM20	74	21.06.11	G2-3	nd	III	Ovary
TAM21	48	29.07.11	G2	pos	III	Ovary
TAM22	71	17.10.11	G2	pos	III	Ovary
TAM23	72	05.01.12	G2	neg	III	Ovary
TAM24	35	09.01.12	G2	neg	I	Ovary
TAM25	76	30.01.12	G2	pos	III	Ovary
TAM26	74	14.02.12	G2	pos	III	Ovary
TAM27	72	05.03.12	G3	pos	III	Ovary
TAM28	71	08.03.12	G3	pos	-	Peritoneum
TAM29	46	13.03.12	G3	pos	III	Ovary
TAM30	54	23.04.12	G3	pos	III	Ovary
TAM31	60	22.05.12	G3	neg	III	Ovary
TAM32	53	20.06.12	G1	neg	III	Ovary
TAM33	65	02.07.12	G2	pos	III	Ovary
TAM36	56	15.08.12	G2-3	neg	III	Ovary
TAM37	61	06.09.12	G2	pos	III	Ovary
TAM38	53	08.10.12	G2	pos	III	Ovary
TAM39	71	18.10.12	G3	pos	III	Ovary
TAM41	39	13.11.12	G3	pos	III	Ovary
TAM42	44	05.12.12	G3	neg	III	Ovary
TAM43	55	24.01.13	G3	nd	III	Ovary
TAM44	72	28.01.13	G2	pos	-	Peritoneum
TAM45	70	29.01.13	G2	nd	III	Ovary
TAM46	61	04.02.13	G3	pos	III	Ovary
TAM47	85	25.02.13	G2	pos	III	Ovary
TAM48	62	28.02.13	G2	pos	II	Ovary
TAM49	75	05.03.13	G3	pos	-	Peritoneum

nd, not determined

Reasons for exclusion of patients after isolation of TAMs were cancer-unrelated death shortly after surgery (n=2), histopathological identification as tumors other than serous ovarian carcinoma (n=3), neoadjuvant chemotherapy (n=1), insufficient TAM recovery (n=1) or extreme deviations in the expression of macrophage markers compared to all other samples (n=2).

Table S2:

Correlation* of CD163 surface expression with ascites cytokines.

Parameter 1	Parameter 2	*n*	r	*p*	significance
$CD14^+$ $CD163^+$	IL-6	28	0.4163	0.0276	*
$CD14^+$ $CD163^+$	IL-10	28	0.4573	0.0144	*
$CD14^+$ $CD163^+$	LIF	28	0.3157	0.1017	*ns*
$CD14^+$ $CD163^+$	CSF-1	19	-0.1998	0.4122	*ns*
$CD14^+$ $CD163^+$	TGFβ	21	0.0083	0.6943	*ns*
$CD14^+$ $CD163^+$	VEGF-A	21	0.0663	0.2730	*ns*
$CD14^+$ $CD163^+$	VEGF-C	24	0.0135	0.5890	*ns*
$CD14^+$ $CD163^+$	ANGPTL4	23	0.0274	0.4504	*ns*

*Pearsons r; significant values ($p<0.05$) indicated in red.

Table S3:

Correlations* of ascites cytokine concentrations.

Cytokine	IL-6	IL-10	LIF
IL-6	-	0,0734	0,0002
IL-10	0,0734	-	0,0079
LIF	0,0002	0,0079	-
Leptin	0,5732	0,3185	0,3850
CSF-1	0,5660	0,2932	0,7576
CCL2	0,8008	0,8041	0,2547
TGFβ	0,0856	0,0224	0,0685
VEGF-A	0,1329	0,0753	0,1747
VEGF-C	0,7717	0,3246	0,8129

**p*-values of Pearsons r, *n*=19-28;
significant values ($p<0.05$) indicated in red.