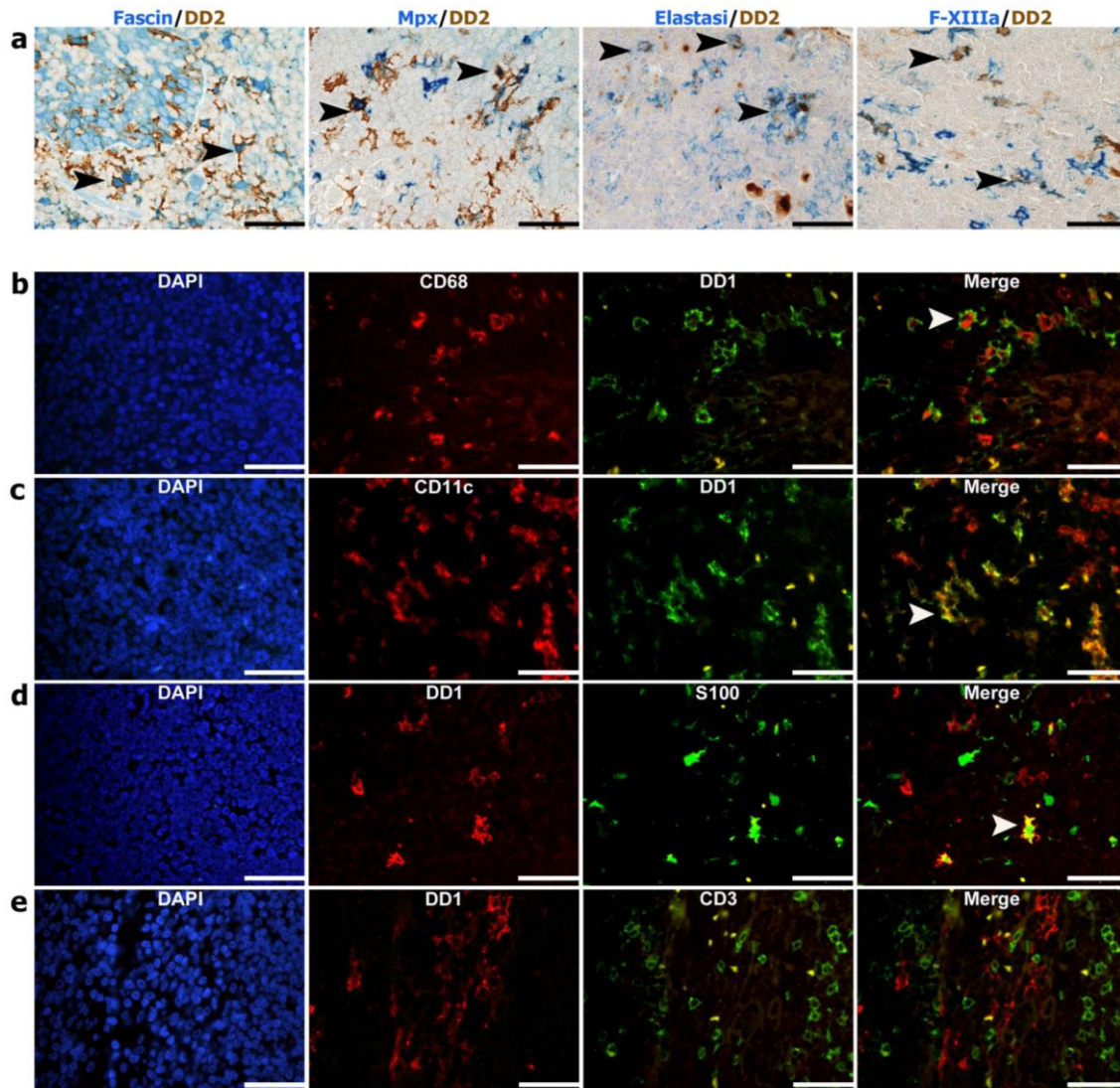
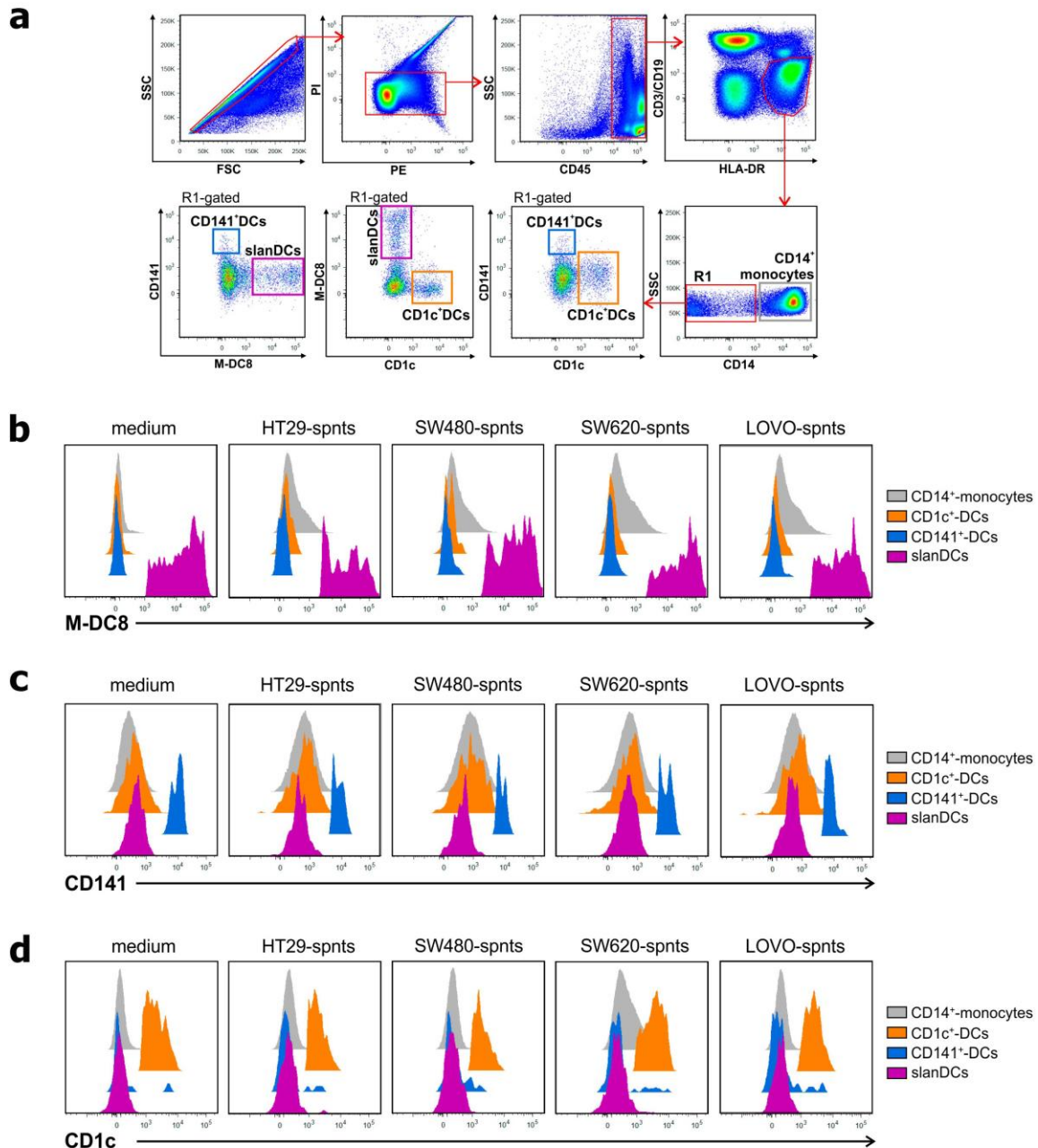


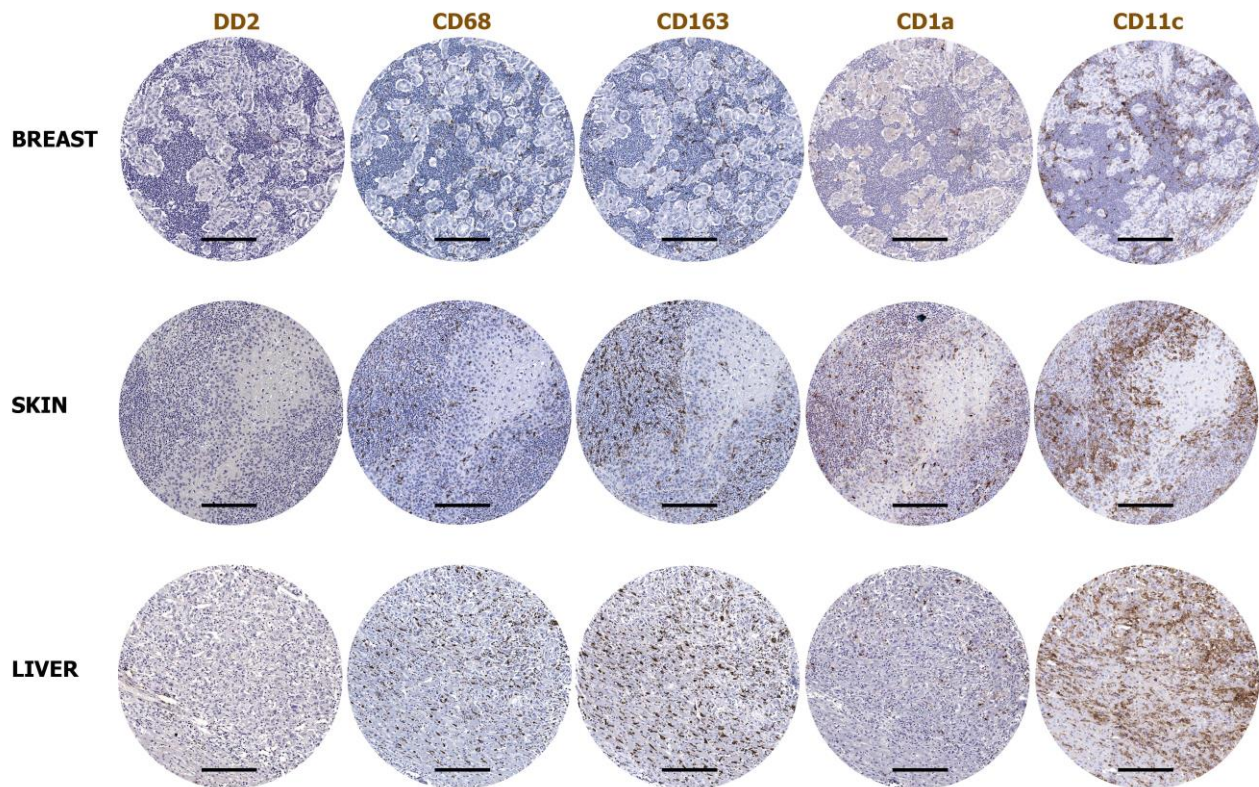
Supplementary Figure S1. DD2 reactivity on human tonsils and analysis of slanDC proliferation. Sections are from a representative human tonsil (**a-f**; n=10) and a representative M-TDLN (**g-h**; n=5) and stained as labeled. (**a, b**) On serial sections DD2 antibody recognizes slanDCs in the tonsil crypt (**a**), whereas the corresponding isotype control antibodies is completely negative (**b**). (**c, d**) DD2 reactivity is also found in the inter-follicular area (**c**) but not on the surface epithelium (**d**). (**e-h**) Double immunohistochemistry for M-DC8 (by DD2 staining) and Ki-67, performed in human tonsils (**e**, low power view; **f**, high power view) as well as in M-TDLN (**g**, low power view; **h**, high power view) show that slanDCs are mostly not proliferating: in fact, strong Ki-67 reactivity is found in the germinal centre (GC) B cells (**e**) and in tumor cells (T in **g** and **h**). Sections were counterstained with Meyer's haematoxylin. Original magnification: 200x (**a-d**; scale bar = 100 microns); 100x (**e** and **g**; scale bar = 200 microns); 400x (**f** and **h**; scale bar = 50 microns).



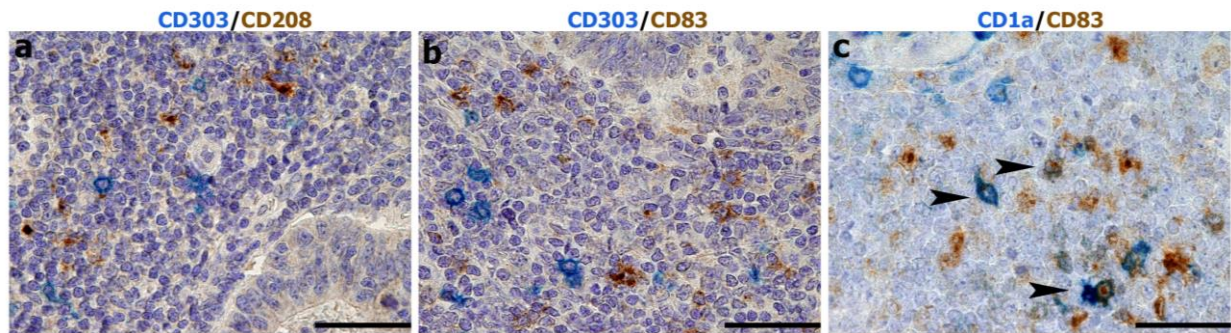
Supplementary Figure S2. Phenotype of slanDCs in human tonsils by immunohistochemistry and immunofluorescence. (a-e) Sections are from a representative human tonsil (n=10) and stained as labelled. For immunohistochemistry experiments, co-expression of the indicated markers is shown by black arrow heads (a). For immunofluorescence experiments, co-expression of M-DC8⁺ slanDCs and CD68 (b), CD11c (c) and S100 (d) is shown by white arrowheads in merged panels, whereas no co-expression is observed for CD3 (e). Sections were counterstained with DAPI. Original magnification: 400x (a; scale bar = 50 microns); 600x (c-e; scale bar = 33 microns).



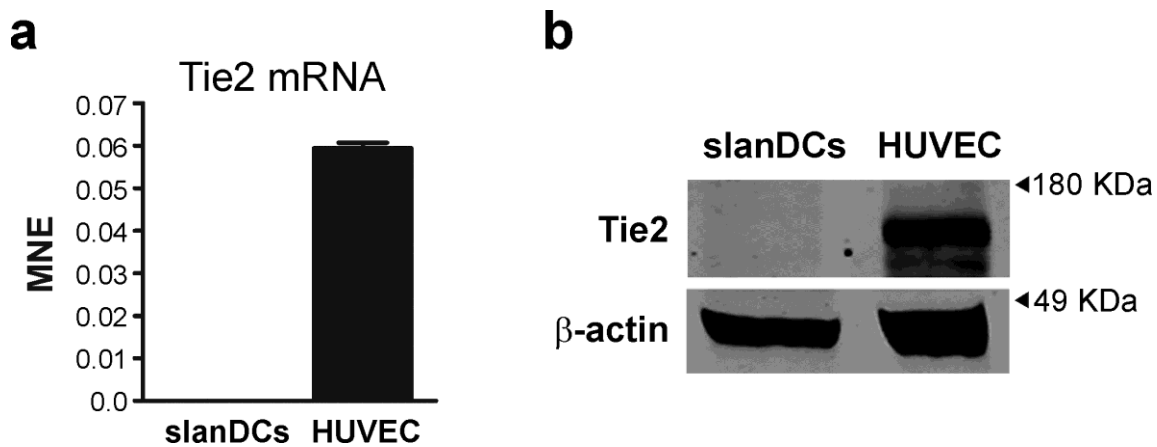
Supplementary Figure S3. Effect of supernatants from colon carcinoma cell lines on M-DC8 expression in slnDCs and DC/monocyte subsets. PBMC from healthy donors were cultured for 18 h in the absence or the presence of supernatants from various colon cancer cell lines at 1/3 dilution prior to marker analysis by flow cytometry. **(a)** Dot plots illustrate the gating strategy used to identify slnDCs, CD1c⁺ DCs, CD141⁺ DCs and CD14⁺ monocytes within PBMC. First, cells are selected via sequential gating by the exclusion of cell doublets and dead cells, in turn followed by the positive gating of CD45⁺ leukocytes. A further gate on HLA-DR⁺/CD3⁻/CD19⁻ cells is set to select both CD14⁺ classical monocytes (gray gate) and CD14^{dim/-} cells, which include CD1c⁺ DCs (orange gate), CD141⁺ DCs (blue gate) and M-DC8⁺ slnDCs (purple gate). **(b-d)** Expression levels of M-DC8 **(b)**, CD1c **(c)** and CD141 **(d)** by monocytes/DC subsets after culture with colon cancer cell line-derived supernatants, as indicated. One representative experiment out of three with identical results is shown.



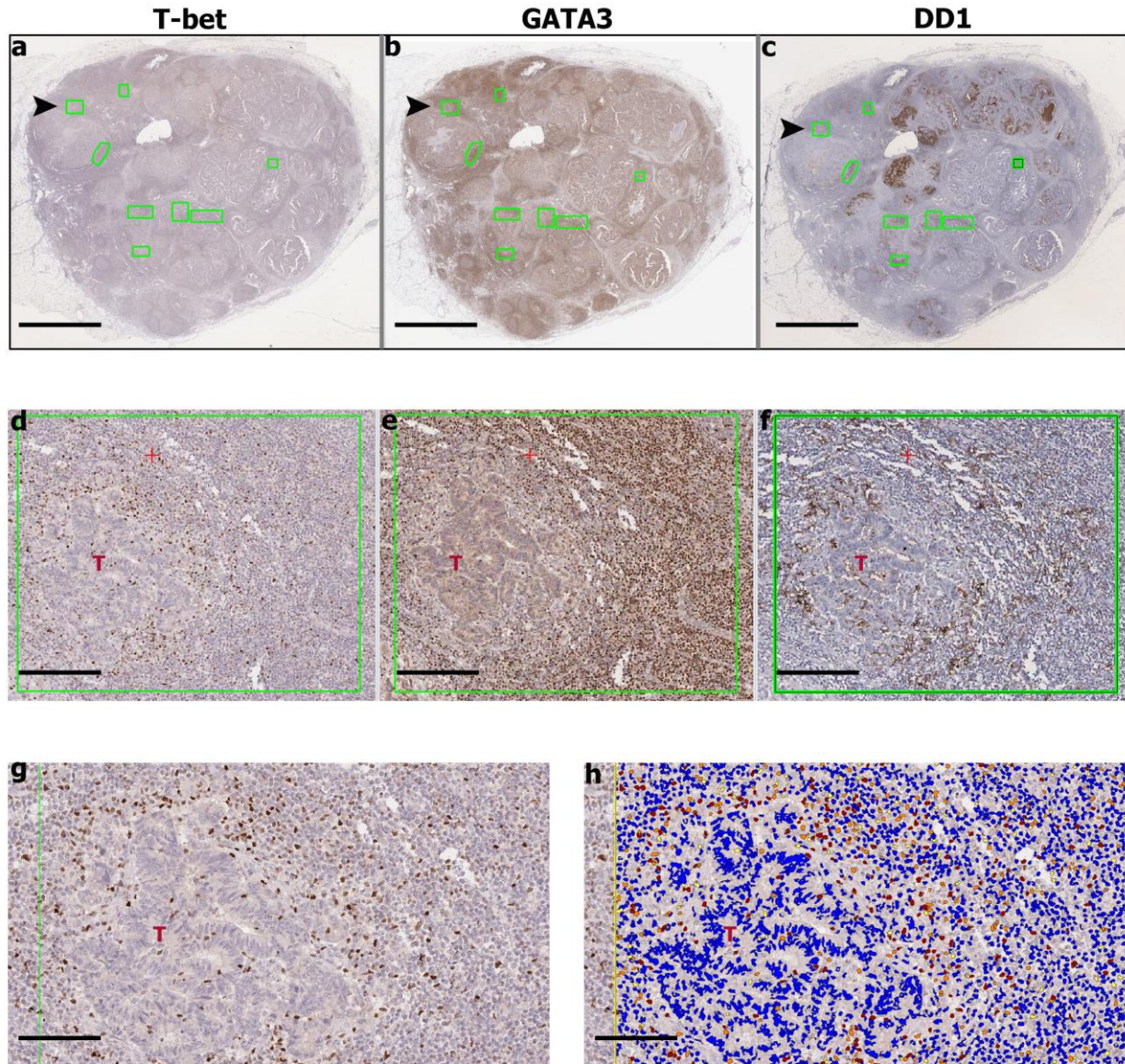
Supplementary Figure S4. Absence of slanDCs in human primary carcinomas. FFPE sections are from a multi-tumor TMA containing carcinomas from different primary sites and stained for different DC and macrophage-associated markers, as indicated. Sections are counterstained with Meyer's haematoxylin. Digital Images were taken as snapshots by using Image Scope software and resized by using Adobe Photoshop. Original Magnification: 100x, scale bar = 200 microns.



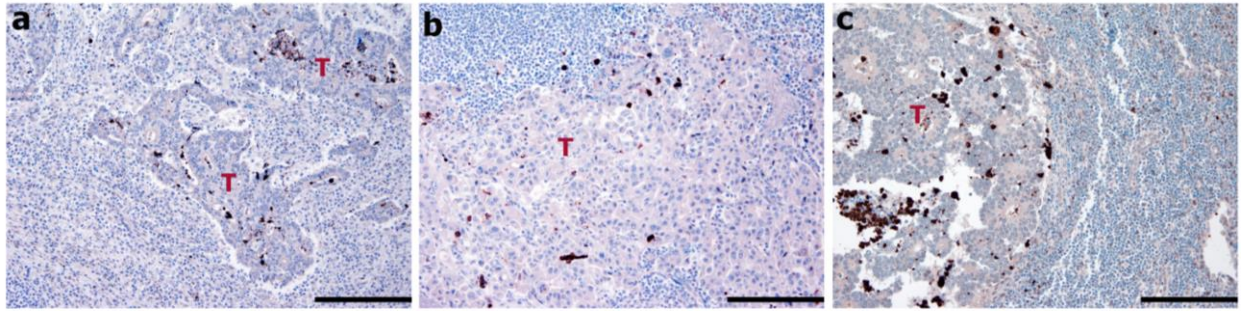
Supplementary Figure S5. DC maturation markers in M-TDLN. Sections are from a human M-TDLN (n = 5) and stained as labeled. CD303⁺ pDCs do not co-express the maturation markers CD208 (a) and CD83 (b), whereas CD83 is frequently co-expressed by CD1a⁺ DCs (c, black arrow heads). Original magnification: 400x, scale bar = 50 microns.



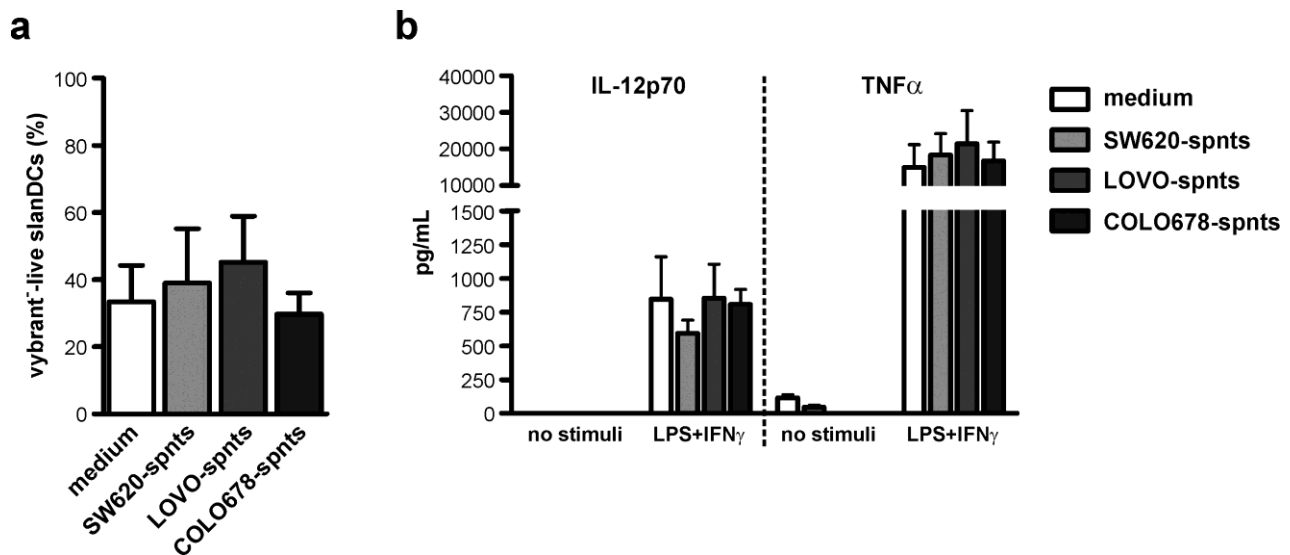
Supplementary Figure S6. Circulating slanDCs do not express Tie2. (a) HUVEC and freshly isolated slanDCs were analyzed for Tie2 mRNA expression by RealTime-PCR analysis. Bar graph shows the mean normalized expression (MNE) \pm SD of Tie2 mRNA from three independent experiments. (b) Whole extracts prepared from HUVEC (0.3×10^6 cells) and freshly isolated slanDCs (1×10^6 cells) were analysed for expression of 140 KDa Tie2 by Western blot. 40 KDa β -actin was also detected for loading normalization. One representative experiment out of three with identical results is shown.



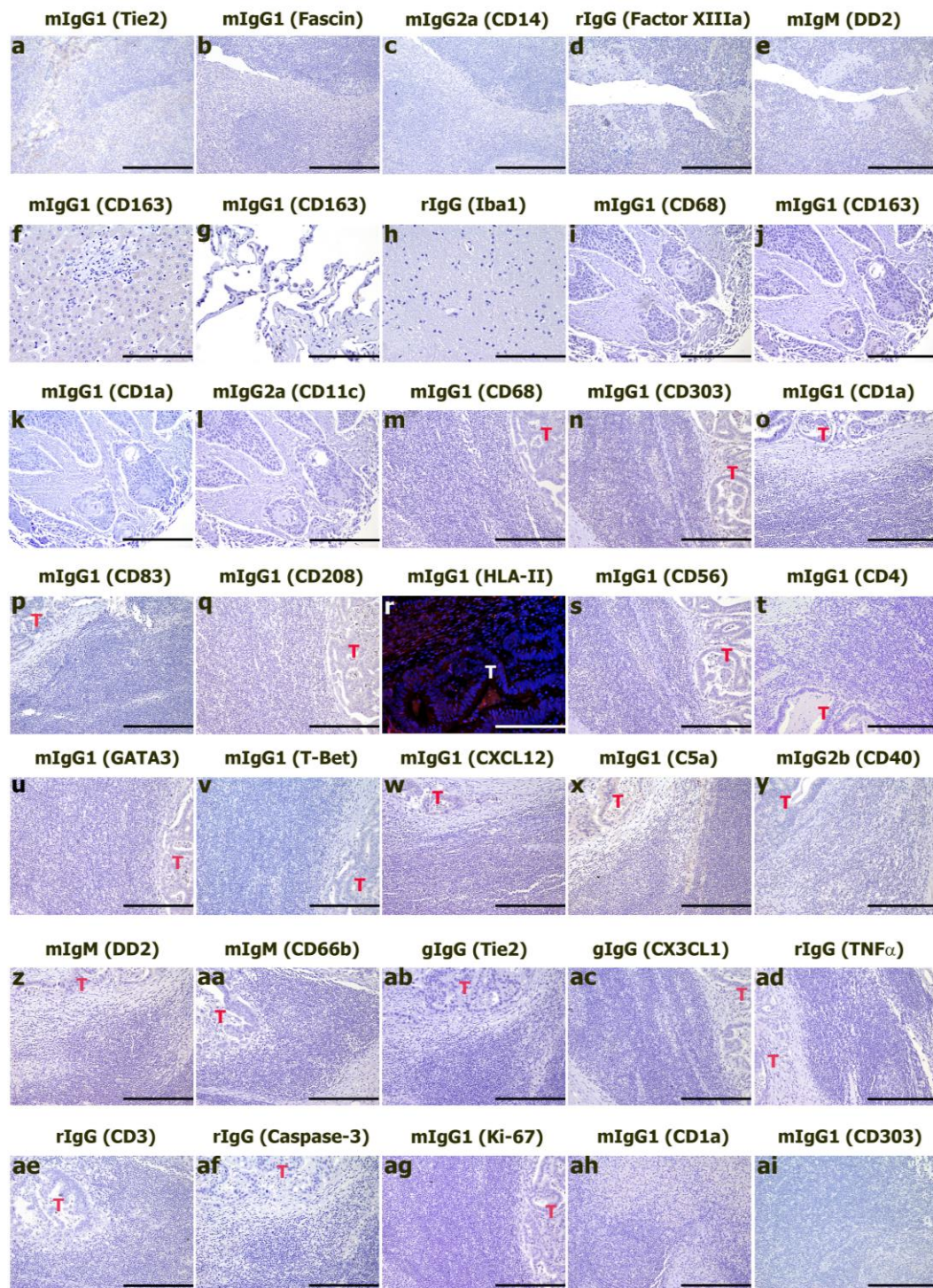
Supplementary Figure S7. Analysis of the Th-type polarization in M-TDLN. (a-h) A representative example illustrating the procedure followed for the automatic cell count of T-bet⁺ and GATA3⁺ cells in M-TDLN. (a-c) Multiple T-cell areas (green rectangles) are captured from digitalized serial sections (by Aperio Scanscope) stained for T-bet (a) and GATA3 (b). Selection is based on the co-occurrence of M-DC8⁺ slanDCs (by DD1 staining) (c) in the same area. (d-e) High power views of a single area (indicated by black arrow heads in a-c) show a lower density of T-bet⁺ cells (d) as compared to GATA3⁺ cells (e). A high density of peri-tumoral slanDCs in this area is shown in f. (g-h) Comparative examples of the native (g) and the corresponding processed (h) images obtained from the T-bet-stained section by using the IHC nuclear algorithm (Aperio Scanscope) are displayed. T-bet⁺ cells are identified by yellow, orange and red colors (respectively for low, moderate and strong positivity), whereas blue highlights T-bet⁻ nuclei in the sections. Images were taken as snapshots by using Image Scope software. Original Magnification: 10x (a-c), scale bar = 2 millimeters; 80x (d-f), scale bar = 250 microns and 300x (g and h), scale bar = 67 microns.



Supplementary Figure S8. Expression of active caspase-3 in M-TDLN. Panels (a), (b) and (c) are representative of three human M-TDLNs (n = 10) stained for active caspase-3 (brown apoptotic bodies). Low power view illustrates a predominant distribution of active caspase-3⁺ apoptotic cells within and around carcinoma nests. Original magnification: 100x, scale bar = 200 microns.



Supplementary Figure S9. Effect of supernatants from colon carcinoma cell lines on survival and cytokine production by slanDCs. slanDCs ($0.5 \times 10^6/\text{mL}$) were cultured with supernatants generated from SW620, LOVO and COLO-678 (at a 1:1 dilution with fresh culture medium), in the absence (**a,b**), or in the presence (**b**) of 100 ng/ml LPS plus 100 U/ml $\text{IFN}\gamma$. After a 24 h-incubation, viability of slanDCs was measured by flow cytometry (**a**), while slanDC-derived IL-12p70 and $\text{TNF}\alpha$ were quantified in cell-free supernatants by specific ELISA kits (both from eBioscience) (**b**). Bar graphs show the mean \pm SD ($n = 3$).



Supplementary Figure S10. Isotype control stains. Sections are from frozen tonsil (**a**) or FFPE tonsil (**b-e**), liver (**f**), lung (**g**), brain (**h**), multi-tumor TMA (**i-l**), M-TDLN (**m-ag**) and reactive lymph node (**ah-ai**), stained as labeled. Microphotographs illustrate isotype controls to the primary antibodies indicated between parentheses. For other tonsil markers, including CD68, pan-cytokeratin, Neutrophil Elastase, Ki-67, CD11c, CD16, Lysozyme, Myeloperoxidase, S100 protein and DD1, isotype controls are not shown since they were used at lower dilutions. Original magnification: 100x for **a-e**, **i-q**, **s-ag** (scale bar = 125 micron); 100x for **f-h** and **r** (scale bar = 63 micron). mIg = mouse Ig; rIg = rabbit Ig; gIg =goat Ig; T= tumor.

Supplementary Table S1. Site of origin and histology of primary carcinomas included in tissue microarrays (TMA).

Primary site	Carcinoma Histology	Number of cases
Lung	squamous	5
	adenocarcinoma	5
Head and Neck	squamous	10
Pancreas	ductal	7
	neuroendocrine	2
	papillary mucinous	1
Thyroid	medullary	2
	papillary	5
	follicular	3
Endometrium	adenosquamous	2
	endometrioid	1
Cervix	squamous	3
Breast	ductal	6
	lobular	2
	mixed	2
Skin	squamous	5
	basal cell carcinoma	5
Stomach	diffuse	3
	mixed	1
	intestinal	6
Liver	epatocellular	6
	cholangiocarcinoma	4
Colo-rectal	adenocarcinoma	7
	mucinous	3
Kidney	clear cell	4
	chromophobe	2
	papillary	4
Bladder	transitional	10
Ovary	serous-papillary	6
	endometrioid	4
Total		126

Supplementary Table S2. Pathology features of the M-TDLN cohort.

M-TDLN cases	Site of the primary tumor	Histology	slanDCs
#1	skin	melanoma	-
#2	skin	melanoma	-
#3	skin	melanoma	-
#4	skin	melanoma	-
#5	skin	melanoma	-
#6	skin	melanoma	+
#7	skin	melanoma	-
#8	skin	melanoma	+
#9	skin	melanoma	-
#10	skin	melanoma	-
#11	lung	oat cell carcinoma	+
#12	lung	squamous cell carcinoma	+
#13	lung	squamous cell carcinoma	-
#14	lung	indifferentiated carcinoma	+
#15	lung	adenocarcinoma	-
#16	lung	adenocarcinoma	-
#17	lung	adenocarcinoma	+
#18	lung	squamous cell carcinoma	+
#19	lung	squamous cell carcinoma	+
#20	lung	squamous cell carcinoma	+
#21	lung	squamous cell carcinoma	-
#22	lung	indifferentiated carcinoma	+
#23	lung	adenocarcinoma	-
#24	lung	adenocarcinoma	-
#25	lung	adenocarcinoma	-
#26	breast	lobular carcinoma	+
#27	breast	ductal carcinoma	-
#28	breast	ductal carcinoma	-
#29	breast	lobular carcinoma	-
#30	breast	indifferentiated carcinoma	+
#31	breast	ductal carcinoma	-
#32	breast	ductal carcinoma	+
#33	breast	ductal carcinoma	-
#34	breast	ductal carcinoma	-
#35	colon	adenocarcinoma	-
#36	colon	adenocarcinoma	+
#37	colon	adenocarcinoma	+
#38	colon	adenocarcinoma	+
#39	colon	adenocarcinoma	+
#40	colon	adenocarcinoma	+
#41	colon	adenocarcinoma	+
#42	colon	adenocarcinoma	-
#43	colon	adenocarcinoma	-
#44	ileum	adenocarcinoma	+
#45	colon	adenocarcinoma	-
#46	stomach	diffuse carcinoma	-
#47	stomach	indifferentiated carcinoma	-
#48	stomach	diffuse carcinoma	+
#49	stomach	mixed carcinoma	+
#50	stomach	diffuse carcinoma	-
#51	stomach- cardias	intestinal carcinoma	+
#52	Liver	cholangiocarcinoma	-
#53	cervix	squamous carcinoma	+
#54	endometrium	endometrioid carcinoma	+
#55	endometrium	serous-papillary and clear cell carcinoma	+
#56	endometrium	endometrioid carcinoma	-
#57	cervix	squamous carcinoma	+
#58	tube	serous-papillary carcinoma	-
#59	bladder	transitional carcinoma	+
#60	bladder	indifferentiated carcinoma	+
#61	bladder	transitional carcinoma	+
#62	pelvi	papillary transitional cell carcinoma	-
#63	bladder	transitional carcinoma	-
#64	kidney	clear cell carcinoma	+
#65	kidney	clear cell carcinoma	-
#66	thyroid	papillary carcinoma	-
#67	thyroid	papillary carcinoma	-
#68	thyroid	medullary carcinoma	-
#69	head neck	squamous carcinoma	+
#70	head neck	squamous carcinoma	+
#71	head neck	squamous carcinoma	+
#72	head neck	indifferentiated carcinoma	-
#73	head neck	squamous carcinoma	+
#74	head neck	squamous carcinoma	-
#75	head neck	squamous carcinoma	+
#76	head neck	squamous carcinoma	-
#77	head neck	squamous carcinoma	-
#78	head neck	squamous carcinoma	-
#79	pancreas	neuroendocrine carcinoma	-
#80	pancreas	ductal carcinoma	-

Supplementary Table S3. Analysis of different Th-cell subsets in MTDLN.

M-TDLN	GATA3 ⁺ /mm ²	T-Bet ⁺ /mm ²	Foxp3 ⁺ /mm ²
Group 1 (G1)-slanDC+ M-TDLN			
#01	9194.8	908.6	2314.5
#02	8398.4	1109.4	621.3
#03	7620.8	1864.1	1354.2
#04	8074.25	677	372
#05	6729.3	953	946.1
Mean(SD)	8003.5(915)	1102.4(453)	1121.6(761.5)
<i>*p</i> (G1)	GATA3 vs T-Bet <0.001 GATA3 vs Foxp3 <0.001 T-Bet vs Foxp3= 0.998		
Group 2 (G2)-slanDC- M-TDLN			
#06	7651.3	5896.6	1354.3
#07	11494	1198.6	1112.3
#08	8910	1925.3	1179
#09	5516.6	2000.6	1952.3
#10	7074.3	1540.3	2363.3
Mean(SD)	8129.2(2241)	2512.2(1919)	1592.2(543.5)
<i>*p</i> (G2)	GATA3 vs T-Bet <0.001 GATA3 vs Foxp3 <0.001 T-Bet vs Foxp3= 0.806		
<i>*p</i> (G1vsG2)	1	0.123	0.436

For each MTDLN case data represent the mean number of cells identified in at least 3 areas.
 * = two-way ANOVA on the log transformed values.

Supplementary Table S4. Clinical data of colon carcinoma patients.

case	sex	age	histology ¹	site ²	stage (AJCC ³)	infections
#01	M	65	AC	RC	II	none
#02	M	75	AC	RC	III	none
#03	M	53	AC	LC	III	none
#04	M	80	AC	RC	III	HBV
#05	M	67	AC	TR	III	none
#06	F	85	AC	RC	III	none
#07	F	74	AC	LC	IV	none
#08	M	65	AC	RC	II	none
#09	F	81	AC	LC	III	none
#10	M	80	AC	TR	I	none
#11	M	79	AC	LC	I	none
#12	F	86	AC	LC	III	none
#13	F	70	AC	TR	II	none
#14	M	72	AC	RC	III	none
#15	M	72	AC	RC	II	none
#16	F	82	AC	RC	III	none
#17	M	75	AC	RC	IV	HCV
#18	M	72	AC	LC	I-II	none
#19	F	58	AC	RC	III	none
#20	M	57	AC	LC	II	none
#21	M	77	AC	RC	I	none
#22	M	68	AC	LC	II	none

¹ AC: adenocarcinoma;

² RC= right colon, LC= left colon, TR= transverse colon;

³ AJCC= American joint committee on cancer

Supplementary Table S5. List of the antibodies used for immunohistochemistry and immunofluorescence.

Reagent	Clone	Dilution	Isotype	Source	
PRIMARY ANTIBODIES (IHC AND IF)					
CD1a	010	1:50	mIgG1	Dako	
CD3	SP7	1:100	rabbitIgG	Thermo Scientific	
CD4	4B12	1:40	mIgG1	Thermo Scientific	
CD11b		1:300	rabbit polyclonal	Sigma-Aldrich	
CD11c	5D11	1:50	mIgG2a	Novocastra Laboratories	
CD14	7	1:50	mIgG2a	Novocastra Laboratories	
CD16	2H7	1:100	mIgG2a	Novocastra Laboratories	
CD40	11E9	1:60	mIgG2b	Novocastra Laboratories	
CD56	123C3.D5	1:30	mIgG1	Thermo Scientific	
CD66b	G10F5	1:200	mIgM	BioLegend	
CD68	KP1	1:300	mIgG1	Dako	
CD68	PG-M1	1:200	mIgG3	Dako	
CD83	1H4b	1:150	mIgG1	Novocastra Laboratories	
CD123	7G3	1:50	mIgG1	BD Biosciences	
CD163	10D6	1:50	mIgG1	Thermo Scientific	
CD169	HSn 7D2	1:50	mIgG1	Novus Biologicals	
CD207/Langerin		1:200	mIgG2b	Vector Laboratories	
CD208/DC-LAMP	104.G4	1:100	mIgG1	Immunotech	
CD303/BDCA2	124B3.13	1:75	mIgG1	Dendritics	
Caspase 3 Active		1:600	rabbit polyclonal	R&D Systems	
CX3CL1/Fractalkine		1:250	goat polyclonal	R&D Systems	
CXCL12/SDF-1	79018	1:50	mIgG1	R&D Systems	
C5a Complement	2952	1:100	mIgG1	Thermo Scientific	
DD1	DD1	1:60	mIgM	K.S (University Hospital Heidelberg)	
DD2	DD2	1:50	mIgM	K.S (University Hospital Heidelberg)	
Factor XIIIa		1:100	rabbit polyclonal	Biogenex	
Fascin	55K-2	1:50	mIgG1	Dako	
Foxp3	PCH101	1:200	rat IgG2a	EBioscience	
GATA3	L50-823	1:300	mIgG1	BD Biosciences	
Keratin (wide spectrum-CKP)	MNF116	1:100	mIgG1	Dako	
HLADR, DP, DQ and DX	V1030	1:250	mIgG1	Biomedica corp.	
Iba1		1:300	rabbit polyclonal	Wako Chemicals	
IC-Mouse IgG1	MG1-45	VD		BioLegend	
IC- Mouse IgG2a	MG2a-53	VD		BioLegend	
IC- Rabbit IgG	DA1E	VD		Cell Signaling	
IC- Mouse IgM	IS5-20C4	VD		Miltenyi Biotech	
IC-Rat IgG2b	MPC-11	VD		BioLegend	
IC-Goat		VD	goat polyclonal	Abcam	
Ki-67	MM1	1:100	mIgG1	Novocastra Laboratories	
Lysozyme		1:1200	rabbit polyclonal	Dako	
Myeloperoxidase		1:6000	rabbit polyclonal	Dako	
Neutrophil Elastase	NP57	1:150	mIgG1	Dako	
S100 protein		1:3000	rabbit polyclonal	Dako	
T-Bet	4B10	1:50	mIgG1	Santa Cruz Biotechnology	
Tie2 (frozen)	TekC1.9	1:100	mIgG1	RELIAtech GmbH	
Tie2 (FFPE)		1:250	goat polyclonal	R&D Systems	
TNFα		1:120	rabbit polyclonal	Abcam	
DETECTION SYSTEM (IF)					
Biotinylated Goat anti-Mouse IgM	NA	1:50		Vector Laboratories	
Streptavidin-FITC	NA	1:75		SouthernBiotech	
Streptavidin Texas Red (TXRD)	NA	1:100		SouthernBiotech	
Goat anti-Mouse IgG1 TXRD	NA	1:75		SouthernBiotech	
Goat anti-Mouse IgG2a TXRD	NA	1:75		SouthernBiotech	
Swine anti-Rabbit FITC	NA	1:75		SouthernBiotech	

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istochemistry; IF= immunofluorescence; IR= irrelevant primary antibody; IC = isotype control antibody;
VD = variable dilution (chosen based on the dilution used for the matched primary mAbs)

Supplementary Table S6. List of the antibodies used for flow cytometry.

Antibody	Clone	Isotype	Dilution	Source
AlexaFluor647 anti-human CX3CR1	2A9-1	rat IgG2b	1:25	BioLegend
AlexaFluor647 rat IgG2b	RTK4530		1:25	BioLegend
APC anti-human CD11c	MJ4-27G12	mIgG2b	1:25	Miltenyi
APC anti-human CD141	AD5-14H12	mIgG1	1:25	Miltenyi
APC anti-human CXCR4	12G5	mIgG2a	1:25	BioLegend
APC mouse IgG2a	MOPC-173		1:25	BioLegend
APC-Cy7 anti-human CD14	MφP9	mIgG2b	1:25	BD Biosciences
APC-Cy7 anti-human HLA-DR	L243	mIgG2a	1:50	BioLegend
Brilliant Violet 510 anti-human CD45	HI30	mIgG1	1:50	BioLegend
FITC anti-human CD11c	MJ4-27G12	mIgG2b	1:25	Miltenyi
FITC anti-human CD303	AC144	mIgG1	1:25	Miltenyi
FITC anti-human HLA-DR	G46-6	mIgG2a	1:10	BD Biosciences
FITC anti-human Slan (M-DC8)	DD1	mIgM	1:25	Miltenyi
PE anti-human C5aR	S5/1	mIgG2a	1:50	BioLegend
PE anti-human CD123	6H6	mIgG1	1:25	BioLegend
PE anti-human CD1c (BDCA-1)	AD5-8E7	mIgG2a	1:25	Miltenyi
PE anti-human CLEC9A	8F9	mIgG2a	1:25	BioLegend
PE anti-human Slan (M-DC8)	DD1	mIgM	1:25	Miltenyi
PE anti-human Tie2	83715	mIgG1	1:10	R&D Systems
PE anti-human Tie2	33.1	mIgG1	1:25	BioLegend
PE mouse IgG1	11711		1:10	R&D Systems
PE mouse IgG1	MOPC-21		1:25	BioLegend
PE-Cy7 anti-human CD19	HIB19	mIgG1	1:20	BioLegend
PE-Cy7 anti-human CD3	UCHT1	mIgG1	1:20	BioLegend
PE-Cy7 anti-human CD45	HI30	mIgG1	1:50	BioLegend
PE-Cy7 anti-human CD56	HCD56	mIgG1	1:25	BioLegend
PerCP-Cy5.5 anti-human CD16	3G8	mIgG1	1:50	BioLegend
Vioblue anti-human CD11c	MJ4-27G12	mIgG2b	1:25	Miltenyi
Vioblue anti-human CD14	TUK4	mIgG2a	1:25	Miltenyi
Vioblue anti-human CD45	5B1	mIgG2a	1:50	Miltenyi