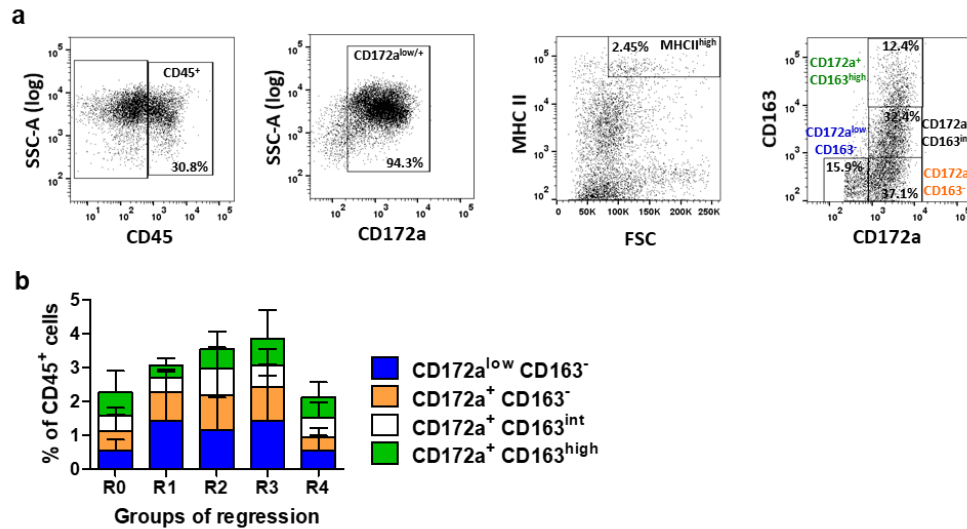
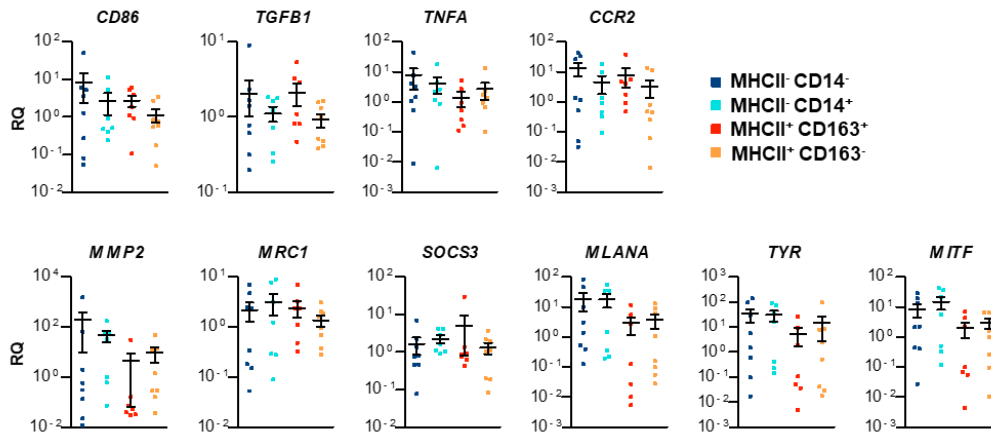




Supplementary Fig. 1 Dendritic cells in melanoma lesions along the regression process. **a** Gating strategy to identify tumor infiltrating DCs. Cells were first gated on the CD45⁺ population. CD172a^{low/+} cells were then selected and DCs were identified as FSC^{high} MHCII^{high} cells within the CD45⁺ CD172a^{low/+} cells. Four subsets were then determined based on their expression of CD172a and CD163. Percentages of the parent populations are shown on each dot plot representing 10000 events. **b** Proportion of the different DC subsets among tumor-infiltrating CD45⁺ cells. In the plots, bars represent the means $\pm$ SEM. (n=16 for R0, n=15 for R1, n=20 for R2, n=17 for R3 and n=14 for R4).



Supplementary Fig. 2 Gene expression analysis from 4 TAMs subsets: MHCII⁻ CD14⁻, MHCII⁻ CD14⁺, MHCII⁺ CD163⁻, and MHCII⁺ CD163⁺. Relative gene expression were calculated by subtracting the Δ Ct(sample) from the Δ Ct(mean) across all samples and the $2^{-\Delta\Delta C_t}$ method.

Supplementay table 1: Pig's individual clinical data and metadata

Tumor burden is defined as low if total number of lesions is comprised between 1 and 3, medium if between 4 and 7 and high if above 8.

Pig	Litter	Sex	CD4 genotype	Number of lesions		tumor burden	Death (day)	Palpable lymphadenopathies		Lesions excised	
				At birth	Total			Observation	Day of appearance	Number	Age of the pig (day)
1331	159	F	Ref/Alt	9	14	high	401	observed	30	8	16, 36, 58, 79, 100, 121, 142, 154
1335	159	F	Ref/Ref	5	8	high	1655	observed	22	5	36, 58, 79, 100, 121
1336	159	F	Ref/Alt	4	7	medium	346	observed	22	6	16, 36, 58, 79, 121, 154
1341	160	M	Ref/Ref	2	3	low	471	observed	14	2	34, 56
1343	160	F	Ref/Alt	4	6	medium	1064	observed	84	2	14, 56
1362	162	F	Ref/Alt	1	12	high	1014	observed	32	4	40, 61, 82, 94
1364	163	M	Ref/Alt	6	11	high	739	observed	26	5	39, 60, 72, 107, 130
1367	163	F	Ref/Alt	2	7	medium	992	observed	38	4	39, 60, 72, 107
1376	164	M	Ref/Ref	1	9	high	301	observed	42	1	42
1377	164	F	Ref/Alt	6	9	high	645	observed	36	4	21, 41, 54, 89
1378	164	F	Ref/Ref	1	10	high	378	observed	42	2	21, 54
1387	165	M	Ref/Ref	4	4	low	415	non-observed		3	29, 49, 69
1396	166	M	Ref/Alt	3	4	low	301	observed	20	2	28, 68
1397	166	F	Ref/Alt	2	9	high	1471	observed	20	1	48
1399	166	M	Ref/Ref	2	4	low	68	observed	27	1	28
1467	173	F	Ref/Alt	4	6	medium	319	observed	36	1	79
1470	174	M	Ref/Ref	2	5	medium	634	observed	33	3	19, 54, 89
1472	174	F	Ref/Ref	4	7	medium	832	observed	33	3	19, 54, 89
1473	174	F	Ref/Ref	2	5	medium	622	observed	56	3	19, 54, 89
1474	174	F	Ref/Ref	5	9	high	712	observed	26	3	19, 54, 89
1475	174	F	Ref/Ref	5	9	high	832	observed	33	3	19, 54, 89
1479	175	M	Ref/Alt	3	9	high	1757	observed	13	3	54, 82, 105
1480	175	M	Ref/Alt	1	4	low	322	observed	48	1	105
1482	175	M	Ref/Alt	1	8	high	119	observed	27	1	82
1483	175	F	Ref/Alt	2	3	low	322	observed	13	2	54, 105
1489	176	F	Ref/Ref	2	3	low	290	observed	45	1	51
1490	176	F	Ref/Alt	1	11	high	890	observed	31	2	51, 73
1506	178	M	Ref/Ref	4	9	high	1278	observed	105	3	28, 69, 69
1510	178	F	Ref/Ref	2	7	medium	1029	observed	105	2	69, 69
1512	178	F	Ref/Ref	6	10	high	797	observed	105	1	28
1516	179	F	Alt/Alt	8	14	high	379	observed	44	6	48, 48, 48, 48, 48, 55
1517	179	M	Alt/Alt	1	3	low	134	observed	44	1	55
1518	179	M	Ref/Alt	2	4	low	134	observed	44	1	48
1519	179	M	Ref/Alt	1	6	medium	1400	observed	44	2	48, 55

Supplementary table 2: Clinical and histological phenotypes for each tumoral
ND: not determined

Clinical observations of the lesions															Histological observations of the tumoral zone										Histological observations of the regressive zone			
Pig_Lesion	Pig	Lesion	Sex	Age of the pig at excision (day)	Presence of a zone of dermal fibrosis	Stage of regression	Lesion size (cm) (Clin_Size)	Lesion size's evolution (Clin_Size_Evolution)	Description (Clin_Description)	Ulceration (Clin_Ulceration)	Grayish lesion (Clin_Grayish)	Palpable adenomegalies (Clin_Adenomegalies)	Profile (Histo_Profile)	Length (cm)	Depth (cm)	Area (mm²)	Clark's level (Histo_Clark)	Presence of Grenz Zone (Histo_Grenz_Zone)	Percentage of connective tissue (Histo_Connective_Tissue)	Ulceration (Histo_Ulceration)	Vascularization (Histo_T_Vascularization)	Clusters of melanophages (Histo_Clustered_melanophages)	Lymphoid infiltration (Histo_T_Lymphoid_infiltration)	Extension (Histo_R_Extension)	Neangiogenesis (Histo_R_Neangiogenesis)	Lymphoid infiltration (Histo_R_Lymphoid_infiltration)		
1331_L3	1331	L3	F	16	no	R0	2.5	increasing	dome	yes (++)	no	no	dome or plateau	0.74	1.08	50.9	V	no	6.6	yes	high	no	yes (perilesional)	-	-	-		
1336_L2	1336	L2	F	14	no	R0	2.7	increasing	plateau	no	no	yes	dome or plateau	1.66	0.49	55.9	V	no	7.1	yes	high	no	yes (perilesional)	-	-	-		
1343_L1	1343	L1	F	14	no	R0	3.0	increasing	dome	yes (++)	no	no	polypoid	1.93	0.71	124.4	V	no	3.9	yes	high	no	no	-	-	-		
1376_L1	1376	L1	M	42	no	R0	2.5	increasing	plateau	yes (+)	no	yes	dome or plateau	2.45	0.97	191.1	V	no	8.5	yes	high	no	no	-	-	-		
1377_L3	1377	L3	F	21	no	R0	1.5	increasing	dome	no	no	no	ND	0.56	0.28	10.7	ND	no	10.3	no	high	no	no	-	-	-		
1378_L1	1378	L1	F	21	no	R0	2.3	increasing	plateau	no	no	no	dome or plateau	1.86	0.94	128.3	V	no	2.8	yes	high	no	no	-	-	-		
1387_L2	1387	L2	M	29	no	R0	2.0	increasing	plateau	no	no	no	dome or plateau	1.66	0.52	66.4	V	no	7.3	yes	high	no	no	-	-	-		
1396_L2	1396	L2	M	28	no	R0	2.5	stable	plateau	yes (+)	no	yes	dome or plateau	2.10	0.61	92.0	V	no	10.2	yes	high	no	no	-	-	-		
1399_L1	1399	L1	M	28	no	R0	3.3	increasing	plateau	yes (+)	no	yes	dome or plateau	2.36	0.69	130.8	V	no	5.2	yes	high	no	no	-	-	-		
1472_L2	1472	L2	F	19	no	R0	3.9	increasing	dome	yes (++)	no	no	polypoid	3.77	1.25	344.0	V	no	4.0	yes	high	no	no	-	-	-		
1474_L2	1474	L2	F	19	no	R0	2.5	increasing	dome	yes (++)	no	no	polypoid	2.82	1.17	184.9	V	no	5.9	yes	high	no	no	-	-	-		
1475_L2	1475	L2	F	19	no	R0	3.0	increasing	dome	yes (+)	no	no	polypoid	2.23	0.71	155.3	IV	no	11.6	yes	high	no	no	-	-	-		
1519_L3	1519	L3	M	48	no	R0	4.7	increasing	dome	yes (++)	no	yes	dome or plateau	4.12	1.55	496.3	V	no	2.8	yes	high	no	yes (intralesional)	-	-	-		
1331_L8	1331	L8	F	36	yes	R1	2.9	stable	dome	yes (+)	no	yes	dome or plateau	1.90	1.01	119.3	IV	no	8.6	yes	high	yes	yes (perilesional)	dermal	yes	no		
1335_L2	1335	L2	F	36	yes	R1	2.5	stable	dome	yes (+)	no	yes	polypoid	ND	ND	ND	V	no	4.0	yes	high	yes	no	dermal	no	no		
1336_L4	1336	L4	F	36	yes	R1	2.8	increasing	dome	yes (+)	no	yes	polypoid	1.09	1.10	101.5	V	no	21.7	yes	high	yes	yes (intralesional)	dermal	yes	yes		
1341_L3	1341	L3	M	34	yes	R1	2.5	increasing	dome	no	no	yes	dome or plateau	0.78	1.02	77.3	ND	no	2.1	yes	high	yes	no	dermal	no	no		
1362_L4	1362	L4	F	40	yes	R1	2.3	increasing	plateau	yes	no	yes	dome or plateau	1.55	0.52	65.5	V	no	17.5	yes	high	yes	no	dermal	yes	no		
1364_L1	1364	L1	M	39	yes	R1	5.1	increasing	dome	yes (+)	no	yes	polypoid	3.33	1.65	303.3	V	no	20.0	yes	high	yes	yes (intralesional)	dermal	yes	no		
1367_L2	1367	L2	F	39	yes	R1	2.7	increasing	dome	yes (+)	no	yes	dome or plateau	2.36	1.16	211.6	V	no	22.9	yes	high	yes	no	dermal	yes	no		
1377_L6	1377	L6	F	42	yes	R1	2.3	stable	plateau	yes (++)	no	yes	polypoid	1.52	0.68	74.5	IV	no	24.1	yes	high	yes	yes (perilesional)	dermal	yes	no		
1387_L1	1387	L1	M	49	yes	R1	2.0	stable	plateau	no	yes	no	dome or plateau	0.79	0.48	25.7	V	no	29.5	no	high	yes	no	dermal	no	no		
1397_L2	1397	L2	F	48	yes	R1	3.2	stable	dome	yes (+)	no	yes	dome or plateau	1.26	0.91	89.2	V	no	21.1	yes	high	yes	no	dermal	yes	no		
1470_L1	1470	L1	M	19	yes	R1	2.8	increasing	dome	yes (++)	no	no	dome or plateau	2.13	0.46	78.2	IV	no	7.0	yes	high	yes	no	dermal	no	no		
1473_L1	1473	L1	F	19	yes	R1	3.0	increasing	exophytic	yes (+)	no	no	polypoid	2.48	1.43	217.3	V	no	28.0	yes	high	yes	no	dermal	no	no		
1506_L3	1506	L3	M	28	yes	R1	3.5	increasing	exophytic	yes (+)	no	yes	polypoid	2.26	0.96	176.8	V	no	12.0	yes	high	yes	no	dermal	yes	no		
1512_L1	1512	L1	F	28	yes	R1	2.9	increasing	exophytic	yes (+)	no	yes	polypoid	2.04	0.77	132.0	V	no	15.5	yes	high	yes	yes (perilesional)	dermal	yes	no		
1516_L1	1516	L1	F	48	yes	R1	3.6	stable	dome	no	no	yes	polypoid	2.67	1.17	234.2	V	no	10.4	yes	high	yes	no	dermal	yes	no		
1516_L11	1516	L11	F	48	yes	R1	2.1	increasing	plateau	yes (+)	no	yes	dome or plateau	1.39	0.74	74.6	V	no	12.1	yes	high	yes	no	ND	no	no		
1516_L2	1516	L2	F	48	yes	R1	3.3	increasing	dome	no	no	yes	polypoid	2.20	1.05	178.9	V	no	22.4	yes	high	yes	no	dermal	yes	yes		
1516_L7	1516	L7	F	48	yes	R1	2.9	increasing	dome	yes (++)	no	yes	polypoid	1.70	1.04	139.6	V	no	13.1	yes	high	yes	yes (perilesional)	dermal	yes	no		
1516_L9	1516	L9	F	48	yes	R1	3.4	stable	plateau	no	no	yes	polypoid	2.59	0.84	150.7	V	no	15.7	yes	high	yes	no	dermal	yes	yes		
1518_L1	1518	L1	M	48	yes	R1	4.9	stable	dome	no	no	yes	polypoid	3.60	1.56	384.8	V	no	26.4	yes	high	yes	yes (intralesional)	dermal	yes	yes		
1331_L9	1331	L9	F	58	yes	R2	2.7	increasing	exophytic	yes (+)	no	yes	dome or plateau	1.59	0.74	86.5	IV	no	14.2	yes	high	yes	yes (perilesional)	dermal	yes	no		
1335_L1	1335	L1	F	58	yes	R2	2.7	increasing	dome	yes (++)	no	yes	dome or plateau	1.37	0.30	38.3	ND	no	12.4	yes	high	yes	no	dermal	yes	no		
1336_L5	1336	L5	F	58	yes	R2	3.2	increasing	plateau	no	yes	yes	dome or plateau	2.08	0.57	106.8	IV	no	23.2	yes	high	yes	yes (intralesional)	dermal	yes	yes		
1341_L1	1341	L1	M	56	yes	R2	2.5	increasing	plateau	no	yes	yes	polypoid	1.77	1.31	169.9	V	no	14.5	no	high	yes	no	dermal	yes	no		
1343_L4	1343	L4	F	56	yes	R2	1.4	stable	dome	no	no	no	polypoid	0.97	0.34	31.7	IV	no	9.6	yes	high	yes	no	dermal	yes	no		
1362_L1	1362	L1	F	61	yes	R2	3.3	increasing	plateau	no	no	yes	dome or plateau	1.98	0.89	146.0	V	no	14.9	yes	low	yes	no	dermal	yes	yes		
1364_L3	1364	L3	M	60	yes	R2	3.6	stable	dome	no	yes	yes	polypoid	2.19	1.72	241.5	V	no	25.2	yes	high	yes	yes (intralesional)	dermal and epidermal	yes	yes		
1367_L4	1367	L4	F	60	yes	R2	2.0	stable	dome	no	no	yes	polypoid	1.82	1.22	166.3	V	no	21.5	yes	high	yes	no	dermal and epidermal	yes	no		
1377_L5	1377	L5	F	54	yes	R2	2.2	stable	plateau	no	yes	yes	dome or plateau	2.05	0.93	149.0	IV	no	23.1	no	high	yes	yes (perilesional)	dermal	yes	yes		
1378_L3	1378	L3	F	54	yes	R2	3.0	increasing	plateau	yes (+)	no	yes	dome or plateau	3.81	0.98	255.8	V	no	18.0	yes	high	yes	no	dermal	yes	no		
1387_L3	1387	L3	M	69	yes	R2	2.0	stable	plateau	no	yes	no	dome or plateau	1.49	0.94	97.1	IV	no	9.5	no	high	yes	yes (intralesional)	dermal	yes	no		
1396_L4	1396	L4	M	68	yes	R2	2.0	stable	plateau	no	no	yes	dome or plateau	1.21	0.27	22.5	V	no	19.3	no	high	yes	no	dermal	yes	no		
1470_L3	1470	L3	M	54	yes	R2	2.1	stable	plateau	no	no	yes	dome or plateau	0.95	0.45	28.7	IV	no	21.2	no	high	yes	no	dermal	yes	no		
1472_L1	1472	L1	F	54	yes	R2	3.9	stable	dome	yes (+)	no	yes	dome or plateau	1.98	1.64	253.9	V	yes	9.2	yes	high	yes	yes (intralesional)	dermal	yes	no		
1473_L2	1473	L2	F	54	yes	R2	2.7	stable	plateau	no	yes	no	dome or plateau	1.27	0.78	86.3	V	no	25.4	no	high	yes	yes (intralesional)	dermal and epidermal	yes	yes		
1474_L3	1474	L3	F	54	yes	R2	2.2	stable	plateau	no	yes	yes	dome or plateau	1.51	0.52	56.0	IV	no	55.0	no	high	yes	yes (intralesional)	dermal and epidermal	yes			

Supplementary table 3: Cytokines assessed in tumoral lesions (expressed in pg/mg of tumor).

ND: not determined

Pig_Lesion	Stage of regression	IL-1 β	IL-2	IL-4	IL-6	IL-8	IL-10	IL-12	IFN γ	TNF α	CCL2	TGF β	IL-17	IFN α
1331_L3	R0	11426	28972	980	8935	1030	6443	6157	9202	17150	2216	742	108.6	606.0
1331_L4	R3	2141	4407	132	547	263	2409	1102	3953	4541	ND	ND	0.0	32.5
1331_L6	R4	7123	15450	632	3242	662	4046	3496	6613	11240	2256	644	63.9	316.8
1331_L9	R2	20077	6998	219	963	1962	2960	957	4595	5242	4178	692	26.6	49.3
1335_L1	R2	15008	4464	169	702	1504	1420	712	4144	2531	5940	1126	111.1	28.3
1335_L2	R1	30427	4823	236	586	1590	773	904	4146	2910	4780	870	134.0	75.4
1335_L3	R4	3891	8245	334	1401	379	1880	1953	5283	5098	1938	472	9.6	125.4
1335_L5	R3	4867	11452	408	2221	412	2751	2555	6058	4323	2446	746	31.5	183.0
1336_L1	R4	1287	1569	56	121	124	606	527	2900	664	334.4	212	0.0	0.0
1336_L2	R0	19079	6147	169	749	3254	2636	719	4298	4120	3370	644	52.8	30.9
1336_L3	R3	7584	12841	504	2622	503	3903	3123	6398	7807	1670	606	39.4	217.5
1336_L5	R2	6407	8187	307	1305	506	2320	1262	5060	5059	3588	792	32.6	88.0
1343_L1	R0	6279	15452	551	2943	615	3170	3472	6687	8610	ND	ND	54.5	271.1
1343_L4	R2	7324	15205	501	3311	673	3476	3398	6887	10015	2654	774	58.2	242.7
1362_L3	R3	10667	22257	835	5112	837	4957	4668	7560	12566	1740	612	63.4	444.5
1362_L4	R1	10450	23934	814	7007	907	5340	4821	8921	15285	2806	812	70.1	458.0
1362_L5	R3	11454	16155	659	3311	676	3856	3072	6977	11152	1778	520	52.4	244.2
1364_L1	R1	10314	23289	813	6377	828	5078	6318	8721	17105	2302	1276	82.3	416.8
1364_L4	R3	14694	31681	1024	8560	935	6805	6395	9577	19226	2050	576	85.5	567.3
1367_L2	R1	8996	20331	707	5677	752	5451	4823	8117	14340	2828	670	62.4	391.5
1367_L4	R2	5598	11079	418	2105	497	3156	2032	5935	7607	2854	528	34.7	153.4
1367_L5	R4	2443	3603	136	383	188	597	726	3587	1588	450	574	0.0	21.5
1367_L6	R3	6445	8487	320	1639	635	2963	2764	5401	9063	4480	576	28.5	131.5
1377_L3	R0	7221	20522	602	7291	954	5570	5538	9286	12508	2446	718	46.1	363.4
1377_L4	R3	14381	9755	302	1813	1123	3429	1771	5618	6973	6760	640	120.8	112.4
1377_L5	R2	9862	20589	656	3864	643	5052	4836	7455	10500	2942	640	53.1	367.7
1378_L3	R2	8280	16337	569	4702	679	4589	3719	7758	10753	4110	542	59.7	294.3
1387_L1	R1	8265	19338	688	5854	728	4142	4258	8324	11374	2536	882	53.3	380.7
1387_L2	R0	10728	21275	664	5260	1449	4525	4397	7842	12318	1960	912	75.4	387.0
1397_L2	R1	4859	4397	141	556	846	1652	726	3703	3248	4660	1038	20.3	32.1
1470_L2	R3	1043	1938	87	105	89	576	472	2918	1024	ND	ND	0.0	0.0
1472_L1	R2	4217	2630	81	427	226	1930	706	3702	2521	670	702	0.0	17.6
1472_L2	R0	8585	18651	682	4956	839	4271	4336	7752	11670	ND	ND	90.0	357.5
1472_L5	R3	4591	12195	397	2974	659	3519	4332	6548	9966	2800	640	27.1	176.1
1474_L2	R0	8253	18338	664	5343	990	4301	4291	8078	11531	2052	702	86.2	86.2
1475_L2	R0	7057	14724	534	2590	581	3280	3563	6544	8060	ND	ND	48.1	48.1

Supplementary table 4: Antibodies used in this study.

A, B and C correspond to the three antibody combinations to identify cell subsets: A for lymphocyte subsets, B for B lymphocytes and C for myeloid cells. Combination D was used to analyze NK cell functionality. (* intracellular stainings)

Specificities	Clones / references	Isotypes	Target species	Fluorochrome	Labelling strategy	Providers	Working dilutions or concentrations	Used in combinations
CD45	K252- 1E4	m IgG1	pig	AF647	Directly conjugated	AbD Serotec	1/20	A, B, C, D
CD3	PPT3	m IgG1	pig	FITC	Directly conjugated	SouthernBiotech	2.5 µg/mL	A
CD8α	76-2-11	m IgG2a	pig	PE-Cy5	Directly conjugated	Abcam	1 µg/mL	A, D
CD4	74-12-4	m IgG2b	pig	APC-Cy7	Secondary antibody ^a	Abcam	2.5 µg/mL	A
gdTCR	PGBL22A	m IgG1	pig	PE-Cy7	Secondary antibody ^b	WSU	2 µg/mL	A
CD16	G7	m IgG1	pig	PE	Directly conjugated	AbD Serotec	1/20	A
MHCII	MSA3	m IgG2a	pig	AF488	Secondary antibody ^c	WSU	2 µg/mL	B
CD21	B-Ly4	m IgG1	human	PE-Cy7	Secondary antibody ^b	BD Biosciences	4 µg/mL	B
CD79a*	HM57	m IgG1	human	PE	Directly conjugated	AbD Serotec	50 µg/mL	B
control	MOPC-21	m IgG1		PE	Directly conjugated	Abcam	1/20	B
PG68A	PG68A	m IgG1	pig	PE-Cy7	Secondary antibody ^b	WSU	5 µg/mL	C
CD163	2A10/11	m IgG1	pig	PE	Directly conjugated	AbD Serotec	1/10	C
CD172a	74-22-15A	m IgG2b	pig	APC-Cy7	Secondary antibody ^a	WSU	1 µg/mL	C
CD14	TUK4	m IgG2a	human	Pacific Blue	Directly conjugated	AbD Serotec	1/10	C
CADM1	3E1	c IgY	human/ mouse	Qdot655	Secondary antibody ^d	MBL	4 µg/mL	C
CD3	8E6-8C8	m IgG2a	pig	PerCp-eFluor710	Secondary antibody ^e	WSU	5 µg/mL	D
CD16	G7	m IgG1	pig	PE-Cy7	Secondary antibody ^b	AbD Serotec	2.5 µg/mL	D
IFNγ*	P2G10	m IgG1	pig	PE	Directly conjugated	BD Biosciences	0.4 µg/mL	D
control	MOPC-21	m IgG1		PE	Directly conjugated	Abcam	1/500	D

^a Goat anti-mouse IgG2b-APC-Cy7, 1.25 µg/mL, Abcam

^b Goat anti-mouse IgG1-PE-Cy7, 0.5 µg/mL, eBioscience

^c Goat anti-mouse IgG2a-AF488, 5 µg/mL, Invitrogen

^d Goat anti-chicken IgY-Qdot655, 2.5nM, Exbio

^e Goat anti-mouse IgG2a-PerCp-eFluor710, 0.5 µg/mL, eBioscience

Supplementary table 6: List of primers used for qRT-PCR.

gene	name	TaqMan assayID or SYBRGreen sequence primers	T°	efficiency (%)	product length
<i>IL10</i>	Interleukin 10	F: GAGCCAACTGCAGCTTCCA R: TCAGGACAAATAGCCCACTAGCTT	62	95	65
<i>IL1B</i>	Interleukin 1 Beta	F: TGCCAACGTGCAGTCTATGG R: TGGGCCAGCCAGCACTAG	60	99	70
<i>CXCL8</i>	C-X-C Motif Chemokine Ligand 8	F: CCGTGTCAACATGACTTCCAA R: GAGCTGCAGAAAGCAGGAAAA	60	101	65
<i>MITF-M</i>	M isoform of Melanocyte Inducing Transcription Factor	F: GCGTGGTTATGCTGGAAATGC R: TGGTTTACCTGCTGCCTTTGG	60	113	109
<i>MLANA</i>	Melan-A	F: CAACTGTGGACCTGTGGTTCC R: CAGAAGACCTGCTGGCTCTCAT	60	118	103
<i>TGFB1</i>	Transforming Growth Factor Beta 1	F: AGGGCTACCATGCCAATTT R: CGGGTTGTGCTGGTTGTAC	58	95	101
<i>TNFA</i>	Tumor Necrosis Factor	F: TGGTGGTGCCGACAGATG R: CAGCCTTGGCCCTGAA	60	109	64
<i>TYR</i>	Tyrosinase	F: GCCTTGGCATCGACTCTTCTT R: CACAATTTCTGCATCTCGCC	58	88	110
<i>VEGFA</i>	Vascular Endothelial Growth Factor A	F: CAACGACGAAGGTCTGGAGTGT R: TCATCTCTCTATGTGCTGGCC	60	123	101
<i>HPRT1</i>	Hypoxanthine Phosphoribosyltransferase 1	F: GGGACTTGAATCATGTTTGTGTCA R: ATGGGACTCCAGATGTTTCCAA	60	107	101
<i>RPL32</i>	Ribosomal Protein L32	F: TGCTCTCAGACCCCTTGTGAAG R: TTTCCGCCAGTTCGCTTA	58	105	106
<i>ARG1</i>	Arginase 1	Ss03391394_m1	60		
<i>CCL2</i>	C-C Motif Chemokine Ligand 2	Ss03394377_m1	60		
<i>CCL5</i>	C-C Motif Chemokine Ligand 5	Ss03648939_m1	60		
<i>CCR2</i>	C-C Motif Chemokine Receptor 2	Ss03378154_u1	60		
<i>CD80</i>	CD80 Molecule	Ss03382507_u1	60		
<i>CD86</i>	CD86 Molecule	Ss03394401_m1	60		
<i>CSF1</i>	Macrophage Colony Stimulating Factor 1	Ss03373560_g1	60		
<i>FCGR2B</i>	Fc Fragment Of IgG Receptor Iib	Ss03392060_m1	60		
<i>AIF1</i>	Allograft Inflammatory Factor 1	Ss03373370_m1	60		
<i>IDO1</i>	Indoleamine 2,3-Dioxygenase 1	Ss04322730_m1	60		
<i>IL4I1</i>	Interleukin 4 Induced 1	Ss06927539_m1	60		
<i>IL6</i>	Interleukin 6	Ss03384604_u1	60		
<i>LYZ</i>	Lysozyme	Ss03394856_m1	60		
<i>MMP2</i>	Matrix Metallopeptidase 2	Ss03394318_m1	60		
<i>MMP9</i>	Matrix Metallopeptidase 9	Ss03392100_m1	60		
<i>MRC1</i>	Mannose Receptor C-Type 1	Ss03373693_m1	60		
<i>MMP14</i>	Matrix Metallopeptidase 14	Ss03394425_g1	60		
<i>NOS2</i>	Nitric Oxide Synthase 2	Ss03374608_u1	60		
<i>CD274</i>	CD274 Molecule	Ss03391948_m1	60		
<i>S100A8</i>	S100 Calcium Binding Protein A8	Ss04246257_g1	60		
<i>SOCS3</i>	Suppressor Of Cytokine Signaling 3	Ss03387992_u1	60		
<i>HPRT1</i>	Hypoxanthine Phosphoribosyltransferase 1	Ss03388274_m1	60		
<i>RPL32</i>	Ribosomal Protein L32	Ss03391553_g1	60		
<i>MPEG1</i>	Macrophage Expressed 1	Ss04954218_s1	60		

Supplementary table 7: Statistical description of groups by cytokines performed using v test after PCA analysis.

	R0		R1		R4	
	v-test	<i>p</i> -value	v-test	<i>p</i> -value	v-test	<i>p</i> -value
IL-8	2.048	0.041			-2.542	0.011
TGFβ			2.247	0.025	-2.628	0.009
IL-6					-2.013	0.044
TNFα					-2.359	0.018
IL-2					-1.986	0.047
IL-1β					-2.594	0.010
IL-10					-2.628	0.009
CCL2					-2.733	0.006

Supplementary table 8: Effect of regression stages on the different proportions of cell populations among immune cells in the tumors. A linear mixed model with sex (two levels) and regression stages (5 levels) as fixed effects was used and pairwise and when a significant effect of regression stages was found, comparisons with Tukey's adjustment were calculated within regression stages. Only significant F and *p*-values are reported in the Table.

Cell subset		effect of regression stage		significant <i>p</i> -values of the post hoc test comparing regression stages									
		F value	<i>p</i> -value	R0 - R1	R0 - R2	R0 - R3	R0 - R4	R1 - R2	R1 - R3	R1 - R4	R2 - R3	R2 - R4	R3 - R4
CD45 ⁺ cells	count/g	3.08	2.06E-02						0.023				
CD3 ⁺ T cells	% of CD45 ⁺	21.29	9.21E-12				<0.001		<0.001		<0.001	<0.001	
γδ T cells	% of CD45 ⁺	10.18	1.62E-06				<0.001		<0.001		<0.001	<0.001	
CD4 ⁺ CD8 ⁺ T cells	% of CD45 ⁺	9.68	2.95E-06				<0.001		<0.001		<0.001	0.009	
CD4 ⁺ CD8 ⁺ T cells	% of CD45 ⁺	9.06	6.33E-06				<0.001		<0.001		<0.001	<0.001	
CD4 ⁺ CD8 ⁺ T cells	% of CD45 ⁺	17.64	5.67E-10				<0.001		<0.001		<0.001	<0.001	
CD4 ⁺ CD8 ⁺ T cells	% of CD45 ⁺	7.23	6.53E-05				<0.001		<0.001		0.003	0.039	
NKT cells	% of CD45 ⁺	6.48	1.77E-04				<0.001		0.003		0.001	0.008	
NK cells	% of CD45 ⁺	14.09	1.19E-08		0.0358	<0.001	<0.001		0.002	<0.001	0.001		
B cells	% of CD45 ⁺	3.68	8.99E-03						0.021		0.043		
PMN	% of CD45 ⁺	5.29	8.25E-04			0.034	0.003		0.007				
macrophages	% of CD45 ⁺	3.94	5.89E-03				0.005		0.020		0.047	0.047	
MHCII ⁺ CD163 ⁺ macrophages	% of CD45 ⁺	5.13	1.05E-03						0.004	0.005			
	% of macrophages	8.59	9.20E-06			0.050	0.0013		0.002	<0.001	0.043		
MHCII ⁺ CD163 ⁺ macrophages	% of CD45 ⁺	16.90	6.43E-10		<0.001	<0.001	<0.001		<0.001	<0.001			
	% of macrophages	22.24	3.89E-12		<0.001	<0.001	<0.001		<0.001	<0.001	0.042	<0.001	
MHCII ⁺ CD14 ⁺ macrophages	% of CD45 ⁺	6.03	2.90E-04				0.0028		0.041	0.001			
	% of macrophages	5.25	8.74E-04				0.015		0.021	0.004			
MHCII ⁺ CD14 ⁺ macrophages	% of CD45 ⁺	8.76	7.35E-06			0.005	<0.001		0.045	<0.001	0.008		
	% of macrophages	8.95	5.78E-06			0.004	<0.001		0.012	<0.001	0.007		

Supplementary table 9: Statistical description of macrophage subsets by gene expression performed using v test after PCA analysis.

	MHCII ⁻ CD14 ⁺		MHCII ⁺ CD163 ⁻		MHCII ⁺ CD163 ⁺	
	v.test	p.value	v.test	p.value	v.test	p.value
<i>IL10</i>			-3.636	0.0003		
<i>CSF1</i>	2.326	0.020			-2.509	0.012
<i>VEGFA</i>			-3.369	0.001	2.282	0.023
<i>IL8</i>			-3.572	0.0004		
<i>IL1B</i>			-3.435	0.001		
<i>LYZ</i>			-3.300	0.001		
<i>ARG1</i>			-3.330	0.001		
<i>CCL2</i>	2.214	0.027	-2.721	0.007		
<i>MPEG1</i>					3.062	0.002
<i>S100A8</i>			-3.045	0.002		
<i>FCGR2B</i>					2.675	0.007
<i>IL4I1</i>			2.727	0.006		
<i>MMP14</i>	1.991	0.047	-1.968	0.049		
<i>CCL5</i>			-2.773	0.006		
<i>CD80</i>			-2.669	0.008		
<i>CD274</i>			-2.227	0.026		
<i>AIF1</i>					1.970	0.049
<i>MMP9</i>					-1.991	0.046
<i>IL6</i>					-2.167	0.030
<i>NOS2</i>					-2.398	0.016