

Appendix

Supplemental Table 1

Gene	NCBI	Upper (5' - 3')	Lower (5' - 3')	Position	Amplicon	Annealing Temp (°C)
ARG1	NM_007482	AAC ACG GCA GTG GCT TTA ACC	GGT TTT CAT CTG GCG CAT TC	958-1075	118	59
CCL2	NM_011333	GCA TCA CAG TCC GAG TCA C	TTC CACAAC CAC CTC AAG C	478-552	75	58
CCL5	NM_013653	CAC TCC CTG CTG CTT TGC	CAC TTG GCG GTT CCT TCG	128-254	127	62
COX2	NM_011198	ACC AGC AGT TCC AGT ATC AG	AGG AGG ATG GAG TTG TTG TAG	1160-1302	143	62
CXCL12	NM_001012477	AAG ATG TGA GAG GTG TGA GTC	GCA GAG GAA GTG GCT ATG G	2864-2961	98	65
ESRA	NM_007956	ATT GAC CAA CCT AGC AGA TAG	CCA GAC GAG ACC AAT CAT C	2996-3158	163	64
ESRB	NM_207707	TGC TGG AGA TGCTGA ATG	CTG TGA CTG GAG GTT CTG	1928-2049	122	58
Flt3L	NM_013520	GCT GTT GCT GCT GCT GAG	CTT TGA AGT TGG AGG AGA TGG G	303-385	83	63
FOXP3	NM_054039	CCC AGG AAA GAC AGC AAC CTT	TTC TCA CAA CCA GGC CAC TTG	783-872	90	62
GAPDH	NM_008084	CCA CTC ACG GCA AAT TCA AC	GTA GAC TCC ACG ACA TAC TCA G	195-340	146	**
GCSF	NM_009971	GCT GCT GCT GTG GCA AAG T	AGC CTG ACA GTG ACC AGG	114-181	68	65
GMCSF	NM_009969	TGG GCA TTG TGG TCT ACA GC	AAG GCC GGG TGA CAG TGA T	315-377	63	68
IDO	NM_008324	GCC CTC AAA TGT GGA AAG C	CTG TGC CCT GAT AGA AGT GG	1192-1325	134	65
IFNg	NM_008337	TCA AGT GGC ATA GAT GTG GAA GAA	TGG CTC TGC AGG ATT TTC ATG	224-315	92	62
IL1-B	NM_008361	GCA GCA GCA CAT CAA CAA G	CCG GAA AGA CAC AGG TAG C	224-311	88	65
IL2	NM_008366	ACT GAC ACT TGT GCT CCT TG	GCT CCT GTA GGT CCA TCA AC	125-219	95	62
IL4	NM_021283	CGA GGT CAC AGG AGA AGG	TTG GAA GCC CTA CAG ACG	200-305	106	62
IL10	NM_010548	GCA CCC ACT TCC CAG TCG	GGA GTC GGT TAG CAG TAT GTT G	164-282	119	62
IL12	NM_008352	ACA TCA AGA GCA GTA GCA GTT C	AGT TGG GCA GGT GAC ATC C	617-759	143	62
IL13	NM_008355	GCT TGC CTT GGT GGT CTC	GTC TGG TCT TGT GTG ATG TTG	101-211	111	62
IL18	NM_008360	CTC TGT GGT TCC ATG CTT TCT G	TTT GAG GCG GCT TTC TTT GTC	26-131	106	62
IL24	NM_053095	GCC TGA GCC TAA TCC TTC TTC	ACA GTG TTC TTC ACA GTC CAG	284-419	136	62
NOS2	NM_010927	AGC CAA GCC CTC ACC TAC	CAA TCT CCT ATC CGT CTC	2639-2743	105	62
TCRzeta	NM_009539	CGC CTC CTT TTC TCC TCA TC	TCC ACT TCA TCT TCT GCT TCC	847-934	88	62
TGFb2	NM_009367	AAT GTG CAG GAT AAT TGC TGC	AAC TCC ATA GAT ATG GGG ATG C	2151-2284	134	58
VEGA	NM_009595	TTA CTG CTG TAC CTC CAC C	ACA GGA CGG CTT GAA GAT G	434-622	189	58
VEGC	NM_009506	CTA CAG ATG TGG GGG TTG CT	GCT GCC TGA CAC TGT GGT AA	618-857	240	65
SCF	NM_013598	CGC CTG CCG AAA TGT ATG ACG	GGT TCT CTG GGT TGG GGT TGC	2706-2867	162	64

Supplemental Table 1 provides the murine primer sequences used in the qRT-PCR. Genes are as identified within the text. The NCBI accession number is provided along with the 5 and 3 prime sequence. The amplicon size is listed as well as the annealing temperature. The housekeeping gene, GAPDH, was used independent of the temperature. The PCR used an initial melting temperature of 95°C for 15 seconds followed by the annealing temperature for 30 seconds and for 30 seconds at 72°. Elongation for forty cycles was used for each primer sequence.

Supplemental Table 2

	Product	Clone	Fluorescence	Company
CD3	anti-mouse CD3e	145-2C11	Phycoerythrin (PE)	eBioscience
CD4	anti-mouse CD4 (L3T4)	GK1.5	Biotin/APC*	eBioscience
CD8	CD8a (Ly-2)	53-6.7	PE, BD Cychrome	BDpharmingen
CD11b	anti-mouse CD11b (integrin alpha M, Mac-1 alpha	M1/70	Biotin/APC*	eBioscience
CD11c	anti-mouse CD11c (integrin aX, p150/90)	N418	FITC	eBioscience
CD25	CD25 (IL-2Ralpha chain, p55)	PC61	FITC	BDpharmingen
CD62L	CD62L (L-selectin, LECAM-1, Ly-22)	MEL-14	BD Cychrome	BDpharmingen
CD83	anti-mouse CD83	Michel-17	PE	eBioscience
NK	CD49b/Pan-NK cells	DX5	FITC	BDpharmingen
GR1	Ly-6G and Ly-6C (Gr-1)	RB6-8C5	FITC	BDpharmingen
B220	CD45R/B220	RA3-6B2	BD Cychrome	BDpharmingen

Supplemental Table 2 lists the antibodies, clone name, fluorescent label and supplier used in our flow analysis. In addition, allo phycocyanin conjugate (APC)-streptavidin was used with the Biotin labeled antibodies and was purchased from Invitrogen.

Supplemental Table 3

a. Absolute numbers (x10⁶ cells)

Cell type	Spleen cells		Ratio
	Wild type mice	Tumor bearing	
Spleen cells	65.0 +/- 4.6	169.6 +/- 17.7*	2.6
Lymphocytes	49.9 +/- 3.6	139.9 +/- 17.7*	2.8
CD3	16.6 +/- 1.4	46.9 +/- 8.4*	2.8
CD3+CD4+	10.3 +/- 1.1	25.8 +/- 5.1*	2.5
CD4+CD25+	0.03 +/- 0.02	0.67 +/- 0.11*	22.3
CD3+CD8+	4.3 +/- 0.5	21.1 +/- 3.5*	4.9
B220	22.6 +/- 2.0	55.8 +/- 10.3*	2.5
NK	1.9 +/- 0.2	2.6 +/- 0.5	1.4
GR1+CD11b+	0.6 +/- 0.1	6.7 +/- 2.4*	11.2
CD11c+CD11b-	1.2 +/- 0.2	3.7 +/- 1.1*	3.1
CD11c+CD11b+	0.7 +/- 0.1	8.3 +/- 1.6*	11.9
CD11c+CD11b-B220+	1.2 +/- 0.2	3.6 +/- 1.0*	3.0

*p<0.05 vs Wild type

b. Frequency (%)

Cell type	Spleen cells		Tumor infiltrating NPCs	Ratio	
	Wild type mice	Tumor bearing		vs w/t	vs TB
Lymphocytes	76.6 +/- 1.0	81.4 +/- 4.4	10.6 +/- 0.2* [#]	0.14	0.13
CD3	25.5 +/- 1.1	26.7 +/- 2.8	1.8 +/- 0.4* [#]	0.07	0.07
CD3+CD4+	16.0 +/- 1.4	14.6 +/- 2.2	0.4 +/- 0.2* [#]	0.03	0.03
CD4+CD25+	0.05 +/- 0.03	0.37 +/- 0.06*	5.9 +/- 0.1* [#]	118.00	15.95
CD3+CD8+	6.6 +/- 0.5	12.1 +/- 0.8*	1.0 +/- 0.3* [#]	0.15	0.08
B220	34.6 +/- 1.3	37.4 +/- 2.5	NA	NA	NA
NK	3.0 +/- 0.3	1.7 +/- 0.4*	4.4 +/- 1.1* [#]	1.47	2.59
GR1+CD11b+	0.8 +/- 0.1	4.0 +/- 0.7*	36.1 +/- 5.9* [#]	45.13	9.03
CD11c+CD11b-	1.9 +/- 0.2	1.9 +/- 0.3	47.5 +/- 3.5* [#]	25.00	25.00
CD11c+CD11b+	1.1 +/- 0.1	4.4 +/- 0.4*	3.2 +/- 0.6*	2.91	0.73
CD11c+CD11b-B220+	1.8 +/- 0.2	1.8 +/- 0.3	45.9 +/- 3.5* [#]	25.50	25.50

*p<0.05 vs Wild type, [#]p<0.05 vs Tumor bearing

Supplemental Table 3 Absolute number of spleen cells and frequency of cellular phenotypes within the spleen and the non-parenchymal cells (NPC) of the tumors in w/t (FVB) and FVB-neuT tumor bearing mice. **a:** The absolute numbers of each cellular phenotype x 10⁶ is identified as well as the fold increase in the tumor bearing mice. **b:** The frequency of spleen cells, in w/t mice and tumor bearing mice as well as the tumor infiltrating NPCs are identified. The last two columns are the fold change of the NPCs as compared to w/t or tumor bearing spleen cells. In both tables, SPSS was used with the student T test to

assess a significant difference. A p value ≤ 0.05 was identified as significantly different. A superscript asterisk (*) represents a significant difference compared to w/t, while (#) identified a significant difference compared to spleen cells from tumor bearing mice.