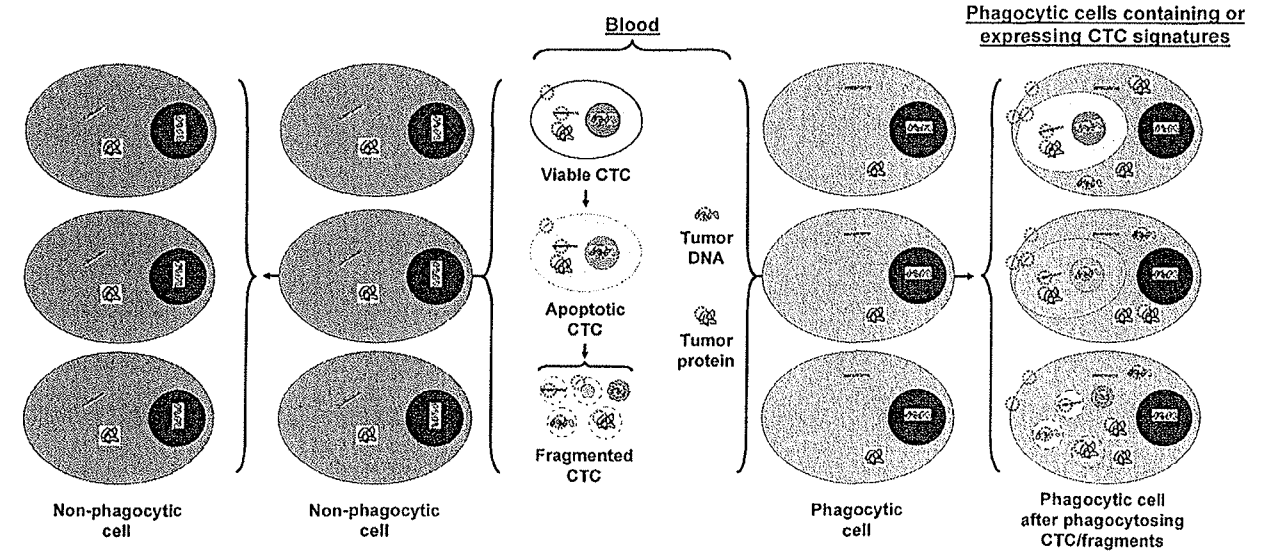


Tumor-specific signatures: DNA ( ), RNA ( ), protein ( ), and lipid ( ).

Figure 1

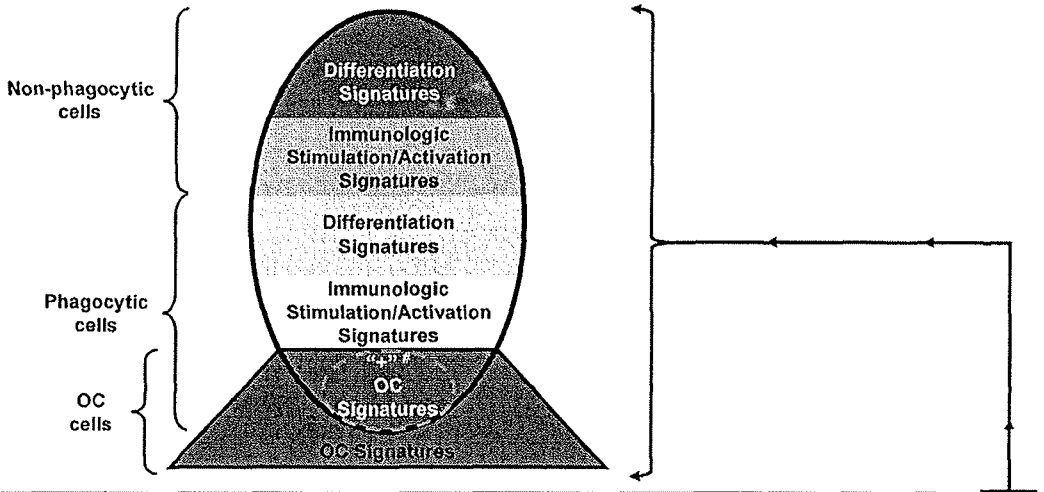


Table 1. Expression of Cancer Signatures in Phagocytic Cells of Patients with Ovarian Cancer

	Patient Genotype-Specific Signatures	Differentiation Signatures			Immunologic Stimulation/ Activation Signatures*		OC Specific Signatures
		P†	NP‡	Normal Ovary	P†	NP‡	
P†	+	+	–	–	+	–	+
NP‡	+	–	+	–	–	+	–
L_1††	0	+	+	–	+	+	+
OC cells	+	–	–	+	–	–	+
Normal ovary	+	–	–	+	–	–	–
L_2††	0	–	–	0	–	–	+

* Expressed NOT as a consequence of phagocytosis of ovarian CTCs and/or other fragments thereof
# Expressed as a consequence of phagocytosis of ovarian CTCs and/or other fragments thereof
† Phagocytic cells (e.g., macrophages, monocytes, neutrophils)
‡ Non-phagocytic cells (e.g., T-cells, B-cells)
†† List of signatures identified (e.g., using dChip software)

Figure 2

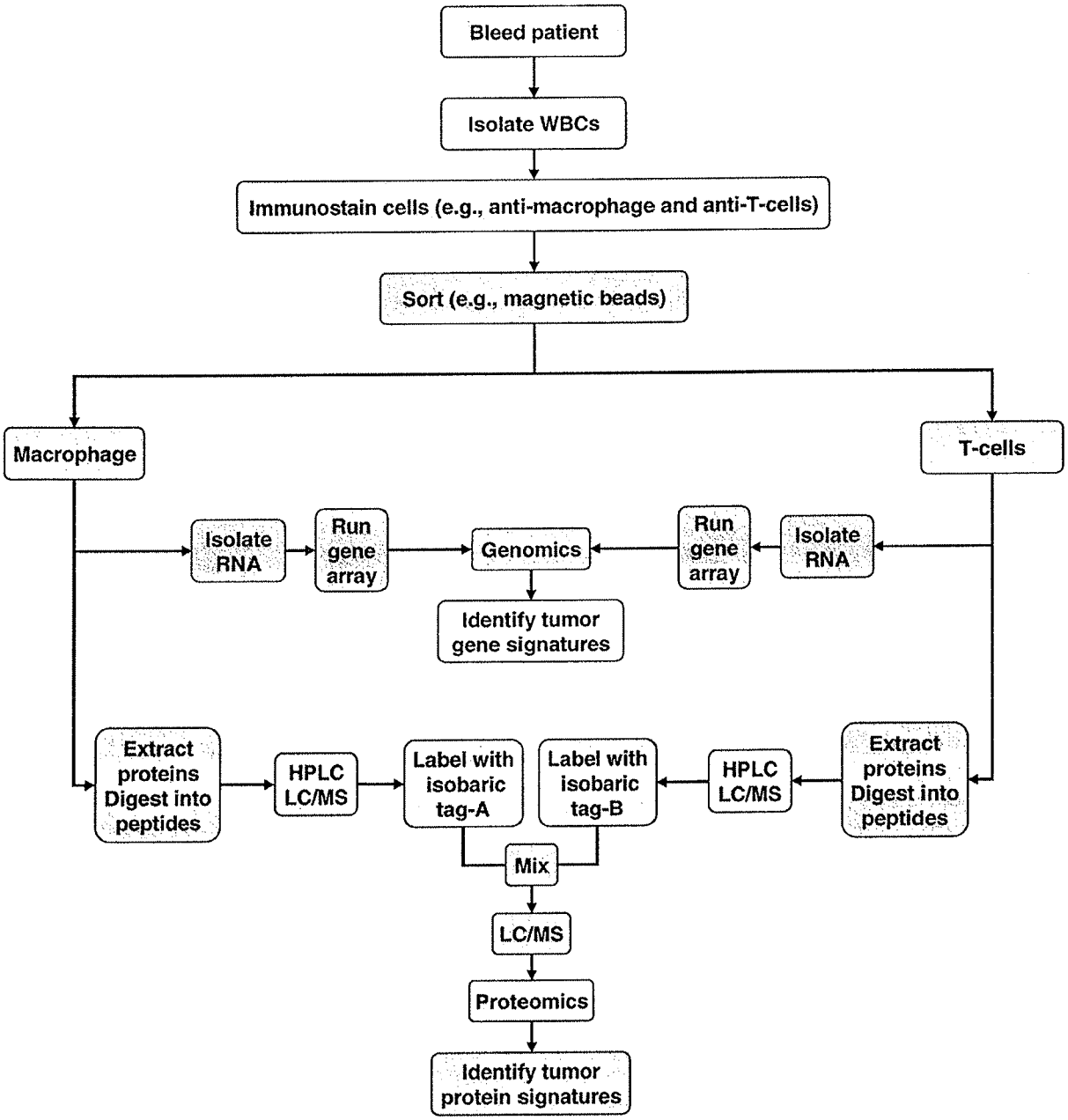

Bleed patient
Isolate WBCs
Immunostain cells (e.g., anti-macrophage and anti-T-cells)
Sort (e.g., magnetic beads)
Macrophage
T-cells
Isolate RNA
Run gene array
Genomics
Run gene array
Isolate RNA
Identify tumor gene signatures
Extract proteins Digest into peptides
HPLC LC/MS
Label with isobaric tag-A
Label with isobaric tag-B
HPLC LC/MS
Extract proteins Digest into peptides
Mix
LC/MS
Proteomics
Identify tumor protein signatures

Figure 3

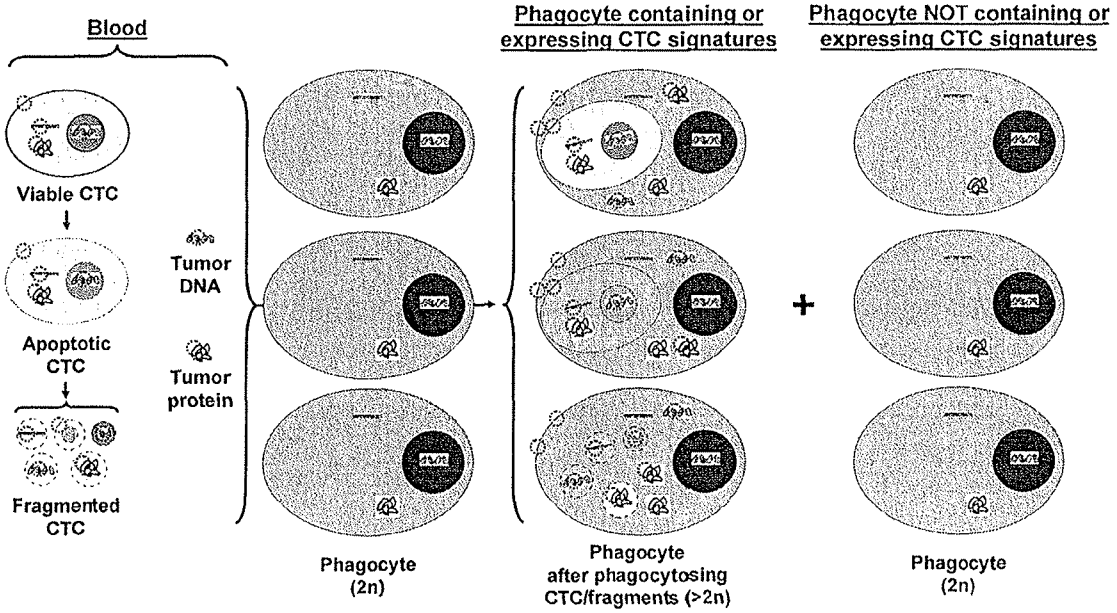

Blood
Viable CTC
Apoptotic CTC
Fragmented CTC
Tumor DNA
Tumor protein
Phagocyte (2n)
Phagocyte containing or expressing CTC signatures
Phagocyte after phagocytosing CTC/fragments (>2n)
Phagocyte NOT containing or expressing CTC signatures
+
Phagocyte (2n)
Tumor-specific signatures: DNA ( ), RNA ( ), protein ( ), and lipid ( ).

Figure 4

Figure 5

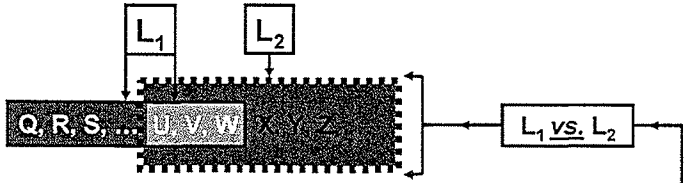


Table 2. Identification of cancer signatures acquired by phagocytic cells of BC-bearing mice

Cell	DNA Content	Mouse Normal Cell Genotype Signatures	Differentiation Signatures		Macrophage Immunologic Stimulation/ Activation Signatures		BC Specific Signatures	
			Macrophage	Normal Breast	Not consequent to phagocytosis	Consequent to phagocytosis‡	Not consequent to phagocytosis	Consequent to phagocytosis#
Macrophages	>2n	A, B, C, ...	E, F, G, ...	NA**	M, N, O, ...	Q, R, S, ...	NA	U, V, W
	2n	A, B, C, ...	E, F, G, ...	NA	M, N, O, ...	—	NA	—
L_1*		0	0	0	0	Q, R, S, ...	0	U, V, W
BC cells	≥2n	A, B, C, ...	NA	I, J, K, ...	NA	NA	U, V, W, X, Y, Z	NA
Normal breast	2n	A, B, C, ...	NA	I, J, K, ...	NA	NA	—	NA
L_2*		0	0	0	0	0	U, V, W, X, Y, Z	0

* List of signatures identified following genomic profiling

** Not Applicable

‡ Expressed as a consequence of phagocytosis of other-than breast CTCs and/or fragments thereof

Expressed as a consequence of phagocytosis of BC CTCs and/or fragments thereof

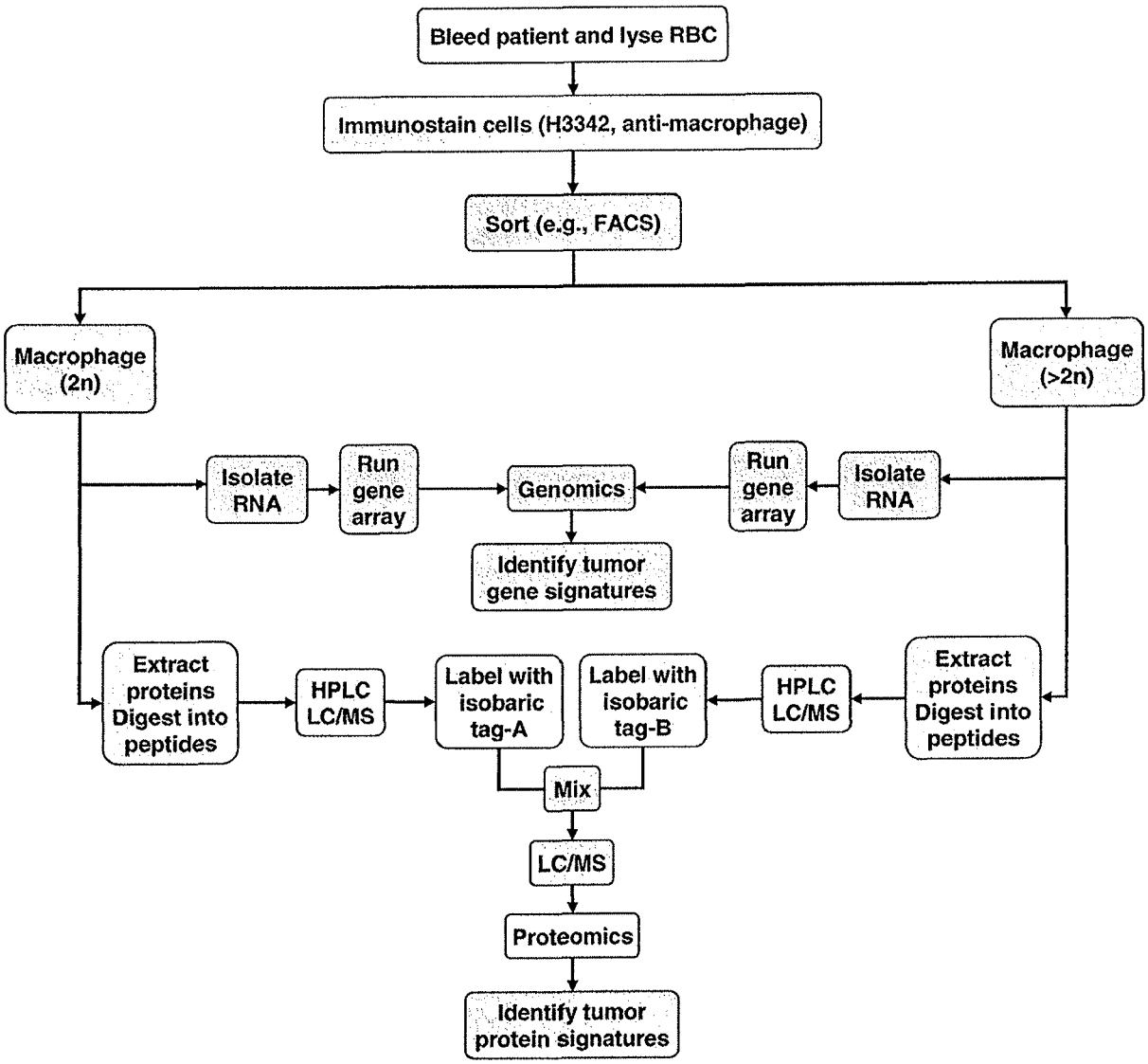

Bleed patient and lyse RBC
Immunostain cells (H3342, anti-macrophage)
Sort (e.g., FACS)
Macrophage (2n)
Macrophage (>2n)
Isolate RNA
Run gene array
Genomics
Run gene array
Isolate RNA
Identify tumor gene signatures
Extract proteins Digest into peptides
HPLC LC/MS
Label with isobaric tag-A
Label with isobaric tag-B
HPLC LC/MS
Extract proteins Digest into peptides
Mix
LC/MS
Proteomics
Identify tumor protein signatures

Figure 6

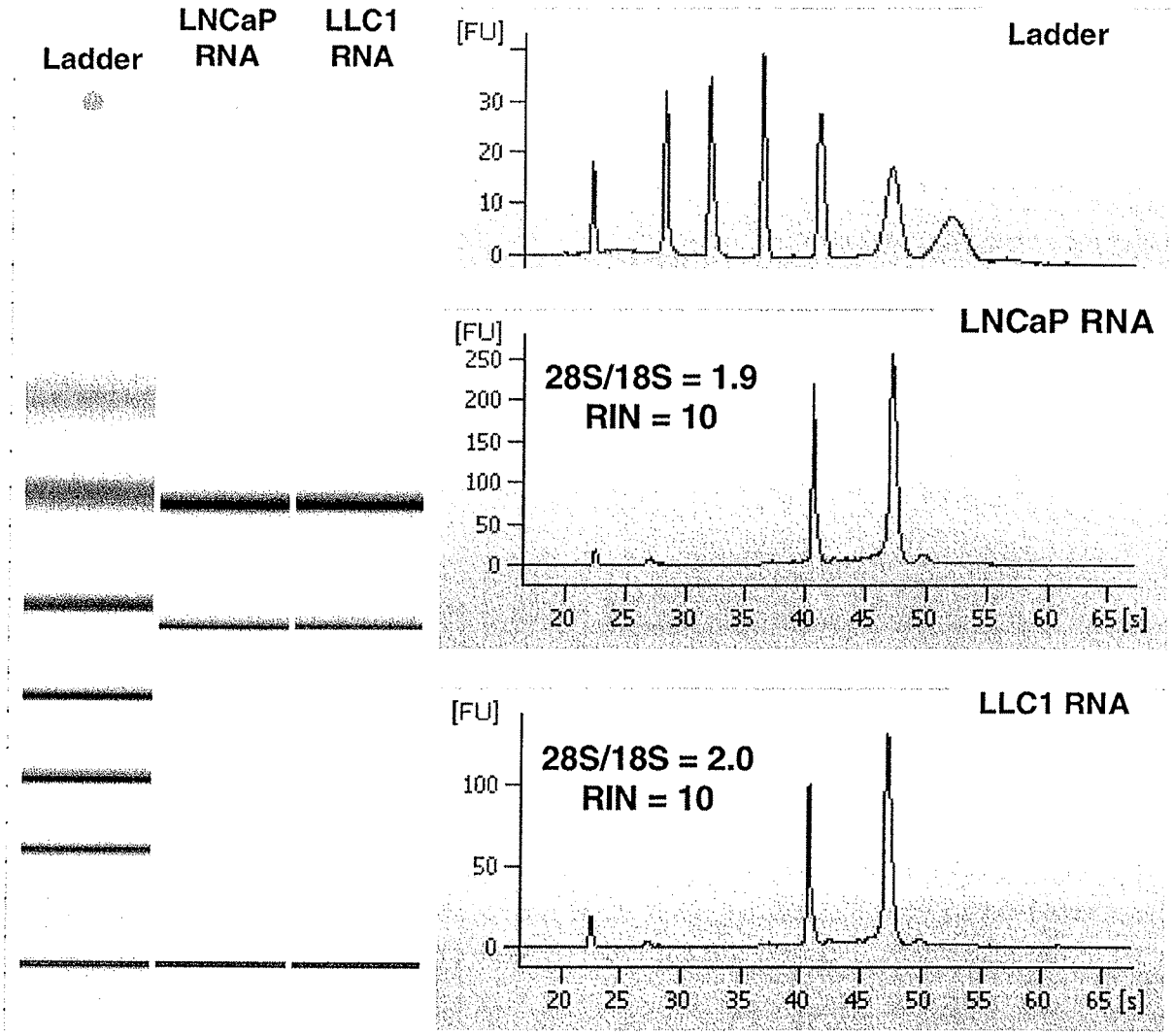

Ladder
LNCaP RNA
LLC1 RNA
[FU]
Ladder
30
20
10
0
[FU]
LNCaP RNA
28S/18S = 1.9
RIN = 10
250
200
150
100
50
0
20 25 30 35 40 45 50 55 60 65 [s]
[FU]
LLC1 RNA
28S/18S = 2.0
RIN = 10
100
50
0
20 25 30 35 40 45 50 55 60 65 [s]

Figure 7

Table 3. Yield and Quality of RNA Obtained from Mouse WBC

	Yield (μg)	28S/18S Ratio	RNA Integrity Number (RIN)
Macrophage*	0.53 ± 0.22	1.9 ± 0.2	9.4 ± 0.8
T cells*	0.42 ± 0.20	1.9 ± 0.3	8.4 ± 0.9
Neutrophils*	0.62 ± 0.16	1.9 ± 0.3	9.6 ± 0.7

*Mean of = 6 cell preparations each.

Figure 8

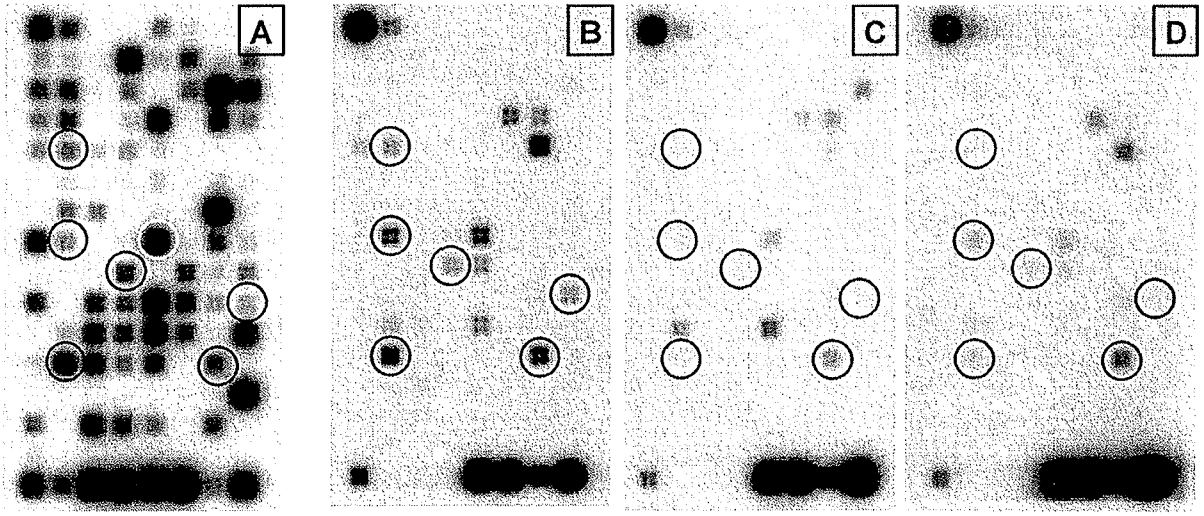


Figure 9

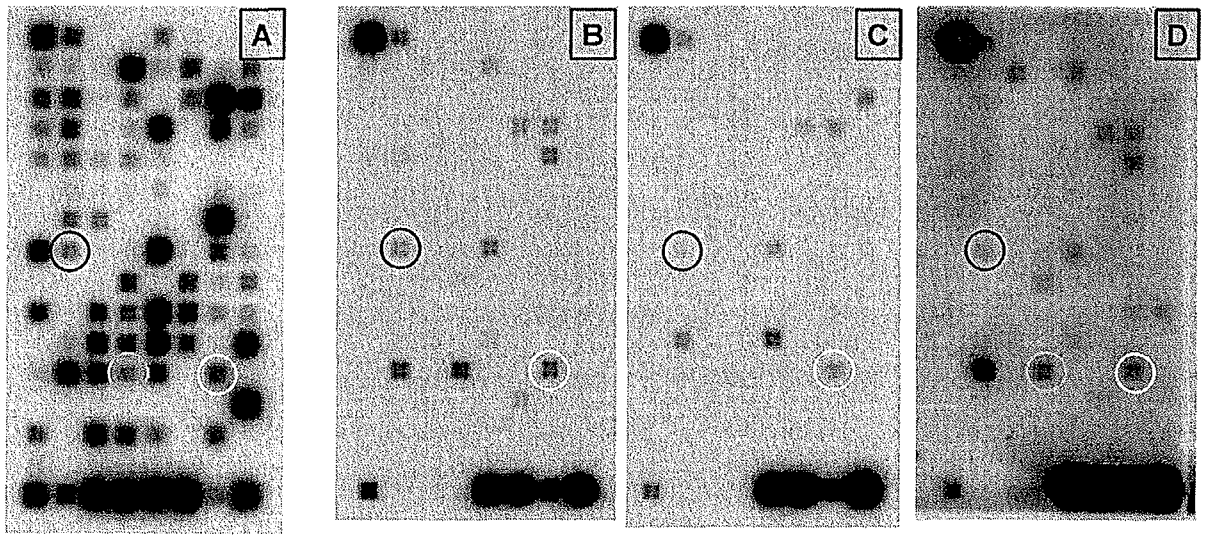


Figure 10

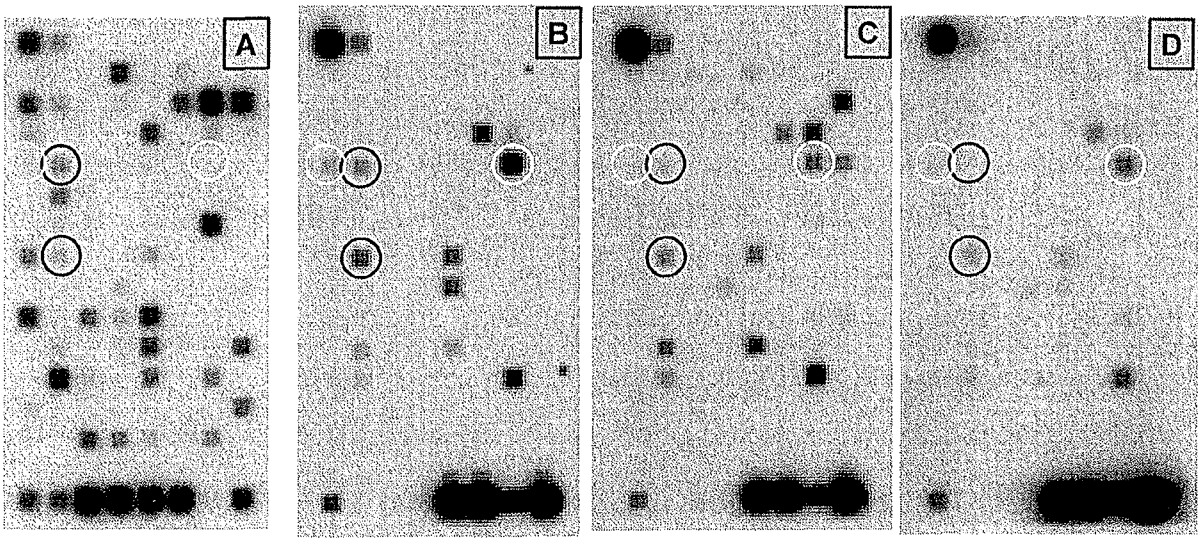


Figure 11

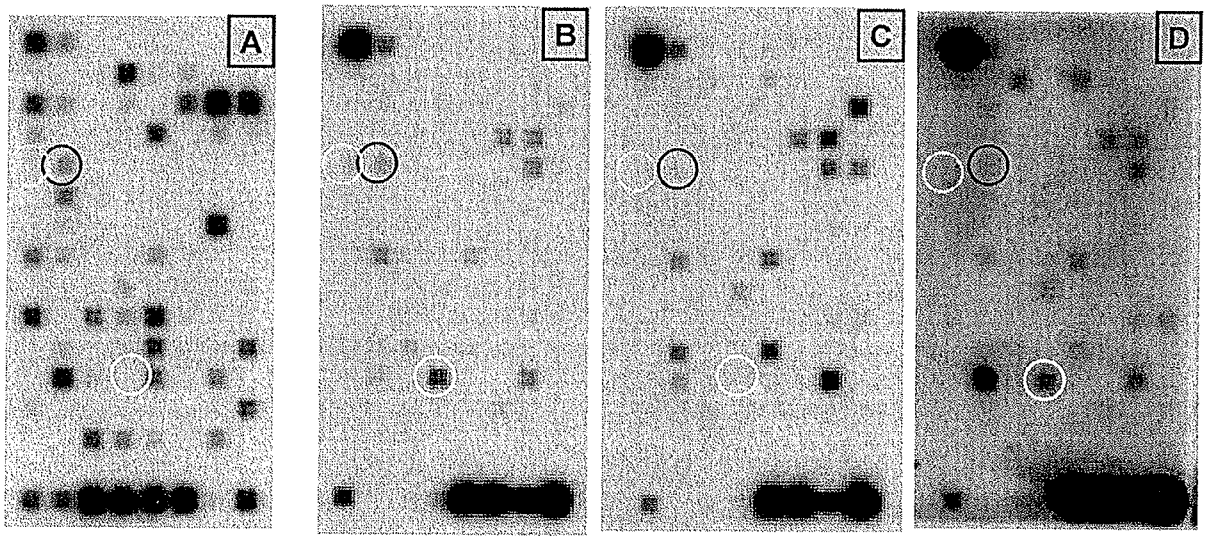


Figure 12

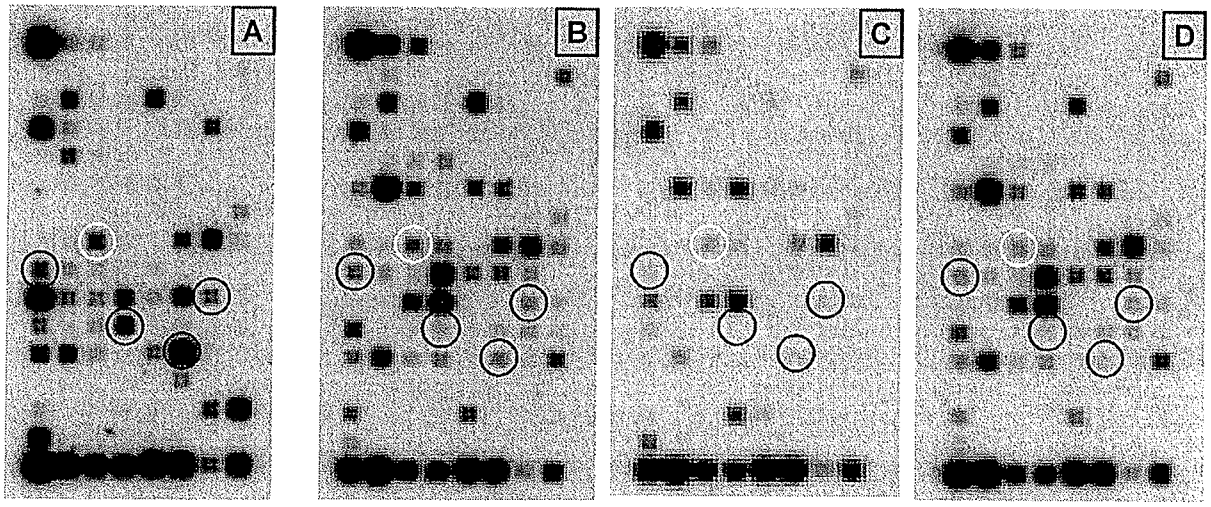


Figure 13

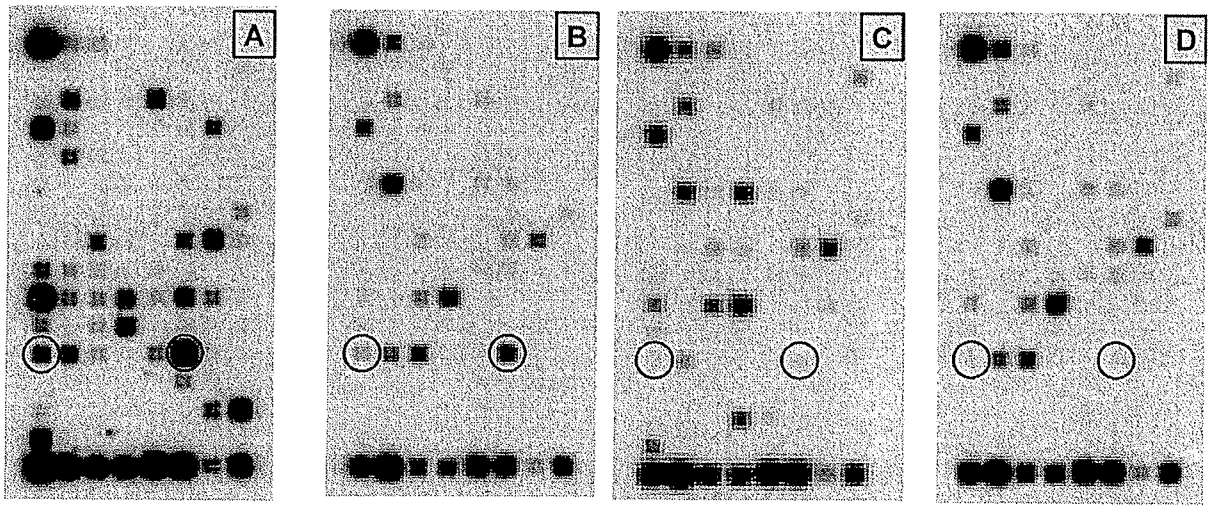


Figure 14

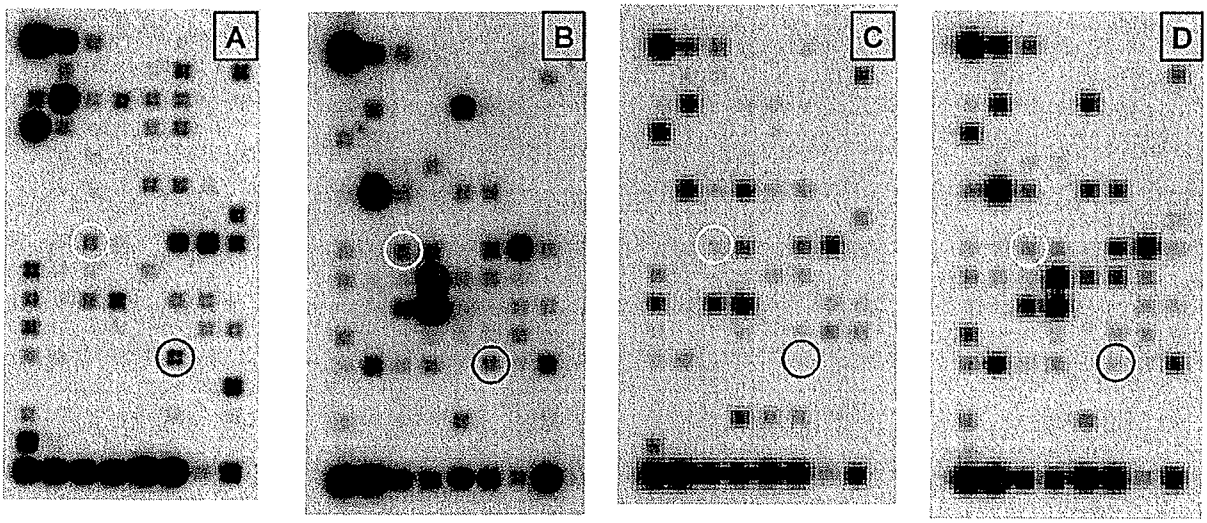


Figure 15

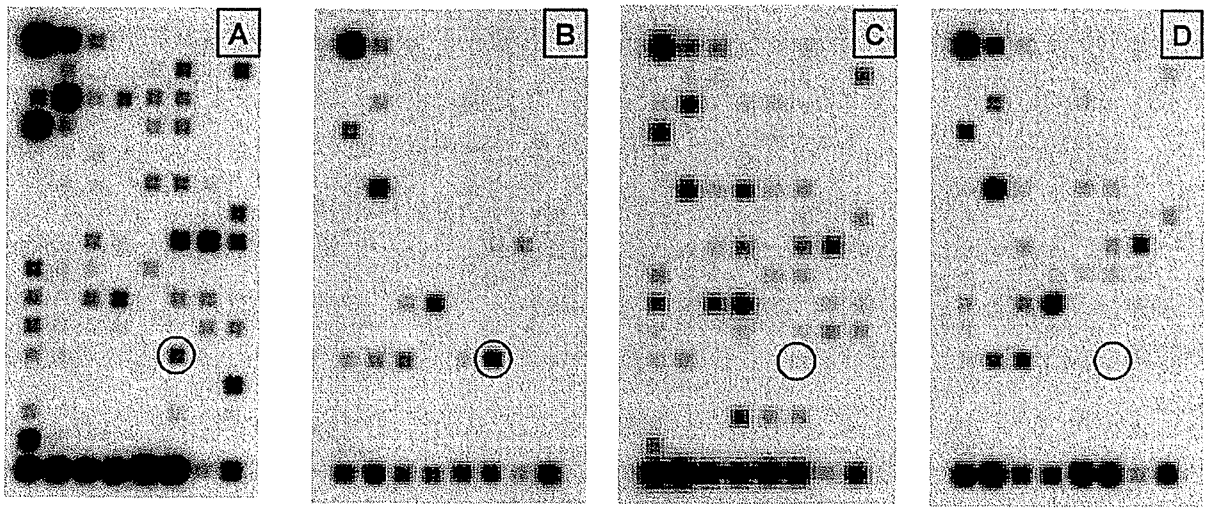


Figure 16

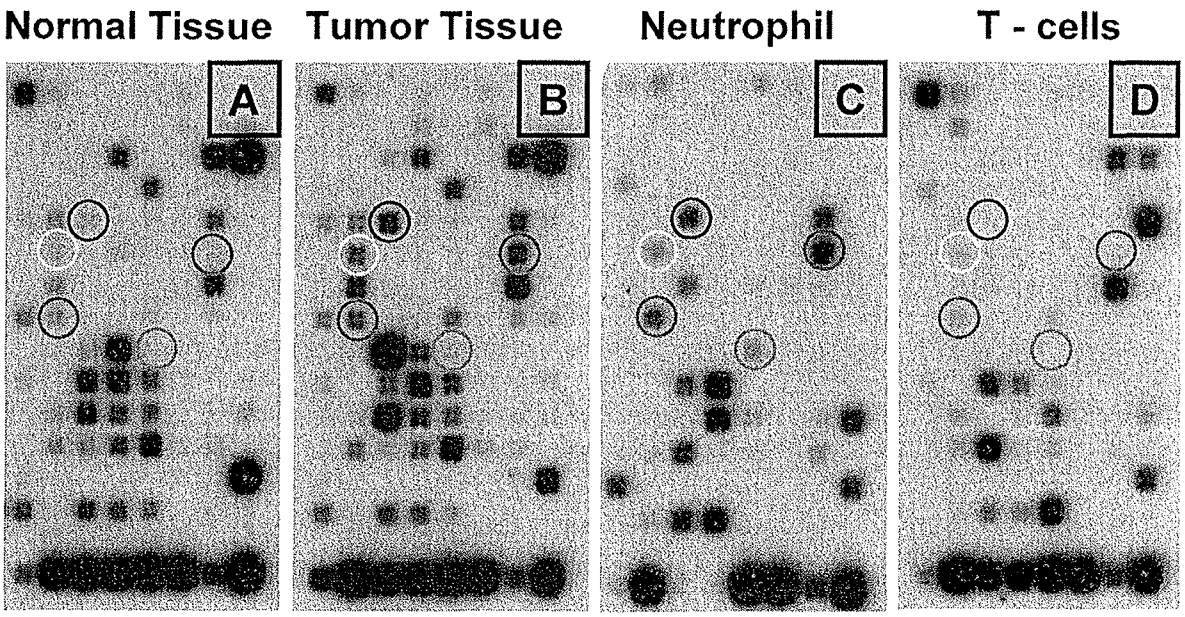


Figure 17

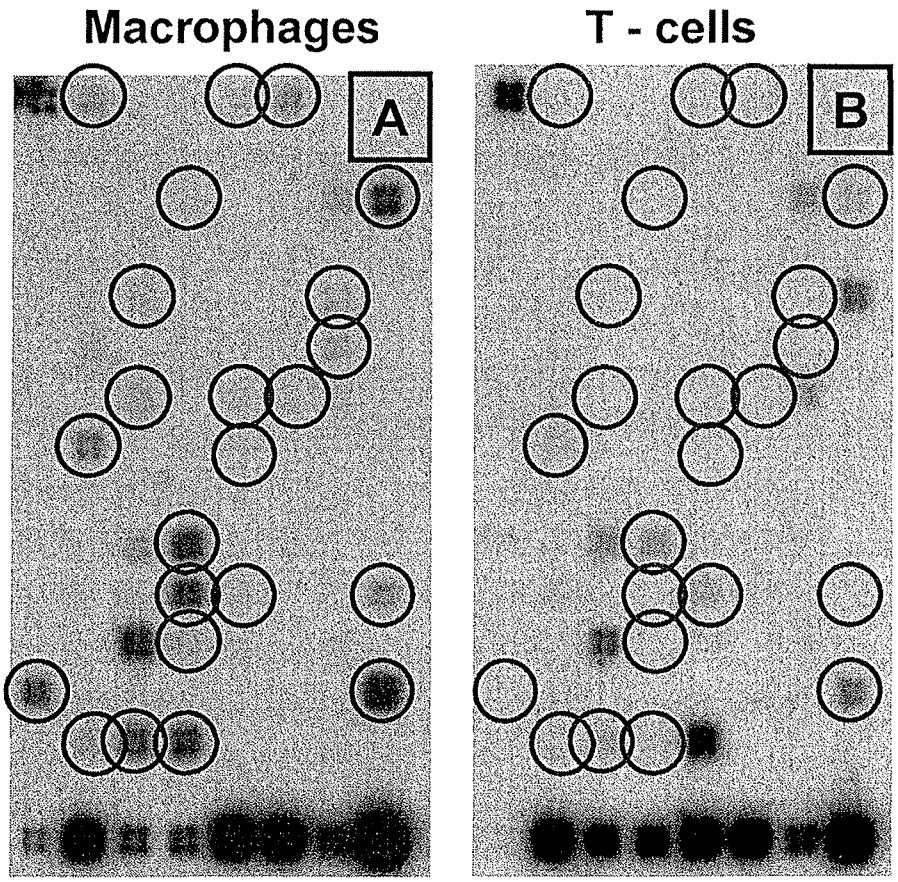


Figure 18

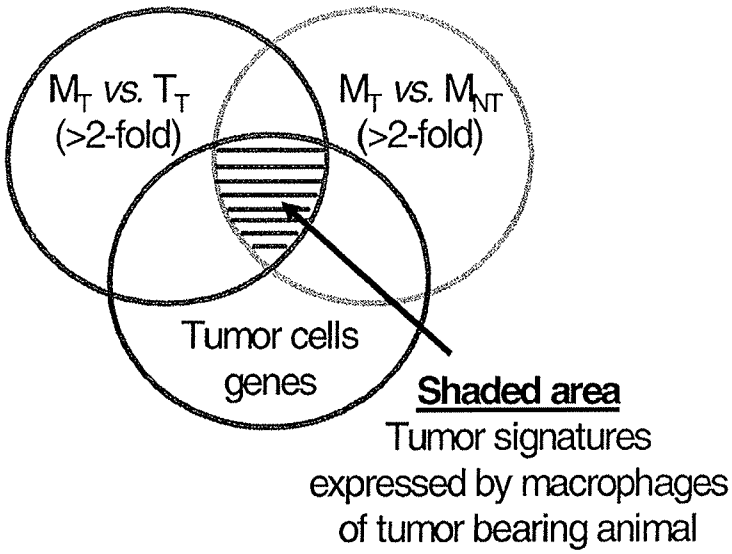

M_T *vs.* T_T
(>2-fold)
M_T *vs.* M_{NT}
(>2-fold)
Tumor cells
genes
Shaded area
Tumor signatures
expressed by macrophages
of tumor bearing animal

Figure 19

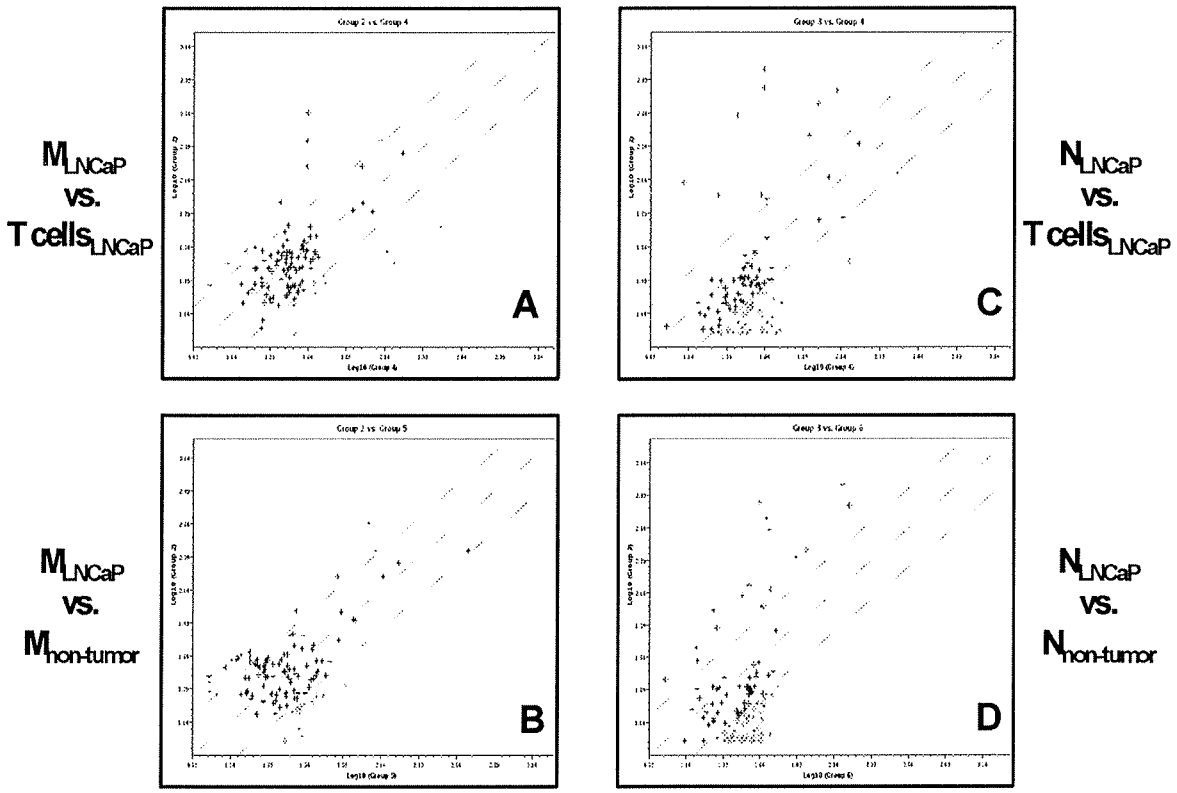

M_{LNCaP} vs. $T cells_{LNCaP}$
A
N_{LNCaP} vs. $T cells_{LNCaP}$
C
M_{LNCaP} vs. $M_{non-tumor}$
B
N_{LNCaP} vs. $N_{non-tumor}$
D

Figure 20

Table 5. Expression of cancer-related genes within phagocytic neutrophils (N) and macrophages (M).

	Over-expressed Genes	LNCaP (human prostate Ca.) (nude mice)		LS174T (human colon Ca.) (nude mice)		B16F10 (mouse melanoma) (black mice)		LLC1 (mouse lung Ca.) (black mice)	
		M	N	M	N	M	N	M	N
	BAK1	–	–	–	–	+	+	–	–
	EGFR	–	–	+	+	–	–	–	–
	ERBB2	–	+	+	+	–	–	–	–
	FOS	–	–	–	+	–	–	–	–
	JUN	+	+	–	+	–	–	–	+
	MAP2K1	–	+	–	–	–	–	–	–
	Mdm2								+
	MMP2	–	+	–	–	–	–	–	–
	PDGFB	–	+	–	–	–	–	–	+
	Plaur								+
	RB1	–	+	–	–	–	–	+	–
	SNCG	+	+	–	–	–	–	–	–
	SERPINB2	+	–	+	–	–	–	–	–
	SPP1	–	–	–	–	–	+	+	+
Total	12	3	7	3	4	1	2	2	5
Genes overexpressed in phagocytes (M+N):		8		5		2		6	

Red = oncogenes; blue = tumor-specific genes; and black = tumor-related genes.
+ = gene acquired/expressed simultaneously by M and N.

Figure 21

Table 6. Upregulated (>2-fold) cancer-related genes in macrophages of ovarian cancer patient

Gene Symbol	*Macrophages/T cells*
*AKT1**	4.62
APAF1	4.95
ATM	5.26
CDC25A	2.01
CDKN1A	4.57
ETS2	3.47
FOS	6.49
IL8	3.62
ITGA4	6.58
ITGA6	3.53
ITGAV	2.50
JUN	3.01
MAP2K1	3.09
NFKBIA	2.77
PLAU	2.13
PLAUR	38.79
RAF1	8.13
SERPINB2	5.37
SYK	20.81
TIMP1	2.04
TNF	3.75
TNFRSF10B	3.67
TNFRSF1A	6.96

* = oncogene

Figure 22

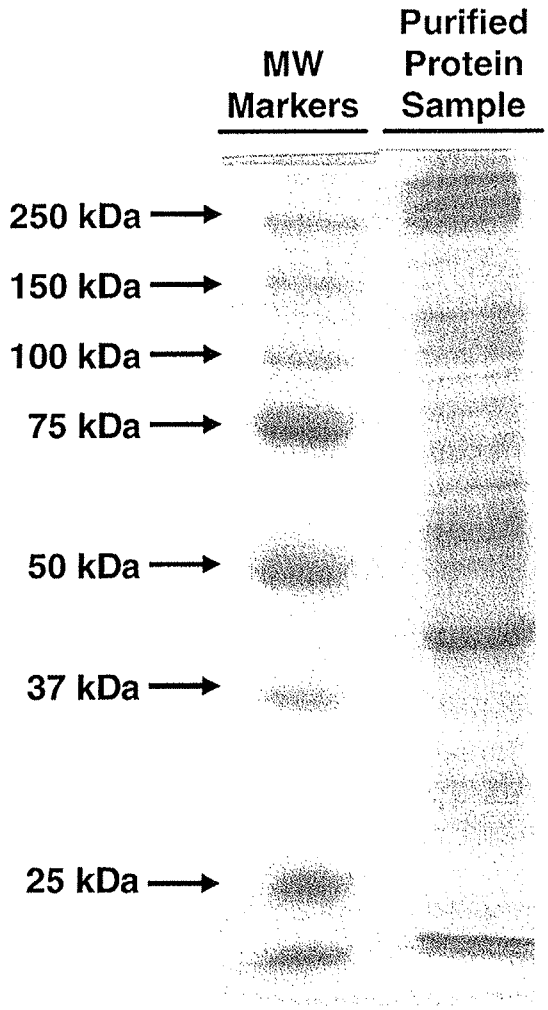

MW Markers
Purified Protein Sample
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa

Figure 23

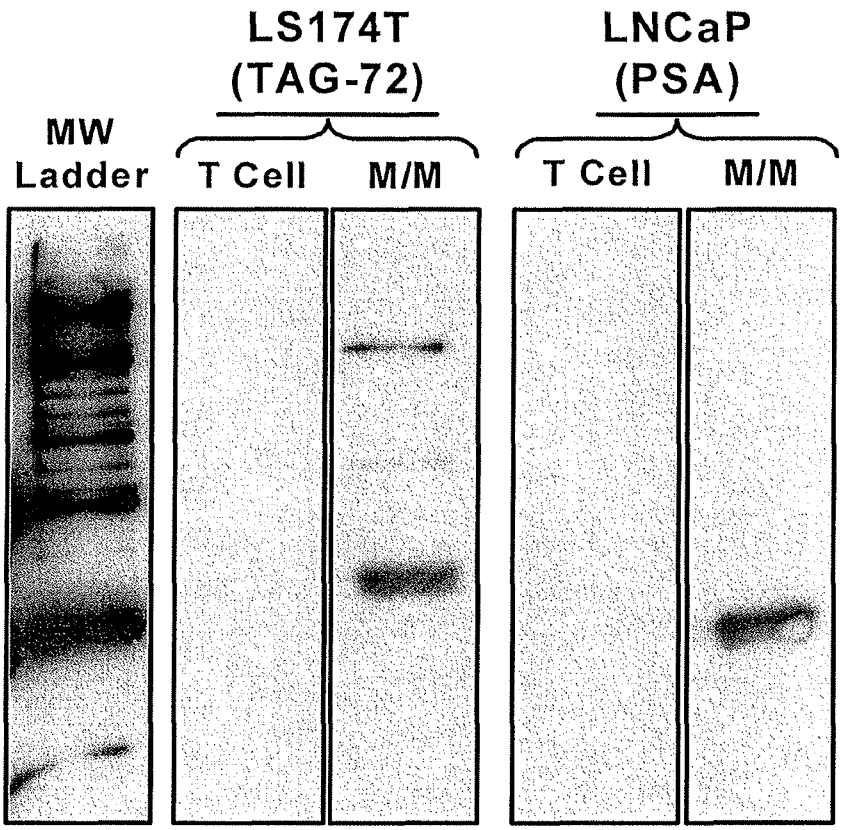


Figure 24