Supplementary Table 1: List of antibodies for flow cytometry

Panel	Antigen	Antibody clone	Supplier	Catalog number	Fluorophore
TRN A	LAG3	11C3C65	BIOLEGEND	369308	FITC
TRN A	CD8	SK1	BIOLEGEND	344742	BV605
TRN A	CD3	UCHT1	BECKMAN COULTER	B00068	KrO
TRN A	CD4	RPA-T4	BD PHARMIGEN	555349	APC
TRN A	CD16	3G8	BIOLEGEND	302042	BV650
TRN A	CD25	B1.49.9	BECKMAN COULTER	B46024	PC5.5
TRN A	CD127	R34.34	BECKMAN COULTER	B49220	PE
TRN A	CD28	CD28.2	BECKMAN COULTER	6607111	ECD
TRN A	PD-1	PD1.3	BECKMAN COULTER	A78885	PC7
TRN A	CD56	N901	BECKMAN COULTER	B46024	APC-Alexa750
TRN A	LIVE/DEAD		MOLECULAR PROBES	L34955	Violet
TRN A	TIM3	344823	R&D	FAB2365N	Alexa700
TRN B	CD14	RM052	BECKMAN COULTER	A86052	Alexa750
TRN B	HLA-DR	Immu-357	BECKMAN COULTER	IM3635	APC
TRN B	LIVE/DEAD		MOLECULAR PROBES	L34955	Violet
TRN B	CD15	80H5	BECKMAN COULTER	B01176	KrO
TRN B	LOX1	15C4	BIOLEGEND	358604	PE
TRN B	CD33	D3HL60.251	BECKMAN COULTER	A70198	PC5.5
TRN B	CD11b	Bear1	BECKMAN COULTER	A54822	PC7
TRN B	CD16	3G8	BECKMAN COULTER	A33098	ECD
TRN B	CD3 CD20 CD19 CD56	UCHT1 2H7 HIB19 B159	BD PHARMIGEN	555332 555622 555412 562794	FITC
TRN C	LIVE/DEAD		MOLECULAR PROBES	L23101	FITC
TRN C	HLA-DR	Immu-357	BECKMAN COULTER	IM3635	APC
TRN C	CD14	RM052	BECKMAN COULTER	A86052	Alexa750
TRN C	CX3CR1	2A9-1	BDBIOSCIENCE	744487	BV510
TRN C	CD192	48607	BDBIOSCIENCE	564067	BV421
TRN C	PDL-1	MIH1	BDBIOSCIENCE	557924	PE
TRN C	CD33	D3HL60.251	BECKMAN COULTER	A70198	PC5.5
TRN C	CD3	OKT3	BDBIOSCIENCE	566699	PECF-594
VAL/Sp	LAG3	11C3C65	BIOLEGEND	369308	FITC
VAL/Sp	CD16	3G8	BIOLEGEND	302042	BV650
VAL/Sp	CD3	UCHT1	BECKMAN COULTER	B00068	KrO
VAL/Sp	CD8	SK1	BIOLEGEND	344742	BV605
VAL/Sp	HLA-DR	Immu-357	BECKMAN COULTER	IM3635	APC
VAL/Sp	CD14	RM052	BECKMAN COULTER	A86052	Alexa750
VAL/Sp	CD56	B159	BDBIOSCIENCE	557919	Alexa700
VAL/Sp	LIVE/DEAD		MOLECULAR PROBES	L34955	Violet
VAL/Sp	CD15	80H5	BECKMAN COULTER	IM2641U	PC5.5
VAL/Sp	PDL-1	MIH1	BDBIOSCIENCE	563742	PECF-594
VAL/Sp	PD1	PD1.3	BECKMAN COULTER	A78885	PC7
VAL/Sp	LOX1	15C4	BIOLEGEND	358604	PE

Supplementary Table 2: List of lymphoid and myeloid immune subpopulations characterized by flow-cytometry

Population	Description	Markers
Lymphoid		
T	T Lymphocytes	CD3+
T $CD8^{+}$	CD8+ T cells	CD3+ CD8+
T $CD3^{+}$ $CD8^{high}$	Activated T cells	CD3+ $CD8^{high}$
T $CD8^{+}$ $TIM3^{+}$	Anergic T cells	CD3+ CD8+ TIM3+
T $CD8^{+}$ $PD\text{-}1^{+}$	Cytotoxic T cells	CD3+ CD8+ PD-1+
T $CD8^{+}$ $PD\text{-}1^{+}$ $LAG3^{+}$	Exhausted T cells	CD3+ CD8+ PD-1+ LAG3+
T $CD8^{+}$ $CD28^{+}$	Memory T cells	CD3+ CD8+ CD28+
T $CD4^{+}$	CD4+ T cells	CD3+ CD4+
T $CD4^{+}$ $CD25^{high}CD127^{+}$	CD4+ T cell effectors	CD3+ CD4+ $CD25^{high}$ CD127+
T reg	T reg	CD3+ CD4+ $CD25^{high}$ $CD127^{neg}$
NK-like T $CD56^{neg}$	NK T cells	CD3+ $CD56^{neg}$ $CD16^{int}$
NK-like T	Activated NK T cells	CD3+ CD56+ $CD16^{int}$
NK-like T $CD16^{high}$	ADCC prone NK T cells	CD3+ CD56+ $CD16^{high}$
NK $CD56^{neg}$	Mature NK	$CD3^{neg}$ $CD56^{neg}$ CD16+
NK $CD56^{neg}CD16^{high}$	ADCC prone NK	$CD3^{neg}$ $CD56^{neg}CD16^{high}$
NK $CD56^{dim}$	Cytotoxic NK	$CD3^{neg}$ $CD56^{dim}$ CD16+
NK $CD56^{dim}$ $CD16^{neg}$	Resting NK	$CD3^{neg}$ $CD56^{dim}$ $CD16^{neg}$
NK TIM3+	Anergic NK	$CD3^{neg}$ $CD56^{dim}$ CD16+ TIM3+
NK $CD16^{neg}$	Th1 cytokine secreting NK	$CD3^{neg}$ $CD56^{high}$ $CD16^{neg}$
Myeloid		
Mo	Monocytes	CD14+
C-Mo	Classical monocytes	CD14+ $CD16^{neg}$
I-Mo	Intermediate monocytes	CD14+ CD16+
M-MDSCs	M-MDSCs	CD14+ HLA-DR^{neg}
Mo $PD\text{-}L1^{+}$	Activated monocytes	CD14+ PD-L1+
Mo $CCR2^{+}$	Migration-prone monocytes	CD14+ CCR2+
Mo $CX3CR1^{+}$	Proangiogenic monocytes	CD14+ CX3CR1+
Mo HLA-DR^{bright}	Protective monocytes	CD14+ HLA-DR^{bright}
Mo $Lox\text{-}1^{+}$	Lox-1 monocytes	$CD14^{high}$ LOX1+
NC-Mo	Non-classical monocytes	$CD14^{dim}$ CD16+
Mo $CD14^{dim}$	Inflammatory monocytes	$CD14^{dim}$ $CD16^{neg}$
PMN-MDSCs	PMN-MDSCs	CD15+ HLA-DR^{neg}
PMN-MDSCs $LOX1^{+}$	Activated PMN-MDSCs	CD15+ HLA-DR^{neg} LOX1+

Supplementary Table 3: List of genes selected as representative of the 4 immune cell subsets discriminating lung cancer patients and controls. Numbers indicate the respective reference/s.

Gene Symbol	Assay ID	Represented immune subset (Ref)				Reliable by RT-qPCR	Filtered in by the calibration set
		T CD8+PD-1+	I-Mo	M-MDSC	PMN-MDSC		
APBA2	*Hs00194072_m1*	X (24)				X	X
ARG1	*Hs00163660_m1*				X (30)		
CCL13	*Hs00234646_m1*			X (30)			
CCL2	*Hs00234140_m1*			X (26)		X	
CCL26	*Hs00171146_m1*		X (30)				
CCR5	*Hs99999149_s1*		X (29)			X	
CD14 *	*Hs02621496_s1*		X	X		X	X
CD274	*Hs01125301_m1*			X (26)		X	X
CD3D *	*Hs00174158_m1*	X				X	
CD8A *	*Hs00233520_m1*	X				X	X
CEACAM8	*Hs00266198_m1*				X (30)		
FCGR3A *	*Hs02388314_m1*		X			X	
FPR1	*Hs04235426_s1*				X (24,25)	X	X
FUT4 *	*Hs01106466_s1*				X	X	X
GFRA2	*Hs00176393_m1*		X (30)			X	X
GZMB	*Hs00188051_m1*	X (25)				X	X
HCAR2	*Hs02341584_s1*				X (27)	X	X
IFNG	*Hs00166223_m1*	X (25)				X	
IL6	*Hs00174131_m1*			X (26)			
OLR1	*Hs01552593_m1*				X (30)		
PDCD1 *	*Hs01550088_m1*	X				X	X
PRF1	*Hs00169473_m1*	X (24,25)				X	X
S100A9	*Hs00610058_m1*				X (28)	X	
S1PR3	*Hs00245464_m1*			X (30)		X	X
SEMA4B	*Hs00384240_m1*			X (26)		X	X
TGFB1	*Hs00998133_m1*			X (26)		X	X

*Gene selected on the basis of immune cell phenotype as determined by flow cytometry analysis.

Supplementary Table 4: Distribution of immune cells subsets according to clinico-pathological and molecular characteristics in the training set. Student's t-test p-value are reported. p-value<0.05 at the univariate level (in bold) is considered significant.

	Gender (F vs. M)	Age (≤60 vs. >60)	Pack-year (<44 vs. ≥44)	MSC (pos vs. neg)	Tumor histology (ADC vs. Others)	Stage (I vs. II-V)
T	0.6487	0.8651	0.7293	0.5096	0.3018	0.1781
T CD8+	0.0583	0.1470	0.6304	0.9648	0.3723	0.4497
T CD3+ CD8^{high}	0.0833	0.2304	0.6548	0.8060	0.5289	0.6051
T CD8+ TIM3+	0.1312	0.3913	0.5252	0.0877	0.6570	0.9506
T CD8+ PD-1+	0.6922	0.8376	0.0972	0.2466	0.0870	**0.0071**
T CD8+ PD-1+ LAG3+	0.1624	0.0823	0.6199	0.3881	0.8815	0.1910
T CD8+ CD28+	0.9123	0.6044	0.6228	0.1904	0.7876	0.3356
T CD4+	0.0598	0.1009	0.6009	0.6684	0.3646	0.3872
T CD4+ CD25^{high} CD127+	0.7973	0.3892	0.2192	0.8755	0.1014	0.1150
T reg	0.5966	0.7664	0.6903	0.4418	0.6074	0.1405
NK-like T CD56^{neg}	**0.0234**	0.1284	0.5575	0.5135	0.0907	0.5927
NK-like T	0.7763	0.5724	0.9999	0.7761	0.7142	0.5954
NK-like T CD16^{high}	0.7119	0.4075	0.1710	0.9403	0.4285	0.3077
NK CD56^{neg}	0.8219	0.1964	0.8469	0.2377	0.1885	0.9219
NK CD56^{neg} CD16^{high}	0.6491	0.6945	0.2911	0.8503	0.0824	0.2112
NK CD56^{dim}	0.6347	0.7867	0.6115	0.0877	0.3720	0.2363
NK CD56^{dim} CD16^{neg}	0.0954	0.6564	0.3759	0.8540	0.3032	0.6814
NK TIM3+	0.1008	0.8727	0.4122	0.2461	0.2840	0.3526
NK CD16^{neg}	0.2569	0.9768	0.9241	**0.0392**	0.5599	0.2673
Mo	0.6771	0.9850	0.4108	0.5207	0.7401	0.1757
C-Mo	0.8158	0.3074	0.1750	0.4202	0.1438	0.5763
I-Mo	0.2800	0.1955	0.9208	0.8737	0.1271	0.8235
M-MDSCs	0.6687	0.8275	0.6479	0.7624	0.2307	0.4793
Mo PD-L1+	0.0973	0.0969	0.7911	0.9443	0.7733	0.8989
Mo CCR2+	0.4810	**0.0378**	0.2443	0.5844	0.7765	0.0606
Mo CX3CR1+	0.4117	0.2321	0.8282	0.6083	0.7137	**0.0070**
Mo HLA-DR^{bright}	0.7219	0.5590	0.5757	0.8413	0.2405	0.0583
Mo Lox-1+	0.0846	0.0840	0.9956	0.9924	0.5589	0.9125
NC-Mo	0.6611	0.6151	0.4311	0.4452	0.5377	0.1774
Mo CD14^{dim}	0.2869	0.4189	0.7134	0.1101	0.3830	0.3999
PMN-MDSC	0.3836	0.3317	**0.0135**	0.3079	0.4849	0.8146
PMN-MDSC LOX1+	0.0999	0.1848	0.1577	0.1131	0.4446	0.5053

Supplementary Table 5: Immune cell subsets whose levels differed at the univariate level (t-test p-value<0.05; fold-change>1.5) in PBMC samples collected from 20 lung cancer patients and 20 matched controls in the training set.

Immune population	Mean value in lung cancer patients	Mean value in control subjects	Fold-change	P-value
PMN-MDSCs	1.06	0.36	2.95	0.0053
M-MDSCs	1.09	0.61	1.78	0.0198
Mo CD14dim	12.18	6.88	1.77	0.0403
T CD8+ PD-1+	12.48	22.68	0.55	0.0003
IMo	2.00	4.17	0.48	0.0063
NK CD56dim	15.61	37.05	0.42	0.0001
NK-like T CD16high	0.30	0.80	0.37	0.0148
NK CD56neg CD16high	0.30	1.25	0.24	0.0031

Supplementary Table 6: Performance of the ISC test in terms of Sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) in the training, validation and LC specificity set considering all, only MSC positive and only MSC negative subjects.

	Training set	**Validation set**	**LC Specificity set**
All			
Se	95%	80%	84%
Sp	85%	60%	75%
PPV	86%	67%	89%
NPV	94%	75%	67%
MSC pos			
Se	93%	80%	78%
Sp	86%	52%	67%
PPV	87%	63%	88%
NPV	92%	75%	50%
MSC neg			
Se	100%	80%	90%
Sp	83%	73%	80%
PPV	86%	75%	90%
NPV	100%	79%	80%

Supplementary Table 7. Performance of the ISC qPCR-based test in terms of Sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) in validation set considering all, only MSC positive and only MSC negative subjects.

	Validation set
All	
Se	79%
Sp	64%
PPV	66%
NPV	78%
MSC pos	
Se	77%
Sp	60%
PPV	63%
NPV	75%
MSC neg	
Se	83%
Sp	71%
PPV	71%
NPV	83%

Supplementary Table 8. Microarray gene expression dataset obtained from PBMC (GSE13255) and whole blood (GSE108375) datasets.

	GSE13255		GSE108375	
	27 NSCLC	6 Non-healthy controls*	29 Pts with malignant nodule	37 Pts with benign nodule
Gender				
Female	17	3	14	20
Male	10	3	15	17
Median age (IQR)	68 (15)	53 (7)	65 (8)	63 (11)
Median Pack-years (IQR)	/	/	60 (43)	50 (20)
Histology				
ADC	13	/	/	/
Other	14	/	/	/
Stage				
I	15	/	21	/
II-IV	12	/	8	/

*2 granulomous inflammation, 1 hamartoma, 1 sarcoidosis, 1 hypertension and 1 cirrhosis