Supplementary Materials

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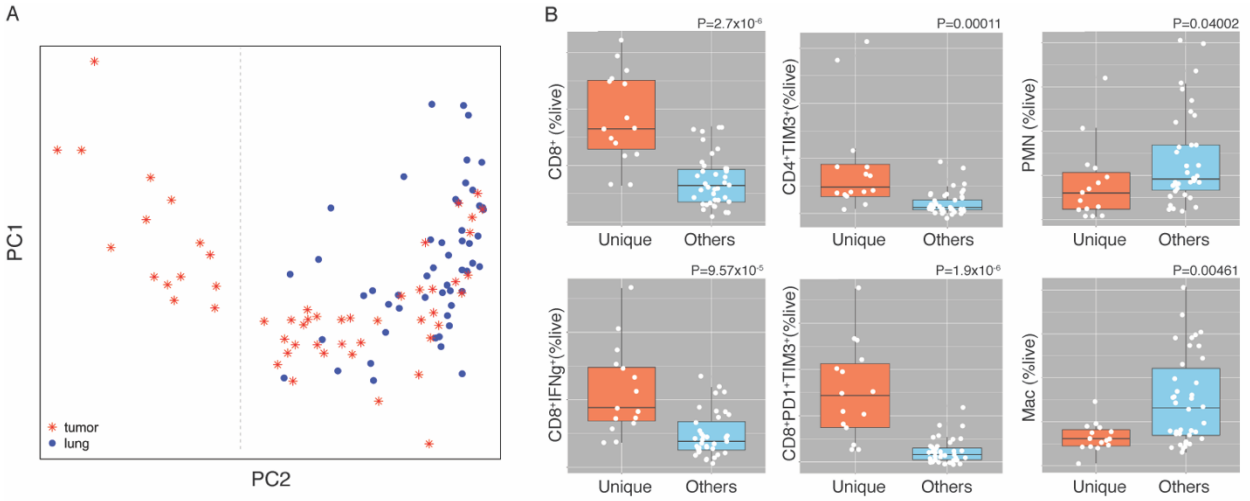

Figure S1. Principle Coordinate Analysis Identified Unique Immune Cell Phenotype. A) Principal Coordinate Analysis (PCoA) of flow cytometric data for lung tumor tissue and non-adjacent lung tissue from N=73 NSCLC resection specimens. The lung tissue and lung cancer profiles segregate together with the exception of N=15 lung cancer cases that clearly separate from the remainder of the group (left). These cases were labled Immune Unique. B) Key immune cell populations that best distinguish between Immune Unique cases and the remainder of the cohort. P values as indicated (as determined by Student's t-test).

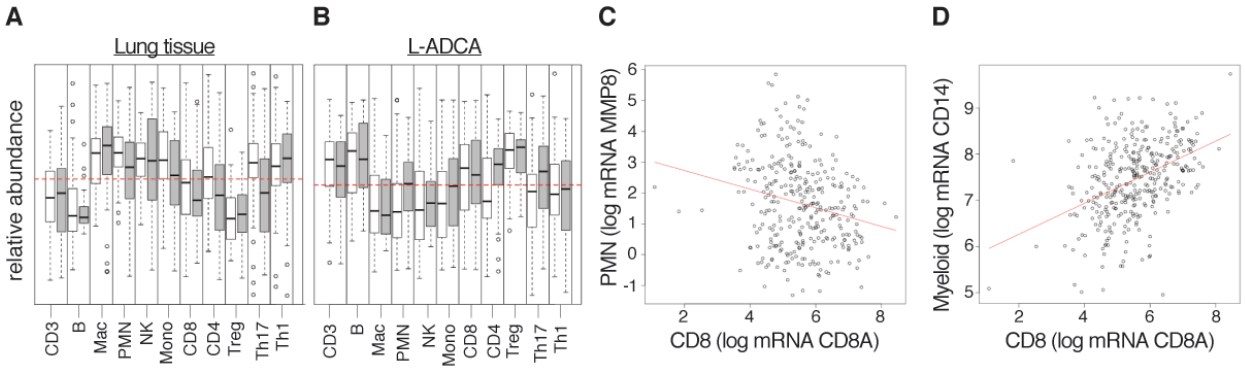

Figure S2. PMN and CD8⁺ T Cell Content in L-ADCA TCGA Dataset. Logarithmic comparison of immune cell composition determined by flow cytometry (white bars) and TCGA data (grey bars) in A) lung tissue and B) L-ADCA tissue. Correlation of log transcript abundance between C) neutrophils (MMP8) and CD8⁺ cells (CD8A) ($P=6.18 \times 10^{-5}$) and between monocytes (CD14) and CD8⁺ cells ($P=2.0 \times 10^{-16}$) using data extracted from the lung adenocarcinoma dataset.

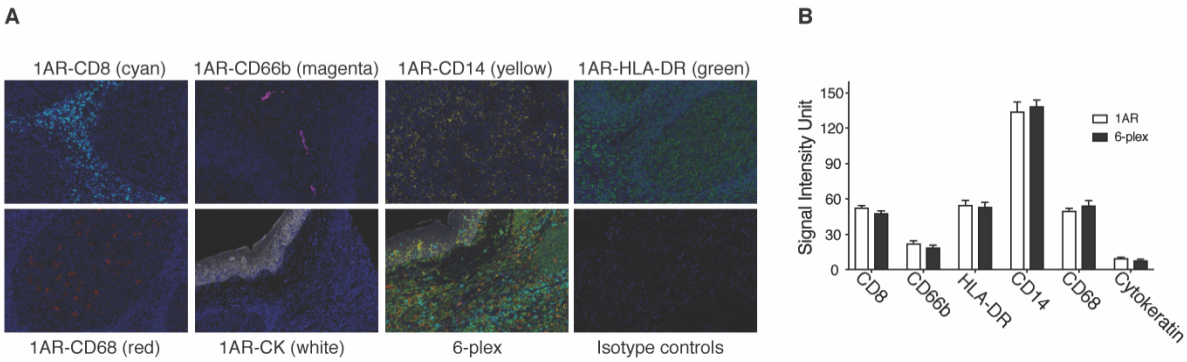

Figure S3. M-IHC Panel Development. Each antibody was tested for staining signal intensity on both single stains (1-antigen retrieval, 1AR) and on a 6-plex slide with multiple cycles of antigen retrieval and staining. A) Representative images of individual marker stained slides, 6-plex staining, and isotype controls on normal tonsil tissue. Isotype controls had gone through six staining cycles. DAPI was used as a nuclear counterstain (blue). B) Comparison of marker signal strength at baseline (1AR) vs. 6-plex. None of these comparisons reached statistical signficance (Student's t-test).

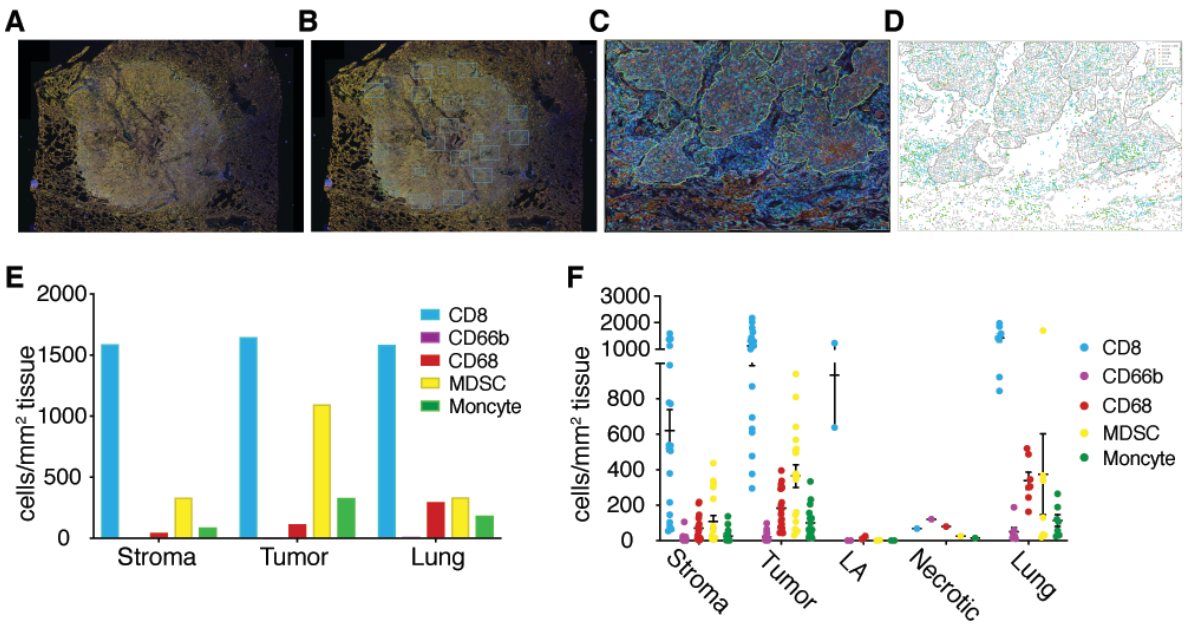

Figure S4. M-IHC Image Analysis Workflow. A) Representative image of whole slide scan (10X) of a multiplex-stained slide. B) Representative example of 15-20 regions of interest (ROI) stamps (20X) placed over the tumor lesion from (A) covering areas across adjacent lung-tumor interface, stroma, tumor, necrotic tumor, and lymphoid aggregate (LA). C) Tumor tissue from a single ROI annotated by tissue compartment. D) Spatial distribution plot of the ROI depicted in (C) showing positive cells gated by threshold. E) Tabluated immune cell content for the individual ROI in (C). F) Tabulated immune cell content for all of the ROIs encompassing the slide in (A). Bars +/- SEM.

Table S1. Histological Subtypes of Resection Cohort

Phenotype	N	LUAD (%)	LUSQ (%)	Other (%)
Inert	10	10 (100%)	0 (0%)	0 (0%)
Myeloid	24	14 (58%)	8 (33%)	2 (8%)
Active	20	15 (75%)	4 (20%)	1 (5%)
Indeterminant	10	10 (71%)	3 (21%)	1 (7%)

*Groups are not statistically different with respect to LUAD and LUSC distribution (Chi-square test).

Table S2. M-IHC immune cell markers

HUMAN					
Antigen	**Color**	**Clone**	**Company**	**Catalog #**	**Panel**
Cytokeratin (panCK)	White	AE1/AE3	Dako	M3515	1,2
CD4	Yellow	EP204	Epitomics	AC0173A	2
CD8	Cyan	C8/144B	Dako	M7103	1,2
CD14	Yellow	EPR3653	Cell Marque	114R-14	1
CD66b	Magenta	G10F5	BD Pharmingen	555723	1,2
CD68	Red	PG-M1	Dako	M0876	1
HLA-DR	Green	TAL.1B5	Dako	M0746	1
MOUSE					
Antigen	**Color**	**Clone**	**Company**	**Catalog #**	**Panel**
Cytokeratin (wide spectrum screening)	Red	WSS	Dako	Z0622	N/A
CD8	Yellow	4SM15	eBiosciences	14-0808-80	N/A
Ly6G	Green	1A8	BioLegend	127601	N/A

Table S3. M-IHC antibody staining sequences

Panel 1: 6-plex (Resection Cohort)					
Position	**Antibody**	**Clone/ Host**	**Company / Item**	**Concentration**	**OPAL Fluor**
1	CD8	144B/Mouse	Dako/M7103	1:800 (0.2ug/mL)	Opal 540
2	CD66b	G10F5/Mouse	BD Pharm/555723	1:250 (2ug/mL)	Opal 520
3	HLA-DR	TAL.1B5/Mouse	Dako/M0746	1:800 (0.03ug/mL)	Opal 570
4	CD14	EPR3653/Rabbit	Cell Marque/114R-14	1:150 (0.125ug/mL)	Opal 620
5	CD68	PG-M1/Mouse	Dako/M0876	1:800 (0.04ug/mL)	Opal 650
6	CK-ae1/3	AE1/AE3/Mouse	Dako/M3515	1:500 (0.33ug/mL)	Opal 690
Panel #2: 4-plex (Treatment Cohort)					
Position	**Antibody**	**Clone/ Host**	**Company / Item**	**Concentration**	**OPAL Fluor**
1	CD8	144B/Mouse	Dako/M7103	1:800 (0.2ug/mL)	Opal 540
2	CD66b	G10F5/Mouse	BD Pharm/555723	1:250 (2ug/mL)	Opal 570
3	CD4	EP204/Rabbit	Epitomics/AC0173A	1:100 (0.165ug/mL)	Opal 520
4	CK-ae1/3	AE1/AE3/Mouse	Dako/M3515	1:500 (0.33ug/mL)	Opal 690
Panel #3: 3-plex (Mouse)					
Position	**Antibody**	**Clone/ Host**	**Company / Item**	**Concentration**	**OPAL Fluor**
1	Ly6G	1A8/Rat	Biolegend/127601	1:4000 (0.125ug/mL)	Opal 520
2	CD8a	4SM15/Rat	eBiosciences/14-0808	1:1500 (0.33ug/mL)	Opal 570
3	CK-wss	WSS/Rabbit	Dako	1:20,000 (0.535ug/mL)	Opal 690

Table S4. NSCLC treatment cohort demographics

Factor	All	CR/PR	SD	PD
N	28	8 (28%)	10 (36%)	10 (39%)
Age	65.1	69.1	62.8	64.2
% Male	57	75	45.5	50
Race				
White	26 (93)	8 (100)	8 (80)	10 (100)
Asian	2 (7)	0	2 (20)	0
Other	0	0	0	0
Smoking				
Current	5 (18)	3 (37.5)	1 (10)	1(10)
Former	15 (53.5)	2 (25)	7 (70)	6 (60)
Never	8 (28.5)	3 (37.5)	2 (20)	3 (30)
Pack Years	30.6	39.6	21.3	34.9
Drug				
pembrolizumab	11 (39.2)	4 (37.5)	5 (50)	2 (20)
nivolumab	9 (32.2)	2 (25)	3 (30)	4 (40)
atezolizumab	6 (21.4)	2 (25)	1 (10)	3 (30)
avelumab	1 (3.6)	0	0	1 (10)
durvalumab	1 (3.6)	0	1 (10)	0