Supplemental Online Content

Supplementary Methods.

Imaging Mass Cytometry

Imaging mass cytometry was performed at the Flow Cytometry and Cellular Imaging Facility at The University of Texas MD Anderson Cancer Center. A tissue microarray was constructed with 6 representative 1-mm^3 formalin-fixed, paraffin-embedded tissue samples: 2 samples obtained from the primary parotid tumor before the first vaccine dose, 2 samples obtained from pulmonary metastases before the first vaccine dose, and 2 samples obtained from pulmonary metastases after the second vaccine dose.

The tissue microarray was deparaffinized and rehydrated prior to heat-induced antigen retrieval performed by microwaving (MW014-MO, EZ-Retriever system, BioGenex) for 15 minutes at 95 °C in Ph 8.5 EZ-AR 2 (EDTA) buffer (HK522-XAK, BioGenex), blocking with 3% bovine serum albumin plus 1% horse serum in phosphate-buffered saline, and incubation with heavy metal-labelled antibodies specified in Supplementary Table 1 overnight at 4 °C. Slides were washed with TBS-T (TBS plus Tween 0.1%) followed by TBS and then incubated with 0.3125 µM Cell-ID Intercalator-Ir (Cat# 201192A, Fluidigm, 1:400 dilution) for the detection of nuclear DNA.

Metal-conjugated antibodies were detected with a Hyperion Imaging Mass Cytometer (Fluidigm). Tissue was laser ablated at 200 Hz. Data were analyzed by custom image analysis scripts. Cells were first segmented based on DNA signal after band-pass Gaussian filtering using Otsu's method, and overlapping cells were divided by seeded watershed. Intensities for each antibody channel were corrected by lateral compensation as described.[1] Marker combinations used to define cell populations are given in Supplementary Table 2. All analyses were performed in Matlab 2019a (Mathworks).

Tumor Molecular Profiling

Primary tumor molecular profiling was performed using a commercial tumor profiling service (CARIS Molecular Intelligence; CARIS Life Sciences, Texas, USA). Targeted-exome sequencing of the patient's primary tumor was also performed using the Oncomine CDx Target test (Thermo Fisher Scientific).

Supplementary Table 1. Antibodies and Metals Used for Imaging Mass Cytometry

Antibody	Metal	Dilution	Vendor	Catalog No	Antibody Clone
Arginase-1	164DY	50	DVS-Fluidigm	3164027D	D4E3M
B7H4	166Er	100	DVS-Fluidigm	3166030D	H74
CD103	146Nd	50	Cell Signaling Technologies[a]	95835S	EP206
CD11b	149Sm	100	DVS-Fluidigm	3149028D	EPR1344
CD11c	171Yb	100	Abcam	ab216655	EP1347Y
CD16	139La	50	Abcam	ab215977	EPR16784
CD163	147Sm	100	DVS-Fluidigm	3147021D	EDHu-1
CD20	161Dy	50	DVS-Fluidigm	3161029D	H1
CD206	163Dy	150	BIORAD	MCA5552Z	5C11
CD3	170Er	75	DVS-Fluidigm	3170019D	Polyclonal, C-terminal
CD31/PECAM	151Eu	75	DVS-Fluidigm	3151025D	EPR3094
CD4	156Gd	200	DVS-Fluidigm	3156033D	EPR6855
CD45	152Sm	75	DVS-Fluidigm	3152018D	D9M8I
CD45RO	173Yb	100	DVS-Fluidigm	3173016D	UCHL1
CD56	145Nd	50	Cell Marque	156R-94	MRQ-42
CD68	165Ho	100	Thermo Fisher	14068882	KP1
CD8a	162Dy	200	DVS-Fluidigm	3162034D	C8/144B
FOXP3	155Gd	50	DVS-Fluidigm	3155016D	236A/E7
Granzyme B	167Er	50	DVS-Fluidigm	3167021D	EPR20129-217
HLA-DR	174Yb	400	DVS-Fluidigm	3174023D	YE2/36 HLK
iNOS	159Tb	50	Abcam	ab239990	SP126
Ki67	168Er	75	DVS-Fluidigm	3168022D	B56
p63	160Gd	100	Cell Signaling Technologies[a]	13109BF	D2K8X
pan-Keratin	148Nd	100	DVS-Fluidigm	3148020D	C11
PD-L1	172Yb	100	Bethyl Laboratories[a]	BLR020E	BLR020E
Siglec-15	169Tm	100	Thermo Fisher	PA572765	Polyclonal
TIGIT	175Lu	25	Abcam	ab243903	BLR047F
TIM3	154Sm	50	DVS-Fluidigm	3154024D	D5D5R
Vimentin	143Nd	400	DVS-Fluidigm	3143027D	D21H3
α-SMA	141Pr	300	DVS-Fluidigm	3141017D	1A4

[a]Bethyl Laboratories and Cell Signaling Technologies graciously provided antibodies to the MD Anderson Flow Cytometry and Cellular Imaging Facility for use in IMC.

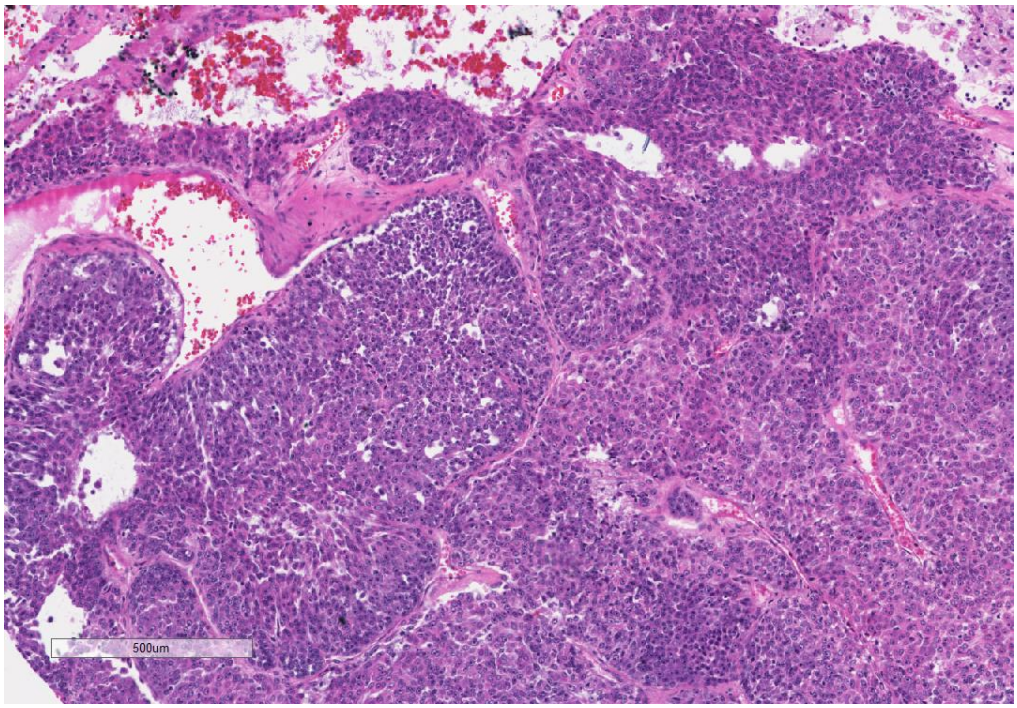
Supplementary Table 2. Cell Populations Analyzed and Corresponding Markers

CD45+ immune Cells	**Markers**
CD8 T cells	CD3+, CD8+
Memory CD8 T cells	CD45RO+ CD8 T cells
CD4 T cells	CD3+, CD4+
Memory CD4 T cells	CD45RO+ CD4 T cells
Regulatory T cells	FoxP3+ CD4 T Cells
All T cells	All CD4 and CD8 T cells
NK cells	CD56+, CD3-
Cytolytic cells	Granzyme B+ and CD56+ or CD8+
B cells	CD20+
Dendritic cells	CD11c+, CD68-
HLA-DR+ dendritic cells	HLA-DR+ dendritic cells
Macrophages	CD68 or CD163+CD206, not T cells, B cells, or dendritic cells
M2 macrophages	CD163+ or CD206+ macrophages
M0/M1 macrophages	Macrophages negative for CD163, CD206, and Arg1
All myeloid cells	All macrophages, dendritic cells, and Arg1+ or CD11b+
Neutrophils	CD16+, not T cells, B cells, or myeloid cells
All immune cells	All immune cells described above
Other cells	
Tumor cells	Pan-cytokeratin+ or p63+, CD31-, non-immune cells
Endothelial cells	CD31+, pan-cytokeratin-, p63-, non-immune cells
Fibroblasts	Vimentin+ or αSMA+, CD31-, pan-cytokeratin-, p63-, non-immune cells
Functional markers	
Proliferating cells	Ki67
T cell function	TIM3, TIGIT

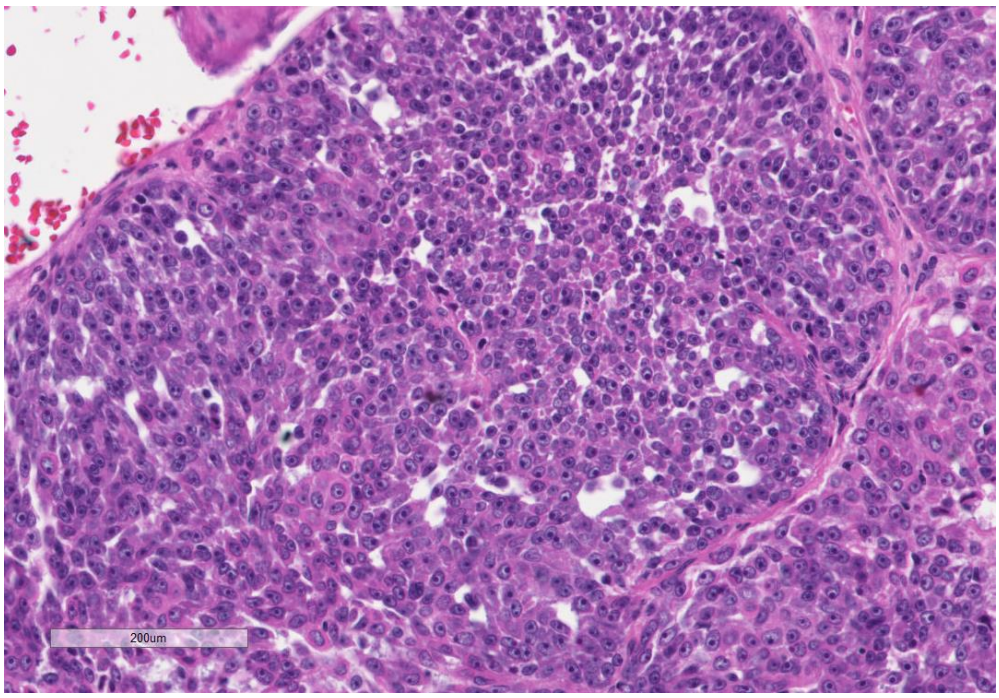
Supplementary Figure 1. Representative hematoxylin and eosin–stained sections.

(A-B) Sections of primary parotid tumor at 4x (A) and 10x (B) magnification. Morphologically, the carcinoma is composed of relatively monotonous round cells with prominent nucleoli and eosinophilic cytoplasm; several areas have a more basophilic and other areas have a rhabdoid/plasmacytic-type appearance of the neoplastic cells. The phenotype is suggestive of a dominant myoepithelial carcinoma. Very rare, scattered mononuclear/lymphocytic elements are noted at the tumor border. (C-D) Section of pre-vaccination lung biopsy specimen at 10x and 4x magnification. The morphology is that of metastatic myoepithelial carcinoma, and entirely resembles the morphology of the primary tumor, including with respect to the immune environment, which has a rare mononuclear/lymphocytic inflammatory infiltrate. (E-F) Sections of post-vaccination lung biopsy specimens at 4x (E) and 10x (F) magnification. There is a massive inflammatory infiltrate with embedded scant tumor clusters (< 5%). The inflammatory infiltrate consists of admixed mononuclear cells (lymphocytes, plasma cells, and monocytes) and a significant number of histiocytes.

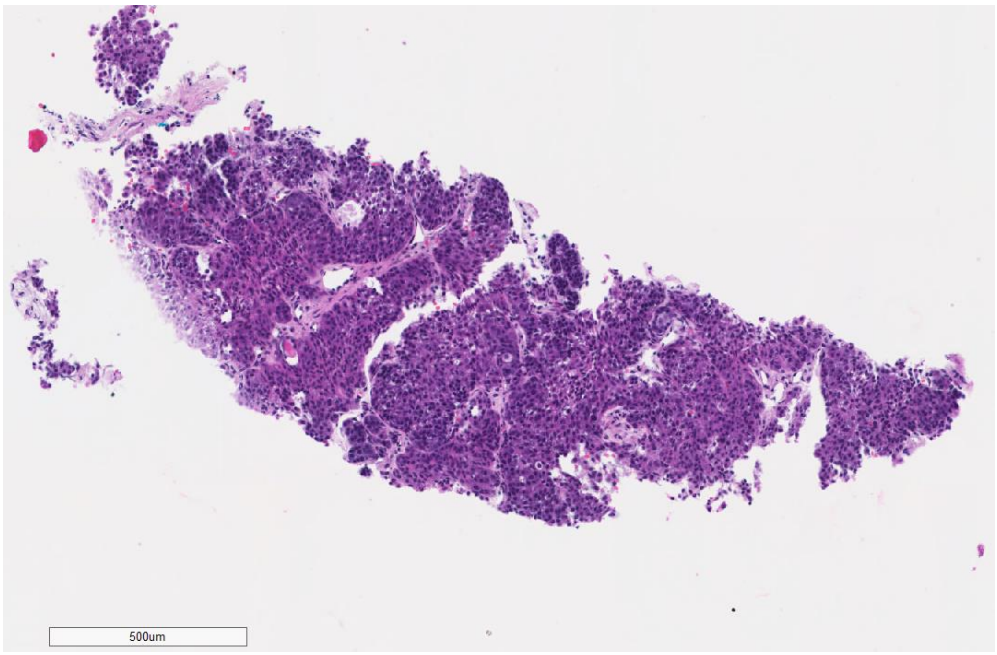
A.



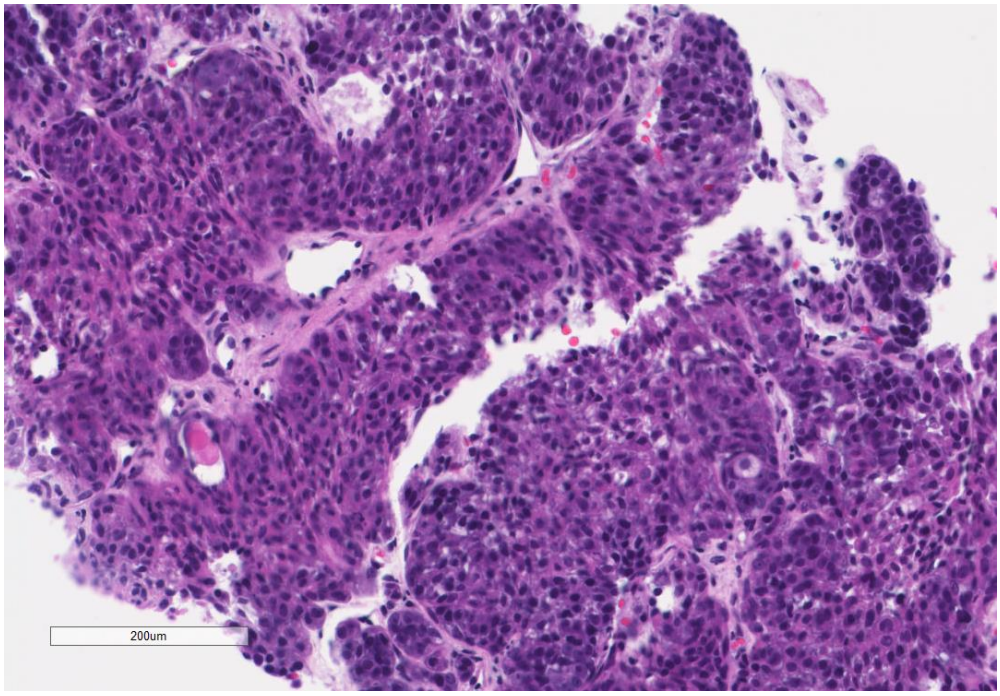
B.



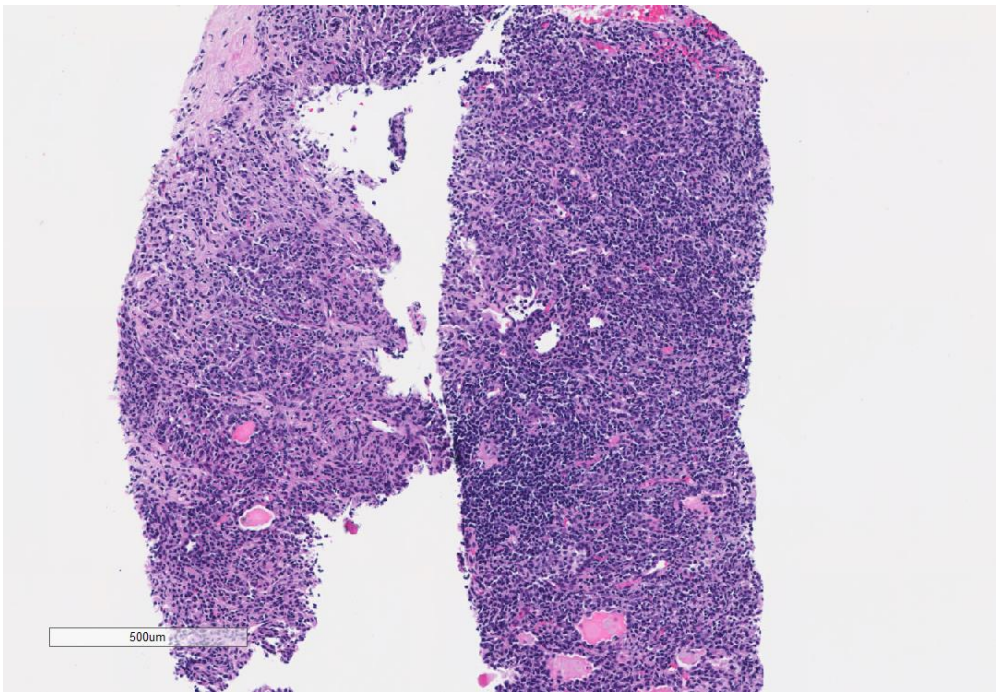
C.



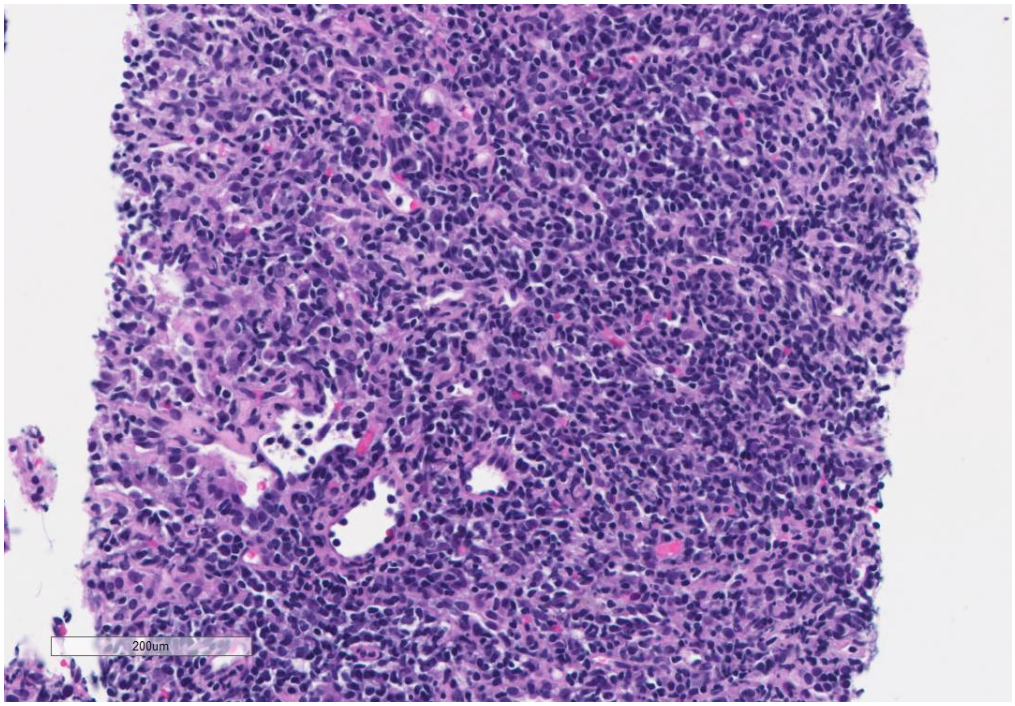
D.



E.



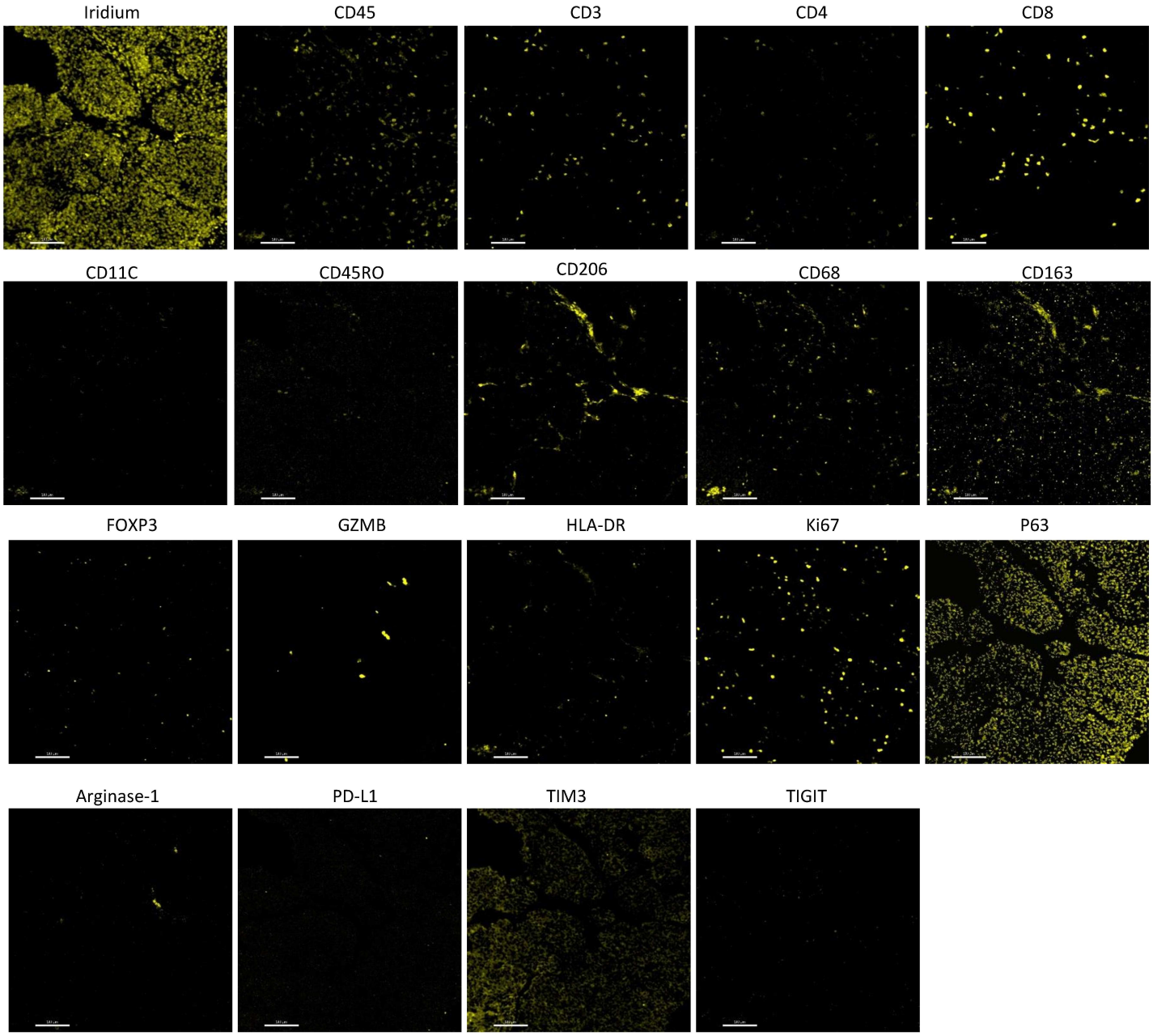
F.



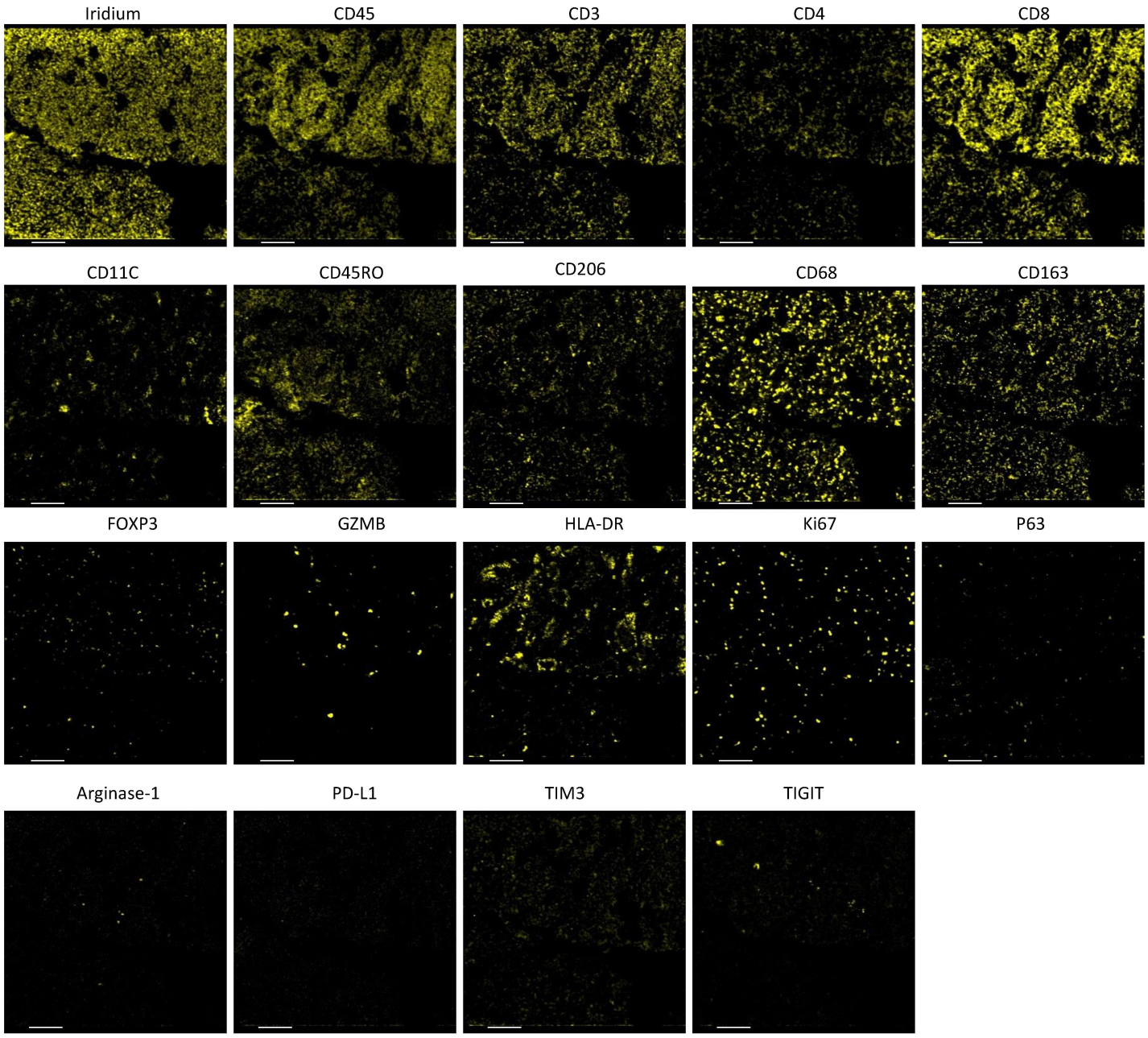
IMAGING MASS CYTOMETRY IMAGES

Supplementary Figure 2. Imaging mass cytometry images showing expression of antibody markers in representative lung biopsy specimens. (A) Pre-vaccine biopsy specimen. All images are of the same region of the same tumor (X, Y 310, 130; height and width, 750 pixels). Scale bars, 100 µm. (B) Post-vaccine biopsy specimen. All images are of the same region of the same tumor (X, Y 5, 85; height and width, 750 pixels). Scale bars, 100 µm.

A.



B.


Iridium
CD45
CD3
CD4
CD8
CD11C
CD45RO
CD206
CD68
CD163
FOXP3
GZMB
HLA-DR
Ki67
P63
Arginase-1
PD-L1
TIM3
TIGIT

Reference

1. Bai Y, Zhu B, Rovira-Clave X, et al. Adjacent Cell Marker Lateral Spillover Compensation and Reinforcement for Multiplexed Images. Front Immunol 2021;12:652631.