FIG. 1

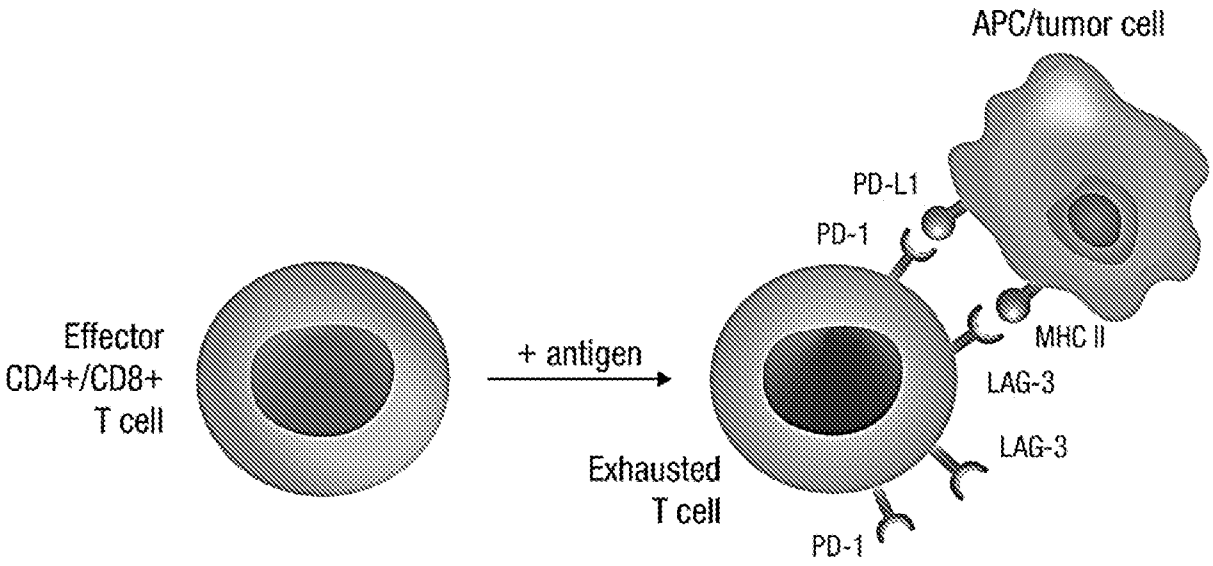

APC/tumor cell
PD-L1
PD-1
MHC II
LAG-3
LAG-3
PD-1
Effector
CD4+/CD8+
T cell
+ antigen
Exhausted
T cell

FIG. 2

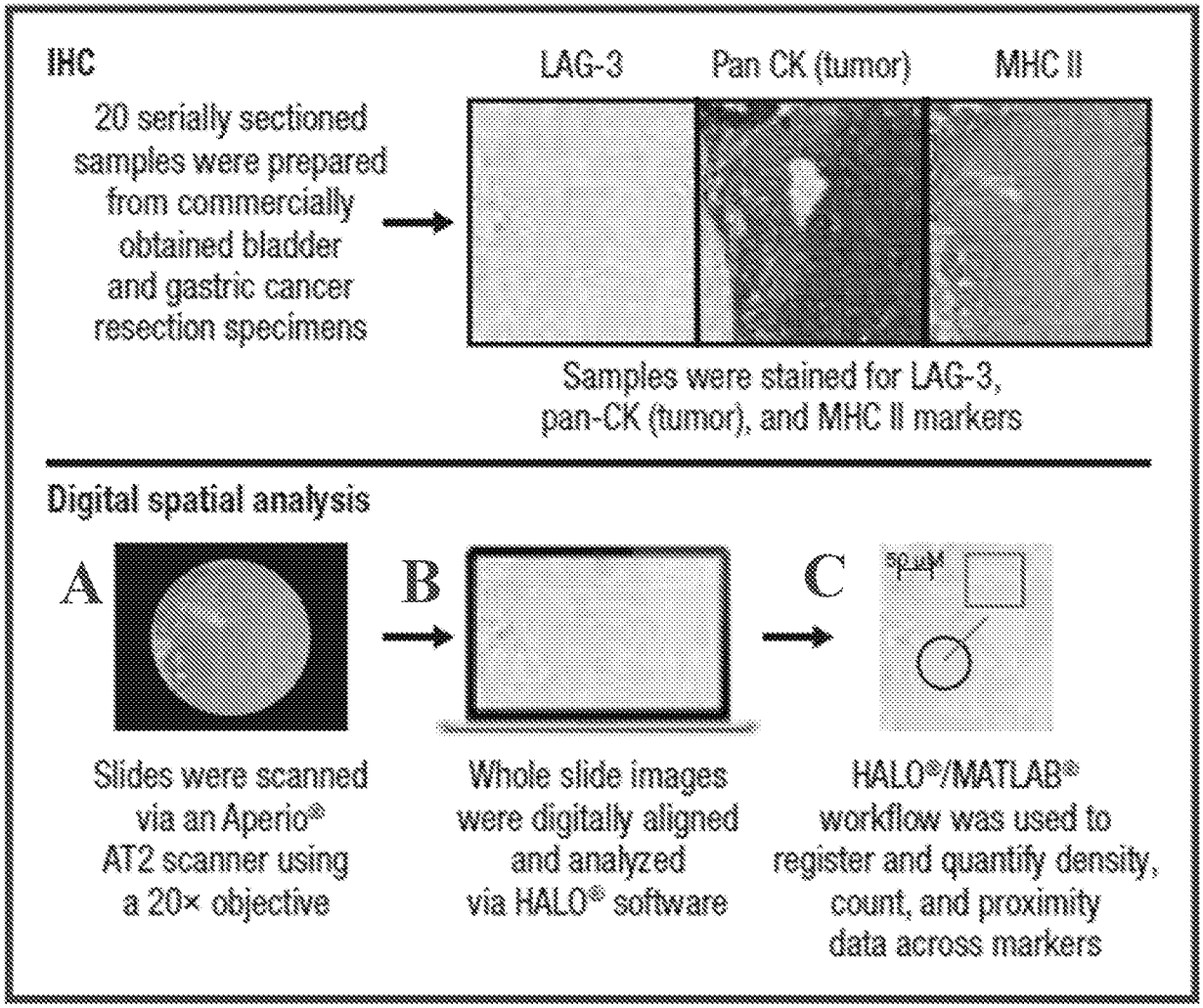

IHC
LAG-3
Pan CK (tumor)
MHC II
20 serially sectioned samples were prepared from commercially obtained bladder and gastric cancer resection specimens
Samples were stained for LAG-3, pan-CK (tumor), and MHC II markers
Digital spatial analysis
A
B
C
Slides were scanned via an Aperio® AT2 scanner using a 20× objective
Whole slide images were digitally aligned and analyzed via HALO® software
HALO®/MATLAB® workflow was used to register and quantify density, count, and proximity data across markers

FIG. 3

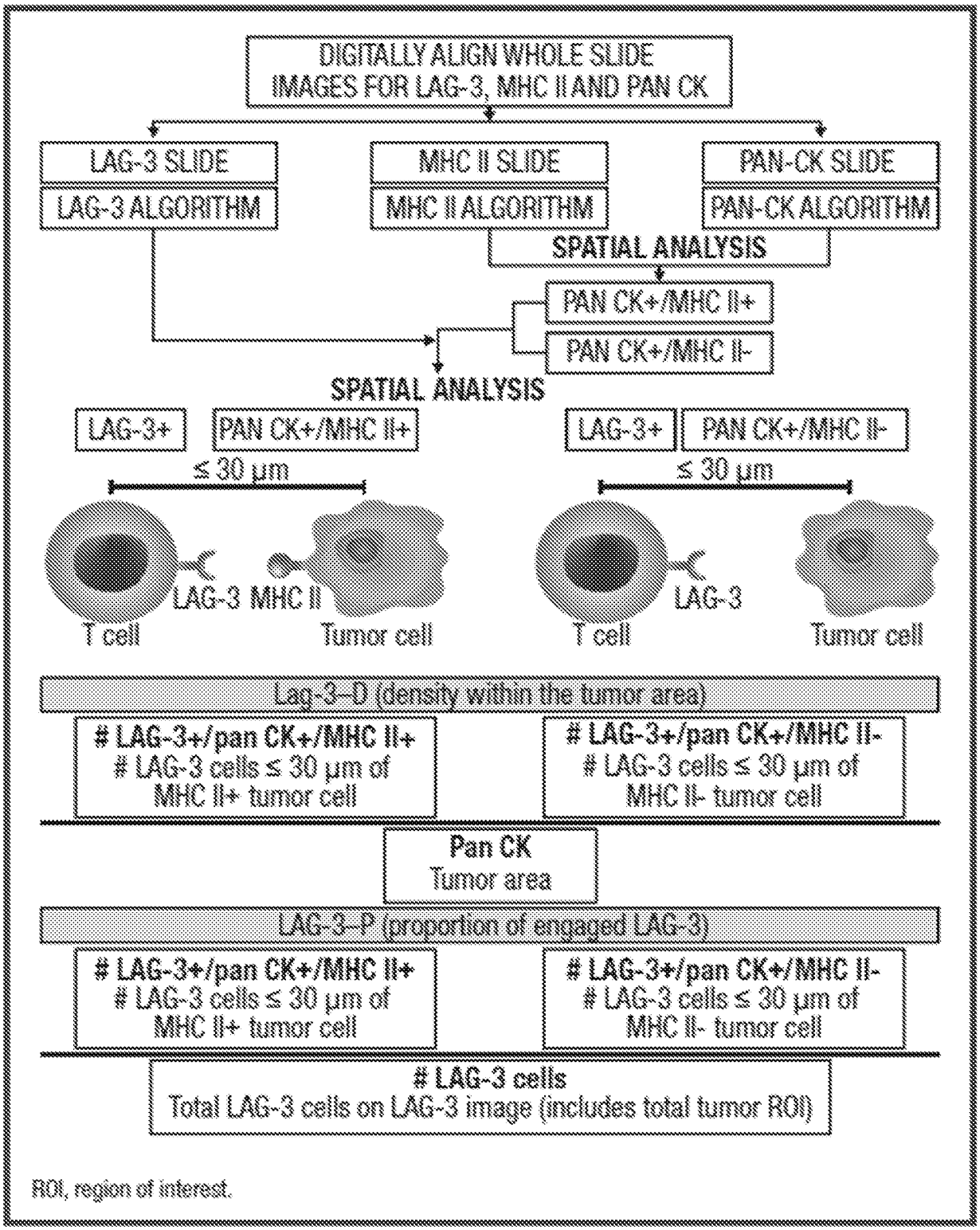

DIGITALLY ALIGN WHOLE SLIDE IMAGES FOR LAG-3, MHC II AND PAN CK
LAG-3 SLIDE
LAG-3 ALGORITHM
MHC II SLIDE
MHC II ALGORITHM
PAN-CK SLIDE
PAN-CK ALGORITHM
SPATIAL ANALYSIS
PAN CK+/MHC II+
PAN CK+/MHC II-
SPATIAL ANALYSIS
LAG-3+
PAN CK+/MHC II+
≤ 30 µm
LAG-3 MHC II
T cell
Tumor cell
LAG-3+
PAN CK+/MHC II-
≤ 30 µm
LAG-3
T cell
Tumor cell
Lag-3–D (density within the tumor area)
LAG-3+/pan CK+/MHC II+
LAG-3 cells ≤ 30 µm of MHC II+ tumor cell
LAG-3+/pan CK+/MHC II-
LAG-3 cells ≤ 30 µm of MHC II- tumor cell
Pan CK
Tumor area
LAG-3–P (proportion of engaged LAG-3)
LAG-3+/pan CK+/MHC II+
LAG-3 cells ≤ 30 µm of MHC II+ tumor cell
LAG-3+/pan CK+/MHC II-
LAG-3 cells ≤ 30 µm of MHC II- tumor cell
LAG-3 cells
Total LAG-3 cells on LAG-3 image (includes total tumor ROI)
ROI, region of interest.

FIG. 4

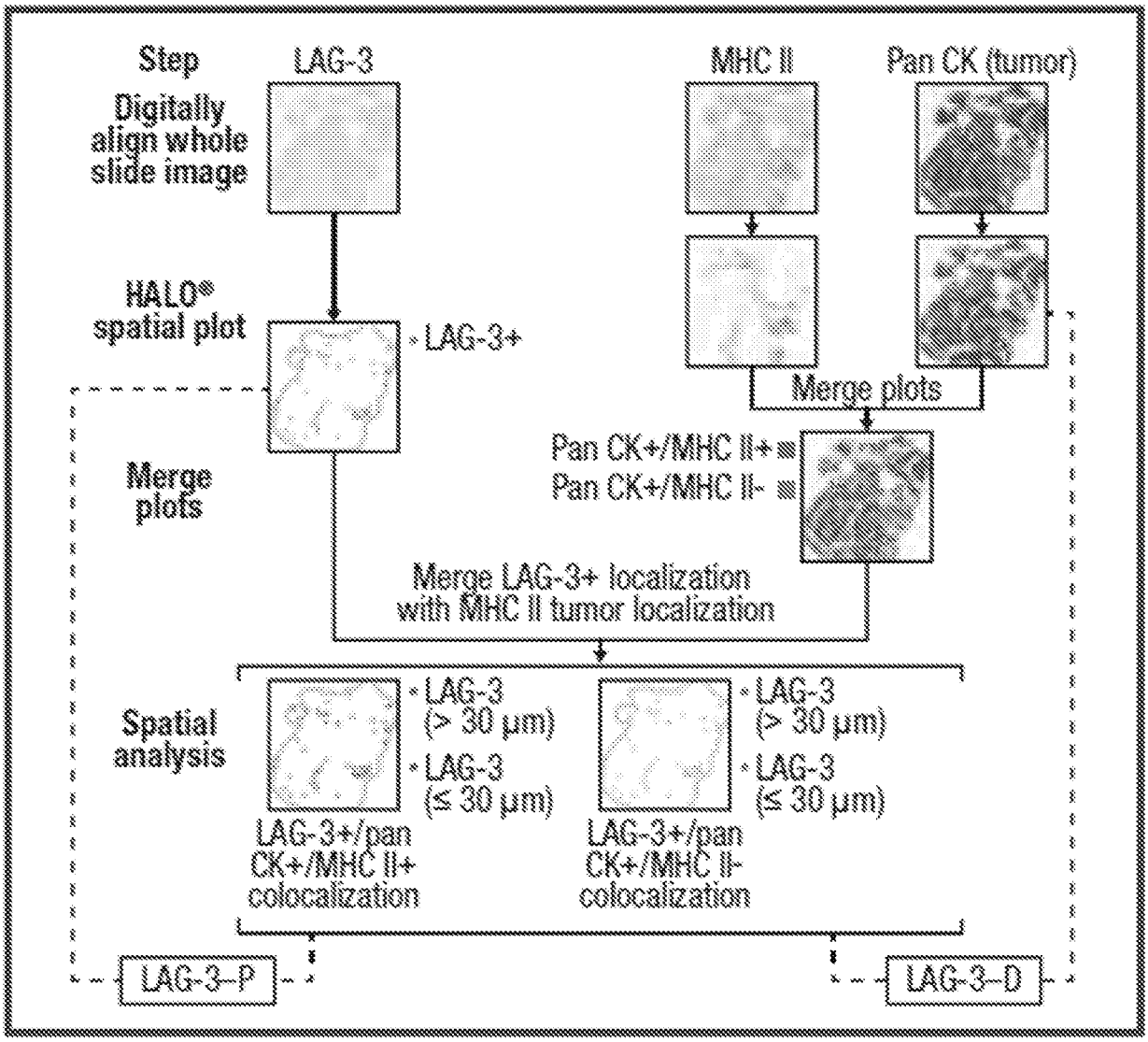

Step
LAG-3
MHC II
Pan CK (tumor)
Digitally align whole slide image
HALO® spatial plot
LAG-3+
Merge plots
Pan CK+/MHC II+
Pan CK+/MHC II-
Merge plots
Merge LAG-3+ localization with MHC II tumor localization
Spatial analysis
LAG-3 (> 30 µm)
LAG-3 (≤ 30 µm)
LAG-3+/pan CK+/MHC II+ colocalization
LAG-3 (> 30 µm)
LAG-3 (≤ 30 µm)
LAG-3+/pan CK+/MHC II- colocalization
LAG-3–P
LAG-3–D

FIG. 5

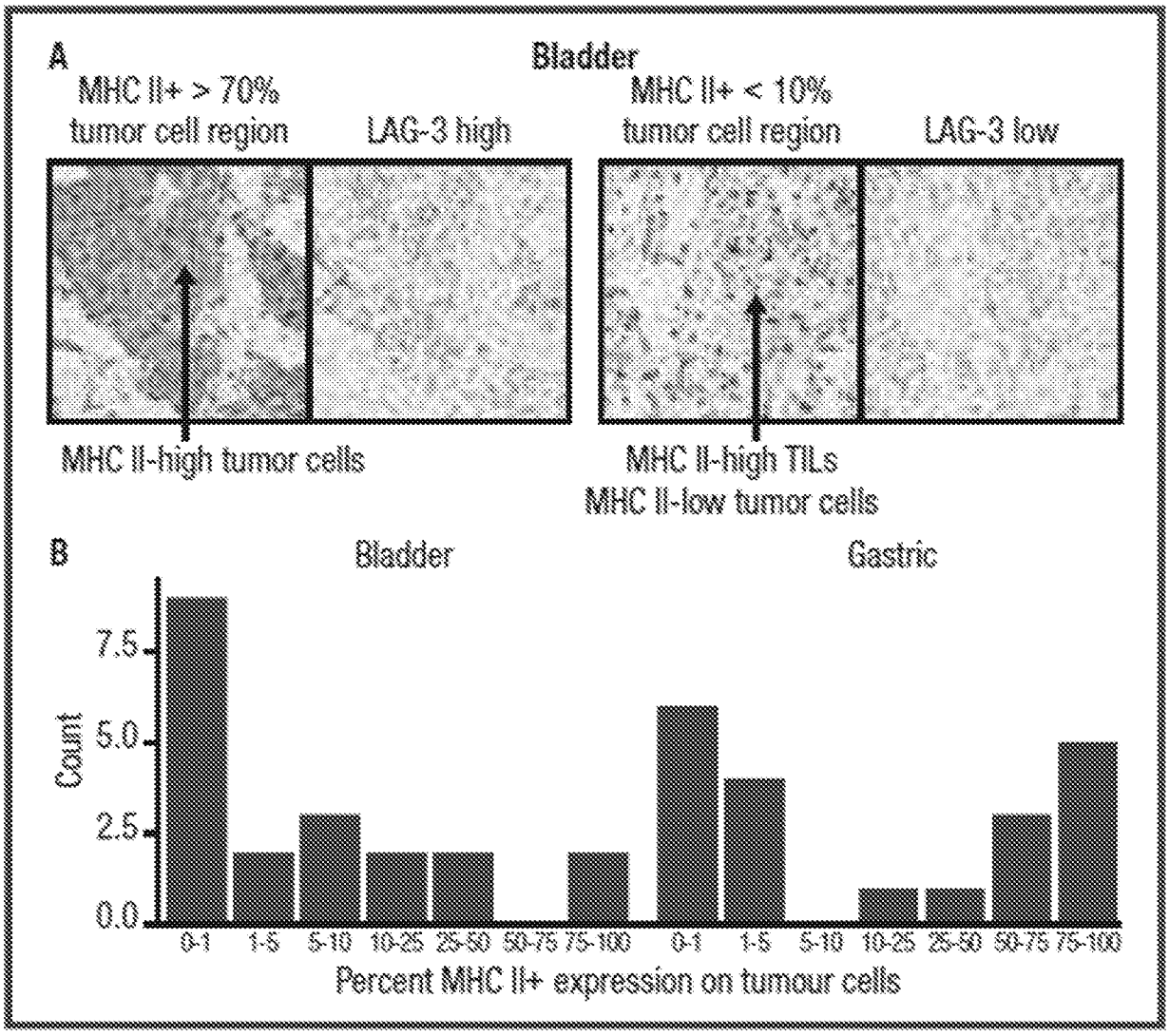

A
Bladder
MHC II+ > 70%
tumor cell region
LAG-3 high
MHC II+ < 10%
tumor cell region
LAG-3 low
MHC II-high tumor cells
MHC II-high TILs
MHC II-low tumor cells
B
Bladder
Gastric
Count
7.5
5.0
2.5
0.0
0-1
1-5
5-10
10-25
25-50
50-75
75-100
Percent MHC II+ expression on tumour cells

FIG. 6

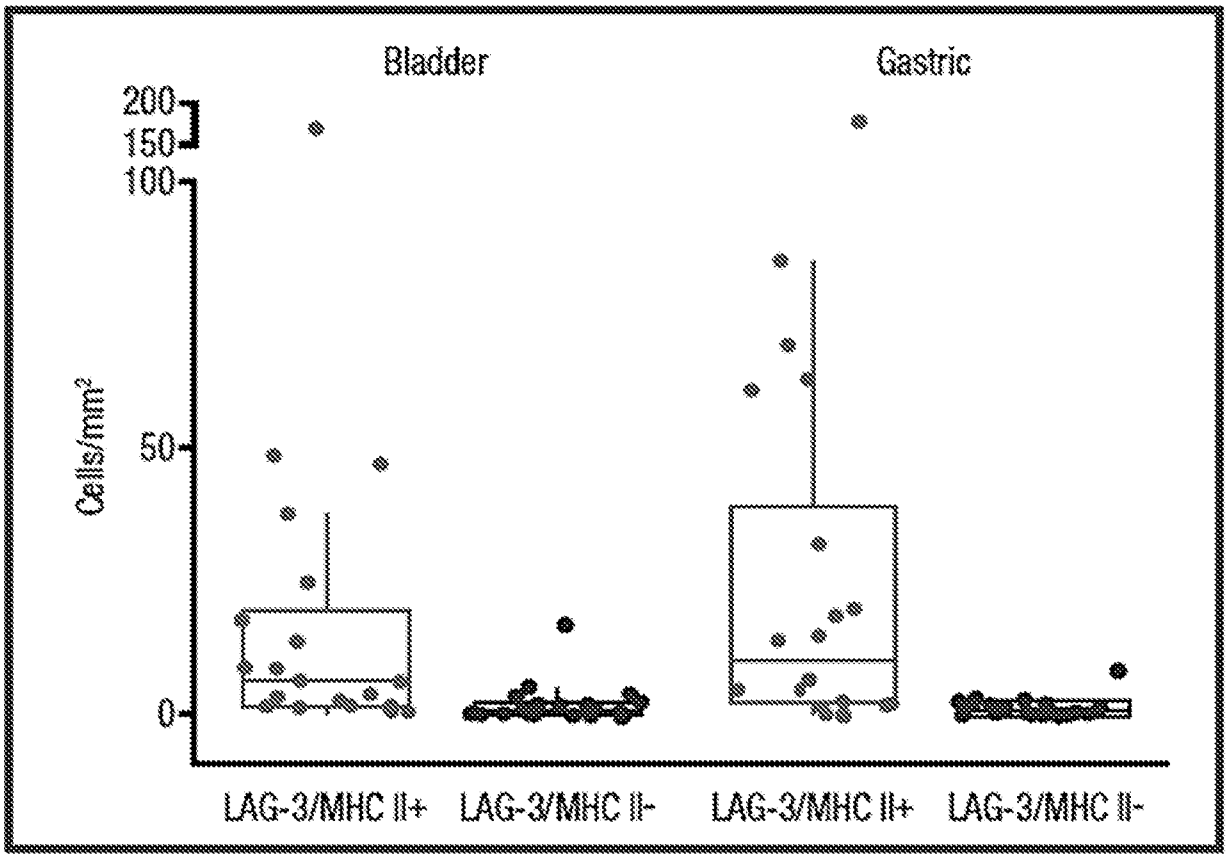
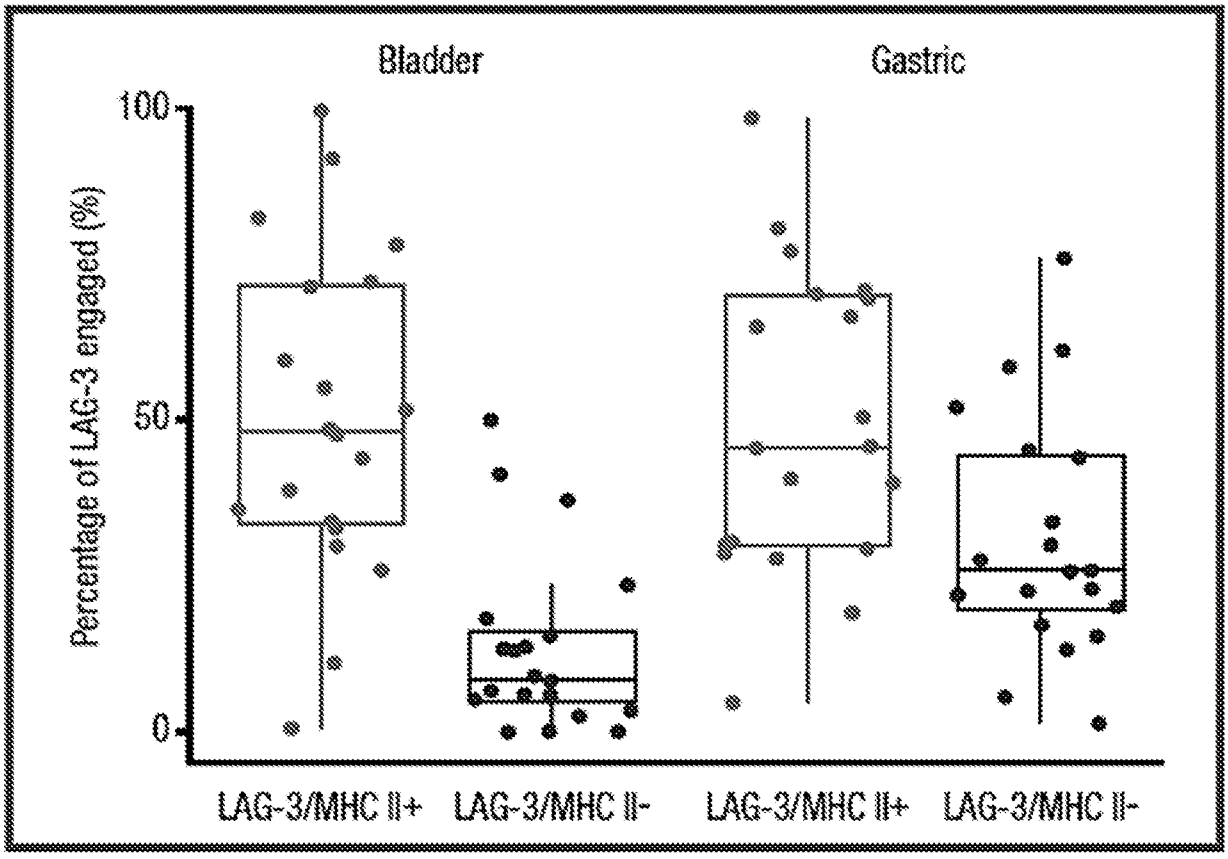

Bladder
Gastric
200
150
100
50
0
Cells/mm²
LAG-3/MHC II+
LAG-3/MHC II-
LAG-3/MHC II+
LAG-3/MHC II-

FIG. 7


Bladder
Gastric
100
50
0
Percentage of LAG-3 engaged (%)
LAG-3/MHC II+
LAG-3/MHC II-
LAG-3/MHC II+
LAG-3/MHC II-