Supplementary Methods

All reagents were purchased from Sigma/Aldrich unless otherwise noted. The IgG3 2^{o} antibody was purchased from Southern Biotec, Alabama, USA (Goat anti-mouse IgG3-AP Human; catalog no. 1100-04). MCF-7 (human breast adenocarcinoma, HTB 24), MDA-MB-231 (human breast adenocarcinoma, HTB-26) and PANC-1 (human pancreatic carcinoma, CRL-1469) were purchased from American Type Culture Collection (ATCC). U251N (human neuroblastoma multiformes) were donated from the Deparment of Neurology/Research-HFHS. RPMI 1640 (Cat# 10-040-CV), L-glutamine 200 mM solution (Cat# 25-005-CI), DMEM (Cat# 10-017-CV), HBSS (Hank's Balanced Salt Solution) 1X (Cat# 21-022-CV), Trypsin EDTA, 1X (Cat# 25-052-CI), MEM (Minimum Essential Medium Eagle) (Cat# 10-011-CV), Sodium Pyruvate (100 mM solution (Cat# 25-000-CI), Nonessential Amino Acids 100 mM solution (Cat# 25-025-CI) and Penicillin-Streptomycin solution (Cat# 30-002-CI) were purchased from MediaTech, Inc., Manassas, VA 20109. Bovine calf serum, (Cat# SH30072.03) was purchased from HyClone, Logan, Utah 84321. Human recombinant insulin, 4 mg/mL (Cat # 12585-014) and Trypan Blue stain 0.4% (Cat# 15250-061) was purchased from Invitrogen, Carlsbad, CA 92008.

Cell Culture and Cell Fixation

For MCF-7, MDA MB-231, and Panc-1 cells, $1x10^{6}$ cells/mL were plated and cultured in standard 6-well plates (one plate set-up for each cell type) (Corning/Costar 3596, Corning, NY 14831). The cells were then counted, and the viability determined by trypan blue exclusion using a hemocytometer and an Olympus Compound Microscope. The media was discarded and 3 mL of phosphate buffered saline (PBS) was added to each well. The wells were gently rinsed and the PBS was discarded. A 0.5 mL of EDTA-PBS solution was added next and the plates were left to stand for 5 min. The cells were detached by gently rinsing using a pipettor and collected in a 15 mL centrifuge tube (plates were checked using a Zeiss inverted microscope to confirm that 95% of the cells were detached). The collected cells were then centrifuged (Thermo IEC CL31 Multi-Speed Centrifuge) at 1100 rpm for 10 min and the EDTA-PBS supernatant was discarded. The cell pellet was resuspended in wash buffer (1X PBS, 5% BSA, and 2.5% sodium azide) and centrifuged as above. Finally, the cells were fixed by adding 4 mL of a 1% paraformaldehyde solution to the cell pellet and mixed thoroughly. Fixed cells were stored at -20 °C for future flow cytometry studies.

General Procedure for Tissue Cell Cuture

After cell confluence was determined and the supernatant removed and discarded, 10 mL of HBSS was added to the flask, swirled around and then also discarded. 5 mL of 0.05% trypsin EDTA, 1X was added to the flask and incubated at 37 ^{o}C for 5 minutes. After the cells were released from the bottom of the flask, 5 mL of the above mentioned culture medium is added to the flask, mixed thoroughly and removed to a 10 mL centrifuge tube. A cell count was performed with trypan blue stain using a hemacytometer and an Olympus Compound Microscope. $2x10^{4}$ cells were pipetted into each well of a 96 well flat bottom Costar/Corning

plate per experiment. The plate was organized according to SI Figure 1 as shown. Plates were then placed in a 37 ^{o}C incubator with 5% CO_2 - 95% humidity until ready for use. After plates were prepared, they were allowed to stand for 48-72 hrs prior to use.

MCF-7 (human breast adenocarcinoma)

Cells were grown in a T-75 tissue culture flask (Corning 430725, Corning, NY 14831) with culture medium consisting of MEM + 15% BCS + 1% sodium pyruvate, 1% non essential amino acids, 1% L-glutamine, 1% Pen+Strep and 12.5 ug/mL human recombinant insulin. When confluent (7 days), the supernatant was removed and discarded.

Culture medium: MEM + 15% BCS + 1% sodium pyruvate, 1% non essential amino acids, 1% L-glutamine, 1% Pen + Strep and 12.5 μg/mL human recombinant insulin.

MDA-MB-231 (human breast adenocarcinoma)

Cells were grown in a T-75 tissue culture flask with cell culture medium RPMI 1640 + 15% BCS+ 1% L-glutamine and 1% Pen+Strep. When confluent (7 days), the supernatant was removed and discarded.

Culture medium: RPMI 1640 + 15% BCS + 1% L-glutamine + 1% Pen + Strep

PANC-1 (human pancreatic carcinoma)

Cells were grown in a T-75 tissue culture flask with culture medium DMEM + 10% BCS+ 1% L-glutamine and 1% Pen+Strep. When confluent (7 days) the supernatant was removed and discarded.

Culture medium: DMEM + 10% BCS + 1% L-glutamine + 1% Pen + Strep (Dulbecco's Modification of Eagle's Medium)

U251N (human neuroblastoma multiformes) (Dept. of Neurology/Research-HFHS) fibroblast-like and neuroblast-like, adherent

Cells were grown in a T-75 tissue culture flask with DMEM + 10% BCS+ 1% L-glutamine and 1% Pen+Strep. When confluent (7 days), the supernatant was removed and discarded.

Culture medium: DMEM + 10% BCS + 1% L-glutamine + 1% Pen + Strep (Dulbecco's Modification of Eagle's Medium)

Jurkat and JurkatTAg

Jurkat and JurkatTAg cells were cultured in T-25 flasks with a cell concentration of $1x10^6$ cells/mL as determined by trypan blue staining using a hemacytometer and an Olympus Compound Microscope. The cells were collected and centrifuged at 1100 rpm for 10 min and the media was then discarded. Next, the cell pellet was resuspended in wash buffer (4 mL of 1X PBS, 5% BSA, and 2.5% sodium azide) and centrifuged at 1100 rpm for 10 min and the supernatant was then discarded. The process was repeated two more times and then the cells were fixed with 4 mL of a 1% parafomaldehyde solution. Subsequently they were stored at -20 °C for flow cytometry analysis.

Isolation of PMBCs

Venous blood was obtained by asceptic venipuncture. Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood using ficoll hypaque separation (GE Healthcare Biosciences, Sweden). Cells were washed three times in 1X PBS then resuspended in 1% paraformaldehyde, diluted in wash buffer (1X PBS, 5% BSA, and 2.5% sodium azide). Cells were kept at 4 ^oC until used for flow cytometry experiments.

Antibody Labeling of Cells for Flow Cytometry

1% Paraformaldehyde fixed cells were centrifuged at 1200 rpm for 10 min and the supernatant was then discarded. Cells were washed in buffer (1X PBS, 5% BSA, and 2.5% sodium azide) and the pellet was resuspended. The cells were centrifuged again at the same speed as noted above and the supernatant discarded. Cell pellets were incubated with different volumes (1, 3, 5 uls) of antiserum obtained from Tn-PS A1 and PS A1 vaccinated mice“ Samples were incubated on ice for 20 min, centrifuged, as above and then washed two times with ice cold wash-buffer. Cell Pellets were incubated with goat anti murine IgG3 monoclonal antibody conjugated to Alexa Fluor® 488 ”for 30 min, on ice in the dark. The samples were centrifuged and washed three times with ice cold wash-buffer. Finally, the cells in each tube were re-suspended in 200 µL of 1% paraformaldehyde and left overnight at 4 ºC in the dark for next day analysis by flow cytometry.

Cytokine Bead Assay

Cytokines (IL10, IL17A, TNF, IFN-γ, IL6, IL4, IL2) were analyzed from sera of immunized and control animals using the BD Biosciences BD™ Cytometric Bead Array (CBA) Mouse Th1/Th2/Th17 Cytokine Kit. Samples were thawed and diluted 1:5 with dilution buffer supplied by the manufacturer. The sample was then incubated with a mixture of cytokine capture beads. Next, detection reagent was added to each sample and incubated for an additional 2 hrs. All samples were washed using wash buffer (supplied by the manufacturer) and centrifuged to pellet the beads. Supernatants were carefully removed using a vacuum trap to prevent disturbance of the bead pellet. All samples were resuspended in wash buffer (supplied by the

manufacturer) and events acquired using an LSR flow cytometer (BD Biosciences, San Jose CA) equipped with dual Argon and HeNe lasers. Machine setup was performed following manufacturer instructions. For each cytokine bead set used, 300 gated events were collected, i.e. the 7 sets used resulted in the acquisition of 2100 gated events to ensure a sufficient number of events for analysis. Data was analyzed using CellQuest Pro v 4.0.2 software (BDIS). Standard curves were generated by including known standards for each cytokine (BDIS) using Excel 2002 software (MS) and plotting mean fluorescence intensity (MFI) vs concentration. Cytokine concentrations for each sample were obtained by using the MFI and calculating the pg/ml level from the standard curve for each cytokine.

Flow Cytometry Measurements

Antibody binding to cancer cell lines and normal human donor cells was detected using an LSR flow cytometer (BD Biosciences). An acquisition gate was created based on light scatter properties of the cells used. A total of 20,000 events was acquired for analysis and the resultant data was analyzed using CellQuest Pro software v5.2 (BD Biosciences). Markers were adjusted using isotype matched controls to accurately determine the percentage of Alexa Fluor® 488 plus cells in each sample.