Supplementary Material

Supplementary Methods

Human Cohorts

All human plasma samples were obtained with informed consent and the respective studies were conducted under protocols approved and supervised by the University of Texas MD Anderson Institutional Review Board. MDACC Cohort #1 consisted of a case-control cohort comprised of 87 TNBC breast cancer cases and 115 cancer-free (minimum of 3 year follow-up) controls (**Supplementary Table 1**). For MDACC cohort #2, prospective plasma samples were collected from women with newly diagnosed (0 ~ 0.8 years) breast cancer. Blood samples were drawn after diagnostic biopsy and prior to neoadjuvant chemotherapy, or prior to definitive surgery in patients who did not receive chemotherapy in the neoadjuvant setting. Plasma samples were included from stage II, and stage III patients, who had no documented distant metastasis at the time of sample collection, as determined by chest x-ray, bone scan and CT of the abdomen, to assess the risk of developing future metastasis (**Supplementary Table 2**). Control plasmas were drawn from patients enrolled in a biomarker discovery trial to aid low-dose-CT-based screening for lung cancer. Plasmas were selected from patients without lung nodules.

Transgenic Mouse Models

All animal experiments were performed under protocols approved and supervised by the University of Texas MD Anderson Cancer Center Institutional Animal Care and Use Committee. Briefly, transgenic FVB/N-Tg(MMTV-PyVT)634Mul/J (PyMT) mice were obtained from the NCI and bred in-house to obtain plasma samples from tumor-bearing mice and control littermates. PyMT heterozygote males were crossed to FVB wild-type females to generate the cohort of PyMT heterozygote and wild-type females for the study. To avoid bias, PyMT transgenic and control mice were paired at weaning and were matched with respect to age, litter, and cage. Beginning at 5 weeks of age, mice were palpated every other day to detect breast tumor growth. Breast tumors were allowed to develop to 1 cm in diameter, after

which each tumor-bearing mouse and corresponding paired control were euthanized on the same day by CO_2 inhalation. Blood was obtained by cardiac puncture, using a 1 ml syringe and 23G needle, and placed in a K_3EDTA tube. Plasma was isolated by centrifugation at 2000 x g for 5 min. Aliquots were transferred to cryovials and frozen at -80°C. Metabolomics profiling was performed on individual plasmas from 10 PyMT mice and 10 littermate controls, respectively. To generate bitransgenic FVB MMTV-rtTA/TetO-NeuNT, mice capable of conditionally expressing the oncogenic form of Neu were generated using the tetracycline regulatory system by cloning the coding sequence of activate *Neu (NeuNT)* downstream of the minimal tet operator. Founder mice harboring this *TetO-NeuNT* transgene, referred to as TAN, were generated and mated to a previously described line of MMTV-rtTA transgenic mice to yield bitransgenic MTB/TAN offspring. For experiments, bitransgenic FVB MMTV-rtTA/TetO-NeuNT "case" and monotransgenic FVB TetO-NeuNT "control" female mice were paired at weaning and maintained in the same cage. Doxycycline (2 mg/ml) was added to drinking water starting at 8 weeks of age. Mice were palpated every other day to detect mammary tumor growth. Mice were sacrificed when tumors were either visibly apparent or predicted to be of specific size and progressive state. Each pair of case and control mice (n=25 pairs) was euthanized on the same day by CO_2 inhalation. Whole blood was collected by heart puncture and immediately emptied into K3EDTA coated 1.5mL microcentrifuge tubes followed by subsequent centrifugation at 5000 rpm for 5min at 4°C. Plasma was thereafter collected and flash frozen in liquid nitrogen. Metabolomics profiling was conducted on individual plasmas from 15 'cases' and 20 'controls'.

Chemicals

Putrescine dihydrochloride and spermidine were purchased from Sigma Aldrich (P5780 and S2626, respectively). Spermine and diminazene (aceturate) were purchased from Cayman Chemical (cat#18041 and cat#18678, respectively).

Cell Culture and Transfection

All breast cancer cell lines were maintained in DMEM (Gibco, #11995065) supplemented with 10% FBS. The identity of each cell line was confirmed by DNA fingerprinting via short tandem repeats at the time of mRNA and total protein lysate preparation using the PowerPlex 1.2 kit (Promega). Fingerprinting results were compared with reference fingerprints maintained by the primary source of the cell line.

Small interfering RNA (siRNA) transfection experiments were performed using the following siRNAs: siControl (Silencer Select Negative Control #1, Life Technologies), si*ODC1* #1 and #2 (s9821 and s9822, Thermo Scientific), si*MYC* #1 and #2 (s9130 and s9131, Thermo Scientific). Cells were transfected at a final concentration of 20nM siRNA using Lipofectamine RNAiMAX (Life Technologies), according to the manufacturer's instructions. Media was exchanged with fresh DMEM + 10% FBS 16 hours post-transfection. Cell lysates were collected 48 hours post-transfection for RNA analyses.

RT-PCR Analysis

RNA was extracted using RNeasy Extraction Kit (Qiagen) according to the manufacturer's protocol. Complementary DNA samples were prepared by combining 10µL of RNA (100ng) with 0.8µL 100mM dNTPs, 1µL 10X multiscribe reverse transcriptase, 1µL of 10X reaction buffer, 2µL random primers, 1µL RNase inhibitor and 3.2µL of ultrapure water (all reagents from Applied Biosciences). PCR cDNA preparation was performed using an Eppendorf Thermal Cycler. Cycling conditions were 25°C/10min, 37°C/120min, 85°C/5min followed by returned to 4°C. TaqMan PCR assay was performed with a 7500 Fast Real-Time PCR System using universal TaqMan PCR master mix (ThermoFisher) and FAM™-labeled probes for MYC (Hs00153408_m1), ODC1 (Hs00159739_m1), SRM (Hs01027696_g1) , SMS (Hs019224834_u1) and SAT1 (Hs00971739_g1) and VIC™-labeled probes for GUSB (Hs_00939627_m1) and ß2M (Hs00187842_m1). PCR reaction was carried out using a BioRad CFX Connect RT System. Cycling conditions were 50°C/2min, 95°C/10min followed by 40 cycles at 95°C/15sec to 60°C/1min. Each sample was run in duplicate. Ct values for each gene were calculated and normalized to CT values for GUSB or ß2M (ΔCT). The ΔΔCT values were then calculated by normalization to the ΔCT values for control.

In vitro viability assays

Cell viability for TNBC cell lines following drug treatment or polyamine treatment was determined using MTS colorimetric cell proliferation assay kits (BioVision Inc) as per the manufacturer's instructions.

Metabolomics Analysis

Exometabolome Experiments

Cells were grown in 1ml of DMEM + 10% FBS in 12-well dishes (Costar) to reach a ~70% (50-80%) confluency, 24 hours post initial seeding. On the day of the experiment, the cells were washed 2 times with 500µL serum free DMEM (Gibco, # 17-207-CV) containing 5mM glucose and 0.5mM glutamine. Then, serum free DMEM (300µL) containing 5mM glucose and 0.5mM glutamine was added to each well and the cells incubated. After the predetermined incubation time (1, 2, 4 and 6 hours), 250µL of the conditioned media was collected. For baseline (T0), 250µL of media was collected directly after the addition of 300µL. All time points were performed in triplicates or quadruplicates. Blank samples containing media only were included and collected at T0 and T6. The 6 hour samples were used to count cell numbers for data normalization. Once all the media samples were collected, the tubes were centrifuged at 2000 ×*g* for 10 min to remove residual debris and the supernatants transferred to 1.5mL tubes (Eppendorf) and stored in -80°C until use for metabolomics analysis.

Primary Metabolites and Biogenic Amines

Plasma metabolites were extracted from pre-aliquoted EDTA plasma (10 µL) with 30µL of LCMS grade methanol (ThermoFisher) in a 96-well microplate (Eppendorf). Plates were heat sealed, vortexed for 5min at 750 rpm, and centrifuged at 2000 × g for 10 minutes at room temperature. The supernatant (10 µL) was carefully transferred to a 96-well plate, leaving behind the precipitated protein. The supernatant was further diluted with 10 µL of 100 mM ammonium formate, pH3. For Hydrophilic Interaction Liquid Chromatography (HILIC) analysis, the samples were diluted with 60 µL LCMS grade acetonitrile (ThermoFisher), whereas samples for C18 analysis were diluted with 60 µL water (GenPure

ultrapure water system, Thermofisher). Each sample solution was transferred to 384-well microplate (Eppendorf) for LCMS analysis.

Frozen media samples were thawed on ice and 30µl transferred to a 96-well microplate (Eppendorf) containing 30µL of 100mM ammonium formate, pH 3.0. The microplates were heat sealed, vortexed for 5min at 750 rpm, and centrifuged at 2000 x g for 10 minutes at room temperature. For Hydrophilic Interaction Liquid Chromatography (HILIC) analysis, 25µL of sample was transferred to a new 96 well microplate containing 75µL acetonitrile, whereas samples for C18 analysis were transferred to a new 96-well microplate containing 75µL water (GenPure ultrapure water system, Thermofisher). Each sample solution was transferred to 384-well microplate (Eppendorf) for LCMS analysis.

For each batch, samples were randomized and matrix-matched reference quality controls and batch-specific pooled quality controls were included.

Untargeted Analysis of Primary Metabolites and Biogenic Amines

Untargeted metabolomics analysis was conducted on Waters Acquity™ UPLC system with 2D column regeneration configuration (I-class and H-class) coupled to a Xevo G2-XS quadrupole time-of-flight (qTOF) mass spectrometer. Chromatographic separation was performed using HILIC (Acquity™ UPLC BEH amide, 100 Å, 1.7 µm 2.1× 100mm, , Waters Corporation, Milford, U.S.A) and C18 (Acquity™ UPLC HSS T3, 100 Å, 1.8 µm,, 2.1×100mm, Water Corporation, Milford, U.S.A) columns at 45°C.

Quaternary solvent system mobile phases were (A) 0.1% formic acid in water, (B) 0.1% formic acid in acetonitrile and (D) 100mM ammonium formate, pH 3. Samples were separated using the following gradient profile: for the HILIC separation a starting gradient of 95% B and 5% D was increase linearly to 70% A, 25% B and 5% D over a 5min period at 0.4mL/min flow rate, followed by 1 min isocratic gradient at 100 % A at 0.4mL/min flow rate. For C18 separation, a chromatography gradient of was as follows: starting conditions, 100% A, with linear increase to final conditions of 5% A, 95% B followed by isocratic gradient at 95% B, 5% D for 1 min.

Binary pump was used for column regeneration and equilibration. The solvent system mobile phases were (A1) 100mM ammonium formate, pH 3, (A2) 0.1 % formic in 2-propanol and (B1) 0.1 % formic acid in acetonitrile. The HILIC column was stripped using 90% A2 for 5 min followed by 2 min equilibration using 100% B1 at 0.3 mL/min flowrate. Reverse phase C18 column regeneration was performed using 95% A1, 5% B1 for 2 min followed by column equilibration using 5% A1, 95% B1 for 5 min.

Mass Spectrometry Data Acquisition

Mass spectrometry data was acquired in sensitivity, positive and negative electrospray ionization mode within 50-1200 Da range for primary metabolites and 100-2000 Da for complex lipids. For the electrospray acquisition, the capillary voltage was set at 1.5 kV (positive), 3.0kV (negative), sample cone voltage 30V, source temperature at 120°C, cone gas flow 50L/h and desolvation gas flow rate of 800L/h with scan time of 0.5 seconds in continuum mode. Leucine Enkephalin: 556.2771 Da (positive) and 554.2615 Da (negative) were used for lockspray correction and scans were performed at 0.5 min. The injection volume for each sample was 3µL, unless otherwise specified. The acquisition was carried out with instrument auto gain control to optimize instrument sensitivity over the samples acquisition time.

Pooled quality control samples were analyzed after a defined number of samples to assess replicate precision and allow LOESS correction by injection order. Additional data was captured using the MSe function for pooled quality control samples.

Data Processing

Peak picking and retention time alignment of LC-MS and MSe data were performed using Progenesis QI software (Nonlinear, Waters). Data processing and peak annotations were performed using an in-house automated pipeline. Annotations were determined by matching accurate mass and retention times using customized libraries created from authentic standards and/or by matching experimental tandem mass spectrometry data against the NIST MSMS, LipidBlast or HMDB v3 theoretical fragmentations. To correct for injection order drift, each feature was normalized using data from repeat injections of quality control samples collected every 10 injections throughout the run sequence.

Measurement data were smoothed by Locally Weighted Scatterplot Smoothing (LOESS) signal correction (QC-RLSC) as previously described (1). Only detected features exhibiting a relative standard deviation (RSD) less than 30 in quality control samples were considered for further statistical analysis. To reduce data matrix complexity, annotated features with multiple adducts or acquisition mode repeats were collapsed to one representative unique feature. Features were selected based on replicate precision (RSD<30), intensity and best isotope similarity matching to theoretical isotope distributions. Values are reported as ratios relative to the median of historical quality control reference samples run with every analytical batch for the given analyte. For exometabolome trials (conditioned media), values were rescaled to the median area of the historical quality control sample, rates (area units per hour) normalized to protein abundance for each cell line were calculated for each cell line and reported as area units per hour per 100µg protein.

Immune Cell Signature Analyses

Specific immune cell infiltration was computationally inferred using RNA-seq data based on gene sets overexpressed in one of 24 immune cell types according to Bindea et al. [1]. Scoring of TCGA cancer samples for each of the immune cell signatures and for expression of Antigen Presentation MHC class I (APM1) genes (HLA-A/B/C, ß2M, TAP1/2, TAPBP) or Antigen Presentation MHC class II (APM2) genes are described elsewhere [2]. For immune cell signature scoring of Hatzis [3] and Curtis [4] cancer samples according to the Bindea criteria [1], aggregate gene expression (median-centered) was used.

Statistical Analyses

In order to find the cut-off point for the covariate that gives the largest difference between individuals in the two already defined groups, we used the method that has been described in Contal and O'Quigley [5]. Using log rank statistic-based on the groups defined by cut-off we have:

$$S_k = \sum_{i=1}^{D} [d_i^+ - d_i \frac{r_i^+}{r_i}]$$

Where D is the total number of distinct death times, d_i is the total number of deaths at each event time (t_i), d_i^+ is the total number of death when DAS value is bigger than the cut-off point. r_i and r_i^+ also define as the total number at risk for all DAS value and DAS value larger than cut-off point respectively. We calculated S_k for all possible cut point in DAS column and the estimated cut point is the value that yields the maximum S_k. In our analysis, the maximum value of S_k is at the top 21.2% of DAS value. In another word, top ~ 21% of DAS values are in the high-risk group and other ~79% are in the low-risk group.

In order to calculate the *p*-value of this test we used the following formula and it gives the value of 0.03. It suggests that DAS level highly relates to survival.

$$p\text{-value} \approx 2\exp(-2Q^2)$$

where:

$$Q = \frac{\max|S_k|}{s\sqrt{D-1}}$$

and

$$s^2 = \frac{1}{D-1} \sum_{i=1}^{D} \{1 - \sum_{j=1}^{i} \frac{1}{D-j+1}\}^2$$

Unsupervised hierarchical clustering was performed using Euclidean distance and Ward's method. Univariate analyses were conducted using Kruskal Wallis for comparisons with more than 2 groups; group specific differences were determined using Dunn's multiple comparison test unless otherwise specified. For two-class comparisons, significance was determined using Wilcoxon rank sum test. Significance was determined at p-values <0.05. Receiver operating characteristic curves were generated using R statistical software. The 95% confidence intervals presented for the individual performance of each biomarker were based on the bootstrap procedure in which we re-sampled with

replacement separately for the controls and the diseased 1000 bootstrap samples. For comparison of gene expression of *SMS* and the tumor immunophenotype, the bottom 25^{th} and top 25^{th} percentiles were chosen as to highlight the effect between the most differential populations. Correlation analyses based on the TCGA TNBC, PPAD and LUSC datasets using continuous variables for *SMS* mRNA expression and gene signatures of tumor immune cell infiltrates are included in **Supplementary Table 4.** The Fischer's exact test was 1-sided as we hypothesize that elevated *SMS* mRNA expression is associated with worse outcome as opposed to variation in *SMS* mRNA expression being associated with worse outcome. Consequently, we test the following hypothesis of H0: $\mu_{\text{top 25th SMS mRNA percentile}} = \mu_{\text{bottom 25th SMS mRNA percentile}}$; Ha: $\mu_{\text{top 25th SMS mRNA percentile}} > \mu_{\text{bottom 25th SMS mRNA percentile}}$.

References

1. Bindea G, Mlecnik B, Tosolini M*, et al.* Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 2013;39(4):782-95.
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3. Hatzis C, Pusztai L, Valero V*, et al.* A genomic predictor of response and survival following taxane-anthracycline chemotherapy for invasive breast cancer. Jama 2011;305(18):1873-81.
4. Curtis C, Shah SP, Chin SF*, et al.* The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 2012;486(7403):346-52.
5. Cecile Contal JOQ. An application of changepoint methods in studying the effect of age on survival in breast cancer. Computational statistics & data analysis 1999;30(3):253-270.

Supplementary Tables

Supplementary Table 1. Patient characteristics for MDACC Cohort #1.

Variable	Controls	TNBC Cases
N	115	87
Risk (N, %)		
Low to Moderate	4 (3.5)	3 (3.4)
Moderate	50 (43.5)	68 (78.2)
High	61 (53.0)	16 (18.4)
Ethnicity (N, %)		
White	84 (73.0)	53 (60.9)
Black	6 (5.2)	13 (14.9)
Hispanic	15 (13.0)	13 (14.9)
Asian Indian	6 (5.2)	4 (4.6)
Other	4 (3.5)	4 (4.6)

Supplementary Table 2. Patient characteristics for MDACC Cohort #2.

Variable	Metastasis	No Metastasis	DCIS	Cancer-free Controls
All Groups, N	90	92	15	30
Age, mean y +/- stdev	49 +/- 12	51 +/- 10	49 +/- 10	62 +/- 4
Stage, N				
I	8	9	-	-
II	30	37	-	-
III	42	46	-	-
IV	10	0	-	-
Menopausal status				
Pre	39	40	10	-
Post	51	52	5	-
Time to Metastasis, mean yrs (range)	2.4 (0.5-8.8)	N/A	-	-
Time of Follow-up, mean yrs (range)	3.5 (0.7-9.3)	4.0 (0.1-8.5)	-	-
ER+ group, N	44	29	15	-
Age, mean y +/- stdev	50 +/- 11	56 +/- 9	49 +/- 10	-
Stage, N				
I	7	9	-	-
II	16	8	-	-
III	17	12	-	-
IV	4	-	-	-
Menopausal status				
Pre	19	8	10	-
Post	25	21	5	-
Time to Metastasis, mean yrs (range)	3.0 (0.6-8.8)	N/A	-	-
Time of Follow-up, mean yrs (range)	4.1 (1.3-9.3)	3.4 (0.1-5.4)	-	-
HER2+ group, N	20	35	-	-
Age, mean y +/- stdev	49 +/- 15	48 +/- 10	-	-
Stage, N				
I	1	-	-	-
II	8	18	-	-
III	7	17	-	-
IV	4	-	-	-
Menopausal status				
Pre	9	17	-	-
Post	11	18	-	-
Time to Metastasis, mean yrs (range)	1.9 (0.5-3.6)	N/A	-	-
Time of Follow-up, mean yrs (range)	3.5 (0.7-9.1)	4.2 (0.9-8.3)	-	-
TNBC group, N	26	28	-	-
Age, mean y +/- stdev	49 +/- 12	50 +/- 11	-	-
Stage, N				
I	-	-	-	-

II	6	11	-	-
III	18	17	-	-
IV	2	-	-	-
Menopausal status				
Pre	11	15	-	-
Post	15	13	-	-
Time to Metastasis, mean yrs (range)	1.7 (0.5-4.1)	N/A	-	-
Time of Follow-up, mean yrs (range)	2.5 (0.8-7.1)	4.3 (1.1-8.5)	-	-

Supplementary Table 3. Detected Polyamines

Polyamine	Assay*	Ret. Time	m/z	Adduct
Spermidine	P_CA	0.43	146.165	[M+H]+
N-acetylspermidine	P_HA	3.92	188.176	[M+H]+
Diacetylspermidine	P_HA	3.13	230.185	[M+H]+
Diacetylspermine	P_HA	3.75	287.244	[M+H]+

*P: Positive Mode Electrospray Ionization; CA-C18 Column; HA-HILIC. Abbrev. P_CA: positive acquisition mode using C18 (Acquity™ UPLC HSS T3 column) method; P_HA: positive mode acquisition using HILIC (Acquity™ UPLC BEH amide column) method (*See Supplementary Material*).

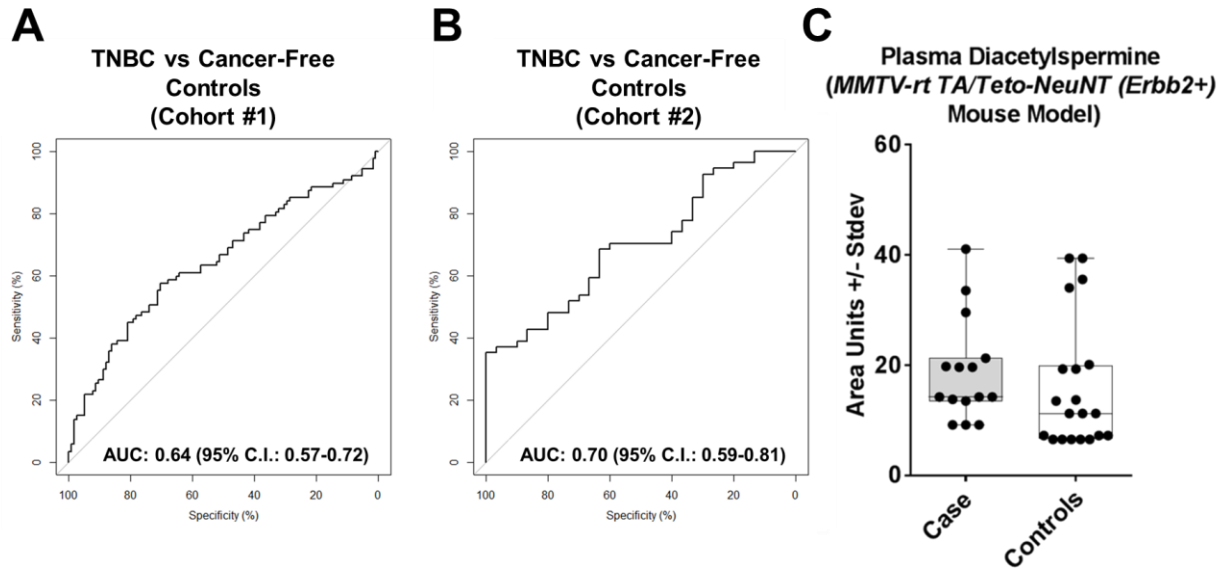
Supplementary Table 4. Spearman correlation coefficients between TCGA-derived mRNA expression of *SMS* and immune-check point related genes and immune cell gene signatures in TNBC, PAAD, CRC and LUSC.

	TNBC		PAAD		LUSC	
Variable	**Spearman Rho**	***P****	**Spearman Rho**	***P****	**Spearman Rho**	***P****
CTAG1B	0.13	.19	0.04	.58	0.12	.005
MAGEA4	0.09	.40	0.06	.41	0.13	.003
SAGE1	0.07	.49	-0.08	.31	-0.02	.59
CD274	0.02	.88	-0.02	.75	0.07	.14
PDCD1	-0.14	.17	-0.16	.03	-0.23	< .001
CD247	-0.14	.16	-0.26	< .001	-0.23	< .001
PDCD1LG2	-0.03	.74	-0.14	.06	-0.04	.33
CTLA4	0.01	.91	-0.19	.01	-0.21	< .001
TNFRSF9	-0.11	.28	-0.11	.16	-0.23	< .001
TNFRSF4	-0.12	.23	-0.06	.42	-0.24	< .001
TLR9	-0.11	.28	-0.16	.03	-0.3	< .001
aDC	0.01	.90	-0.09	.24	-0.24	< .001
APM1 (antigen Presenting MHC1)	-0.02	.84	0.32	< .001	-0.21	< .001
APM2 (Antigen Presenting MHC2)	-0.22	.03	-0.13	.10	-0.28	< .001
B cells	-0.06	.55	-0.3	< .001	-0.19	< .001
CD8 T cells	-0.28	.005	-0.32	< .001	-0.22	< .001
Cytotoxic cells	-0.23	.02	-0.26	< .001	-0.23	< .001
DC	-0.24	.02	-0.31	< .001	-0.21	< .001
Eosinophils	-0.38	< .001	-0.39	< .001	-0.21	< .001
iDC	-0.26	.008	-0.22	.003	-0.22	< .001
Lymph vessels	-0.22	.03	-0.15	.05	-0.14	.001
Macrophages	-0.13	.20	-0.1	.21	-0.25	< .001
Mast cells	-0.35	< .001	-0.36	< .001	-0.23	< .001
Neutrophils	-0.27	.008	-0.06	.40	-0.31	< .001
NK CD56bright cells	-0.29	.004	0.09	.26	-0.14	.002
NK CD56dim cells	-0.11	.29	0.02	.77	-0.26	< .001
NK cells	-0.34	.001	-0.39	< .001	-0.18	< .001
pDC	-0.31	.002	-0.23	.002	-0.27	< .001
T cells	-0.19	.06	-0.32	< .001	-0.25	< .001
T helper cells	-0.05	.65	-0.04	.57	-0.04	.34
Tcm cells	-0.29	.003	-0.37	< .001	-0.19	< .001
Tem cells	-0.18	.08	-0.24	.001	-0.13	.003
Tfh cells	-0.23	.02	-0.43	< .001	-0.19	< .001
Tgd cells	-0.36	< .001	-0.23	.002	-0.1	.02
Th1 cells	-0.11	.27	-0.2	.007	-0.3	< .001
Th17 cells	-0.04	.66	-0.22	.003	-0.18	< .001
Th2 cells	0.13	.19	0.13	.09	0.08	.06

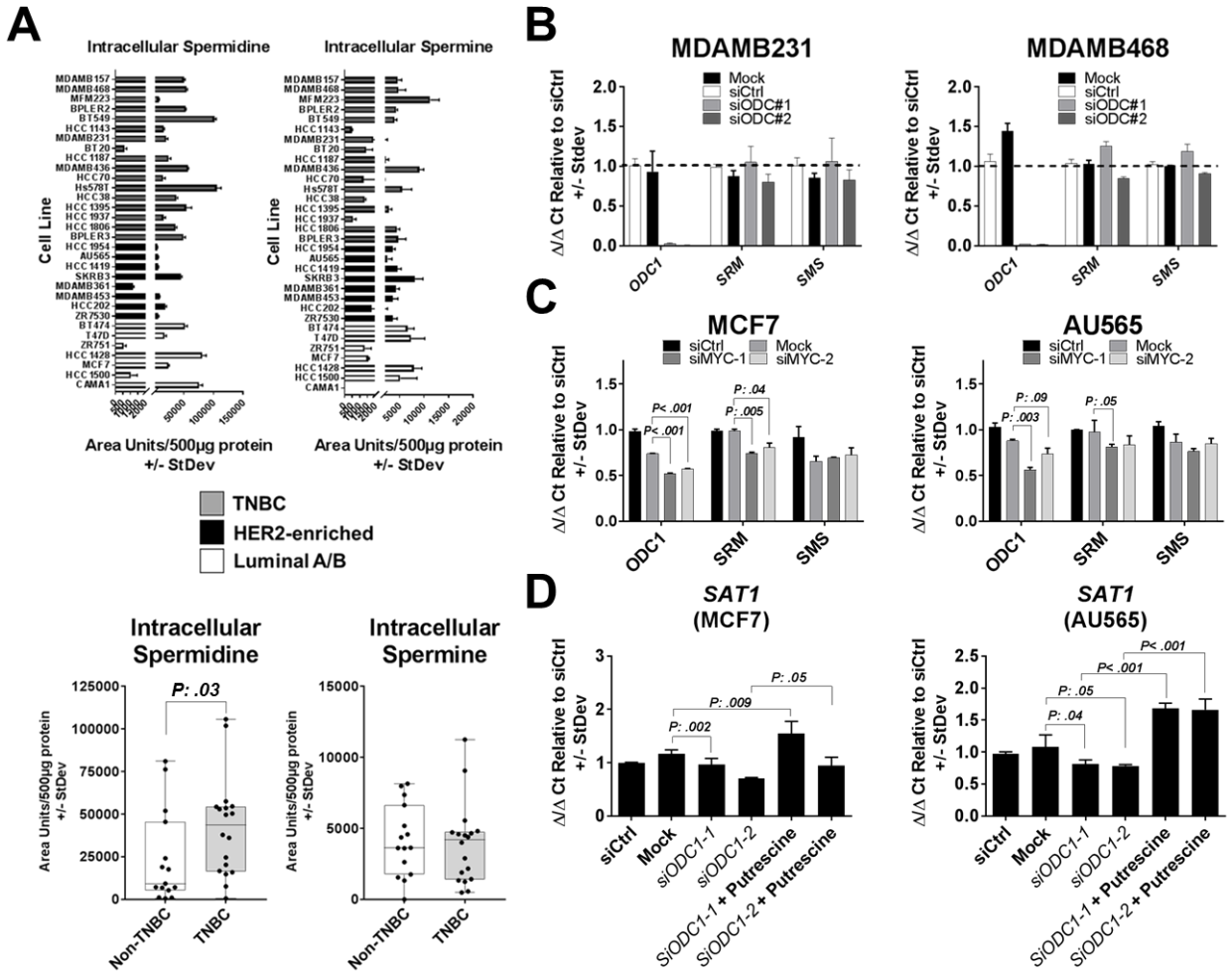
Treg cells	0	.96	-0.24	.002	-0.27	< .001

* Values depict spearman correlation coefficients between TCGA-derived mRNA expression of *SMS* and immune-check point related genes and immune cell gene signatures in TNBC, PAAD, CRC and LUSC. Significance was determined as 2-sided

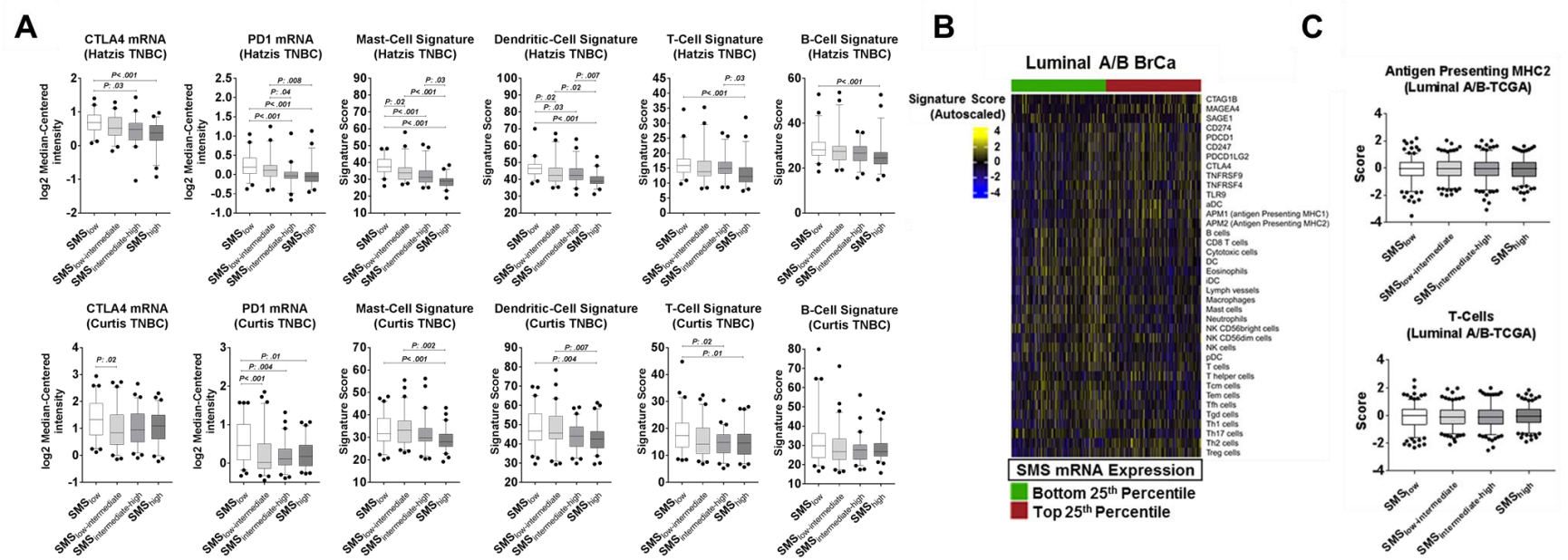
Supplementary Figures



Supplementary Figure 1. Classification performance of plasma DAS in distinguishing TNBC cases from cancer-free controls and plasma levels of DAS in bitransgenic *MMTV-rt TA/Teto-NeuNT (Erbb2+)* mice. A) ROC curves illustrating classification performance of plasma DAS in distinguishing TNBC cases (n=87) from cancer-free controls (n=115) in MDACC Cohort #1. **B)** ROC curves depicting classification performance of plasma DAS in delineating TNBC cases (n=54) from cancer-free controls (n=30) in MDACC Cohort #2. **C)** Plasma DAS levels in *MMTV-rt TA/Teto-NeuNT (Erbb2+)* mice (n=15) compared to littermate controls (n=20).



Supplementary Figure 2. Maintenance of and regulation of homeostatic intracellular polyamine pools. A) Intracellular polyamine levels of spermidine and spermine in 32 breast cancer cell lines. Putrescine was not detected (data not shown). Each cell line was analyzed in biological triplicate. **B)** Relative fold change in mRNA expression ($2^{-\Delta/\Delta\ CT}$) of *ODC1*, *SRM* and *SMS* in TNBC cell lines MDAMB231 and MDAMB468 following transient knockdown of *ODC1*. **C)** Relative fold change in mRNA expression ($2^{-\Delta/\Delta\ CT}$ of *ODC1*, *SRM*, and *SMS* following transient knockdown of *MYC* in non-TNBC cell lines MCF7 and AU565. **D)** Relative fold change in mRNA expression ($2^{-\Delta/\Delta\ CT}$) of *SAT1* following transient knockdown of *ODC1* and rescue by putrescine in non-TNBC cell lines MCF7 and AU565. Significance was determined using 2-sided student T-test.



Supplementary Figure 3. Checkpoint blockade and Immune cell gene signatures in TNBC datasets and Luminal A/B breast cancers. A) Box and whisker plots depicting distribution of *CTLA4*, *PD1* and immune cell gene signatures for Mast Cells, Dendritic Cells, T-cells and B-cells in TNBC tumors stratified into *SMS* mRNA quartiles in the Hatzis [3] (top) and Curtis [4] (bottom) breast cancer cohorts. **B)** Heatmap depicting distribution of TCGA-derived mRNA expression for checkpoint-blockade related genes and immune cell gene signatures in Luminal A/B breast cancer tumors stratified into the bottom 25th and top 75th percentile of *SMS* mRNA expression. **C**) Box and whisker plots depicting distribution of TCGA-derived immune cell gene signatures for antigen presenting MHC class II and T-cells in Luminal A/B breast cancers. HER2-enriched breast cancers were excluded due to insufficient sample size. Statistical significance was determined by Kruskal Wallis test; specific group differences were determined by Dunn's multiple comparison test.