

# **Reporting Summary**

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When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main

## Statistical parameters

text,	text, or Methods section).					
n/a	Cor	nfirmed				
	$\boxtimes$	The $\underline{\text{exact sample size}}(n)$ for each experimental group/condition, given as a discrete number and unit of measurement				
	$\boxtimes$	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly				
	$\boxtimes$	The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.				
	$\boxtimes$	A description of all covariates tested				
	$\boxtimes$	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons				
	$\boxtimes$	A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals)				
	$\boxtimes$	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>				
$\boxtimes$		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings				
X		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes				
	$\boxtimes$	Estimates of effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated				
		Clearly defined error bars				

Our web collection on <u>statistics for biologists</u> may be useful.

### Software and code

Policy information about availability of computer code

State explicitly what error bars represent (e.g. SD, SE, CI)

Data collection No software was used.

Data analysis

Statistical comparisons of all glycan abundances within different annotated regions were performed through Prism9, using the Two-stage step-up method (Benjamini, Krieger, and Yekutieli) with false discovery rate (FDR) of 5%. Multi-level model (mixed-effects model) was used in R Studio to account for potential correlations among different tumor regions within each patient to validate select glycans that were significant by T-test.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

#### Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that supports the findings of this study are available from the corresponding authors upon reasonable request.

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## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
The number of patient cases for each group was dictated based on the # of available tissue samples in Pathology since this study involved rarely resected tissue. A statistician (co-author on this paper) was heavily involved to make sure the number of samples per group would generate valid conclusions from the data by T-test. In experiments with lower number of samples per group (< 5 samples), a mixed effects model was used to control for patient variability in the validation of select glycan markers.

Data exclusions No data was excluded from the analysis.

Replication All analyses involved multiple patients. Major findings central to the conclusions of the paper were validated in vitro.

Randomization Due to the nature of the study, samples were allocated into groups based on disease stage and treatment status (i.e. hormone-sensitive, hormonally-treated, CRPC-adenocarcinoma, CRPC-neuroendocrine).

Blinding Investigators were blinded to group allocation during analyses.

# Reporting for specific materials, systems and methods

Ma:	terials & experimental systems	Methods			
n/a	Involved in the study	n/a	Involved in the study		
$\boxtimes$	Unique biological materials	$\boxtimes$	ChIP-seq		
	Antibodies		Flow cytometry		
	Eukaryotic cell lines	$\boxtimes$	MRI-based neuroimaging		
$\boxtimes$	Palaeontology				
$\boxtimes$	Animals and other organisms				
$\boxtimes$	Human research participants				

### **Antibodies**

Antibodies used

Phaseolus vulgaris Lectin (PHA-L) - FITC, GlycoMatrix, SKU #: 30330033-1, Lot #: L21012703DVAK

Validation

The lectin was validated by the manufacturer with certificate of analysis and relevant citations available on the GlycoMatrix website. Furthermore, since MGAT5 induces formation of the product of PHA-L, we have tested the lectin in MGAT5-overexpressing cells which further confirmed the ability of PHA-L to detect complex, branched glycans.

## Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

ATCC

Authentication

All cell lines used were obtained from ATCC which follows rigorous authentication procedures (sterility testing, CO1, and STR profiling)

Mycoplasma contamination

All cell lines used tested negative for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

### Flow Cytometry

#### Plots

Confirm that:

The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

All plots are contour plots with outliers or pseudocolor plots.

A numerical value for number of cells or percentage (with statistics) is provided.

#### Methodology

Sample preparation

1x106 cells from each cell line (obtained from ATCC) were harvested, washed twice with PBS, re-suspended in staining buffer (PBS + 0.2% FBS) and blocked (FcR Blocking Reagent, Miltenyi Biotec). Cells were then incubated with PHA-L lectin (GlycoMatrix) at a concentration of 10 ug/mL for 30 minutes at 4 °C. Following incubation, cells were washed twice with PBS, and resuspended in staining buffer. 30,000 events were recorded for each tube using a BD LSRII Cell Analyzer. All data was further processed using FlowJo software.

Instrument

BD LSRII Cell Analyzer

Software

DiVa was used for collecting data on the BD LSRII Cell Analyzer. FlowJo software was used for subsequent analysis.

Cell population abundance

Cells were not sorted. Flow cytometry was only used to determine the

Gating strategy

FSC/SSC gating followed by FSC-H/FSC-A gating was used to select only single, viable cells for analysis. An unstained control was subsequently used for determining the FITC+ population which corresponded to cells binding PHA-L.

| Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.