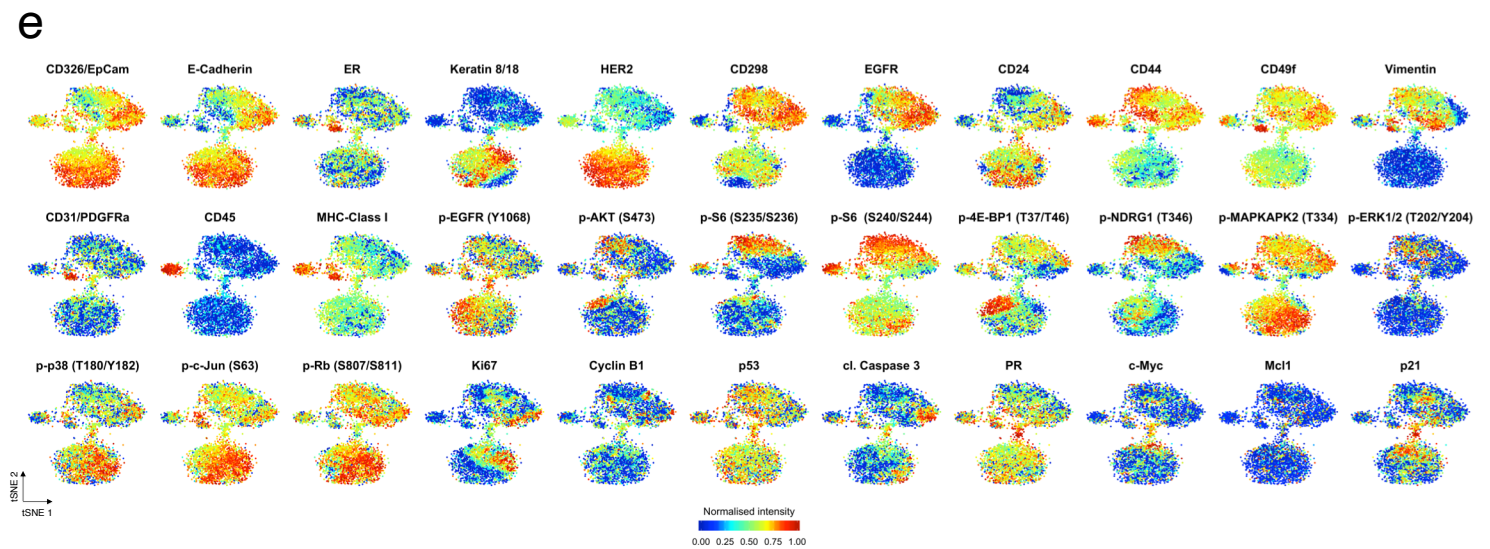
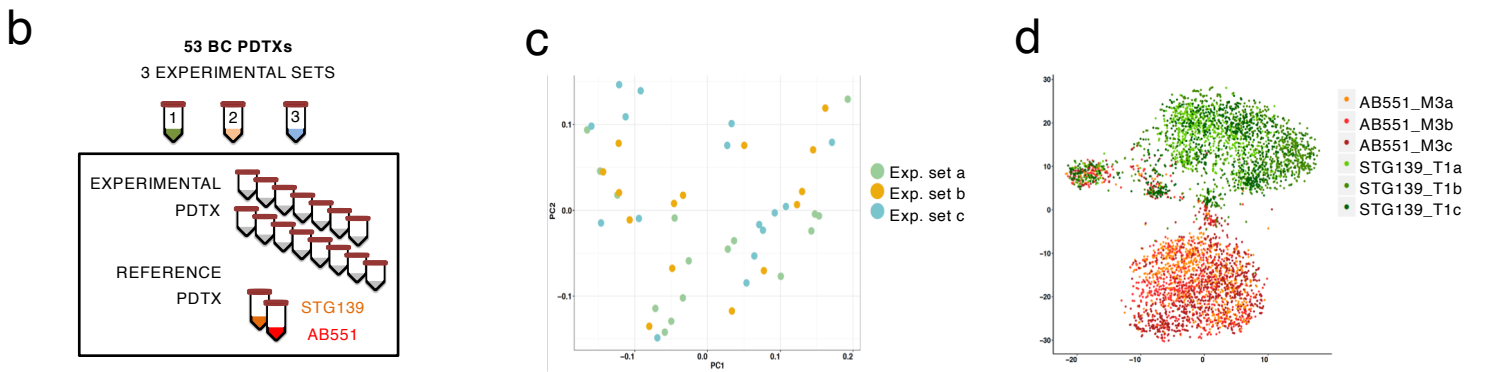
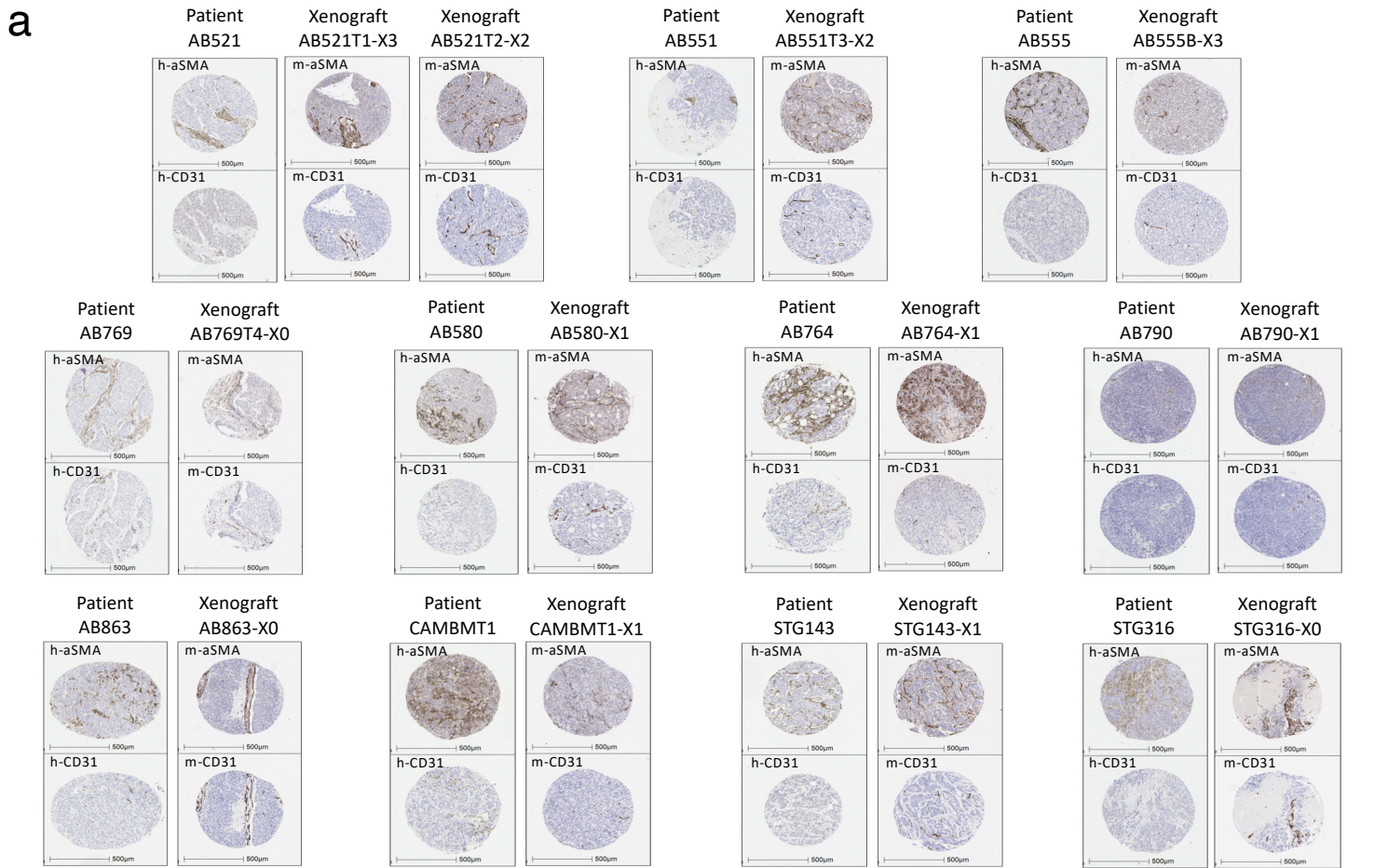
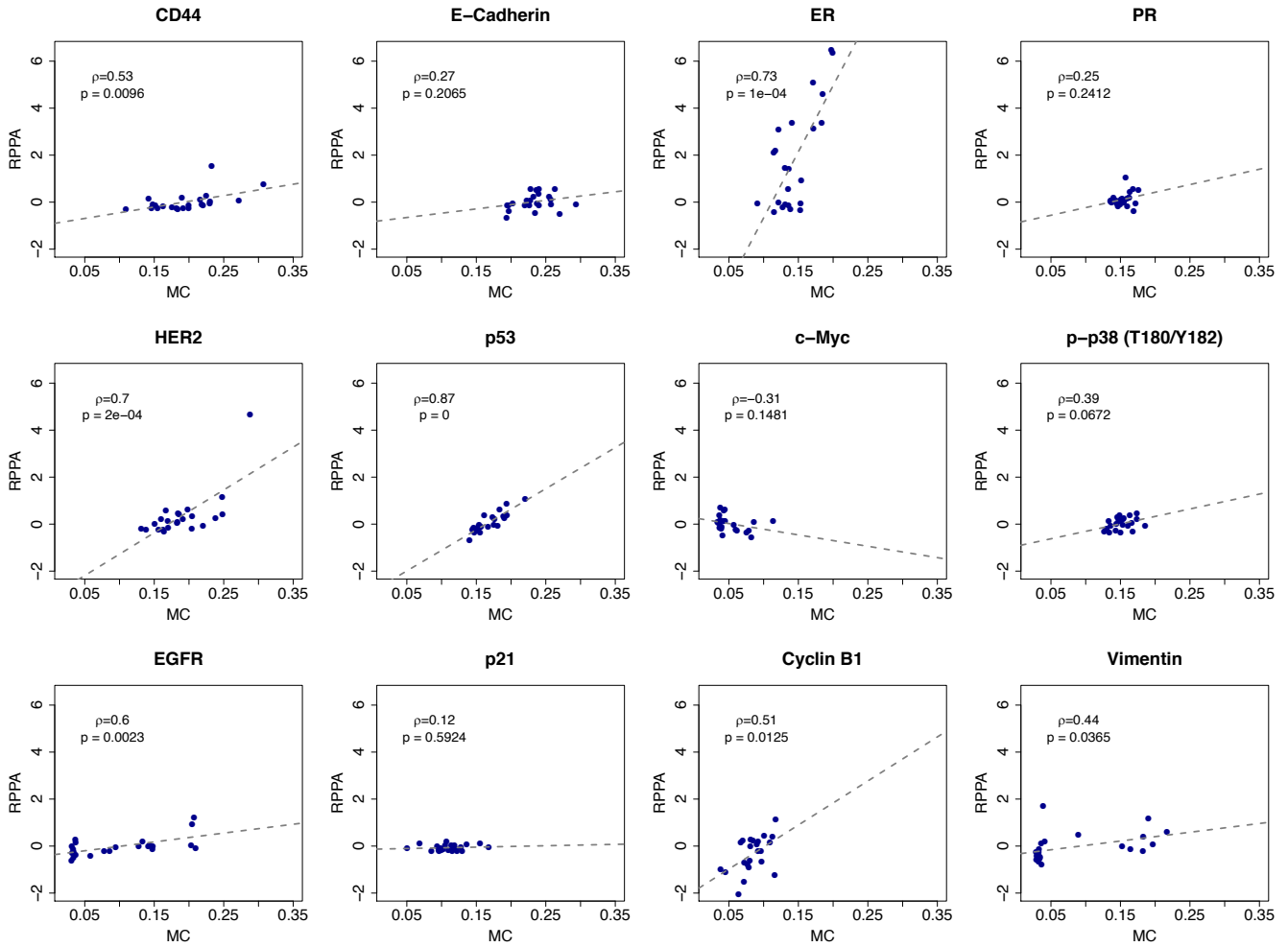
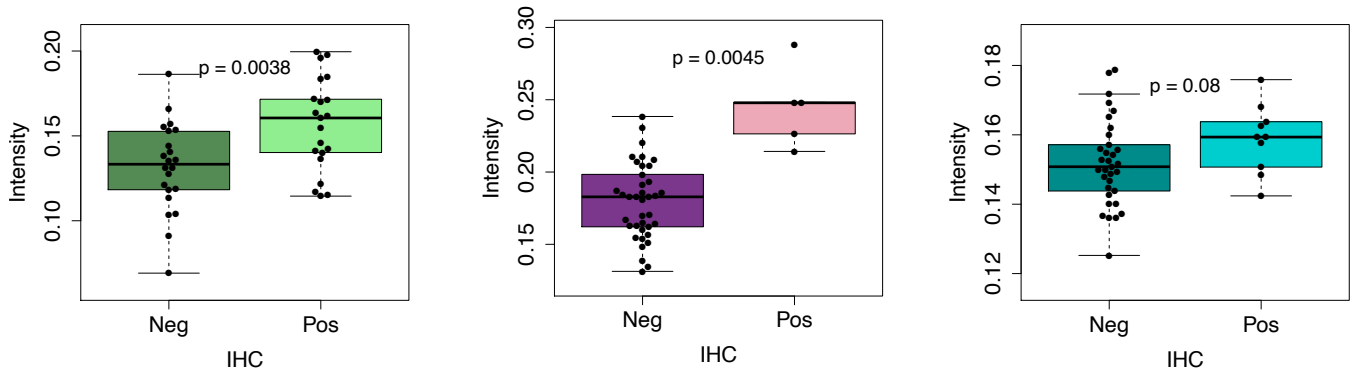


Supplementary Figure 1 - BCMC gating and validation strategy. a) LEFT- Gating for intact cells used cytometry bi-plots (intercalator Iridium (^{191}Ir and ^{193}Ir), and ^{140}Ce , one of the elements in the EQ four element calibration beads ($^{140/142}\text{Ce}$, $^{151/153}\text{Eu}$, ^{165}Ho and ^{175}Lu)). MIDDLE- Gating for single cells using cytometry bi-plots of intercalator Iridium (^{191}Ir and ^{193}Ir) versus event length. RIGHT- Gating for live cells using cytometry bi-plots of intercalator Rhodium (^{103}Rh), a live-dead exclusion marker for CyTOF, and Iridium. Good quality events were then imported in R and processed as detailed in the Methods. **b)** tSNE plots of 7 breast cancer cell lines shown in Fig 1b. Intensity of each BCMC marker is indicated by colour gradient. ($N_{\text{cells}}=20,846$). **c)** Mass Cytometry (MC)-based intensity distribution of Oncogenic Signalling Activation (OSA) and Cell Cycle and Apoptosis (CCA) markers in MCF7 cells treated for 1 hour with: vistusertib ($1\mu\text{M}$) or DMSO as a control ($N_{\text{cells}}= 20478$). Differences were evaluated by Earth Mover's Distance (indicated as 'e' in the graph). **d)** MC-based intensity distribution of OSA and CCA markers in MCF7 cells treated for 1 hour with palbociclib ($1\mu\text{M}$) or DMSO as a control ($N_{\text{cells}}= 27859$). Differences were evaluated by Earth Mover's Distance (indicated as 'e' in the graph). **e)** Schematic representation of PI3K/mTOR and MAPK signalling pathways. Pathway components analysed by the BCMC panel are indicated (red solid circle).

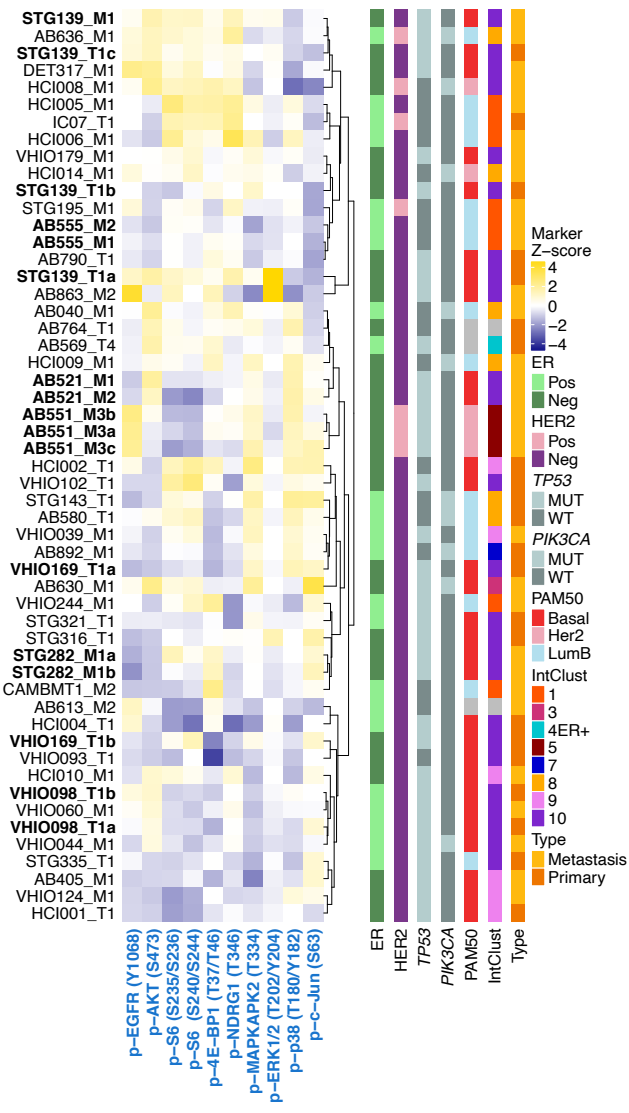


Supplementary Figure 2 - Analysis of a cohort of breast cancer PDXs by mass cytometry. a) Matched clinical human and PDX samples staining for aSMA and CD31. Human and mouse specific antibodies were used, respectively (indicated with 'h' and 'm', respectively). **b)** 53 samples were processed for mass cytometry in 3 experimental sets randomised for ER and HER2 status. Two PDXs (STG139 and AB551) were processed across batches to assess reproducibility and for normalization. **c)** PCA plot of the average mass cytometry profile of each sample across the three experimental batches. **d)** tSNE plot of the reference samples (STG139 and AB551) coloured by model and batch. **e)** tSNE plots as in d) showing the cell-level intensity of each BCMC protein marker.

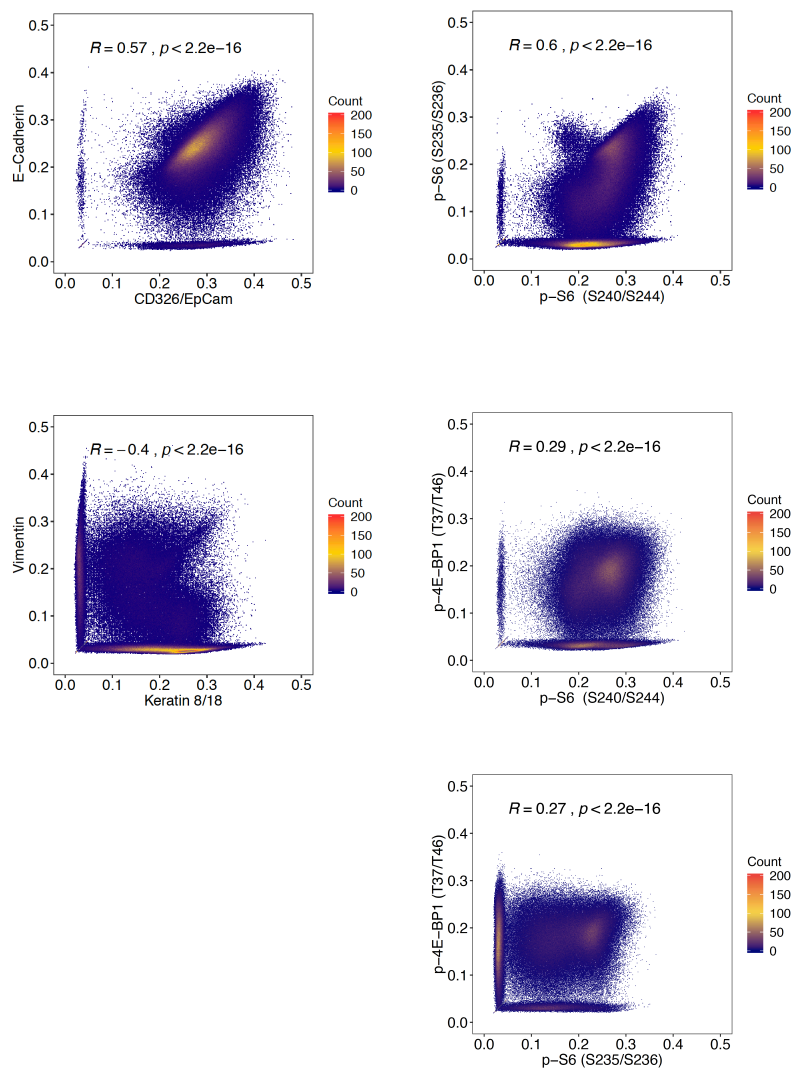
a**b**

Supplementary Figure 3 - Additional validation of BCMC panel using RPPA and IHC. a) Scatter plots of the cell population-median Mass Cytometry (MC)-quantified versus Reverse Phage Protein Array (RPPA)-quantified expression of 12 protein markers in common between the two platforms. Pearson's ρ and two-sided p-values are indicated. **b)** Boxplots of median expression of ER, HER2 and PR by mass cytometry in samples stratified by immunohistochemistry (IHC)-determined ER, HER2 and PR status ($p=0.0038$, $p=0.0045$ and $p=0.08$, respectively; two-sided t-test). Boxplot elements are defined as follows: center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; points, outliers. **c)** Heatmap of median expression of Oncogenics Signaling Activation (OSA) proteins across 53 PDTXs. PDTXs used as experimental set reference samples or originating from the same patient are represented in bold. Histological and molecular data indicated in the right panels. **d)** Scatterplots of selected HTC, MSC and OSA protein marker expression levels (EpCAM/E-Cadherin, Keratin8/18/Vimentin, p-4E-BP1 (T37/T46) and pS6(S240/244)/pS6(S235/236)). Spearman's R and two-sided p-values are indicated. **e)** Heatmap of the pairwise DREMI score values of all non mouse specific markers across all PDTX cells ($N_{\text{cells}}=405,827$). Marker subpanel is colour coded as in Fig. 1a.

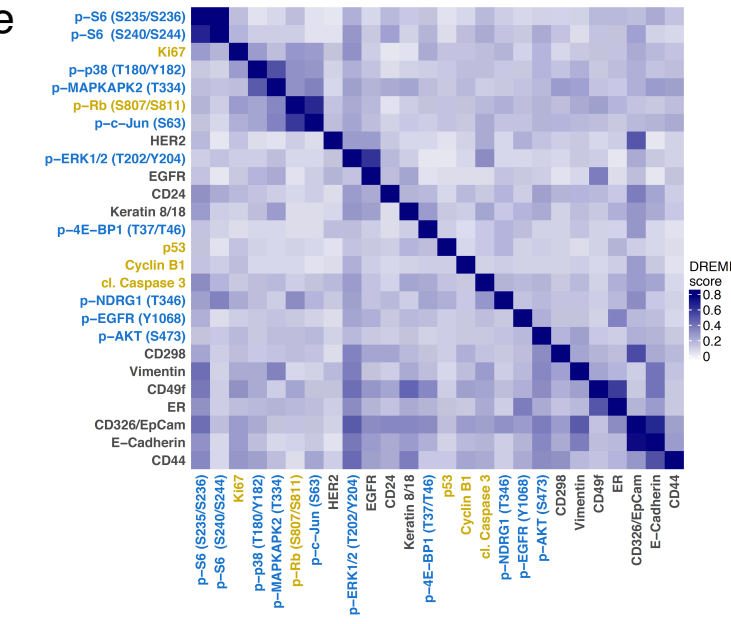
c



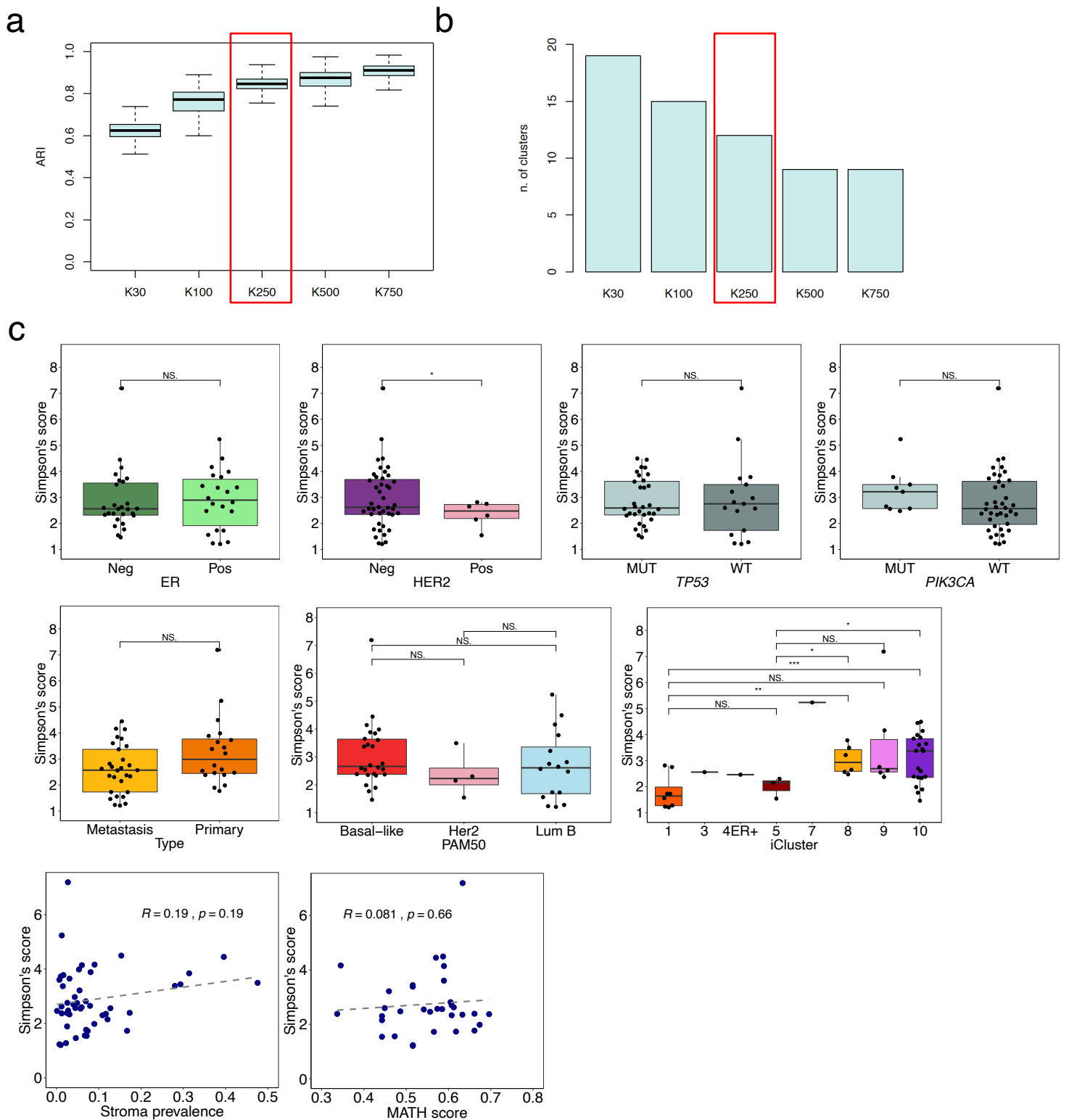
d



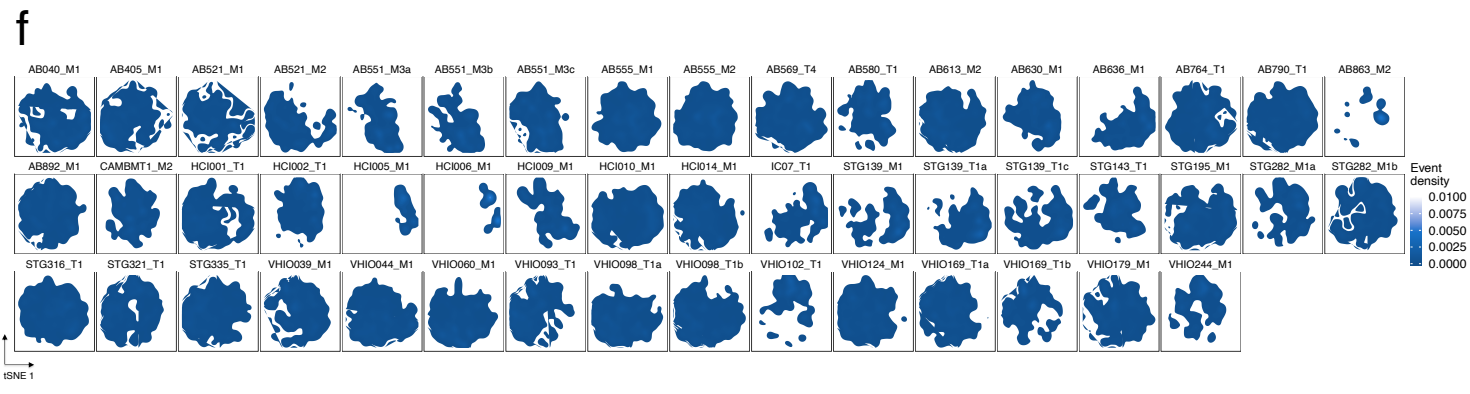
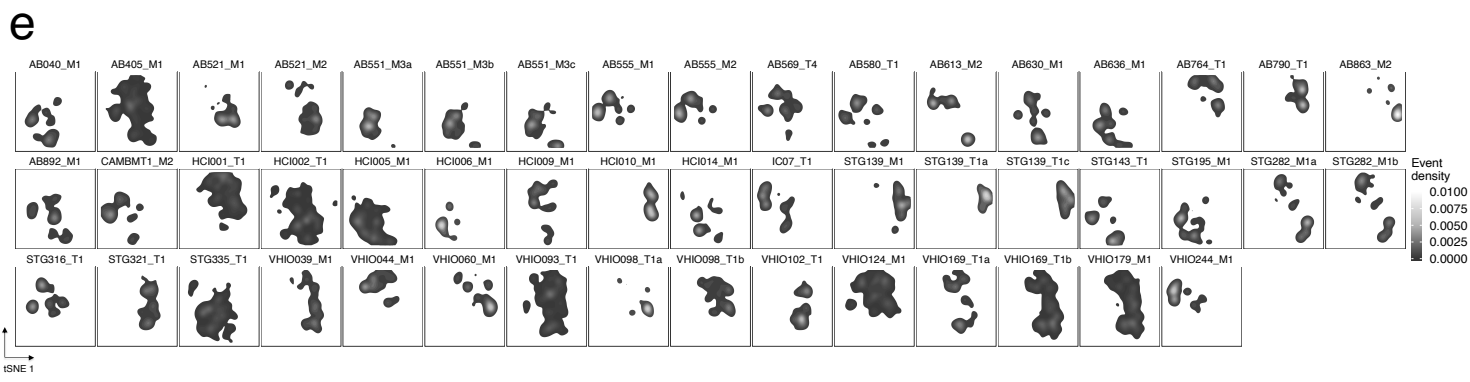
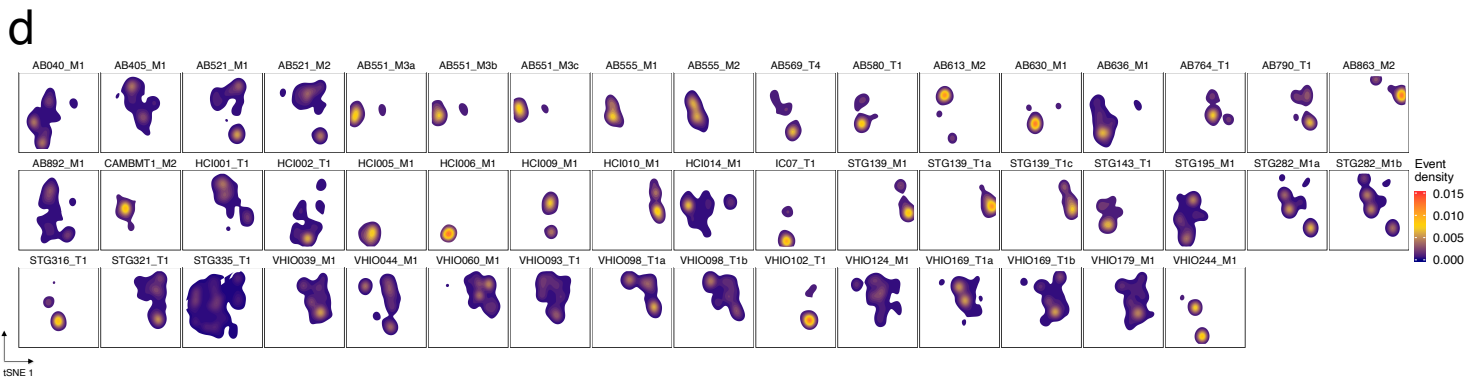
e



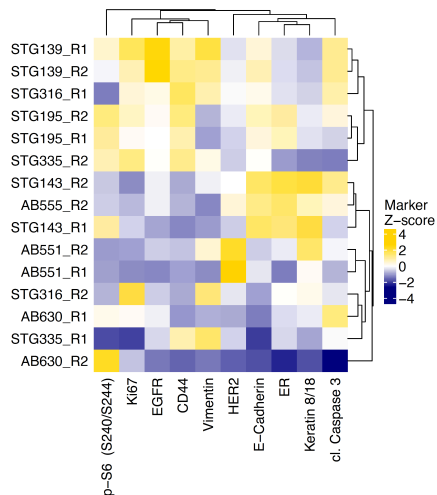
Y



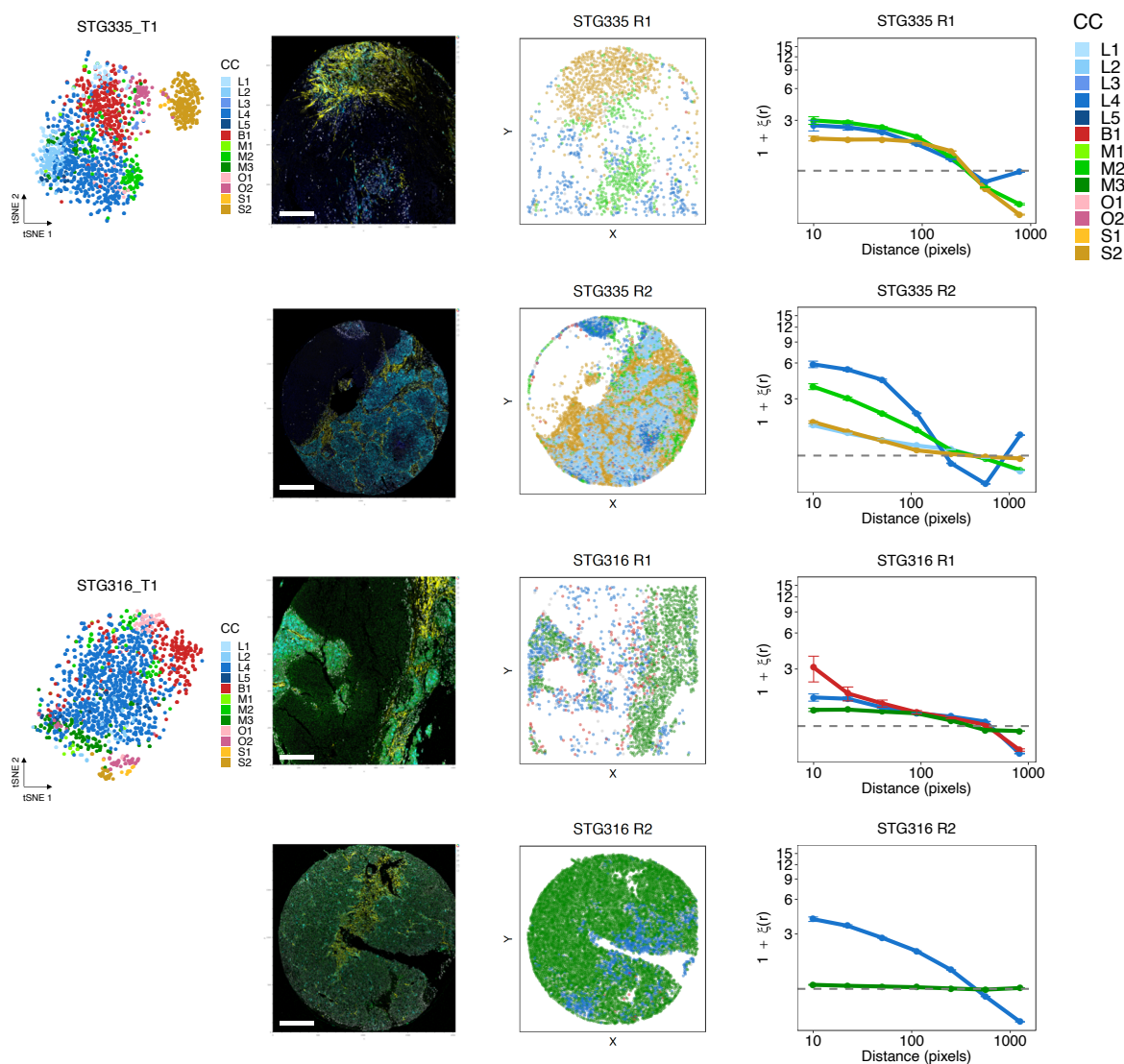
Supplementary Figure 4 - Phenotypic diversity across PDTXs. **a**) Adjusted Rand Index (ARI) for a range of values of PhenoGraph parameter K. Each boxplot shows ARI distribution for all pairwise clustering label comparisons ($n = 100$ clustering iterations). Red box indicates the results for the selected K used in this study. In the box plots, the lower and upper hinges correspond to the first and third quartiles. The upper and lower whisker extends from the hinge to the largest value no further than $1.5 * IQR$ from the hinge. Data beyond the end of the whiskers are plotted individually. **b**) Median number of clusters obtained for each value of K across 100 iterations. **c**) Boxplots of Simpson's score distribution in different subgroups of PDTX samples ($n = 49$; models with specific annotation missing were excluded, see Source Data file for details) as defined by ER and HER2 status, IntClust and PAM50, somatic mutation in *TP53* and *PIK3CA* and PDTXs originating tissue (primary tumour or metastasis). In the box plots, the lower and upper hinges correspond to the first and third quartiles. The upper and lower whisker extends from the hinge to the largest value no further than $1.5 * IQR$ from the hinge. Data beyond the end of the whiskers are plotted individually. Differences were evaluated by two-sided t-test; NS = not statistically significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Association between Simpson's score and PDTX stroma content (computed as prevalence of S1+S2 CCs) or PDTX genomic heterogeneity (MATH score) was evaluated by linear regression analysis. **d**) tSNE cell density plots of each of the 49 PDTXs based on all markers. Only cells falling in one of the 11 human CCs were included. **e**) tSNE cell density plots (same cells as in d)) based on Human Tumour Compartment (HTC) markers only. **f**) tSNE cell density plots (same cells as in d)) based on Oncogenics Signaling Activation (OSA) markers only.



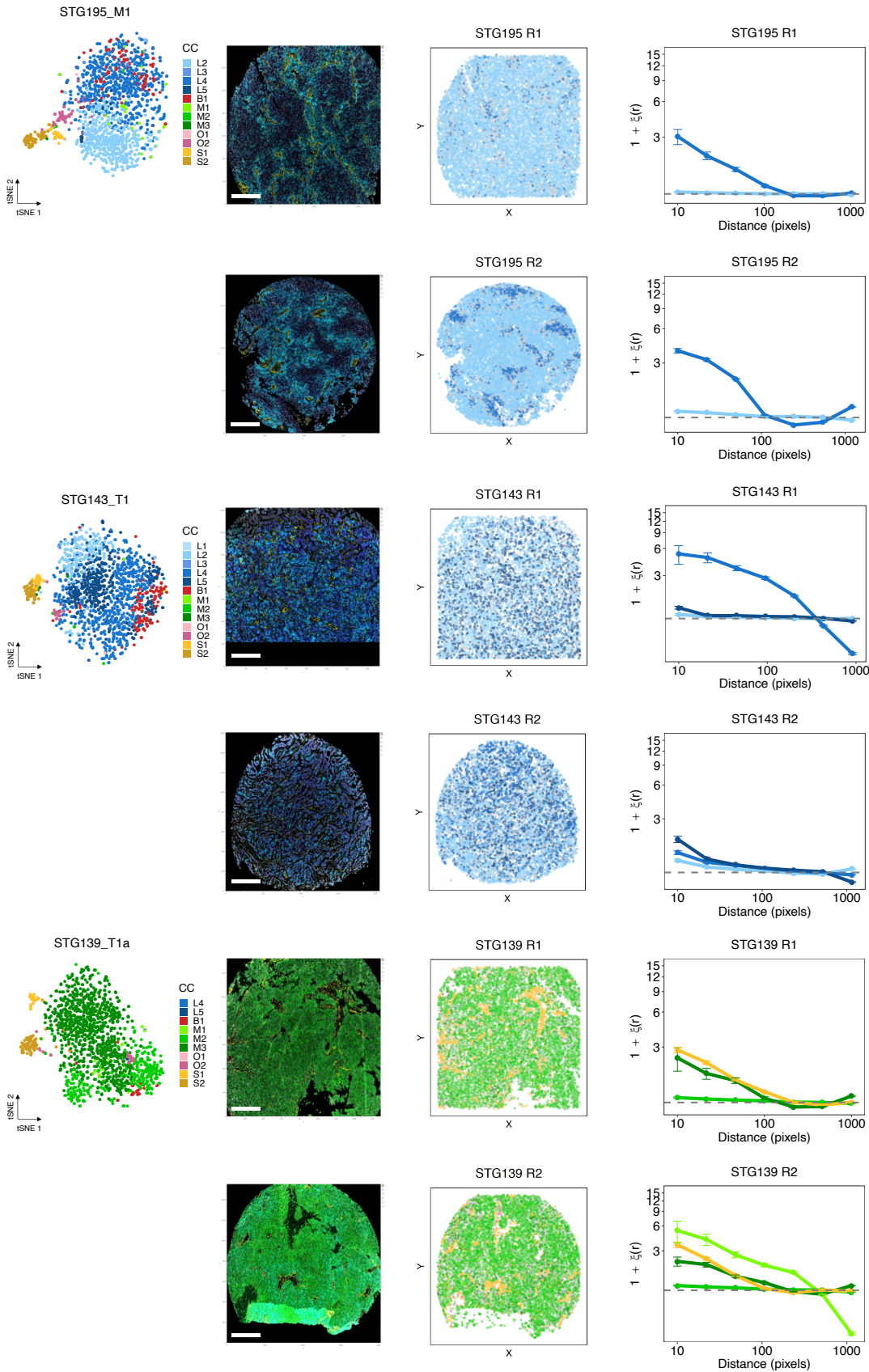
a

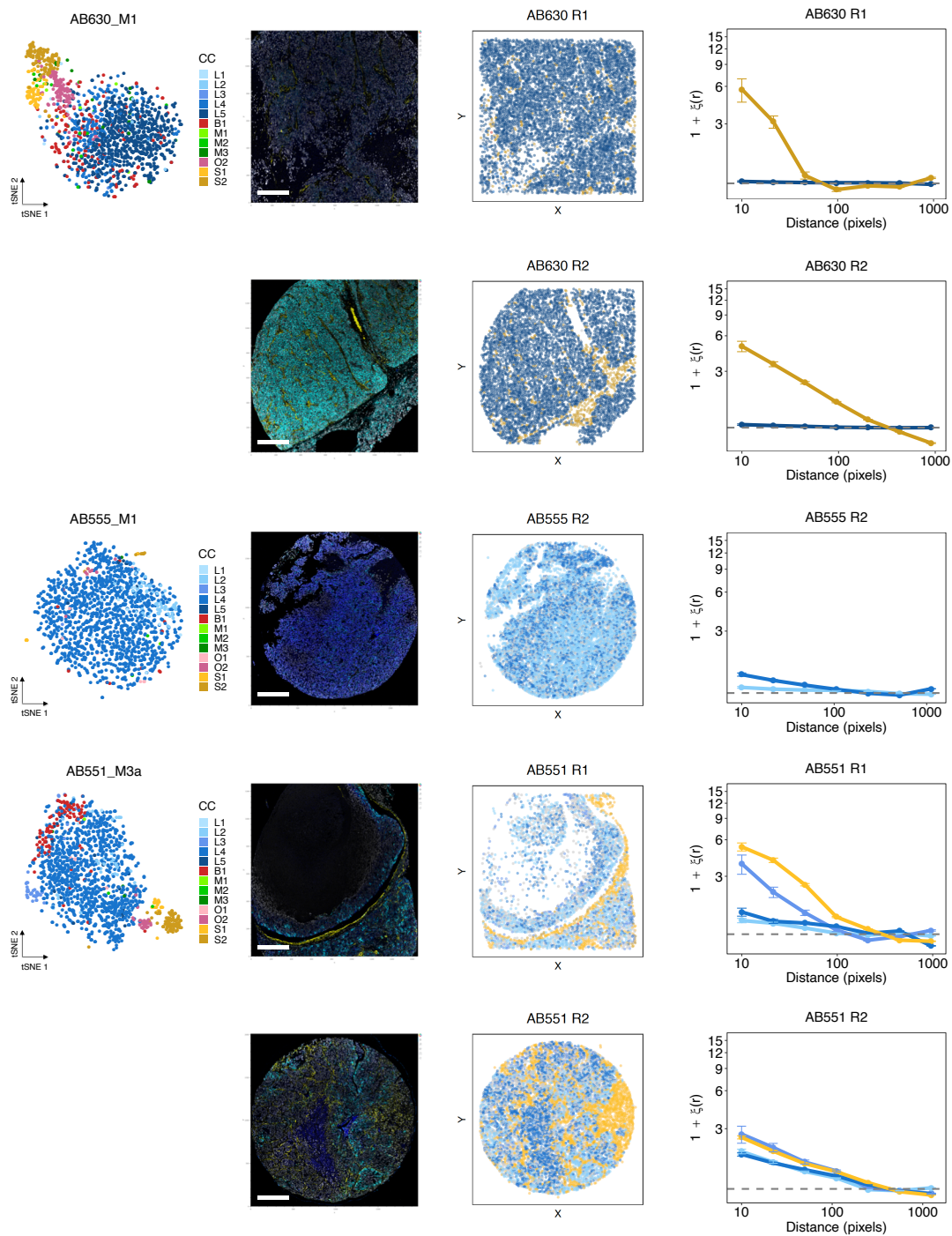


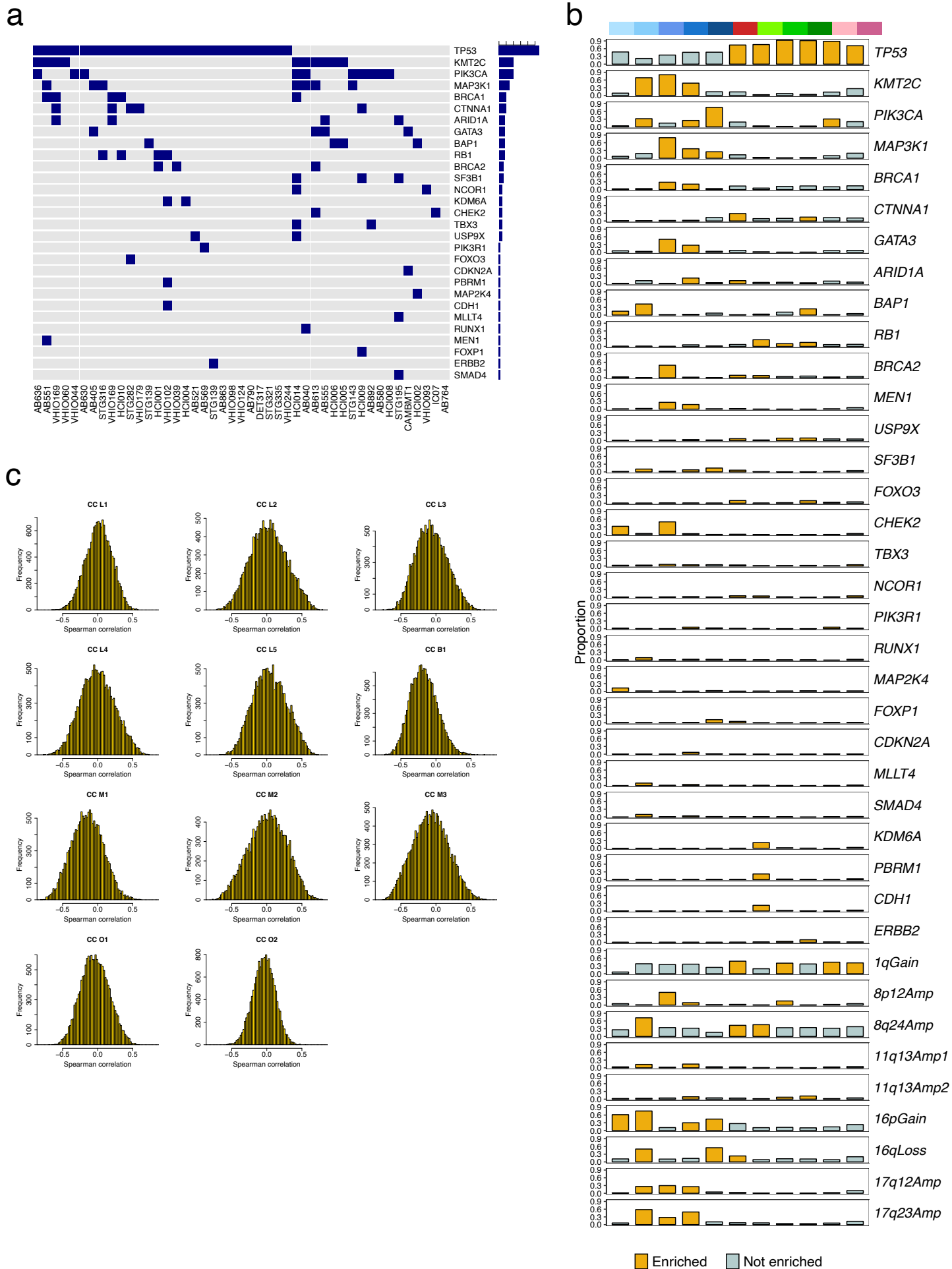
b



Supplementary Figure 5 - Spatial distribution of BCMC phenotypes across PDTXs. a) Median signals of 10 markers in cells segmented from Imaging Mass Cytometry (IMC) images ($N_{\text{samples}}=15$, $N_{\text{markers}}=10$, $N_{\text{cells}}=99,336$). **b)** Results of mass cytometry Cell-Cluster (CC) mapping to IMC segmented cells for all models analysed. For each model, from left to right: tSNE plot based on Mass Cytometry (MC) profiling; IMC image (for one or two areas) coloured for a subset of relevant markers representative of distinct CCs; pseudo-image with segmented cells labelled according to the classifier; two-point autocorrelation analysis results for each CC showing deviation from a random cell distribution as a function of distance. Data are presented as mean values \pm SD.

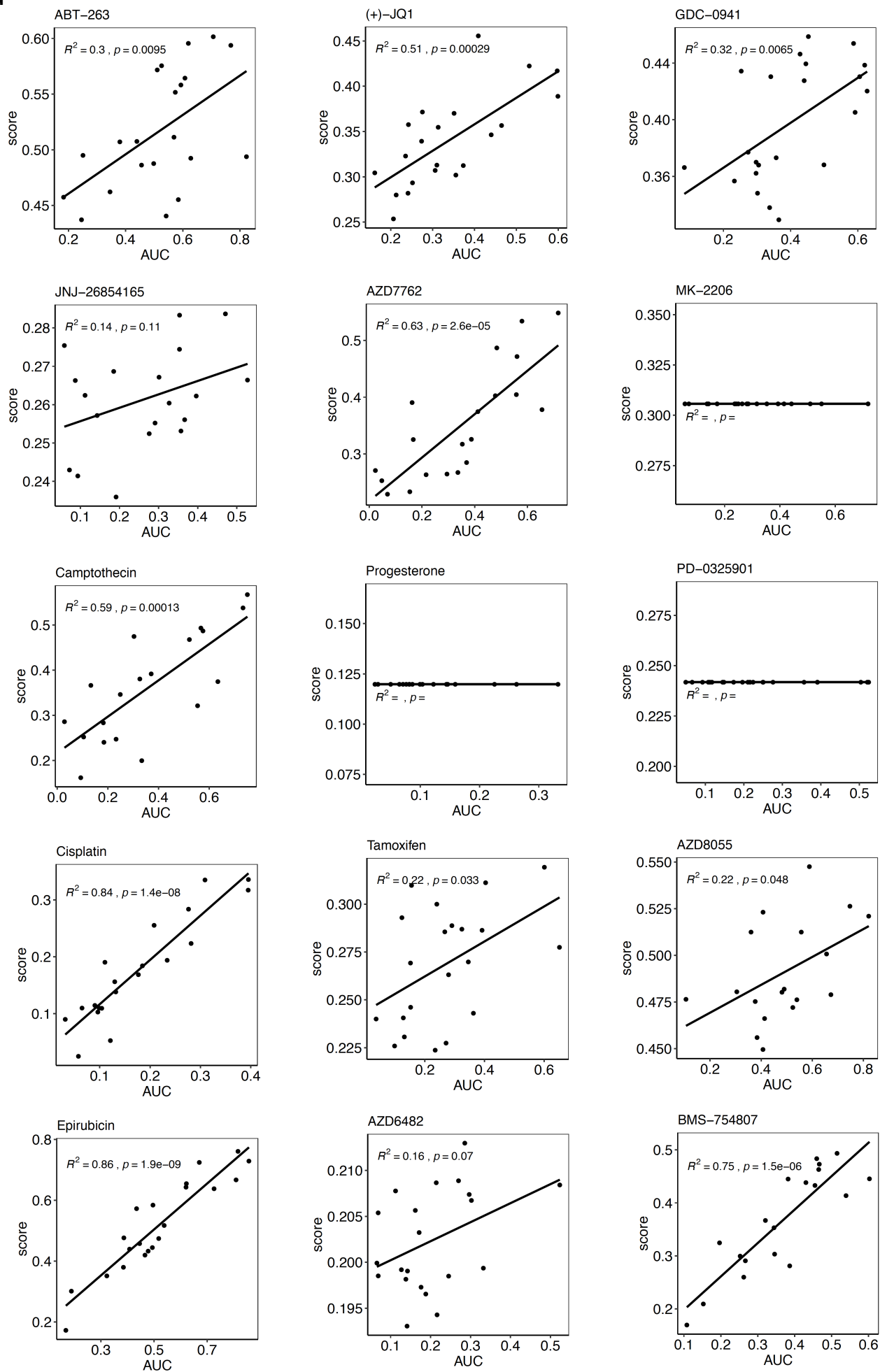


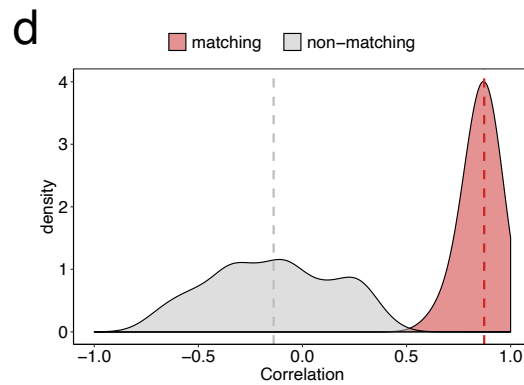
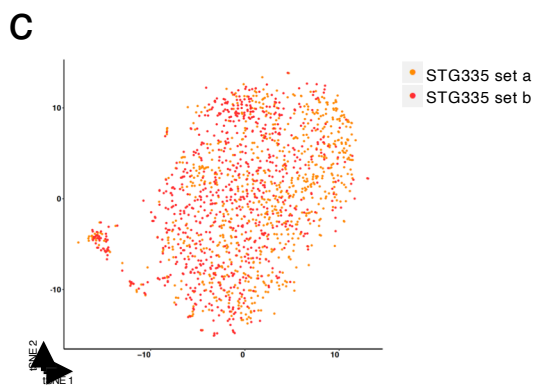
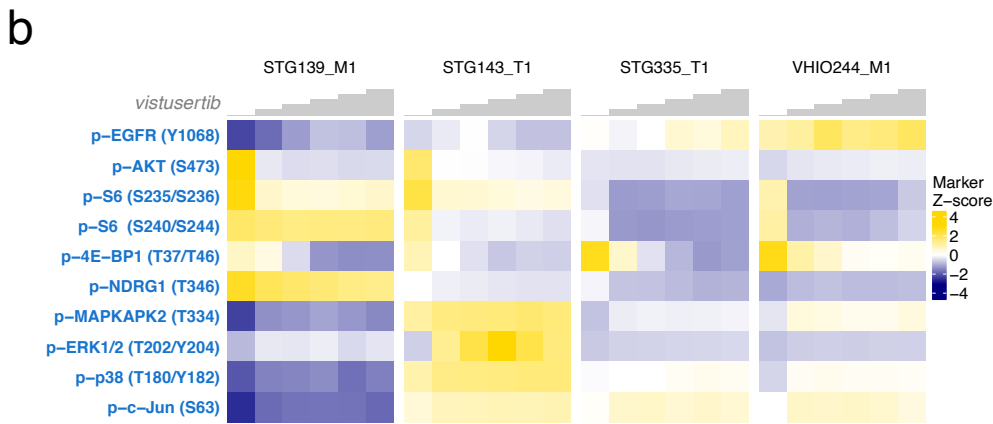
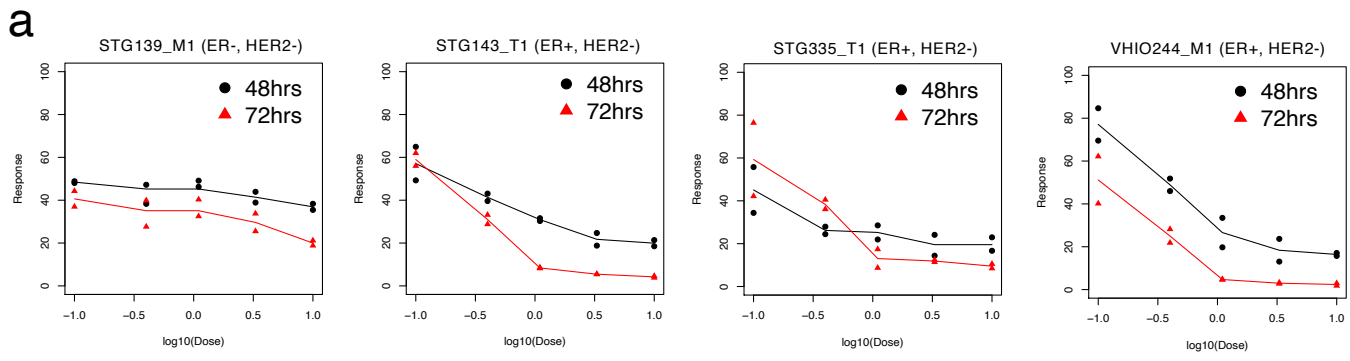




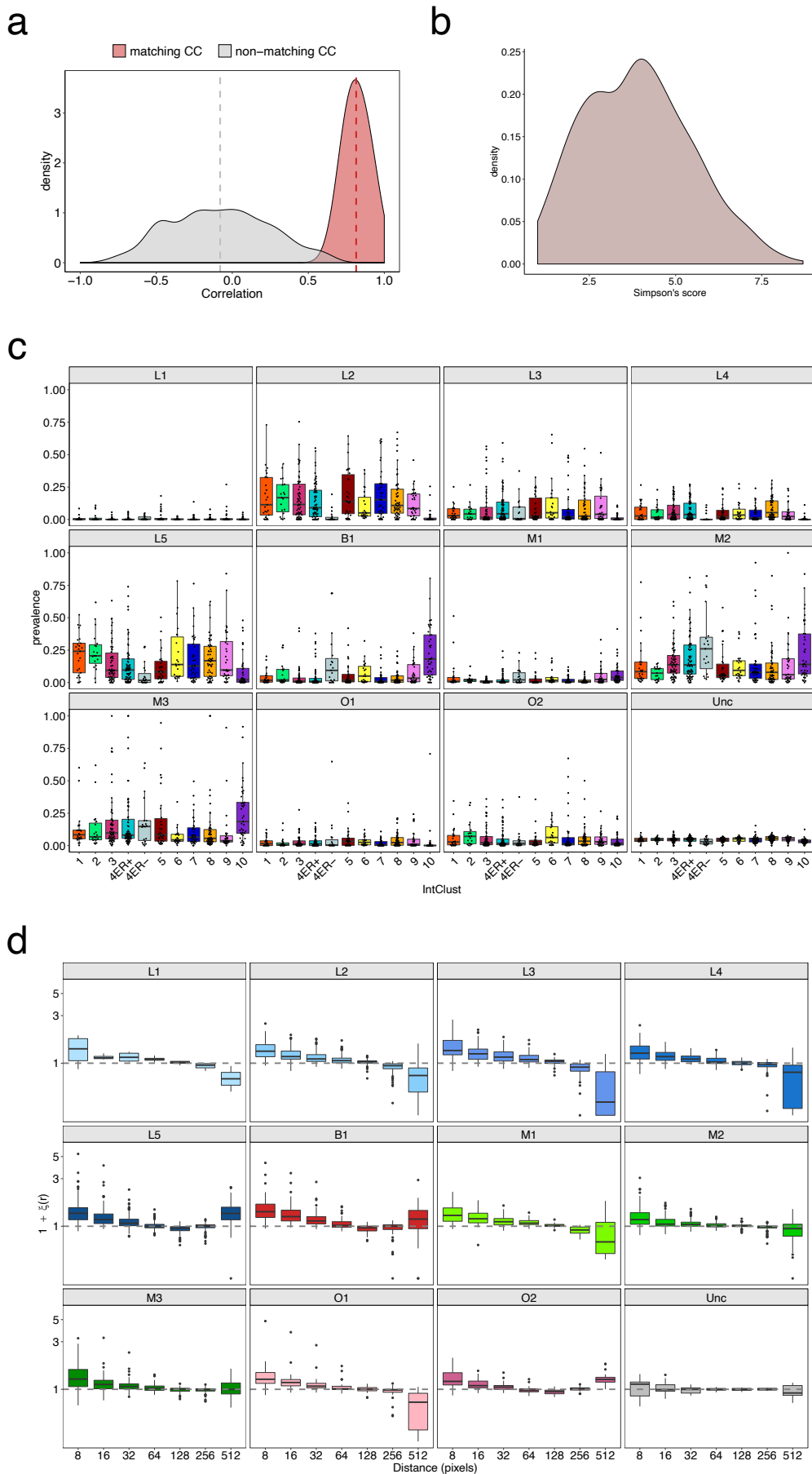
Supplementary Figure 6 - Multidimensional molecular data integration. **a**) Oncoprint showing somatic mutations identified in the 29 breast cancer driver genes mutated in at least one PDTX included in this study. **b**) Prevalence of major breast cancer driving genes and Copy Number Aberrations (CNAs) across the 11 human Cell-Clusters (CCs). Yellow bars - significant enrichment (hypergeometric test, two-sided adjusted $p < 0.01$). **c**) Distribution of correlation values between all genes and the prevalence of each of the 11 human CCs. **d**) Scatterplots of regularised linear model prediction against observed Area Under the Curve (AUC) for 15 compounds. Linear regression R^2 and associated p -values (two-sided) are reported.

d





Supplementary Figure 7 - Drug response and cellular phenotypic dynamics in PDTXs. a) Dose-response curves, using viability Cell-Titre Glo (CTG) assay, of 4 models treated with vistusertib for 48h and 72h (dosed from 0.1 to 10 μ M). **b)** Heatmap of median signal of Oncogenic Signaling Activation (OSA) markers in 4 PDTX models untreated or vistusertib-treated (dosed from 0.1 to 10 μ M). **c)** tSNE plot of reference sample STG335 using all protein markers evaluated by the Breast Cancer Mass Cytometry (BCMC). Cells labelled according to the sample of origin. **d)** Distribution of MC-MC centroid correlation [median for matching Cell-Cluster (CC) centroids = 0.87, in red; median for non-matching CC centroids = -0.14, in grey].



Supplementary Figure 8 - Mapping of the 11 CCs onto IMC data from clinical samples. a) Distribution of MC-MC centroid correlation [median for matching Cell-Cluster (CC) centroids = 0.81, in red; median for non-matching CC centroids = -0.08, in grey]. **b)** Simpson's score distribution computed across 481 Imaging Mass Cytometry (IMC) METABRIC cases and based on 11 CC proportions. **c)** Distribution of prevalence of each CC across the 11 IntClust subtypes (number of samples: IC1 = 25, IC2 = 19, IC3 = 58, IC4ER- = 19, IC4ER+ = 68, IC5 = 30, IC6 = 22, IC7 = 37, IC8 = 51, IC9 = 32, IC10 = 41). In the box plots, the lower and upper hinges correspond to the first and third quartiles. The upper and lower whisker extends from the hinge to the largest value no further than 1.5 * IQR from the hinge. Data beyond the end of the whiskers are plotted individually. **d)** Two-point autocorrelation analysis results for each CC across all tumours quantifying the deviation from a random cell distribution as a function of distance. In the box plots, the lower and upper hinges correspond to the first and third quartiles. The upper and lower whisker extends from the hinge to the largest value no further than 1.5 * IQR from the hinge. Data beyond the end of the whiskers are plotted individually.

Supplementary Table 1 - MC and IMC antibodies

Metal	protein/target	modification	Clone	Catalogue No	Supplier	Reactivity*	Positive control	Negative control	Validation status	Subpanel	Method	Dilution
141Pr	CD326/EpCam		9C4	3141006	Fluidigm	H	MCF7	MDA-MB-231	validated	ETC	MC	1in200
142Nd	p-S6 (S240/S244)	Ser240/Ser244	D57.2.2E	custom	Fluidigm	H, M	MCF7	MCF7 Vistusertib-treat	validated	OSA	MC	1in200
143Nd	p53		7F5	3143018	Fluidigm	H	RPPA	RPPA	validated	CCA	MC	1in50
	FITCH-MHC-Class I		28-8-6	114606	BD Biosciences	M	Mouse peritoneum	Human breast cancer c	validated	MSC	MC	1in100
146Nd	p-EGFR (Y1068)	Tyr1068	D7A5	3146007	Fluidigm	H, M	RPPA	RPPA	validated	OSA	MC	1in50
147Sm	CD45		30-F11	3147003	Fluidigm	M	Mouse peritoneum	Human breast cancer c	validated	MSC	MC	1in200
148Nd	HER2		29D8	3148011	Fluidigm	H, M	SK-BR-3	MDA-MB-231	validated	ETC	MC	1in100
149Sm	p-4E-BP1 (T37/T46)	Thr37/Thr46	236B4	3149005	Fluidigm	H, M	MCF7	MCF7 Vistusertib-treat	validated	OSA	MC	1in50
150Nd	CD44		IM7	3150018	Fluidigm	H, M	MDA-MB-231	MCF7	validated	ETC/MSC	IMC/MC	1in50
152Sm	p-AKT (S473)	Ser473	D9E	3152005	Fluidigm	H, M	MCF7	MCF7 Vistusertib-treat	validated	OSA	MC	1in50
153Eu	Cyclin B1		GNS-1	3153009	Fluidigm	H, M	MCF7	MCF7 Palbociclib-trea	validated	CCA	MC	1in50
154Sm	Vimentin		D21H3	3154014	Fluidigm	H, M	MDA-MB-231	MCF7	validated	ETC/MSC	MC	1in50
156Gd	p-p38 (T180/Y182)	Thr180/Tyr182	D3F9	3156002	Fluidigm	H, M	MDA-MB-231	MCF7	validated	OSA	MC	1in50
158Gd	E-Cadherin		24E10	3158021	Fluidigm	H, M	MCF7	MDA-MB-231	validated	ETC	MC	1in200
159Tb	p-MAPKAPK2 (T334)	Thr334	27B7	3159010	Fluidigm	H, M	MDA-MB-231	MCF7	validated	OSA	MC	1in50
160Gd	p-NDRG1 (T346)	Thr346	D98G11	custom	Fluidigm	H, M	MCF7 vehicle	MCF7 Vistusertib-treat	validated	OSA	MC	1in100
	APC-CD31		390	17-0311-80	BD Biosciences	M	Mouse peritoneum	Human cell lines	validated	MSC	MC	1in200
	APC-PDGFRa		APAS	135907	BD Biosciences	M	Mouse peritoneum	Human cell lines	validated	MSC	MC	1in200
163Dy	ER		D8H8	3163024	Fluidigm	H	MCF7	MDA-MB-231	validated	ETC	IMC/MC	1in50
164Dy	CD49f		GoH3	3164006	Fluidigm	H, M	MDA-MB-231	MCF7	validated	ETC/MSC	MC	1in100
	PE-CD298		LNH-94	341704	BD Biosciences	H	Mouse peritoneum	Human cell lines	validated	ETC	IMC/MC	1in50
166Er	p-Rb (S807/S811)	Ser807/Ser811	J112-906	3166011	Fluidigm	H	MCF7 vehicle	MCF7 Palbociclib-trea	validated	CCA	MC	1in200
168Er	Ki67		B56	3168007	Fluidigm	H, M	MCF7 vehicle	MCF7 Palbociclib-trea	validated	CCA	MC	1in200
169Tm	CD24		ML5	3169004	Fluidigm	H	MCF7	MDA-MB-231	validated	ETC	MC	1in100
170Er	EGFR		AY13	3170009	Fluidigm	H	MDA-MB-231	MCF7	validated	ETC	MC	1in100
171Yb	p-ERK1/2 (T202/Y204)	Thr202/Tyr204	D13.14.4E	3171010	Fluidigm	H, M	MDA-MB-231/468	ZR-75-1	validated	OSA	MC	1in50
172Yb	Cleaved caspase 3		5A1E	3172023	Fluidigm	H, M	NA	NA	validated	CCA	MC	1in50
173Yb	p-c-Jun (S63)	Ser63	54B3	custom	Fluidigm	H, M	MDA-MB-231	ZR-75-1	validated	OSA	MC	1in50
174Yb	Keratin 8/18		C51	3174014	Fluidigm	H	MCF7	MDA-MB-231	validated	ETC	MC	1in50
175Lu	p-S6 (S235/S236)	Ser235/Ser236	N7-548	3175009	Fluidigm	H, M	MCF7 vehicle	MCF7 Vistusertib-treat	validated	OSA	MC	1in50
145Nd	PR		D8Q2J	3145011	Fluidigm	H	RPPA	RPPA	not validated	NA	MC	1in50
167Er	p21 Waf1/Cip1		12D1	custom	Fluidigm	H	RPPA	RPPA	not validated	CCA	MC	1in50
176Yb	c-Myc		9E10	3176012	Fluidigm	H	RPPA	RPPA	not validated	NA	MC	1in50
144Nd	a-FITCH		FIT-22	3144006	Fluidigm	NA	NA	NA	NA	NA	MC	1in300
162Dy	a-APC		APC003	3162006	Fluidigm	NA	NA	NA	NA	NA	MC	1in300
165Ho	a-PE		PE001	3165015	Fluidigm	NA	NA	NA	NA	NA	IMC/MC	1in300

Metal	protein/target	modification	Clone	Catalogue No	Supplier	Reactivity*	Validation status	Panel arm	Method	Dilution		
143Nd	Vimentin		D21H3	3143027D	Fluidigm	H, M	PDTX TMA	PDTX TMA	validated	HTC/MSC	IMC/MC	1in200
148Nd	SMA		1A4	MA511547	custom	H, M	PDTX TMA	PDTX TMA	validated	MSC	IMC	1in100
151Eu	HER2		D8F12	4290BF	custom	H	PDTX TMA	PDTX TMA	validated	HTC	IMC/MC (differe	1in70
160Gd	CD44		IM7	14-0441-82	custom	H, M	PDTX TMA	PDTX TMA	validated	HTC	IMC/MC	1in50
163Dy	ER		D8H8	3163024A	Fluidigm	H	PDTX TMA	PDTX TMA	validated	HTC	IMC/MC	1in100
165Ho	a-PE		PE001	3165015	Fluidigm	NA	PDTX TMA	PDTX TMA	validated	NA	IMC/MC	1in30
	PE-CD298		LNH-94	341704	BD Biosciences	H	PDTX TMA	PDTX TMA	validated	HTC	IMC/MC	1in30
167Er	E-Cadherin		36/E-Cadherin	610182	custom	H, M	PDTX TMA	PDTX TMA	validated	HTC	IMC/MC (differe	1in100
168Er	Ki67		B56	3168022D	Fluidigm	Cross	PDTX TMA	PDTX TMA	validated	CCA	IMC/MC	1in100
169Tm	EGFR		AY13	352901	custom	H	PDTX TMA	PDTX TMA	validated	HTC	IMC/MC	1in100
170Er	pS6 (S240/S244)	S240/S244	D57.2.2E	4858BF	custom	H, M	PDTX TMA	PDTX TMA	validated	OSA	IMC/MC	1in200
172Yb	cl.caspase 3		5A1E	3172027D	Fluidigm	H, M	PDTX TMA	PDTX TMA	validated	CCA	IMC/MC	1in50
174Yb	Keratin 8/18		C51	3174022D	Fluidigm	H	PDTX TMA	PDTX TMA	validated	HTC	IMC/MC	1in50

Metal	protein/target	modification	Clone	Catalogue No	Supplier	Reactivity*	Validation status	Panel arm	Method	Dilution/Concentration	Antigen retrieval	
	CD31		D8V9E	77699	Cell Signaling	M	PDTX TMA	HUMAN TMA	validated	IHC	1in100	Tris-EDTA, 20' at 100C
	ASMA		polyclonal	ab5694	Abcam	M	PDTX TMA	HUMAN TMA	validated	IHC	1in500	Tris-EDTA, 10' at 100C
	ASMA		1A4	A2547	Sigma Aldrich	H	HUMAN TMA	PDTX TMA	validated	IHC	1in2000	None
	CD31		JC70A	M0823	Dako	H	HUMAN TMA	PDTX TMA	validated	IHC	4.1ug/ml	Sodium Citrate, 20' at 100C

*according to the manufacturer

Supplementary Table 2 - Cell line metadata

Model_ID	Species	Experiment	Experimental Set	Treatment	Dose (uM)	Viability (%)	ER_IHC	HER2_IHC	PR_IHC	PAM50
mPER	mouse	MSC validation	a	NA	NA	NT	NA	NA	NA	NA
4t09	mouse	MSC validation	a	NA	NA	>90	NA	NA	NA	NA
MCF7	human	MSC validation	a	NA	NA	>90	Pos	Neg	NA	Luminal
MDA-MB-231	human	MSC validation	a	NA	NA	>90	Neg	Neg	NA	Basal
MCF7	human	HTC validation	a	NA	NA	>90	Pos	Neg	NA	Luminal
MDA-MB-231	human	HTC validation	a	NA	NA	>90	Neg	Neg	NA	Basal
ZR-75-1	human	HTC validation	a	NA	NA	>90	Pos	Neg	NA	Luminal
CAMA1	human	HTC validation	a	NA	NA	>90	Pos	Neg	NA	Luminal
T47D	human	HTC validation	a	NA	NA	>90	Pos	Neg	NA	Luminal
SK-BR-3	human	HTC validation	a	NA	NA	>90	Neg	Pos	NA	Her2
MDA-MB-468	human	HTC validation	a	NA	NA	>90	Neg	Neg	NA	Basal
MCF7	human	CCA/OSA validation	a	DMSO	NA	>90	Pos	Neg	NA	Luminal
MCF7	human	CCA/OSA validation	a	Vistusertib	1	>90	Pos	Neg	NA	Luminal
MCF7	human	CCA/OSA validation	a	Palbociclib	1	>90	Pos	Neg	NA	Luminal

Supplementary Table 4 - Number of samples and cells analysed

Experiment	N in-gate events	N events after filtering	N samples (inc. replicates)	N samples removed post QC	N downsampled events per sample	N events after downsampling
HTC validation	21000	20846	7	0	2900	20300
CCA/OSA validation	38542	36582	3	0	2900	8700
MSC validation	27900	26154	4	0	NA	NA
PDTX characterisation	435956	405827	53	4	1600	78400
PDTX target engagement	923269	461634	31	0	1700	40800
IMC in PDTX	106569	99336	15	0	NA	NA
TOTAL	1553236	1050379	113			