

# Tissue-dependent mechanosensing by cells derived from human tumors

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Table 1: Number of single cell measurements of spread area, aspect ratio, and circularity for each cell line-substrate combination.

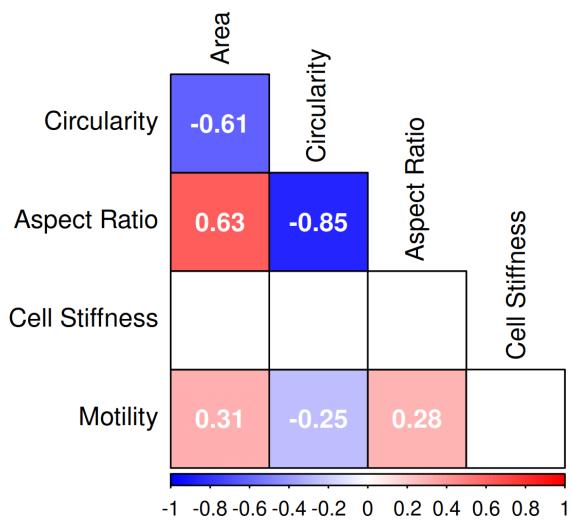
	30kPa Coll	30kPa FN	500Pa Coll	500Pa FN	Glass	HA Coll	HA FN
SK-MEL-2	100	100	101	100	100	101	100
A375	100	100	101	100	100	100	100
WM266-4	100	100	100	100	100	100	100
MeWo	50	50	100	96	100	100	80
RWPE-1(N)	102	103	100	103	100	101	101
22Rv1	99	100	99	81	103	103	98
LnCaP	41	49	34	59	90	32	46
DU145	77	99	67	101	81	102	103
PC-3	101	100	100	90	100	100	40
hTERT-HPNE(N)	94	100	106	97	100	77	76
Panc-1	100	102	72	21	101	103	105
Capan-1	105	100	102	100	101	101	100
SKOV-3	108	101	82	104	102	100	92
Caov-3	85	34	37	16	81	59	43
OVCAR-3	100	86	94	89	90	100	97
NL20(N)	168	257	247	284	248	126	150
NCI-H2126	100	100	100	100	100	100	100
NCI-H2087	100	100	100	100	100	100	100
HCT116	100	100	107	101	101	100	100
HT29	82	55	93	40	165	95	49
SW480	100	100	100	100	100	100	100
SW620	101	100	100	100	101	100	100
hTERT-HME1(N)	100	100	101	100	100	74	51
MCF10A(N)	100	100	100	100	91	88	62
T-47D	100	100	100	86	100	84	97
MCF7	100	68	100	82	100	45	29
MDA-MB-231	121	218	145	164	193	77	160
HCC1937	100	100	100	100	100	100	100
U-87	101	101	102	100	101	101	100
T98G	100	101	100	101	101	100	101

Table 2: Number of measurements of cell stiffness for each cell line-substrate combination.

	30kPa Coll	30kPa FN	500Pa Coll	500Pa FN	Glass	HA Coll	HA FN
SK-MEL-2	45	44	43	47	45	50	43
A375	45	30	39	44	45	42	48
WM266-4	63	44	42	41	44	41	48
MeWo	29	31	30	33	30	33	30
RWPE-1(N)	30	36	43	48	67	45	30
22Rv1	30	30	45	30	45	42	30
LnCaP	29	15	27	12	48	NA	38
DU145	47	44	51	51	49	41	50
PC-3	45	39	35	29	45	30	28
hTERT-HPNE(N)	45	49	50	42	45	45	45
Panc-1	36	43	30	39	45	43	44
Capan-1	44	31	29	27	33	32	29
SKOV-3	44	39	29	27	28	30	26
Caov-3	44	45	44	45	45	44	28
OVCAR-3	45	44	45	44	46	47	45
NL20(N)	30	30	30	33	33	30	32
NCI-H2126	30	45	29	30	30	30	30
NCI-H2087	30	29	32	30	30	51	30
HCT116	65	45	49	42	67	66	54
HT29	45	39	45	32	45	45	48
SW480	44	53	45	59	63	45	45
SW620	31	29	45	32	31	30	48
hTERT-HME1(N)	45	45	30	47	47	27	45
MCF10A(N)	45	44	44	45	60	45	45
T-47D	48	44	45	45	48	39	42
MCF7	52	42	47	42	29	45	45
MDA-MB-231	45	45	30	30	32	30	45
HCC1937	42	30	45	44	39	42	42
U-87	51	35	59	54	72	54	45
T98G	63	64	59	61	61	57	56

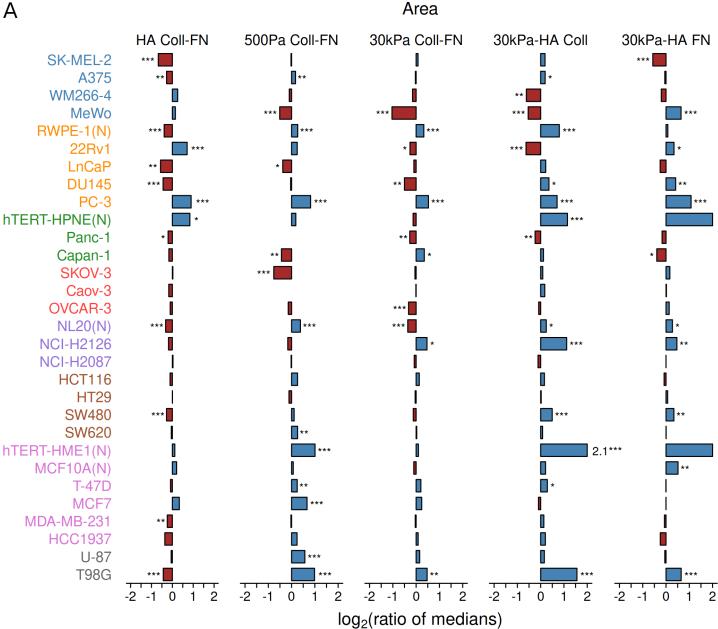
Table 3: Number of single cell measurements of cell speed for each cell line-substrate combination.

	30kPa Coll	30kPa FN	500Pa Coll	500Pa FN	Glass	HA Coll	HA FN
SK-MEL-2	71	72	61	141	129	70	68
A375	50	50	50	50	51	52	51
WM266-4	41	46	54	51	50	51	51
MeWo	176	58	255	53	159	30	72
RWPE-1(N)	119	109	114	102	66	108	105
22Rv1	36	42	50	45	49	50	56
LnCaP	48	50	29	50	50	48	53
DU145	41	11	27	43	32	50	40
PC-3	13	21	13	99	101	24	14
hTERT-HPNE(N)	55	52	51	51	52	47	50
Panc-1	45	44	33	8	57	62	55
Capan-1	52	46	79	17	56	53	30
SKOV-3	33	26	39	49	51	62	44
Caov-3	22	17	25	4	24	30	27
OVCAR-3	29	52	49	53	58	51	50
NL20(N)	61	70	54	67	26	45	44
NCI-H2126	51	56	72	75	85	56	103
NCI-H2087	87	27	103	114	104	93	69
HCT116	47	36	44	9	70	26	23
HT29	50	36	57	15	50	50	50
SW480	56	50	55	83	NA	40	65
SW620	25	25	51	51	25	51	36
hTERT-HME1(N)	51	29	50	54	52	20	32
MCF10A(N)	50	50	50	60	50	50	52
T-47D	30	56	31	47	15	81	27
MCF7	22	15	37	3	50	50	36
MDA-MB-231	27	25	30	29	34	12	26
HCC1937	51	49	54	50	50	53	48
U-87	59	54	52	102	70	58	47
T98G	74	82	123	94	62	75	86

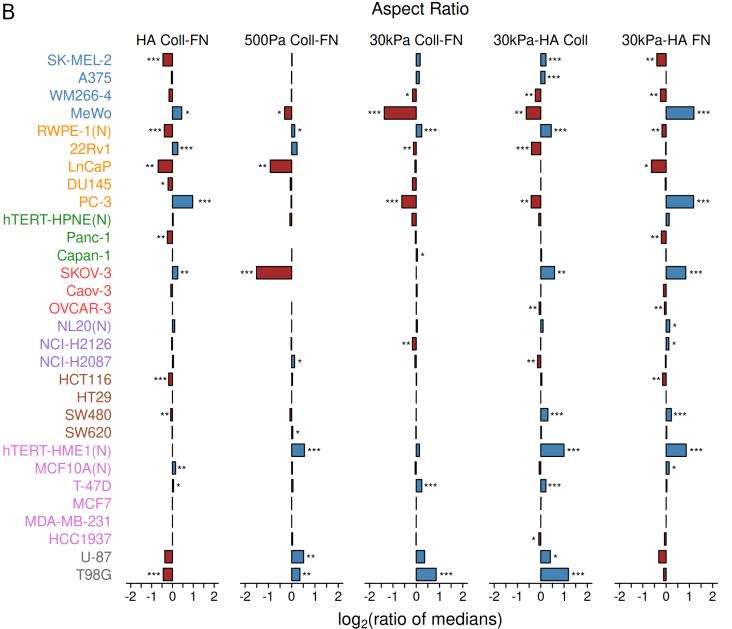


Supplementary Figure 1: Spearman correlation matrix using the median values of the phenotypic features for the cell line-substrate pairs. Only statistically significant correlations are shown. Note that in this analysis only those cell line-substrate pairs which have at least 25 data points for each of the physical feature are considered.

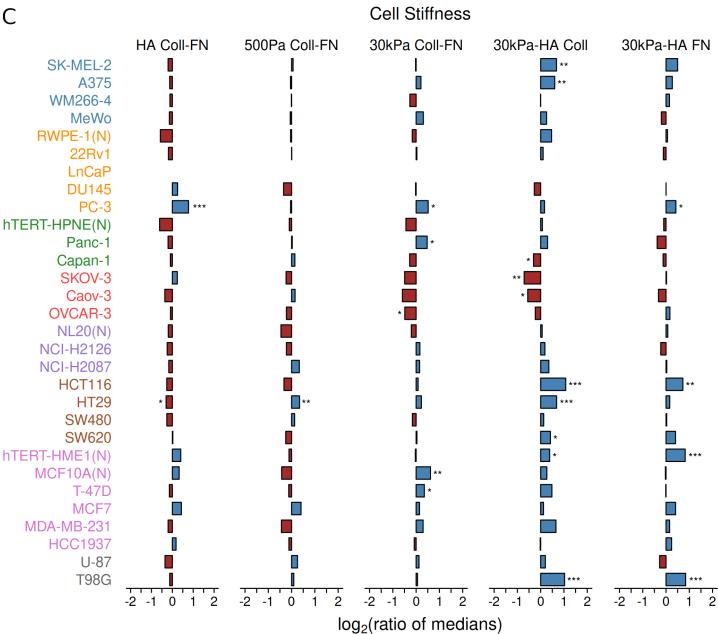
A



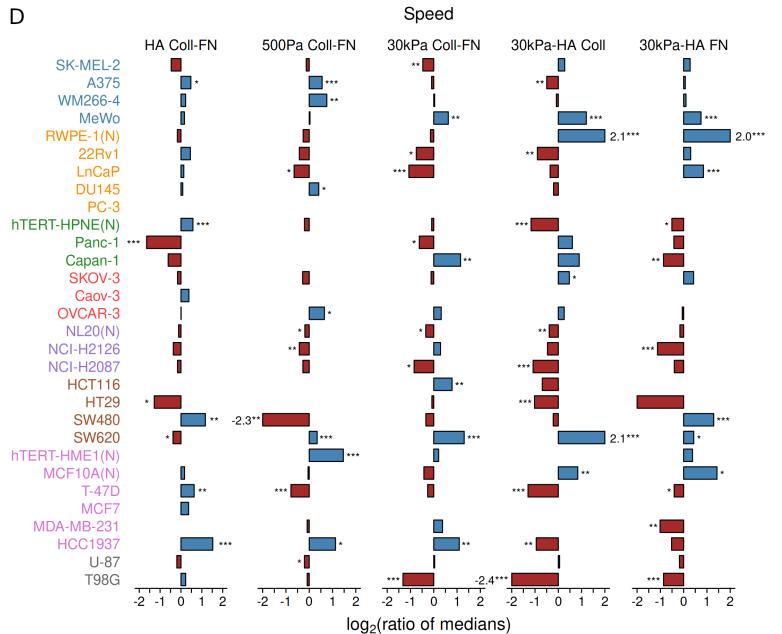
B



C

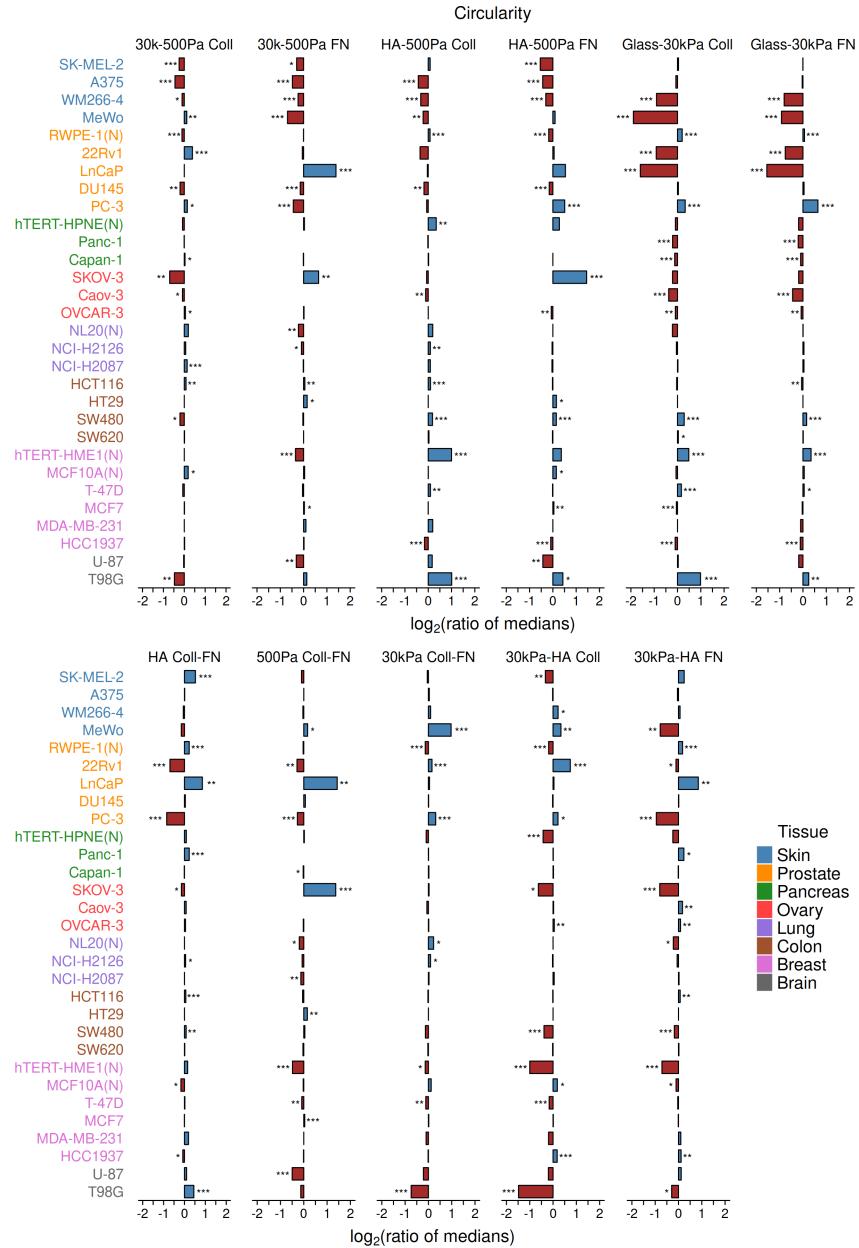


D

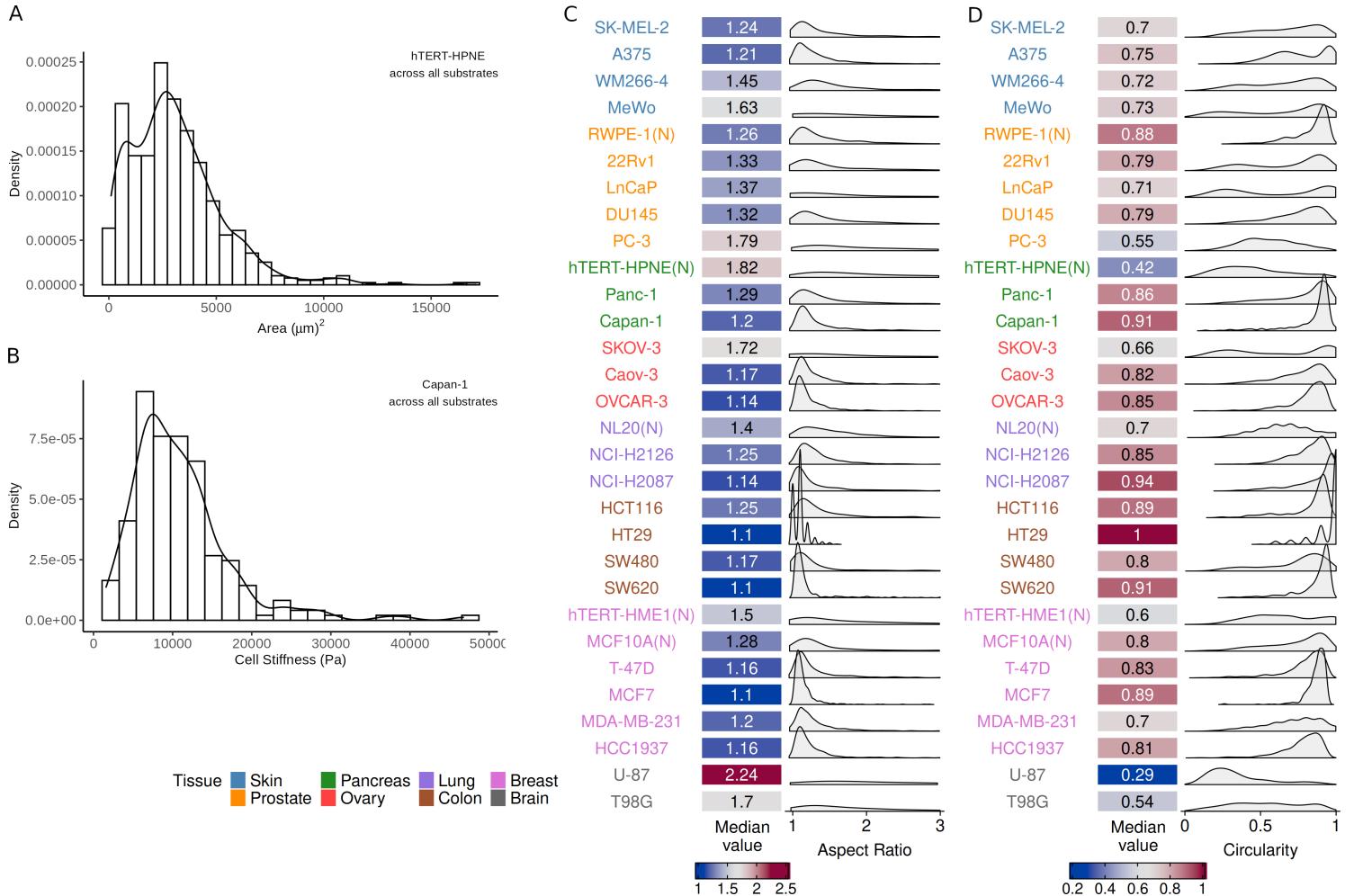


Tissue  
█ Skin   █ Pancreas   █ Lung   █ Breast  
█ Prostate   █ Ovary   █ Colon   █ Brain

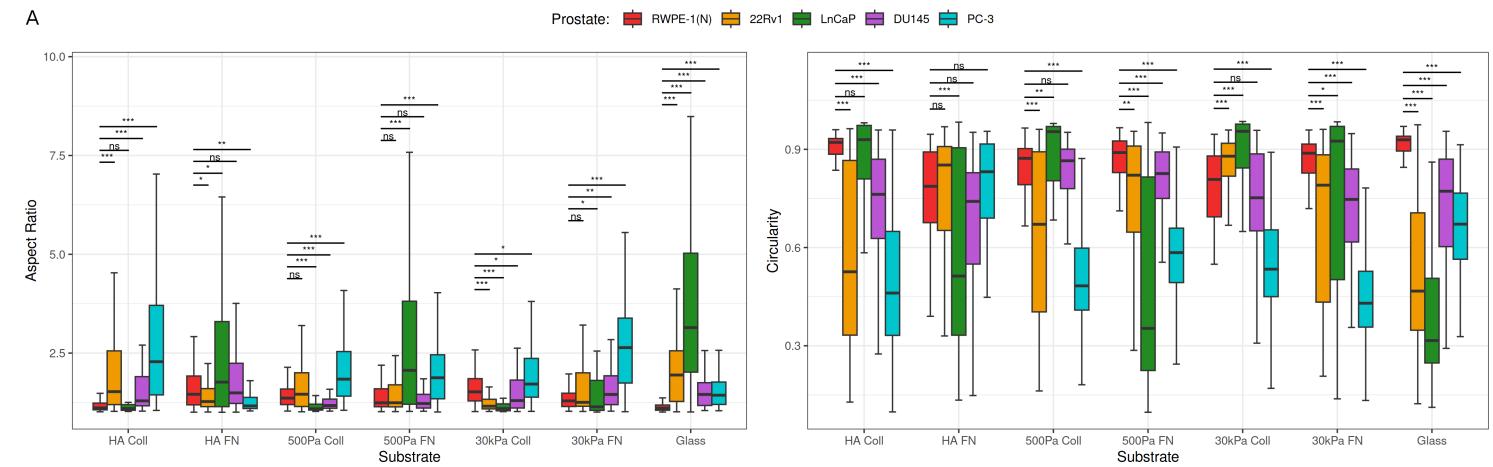
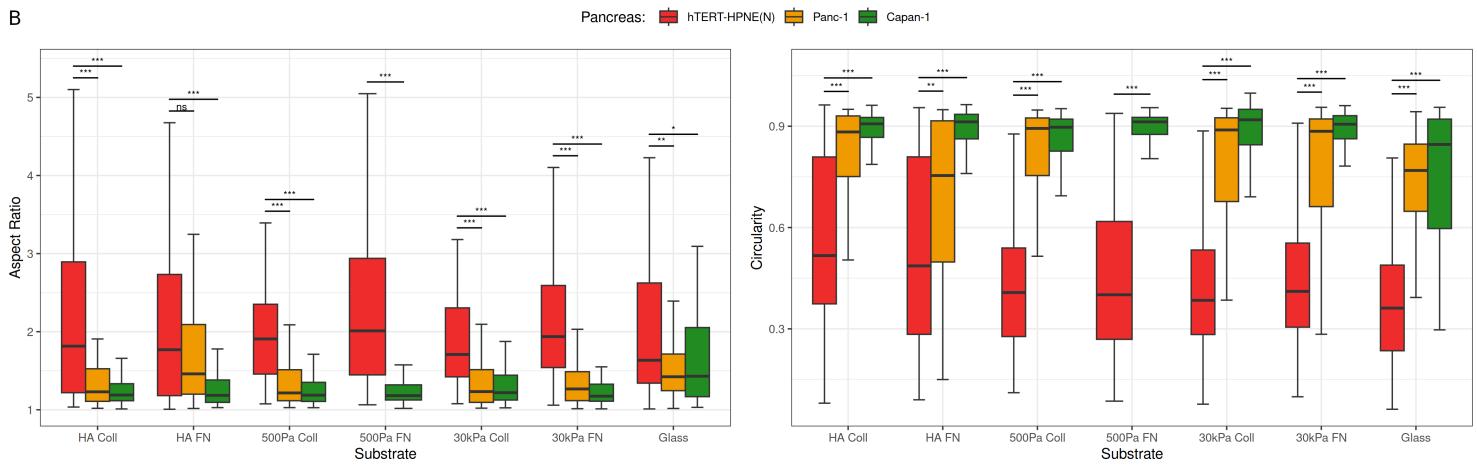
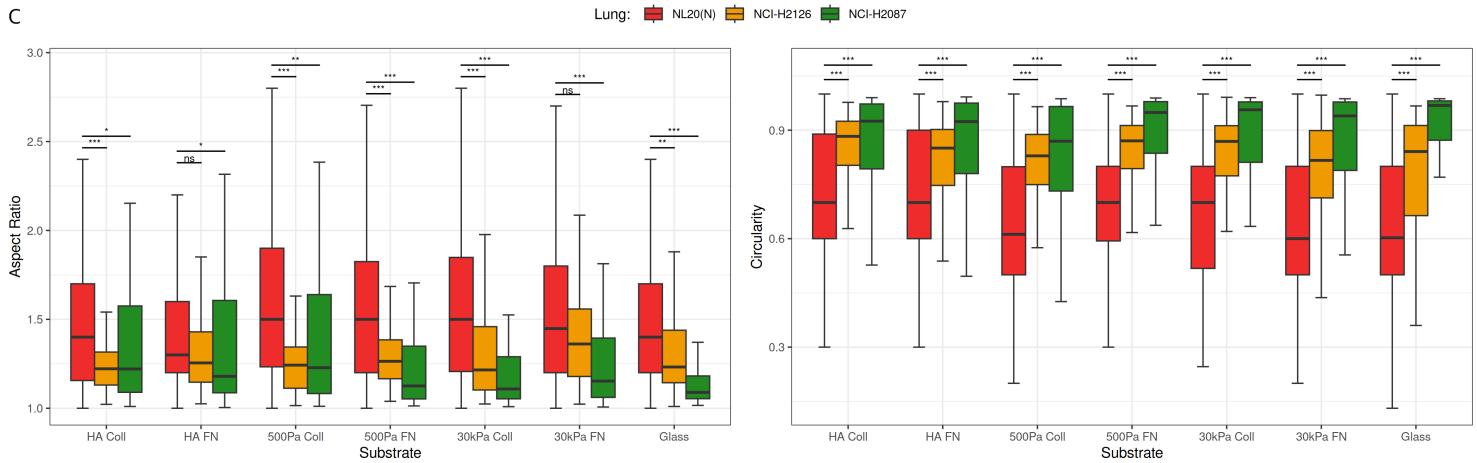
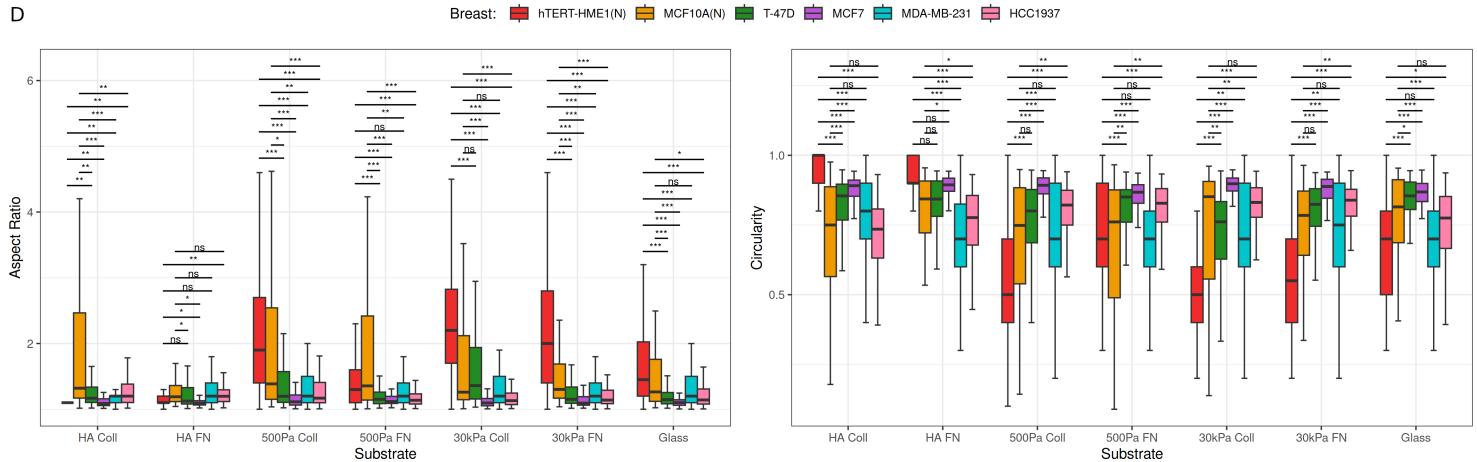
Supplementary Figure 2: For each cell line, the ratio of the median values for (A) area, (B) shape (aspect ratio), (C) cell stiffness, and (D) speed as a measure of the phenotypic sensitivity to substrate change. (HA Coll-FN: HA Coll/HA FN, 500Pa Coll-FN: 500Pa FN/500Pa Coll, 30kPa Coll-FN: 30kPa Coll/30kPa FN, 30kPa-HA Coll: 30kPa Coll/HA Coll, 30kPa-HA FN: 30kPa FN/HA FN). (N) refers to non-malignant (normal) cell lines. \*\*\*p-value < 0.001; \*\*p-value < 0.01; \*p-value < 0.05. For each cell line on a particular substrate,  $n > 25$  cells. See supplementary tables 1-3 for the exact value of  $n$  for the cell lines.



Supplementary Figure 3: For each cell line, the ratio of the median values for circularity as a measure of the phenotypic sensitivity to substrate change. (30k-500Pa Coll: 30kPa Coll/500Pa Coll, 30k-500Pa FN: 30kPa FN/500Pa FN, HA-500Pa Coll: HA Coll/500Pa Coll, HA-500Pa FN: HA FN/500Pa FN, Glass-30kPa Coll: Glass/30kPa Coll, Glass-30kPa FN: Glass/30kPa FN, HA Coll-FN: HA Coll/HA FN, 500Pa Coll-FN: 500Pa FN/500Pa Coll, 30kPa Coll-FN: 30kPa Coll/30kPa FN, 30kPa-HA Coll: 30kPa Coll/HA Coll, 30kPa-HA FN: 30kPa FN/HA FN). (N) refers to non-malignant (normal) cell lines. \*\*\*p-value < 0.001; \*\*p-value < 0.01; \*p-value < 0.05. For each cell line on a particular substrate,  $n > 25$  cells. See supplementary tables 1-3 for the exact value of  $n$  for the cell lines.

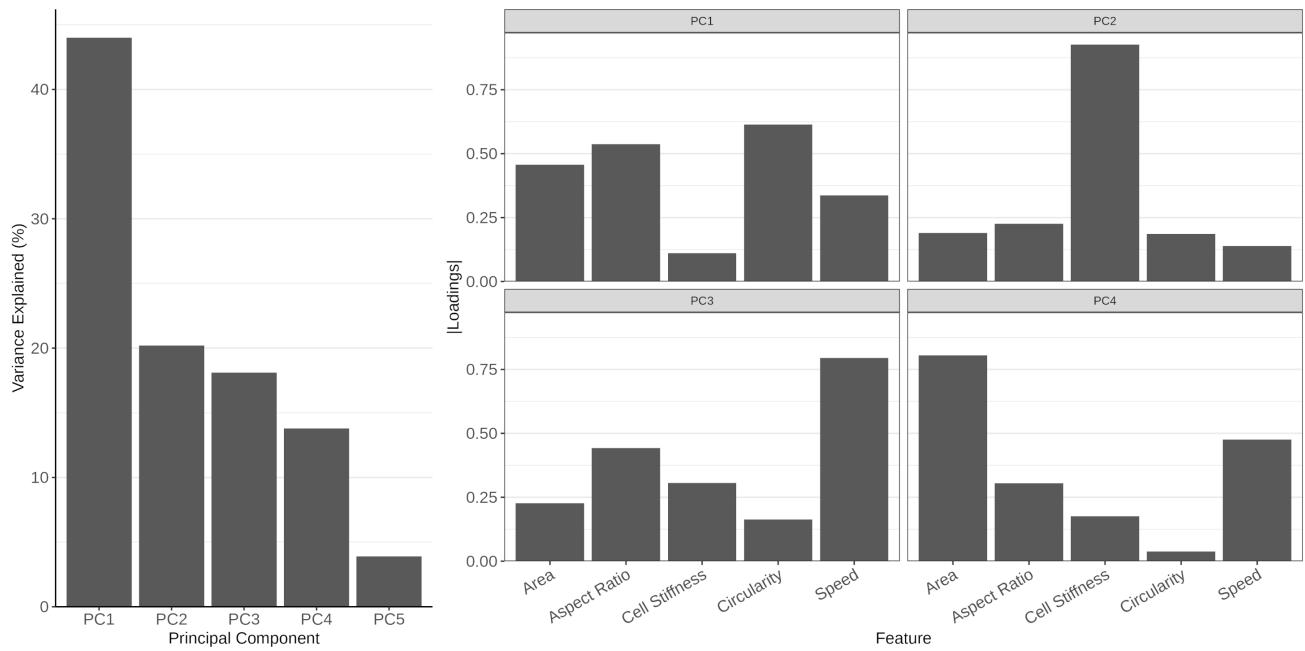


Supplementary Figure 4: KDEs for outlier-like distributions of (A) hTERT-HPNE cellular area across all substrates, and (B) Capan-1 cell stiffness across all substrates. Median values of cell (A) aspect ratio, and (B) circularity across all the 7 different substrates, along with the kernel density estimates (KDE) for summarizing the distribution of feature values for each cell line. (N) refers to non-malignant (normal) cell lines. Measurements from  $n > 200$  cells for each of the cell lines. See supplementary tables 1-3 for the exact value of  $n$  for the cell lines.

**A****B****C****D**

Supplementary Figure 5: Comparing tissue-specific normal and cancer cell behavior in terms of aspect ratio, and circularity for (A) prostate, (B) pancreas, (C) lung, and (D) breast cell lines soft (500 Pa) and stiff (30 kPa) PAAm Coll and FN substrates, soft (500 Pa) HA substrates coated with Coll and FN, and glass. (N) refers to non-malignant (normal) cell lines. \*\*\*p-value < 0.001; \*\*p-value < 0.01; \*p-value < 0.05. For each cell line on a particular substrate,  $n > 25$  cells. See supplementary tables 1-3 for the exact value of  $n$  for the cell lines.

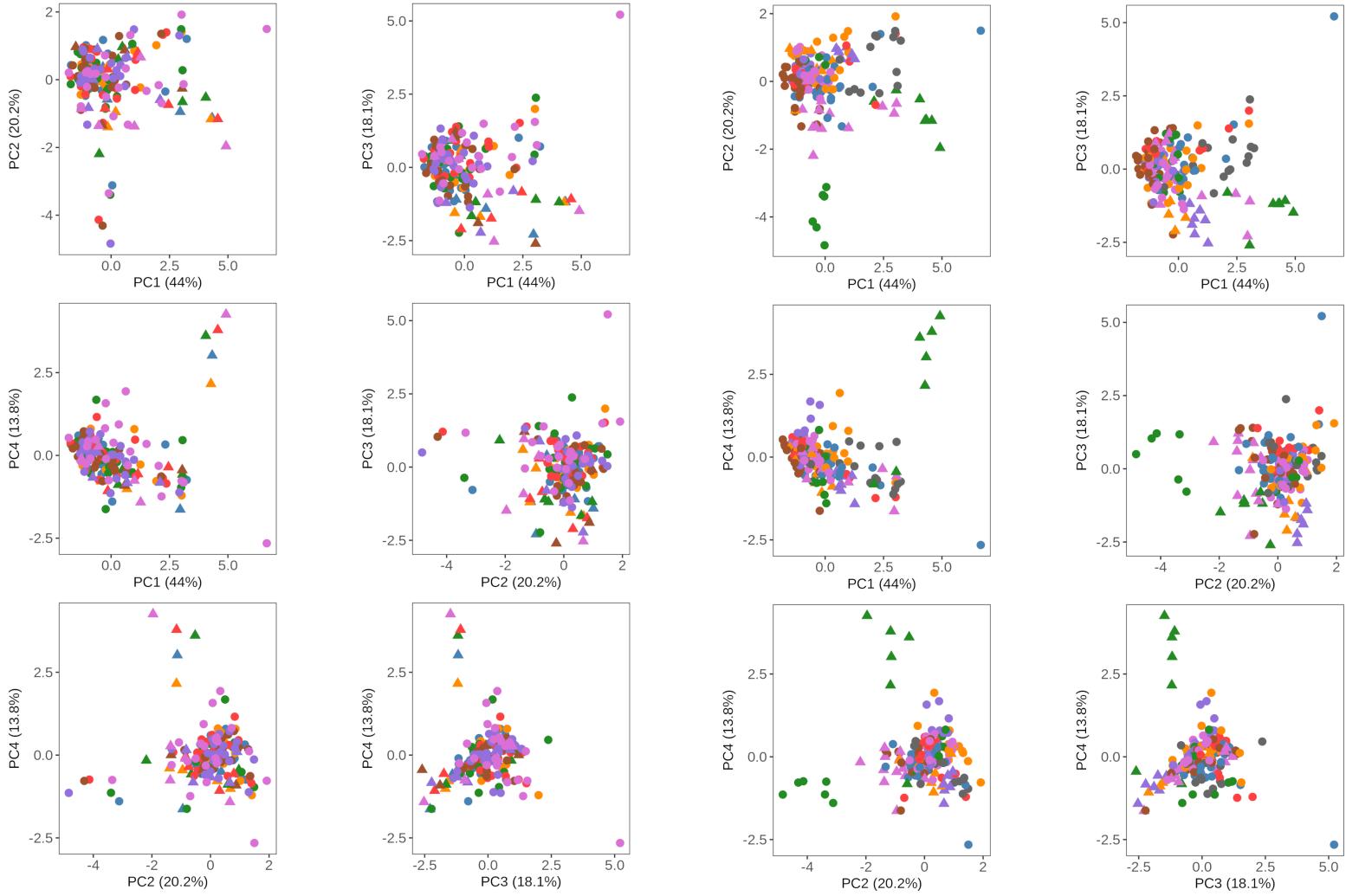
A



B

Legend:

- 30kPa Coll (green circle)
- 500Pa Coll (blue circle)
- Glass (pink circle)
- HA FN (purple circle)
- Cancer (black circle)
- Non-cancer (black triangle)
- 30kPa FN (red circle)
- 500Pa FN (orange circle)
- HA Coll (brown circle)

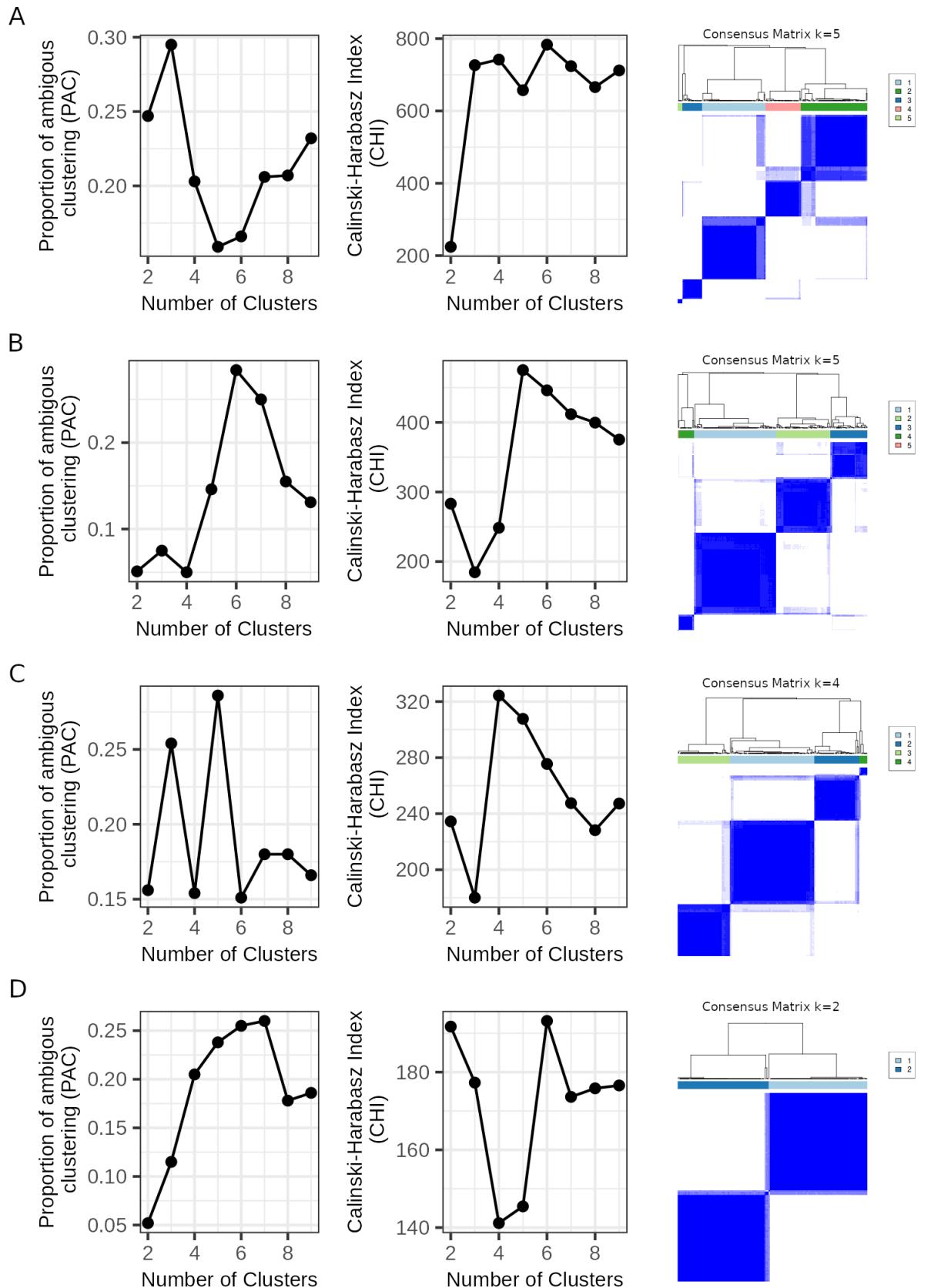


C

Legend:

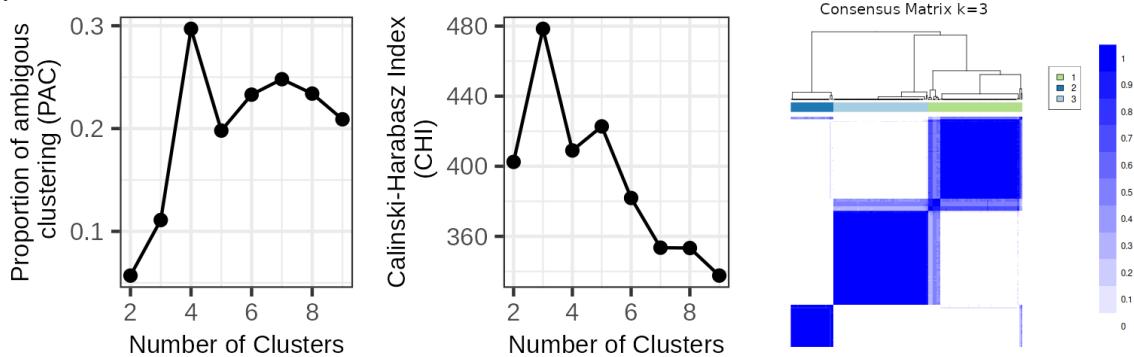
- Brain (grey circle)
- Colon (brown circle)
- Ovary (red circle)
- Prostate (orange circle)
- Cancer (black circle)
- Non-cancer (black triangle)
- Breast (pink circle)
- Lung (purple circle)
- Pancreas (green circle)
- Skin (blue circle)

Supplementary Figure 6: Principal component analysis (PCA) performed using the median values of cell area, aspect ratio, circularity, stiffness, and speed from all the cell line-substrate pairs. Note that in this analysis only those cell line-substrate pairs which have at least 25 data points for each of the physical feature are considered. (A) Variance explained by each of the PCs, and the loadings of the physical features on the first four PCs. Pairwise scatter plots for the first four PCs, with the points colored by (B) the substrate type and (C) tissue type.



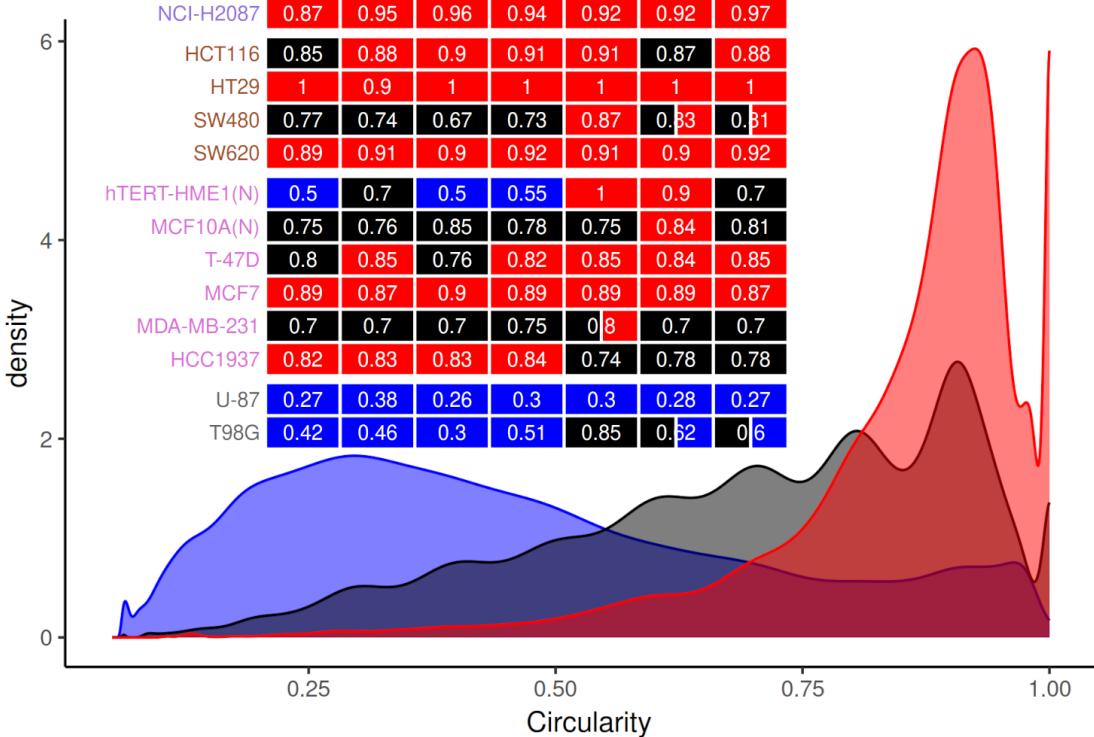
Supplementary Figure 7: Clustering statistics (PAC and CHI) used for identifying the optimal number of clusters (mechanotypes) based on Wasserstein-1 distance and the corresponding consensus matrix for optimal cluster count  $k$  of cell (A) area, (B) aspect ratio, (C) stiffness, and (D) speed.

A

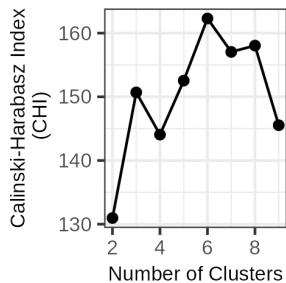
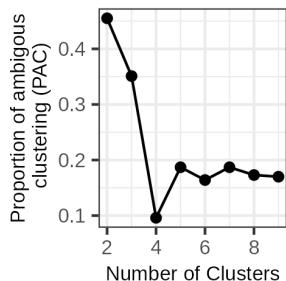


B

	500Pa Coll	500Pa FN	30kPa Coll	30kPa FN	HA Coll	HA FN	Glass
SK-MEL-2	0.77	0.82	0.65	0.67	0.81	0.57	0.67
A375	0.95	0.95	0.7	0.68	0.71	0.7	0.66
WM266-4	0.8	0.32	0.74	0.7	0.64	0.67	0.4
MeWo	0.83	0.74	0.9	0.46	0.71	0.79	0.24
RWPE-1(N)	0.87	0.89	0.31	0.89	0.92	0.79	0.93
22Rv1	0.67	0.82	0.88	0.79	0.53	0.85	0.47
LnCaP	0.95	0.35	0.96	0.92	0.93	0.51	0.32
DU145	0.86	0.83	0.75	0.75	0.76	0.74	0.77
PC-3	0.48	0.58	0.53	0.43	0.46	0.83	0.67
hTERT-HPNE(N)	0.41	0.4	0.38	0.41	0.52	0.49	0.36
Panc-1	0.89		0.99	0.98	0.88	0.75	0.77
Capan-1	0.9	0.91	0.92	0.91	0.91	0.91	0.85
SKOV-3	0.89	0.34	0.55	0.54	0.85	0.94	0.47
Caov-3	0.9		0.84	0.88	0.83	0.79	0.65
OVCAR-3	0.84	0.86	0.88	0.87	0.84	0.82	0.32
NL20(N)	0.61	0.7	0.7	0.6	0.7	0.7	0.6
NCI-H2126	0.83	0.87	0.87	0.82	0.88	0.85	0.34
NCI-H2087	0.87	0.95	0.96	0.94	0.92	0.92	0.97
HCT116	0.85	0.88	0.9	0.91	0.91	0.87	0.88
HT29	1	0.9	1	1	1	1	1
SW480	0.77	0.74	0.67	0.73	0.87	0.83	0.31
SW620	0.89	0.91	0.9	0.92	0.91	0.9	0.92
hTERT-HME1(N)	0.5	0.7	0.5	0.55	1	0.9	0.7
MCF10A(N)	0.75	0.76	0.85	0.78	0.75	0.84	0.81
T-47D	0.8	0.85	0.76	0.82	0.85	0.84	0.85
MCF7	0.89	0.87	0.9	0.89	0.89	0.89	0.87
MDA-MB-231	0.7	0.7	0.7	0.75	0.8	0.7	0.7
HCC1937	0.82	0.83	0.83	0.84	0.74	0.78	0.78
U-87	0.27	0.38	0.26	0.3	0.3	0.28	0.27
T98G	0.42	0.46	0.3	0.51	0.85	0.62	0.6

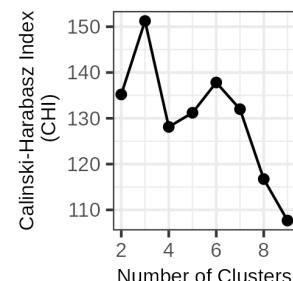
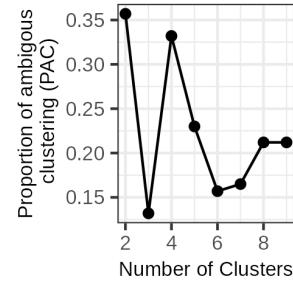


Supplementary Figure 8: (A) Clustering statistics (PAC and CHI) used for identifying the optimal number of clusters (mechanotypes) based on Wasserstein-1 distance and the corresponding consensus matrix for optimal cluster count  $k$  of circularity. (B) Mechanotypes for circularity, whereby the heatmap shows the phenotypic class for each cell line-substrate pair and the KDEs correspond to characteristic density function for each class. The numeric values shown in the heatmap correspond to median values of circularity for each cell line-substrate pair. (N) refers to non-malignant (normal) cell lines. Note that in this analysis only those cell line-substrate pairs which have at least 25 data points for the physical feature of interest are considered.

**A**

Tissue	Skin	Pancreas	Lung	Breast	U-87	T98G	Glass
Prostate							
Ovary							
Colon							
Brain							

	500Pa Coll	500Pa FN	30kPa Coll	30kPa FN	HA Coll	HA FN	Glass
SK-MEL-2	291	296	361	341	317	505	652
A375	314	279	539	551	472	571	672
WM266-4	277	296	318	354	487	408	755
MeWo	263	374	244	501	354	320	664
RWPE-1(N)	409	340	448	356	257	348	267
22Rv1	351	297	254	305	392	240	396
LnCaP	350	455	396	424	341	505	581
DU145	483	496	653	926	510	695	1054
PC-3	1611	914	1564	1084	959	514	1388
hTERT-HPNE(N)	3017	2659	3104	3395	1398	781	3738
Panc-1	437		406	491	480	552	727
Capan-1	406	549	480	376	441	495	412
SKOV-3	252	429	339	351	319	313	440
Caov-3	392		373	376	330	371	852
OVCAR-3	292	312	316	396	345	360	390
NL20(N)	550	423	518	664	439	549	446
NCI-H2126	561	628	1054	762	486	551	1168
NCI-H2087	458	454	410	433	443	434	369
HCT116	298	250	299	273	268	289	377
HT29	302	326	304	313	299	298	327
SW480	34	319	374	407	265	322	349
SW620	233	195	204	200	193	200	220
hTERT-HME1(N)	904	450	1229	1148	283	260	694
MCF10A(N)	409	389	464	497	401	348	539
T-47D	265	224	359	312	294	313	258
MCF7	386	244	366	310	391	311	354
MDA-MB-231	404	412	372	382	338	400	396
HCC1937	642	546	578	544	502	646	509
U-87	744	501	580	522	520	541	753
T98G	1077	543	1105	797	375	507	1137

**B**

Tissue	Skin	Pancreas	Lung	Breast	U-87	T98G	Glass
Prostate							
Ovary							
Colon							
Brain							

	500Pa Coll	500Pa FN	30kPa Coll	30kPa FN	HA Coll	HA FN	Glass
SK-MEL-2	2839	2715	4325	4391	2701	3110	5364
A375	3521	3635	4083	3539	2688	2934	5589
WM266-4	3632	3655	3678	4430	3690	4000	4216
MeWo	2731	2843	2944	2373	2469	2726	5269
RWPE-1(N)	2017	2077	1583	1776	1143	1701	4546
22Rv1	1526	1518	1643	1605	1526	1738	2037
LnCaP	956		1215			1064	1686
DU145	2305	2926	2724	2770	3293	2766	3285
PC-3	2031	2143	2403	1678	2142	1252	2382
hTERT-HPNE(N)	4252	4555	3052	4133	2898	4422	5193
Panc-1	2744	2689	2349	1692	1912	2203	1834
Capan-1	8255	7482	8826	10700	10927	11670	9483
SKOV-3	2434	2868	2156	3033	3525	2988	5858
Caov-3	3070	2766	1748	2632	2567	3296	2413
OVCAR-3	2458	2865	2394	3355	2809	2987	2766
NL20(N)	1164	1597	1186	1369	1133	1298	1454
NCI-H2126	2171	2531	1769	1582	1558	1851	2444
NCI-H2087	2332	1854	2746	2477	2152	2421	2023
HCT116	2086	2617	3714	3501	1762	2120	3537
HT29	1988	1569	3115	2664	1939	2386	2511
SW480	3853	3517	5322	5955	4859	5838	3438
SW620	2529	3010	3716	3627	2780	2735	4456
hTERT-HME1(N)	4569	4931	4387	4488	3357	2537	5357
MCF10A(N)	4389	5896	7198	4691	5989	4781	5737
T-47D	1401	1523	1822	1427	1303	1439	2145
MCF7	3276	2472	3257	2948	2993	2219	2451
MDA-MB-231	1613	2184	3129	2541	1984	2284	4216
HCC1937	2173	2352	2723	2881	2759	2438	2763
U-87	2494	2090	1926	1767	1676	2139	4747
T98G	4681	4381	4474	4293	2195	2395	3470

Supplementary Figure 9: Clustering statistics (PAC and CHI) used for identifying the optimal number of clusters based on Kolomogrov-Smirnov distance and the heatmap showing the corresponding classes for each of the cell line-substrate pairs for cell (A) area, and (B) stiffness. The numeric values shown in the heatmap correspond to median values of circularity for each cell line-substrate pair. (N) refers to non-malignant (normal) cell lines. Note that in this analysis only those cell line-substrate pairs which have at least 25 data points for the physical feature of interest are considered.