

# Proteomic Profile Distinguishes New Subpopulations of Breast Cancer Patients with Different Survival Outcomes

Joanna Tobiasz <sup>1,2</sup> and Joanna Polanska <sup>1,\*</sup>

<sup>1</sup> Department of Data Science and Engineering, Silesian University of Technology, 44-100 Gliwice, Poland; joanna.tobiasz@polsl.pl

<sup>2</sup> Department of Computer Graphics, Vision and Digital Systems, Silesian University of Technology, 44-100 Gliwice, Poland

\* Correspondence: joanna.polanska@polsl.pl

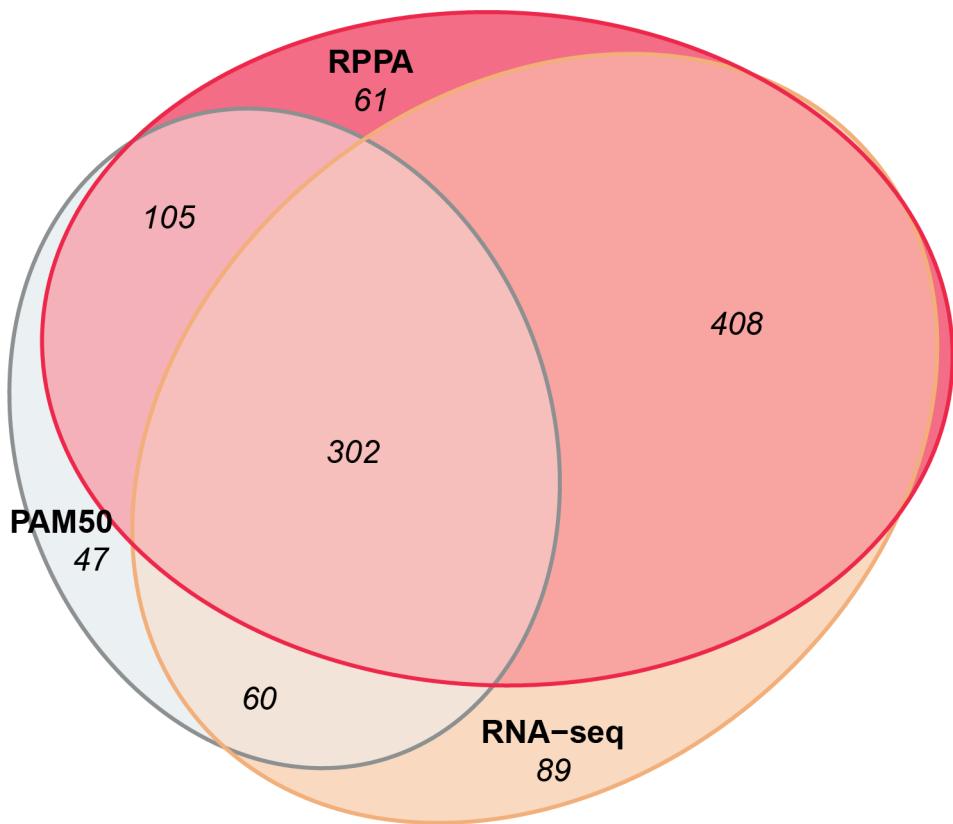
## Supplementary Materials

**Table S1.** The list of 86 proteins selected with the Gaussian-mixture-model-based feature filtration.

Selected proteins
14-3-3_zeta-R-V
4E-BP1-R-V
4E-BP1_pT37_T46-R-V
ACC_pS79-R-V
ADAR1-M-V
Akt-R-V
Akt_pS473-R-V
Akt_pT308-R-V
AMPK_pT172-R-V
Annixin-1-M-E
AR-R-V
ASNS-R-V
Bcl-2-M-V
Bcl2A1-R-V
beta-Catenin-R-V
Bim-R-V
B-Raf_pS445-R-V
BRD4-R-V
Caspase-7_cleavedD198-R-C
Caveolin-1-R-V
CD31-M-V
CDK1_pY15-R-C
c-Kit-R-V
Claudin-7-R-V
c-Myc-R-C
COG3-R-V
Collagen_VI-R-V

<b>Selected proteins</b>
Cyclin_B1-R-V
Cyclin_D1-R-V
Cyclin_E1-M-V
DUSP4-R-V
E-Cadherin-R-V
eEF2K-R-V
EGFR_pY1068-R-C
eIF4G-R-C
ER-alpha-R-V
ER-alpha_pS118-R-V
ERK2-R-E
FASN-R-V
Fibronectin-R-V
FoxM1-R-V
G6PD-M-V
GAB2-R-V
GAPDH-M-C
GATA3-M-V
GSK3-alpha-beta-M-V
GSK3-alpha-beta_pS21_S9-R-V
GSK3_pS9-R-V
HER2-M-V
HSP70-R-C
IGFBP2-R-V
Ku80-R-C
Lck-R-V
MAPK_pT202_Y204-R-V
MEK1-R-V
mTOR-R-V
MYH11-R-V
Myosin-IIa_pS1943-R-V
NDRG1_pT346-R-V
NF-kB-p65_pS536-R-C
p16_INK4a-R-V
p27-R-V
p38_pT180_Y182-R-V
p70S6K-R-V
PDCD4-R-C
PKC-pan_BetaII_pS660-R-V
PR-R-V
PREX1-R-E
PTEN-R-V
Rab25-R-V
RBM15-R-V

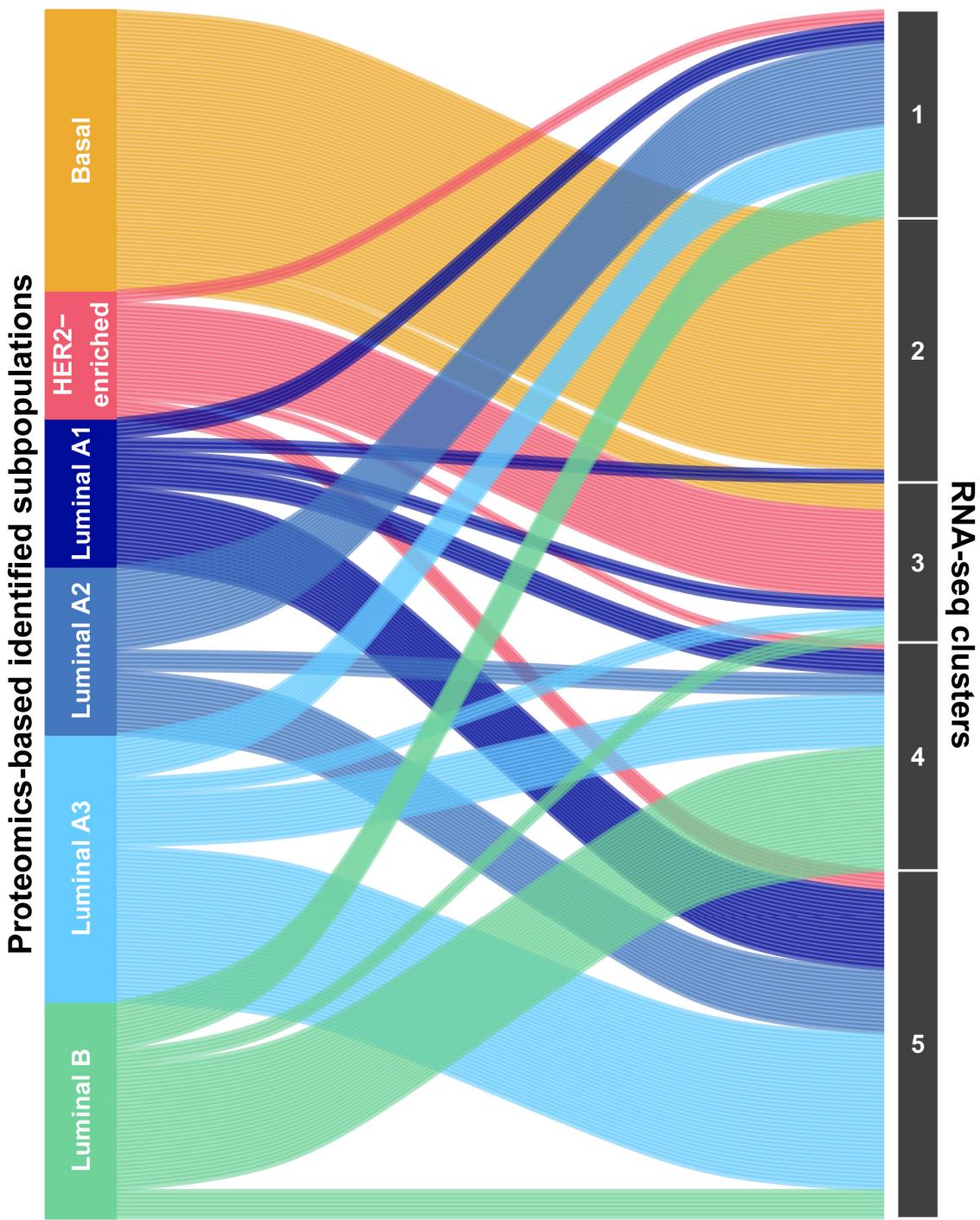
Selected proteins
Rb_pS807_S811-R-V
Rictor-R-C
S6-R-E
S6_pS235_S236-R-V
S6_pS240_S244-R-V
SCD-M-V
SHP-2_pY542-R-C
Smac-M-E
Src_pY416-R-C
Src_pY527-R-V
STAT3_pY705-R-V
STAT5-alpha-R-V
Syk-M-V
TFRC-R-V
TSC1-R-C



**Figure S1.** The overlapping of patients between the data sets of all available PAM50 labels, protein levels measured with Reverse Phase Protein Arrays (RPPA), and RNA sequencing measurements used by Tobiasz et al. [30].

**Table S2.** Number and percentage of patients in proteomics-based subpopulations referred to clusters obtained based on the RNA sequencing results [30].

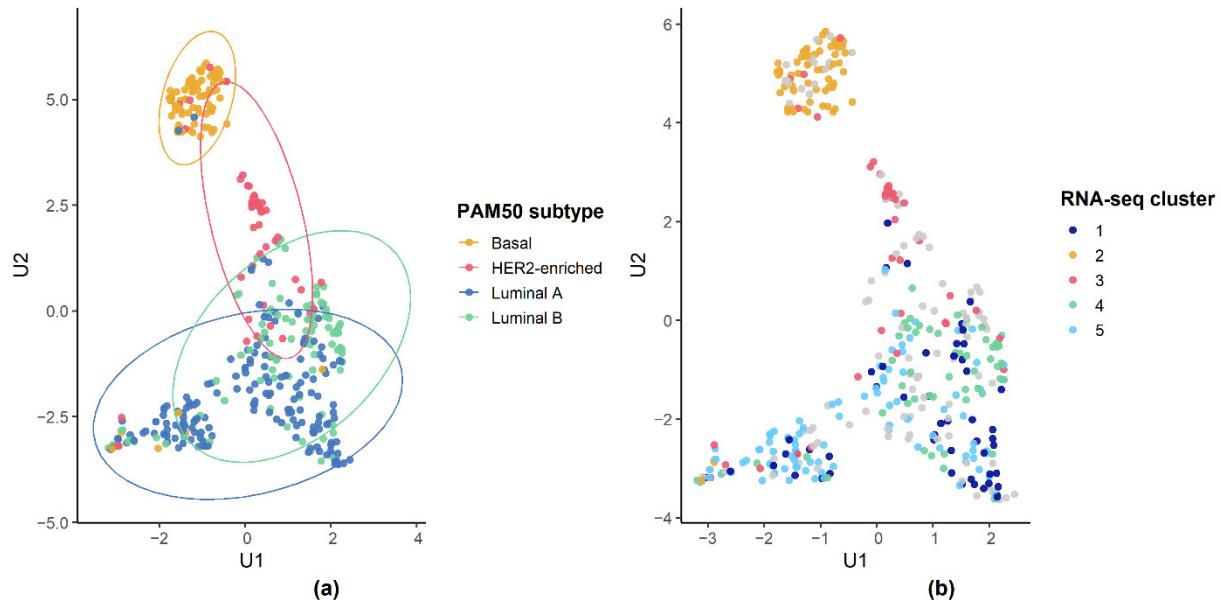
RNA-seq-based cluster	Proteomics-based subpopulation						TOTAL	
	Basal	HER2-enriched	Luminal					
			A1	A2	A3	B		
1	0 (0.00%)	3 (9.38%)	5 (13.51%)	21 (50.00%)	11 (16.42%)	12 (22.22%)	52 (17.22%)	
2	63 (90.00%)	0 (0.00%)	3 (8.11%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	66 (21.85%)	
3	7 (10.00%)	22 (68.75%)	3 (8.11%)	0 (0.00%)	4 (5.97%)	4 (7.41%)	40 (13.25%)	
4	0 (0.00%)	2 (6.25%)	6 (16.22%)	5 (11.90%)	13 (19.40%)	31 (57.41%)	57 (18.87%)	
5	0 (0.00%)	5 (15.62%)	20 (54.05%)	16 (38.10%)	39 (58.21%)	7 (12.96%)	87 (28.81%)	
<b>TOTAL</b>	<b>70</b> (100.00%)	<b>32</b> (100.00%)	<b>37</b> (100.00%)	<b>42</b> (100.00%)	<b>67</b> (100.00%)	<b>54</b> (100.00%)	<b>302</b> (100.00%)	



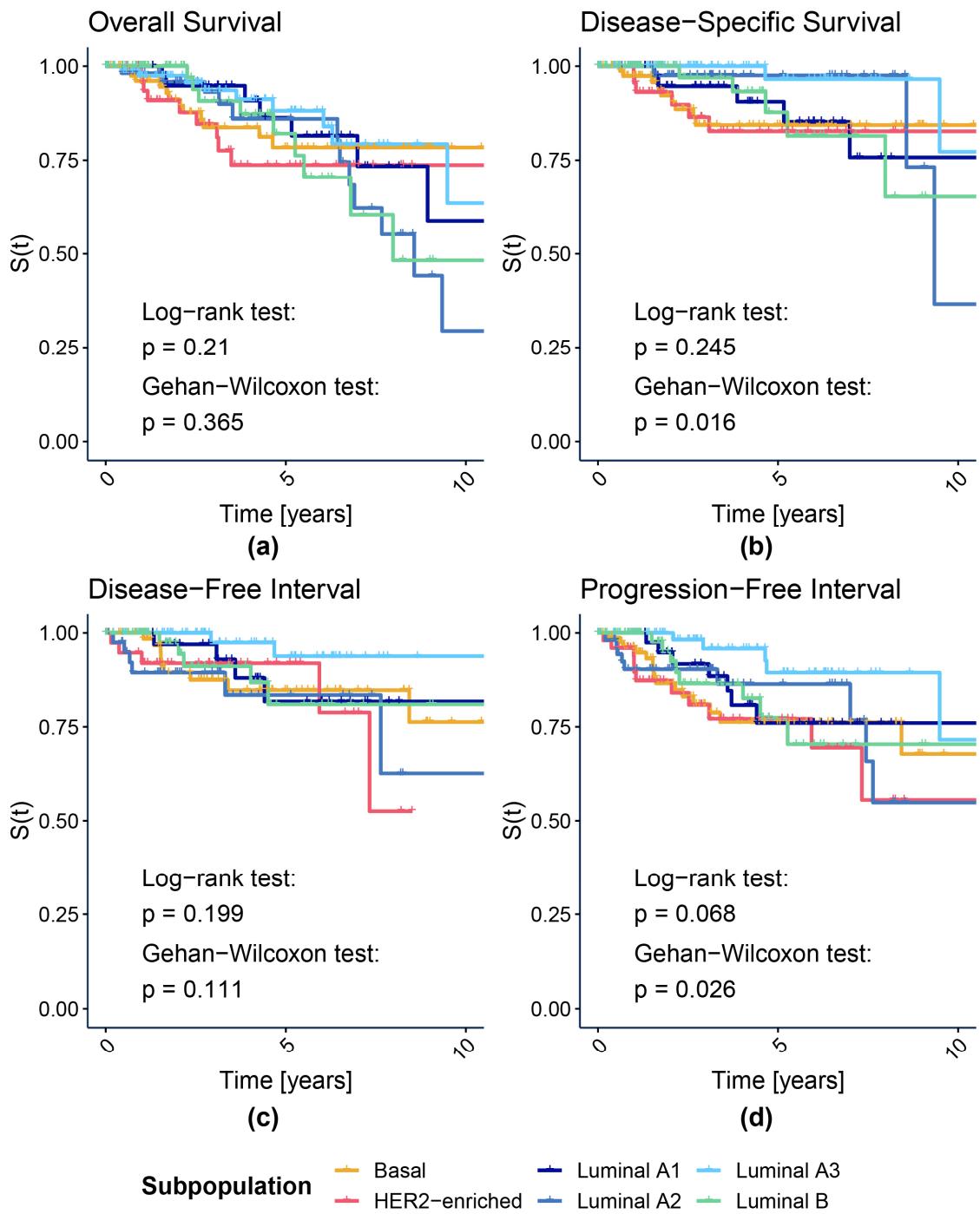
**Figure S2.** Proteomics-based subpopulations related to clusters obtained based on the RNA sequencing results [30].

**Table S3.** Number and percentage of patients in clusters obtained based on the RNA sequencing results [30] referred to PAM50 subtypes.

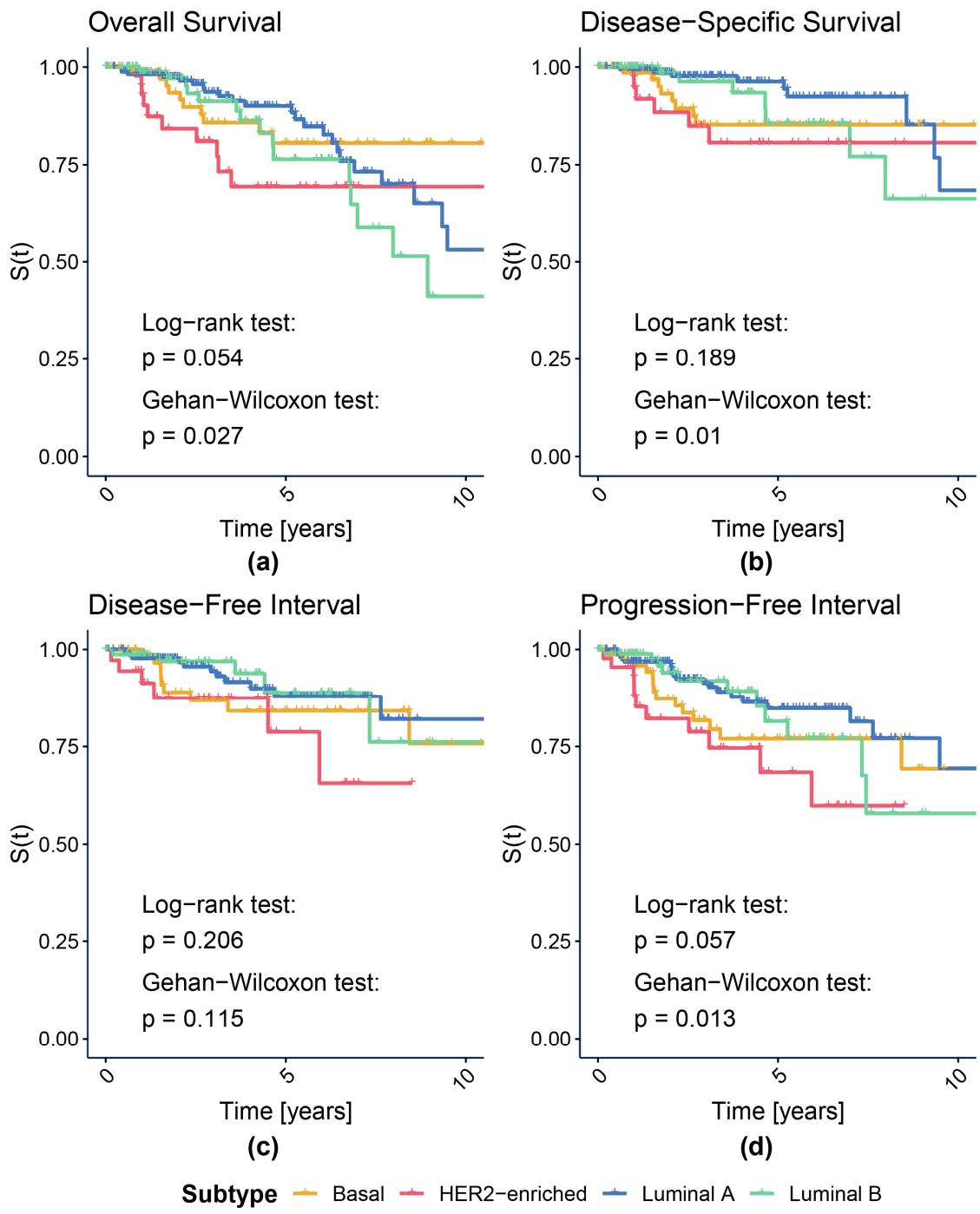
PAM50 subtype	RNA-seq-based cluster				
	1	2	3	4	5
<b>Basal</b>	<b>1</b> (1.92%)	<b>65</b> (98.48%)	<b>2</b> (5.00%)	<b>1</b> (1.75%)	<b>1</b> (1.15%)
<b>HER2-enriched</b>	<b>4</b> (7.69%)	<b>0</b> (0.00%)	<b>24</b> (60.00%)	<b>0</b> (0.00%)	<b>3</b> (3.45%)
<b>Luminal A</b>	<b>32</b> (61.54%)	<b>1</b> (1.52%)	<b>6</b> (15.00%)	<b>24</b> (42.11%)	<b>74</b> (85.06%)
<b>Luminal B</b>	<b>15</b> (28.85%)	<b>0</b> (0.00%)	<b>8</b> (20.00%)	<b>32</b> (56.14%)	<b>9</b> (10.34%)
<b>TOTAL</b>	<b>52</b> (100.00%)	<b>66</b> (100.00%)	<b>40</b> (100.00%)	<b>57</b> (100.00%)	<b>87</b> (100.00%)



**Figure S3.** Protein-level-based UMAP visualization with data points colored with regard to breast cancer subtypes. **(a)** Breast cancer subtypes obtained with the PAM50 transcriptomic-based predictor. **(b)** Breast cancer patients' subtypes resulting from clustering of RNA sequencing results [30].



**Figure S4.** Kaplan-Meier survival curves of all subpopulations identified with k-means clustering of protein levels. (a) Overall Survival; (b) Disease-Specific Survival; (c) Disease-Free Interval; (d) Progression-Free Interval.



**Figure S5.** Kaplan-Meier survival curves of all PAM50 subtypes. (a) Overall Survival; (b) Disease-Specific Survival; (c) Disease-Free Interval; (d) Progression-Free Interval.

**Table S4.** Results of log-rank and Gehan-Wilcoxon tests for luminal subtypes identified with k-means clustering or PAM50 classifier.

Endpoint type	$\chi^2$		p-value	
	Log-rank test	Gehan-Wilcoxon test	Log-rank test	Gehan-Wilcoxon test
<b>Proteomics-based subpopulations</b>				
Overall Survival	4.99	0.68	0.1724	0.8788
Disease-Specific Survival	4.06	5.08	0.2552	0.1661
Disease-Free Interval	6.97	9.12	0.0730	0.0277
Progression-Free Interval	4.87	6.41	0.1818	0.0932
<b>PAM50-based subtypes</b>				
Overall Survival	2.32	0.57	0.1280	0.4521
Disease-Specific Survival	3.01	0.70	0.0828	0.4043
Disease-Free Interval	0.01	0.10	0.9333	0.7488
Progression-Free Interval	0.56	0.003	0.4530	0.9512

**Table S5.** Results of log-rank and Gehan-Wilcoxon tests for all subtypes identified with k-means clustering or PAM50 classifier.

Endpoint type	$\chi^2$		p-value	
	Log-rank test	Gehan-Wilcoxon test	Log-rank test	Gehan-Wilcoxon test
<b>Proteomics-based subpopulations</b>				
Overall Survival	:148	8177	3153<<	31697;
Disease-Specific Survival	919<	461<7	31577:	313493
Disease-Free Interval	:164	;1<9	314<;9	31443;
Progression-Free Interval	4315;	451:3	3139::	313597
<b>PAM50-based subtypes</b>				
Overall Survival	:199	<14;	313869	3135:3
Disease-Specific Survival	71:;	4415:	314:;;	313436
Disease-Free Interval	718:	81<6	315393	314486
Progression-Free Interval	:185	431:5	3138:4	313466

**Table S6.** Cox proportional hazard analysis of all identified subpopulations.

Subpopulation	N	Ne	Nc	HR	HR effect	HR allocation adjusted critical value			
						$\alpha = 0.1$ small effect	$\alpha = 0.3$ medium effect	$\alpha = 0.5$ large effect	
<b>Overall Survival</b>									
<b>Basal</b>	89	13	76	Reference					
<b>HER2-enriched</b>	54	11	43	1.836	Small	HR < 0.773; HR > 1.294	HR < 0.468; HR > 2.135	HR < 0.274; HR > 3.648	
<b>Luminal A1</b>	44	8	36	1.050	No effect	HR < 0.749; HR > 1.336	HR < 0.436; HR > 2.295	HR < 0.249; HR > 4.023	
<b>Luminal A2</b>	61	14	47	2.016	Small	HR < 0.785; HR > 1.273	HR < 0.487; HR > 2.054	HR < 0.289; HR > 3.459	
<b>Luminal A3</b>	87	9	78	0.837	No effect	HR < 0.816; HR > 1.225	HR < 0.536; HR > 1.867	HR < 0.331; HR > 3.023	
<b>Luminal B</b>	72	9	63	1.445	Small	HR < 0.801; HR > 1.248	HR < 0.511; HR > 1.958	HR < 0.309; HR > 3.236	
<b>Disease-Specific Survival</b>									
<b>Basal</b>	87	9	78	Reference					
<b>HER2-enriched</b>	52	7	45	1.579	Small	HR < 0.771; HR > 1.297	HR < 0.466; HR > 2.146	HR < 0.272; HR > 3.673	
<b>Luminal A1</b>	43	5	38	0.957	No effect	HR < 0.749; HR > 1.336	HR < 0.436; HR > 2.296	HR < 0.249; HR > 4.023	
<b>Luminal A2</b>	58	4	54	0.817	No effect	HR < 0.783; HR > 1.278	HR < 0.483; HR > 2.071	HR < 0.286; HR > 3.5	
<b>Luminal A3</b>	85	2	83	0.241	Large	HR < 0.816; HR > 1.225	HR < 0.536; HR > 1.867	HR < 0.331; HR > 3.024	
<b>Luminal B</b>	72	5	67	1.002	No effect	HR < 0.803; HR > 1.245	HR < 0.514; HR > 1.946	HR < 0.312; HR > 3.208	
<b>Disease-Free Interval</b>									
<b>Basal</b>	79	9	70	Reference					
<b>HER2-enriched</b>	41	5	36	1.315	No effect	HR < 0.755; HR > 1.325	HR < 0.444; HR > 2.254	HR < 0.255; HR > 3.927	
<b>Luminal A1</b>	38	4	34	0.819	No effect	HR < 0.745; HR > 1.342	HR < 0.431; HR > 2.32	HR < 0.245; HR > 4.079	
<b>Luminal A2</b>	46	6	40	1.516	Small	HR < 0.768; HR > 1.302	HR < 0.462; HR > 2.165	HR < 0.269; HR > 3.717	
<b>Luminal A3</b>	79	2	77	0.225	Large	HR < 0.818; HR > 1.222	HR < 0.538; HR > 1.857	HR < 0.333; HR > 3	
<b>Luminal B</b>	64	5	59	0.884	No effect	HR < 0.801; HR > 1.248	HR < 0.511; HR > 1.958	HR < 0.309; HR > 3.234	
<b>Progression-Free Interval</b>									
<b>Basal</b>	89	15	74	Reference					
<b>HER2-enriched</b>	54	12	42	1.483	Small	HR < 0.773; HR > 1.294	HR < 0.468; HR > 2.135	HR < 0.274; HR > 3.648	
<b>Luminal A1</b>	44	7	37	0.738	Small	HR < 0.749; HR > 1.336	HR < 0.436; HR > 2.295	HR < 0.249; HR > 4.023	

Subpopulation	N	Ne	Nc	HR	HR effect	HR allocation adjusted critical value		
						$\alpha = 0.1$ small effect	$\alpha = 0.3$ medium effect	$\alpha = 0.5$ large effect
Luminal A2	61	9	52	0.965	No effect	HR < 0.785; HR > 1.273	HR < 0.487; HR > 2.054	HR < 0.289; HR > 3.459
Luminal A3	87	5	82	0.308	Large	HR < 0.816; HR > 1.225	HR < 0.536; HR > 1.867	HR < 0.331; HR > 3.023
Luminal B	72	8	64	0.846	No effect	HR < 0.801; HR > 1.248	HR < 0.511; HR > 1.958	HR < 0.309; HR > 3.236

**Table S7.** Cox proportional hazard analysis of all PAM50 subtypes.

PAM50 subtype	N	Ne	Nc	HR	HR effect	HR allocation adjusted critical value		
						$\alpha = 0.1$ small effect	$\alpha = 0.3$ medium effect	$\alpha = 0.5$ large effect
<b>Overall Survival</b>								
Basal	86	11	75			Reference		
HER2	50	11	39	2.830	Medium	HR < 0.768; HR > 1.302	HR < 0.462; HR > 2.166	HR < 0.269; HR > 3.72
LumA	173	26	147	1.408	Small	HR < 0.857; HR > 1.166	HR < 0.609; HR > 1.642	HR < 0.4; HR > 2.497
LumB	98	16	82	2.137	Medium	HR < 0.827; HR > 1.209	HR < 0.554; HR > 1.805	HR < 0.348; HR > 2.878
<b>Disease-Specific Survival</b>								
Basal	84	8	76			Reference		
HER2	48	6	42	1.977	Small	HR < 0.766; HR > 1.306	HR < 0.459; HR > 2.179	HR < 0.267; HR > 3.75
LumA	169	10	159	0.691	Small	HR < 0.857; HR > 1.166	HR < 0.609; HR > 1.642	HR < 0.4; HR > 2.497
LumB	96	8	88	1.326	Small	HR < 0.828; HR > 1.208	HR < 0.554; HR > 1.804	HR < 0.348; HR > 2.875
<b>Disease-Free Interval</b>								
Basal	76	9	67			Reference		
HER2	42	6	36	1.723	Small	HR < 0.762; HR > 1.312	HR < 0.454; HR > 2.204	HR < 0.262; HR > 3.81
LumA	147	11	136	0.664	Small	HR < 0.856; HR > 1.169	HR < 0.606; HR > 1.65	HR < 0.397; HR > 2.517
LumB	82	5	77	0.624	Small	HR < 0.824; HR > 1.214	HR < 0.548; HR > 1.826	HR < 0.342; HR > 2.927
<b>Progression-Free Interval</b>								
Basal	86	14	72			Reference		
HER2	50	11	39	1.840	Small	HR < 0.768; HR > 1.302	HR < 0.462; HR > 2.166	HR < 0.269; HR > 3.72
LumA	173	20	153	0.679	Small	HR < 0.857; HR > 1.166	HR < 0.609; HR > 1.642	HR < 0.4; HR > 2.497
LumB	98	11	87	0.858	No effect	HR < 0.827;	HR < 0.554;	HR < 0.348;

PAM50 subtype	N	N <sub>e</sub>	N <sub>c</sub>	HR	HR effect	HR allocation adjusted critical value		
						$\alpha = 0.1$ small effect	$\alpha = 0.3$ medium effect	$\alpha = 0.5$ large effect
						HR > 1.209	HR > 1.805	HR > 2.878

"HER2" denotes HER2-enriched subtype, "LumA" luminal A subtype, and "LumB" luminal B subtype.

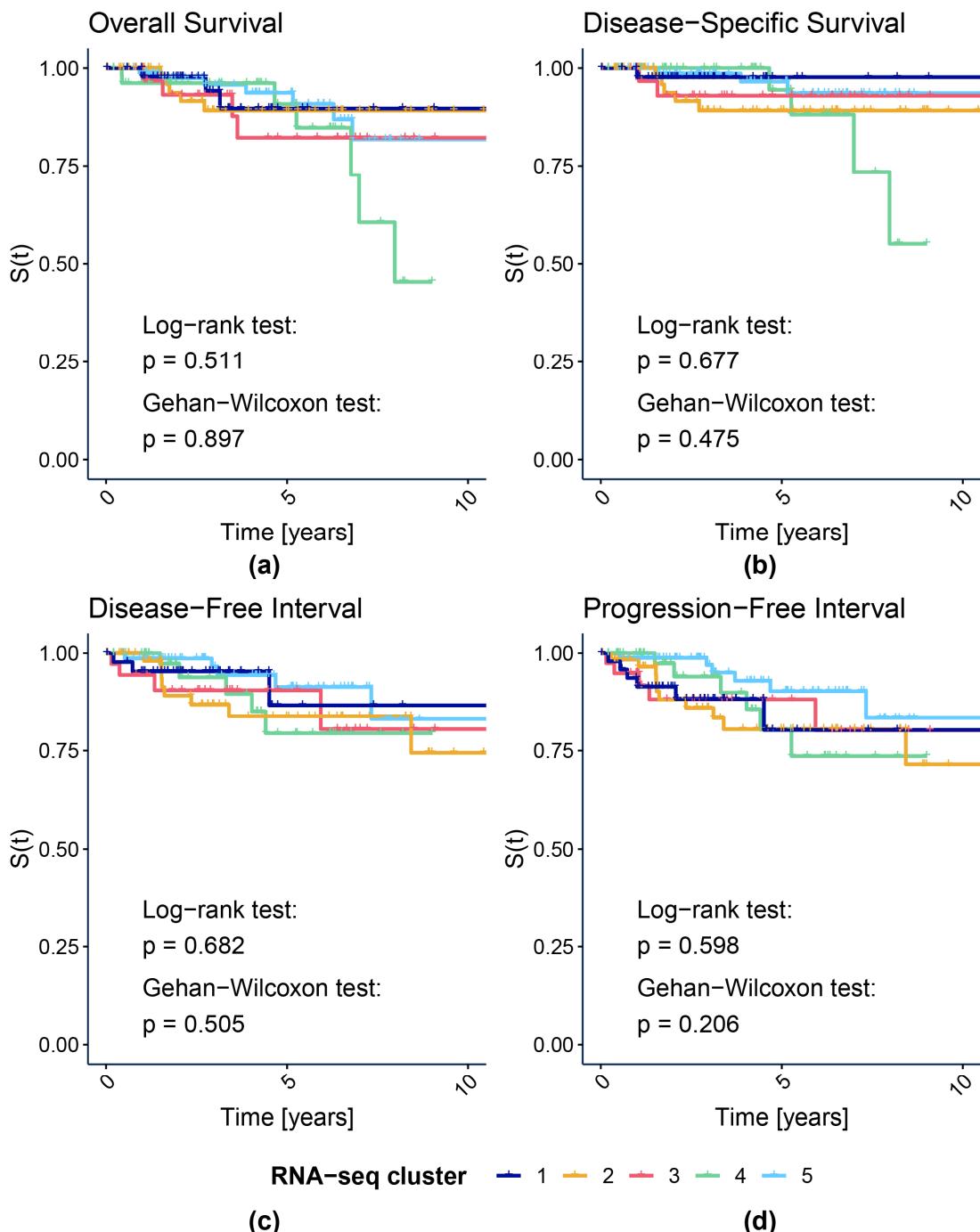
**Table S8.** Results of log-rank and Gehan-Wilcoxon tests for pairwise comparisons of luminal subpopulations identified with k-means clustering.

Comparison	$\chi^2$		p-value	
	Log-rank test	Gehan-Wilcoxon test	Log-rank test	Gehan-Wilcoxon test
<b>Overall Survival</b>				
<b>A1 versus A3</b>	0.37	0.04	0.5432	0.8430
<b>A2 versus A3</b>	3.80	0.43	0.0511	0.5138
<b>B versus A3</b>	1.38	0.10	0.2395	0.7578
<b>A2 versus A1</b>	2.93	0.61	0.0872	0.4358
<b>B versus A1</b>	0.54	0.10	0.4629	0.7518
<b>A2 versus B</b>	0.15	0.09	0.7020	0.7632
<b>Disease-Specific Survival</b>				
<b>A1 versus A3</b>	2.83	4.70	0.0925	0.0302
<b>A2 versus A3</b>	4.04	1.23	0.0444	0.2670
<b>B versus A3</b>	3.84	4.10	0.0499	0.0428
<b>A2 versus A1</b>	0.00	0.93	0.9867	0.3337
<b>B versus A1</b>	0.00	0.25	0.9812	0.6187
<b>A2 versus B</b>	0.23	0.43	0.6334	0.5104
<b>Disease-Free Interval</b>				
<b>A1 versus A3</b>	2.59	3.02	0.1074	0.0824
<b>A2 versus A3</b>	6.74	7.52	0.0094	0.0061
<b>B versus A3</b>	3.59	4.35	0.0583	0.0370
<b>A2 versus A1</b>	0.85	1.34	0.3567	0.2474
<b>B versus A1</b>	0.02	0.04	0.8937	0.8359
<b>A2 versus B</b>	0.86	2.18	0.3525	0.1398
<b>Progression-Free Interval</b>				
<b>A1 versus A3</b>	2.28	4.18	0.1313	0.0409
<b>A2 versus A3</b>	4.30	5.41	0.0382	0.0201
<b>B versus A3</b>	4.24	5.36	0.0394	0.0207
<b>A2 versus A1</b>	0.17	0.22	0.6803	0.6363
<b>B versus A1</b>	0.04	0.01	0.8333	0.9235
<b>A2 versus B</b>	0.01	0.62	0.9033	0.4297

**Table S9.** Cox proportional hazard pairwise analysis of luminal subpopulations identified with k-means clustering.

Compar- son	Subpo- pulation	N	Ne	Nc	HR	HR effect	HR allocation adjusted critical value		
							$\alpha = 0.1$	$\alpha = 0.3$	$\alpha = 0.5$
							small effect	medium effect	large effect
<b>Overall Survival</b>									
<b>A1 vs A3</b>	<b>A1</b>	44	8	36	1.344	Small	HR < 0.751; HR > 1.331	HR < 0.439; HR > 2.276	HR < 0.251; HR > 3.977
	<b>A3</b>	87	9	78			Reference		
<b>A2 vs A3</b>	<b>A2</b>	61	14	47	2.278	Medium	HR < 0.788; HR > 1.27	HR < 0.49; HR > 2.04	HR < 0.292; HR > 3.426
	<b>A3</b>	87	9	78			Reference		
<b>B vs A3</b>	<b>A3</b>	87	9	78			Reference		
	<b>B</b>	72	9	63	1.740	Small	HR < 0.803; HR > 1.245	HR < 0.514; HR > 1.946	HR < 0.312; HR > 3.208
<b>A2 vs A1</b>	<b>A1</b>	44	8	36			Reference		
	<b>A2</b>	61	14	47	2.196	Medium	HR < 0.839; HR > 1.191	HR < 0.575; HR > 1.738	HR < 0.367; HR > 2.721
<b>B vs A1</b>	<b>A1</b>	44	8	36			Reference		
	<b>B</b>	72	9	63	1.448	Small	HR < 0.848; HR > 1.179	HR < 0.592; HR > 1.69	HR < 0.383; HR > 2.611
<b>A2 vs B</b>	<b>A2</b>	61	14	47	1.180	No effect	HR < 0.805; HR > 1.242	HR < 0.517; HR > 1.934	HR < 0.314; HR > 3.18
	<b>B</b>	72	9	63			Reference		
<b>Disease-Specific Survival</b>									
<b>A1 vs A3</b>	<b>A1</b>	43	5	38	3.725	Medium	HR < 0.751; HR > 1.331	HR < 0.439; HR > 2.276	HR < 0.251; HR > 3.977
	<b>A3</b>	85	2	83			Reference		
<b>A2 vs A3</b>	<b>A2</b>	58	4	54	5.078	Large	HR < 0.785; HR > 1.274	HR < 0.486; HR > 2.057	HR < 0.289; HR > 3.466
	<b>A3</b>	85	2	83			Reference		
<b>B vs A3</b>	<b>A3</b>	85	2	83			Reference		
	<b>B</b>	72	5	67	4.668	Large	HR < 0.805; HR > 1.242	HR < 0.517; HR > 1.935	HR < 0.314; HR > 3.181
<b>A2 vs A1</b>	<b>A1</b>	43	5	38			Reference		
	<b>A2</b>	58	4	54	1.011	No effect	HR < 0.838; HR > 1.193	HR < 0.573; HR > 1.746	HR < 0.365; HR > 2.741
<b>B vs A1</b>	<b>A1</b>	43	5	38			Reference		
	<b>B</b>	72	5	67	1.015	No effect	HR < 0.849; HR > 1.177	HR < 0.594; HR > 1.685	HR < 0.385; HR > 2.597
<b>A2 vs B</b>	<b>A2</b>	58	4	54	0.725	Small	HR < 0.801; HR > 1.249	HR < 0.51; HR > 1.961	HR < 0.309; HR > 3.241
	<b>B</b>	72	5	67			Reference		
<b>Disease-Free Interval</b>									
<b>A1 vs A3</b>	<b>A1</b>	38	4	34	3.681	Medium	HR < 0.745; HR > 1.342	HR < 0.431; HR > 2.32	HR < 0.245; HR > 4.079
	<b>A3</b>	79	2	77			Reference		
<b>A2 vs A3</b>	<b>A2</b>	46	6	40	6.407	Large	HR < 0.768; HR > 1.302	HR < 0.462; HR > 2.165	HR < 0.269; HR > 3.717

Compari- son	Subpo- pulation	N	Ne	Nc	HR	HR effect	HR allocation adjusted critical value		
							$\alpha = 0.1$	$\alpha = 0.3$	$\alpha = 0.5$
							small effect	medium effect	large effect
	A3	79	2	77			Reference		
B vs A3	A3	79	2	77			Reference		
	B	64	5	59	4.291	Large	HR < 0.801; HR > 1.248	HR < 0.511; HR > 1.958	HR < 0.309; HR > 3.234
A2 vs A1	A1	38	4	34			Reference		
	A2	46	6	40	1.803	Medium	HR < 0.831; HR > 1.203	HR < 0.561; HR > 1.783	HR < 0.354; HR > 2.826
B vs A1	A1	38	4	34			Reference		
	B	64	5	59	1.094	No effect	HR < 0.85; HR > 1.177	HR < 0.594; HR > 1.683	HR < 0.386; HR > 2.594
A2 vs B	A2	46	6	40	1.751	Small	HR < 0.79; HR > 1.266	HR < 0.494; HR > 2.025	HR < 0.295; HR > 3.391
	B	64	5	59			Reference		
<b>Progression-Free Interval</b>									
A1 vs A3	A1	44	7	37	2.361	Medium	HR < 0.751; HR > 1.331	HR < 0.439; HR > 2.276	HR < 0.251; HR > 3.977
	A3	87	5	82			Reference		
A2 vs A3	A2	61	9	52	3.056	Medium	HR < 0.788; HR > 1.27	HR < 0.49; HR > 2.04	HR < 0.292; HR > 3.426
	A3	87	5	82			Reference		
B vs A3	A3	87	5	82			Reference		
	B	72	8	64	3.098	Medium	HR < 0.803; HR > 1.245	HR < 0.514; HR > 1.946	HR < 0.312; HR > 3.208
A2 vs A1	A1	44	7	37			Reference		
	A2	61	9	52	1.231	Small	HR < 0.839; HR > 1.191	HR < 0.575; HR > 1.738	HR < 0.367; HR > 2.721
B vs A1	A1	44	7	37			Reference		
	B	72	8	64	1.115	No effect	HR < 0.848; HR > 1.179	HR < 0.592; HR > 1.69	HR < 0.383; HR > 2.611
A2 vs B	A2	61	9	52	1.062	No effect	HR < 0.805; HR > 1.242	HR < 0.517; HR > 1.934	HR < 0.314; HR > 3.18
	B	72	8	64			Reference		



**Figure S6.** Kaplan-Meier survival curves of the clusters obtained based on the RNA sequencing results [30]. **(a)** Overall Survival; **(b)** Disease-Specific Survival; **(c)** Disease-Free Interval; **(d)** Progression-Free Interval.

**Table S10.** Association between categorical demographic and clinical factors and all subtypes identified with k-means clustering or based on PAM50 classifier.

Feature	$\chi^2$	p-value	Cramér's V	Cramér's V effect threshold		
				Small	Medium	Large
<b>Proteomics-based subpopulations</b>						
Race	29.13	0.0155	0.1661	0.0577	0.1732	0.2887
Ethnicity	1.14	0.9508	0.0609	0.1	0.3	0.5
AJCC Stage	29.32	0.0146	0.1557	0.0577	0.1732	0.2887

Feature	$\chi^2$	p-value	Cramér's V	Cramér's V effect threshold		
				Small	Medium	Large
AJCC Tumor	29.33	0.0146	0.1554			
AJCC Node	28.79	0.0171	0.1538			
AJCC Tumor Binarized	18.72	0.0022	0.2150	0.1	0.3	0.5
AJCC Node Binarized	13.09	0.0225	0.1796			
AJCC Metastasis	2.58	0.7649	0.0798			
Radiotherapy	5.45	0.3634	0.1157			
Chemotherapy	19.39	0.0016	0.2183			
Hormone Therapy	69.76	<0.0001	0.4140			
<b>PAM50-based subtypes</b>						
Race	34.68	0.0001	0.1812	0.0577	0.1732	0.2887
Ethnicity	4.09	0.2514	0.1155	0.1	0.3	0.5
AJCC Stage	20.13	0.0172	0.1290	0.0577	0.1732	0.2887
AJCC Tumor	24.28	0.0039	0.1414			
AJCC Node	13.42	0.1447	0.1049			
AJCC Tumor Binarized	19.58	0.0002	0.2199			
AJCC Node Binarized	8.28	0.0406	0.1428			
AJCC Metastasis	2.78	0.4263	0.0829			
Radiotherapy	1.18	0.7572	0.0539			
Chemotherapy	8.83	0.0316	0.1473			
Hormone Therapy	62.85	<0.0001	0.3930			

Table cells with Cramér's V values are colored based on the effect size interpretation. Cramér's V effect interpretation thresholds were adjusted for the size of corresponding contingency tables.

**Table S11.** Summary of demographic and clinical categorical data.

Feature	Number of patients with records	Percentage of available records	Number of categories
Race	352	86.49%	4/3*
Ethnicity	307	75.43%	2
AJCC Cancer Stage	403	99.02%	4
AJCC T	405	99.51%	4
AJCC T binarized	405	99.51%	2
AJCC N	406	99.75%	4
AJCC N binarized	406	99.75%	2
AJCC M	405	99.51%	2

\* The number of race categories was equal to 4 for all subtypes considered and 3 for luminal or luminal A subpopulations only.

**Table S12.** The list of proteins with at least a large  $\eta^2$  effect between the identified four luminal subpopulations.

Selected proteins
14-3-3_beta-R-V
14-3-3_epsilon-M-C
ACVRL1-R-C
ADAR1-M-V
Akt_pS473-R-V
Akt_pT308-R-V
Annexin-1-M-E
Annexin_VII-M-V
ASNS-R-V
Bcl2A1-R-V
beta-Catenin-R-V
Bid-R-C
BRD4-R-V
c-Kit-R-V
c-Met-M-E
C-Raf-R-V
Caspase-8-M-E
Caveolin-1-R-V
CD20-R-C
cIAP-R-V
Claudin-7-R-V
COG3-R-V
Collagen_VI-R-V
Cyclin_B1-R-V
Cyclin_D1-R-V
DIRAS3-M-E
DUSP4-R-V
Dvl3-R-V
E-Cadherin-R-V
eEF2K-R-V
EGFR_pY1173-R-V
eIF4G-R-C
ER-alpha-R-V
ER-alpha_pS118-R-V
ETS-1-R-V
FoxM1-R-V
FOXO3a-R-C
GATA3-M-V
GSK3-alpha-beta-M-V
Heregulin-R-V
HSP70-R-C
Jak2-R-V
Ku80-R-C

<b>Selected proteins</b>
Lck-R-V
mTOR-R-V
MYH11-R-V
N-Cadherin-R-V
N-Ras-M-V
NDRG1_pT346-R-V
Notch1-R-V
p70S6K-R-V
PDCD4-R-C
PDK1_pS241-R-V
PKC-alpha-M-V
PR-R-V
PREX1-R-E
Raptor-R-V
Rb-M-E
Rb_pS807_S811-R-V
RBM15-R-V
S6-R-E
SCD-M-V
Src_pY527-R-V
STAT3_pY705-R-V
Stathmin-R-V

**Table S13.** The list of 40 proteins selected with the Gaussian-mixture-model-based feature filtration and with at least a large  $\eta^2$  effect between the identified four luminal subpopulations.

<b>Selected proteins</b>
ADAR1-M-V
Akt_pS473-R-V
Akt_pT308-R-V
Annexin-1-M-E
ASNS-R-V
Bcl2A1-R-V
beta-Catenin-R-V
BRD4-R-V
c-Kit-R-V
Caveolin-1-R-V
Claudin-7-R-V
COG3-R-V
Collagen_VI-R-V
Cyclin_B1-R-V
Cyclin_D1-R-V
DUSP4-R-V
E-Cadherin-R-V
eEF2K-R-V

<u>Selected proteins</u>
eIF4G-R-C
ER-alpha-R-V
ER-alpha_pS118-R-V
FoxM1-R-V
GATA3-M-V
GSK3-alpha-beta-M-V
HSP70-R-C
Ku80-R-C
Lck-R-V
mTOR-R-V
MYH11-R-V
NDRG1_pT346-R-V
p70S6K-R-V
PDCD4-R-C
PR-R-V
PREX1-R-E
Rb_pS807_S811-R-V
RBM15-R-V
S6-R-E
SCD-M-V
Src_pY527-R-V
STAT3_pY705-R-V