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Supplementary Information for

Extracellular RNA in a single droplet of human serum reflects physiologic and disease states

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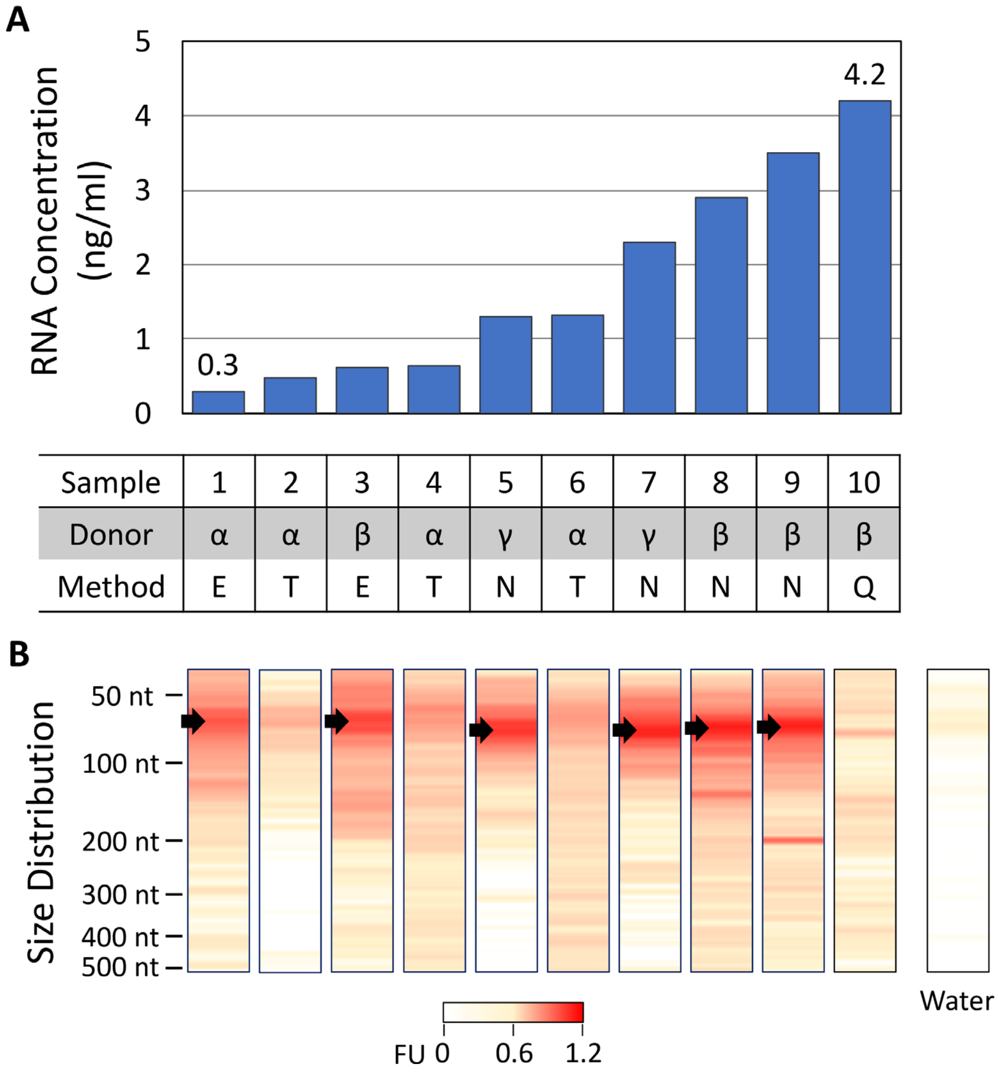


Sample	1	2	3	4	5	6	7	8	9	10
Donor	α	α	β	α	γ	α	γ	β	β	β
Method	E	T	E	T	N	T	N	N	N	Q

Fig. S1. Concentrations and lengths of exRNA in human serum. (A) Measured concentrations of exRNA (y axis) in ten serum samples (columns) from three donors (indexed by α, β, γ). The exRNA of each sample was purified by one of the four commercially available methods (indexed by E, N, Q, T). E: exoRNeasy by QIAGEN, N: NORGEN RNA purification kit, Q: QIAzol by QIAGEN, T: TRIzol$^{\text{TM}}$ LS by ThermoFisher. (B) Length distributions of exRNA. The relative densities in arbitrary fluorescent unit (FU, scale bar) are plotted against the length separated exRNA (nucleotides (nt), y axis) in each sample (lane). Arrows: density peaks. As a control, 1 µL Ultrapure water was given as input to bioanalyzer (Water lane), which yielded smaller than 0.2 FU at any size. Nearly all detected exRNA are within the size range of 20 to 200 nt (FU > 0.2).

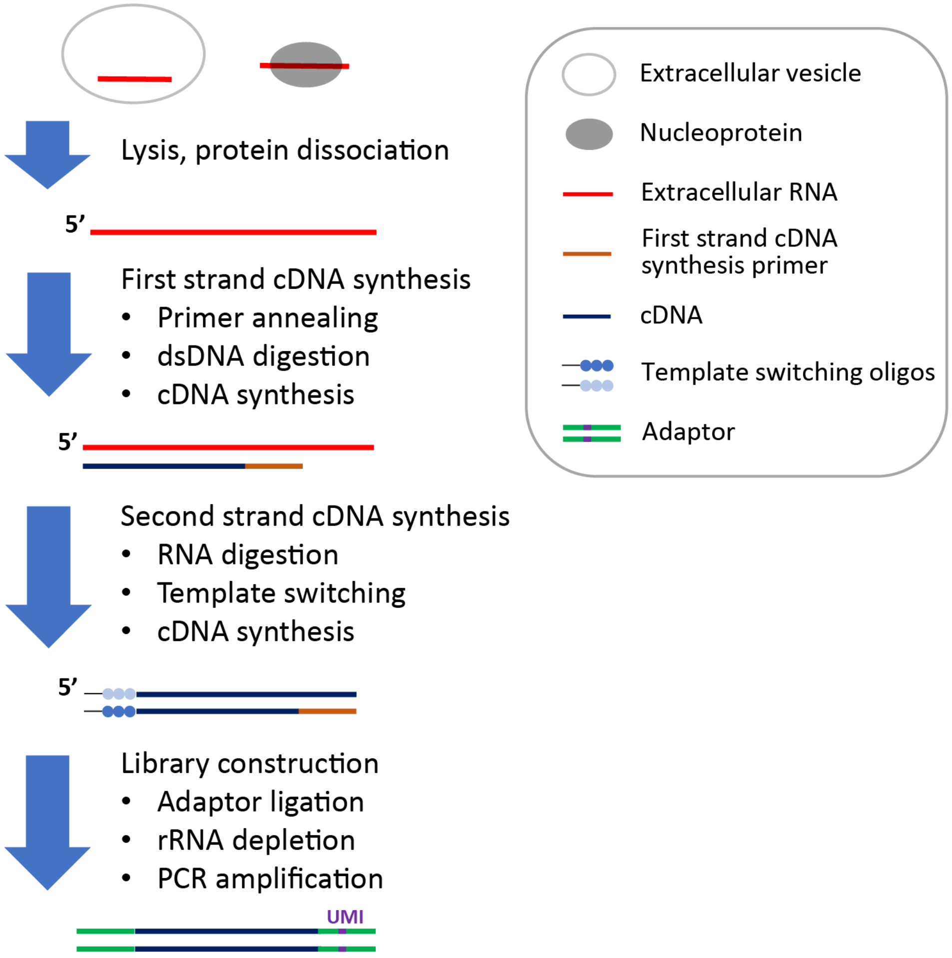


Fig. S2. Flowchart of the SILVER-seq pipeline. UMI: Unique molecular identifiers.

Volume (µL)	3	3	3	3	3	3	3	3	3	7	7	7	7	7	7
Donor #	2	2	2	2	3	3	3	3	3	2	2	3	3	3	3
Aliquot #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

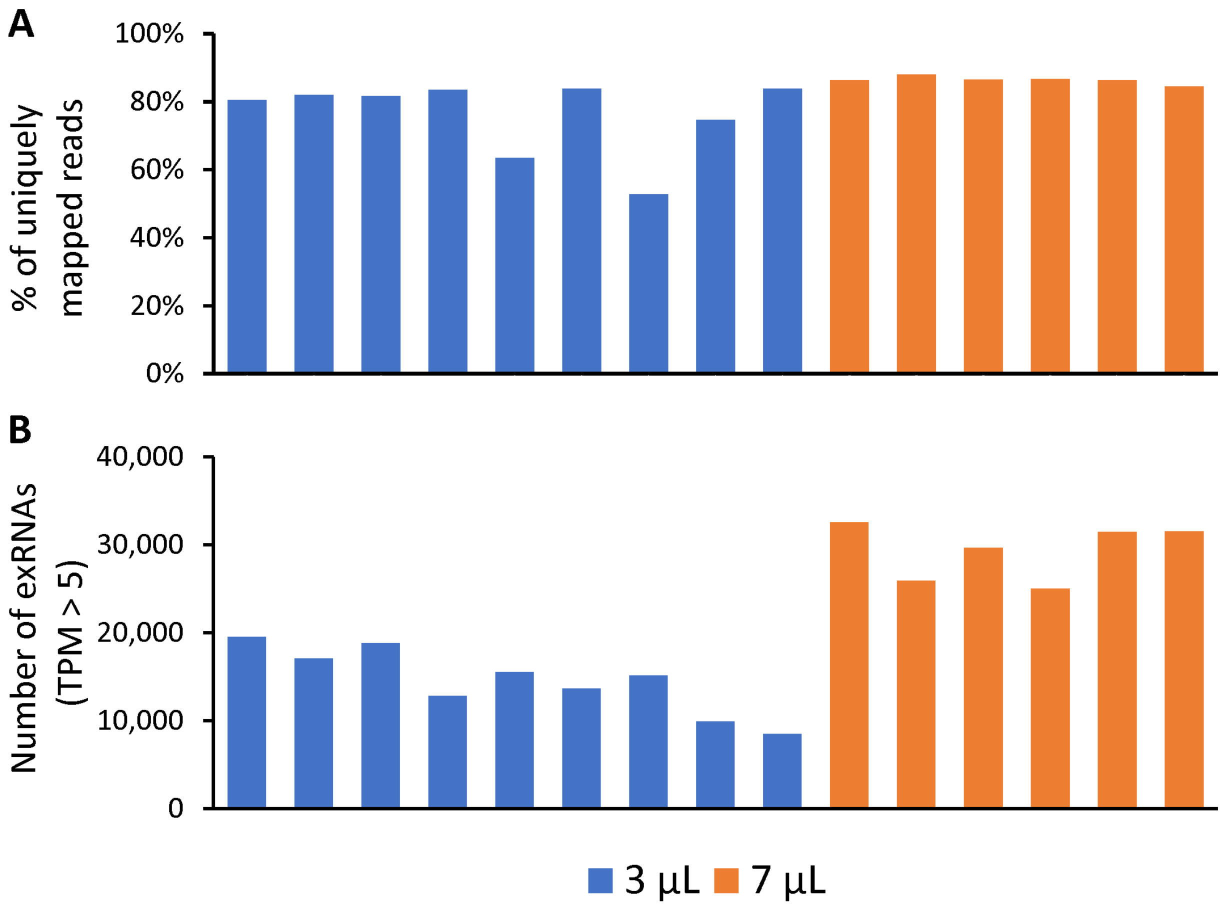
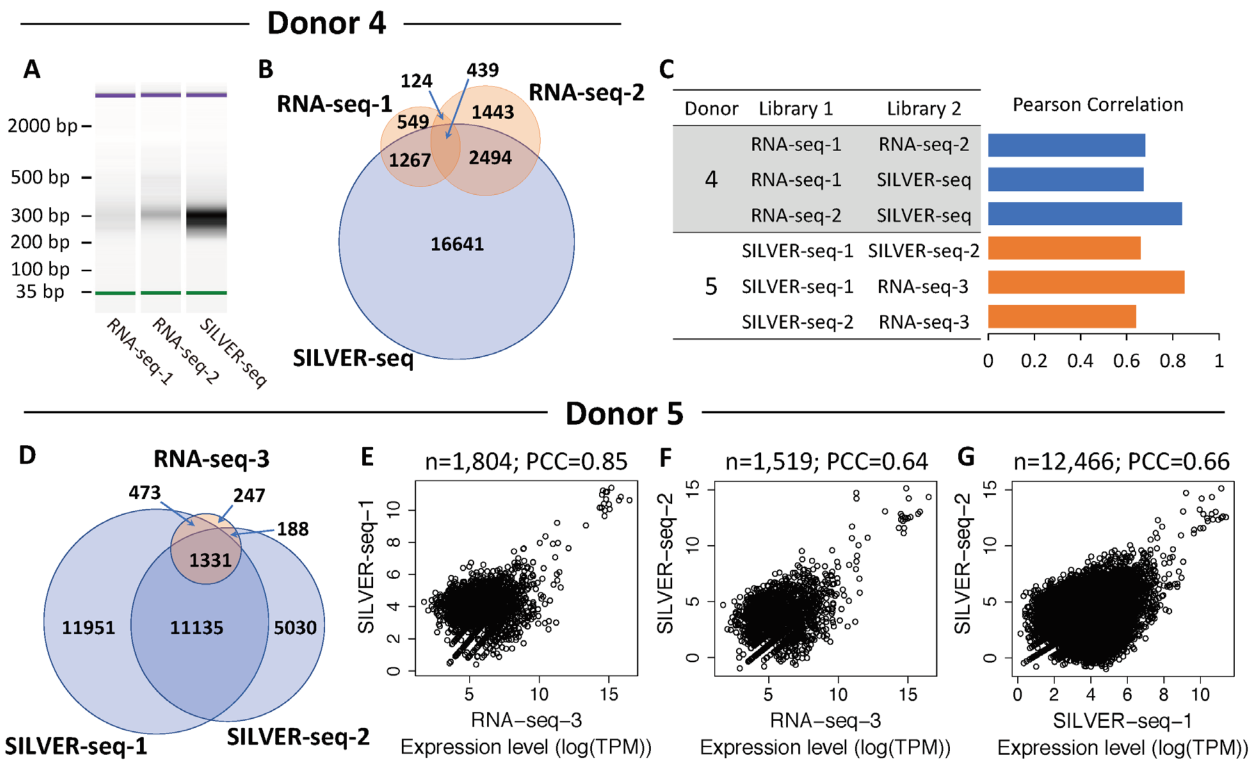


Fig. S3. Comparison of input volumes with serum from two additional donors. (A) Percentage of uniquely mapped reads (y axis) for each library. (B) Number of exRNAs with 5 or more Transcripts Per Million (TPM) in each library (column).



Donor	Library 1	Library 2
4	RNA-seq-1	RNA-seq-2
	RNA-seq-1	SILVER-seq
	RNA-seq-2	SILVER-seq
5	SILVER-seq-1	SILVER-seq-2
	SILVER-seq-1	RNA-seq-3
	SILVER-seq-2	RNA-seq-3

Fig. S4. Comparison of SILVER-seq and standard RNA-seq. (A-B) Comparison of standard RNA-seq libraries (RNA-seq-1, RNA-seq-2) and a SILVER-seq library generated from different aliquots of the same serum sample (Donor 4). (A) Bioanalyzer derived size distributions of the final sequencing libraries. (B) Venn diagram of the numbers of detected exRNA genes from the three libraries. (C) Pairwise Pearson correlation coefficient (PCC) of measured exRNA levels between two sequencing libraries, based on serum aliquots from Donor 4 (blue bars) and 5 (orange bars). (D-G) Variability of SILVER-seq measurements. (D) Venn diagram of the numbers of detected exRNA genes from two SILVER-seq libraries (SILVER-seq-1, SILVER-seq-2) and a standard RNA-seq library (RNA-seq-3). (E-G) Scatter plots of exRNAs (dots) with expression levels (log(TPM)) derived from RNA-seq-3 (x axis) and SILVER-seq-1 (y axis) (E), RNA-seq-3 and SILVER-seq-2 (F), as well as SILVER-seq-1 and SILVER-seq-2 (G).

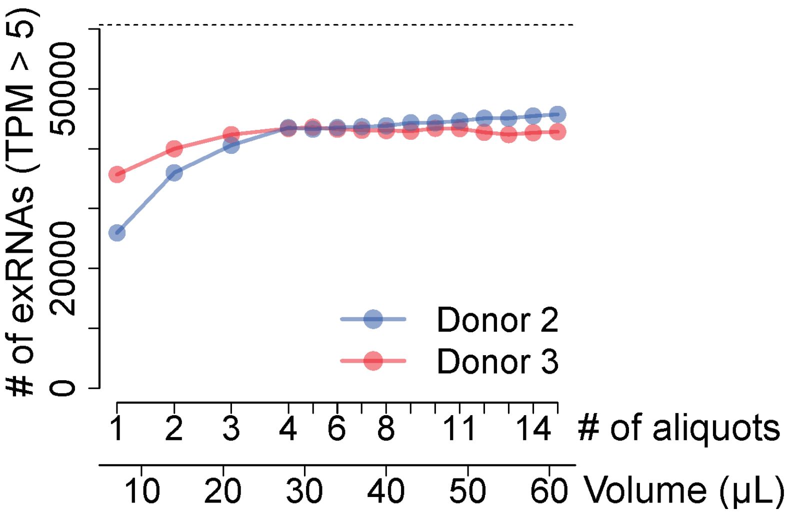


Fig. S5. Saturation analysis. The number of exRNAs (y axis) detected from the pooled sequencing data of the number of aliquots (# of aliquots, x axis). Volume: the cumulative volume of the corresponding number of aliquots.

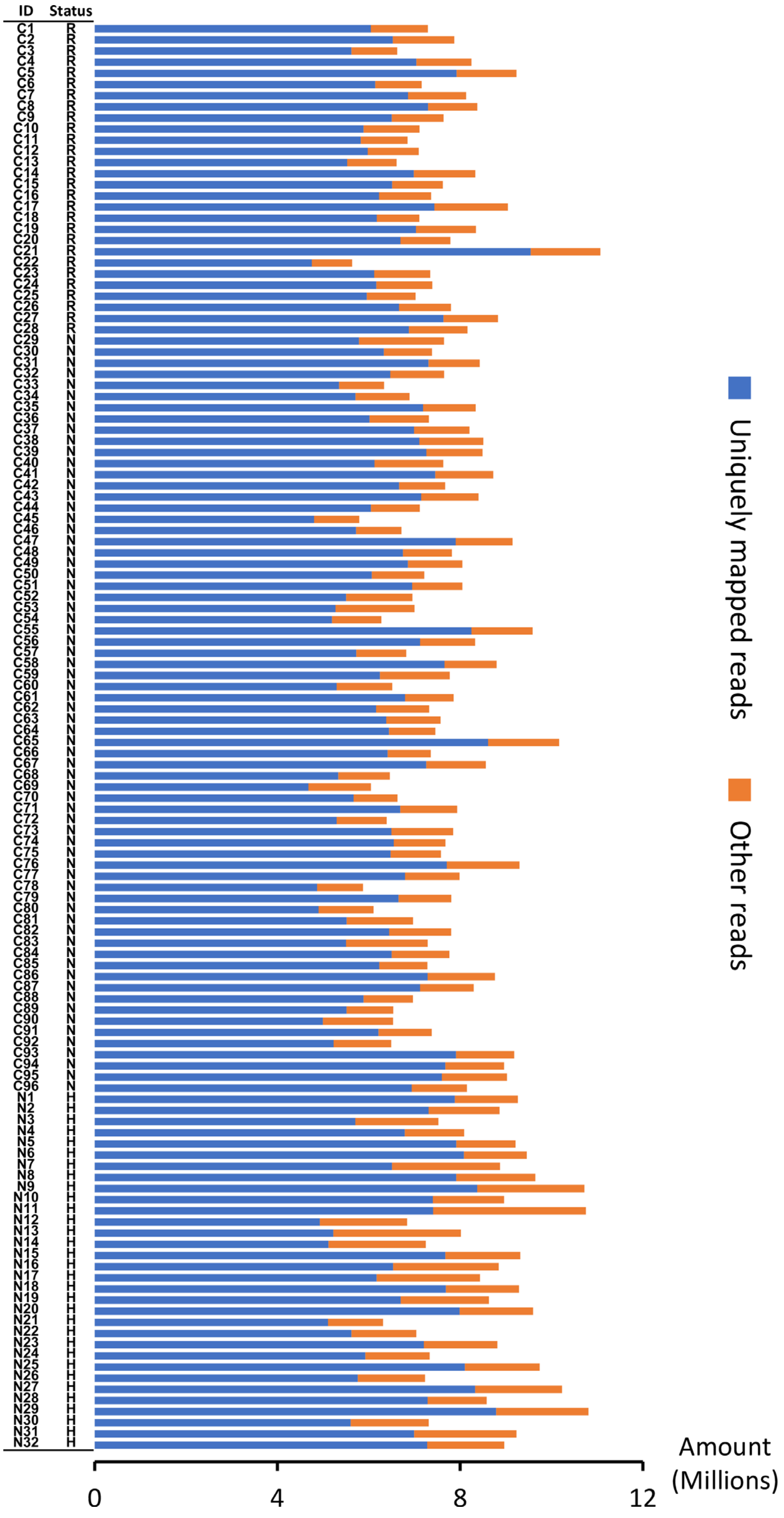


Fig. S6. Summary of 128 serum samples by sequencing depth (x axis). Status R: cancer with recurrence; N: cancer without recurrence, H: normal. Also see Table S3.

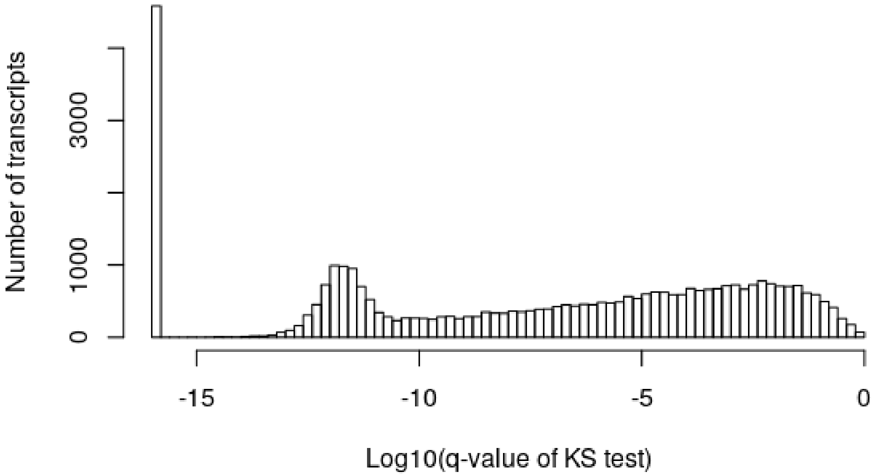


Fig. S7. Histogram of q-values derived from KS tests for uniform distribution. Each full-length transcript that is longer than 500 nt and covered by at least 100 sequencing reads was subjected to a KS test. The null hypothesis is that the sequencing reads are uniformly distributed across the full-length transcript. The alternative hypothesis is that the sequencing reads are not uniformly distributed. The KS test q-value indicates the degree of deviation of the reads from uniformly covering this full-length transcript.

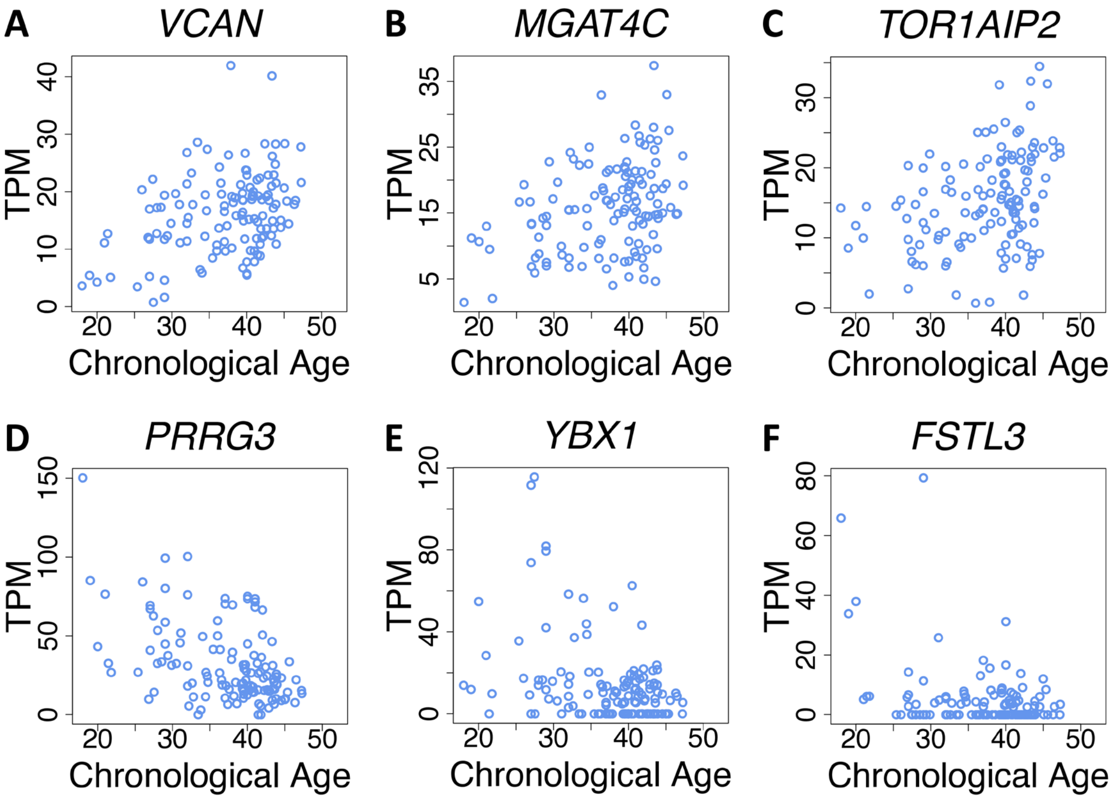


Fig. S8. Age associated exRNAs. exRNAs with expression levels (y axis) positively (A-C) and negatively (D-F) correlated with chronological age (x axis).

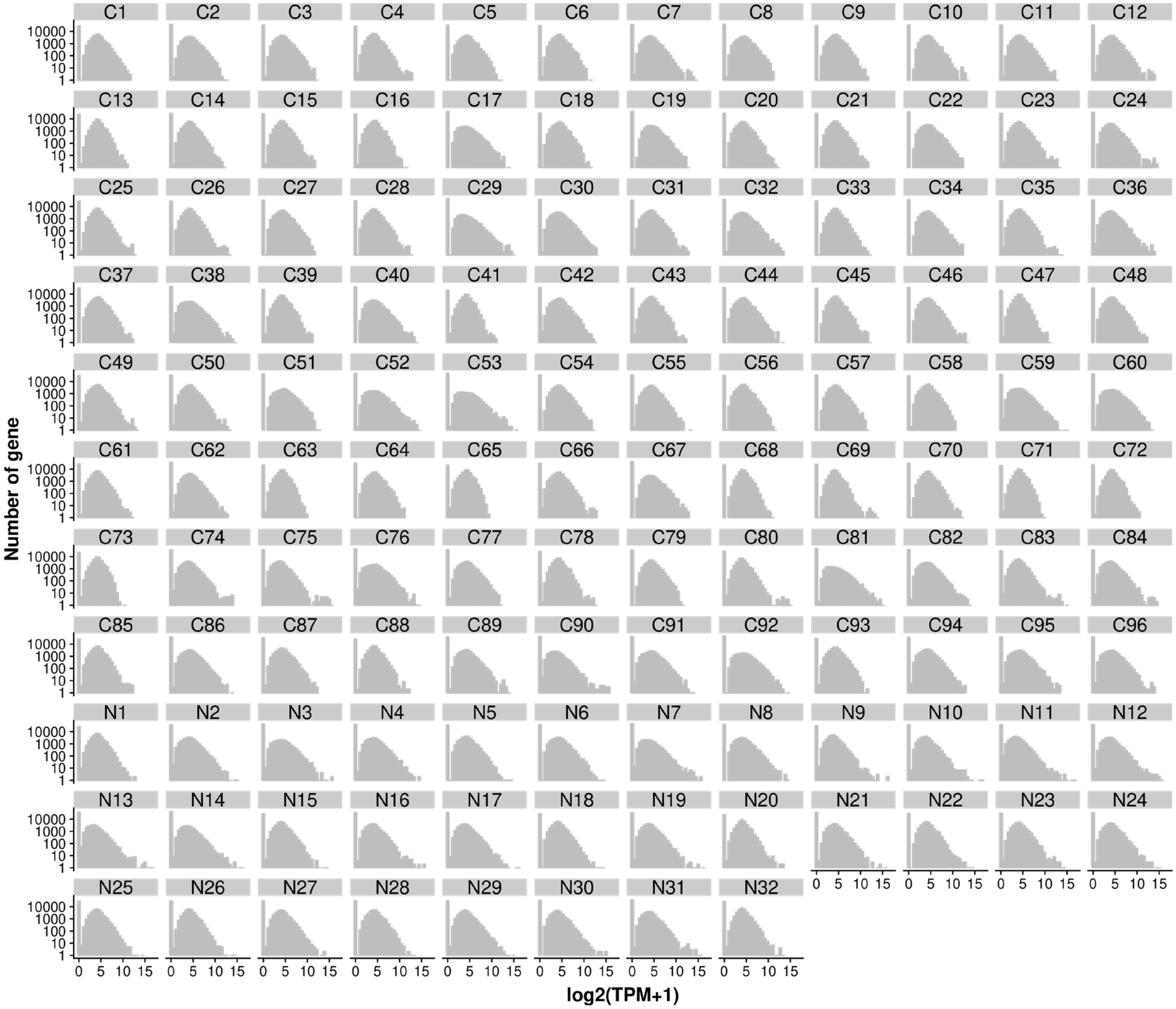


Fig. S9. Histograms exRNA expression level (Log2(TPM+1), x axis) in every sample. C1 – C96: cancer samples. N1 – N32: normal samples.

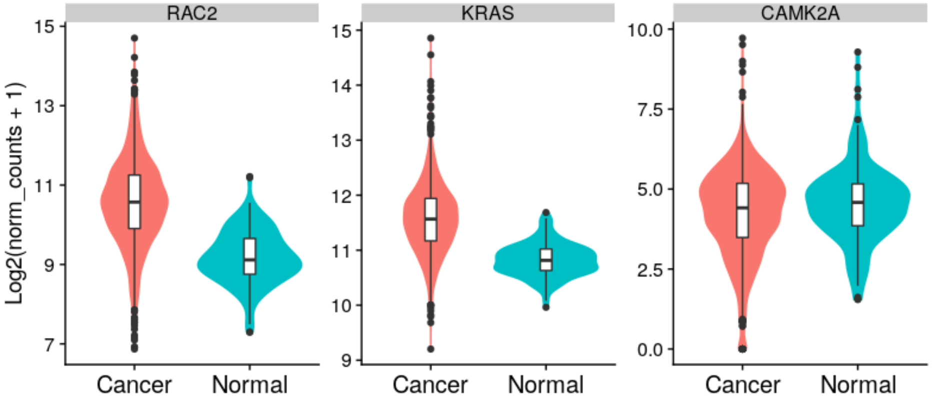


Fig. S10. Comparison of expression levels between breast cancer and normal breast. The RNA-seq data of 1,099 breast cancer tissue samples were generated by TCGA (red) and 178 normal breast tissues were generated by GTEx (blue) and were uniformly processed by UCSC's Xena platform. The normalized read counts (y axis) produced by Xena removed data processing differences and minimized batch efforts.

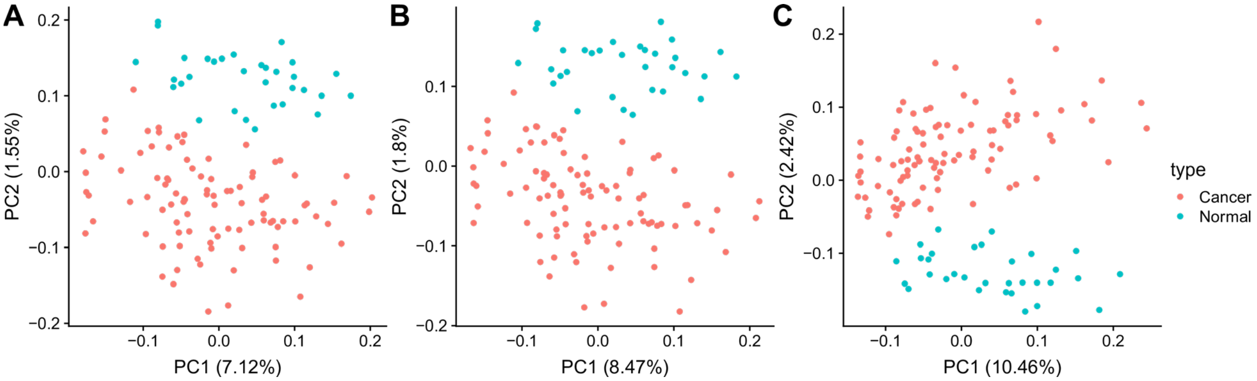


Fig. S11. Sensitivity of the PCA analysis to the choice of genes. (A) PCA with all genes (60,675 annotated genes, Hg38). (B) PCA analyses with the exRNAs that were detected in either 25% or more cancer samples or 25% or more normal samples (25%-recurring exRNAs). (C) PCA analyses with the exRNAs that were detected in either 50% or more cancer samples or 50% or more normal samples (50%-recurring exRNAs). Cancer and normal samples remained mixed in PC1 but somewhat separated in PC2. Red: cancer samples. Blue: normal samples.

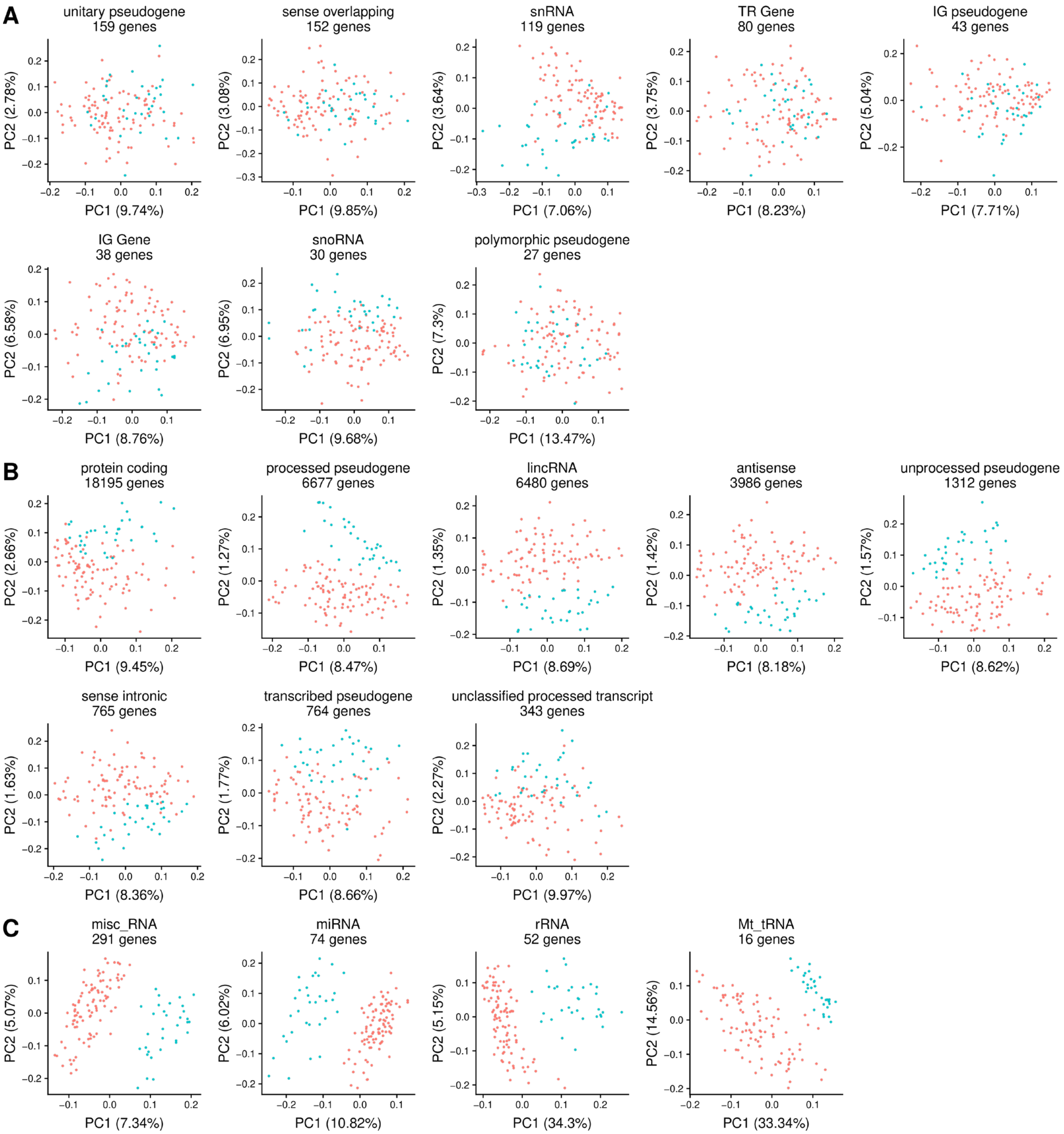


Fig. S12. PCA plots by RNA type. The genes of each RNA type (plot title) that were detected in either 25% or more cancer samples or 25% or more normal samples were used for PCA analysis. (A-C) The RNA types that poorly (A), moderately (B), and clearly separated (C) cancer (red) and normal samples (blue) by the first two principal components.

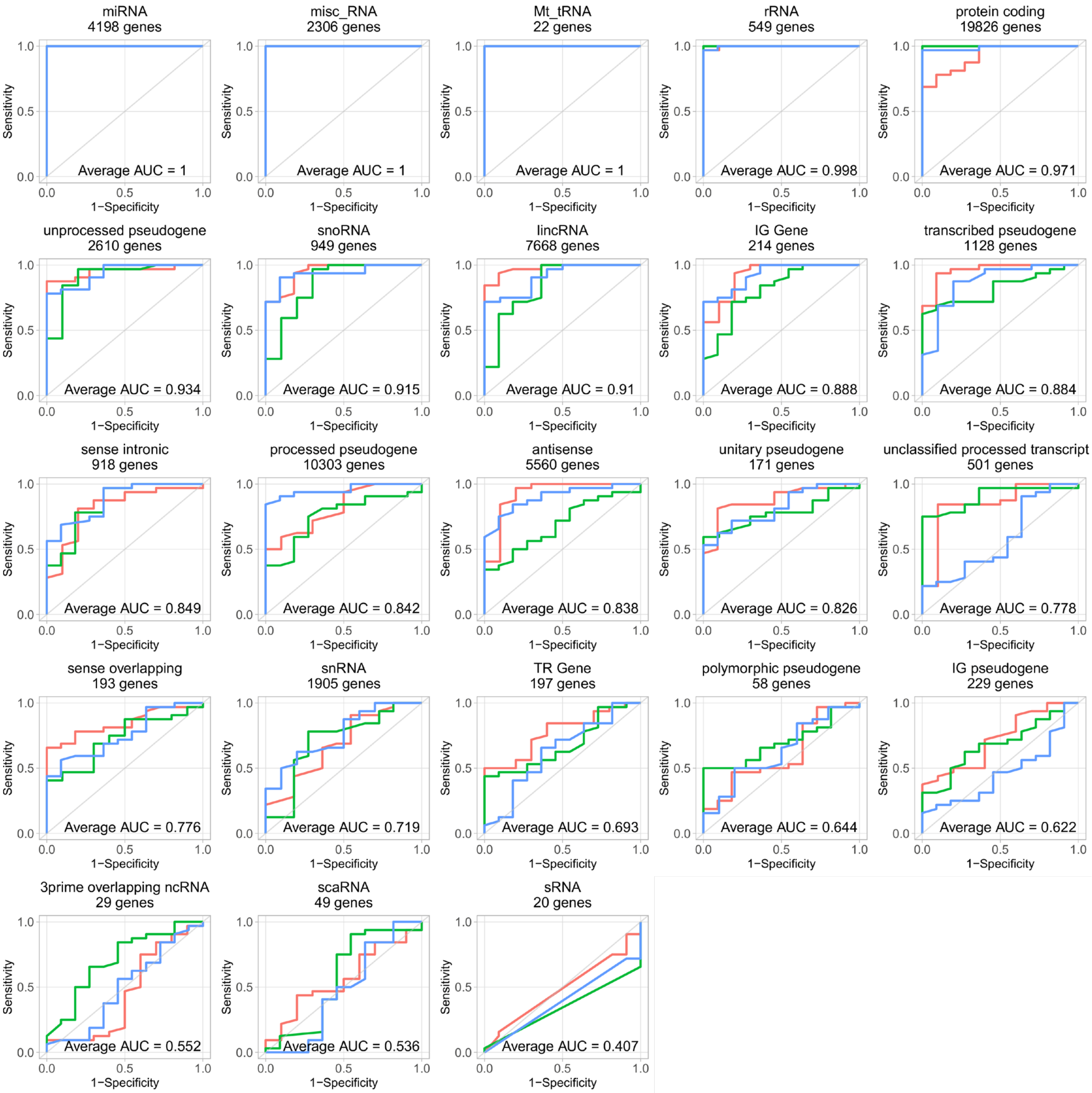


Fig. S13. Classification of cancer and normal serums by random forest. All the genes of each RNA type (panel title) were used as the features. Three ROC curves were generated from 3-fold cross validations.

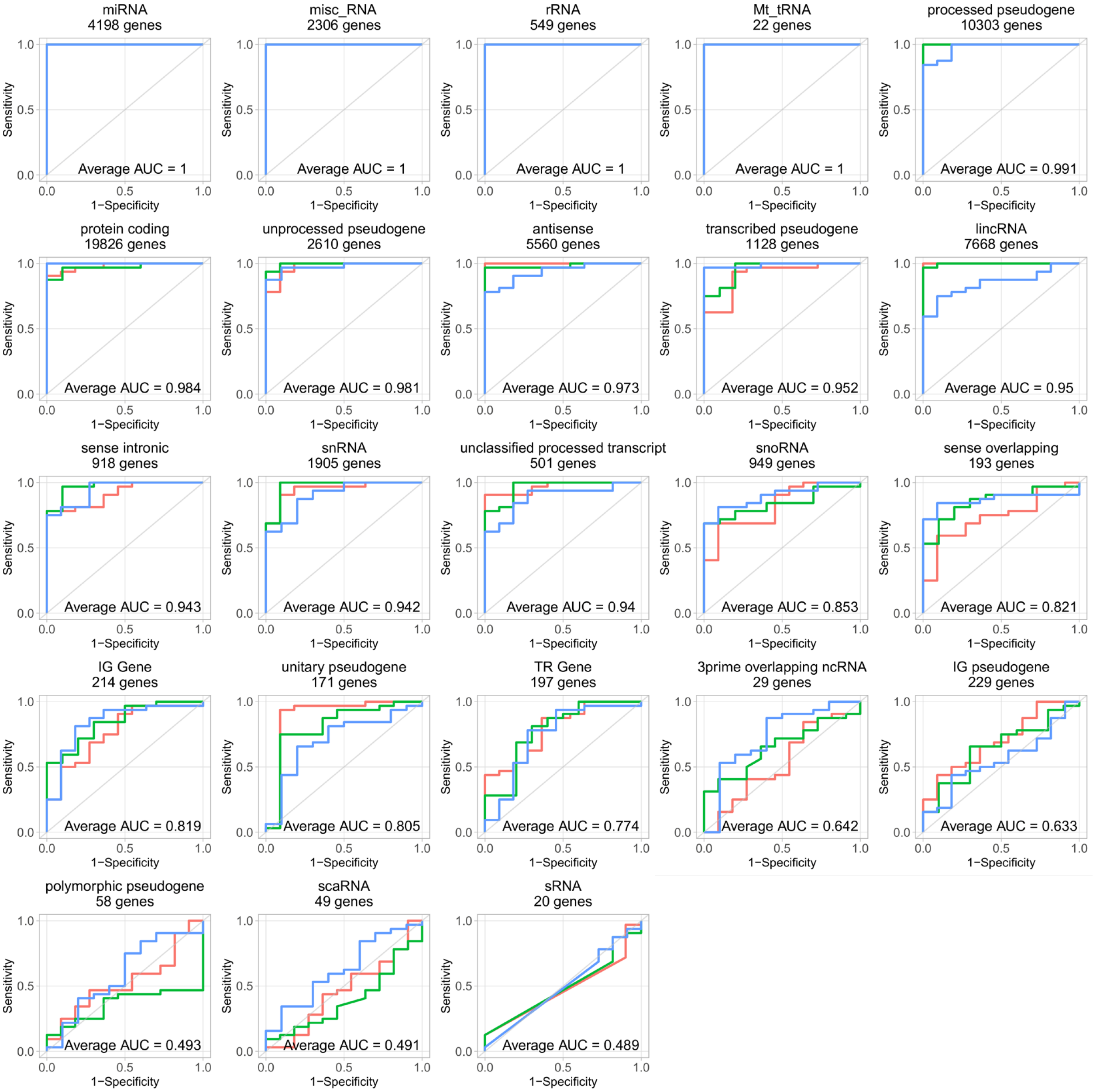


Fig. S14. Classification of cancer and normal serums by SVM. All the genes of each RNA type (panel title) were used as the features. Three ROC curves were generated from 3-fold cross validations.

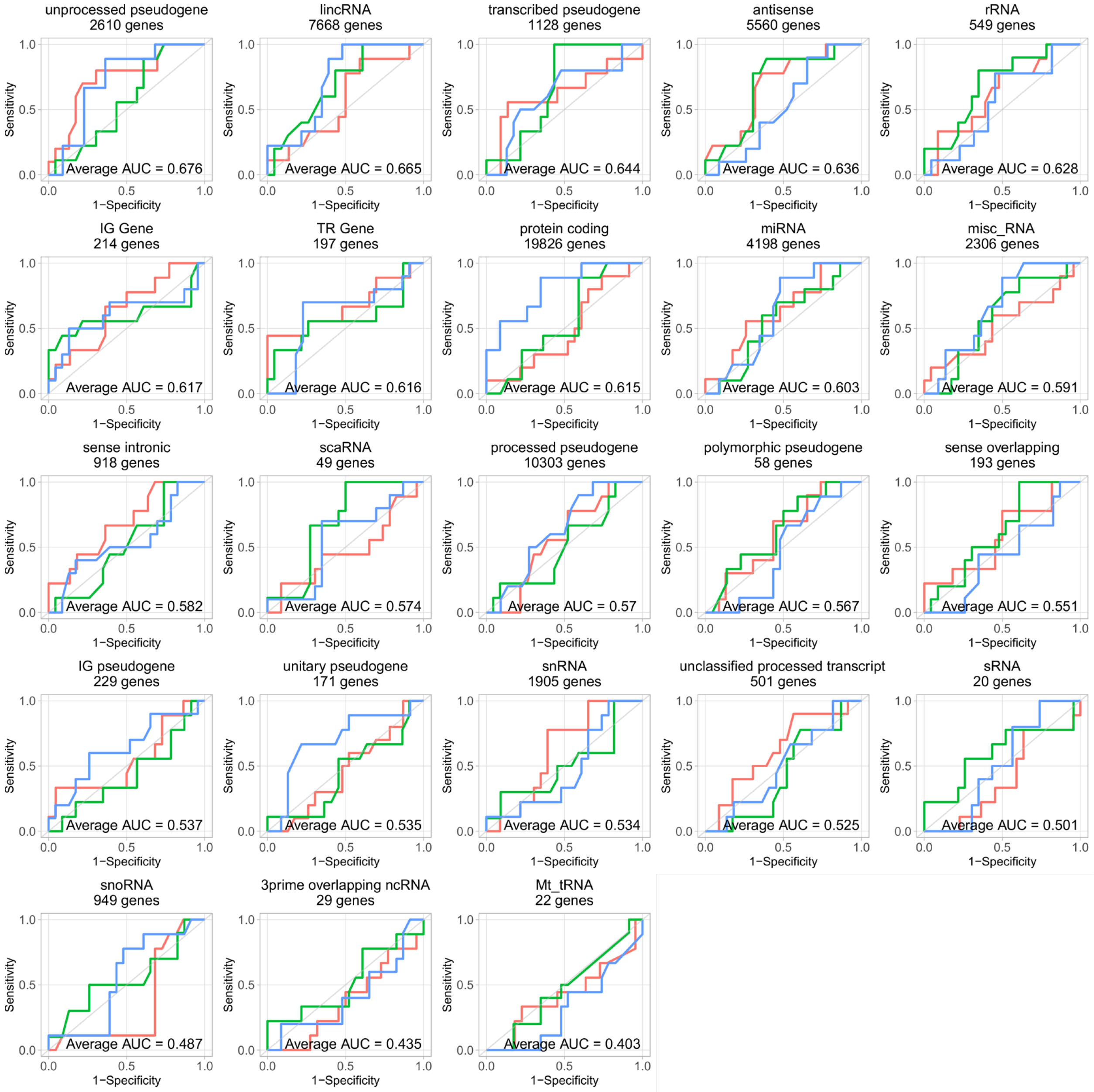


Fig. S15. Classification of recurrence and non-recurrence cancer samples by random forest. All the genes of each RNA type (panel title) were used as the features. Three ROC curves were generated from 3-fold cross validations.

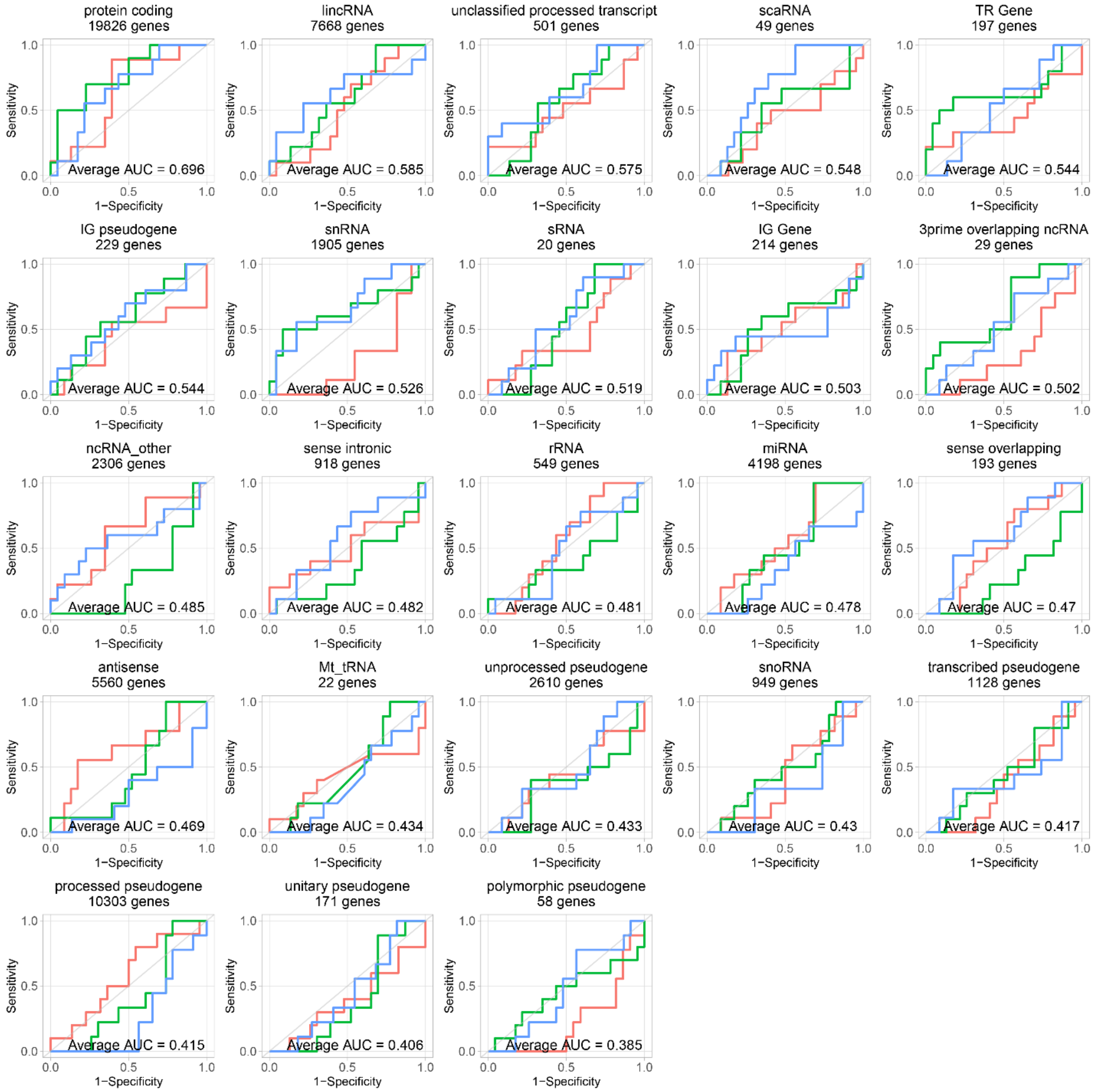


Fig. S16. Classification of recurrence and non-recurrence cancer samples by SVM. All the genes of each RNA type (panel title) were used as the features. Three ROC curves were generated from 3-fold cross validations.

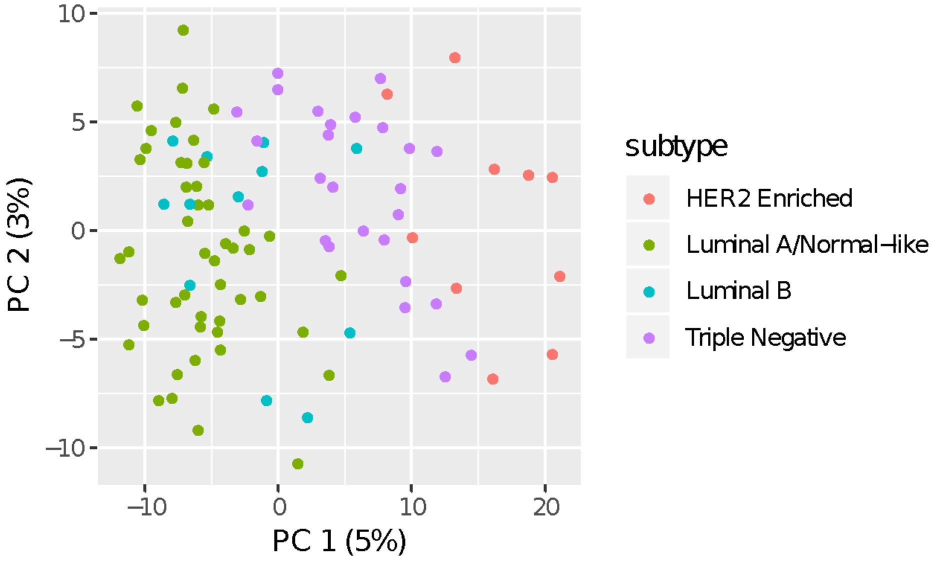


Fig. S17. Principle component analysis of the 96 samples (dots) based on 100 exRNAs. Her2-enriched, luminal A or normal-like, luminal B, and triple negative samples are colored coded in pink, green, blue, and purple, respectively.

Table S1. Contingency tables of exRNAs detected in RNA-seq-1, RNA-seq-2, and SILVER-seq. Corresponding to Figure S4B.

A

	SILVER-seq +	SILVER-seq -	Total
RNA-seq-1 +	1,706	673	2,379
RNA-seq-1 -	19,135	39,161	58,296
Total	20,841	39,834	60,675

Odds ratio = 5.188, Chi-squared p-value = 0

B

	SILVER-seq +	SILVER-seq -	Total
RNA-seq-2 +	2,933	1,567	4,500
RNA-seq-2 -	17,908	38,267	56,175
Total	20,841	39,834	60,675

Odds ratio = 4.000, Chi-squared p-value = 0

C

	SILVER-seq +	SILVER-seq -	Total
RNA-seq-1 + OR RNA-seq-2 +	4,200	2,116	6,316
Other genes	16,641	37,718	54,359
Total	20,841	39,834	60,675

Odds ratio = 4.499, Chi-squared p-value = 0

D

	SILVER-seq +	SILVER-seq -	Total
RNA-seq-1 + AND RNA-seq-2 +	439	124	563
Other genes	20,402	39,710	60,112
Total	20,841	39,834	60,675

Odds ratio =6.891, Chi-squared p-value = 2.572e-106

Table S2. Contingency tables of exRNAs detected in SILVER-seq-1, SILVER-seq-2, and RNA-seq-3. Corresponding to Figure S4D.

A

	RNA-seq-3 +	**RNA-seq-3 -**	**Total**
SILVER-seq-1 +	1,804	23,086	24,890
SILVER-seq-1	435	35,350	35,785
Total	2,239	58,436	60,675

Odds ratio = 6.350, Chi-squared p-value = 0

B

	RNA-seq-3 +	**RNA-seq-3 -**	**Total**
SILVER-seq-2 +	1,519	16,165	17,684
SILVER-seq-2 -	720	42,271	42,991
Total	2,239	58,436	60,675

Odds ratio = 5.517, Chi-squared p-value = 0

C

	RNA-seq-3 +	**RNA-seq-3 -**	**Total**
SILVER-seq-1 + OR SILVER-seq-2 +	1,992	28,116	30,108
Other genes	247	30,320	30,567
Total	2,239	58,436	60,675

Odds ratio = 8.697, Chi-squared p-value = 4.89e-315

D

	RNA-seq-3 +	**RNA-seq-3 -**	**Total**
SILVER-seq-1 + AND SILVER-seq-2 +	1,331	11,135	12,466
Other genes	908	47,301	48,209
Total	2,239	58,436	60,675

Odds ratio = 6.227, Chi-squared p-value = 0

Table S3. Summary of SILVER-seq data from 128 serum samples. C-R Status: Serum collected from the cancer patients who had cancer recurrences within 5 years of chemotherapy. C-N: Serum collected from the cancer patients who did not exhibit cancer recurrence during a 5-year follow-up after chemotherapy. N: serum from normal donors.

Sample ID	Status	Total number of reads	Number of uniquely mapped reads	Uniquely mapped reads %
C1	C-R	7,293,272	6,044,825	82.88%
C2	C-R	7,870,895	6,526,361	82.92%
C3	C-R	6,622,312	5,620,525	84.87%
C4	C-R	8,248,171	7,041,599	85.37%
C5	C-R	9,233,397	7,922,603	85.80%
C6	C-R	7,156,841	6,140,656	85.80%
C7	C-R	8,130,653	6,858,594	84.35%
C8	C-R	8,374,268	7,298,256	87.15%
C9	C-R	7,637,570	6,504,379	85.16%
C10	C-R	7,110,637	5,885,638	82.77%
C11	C-R	6,848,186	5,824,106	85.05%
C12	C-R	7,093,422	5,976,848	84.26%
C13	C-R	6,609,194	5,525,581	83.60%
C14	C-R	8,330,933	6,986,899	83.87%
C15	C-R	7,620,354	6,510,882	85.44%
C16	C-R	7,365,425	6,222,656	84.48%
C17	C-R	9,045,224	7,435,542	82.20%
C18	C-R	7,105,263	6,173,926	86.89%
C19	C-R	8,346,173	7,032,824	84.26%
C20	C-R	7,786,528	6,693,048	85.96%
C21	C-R	11,070,218	9,545,714	86.23%
C22	C-R	5,636,176	4,752,813	84.33%
C23	C-R	7,344,237	6,119,400	83.32%
C24	C-R	7,389,168	6,166,270	83.45%
C25	C-R	7,023,051	5,955,431	84.80%
C26	C-R	7,799,765	6,661,309	85.40%
C27	C-R	8,827,999	7,634,037	86.48%
C28	C-R	8,161,797	6,876,973	84.26%
C29	C-N	7,646,966	5,784,958	75.65%
C30	C-N	7,382,725	6,330,528	85.75%
C31	C-N	8,429,401	7,308,056	86.70%
C32	C-N	7,649,365	6,471,495	84.60%
C33	C-N	6,335,158	5,350,491	84.46%

C34	C-N	6,891,717	5,709,138	82.84%
C35	C-N	8,340,748	7,187,823	86.18%
C36	C-N	7,315,306	6,017,481	82.26%
C37	C-N	8,203,775	6,991,452	85.22%
C38	C-N	8,505,871	7,105,154	83.53%
C39	C-N	8,491,263	7,259,373	85.49%
C40	C-N	7,630,757	6,124,622	80.26%
C41	C-N	8,724,735	7,454,713	85.44%
C42	C-N	7,672,233	6,663,618	86.85%
C43	C-N	8,401,167	7,147,021	85.07%
C44	C-N	7,116,876	6,046,207	84.96%
C45	C-N	5,791,185	4,802,272	82.92%
C46	C-N	6,715,321	5,721,536	85.20%
C47	C-N	9,147,154	7,901,213	86.38%
C48	C-N	7,820,560	6,747,814	86.28%
C49	C-N	8,047,948	6,857,495	85.21%
C50	C-N	7,215,702	6,066,533	84.07%
C51	C-N	8,048,885	6,949,432	86.34%
C52	C-N	6,955,775	5,502,735	79.11%
C53	C-N	7,002,006	5,271,164	75.28%
C54	C-N	6,274,345	5,195,457	82.80%
C55	C-N	9,586,903	8,247,657	86.03%
C56	C-N	8,328,434	7,122,153	85.52%
C57	C-N	6,820,883	5,725,572	83.94%
C58	C-N	8,798,580	7,656,525	87.02%
C59	C-N	7,772,216	6,245,079	80.35%
C60	C-N	6,514,249	5,296,154	81.30%
C61	C-N	7,857,570	6,793,867	86.46%
C62	C-N	7,322,319	6,158,949	84.11%
C63	C-N	7,570,788	6,382,468	84.30%
C64	C-N	7,458,148	6,442,717	86.38%
C65	C-N	10,165,886	8,612,608	84.72%
C66	C-N	7,355,997	6,413,125	87.18%
C67	C-N	8,561,951	7,258,003	84.77%
C68	C-N	6,459,683	5,333,914	82.57%
C69	C-N	6,047,318	4,679,201	77.38%
C70	C-N	6,628,185	5,665,939	85.48%
C71	C-N	7,935,080	6,688,680	84.29%
C72	C-N	6,392,908	5,296,188	82.84%

C73	C-N	7,846,486	6,495,130	82.78%
C74	C-N	7,677,529	6,546,753	85.27%
C75	C-N	7,578,732	6,479,616	85.50%
C76	C-N	9,298,701	7,706,716	82.88%
C77	C-N	7,986,157	6,793,437	85.07%
C78	C-N	5,873,688	4,867,513	82.87%
C79	C-N	7,804,645	6,651,239	85.22%
C80	C-N	6,103,776	4,900,734	80.29%
C81	C-N	6,968,233	5,516,174	79.16%
C82	C-N	7,802,809	6,445,292	82.60%
C83	C-N	7,288,621	5,502,550	75.50%
C84	C-N	7,764,460	6,500,442	83.72%
C85	C-N	7,283,528	6,229,155	85.52%
C86	C-N	8,761,199	7,287,353	83.18%
C87	C-N	8,296,427	7,121,670	85.84%
C88	C-N	6,965,863	5,885,477	84.49%
C89	C-N	6,536,128	5,514,069	84.36%
C90	C-N	6,532,672	4,995,183	76.46%
C91	C-N	7,377,949	6,212,975	84.21%
C92	C-N	6,490,139	5,229,779	80.58%
C93	C-N	9,183,238	7,910,546	86.14%
C94	C-N	8,961,017	7,675,137	85.65%
C95	C-N	9,025,676	7,600,017	84.20%
C96	C-N	8,148,798	6,940,255	85.17%
N1	N	9,263,850	7,883,088	85.10%
N2	N	8,862,600	7,309,574	82.48%
N3	N	7,524,176	5,704,954	75.82%
N4	N	8,087,304	6,787,790	83.93%
N5	N	9,212,857	7,916,254	85.93%
N6	N	9,459,977	8,084,035	85.46%
N7	N	8,873,306	6,506,505	73.33%
N8	N	9,647,151	7,914,755	82.04%
N9	N	10,720,753	8,372,183	78.09%
N10	N	8,960,995	7,402,048	82.60%
N11	N	10,753,306	7,408,864	68.90%
N12	N	6,838,430	4,927,597	72.06%
N13	N	8,016,497	5,224,664	65.17%
N14	N	7,248,737	5,116,580	70.59%
N15	N	9,317,317	7,675,978	82.38%

N16	N	8,843,226	6,532,444	73.87%
N17	N	8,434,973	6,172,272	73.17%
N18	N	9,288,594	7,687,495	82.76%
N19	N	8,630,942	6,698,816	77.61%
N20	N	9,595,997	7,987,941	83.24%
N21	N	6,312,539	5,105,886	80.88%
N22	N	7,039,294	5,621,452	79.86%
N23	N	8,815,317	7,204,811	81.73%
N24	N	7,331,502	5,925,628	80.82%
N25	N	9,740,427	8,104,113	83.20%
N26	N	7,231,993	5,758,838	79.63%
N27	N	10,230,936	8,327,144	81.39%
N28	N	8,579,794	7,286,744	84.93%
N29	N	10,808,607	8,789,442	81.32%
N30	N	7,312,205	5,604,035	76.64%
N31	N	9,232,461	6,992,056	75.73%
N32	N	8,964,442	7,278,097	81.19%

Table S4. Summary of the 96 breast cancer serum samples. Recurrence status R: Serum collected from the cancer patients who had cancer recurrences within 5 years of chemotherapy. Recurrence Status N: Serum collected from the cancer patients who did not exhibit cancer recurrence during a 5-year follow-up after chemotherapy. Chemo-, GnRH (Gonadotropin-releasing hormone), TAM (Tamoxifen), AI (Aromatase inhibitors) and BSO (Bilateral salpingo-oophorectomy) therapy status B: Serum was collected before the treatment started. Therapy status D: Serum was collected while the treatment was in progress. Therapy status A: Serum was collected after the treatment ended. Therapy status N/A: No record or not applicable.

Sample ID	Recurrence status	Cancer subtype	Chemo-therapy status	GnRH therapy status	TAM therapy status	AI therapy status	BSO therapy status
C1	R	Triple Negative	D	N/A	N/A	N/A	N/A
C2	R	Triple Negative	A	N/A	N/A	N/A	N/A
C3	R	Triple Negative	A	N/A	N/A	N/A	N/A
C4	R	Triple Negative	A	N/A	N/A	N/A	N/A
C5	R	Luminal A/Normal-like	D	B	B	N/A	N/A
C6	R	Luminal A/Normal-like	A	B	N/A	N/A	N/A
C7	R	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C8	R	Luminal A/Normal-like	A	N/A	B	N/A	N/A
C9	R	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C10	R	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C11	R	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C12	R	Triple Negative	D	N/A	N/A	N/A	N/A
C13	R	Triple Negative	A	N/A	N/A	N/A	N/A
C14	R	Triple Negative	A	N/A	N/A	N/A	N/A
C15	R	Triple Negative	A	N/A	N/A	N/A	N/A
C16	R	Triple Negative	A	N/A	N/A	N/A	N/A
C17	R	Triple Negative	D	N/A	N/A	N/A	N/A
C18	R	Triple Negative	A	N/A	N/A	N/A	N/A
C19	R	Triple Negative	A	N/A	N/A	N/A	N/A
C20	R	Luminal A/Normal-like	A	N/A	B	N/A	N/A
C21	R	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C22	R	HER2 Enriched	A	A	N/A	N/A	N/A
C23	R	Luminal A/Normal-like	A	B	N/A	N/A	N/A
C24	R	HER2 Enriched	A	A	N/A	N/A	N/A
C25	R	HER2 Enriched	D	D	N/A	N/A	N/A
C26	R	HER2 Enriched	A	A	N/A	N/A	N/A
C27	R	HER2 Enriched	A	A	N/A	N/A	N/A
C28	R	HER2 Enriched	A	A	N/A	N/A	N/A
C29	N	Luminal B	A	N/A	B	N/A	N/A

C30	N	Luminal B	A	N/A	N/A	N/A	N/A
C31	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C32	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C33	N	Luminal A/Normal-like	D	N/A	B	N/A	N/A
C34	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C35	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C36	N	Luminal A/Normal-like	B	B	N/A	N/A	N/A
C37	N	Triple Negative	A	N/A	N/A	N/A	B
C38	N	Triple Negative	A	N/A	N/A	N/A	B
C39	N	Luminal B	A	N/A	B	N/A	N/A
C40	N	Luminal B	A	N/A	N/A	N/A	N/A
C41	N	Triple Negative	A	D	N/A	N/A	N/A
C42	N	Triple Negative	A	A	N/A	N/A	N/A
C43	N	Luminal A/Normal-like	D	N/A	N/A	N/A	B
C44	N	Luminal A/Normal-like	A	N/A	N/A	N/A	B
C45	N	Luminal A/Normal-like	D	N/A	B	N/A	N/A
C46	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C47	N	Luminal A/Normal-like	D	N/A	N/A	N/A	N/A
C48	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C49	N	Triple Negative	D	N/A	N/A	N/A	N/A
C50	N	Triple Negative	A	N/A	N/A	N/A	N/A
C51	N	Triple Negative	A	N/A	N/A	N/A	N/A
C52	N	Triple Negative	A	N/A	N/A	N/A	N/A
C53	N	Luminal B	D	N/A	B	N/A	N/A
C54	N	Luminal B	A	N/A	N/A	N/A	N/A
C55	N	Luminal A/Normal-like	D	D	B	N/A	N/A
C56	N	Luminal A/Normal-like	A	A	B	N/A	N/A
C57	N	Luminal A/Normal-like	D	N/A	N/A	N/A	B
C58	N	Luminal A/Normal-like	A	N/A	N/A	N/A	B
C59	N	Luminal A/Normal-like	D	N/A	B	N/A	N/A
C60	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C61	N	Luminal B	D	N/A	B	N/A	N/A
C62	N	Luminal B	A	N/A	B	N/A	N/A
C63	N	Luminal A/Normal-like	A	B	N/A	N/A	N/A
C64	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C65	N	Luminal A/Normal-like	A	B	N/A	B	N/A
C66	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C67	N	HER2 Enriched	A	N/A	N/A	N/A	N/A
C68	N	HER2 Enriched	A	N/A	N/A	N/A	N/A

C69	N	Luminal B	D	N/A	B	N/A	N/A
C70	N	Luminal B	A	N/A	B	N/A	N/A
C71	N	HER2 Enriched	D	N/A	N/A	N/A	N/A
C72	N	HER2 Enriched	A	N/A	N/A	N/A	N/A
C73	N	Luminal A/Normal-like	D	N/A	B	N/A	N/A
C74	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C75	N	Triple Negative	D	N/A	N/A	N/A	N/A
C76	N	Triple Negative	A	N/A	N/A	N/A	N/A
C77	N	Luminal A/Normal-like	A	N/A	B	N/A	N/A
C78	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C79	N	Triple Negative	D	N/A	N/A	N/A	N/A
C80	N	Triple Negative	A	N/A	N/A	N/A	N/A
C81	N	Luminal A/Normal-like	A	B	D	N/A	N/A
C82	N	Luminal A/Normal-like	A	B	D	N/A	N/A
C83	N	Luminal A/Normal-like	D	N/A	B	N/A	N/A
C84	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C85	N	Luminal A/Normal-like	D	N/A	B	N/A	N/A
C86	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C87	N	Triple Negative	D	N/A	N/A	N/A	N/A
C88	N	Triple Negative	A	N/A	N/A	N/A	N/A
C89	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C90	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C91	N	Luminal A/Normal-like	A	N/A	B	N/A	N/A
C92	N	Luminal A/Normal-like	A	N/A	N/A	N/A	N/A
C93	N	Luminal A/Normal-like	D	N/A	B	N/A	N/A
C94	N	Luminal A/Normal-like	A	N/A	B	N/A	N/A
C95	N	Luminal B	D	N/A	B	N/A	N/A
C96	N	Luminal B	A	N/A	B	N/A	N/A

Table S5. Prior-association genes. The genes that have been associated with breast cancer by prior literature.

Category	Gene name
H/I™ [1]	*HOXB13, IL17BR*
PAM50 [2]	*UBE2T, BIRC5, NUF2, CDC6, CCNB1, TYMS, MYBL2, CEP55, MELK, NDC80, RRM2, UBE2C, CENPF, PTTG1, EXO1, ORC6L, ANLN, CCNE1, CDC20, MKI67, KIF2C, ACTR3B, MYC, EGFR, KRT5, PHGDH, CDH3, MIA, KRT17, FOXC1, SFRP1, KRT14, ESR1, SLC39A6, BAG1, MAPT, PGR, CXXC5, MLPH, BCL2, MDM2, NAT1, FOXA1, BLVRA, MMP11, GPR160, FGFR4, GRB7, TMEM45B, ERBB2*;
Onco*type* DX™ [3]	*BAG1, BCL2, CCNB1, CD68, SCUBE2, CTSL2, ESR1, GRB7, GSTM1, ERBB2,* *MKI67, MYBL2, PGR, AURKA, MMP11, BIRC5, ACTB, GAPDH, GUSB, RPLP0, TFRC*;
BreastOncPx™ [4]	*BUB1, CCNB1, CENPA, DC13, DIAPH3, MELK, MYBL2, ORC6L, PKMYT1, PRR11, RACGAP1, RFC4, TK1, UBE2S*;
MammaPrint™ [5]	*BBC3, EGLN1, TGFB3, ESM1, IGFBP5, FGF18, SCUBE2, TGFB3, WISP1, FLT1, HRASLS, STK32B, RASSF7, DCK, MELK, EXT1, GNAZ, EBF4, MTDH, PITRM1, QSCN6L1, CCNE2, ECT2, CENPA, LIN9, KNTC2, MCM6, NUSAP1, ORC6L, TSPYL5, RUNDC1, PRC1, RFC4, RECQL5, CDCA7, DTL, COL4A2, GPR180, MMP9, GPR126, RTN4RL1, DIAPH3, CDC42BPA, PALM2, ALDH4A1, LPCAT1, LPCAT2, LPCAT4, OXCT1, PECI, GMPS, GSTM3, SLC2A3, FLT1, FGF18, COL4A2, GPR180, EGLN1, MMP9, C9orf30, ZNF533, C16orf61, SERF1A, C20orf46, LGP2, NMU, UCHL5, JHDM1D, AP2B1, MS4A7, RAB6B*;
HDPP [6]	*ABCD4, ADD3, AFP, AFTIPHILIN, ALAS2, ANPEP, ARHGAP19, ARIH2, ATOH8, ATP8B2, AXIN2, BIN3, BMP4, C11orf57, C12orf29, C14orf49, C17orf68, C6orf188, C6orf190, C7orf25, CASP6, CD3D, CD55, CD69, CDON, CFL2, CLCN7, CLSTN3, COL8A1, COLEC12, COMP, CRMP1, CX3CR1, CXCR4, CYorf15B, DCBLD1, DDEF2, DKFZp686E10196, DMN, DPYSL2, DRCTNNB1A, EDG5, EDNRB, ELMO3, EPB41, ETS1, FAIM3, FAM46A, FAM89A, FGF2, FLNC, FYN, GAS7, GLRX2, GLTSCR1, GPC3, GPR172A, GPX3, GRB7, GRHL1, GYPC, HEMGN, HHIP, HMHA1, HSPBP1, IL17RD, INHBA, IRF6, ITGA4, ITM2A, KIAA1549, KIAA1754L, KIAA1826, KIRREL, KLF9, KPNA3, LAD1, LAT, LOC116143, LOC133308, LOC90624, LONRF1, LPGAT1, LTBP1, LTBP4, MAP4, MAP7D3, MARCH8, MED19, MFAP4, MGC40579, MLXIPL, MTHFR, NDN, NLGN2, NUDT9, OSBPL3, PDE2A, PELI2, PERLD1, PEX11B, PGM2L1, PIM1, PKP3, PLAC8, PLAU, PLXNA4B, PPP1R14A, PPP5C, PRKCA, PRSS12, PTPRCAP, PXDN, RAC2, RASIP1, RBM5, RBP4, RFT1, RNF166, S100A11, S100B, SDPR, SEMA6A, SEMA7A, SENP5, SFRS5, SH3BP5, SH3KBP1, SH3YL1, SLC16A7, SLC25A42, SLC38A5, SLK, SNCA, SNF1LK2, SOCS1, SOX10, SPTBN2, ST3GAL2, ST6GALNAC6, STARD4, STAT5A, STON2, STXBP1, TCF7, TF, TGFBR3, TIGA1, TLE4, TMEFF1, TSPAN7, UBE2G1, WDHD1, ZAP70, ZNF276, ZNF281, ZNF609, ZNF690*;

HTICS [7]	*AURKB*, *CCNA2*, *SCRN1*, *NPY*, *ATP7B*, *CHAF1B*, *CCNB1*, *CLDN8*, *NRP1*, *CCR2*, *C1QB*, *CD74*, *VCAM1*, *CD180*, *ITGB2*, *CD72*, *ST8SIA4*;
eXagen*BC*™ [8]	*CYP2D6*, *CYP24*, *PDCD6IP*, *BIRC5*, *NR1D1*, *SMARCE1*;
Mammostrat™ [9]	*TP53*, *NDRG1*, *CEACAM5*, *SLC7A2*, *SLC7A5*, *TRMT2A*;
ProEx™Br [10]	*E2F1*, *RASA1*, *PSMB1*;
Rotterdam [11]	*CLN8*, *GOLM1*, *YIF1A*, *IL18*, *CBX3*, *DUSP4*, *PPP1CC*, *ABLIM1*, *TNFSF13*, *TMEM8A*, *C3*, *NCAPG2*, *C11orf9*, *MAP4*, *TACC2*, *SMC4*, *OR12D2*, *ORC3*, *ATAD2*, *SMC4*, *DUSP4*, *CD44*, *PLK1*, *CNKSR1*, *Hs.591790*, *FEN1*, *FKBP2*, *KPNA2*, *RRNAD1*, *GFOD2*, *AP2A2*, *MLF1IP*, *NEURL*, *SUPT16H*, *ZFP36L2*, *ARHGDIB*, *PSMC2*, *ZCCHC8*, *ANKHD1-EIF4EBP3*, *CD44*, *SLC35A1*, *CCNE2*, *PHF11*, *POLQ*, *CAPN2*, *ETV2*, *UCKL1*, *LST1*, *ACACB*, *Hs.505202*, *EEF1A2*, *NEFL*, *TNFSF13*, *MMP23B*, *GTSE1*, *RFX7*, *COL2A1*, *GAS2*, *ZNF362*, *FUT3*, *MYH2*, *TNFSF10*, *RPL23AP7*, *GABRQ*, *PARP4*, *C11orf51*, *BCL2L14*, *CEP57*, *RPS4XP3*, *ACOT11*;
MapQuant Dx™ [12]	*UBE2C*, *KPNA2*, *TPX2*, *FOXM1*, *STK6*, *CCNA2*, *BIRC5*, *MYBL2*;
IGS™ [13]	*DPF2*, *CASP8*, *BCL2*, *SCGN*, *SWAP70*, *KIAA0276*, *C10orf9*, *C10orf7*, *TOB2*, *XPR1*, *CD59*, *LRP2*, *PLP2*, *MAPK14*, *CXCL2*, *MMP7*, *MGP*, *MLF1*, *FLNB*, *SCNM1*, *HSPC163*, *C5orf18*, *MGC4399*, *CDW92*, *TMC4*, *ZDHHC2*, *TICAM2*, *KDELR3*, *GNPDA1*, *THEM2*, *DBR1*, *FLJ90709*, *FLJ10774*, *C16orf33*, *GAPD*, *LDHA*, *MR1*, *LARS*, *GTPBP1*, *PRSS16*, *WFDC2*, *AIM1*, *DHRS6*, *DHRS4*, *MGC15429*, *MGC45840*, *ECHDC2*, *GOLGA8B*, *GOLGA8A*, *KIAA0436*, *CYP4V2*, *JTV1*, *ICMT*, *DNMT3A*, *HNMT*, *METTL7A*, *METTL2*, *VIL2*, *TPD52*, *ARPC5*, *NOL8*, *NSF*, *RAD23B*, *SRP54*, *HSPA2*, *THAP2*, *CIRBP*, *SNRPN*, *KIAA0052*, *DUSP10*, *SSR1*, *ERBB4*, *EMP1*, *CHPT1*, *LRPAP1*, *FLJ11752*, *CSTF1*, *KLHL20*, *DNAJC13*, *APLP2*, *ARGBP2*, *DNAJB1*, *NEBL*, *SH3BGRL*, *NUDT5*, *GABARAPL1*, *MAPT*, *DCBLD1*, *STK39*, *PAK2*, *CSNK2A1*, *PILRB*, *ERN1*, *SGK3*, *WEE1*, *MAST4*, *C11orf17*, *NUP37*, *CLTC*, *COPB2*, *SLC25A25*, *ECOP*, *PDE8A*, *STAM*, *TUBB*, *SNX6*, *RAB23*, *PLAA*, *STC2*, *LTF*, *ISGF3G*, *ATXN3*, *GTF3C3*, *GSK3B*, *KLF10*, *ELL2*, *ZBTB20*, *IRX3*, *ETS1*, *SERTAD1*, *MGC4251*, *MAFF*, *SFPQ*, *CITED4*, *CEBPD*, *EIF4E2*, *HS2ST1*, *AGPS*, *PGK1*, *ATIC*, *ETNK1*, *ALG2*, *NCE2*, *8-Mar*, *CNOT4*, *RNF8*, *PSMA5*, *DPF2*, *AMMECR1*, *KIAA1287*, *LOC144233*, *NPAS*-4, *FLJ20530*, *THUMPD3*, *MGC45564*, *CAP350*, *ETAA16*, *HAN11*, *DNAPTP6*, *C7orf25*, *FLJ37953*, *FLJ10587*, *C7orf36*, *ELP4*, *NDEL1*, *NPD014*, *DKFZP564D172*, *FAM53C*, *IER5*, *LOC255783*, *KIAA0146*, *KIAA0792*, *LOC283481*, *CG018*, *LOC130576*, *NGFRAP1L1*, *KIAA1217*, *C4orf7*, *C21orf86*, *C9orf64*, *FLJ13456*, *KIAA1600*, B7-H4, *C7orf2*, *NUCKS*, *DKFZP566D1346*, *LOC388279*, *FLJ31795*, *C6orf107*, *FLJ12439*, *FLJ12806*, *FLJ39370*;
NuvoSelect™ [14]	*DD96*, *FCGR3A*, *PLD3*, *CCNL2*, *PP591*, *SEC61A1*, *KPNA2*, *NFAT5*, *HN1*, *DKFZP564I1171*, *SLC35B1*, *PCDHB2*, *NME1*, *EFEMP1*, *SLC35B1*, *RALGDS*, *SURF4*, *KRT13*, *FLJ13855*, *GSR*, *ATF5*, *LCHN*, *PTPRC*, *ACLY*, *FAAH*, *RTKN*, *FLJ20323*, *STOML1*, *MGST1*, *CREM*, *PB1*, *ANXA8*, *FLJ11196*, *HSPC134*, *HBOA*, *TFRC*, *TCEB3*, *APH1A*, *KIAA1041*, *PRPSAP1*, *EPB42*, *KIAA0742*, *NME2*, *TBPL1*, *GTF2H3*, *SLC7A10*, *NDP*, *SCARA3*, *APOE*, *DKFZP434F195*, *KIAA1155*;

Protein expression associated with breast cancer	*TOP2A*, *EGFR*; [15] *FOXA1*; [16] *PIP*; [17] *BigH3*; [18] *MCM2*; [19] *HERV-K*; [20] *KI67*; [21] *CAP2*; [22] *SNCG*; [23] *DAP*; [24] *CENP-A*; [25] *MDC1*; [26] *PRKCA*; [27] *YB-1*; [28] *5-LOX*; [29] *GRB7*, *HER2*; [30] *MKI67*, *CCNA1*, *CCNA2*, *CCNB1*, *CCNB2*, *CCNB3*, *CCNC*, *CCND1*, *CCND2*, *CCND3*, *CCNE1*, *CCNE2*, *CCNF*, *CCNG1*, *CCNG2*, *CCNH*, *CCNI*, *CCNI2*, *CCNJ*, *CCNJL*, *CCNK*, *CCNL1*, *CCNL2*, *CCNO*, *CCNT1*, *CCNT2*, *CCNY*, *CCNYL1*, *CCNYL2*, *CCNYL3*, *PLAU*, *TP53*, *CDKN1A*, *CASP9*, *BAK1*, *BAD*, *BCL2L11*, *BCL2A1*, *BCL2L1*, *CASP4*, *AIFM1*, *TIAF1*, *BCL2*, *CASP3*, *SIVA1*, *BAX*, *PAWR*, *BRCA1*, *BRCA2*; [31] *ESR1*, *ESR2*, *PGR*, *AR*; [32] *NME1*; [33]
RNA expression associated with breast cancer	*PTPN13*, *PTPL1*; [34] *EPS15*; [35] *WDR5*; [36] *PVRL4*; [37] *DLC1*, *CDK6*; [38] *INPP4B*; [39] *CCNE1*; [40] *LINC00472*; [41] *EPB41L-AS2*; [42] *MEG3*; [43] *CCAT1*; [44] *BCYRN1*; [45] *FGF14-AS2*; [46] *H19*; [47] *CCAT2*; [48] *GAS5*; [49] *BCAR4*; [50] *MALAT1*; [51] *BTG3*; [52] *ABCB10*; [53] *LINC-ROR*; [54] *TWIST2*; [55] *TWISTNB*; [56]

DNA sequence associated with breast cancer	*AATK*, *USP32*, *ACTB*, *C20orf112*, *APOL1*, *CD74*, *MBD6*, *GNB2*, *CTSD*, *KRT18*, *PLEC*, *TSPAN14*, *YWHAZ*, *HLA-E*, *ZBTB33*, *COL1A1*, *FMNL3*, *CYB5R3*, TXNIP, *DCLK1*, *COL3A1*, *ELF3*, *SLC39A6*, *MGP*, *NCRNA00188*, *STC2*, *RNF11*, *TAX1BP1*, *MALAT1*; [57] *ACHE*, *EPHB4*, *BCHE*, *MME*; [58] *TOP2A*; [15] *ADPRHL1*, *DCUN1D2*, *TMCO3*, *TFDP1*; [59] *TP53*; [60] *BRCA1*, *APC*, *RASSF1A*, *WIF1*, *MGMT*, *MAL*, *CDH13*, *RARβ*, *CDKN2A*, *TP73*, *GSTP1*, *CDH1*; [61] *CCND2*, *RARB*, *TWIST1*; [62] *CYP1B1*, *ARHI*; [63] *PIK3CA*; [64] *BRCA2*; [65] *PTEN*; [66] *RAD50*; [67] *CHEK2*, *PALB2*; [68] *RAD51C*; [69] *NBN*; [70] *ATM*; [71] *FANCM*; [72] *FGFR2*, *TOX3*, *MAP3K1*, *LSP1*, *HCN1*; [73] *BRIP1*; [74] *SULT1A1*; [75] *RB1*; [76] *VEGF*; [77] *MDM2*; [78] *HLA-G*; [79] *IL1A*; [80] *PDCD6IP*; [81] *RAD51*; [82] *APOBEC3A*, *APOBEC3AP1*, *APOBEC3B*, *APOBEC3B-AS1*, *APOBEC3C*, *APOBEC3D*, *APOBEC3F*, *APOBEC3G*, *APOBEC3H*; [83] *KRAS*, *NRAS*; [84]

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