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Supplemental Information

Rapid and Sensitive Detection of Breast Cancer Cells in Patient Blood with Nuclease-Activated Probe Technology

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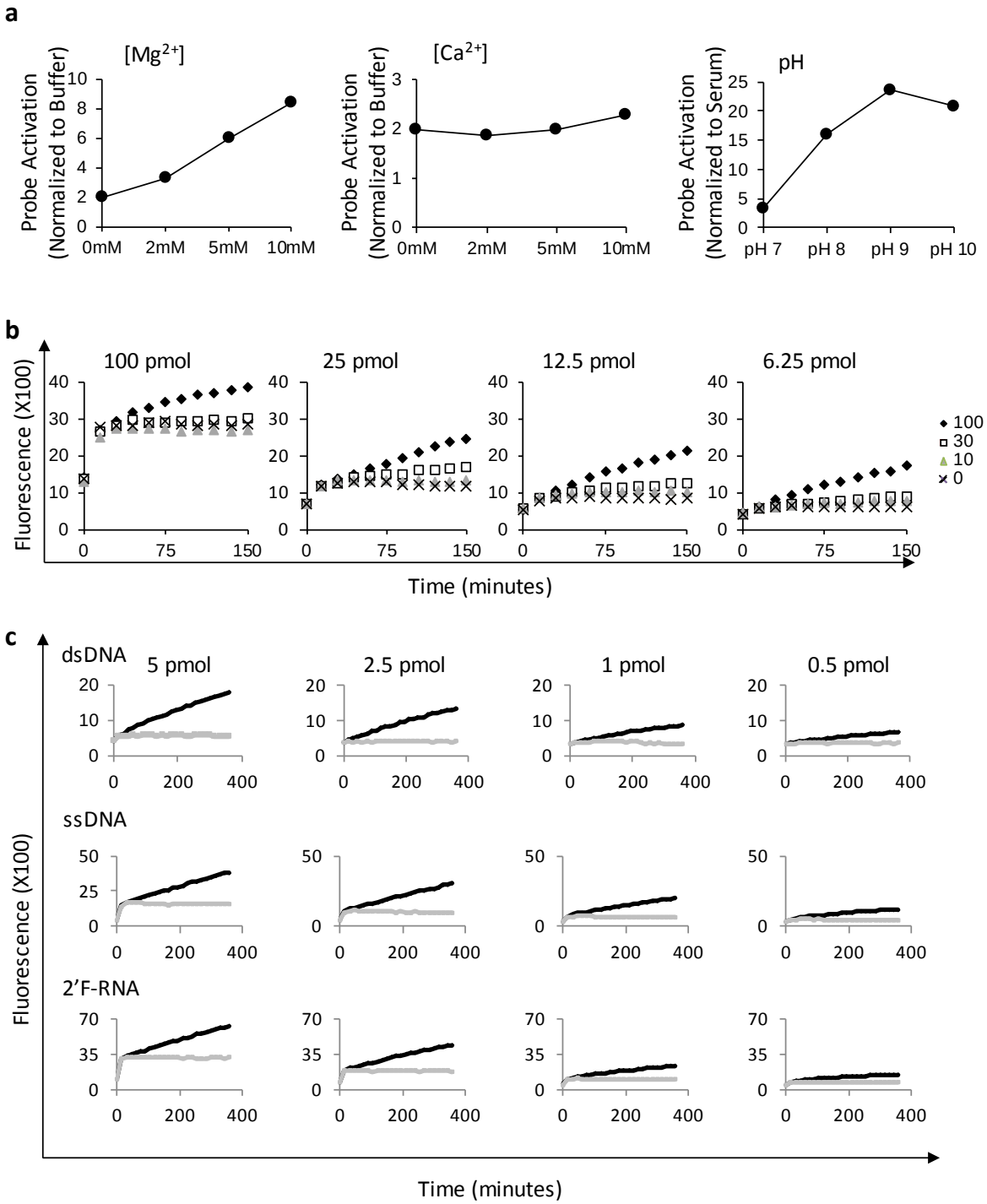
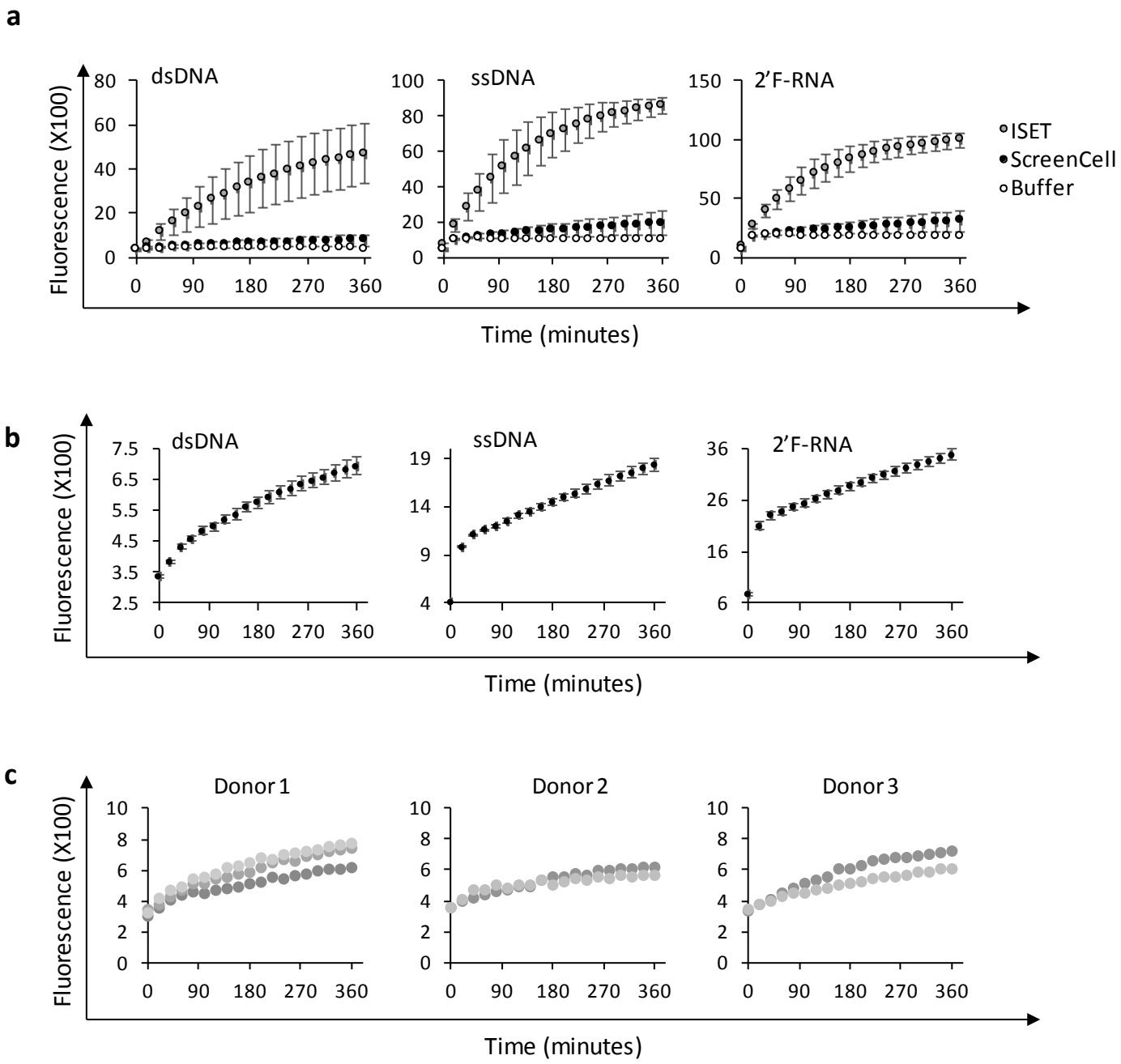
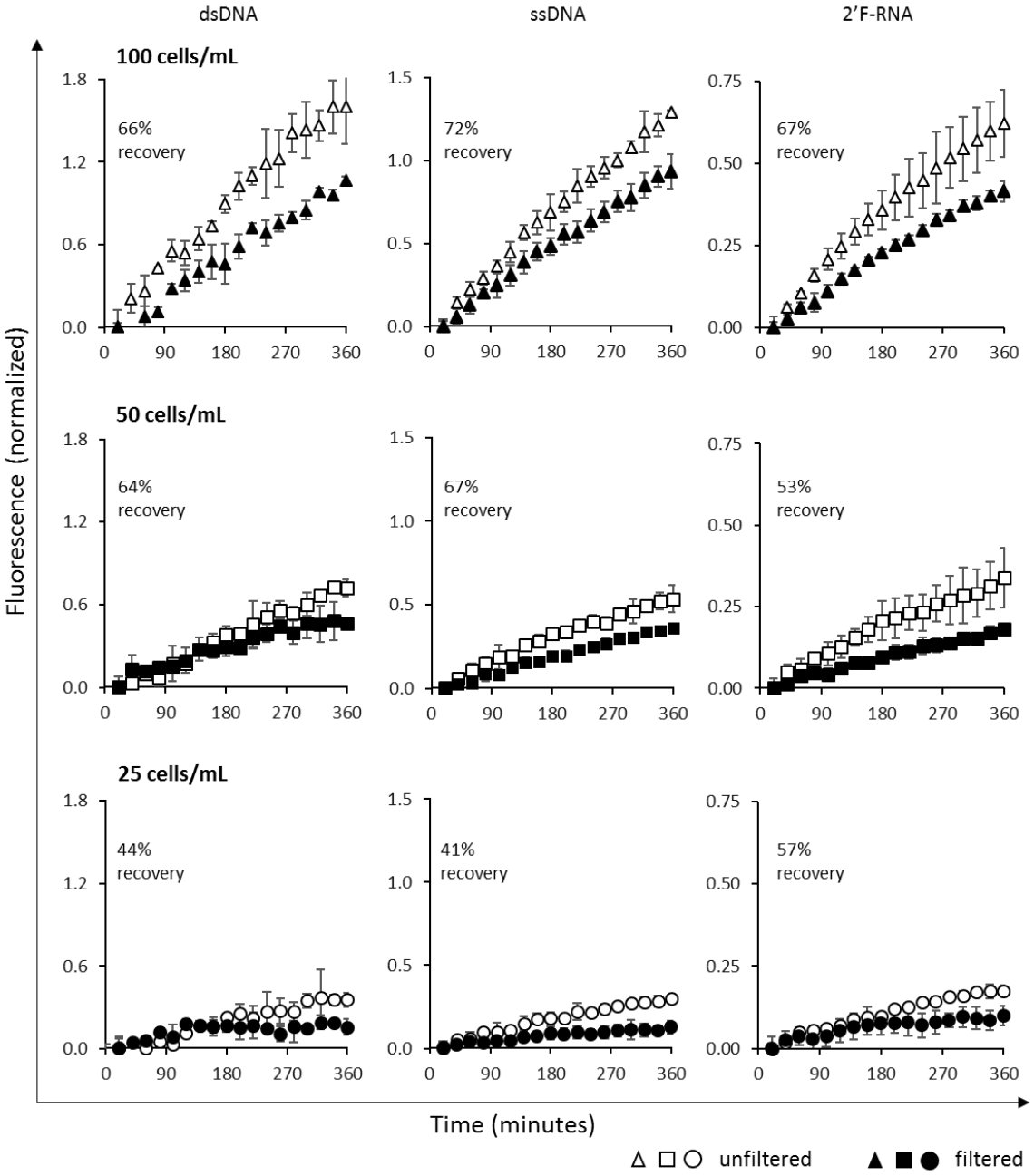
Supplementary Materials



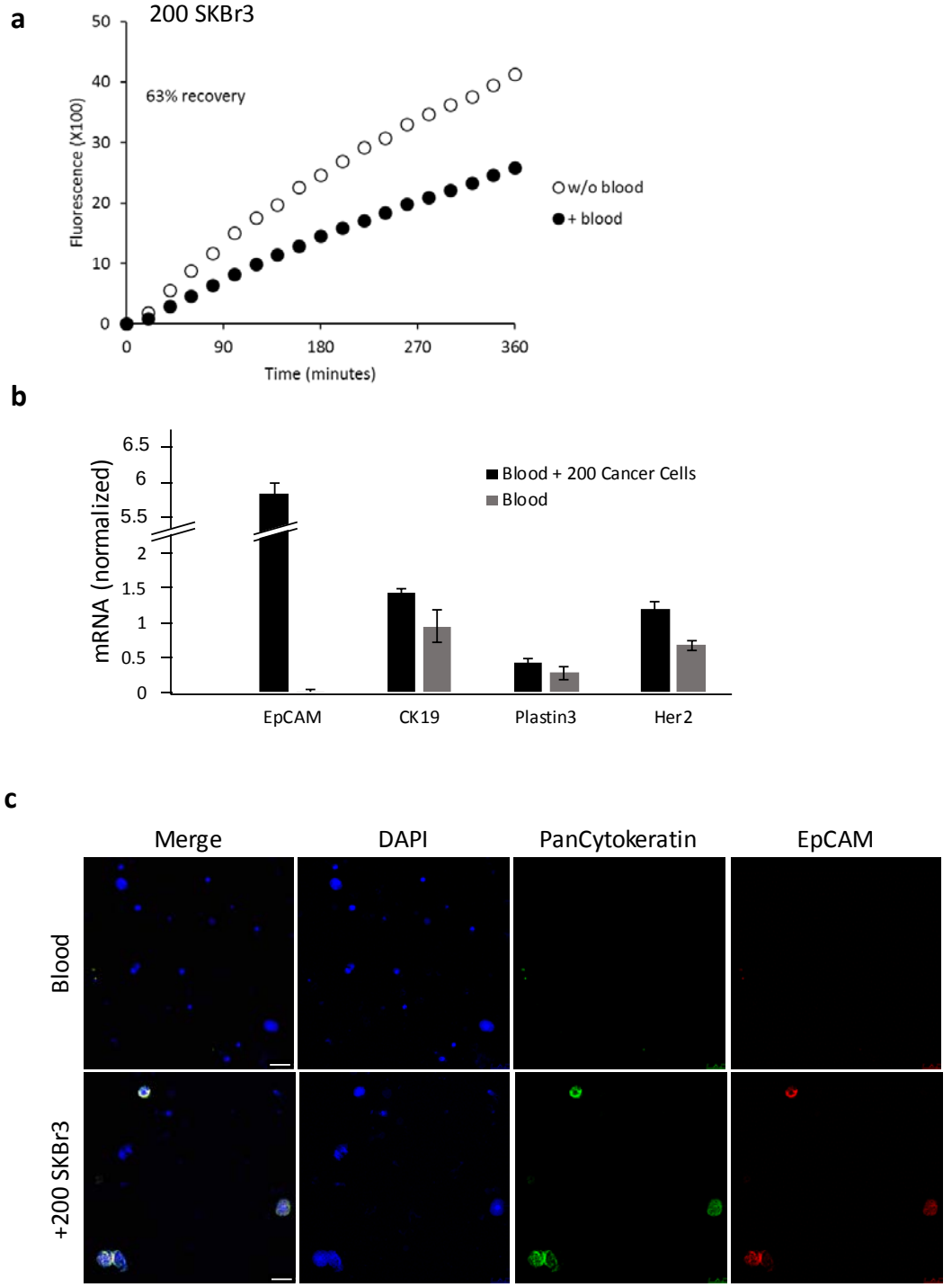
Supplementary Fig. 1. Optimal conditions for nuclease activity assay. (a) Effect of magnesium cation (Mg^{+2}) or calcium cation (Ca^{+2}) concentration and pH on nuclease-activatable probe activity. **(b)** Varying amounts of ssDNA nuclease-activated probe were used in the nuclease activity assay for lysates generated from increasing amounts of SkBR3 breast cancer cells (0 to 100). **(c)** Lysates from 100 Iowa 1T cells (black) or lysis buffer (gray) were mixed with 5, 2.5, 1, or 0.5 pmol of dsDNA, ssDNA, or 2'F-RNA probe, and incubated for 6 hours at 37°C. Fluorescence was measured every 20 minutes over the course of several hours using a microplate reader.



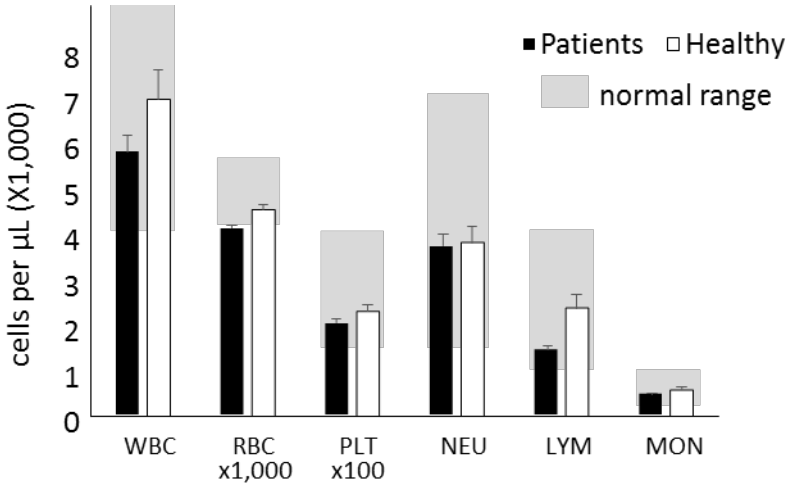
Supplementary Fig. 2. Microfilter capture systems. (a) Background fluorescence signal from blood using ISET and ScreenCell filters. **(b)** Average nuclease-activated probe activity of all healthy donors following ScreenCell filtration. **(c)** Variability of fluorescent signal between blood draws performed on different days from the same donor (n=3 donors). Each graph represents a different donor blood sample tested with the dsDNA nuclease-activated probe.



Supplementary Fig. 3. Cancer cell retention/capture efficiency using the ScreenCell filters. Breast cancer cells (HCC1937) were either lysed prior to filtration/enrichment (open symbols) or filtered through ScreenCell filtration units and then lysed directly on the filter (closed symbols). Lysates were incubated with the nuclease-activated probes and fluorescence measurements were obtained as described above. Varying number of cells (0–100 cells) were either directly lysed in nuclease lysis buffer or subjected to the filtration/enrichment/capture step. The signal intensity of the retained/captured cell lysates is compared to that of the straight cell lysates. Top row: 100 cells/mL. Middle row: 50 cells/mL. Bottom row: 25 cells/mL. Percent recovery at each cell density was between 66–72% for 100 cells/mL, 53–67% for 50 cells/mL and 41–57% for 25 cells/mL.



Supplementary Fig. 4. Efficiency of ScreenCell filter units. (a) Blood samples from a healthy donor were spiked with the addition of 200 SKBr3 breast cancer cells per 1 mL of blood and evaluated using the nuclease-activated probe assay. Blood without cancer cells spiked in served as a control (blood). Percent recovery of cells on the ScreenCell microfilter was calculated as in Fig 4. **(b)** mRNA expression of genes implicated in cancer (EpCAM, CK19, Plastin3 and Her2) was determined from samples in part A. **(c)** Immunostaining of cells captured by ScreenCell filtration from healthy blood with and without the addition of 200 SKBr3 breast cancer cells per 1 mL. Sections stained with DAPI (4',6-diamidino-2-phenylindole), PanCytokeratin antibody and antibody to human EpCAM. Scale bar: 50 µm.



Supplementary Fig. 5. Blood cell counts from breast cancer patients and healthy donors. Patient blood showed a reduced number of all blood cells types in comparison to healthy donor blood. Grey shaded areas present the reference range considered as healthy. (ref: https://labtestsonline.org, American Association for Clinical Chemistry; Accessed October 2016)

Number	Gene Name	Relative RNA amount to Whole Blood (Log2)
1	AZGP1	9.31
2	EXO1	7.03
3	ZC3H12C	6.07
4	EME1	6.04
5	NEIL3	5.94
6	NME1	5.56
7	TATDN1	4.95
8	POP1	4.68
9	HRSP12	4.68
10	RPP40	4.64
11	POLA1	4.60
12	FANCM	4.53
13	ERI2	4.44
14	FEN1	4.39
15	AC004381.6	4.30
16	CKAP5	4.19
17	DNA2	4.13
18	RNASEH2A	4.09
19	ERCC4	4.09
20	DROSHA	4.06
21	EXD2	4.04
22	DCLRE1A	4.04
23	RBBP8	4.04
24	RPP25	4.02
25	RAD51C	4.01
26	PNPT1	3.98
27	BVES	3.97
28	WRN	3.90
29	RAD1	3.89
30	RNASEH1	3.83
31	RAD50	3.77
32	PGAP1	3.74
33	DDX1	3.69
34	RDH14	3.49
35	GEN1	3.44
36	TSEN15	3.38
37	DBR1	3.34
38	CNOT6	3.23
39	TSEN2	3.23
40	G3BP1	3.15
41	UBXN8	3.12
42	DCLRE1B	3.04
43	SETMAR	3.03
44	N4BP2	3.02
45	DIS3L	2.98
46	PTER	2.98
47	REXO2	2.91
48	POLE	2.90
49	PMS2	2.89
50	TSN	2.88

Number	Gene Name	Relative RNA amount to Whole Blood (Log2)
51	EXOSC2	2.76
52	DGCR8	2.75
53	PDE12	2.70
54	APLF	2.70
55	DIS3	2.68
56	ENDOG	2.67
57	HMGA1	2.64
58	POP7	2.60
59	RCL1	2.60
60	APEX1	2.60
61	FAN1	2.57
62	ELAC2	2.56
63	TDP1	2.49
64	APTX	2.47
65	REV3L	2.45
66	NEIL2	2.44
67	ZRANB3	2.43
68	EXOSC3	2.43
69	NOB1	2.41
70	ELAC1	2.40
71	ENDOD1	2.40
72	NTHL1	2.37
73	EXD1	2.36
74	CPSF3	2.35
75	EXO5	2.34
76	ERI3	2.30
77	CNOT7	2.28
78	EXOSC9	2.24
79	SND1	2.24
80	TSNAX	2.22
81	REXO4	2.17
82	PPP1R8	2.16
83	RPP38	2.13
84	EXOSC5	2.13
85	PLD6	2.12
86	AEN	2.09
87	MGME1	2.05
88	POLD1	1.99
89	ENDOV	1.99
90	EXOSC7	1.99
91	CNOT1	1.98
92	APEX2	1.97
93	PARN	1.92
94	RPP14	1.92
95	ISG20L2	1.85
96	POP5	1.83
97	HARBI1	1.75
98	YBX1	1.70
99	RPP30	1.69
100	POP4	1.69

Number	Gene Name	Relative RNA amount to Whole Blood (Log2)
101	NOCT	1.66
102	RPP21	1.65
103	DIS3L2	1.63
104	SMG6	1.63
105	MRE11A	1.61
106	CNOT2	1.60
107	EXOSC10	1.58
108	RPS3	1.52
109	EXOG	1.52
110	MRPL44	1.48
111	SLX4	1.44
112	ASTE1	1.43
113	ERI1	1.39
114	DCLRE1C	1.38
115	DNASE1L2	1.35
116	ALKBH1	1.30
117	TATDN3	1.20
118	DFFB	1.13
119	DNASE2	1.07
120	ERCC1	0.98
121	ZC3H12B	0.89
122	EME2	0.87
123	TATDN2	0.86
124	XRN2	0.84
125	PAN2	0.72
126	DXO	0.72
127	HMGB2	0.71
128	DICER1	0.70
129	REXO1	0.62
130	PELO	0.56
131	XRN1	0.52
132	TREX1	0.48
133	ERCC5	0.46
134	RAD9A	0.45
135	EXOSC4	0.45
136	EXD3	0.38
137	MBD4	0.29
138	PAN3	0.26
139	OGG1	0.25
140	AGO2	0.21
141	DCP2	0.14
142	CNOT8	0.10
143	MUS81	0.00
144	DNASE1L1	-0.03
145	TREX2	-0.09
146	XRCC3	-0.23
147	ANG	-0.24
148	DNASE1	-0.24
149	NEIL1	-0.26
150	PNKP	-0.26
151	TDP2	-0.30
152	RNASEL	-0.75
153	YIPF1	-0.76
154	TSEN34	-0.80
155	USB1	-1.36
156	ERN1	-1.43
157	ZC3H12A	-1.63
158	RNASEK	-1.73
159	ISG20	-2.50
160	RNASET2	-3.70

Supplementary Table 1. Nuclease genes and genes of nucleic acid binding proteins with enriched mRNA expression in breast cancer cells. Curated RNA sequencing data was queried from the data sets RNAseq of 675 commonly used human cancer cell lines and RNAseq from 53 human tissue samples from the Genotype-Tissue Expression (GTEx) Project located on the EMBL-EBI expression atlas. From these dataset 160 genes that were identified as DNA or RNA binding proteins were enquired in 60 different breast cancer cell lines and whole blood. The fold change of the different genes was determined by dividing the gene of the breast cancer cells by the whole blood.