

Novel RNA aptamer identifies plasma membrane ATP synthase beta subunit as an early marker and therapeutic target in aggressive cancer

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Online Resource 1. cDNA oligomer sequences of 5 aptamer candidates used for T7 in vitro transcription

aptamer	oligomer sequence
Apt413	5'-GGG GGA ATT CTA ATA CGA CTC ACT ATA GGG AGG ACG ATG CGG ATT ACC AAC TCG AAC GCC GAG AGTGTG GTC ACG TGT TCT GCA GAC GAC TCG CTG AGG ATC CGA GA-3'
Apt63	5'-GGG GGA ATT CTA ATA CGA CTC ACT ATA GGG AGG ACG ATG CGG GGA AGC AAC ACT TAG TCG CGA TTG ATA CGT GCG CAG TCA TCA GAC GAC TCG CTG AGG ATC CGA GA-3'
Apt 41	5'-GGG GGA ATT CTA ATA CGA CTC ACT ATA GGG AGG ACG ATG CGG ACG GAG GAT AGT TGC TAA TCG AGC GCT GCC GAC GCT CCA GAC GAC TCG CTG AGG ATC CGA GA-3'
Apt 132	5'-GGG GGA ATT CTA ATA CGA CTC ACT ATA GGG AGG ACG ATG CGG TCT CGA CTT TCC ACA CGC GTG TTC TTCGTC TGA TCT GGC CCA GAC GAC TCG CTG AGG ATC CGA GA-3'
Apt 521	5'-GGG GGA ATT CTA ATA CGA CTC ACT ATA GGG AGG ACG ATG CGG CCC ATC ACT CCC GCG TAT TGC GAA CGC ATC GTT ATT TAG CCA GAC GAC TCG CTG AGG ATC CGA GA-3'
Scr	5'-GGG GGA ATT CTA ATA CGA CTC ACT ATA GGG AGG ACG ATG CGG GGA AGC AAC ACT TAG TCG GTT AAA GCA ACA CTT AGC CAC CCA GAC GAC TCG CTG AGG ATC CGA GA-3'

Online Resource 2 (Supplemental Methods)

In vitro aptamer binding affinity and cytotoxicity assays

Two independent assays were carried out to evaluate Apt63 cytotoxicity in vitro: **(1)** direct visualization of Apt 63 cytotoxicity was performed in the IncuCyte® S3 Live-Cell Analysis System. LN3 and Pro 5 cells were seeded at 1×10^3 cells per well in 24-well, black-walled, clear-bottomed tissue culture plates (Corning, Corning, NY) in 300 μ l complete growth medium. Cells were allowed to grow for 24 hours. Cells were then treated with 1 nM of unlabeled Apt63, AptScr and 5 nM of SYTOX™ Green. The time-lapse video was recorded every 5 min for 1h 40min post aptamer treatment. **(2)** Apt63 cytotoxicity was evaluated by SYTOX™ Green uptake. PC3, PC3ML, RWPE, MDA-MB-23, MDA-MB-436, MCF7, MCF10A, T41, NR 67, LN3, Pro5 cell lines were seeded in 96-well, black-walled, clear-bottomed tissue culture plates (Corning, Corning, NY) in 100 μ l complete growth medium. The number of seeded cells per well was experimentally determined for each cell line to be 60-70% confluent in 24 hours for all tested cultures. Cells were treated with 1 nM of unlabeled Apt63 and 5nM of SYTOX™ Green and endpoint readings were performed at 3 hours post aptamer treatment by recording SYTOX™ Green fluorescence. Readings were normalized to untreated cells and plotted using Excel.

Binding affinity of Apt63 to its membrane target was measured using the CellTiter-Glo® luminescent assay (Promega, Madison, WI). LN3 cells were seeded at 4×10^3 cells per well in 96-well, black-walled, clear-bottomed tissue culture plates (Corning, Corning, NY) in 100 μ l complete growth medium and allowed to grow for 24 hours. For the experiment cells were treated in triplicate with unlabeled Apt63 at different concentrations (0.35 nM, 0.7 nM, 1nM, 1.4 nM, 2.3 nM, 7.0 nM, 10 nM, 15 nM). CellTiter-Glo® reagent was added to the wells at 1:1 vol/vol ratio as recommended by manufacturer and endpoint readings were performed at 60 min post aptamer treatment. The raw relative luminescence units (RLUs) from the plate reader were normalized to nontreated cells and plotted using GraphPad Prism 8 Software.

Both, luminescent and fluorescent readings were obtained using EnVision™ (PerkinElmer, USA) plate reader.

Mouse xenograft models and aptamer cytotoxicity in vivo

For visualization of Apt63 uptake and retain by xenograft tumor 14 male NOD.CB17-Prkdcscid/J mice (10 weeks old) were injected orthotopically into the right anterior lobe of the prostate with 2×10^6 LNCapLN3 and 2×10^6 LNCapPro5 cells. Each mouse received one type of cells. When xenograft tumors reach a palpable/visual size, each mouse received single tail vein injection with 1nmol of Alexa Fluor™ 647 labeled Apt63 and AptScr in 200 μ l of PBS, 3 mice per each treatment. One mouse was injected with PBS only to establish auto fluorescence baseline. Live imaging of Alexa Fluor™ 647 labeled Apt63, and AptScr uptake was recorded in vivo on Xenogen IVIS-200 imaging system immediately after completed injections of all mice in the groups. Mice were re-imaged 3 hours post injection to record retention of labeled Apt63 and Apt Scr. Mouse IVIS imaging was performed by IUCAC approved standard procedure, were each mouse anesthetized by vaporized 2% isoflurane during imaging. After first imaging session mice were returned into resting cage and re-anesthetized for re-imaging. Mice were sacrificed after the last imaging session; xenograft tumors were recovered, frozen, sectioned, fixed and counterstained with DAPI as described in the following section. Sections were imaged on the confocal microscope to confirm aptamer retention by LN3 primary tumor xenografts.

For in vivo experiments to determine retention of Apt63 by mammary xenograft tumors, 21 NOD.CB17-Prkdcscid/J female mice (6-8 weeks old) were injected orthotopically into the mammary fat pad with 10^6 of MDA-MB-231 cells. When xenograft tumors reach a palpable/visual size, each mouse received single tail vein injection or 1 nmol of Alexa Fluor™ 647 labeled Apt63 and AptScr in 200 μ l PBS, 9 mice with Apt63, 9 mice with AptScr. 3 mice were injected with PBS only for control. Mice were sacrificed at 6, 24 and 48 hours post injection. Control mice were sacrificed at one time point, 48 hours post injection. Xenograft tumors were recovered, frozen, processed for histology and immunofluorescent analysis as described in the following section. Small tumor samples were separated prior to fixation for DNA extraction.

Online Resource 2 continued (Supplemental Methods)

Tumor tissue and FFPE human biopsy arrays fluorescent staining and analysis

Xenograft tumors were removed from sacrificed mouse, oriented in cryomold with Tissue-Tek® O.C.T. Compound (Sakura® Finetek, USA) positioned flat on dry ice, and left to freeze. Frozen tissue blocks were sectioned on a Leica Cryomicrotome, -20°C. 10 µm thick sections were attached to glass slides and kept in -20 °C until being processed for staining. For Cy3-Apt63 staining, slides with frozen sections were removed from the freezer and placed immediately into 2% paraformaldehyde in PBS and fixed for 10 min. Next, slides were washed with PBS (3 times 1 min each). To block nonspecific RNA and DNA signals, sections were incubated 30 min in 10% dextran sulfate (Sigma) in PBS. Next, sections were washed (3 times 1 min each) and incubated with 1nM of Cy3-Apt63 for 30 min in the humidity chamber. After incubation, sections were briefly washed with PBS (3 times 1 min each) and counterstained with DAPI (Sigma) at 1 µg final concentration for 5 minutes. For Alexa Fluor® 647 labeled Apt63 uptake study xenograft tumors were removed, frozen, sectioned, counterstained with DAPI (Sigma), 1 µg final concentration for 5 minutes, washed and mounted with ProLong®Gold antifade reagent (Life Technologies).

Prostate and breast tissue biopsies microarrays were purchased from US Biomax, Inc (Rockville, MD). List of microarrays used in this study summarized in Online Resource 3. Prior staining with Cy3-Apt63, sections were deparaffinized by standard histological procedure and heated in 1X Citrate Buffer, pH 6.0 (Life Technologies, USA) for 30 minutes for antigen retrieval in the microwave. Next, sections were thoroughly washed with PBS (3 times 10 min each), incubated 30 min in 10% dextran sulfate (Sigma) in PBS to block nonspecific RNA and DNA signals, and incubated with 1nM of Cy3-Apt63 in PBS or 30 min in a humidified chamber. After incubation, sections were washed with PBS (3 times 5 min each), counterstained with DAPI (Sigma), 1 µg final concentration for 5 minutes, washed and mounted with ProLong®Gold antifade reagent (Life Technologies).

For co-localization of Alexa Fluor® 647 ATP5B antibodies (ab223436, ABCAM) and Cy3-Apt63, the tissue arrays were processed for antigen retrieval as described above than stained with 1:200 diluted ATP5B antibodies for 1 hour in RT. Next, sections were thoroughly washed with PBS (3 times 10 min each) and stained with 1 nM of Cy3-Apt63, than washed, counterstained with DAPI and mounted. To confirm the specificity of Apt63 and ATP5B co-localization, staining was also performed in reverse, when sections were first stained with Cy3-Apt63, than with ATP5B antibodies, counterstained with DAPI and mounted.

Primary rabbit polyclonal EndoG antibodies (ab9647, ABCAM) were used on xenograft frozen sections to determine the localization of endonuclease G. Sections were incubated for 2 hours in RT with 1:500 dilution for primary antibodies and 1:500 for Alexa Fluor® 488 Goat anti-Rabbit secondary antibodies (A-11034, Thermo Fisher Scientific). Sections were counterstained with DAPI as described above and mounted.

Fluorescent images were obtained on a confocal microscope (Leica SP5) using 20x dry objective (Leica PL APO CS).

Online Resource 3. List of breast cancer and normal tissue biopsies. Pathology diagnosis, TNM, grade, stage, type of tumor were provided by Biomax, Inc. Apt63 score ("0" - no specific staining, "1" - yes specific staining) was assigned to each biopsy based on staining criteria listed in Methods. Total of 416 cases.

number	Organ	Pathology diagnosis	TNM	Grade	Stage	Type	aptamer staining 0/1	TMA ID number
1	Breast	Cancer adjacent normal breast tissue	-	-	-	NAT	0	BR723
2	Breast	Cancer adjacent normal breast tissue	-	-	-	NAT	0	BR723
3	Breast	Cancer adjacent normal breast tissue	-	-	-	NAT	0	BR723
4	Breast	Normal breast tissue (sparse)	-	-	-	normal	0	BR723
5	Breast	Normal breast duct tissue	-	-	-	normal	0	BR723
6	Breast	Adenosis (sparse)	-	-	-	benign	0	BR723
7	Breast	Adenosis	-	-	-	benign	0	BR723
8	Breast	Adenosis	-	-	-	benign	0	BR723
9	Breast	Adenosis (fibrofatty tissue)	-	-	-	benign	0	BR723
10	Breast	Adenosis	-	-	-	benign	0	BR723
11	Breast	Adenosis	-	-	-	benign	1	BR723
12	Breast	Adenosis	-	-	-	benign	0	BR723
13	Breast	Adenosis (fibrofatty tissue)	-	-	-	benign	0	BR723
14	Breast	Adenosis	-	-	-	benign	0	BR723
15	Breast	Fibroadenoma	-	-	-	benign	0	BR723
16	Breast	Fibroadenoma	-	-	-	benign	0	BR723
17	Breast	Fibroadenoma	-	-	-	benign	0	BR723
18	Breast	Fibroadenoma	-	-	-	benign	0	BR723
19	Breast	Hyperplasia of lobule	-	-	-	benign	0	BR723
20	Breast	Hyperplasia of lobule	-	-	-	benign	1	BR723
21	Breast	Hyperplasia of lobule	-	-	-	benign	0	BR723
22	Breast	Hyperplasia of lobule	-	-	-	benign	0	BR723
23	Breast	Adenosis with mild hyperplasia of ductal epithelium	-	-	-	benign	0	BR723
24	Breast	Hyperplasia (fibrofatty tissue)	-	-	-	benign	0	BR723
25	Breast	Mild hyperplasia of ductal epithelium	-	-	-	benign	0	BR723
26	Breast	Papillary hyperplasia of ductal epithelium	-	-	-	benign	1	BR723
27	Breast	Hyperplasia (fibrous tissue and blood vessel tissue)	-	-	-	benign	0	BR723
28	Breast	Mild atypical hyperplasia of lobular epithelium (sparse)	-	-	-	benign	0	BR723
29	Breast	Mild atypical hyperplasia of lobular epithelium	-	-	-	benign	0	BR723
30	Breast	Mild atypical hyperplasia of lobular epithelium (sparse)	-	-	-	benign	0	BR723
31	Breast	Mild atypical hyperplasia of ductal epithelium	-	-	-	benign	0	BR723
32	Breast	Mild atypical hyperplasia of lobular epithelium	-	-	-	benign	0	BR723
33	Breast	Moderate atypical hyperplasia of ductal epithelium	-	-	-	benign	0	BR723
34	Breast	Hyperplasia (fibrofatty tissue)	-	-	-	benign	0	BR723
35	Breast	Mild atypical hyperplasia of lobular epithelium	-	-	-	benign	0	BR723
36	Breast	Mild atypical hyperplasia of lobular epithelium	-	-	-	benign	1	BR723
37	Breast	Intraductal carcinoma	T1N0M0	-	1	malignant	0	BR723
38	Breast	Intraductal carcinoma	TisN0M0	-	0	malignant	0	BR723
39	Breast	Intraductal carcinoma	T2N0M0	-	2	malignant	0	BR723
40	Breast	Invasive ductal carcinoma	T2N0M0	1	2	malignant	0	BR723
41	Breast	Invasive ductal carcinoma	T4cN0M0	2	3	malignant	1	BR723
42	Breast	Invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR723
43	Breast	Invasive ductal carcinoma	T1N0M0	2	1	malignant	1	BR723
44	Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR723
45	Breast	Invasive ductal carcinoma (adenosis)	T2N0M0	2	2	malignant	0	BR723
46	Breast	Invasive ductal carcinoma	T2N0M0	-	2	malignant	1	BR723
47	Breast	Invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR723
48	Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR723
49	Breast	Invasive ductal carcinoma	T3N1M0	2	2	malignant	1	BR723
50	Breast	Invasive ductal carcinoma (sparse)	T2N0M0	-	2	malignant	0	BR723
51	Breast	Invasive ductal carcinoma	T3N1M1	1--2	4	malignant	0	BR723
52	Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR723
53	Breast	Invasive ductal carcinoma	T2N0M0	3	2	malignant	1	BR723
54	Breast	Invasive ductal carcinoma	T2N0M0	3	2	malignant	1	BR723
55	Breast	Invasive ductal carcinoma	T4N1M0	3	3	malignant	1	BR723
56	Breast	Invasive ductal carcinoma	T2N0M0	3	2	malignant	0	BR723
57	Breast	Invasive ductal carcinoma	T2N0M0	3	2	malignant	1	BR723
58	Breast	Medullary carcinoma	T2N0M0	-	2	malignant	1	BR723

59 Breast	Medullary carcinoma	T1N0M0	-	1	malignant	0	BR723
60 Breast	Medullary carcinoma	T1N0M0	-	1	malignant	1	BR723
61 Breast	Mucinous carcinoma	T2N0M0	-	2	malignant	0	BR723
62 Breast	Mucinous carcinoma	T1N0M0	-	1	malignant	1	BR723
63 Breast	Invasive lobular carcinoma	T2N0M0	-	2	malignant	0	BR723
64 Breast	Invasive lobular carcinoma	T4N0M0	-	3	malignant	0	BR723
65 Breast	Invasive lobular carcinoma	T4N1M0	-	3	malignant	0	BR723
66 Breast	Metastatic invasive ductal carcinoma	-	-	-	MET.	1	BR723
67 Breast	Metastatic invasive ductal carcinoma	-	-	-	MET.	1	BR723
68 Breast	Metastatic invasive ductal carcinoma	-	-	-	MET.	1	BR723
69 Breast	Metastatic invasive ductal carcinoma	-	-	-	MET.	0	BR723
70 Breast	Metastatic invasive ductal carcinoma	-	-	-	MET.	1	BR723
71 Breast	Metastatic invasive ductal carcinoma	-	-	-	MET.	1	BR723
72 Breast	Invasive ductal carcinoma	T2N0M0	1	2	malignant	0	BR8017
73 Breast	Invasive ductal carcinoma	T3N0M0	1--2	2	malignant	1	BR8017
74 Breast	Invasive ductal carcinoma	T2N0M0	1	2	malignant	0	BR8017
75 Breast	Invasive ductal carcinoma	TisN0M0	1	0	malignant	1	BR8017
76 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
77 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
78 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
79 Breast	Invasive ductal carcinoma	T4cN0M0	2	2	malignant	0	BR8017
80 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
81 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
82 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
83 Breast	Invasive ductal carcinoma	T3N1M0	2	2	malignant	1	BR8017
84 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
85 Breast	Invasive ductal carcinoma	T3N0M0	2	2	malignant	0	BR8017
86 Breast	Invasive ductal carcinoma	T3N0M0	2	2	malignant	1	BR8017
87 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
88 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
89 Breast	Invasive ductal carcinoma	T2N0M0	1	2	malignant	1	BR8017
90 Breast	Invasive ductal carcinoma	T3N1M0	2	2	malignant	0	BR8017
91 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
92 Breast	Invasive ductal carcinoma	T4bN0M0	2	2	malignant	1	BR8017
93 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
94 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
95 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
96 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
97 Breast	Invasive ductal carcinoma	T3N0M0	2	2	malignant	1	BR8017
98 Breast	Invasive ductal carcinoma	T3N0M0	2	2	malignant	0	BR8017
99 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
100 Breast	Invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8017
101 Breast	Invasive ductal carcinoma	T2N1M0	2	2	malignant	1	BR8017
102 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
103 Breast	Invasive ductal carcinoma	T3N0M0	2	2	malignant	0	BR8017
104 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
105 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
106 Breast	Invasive ductal carcinoma	T2N1M0	1	2	malignant	1	BR8017
107 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
108 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
109 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
110 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
111 Breast	Invasive ductal carcinoma	T2N0M0	1	2	malignant	1	BR8017
112 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
113 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
114 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	1	BR8017
115 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
116 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
117 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017
118 Breast	Invasive ductal carcinoma	T2N0M0	2	2	malignant	0	BR8017

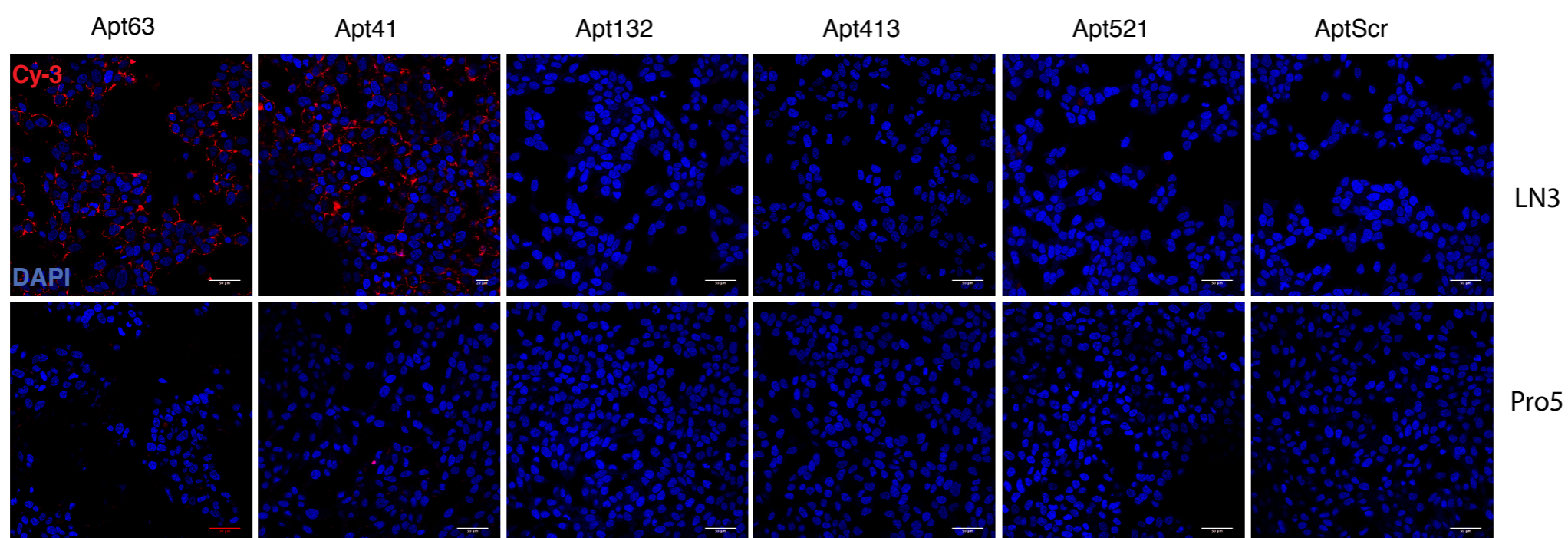
179	Lymph node	Metastatic invasive ductal carcinoma from breast	-	2	-	Metastasis	1	BR8017
180	Lymph node	Metastatic invasive ductal carcinoma from breast	-	2	-	Metastasis	1	BR8017
181	Lymph node	Metastatic invasive ductal carcinoma from breast	-	2	-	Metastasis	1	BR8017
182	Lymph node	Metastatic invasive ductal carcinoma from breast (fibrous -	-	-	-	Metastasis	1	BR8017
183	Lymph node	Metastatic invasive ductal carcinoma from breast	-	2	-	Metastasis	1	BR8017
184	Breast	Invasive ductal carcinoma	T3N0M0	1	2	Malignant	1	BR2082b
185	Breast	Invasive ductal carcinoma	T1N0M0	1	1	Malignant	0	BR2082b
186	Breast	Invasive ductal carcinoma	T2N0M0	1	2	Malignant	0	BR2082b
187	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
188	Breast	Invasive ductal carcinoma	T2N2M0	2	3	Malignant	1	BR2082b
189	Breast	Invasive ductal carcinoma	T2N0M0	1	2	Malignant	0	BR2082b
190	Breast	Invasive ductal carcinoma	T1CN0M0	2	1	Malignant	1	BR2082b
191	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	1	BR2082b
192	Breast	Invasive ductal carcinoma	T4N0M0	2	3	Malignant	1	BR2082b
193	Breast	Invasive ductal carcinoma	T2N1M0	2	2	Malignant	0	BR2082b
194	Breast	Invasive ductal carcinoma of No. 44	T2N1M0	2	2	Malignant	0	BR2082b
195	Breast	Invasive ductal carcinoma	T1CN0M0	2	1	Malignant	0	BR2082b
196	Breast	Invasive ductal carcinoma	T3N2M0	2	3	Malignant	0	BR2082b
197	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
198	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
199	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	1	BR2082b
200	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
201	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
202	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
203	Breast	Invasive ductal carcinoma	T2N1M0	2	2	Malignant	1	BR2082b
204	Breast	Invasive ductal carcinoma	T4N2M0	2	3	Malignant	0	BR2082b
205	Breast	Invasive ductal carcinoma	T4BN0M0	2	3	Malignant	0	BR2082b
206	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
207	Breast	Invasive ductal carcinoma	T1N0M0	2	1	Malignant	0	BR2082b
208	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
209	Breast	Invasive ductal carcinoma	T4N0M0	2	3	Malignant	0	BR2082b
210	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
211	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	1	BR2082b
212	Breast	Invasive ductal carcinoma	T1N0M0	2	1	Malignant	1	BR2082b
213	Breast	Invasive ductal carcinoma	T2N0M1	2	2	Malignant	1	BR2082b
214	Breast	Invasive ductal carcinoma	T2N1M0	2	2	Malignant	1	BR2082b
215	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	1	BR2082b
216	Breast	Invasive ductal carcinoma	T1N0M0	2	1	Malignant	1	BR2082b
217	Breast	Invasive ductal carcinoma	T1N2M0	2	3	Malignant	0	BR2082b
218	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
219	Breast	Invasive ductal carcinoma (brease tissue)	T1N0M0	-	1	Malignant	1	BR2082b
220	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	1	BR2082b
221	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	1	BR2082b
222	Breast	Invasive ductal carcinoma (fatty tissue and blood vessel)	T2N2M0	-	3	Malignant	0	BR2082b
223	Breast	Invasive ductal carcinoma	T4N0M0	2	3	Malignant	0	BR2082b
224	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
225	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	1	BR2082b
226	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	1	BR2082b
227	Breast	Invasive ductal carcinoma	T1N0M0	2	1	Malignant	1	BR2082b
228	Breast	Invasive ductal carcinoma	T1N1M0	2	2	Malignant	1	BR2082b
229	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	1	BR2082b
230	Breast	Invasive ductal carcinoma	T2N0M0	3	2	Malignant	1	BR2082b
231	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
232	Breast	Invasive ductal carcinoma	T2N2M0	2	3	Malignant	1	BR2082b
233	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
234	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
235	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
236	Breast	Invasive ductal carcinoma	T2N1M0	2	2	Malignant	0	BR2082b
237	Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	1	BR2082b
238	Breast	Invasive ductal carcinoma	T1N0M0	2	1	Malignant	1	BR2082b

239 Breast	Invasive ductal carcinoma	T4N1M0	2	3	Malignant	1	BR2082b
240 Breast	Invasive ductal carcinoma	T2N1M0	2	2	Malignant	0	BR2082b
241 Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
242 Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
243 Breast	Invasive ductal carcinoma	T3N2M0	3	3	Malignant	0	BR2082b
244 Breast	Invasive ductal carcinoma	T2N0M0	2	2	Malignant	0	BR2082b
245 Breast	Invasive ductal carcinoma	T3N0M0	2	3	Malignant	0	BR2082b
246 Breast	Invasive ductal carcinoma	T2N1M0	3	2	Malignant	1	BR2082b
247 Breast	Invasive ductal carcinoma	T2N2M0	3	3	Malignant	1	BR2082b
248 Breast	Invasive ductal carcinoma	T2N1M0	3	2	Malignant	1	BR2082b
249 Breast	Invasive ductal carcinoma	T4N0M0	3	3	Malignant	0	BR2082b
250 Breast	Invasive lobular carcinoma	T2N0M0	-	2	Malignant	0	BR2082b
251 Breast	Invasive lobular carcinoma	T2N2M0	-	3	Malignant	0	BR2082b
252 Breast	Invasive lobular carcinoma	T2N1M0	-	2	Malignant	1	BR2082b
253 Breast	Invasive lobular carcinoma (sparse)	T2N1M0	-	2	Malignant	0	BR2082b
254 Breast	Invasive lobular carcinoma	T2N0M0	-	2	Malignant	1	BR2082b
255 Breast	Invasive lobular carcinoma	T2N1M0	-	2	Malignant	1	BR2082b
256 Breast	Invasive lobular carcinoma	T4N0M0	-	3	Malignant	1	BR2082b
257 Breast	Invasive lobular carcinoma	T4N0M0	-	3	Malignant	0	BR2082b
258 Breast	Invasive lobular carcinoma	T2N0M0	-	2	Malignant	1	BR2082b
259 Breast	Invasive lobular carcinoma	T2N1M0	-	2	Malignant	1	BR2082b
260 Breast	Invasive lobular carcinoma	T2N0M0	-	2	Malignant	1	BR2082b
261 Breast	Invasive lobular carcinoma	T4N0M0	-	3	Malignant	1	BR2082b
262 Breast	Invasive lobular carcinoma	T2N1M0	-	2	Malignant	0	BR2082b
263 Breast	Invasive lobular carcinoma	T2N1M0	-	2	Malignant	0	BR2082b
264 Breast	Invasive lobular carcinoma	T2N1M0	-	2	Malignant	0	BR2082b
265 Breast	Invasive lobular carcinoma (breast tissue)	T2N0M0	-	2	Malignant	0	BR2082b
266 Breast	Invasive lobular carcinoma (sparse)	T2N1M0	-	2	Malignant	0	BR2082b
267 Breast	Invasive lobular carcinoma (sparse)	T2N1M0	-	2	Malignant	1	BR2082b
268 Breast	Invasive lobular carcinoma	T2N1M0	-	2	Malignant	0	BR2082b
269 Breast	Invasive lobular carcinoma	T3N1M0	-	3	Malignant	0	BR2082b
270 Breast	Invasive lobular carcinoma	T2N0M0	-	2	Malignant	1	BR2082b
271 Breast	Invasive lobular carcinoma	T2N1M0	-	2	Malignant	1	BR2082b
272 Breast	Squamous cell carcinoma	T1N0M0	1--2	1	Malignant	1	BR2082b
273 Breast	Squamous cell carcinoma	T3N0M0	2	2	Malignant	0	BR2082b
274 Breast	Squamous cell carcinoma	T4N0M0	2	3	Malignant	0	BR2082b
275 Breast	Squamous cell carcinoma	T1N0M0	2	1	Malignant	0	BR2082b
276 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	0	BR2082b
277 Breast	Intraductal carcinoma (sparse)	TisN0M0	-	0	Malignant	1	BR2082b
278 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	1	BR2082b
279 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	1	BR2082b
280 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	1	BR2082b
281 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	1	BR2082b
282 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	1	BR2082b
283 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	1	BR2082b
284 Breast	Intraductal carcinoma (adenosis)	TisN0M0	-	0	Malignant	0	BR2082b
285 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	1	BR2082b
286 Breast	Intraductal carcinoma	T2N0M0	-	2	Malignant	0	BR2082b
287 Breast	Intraductal carcinoma (breast tissue)	TisN0M0	-	0	Malignant	0	BR2082b
288 Breast	Intraductal carcinoma with early infiltration	T2N0M0	-	2	Malignant	1	BR2082b
289 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	1	BR2082b
290 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	1	BR2082b
291 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	0	BR2082b
292 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	0	BR2082b
293 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	0	BR2082b
294 Breast	Intraductal carcinoma (duct tissue)	TisN0M0	-	0	Malignant	0	BR2082b
295 Breast	Intraductal carcinoma	T2N1M0	-	2	Malignant	0	BR2082b
296 Breast	Intraductal carcinoma	TisN0M0	-	0	Malignant	0	BR2082b
297 Breast	Intraductal carcinoma	T2N0M0	-	2	Malignant	0	BR2082b
298 Breast	Lobular carcinoma in situ (adenosis)	TisN0M0	-	0	Malignant	0	BR2082b

299 Breast	Lobular carcinoma in situ	TisNOM0	-	0	Malignant	0	BR2082b
300 Breast	Lobular carcinoma in situ	TisNOM0	-	0	Malignant	0	BR2082b
301 Breast	Fibroadenoma	-	-	-	Benign	1	BR2082b
302 Breast	Fibroadenoma	-	-	-	Benign	1	BR2082b
303 Breast	Fibroadenoma	-	-	-	Benign	1	BR2082b
304 Breast	Fibroadenoma	-	-	-	Benign	0	BR2082b
305 Breast	Fibroadenoma	-	-	-	Benign	0	BR2082b
306 Breast	Fibroadenoma	-	-	-	Benign	0	BR2082b
307 Breast	Fibroadenoma	-	-	-	Benign	0	BR2082b
308 Breast	Fibroadenoma	-	-	-	Benign	0	BR2082b
309 Breast	Adenosis with hyperplasia of breast duct	-	-	-	Hyperplasia	1	BR2082b
310 Breast	Adenosis with hyperplasia of breast duct	-	-	-	Hyperplasia	0	BR2082b
311 Breast	Adenosis with hyperplasia of breast duct	-	-	-	Hyperplasia	1	BR2082b
312 Breast	Moderate hyperplasia of breast duct	-	-	-	Hyperplasia	1	BR2082b
313 Breast	Atypical hyperplasia in duct (grade II)	-	-	-	Hyperplasia	1	BR2082b
314 Breast	Adenosis with hyperplasia of breast duct	-	-	-	Hyperplasia	0	BR2082b
315 Breast	Adenosis with hyperplasia of breast duct	-	-	-	Hyperplasia	0	BR2082b
316 Breast	Hyperplasia of breast duct (hyalinosis of fibrous tissue)	-	-	-	Hyperplasia	0	BR2082b
317 Breast	Adenosis with moderate hyperplasia of duct	-	-	-	Hyperplasia	0	BR2082b
318 Breast	Hyperplasia (sparse breast tissue)	-	-	-	Hyperplasia	0	BR2082b
319 Breast	Hyperplasia (adenosis)	-	-	-	Hyperplasia	0	BR2082b
320 Breast	Hyperplasia (adenosis)	-	-	-	Hyperplasia	1	BR2082b
321 Breast	Hyperplasia (adenosis)	-	-	-	Hyperplasia	1	BR2082b
322 Breast	Hyperplasia (adenosis)	-	-	-	Hyperplasia	1	BR2082b
323 Breast	Hyperplasia (fibrous tissue and blood vessel)	-	-	-	Hyperplasia	0	BR2082b
324 Breast	Hyperplasia (adenosis)	-	-	-	Hyperplasia	0	BR2082b
325 Breast	Cancer adjacent normal breast tissue	-	-	-	NAT	1	BR2082b
326 Breast	Cancer adjacent normal breast tissue (fatty tissue and fibr -	-	-	-	NAT	0	BR2082b
327 Breast	Cancer adjacent normal breast tissue (fatty tissue and fibr -	-	-	-	NAT	0	BR2082b
328 Breast	Cancer adjacent normal breast tissue (ductal ectasia)	-	-	-	NAT	1	BR2082b
329 Breast	Cancer adjacent normal breast tissue	-	-	-	NAT	1	BR2082b
330 Breast	Cancer adjacent normal breast tissue	-	-	-	NAT	0	BR2082b
331 Breast	Cancer adjacent normal breast tissue (fibrous tissue)	-	-	-	NAT	0	BR2082b
332 Breast	Cancer adjacent normal breast tissue	-	-	-	NAT	0	BR2082b
333 Breast	Cancer adjacent normal breast tissue	-	-	-	NAT	0	BR2082b
334 Breast	Cancer adjacent normal breast tissue with ductal epithelia -	-	-	-	Normal	0	BR2082b
335 Breast	Cancer adjacent normal breast tissue	-	-	-	NAT	0	BR2082b
336 Breast	Cancer adjacent normal breast tissue (fibrous tissue and b -	-	-	-	NAT	0	BR2082b
337 Breast	Cancer adjacent normal breast tissue (fibrous tissue and b -	-	-	-	NAT	0	BR2082b
338 Breast	Cancer adjacent normal breast tissue of No. 9	-	-	-	NAT	0	BR2082b
339 Breast	Cancer adjacent normal breast tissue	-	-	-	NAT	0	BR2082b
340 Breast	Cancer adjacent normal breast tissue	-	-	-	NAT	1	BR2082b
341 Breast	Breast Breast invasive ductal carcinoma	T2NOM0	1	2	malignant	0	BR8013
342 Breast	Breast invasive ductal carcinoma	T2NOM0	1	2	malignant	0	BR8013
343 Breast	Breast invasive ductal carcinoma	T1N1M0	2	2	malignant	1	BR8013
344 Breast	Breast invasive ductal carcinoma	T2NOM0	2	2	malignant	1	BR8013
345 Breast	Breast invasive ductal carcinoma	T1N1M0	2	2	malignant	1	BR8013
346 Breast	Breast invasive ductal carcinoma	T2NOM0	2	2	malignant	1	BR8013
347 Breast	Breast invasive ductal carcinoma	T1N1M0	2	2	malignant	0	BR8013
348 Breast	Breast invasive ductal carcinoma	T2NOM0	2	2	malignant	0	BR8013
349 Breast	Breast invasive ductal carcinoma (fibrofatty tissue)	T2NOM0	-	2	malignant	1	BR8013
350 Breast	Breast invasive ductal carcinoma	T2NOM0	2	2	malignant	1	BR8013
351 Breast	Breast invasive ductal carcinoma	T2NOM0	3	2	malignant	1	BR8013
352 Breast	Breast invasive ductal carcinoma	T3N1M0	2	2	malignant	0	BR8013
353 Breast	Breast invasive ductal carcinoma	T2NOM0	2	2	malignant	1	BR8013
354 Breast	Breast invasive ductal carcinoma	T2NOM0	2	2	malignant	0	BR8013
355 Breast	Breast invasive ductal carcinoma (breast tissue)	T2N1M0	-	2	malignant	1	BR8013
356 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
357 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	1	BR8013
358 Breast	Breast invasive ductal carcinoma	T4N1M0	2	3	malignant	0	BR8013

359 Breast	Breast invasive ductal carcinoma (hyperplasia of breast du	T2N1M0	-	2	malignant	1	BR8013
360 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	1	BR8013
361 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
362 Breast	Breast invasive ductal carcinoma	T4N1M0	2	3	malignant	1	BR8013
363 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	1	BR8013
364 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
365 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
366 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
367 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
368 Breast	Breast invasive ductal carcinoma	T3N1M0	2	3	malignant	1	BR8013
369 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
370 Breast	Breast invasive ductal carcinoma	T2N1M0	2	3	malignant	1	BR8013
371 Breast	Breast invasive ductal carcinoma	T4N2M0	2	3	malignant	1	BR8013
372 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
373 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
374 Breast	Breast invasive ductal carcinoma	T2N2M0	2	3	malignant	0	BR8013
375 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
376 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	1	BR8013
377 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
378 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	1	BR8013
379 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	1	BR8013
380 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
381 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	1	BR8013
382 Breast	Breast invasive ductal carcinoma	T4N2M0	2	3	malignant	1	BR8013
383 Breast	Breast invasive ductal carcinoma	T3N2M0	2	3	malignant	1	BR8013
384 Breast	Breast invasive ductal carcinoma	T4N1M0	2	3	malignant	1	BR8013
385 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	1	BR8013
386 Breast	Breast invasive ductal carcinoma	T4N2M1	2	4	malignant	0	BR8013
387 Breast	Breast invasive ductal carcinoma (breast tissue)	T4N0M0	-	3	malignant	1	BR8013
388 Breast	Breast invasive ductal carcinoma (sparse)	T2N1M0	-	2	malignant	0	BR8013
389 Breast	Breast invasive ductal carcinoma	T2N1M0	1--2	2	malignant	1	BR8013
390 Breast	Breast invasive ductal carcinoma	T2N1M0	3	2	malignant	1	BR8013
391 Breast	Breast invasive ductal carcinoma	T2N1M0	3	2	malignant	1	BR8013
392 Breast	Breast invasive ductal carcinoma	T2N2M0	3	2	malignant	1	BR8013
393 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	0	BR8013
394 Breast	Breast invasive ductal carcinoma	T2N2M0	2	3	malignant	1	BR8013
395 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	1	BR8013
396 Breast	Breast invasive ductal carcinoma	T3N1M0	3	3	malignant	1	BR8013
397 Breast	Breast invasive ductal carcinoma	T2N1M0	2	2	malignant	1	BR8013
398 Breast	Breast invasive ductal carcinoma	T2N1M0	3	2	malignant	0	BR8013
399 Breast	Breast invasive ductal carcinoma	T3N1M0	3	3	malignant	1	BR8013
400 Breast	Breast invasive ductal carcinoma	T2N1M1	3	2	malignant	0	BR8013
401 Breast	Breast invasive ductal carcinoma	T2N1M0	3	2	malignant	1	BR8013
402 Breast	Breast invasive lobular carcinoma	T2N1M0	-	2	malignant	0	BR8013
403 Breast	Breast invasive lobular carcinoma	T2N1M0	-	2	malignant	0	BR8013
404 Breast	Breast invasive lobular carcinoma	T3N1M0	-	3	malignant	0	BR8013
405 Breast	Breast invasive lobular carcinoma	T2N1M0	-	2	malignant	0	BR8013
406 Breast	Breast invasive lobular carcinoma	T2N1M0	-	2	malignant	0	BR8013
407 Breast	Normal breast tissue (fibrofatty tissue)	-	-	-	normal	0	BR8013
408 Breast	Normal breast tissue	-	-	-	normal	0	BR8013
409 Breast	Normal breast tissue (breast duct tissue)	-	-	-	normal	0	BR8013
410 Breast	Normal breast tissue	-	-	-	normal	0	BR8013
411 Breast	Normal breast tissue (breast duct tissue)	-	-	-	normal	0	BR8013
412 Breast	Normal breast tissue	-	-	-	normal	0	BR8013
413 Breast	Normal breast tissue	-	-	-	normal	0	BR8013
414 Breast	Normal breast tissue	-	-	-	normal	0	BR8013
415 Breast	Normal breast tissue	-	-	-	normal	0	BR8013
416 Breast	Normal breast tissue (sparse)	-	-	-	normal	0	BR8013

Online Resource 4. Aptamer #63 and #41 show high specificity to aggressive prostate LN3 cell line. LN3 (top) and Pro5 (bottom) cancer cell lines were incubated with 1nmol of representative Cy3-labeled aptamers from 5 sequenced families. Aggressive LN3 cell line show positive staining by Apt63 and 41; Pro5 cell line doesn't show positive staining. Original confocal imaging magnification: 20x



Online Resource 5. Apt63 efficiently discriminates between murine breast cancer cell lines with different metastatic potential, indicating inter-spe-cies conservation of the binding target. E0771.LMB and 4T1, highly metastatic cell lines show positive staining by Apt63; E0771 and 67 NR doesn't show positive staining. Original confocal imaging magnification: 20x

