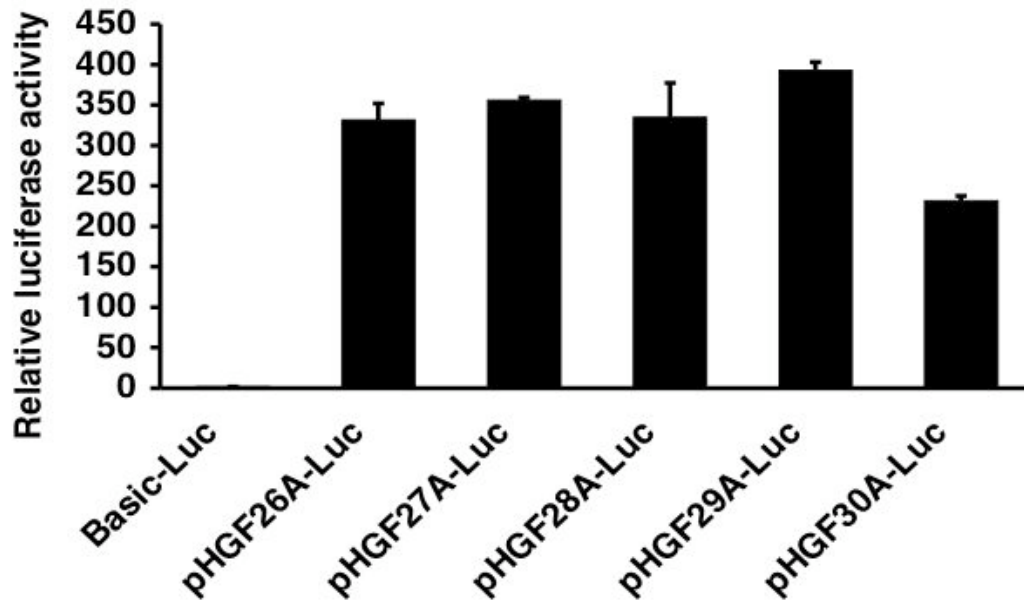
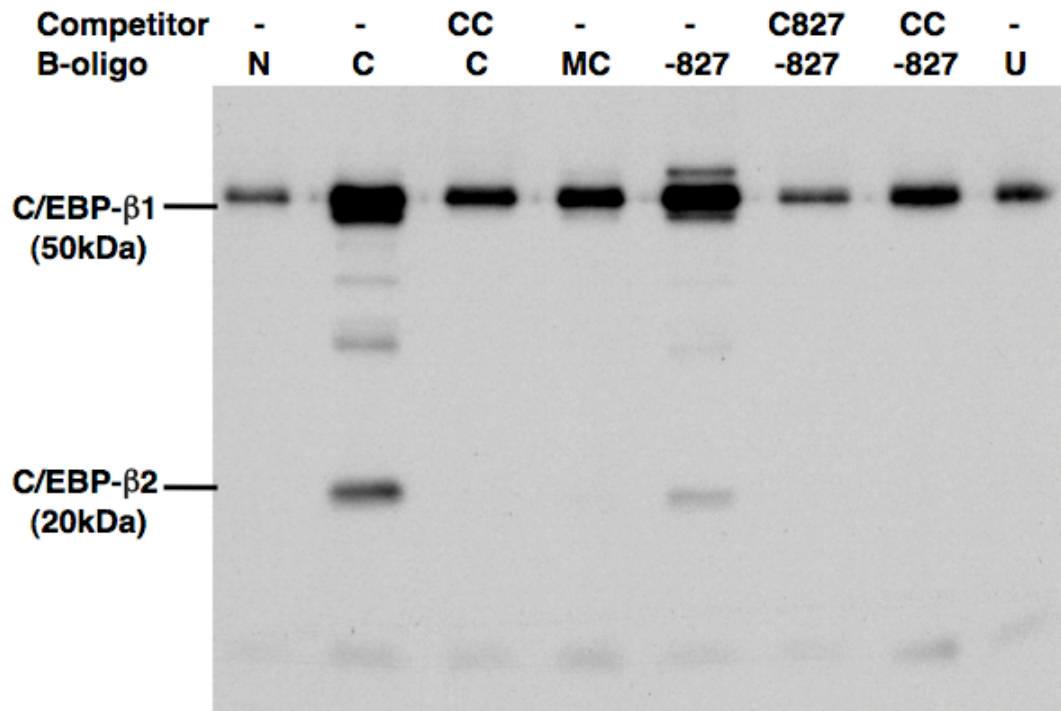




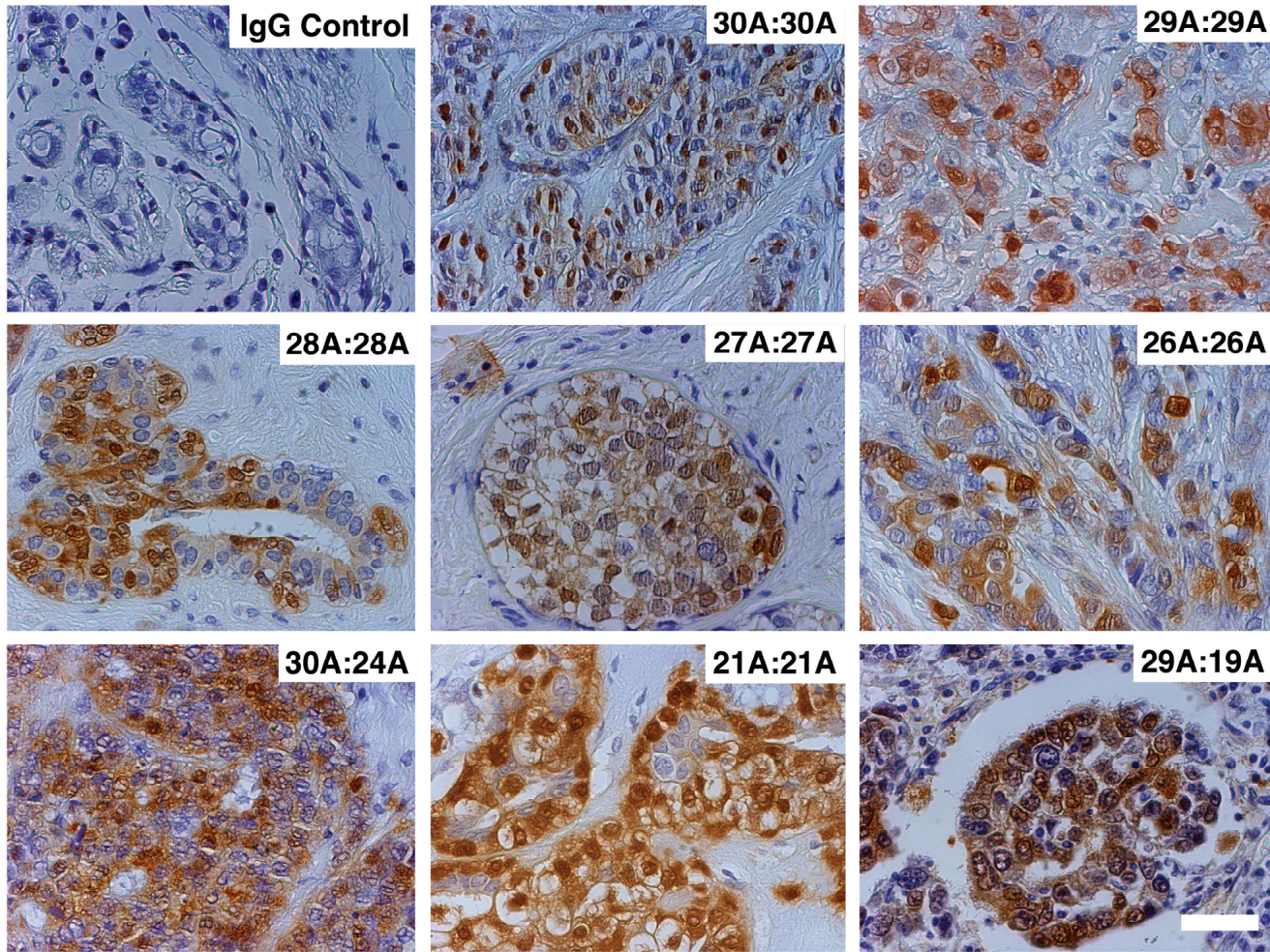
Supplemental Figure 1

Effects of DATE shortening on HGF promoter activity. The *HGF* promoter region (-1037 to +56) containing wild-type (30As) or truncated DATE (26As, 27As, 28A, 29As) from breast cancer cases was inserted into the upstream of the luciferase report gene in pGL2-basic vector (namely, pHGF30A-Luc, pHGF26A-Luc, pHGF27A-Luc, pHGF28A-Luc and pHGF29A-Luc). PGL2-basic (Basic-Luc) was used as a negative control vector. The above plasmids were verified by DNA sequencing of the entire insert and transiently transfected into HeLa cells. Luciferase activity in pHGF30A-Luc is significantly lower than those constructs with truncated DATE (30As vs. 26As, ***P*1** < 0.01; 30As vs. 27As, ***P*2** < 0.01; 30As vs. 28As, ***P*3** < 0.05; 30As vs. 29As, ***P*4** < 0.01, two tail paired t-test).



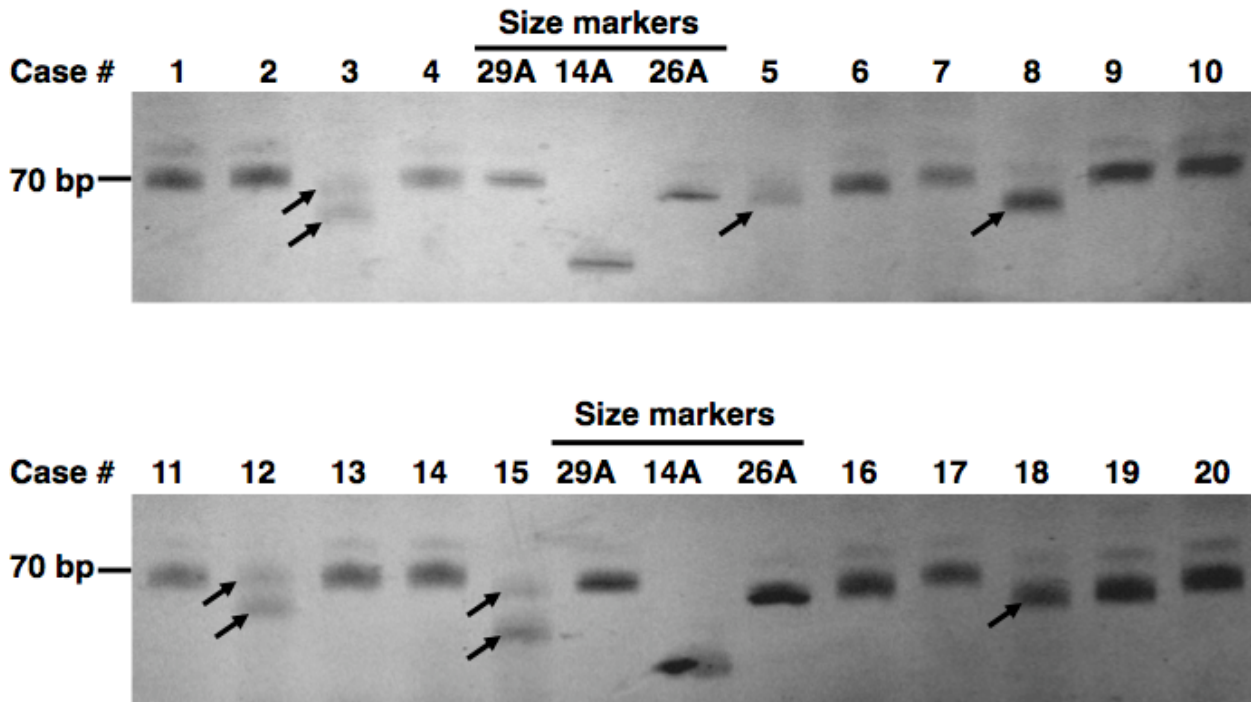
Supplemental Figure 2

Identification of C/EBP- β binding site in *HGF* promoter region. Biotin labeled synthetic oligo DNA (B-oligo) corresponding to the C/EBP- β site upstream of DATE (at -827) was used for DNA affinity purification assay to check C/EBP- β binding ability employing C/EBP- β antibody and western blot. Commercially available C/EBP- β oligos (C) were used as a positive control; 50X of unlabeled -827 oligo (C 827) and C/EBP- β oligo (CC) were used as competitors to show specific binding. N: no oligo added in the pull down reaction; MC: mutant C/EBP- β oligo; U: unrelated oligos; Arrows point to C/EBP- β (C/EBP- β 1 and C/EBP- β 2) bands binding to the site present at -827 in the *HGF* promoter and to C/EBP- β oligo.



Supplemental Figure 3

Representative Profile of Met protein expression in eight different breast cancer cases having various DATE length (genotype). Met expression was noted in all breast cancer tissues. A pattern of high Met expression (+++) was apparent in breast cancer tissues having the truncated DATE genotype (i.e. 30A:24A, 21A:21A and 29A:19A also see Supplemental Table 2) as opposed to those with wild type DATE which exhibited weak (+) to moderate (++) Met expression. Bar indicates 40 μ M.



Supplemental Figure 4

Truncated DATEs are present in normal human breast tissues. Genotyping was done by PCR and the resulting 70 bp PCR products were analyzed on 15% denaturing polyacrylamide gel containing 8M urea. The arrows show truncated DATE. Cloned and sequenced 70 bp PCR products containing 14As, 26As, and 29As and were used as markers as indicated.

Supplemental Table 1 Patient demographics and genotype analysis of DATE in breast cancer and its corresponding adjacent normal tissues

Case#	Type of tumor	ER	PR	Size of tumors	Age (yr)	Race	Length of DATE (As)		Lymph node invasion
							Breast tumor	Normal adjacent	
1	Ductal carcinoma	-	-	2.5x2.5x2.5cm	43	AA	17	17	-
2	/	/	/	/	/	/	26:17	26	/
3	Ductal carcinoma Invasive	/	/	3.0x2.8x2.6 cm	59	AA	30:18	30	+
4	Ductal carcinoma Invasive	/	/	3.5x3.5x2.8 cm	46	AA	19	19	+
5	Ductal carcinoma	+	+	1.5X0.8X0.5 cm	53	AA	22:19	22:19	-
6	Ductal carcinoma Invasive	-	-	8.0x5.5x4.7 cm	67	AA	29:19	29	+
7	Ductal carcinoma Invasive	+	+	1.5X1.5X1.0cm	42	AA	27:20	27:20	+
8	Ductal carcinoma	/	/	9.5x5.3x3.0 cm	55	CC	30:20	30:20	-
9	Ductal carcinoma	/	/	2.6X2.0X2.4 cm	38	AA	30:20	30:20	+
10	Ductal carcinoma Metaplastic	+	+	2.0x2.0x1.8 cm	51	AA	21	21	-
11	Ductal carcinoma	-	-	2.5X2.0X1.9cm	44	AA	30:22	30:22	-
12	Lobular carcinoma Invasive	/	/	2.2x3.5x1.8 cm	62	AA	23	23	+
13	Ductal carcinoma Invasive	/	/	2.0x1.7x1.5 cm	63	CC	30:23	30	+
14	Ductal carcinoma	/	/	28x24x6.5 cm	46	AA	30:23	30:23	/
15	Ductal carcinoma	/	/	2.8x2.0x2.0 cm	58	AA	24	24	-
16	Ductal carcinoma Invasive	/	/	4.0x2.0x3.0 cm	71	CC	24	24	+
17	/	/	/	/	36	CC	24	24	/
18	Ductal carcinoma	/	/	6.0x5.0x4.0 cm	33	AA	24	24	-
19	Ductal carcinoma	+	+	1.7x1.7x1.7 cm	53	CC	30:24	30	-
20	Ductal carcinoma	+	+	1.9x1.8x1.6 cm	51	CC	30:24	30	-
21	Ductal carcinoma	/	/	/	47	AA	30:24	30:24	/
22	Ductal carcinoma	-	-	1.7X1.7X1.5 cm	53	AA	30:24	30:24	-
23	Ductal carcinoma	/	/	2.5x2.2x2.0 cm	62	CC	25	25	+
24	Ductal carcinoma Invasive	/	/	3.5x2.5x4.0 cm	73	AA	25	25	+
25	Ductal carcinoma Invasive	/	/	10.5x6.5x3.5 cm	48	CC	25	25	+
26	Ductal carcinoma Invasive	+	-	9.5X6.5X2.5 cm	52	AA	25	25	+
27	Ductal carcinoma Invasive	-	-	5.0X5.0X3.0 cm	/	AA	25	25	-
28	Ductal carcinoma Invasive	/	/	2.0X1.0X1.0 cm	34	AA	25	25	+
29	Lobular carcinoma Invasive	/	/	3.0x2.4x1.5 cm	80	CC	26	26	-
30	Ductal carcinoma Poorly differentiated	/	/	1.8x1.5x1.0 cm	47	CC	26	26	-
31	Ductal carcinoma Invasive	/	/	2.5x2.3x2.0 cm	61	CC	26	26	-

Case#	Type of tumor	ER	PR	Size of tumors	Age (yr)	Race	Length of DATE (As)		
							Breast tumor	Normal adjacent	Lymph node invasion
32	Ductal carcinoma Invasive	/	/	2.1X1.6X1.6 cm	46	AA	26	26	+
33	Ductal carcinoma Invasive	+	+	2.5x2.0x1.0 cm	90	CC	28:26	28	+
34	Ductal carcinoma Invasive	/	/	3.0x2.4x1.5 cm	50	AA	30:26	30:26	+
35	Ductal carcinoma	+	+	1.3X1.2X1.0cm	62	AA	30:26	30:26	-
36	Ductal carcinoma Invasive	/	/	2.5x2.0x1.8 cm	58	CC	27	27	+
37	Ductal carcinoma Metastatic	/	/	2.5x2.5x2.2 cm	67	CC	27	27	-
38	Ductal carcinoma Invasive	-	-	8.0x3.0x1.5 cm	55	CC	27	27	+
39	Ductal carcinoma Invasive	/	/	4.5x2.0x1.5 cm	59	CC	27	27	+
40	Ductal carcinoma Invasive	+	+	3.0x3.0x2.5 cm	50	CC	27	27	+
41	Ductal carcinoma Invasive	/	/	9.6x8.0x8.0 cm	54	CC	27	27	+
42	Ductal carcinoma Invasive	/	/	2.9x2.5x1.7 cm	42	CC	27	27	+
43	Ductal carcinoma Invasive	/	/	2.5X2.0X1.5 cm	47	AA	27	27	+
44	Ductal carcinoma Invasive	/	/	2.8x2.5x2.4 cm	40	CC	28	28	+
45	Ductal carcinoma	/	/	16.0x14.0 x3 cm	53	AA	28	28	/
46	Adenocarcinoma	+	+	2.3x1.5x0.8 cm	69	CC	28	28	+
47	Ductal carcinoma Invasive	/	/	1.5x1.0x1.0 cm	43	CC	28	28	+
48	Ductal carcinoma Invasive	/	/	3.0x2.8x1.4 cm	52	CC	28	28	+
49	Ductal carcinoma Invasive	/	/	4.7x2.5x2.0 cm	55	CC	28	28	+
50	Ductal carcinoma Invasive	/	/	10.0x6.5x4.5 cm	40	CC	28	28	+
51	Ductal carcinoma Invasive	+	+	2.0x2.0x2.0 cm	80	/	28	28	+
52	Ductal carcinoma Invasive	/	/	32.5x25x3.4 cm	37	CC	28	28	+
53	Ductal carcinoma Invasive	-	-	3.0x 3.0x2.5 cm	71	CC	28	29	+
54	Ductal/Lobular Invasive	/	/	3.1x3.8x2.2 cm	63	CC	28	28	+
55	Ductal carcinoma Invasive	-	-	3.5x3.2x3.0 cm	54	CC	28	29	+
56	Ductal carcinoma Invasive	/	/	4.7x4.0x3.2 cm	50	CC	28	29	+
57	Ductal carcinoma	/	/	4.0x3.0x3.5 cm	66	CC	29	29	-
58	Ductal carcinoma	/	/	2.5x1.8x1.4 cm	54	/	29	29	+

Case#	Type of tumor	ER	PR	Size of tumors	Age (yr)	^Race	Length of DATE (As)		Lymph node invasion
							Breast tumor	Normal adjacent	
59	Ductal carcinoma Invasive	+	+	4.5x3.5x3.0 cm	59	CC	29	29	+
60	Ductal carcinoma Invasive	/	/	5.0x5.5x4.0 cm	71	AS	29	29	+
61	Ductal carcinoma Invasive	-	-	13.0x11.0x7 cm	78	AA	29	29	+
62	Ductal carcinoma	+	+	2.2x2.0x1.6 cm	73	AA	29	29	-
63	Ductal carcinoma	/	/	0.8x0.8x0.7 cm	44	CC	29	30	-
64	Ductal carcinoma Invasive	/	/	3.0x2.5x1.0 cm	57	CC	29	30	+
65	Ductal carcinoma Invasive	/	/	2.5x1.5x1.0 cm	64	CC	29	30	+
66	Ductal carcinoma	/	/	2.0x1.5x1.0 cm	53	CC	30	30	-
67	Ductal carcinoma	+	-	2.2x1.5x1.3 cm	55	CC	30	30	-
68	Ductal carcinoma	/	/	2.5x2.0x1.5 cm	68	AS	30	30	-
69	Ductal carcinoma Invasive	+	+	4.5x2.5x2.0 cm	44	CC	30	30	+
70	Ductal carcinoma Invasive	/	/	2.7x2.3x2.5 cm	78	CC	31	30	+
71	Ductal carcinoma Invasive	/	/	2.2x2.0x1.5 cm	64	CC	30	30	-
72	Ductal carcinoma Invasive	/	/	1.7x1.7x1.0 cm	54	CC	30	30	-
73	Ductal carcinoma	/	/	1.8x1.8x1.5 cm	78	CC	30	30	/
74	Ductal carcinoma Invasive	/	/	2.5x1.7x2.0 cm	45	AA	30	30	-
75	Ductal carcinoma Invasive	+	+	2.8x1.2x1.5 cm	48	CC	30	30	-
76	Ductal carcinoma Invasive	+	+	3.0x3.0x2.7 cm	43	CC	30	30	+
77	Ductal carcinoma Invasive	/	/	6.0x4.0x3.0 cm	34	CC	30	30	+
78	Ductal carcinoma Invasive	/	/	2.7x2.3x2.5 cm	68	CC	30	30	+
79	Ductal carcinoma Invasive	+	+	11.0x6.0x2.0 cm	95	CC	30	30	-
80	Lobular carcinom	/	/	2.2x1.5x0.5 cm	78	CC	30	30	-
81	Ductal carcinoma Invasive	/	/	2.5x1.0x1.0 cm	70	CC	30	30	+
82	Ductal carcinoma Invasive	/	/	4.0x3.8x2.5 cm	78	CC	30	30	+
83	Ductal carcinoma Invasive	/	/	3.5x3.0x2.0 cm	42	CC	30	30	+
84	Ductal carcinoma	-	-	6.5x5.5x4.0 cm	81	CC	30	30	-
85	Ductal carcinoma Invasive	/	/	7.0x4.0x2.0 cm	59	CC	30	30	+
86	Ductal / Lobular Invasive	-	-	6.5x6.0x6.5 cm	33	AA	30	30	+
87	Ductal / Lobular Invasive	+	+	3.0x2.4x1.5 cm	53	AA	30	30	+

Case#	Type of tumor	ER	PR	Size of tumors	Age (yr)	^A Race	Length of DATE (As)		Lymph node invasion
							Breast tumor	Normal adjacent	
88	Ductal carcinoma Invasive	-	-	18.0x15.0x11cm	53	AA	30	30	-
89	Ductal carcinoma Invasive	+	+	3.0x1.7x1.6 cm	43	AA	30	30	+
90	Ductal / Lobular Invasive	+	+	6.0x3.0x2.5 cm	73	AA	30	30	+
91	Ductal carcinoma	-	-	4.0x2.0x1.5 cm	57	AA	30	30	-
92	Ductal carcinoma	+	+	1.2x1.3x0.9 cm	53	AA	30	30	-
93	Ductal carcinoma	+	+	8x4.6x4.0 cm	68	AA	30	30	-
94	Ductal / Lobular Invasive	+	-	2.1x1.0x1.0 cm	53	AA	30	30	+
95	Ductal carcinoma	/	/	3.0x1.5x1.3 cm	37	AA	30	30	/

^A Race: CC: Caucasian; AA: African American; AS: Asia; /: Not available

Supplemental Table 2 Profile of HGF and Met protein expression in human breast cancer cases as determined by immunohistochemistry

Cases	Type of tumors	Age	^A Race	Length of DATE (As)		^B HGF expression		Met expression	
				Tumor	Adjacent	Tumor	Adjacent	Tumor	Adjacent
1	Ductal carcinoma	59	AA	30:18	30	+++	++	+++	+
2	Ductal carcinoma	67	AA	29:19	29	+++	+++	+++	++
3	Ductal carcinoma	46	AA	19	19	+++	+++	+++	+++
4	Ductal carcinoma	55	CC	30:20	30:20	+++	++	++	++
5	Ductal carcinoma	51	AA	21	21	+++	+++	+++	++
6	Ductal carcinoma	63	CC	30:23	30	+++	-	++	+
7	Ductal carcinoma	46	AA	30:23	30:23	+++	++	+	+
8	Ductal carcinoma	51	CC	30:24	30	++	++	+++	++
9	Ductal carcinoma	53	CC	30:24	30	++	++	+	+
10	Ductal carcinoma	50	AA	30:26	30:26	+++	+++	++	++
11	Ductal carcinoma	90	CC	28:26	28	+	-	+	+
12	Lobular carcinoma	80	CC	26	26	++	+	++	+
13	Ductal carcinoma	47	CC	26	26	+	-	++	+
14	Ductal carcinoma	61	CC	26	26	+	++	+	+
15	Ductal carcinoma	58	CC	27	27	-	-	+	+
16	Ductal carcinoma	67	CC	27	27	++	+	+++	++
17	Ductal carcinoma	55	CC	27	27	+	-	++	+
18	Ductal carcinoma	59	CC	27	27	++	++	++	+
19	Ductal carcinoma	40	CC	28	28	+	++	+	+
20	Ductal carcinoma	53	AA	28	28	+	+	+	++
21	Adenocarcinoma	69	CC	28	28	+	-	++	+
22	Ductal carcinoma	43	CC	28	28	+	-	+	+
23	Ductal carcinoma	52	CC	28	28	+	-	+	+
24	Ductal carcinoma	55	CC	28	28	++	++	++	+
25	Ductal carcinoma	40	CC	28	28	++	++	+	++
26	Ductal carcinoma	66	CC	29	29	+	++	+	+
27	Ductal carcinoma	44	CC	29	30	+++	++	++	+++
28	Ductal carcinoma	57	CC	29	29	+	++	++	++
29	Ductal carcinoma	54	/	29	29	++	++	+	++
30	Ductal carcinoma	64	CC	29	30	++	+	++	+
31	Ductal carcinoma	59	CC	29	29	+++	+++	++	+
32	Ductal carcinoma	53	CC	30	30	+	+	++	+
33	Ductal carcinoma	55	CC	30	30	+	+	+	+
34	Ductal carcinoma	68	AA	30	30	+	-	++	+
35	Ductal carcinoma	44	CC	30	30	+	+	+	+
36	Ductal carcinoma	78	CC	31	30	-	-	+	++
37	Ductal carcinoma	64	CC	30	30	+	+	++	+
38	Ductal carcinoma	54	CC	30	30	+	+	++	++
39	Ductal carcinoma	78	CC	30	30	++	++	++	+
40	Ductal carcinoma	45	AA	30	30	++	++	+++	++
41	Ductal carcinoma	43	CC	30	30	++	++	++	++
42	Ductal carcinoma	42	CC	30	30	++	++	++	++

^ARace: AA: African American; CC: Caucasian; /: Not available

^BHGF & Met expression: Intensity of HGF or Met immunoreactivity in tumors and adjacent tissues was assessed by employing an arbitrary score of "-" to "+++". "-" = no staining/negative. "+" = faint staining with 25% or less of cells positive. "++" = 50% of cells staining with moderate intensity. "+++" = intense staining in which at least 80% of cells positive for HGF or Met (Figure 8A; Figure 8C; Supplemental Figure 3).