

Supplementary Materials

# The Tumor-Specific Expression of L1 Retrotransposons Independently Correlates with Time to Relapse in Hormone-Negative Breast Cancer Patients

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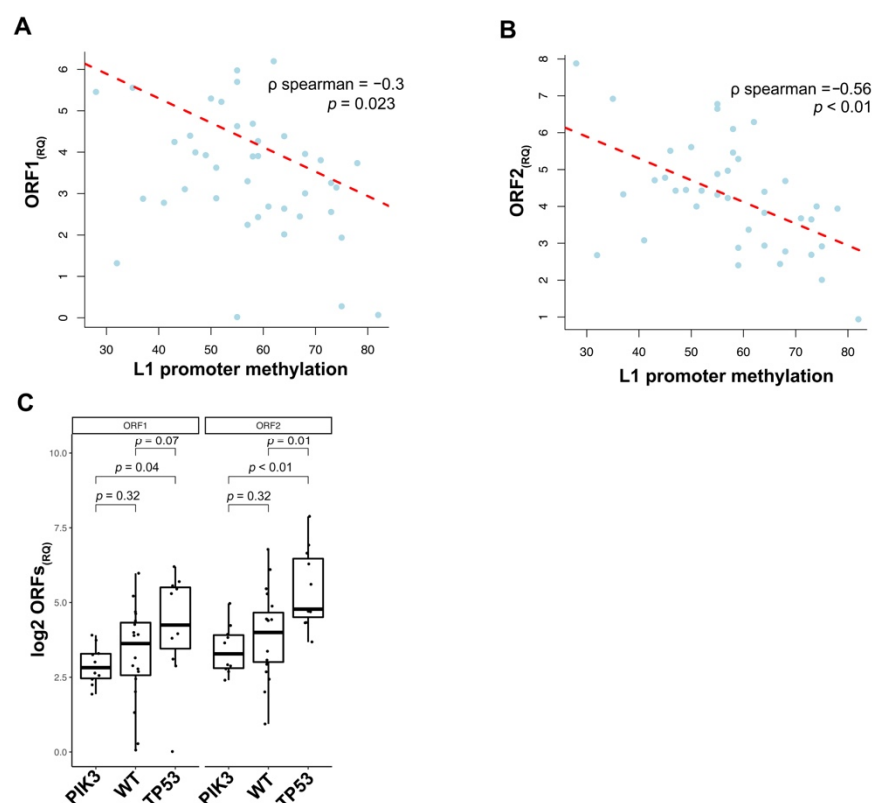
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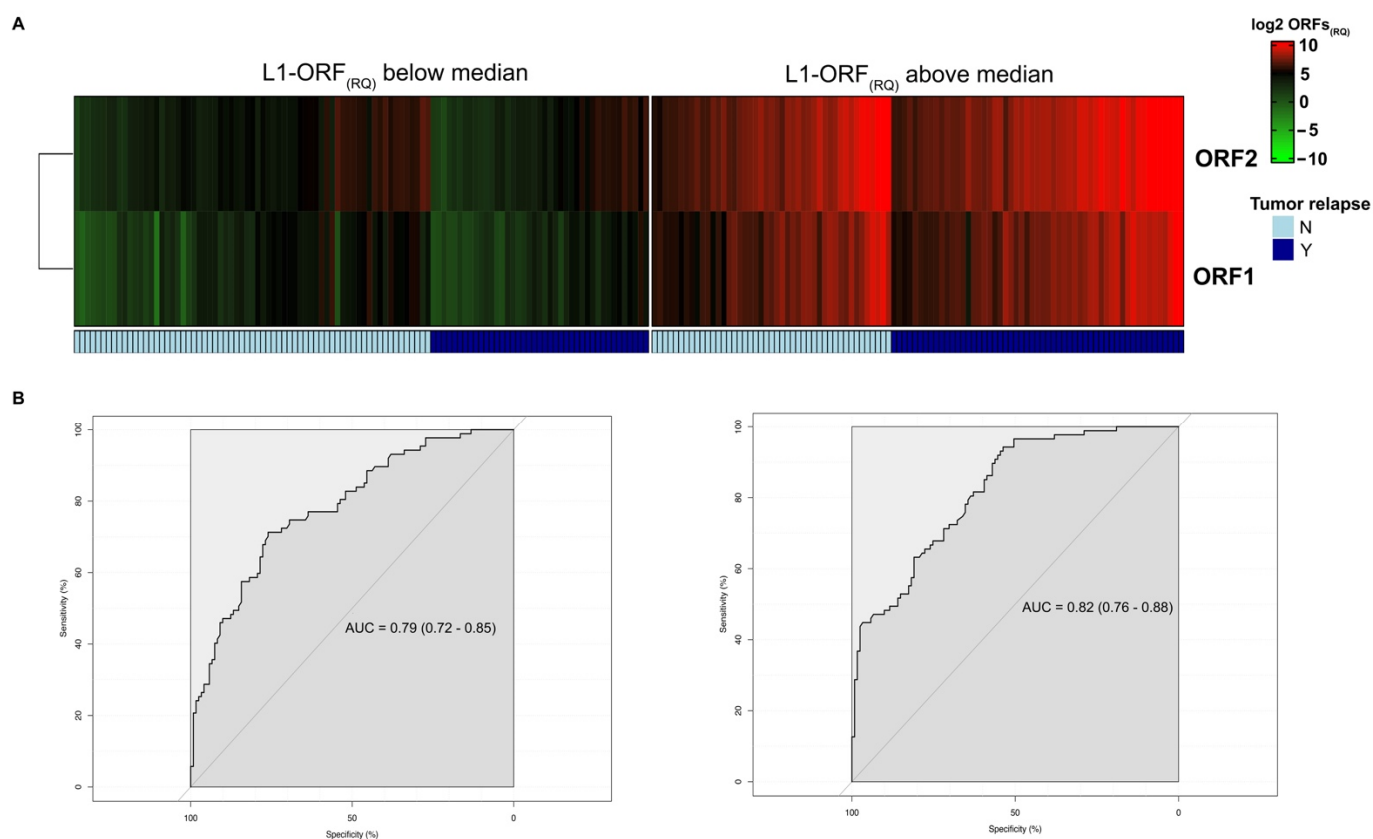
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**Figure S1.** (A) Correlation plot for ORF1 and methylation levels in the 41 BC tumors. (B) Correlation plot for ORF2 and methylation levels in the 41 BC tumors. (C) Boxplot reporting the expression of both ORF1 and ORF2 in *PIK3CA/AKT* mutated, WT or *TP53* mutated BCs ( $n = 41$ ).



**Figure S2. (A)** Heatmap of ORF1<sub>(RQ)</sub> and ORF2<sub>(RQ)</sub> within BC, with recurrence (N = light blue, Y = dark blue) annotation, clustered for the median level (above or below). Green-to-red scale for the log2 expression revealed a sensible increased expression in patients with tumor relapse. **(B)** Patient relapse ROC curves for both ORF1 and ORF. Plots also reporting the area under the curve (AUC) with range.

**Table S1.** BC cell lines analyzed in the study.

Cell Line	PAM50	Histology	ER	PR	HER2	Metastatic Potential	ORF1 (dCT)	ORF2(dCT)	L1(dCT)
Invasive									
BT474	Luminal B	Ductal Carcinoma	+	+	+	1	0.29	0.09	0.19
BT549	Basal-like	Adenocarcinoma	-	-	-	3	0.55	0.39	0.47
HCC1187	Basal-like	Ductal Carcinoma	-	-	-	2	2.54	1.83	2.185
HCC70	Basal-like	Ductal Carcinoma	-	-	-	2	0.44	0.31	0.375
HMEC	Non transformed	Human Mammary	na	na	na	0	0.05	0.03	0.04

		Epitheli al Cell								
		Invasiv e								
HS578T	Basal- like	Ductal Carcino ma	-	-	-	3	1.42	0.88	1.15	
MCF-7	Luminal A	Ductal Carcino ma	+	+	-	1	0.54	0.41	0.475	
MCF10A	Non transfor med	Fibrocy stic disease	na	na	na	0	0.09	0.14	0.115	
MDA-MB 231	Basal- like	Adenoc arcino ma	-	-	-	1	0.59	0.31	0.45	
MDA-MB 453	HER2	Adenoc arcino ma	-	-	+	3	1.04	0.91	0.975	
MDA-MB- 468	Basal- like	Ductal Carcino ma	-	-	-	1	1.62	0.89	1.255	
SKBR3	HER2	Adenoc arcino ma	-	-	+	1	0.25	0.11	0.18	
T47D	Luminal A	Invasiv e Ductal Carcino ma	+	+	-	1	0.47	0.4	0.435	
ZR751	Luminal A	Invasiv e Ductal Carcino ma	+	+/-	-	1	0.09	0.03	0.06	

**Table S2.** BC cohort features.

Age ( <i>n</i> = 210)	Mean
	58 (range 30-89)
Ki67 ( <i>n</i> = 210)	Mean (%)
	38.5 (range 3-95)
TILs ( <i>n</i> = 210)	Mean (%)
	10 (range 2-60)
Tumor Size ( <i>n</i> = 210)	Mean (mm)
	26.5 (range 2-90)
Histotype ( <i>n</i> = 210)	# Patients (%)
Lobular	16 (7.5%)
Ductal	171 (81.5%)
Other	23 (11%)
Grading ( <i>n</i> = 210)	
G1	5 (2.5%)
G2	64 (30.5%)
G3	141 (67%)
Staging ( <i>n</i> = 210)	
pT1	69 (33%)
pT2	116 (55%)
pT3	17 (8%)
pT4	8 (4%)
Lymph node metastases ( <i>n</i> = 210)	
pN0	69 (33%)
pN1	114 (54%)
pN2	14 (6.5%)
pN3	13 (6%)
Molecular subtype ( <i>n</i> = 210)	
Luminal A	67 (32%)
Luminal B	51 (24%)
HER2	52 (25%)
Basal-like	40 (19%)
Recurrence ( <i>n</i> = 210)	
Yes	112 (53%)
No	96 (46%)
Unknown	2 (1%)
Treatment ( <i>n</i> = 209)	
Chemotherapy	160 (76%)
Radiotherapy	157 (75%)
Hormone therapy	156 (74%)
Anti-HER2 therapy	48 (23%)
Relapse ( <i>n</i> = 208)	
Yes	96 (46%)
No	112 (54%)

**Table S3.** Primers, thermal profiles and protocols for L1 analysis.

qRT-PCR and Methylation Primers	
	5'-GGC CAA CGT TCA GAT TCA GG-3'
ORF1-R	5'-AAC CCG ACC TTT CTC TCT GG-3'
ORF1-RA	5'-AAC CCG ACC TTT CTC TCT GG-3'
ORFA-FA	5'-GGC CAA CGT TCA GAT TCA GG-3'
GAPDH-F	5'-GAA GGT GAA GGT CGG AGT C-3'
GAPDH-R	5'-GAA GAT GGT GAT GGG ATT TC-3'
18S-F	5'-AGTCCCTGCCCTTTGTACACA-3'

18S-R	5'-GATCCGAGGGCCTCACTAAAC-3'		
L1 methylation-F	5'-GAGTTAGGTGTGGGATATAGT-3'		
L1 methylation-R	5'biotin-CAAAAAATCAAAAAA TTCCCTTTC-3'		
L1 methylation sequencing	5'-GGTGTGGGATATAGTT-3'.		
L1-ORF1	Concentration		95°C (3 minutes)
cDNA	50 ng		95°C (3 minutes)
H2O	to 25 ul		95°C (30 seconds)
MgCl2	3mM	30 cycles	63°C (30 seconds)
dNTPs	0,2mM		72°C (45 seconds)
Buffer	1x		72°C (5 minutes)
PF	0,4 uM		
PR	0,4 uM		
TakaraTaq	1U		
EvaGreen	2X		
L1-ORF2	Concentration		95°C (3 minutes)
cDNA	50 ng		95°C (3 minutes)
H2O	to 25 ul	30 cycles	95°C (30 seconds)
MgCl2	3mM		60°C (30 seconds)
dNTPs	0,2mM		72°C (45 seconds)
Buffer	1x		72°C (5 minutes)
PF	0,4 uM		
PR	0,4 uM		
TakaraTaq	1U		
EvaGreen	2X		
18S	Concentration		95°C (3 minutes)
cDNA	50 ng		95°C (3 minutes)
H2O	to 25 ul		95°C (30 seconds)
MgCl2	2mM	25 cycles	58°C (30 seconds)
dNTPs	0,2mM		72°C (45 seconds)
Buffer	1x		72°C (5 minutes)
PF	0,4 uM		
PR	0,4 uM		
TakaraTaq	1U		
EvaGreen	2X		
GAPDH	Concentration		95°C (3 minutes)
cDNA	50 ng		95°C (3 minutes)
H2O	to 25 ul		95°C (30 seconds)
MgCl2	2mM	30 cycles	58°C (30 seconds)
dNTPs	0,2mM		72°C (45 seconds)
Buffer	1x		72°C (5 minutes)
PF	0,4 uM		
PR	0,4 uM		
TakaraTaq	1U		
EvaGreen	2X		
<b>L1-promoter methylation</b>			

Concentration		
Coverted DNA	50 ng	95°C (3 minutes)
H2O	to 25 ul	95°C (25 seconds)
MgCl <sub>2</sub>	3mM	40 cycles 50°C (25 seconds)
dNTPs	0.2 mM	72°C (25 seconds)
Buffer	1x	72°C (5 minutes)
PF	0,4 uM	
PR	0,4 uM	
GoTaq	1.25U	
EvaGreen	2X	

**Table S4.** Mutation identified in the 41 BC patients.

Patient ID	Mutated Gene	Coding Mutation	Protein Mutation
1	PIK3CA	c.1035T>A	p.N345K
2	TP53	c.574C>T/743G>A	p.Q192*/p.R248Q
3	WT	/	/
4	PIK3CA	c.3140A>G	p.H1047R
5	PIK3CA	c.1637A>C	p.Q546P
6	PIK3CA	c.3140A>T	p.H1047L
7	AKT	c.49G>A	p.E17K
8	PIK3CA	c.1634A>G	p.E545G
9	PIK3CA	c.3140A>T	p.H1047L
10	TP53	c.818G>A	p.R273H
11	WT	/	/
12	TP53	c.586C>T	p.R196Ter
13	WT	/	/
14	AKT	c.49G>A	p.E17K
15	WT	/	/
16	WT	/	/
17	WT	/	/
18	TP53	c.578A>G	p.H193r
19	PIK3CA	c.1634A>G	p.E545G
20	WT	/	/
21	PIK3CA	c.3140A>G	p.H1047R
22	WT	/	/
23	TP53	c.711G>A	p.M237I
24	WT	/	/
25	WT	/	/
26	WT	/	/
27	WT	/	/
28	WT	/	/
29	CDH1	c.1774G>A	p.A542T
30	WT	/	/
31	WT	/	/
32	MAP2K4	c.946C>T	p.Q316*
33	TP53	c.637C>T	p.R213Ter
34	TP53	c.586C>T	p.R196Ter
35	TP53	c.584T>C	p.I195T
36	WT	/	/
37	TP53	c.742C>T	p.R248W
38	WT	/	/
39	TP53	c.742C>T/c.574C>T	p.Q192Terp.R248W

40	TP53	c.637C>T	p.R213Ter
41	TP53	c.637C>T	p.R213Ter

**Table S5.** L1 expression stratified by BC treatment ( $n = 207$ ).

	Relapse	No Relapse	
Chemotherapy alone ( $n = 50$ )	$n=29$	$n=21$	p-value
ORF1(RQ)	35.18	22.23	<b>0.03</b>
ORF2(RQ)	57.32	35.55	<b>0.03</b>
Chemo + Hormone therapy ( $n = 80$ )	$n = 38$	$n = 52$	p-value
ORF1(RQ)	16.84	11.58	0.9
ORF2(RQ)	23.21	16.18	0.9
Hormone therapy alone ( $n = 31$ )	$n=13$	$n=16$	p-value
ORF1(RQ)	7.9	10.74	0.4
ORF2(RQ)	13.86	17.74	0.9
Anti-HER2 therapy ( $n = 46$ )	$n = 25$	$n = 21$	p-value
ORF1(RQ)	23.71	11.19	0.2
ORF2(RQ)	33.59	28.19	0.5