

The high protein expression of FOXO3, but not that of FOXO1, is associated with markers of good prognosis.

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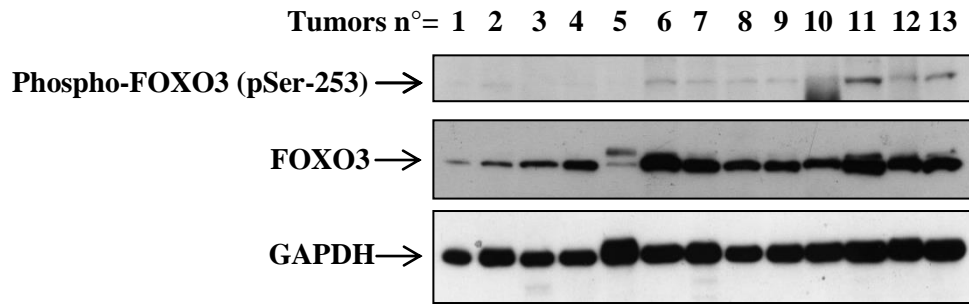


Figure S1: FOXO3 and Phospho-FOXO3 (ser-253) expressions in breast tumors. Breast tumor extracts were analysed by immunoblotting for their expressions of FOXO3, Phospho-FOXO3 (Ser-253), and GAPDH (loading control).

Table S1: Characteristics of the 218 breast tumours

	Number of patients (%)	Number with metastases (%)	<i>p</i> -value ^a
<i>Total</i>	218 (100)	100 (45.9)	
<i>Age</i>			
≤50	54 (24.8)	27 (50.0)	0.35 (NS)
>50	164 (75.2)	73 (44.5)	
<i>SBR histological grade</i> ^{b,c}			
I	22 (10.4)	7 (31.8)	0.22 (NS)
II	86 (40.8)	41 (47.7)	
III	103 (48.8)	51 (49.5)	
<i>Lymph node status</i> ^d			
0	69 (31.9)	20 (29.0)	< 0.0001
1-3	84 (38.9)	37 (44.0)	
>3	63 (29.2)	42 (66.7)	
<i>Macroscopic tumor size</i> ^e			
≤25mm	82 (38.5)	26 (31.7)	0.0009
>25mm	131 (61.5)	73 (55.7)	
<i>ERα status</i>			
Negative	89 (40.8)	42 (47.2)	0.34 (NS)
Positive	129 (59.2)	58 (45.0)	
<i>PR status</i>			
Negative	117 (53.7)	56 (47.9)	0.24 (NS)
Positive	101 (46.3)	44 (43.6)	
<i>ERBB2 status</i>			
Negative	156 (71.6)	68 (43.6)	0.18 (NS)
Positive	62 (28.4)	32 (51.6)	
<i>Molecular subtypes</i>			
HR- ERBB2-	44 (20.2)	17 (38.6)	0.12 (NS)
HR- ERBB2+	42 (19.3)	24 (57.1)	
HR+ ERBB2-	112 (51.4)	51 (45.5)	
HR+ ERBB2+	20 (9.2)	8 (40.0)	

NS: not significant.

^a Log-rank test.

^b Scarff Bloom Richardson classification.

^c Information available for 211 patients.

^d Information available for 216 patients.

^e Information available for 213 patients.

Table S2: mRNA expression levels of *FOXO1*, *FOXO3*, *FOXO4* and *FOXO6* in normal breast samples and in the series of 218 breast tumours

	n=	<i>FOXO1</i>	<i>FOXO3</i>	<i>FOXO4</i>	<i>FOXO6</i>
<i>Normals</i>					
Median (range)	15	1.0 (0.51-1.85)	1.0 (0.71-1.86)	1.0 (0.44-1.49)	1.0 (0.36-2.34)
Under-expressed (%) ^a		0 (0)	0 (0)	0 (0)	0 (0)
Over-expressed (%) ^a		0 (0)	0 (0)	0 (0)	0 (0)
<i>Total population</i>					
Median (range)	218	0.41 (0.04-1.36)	1.14 (0.31-7.88)	0.59 (0.14-3.32)	1.77 (0.19-23.1)
Under-expressed (%) ^a		73 (33.5)	1 (0.5)	30 (13.8)	4 (1.8)
Over-expressed (%) ^a		0 (0)	9 (4.1)	1 (0.5)	51 (23.4)
<i>p</i> -value ^b		< 0.0001	0.55 (NS)	0.0002	0.0004
<i>HR- ERBB2-</i>					
Median (range)	44	0.45 (0.13-1.36)	1.26 (0.49-6.59)	0.56 (0.14-3.32)	1.29 (0.19-7.74)
Under-expressed (%) ^a		14 (31.8)	0 (0)	11 (25.0)	4 (9.1)
Over-expressed (%) ^a		0 (0)	4 (9.1)	1 (2.3)	7 (15.9)
<i>p</i> -value ^b		< 0.0001	0.12 (NS)	0.0073	0.18 (NS)
<i>HR- ERBB2+</i>					
Median (range)	42	0.55 (0.07-1.28)	1.17 (0.41-4.67)	0.54 (0.15-2.90)	1.75 (0.47-11.1)
Under-expressed (%) ^a		11 (26.2)	0 (0)	7 (16.7)	0 (0)
Over-expressed (%) ^a		0 (0)	3 (7.1)	0 (0)	9 (21.4)
<i>p</i> -value ^b		0.0002	0.36 (NS)	0.0002	0.0005
<i>HR+ ERBB2-</i>					
Median (range)	112	0.38 (0.04-1.28)	1.01 (0.31-4.43)	0.58 (0.14-1.81)	1.82 (0.38-18.4)
Under-expressed (%) ^a		43 (38.4)	1 (0.9)	10 (8.9)	0 (0)
Over-expressed (%) ^a		0 (0)	1 (0.9)	0 (0)	28 (25.0)
<i>p</i> -value ^b		< 0.0001	0.67 (NS)	< 0.0001	< 0.0001
<i>HR+ ERBB2+</i>					
Median (range)	20	0.46 (0.18-1.14)	1.39 (0.78-7.88)	0.86 (0.20-2.45)	2.43 (0.62-23.1)
Under-expressed (%) ^a		5 (25.0)	0 (0)	2 (10.0)	0 (0)
Over-expressed (%) ^a		0 (0)	1 (5.0)	0 (0)	7 (35.0)
<i>p</i> -value ^b		0.0001	0.015	0.49 (NS)	0.0001

mRNA expression levels were normalized so that the median of values for normal breast tissues (n=15) was 1. ^a Over- and under-expressions were defined as threefold variations of expression relative to the median expression of normal samples. ^b Mann-Whitney test relative to normals.

Supplemental Table S3: Multivariate COX analysis of MFS for FOXO3 protein level in the series of 218 breast tumours

Characteristics		HR ^a	95% CI ^b	<i>p</i> -value ^c
<i>Lymph node status</i>	0	1		< 0.0001
	1-3	1.75	1.34-2.28	
	> 3	3.07	1.81-5.21	
<i>Macroscopic tumor size</i>	≤ 25mm	1		0.0037
	> 25mm	1.97	1.24-3.11	
<i>FOXO3 protein level</i>	Low	1		0.0055
	High	0.55	0.36-0.84	

^a Hazard ratio.

^b 95% Confidential Interval.

^c Multivariate COX analysis.

Supplemental Table S4: Antibodies used in this study

Antibody	From	Ref.
Phospho-S6K (Thr-421/Ser-424)	Upstate (Millipore)	04-393
Phospho-S6K (Thr-389)	Upstate (Millipoe)	04-392
Phospho-S6 Rib (Ser-235/236)	CST	2211
Phospho-S6 Rib (Ser-240/244)	CST	2215
AKT	CST	9272
PDK1	CST	5662
mTor	Abcam	Ab51089
S6 Rib	CST	2212
IRS1	Upstate (Millipoe)	06-248
Ku80	CST	2180
DNA.PK	Upstate (Millipoe)	05-907
PARP	Epitomics	1077-1
NBS1	CST	3002
RAD50	CST	3427
Mre11	CST	4847
p15	CST	4822
p53	CST	9282
Phospho-p53 (Ser-15)	CST	9286
Phospho-p53 (Ser-392)	Epitomics	2326-1
KI67	Dako	M7240
FOXO1	CST	2880
FOXO3	CST	9467