THE LANCET Oncology

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Luen SJ, Salgado R, Fox S, et al. Tumour-infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab or placebo in addition to trastuzumab and docetaxel: a retrospective analysis of the CLEOPATRA study. *Lancet Oncol* 2016; published online Dec 7. http://dx.doi.org/10.1016/S1470-2045(16)30631-3.

Supplementary appendix

Table S1. Proportions of primary versus metastatic tissue biopsies in fresh and archival specimens.

	Fresh	Archival	Chi squared test *P* value
Primary	144 (93%)	485 (93%)	0.96
Metastasis	11 (7%)	34 (7%)	

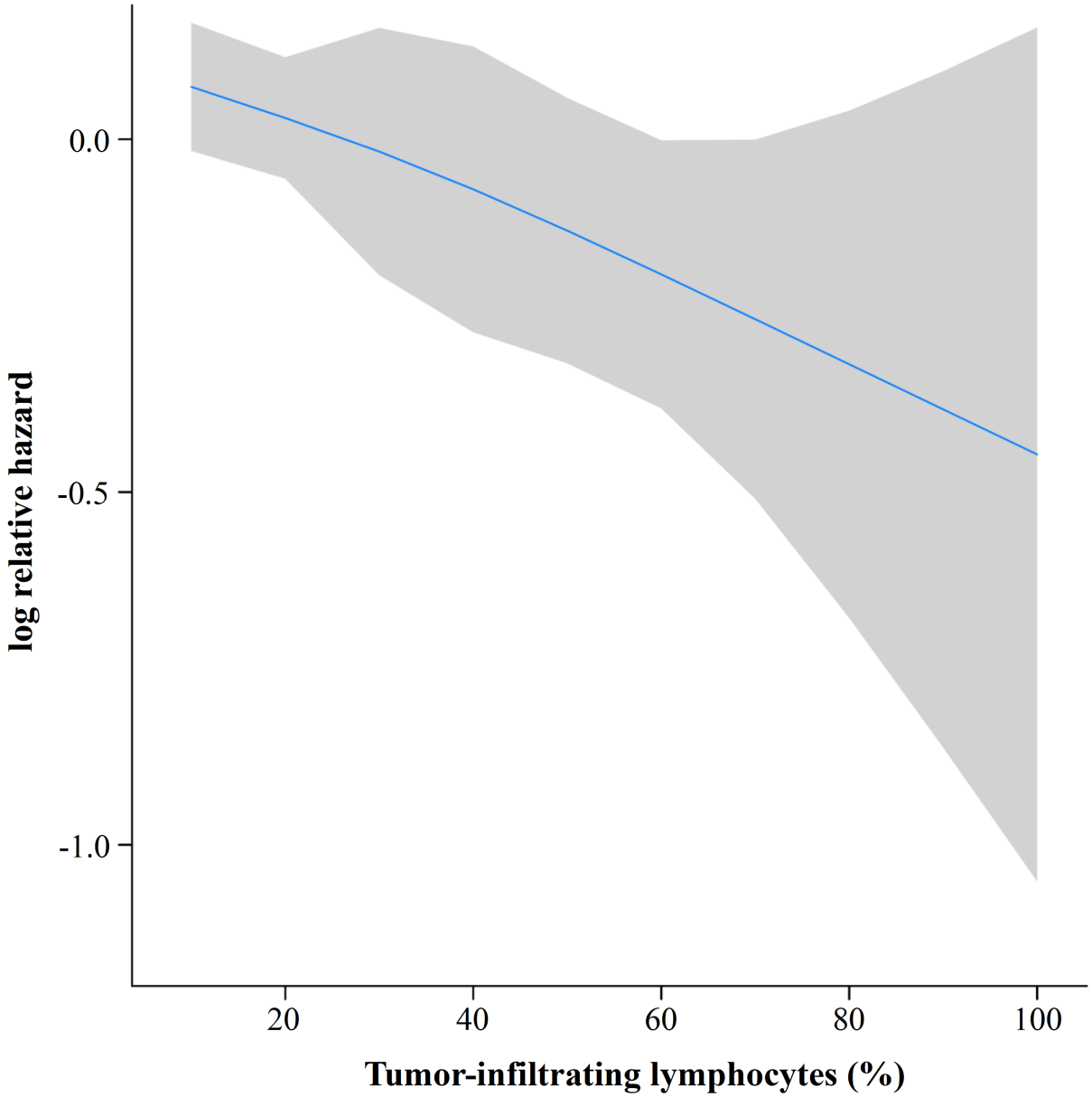
Table S2. Proportions of primary versus metastatic tissue biopsies and fresh and archival tissue specimens by ethnicity.

	White	Asian	Black	Chi squared test *P* value - asian vs white
Primary	361 (92%)	217 (94%)	21 (88%)	0.43
Metastasis	30 (8%)	13 (6%)	3 (13%)	
Fresh	86 (22%)	62 (27%)	2 (8%)	0.22
Archival	301 (78%)	168 (73%)	22 (92%)	

Table S3. Proportions of fresh versus archival tissue biopsies and ER positive versus ER negative by *PIK3CA* genotype.

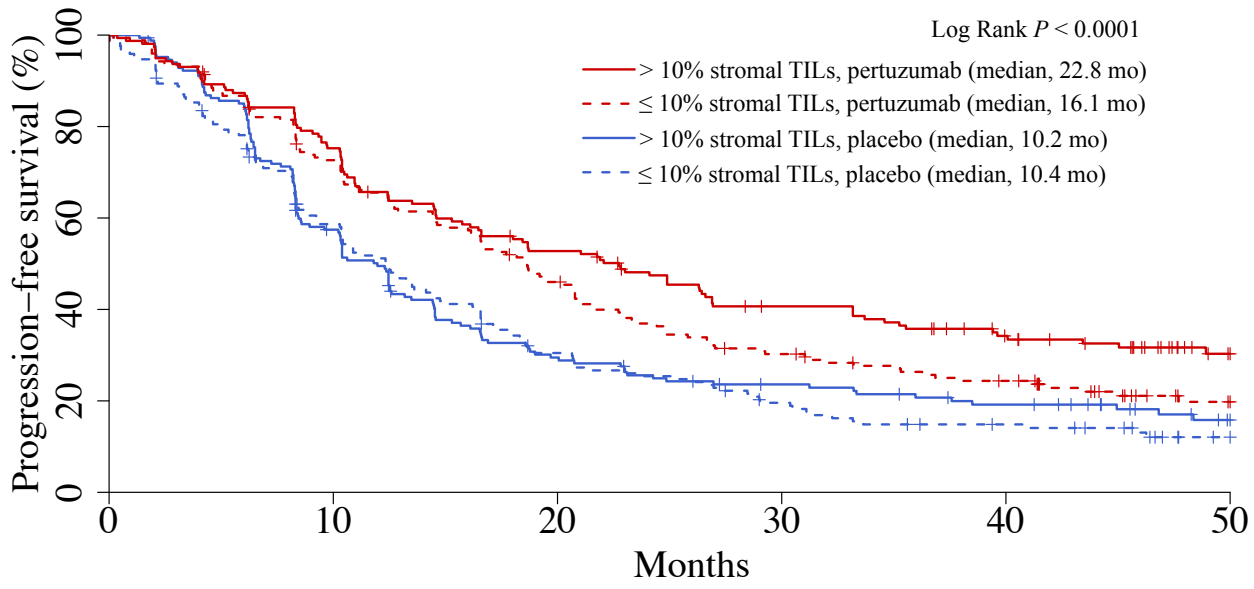
	PIK3CA mutated	*PIK3CA* wild type	Chi squared test *P* value
Fresh	35 (24%)	88 (28%)	0.46
Archival	111 (76%)	229 (72%)	
ER positive	71 (48%)	161 (51%)	0.67
ER negative	74 (50%)	155 (49%)	

Figure S1. Log relative hazard for death verus stromal TIL level



Plot demonstrating log-relative hazard for death (5 year overall survival) versus stromal TIL level (by 10% increment) after fitting a cubic smoothing spline, to determine if stromal TIL levels as a continuous meausre are linearly related to prognosis.

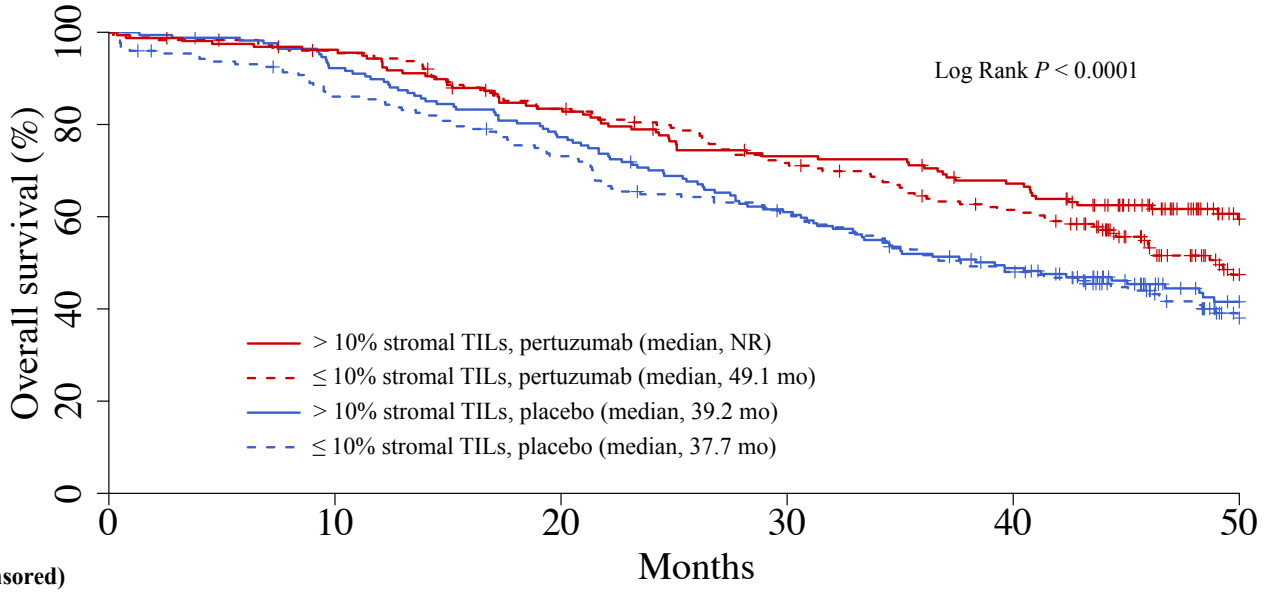
Figure S4. Progression-free survival by median stromal TILs cut-off and treatment arm



No. at risk (censored)						
> 10% TILs, pertuzumab	159 (0)	118 (2)	81 (4)	58 (9)	43 (15)	20 (34)
≤ 10% TILs, pertuzumab	177 (0)	123 (7)	77 (8)	49 (10)	36 (14)	14 (31)
> 10% TILs, placebo	168 (0)	94 (3)	46 (6)	33 (10)	25 (12)	11 (23)
≤ 10% TILs, placebo	174 (0)	94 (11)	48 (12)	29 (14)	19 (17)	6 (27)

Survival analysis showing Kaplan-Meier estimates for progression-free survival using the treatment arm and median stromal TIL level as a cut-off value for illustration. All analyses were performed in the intention-to-treat population of the TILs-evaluable cohort. The tick marks indicate censoring events. Abbreviations: TIL, tumour-infiltrating lymphocytes; mo, months.

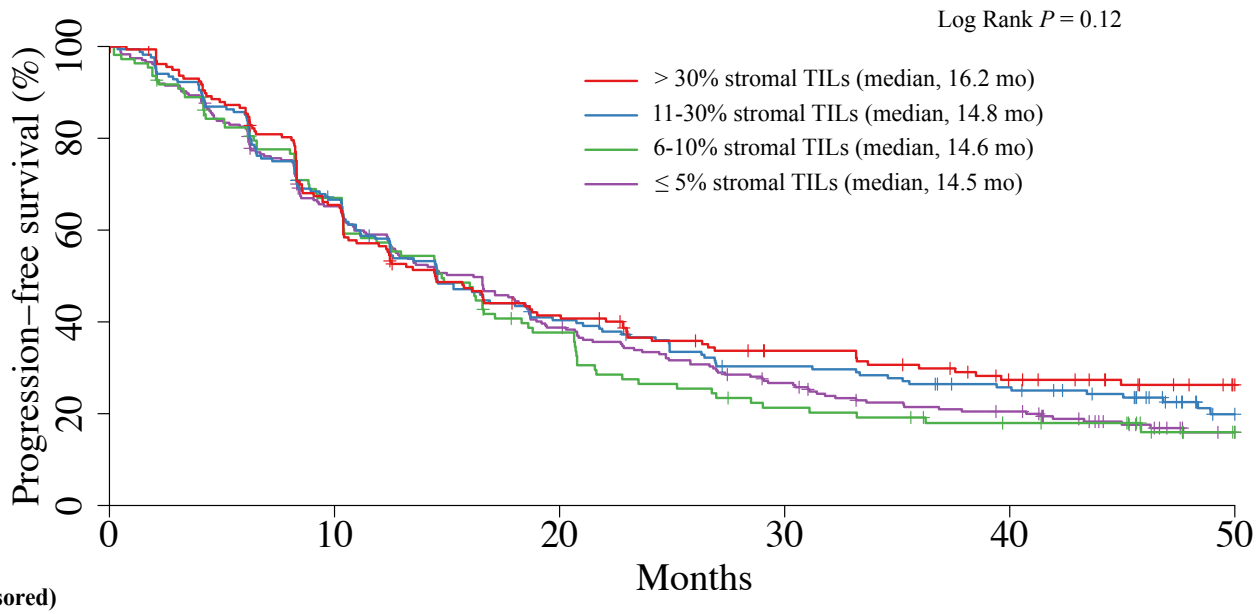
Figure S5. Overall survival by median stromal TIL TILs cut-off and treatment arm



No. at risk (censored)						
> 10% TILs, pertuzumab	159 (0)	151 (2)	130 (3)	112 (5)	101 (7)	52 (46)
≤ 10% TILs, pertuzumab	177 (0)	167 (2)	144 (4)	122 (6)	101 (10)	42 (51)
> 10% TILs, placebo	168 (0)	154 (1)	129 (1)	101 (2)	78 (5)	42 (32)
≤ 10% TILs, placebo	174 (0)	147 (3)	124 (4)	101 (6)	79 (7)	36 (37)

Survival analysis showing Kaplan-Meier estimates for overall survival using the treatment arm and median stromal TIL level as a cut-off value for illustration. All analyses were performed in the intention-to-treat population of the TILs-evaluable cohort. The tick marks indicate censoring events. Abbreviations: TIL, tumour-infiltrating lymphocytes; mo, months; NR, not reached.

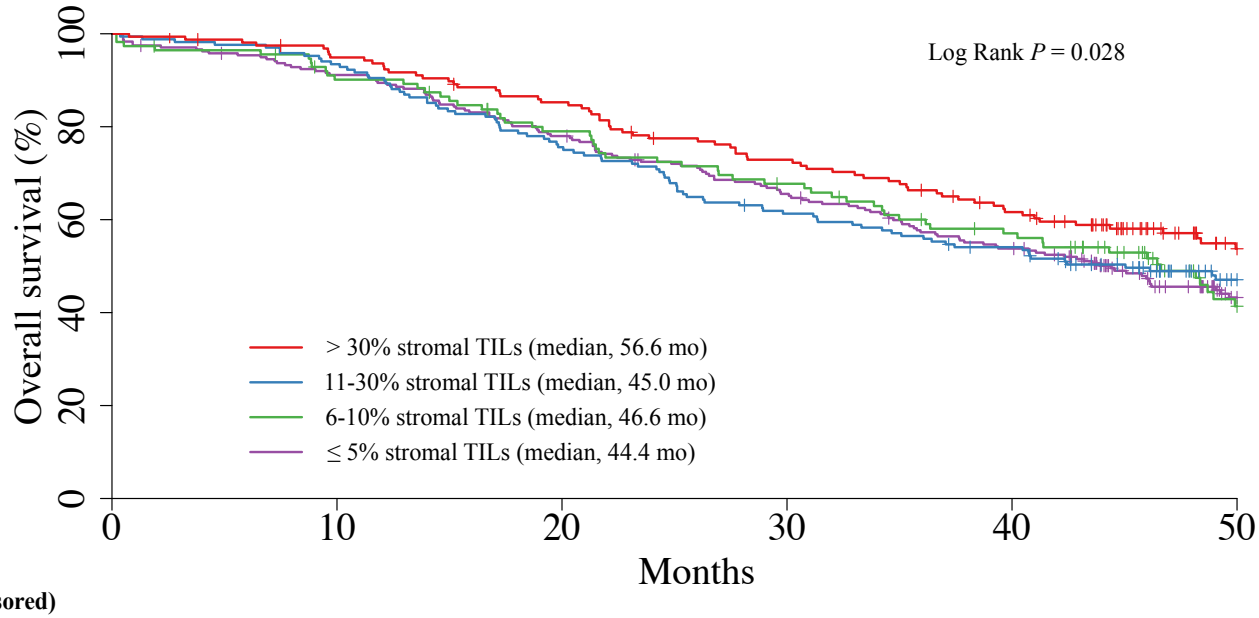
Figure S6. Progression-free survival by stromal TIL quartiles



No. at risk (censored)						
Fourth quartile	159 (0)	102 (3)	62 (6)	44 (13)	31 (18)	17 (31)
Third quartile	168 (0)	110 (2)	65 (4)	47 (6)	37 (9)	14 (26)
Second quartile	113 (0)	69 (9)	37 (11)	20 (12)	14 (15)	5 (23)
First quartile	238 (0)	148 (9)	88 (9)	58 (12)	41 (16)	15 (35)

Survival analysis showing Kaplan-Meier estimates for progression-free survival using stromal TIL population quartiles as cut-off values for illustration. All analyses were performed in the intention-to-treat population of the TILs-evaluable cohort. The tick marks indicate censoring events. Log Rank *P* value calculated comparing the first quartile with fourth quartile. Abbreviations: TIL, tumour-infiltrating lymphocytes; mo, months.

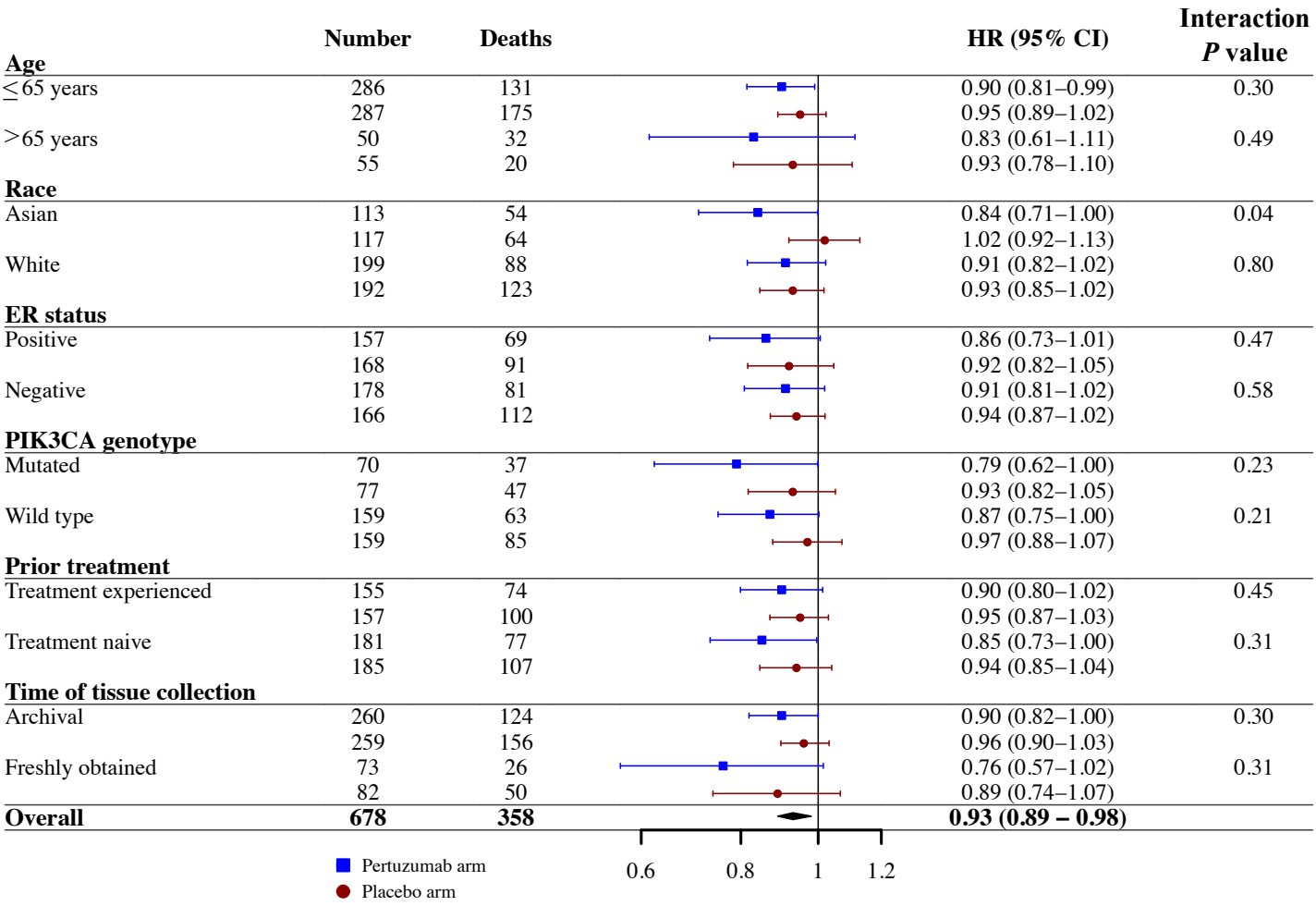
Figure S7. Overall survival by stromal TIL quartiles



No. at risk (censored)						
Fourth quartile	159 (0)	148 (3)	132 (4)	111 (6)	91 (9)	46 (45)
Third quartile	168 (0)	157 (0)	127 (0)	102 (1)	88 (3)	48 (33)
Second quartile	113 (0)	99 (3)	84 (6)	71 (7)	57 (10)	27 (28)
First quartile	238 (0)	215 (2)	184 (2)	152 (5)	123 (7)	51 (60)

Survival analysis showing Kaplan-Meier estimates for overall survival using stromal TIL population quartiles as cut-off values for illustration. All analyses were performed in the intention-to-treat population of the TILs-evaluable cohort. The tick marks indicate censoring events. Log Rank *P* value calculated comparing the first quartile with fourth quartile. Abbreviations: TIL, tumour-infiltrating lymphocytes; mo, months.

Figure S8. Subgroup analysis for overall survival



Subgroup analysis showing hazard ratios (per 10% stromal tumour-infiltrating lymphocyte increment) and 95% confidence intervals for overall survival by specified subgroups and stratified by treatment arm. Treatment interaction for each subgroup is shown. Treatment naive is defined as no prior treatment with chemotherapy or trastuzumab (endocrine therapy was allowed), and treatment experienced is defined as prior (neo)adjuvant chemotherapy and/or trastuzumab as described in the methods secion. Definitions for freshly obtained tissue and archival tissue are described in the methods section. Abbreviations: HR, hazard ratio; 95% CI, 95% confidence intervals; ER, estrogen receptor.