Supplemental Figures and Tables

Figure S1. Tamoxifen sensitivity in breast cancer cell lines. Indicated cell lines were treated with different concentrations of tamoxifen for 72 hours and then were assayed for cell viability.

Figure S2. Knockdown of Aurora-A sensitizes cells to tamoxifen in MCF7-TamR cells. The cells were transfected with two siRNAs of Aurora-A and control siRNA. Following 72 hours of incubation, cells were immunoblotted with indicated antibodies (**A**). The control siRNA and Aurora-A-knockdown MCF7-TamR cells were treated with and without tamoxifen for 5 days and then assessed apoptosis (**B**), and focus formation (**C**) as described in the "Materials and Methods". The asterisks denote significance (*$P <$ 0.05 and ** $P < 0.01$).

Figure S3. Aurora-A has no effects on tamoxifen sensitivity in ERα-negative cells. MDA-MB-468 cells were transfected with 2 Aurora-A siRNAs and control siRNA (**A**) whereas MDA-MB-231 cells were introduced with HA-Aurora-A and vector control (**D**) . After 72 hours of incubation, cells were treated with different concentration of tamoxifen and then were assayed for cell survival (**B**, **E**) and focus formation (**C**, **F**).

Figure S4. Aurora-A inhibitor MLN8237 synergizes with tamoxifen. Tamoxifen resistant BT474 and MCF7-TamR cells were seeded in 96-well plate and treated with different concentrations of MLN8237 and tamoxifen for 72 hours. The cell viability was

evaluated by MTT assay (**A** and **B**). The effects of drug combinations were evaluated with Calcusyn software (Biosoft). CI analysis to determine synergy (defined as CI values < 1) was carried out using Calcusyn software as described in Methods (**C** and **D**). The curves were generated by Calcusyn software to fit the experimental points. The effect ranges from 0 (no inhibition) to 1 (complete inhibition). Each data point is the average of 4 wells each from 3 independent experiments. Error bars indicate standard error.

Figure S5. Orthotopic breast tumours from MCF7 cells are sensitive to tamoxifen. MCF7 cells (5×10^6) were injected into mammary fat pad of nude mice. When tumour reached $100mm^3$, mice were treated with tamoxifen and vehicle as described in "Materials and Methods". The tumour growth (**A**) and tumour weight (**B**) were examined.

Figure S6. Aurora-A increases E2-stimulated ERE reporter activity but Aurora-A-induced reporter activation could not be inhibited by tamoxifen. (A and **B)** MCF7 cells were transfected and treated with indicated plasmids and agents and then were subjected to luciferase assay.

Figure S7. Aurora-A phosphorylates ERα. (**A**) GST-fused truncation mutations of ERα and location of three putative Aurora-A phosphorylation residues of ERα. (**B** and **C**) *In vitro* Aurora-A kinase was carried out by incubation of recombinant Aurora-A with indicated GST-ERα fusion proteins (top panel). Panel 2 shows GST-ERα proteins and panel 3 is recombinant Aurora-A used for *in vitro* kinase assay. (**D**) Cold *in vitro* Aurora-A kinase. Recombinant ERα was incubated with and without Aurora-A and then

immunoblotted with phospho-ERα-Ser167 and –Ser305 antibodies (panel 1 and 2). Panels 3 and 4 are Western blot showing ERα and Aurora-A used for *in vitro* kinase.

Figure S8. Aurora-A inhibitor MLN8237 inhibits p-ERα-Ser167 and -Ser-305. Tamoxifen resistant BT474 (**A**) MCF7-TamR (**B**) cells were treated with MLN8237 and then immunoblotted with indicated antibodies. (**C**) Western blot analysis of orthotopic tumours, which were treated with and without MLN8237, with indicated antibodies.

Figure S9. Aurora-A does not induce CCND1 expression in ERα-negative cells. MDA-MB-231 cells were transfected with Aurora-A or pHM6 vector alone. After 72 hours of incubation, cells were subjected to semi-quantitative RT-PCR (top 1 and 2 panels) and Western blot (panels 3-5) analyses.

Figure S10. Aurora-A is positively correlated with CCND1 levels in ERα-positive breast tumours. The expression of Aurora-A and CCND1 was analyzed in 2 independent datasets. CCND1 levels were significantly correlated with Aurora-A expression in ERα-positive (left panels) but not ERα-negative tumours (right panels).

Figure S11. Relationship of p-ERα-Ser167 or/and p-ERα-Ser305 with disease-free survival. Kaplan-Meier curves revealed that phosphorylation of both Ser167 and Ser305 (**A**), especially co-occurrence with elevated Aurora-A (**D**) was significantly associated with short DFS. The p-ERα-Ser167 (**B)** but not p-ERα-Ser305 (**C**) alone was also associated with DFS.

Figure S12. Analysis for the Aurora-A expression levels with the recurrence in ERα-positive breast cancers. The analysis was conducted on a panel of 4 independent datasets summing-up more than ERα-positive 854 tumours (note: 3 of these datasets are also included in Kaplan-Meier Plotter database). Aurora-A expression values were used to separate tumour samples into "high" and "low" groups (see Method). Kaplan-Meier graphs represent the probability of cumulative recurrence-free survival in breast cancer datasets. The p-value of the log-rank test reflects a significant association between Aurora-A high and shorter survival.

Figure S13. The Receiver Operating Characteristic (ROC) curves for Aurora-A expression for prediction of recurrence from endocrine therapy. The curve describes the association between Aurora-A expression and the recurrence from endocrine therapy in ERα-positive breast cancers in 4 databases. Area under the curves (AUC) for *Aurora-A* range from 0.666 to 0.806.

Figure S14. Aurora-A is associated with disease-free survival in ERα-positive but not ERα-negative and basal-like breast cancers. Kaplan-Meier curves show the recurrence free-survival in breast cancer patients based on Aurora-A expression.

Figure S15. Co-localization of Aurora-A with p-ERα-Ser167 and -Ser-305. (**A**) MCF7-TamR cells were immunostained with anti-Aurora-A (green; *b* and *f*) and -p-ERα-Ser167 and -Ser-305 (red; *c* and *g*) antibodies, and counterstained with DAPI (blue; *a* and

e). The merged pictures (green, red and blue) were shown as *d* and *h*. The magnified images of the indicated areas in panels i-iii are shown at the right side; Aurora-A: top panel (green), p-ERα: middle panel (red), overlay: bottom panel. (**B**) MCF10A cells were transfected with GFP-ERα (*a*) and Aurora-A, and then were immunostained with antibody against γ-tubulin (*b*). Panel *c* is counterstained with DAPI and panel *d* shows the merged image.

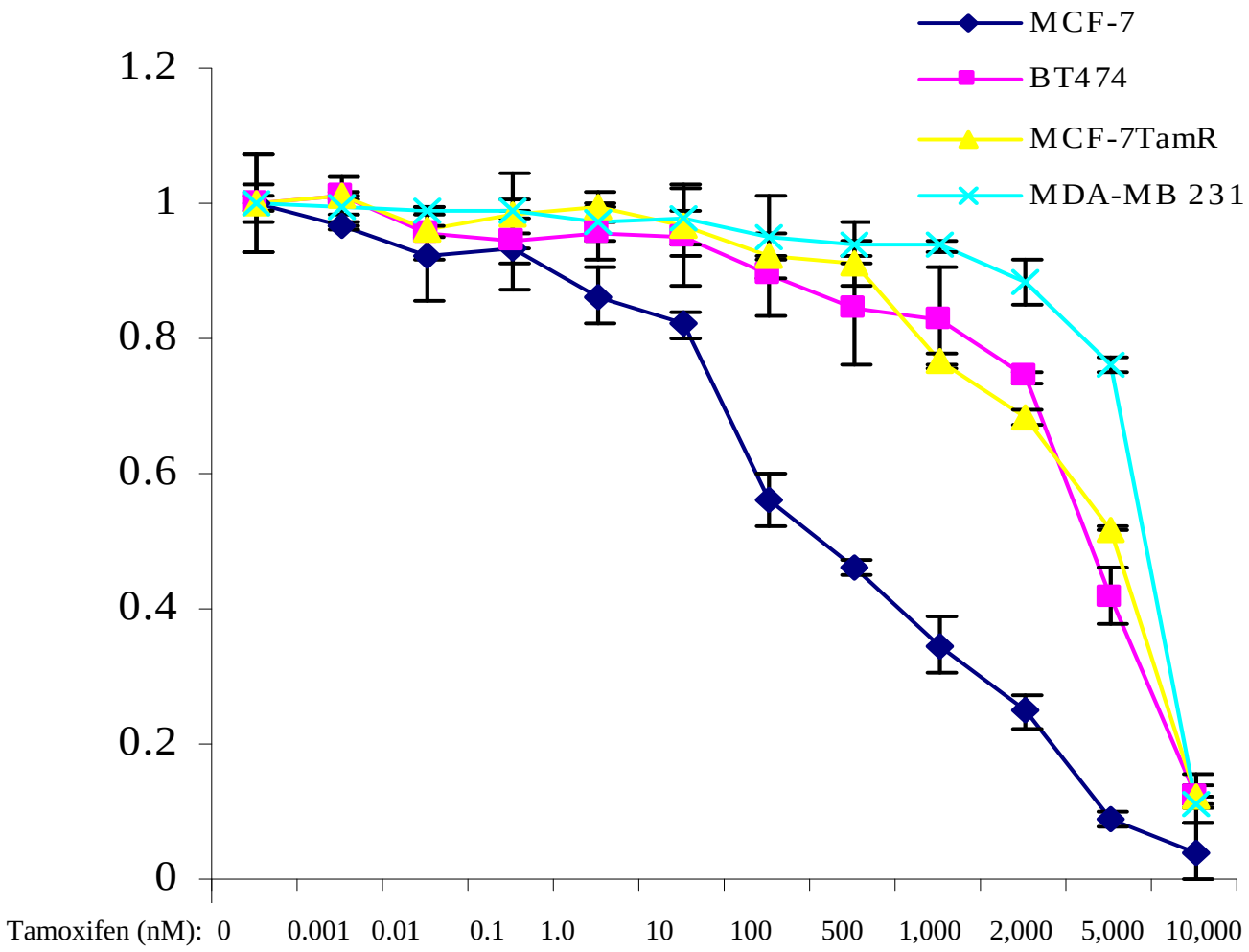

MCF-7
BT474
MCF-7TamR
MDA-MB 231
1.2
1
0.8
0.6
0.4
0.2
0
Tamoxifen (nM): 0 0.001 0.01 0.1 1.0 10 100 500 1,000 2,000 5,000 10,000

Figure S1

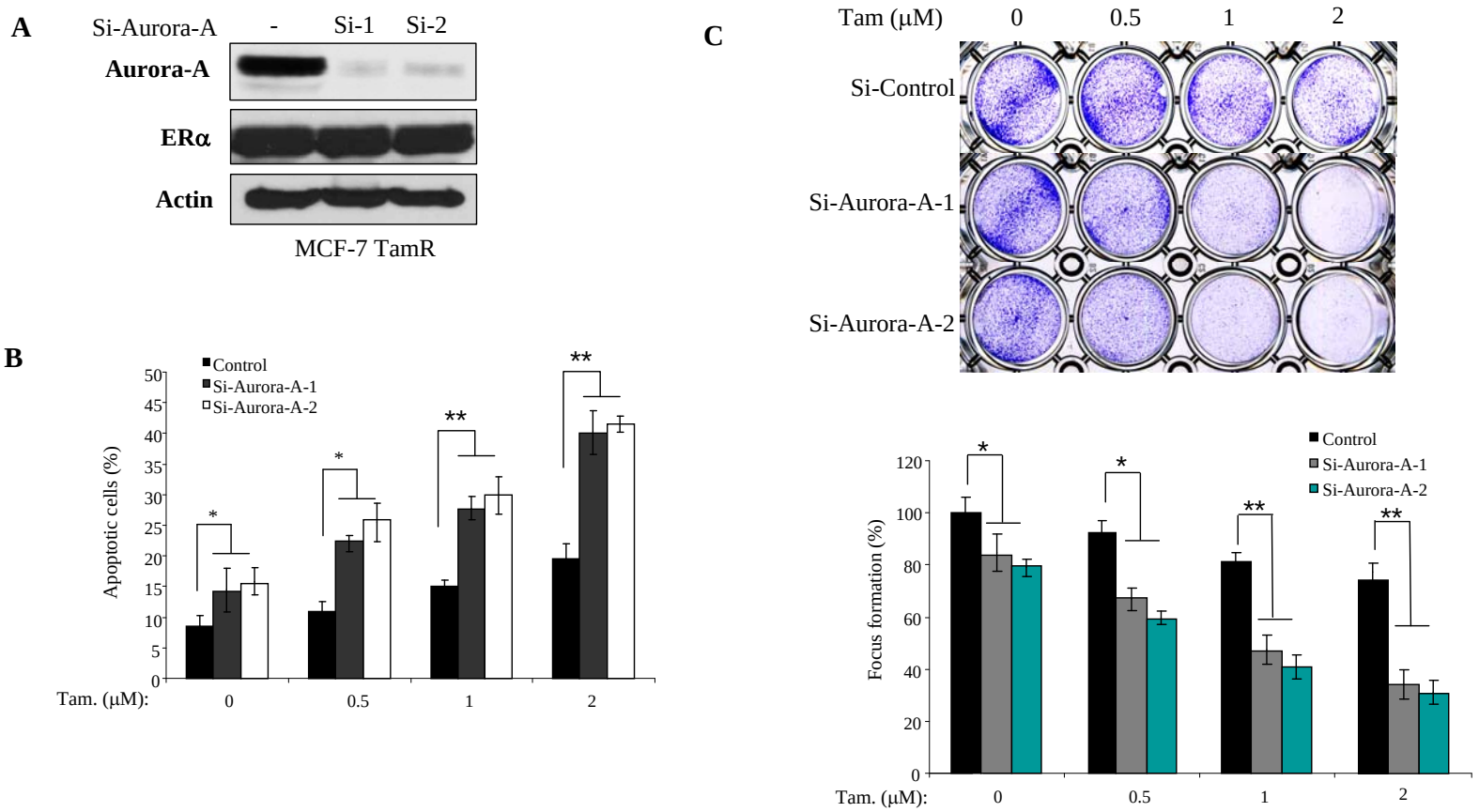

A
Si-Aurora-A
-
Si-1
Si-2
Aurora-A
ERα
Actin
MCF-7 TamR
B
Control
Si-Aurora-A-1
Si-Aurora-A-2
Apoptotic cells (%)
Tam. (μM):
0
0.5
1
2
C
Tam (μM)
0
0.5
1
2
Si-Control
Si-Aurora-A-1
Si-Aurora-A-2
Focus formation (%)
Control
Si-Aurora-A-1
Si-Aurora-A-2
Tam. (μM):

Figure S2

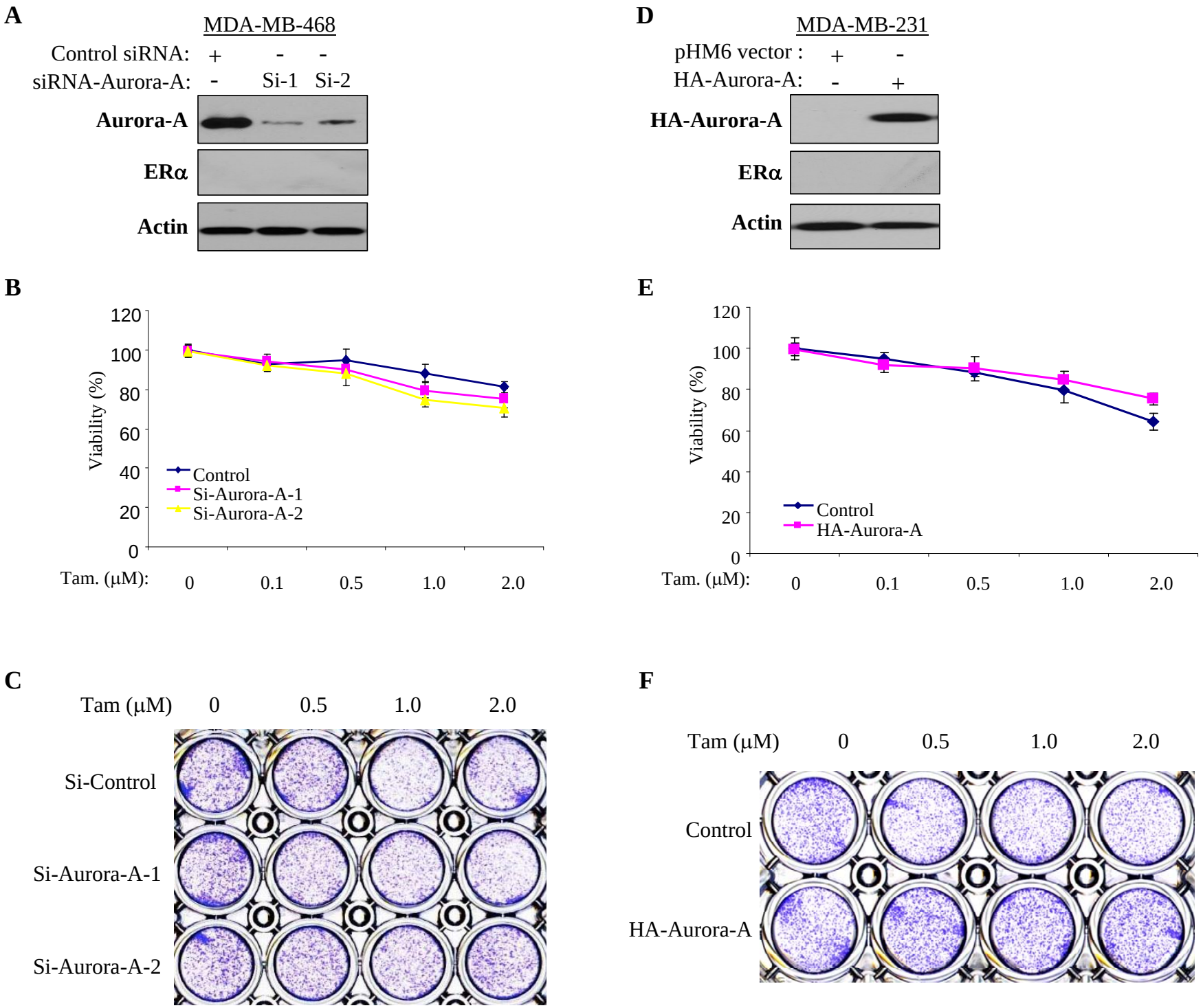

A
MDA-MB-468
Control siRNA: + - -
siRNA-Aurora-A: - Si-1 Si-2
Aurora-A
ERα
Actin
D
MDA-MB-231
pHM6 vector : + -
HA-Aurora-A: - +
HA-Aurora-A
ERα
Actin
B
Viability (%)
120
100
80
60
40
20
0
Control
Si-Aurora-A-1
Si-Aurora-A-2
Tam. (μM): 0 0.1 0.5 1.0 2.0
E
Viability (%)
120
100
80
60
40
20
0
Control
HA-Aurora-A
Tam. (μM): 0 0.1 0.5 1.0 2.0
C
Tam (μM) 0 0.5 1.0 2.0
Si-Control
Si-Aurora-A-1
Si-Aurora-A-2
F
Tam (μM) 0 0.5 1.0 2.0
Control
HA-Aurora-A

Figure S3

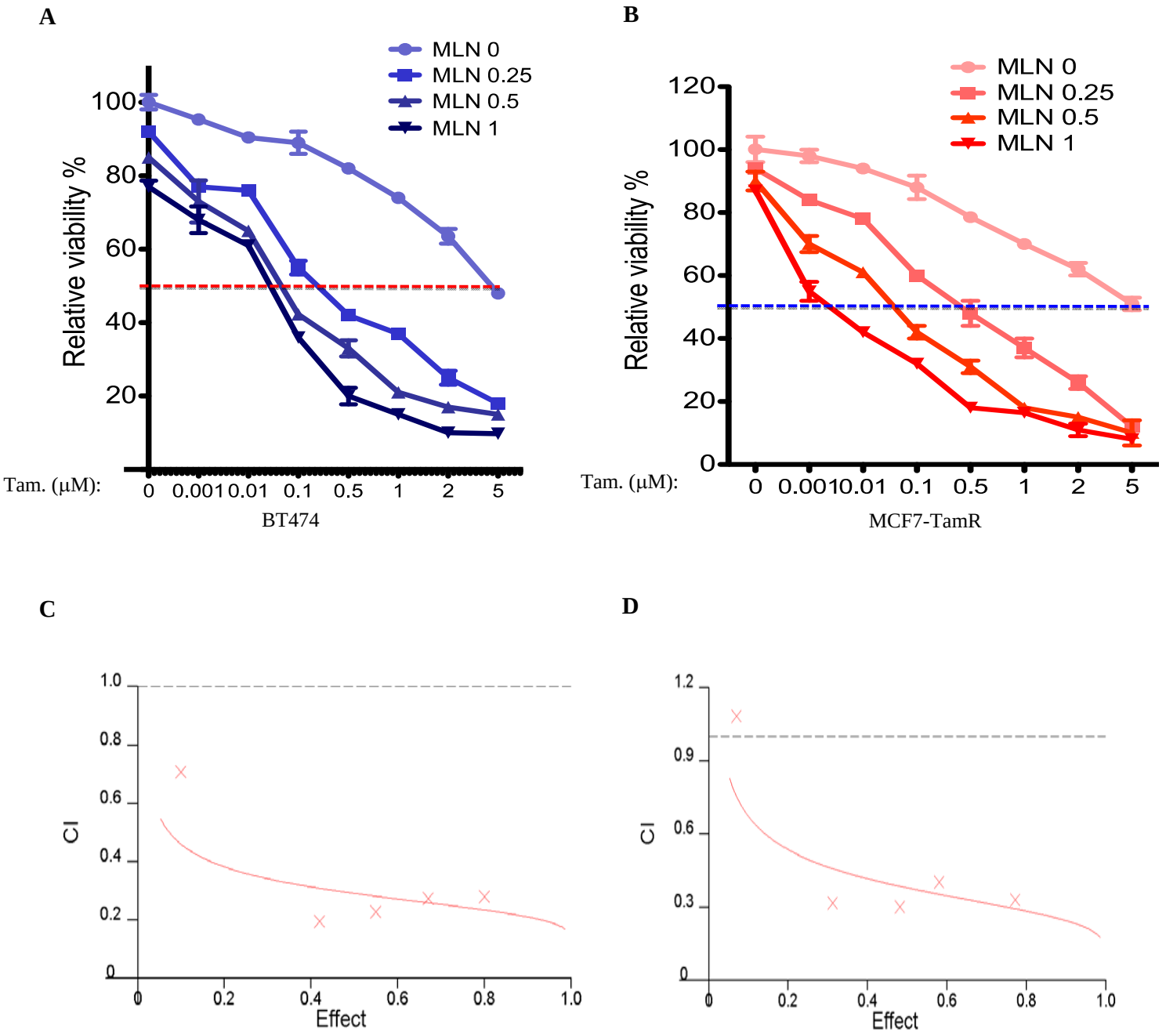

A
MLN 0
MLN 0.25
MLN 0.5
MLN 1
Relative viability %
100
80
60
40
20
Tam. (μM):
0 0.001 0.01 0.1 0.5 1 2 5
BT474
B
MLN 0
MLN 0.25
MLN 0.5
MLN 1
Relative viability %
120
100
80
60
40
20
0
Tam. (μM):
0 0.001 0.01 0.1 0.5 1 2 5
MCF7-TamR
C
CI
1.0
0.8
0.6
0.4
0.2
0
0 0.2 0.4 0.6 0.8 1.0
Effect
D
CI
1.2
0.9
0.6
0.3
0
0 0.2 0.4 0.6 0.8 1.0
Effect

Figure S4

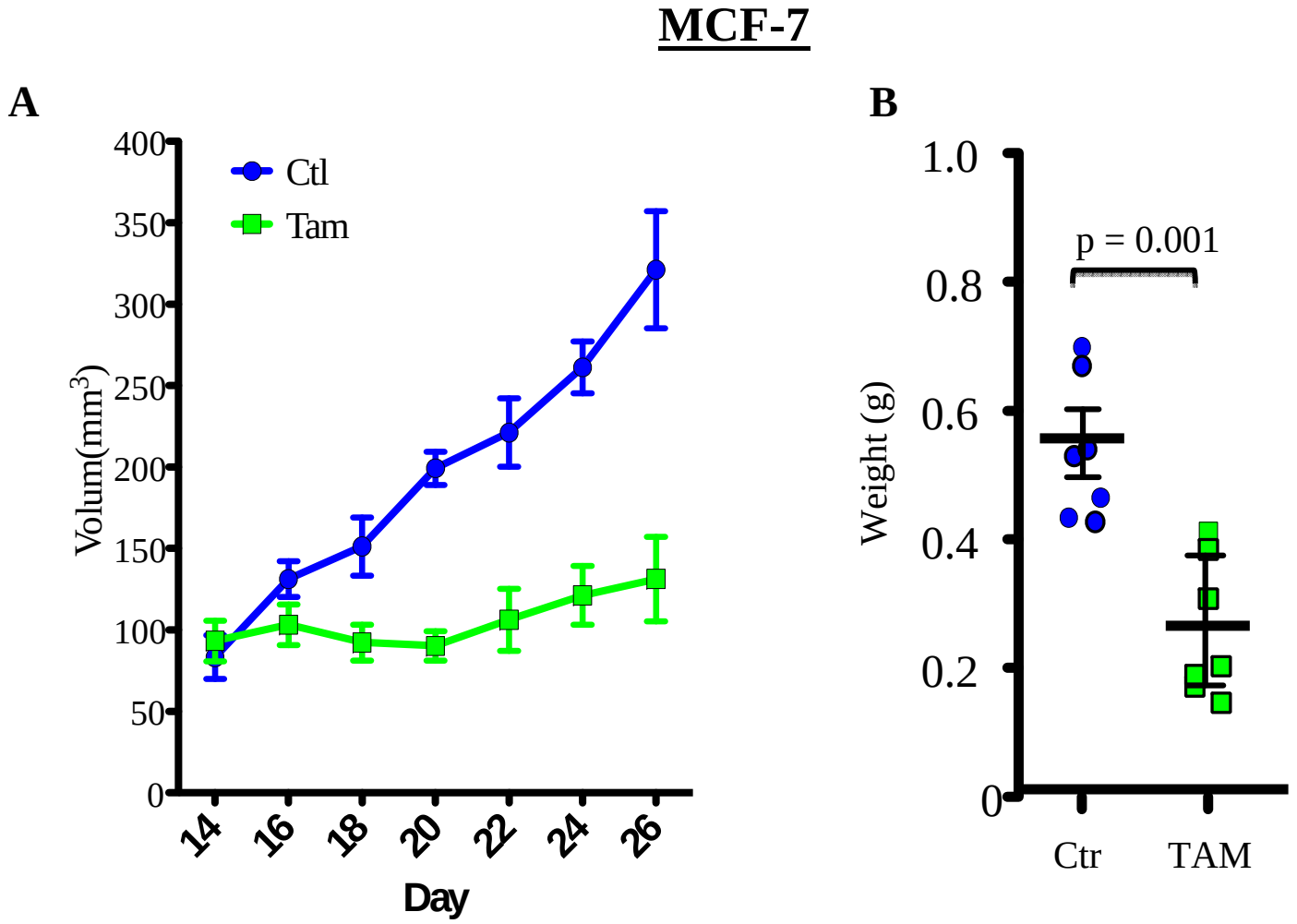


Figure S5

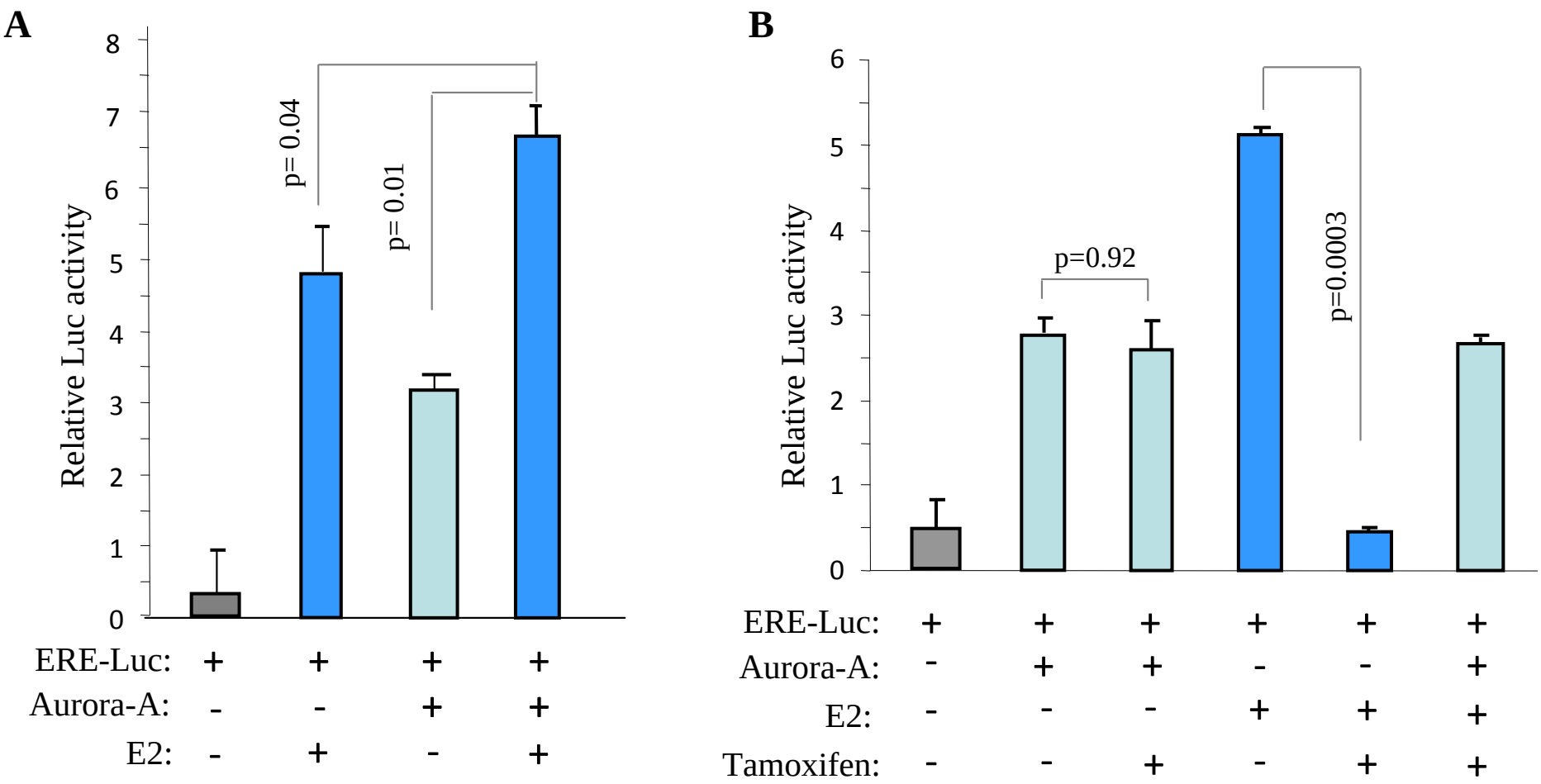

A
Relative Luc activity
0
1
2
3
4
5
6
7
8
p= 0.04
p= 0.01
ERE-Luc: + + + +
Aurora-A: - - + +
E2: - + - +
B
Relative Luc activity
0
1
2
3
4
5
6
p=0.92
p=0.0003
ERE-Luc: + + + + + +
Aurora-A: - + + - - +
E2: - - - + + +
Tamoxifen: - - + - + +

Figure S6

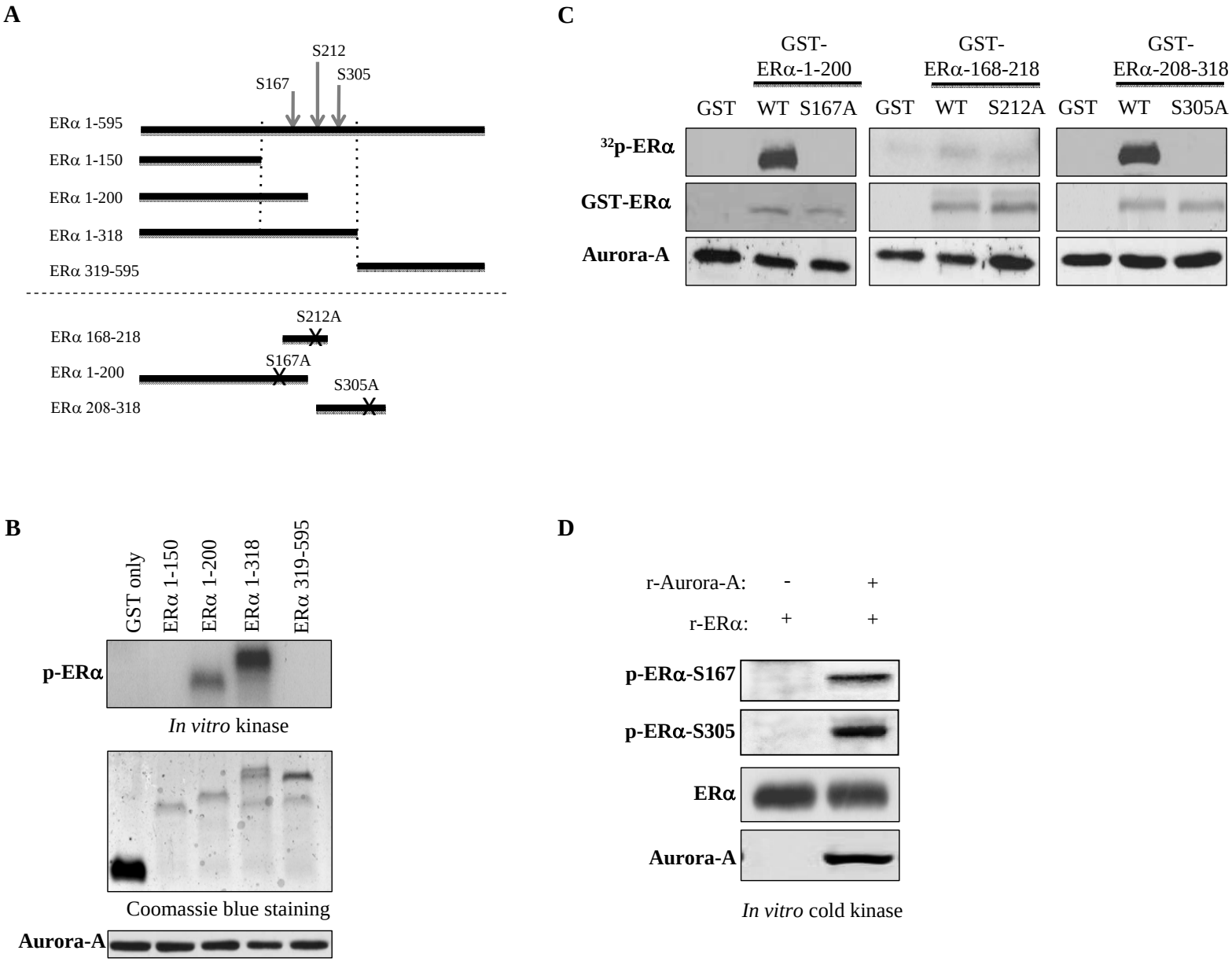

A
S212
S167
S305
ERα 1-595
ERα 1-150
ERα 1-200
ERα 1-318
ERα 319-595
S212A
ERα 168-218
S167A
ERα 1-200
S305A
ERα 208-318
B
GST only
ERα 1-150
ERα 1-200
ERα 1-318
ERα 319-595
p-ERα
In vitro kinase
Coomassie blue staining
Aurora-A
C
GST-ERα-1-200
GST-ERα-168-218
GST-ERα-208-318
GST WT S167A GST WT S212A GST WT S305A
32p-ERα
GST-ERα
Aurora-A
D
r-Aurora-A: - +
r-ERα: + +
p-ERα-S167
p-ERα-S305
ERα
Aurora-A
In vitro cold kinase

Figure S7

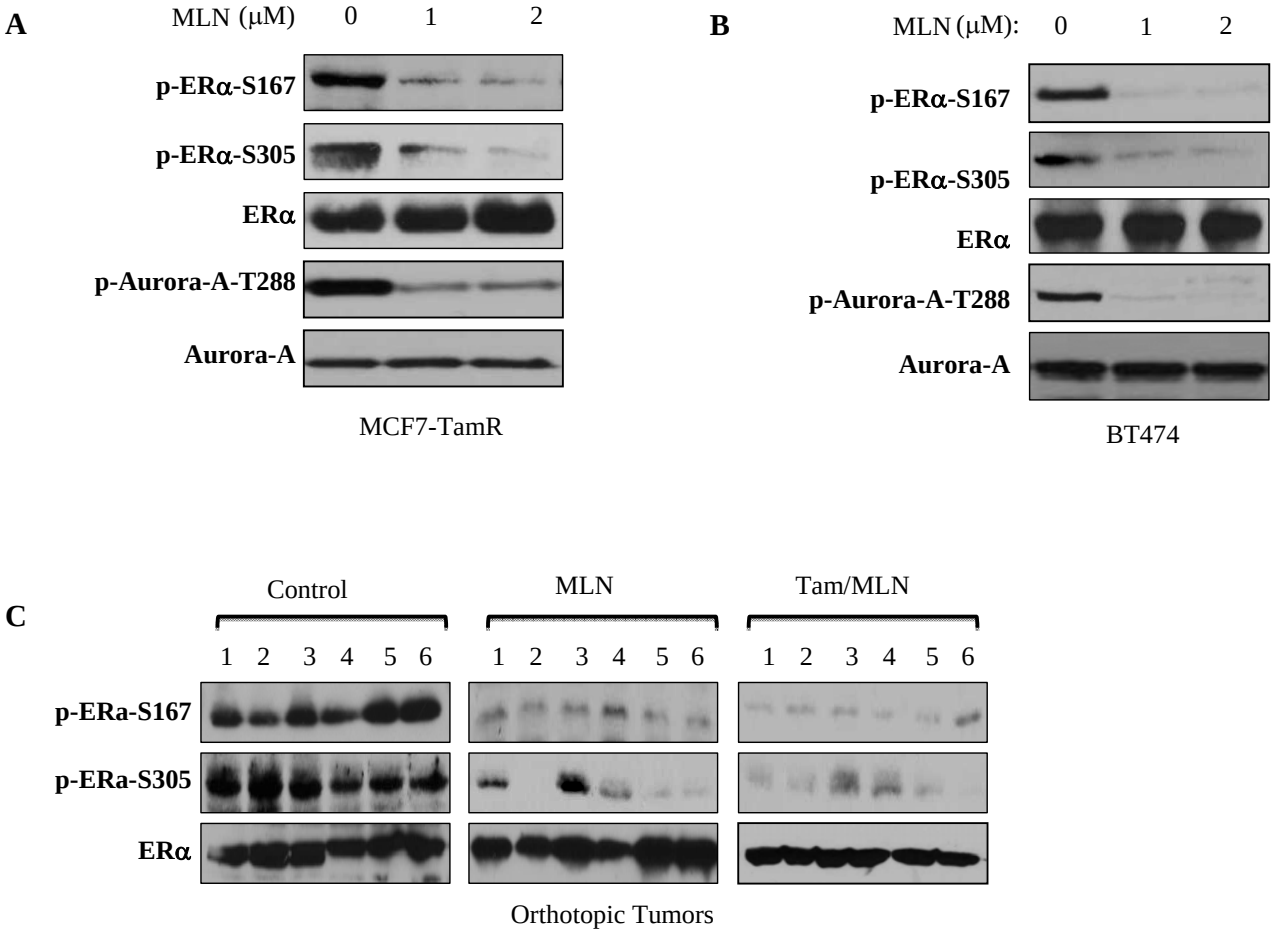

A
MLN (μM) 0 1 2
p-ERα-S167
p-ERα-S305
ERα
p-Aurora-A-T288
Aurora-A
MCF7-TamR
B
MLN (μM): 0 1 2
p-ERα-S167
p-ERα-S305
ERα
p-Aurora-A-T288
Aurora-A
BT474
C
Control
MLN
Tam/MLN
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
p-ERa-S167
p-ERa-S305
ERα
Orthotopic Tumors

Figure S8

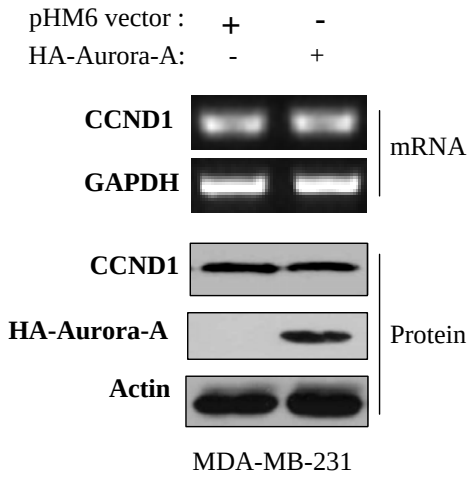

pHM6 vector : + -
HA-Aurora-A: - +
CCND1
GAPDH
mRNA
CCND1
HA-Aurora-A
Actin
Protein
MDA-MB-231

Figure S9

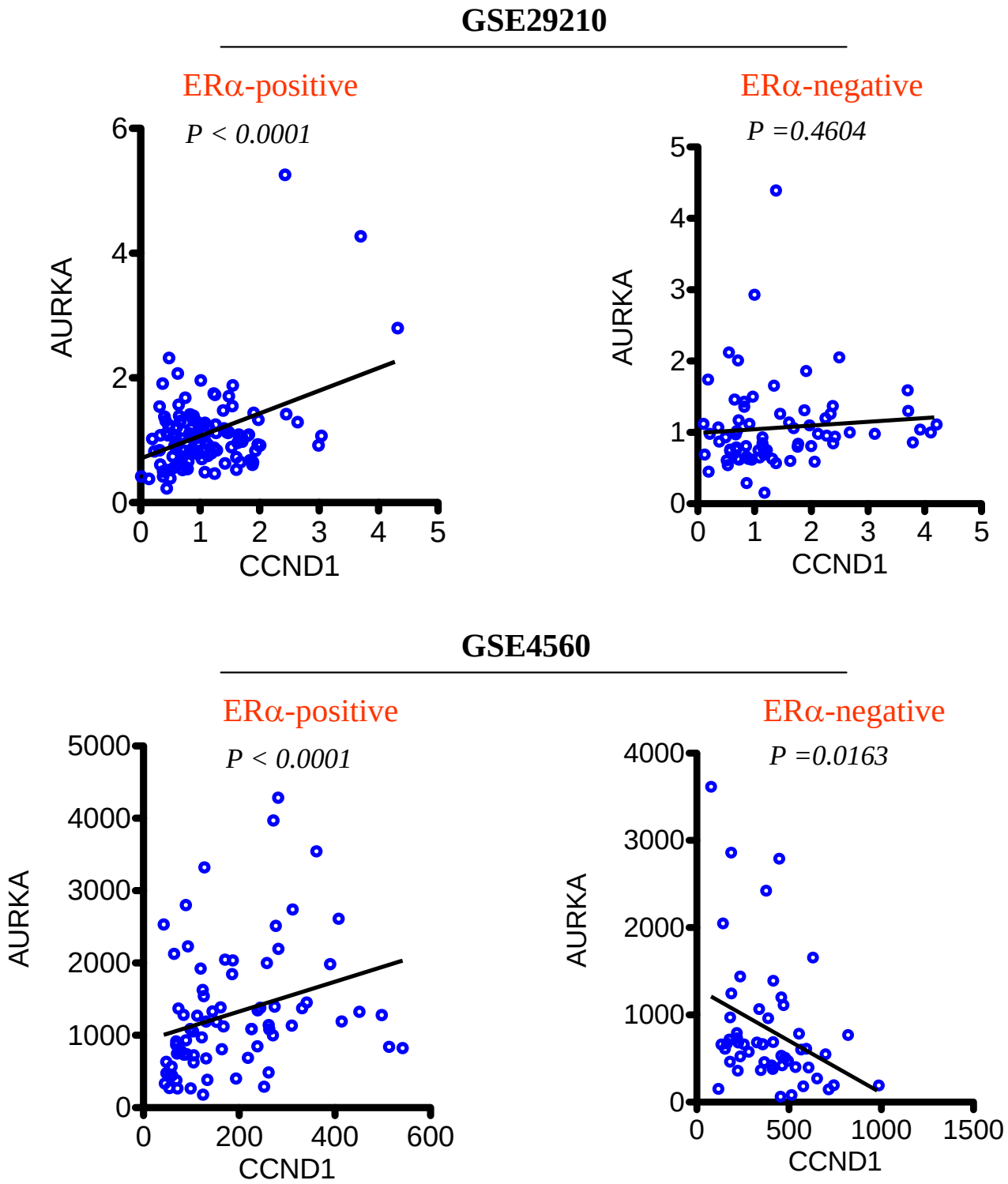

GSE29210
ERα-positive
P < 0.0001
AURKA
CCND1
ERα-negative
P =0.4604
AURKA
CCND1
GSE4560
ERα-positive
P < 0.0001
AURKA
CCND1
ERα-negative
P =0.0163
AURKA
CCND1

Figure S10

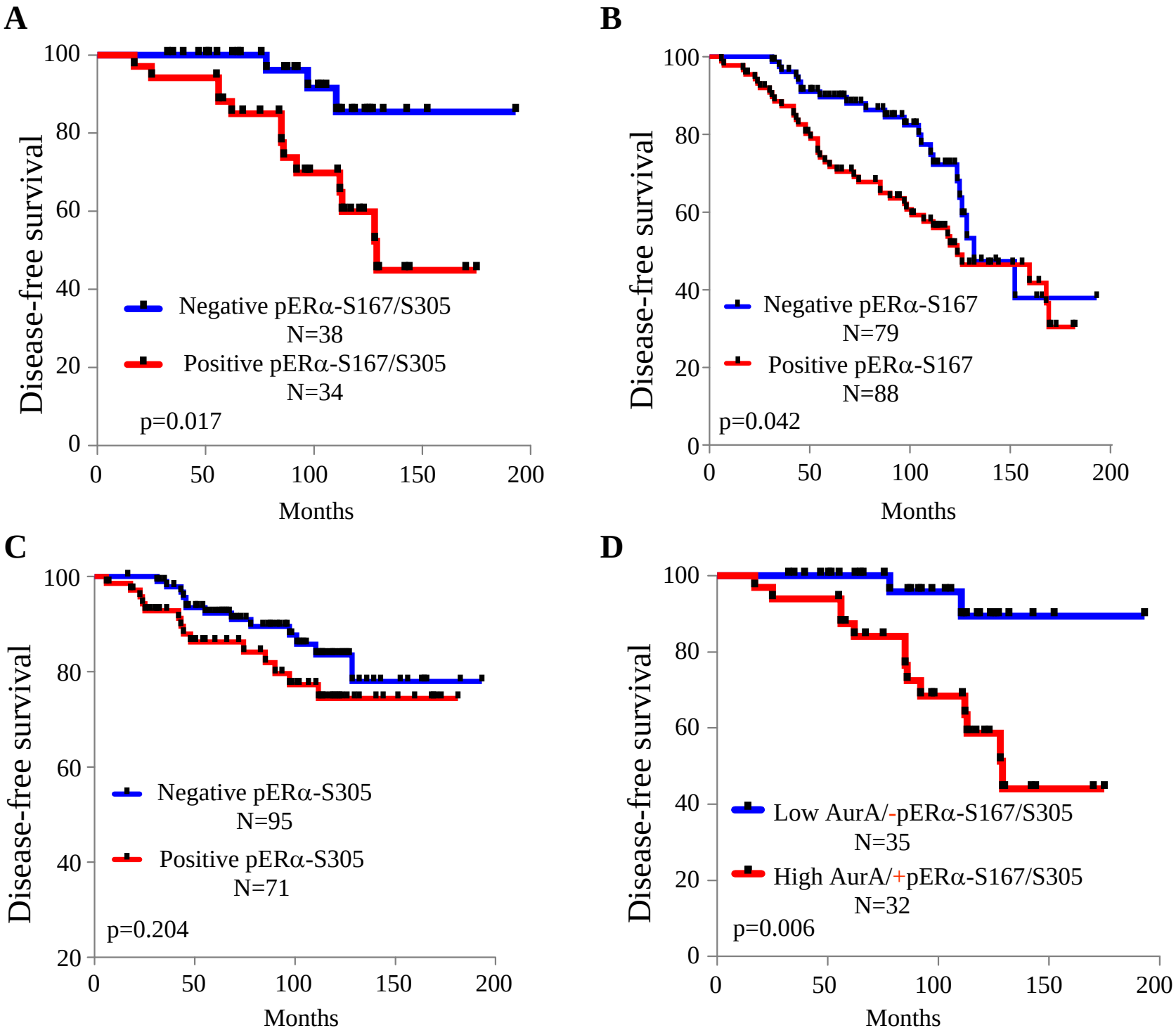

A
Disease-free survival
100
80
60
40
20
0
0
50
100
150
200
Months
Negative pERα-S167/S305
N=38
Positive pERα-S167/S305
N=34
p=0.017
B
Disease-free survival
Months
Negative pERα-S167
N=79
Positive pERα-S167
N=88
p=0.042
C
Disease-free survival
Months
Negative pERα-S305
N=95
Positive pERα-S305
N=71
p=0.204
D
Disease-free survival
Months
Low AurA/-pERα-S167/S305
N=35
High AurA/+pERα-S167/S305
N=32
p=0.006

Figure S11

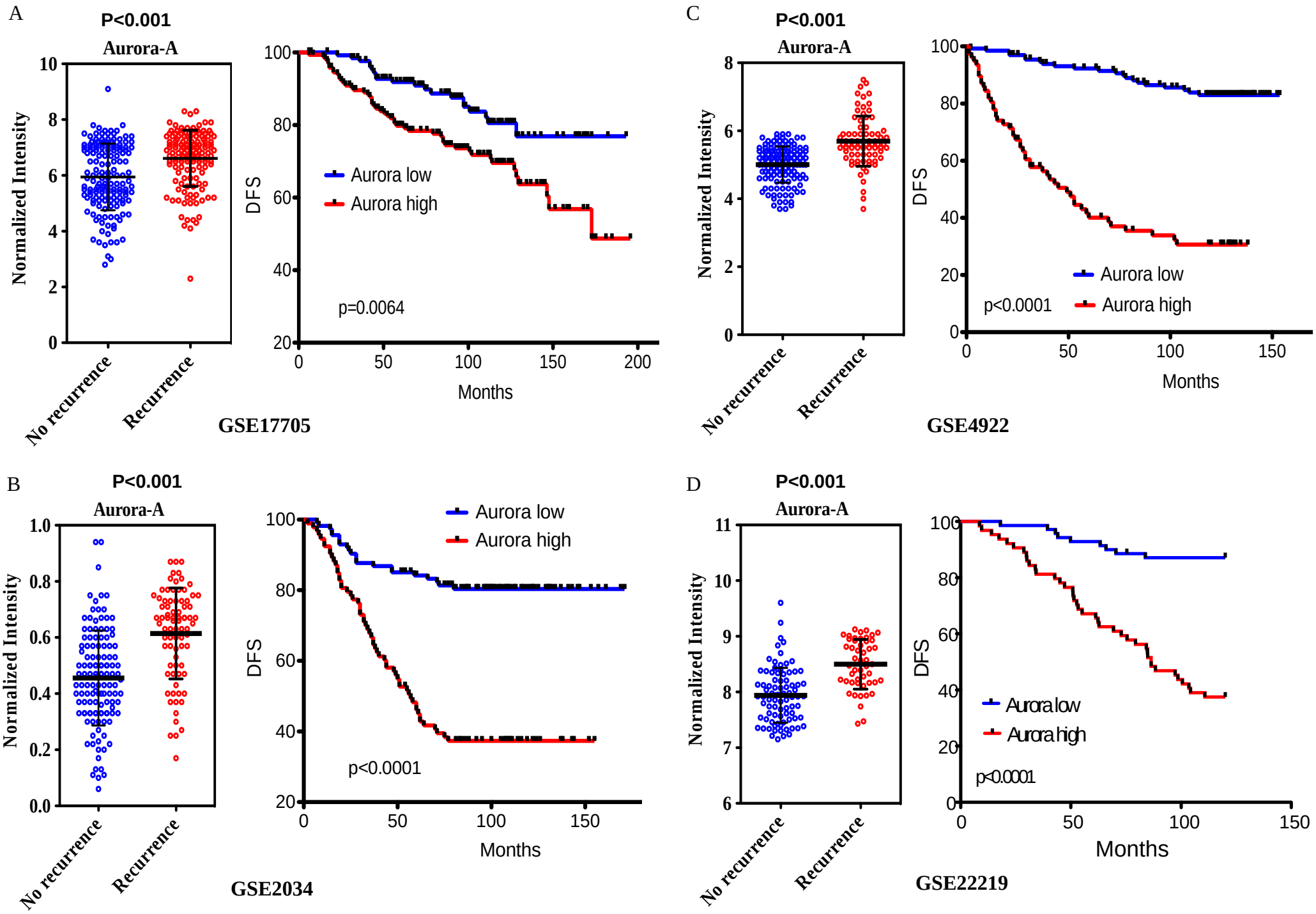

A
P<0.001
Aurora-A
Normalized Intensity
No recurrence
Recurrence
DFS
Aurora low
Aurora high
p=0.0064
Months
GSE17705
C
P<0.001
Aurora-A
Normalized Intensity
No recurrence
Recurrence
DFS
Aurora low
Aurora high
p<0.0001
Months
GSE4922
B
P<0.001
Aurora-A
Normalized Intensity
No recurrence
Recurrence
DFS
Aurora low
Aurora high
p<0.0001
Months
GSE2034
D
P<0.001
Aurora-A
Normalized Intensity
No recurrence
Recurrence
DFS
Aurora low
Aurora high
p<0.0001
Months
GSE22219

Figure S12

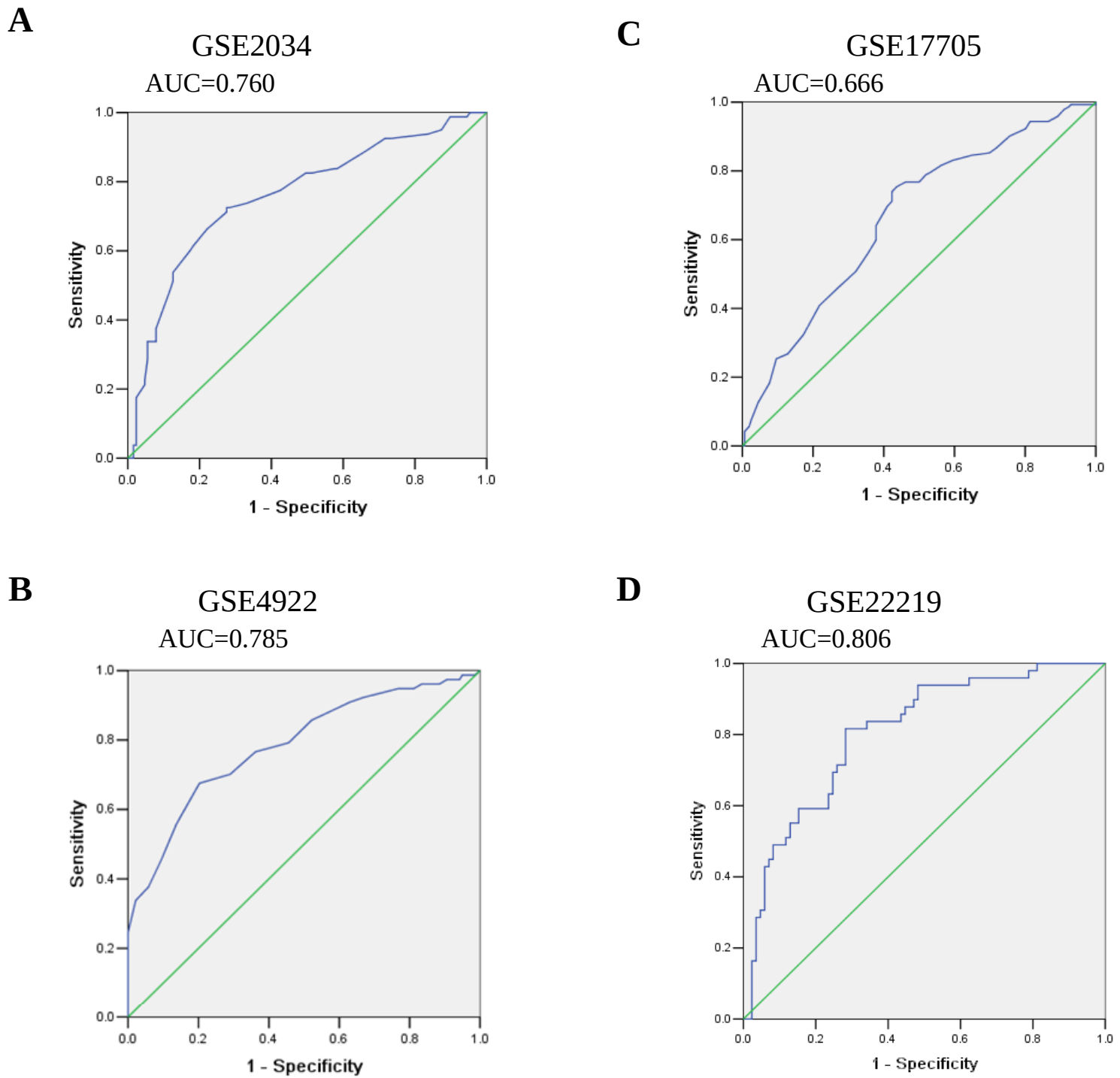
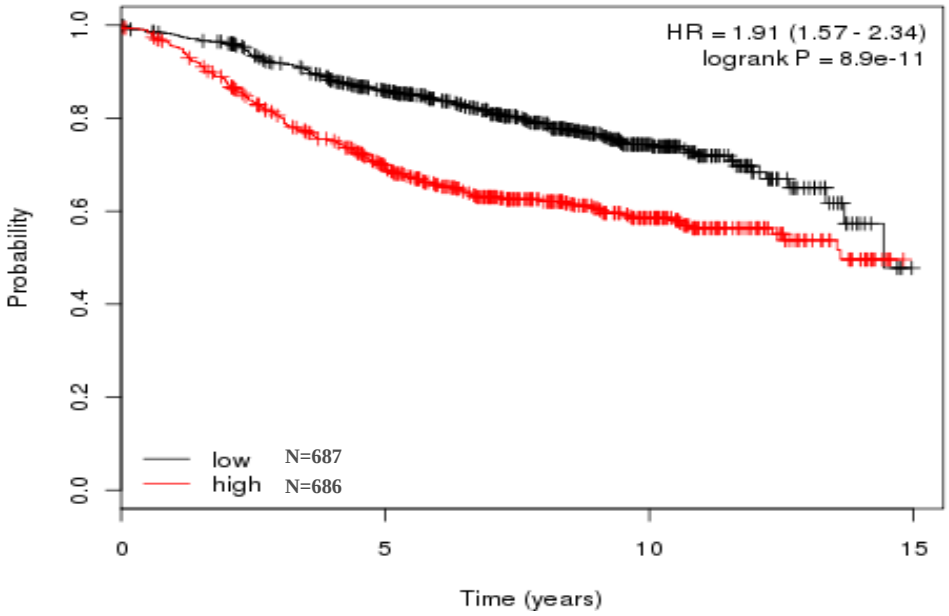

A
GSE2034
AUC=0.760
Sensitivity
1 - Specificity
C
GSE17705
AUC=0.666
Sensitivity
1 - Specificity
B
GSE4922
AUC=0.785
Sensitivity
1 - Specificity
D
GSE22219
AUC=0.806
Sensitivity
1 - Specificity

Figure S13

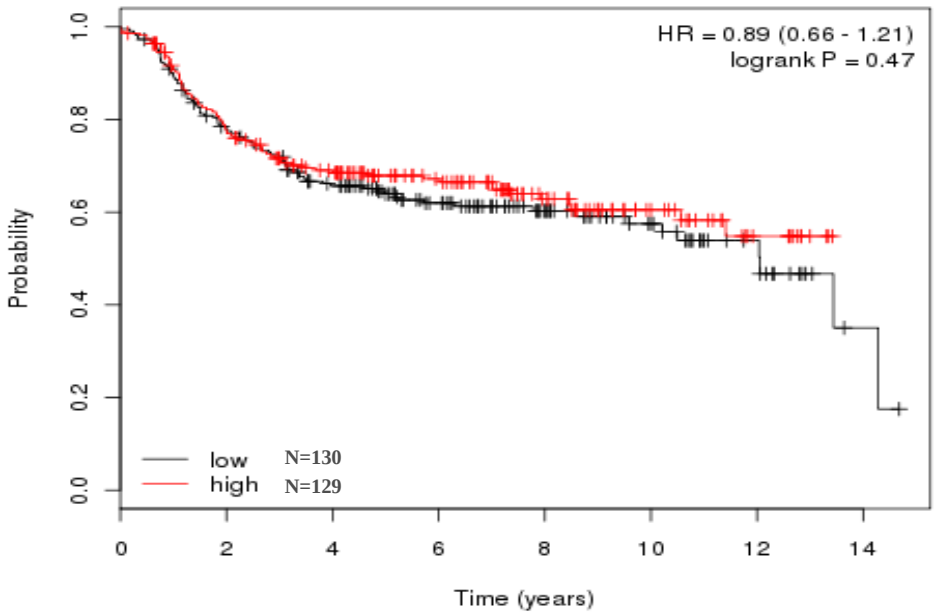
ERα-positive Breast Cancer/Endocrine Therapy

Recurrence-free survival



ERα-negative Breast Cancer

Recurrence-free survival



Basal-like Breast Cancer

Recurrence-free survival

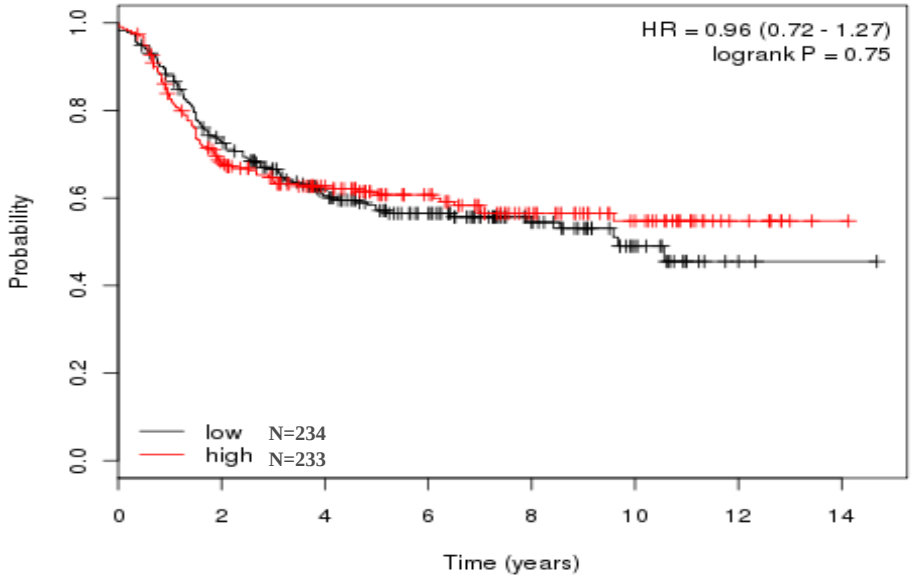


Figure S14

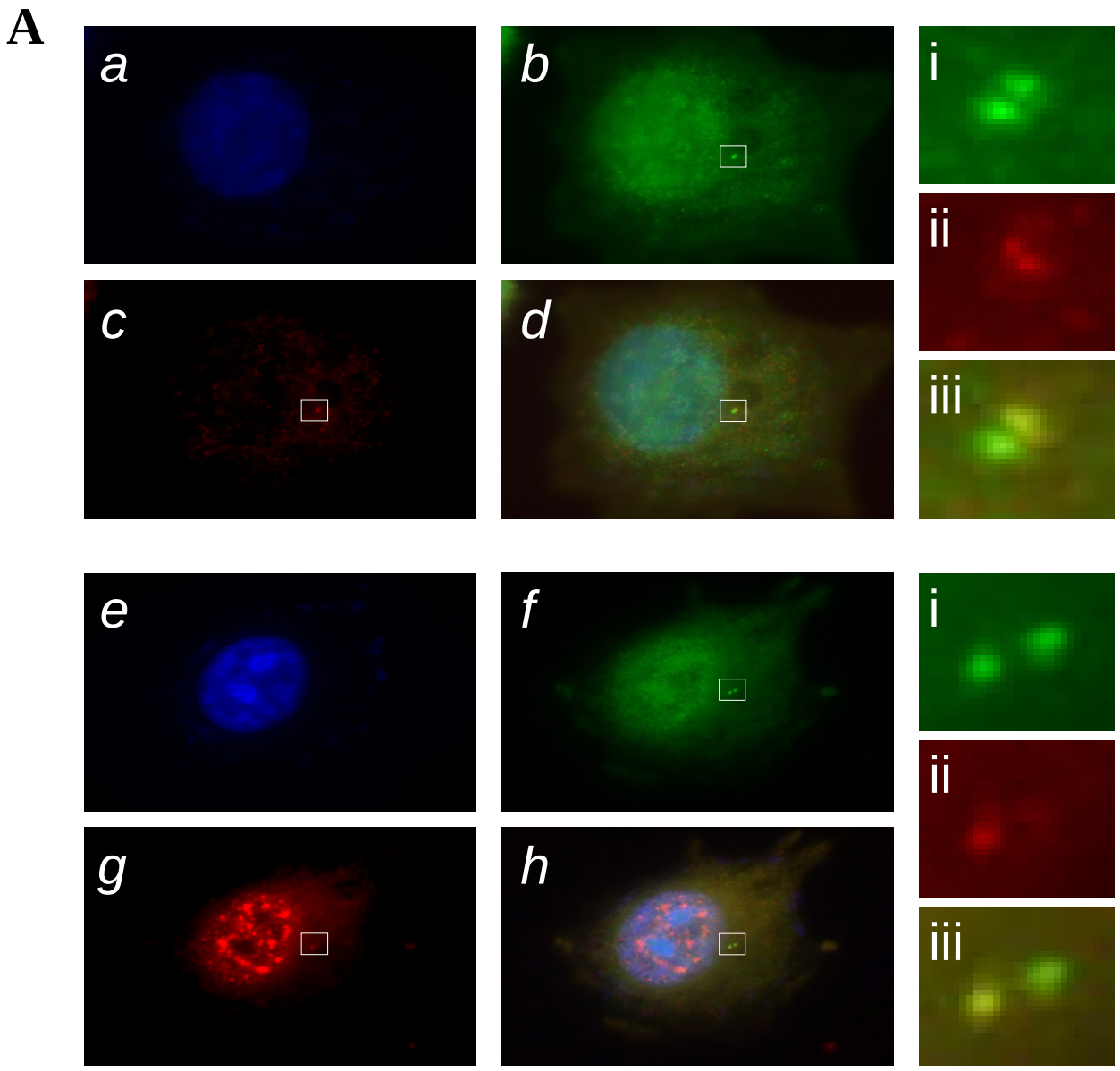


Figure S15

Table S1. Correlation of Aurora-A expression and ERα phosphorylation with clinicopathological characteristics in ERα-positive breast tumor patients

Variables	*Cases (n)*	*Aurora-A Positive*		*p-S167-ERα Positive*		*p-S305-ERα Positive*	
		n (%)	*P **	*n (%)*	*P **	*n (%)*	*P **
Age							
<50	61	29 (48)		34 (56)		23 (37)	
≥50	106	44 (42)	0.276	54 (51)	0.332	48 (45)	0.240
Tumor size							
<2 cm	112	45 (40)		54 (48)		47 (42)	
≥2 cm	55	28 (51)	0.126	34 (62)	0.068	24 (44)	0.483
Lymph node status							
Negative	98	38 (39)		46 (47)		41 (42)	
Positive	69	35 (51)	0.085	42 (61)	0.053	30 (43)	0.479
Stage							
I/II	132	57 (43)		67 (51)		54 (41)	
III/IV	35	16 (46)	0.468	21 (60)	0.217	17 (49)	0.266
Grade							
I/II	119	49 (41)		53 (45)		46 (39)	
III/IV	58	22 (38)	0.403	35 (60)	0.035	25 (43)	0.342

* *p*-value

Table S2. Multivariate analysis of recurrence-free survival

Variables	*Recurrence-free survival*			
	Univariate analysis		*Multivariate analysis*	
	HR (95% CI)	*p-value*	*HR (95% CI)*	*p-value*
Age				
<50 *vs.* ≥50	0.881 (0.583-1.493)	0.861	1.101 (0.789-1.672)	0.756
Tumor size				
≥2 cm *vs.* <2 cm	1.762 (1.091-2.531)	0.01	1.489 (0.846-3.271)	0.044
Lymph node status				
Positive *vs.* Negative	2.472 (1.456-4.134)	0.001	2.285 (1.270-3.531)	0.012
Stage				
III/IV *vs. I*/II	2.310 (1.211-4.219)	0.003	2.116 (1.412-4.010)	0.001
Grade				
III/IV *vs. I*/II	0.912 (0.678-1.231)	0.892	0.981 (0.579-1.872)	0.612
Aurora-A				
Positive *vs.* negative	2.313 (1.112-4.147)	0.0012	1.99 (1.012-4.002)	0.006
p-S167-ERα				
Positive *vs.* negative	1.892 (0.981-3.257)	0.035	1.451 (0.891-2.190)	0.042
p-S305-ERα				
Positive *vs.* negative	0.972 (0.687-2.192)	0.421	1.108 (0.891-1.987)	0.362

Supplemental Table S3

Cohort	Platforms	Samples	Data source	References
Nuvera Biosciences	HG-U133A	298*	GEO GSE17705	Symmans WF, et, al 2010
Veridex	HG-U133A	208* (286†)	GEO GSE2034	Wang Y, et, al 2005
Genome Institute of Singapore	HG-U133A	214* (249†)	GEO GSE4922	Ivshina AV, et, al 2006
University of Oxford	HumanRef-8 v1.0	134* (216†)	GEO GSE22219	Buffa FM, et, al 2011

†Total sample number. *ERα-positive tumours.

Symmans WF, Hatzis C, Sotiriou C, Andre F et al. Genomic index of sensitivity to endocrine therapy for breast cancer. *J Clin Oncol* 2010;28:4111-9.

Wang Y, Klijn JG, Zhang Y, Sieuwerts AM et al. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* 2005; 365:671-9.

Ivshina AV, George J, Senko O, Mow B et al. Genetic reclassification of histologic grade delineates new clinical subtypes of breast cancer. *Cancer Res* 2006;66:10292-301.

Buffa FM, Camps C, Winchester L, Snell CE et al. microRNA-associated progression pathways and potential therapeutic targets identified by integrated mRNA and microRNA expression profiling in breast cancer. *Cancer Res* 2011;71:5635-45.

Table S4. ROC analysis of using Aurora-A expression for prediction of endocrine therapy outcome

Data source	AUC*	p value
GEO GSE17705	0.666	0.001
GEO GSE2034	0.760	0.0001
GEO GSE4922	0.785	0.0001
GEO GSE22219	0.806	0.0001

*AUC, area under the curve

Table S5. Association of Aurora-A expression levels with 15-years recurrence-free survival of breast cancer patients from the KM Plotter Database[†]

		Aurora-A Probe 1 204092_s_at			Aurora-A Probe 2 208079_s_at		
		Number[¶]	HR[#]	P value	Number	HR	P value
ERα-positive	Endocrine therapy	687/686	1.91 (1.57-2.34)	**8.9e-11**	687/686	1.94 (1.58-2.36)	**4.3e-11**
	Grade 1	94/94	1.55 (0.79-3.05)	**0.2**	94/94	2.27 (1.11-4.62)	**0.02**
	Grade 2	178/178	1.48 (1.02-2.14)	0.036	178/178	1.43 (0.99-2.07)	0.054
	Grade 3	98/98	1.29 (0.81-2.05)	0.27	98/98	1.2 (0.76-1.90)	0.43
ERα-negative		222/222	0.89 (0.66-1.21)	0.47	222/222	0.85 (0.63-1.16)	0.32
Basal[§]		234/234	0.96 (0.72-1.27)	0.75	234/234	1.10 (0.83-1.46)	0.51

[†](http://www.kmplot.com) [¶]Number of Aurora-A low/high patients. [#]HR, hazard ratio with 95% confidence interval. [§]Basal-like breast cancer.