Supplementary Information

Heat shock proteins create a signature to predict the clinical outcome in breast cancer

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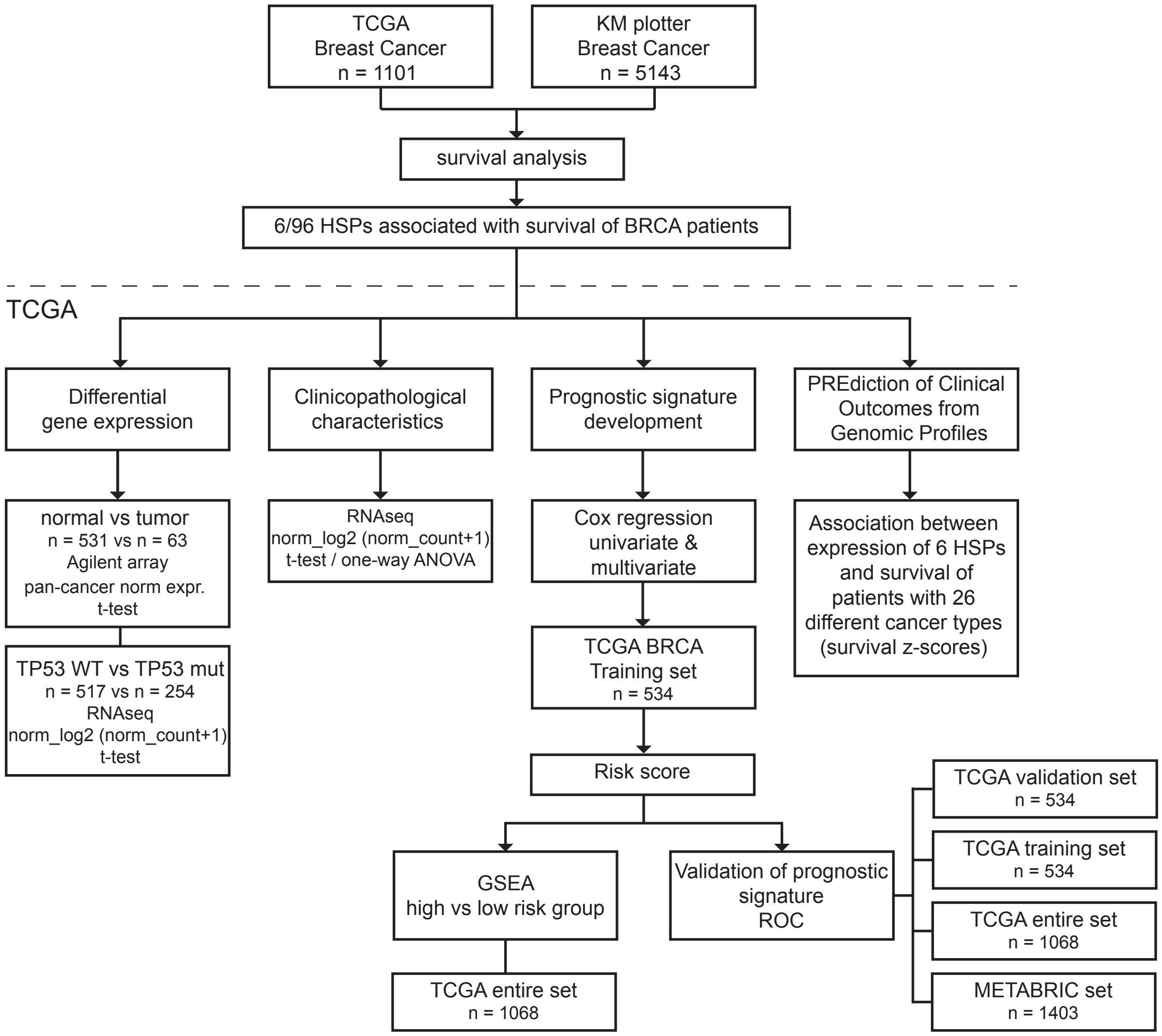
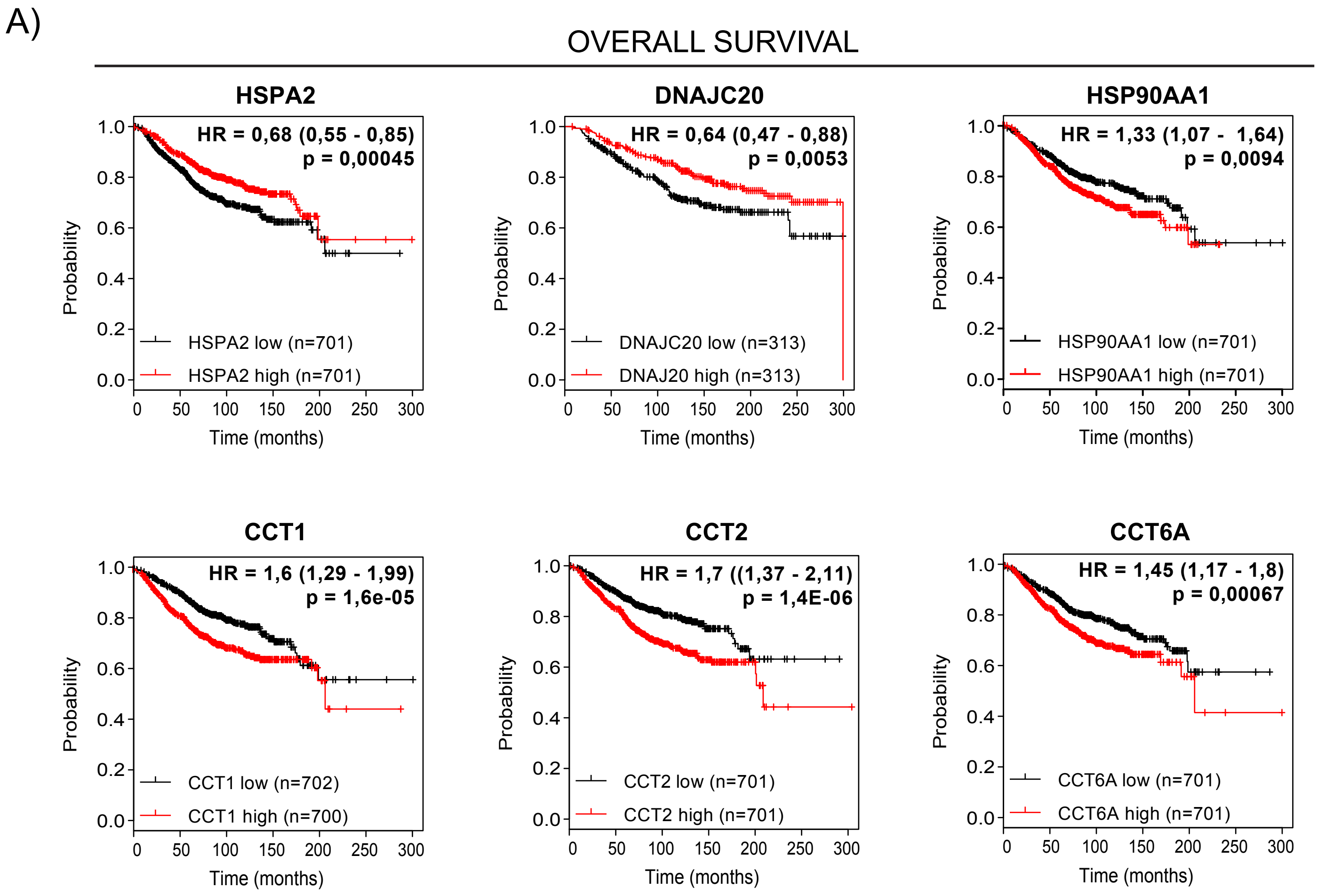


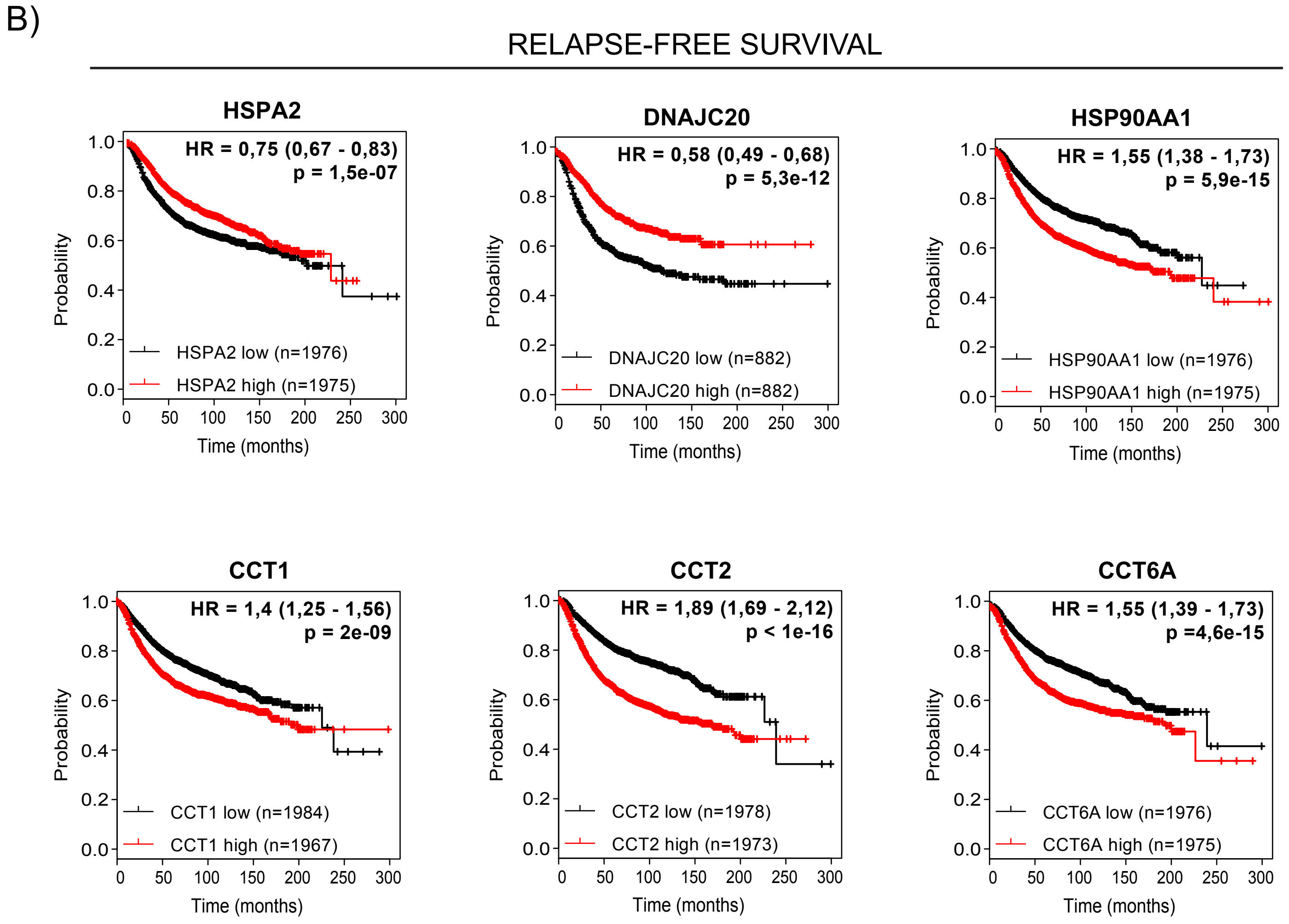
Supplementary Fig. S1. Workflow of the present study.

Gene	Coefficient	Coeficient SE	P-value
CCT1	**0,52**	**0,13**	**0,0001**
DNAJC23	**0,42**	**0,12**	**0,0005**
HSPA9	**0,54**	**0,16**	**0,0007**
HSP90AA1	**0,38**	**0,11**	**0,0009**
HSPA8	**0,41**	**0,13**	**0,0012**
DNAJB5	**-0,30**	**0,11**	**0,0064**
HSPA2	**-0,13**	**0,05**	**0,0065**
HSPH1	**0,26**	**0,10**	**0,0130**
CCT4	**0,39**	**0,16**	**0,0140**
DNAJC13	**0,39**	**0,16**	**0,0160**
HSCB	**-0,29**	**0,13**	**0,0330**
DNAJC30	**-0,31**	**0,15**	**0,0360**
CCT5	**0,25**	**0,12**	**0,0440**
CCT2	**0,24**	**0,12**	**0,0460**
DNAJC4	**-0,22**	**0,11**	**0,0500**
CCT8	0,29	0,16	0,0630
HSPD1	0,21	0,12	0,0720
HSP90AB1	0,24	0,13	0,0740
HSPB8	0,07	0,04	0,0760
HSPA4	0,28	0,16	0,0770
DNAJA2	0,24	0,14	0,0850
DNAJB13	-0,12	0,08	0,1100
CCT7	0,28	0,18	0,1200
DNAJB12	-0,30	0,19	0,1300
DNAJC6	0,07	0,05	0,1500
HSPA14	0,16	0,13	0,2000
DNAJB8	0,56	0,44	0,2000
DNAJC5	0,19	0,15	0,2000
HSPA7	-0,07	0,06	0,2100
HSPA13	0,13	0,10	0,2100
HSPE1	0,15	0,12	0,2100
DNAJB14	0,14	0,11	0,2100
DNAJC14	0,26	0,21	0,2100
DNAJC12	-0,04	0,03	0,2400
HSPB4	0,14	0,12	0,2500
HSPB11	-0,15	0,13	0,2500
DNAJB7	-0,12	0,11	0,2500
DNAJC1	-0,09	0,08	0,2500
DNAJC22	0,05	0,04	0,2500
DNAJA4	0,09	0,08	0,2600
HSPA6	-0,07	0,07	0,2700
DNAJC11	0,22	0,21	0,2800
DNAJC21	0,20	0,19	0,2800
CCT3	0,15	0,14	0,3000
DNAJC28	-0,12	0,11	0,3100
DNAJC3	0,13	0,13	0,3200
DNAJC19	-0,14	0,14	0,3200
DNAJC17	-0,11	0,12	0,3300

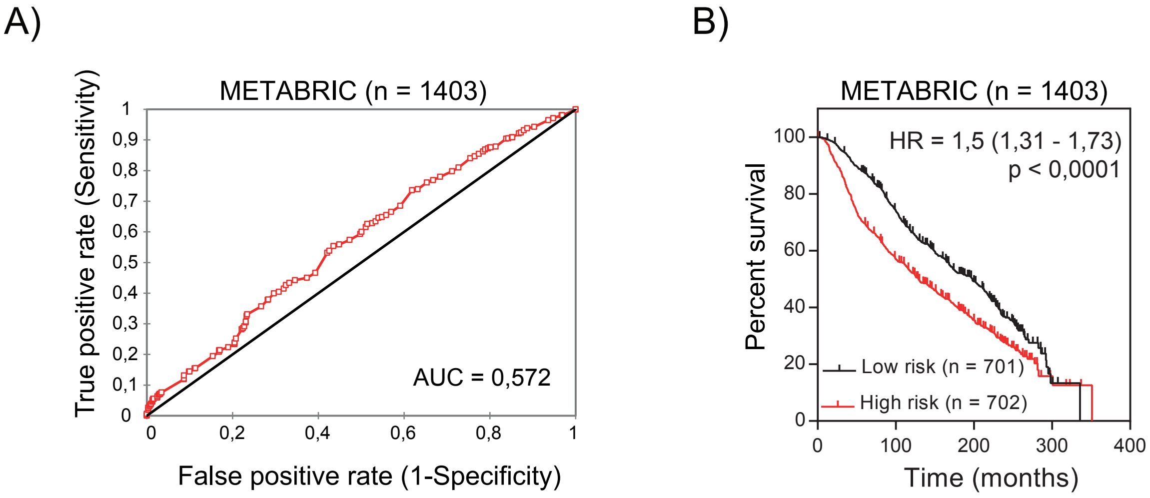
Gene	Coefficient	Coeficient SE	P-value
HSPB3	0,07	0,07	0,3600
HSPB5	-0,04	0,04	0,3600
DNAJC15	-0,09	0,10	0,3600
DNAJC2	-0,14	0,15	0,3700
DNAJC16	-0,13	0,15	0,3800
CCT6A	0,11	0,13	0,3900
DNAJC27	0,13	0,16	0,4000
HSPB2	-0,05	0,06	0,4100
DNAJB4	0,08	0,10	0,4200
HYOU1	0,09	0,12	0,4400
HSPA12B	-0,05	0,07	0,4500
DNAJB3	0,15	0,22	0,5000
DNAJA3	0,11	0,17	0,5100
HSPA4L	0,03	0,05	0,5200
DNAJC7	-0,11	0,18	0,5300
DNAJC24	-0,11	0,17	0,5300
HSPB6	-0,03	0,05	0,5500
DNAJC25	0,11	0,18	0,5500
DNAJB2	0,08	0,13	0,5600
HSPB10	-0,14	0,27	0,5900
CCT6B	-0,05	0,09	0,6000
MKKS	0,10	0,19	0,6000
HSPB7	0,02	0,05	0,6200
DNAJC5B	-0,03	0,05	0,6200
DNAJC29	-0,04	0,08	0,6200
DNAJC8	-0,11	0,23	0,6300
DNAJC26	0,07	0,15	0,6300
BBS12	-0,06	0,12	0,6500
DNAJA1	0,06	0,15	0,6700
DNAJB9	0,05	0,13	0,6800
BBS10	-0,05	0,13	0,7100
HSPA12A	0,03	0,08	0,7400
DNAJB11	-0,05	0,14	0,7400
HSPA1B	0,03	0,10	0,7800
DNAJC10	0,04	0,14	0,7900
DNAJB6	-0,03	0,16	0,8300
HSPB1	0,01	0,06	0,8400
HSP90L	0,02	0,12	0,8600
HSPA1A	0,01	0,09	0,8900
DNAJC18	-0,02	0,13	0,8900
HSP90B1	-0,02	0,16	0,9000
DNAJB1	0,02	0,15	0,9100
HSPA1L	-0,01	0,13	0,9200
HSPB9	0,01	0,08	0,9200
DNAJC5G	0,03	0,29	0,9300
HSPA5	0,01	0,15	0,9600
DNAJC9	0,00	0,15	0,9800

Supplementary Fig. S2. Univariate Cox proportional hazards analysis of overall survival for TCGA BRCA patients based on HSP expression.

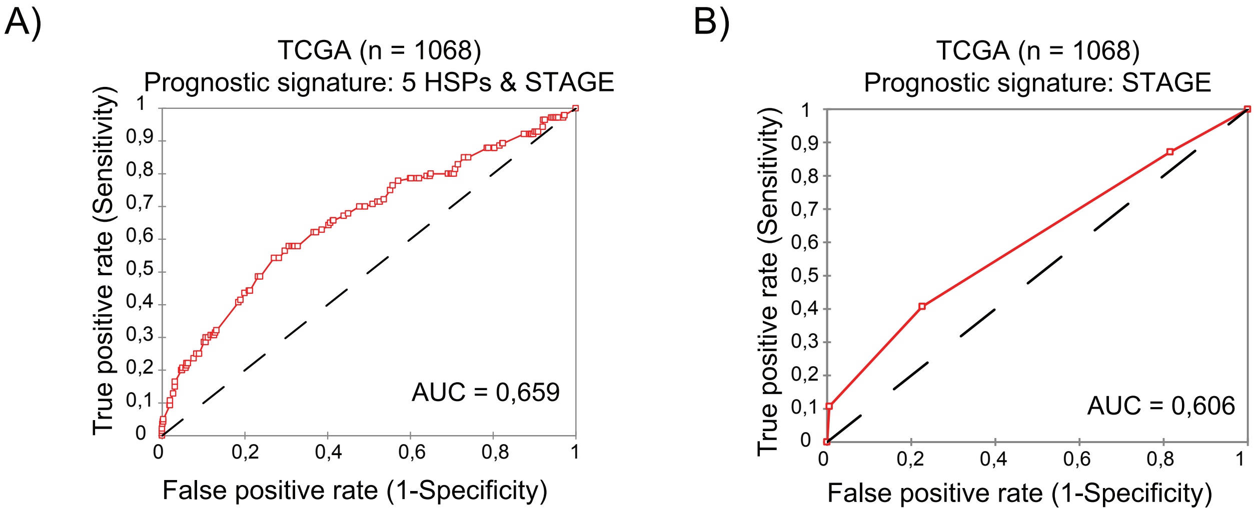




Supplementary Fig. S3. Expression of six identified HSPs predicts survival of breast cancer patients. Kaplan-Meier analyses of overall survival **(A)** and relapse-free survival **(B)** based on the gene expression in cohort of KM plotter patients. Hazard ratios (HR) with 95% confidence intervals and p-values (log-rank test, Mantel-Haenszel) were calculated. Data for survival curves were obtained from KM plotter (http://kmplot.com/analysis/).



Supplementary Fig. S4. (A) Diagnostic value of five candidate HSPs and cancer stage in METABRIC dataset (n = 1403). The areas under curve (AUC) were calculated for ROC curves, and sensitivity and specificity were calculated to assess the score performance. **(B)** Kaplan-Meier survival curves for five-HSP and stage signature in METABRIC dataset (n = 1403). Patients were stratified into high-risk and low-risk groups based on median of risk score. Hazard ratios (HR) with 95% confidence intervals and log-rank p-values were calculated.

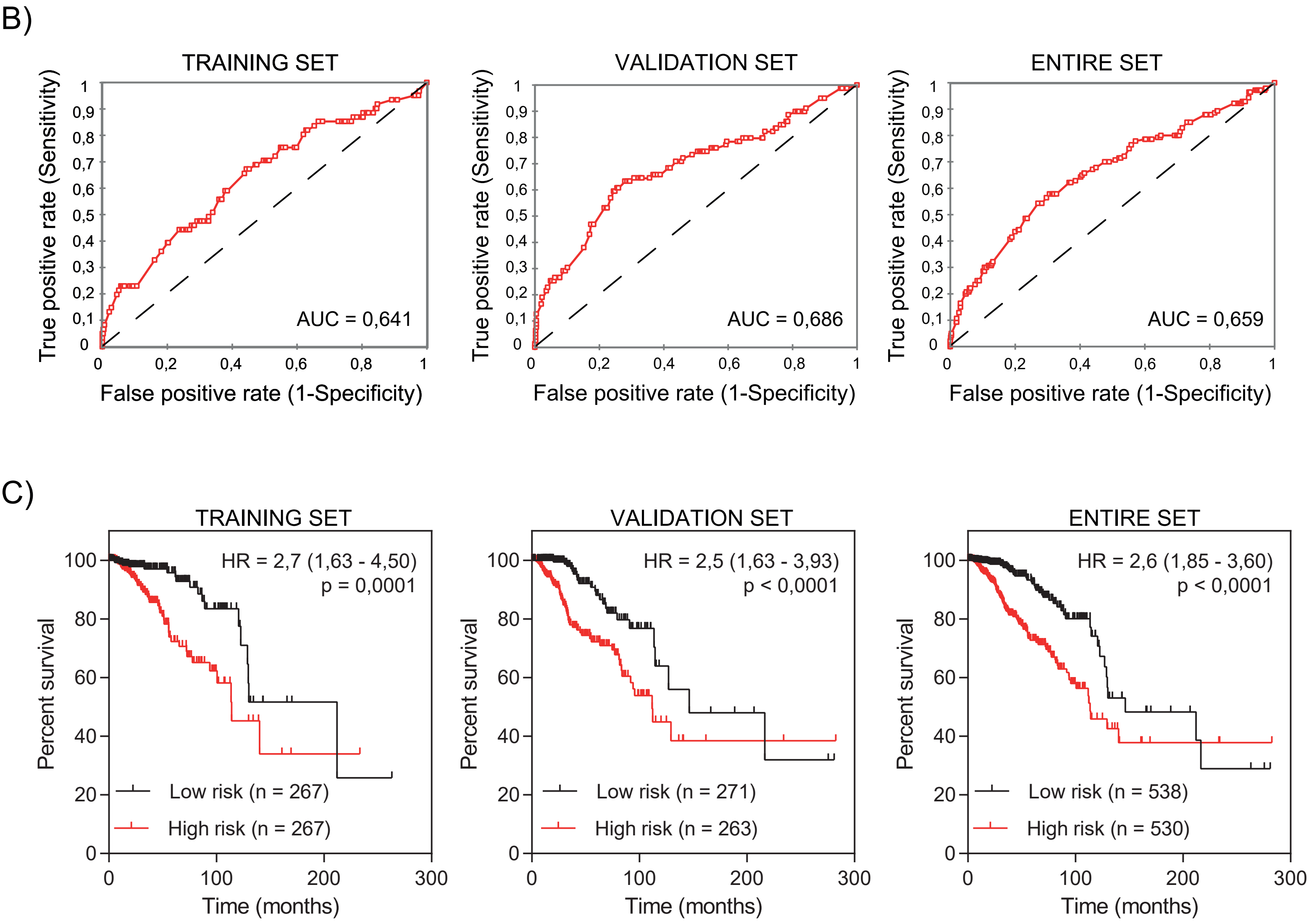


Supplementary Fig. S5. Comparison of signatures for survival prediction of TCGA breast cancer patients based on **A)** expression of 5 HSPs and stage, **B)** stage only. The areas under curve (AUC) were calculated for ROC curves, and sensitivity and specificity were calculated to assess the score performance.

A)

Multivariate analysis					
Variable	**Coefficient**	**p-value**	**HR**	**95% CI**	
				Lower	**Upper**
Training set (n = 534)					
HSPA2	-0,461	0,084	0,631	0,374	1,063
DNAJC20	-0,180	0,502	0,835	0,494	1,413
HSP90AA1	0,703	**0,036**	2,020	1,048	3,896
CCT1	0,127	0,661	1,135	0,644	2,002
CCT2	0,160	0,625	1,173	0,619	2,223
STAGE I	-3,357	**< 0,0001**	0,035	0,011	0,111
STAGE II	-3,238	**< 0,0001**	0,039	0,014	0,107
STAGE III	-2,426	**< 0,0001**	0,088	0,032	0,249
Validation set (n = 534)					
HSPA2	-0,625	**0,015**	0,535	0,323	0,887
DNAJC20	-0,064	0,791	0,938	0,583	1,509
HSP90AA1	0,391	0,146	1,478	0,873	2,502
CCT1	0,558	**0,0315**	1,747	1,051	2,906
CCT2	-0,1416	0,584	0,868	0,523	1,441
STAGE I	-2,7548	**< 0,0001**	0,064	0,025	0,165
STAGE II	-2,3064	**< 0,0001**	0,010	0,046	0,218
STAGE III	-1,7115	**< 0,0001**	0,180	0,082	0,397
Entire TCGA set (n = 1068)					
HSPA2	-0,549	**0,0025**	0,578	0,405	0,824
DNAJC20	-0,123	0,492	0,885	0,624	1,255
HSP90AA1	0,509	**0,014**	1,663	1,080	2,495
CCT1	0,401	**0,035**	1,494	1,029	2,170
CCT2	-0,0005	0,998	1,001	0,677	1,478
STAGE I	-2,807	**< 0,0001**	0,060	0,030	0,123
STAGE II	-2,548	**< 0,0001**	0,078	0,043	0,143
STAGE III	-1,891	**< 0,0001**	0,150	0,082	0,279

HR, hazard ratio; CI, confidence interval



Supplementary Fig. S6. Survival prediction model for breast cancer patients calculated using formula with binarized Stage variables:

Risk score = (-0,461 x HSPA2 0/1) + (-0,180 x DNAJC20 0/1) + (0,703 x HSP90AA1 0/1) + (0,127 x CCT1 0/1) + (0,16 x CCT2 0/1) + (-3,357 x Stage I 0/1) + (-3,238 x Stage II 0/1) + (-2,426 x Stage III 0/1).

Expression data were converted to a binary format as follows: low expression = 0, high expression = 1 and stage was binarized as follows: e.g. Stage I NO = 0, YES = 1.

(A) Multivariate Cox proportional hazards analysis of overall survival for TCGA BRCA patients. **(B)** Diagnostic value of five candidate HSPs and cancer stage (I/II/III) in the training (n = 534), validation (n = 534) and entire TCGA BRCA dataset (n = 1068). The areas under curve (AUC) were calculated for ROC curves, and sensitivity and specificity were calculated to assess the score performance. **(C)** Kaplan-Meier survival curves for five-HSP and stage signature in the training (n = 534), validation (n = 534) and entire TCGA BRCA dataset (n = 1068). Patients were stratified into high-risk and low-risk groups based on median of risk score. Hazard ratios (HR) with 95% confidence intervals and log-rank test p-values were calculated.