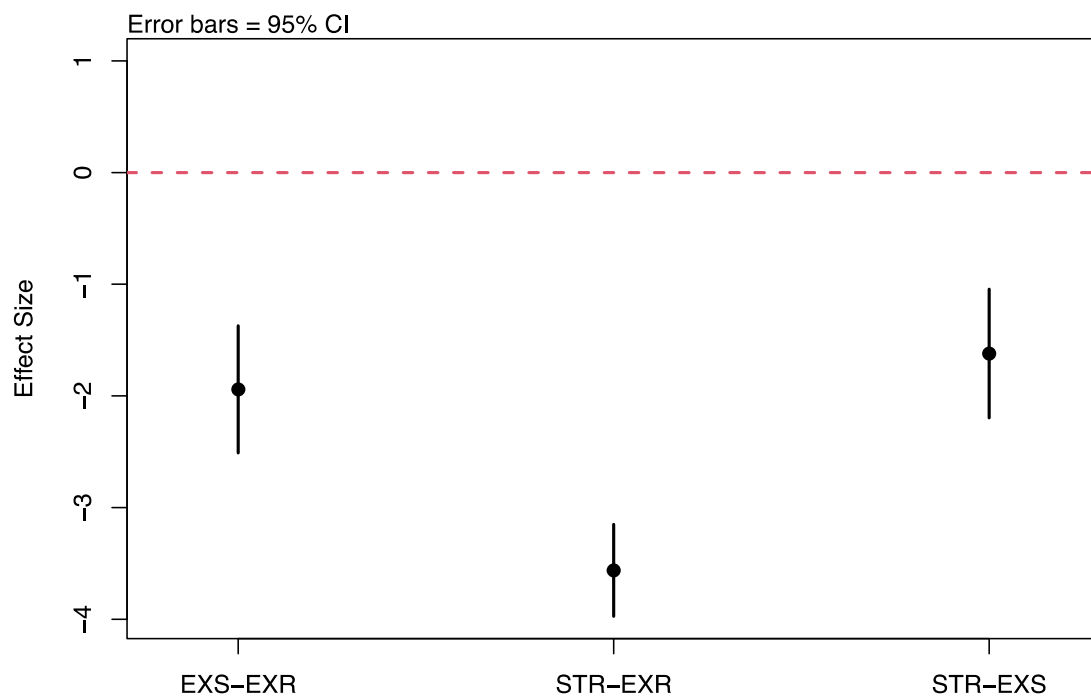


Supplementary Figure S1. Overview of patient selection and sequencing data.

A total of 243 patients with HER2-positive MBC were enrolled, from whom 29 who never progressed were identified as ExR with an OS > 60 months, 54 were identified as ExS with an OS > 60 months and 160 were identified as STR with an OS < 60 months at the database cut-off (December 2020 and follow-up updated in January 2024).



	diff	lwr	upr	p adj
EXS-EXR	-1.941572	-2.510722	-1.372422	0
STR-EXR	-3.562167	-3.973662	-3.150672	0
STR-EXS	-1.620595	-2.196177	-1.045013	0

Figure S2. Tukey comparisons of centromeric CN means between ExR, ExS and STR.
 Post hoc ANOVA test (Tukey) performed using R (V4.2.0) and R packages rstatix, AICcmodavg, ggplot2, ggpubr, tidyverse, broom and multcompView.

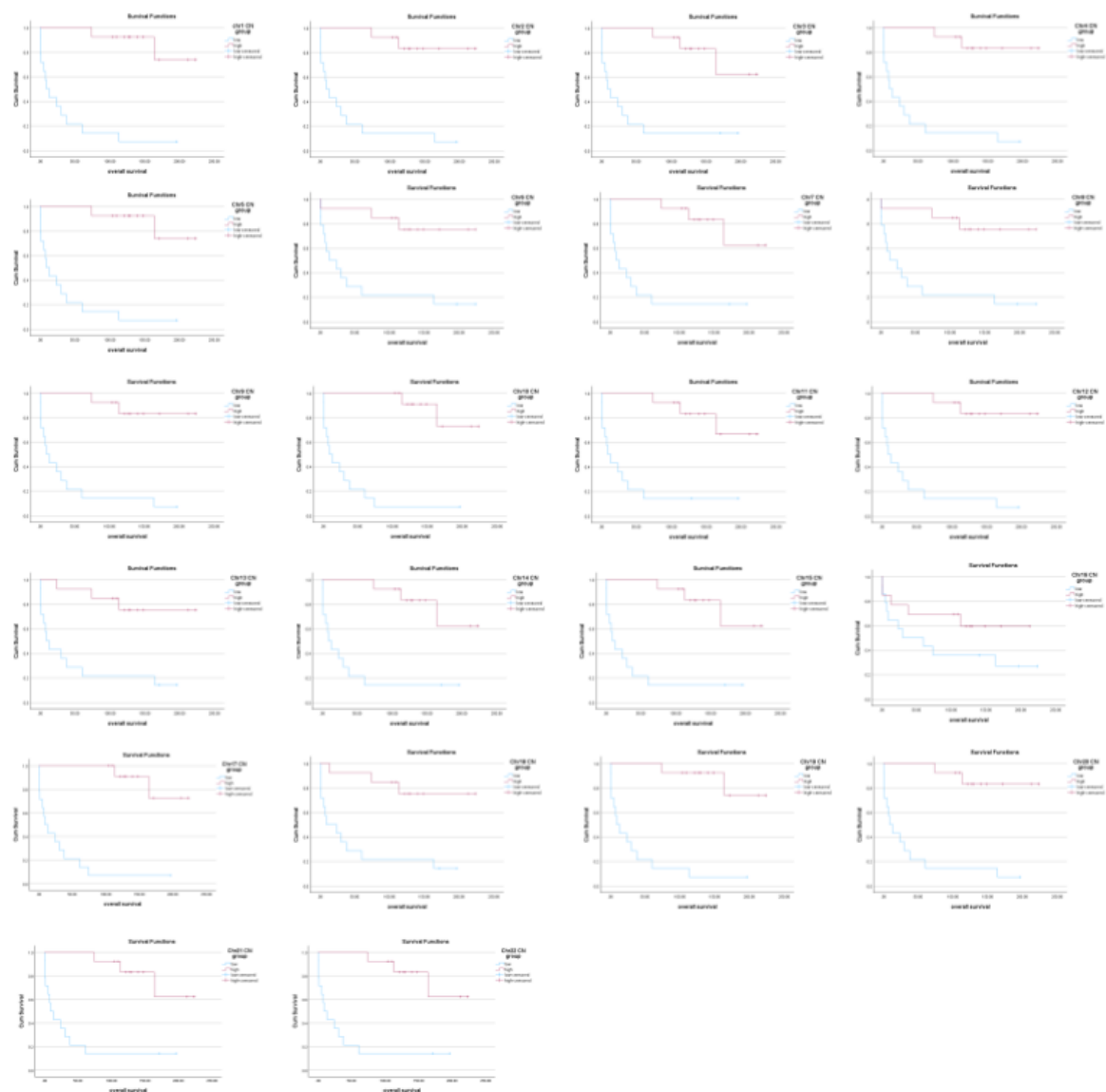


Figure S3. Kaplan Meier curves of overall survival in ExR, ExS, and STR patients divided by centromeric copy number. Patients were divided into a low or high CCN group based on the median CCN.

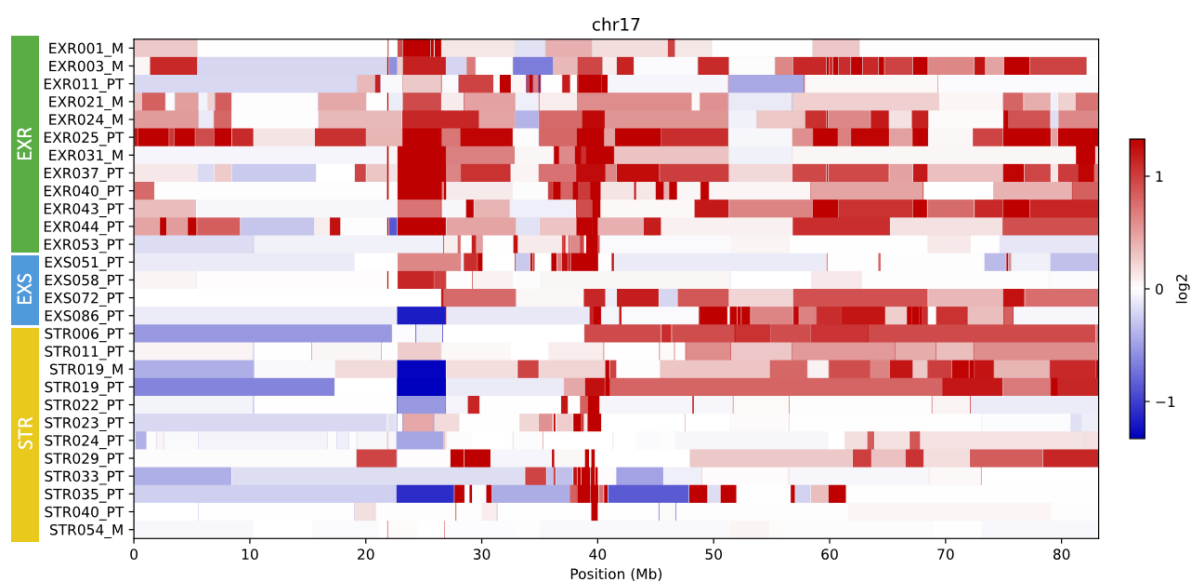


Figure S4. Copy number aberration profiles of chromosomes 17. Heatmap of the log2 normalized CNA profiles of the whole genome of the 12 EXR, 4 EXS and 11 STR samples. Red color indicates a gain of copy, blue indicates a loss of copy and white indicates a normal copy number (2 copies)

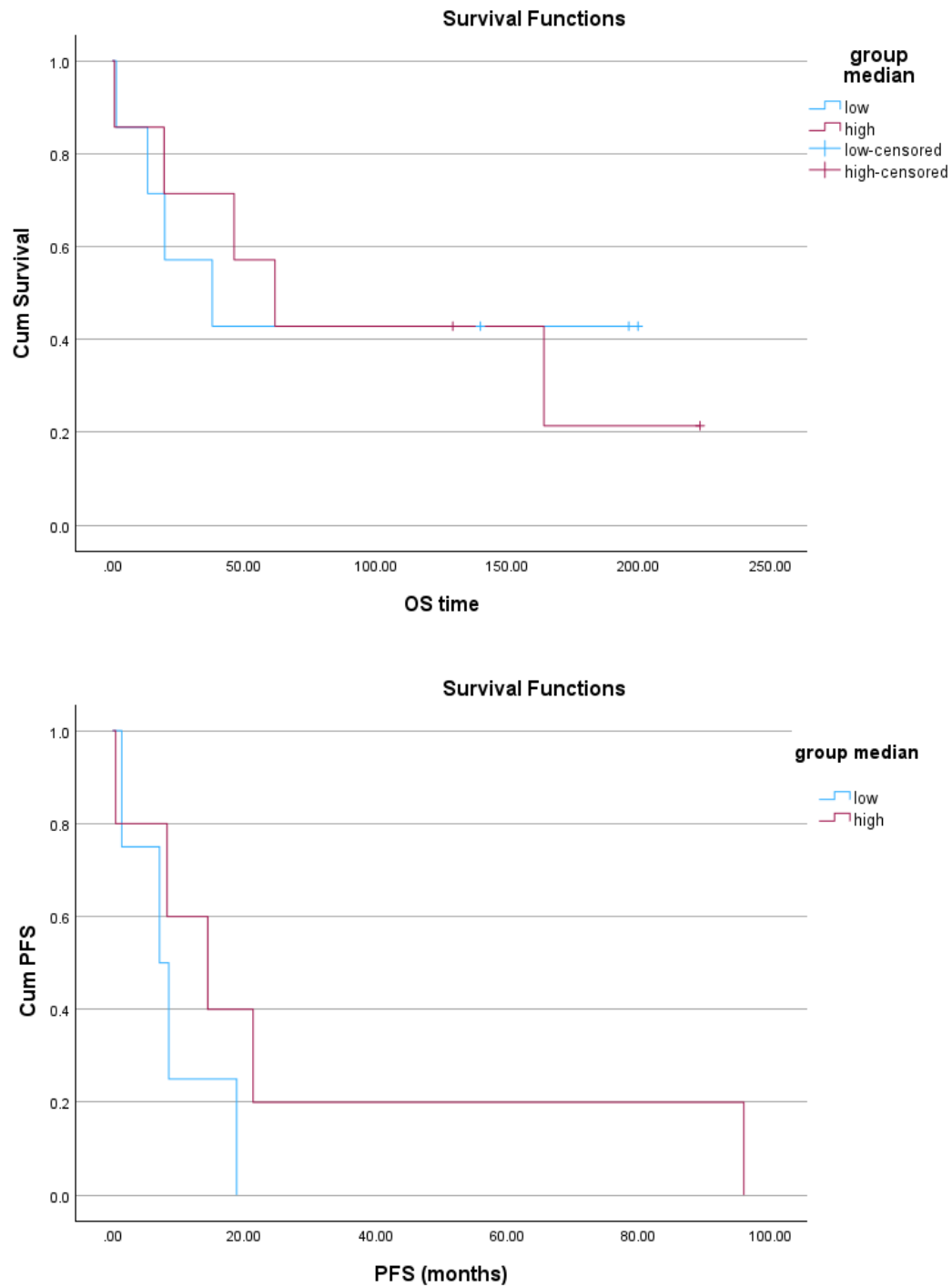


Figure S5. Kaplan Meier curves of overall survival and progression-free survival in ExR, ExS, and STR patients grouped by D4Z1 copy number.

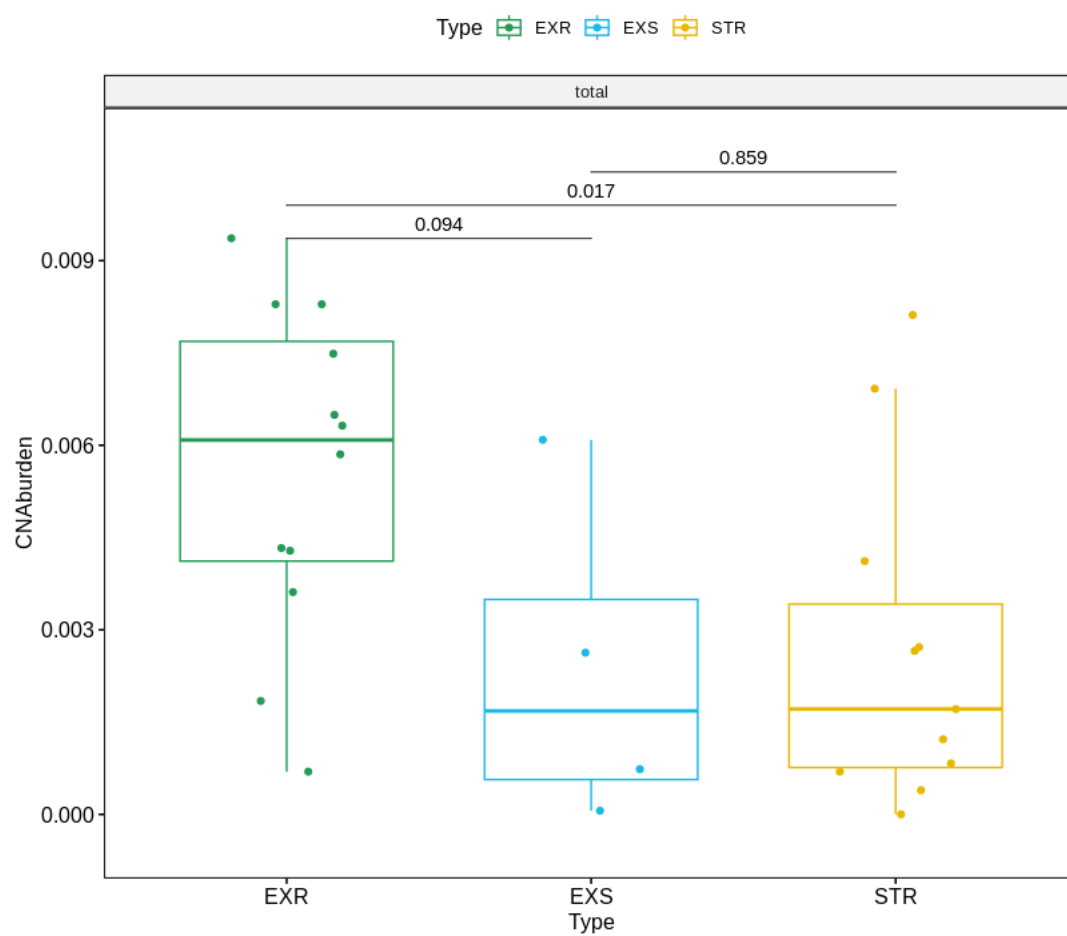


Figure S6. CNA burden of the coding regions. Fractions of the genome altered by CNA in terms of loss and gain (total) for the EXR, EXS and STR groups.

Table S1. Coordinates of the selected centromeric regions (hg38). Coordinates used to estimate the copy-number status of the centromeric regions.

<i>chromosome</i>	<i>start</i>	<i>end</i>
chr1	122500000	124000000
chr2	93000000	94000000
chr3	91551000	93400000
chr4	49650000	49700000
chr5	47251000	48600000
chr6	58451000	58600000
chr7	59851000	60000000
chr8	44100000	44500000
chr9	44900000	45400000
chr10	39700000	39900000
chr11	53951000	54300000
chr12	35400000	37200000
chr13	17500000	18000000
chr14	17651000	18200000
chr15	18400000	19700000
chr16	36110000	36210000
chr17	25950000	26500000
chr18	20610000	20800000
chr19	26300000	26900000
chr20	26551000	28000000
chr21	12310000	12400000
chr22	14500000	15000000

Table S2. PFS and OS statistics. *Kaplan Meier survival analysis performed using R (V4.2.0) and R packages survival and survminer.*

	EXR (n=29)	EXS (n=54)	STR (n=160)
Progression-Free Survival			
records	29	54	159
events	0	54	156
mean, months	NA	46.5	15.1
median, months	NA	34.4	10.9
0.95 LCL, months	NA	27.7	8.54
0.95 UCL, months	NA	43.4	12.7
Overall Survival			
records	29	54	159
events	3	36	154
mean, months	157	139	23.8
median, months	148	113	21.1
0.95 LCL, months	NA	91.5	19.3
0.95 UCL, months	NA	152	26.4

Table S3. Clinical characteristics of the sequenced cases. Kaplan Meier survival analysis performed using R (V4.2.0) and R packages survival and survminer.

	EXR (n=12)	EXS (n=4)	NR (n=11)
Death event	3	4	11
Mean PFS, in months	NA	62.8	5.89
Mean OS, in months	158	102	11.9
de novo metastasis			
yes	6	2	0
no	6	2	11
Best treatment response			
complete response (CR)	9	2	0
partial response (PR)	1	1	2
stable disease (SD)	2	1	1
other	0	0	8
Drug category			
single agent trastuzumab	0	0	2
trastuzumab with taxane agent	12	2	3
other / unknown	0	2	6
Trastuzumab pretreated			
yes	1	2	6
no	11	2	5

Table S4. D4Z1 copy number as measured by dPCR and associated PFS and OS.

ID	D4Z1_avg	OS	PFS	WGS
EXR004	665.88	199.69	NA	N
EXR021	602.49	139.79	NA	Y
EXR024	1557.74	129.31	NA	Y
EXR043	4405.96	223.28	NA	Y
EXR051	2917.61	163.94	95.97	Y
EXR053	874.31	196.17	NA	Y
EXR085	66885.45	61.7	21.36	N
NR005	899.56	13.31	7.13	N
NR023	2711.97	0.72	0.46	Y
NR024	1170.8	1.38	1.38	Y
NR049	3459.9	19.55	8.28	N
NR052	555.08	19.81	8.54	N
NR053	2092.07	46.19	14.46	N
NR054	523.73	37.88	18.86	Y