SUPPLEMENTARY MATERIAL

Tracking response to neoadjuvant systemic therapy through circulating tumor DNA analysis in breast cancer

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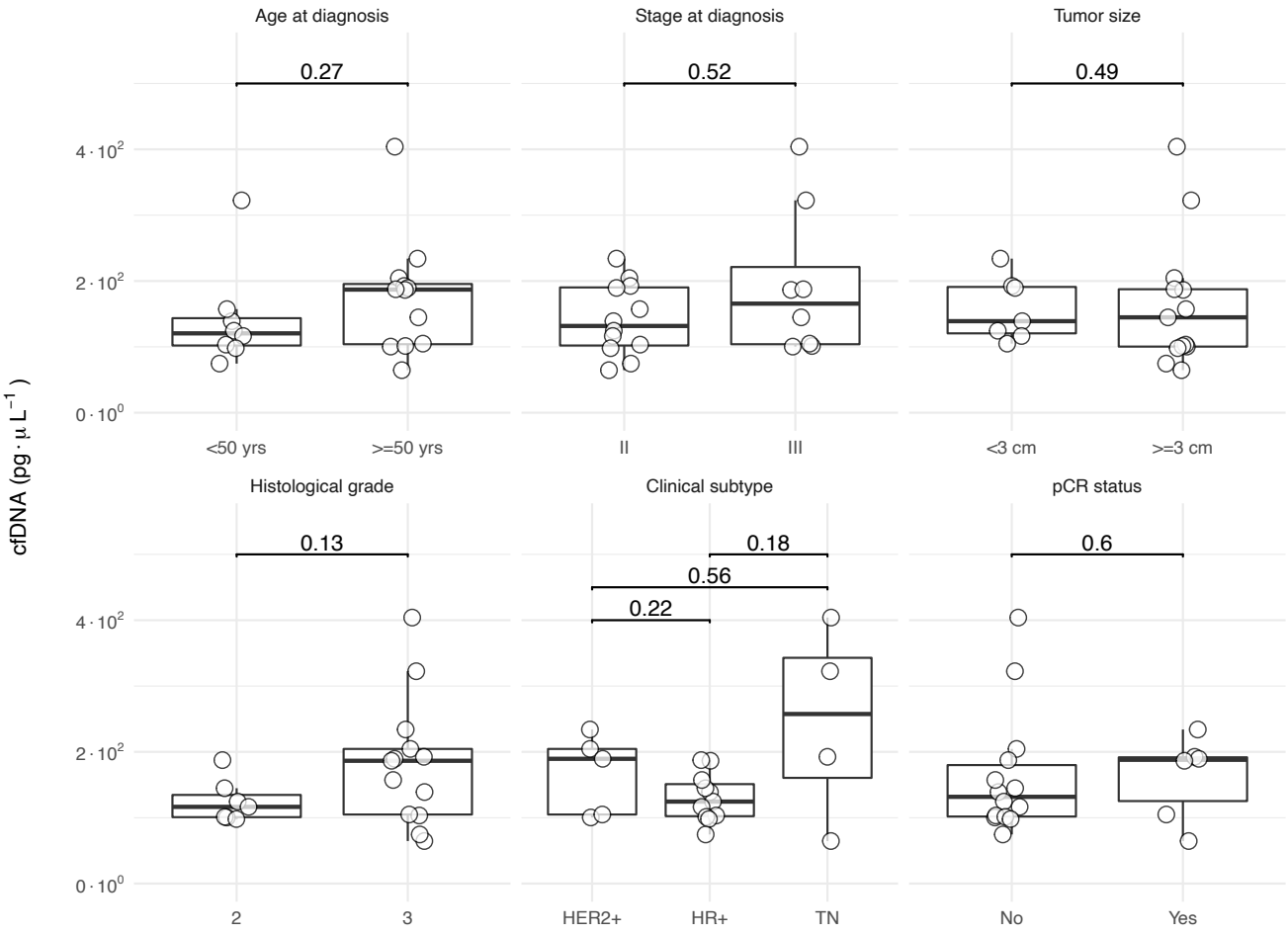
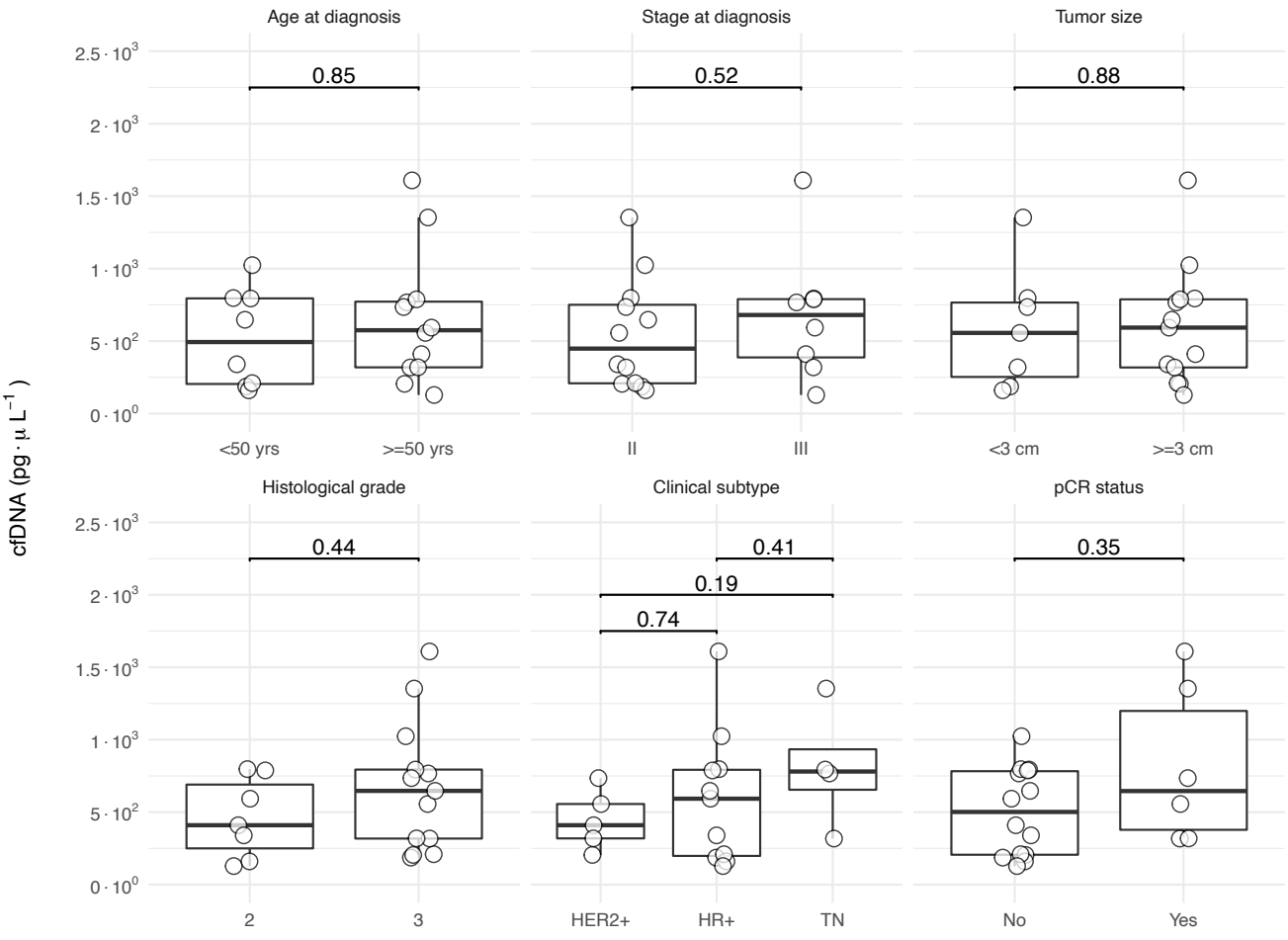
Supplementary Table S1. Detection of ctDNA in baseline plasma by pathologic complete response (pCR) and breast cancer subtype.

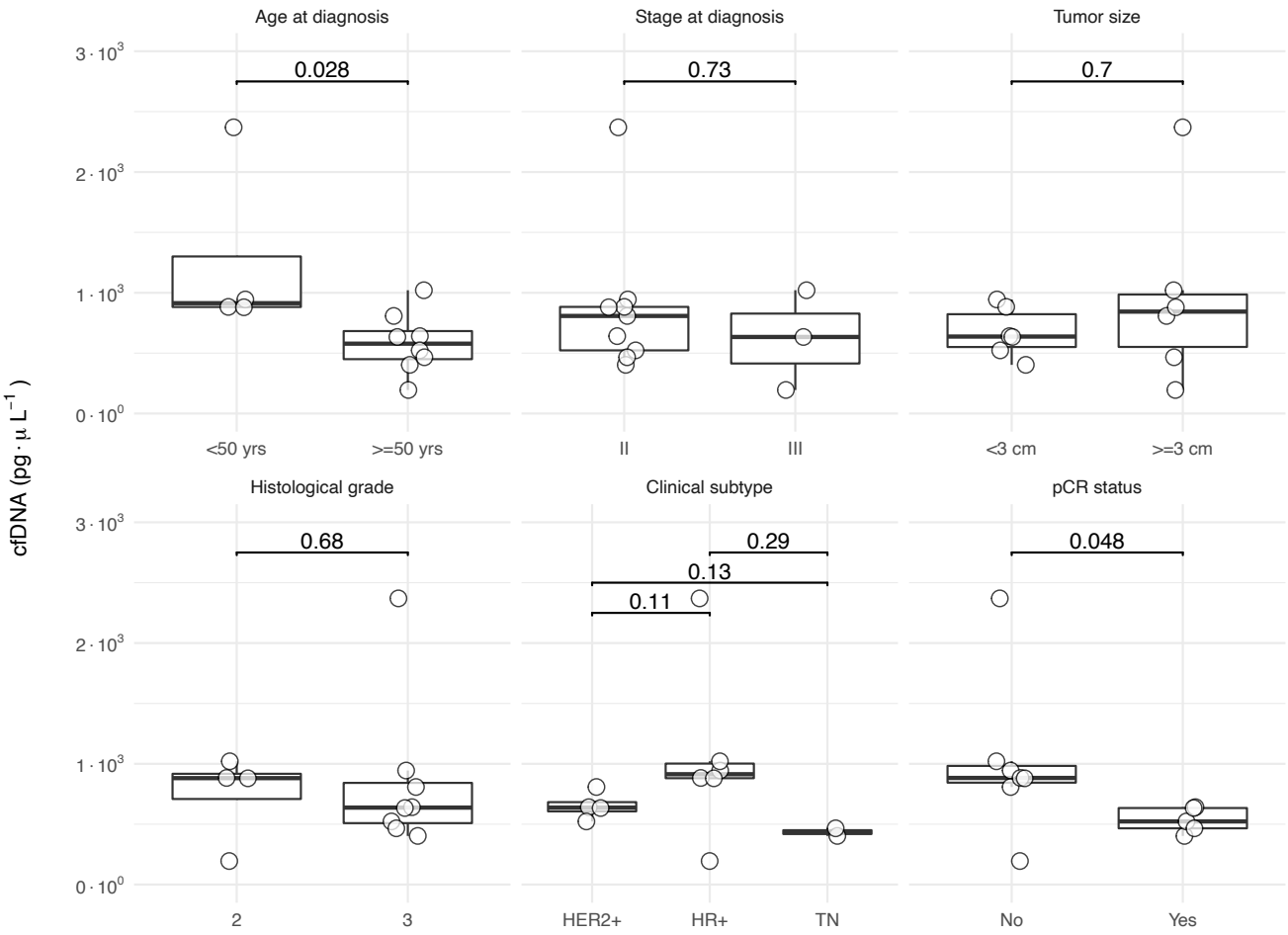
		Baseline ctDNA detection		
Breast cancer subtype	**pCR**	**No (n=3)**	**Yes (n=15)**	**Total (n=18)**
HR-positive/HER2-negative	No	2 (100.0%)	5 (83.3%)	7 (87.5%)
	Yes	0 (0.0%)	1 (16.7%)	1 (12.5%)
HER2-positive	No	0 (0.0%)	3 (60.0%)	3 (50.0%)
	Yes	1 (100.0%)	2 (40.0%)	3 (50.0%)
Triple-negative	No	0	2 (50.0%)	2 (50.0%)
	Yes	0	2 (50.0%)	2 (50.0%)



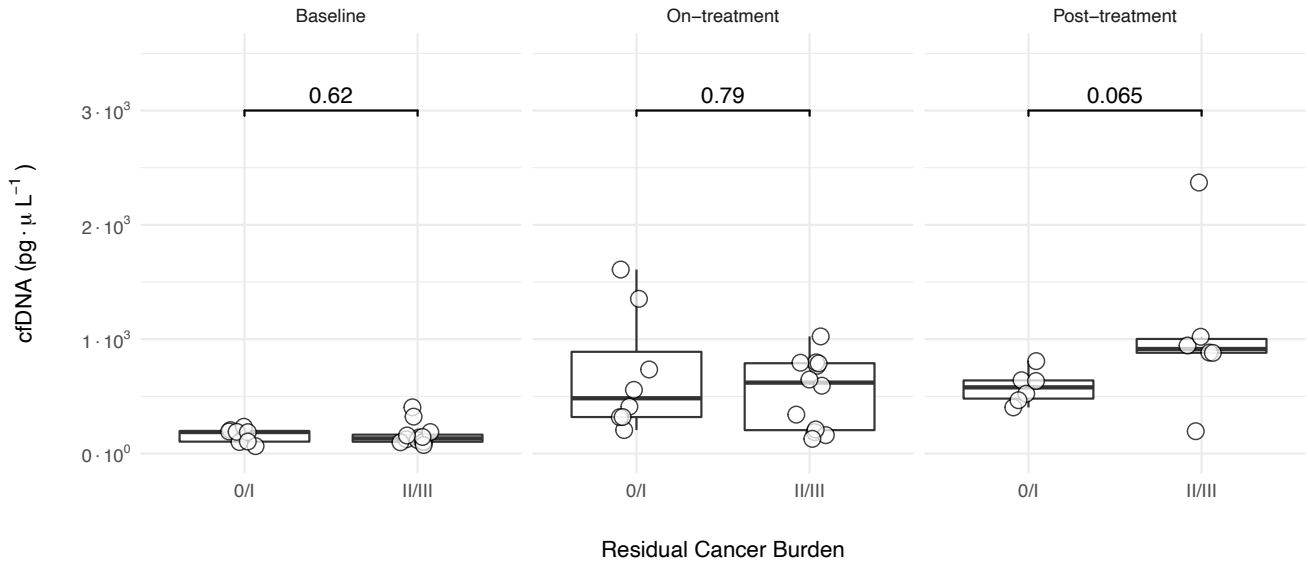
Supplementary Figure S1: Circulating cell-free DNA (cfDNA) concentration at baseline by clinicopathologic variables. Box plots showing the association between cfDNA concentration at baseline with clinical and pathological variables of breast cancer patients. In all panels, the boxes show the median and interquartile range. The whiskers extend to the full range of the data points excluding outliers. In all panels, the *p*-values were calculated using the Wilcoxon rank sum test. All tests were two-sided. HR+, hormone receptor-positive; pCR, pathological complete response; TN, triple-negative; yrs, years.



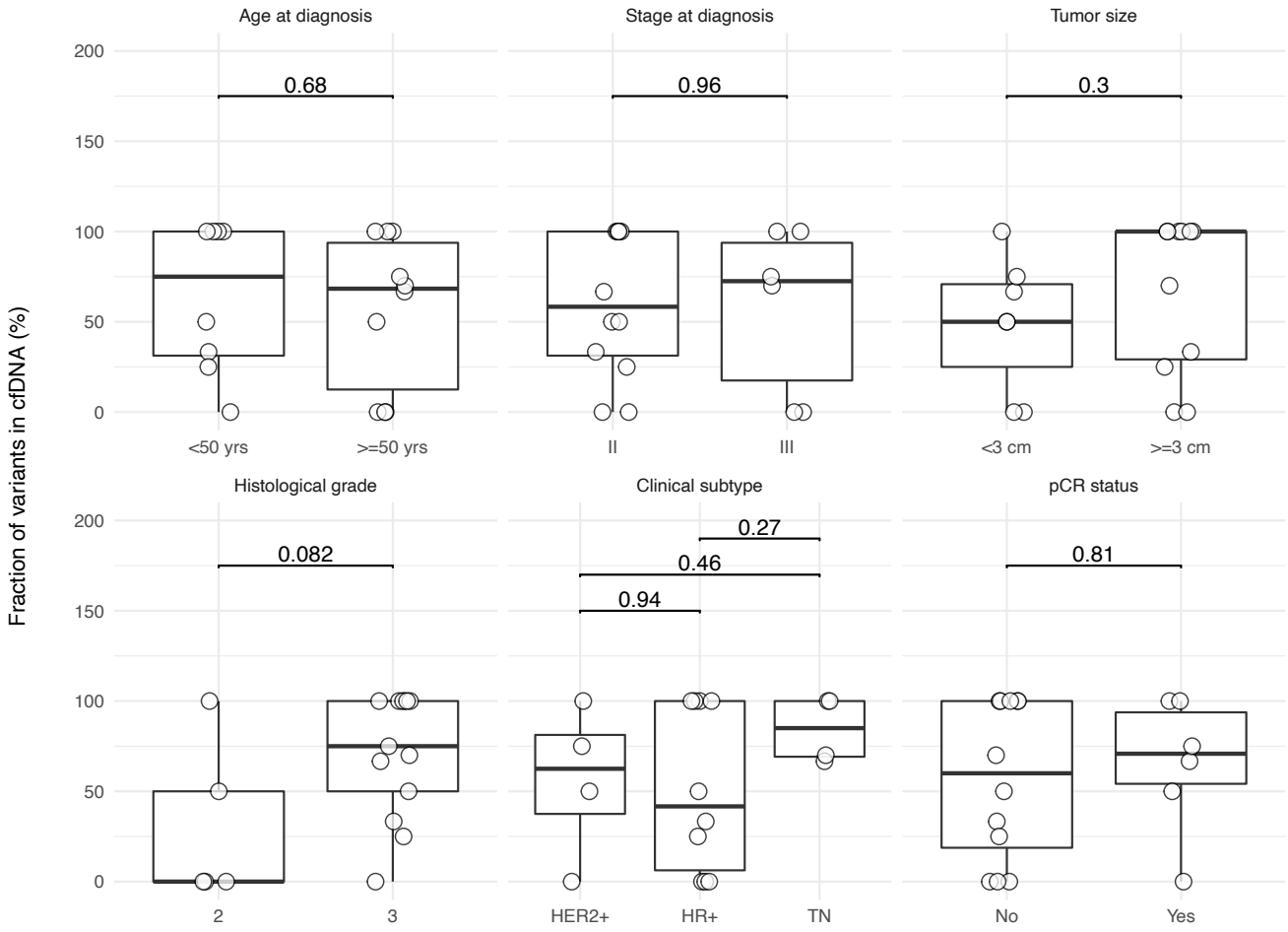
Supplementary Figure S2: Circulating cell-free DNA (cfDNA) concentration on-treatment by clinicopathologic variables. Box plots showing the association between cfDNA concentration on-treatment with clinical and pathologic variables of breast cancers. In all panels, the boxes show the median and interquartile range. The whiskers extend to the full range of the data points excluding outliers. In all panels, the *p*-values were calculated using the Wilcoxon rank sum test. All tests were two-sided. HR+, hormone receptor-positive; pCR, pathological complete response; TN, triple-negative; yrs, years.



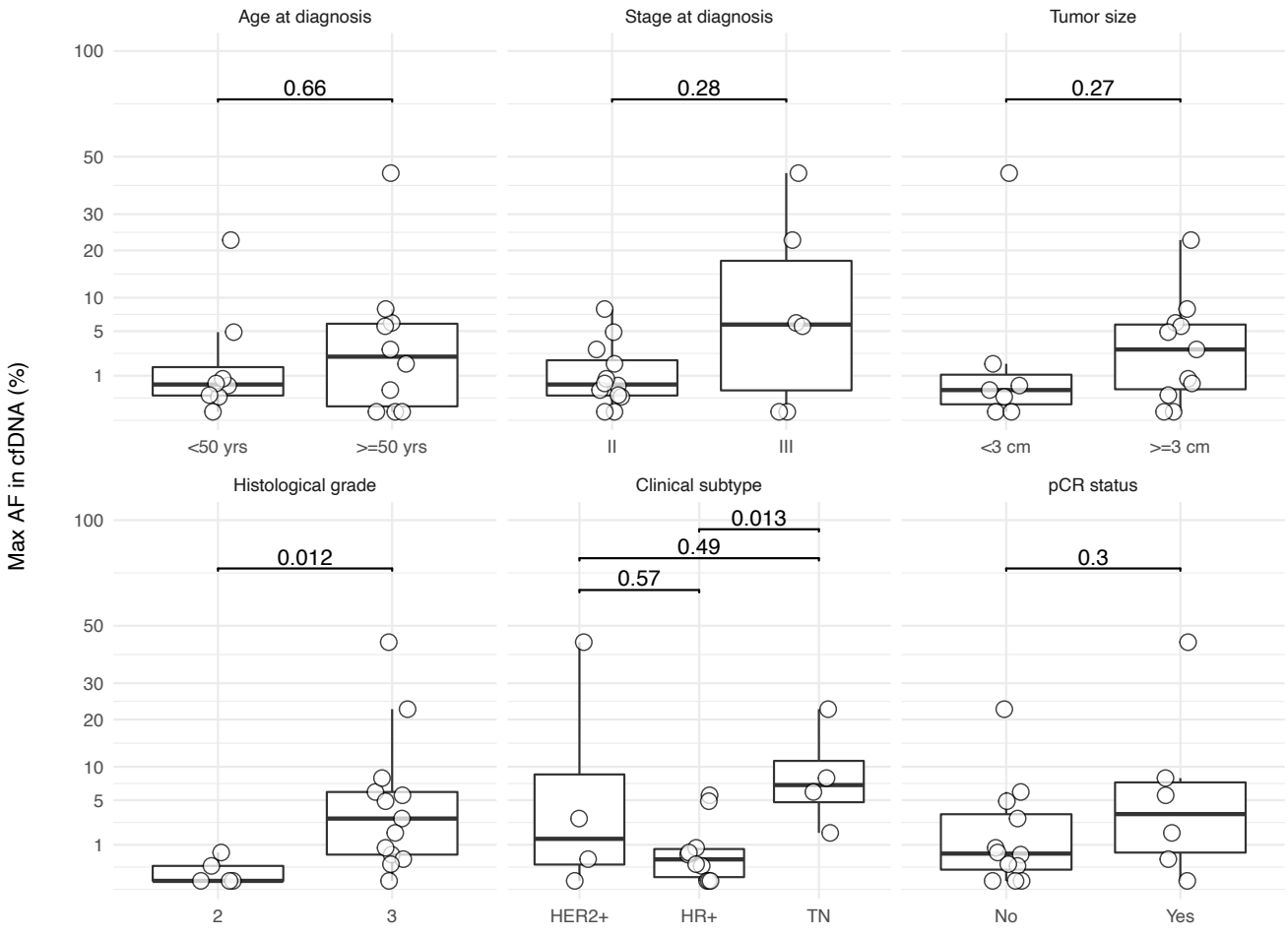
Supplementary Figure S3: Circulating cell-free DNA (cfDNA) concentration post-treatment by clinicopathologic variables. Box plots showing the association between cfDNA concentration post-treatment with clinical and pathologic variables of breast cancer patients. In all panels, the boxes show the median and interquartile range. The whiskers extend to the full range of the data points excluding outliers. In all panels, the *p*-values were calculated using the Wilcoxon rank sum test. All tests were two-sided. HR+, hormone receptor-positive; pCR, pathological complete response; TN, triple-negative; yrs, years.



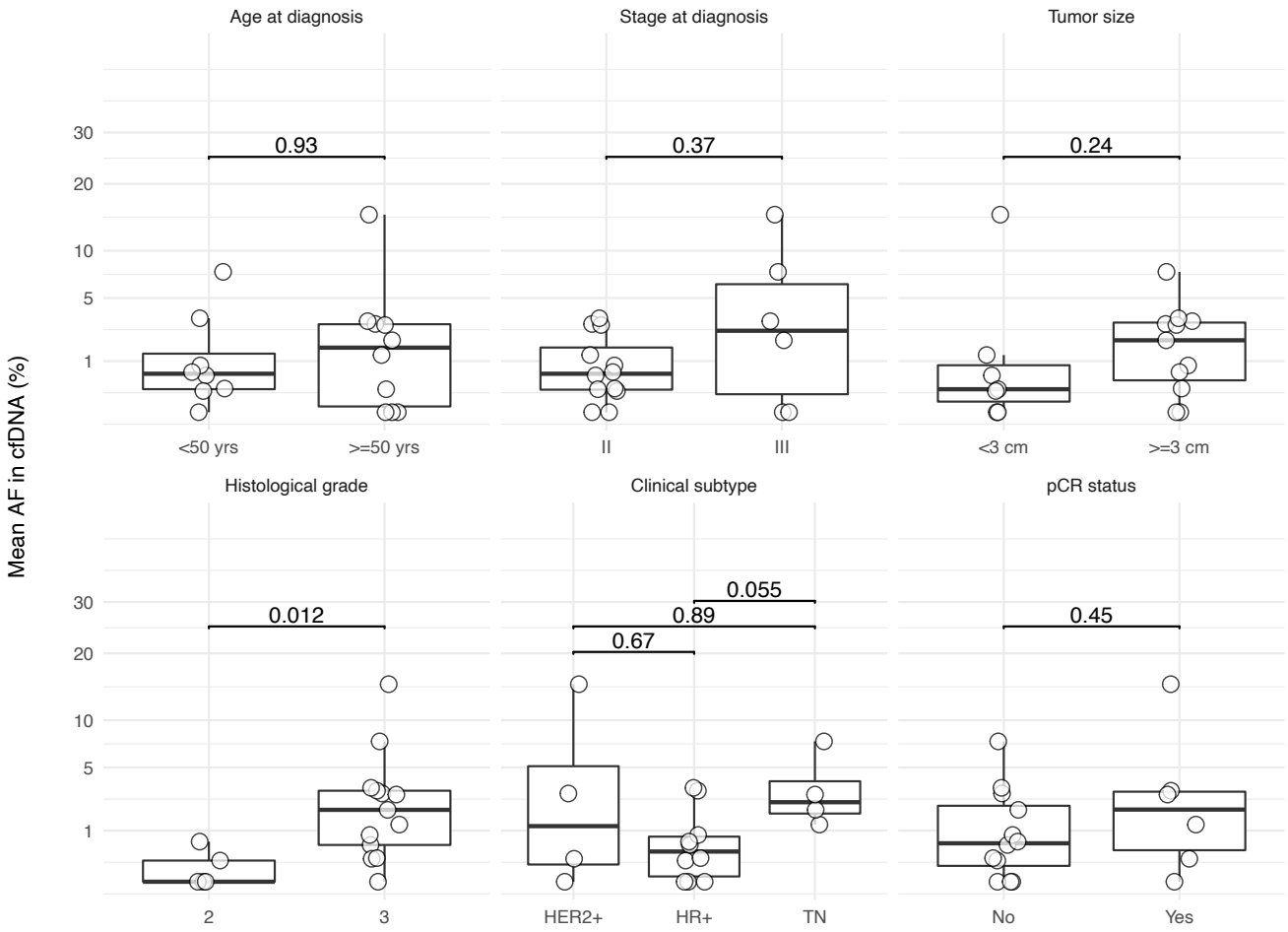
Supplementary Figure S4: Distribution of circulating cell-free DNA (cfDNA) concentration by residual cancer burden (RCB). Box plots comparing the distribution of cfDNA concentration at various time points between patients with no/low (0/I) and high (II/III) RCB. In all panels, the boxes show the median and interquartile range. The whiskers extend to the full range of the data points excluding outliers. In all panels, the *p*-values were calculated using the Wilcoxon rank sum test. All tests were two-sided.



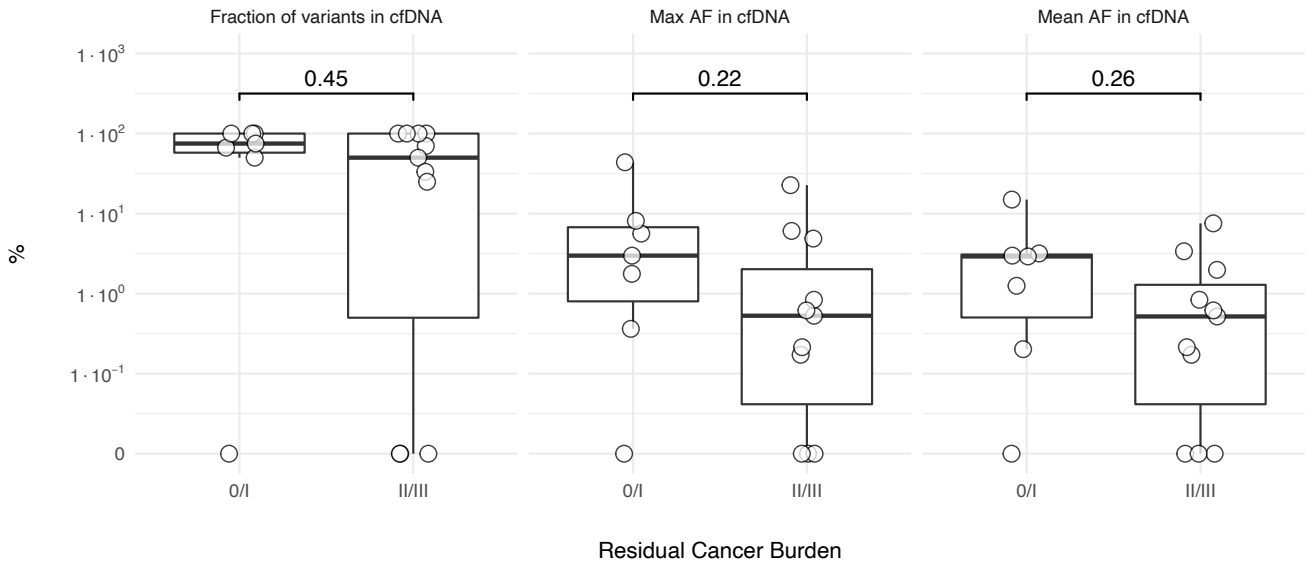
Supplementary Figure S5: Circulating tumor DNA (ctDNA) fraction defined as fraction of variants at baseline by clinicopathologic variables. Box plots showing the association between ctDNA fraction, defined as the number of variants detected in cfDNA as a fraction of the aggregate set of variants detected in the given breast cancer patient, at baseline with clinical and pathologic variables. In all panels, the boxes show the median and interquartile range. The whiskers extend to the full range of the data points excluding outliers. In all panels, the *p*-values were calculated using the Wilcoxon rank sum test. All tests were two-sided. HR+, hormone receptor-positive; pCR, pathological complete response; TN, triple-negative; yrs, years.



Supplementary Figure S6: Circulating tumor DNA (ctDNA) fraction defined as maximum allele fraction at baseline by clinicopathologic variables. Box plots showing the association between ctDNA fraction, defined as the maximum allele fraction (AF) of any detected variant, at baseline with clinical and pathologic variables of breast cancer patients. In all panels, the boxes show the median and interquartile range. The whiskers extend to the full range of the data points excluding outliers. In all panels, the *p*-values were calculated using the Wilcoxon rank sum test. All tests were two-sided. HR+, hormone receptor-positive; pCR, pathological complete response; TN, triple-negative; yrs, years.



Supplementary Figure S7: Circulating tumor DNA (ctDNA) fraction defined as mean allele fraction at baseline by clinicopathologic variables. Box plots showing the association between ctDNA fraction, defined as the mean allele fraction (AF) of all detected variants, at baseline with clinical and pathologic variables of breast cancer patients. In all panels, the boxes show the median and interquartile range. The whiskers extend to the full range of the data points excluding outliers. In all panels, the *p*-values were calculated using the Wilcoxon rank sum test. All tests were two-sided. HR+, hormone receptor-positive; pCR, pathological complete response; TN, triple-negative; yrs, years.



Supplementary Figure S8: Distribution of circulating tumor DNA (ctDNA) by residual cancer burden (RCB). Box plots comparing the distribution of ctDNA fraction at various time points between patients with low (0/I) and high (II/III) RCB. In all panels, the boxes show the median and interquartile range. The whiskers extend to the full range of the data points excluding outliers. In all panels, the *p*-values were calculated using the Wilcoxon rank sum test. All tests were two-sided.