Table SI. Thermocycling protocol.

Protocol step	Temperature	Time	Number of cycles
Step 1	95˚C	10 min	
Step 2a	95˚C	15 sec	44 cycles
Step 2b	56˚C	1 min	44 cycles
Step 3	98˚C	10 min	
Sample ramp rate: 1.7°C/sec.			

Table SII. Details on primers and probes.

Targeted sequence	Fluorophore	Probe sequence, 5'-3'	Final conc., µM	Forward primer sequence, 5'-3' (stock conc. 4 µM)	Reverse primer sequence, 5'-3' (stock conc. 4 µM)
Albumin	VIC	AGGGTTTTTATAATTTA	0.4	GGGATGGAAAGAATTTTATGTT	AAACAAACTAACCCCAAATTCT
HOXA9	FAM	TTAGTTTAAGGCGACGGTGTT	0.2	GAGTATTTCGATTTTAGTTCGTGT	CGCGTACACTAAATTCCAC

Primers and *HOXA9* probes were purchased from LGC Biosearch Technologies (Aarhus, Denmark), and the albumin probe from Thermo Fisher Scientific (Waltham, MA, USA).

Table SIII. HER2 assessment criteria in breast cancer.

A, HER2 IHC	
Result	Assessment
Score 0	No staining or membrane staining that is faint or barely perceptible and within ≤ 10% of the invasive tumor cells.
Score 1+	Incomplete membrane staining that is faint or barely perceptible and within ≥ 10% of the invasive tumor cells.
Score 2+	Weak to moderate complete membrane staining > 10% of invasive tumor cells.
Score 3+	Circumferential membrane staining that is complete, intense, and in >10% of invasive tumor cells.
B, HER2 SISH	
Result	Assessment
Positive	HER2 gene copy number and chromosome 17 centromere ratio ≥2.0.
Negative	HER2 gene copy number and chromosome 17 centromere ratio <2.0.

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; SISH, silver *in situ* hybridization.

Table SIV. Estrogen receptor assessment criteria in breast cancer.

Result	Assessment
Positive	≥1% of tumor cell nuclei are immunoreactive, but if 1-9% of tumor cell nuclei are immunoreactive, the sample is reported with an additional comment of 'low positive'.
Negative	<1% of tumor cell nuclei are immunoreactive.

Table SV. Subgroup analysis of meth-*HOXA9* in primary breast-cancer tissue. Log-rank test of the association between tumor meth-*HOXA9* and mortality in ER-positive/HER2-negative disease.

	Events		
Status	Observed	Expected	P-value
Meth-*HOXA9* in plasma +	28	26.4	0.476
Meth-*HOXA9* in plasma -	5	6.6	

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; meth-*HOXA9*, methylated homeobox A9.

Table SVI. Subgroup analysis of meth-*HOXA9* in primary breast-cancer tissue. Log-rank test of the association between tumor meth-*HOXA9* and mortality in HER2-positive disease.

	Events		
Status	Observed	Expected	P-value
Meth-*HOXA9* in plasma +	4	2.6	0.126
Meth-*HOXA9* in plasma -	0	1.4	

HER2, human epidermal growth factor receptor 2; meth-*HOXA9*, methylated homeobox A9.

Table SVII. Subgroup analysis of meth-*HOXA9* in primary breast-cancer tissue. Log-rank test of the association between tumor meth-*HOXA9* and mortality in triple-negative disease.

	Events		
Status	Observed	Expected	P-value
Meth-*HOXA9* in plasma +	2	1.3	0.433
Meth-*HOXA9* in plasma -	2	2.7	

meth-*HOXA9*, methylated homeobox A9.

Table SVIII. Sensitivity analysis of meth-*HOXA9* in primary breast-cancer tissue. HR with 95% CI of breast-cancer mortality according to breast-cancer tissue meth-*HOXA9* status (n=44) after exclusion of patients who received neoadjuvant chemotherapy.

Status	Mortality, n	Incidence, % (95% CI)	Unadjusted HR (95% CI)	Adjusted[a] HR (95% CI)
Undetectable meth-*HOXA9*	7	87.50 (44.77-98.37)	Ref.	Ref.
Detectable meth-*HOXA9*	30	83.33 (66.99-92.49)	0.99 (0.43-2.29)	1.07 (1.01-1.12)

[a]Adjusted for age at primary operation. CI, confidence interval; HR, hazard ratio; meth-*HOXA9*, methylated homeobox A9.

Table SIX. Subgroup analysis of meth-*HOXA9* in plasma at the time of breast cancer recurrence. Log-rank test of the association between plasma meth-*HOXA9* and mortality in ER-positive/HER2- negative disease according to receptor status in the metastasis.

	Events		
Status	Observed	Expected	P-value
Meth-*HOXA9* in plasma +	14	6.7	0.180
Meth-*HOXA9* in plasma -	4	11.3	

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; meth-*HOXA9*, methylated homeobox A9.

Table SX. Subgroup analysis of meth-*HOXA9* in plasma at the time of breast cancer recurrence. Log-rank test of the association between plasma meth-*HOXA9* and mortality in HER2-positive disease according to receptor status in the metastasis.

	Events		
Status	Observed	Expected	P-value
Meth-*HOXA9* in plasma +	1	0.3	0.157
Meth-*HOXA9* in plasma -	0	0.7	

HER2, human epidermal growth factor receptor 2; meth-*HOXA9*, methylated homeobox A9.

Table SXI. Subgroup analysis of meth-*HOXA9* in plasma at the time of breast cancer recurrence. Log-rank test of the association between plasma meth-*HOXA9* and mortality in triple-negative disease according to receptor status in the metastasis.

	Events		
Status	Observed	Expected	P-value
Meth-*HOXA9* in plasma +	2	1.6	0.707
Meth-*HOXA9* in plasma -	4	4.4	

meth-*HOXA9*, methylated homeobox A9.

Table SXII. Sensitivity analysis of meth-*HOXA9* in plasma at the time of breast cancer recurrence. HR with 95% CI of mortality after breast cancer recurrence according to plasma meth-*HOXA9* status (n=34) after re-classification of patients with ≤200 µl plasma.

Status	Mortality, n	Incidence, % (95% CI)	Unadjusted HR (95% CI)	Adjusted[a] HR (95% CI)
Undetectable meth-*HOXA9*	8	72.72 (40.21-91.36)	Ref.	Ref.
Detectable meth-*HOXA9*	18	78.26 (56.28-90.97)	1.57 (0.68-3.64)	2.23 (0.92-5.44)

[a]Adjusted for age at recurrence. CI, confidence interval; HR, hazard ratio; meth-*HOXA9*, methylated homeobox A9.

Table SXIII. Sensitivity analysis of meth-*HOXA9* in plasma and metastatic tissue at breast cancer recurrence. Comparison of metastatic tissue and plasma meth-*HOXA9* using Fisher's exact test (n=20) after re-classification of patients with ≤200 µl plasma.

	Meth-*HOXA9* in metastatic tissue		
Status	+	-	P-value
Meth-*HOXA9* in plasma +	14 (93.3%)	1 (6.7%)	0.447
Meth-*HOXA9* in plasma -	4 (80.0%)	1 (20.0%)	

meth-*HOXA9*, methylated homeobox A9.