

Supplementary material

Low 5-hydroxymethylcytosine level is an independent predictor of high histological grade in locally advanced breast cancer

Diddier Prada, José Díaz-Chávez, Omar Peña-Curiel, Miguel Ramírez, Elena Colicino, Cynthia Villarreal-Garza, Paula Cabrera-Galeana, Thania Castro-Belio, Nancy Reynoso, Marco Andonegui, Guiselle Navarro, David Cantú de León, Yolanda Villaseñor, Alejandro López-Saavedra, Cristian Arriaga-Canon, Carlo C. Cortés, Claudia Caro, Abelardo Meneses Garcia, Enrique Bargalló, and Luis A. Herrera

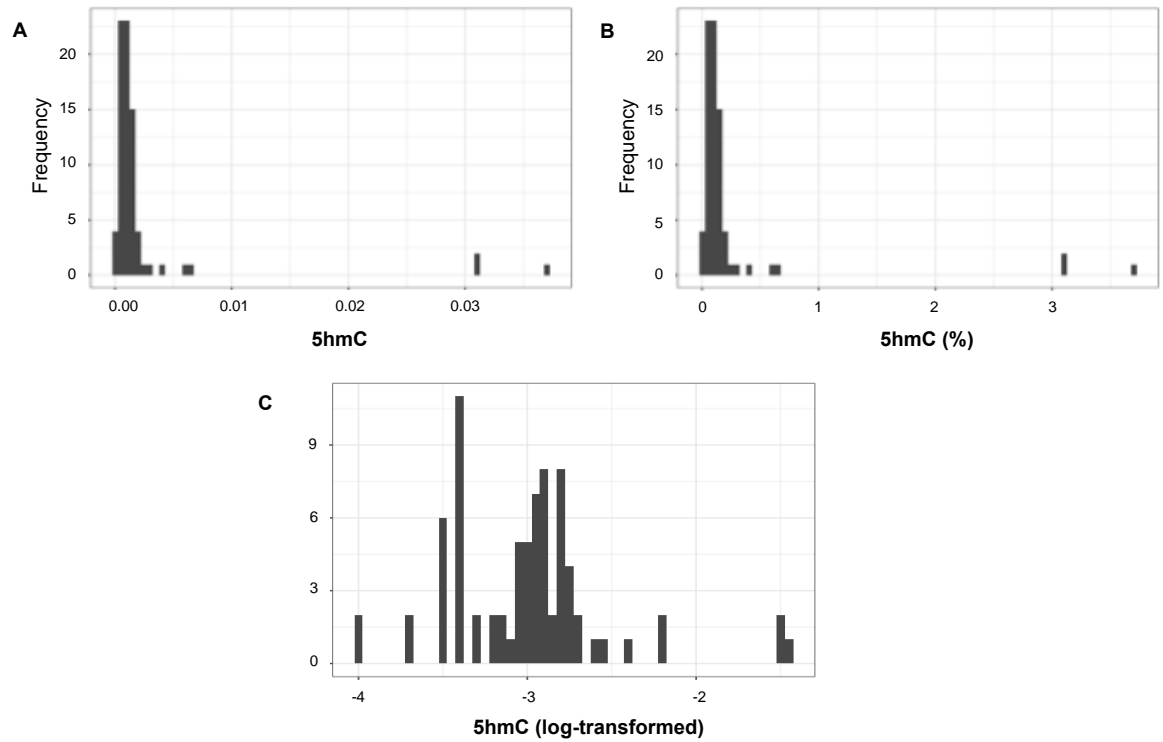
Supplementary Table

Supplementary Table Mixed effects linear model (unadjusted) for the association between 5hmC levels^a and age^b in patients attended in the National Cancer Institute - Mexico, 2013-2015 (N=141).

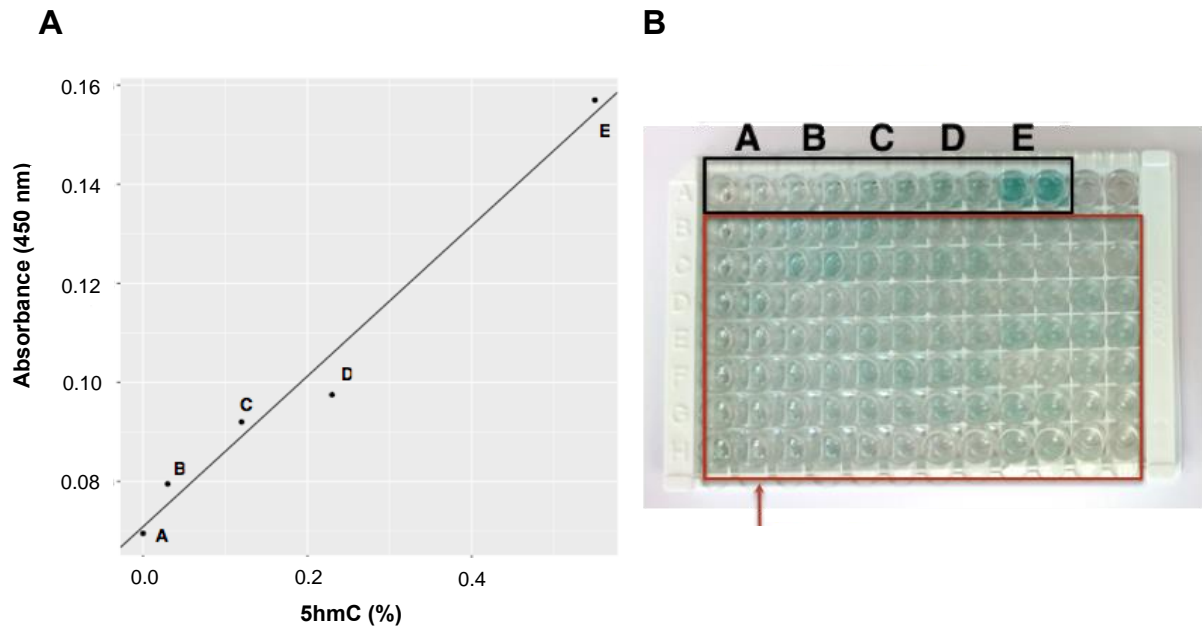
	<i>n</i>	β^c	<i>CI 95%</i>	<i>p-value</i>
All samples	141	-0.006	(-0.015, 0.003)	0.256
ER/PR (+)	114	-0.002	(-0.013, 0.009)	0.686
ER/PR (-)	27	-0.028	(-0.045, -0.010)	0.005*
HER2 (+)	8	-0.040	(-0.432, 0.352)	0.063
Triple negative	19	-0.023	(-0.044, -0.001)	0.046*

^aLog-transformed; ^bAs continuous variables. ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal receptor 2; CI 95%: confidence interval 95%. ^cEstimate for the association using mixed linear model including random intercept for duplicate samples; *: Statistically significant variables associated with the dependent variable.

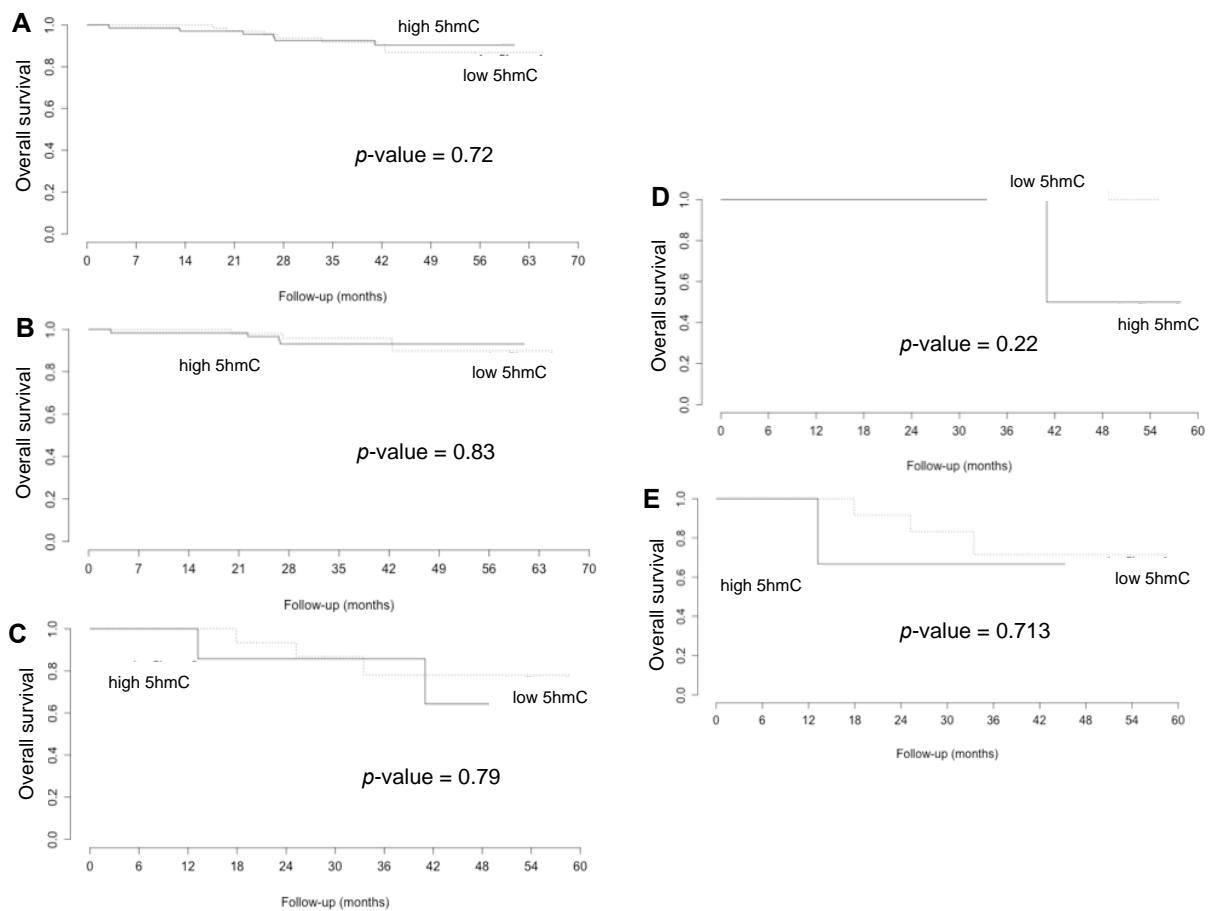
Supplementary Figures



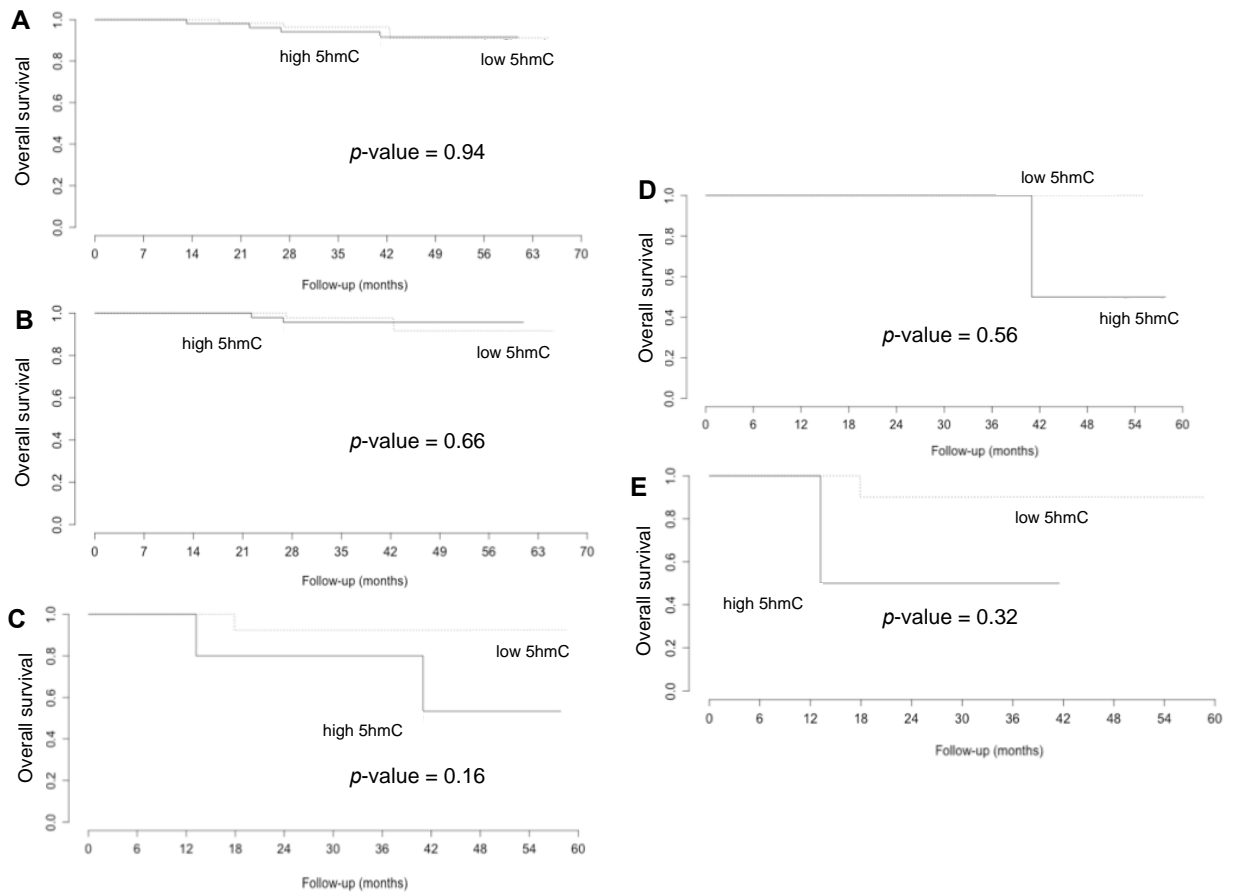
Supplementary Figure 1 Distribution of 5hmC levels in breast cancer samples at first biopsy. (A) Raw 5-hydroxymethylation (5hmC) levels. (B) Percentage levels of 5hmC. (C) Log-transformed values of 5hmC. Figures include outlier values.



Supplementary Figure 2 Determination of 5hmC levels in breast cancer samples. Panel A. Correlation between samples with known concentration of 5hmC and 450 nm absorbance, determined using immunoabsorbance (ELISA) y-axis: Mean immunoabsorbance (2 samples), x-axis: 5hmC percentage. Panel B. Immunoabsorbance plate with samples with known concentration of 5hmC (first ten rows, from A to E) and samples from breast cancer patients (red square and arrow).



Supplementary Figure 3 Overall survival according to 5hmC level (high vs. low, using the median value) in the initial biopsy samples from patients treated in the National Cancer Institute – Mexico, 2013-2015. (A) All samples (N = 141). (B) ER/PR (+): Tumors positive for estrogen and progesterone receptors (n = 113). (C) ER/PR (-): Tumors negative for estrogen and progesterone receptors (n = 28). (D) HER2+: Tumors positive for HER2 (n = 15). (E) Triple negative tumors (n = 24). The p -values were calculated using the log-rank test.



Supplementary Figure 4 Relapse-free survival according to 5hmC level (high vs. low, using the median value) in the initial biopsy samples from patients treated in the National Cancer Institute – Mexico, 2013-2015. (A) Locally advanced breast cancer (LABC) samples (N = 111). (B) LABC - ER/PR (+): Tumors positive for estrogen and progesterone receptors (n=92). (C) LABC - ER/PR (-): Tumors negative for estrogen and progesterone receptors (n = 19). (D) LABC - HER2+: Tumors positive for HER2 (n = 6). (E) LABC - Triple negative tumors (n = 13). The *p*-values were calculated using the log-rank test.