

# **Supplemental Material**

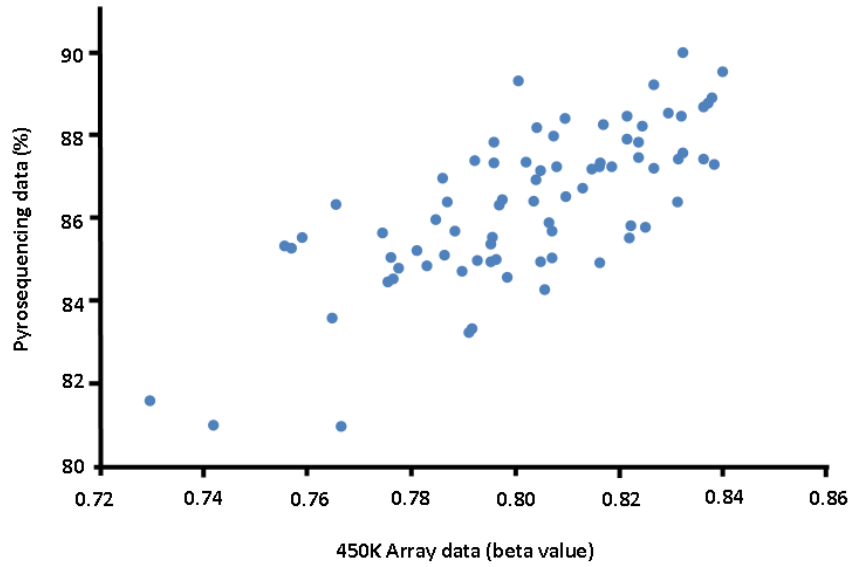
## **Hormone therapy use and breast tissue DNA methylation: analysis of epigenome wide data from the normal breast study**

Sophia Harlid, Zongli Xu, Erin Kirk, Lauren E. Wilson, Melissa A. Troester, Jack A. Taylor\*

\*Correspondence to: Jack A. Taylor, MD, PhD, National Institute of Environmental Health Sciences, MD A3-05, 111 T.W. Alexander Drive, PO Box 12233, Research Triangle Park, NC 27709. E-mail: [taylor@niehs.nih.gov](mailto:taylor@niehs.nih.gov). Phone: (919) 541-4631, Fax : (919) 541-2511

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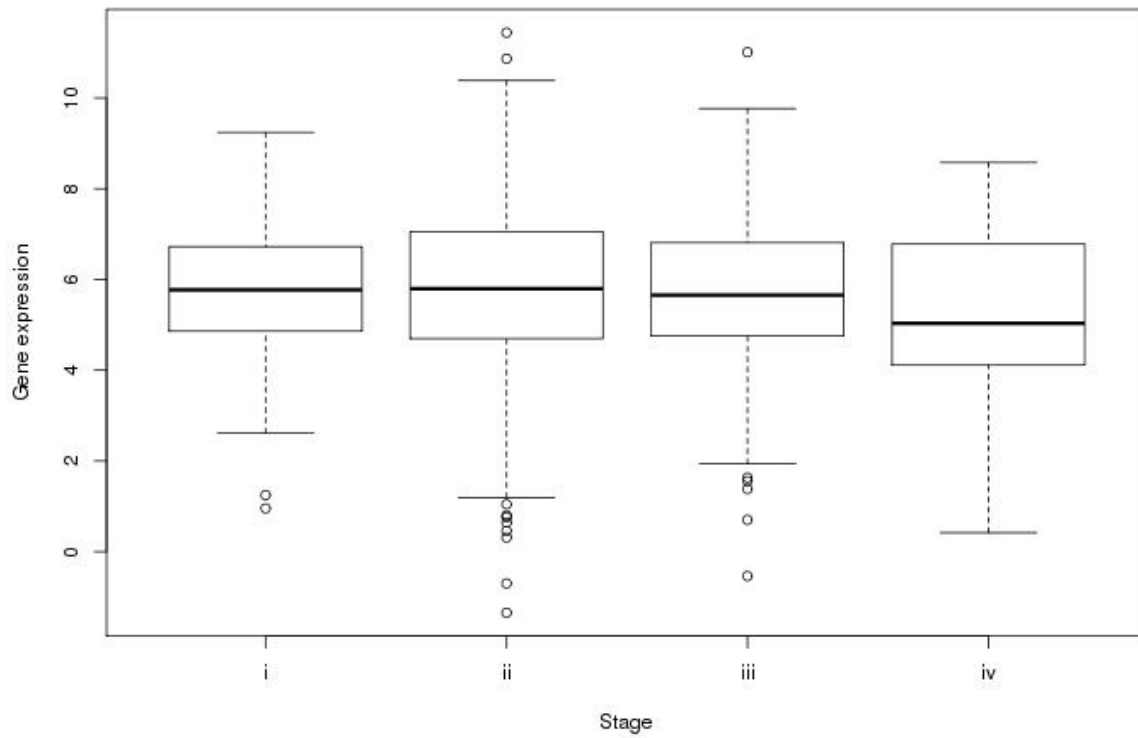
**Fig. S1**



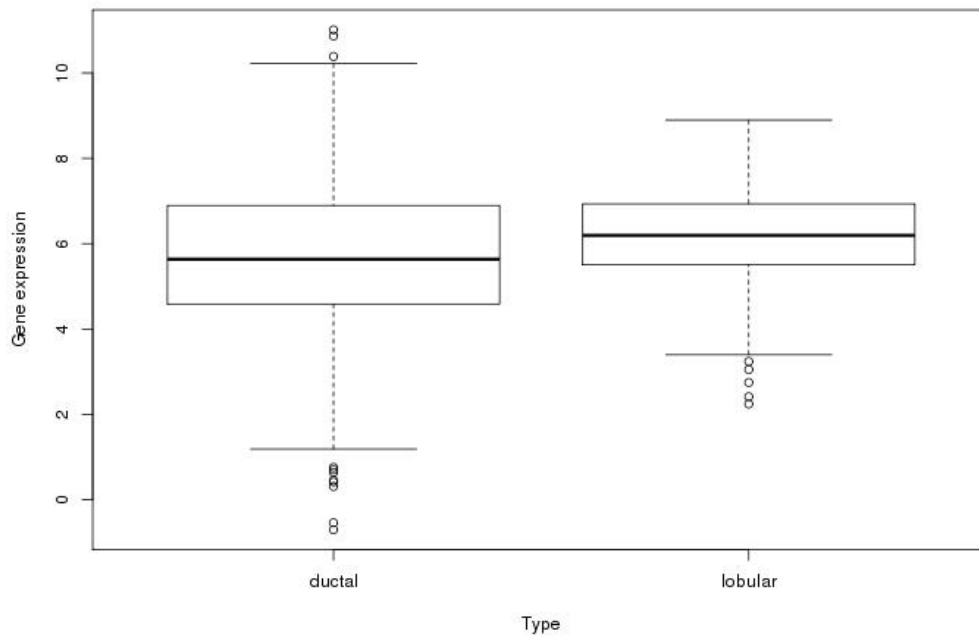
**Fig. S1** Correlation between raw 450K beta values and % methylation derived using pyrosequencing. Pearson's correlation = 0.73

**Fig. S2**

**a**

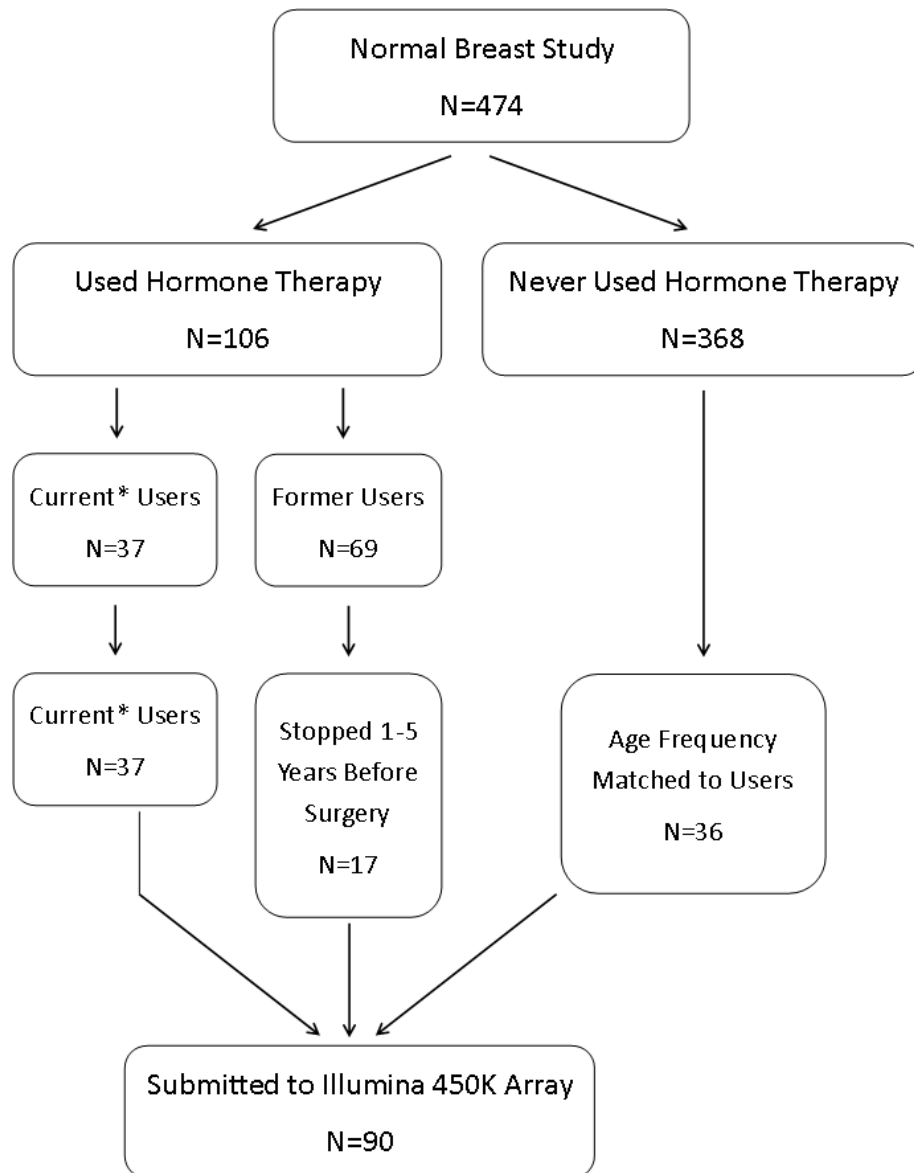


**b**



**Fig. S2** *ARHGEF4* gene expression related to breast cancer stage and subtype (data from TCGA). a) Expression of *ARHGEF4* in tumor samples from different stages of breast cancer (I-IV), b) Expression of *ARHGEF4* in tumor samples from ductal and lobular breast cancer.

**Fig. S3**



**Fig. S3** Flowchart describing the selection of samples for DNA methylation analysis.

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**Table S1.** Participant characteristics (HT-users only)

Characteristic	HT users N (%)
<b>Time since quitting HT</b>	
0 years*	4 (7)
<1 year	33 (61)
1-5 years	17 (32)
<b>Type of HT</b>	
Combined	26 (48)
Estrogen only	12 (22)
Progesterone only	2 (4)
Missing	14 (26)
<b>Duration of HT use</b>	
<1 year	1
1-5 years	18
6-10 years	9
>10 years	26

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**Table S2.** CpGs significant in at least one test

*Available as excel sheet*

**Table S3.** Mean methylation values (%) from pyrosequencing and Illumina450K bead array of cg26334888 (FAM3D)

Probe	CHR	Gene	Method	EVER/NEVER		Duration of use			Recency of use		
				Ever	Never	1-5 years	6-10 years	>10 years	Current	Former	Never
cg26334888	3	FAM3D	<b>Pyroseq. (%)</b>	86.6	86.0	86.9	86.4	86.5	86.8	86.1	86.0
cg26334888	3	FAM3D	<b>Array (%)</b>	81.0	79.0	81.2	80.8	80.9	81.4	80.0	79.0

**Table S4.** Mean beta-values for the twelve CpGs that were significant in all three analyses

Probe	CHR	Gene / Location	Ever / Never		Duration of use			Recency of use		
			Ever	Never	1-5 years	6-10 years	>10 years	Current	Former	Never
cg01382688	2	ARHGEF4	0.930	0.914	0.928	0.932	0.932	0.930	0.930	0.914
cg01571735	2	SP5	0.037	0.032	0.037	0.037	0.038	0.037	0.037	0.032
cg02245004	15	15q24	0.076	0.102	0.076	0.082	0.073	0.076	0.075	0.102
cg08460153	7	TNRC18	0.014	0.011	0.014	0.012	0.014	0.013	0.014	0.011
cg08975834	10	TCERG1L	0.886	0.863	0.879	0.881	0.892	0.887	0.885	0.863
cg09742643	7	PTPRN2	0.910	0.867	0.908	0.917	0.909	0.913	0.906	0.867
cg11034191	4	INPP4B	0.021	0.017	0.019	0.023	0.022	0.020	0.022	0.017
cg15028339	5	RAI14	0.915	0.891	0.914	0.915	0.916	0.918	0.912	0.891
cg16416165	4	RGS12	0.962	0.950	0.963	0.963	0.961	0.962	0.961	0.950
cg26098117	6	6q22	0.879	0.846	0.876	0.876	0.882	0.885	0.872	0.846
cg26185079	18	SLMO1	0.856	0.839	0.852	0.864	0.856	0.866	0.843	0.839
cg26705583	5	SGTB	0.027	0.022	0.028	0.025	0.027	0.028	0.025	0.022

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**Table S5.** Pyrosequencing primers.

<b>Primers for FAM3D, cg26334888</b>	<b>Sequence</b>
Forward Primer	5'- TGGGGTATATTGAATTAGAGGTTAAAGG-3'
Reverse Primer	5'-Biotin- CCACTAAACAACCCTTCTTTTCTT-3'
Sequencing Primer	5'- GTGTGGAGAAAGGGA-3'