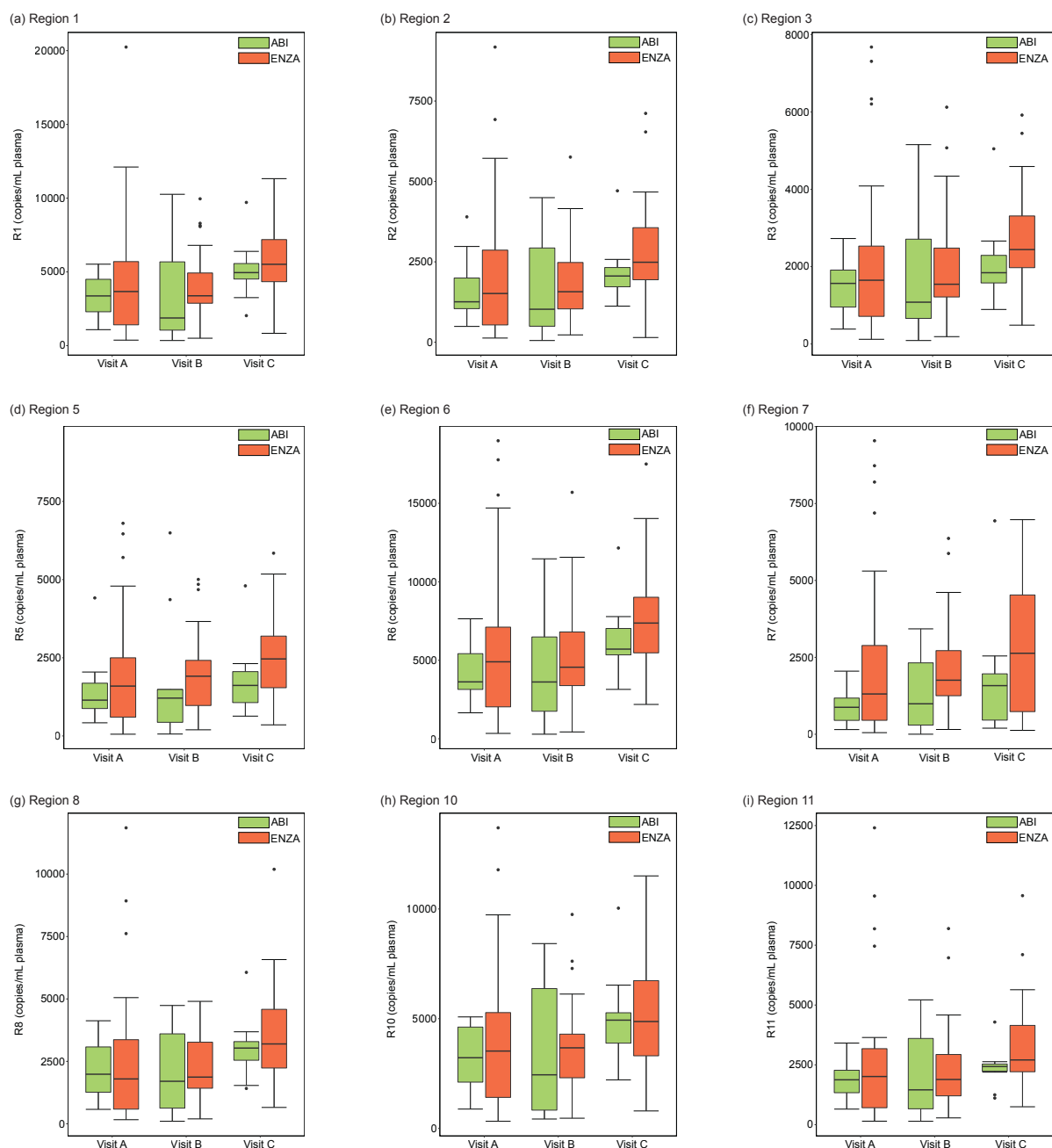


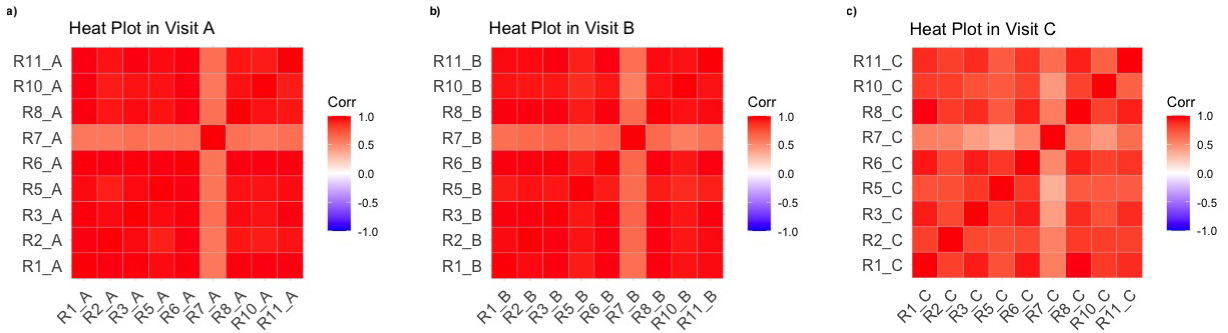
**Supplementary Figure 1. Overview of the changes in methylation levels of each region from visit A to C for each patient.**

For each region analyzed, methylation levels (copies/mL) are plotted for patients with all study visits analyzed. Each line represents a single patient. Changes in methylation over time was assessed using the Friedman test, with pairwise comparisons between study visits. N= 26 patients with cfDNA from visits A, B and C.

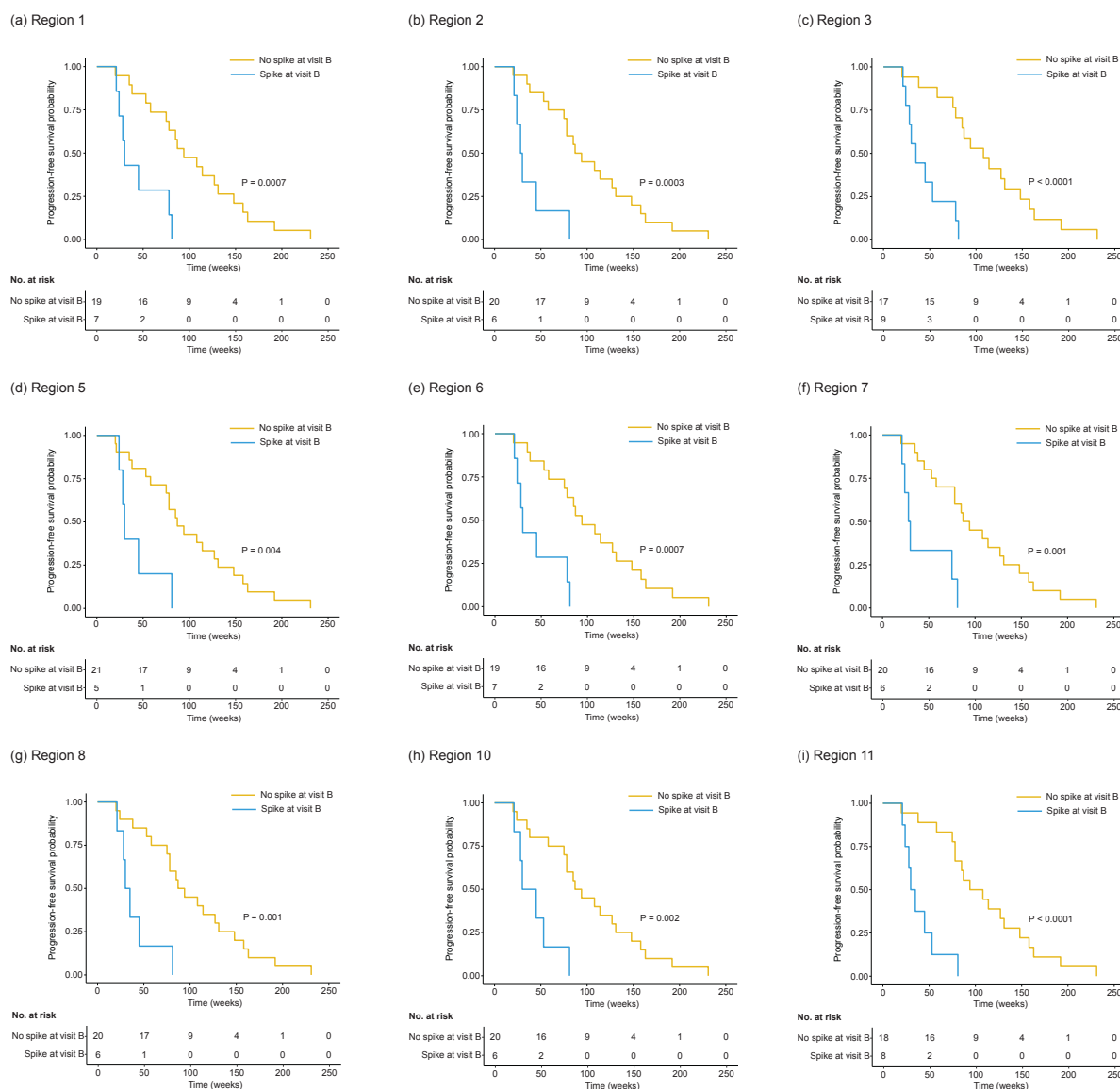


## Supplementary Figure 2. Methylation levels of each candidate region stratified by treatment type.

Boxplots show the median, first and third quartile methylation levels (copies/mL) for (a) Region 1, (b) Region 2, (c) Region 3, (d) Region 5, (e) Region 6, (f) Region 7, (g) Region 8, (h) Region 10, and (i) Region 11. Mann-Whitney U test was used to compare methylation levels between enzalutamide (ENZA)-treated and abiraterone acetate (ABI)-treated patients for each visit. N = 37 ENZA-treated patients, N = 12 ABI-treated patients.

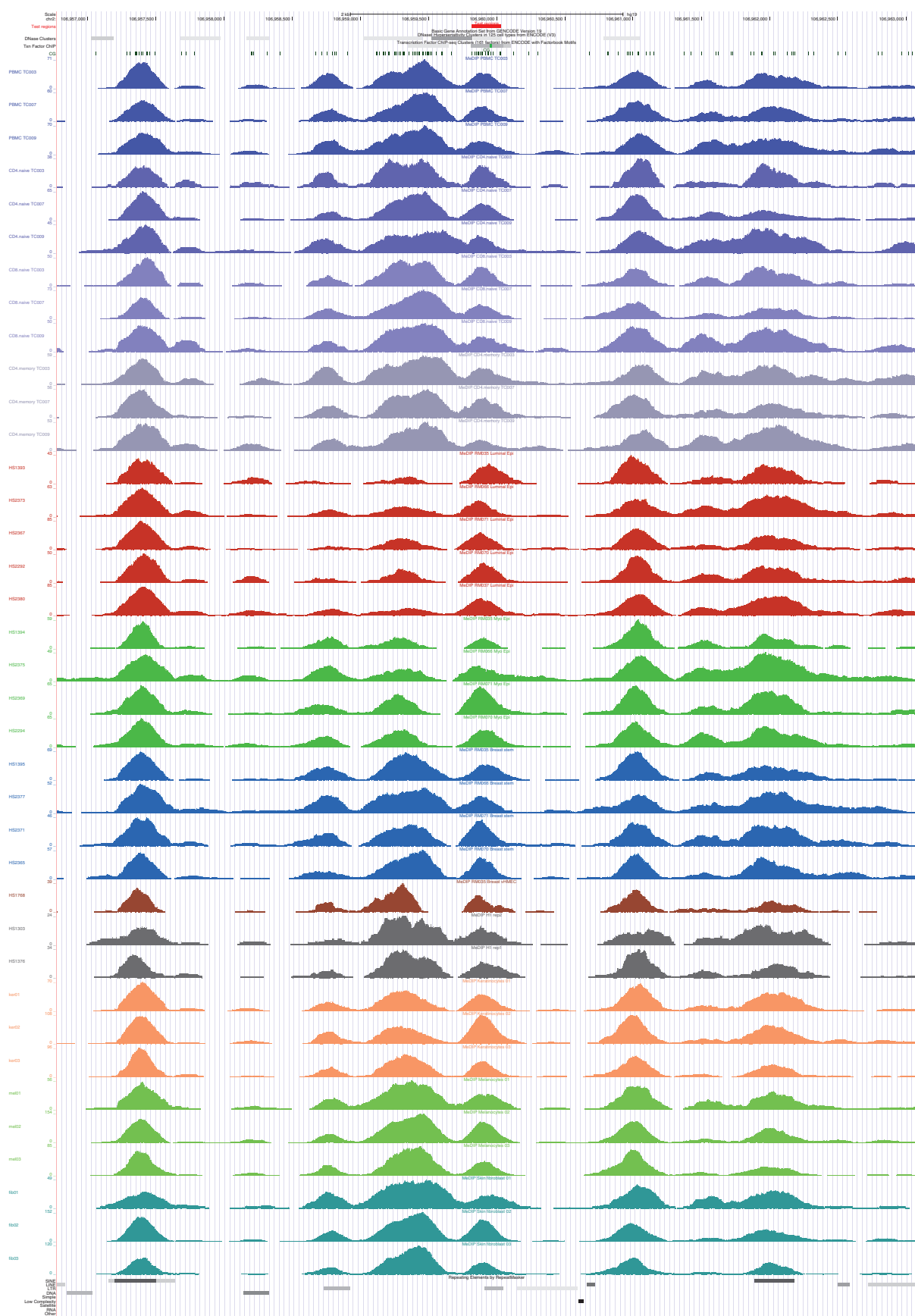


**Supplementary Figure 3. Correlation matrices of methylation levels for all regions.**  
Heatmaps show correlation matrices of methylation values (copies/mL) between all regions for (a) visit A, (b) visit B, (c) and visit C.



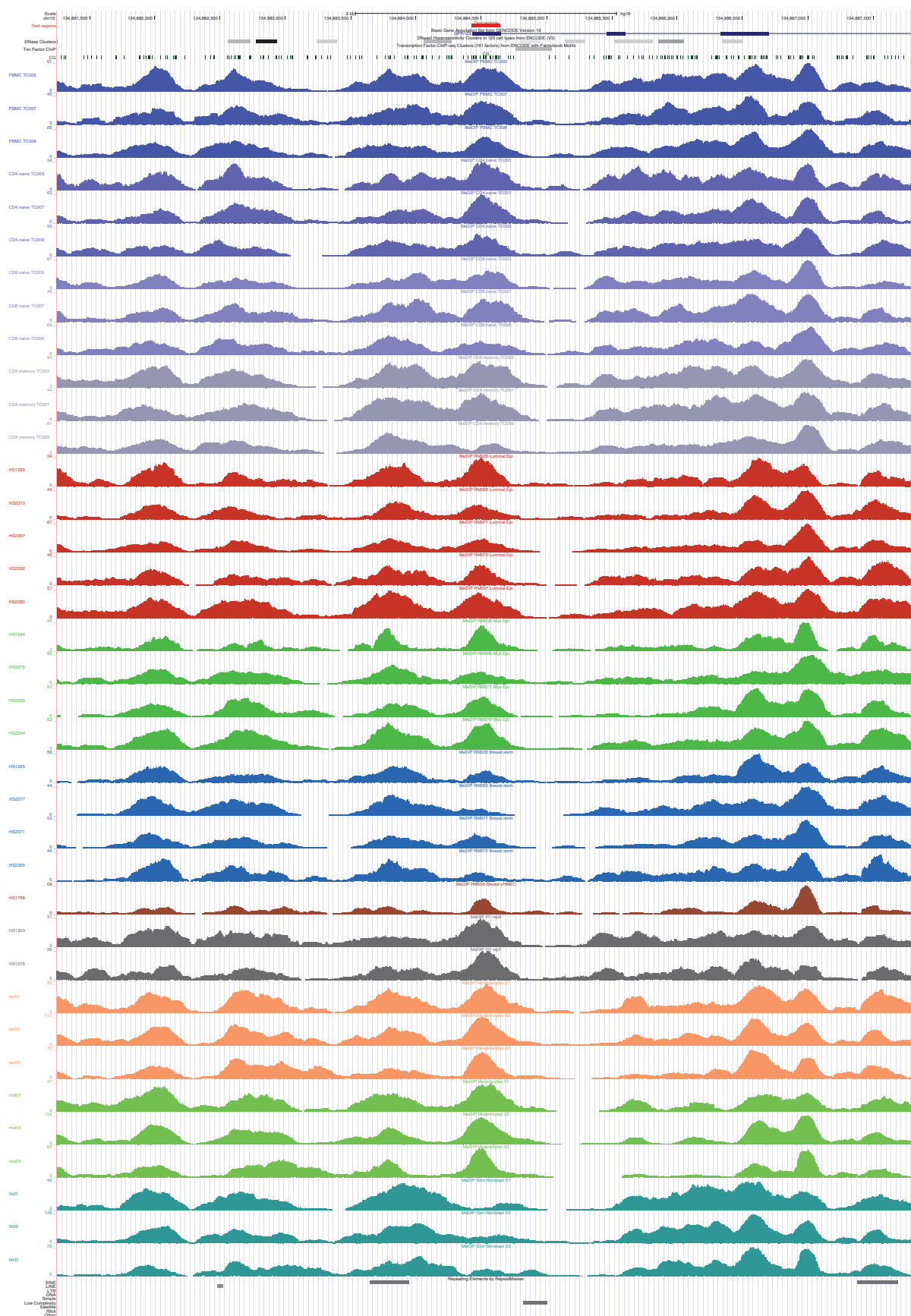
**Supplementary Figure 4. Kaplan–Meier analysis of association between methylation spike status at visit B and TTCP.**

For each region, patients were stratified based on whether they demonstrated a spike in methylation at visit B compared to visits A and B (No spike vs. Spike). Kaplan-Meier analysis was performed for TTCP (progression-free survival) and log-rank test results are shown for each region.



**Supplementary Figure 5. DNA methylation patterns of Region 1 in other tissue/cell types.**

Methylation of R1 (red bar/test region on top) is shown in different cell/tissue types from the Roadmap Epigenomics Consortium: peripheral blood mononuclear cells (PBMCs), CD4+ naive T cells, CD8+ naive T cells, CD4+ memory T cells, luminal epithelial cells (red), myoepithelial cells (green), progenitor/stromal mammary cells (breaststem) (light blue), in-vitro transformed breast epithelial cells (vHMEC) (brown), H1 human embryonic stem cells (dark grey), keratinocytes (orange), melanocytes (light green), skin fibroblasts (navy green).



**Supplementary Figure 6. DNA methylation patterns of Region 5 in other tissue/cell types.**

Methylation of R5 (red bar/test region on top) is shown in different cell/tissue types from the Roadmap Epigenomics Consortium: peripheral blood mononuclear cells (PBMCs), CD4+ naive T cells, CD8+ naive T cells, CD4+ memory T cells, luminal epithelial cells (red), myoepithelial cells (green), progenitor/stromal mammary cells (breaststem) (light blue), in-vitro transformed breast epithelial cells (vHMEC) (brown), H1 human embryonic stem cells (dark grey), keratinocytes (orange), melanocytes (light green), skin fibroblasts (navy green).