Supplementary Figure S1.   Unpaired 2-class SAM comparing benign adjacent prostate tissues and tumors.   Benign adjacent vs tumor unpaired 2-class SAM analysis of the 181 prostate samples. False discovery rate of 0.78% resulted in 8,063 differentially methylated CpGs including 5,912 hypermethylated CpGs (red) and 2,151 hypomethylated CpGs (green).

Supplementary Figure S2.   Paired 2-class SAM comparing benign adjacent prostate tissues and tumors.   Benign adjacent vs tumor paired 2-class SAM analysis of the 181 prostate samples. False discovery rate of 0.78% resulted in 7,741 differentially methylated CpGs including 5,556 hypermethylated CpGs (red) and 2,185 hypomethylated CpGs (green).

Supplementary Figure S3.   *APC* proximal promoter hypermethylation in prostate tumors. (*A*) Diagram of the RefSeq annotation of the *APC* gene. There are no CpG islands, calculated by the UCSC Genome Browser, in this window.   Circles are CpG sites assayed by HumanMethylation27: red circles represent probes that were identified to be hypermethylated in prostate tumors by 2-class SAM. The numbers above and below the circles indicate the relative distance in base pairs from the predicted TSS. (*B*) Heatmap depicts DNA methylation pattern of the 6 probes near *APC*. The dendrogram is based on the hierarchical clustering from Figure 2. Red branches represent tumor samples and blue branches represent benign adjacent samples. Coordinates are based on NCBI36/hg18 human genome assembly.

Supplementary Figure S4.   *RASSF1* proximal promoter hypermethylation in prostate tumors. (*A*) Diagram of the RefSeq annotation of the *RASSF1* gene. Green boxes represent the CpG islands calculated by UCSC Genome Browser.   Circles are CpG sites assayed by HumanMethylation27: red circles represent probes that were identified to be hypermethylated in prostate tumors by 2-class SAM and the gray circles represent probes that showed no significant

change. The numbers above and below the circles indicate the relative distance in basepairs from the predicted TSS. (*B*) Heatmap depicts DNA methylation pattern of the 9 probes near *RASSF1*. The dendrogram is based on the hierarchical clustering from Figure 2. Red branches represent tumor samples and blue branches represent benign adjacent samples. Coordinates are based on NCBI36/hg18 human genome assembly.

Supplementary Figure S5. Diagnostic markers of prostate cancer identified by PAM. Unsupervised hierarchical clustering of 181 prostate samples based on the 87 diagnostic CpG sites identified by PAM. Red branches represent tumor samples and blue branches represent benign adjacent samples. Red pixels represent high DNA methylation while green pixels represent low DNA methylation.

Supplementary Figure S6. PyroMark validates HumanMethylation27 results. PyroMark sequencing results compared to HumanMethylation27 beta scores at 9 diagnostic CpGs identified by PAM. Blue circles are benign adjacent samples and red circles are tumor samples. Y-axis: fraction methylation calculated from PyroMark. X-axis: fraction methylation calculated from HumanMethylation27 (beta scores). Black line: linear regression. (*A*) *CYBA* (cg19790294). (*B*) *GDAP1L1* (cg04448487). (*C*) *HIF3A* (cg02879662). (*D*) *LGLS1* (cg19853760). (*E*) *LOC387758* (cg04622802). (*F*) *MCAM* (cg21096399). (*G*) *RPIP8* (cg13102585). (*H*) *RAB33A* (cg24340926). (*I*) *SCGB2A2* (cg22862656).

Supplementary Figure S7. Comparison of neighboring CpGs by PyroMark. PyroMark sequencing results comparing neighboring CpGs of the 9 diagnostic CpGs identified by PAM. Each diamond represents a CpG methylation level for an individual sample. Lines connect CpGs from each sample. Blue lines are benign adjacent samples, red lines are tumor samples. Y-axis:

fraction methylation calculated from PyroMark. X-axis: relative coordinates in basepairs. Box indicates CpG assayed by HumanMethylation27. (*A*) *CYBA* (cg19790294). (*B*) *GDAP1L1* (cg04448487). (*C*) *HIF3A* (cg02879662). (*D*) *LGLS1* (cg19853760). (*E*) *LOC387758* (cg04622802). (*F*) *MCAM* (cg21096399). (*G*) *RPIP8* (cg13102585). (*H*) *RAB33A* (cg24340926). (*I*) *SCGB2A2* (cg22862656).

Supplementary Table S1. Clinical information associated with prostate samples . For each sample: PC# (unique sample-specific ID), Age (patient age at time of surgery), Pre-treatment PSA score, Pathology Grade (Gleason score), Months followed-up (after time of surgery), Recurrence (biochemical), Days to recurrence (after time of surgery).

Supplementary Table S2. Differentially methylated CpGs identified by unpaired 2-class SAM. CpG ID: Designated by Illumina. Chr/MapInfo: chromosome number and coordinates based on NCBI36/hg18. SourceSequence: sequence upstream of the CpG. RefSeq Gene_ID/GID/Accession/Symbol/Gene_Strand/TSS_Coordinate: annotatino of nearest gene provided by Illumina. Fold Change: fold change in beta score between benign adjacent and tumor calculated by SAM. q-value (%): indicates lowest FDR at which the site is called significant.

Supplementary Table S3. Promoter with differentially methylated CpGs identified by unpaired 2-class SAM. List of 4,004 gene promoters with only hypermethylated CpGs, 1,572 gene promoters with only hypomethylated CpGs and 223 gene promoters with at least one hypermethylated and at least one hypomethylated CpG. Both RefSeq GeneID and gene symbol are listed for each promoter.

Supplementary Table S4. Diagnostic methylation markers of prostate cancer identified by PAM. CpG ID: Designated by Illumina. Chr/MapInfo: chromosome number and coordinates based on NCBI36/hg18. SourceSequence: sequence upstream of the CpG. RefSeq Gene_ID/GID/Accession/Symbol/Gene_Strand/TSS_Coordinate: annotatino of nearest gene provided by Illumina.

Supplementary Table S5. CpG sites with methylation patterns that correlated with time-to-recurrence after radical prostatectomy identified by SAM. CpG ID: Designated by Illumina. Chr/MapInfo: chromosome number and coordinates based on NCBI36/hg18. SourceSequence: sequence upstream of the CpG. RefSeq Gene_ID/GID/Accession/Symbol/Gene_Strand/TSS_Coordinate: annotation of nearest gene provided by Illumina. q-value (%): indicates lowest FDR at which the site is called significant.

Supplementary Table S6. Primer sequences used in PyroMark assays. Target CpG (CpG ID designated by Illumina to be assayed), Promoter (gene promoter associated with the CpG), Primer (orientation/function), Sequence (in 5' to 3' orientation).

Supplementary Table S7. Differentially methylated CpGs identified by paired 2-class SAM. CpG ID: Designated by Illumina. Chr/MapInfo: chromosome number and coordinates based on NCBI36/hg18. SourceSequence: sequence upstream of the CpG. RefSeq Gene_ID/GID/Accession/Symbol/Gene_Strand/TSS_Coordinate: annotatino of nearest gene provided by Illumina. Fold Change: fold change in beta score between benign adjacent and tumor calculated by SAM. q-value (%): indicates lowest FDR at which the site is called significant.