Supplementary Information

of

Epigenetically reprogrammed methylation landscape drives the DNA self-assembly and serves as a universal cancer biomarker

Abu Ali Ibn Sina†[1], Laura G. Carrascosa†*[1], Ziyu Liang,[1] Yadveer S. Grewal,[1] Andri Wardiana,[1] Muhammad J. A. Shiddiky,[1] , Frank Gardiner[4], Hemamali Samaratunga[4], Maher Gandhi[5], Rodney Scott[3], Darren Korbie,[1] and Matt Trau*[1,2]

[1]Center for Personalised Nanomedicine, Australian Institute for Bioengineering and Nanotechnology (AIBN), Corner of College and Cooper Roads (Bldg 75), The University of Queensland, Brisbane QLD 4072, Australia

[2]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD 4072, Australia.

[3]School of Biomedical Science and Pharmacy, The University of Newcastle, Callaghan NSW 2308 Australia

[4]School of Medicine, The University of Queensland, Brisbane, QLD 4072, Australia

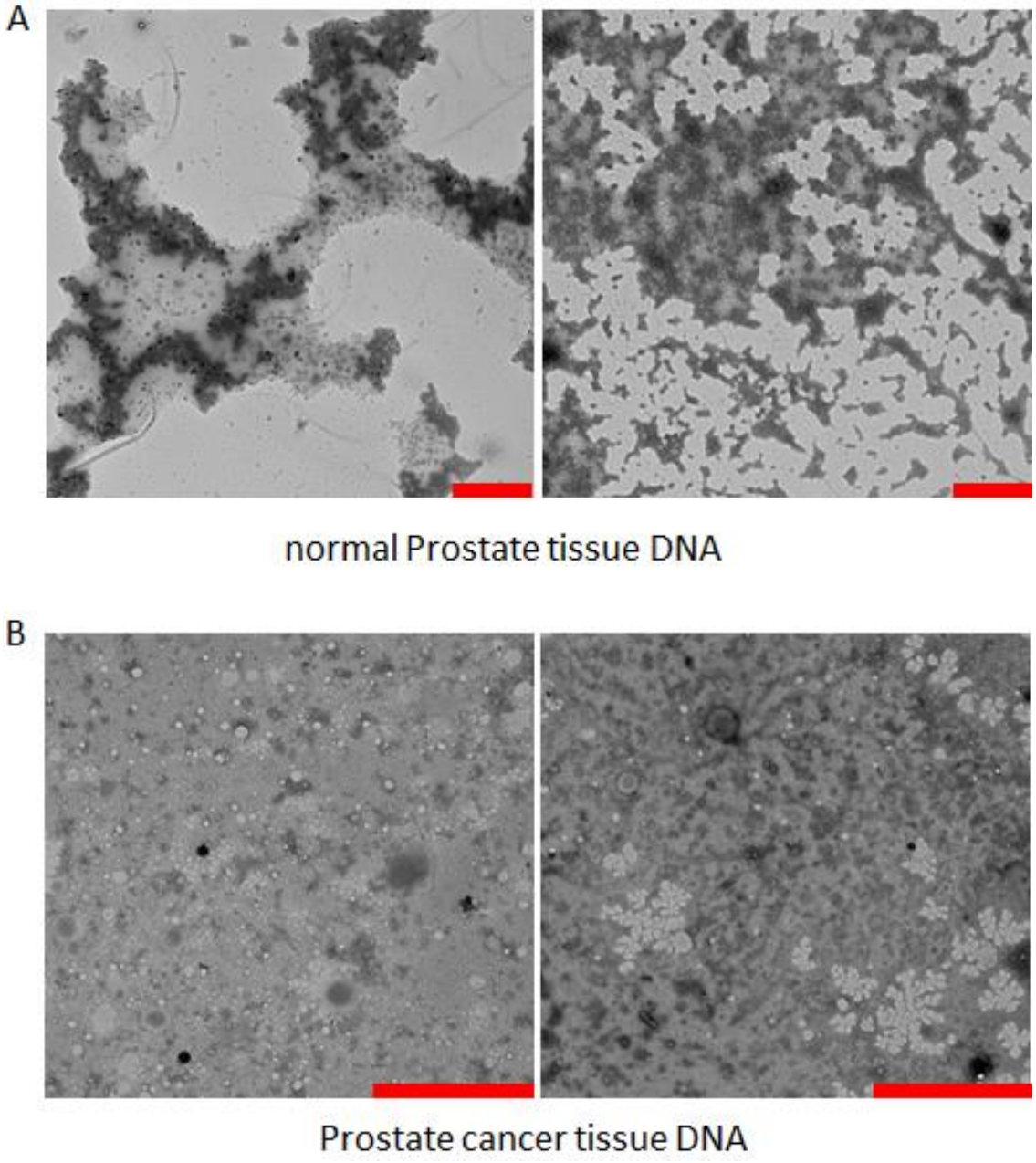
[5]Diamantina Institute, The University of Queensland, Brisbane, QLD 4072, Australia

Tel: +61-7-33464178; Fax: +61-7-33463973

*Corresponding authors

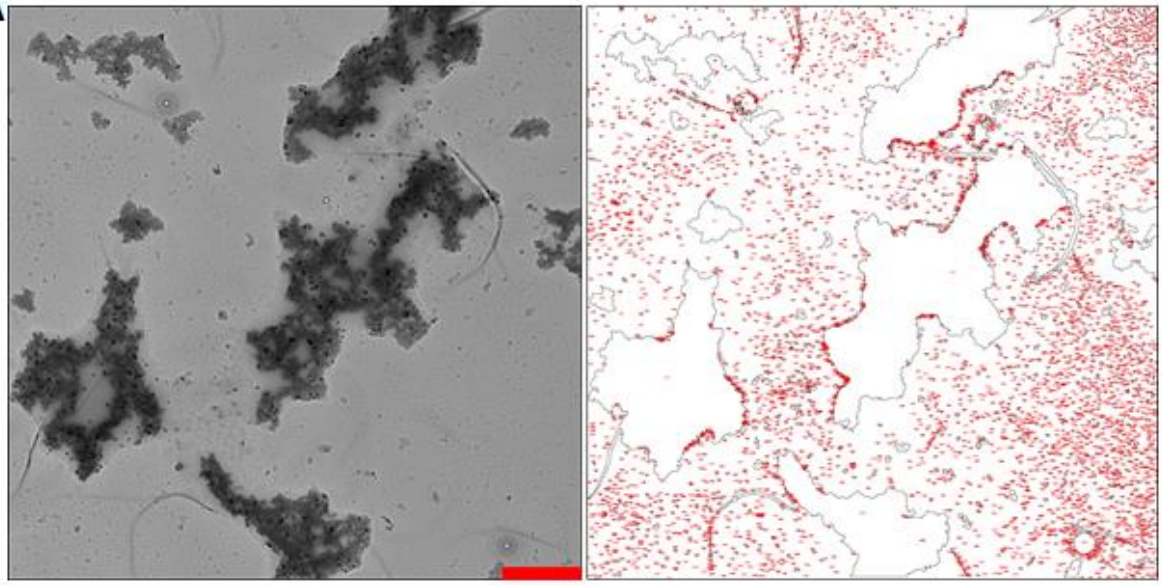
†Joint first-authors

Supplementary Figures



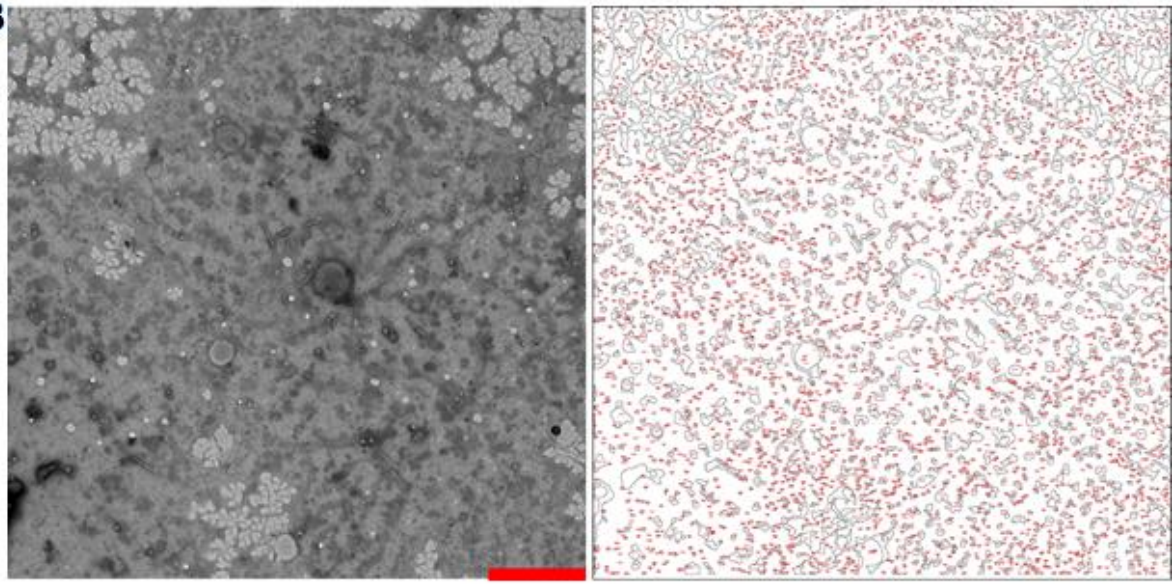
Supplementary Figure 1. Additional TEM image of gDNA. A) gDNA derived from normal prostate tissue and B) gDNA derived from prostate cancer tissue. Scale bars are 2000nm for all the supplementary figures.

A



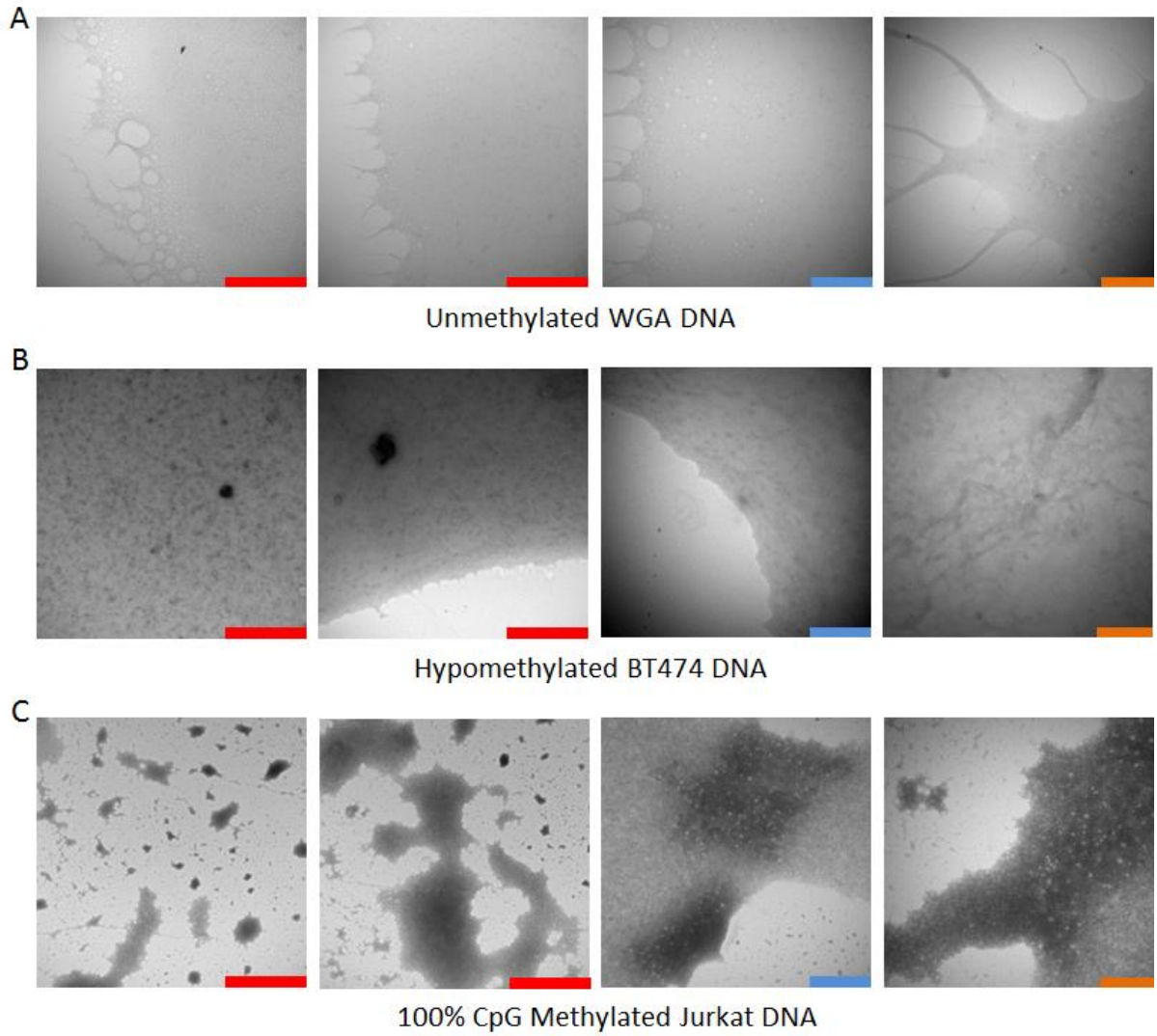
Sample	Aggregates Count	Total Area (nm^2)	Average Size (nm^2)	%Area
gDNA (Normal Prostate tissue)	5464	45341257.06	8298.18	25.369

B



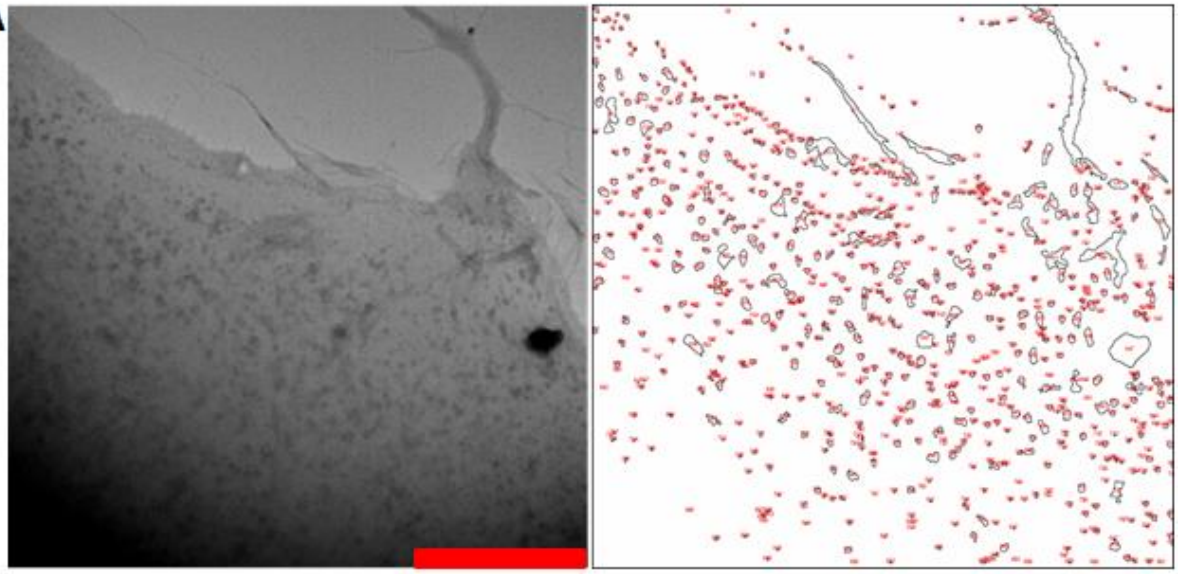
Sample	Aggregates Count	Total Area (nm^2)	Average Size (nm^2)	%Area
gDNA (Cancer Prostate tissue)	3933	6057435.111	1540.156	14.608

Supplementary Figure 2. ImageJ analysis for the TEM image of gDNA A) gDNA from normal tissue and B) gDNA from cancer tissue. Scale bars are 2000nm for all the supplementary figures.



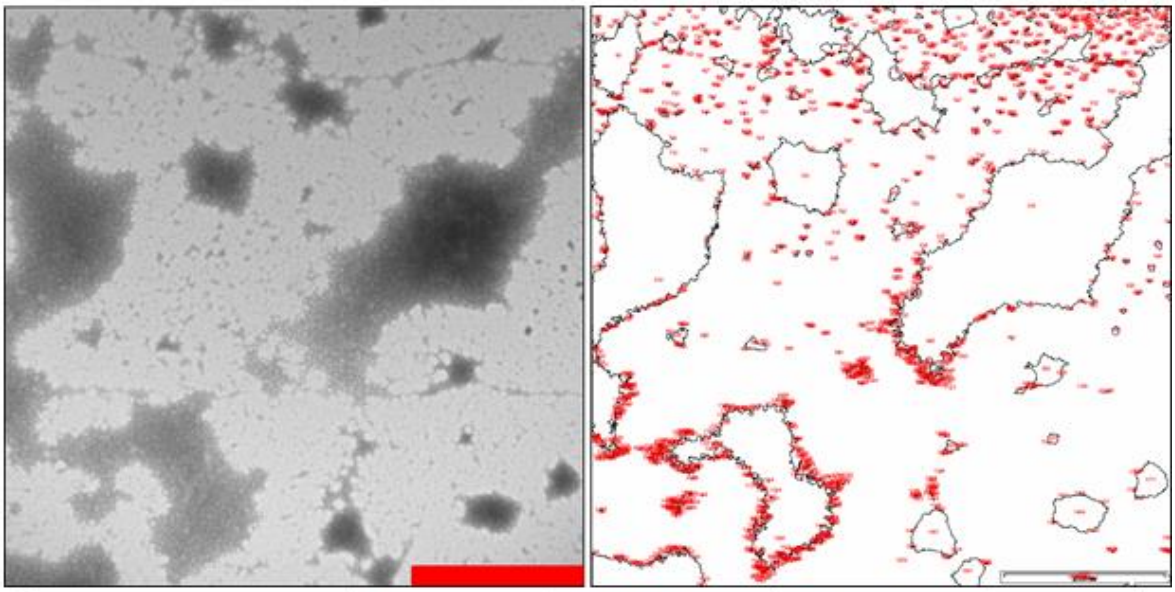
Supplementary Figure 3. Additional TEM images of gDNA with different methylation status. A) Unmethylated WGA DNA B) Hypomethylated BT474 breast cancer cell line DNA (43% global methylation) C) 100% Methylated Jurkat DNA. Scale bars are 2000nm (red) 1000nm (blue) and 500nm (orange) for all the supplementary figures.

A



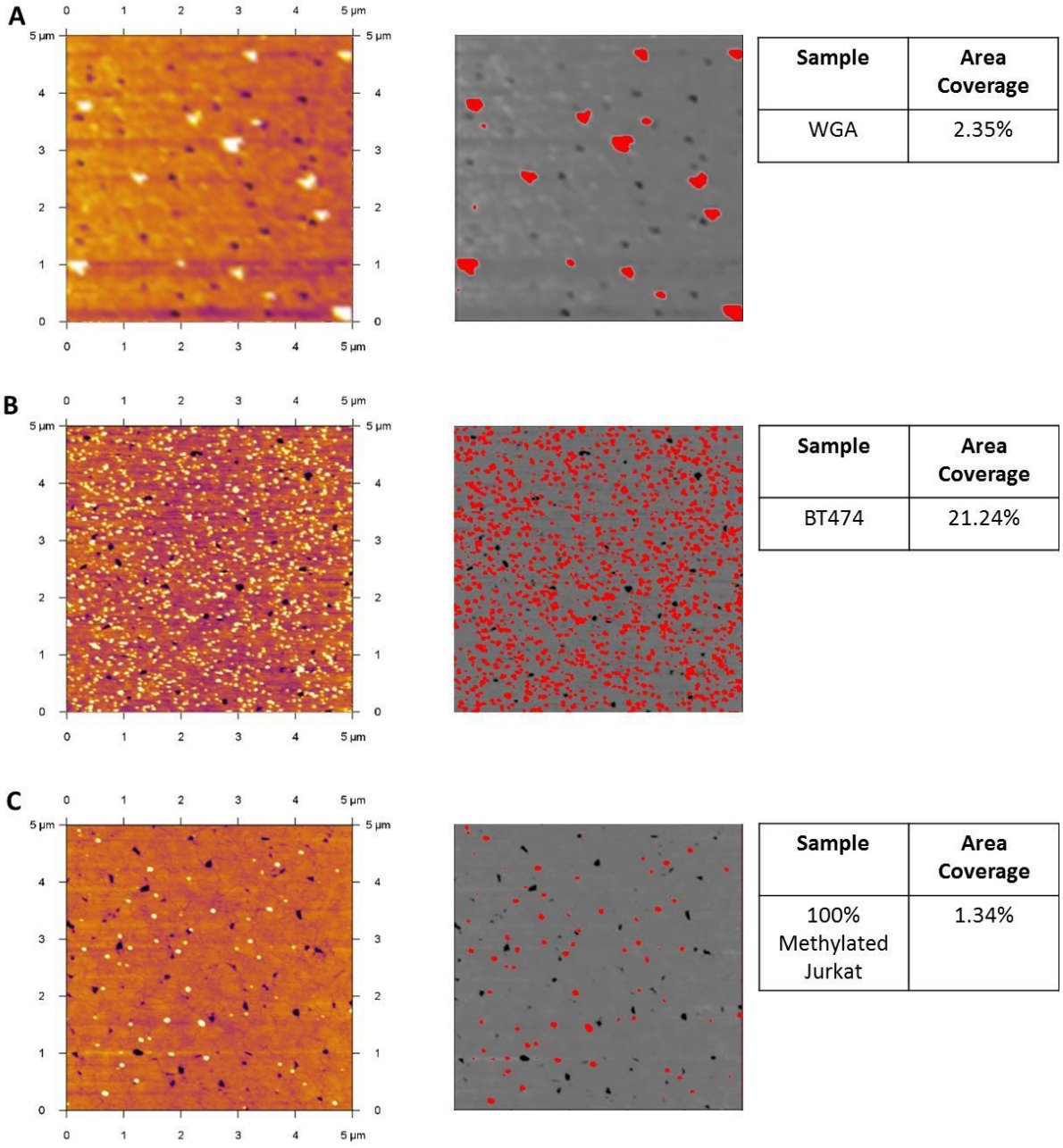
Sample	Aggregates Count	Total Area (nm^2)	Average Size (nm^2)	%Area
gDNA (BT474 Breast Cancer Cell Line)	781	2461565.146	3151.812	4.734

B



Sample	Aggregates Count	Total Area (nm^2)	Average Size (nm^2)	%Area
gDNA (100% CpG Methylated Jurkat)	1623	13501773.07	8319.02	25.99

Supplementary Figure 4. ImageJ analysis for the TEM image of DNA with different methylation status. A) gDNA from BT474 breast cancer cell line B) 100% CpG methylated Jurkat DNA. Scale bars are 2000nm for all the supplementary figures.



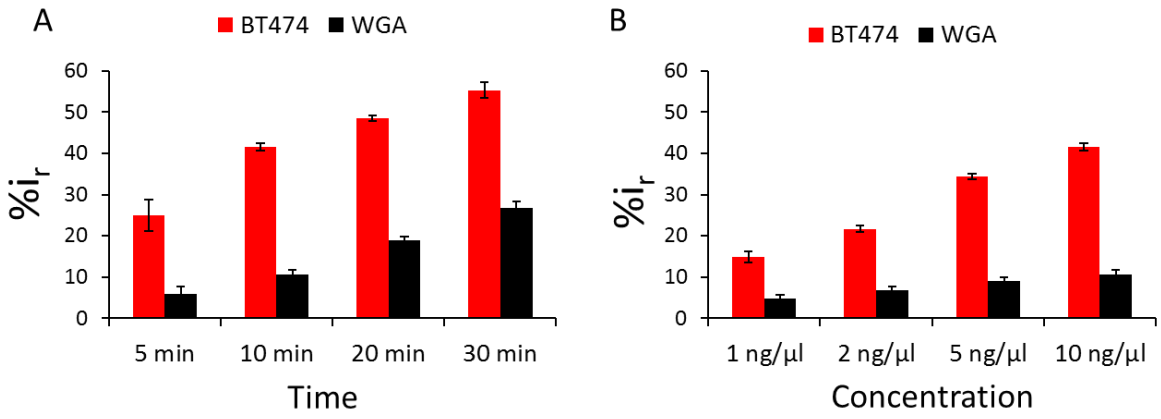
Sample	Area Coverage
WGA	2.35%

Sample	Area Coverage
BT474	21.24%

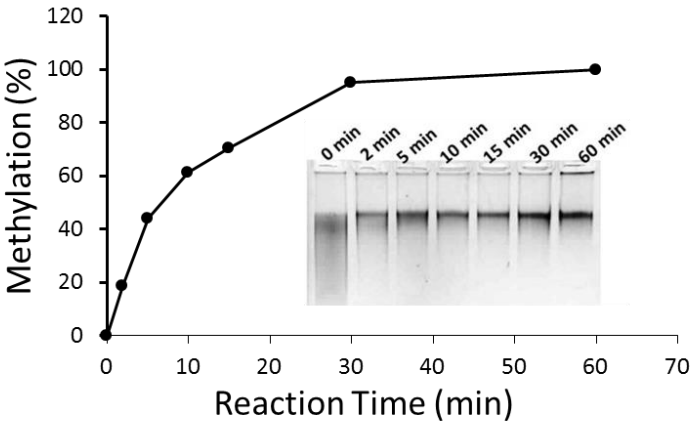
Sample	Area Coverage
100% Methylated Jurkat	1.34%

Supplementary Figure 5. 2D-AFM image of DNA with different methylation status. A) Unmethylated WGA DNA B) Hypomethylated BT474 breast cancer cell line DNA (43% global methylation) C) 100% Methylated Jurkat DNA. Right tables show the approximate %area coverage obtained from ImageJ analysis

Optimisation of the operating parameters for electrochemistry experiments: We first optimised the time to obtain maximum current difference for the adsorption of BT474 breast cancer cell derived DNA and fully unmethylated WGA DNA. As shown in supplementary figure 6A, adsorption of both the DNA increases with increasing time. However, 10 min provided the maximum differences between the relative DPV current for BT474 and WGA DNA. Similarly, we optimised the DNA concentration and found that 10ng/µl sample provided significant difference between the relative DPV current for the BT474 and WGA DNA (supplementary figure 6B).

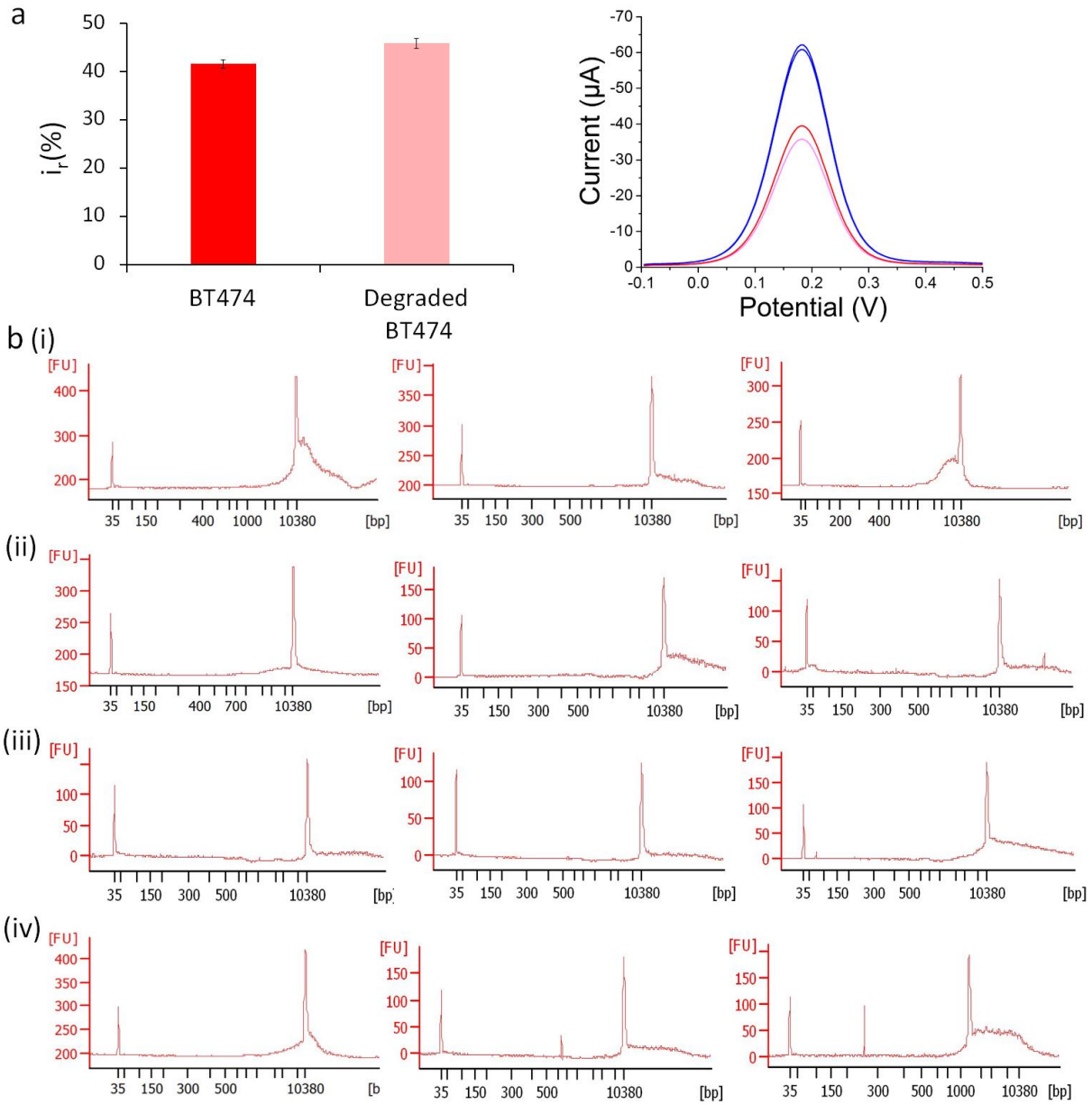


Supplementary Figure 6. Optimisation of the operating parameters for cell line DNA. Mean values of the relative DPV current obtained for the adsorption of WGA and BT474 cell derived DNA at (A) different time (DNA concentration, 10ng/µl) and (B) different concentration (adsorption time, 10 min). Each bar represents the average of three separate trials (n = 3). Error bars represent the standard deviation of measurements (relative standard deviation (%RSD) was found to be <5% for n = 3).

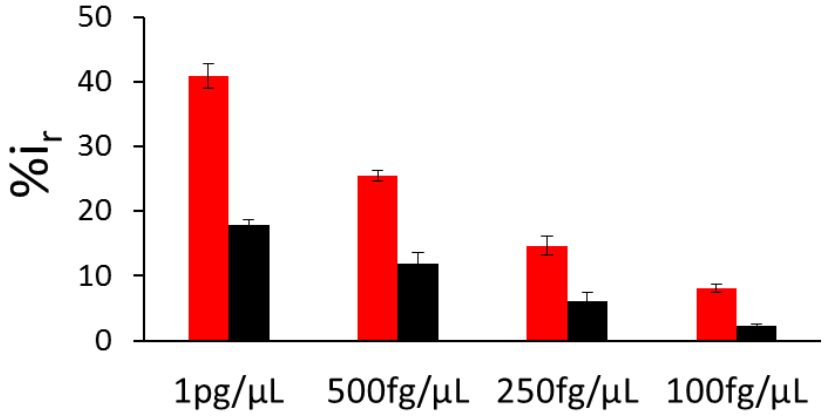


Reaction Time (Min)	Methylation (%)
0	0
2	18.85
5	43.99
10	61.36
15	70.59
30	95.09
60	100

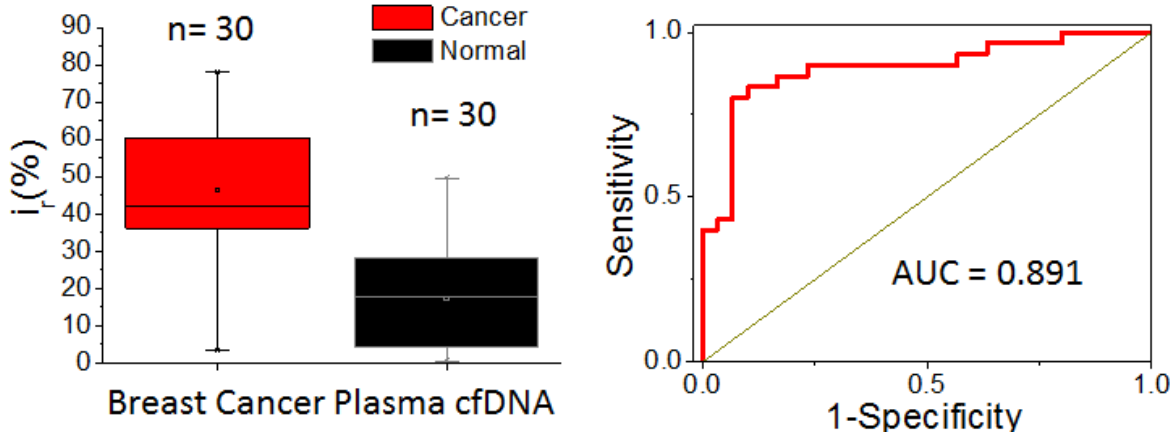
Supplementary Figure 7. ImageJ analysis of the electrophoresis gel picture showing the methylation levels for each of the samples treated with *M.SssI* enzyme with different reaction time (methylation level is assumed 100% for 60 min reaction times as suggested by the manufacturer instruction for the kit used).



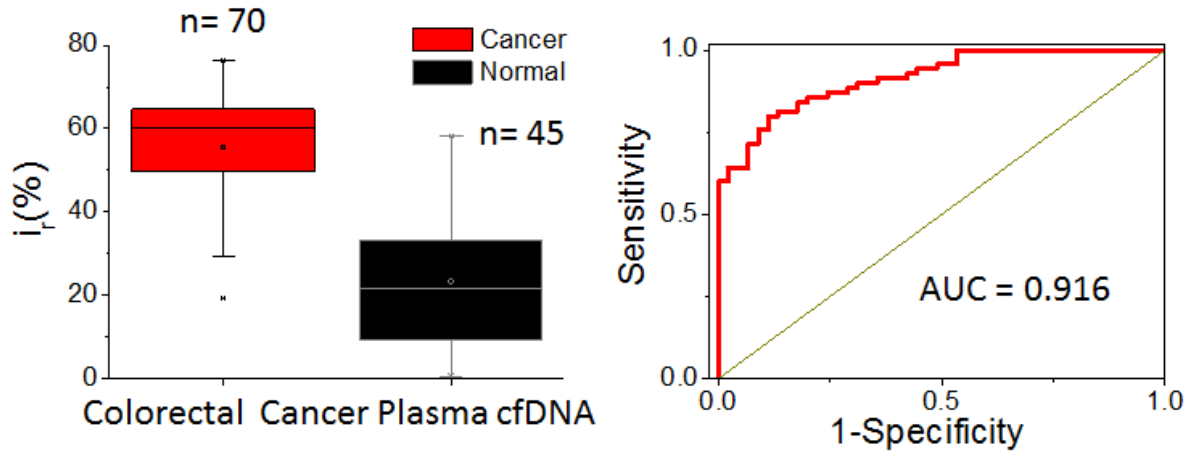
Supplementary Figure 8. DNA fragmentation measurement and it's effect on adsorption (a) Bars represent the relative current mean values (%i_r) for BT474 Breast cancer cell derived DNA (Red Bar) and fragmented BT474 DNA (Pink Bar). Each data point represents the average of three separate trials, and error bars represent the standard deviation of measurements (%RSD =<5% for n = 3). Right Panel: Corresponding DPV graphs and their respective baselines. (b)Bioanalyser sensogram showing the size (no. of base pairs) of the DNA. (i) Three breast cancer patient tissue DNA, Supplementary Table 6, Sample No. 1-3, (ii) Three lymphoma cancer patient tissue DNA, Supplementary Table 7, Sample No. 55-57,(iii) Three prostate cancer patient tissue DNA, Supplementary Table 8, Sample No. 65-67, iv) Three normal tissue DNA, Supplementary Table 9, Sample No. 1 (Normal Breast), 20 (Normal Lymphnode), 23 (Normal Prostate).



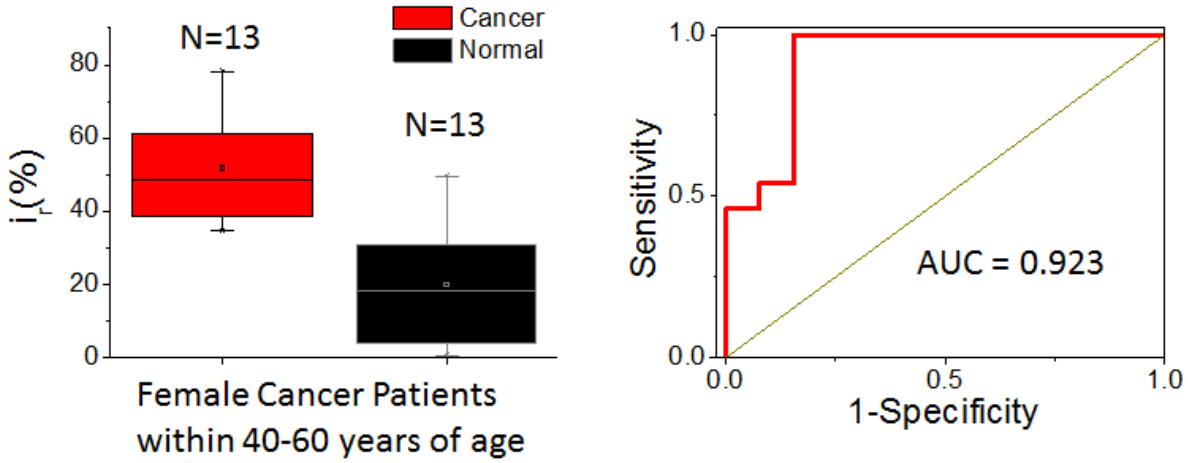
Supplementary Figure 9. Sensitivity for the detection of DNA methylation landscape in cfDNA. Mean values of the relative DPV current obtained for the adsorption of 5 µl of cfDNA samples derived from the blood plasma of patient (Supplementary Table 10, Sample number 2) and healthy (Supplementary Table 11, Sample number 7) individuals at different concentration. Each bar represents the average of three separate trials (n = 3). Error bars represent the standard deviation of measurements (relative standard deviation (%RSD) was found to be <5% for n = 3).



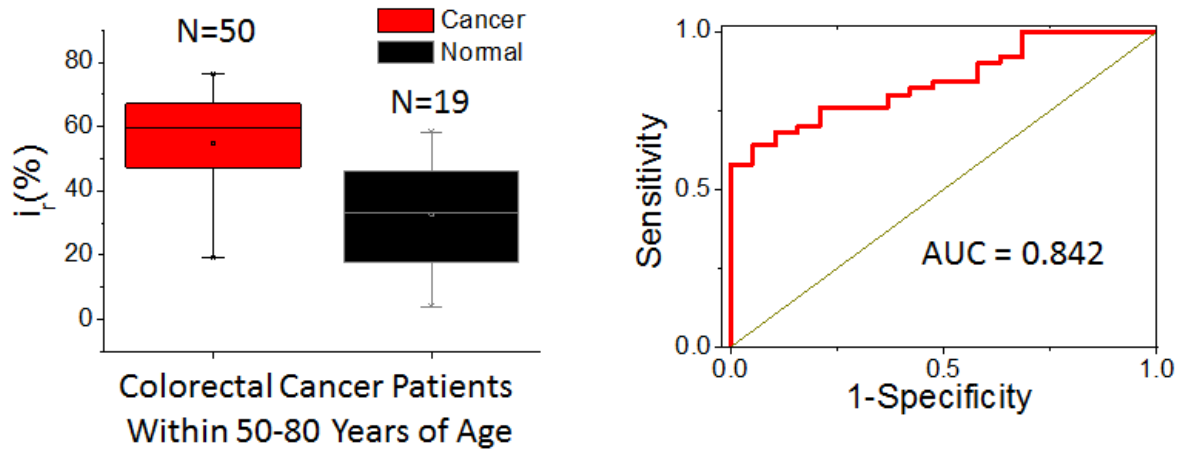
Supplementary Figure 10. Box plot showing the mean relative current values generated by electrochemical detection of plasma cfDNAs extracted from 30 normal and 30 breast cancer patients. The ROC analysis is shown on the right. In the box and whisker plots, the middle lines of the boxes represent the median (50th percentile) and the terminal line of the boxes represents the 25th to 75th percentile. The whiskers represent the lowest and the highest value.



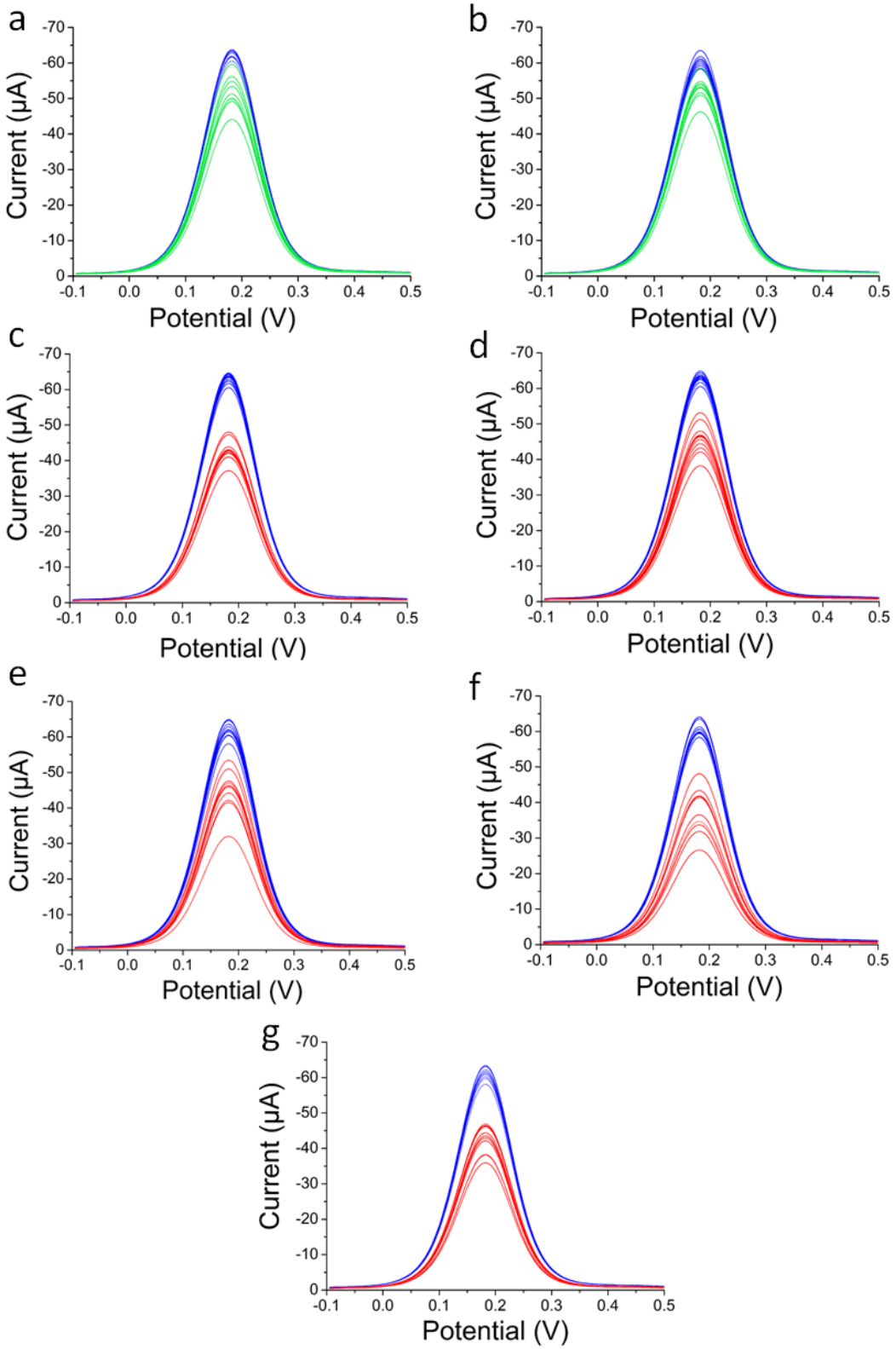
Supplementary Figure 11. Box plot showing the mean relative current values generated by electrochemical detection of plasma cfDNAs extracted from 45 normal and 70 colorectal cancer patients. The ROC analysis are shown on the right. In the box and whisker plots, the middle lines of the boxes represent the median (50th percentile) and the terminal line of the boxes represents the 25th to 75th percentile. The whiskers represent the lowest and the highest value.



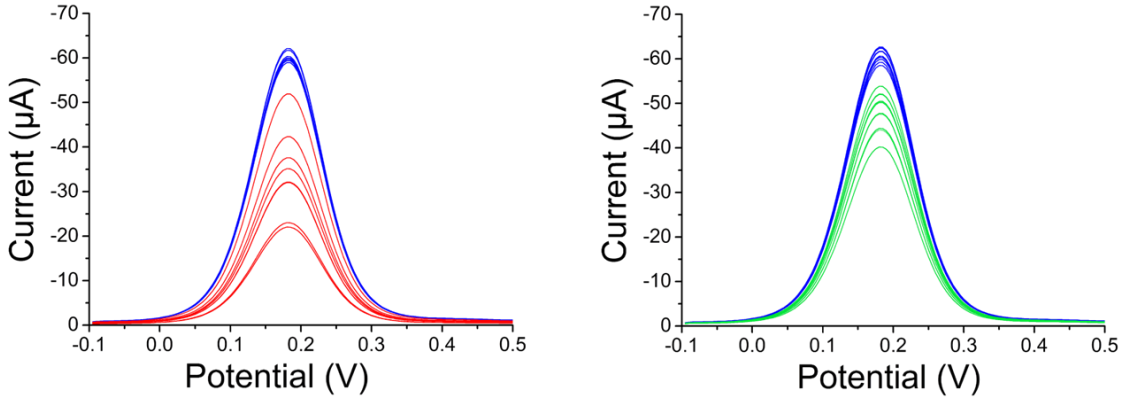
Supplementary Figure 12. Box plot showing the mean relative current values generated by electrochemical detection of plasma cfDNAs extracted from 13 normal and 13 female cancer patients with the age above 40 years. The ROC analysis is shown on the right. In the box and whisker plots, the middle lines of the boxes represent the median (50th percentile) and the terminal line of the boxes represents the 25th to 75th percentile. The whiskers represent the lowest and the highest value.



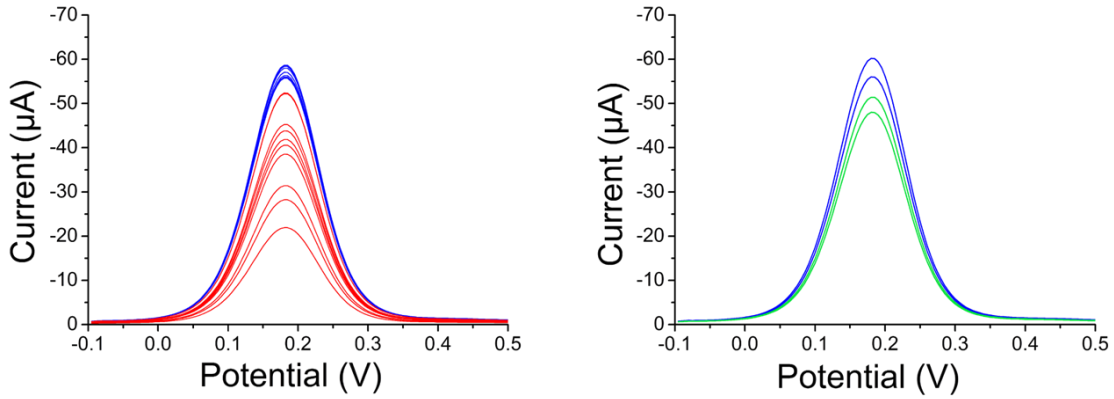
Supplementary Figure 13. Box plot showing the mean relative current values generated by electrochemical detection of plasma cfDNAs extracted from 19 healthy individuals and 50 colorectal cancer patients within 50-80 years of age. The ROC analysis is shown on the right. In the box and whisker plots, the middle lines of the boxes represent the median (50th percentile) and the terminal line of the boxes represents the 25th to 75th percentile. The whiskers represent the lowest and the highest value.



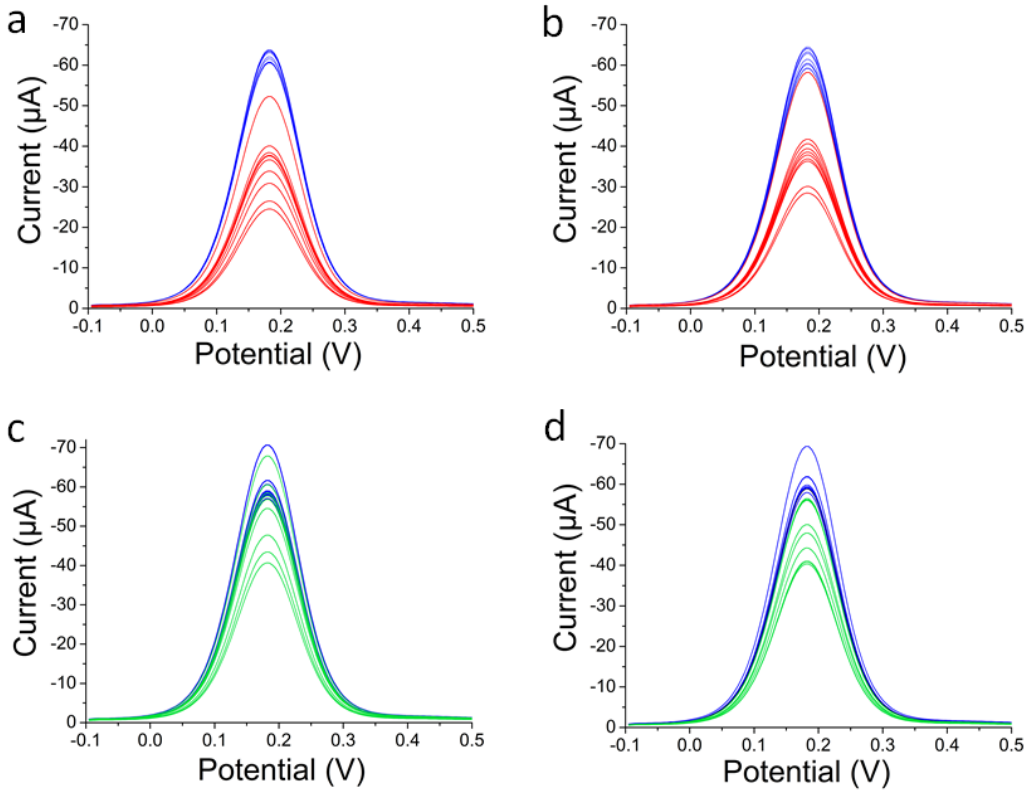
Supplementary Figure 14: DPV signals for the adsorption of gDNA derived from 31 normal breast tissues (green), 54 breast cancer tissues (red), and their corresponding baselines (blue). a) Normal Samples 1-15 b) Normal Samples 16-31 c) Patient Sample 1-11 d) Patient Samples 12-22 e) Patient Samples 22-33 f) Patient Samples 34-45 g) Patient sample 34-54.



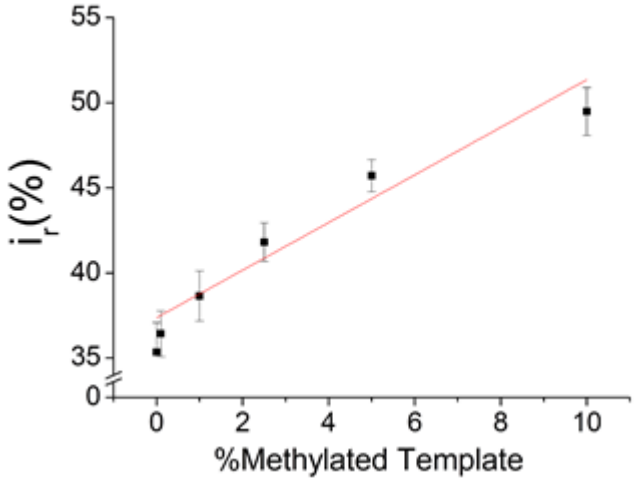
Supplementary Figure 15: DPV signals for the adsorption of gDNA derived from 8 prostate cancer tissues (red), 10 normal prostate tissues (green) and their corresponding baselines (blue).



Supplementary Figure 16: DPV signals for the adsorption of gDNA derived from 10 lymphoma cancer tissues (red), 2 normal lymphoma tissues (green) and their corresponding baselines (blue).

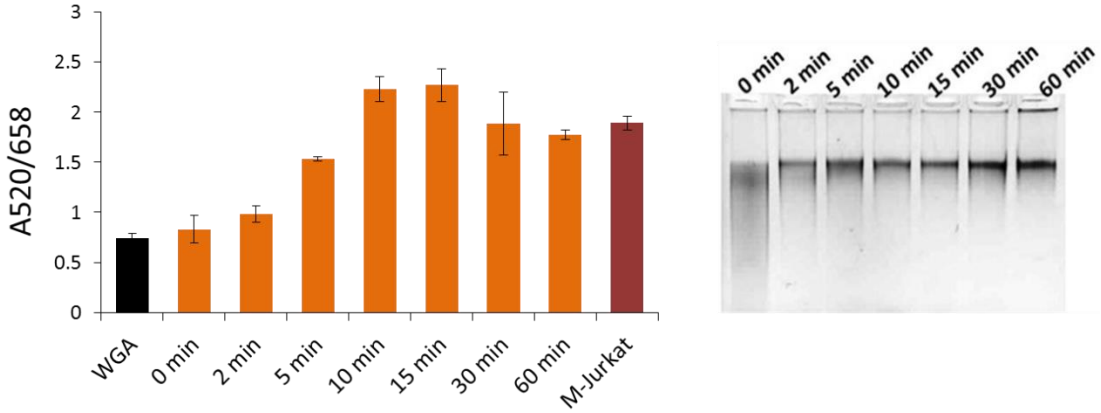


Supplementary Figure 17: DPV signals for the adsorption of cfDNA derived from 20 breast cancer plasma (red), 20 healthy plasma (green) and their corresponding baselines (blue).a) Patient samples 1-10 b) Patient samples 11-20 c) Normal samples 1-10 d) normal samples 11-20

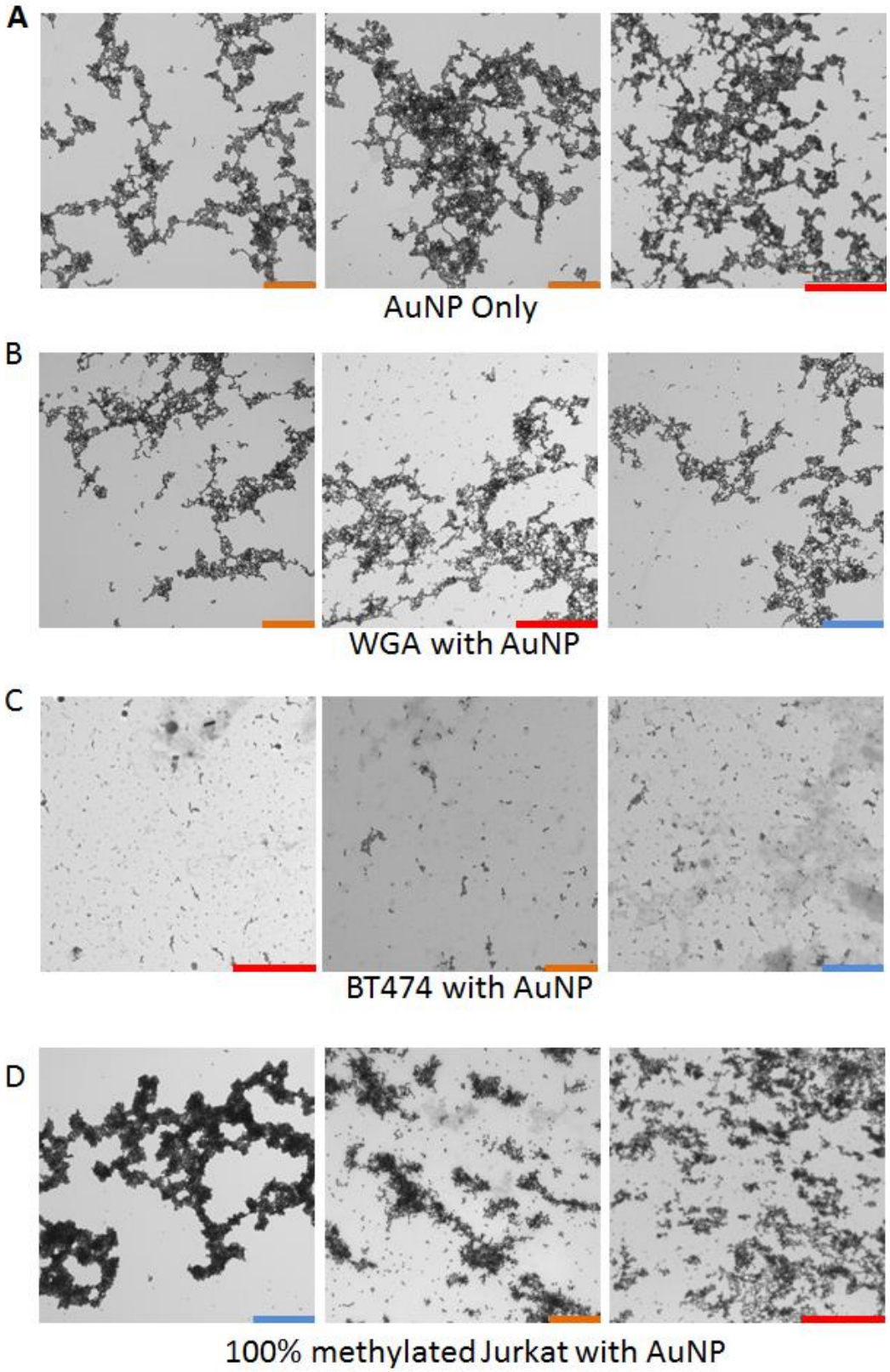


Supplementary Figure 18: relative adsorption (%i_r) vs % Methylated template DNA plot showing that the adsorption of normal cfDNA increases with the increased amount of clustered methylated DNA spiked in the normal cfDNA sample solution. Each point

represents the average of three separate trials (n = 3). Error bars represent the standard deviation of measurements (relative standard deviation (%RSD) was found to be <5% for n = 3).

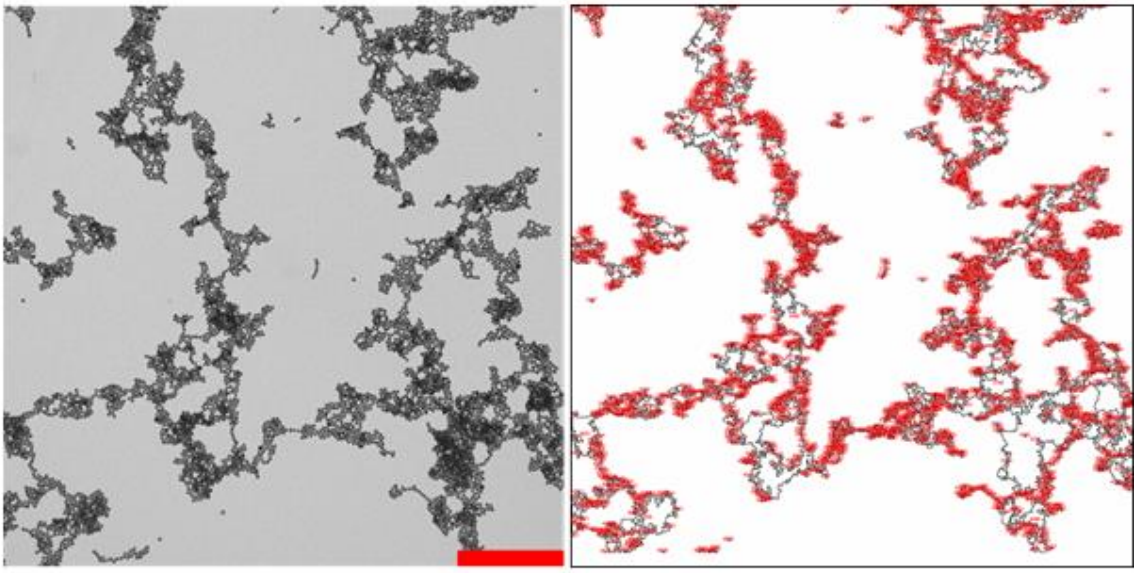


Supplementary Figure 19: Effect of non-clustered CpG methylation on gold-DNA adsorption. Relative absorbance ($A_{520/658}$) values for unmethylated (WGA), fully methylated (Jurkat) DNAs, and various genomic DNAs prepared from WGA DNA by enzymatic reaction using the M.SssI CpG methyltransferase enzyme for increasing time periods up to 60 min. Each bar represents the average of three separate trials (n = 3). Error bars represent the standard deviation of measurements (relative standard deviation (%RSD) was found to be <5% for n = 3). The inset panel shows an electrophoresis gel of the enzymatically methylated DNA samples digested with methylation sensitive HpaII restriction enzyme.



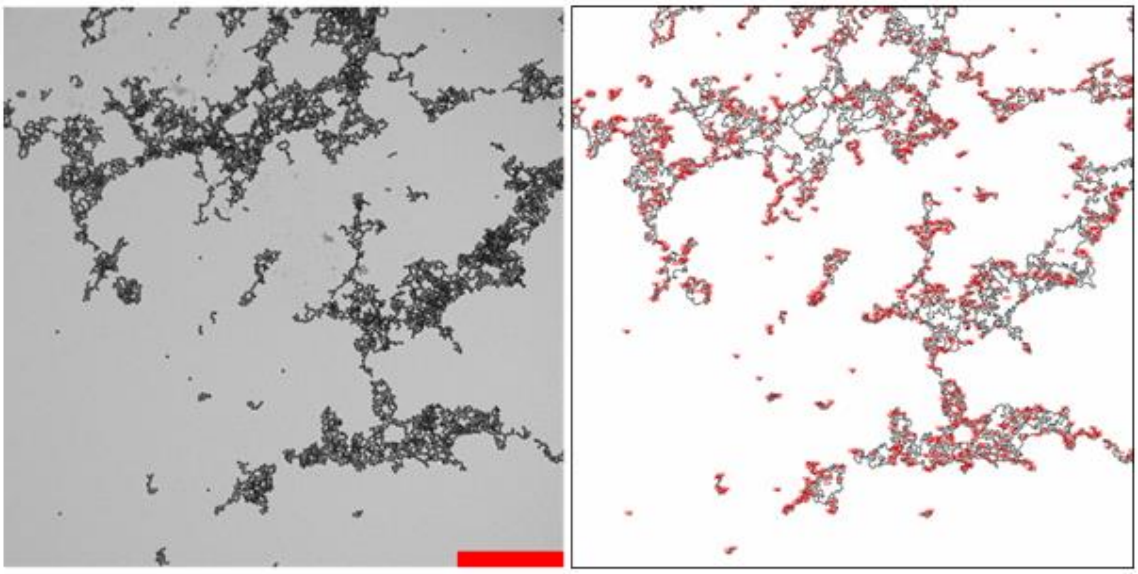
Supplementary Figure 20. TEM images for gold Nanoparticles and their interaction with DNA. Scale bars are 2000nm (red) 1000nm (blue) and 500nm (orange) for all the supplementary figures.

A



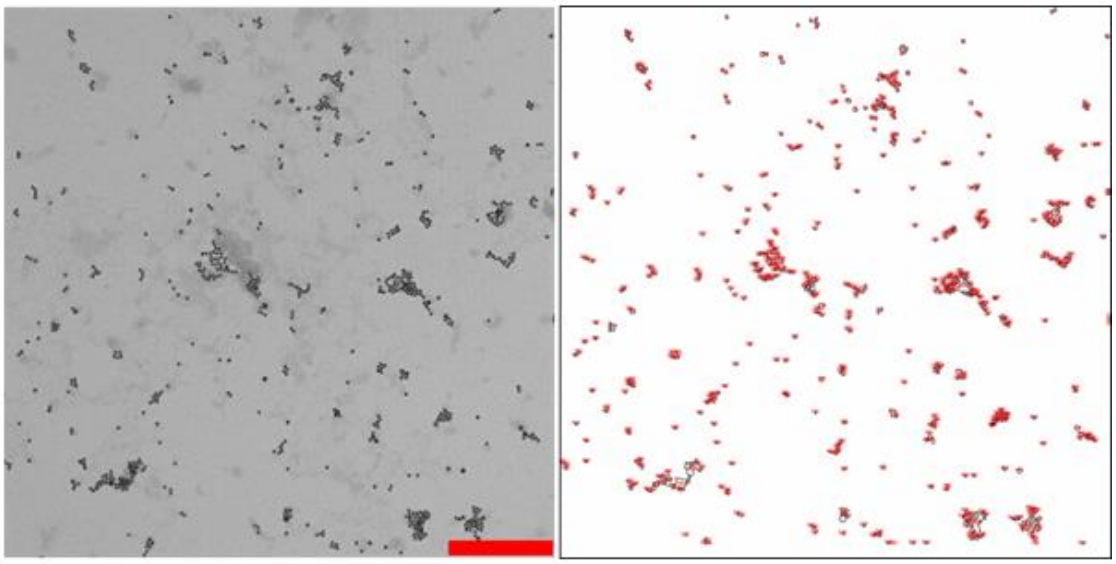
Sample	Aggregates Count	Total Area (nm^2)	Average Size (nm^2)	%Area
AuNP	2270	1067996.107	470.483	13.096

B



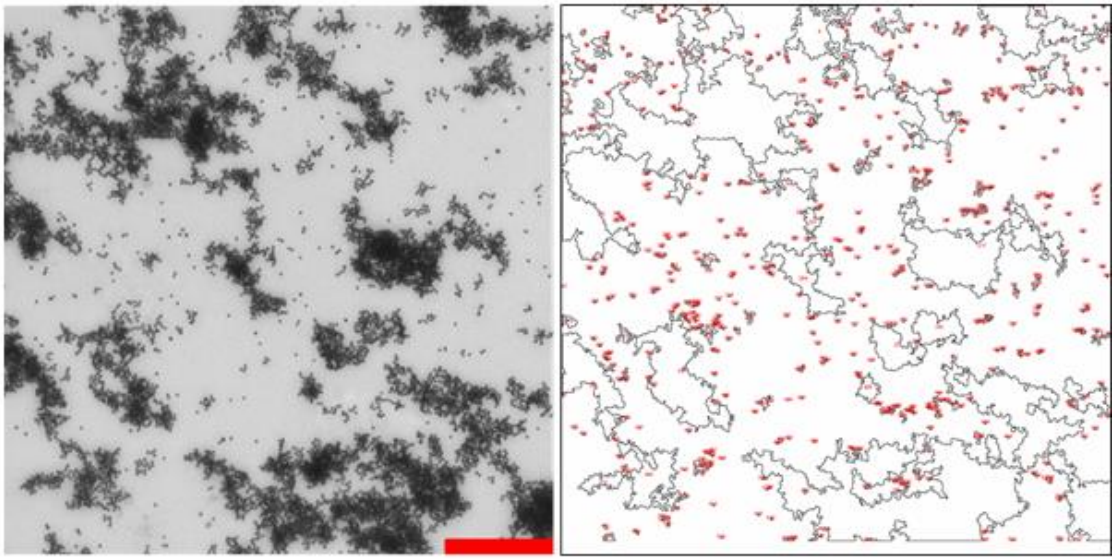
Sample	Aggregates Count	Total Area (nm^2)	Average Size (nm^2)	%Area
WGA+ AuNP	979	808571.931	825.916	9.915

C



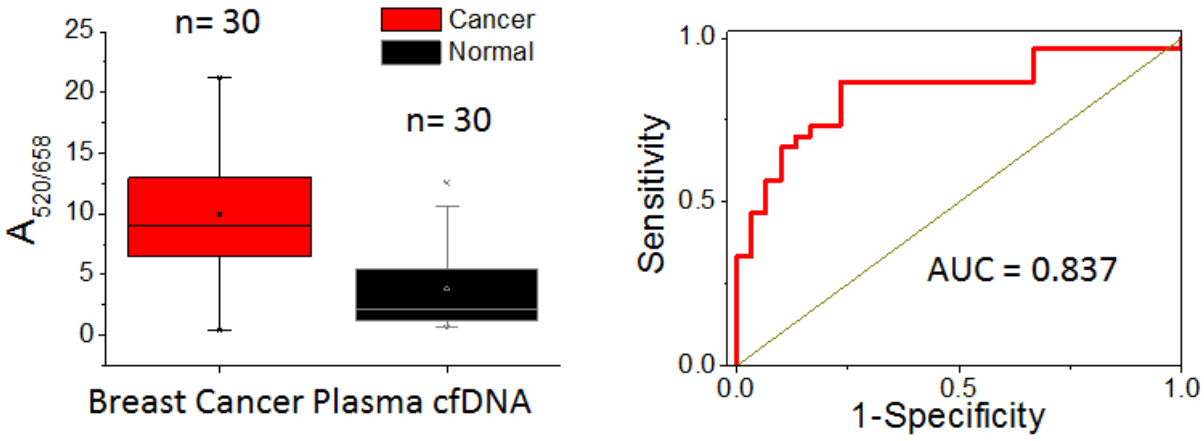
Sample	Aggregates Count	Total Area (nm^2)	Average Size (nm^2)	%Area
BT474+ AuNP	559	120000	214.47	1.47

D

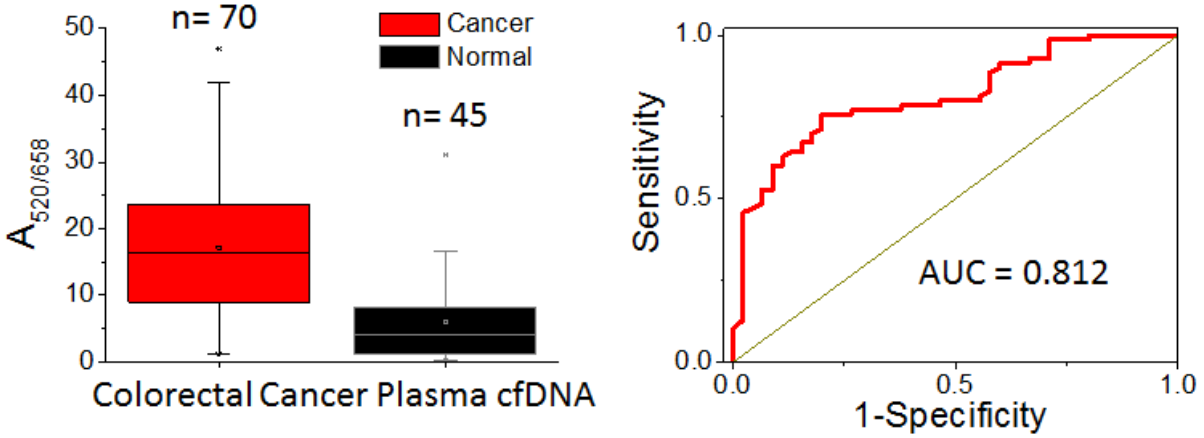


Sample	Aggregates Count	Total Area (nm^2)	Average Size (nm^2)	%Area
100% Meth Jurkat+ AuNP	557	2110000	3791.893	25.999

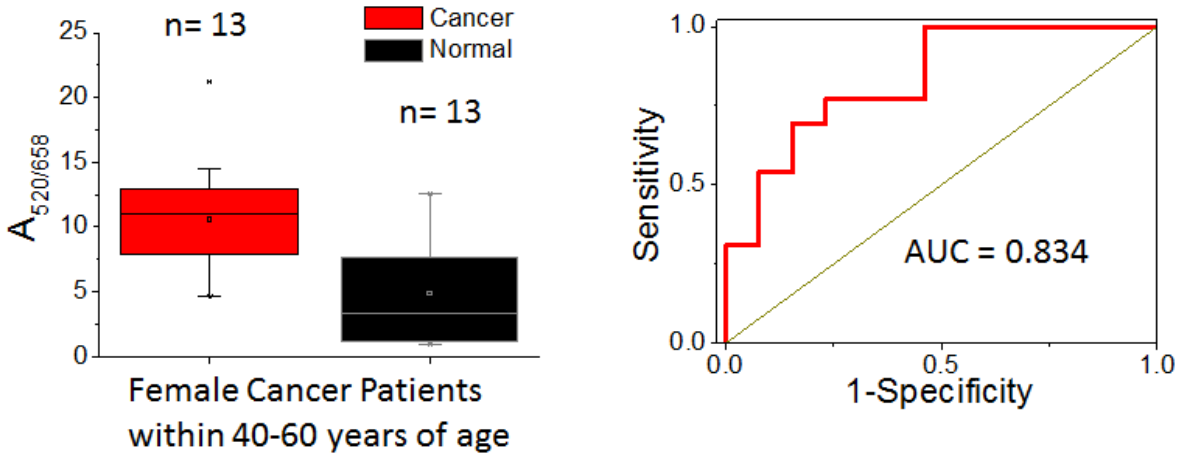
Supplementary Figure 21. ImageJ analysis for TEM image for AuNP and its interaction with gDNA having different methylation status. (A) AuNP (B) AUNP with WGA DNA (C)AuNP with BT474 DNA (D) AuNP with Jurkat 100% methylated DNA. Scale bars are 500nm for all the supplementary figures.



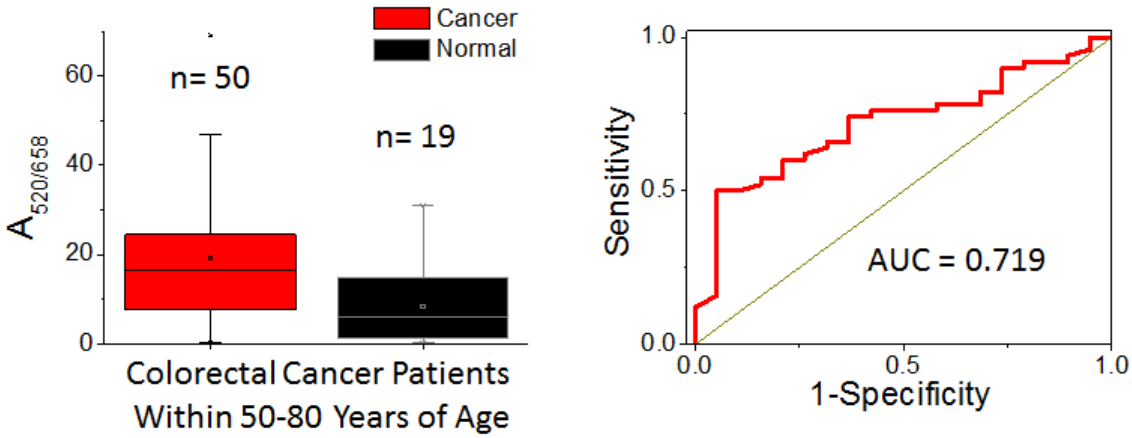
Supplementary Figure 22. Box plot showing the mean relative absorbance values $A_{520/658}$ of AuNP-cfDNA solution for cfDNA samples derived from the plasma of breast cancer patients or healthy donors. The ROC analysis is shown on the right. In the box and whisker plots, the middle lines of the boxes represent the median (50th percentile) and the terminal line of the boxes represents the 25th to 75th percentile. The whiskers represent the lowest and the highest value.



Supplementary Figure 23. Box plot showing the mean relative absorbance values $A_{520/658}$ of AuNP-cfDNA solution for cfDNA samples derived from the plasma of colorectal cancer patients or healthy donors. The ROC analysis is shown on the right. In the box and whisker plots, the middle lines of the boxes represent the median (50th percentile) and the terminal line of the boxes represents the 25th to 75th percentile. The whiskers represent the lowest and the highest value.



Supplementary Figure 24. Box plot showing the mean relative absorbance values $A_{520/658}$ of AuNP-cfDNA solution for cfDNA samples derived from the plasma of female cancer patients or healthy donors within 40-60 years of age. The ROC analysis is shown on the right. In the box and whisker plots, the middle lines of the boxes represent the median (50th percentile) and the terminal line of the boxes represents the 25th to 75th percentile. The whiskers represent the lowest and the highest value.



Supplementary Figure 25. Box plot showing the mean relative absorbance values $A_{520/658}$ of AuNP-cfDNA solution for cfDNA samples derived from the plasma of colorectal cancer patients or healthy donors within 50-80 years of age. The ROC analysis is shown on the right. In the box and whisker plots, the middle lines of the boxes represent the median (50th percentile) and the terminal line of the boxes represents the 25th to 75th percentile. The whiskers represent the lowest and the highest value.

Supplementary Tables

Supplementary Table 1. P value obtained from the student's t- test for the analysis of paired normal and cancer DNA derived from tissue and plasma samples

Sample Tissue DNA-Electrochemistry (Methylscape)	**P value**
All Cancer tissue DNA vs All Normal tissue DNA	0.0015
Breast Cancer tissue DNA vs normal tissue DNA	0.0001
Lymphoma tissue DNA vs normal tissue DNA	0.2547
Prostate cancer tissue DNA vs normal tissue DNA	0.0012
Sample Plasma DNA-Electrochemistry	
Breast and Colorectal Cancer Plasma DNA vs Normal Plasma DNA	0.0001
Sample **Tissue and Plasma DNA- Nanoparticle (Nanomethylscape)**	
All Cancer tissue DNA vs Normal tissue DNA	0.0005
Breast and Colorectal Cancer Plasma DNA vs Normal Plasma DNA	0.0001

Supplementary Table 2. Statistical Diagnostic Test evaluation for electrochemical analysis of tissue derived gDNA. (Cut off $\%i_r$ = 20, Cancer>20<normal)

Statistic	**Value for electrochemical gDNA test**
Sensitivity	93.06%
Specificity	80.65%
Disease Prevalence (DP)	69.90%
Positive Predictive Value (PPV)	91.78%
Negative Predictive Value(NPV)	83.33%
Accuracy	89.32%

Supplementary Table 3. Statistical Diagnostic Test evaluation for electrochemical analysis of plasma derived cfDNA. (Cut off $\%i_r$ = 35.7, Cancer>35.7<normal)

Statistic	Value for electrochemical cfDNA test
Sensitivity	84.00%
Specificity	82.22%
Disease Prevalence (DP)	68.97%
Positive Predictive Value (PPV)	91.30%
Negative Predictive Value(NPV)	69.81%
Accuracy	83.45%

Supplementary Table 4. Statistical Diagnostic Test evaluation for nanoparticle based analysis of of tissue derived gDNA. (Cut off $\%i_r$ = 35.7, Cancer>35.7<normal)

Statistic	Value for colorimetric gDNA test
Sensitivity	70.83%
Specificity	83.33%
Disease Prevalence (DP)	50.00%
Positive Predictive Value (PPV)	80.95%
Negative Predictive Value(NPV)	74.07%
Accuracy	77.08%

Supplementary Table 5. Statistical Diagnostic Test evaluation for nanoparticle based analysis of plasma derived cfDNA. (Cut off $A_{520/658}$ = 8.7, Cancer>8.7<normal)

Statistic	Value for colorimetric cfDNA test
Sensitivity	70.00%
Specificity	80.00%
Disease Prevalence (DP)	68.97%
Positive Predictive Value (PPV)	88.61%
Negative Predictive Value(NPV)	54.55%
Accuracy	73.10%

Supplementary Table 6. Clinical Information of Breast Cancer Patients (for tissue derived gDNA samples) *N/A= Not Available

Sample Number	Gender	Age (Yrs)	Cancer Type	Cancer Stage	Global Methylation (%)	Mean Relative Adsorption (%i_r)
1	Female	56	ER+ Breast	Metastatic	33.21	30.98
2	Female	76	ER+ Breast	Metastatic	41.46	39.69
3	Female	65	ER+ Breast	Metastatic	35.64	34.25
4	Female	71	ER+ Breast	Metastatic	39.25	33.66
5	Female	53	ER+ Breast	Metastatic	48.70	26.64
6	Female	44	ER+ Breast	Metastatic	45.32	32.02
7	Female	N/A	ER+ Breast	Metastatic	47.61	28.96
8	Female	63	ER+ Breast	Metastatic	34.28	32.69
9	Female	63	ER+ Breast	Metastatic	35.26	25.95
10	Female	59	ER+ Breast	Metastatic	46.14	35.97
11	Female	55	ER+ Breast	Metastatic	57.70	27.90
12	Female	85	ER+ Breast	Metastatic	48.93	32.03
13	Female	69	ER+ Breast	Metastatic	43.52	15.28
14	Female	66	ER+ Breast	Metastatic	34.61	25.95
15	Female	60	ER+ Breast	Metastatic	Error reading	32.11
16	Female	49	ER+ Breast	Metastatic	54.61	19.29
17	Female	66	ER+ Breast	Metastatic	43.68	27.42
18	Female	84	ER+ Breast	Metastatic	33.50	37.83
19	Female	60	ER+ Breast	Metastatic	39.20	26.10
20	Female	71	ER+ Breast	Metastatic	51.64	29.96
21	Female	64	ER+ Breast	Metastatic	53.61	22.81
22	Female	76	ER+ Breast	Metastatic	31.48	33.37
23	Female	66	ER+ Breast	Metastatic	56.82	22.11
24	Female	58	ER+ Breast	Metastatic	49.62	18.23
25	Female	64	ER+ Breast	Metastatic	36.59	30.42
26	Female	60	ER+ Breast	Metastatic	37.43	27.51
27	Female	46	ER+ Breast	Metastatic	42.90	25.92
28	Female	73	ER+ Breast	Metastatic	45.27	24.52
29	Female	52	ER+ Breast	Metastatic	40.20	23.62
30	Female	66	ER+ Breast	Metastatic	No Sample left	43.05
31	Female	53	ER+ Breast	Metastatic	37.63	31.28
32	Female	63	ER+ Breast	Metastatic	46.29	26.22
33	Female	66	ER+ Breast	Metastatic	No Sample left	30.70
34	Female	55	ER+ Breast	Metastatic	34.57	44.91
35	Female	57	ER+ Breast	Metastatic	62.80	25.66
36	Female	N/A	ER+ Breast	Metastatic	No Sample left	39.66
37	Female	N/A	ER+ Breast	Metastatic	No Sample left	32.38

38	Female	N/A	ER+ Breast	Metastatic	No Sample left	57.44
39	Female	N/A	ER+ Breast	Metastatic	No Sample left	34.05
40	Female	N/A	ER+ Breast	Metastatic	No Sample left	44.28
41	Female	N/A	ER+ Breast	Metastatic	No Sample left	47.56
42	Female	N/A	ER+ Breast	Metastatic	No Sample left	21.84
43	Female	N/A	ER+ Breast	Metastatic	No Sample left	24.21
44	Female	N/A	ER+ Breast	Metastatic	28.23	32.02
45	Female	N/A	ER+ Breast	Metastatic	No Sample left	28.96
46	Female	N/A	ER+ Breast	Metastatic	No Sample left	26.48
47	Female	N/A	ER+ Breast	Metastatic	No Sample left	26.51
48	Female	N/A	ER+ Breast	Metastatic	No Sample left	40.34
49	Female	N/A	ER+ Breast	Metastatic	No Sample left	21.50
50	Female	N/A	ER+ Breast	Metastatic	27.33	37.59
51	Female	N/A	ER+ Breast	Metastatic	No Sample left	32.16
52	Female	N/A	ER+ Breast	Metastatic	No Sample left	25.73
53	Female	N/A	ER+ Breast	Metastatic	No Sample left	40.63
54	Female	N/A	ER+ Breast	Metastatic	No Sample left	30.28

Supplementary Table 7. Clinical Information of Lymphoma Patients (for tissue derived gDNA samples)

55	Female	78	Lymphoma	Metastatic	45.77	9.57
56	Female	65	Lymphoma	Metastatic	43.89	30.25
57	Female	75	Lymphoma	Metastatic	47.22	8.58
58	Male	72	Lymphoma	Metastatic	59.40	27.92
59	Female	71	Lymphoma	Metastatic	45.63	62.43
60	Female	81	Lymphoma	Metastatic	33.60	33.51
61	Female	40	Lymphoma	Metastatic	30.48	45.14
62	Female	73	Lymphoma	Metastatic	40.26	50.56
63	Male	49	Lymphoma	Metastatic	42.67	24.18
64	Female	73	Lymphoma	Metastatic	37.24	22.73

Supplementary Table 8. Clinical Information of Prostate Cancer Patients (for tissue derived gDNA samples)

65	Male	60	Prostate	Metastatic	31.20	62.80
66	Male	67	Prostate	Metastatic	37.63	31.65
67	Male	58	Prostate	Metastatic	42.54	12.86
68	Male	77	Prostate	Metastatic	41.87	37.16
69	Male	60	Prostate	Metastatic	56.21	41.95
70	Male	79	Prostate	Metastatic	32.83	46.42
71	Male	68	Prostate	Metastatic	34.59	61.39
72	Male	65	Prostate	Metastatic	37.68	48.51

Supplementary Table 9. Clinical Information of Healthy Individuals (for tissue derived gDNA samples)

Sample Number	Gender	Age	Tissue Type	Global Methylation (%)	Mean Relative Adsorption (%i_r)
1	Female	38	Normal Breast	74.29	17.96
2	Female	22	Normal Breast	No Sample left	12.66
3	Female	38	Normal Breast	No Sample left	5.47
4	Female	53	Normal Breast	63.51	16.60
5	Female	58	Normal Breast	No Sample left	10.09
6	Female	46	Normal Breast	No Sample left	11.14
7	Female	38	Normal Breast	No Sample left	17.68
8	Female	21	Normal Breast	56.74	27.50
9	Female	50	Normal Breast	No Sample left	19.38
10	Female	53	Normal Breast	60.91	17.15
11	Female	56	Normal Breast	No Sample left	16.70
12	Female	34	Normal Breast	No Sample left	21.99
13	Female	N/A	Normal Breast	No Sample left	11.65
14	Female	N/A	Normal Breast	No Sample left	7.98
15	Female	N/A	Normal Breast	No Sample left	10.71
16	Female	N/A	Normal Breast	No Sample left	11.28
17	Female	N/A	Normal Breast	No Sample left	7.87
18	Female	N/A	Normal Breast	No Sample left	10.89
19	Female	N/A	Normal Breast	60.59	10.95
20	Female	30	Normal lymph node	58.21	17.32
21	Male	56	Normal lymph node	61.24	15.10
22	Male	82	Normal Prostate	68.20	16.80
23	Male	72	Normal Prostate	51.43	35.72
24	Male	79	Normal Prostate	62.37	16.70
25	Male	44	Normal Prostate	55.81	26.48
26	Male	69	Normal Prostate	51.29	19.60
27	Male	91	Normal Prostate	49.23	17.08
28	Male	72	Normal Prostate	71.48	24.14
29	Male	59	Normal Prostate	42.65	13.68
30	Male	62	Normal Prostate	48.28	22.61
31	Male	76	Normal Prostate	57.60	15.43

Supplementary Table 10. Clinical information of Breast cancer patient samples (for cfDNA samples extracted from the plasma)

Sample Number	Gender	Age	Cancer Type	Cancer Stage	Mean Relative Adsorption (%i_r)	Mean Relative Absorbance ($A_{520/658}$)
1	Female	85	ER+ Breast	Metastatic	42.43	6.75
2	Female	76	ER+ Breast	Metastatic	41.67	5.85
3	Female	44	ER+ Breast	Metastatic	56.78	7.88
4	Female	66	ER+ Breast	Metastatic	16.23	1.27
5	Female	66	ER+ Breast	Metastatic	39.18	6.56
6	Female	58	ER+ Breast	Metastatic	38.56	11.50
7	Female	64	ER+ Breast	Metastatic	36.17	1.19
8	Female	59	ER+ Breast	Metastatic	35.77	4.61
9	Female	85	ER+ Breast	Metastatic	51.14	6.00
10	Female	76	ER+ Breast	Metastatic	60.49	5.63
11	Female	57	ER+ Breast	Metastatic	39.01	4.88
12	Female	66	ER+ Breast	Metastatic	8.69	1.13
13	Female	66	ER+ Breast	Metastatic	52.50	6.00
14	Female	58	ER+ Breast	Metastatic	42.16	4.88
15	Female	64	ER+ Breast	Metastatic	42.30	5.75
16	Female	59	ER+ Breast	Metastatic	48.60	10.05
17	Female	76	ER+ Breast	Metastatic	29.31	8.46
18	Female	60	ER+ Breast	Metastatic	34.67	11.00
19	Female	60	ER+ Breast	Metastatic	36.52	9.17
20	Female	85	ER+ Breast	Metastatic	30.58	9.75
21	Female	49	ER+ Breast	Metastatic	73.64	14.5
22	Female	63	ER+ Breast	Metastatic	37.94	10.83
23	Female	71	ER+ Breast	Metastatic	3.37	0.38
24	Female	71	ER+ Breast	Metastatic	77.14	15.83
25	Female	60	ER+ Breast	Metastatic	57.58	12.00
26	Female	64	ER+ Breast	Metastatic	74.93	6.31
27	Female	58	ER+ Breast	Metastatic	78.26	13.00
28	Female	60	ER+ Breast	Metastatic	74.32	20.00
29	Female	46	ER+ Breast	Metastatic	61.13	13.00
30	Female	46	ER+ Breast	Metastatic	70.96	21.25

Supplementary Table 11. Clinical information of Colorectal cancer patient samples (for cfDNA samples extracted from the plasma)

Sample Number	Gender	Age	Cancer Type	Cancer Stage	Mean Relative Adsortion (%i_r)	Mean Relative Absorbance ($A_{520/658}$)
1	F	23*	Colorectal	Metastatic	49.71	14.5
2	M	43	Colorectal	Metastatic	63.06	23.7
3	M	49	Colorectal	Metastatic	50.91	16.3
4	M	51	Colorectal	Metastatic	63.85	60
5	M	54	Colorectal	Metastatic	52.65	15
6	F	56	Colorectal	Metastatic	72.30	22.3
7	M	56	Colorectal	Metastatic	61.33	21
8	F	56	Colorectal	Metastatic	68.05	47
9	F	56	Colorectal	Metastatic	61.10	66
10	M	59	Colorectal	Metastatic	63.30	0.4
11	M	61	Colorectal	Metastatic	50.40	10.8
12	M	61	Colorectal	Metastatic	29.23	14.8
13	M	61	Colorectal	Metastatic	67.08	36
14	F	62	Colorectal	Metastatic	61.96	28.5
15	F	62	Colorectal	Metastatic	62.70	16.3
16	F	63	Colorectal	Metastatic	62.00	10.6
17	F	63	Colorectal	Metastatic	73.43	22
18	M	63	Colorectal	Metastatic	69.64	15.7
19	M	64	Colorectal	Metastatic	70.14	21.7
20	M	64	Colorectal	Metastatic	63.70	67
21	M	65	Colorectal	Metastatic	58.03	9
22	M	65	Colorectal	Metastatic	54.45	31
23	F	65	Colorectal	Metastatic	47.19	0.7
24	M	65	Colorectal	Metastatic	20.40	2
25	F	66	Colorectal	Metastatic	35.85	0.9
26	M	69	Colorectal	Metastatic	25.70	2.1
27	M	69	Colorectal	Metastatic	26.80	1.8
28	F	70	Colorectal	Metastatic	76.16	3
29	F	70	Colorectal	Metastatic	35.96	69
30	F	70	Colorectal	Metastatic	59.55	15.3
31	M	71	Colorectal	Metastatic	63.66	30
32	F	71	Colorectal	Metastatic	59.23	18
33	M	71	Colorectal	Metastatic	75.39	12.3
34	M	71	Colorectal	Metastatic	61.40	17
35	F	71	Colorectal	Metastatic	30.20	2.6
36	F	71	Colorectal	Metastatic	35.84	0.4
37	M	72	Colorectal	Metastatic	71.03	11.3

38	M	73	Colorectal	Metastatic	68.25	13.4
39	M	73	Colorectal	Metastatic	58.16	31
40	M	73	Colorectal	Metastatic	58.70	21
41	F	73	Colorectal	Metastatic	34.26	17.3
42	F	74	Colorectal	Metastatic	48.25	7.8
43	M	74	Colorectal	Metastatic	67.53	23.7
44	F	74	Colorectal	Metastatic	64.30	4.3
45	M	75	Colorectal	Metastatic	76.41	23
46	M	75	Colorectal	Metastatic	49.19	16.5
47	F	75	Colorectal	Metastatic	59.32	14.5
48	M	76	Colorectal	Metastatic	64.80	23.7
49	M	76	Colorectal	Metastatic	19.14	1.8
50	M	77	Colorectal	Metastatic	55.69	29
51	M	77	Colorectal	Metastatic	68.35	35.5
52	M	77	Colorectal	Metastatic	20.23	1.1
53	M	78	Colorectal	Metastatic	57.40	28
54	M	79	Colorectal	Metastatic	32.32	17.3
55	F	79	Colorectal	Metastatic	64.69	24.5
56	F	79	Colorectal	Metastatic	53.00	18.7
57	F	79	Colorectal	Metastatic	76.00	3.3
58	F	80	Colorectal	Metastatic	55.59	9.5
59	M	80	Colorectal	Metastatic	60.01	21.7
60	F	81	Colorectal	Metastatic	45.62	11.6
61	F	81	Colorectal	Metastatic	63.19	29
62	M	81	Colorectal	Metastatic	60.21	16.3
63	F	83	Colorectal	Metastatic	63.36	2.3
64	F	84	Colorectal	Metastatic	60.91	9.3
65	F	85	Colorectal	Metastatic	60.02	15.8
66	F	85	Colorectal	Metastatic	60.05	23
67	M	88	Colorectal	Metastatic	61.01	6.7
68	F	89	Colorectal	Metastatic	22.31	1.1
69	F	92	Colorectal	Metastatic	65.56	16.7
70	M	92	Colorectal	Metastatic	68.27	22.7

*It is very unlikely to develop colorectal cancer at the age of 23. However, we assume this patient may have a heritable predisposition.

Supplementary Table 12. Normal cfDNA samples extracted from the plasma of healthy individuals. *NA= Not Applicable

Sample Number	Gender	Age	Sample Type	Cancer Stage	Mean Relative Adsorption (%i_r)	Mean Relative Absorbance ($A_{520/658}$)
1	F	54	Normal	NA	3.98	1.02
2	F	31	Normal	NA	9.30	1.22
3	F	57	Normal	NA	33.31	7.53
4	F	18	Normal	NA	27.89	5.50
5	F	27	Normal	NA	17.95	7.15
6	F	54	Normal	NA	18.01	5.05
7	F	21	Normal	NA	18.53	2.14
8	F	23	Normal	NA	29.98	3.99
9	F	18	Normal	NA	5.60	0.96
10	F	43	Normal	NA	30.90	7.67
11	F	41	Normal	NA	16.93	2.13
12	F	25	Normal	NA	2.37	1.09
13	F	34	Normal	NA	0.97	1.15
14	F	47	Normal	NA	3.11	1.05
15	F	23	Normal	NA	4.22	0.86
16	F	41	Normal	NA	5.43	0.95
17	F	25	Normal	NA	22.76	4.03
18	F	46	Normal	NA	1.31	2.04
19	F	35	Normal	NA	28.17	5.08
20	F	49	Normal	NA	0.52	1.21
21	F	26	Normal	NA	21.3	2.27
22	F	46	Normal	NA	29.71	8.40
23	F	38	Normal	NA	35.34	4.00
24	F	48	Normal	NA	21.8	3.30
25	F	55	Normal	NA	43.83	10.60
26	F	60	Normal	NA	49.51	12.60
27	F	N/A	Normal	NA	22.4	7.00
28	F	N/A	Normal	NA	10.54	1.70
29	F	N/A	Normal	NA	0.59	2.08
30	F	N/A	Normal	NA	5.1	0.63
31	F	40	Normal	NA	56.2	8.40
32	M	56	Normal	NA	51.1	12.40
33	M	58	Normal	NA	18.31	2.40
34	F	59	Normal	NA	17.94	3.70
35	M	63	Normal	NA	10.37	0.30
36	M	63	Normal	NA	46.09	16.50
37	M	65	Normal	NA	33.26	11.50

38	M	65	Normal	NA	29.21	7.70
39	M	67	Normal	NA	25.81	16.20
40	F	70	Normal	NA	14.25	6.20
41	M	70	Normal	NA	58.24	0.70
42	M	75	Normal	NA	34.96	16.70
43	M	77	Normal	NA	56.84	31.00
44	M	77	Normal	NA	42.57	8.30
45	M	78	Normal	NA	35.65	12.70