Supplementary Data

Table S1. Assay performance and sequencing quality metrics comparing the AnchorIRIS™ (IRM) assay to the SWIFT® Accel-NGS Methyl-Seq™ (SWT) assay.

Sample Name	Assay	Input of cfDNA (ng)	Mapping%	Total PF reads (M)	On target%	Pre-deduped mean bait coverage	Deduped mean bait coverage	Deduped mean target coverage	% target bases > 30X	Uniformity (0.2X mean)
1ng_IRM_Rep1	IRIS	1	65%	11	36%	670	155	92	80%	90%
1ng_IRM_Rep2	IRIS	1	65%	10	37%	638	151	90	80%	91%
3ng_IRM_Rep1	IRIS	3	68%	11	35%	643	268	161	91%	91%
3ng_IRM_Rep2	IRIS	3	67%	13	36%	762	288	172	92%	91%
5ng_IRM_Rep1	IRIS	5	69%	11	39%	738	350	211	95%	92%
5ng_IRM_Rep2	IRIS	5	67%	10	39%	626	318	191	93%	91%
10ng_IRM_Rep1	IRIS	10	70%	12	45%	881	481	290	97%	92%
10ng_IRM_Rep2	IRIS	10	70%	11	44%	815	447	270	97%	91%
1ng_SWT_Rep1	SWIFT	1	66%	11	63%	1427	20	12	8%	82%
1ng_SWT_Rep2	SWIFT	1	66%	13	63%	1704	21	12	9%	83%
3ng_SWT_Rep1	SWIFT	3	68%	12	79%	2083	48	29	40%	85%
3ng_SWT_Rep2	SWIFT	3	68%	12	79%	1988	41	25	34%	84%
5ng_SWT_Rep1	SWIFT	5	68%	12	59%	1542	65	40	52%	85%
5ng_SWT_Rep2	SWIFT	5	67%	12	59%	1488	64	39	51%	85%
10ng_SWT_Rep1	SWIFT	10	69%	12	64%	1608	113	69	68%	85%
10ng_SWT_Rep2	SWIFT	10	68%	13	64%	1763	107	65	67%	84%
QC requirements			> 50%	>10	>30%	>500	>100	>50	>50%	>85%

Table S2. Assay performance metrics of serial dilution samples.

Sample ID	Mapping%	Total PF reads (M)	On target%	Pre-dedup mean bait coverage	Deduped mean bait coverage	Deduped mean target coverage	% target bases > 30X	Uniformity (0.2X mean)	C methylated in CHG context	C methylated in CHH context
100%-rep1	70.34%	44	48.75%	1775	1006	629	99.59%	94.62%	0.7%	0.7%
100%-rep2	71.51%	29	47.27%	1152	762	473	99.24%	94.16%	0.8%	0.8%
10%-rep1	69.75%	44	50.70%	1845	1043	657	99.79%	96.62%	0.7%	0.7%
10%-rep2	68.89%	47	49.88%	1925	1108	705	99.79%	96.69%	0.7%	0.7%
3.3%-rep1	69.40%	46	48.52%	1827	1023	656	99.73%	95.08%	0.8%	0.8%
3.3%-rep2	69.13%	39	47.05%	1493	877	561	99.60%	94.97%	0.8%	0.8%
1%-rep1	69.68%	39	49.66%	1581	924	596	99.65%	95.30%	0.8%	0.8%
1%-rep2	71.00%	35	51.53%	1467	835	540	99.57%	95.29%	0.7%	0.7%
0.33%-rep1	67.97%	40	47.35%	1546	902	580	99.59%	94.97%	0.8%	0.8%
0.33%-rep2	71.43%	32	53.71%	1418	811	529	99.54%	95.02%	0.7%	0.7%
0.1%-rep1	69.33%	42	49.10%	1698	980	632	99.73%	95.99%	0.8%	0.8%
0.1%-rep2	71.28%	41	53.15%	1772	954	621	99.70%	96.00%	0.7%	0.7%
0.033%-rep1	69.64%	44	46.78%	1660	946	619	99.56%	93.46%	0.8%	0.8%
0.033%-rep2	70.76%	35	51.14%	1460	824	541	99.35%	93.08%	0.8%	0.8%
WBC1-rep1	70.54%	39	50.34%	1617	931	603	99.71%	96.33%	0.8%	0.8%
WBC1-rep2	70.23%	34	46.69%	1289	786	512	99.60%	96.22%	0.8%	0.8%
WBC2-rep1	70.95%	33	50.14%	1330	809	527	99.59%	95.66%	0.8%	0.8%
WBC2-rep2	71.86%	37	50.28%	1522	885	577	99.66%	95.64%	0.8%	0.8%
QC requirements	> 50%	>10	>30%	>500	>100	>50	>50%	>85%	<3%	<3%

Table S3. Prediction performance of non-invasive adenocarcinoma malignant tissue samples using the tissue classifier trained based on IA specimens versus benign lesions.

Malignant subtypes	Negative	Positive	Total	sensitivity
MIA	15	20	35	57.1%
AIS	8	6	14	42.9%
SC		7	7	100.0%
Others	2	6	8	75.0%
Sum	25	39	64	60.9%

Table S4. Enrolled plasma samples were randomly divided into the training and independent test data sets for building a plasma diagnostic prediction model.

Plasma samples	Training (66)	Test (66)	Sum (132)
Malignant	**40**	**39**	**79**
IA	27	23	50
MIA	5	9	14
SC	2	6	8
LC	2		2
SCLC	2		2
MC	2		2
ANG		1	1
Benign	**26**	**27**	**53**
INF	3	1	4
GRAN	4	3	7
HAM	6	7	13
TB	12	13	25
FUN		3	3
FSN	1		1

Table S5. Univariate (left) and multivariate (right) analyses were performed using logistic regression to determine significant clinical co-variates of malignancy for early stage lung cancers.

	Univariable analyses		Multivariable analyses	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Clinical variable				
DNA methylation	116.54 (25.12-685.49)	1.32E-08	58.96 (11.52-384.77)	4.32E-06
Age	1.02 (0.99-1.05)	0.136		
Smoking history (non-smoker vs smoker)	0.44 (0.19-0.99)	0.054	0.62 (0.22-1.70)	0.352
Gender (male vs female)	1.35 (0.67-2.75)	0.403		
Nodule density (P vs S)	3.24 (0.56-61.54)	0.279		
Nodule density (G vs S)	3.53 (0.62-66.90)	0.244		
Nodule size	1.69 (1.15-2.62)	0.012	1.46 (0.94-2.52)	0.126

Table S6. Malignancy prediction performance of plasma samples according to nodule sizes.

Nodule size (cm)	Plasma samples	Negative	Positive	Total	Sensitivity
≤1.0	malignant	2	8	10	80.0%
1.1 - 2.0	malignant	2	9	11	81.8%
2.1 - 3.0	malignant	3	6	9	66.7%
≥3.1	malignant	0	4	4	100.0%
Unknown size		1	4	5	80.0%
Sum		8	31	39	79.5%

Table S7. Performance of Four Overlapping Markers in Ooki et al. study and Our Testing Panel

Feature	AUC	AUC.lower	AUC.upper	sensitivity	specificity
cg10474350	55.08594	43.58117	66.59071	28.20513	91.20879
cg07074316	54.38152	43.6046	65.15843	79.48718	37.36264
cg26365299	52.57819	41.50167	63.65471	20.51282	92.30769
cg02483484_cg14218042	50.4931	37.76393	63.22226	48.71795	73.62637

Table S8. Performance of Nine Markers Selected in Our Final Diagnostic Model

Feature	AUC	AUC.lower	AUC.upper	sensitivity	specificity
cg19864007_cg22636429_cg15542994	83.82643	76.34367	91.30919	74.35897	83.51648
cg26970841_cg03978375_cg24826867	79.96619	71.24841	88.68396	89.74359	60.43956
cg04175417	76.64131	68.44905	84.83357	89.74359	52.74725
cg21962423	76.1623	66.97135	85.35325	64.10256	80.21978
cg23156742	75.68329	65.76784	85.59874	61.53846	84.61538
cg06287318	69.73795	60.11728	79.35863	69.23077	61.53846
cg21963643	61.67935	49.94657	73.41213	53.84615	79.12088
cg07568344	57.39645	45.39097	69.40193	43.58974	80.21978
cg12545252	53.19808	39.94905	66.44711	30.76923	96.7033

Supplementary Table 9. The summary of tumor size, cfDNA yield, sequencing QC, and enrollment/exclusion decisions for the plasma study cohort and asymptomatic normal participants.

Sample ID	Excluded/Enrolled samples	Matched tissue sample	Tumor size (cm)	Pathology	Plasma volume (ml)	cfDNA yield (ng/ml)	Mapping%	Total PF reads (M)	On target%	Pre-dedup mean bait coverage	Deduped mean bait coverage	Deduped mean target coverage	% target bases > 30X	Uniformity (0.2X mean)	C methylated in CHG context	C methylated in CHH context
Cohort1-P-017	enrolled	Yes	2.2	Benign	2.4	7.66	80.40%	45	52.40%	2056	662	425	99.53%	96.16%	0.60%	0.60%
Cohort1-P-018	enrolled	Yes	2.7	Benign	2	9.23	77.80%	36	48.92%	1530	475	305	98.55%	94.28%	0.60%	0.60%
Cohort1-P-055	enrolled	Yes	2	Benign	1.8	7.71	78.60%	38	55.72%	1854	597	381	99.35%	95.94%	0.60%	0.60%
Cohort1-P-095	enrolled	Yes	0.7	Benign	2.8	1.30	70.58%	20	56.87%	1017	370	223	95.98%	92.15%	0.40%	0.40%
Cohort1-P-099	enrolled	Yes	1.6	Benign	2.8	3.26	72.46%	29	63.34%	1683	563	333	98.32%	92.78%	0.30%	0.30%
Cohort1-P-100	enrolled	Yes	1	Benign	2.8	1.50	69.48%	34	56.20%	1700	344	198	93.13%	89.73%	0.40%	0.40%
Cohort1-P-103	enrolled	Yes	2.3	Benign	3	1.35	68.07%	21	49.06%	904	121	71	87.51%	97.66%	0.70%	1.00%
Cohort1-P-097	No pathological reports	No	NA	NA	3	10.73	50.79%	17	62.74%	994	428	243	98.22%	96.62%	0.30%	0.30%
Cohort1-P-109	enrolled	Yes	1.2	Benign	3	2.00	70.71%	28	48.73%	1186	464	276	97.35%	92.97%	0.30%	0.30%
Cohort1-P-116	enrolled	Yes	0.8	Benign	3	3.11	69.14%	29	43.37%	1108	274	159	95.98%	96.41%	0.40%	0.40%
Cohort1-P-126	enrolled	Yes	1	Benign	3	1.93	74.77%	33	49.96%	1412	186	117	92.63%	96.45%	0.40%	0.50%
Cohort1-P-193	enrolled	Yes	5	Benign	1.5	5.23	79.10%	39	53.60%	1806	489	310	99.00%	95.94%	0.70%	0.70%
Cohort1-P-102	Insufficient cfDNA	No	0.9	NA	1.3											
Cohort1-P-208	enrolled	Yes	1	Benign	2	5.20	79.20%	39	51.79%	1754	566	359	99.53%	97.88%	0.60%	0.60%
Cohort1-P-215	enrolled	Yes	1.5	Benign	2.2	6.74	79.70%	45	56.80%	2220	647	411	99.32%	94.81%	0.60%	0.60%
Cohort1-P-105	QC Failed	Yes	1.5	NA	3	3.43	60.43%	14	48.23%	582	57	26	29.84%	83.89%	8.30%	8.10%
Cohort1-P-106	QC Failed	Yes	1.6	NA	3	3.73	65.24%	28	48.81%	1130	94	43	46.20%	83.59%	8.50%	8.60%
Cohort1-P-107	Hemolysis	No	1.3	NA												
Cohort1-P-108	QC Failed	No	NA	NA	3	1.29	68.92%	19	51.01%	866	84	47	72.07%	97.72%	0.40%	0.40%
Cohort1-P-216	enrolled	Yes	1.2	Benign	2.3	5.36	78.80%	55	51.73%	2461	751	476	99.67%	97.00%	0.70%	0.70%
Cohort1-P-110	enrolled	No	1.8	malignant	1.7	2.28	59.73%	22	43.16%	833	322	177	90.23%	88.43%	0.40%	0.40%
Cohort1-P-111	Genomic DNA contamination	Yes	2	NA	3	1.21										
Cohort1-P-218	enrolled	Yes	1.2	Benign	2	4.29	78.50%	44	51.07%	1894	564	367	99.37%	96.67%	0.70%	0.70%
Cohort1-P-113	No pathological reports	No	NA	NA	3	1.43	72.76%	11	42.24%	402	133	82	86.44%	96.55%	0.50%	0.50%
Cohort1-P-226	enrolled	Yes	1	Benign	1.8	2.52	78.00%	40	54.74%	1944	459	285	99.39%	97.83%	0.80%	0.80%
Cohort1-P-229	enrolled	Yes	1.1	Benign	2	3.62	77.90%	40	54.22%	1923	565	350	99.60%	98.32%	0.80%	0.80%
Cohort1-P-279	enrolled	Yes	1.3	Benign	2.2	7.96	77.70%	41	52.76%	1852	530	337	99.33%	96.98%	0.80%	0.80%
Cohort1-P-117	QC Failed	Yes	0.8	NA	3	1.31	67.07%	14	37.30%	467	89	50	60.93%	93.76%	3.70%	5.40%
Cohort1-P-285	enrolled	Yes	0.9	Benign	2	5.68	76.80%	39	52.18%	1779	571	360	99.31%	96.13%	0.80%	0.80%
Cohort1-P-358	enrolled	Yes	1.1	Benign	0.85	1.65	72.00%	44	47.15%	1801	433	267	97.05%	92.34%	0.70%	0.70%
Cohort1-P-359	enrolled	Yes	0.6	Benign	0.8	2.73	70.80%	37	44.68%	1404	209	128	93.34%	95.79%	0.80%	0.80%
Cohort1-P-121	Insufficient cfDNA	Yes	1.8	NA	3	0.83										
Cohort1-P-360	enrolled	Yes	1	Benign	1	1.98	72.50%	37	46.15%	1469	345	214	97.80%	95.85%	0.70%	0.70%
Cohort1-P-123	No pathological reports	No	NA	NA	3	5.97	68.99%	23	44.91%	914	208	115	94.46%	97.40%	0.30%	0.30%
Cohort1-P-361	enrolled	Yes	1.5	Benign	0.87	3.31	70.40%	38	43.04%	1383	413	260	98.51%	95.51%	0.80%	0.80%
Cohort1-P-125	No pathological reports	No	NA	NA	3	1.92	77.35%	59	51.15%	2656	468	295	98.79%	95.26%	0.70%	1.00%
Cohort1-P-362	enrolled	Yes	1.8	Benign	1	10.50	70.90%	41	43.59%	1540	349	217	97.75%	95.83%	0.90%	0.90%
Cohort1-P-363	enrolled	Yes	2.8	Benign	1	3.96	72.20%	41	49.08%	1723	511	316	99.18%	96.27%	0.80%	0.80%
Cohort1-P-128	Low pre-library yield	No	NA	NA	3	2.17										
Cohort1-P-364	enrolled	Yes	1	Benign	1.2	1.73	69.00%	24	47.00%	993	338	196	95.11%	93.07%	0.80%	0.80%
Cohort1-P-365	enrolled	Yes	2.3	Benign	1.2	3.57	69.10%	25	45.69%	997	390	225	95.27%	91.71%	0.80%	0.80%
Cohort1-P-131	Low pre-library yield	Yes	2.5	NA	3	1.36										
Cohort1-P-132	Low pre-library yield	Yes	1.4	NA	3	1.53										
Cohort1-P-366	enrolled	Yes	3	Benign	1	8.64	70.20%	30	46.02%	1200	631	362	97.84%	90.64%	0.70%	0.70%
Cohort1-P-134	enrolled	No	1.2	malignant	3	19.33	49.33%	37	49.33%	1633	598	357	99.22%	96.69%	0.30%	0.30%
Cohort1-P-135	QC Failed	Yes	2.5	NA	3	1.95	72.15%	27	51.93%	1258	76	45	70.44%	98.36%	0.60%	0.70%
Cohort1-P-093	enrolled	Yes	4	malignant	3	1.59	54.60%	23	59.59%	1208	500	294	97.93%	93.31%	0.30%	0.30%
Cohort1-P-094	enrolled	Yes	3.2	malignant	2.9	3.21	72.07%	17	54.97%	805	353	212	91.55%	85.87%	0.40%	0.40%
Cohort1-P-138	QC Failed	Yes	3	NA	3	1.25	73.84%	27	51.05%	1194	61	37	55.94%	97.25%	0.60%	0.70%
Cohort1-P-139	No pathological reports	No	NA	NA	3	3.11	76.19%	50	50.61%	2274	98	59	82.62%	98.06%	0.60%	0.70%
Cohort1-P-096	enrolled	Yes	3	malignant	3	3.45	68.10%	12	56.85%	611	157	91	88.32%	96.17%	0.30%	0.40%
Cohort1-P-098	enrolled	Yes	2	malignant	2	2.68	68.02%	18	61.68%	982	281	166	91.87%	90.95%	0.30%	0.40%
Cohort1-P-101	enrolled	Yes	0.9	malignant	1.2	2.53	68.19%	14	60.98%	763	206	121	87.62%	92.59%	0.40%	0.40%
Cohort1-P-143	Insufficient cfDNA	Yes	2	NA	3	0.69										
Cohort1-P-104	enrolled	Yes	1.5	malignant	3	1.48	69.16%	27	52.78%	1276	145	81	92.30%	98.68%	0.40%	0.50%
Cohort1-P-145	enrolled	No	NA	malignant	1.4	4.06	68.07%	35	37.78%	1155	339	203	93.05%	89.15%	0.80%	0.80%
Cohort1-P-112	enrolled	Yes	2	malignant	3	1.55	72.61%	38	45.02%	1384	224	141	96.85%	97.84%	0.50%	0.50%
Cohort1-P-111	enrolled	Yes	1.8	malignant	3	3.07	70.13%	17	42.89%	648	171	100	95.00%	98.36%	0.50%	0.50%
Cohort1-P-149	enrolled	No	1.2	malignant	3.8	1.47	65.82%	44	51.10%	1969	227	135	98.11%	98.97%	0.50%	0.50%
Cohort1-P-150	enrolled	No	NA	malignant	4.2	0.70	73.10%	63	49.99%	2689	309	185	98.99%	99.12%	0.50%	0.50%
Cohort1-P-151	No pathological reports	No	0.7	NA	4.6	0.84	70.82%	50	53.15%	2307	352	212	99.15%	98.97%	0.40%	0.40%
Cohort1-P-152	enrolled	No	1	malignant	3	0.97	67.46%	43	49.77%	1913	167	98	96.20%	99.14%	0.50%	0.50%
Cohort1-P-153	enrolled	No	1.8	malignant	3.5	1.85	75.08%	60	49.44%	2599	399	241	99.59%	99.48%	0.50%	0.60%
Cohort1-P-154	enrolled	No	2.3	Benign	3.8	1.13	68.64%	50	50.61%	2226	290	174	98.94%	99.09%	0.50%	0.50%
Cohort1-P-155	enrolled	No	1.5	malignant	3.2	1.49	63.63%	29	51.03%	1297	194	115	97.65%	99.13%	0.40%	0.50%
Cohort1-P-156	enrolled	No	6	malignant	3.8	0.91	65.51%	25	54.32%	1203	133	79	93.51%	98.90%	0.70%	0.90%
Cohort1-P-157	enrolled	No	1.7	malignant	3.8	1.64	54.01%	29	43.91%	1516	314	194	98.94%	98.73%	0.50%	0.50%
Cohort1-P-158	enrolled	No	3	malignant	3.8	1.39	69.45%	43	48.57%	1809	250	154	98.38%	98.73%	0.50%	0.50%
Cohort1-P-159	enrolled	No	2.5	malignant	3.2	1.58	68.69%	17	42.89%	639	167	102	93.19%	97.86%	1.20%	1.60%
Cohort1-P-160	No pathological reports	No	NA	NA	4.2	4.26	71.66%	39	47.30%	1606	405	245	99.24%	98.44%	0.70%	0.90%
Cohort1-P-161	QC Failed	Yes	NA	NA	3.8	0.71	62.00%	12	50.51%	509	63	37	51.85%	97.87%	0.60%	0.70%
Cohort1-P-162	enrolled	No	1	malignant	2.8	1.86	69.01%	98	41.21%	3411	433	267	97.59%	92.63%	0.70%	0.90%
Cohort1-P-163	QC Failed	No	1.3	NA	3.3	3.13	52.44%	12	44.03%	441	46	28	32.26%	95.83%	1.00%	1.20%
Cohort1-P-165	QC Failed	No	1.3	NA	2.5	3.62	54.41%	35	34.60%	1029	61	36	48.34%	96.32%	1.50%	1.80%
Cohort1-P-166	enrolled	No	3	malignant	3.8	2.51	58.29%	44	33.47%	1253	94	55	71.98%	96.94%	1.20%	1.50%
Cohort1-P-167	QC Failed	No	1.2	NA	3.8	1.44	54.01%	29	31.91%	774	69	42	54.97%	95.61%	1.30%	1.50%
Cohort1-P-168	enrolled	No	NA	malignant	1.8	3.00	66.23%	31	30.02%	761	170	105	80.78%	89.32%	0.90%	0.90%
Cohort1-P-169	enrolled	No	0.6	malignant	4.6	2.48	70.20%	30	31.58%	787	316	197	90.71%	86.68%	2.30%	2.90%
Cohort1-P-170	enrolled	No	0.8	Benign	5	1.20	74.40%	29	44.07%	1085	98	61	73.95%	94.93%	1.00%	1.20%
Cohort1-P-171	enrolled	No	2.5	malignant	4.5	2.35	70.68%	34	47.56%	1359	392	250	96.77%	91.79%	0.70%	0.70%
Cohort1-P-172	enrolled	No	2	Benign	5	3.97	67.98%	22	43.11%	809	211	132	91.17%	94.25%	0.80%	0.90%
Cohort1-P-173	enrolled	No	1.2	malignant	4	3.57	72.07%	38	33.15%	1036	477	303	97.40%	89.45%	0.70%	0.70%
Cohort1-P-174	enrolled	No	2.5	malignant	3.2	4.28	70.55%	31	32.79%	842	398	250	94.65%	87.09%	0.80%	0.80%
Cohort1-P-175	enrolled	No	2	malignant	3.8	1.69	71.97%	43	48.59%	1760	452	284	95.69%	88.00%	0.80%	0.80%
Cohort1-P-176	enrolled	No	0.9	malignant	3.2	1.35	68.55%	28	39.37%	946	203	128	86.81%	90.37%	0.90%	0.90%
Cohort1-P-177	No pathological reports	No	NA	NA	4.5	2.59	70.03%	34	32.78%	946	398	247	95.13%	89.08%	0.80%	0.80%
Cohort1-P-178	enrolled	No	1.1	Benign	3.8	1.05	69.08%	28	38.80%	895	209	132	85.32%	88.43%	1.20%	1.30%
Cohort1-P-115	enrolled	Yes	4	malignant	2.95	7.05	71.16%	23	44.92%	895	178	103	95.29%	98.34%	0.50%	0.50%
Cohort1-P-118	enrolled	Yes	NA	malignant	3	1.72	68.19%	30	43.31%	1109	167	97	87.79%	95.71%	0.40%	0.40%
Cohort1-P-119	enrolled	Yes	1.3	malignant	3	1.56	68.06%	25	42.63%	915	165	95	88.60%	96.34%	0.60%	0.70%
Cohort1-P-120	enrolled	Yes	3.2	malignant	3	2.19	69.54%	20	43.60%	732	293	173	95.77%	95.25%	0.30%	0.30%
Cohort1-P-122	enrolled	Yes	1.2	malignant	3	1.75	67.97%	25	48.45%	1096	184	101	93.79%	97.73%	0.40%	0.40%
Cohort1-P-124	enrolled	Yes	2	malignant	3	2.88	71.93%	29	45.23%	1125	432	255	96.54%	90.95%	0.30%	0.30%
Cohort1-P-127	enrolled	Yes	1.5	malignant	3	2.63	76.17%	48	48.29%	2032	325	203	97.83%	96.27%	0.50%	0.60%
Cohort1-P-129	enrolled	Yes	3	malignant	3	1.43	78.53%	70	52.83%	3274	497	310	98.81%	94.66%	0.40%	0.40%
Cohort1-P-130	enrolled	Yes	1	malignant	3	3.25	74.22%	40	51.29%	1854	189	113	96.82%	98.76%	0.40%	0.40%
Cohort1-P-290	Low pre-library yield	No	NA	NA	3.8	3.83										
Cohort1-P-291	enrolled	No	2.3	malignant	3	4.51	59.91%	37	49.55%	1567	105	63	83.54%	98.06%	1.40%	1.60%
Cohort1-P-292	Genomic DNA contamination	No	NA	NA	3.9	2.94										
Cohort1-P-293	enrolled	No	1.2	malignant	2.6	7.77	69.38%	35	50.50%	1494	517	334	98.33%	91.32%	0.90%	0.90%
Cohort1-P-294	Genomic DNA contamination	No	NA	NA	4	1.31										
Cohort1-P-295	QC Failed	No	1.2	NA	3.8	2.99	57.80%	28	48.77%	1163	118	73	82.49%	96.81%	2.50%	3.30%
Cohort1-P-296	QC Failed	No	4.5	NA	4	2.71	50.46%	11	60.25%	582	54	32	47.78%	97.00%	2.90%	3.90%
Cohort1-P-297	enrolled	No	NA	Benign	4	1.95	66.33%	25	44.16%	994	284	181	94.45%	92.34%	1.10%	1.30%
Cohort1-P-298	enrolled	No	1	malignant	3.8	0.93	64.98%	33	39.87%	1112	261	163	87.54%	86.35%	1.20%	1.30%
Cohort1-P-299	enrolled	No	NA	malignant	3.8	1.96	71.18%	35	41.11%	1192	378	245	97.15%	92.98%	1.10%	1.30%
Cohort1-P-300	enrolled	No	3.5	malignant	3.8	1.25	65.52%	34	42.30%	1210	253	159	88.59%	87.96%	1.20%	1.40%
Cohort1-P-301	enrolled	No	0.6	malignant	3.8	2.05	67.22%	39	45.09%	1441	338	215	96.83%	93.58%	1.30%	1.50%
Cohort1-P-302	enrolled	No	2.6	malignant	3.2	1.76	69.10%	33	40.61%	1137	263	164	86.60%	85.42%	0.90%	1.00%
Cohort1-P-303	enrolled	No	0.8	malignant	3	2.76	67.43%	30	37.46%	918	237	151	88.20%	88.54%	1.10%	1.20%
Cohort1-P-304	enrolled	No	NA	Benign	3.6	1.27	65.60%	29	29.51%	731	201	122	90.10%	94.33%	0.90%	0.90%
Cohort1-P-305	No pathological reports	No	NA	NA	3.8	1.13	68.83%	24	42.36%	865	270	165	87.33%	85.97%	0.80%	0.80%
Cohort1-P-306	enrolled	No	3.3	malignant	4	1.63	70.45%	41	32.55%	1110	446	275	97.20%	90.86%	0.80%	0.80%
Cohort1-P-307	enrolled	No	0.5	malignant	3.3	0.99	65.14%	23	38.73%	763	266	161	92.43%	92.37%	0.80%	0.80%
Cohort1-P-308	Genomic DNA contamination	No	NA	NA	4	1.05										
Cohort1-P-309	enrolled	No	2	malignant	4	2.19	67.82%	21	29.20%	498	216	133	89.42%	92.39%	0.90%	0.90%
Cohort1-P-310	enrolled	No	1.5	Benign	3.2	1.09	70.45%	59	36.10%	1810	479	293	98.83%	95.59%	0.80%	0.80%
Cohort1-P-311	enrolled	No	NA	malignant	3.8	3.13	71.22%	38	44.48%	1438	598	367	99.05%	94.95%	0.70%	0.70%
Cohort1-P-312	No pathological reports	No	NA	malignant	3.8	2.95	70.25%	45	37.55%	1425	592	368	98.81%	93.18%	0.80%	0.90%
Cohort1-P-313	enrolled	No	2.5	malignant	3.6	1.88	72.08%	40	38.81%	1302	441	273	96.18%	89.68%	0.80%	0.80%
Cohort1-P-314	Low pre-library yield	No	NA	NA	2.2	3.07										
Cohort1-P-315	No pathological reports	No	NA	NA	4	1.88	70.45%	42	33.36%	1172	424	261	96.50%	90.16%	0.80%	0.80%
Cohort1-P-316	enrolled	No	0.6	malignant	3.8	1.61	68.99%	56	33.84%	1592	459	284	98.15%	93.06%	0.80%	0.80%
Cohort1-P-317	enrolled	No	0.6	malignant	3.8	4.19	69.87%	63	34.60%	1823	725	446	99.14%	92.63%	0.80%	0.80%
Cohort1-P-318	enrolled	No	NA	malignant	3.8	1.75	67.78%	26	35.18%	775	269	165	87.97%	86.29%	0.80%	0.90%
Cohort1-P-319	enrolled	No	0.5	malignant	3.8	1.25	66.97%	25	36.53%	749	163	103	84.08%	92.61%	0.90%	0.90%
Cohort1-P-320	No pathological reports	No	NA	NA	4	2.17	72.05%	49	40.86%	1691	457	283	96.79%	90.03%	0.70%	0.80%
Cohort1-P-321	enrolled	No	3.5	malignant	4	1.64	74.74%	32	30.99%	823	271	170	87.71%	85.62%	0.80%	0.80%
Cohort1-P-322	enrolled	No	1	Benign	2.8	2.63	72.94%	40	36.62%	1262	427	263	97.55%	92.83%	0.70%	0.70%
Cohort1-P-323	enrolled	No	1.4	malignant	3	1.64	69.56%	35	42.66%	1287	336	191	93.67%	90.77%	0.80%	0.80%
Cohort1-P-324	enrolled	No	0.3	Benign	3.7	2.38	71.31%	35	39.10%	1186	436	269	96.74%	90.99%	0.70%	0.70%
Cohort1-P-325	No pathological reports	No	NA	malignant	3	1.54	69.85%	33	41.86%	1187	296	180	91.37%	88.72%	0.80%	0.80%
Cohort1-P-326	enrolled	No	2.5	malignant	3.9	2.28	68.01%	26	50.99%	1189	493	279	95.24%	88.66%	0.80%	0.80%
Cohort1-P-327	enrolled	No	2.5	malignant	3.9	1.79	71.54%	36	39.59%	1206	369	229	94.52%	88.98%	0.80%	0.80%
Cohort1-P-328	QC Failed	No	2.8	NA	3.3	1.59	63.93%	23	45.87%	918	76	45	57.64%	93.57%	1.30%	1.60%
Cohort1-P-329	QC Failed	No	3.5	NA	4	1.62	64.31%	22	44.97%	851	74	44	59.64%	95.56%	1.30%	1.60%
Cohort1-P-330	No pathological reports	No	NA	NA	4.5	2.60	71.39%	37	35.47%	1067	428	272	97.07%	90.71%	1.60%	2.00%
Cohort1-P-331	enrolled	No	0.5	malignant	3.7	2.97	73.54%	54	36.96%	1662	623	393	97.92%	89.24%	0.80%	0.80%
Cohort1-P-332	enrolled	No	4.3	malignant	4.5	2.38	71.95%	50	34.22%	1402	534	336	97.65%	89.94%	0.90%	0.90%
Cohort1-P-333	enrolled	No	2.5	malignant	3.8	4.95	73.45%	49	35.23%	1441	675	420	97.85%	88.30%	0.80%	0.80%
Cohort1-P-334	No pathological reports	No	1.2	malignant	3	1.64	69.09%	46	42.83%	1677	419	260	98.18%	94.43%	0.90%	1.00%
Cohort1-P-335	enrolled	No	NA	malignant	3.8	1.43	72.69%	31	35.96%	953	327	200	89.77%	85.28%	0.80%	0.90%
Cohort1-P-336	QC Failed	No	0.7	NA	3.6	1.71	64.92%	10	27.58%	235	76	48	55.09%	93.80%	26.20%	34.60%
Cohort1-P-337	enrolled	No	1.2	malignant	3.6	1.91	71.86%	38	38.23%	1221	431	267	97.76%	93.23%	0.80%	0.80%
Cohort1-P-338	enrolled	No	2.7	malignant	4.4	2.84	66.33%	46	44.73%	1751	152	90	89.06%	96.91%	1.20%	1.30%
Cohort1-P-339	enrolled	No	1.5	malignant	4.6	1.41	70.39%	37	42.26%	1309	300	186	90.30%	87.23%	0.80%	0.90%
Cohort1-P-340	enrolled	No	1.7	malignant	1.8	4.71	73.16%	37	35.13%	1088	439	269	93.73%	85.78%	0.80%	0.80%
Cohort1-P-341	enrolled	No	3	malignant	2	2.02	67.15%	31	44.01%	1143	211	130	85.33%	88.44%	0.90%	0.90%
Cohort1-P-342	enrolled	No	0.8	malignant	4	1.85	67.89%	55	45.22%	2063	192	119	87.71%	92.44%	1.00%	1.10%
Cohort1-P-343	QC Failed	No	NA	NA	4	2.18	68.66%	47	59.75%	2414	71	44	56.15%	94.06%	1.10%	1.40%
Cohort1-P-344	enrolled	No	1.5	malignant	4.6	1.17	74.98%	50	61.33%	2574	136	87	82.91%	94.40%	1.00%	1.20%

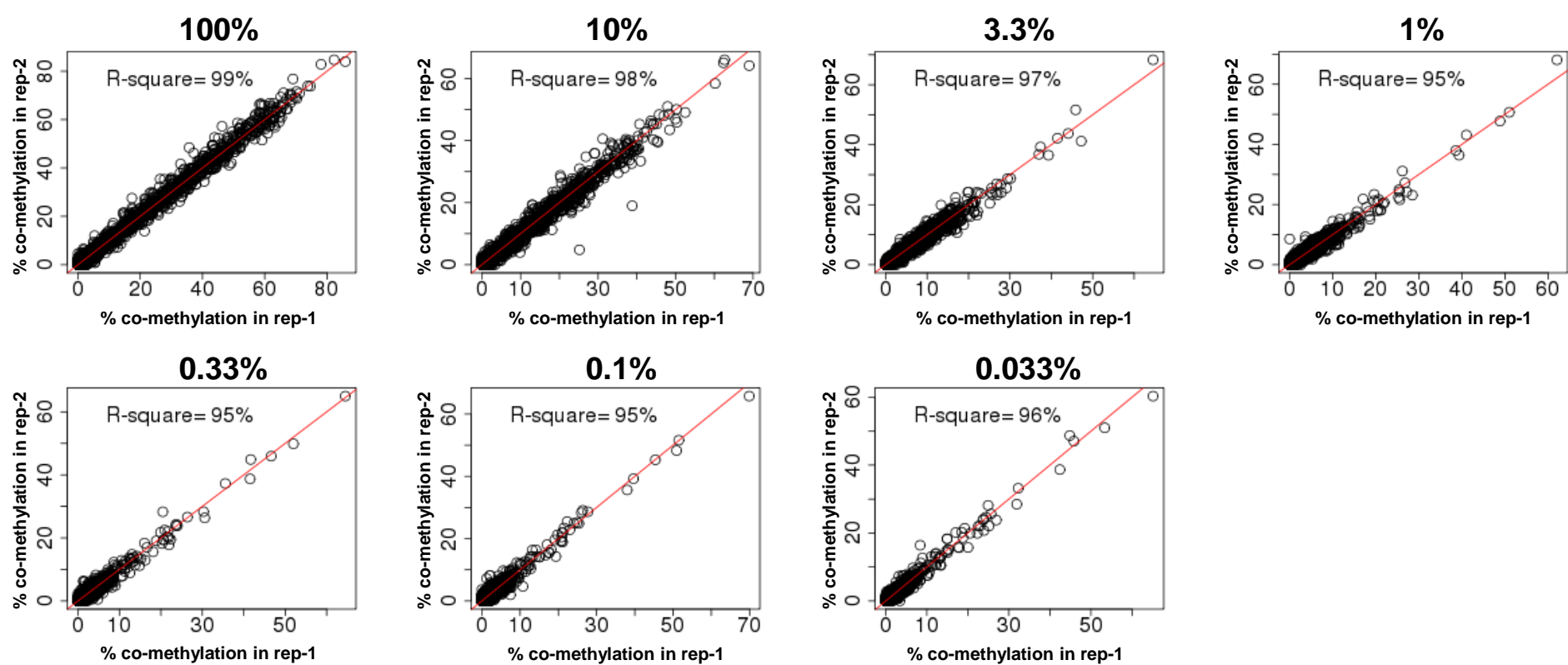|																
Cohort1-P-345	enrolled	No	0.5	malignant	4	3.40	75.64%	57	56.99%	2756	180	114	84.36%	91.13%	1.00%	1.20%
Cohort1-P-346	Genomic DNA contamination	No	NA	NA	2.8	22.00										
Cohort1-P-347	enrolled	No	1.3	malignant	3.6	3.01	69.05%	52	66.10%	3020	88	54	70.50%	96.56%	1.20%	1.40%
Cohort1-P-348	QC Failed	No	NA	NA	3.5	1.61	64.76%	34	62.65%	1865	54	33	42.19%	94.30%	1.40%	1.90%
Cohort1-P-349	enrolled	No	1.1	malignant	3.2	2.43	72.81%	42	59.60%	2162	124	79	82.50%	95.92%	1.10%	1.20%
Cohort1-P-350	QC Failed	No	NA	NA	3.2	1.99	66.31%	36	66.13%	2035	51	31	37.95%	92.08%	1.40%	1.80%
Cohort1-P-351	No pathological reports	No	1.2	NA	3	3.19	75.23%	31	60.94%	1666	159	99	88.30%	95.95%	0.90%	1.00%
Cohort1-P-352	Low pre-library yield	No	NA	NA	2	2.68										
Cohort1-P-353	No pathological reports	No	NA	NA	4	3.20	78.26%	67	59.32%	3451	673	442	99.03%	91.76%	0.70%	0.80%
Cohort1-P-354	No pathological reports	No	2.5	NA	5	2.51	77.07%	47	56.26%	2218	305	199	91.08%	86.46%	0.70%	0.70%
Cohort1-P-355	Low pre-library yield	No	NA	NA	4	2.16										
Cohort1-P-356	Low pre-library yield	No	NA	NA	2.5	2.21										
Cohort1-P-357	Low pre-library yield	No	NA	NA	4.5	1.68										
Cohort1-P-133	enrolled	Yes	3.9	malignant	3	1.91	78.66%	53	50.33%	2272	228	145	91.54%	92.82%	0.30%	0.30%
Cohort1-P-136	enrolled	Yes	NA	malignant	3	4.29	75.47%	31	45.77%	1246	281	172	97.34%	97.09%	0.40%	0.40%
Cohort1-P-137	enrolled	Yes	0.9	malignant	3	3.81	77.63%	32	48.39%	1355	229	143	95.29%	96.69%	0.40%	0.40%
Cohort1-P-140	enrolled	Yes	2.5	malignant	2.7	1.38	64.65%	25	69.98%	1603	130	74	89.43%	98.34%	0.40%	0.40%
Cohort1-P-141	enrolled	Yes	2.1	malignant	3	1.31	66.79%	37	66.60%	2263	292	175	98.30%	98.29%	0.50%	0.50%
Cohort1-P-142	enrolled	Yes	1.5	malignant	3	2.40	67.22%	41	54.96%	1961	468	289	99.24%	97.44%	0.40%	0.40%
Cohort1-P-144	enrolled	Yes	1.3	malignant	3	1.09	63.08%	24	64.37%	1362	142	84	87.92%	97.35%	0.50%	0.50%
Cohort1-P-146	enrolled	Yes	1.5	malignant	3.2	1.56	73.99%	82	48.27%	3380	511	309	99.65%	99.21%	0.40%	0.40%
Cohort1-P-148	enrolled	Yes	1.2	malignant	3.6	1.33	70.47%	41	54.14%	1928	244	149	98.55%	99.02%	0.40%	0.50%
Cohort1-P-367	enrolled	no	1.7	Benign	2.5	2.30	77.00%	43	50.83%	1921	496	306	99.34%	97.58%	1.10%	1.10%
Cohort1-P-368	enrolled	no	1.7	Benign	2	3.77	77.90%	45	50.56%	2007	562	346	99.05%	95.69%	0.90%	0.90%
Cohort1-P-369	enrolled	no	1.4	Benign	2	1.29	77.70%	45	54.93%	2202	387	236	98.58%	96.91%	1.20%	1.40%
Cohort1-P-370	enrolled	no	1.2	Benign	2.1	3.28	78.30%	53	54.13%	2511	641	395	99.54%	97.09%	0.90%	0.90%
Cohort1-P-371	Insufficient cfDNA	no	2.6	Benign	1.2	too low										
Cohort1-P-372	Insufficient cfDNA	no	2.5	Benign	2	0.84										
Cohort1-P-373	enrolled	no	1.8	Benign	2.1	2.21	79.74%	34	59.43%	1781	409	255	98.99%	97.10%	1.00%	1.00%
Cohort1-P-374	enrolled	no	1.5	Benign	1.7	1.76	80.08%	32	58.61%	1638	332	207	97.46%	95.85%	0.90%	0.90%
Cohort1-P-375	enrolled	no	2.5	Benign	1.8	6.45	79.85%	36	60.53%	1927	553	348	99.26%	96.13%	1.00%	1.00%
Cohort1-P-376	enrolled	no	0.7	Benign	2.2	5.00	79.58%	41	58.87%	2109	634	397	99.36%	95.88%	1.00%	1.00%
Cohort1-P-377	enrolled	no	0.8	Benign	2.1	1.25	77.77%	28	50.27%	1239	266	165	97.75%	97.64%	0.90%	0.90%
Cohort1-P-378	enrolled	no	1	Benign	2.3	2.59	79.16%	34	51.90%	1541	426	260	99.10%	97.66%	0.90%	0.90%
Cohort1-P-379	enrolled	no	1.7	Benign	2.2	1.15	78.39%	34	54.63%	1574	268	169	97.68%	97.53%	1.10%	1.20%
Cohort1-P-380	enrolled	no	1.7	Benign	2	1.86	79.05%	35	55.13%	1669	374	230	98.29%	96.33%	1.00%	1.00%
Cohort1-P-381	enrolled	no	3	Benign	3	3.18	79.98%	35	61.70%	1898	476	303	99.19%	96.99%	0.90%	0.90%
Cohort1-P-382	enrolled	no	NA	Benign	1.5	2.79	80.48%	41	62.83%	2325	416	257	98.66%	96.28%	0.90%	0.90%
Cohort1-P-383	Insufficient cfDNA	no	NA	Benign	1.2	too low										
Cohort1-P-384	enrolled	no	NA	Benign	1.3	5.28	80.46%	31	61.91%	1729	422	263	97.97%	94.73%	0.90%	0.90%
Cohort1-P-385	Insufficient cfDNA	no	NA	Benign	1.1	1.20										

Supplementary Table 9. The summary of tumor size, cfDNA yield, sequencing QC, and enrollment/exclusion decisions for the plasma study cohort and asymptomatic normal participants.

Sample ID	Excluded/Enrolled samples	Plasma volume (ml)	cfDNA yield (ng/ml)	Mapping%	Total PF reads (M)	On target%	Pre-dedup mean bait coverage	Deduped mean bait coverage	Deduped mean target coverage	% target bases > 30X	Uniformity (0.2X mean)	C methylated in CHG context	C methylated in CHH context
NLCTL-P-055	Enrolled	2.6	1.46	78.7%	42	54.84%	2031	452	284	98.61%	95.40%	0.8%	0.8%
NLCTL-P-072	Enrolled	2.6	1.11	78.8%	40	52.74%	1844	411	260	98.42%	95.31%	0.8%	0.8%
NLCTL-P-074	Enrolled	2.6	1.74	79.4%	42	50.35%	1813	509	323	98.42%	93.92%	0.9%	0.9%
NLCTL-P-075	Enrolled	2.6	2.09	80.4%	59	50.12%	2542	722	462	99.76%	98.49%	0.8%	0.8%
NLCTL-P-077	Enrolled	2.6	3.73	79.0%	43	47.59%	1767	710	456	99.64%	97.34%	0.9%	0.9%
NLCTL-P-078	Enrolled	2.8	5.31	79.4%	43	45.57%	1682	656	414	98.90%	93.35%	0.8%	0.8%
NLCTL-P-079	Enrolled	2.7	2.46	77.9%	33	42.02%	1186	497	320	99.28%	97.25%	0.9%	0.9%
NLCTL-P-080	Enrolled	2.6	1.12	77.6%	31	46.81%	1223	371	240	98.56%	96.81%	1.0%	1.0%
NLCTL-P-081	Enrolled	2.5	1.22	78.4%	30	44.59%	1122	349	227	98.20%	96.26%	0.9%	0.9%
NLCTL-P-084	Enrolled	3	1.81	78.8%	36	47.78%	1468	479	312	98.83%	94.98%	0.9%	0.9%
NLCTL-P-085	Enrolled	2.6	1.15	76.0%	37	44.47%	1394	344	217	97.93%	96.22%	0.9%	1.0%
NLCTL-P-087	Enrolled	3	2.01	75.4%	33	42.84%	1233	488	304	98.57%	94.83%	0.9%	0.9%
NLCTL-P-088	Enrolled	3	1.51	78.3%	29	49.68%	1228	410	263	99.11%	97.52%	1.0%	1.0%
NLCTL-P-089	Enrolled	2.6	1.14	76.2%	44	48.82%	1896	491	303	98.83%	95.66%	0.9%	1.0%
NLCTL-P-090	Enrolled	3	0.71	74.9%	25	47.28%	1031	319	199	95.61%	93.31%	0.9%	1.0%
NLCTL-P-092	Enrolled	2.8	2.93	78.5%	32	49.23%	1350	488	317	98.80%	95.50%	0.9%	0.9%
NLCTL-P-093	Enrolled	3.9	5.22	77.7%	58	54.05%	2788	709	435	99.64%	97.66%	0.7%	0.7%
NLCTL-P-094	Enrolled	2.8	1.57	60.3%	46	36.21%	1436	210	147	93.11%	95.43%	1.3%	1.4%
NLCTL-P-095	Enrolled	2.8	2.11	70.4%	26	47.94%	1076	295	184	98.08%	97.34%	0.6%	0.6%
NLCTL-P-096	Enrolled	2.4	4.68	73.3%	22	50.24%	961	345	216	98.23%	96.41%	0.4%	0.4%
NLCTL-P-097	Enrolled	2.6	3.03	71.4%	32	49.46%	1381	421	259	98.76%	96.40%	0.5%	0.5%
NLCTL-P-098	Enrolled	2.6	4.88	70.4%	36	51.46%	1577	583	361	99.46%	97.64%	0.4%	0.4%
NLCTL-P-107	Enrolled	2.8	3.70	71.7%	34	66.14%	1981	618	387	99.53%	97.93%	0.5%	0.5%
NLCTL-P-108	Enrolled	2.8	2.81	69.1%	18	55.65%	862	345	212	97.74%	95.88%	0.4%	0.5%
NLCTL-P-112	Enrolled	2.7	9.11	69.7%	22	55.02%	1053	538	336	99.28%	96.66%	0.4%	0.4%
NLCTL-P-113	Enrolled	2.8	3.93	69.1%	30	52.42%	1357	510	315	99.15%	96.29%	0.4%	0.4%
NLCTL-P-117	Enrolled	3	4.99	69.0%	24	52.44%	1072	421	260	98.57%	95.82%	0.4%	0.4%
NLCTL-P-120	Enrolled	2.8	4.06	66.9%	21	53.27%	957	482	295	99.01%	96.51%	0.4%	0.4%
NLCTL-P-121	Enrolled	2.8	1.51	66.7%	18	51.72%	811	285	177	97.41%	96.89%	0.5%	0.5%
NLCTL-P-122	Enrolled	2.8	2.21	71.2%	29	51.25%	1273	488	304	98.95%	95.48%	0.5%	0.5%
NLCTL-P-126	Enrolled	2.8	2.16	69.9%	33	51.16%	1457	548	339	99.38%	97.44%	0.4%	0.4%
NLCTL-P-127	Enrolled	2.4	6.13	70.5%	16	49.54%	680	383	238	97.82%	94.50%	0.4%	0.4%
NLCTL-P-128	Enrolled	2.8	6.46	79.0%	47	52.70%	2107	621	400	98.28%	90.35%	0.6%	0.6%
NLCTL-P-130	Enrolled	2.8	4.41	79.2%	36	57.72%	1765	400	260	96.60%	91.69%	0.7%	0.7%
NLCTL-P-131	Enrolled	2.8	4.24	79.0%	42	53.72%	1932	591	384	99.08%	93.60%	0.7%	0.7%
NLCTL-P-133	Enrolled	2.8	6.99	77.8%	45	48.04%	1862	689	433	97.86%	88.51%	0.7%	0.7%
NLCTL-P-135	Enrolled	2.4	2.88	78.2%	42	57.66%	2073	410	261	98.37%	94.97%	0.8%	0.8%
NLCTL-P-136	Enrolled	2.8	6.80	80.4%	43	53.37%	2002	702	448	98.70%	90.48%	0.8%	0.8%
NLCTL-P-138	Enrolled	2.8	6.96	77.2%	41	53.67%	1861	582	381	98.90%	92.77%	0.5%	0.5%
NLCTL-P-140	Enrolled	2.8	6.13	77.9%	48	50.99%	2083	653	421	98.51%	90.29%	0.7%	0.7%
NLCTL-P-141	Enrolled	3	2.16	77.5%	43	53.18%	1965	467	298	97.27%	90.96%	0.5%	0.6%
NLCTL-P-142	Enrolled	2.8	3.79	78.7%	42	52.58%	1865	536	341	97.58%	89.45%	0.5%	0.5%
NLCTL-P-143	Enrolled	2.8	2.24	77.7%	42	52.94%	1889	415	263	97.53%	93.06%	0.5%	0.5%
NLCTL-P-145	Enrolled	2.8	3.01	79.0%	35	59.33%	1817	403	254	96.44%	91.53%	0.6%	0.7%
NLCTL-P-146	Enrolled	2.8	4.69	79.1%	36	55.27%	1683	607	401	99.64%	97.86%	0.8%	0.8%
NLCTL-P-147	Enrolled	2.8	5.50	78.6%	45	51.39%	1947	660	423	98.99%	92.37%	0.5%	0.5%
NLCTL-P-149	Enrolled	2.8	2.76	77.1%	50	53.76%	2328	596	380	99.13%	94.23%	0.7%	0.8%
NLCTL-P-152	Enrolled	2.8	2.86	78.9%	45	49.45%	1902	370	234	96.71%	92.71%	0.9%	1.0%
NLCTL-P-155	Enrolled	2.8	3.74	80.0%	43	48.85%	1762	463	300	97.46%	91.19%	1.0%	1.1%
NLCTL-P-158	Enrolled	2.8	4.27	79.0%	38	56.01%	1848	568	363	98.87%	94.15%	0.6%	0.6%
NLCTL-P-159	Enrolled	2.8	3.14	76.3%	43	53.54%	2030	500	310	97.74%	92.21%	0.6%	0.6%
NLCTL-P-161	Enrolled	2.8	3.73	74.0%	32	48.74%	1352	485	305	98.05%	92.60%	0.6%	0.6%
NLCTL-P-169	Enrolled	2.8	5.83	74.2%	40	52.15%	1799	704	448	99.39%	94.95%	0.7%	0.7%
NLCTL-P-172	Enrolled	2.8	4.60	79.4%	34	55.68%	1607	527	345	98.62%	93.45%	0.5%	0.5%
NLCTL-P-174	Enrolled	2.9	3.23	75.1%	36	54.03%	1723	537	329	96.85%	89.83%	0.9%	0.9%
NLCTL-P-175	Enrolled	2.9	1.93	77.1%	27	47.22%	1102	373	242	96.64%	92.17%	0.9%	0.9%
NLCTL-P-185	Enrolled	2.7	5.05	77.3%	44	54.68%	2072	590	379	99.56%	97.82%	0.9%	0.9%
NLCTL-P-187	Enrolled	2.8	4.63	78.8%	32	52.63%	1435	578	375	98.89%	94.29%	0.9%	0.8%
NLCTL-P-189	Enrolled	2.8	14.71	80.2%	39	55.32%	1882	705	455	98.90%	92.69%	0.9%	0.9%
NLCTL-P-192	Enrolled	2.8	5.83	74.7%	35	52.94%	1650	616	381	99.28%	95.70%	0.8%	0.8%
NLCTL-P-193	Enrolled	2.8	3.76	75.4%	52	51.67%	2329	862	541	99.69%	96.36%	0.8%	0.8%
NLCTL-P-198	Enrolled	2.8	6.24	79.4%	33	55.36%	1553	711	457	99.51%	96.05%	0.7%	0.7%
NLCTL-P-202	Enrolled	2.8	3.93	75.5%	44	47.86%	1847	676	421	99.29%	94.96%	1.0%	1.0%
NLCTL-P-205	Enrolled	2.8	4.71	74.7%	51	55.14%	2484	712	449	99.62%	97.26%	0.7%	0.8%
NLCTL-P-210	Enrolled	2.7	32.59	79.9%	37	54.88%	1819	793	486	99.12%	93.17%	0.6%	0.7%
NLCTL-P-211	Enrolled	3	4.51	74.6%	24	56.41%	1167	525	333	99.29%	96.98%	0.4%	0.4%
NLCTL-P-216	Enrolled	3	3.67	72.5%	31	55.88%	1503	626	390	99.50%	97.34%	0.4%	0.4%
NLCTL-P-220	Enrolled	3	2.40	75.4%	46	57.42%	2201	393	234	96.25%	95.79%	0.3%	0.3%
NLCTL-P-223	Enrolled	3	9.27	77.5%	33	54.62%	1569	750	430	95.90%	88.28%	0.3%	0.3%
NLCTL-P-233	Enrolled	3	1.43	74.5%	20	56.34%	1008	306	180	92.11%	91.14%	0.3%	0.3%
NLCTL-P-236	Enrolled	3	2.48	74.0%	20	49.26%	823	330	200	92.91%	90.84%	0.3%	0.3%
NLCTL-P-238	Enrolled	3	8.13	78.3%	46	54.12%	2119	808	471	96.71%	91.11%	0.2%	0.2%
NLCTL-P-240	Enrolled	3	2.44	66.3%	38	55.14%	1841	455	279	99.15%	97.16%	0.4%	0.4%
NLCTL-P-241	Enrolled	3	2.57	71.7%	22	56.64%	1086	386	238	98.39%	96.14%	0.4%	0.4%
NLCTL-P-251	Enrolled	3	3.65	66.4%	20	47.01%	805	322	200	97.15%	95.24%	0.4%	0.4%
NLCTL-P-254	Enrolled	3	2.64	75.1%	27	54.10%	1240	486	286	95.69%	92.45%	0.2%	0.2%
NLCTL-P-256	Enrolled	2.6	7.85	71.1%	45	46.02%	1806	845	512	99.60%	96.14%	0.7%	0.7%
NLCTL-P-257	Enrolled	2.5	4.33	70.7%	37	48.98%	1597	704	429	99.39%	95.69%	0.7%	0.7%
NLCTL-P-258	Enrolled	3	4.96	72.5%	45	51.75%	2038	796	479	99.26%	92.69%	0.7%	0.7%
NLCTL-P-264	Enrolled	2.6	5.52	70.2%	41	52.27%	1888	808	485	99.57%	95.70%	0.7%	0.7%
NLCTL-P-265	Enrolled	3	1.92	70.3%	37	50.61%	1637	580	353	98.53%	93.18%	0.7%	0.7%
NLCTL-P-266	Enrolled	2.8	0.81	70.1%	41	51.38%	1833	479	291	98.35%	94.83%	0.7%	0.8%
NLCTL-P-269	Enrolled	2.4	1.39	71.6%	42	51.42%	1880	536	325	99.02%	95.60%	0.7%	0.7%
NLCTL-P-273	Enrolled	1.6	4.71	68.7%	43	46.41%	1729	718	434	99.54%	96.60%	0.8%	0.8%
NLCTL-P-275	Enrolled	3	16.73	70.8%	53	49.36%	2262	977	589	99.70%	96.18%	0.8%	0.8%
NLCTL-P-278	Enrolled	3	9.00	70.9%	57	49.68%	2463	986	604	99.72%	96.56%	0.8%	0.8%
NLCTL-P-279	Enrolled	3	4.94	73.9%	51	53.83%	2402	967	590	99.57%	94.44%	0.8%	0.8%
NLCTL-P-281	Enrolled	3	4.05	68.5%	36	42.27%	1331	617	382	99.21%	95.29%	0.9%	0.9%
NLCTL-P-285	Enrolled	3	3.62	72.2%	32	50.51%	1407	606	379	98.92%	94.28%	0.9%	0.9%
NLCTL-P-288	Enrolled	3	6.36	72.7%	42	50.93%	1852	848	524	99.68%	96.82%	0.8%	0.8%
NLCTL-P-289	Enrolled	3	6.12	72.3%	48	49.32%	2046	862	527	99.28%	93.56%	0.8%	0.8%
NLCTL-P-290	Enrolled	3	2.00	70.4%	36	46.75%	1467	557	341	98.97%	95.17%	0.8%	0.8%
NLCTL-P-295	Enrolled	3	2.39	71.2%	45	49.79%	1976	658	398	99.39%	95.79%	0.8%	0.8%
NLCTL-P-302	Enrolled	3	3.79	70.5%	46	47.36%	1893	696	426	99.36%	95.06%	0.8%	0.8%
NLCTL-P-303	Enrolled	3	4.65	71.5%	38	47.18%	1545	469	284	96.87%	91.10%	0.8%	0.8%
NLCTL-P-311	Enrolled	3	3.82	70.4%	43	49.53%	1876	708	433	99.22%	94.35%	0.8%	0.8%
NLCTL-P-315	Enrolled	2.4	3.86	76.5%	33	50.09%	1413	566	359	98.88%	94.62%	0.7%	0.7%
NLCTL-P-316	Enrolled	3	1.81	73.4%	31	47.41%	1236	459	296	99.10%	97.01%	0.8%	0.9%
NLCTL-P-336	Enrolled	3	1.23	75.2%	36	49.38%	1504	462	290	98.80%	95.88%	0.9%	0.9%
NLCTL-P-337	Enrolled	2.4	1.92	75.5%	39	49.51%	1638	463	292	98.54%	95.00%	0.9%	1.1%
NLCTL-P-349	Enrolled	3	13.53	75.4%	34	45.89%	1345	556	346	99.01%	95.07%	0.8%	0.9%
NLCTL-P-364	Enrolled	3	2.31	75.7%	39	50.93%	1702	607	391	99.43%	96.11%	0.8%	0.9%
NLCTL-P-365	Enrolled	3	3.01	73.8%	29	43.79%	1090	477	308	98.63%	95.01%	1.0%	1.1%
NLCTL-P-373	Enrolled	3	2.94	73.5%	32	42.70%	1151	486	310	98.61%	95.00%	0.9%	1.0%
NLCTL-P-374	Enrolled	3	4.43	76.0%	32	49.08%	1334	542	348	99.05%	95.02%	0.9%	0.9%
NLCTL-P-376	Enrolled	3	5.33	75.6%	32	48.56%	1298	552	355	96.24%	94.24%	1.1%	1.4%
NLCTL-P-378	Enrolled	2.6	4.34	76.1%	38	50.25%	1634	643	411	99.33%	95.32%	0.7%	0.7%
NLCTL-P-416	Enrolled	3	3.21	75.6%	34	46.36%	1312	518	336	98.88%	95.11%	0.8%	0.8%
NLCTL-P-417	Enrolled	3	2.08	74.3%	29	44.86%	1124	438	279	98.50%	95.63%	0.9%	0.9%
NLCTL-P-418	Enrolled	3	2.76	75.7%	39	49.49%	1672	628	399	99.61%	97.57%	0.9%	0.9%
NLCTL-P-419	Enrolled	3	7.99	76.0%	42	49.21%	1749	709	457	99.67%	97.97%	0.8%	0.8%
NLCTL-P-431	Enrolled	3	2.87	75.3%	34	47.65%	1371	517	330	98.75%	94.53%	0.8%	0.8%
NLCTL-P-438	Enrolled	3	2.79	75.3%	36	52.32%	1622	607	387	99.24%	95.50%	0.8%	0.8%
NLCTL-P-442	Enrolled	3	4.68	74.6%	33	46.20%	1289	566	362	99.17%	95.16%	0.9%	0.9%
NLCTL-P-454	Enrolled	2	5.03	72.8%	32	42.59%	1143	529	344	99.02%	95.39%	0.8%	0.8%
NLCTL-P-457	Enrolled	3	9.53	75.9%	34	55.50%	1621	607	387	99.40%	96.41%	0.6%	0.7%
NLCTL-P-482	Enrolled	2	8.51	73.6%	44	45.67%	1706	614	391	99.37%	95.77%	0.7%	0.7%
NLCTL-P-486	Enrolled	3	3.26	74.4%	35	41.45%	1241	506	326	98.95%	95.15%	0.7%	0.7%

Figure S1

Figure S1. Technical replicates of each dilution were highly correlated. We define co-methylated reads as those reads with 3 methylated CpGs within a sliding window containing 3 to 5 CpGs. The percentage of co-methylated reads at each target region was calculated for each sample and Pearson correlation was examined.

Figure S2

A

B
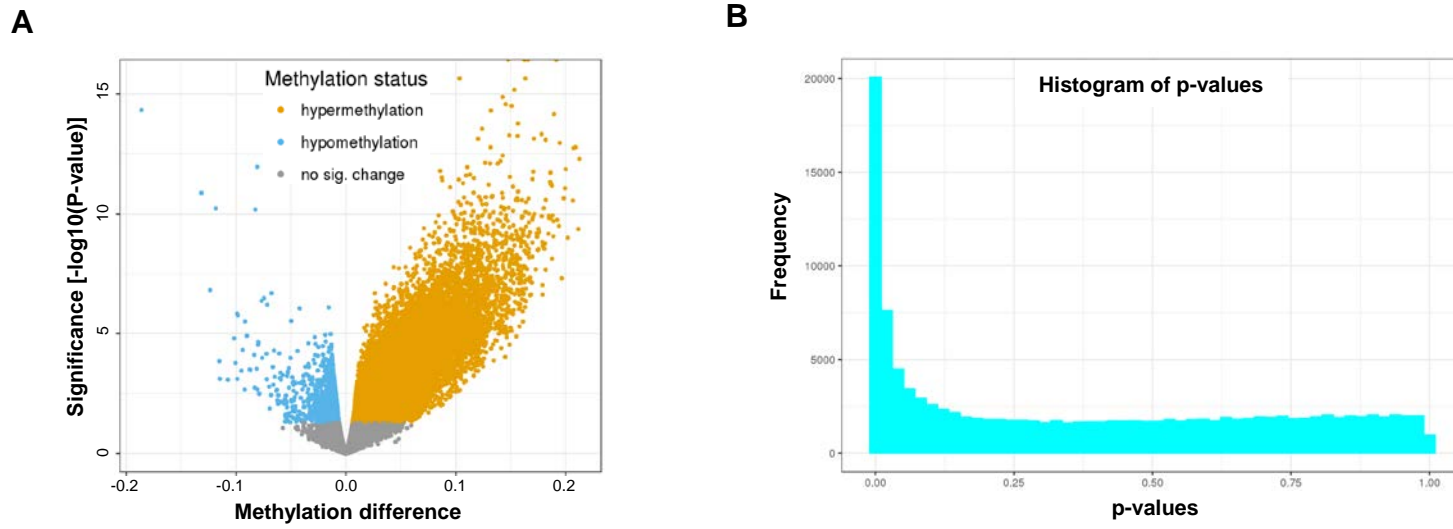
Figure S2. Differentially methylated regions were identified using the Wilcoxon rank sum test comparing 33 IA samples to 78 benign lesions. (A) Hypermethylated regions were determined with P value < 0.05 in the volcano plot. (B) The histogram displays the distribution of p-values.

Figure S3

TCGA validation
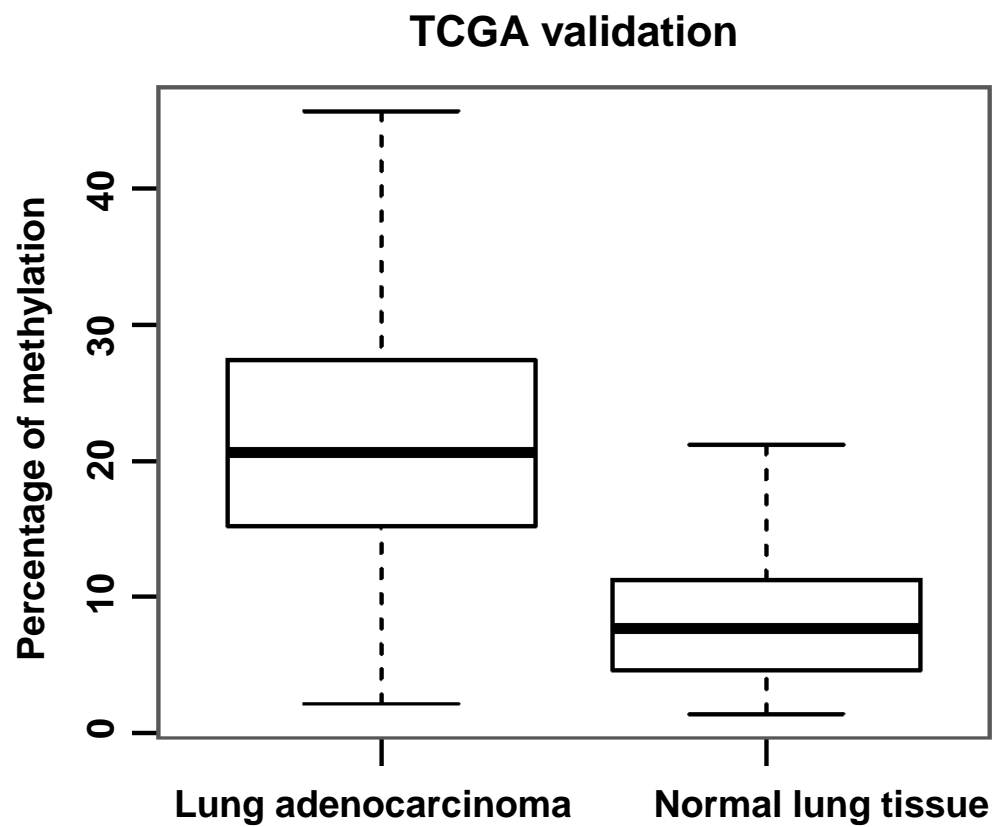
Figure S3. Hypermethylated CpG sites that were identified in lung cancer tissue were examined using the TCGA DNA methylation microarray data independently generated from lung adenocarcinoma tumor samples (n=31) and normal lung tissues (n=31). The boxplot shows consistent hypermethylation between our sequencing data and the TCGA microarray data.

Figure S4


```
┌─────────────────────────────────────────────────────┐
│      192 collected plasma samples (65 paired)        │
└─────────────────────────────────────────────────────┘

   13 samples excluded due to hemolysis, insufficient
        cfDNA yield, or genomic DNA contamination

┌─────────────────────────────────────────────────────┐
│   179 samples for library construction (62 paired)   │
└─────────────────────────────────────────────────────┘

        9 samples excluded due to low pre-library yield

┌─────────────────────────────────────────────────────┐
│      170 samples for sequencing (60 paired)          │
└─────────────────────────────────────────────────────┘

        18 samples excluded due to failed sequencing QC

┌─────────────────────────────────────────────────────┐
│       152 samples for analysis (54 paired)           │
└─────────────────────────────────────────────────────┘

   20 samples excluded due to unavailable pathological reports

┌─────────────────────────────────────────────────────┐
│     132 samples enrolled for study (54 paired)       │
└─────────────────────────────────────────────────────┘

                        +

┌─────────────────────────────────────────────────────┐
│  118 enrolled asymptomatic normal plasma samples     │
└─────────────────────────────────────────────────────┘
```

Figure S4. Patient enrollment to study the plasma-level DNA methylation-based malignancy classifier. Quality controls were performed at each indicated stage to ensure reliable high quality data for subsequent analyses.

Figure S5

A

Diversity estimation:

Let

S = sequencing depth (pre − deduplication mean bait coverage)
D_t = true diversity (estimated diverstity)
D_o = observed diversity (post − deduplication mean bait coverage)
r = duplication rate
f = fraction of diversity observed

Then

$$f = \frac{D_o}{D_t}$$

$$r = 1 - \frac{D_o}{S} = 1 - \frac{f*D_t}{S}$$

The missed diversity ($D_t - Do$) corresponds to those molecules observed 0 times during the S times observation events. For each molecule, the chance of being observed 0 times could be define by Poisson distribution:

$$P(k = 0) = \frac{\lambda^k * e^{-\lambda}}{k!}$$

where

$$\lambda = S/D_t$$

Thus

$$D_o = Dt * [1 - P(k = 0)] = Dt * (1 - e^{-\frac{S}{D_t}})$$

Thus

$$f = \frac{D_o}{D_t} = 1 - e^{-\frac{f}{1-r}}$$

Library conversion efficiency estimation:

- Each haploid human genome is about 3.3 pg. So each ng of genomic DNA contains 300 copies of genome.

- $Conversion\ efficiency = \frac{Estimated\ DNA\ molecule\ number}{Input\ DNA\ molecule\ number} = \frac{\frac{Mean\ bait\ coverage}{f}}{300 * Input\ DNA\ in\ ng}$

B

Duplicate rate (r)	Fraction of diversity observed (f)
0.5%	1%
2.5%	5%
4.6%	9%
6.7%	13%
8.8%	17%
10.9%	21%
13.1%	25%
15.3%	29%
17.6%	33%
19.9%	37%
22.3%	41%
24.7%	45%
27.2%	49%
29.8%	53%
32.5%	57%
35.2%	61%
38.1%	65%
41.1%	69%
44.2%	73%
47.6%	77%
51.2%	81%
55.2%	85%
59.7%	89%
65.0%	93%
72.3%	97%
78.5%	99%

Figure S5. Library conversion efficiency estimation using sequencing depth and post-deduplication mean bait coverage based on the Poisson Distribution. (A) The detailed derivation of library conversion efficiency. (B) A list of duplicate rates and their corresponding fractions of observed diversity.

Figure S6
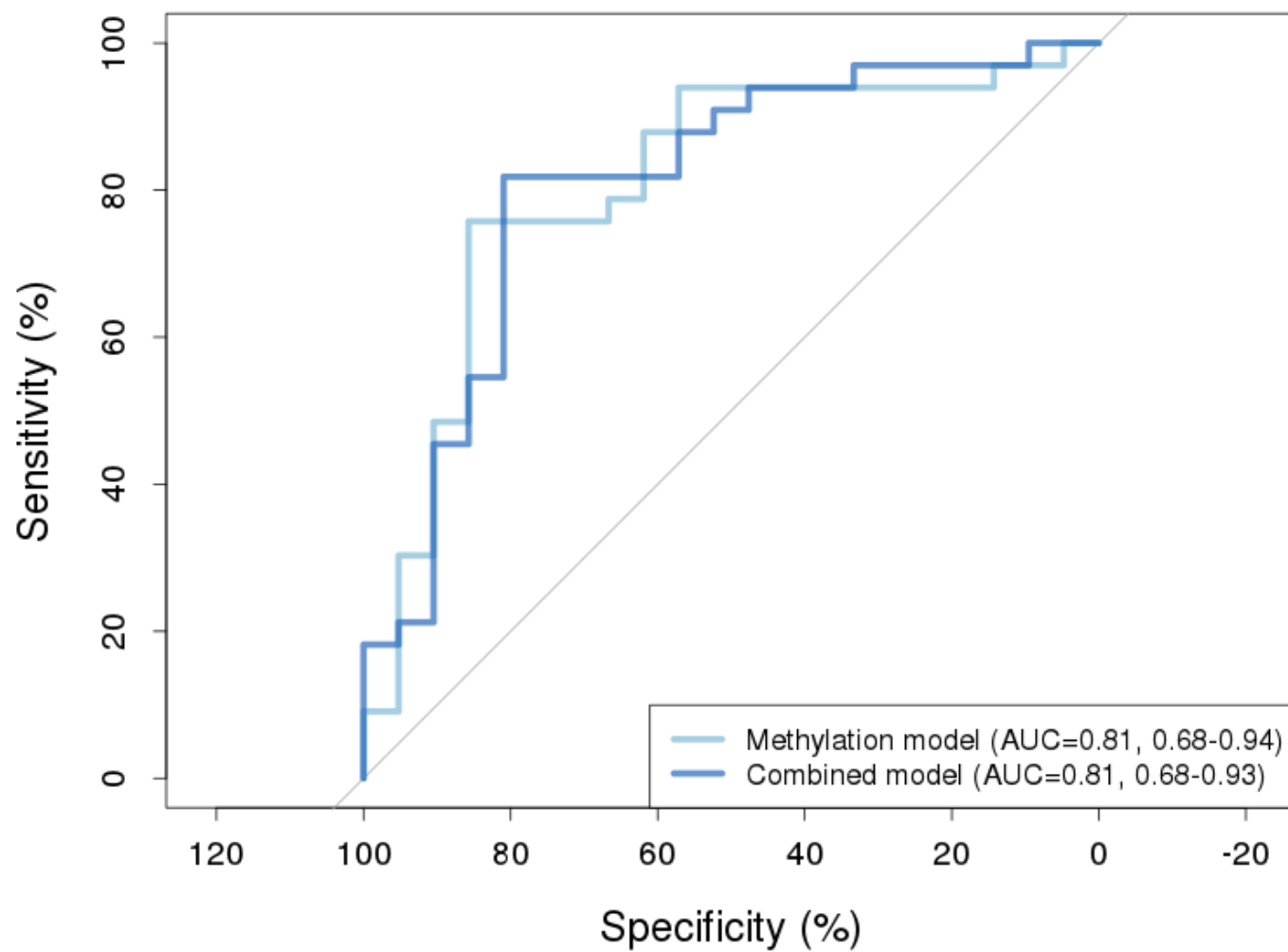
Figure S6. ROC plot of methylation signature model and combined model by integrating methylation signature model with Mayo model.
For performance comparison,we show the ROC-AUC and its 95% confidence interval for two models.