

Supplementary figures

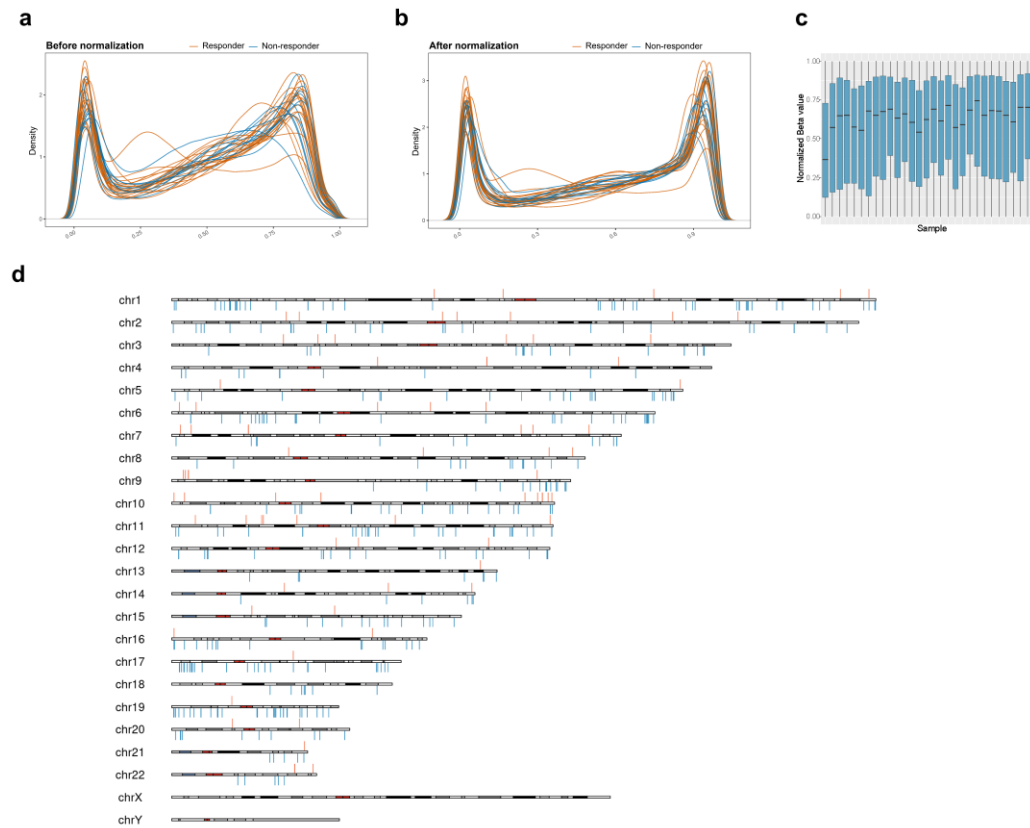


Fig. S1: Infinium MethylationEPIC BeadChip on 30 gastric cancer samples. (a) Density curves of the 30 samples before data normalization. (b) Density curves of the 30 samples after data normalization. (c) Box plot of β value distribution of 30 samples after normalization. (d) Distribution of differential methylation probes on chromosomes

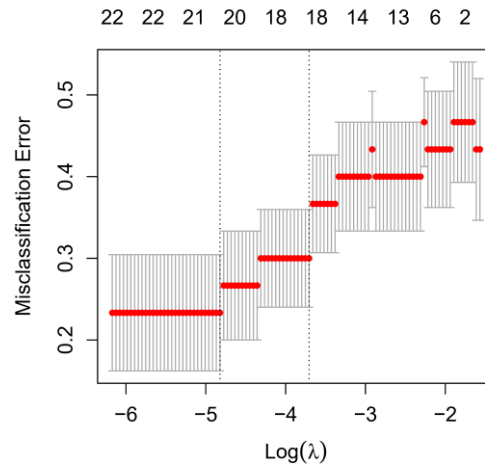


Fig. S2: Cross-validation of LASSO regression parameter selection.

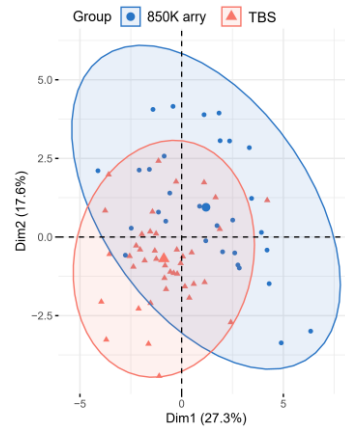


Fig. S3: Principal Component Analysis (PCA) of DNA methylation levels from 20 differential methylation CpG probes (DMPs) measured by the Infinium MethylationEPIC BeadChip (850K array) and Targeted Bisulfite Sequencing (TBS).

Supplementary tables

Table S1. Specific parameters of each machine learning method

Method	Parameters	Value
SVM-RFE	nfold	5
	halve.above	100
LASSO	alpha	1
	nfold	10
NB	fL	0, 0.5, 1, 1.5, 2.0
	usekernel	TRUE
	adjust	0.5, 0.75, 1, 1.25, 1.5
	sigma	0.0005, 0.001, 0.005, 0.01, 0.05
SVM	C	1, 3, 5, 10, 20
	Weight	0.1, 0.5, 1, 2, 3, 5, 10
RF	mtry	2, 42, 83, 124, 165, 205, 246, 287, 328, 369
	kmax	5, 7, 9, 11, 13
KNN	distance	2
	kernel	optimal
AdaBoost	nIter	50, 100, 150, 200, 250
	method	Adaboost.M1, Real adaboost
LogitBoost	nIter	11, 21, 31, 41, 51, 61, 71, 81, 91, 101

Other parameters were set as default values.

Table S2. Comparison of clinicopathological characteristics between responders and non-responders to anti-PD-1-based treatment

Characteristic	<i>Non-responder</i>	<i>Responder</i>	p value
	<i>n=24</i>	<i>n=47</i>	
Age [mean (SD)]	56.1 (12.3)	59.6 (11.8)	0.26
Gender [n (%)]			0.41
Male	16 (66.7)	37 (78.7)	
Female	8 (33.3)	10 (21.3)	
BMI [mean (SD), Kg/m2]	23.2 (4.0)	22.7 (3.8)	0.63
Smoking [n (%)]			0.50
Yes	7 (29.2)	19 (40.4)	
No	17 (70.8)	28 (59.6)	
Alcohol consumption [n (%)]			0.76
Yes	4 (16.7)	10 (21.3)	
No	20 (83.3)	37 (78.7)	
Location [n (%)]			0.06
Upper 1/3	5 (20.8)	23 (48.9)	
Middle 1/3	8 (33.3)	12 (25.5)	
Lower 1/3	8 (33.3)	11 (23.4)	
Whole	2 (8.3)	0 (0.0)	
Remnant	1 (4.2)	1 (2.1)	
Tumor invasion [n (%)]			0.52
T2	2 (8.3)	2 (4.3)	
T3	5 (20.8)	5 (10.6)	
T4a	14 (58.3)	33 (70.2)	
T4b	3 (12.5)	7 (14.9)	
Lymph node [n (%)]			>0.99
N0	1 (4.2)	1 (2.1)	
N1-3	23 (95.8)	46 (97.9)	
Distant metastasis [n (%)]			>0.99
M0	11 (45.8)	23 (48.9)	
M1	13 (54.2)	24 (51.1)	

(continued)			
Characteristic	<i>Non-responder</i>	<i>Responder</i>	p value
	<i>n=24</i>	<i>n=47</i>	
cTNM stage [n (%)]			0.97
II	1 (4.2)	2 (4.3)	
III	8 (33.3)	18 (38.3)	
IVA	2 (8.3)	3 (6.4)	
IVB	13 (54.2)	24 (51.1)	
Differentiation [n (%)]			0.045
Well or Moderate	0 (0.0)	8 (17.0)	
Poor	24 (100.0)	39 (83.0)	
Anti-PD-1 agent [n (%)]			0.32
Camrelizumab	3 (12.5)	6 (12.8)	
Pembrolizumab	2 (8.3)	0 (0.0)	
Nivolumab	5 (20.8)	10 (21.3)	
Sintilimab	14 (58.3)	31 (66.0)	
Chemotherapy [n (%)]			0.43
SOX	20 (83.3)	43 (91.5)	
CapeOx	3 (12.5)	2 (4.3)	
Others	1 (4.2)	2 (4.3)	
CPS* [n (%)]			0.32
<5	14 (82.4)	19 (65.5)	
≥5	3 (17.6)	10 (34.5)	
MSI* [n (%)]			0.29
MSI-H	0 (0.0)	4 (11.8)	
MSS	17 (100)	30 (88.2)	

SOX: S-1 + Oxaliplatin; CapeOx: Capecitabine + Oxaliplatin; Others: Paclitaxel + Carboplatin, mFOLFOX6 and S-1 + Carboplatin; CPS: combined positive score; MSI: microsatellite instability.

*Not available for some patients.

Table S3 Comparison of clinicopathological characteristics of patients sequenced by different methods

Characteristic	<i>Infinium MethylationEPIC BeadChip</i>	<i>Targeted Bisulfite Sequencing</i>	p value
	<i>n=30</i>	<i>n=41</i>	
Age [mean (SD)]	54.2 (10.9)	61.5 (12.0)	0.01
Gender [n (%)]			0.95
Male	23 (76.7)	30 (73.2)	
Female	7 (23.3)	11 (26.8)	
BMI [mean (SD), Kg/m2]	22.9 (4.2)	22.9 (3.6)	0.98
Smoking [n (%)]			0.46
Yes	9 (30.0)	17 (41.5)	
No	21 (70.0)	24 (58.5)	
Alcohol consumption [n (%)]			0.80
Yes	5 (16.7)	9 (22.0)	
No	25 (83.3)	32 (78.0)	
Location [n (%)]			0.53
Upper 1/3	13 (43.3)	15 (36.6)	
Middle 1/3	7 (23.3)	12 (29.3)	
Lower 1/3	7 (23.3)	13 (31.7)	
Whole	1 (3.3)	1 (2.4)	
Remnant	2 (6.7)	0 (0.0)	
Tumor invasion [n (%)]			0.05
T2	4 (13.3)	0 (0.0)	
T3	2 (6.7)	8 (19.5)	
T4a	19 (63.3)	28 (68.3)	
T4b	5 (16.7)	5 (12.2)	
Lymph node [n (%)]			>0.99
N0	1 (3.3)	1 (2.4)	
N1-3	29 (96.7)	40 (97.6)	
Distant metastasis [n (%)]			0.68
M0	13 (43.3)	21 (51.2)	
M1	17 (56.7)	20 (48.8)	

(continued)

Characteristic	<i>Infinium MethylationEPIC BeadChip</i>	<i>Targeted Bisulfite Sequencing</i>	p value
	<i>n=30</i>	<i>n=41</i>	
cTNM stage [n (%)]			0.12
II	3 (10.0)	0 (0.0)	
III	8 (26.7)	18 (43.9)	
IVA	2 (6.7)	3 (7.3)	
IVB	17 (56.7)	20 (48.8)	
Differentiation [n (%)]			0.71
Well or Moderate	4 (13.3)	4 (9.8)	
Poor	26 (86.7)	37 (90.2)	
Anti-PD-1 agent [n (%)]			<0.01
Camrelizumab	9 (30.0)	0 (0.0)	
Pembrolizumab	2 (6.7)	0 (0.0)	
Nivolumab	12 (40.0)	3 (7.3)	
Sintilimab	7 (23.3)	38 (92.7)	
Chemotherapy [n (%)]			0.01
SOX	23 (76.7)	40 (97.6)	
CapeOx	5 (16.7)	0 (0.0)	
Others	2 (6.7)	1 (2.4)	
CPS* [n (%)]			0.74
<5	22 (73.3)	11 (68.8)	
≥5	8 (26.7)	5 (31.2)	
MSI* [n (%)]			0.57
MSI-H	0 (0.0)	4 (9.8)	
MSS	10 (100.0)	37 (90.2)	
Treatment Response [n (%)]			0.86
Responder	19 (63.3)	28 (68.3)	
Non-responder	11 (36.7)	13 (31.7)	

SOX: S-1 + Oxaliplatin; CapeOx: Capecitabine + Oxaliplatin; Others: Paclitaxel + Carboplatin, mFOLFOX6 and S-1 + Carboplatin; CPS: combined positive score; MSI: microsatellite instability.

*Not available for some patients.

Table S5. Comparison of clinicopathological characteristics between responders and non-responders to anti-PD-1-based treatment in validation set

Characteristic	<i>Non-responder</i>	<i>Responder</i>	p value
	<i>n=9</i>	<i>n=19</i>	
Age [mean (SD)]	53.2 (13.7)	61.7 (11.3)	0.13
Gender [n (%)]			0.06
Male	5 (55.6)	17 (89.5)	
Female	4 (44.4)	2 (10.5)	
BMI [mean (SD), Kg/m2]	22.0 (2.3)	22.1 (2.3)	0.91
Smoking [n (%)]			0.69
Yes	4 (44.4)	11 (57.9)	
No	5 (55.6)	8 (42.1)	
Alcohol consumption [n (%)]			>0.99
Yes	2 (22.2)	6 (31.6)	
No	7 (77.8)	13 (68.4)	
Location [n (%)]			0.06
Upper 1/3	0 (0.0)	6 (31.6)	
Middle 1/3	5 (55.6)	4 (21.1)	
Lower 1/3	4 (44.4)	9 (47.4)	
Tumor invasion [n (%)]			0.36
T3	0 (0.0)	2 (10.5)	
T4a	6 (66.7)	15 (78.9)	
T4b	3 (33.3)	2 (10.5)	
Lymph node [n (%)]			-
N0	0 (0.0)	0 (0.0)	
N1-3	9 (100.0)	19 (100.0)	
Distant metastasis [n (%)]			0.25
M0	2 (22.2)	9 (47.4)	
M1	7 (77.8)	10 (52.6)	
cTNM stage [n (%)]			0.60
III	2 (22.2)	8 (42.1)	
IVA	0 (0.0)	1 (5.3)	
IVB	7 (77.8)	10 (52.6)	

(continued)

Characteristic	<i>Non-responder</i> <i>n=9</i>	<i>Responder</i> <i>n=19</i>	p value
Differentiation [n (%)]			>0.99
Well or Moderate	0 (0.0)	2 (10.5)	
Poor	9 (100.0)	17 (89.5)	
Anti-PD-1 agent [n (%)]			>0.99
Nivolumab	1 (11.1)	2 (10.5)	
Sintilimab	8 (88.9)	17 (89.5)	
Chemotherapy [n (%)]			0.32
SOX	8 (88.9)	19 (100.0)	
Raltitrexed + Oxaliplatin	1 (11.1)	0 (0.0)	
CPS* [n (%)]			-
<5	0 (0.0)	0 (0.0)	
≥5	1 (100.0)	0 (0.0)	
MSI* [n (%)]			-
MSI-H	0 (0.0)	0 (0.0)	
MSS	7 (100.0)	11 (100.0)	

SOX: S-1 + Oxaliplatin; CPS: combined positive score; MSI: microsatellite instability.

*Not available for some patients.