Additional File 1
Supplementary Materials

The file includes:

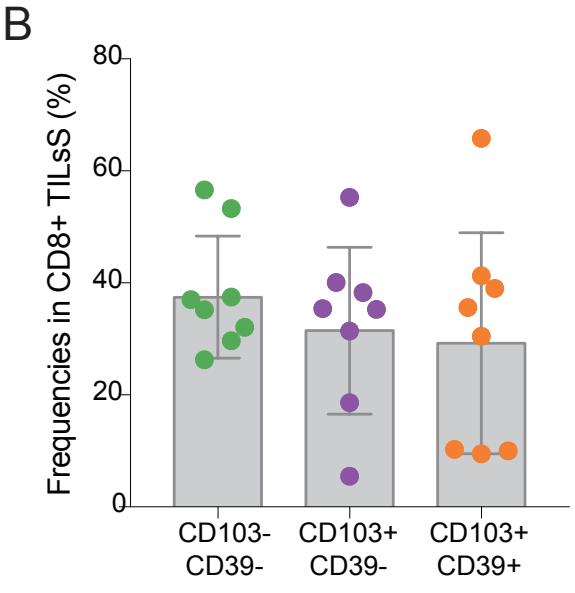
Fig. S1-S8

Table S1

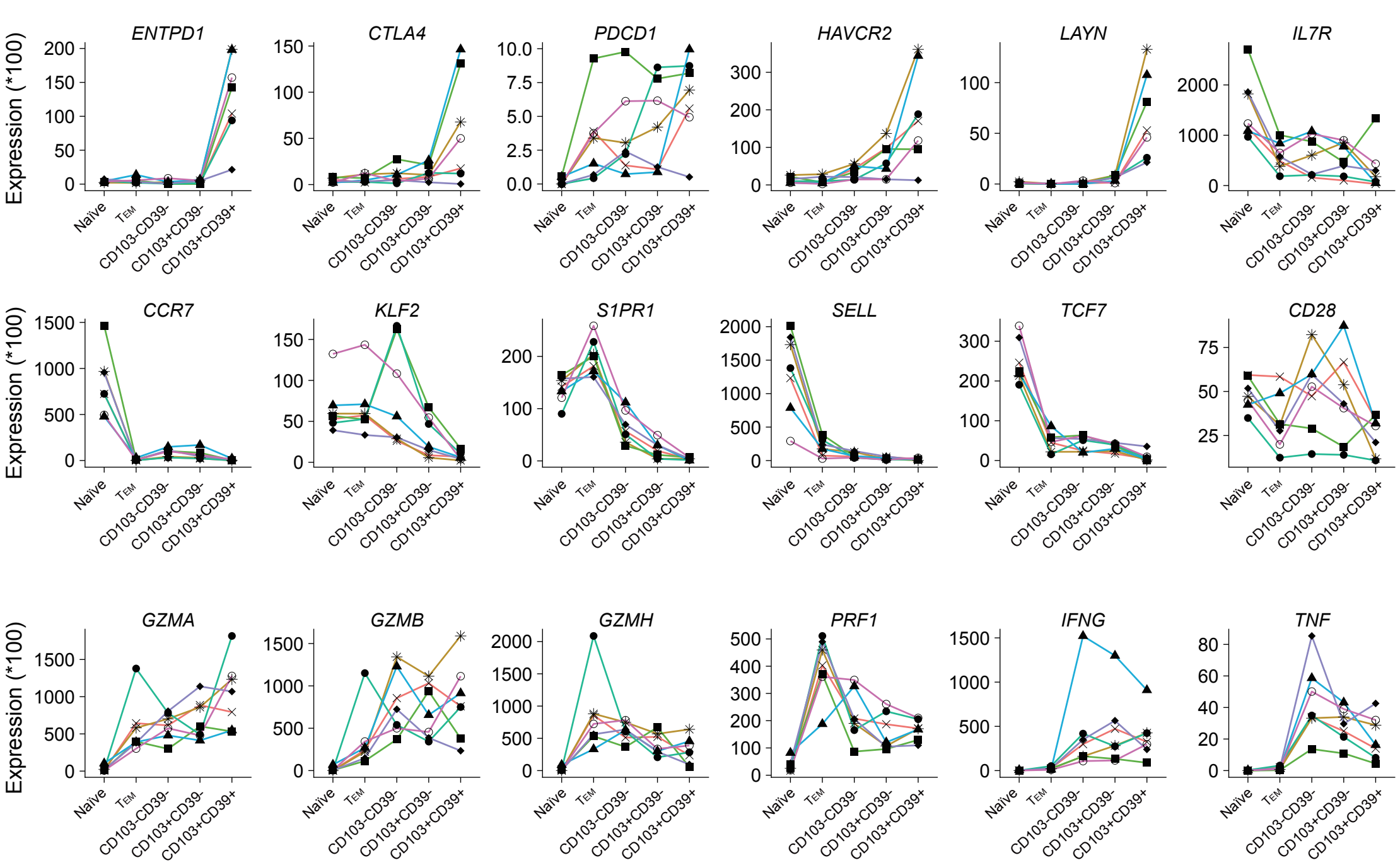
A

Patient ID	Clinical Information				Sequencing methods used	
	Age	Sex	Location	Stage	Smart-seq2	Bisulfite-seq
P0929	58	M	Right colon	IIA	√	√
P1030	58	M	Rectum	IIB	√	√
P1031	79	F	Colon sigmoideum	IVA	√	√
P1101	81	F	Colon transversum	IIIB	√	√
P1112	83	F	Rectum	I	√	√
P1113	83	F	Colon sigmoideum	IIIC	√	√
P1120	74	M	Rectum	I	√	√
P0117	60	F	Right colon	IIIB	√	√

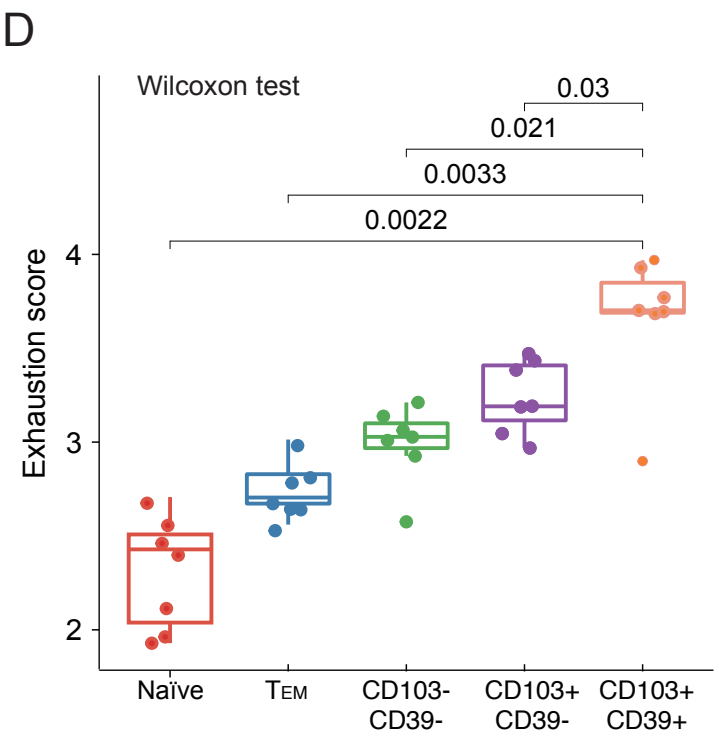
B



C



D



E

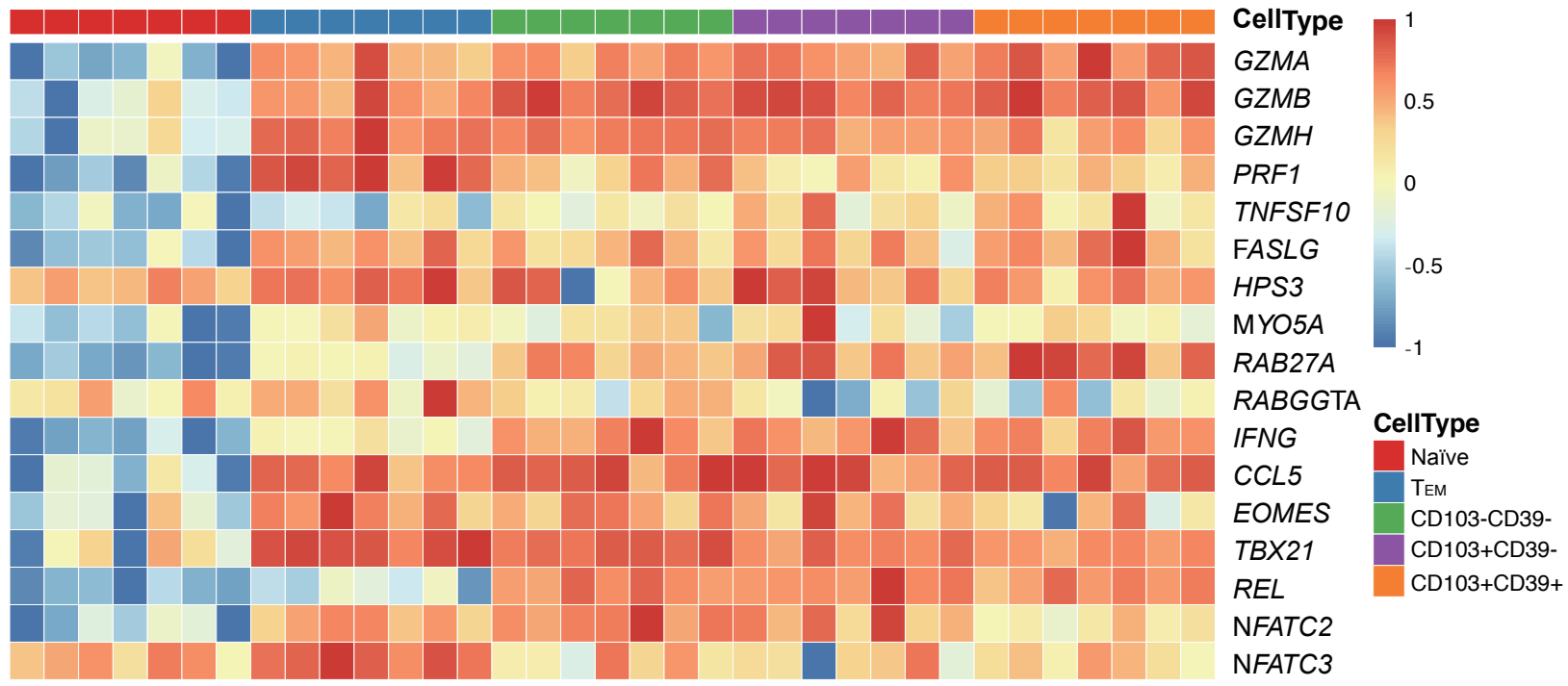


Figure S1. Gene expression analysis revealed a distinct transcriptome of tumor-reactive CD8+ T subtype. (A) The clinical information of CRC patients and sequencing methods used for each patient. (B) Frequencies of Three TIL subtypes in total CD8+ TILs for 8 CRC patients. (C) Examples of immune-related gene expression in five CD8+ T cell populations. Each symbol/line represents one patient, which is connected by a line. (D) Exhaustion levels of five CD8+ T cell populations were analyzed by Wilcoxon test. (E) Heat map displays expression of T_{EM} -related genes in five CD8+ T cell populations.

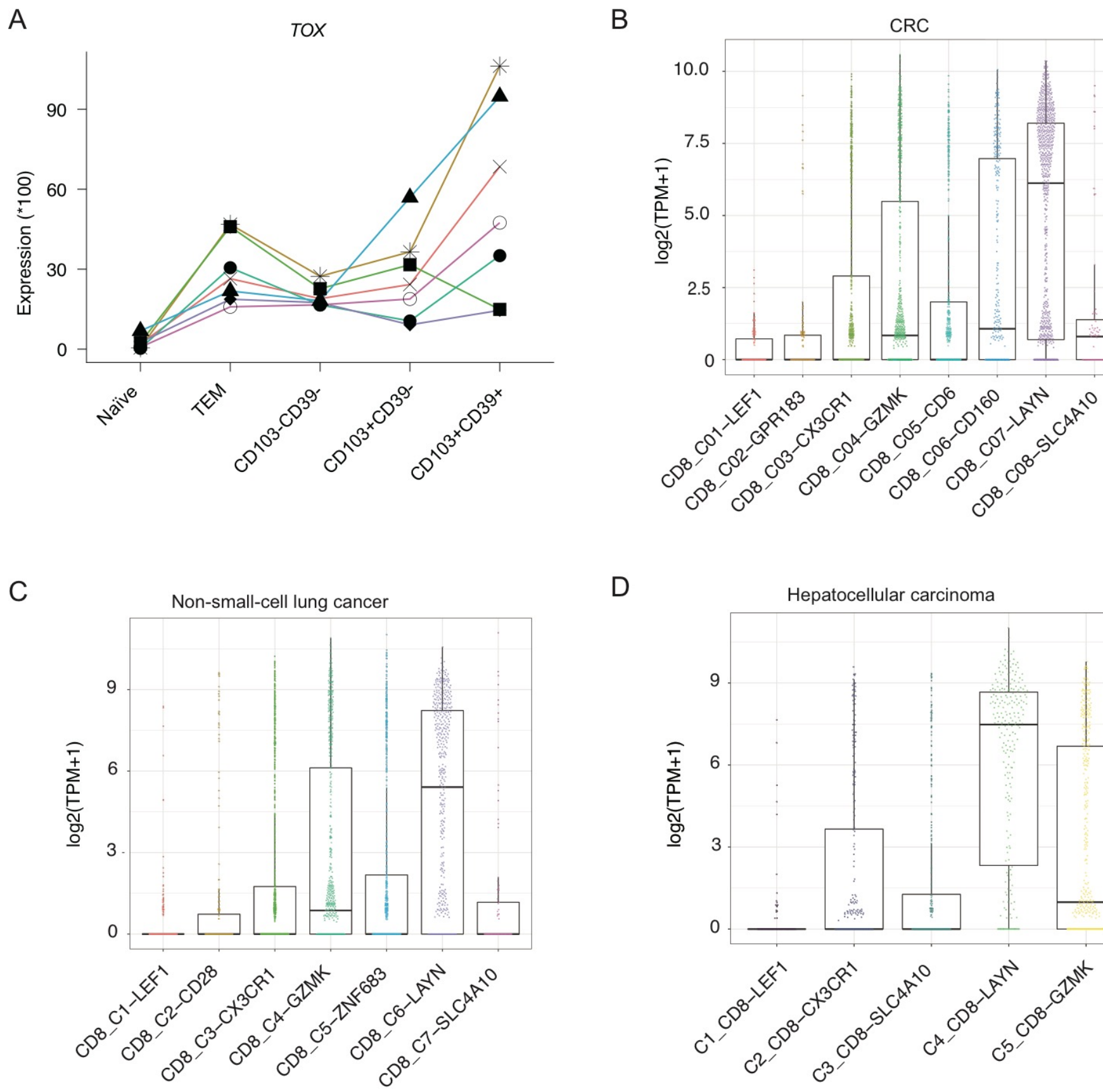


Figure S2. *TOX* is induced in exhausted CD8+ T cells. (A) RNA expression of *TOX* in five subtypes. (B-D) Box plots showing *TOX* expression profiles in different CD8+ T cell clusters from our previous scRNA-seq data of CD8+ T cells in (B) CRC, (C) non-small-cell lung cancer and (D) hepatocellular carcinoma, respectively. Notably, LAYN cluster represents exhausted CD8+ T cells.

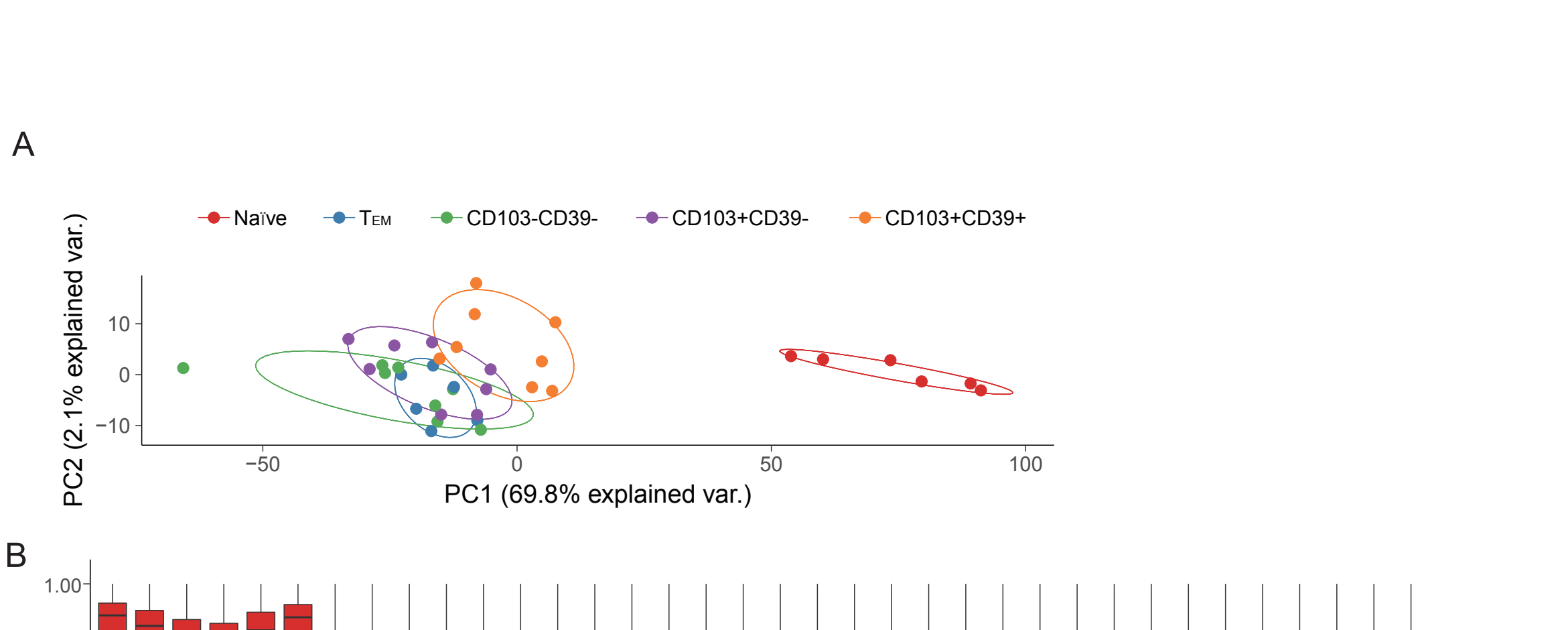


Figure S3. Whole-Genome methylation profiling across multiple CD8+ T cell subtypes. (A) PCA analysis based on methylation profiles of CD8+ T cells in five subtypes. (B) Box plots for the comparison of DNA methylation levels across five subtypes.

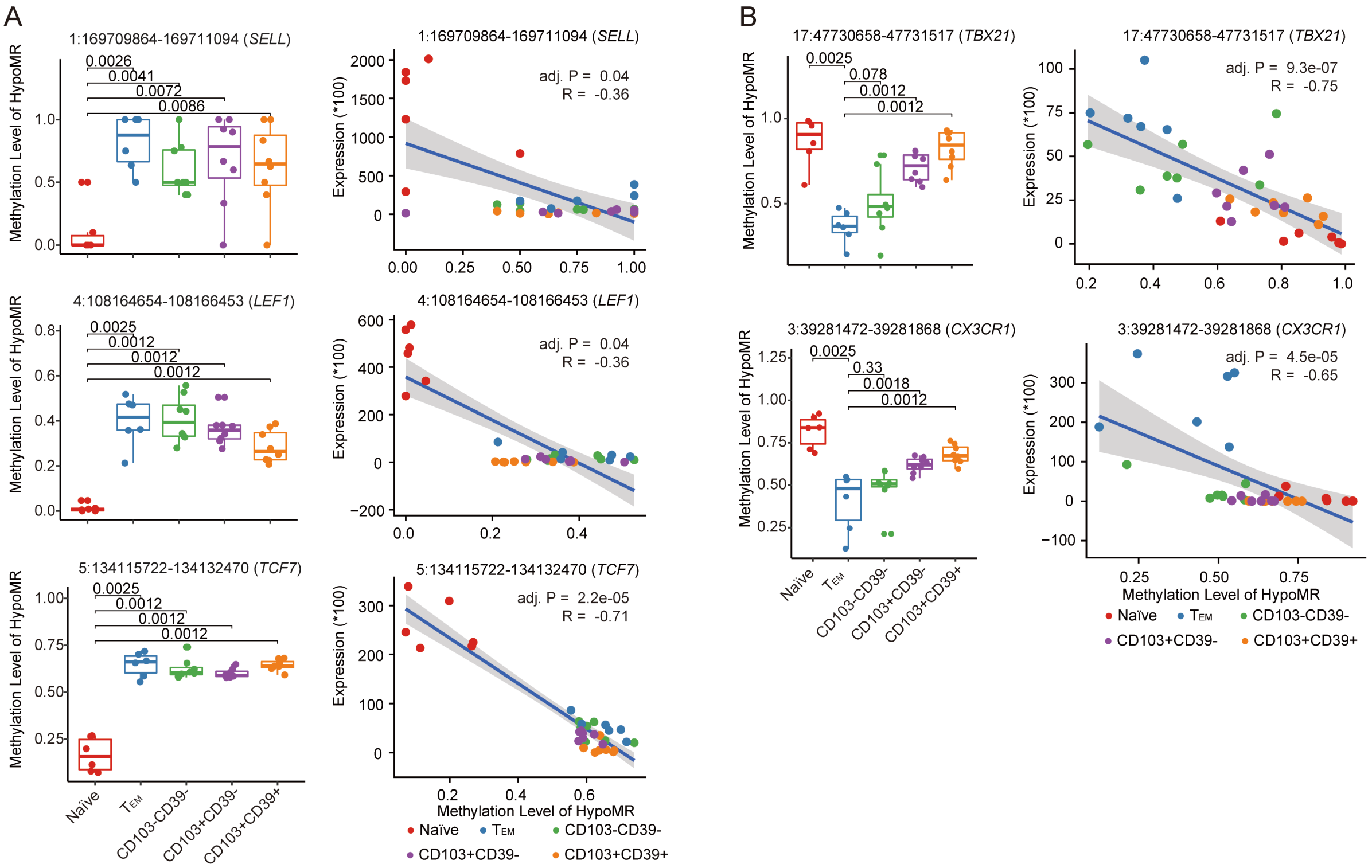


Figure S4. The HypoMR methylation levels of representative genes in naïve and T_{EM} CD8 T cells and their correlations with RNA expression levels across five populations. (A) Representative genes affected by HypoMRs in naïve CD8 T cells. The left panel showed the methylation level of specific HypoMR across the five T cell subtypes. One-tailed wilcoxon test was used to analyze the statistical significance between CD103+CD39+ subtype and other four subtypes. The right panel showed the correlation between RNA expression and HypoMR methylation level. adj.P: Benjamini-Hochberg adjusted p-value. R: regression coefficient. The title of each sub-figure was formatted as “chromosome:start-end(affected gene)”. (B) Representative genes affected by HypoMRs in T_{EM} CD8 T cells. The left and right panels were the same as (A).

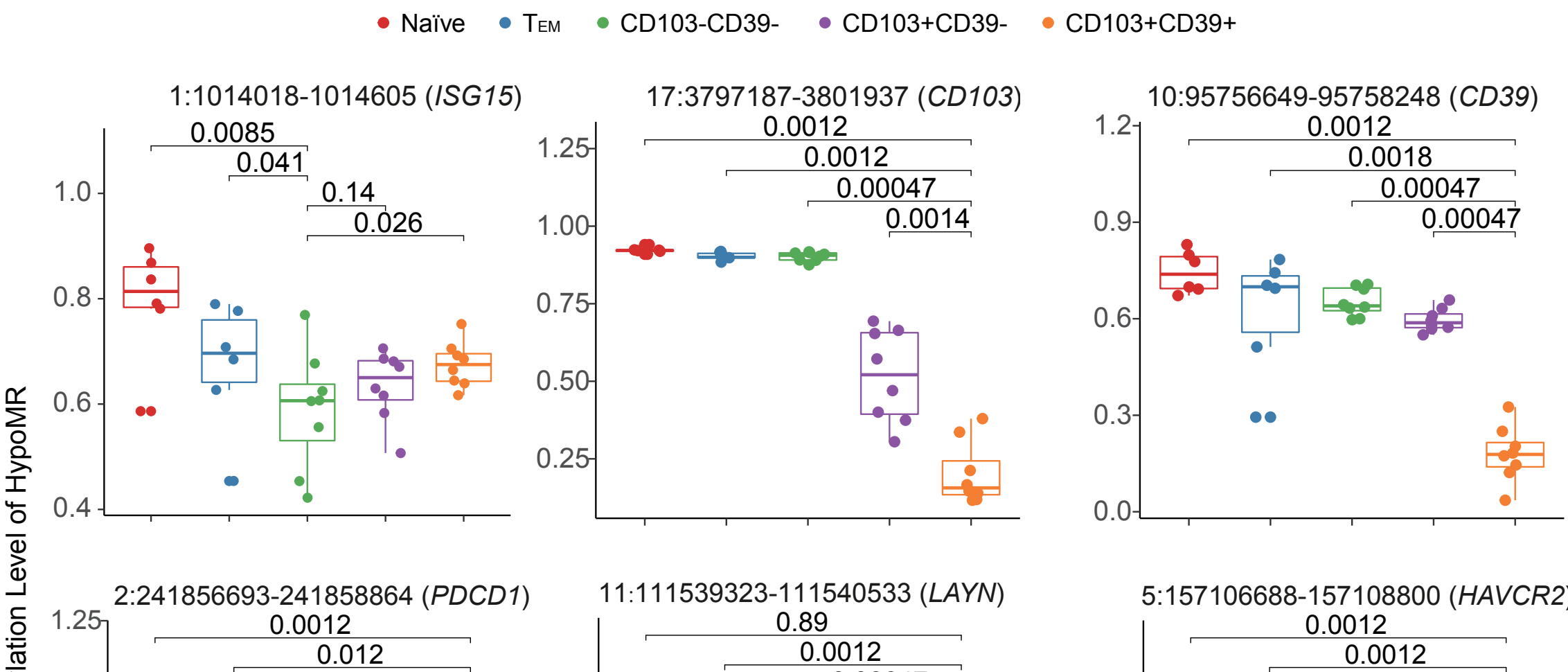


Figure S5. The HypoMR methylation levels of of representative genes across five CD8 T cell populations. One-tailed wilcoxon test was used to analyze the statistical significance between different subtypes. The title of each sub-figure was formatted as “chromosome:start-end(affected gene)”.

Naïve TEM CD103-CD39- CD103+CD39- CD103+CD39+

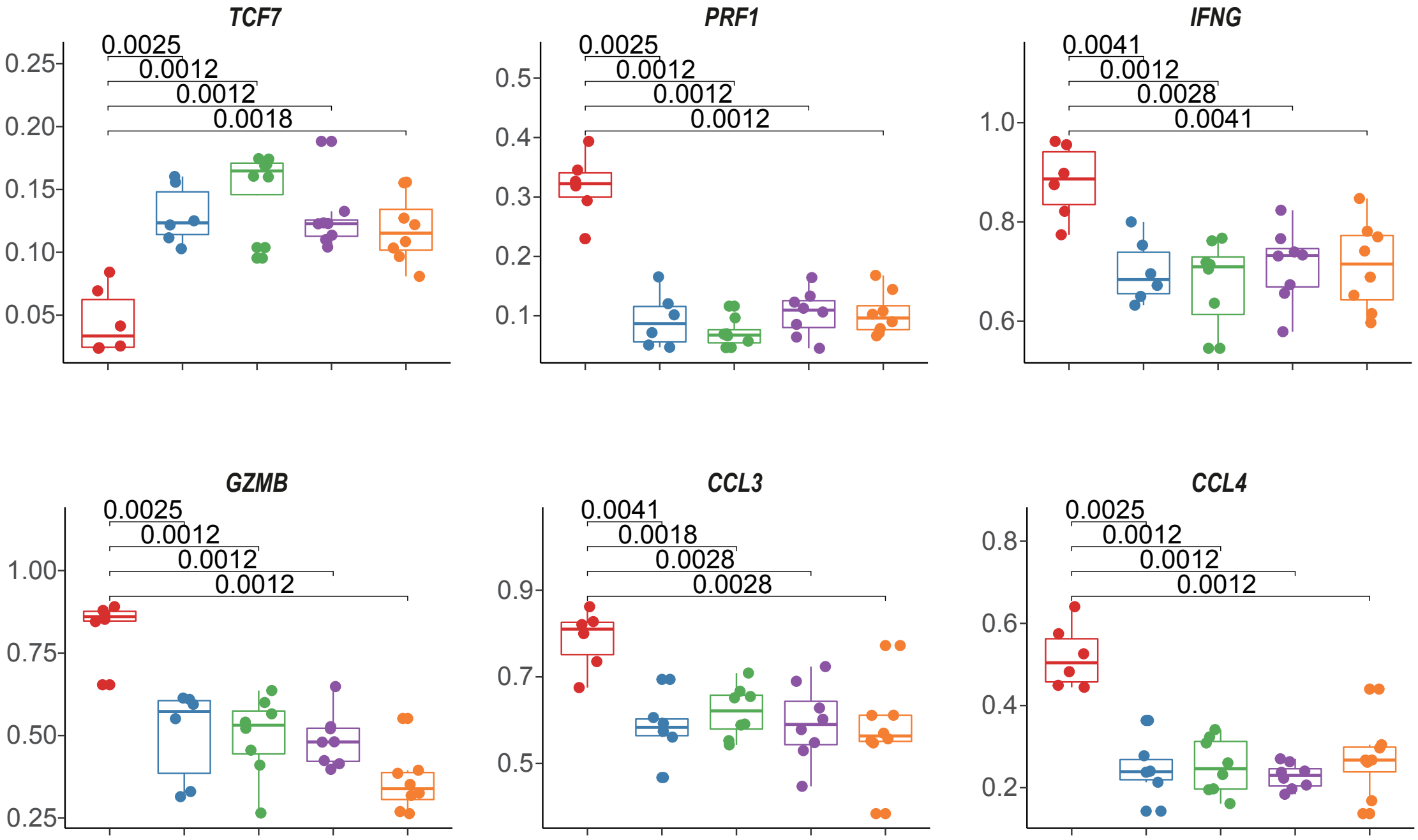


Figure S6. The methylation levels of gene promoters across five CD8 T cell subtypes. One-tailed wilcoxon test was used to analyze the statistical significance between different subtypes.

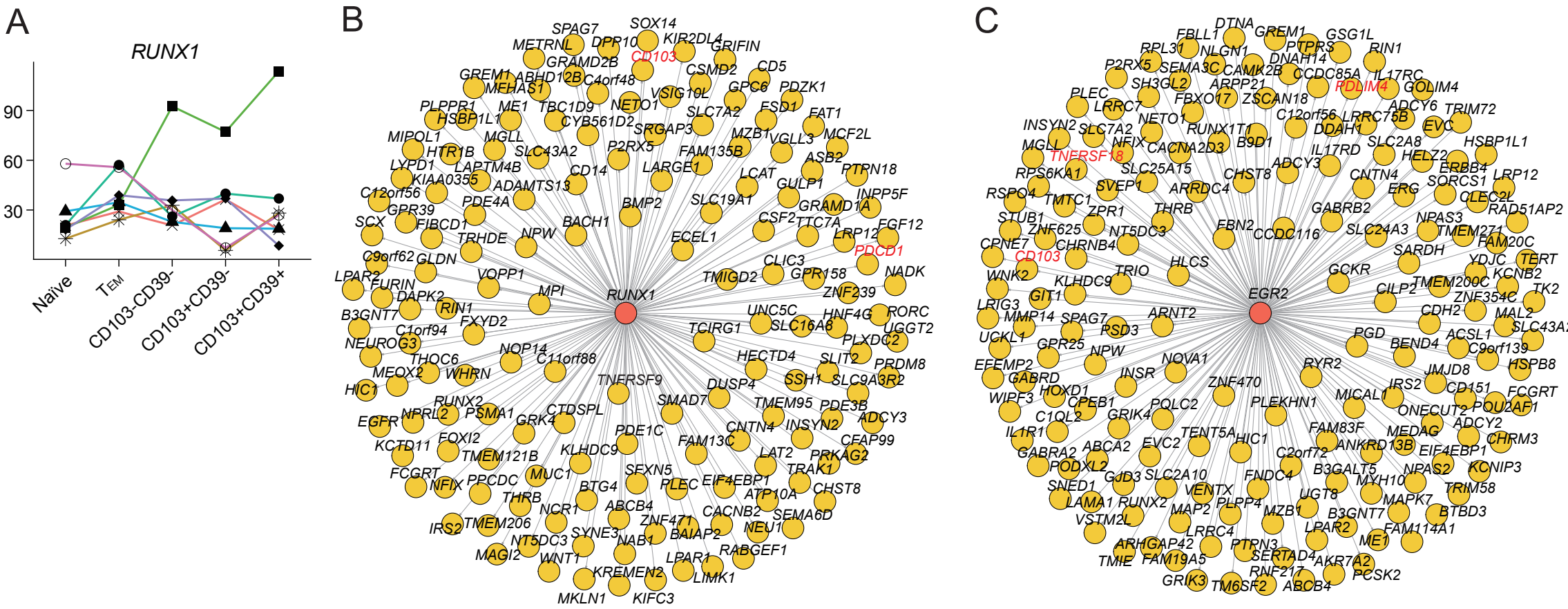


Figure S7. Key transcription factors for CD103+CD39+ subtype. (A) RNA expression of *RUNX1* in five subtypes. (B, C) Predicted targets of (B) *RUNX1* and (C) *EGR2* were displayed. Targets of interest were labeled in red.

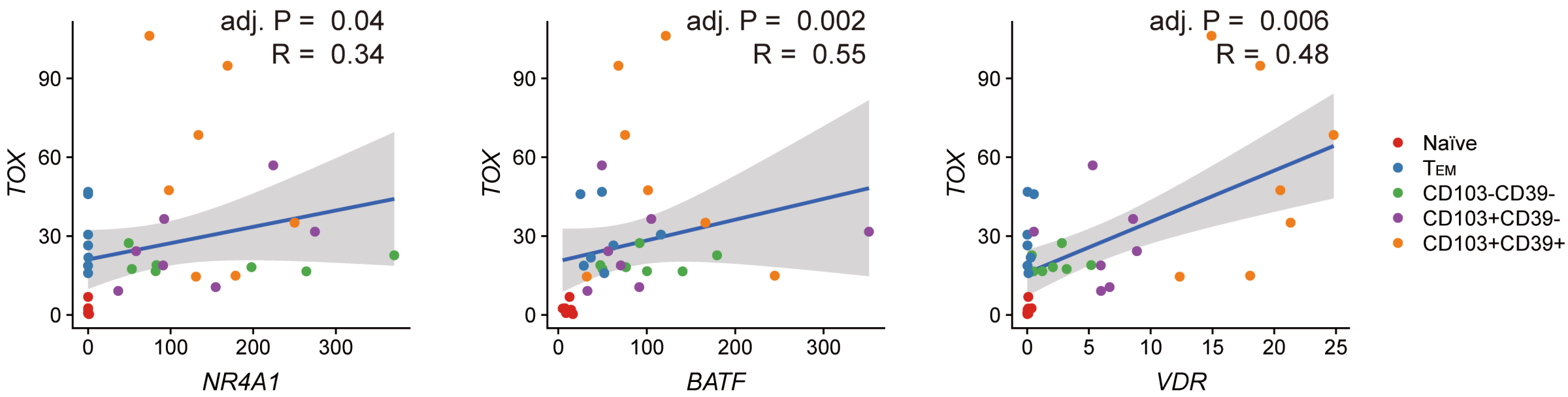


Figure S8. Expression correlation of *TOX* and *NR4A1*, *BATF*, *VDR* in five CD8 T cell populations.

median of covered CpG: 26624899
median of covered CpG by fraction: 0.451475

Naïve T cells
TEM T cells
CD103+CD39+
CD103+CD39-
CD103-CD39-

Patient	Sample	Total_reads	Total_GB	QC_Reads	Mapped_reads	Mapped_ratio	non-CpG_bs	Lambda_bs	Covered_CpGs	Covered_frac
P0929	P1-1_L23_A003	144857804	21.73	143481270	35819145	24.96	0.985187	0.996325	25316945	0.429297
	P1-2_L23_A008	160369528	24.06	158211594	37827232	23.91	0.98311	0.996198	25753434	0.436698
	P2-1_L23_A004	160807964	24.12	158727662	38278395	24.12	0.985238	0.996295	25691277	0.435647
	P2-2_L23_A009	155499252	23.32	151361068	41227040	27.24	0.989246	0.996307	27288506	0.462731
	P3-1_L23_A005	189868220	28.48	187244590	46906145	25.05	0.986103	0.996225	28680269	0.486329
	P3-2_L23_A010	192858134	28.93	190085016	44595736	23.46	0.98534	0.996281	27795898	0.471329
	P4-1_L23_A006	188797732	28.32	185918660	44008803	23.67	0.984573	0.996225	27314056	0.463162
	P4-2_L23_A011	165394086	24.81	161737006	39372608	24.34	0.986713	0.996426	26624899	0.451475
	P5-1_L23_A007	222740972	33.41	219163612	55212634	25.19	0.986483	0.996293	31089377	0.52718
	P5-2_L23_A012	207165724	31.07	203526662	47519057	23.35	0.986125	0.996299	28950498	0.49091
P1030	P1030-1-1-1_HTGW7C	179180718	26.88	174024106	26432757	15.19	0.975764	0.995501	22246378	0.377229
	P1030-1-2-1_HTGW7C	243923314	36.59	234993222	37748251	16.06	0.975639	0.995367	26688835	0.452558
	P1030-1-3-1_HTGW7C	206318860	30.95	197070662	32507057	16.5	0.9757	0.995482	24969435	0.423406
	P1030-1-4-1_HTGW7C	228038518	34.21	219510606	32381554	14.75	0.970754	0.995452	24810411	0.420707
	P1030-1-5-1_HTGW7C	201846596	30.28	192649544	30755469	15.96	0.971685	0.995513	24174702	0.409926
	P1030-2-1-2_HTGW7C	237259734	35.59	223781384	32813064	14.66	0.976926	0.993604	24515052	0.415699
	P1030-2-2-2_HTGW7C	287206650	43.08	271876786	39648695	14.58	0.977548	0.993422	26787026	0.454222
	P1030-2-3-2_HTGW7C	285058550	42.76	264491812	41476278	15.68	0.980722	0.993605	27671885	0.469226
	P1030-2-4-2_HTGW7C	224148840	33.62	205613538	32804166	15.95	0.980194	0.993729	24443574	0.414486
	P1030-2-5-2_HTGW7C	204455744	30.67	180633362	26208704	14.51	0.976197	0.993073	21632656	0.366821
	P1030-3-1-3_HTGW7C	243894334	36.58	235889000	40119826	17.01	0.977359	0.995533	27689832	0.469531
	P1030-3-2-3_HTGW7C	266111622	39.92	256195768	44781917	17.48	0.978338	0.995494	29034828	0.492339
	P1030-3-3-3_HTGW7C	253536002	38.03	244652982	40128840	16.4	0.977528	0.995534	27975490	0.474377
	P1030-3-4-3_HTGW7C	214376246	32.16	205870062	34780240	16.89	0.978685	0.995402	25599763	0.43409
	P1030-3-5-3_HTGW7C	196830880	29.52	183131452	33894297	18.51	0.980547	0.995266	25301285	0.42903
P1031	P1031-1-P1-1_HTGVKC	186765460	28.01	181080936	24554562	13.56	0.978987	0.993377	20658256	0.350301
	P1031-1-P2-1_HTGVKC	222399864	33.36	215757764	30925016	14.33	0.975036	0.993439	23647530	0.40099
	P1031-1-P3-1_HTGVKC	214769116	32.22	207718086	27318414	13.15	0.971912	0.99341	22230004	0.376954
	P1031-1-P4-1_HTGVKC	238155942	35.72	232028242	29761976	12.83	0.969261	0.993491	22979693	0.389665
	P1031-1-P5-1_HTGVKC	198387742	29.76	192542854	27626045	14.35	0.974132	0.993577	22230655	0.376968
	P1031-2-P1-2_HTGW7	255724358	38.36	247849982	37281861	15.04	0.974197	0.994007	26297050	0.445916
	P1031-2-P2-2_HTGW7	215396726	32.31	206860786	33343137	16.12	0.970856	0.994175	25224221	0.427728
	P1031-2-P3-2_HTGW7	219463238	32.92	210620196	32542767	15.45	0.974446	0.994042	24877871	0.421849
	P1031-2-P4-2_HTGW7	166269210	24.94	157369478	23663822	15.04	0.974359	0.994165	20591649	0.349169
	P1031-2-P5-2_HTGW7	219819942	32.97	208637178	32545100	15.6	0.973782	0.99407	24535877	0.416048
	P1031-3-P1-3_HTGVKC	285920448	42.89	275119678	43575495	15.84	0.977364	0.994269	28726383	0.487111
	P1031-3-P2-3_HTGVKC	221728678	33.26	212162626	32888575	15.5	0.977707	0.994259	25175998	0.426904
	P1031-3-P3-3_HTGVKC	234868086	35.23	219986554	33596020	15.27	0.975916	0.994092	25075563	0.425205
	P1031-3-P4-3_HTGVKC	198136754	29.72	187116002	30135799	16.11	0.978597	0.994147	23659312	0.401188
	P1031-3-P5-3_HTGVKC	177753906	26.66	169211880	28466741	16.82	0.979439	0.994206	22850387	0.387474
P1101	P3-1_L4_A008	233050326	34.96	218998162	35386841	16.16	0.987724	0.994789	26508593	0.449509
	P3-2_L3_A010	335491570	50.32	315955066	32221393	10.2	0.979048	0.994939	24529359	0.415946
	P3-3_L2_A009	278435562	41.77	244080018	31521419	12.91	0.974932	0.987718	22217071	0.376735
	P4-1_L4_A009	332620464	49.89	308180738	46639119	15.13	0.98615	0.99483	29885556	0.506767
	P4-2_L3_A011	336890966	50.53	318188084	34689128	10.9	0.980846	0.995197	25804696	0.43757
	P4-3_L2_A011	249155338	37.37	227001400	24749148	10.9	0.972928	0.988586	19325543	0.327704
	P5-1_L4_A010	378938026	56.84	343897778	50139444	14.58	0.985633	0.994945	30683450	0.520289
	P5-2_L3_A012	178567094	26.79	171375332	17934792	10.47	0.984376	0.995371	17796608	0.301779
	P5-3_L2_A012	221684686	33.25	201043102	21755324	10.82	0.969471	0.991765	17658838	0.299442
P1112	1112P1-1_L2_A001	242329132	36.35	241067850	42208985	17.51	0.980843	0.99616	28944875	0.49082
	1112P1-2_L2_A002	272826982	40.92	271286216	49892042	18.39	0.983779	0.996007	30148368	0.511225
	1112P2-1_L2_A004	394821502	59.22	392763952	69909836	17.8	0.984606	0.996024	35274135	0.598141
	1112P2-3_L2_A006	450490368	67.57	448532652	75045663	16.73	0.98093	0.996022	35306024	0.598681
	1112P3-1_L2_A007	293962202	44.09	290997588	43960227	15.11	0.978401	0.995898	22674393	0.384487
	1112P3-2_L2_A008	265061094	39.76	263674088	48997962	18.58	0.976825	0.996262	30833754	0.52285
	1112P4-1_L2_A009	240535982	36.08	239191962	44134368	18.45	0.983778	0.996105	29674868	0.503199
	1112P4-2_L2_A010	358150206	53.72	356333106	63789129	17.9	0.98373	0.9962	33970573	0.57603
	1112P5-1_L2_A011	292194296	43.83	290686054	50875245	17.5	0.985337	0.996218	31442770	0.533175
P1113	1113P1-1_L4_A001	134940296	20.24	134133872	21908545	16.33	0.976897	0.99574	20385280	0.345675
	1113P1-2_L4_A002	395442446	59.32	393072938	62736932	15.96	0.977719	0.995697	32777776	0.555805
	1113P2-1_L4_A004	293259918	43.99	291596862	47267861	16.21	0.979476	0.99572	29586224	0.501694
	1113P2-2_L4_A005	271504624	40.73	270061644	45075982	16.69	0.98055	0.995913	29324470	0.497252
	1113P3-1_L4_A007	265529908	39.83	264083918	46942354	17.78	0.984474	0.996653	30008336	0.508849
	1113P3-2_L4_A008	293202510	43.98	291322458	51260723	17.6	0.982148	0.996525	30451029	0.516359
	1113P4-1_L4_A009	295772280	44.37	294159104	60514368	20.57	0.985819	0.996571	33288340	0.564471
	1113P4-2_L4_A010	444280324	66.64	440645406	76693772	17.4	0.985238	0.996431	35868200	0.608213
	1113P5-1_L4_A011	300581034	45.09	297996062	50636902	16.99	0.982603	0.996554	30754303	0.521501
	1113P5-2_L4_A012	237429208	35.61	234199370	39547488	16.89	0.983056	0.996533	27173305	0.460781

P1120	1113P1-1_L4_A001	134940296	20.24	134133872	21908545	16.33	0.976897	0.99574	20385280	0.345675
	1113P1-2_L4_A002	395442446	59.32	393072938	62736932	15.96	0.977719	0.995697	32777776	0.555805
	1113P2-1_L4_A004	293259918	43.99	291596862	47267861	16.21	0.979476	0.99572	29586224	0.501694
	1113P2-2_L4_A005	271504624	40.73	270061644	45075982	16.69	0.98055	0.995913	29324470	0.497252
	1113P3-1_L4_A007	265529908	39.83	264083918	46942354	17.78	0.984474	0.996653	30008336	0.508849
	1113P3-2_L4_A008	293202510	43.98	291322458	51260723	17.6	0.982148	0.996525	30451029	0.516359
	1113P4-1_L4_A009	295772280	44.37	294159104	60514368	20.57	0.985819	0.996571	33288340	0.564471
	1113P4-2_L4_A010	444280324	66.64	440645406	76693772	17.4	0.985238	0.996431	35868200	0.608213
	1113P5-1_L4_A011	300581034	45.09	297996062	50636902	16.99	0.982603	0.996554	30754303	0.521501
	1113P5-2_L4_A012	237429208	35.61	234199370	39547488	16.89	0.983056	0.996533	27173305	0.460781
P0117	0117P3-1_L2_A004	335771944	50.37	293869202	35255255	12	0.984836	0.995065	25601252	0.434121
	0117P3-2_L2_A005	297634670	44.65	242853254	25966323	10.69	0.984749	0.994775	21528474	0.36506
	0117P3-3_L2_A006	431734528	64.76	345332060	33385454	9.67	0.984306	0.994531	23713703	0.402109
	0117P4-1_L2_A007	324550082	48.68	274836624	32343072	11.77	0.986167	0.994355	24485619	0.415204
	0117P4-2_L2_A008	433115020	64.97	356651126	37400202	10.49	0.984414	0.994867	25932228	0.439726
	0117P4-3_L2_A009	353761964	53.06	306454906	39440724	12.87	0.986059	0.995162	27025106	0.458264
	0117P5-1_L2_A010	423548370	63.53	355336208	39960613	11.25	0.986299	0.994806	26781800	0.454139
	0117P5-2_L2_A011	354669536	53.2	306690600	34974260	11.4	0.985873	0.994601	25735180	0.436387
	0117P5-3_L2_A012	788890	0.12	657704	63440	9.65	0.979232	0.993103	109364	0.00185778

Table S1. Summary of bisulfite-seq data in eight patients.