

Supplementary Table 1. Information regarding the horses evaluated in this study.

Horse name	Life number	Age	Sex	Hair color	Price of the horse (KRW)
JIGUSANG SERYEOK	KOR05218979	8	Female	Chestnut	---
GEUMBIT SESANG	KOR06120650	7	Male	Brown	₩149,645,000

Supplementary Table 2. Information regarding the primers used to validate the methylation state of target gene promoter region by BSP, and assess expression by using real-time RT-PCR.

Gene	Accession number	Direction	Sequences (5'-3')	Amplify size (bp)
<i>ZNF80</i> (for BSP)	ENSECAT000000506 2	Forward	GGTTTTGGGATTTGTAGTTTATTG	203
		Reverse	AACCTAATCCAACCTACCCCAACT	
<i>POU1F1</i> (for BSP)	ENSECAT000000961 5	Forward	TAAAGGGATTTTTTTGTAGTATAAG	160
		Reverse	AAAAAAAATACTACTCCCAAATC	
<i>MFGES</i> (for BSP)	ENSECAT000002028 5	Forward	TAGGGGAGGGGATTAATTTGTTA	292
		Reverse	CACCTTAACATCCCAAAAAACTAC	
<i>ANAPC1</i> (for BSP)	ENSECAT000002281 0	Forward	TGTAGTAATAGTTGTTAATGTAGAGATTTG	421
		Reverse	AACCTTTCTACTCATAAAATCCAAAC	
<i>ANAPC1</i> (for expression)	ENSECAT000002281 0	Forward	GCAAACACCACCCTAATGCT	142
		Reverse	TGCCAGCTTTCCTTCTGTTT	
<i>GAPDH</i>	ENSECAT000002372 1	Forward	ATCTGACCTGCCGCCTGGAG	68
		Reverse	CGATGCCTGCTTCACCACTTC	

Supplementary Table 3. The general information of read alignment in the genomes of the blood samples collected from the two thoroughbred horses before and after trotting.

	JIGUSANG SERYEOK		GEUMBIT SESANG	
	Before exercise	After exercise	Before exercise	After exercise
Data size (Gb)	1.20	1.19	1.15	1.20
Total sequenced reads (No.)	24,489,796	24,251,782	23,506,720	24,489,796
Mapped reads (No.)	20,888,843	20,850,727	19,905,128	21,304,452
Mapping rate (%)	85.30	85.98	84.68	86.99
Effective chain depth ¹	0.43	0.43	0.41	0.44
Uniquely mapped reads (No.)	17,847,159	17,745,354	16,700,660	18,540,301
Unique mapping rate (%) ²	72.88	73.17	71.05	75.71
Total predicted peaks (No.)	105,303	92,969	81,140	98,210
Average peak length (bp)	860	878	889	883
Median peak length (bp)	774	791	802	796
Total length of peaks (bp)	90,566,979	81,629,516	72,173,605	86,699,687
Peak-covered size in the reference genome (%)	3.83	3.45	3.05	3.66

¹Effective chain depth = total length of mapped reads / the size of the reference genome

²Unique mapping rate = total uniquely mapped reads / total sequenced reads

Supplementary Table 4. Number of different genomic regions in the reference genome (EquCab2).

	Promoter	5' UTR	Exon	Intron	3' UTR	Downstream	Repeat	Others
EquCab2	27,554	12,441	205,323	178,583	11,862	27,488	2,501,645	7,576,494

Supplementary Table 5. Frequency of methylation peaks in different genomic regions.

		Promoter	5' UTR	Exon	Intron	3' UTR	Downstream	Repeat	Others
JIGUSANG SERYEOK	Before exercise	0.075	0.005	0.023	0.168	0.013	0.073	0.012	0.005
	After exercise	0.067	0.006	0.021	0.146	0.011	0.066	0.010	0.004
GEUMBIT SESANG	Before exercise	0.056	0.004	0.019	0.128	0.009	0.056	0.009	0.004
	After exercise	0.069	0.005	0.020	0.156	0.013	0.071	0.011	0.005

Frequency of peaks = Peak distribution / Numbers of different genomic regions in the EquCab2

Supplementary Table 7. The list of negatively-correlated genes between expression level and methylation level.

Differentially expressed genes (DEGs) from RNA-Seq

Gene	Locus	GS FPKM (A)	GS FPKM (B)	Log2(B/A)	P-value	Expression change after exercise in GS
FOXP2	Chr4:71764132-72012020	0.0680683	0	-1.79769e+308	0.0369233	Low-expressed
GXYLT2	Chr16:17474065-17553670	0.223271	0	-1.79769e+308	0.0335125	Low-expressed
SH2D4B	Chr1:88561674-88654379	0.131498	0	-1.79769e+308	0.0335125	Low-expressed
SLC5A3	Chr26:30639217-30670146	6.52548	10.0874	0.628406	0.0433971	High-expressed
TMEM150B	Chr10:24471616-24498340	11.2547	18.1067	0.685998	0.0489095	High-expressed
DEDD2	Chr10:13553003-13573408	17.423	30.137	0.790539	0.00105037	High-expressed
C1orf21	Chr5:21315334-21458065	6.19695	12.24	0.98197	0.0135391	High-expressed
NLRP12	Chr10:22834672-22866121	2.2363	6.33784	1.50288	0.000012507	High-expressed
LRP11	Chr31:16978002-17011735	0.240771	0.700482	1.54069	0.027201	High-expressed
ANGPT1	Chr9:51942202-52181014	0	0.0859218	1.79769e+308	0.0345177	High-expressed
FSTL4	Chr14:42281899-42486963	0	0.0164148	1.79769e+308	0.0384396	High-expressed
Gene	Locus	JS FPKM (A)	JS FPKM (B)	Log2(B/A)	P-value	Expression change after exercise in JS
NCKAP5	Chr18:16876570-17702260	0.125461	0.0128215	-3.2906	0.0245383	Low-expressed
PHC3	Chr19:10518569-10590129	15.2939	6.11646	-1.32218	0.00593945	Low-expressed
SLC16A6	Chr11:12371268-12389919	23.1525	10.8843	-1.08892	0.0121751	Low-expressed
CACNA1D	Chr16:34062865-34381927	0.0377868	0	-1.79769e+308	0.0436843	Low-expressed
GNAL	Chr8:38096950-38193302	0.238902	0	-1.79769e+308	0.0371067	Low-expressed
SCUBE2	Chr7:78361864-78421344	0.0569065	0	-1.79769e+308	0.0433786	Low-expressed
TNFRSF21	Chr20:45737669-45803833	2.8901	6.04327	1.06421	0.0259256	High-expressed
CCS	Chr12:26545317-26556416	17.7861	37.2994	1.0684	0.0297999	High-expressed
TGM2	Chr22:28368451-28401055	11.1224	25.4011	1.19142	0.00117231	High-expressed
UNC93A	Chr31:7737541-7780641	12.8184	30.5082	1.25099	0.0287247	High-expressed
CYSTM1	Chr14:36525183-36642135	24.9577	75.159	1.59046	0.0483555	High-expressed
NFKBIA	Chr1:172073462-172075841	204.95	899.854	2.13442	9.07634E-06	High-expressed
CORO6	Chr11:43380047-43389316	0.139187	0.678452	2.28522	0.0043599	High-expressed

Differentially methylated regions (DMRs) from MeDIP-Seq

Locus	Region	GS #No. of nomalized tag (C)	GS #No. of nomalized tag (D)	log2(D/C)	Methylation change after exercise in GS
chr4:71883226-71884016	Intron	8.382902233	14.0235048	0.742325248	Hypermethylation
chr16:17523312-17524090	Intron	11.97557462	25.88954732	1.112274858	Hypermethylation
chr1:88634905-88636084	Intron	19.16091939	29.12574073	0.604127954	Hypermethylation
chr26:30659825-30660957	CDS	22.75359177	14.0235048	-0.698247343	Hypomethylation
chr10:24493821-24494734	Intron	25.1487067	11.86604252	-1.083645352	Hypomethylation
chr10:13551779-13552578	Promoter	17.3645832	9.169214675	-0.921277702	Hypomethylation
chr5:21365884-21366919	Intron	23.35237051	12.94477366	-0.851199266	Hypomethylation
chr10:22835202-22837013	Intron	46.10596228	29.6651063	-0.636186375	Hypomethylation
chr31:17006150-17006808	CDS	17.3645832	10.78731138	-0.686812448	Hypomethylation
chr9:52167932-52168543	Intron	16.76580447	8.629849105	-0.95811447	Hypomethylation
chr14:42281774-42282989	Intron	24.54992797	14.56287037	-0.75342405	Hypomethylation
Chr	Region	JS #No. of nomalized tag (C)	JS #No. of nomalized tag (D)	log2(D/C)	Methylation change after exercise in JS
chr18:17202202-17202867	Intron	15.12845826	23.66816689	0.645683003	Hypermethylation
chr19:10559357-10560782	Intron	13.44751845	27.61286137	1.038000426	Hypermethylation
chr11:12380345-12382804	CDS	19.61096441	47.89986156	1.288361001	Hypermethylation
chr16:34245727-34246767	Intron	10.64595211	18.59641684	0.804719688	Hypermethylation
chr8:38130307-38130965	Intron	10.08563884	15.77877793	0.645683003	Hypermethylation
chr7:78395986-78397691	Intron	13.44751845	28.17638916	1.067146771	Hypermethylation
chr20:45739955-45740750	CDS	17.93002461	11.83408345	-0.599429495	Hypomethylation
chr12:26546342-26548572	Intron	57.7122667	32.68461142	-0.82026645	Hypomethylation
chr22:28401186-28402536	Promoter	40.90286863	26.48580581	-0.626982625	Hypomethylation
chr31:7775322-7776231	Intron	28.57597671	18.03288906	-0.66417226	Hypomethylation
chr14:36605515-36606545	Intron	17.36971134	11.27055566	-0.624015133	Hypomethylation
chr1:172074178-172075231	Intron	19.61096441	10.1435001	-0.951104933	Hypomethylation
chr11:43384670-43385728	CDS	26.33472364	16.90583349	-0.639445174	Hypomethylation

Supplementary Table 8. Distribution and frequency of methylation peaks over repeats in the genome.

Repeat class	Repeats in the genome	No. of peaks				Frequency of peaks			
		JIGUSANG SERYEOK		GEUMBIT SESANG		JIGUSANG SERYEOK		GEUMBIT SESANG	
		Before exercise	After exercise	Before exercise	After exercise	Before exercise	After exercise	Before exercise	After exercise
DNA	364,428	2,458	2,227	1,787	2,407	0.007	0.006	0.005	0.007
LINE	1,246,366	18,338	15,468	13,565	17,457	0.015	0.012	0.011	0.014
Low complexity	252,740	393	343	298	358	0.002	0.001	0.001	0.001
LTR	436,921	3,826	3,743	3,042	3,779	0.009	0.009	0.007	0.009
RC	2,424	12	19	10	12	0.005	0.008	0.004	0.005
RNA	696	4	4	6	3	0.006	0.006	0.009	0.004
rRNA	445	13	10	11	11	0.029	0.022	0.025	0.025
Satellite	1,344	59	61	65	71	0.044	0.045	0.048	0.053
scRNA	73	1	1	-	1	0.014	0.014	-	0.014
Simple repeat	199,308	1,213	1,024	800	1,032	0.006	0.005	0.004	0.005
SINE	990,165	16,339	13,931	12,763	14,225	0.017	0.014	0.013	0.014
snRNA	989	9	10	8	16	0.009	0.010	0.008	0.016
srpRNA	25	2	1	2	1	0.080	0.040	0.080	0.040
tRNA	14,097	51	56	49	55	0.004	0.004	0.003	0.004
Unknown	8,133	40	42	30	46	0.005	0.005	0.004	0.006
Total	3,518,154	42,758	36,940	32,436	39,474	0.012	0.010	0.009	0.011

Supplementary Table 9. DMR-containing gene lists after GO and KEGG analysis of the two horses before and after exercise.

Category	Term	P-value	Genes	Count
GOTERM_BP_FAT	Metal ion transport	0.004663668	SLC12A8, SLC8A2, SLC39A11, KCNB1, TRPC7, SFXN4, SFXN1, TCN2, KCNIP1, SFXN5, CACNA2D4, KCNMB2, KCNQ5, ABCC9, KCNN3, SCN8A, CHRNA10	17
GOTERM_BP_FAT	Protein amino acid phosphorylation	7.03E-04	PRKCA, PIK3CG, OBSCN, CDK5R1, FLT3, NLK, STRADA, PRKCH, EIF2A, TTN, CDKL5, CAMKK1, DAPK1, MAP4K3, IRAK3, PTK2, COL4A3BP, PDGFRA, SPTBN1, PDGFRB, GRK4, EIF2AK4, AKT3, NEK7	24
GOTERM_BP_FAT	Regulation of small GTPase mediated signal transduc	2.96E-04	OBSCN, ARHGEF7, KIAA1244, PREX1, IQGAP2, PSD4, TTN, DNMBP, ADAP1, TBC1D16, GARNL3, RASGRF2, GIT2, RASA3	14
GOTERM_CC_FAT	Contractile fiber	0.003515576	OBSCN, CDK5R1, SYNE1, TNNT1, MYH11, SPTBN1, MYOM1, TTN	8
GOTERM_CC_FAT	M band	3.27E-04	OBSCN, SPTBN1, MYOM1, TTN	4
GOTERM_CC_FAT	Cytoskeleton	8.54E-05	DNAH11, MAD1L1, PARD3, LMNB1, CNN3, CDC14A, DNAH14, FAM110B, IQGAP2, DNAH2, TTN, STAU1, PTK2, SORBS1, CDK5RAP2, MYOM1, IFNGR1, ZW10, NF2, MICAL2, KIF18B, SPATC1, SEPT10, DNMBP, DAPK1, ARPC1A, SYNE1, EPB41L1, TNNT1, CST6, TBCA, SPAG5, CGN, FRMD4A, MCPH1, NINL, MYH11, PEBP1, CLIP1, SPTBN1, PDE4DIP, ADD1	42
GOTERM_MF_FAT	Small GTPase regulator activity	4.74E-04	OBSCN, ARHGEF7, KIAA1244, PREX1, IQGAP2, PSD4, TTN, DNMBP, ADAP1, TBC1D16, MAP4K3, GARNL3, RASGRF2, GIT2	14
GOTERM_MF_FAT	Calmodulin binding	4.19E-04	SLC8A2, CNN3, KCNN3, MYH11, IQGAP2, SPTBN1, TTN, CAMKK1, ADD1, DAPK1	10
GOTERM_MF_FAT	Vascular endothelial growth factor receptor activity	2.14E-04	NRP2, FLT3, PDGFRA, PDGFRB	4
KEGG_PATHWAY	Tight junction	0.011503665	PRKCA, INADL, PARD3, EPB41L1, CGN, MYH11, PRKCH, AKT3	8
KEGG_PATHWAY	Glioma	0.028459795	PRKCA, PIK3CG, PDGFRA, PDGFRB, AKT3	5