

1 **Exercise-related genes analysis of Mongolian Horse**

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17 **Exercise-related genes analysis of Mongolian Horse - Abaga horse and Wushen**
18 **horse**

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20 **Keywords:** genome, Mongolian horse, Abaga horse, Wushen horse, exercise

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27 **ABSTRACT**

28 The Mongolian horses, as a neglected scientific resource, have excellent endurance
29 and stress resistance to adapt to the cold and harsh plateau conditions. Intraspecific
30 genetic diversity is mainly embodied in various genetic advantages of different
31 branches of Mongolian horse. Abaga horse is better than Wushen horse in running
32 speed, for example. Because people pay progressively attention to the athletic
33 performance of horse, such as horse racing in Mongolia's Naadam festival, we expect
34 to guide the exercise-oriented breeding of horses through genomics research. We
35 obtained the clean data of 630,535,376,400 bp through the entire genome
36 second-generation sequencing for the whole blood of 4 Abaga horses and 10 Wushen
37 horses. Based on the data analysis of single nucleotide polymorphism (SNP), we
38 severally detected that 479 and 943 positively selected genes, particularly
39 exercise-related, were mainly enriched on equine chromosome 4 in Abaga horses and
40 Wushen horses, which implied that the chromosome 4 may be associated with the
41 evolution of the Mongolian horse and athletic performance. Four hundred and forty
42 genes of positive selection were enriched in 12 exercise-related pathways and
43 narrowed in 21 exercise-related genes in Abaga horse, which were distinguished from
44 Wushen horse. So, we speculated that the Abaga horse may have oriented genes for
45 the motorial mechanism and 21 exercise-related genes also provided molecular
46 genetic basis for exercise-directed breeding of Mongolian horse.

47 **INTRODUCTION**

48 As an ancient breed, Mongolian horse has gone through a long breeding period (China

49 National Commission of Animal Genetic Resources 2011). With a view to research
50 tendentiousness, researchers pay more attention to traits of Thoroughbred horse (Gim
51 *et al.* 2004; Park *et al.* 2012; Capomaccio *et al.* 2013) and Quarter horse (Doan *et al.*
52 2012; Meira *et al.* 2014), but not the Mongolian horse and its diverse sub-branch. The
53 preminent endurance and stress-resistance of Mongolia horse are important factors
54 for it to well adapt to the cold and harsh plateau environment (Li *et al.* 2009). Natural
55 factors may have enormous impacts on evolution owing to the rough domestication of
56 Mongolian horse (Hund 2008). Due to various geographic conditions and human
57 necessities, Mongolian horse gradually formed several specific traits. Some horses
58 which adapt to the desert climate have larger feet, for instance, some horses which
59 adjust to mountain road with rocks have supple body and hard hoof; in addition, the
60 features of horse which accommodate to the grassland climate are tall physique and
61 good at running (Elisabeth 2011). Living in the Xilin Gol grassland of Inner Mongolia,
62 Abaga horse belongs to the steppe horse and speeds up to 1600 meters every 91.47
63 seconds (China National Commission of Animal Genetic Resources 2011). Wushen
64 horse, which is small build and has broad-flat horseshoe, as symbol of the desert horse
65 in the south of Maowusu desert of Ordos City in Inner Mongolia, can hoof steadily in
66 the desert, albeit noffast running speed of 13 to 15 kilometers per hour (Dugarjaviin
67 2009).

68 In Mongolia, the herdsmen depend on horses by reason that they are the
69 indispensable sources of pastoral rations, such as meat and dairy products, and used to
70 be one of the means of transport by herders (Hund 2008). Furthermore, Mongolian

71 horse was an essential and distinguished war-horse in history (Article: The Horse in
72 Mongolian Culture 2018). For the Naadam of traditional festivals in Mongolia, horse
73 racing is one of the entertaining activities for herds and regarded as the second most
74 popular sporting events after wrestling (Davis 2010). So, the running speed of
75 Mongolian horses has been one of the focuses of attention. Despite not the fastest
76 horse in the world, people still endeavor to improve running speed of the Mongolian
77 horse through unremitting consideration and breeding.

78 In order to discuss the genetic variation between Abaga horse and Wushen horse
79 in Mongolian horse strains, we planned to analyze data of the entire genome with
80 second-generation sequencing technology to seek out exercise-related single
81 nucleotide polymorphism (SNP) locus of Mongolian horse and offer a reference for
82 identification and improvement of Mongolian horse varieties.

83 **MATERIALS AND METHODS**

84 **Experimental animals and sample preparation**

85 We collected jugular blood of 4 Abaga horses in the Inner Mongolia Abaga County
86 and 10 Wushen horses in the Inner Mongolia Ordos City Wushen County. Adhering to
87 the manufacturer's instructions for the extraction of DNA from the whole blood,
88 genome was extracted by the AxyPrep blood genomic DNA kit. Then we used the
89 NanoDrop™ 1000 spectrophotometer and polyacrylamide gel electrophoresis to
90 detect the concentration and integrity of the genome. The concentration of extracted
91 DNA was between 26.4 ng/μL and 34.4 ng/μL for subsequent library construction.

92 **Library preparation and whole-genome sequencing**

93 A TruSeq DNA Sample Prep Kit was used to construct a sequencing library.
94 Whole-genome sequencing of the horses was performed using the Illumina HiSeq X
95 TM Ten Sequencing System.

96 **Data quality control and comparison to reference genome**

97 The raw data of 639,723,611,100 bp was sequenced from 14 samples. The inferior
98 quality reads which have sequencing adapter, higher than 10% of N (base of
99 uncertainty) content or inferior mass base ($Q \leq 5$) content of higher than 50% were
100 filtered out by in-house Perl/Python scripts to achieve clean data of 630,535,376,400
101 bp. The Q20, Q30, error rate, GC content and other information of these data were
102 counted by in-house Perl/Python scripts (Table 1). The sequencing reads were mapped
103 to reference genomes (Ensembl release 82) by BWAmem (bwa-0.7.8) (Li 2013),
104 which PCR and optical repetition of results were removed by using Picard (Broad
105 Institute 2018). Statistics of mapping rate, average depth and coverage of the data
106 after comparison were computed by in-house Perl/Python scripts (Table 2).

107 **Single nucleotide polymorphism calling and annotation**

108 SNP calling was performed using the GATK HaplotypeCaller (v3.5) (McKenna *et al.*
109 2010). In order to evaluate the reliability of the detected SNP sites and filter inferior
110 quality SNP, we used SAMTools for SNP detection (Li 2011). Simultaneously, the
111 dbSNP database 5,019,393 SNPs and 670K chip site information were downloaded.
112 The data was used as training set, and the detected SNPs were evaluated and filtered
113 by using the GATK VQSR process. The standard for the retention of the final site is
114 the tranche value of 99 ($T_i / T_v = 2.02$). Finally, the SNPs of equine population were

115 filtered: $GQ > 10$, $MAF > 0.05$, $call\ rate > 0.9$ (Figure 1). The variants after filtering
116 were annotated by ANNOVAR (v2016-02-01) (Wang *et al.* 2010) (Table 3).

117 **Selective sweep analysis**

118 To identify potential selective sweeps between Abaga horse (fast) and Wushen horse
119 (slow), $Pi\ log_2(slow/fast)$ and F_{ST} was calculated together using VCFtools with a
120 20kb sliding window and a step size of 10 kb. Windows that contained less than 10
121 SNPs were excluded from further analysis. The windows that were simultaneously 1)
122 in the top 5% of Z-transformed F_{ST} values and 2) in the bottom 5% $Pi\ log_2(slow/fast)$
123 were considered to be candidate selective regions in Abaga horse (Figure 2A; Table
124 S1 <https://doi.org/10.6084/m9.figshare.6289523.v1>). The same applies to Wushen
125 horse (Figure 2B; Table S2 <https://doi.org/10.6084/m9.figshare.6289532.v1>).

126 **Statistic and advanced analysis of positively selected and candidate genes**

127 We annotated the positively selected genes via GO (GOseq) to further screen out the
128 major enriched functions (Young *et al.* 2010). The pathways which included these
129 selected genes were enriched by KEGG (KOBAS) (Xie *et al.* 2011). Many positively
130 selected genes which are in Abaga horse were analyzed further without overlapped
131 genes between Abaga horse and Wushen horse.

132 **RESULTS AND DISCUSSION**

133 **Related-clean data stated**

134 We performed the entire genome second-generation sequencing for the whole blood
135 of four Abaga horses (Figure 3A) and ten Wushen horses (Figure 3B) with the
136 Illumina HiSeqX ten sequencing platform. The clean data of 630,535,376,400 bp

137 (effective rate of data: 98.56%, error rate of data: 0.03%, mean of Q20: 94.95% and
138 mean of Q30: 89.80%) was sequenced by filtration and 41.95G as the mean of clean
139 data was generated in each sample (Table 1). Then, the data was mapped to the
140 reference genome (Ensembl release 82) via BWAmem (Li 2013). Without PCR and
141 optical repetition, the successful mapping rate of data was 98.36%. For 14 samples,
142 the average sequencing depth was 16.75× coverage and average cover degree was
143 99.55% on reference sequences (Table 2).

144 **Distribution of positive selection genes on chromosomes**

145 Based on the data of SNP following SNP calling (Figure 1), we obtained the genes of
146 significantly genetic differences by using F-statistics between Abaga horses and
147 Wushen horses, and narrowed the above genes down to 479 and 943 positively
148 selected genes combined with SNP polymorphism analysis in Abaga horses and
149 Wushen horses, respectively (Figure 2A, B; Table S1, S2). We discovered that these
150 selected genes were mainly distributed on chromosome 4, 7 and 10 in Abaga horses,
151 and chromosome 1, 4, 8 and 16 in Wushen horses with the analysis of genes
152 distribution on chromosome (Figure 4).

153 Many genes of the positively selected 479 and 943 genes were enriched on
154 chromosome 4, and the enrichment quantity of positively selected genes was
155 secondary by the chromosome enrichment analysis both in Abaga horse and Wushen
156 horse. In the statement of Schröder (Schröder *et al.* 2011), athletic
157 performance-related genes were significantly enriched on chromosomes 4 and 12 of
158 horses, which coincided with the different traits of running speed in our exploring

159 direction. Possibly, we will take equine chromosome 4 as the exercise-related
160 emphasis of scientific research.

161 **Gene Ontology, KEGG pathways and exercise-related genes**

162 Above these positively selected genes were functional annotation by Gene Ontology
163 (GO). The selected 479 genes of Abaga horse were mainly enriched in neuron part
164 (GO:0097458), neuron projection (GO:0043005), regulation of membrane potential
165 (GO:0045838), positive regulation of cell projection organization (GO:0031346),
166 neuron-neuron synaptic transmission (GO:0007270), synaptic transmission,
167 glutamatergic (GO:0035249), neurotransmitter secretion (GO:0007269), antigen
168 processing and presentation (GO:0019882), telencephalon cell migration
169 (GO:0022029) and forebrain cell migration (GO:0021885). The selected 943 genes of
170 Wushen horse were mainly enriched in membrane part (GO:0044425), intrinsic
171 component of membrane (GO:0031224), integral component of membrane
172 (GO:0016021), cell projection (GO:0042995), neuron part (GO:0097458), neuron
173 projection (GO:0043005), synapse (GO:0045202), cilium (GO:0005929) and cell
174 projection assembly (GO:0030031) (Figure 5A, B).

175 The athletic ability of the horse may be influenced not only by physiology, but
176 also thought and motive. According to the previous studies, equine exercise-related
177 genes included *DRD1-5*, *SLC6A4* and *BDNF*, the three genes functions were related
178 to many neurological processes, involving motivation, pleasure, cognition, memory,
179 learning, fine motor control, modulation of neuroendocrine signaling, adaptive ability
180 of controlling emotions, supporting the survival of existing neurons, encouraging the

181 growth and differentiation of new neurons and synapses (Momozawa *et al.* 2005;
182 Bryan *et al.* 2007; Kulikova *et al.* 2007; Lippi *et al.* 2010). The GO analysis results of
183 Abaga horse were also preferentially enriched in neuronal composition,
184 neurotransmission and brain cell migration. These genes may allow Abaga horse to
185 quickly observe and distinguish the surrounding during moving with high-speed,
186 timely rectify status to respond to the various circumstances.

187 By pathways enrichment analysis of Kyoto Encyclopedia of Genes and Genomes
188 (KEGG) with the positively selected 479 genes in Abaga horse and 943 genes in
189 Wushen horse, the enriched pathways ($P \leq 0.05$) of Abaga horse included Propanoate
190 metabolism, Viral myocarditis, Phototransduction, PI3K-Akt signaling pathway,
191 Glycerolipid metabolism, Morphine addiction and mRNA surveillance pathway in
192 which the pathway with the largest number of enriched genes (13 genes) was
193 PI3K-Akt signaling pathway. As intracellular basal signaling pathways, PI3K-Akt
194 signaling pathway involves lots of vital movement, such as exercise-induced
195 physiologic hypertrophy (Shioi *et al.* 2002; Luo *et al.* 2005; Sagara *et al.* 2012; Song
196 *et al.* 2015) and protecting mitochondria of skeletal muscle by aerobic endurance
197 training (Liu *et al.* 2016), further explaining the excellent athletic performance of
198 Abaga horse. Besides that, the enriched pathways ($P \leq 0.05$) of Wushen horse
199 contained Base excision repair, Glutamatergic synapse, Endometrial cancer,
200 Glycolysis / Gluconeogenesis, Propanoate metabolism and ABC transporters (Figure
201 6A, B).

202 Further on SNPs, we analyzed functions of 440 genes of Abaga horse without 39

203 overlapped genes of positively selected genes between Abaga horse and Wushen
204 horse. We focused on the enriched exercise-related pathways which referred to
205 Metabolic pathways (Hill *et al.* 2010), Ras signaling pathway (Shioi *et al.* 2002; Xie
206 *et al.* 2007), PI3K-Akt signaling pathway (Shioi *et al.* 2002; Luo *et al.* 2005; Sagara
207 *et al.* 2012; Standard *et al.* 2014; Song *et al.* 2015; Liu *et al.* 2016; Vega *et al.* 2017),
208 MAPK signaling pathway (Schröder *et al.* 2011), Hippo signaling pathway (Gabriel *et*
209 *al.* 2016), Valine, leucine and isoleucine degradation (McGivney *et al.* 2010), Cardiac
210 muscle contraction (Do *et al.* 2015), NF-kappa B signaling pathway (Kramer and
211 Goodyear 2007), Arachidonic acid metabolism (Hill *et al.* 2010), Regulation of actin
212 cytoskeleton (Hill *et al.* 2010; Schröder *et al.* 2011), Insulin signaling pathway (Gim
213 *et al.* 2004; Hill *et al.* 2010) and Fatty acid metabolism (Gim *et al.* 2004; Hill *et al.*
214 2010) in the 440 positively selected genes of Abaga horse that distinguished from
215 Wushen horse (Figure 7). These enriched pathways comprised some recurrent genes
216 (Figure 7). Taking repeated genes as pivots, we speculated that the synergistic effect
217 of pathways enabled faster running speed of Abaga horse compared with Wushen
218 horse. But, in our studying, the enriched genes of positive selection were different
219 from the previous studied genes in the above exercise-related pathways (Table 4),
220 which indicated species-specific genes of positive selection in Abaga horse compared
221 with other species (human, rat, mouse, leopard, thoroughbred horse etc.).

222 According to the analysis of GO, KEGG and individual gene function, we
223 subsequently put our interest in exercise-related genes of Abaga horse. Twenty-one
224 genes may involve in exercise of Abaga horse while their functions embodied

225 vasoconstriction (*HTR2B*) (Launay *et al.* 2002; Bevilacqua *et al.* 2010; Meira *et al.*
226 2014), angiogenesis (*CDH5*) (Sauteur *et al.* 2014), cardiac contraction (*KCNQ1*)
227 (Jespersen *et al.* 2005; Brown *et al.* 2015; Pedersen *et al.* 2017), cardiac development
228 and muscle structure (*ENAH*) (Franzini-Armstrong 1973; Benz *et al.* 2013), muscle
229 growth (*PIH1D1*, *SMURF1*) (Inoue *et al.* 2010; Ponsuksili *et al.* 2014; Dalbo *et al.*
230 2013), myogenic differentiation (*UNC13C*) (Meyer *et al.* 2015; Langlois and Cowan
231 2017), skeletal muscle function (*ATPIA3*) (Aughey *et al.* 2007; Brashear *et al.* 2007),
232 femur strength and bone mineral density (*PPP2R5B*, *PPP6R3*) (Alam *et al.* 2009;
233 Medina-Gomez *et al.* 2017), osteoclast growth (*PTPRE*, *RHOBTB1*) (Chiusaroli *et al.*
234 2004; Song *et al.* 2014), chondrogenesis (SCFD) (DeLise *et al.* 2000; Hou *et al.* 2017),
235 lipid and carbohydrate metabolism (*PPARD*, *GCG*, *TCF7L2*, *GALNT13*) (Yi *et al.*
236 2005; Bevilacqua *et al.* 2010; Park *et al.* 2012; Ahmetov and Fedotovskaya 2015;
237 Giordano Attianese and Desvergne 2015; Ropka-Molik *et al.* 2017), exercise
238 stress-induced response (*CD69*, *EIF4G3*) (Testi *et al.* 1989; Gradi *et al.* 1998;
239 Cappelli *et al.* 2007; Morabito *et al.* 2016), exercise coordination (*GRM1*) (Conquet *et*
240 *al.* 1994; Bossi *et al.* 2017) and height (*VGLLA*) (Gabriel *et al.* 2016). These genes of
241 positive selection were presented simultaneously in Abaga horse, which may be a
242 reason that it runs rapider than Wushen horse.

243 Counting on exercise-related genes of previous studies, the equine athletic
244 performance is related to glucose metabolism, stress immune response, angiogenesis
245 and muscle supply, insulin signal transduction, fat substrate application, muscle
246 strength and the formation of bones and cartilage with growth (Gim *et al.* 2004; Hill

247 *et al.* 2010; Park *et al.* 2012; Capomaccio *et al.* 2013; Kamm *et al.* 2013). We picked
248 up exercise-related genes as candidate genes in positively selected genes, further,
249 presented enriched KEGG pathways and functions with selected exercise-related
250 genes (Figure 7). *HTR2B* (encoding 5-hydroxytryptamine receptor 2B), has been
251 identified in the genome of Quarter horses of the racing line (Meira *et al.* 2014) and
252 associated with impulsive behavior (Bevilacqua *et al.* 2010) and vasoconstriction
253 (Launay *et al.* 2002). In zebrafish, vascular endothelial cadherin (encoded by *CDH5*)
254 can promote elongation of the endothelial cell interface during angiogenesis (Sauteur
255 *et al.* 2014). *KCNQ1* (encoding KvLQT1, a potassium channel protein) is related to
256 exercise, and mutation of *KCNQ1* and *KCNE1* can casus susceptibility of sudden
257 cardiac death (SCD) for horse (Jespersen *et al.* 2005; Brown *et al.* 2015; Pedersen *et*
258 *al.* 2017). Mena (encoded by *ENAH*) which located in Z line that the borders of the
259 sarcomere, VASP, and α II-Spectrin assemble cardiac multi-protein complexes to
260 regulate cytoplasmic actin networks (Franzini-Armstrong 1973; Benz *et al.* 2013).
261 *PIH1D1* (encoding the components of the apoptotic regulatory complex R2TP) is
262 relevant to muscle mass (Inoue *et al.* 2010; Ponsuksili *et al.* 2014). Because E3
263 ubiquitin-protein ligase SMURF1 (encoded by *SMURF1*) function as negative
264 regulators of myostatin pathway activity and myostatin is negative regulator of
265 skeletal muscle mass, up-regulated expression of *SMURF1* may link to skeletal
266 muscle growth following prolonged training (Dalbo *et al.* 2013). *UNC13C* is
267 connected with differentiation of myoblast while integral myotubes originate in
268 myoblast differentiation and raise the distinct muscle fiber types to build the complex

269 skeletal muscle architecture for body movement, postural behavior and breathing
270 (Meyer *et al.* 2015; Langlois and Cowan 2017). *ATPIA3* encodes subunit alpha-3 of
271 sodium/potassium-transporting ATPase, which increased expression may be
272 conducive to decrease fatigue after training (Brashear *et al.* 2007; Aughey *et al.* 2007).
273 *PPARD* (encoding peroxisome proliferator-activated receptor delta) participate in
274 regulation of energy metabolism, cell proliferation and differentiation, protection in
275 stress conditions such as oxidative stress and inflammation and other important life
276 activities (Giordano Attianese and Desvergne 2015). The antecedent studies have
277 shown that Arabian horse will change the expression of *PPARD* and other genes of
278 PPAR signaling pathway genes in skeletal muscle during exercise, and improve
279 coefficient of utilization of fatty acids by energy conversion (Ropka-Molik *et al.*
280 2017). The up-regulated *PPARD* are also found after exercise in Thoroughbred horse
281 (Park KD *et al.* 2012). So, we speculated that positively selected *PPARD* improved
282 athletic ability by a similar mechanism in Abaga horse. Besides counter-regulatory
283 hormone of insulin, *GCG* (encoding glucagon) is deemed to be involved in adipose
284 metabolism and energy balance (Bevilacqua *et al.* 2010). Transcription factor 7-like 2
285 (encoded by *TCF7L2*) not only affects the metabolism of adipocytes by DNA
286 methylation, but also activates the corresponding target genes through the Wnt
287 signaling pathway to specifically inhibit glucagon synthesis in enteroendocrine cells
288 (Yi *et al.* 2005). *GALNT13* may be involved in metabolic and energy pathways
289 (Ahmetov and Fedotovskaya 2015).

290 Exercise has a great influence on the composition of the developing horse joints,

291 the thickness of the hyaline cartilage of the adult horse, the calcified cartilage and
292 subchondral bone (van de Lest *et al.* 2002; Tranquille *et al.* 2009). We found several
293 genes associated with skeleton and cartilage development among candidate genes of
294 Abaga horse. *PPP2R5B* and *PPP6R3* are closely related to femur strength in rats and
295 bone mineral density in humans, respectively (Alam *et al.* 2009; Medina-Gomez *et al.*
296 2017). *PTPRE* encodes receptor-type tyrosine-protein phosphatase epsilon which is a
297 positive regulator of osteoclast function (Chiusaroli *et al.* 2004). *RHOBTBI* is
298 involved in osteoclast-mediated bone absorption activity (Song *et al.* 2014).
299 Chondrogenesis demands transformation of chondrocytes from a simple mesenchymal
300 condensation to cells with a highly enriched extracellular matrix (ECM) in the
301 developing skeleton in which *SCFD1* plays an important role in the secretion of ECM
302 protein during chondrogenesis (DeLise *et al.* 2000; Hou *et al.* 2017). So far there are
303 no studies of association between these genes and the motor function of horses, but
304 these skeleton- and cartilage-related genes provide new inspiration into the
305 correlational research between ossature and exercise.

306 After exercise, the equine stress reaction will involve inflammation, cell
307 signaling, and immune interactions (Capomaccio *et al.* 2013). Cell activation is the
308 first step in the proliferation of immune cells, and CD69 is firstly detected in cell
309 surface glycoproteins after activation (Testi *et al.* 1989). The low- to
310 moderate-intensity aerobic trekking induces activation of CD69 T cell and promotes
311 anti-stress effects on the oxidative balance and the high-altitude-induced injury of the
312 immune responses among women (Morabito *et al.* 2016). *EIF4G3* encodes eukaryotic

313 translation initiation factor 4 gamma 3 which is indispensable for triggering protein
314 synthesis and is thought to be involved in exercise stress-induced response in horses
315 (Gradi *et al.* 1998; Cappelli *et al.* 2007). We hypothesized that these genes may be
316 involved in the ability of Abaga horses to enhance certain diseases resistance through
317 exercise, but more data and experiments are needed to verify.

318 *GRM1* encodes metabotropic glutamate receptor 1, which deficiency can lead to
319 serious deficits of motor coordination and spatial learning in mice (Conquet *et al.*
320 1994; Bossi *et al.* 2017). The effectors of Hippo signal pathway regulate several
321 motor-related genes and adaptations while *VGLL4* is Hippo-signal-related to body
322 height (Gabriel *et al.* 2016). These exercise-related genes were positively selected in
323 Abaga horse, indicating that Abaga horse has exercise-related genetic potential
324 compared with Wushen horse.

325 In conclusion, we analyzed the genomic data of Abaga horse and Wushen horse
326 by sequencing. We uncovered that most of the positively selected genes, particularly
327 exercise-related, of Abaga horse and Wushen horse were concentrated on
328 chromosome 4, which implied that the chromosome 4 may be associated with the
329 evolution of the Mongolian horse, and athletic performance may be the future
330 research direction. The positively selected genes of Abaga horse were enriched in
331 exercise-related pathways that were different from some selected genes of other
332 horses or species, suggesting that the Abaga horse may have exclusively physiological
333 mechanism for the motorial process. Twenty-one exercise-related genes were detected,
334 which provided molecular genetic basis for further research on athletic performance

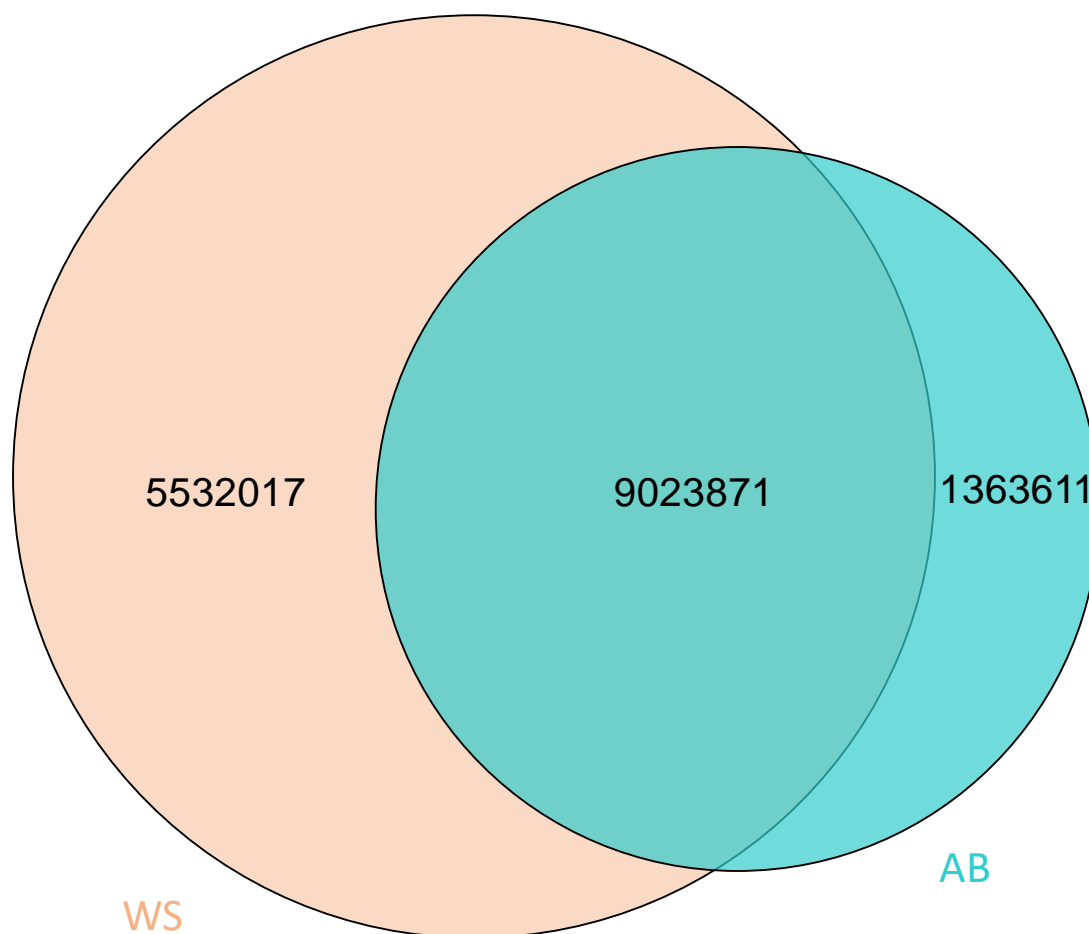
335 and breeding of Mongolian horse.

336 ACKNOWLEDGMENTS

337 We thank Y.R. Zhang, K.F. Wu, S.Y. Wang, Z.Z. Liu, W. Wei, P.W. Shi and C.H. Cao

338 for sampling assistance.

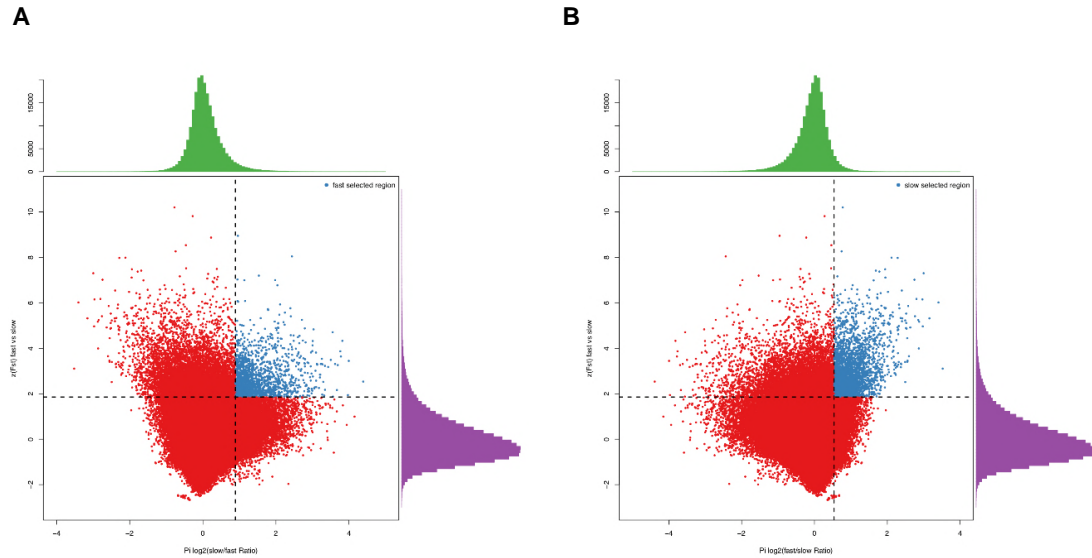
339 FIGURE LEGENDS



340

341 **Figure 1** SNP following SNP calling. High quality SNPs were evaluated and indentified. AB, WS

342 indicated Abaga horse and Wushen horse, respectively.



343

344 **Figure 2** Identification of selected regions in Abaga horse and Wushen horse. To identify potential

345 selective sweeps between Abaga horse (fast) and Wushen horse (slow), $\log_2(\pi_{\text{slow}}/\pi_{\text{fast}})$ and F_{ST} was

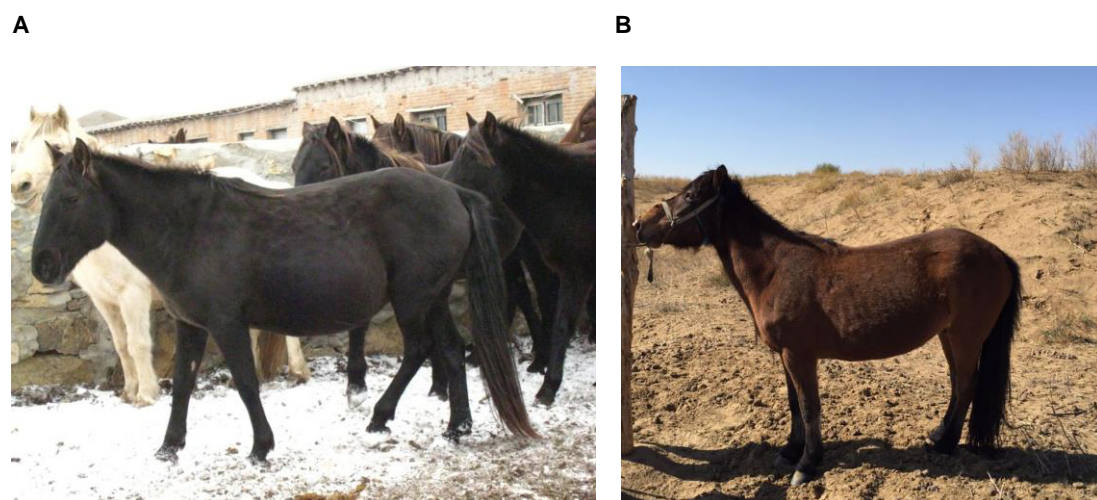
346 calculated together using VCFtools with a 20kb sliding window and a step size of 10 kb. Windows that

347 contained less than 10 SNPs were excluded from further analysis. The windows that were

348 simultaneously 1) in the top 5% of Z-transformed F_{ST} values and 2) in the bottom 5%

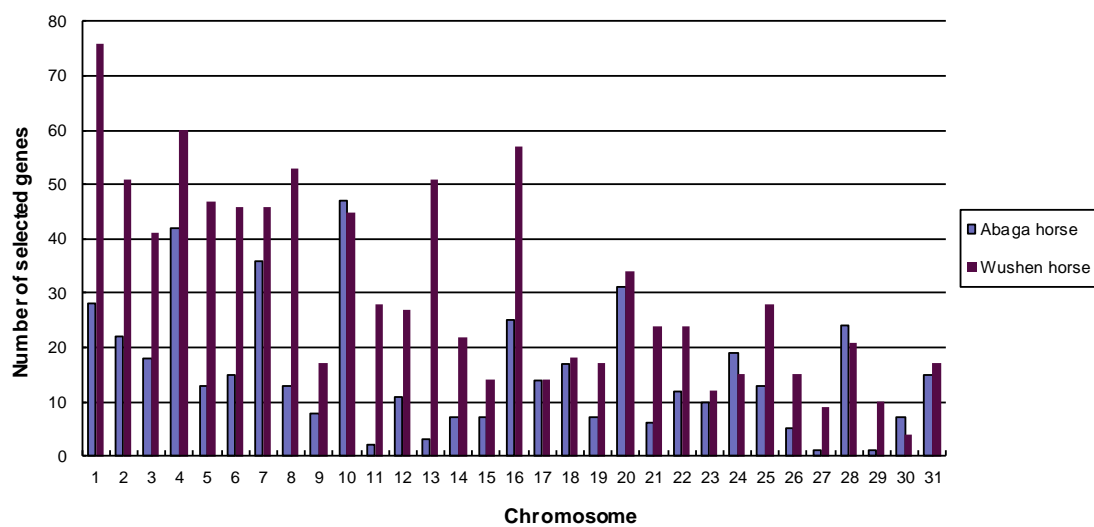
349 $\log_2(\pi_{\text{fast}}/\pi_{\text{slow}})$ were considered to be candidate selective regions in (A) Abaga horse and (B)

350 Wushen horse.



351

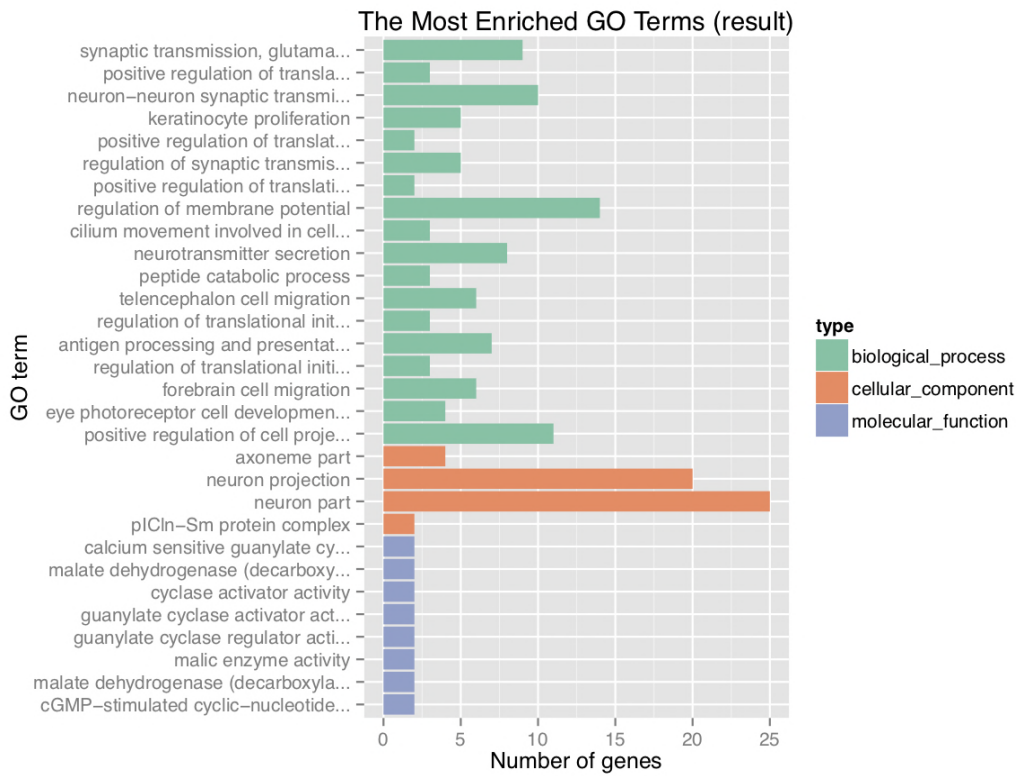
352 **Figure 3** Actual Photos of Mongolian horses, (A) Abaga horse and (B) Wushen horse.



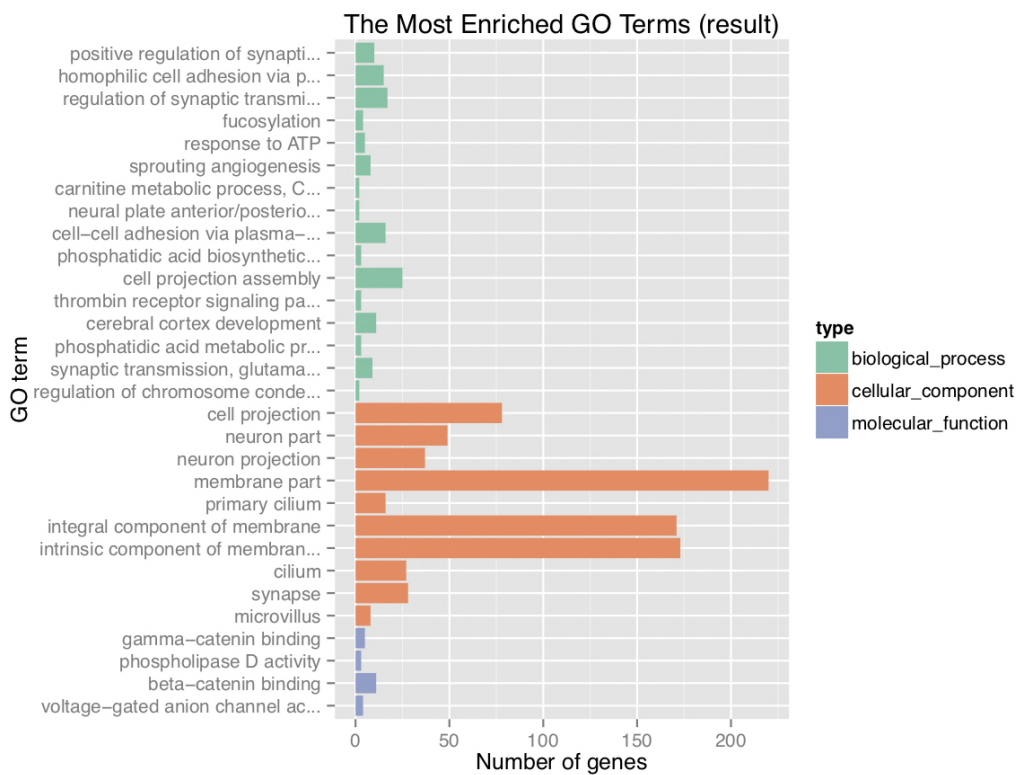
353

354 **Figure 4** Distribution of selected genes on chromosomes.

A



B

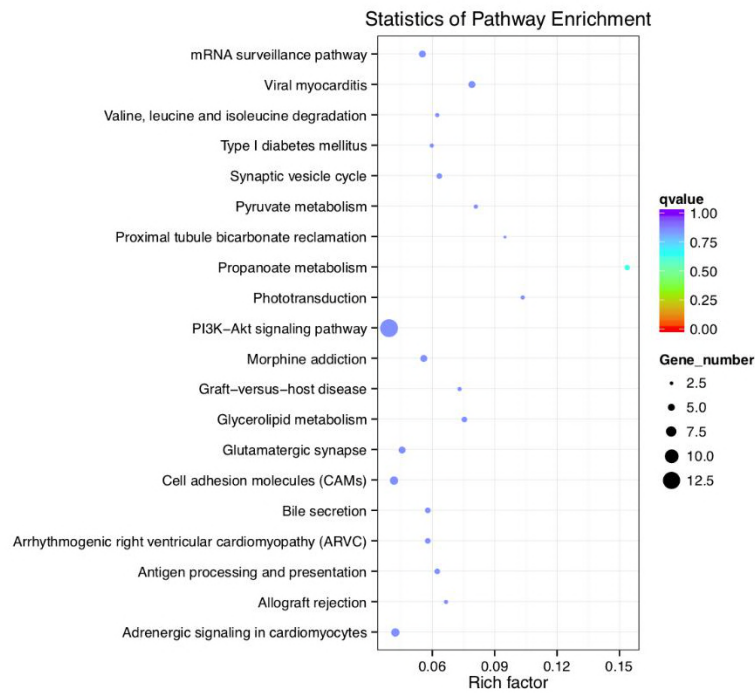


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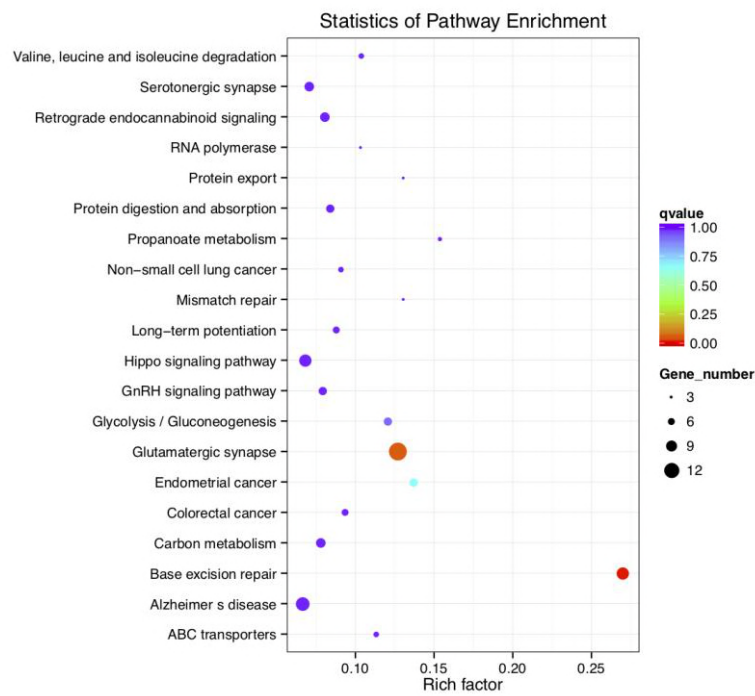
356 **Figure 5** Function analysis based on Gene Ontology. The most enriched GO terms in (A) Abaga horse

357 and (B) Wushen horse.

A



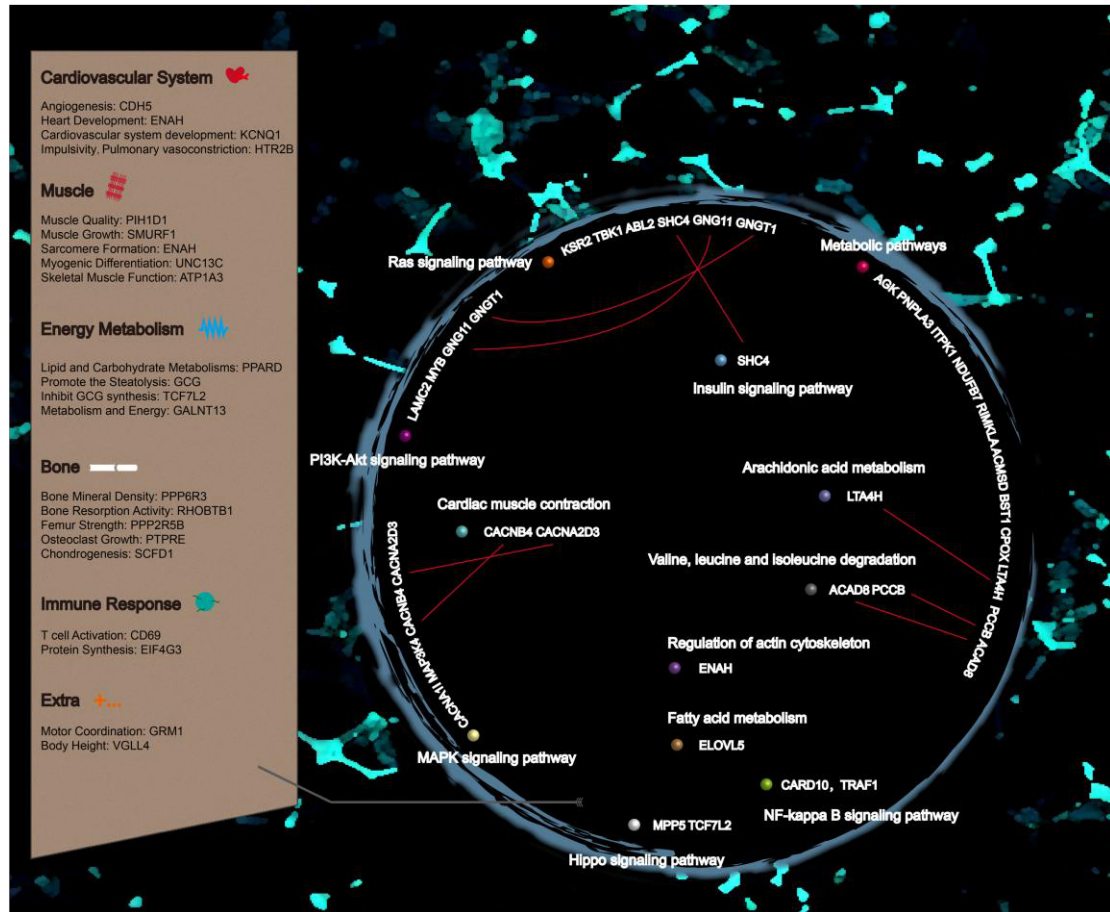
B



358

359 **Figure 6** The KEGG pathway enrichment analysis. Top 20 of enriched pathways by statistics in (A)

360 Abaga horse and (B) Wushen horse.



361

362 **Figure 7** The exercise-related candidate genes and pathways of Abaga horse.

363 **Tables**

364

Table 1 Data volume and quality

Sample	Sample Type	RawData	CleanData	EffectiveRat	ErrorRate	Q20	Q30	GC Content
AB01	Abaga Horse	48,216,007,500	47,152,467,900	97.79	0.03	96.21	92.24	42.89
AB02	Abaga Horse	43,047,445,800	42,340,444,800	98.36	0.03	96.17	92.1	42.65
AB03	Abaga Horse	47,273,694,300	46,714,855,800	98.82	0.03	95.76	91.36	42.55
AB04	Abaga Horse	48,416,284,200	47,906,212,800	98.95	0.03	95.96	91.47	42.13
WS01	Wushen Horse	49,415,626,200	48,784,894,800	98.72	0.03	95.69	91.23	41.97
WS02	Wushen Horse	53,074,008,900	52,417,962,000	98.76	0.03	96.09	92.19	42.35
WS03	Wushen Horse	45,358,642,200	44,789,835,900	98.75	0.03	95.87	91.47	41.97
WS04	Wushen Horse	47,681,665,800	47,139,254,400	98.86	0.03	95.94	91.6	41.66
WS05	Wushen Horse	50,647,742,700	50,012,300,700	98.75	0.03	95.86	91.47	41.84
WS06	Wushen Horse	45,072,226,800	44,633,740,200	99.03	0.03	95.79	91.61	42.19
WS07	Wushen Horse	34,681,049,100	34,040,596,500	98.15	0.05	92.2	84.65	43.07
WS08	Wushen Horse	45,212,322,600	44,350,535,700	98.09	0.04	92.41	84.97	43.02
WS09	Wushen Horse	44,382,563,100	43,593,247,800	98.22	0.04	92.47	85.07	43.23
WS10	Wushen Horse	37,244,331,900	36,659,027,100	98.43	0.04	92.91	85.77	43.12
Average		45,694,543,650	45,038,241,171		0.03	94.95	89.80	42.47
Total		639,723,611,100	630,535,376,400	98.56%				

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Table 2 Data comparison

Sample	Total Reads	Mapping Rate(%)	Average Depth	Coverage at least 1X(%)	Coverage at least 4X(%)	Coverage at least 10X(%)
AB01	315216417	98.55	17.46	99.59	99.32	93.57
AB02	282990429	98.49	15.82	99.56	99.24	89.18
AB03	312280472	98.49	16.93	99.52	98.98	90.17
AB04	320340217	98.22	18.25	99.57	99.15	93.1
WS01	326298749	98.6	17.63	99.54	99.25	94.36
WS02	350423462	98.41	19.65	99.57	99.24	94.72
WS03	299507485	98.63	16.45	99.51	99.16	92.2
WS04	315038236	98.52	17.28	99.51	98.98	91.77
WS05	334519623	98.65	18.19	99.54	99.24	95.43
WS06	298331035	98.52	16.88	99.53	99.23	92.89
WS07	227540381	97.88	12.85	99.56	98.73	68.52
WS08	296433759	97.97	16.75	99.61	99.32	90.53
WS09	291390679	98	16.44	99.51	99.18	88.97
WS10	245088985	98.13	13.85	99.6	99.05	76.55
Average	301099994.9	98.36142857	16.745	99.55142857	99.14785714	89.42571429

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Table 3 Single nucleotide polymorphism annotation

sample	total	intergenic	upstream	downstream	upstream;downstream	UTR	intronic	splicing	exonic	ncRNA intronic	ncRNA splicing	ncRNA exonic	
AB01	5941934	4312213	53776	48084	1290	5461	1470340	382	44314	0	58	6016	
AB02	5980256	4328671	54333	49203	1132	5508	1489605	372	45223	1	57	6151	
AB03	5997232	4335631	54186	48901	1150	5562	1499169	370	46143	1	54	6065	
AB04	6001989	4357097	53432	49324	1155	5565	1482717	371	46136	0	55	6137	
WS01	6064076	4396385	54982	49523	1205	5500	1504242	381	45597	0	58	6203	
WS02	6090471	4401130	55567	49597	1208	5739	1523751	398	46839	0	63	6179	
WS03	6020664	4356630	53850	48718	1233	5456	1503968	379	44501	0	52	5877	
WS04	6085300	4412074	54616	49554	1213	5585	1509129	366	46585	2	57	6119	
WS05	6068043	4390157	54591	48846	1243	5508	1516293	385	44908	2	52	6058	
WS06	6040564	4375737	54833	49476	1233	5552	1502532	394	44718	1	57	6031	
WS07	5768919	4180511	50791	46623	1152	5073	1436192	364	42507	1	54	5651	
WS08	6036395	4368807	53612	49117	1212	5410	1506878	378	44892	1	61	6027	
WS09	5541660	4011569	49104	45086	973	5031	1382618	358	41503	1	50	5367	
WS10	5952039	4316455	52643	47956	1120	5423	1478539	357	43679	0	48	5819	
sample	stopgain	stoploss	synonymous SNV	nonsynonymous SNV	non/sys	Het in exon heterozygous	Het rate in exon(%)	Ts	Tv	Ts/Tv	Het	Het_rate(%)	Freq(1kb)
AB01	188	16	23564	20497	0.87	28681	0.919	3881220	1930876	2.01	3984470	1.641	2.446
AB02	184	10	23969	21009	0.877	29228	0.936	3907370	1942215	2.012	4032261	1.66	2.462
AB03	206	9	24629	21248	0.863	29837	0.956	3918705	1947553	2.012	3964342	1.632	2.469
AB04	182	13	24558	21354	0.87	30205	0.967	3921646	1948790	2.012	3997852	1.646	2.471
WS01	189	16	24256	21104	0.87	28987	0.928	3965532	1966966	2.016	4014979	1.653	2.497
WS02	204	11	24947	21670	0.869	29835	0.956	3978988	1978429	2.011	3948266	1.626	2.508

WS03	166	11	23690	20583	0.869	27712	0.888	3938680	1952297	2.017	3928887	1.618	2.479
WS04	187	13	24873	21452	0.862	29601	0.948	3980030	1974155	2.016	3960924	1.631	2.506
WS05	181	11	23914	20778	0.869	28242	0.905	3969915	1967229	2.018	4023060	1.656	2.498
WS06	181	12	23838	20644	0.866	27812	0.891	3949609	1959971	2.015	3962792	1.632	2.487
WS07	168	13	22814	19503	0.855	27110	0.868	3777434	1866307	2.024	3850010	1.585	2.375
WS08	193	13	24013	20632	0.859	28452	0.911	3950543	1955509	2.02	4008283	1.65	2.485
WS09	155	10	22172	19118	0.862	21714	0.696	3624488	1796797	2.017	3022587	1.244	2.282
WS10	187	14	23361	20075	0.859	27460	0.88	3895339	1927308	2.021	3905744	1.608	2.451

371 **Table 4** Comparison of enriched genes in candidate pathways in Abaga horse and previous studies

KEGG pathway	ID	Selected genes in Abaga horse	Selected genes or proteins in previous studies
Metabolic pathways	ecb01100	<i>LTA4H AGK PNPLA3 ITPK1 NDUFB7 RIMKLA PCCB ACAD8 ACMSD BST1 CPOX</i>	<i>CYP51A1</i>
Ras signaling pathway	ecb04014	<i>SHC4 GNG11 GNGT1 KSR2 TBK1 ABL2</i>	<i>APOA1 IGF-1 HRAS</i>
PI3K-Akt signaling pathway	ecb04151	<i>LAMC2 GNG11 GNGT1 MYB</i>	PGC-1a IGF-1 IGF-1R ErbB2 ErbB4
MAPK signaling pathway	ecb04010	<i>CACNA11 CACNA2D3 CACNB4 MAP3K4</i>	ERK AP-1
Hippo signaling pathway	ecb04390	<i>MPP5 TCF7L2</i>	<i>WWTR1 LATS2 TEAD YAP1 VGLL2 VGLL3 VGLL4</i>
Cardiac muscle contraction	ecb04260	<i>CACNA2D3 CACNB4</i>	<i>CK-M</i>
NF-kappa B signaling pathway	ecb04064	<i>CARD10 TRAF1</i>	MnSOD iNOS
Arachidonic acid metabolism	ecb00590	<i>LTA4H</i>	<i>PTGS1</i>
Regulation of actin cytoskeleton	ecb04810	<i>ENAH</i>	<i>GSN BDKRB2 CHRM MYLK ACTN3</i>
Insulin signaling pathway	ecb04910	<i>SHC4</i>	<i>GYS1 PPARGC1A</i>
Fatty acid metabolism	ecb01212	<i>ELOVL5</i>	<i>ADHFE1 SREBP2</i>

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373 SUPPLEMENTAL MATERIALS

374 **Table S1** The genes of the selective regions in Abaga horse.

375 **Table S2** The genes of the selective regions in Wushen horse.

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377 **LITERATURE CITED**

378 Ahmetov I.I., Fedotovskaya O.N., 2015. Current Progress in Sports Genomics. Adv
379 Clin Chem. 70:247-314.

380 Article: The Horse in Mongolian Culture, 2018.
381 [https://www.amnh.org/explore/science-bulletins/bio/documentaries/the-last-wild-](https://www.amnh.org/explore/science-bulletins/bio/documentaries/the-last-wild-horse-the-return-of-takhi-to-mongolia/article-the-horse-in-mongolian-culture)
382 [horse-the-return-of-takhi-to-mongolia/article-the-horse-in-mongolian-culture.](https://www.amnh.org/explore/science-bulletins/bio/documentaries/the-last-wild-horse-the-return-of-takhi-to-mongolia/article-the-horse-in-mongolian-culture)
383 Accessed 10 Jan 2018.

384 Alam I., Sun Q., Koller D.L., Liu L., Liu Y., Edenberg H.J., Li J., Foroud T., Turner
385 C.H., 2009. Differentially expressed genes strongly correlated with femur
386 strength in rats. Genomics 94:257-62.

387 Aughey R.J., Murphy K.T., Clark S.A., Garnham A.P., Snow R.J., Cameron-Smith D.,
388 Hawley J.A., McKenna M.J., 2007. Muscle Na⁺-K⁺-ATPase activity and isoform
389 adaptations to intense interval exercise and training in well-trained athletes. J
390 Appl Physiol (1985). 103:39-47.

391 Benz P.M, Merkel C.J, Offner K., Abeßer M., Ullrich M., Fischer T., Bayer B.,
392 Wagner H., Gambaryan S., Ursitti J.A., Adham I.M., Linke W.A., Feller S.M.,
393 Fleming I., Renné T., Frantz S., Unger A., Schuh K., 2013. Mena/VASP and
394 α II-Spectrin complexes regulate cytoplasmic actin networks in cardiomyocytes
395 and protect from conduction abnormalities and dilated cardiomyopathy. Cell
396 Commun Signal. 11:56.

397 Bevilacqua L., Doly S., Kaprio J., Yuan Q., Tikkanen R., Paunio T., Zhou Z.,
398 Wedenoja J., Maroteaux L., Diaz S., Belmer A., Hodgkinson C.A., Dell'osso L.,

399 Suvisaari J., Coccaro E., Rose R.J., Peltonen L., Virkkunen M., Goldman D.,
400 2010. A population-specific *HTR2B* stop codon predisposes to severe impulsivity.
401 Nature. 468:1061-6.

402 Bossi S., Musante I., Bonfiglio T., Bonifacino T., Emionite L., Cerminara M.,
403 Cervetto C., Marcoli M., Bonanno G., Ravazzolo R., Pittaluga A., Puliti A., 2017.
404 Genetic inactivation of mGlu5 receptor improves motor coordination in the
405 *Grm1^{crv4}* mouse model of SCAR13 ataxia. Neurobiol Dis. 109:44-53.

406 Brashear A., Dobyens W.B., de Carvalho Aguiar P., Borg M., Frijns C.J., Gollamudi S.,
407 Green A., Guimaraes J., Haake B.C., Klein C., Linazasoro G., Münchau A.,
408 Raymond D., Riley D., Saunders-Pullman R., Tijssen M.A., Webb D., Zaremba
409 J., Bressman S.B., Ozelius L.J., 2007. The phenotypic spectrum of rapid-onset
410 dystonia-parkinsonism (RDP) and mutations in the *ATPIA3* gene. Brain.
411 130:828-35.

412 Broad Institute. Picard Tools - By Broad Institute.
413 <http://broadinstitute.github.io/picard>. Accessed 11 Jan 2018.

414 Brown W.M., 2015. Exercise-associated DNA methylation change in skeletal muscle
415 and the importance of imprinted genes: a bioinformatics meta-analysis. Br J
416 Sports Med. 49:1567-78.

417 Bryan A., Hutchison K.E., Seals D.R., Allen D.L., 2007. A transdisciplinary model
418 integrating genetic, physiological, and psychological correlates of voluntary
419 exercise. Health Psychol. 26:30-9.

420 Capomaccio S., Vitulo N., Verini-Supplizi A., Barcaccia G., Albiero A., D'Angelo M.,

- 421 Campagna D., Valle G., Felicetti M., Silvestrelli M., Cappelli K., 2013. RNA
422 sequencing of the exercise transcriptome in equine athletes. *PLoS One*.
423 8:e83504.
- 424 Cappelli K., Verini-Supplizi A., Capomaccio S., Silvestrelli M., 2007. Analysis of
425 peripheral blood mononuclear cells gene expression in endurance horses by
426 cDNA-AFLP technique. *Res Vet Sci*. 82:335-43.
- 427 China National Commission of Animal Genetic Resources., 2011. *Animal Genetic
428 Resources in China: Horse, Donkeys, Camels*. China Agriculture Press, Beijing,
429 pp. 28-37.
- 430 Chiusaroli R., Knobler H., Luxenburg C., Sanjay A., Granot-Attas S., Tiran Z.,
431 Miyazaki T., Harmelin A., Baron R., Elson A., 2004. Tyrosine phosphatase
432 epsilon is a positive regulator of osteoclast function in vitro and in vivo. *Mol
433 Biol Cell*. 15:234-44.
- 434 Conquet F., Bashir Z., Davies C.H., Daniel H., Ferraguti F., Bordi F., Franz-Bacon K.,
435 Reggiani A., Matarese V., Condé F., Collingridge G.L., Crépel F., 1994. Motor
436 deficit and impairment of synaptic plasticity in mice lacking mGluR1. *Nature*.
437 372:237-43.
- 438 Dalbo V.J., Roberts M.D., Hassell S., Kerksick C.M. 2013. Effects of pre-exercise
439 feeding on serum hormone concentrations and biomarkers of myostatin and
440 ubiquitin proteasome pathway activity. *Eur J Nutr*. 52:477-87.
- 441 Davis M., 2010. *When Things Get Dark: A Mongolian Winter's Tale*. St. Martin's
442 Press, NY, pp. 169.

- 443 DeLise A.M., Fischer L., Tuan R.S., 2000. C Cellular interactions and signaling in
444 cartilage development. *Osteoarthritis Cartilage*. 8:309-34.
- 445 Do K.T., Cho H.W., Badrinath N., Park J.W., Choi J.Y., Chung Y.H., Lee H.K., Song
446 K.D., Cho B.W., 2015. Molecular Characterization and Expression Analysis of
447 Creatine Kinase Muscle (*CK-M*) Gene in Horse. *Asian-Australas J Anim Sci*.
448 28:1680-5.
- 449 Doan R., Cohen N.D., Sawyer J., Ghaffari N., Johnson C.D., Dindot S.V., 2012.
450 Whole-genome sequencing and genetic variant analysis of a Quarter Horse mare.
451 *BMC Genomics*. 13:78.
- 452 Dugarjaviin M., 2009. *Horse in China*. Hong Kong Cultural/China Horstry Publishing
453 CO., Ltd, HK.
- 454 Elisabeth Y., 2011. *The Mongolian Horse and Horseman*. In: SIT Graduate
455 Institute/SIT Study Abroad, SIT Digital Collections.
456 [http://digitalcollections.sit.edu/cgi/viewcontent.cgi?article=2074&context=isp_c](http://digitalcollections.sit.edu/cgi/viewcontent.cgi?article=2074&context=isp_collection)
457 [ollection](http://digitalcollections.sit.edu/cgi/viewcontent.cgi?article=2074&context=isp_collection). Accessed 11 Jan 2018.
- 458 Franzini-Armstrong C., 1973. The structure of a simple Z line. *J Cell Biol*. 58:630-42.
- 459 Gabriel B.M., Hamilton D.L., Tremblay A.M., Wackerhage H., 2016. The Hippo
460 signal transduction network for exercise physiologists. *J Appl Physiol*. (1985)
461 120:1105-17.
- 462 Gim J.A., Ayarpadikannan S., Eo J., Kwon Y.J., Choi Y., Lee H.K., Park K.D., Yang
463 Y.M., Cho B.W., Kim H.S., 2014. Transcriptional expression changes of glucose
464 metabolism genes after exercise in thoroughbred horses. *Gene*. 547: 152-8.

- 465 Giordano Attianese G.M., Desvergne B., 2015. Integrative and systemic approaches
466 for evaluating PPAR β/δ (PPARD) function. *Nucl Recept Signal*. 13:e001.
- 467 Gradi A., Imataka H., Svitkin Y.V., Rom E., Raught B., Morino S., Sonenberg N.,
468 1998. A novel functional human eukaryotic translation initiation factor 4G. *Mol*
469 *Cell Biol*. 18:334-42.
- 470 Hill E.W., Gu J., McGivney B.A., MacHugh D.E., 2010. Targets of selection in the
471 Thoroughbred genome contain exercise-relevant gene SNPs associated with elite
472 racecourse performance. *Anim Genet* 41 Suppl. 2:56-63.
- 473 Hou N., Yang Y., Scott I.C., Lou X., 2017. The Sec domain protein Scfd1 facilitates
474 trafficking of ECM components during chondrogenesis. *Dev Biol*. 421:8-15.
- 475 Hund A. 2008. The Stallion's Mane The Next Generation of Horses in Mongolia.
476 *Parasite Immunology*. 20:73-80.
- 477 Inoue M., Saeki M., Egusa H., Niwa H., Kamisaki Y., 2010. PIH1D1, a subunit of
478 R2TP complex, inhibits doxorubicin-induced apoptosis. *Biochem Biophys Res*
479 *Commun*. 403:340-4.
- 480 Jespersen T., Grunnet M., Olesen S.P., 2005. The KCNQ1 Potassium Channel: From
481 Gene to Physiological Function. *Physiology (Bethesda)*. 20:408-16.
- 482 Kamm J.L., Frisbie D.D., McIlwraith C.W., Orr K.E., 2013. Gene biomarkers in
483 peripheral white blood cells of horses with experimentally induced osteoarthritis.
484 *Am J Vet Res*. 74:115-21.
- 485 Kramer H.F., Goodyear L.J., 2007. Exercise, MAPK, and NF-kappaB signaling in
486 skeletal muscle. *J Appl Physiol* (1985). 103:388-95.

- 487 Kulikova M.A., Maliuchenko N.V., Timofeeva M.A., Shleptsova V.A., Tschegol'kova
488 IuA., VEDIKOV A.M., TONEVITSKIĀ A.G., 2007. Polymorphisms of the main genes
489 of neurotransmitter systems: I. the dopaminergic system. *Fiziol Cheloveka*.
490 33:105-12.
- 491 Langlois S., Cowan K.N., 2017. Regulation of Skeletal Muscle Myoblast
492 Differentiation and Proliferation by Pannexins. *Adv Exp Med Biol*. 925:57-73.
- 493 Launay J.M., Hervé P., Peoc'h K., Tournois C., Callebert J., Nebigil C.G., Etienne N.,
494 Drouet L., Humbert M., Simonneau G., Maroteaux L., 2002. Function of the
495 serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. *Nat Med*.
496 8:1129-35.
- 497 Li H., 2011. A statistical framework for SNP calling, mutation discovery, association
498 mapping and population genetical parameter estimation from sequencing data.
499 *Bioinformatics*. 27:2987-93.
- 500 Li H., 2013. Aligning sequence reads, clone sequences and assembly contigs with
501 BWA-MEM. <https://arxiv.org/abs/1303.3997>. Accessed 26 Dec 2016.
- 502 Li L.F., Guan W.J., Hua Y., Bai X.J., Ma Y.H., 2009. Establishment and
503 characterization of a fibroblast cell line from the Mongolian horse. *In Vitro Cell*
504 *Dev Biol Anim*. 45:311-6.
- 505 Lippi G., Longo UG., Maffulli N., 2010, Genetics and sports. *Br Med Bull*, 93:27-47,
- 506 Liu S.D., Zhang Y.Q., Cao J., 2016. The influence of the aerobic endurance training
507 on the skeletal muscular mitochondria function and PI3K-Akt protein expression.
508 *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 32:55-8.

- 509 Luo J., McMullen J.R., Sobkiw C.L., Zhang L., Dorfman A.L., Sherwood M.C.,
510 Logsdon M.N., Horner J.W., DePinho R.A., Izumo S., Cantley L.C., 2005. Class
511 I_A Phosphoinositide 3-Kinase Regulates Heart Size and Physiological Cardiac
512 Hypertrophy. *Mol Cell Biol.* 25:9491-502.
- 513 McGivney B.A., McGettigan P.A., Browne J.A., Evans A.C., Fonseca R.G., Loftus
514 B.J., Lohan A., MacHugh D.E., Murphy B.A., Katz L.M., Hill E.W., 2010.
515 Characterization of the equine skeletal muscle transcriptome identifies novel
516 functional responses to exercise training. *BMC Genomics.* 11:398.
- 517 McKenna A., Hanna M., Banks E., Sivachenko A., Cibulskis K., Kernytzky A.,
518 Garimella K., Altshuler D., Gabriel S., Daly M., DePristo M.A., 2010. The
519 Genome Analysis Toolkit: a MapReduce framework for analyzing
520 next-generation DNA sequencing data. *Genome Res.* 20:1297-303.
- 521 Medina-Gomez C., Kemp J.P., Dimou N.L., Kreiner E., Chesi A., Zemel B.S.,
522 Bønnelykke K., Boer C.G., Ahluwalia T.S., Bisgaard H., Evangelou E., Heppe
523 D.H.M., Bonewald L.F., Gorski J.P., Ghanbari M., Demissie S., Duque G.,
524 Maurano M.T., Kiel D.P., Hsu Y.H., C. J. van der Eerden B., Ackert-Bicknell C.,
525 Reppe S., Gautvik K.M., Raastad T., Karasik D., van de Peppel J., Jaddoe V.W.V.,
526 Uitterlinden A.G., Tobias J.H., Grant S.F.A., Bagos P.G., Evans D.M.,
527 Rivadeneira F., 2017. Bivariate genome-wide association meta-analysis of
528 pediatric musculoskeletal traits reveals pleiotropic effects at the
529 *SREBF1/TOMIL2* locus. *Nat Commun.* 8:121.
- 530 Meira C.T., Curi R.A., Farah M.M., de Oliveira H.N., Béltran N.A., Silva J.A. 2nd.,

- 531 2014. Prospection of genomic regions divergently selected in racing line of
532 Quarter Horses in relation to cutting line. *Animal*. 8:1754-64.
- 533 Meyer S.U., Krebs S., Thirion C., Blum H., Krause S., Pfaffl M.W., 2015. Tumor
534 Necrosis Factor Alpha and Insulin-Like Growth Factor 1 Induced Modifications
535 of the Gene Expression Kinetics of Differentiating Skeletal Muscle Cells. *PLoS*
536 *One*. 10:e0139520.
- 537 Momozawa Y., Takeuchi Y., Kusunose R., Kikusui T., Mori Y., 2005. Association
538 between equine temperament and polymorphisms in dopamine D4 receptor gene.
539 *Mamm Genome*. 16:538-44.
- 540 Morabito C., Lanuti P., Caprara G.A., Guarnieri S., Verratti V., Ricci G., Catizone A.,
541 Marchisio M., Fanò-Illic G., Marigliò M.A., 2016. Responses of peripheral
542 blood mononuclear cells to moderate exercise and hypoxia. *Scand J Med Sci*
543 *Sports*. 26:1188-99.
- 544 Park K.D., Park J., Ko J., Kim B.C., Kim H.S., Ahn K., Do K.T., Choi H., Kim H.M.,
545 Song S., Lee S., Jho S., Kong H.S., Yang Y.M., Jhun B.H., Kim C., Kim T.H.,
546 Hwang S., Bhak J., Lee H.K., Cho B.W., 2012. Whole transcriptome analyses of
547 six thoroughbred horses before and after exercise using RNA-Seq. *BMC*
548 *Genomics*. 13:473.
- 549 Pedersen P.J., Thomsen K.B., Flak J.B., Tejada M.A., Hauser F., Trachsel D., Buhl R.,
550 Kalbfleisch T., DePriest M.S., MacLeod J.N., Calloe K., Klaerke D.A., 2017.
551 Molecular cloning and functional expression of the K⁺ channel Kv7.1 and the
552 regulatory subunit KCNE1 from equine myocardium. *Res Vet Sci*. 113:79-86.

- 553 Ponsuksili S., Murani E., Trakooljul N., Schwerin M., Wimmers K., 2014. Discovery
554 of candidate genes for muscle traits based on GWAS supported by
555 eQTL-analysis. *Int J Biol Sci.* 10:327-37.
- 556 Ropka-Molik K., Stefaniuk-Szmukier M., Z Ukowski K., Piórkowska K.,
557 Bugno-Poniewierska M., 2017. Exercise-induced modification of the skeletal
558 muscle transcriptome in Arabian horses. *Physiol Genomics.* 49:318-326.
- 559 Sagara S., Osanai T., Itoh T., Izumiyama K., Shibutani S., Hanada K., Yokoyama H.,
560 Yamamoto Y., Yokota T., Tomita H., Magota K., Okumura K., 2012.
561 Overexpression of coupling factor 6 attenuates exercise-induced physiological
562 cardiac hypertrophy by inhibiting PI3K/Akt signaling in mice. *J Hypertens.*
563 30:778-86.
- 564 Sauter L., Krudewig A., Herwig L., Ehrenfeuchter N., Lenard A., Affolter M.,
565 Belting H.G., 2014. Cdh5/VE-cadherin promotes endothelial cell interface
566 elongation via cortical actin polymerization during angiogenic sprouting. *Cell*
567 *Rep.* 9:504-13.
- 568 Schröder W., Klostermann A., Distl O., 2011. Candidate genes for physical
569 performance in the horse. *Vet J.* 190:39-48.
- 570 Shioi T., McMullen J.R., Kang P.M., Douglas P.S., Obata T., Franke T.F., Cantley L.C.,
571 Izumo S., 2002. Akt/protein kinase B promotes organ growth in transgenic mice.
572 *Mol Cell Biol.* 22:2799-809.
- 573 Song H.K., Kim J., Lee J.S., Nho K.J., Jeong H.C., Kim J., Ahn Y., Park W.J., Kim
574 D.H., 2015. Pik3ip1 modulates cardiac hypertrophy by inhibiting PI3K pathway.

- 575 PLoS One. 10:e0122251.
- 576 Song R., Gu J., Liu X., Zhu J., Wang Q., Gao Q., Zhang J., Cheng L., Tong X., Qi X.,
577 Yuan Y., Liu Z., 2014. Inhibition of osteoclast bone resorption activity through
578 osteoprotegerin-induced damage of the sealing zone. *Int J Mol Med.* 34:856-62.
- 579 Standard J., Jiang Y., Yu M., Su X., Zhao Z., Xu J., Chen J., King B., Lu L., Tomich J.,
580 Baybutt R., Wang W., 2014. Reduced signaling of PI3K-Akt and RAS-MAPK
581 pathways are the key targets for weight loss-induced cancer prevention by
582 dietary calorie restriction and/or physical activity. *J Nutr Biochem.* 25:
583 1317–1323.
- 584 Testi R., Phillips J.H., Lanier L.L., 1989. Leu 23 induction as an early marker of
585 functional CD3/T cell antigen receptor triggering. Requirement for receptor
586 cross-linking, prolonged elevation of intracellular [Ca⁺⁺] and stimulation of
587 protein kinase C. *J Immunol.* 142:1854-60.
- 588 Tranquille C.A., Blunden A.S., Dyson S.J., Parkin T.D., Goodship A.E., Murray R.C.,
589 2009. Effect of exercise on thicknesses of mature hyaline cartilage, calcified
590 cartilage, and subchondral bone of equine tarsi. *Am J Vet Res.* 70:1477-83.
- 591 van de Lest C.H., Brama P.A., Van Weeren P.R., 2002. The influence of exercise on
592 the composition of developing equine joints. *Biorheology.* 39:183-91.
- 593 Vega R.B., Konhilas J.P., Kelly D.P., Leinwand L.A., 2017. Molecular Mechanisms
594 Underlying Cardiac Adaptation to Exercise. *Cell Metab.* 25:1012-1026.
- 595 Wang K., Li M., Hakonarson H., 2010. ANNOVAR: functional annotation of genetic
596 variants from high-throughput sequencing data. *Nucleic Acids Res.* 38:e164.

- 597 Xie C., Mao X., Huang J., Ding Y., Wu J., Dong S., Kong L., Gao G., Li C.Y., Wei L.,
598 2011. KOBAS 2.0: a web server for annotation and identification of enriched
599 pathways and diseases. *Nucleic Acids Res.* 39:W316-22.
- 600 Xie L., Jiang Y., Ouyang P., Chen J., Doan H., Herndon B., Sylvester J.E., Zhang K.,
601 Molteni A., Reichle M., Zhang R., Haub M.D., Baybutt R.C., Wang W., 2007.
602 Effects of dietary calorie restriction or exercise on the PI3K and Ras signaling
603 pathways in the skin of mice. *J Biol Chem.* 282:28025-35.
- 604 Yi F., Brubaker P.L., Jin T., 2005. TCF-4 Mediates Cell Type-specific Regulation of
605 Proglucagon Gene Expression by β -Catenin and Glycogen Synthase Kinase-3 β . *J*
606 *Biol Chem.* 280:1457-64.
- 607 Young M.D., Wakefield M.J., Smyth G.K., Oshlack A., 2010. Gene ontology analysis
608 for RNA-seq: accounting for selection bias. *Genome Biol.* 11:R14.