The Japanese wolf is most closely related to modern dogs and its ancestral genome has been widely inherited by dogs throughout East Eurasia

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Abstract

The Japanese wolf (*Canis lupus hodophilax* Temminck, 1839) was a subspecies of the gray wolf that inhabited the Japanese Archipelago and became extinct 100-120 years ago. In this study, we determined the whole genomes of nine Japanese wolves from the 19th- early 20th centuries and 11 Japanese dogs and analyzed them along with both modern and ancient wolves and dogs. Genomic analyses indicate that the Japanese wolf was a unique subspecies of the gray wolf that was genetically distinct from both modern and ancient gray wolves, lacking gene flow with other gray wolves. A Phylogenetic tree that minimizes the effects of introgression shows that Japanese wolves are closest to the dog monophyletic group among the gray wolves. Moreover, Japanese wolves show significant genetic affinities with East Eurasian dogs. We estimated the level of introgression from the ancestor of the Japanese wolves to the ancestor of East Eurasian dogs that had occurred in the transitional period from the Pleistocene to the Holocene, at an early stage after divergence from West Eurasian dog lineages. Because of this introgression, Japanese wolf ancestry has been inherited by many dogs through admixture between East Eurasian dog lineages. As a result of this heredity, up to 5.5% of modern dog genomes throughout East Eurasia are derived from Japanese wolf ancestry.

Introduction

The phylogeny of gray wolves (*Canis lupus*) attracts wide attention from researchers and the public because wolves are the closest relatives to one of the most familiar animal species to humans, i.e., dogs. The extant gray wolves (*Canis lupus*) are divided into three lineages: the North American, Eurasian, and domestic dog lineages, including several now-extinct lineages that inhabited Eurasia during the Pleistocene (Ramos-Madrigal, et al. 2021). Recent phylogenomic analyses of gray wolves have shown that the North American gray wolf diverged at the basal ancestral position, followed by the Eurasian lineage (Fan, et al. 2016; Leathlobhair, et al. 2018). Dogs form a monophyletic clade which is the sister group to the Eurasian lineage of the gray wolf (Freedman, et al. 2014; Fan, et al. 2016; Leathlobhair, et al. 2018). Therefore, the hypothesis that the dog lineages have originated in Eurasia has been widely accepted. But there is still much debate concerning when, where, how many times, and from which population, the ancestor of dogs was domesticated (Leonard, et al. 2002; Savolainen, et al. 2002; Germonpré, et al. 2009; Pang, et al. 2009; Vonholdt, et al. 2010; Larson, et al. 2012; Axelsson, et al. 2013; Thalmann, et al. 2013; Freedman, et al. 2014; Perri 2016; Janssens, et al. 2018; Leathlobhair, et al. 2018; Perri, et al. 2021). Because no extant population of gray wolves has been reported to be more closely related to dogs than the other wolf populations, it is believed that the dog lineage has been domesticated from an extinct population of gray wolves (Larson, et al. 2012; Thalmann, et al. 2013; Freedman, et al. 2014; Skoglund, et al. 2015; Fan, et al. 2016; Frantz, et al. 2016). However, no information is available about this extinct population.

Many regions in Eurasia, including southern East Asia (Savolainen, et al. 2002; Pang, et al. 2009; Wang, et al. 2013; Wang, et al. 2016), Middle East (Vonholdt, et al. 2010), Central Asia

(Shannon, et al. 2015), Europe (Thalmann, et al. 2013), and both West and East Eurasia (dual origin) (Frantz, et al. 2016), have been proposed as candidates for the origin of dogs, but the debate on the origin (single, or dual) as well as the timing of domestication still continues. Divergence between the Eurasian gray wolf and dog lineages has been estimated to be 20,000-40,000 years ago (Skoglund, et al. 2015; Botigué, et al. 2017). Based on phylogenomic analyses, dogs were initially reported to be genetically divided into two distinct lineages, i.e., the West and East Eurasian lineages (Freedman, et al. 2014; Shannon, et al. 2015; Frantz, et al. 2016; Botigué, et al. 2017; Leathlobhair, et al. 2018). Subsequent reports suggested an ancient divergence of the Arctic sled dog lineage (Sinding, et al. 2020), which is closely related to the pre-contact American dogs (Leathlobhair, et al. 2018). The phylogenetic relationship between the Arctic sled dog lineage and the West and East Eurasian lineages is conflicting (Larson, et al. 2012; Frantz, et al. 2016; Wang, et al. 2016), and these inconsistent topologies can be explained by either a high degree of admixture after the divergence of the three lineages, or by nearly simultaneous divergence (Frantz, et al. 2016; Zhang, et al. 2020). The West Eurasian and East Eurasian lineages diverged 17,000-24,000 years ago (Botigué, et al. 2017), and the Arctic sled dog lineage is estimated to have existed at least 9500 years ago (Sinding, et al. 2020).

Studies have suggested that wolf populations in Europe (Thalmann, et al. 2013), the Middle East (Vonholdt, et al. 2010), Central Asia (Shannon, et al. 2015), Siberia (Sinding, et al. 2020; Ramos-Madrigal, et al. 2021), and East Asia (Savolainen, et al. 2002; Wang, et al. 2016) have undergone introgression or bidirectional gene flow (Freedman, et al. 2014) with dogs. However, genomic introgression from gray wolves to dogs has been considered to be limited due to the presence of Eurasian wolves that do not show genetic affinity to any dog breed (Bergström, et al. 2020).

81 The Japanese wolf (*Canis lupus hodophilax* Temminck, 1839) was a subspecies of the gray wolf
82 that inhabited Honshu, Shikoku, and Kyushu Islands in the Japanese Archipelago and became
83 extinct 100-120 years ago (Ishiguro, et al. 2009). Molecular phylogenetic analysis of the
84 mitochondrial genome suggests that the Japanese wolf diverged at the basal position of the extant
85 gray wolf clade (Matsumura, et al. 2014; Matsumura, et al. 2020). Recent genome analysis of a
86 "Honshu wolf" (one of the common names for the Japanese wolf) from the collection of the
87 British Museum suggests that this individual is closely related to a lineage of Siberian wolves that
88 existed in the Late Pleistocene and shows significant gene flow with Japanese dogs (Niemann, et
89 al. 2021).
90 In this study, the genomes of nine Japanese wolves, including the type specimens, and 11
91 Japanese dogs were newly determined and analyzed. The analyses showed that 1) the Japanese
92 wolf was a unique subspecies of the gray wolf that is genetically distinct from both extant and
93 ancient gray wolves known to date, 2) the Japanese wolf is most closely related to a monophyletic
94 group of dogs, and 3) Japanese wolf ancestry has introgressed into the ancestor of East Eurasian
95 dogs at an early stage of their history after diverging from the West Eurasian lineages, and the
96 genome derived from Japanese wolf ancestry has been inherited by many modern dogs, even in
97 the West Eurasian lineages, through their historical admixture with East Eurasian lineages.
98
99 **Results**
100 **Relationships between Japanese wolves and other dogs and gray wolves**
101 In this study, original genomic DNA sequences of nine Japanese wolves (22-282 Gb) and 12
102 Japanese dogs (70-127 Gb) were determined (Table S1, Fig. S1). For the present study, we treated

103 nine individuals with Japanese wolf type mitochondrial DNA haplotypes (Matsumura, et al. 2020)
104 as the Japanese wolf. In addition, we used sequencing data of modern gray wolves with depth of
105 coverage >20x in the database, ancient canids with relatively high coverage, and outgroup species
106 from the database (Table S2). All sequence data were mapped to the reference genome sequence
107 (CanFam3.1). After haplotype calling and gvcf file merging, single nucleotide polymorphisms
108 (SNPs) were genotyped. To examine the genetic relationship among the individuals used in this
109 study, a principal component analysis (PCA) was performed using individuals with high coverage
110 (Fig. 1A). In the PCA, Japanese wolves formed a distinct cluster, suggesting that Japanese wolves
111 were genetically separated from dogs, gray wolves, and any of the outgroup species. The gray
112 wolves were clustered into two groups, i.e., a North American/Arctic group and a Eurasian group
113 along the PC1 axis. Dogs show an East-West cline along the PC2 axis. Dingoes and New Guinea
114 singing dogs (NGSD) were the closest to Japanese wolves among dogs along the PC2 axis,
115 followed by a cluster of Japanese dogs (Fig. 1A). Using the same data set we also generated an
116 ADMIXTURE result with the lowest CV error at K=4 (Fig. 1B). In this analysis, Japanese wolves
117 also formed clusters with higher K such as K=5 or K=6, indicating that their genetic composition
118 was unique compared with that of the other dogs and gray wolves (Fig. 1B and S2).
119 Next, we added the three Japanese wolf individuals with low coverage [Leiden b and c, (Jentink
120 1887), and a "Honshu wolf" (Niemann, et al. 2021), see Table S2] into the analysis. PCA showed
121 that Leiden b and Honshu wolf were very close to the Japanese wolf cluster, while Leiden c was
122 placed at an intermediate position between dogs and Japanese wolves (Fig. S3). ADMIXTURE
123 analysis showed that Leiden b and Honshu wolf exhibit the same ancestry pattern as the other
124 Japanese wolf individuals, while Leiden c seemed to be admixed with other dogs (Fig. S4). We

125 used Patterson's *f*4 statistic (Patterson, et al. 2012) to identify dog individuals with high genetic
126 affinity to Leiden c to see which dog population was the source of the introgression. The dog that
127 showed the highest affinity to Leiden c was the Japanese dog Shiba (Fig. S5A), and Leiden c
128 contained 39% of the Shiba's genome (Fig. S5B). In contrast, Leiden b showed no affinity with
129 dogs (Fig. S5C). These results indicate that Leiden b and Honshu wolf are included in the group
130 of Japanese wolves with a unique genetic composition, while Leiden c is a hybrid individual
131 between Japanese wolves and dogs. Subsequently, we removed Leiden b, c and Honshu wolf
132 individuals from further analyses due to their low coverage.
133 A previous study (Niemann, et al. 2021) suggested that Honshu wolf has a close relationship
134 with a lineage of Siberian wolves that existed in the Late Pleistocene and was likely to be admixed
135 with dogs. Therefore, we added the Pleistocene wolves to our dataset which are comprised of the
136 modern gray wolves and dogs and performed PCA (Fig. S6). Pleistocene wolves were placed
137 closely related to Eurasian wolves, while Japanese wolves formed a distinct cluster. Our
138 ADMIXTURE analysis (Fig. S4) suggests that Honshu wolf does not contain more DNA
139 components of dogs than the other Japanese wolf individuals. The differences between the results
140 of Niemann et al. 2020 and this study could be caused by differences in the number of Japanese
141 wolves used in the analyses and/or in the coverage of DNA sequencing.
142
143 **Phylogenetic position of the Japanese wolves**
144 To determine the phylogenetic position of the Japanese wolves, a phylogenetic tree was
145 constructed using the maximum likelihood (ML) method (Fig, 2A). Among gray wolves, North
146 American/Arctic individuals branched off first at the basal position of the tree, followed by

147 European/Middle Eastern and East Asian gray wolves (also see Fig. S7). Dogs formed a
148 monophyletic clade including East Eurasian, West Eurasian, and sled dog lineages (Fig. 2A, S7),
149 as shown in previous studies (Freedman, et al. 2014; Shannon, et al. 2015; Frantz, et al. 2016;
150 Botigué, et al. 2017; Leathlobhair, et al. 2018; Sinding, et al. 2020). Japanese wolves formed a
151 monophyletic clade that was a sister group to the monophyletic clade of dogs (Fig. 2A, S7). The
152 sister group relationship between Japanese wolves and dogs was also supported by a tree inferred
153 by SVDQuartets and a Neighbor-Joining tree based on identity-by-state (IBS) distance (Figs. S8,
154 S9). Analysis using outgroup-*f3* statistics (Patterson, et al. 2012) also showed that the Japanese
155 wolf was the most closely related to dogs among wolves (Fig. 2B). When we further divide the
156 dogs into subpopulations, outgroup-*f3* statistics showed different results between dingo/NGSD
157 and African dogs; dingo/NGSD was related most closely to Japanese wolf while African dog is
158 related most closely to the Middle Eastern gray wolves (Fig. S10A and S10B). The different
159 genetic affinities of dog populations to the Japanese wolf may have resulted from introgression
160 between African dogs and Middle Eastern gray wolves (Vonholdt, et al. 2010; Bergström, et al.
161 2020).

162 Since the tree topology in phylogenetic analyses could be affected by introgression between taxa,
163 a phylogenetic tree using taxa showing minimal introgression effects is expected to be the most
164 accurate representation of population branching. Therefore, in order to obtain such a tree, we
165 examined introgression between Japanese wolves and other dogs and wolves. We compared the
166 genetic affinity of each dog with the Japanese wolves using *f3* and *f4* statistics, and found that
167 dogs of the East Eurasian lineage (Fig. S7), in particular, dingoes, NGSDs, and Japanese dogs,
168 showed significant affinity with the Japanese wolves (Z score > 3) (Fig. 2C, S11). In contrast,

169 dogs of the west Eurasian lineage, in particular dogs from Africa, showed low affinity to Japanese
170 wolves (Fig. 2C, S11). *f4* statistics showed no affinity between any of the gray wolf populations
171 and Japanese wolves (Fig. 2D). Possibilities of gene flow between gray wolves except for the
172 Japanese wolf and dogs were also examined using *f4* statistics. Gray wolves in the Middle East
173 showed strong affinity with dogs (Fig. S12), consistent with previous reports (Vonholdt, et al.
174 2010; Bergström, et al. 2020). Based on these results, we reperformed phylogenetic analysis to
175 confirm the relationship between Japanese wolves and dogs. To minimize the effect of
176 introgression between wolves and dogs, we included African dogs as the sole representatives of
177 dogs, and excluded gray wolves from the Middle East. Even in the phylogenetic tree obtained
178 from this analysis, the Japanese wolf still formed a sister clade with African dogs (Fig. S13). Thus,
179 we concluded that the most closely related wolves to the dog lineage are the Japanese wolves.
180

181 **The genome of the Japanese wolf ancestor in the dog genome**

182 Japanese wolves showed strong affinity with many East Eurasian dogs (*f3*, *f4* statistics) (Figs. 2C,
183 S11), which may be caused by the introgression of dog genomes into Japanese wolves (Japanese
184 wolf or its ancestors, hereafter simply referred to as the "the Japanese wolf genome") or vice versa.
185 A recent report suggested widespread gene flow from dogs to gray wolves, but little gene flow
186 from gray wolves to dogs, based on the existence of gray wolves that have no affinity with dogs
187 (Bergström, et al. 2020). We investigated the direction of gene flow between Japanese wolves and
188 East Eurasian dogs using the *f4*-ratio (Patterson, et al. 2012). We found that the degree of genome
189 introgression from the Japanese wolf lineage to dogs was the highest in dingoes and NGSDs
190 (5.5%) followed by Japanese dogs (3-4%), as well as in dogs of other East Eurasian lineages (Fig.

191 3A). In contrast, genomic introgression from dogs to the Japanese wolf genome was not supported
192 (Fig. S14). We further analyzed the possibility of a small proportion of genomic introgression
193 from dogs to the Japanese wolf genome by the *f4* statistics. If the dog genome is introgressed into
194 the Japanese wolf genome, the genetic affinities between the Japanese wolf and dogs would be
195 different between individuals. Indeed, the degree of affinity of Japanese wolves with
196 dongo/NGSD varied among individuals (Fig. S15) and Jw258, Jw269, and Jw271 showed
197 significant affinity to Japanese dogs (Fig. S16). This difference in affinity suggests that the
198 Japanese wolf genome contains a small proportion of the dog genome that is undetectable by *f*4-
199 ratio.

200 The degree of genomic introgression from Japanese wolves to dogs was higher in East Eurasian
201 than in West Eurasian dogs. It also varied among the dogs of East Eurasia. This variation may
202 have been caused by multiple introgression events between the ancestors of Japanese wolves and
203 dogs in different regions, or by a single introgression followed by diffusion of the Japanese wolf
204 genome into various dog populations. To determine which hypothesis is more likely, we first
205 examined the degree of gene flow among dogs in different regions. African dogs and dingo/NGSD
206 represent opposite edges of the dog cluster in the PCA (Fig. 1A), and show the lowest and highest
207 affinities with Japanese wolves, respectively (Fig. 2C). Among dogs, African dogs show the
208 lowest affinity with dingo/NGSD, while dingo/NGSDs show the lowest affinity with African dogs
209 (Fig. S17, S18). Dingoes are estimated to have been introduced to Australia between 4600 and
210 18300 years ago based on their mitochondrial DNA (Oskarsson, et al. 2012), with archaeological
211 evidence supporting the arrival of at least 3500 years ago (Milham and Thompson 1976). It is
212 considered that the dingoes have been isolated in Australia since then (Larson, et al. 2012; Fan,

213 et al. 2016). African dogs are estimated to have migrated to Africa 14,000 years ago (Liu, et al.
214 2018) and have been isolated since then (Larson, et al. 2012; Fan, et al. 2016). The African Dog
215 and Dingo/NGSD are included in the West Eurasian and East Eurasian lineages, respectively (Fig.
216 S7). Therefore, they are likely to be the oldest dogs of their respective lineages. The *f4* statistics
217 biplot showed that dogs showing higher affinity with dingo/NGSD show lower affinity with
218 African dogs while dogs showing higher affinity with African dogs show lower affinity with
219 Dingo/NGSD (Fig. S19). This negative correlation suggests most of dog populations were formed
220 through extensive past mixing between East and West Eurasian lineages represented by
221 dingo/NGSD and African dogs, respectively. Indeed, several dogs in South and East Asia are
222 genomically characterized as dingo/NGSD admixed with African dogs by negative values of *f3*
223 statistics (Patterson, et al. 2012) (Fig. S20).
224 Next, we examined the degree of introgression between dogs from different regions and
225 Japanese wolves. TreeMix analysis indicates an introgression from the ancestor of Japanese
226 wolves into the common ancestor of dingo/NGSD and Japanese dogs (Fig. 3B). The *f3* biplot of
227 affinities with dingo/NGSD and with Japanese wolves shows a positive correlation among dogs,
228 indicating that the Japanese wolf genome has become widespread through the admixture between
229 West and East Eurasian dog lineages and persists in the modern genomes of the East Eurasian
230 lineage (Fig. 3C). Therefore, it is likely that the genome of the Japanese wolf ancestor was
231 introgressed into an ancestral lineage of the East Eurasian lineage after the split of West and East
232 Eurasian lineages. Subsequently, the East Eurasian lineage containing the Japanese wolf ancestry
233 admixed with the West Eurasian lineage, resulting in differences in affinities with the Japanese
234 wolves. The Southeast Asian dogs (Fig. 3C green) have a relatively higher affinity with

235 Dingo/NGSD compared to their affinity with Japanese wolves, suggesting gene flow between the
236 Southeast Asian dogs and dogs that carried no Japanese wolf genome after the first admixture
237 event. Similarly, Japanese dogs (Fig. 3C blue) have a relatively higher affinity with Japanese
238 wolves compared to their affinity with Dingo/NGSD, suggesting subsequent gene flow between
239 Japanese dogs and Japanese wolves. Hence, it is likely that the difference in the degree of genomic
240 introgression from Japanese wolves to dogs was caused by a single introgression followed by
241 diffusion of the Japanese wolf genome into various dog populations.
242
243 **Genomic regions derived from Japanese wolf that contribute to the traits of Japanese dogs**
244 The results of *f4*-ratio analysis showed that Akita, Shiba, and Kishu dogs contain 36-45% West
245 Eurasian dog genomes (Table S3). It is expected that the genomic regions containing genes
246 responsible for traits of the Japanese dogs are highly differentiated from those of West Eurasian
247 dogs. If such regions overlap with the Japanese wolf-derived genomic regions (3-4%) in the
248 modern Japanese dog genome, it could be that Japanese wolf-derived genes are responsible for
249 the characteristic traits of the Japanese dog. To investigate such regions, we examined genomic
250 regions derived from Japanese wolves using a f_{dM} sliding window and regions differentiated
251 between Japanese dogs and West Eurasian dogs using an F_{ST} sliding window, and extracted
252 overlapping regions. Six regions were found, which included four genes and the upstream or
253 downstream regions of four genes (Fig. 3D, Fig. S21, Table S5). These regions may be candidates
254 of genomic regions derived from Japanese wolf that contribute to the traits of Japanese dogs.
255
256 **Discussion**

The Japanese wolves are likely to have been isolated in the Japanese archipelago until their extinction only 100 years ago. This study reveals that they form a monophyletic group with no evidence for gene flow with other Eurasian gray wolves.

One notable aspect of Japanese wolves is their phylogenetic position. In Eurasia, our phylogenetic analysis showed that the European lineage of the gray wolves diverged at the basal position, followed by the Middle East and East Asian lineages. In the East Asian lineage, the monophyletic group of Japanese wolves and the dog lineage form a sister-group relationship. The order in which Eurasian wolf lineages diverged is from west to east in geographical order on the Eurasian continent. Considering these phylogenetic and geographic relationships, it is most likely that it was in East Asia that the divergence between the Japanese wolf and the dog lineages has occurred. In other words, the extinct population of the gray wolf from which dogs are suspected to have been domesticated (Larson, et al. 2012; Thalmann, et al. 2013; Freedman, et al. 2014; Skoglund, et al. 2015; Fan, et al. 2016; Frantz, et al. 2016) is closely related to the ancestor of the Japanese wolf and is likely to inhabit East Asia. This hypothesis does not directly imply that the origin of dog domestication was in East Asia, nor does it directly imply that the earliest dogs descended from the ancestor of Japanese wolves. Although the domestication process would have been initiated with the animals' association with humans (Larson, et al. 2012; Perri 2016; Janssens, et al. 2018), our phylogenetic analyses provide no evidence for when dog lineages began to associate with humans. Further archaeological evidence in the studies of ancient "proto-dog" populations are required to clarify the beginnings of the dog-human relationship.

This study suggests ancient genomic introgression from the Japanese wolf to dogs, most likely to the ancestor of the East Eurasian lineage. The divergence between the dog lineage and the

279 Eurasian gray wolves has been estimated to be 20,000-40,000 years ago (Skoglund, et al. 2015;
280 Botigué, et al. 2017). Dogs have been reported to have split into West Eurasian, East Eurasian,
281 and sled dog lineages in their early divergence (Freedman, et al. 2014; Shannon, et al. 2015;
282 Frantz, et al. 2016; Botigué, et al. 2017; Leathlobhair, et al. 2018). Because a 9500-year-old sled
283 dog (Sinding, et al. 2020) already contained the same proportion of the Japanese wolf genome as
284 the modern sled dog (2%: Table S4), the genomic introgression of the ancestor of the Japanese
285 wolf to the East Eurasian lineage of dogs must have occurred before the establishment of the sled
286 dog lineage at least 9500 years ago during the transitional period from the Pleistocene to the
287 Holocene and shortly after the divergence of the East and West Eurasian dog lineages. The
288 genome of NGSD was estimated to contain the Japanese wolf genome (5.5%). It is estimated that
289 the NGSD lineage already existed by 10,900 years ago (Bergström, et al. 2020), which also
290 supports the hypothesis that the introgression from the ancestor of Japanese wolves into dogs had
291 occurred in the Pleistocene. The ancient dog genome data from two European individuals (4,800
292 and 7,000 years ago) already contained about 1.6% of the Japanese wolf genome (Table S4). Since
293 the gene flow from the Southeast Asian dog ancestry to the ancestor of these two ancient European
294 dogs has been reported (Botigué, et al. 2017), the genome of the Japanese wolf may have been
295 transmitted to European dogs via the Southeast Asian dog ancestry more than 7,000 years ago.
296 Although the Japanese wolf has only ever been found living in the Japanese archipelago, it is
297 unlikely that the introgression between the ancestor of the Japanese wolf and dogs of the East
298 Eurasian lineage occurred in the Japanese archipelago. An ancient sled dog (Sinding, et al. 2020)
299 excavated at the same time as the excavation of the oldest dog in Japan (9600 years ago)
300 (Shigehara and Hongo 2000) already possessed the Japanese wolf genome, suggesting that

301 introgression between the ancestor of Japanese wolves and dogs of the East Eurasian lineage had
302 occurred before dogs were brought to the Japanese archipelago. Therefore, the introgression
303 between the ancestral Japanese wolf and the East Eurasian lineage of dogs is most likely to have
304 occurred somewhere in East Asia.

305 The dogs with a high proportion of the Japanese wolf genome are the dingo/NGSD (5.5%) and
306 the Japanese dogs (3-4%). The high proportion of the dingo/NGSD is inferred to be due to their
307 isolation in the islands of Southeast Asia and Australia, where they have escaped admixture with
308 the West Eurasian dog lineage. NGSD is estimated to be an admixture of two lineages (Bergström,
309 et al. 2020), and thus one of the admixed lineages may have possessed a higher proportion (~10%)
310 of the Japanese wolf genome than NGSD. In contrast, Japanese dogs contain about 36-45% of the
311 West Eurasian genome (Table S3), even though a high proportion of the Japanese wolf genome
312 persists in their genomes. After the first introgression with the East Eurasian dog ancestry, the
313 Japanese wolf genome may have introgressed into the Japanese dog genomes in the Japanese
314 archipelago. This hypothesis is supported by the ratio of affinity with Japanese wolves and with
315 dingoes/NGSDs, which tends to be higher in Japanese dogs (Fig. 3C). In addition, the Japanese
316 dogs have the highest affinity to the Japanese wolf among all dogs (Fig. S16), suggesting that the
317 Japanese wolf genome was recently introgressed into the Japanese dog genome.

318 Although only a small proportion of the Japanese wolf genome persists in modern dog genomes,
319 the Japanese wolf genome might have an effect on dog characteristic traits. We searched for
320 genomic regions in Japanese dogs that derived from the Japanese wolf, and that were highly
321 differentiated between Japanese dogs and West Eurasian dogs. Six regions were found with these
322 criteria (Fig. 3D, Fig. S21, Table S5). Despite the gene flow from Eurasian dogs, these regions

323 were differentiated between the genomes of Japanese and West Eurasian dogs. Therefore, these
324 regions are expected to have been selected in the Japanese dogs and are the candidates of genomic
325 regions potentially responsible for phenotypic characteristics of Japanese dogs. Further analysis
326 of the regions selected in East Eurasian dogs and a genome-wide association study for East
327 Eurasian dog traits should reveal the effects of the Japanese wolf genome on dog traits.
328 In this study, we demonstrated that the Japanese wolf is a sister group with the monophyletic
329 clade of dogs. Our original results support the hypothesis that the modern dog lineage was
330 domesticated from an extinct population of gray wolves (Larson, et al. 2012; Thalmann, et al.
331 2013; Freedman, et al. 2014; Skoglund, et al. 2015; Fan, et al. 2016; Frantz, et al. 2016), and the
332 Japanese wolf is the closest to this now-extinct gray wolf population. In addition, we estimated
333 the levels of introgression from the ancestor of Japanese wolves to the ancestor of East Eurasian
334 dogs. Accordingly, the Japanese wolf genome is expected to be involved in the early stages of
335 dog domestication. Further analysis of the genome of the Japanese wolf and ancient dog genomes,
336 in particular from East Eurasia, will continue to shed light on the origins of dog domestication.
337
338
339 **Materials and methods**
340 **Samples, DNA extraction, and sequencing**
341 Japanese Wolf and Japanese dog DNAs were extracted and used in our previous studies
342 (Matsumura, et al. 2014; Matsumura, et al. 2020). The sample locations are listed in Table S1.
343 DNAs of two individuals of Shiba (Shiba_shiro and Shiba_kuro) were extracted from blood
344 samples using a DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions.

345 The NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Bio Labs, Ipswich, MA,
346 USA) was used to construct libraries from genomic DNA following the manufacturer's
347 instructions. Paired-end (2 × 150 bp) sequencing was performed on the Illumina HiSeq X or
348 NovaSeq 6000 platforms.
349 For Leiden b and Leiden c, the genome capture was performed using the SeqCap EZ
350 Hybridization and Wash Kit (Roche, Basel, Switzerland), SeqCap EZ Accessory Kit v2 (Roche),
351 SeqCap HE-Oligo Kit (Roche), and SeqCap EZ Pure Capture Bead Kit (Roche) following the
352 manufacturer's instructions for SeqCap EZ Library SR (Roche), with minor modifications.
353 Briefly, Biotin-labeled genomic DNA fragments from Shiba were used as hybridization probes,
354 instead of the SeqCap EZ library (Roche). Leiden b and Leiden c libraries were mixed with 135
355 ng of Biotin-labeled genomic DNA fragments and were hybridized at 47°C for 72 h. Other
356 procedures were performed in accordance with the manufacturer's instructions. Total mapped
357 reads are listed in Table S1.
358
359 **Extraction of SNPs and vcf file preparation**
360 Sequence reads from the genomic DNA libraries of nine Japanese wolves, eleven Japanese dogs
361 (Table S1) as well as 88 wolves/dogs and six outgroup species from the database (Table S2) were
362 trimmed to remove adaptor sequences and mapped to the dog reference genome (CanFam3.1)
363 using CLC Genomics Workbench (https://www.qiagenbioinformatics.com/). Reads showing high
364 similarity (> 90% in > 90% of read length) were mapped to the reference genome sequences and
365 reads mapped to more than one position were removed ("ignore" option for reads mapped to
366 multiple positions). The mapping data was exported in bam file format and sorted and indexed

367 using samtools (Li, et al. 2009). The duplicated reads in bam files were marked by the
368 MarkDuplicates algorithm implemented in GATK v4.2 (https://gatk.broadinstitute.org/hc/en-us).
369 We performed genotype calling on all individuals analyzed in this study using the
370 HaplotypeCaller algorithm in GATK v4.2. Genotypes of all individuals were output as gvcf
371 format (-ERC GVCF option) and filtered by the VariantFiltration algorithm in GATK v4.2 with
372 default parameters. All gvcf files were combined into a single gvcf format file by the
373 CombineGVCFs algorithm in GATK v4.2. The combined file was genotyped by the
374 GenotypeGVCFs algorithm and filtered by Filtervcf in GATK v4.2 with parameters; --filter-
375 expression "QD < 2.0" --filter-name "QD2" --filter-expression "QUAL < 30.0" --filter-name
376 "QUAL30" --filter-expression "FS > 200.0" --filter-name "FS200" --filter-expression "SOR >
377 10.0" -filter-name "SOR10" --filter-expression "ReadPosRankSum < -20.0" --filter-name
378 "ReadPosRankSum-20".
379 To maximize the number of SNPs for analyses without missingness, we prepared vcf files for
380 each analysis using vcftools (Danecek, et al. 2011). When including all individuals except for
381 ancient samples and Leiden b, Leiden c, and Honshu wolf (see the sample combination in Table
382 S2), we removed all indels, singleton, and doubleton sites to eliminate PCR and sequencing errors
383 that may have occurred in one individual, and extracted bi-allelic sites with coverage equal to or
384 more than three in all individuals and with GQ values equal to or more than eight in all individuals.
385 Mutations due to DNA damage at both ends of fragments were less than 1% in Japanese wolves
386 (Fig. S1), therefore we can infer that mutations by DNA damage in the sequences of Japanese
387 wolves were removed by this filtration. For analyses with ancient samples, sites were filtered
388 using the same conditions as with modern samples. In addition, we used only transversion sites

389 for the analyses of ancient samples. For the PCA and ADMIXTURE analyses with Liden b and
390 Liden c, sites with missingness greater than 3% and minor allele frequency (MAF) < 0.04 were
391 excluded. Sites were filtered using the same conditions as with modern samples, and only
392 transversion sites were used.
393
394 **Phylogenetic analysis**
395 The SNPs in a vcf file including dogs, wolves and Japanese wolves (see Table S2) were converted
396 to PHYLIP format. 10 kb sequences from the 5' end of the PHYLIP format file were extracted
397 and a model for Maximum Likelihood method was selected using MEGA ver. X (Kumar, et al.
398 2018). A phylogenetic tree was constructed using the Maximum Likelihood (ML) method using
399 PhyML ver. 3.2 (Guindon, et al. 2010) with a model selection option "-m GTR" and with 100
400 bootstrap replications. ML trees were constructed using all individuals (Fig. S7: 489,524 SNPs),
401 selected individuals (Fig. 2A: 1,971,890 SNPs), and outgroup species (Table S2), wolves,
402 Japanese wolves, and African dogs (Fig. S13: 2,065,200 SNPs).
403 The same vcf file used in the ML method (Fig. S7: 489,524 SNPs) was converted to NEXUS
404 format. A phylogenetic tree was constructed by the svdq algorithm (Chifman and Kubatko 2014)
405 in PAUP* ver. 4a (Wilgenbusch and Swofford 2003) with 100 bootstrap replications (Fig. S8).
406 We used PLINK ver. 1.9 (Purcell, et al. 2007) with an option "—distance 1-ibs" to calculate an
407 Identity By State (IBS) distance matrix using 1,992,260 SNPs. A neighbour joining tree was
408 constructed from the IBS distance matrix using MEGA ver. X (Kumar, et al. 2018)(Fig. S9).
409
410 **Principal component analysis and ADMIXTURE**

411 We performed a principal component analysis (PCA) using PLINK ver. 1.9 (Purcell, et al. 2007)
412 with an option "--indep-pairwise 50 10 0.1" to explore the affinity among gray wolves, Japanese
413 wolves, and dogs (Figure 1A). We also performed PCA with type specimens of Japanese wolf
414 (Fig. S3), and with ancient canids (Fig. S6).
415 ADMIXTURE ver. 1.3 (Alexander and Lange 2011) was run on the dataset of modern samples
416 (Fig. 1B and Fig. S2) and modern specimens with type specimens of Japanese wolf (Fig. S4)
417 assuming 2 to 8 clusters (K=2-8).
418
419 ***f3*, *f4* statistics, and *f4*-ratio**
420 *f*3, *f4* statistics, and *f4*-ratio implemented in ADMIXTOOLS ver. 7.0.1 (Patterson, et al. 2012)
421 were used to evaluate the shared genetic drift among gray wolves, Japanese wolves, and dogs.
422 We used the same SNPs data set used for the phylogenetic analysis for all modern samples
423 (489,524 SNPs). For Liden b and Leiden c analyses, vcf files were prepared to maximize the
424 number of SNPs (Table S2).
425 Outgroup *f3* statistics were calculated to explore shared genetic drift between all dogs and each
426 of the wolves (Fig. 2B), African dogs and each of the wolves (Fig. S10A), dingo/NGSD dogs and
427 each of the wolves (Fig. S10B), Japanese wolf and each of the dogs (Fig. 2C), African dogs and
428 each of the other dogs (Fig. S17A), and dingo/NGSD dogs and each of the other dogs (Fig. S17B).
429 *f3* statistics were calculated to test the genomic mixture of African and dingo/NGSD dogs in all
430 dogs (Fig. S20).
431 *f*4 statistics were calculated to explore shared genetic drift between each of the dogs and Leiden
432 c (Fig. S5A: 38,254 SNPs), each of the dogs and Leiden b (Fig. S5C: 83,259 SNPs), each of the

433 wolves and Japanese wolves (Fig. 2D), each of the dogs and Japanese wolves (Fig. S11), each of
434 the dogs and each of the wolves (Fig. S12), dingo/NGSD and Japanese wolves (Fig. S15), each
435 of the dogs and Japanese wolves (Fig. S16), NGSD1 or Basenji and each of the dogs (Fig. S18),
436 and dingoes and each of the other dogs, and African dogs and each of the other dogs (Fig. S19)
437
438 **TreeMix**
439 To examine the admixture events, we used TreeMix (Pickrell and Pritchard 2012) to build a tree
440 with admixture edges. We used major groups of gray wolves and dogs as follows; Gray Wolves
441 (North America: n = 2), Gray Wolves (Canada/Arctic: n = 8), Gray Wolves (East Asia: n =5),
442 Gray Wolves (West Eurasia: n = 7), Japanese Wolves (Japan: n = 7), Dogs (Central Asian: n = 4),
443 Dogs (Europe: n = 11), Dogs (Africa: n = 10), Dogs (sled dogs: n = 4), Dogs (Vietnamese
444 Indigenous: n = 5), Ding/NGSD (Oceania: n = 7), Japanese Dogs (Japan: n = 11), and Dogs
445 (Korea: n = 6). The SNPs were pruned based on linkage disequilibrium (LD-pruning) by using
446 plink with an option "--indep-pairwise 50 10 0.12. As a result of LD-pruning, 150,502 SNPs were
447 used for TreeMix.
448
449 **Assessing DNA damage patterns**
450 We used mapDamage ver. 2.2.0 (Ginolhac, et al. 2011) to assess DNA damage patterns in the
451 Japanese wolf samples sequenced in this study. Mapped reads from the Japanese wolf samples
452 showed slightly increased proportion (equal to or less than 1%) of C to T and G to A substitutions
453 at the 5' and 3' read ends, respectively (Fig. S1).
454

455 **Calculation of maximum contamination rate**

456 We used substitutions in mitochondria DNA specific to Japanese wolf to assess the contamination
457 rate of the other animal DNA in Japanese wolf DNA. Fifteen fixed substitutions unique to
458 Japanese wolf were selected using an alignment of mitochondria DNA sequences including gray
459 wolves, Japanese wolves, and dogs used in previous studies (Matsumura, et al. 2014; Matsumura,
460 et al. 2020). The lowest coverage at fifteen sites was 48 (highest was 28,324) in the mapping
461 result to the mitochondria genome in CanFam3.1. We calculated the average mapping ratio in
462 fifteen sites. The ratio of the reads mapped to the fifteen sites without substitutions specific to
463 Japanese wolf were assumed as the maximum contamination rate (Table S6), because the
464 mitochondria DNA like sequences are found in the nuclear genome.

465

466 **Detection of genomic regions that are derived from Japanese wolf and may have contributed**
467 **to Japanese dog characteristics**

468 The genomic regions containing genes that form the characteristics of the Japanese dog are
469 expected to be largely differentiated between the Japanese dog and the West Eurasian dog. To
470 detect such regions, we examined sequences differentiated between Japanese dogs and West
471 Eurasian dogs by using F_{ST} sliding window analysis. Using SNPs of Japanese dogs and African
472 dogs (Table S2) in a vcf file, F_{ST} values for all sites were computed by vcftools v0.1.16 (Danecek,
473 et al. 2011). The average F_{ST} values in windows of 50 SNPs with a 25 SNPs slide across
474 chromosomes were calculated by RStudio version (1.4.1106) (RStudio Team 2020. RStudio:
475 Integrated Development for R. RStudio, PBC, Boston, MA). The Japanese wolf-derived genomic
476 regions were calculated by f_{dM} values in windows of 50 SNPs with a 25 SNPs slide across

477 chromosomes using Dsuite (Malinsky, et al. 2021). Then, we extracted regions of overlap between
478 high F_{ST} regions (top 1%) and the Japanese wolf-derived genomic regions (top 1% of f_{dM} values).
479
480
481 **Availability of data**
482 The nucleotide sequences were deposited in the DDBJ Sequenced Read Archive.
483
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487
488 **Acknowledgments**
489
490 **Author contributions**
491 JG: data analyses and manuscript writing; NA: next generation sequencing and vcf file
492 preparation of modern samples; XX: assessing DNA damage patterns; YM: genomic DNA
493 extraction of Shiba individuals and next generation sequencing; SM: preparation of an alignment
494 of mitochondrial DNA sequences and manuscript editing; HT: archeological information for
495 discussion and manuscript editing; NI preparation of genomic DNA of the Japanese wolves and
496 Japanese dogs and manuscript editing; YT: research concept, research plan, next generation
497 sequencing, vcf file preparation, data analyses, and manuscript writing.

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621

622 **Figure legends**

623 Figure 1 Relationships between Japanese wolves and other canids

624 (A) Principal Components Analysis (PC1 versus PC2) of 100 samples based on 2,065,200 SNPs

625 (see Table S2 for sample information). Colored circle, square, and triangle correspond to the

626 names of dogs or wolves in the panel. (B) ADMIXTURE results based on SNP data for K = 4
627 (see Table S2 for sample information).
628
629 Figure 2 Phylogenetic relationships and genetic affinity between Japanese wolves and other
630 canids. (A) Maximum likelihood tree based on 1,971,890 SNPs. Node labels shown in blue
631 squares indicate bootstrap values out of 100. (B) Shared genetic drift between dogs and gray
632 wolves measured by outgroup *f*3 statistics. Each of all dogs and Japanese wolves were used as
633 populations. Each *f3* statistical value is plotted in order of highest to lowest value from the top,
634 and the names of the wolves are shown on the right side of the panel. Error bars represent standard
635 errors. (C) Shared genetic drift between Japanese wolf and all dogs measured by outgroup *f*3
636 statistics. Each of the African dogs and Dingo/NGSD individuals were used as populations. Each
637 *f3* statistical value is plotted in order of highest to lowest value from the top, and the names of the
638 dogs are shown on the left side of the panel. Error bars represent standard errors. (D) *f4* statistics
639 testing the relationships between the Japanese wolf and all other wolves compared with NGSD1
640 (left panel) and Basenji (right panel). Each Z score is plotted in order of highest to lowest value
641 from the top, and the names of wolves are shown on the left and right sides of the left and right
642 panels, respectively. Gray lines show the Z score -3 and 3.
643
644 Figure 3 Admixture between Japanese wolves and the other canids.
645 (A) *f4*-ratio test to estimate proportion of genome introgression from the Japanese wolf to
646 dogs. Each *f4*-ratio α value is plotted in order of highest to lowest value from the top, and the
647 names of the dogs are shown on the right side of the panel. Error bars represent standard errors.

648 Z score above 3 is colored in blue. (B) TreeMix admixture graph built using LD-pruned data
649 (150,502 sites) on a dataset consisting of 88 dogs/wolves merged into 13 groups according to their
650 phylogenetic relationships. (C) *f3* statistics testing whether dogs share more alleles with
651 dingo/NGSD (x-axis) or Japanese wolf (y-axis). Dots show the *f3* statistics, and horizontal and
652 vertical error bars represent standard errors for tests with the African dogs (x-axis) and
653 dingo/NGSD (y-axis), respectively. Each of the Japanese wolves and dingo/NGSD individuals
654 were used as populations. (D) Sliding window analyses of the F_{ST} values (y-axis: upper panel)
655 and f_{dM} (y-axis: lower panel) in windows of 50 SNPs using a 25 SNPs slide across chromosome
656 (x-axis). Dashed lines show the 99th percentiles. Blue arrow indicates overlapping regions above
657 99th percentiles between upper and lower panels. The gene name above the arrow is the gene in
658 the overlapped region.
659
660

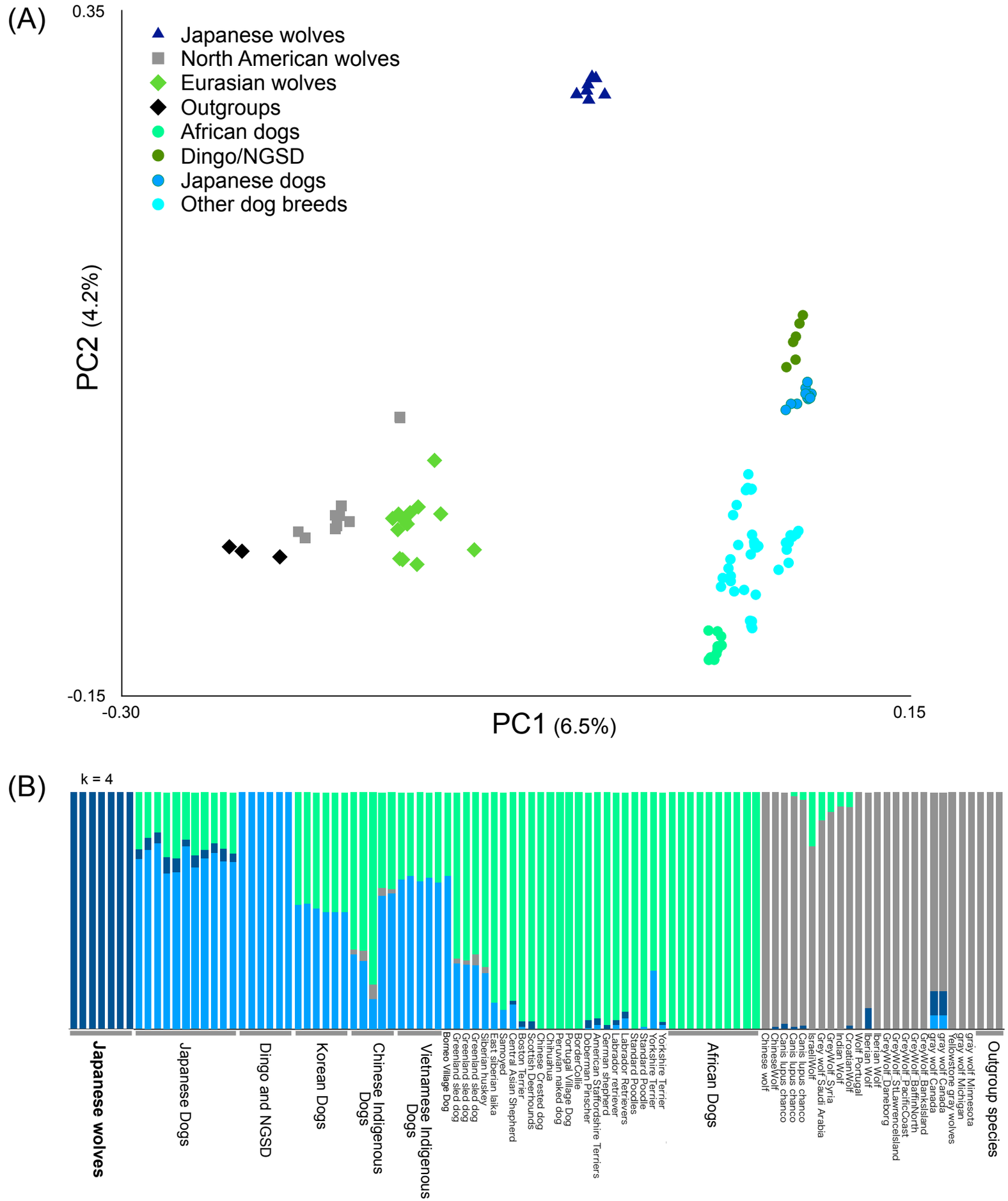


Figure 1 Relationships between Japanese wolves and other canids

(A) Principal Components Analysis (PC1 versus PC2) of 100 samples based on 2,065,200 SNPs (see Table S2 for sample information). Colored circle, square, and triangle correspond to the names of dogs or wolves in the panel. (B) ADMIXTURE results based on SNP data for K = 4 (see Table S2 for sample information).

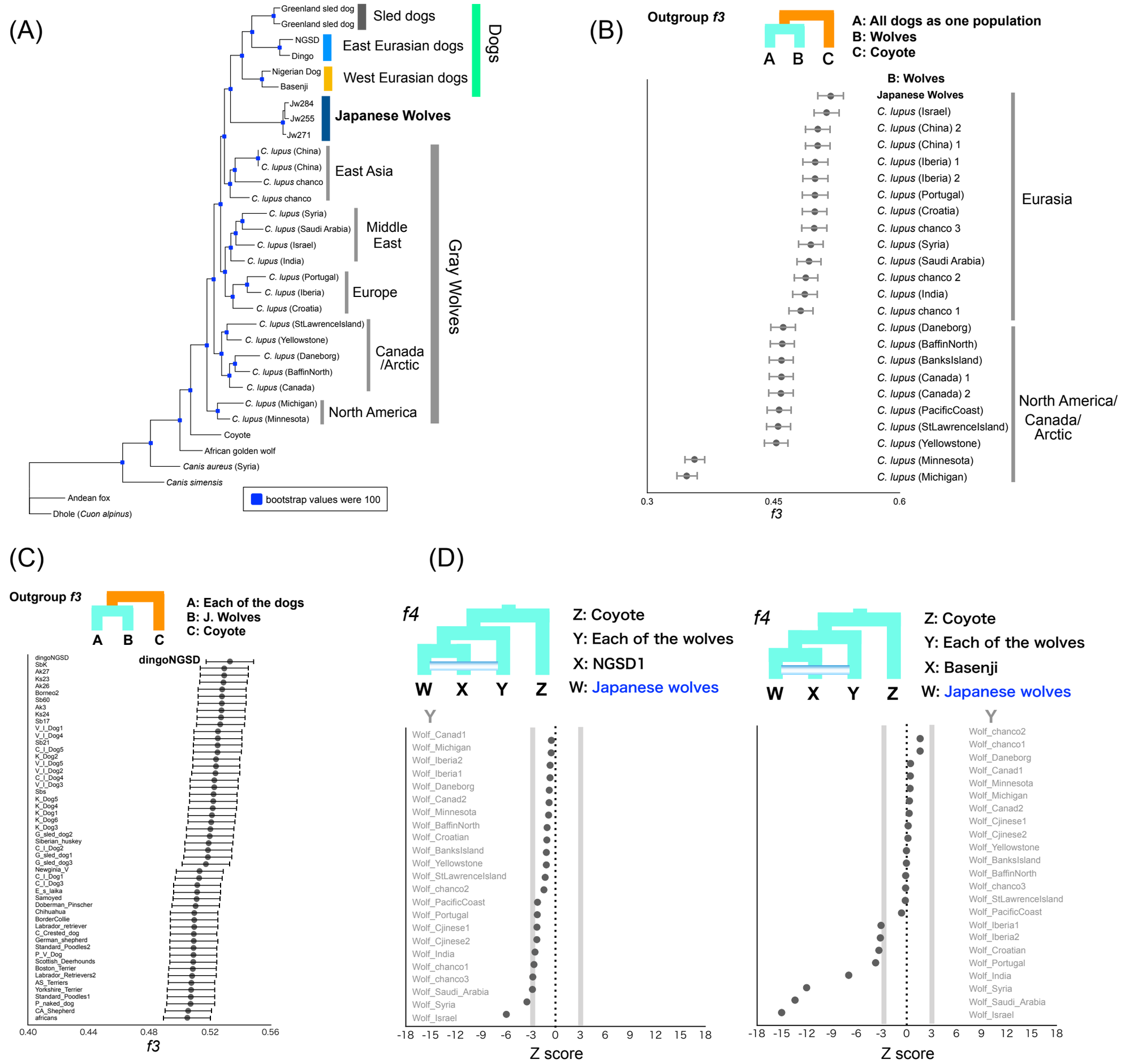


Figure 2 Phylogenetic relationships and genetic affinity between Japanese wolves and other canids.
(A) Maximum likelihood tree based on 1,971,890 SNPs. Node labels shown in blue squares indicate bootstrap values out of 100. (B) Shared genetic drift between dogs and gray wolves measured by outgroup *f*3 statistics. Each of all dogs and Japanese wolves were used as populations. Each *f3* statistical value is plotted in order of highest to lowest value from the top, and the names of the wolves are shown on the right side of the panel. Error bars represent standard errors. (C) Shared genetic drift between Japanese wolf and all dogs measured by outgroup *f*3 statistics. Each of the African dogs and Dingo/NGSD individuals were used as populations. Each *f3* statistical value is plotted in order of highest to lowest value from the top, and the names of the dogs are shown on the left side of the panel. Error bars represent standard errors. (D) *f4* statistics testing the relationships between the Japanese wolf and all other wolves compared with NGSD1 (left panel) and Basenji (right panel). Each Z score is plotted in order of highest to lowest value from the top, and the names of wolves are shown on the left and right sides of the left and right panels, respectively. Gray lines show the Z score -3 and 3.

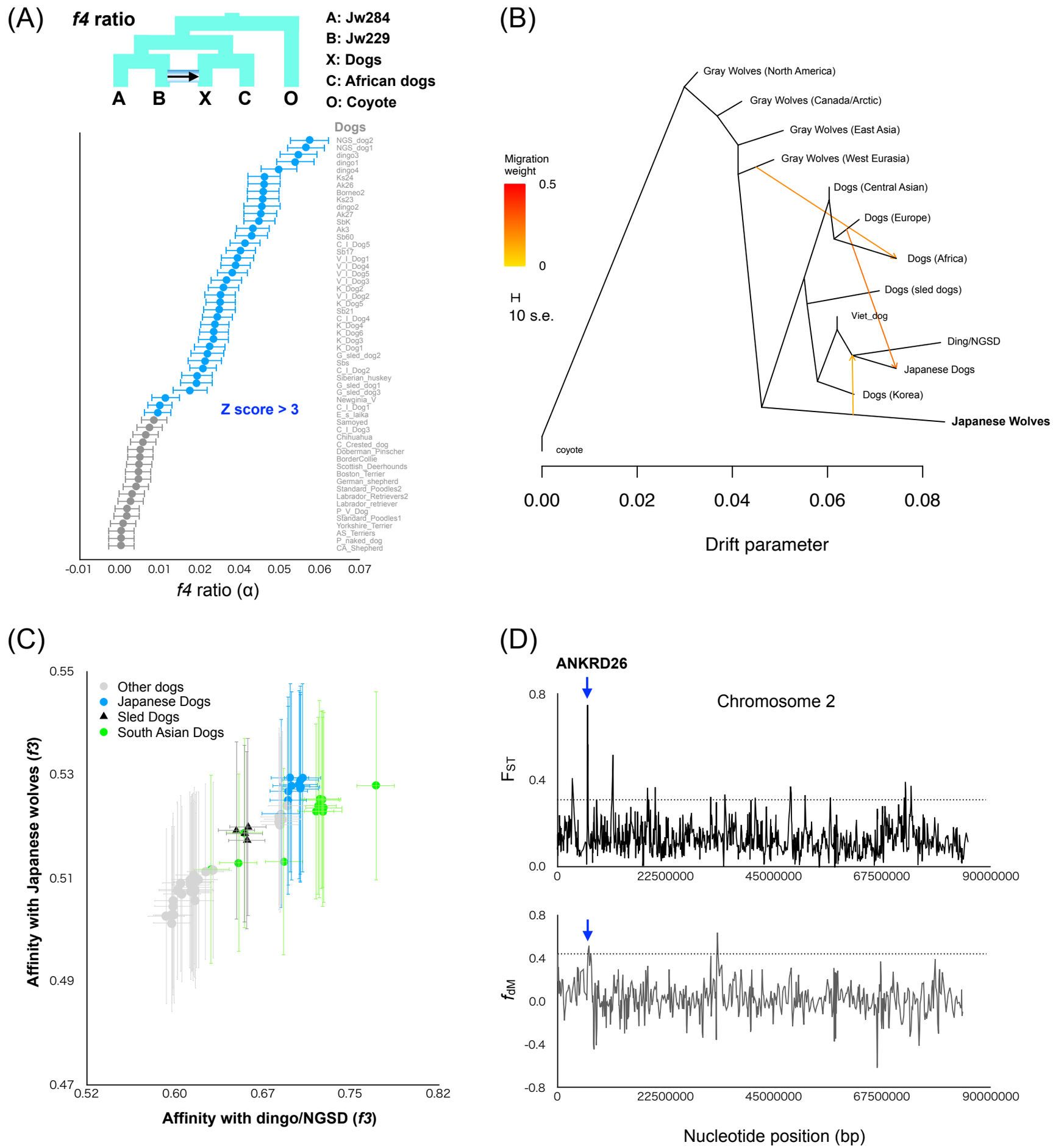


Figure 3 Admixture between Japanese wolves and the other canids.

f4-ratio test to estimate proportion of genome introgression from the Japanese wolf to dogs. Each *f4*-ratio α value is plotted in order of highest to lowest value from the top, and the names of the dogs are shown on the right side of the panel. Error bars represent standard errors. Z score above 3 is colored in blue. (B) TreeMix admixture graph built using LD-pruned data (150,502 sites) on a dataset consisting of 88 dogs/wolves merged into 13 groups according to their phylogenetic relationships. (C) *f3* statistics testing whether dogs share more alleles with dingo/NGSD (x-axis) or Japanese wolf (y-axis). Dots show the *f3* statistics, and horizontal and vertical error bars represent standard errors for tests with the African dogs (x-axis) and dingo/NGSD (y-axis), respectively. Each of the Japanese wolves and dingo/NGSD individuals were used as populations. (D) Sliding window analyses of the F_{ST} values (y-axis: upper panel) and f_{dM} (y-axis: lower panel) in windows of 50 SNPs using a 25 SNPs slide across chromosome (x-axis). Dashed lines show the 99th percentiles. Blue arrow indicates overlapping regions above 99th percentiles between upper and lower panels. The gene name above the arrow is the gene in the overlapped region.

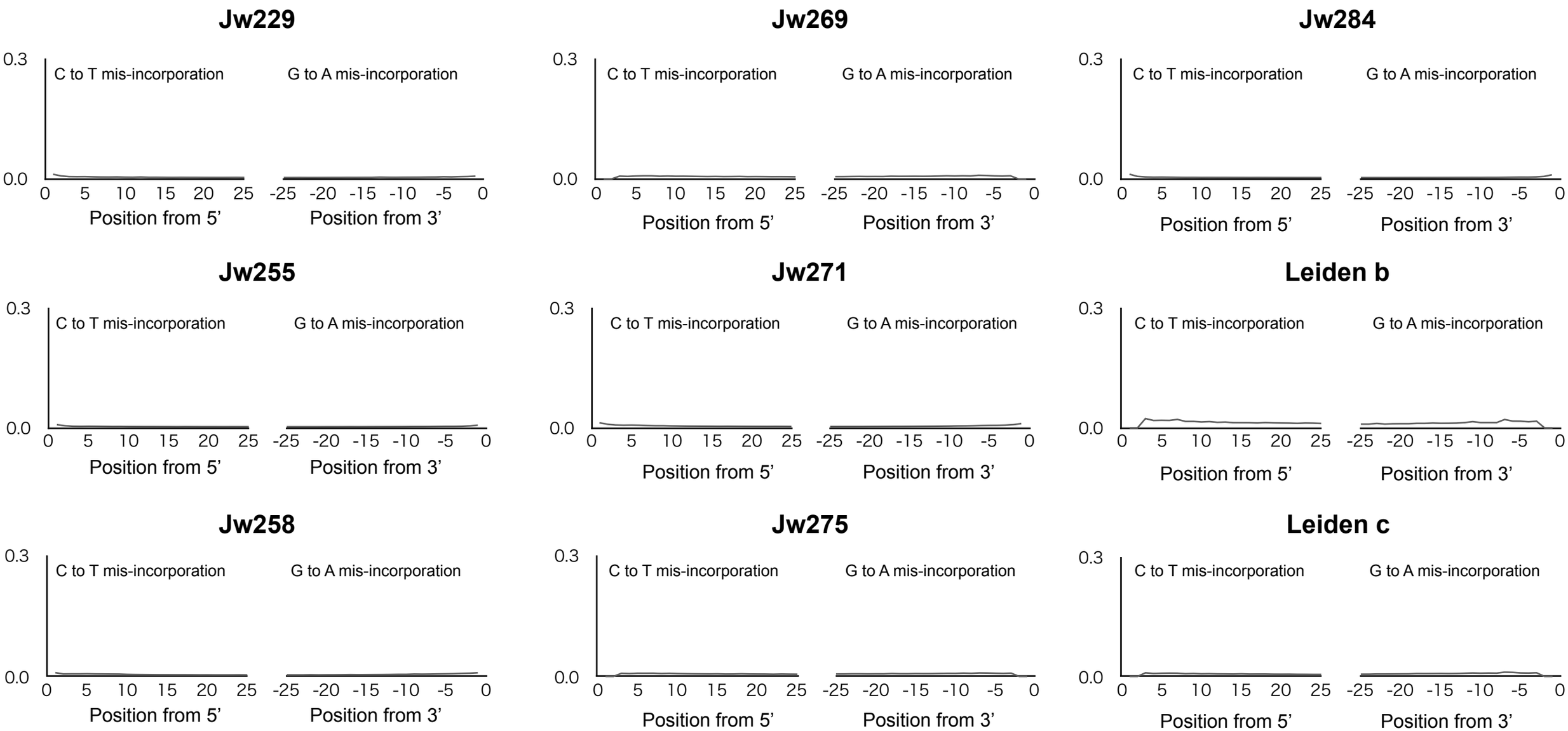


Figure S1

C to T and G to A frequency of mis-incorporation at 3' and 5' end of reads determined in this study.

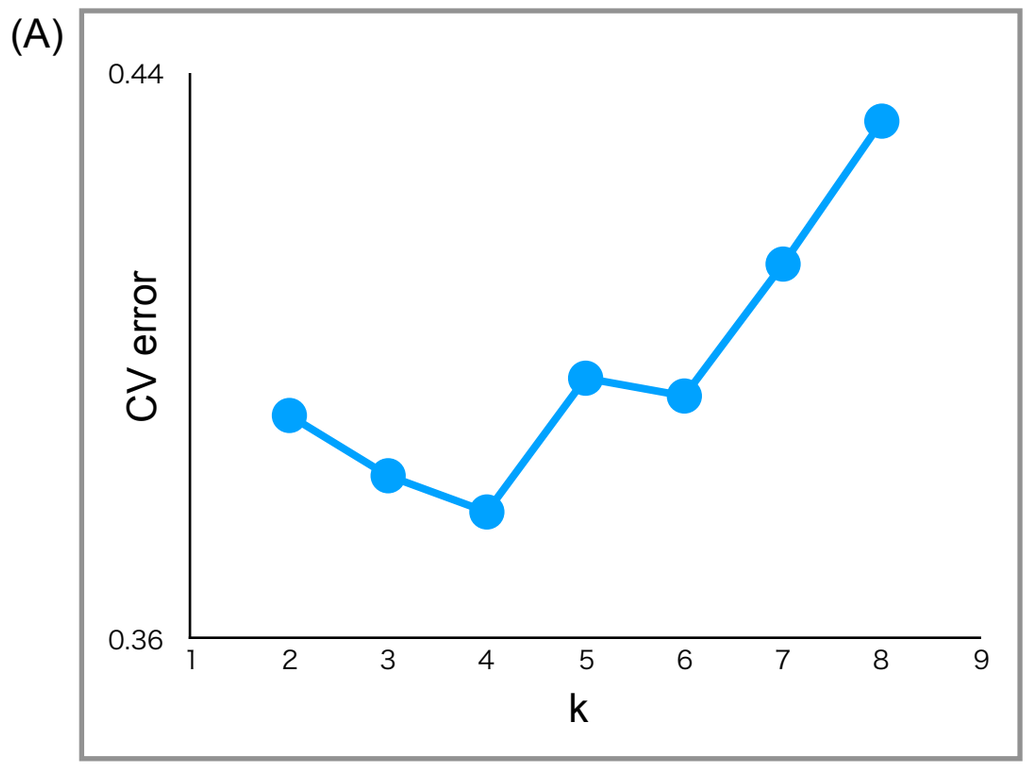


Figure S2
(A) Cross validation (CV) values for ADMIXTURE analysis of SNP data.
(B) ADMIXTURE results based on SNP data for K = 2-6 (see Table S2 for sample information).

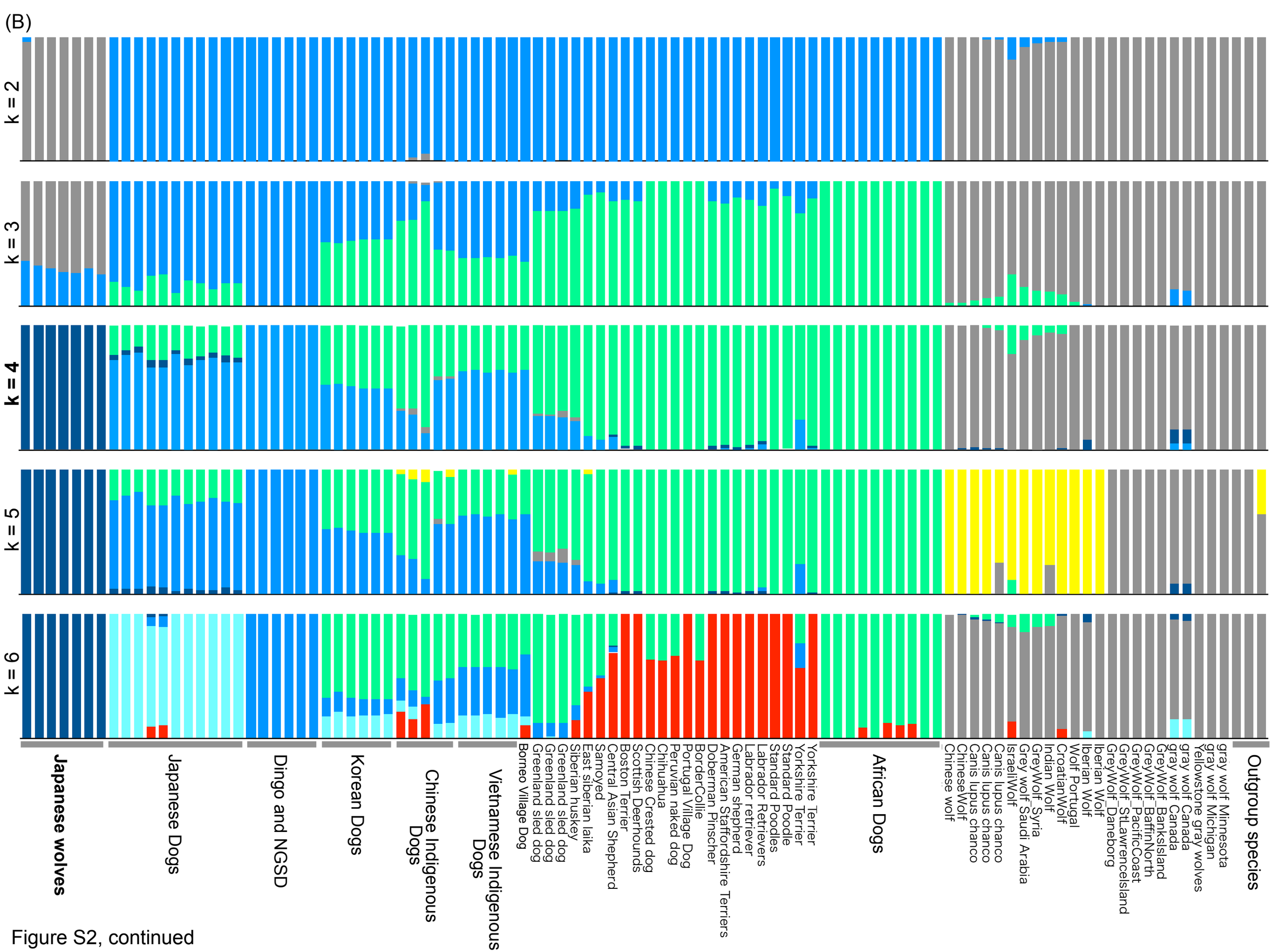


Figure S2, continued

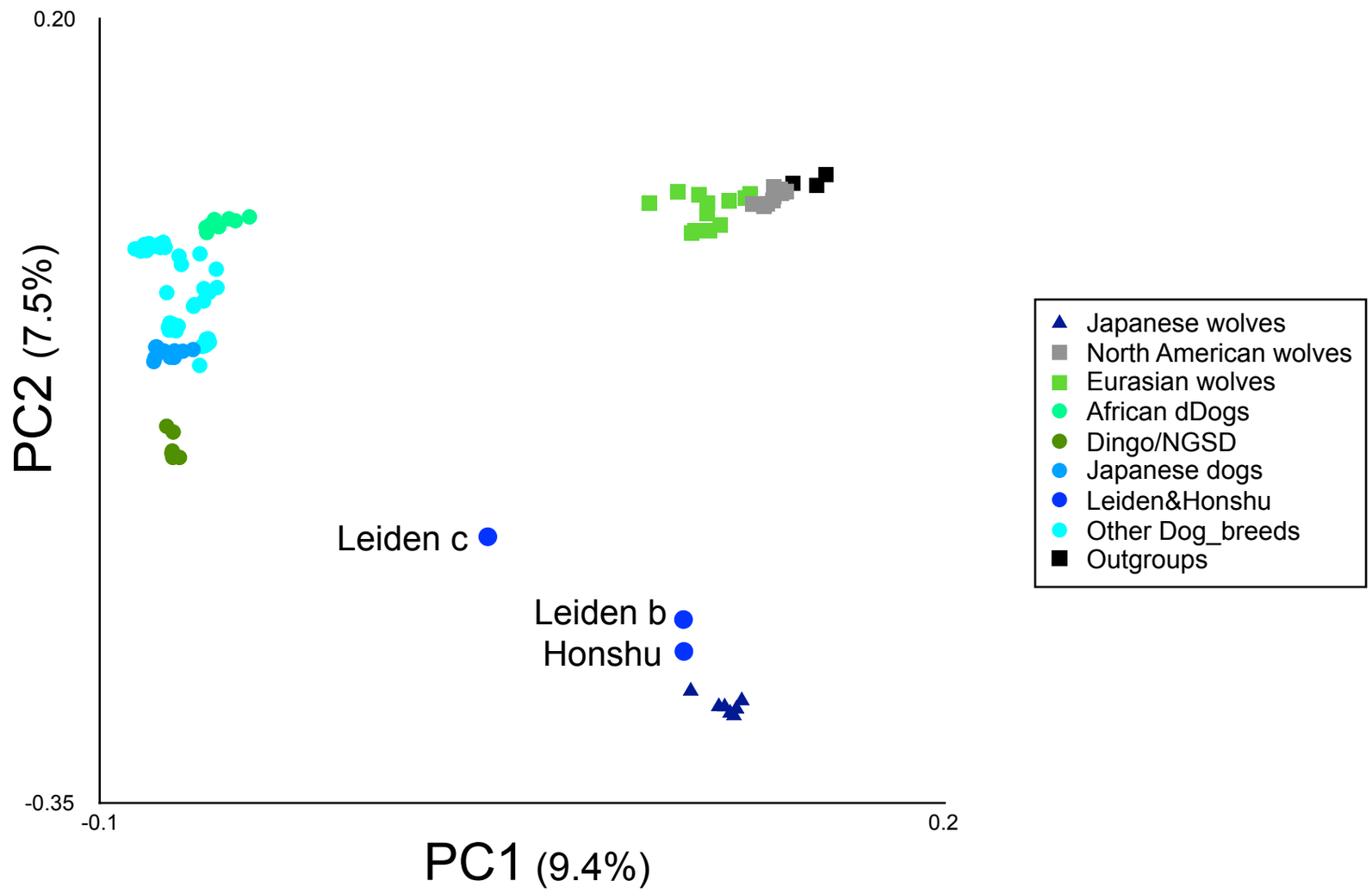


Figure S3
Principal Components Analysis (PC1 versus PC2) of 103 samples based on 103,432 SNPs (see Table S2 for sample information). Colored circle, square, and triangle correspond to the names of dogs or wolves in the panel.

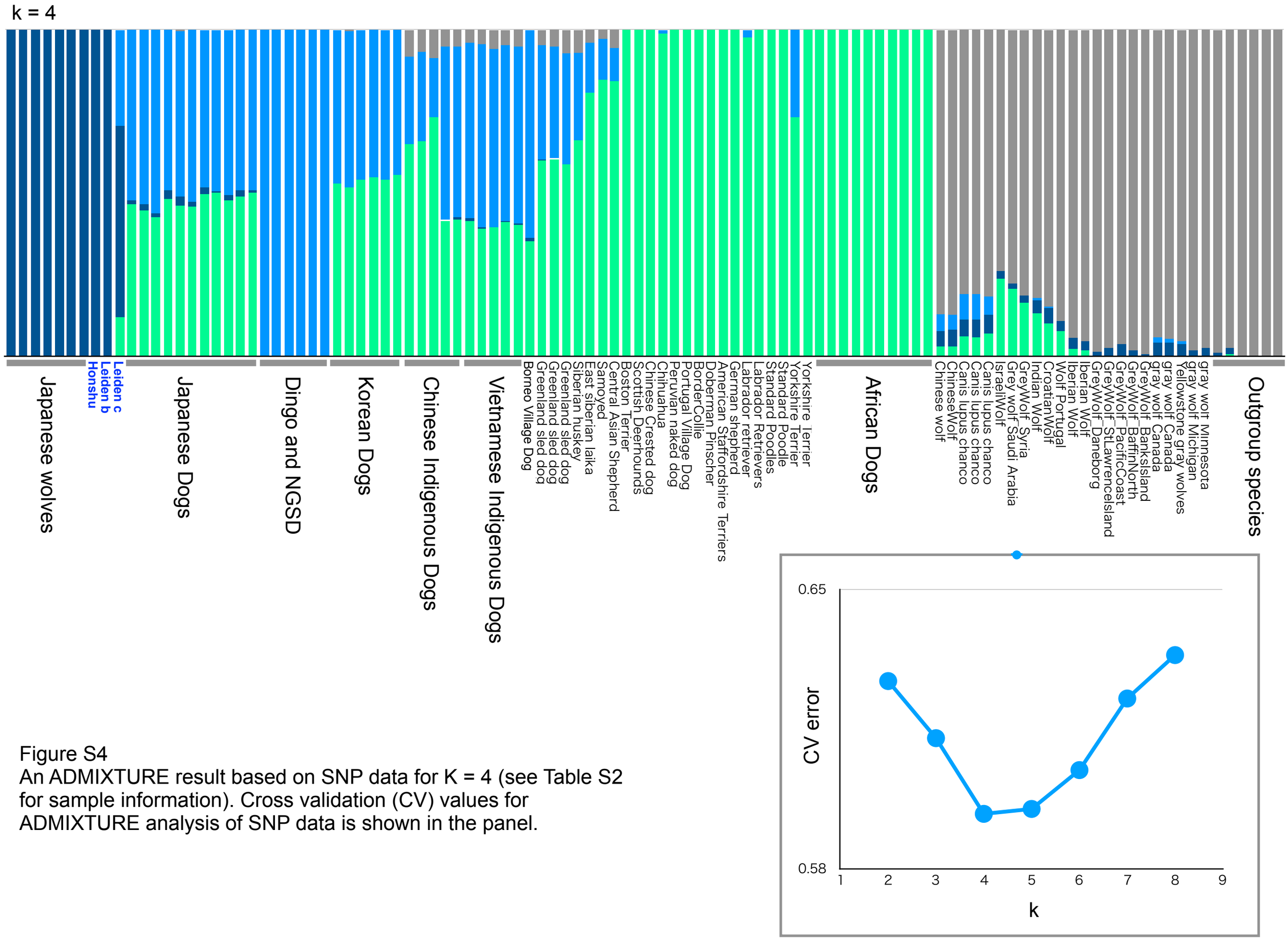
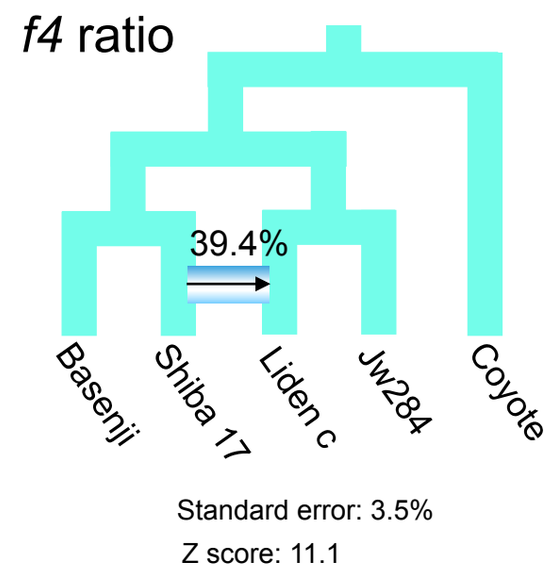


Figure S4
An ADMIXTURE result based on SNP data for K = 4 (see Table S2 for sample information). Cross validation (CV) values for ADMIXTURE analysis of SNP data is shown in the panel.



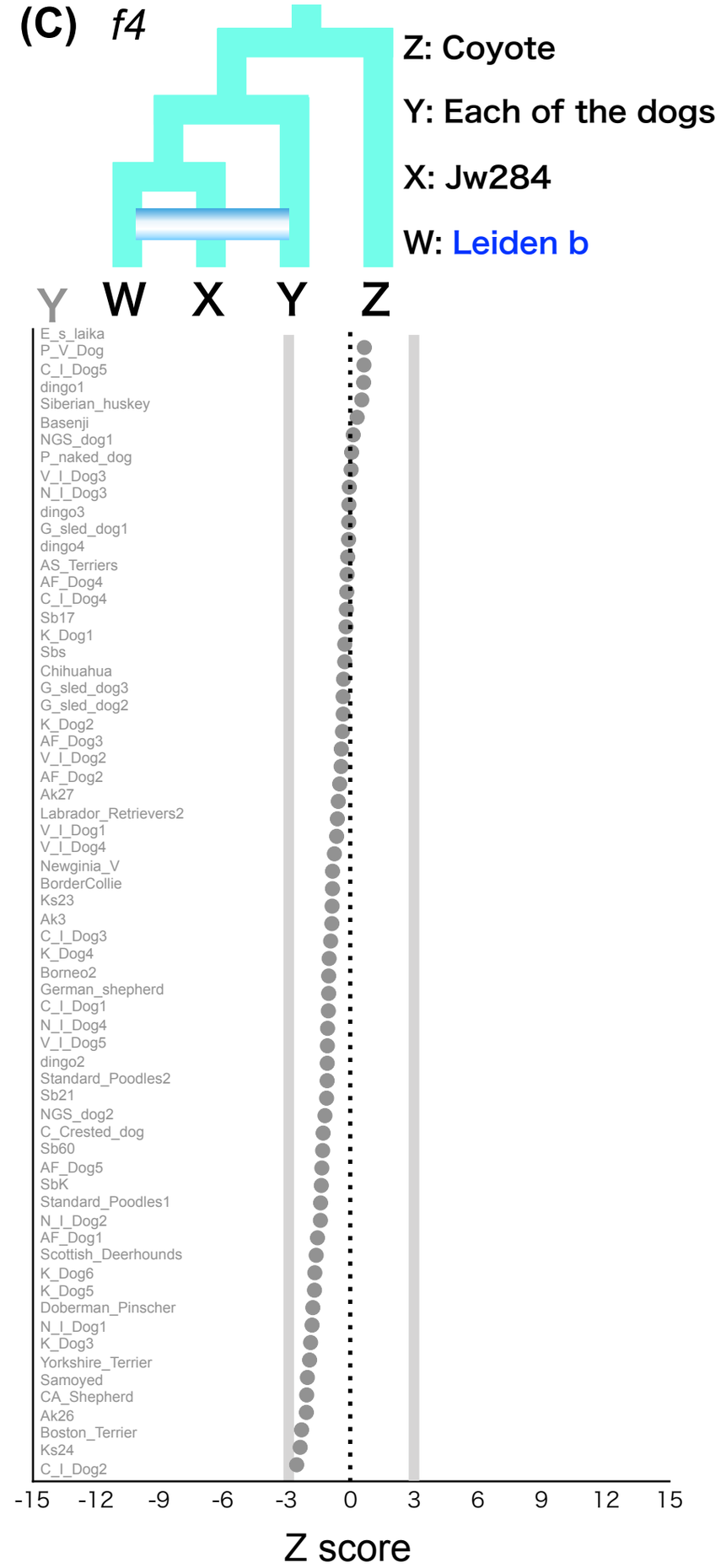


Figure S5
(A) *f4*-statistics testing the relationships between the Japanese wolf (Jw284), Leiden c, and all other dogs. Each Z score is plotted in order of highest to lowest value from the top, and the names of dogs are shown on the left side of each panel. Gray lines show the Z score -3 and 3. Leiden c shows genetic affinity with all other dogs (Z score > 3). (B) *f4*-ratio test to estimate proportion of genome introgression from a Japanese dog (Shiba 17) to Leiden C. (C) *f4*-statistics testing the relationships between the Japanese wolf (Jw284), Leiden b, and all other dogs. The genetic affinities of Leiden b with all other dogs are rejected (-3 < Z score < 3).

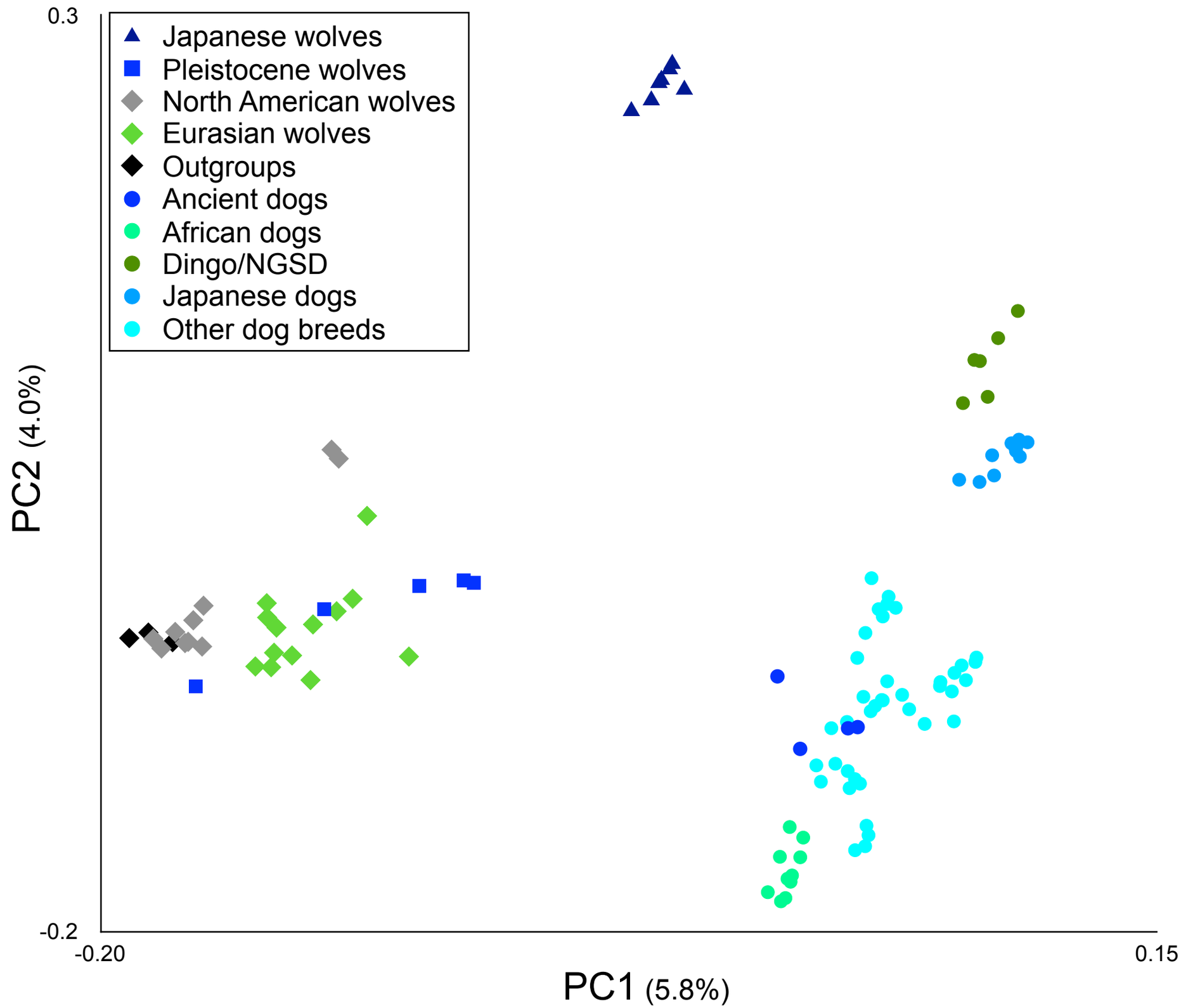


Figure S6
Principal Components Analysis (PC1 versus PC2) of 109 samples based on 100,588 SNPs (transversion sites, see Table S2 for sample information). Colored circle, square, and triangle correspond to the names of dogs or wolves in the panel.

Figure S7
Maximum likelihood tree based on 489,524 SNPs. Node labels indicate bootstrap replicates.

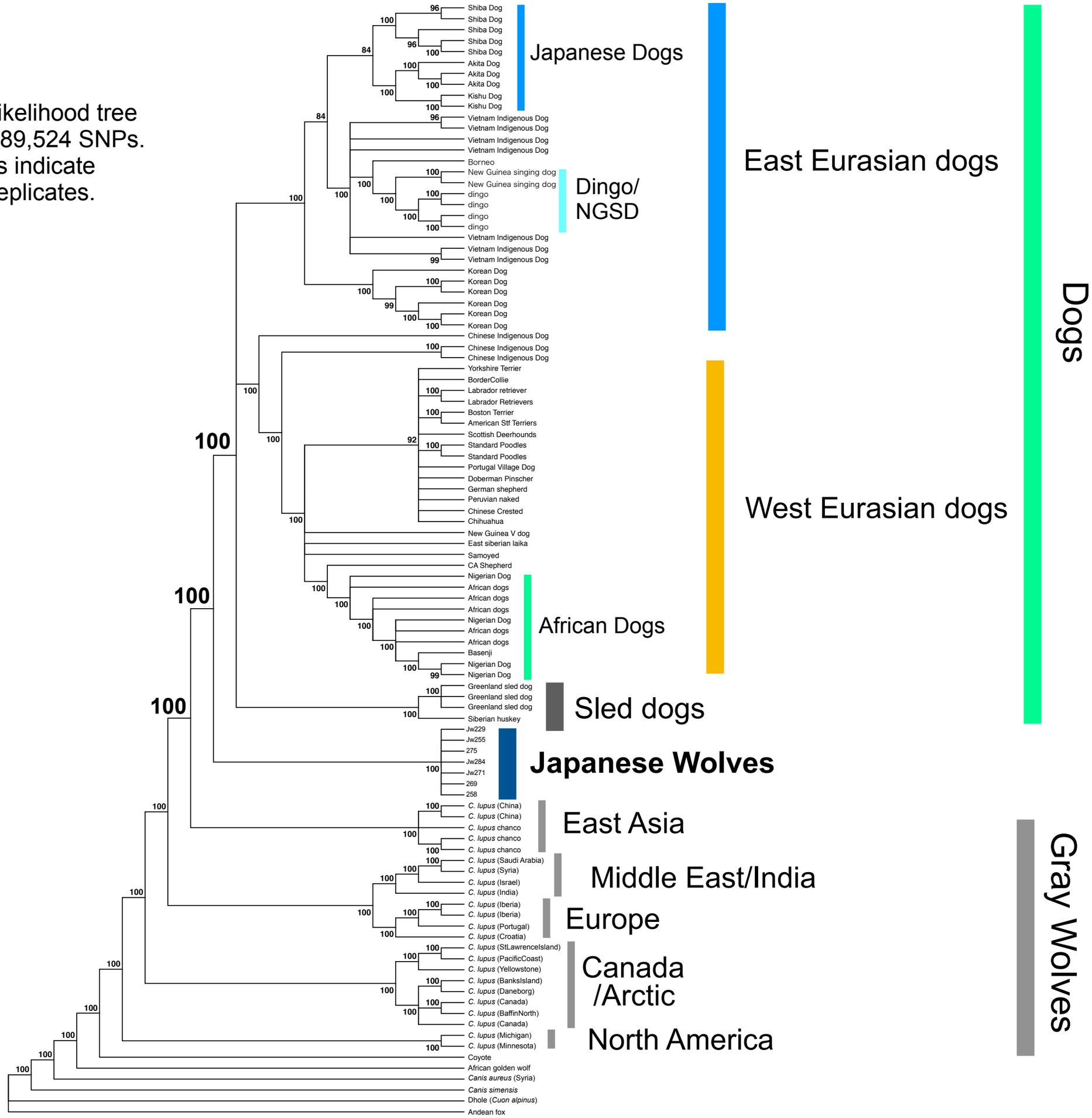


Figure S8
Phylogenetic tree constructed by SVDquartets based on 489,524 SNPs. Node labels indicate bootstrap replicates.

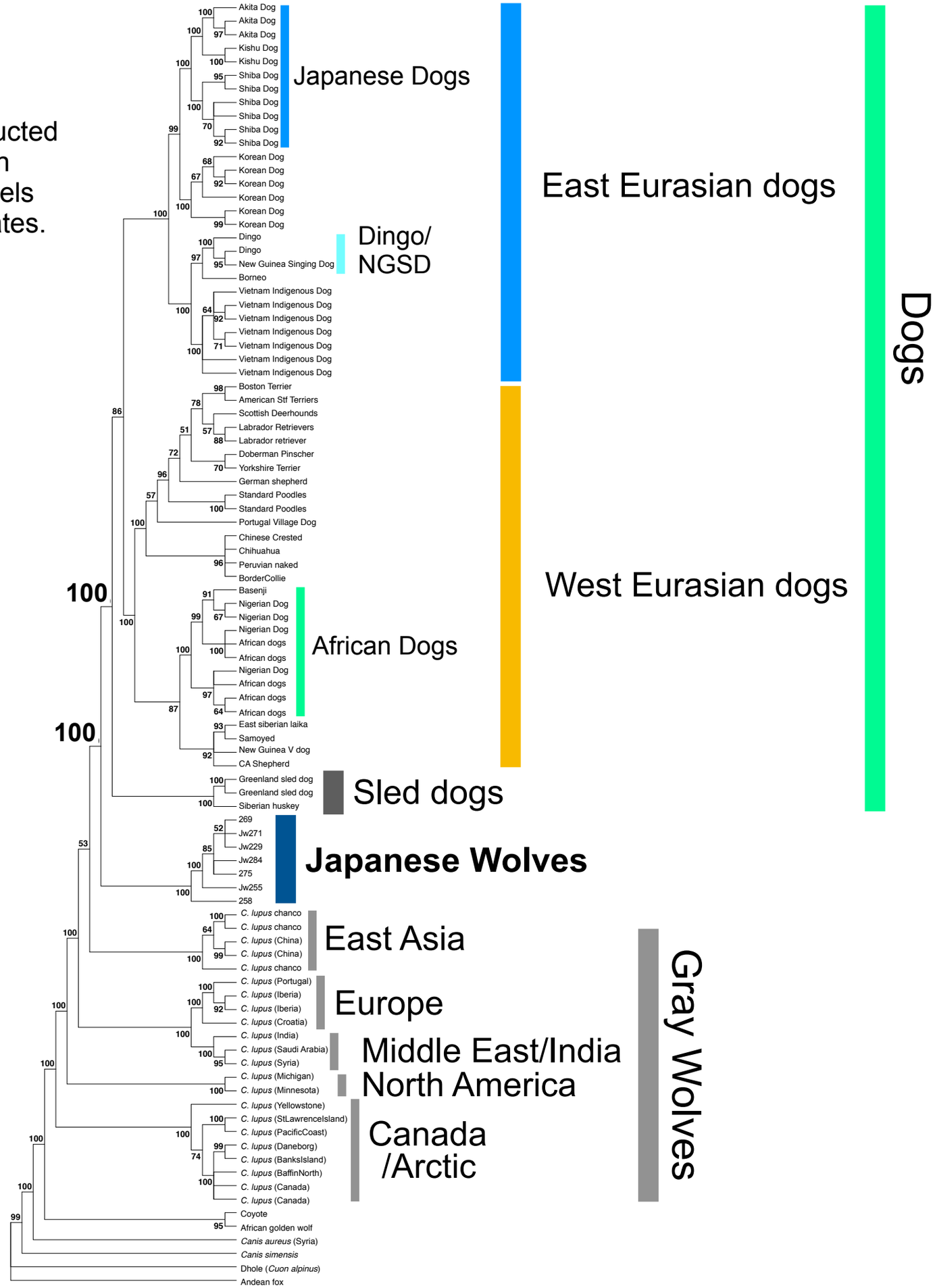
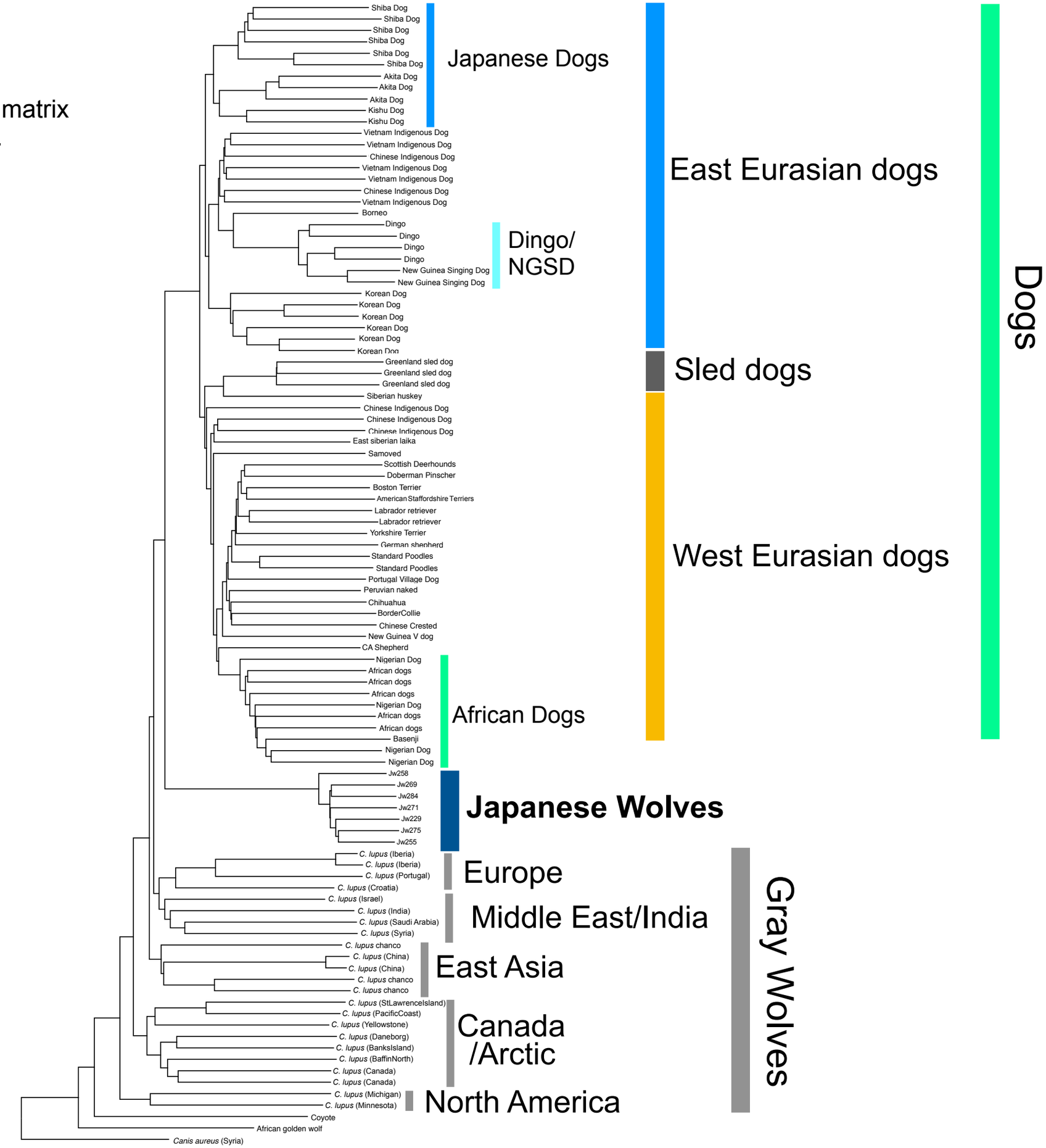


Figure S9
NJ tree based on IBS matrix from 1,992,260 SNPs.



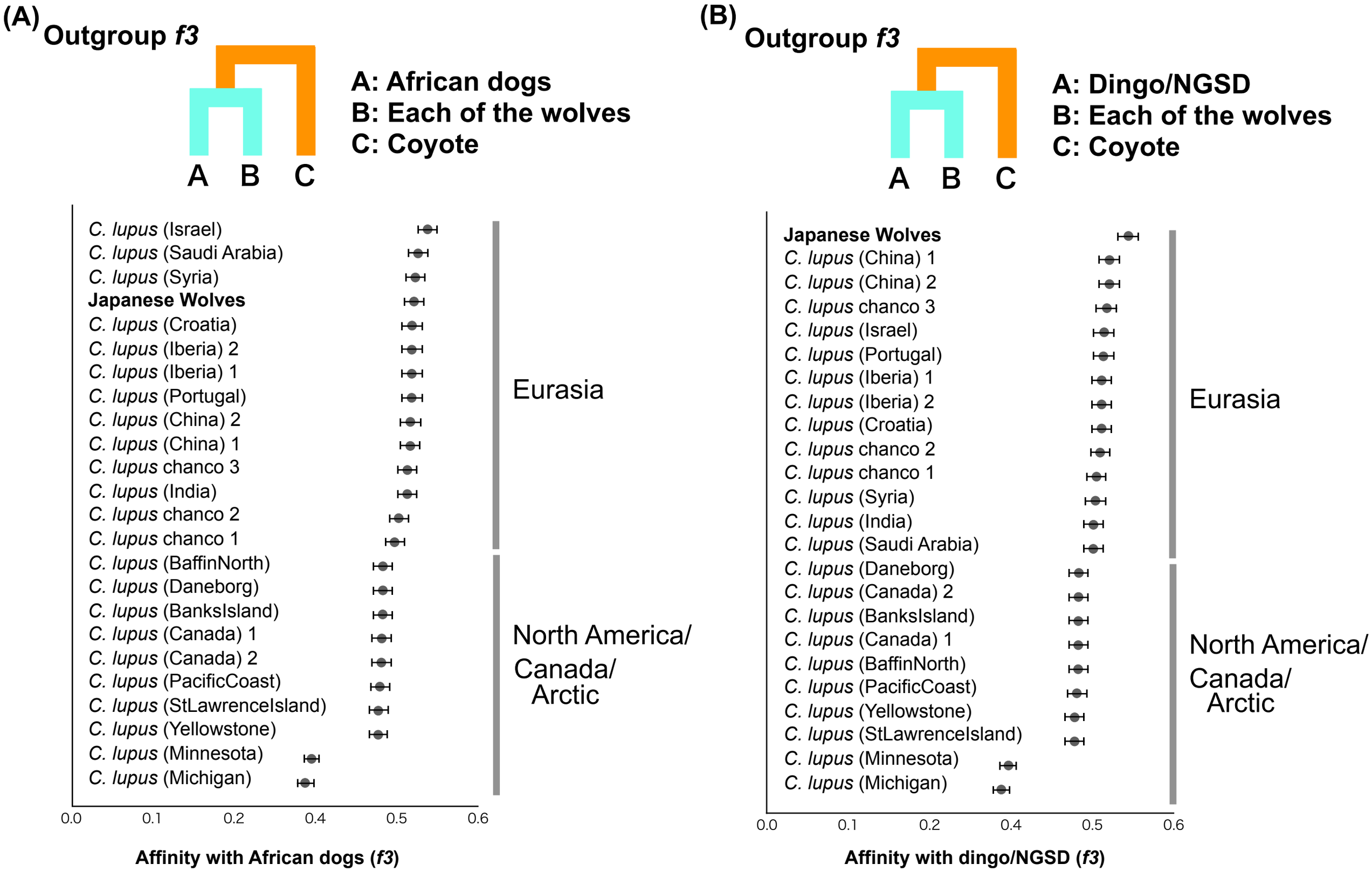
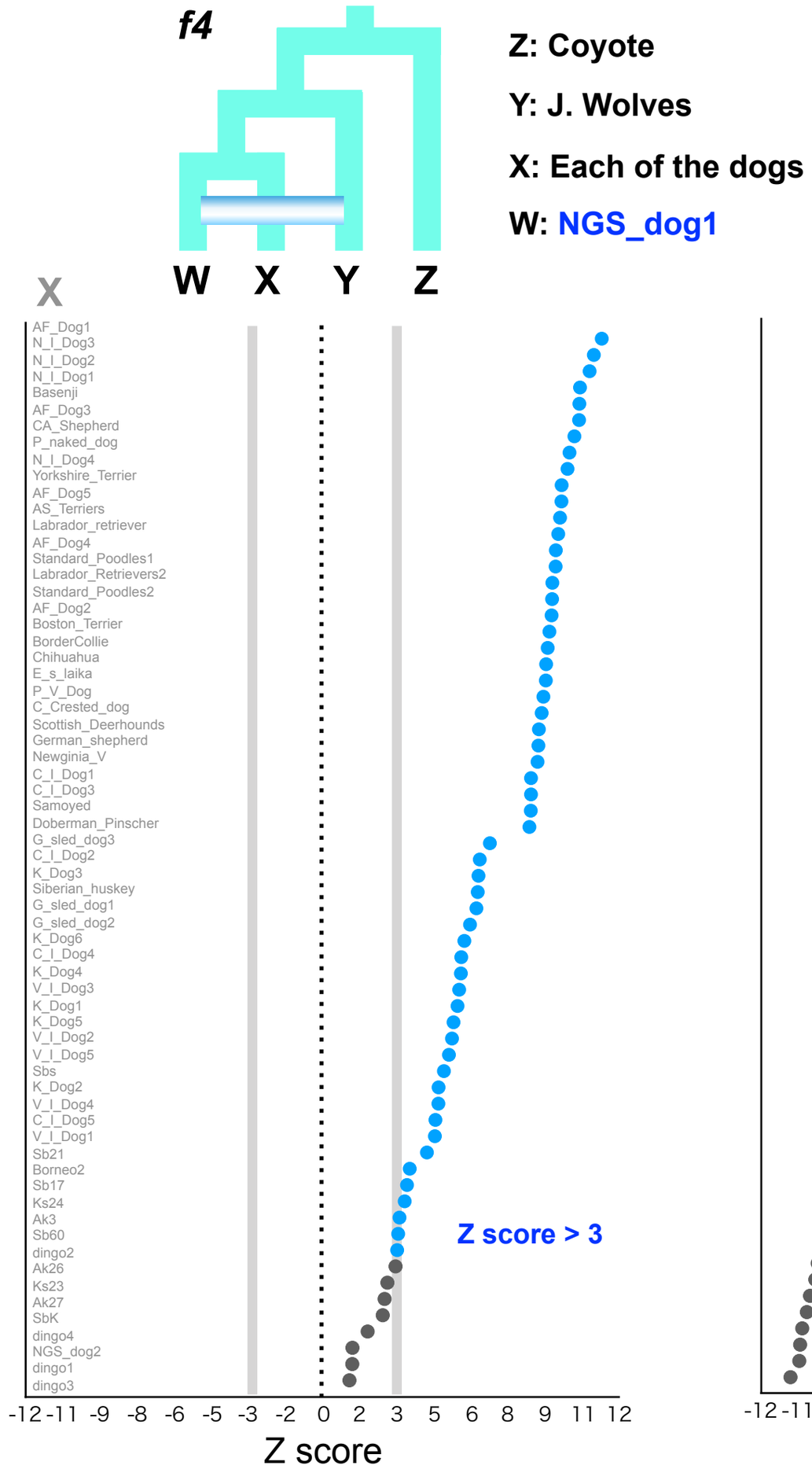


Figure S10
Shared genetic drift between African dogs (A) and Dingo/NGSD (B) and gray wolves measured by outgroup *f3* statistics. Each of the African dogs and Dingo/NGSD individuals were used as populations. Each *f3* statistical value is plotted in order of highest to lowest value from the top, and the names of the wolves are shown on the left side of each panel. Error bars represent standard errors.

Figure S11
f4 statistics testing the genetic affinity of the Japanese wolf with all other dogs. All Japanese wolves were used as a population. Z scores for each combination are plotted. We computed *f4* statistics where W in the schematic representation is shown and fixed in each panel and X represented any possible other dogs. Each Z score is listed in order of highest to lowest value from the top. Dotted line shows the Z score 3. Large size graphs of highest (NGS_dog1) and lowest (Nigerian Dog2) affinity to the Japanese wolf are show in the first two panels. The names of the dogs are shown on the left or right sides of the panels. Small size panels of these two are surrounded by gray squares.



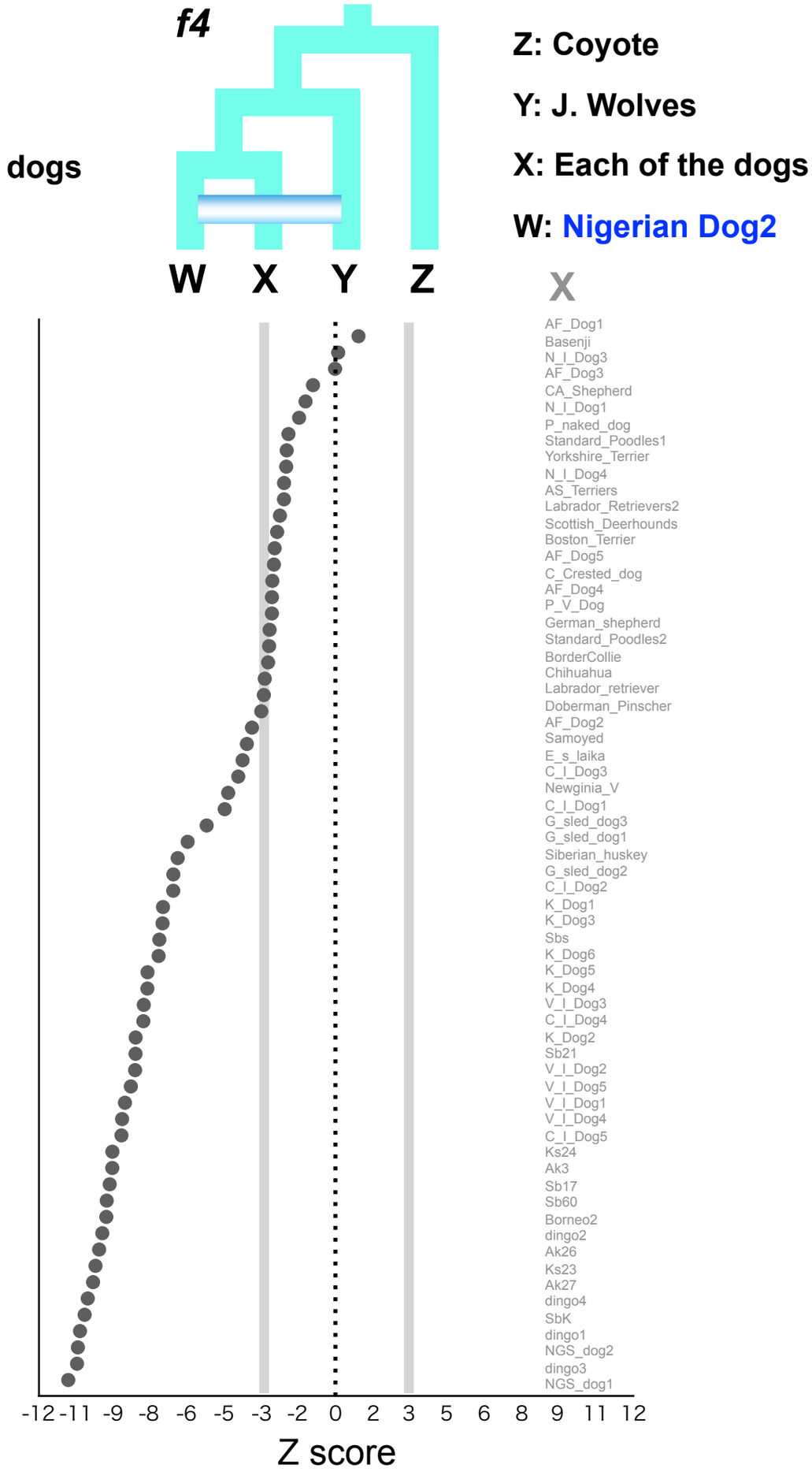
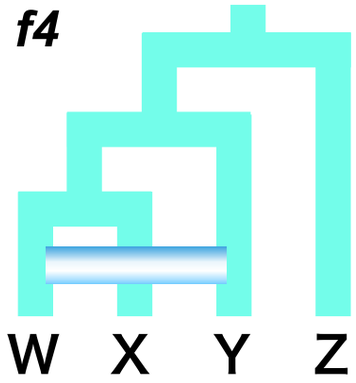


Figure S11



Z: Coyote

Y: J. Wolves

X: Each of the dogs

W: Dog shown in the panel

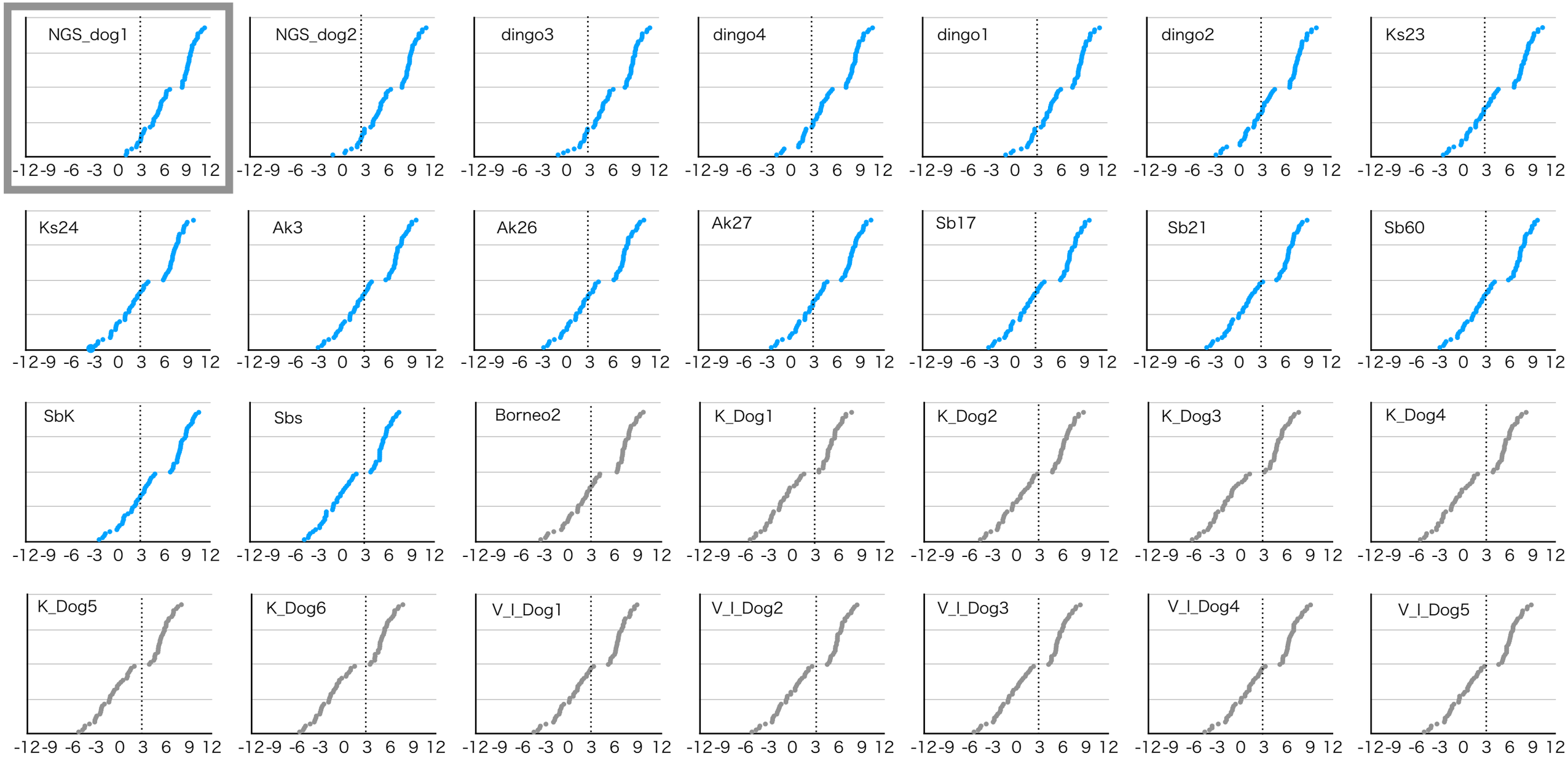


Figure S11 continued

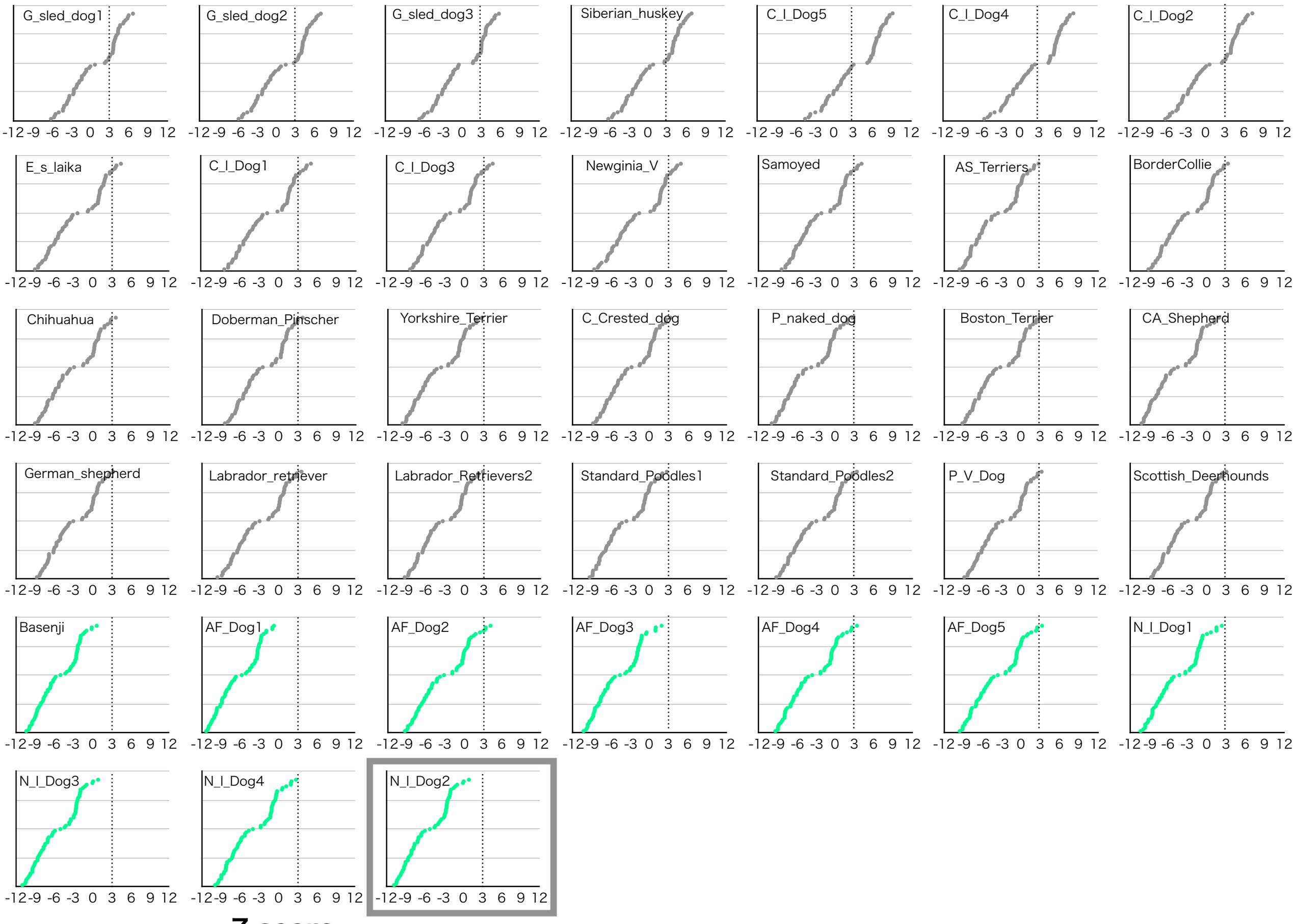


Figure S11 continued

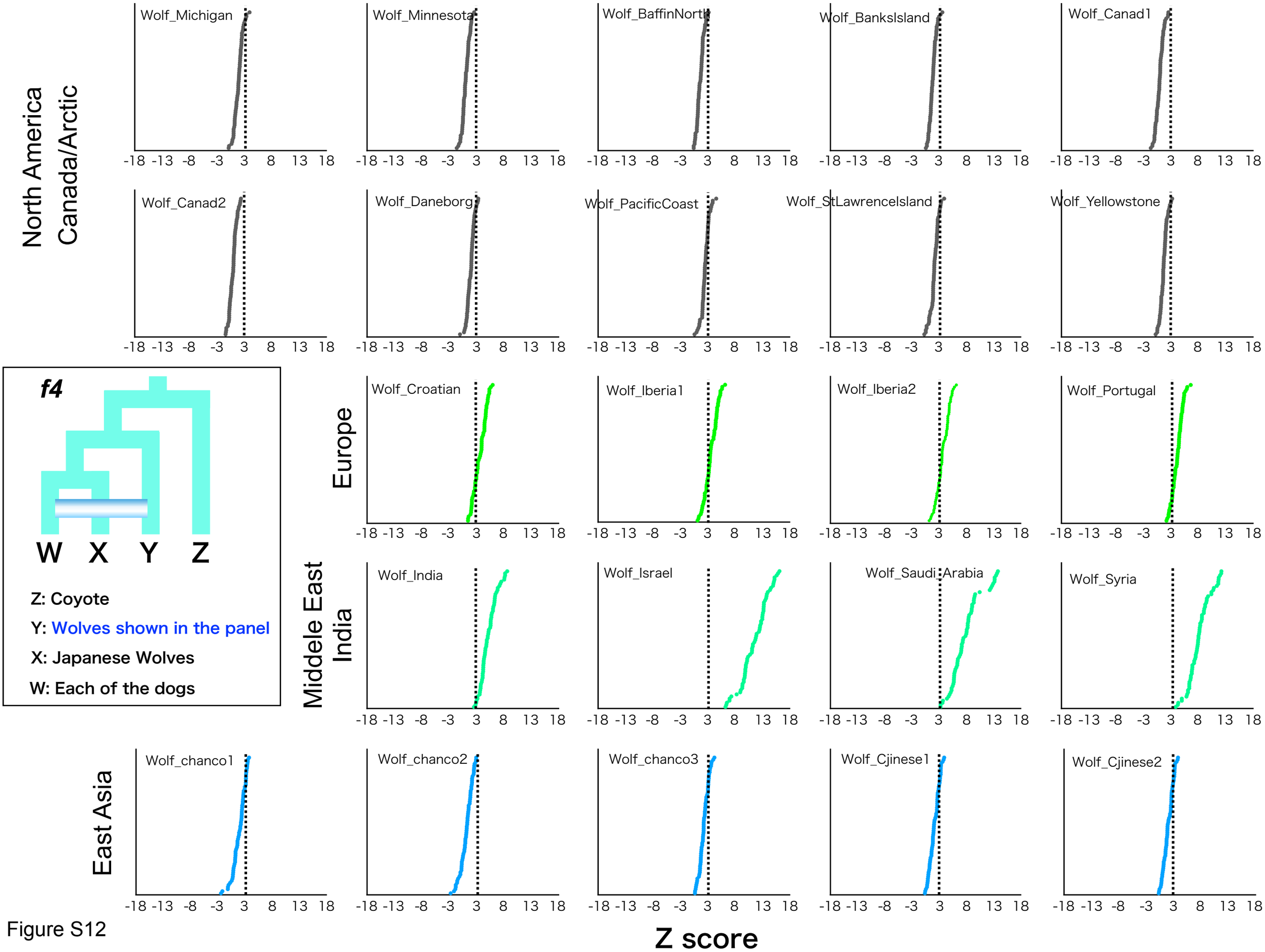
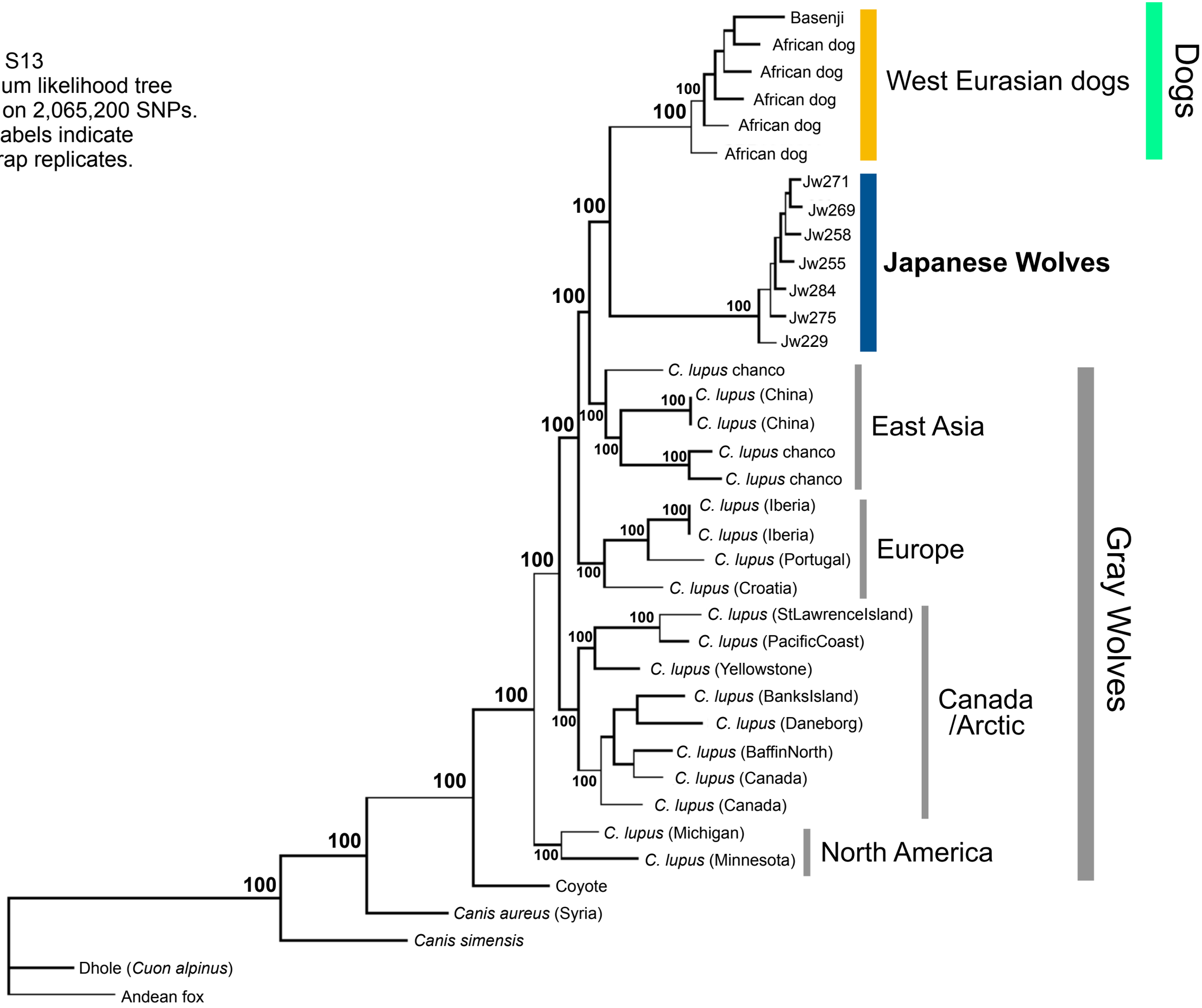

North America
Canada/Arctic
Wolf_Michigan
Wolf_Minnesota
Wolf_BaffinNorth
Wolf_BanksIsland
Wolf_Canad1
Wolf_Canad2
Wolf_Daneborg
Wolf_PacificCoast
Wolf_StLawrenceIsland
Wolf_Yellowstone
f4
W X Y Z
Z: Coyote
Y: Wolves shown in the panel
X: Japanese Wolves
W: Each of the dogs
Europe
Wolf_Croatian
Wolf_Iberia1
Wolf_Iberia2
Wolf_Portugal
Middele East
India
Wolf_India
Wolf_Israel
Wolf_Saudi_Arabia
Wolf_Syria
East Asia
Wolf_chanco1
Wolf_chanco2
Wolf_chanco3
Wolf_Cjinese1
Wolf_Cjinese2
-18 -13 -8 -3 3 8 13 18
Z score

Figure S12

Figure S12

f4 statistics testing the genetic affinity of gray wolves shown in each panels with all dogs. Z scores for each combination are plotted in order of highest to lowest value from the top. We computed *f*4 statistics where Y in the schematic representation is shown and fixed in each panel and W represented any possible other dogs. Dotted line shows the Z score 3.

Figure S13
Maximum likelihood tree based on 2,065,200 SNPs. Node labels indicate bootstrap replicates.



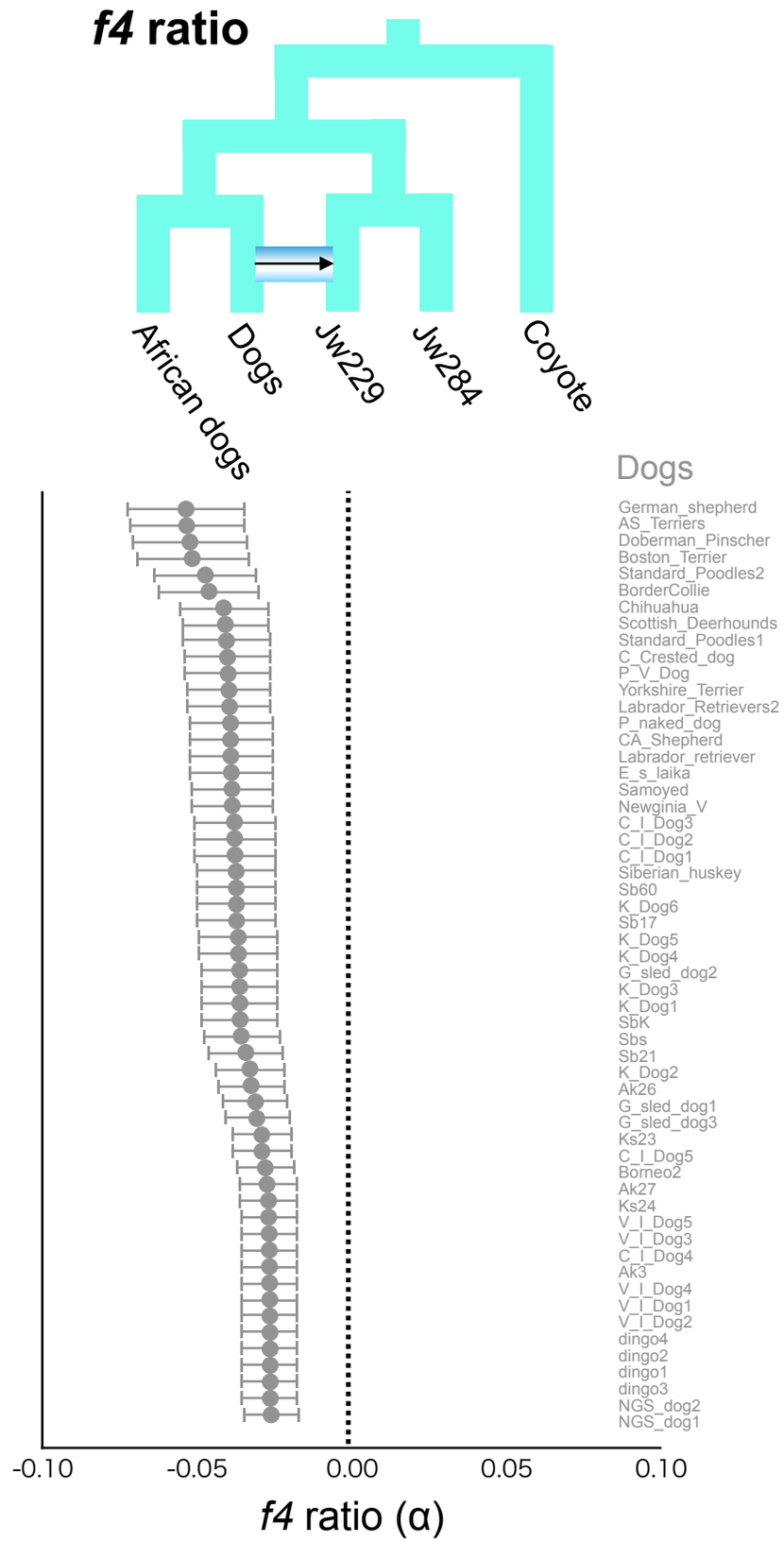


Figure S14
f4-ratio test to estimate proportion of genome introgression from dogs to the Japanese wolf. Each *f4*-ratio α value is plotted in order of lowest to highest value from the top, and the names of the dogs are shown on the right side of the panel. Error bars represent standard errors.

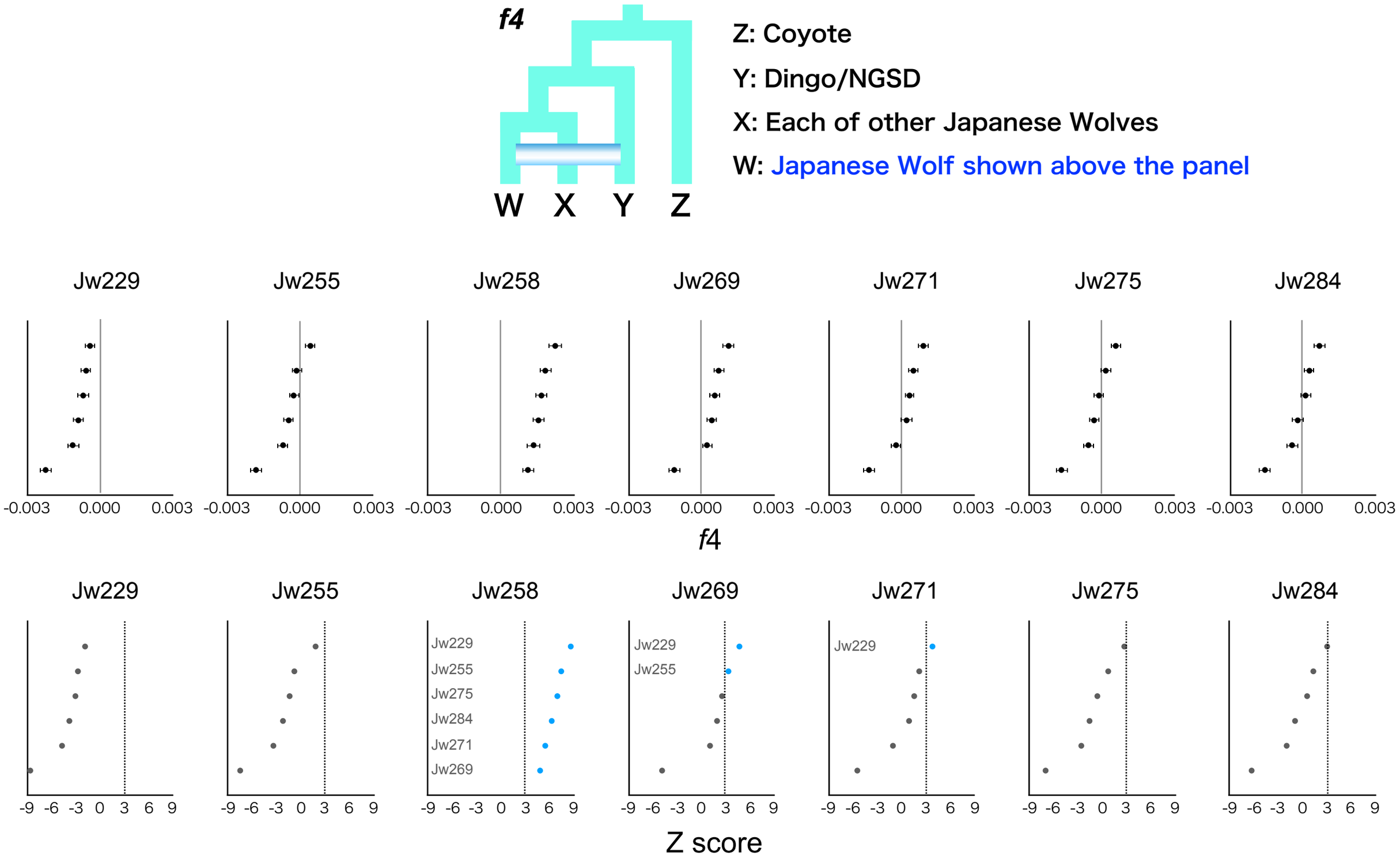


Figure S15
(A) *f4* statistics testing the difference of the affinity to dingo/NGSD between the Japanese wolf individuals. *f4* statistics (upper panels) and Z score (lower panels) value is plotted in order of highest to lowest value from the top. Z score above 3 is colored in blue. When the Japanese wolf individual showing a significant affinity to dingo/NGSD, the names of the Japanese wolf individual at the position X in the schematic representation are shown on the left side of Z score panel.

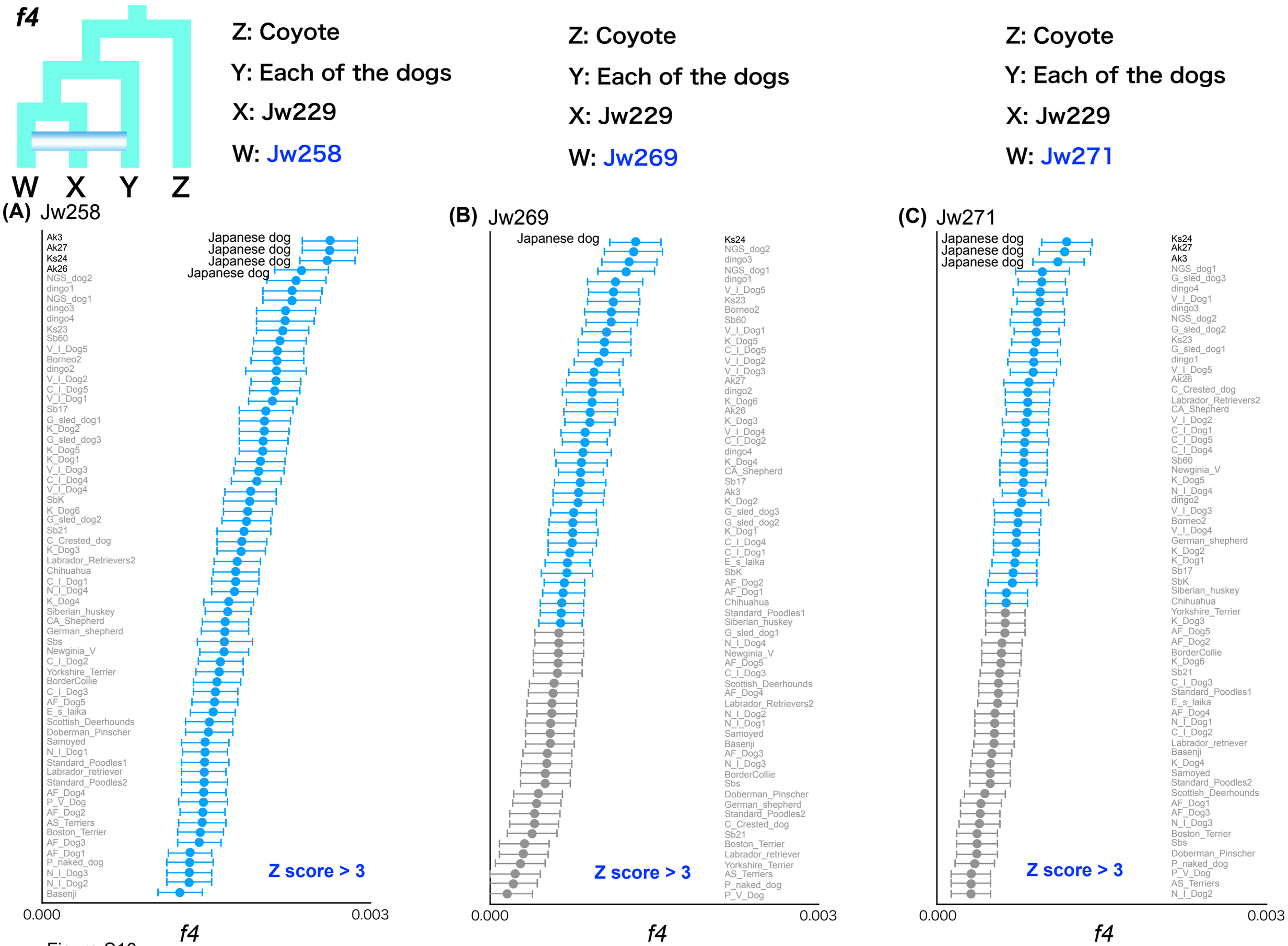


Figure S16

(A) *f4* statistics testing the relationships between (A) Jw258, (B) Jw269, and (C) Jw271 and all dog individuals. Each Z score is plotted in order of highest to lowest value from the top, and the names of dogs are shown on theft or right sides of each panel (see table S2). Z score above 3 is colored in blue.

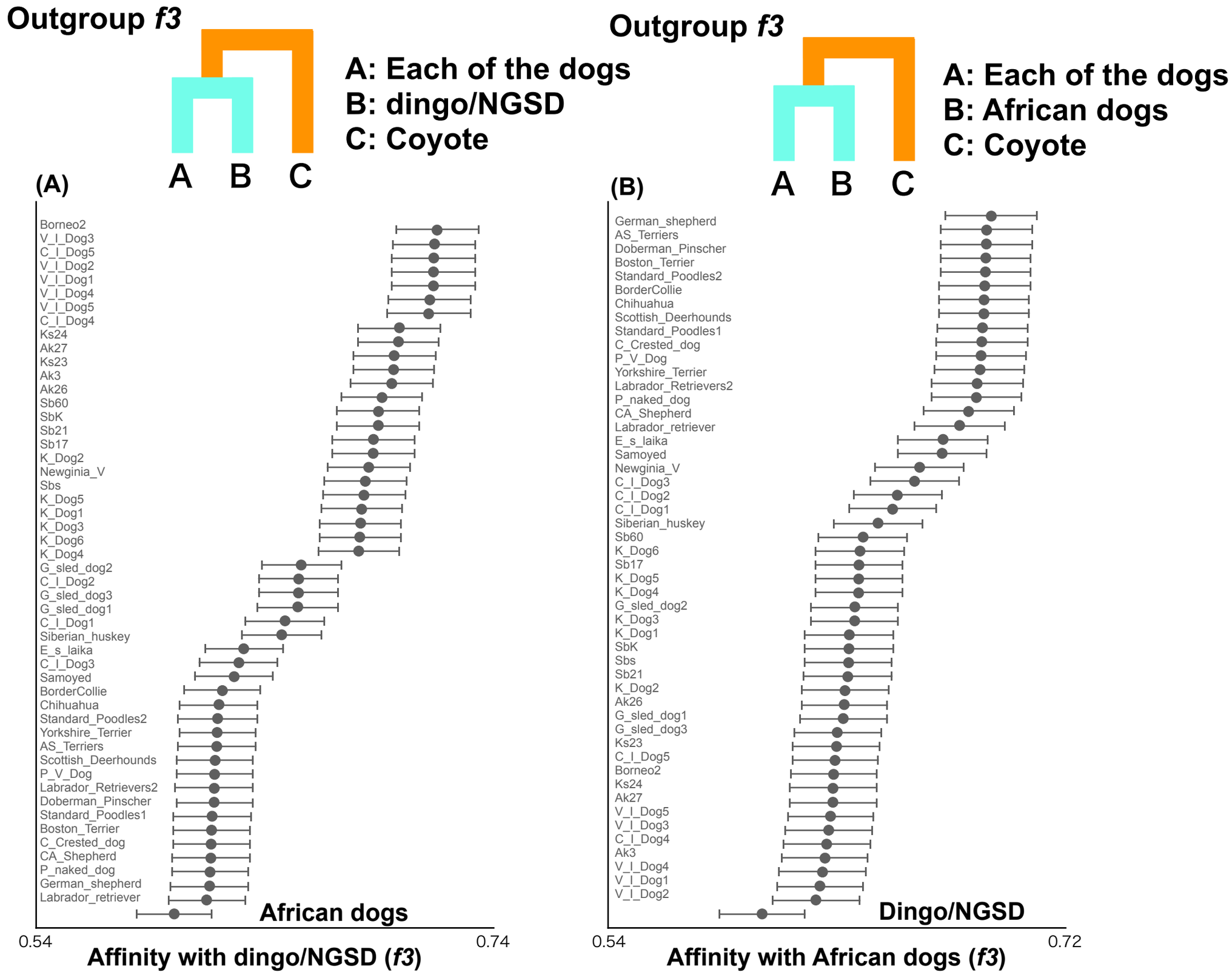
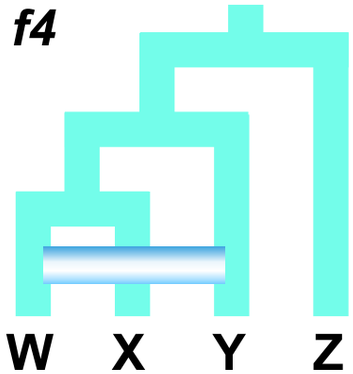


Figure S17
Shared genetic drift between (A) dingo/NGSD and (B) African dogs and all dogs measured by outgroup *f*3 statistics. Each of the African dogs and dingo/NGSD individuals were used as populations. Each *f3* value is plotted in order of highest to lowest value from the top, and the names of the dogs are shown on the left side of the panel. Error bars represent standard errors.

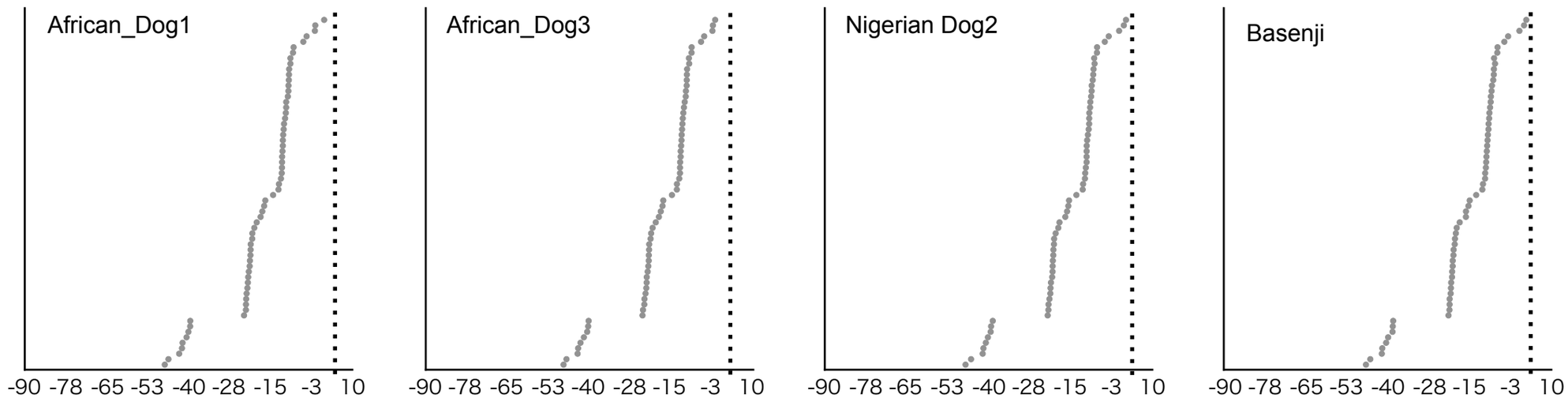


Z: Coyote

Y: NGSD1

X: Each of the dogs

W: Dog shown in the panel



Z: Coyote

Y: Basenji

X: Each of the dogs

W: Dog shown in the panel

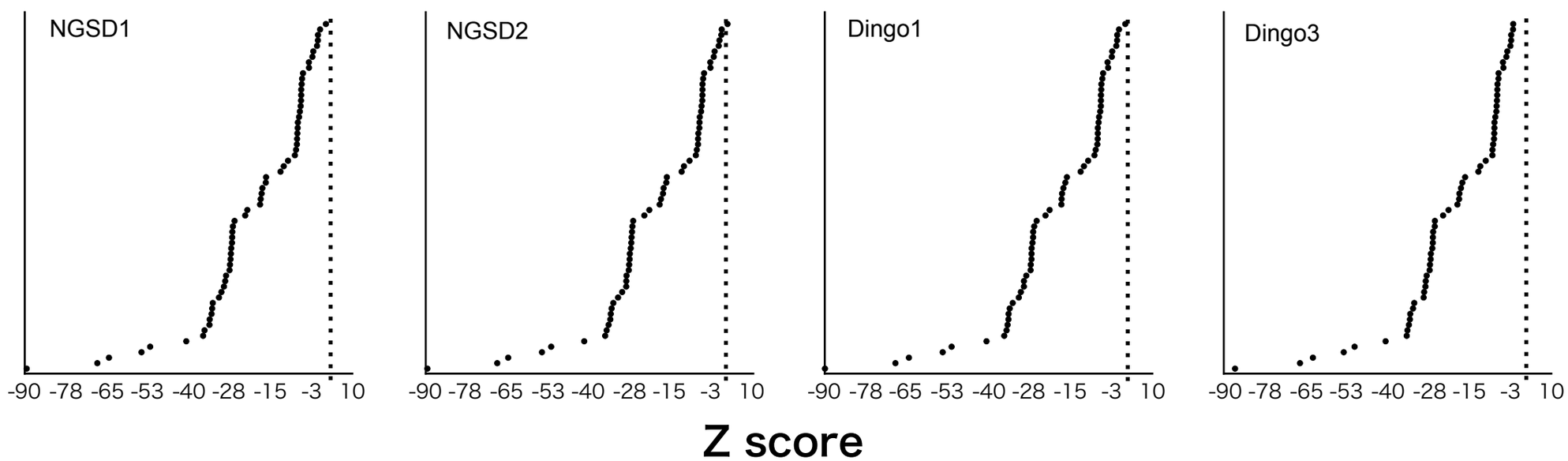
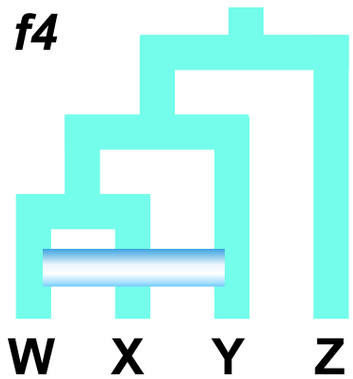


Figure S18
f4 statistics testing the affinity of NGSD1 with African dogs (upper panels) and that of Basenji with dingo/NGSD dogs (lower panels). All Z scores were under 3.



Z: Coyote

Y: Dingo/NGSD

X: African dogs

W: Each of the dogs

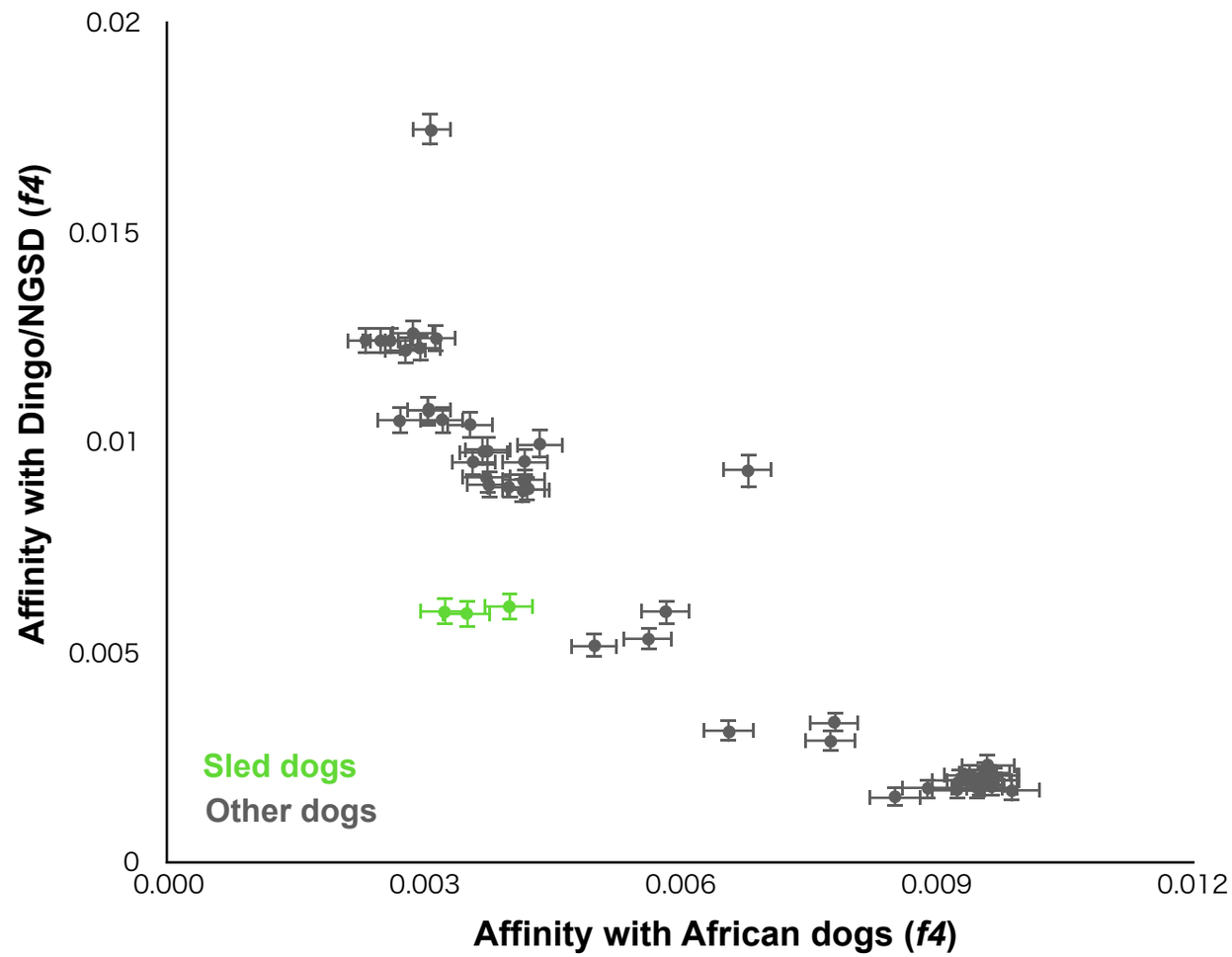
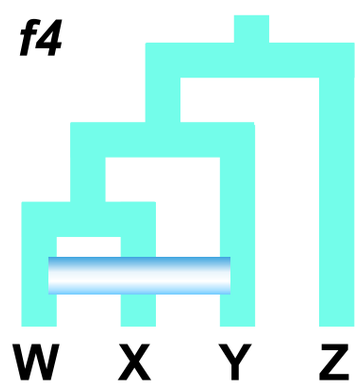


Figure S19
f4 statistics testing whether dogs share more alleles with African dogs (x-axis) or dingo/NGSD (y-axis) compared with dingo/NGSD and African dogs, respectively. Dots show the *f4* statistics, and horizontal and vertical error bars represent standard errors for the test with the African dogs (x-axis) and dingo/NGSD (y-axis), respectively. Each of the African dogs and dingo/NGSD individuals were used as populations.



Z: Coyote

Y: African dogs

X: Dingo/NGSD

W: Each of the dogs

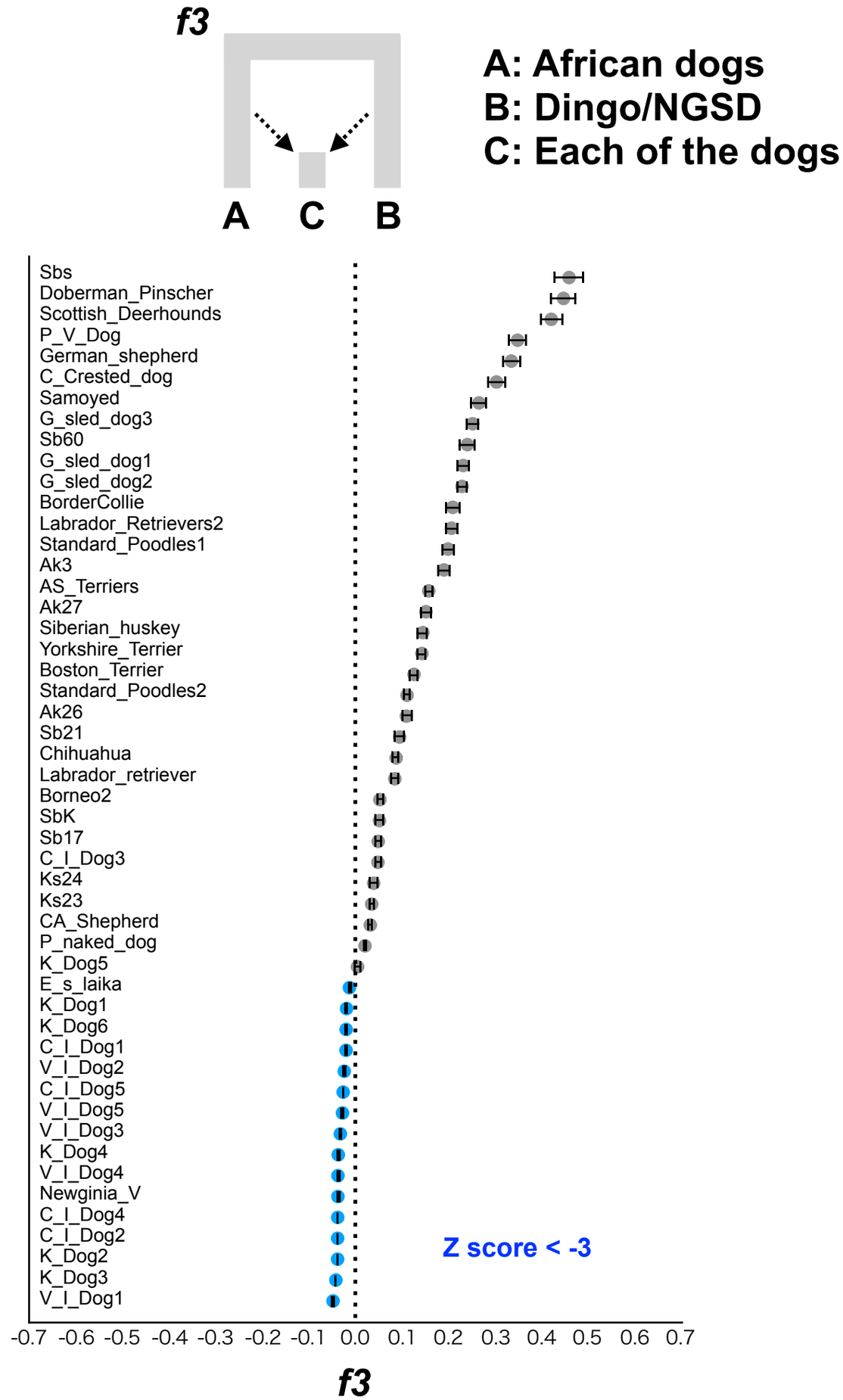


Figure S20
f3 statistics testing the genomic mixture of African and Dingo/NGSD dogs in all dogs. Z score under -3 is colored in blue. Each of the African dogs and Dingo/NGSD individuals were used as populations. Each *f3* value is plotted in order of highest to lowest value from the top, and the names of the dogs are shown on the left side of the panel. Error bars represent standard errors.

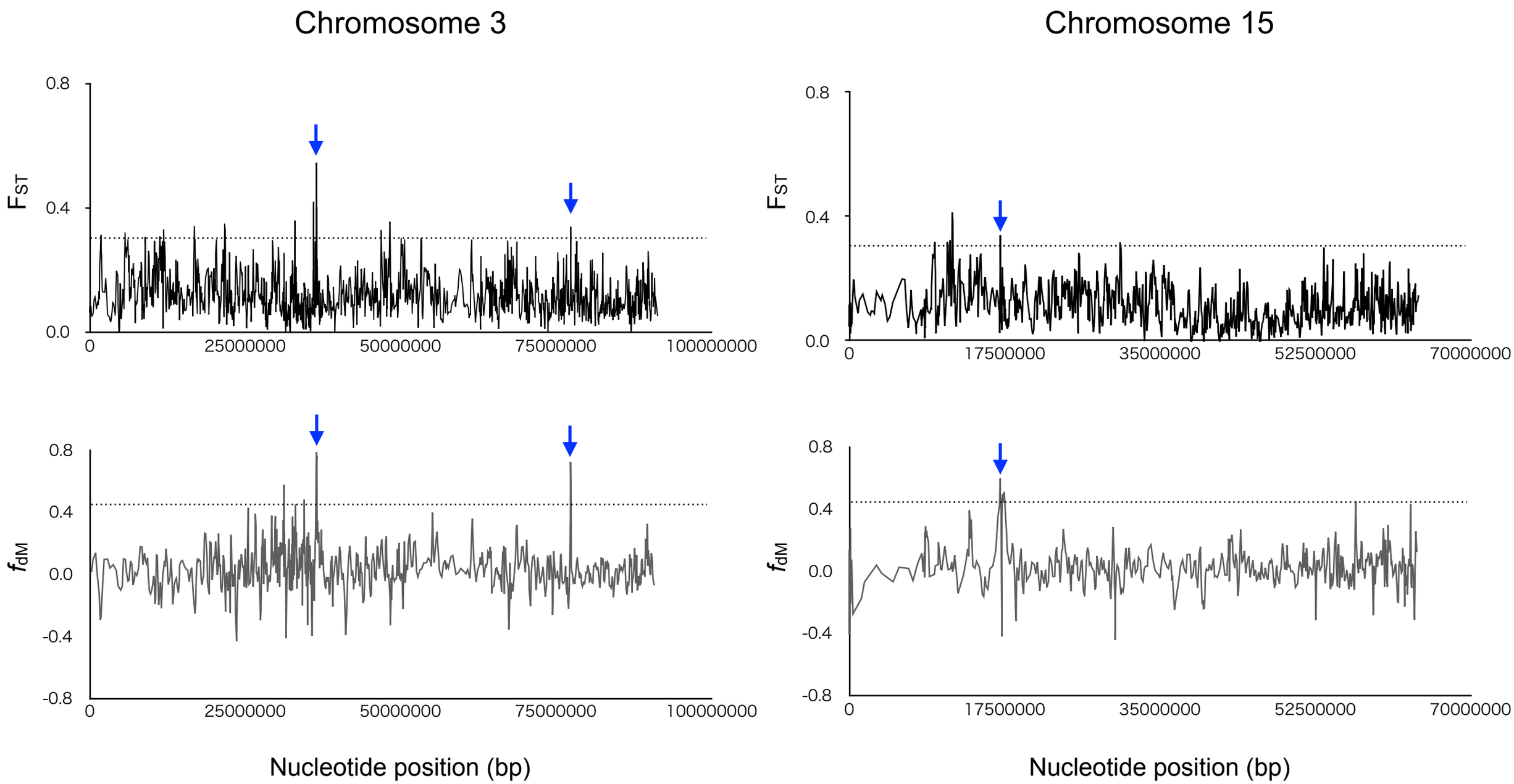


Figure S21
Sliding window analyses of the F_{ST} values (y-axis: upper panel) and f_{dM} (y-axis: lower panel) in windows of 50 SNPs using a 25 SNPs slide across scaffolds (x-axis). Dashed lines show the 99th percentiles. Blue arrow indicate overlapping regions above 99th percentiles between upper and lower panels.

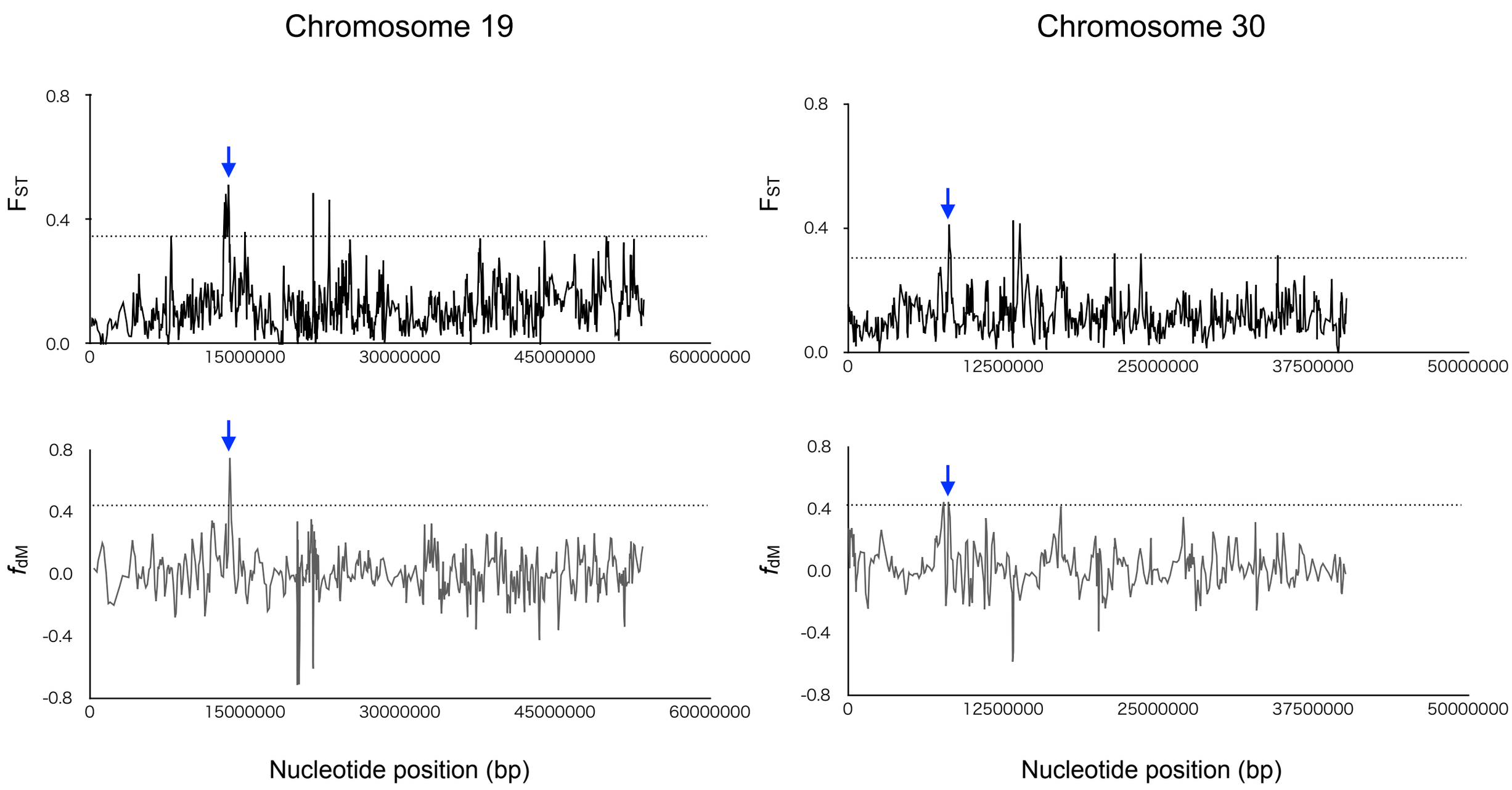


Figure S21 continued

Table S1 Determined sequences in this study

Wolf/Dog	ID	Period	Isolation site (Prefecture)	Mapped reads count	Total mapped reads
Japanese Wolves	Leiden_b (Jentink 1887 b RMNH.MAM.39183)	Edo	Leiden	149 M	22.35 Gb
Japanese Wolves	Leiden_c (Jentink 1887 c RMNH.MAM.39181)	Edo	Leiden	188 M	28.2 Gb
Japanese Wolves	Jw255	Edo-Meiji	Yamanashi Pref.	1881 M	282.2 Gb
Japanese Wolves	Jw271	Edo-Meiji	Iwate Pref.	820 M	123 Gb
Japanese Wolves	Jw284 (ZMB_Mam_048817)	Meiji	Berlin	1237 M	185.6 Gb
Japanese Wolves	Jw275	Edo-Meiji	Shimane Pref.	134 M	46.5 Gb
Japanese Wolves	Jw229	Edo	Kochi Pref.	661 M	99.2 Gb
Japanese Wolves	Jw258	Edo-Meiji	Nagano Pref.	262 M	110 Gb
Japanese Wolves	Jw269	Edo-Meiji	Nagano Pref.	193 M	61.8 Gb
Dog (Akita)	Akita26	Modern	—	847 M	127.1 Gb
Dog (Akita)	Akita27	Modern	—	808 M	121.2 Gb
Dog (Akita)	Akita3	Modern	—	772 M	115.8 Gb
Dog (Kishu)	Kishu23	Modern	—	832 M	124.8 Gb
Dog (Kishu)	Kishu24	Modern	—	634 M	95.1 Gb
Dog (Shiba)	Shiba17	Modern	—	632 M	94.8 Gb
Dog (Shiba)	Shiba21	Modern	—	517 M	77.6 Gb
Dog (Shiba)	Shiba60	Modern	—	668 M	100.2 Gb
Dog (Shiba)	ShibaKuro	Modern	—	472 M	70.8 Gb
Dog (Shiba)	ShibaShiro	Modern	—	697 M	104.6 Gb
Dog (Shiba)	Jm	Modern	—	391 M	58 Gb

Table S2 Sample information

Accession#	Sub-species	Common name	IDs in analyses	PCA & ADMIXTURE (modern)	PCA with Pleistocene canid	PCA & ADMIXTURE with Leiden b, c and Honshu Wolf	Phylogenetic analysis	f4 and f4-ratio with Leiden c	f4 with Leiden b	f3, f4, and f4-ratio	Fst sliding window	fdM sliding window	TreeMix
				Without missing data	Without missing data	With missing data	Without missing data	Without missing data	Without missing data	Without missing data	Without missing data	Without missing data	Without missing data
Depositing	Japanese Wolf	Japanese Wolf	Jw255	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw271	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw284	O	O	O	O	O	O	O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw275	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw229	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw258	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Jw269	O	O	O	O			O		O	O
Depositing	Japanese Wolf	Japanese Wolf	Leiden_b			O			O				
Depositing	-	Jama-inu	Leiden_c			O		O					
ERS5374233	Japanese Wolf	Honshu Wolf				O							
Depositing	Dog	Kishu	Ks23	O	O	O	O	O	O	O	O	O	O
Depositing	Dog	Kishu	Ks24	O	O	O	O	O	O	O	O	O	O
Depositing	Dog	Akita	Ak26	O	O	O	O	O	O	O	O	O	O
Depositing	Dog	Akita	Ak27	O	O	O	O	O	O	O	O	O	O
Depositing	Dog	Akita	Ak3	O	O	O	O	O	O	O	O	O	O
Depositing	Dog	Shiba	Sb17	O	O	O	O	O	O	O	O	O	O
Depositing	Dog	Shiba	Sb21	O	O	O	O	O	O	O	O	O	O
Depositing	Dog	Shiba	Sb60	O	O	O	O	O	O	O	O	O	O
Depositing	Dog	Shiba	SbK	O	O	O	O	O	O	O	O	O	O
Depositing	Dog	Shiba	Sbs	O	O	O	O	O	O	O	O	O	O
Depositing	Dog	Shiba	Jm	O	O	O	O				O	O	O
SRR10596312	Dog	Dingo	dingo1	O	O	O	O	O	O	O			O
SRR10596315	Dog	Dingo	dingo2	O	O	O	O	O	O	O			O
SRR10596318	Dog	Dingo	dingo3	O	O	O	O	O	O	O			O
SRR10596321_1	Dog	Dingo	dingo4	O	O	O	O	O	O	O			O
SRR10597858	Dog	New Guinea Singing Dog	NGS_dog1	O	O	O	O	O	O	O			O
SRR10597859	Dog	New Guinea Singing Dog	NGS_dog2	O	O	O	O	O	O	O			O
SRR2827578_1	Dog	Chinese Indigenous Dog	C_I_Dog1	O	O	O	O	O	O	O			
SRR2827583_1	Dog	Chinese Indigenous Dog	C_I_Dog2	O	O	O	O	O	O	O			
SRR2827587_1	Dog	Chinese Indigenous Dog	C_I_Dog3	O	O	O	O	O	O	O			
SRR2827588_1	Dog	Chinese Indigenous Dog	C_I_Dog4	O	O	O	O	O	O	O			
SRR2827589_1	Dog	Chinese Indigenous Dog	C_I_Dog5	O	O	O	O	O	O	O			
SRR2827595_1	Dog	Vietnamese Indigenous Dog	V_I_Dog1	O	O	O	O	O	O	O			O
SRR2827596_1	Dog	Vietnamese Indigenous Dog	V_I_Dog2	O	O	O	O	O	O	O			O
SRR2827597_1	Dog	Vietnamese Indigenous Dog	V_I_Dog3	O	O	O	O	O	O	O			O
SRR2827598_1	Dog	Vietnamese Indigenous Dog	V_I_Dog4	O	O	O	O	O	O	O			O
SRR2827599_1	Dog	Vietnamese Indigenous Dog	V_I_Dog5	O	O	O	O	O	O	O			O
SRR5177184_1	Dog	DongGyeongi	K_Dog1	O	O	O	O	O	O	O			O
SRR5177186_1	Dog	DongGyeongi	K_Dog2	O	O	O	O	O	O	O			O
SRR5177187_1	Dog	DongGyeongi	K_Dog3	O	O	O	O	O	O	O			O
SRR5177188_1	Dog	DongGyeongi	K_Dog4	O	O	O	O	O	O	O			O
SRR5177189_1	Dog	DongGyeongi	K_Dog5	O	O	O	O	O	O	O			O
SRR5177190_1	Dog	DongGyeongi	K_Dog6	O	O	O	O	O	O	O			O
SRR11193488_1	Dog	Greenland sled dog	G_sled_dog1	O	O	O	O	O	O	O			O
SRR11193493_1	Dog	Greenland sled dog	G_sled_dog2	O	O	O	O	O	O	O			O
SRR11193499_1	Dog	Greenland sled dog	G_sled_dog3	O	O	O	O	O	O	O			O
SRR2827574_1	Dog	Siberian huskey	Siberian_huskey	O	O	O	O	O	O	O			O
SRR2827565_1	Dog	East siberian laika	E_s_laika	O	O	O	O	O	O	O			O
SRR2827573_1	Dog	Samoyed	Samoyed	O	O	O	O	O	O	O			O
SRR5381785_1	Dog	Central Asian Shepherd	CA_Shepherd	O	O	O	O	O	O	O			O
SRR1594157_1	Dog	Chinese Crested dog	C_Crested_dog	O	O	O	O	O	O	O			
SRR2827564_1	Dog	Chihuahua	Chihuahua	O	O	O	O	O	O	O			
SRR2827572_1	Dog	Peruvian naked dog	P_naked_dog	O	O	O	O	O	O	O			
SRR3384031_1	Dog	Portugal Village Dog	P_V_Dog	O	O	O	O	O	O	O			O
SRR3466766_1	Dog	BorderCollie	BorderCollie	O	O	O	O	O	O	O			
SRR6297982_1	Dog	Doberman Pinscher	Doberman Pinscher	O	O	O	O	O	O	O			O
SRR6376929_1	Dog	American Staffordshire Terriers	AS_Terriers	O	O	O	O	O	O	O			O
SRR7107687	Dog	Borneo Village Dog	Borneo2	O	O	O	O	O	O	O			O
SRR10208218_1	Dog	Boston Terrier	Boston Terrier	O	O	O	O	O	O	O			O
SRR10250959_1	Dog	Scottish Deerhounds	Scottish Deerhounds	O	O	O	O	O	O	O			O
SRR9117685_1	Dog	German shepherd	German shepherd	O	O	O	O	O	O	O			O
SRR11787131_1	Dog	Labrador retriever	Labrador retriever	O	O	O	O	O	O	O			O
SRR9962250_1	Dog	Labrador Retriever	Labrador_Retrievers2	O	O	O	O	O	O	O			O
SRR7107697	Dog	Newginia Village Dog	Newginia_V	O	O	O	O	O	O	O			O
SRR7764562_1	Dog	Yorkshire Terrier	Yorkshire Terrier	O	O	O	O	O	O	O			O
SRR8163589_1	Dog	Standard Poodle	Standard_Poodles1	O	O	O	O	O	O	O			O
SRR8163591_1	Dog	Standard Poodle	Standard_Poodles2	O	O	O	O	O	O	O			O
SRR2827591_1	Dog	Nigerian Indigenous Dog	N_I_Dog1	O	O	O	O	O	O	O	O	O	O
SRR2827592_1	Dog	Nigerian Indigenous Dog	N_I_Dog2	O	O	O	O	O	O	O	O	O	O
SRR2827593_1	Dog	Nigerian Indigenous Dog	N_I_Dog3	O	O	O	O	O	O	O	O	O	O
SRR2827594_1	Dog	Nigerian Indigenous Dog	N_I_Dog4	O	O	O	O	O	O	O	O	O	O
SRR2149861_1	Dog	Basenji	Basenji	O	O	O	O	O	O	O	O	O	O
SRR3603090_1	Dog	African dogs	AF_Dog1	O	O	O	O	O	O	O	O	O	O
SRR3603171_1	Dog	African dogs	AF_Dog2	O	O	O	O	O	O	O	O	O	O
SRR3603205_1	Dog	African dogs	AF_Dog3	O	O	O	O	O	O	O	O	O	O
SRR3603206_1	Dog	African dogs	AF_Dog4	O	O	O	O	O	O	O	O	O	O
SRR3603236_1	Dog	African dogs	AF_Dog5	O	O	O	O	O	O	O	O	O	O
SRR2017901_1	Gray Wolf	Himalayan Wolf	Wolf_chanco1	O	O	O	O			O			O
SRR2017902_1	Gray Wolf	Himalayan Wolf	Wolf_chanco2	O	O	O	O			O			O
SRR2017904_1	Gray Wolf	Himalayan Wolf	Wolf_chanco3	O	O	O	O			O			O
SRR1518523_1	Gray Wolf	Gray Wolf (Portugal)	Wolf Portugal	O	O	O	O			O			O
SRR2149870_1	Gray Wolf	Israeli Wolf	Wolf_Israel	O	O	O	O			O			
SRR2149873_1	Gray Wolf	Croatian Wolf	Wolf_Croatian	O	O	O	O			O			O
SRR2149879_1	Gray Wolf	Chinese Wolf	Wolf_Chinese1	O	O	O	O			O			O
SRR2149880_1	Gray Wolf	Chinese Wolf	Wolf_Chinese2	O	O	O	O			O			O
SRR3384029_1	Gray Wolf	Iberian Wolf	Wolf_Iberia1	O	O	O	O			O			O
SRR3384030_1	Gray Wolf	Iberian Wolf	Wolf_Iberia2	O	O	O	O			O			O
SRR3574809	Gray Wolf	Indian Wolf	Wolf_India	O	O	O	O			O			O
SRR8049193_1	Gray Wolf	Grey Wolf (Saudi Arabia)	Wolf_Saudi_Arabia	O	O	O	O			O			O
SRR8049194_1	Gray Wolf	Grey Wolf (Syria)	Wolf_Syria	O	O	O	O			O			O
SRR8049195_1	Gray Wolf	Grey Wolf (Daneborg)	Wolf_Daneborg	O	O	O	O			O			O
SRR8066603_1	Gray Wolf	Grey Wolf (StLawrenceIsland)	Wolf_StLawrenceIsland	O	O	O	O			O			O
SRR8066604_1	Gray Wolf	Grey Wolf (PacificCoast)	Wolf_PacificCoast	O	O	O	O			O			O
SRR8066607_1	Gray Wolf	Grey Wolf (BaffinNorth)	Wolf_BaffinNorth	O	O	O	O			O			O
SRR8066615_1	Gray Wolf	Grey Wolf (BanksIsland)	Wolf_BanksIsland	O	O	O	O			O			O
SRR8380700_1	Gray Wolf	Grey Wolf (Michigan)	Wolf_Michigan	O	O	O	O			O			O
SRR8380701_1	Gray Wolf	Grey Wolf (Minnesota)	Wolf_Minnesota	O	O	O	O			O			O
SRR8380706_1	Gray Wolf	Grey Wolf (Canada)	Wolf_Canad1	O	O	O	O			O			O
SRR8380708_1	Gray Wolf	Grey Wolf (Canada)	Wolf_Canad2	O	O	O	O			O			O
SRR9095639_1	Gray Wolf	Grey Wolf (Yellowstone)	Wolf_Yellowstone	O	O	O	O			O			O
SRR3417116	Ancient dog	Ancient dog	Cherry Tree Cave Dog		O								
SRR3417117	Ancient dog	Ancient dog	Herxheim Dog		O								
SRR11193507	Ancient dog	Ancient dog	Zhokhov Dog		O								
ERR1341514	Ancient dog	Ancient dog	Newgrange Dog		O								
ERR4805960	Pleistocene canid	Pleistocene canid	Bunge-Toll-1885		O								
ERR4805984	Pleistocene canid	Pleistocene canid	Tirekhtyakh		O								
ERR4805988	Pleistocene canid	Pleistocene canid	Tumat 2		O								
ERR4805972	Pleistocene canid	Pleistocene canid	Ulakhan Sular		O								
SRR11193495	Pleistocene canid	Pleistocene canid	Yana RHS		O								
SRR7107770	out group	Coyote	Coyote	O	O	O	O	O	O	O		O	O
SRR8049189	out group	Dhole	Dhole				O						
SRR8049190	out group	Ethiopian wolf	Ethi_W				O						
SRR8049192	out group	Golden Jackal (Syria)	G_Jackal_Syria	O	O	O	O						
PRJCA000335	out group	African Golden Wolf	AGW	O	O	O	O						
SRR1066702	out group	Andean fox	ad_fox				O						

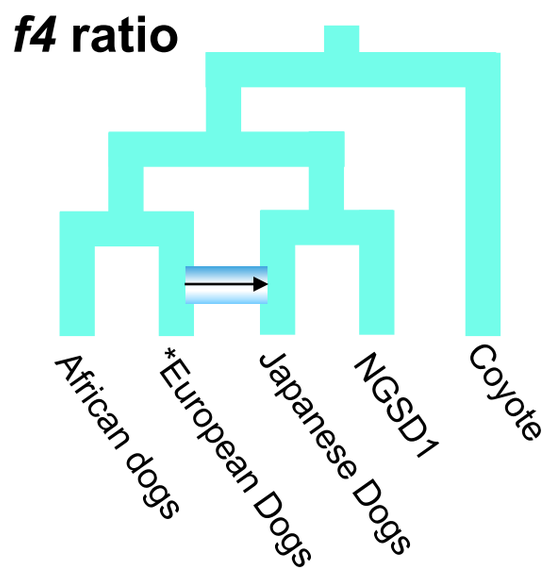


Table S3 The degree of mixing of the European dog genome into the Japanese dog genome

Japanese Dogs	*f4*-ratio (alpha)	Standard error	Z score
Akita	**0.363**	0.019328	18.768
Ks	**0.366**	0.020174	18.133
Shiba	**0.445**	0.017633	25.237

*European Dogs: Yorkshire_Terrier, P_V_Dog, Standard_Poodles1, Standard_Poodles2, Scottish_Deerhounds, BorderCollie, Boston_Terrier, Doberman_Pinscher, AS_Terriers, German_shepherd.
See Table S2 for the name of the dogs.

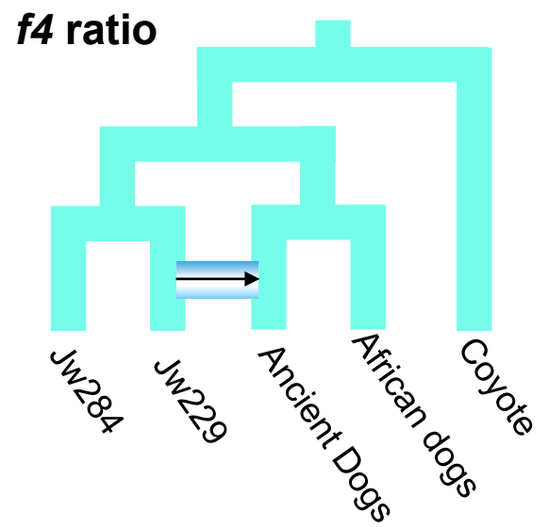


Table S4 Gene flow between Japanese Wolf and ancient dogs

Ancient Dogs	**Years ago**	***f4***	**stderr**	**Zscore**	***f4*-ratio(alpha)**	**stderr**	**Zscore**
Newgrange	4800	0.003171	0.000614	***5.163***	0.015764	0.00305	***5.168***
Cherry Tree Cave	5000	0.001028	0.000568	1.81	0.005231	0.002874	1.82
Herxheim	7000	0.003199	0.000606	***5.278***	0.016101	0.003041	***5.295***
Zhokhov	9500	0.004214	0.000715	***5.897***	0.021478	0.003612	***5.947***

Table S5 Genomic regions differentiated from Western Eurasian dogs and derived from Japanese Wolf

Chromosome	5' end	3' end	Genes in the region
2	6257975	6307631	*Ankyrin repeat domain 26 (ANKRD26)
3	36520617	36555078	Upstream of MKRN3, Downstream of CHRNA7
3	77467500	77472016	-
15	17047771	17058407	Upstream of FAM183A
19	13299716	13485166	**Upstream of INTU
30	8023335	8222693	INO80, ***DLL4, VPS18

*Mice mutant in one of these genes, ANKRD26, become hyperphagia and also have enhanced adipocyte differentiation (Bera, et al. 2008; Fei, et al. 2011). The traits resulting from the mutation of this gene may have been advantageous to the diet in the Japanese archipelago during Japanese dog evolution.
**INTU is part of a protein network called CPLANE, and mutations in INTU cause a number of skeletal morphological abnormalities, one of which is a flat nasal bridge (Toriyama, et al. 2016). Japanese wolves and Japanese dogs of the Jomon period (still found in a small number of Japanese dogs today) are known to have shallow stops, and perhaps mutations in this gene are related to the formation of shallow stops.
***Notch signaling plays an important role in the development and differentiation of many cell types in diverse organisms, and DLL4, the ligand for the Notch receptor, is known to have many roles during development (Benedito and Duarte 2005). Although this gene is multifunctional and therefore traits could not be identified, it may be involved in shaping the characteristics of Japanese dogs through the Notch signaling pathway.

Reference

- Benedito R, Duarte A. 2005. Expression of Dll4 during mouse embryogenesis suggests multiple developmental roles. Gene expression patterns 5:750-755.
- Bera TK, Liu X-F, Yamada M, Gavrilova O, Mezey E, Tessarollo L, Anver M, Hahn Y, Lee B, Pastan I. 2008. A model for obesity and gigantism due to disruption of the Ankrd26 gene. Proceedings of the National Academy of Sciences 105:270-275.
- Fei Z, Bera TK, Liu X, Xiang L, Pastan I. 2011. Ankrd26 gene disruption enhances adipogenesis of mouse embryonic fibroblasts. Journal of Biological Chemistry 286:27761-27768.
- Toriyama M, Lee C, Taylor SP, Duran I, Cohn DH, Bruel A-L, Tabler JM, Drew K, Kelly MR, Kim S. 2016. The ciliopathy-associated CPLANE proteins direct basal body recruitment of intraflagellar transport machinery. Nature genetics 48:648-656.

Table S6 Mapping rate (%) of reads with Japanese Wolf specific substitutions in the mitochondria DNA

Position in CanFam3.1	2730	3346	4400	4766	5828	6461	7732	8473	9514	10464	10701	11461	12062	15225	15490	Mean (%)	Estimated maximum contamination (%)
Jw258	97.0	97.0	94.9	96.3	99.8	100.0	100.0	99.8	100.0	100.0	100.0	100.0	96.4	99.8	100.0	98.7	1.3
Jw269	70.3	80.3	75.0	76.7	86.7	100.0	97.9	83.8	84.2	89.3	91.0	84.9	81.8	86.0	75.0	84.2	15.8
Jw229	92.5	93.9	94.8	94.4	97.2	99.7	97.0	96.3	95.9	97.8	97.6	97.0	90.9	95.2	95.8	95.7	4.3
Jw255	95.3	95.9	95.3	95.4	97.9	99.9	98.8	98.3	97.8	98.1	98.1	98.5	96.8	98.0	97.9	97.5	2.5
Jw284	99.6	99.6	99.7	99.7	99.9	99.8	99.8	99.8	99.7	99.8	99.9	99.9	99.7	99.8	99.7	99.8	0.2
Jw271	98.5	98.7	98.5	98.6	99.2	99.9	99.4	99.2	99.1	99.0	99.2	99.1	98.9	99.5	98.3	99.0	1.0
Jw275	98.6	98.4	98.1	98.0	99.3	100.0	99.5	99.5	99.0	99.0	98.6	98.6	97.7	99.0	99.0	98.8	1.2
Leiden_c	100.0	98.8	95.6	100.0	99.2	100.0	99.3	100.0	99.2	99.0	100.0	100.0	98.9	100.0	100.0	99.3	0.7
Leiden_b	100.0	100.0	99.9	100.0	100.0	100.0	100.0	100.0	100.0	99.9	95.7	100.0	94.5	98.8	100.0	99.3	0.7