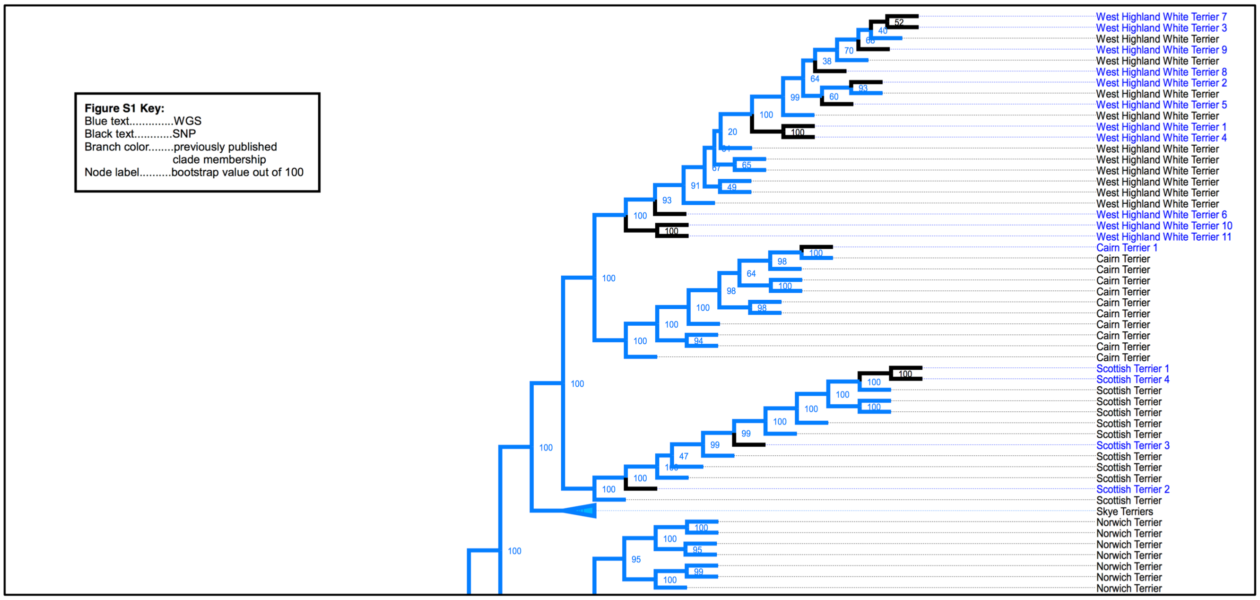
Whole Genome Sequencing of Canids Reveals Genomic Regions Under Selection and Variants Influencing Morphology

Plassais *et al*.

SUPPLEMENTARY FIGURES

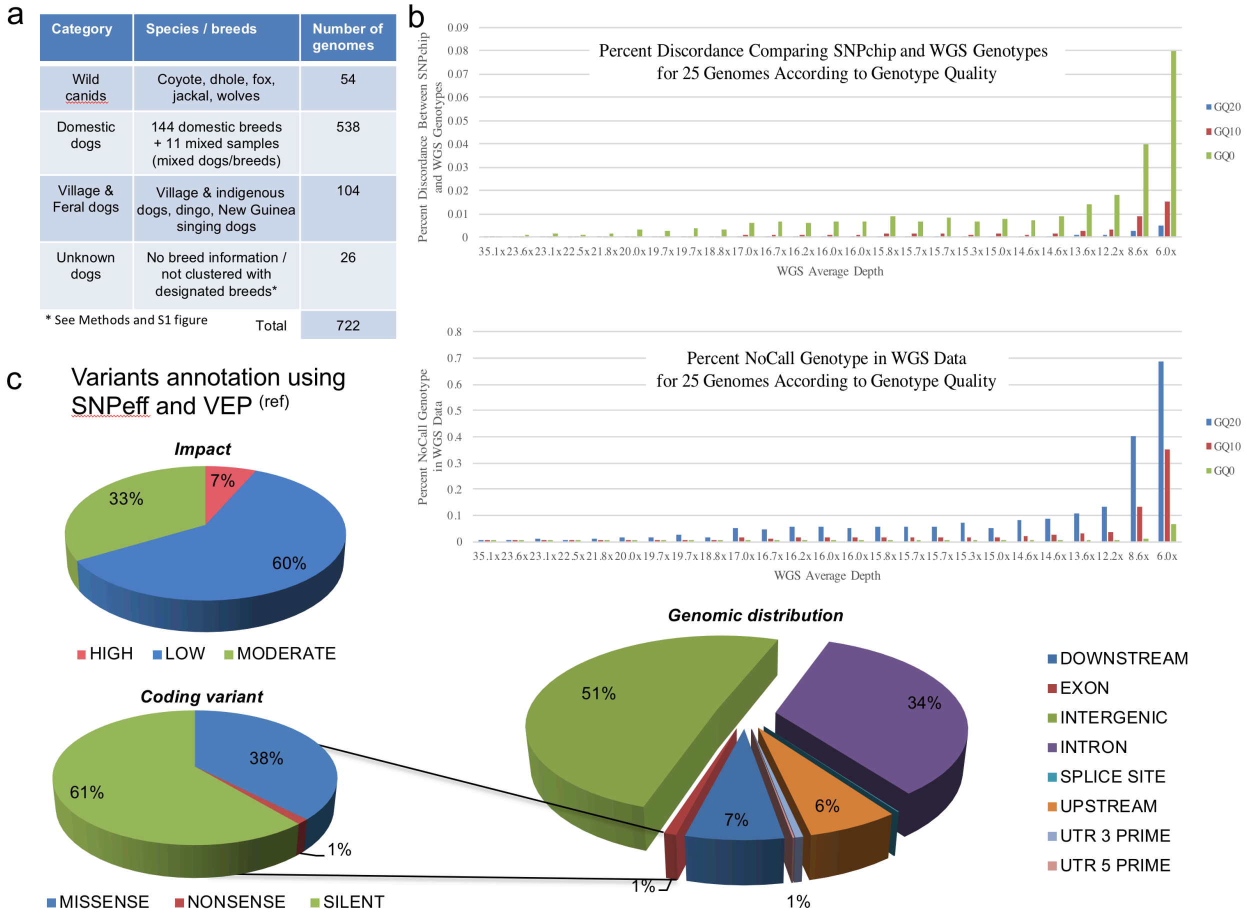


Example: Zoom in on the top of the phylogenic tree fully available on our website: https://research.nhgri.nih.gov/dog_genome/data_release/index.shtml

Supplementary Figure 1. Neighbor joining tree of 2080 canids. The tree is rooted with the Golden Jackal found at the bottom of the figure. Blue text highlights the WGS breed samples present in our canid catalog. The branches of the SNP genotyped individuals are colored to represent the clades identified in Parker et al[1]. Individuals from breeds without sequenced samples have been reduced to a single line. The number at each node is the bootstrap value out of 100 replicates.

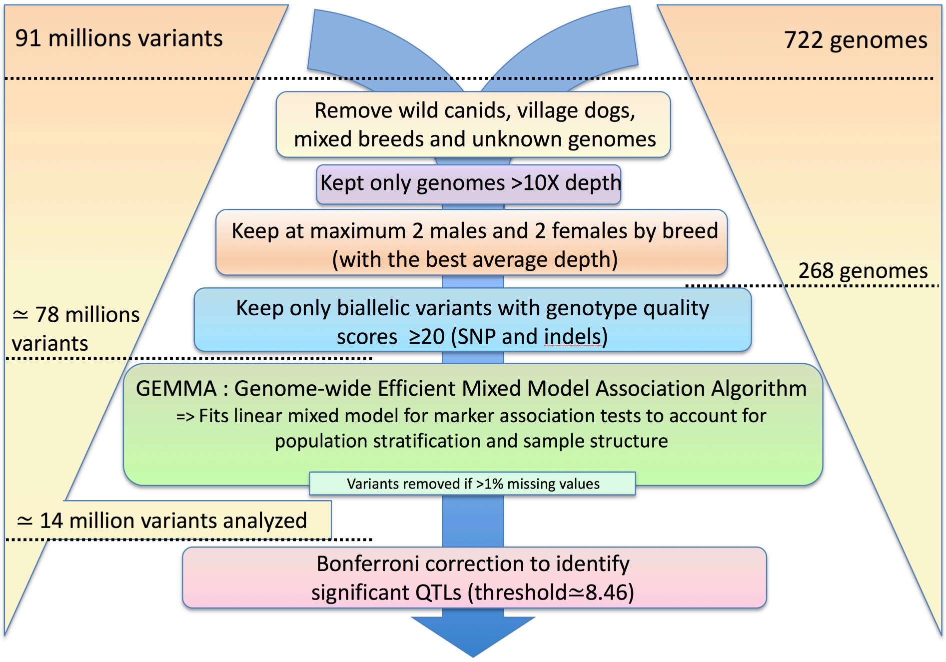
a

Category	Species / breeds	Number of genomes
Wild canids	Coyote, dhole, fox, jackal, wolves	54
Domestic dogs	144 domestic breeds + 11 mixed samples (mixed dogs/breeds)	538
Village & Feral dogs	Village & indigenous dogs, dingo, New Guinea singing dogs	104
Unknown dogs	No breed information / not clustered with designated breeds*	26
* See Methods and S1 figure	Total	722

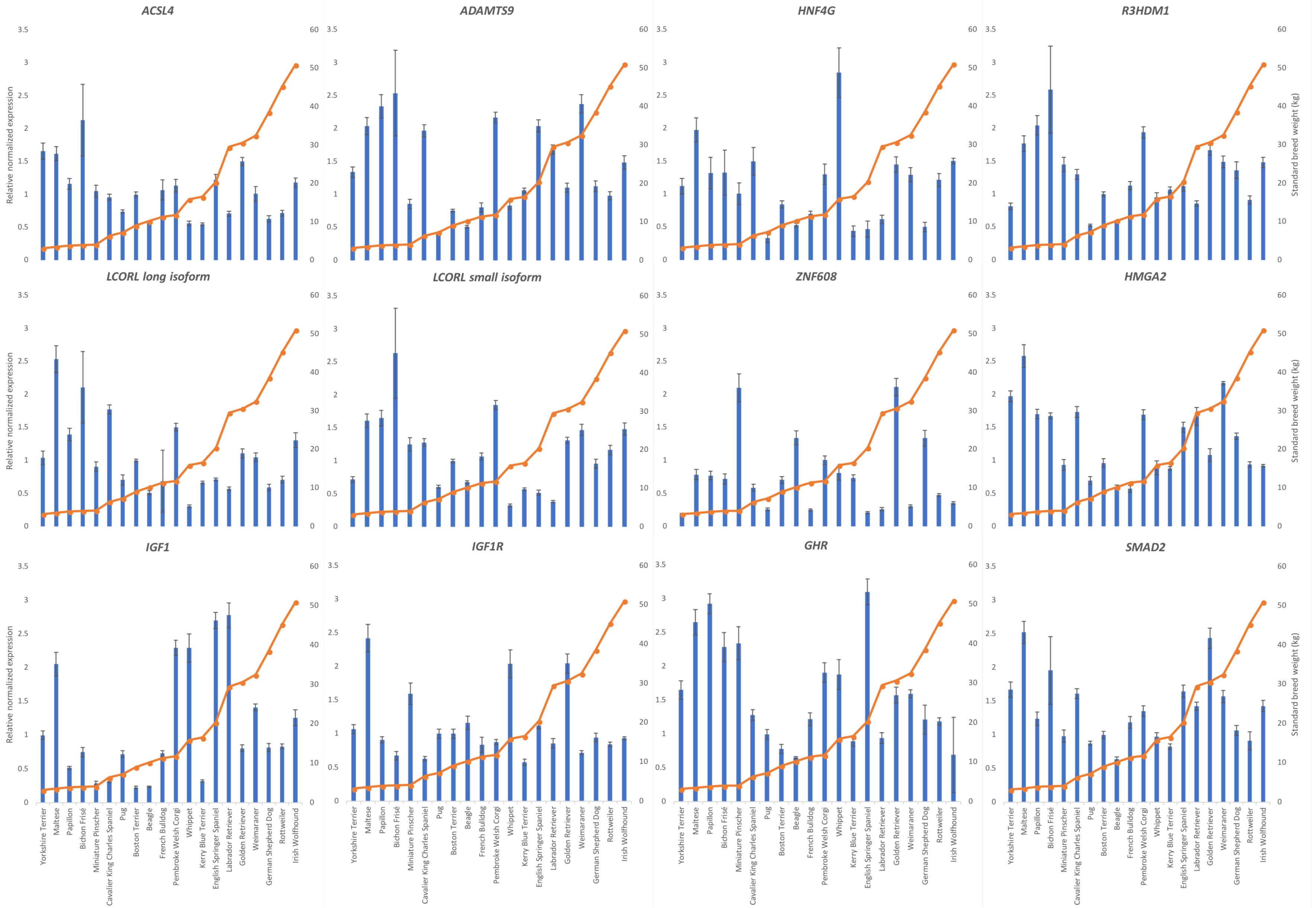


Supplementary Figure 2. Summary of the canids catalog and the distribution of the 91 million variants. (a) List of genomes used to build the catalog. (b) Percent discordance and WGS "no call" genotype according to genotype quality (GQ). Following these observations, we chose to filter WGS for a GQ of 20 keeping only WGS with an average depth >10x before performing GWAS. (c) Variants annotations combining two effect prediction tools: snpEFF version 4.3T[2] and VEP 93[3].

Pipeline to run GWAS on the canids catalog using VCFtools[4] and GEMMA[5]

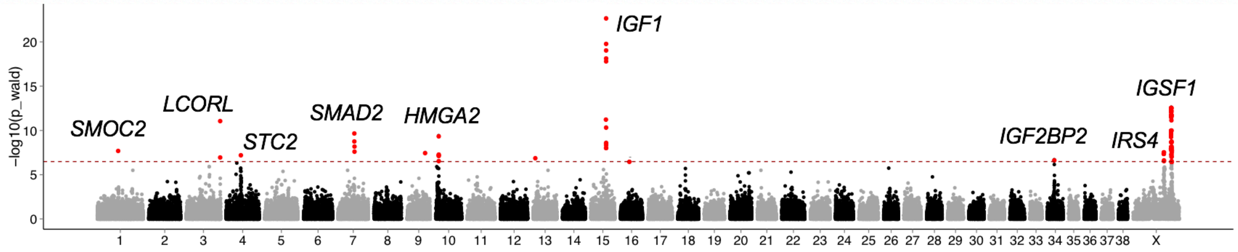


Supplementary Figure 3. **Whole genome sequencing assembly, variant calling and GWAS pipeline**. Sequence reads were aligned to the CanFam 3.1 reference genome (http://genome.ucsc.edu/cgi-bin/hgGateway?db=canFam3) using the BWA-MEM algorithm[6] (current version BWA 0.7.17) and sorted with SAMtools[7] (current version SAMtools 1.6). For non-PCR-free libraries, PCR duplicates were marked as secondary reads using PicardTools (http://github.com/broadinstitute/picard; current version PicardTools 2.9.2). GATK[8,9] (current version GATK 3.7) was used to perform local realignment around putative indels events using 714,278 variants previously published as a training set[10]. GATK base recalibration was performed using 172,254 Illumina Canine HD Chip positions and 2,738,537 dbSNP v131 variants. SNVs and small indels were called using GATK HaplotypeCaller, which first calls variants per-individual in gVCF mode with subsequent joint-calling utilizing all individuals[11]. Variant quality score recalibration was conducted with GATK best practices and default parameters for SNV and indels separately as follows. SNV recalibration: 172,254 Illumina Canine HD Chip variants (training, true, prior = 15); 2,738,537 dbSNP v131 variants (known, training, prior = 6); 3,627,539 published variants from Axelsson *et al*[10] (known, prior = 6). Indel recalibration: 714,278 variants as known, training and truth sets with a prior of six[10] and maxGaussians set to 4.

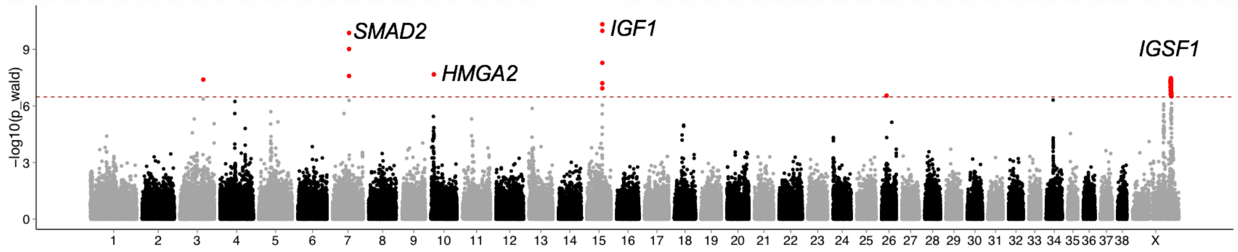


Supplementary Figure 4. Relative normalized expressions of body size genes in testes. No distinct correlations could be observed between relative normalized expressions level measured by qRT-PCR (blue) and standard breed weight (orange curve). Concerning *ACSL4*, *ADAMTS9*, *HNF4G*, *R3HDM1* and *ZNF608* associated with the bulky phenotype, our panel does not allow us to deduce any results. Indeed, our panel contains only one "bulky breed ("one Rottweiler). In addition, comparing these results to the RNA-Seq analyses described in the supplementary table 6, we can make the hypothesis that testes are probably not the best tissue to test the different *LCORL* isoforms and *GHR*, *HNF4G*, *IGF1* (weakly expressed), as well as *ADAMTS9-AS*, *IGSF1*, *IRS4* and *STC2* not detected by qRT-PCR. Source data are provided as a Source Data file.

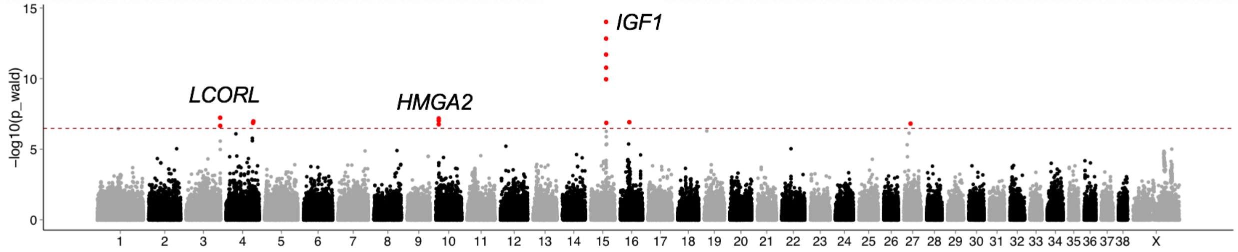
a) Manhattan plot showing GWAS results for standard breed weight



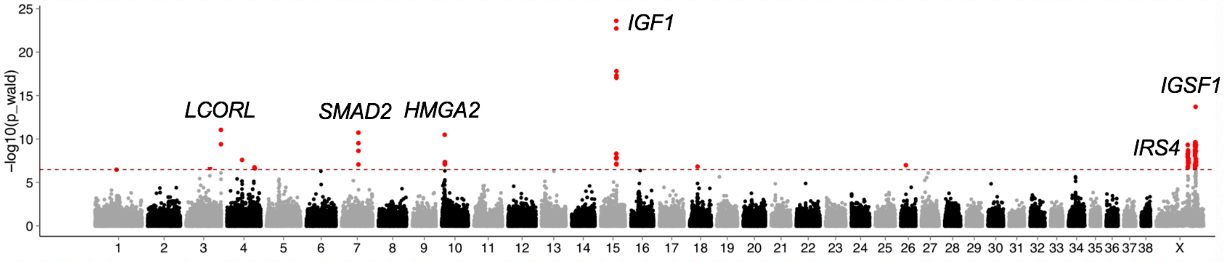
b) Manhattan plot showing GWAS results for standard breed life span



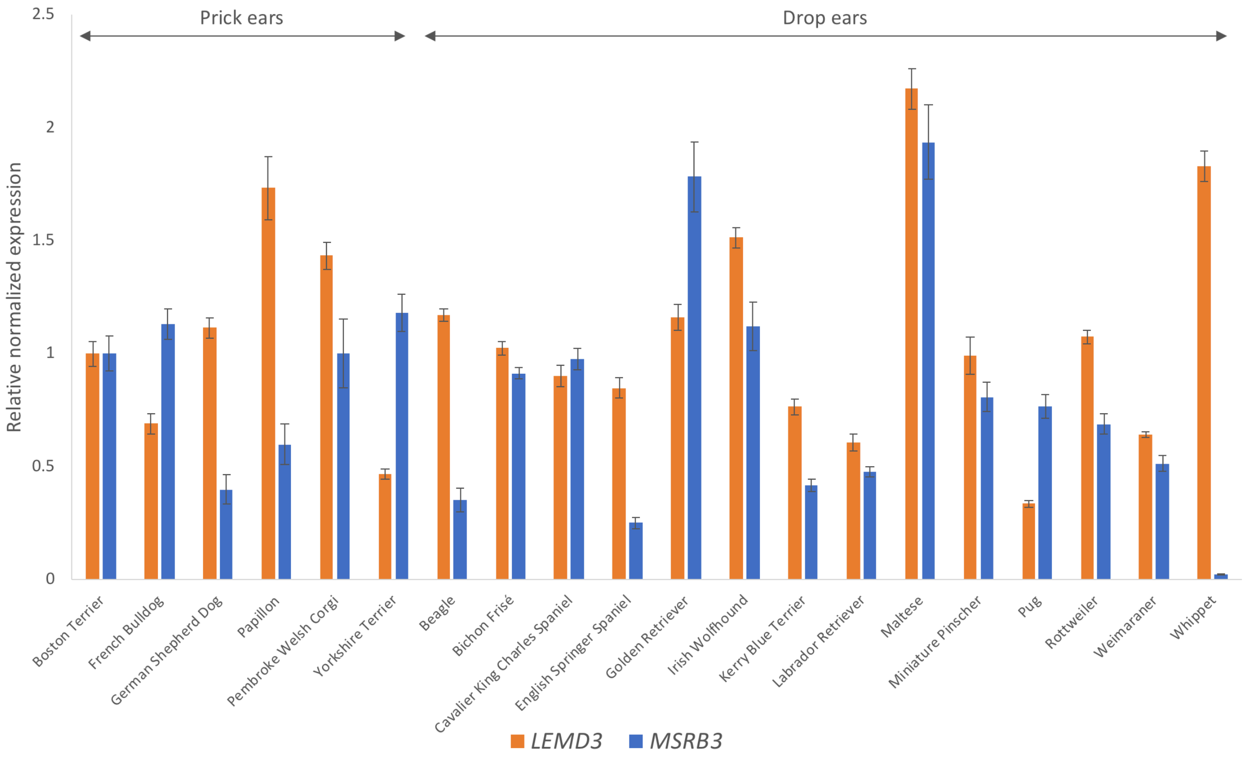
c) Manhattan plot showing GWAS results for SBW using life span as covariate



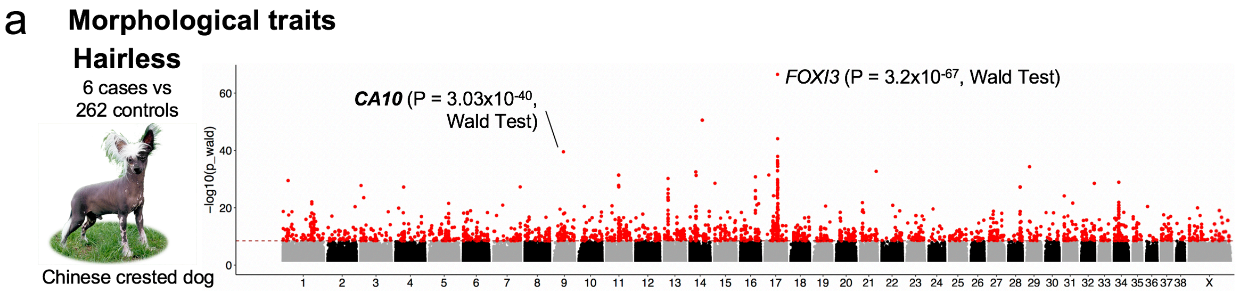
d) Manhattan plot showing multivariate GWAS results for SBW and life span



Supplementary Figure 5. Manhattan plots showing GWAS for standard breed weight (SBW) and longevity using 714 dogs genotyped on Illumina canine HD SNP array (170k). We observe that signals on CFA3, 7, 10, 15 and X loci are common between all GWAS, but decrease when life span is used as a covariable (c). As shown in figure D, multivariable GWAS confirmed that these loci are involved in both phenotypes (SBW and life span) tightly correlated, with higher p-values than in other GWAS (a, b and c). These results confirmed signals identified with the catalog of 722 canids genomes.

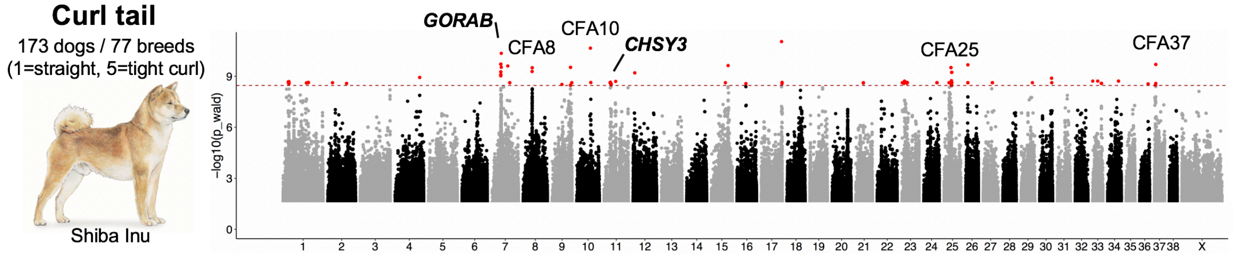


Supplementary Figure 6. Relative normalized expressions of candidate genes associated with ears morphology. No distinct correlations could be observed between ear morphology and the expressions level measured by qRT-PCR for *LEMD3* (orange) and *MSRB3* (blue). We did not detected signals for *WIF1 and* the mutated lncRNA on chromosome 10 identified in this paper, as well as the two candidate genes *RIMS1* and *KCNQ5* located on chromosome 12 associated with the "large and round ears" phenotype. Comparing these results to the RNA-Seq analyses described in the supplementary table 6, we can conclude, as expected, that testes are probably not the best tissue to analyze the ear morphology. Future investigations should focus on ear cartilages to check all gene expressions (*WIF1*, *LEMD3*, *MSRB3*, *RIMS1*, *KCNQ5*) and the lncRNA on chromosome 10. Source data are provided as a Source Data file.



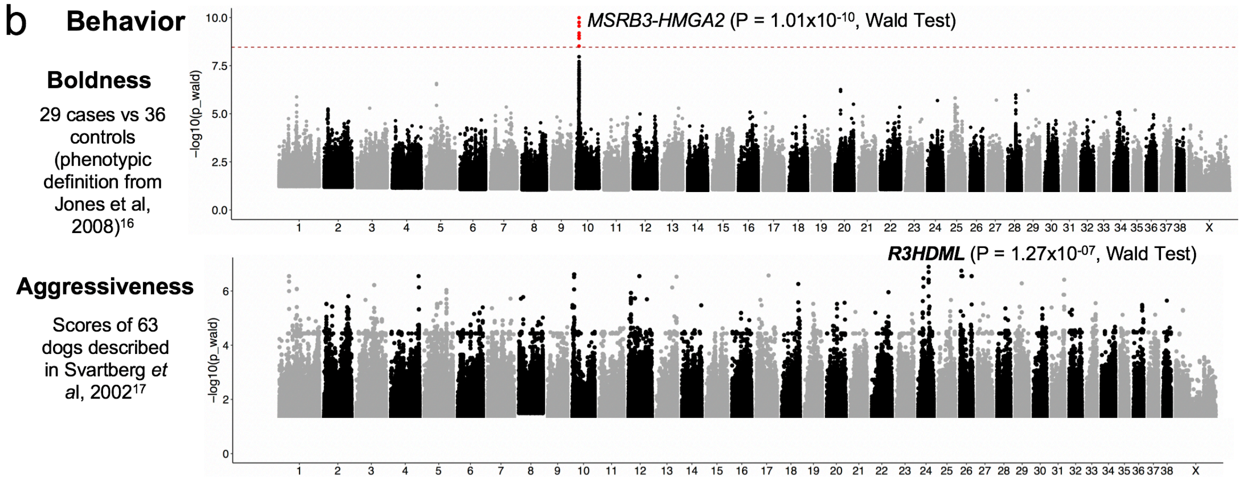
FOXI3 (*forkhead box protein I3*): Hairless gene in dogs[12]

CA10 (*carbonic anhydrase-related protein 10*) : involved in metabolic syndrome[13]. Could be involved in overweight. Mutation only found in Chinese Crested dog, Peruvian and Mexican hairless dogs and Chihuahua



GORAB (*RAB6-interacting golgin*) : involved in bones morphology[14]

CHSY3 (*chondroitin sulfate synthase 3*): mechanical function in cartilage[15]

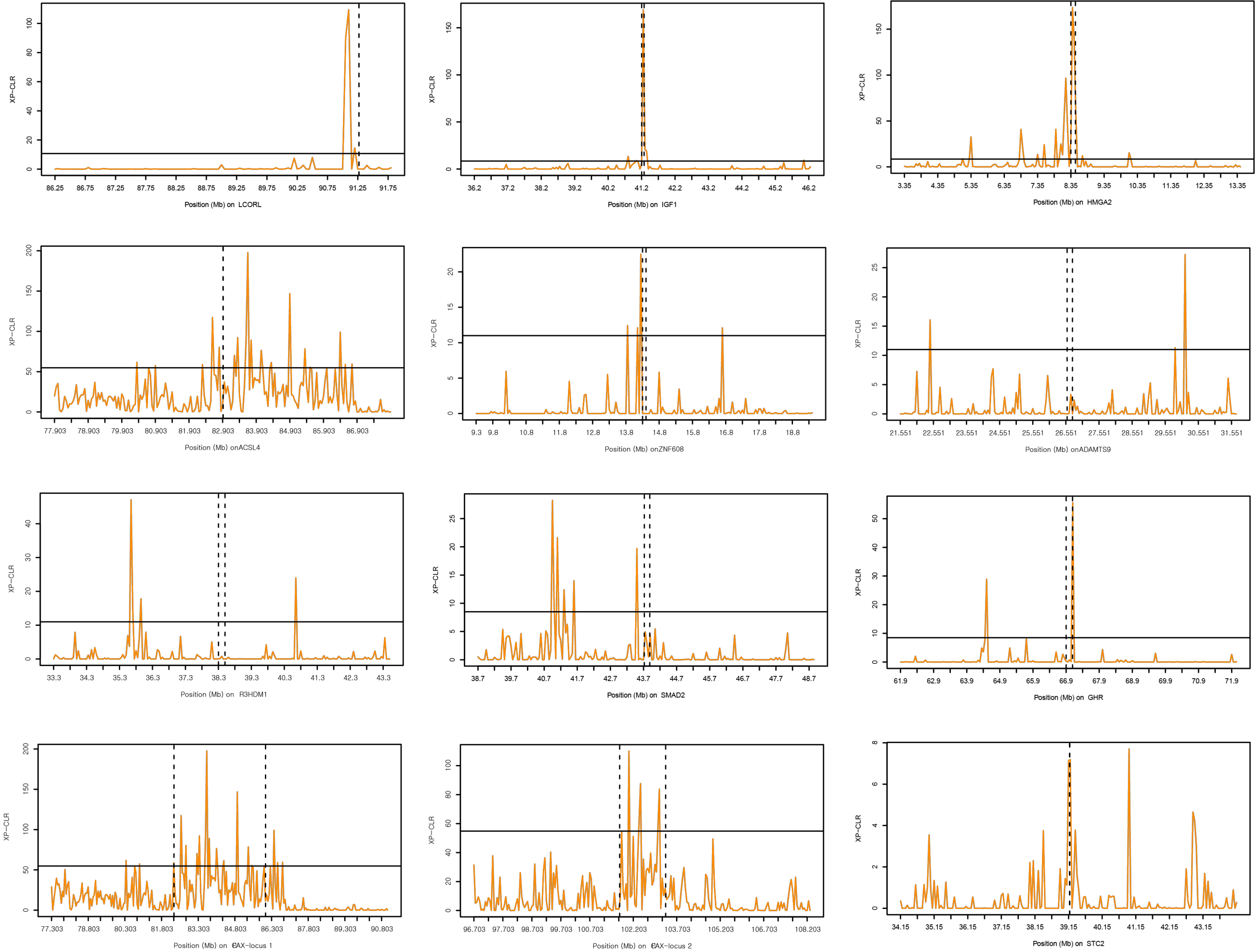


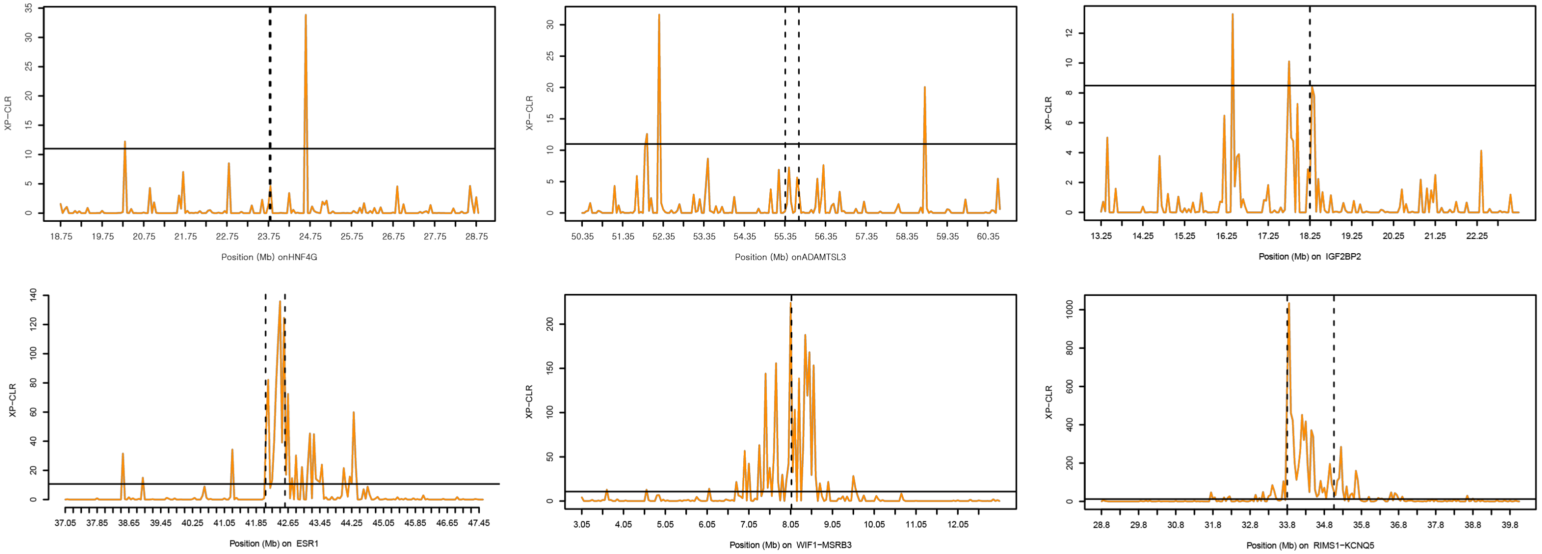
HMGA2 (high mobility group protein): previously associated with body size and boldness in dogs[18]

R3HDML (R3H domain containing like): uncharacterized gene associated with psychotic illness in humans[19]

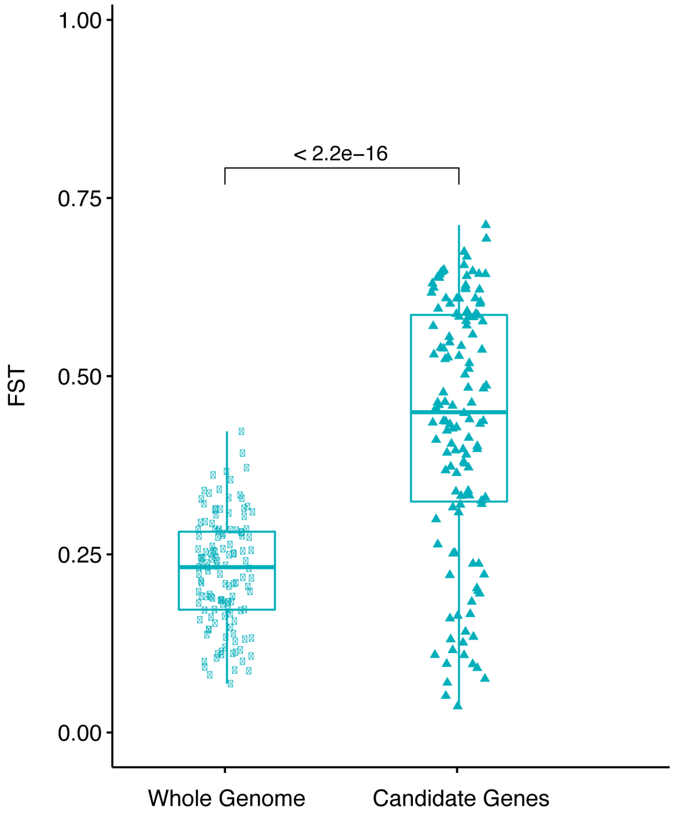
Supplementary Figure 7. Manhattan plots showing association results for hairless, curl tail and behaviors.

Supplementary Figure 8. XP-CLR plots on candidate gene regions for body size trait, long legs (Sighthounds) and ears morphology.

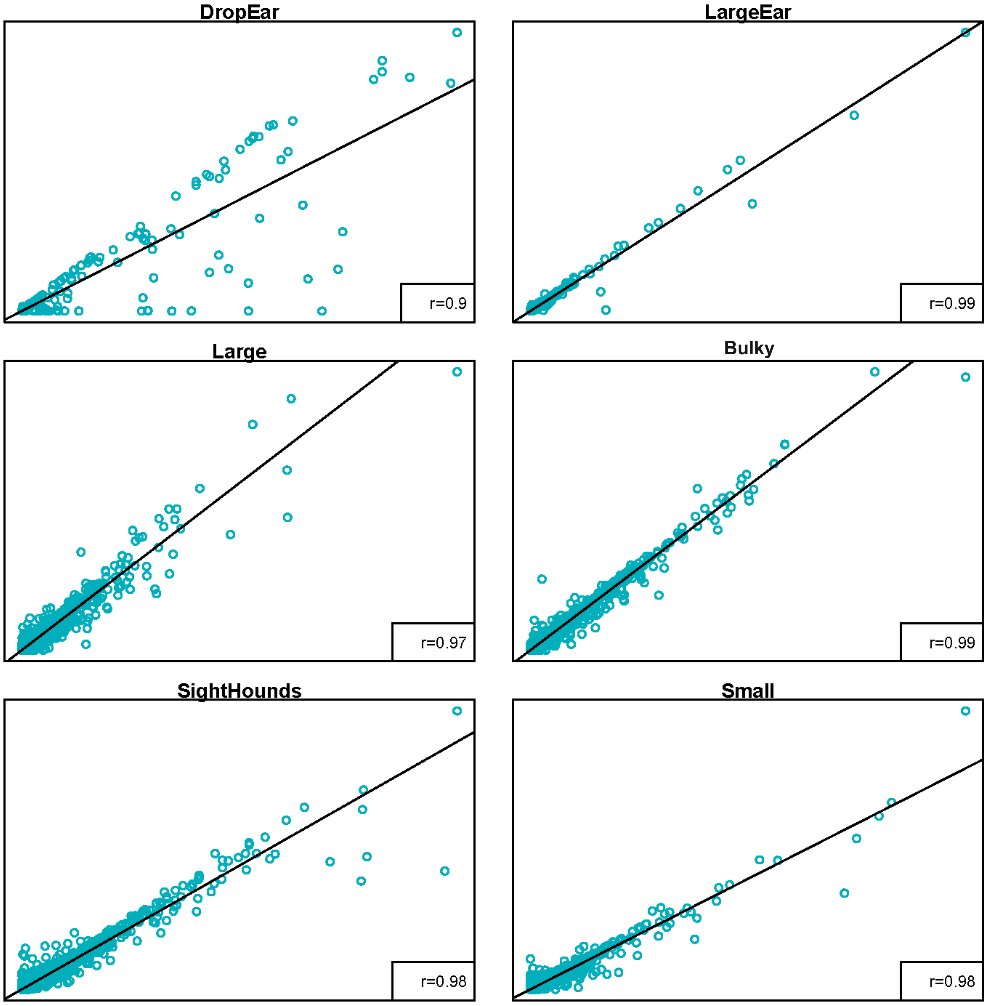




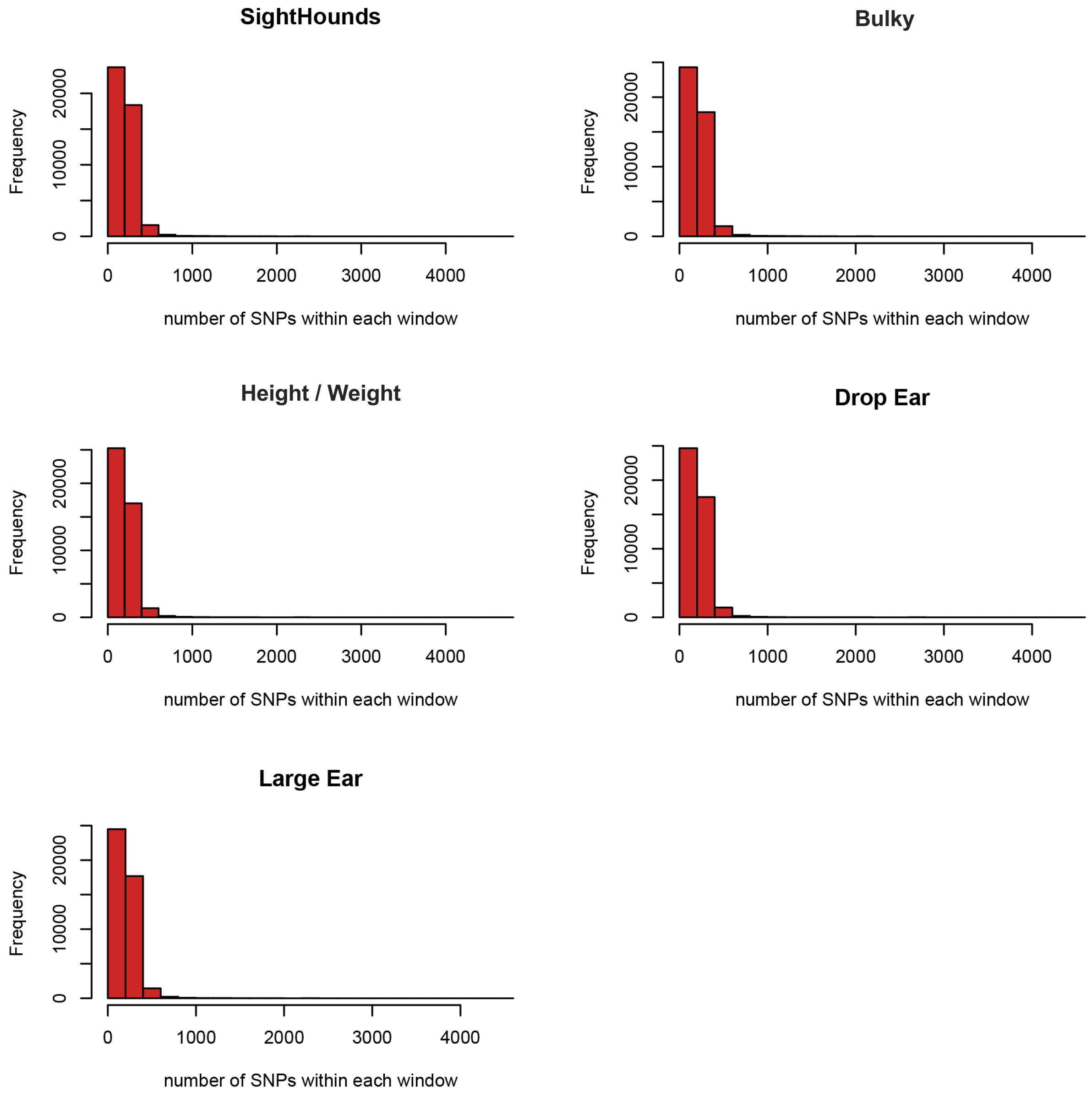
Vertical lines correspond to the candidate gene or variant identified by GWAS. Horizontal lines represent the empirical top 1% of genomic regions. Most of the examples illustrate the human-driven selection pressure to create breeds harboring a particular phenotype like the small size or the drop ears. The values and comparison with village dogs are also presented in the supplementary table 10.



Supplementary Figure 9. Boxplot of FST measured within candidate genes and whole genome between small and large breeds. As expected, we observed a significant difference when we run selection scans on the entire genome or only focused on the 14 body size genes identified by GWAS and described in this paper.



Supplementary Figure 10. Distribution of XP-CLR scores based on phased *versus* unphased genotypes.



Supplementary Figure 11. Distribution of number of SNPs within each 50kb window.

SUPPLEMENTARY TABLES

Supplementary Table 1. Summary of variants detected in the 722 canids genomes using SnpEFF[2] and VEP[3]

	SnpEFF		VEP	
	Number	%	Number	%
HIGH	69696	6.6%	60527	6.6%
LOW	629883	59.9%	549222	52.2%
MODERATE	352515	33.5%	311387	29.6%
MISSENSE	332559	37.6%	297671	37.6%
NONSENSE	11273	1.3%	9403	1.2%
SILENT	540063	61.1%	484307	61.2%
DOWNSTREAM	6318075	6.1%	5637219	6.5%
EXON	883895	0.8%	791381	0.8%
INTERGENIC	55256838	53.1%	41872987	40.2%
INTRON	34330953	33.0%	31237519	30.0%
SPLICE_SITE_REGION	97320	0.1%	81286	0.1%
UPSTREAM	6544634	6.3%	5849265	5.6%
UTR_3_PRIME	565967	0.5%	511288	0.5%
UTR_5_PRIME	139638	0.1%	112603	0.1%

Supplementary Table 2. List of the breeds genotyped on the most associated SNP on *ESR1* locus using the Illumina HD canine SNP array (170k)

A/A: Homozygote for the ancestral allele (blue); D/D: Homozygous for the derived allele (red);
A/D: Heterozygous (yellow)
- : Phenotype not applicable

			chr1:42518863		
Breed	**Nb dogs**	**longlegs**	**A/A**	**A/D**	**D/D**
Afghan Hound	10	yes		1	9
Akita	10	no	1	2	7
Alaskan Malamute	10	-	5	5	
American Cocker Spaniel	10	-	10		
Anatolian Shepherd Dog	6	no	6		
Australian Shepherd Dog	10	-	6	4	
Australian Terrier	10	-	6	3	1
Basenji	10	-	6	2	2
Basset Hound	10	-	10		
Beagle	11	-	11		
Belgian Shepherd	10	-	2	3	5
Belgian Tervuren	10	-	6	4	
Bernese Mountain Dog	10	no	10		
Black Russian Terrier	4	yes		2	2
Bloodhound	10	-	10		
Border Collie	10	-	7	3	
Borzoi	10	yes			10
Boston Terrier	10	-	10		
Boxer	10	-	10		
Briard	10	-	6	3	1
Brittany	10	-	3	5	2
Bulldog	10	-	10		
Bullmastiff	10	no	8	2	
Bullterrier	9	-	10		
Cairn Terrier	10	-	10		
Cardigan Welsh Corgi	10	-	10		
Cavalier King Charles Spaniel	10	-	10		
Chihuahua	10	-	8	2	
Chinese Shar-Pei	10	-	8	2	
Chow Chow	10	-	4	4	2
Collie	10	-	8	2	
Dachshund	10	-	10		
Doberman Pinscher	10	-	7	3	
Dogue De Bordeaux	6	no	4	2	

English Cocker Spaniel	10	-	9		
English Mastiff	10	no	5	5	
English Springer Spaniel	10	-	5	4	1
Flat-Coated Retriever	10	-	10		
French Bulldog	10	-	10		
German Shepherd Dog	10	-	10		
German Shorthaired Pointer	10	-	8	2	
Giant Schnauzer	10	-	7	3	
Glen of Imaal Terrier	9	-	6	3	
Golden Retriever	10	-	8	2	
Great Dane	10	yes		3	7
Great Pyrenees	10	yes	1	5	4
Greater Swiss Mountain Dog	6	no	6		
Greyhound	10	yes	2	2	6
Havanese	10	-	7	3	
Ibizan Hound	10	-		2	8
Irish Water Spaniel	10	-	10		
Irish Wolfhound	10	yes		4	6
Italian Greyhound	10	-	10		
Kuvasz	10	no	4	6	
Labrador Retriever	10	-	8	2	
Leonberger	10	no		1	9
Miniature Bull Terrier	10	-	10		
Miniature Pinscher	10	-	10		
Miniature Poodle	10	-	8	2	
Miniature Schnauzer	10	-	10		
Neapolitan Mastiff	6	-	1	3	2
Newfoundland	10	no	7	2	1
Norwich Terrier	10	-	4	5	1
Old English Sheepdog	10	-	9	1	
Papillon	10	-	7	3	
Pekingese	10	-	6	4	
Pembroke Welsh Corgi	10	-	9	1	
Petit Basset Griffon Vendéen	10	-	10		
Pomeranian	10	-	9	1	
Portuguese Water Dog	10	-	10		
Pug	10	-	8	2	
Rottweiler	11	no	11		
Saint Bernard	10	no	5	5	
Saluki	11	yes	2	2	7

Samoyed	10	-	10		
Scottish Deerhound	10	no	10		
Scottish Terrier	10	-	10		
Shetland Sheepdog	10	-	10		
Shih Tzu	10	-	8	2	
Siberian Husky	10	-	8	2	
Staffordshire Bull Terrier	10	-	10		
Standard Poodle	10	-	9	1	
Standard Schnauzer	10	-	10		
Tibetan Mastiff	6	no	1	5	
Toy Poodle	10	-	8	1	1
West Highland White Terrier	10	-	7	3	
Whippet	10	-	6	4	
Yorkshire Terrier	10	-	9	1	
Total	855	200	610	151	94

	General population	**Long legs**	**Normal legs**
Ancestral allele frequency	0.80	0.19	0.74
Derived allele frequency	0.20	0.81	0.26

Supplementary Table 3. Validation of the mutation on CFA10 and genotype of the most associated variant on CFA12 associated with the shape of ears

For CFA12, we genotyped the most associated marker identified by GWAS using the 722 WGS catalog.
A/A: Homozygote for the ancestral allele (blue); D/D: Homozygous for the derived allele (red);
A/D: Heterozygous (yellow)
- : Phenotype not applicable

				chr10:8070103			chr12:34439600		
Breed	**Nb dogs**	**Drop ears**	**Large ears**	**A/A**	**A/D**	**D/D**	**A/A**	**A/D**	**D/D**
Afghan Hound	10	-	no	3	4	3	3	7	
Akita	10	no	no	10			8	2	
Alaskan Malamute	10	no	no	10			9	1	
American Cocker Spaniel	10	yes	yes			10			10
Anatolian Shepherd Dog	6	yes	no	4	1	1	6		
Australian Shepherd Dog	10	no	no	8	2		10		
Australian Terrier	10	no	no	10			10		
Basenji	10	no	no	10			10		
Basset Hound	10	yes	yes			10	1	5	4
Beagle	11	yes	yes		2	8			11
Belgian Shepherd	10	no	no	10			10		
Belgian Tervuren	10	no	no	10			10		
Bernese Mountain Dog	10	yes	-		2	8	3	4	3
Black Russian Terrier	4	no	no	3	1		3	1	
Bloodhound	10	yes	-			10	10		
Border Collie	10	no	no	6	4		8	2	
Borzoi	10	-	no	10			10		
Boston Terrier	10	no	no	10			9	1	
Boxer	10	yes	no	10			10		
Briard	10	yes	no		8	2	10		
Brittany	10	yes	no			10	10		
Bulldog	10	-	no	6	4		10		
Bullmastiff	10	yes	no	10			10		
Bullterrier	9	no	no	10			9		
Cairn Terrier	10	no	no	6	3	1	10		
Cardigan Welsh Corgi	10	no	yes	8	2				10
Cavalier King Charles Spaniel	10	yes	yes			10			10
Chihuahua	10	no	no	4	4	2	5	5	
Chinese Shar-Pei	10	-	no	10			9	1	
Chow Chow	10	no	no	10			10		

Collie	10	no	no	10			10		
Dachshund	10	yes	yes		3	7		3	7
Doberman Pinscher	10	-	no	5	3	2	10		
Dogue De Bordeaux	6	yes	no		2	4	6		
English Cocker Spaniel	10	yes	yes			10			10
English Mastiff	10	yes	no	4	3	3	10		
English Springer Spaniel	10	yes	yes		2	8	2	4	4
Flat-Coated Retriever	10	yes	no			10	10		
French Bulldog	10	no	yes	5	4	1		2	8
German Shepherd Dog	10	no	no	10			9	1	
German Shorthaired Pointer	10	yes	-			10	5	4	1
Giant Schnauzer	10	yes	no	4	3	3	9	1	
Glen of Imaal Terrier	9	no	no	10			9		
Golden Retriever	10	yes	no		4	6	10		
Great Dane	10	yes	no	1	2	7	7	3	
Great Pyrenees	10	yes	no		3	7	10		
Greater Swiss Mountain Dog	6	yes	no		2	4	6		
Greyhound	10	-	no	10			10		
Havanese	10	yes	no	2	6	2	5	4	1
Ibizan Hound	10	no	no	3	6	1	8	2	
Irish Water Spaniel	10	yes	no			10	9	1	
Irish Wolfhound	10	-	no	10			10		
Italian Greyhound	10	-	no	8	2		9	1	
Kuvasz	10	yes	no	4	6		8	2	
Labrador Retriever	10	yes	no	2	4	4	10		
Leonberger	10	yes	no			10	8	2	
Miniature Bull Terrier	10	no	no	10			10		
Miniature Pinscher	10	-	no	10			8	2	
Miniature Poodle	10	yes	yes	9	1		2	1	7
Miniature Schnauzer	10	-	no	10			10		
Neapolitan Mastiff	6	yes	no		2	4	6		
Newfoundland	10	yes	-		2	8	3	3	4
Norwich Terrier	10	no	no	10			10		
Old English Sheepdog	10	yes	no		1	9	8	2	
Papillon	10	no	yes	9	1		1	3	6
Pekingese	10	-	-	1	4	5		3	7
Pembroke Welsh Corgi	10	no	yes	10					10
Petit Basset Griffon Vendéen	10	yes	yes			10	3	4	3

Pomeranian	10	no	no	8	1	1	10		
Portuguese Water Dog	10	yes	no	2		8	8	2	
Pug	10	-	no			10	10		
Rottweiler	11	yes	no	7	2	2	10	1	
Saint Bernard	10	yes	-	2	3	5	5	5	
Saluki	11	-	no		1	9	8	3	
Samoyed	10	no	no	10			10		
Scottish Deerhound	10	-	no	10			8	2	
Scottish Terrier	10	no	-	10			2	5	3
Shetland Sheepdog	10	no	no	2	5	3	10		
Shih Tzu	10	yes	no	5	4	1	2	6	2
Siberian Husky	10	no	no	10			10		
Staffordshire Bull Terrier	10	no	no	10			10		
Standard Poodle	10	yes	yes	1	1	8	10		
Standard Schnauzer	10	-	no	6	4		10		
Tibetan Mastiff	6	-	no		2	4	5	1	
Toy Poodle	10	yes	yes	8	2		1	3	6
West Highland White Terrier	10	no	no	10			10		
Whippet	10	-	no	10			8	2	
Yorkshire Terrier	10	no	no	10			10		
Total	855	688	785	456	128	271	621	107	127

	Chr10:8070103			**Chr12:34439600**		
	General population	**Drop ears breeds**	**Prick ears breeds**	**General population**	**Large ears breeds**	**Normal ears breeds**
Ancestral allele frequency	0.61	0.29	0.92	0.79	0.22	0.95
Derived allele frequency	0.39	0.71	0.08	0.21	0.78	0.05

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