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Supporting Information
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Genetic Architecture of Tameness in a Rat Model of Animal Domestication

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FILE S1

Materials and Methods

Mapping pedigree: The pedigree described in this study was initiated from four unrelated tame and four aggressive individuals (two females each; the "F_{-1}" or "F minus one" generation) mated within line to yield 11 (five tame and six aggressive rats, one male each) F_0 animals. The F_{-1} animals did not have common parents and at most one common grandparent. The F_0 animals were crossed reciprocally, i.e. both sexes were used from each line. Two out of four tame females and two out of five aggressive females were full siblings of the respective F_0 founder males. Six hybrid F_1 males (three sired by the tame, and three sired by the aggressive F_0 male) were repeatedly mated to 37 F_1 females to produce a total of 733 F_2 rats (383 females). F_1 animals sired by the aggressive F_0 male were mated only to F_1 animals sired by the tame F_0 male, and vice versa.

Behavioral testing: A list of all traits we collected can be found in Table 1. At six to eight weeks of age, F_2 rats performed the "glove test", which measures an animal's level of tameness/aggression by confronting it with an approaching human hand and attempts at handling (see (ALBERT *et al.* 2008) for details on the testing procedure). Within two weeks, but at least after a four-day break, rats performed an open-field test, followed three days later by a light-dark test. These tests provide various measures of exploratory and anxiety-related behavior. After nine days, rats performed a startle response test, which measures the behavioral response to a sudden acoustic stimulus. 470 (64%) of the F_2 rats then performed a second glove test trial after maximally two weeks. F_1 rats were tested once in the glove test at 12 – 14 weeks of age, and then followed the testing schedule described in (ALBERT *et al.* 2008). All tests were performed with minimal handling following the procedures described in (ALBERT *et al.* 2008). Open field, light-dark and startle response test data were recorded using automated measuring technology (TSE Systems, Bad Homburg, Germany). Glove test trials were videotaped and later analyzed by two independent observers (5% of trials only by one observer) using the software "Interact" (Mangold Software, Arstorf, Germany). Experimenters and observers were blind to the animals' identity and to further data processing. A set of 11 behaviors (e.g. "attack" or "tolerate handling") were scored (see (ALBERT *et al.* 2008) for detailed descriptions of the behaviors), and each converted to a numeric measure (e.g. "number of occurrences" or "duration in seconds"; Table 1).

Serological phenotypes: We measured a series of serum traits, most of which had earlier been found (ALBERT *et al.* 2008) to differ between the tame and the aggressive rats (Table 1). Serum was analyzed in 684 F_2 rats (357 females) as described in the main text. Corticosterone levels reflect the rats' response to handling and sacrifice, rather than baseline (undisturbed) levels. This is consistent with their high values compared to basal measures reported in the literature (VAHL *et al.* 2005). Twenty-two F_2 individuals were excluded from further analysis of all serum traits because they appeared to be outliers with respect to the remaining F_2 population: two individuals with chloride values < 90 mmol / l and 16 individuals with chloride values > 110 mmol / l, and four individuals with triglyceride levels > 4 mmol / l.

Glove test analyses: To summarize a rat's behavior in the glove test, we performed principal component analysis (PCA) on the individual glove test measures. The resulting principal component (PC) scores represent a linear, weighted combination of individual behaviors. Combining animals from several generations when performing PCA could potentially distort the results. Hence, we used only F_2 animals in the analysis.

F_0 and F_1 animals performed only one glove test trial and were scored by a single observer. For comparing F_2 animals' glove tests to those of the F_0 and F_1 generations, we used only observations from the first trial, and from the same observer who scored the F_0 and F_1 animals. We applied the PCA regression coefficients obtained in F_2 animals to the single observations from F_0, F_1, and the comparable subset of single F_2 observations. A small number of F_2 animals had PC scores that exceeded the range defined by the most tame and the most aggressive F_0 animals. Since QTL mapping can be sensitive to phenotypic outliers, we set these extreme individuals' phenotypes to the most extreme values observed in F_0 animals. Adjusting outliers this way did not greatly alter the mapping results.

Marker ascertainment and genotyping procedures: Animals were genotyped with 152 microsatellite and 49 single nucleotide polymorphism (SNP) markers. SNPs were added after a preliminary scan for QTLs using only the microsatellite data (not shown), to improve genome coverage and to obtain more data in putative QTL regions. Microsatellite markers were ascertained from public databases. SNP markers were ascertained from a panel of 16,927 SNPs under study at the CEA/IG-Centre National de Genotypage, Evry, France. All markers were selected based on their segregation patterns in the outbred tame and aggressive rat lines as determined from preliminary genotyping of a panel of F0 animals. All markers used in the QTL mapping are listed in Table S2. DNA was isolated either from lung tissue according to the NucPrep Protocol for animal tissues (Applied Biosystems, Foster City, CA, USA) or from spleen tissue using the DNeasy Blood & Tissue Kit (Quiagen GmbH, Hilden, Germany) according to the manual's section "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)". The extraction protocols were slightly modified. For NucPrep, digestion at 65 °C for 1 h was preceded by overnight incubation of the tissue samples at room temperature in NucPrep Digestion Buffer and Proteinase K (both Applied Biosystems), and the pre-filtration step was skipped. For DNeasy, two final elution steps were performed, each with 100 µl of Buffer AE. For all markers, polymerase chain reaction (PCR) was performed in 384-well format. Microsatellite marker PCRs were performed using the M13-primer PCR system (SCHUELKE 2000). 15 ng of dried DNA per sample were used in a 10 µl PCR mix containing (per sample) 1.0 µl of PCR buffer (Quiagen), 0.5 µl DMSO (Merck KG, Darmstadt, Germany), 0.4 µl dNTPs (5 mM) (Amersham Biosciences, Buckinghamshire, England), 0.2 µl forward and reverse primer in a 1 / 10 ratio (1 / 10 µM) (Thermo Electron, Ulm, Germany), 0.2 µl M13-tail (10 µM) labeled with one of three fluorophores [6-fam (Thermo Electron), Ned or Vic (both Applied Biosystems)], 7.66 µl of water and 0.039 µl of HotStarTaq® Plus DNA polymerase (5 U / µl) (Quiagen). The M13-tail used in all reactions was 5'-[fluorophore]-CACGACGTTGTAAAACGAC. PCR was performed on Eppendorf Mastercycler ep384 thermocyclers (Eppendorf, Hamburg, Germany) according to the following touch-down PCR protocol: 95 °C for 5 min., 44 cycles of each at 94 °C for 30 s, a variable temperature for 30 s

and 72 °C for 30 s. The variable temperature was 65 °C in the first cycle and decreased by 1 °C in the following 5 cycles, then decreased by 0.5 °C for 17 cycles and then held constant at 52 °C for the remaining 21 cycles. PCR products were held at 8 °C after program completion. Up to 10 PCR products of different expected lengths and labeled with different dyes were pooled and analyzed on an ABI3730 DNA Analyzer (Applied Biosystems). Microsatellite genotypes were determined using the software GeneMapper Version 4.0 (Applied Biosystems) and all genotypes were manually double-checked. SNP assays were ordered from ABI Applied Biosystems. PCR cocktails containing 10 ng of dried DNA / sample were prepared according to the assay manufacturer's specifications, but using "ABgene Absolute QPCR Rox Mix X2" (Applied Biosystems) instead of "TaqMan Universal PCR Master Mix". PCR was performed on Eppendorf Mastercycler ep384 thermocyclers (Eppendorf) as follows: 10 min. at 95 °C, followed by 40 cycles of 15 s at 92 °C and 1 min. at 60 °C; reactions were held at 8 °C after program completion. Intensity scans of PCR products were performed on an ABI 7900HT Sequence Detection System v2.2 (Applied Biosystems). SNP genotypes were called automatically as part of the scanning process, and genotype plots inspected visually. Preliminary genotyping of 16,927 SNPs had been performed using the Illumina GoldenGate assay according to manufacturer's instruction in an Illumina Beadlab, as described (SAAR *et al.* 2008).

Details on linkage map construction: We constructed sex-averaged linkage maps for each chromosome using a version of the program crimap (GREEN *et al.* 1990) modified to handle large pedigrees by the University of California Davis, Veterinary Genetics Laboratory. We used the option FIXED to estimate marker distances in centiMorgan (cM) on markers ordered according to their physical chromosomal locations as annotated in the UCSC Genome Browser. Marker order was confirmed using crimap's FLIPS option. Markers that were not annotated in the genome browser were placed on the linkage map using the option ALL. We used the option CHROMPIC to identify inferred double recombination events. For all such events, genotype data was double-checked for errors.

Computation of linkage map information content: We estimated the information content at marker positions from the respective gametic identical-by-decent (IBD) matrices X_g (see below for the matrix estimation procedure). For a given position, the IBD matrices specify which individuals have inherited identical alleles. At informative positions, many allele pairs can be assigned unambiguously (IBD = 1 or IBD = 0). At less informative positions, some estimated IBD estimates will tend towards 0.5. We estimated the information content at a position as $2\frac{1}{n^2}\sum\left|0.5 - X_g\right|$, where n is the number of alleles in the pedigree. The information content statistic takes a value of one for perfectly informative markers, and zero if no allele can be traced to its pedigree origin. On the X-chromosome, we calculated a lower limit for the information content at marker positions as the average difference in allele frequency between tame and aggressive F_{-1} founders, weighted by the respective allele frequency across all F_{-1} founders.

Haplotype-based inference of missing genotypes: Missing F_0 genotypes can lead to a loss of power in QTL mapping because some alleles in F_2 animals might not be traced reliably to parental lines. We sought to overcome this limitation by including the genotyped parents of F_0 animals (the F_{-1}) in the analysis, and by tracing alleles back to them. This way, we sought to infer missing genotypes of F_0 and F_1 animals based on their ancestors' and offspring genotypes. We first determined the phase of informative markers for all individuals in the pedigree. Then, we used a genetic algorithm to iteratively generate chromosome segments carrying putative haplotypes. For each such segment, the algorithm calculates the likelihood that the segment is compatible with the phase of the informative markers, and keeps the most likely haplotype from each iteration until it converges to an optimum. We then inferred the missing genotype information in F_0 and F_1 animals from the haplotypes of related F_{-1} and F_2 individuals. Details of this procedure for inference of haplo- and genotypes will be published elsewhere.

Single QTL mapping on sex chromosomes: The analyses described thus far were designed to handle autosomal data. To analyze the X-chromosome, we used the software QxPak (PEREZ-ENCISO and MISZTAL 2004), which is able to handle sex chromosomal data appropriately. For each trait, we first fitted an additive QTL using the option 'fix_a'. Where this yielded a significant ($p < 0.005$) QTL, we then tested for dominance using option 'fix_ad'. Since the permutation-based significance thresholds derived for the autosomes cannot be directly applied to the X chromosome, we assumed QTLs with a nominal p-value < 0.001 (0.005) to be significant (suggestive) at a genome-wide level, as suggested in the QxPak manual.

To test for linkage to the Y chromosome, we note that F_2 males in our pedigree carry one of only two Y chromosomes, one derived from the tame F_0 male, one from the aggressive F_0 male. Due to the design of our cross, F_2 males carrying the Y chromosome from one parental line always carry mitochondria (and potentially other maternally inherited factors) from the same line. However, while F_2 females share such maternal factors with F_2 males, they do not carry the Y chromosomes. This allows us to disentangle the effect of the Y chromosome by comparing the phenotypes of F_2 males carrying the Y chromosome inherited from the tame F_0 male, of males carrying the Y chromosome inherited from the aggressive F_0 male, and of F_2 females that were sired by the respective F_1 males. A Y-linked locus should lead to a phenotype difference between the two groups of F_2 males, while the two groups of F_2 females should not differ. In an analysis of variance (ANOVA), we tested for interactions between the sex of F_2 animals and the strain origin of the Y chromosome carried by their fathers. Covariates were included in the ANOVA as listed in Table S6. We assumed linkage to the Y chromosome if the interaction term was significant ($p < 0.005$) and if a post-hoc T-test showed a significant ($p < 0.05$) difference between F_2 males, but not between F_2 females.

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FILE S2

A compressed folder is available at http://www.genetics.org/cgi/content/full/genetics.109.102186/DC1.

This folder contains four text files that contain raw data (as described below). This description is also available in the **Read Me.txt** file located in the compressed folder.

1. "genotypes.txt" – contains genotypes and marker info
    - first line contains marker names
    - second line contains markers' chromosomes
    - third line contains markers' genetic position on a given chromosome
    - the rest of the file contains the genotypes:
        - first column is animal id
        - there are *two* columns per marker, one for each allele
        - allele codes are arbitrary numbers, and do not by themselves contain information about strain origin or the genotype
        - the two allele columns per marker do *not* indicate whether an allele was inherited from the mother or the father
        - missing genotypes are coded as '0'
        - on the X-chromosome, '9' indicates the "missing" allele in hemizygous males

2. "pedigree_fixed_effects_covariates.txt"
    - first column is animal id
    - second column is an animal's father ('0' for the first generation)
    - third column: is an animal's mother ('0' for the first generation)
    - fourth column is sex: males are '1', females are '2'
    - fifth column indicates an animals strain (tame or aggressive) or the respective generation in the cross (F1 or F2)
    - remaining columns are numerical covariates (measured only in F2 animals)

3. "phenotypes.txt"
    - first column is animal id
    - only F2 animals were measured (beginning with animal ID 63), and only F2s are listed in this file
    - with the exception of glove test traits, all traits are "raw", i.e. not adjusted for family effects or covariates
    - glove test behaviors in this file *are* adjusted for observer, trial and family effects - see "raw_glove_data.txt" for raw glove test data

4. "raw_glove_data.txt"
    - data from the glove test, directly as scored from the video tapes
    - this data forms the basis for the principal components used to calculate tameness and aggression scores
    - only F2 animals are listed (beginning with animal ID 63)

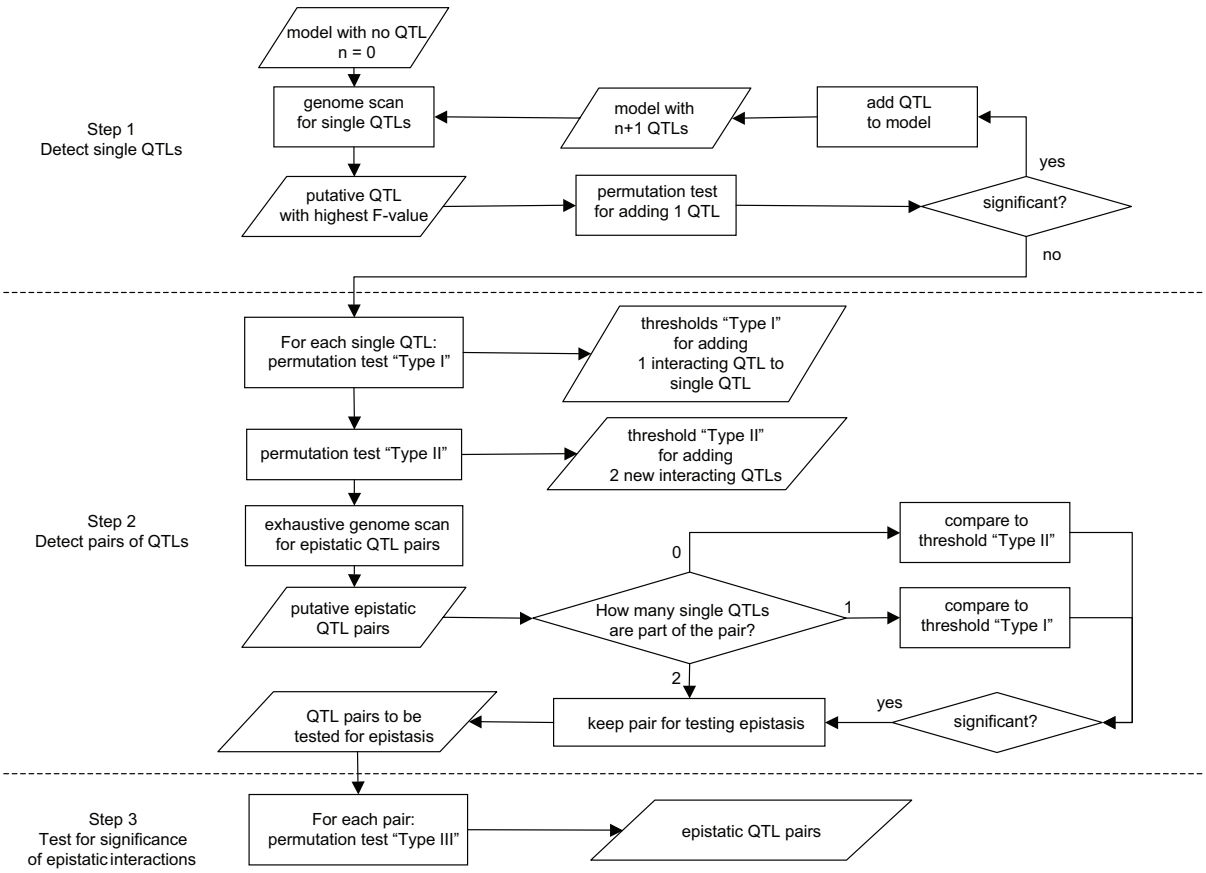

FIGURE S1.—Flow diagram of the search strategy for single QTLs and epistatic QTL pairs.

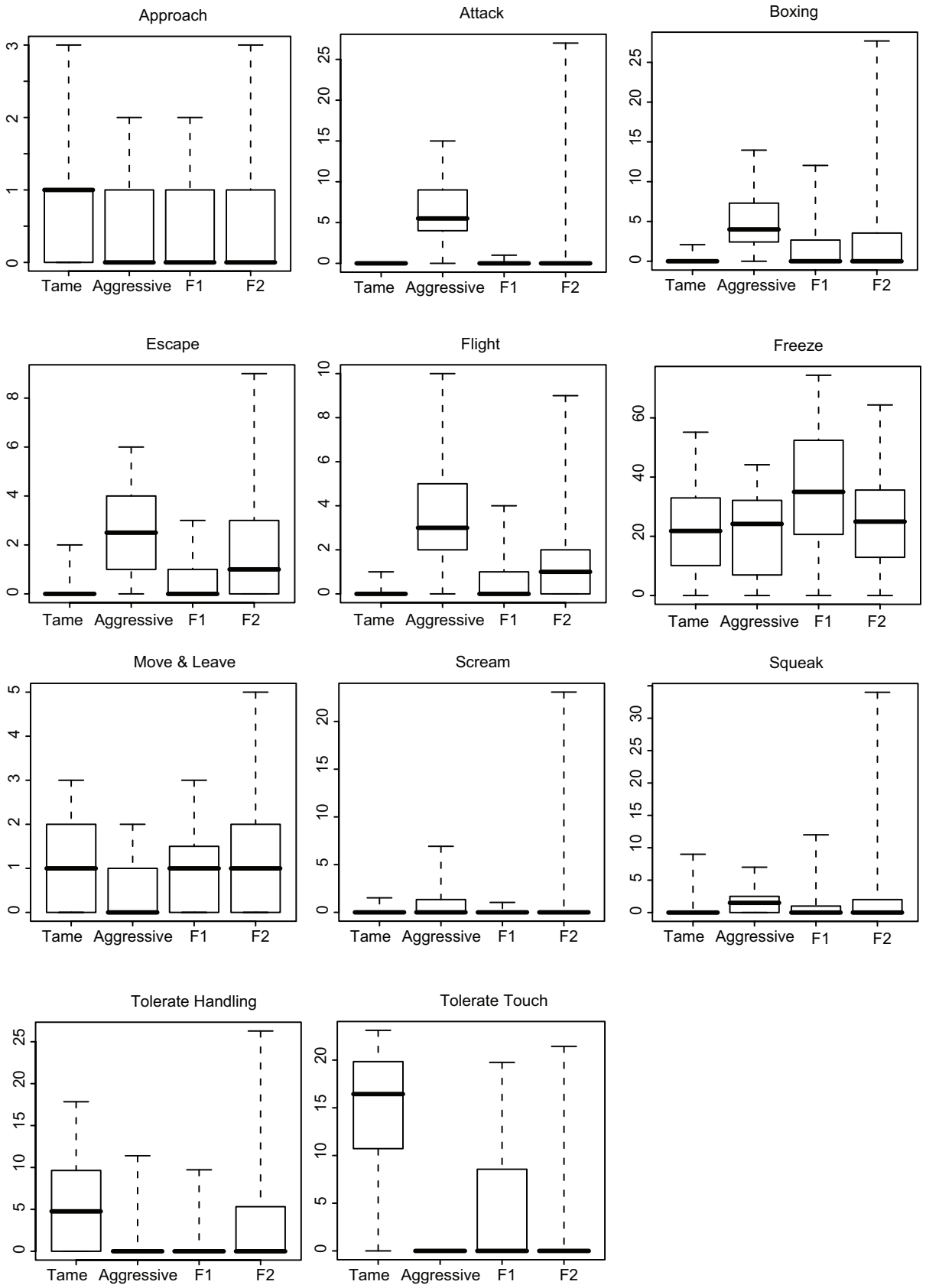

FIGURE S2.—Phenotype ranges for traits recorded in the glove test. Solid boxes represent the 50% of trait values closest to the median (bold line). Whiskers represent the low and high 25% of trait values.

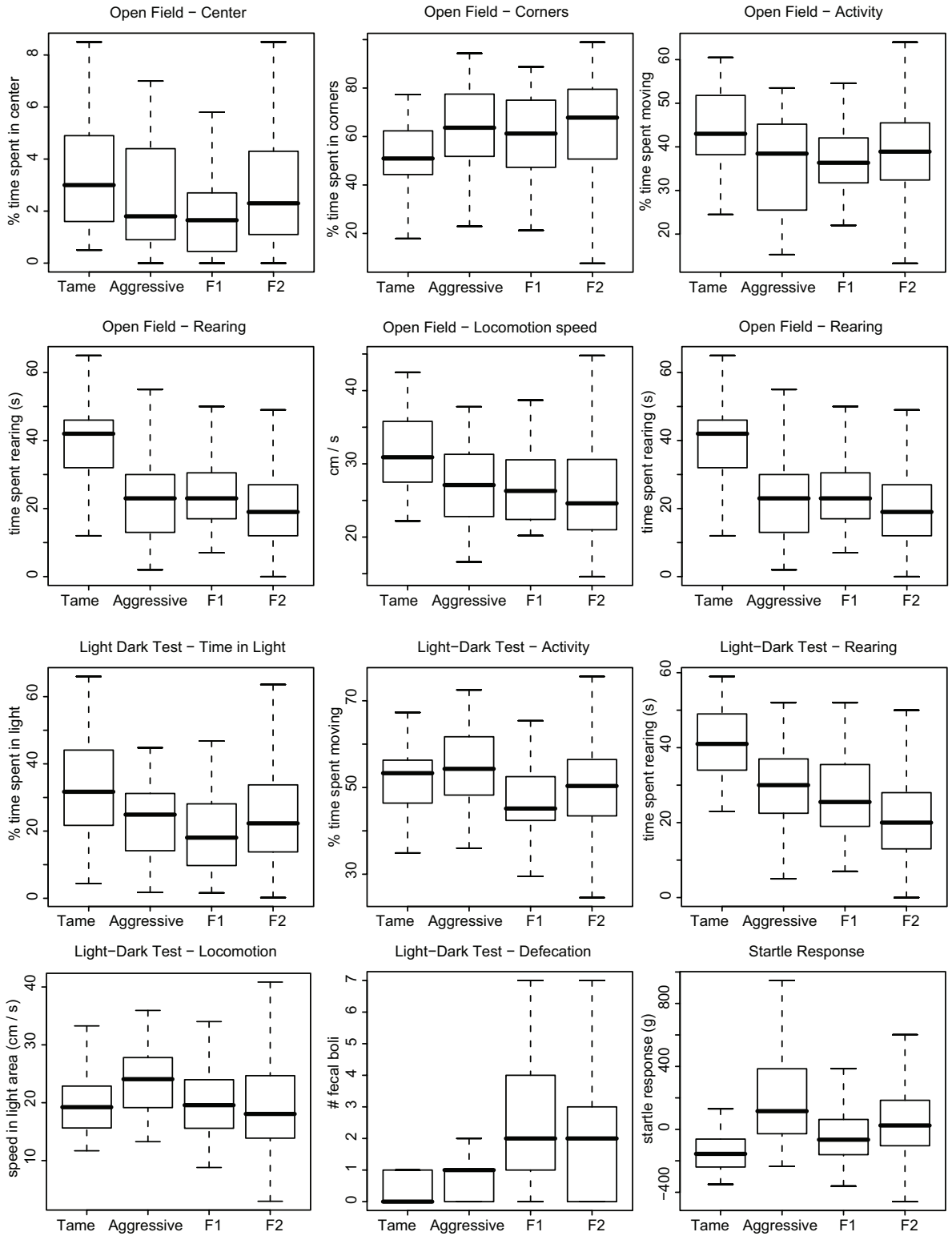

FIGURE S3.—Phenotype ranges for traits recorded in the open field, light-dark and startle response tests. Solid boxes represent the 50% of trait values closest to the median (bold line). Whiskers represent the low and high 25% of trait values. For clarity, potential outliers are not shown.

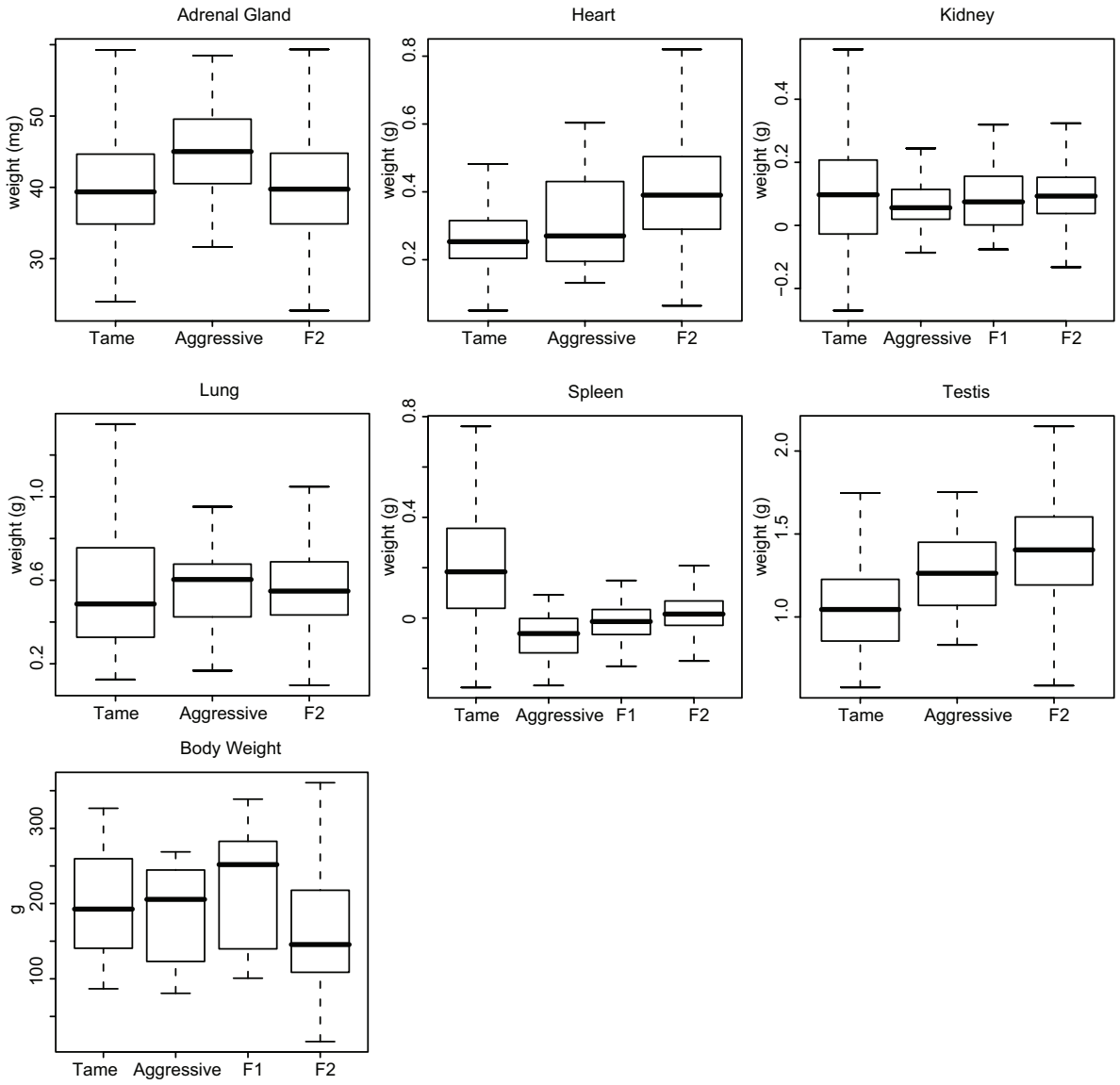

FIGURE S4.—Phenotype ranges for body and organ weights. Solid boxes represent the 50% of trait values closest to the median (bold line). Whiskers represent the low and high 25% of trait values. For clarity, potential outliers are not shown.

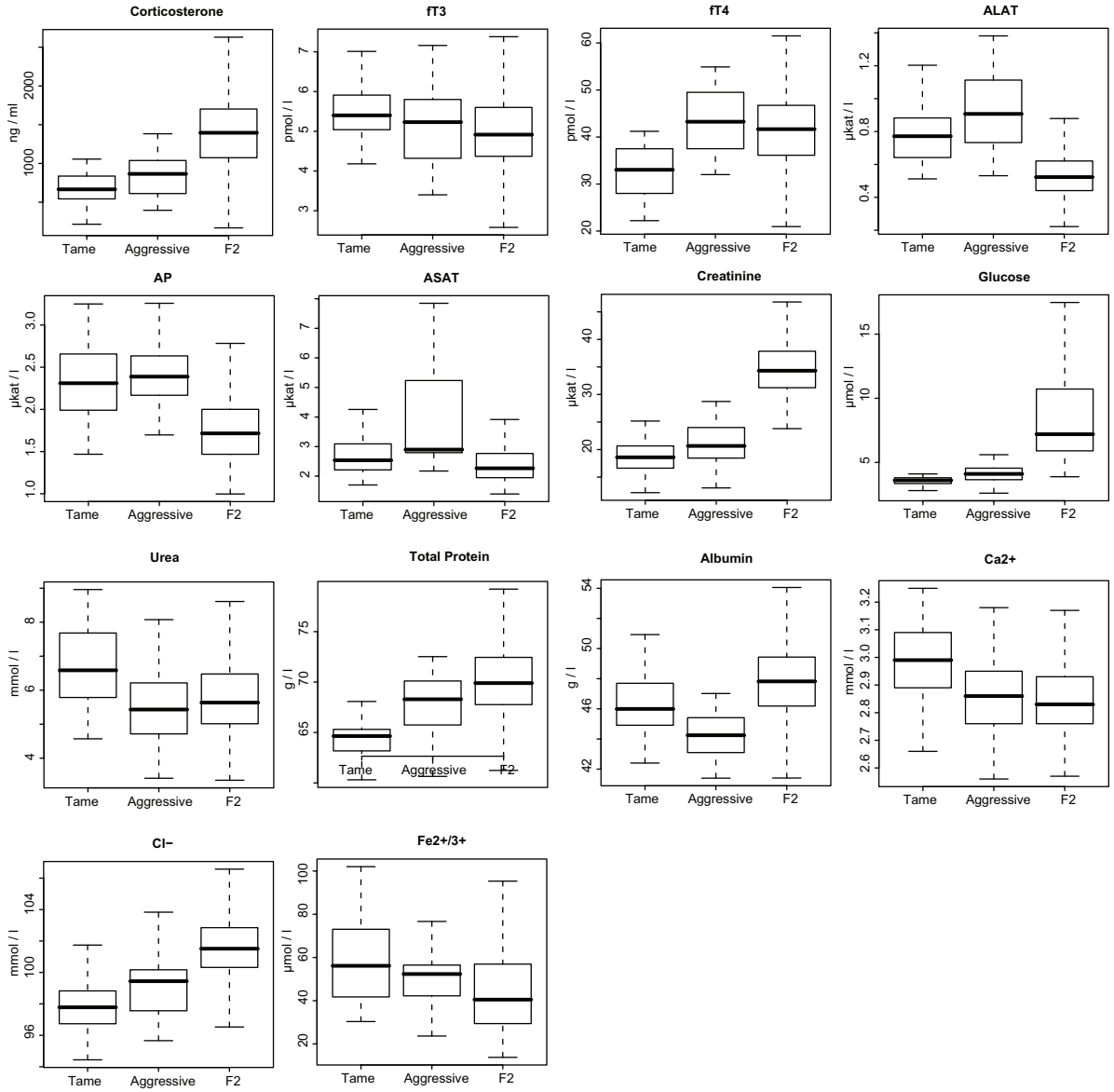

FIGURE S5.—Phenotype ranges for serum traits. Solid boxes represent the 50% of trait values closest to the median (bold line). Whiskers represent the low and high 25% of trait values. For clarity, potential outliers are not shown.

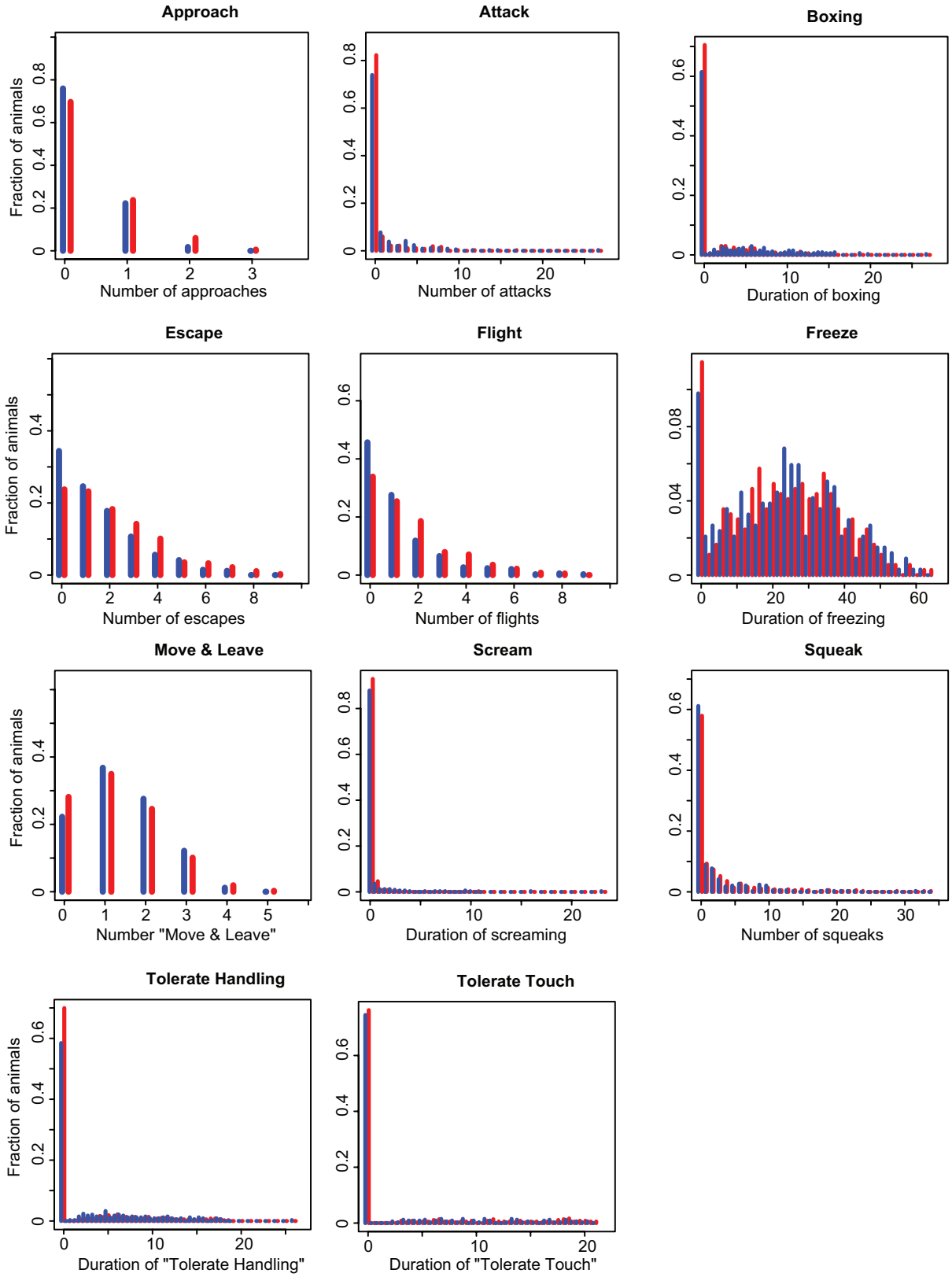

FIGURE S6.—Phenotype distributions for traits recorded in the glove test. Red: female F2 rats, blue: male F2 rats

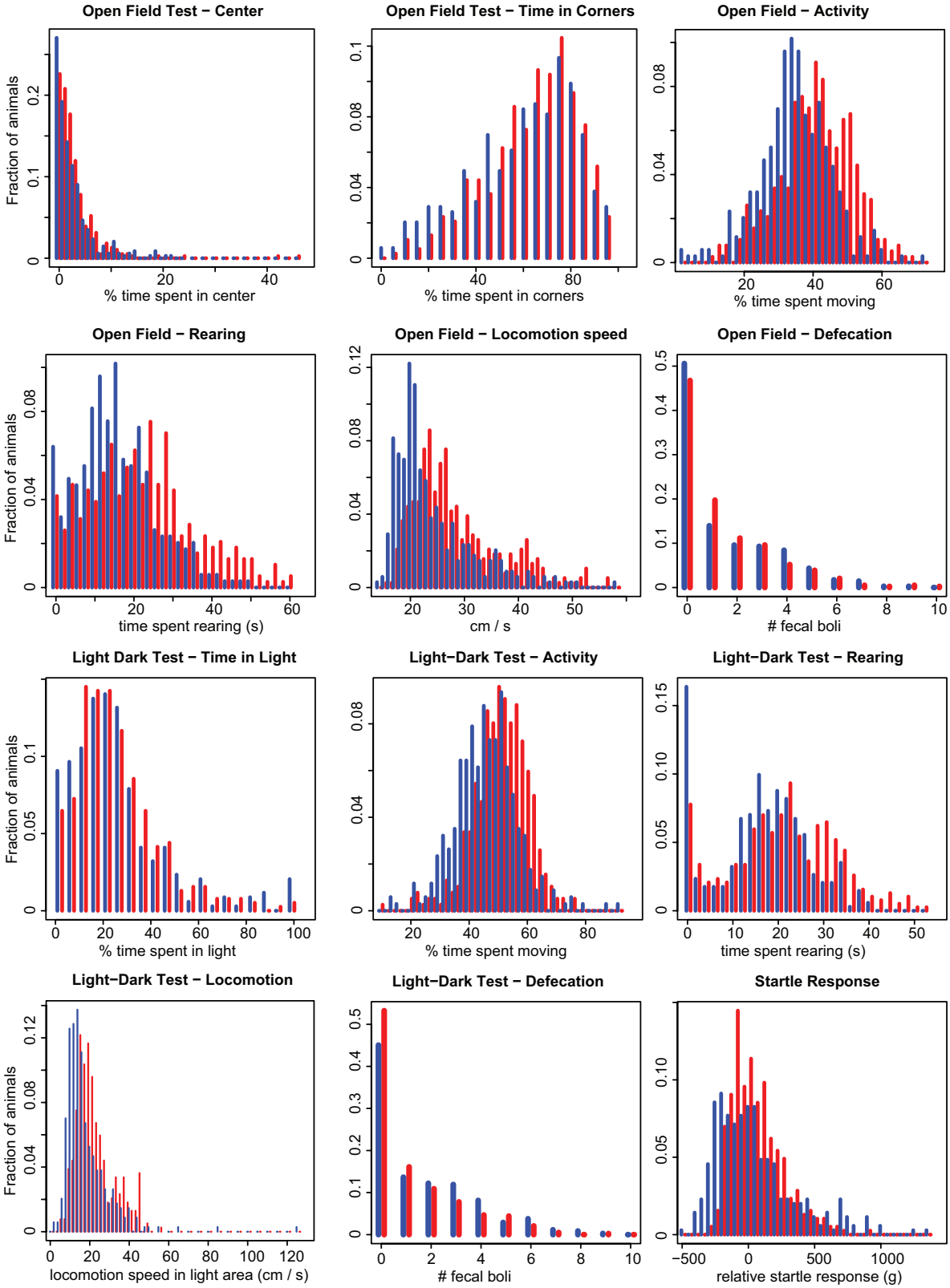

FIGURE S7.—Phenotype distributions for traits recorded in the open field, light-dark, and startle response tests. Red: female F2 rats, blue: male F2 rats.

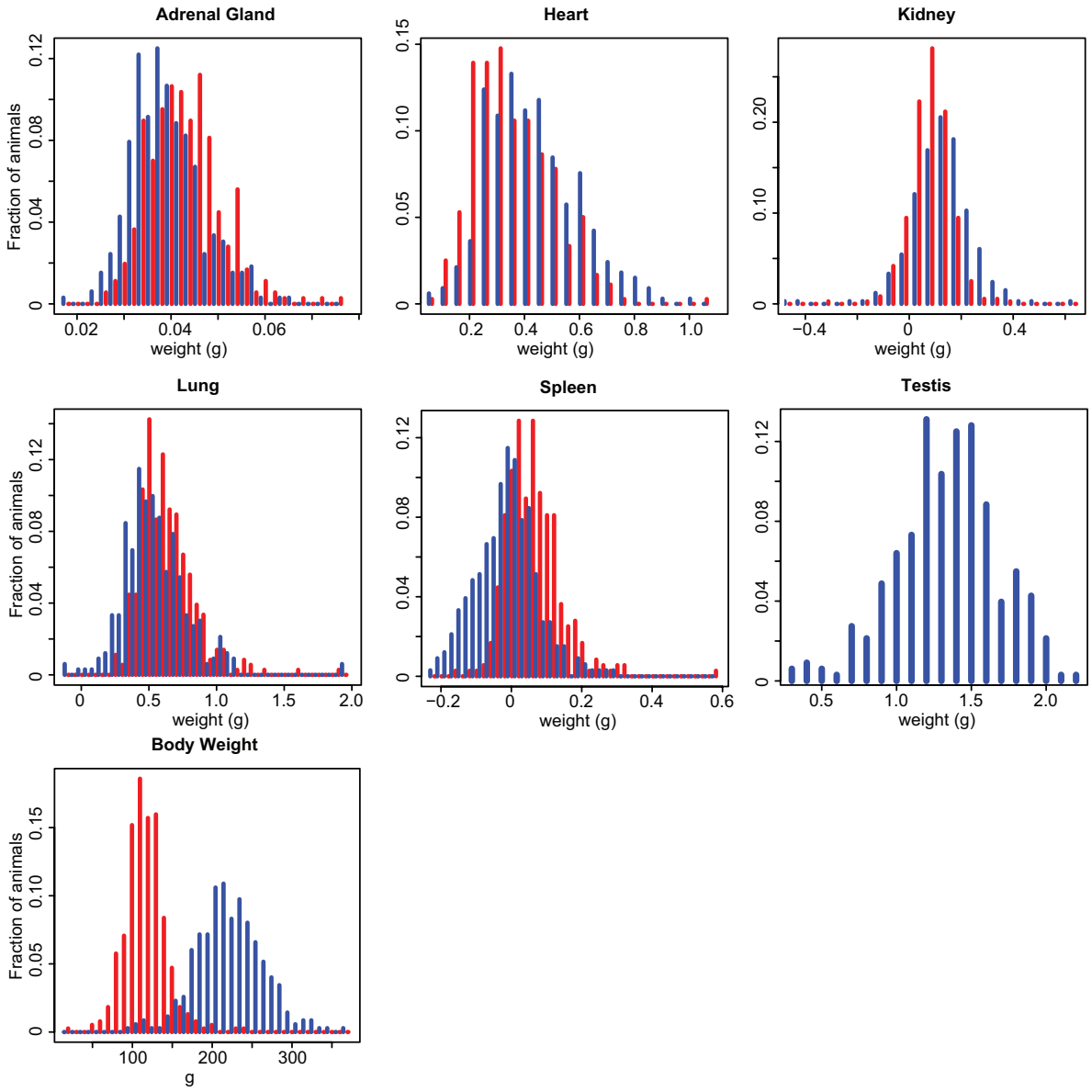

FIGURE S8.—Phenotype distributions for body and organ weights. Organ weights are shown relative to body weight. Red: female F2 rats, blue: male F2 rats

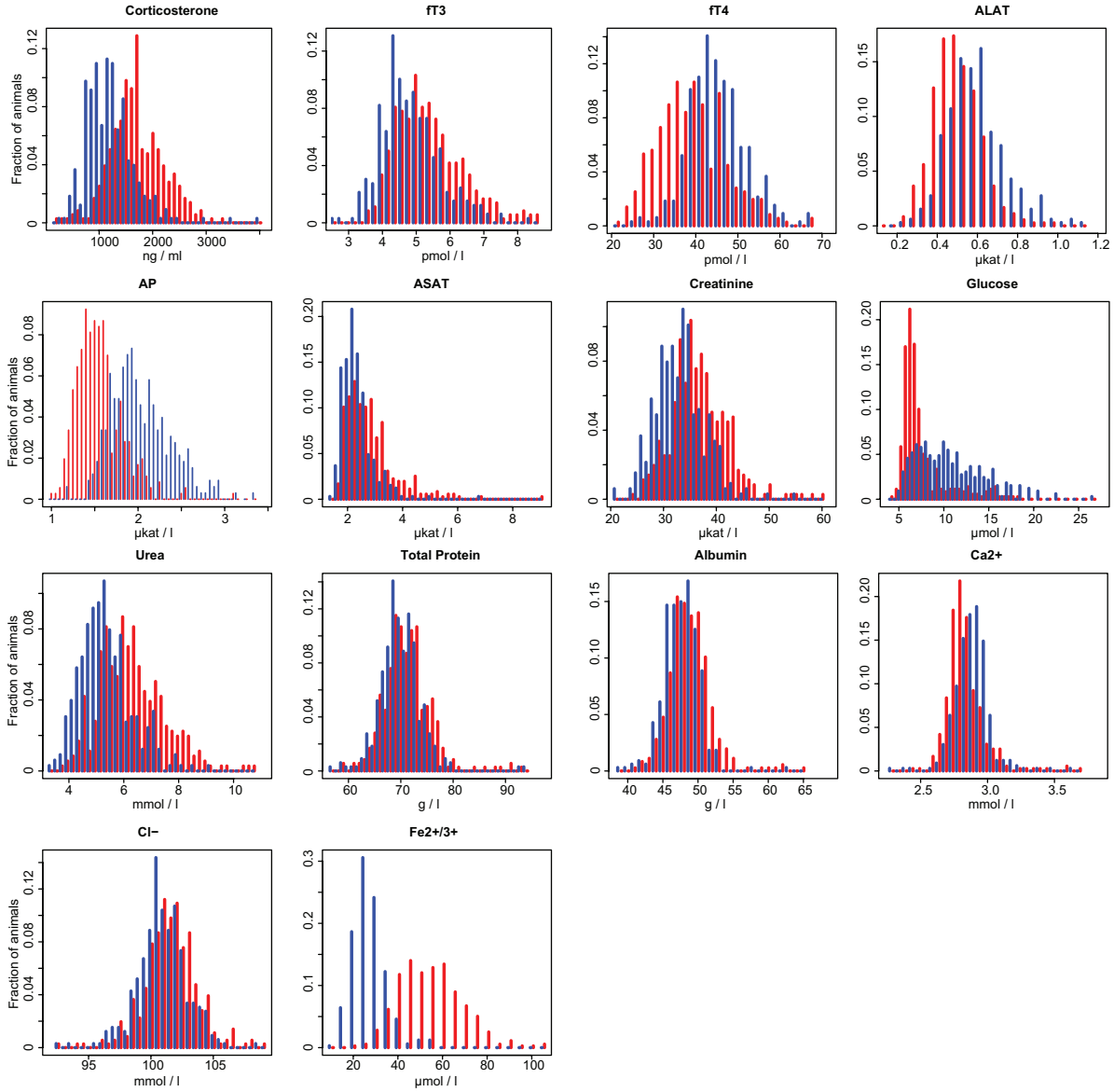

FIGURE S9.—Phenotype distributions for serum traits. Red: female F2 rats, blue: male F2 rats

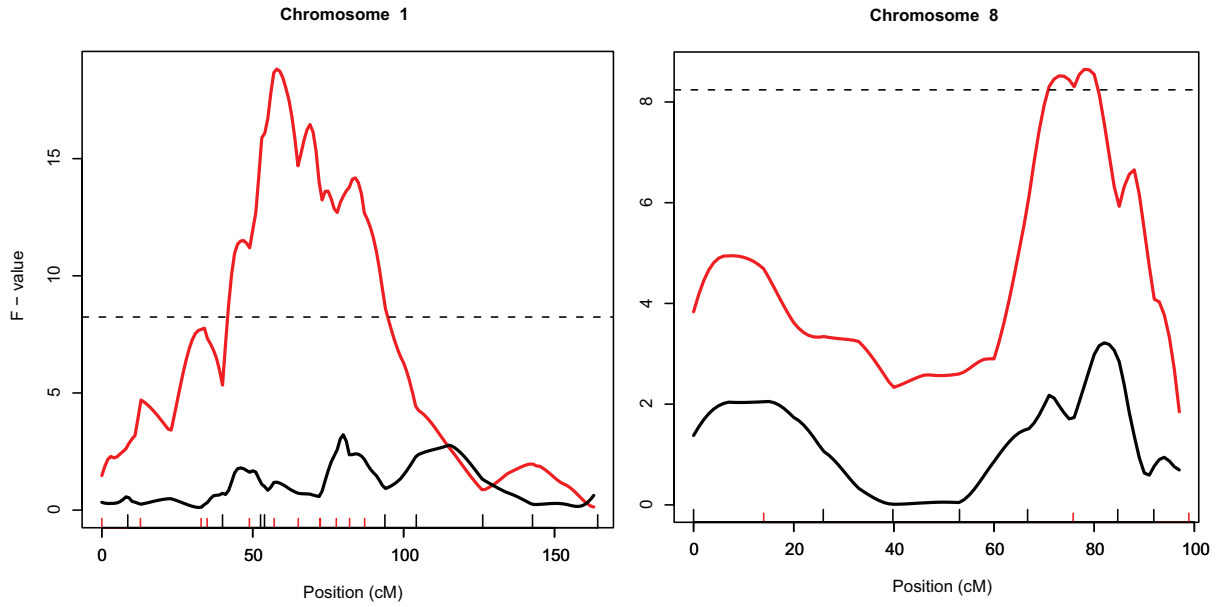

FIGURE S10.—QTLs for tameness do not influence white coat spotting. Red line: tameness. Black line: spotting. Black upward tickmarks: microsatellite marker positions. Red tickmarks: SNP marker positions

TABLE S1

Phenotype modelling

Phenotype group	Fixed effects	Random effects	Covariates	Adjusted effects	Effects included in QTL model
Glove test*	sex, trial	litter, observer	-	litter, trial, observer	sex
Open field & light-dark tests	sex	litter	-	litter	sex
Startle response	sex	litter	body weight	litter	sex, body weight
Organ weights	sex	litter	body weight	litter	sex, body weight
Body weight	sex	litter	age	litter	sex, age
Serum phenotypes	sex	litter	age§	litter, batch	sex, age§

To account for repeated observations of the same rat, trial and observer were modeled as being nested within individual rats. § age was included for traits for which a significant correlation (Pearson's product momentum correlation, $p < 0.05$) between trait and age was found (ALAT, ASAT, AP, creatinine, urea, chloride, iron, protein, albumin and fT4, but not for glucose, calcium, fT3 and corticosterone).

TABLE S2

Genetic markers used in QTL mapping

Table S2 is available as an Excel file at http://www.genetics.org/cgi/content/full/genetics.109.102186/DC1.

TABLE S3

Sex differences

Trait	p-value	females		males		Unit
Open Field Test		mean	s.e.m.[1]	mean	s.e.m.[1]	
time spent in center	0.4	3.46	0.2	3.43	0.2	%
time spent in corner	0.06	0.65	0.01	0.62	0.01	%
time spent moving	< 0.0001	41	0.5	36	0.6	%
time spent rearing	< 0.0001	23	0.7	17	0.6	s
locomotion speed	< 0.0001	29	0.4	25	0.4	cm / s
fecal boli	0.6	2.1	0.1	2.2	0.1	count
Light - Dark Test						
time spent in light compartment	0.6	26	0.9	26	1.1	%
time spent moving	< 0.0001	52	0.5	48	0.6	%
time spent rearing	< 0.0001	22	0.6	18	0.6	s
locomotion speed in light compartment	< 0.0001	22	0.5	19	0.6	cm / s
fecal boli	0.01	1.9	0.1	2.4	0.1	count
Startle Response Test						
startle response	0.01	76	9.9	72	16.4	g
Anatomy						
body weight	< 0.0001	111	1.3	222	2.2	g
adrenal gland weight	< 0.0001	42	0.4	40	0.4	mg
heart weight	< 0.0001	0.33	0.008	0.38	0.009	g
kidney weight	< 0.0001	0.04	0.004	0.07	0.007	g
lung weight	< 0.0001	0.67	0.01	0.64	0.01	g
spleen weight	< 0.0001	0.16	0.004	0.15	0.005	g
Serum traits						
Hormones						
corticosterone	< 0.0001	1650	26	1192	25	ng / ml
fT3	< 0.0001	5.3	0.05	4.8	0.05	pmol / l
fT4	< 0.0001	38.5	0.4	45.3	0.4	pmol / l
Enzymes						
ALAT	< 0.0001	0.49	0.006	0.6	0.008	µkat / l
AP	< 0.0001	1.5	0.01	2	0.02	µkat / l
ASAT	< 0.0001	2.7	0.05	2.3	0.03	µkat / l
Substrates						
creatinine	< 0.0001	36	0.3	33	0.3	µkat / l
glucose	< 0.0001	7.00	0.1	10.5	0.2	µmol / l

urea	< 0.0001	6.2	0.06	5.3	0.05	mmol / l
Serum protein						
total protein	0.014	71	0.2	70	0.2	g / l
albumin	0.0003	48.3	0.2	47.5	0.1	g / l
Electrolytes						
Ca^{2+}	< 0.0001	2.82	0.008	2.89	0.007	mmol / l
Cl^-	< 0.0001	102.5	0.3	101.3	0.2	mmol / l
$Fe^{2+/3+}$	< 0.0001	57	0.7	30	0.4	µmol / l

[1] standard error of the mean

TABLE S4

Correlations between phenotypes

Table S4 is available as an Excel file at http://www.genetics.org/cgi/content/full/genetics.109.102186/DC1.

TABLE S5

Suggestive autosomal QTLs

Trait	unit for genetic effects	Chromosome	peak position (cM)	1 LOD support interval (cM)	F-value	additive effect	dominance effect	% residual phenotypic variance explained	females?	males?
Glove Test										
Tameness (PC1)	-	3	109	93 - 117	5.4	0.12	0.73	1.4	-	-
Tameness (PC1)	-	10	42	11 - 69	5.4	0.4	-0.08	1.4	suggestive	-
Tameness (PC1)	-	12	5	2 - 9	5.4	0.13	-0.69	1.4	-	-
Tameness (PC1)	-	19	61	45 - 62	5	0.14	-0.75	1.3	-	-
PC2	-	14	5	0 - 25	6	0.32	-0.12	1.7	-	suggestive
PC2	-	11	49	36 - 54	4.8	-0.1	0.46	1.4	-	-
PC3	-	19	15	7 - 25	6	0.2	-0.26	1.7	-	-
PC3	-	10	59	41 - 67	5.1	-0.16	0.39	1.5	-	-
PC3	-	17	0	0 - 16	4.9	0.2	0.26	1.4	suggestive	-
approach	count	3	52	46 - 63	6.8	0.04	-0.15	1.9	suggestive	-
attack	count	8	72	67 - 90	6.6	-0.42	-0.19	1.8	-	-
attack	count	12	6	3 - 10	5.9	-0.18	0.68	1.6	-	-
attack	count	17	15	4 - 24	5.8	0.05	0.83	1.6	-	-
escape	count	10	94	89 - 94	7.8	-0.03	0.52	2.2	-	-
escape	count	18	24	16 - 38	6	-0.09	0.33	1.7	-	-
flight	count	10	49	36 - 71	6.8	-0.33	-0.16	1.9	suggestive	-
flight	count	9	27	16 - 41	5.7	-0.26	0.75	1.6	-	-
move & leave	count	7	1	0 - 5	6.3	0.01	0.38	1.8	-	-
move & leave	count	15	25	21 - 46	6	0.09	-0.36	1.7	-	-
move & leave	count	19	1	0 - 15	5.3	-0.19	-0.15	1.5	-	-
move & leave	count	11	43	33 - 52	5	0.03	-0.36	1.4	-	-
squeak	count	8	97	96 - 97	7.5	-0.02	-5	2.1	-	suggestive
squeak	count	17	1	0 - 11	6.8	1.1	1.5	1.9	-	-
squeak	count	3	63	51 - 72	5.5	-1.2	-0.5	1.6	-	-
boxing	s	6	15	6 - 26	7.3	-0.5	-0.3	2	-	-
boxing	s	1	78	72 - 102	7.1	-0.4	-0.5	2	-	-
boxing	s	19	54	42 - 62	6.8	-0.2	1.1	1.9	suggestive	-

screaming	s	8	72	63 - 82	8.6	-0.73	-0.46	2.3	-	-
screaming	s	19	60	42 - 62	5.4	0.14	1.1	1.4	suggestive	-
screaming	s	10	15	6 - 60	5.3	-0.65	0.17	1.4	-	-
screaming	s	12	8	2 - 23	5.1	-0.4	0.76	1.4	-	-
freezing	s	10	84	68 - 91	6.2	2	-0.7	1.7	-	suggestive
tolerate touch	s	18	30	23 - 39	7.2	0.35	-0.22	1.9	significant	-
tolerate touch	s	6	86	73 - 93	6.6	1.05	-1.09	1.8	-	-
tolerate touch	s	8	0	0 - 17	5.5	0.46	-0.21	1.5	-	-
tolerate touch	s	8	80	67 - 92	5.2	0.78	0.99	1.5	-	significant
tolerate handling	s	19	17	0 - 27	7	0.79	-0.17	2	-	-
tolerate handling	s	14	11	0 - 27	5.6	0.83	0.25	1.6	-	-
tolerate handling	s	6	61	51 - 80	5.3	0.68	-0.17	1.5	-	suggestive
tolerate handling	s	13	52	32 - 52	5.2	0.46	1.2	1.5	-	-
tolerate handling	s	11	49	40 - 54	5	-0.22	1.3	1.4	-	-
Open field										
time spent in center	%	8	27	18 - 56	7.8	0.0095	-0.22	2.2	-	suggestive
time spent in center	%	10	71	43 - 81	5.9	0.0079	0.0046	1.7	-	-
time spent moving	%	2	103	96 - 118	6.3	0.011	0.028	1.8	-	-
time spent moving	%	8	94	85 - 97	6.3	0.024	0.004	1.8	-	-
time spent moving	%	1	70	62 - 87	6.1	0.021	0.012	1.8	-	-
rearing	s	8	93	85 - 97	4.9	2.3	-0.2	1.4	-	-
locomotion speed	cm / s	2	86	83 - 95	6.9	0.5	2.7	2	-	suggestive
locomotion speed	cm / s	13	52	48 - 52	5.1	-1.1	-2.4	1.5	-	-
fecal boli	count	2	63	36 - 79	7.6	0.5	-0.2	2.2	suggestive	-
fecal boli	count	9	0	0 - 10	6.6	-0.5	-0.04	1.9	-	suggestive
fecal boli	count	10	80	68 - 91	6.6	0.6	-0.3	1.9	-	-
fecal boli	count	17	18	6 - 25	6.3	0.4	0.6	1.8	-	-

Light Dark Test										
time spent in light	%	6	38	27 - 54	7.6	0.044	-0.013	2.2	-	suggestive
time spent in light	%	19	53	43 - 62	7	-0.028	-0.097	2	significant	-
time spent in light	%	18	0	0 - 23	6	0.035	-0.025	1.7	-	suggestive
time spent in light	%	2	82	72 - 86	5.9	0.0009	0.064	1.7	-	-
time spent moving	%	3	0	0 - 2	7.1	0.015	0.036	2	-	-
time spent moving	%	7	35	18 - 73	6.2	-0.023	-0.027	1.8	suggestive	-
time spent moving	%	2	66	58 - 77	6	0.012	0.029	1.7	-	-
rearing	s	2	76	62 - 82	6	0.9	4	1.7	-	-
locomotion speed in light compartment	cm / s	7	24	7 - 52	5.4	2.2	-3.3	1.5	-	suggestive
locomotion speed in light compartment	cm / s	18	0	0 - 14	5.3	-0.3	2.5	1.5	-	-
fecal boli	count	18	21	2 - 25	8.3	0.5	0.3	2.4	suggestive	suggestive
fecal boli	count	4	58	52 - 71	6.5	0.5	-0.06	1.9	-	-
fecal boli	count	13	19	8 - 28	5	-0.3	-0.5	1.4	-	-
Startle response										
startle response	g	2	47	26 - 57	7.6	30	-81	2.1	-	-
startle response	g	4	73	63 - 86	6	-32	-64	1.6	-	significant
startle response	g	8	26	17 - 38	5.7	43	-32	1.6	-	-
startle response	g	13	15	3 - 22	5.2	50	23	1.4	-	-
Anatomy										
adrenal gland	mg	11	42	34 - 54	7.1	1.4	-1.9	1.8	suggestive	-
adrenal gland	mg	2	48	37 - 56	6.1	0.6	2.1	1.6	-	-

adrenal gland	mg	6	65	47 - 82	6.2	-1.4	1.3	1.6	-	-
adrenal gland	mg	8	64	42 - 79	6.1	1.5	0.4	1.6	-	suggestive
adrenal gland	mg	16	18	15 - 26	5.6	1.4	1.5	1.4	-	-
adrenal gland	mg	12	11	6 - 25	5	-1.1	1.3	1.3	-	-
body weight	g	18	16	0 - 22	6.8	6.3	-3.8	1.8	suggestive	-
heart	g	18	20	6 - 26	5.4	0.03	-0.005	1.5	-	-
kidney	g	3	54	31 - 66	6.9	0.02	0.01	2	-	-
kidney	g	7	13	0 - 25	5.9	0.02	0.02	1.8	-	-
kidney	g	8	88	85 - 97	5.9	0.02	0.01	1.7	-	-
kidney	g	20	26	11 - 42	4.3	0.003	0.007	1.3	-	suggestive
lung	g	10	53	35 - 67	5.8	0.02	-0.1	1.7	-	-
lung	g	6	35	19 - 43	5.2	-0.007	0.06	1.5	-	-
spleen	g	20	21	10 - 31	6.9	0.007	0.019	1.8	suggestive	-
spleen	g	3	63	51 - 75	6.6	-0.015	0.014	1.8	-	-
spleen	g	18	17	11 - 24	5.4	0.014	-0.005	1.5	-	suggestive
testis	g	10	92	86 - 94	6.4	0.06	0.26	3.9	n/a	n/a
testis	g	17	21	5 - 25	7.2	0.09	-0.09	4.3	n/a	n/a
spotting	-	6	48	39 - 56	5.8	0.06	-0.14	1.8	-	suggestive
Serum traits										
albumin	g / l	3	5	0 - 22	7.5	0.47	-0.66	2.3	significant	-
albumin	g / l	4	62	52 - 85	5.4	-0.46	-0.17	1.6	-	-
AP	µkat / l	10	52	8 - 72	5.8	-0.06	0.15	1.7	-	-
AP	µkat / l	7	92	78 - 103	5.3	0.02	-0.11	1.6	-	-
creatinine	µkat / l	15	23	15 - 28	6.9	-0.97	-0.67	2.1	-	-
creatinine	µkat / l	19	51	30 - 62	5	-1.03	-1.10	1.5	-	-
corticostero ne	ng / ml	5	18	1 - 30	6	-23.8	201	1.9	significant	-
corticostero ne	ng / ml	6	88	64 - 94	6	-110	29	1.9	-	-
fT4	pmol / l	5	94	76 - 109	8.5	-2.2	0.4	2.6	-	suggestive
fT4	pmol / l	14	34	3 - 47	7.1	-1.8	-0.5	2.2	suggestive	-
fT4	pmol / l	13	2	0 - 22	5.3	1.3	-1	1.6	-	-
ALAT	µkat / l	5	67	57 - 77	7.3	-0.015	-0.045	2.3	-	-
ALAT	µkat / l	12	21	10 - 42	5.8	0.026	-0.024	1.8	-	-
ASAT	µkat / l	8	87	81 - 92	7.8	-0.14	0.25	2.4	suggestive	-
glucose	µmol / l	2	49	37 - 57	6.6	0.67	-0.09	2	-	-
urea	mmol / l	7	106	94 - 106	5.8	-0.06	-0.37	1.8	-	-
total protein	g / l	3	117	112 - 17	6.3	0.75	0.38	2	-	significant

total protein	g / l	11	15	7 - 19	6	0.28	-1.2	1.9	suggestive	-
total protein	g / l	7	64	50 - 103	5.8	0.6	0.65	1.8	-	-
total protein	g / l	8	2	0 - 36	5.6	-0.72	-0.86	1.7	-	-
total protein	g / l	6	89	66 - 94	5.2	-0.64	-0.56	1.6	-	-
Cl-	mmol / l	2	144	134 - 147	6.3	-0.7	0.67	1.9	suggestive	-
Cl-	mmol / l	10	46	10 - 71	5.4	-0.51	-0.27	1.7	-	-
Fe2+/3+	µmol / l	2	96	87 - 117	7	-2.4	-0.1	2.2	suggestive	-
Fe2+/3+	µmol / l	18	22	0 - 26	5.4	-0.02	1.8	1.7	-	-

Shown are QTLs with chromosome-wide (but not genome-wide) significance

TABLE S6

X-Chromosomal QTLs

Trait	Peak (cM)	d.f.[1]	LR[2]	p value	additive effect	% variance[3]
urea	47	2	13.9	0.00096	-0.34	2.2

[1] degrees of freedom; [2] likelihood ratio; [3] residual phenotypic variance explained after accounting for fixed effects

TABLE S7

Sex-specific QTLs identified at genome-wide significance

Trait	sex	chr[1]	peak[2]	1 LOD c.i.[2, 3]	F	additive effect	dominance	% variance[4]
adrenal gland	females	5	48	44 – 58	9.5	-2.7 mg	-1.3 mg	5.3
corticosterone	females	12	0	0 - 2	8.5	12 ng / ml	280 ng / ml	4.9

Only significant QTLs that do not overlap with QTLs found in the sex-combined analyses are shown. See Table 3 and Table S5 for sex-specific QTLs overlapping QTLs found in the sex-combined analyses. [1] chromosome; [2] cM; [3] confidence interval; [4] residual phenotypic variance explained after accounting for fixed effects;