Supplemental Table 1. Assembly statistics for Dovetail genome

	Short-read assembly	**Final Assembly**
Total Length (Mbp)	2,113.27	2,119.20
L50 (scaffold count)	7016	5
N50 (Mbp)	0.08	165.747
L90 (scaffold count)	30,428	14
N90 (Mbp)	0.015	30.611
Busco (Eukaryota)		
Single copy	246	263
Duplicated	7	7
Fragmented	25	9
Missing	25	24
Total	303	303

Supplemental Table 2. Chromosome-level anchoring of genome using *Phodopus* genetic map

Chromosome	Mbp	cM	Marker count	All scaffolds	Scaffolds > 1Mbp
1	317.65	122.51	208	3	2
2	288.91	120.25	174	4	1
3	258.08	96.92	148	3	2
4	155.67	97.42	137	2	1
5	291.62	122.99	134	6	5
6	129.1	107.65	135	4	1
7	224.74	86.16	118	1	1
8	125.09	67.23	74	2	1
9	32.54	48.68	68	3	1
10	64.22	52.51	52	2	2
11	37.12	54.88	46	2	2
12	24.28	54.05	47	2	1
13	30.61	45.14	42	1	1
X	119.12	42.79	42	2	1
unplaced	20.41	NA	NA	7719	0

Supplemental Table 3: Comparison of features on the X chromosomes of dwarf hamster and rat

	Length (Mbp)	Prop. X	gene count, gene models	gene count, with pseudo-genes	Prop. genes	average intergenic distance, gene model genes only	median intergenic distance, gene model genes only	median exon length
Phodopus X	119.1		697	896		136402	41537.5	238
Xq	41.69	0.350	318	385	0.544	105144	29915.5	236.5
Xp	77.42	0.650	379	511	0.456	162698	56238.5	238
Rat X	159.89		824	1048		156894	51211	282
Xq	55.50	0.347	352	388	0.427	125683	40508	279
Xp	104.39	0.653	472	660	0.573	180086	62730	291.5

Supplemental Table 4. Transposable element enrichment on X chromosome arms

		Phodopus				*Rattus*			
Repeat genus	**Repeat species**	**recomb. count**	**suppress. count**	**P value**	**enriched arm**	**recomb. count**	**suppress. count**	**P value**	**enriched arm**
DNA	Academ	1	1	1		N/A	N/A		
	CMC-EnSpm	526	1270	**0.00261**	**Xp**	1668	2594	4.30E-08	recomb.
	DNA	64	149	1		242	416	1	
	Harbinger	8	12	1		27	49	1	
	hAT	5	5	1		14	9	0.22272	
	hAT-Ac	123	185	0.4346		442	829	1	
	hAT-Charlie	731	1399	1		1029	1755	0.21794	
	Mariner	1	3	1		3	5	1	
	Maverick	14	21	1		58	76	0.63574	
	Tigger	228	353	0.0946		296	412	0.00181	Xq
	Tip99	27	27	0.29001		26	29	0.7734	
LINE	CR1	0	2	1		#N/A	#N/A		
	L0	9889	19087	1		21923	38221	6.38E-15	Xq
	L2	41	30	0.00113	Xq	36	20	0.00017	Xq
LTR	ERV1	642	1753	2.83E-11	Xp	1259	2659	0.01409	Xp
	ERVK	1842	3739	1		1358	3234	3.95E-12	Xp
	ERVL	777	1617	1		1904	3474	1	

	ERVL-MaLR	3212	6783	**0.04414**	**suppress.**	7720	13691	0.00068	recomb.
	Gypsy	31	82	1		56	118	1	
	Ngaro	9	170	**7.48E-15**	**Xp**	18	19	0.92928	
Retro-transposon	L1	287	575	1		459	706	0.01585	Xq
rRNA	rRNA	15	28	1		10	38	0.62725	
SINE	Alu	10451	19278	5.02E-07	Xq	18038	30379	6.38E-15	Xq
	B4	951	1603	0.00300	Xq	2342	3490	6.38E-15	Xq
	ID	331	666	1		2888	5162	0.4038	
	MIR	151	165	7.16E-06	Xq	211	227	2.02E-07	Xq
	SB1	1577	2961	1		6155	9844	6.38E-15	Xq
snRNA	snRNA	25	54	1		73	125	1	
tRNA	tRNA	2	0	1		7	7	1	
Satellite	Satellite	3	3	1		56	129	1	
Simple repeat	Simple repeat	28456	58384	**6.06E-06**	**Xp**	22316	35728	6.38E-15	Xq
Low complexity	Low complexity	5483	11451	**0.01818**	**Xp**	3136	5189	3.61E-07	Xq
Unknown	Unknown	1305	3129	**9.50E-08**	**Xp**	992	1870	1	
tRNA core	tRNA core	101	122	0.00722	Xq	N/A	N/A		

Supplemental Table 5: Counts of tissue-enriched genes by chromosome, and BH corrected p-values

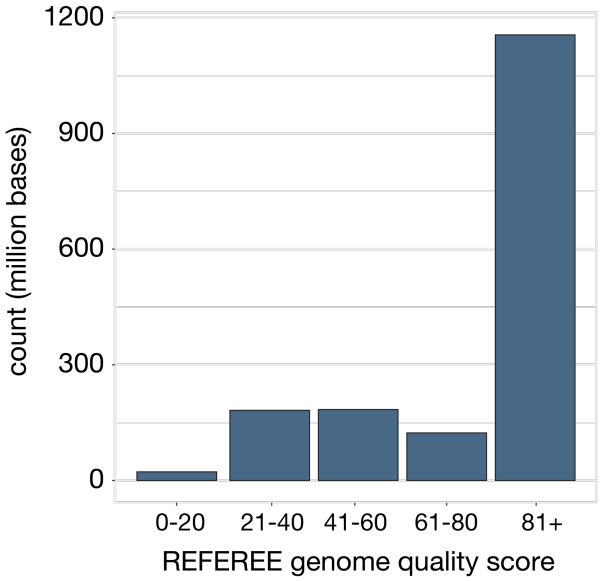
Count

Chr.	Total	Brain	Heart	Kidney	Liver	Muscle	Placenta	Spleen	Testis	Uterus
1	1233	285	36	99	56	30	86	198	342	101
2	1250	287	41	107	60	41	98	106	409	101
3	917	184	33	90	43	36	55	89	314	73
4	883	172	29	60	34	37	76	72	317	86
5	1029	199	37	70	67	23	105	112	349	67
6	723	177	31	42	40	24	47	67	241	54
7	869	181	16	86	45	43	59	83	281	75
8	475	101	20	31	21	14	35	76	142	35
9	405	105	9	18	25	12	28	56	128	24
10	281	43	11	21	7	13	17	77	73	19
11	88	21	6	11	4	2	3	11	29	1
12	152	42	4	15	6	9	1	27	39	9
13	154	33	3	12	14	7	6	17	49	13
X	370	70	10	13	7	9	50	19	164	28
unplaced	66	8	0	1	3	3	1	9	41	0
total	8895	1908	286	676	432	303	667	1019	2918	686
Xq	171	39	1	5	3	3	29	7	67	17
Xp	199	31	9	8	4	6	21	12	97	11

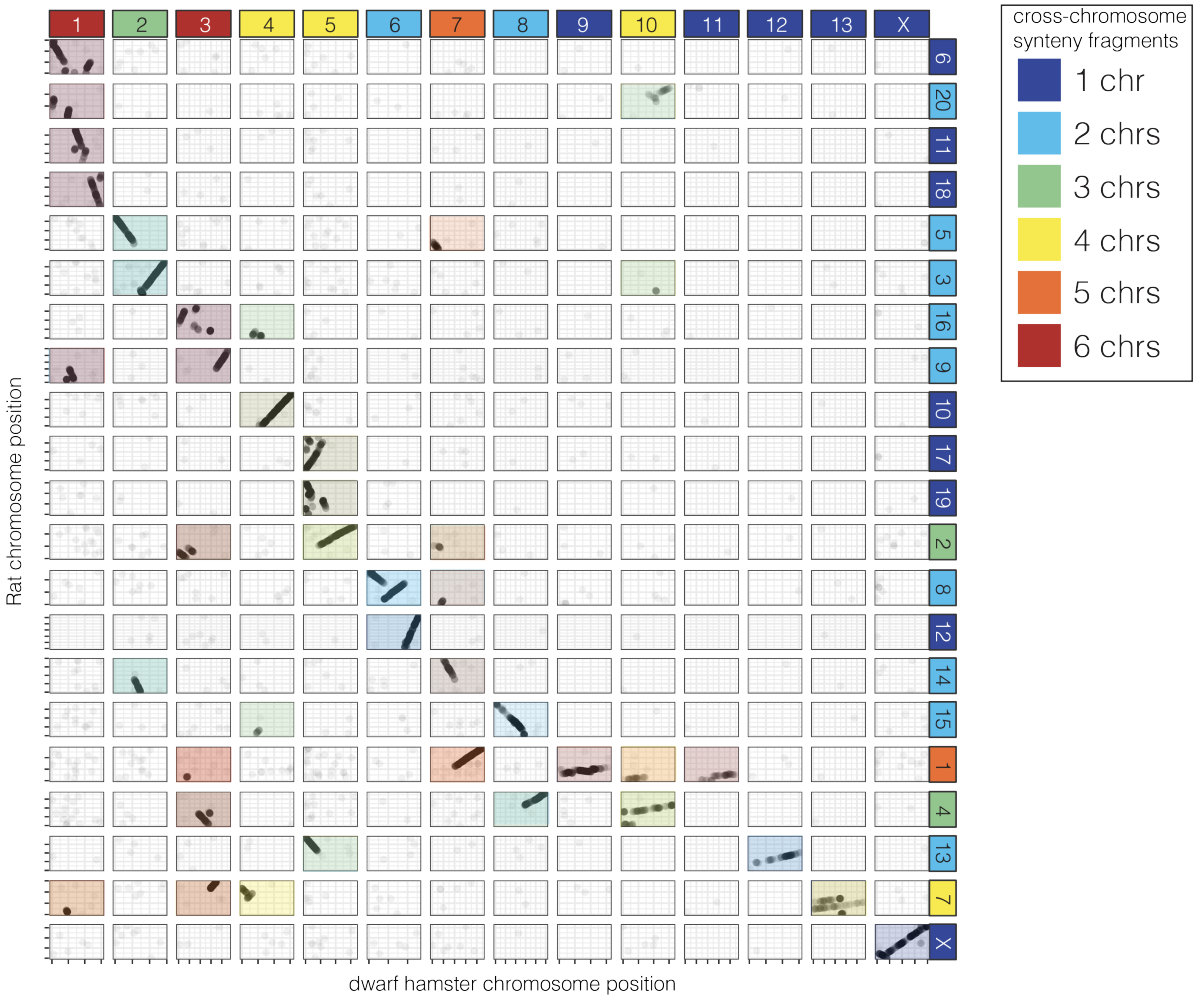
Adjusted Pval

Chromosome	Brain	Heart	Kidney	Liver	Muscle	Placenta	Spleen	Testis	Uterus
1	1	1	1	1	1	1	0.0011846	1	1
2	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	0.370845	1	1	1
6	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	0.315248	1	1
9	**0.00055772**	1	1	0.370912	1	1	**0.0197259**	0.97726	1
10	1	1	1	1	1	1	**2.49E-14**	1	1
11	1	1	1	1	1	1	1	1	1
12	**0.0025056**	1	0.489456	1	0.201204	1	**0.0027027**	1	1
13	1	1	1	1	1	1	1	1	1

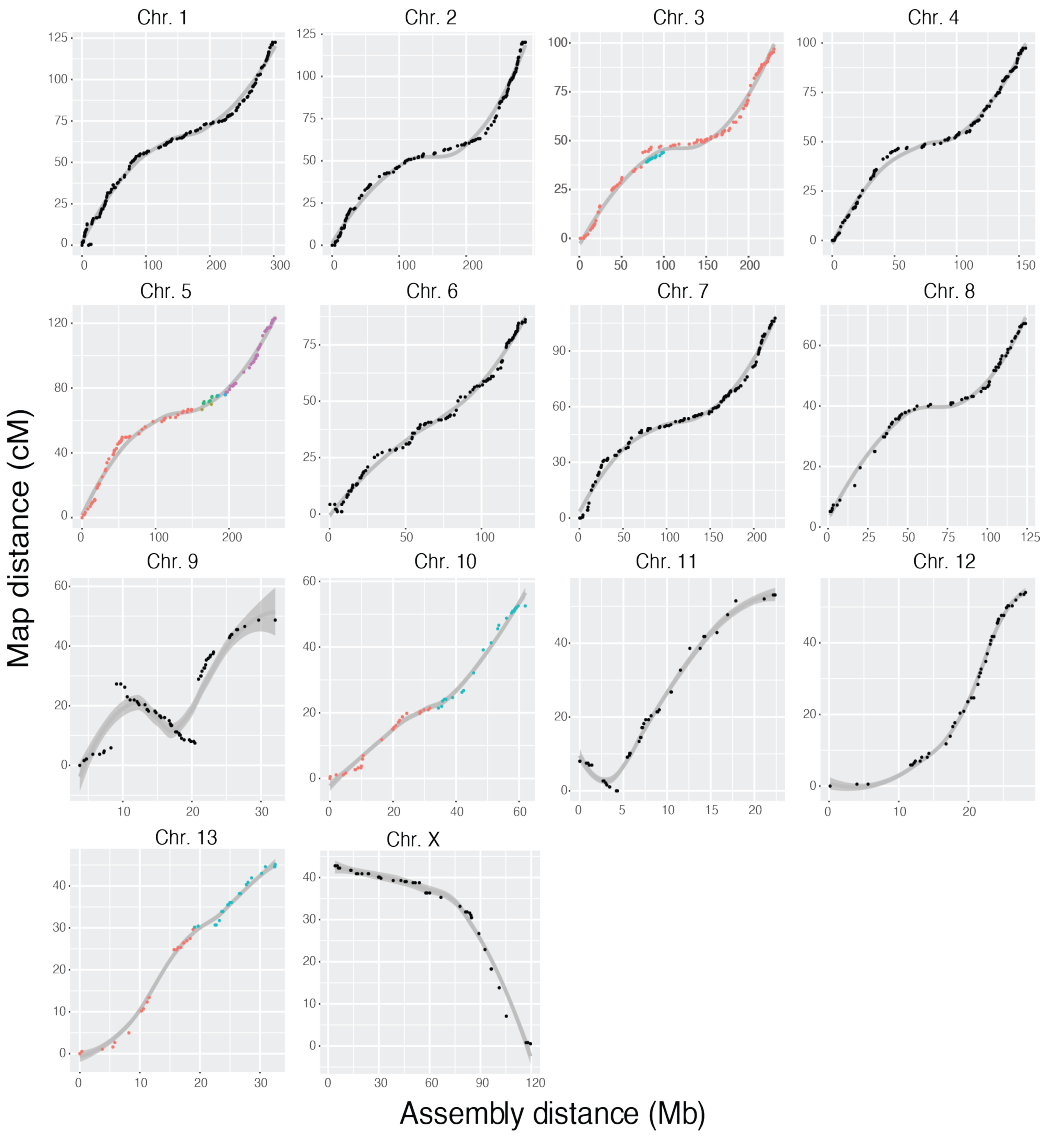
X	1	1	1	1	1	**1.44E-08**	1	**3.43E-15**	1
unplaced	1	1	1	1	0.16624 4	1	**0.0060634**	**2.11E-13**	1
Xq	0.230845	1	1	1	1	**8.84E-07**	1	**0.0004527 6**	0.44209 2
Xp	1	1	1	1	1	0.05699 4	1	**4.31E-11**	1



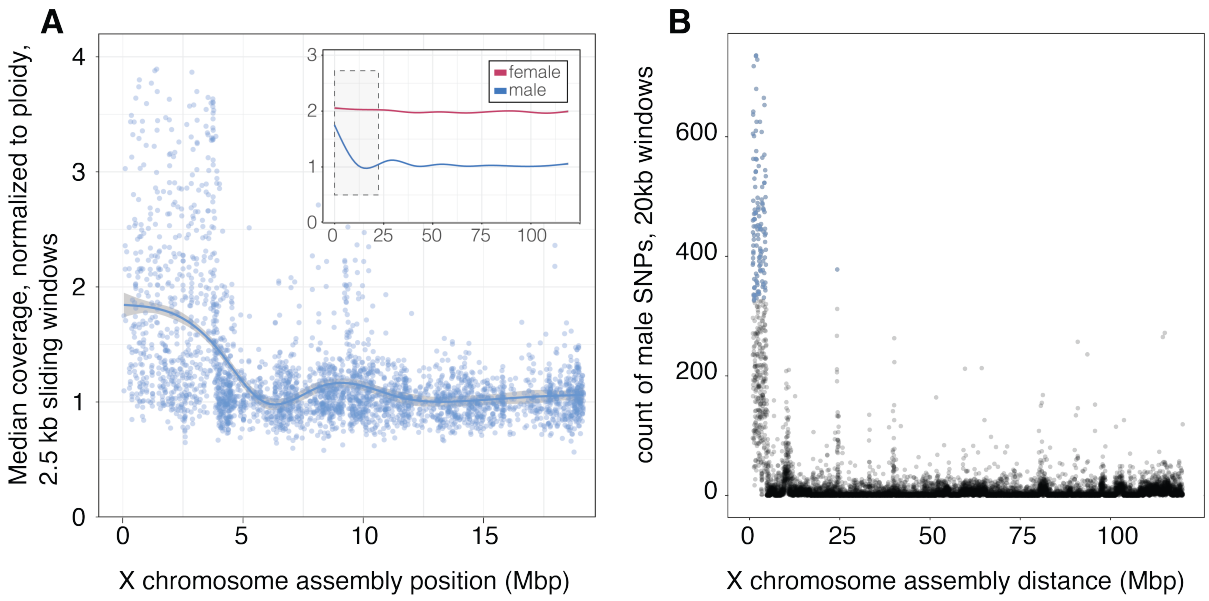
Supplemental Figure 1. Distribution of site-based quality scores from the largest scaffold per chromosome from Referee.



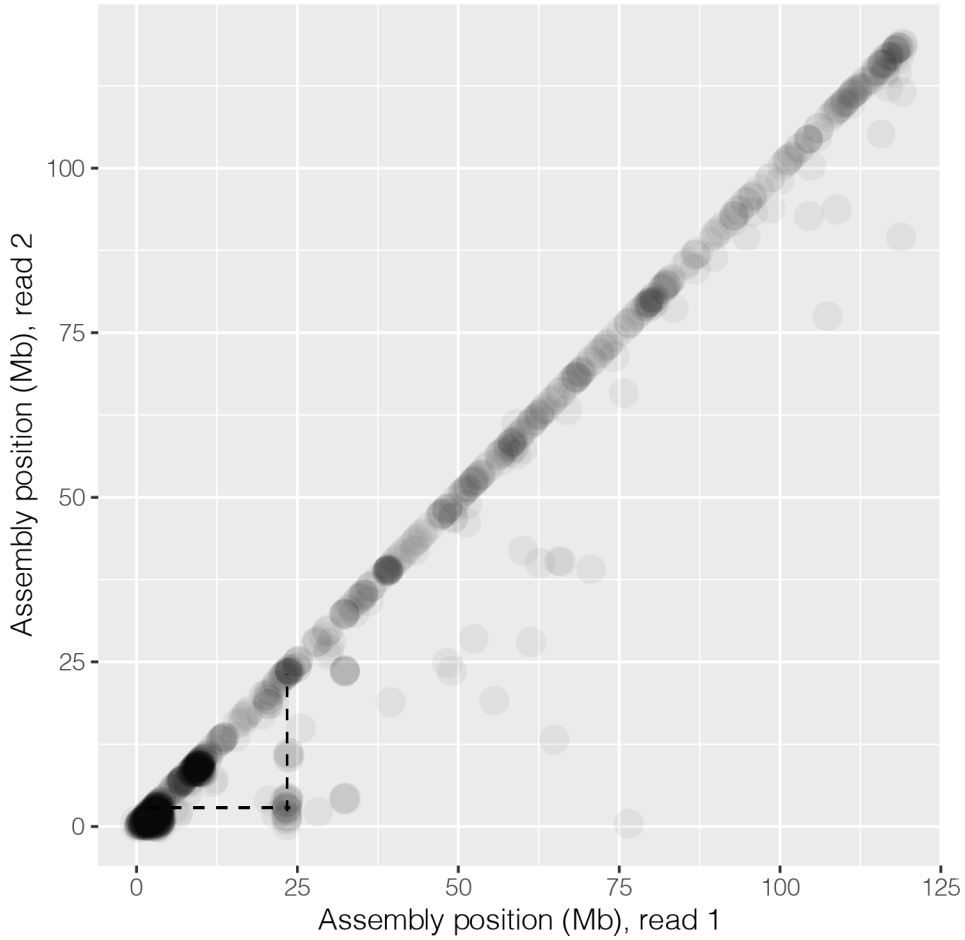
Supplemental Figure 2. Synteny between rat and dwarf hamster chromosomes. Gene positions for dwarf hamster chromosomes (x-axis) and rat (y-axis), reflecting levels of conservation in chromosomal content between species. The number of chromosomes with syntenic content in the other species is emphasized by color, with warmer colors indicating that the same content is spread across multiple chromosomes. Dwarf hamster chromosomes (columns) are ordered chromosome number, and rat chromosomes (rows) are ordered relative to their shared identity with dwarf hamster.



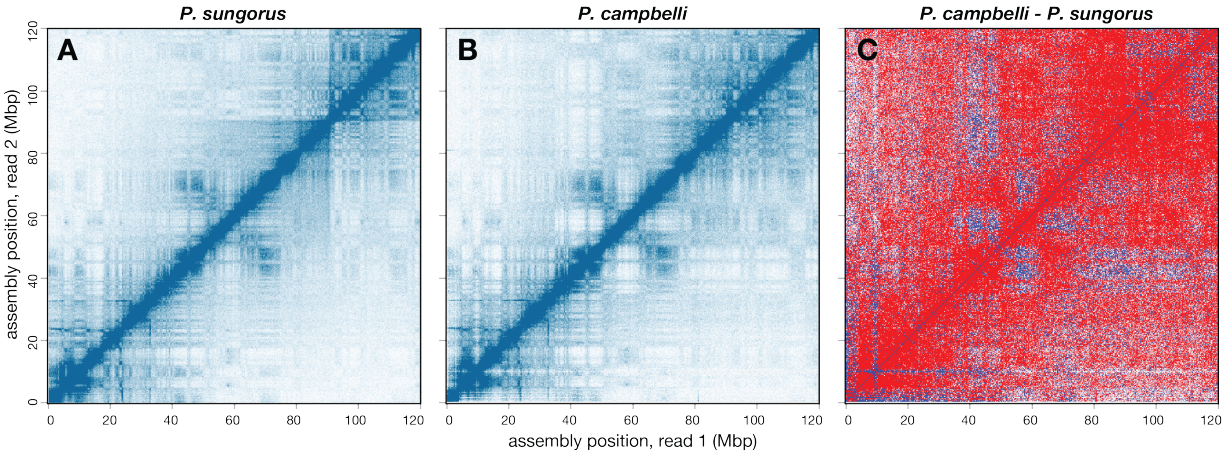
Supplemental Figure 3. Marker positions by genetic map (cM) and assembly positions (Mb) for all chromosomes. Genetic and physical marker locations show recombination across each chromosome for all anchored scaffolds over 1Mb, with the line showing a smoothed spline best fit. The slope of this line reflects recombination rate, with a steeper slope indicating a higher recombination rate. Metacentric chromosomes 1-8, 10, and 13 show an expected reduction in recombination rate near the centromere, whereas chromosome 12 shows an initial reduction in recombination that likely reflect acrocentric centromeres. Despite being metacentric, the X chromosome never recovers recombination on the Xp arm. Regions of chromosome 9 and chromosome 13 with negative slopes likely reflect assembly errors. Colored points on chromosomes 3, 5, 10, and 13 indicate scaffolds, all other chromosomes consist of one major scaffold.



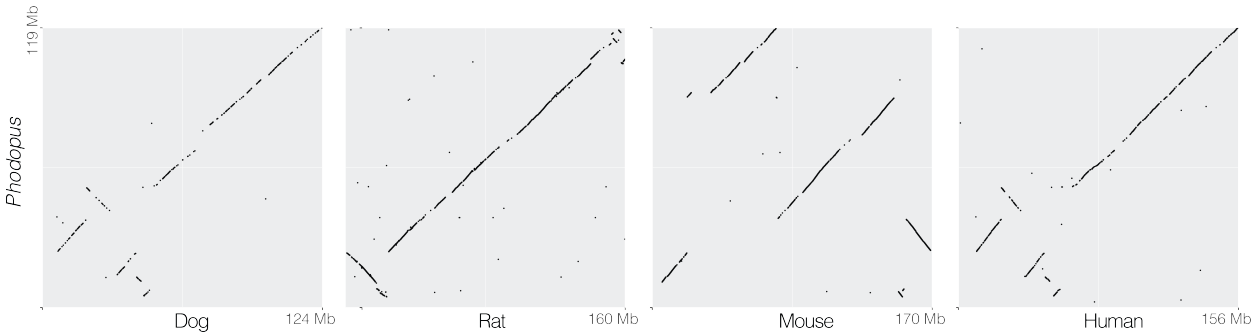
Supplemental Figure 4. Identification of the pseudoautosomal region of the X chromosome. (A) Inset, median coverage normalized to ploidy along the entire *P. sungorus* X chromosome for males (blue) and females (female). The main panel shows the start of the X chromosome, where the pseudoautosomal region is indicated by an increase in coverage where reads from the Y map to homologous sequence on the X. Mean coverage calculated in 2.5 kbp sliding windows. (B) The start of the chromosome also shows an increase in the number of SNPs called from the male sequence, suggesting that these reads come from divergent Y sequence. Count of SNPs in 20 kbp windows. For (B), blue points indicate windows in the top and bottom 1% of the distribution of values.



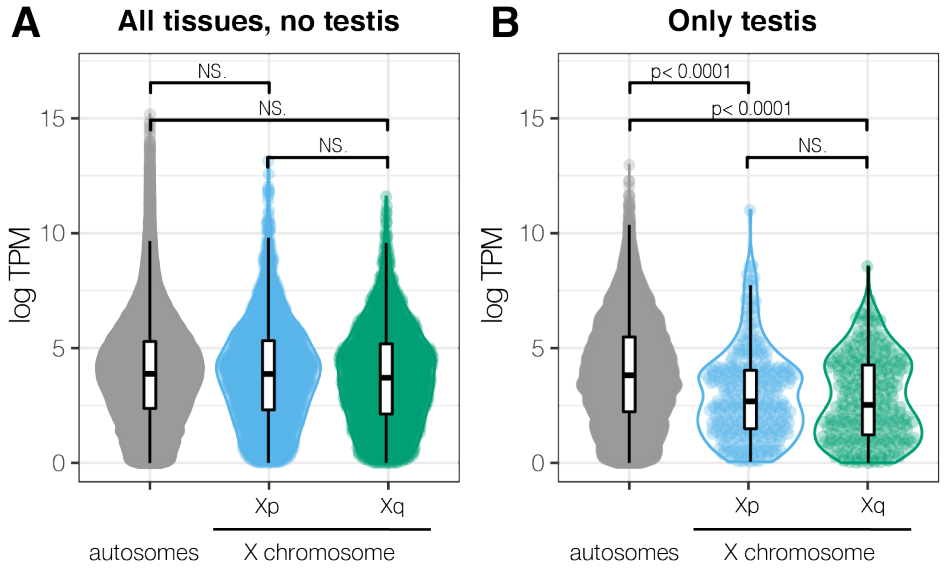
Supplemental Figure 5. Short-read support an inversion between *P. sungorus* and *P. campbelli* on the X chromosome. Differences in the mapping location of read 1 (x-axis) and read 2 (y-axis) in a read-pair indicate an inversion in *P. campbelli*, using short-reads from *P. sungorus* as a baseline. Darker colors indicate more support for an inversion in this location. Dashed lines show edges of breakpoints.



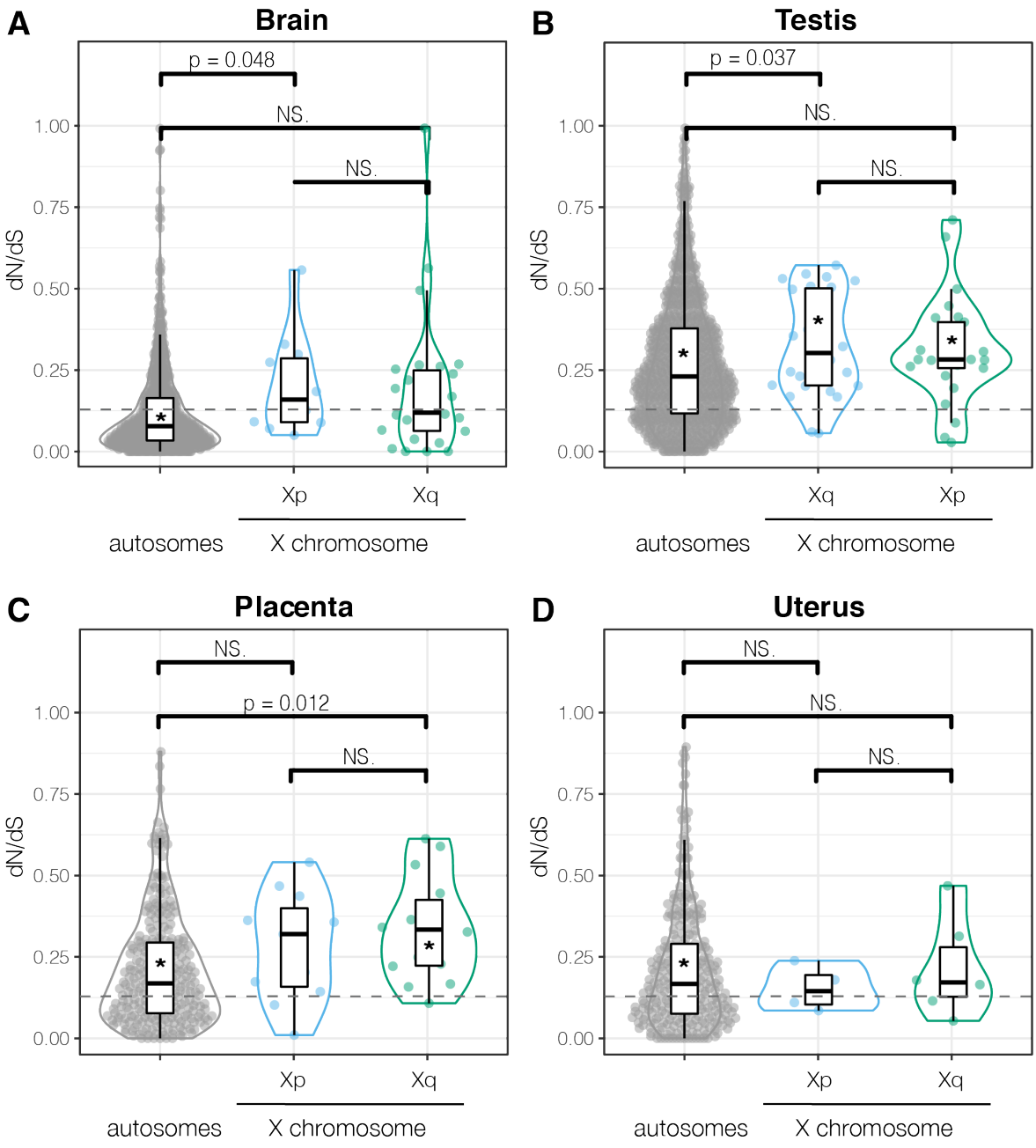
Supplemental Figure 6. Comparative chromatin configuration of the X chromosome between *P. sungorus* and *P. campbelli*. HiC chromatin interactions, show short- and long-range interactions between points on the X chromosome, 250 Kbp resolution, square root coverage normalization for (A) *P. sungorus* and (B) *P. campbelli*. (C) The difference between the two , where blue indicates increased contact in the *P. sungorus* chromatin map relative to the *P. campbelli* map.



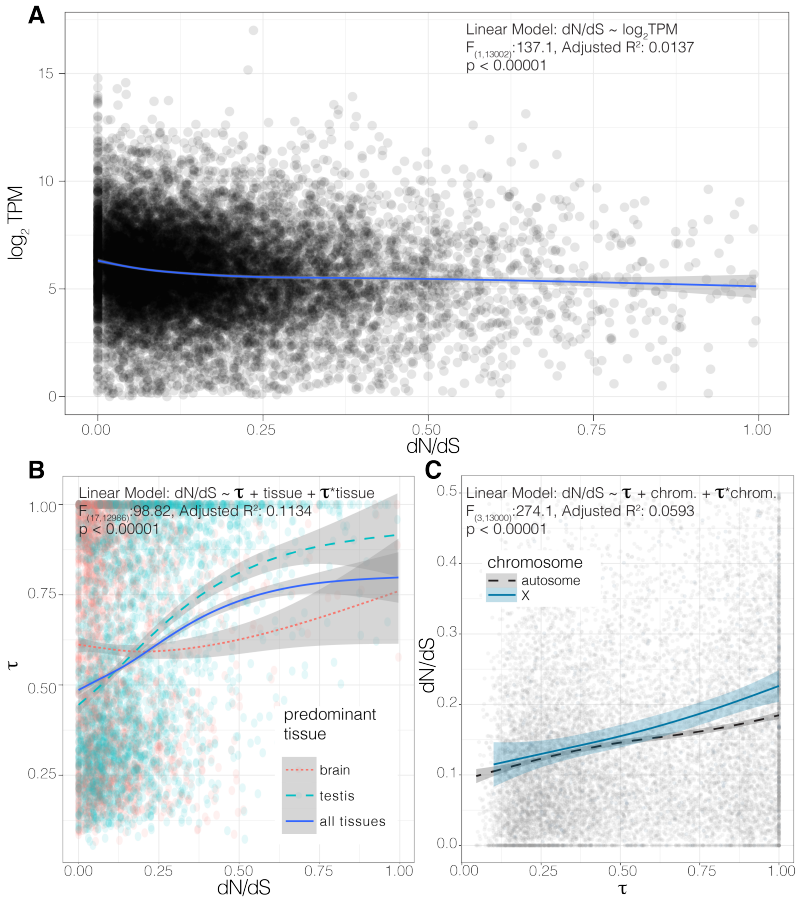
Supplemental Figure 7. X chromosome synteny between mammalian species. Alignments of the X chromosomes of dwarf hamster to domestic dog (CanFam3.1), rat (rnor6), mouse (mm10), and human (GRCh38) show broad conservation of synteny across mammals with the exception of mouse.



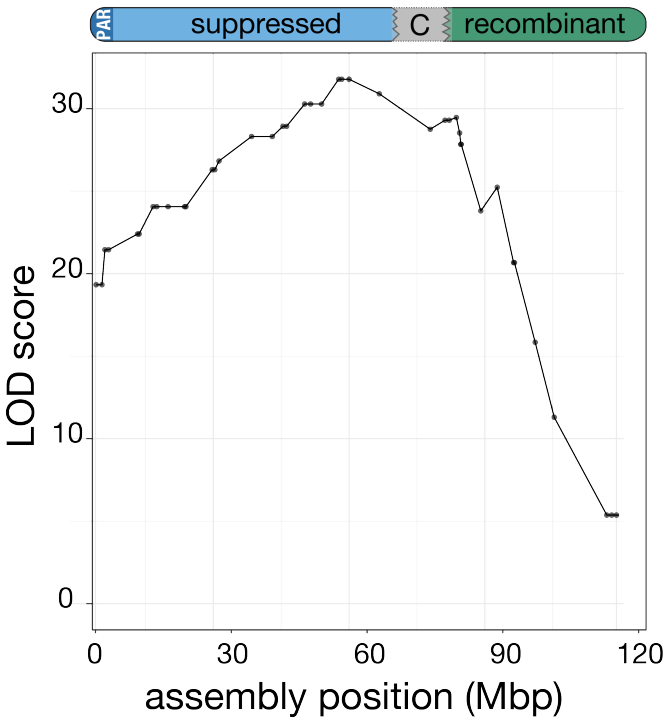
Supplemental Figure 8. Gene expression levels on the X chromosome arms vs autosomes. Gene expression levels (log_2TPM [transcripts per million] for (A) all tissues expect testis and (B) testis (significance, pairwise Wilcoxon).



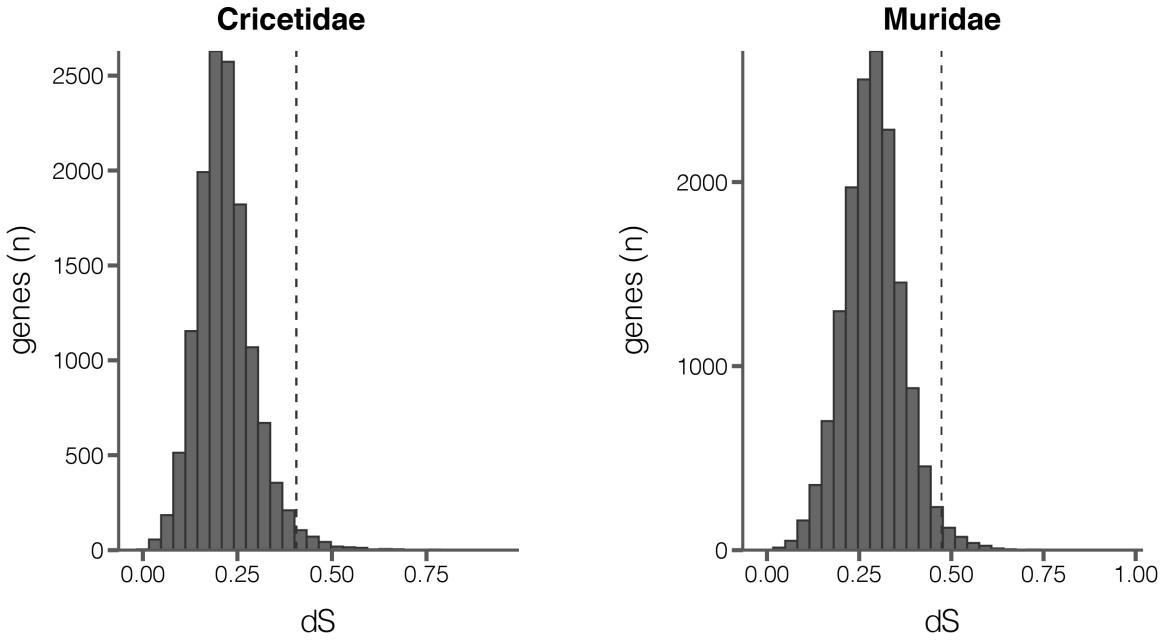
Supplemental Figure 9. Evolutionary rates in genes with high reproductive tissue specificity. dN/dS for genes with Tau greater than 0.8, for four tissues with sufficient numbers of genes on both X chromosome arms. Significance between autosomes and X arms indicated with bars and corrected p-values (pairwise Wilcoxon). Dashed line indicates genome-wide dN/dS for all genes, all tissues; significant deviation from the genome-wide median dN/dS values is indicated with an asterisk (*) in the box plot (pairwise Wilcoxon).



Supplemental Figure S10. Relative relationships between gene expression levels, tissue specificity, and evolutionary rate. A) For all genes passing filters as described in text, the relationship between levels of expression (transcripts per million, $\log_2$TPM) and ratio of rates of change in non-synonymous to synonymous nucleotides (dN/dS), GLM p<0.00001. B) Evolutionary rate (dN/dS) as a function of tissue specificity (τ, all genes represented with a solid blue line, GLM p<0.00001) with genes most highly expressed in two tissues highlighted; brain (dotted pink line) and testis (dashed green line). C) Tissue specificity (τ) as a function of evolutionary rate (dN/dS), comparing the autosomes to the X chromosome. All model results reported by panel.



Supplemental Figure 11: Placental hybrid incompatibility QTL position on the X chromosome genome build. Significance of association (LOD score) for X-linked hybrid incompatibility QTL shown according to marker assembly location (in Mbp), rather than genetic map (data from Brekke *et al* 2021)



Supplemental Figure 12: Distributions of dS among single copy orthogroups identified in four species in Cricetidae and four species in Muridae. The vertical dashed line represents the 98th percentile, above which genes were removed from subsequent analysis as a control for possible alignment error.