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)

|                | Short-read assembly | Final Assembly |
|----------------|---------------------|----------------|
| 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** |              |         |                         |                               |             |                                               |                                               |                   |
| Xq     | 119.1        | 0.350   | 697                     | 896                           | 136402      | 41537.5                                       | 238                                           |                   |
| Xp     | 77.42        | 0.650   | 379                     | 511                           | 162698      | 56238.5                                       | 238                                           |                   |
| **Rat X**   |              |         |                         |                               |             |                                               |                                               |                   |
| Xq     | 159.89       | 0.347   | 352                     | 388                           | 125683      | 40508                                         | 279                                           |                   |
| Xp     | 104.39       | 0.653   | 472                     | 660                           | 180086      | 62730                                         | 291.5                                         |                   |
Supplemental Table 4. Transposable element enrichment on X chromosome arms

| Repeat genus | Repeat species | Phodopus recomb. count | Phodopus suppress. count | Phodopus P value | Rattus recomb. count | Rattus suppress. count | Rattus P value | enriched arm |
|-------------|----------------|------------------------|--------------------------|-----------------|----------------------|------------------------|-----------------|--------------|
| DNA         | Academ         | 1                      | 1                        | 1               | N/A                  | N/A                    | 1               | N/A          |
|             | CMC-EnSpm 526 | 1270                   | 0.00261                  | Xp              | 1668                 | 2594                   | 4.30E-08        | recomb.      |
| DNA         | DNA            | 64                     | 149                      | 1               | 242                  | 416                    | 1               | N/A          |
| DNA         | Harbinger      | 8                      | 12                       | 1               | 27                   | 49                     | 1               | N/A          |
| DNA         | hAT            | 5                      | 5                        | 1               | 14                   | 9                      | 0.22272         |
| DNA         | hAT-Ac         | 123                    | 185                      | 0.4346          | 442                  | 829                    | 1               | N/A          |
| DNA         | hAT-Charlie    | 731                    | 1399                     | 1               | 1029                 | 1755                   | 0.21794         |
| DNA         | Mariner        | 1                      | 3                        | 1               | 3                    | 5                      | 1               | N/A          |
| DNA         | Maverick       | 14                     | 21                       | 1               | 58                   | 76                     | 0.63574         |
| DNA         | Tigger         | 228                    | 353                      | 0.0946          | 296                  | 412                    | 0.00181         | Xq           |
| DNA         | Tip99          | 27                     | 27                       | 0.29001         | 26                   | 29                     | 0.7734          |
| LINE        | CR1            | 0                      | 2                        | 1               | #N/A                 | #N/A                   | 1               | N/A          |
| LINE        | L0             | 9889                   | 19087                    | 1               | 21923                | 38221                  | 6.38E-15         | Xq           |
| LINE        | L2             | 41                     | 30                       | 0.00113         | Xq                   | 36                     | 20              | 0.00017      | Xq           |
| LTR         | ERV1           | 642                    | 1753                     | 2.83E-11        | Xp                   | 1259                   | 2659            | 0.01409      | Xp           |
| LTR         | ERVK           | 1842                   | 3739                     | 1               | 1358                 | 3234                   | 3.95E-12        | Xp           |
| LTR         | ERVL           | 777                    | 1617                     | 1               | 1904                 | 3474                   | 1               | N/A          |
| Type                  | Accession | Length | Start | End   | TSS (kb) | Strand | P-value |
|----------------------|-----------|--------|-------|-------|----------|--------|---------|
| ERVL-MaLR            | 3212      | 6783   | 0.04414 | suppress. 7720 | 13691 | 0.00068 | recom. |
| 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                 | 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                | 25        | 54     | 1     | 73    | 125      | 1      |         |
| tRNA                 | 2         | 0      | 1     | 7     | 7        | 1      |         |
| 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       | 5483      | 11451  | 0.01818 Xp | 3136 | 5189 | 3.61E-07 Xq |         |
| Unknown              | 1305      | 3129   | 9.50E-08 Xp | 992 | 1870 | 1      |         |
| tRNA core            | 101       | 122    | 0.00722 Xq | N/A  | N/A      | N/A    |         |
Supplemental Table 5: Counts of tissue-enriched genes by chromosome, and BH corrected p-values

| 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 |

| Chromosome | Brain | Heart | Kidney | Liver | Muscle | Placenta | Spleen | Testis | Uterus |
|------------|-------|-------|--------|-------|--------|----------|--------|--------|--------|
| Xq         | 171   | 39    | 1      | 5     | 3      | 3        | 29     | 7      | 67     | 17     |
| Xp         | 199   | 31    | 9      | 8     | 4      | 6        | 21     | 12     | 97     | 11     |
| Adjusted Pval | | | | | | | | | | |
| 1           | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 5      | 1      | 1      |
| 2           | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 1      | 1      | 1      |
| 3           | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 1      | 1      | 1      |
| 4           | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 1      | 1      | 1      |
| 5           | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 5      | 1      | 1      |
| 6           | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 1      | 1      | 1      |
| 7           | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 1      | 1      | 1      |
| 8           | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 1      | 1      | 1      |
| 9           | 2     | 1     | 1      | 2     | 1      | 1        | 1      | 1      | 0.0197259 | 0.97726 |
| 10          | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 1      | 1      | 1      |
| 11          | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 1      | 1      | 1      |
| 12          | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 1      | 1      | 1      |
| 13          | 1     | 1     | 1      | 1     | 1      | 1        | 1      | 1      | 1      | 1      |
|     | X   | 1 | 1 | 1 | 1 | 1.44E-08 | 1  | 3.43E-15 | 1  |
|-----|-----|---|---|---|---|----------|---|----------|---|
| unplace | d   | 1 | 1 | 1 | 1 | 0.16624  | 1  | 0.0060634 | 1  |
|     |     | 1 | 1 | 1 | 1 | 4        | 1  | 0.0004527 | 0.44209 |
| Xq  |     | 0.230845 | 1 | 1 | 1 | 1 | 8.84E-07 | 1  | 6        | 2  |
| Xp  |     | 1 | 1 | 1 | 1 | 1 | 0.05699  | 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_2 TPM [transcripts per million]) for (A) all tissues except 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 ($\tau$, 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 ($\tau$) 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.