Supplementary Note 1: BAC selection strategy and assembly of the rhesus MSY sequence

The CHORI-250 BAC library had been fingerprinted and assembled into fingerprint contigs at the Michael Smith Genome Sciences Centre (http://www.bcgsc.ca/downloads/rhesusmap.tar.gz). We identified Y chromosome fingerprint contigs by searching for contigs containing multiple BACs with end sequences that did not match the female whole-genome shotgun sequence. We then verified the male-specificity of these contigs using +/- PCR assays on male and female genomic DNA and selected tiling paths of clones for sequencing. We used high-density filter hybridization with pools of overgo probes to identify clones from the CHORI-250 and RMAEX libraries to fill gaps.

The assembled rhesus MSY sequence spans 11.0 megabases (Mb) in three contigs (Supplemenary File 1). The largest contig (7.9 Mb) is anchored by the pseudoautosomal region, which was confirmed to be located at the distal end of the chromosome by extended metaphase fluorescence in situ hybridization (EM-FISH) (Supplementary Fig. 2). The smallest contig (1.1 Mb) is bounded by the centromere. The rhesus Y is acrocentric, as determined by EM-FISH (Supplementary Fig. 3). Therefore, the third contig (1.9 Mb) is located between the 7.9 Mb and 1.1 Mb contigs. We determined the orientation of the 1.9 Mb contig by EM-FISH (Supplementary Fig. 4) and radiation hybrid mapping (Supplementary Fig. 5 and Supplementary File 2). We conclude that our assembled sequence is nearly complete based on the small size of the gaps between the three contigs as determined by interphase FISH (Supplementary Fig. 6).

Supplementary Note 2: Determination of X-Y ancestral gene content within strata 1-5.

For this analysis, we used the human X-linked protein-coding gene set from Ensembl. We ordered the genes according to their position on the human X chromosome, which is an approximation of the gene order on the ancestral chromosome, and divided the genes into strata according to boundaries given in Lahn and Page[1] and Ross, *et al*.[2]. We then determined whether each gene was present in the X-Y common ancestor or added to the X chromosome after X-Y differentiation according to the following criteria (see Supplementary Table 4):

1. From Bellott *et al*.[3]: Autosomal progenitors of the human X and Y chromosomes are represented in chicken chromosomes 1 and 4, so a gene with a homolog in a syntenic location within one of these chicken chromosomes is ancestral. (Note: all strata 4 and 5 genes have homologs on chicken 4).
2. From Bellott *et al*.[3] If a gene is absent from chicken 1 or 4, it may have been added to the X or lost in chicken. If such a gene is present in a syntenic location in at least one outgroup species (*Xenopos tropicalis* or *Anolis carolinensis*), it is ancestral.
3. The remaining genes were presumably added to the X sometime during mammalian evolution. We determined the approximate timing of introduction to the X by searching for syntenic loci in various other mammalian X chromosome sequences: rhesus, marmoset, mouse, rat, dog, bovine and opossum. Specifically, we used TBLASTX to search for hits for each gene in question as well as its two neighboring genes. (Note: All other X chromosome assemblies, with the exception of mouse, are draft sequences, so some genes will be absent because of missing data.) For strata 1 and 2, which formed prior to the eutherian (placental mammals) – metatherian (marsupial mammals) split, a gene was considered ancestral only if it was found in opossum. For stratum 3, which was formed prior to the eutherian radiation, a gene was considered ancestral if it was found in at least two non-primate species.

Each MSY gene and pseudogene was in turn considered ancestral if its X-linked counterpart was classified as ancestral. All but two MSY genes, *TSPY* and *AMELY*, have X-linked counterparts with homologs on chicken 1 and 4. A homolog of *TSPYL2*, which is the X counterpart of *TSPY*, is present in *Xenopus tropicalis* in the same sequence contig as homologs of two syntenic genes: *KDM5C* and *GPR173.* A homolog of *AMELX* is present in *Xenopus tropicalis* in the same sequence contig as homologs of two syntenic genes: *ARHGAP6* and *CXorf22.*

Supplementary Note 3: Human/chimpanzee/rhesus ancestral MSY gene content.

We deduce that within strata 1-4, the MSY of the human/chimpanzee/rhesus common ancestor had the same set of ancestral genes as that of the present-day rhesus and human MSY's and, therefore, there has been no gene loss in either species within these strata. While it is formally possible that the human/chimpanzee/rhesus common ancestor had additional ancestral genes and the same ancestral genes were lost independently in each lineage, we believe that this is highly unlikely for the following reason: The X-degenerate regions of the three species are composed of the same segments of X-homologous sequence (Supplementary Figs. 8 and 9), implying that the deletion events that removed all but the remaining few X-homologous genes occurred in the common ancestor of these species. The likelihood of the alternative scenario – that the same exact series of deletion events occurred independently in each lineage – is extremely low.

Supplementary Note 4: Discussion of dN/dS values for ancestral genes.

The dN/dS ratios for the ancestral genes were calculated from alignments of rhesus and human homologs and are given in Supplementary Table 3. These values range widely, from 0.09 for *NLGN4Y* to 1.33 for *AMELY*. Of the 19 ancestral genes, 12 display evidence for purifying selection, with dN/dS ratios of 0.33 or less and statistically significant deviation from neutrality by Fisher's exact test. Of the remaining 7 genes, only *AMELY* has a dN/dS ratio greater than 0.7. This gene is one of the smallest ancestral genes so the confidence intervals for this calculation are large (0.61-2.98). Therefore, we would not conclude that this gene has experienced the effects of positive selection or relaxed constraint. We also calculated dN/dS for *MXRA5Y*, which is intact in rhesus but a pseudogene in human. As expected, the dN/dS ratio at this locus is high (0.99) reflecting its neutral evolution in the human lineage. We performed sliding window dN/dS analyses to determine if selection was operating differently at distinct locations with each gene, and the results are shown in Supplementary Figure 16. In most cases, the dN/dS value varies along the length of the gene. However, most genes also have an extended region that displays a very low dN/dS value, confirming the operation of purifying selection on these genes.

Supplementary Note 5: Determining time of pseudogene inactivation.

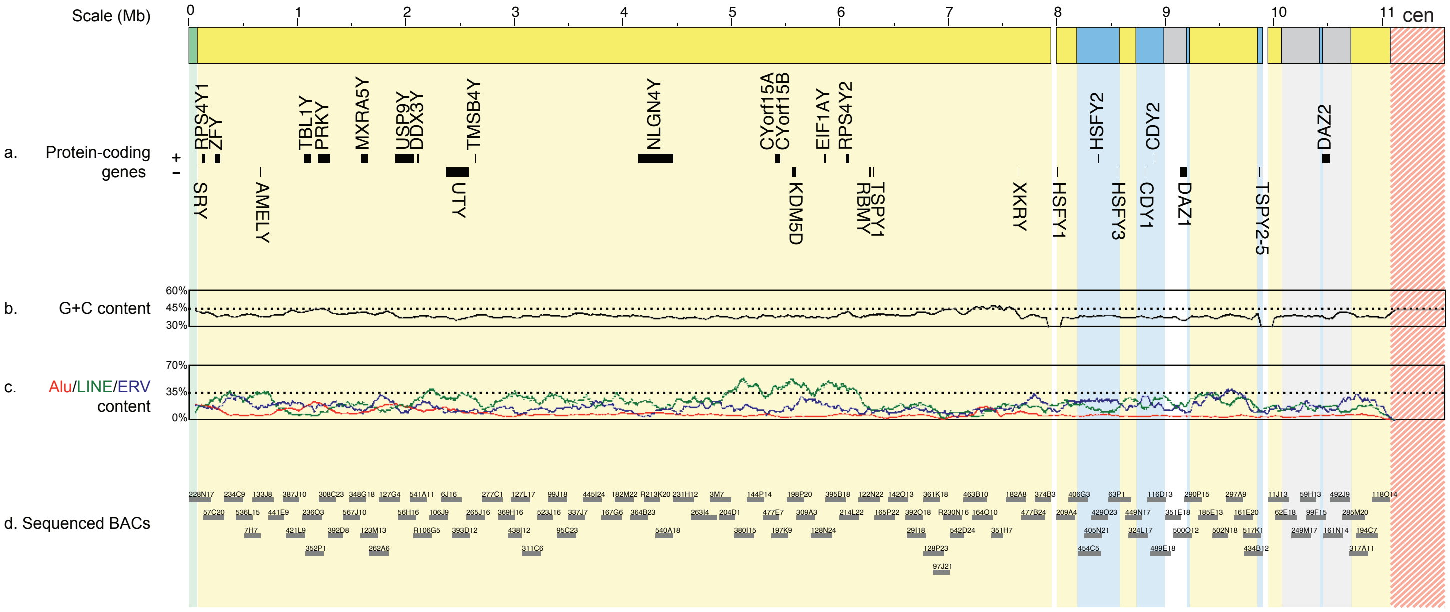
Within the older strata, five of six pseudogenes display at least one inactivating mutation shared between rhesus, human and chimpanzee, so their inactivation predates the OWM-ape split (see Supplementary Fig. 15). We cannot date with certainty the inactivation of the four pseudogenes within stratum 5 or the sixth pseudogene in the older strata, however, because these loci have been deleted outright in rhesus. All five of these pseudogenes are located within a single 280-kb region in human, so they were likely removed by one large deletion in the rhesus lineage.

LITERATURE CITED

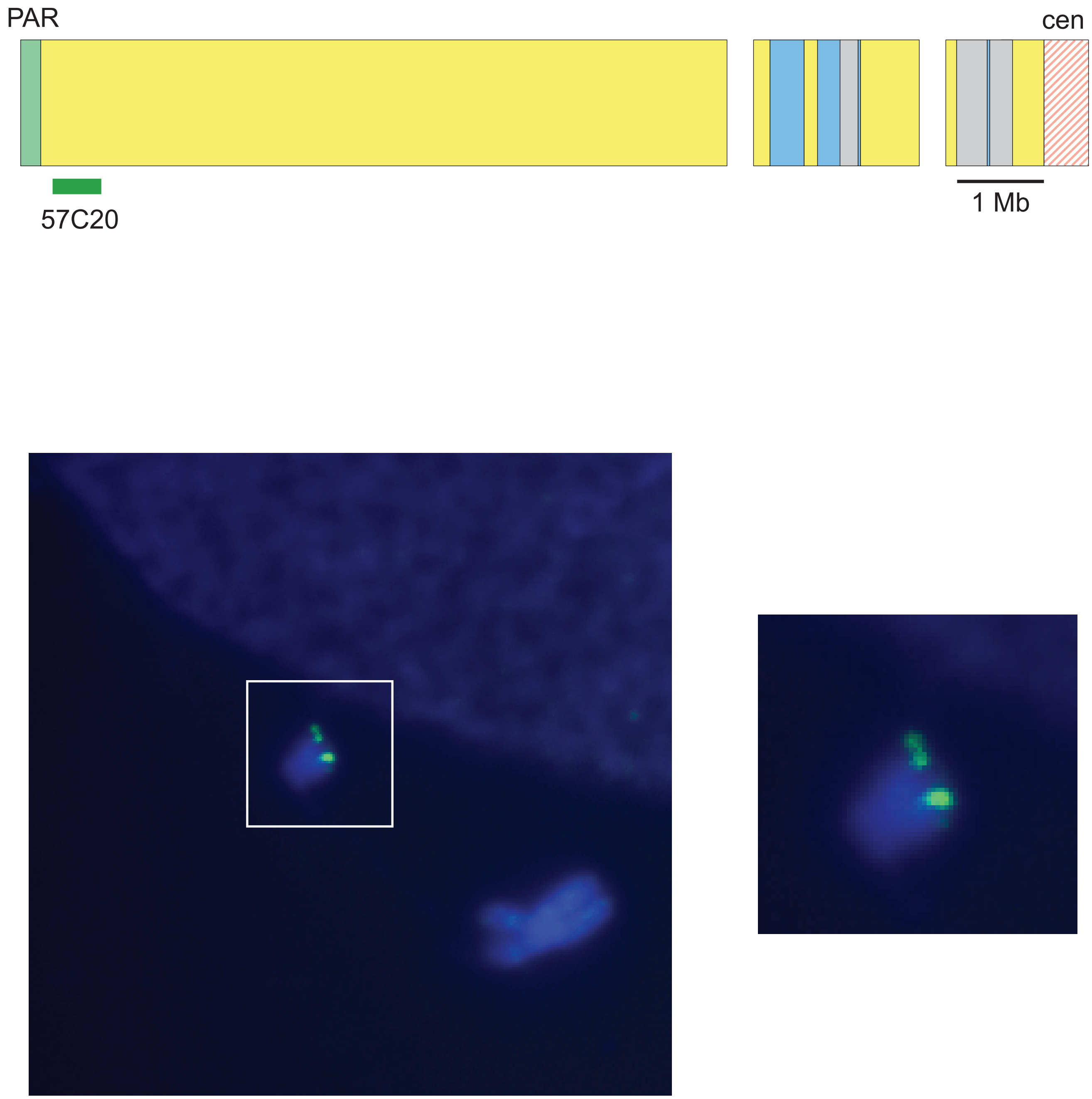
[1] Lahn, B. T. & Page, D. C. Four evolutionary strata on the human X chromosome. *Science* **286**, 964-967 (1999).

[2] Ross, M. T. *et al.* The DNA sequence of the human X chromosome. *Nature* **434**, 325-337 (2005).

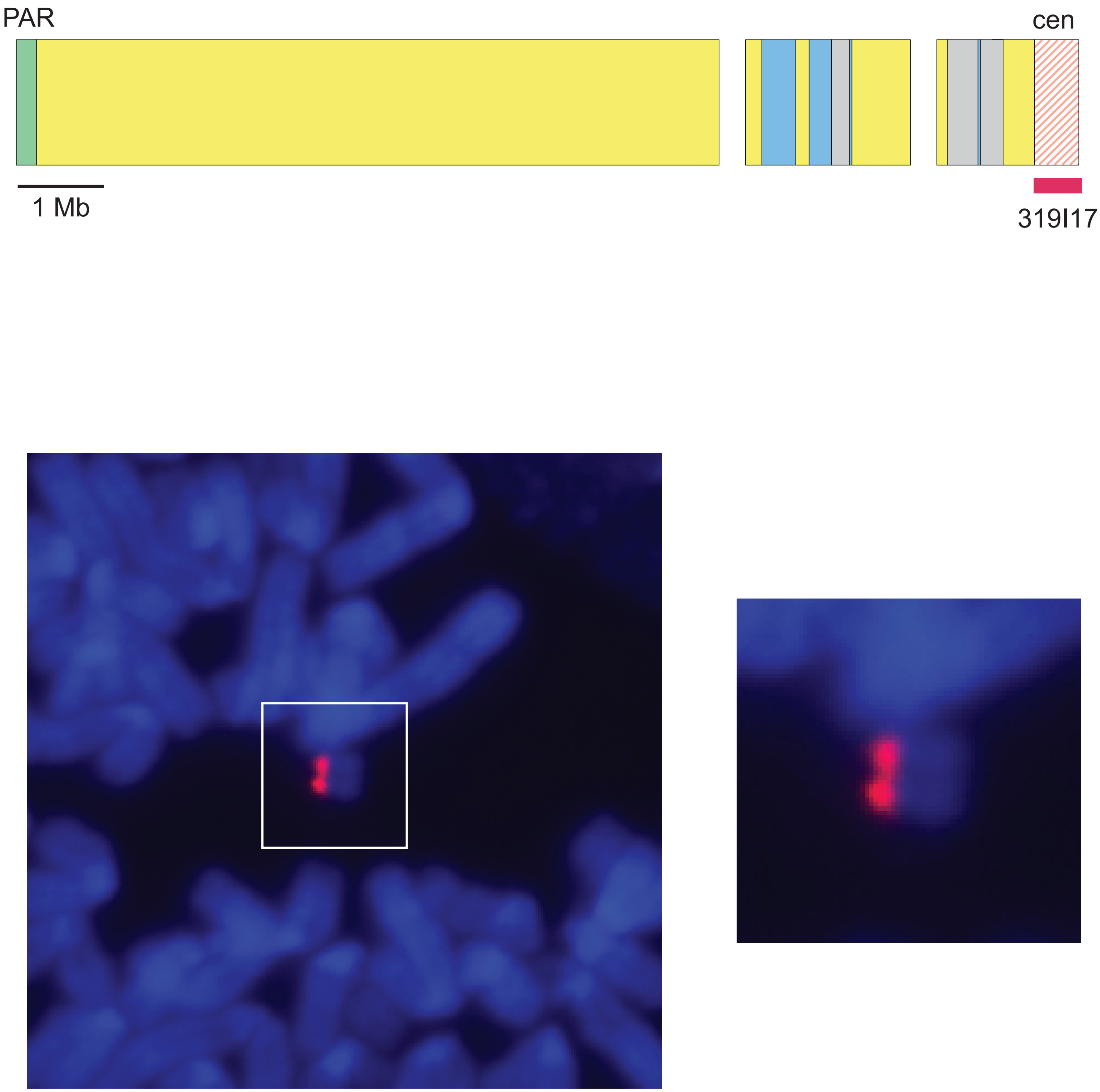
[3] Bellott, D. W. *et al.* Convergent evolution of chicken Z and human X chromosomes by expansion and gene acquisition. *Nature* **466**, 612-616 (2010).



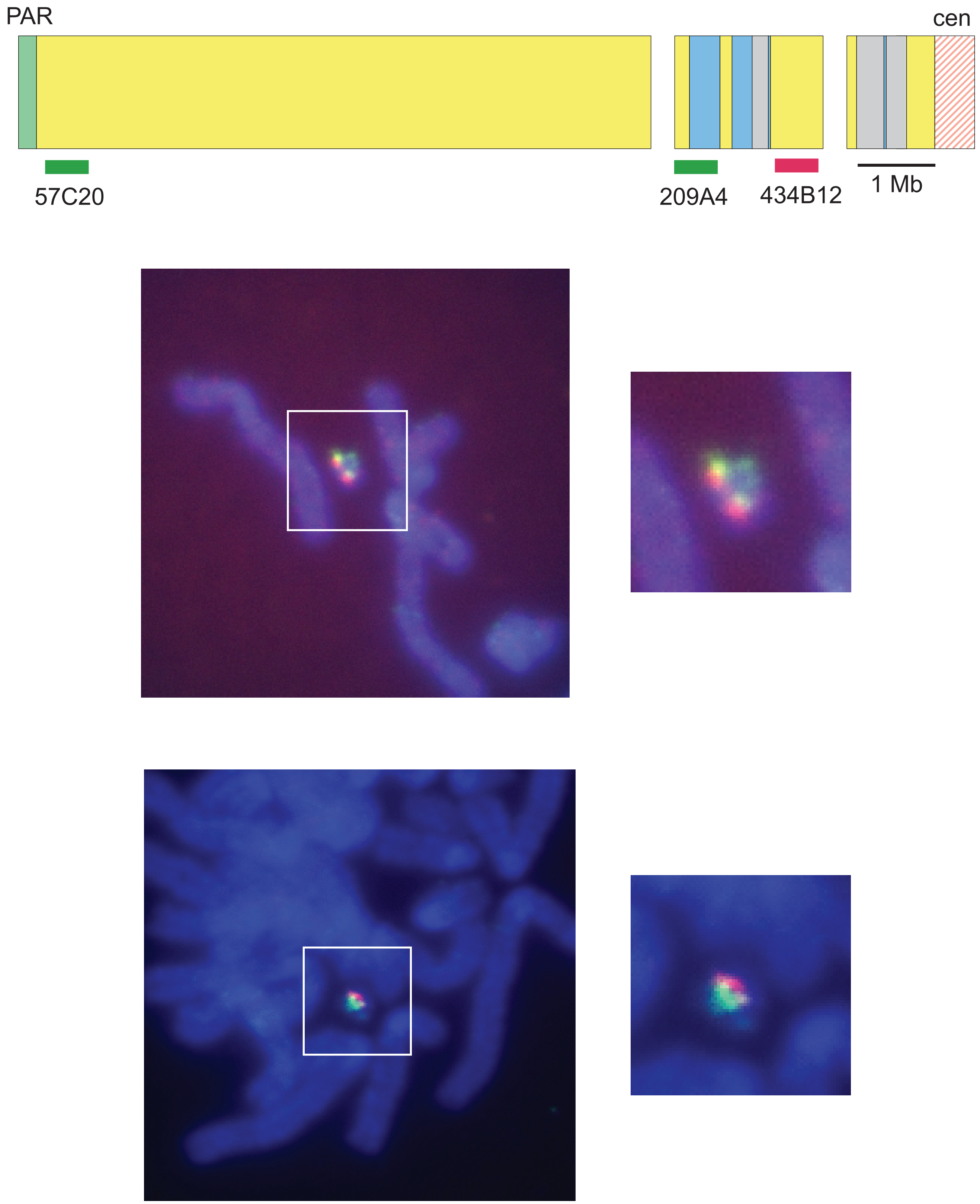
Supplementary Figure 1 Annotated sequence of rhesus MSY. Background colors represent five different sequence classes: pseudoautosomal (green); X-degenerate (yellow); ampliconic (blue); other (gray); and heterochromatic (pink stripes). Gaps shown in white. All sequence features and BACs drawn to scale. a. Positions of all intact, actively transcribed genes. Plus (+) strand above, minus (-) strand below. b. G+C content (%) calculated in a 100-kb sliding window with 1-kb steps. c. Alu, LINE, and endogenous retrovirus (ERV) densities, expressed as percentage of nucleotides, calculated in a 200-kb sliding window with 1-kb steps. d. Sequenced MSY BACs. Each bar represents the size and position of one BAC clone, labeled with the library identifier. BAC clones with no prefix are from the CHORI-250 library. BAC clones with prefix "R" are from the RMAEX library.



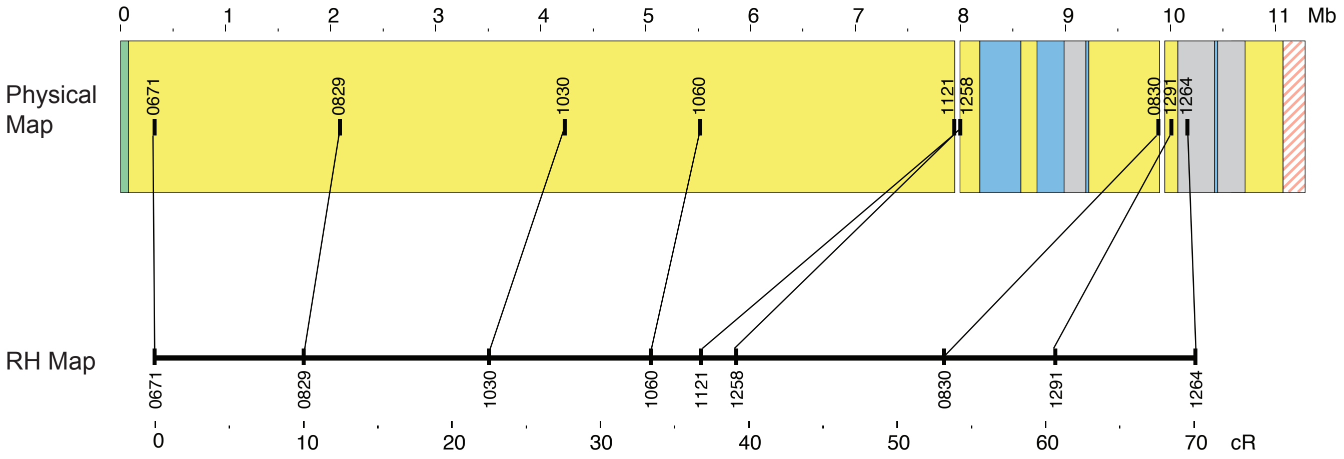
Supplementary Figure 2 Extended metaphase FISH analysis of pseudoautosomal boundary (PAB). Confirms that PAB is located at one end of rhesus Y chromosome. Probe 57C20 (not drawn to scale) is a BAC clone from rhesus CHORI-250 library. PAR = pseudoautosomal region; cen = centromere.



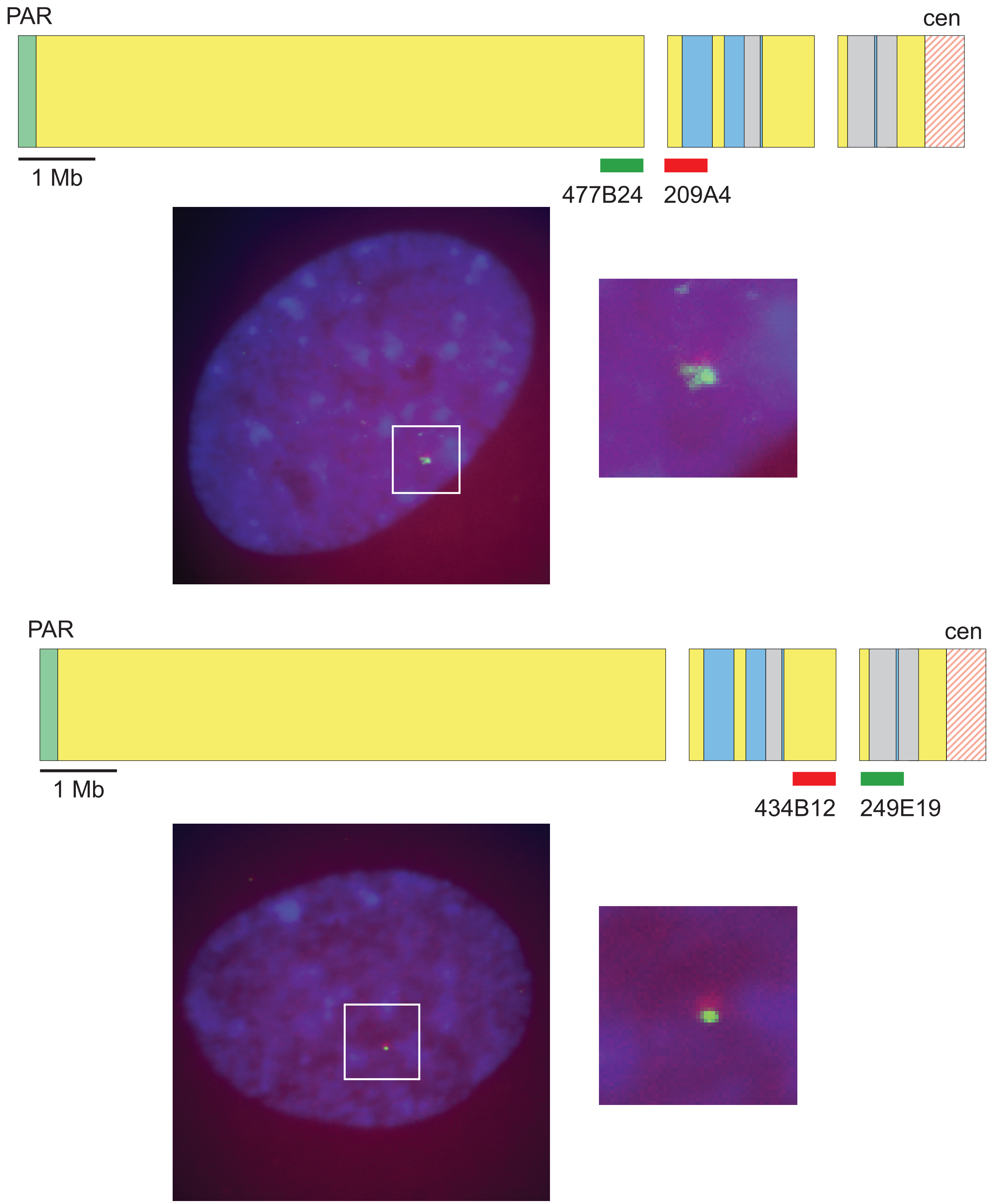
Supplementary Figure 3 Extended metaphase FISH analysis of centromere. Confirms that centromere is located at one end of rhesus Y chromosome. Probe 319I17 (not drawn to scale) is a BAC clone from rhesus CHORI-250 library. PAR = pseudoautosomal region; cen = centromere.



Supplementary Figure 4 Extended metaphase FISH analysis reveals orientation of middle contig. Probes 57C20, 209A4 and 434B12 (not drawn to scale) are BAC clones from rhesus CHORI-250 library. PAR = pseudoautosomal region; cen = centromere.

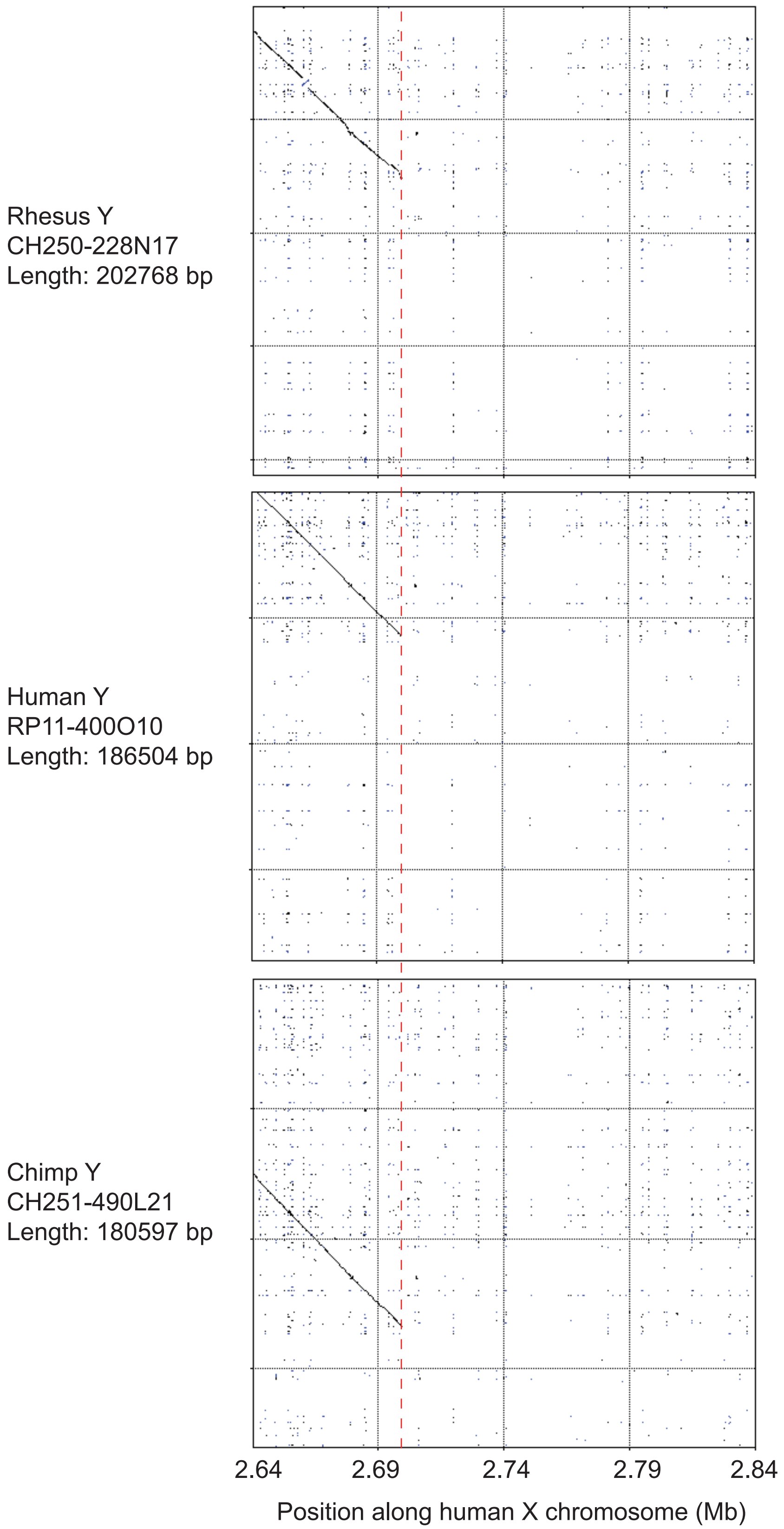


Supplementary Figure 5 Comparison of radiation hybrid (RH) and physical maps of rhesus MSY. Locations of nine RH markers on physical map are shown on schematic representation of chromosome at top. Raw data vectors are given in Supplementary File 2. Scale is shown with distances in megabases (Mb). RH map of nine markers (listed in Supplementary Table 7) is shown at bottom. Scale is shown with distances in centirays (cR). 1 cR = 1% recombination frequency.



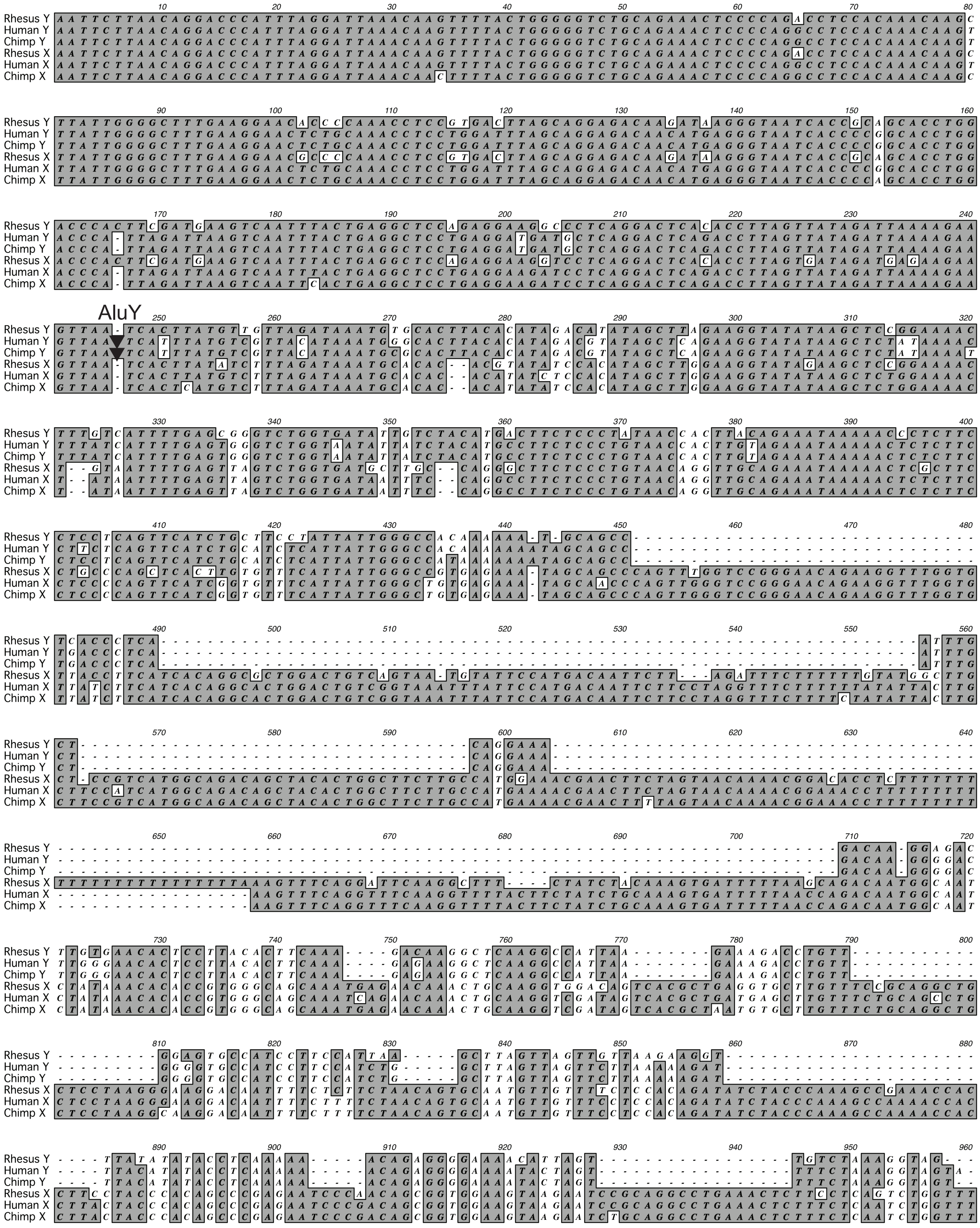
Supplementary Figure 6 Interphase FISH analysis of gaps. Probes 477B24, 209A4, 434B12, and 249E19 (not drawn to scale) are BAC clones from rhesus CHORI-250 library. PAR = pseudoautosomal region; cen = centromere.

a.

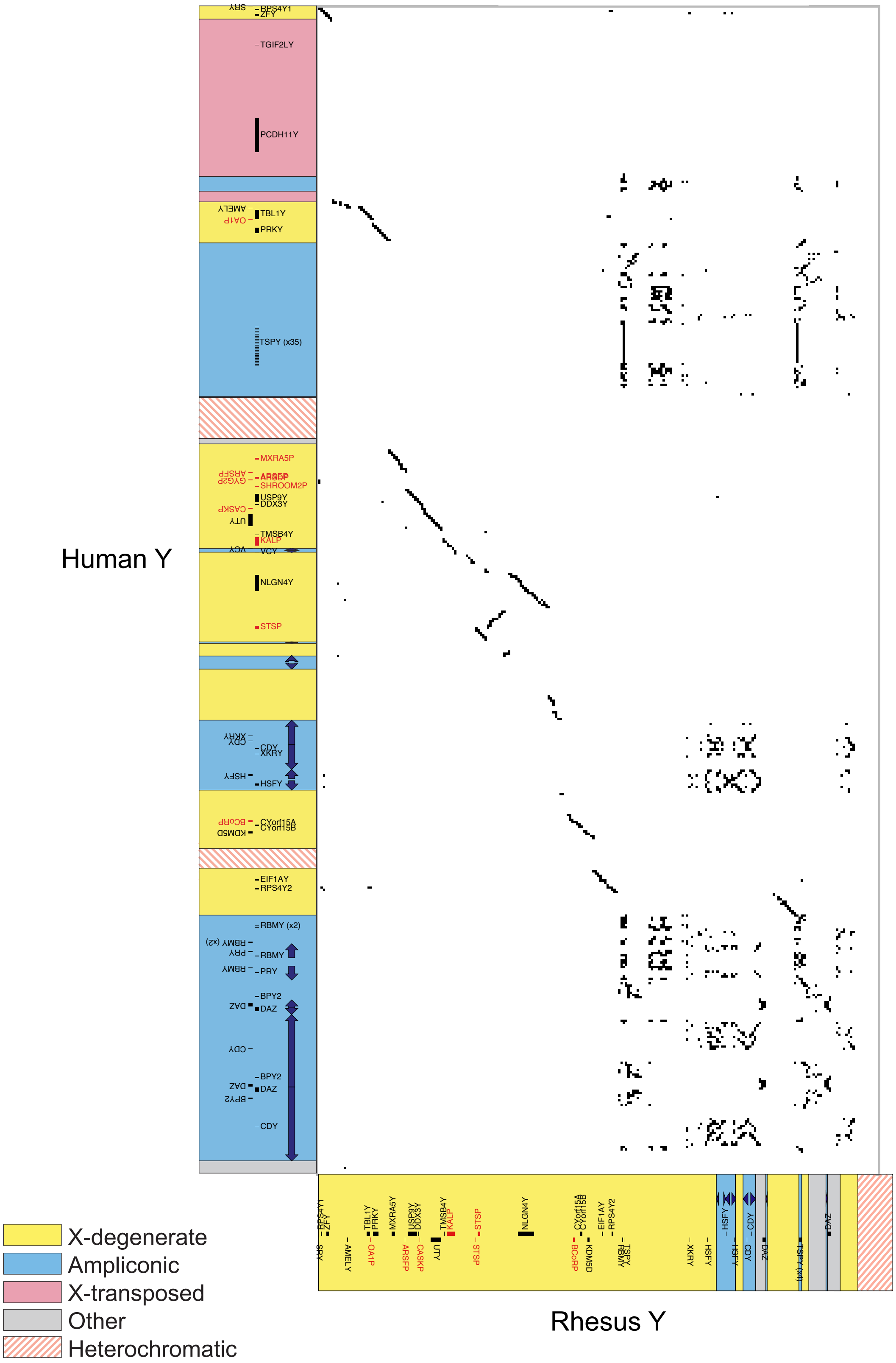


Supplementary Figure 7a Comparison of pseudoautosomal boundaries (PABs) in rhesus, human and chimpanzee. Dot plot analyses to demonstrate that position of PAB is identical in all three species. 200-kb sequence spanning PAB of human X chromosome is represented on each X axis. Each Y axis represents sequence from a BAC spanning the Y chromosome PAB in human (top), chimpanzee (middle), and rhesus (bottom). Red dotted line shows position of PAB relative to human X sequence. Each dot plot was constructed in MacVector using a 100-bp window and 85% identity.

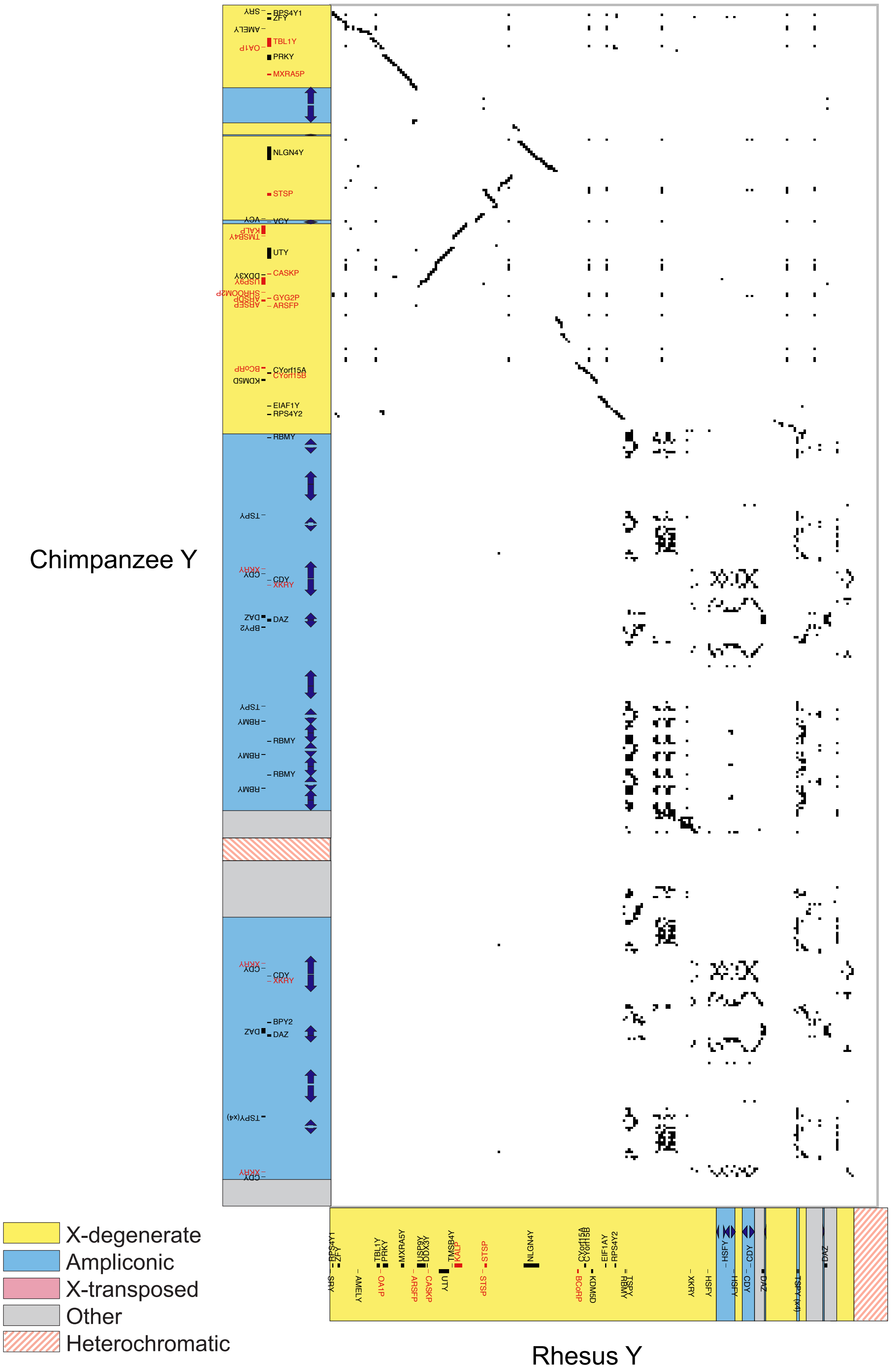
b.



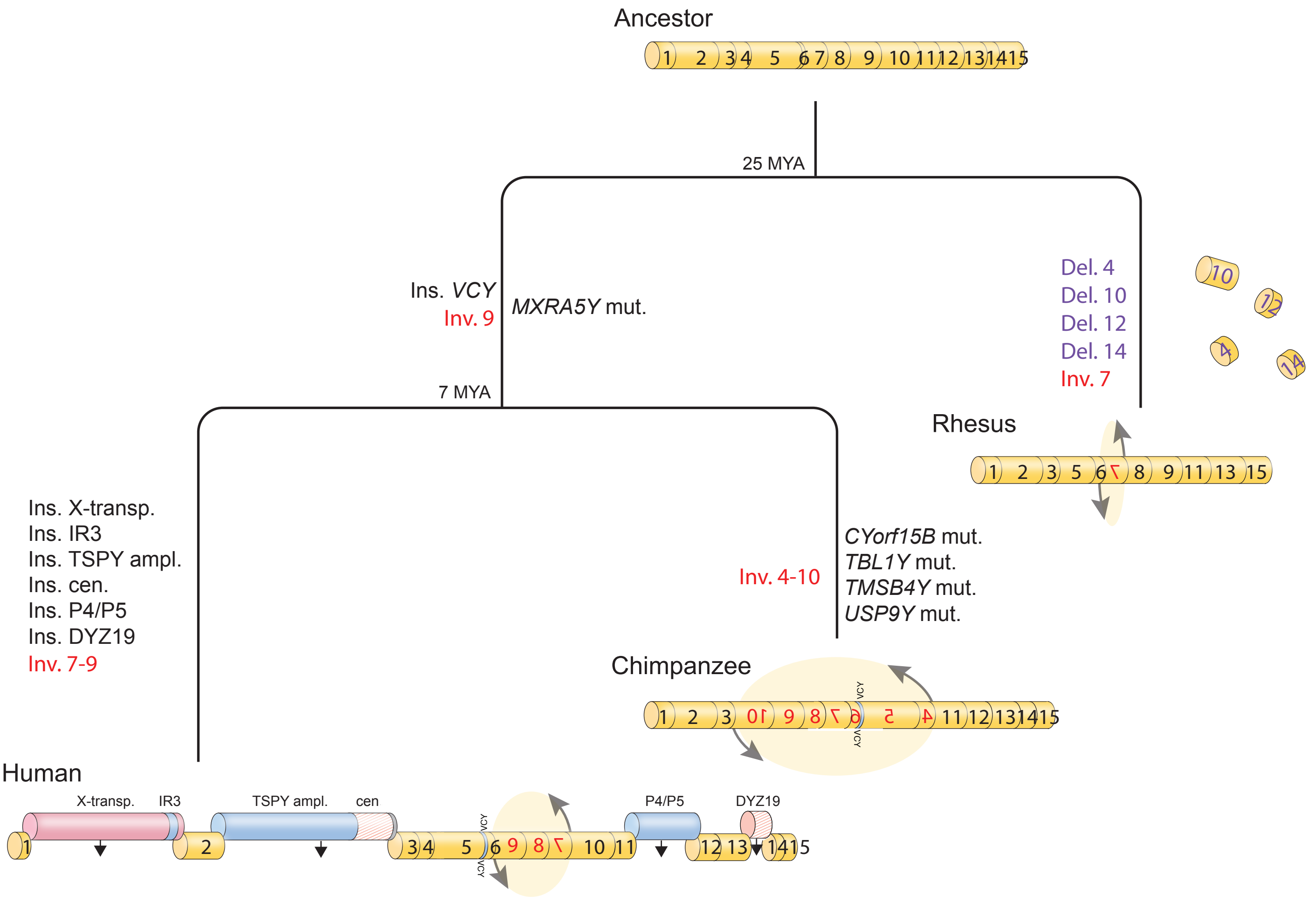
Supplementary Figure 7b Comparison of pseudoautosomal boundaries (PABs) in rhesus, human and chimpanzee. Alignment of PAB region of X and Y chromosomes of all three species. Alignment was generated in MacVector using ClustalW. Identical nucleotides are shaded in gray. Position of Alu insertion in human and chimpanzee Y sequences, which marks the location of the PAB, is shown (position 246 in the alignment). Accession numbers for sequences are as follows: Human Y - AC006040; Chimp Y - AC146189; Rhesus Y - AC217136; Human X - NT_167197; Chimp X - NW_001251668; Rhesus X - NW_001218091.



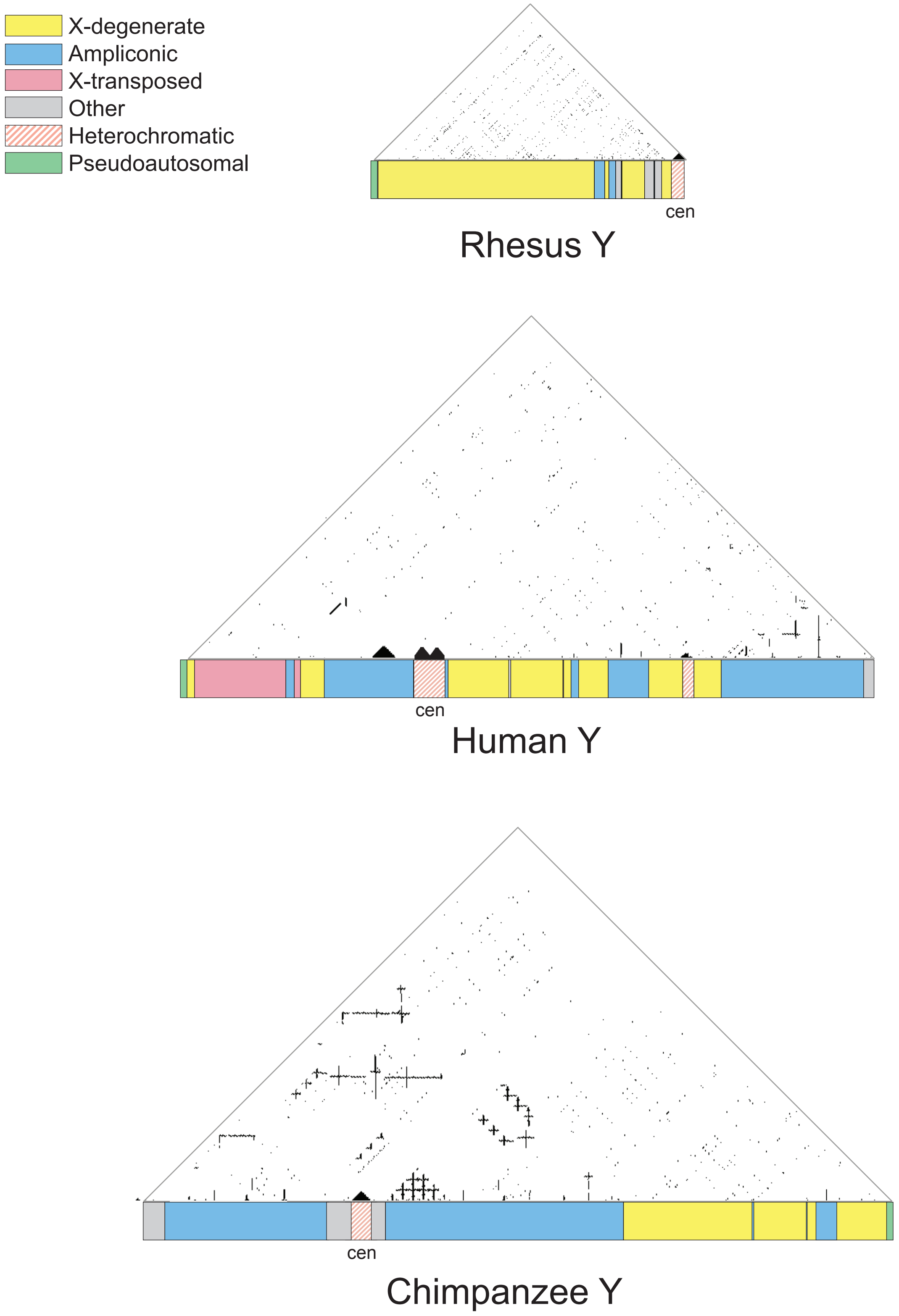
Supplementary Figure 8 Dot-plot comparison of rhesus and human MSYs. Major features of chromosomes are shown schematically along the axes. Intact genes are in black and pseudogenes are in red. Rhesus sequence was masked prior to analysis using RepeatMasker (www.repeatmasker.org) with default parameters. Each dot on the plot represents 100% identity within a 40-bp window.



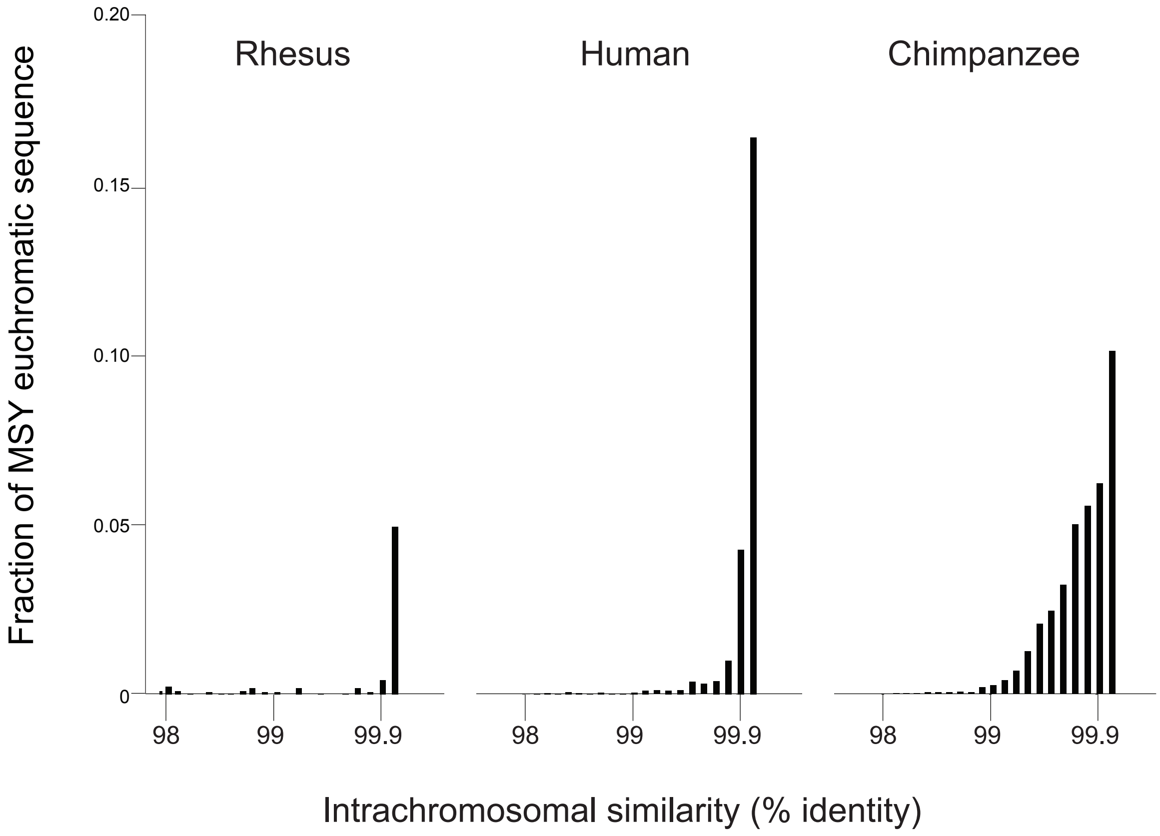
Supplementary Figure 9 Dot-plot comparison of rhesus and chimpanzee MSYs. Major features of chromosomes are shown schematically along the axes. Intact genes are in black and pseudogenes are in red. Rhesus sequence was masked prior to analysis using RepeatMasker (www.repeatmasker.org) with default parameters. Each dot on the plot represents 100% identity within a 40-bp window.



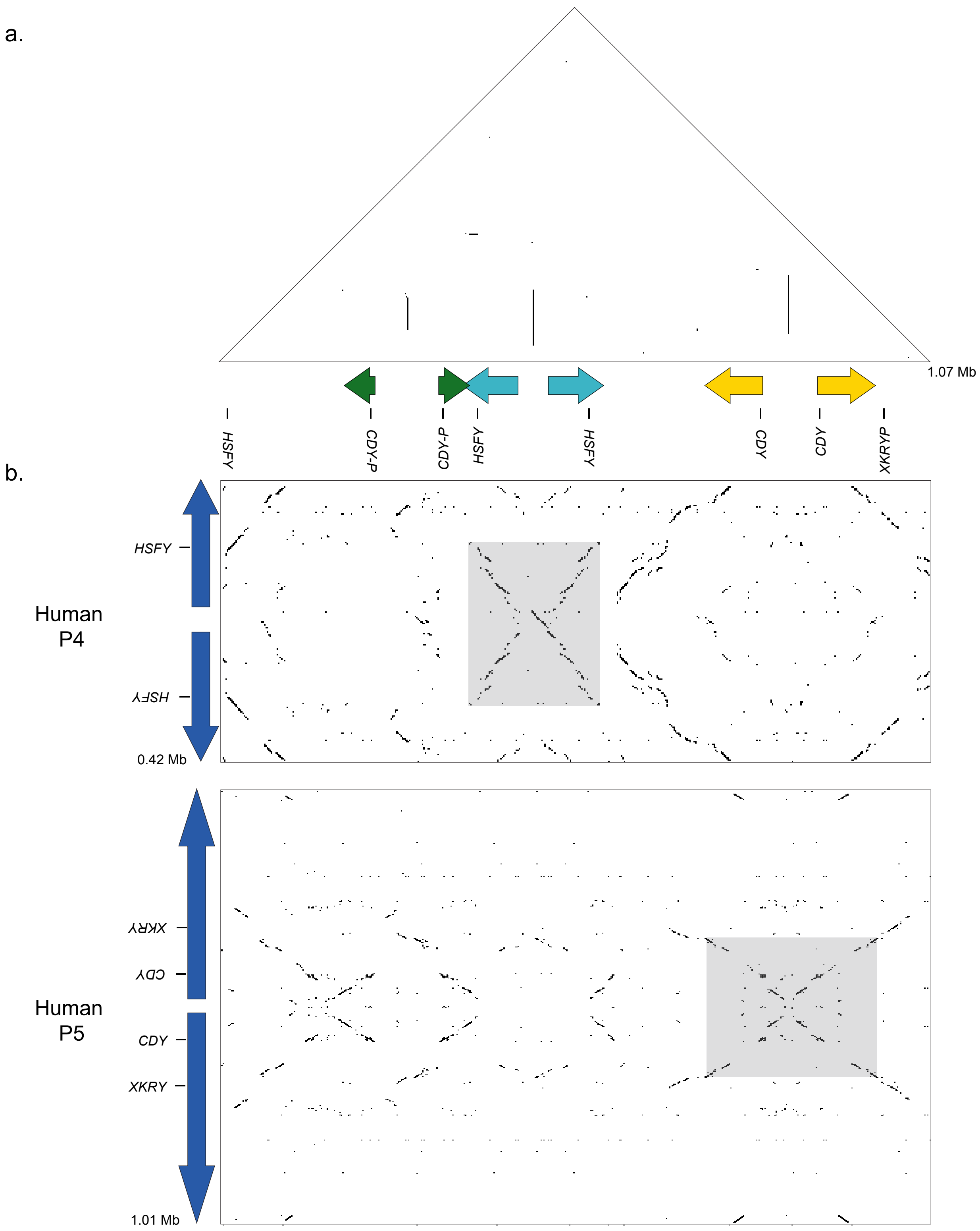
Supplementary Figure 10 Rearrangements during evolution of rhesus, human and chimpanzee Y chromosomes. Dot-plot analyses (Supplementary Figs 8 & 9) revealed large-scale rearrangment events that occurred in each lineage, enabling the reconstruction of a hypothetical ancestral chromosome. Sequence in ancestral chromosome is divided into 15 synteny blocks, each > 200 kb in size, which are labeled in each species. Bracketed lines (not to scale) show evolutionary relationships between three extant species and common ancestor. Approximate divergence times are indicated at intersections. Observed large-scale rearrangments (left) and gene changes (right) are indicated on each vertical line. Insertions and deletions < 200 kb in size are not depicted.



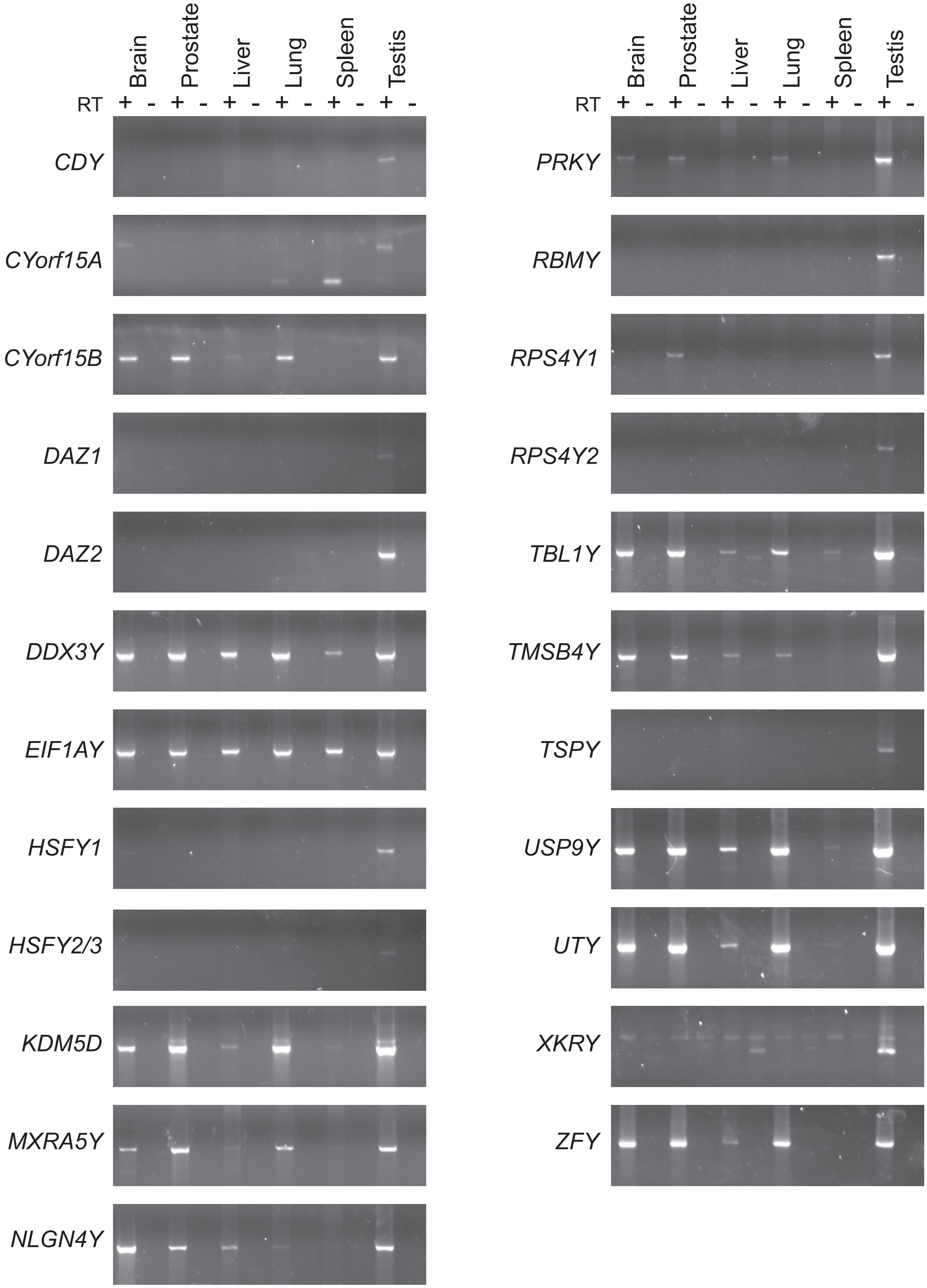
Supplementary Figure 11 Triangular dot plots of DNA sequence identities within the rhesus, human, and chimpanzee MSYs. Each dot represents 100% identity within a 200-bp window. Direct repeats appear as horizontal lines, inverted repeats as vertical lines, and palindromes as vertical lines that nearly intersect the baseline. Immediately below the plots are schematic depictions of the MSY sequences, with colors representing MSY sequence classes. cen = centromere.



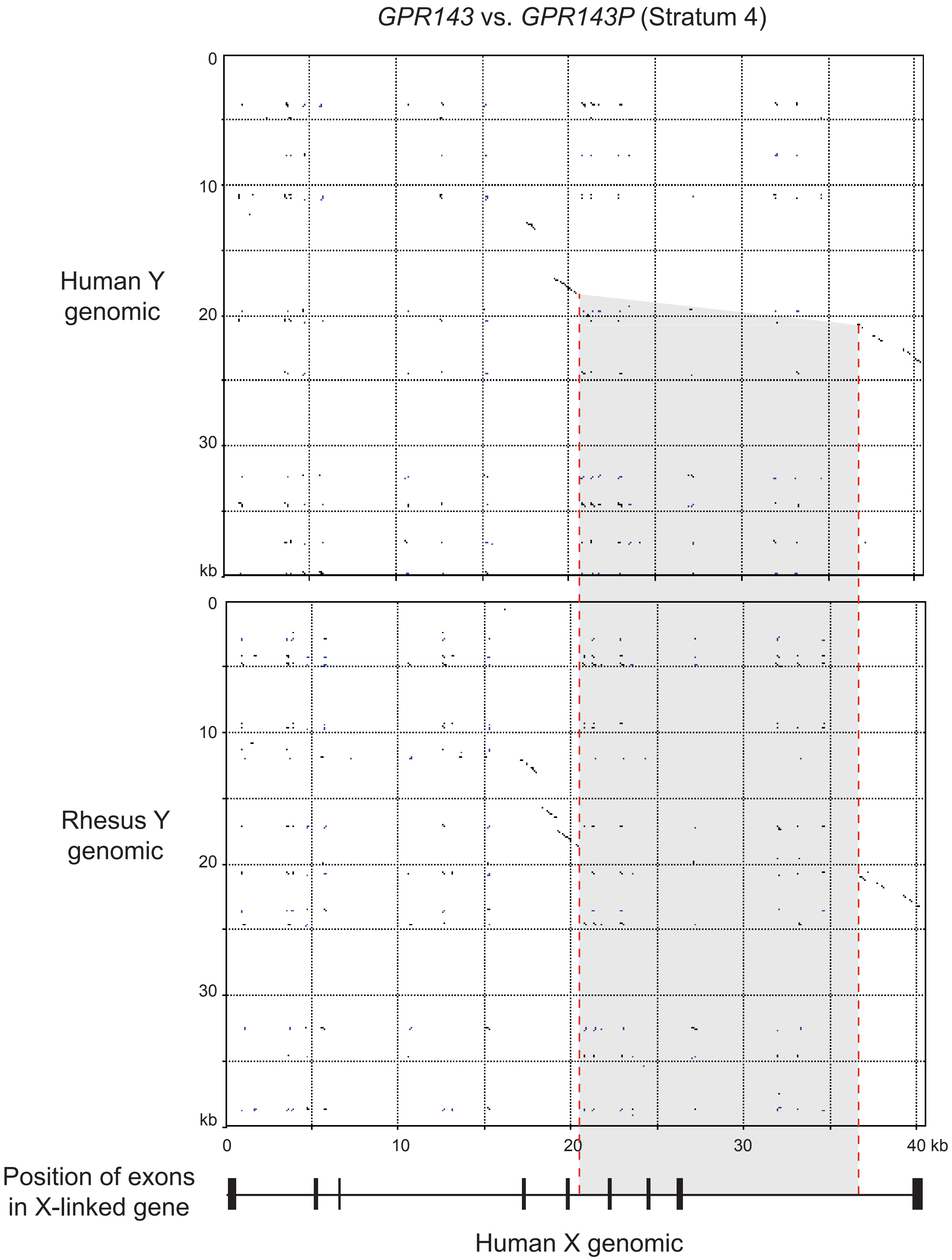
Supplementary Figure 12 Electronic fractionation of rhesus, human and chimpanzee MSY sequences according to intrachromosomal similarity. Percent identity to other MSY sequences is plotted on a logarithmic scale. Values < 98% are not shown. Analyses were performed using custom Perl code that used BLAST to compare all 5-kb sequence segments, in 2-kb steps, to the entire remainder of the MSY sequence.



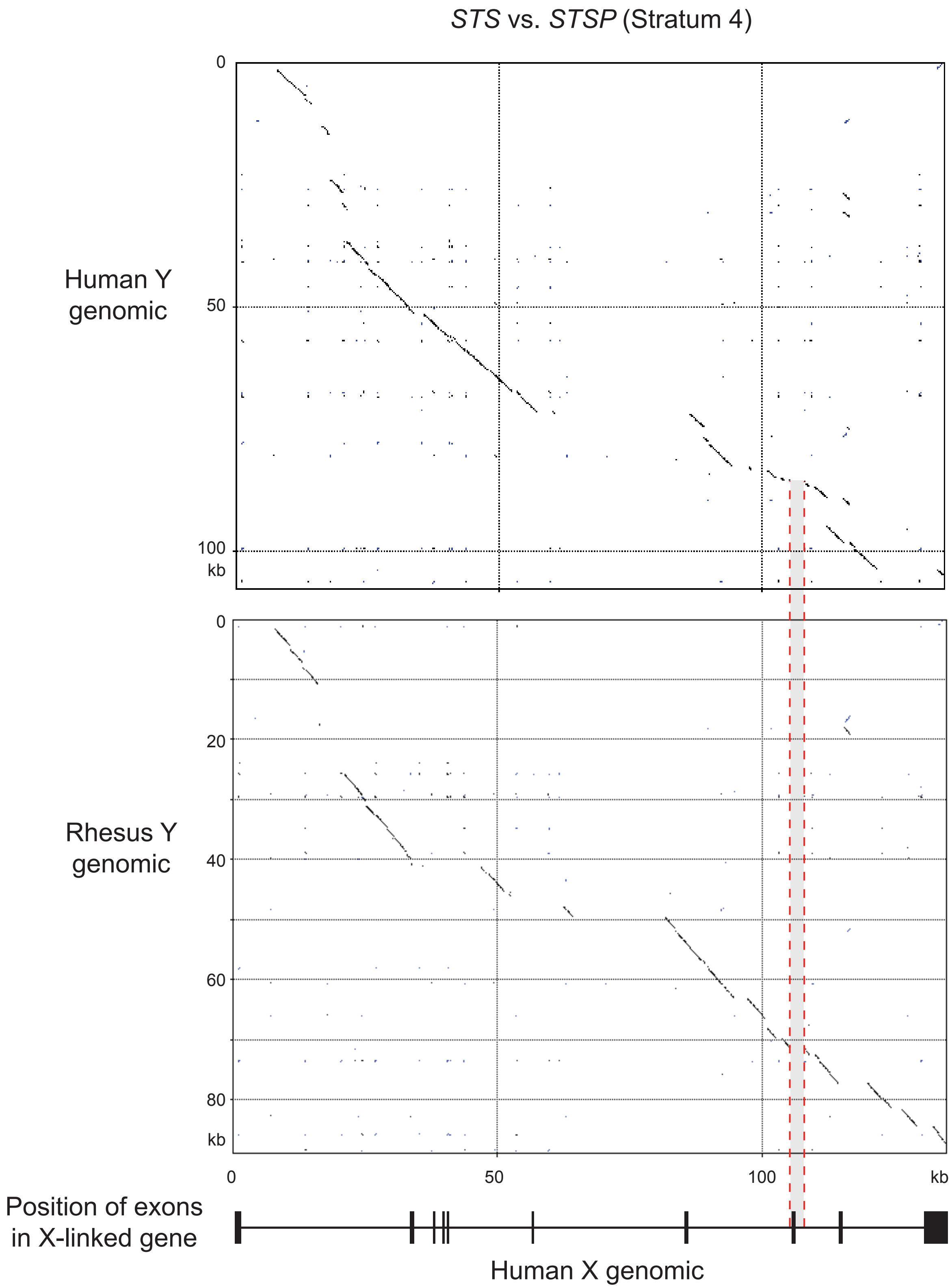
Supplementary Figure 13 a. Triangular dot plot of DNA sequence identities within major rhesus ampliconic region. The region represented corresponds to the first 1.07 Mb of the middle contig (Supplementary Figure 1). Each dot represents 100% identity within a 200-bp window. Direct repeats appear as horizontal lines and palindromes as vertical lines that nearly intersect the baseline. Immediately below the plot is a schematic depiction of the organization of palindromes. Positions of genes are shown below palindromes; "P" after gene name indicates pseudogene. b. Dot plot comparisons of same 1.07 Mb region of rhesus sequence from part a to orthologous palindromes P4 and P5 in human. Human sequence was masked with RepeatMasker prior to analyses. Each dot represents 100% identity within a 20-bp window. Gray shading indicates regions of homology between rhesus and human palindromes, indicative of the shared ancestry of these structures.



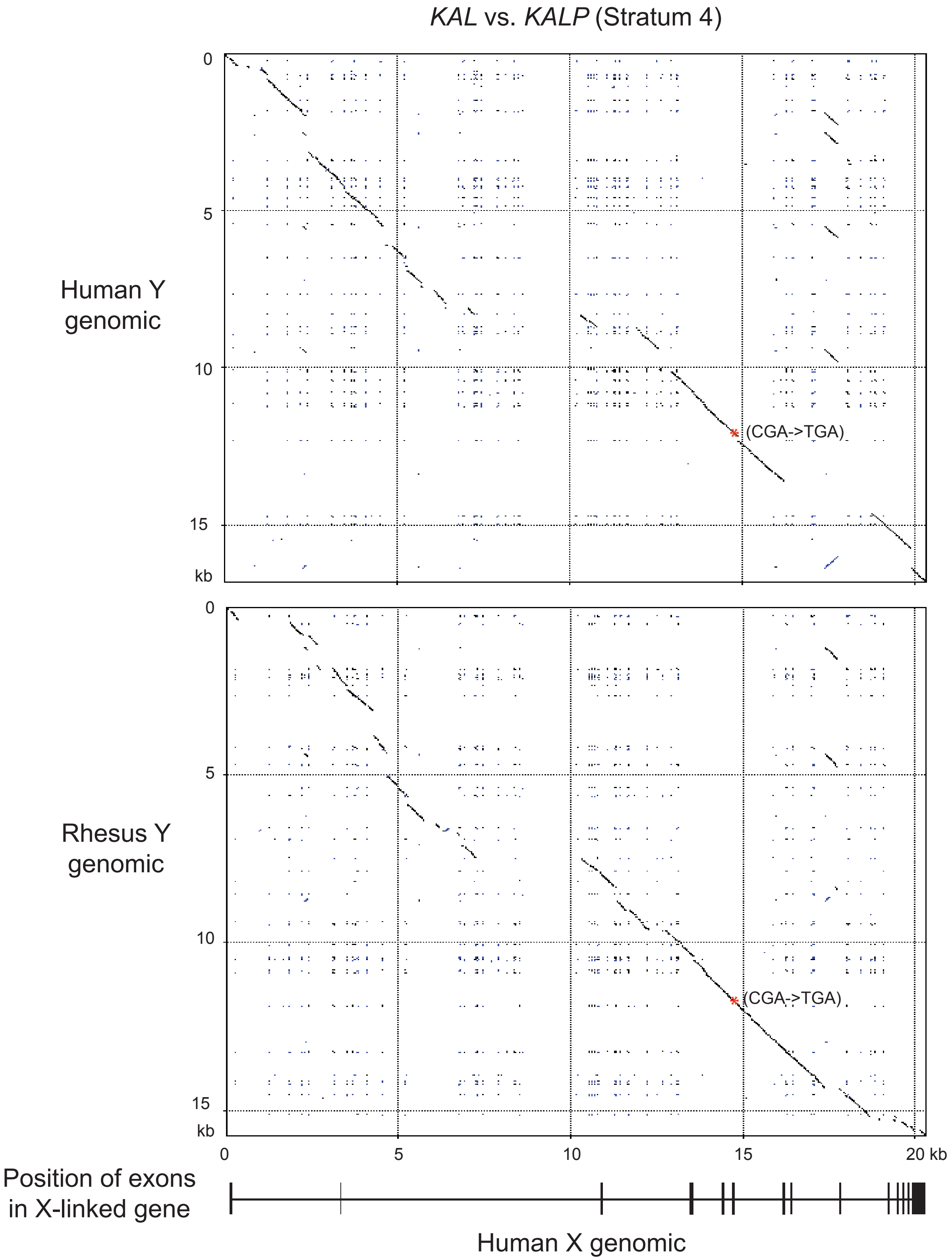
Supplementary Figure 14 RT-PCR analysis of rhesus MSY genes. Rhesus tissues were used to isolate RNA for cDNA generation: reactions were performed in parallel in the presence or absence of reverse transcriptase (RT). PCR products were sequenced to confirm their identity. Primer sequences and product sizes are listed in Supplementary Table 8. We were not able to detect transcription of *AMELY* and *SRY* in the tissues tested. Expression of these genes in human is restricted to tooth enamel and embryonic testes, respectively.



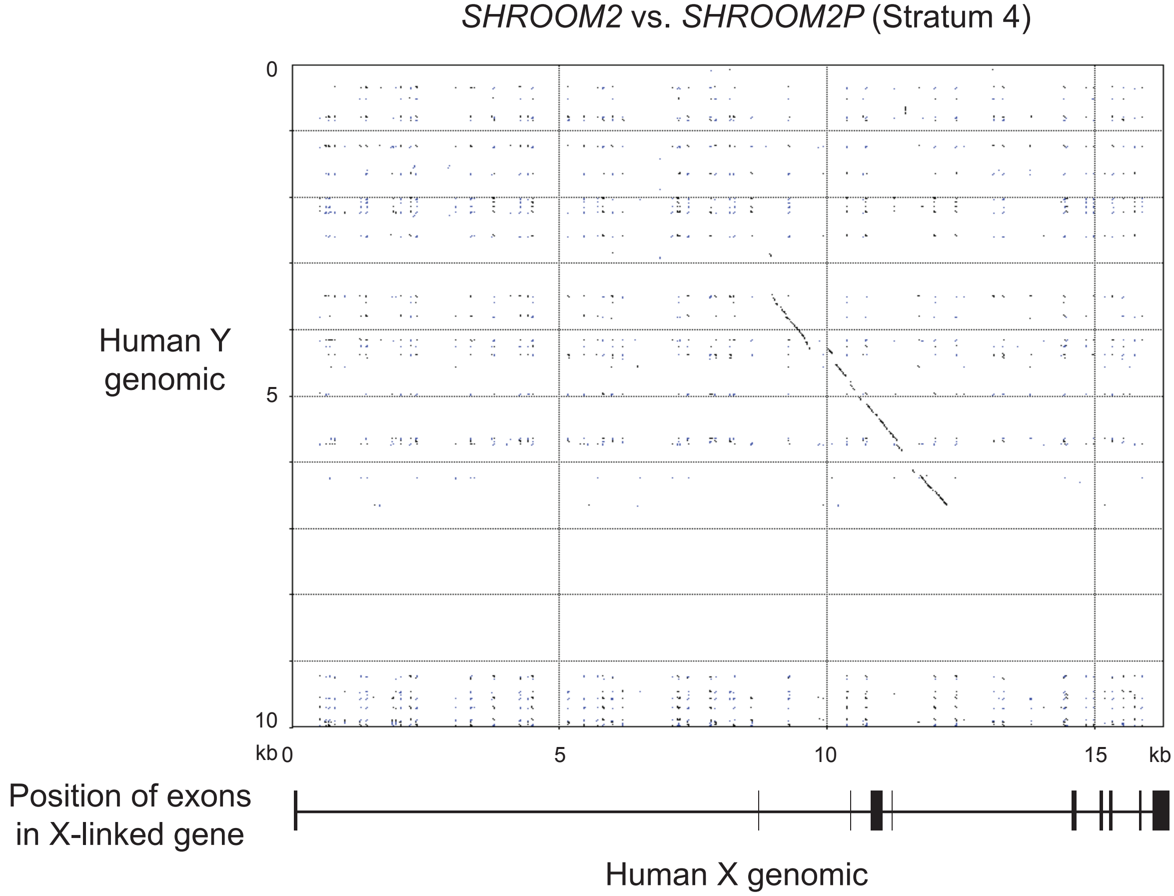
Supplementary Figure 15a Analysis of MSY ancestral pseudogenes in rhesus and human. Y-linked *GPR143P* vs. X-linked *GPR143* dot-plot analyses. On X-axis, human *GPR143* genomic sequence. Exon-intron structure of gene is depicted below. On Y-axes, genomic Y sequence from human (top) and rhesus (bottom). For each plot, a dot represents 70% identity within a 100-bp window. *GPR143P* is a stratum 4 pseudogene. One large deletion removing exons 6-8 is common in human and rhesus (deleted region shaded, boundaries indicated by red-dotted lines), indicating inactivation prior to OWM-ape split. Pattern in chimpanzee is nearly identical to that in human (not shown).



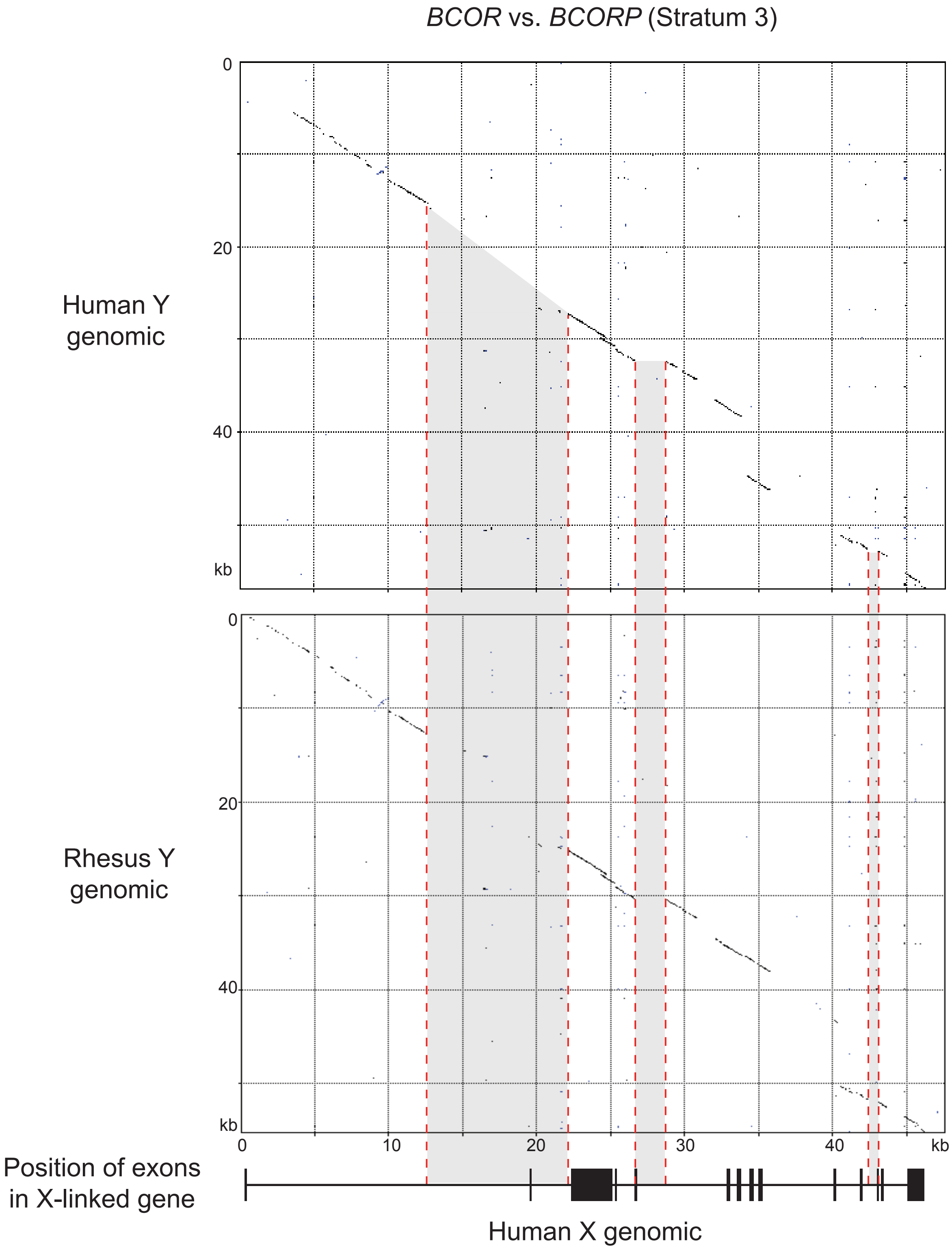
Supplementary Figure 15b Analysis of MSY ancestral pseudogenes in rhesus and human. Y-linked *STSP* vs. X-linked *STS* dot-plot analyses. On X-axis, human *STS* genomic sequence. Exon-intron structure of gene is depicted below. On Y-axes, genomic Y sequence from human (top) and rhesus (bottom). For each plot, a dot represents 70% identity within a 100-bp window. *STSP* is a stratum 4 pseudogene. One deletion removing exon 8 (causing a frameshift and truncation of >200 amino acids in encoded protein) is common in human and rhesus (deleted region shaded, boundaries indicated by red dotted lines), indicating inactivation prior to OWM-ape split. Pattern in chimpanzee is nearly identical to that in human (not shown).



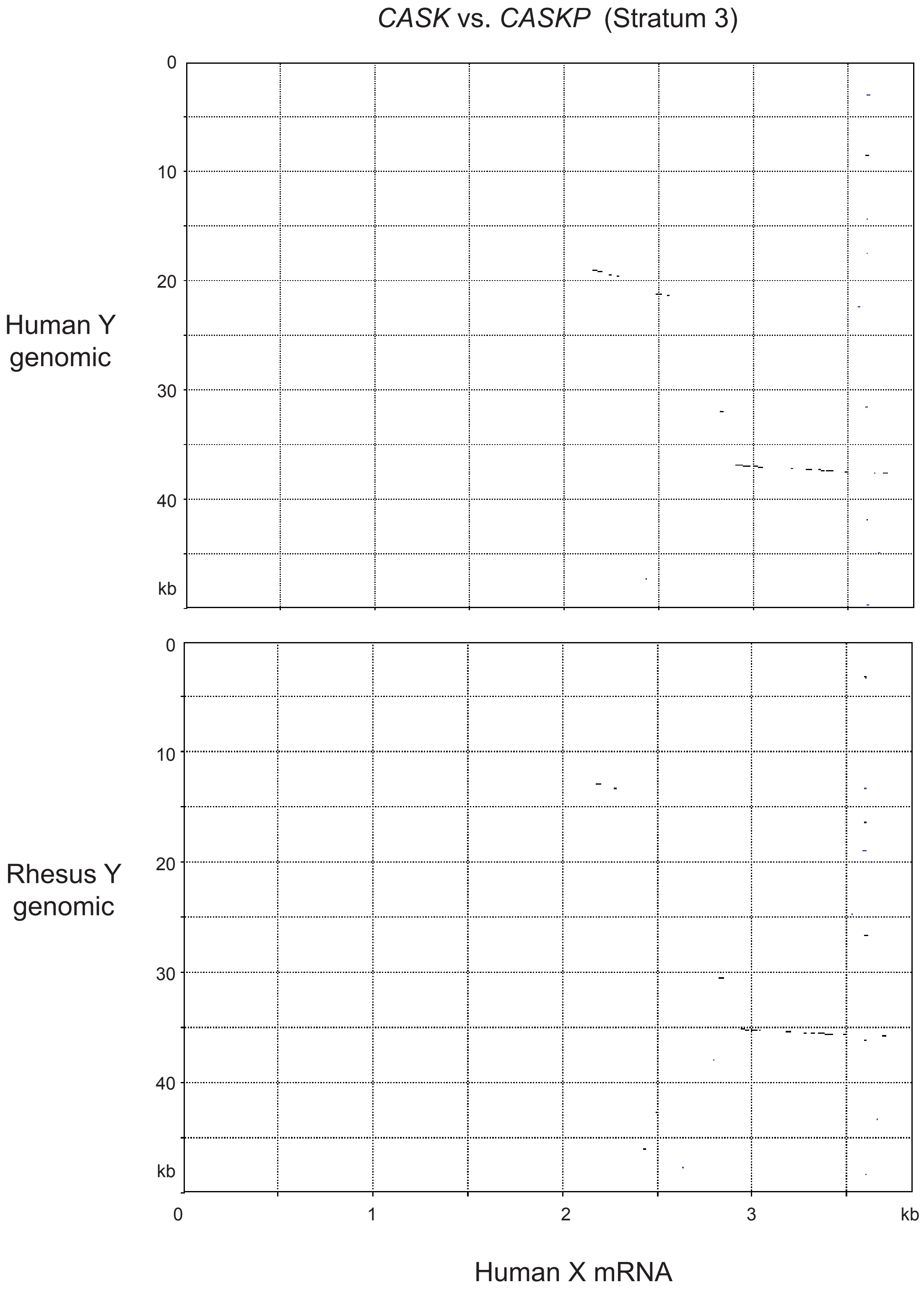
Supplementary Figure 15c Analysis of MSY ancestral pseudogenes in rhesus and human. Y-linked *KALP* vs. X-linked *KAL* dot-plot analyses. On X-axis, human *KAL* genomic sequence. Exon-intron structure of gene is depicted below. On Y-axes, genomic Y sequence from human (top) and rhesus (bottom). For each plot, a dot represents 70% identity within a 100-bp window. *KALP* is a stratum 4 pseudogene. One premature stop mutation (truncates encoded protein by >400 amino acids) is common in human and rhesus (position indicated by red asterisk), indicating inactivation prior to OWM-ape split. Pattern in chimpanzee is nearly identical to that in human (not shown).



Supplementary Figure 15d Analysis of MSY ancestral pseudogenes in human. Y-linked *SHROOM2P* vs. X-linked *SHROOM2* dot-plot analysis. On X-axis, human *SHROOM2* genomic sequence. Exon-intron structure of gene is depicted below. On Y-axis, genomic Y sequence from human (pseudogene is deleted in rhesus). A dot represents 70% identity within a 100-bp window. *SHROOM2P* is a stratum 4 pseudogene, and is highly degenerated compared to its X homolog (to an even greater extent than other Stratum 4 pseudogenes -- Supplementary Figures 15a-c), indicating that inactivation likely occurred prior to OWM-ape split. Pattern in chimpanzee is nearly identical to that in human (not shown).



Supplementary Figure 15e Analysis of MSY ancestral pseudogenes in rhesus and human. Y-linked *BCORP* vs. X-linked *BCOR* dot-plot analyses. On X-axis, human *BCOR* genomic sequence. Exon-intron structure of gene is depicted below. On Y-axes, genomic Y sequence from human (top) and rhesus (bottom). For each plot, a dot represents 30% identity within a 60-bp window. *BCORP* is a highly diverged stratum 3 pseudogene. Several deletions removing or disrupting exons are common in human and rhesus (deleted regions shaded, boundaries indicated by red-dotted lines), indicating inactivation prior to OWM-ape split. Pattern in chimpanzee is nearly identical to that in human (not shown).

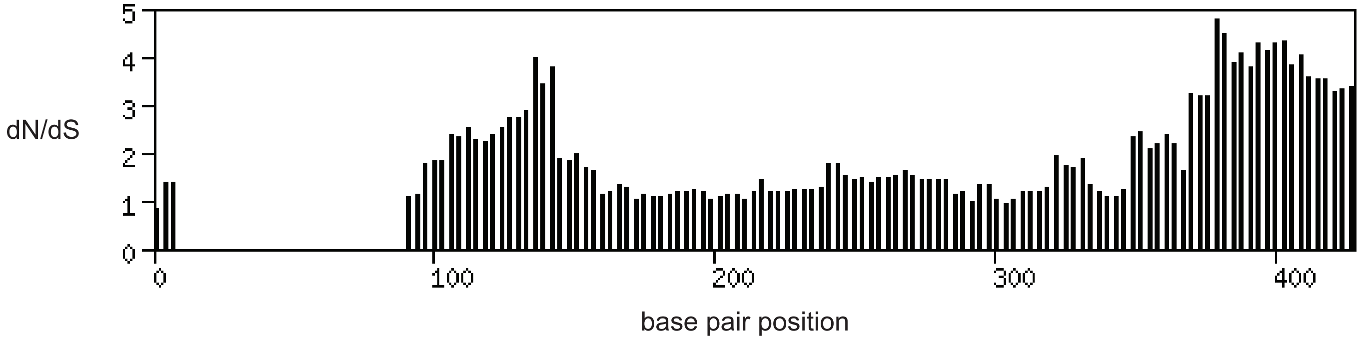


Supplementary Figure 15f Analysis of MSY ancestral pseudogenes in rhesus and human. Y-linked *CASKP* vs. X-linked *CASK* dot-plot analyses. On X-axis, human *CASK* mRNA sequence (if genomic sequence is used, X-Y identities are very difficult to visualize). On Y-axes, genomic Y sequence from human (top) and rhesus (bottom). For each plot, a dot represents 30% identity within a 60-bp window. *CASKP* is a highly diverged stratum 3 pseudogene. Overall divergence patterns in human and rhesus are similar, indicating inactivation prior to OWM-ape split. Pattern in chimpanzee is nearly identical to that in human (not shown).

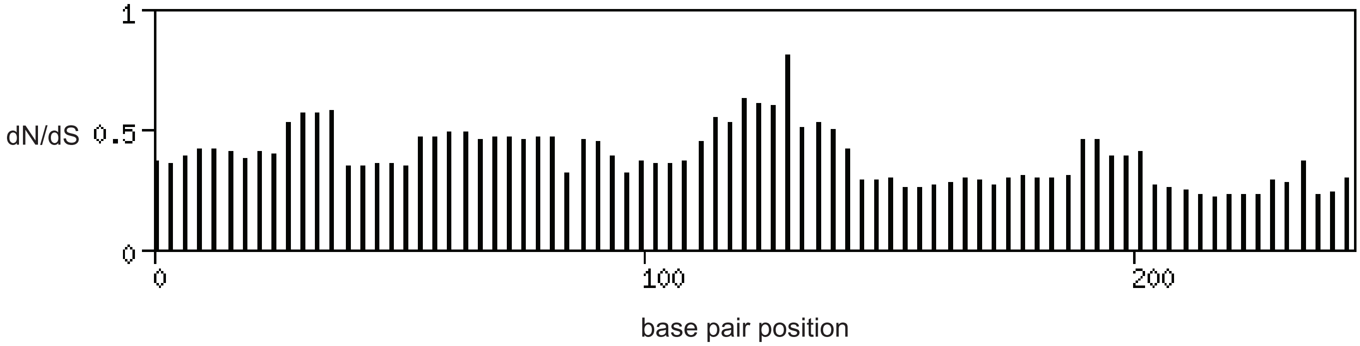
Supplementary Figure 16 Sliding-window analyses for rhesus-human ancestral gene pairs. For each ancestral gene, translated ORF sequences for rhesus and human were aligned using stretcher (http://emboss.sourceforge.net) with gap-open penalty of 20 and gap-extend penalty of 1. Resulting amino-acid alignments were used to convert corresponding rhesus and human mRNA sequences into codon alignments using PAL2NAL (http://www.bork.embl.de/pal2nal). A custom perl code was used to implement the CODEML program in PAML to calculate dN/dS ratios over a sliding window with a 3-bp step. Coding sequence (CDS) length determined window size: if CDS < 500 bp, window size = 150 bp; if CDS > 500 bp, window size = 300 bp. dN/dS values for each gene as a whole are given in Supplementary Table 3.

Supplementary Figure 16

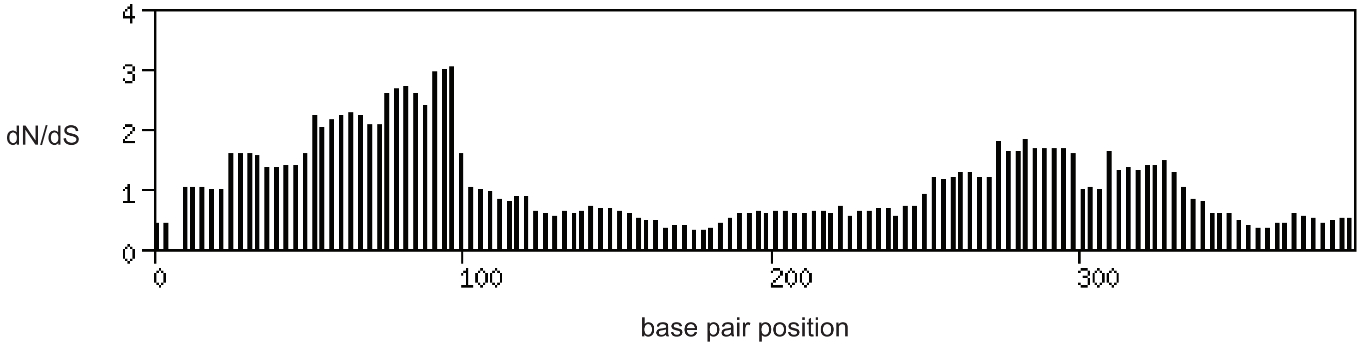
AMELY window size = 150 bp



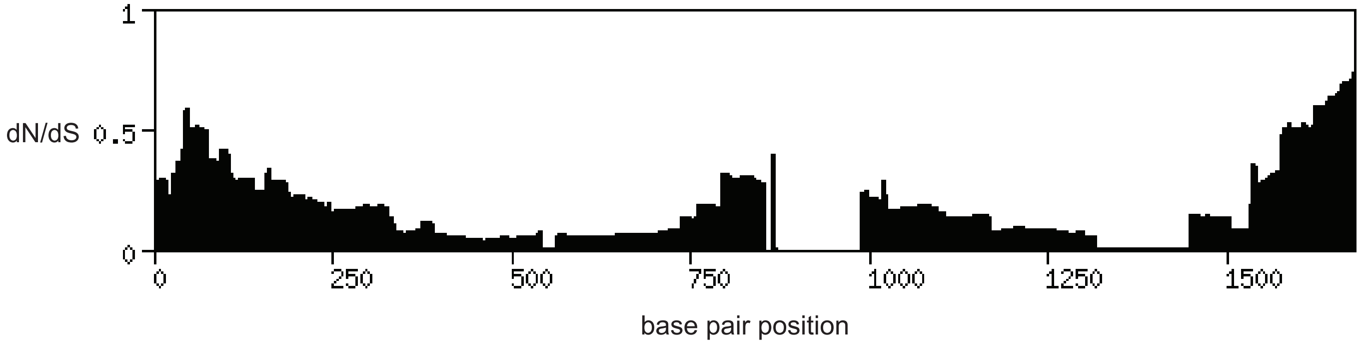
CYorf15a window size = 150 bp



CYorf15b window size = 150 bp

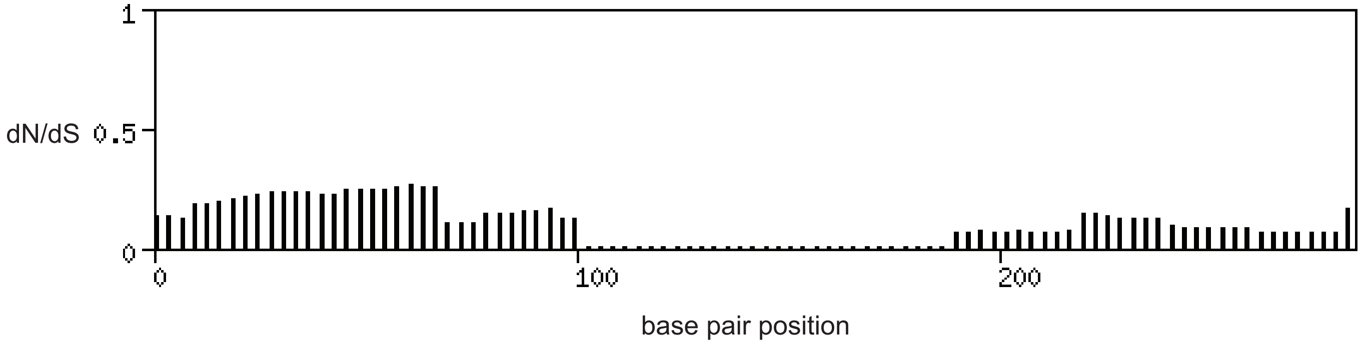


DDX3Y window size = 300 bp

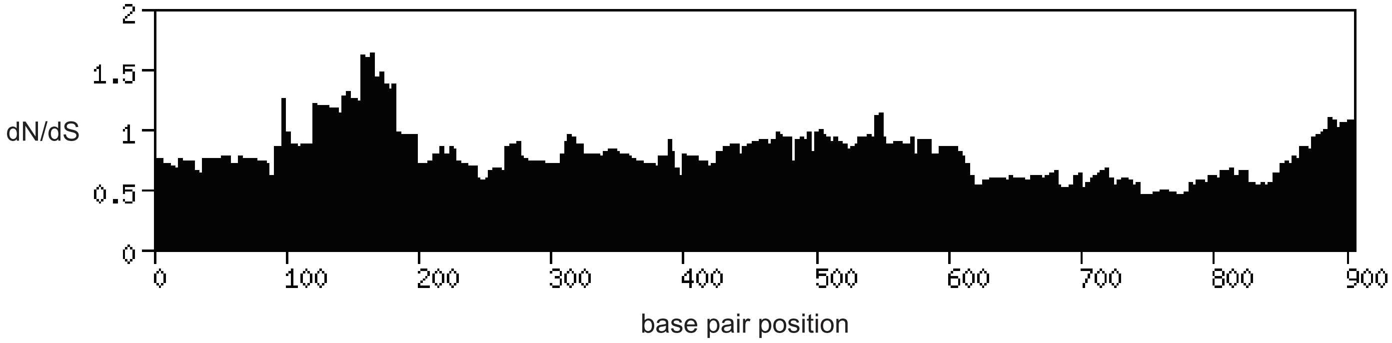


Supplementary Figure 16

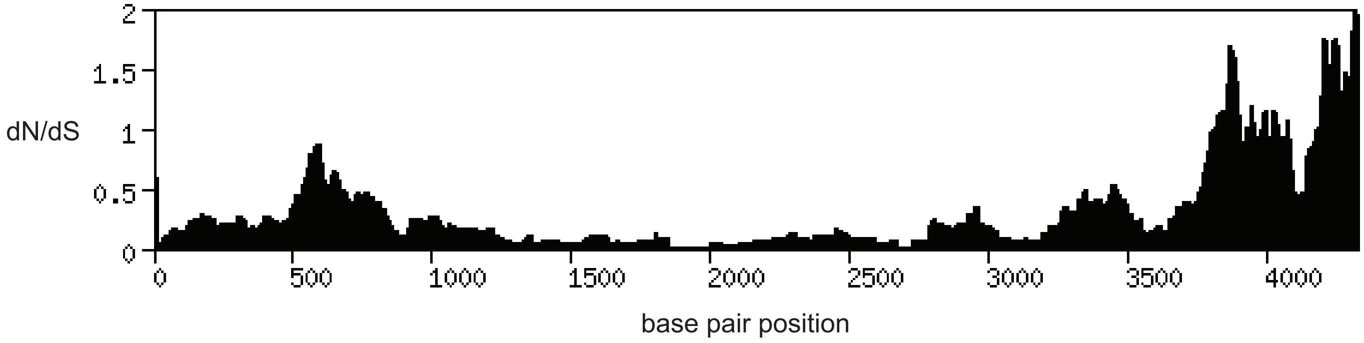
EIF1AY window size = 150 bp



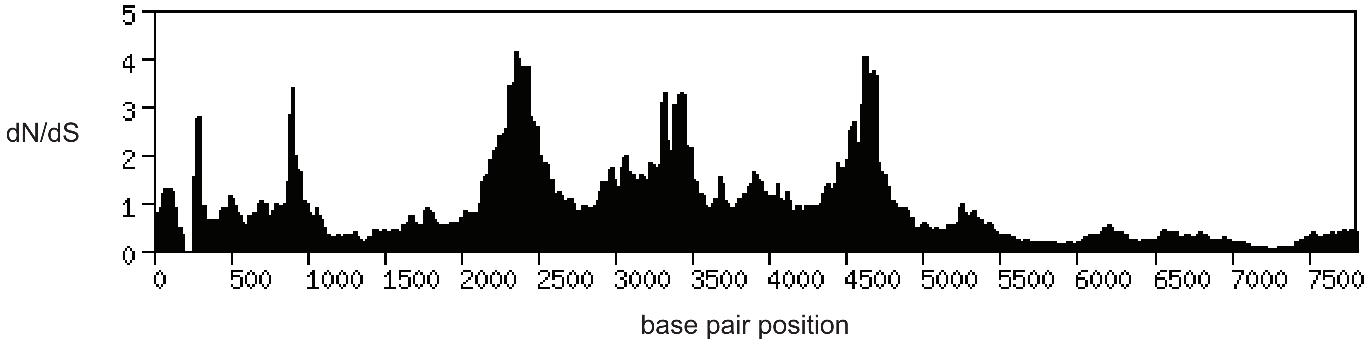
HSFY window size = 300 bp



KDM5D window size = 300 bp

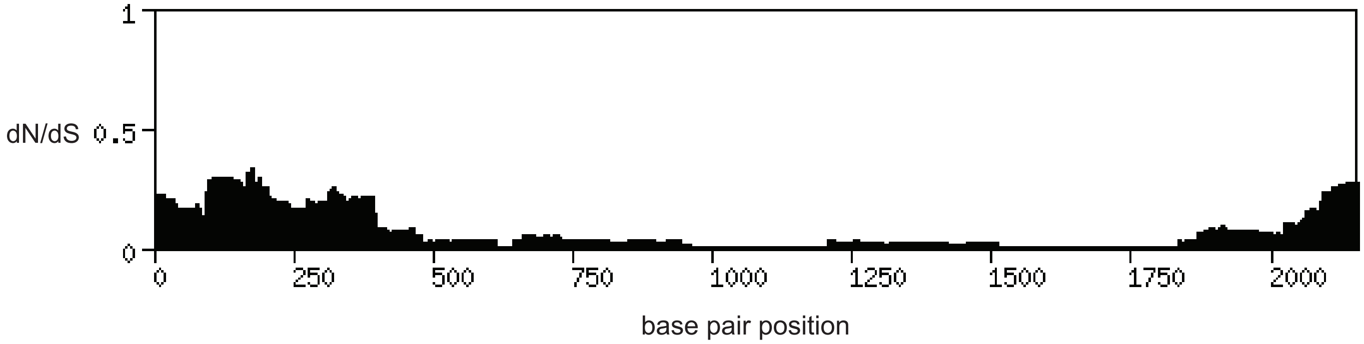


MXRA5Y window size = 300 bp

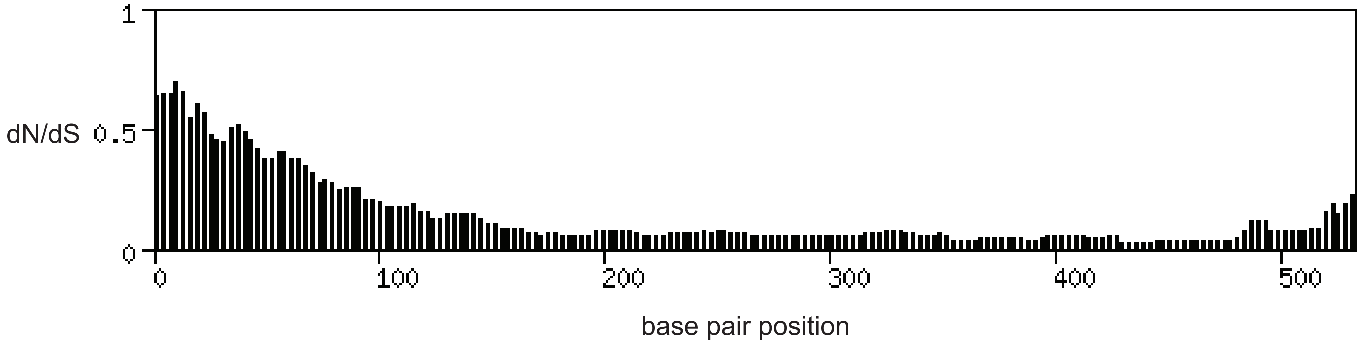


Supplementary Figure 16

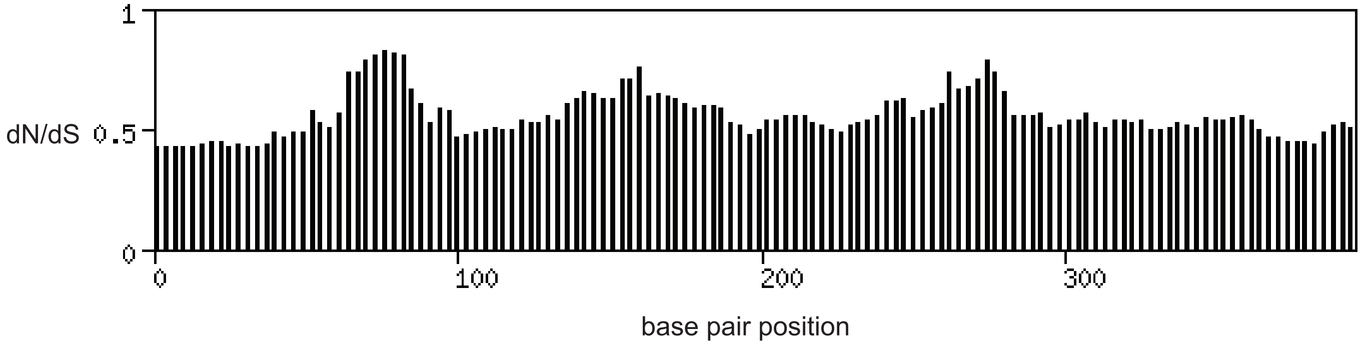
NLGN4Y window size = 300 bp



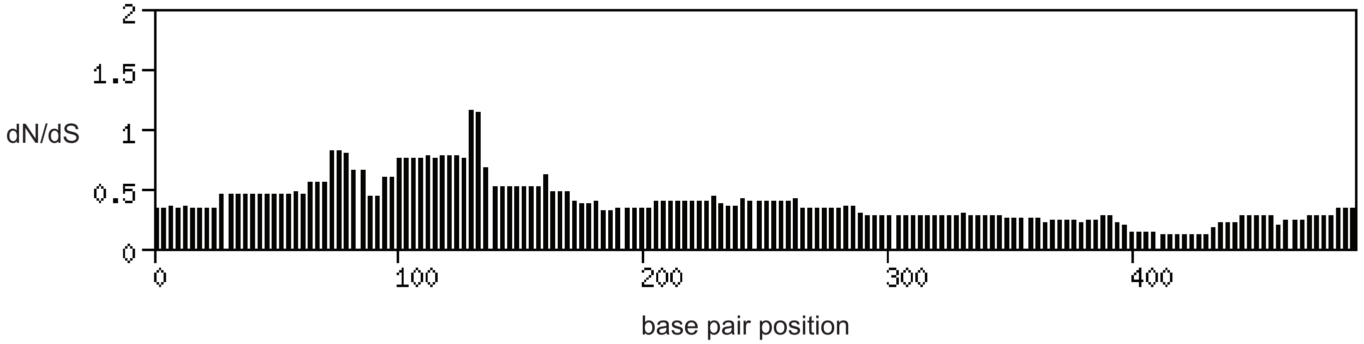
PRKY window size = 300 bp



RBMY window size = 300 bp

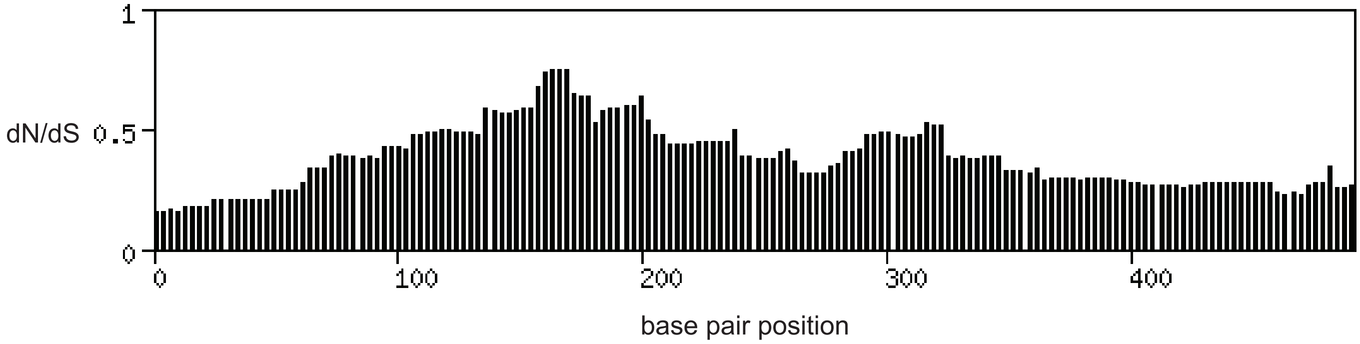


RPS4Y1 window size = 300 bp

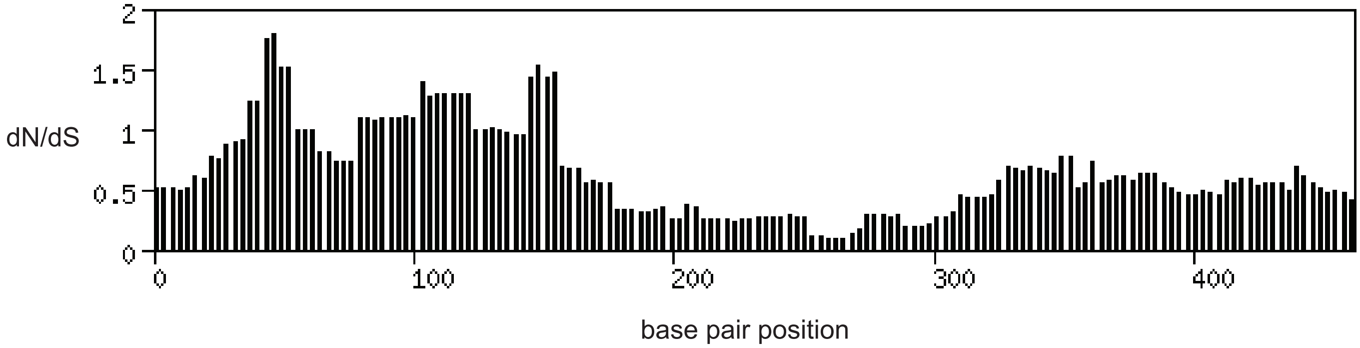


Supplementary Figure 16

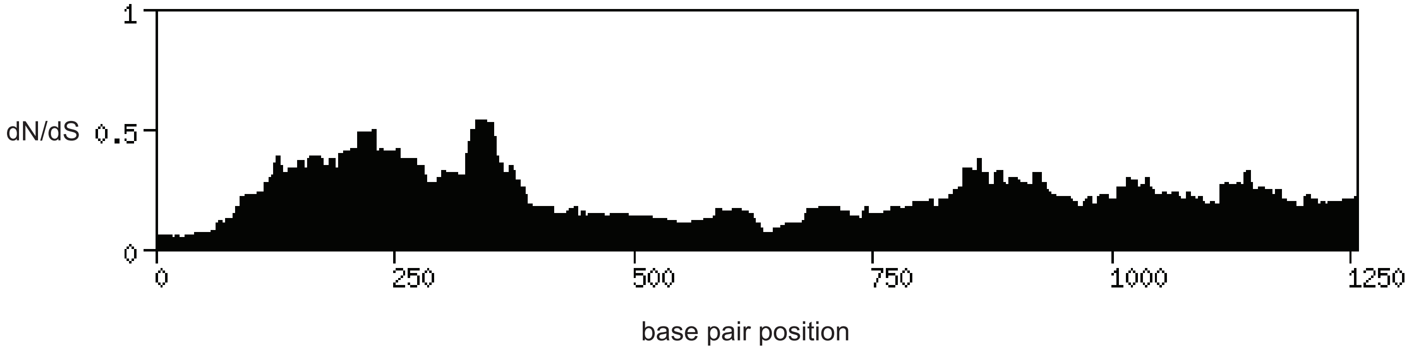
RPS4Y2 window size = 300 bp



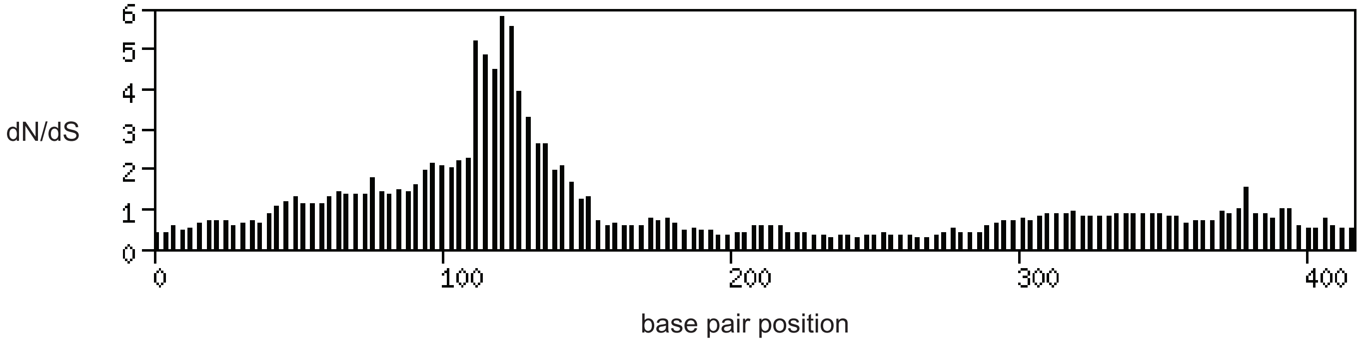
SRY window size = 150 bp



TBL1Y window size = 300 bp

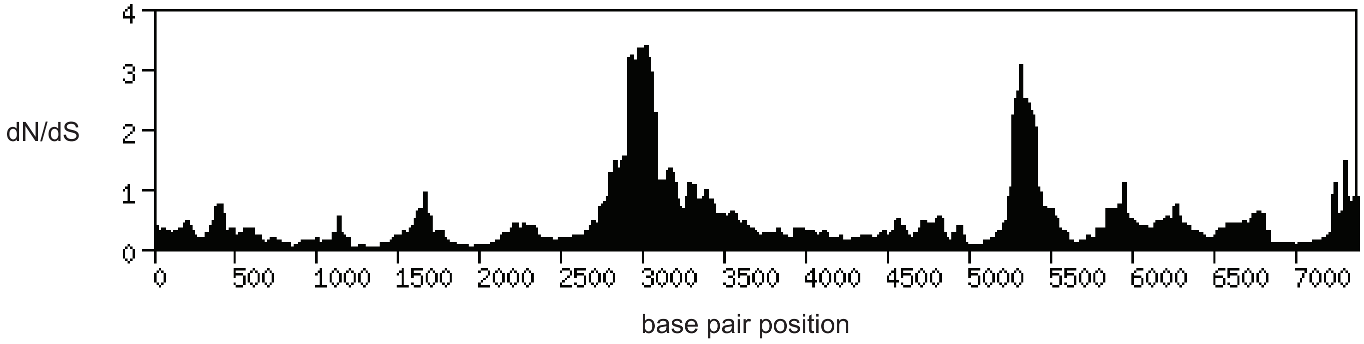


TSPY window size = 150 bp

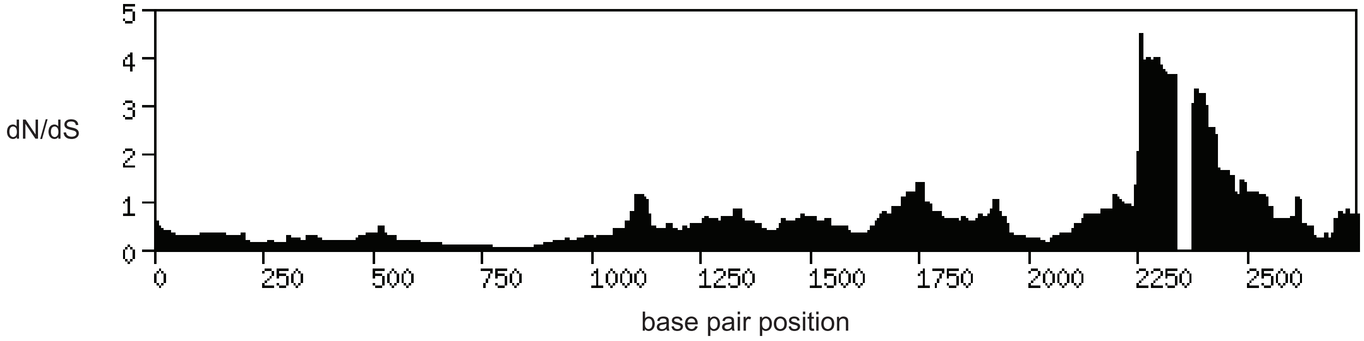


Supplementary Figure 16

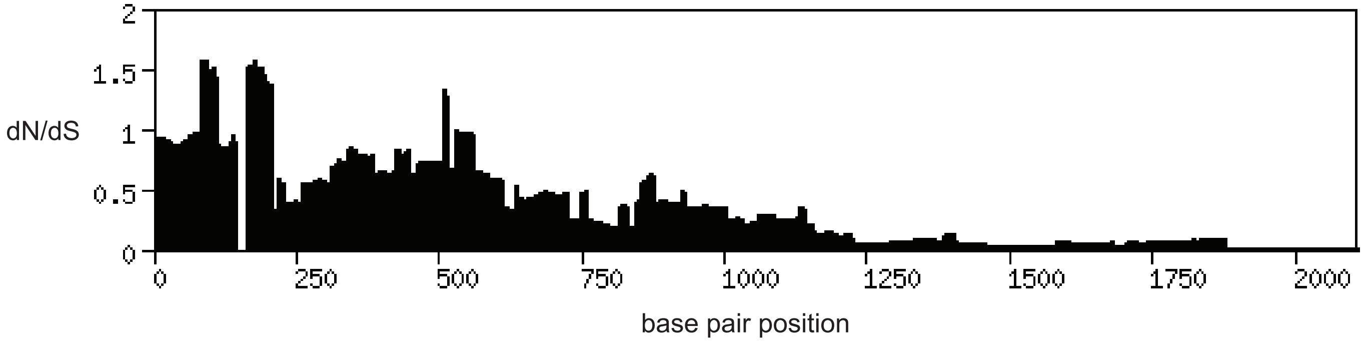
USP9Y window size = 300 bp

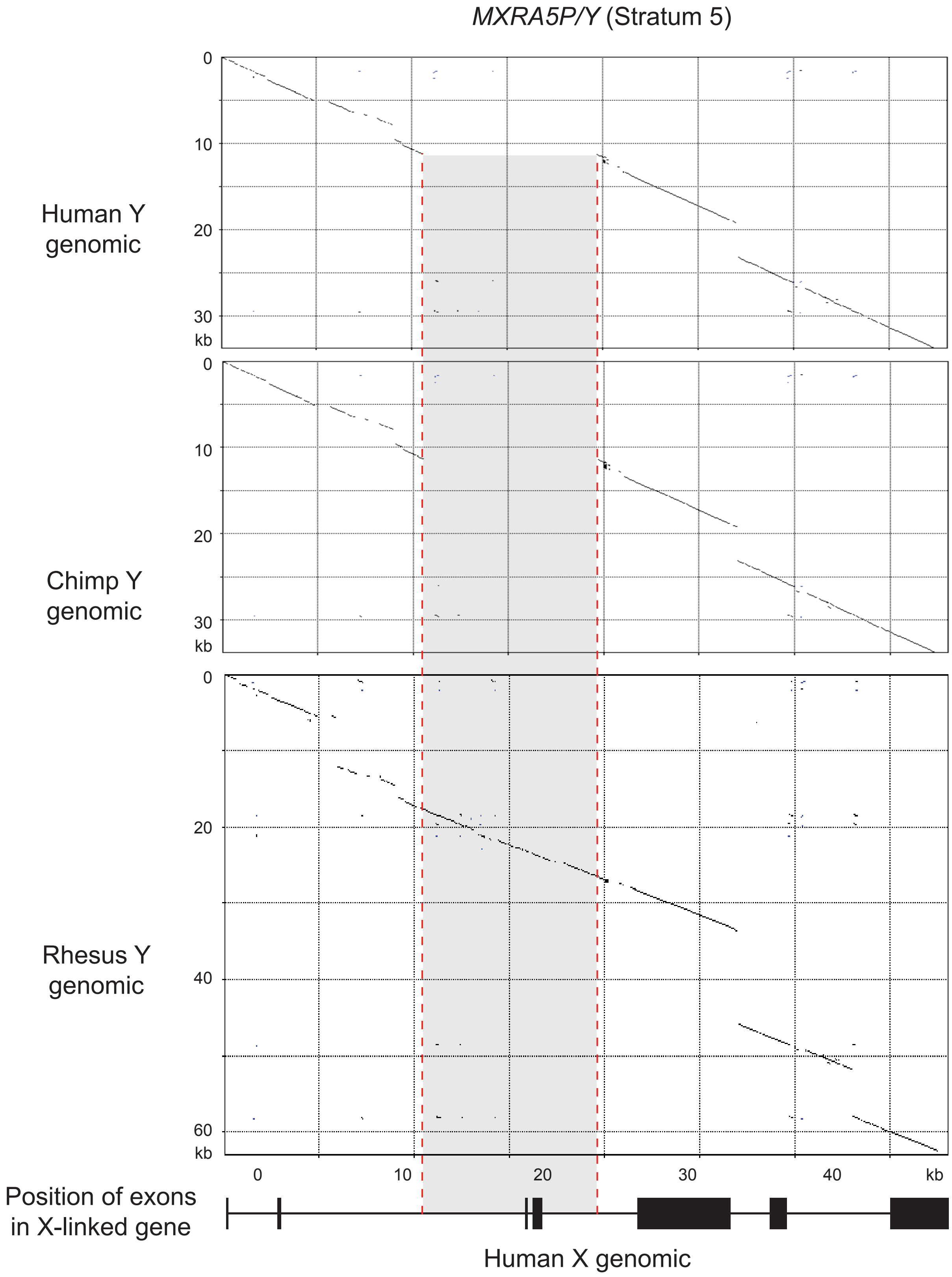


UTY window size = 300 bp



ZFY window size = 300 bp



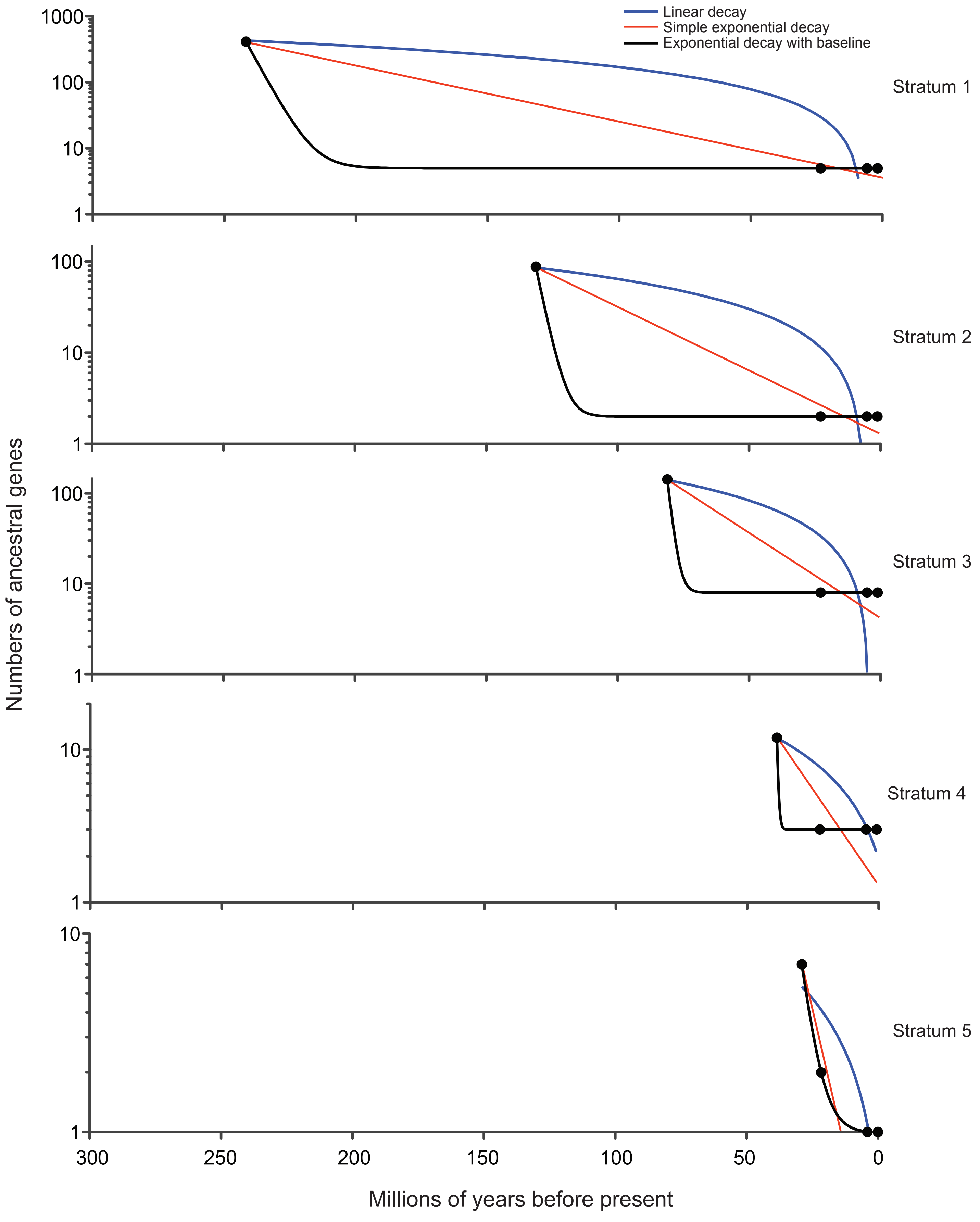


Supplementary Figure 17 Y-linked *MXRA5P/Y* vs. X-linked *MXRA5* dot-plot analyses in rhesus, human and chimpanzee. On X-axis, human *MXRA5* genomic sequence. Exon-intron structure of gene is depicted below. On Y-axes, genomic Y sequence from human (top), chimpanzee (middle), and rhesus (bottom). For each plot, a dot represents 70% identity within a 100-bp window. One deletion removing exons 4 and 5 (causing a truncation of >160 amino acids in the encoded protein) is common in human and chimpanzee (deleted region shaded, boundaries indicated by red dotted lines), but corresponding region is intact in rhesus MSY, indicating inactivation after OWM-ape split but prior to human-chimpanzee split.

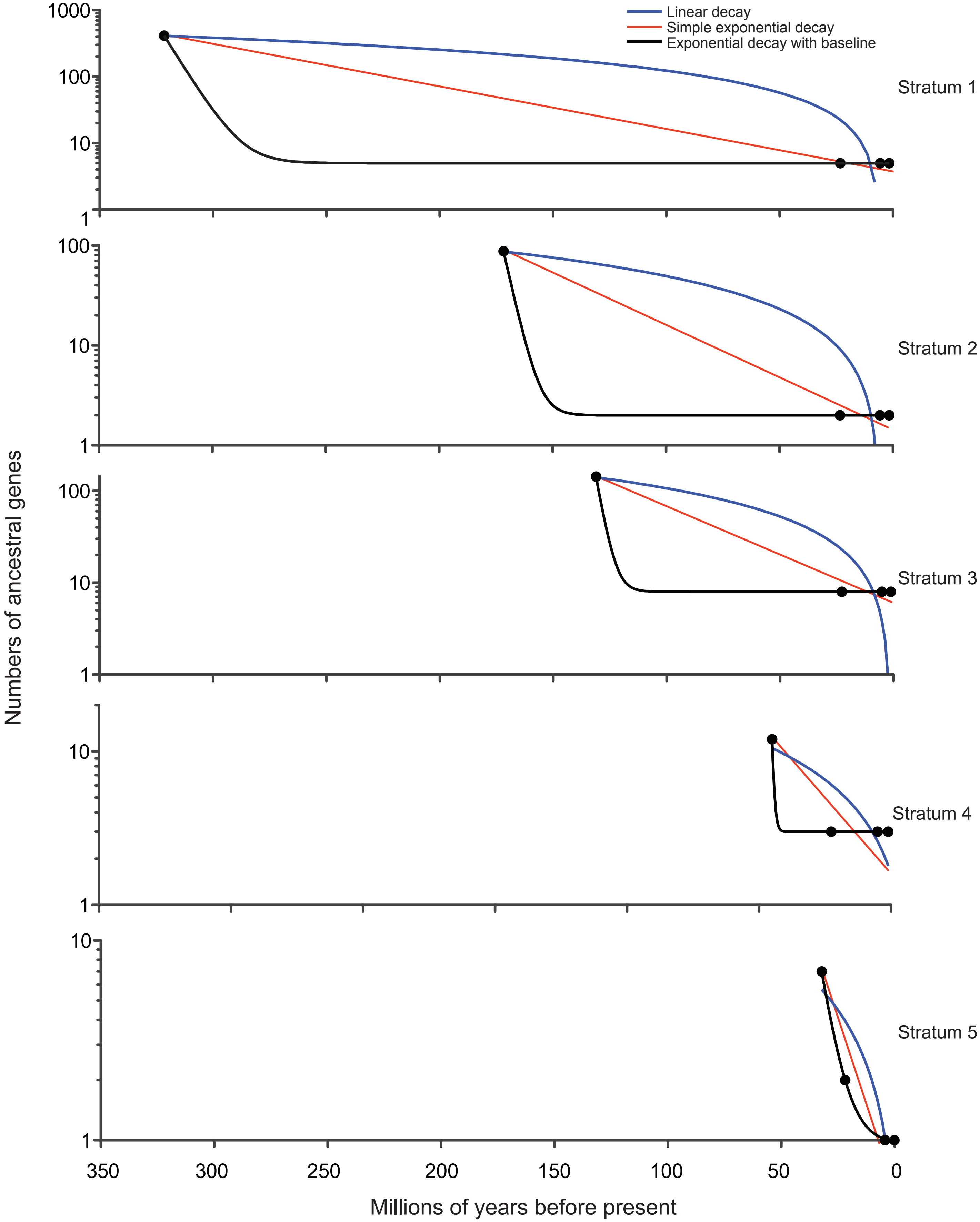
Supplementary Figure 18 Gene decay plots using alternative dates. Minimum (a) and maximum (b) estimations for strata formation dates and lineage divergence dates (given in table below) were used to fit curves using the same method as in Figure 3. Strata date ranges are from main text references 2 and 3. Lineage divergence date ranges are from http://www.timetree.org.

	Minimum date (millions of years ago)	Maximum date (millions of years ago)
Stratum 1 formation	240	320
Stratum 2 formation	130	170
Stratum 3 formation	80	130
Stratum 4 formation	38	44
Stratum 5 formation	29	32
Rhesus/human/chimp ancestor	21.6	38.1
Human/chimp ancestor	4	8.4

Supplementary Figure 18a



Supplementary Figure 18b



Supplementary Table 1. BAC clones, GenBank accession numbers and nucleotide positions in rhesus MSY sequence assembly

BAC name	GenBank accession number	Start position	End position
CH250-228N17	AC217136	1	207268
CH250-57C20	AC217135	133076	325216
CH250-234C9	AC218138	322828	501380
CH250-536L15	AC209790	431875	584730
CH250-7H7	AC225167	512927	663373
CH250-133J8	AC208953	586819	782238
CH250-441E9	AC207029	730115	876799
CH250-387J10	AC233579	864994	1013294
CH250-421L9	AC209415	892911	1069757
CH250-236O3	AC215551	1046643	1230612
CH250-352P1	AC209450	1076356	1242918
CH250-308C23	AC209448	1197004	1352770
CH250-392D8	AC232277	1280648	1422345
CH250-567J10	AC232812	1418007	1578821
CH250-348G18	AC225626	1477163	1632288
CH250-123M13	AC214072	1579682	1743196
CH250-262A6	AC213330	1658191	1842010
CH250-127G4	AC206102	1752845	1943979
CH250-56H16	AC206891	1925142	2091810
CH250-541A11	AC213321	2035026	2191714
RMAEX-106G5	AC237431	2068941	2238849
CH250-106J9	AC213381	2215041	2384793
CH250-6J16	AC212894	2324975	2515799
CH250-393D12	AC212004	2427003	2593199
CH250-265J16	AC217134	2557979	2729056
CH250-277C1	AC212744	2702320	2894742
CH250-369H16	AC217132	2847613	3006214
CH250-438I12	AC212027	2943049	3067481
CH250-127L17	AC212387	2970977	3146470
CH250-311C6	AC218137	3070618	3248221
CH250-523J16	AC233736	3209673	3350882
CH250-99J18	AC205186	3308293	3487624
CH250-95C23	AC213328	3390177	3567856
CH250-337J7	AC206885	3495927	3653839
CH250-445I24	AC207030	3632981	3806382
CH250-167G6	AC215694	3801429	3987257
CH250-182M22	AC206433	3927771	4100401
CH250-364B23	AC207035	4071824	4230386
RMAEX-213K20	AC237222	4194948	4340225
CH250-540A18	AC209796	4299833	4462069
CH250-231H12	AC229687	4460022	4656116
CH250-263I4	AC217137	4628633	4869929
CH250-3M7	AC207616	4802760	4999152

BAC name	GenBank accession number	Start position	End position
CH250-204D1	AC225831	4890266	5034651
CH250-380I15	AC217133	5026232	5211571
CH250-144P14	AC205194	5143525	5303607
CH250-477E7	AC225635	5291502	5442191
CH250-197K9	AC225612	5368446	5523328
CH250-198P20	AC215644	5515066	5672573
CH250-309A3	AC215643	5600065	5770585
CH250-128N24	AC215552	5733408	5930884
CH250-395B18	AC215642	5868056	6054638
CH250-214L22	AC217166	5998274	6175965
CH250-122N22	AC215550	6169834	6366283
CH250-165P22	AC206800	6320200	6506534
CH250-142O13-2	AC207049	6444445	6629531
CH250-392O18	AC215553	6604213	6767217
CH250-29I18	AC215641	6619972	6795425
CH250-361K18	AC215549	6769616	6921226
CH250-128P23	AC215640	6771158	6960166
CH250-97J21	AC231831	6859263	7014682
RMAEX-230N16	AC237223	6949034	7126320
CH250-542D24	AC209262	7016219	7146999
CH250-463B10	AC208130	7138967	7354612
CH250-164O10	AC208133	7218549	7411027
CH250-351H7	AC212790	7396859	7505560
CH250-182A8	AC209264	7529592	7712304
CH250-477B24	AC213057	7672571	7888031
CH250-374B3	AC225627	7797384	7945558
CH250-209A4	AC232762	7995862	8170559
CH250-406G3	AC217131	8107991	8283493
CH250-405N21	AC217130	8252767	8417927
CH250-429O23	AC234330	8321309	8475535
CH250-454C5	AC234329	8220669	8390741
CH250-63P1	AC231654	8477122	8686768
CH250-449N17	AC217129	8632679	8786585
CH250-324L17	AC217105	8661406	8838469
CH250-116D13	AC232761	8835736	9002807
CH250-489E18	AC225837	8864514	9051478
CH250-351E18	AC225636	9002180	9141063
CH250-500O12	AC209263	9072191	9243731
CH250-290P15	AC208132	9178269	9338264
CH250-185E13	AC208134	9303517	9488431
CH250-502N18	AC208128	9434149	9579023
CH250-297A9	AC211243	9556265	9716395
CH250-161E20	AC208131	9631825	9811209
CH250-517K1	AC208129	9715405	9885628
CH250-434B12	AC240711	9800240	9961034
CH250-11J13	AC240710	10126901	10322331
CH250-62E18	AC234913	10191476	10395727

BAC name	GenBank accession number	Start position	End position
CH250-249M17	AC233735	10343539	10527567
CH250-59H13	AC212028	10421737	10571823
CH250-99F15	AC212487	10480478	10664291
CH250-161N14	AC217138	10638430	10814598
CH250-492J9	AC208955	10699854	10878866
CH250-285M20	AC212789	10812514	11021146
CH250-317A11	AC209447	10879191	11053172
CH250-194C7	AC209446	10938528	11137503
CH250-118O14	AC219066	11091848	11253688

Supplementary Table 2. Palindromes in rhesus, human and chimpanzee MSYs

	Number of palindromes	Average arm length (kb)	Total arm length (kb)	% of total euchromatic sequence	Total # of genes in palindromes
Rhesus	3	73	437	3.9	4
Human	8	344	5507	24.2	20
Chimpanzee	19	198	7516	29.2	15

Supplementary Table 3. Comparative analysis of rhesus and human MSY genes

		Nucleotide position in rhesus MSY assembly			Coding sequence		% divergence (rhesus - human)			Detecting purifying selection			
	Ancestral				ORF	% of human					Fisher's exact test		
Stratum	genes	Start	End	Strand	length	ORF length	mRNA	Coding	Introns	dN/dS^1	95% C.I.		p-value
1	*SRY*	81482	82385	R	611	100	6.60	6.04	n/a	0.55	0.29	0.98	0.06
	RBMY	6271571	6284071	R	695	47	11.32	9.04	12.21	0.54	0.32	0.99	0.07
	RPS4Y1	122092	147116	F	791	100	3.90	3.04	8.74	0.31	0.14	0.70	0.005
	RPS4Y2	6055088	6082902	F	791	100	5.31	4.55	9.58	0.26	0.13	0.48	<0.0001
	HSFY1	8006371	8008261	R	1205	100	7.76	7.79	10.55	0.62	0.42	0.91	0.02
	HSFY2	8384853	8386745	F	1205	100	9.16	8.70	12.11	0.81	0.55	1.19	0.3
	HSFY3	8553282	8555173	R	1205	100	9.29	8.78	12.11	0.77	0.53	1.15	0.2
2	*KDM5D*	5560125	5599597	R	4619	100	5.09	4.31	9.11	0.26	0.20	0.35	<0.0001
	TSPY1	6307721	6309591	R	566	61	11.27	9.48	15.54	0.77	0.41	1.26	0.3
	TSPY2	9880248	9881875	R	821	89	11.69	9.76	15.67	0.69	0.50	1.25	0.3
	TSPY3	9896624	9898258	R	821	89	11.69	9.76	15.67	0.69	0.50	1.25	0.3
	TSPY4	9913025	9914659	R	821	89	11.69	9.76	15.67	0.69	0.50	1.25	0.3
	TSPY5	9929422	9931056	R	821	89	11.69	9.76	15.67	0.69	0.50	1.25	0.3
3	*ZFY*	239742	287462	F	2405	100	3.71	2.08	7.63	0.17	0.09	0.30	<0.0001
	DDX3Y	2107890	2124485	F	1979	100	3.52	2.28	9.01	0.19	0.11	0.34	<0.0001
	UTY	2366565	2577315	R	4043	100	4.38	3.49	8.19	0.33	0.25	0.46	<0.0001
	EIF1AY	5853306	5870788	F	434	100	7.85	2.75	8.55	0.10	0.03	0.40	0.0003
	CYorf15A	5408162	5432524	F	395	100	6.74	5.79	9.10	0.33	0.15	0.69	0.005
	CYorf15B	5434583	5448447	F	539	99	6.26	5.74	8.61	0.54	0.27	1.05	0.07
	USP9Y	1904430	2077520	F	7682	100	3.67	3.02	8.22	0.29	0.23	0.37	<0.0001
	TMSB4Y	2640014	2642403	F	134	100	10.22	10.29	9.57	0.47	0.17	1.12	0.1
4	*AMELY*	659259	667900	R	578	100	4.89	5.00	7.19	1.33	0.61	2.98	0.5
	NLGN4Y	4143178	4465183	F	2510	102	6.90	4.04	9.95	0.09	0.05	0.13	<0.0001
	TBL1Y	1058868	1127299	F	1562	100	6.78	6.14	9.10	0.18	0.12	0.26	<0.0001

		Nucleotide position in rhesus MSY assembly			Coding sequence		% divergence (rhesus - human)			Detecting purifying selection			
											Fisher's exact test		
Stratum	Ancestral genes	Start	End	Strand	ORF length	% of human ORF length	mRNA	Coding	Introns	dN/dS*	95% C.I.		p-value
5	*PRKY*	1189854	1297038	F	1076	129	8.28	6.31	9.64	0.28	0.16	0.46	<0.0001
	MXRA5Y	1583565	1646589	F	8405	642	7.17	7.35	8.67	0.99	0.80	1.24	1.0
	Average[2,3] (ancestral):						6.55	5.32	9.47	0.30	0.27	0.34	<0.0001
	Average[3] (ancestral; single-copy in rhesus and human only):						5.88	4.68	8.81	0.26	0.23	0.29	<0.0001
	Added genes												
	DAZ1	9133192	9195151	R	1796	80	9.24	7.53	11.00	0.92	0.53	1.59	0.75
	DAZ2	10630299	10698472	R	1742	78	9.53	11.03	11.27	0.62	0.40	0.96	0.06
	CDY1	8812938	8815149	R	1626	100	10.92	10.22	n/a	0.41	0.31	0.53	<0.0001
	CDY2	8902500	8904710	F	1625	100	10.87	10.16	n/a	0.41	0.32	0.54	<0.0001
	XKRY	7641603	7643120	R	749	212	16.06	14.40	n/a	1.08	0.75	1.57	0.7
	Average[2] (added):						11.44	10.79	11.13	0.60	0.50	0.72	<0.0001

[1] dN/dS = Ratio of # of non-synonymous substitutions per non-synonymous site (dN) to # of synonymous substitutions per synonymous site (dS)

(Calculated using the CODEML program in the PAML package: Yang Z (1998) Mol Biol Evol 15:568-573)

(Fisher's exact test used to detect deviation of dN/dS ratio from neutrality or deviation from dN/dS = 1)

[2] Averages calculated using only one member of multicopy gene families with >99% identity

[3] Ancestral average does not include *MXKRA5Y*, which is a pseudogene in human

Supplementary Table 4. List of human X genes, organized by stratum, and conserved synteny in other vertebrates.

					Most distant conserved synteny of genes without chicken syntenic homologs		
	Ensembl gene ID	Gene symbol	Human X Position	Syntenic chicken homolog	Anolis or Xenopus	Metatherians	Eutherians
Pseudo-autosomal region 1	ENSG00000182378	PLCXD1	132989	▨			
	ENSG00000178605	GTPBP6	160025	▨			
	ENSG00000178605	GTPBP6	168295	▨			
	ENSG00000167393	PPP2R3B	214970	▨			
	ENSG00000185960	SHOX	505079	▨			
	ENSG00000205755	CRLF2	1274885	▨			
	ENSG00000198223	CSF2RA	1347693	▨			
	ENSG00000185291	IL3RA	1415509	▨			
	ENSG00000169100	SLC25A6	1465144	▨			
	ENSG00000169093	ASMTL	1482032	▨			
	ENSG00000182162	P2RY8	1541468	▨			
	ENSG00000197976	SFRS17A	1670486	▨			
	ENSG00000196433	ASMT	1694024	▨			
	ENSG00000169084	DHRSX	2147557	▨			
	ENSG00000214717	ZBED1	2414455	▨			
	ENSG00000002586	CD99	2619228	▨			
	ENSG00000124343	XG	2680115	▨			
Stratum 5	ENSG00000056998	GYG2	2756859	▨			
	ENSG00000006756	ARSD	2832011	▨			
	ENSG00000157399	ARSE	2862673	▨			
	ENSG00000205667	ARSH	2934654	▨			
	ENSG00000062096	ARSF	2969512	▨			
	ENSG00000101825	MXRA5	3236606	▨			
	ENSG00000183943	PRKX	3532415	▨			
Stratum 4	ENSG00000146938	NLGN4X	5818085	▨			
	ENSG00000130021	HDHD1A	6976961	▨			
	ENSG00000101846	STS	7147472	▨			
	ENSG00000006757	PNPLA4	7826804	▨			
	ENSG00000011201	KAL1	8456915	▨			

Stratum 4	ENSG00000183304	FAM9A	8718836	■			
	ENSG00000101849	TBL1X	9391352	■			
	ENSG00000101850	GPR143	9653454	■			
	ENSG00000146950	SHROOM2	9714496	■			
	ENSG00000047644	WWC3	9943795	■			
	ENSG00000073464	CLCN4	10084985	■			
	ENSG00000101871	MID1	10373596	■			
Stratum 3	ENSG00000004961	HCCS	11039342				■
	ENSG00000047648	ARHGAP6	11065584	■			
	ENSG00000125363	AMELX	11221454		■		
	ENSG00000005302	MSL3	11686199	■			
	ENSG00000169933	FRMPD4	12066506	■			
	ENSG00000101911	PRPS2	12719414	■			
	ENSG00000196664	TLR7	12795123	■			
	ENSG00000101916	TLR8	12834679	■			
	ENSG00000205542	TMSL2	12903150	■			
	ENSG00000198759	EGFL6	13497645	■			
	ENSG00000176896	TCEANC	13581146	■			
	ENSG00000123595	RAB9A	13617262	■			
	ENSG00000196459	TRAPPC2	13640282	■			
	ENSG00000046651	OFD1	13662785	■			
	ENSG00000046653	GPM6B	13698983	■			
	ENSG00000046647	GEMIN8	13934766	■			
	ENSG00000101958	GLRA2	14457565	■			
	ENSG00000181544	FANCB	14771450	■			
	ENSG00000130150	MOSPD2	14801484	■			
	ENSG00000102048	ASB9	15172030	■			
	ENSG00000165192	ASB11	15209759	■			
	ENSG00000165195	PIGA	15247503	■			
	ENSG00000165197	FIGF	15273640	■			
	ENSG00000087842	PIR	15312847	■			
	ENSG00000102010	BMX	15392290	■			
	ENSG00000130234	ACE2	15489077	■			
	ENSG00000147003	TMEM27	15555372	■			
	ENSG00000186312	CA5BP	15602976				■

Stratum 3	ENSG00000169239	CA5B	15666313				■
	ENSG00000169249	ZRSR2	15718495	■			
	ENSG00000212643	ZRSR1	15743719	■			
	ENSG00000182287	LOC653653	15753850	■			
	ENSG00000126010	GRPR	16051345	■			
	ENSG00000182798	MAGEB17	16095525		■		
	ENSG00000047230	CTPS2	16516047	■			
	ENSG00000169906	S100G	16578202				■
	ENSG00000169895	SYAP1	16647676	■			
	ENSG00000086712	CXORF15	16714526	■			
	ENSG00000102054	RBBP7	16772385	■			
	ENSG00000169891	REPS2	16874735	■			
	ENSG00000188158	NHS	17303802	■			
	ENSG00000047634	SCML1	17665509	■			
	ENSG00000131831	RAI2	17728092	■			
	ENSG00000177324	BEND2	18090974	■			
	ENSG00000102098	SCML2	18167355	■			
	ENSG00000008086	CDKL5	18353646	■			
	ENSG00000102104	RS1	18567733	■			
	ENSG00000086717	PPEF1	18618967	■			
	ENSG00000044446	PHKA2	18820802	■			
	ENSG00000173698	GPR64	18917348	■			
	ENSG00000131828	PDHA1	19271972	■			
	ENSG00000163114	PDHA2	19283385	■			
	ENSG00000180815	MAP3K15	19288095	■			
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	ENSG00000184481	FOXO4	70232751	▨			
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	ENSG00000133149	TEX13A	104350269				■
	ENSG00000123572	NRK	104953192	■			
	ENSG00000123561	SERPINA7	105163853				■
	ENSG00000157502	MUM1L1	105298954				■
	ENSG00000147231	CXORF57	105741858	■			
	ENSG00000133135	RNF128	105823724	■			
	ENSG00000133138	TBC1D8B	105932566	■			
	ENSG00000147223	RIPPLY1	106029951	■			

Stratum 1	ENSG00000165376	CLDN2	106050290	▨			
	ENSG00000133131	MORC4	106070620	▨			
	ENSG00000089682	RBM41	106194306	▨			
	ENSG00000198088	NUP62CL	106253367	▨			
	ENSG00000213024	NUP62	106284125	▨			
	ENSG00000080572	CXORF41	106336518	▨			
	ENSG00000147234	FRMPD3	106652336	▨			
	ENSG00000147224	PRPS1	106758403	▨			
	ENSG00000157514	GRAMD4	106843107				▨
	ENSG00000170935	NCBP2L	106924107	▨			
	ENSG00000080561	MID2	106956116	▨			
	ENSG00000170925	TEX13B	107110750				▨
	ENSG00000101842	VSIG1	107174856	▨			
	ENSG00000101843	PSMD10	107214093	▨			
	ENSG00000101844	ATG4A	107221590	▨			
	ENSG00000197565	COL4A6	107285494	▨			
	ENSG00000188153	COL4A5	107569810	▨			
	ENSG00000133124	IRS4	107862368	▨			
	ENSG00000101890	GUCY2F	108502791	▨			
	ENSG00000101888	NXT2	108665666	▨			
	ENSG00000176076	KCNE1L	108753585	▨			
	ENSG00000068366	ACSL4	108771222	▨			
	ENSG00000157600	TMEM164	109132979	▨			
	ENSG00000101935	AMMECR1	109324071	▨	▨		
	ENSG00000181110	RGAG1	109548941				
	ENSG00000101938	CHRDL1	109803740	▨			
	ENSG00000077264	PAK3	110226244	▨			
	ENSG00000077274	CAPN6	110374987	▨			
	ENSG00000077279	DCX	110423663	▨			
	ENSG00000101901	ALG13	110811069	▨			
	ENSG00000072315	TRPC5	110904198	▨			
	ENSG00000204025	RP1-269O5.2	111011781				▨
	ENSG00000187823	ZCCHC16	111584383		▨		
	ENSG00000182508	LHFPL1	111760535	▨			
	ENSG00000126016	AMOT	111904387	▨			

Stratum 1	ENSG00000147246	HTR2C	113724807	■			
	ENSG00000165502	RPL36AL	113730376	■			
	ENSG00000123496	IL13RA2	114144794	■			
	ENSG00000130224	LRCH2	114251441	■			
	ENSG00000170748	RBMXL2	114330261	■			
	ENSG00000213516	CCBL2	114330261	■			
	ENSG00000102024	PLS3	114701740	■			
	ENSG00000180772	AGTR2	115215986	■			
	ENSG00000087916	SLC6A14	115481818	■			
	ENSG00000003096	KLHL13	116915804	■			
	ENSG00000198642	KLHL9	116928087	■			
	ENSG00000131725	WDR44	117364070	■			
	ENSG00000147251	DOCK11	117513900	■			
	ENSG00000131724	IL13RA1	117745587	■			
	ENSG00000174460	ZCCHC12	117841774		■		
	ENSG00000175556	LONRF3	117992609	■			
	ENSG00000175553	KIAA1210	118096627				■
	ENSG00000101856	PGRMC1	118254279	■			
	ENSG00000214992	AC004835.2	118270637	■			
	ENSG00000077713	SLC25A43	118397679	■			
	ENSG00000005022	SLC25A5P8	118486436	■			
	ENSG00000018610	CXORF56	118556140	■			
	ENSG00000077721	UBE2A	118592527	■			
	ENSG00000186416	NKRF	118606328	■			
	ENSG00000125354	SEPT6	118633715	■			
	ENSG00000187808	ANKRD58	118776604	■			
	ENSG00000198918	RPL39	118804497	■			
	ENSG00000214289	RPL39P5	118808002	■			
	ENSG00000125351	UPF3B	118852017	■			
	ENSG00000125352	RNF113A	118888466				■
	ENSG00000125356	NDUFA1	118889762	■			
	ENSG00000186471	AKAP14	118913828	■			
	ENSG00000101882	NKAP	118943052	■			
	ENSG00000189134	NKAPL	118943390	■			
	ENSG00000177485	ZBTB33	119268635	■			

Stratum 1	ENSG00000125355	FAM70A	119276534	▨			
	ENSG00000101892	ATP1B4	119379995	▨			
	ENSG00000005893	LAMP2	119446367	▨			
	ENSG00000158290	CUL4B	119542476	▨			
	ENSG00000101898	MCTS1	119622049	▨			
	ENSG00000171155	C1GALT1C1	119643564	▨			
	ENSG00000125675	GRIA3	122145687	▨			
	ENSG00000125676	THOC2	122562094	▨			
	ENSG00000101966	XIAP	122821729	▨			
	ENSG00000180152	BIRC8	122868524	▨			
	ENSG00000101972	STAG2	122921743	▨			
	ENSG00000183918	SH2D1A	123307831	▨			
	ENSG00000009694	ODZ1	123339504	▨			
	ENSG00000198354	DCAF12L2	125126195		▨		
	ENSG00000198889	DCAF12L1	125511050		▨		
	ENSG00000183631	CXORF64	125781432				▨
	ENSG00000123165	ACTRT1	127012622				▨
	ENSG00000102038	SMARCA1	128408159	▨			
	ENSG00000122126	OCRL	128501933	▨			
	ENSG00000171388	APLN	128607007			▨	
	ENSG00000122121	XPNPEP2	128700627	▨			
	ENSG00000122122	SASH3	128741641	▨			
	ENSG00000188706	ZDHHC9	128766596	▨			
	ENSG00000156697	UTP14A	128866195	▨			
	ENSG00000214320	UTP14C	128881052	▨			
	ENSG00000085185	BCORL1	128944350	▨			
	ENSG00000102034	ELF4	129026539	▨			
	ENSG00000156709	AIFM1	129091018	▨			
	ENSG00000134594	RAB33A	129133454	▨			
	ENSG00000056277	ZNF280C	129164372				▨
	ENSG00000102078	SLC25A14	129301699	▨			
	ENSG00000147262	GPR119	129346095	▨			
	ENSG00000134597	RBMX2	129363624	▨			
	ENSG00000165675	ENOX2	129585031	▨			
	ENSG00000147256	ARHGAP36	130019897	▨			

Stratum 1	ENSG00000147255	IGSF1	130235161				■
	ENSG00000171054	OR13H1	130505729			■	
	ENSG00000134602	MST4	130984926	■			
	ENSG00000165694	FRMD7	131038702	■			
	ENSG00000123728	RAP2C	131164734	■			
	ENSG00000076770	MBNL3	131339862	■			
	ENSG00000171004	HS6ST2	131587719	■			
	ENSG00000134588	USP26	131986325				■
	ENSG00000183434	TFDP3	132178363	■			
	ENSG00000076716	GPC4	132262730	■			
	ENSG00000147257	GPC3	132497448	■			
	ENSG00000203952	CCDC160	133198743			■	
	ENSG00000156531	PHF6	133335008	■			
	ENSG00000165704	HPRT1	133421923	■			
	ENSG00000170965	PLAC1	133527539				■
	ENSG00000156504	FAM122B	133731262	■			
	ENSG00000156500	FAM122C	133768890	■			
	ENSG00000101928	MOSPD1	133849323	■			
	ENSG00000184785	LOC644538	133952634				■
	ENSG00000212747	LOC10012894	133982209				■
	ENSG00000203950	FAM127B	133983721				■
	ENSG00000134590	FAM127A	133993999				■
	ENSG00000169551	LOC650024	134118129	■			
	ENSG00000186376	ZNF75D	134247386	■			
	ENSG00000173275	ZNF449	134306387	■			
	ENSG00000165359	DDX26B	134482250	■			
	ENSG00000187267	CT45A1	134674851		■		
	ENSG00000203948	CT45A2	134693880		■		
	ENSG00000213444	CT45A3	134711154		■		
	ENSG00000203947	CT45A6	134756363		■		
	ENSG00000213441	CT45A5	134773630		■		
	ENSG00000203946	CT45A6	134790881		■		
	ENSG00000181433	SAGE1	134803451	■			
	ENSG00000169446	MMGT1	134871897	■			
	ENSG00000198689	SLC9A6	134895252	■			

Stratum 1	ENSG00000022267	FHL1	135057346	▨			
	ENSG00000129680	MAP7D3	135123047	▨			
	ENSG00000156920	GPR112	135210788	▨			
	ENSG00000102239	BRS3	135397791	▨			
	ENSG00000102241	HTATSF1	135407337	▨			
	ENSG00000102243	VGLL1	135441977	▨			
	ENSG00000102245	CD40LG	135558002	▨			
	ENSG00000129675	ARHGEF6	135575372	▨			
	ENSG00000147274	RBMX	135783288	▨			
	ENSG00000165370	GPR101	135939973		▨		
	ENSG00000156925	ZIC3	136476012	▨			
	ENSG00000129682	FGF13	137541401	▨			
	ENSG00000101981	F9	138440561	▨			
	ENSG00000101977	MCF2	138491596	▨			
	ENSG00000101974	ATP11C	138636171	▨			
	ENSG00000203933	CXORF66	138865550				▨
	ENSG00000134595	SOX3	139412818	▨			
	ENSG00000184258	CDR1	139692236				▨
	ENSG00000182195	LDOC1	140097596				▨
	ENSG00000165509	MAGEC3	140753768		▨		
	ENSG00000155495	MAGEC1	140819346		▨		
	ENSG00000046774	MAGEC2	141117797		▨		
	ENSG00000179542	SLITRK4	142543610	▨			
	ENSG00000102069	UBE2NL	142794839	▨			
	ENSG00000185985	LOC10012909	144707381	▨			
	ENSG00000221870	CXORF1	144716888			▨	
	ENSG00000102081	FMR1	146801173	▨			
	ENSG00000176988	FMR1NB	146870541				▨
	ENSG00000155966	AFF2	147389831	▨			
	ENSG00000010404	IDS	148368206	▨			
	ENSG00000176289	IDSP1	148414420	▨			
	ENSG00000197620	CXORF40A	148430463	▨			
	ENSG00000123584	MAGEA9B	148471105		▨		
	ENSG00000171129	HSFX2	148481974	▨			
	ENSG00000155984	TMEM185A	148486016	▨			

Stratum 1	ENSG00000185247	MAGEA11	148575479		■		
	ENSG00000171116	HSFX1	148664132	■			
	ENSG00000166008	MAGEA9B	148671395		■		
	ENSG00000156009	MAGEA8	148770653		■		
	ENSG00000197021	CXORF40A	148848949	■			
	ENSG00000013619	MAMLD1	149282209	■			
	ENSG00000171100	MTM1	149487727	■			
	ENSG00000063601	MTMR1	149612527	■			
	ENSG00000102181	CD99L2	149685470	■			
	ENSG00000029993	LOC729952	149902421	■			
	ENSG00000102195	GPR50	150095717	■			
	ENSG00000160131	VMA21	150315696	■			
	ENSG00000166049	PASD1	150482663	■			
	ENSG00000130032	PRRG3	150614607	■			
	ENSG00000147378	FATE1	150635164				■
	ENSG00000183862	CNGA2	150653874	■			
	ENSG00000147381	MAGEA4	150831652				
	ENSG00000102287	GABRE	150872253	■			
	ENSG00000183686	MAGEA5	151033182				■
	ENSG00000124260	MAGEA10	151053566				
	ENSG00000011677	GABRA3	151086290	■			
	ENSG00000147402	GABRQ	151557293	■			
	ENSG00000221867	MAGEA6	151617901		■		
	ENSG00000183305	MAGEA2B	151633746		■		
	ENSG00000197172	MAGEA12	151649952		■		
	ENSG00000184750	MAGEA2B	151669043		■		
	ENSG00000213401	MAGEA3	151685309		■		
	ENSG00000147400	CETN2	151746529	■			
	ENSG00000177143	CETN1	151748963	■			
	ENSG00000147383	NSDHL	151750167	■			
	ENSG00000147394	ZNF185	151833653	■			
	ENSG00000198883	PNMA5	151908025		■		
	ENSG00000183837	PNMA3	151975530		■		
	ENSG00000198013	PNMA6A	151991521		■		
	ENSG00000203902	PNMA6A	151994652		■		

Stratum 1	ENSG00000198681	MAGEA1	152134716		■		
	ENSG00000063587	ZNF275	152252807	■			
	ENSG00000189420	ZFP92	152336975	■			
	ENSG00000183479	TREX2	152363372				
	ENSG00000213397	TREX2	152366318				
	ENSG00000182492	BGN	152413605				■
	ENSG00000067842	ATP2B3	152436328	■			
	ENSG00000147382	LOC401218	152506579				
	ENSG00000130829	DUSP9	152561182	■			
	ENSG00000130822	PNCK	152588379	■			
	ENSG00000130821	SLC6A8	152606586	■			
	ENSG00000185825	BCAP31	152619146	■			
	ENSG00000101986	ABCD1	152643517	■			
	ENSG00000198753	PLXNB3	152682845	■			
	ENSG00000184343	SRPK3	152699656	■			
	ENSG00000067829	IDH3G	152704416			■	
	ENSG00000180879	SSR4	152712165				
	ENSG00000067840	PDZD4	152720817	■			
	ENSG00000198910	L1CAM	152780163	■			
	ENSG00000126895	AVPR2	152821179	■			
	ENSG00000089820	ARHGAP4	152826015	■			
	ENSG00000102030	NAA10	152848561				
	ENSG00000102032	RENBP	152853910				
	ENSG00000172534	HCFC1	152866204	■			
	ENSG00000177854	TMEM187	152891185				
	ENSG00000184216	IRAK1	152929154				
	ENSG00000169057	MECP2	152940218				
	ENSG00000102076	OPN1LW	153062939			■	
	ENSG00000102080	TEX28	153077878				
	ENSG00000182242	TEX28	153115008				
	ENSG00000185254	TEX28	153152124				
	ENSG00000007350	TKTL1	153177345			■	
	ENSG00000196924	FLNA	153230091				■
	ENSG00000102119	EMD	153260917				■
	ENSG00000147403	RPL10P6	153278689		■		

Stratum 1	ENSG00000013563	DNASE1L1	153282773		■		
	ENSG00000102125	TAZ	153293071		■		
	ENSG00000071553	ATP6AP1	153310172	■			
	ENSG00000203879	GDI1	153318705	■			
	ENSG00000071859	FAM50A	153325698	■			
	ENSG00000130827	PLXNA3	153339817	■			
	ENSG00000196976	LAGE3	153358435				■
	ENSG00000102178	UBL4A	153365254		■		
	ENSG00000126903	SLC10A3	153368842		■		
	ENSG00000071889	FAM3A	153387696	■			
	ENSG00000160211	G6PD	153412800		■		
	ENSG00000073009	IKBKG	153423672		■		
	ENSG00000160219	GAB3	153556725	■			
	ENSG00000130826	DKC1	153637340	■			
	ENSG00000100721	TCL1A	153646932	■			
	ENSG00000130830	MPP1	153660162	■			
	ENSG00000185010	F8	153717257	■			
	ENSG00000198082	H2AFB1	153766511	■			
	ENSG00000197932	F8A1	153767829			■	
	ENSG00000165775	FUNDC2	153908258	■			
	ENSG00000165775	FUNDC2	153938384	■			
	ENSG00000182712	MTCP1	153943091	■			
	ENSG00000214827	MTCP1	153943098	■			
	ENSG00000185515	BRCC3	153952889	■			
	ENSG00000155959	VBP1	154097744	■			
	ENSG00000155961	RAB39B	154140721	■			
	ENSG00000155962	CLIC2	154158720	■			
	ENSG00000198307	H2AFB2	154263622	■			
	ENSG00000185978	H2AFB2	154342270	■			
	ENSG00000185973	TMLHE	154372967	■			
Pseudo-autosomal region 2	ENSG00000168939	SPRY3	154650645	■			
	ENSG00000124333	VAMP7	154764207	■			
	ENSG00000182484	WASH6P	154903161	■			

Supplementary Table 5. Copy numbers of rhesus, human, and chimpanzee MSY genes

			Copy number of gene in indicated species		
Origin	Stratum	Gene	Rhesus	Human[1]	Chimpanzee[2]
Ancestral	1	*SRY*	1	1	1
		RBMY	1	6	6
		RPS4Y1	1	1	1
		RPS4Y2	1	1	1
		HSFY	3	2	0
	2	*KDM5D*	1	1	1
		TSPY	5	35	6
	3	*ZFY*	1	1	1
		DDX3Y	1	1	1
		UTY	1	1	1
		EIF1AY	1	1	1
		CYorf15A	1	1	1
		CYorf15B	1	1	0
		USP9Y	1	1	0
		TMSB4Y	1	1	0
	4	*AMELY*	1	1	1
		NLGN4Y	1	1	1
		TBL1Y	1	1	0
	5	*PRKY*	1	1	1
		MXRA5Y	1	0	0
		Total:	**26**	**59**	**24**
Added		*DAZ*	2	4	4
		CDY	2	4	5
		XKRY	1	2	0
		VCY	0	2	2
		BPY2	0	3	2
		PRY	0	2	0
		PCDH11Y	0	1	0
		TGIF2LY	0	1	0
		Total:	**5**	**19**	**13**
		Grand Total:	**31**	**78**	**37**

[1] Gene numbers for human from Skaletsky *et al*., 2003, Nature 423:825-37

[2] Gene numbers for chimpanzee from Hughes, *et al*. 2010, Nature 463:536-9

Supplementary Table 6. Parameters of one-phase exponential decay models of gene numbers in MSY strata

Stratum	N_0	K (my^{-1})	Half-life (my)	Baseline	R squared
1	414	0.144	4.8	5	1.00
2	88	0.288	2.4	2	1.00
3	143	0.433	1.6	8	1.00
4	12	2.020	0.34	3	1.00
5	7	0.325	2.1	1	1.00

N_0	Starting number of genes
K	Decay constant
my	Millions of years

Supplementary Table 7. PCR primers for radiation hybrid mapping

Marker	BAC location of marker	Product size (bp)	Left primer	Right primer
0671	CH250-57C20	200	CATGAATGTTACTGAATCTTCAACG	GGAATTAGAAGTGCCTGCGA
0829	CH250-56H16	253	TCTGAACAAAGGACTGACAGGA	CTTGAGGCTTGCAACTTGAT
1030	CH250-364B23	327	CTTTGTCCCTCACTGGTGGT	AGTCAAGGGGTGTTGGACTG
1060	CH250-198P20	337	GCTCTCAGTGACTGCTTCCA	TCTGCACATGTACCCCAGAA
1121	CH250-374B3	325	GCCATTGTCAAGAGAGCACA	ACACTTGCTGTCAGGGGAAA
1258	CH250-209A4	341	CTGTGAGCAGTTGTAGGCCA	ACTTTCTCCCGCTTTCCTTC
0830	CH250-517K1	345	GGAATTCTCCAGCAAACACG	CCCACATCTCTCTCTCACACC
1291	CH250-62E18	263	CCTTCCAGGGAGAGATTTCA	AGGGGGAGTTGTAACCAAGG
1264	CH250-249M17	311	TTTGGATGCAGTGTTTGGAA	TCAAACCACAACATGTGCAG

Supplementary Table 8. Primers for RT-PCR analysis of rhesus MSY genes

Gene	Product size (bp)	Left primer	Right primer
CDY	736	AACAACACGGATCCAAAAGC	TCAAGACCGCAACAAAACAC
CYorf15A	595	GAAGTAGGGAAAGAGGTAATACCCA	CAAAGGCTGGTAGGGTTTCC
CYorf15B	495	TGCTTCTAGGCTACTTTTTCTCC	TTCTCTTCAGCCATTTGCAG
DAZ1	782	CCAAATCCACCAGGTGCA	CAGATTTCCCTTTTGCTCCC
DAZ2	677	GGTCCGCTTTTAGAGGCTCT	GTTATAGGATTCAGCGCGACTC
DDX3Y	661	GCCACCTCATTCTTTAATGAAAAA	CGAACGATCCTCAGTCACAA
EIF1AY	690	TGAGGCATCTTAGGTCAGCC	GGGTCATCAGCTTCCAAAGA
HSFY1/2	687	CCACCTTTCTGGCTGAAGAG	GCCTGGAAAGAGGGCTAGAT
HSFY3	604	TGAAGATTTCAACAGGAAGCA	TGGTACTCTTTTAATGGAAGATCAG
JARID1D	617	CACTGACACCATCCCTCATG	CGGCAACAGTGAGGACAGTA
MXRA5Y	827	TGAGCACAAGGAGGAGAGCA	GCGCCATGCATACTAGCTCA
NLGN4Y	739	GTACCCGCCGATCTCCTG	TGCCAATGAGAAGTGTCTGC
PRKY	988	AAGCTCACGGACTTTGGATTT	TGTTGTCCCCAAGAGAAGAAAA
RBMY	666	TGCATACGACCTGAGGACTG	GAAGAGGTCGGCTCTCTGTG
RPS4Y1	963	TTCCAGGCTGTGAACTCTATAGC	GGCAAAGAAAAAAGATACACTGC
RPS4Y2	917	CGGATTACGACCACAGAGTACG	GAAACAGGAATTCACTGTTTATTTGT
TBL1Y	704	TGTTGAAATTCCACCCAACA	CCTGAAGATTGTGAACGCAT
TMSB4Y	665	ATTCGCAGTGGTCAATTTCC	ATTTCACTGCCATCCCACTC
TSPY	458	GCAAACCACCCTCAGATGTC	TCAGCTCTGATCACTCCATCA
USP9Y	675	TCCCTCATCCGAGTGCATA	AGGTGGCCTTCCAGTATCAG
UTY	559	CAATTAGGTCACTTCAACCTATTGC	CAGAATTAGGATCTGCCTCCAGT
XKRY	333	TGTGTACCATTGTTGGCACC	GATGAGCTGCATGGCAATTA
ZFY	572	ATCATTACTGATCCTCTGACCAGT	ACGAAGGTTTTCATTTCATCC