Supplemental Note

Hominoid fission of chromosome 14/15 and role of segmental duplications

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1. Analysis of the macaque contig spanning the hominoid 14/15 fission site

We grouped contig clones based on their FISH pattern on human and macaque chromosomes (Groups F1, F2, F3, G1, G2, G3, and G4). Group F2 clones (Hsa15b- and Hsa15c-positive) showed a single signal on macaque 7q and three signal clusters on human chromosome 15: at the orthologous 15q26, as expected, as well as at 15q11–14 and 15q24–25, which correspond to the actual and ancestral pericentromeric regions, respectively (Ventura et al. 2003). Indeed, this locus contains an LCR15 copy (Pujana et al. 2001) in both the macaque and human genomes. Most BAC clones were one-end anchored in the human genome (chr15:100,028–100,071 kb). In macaque this region experienced a 64 kb duplicative insertion from chromosome 17 (orthologous to human chromosome 13) (**Figure 2**), with the human configuration (absence of insertion) likely being the ancestral state because it is identical in orangutan and marmoset. Four Hsa15b-positive clones mapped on macaque and human chromosome 19, but neither human nor macaque assemblies report the STS Hsa15b duplicated at this locus. The presence of assembly gaps in macaque may explain why the STS is not annotated in this region. We aligned the sequence of macaque CH250-70H12 (AC187495.2) versus its human orthologous sequence (hg18 chr15:100,039k-100,170k) and found a 12 kb human expansion through tandem duplication of a ~100 bp unit corresponding to a portion of exon 20 of *DNM1* (**Figure S3**). We detected the expansion in the gorilla and chimpanzee orthologous sequences, whereas no expansion was found in the orangutan sequence.

The 15q most terminal segment (Group F3, STS 177K24sp6- and Hsa15d-positive) is duplicated to the subterminal regions of other chromosome arms (1p, 3q, 5q, 6p, 6q, 8p, 9q, 11p, and 19p) preserving the same centromere-telomere orientation except for 1p (UCSC Chained Alignments, http://genome.ucsc.edu/). In the human genome, most clones had one end mapping at chr15:100.2 Mb and the other scoring no similarity due to the proximity to the fission point. The subsequent clones (STS 197J20t7 and 265J10t7) showed a single signal on MMU7 and an absent or faint signal on human 14q11 and corresponded to the evolutionary fission point as confirmed by BAC-end mapping (one-end anchored on chromosome 14:19.6 Mb).

We continued the contig in the macaque sequence orthologous to human 14q pericentromeric region, showing a hominoid-specific inversion of the first 400 kb (based on the comparison with marmoset, orangutan, and chimpanzee genomes). In human, the first segment (Group G2, STS from 250G8sp6 to 250G8t7) was duplicated to 18p11 and

21q11 whereas the next one (Group G3, STS from 279G5t7 to 237P11sp6) was duplicated to 15q11. We dated all duplication events (14-15, 14-18, and 14-21) prior to human-orangutan divergence (**section "Duplication events dating"**).

We obtained an average of 16 positive clones using the STS from 98G3t7 to Hsa14c, which provides evidence of duplication of this region in macaque. We distinguished the clones into two groups based on diagnostic sequence differences (**Figure 2**). Using Illumina technology, we sequenced five BAC clones (CH250-152K7, CH250-213D20, CH250-240C18, CH250-265P9, and CH250-279G5) representing the two copies and detected locations on the macaque and human genomes consistent with the end-based placements (**Table S2**). We detected the same orientation of human and macaque genomes and an absence of duplication in both genomes when we used the STS 21D8sp6 (Group G4).

According to the RefSeq gene annotation, SDs from the subtelomeric region of chromosome 15 generated *OR4F29*, *OR4F16*, and *OR4F3* on chromosomes 1 and 5; *OR4F21* on chromosome 8; *OR4F5* on chromosome 1; and *OR4F17* on chromosome 19. SDs from the pericentromeric region of chromosome 14 generated *OR4N3P*, *OR4N4*, and *OR4M2* on the pericentromeric region of chromosome 15.

Macaque clone CH250-22K1 was positive to two probes (197J20sp6 and 271H18sp6) but did not show PCR amplification product when used as a template with the same primers. This clone showed a tandem duplicated signal on macaque chromosome 10p and no signal on human chromosome metaphases, thus we considered it a false positive. The sp6 end sequence was repetitive while the t7 end sequence had several matches in the human genome mainly at chr2:130–132 Mb, chr8:48 and 43 Mb, chr14:19 Mb, and chr22:14 Mb, and match in the rhesus genome at chr8:44Mb (orthologous to human chromosome 8) and chr10:59 Mb, the transition region from human chromosome 20 orthologous region to the centromere. We noted the t7 sequence aligns with intronic fragments of *POTE* genes.

We identified a macaque-specific duplication in tandem (haploid copy = 2) at chr7:82.7–82.8 Mb. Sequence data show a high degree of sequence identity (99.85%) between the duplicated blocks possibly due to genomic conversion and/or the recent origin of the duplication event in the macaque lineage. Read-depth analysis predicts the entire region of MMU7 as single copy, in contrast to library coverage, sequencing data, and FISH pattern, which all support the duplication. As previously observed by Alkan et al. (Alkan et al. 2009), since the Sanger read-based WSSD method uses a three standard deviation threshold that corresponds to a diploid copy number of ~3.5, some truly duplicated segments may fall below the threshold. In this regard, more recent depth-of-coverage analyses using next-generation sequencing reads predicted the absolute copy number of duplicated sequences using the ratio of their read depth and the read depth calculated in unique loci (Alkan et al. 2009).

2. Duplication events dating

We compared the pairwise identity between the sequences on chromosome 14 (the ancestral ones) and duplicated sequences on chromosomes 15, 18, and 21 (the derivative ones) with estimates of the genetic distance between human and chimpanzee, gorilla, orangutan, and baboon, respectively (Elango et al. 2006), to date back the duplication

events in the evolutionary time. Since we compared SDs in the human genome, we used estimates of the genetic distance calculated for the human lineage (5.4, 6.47, 14.09, and 29.02 subst/site/10^3, for human-chimpanzee, human-gorilla, human-orangutan, and human-baboon, respectively) and doubled them to consider branches of both duplications. Based on these values, we assigned whether the duplication event occurred in the human lineage after divergence from chimpanzee or in the human-chimpanzee, human-chimpanzee-gorilla, hominoid, or Catarrhini common ancestor.

3. Study of LCR15

The F2 locus has an LCR15 copy in both macaque and human genomes, indicating the copy was already present in their common ancestor. Zody and colleagues showed that the LCR15 family consists of a 15 kb combined unit of *GOLGA2* (golgin A2) and *ITSN2* (intersectin 2) UTR (untranslated region), whose ancestral loci map to human 9q34 and 2p23.3, respectively (Zody et al. 2006). We noted that the *GOLGA2* module of LCR15 duplications comprises part of *DNM1* (dynamin 1) (Makrinou et al. 2004), and *GOLGA2* and *DNM1* ancestral loci are associated at 9q34. Therefore, two genomic segments, *DNM1-GOLGA2* and *ITSN2*, duplicated and formed the *ITSN2/DNM1-GOLGA2* ancestral duplication, then expanded in the human genome mainly on chromosome 15 (**Figure 3**), with *GOLGA* copies on chromosome 15 named *GOLGA6* and *GOLGA8*.

Through a BLAT search (Kent 2002) on chromosome 15, we identified the genomic locations of *ITSN2*, *DNM1*, and *GOLGA2* duplications and found that only a few LCR15 lacked in one of the three components. We performed three-color FISH experiments on human, macaque, and marmoset chromosome metaphases: ten co-hybridizations of human RP11 BAC clones and one co-hybridization of macaque CH250 BAC clones retrieved from the contig. We chose the BAC triplets as follows: one BAC (in red) spanning the LCRs and the other two at the single-copy regions immediately upstream (in green) and downstream (in blue) of the duplication cluster (**Figure 3**). The ten RP11 triplets were designed at 23, 28, 30, 32, 34, 75, 78, 79, 82, and 84 Mb. We used a CH250 triplet for LCR15 on 15q26.3 because none of the RP11 clones map downstream of the duplication. Since BAC clones spanning the duplication showed signals at several loci, we considered only signals co-mapping with signals of single-copy clones.

The LCR15 duplication cluster at locus A (15q11.2) does not contain the *DNM1* module and the corresponding BAC clone showed FISH signal in macaque and marmoset besides a tandem duplicated pattern in human, where it is part of the pericentromeric region (**Figure 1**). BAC clones designed in single-copy segments of loci AB and F hybridized to marmoset chromosomes 6 and 10, thus these loci correspond to the Catarrhini inversion breakpoints (**Figures 1 and 3**).

BAC clones at loci D1, D2, DE1, and DE2 (15q24-q25) mapped to MMU7 and marmoset chromosome 6 and 10 pericentromeric regions. At locus DE1, BAC clones selected in single-copy regions mapped to marmoset chromosomes 10 and 6, respectively, whereas the middle probe at LCR15 showed signal on both chromosomes, suggesting that locus DE1 corresponds to the fission breakpoint in the marmoset lineage (**Figures 1 and 3**). Single-copy segments at locus DE2 pinpoint pericentromeric sequences of MMU7 since BAC clones hybridized to the macaque 7p and 7q pericentromeric regions, respectively.

We developed a phylogenetic analysis of the same duplications collecting human, chimpanzee, gorilla, orangutan, macaque, and marmoset *GOLGA2* and *ITSN2* sequences through a BLAT search on the reference genomes (hg19, panTro3, gorGor3, ponAbe2, rheMac2, and calJac3) using as a query a 2.5 kb segment of the *GOLGA2* module and a 3.3 kb segment of the *ITSN2* module from the LCR15 copy at 15q26.3, proximal to the fission breakpoint. We retrieved human sequences from the most recent hg19 release because, here, unlike the previous hg18 (used in other analyses of this work), all human LCR15 duplications are assigned and placed on chromosome 15. We aligned the sequences and built neighbor-joining phylogenetic trees (**Figure 4**). As previously shown (Zody et al. 2006), *GOLGA2* and *ITSN2* ancestral loci map on chromosomes 9q34.11 and 2p23.3, respectively.

4. Copy number data in humans and apes

We retrieved copy number data in humans (Sudmant et al. 2010) for the 15q subterminal and 14q pericentromeric regions orthologous to the ancestral fission site together with the newly acquired 14q pericentromeric region (**Figure S4**). As already shown by the FISH pattern of macaque BAC clones on human chromosome metaphases, the region is single copy (copy number equal to 2, because the diploid genome is considered) at its extremes. On the chromosome 15 side, there are two segments with a copy number greater than 2: the *ITSN2*-*GOLGA2* segment and the most terminal one, which is duplicated on the terminal portions of other chromosomes. On the chromosome 14 side, the newly minted pericentromeric region is made up of highly duplicated sequences. The first portion derived from the ancestral submetacentric chromosome is duplicated with a copy number around 3–4, in agreement with the presence of a 130 kb segment (chr14:19.2–19.4 Mb, Group G3 of the contig) duplicated to 15q11.2. This region is a copy number variant not stratified in human populations. CH250-98G3 showed interchromosomal duplication on chromosomes 14 and 15 in chimpanzee and gorilla but not in orangutan. Since we dated the duplication event in the hominoid common branch, the duplication was lost in the orangutan genome. The weak signals on chromosomes and nuclei along with the low coverage of sequence reads revealed a partial deletion for this genomic region in chimpanzee. In the orangutan lineage, intrachromosomal and tandem duplications expanded the same region, as shown by both FISH signals and depth-of-coverage data (**Figure S5**). Copy number data in apes confirm this region is deleted in chimpanzees and bonobos and is duplicated in orangutan (copy number equal to 4 or 5); in gorilla most of the region has a copy number equal to 2 or 3 (Sudmant et al. 2013).

5. Additional note about human chromosome 14 newly minted pericentromeric region

In order to investigate the rearrangements that shaped the 14q pericentromeric region, we screened the macaque CH250 high-density filters using three STS at chr14:19 Mb (hg18)—Hsa14d (19239 kb), Hsa14e (19210 kb), and Hsa14f (19180 kb). The number of positive clones in each screening suggested duplication of these STS in the macaque genome (**Table S3**). BAC clones of Groups II, III, and IV mapped to 14q11, as expected, whereas those of Groups I, V, and VI showed a secondary and faint signal or no signal at this cytogenetic band.

In particular, clones of Groups I–III mapped to macaque 12p and human 2q21, both euchromatic regions orthologous to an ancestral pericentromeric region (Yunis and Prakash 1982; Wienberg et al. 1994; Roberto et al. 2008), as well as to the pericentromeric regions of macaque 9q (orthologous to human chromosome 10) and human 14q. Clones of Group III further hybridized to macaque 16p (orthologous to human 17p) as a hemizygous signal. In addition, CH250-21M16 (Group III) revealed faint signals at the pericentromeric and subterminal regions of other macaque and human chromosomes. Clone end placements were concordant, inconsistent, or one-end anchored at macaque chr13:136 Mb along with human orthologous chr2:130–131 Mb. Using Illumina technology, we sequenced CH250-82A10 (Group I) and CH250-2F9 (Group II) and confirmed their mapping at macaque chr13:136.2–136.4 Mb (rheMac2). (Note: The discrepancy between cytogenetic and sequence mappings in macaque derives from an annotation error in the rheMac2 reference for chromosomes 12 and 13, orthologous to ape 2B (human 2q) and ape 2A (human 2p-2q), respectively (Roberto et al. 2008).) These clones showed in all three apes (orangutan, gorilla, and chimpanzee) a pericentromeric signal on chromosome 2B (orthologous to macaque 12p and human 2q21) and, in some cases, additional faint pericentromeric signals on other chromosomes. Only in chimpanzee did these clones show a strong or faint signal on chromosome 14 as in human metaphases. The clone of Group III further hybridized to the pericentromeric region of 10q only in orangutan, as observed in macaque.

Notably, Group IV clones (n = 16) mapped to nonorthologous chromosomes in macaque and human. These clones showed a tandem duplicated signal at macaque 9q pericentromeric region, some having additional faint signals on 12p and 10q (orthologous to human chromosome 20), and 16p in a hemizygous state. On human metaphases we observed major signals at 2q21 and 14q11, along with minor signals at 15q11, 18p, 21q11, and 22q11 in some cases. Of note, no signal was detected at the pericentromeric region of human chromosome 10. The sp6 and t7 end sequences had inconsistent or no homologous placement on both the macaque and human genome references, suggesting that the macaque 9q pericentromeric sequence is species-specific and incomplete in the macaque reference. This finding was confirmed by the Illumina sequencing of two clones (CH250-48F1 and CH250-34C6). The inserts could not be assigned to any location on rheMac2 and, in particular, no sequence reads matched the pericentromeric region of chromosome 9. CH250-48F1 showed pericentromeric FISH-signals in ape chromosomes as well (**Figure S7 and Table S3**). However, the clone hybridized only in orangutan to the ortholog of macaque chromosome 9; in all apes a main signal was on chromosome 2B (orthologous to human 2q21). We hybridized this clone on marmoset chromosome metaphases and observed no signal.

In contrast to the previous groups, Group V comprises BAC clones mapping to a nonpericentromeric region, i.e., macaque 9q (57.1–57.5 Mb, rheMac2) and human orthologous 10q22.3 (80.9–82.1 Mb, hg18). In human we further observed weak signals at 2q21 and 14q11. Finally, Group VI clones (n = 8) mapped at a different pericentromeric region: the orthologous macaque 11p (29 Mb, rheMac2) and human 12p11 (29 Mb, hg18).

As a second approach to analyze human 14q pericentromeric sequence acquired after the fission event, we analyzed the 1.3 Mb assembled sequence (chr14:18070000-19252885, hg18) at this position and found a 400 kb segment showing an inverted duplication (**Figure S6A**). We designed a tiling path of eight human RP11 BAC clones spanning this region and compared their FISH pattern in human, orangutan, and macaque (**Figure S8**). In human, all clones showed a tandem duplicated signal at 14q11 and a strong or weak

signal at 22q11. Clones had an additional faint signal at 2q21. In orangutan all clones showed pericentromeric signal on chromosome 2B; additional pericentromeric signals were observed, mainly on chromosomes 14, 15, and 21, and in two cases at 10q.

We retrieved SD data (Bailey et al. 2001; Bailey et al. 2002) for this region from the UCSC Genome Browser and analyzed the underlying 245 pairwise alignments (**Table 1**). We classified them according to the chromosomal location of the duplicate copy, further distinguishing those mapping to human (13q11, 14q11, 15q11, 21q11, and 22q11) and ancestral hominoid (2q21, 9p11, and 18p11) q arm pericentromeric regions of acrocentric chromosomes versus those mapping to other chromosomal locations. (Note: Chromosomes 13 (Cardone et al. 2006), 21, and 22 (Yunis and Prakash 1982) have been acrocentric chromosomes for the last 25 million years; chromosomes 2A, 2B, 9, 11, and 18 were acrocentric in the hominoid ancestor; within the human lineage chromosomes 2B and 11 are no longer acrocentric in the African ape ancestral branch (Yunis and Prakash 1982; Wienberg et al. 1994; Roberto et al. 2008; Sudmant et al. 2013); chromosomes 2A (Yunis and Prakash 1982; Wienberg et al. 1994; Roberto et al. 2008) and 9 (Montefalcone et al. 1999) are no longer acrocentric in the human-chimpanzee common branch; and chromosome 18 is no longer acrocentric in the human lineage due to a pericentric inversion that occurred after divergence of chimpanzee from human (Dennehey et al. 2004).)

6. Illumina sequencing of BAC clones

To isolate BAC clone DNA, a cell pellet was resuspended in 250 µL Qiagen buffer P1 with RNAse and lysed with 250 µL of NaOH 0.2 M/ SDS 1% solution for 5 min. Lysis was neutralized with 250 µL of NaOAc 3 M, pH 4.8. Neutralized lysate was incubated on ice for 40 min, collected by centrifugation for 15 min at 13,000 rpm and 4°C, concentrated by standard ethanol precipitation, and resuspended in 50 µL of Tris-Cl pH 8.5 10 mM. Libraries were prepared from BAC clone DNA using Illumina-compatible Nextera DNA sample prep kits (Epicentre, Cat. No. GA09115). The manufacturer's protocol was followed with modifications, including a set of barcoded oligos as described by Adey *et al.* (Adey et al. 2010). Barcoded libraries were combined for size selection in 6% pre-cast polyacrylamide gels (Invitrogen, Cat. No. EC6265BOX). The band spanning 500-650 bp was excised, diced, incubated in Tris-Cl pH 8.5 10 mM at 65°C for 2 hr, purified through a Nanosep MF 0.2 µm centrifugal filter (Pall, Cat. No. ODM02C33), and amplified via limited-cycle PCR with iProof High-Fidelity polymerase (BioRad) with the following program: initial denaturation at 98°C for 30 sec, followed by 6–12 cycles of denaturation at 98°C for 10 sec, annealing at 64°C for 30 sec, and extension at 72°C for 40 sec. Amplified, size-selected libraries were then purified with QIAquick PCR Purification Kit (Cat. No. 28104), quantified on an Invitrogen Qubit fluorometer, and paired-end sequenced (101 bp reads) on an Illumina HiSeq 2000.

Supplemental Figures

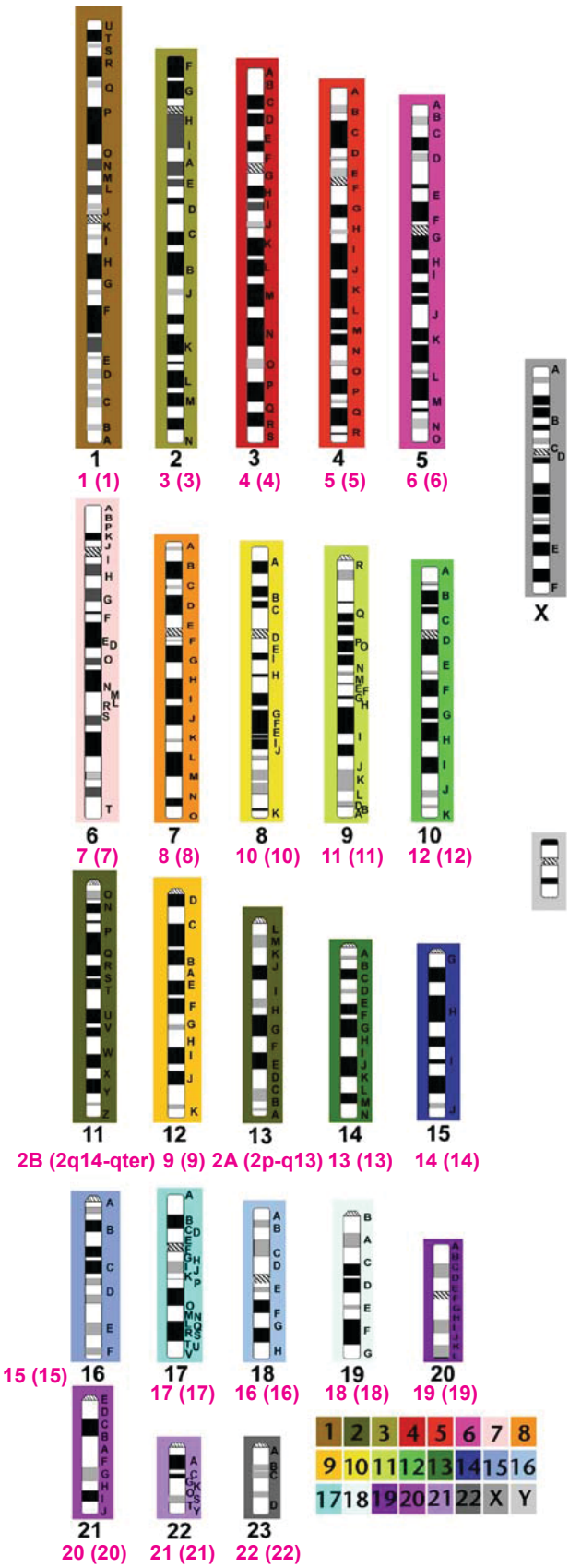

Figure S1: Idiogram of the ancestral hominoid karyotype, 2n = 48. Figure modified from Stanyon R, Rocchi M, Capozzi O, Roberto R, Misceo D, Ventura M, Cardone MF, Bigoni F, Archidiacono N. 2008. Primate chromosome evolution: ancestral karyotypes, marker order and neocentromeres. *Springer Chromosome Res* 16(1): 17-39. Figure 9. Idiogram of the ancestral hominoid karyotype (AHK), 2n = 48. The hypothesis is that this karyotype was present in the last common ancestor of all hominoids (lesser apes, great apes and humans). Chromosomes are arranged according to size and numbered below. Chromosomes are colour coded (USC standard colours) in reference to human chromosome syntenies. Marker order is in reference to the order in humans (p to q) and is indicated by letters to the right. BAC hybridizations between letters in the idiogram are considered to be collinear with the human karyotype (see Supplementary Tables for index to BACs). *With kind permission from Springer Science and Business Media*. The chromosome nomenclature used in the text, as proposed by (McConkey 2004), with the nomenclature of orthologous human chromosomes in brackets is added in magenta.

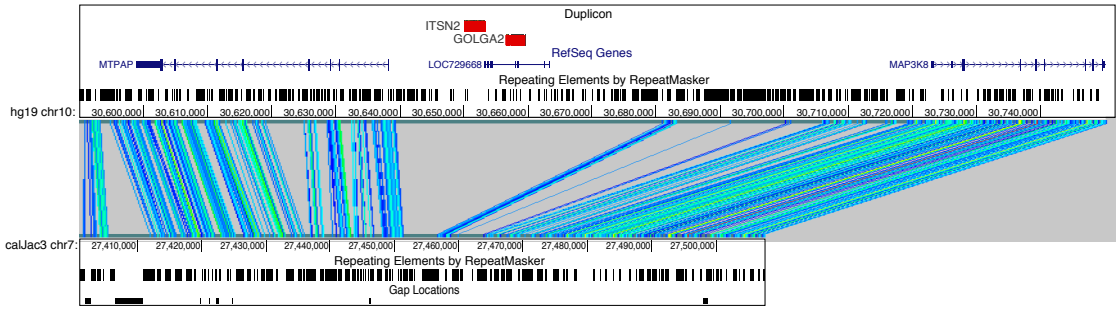

Figure S2: Sequence comparison of hg19_chr10:30590000-30750000 versus calJac3_chr7:27401125-27508054. All gaps in the marmoset sequence are bridged. The comparison shows the absence of the *ITSN2-GOLGA2* duplication on chr7 in the marmoset genome, orthologous to human chromosome 10, suggesting the duplication on chromosome 10 happened in the Catarrhini ancestor.

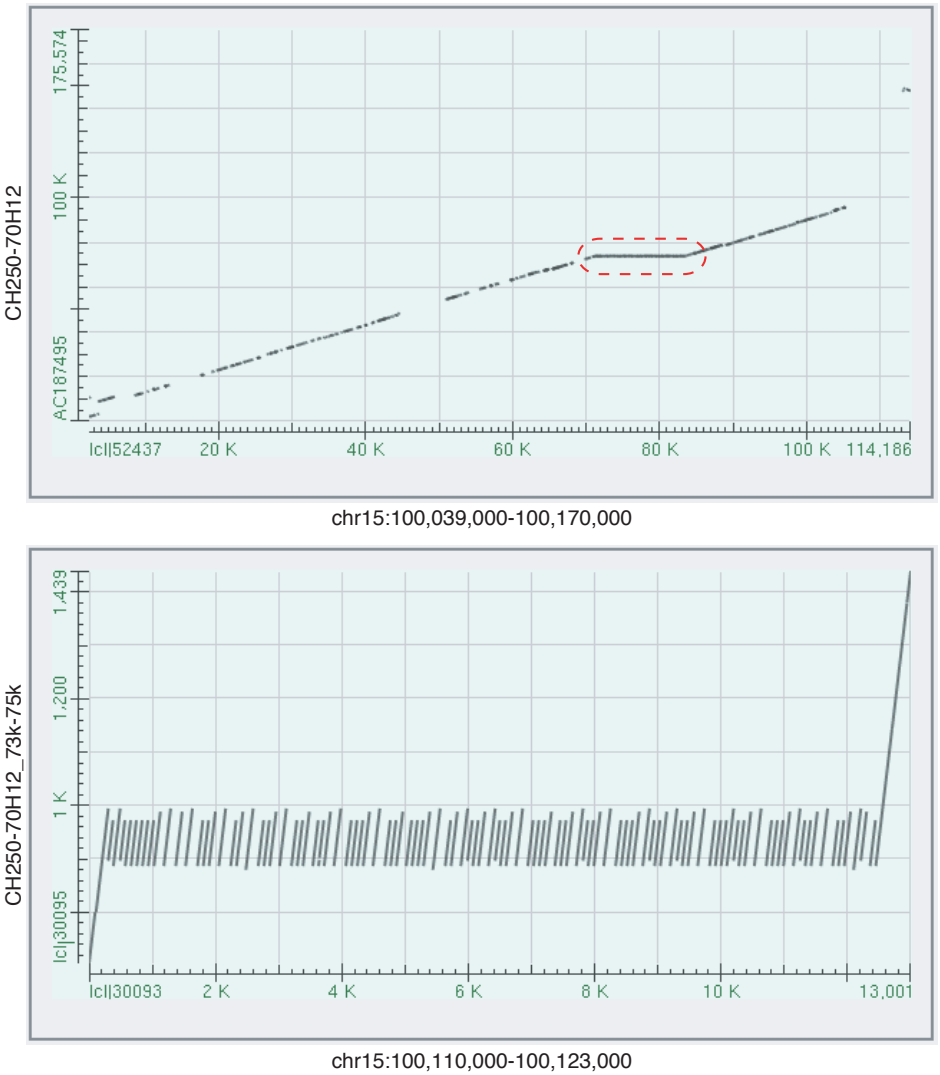

Figure S3: Comparison of CH250-70H12 and its human orthologous region. (*top*) Sequence comparison of the whole CH250-70H12 sequence (AC187495.2) and chr15:100,039,000-100,170,000 (hg18) using discontiguous megablast (Altschul et al. 1990). (*bottom*) Details of part of the alignment (red circle), showing the expansion of a ~100 bp unit, are illustrated.

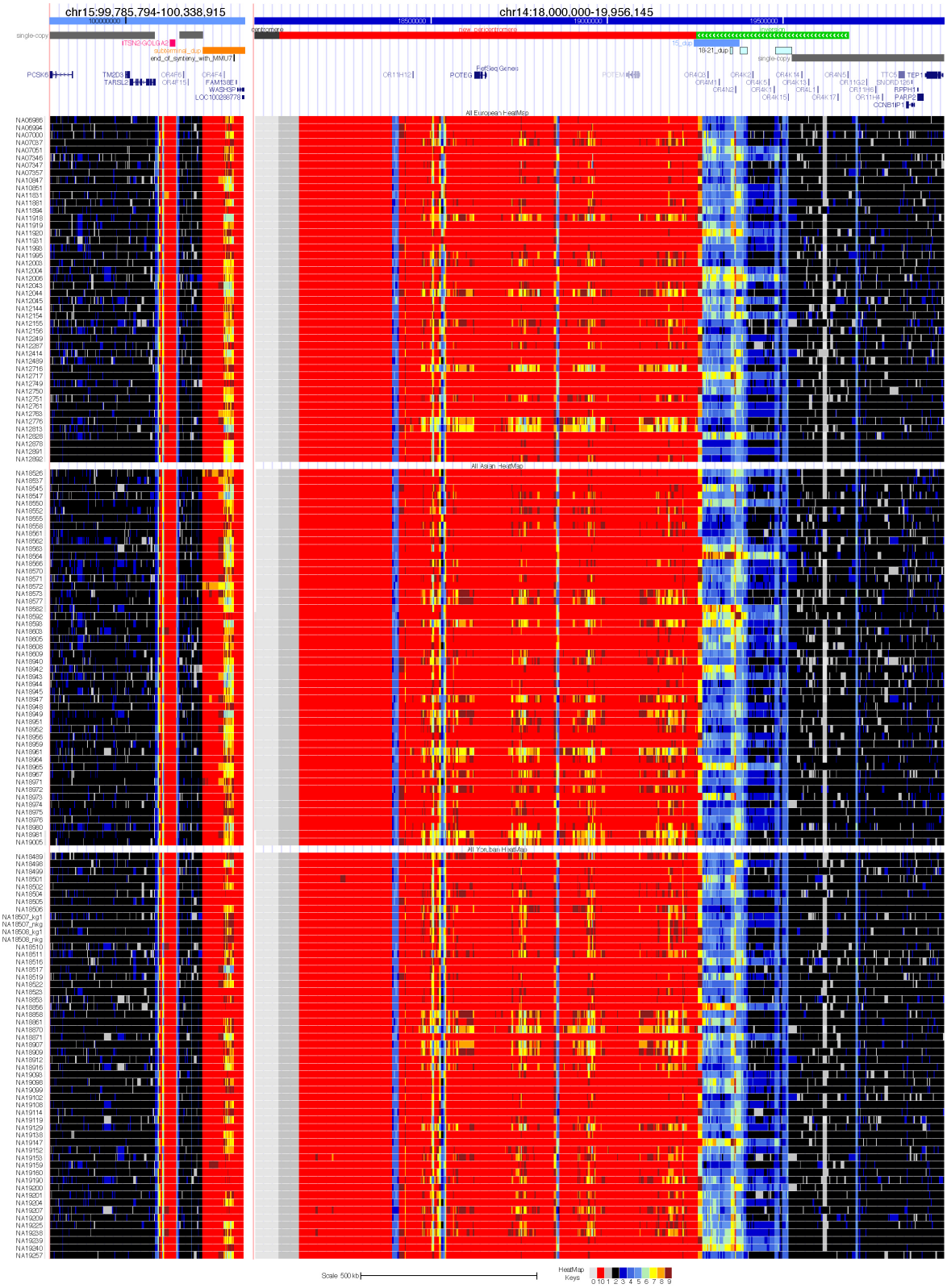

Figure S4: Copy number data in human populations for orthologous MMU7 region analyzed. Single-copy regions (gray), duplications (*ITSN2-GOLGA2* in pink, subterminal in orange, 14-15 in light blue, 14-18-21 in aqua), inversion in respect to the macaque (green), novel chromosome 14 pericentromeric sequences (red), end of synteny with MMU7 on chromosome 15, and RefSeq genes are indicated.

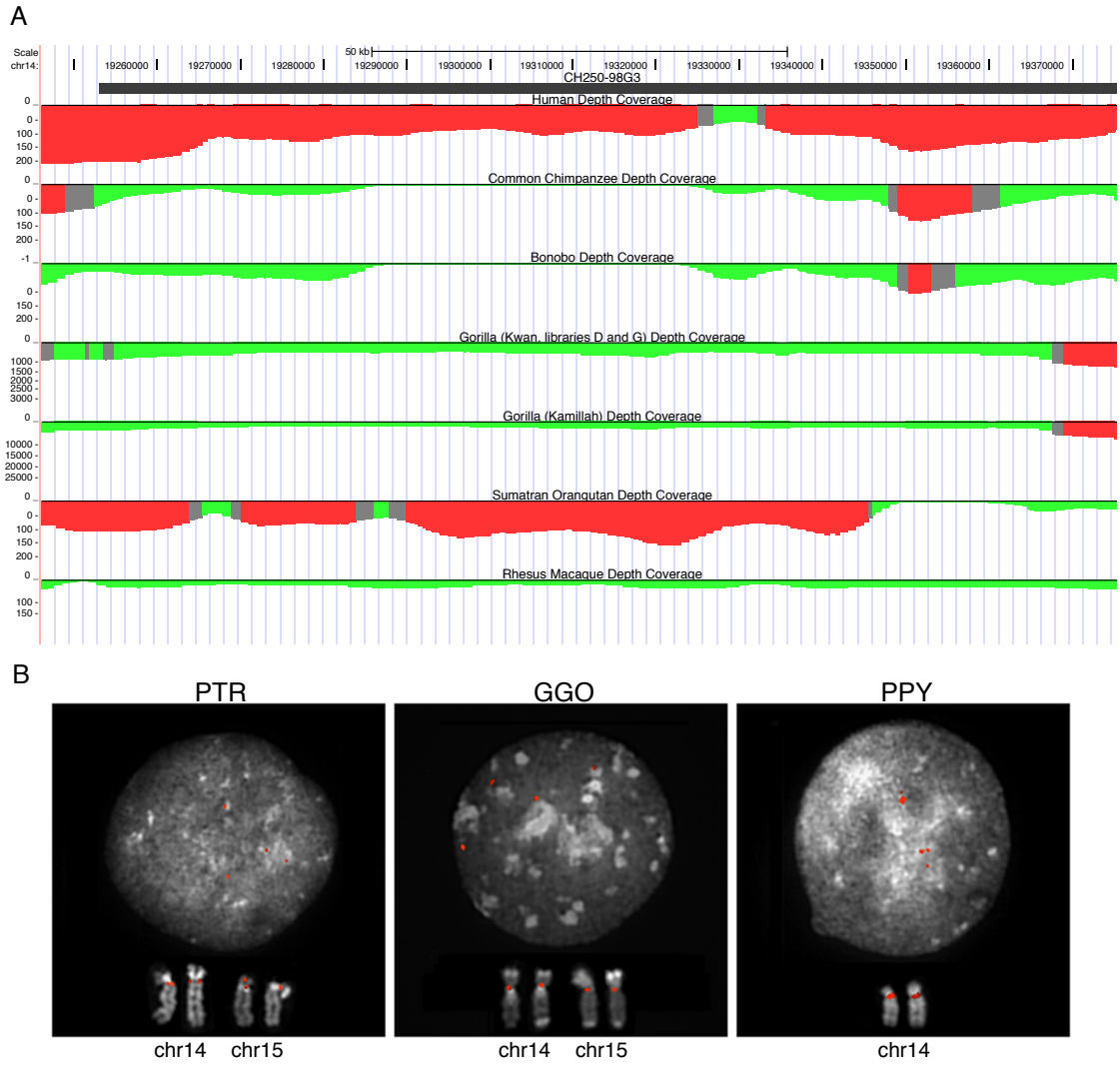

Figure S5: Comparison of the 14q11.2-15q11.2 duplication among apes. A) Depth-of-coverage data of the chr14:19,246,159-19,375,262 (hg18) region are shown for human, chimpanzee, bonobo, gorilla (Kwan and Kamillah), orangutan, and macaque. B) FISH results of BAC probe CH250-98G3 on chimpanzee, gorilla, and orangutan chromosomes and interphase nuclei.

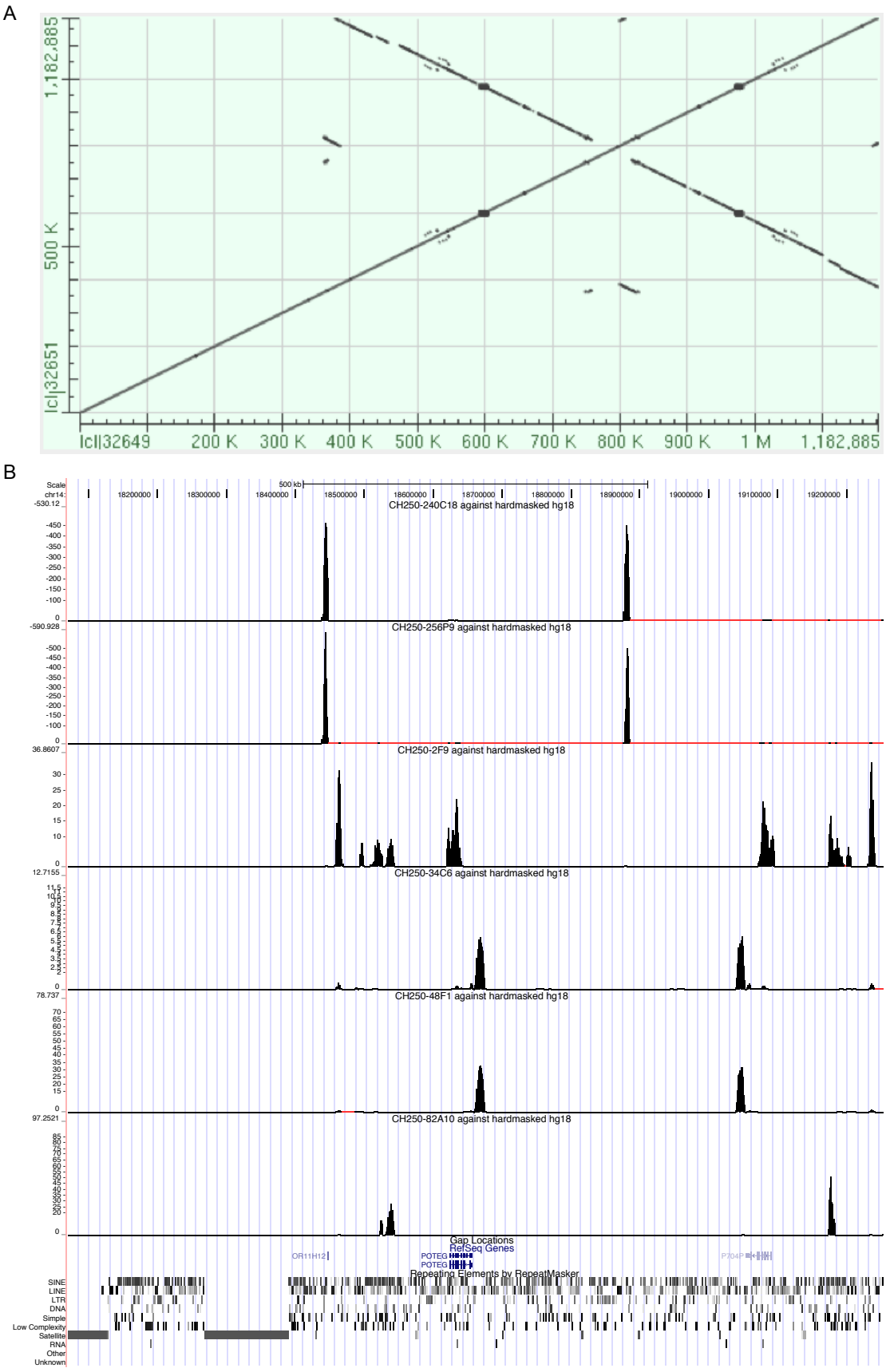

Figure S6: Chromosome 14 pericentromeric region. A) Self-comparison of chr14:18,070,001–19,252,885 (hg18) genomic sequence. B) Coverage data for the same region of CH250-240C18, 256P9, 2F9, 34C6, 48F1, and 82A10 Illumina reads.

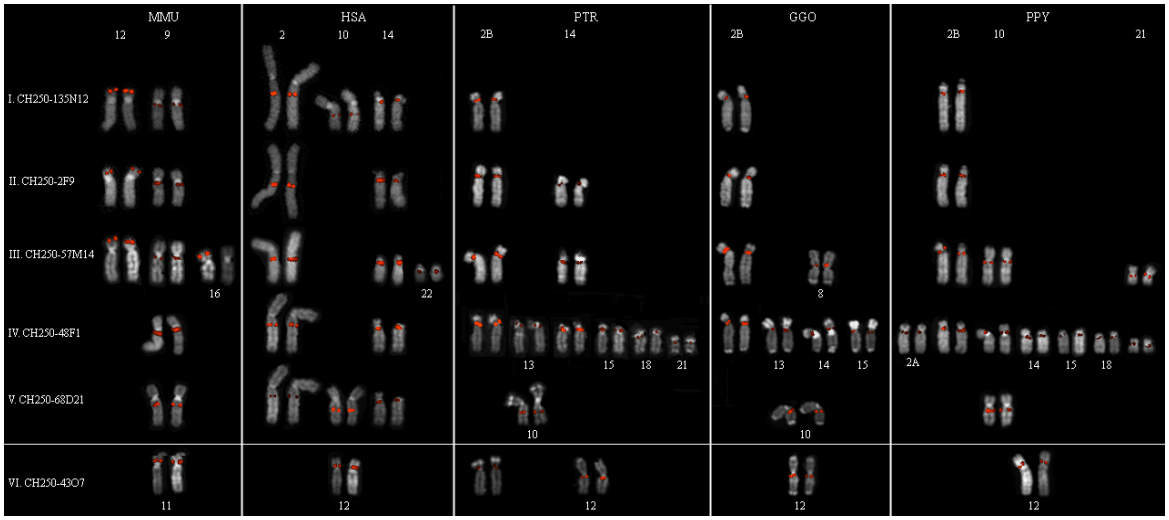

Figure S7: FISH results of representative BAC clones positive to the STS Hsa14d, Hsa14e, and Hsa14f on macaque (MMU), human (HSA), chimpanzee (PTR), gorilla (GGO), and orangutan (PPY) chromosome metaphases.

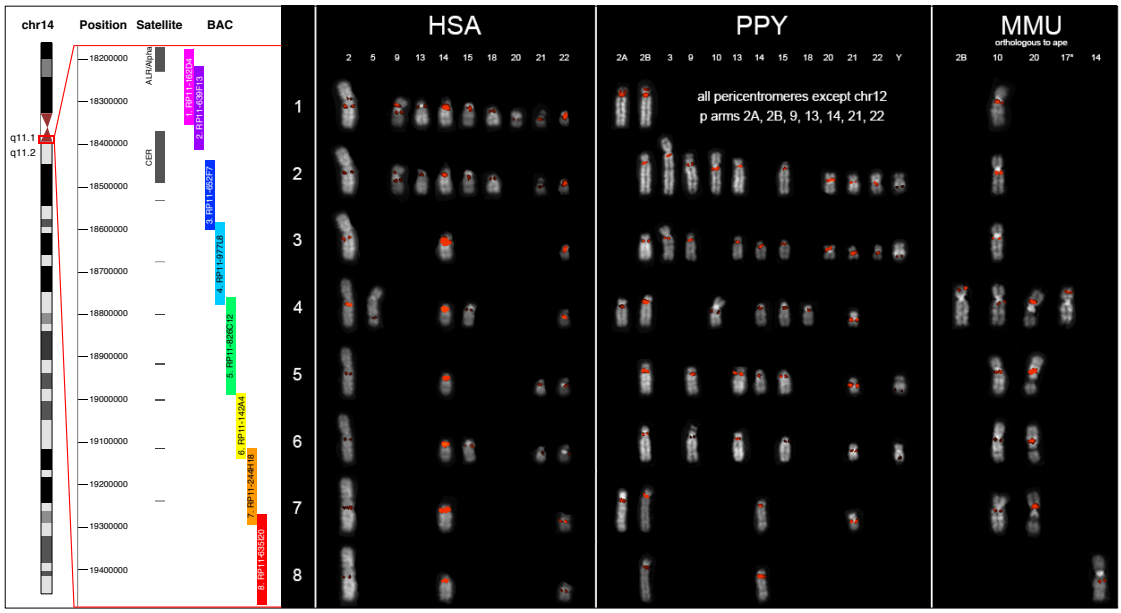

Figure S8: Comparative molecular cytogenetic analysis of the human chr14:18,075,982-19,382,135 (hg18) in human, orangutan, and macaque. FISH results of a tiling path of eight human RP11 BAC clones (left panel) on human, orangutan, and macaque chromosomes (right panel). Orthologous human chromosomes are indicated for macaque chromosomes.

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