Supplementary Information

(1) Comparison of T/S measurements between the two captive chimpanzee populations

We treated the two sources of chimpanzee samples as equivalent, even though the relatedness and infectious disease load (based on medical records) varies between the two research centers. To test whether we erred in this assumption, we regressed T/S ratio on age in the two groups separately (See **Supplementary Figure (SF) 3**). While the Yerkes samples showed a steeper decline with age, the 95% confidence intervals for both the y-intercept and slope of the regression lines overlap. This result suggests that the various HIV/hepatitis infections of the chimpanzee females from the Southwest National Primate Research Center did not alter our general conclusions for chimpanzees (All individuals from Yerkes reportedly tested serology negative for HIV/HBV/HCV). This might be expected for HIV, as chimpanzees can be infected but rarely develop immunodeficiency and so should not display increased TROC. This was shown in an earlier, albeit statistically underpowered, study on chimpanzee telomere dynamics (Feng et al., 1998). This is in contrast to SIV-infected macaques who do show increased TROC (Shibata et al., 1999). Chimpanzees are the only available animal model that can be infected with and suffer from HBV/HCV, though the effect on telomeres remains unknown. We note that our Utah CEPH human DNAs were collected from blood in the 1980s without regard to any disease phenotypes or serological tests.

(2) Comparison of Tackney et al T/S results to Cawthon 2009

In this paper we modified the telomere monochrome multiplex qPCR relative to the previously published assay in Cawthon (2009). As Cawthon (2009) provides not only an additional 47 female T/S results to compare to, but also the correlation between mean TRF lengths and relative T/S ratios we used to convert our measurements, it was imperative to confirm consistency of results between the two papers. We happened to analyze six individuals from the CEPH panel who were also included in Cawthon (2009). Using the six replicates (triplicates from two PCRs) from the earlier study, we compared the average T/S values and 99% confidence intervals of each individual (**SF 4**). For all six individuals the confidence intervals from the two studies overlapped. For four individuals the average T/S value from Cawthon (2009) fell within the confidence intervals calculated from our dataset. We calculated the mean coefficient of variation (CV) as 11.45% for these individuals, treating all the measurements for each individual as though they came from a single assay. The correlation coefficient between these six points from both studies is 0.85 (Pearson's product-moment correlation; p=0.02; 95% Confidence Interval: 0.28, 1.00).

We next compared the linear regressions of T/S ratio on age for the 35 females from the Cawthon (2009) runs that fell within the age range (5.2 – 48.2 yrs) covered by our human results (43 females; **SF 5**). The 95% confidence intervals for both the y-intercept and slope of the regression lines overlap, indicating that at least with this comparison both methods give the same result for human age-related telomere decline. Congruency between the two methods justifies using the regression equation from Figure 5 of Cawthon (2009) to convert T/S ratios in our study to bp lengths. Additionally, the CEPH females from the earlier study included related individuals from six families, while our 43 human females were specifically chosen to be unrelated (based

on available three generation pedigrees). The similar regression lines indicate that the relatedness of the earlier study cohort does not influence age-related TROC measurement.

(3) An expanded human data set

We explored whether our calculated TROC for human females would change if we included the female T/S measurements from Cawthon (2009). Since we knew the two data sets (compiled using slightly different assay methodologies) yielded overlapping regression lines **(SF 5),** we were unsurprised that for all 72 of the female CEPH samples under 60 years of age the linear regressions of T/S ratio on age gave a y-intercept (1.565) and β coefficient (-0.010) within the confidence intervals of our previous human female calculations (1.648 and -0.012 respectively; see **SF 6** and **Figure 1**). This updated TROC corresponds to an attrition rate of ~33 nt/year. This new β coefficient still has overlapping confidence intervals with the chimpanzee regression (**Figure 1**), though we point out that the value calculated from these mixed samples is half that of the chimpanzee slope (-.022).

We next included twelve older females from Cawthon (2009), aged 61.2 to 84.4 years (dotted line of **SF 6**). While the slope of the regression using older females appears less steep, the confidence intervals of the two lines overlapped. We anticipated slight flattening due to mortality selection in the cohort >60 years of age (only the people with relatively long telomeres are alive and available for blood draws; the people with the shorter telomeres have died) and/or due to changes in the distribution of leukocyte subpopulations in older individuals (Aviv et al 2006). However, given the tight similarity of the slopes, mortality selection doesn't seem to be a confounding problem in the linear model of the expanded data set.

(4) Statistical Methods

We fitted three distinct statistical models (linear, exponential, and quadratic) to human and chimpanzee data in an effort to understand the relationship between age and T/S ratios in these species. After fitting each model to the data, we compared goodness of fit using the Akaike Information Criterion or AIC (Akaike, 1974; Burnham and Anderson, 2002). AIC showed that the linear model fit both human and chimpanzee data best. The linear model has the added benefit of allowing intuitive cross-species comparisons of TROC and telomere length across ages. As we note in **Supplementary Information** (**SI) 6**, the rate of leukocyte telomere shortening is not likely to be constant over the lifespan of an individual and it is well established that very rapid attrition likely occurs early in life (Baerlocher et al., 2007; Frenck Jr et al., 1998). Additionally, in cross-sectional studies there might be cohort effects at the older age ranges that might give the impression of a slower attrition rate (see **SI 3**). Since our human sample excludes both young and old age cohorts, we expected the linear model to fit best. Our youngest chimpanzee was 6.2 years of age so that sample too is unbiased by infant telomere loss.

Instead of reporting p values for our parameter estimates, we have chosen to report confidence intervals. Confidence intervals and effect sizes are both informative and more consistent with our methodology (Anderson et al., 2000; Gigerenzer et al., 2004; Johnson and Omland, 2004).

(5) Chimpanzee and Human Telomere Lengths: Details and Comparisons

TL varies between age-matched individuals and between tissues within individuals (Aviv, 2008; Aviv et al., 2011; Gadalla et al., 2010). We analyzed whole blood DNA, for which the vast majority of the genomic material comes from sub-populations of leukocytes. Leukocyte telomere biology is complex, but it is the sample of choice in clinical epidemiology and is well studied. An individual's leukocyte TL at a certain age will be determined both by their TL at birth and the subsequent rate of change up to the collection point. For investigating the use of replicative aging in the great apes, the high proliferation rate of hematopoietic stem cells / progenitor cells (HSCs/PCs) and their involvement in somatic maintenance and immune response potentially make leukocyte telomere dynamics an excellent marker of lifespan inflammation and oxidative stress (reviewed in Aviv, 2008; Aviv et al., 2006). Leukocytes are a diverse population of cells and we note for referenced material when cell sorting was used prior to analysis either generally to isolate peripheral blood mononuclear cells (PBMCs) or more specifically with antibody staining/flow cytometry. To maximize our nonhuman primate comparative data we included non-leukocyte tissue and cultured cell line results where these have been published.

Our monochrome multiplex qPCR method directly assays telomere repeats and, in conjunction with a reference DNA sample, telomere (T) and single-copy gene (S) signals yield relative T/S ratios that are proportional to the mean telomere length of all the cells in the sample. Our results are not affected by any subtelomeric length or restriction site polymorphisms when comparing individuals intra or inter species (in contrast to the TRF Southern blot method).

However, interstitial telomeric repeats (ITRs) will be amplified by our assay and differences in repeat count between species under analysis could cause qPCR average telomere lengths to be falsely high. With current short-read next-generation sequencing technologies, long repetitive portions of genomes (like telomere repeats) are difficult to sequence and/or map to a reference. As more nonhuman primate genomes are sequenced in this way, true ITR count differences might not be detected. The magnitude of this false identification needs to be quite large to see a measurable effect here, however. In a recent search of the human and chimpanzee genomes, 100 and 110 loci of ≥ 4 telomere repeats were found, respectively. These loci were highly conserved between the two species (> 90%) and were on average 46 nucleotides long (Nergadze et al., 2007). Telomeric repeat differences at this scale cannot explain our length results.

We assume ITRs are relatively constant within each species. While ITR polymorphisms have been discovered in humans and mice (Lin and Yan, 2008), they have so far only been described at a few loci, with alleles of small size (<100bp). This degree of polymorphism would be outside the resolution of this assay. Chimpanzees have an extra pair of chromosomes, which fused into Chromosome 2 in our lineage. The four extra telomere ends compared to the full human set of 92 cannot explain our observed difference in telomeric repeats between the species. In humans, some of those ancestral telomeres are actually retained in fusion head-to-head arrays of interstitial telomere repeats within Chromosome 2 (Ijdo et al., 1991).

Ignoring individual ages, our chimpanzee females had an average T/S ratio approximately 2X greater than our human females (**Table 2**). Since we used the same Standard DNA pool (whose T/S ratio is, by definition, 1.00) on both samples, these results are directly comparable. There

were some humans (aged <40) who had particularly long telomeres (>T/S ratio of 2.0) and there were some chimpanzees (mostly aged >40, with one young exception) who had particularly short telomeres (≤T/S ratio of 2.0), with the expected age-matched variability in both species (**Figure 2**).

The standard method in quantifying telomere length is to report the TRF length (or equivalent from a mean TRF to T/S linear regression) and this is reflected in the published literature (**Table 1**). TL variability can therefore be the result of actual TTAGGG repeat changes or of subtelomere length variability. Subsumed under subtelomere length variability are true restriction site polymorphisms and/or different subtelomere lengths due to different restriction enzymes used in the TRF protocol. The more restriction enzymes one uses, the shorter the subtelomere length should be. Throughout this paper when reporting length in basepairs we avoided this issue (see Experimental Procedures). However, to compare our results to the published telomere lengths, the subtelomeric component needs to be addressed.

Our equivalent human TRF lengths are based on a mean TRF assay using *HaeIII*, for which Cawthon (2002) observed non-telomeric DNA to be ~4.2 Kb in length and vary by upwards of 2 Kb between individuals. Leukocyte subtelomere length has also been approximated at 4 Kb (Figure 2A: Aviv et al., 2011) or 6 Kb (Statistical analyses subsection: Ehrlenbach et al., 2009) using *RsaI/HinfI*. Incorporating our observed telomere repeat lengths (**Table 2;** See Experimental Procedures), the human female leukocyte TRFs here are between 5.3 and 13.9 Kb long, with a mean of 8.4 Kb. This range includes most of the reported lengths from the literature (**Table 1**), though is not as wide as 5.1-18.6 Kb reported using a similar qPCR assay (Eisenberg et al., 2011). Our Utah CEPH samples, therefore, show expected lengths for humans and no indication of departure from other samples in the literature. Telomere length from cultured fibroblasts have been shown to be ~35% longer than leukocyte TL (Gadalla et al., 2010). On those grounds we hypothesize that our reported lengths would shift upward in that tissue.

Our equivalent chimpanzee TRF length is harder to determine as we did not have reference *HaeIII* TRF results for this species. If we assume a similarly sized subtelomere length, chimpanzee mean telomere length would be 13.2 Kb, with a min/max range of 7.9 to 18.6 Kb (using the same TRF-to-T/S linear regression as we did for the human samples; **SI 8** addresses concerns on this decision). The only published chimpanzee TRF result using only *HaeIII* that we are aware of was from Bhatnagar and colleagues (1995) and there an individual of unreported age or sex resolved into two distinct telomere bands of 20.6 Kb and 14.5 Kb. The human sample from that study resolved at ~8 Kb. This paper also nicely displays the variant lengths one can expect from using different restriction enzymes. Other published TRF results we are aware of place telomere lengths at 9.3-10.1 Kb for bonobo cultured skin fibroblasts (Steinert et al., 2002) and 11.6 ±0.61 Kb for chimpanzee PBMCs (Feng et al., 1998) (**Table 1**). We have not incorporated data from Kakuo et al. (1999), as unacknowledged experimental error may have affected their TRF results. They calculated extremely long (>23kb) and unusually similar telomere lengths from various tissue samples of five nonhuman primate species, including one newborn male chimpanzee, and they observed no change in telomere length in these species using different combinations of restriction enzymes. This telomere phenotype was not observed in a subsequent study that included two of the same species found in Kakuo et al. (1999) (rhesus

monkey and orangutan from cultured skin fibroblasts) (Steinert et al., 2002) nor have we observed it in our study of chimpanzees.

If chimpanzee subtelomeres have restriction sites different from those of humans, then we would be wrong to approximate their *HaeIII* TRF values from our T/S results. Chimpanzees could have a very large subtelomere fragment when cut, so a TRF value of 17 Kb could theoretically mask the same sized telomere length as a human TRF of 8 Kb. A good example is the recent publication by Gardner and colleagues (2007) where the older non-multiplex version of this assay was used (Cawthon, 2002) and different single-copy genes were chosen for macaques and humans. TRF values using *HinfI* and *RsaI* were calculated for both species and mean TRFs were regressed against T/S values. Macaque subtelomeres had a length of 10.7 Kb and we calculated TRF equivalent lengths from T/S values at ~13-18 Kb. Human subtelomeres had a length of 5.9 Kb and TRF equivalent lengths of ~8-11 Kb (Figure 5: Gardner et al., 2007). From these approximations, while TRF values are larger in macaques, this study suggests that the telomere repeat length of macaques and humans may actually be quite similar.

Our results and the published literature (**Table 1**) support the observation that primates retain the ancestral phenotype of <20Kb telomeres (Gomes et al., 2011). Lemurs are one exception, in that they seem to have a large amount of telomere length heterogeneity – very long telomeres, very short telomeres and large blocks of pericentromeric telomere repeats (Steinert et al., 2002). Baboons are another exception, in that they too show marked heterogeneity. Leukocyte TL at birth in 4 individuals fell into two size ranges: ~25-28 Kb and ~13-15Kb; this difference was maintained at 200 weeks of age (Baerlocher et al., 2007). Based solely on previous TRF (and equivalent) lengths, humans do seem to have telomeres on the shorter end of the range of primate-like short telomeres, but length overlap between species using this measure is substantial. It was only by excluding subtelomere variation with a canonical telomere repeat-specific qPCR assay that we determined humans indeed have about half the length of telomere repeats as chimpanzees.

(6) Chimpanzee and Human Telomere Attrition: Details and Comparisons

Applicable human and nonhuman primate published rates of telomere attrition are outlined in **Table 1.** Like TL, telomere attrition dynamics vary between tissues and between individuals. TROC variation likely contributes much to the observed TL age-matched variation. Human TROC consistently falls below 100 nt/yr for a range of tissue types as reported from a 2003 review of mammalian literature (Haussmann et al., 2003) and is usually around 30 nt/year for leukocytes in human cross-sectional data (Aviv et al., 2011). Longitudinal studies (noted by 'L' in **Table 1)** would be expected to give more fine grained rates of shortening, but these are complicated by TL stasis or growth in certain individuals (Aviv et al., 2009; Bendix et al., 2013; Ehrlenbach et al., 2009; Farzaneh-Far et al., 2010; Nordfjäll et al., 2009). The average leukocyte TL attrition rate hovers near 40 nt/year for these longitudinal studies, though this includes the 10-30% of individuals in each study who are not shortening. However, recent analyses suggest that at least some portion of the observed elongation of TL in these studies is due to measurement error and regression to the mean (see Bendix et al. (2013); Steenstrup et al. (2013); Verhulst et al. (2013)). Telomere attrition in leukoctyes is expected to result from damage to/replication of

HSCs/PCs; and somatic damage in other tissues would likely call for increased cellular divisions of these cells and a concomitant increased leukocyte telomere attrition (Aviv, 2008).

Attrition data for nonhuman primates is limited and inconsistent. PBMCs from cross-sectional data of young cynomolgus macaques (aged 3.9-8.2 yrs) and pig tailed macaques (aged 1.7-9.0) gave an average TROC of 140 nt/year and 440 nt/year, respectively (Shibata et al., 1999). Baerlocher and colleagues, using flow FISH and immunostaining, were able to differentiate shortening rates between subpopulations of leukocytes in baboons. Lymphoctyes had higher attrition rates than granulocytes, driven in particular by T-cell telomere shortening. Actual rates were not provided, but a rough calculation from **Table 1** for male baboons aged 6.5-26.5 sets lymphocyte TROC at ~280 nt/year and granulocyte TROC at ~100 nt/year (Baerlocher et al., 2003). These are much higher than similar measures of ~52 nt/year and 36 nt/year, respectively, for humans (Rufer et al., 1999) or ~63 nt/year for PBMCs of cynomolgus macaques (Lee et al., 2002), though they are of the same order of magnitude as the Shibata et al data set. However, since Baerlocher and colleagues reported that baboon leukocytes as a whole, and lymphocytes in particular, showed extreme inter-individual variation in telomere fluorescence, cross-sectional data from this data set (and possibly species) might be particularly ill suited for calculating comparative attrition rates. There is a baboon longitudinal data set but it is not helpful for our comparisons since only very young individuals were sampled (newborn to 200 weeks) (Baerlocher et al., 2007; see below). Outside of blood, TRF attrition rates from cultured fibroblast population doublings (PD) have been observed at 120-200 bp/PD for old world primates (Steinert et al., 2002), or 2-4X greater than a similar measure of 48 bp/PD in humans (Harley et al., 1990).

Our calculated TROC for chimpanzees was -.022 T/S per year or ~73 nt/year (95% confidence: 97 nt/year - 47 nt/year). This rate was based on a sampling of 65 female chimpanzees representing a ~50 year age span. This sample size and age distribution is unprecedented in scope in the nonhuman primate literature and should give an accurate picture of cross-sectional chimpanzee TL attrition. The measurement for humans was similarly calculated at -.012 T/S per year or ~40 nt/year (95% confidence: 70 nt/year - 13 nt/year). The confidence intervals for the two measurements overlap, and the rate for our human samples matches nicely with other recent human studies using leukocytes (**Table 1**). We note that for the age range studied in both species, our sample count for each species exceeds the number that Aviv and colleagues (2006) estimated as necessary to calculate a significantly informative species attrition rate. However, the sample count falls below what that group estimated is needed to distinguish rate differences between two groups if they are less than three-fold. Their results imply that more chimpanzee samples are necessary to detect smaller differences in attrition rate.

We also calculated TROC in an expanded set of human females by combining our results with those from Cawthon (2009) (see **SI 2 and 3**). For 72 human females aged 5.2 years to 57.3 years our telomere attrition rate was similar to above at .010 T/S per year. When a further twelve females were added with ages from 61.2 years to 84.4 years the attrition was less steep at .009 T/S per year, though the confidence intervals of both measures still overlapped (see **SF 6**).

The picture from most of the published nonhuman primate data for which attrition rates were calculated seems to be a quicker TROC than in humans, and yet rates are quite similar in our

chimpanzee and human samples. Hominids are notably longer lived than other primates, which are grounds for expecting differences between monkeys and apes. Another possibility is that the previously published data sets were biased towards younger individuals, except for Lee et al. (2002). Lee and colleagues sampled from a wider age range of cynomolgus macaques (who may live to ~40 years of age in captivity) and they reported an attrition rate that matches humans (and our chimpanzee results). This underlines the importance of the ages sampled. The rate of telomere shortening is much more rapid early in life, as seen in whole blood/leukocyte telomere length measurements from baboons (Baerlocher et al., 2007) and humans (Frenck Jr et al., 1998; Rufer et al., 1999; Zeichner et al., 1999), possibly due to increased cell division early in life if loss per replication is constant (Baerlocher et al., 2003). Our youngest human and chimpanzee females are 7.4 and 6.2 years of age respectively, thereby excluding the years of steepest change. As noted in **SI 4** we compared several models and found linear models the best fit for the rate of change across our range of ages. However, cross sectional samples, which we use here, may underestimate shifts in rate of change with age.

In **SI 5** we discussed the possible impact of interstitial telomeric repeats (ITRs) on our assay for telomere length measurements and we note them again here for attrition measurements. Telomere attrition rate measurements by qPCR should not be affected by species ITR differences unless interstitials (which would never shorten) are contributing the vast majority of the telomere signal in the assay. This might occur if at a certain age senescent cells with critically short 'true' telomeres make up the majority of the blood sample and would result in a gradual flattening of telomere attrition with increasing age. Such flattening is absent in the age-matched chimpanzee and human samples (though see **SI 3** when we incorporate older human females) and the magnitude of that flattening would be independent of the load of interstitials per cell. Whereas Foote and colleagues (2013) have shown that attrition rates are underestimated when measured by Southern blot due to the contribution of ITRs in calculating the average telomere length, this is not a problem for qPCR as the results are normalized to cell count using the single copy gene as a proxy and ITRs always contribute a constant, additive factor.

(7) Birds and mice are not appropriate model organisms for human telomeres

Our phylogenetic approach to telomere dynamics and life history comparisons between humans and chimpanzees makes conclusions about telomere lengths, longevity and mortality/morbidity drawn from distantly related vertebrates less relevant. Gomes and colleagues (2011) consider the order Rodentia analyzed by Seluanov and others (2007) and note that *Mus musculus* has apparently abandoned replicative aging. The different telomere biology of mice (and in particular laboratory mice) and humans has long been noted. They have much longer telomere lengths, greater telomerase activity and expression, and their cells easily immortalize in culture (See Baerlocher et al., 2003; Eisenberg, 2011 and references therein; Shay and Wright, 2007). Small model animals usually have high extrinsic mortality rates, which life history theory suggests would have selected for less investment in somatic maintenance, making them unlikely models for many aspects of human physiology (Bolker, 2012; Selman et al., 2012). Gomes and colleagues (2011) confirm that many of these smaller, shorter-lived mammals independently abandoned replicative aging (and its cancer suppression) in favor of some other compensating mechanism.

Phylogentic distance and likely differences in telomere biology between mammals and birds also limit the relevance of the rich avian literature to our own great ape radiation. Gomes et al. (2011) hypothesize that the ancestral mammalian telomere phenotype was a result of the larger therapsid precursors to mammals evolving into smaller endothermic homeotherms. Endothermy would have carried with it a concomitant increase in free radical production and ancestral mammals were therefore under selection for mechanisms protecting against cancer. As the therapsids (via synapsids) diverged from the sauropsids millions of years earlier, the eventual endothermy in birds and their mechanisms for tumor-suppression might be quite different. Birds live longer than similarly sized mammals, even though they do seem to express telomerase in somatic tissue (Haussmann et al., 2007), and have high body temperature and metabolic rates (Holmes and Austad, 1995), which might be expected to increase oxidative load. Some long-lived avian species even increase their TL as they age, suggesting that they have abandoned replicative aging (Haussmann et al., 2003). Their small mass might have allowed them to express telomerase without increased cancer risk and/or they may have been selected for especially high somatic maintenance to keep flying (Monaghan, 2010). Finally, in some bird species interstitial telomeric sequences can make up a large percentage of total telomeric signal and there might be substantial differences in interstitial repeats between individuals and between species. For these reasons birds might not be good candidates for our qPCR methodology (see discussion in **SI 5**)(Foote et al., 2013).

(8) Comparability of the qPCR assay in the human and chimpanzee DNA samples

We used the linear regression formula from Cawthon (2009) to correlate our T/S ratios to basepair lengths; a sample with a relative T/S ratio of 1.0 would have a cellular average telomere length of ~3330 bp. Since the human samples in that study only displayed T/S ratios from ~0.5 to ~2.0, we are interpolating base pair measurements beyond the validated bounds when applying the same equation to most of the chimpanzee samples. Aviv and colleagues (2011) have made a case that there is potential non-linearity between TRF measurements and qPCR measurements at high T/S ratios (>1.4), where T/S ratios may continue to increase with little increase in the TRF measured (see Figure 2 of that study). The addition of a quadratic term as a better fit for their data seems to be driven by four samples with T/S ratios of 1.4-1.6 and TRF measured lengths of 6.25-7.25 Kb. These results do not demonstrate a general phenomenon, as many samples with T/S ratios at or above this range show a linear relationship with TRF. If we allow that this might be an occasional error, its effect on our chimpanzee measurements would only further highlight similarities in chimpanzee and human attrition rate. Older chimpanzee telomere lengths fall within the range of younger humans (~2 T/S), so it would be younger chimpanzees whose large T/S ratios might be masking relatively shorter TRF determined lengths. This would suggest that chimpanzee TROC is actually even slower than we calculated, with chimpanzee lengths approaching double that of humans only in old age. The human derived state of shorter telomeres would become more pronounced the older we get.

More likely, the phenomenon of non-linearity is based on DNA quality issues. Aviv et al. (2011) extracted DNA with Qiagen QIAamp blood kits and analyzed DNA integrity on agarose gels; specifically they checked for low molecular weight DNA fragments. These, in particular, would throw off TRF measurements, though it is unclear how they would influence qPCR determined lengths. Our chimpanzee whole blood samples were purified with a Qiagen QIAamp DNA

Blood Mini Kit, while the long-term storage panel of human DNA was phenol-chloroform (a total of 24 DNA samples were selected from this group) or PureGene (a total of 19 DNA samples were selected from this group) extracted from whole blood samples.

A recent study by Cunningham et al. (2013) reports that telomere length measurements may be influenced by the method of DNA extraction. Specifically, peripheral blood leukocyte column-based purifications lead to shorter (though less variable) lengths than liquid-to-liquid phase methods. They hypothesize that DNA shearing during column purification or DNA quality might be a contributing factor. qPCR inhibition was unlikely, as telomere length assessed by Southern blot analysis and reported in their supplemental material showed a similar shorter size post-QIAamp purifications vs phenol/chloroform or PureGene methods. Unless column purification had an opposite effect in our chimpanzee samples, we would expect our calculated T/S ratios to go down relative to the liquid-to-liquid phase method extracted human samples and not up. If the extraction method bias is confirmed, however, this would suggest that our chimpanzee telomere lengths are potentially even longer than we report here.

Since DNA quality might be a confounding factor in telomere length measurements, we explored the association of predicted DNA concentrations based on the albumin amplification to input DNA based on absorbance with a NanoDrop 1000 Spectrophotometer (**SF 7 a,b,c**). We calculated linear regressions of albumin s-signal on input DNA in nanograms for each set of chimpanzee DNA extractions from each research center (Yerkes and Southwest) and for 21 of the 45 CEPH human females. We did not collect new NanoDrop data for the rest of the human samples, as we used previously recorded DNA concentrations to determine input. The confidence intervals for the y-intercepts of the five data sets were large, but all overlapped. All confidence intervals overlapped for the slopes of the regressions. The second extraction of samples from Yerkes barely overlapped with the human beta coefficient (it had a steeper slope). However, since the human samples spanned a much greater range of DNA input amount, the confidence interval for the human set was much tighter than for the chimpanzees. The Yerkes second extraction beta coefficient confidence interval overlapped with the other 3 chimpanzee sets substantially. These results together suggest comparable amplification of the single-copy gene across both species.

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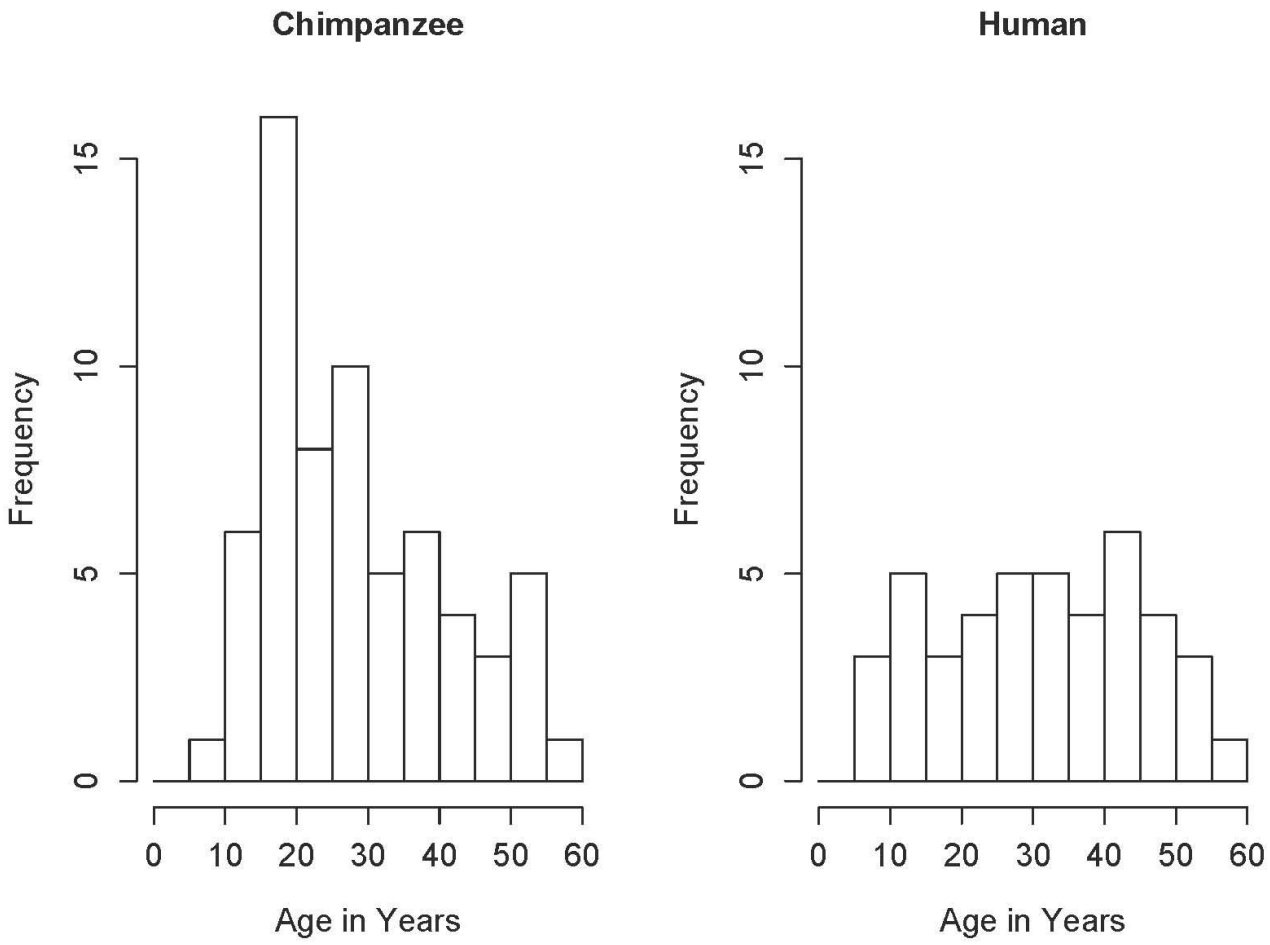
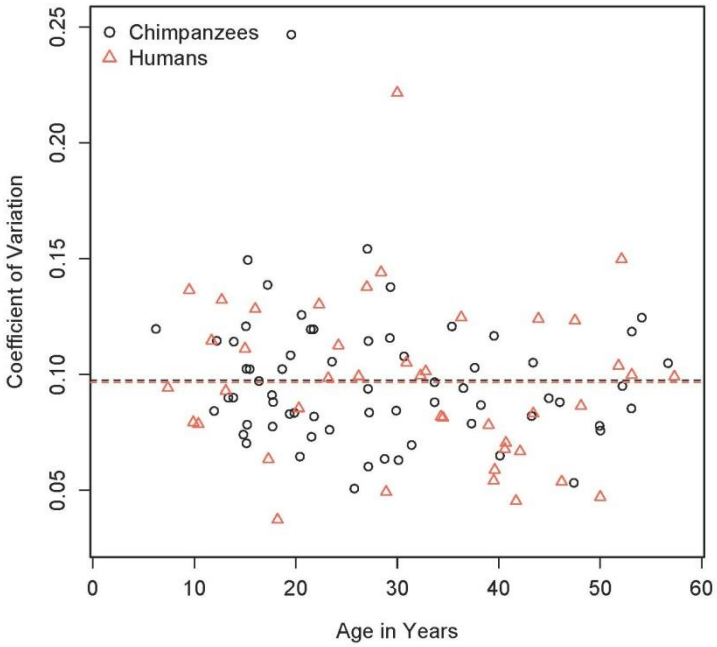
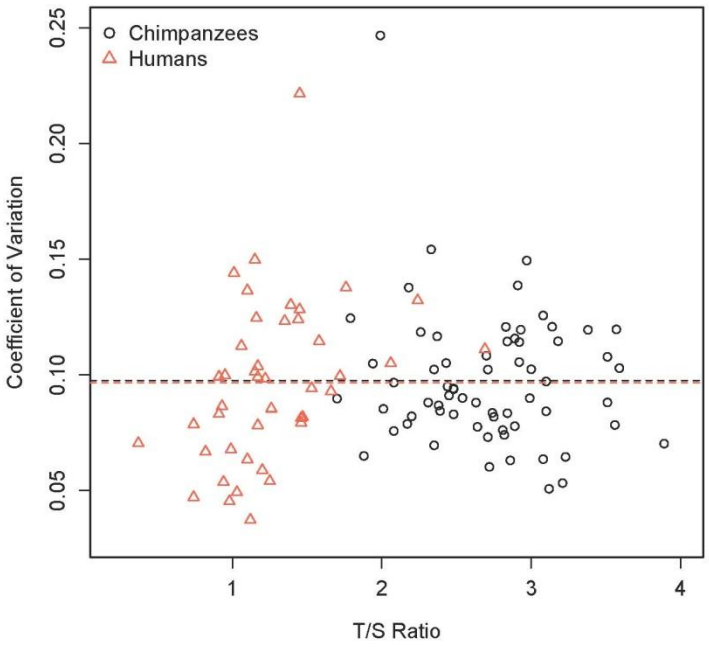
Supplementary Information Figures and Table

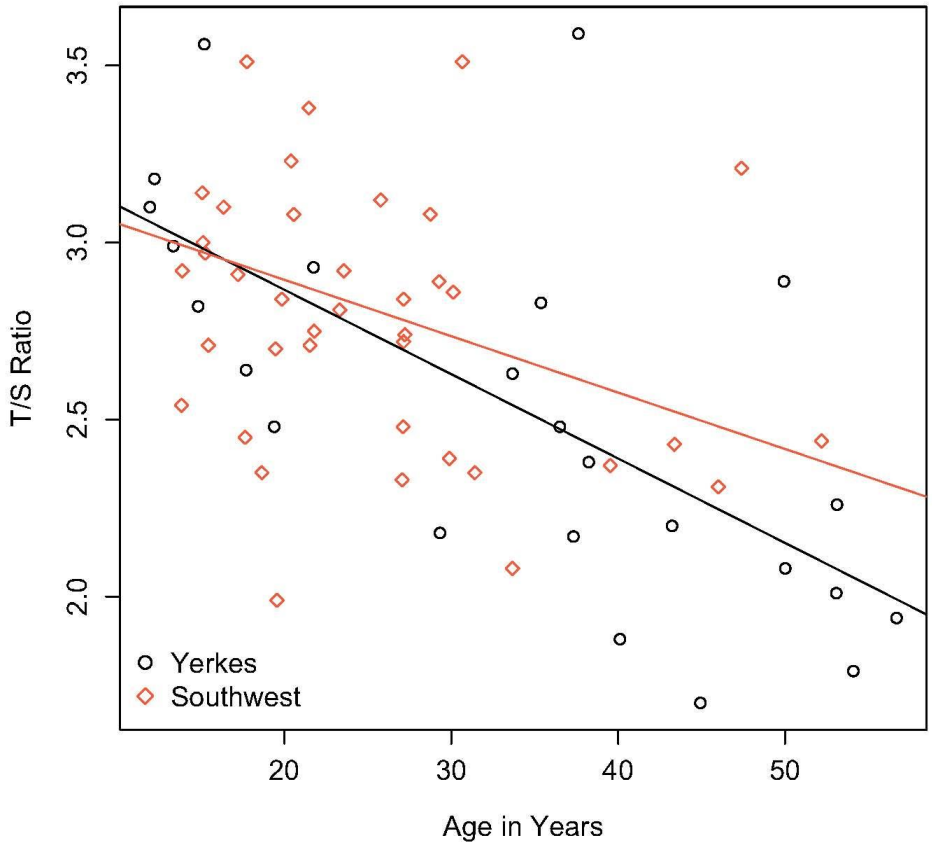


Supplementary Figure 1
Histograms of the chimpanzee and primary human samples used in this study. Bins are designated every 5 years from birth to age 60. The human samples were easier to acquire and so bin counts are more similar to each other than for the chimpanzee samples.





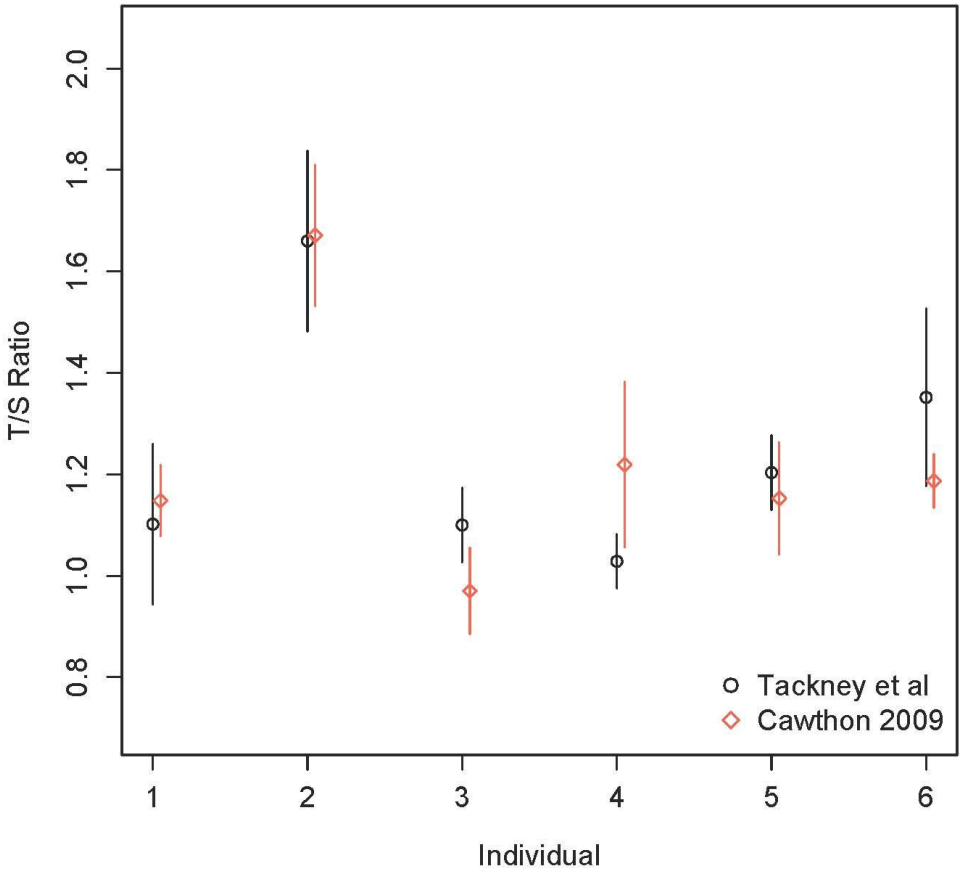
Supplementary Figures 2a and 2b
Coefficient of variation for each of the 110 samples assayed in this paper with the same monochrome multiplex qPCR assay. Figure 2a is relative to age of the individual and Figure 2b is relative to the calculated T/S ratio of the individual. Dashed lines are the mean CV for each species.



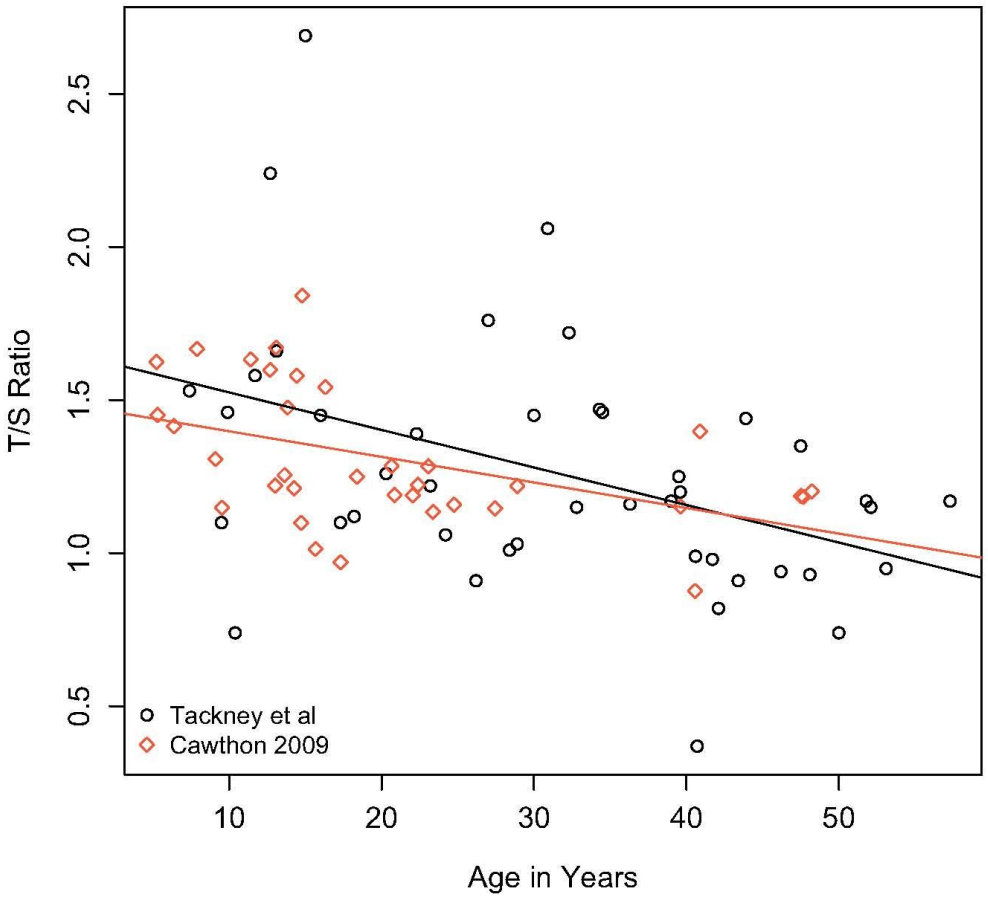
Data Source	Intercept	Slope
Yerkes	3.344 (2.910, 3.777)	-0.024 (-0.035, -0.012)
Southwest	3.213 (2.879, 3.548)	-0.016 (-0.028, -0.003)

Supplementary Figure 3

Telomere length change as a function of age for cross-sectional chimpanzee samples grouped by location of residence (Southwest National Primate Research Center or the Yerkes National Primate Research Center). All estimates for the slope and y-intercept of the two regression lines are given as: mean value (95% confidence interval).

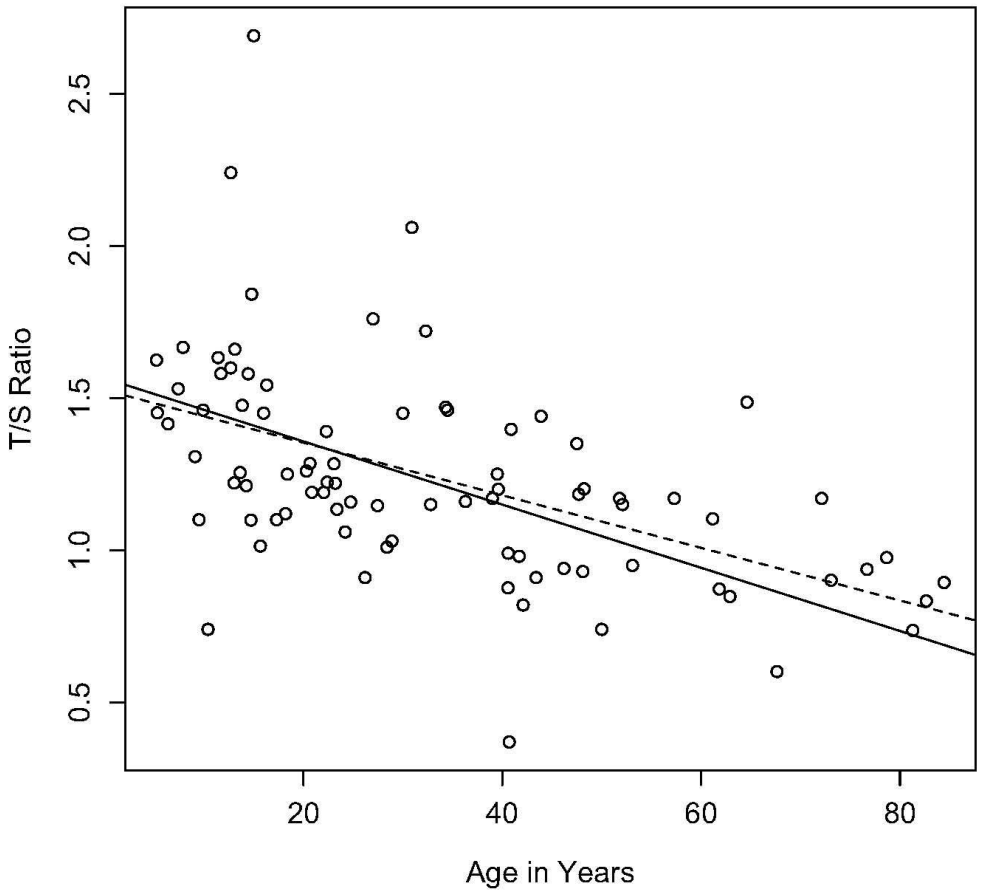


Supplementary Figure 4
Plots of T/S results for six human females reported in Cawthon (2009) and assayed in this paper using the modified protocol. Lines represent 99% confidence intervals calculated using the following equation: z-score * Standard Deviation of the replicates / Sqrt (# of replicates). The correlation coefficient between these six points from both studies is 0.85 (Pearson's product-moment correlation; p=0.02; 95% Confidence Interval: 0.28, 1.00).



Data Source	Age Range	N	Intercept	Slope
Tackney et al.	7.4 - 57.3	43	1.648 (1.358, 1.938)	-0.012 (-0.021, -0.004)
Cawthon 2009	5.22 - 48.23	35	1.482 (1.345, 1.618)	-0.008 (-0.014, -0.003)

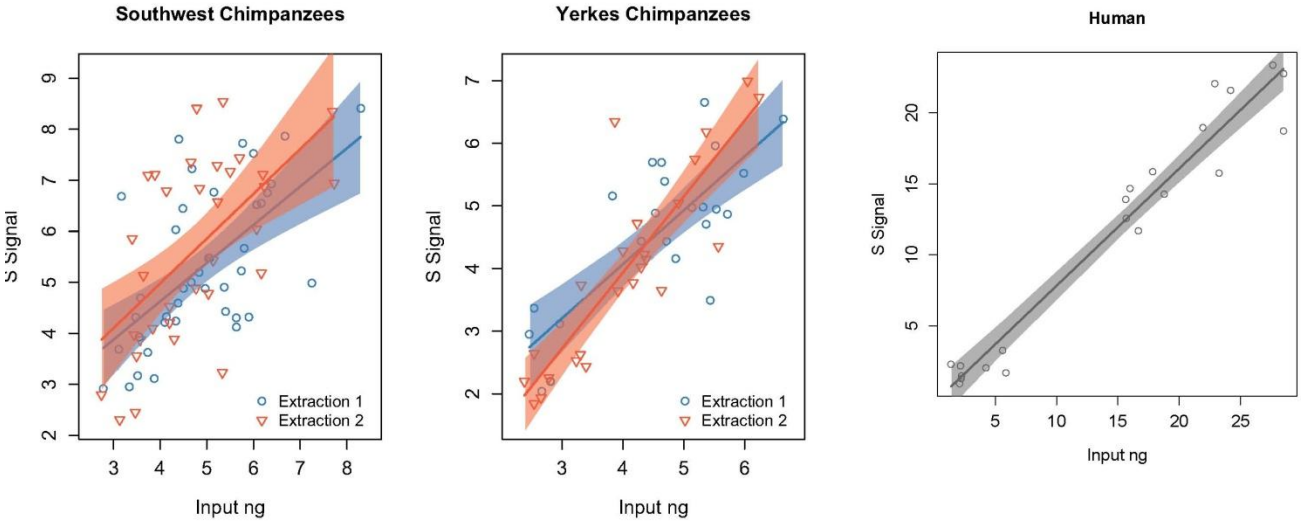
Supplementary Figure 5
Telomere length change as a function of age for two cross-sectional data sets of human samples measured with two different assays (the one reported here and Cawthon 2009). All estimates for the slope and y-intercept of the two regression lines are given as: mean value (95% confidence interval).



Data	Age Range	N	Intercept	Slope
Humans Aged <60	5.22 - 57.3	72	1.565 (1.406, 1.724)	-0.010 (-0.016, -0.005)
All Humans	5.22 - 84.44	84	1.526 (1.402, 1.651)	-0.009 (-0.012, -0.005)

Supplementary Figure 6

Telomere length change as a function of age for an expanded human data set of T/S measurements from this study and Cawthon 2009. For the six individuals who overlapped between the two studies, the average T/S value from this study was used. The solid line indicates the regression for all samples under 60 years of age and the dotted line includes the twelve older females. All estimates for the slope and y-intercept of the two regression lines are given as: mean value (95% confidence interval).



Species	Data Source	Extraction	N	Intercept	Beta
Chimpanzee	Southwest	1	41	1.626 (0.0431, 3.209)	0.750 (0.437, 1.063)
Chimpanzee	Southwest	2	33	1.469 (-0.650, 3.587)	0.878 (0.446, 1.310)
Chimpanzee	Yerkes	1	24	0.621 (-0.735, 1.976)	0.862 (0.571, 1.152)
Chimpanzee	Yerkes	2	24	-0.913 (-2.184, 0.359)	1.212 (0.910, 1.515)
Human	CEPH	1	21	-0.414 (-2.018, 1.190)	0.824 (0.732, 0.916)

Supplementary Figures 7a, 7b, and 7c
Albumin single-copy gene amplification (s-signal) relative to calculated input nanograms of DNA via NanoDrop for chimpanzee DNA samples from Southwest (**7a**) and Yerkes (**7b**), and for 22 human CEPH samples analyzed in this investigation (**7c**). All estimates for the slope and y-intercept of the regression lines are given as: mean value (95% confidence interval); confidence intervals are plotted for each regression as shaded areas.

Supplementary Table 1: Primer sequences and concentrations used in this study

Name	Sequence (5'-3')	Final Concentration (nM)
telg	acactaaGGTTtGGGTTtGGGTTtGGGTTtGGGTTAGtGT	100
telc	tgttaggTAtCCCTAtCCCTAtCCCTAtCCCTAtCCCTAACa	900
Albugcr2	cggcggcgggcggcgcgggctgggcggccATGCTTTTCAGCTCTGcAAGTC	700
Albdgcr2	gcccggcccgccgcgcccgtcccgccgAGCATTAAgCTCTTTGGcAACGTAgGTTTC	500