Original Research

Identification of multiple TAR DNA Binding Protein retropseudogene lineages during the evolution of primates

Juan C. Opazo^{1,2,3*}, Kattina Zavala², Luis Vargas-Chacoff^{1,4,5}, Francisco J. Morera^{1,6}, Gonzalo A. Mardones^{1,7,8*}

¹Integrative Biology Group, Universidad Austral de Chile, Valdivia, Chile.

²Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.

³Millennium Nucleus of Ion Channel-Associated Diseases (MiNICAD), Valdivia, Chile.

⁴Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile.

⁵Centro Fondap de Investigación de Altas Latitudes (IDEAL), Universidad Austral de Chile, Valdivia, Chile.

⁶Applied Biochemistry Laboratory, Instituto de Farmacología y Morfofisiología, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile.

⁷Department of Physiology, School of Medicine, Universidad Austral de Chile, Valdivia, Chile.

⁸Center for Interdisciplinary Studies of the Nervous System (CISNe), Universidad Austral de Chile, Valdivia, Chile.

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*Correspondence:

Juan C. Opazo

jopazo@gmail.com

Gonzalo A. Mardones

gonzalo.mardones@uach.cl

Abstract

The TAR DNA Binding Protein (TARDBP) gene has gained attention in biomedicine after the discovery of several pathogenic mutations. The lack of knowledge about its evolutionary history contrasts with a large number of studies in the biomedical area. This study aimed to investigate the retrotransposition evolutionary dynamics associated with this gene in primates. We identified retropseudogenes that originated in the ancestors of anthropoids, catarrhines, and lemuriformes, i.e. the strepsirrhine clade that inhabit Madagascar. We also found species-specific retropseudogenes in the philippine tarsier, Bolivian squirrel monkey, capuchin monkey and vervet. Although retropseudogenes are not able to produce a functional protein, we can not rule out that they may represent genetic material upon which evolution acts on, especially with regulatory functions.

Introduction

Today, the availability of whole-genome sequences allows performing more detailed research on the evolution of different genetic elements. An important source of evolutionary innovation are the events of RNA retrotranscription and its insertion into the genome (Kaessmann et al. 2009; Casola & Betrán 2017). These events depend on the enzymatic machinery encoded by retrotransposable elements, and they produce an intronless gene duplicate that could produce a protein similar to the parental gene (Zhang 2003). However, most retrotranscribed sequences are inserted at a random position in the genome, lacking all necessary transcription elements and becoming a pseudogene, a phenomenon called “dead on arrival” (Zhang 2003). However, retroseudogenes are not necessarily a dead end. There are cases in which they encode small interfering RNAs with regulatory functions (Tam et al. 2008; Watanabe et al. 2008). Thus, identifying the presence of retrocopies/retroseudogenes is an important piece of information to have a complete picture of the evolution of any particular gene.

The TAR DNA Binding Protein (TARDBP) gene, which encodes the Transactive response DNA-binding protein 43 kDa (TDP-43), has gained considerable attention after the initial discovery that its mutations can cause familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), two major forms of neurodegenerative disorders (Sreedharan et al. 2008; Kabashi et al. 2008). Up to date, more than 50 pathogenic missense mutations have been characterized (Sreedharan et al. 2008; Kabashi et al. 2008). TDP-43 is an RNA-binding protein with a variety of RNA metabolism functions, including transcription, mRNA transport and stabilization, miRNA biogenesis, long noncoding RNA processing, and translation (Hanson et al. 2012). More recent findings indicate that TDP-43 participates in the pathogenesis of other neurodegenerative disorders of several other proteinopathies, such as Parkinson’s disease and Alzheimer’s disease, which are conditions characterized by toxic protein aggregation (Klim et al. 2021). In human cells, under physiological conditions, TDP-43 mainly localizes in the nucleus, but in neurons and glial cells of ALS and FTD patients it shuttles and accumulates in the cytoplasm where eventually aggregates and contribute to the onset and progression of these diseases (Neumann et al. 2006; Arai et al. 2006; Robberecht & Philips 2013; Heyburn & Moussa 2017; Pinarbasi et al. 2018). The TARDBP gene is conserved in species that share a common ancestor deep in time (Wang et al. 2004), suggesting that this gene carries out essential functions. This gene underwent an event of

positive selection in the ancestor of mammals (Zhao et al. 2020), suggesting functional adaptations for the group. More recently in evolutionary time, it has been shown that during the evolution of humans, genes related to diseases like Alzheimer's also underwent positive selection (Vamathevan et al. 2008). Although events of positive selection are seen as conferring selective advantage, as a by-product, they can also have adverse effects (Holt et al. 1996). In this regard, it is proposed that human susceptibility to neurodegenerative disorders could be a consequence of improving our cognitive function (Gearing et al. 1994; Keller & Miller 2006).

The aim of this study is to investigate the retrotransposition dynamics associated with the TARDBP gene in primates. According to our phylogenetic and synteny analyses, we identified retropseudogenes that originated at different times during the evolution of primates. TARDBP retropseudogenes originated in the anthropoid ancestor, between 67 and 43.2 million years ago, in the ancestor of catarrhines, between 43.2 and 29.44 million years ago, and in the ancestor of lemuriformes, i.e. the strepsirrhine clade that inhabit madagascar, between 59.3 and 55 million years ago. We also found species-specific retropseudogenes in the philippine tarsier (*Carlito syrichta*), Bolivian squirrel monkey (*Saimiri boliviensis*), capuchin monkey (*Cebus capucinus imitator*) and vervet (*Chlorocebus sabaeus*). Although annotated sequences are not putatively functional, given the presence of indels and premature stop codons, we can not rule out that these retrotransposed sequences may represent material on which evolution acts, especially to regulate the expression of their parental gene.

Results

Multiple retropseudogenes lineages characterize the evolution of the TARDBP gene in primates.

According to our phylogenetic and synteny analyses, we identified retropseudogenes of the TARDBP gene that originated at different times during the evolution of primates. We identified retropseudogenes originated in the ancestor of anthropoids, between 67 and 43.2 million years ago, in the ancestor of catarrhines, between 43.2 and 29.44 million years ago, and in the ancestor of lemuriformes, i.e. the strepsirrhine clade that inhabit madagascar, between 59.3 and 55 million years ago (Fig. 1). More recently in evolutionary time, we found species-specific retropseudogenes in the philippine tarsier (*Carlito syrichta*), Bolivian squirrel monkey (*Saimiri boliviensis*), capuchin monkey (*Cebus capucinus imitator*) and vervet

(*Chlorocebus sabaeus*). All of them did not have intron sequences and were identified on a different autosome in comparison to the chromosomal location of the functional copy (Fig. 1). Our gene tree did not significantly deviate from the most updated phylogenetic hypotheses for the main group of primates (Pozzi et al. 2014; Finstermeier et al. 2013; Perelman et al. 2011), suggesting that the functional copy of the TARDBP gene was present as a simple copy gene in the ancestor of the group (Fig. 1).

We recovered three highly supported monophyletic groups containing representative species of all major groups of anthropoids i.e. apes, Old World monkeys and New World monkeys (light blue, brown and green lineages, Fig. 1), indicating that these retropseudogenes originated in the ancestor of the group, between 67 and 43.2 millions of years ago, and were maintained in representative species of all descendant primate groups. The retropseudogene lineage depicted with the purple shading (Fig. 1), although it was not recovered monophyletic, our synteny analyses suggest that it indeed belongs to a single lineage (Fig. 1). Representative species of the three purple clades possess the same flanking genes, DIAPH3 at the 5' side and TDRD3 at the 3' side of the retropseudogene, strongly suggesting that the lack of monophyly could be attributed to a phylogenetic artifact (Fig. 1). The small number of changes, as illustrated by the short branches that define the sister group relationships of the main clades, could be the main cause (Fig. 1). We also found a retropseudogene lineage that according to our phylogenetic tree originated in the ancestor of catarrhine primates, the group that includes apes and Old World monkeys (blue lineage, Fig. 1), between 43.2 and 29.44 million years ago. In this case, we recovered a clade containing the functional copy of the TARDBP gene in catarrhines (upper pink lineage, Fig. 1), sister to a group containing a retropseudogene in the same primate group (blue lineage, Fig. 1). The clade containing TARDBP functional sequences from New World monkeys was recovered sister to the above mentioned clade (Fig. 1). In this clade in addition to the functional TARDBP copy, we found New World monkey specific retropseudogenes for which the evolutionary history is difficult to resolve given the shortness of the branches (Fig. 1). We identified three retropseudogenes, two in the capuchin monkey (*Cebus capucinus imitator*) and one in the Bolivian squirrel monkey (*Saimiri boliviensis*). Finally, we recovered a sequence from the Angola colobus (*Colobus angolensis*) (yellow branch, Fig. 1), which was recovered sister to a clade containing the TARDBP functional copy (pink lineage, Fig. 1) and three retropseudogenes lineages

(blue, purple and green clades, Fig. 1). The phylogenetic position of this branch in our gene tree suggests that it represents a retropseudogene originated in the anthropoid ancestor, but only conserved in this species. In support of this claim, the single flanking gene (PRMT2) found in the genomic piece containing the TARDBP retropseudogene in the Angola colobus is not shared with any other gene lineage described in this study (Fig. 1). In all cases, the identified retropseudogenes during the evolutionary history of anthropoid primates have premature stop codons and indels (supplementary figures 1 to 5).

We also identified retropseudogenes in tarsiers and strepsirrhines (Fig. 1). We found a single retropseudogene in the Philippine tarsier (*Carlito syrichta*), which shows the hallmark of a sequence free from selective constraints, i.e., a long branch as a signal of an accelerated rate of evolution in comparison to the functional copy (Fig. 1). In the strepsirrhine clade we identified a highly supported lineage containing the TARDBP functional copy in three species, greater bamboo lemur (*Prolemur simus*), coquerel's sifaka (*Propithecus coquereli*) and the mouse lemur (*Microcebus murinus*), which in turn was recovered sister to an also highly supported clade containing retropseudogenes in the greater bamboo lemur (*Prolemur simus*) and coquerel's sifaka (*Propithecus coquereli*) (Fig.1). This tree topology suggests that this retrocopy originated in the ancestor of lemuriformes, i.e. the strepsirrhine clade that inhabits Madagascar, between 59.3 and 55 million years ago, and it has been maintained in the genome of descendant species. Finally, the functional copy of the bushbaby (*Otolemur garnettii*) was recovered sister to the lemuriformes clade. Similar to the case of anthropoids, all retropseudogenes identified in tarsiers and strepsirrhines have premature stop codons and indels (supplementary figures 6 and 7).

Discussion

In this study we revealed that the evolutionary history of TARDBP, an RNA-binding protein involved in several human neurodegenerative disorders (Sreedharan et al. 2008; Kabashi et al. 2008), is characterized by the presence of retropseudogenes that originated at different ages during the evolutionary history of primates. An important fraction of the retropseudogenes originated in the anthropoid ancestor, between 67 and 43.2 million years ago, and has remained in the genome of the species (Fig. 2). This phenomenon fits the expectation of a peak of retrocopy formation around 40 million years ago, which coincides with an increased activity of L1

retroelements that produced an explosion in SINE/Alu repeat amplification (Ohshima et al. 2003; Marques et al. 2005). Interestingly, this period of time represents a key moment during the evolutionary history of primates, the radiation of the anthropoid lineage, where significant morphological and physiological traits arose (Kay et al. 1997).

Although we did not find retrocopies, i.e. intronless copies with the potential to produce a similar protein compared to the parental gene, it has been suggested that retropseudogenes could encode small interfering RNAs that can regulate the expression of their parental genes (Tam et al. 2008; Watanabe et al. 2008). Thus, this period of Vesuvian mode of evolution could be seen as a source of evolutionary novelty that fueled the origin of the phenotypes that define the anthropoid lineage (Long et al. 2003; Kaessmann et al. 2009; Casola & Betrán 2017). Other retropseudogenes originated in the catarrhine ancestor, between 43.2 and 29.44 million years ago, and in other primate groups (Fig. 2).

In agreement with the literature, and given the nature of the process originating retrocopies, all of them seem to be non-functional as canonical TARDBP (Zhang 2003), which can be verified by the presence of indels and premature stop codons (supplementary figures 1 and 7). The identification of several retropseudogenes for the TARDBP gene in primates appears to be not a surprise as this gene complies with all the requisites to be a gene with multiple retropseudogenes (Zhang & Gerstein 2004; McDonnell & Drouin 2012; Gonçalves et al. 2000), i.e., short transcripts (61 to 414 amino acids) (Yates et al. 2020), widely and highly expressed (Uhlén et al. 2015), low GC-content (47%, average among 23 primate species) and highly conserved (3.37%, maximal divergence among primates). Furthermore, in agreement with slow rate of length abridgment, the identified retropseudogenes possess a length (mean 1128 bp, median 1193 bp) similar to the functional TARDBP gene (1245 bp).

Among apes, the number of TARDBP retrotransposition events appear to be higher in comparison to the average number of retrocopies per parental gene in their genomes (Navarro & Galante 2015). On average, ape genomes possess 2.91 retrocopies per parental gene (Navarro & Galante 2015), however in our study we identified five TARDBP retropseudogenes in each examined ape species. Coincident with previous evidence, we also found a higher number of retropseudogenes in New World monkeys (Navarro & Galante 2015). Although it is not clear the reason why this primate group possesses more retrocopies in comparison to catarrhines, it is suggested

that a lineage specific expansion of L1PA1 and L1P3 subelements could be linked to the observed pattern (Navarro & Galante 2015; Casola & Betrán 2017).

In conclusion, in this work, we demonstrate that the TARDBP gene in primates has an evolutionary history characterized by the presence of multiple retropseudogene lineages. In the ancestor of anthropoids occurred a burst of activity, which led to intronless sequences that can not give rise to functional proteins. However, we can not discard that these DNA retrotransposed sequences could represent the raw genetic material for the evolution to act on, especially to regulate the expression of their parental gene, as it has been described for other genes.

Material and Methods

DNA sequences

DNA sequences and phylogenetic analyses

We performed searches for TAR DNA Binding Protein (TARDBP) genes in primate genomes in Ensembl v.102 (Yates et al. 2020). We retrieved primate orthologs, using the human (*Homo sapiens*) entry, based on the ortholog prediction function of Ensembl v.102 (Yates et al. 2020). We identified TARDBP retropseudogenes in primate species by performing BLASTN searches (Altschul et al. 1990), against the whole genome sequence in Ensembl v.102 (Yates et al. 2020) using default settings. In each case the query sequence (TARDBP) was from the same species of the genome in which retropseudogenes were looking for. In our searches, a retropseudogene was recognized as a sequence containing all exons together and found in a different chromosome in comparison to the functional copy. Genomic fragments containing retropseudogenes were extracted and manually annotated by comparing the coding sequence of the same species using the program Blast2seq v2.5 (Tatusova & Madden 1999) with default parameters. Accession numbers and details about the taxonomic sampling are available in Supplementary Table S1.

Nucleotide sequences were aligned using MAFFT v.7 (Katoh & Standley 2013), allowing the program to choose the alignment strategy (FFT-NS-i). We used the proposed model tool of IQ-Tree v.1.6.12 (Kalyaanamoorthy et al. 2017) to select the best-fitting model of nucleotide substitution, which selected GTR+F+R3. We used the maximum likelihood method to obtain the best tree using the program IQ-Tree v1.6.12 (Trifinopoulos et al. 2016). We assessed support for the nodes using three strategies: a Bayesian-like

transformation of aLRT (aBayes test) (Anisimova et al. 2011), SH-like approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010) and the ultrafast bootstrap approximation (Hoang et al. 2018). We carried out 20 independent runs to explore the tree space, and the tree with the highest likelihood score was chosen. TARDBP sequences from the African elephant (*Loxodonta africana*), blue whale (*Balaenoptera musculus*) and red fox (*Vulpes vulpes*) were used as outgroups.

Assessment of conserved synteny

We examined genes found upstream and downstream of functional copies and retropseudogenes. We used the estimates of orthology and paralogy derived from the Ensembl Compara database (Herrero et al. 2016); these estimates are obtained from a pipeline that considers both synteny and phylogeny to generate orthology mappings. These predictions were visualized using the program Genomicus v100.01 (Nguyen et al. 2018). Our assessments were performed in representative species for each lineage.

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Author contributions

JCO and GAM designed the study. KZ, JCO collected and analyzed data. JCO and GAM wrote the manuscript. LV-C, FJM reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

Competing Interests

The authors declare no competing of interest

Figure legends

Figure 1. Maximum likelihood tree showing sister group relationships between the functional copy of TARDBP and primate retropseudogenes. Numbers on the nodes correspond to support values from the ultrafast bootstrap routine. TARDBP sequences from the African elephant (*Loxodonta africana*), blue whale (*Balaenoptera musculus*) and red fox (*Vulpes vulpes*) were used as outgroups (not shown). The scale denotes substitutions per site and colors represent lineages. The pink lineage represents the TARDBP functional copy. Synteny information is provided for each lineage at the right side of the figure.

Figure 2. Time calibrated primate phylogeny showing the origin of the different retropseudogene lineages depicted in figure 1. Numbers on the triangles correspond to retropseudogenes originated within a particular group of primates. Silhouette images were obtained from PhyloPic (<http://phylopic.org/>). Divergence times were obtained from timetree (<http://www.timetree.org/>)(Kumar et al. 2017).

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427

Human Chr1 TARDBP	1 ATG M	TCT S	GAA E	10 TAT Y	ATT I	CGG R	20 GTA V	ACC T	GAA E	30 GAT D	GAG E	AAC N	40 GAT D	GAG E	CCC P	ATT I	50 GAA E	ATA I	CCA P	60 TCG S	GAA E	GAC D	GAT D	70 -GG -G	GAC T	GGT V	80 GCT L	GCT L	CTC S	90 CAC T
Human Chr2	ATG M	TCT S	GAA E	TAT Y	ATT I	CGG R	GTA V	ACC T	GAA E	GAT D	GAG E	AAC N	GAT D	GAG E	CCC P	ATT I	GAA E	ATA I	CCA P	TCG S	GAA E	GAC D	GAT D	-GG -G	GAC T	GGT V	GCT L	GCT L	GTC S	CAC T
Macaque Chr12	GTG V	TCT S	GAA E	TAT Y	ATT I	CAG Q	GTA V	ACC T	GAA E	GAT D	GAG E	AAC N	GAT D	GAG E	CCC P	ATT I	GAA E	ATA I	TCA S	TCG S	CAA Q	GAC D	GAT D	GGG G	GAC D	AGT S	GCT A	GCT A	GTC V	CAC H
Human Chr1 TARDBP	GGT V	TAC T	AGC A	100 CCA Q	GTT F	TCC P	110 AGG G	GGC A	GTG C	120 TGG G	GCT L	TCG R	130 CTA Y	CAG R	GAA N	TCC P	140 AGT V	GTC S	TCA Q	150 GTG C	TAT M	GAG R	160 AGG G	TGT V	CCG R	GCT L	GGT V	AGA E	AGG G	180 AAT I
Human Chr2	GGT V	TAC T	AGC A	CCA Q	GTT F	TCC P	AGG G	GGC A	GTG C	TGG G	GCT L	TCG R	CTG C	CAG R	GAA N	TCC P	GGT V	GTC S	TCA Q	ATG C	TAT M	GAG R	AGG G	TGT V	CCG R	GCT L	GGT V	AGA E	AGG G	AAT I
Macaque Chr12	GCT A	TAT Y	GGC G	ACA T	GTT V	TCC S	AGG R	GGC G	GTG V	TGG W	GCT A	TCG S	CTA L	CAG Q	AAA K	TCC S	AGC S	GTC V	TCA S	ATG M	TAT Y	GAG E	AGG R	TGT C	ACG T	GCT A	GGT G	AGA R	AGG R	AAT N
Human Chr1 TARDBP	TCT L	GCA H	TGC A	190 CCC P	AGA D	TGC A	200 TGG G	CTG W	GGG G	210 AAA N	TCT L	GGT V	220 GTA Y	TGT V	TGT V	CAA N	230 CTA Y	TCC P	AAA K	240 AGA D	TAA N	CAA K	250 AAG R	AAA K	AAT M	GGA D	260 TGA E	GAC T	AGA D	TGC A
Human Chr2	TCT L	GCA H	TGC A	CCC P	CGA D	TGC A	TGG G	CTG W	GAG R	AAA N	TCC P	GGT V	GTA Y	TGT V	TGT V	CAA N	CTA Y	TCC P	GAA K	AGA D	TAA N	CAA K	AAG R	AAA K	AAT M	GGG G	TGA E	GAC T	AGA E	AGC A
Macaque Chr12	TCT S	GCC A	TGC C	CCC P	CGA R	TGC C	TGG W	CTG L	GGG G	AAA K	TCT S	GGT G	GTA V	TGT C	TGT C	CAA Q	CTA L	TCC S	GAA E	AGA R	TAA *	CAA Q	AAG K	AAA K	AAT N	GGA G	TGA *	GAC D	AGA R	TGC C
Human Chr1 TARDBP	TTC S	ATC S	AGC A	280 AGT V	GAA K	AGT V	290 GAA K	AAG R	AGC A	300 AGT V	CCA Q	GAA K	310 AAC T	ATC S	CGA D	TTT L	320 AAT I	AGT V	GTT L	330 GGG G	TCT L	CCC P	ATG W	340 GAA K	AAC T	AAC T	350 CGA E	ACA Q	GGA D	360 CCT T
Human Chr2	TTC S	ATC S	AGC A	AGT V	GAA K	AGT V	GAA K	AAG R	ACC P	AG- -	---- ----	---- ----	---- ----	-TC -V	CGA R	TTT F	AAT N	AGT S	GTT V	GGG G	TCT S	CCC P	ATG M	GAA E	AAC N	AAC N	TGA *	ACA T	GGA G	CCT P
Macaque Chr12	TTC F	ATG M	AGC S	AGT S	GAA E	AGT S	GAA E	AAG K	AGT S	AGT S	CTA L	GAA E	AAC N	ATC I	CGA R	TTT F	AAC N	AGT S	GTT V	GGG G	TCT S	CCC P	GTG V	GGG G	AAC N	GAC D	TGA *	ACA T	GGG G	TCT S

Supplementary figure 1. Nucleotide alignment of the TARDBP functional copy of humans (*Homo sapiens*) and TARDBP retrocopies in representative species of primates in which the retrocopy was identified corresponding to the blue lineage on figure 1. The shading highlights the mutations that make the retrocopies non-functional.

Human Chr1 TARDBP	1 ATG M			10 TAT Y			20 GTA V			30 GAT D			40 TGA E			50 TGA E			60 ATC S			70 CGA D			
Human Chr13	ATA I	TCT S	GAA E	TAT Y	ATT I	CGC R	GT- V-	ACC P	GAA K	GAT M	GAG R	AAG R	GA- -	TGA M	GCC S	CAT P	TGA L	AAT K	ACC Y	ATC H	GGA R	AGA K	CGA T	TGG M	
Macaque Chr17	ATA I	TCT S	GAA E	TAT Y	ATT I	CGG R	GTA V	ACC T	AAA K	GAT D	GAG E	AAC N	GGT G	TGA *	GCC A	CGT R	TGA *	AAT N	ACC T	ATT I	GGA G	AGA R	CGA R	TGG W	
Marmoset NTIC01000001	--- ---	ACT T	GAA E	TAT Y	AAT N	TGG W	GTA V	AGC S	GAA E	GAT D	GAG E	AAT N	GA- D-	TGA E	GCC P	CAT I	TGA D	CAT I	ACC P	ATC S	AGA E	AGA D	CAA N	TGG G	
Human Chr1 TARDBP				80 GCT L			90 GCT L			100 GTT F			110 AGG G			120 TGG G			130 CAG R			140 AGT V			
Human Chr13	GAC G	GGT R	GCT C	GCT C	GTC C	CAT P	GGT W	TAC L	AGC Q	CCA P	GTT S	TCC F	AGG Q	GGT G	GTG C	TGG V	GCT G	TCG F	CTA A	CAG T	AAA E	TCC I	AGT Q	GTC C	
Macaque Chr17	GAC D	AGT S	GCT A	GCT A	GTC V	CAC H	GGT G	TAC Y	AGC S	CCA P	GTT V	TCC S	AGG R	GGC G	ATG M	TGG W	GCT A	TCG S	CGA R	CAG Q	GAA E	TCC S	AGT S	GTC V	
Marmoset NTIC01000001	GAC T	AGT V	GCT L	TCT L	GTC S	CAT M	GGT V	TAC T	AGC A	CCA Q	GTT F	TCC P	AGG G	GGC A	ATG C	TGG G	GCT L	TCC P	TTA Y	CAG R	AAA N	TCC P	ACC P	GTC S	
Human Chr1 TARDBP				150 TA- -			160 TGA M			170 TCC V			180 TAG V			190 TGC L			200 CAG P			210 CTG A			
Human Chr13	TCA L	GTG S	TA- V	--- -	--- -	--- -	TGA *	GAG E	GTG V	TCC S	GGT G	TGG W	TAG *	AAG K	GAA E	TTC F	TTC F	ATG M	CCC P	CCG P	ATG M	CTG L	GCT A		
Macaque Chr17	TCA S	ATG M	TA- Y	--- -	--- -	--- -	TGA E	GAG R	GTG C	TCC P	GGC A	TGG G	TGG G	AAG R	GAA N	TTC S	TTC S	ATG C	CCC P	CCG R	GTG C	CTG W	GCT W		
Marmoset NTIC01000001	ACA Q	ATG C	TAT M	T G	GAG R	AGG G	TGA E	GAG R	GTG C	TCC P	GAC T	TGG G	TAG R	AAG R	GAA N	TGC A	TGC A	ATG C	CCC P	CTG *	ATG C	CTG W	GCT W		

Supplementary figure 2. Nucleotide alignment of the TARDBP functional copy of humans (*Homo sapiens*) and TARDBP retrocopies in representative species of primates in which the retrocopy was identified corresponding to the purple lineage on figure 1. The shading highlights the mutations that make the retrocopies non-functional.

	1	10	20	30	40	50	60														
Human Chr1 TARDBP	A T G M	T C T S	G A A E	T A T Y	A T T I	C G G R	G T A V	A C C T	G A A E	G A T D	G A G E	A A C N	G A T D	G A G E	C C C P	A T T I	G A A E	A T A I	C C A P	T C G S	G A A E
Human Chr8	A T G M	T C T S	G A A E	T A T Y	A T T I	C G G R	G T A V	A C C T	A A A K	G A T D	G A G E	A A C N	A A T N	G A G E	C C C P	A T T I	T A A *	A T A I	C C T P	T C A S	G A G E
Macaque Chr8	A T G M	T C T S	G A A E	T A G *	A T T I	C A G Q	G T A V	A C C T	G A A E	G A T D	G A G E	A A C N	A A T N	T A G *	C C C P	A T T I	T A A *	A T A I	C C A P	C C A P	G A A E
Ma's night monkey KZ195588	A T A I	T T T F	G A A E	T A T Y	A C T T	C - G - R	G T A *	A C C P	A A A K	G A T M	G A G R	A A C T	A A T M	G A G S	C C C P	A - T -	G A A *	A T A N	C C A T	T T G I	G A A G
			70			80			90				100	105							
Human Chr1 TARDBP	G A C D	G A T D	G G G G	A C G T	G T G V	C T G L	C T C L	T C C S	A C G T	G T T V	A C A T	G C C A	C A G Q	T T T F							
Human Chr8	G A C D	G A A E	G G G G	A C G T	G T G V	C T G L	C T G L	T C C S	A C T T	G T T V	A C A T	G C - -	- - - -	- - - -							
Macaque Chr8	A A C N	A A T N	G G G G	A C G T	G C G A	C T G L	C T G L	T C C S	A T G M	A T T I	A C G T	G C - -	- - - -	- - - -							
Ma's night monkey KZ195588	G A T R	G A T *	G G G W	A C G D	G T G G	C T G A	C T G A	T C C V	A C A H	G T T S	A C A Y	G C - S	- - - -	- - - -							

Supplementary figure 3. Nucleotide alignment of the TARDBP functional copy of humans (*Homo sapiens*) and TARDBP retrocopies in representative species of primates in which the retrocopy was identified corresponding to the green lineage on figure 1. The shading highlights the mutations that make the retrocopies non-functional.

Human Chr1 TARDBP	1	ATG	TCT	GAA	TAT	ATT	CGG	GTA	ACC	GAA	GAT	GAG	AAC	GAT	GAG	CCC	ATT	GAA	ATA	CCA	TCG	GAA	GAC	GAT	GGG	ACG	GTG	CTG	CTC	TCC	ACG		
		M	S	E	Y	I	R	V	T	E	D	E	N	D	E	P	I	E	I	P	S	E	D	D	G	T	V	L	L	S	T		
Human Chr20		ATG	TCT	GAA	TAC	ACT	AGG	GTA	AGT	GAA	GTT	GAG	AAT	AAG	GAG	CTC	GTT	GAA	ATA	TCA	TCA	GAA	GAC	AAT	GGG	GTG	GTG	CTG	CTG	TCC	ACA		
		M	S	E	Y	T	R	V	S	E	V	E	N	K	E	L	V	E	I	S	S	E	D	N	G	V	V	L	L	S	T		
Macaque Chr10		ATG	TTT	GAA	TAC	ACT	TGG	GTA	AGT	GAA	GAT	GAG	AAT	AAG	GAG	CTC	ATG	GAA	ATA	TCA	TCA	GAA	TAC	AAT	GGG	GTA	GTG	CTG	CCG	TCC	ACA		
		M	F	E	Y	T	W	V	S	E	D	E	N	K	E	L	M	E	I	S	S	E	Y	N	G	V	V	L	P	S	T		
Marmoset NTIC01034389		ATG	TCT	GAA	TAT	ACT	CAA	GTA	ACT	GAA	GAT	GAG	AAT	AAG	GAA	CTC	TTT	GAA	AT	---	---	A	TCA	GAA	GAC	AAT	GGG	ATG	GTG	CTG	CTG	TCC	ATG
		M	S	E	Y	T	Q	V	T	E	D	E	N	K	E	L	F	E	I	---	---	S	E	D	N	G	M	V	L	L	S	M	
Human Chr1 TARDBP	100	GTT	ACA	GCC	CAG	TTT	CCA	GGG	GCG	TGT	GGG	CTT	CGC	TAC	AGG	AAT	CCA	GTG	TCT	CAG	TGT	ATG	AGA	GGT	GTC	CGG	CTG	GTA	GAA	GGA	ATT		
		V	T	A	Q	F	P	G	A	C	G	L	R	Y	R	N	P	V	S	Q	C	M	R	G	V	R	L	V	E	G	I		
Human Chr20		GTT	ACA	GCC	CAG	TTT	CCA	GGG	GCA	TGT	GGA	CTT	CAC	TAC	AAG	AAT	CCA	GTG	TCT	CAG	TGT	ATG	AGA	GGT	ATC	CAG	CTG	GTA	GAA	GGA	ATT		
		V	T	A	Q	F	P	G	A	C	G	L	H	Y	K	N	P	V	S	Q	C	M	R	G	I	Q	L	V	E	G	I		
Macaque Chr10		GTT	ACA	GCC	CAG	TTT	CCA	GGG	GCA	CGT	GAG	CTC	CAC	CAC	AAG	AAT	GCA	GTG	TCT	CAA	TGT	ATG	AAA	GGT	ATC	CTG	CTG	GTA	GAA	GGA	ATT		
		V	T	A	Q	F	P	G	A	R	E	L	H	H	K	N	A	V	S	Q	C	M	K	G	I	L	L	V	E	G	I		
Marmoset NTIC01034389		GGT	ACA	GTC	CAG	TTT	CCA	GGG	GCA	TGT	GGG	CTT	CAC	TAC	AAG	AAT	CCA	GTG	TCT	CAA	TGT	ATG	AGA	GGT	ATC	TAG	TTG	GCA	GAA	GGA	ATT		
		G	T	V	Q	F	P	G	A	C	G	L	H	Y	K	N	P	V	S	Q	C	M	R	G	I	*	L	A	E	G	I		
Human Chr1 TARDBP	190	CTG	CAT	GCC	CCA	GAT	GCT	GGC	TGG	GGA	AAT	CTG	GTG	TAT	GTT	GTC	AAC	TAT	CCA	AAA	GAT	AAC	AAA	AGA	AAA	ATG	GAT	GAG	ACA	GAT	GCT		
		L	H	A	P	D	A	G	W	G	N	L	V	Y	V	V	N	Y	P	K	D	N	K	R	K	M	D	E	T	D	A		
Human Chr20		CTG	CAT	G-C	CCT	GAT	GCT	GAC	TGT	GGA	AAT	CTG	GTA	TAT	GTT	GTC	AAC	TGT	CCC	AAA	GAT	AAC	AAA	AGA	AAA	ATG	GGT	GAG	A	---	---		
		L	H	-A	L	M	L	T	V	E	I	W	Y	M	L	S	T	V	P	K	I	T	K	E	K	W	V	R	---	---	---		
Macaque Chr10		CTG	CAT	GCC	CCT	GAT	GCT	GAC	AGT	GGA	AAT	CTG	GTA	TAT	GTT	GTC	AAC	TGT	CCC	AAA	GAC	AAC	AAA	AGA	AAA	ATG	GGT	GAG	A	---	---		
		L	H	A	P	D	A	D	S	G	N	L	V	Y	V	V	N	C	P	K	D	N	K	R	K	M	G	E	---	---	---		
Marmoset NTIC01034389		CTG	CAT	GCC	CCT	CAT	GCT	GCC	TGT	GGA	AAT	CTG	GTC	TAT	GTT	GTC	AAC	TAT	CCC	AAA	GCC	AAC	AAA	AGA	AAA	ATG	GAT	GAG	A	---	---		
		L	H	A	P	H	A	A	C	G	N	L	V	Y	V	V	N	Y	P	K	A	N	K	R	K	M	D	E	---	---	---		
Human Chr1 TARDBP	280	TCA	TCA	GCA	GTG	AAA	GTG	AAA	AGA	GCA	GTC	CAG	AAA	ACA	TCC	GAT	TTA	ATA	GTG	TTG	G	---	---	---	---	---	---	---	---	---			
		S	S	A	V	K	V	K	R	A	V	Q	K	T	S	D	L	I	V	L	--	---	---	---	---	---	---	---	---	---			
Human Chr20		---	---	CG	GCA	GTG	AAA	GTG	---	AGA	GCA	GTC	CAG	AAA	ATA	TCT	TAT	TTA	ATA	GCG	TTG	GTA	ATT	AGT	GAG	TTT	TTG	CTC	TC				
		---	---	R	Q	*	K	*	--	R	A	V	Q	K	I	S	Y	L	I	A	L	V	I	S	E	F	L	L	---				
Macaque Chr10		---	---	CA	GCA	GTG	AAA	GTG	---	AGA	GCA	GTC	CAG	AAA	ATA	TCT	TAC	TTA	ATA	GTA	CTG	GTA	ATT	AGT	GAG	TTT	TTG	CTC	TG				
		---	---	T	A	V	K	V	---	K	S	S	P	E	N	I	L	L	N	S	T	G	N	*	*	V	C	A	L				
Marmoset NTIC01034389		---	---	CA	TCA	GTG	AAA	GTG	---	AGA	GCA	GCC	CAG	AAA	ACA	TCT	TAT	TTA	ATA	GTG	TTG	GTA	ATT	AGC	GAG	TTT	GTG	--	C	TG			
		---	---	T	S	V	K	V	--	K	S	S	P	E	N	I	L	F	N	S	V	G	N	*	R	V	C	--	A	---			

Supplementary figure 4. Nucleotide alignment of the TARDBP functional copy of humans (*Homo sapiens*) and TARDBP retrocopies in representative species of primates in which the retrocopy was identified corresponding to the brown lineage in figure 1. The shading highlights the mutations that make the retrocopies non-functional.

Human Chr1 TARDBP	1 A T G	T C T	G A A	10 T A T	A T T	C G G	20 G T A	A C C	G A A	30 G A T	G A G	A A C	G A T	40 G A G	C C C	A T T	50 G A A	A T A	C C A	60 T C G	G A A	G A C	G A T	70 G G G	A C G	G T G	80 C T G	C T C
Human Chr6	A T A	T C T	G C A	T G T	A T T	C A G	G T A	A C T	A G A	G A C	A A G	A A T	T A T	G A G	C C C	A T T	G A A	A T A	C C A	T C A	G A A	G A T	G A T	A G A	A C A	G T G	C T G	C T G
Macaque Chr4	---	---	---A	T G T	A T T	C A G	G T A	A C C	A G A	G A T	A A G	A A T	T A T	G A G	C C C	A T T	G A A	A T A	C C A	T C G	G A A	G A C	G A T	G G A	A C A	G T G	C T G	C T G
Marmoset NTIC01022223	---	---	---	M	Y	S	G	N	Q	R	*	E	L	*	A	H	*	N	T	I	G	R	R	W	N	S	A	A
	---	T C T	G A A	T A T	A T T	T A G	G T A	A C A	A T A	G A C	T A G	A A T	A A T	G A A	C C C	A T T	G A A	A T A	C C A	T C A	G A A	G T C	A A T	G G A	A T G	A T G	C T T	C T G
	---	S	E	Y	I	*	V	T	I	D	*	N	N	E	P	I	E	I	P	S	E	V	N	G	M	M	L	L
Human Chr1 TARDBP	90 T C C	A C G	G T T	A C A	G C C	100 C A G	T T T	C C A	G G G	110 G C G	T G T	G G G	C T T	120 C G C	T A C	130 A G G	A A T	C C A	140 G T G	T C T	C A G	150 T G T	A T G	A G A	G G T	160 G T C	C G G	C T G
Human Chr6	C C C	A C G	G T T	A C A	G T C	C A G	T T T	C C A	G G G	G C A	T G T	G G G	C T C	C C C	T A C	A G G	A T T	C C A	G T G	T C T	C A G	-G T	A T T	A G G	A G T	G T C	T-G	C T G
Macaque Chr4	C C C	A C G	G T T	G C A	G T T	C A G	T T T	C C A	G G G	G C A	T G T	G G G	C T T	C C C	T A C	A G G	A T T	C C A	G T G	T C C	C A G	G G T	A T T	A G G	G G T	G T C	T-G	C T G
Marmoset NTIC01022223	C C C	A T G	G T T	A--	---	---	---	-C A	G A G	G T A	T G T	G G G	C T T	C C C	T A C	A G G	A T T	C C T	A T G	T C T	T G G	G T T	A T T	A G A	G G T	A T C	T G G	C T G
	P	M	V	--	---	---	---	-T	E	V	C	G	L	P	Y	R	I	P	M	S	W	V	I	R	G	I	W	L
Human Chr1 TARDBP	170 G T A	G A A	G G A	A T T	C T G	C A T	G C C	C C A	G A T	G C T	G G C	T G G	G G A	210 A A T	C T G	G T G	T A T	G T T	G T C	A A C	230 T A T	C C A	A A A	G A T	A A C	A A A	A G A	A
Human Chr6	G T A	G A A	G G A	A T T	C T G	C A T	G C C	C C C	A G T	G C T	G G C	T G G	G G A	A A T	T T G	G T G	T A C	G T T	G T C	A A C	T A T	T C T	G T A	G A T	A A C	T A C	A G A	A
Macaque Chr4	G T A	G A A	G G A	A T T	C T G	C A T	G C C	C C C	A A T	G C T	G G C	T G G	G G A	A A T	T T G	A T A	T A T	G T T	G T C	A A C	T A T	T C T	G T A	G A T	A A C	T A C	A G A	A
Marmoset NTIC01022223	G T A	G A A	G G A	A T T	C T G	C A T	G G T	C C C	A A T	G A T	G G C	T G G	G G A	A A T	T T G	G T A	T A T	G T T	G T C	A A C	T A T	C C C	A A A	G A T	A A C	T A A	A G A	A
	V	E	G	I	L	H	G	P	N	D	G	W	G	N	L	V	Y	V	V	N	Y	P	K	D	N	*	R	

Supplementary figure 5. Nucleotide alignment of the TARDBP functional copy of humans (*Homo sapiens*) and TARDBP retrocopies in representative species of primates in which the retrocopy was identified corresponding to the light blue lineage in figure 1. The shading highlights the mutations that make the retrocopies non-functional.

Tarsier KE931768 TARDBP	1 A T G M	TCT S	GAA E	10 TAT Y	ATT I	CGG R	20 GTA V	ACT T	GAA E	30 GAC D	GAG E	AAT N	GAT D	40 GAG E	CCC P	ATT I	50 GAG E	ATA I	CCA P	60 TCA S	GAA E	GAT D	GAT D	70 GGG G	ACG T	GTA V	80 CTG L	CTG L	TCC S	90 ACG T
Tarsier KE944022	---	TCT S	GAA E	TAT Y	ATT I	CGG R	GTA V	ACT T	GAA E	GAC D	---	---	---	---	---	---	AA K	ATA Y	CCA Q	GCA Q	GAA K	GAT M	GAT M	GGG G	GAG R	GTA Y	CTA Y	CTG C	TTC S	CTG W
Tarsier KE931768 TARDBP	GTC V	ACG T	GCC A	100 CAG Q	TTT F	CCA P	110 GGA G	GCA A	TGT C	120 GGG G	CTG L	CGC R	TAC Y	130 CGG R	AAT N	CCA P	140 GTG V	TCG S	CAG Q	150 TGT C	ATG M	AGA R	GGT G	160 GTC V	CGA R	CTG L	170 GTG V	GAA E	GGA G	180 ATC I
Tarsier KE944022	GTC S	ACA Q	GCC P	CAG S	TTT F	CCA Q	GGG G	GCA H	TGT V	GGG G	CCG R	TGC A	TAC T	AGG G	AAT I	TCA Q	GTG C	TCT L	CAA N	TGT V	ATG *	AGA E	GAT I	ATC S	TGA D	CTG W	GAA K	GAA K	GGA E	ATC

Supplementary figure 6. Nucleotide alignment of the TARDBP functional copy of the philippine tarsier (*Carlito syrichta*) and TARDBP retrocopy identified in the same species. The shading highlights the mutations that make the retrocopy non-functional.

Bamboo lemur MPIZ01001738 TARDBP	1	ATG	TCT	GAA	TAT	ATT	CGG	GTA	ACC	GAA	GAT	GAG	AAT	GAT	GAA	CCC	ATT	GAA	ATA	CCA	TCA	GAA	GAC	GAT	GGG	ACC	GTA	CTG	CTG	TCC	ACA	90	
Bamboo lemur MPIZ01000479		ATG	TCT	GAA	TAT	GTT	CGG	GTA	ACT	GAA	GAC	GAG	AAT	GAC	GAG	CCC	ACG	GAA	ACG	CCA	TCG	GGA	GGT	GAC	GGG	ACG	GTG	CTG	CTG	GCC	GCA		
Mouse lemur Chr2		ATG	TCT	GAA	TAT	ATT	CGG	GTA	ACC	GAA	GAT	GAG	AAC	GAC	GAA	CCC	ATT	GAA	ATA	CCA	TCA	GAA	GAC	GAT	GGG	ACG	GTG	CTG	CTA	TCC	ACA		
Coquerel's sifaka KQ023781		ATG	TCT	GAA	TAT	ATT	CGG	GTA	ACT	GGA	GAT	GAG	----	----	----	CCC	ATT	GAA	ATG	CCA	TCC	AGA	GAG	GAC	AGG	ACG	GTG	CTG	CTG	TCA	GCA		
Bamboo lemur MPIZ01001738 TARDBP	100	GTT	ACA	GCC	CAG	TTT	CCA	GGG	GCC	TGT	GGG	CTG	CGC	TAC	AGG	AAT	CCA	GTG	TCT	CAA	TGT	ATG	AGA	GGT	GTC	CGG	CTG	GTA	GAA	GGA	ATT	180	
Bamboo lemur MPIZ01000479		GCT	GCA	GCC	CAG	TTC	CCA	GCG	CCA	TGT	CGG	CTG	CGC	TGC	GGG	AGT	CCC	GGG	TCG	CAG	TGT	AGG	AGA	GGC	C-T	CGG	CTG	GTA	GAA	GGG	ATT		
Mouse lemur Chr2		GTT	ACA	GCC	CAG	TTT	CCA	GGG	GCA	TGT	GGG	TTG	CGC	TAC	AGG	AAT	CCA	GTG	TCT	CAA	TGT	ATG	AGA	GGT	GTT	CGG	CTG	GTA	GAA	GGA	ATT		
Coquerel's sifaka KQ023781		GCT	GCA	GCC	CAG	TTT	CCA	GCG	GCA	TGT	GGG	CTG	TGC	TGC	AGG	GAT	CCC	AGG	TCT	CAA	TGT	ACG	AGA	GGC	A-T	CGG	CTG	GTA	GAA	GGA	ATT		
Bamboo lemur MPIZ01001738 TARDBP	190	CTG	CAT	GCC	CCC	GAT	GCT	GGT	TGG	GGG	AAT	CTG	GTA	TAT	GTT	GTG	AAC	TAT	CCC	AAA	GAT	AAC	AAA	AGA	AAA	ATG	GAT	GAG	ACA	GAT	GCT	270	
Bamboo lemur MPIZ01000479		CTG	CAG	GCG	CCC	GAT	GCT	GGT	TGG	GGA	AAT	CTG	ACA	-AT	GTT	GTC	AAC	TAT	CCC	AAA	GGT	AAC	AAA	AGA	AAA	ATG	GAA	GAG	ACA	GAA	GCT		
Mouse lemur Chr2		CTG	CAT	GCC	CCG	GAT	GCT	GGT	TGG	GGG	AAT	CTG	GTG	TAT	GTT	GTG	AAC	TAT	CCT	AAA	GAT	AAC	AAA	AGA	AAA	ATG	GAT	GAG	ACA	GAT	GCT		
Coquerel's sifaka KQ023781		CTG	CAG	GTC	CCT	GAT	GCT	GGT	TAG	GGA	AAT	CTG	ATA	TAT	GTT	GTC	AAC	TAT	CCC	AAA	GAT	AAC	AAA	AGA	AAA	ATG	GAC	GAG	ACA	GAA	GCT		
Bamboo lemur MPIZ01001738 TARDBP	280	TCA	TCA	GCA	GTG	AAG	GTG	AAA	AGA	GCA	GTC	CAG	AAA	ACA	TCT	GAT	TTA	ATC	GTG	TTG	GGT	CTC	CCA	TGG	AAA	ACA	ACT	GAA	CAG	GAT	CTA	360	
Bamboo lemur MPIZ01000479		TCG	CCA	GCA	GTG	AAA	GTG	ACC	AGA	GCA	GTC	CGG	AAA	ACA	TCT	GAC	TTA	ATA	GAG	TCG	CAT	CCC	AC-	--G	GAA	GAC	GCT	GCA	CAG	GAT	CTA		
Mouse lemur Chr2		TCA	TCG	GCA	GTG	AAG	GTG	AAA	AGA	GCA	GTC	CAG	AAA	ACA	TCT	GAT	TTA	ATC	GTG	TTG	GGT	CTC	CCG	TGG	AAA	ACA	ACT	GAA	CAG	GAT	CTA		
Coquerel's sifaka KQ023781		TCA	CCA	GCA	GTG	AAA	GTG	ACA	AGA	GCA	GTC	CGG	AAA	GCA	TCT	GAT	TTA	ATA	GAG	TTG	CAT	CCC	C--	---	---	---	---	---	---	---	---	---	
Bamboo lemur MPIZ01001738 TARDBP	370	AAG	GAA	TAT	TTT	AGT	ACC	TTT	GGT	GAA	GTT	CTT	ATG	GTG	CAG	GTC	AAG	AAA	GAT	ATT	AAA	ACT	GGT	CAT	TCA	AAA	GGG	TTT	GGC	TTT	GTT	450	
Bamboo lemur MPIZ01000479		AAG	GGA	---	--T	AGT	ACC	TTT	GGA	GAA	GTT	CTT	ATG	GTG	CAG	GTC	AAG	AAA	GAT	GTT	AAA	ATT	CCT	CAT	TCA	AAA	GGG	TTT	GGC	TTC	ATT		
Mouse lemur Chr2		AAG	GAA	TAT	TTT	AGT	ACC	TTT	GGT	GAA	GTT	CTT	ATG	GTG	CAG	GTC	AAG	AAA	GAT	ATT	AAG	ACT	GGT	CAT	TCA	AAA	GGA	TTT	GGC	TTT	GTT		
Coquerel's sifaka KQ023781		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
Bamboo lemur MPIZ01001738 TARDBP	460	CGT	TTT	ACG	GAA	TAT	GAA	A-C	GCA	GGT	GAA	AGT	AAT	GTC	ACA	GCG	ACA	TAT														501	
Bamboo lemur MPIZ01000479		CTT	TTC	ACA	GAA	TAT	GAA	ACC	CCA	GGT	GAA	AGT	GAT	GTC	ACA	GCA	ACA	TAT															
Mouse lemur Chr2		CTT	TAC	---	GGA	TAT	GAA	--C	CCA	GGT	GAA	AGT	AAT	GTC	ACA	GCG	ACA	TAT															
Coquerel's sifaka KQ023781		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---														

Supplementary figure 7. Nucleotide alignment of the TARDBP functional copy of the bamboo lemur (*Prolemur simus*) and TARDBP retrocopies in representative species of strepsirrhines in which the retrocopy was identified on figure 1. The shading highlights the mutations that make the retrocopies non-functional.