

**SUPPLEMENTAL MATERIAL**

**Figure S1. The *TBC1D3* locus is duplicated several times on chromosome 17.** (A) Snapshot adapted from the UCSC Genome Browser (<http://genome.ucsc.edu/>) showing the position of the *TBC1D3* loci found duplicated in chromosome 17. BAC clones position in the assembly and their accession numbers are shown; specific BACs containing the *TBC1D3* loci are highlighted in red, and their sequences are complete. Gene composition of the region is also provided. The possibility that these duplicated loci are the consequence of a wrong assembly of the human genome can be excluded for several reasons. Firstly, all copies of the *TBC1D3* gene are entirely contained in BAC clones whose sequences are complete. Four BACs contain two copies each of the *TBC1D3* gene, which represents the only common portion of sequence. Secondly, (B) two copies of the *TBC1D3* gene lay in a region of the chromosome 17 containing also the *CCL3* and *CCL4* genes, which also underwent several rounds of segmental duplication during evolution (Modi, 2004). Lastly, (C) a recent duplication of the *TBC1D3* ancestral locus that preceded a fusion event with a duplicated *USP32*, gene must be invoked to account for the generation of *USP6* (Paulding *et al.*, 2003). In line with this, *TBC1D3* duplicated locus at 17q23.2 (chr17:55440265-55451181) maps very closely to the *USP32* gene (~200 Kbp), suggesting that proximity of the two “ancestral genes” might have favored a recombination event during the evolution of chromosome 17 in the primate lineage.

**Figure S2. Exon and intron length conservation among the loci of *TBC1D3* and *RNTRE* from different organisms, and their protein products.**

(A) Exon and intron length of Human *TBC1D3* and *RNTRE*, and of Mouse and Xenopus *RNTRE*. Length of intron 5 and 6 of the Xenopus *RNTRE* locus cannot be assessed due to gaps in the genomic assembly. (B) Multiple alignment of the protein product of the above mentioned loci. ClustalX colors (reflecting the chemical properties of the aminoacids) are applied whenever group conservation exceeds 50%. GenBank protein accession numbers are as following: Hs\_RNTRE: AAH42943; Hs\_TBC1D3; NP\_115634; Mm\_RNTRE: BAC97847; Xt\_RNTRE: ORF predicted from mRNA CT030299.

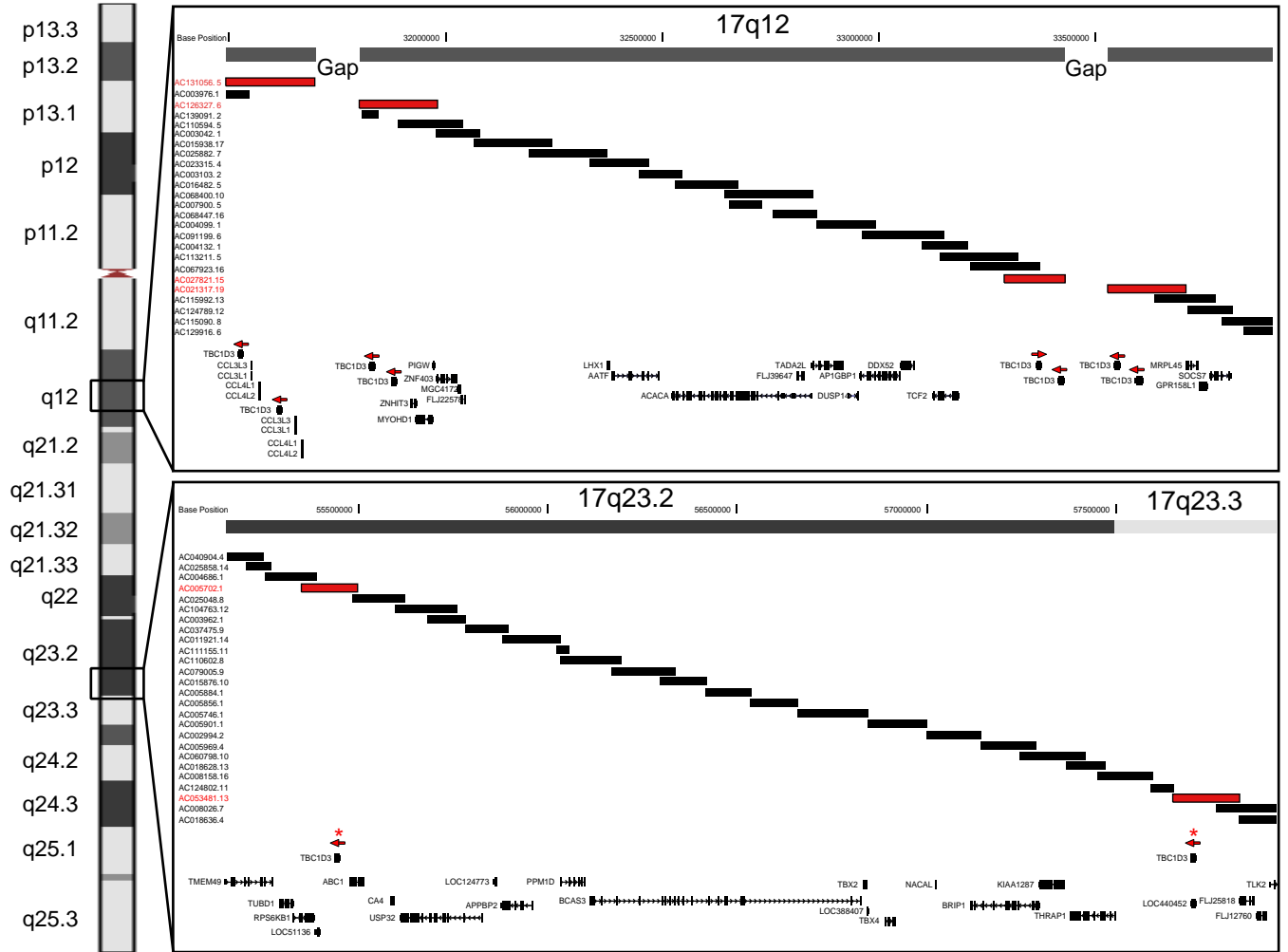
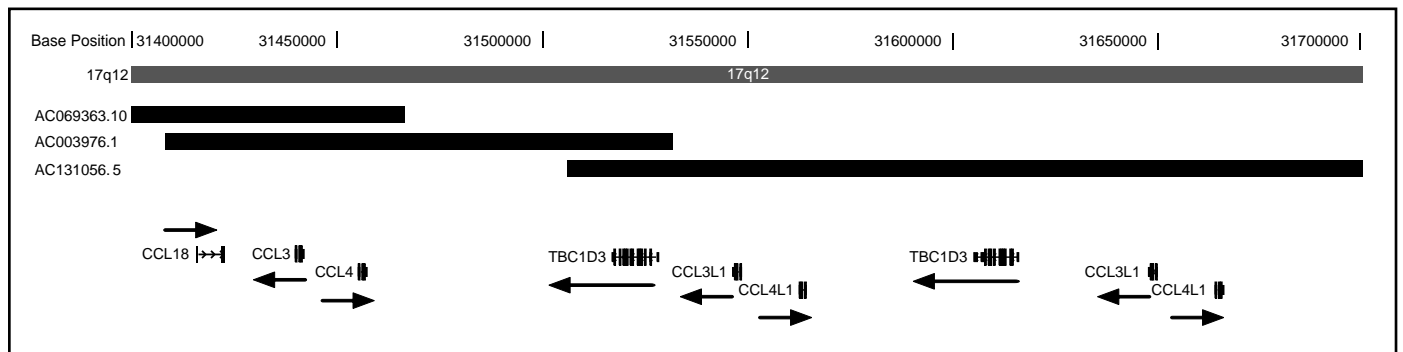
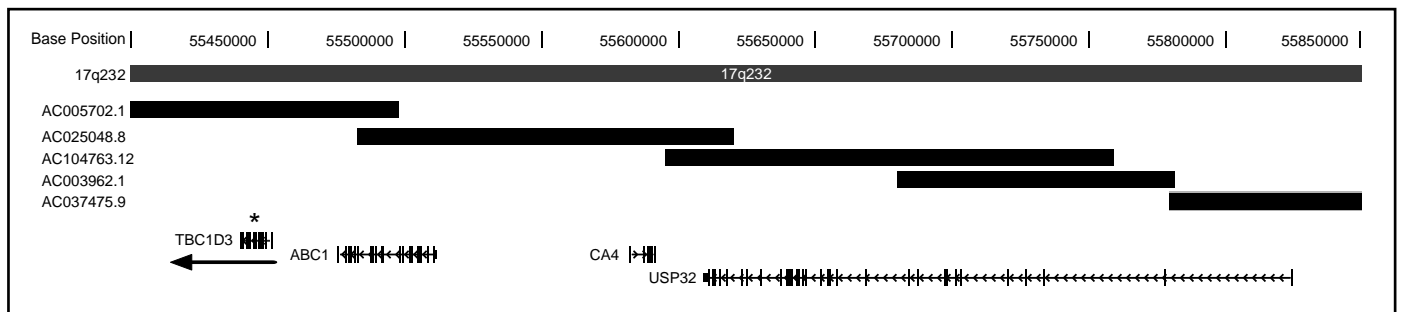
**Figure S3. Quantification of colocalization between TBC1D3, GGA3 and ARFQ67L.**

The extent of colocalization of coexpressed proteins in the experiments shown in Fig. 6 B-C of the main manuscript is reported at the left of each image, and was monitored using ImageJ (<http://rsb.info.nih.gov/ij/>, Colocalization Threshold plugin). After fixing the threshold values for each channel, spatial correlation was calculated and colocalizing pixels highlighted in white. The tables report the Manders Coefficients (tM1 and tM2, corresponding to the indicated color-coded signals of the expressed proteins), calculated over intensities higher than the set threshold.

## REFERENCES

Modi, W.S. (2004). CCL3L1 and CCL4L1 chemokine genes are located in a segmental duplication at chromosome 17q12. *Genomics* 83, 735-738.

Paulding, C.A., Ruvolo, M., and Haber, D.A. (2003). The Tre2 (USP6) oncogene is a hominoid-specific gene. *Proc Natl Acad Sci U S A* 100, 2507-2511.

**A****B****C****Figure S1**

**A**

H. Sapiens <i>TBC1D3</i> locus, 10898 bp			H. Sapiens <i>RNTRE</i> locus, 149574 bp			M. Musculus <i>RNTRE</i> locus 120590 bp			X. tropic. <i>RNTRE</i> locus, 79268 bp		
Exon	Exon length	Intron Length	Exon	Exon length	Intron Length	Exon	Exon length	Intron Length	Exon	Exon length	Intron Length
			1	185	13725	1	50	13413			
1	97	1428	2	87	70063	2	92	54596	1	59	30011
2	73	162	3	68	2031	3	68	3350	2	74	1007
3	86	1283	4	83	6811	4	83	5724	3	83	13679
4	40	482	5	40	8859	5	40	8609	4	40	1628
5	81	443	6	81	8386	6	81	5923	5	81	n.a.
6	108	329	7	108	7872	7	108	5862	6	108	n.a.
7	110	1255	8	110	2238	8	110	2409	7	110	384
8	49	543	9	49	1609	9	49	801	8	49	2664
9	121	713	10	121	3190	10	121	2502	9	121	1121
10	95	384	11	95	582	11	95	562	10	95	6196
11	66	463	12	66	98	12	66	94	11	66	2944
12	100	370	13	100	3048	13	100	2614	12	100	4671
13	153	981	14	153	17920	14	153	9782	13	153	8694
14	884		15	1797		15	3135		14	3945	

**B**

Hs_RNTRE	1	MN--DDQVALKLAQERAEIVAKYDRGREGAIEPWEADYLVYK-VDRDFGLHEEELFDHNAVERGRHLEIERITKWLKMLKWKERYKNTKPFERRIYKGIPLQLRGEVWALLLEIP	117
Mm_RNTRE	1	MN--DDQVALKLAQERAEIVAKYDRGREGAIEPWEADYLVYK-VDRDFGLHEEELPYHNAADRGRQLEIERITKWLKMLKWKERYKNTKPFERRIYKGIPLQLRGEVWALLLEIP	117
Xt_RNTRE	1	MNTEDDQASVRAAQRERAEIVAKYDRGREGAKIDPWEADFLLYK-VDRDFGLHKEELPIHDEVAQKQLLEIERITKWKVMKRSWDRYKNSKLEHRRRIYKGIPLQLRGEVWALLLEIVS	119
Hs_TBC1D3	1	MD--VVVAVASGAWAQRDEIDIMKYEGRHRAELPEDKGKPFRRYNNNVHLGIVHETLPELPIAREAKQIRRSISSKSKVMDLGDWEKYKSKRLLIDRAYKGMFNRRGEMMSVLLNTE	118
Hs_RNTRE	118	KMKEETRDLYSKLHFRAGCSFDIRQIDLDVNRTRFRDHIMFRDRYGVKQQSLFHVLAAYSIYNTYEVGYCGGMSQITALLLMYMNEDDAFWALVKLFSGPKHAMHGFFVQGFPKLLRFQEH	237
Mm_RNTRE	118	KMKEETRDLYSKLHFRAGCSFDIRQIDLDVNRTRFRDHIMFRDRYGVKQQSLFHVLAAYSIYNTYEVGYCGGMSQITALLLMYMNEDDAFWALVKLFSGPKHAMHGFFVQGFPKLLRFQEH	237
Xt_RNTRE	120	KLAEERKDLVLVLCQARRLSADIRQIDLDVNRTRFRDHIMFRDRYGVKQQALFHVLAAYSIYNTYEVGYCGGMSQITALLLMYMNEDDAFWALVKLFSGPKHAMHGFFVQGFPKLLRFQEH	239
Hs_TBC1D3	119	EMLKNLNGRQIMREKCKKSEHFKRIRDFVSGTLKHKIFFNDRYGTKRELLHILLAAEYNYEVGYGRDLHIAALFLLYLPEEADFWALVQLLASEHSLGQFHSNGGTVQGLDQ	238
Hs_RNTRE	238	HEKILNKFLSKLQHLDSOIEYTSFYTMKWFQCFPLDRTPPTLNRIRWIDYIFEGEVRTLTAMSYTILKHLKHLKMLKSMBELVEFFLOETLAKDFFPEDDFVIEQLQVSMTELKRAKRLDLP	357
Mm_RNTRE	238	HEKILNKFLSKLQHLDSOIEYTSFYTMKWFQCFPLDRTPPTLNRIRWIDYIFEGEVRTLTAMSYTILKHLKHLKMLKSMBELVEFFLOETLAKDFFPEDDFVIEQLQVSMTELKRAKRLDLP	357
Xt_RNTRE	240	HDRIILKFMFKLQHFETQELYTSFYTMKWFQCFPLDRTPPTLNRIRWIDYIFEGEVRTLTAMSYTILKHLKHLKMLKSMBELVEFFLOETLAKDFFPEDDFVIEQLQVSMTELKRAKRLDLP	359
Hs_TBC1D3	239	QEHVYVATQPKMGMHQKDKLGCQSPGLCLIRILLDIGLGLTLRLWVYLVGEQALMLETIRIAFKVQKRLTNTRCGEWARFCNRFVDTWARDIEDVLKHLRASMKKLTKKKGLDF	358
Hs_RNTRE	358	EPGKEDEYPKKPLGLQPPLEQSGWVHHLNGQRSVGRSPFLASGRRESGAPHRREKSPHEQSTGTGTPER-AQEPERRASVEEEKLKLDEADFOFKLSEGE-QDSFRQYNHAAAANSNSA	475
Mm_RNTRE	358	EPGKEDEYPKKPLGLQPPLEQSGWVHHLNGQRSVGRSPFKTSSRNEDGSPKKNHESPVHHSNNGTPEP-AGQSRNASVDEGSKNLHRAEASQKFKSPGM-QDSFRHYNHAAAANSNSA	473
Xt_RNTRE	360	PPKDEEFPKPLGLQIPPEPQPTLVLNHVNGRTRHRLPQVKSDYSPSPSLLHNSNNRNOQKNSMEXENLKMKAQGHASRFQNKVEIYRYSDDYSLNKGDKLYNHAANNSNSA	479
Hs_TBC1D3	359	PPAK--PPCGSASREVPARGGKTLCKDHRQAPPPPE-----ARFPRFLNSASPPRAPSSSTLCP-----GGAVEDTIVFGTGVGPF	435
Hs_RNTRE	476	TSIRKREFEVKNWKEFSDVSAETRTAKYITMECKGRAAHFALAVTVPGEAEVRSVNRPKMKALDAEDGKRGTSASQYDINVPVGGESASVSEALERAYSQSERHALYPPSPR-KHAEFSS	594
Mm_RNTRE	474	ISNRKREFMKWRKPSDASAIEFTTKYAVCKSHSALPALEVALGSAETRLNENSGQMMLDGGCKRGNASQYDINVPVGGESASVSEALERAYSQSERHALYPPSPR-KHAEFSS	583
Xt_RNTRE	480	TPASAGSITLWKMSPEE-KNLDLLNIAECKGKPEDNANALQTSQAVI----VQKSRVDTE-CGRKSNASLDVAVPGE-----DSENTDENRHSSDSFTFRNTVYSTTASENPSKGS	590
Hs_TBC1D3	436	HALAGGGGQSGNHFQWNNMELRFTLDDVGE-----PWRHYDPRQSCWVRALSGPD-----	487
Hs_RNTRE	595	SPSKVSN--KFTFKVQPPSHARYSPSLDGEARGLAHPPSYSNPPVYHGNSPKHFFPTANSPFASPOFSPGTDLNPSRRRPHGSLTYSVA--PEKYSRSPSPVLVPSRIEVLVDTGAGGY	710
Mm_RNTRE	584	SPPKVSN--KFTFKVQPPSHVRYPPQLPEEDHRAAYPPSYSNPPVYHGNSPKHVFPTAHGFGVSTQISPRPQINPSRRRPHGSLTYSVDS--PEKYSRSPSPVLVPSRIEVLVDTGAGGY	699
Xt_RNTRE	591	SPSLSSENSIVFVHSPHSHVQIQEEDDVFSIKQPPPIYSNPPYIYENS---VTKPHE---FOYSRINLMSPSKRSYVQSFRLPPDKMPSANITATQYNTVTSFHRIEVLVPADESATL	703
Hs_TBC1D3	488	-----QLACQWAEHFAERVRSFAAPSTSDQGFPRARDE-----QCAPISGRCCLGLHLESQFFPGE-----	549
Hs_RNTRE	711	SGNSGSPKNGKLIIPVDYLPNRTMSEVSYTYRETCGQSWTRDAHS---ENLPKYSAFQLAFFDHLGLFAVSVDPVRYKMSAAEDASGSPGYVSGPPPPAYHYRN-RDGLSIQSSV	826
Mm_RNTRE	700	SSSGSKNGQILIPVDYLPENRKNSEVSYTYRETCGQSWTRDAHS---ENLPKYSAFQLAFFDHLGLFAVSVDPVRYKMSAAEDASGSPGYVSGPPPPAYHYRN-RDGLSIQSSV	817
Xt_RNTRE	704	LSSFNQKNSQVILIPVDYHTHTFPPDAAPG---FICGPLNREVSQSYLSSLQKSENLPSTGCHYNTSSVSAISVRLRITSRSNKNPELQYLVSTSGATQFFRNPFDGIIMHESV	819

**Figure S2**

ARF6-Q67L + TBC1D3

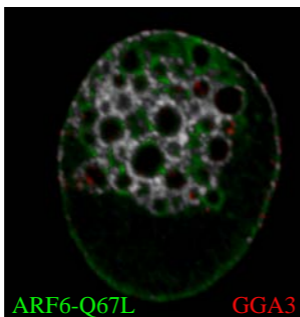


ARF6-Q67L

TBC1D3

tM1	0.6764
tM2	0.5285

ARF6-Q67L + GGA3

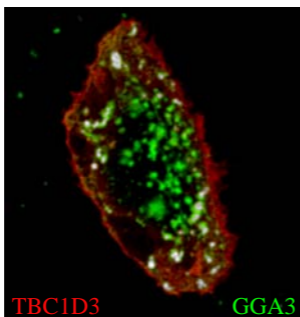


ARF6-Q67L

GGA3

tM1	0.9295
tM2	0.7087

TBC1D3 + GGA3

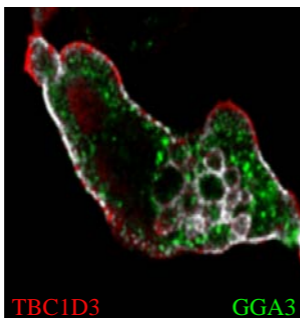


TBC1D3

GGA3

tM1	0.1948
tM2	0.2040

ARF6-Q67L +  
TBC1D3 + GGA3



TBC1D3

GGA3

tM1	0.8557
tM2	0.8438

Figure S3