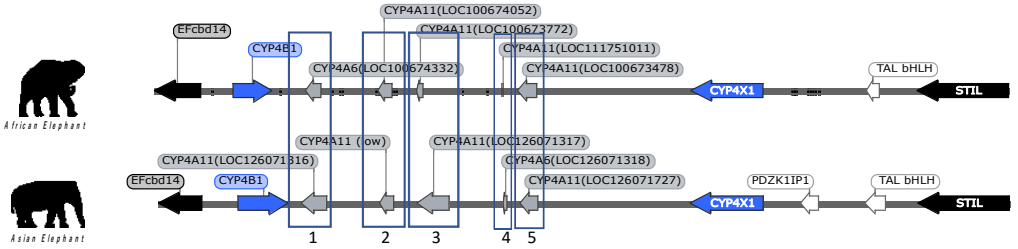
Confirmation of pseudonization in CYP4As gene



Group 1

number of aligned nucleotide	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369
translated to animo acid Human CYP4A11	M			K			V			I			L			G			R			S			P			K			S			H			G			S			Y			R			F			L		
H. sapiens CYP4A11 NM 000778	A	T	G	A	A	G	G	T	G	A	T	T	C	T	G	G	G	G	A	G	A	T	C	A	G	A	C	C	C	G	A	A	A	T	C	C	C	A	T	G	G	T	T	C	C	T	A	C	A	G	A	T	T	C	C	T	G
H. sapiens CYP4A22 NM 001010969 XM 208213	A	T	G	A	A	G	G	T	G	A	T	T	C	T	G	G	G	G	A	G	A	T	C	A	G	A	C	C	C	G	A	A	A	T	C	C	C	A	T	G	G	A	T	C	C	T	A	C	A	A	A	T	T	C	C	T	G
O. afer afer XM 007946703 LOC103201934	A	T	G	A	A	G	G	T	G	A	T	T	C	T	A	G	G	G	A	G	A	T	C	A	G	A	C	C	C	A	A	A	G	T	T	A	A	G	A	G	A	A	T	A	C	C	T	A	C	A	G	A	T	T	C	A	T	G
E. edwardii XM 006902916 LOC102867299	A	T	T	A	A	G	G	T	G	A	T	T	C	T	G	G	G	G	A	G	A	T	C	A	G	A	C	C	C	A	A	A	G	T	C	T	A	C	T	A	G	A	A	C	C	T	A	C	A	G	A	T	T	C	C	T	G
E. edwardii XM 006902913 LOC102862835	A	T	G	A	A	G	G	T	G	G	T	T	C	T	G	G	C	G	A	G	A	T	C	A	G	A	T	G	T	A	A	A	G	A	C	T	C	A	T	G	A	G	C	G	C	T	A	C	C	G	A	T	T	C	C	T	A
T. manatus latirostris XM 004371774 LOC101346203	G	C	G	A	A	G	G	T	G	A	T	T	C	T	G	G	G	G	A	G	A	C	C	A	G	A	C	C	C	A	A	A	A	T	C	T	C	A	T	G	A	G	C	C	C	T	A	C	A	G	A	T	T	C	C	T	G
T. manatus latirostris XM 012554256 LOC101345950	A	T	G	A	A	G	G	T	G	A	T	T	A	T	G	G	G	G	A	G	A	T	C	A	G	A	C	C	C	A	A	G	G	C	T	A	A	T	G	A	T	G	C	T	T	A	T	A	G	A	T	T	C	C	T	G
L. Africana CYP4A11 LOC100673772 NW 003573437	T	G	A	A	G	G	T	G	G	T	T	A	T	G	G	A	G	A	G	A	T	C	A	G	A	C	A	C	A	A	A	G	G	C	T	A	A	C	G	A	T	G	C	C	T	A	C	G	G	A	T	T	C	C	T	A	G
E. maximus CYP4A11 LOC126071317 NC 064821	T	G	A	A	G	G	T	G	G	T	T	A	T	G	G	A	G	A	G	A	T	C	A	G	A	C	A	C	A	A	A	G	G	C	T	A	A	C	G	A	T	G	C	C	T	A	C	G	G	A	T	T	C	C	T	A	G
translated to animo acid E.maximus CYP4A11	*			R			W			L			W			R			D			Q			T			Q			R			L			T			M			P			T			D			S			*		

EXON2 EXON3

Group 2

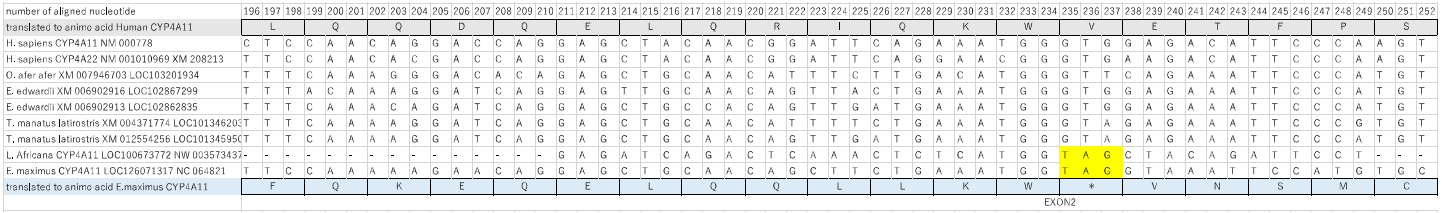
number of aligned nucleotide	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141
translated to animo acid Human CYP4A11	L			L			L			L			L			I			K			A			V			Q			L			Y			L			H			R			Q			W			L			L			K			A		
H. sapiens CYP4A11 NM 000778	C	T	G	C	T	T	C	T	G	C	T	G	C	T	G	A	T	C	A	A	G	G	C	A	G	T	T	C	A	G	C	T	C	T	A	C	C	T	G	C	A	C	A	G	G	C	A	G	T	G	G	C	T	G	C	T	C	A	A	A	G	C	C
H. sapiens CYP4A22 NM 001010969 XM 208213	C	T	G	C	T	T	C	T	G	C	T	G	C	T	G	A	T	C	A	A	G	G	C	A	G	C	T	C	A	G	C	T	C	T	A	C	C	T	G	C	A	T	A	G	G	C	A	G	T	G	G	C	T	G	C	T	C	A	A	A	G	C	C
O. afer afer XM 007946703 LOC103201934	C	T	G	G	T	T	C	T	G	C	T	G	T	T	G	C	T	C	A	A	G	G	C	A	G	C	C	C	A	G	T	T	C	T	A	C	T	T	G	C	G	C	A	G	G	C	A	A	T	G	G	C	T	A	C	T	C	A	A	A	G	C	C
E. edwardii XM 006902916 LOC102867299	C	T	T	G	T	T	C	T	G	C	T	G	C	T	G	T	T	C	A	A	G	G	C	A	A	T	C	C	A	A	T	T	A	T	A	C	C	T	G	C	G	C	A	G	A	C	A	G	T	G	G	C	T	G	T	T	G	A	A	A	G	T	C
E. edwardii XM 006902913 LOC102862835	C	T	A	G	T	T	C	T	A	C	T	G	C	T	G	C	T	C	A	A	G	G	C	A	G	C	T	C	A	G	T	T	C	T	A	C	C	T	G	G	A	C	A	G	G	C	A	G	T	G	G	C	T	A	C	T	C	A	A	A	G	C	T
T. manatus latirostris XM 004371774 LOC101346203	C	T	G	G	T	T	C	T	G	C	T	G	C	T	G	C	T	C	A	A	G	G	C	A	G	C	C	C	A	G	C	T	C	T	A	C	C	T	G	C	G	C	A	G	G	C	A	G	T	G	G	C	T	A	C	T	C	A	A	G	G	C	C
T. manatus latirostris XM 012554256 LOC101345950	C	T	G	G	T	T	C	T	G	C	T	G	C	T	G	C	T	C	A	A	A	G	C	A	G	C	C	C	A	G	C	T	C	T	A	C	C	T	G	T	G	C	A	G	G	C	T	A	T	G	G	C	T	A	C	T	C	A	A	A	G	C	C
L. Africana CYP4A11 LOC100673772 NW 003573437	C	T	G	G	C	T	C	T	G	C	T	A	c	T	T	G	C	T	C	A	A	G	G	C	A	G	C	C	C	A	G	C	T	C	T	A	T	C	T	G	C	G	C	A	G	G	C	G	T	T	G	G	T	T	A	C	T	C	A	A	A	G	C
E. maximus CYP4A11 LOC126071317 NC 064821	C	T	G	G	C	T	C	T	G	C	T	A	T	T	G	C	T	C	A	A	G	G	C	A	G	C	C	C	A	G	C	T	C	T	A	T	C	T	G	C	G	C	A	G	G	C	G	T	T	G	G	T	T	A	C	T	C	A	A	A	G	C	C
translated to animo acid E.maximus CYP4A11	L			A			L			L			L			L			K			A			A			Q			L			Y			L			R			R			R			W			L			L			K			A		

EXON2

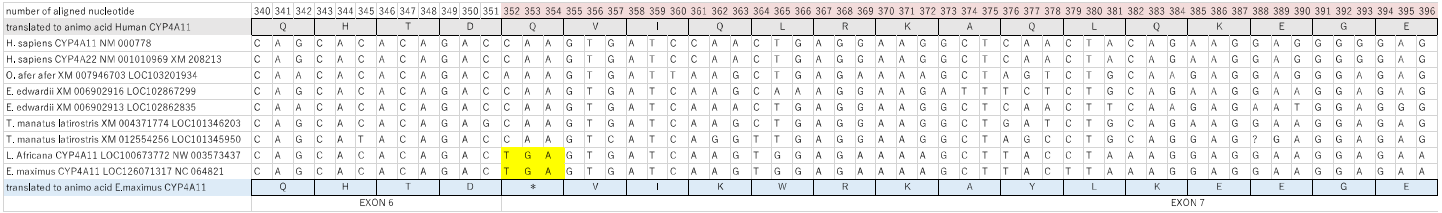
number of aligned nucleotide	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343
translated to animo acid Human CYP4A11	V			Q			L			Y			D			P			D			Y			M			K			V			I			L			G			R			S			D			P		
H. sapiens CYP4A11 NM 000778	G	T	C	C	A	G	C	T	C	T	A	T	G	A	C	C	C	T	G	A	C	T	A	T	A	T	G	A	A	G	G	T	G	A	T	T	C	T	G	G	G	G	A	G	A	T	C	A	G	A	C	C	C	G	-
H. sapiens CYP4A22 NM 001010969 XM 208213	G	T	C	C	A	G	C	T	C	T	A	T	G	A	C	C	C	T	G	A	C	T	A	T	A	T	G	A	A	G	G	T	G	A	T	T	C	T	G	G	G	G	A	G	A	T	C	A	G	A	C	C	C	G	G
O. afer afer XM 007946703 LOC103201934	T	T	C	C	A	T	G	T	T	T	A	T	G	A	T	C	C	T	G	A	C	T	A	C	A	T	G	A	A	G	G	T	G	A	T	T	C	T	A	G	G	G	A	G	A	T	C	A	G	A	C	C	C	A	-
E. edwardii XM 006902916 LOC102867299	G	T	C	A	T	G	A	T	C	T	A	T	G	A	T	C	C	T	G	A	C	T	A	C	A	T	G	A	A	G	G	T	G	A	T	T	C	T	G	G	G	G	A	G	A	T	C	A	G	A	C	C	C	A	-
E. edwardii XM 006902913 LOC102862835	A	T	C	T	C	A	A	T	A	T	A	T	G	A	T	C	C	T	G	A	C	T	A	C	A	T	G	A	A	G	G	T	G	G	T	T	C	T	G	G	C	G	A	G	A	T	C	A	G	A	T	G	T	A	-
T. manatus latirostris XM 004371774 LOC101346203	T	T	C	C	A	G	G	T	C	T	A	T	G	A	T	C	C	C	G	A	C	T	A	C	G	C	G	A	A	G	G	T	G	A	T	T	C	T	G	G	G	G	A	G	A	C	C	A	G	A	C	C	C	A	-
T. manatus latirostris XM 012554256 LOC101345950	A	T	C	C	T	G	G	T	C	A	A	T	G	A	C	C	C	T	G	A	C	T	A	C	A	T	G	A	A	G	G	T	G	A	T	T	A	T	G	G	G	G	A	G	A	T	C	A	G	A	C	C	C	A	-
L. Africana CYP4A11 LOC100673772 NW 003573437	C	T	A	C	G	C	T	G	A	C	T	A	G	A	T	G	A	A	G	G	T	G	A	T	T	C	T	G	G	G	G	A	G	A	T	C	A	G	A	C	C	C	A	A	A	G	C	T	T	C	A	T	G	A	T
E. maximus CYP4A11 LOC126071317 NC 064821	A	T	G	G	C	T	G	T	C	T	A	C	-	-	-	G	C	T	G	A	C	T	A	G	A	T	G	A	A	G	G	T	G	A	T	T	C	T	G	G	G	G	A	G	A	T	C	A	G	A	C	C	C	A	-
translated to animo acid E.maximus CYP4A11	M			A			V			Y						A			D			*			M			K			V			I			L			G			R			S			D			P		

EXON2

Group 3



Group 4



Group 5

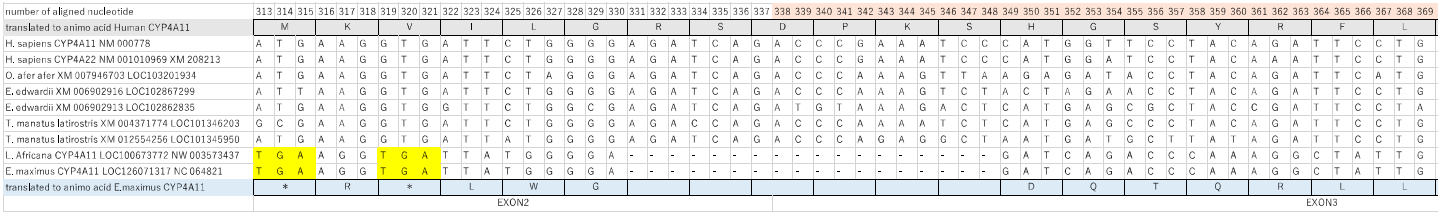


Figure S1. The loss of the CYP4A gene in African and Asian elephants was confirmed by MEGAX alignment. CYP4A gene sequences were visualized and compared to confirm pseudogene. Analysis was performed separately for each homologous gene as described in the synteny map. Human CYP4A11 (NM_000778) were referenced for nucleotide number of aligned genes. Different exon regions were described and highlighted in the manuscript. In addition, human and Asian elephant amino acids are listed at the top and bottom of the table. As a result, in all sequences stop codons (*) were found. Groups 1, 4, and 5 identified several stop codons in positions conserved in African and Asian elephants. Groups 2 and 3 are less similar in sequence, particularly in Group 2, a gene insertion was identified in African elephant. Stop codon detected in all group was located on the loci at less than 50% of the full length of the gene sequence.