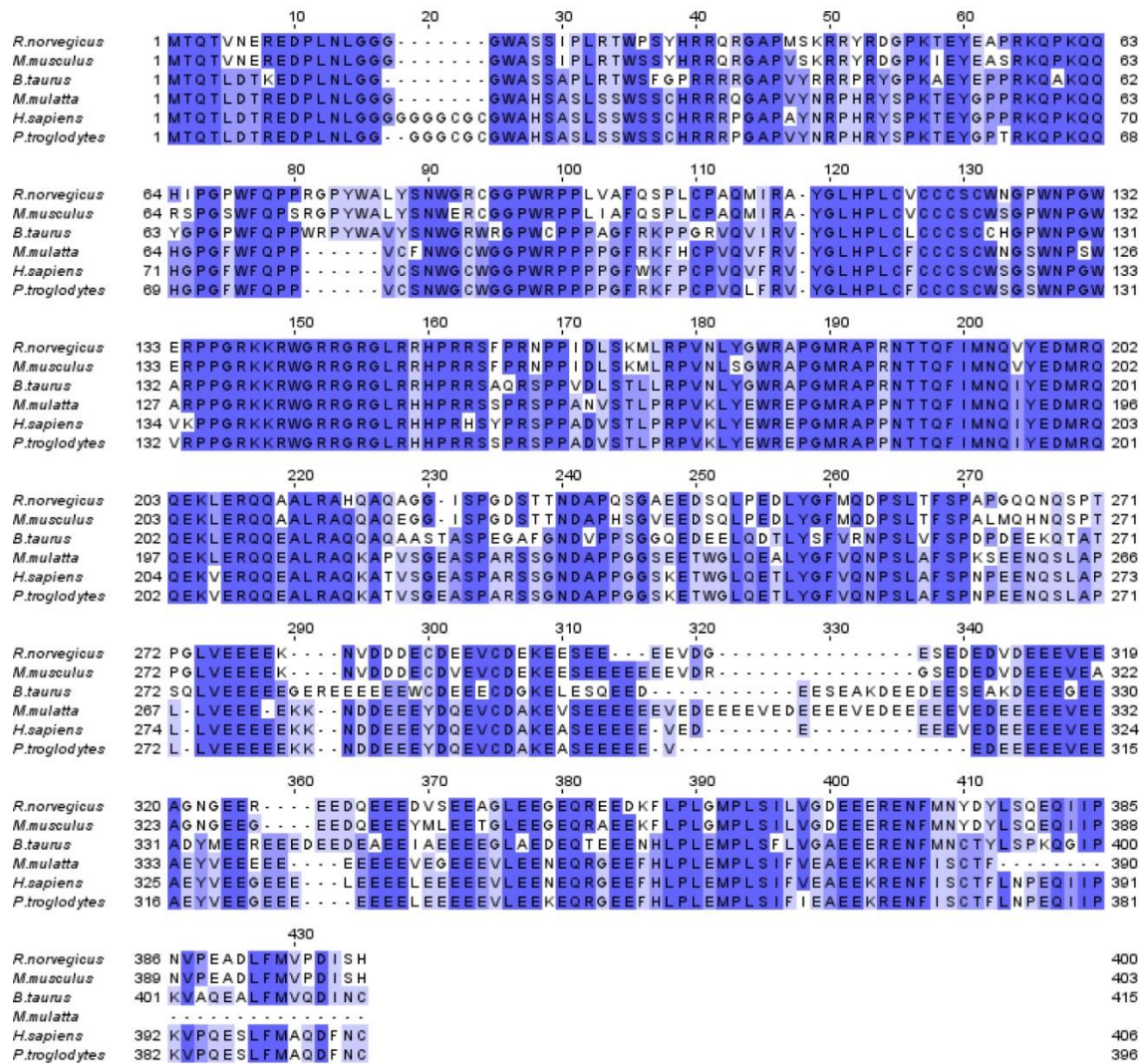


# Supplementary Material

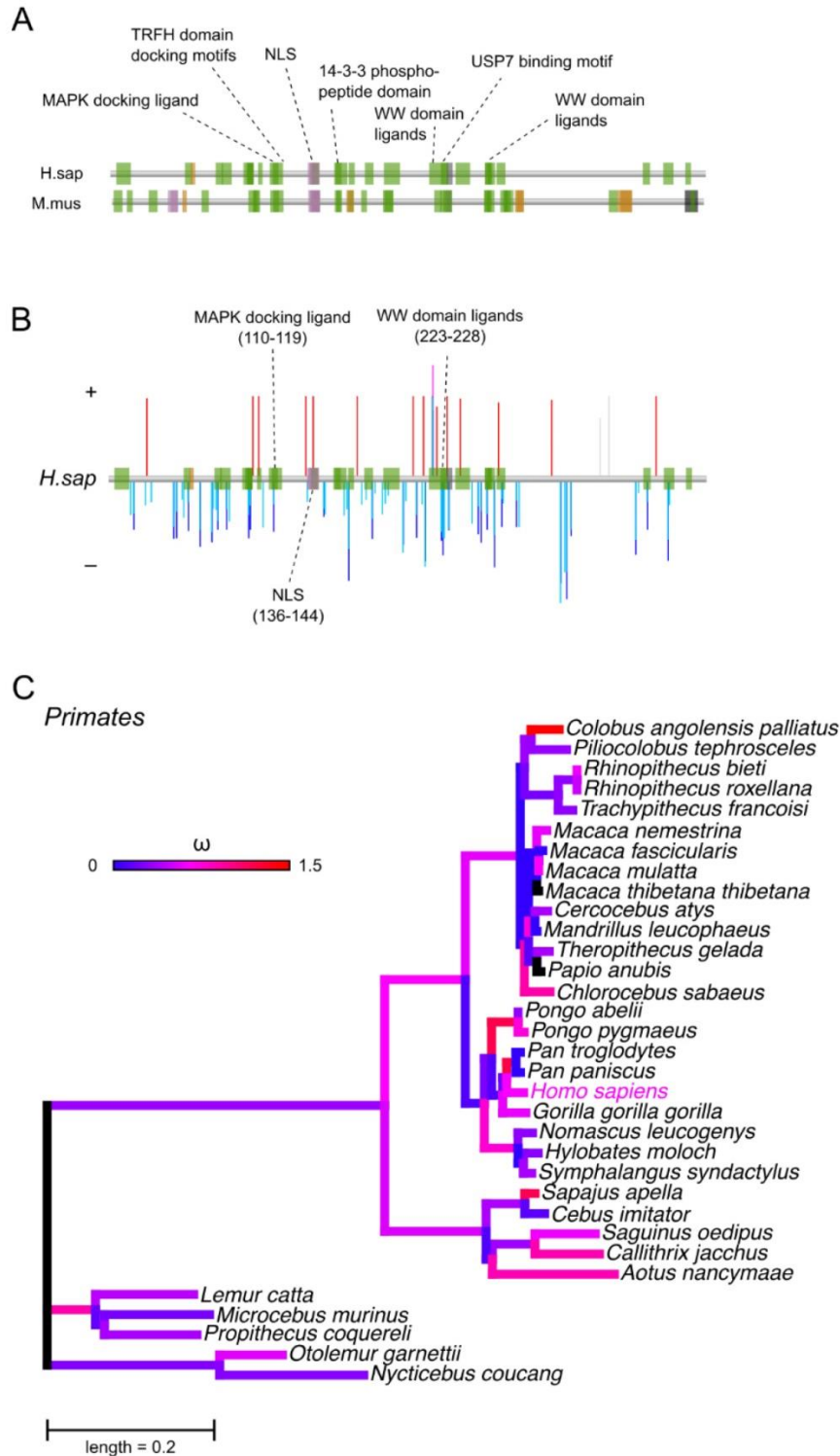
corresponding to:

## ***Ccer1* is a spermatid-specific gene required for spermatogenesis and male fertility**

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ANNA-LIISA HONKIMAA, PETAR PETROV, EMMI KAPIAINEN, ILKKA MIINALAINEN,  
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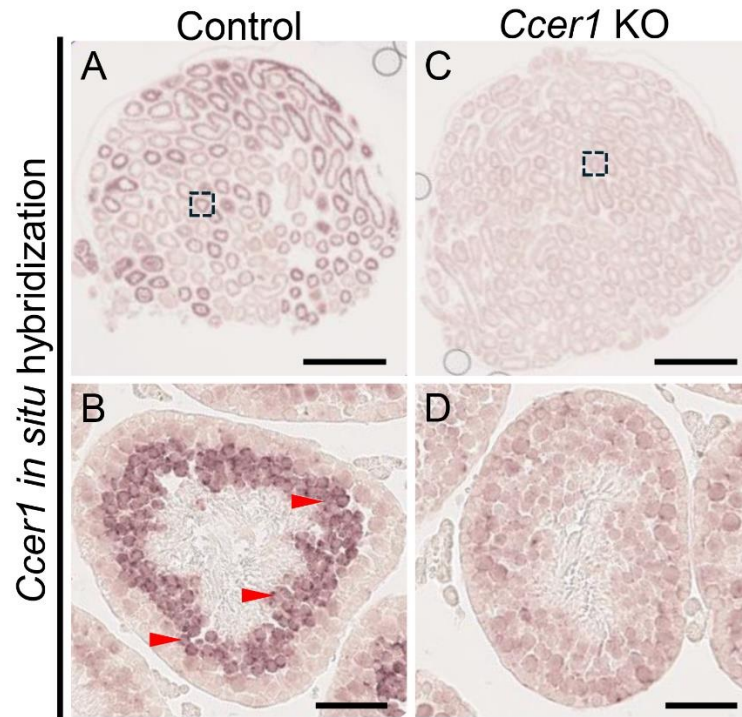
**Fig. S1. *In silico* CCER1 protein sequence analysis in mammals.** Sequence similarity of CCER1 protein amongst mammals. The deep purple fill indicates similarity in all shown species and the light purple fill indicates partial similarity.



**Fig. S2. *In silico* CCER1 analysis.** (A) Short function motifs from human and mouse. Overlapping motifs are indicated. (B) Evolutionary selection on short functional motifs from human. Nuclear localization signal (NLS) is indicated. Motifs with sites under negative selection are pointed out: a MAPK docking ligand motif and a WW domain ligands motif. Amino acid coordinates are shown in brackets. (C) Evolutionary selection on branches. Branch length corresponds to the number of nucleotide substitutions per codon, while omega values ( $\omega$ ) are shown in the color gradient.



**Fig. S3. *CCER1* expression in human tissues and cells.** *CCER1* expression across all the cell types available at The Human Protein Atlas. Note the specific enrichment in spermatids and spermatocyte cells.



**Fig. S4. *Ccer1* expression in mouse testis.** (A-D) *In situ* hybridization with a *Ccer1* antisense probe in control (A, B) and *Ccer1* KO (C, D) testis cross-sections. Framed areas in A and C are magnified in B and D, respectively. Control seminiferous tubules contain *Ccer1*-expressing cells (purple staining; red arrowheads in B), while *Ccer1* KO seminiferous tubules show only unspecific background staining.  $n = 3$  mice/genotype. Scale bars: (A, C) 1 mm; (B, D) 50  $\mu$ m.



**Table S1. Species nomenclature used in this article.** *CCER1* coding sequence and *CCER1* amino acid sequence identifiers. N/U – sequences not used for analysis. Unless otherwise specified, accession numbers are from NCBI.

Species	Common Name	Group	CDS acc. number	Protein acc. number
<i>Geotrypetes seraphini</i>	Gaboon caecilian	Amphibian	N/U	XP_033807704.1
<i>Vidua chalybeata</i>	Village indigobird	Birds	N/U	XP_053800053.1
<i>Gallus gallus</i>	Red junglefowl	Birds	N/U	XP_025010451.2
<i>Hypomesus transpacificus</i>	Delta smelt	Bony fishes	N/U	XP_046881158.1
<i>Neoarius graeffei</i>	Blue salmon catfish	Bony fishes	N/U	XP_060766740.1
<i>Salmo salar</i>	Atlantic salmon	Bony fishes	N/U	NP_001139947.1
<i>Amblyraja radiata</i>	Thorny skate	Cartilaginous fish	no sequence found	no sequence found
<i>Callorhynchus milii</i>	Australian ghostshark	Cartilaginous fish	no sequence found	no sequence found
<i>Scyliorhinus canicula</i>	Small-spotted catshark	Cartilaginous fish	N/U	XP_038636413.1
<i>Bos taurus</i>	Domestic cattle	Mammals	N/U	Q2T9P9 (UniProt)
<i>Mus musculus</i>	House mouse	Mammals	N/U	Q9CQL2 (UniProt)
<i>Rattus norvegicus</i>	Brown rat	Mammals	N/U	Q6AY45 (UniProt)
<i>Aotus nancymaeae</i>	Nancy Ma's night monkey	Mammals/Primate	XM_064376698.1	XP_064232768.1
<i>Callithrix jacchus</i>	Common marmoset	Mammals/Primate	XM_035257177.2	XP_035113068.1
<i>Cebus imitator</i>	Panamanian white-faced capuchin	Mammals/Primate	XM_037745752.1	XP_037601680.1
<i>Cercocebus atys</i>	Sooty mangabey	Mammals/Primate	XM_012084241.1	XP_011939631.1
<i>Chlorocebus sabaeus</i>	Green monkey	Mammals/Primate	XM_008004226.2	XP_008002417.2
<i>Colobus angolensis palliatus</i>	Angola colobus	Mammals/Primate	XM_011933584.1	XP_011788974.1
<i>Gorilla gorilla gorilla</i>	Western lowland gorilla	Mammals/Primate	XM_055357447.2	XP_055213422.1
<i>Homo sapiens</i>	Modern human	Mammals/Primate	NM_152638.4	NP_689851.1
<i>Hylobates moloch</i>	Silvery gibbon	Mammals/Primate	XM_058425625.1	XP_058281608.1
<i>Lemur catta</i>	Ring-tailed lemur	Mammals/Primate	XM_045555125.1	XP_045411081.1
<i>Macaca fascicularis</i>	Crab-eating macaque	Mammals/Primate	XM_045366838.2	XP_045222773.2
<i>Macaca mulatta</i>	Rhesus macaque	Mammals/Primate	XM_015152441.2	XP_015007927.2
<i>Macaca nemestrina</i>	Southern pig-tailed macaque	Mammals/Primate	XM_011744724.1	XP_011743026.1
<i>Macaca thibetana thibetana</i>	Tibetan macaque	Mammals/Primate	XM_050746565.1	XP_050602522.1
<i>Mandrillus leucophaeus</i>	Drill	Mammals/Primate	XM_011988912.1	XP_011844302.1
<i>Microcebus murinus</i>	Gray mouse lemur	Mammals/Primate	XM_012753885.2	XP_012609339.1
<i>Nomascus leucogenys</i>	Northern white-cheeked gibbon	Mammals/Primate	XM_030821832.1	XP_030677692.1
<i>Nycticebus coucang</i>	Sunda slow loris	Mammals/Primate	XM_053582254.1	XP_053438229.1
<i>Otolemur garnettii</i>	Northern greater galago	Mammals/Primate	XM_003783363.2	XP_003783411.1
<i>Pan paniscus</i>	Bonobo	Mammals/Primate	XM_055095975.2	XP_054951950.1
<i>Pan troglodytes</i>	Chimpanzee	Mammals/Primate	XM_009425983.5	XP_009424258.3
<i>Papio anubis</i>	Olive baboon	Mammals/Primate	XM_003906940.5	XP_003906989.3
<i>Ptilocolobus tephroscelens</i>	Ugandan red colobus	Mammals/Primate	XM_026454598.1	XP_026310383.1
<i>Pongo abelii</i>	Sumatran orangutan	Mammals/Primate	XM_009248088.3	XP_009246363.1
<i>Pongo pygmaeus</i>	Bornean orangutan	Mammals/Primate	XM_054443515.2	XP_054299490.1
<i>Propithecus coquereli</i>	Coquerel's sifaka	Mammals/Primate	XM_012664010.1	XP_012519464.1
<i>Rhinopithecus bieti</i>	Black-and-white snub-nosed monkey	Mammals/Primate	XM_017868368.1	XP_017723857.1
<i>Rhinopithecus roxellana</i>	Golden snub-nosed monkey	Mammals/Primate	XM_010383946.2	XP_010382248.2
<i>Saguinus oedipus</i>	Cotton-top tamarin	Mammals/Primate	JASSZA01000009.1 position 98554264 - 98555436	KAK2102349.1

<i>Sapajus apella</i>	Tufted capuchin	Mammals/Primate	XM_032286534.1	XP_032142425.1
<i>Symphalangus syndactylus</i>	Siamang	Mammals/Primate	XM_055237368.2	XP_055093343.1
<i>Theropithecus gelada</i>	Gelada	Mammals/Primate	XM_025402887.1	XP_025258672.1
<i>Trachypithecus francoisi</i>	François' langur	Mammals/Primate	XM_033221096.1	XP_033076987.1
<i>Phascolarctos cinereus</i>	Koala	Marsupials	N/U	XP_020862117.1
<i>Sarcophilus harrisii</i>	Tasmanian devil	Marsupials	N/U	XP_031796004
<i>Ornithorhynchus anatinus</i>	Platypus	Monotremes	N/U	XP_028934500.1
<i>Chelydra serpentina</i>	Common snapping turtle	Reptiles	N/U	KAG6930038.1
<i>Pelodiscus sinensis</i>	Chinese softshell turtle	Reptiles	N/U	XP_025044434.1
<i>Sceloporus undulatus</i>	Eastern fence lizard	Reptiles	N/U	XP_042326327.1

**Table S2. Positive (episodic) selection detected by MEME on sites.** Position in the multiple sequence alignment (MSA). Amino acid (*H. sapiens*): amino acid corresponding to reference organism, *Homo sapiens*. Position (*H. sapiens*): Position in the unaligned sequence of the reference organism, *Homo sapiens*. p+: Mixture distribution weight allocated to beta+ (roughly; the proportion of the phylogeny for which positive selection was detected at that particular site). P value: Asymptotic P value for episodic diversification.

MSA position	Amino acid ( <i>H. sapiens</i> )	Position ( <i>H. sapiens</i> )	p+	P value
34	W	26	825.875	0.03592
113	G	98	1000	0.07457
117	F	102	1000	0.02298
149	V	134	994.394	0.03017
154	R	139	1000	0.08946
190	L	169	1000	0.06607
228	V	207	1000	0.06782
235	L	214	1000	0.06832
241	T	220	1000	0.09367
244	G	223	410.589	0.05518
251	S	230	1000	0.00977
260	G	239	806.487	0.03035
287	P	265	583.051	0.05022
326	S	301	718.765	0.00528
440	E	372	1000	0.08355



**Table S3. Selection detected by SLAC on sites.** Position in the multiple sequence alignment (MSA). Amino acid (*H. sapiens*): amino acid corresponding to reference organism, *Homo sapiens*. Position (*H. sapiens*): Position in the unaligned sequence of the reference organism, *Homo sapiens*. dN-dS: difference between inferred nonsynonymous and synonymous substitution rates. P value (pos): P value for positive selection at site. P value (neg): P value for negative selection at site. Selection: type of selection at site. Negative selection in black and positive in blue.

MSA position	Amino acid ( <i>H. sapiens</i> )	Position ( <i>H. sapiens</i> )	dN-dS	P value (pos)	P value (neg)	Selection
17	G	17	-3.5295	1	0.03704	negative
52	G	44	-4.1585	0.99605	0.04395	negative
54	P	46	-4.1177	0.99588	0.04527	negative
62	Y	54	-3.6091	1	0.06044	negative
70	P	62	-4.7060	1	0.01235	negative
78	Q	70	-4.3936	0.99383	0.06956	negative
82	G	74	-3.5295	1	0.03765	negative
88	P	80	-3.5295	1	0.03704	negative
110	P	95	-4.1177	0.99588	0.04527	negative
111	P	96	-3.5295	1	0.03704	negative
127	Y	112	-3.6667	1	0.05901	negative
161	R	146	-2.6395	1	0.08884	negative
181	P	163	-7.0590	1	0.00137	negative
200	R	179	-4.1705	1	0.02453	negative
221	D	200	-4.5579	1	0.02961	negative
230	R	209	-4.0824	0.99574	0.04645	negative
236	R	215	-5.7114	1	0.00486	negative
241	T	220	4.73854	0.08342	0.98675	positive
247	S	226	-4.2939	0.99647	0.04048	negative
248	P	227	-5.2942	0.99863	0.01783	negative
252	S	231	-3.5713	1	0.03784	negative
272	Y	251	-3.8500	1	0.05478	negative
274	F	253	-4.4234	0.99678	0.04442	negative
278	P	257	-3.5295	1	0.03704	negative
284	S	262	-6.1311	0.99927	0.01260	negative
289	E	267	-3.3377	1	0.05987	negative
301	E	277	-3.3498	1	0.06085	negative
333	V	307	-7.2259	0.99745	0.01939	negative
338	E	311	-8.3357	1	0.00092	negative
341	V	314	-3.9623	0.98464	0.09179	negative
426	H	358	-5.2643	1	0.03261	negative
434	S	366	-3.5295	1	0.05418	negative
448	S	380	-5.6810	0.99867	0.02381	negative

**Table S4. Selection detected by FUBAR on sites.** MSA position: position in the multiple sequence alignment. Amino acid (*H. sapiens*): amino acid corresponding to reference organism, *Homo sapiens*. Position (*H. sapiens*): Position in the unaligned sequence of the reference organism, *Homo sapiens*. beta-alpha: Difference between mean posterior non-synonymous and synonymous substitution rate at a site. Bayes (pos): empirical Bayes factor for positive selection at the site. Bayes (neg): empirical Bayes factor for negative selection at the site. Negative selection in black and positive in blue.

MSA position	Amino acid ( <i>H. sapiens</i> )	Position ( <i>H. sapiens</i> )	beta-alpha	Bayes (neg)	Bayes (pos)	Selection
15	G	15	-4.3020	0.91607	0.03787	negative
17	G	17	-4.1085	0.98353	0.00960	negative
33	G	25	-3.1448	0.95364	0.03184	negative
37	S	29	-2.7287	0.94339	0.03825	negative
52	G	44	-5.7342	0.99201	0.00156	negative
54	P	46	-3.9017	0.97760	0.00739	negative
58	R	50	-2.3980	0.93190	0.03839	negative
59	P	51	-4.6277	0.94090	0.01461	negative
62	Y	54	-2.7830	0.94548	0.03765	negative
70	P	62	-6.1222	0.99691	0.00121	negative
78	Q	70	-6.4077	0.97246	0.00919	negative
79	H	71	-3.5219	0.93034	0.04508	negative
81	P	73	-3.6992	0.95898	0.02821	negative
82	G	74	-5.0583	0.98865	0.00637	negative
88	P	80	-3.5729	0.97899	0.01258	negative
97	C	82	-5.1017	0.95092	0.03656	negative
110	P	95	-4.3012	0.98331	0.00483	negative
111	P	96	-3.1499	0.97590	0.01453	negative
120	P	105	-5.8415	0.97758	0.00237	negative
127	Y	112	-2.9782	0.94817	0.03572	negative
150	K	135	-2.6988	0.92480	0.05084	negative
161	R	146	-3.5100	0.96400	0.02506	negative
165	G	147	-4.2405	0.95652	0.02574	negative
178	R	160	-4.3708	0.96988	0.01505	negative
180	P	162	-3.6068	0.96194	0.01871	negative
181	P	163	-8.4346	0.99965	3.1E-05	negative
193	P	172	-3.0465	0.91330	0.05681	negative
196	L	175	-2.4557	0.91370	0.04096	negative
200	R	179	-4.7557	0.99427	0.00255	negative
202	P	181	-1.6757	0.91353	0.06173	negative
207	P	186	-4.2293	0.96836	0.02142	negative
209	N	188	-2.9289	0.90402	0.07284	negative
211	T	190	-1.9560	0.92857	0.05069	negative
221	D	200	-8.9155	0.98251	0.01162	negative
223	R	202	-2.6142	0.94287	0.04023	negative
230	R	209	-4.2847	0.98614	0.00471	negative
234	A	213	-2.9903	0.90445	0.05991	negative
236	R	215	-10.61	0.99966	7.5E-05	negative
241	T	220	10.1293	0.00418	0.96687	positive
247	S	226	-6.3549	0.99159	0.00202	negative
248	P	227	-7.0649	0.99497	0.00064	negative
249	A	228	-7.1537	0.92730	0.00290	negative
252	S	231	-6.0826	0.99340	0.00345	negative
268	Q	247	-5.3611	0.93766	0.03346	negative
272	Y	251	-3.2340	0.95525	0.03050	negative
274	F	253	-3.8459	0.97109	0.01503	negative
278	P	257	-4.1969	0.98532	0.00846	negative
284	S	262	-6.7526	0.99553	0.0016	negative

**Table S5. Selection detected by CodeML (M8) on sites.** MSA position: position in the multiple sequence alignment. Amino acid (*H. sapiens*): amino acid corresponding to reference organism, *Homo sapiens*. Position (*H. sapiens*): Position in the unaligned sequence of the reference organism, *Homo sapiens*. Omega: postmean omega (dN/dS ratio). BEB: Bayes empirical Bayes analysis for the posterior probability of omega > 1 at the site.

MSA position	Amino acid ( <i>H. sapiens</i> )	Position ( <i>H. sapiens</i> )	Omega	BEB
241	T	220	1.567	0.97294

**Table S6. CodeML nested models comparison (*pchisq*).**

CodeML nested models	2ΔL	P value
M7 vs M8	11.70176	0.00287736
M8A vs M8	23.41225	8.243175e-06

**Table S7. Differential expressed gene list.**

*See the separate Excel file*

**Table S8. Detailed ontology analysis list.**

*See the separate Excel file*

**Table S9. List of gRNAs and primers used for genotyping, RT-PCR, RT-qPCR, and *in situ* hybridization probe template plasmid cloning.**

Gene	Forward (5'—3')	Reverse (5'—3')
<b>gRNA</b>		
gRNA	CCAGACGGTCAACGAAAGGG	GCCACAACAGACTGCAAACG
<b>Genotyping</b>		
<i>WT</i>	TATGGTCTGCACCCACTCTG	CTCCTCCTCGCTCTCCTCTT
<i>Ccer1KO</i>	GGGAGTGGCTCTCACATCTC	CGAAGACAAACTGCGAAACA
<b>RT-PCR</b>		
<i>Ccer1</i>	AGCAACAGCGCAGCCCTGGC	GGTCCAGGGTCCGCTCCA
<i>Actb</i>	TTCTACAATGAGCTGCGTGTGGCCCC	GTGGTACGACCAGAGGCATACAGGGAC
<b>RT-qPCR</b>		
<i>Ccer1</i>	AGCAACAGCGCAGCCCTGGC	GGTCCAGGGTCCGCTCCA
<i>Gapdh</i>	TGTGTCCGTCGTGGATCTGA	TTGCTGTTGAAGTCGCAGGAG
<b><i>In situ</i> hybridization probe template cloning</b>		
<i>Ccer1</i>	AAAAGCTTGGTAGAAAGAAGCGCTG	AATCTAGATGGCTAATGTCCGGTACCAT