Supporting Information

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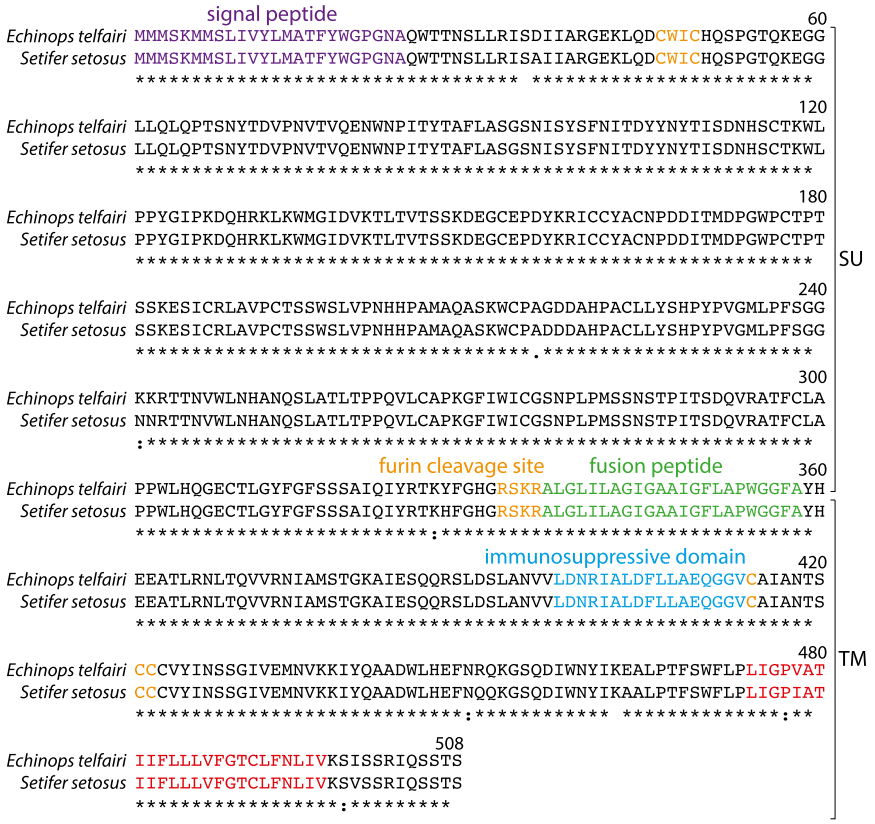
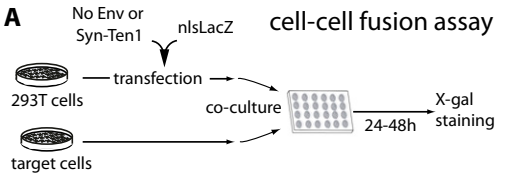
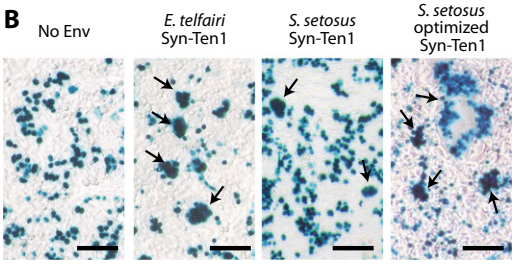


Fig. S1. Primary sequences and alignment of *Echinops telfairi* and *Setifer setosus* syncytin-Ten1 (Syn-Ten1) proteins. Primary amino acid sequence and characteristic structural features of the orthologous envelope (env) proteins, including the putative furin cleavage site and immunosuppressive domain; the color code is as in Fig. 2; asterisks indicate amino acid identity, and colons indicate amino acid similarity. SU, surface subunit; TM, transmembrane subunit.





C

Species	Cell	fusion efficiency: *E. telfairi* Syn-Ten1	*S. setosus* Syn-Ten1	*S. setosus* optimized Syn-Ten1	No Env
Human	293T	-	-	-	-
	HeLa	-	-	-	-
	HuH7	-	-	-	-
	SHSY-5Y	-	-	-	-
Mouse	MCA205	-	-	-	-
	WOP	-	-	-	-
Rat	208F	-	-	-	-
Hamster	A23	++	+	+++	-
Cat	G355.5	-	-	-	-
Dog	MDCK	-	-	-	-
Tenrec (*O. hova*)	primary fibroblast	+	+	++	-

Fig. S2. Syncytin-Ten1 is a fusogenic retroviral env protein. (*A*) Schematic representation of the coculture assay for cell–cell fusion with *Syncytin-Ten1*. Human 293T cells were transfected with an expression vector for syncytin-Ten1 or with an empty vector (No Env) as a control and a plasmid expressing a nuclear β-galactosidase (nlsLacZ). After transfection, the 293T cells were cocultured with a panel of target cells and were stained with X-Gal 24–48 h later. (*B*) Syncytium formation (arrows) with *E. telfairi* and *S. setosus* Syn-Ten1, using A23 cells as the target (no syncytium formed with the No Env control). (Scale bars: 200 μm.) (*C*) Fusogenic activity of *E. telfairi* and *S. setosus* Syn-Ten1 on the indicated target cells, including primary fibroblasts from the *Oryzorictes hova* Tenrecidae species shown in Fig. 5. Experimental conditions were the same as in *B*. Fusion efficiency was determined as indicated in *Materials and Methods*, with the following scale: –, <5; +, 5–10; ++, 10–30; +++, >30.

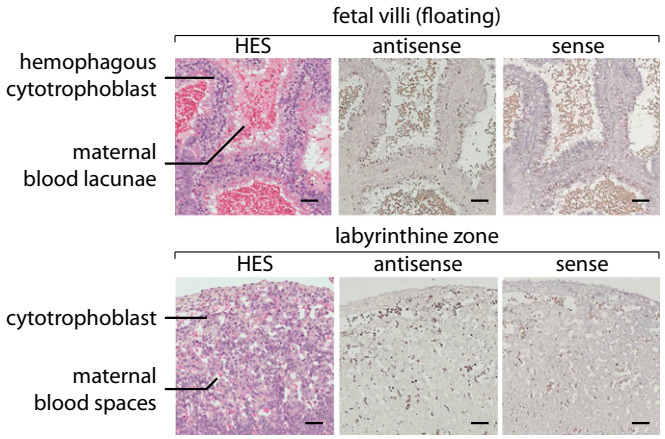


Fig. S3. Lack of *syncytin-Ten1* expression in the hemophagous cytotrophoblasts and the labyrinthine zone. Hematoxylin eosin saffron (HES) staining and in situ hybridization on the serial sections shown in Fig. 6 of the columnar hemophagous cytotrophoblasts of the central region (*Upper*) and of the labyrinthine zone of the placental pad (*Lower*). Experimental conditions were the same as in Fig. 6. Digoxigenin-labeled antisense or sense riboprobes are revealed with an alkaline phosphatase-conjugated anti-digoxigenin antibody. No signal was observed with either of the two probes. (Scale bars: 50 μm.)

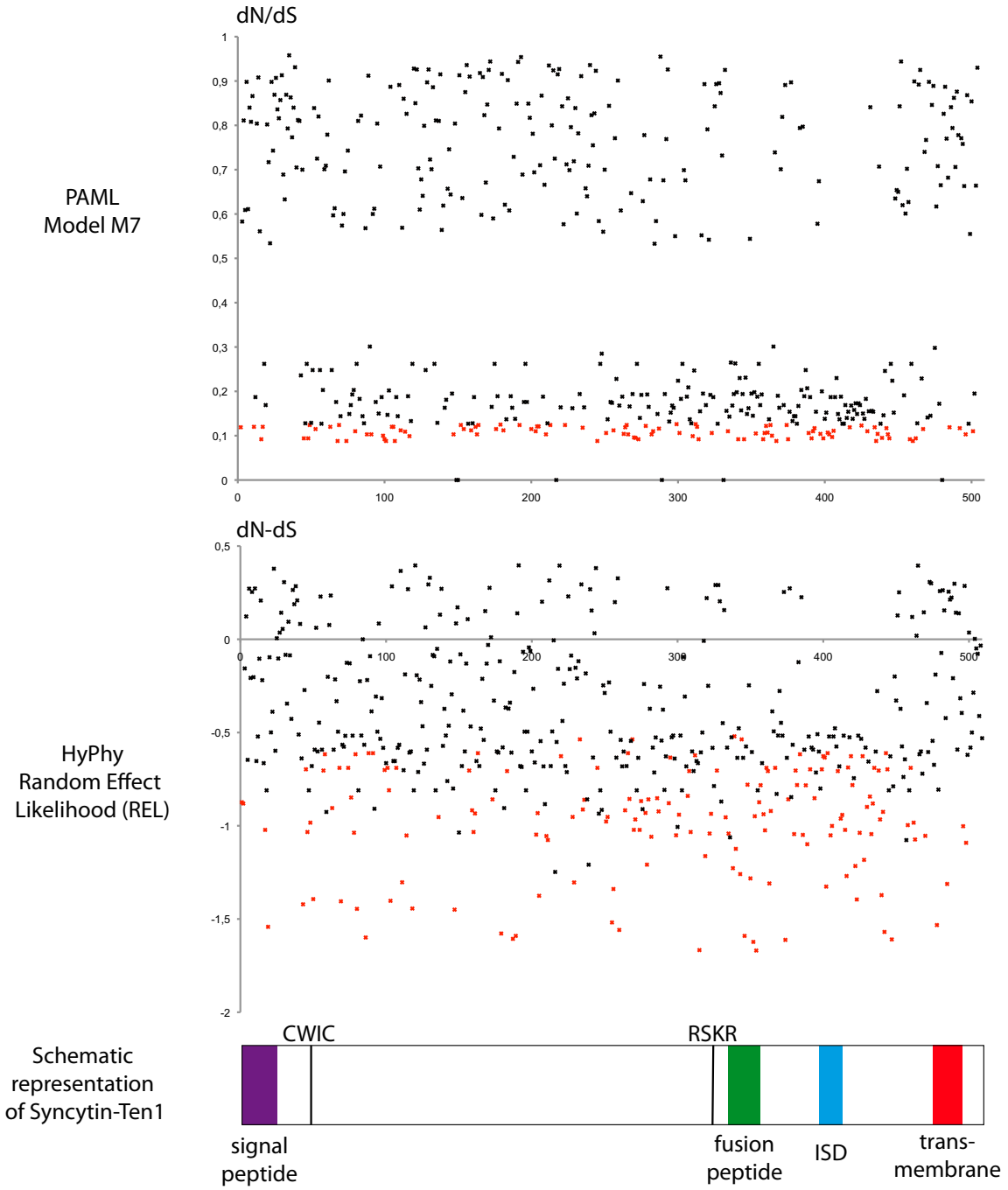


Fig. S4. Analysis of site-specific selections within *syncytin-Ten1*. Site-specific analysis of selection on *syncytin-Ten1* gene codons. Two independent analyses were performed using the phylogenetic analysis by the maximum likelihood (PAML) M7 model (*Top*) or the HyPhy random effect likelihood (REL) model (*Middle*) packages. The HyPhy package was run on the web server www.datamonkey.org. The relevant indexes for selective pressure are provided for each codon. (*Bottom*) A schematic representation of syncytin-Ten1 protein domains with the conventions used in Fig. 2. At variance with the PAML model (M7), in which dS is estimated for the entire sequence, the HyPhy REL model estimates nonsynonymous mutations (dN) and synonymous mutations (dS) values independently for each codon, allowing the dS value to be null. Consequently, for the HyPhy model, dN − dS values are represented instead of dN/dS values. Significant values (probability ≥ 0.95) are represented in red; nonsignificant ones are represented in black. No model predicts codons to be significantly under positive selection (i.e., dN/dS > 1 or dN − dS > 0). Moreover, no specific domain emerges, and in the HyPhy model weakly positive codons are distributed rather equally throughout the entire sequence, with the noticeable exception of the immunosuppressive domain within the transmembrane subunit.

Table S1. Genomic coordinates for *E. telfairi* env protein-coding sequences

Env coding gene	Scaffold no.	Strand orientation	Coordinates
Ten-env1	JH980362	–	6222281–6223765
Tenv-env2	JH980315	+	5506373–5507719
Tenv-env3	AAIY02274049	+	508–1542
Ten-env4-1	JH980293	+	31474421–31475623
Ten-env4-2	JH981068	+	3358–4647
Ten-env5	JH980329	+	6614307–6616253
Ten-env6-1	JH980338	+	9151416–9153191
Ten-env6-2	JH980338	–	9265470–9267248
Ten-env7	JH980343	–	10358315–10359964

Scaffold number and coordinates are given according to the reference Tenrec genome (echTel2) from the University of California, Santa Cruz genome database (http://genome-euro.ucsc.edu/cgi-bin/hgGateway?hgsid=197847906_BRNKiNWDwFbBcGBhcCnQJYmoqmgg&clade=mammal&org=Tenrec&db=0).

Table S2. Primers used in this study

Primer names	Primer sequences
To search for *E. telfairi* genes in *S. setosus*	
Full-length genes	
Ten-Env1-Full-F	5′-CTCATCGGTTAGATCTGAGTT
Ten-Env1-Full-R	5′-ATTGCCAGGGAAGAGGAG
Ten-Env2-Full-F	5′-TAAGCCCCCTAAAGCCGT
Ten-Env2-Full-R	5′-GCGATGCTACTGGACCTG-T
Ten-Env3-Full-F	5′-ATACCAGGTCACTGAAATCAGG
Ten-Env3-Full-R	5′-AATAATACCAGCAGTACCAGGG
Ten-Env4-Full-F	5′-CCGCGGAAGAAGCTAAAA
Ten-Env4-Full-R	5′-GGGCAATCTCGTACATCTCT
Ten-Env5-Full-F	5′-CCGGGAATGTGTGTGTTTTT
Ten-Env5-Full-R	5′-CCTTGCCATGTCCTCTCC
Ten-Env6-Full-F	5′-GTGCGGACAGATTAAGAAGTG
Ten-Env6-Full-R	5′-AATGTGCATCCTAAAAGCCTT
Ten-Env7-Full-F	5′-AGACTGGCCCCGTCATATT
Ten-Env7-Full-R	5′-GCTCCTCTGCGAGAAAATG
Internal fragments	
Ten-Env2-Int-F	5′-GGGAAGAACTAAAAGAGCAGTA
Ten-Env2-Int-R	5′-GCTGTAAGTAAATCTCGACCTC
Ten-Env4-Int-F	5′-GAAGTCCTGCGCCGGTC
Ten-Env4-Int-R	5′-GAAACAACAAATCTAATCCTCG
Ten-Env5-Int-F	5′-CATGGATTAATGGAAAGACC
Ten-Env5-Int-R	5′-CTCTCATATTCTGCGTGACA
For quantitative RT-PCR	
RPL19-F	5′-GCTGTGGCAAGAAGAAAGT
RPL19-R	5′-GCCTAAGAATGGACGGTCA
Ten-Env1-qRT-PCR-F	5′-TGGATGGGTATTGATGTGAAA
Ten-Env1-qRT-PCR-R	5′-GCTAGTCTGCATATGGATTCTT
Ten-Env2-qRT-PCR-F	5′-GGGAAGAACTAAAAGAGCAGTA
Ten-Env2-qRT-PCR-R	5′-GCTGTAAGTAAATCTCGACCTC
Ten-Env3-qRT-PCR-F	5′-TAGATGGATGGTGGGCTTG
Ten-Env3-qRT-PCR-R	5′-AGCTGGGGCCTGGTAATAA
Ten-Env5-qRT-PCR-F	5′-CATGGATTAATGGAAAGACC
Ten-Env5-qRT-PCR-R	5′-CTCTCATATTCTGCGTGACA
Ten-Env6-qRT-PCR-F	5′-GATACTCAGTTTCCTTGGGC
Ten-Env6-qRT-PCR-R	5′-CGCCATCAACCTTTTCTAAT
Ten-Env7-qRT-PCR-F	5′-GGATGGCATGGAAGGATTAG
Ten-Env7-qRT-PCR-R	5′-TCACGGTGTTTGCCTTATAC
For 5′ RACE	
Ten-Env1-5′RACE-R	5′-CGCGTATCTCACTCACTCCTCTCA
For in situ hybridization	
Ten-Env1-ISH-1-F	5′-TTTTATTGGGGCCCTGGAA
Ten-Env1-ISH-1-R	5′-AAGCATAGCAACAAATTCG
Ten-Env1-ISH-2-F	5′-CCTTGCACTCCGACTTCTA
Ten-Env1-ISH-2-R	5′-TGAAAAGCCAAAATAACCT
Ten-Env1-ISH-3-F	5′-ACTTTGGCCACGGTAGATC
Ten-Env1-ISH-3-R	5′-CAGAGGGAGGAACCAGCTAAA
For amplification of *syncytin-Ten1* in Malagasy tenrecs	
Syncytin-Ten1 locus F1	5′-CAGCTGATTCTTCTCATTTTGA
Syncytin-Ten1 locus R1	5′-TTGACACAACTCCATGAATATA
Syncytin-Ten1 locus F2	5′-CTGGTTTGTGATTTGGTGTCTC
(5/6 *Microgale* species)	
Syncytin-Ten1 locus R2 (5/6 *Microgale* species)	5′-TRGTATCATTAGGRTTRRTACCCA
Syncytin-Ten1 ORF F1	5′-ATACATCTCGAGCTCWTYRGTYAGATCTGAGTT
Syncytin-Ten1 ORF R1	5′-ATACATACGCGTATTGCCAGGGAAGAGGAG
Syncytin-Ten1 ORF R2 (*Micropotamogale*)	5′-TCACATTGCCAGGGAAGA
Syncytin-Ten1 internal F	5′-CCTCAAGTTCTTTGTGCTCC
Syncytin-Ten1 internal R	5′-AATTCRTGTAGCCAATCAGC

Table S3. Origin of tissues used for genomic DNA extraction

Species	Provider	Institution	Preservation condition
Echinops telfairi	M. Milinkovitch	Laboratory of Artificial and Natural Evolution, University of Geneva, Geneva	Frozen
Setifer setosus	M. Milinkovitch	Laboratory of Artificial and Natural Evolution, University of Geneva, Geneva	Frozen
Tenrec ecaudatus	F. Catzeflis	Collection de Tissus de Mammifères de Montpellier, Montpellier	Ethanol
Hemicentetes semispinosus	S. Goodman V. Soarimalala	Field Museum of Natural History, Chicago Université d'Antananarivo, Département de Biologie Animale, Madagascar	EDTA
Oryzorictes tetradactylus	S. Goodman V. Soarimalala	Field Museum of Natural History, Chicago Université d'Antananarivo, Département de Biologie Animale, Madagascar	EDTA
Geogale aurita	S. Goodman V. Soarimalala	Field Museum of Natural History, Chicago Université d'Antananarivo, Département de Biologie Animale, Madagascar	EDTA
Microgale brevicaudata	S. Goodman V. Soarimalala	Field Museum of Natural History, Chicago Université d'Antananarivo, Département de Biologie Animale, Madagascar	EDTA
Microgale parvula	S. Goodman V. Soarimalala	Field Museum of Natural History, Chicago Université d'Antananarivo, Département de Biologie Animale, Madagascar	EDTA
Microgale dobsoni	S. Goodman V. Soarimalala	Field Museum of Natural History, Chicago Université d'Antananarivo, Département de Biologie Animale, Madagascar	EDTA
Microgale soricoides	S. Goodman V. Soarimalala	Field Museum of Natural History, Chicago Université d'Antananarivo, Département de Biologie Animale, Madagascar	EDTA
Microgale gymnoryncha	S. Goodman V. Soarimalala	Field Museum of Natural History, Chicago Université d'Antananarivo, Département de Biologie Animale, Madagascar	EDTA
Microgale thomasi	S. Goodman V. Soarimalala	Field Museum of Natural History, Chicago Université d'Antananarivo, Département de Biologie Animale, Madagascar	EDTA
Micropotamogale lamottei	J. Decher	Zoologisches Forschungsmuseum Alexander Koenig, Bonn	Ethanol
Amblysomus hottentotus	F. Catzeflis	Collection de Tissus de Mammifères de Montpellier, Montpellier	Ethanol
Chrysochloris asiatica	F. Catzeflis	Collection de Tissus de Mammifères de Montpellier, Montpellier	Ethanol
Macroscelides proboscideus	U. Zeller	Humbolt Universität, Berlin	Frozen