Supplementary Information

A novel human endogenous retroviral protein inhibits cell-cell fusion

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Supplementary Figures:

a

Nucleotide alignment

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Human ATGGCCTGTATCTACCCAACCACTTTCTATACCTCTCTTCCAACCAAAAGTCTTAATATGGGAATATCCCTCACCACGATCCTA 84
Chimp ..........................................................C....................C.... 84

Human ATACTGTCAGTAGCTGTCCTGCTGTCCACAGCAGCCCCTCCGAGCTGCCGTGAGTGTTATCAGTCTTTGCACTACAGAGGGGAG 168
Chimp .................................................................................... 168

Human ATGCAACAATACTTTACTTACCATACTCATATAGAAAGATCCTGTTATGGAAACTTAATCGAGGAATGTGTTGAATCAGGAAAG 252
Chimp .................................................................................... 252

Human AGTTATTATAAAGTAAAGAATCTAGGAGTATGTGGCAGTCGTAATGGGGCTATTTGCCCCAGAGGGAAGCAGTGGCTTTGCTTC 336
Chimp .................................................................................... 336

Human ACCAAAATTGGACAATGGGGAGTAAACACTCAGGTGCTTGAGGACATAAAGAGAGAACAGATTATAGCCAAAGCCAAAGCCTCA 420
Chimp ..............G...........................................................------.... 414

Human AAACCAACAACTCCCCCTGAAAATCGCCCGCGGCATTTCCATTCCTTTATACAAAAACTATAA                      483
Chimp ...............................................................                      477
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Amino acid alignment

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Human MACIYPTTFYTSLPTKSLNMGISLTTILILSVAVLLSTAAPPSCRECYQSLHYRGEMQQYFTYHTHIERSCYGNLIEECVESGKS 85
Chimp ...................T......T.......................................................... 85

Human YYKVKNLGVCGSRNGAICPRGKQWLCFTKIGQWGVNTQVLEDIKREQIIAKAKASKPTTPPENRPRHFHSFIQKL.          161
Chimp ....................................................--......................          159
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b

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Suppressyn  40  APPSCR-----ECYQSLHYRGEMQQ-----------------YFTYHTHIERSCYGNLIEECVESG  83
                *** **       **   .*  **.                  ** ***. *.** .    *. .
Syncytin-1  21  APPPCRCMTSSSPYQEFLWR--MQRPGNIDAPSYRSLSKGTPTFTAHTHMPRNCYHS-ATLCMHAN  83

Suppressyn  84  KSYY--KVKNLGVCGSRNGAICPRGKQWLCFTKIGQ---WGVNTQVLEDIKREQIIAKAKASKPTT  144
                  *.  *. *   *      .*     *  **. *     **  *  *   .* *    .    ..
Syncytin-1  84  THYWTGKMINPSCPGGLGVTVC-----WTYFTQTGMSDGGGVQDQAREKHVKEVISQLTRVHGTSS  144
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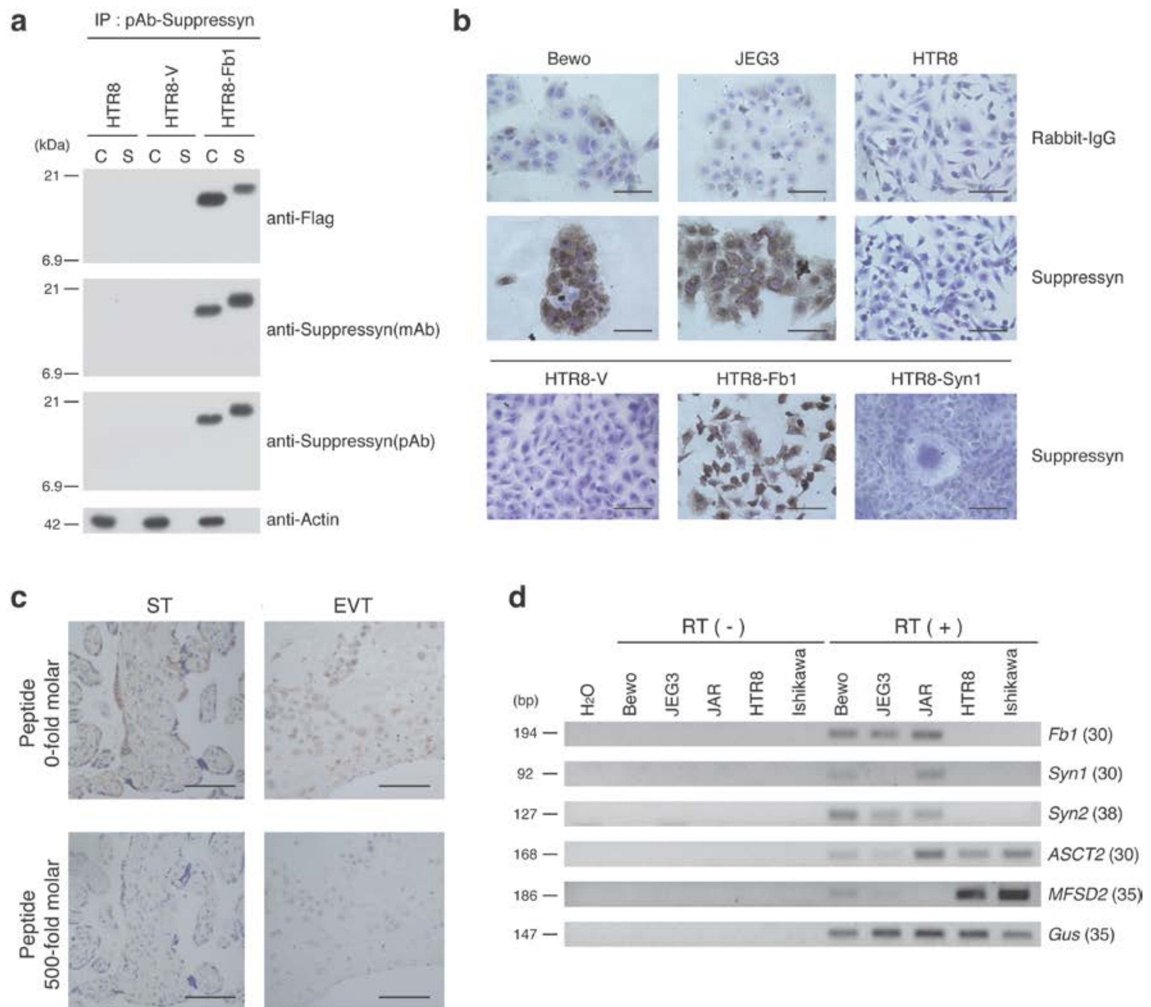
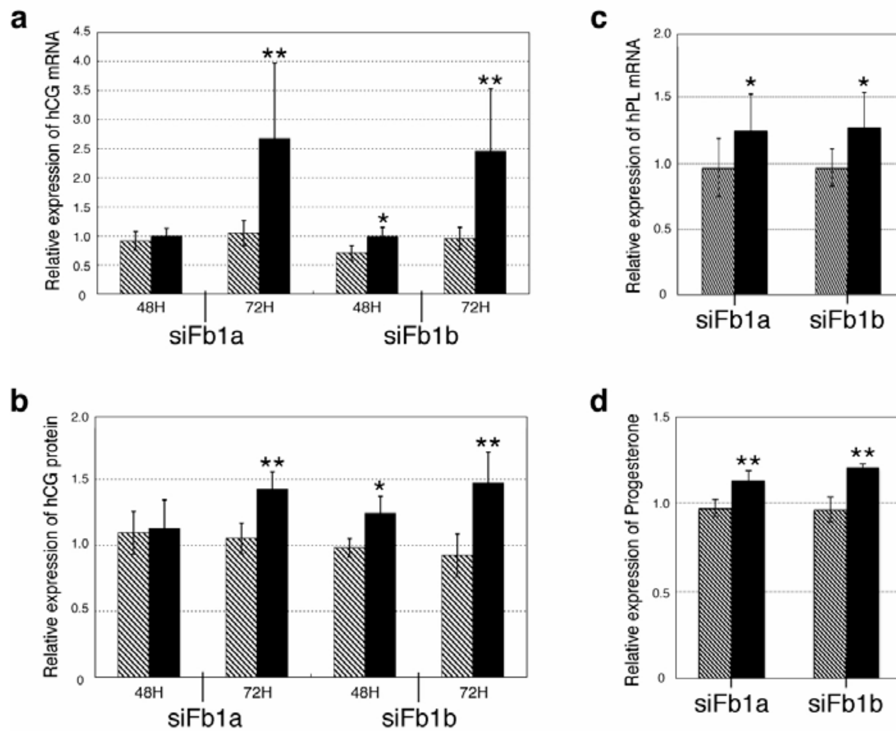
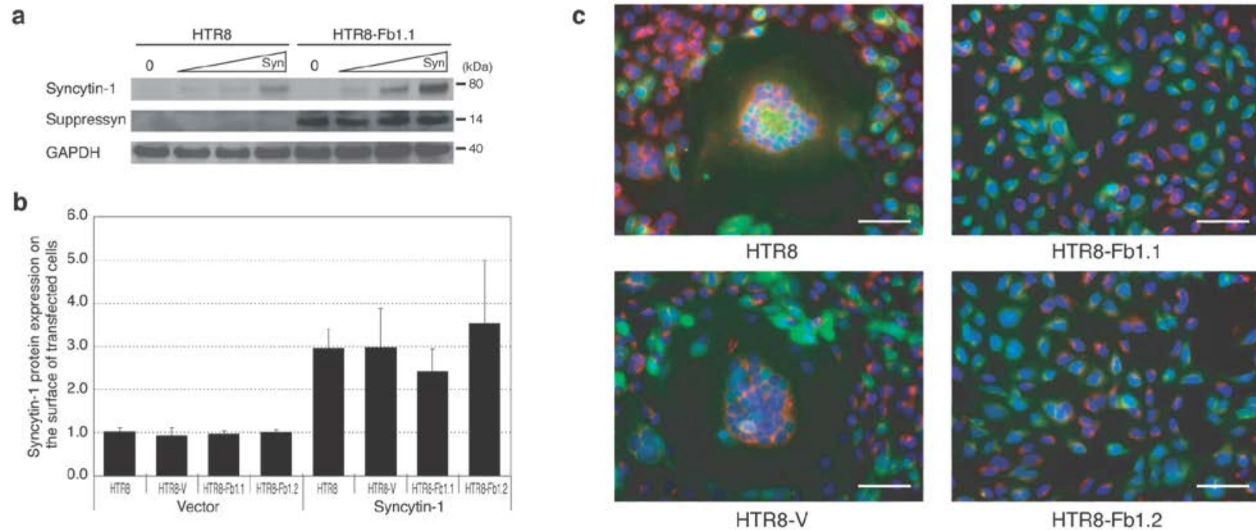
Supplementary Figure S1: Nucleotide and amino acid alignments for *HERV-Fb1* vs. a chimpanzee suppressyn homolog and suppressyn vs. syn1, respectively (a) Human and chimpanzee nucleotide and amino acid sequences are aligned to demonstrate homology. The chimpanzee amino acid sequence was derived from 28971776-28972252 (database; CGSC Build 2.2.3) genomic sequences on chromosome 21. (**b**) The 124 amino acid SU of syn1 is aligned with that of suppressyn. (*) identical amino acids ; (.) similar amino acids. The reported ASCT-2 binding sites[22, 27] are delimited by arrowheads. The conserved amino acid motif directly involved in syn1/ASCT-2 receptor interactions is within the box.



Supplementary Figure S2: Anti-suppressyn antibody specificity and suppressyn expression in human cell lines and primary tissues. (a) HTR8, HTR8-V and HTR8-Fb1 cell lysates (C) and supernatants (S) were immunoprecipitated with the polyclonal anti-suppressyn antibody. After separation over a 15% SDS-PAGE gel and protein transfer, suppressyn-specific detection was performed using anti-Flag, and monoclonal and polyclonal anti-suppressyn antibodies. **(b)** Trophoblast cell lines (Bewo, JEG3 and HTR8) were cultured under standard conditions and exposed to the suppressyn polyclonal antibody or to Rabbit-IgG (negative control) as primary reagents, then to a secondary, biotinylated anti-rabbit IgG antibody and finally to DAB for detection. Cells were counterstained with hematoxylin, mounted and visualized using standard microscopy. Transfected cells (HTR8-V, -Fb1 and –syn1) were used as additional positive and negative controls. Scale bar indicates 100μm. **(c)** The anti-suppressyn polyclonal antibody was incubated with 0- or 500-molar excess of a blocking peptide overnight. These antibody preparations were then used as primary antibodies for immunohistochemical analysis of third trimester villous and extravillous placental samples as in **Figure 1g-1j**. ST-syncytiotrophoblast, EVT-extravillous trophoblast. Scale bar indicates 100μm. **(d)** Conventional RT-PCR was performed using gene-specific primers (**Supplementary Table 1**). Reactions lacking reverse transcriptase (-) assess genomic DNA contamination. The number of amplification cycles used in a given amplification is provided in parentheses.



Supplementary Figure S3: *Fb1* knock-down increases BeWo cell transcription and translation of secretory hormones. hCG transcription **(a)** and secreted protein **(b)** were increased 2.5-fold (mRNA) and 1.5-fold (secreted protein) 72 hours after suppressyn knock-down when compared to parental cells or to those treated with control siRNA. Human placental lactogen (*hPL*) transcription **(c)** and progesterone secretion **(d)** were likewise increased at 72 hours post suppressyn siRNA transfection. Black bars- *Fb1* siRNA exposed; hatched bars-control siRNA exposed. Data in (**a, b, c, d**) are representative of three independent experiments performed in duplicate. Expression is normalized to unexposed samples cultured for similar time periods (48 or 72H), $*p<0.05$ and $**p<0.01$ when compared to matched siRNA control. Statistical comparisons used Kruskal-Wallis and Mann-Whitney U-testing without Bonferroni corrections.



Supplementary Figure S4: Suppressyn inhibits syn1-medited cell fusion and co-transfection does not inhibit suppressyn translation or syn1 surface expression. (**a**) HTR8 parent cells and HTR8 cells stably-transfected with a vector driving suppressyn expression (HTR8-Fb1.1) were transiently transfected with increasing amounts of a vector driving expression of syn1 for 72 hours. Lysates were separated by PAGE and immunoblotted with polyclonal antibodies specific for syn1 and suppressyn and a monoclonal antibody detecting GAPDH. **(b)** HTR8 parental cells and those stably-transfected with two independent vectors driving suppressyn expression (HTR8-Fb1.1 or –Fb1.2) or vector-only control (HTR8-V) were transiently transfected with syn1. Surface expression of syn1 was confirmed using a polyclonal antibody specific for syn1 and analyzed using flow cytometry. The relative syn1 expression ratio was normalized to that in non-transfected HTR8 cells. Data are representative of three independent experiments performed in duplicate. (**c**) Cells stably transfected with two independent vectors driving suppressyn expression (HTR8-Fb1.1 or -Fb1.2) or vector-only control (HTR8-V) were stained with either Cell Vue Claret (Red) or CFSE (Green) (MINCLARET and 21888; Sigma-Aldrich). Color-mixed cells were transiently transfected with a vector driving syn1 expression, fixed at 24 hrs and analyzed using a Leica DMI 6000 B fluorescence microscope. Scale bar indicates 100μm.

Supplementary Table 1: Primer and siRNA sequences.

< Cloning >	
Fb1-1st-S	GATATCCAGGTGCTTATTAAAACA (21)
Fb1-1st-AS	GTATCCATCGTGCCGCTGTAG (2315)
Fb1flag-2nd-S	GGATATCCACAAGGAAGACTAACCACG (528)
Fb1flag-2nd-AS	CGGATCCGGATATAGTTTTTGTATAAAGG (1040)
Fb1-2nd-S	GGATATCCACAAGGAAGACTAACCACG (528)
Fb1-2nd-AS	CGGGATCCCAGGAGGTTAACTGTAGTTT (2273)
Syn1-1st-S	AACTGCGGTTAAAGTGGCTGGAGT (860)
Syn1-1st-AS	TTGGTCAGGTGTGAGCTAAGTTGC (2835)
Syn1-2nd-S	CGGATATCAGGATTTGCGCCTGCTCTTCAAAC (982)
Syn1-2nd-AS	CGGGATCCCGTGTTTAAAGGTGGATGTGGT (2807)
Syn2-1st-S	ACTTGTACACCACCAGGAGTTCCA(308)
Syn2-1st-AS	AGCGGGTGACTTGAGAGATCCAAT(2751)
Syn2-2nd-S	CGGGTACCATGGGCCTGCTCCTGCTGG(368)
Syn2-2nd-AS	CCTGATCAAAGAAGGGTGACTCTTGA(1985)
ASCT2-1st-S	AAGTTCCAGTCTCCAGGTGCTGTT(23)
ASCT2-1st-AS	ACAGCAGGTATTTGTCCTCAGCCT(2338)
ASCT2-2nd-S	CGGATATCATGGTGGCCGATCCTCCTC(138)
ASCT2-2nd-AS	CCAGATCTATGACTGATTCCTTCTCA(1762)
< Conventional and Real time PCR >	
Fb1-S	TCCGGGTTCCAACCAATGCAAGA
Fb1-AS	TGTGCCAGTAGGCGAGATCAGT
Gus-S	AGCAGTACCA TCTGGGTCTG
Gus-AS	TTGGTTGTCT CTGCCGAGTG
Syn1-S	CCACGAACGGACATCCAA
Syn1-AS	TCCACTCCAGCCACTTTAAC
Syn2-S	TCTCAAATGGTGCAGTGACTCGGA
Syn2-AS	TGCTGGTTCTGGCTCTGGAGTTTA
ASCT2-S	TCGATTCGTTCCTGGATCTTGCGA
ASCT2-AS	ACACTACCAAGCCCAGGATGTTCA
MFSD2-S	TCGCCTTATGCCCTGGATCATCTT
MFSD2-AS	TCGGTGCTGATGAACATGGTGAGA
hCG-S	CATCACCGTCAACACCACCATCT
hCG-AS	AGGAGACCACGGGGTTCACG
hPL-S	TGGACAGCTCACCTAGTGGCAAT
hPL-AS	AAGCCTGGATAAGGGAACGGTTTG
< siRNA >	
siFb1a	UGUAUCUACCCAACCACUUUCUAUA
siFb1b	CCCGCGGCAUUUCCAUUCCUUUAUA
siFb1a-C	Stealth RNAi negative low GC control : 12935-200
siFb1b-C	CCCGGACUUCUACUUCCUUUCGAUA

Numbers in parentheses indicate the 5’ sequence positions of the respective genes. (*HERV-Fb1*: c21orf105, *Syn1*: NM_014590.3, *Syn2*: BC068585, *ASCT2*: BC000062)