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Supporting Information

A systematic proteogenomic approach to exploring a novel function for NHERF1 in human reproductive disorder: lessons for exploring missing proteins

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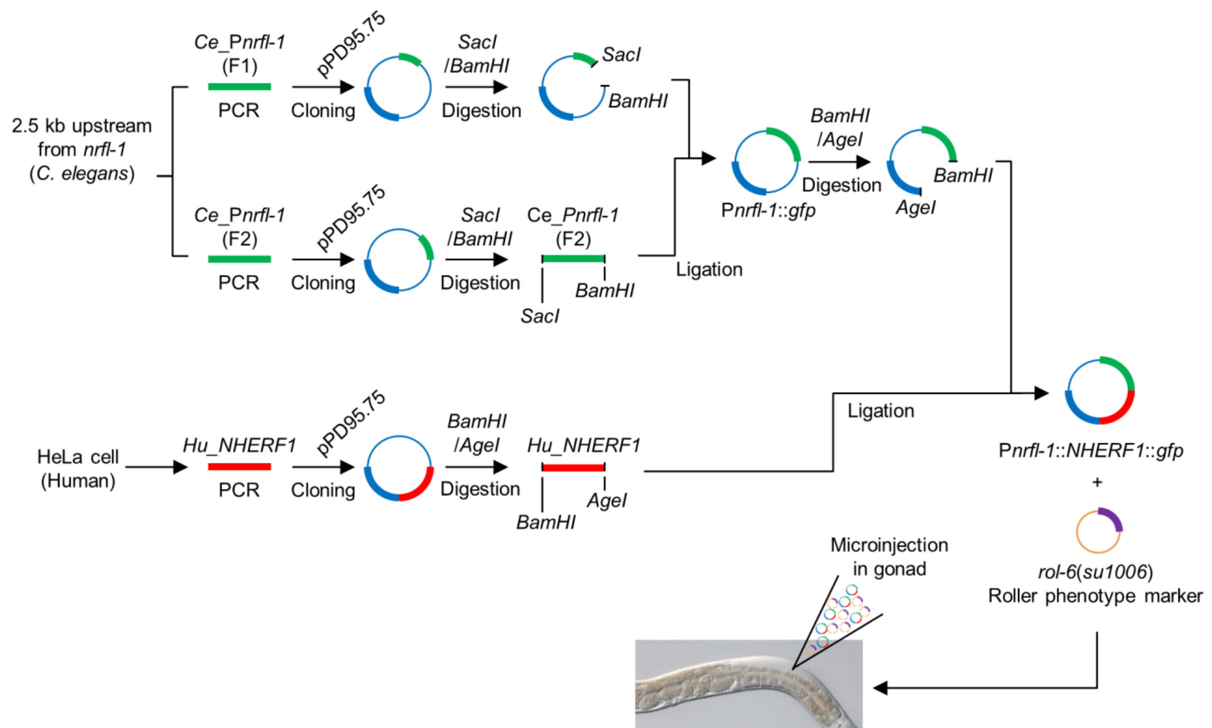

Figure S1. Generation of transgenic *C. elegans* which contains an extra-chromosomal human *NHERF1* gene.

A part of upstream fragment (2.5 kb) of the *nrfl-1* gene promoter (F1) was amplified by PCR using the oligonucleotide primers that contain not only the terminal ends of the target gene but also the sequence of restriction enzyme sites (*HindIII*, *SacI*). The newly created fragments were digested with restriction enzyme (*HindIII*, *SacI*) and inserted into the same restriction sites of pPD95.75 vector. We also amplified the other promoter region of *nrfl-1* (F2) which contains restriction enzyme sequence (*SacI*, *BamHI*) sites in the same manner. These two fragments were then combined into one expression vector by inserting F2 into restriction sites (*SacI*, *BamHI*) of the pPD95.75 vector containing F1. In a separate reaction, cDNA of NHERF1 was prepared from the total mRNA from human HeLa cells. Linker of restriction enzyme sequence (*BamHI*, *AgeI*) was attached to this cDNA of *Hu_NHERF1* and the resulting DNA fragment was amplified by PCR. The *Hu_NHERF1* gene was digested by restriction enzymes (*BamHI*, *AgeI*) and then inserted into the expression vector as prepared previously. The sequences of this recombinant DAN was selected by colony PCR. When transfected into cells, this recombinant vector (50 ng/μL) and *rol-6*(*su1006*) (10 ng/μL), a roller phenotype marker, were microinjected into *C. elegans* gonad.

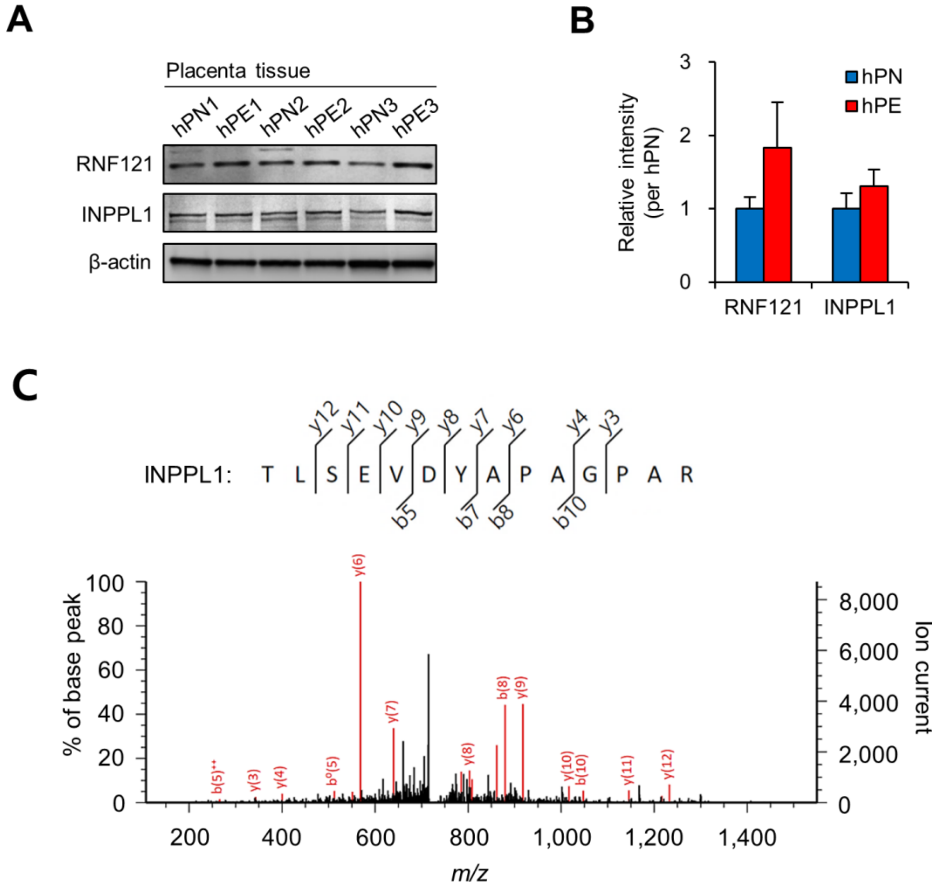

Figure S2. Validation by immunoblotting of RNF121 and INPPL1.

(A) Twenty micrograms of protein lysates for individual placenta tissues were used for detection of RNF121 and INPPL1 by Western blot. (B) The relative band intensity was estimated based on the average level for three hPN samples. Their differences between hPN and hPE were not significant ($P > 0.05$). (C) The antibody-captured target band was sliced, tryptic-digested, and identified as only INPPL1; RNF121 was not identified. The MS data show the representative detected INPPL1 peptide sequence “TLSEVDYAPAGPAR.”

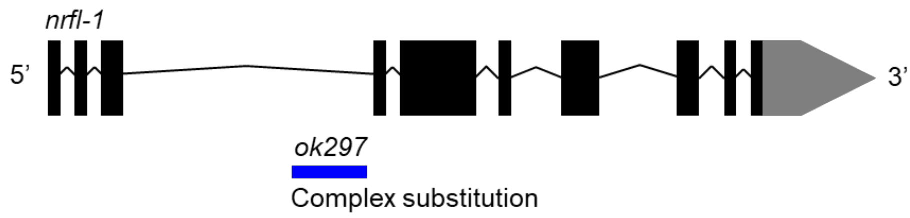

Figure S3. Illustration of complex substitution position of *nrfl-1*(*ok297*) mutants (from WormBase).

The *nrfl-1*(*ok297)* mutant has an *indel* mutation within the *nrfl-1* gene (724 bp insertion/16 bp deletion), which is located at intron 3 between exon 3 and exon 4 (blue line). Black box = exon; line = intron.