Supplementary information

Materials and Methods

Preparation of tissue samples from an E. nilssonii

A northern bat (*E. nilssonii*) was caught in Obihiro, Hokkaido, Japan and euthanized. The liver, kidney, spleen and lung were removed from the bat, and kept on ice until use.

Cell culture

The tissues were minced, and treated with PBS containing 0.05 % trypsin and 0.025 % EDTA at room temperature for 20 min. The trypsinized tissues were mixed with equal amount of Dulbecco's Modified Eagle's Medium/ Ham's F-12 (DMEM/F-12) containing 10% fetal calf serum (FCS), and centrifuged at 1,000 × g for 5 min. Debris was removed using cell strainers (EASYstrainer, Greiner Bio-One, Kremsmünster, Austria), and the cells were seeded on 6-well plates with DMEM/F12 or RPMI-1640 medium supplemented with 10% FCS, non-essential amino acids (Wako, Osaka, Japan), sodium pyruvate (Wako), gentamycin (Wako), penicillin/ streptomycin (Wako) and amphotericin B (Wako). The cells were passaged every two to six days depending on the cell density: the cells were washed with PBS and detached with PBS containing 0.25% trypsin and 1 mM EDTA. The detached cells were mixed with growth medium, and centrifuged at 1,000 × g for 3 min. The supernatant was removed, and the cell pellet was resuspended in the growth medium. The cells were seeded into a fresh culture dish at a ratio of 1:3 to 1:8 depending on the cell densities. For the kidney-derived cells HAMOI-EnK cells, the growth medium was changed to DMEM containing 10% FCS at the 40th passage, and the cells were subcultured as described above.

Sequencing and phylogenetic analysis of partial cytochrome b genes

To determine nucleotide sequence of partial cytochrome b (*cytb*) gene from the HAMOI-EnK cell, cellular DNA was isolated from the cells using Quick-gDNA™ MiniPrep (Zymo Research Corporation, Irvine, CA, USA). PCR was conducted with Phusion Hot Start II DNA Polymerase (Thermo Fisher Scientific, Waltham, MA USA) using primers that were designed based on highly conserved regions among *cytb* genes in bats of genus *Eptesicus* (5'-ACTACGGCTGAGTATTGCGC-3' and 5'-AGGCATTGGCTGATAGGACG-3'). PCR conditions were as follows: first denaturation at 94°C for 5 min, 35 cycles of 98°C for 10 sec, 63°C for 30 sec, 72°C for 30 sec, then final extension at 72°C for 5 min. The amplicon was purified with NucleoSpin Gel and PCR Clean-up (MACHEREY-NAGEL, Düren, Germany), and then sequenced at FASMAC (Atsugi, Japan).

For phylogenetic analysis, nBLAST was performed using the determined nucleotide sequence of *cytb* gene of HAMOI-EnK cells as a query. Nucleotide sequences of the top 50 hits in the BLAST search were collected, and were used for phylogenetic analysis. The nucleotide sequences of *cytb* genes were aligned with MUSCLE [2]. A phylogenetic tree was constructed using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model [5] and Gamma distribution by MEGA6 [13]. Reliability of the each interior branch was assessed by 100 bootstrap replications.

Cell growth

To investigate growth kinetics of HAMOI-EnK cells, 1×10^5 cells at 47th passages were seeded into a 12-well plate. The cells were detached with PBS containing 0.25%

trypsin and 1 mM EDTA, and cell numbers were counted by manual 26, 48, 76 hours after the seeding.

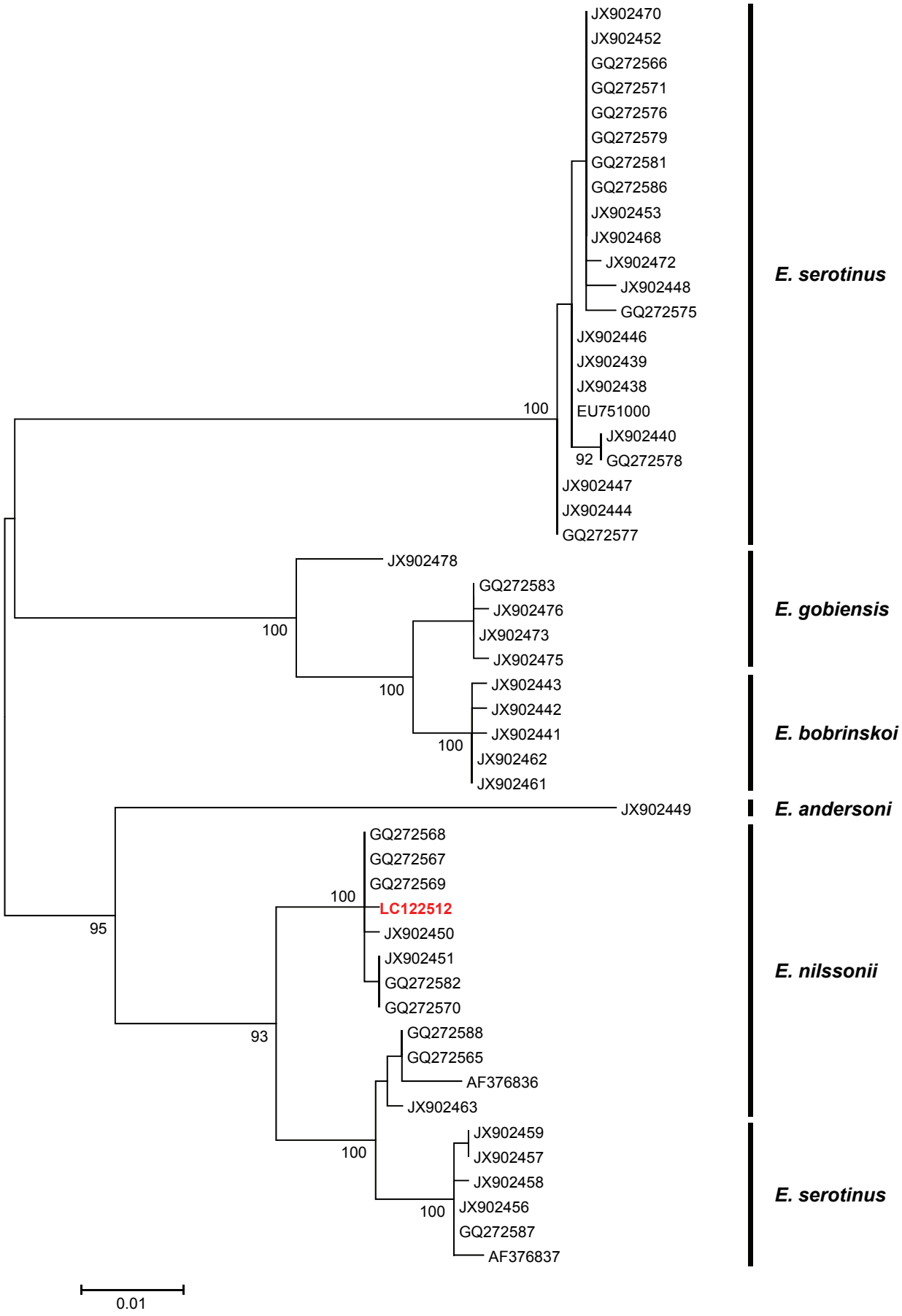
Detection and sequencing of eEBLL-1 in the genome of HAMOI-EnK cells

To detect eEBLL-1 in the genome of HAMOI-EnK cells, genomic PCR analyses were performed. PCR was conducted using 30 ng of the cellular DNA, primers 5'-CAATGCAGGTCTCTTTTACAGGTG-3' and 5'-TGTCAGTATAGCAAGTGGATCTCG-3' and Phusion Hot Start II DNA Polymerase according to the manufacturer's instruction. PCR conditions were as follows: first denaturation at 94°C for 5 min, 35 cycles of 98°C for 10 sec, 62°C for 30 sec, 72°C for 30 sec, then final extension at 72°C for 5 min (primer sequences are available in Table S1). The PCR products were analyzed by agarose gel electrophoresis. Bands of expected sizes were extracted and purified with NucleoSpin Gel and PCR Clean-up, which were subject to direct sequencing at FASMAC. When direct sequencing did not work, the amplicons were cloned by Zero Blunt TOPO PCR Cloning Kit (Thermo Fisher Scientific), and then sequenced at FASMAC.

Detection of eEBLL-1 transcripts

Strand-specific RT-PCR analysis was conducted to detect eEBLL-1 transcripts. RNA was extracted from HAMOI-EnK cells using NucleoSpin RNA plus (MACHEREY-NAGEL). cDNA was synthesized from 5 μg of the isolated RNA using SuperScript III Reverse Transcriptase (Thermo Fisher Scientific) and Oligo (dT), sense primer A (5'-GTGTCATGCTCATATGGAAAGCAGTTG-3') or antisense primer B (5'-TGTAGCAGTCTCCTGAAGGTGAAATTG-3') according to the manufacturer's

protocol. PCR was carried out using primrers 5'-CATAGAGCTGATGAAAGAGGGGTTCAG-3' and 5'-AAAACAATCCGAGCCAAACATTCTGTC-3' and Ex Taq Hot Start Version (TaKaRa, Kusatsu, Japan) as follows: first denaturation at 94°C for 2 min, 40 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec, then final extension at 72°C for 3 min. The PCR products were analyzed by agarose gel electrophoresis.



Supplementary Figure 1. A phylogenetic tree of cytochrome b genes. Nucleotide sequences of cytochrome b genes of *Eptesicus* bats were aligned with MUSCLE. The maximum likelihood tree was constructed using HKY + G model of nucleotide substitution. The reliability of the each interior branch was assessed by 100 bootstrap resamplings. The scale bar shows the number of amino acid substitutions per site. The cytochrome b gene in HAMOI-EnK cells was shown in red.