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Supplementary Information for

Inefficient development of syncytiotrophoblasts in the *Atp11a*-deficient mouse placenta.

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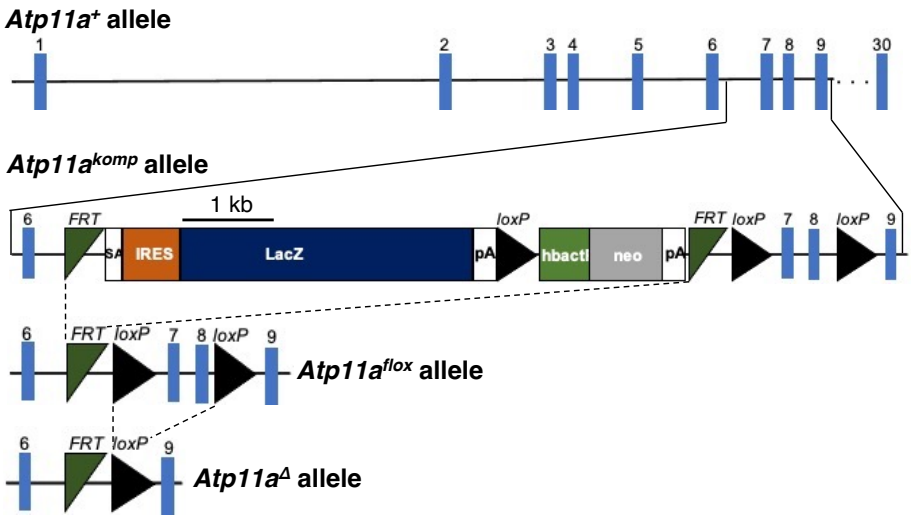

Fig. S1. Null and floxed alleles of the *Atp11a* gene.
The alleles of the wild-type (*Atp11a*$^{+/+}$), knockout first (*Atp11a*^{komp}), floxed (*Atp11a*^{flox}), and exons deleted (*Atp11a*$^{\Delta}$) *Atp11a* gene are schematically shown. The mutant mice carry the *Atp11a*^{komp} allele, which holds an approximately 10-kb DNA fragment containing a gene trap sequence in intron 6 and the exons 7 and 8 flanked by loxP sequences. Blue boxes represent exons. FRT, yeast flippase (FLPe) recognition site; SA, splicing acceptor sequence; IRES, internal ribosome entry site; LacZ, β-*galactosidase* gene; pA, polyadenylation signal; loxP, Cre recombinase recognition site; hbactP, human β-actin promoter; neo, the neomycin-resistant gene. *Atp11a*^{flox} mice were generated by crossing *Atp11a*^{komp} mice with CAG-FLPe mice. *Atp11a*^{flox} mice were then crossed with transgenic mice expressing the *Cre* gene in a tissue-specific manner to generate mice carrying the tissue-specific *Atp11a*$^{\Delta}$ allele.

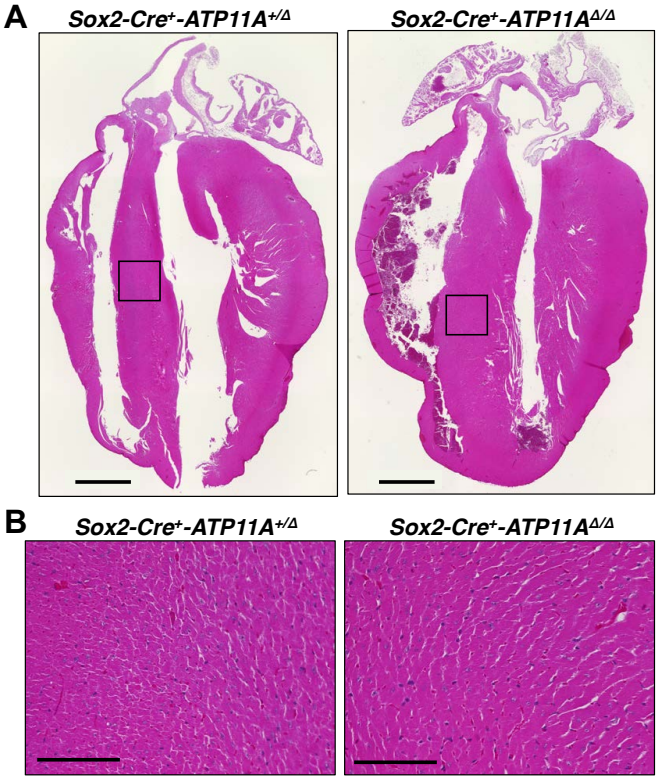

Fig. S2. No abnormalities in the heart of *Sox2-Cre*$^{+}$*-Atp11a*$^{\Delta/\Delta}$ mice.
The heart sections of 16 months-old *Sox2-Cre*$^{+}$*-Atp11a*$^{+/\Delta}$ or *Atp11a*$^{\Delta/\Delta}$ littermate mice were stained with H&E. The boxed area in (A) was enlarged in (B). The scale bar; 1000 mm (A) and 100 mm (B)

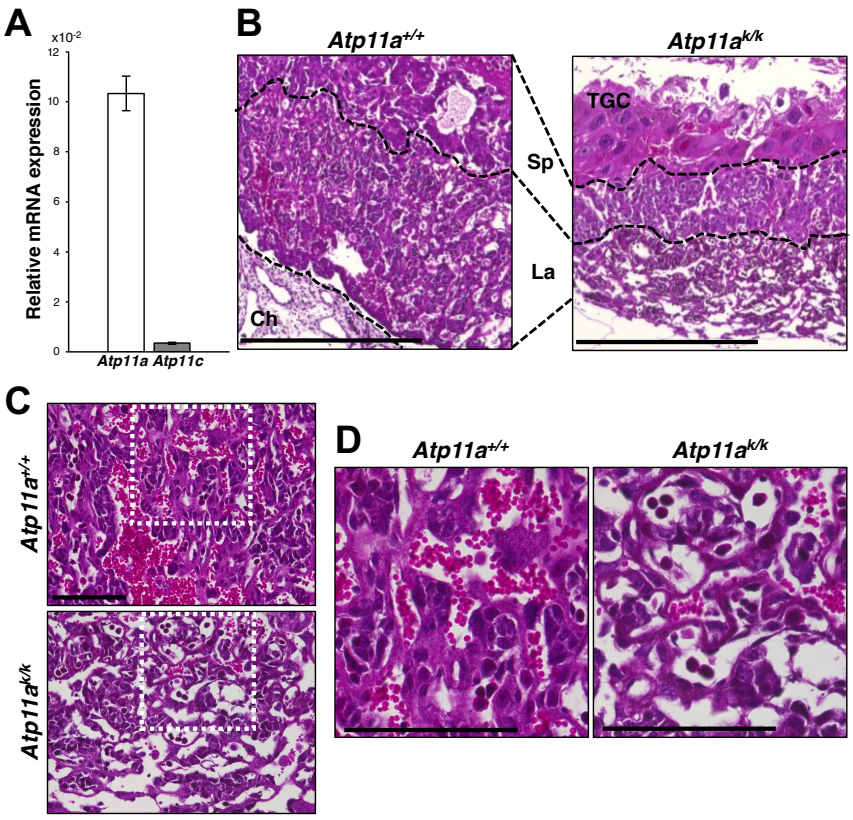

Fig. S3. The abnormal labyrinthine layer of the E11.5 *Atp11a*-deficient placenta.
(A) mRNAs for *Atp11a* and *Atp11c* in the E11.5 wild-type mouse placenta (n=3) were quantified by real-time RT-PCR, and their relative levels against *Gapdh* mRNA were plotted with SD. (B-D) Histological analysis of the E11.5 placenta (B) and labyrinthine layer (C and D). *Atp11a*$^{+/komp}$ mice were intercrossed. Paraffin sections from the *Atp11a*$^{+/+}$ or *Atp11a*$^{k/k}$ placenta were stained with H&E and observed by microscopy. Higher magnification images of the boxed regions are shown in (D). The scale bar is 500 mm in (B) and 100 mm in (C and D). TGC; Trophoblasts giant cells, Sp; Spongiotrophoblasts layer, La; Labyrinthine layer, Ch; Chorion.

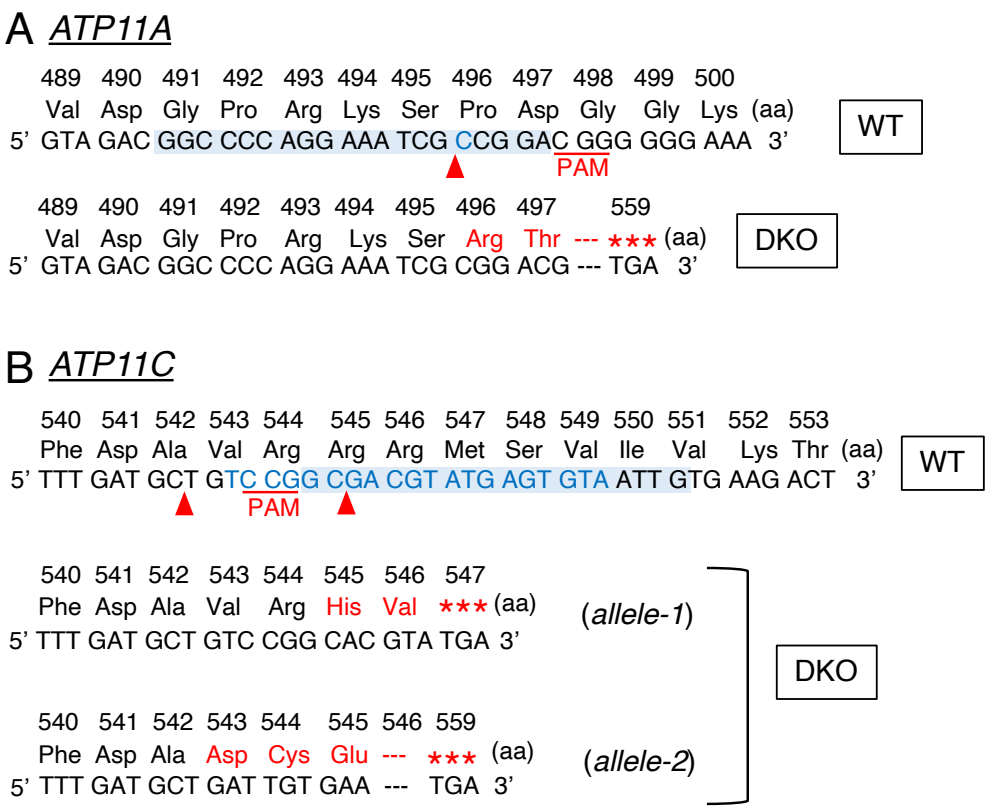

Fig. S4. Gene knockout in human BeWo cells by the CRISPR/Cas9 system.
The wild-type and mutated alleles of the *ATP11A* gene (A) and *ATP11C* gene (B) in *ATP11A*$^{-/-}$ *ATP11C*$^{-/-}$*(DKO)* BeWo cells are shown. Protospacer sequences are highlighted in light blue, protospacer-adjacent motifs (PAM) are underlined in red, and red arrowheads point to cleavage sites. Deleted and inserted sequences are shown in blue and red, respectively.

Table S1. No effect of the engulfment system on the lethality of the *Atp11a*$^{k/k}$ embryo

genotype	*MerTK*$^{-/-}$*Axl*$^{-/-}$ *Atp11a*$^{+/+}$	*MerTK*$^{-/-}$*Axl*$^{-/-}$ *Atp11a*$^{+/k}$	*MerTK*$^{-/-}$*Axl*$^{-/-}$ *Atp11a*$^{k/k}$	total
Number	18	14	0	32

MerTK$^{-/-}$*Axl*$^{-/-}$*Atp11a*$^{+/komp}$ mice were intercrossed, and the genotypes of 32 pups were identified at the age of 4 weeks. The number of mice with the indicated genotype is shown.

Table S2. Primers used for genotyping and real-time RT-PCR.

Primers for mouse genotyping

	Forward primer	Reverse primer
*Atp11a*_wild	5'-CACGTCTGTGTTCCTGTGTCC-3'	5'-TATTTGATGCACCTGCCCTG-3'
*Atp11a*_mutant	5'-CACGTCTGTGTTCCTGTGTCC-3'	5'-TCGTGGTATCGTTATGCGCC-3'
*Atp11a*_wild 2	5'-AGTTTGGGAAACGGGACCAA-3'	5'-GTGAGTCCTACTGGGGGTCT-3'
Atp11a^{flox} and Atp11a$^{\Delta}$	5'-ACTTCGTCGAGATAACTTCGTAT-3'	5'-CGGGTAGGCTAATCTGCTGT-3'
CAG-Flpe	5'-CCTACAGCTCCTGGGCAACGTGC-3'	5'-CTGCTTCTTCCGATGATTCG-3'
Mhc-Cre	5'-ATGACAGACAGATCCCTCCTATCTCC-3'	5'-CTCATCACTCGTTGCATCATCGAC-3'
Sox2-Cre	5'-CCAGTGCAGTGAAGCAAATC-3'	5'-TAGTGCCCCATTTTTGAAGG-3'
Sox2-Cre non-transgenic	5'-CTTGTGTAGAGTGATGGCTTGA-3'	5'-TAGTGCCCCATTTTTGAAGG-3'
Zfy	5'-GAACGGAAAGACTGGTGAGC-3'	5'-CACTCAGAACTCACCAGACC-3'

Primers for Real-Time RT-PCR

Mouse genes

Atp8a2	5'-ATTGTTCCTGGTTCCGACTG-3'	5'-GGAGGTGTCTTCCTGCTGAG-3'
Atp11a (Probe 1)	5'-GCCAGCTTAGACGGAGAGTC-3'	5'-TGACCCCAAAGGCCTCACTA-3'
Atp11a (Probe 2)	5'-CTGGCGGGTGTTCATTTACT-3'	5'-TGACAGTGAGCACCATCACA-3'
Atp11c	5'-TTACAGTTGGGGCCCTTCTT-3'	5'-TCTGAAGCTCGCCTTGGATA-3'
Gapdh	5'-AACGACCCCTTCATTGAC-3'	5'-TCCACGACATACTCAGCAC-3'
Tfrc	5'-GTTCGTACAGCAGCGGAAGT-3'	5'-GGAAGTAGTCTCCACGAGCG-3'
Mct4	5'-ATACAGCGGCTGGCGGTAA-3'	5'-GCTGCTTTCACCAAGAACTGA-3'
Gcm1	5'-AGATTTACCTGAGACCCGCC-3'	5'-GTCGTGCCTCCAGAAGTTGG-3'
Mct1	5'-AGTGCAACGACCAGTGAAGT-3'	5'-GCGATCATTACTGGACGGCT-3'

Human genes

ATP8A2	5'-GTTGGTTCATGGAGCCTGGA-3'	5'-GTTGATGCAGTGACCCCAGA-3'
ATP11A	5'-TCCAGTGACAAGCGGACTTC-3'	5'-TCAAGTCGCAGGGAAAGGTC-3'
ATP11C	5'-GCCGGAGCTTGAATCGTTTT-3'	5'-TGGTGTGTCTACTGTGACCTG-3'
ERVFRD-1 (SYNCYTIN-2)	5'-GCCTGCAAATAGTCTTCTTT-3'	5'-ATAGGGGCTATTCCCATTAG-3'
DYSF (DYSFERLIN)	5'-CCAAGCATGCTGAGGGTCTT-3'	5'-TCCCCATCGTCTCATGGTCT-3'
ACTB (BETA-ACTIN)	5'-GATTCCTATGTGGGCGACGA-3'	5'-AGGTCTCAAACATGATCTGGGT-3'

Oligonucleotides to generate sgRNA for human *ATP11A* and *ATP11C* genes

ATP11A	5'-CACCGGGCCCCAGGAAATCGCCGGA-3'	5'-AAACTCCGGCGATTTCCTGGGGCCC-3'
ATP11C	5'-CACCGCAATTACACTCATACGTCGC-3'	5'-AAACGCGACGTATGAGTGTAATTGC-3'