# Deficiency of a retinal dystrophy protein ACBD5 impairs peroxisomal β-oxidation of very-long-chain fatty acids

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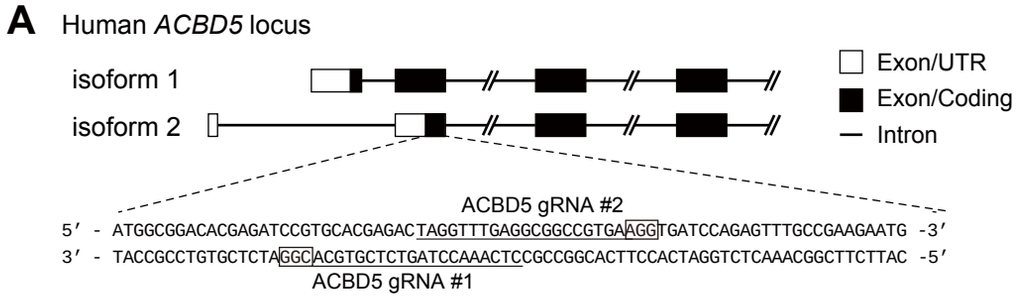
Figure S1. **Generation of ACBD5-KO HeLa cells via the CRISPR/Cas9-mediated genome editing.**
*A*, Schematic representation of the genomic structure of the human *ACBD5* locus. The partial genomic structures for ACBD5 isoform 1 and isoform 2 (NCBI reference sequences: NP_663736.2 and NP_001035938.1, respectively) are shown. Boxes represent exons, and horizontal lines connecting the exons indicate introns. Open and filled regions in the exons are untranslated and coding regions, respectively. The 20-bp target sequences of ACBD5 gRNA#1 and ACBD5 gRNA#2 are underlined. The PAM sequences are depicted and boxed. *B*, The genomic DNA was extracted from ACBD5-KO#1 (a) and ACBD5-KO#2 (b) cell lines, respectively, and a genomic region containing the target sites of the *ACBD5*-specific gRNAs was amplified, subcloned, and sequenced. The 20-bp target sequences and the PAM sequences are indicated as in A. Overlined letters and dashes depict the identified insertions and deletions, respectively The numbers of insertions and deletions (+, insertions; Δ, deletions) as well as the frequency of the mutated alleles detected are shown on the right.


A
Human ACBD5 locus
isoform 1
isoform 2
Exon/UTR
Exon/Coding
Intron
ACBD5 gRNA #2
5′ - ATGGCGGACACGAGATCCGTGCACGAGACTAGGTTTGAGGCGGCCGTGAAGGTGATCCAGAGTTTGCCGAAGAATG -3′
3′ - TACCGCCTGTGCTCTAGGCACGTGCTCTGATCCAAACTCCGCCGGCACTTCCACTAGGTCTCAAACGGCTTCTTAC -5′
ACBD5 gRNA #1

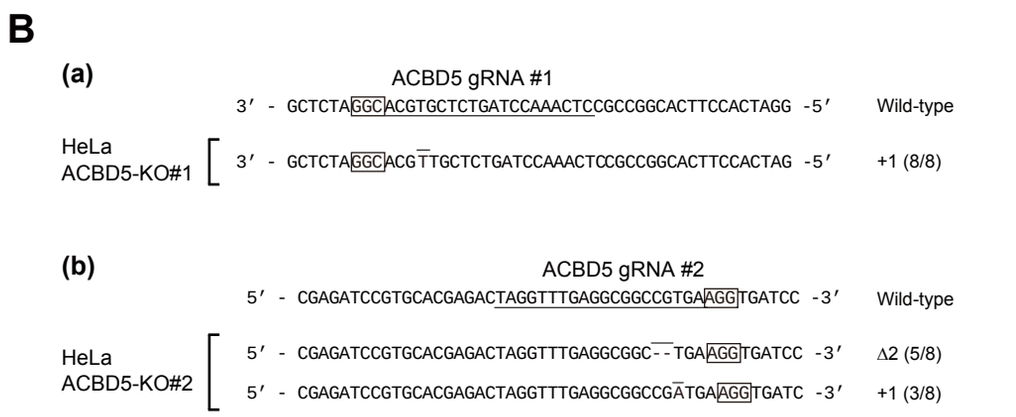

B
(a)
ACBD5 gRNA #1
3′ - GCTCTAGGCACGTGCTCTGATCCAAACTCCGCCGGCACTTCCACTAGG -5′
Wild-type
HeLa ACBD5-KO#1
3′ - GCTCTAGGCACG–TTGCTCTGATCCAAACTCCGCCGGCACTTCCACTAG -5′
+1 (8/8)
(b)
ACBD5 gRNA #2
5′ - CGAGATCCGTGCACGAGACTAGGTTTGAGGCGGCCGTGAAGGTGATCC -3′
Wild-type
HeLa ACBD5-KO#2
5′ - CGAGATCCGTGCACGAGACTAGGTTTGAGGCGGC--TGAAGGTGATCC -3′
Δ2 (5/8)
5′ - CGAGATCCGTGCACGAGACTAGGTTTGAGGCGGCCGATGAAGGTGATC -3′
+1 (3/8)

Fig. S1