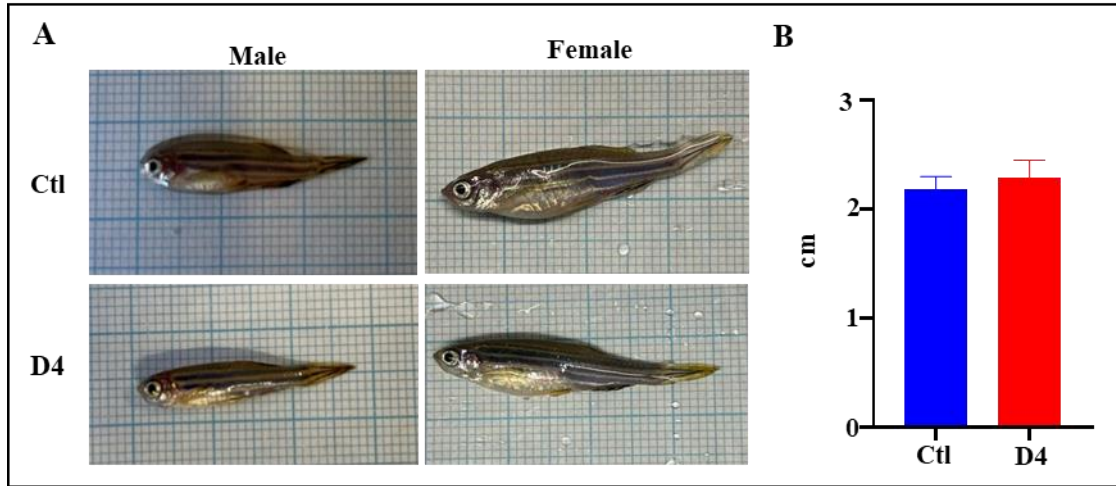
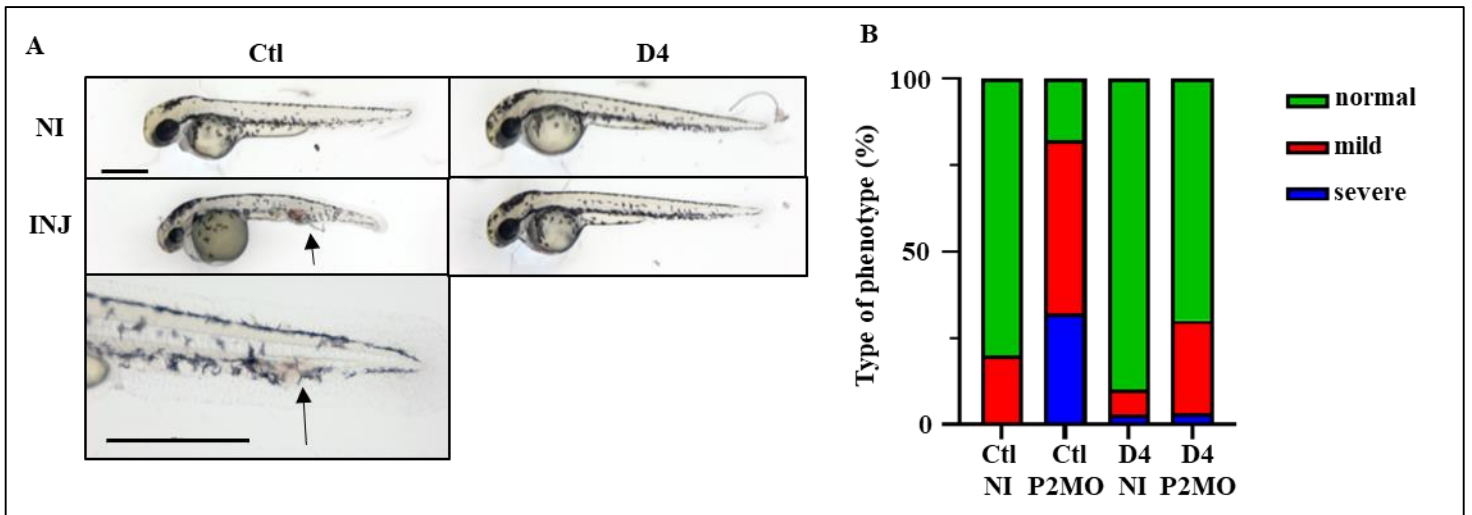


Supplementary Figure S1. Description of the CRISPR/Cas9-induced mutation in *pank2* gene
(A) Chromatograms (Sanger sequencing) of D4 and wild-type *pank2* exon 1. **(B)** Alignment of the wild-type amino acid sequence with the predicted D4 mutant sequence, obtained by the Expasy translate tool (<https://web.expasy.org/translate/>). **(C)** Graphic representation of the predicted mutant protein; the yellow area indicates conserved amino acids; the white one different amino acids.

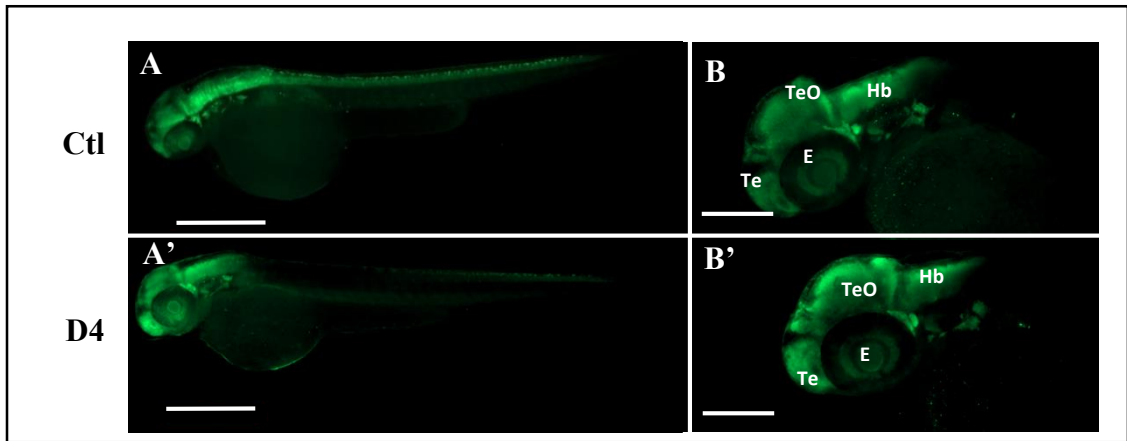


Supplementary Figure S2. Morphological characterization of 1-year-old D4 mutant fish. (A) Lateral view of representative 1-year-old adult mutant fish on graph paper. Left panels = females. Right panels = males. (B) Measurement of total body length of adult fish; N = 8/group.

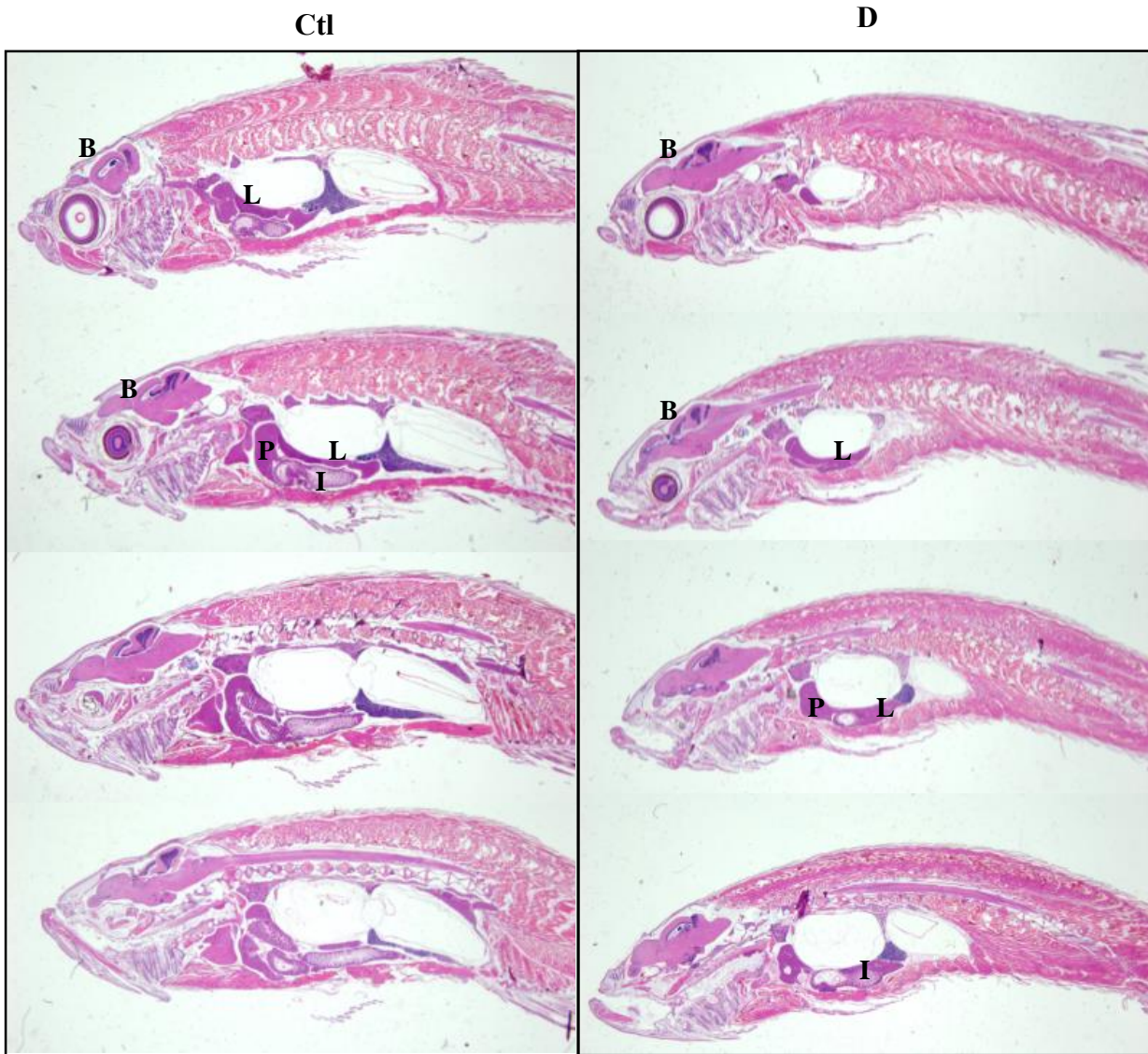


Supplementary Figure S3. Mutant line validation by injection of a *pank2*-specific morpholino (P2MO).

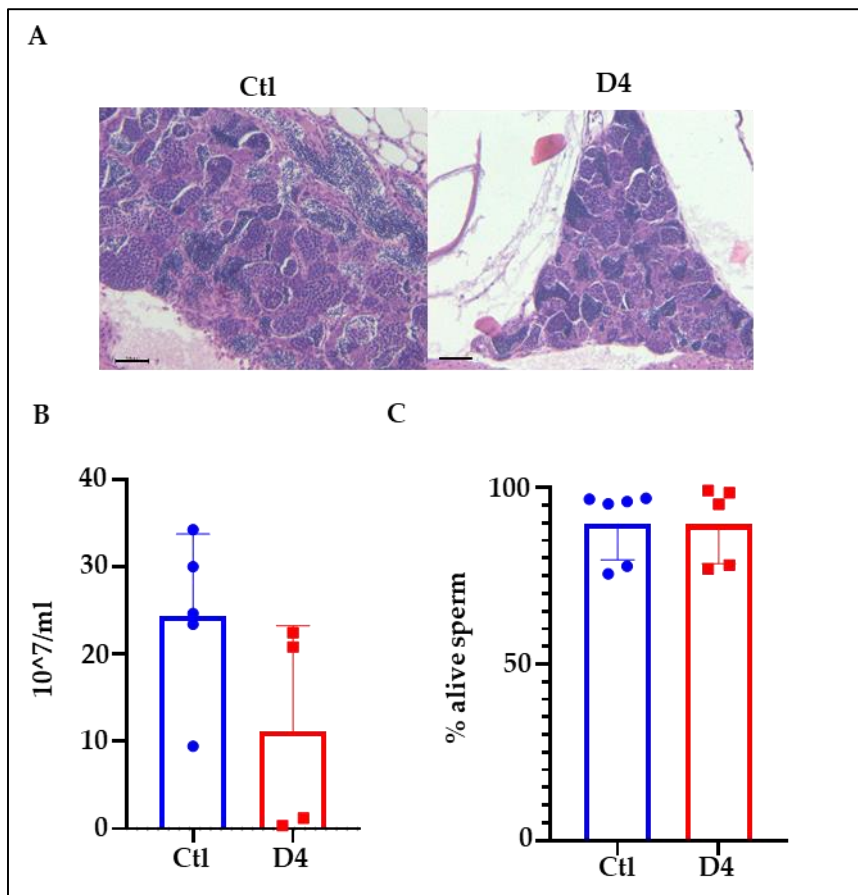
(A) Lateral view of 48 hpf embryos; arrows indicate blood accumulation. (N = 1, D4 NI = 50 embryos, D4 P2MO = 63 embryos, Ctl NI = 57 embryos, Ctl P2MO = 59 embryos); Size bar = 500 μ m. (B) Graph with the phenotype distribution in not injected and injected embryos. NI, not injected; P2MO, *pank2*-specific morpholino-injected embryos.



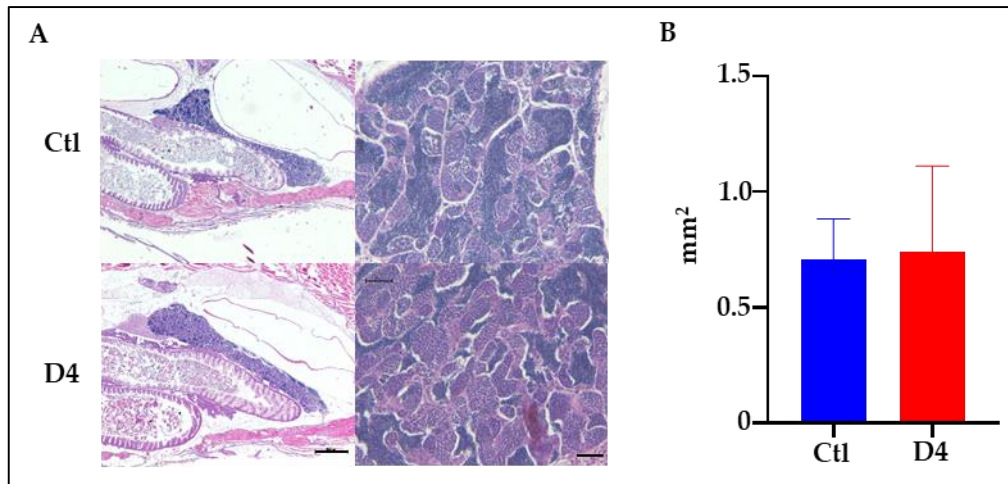
Supplementary Figure S4. Immunofluorescence staining for the pan-neuronal marker Elavl3+4. (A, A') Lateral view of stained embryos at 48 hpf. N = 2; D4 (37/40) and control embryos (37/38). Size bar = 500 μ m. **(B, B')** Magnification of the lateral views of the head of 48 hpf embryos. E, eye; Hb, hindbrain; Te, telencephalon, TeO, tectum opticum.



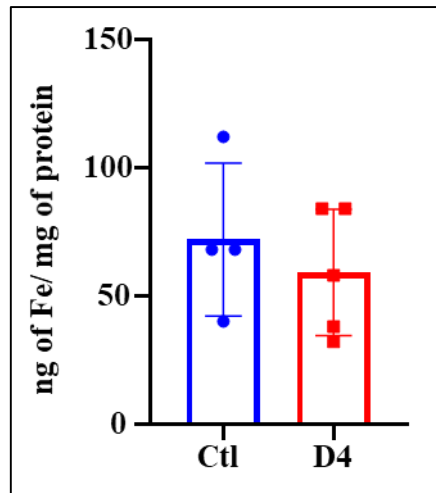
Supplementary Figure S5. Histological sections stained with hematoxylin-eosin of 1-year-old fish. B, brain; I, intestine; L, liver; P, pancreas. N = 7 for each group



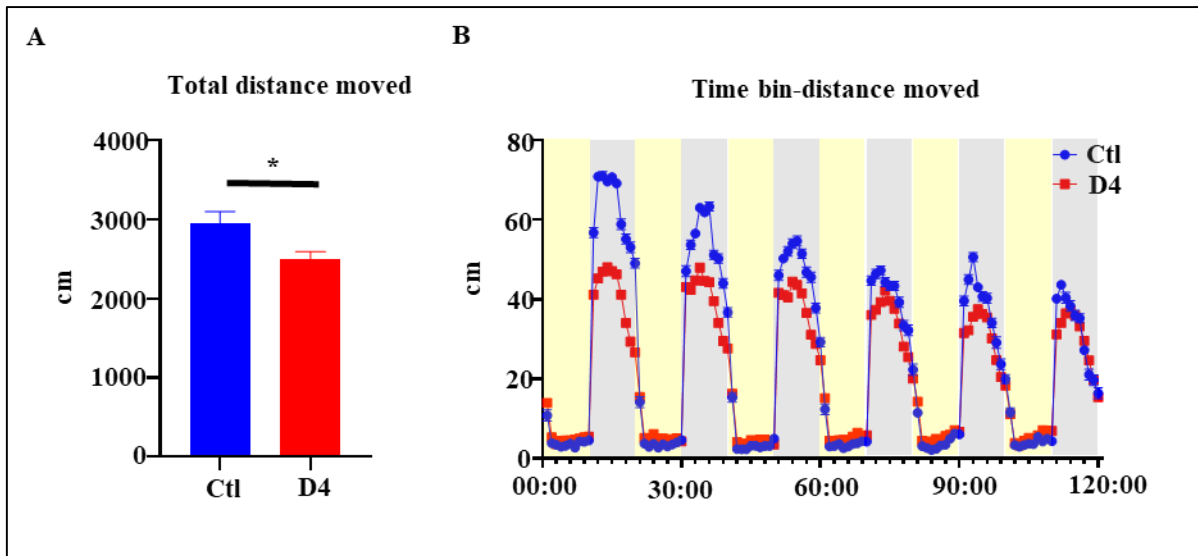
Supplementary Figure S6. Number and vitality of spermatozoa in 1-year-old fish. (A) Representative histological sagittal sections of 1-year-old fish, stained with hematoxylin-eosin. N = 5 for each group. Magnification 110X. Size bar = 50 μm . (B) Concentration of ejaculated sperm from 1-year-old fish. Ctl = 5, D4 = 4; Ctl mean = $24.35 \times 10^7/\text{ml}$, D4 mean = $11.20 \times 10^7/\text{ml}$. (C) Viability of ejaculated sperm from 1-year-old fish. Ctl = 5, D4 = 5; Ctl mean = 89.90%; D4 mean = 89.66%.



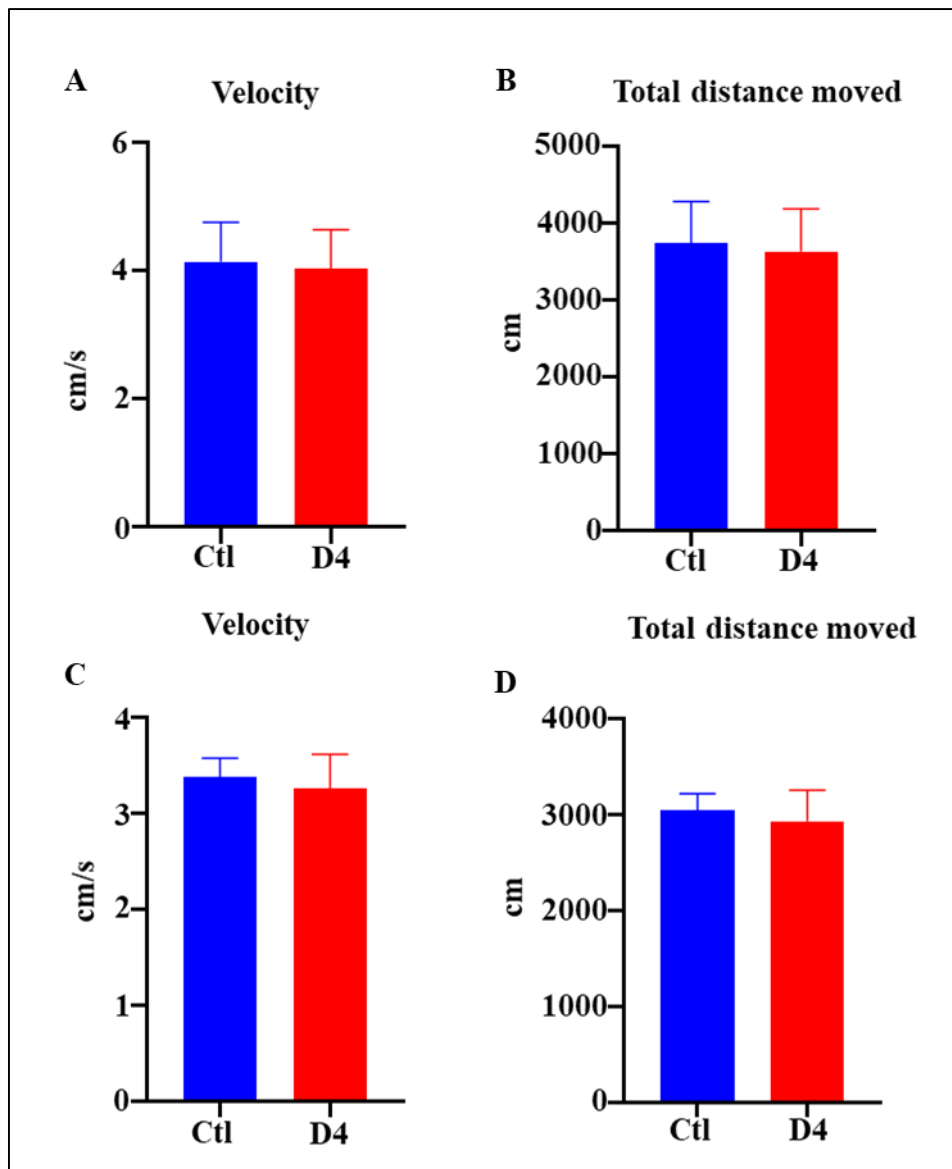
Supplementary Figure S7. Investigation of the testis in 3-month-old fish. (A) Representative histological sagittal sections of 3-month-old fish, stained with hematoxylin-eosin. N = 3 for each group. Magnification 40X and 110X. Size bar = 500 μ m and 50 μ m (B) Quantification of testis area performed on histological sections; Ctl mean = 0.70 mm²; D4 mean = 0.74 mm².



Supplementary Figure S8. Iron quantification in the brain. Iron quantification by ICP-MS in brains from 1-year-old control (Ctl).and mutant (D4) fish. N = 4 for controls and N = 5 for D4.



Supplementary Figure S9. Behavioral study in control and mutant embryos at 5 dpf. (A) The graph shows the mean plus standard deviation of the total distance swam by control (Ctl) and mutant (D4) fish. (B) Time bin analysis of the movement. Yellow indicates light on, gray indicates light off. Ctl = 67, D4 = 125, two biological replicates. * $P < 0.05$. Student's t-test.



Supplementary Figure S10. Behavioural analysis in adult fish. (A) Total distance swam and (B) mean velocity of 1-year-old fish; N = 11 for Ctl and 12 for D4. (C) Total distance swam and (D) mean velocity of 3-month-old fish. N = 3.

qPCR primers		
Gene		Sequence
coasy	Forward:	5'- CATTGGCTCTTCAGTCTCCTC-3'
coasy	Reverse:	5'- AGGTTTGGGTCGCAACTATC-3'
pank2	Forward:	5'- TCCATGAAGTTGCTCGCGTA-3'
pank2	Reverse:	5'- CAGCTCAGGATGGTTTGGT-3'
pank1a	Forward:	5'-TACCTCACGTCCAACACAGC-3'
pank1a	Reverse:	5'-AGTCAATGTACAGCAGGCC-3'
pank1b	Forward:	5'-CGGAGGTGGTCTGGGTACTA-3'
pank1b	Reverse:	5'-CGGCCCATTTGGATAAAGCG-3'

Genotyping		
Gene		Sequence
pank2	Forward:	5-AGCTGCCTCGGTCAAAGAC-3'
pank2	Reverse:	5'-AGTGAGTCGAGCCTCAGCAT-3'

sgRNA		
Gene		Sequence
pank2		GGGGAAGTGCGCTCAGAGAG

Supplementary Table S1. Sequences of primers and sgRNA.