Supplementary information:

MAP11 (C7orf43) is a microtubule-associated protein whose mutations cause microcephaly in humans and zebrafish

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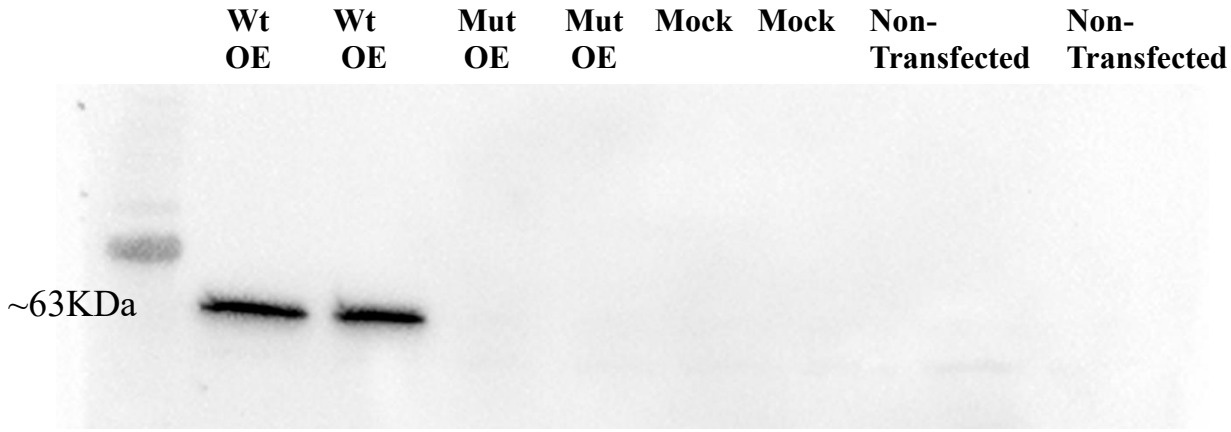
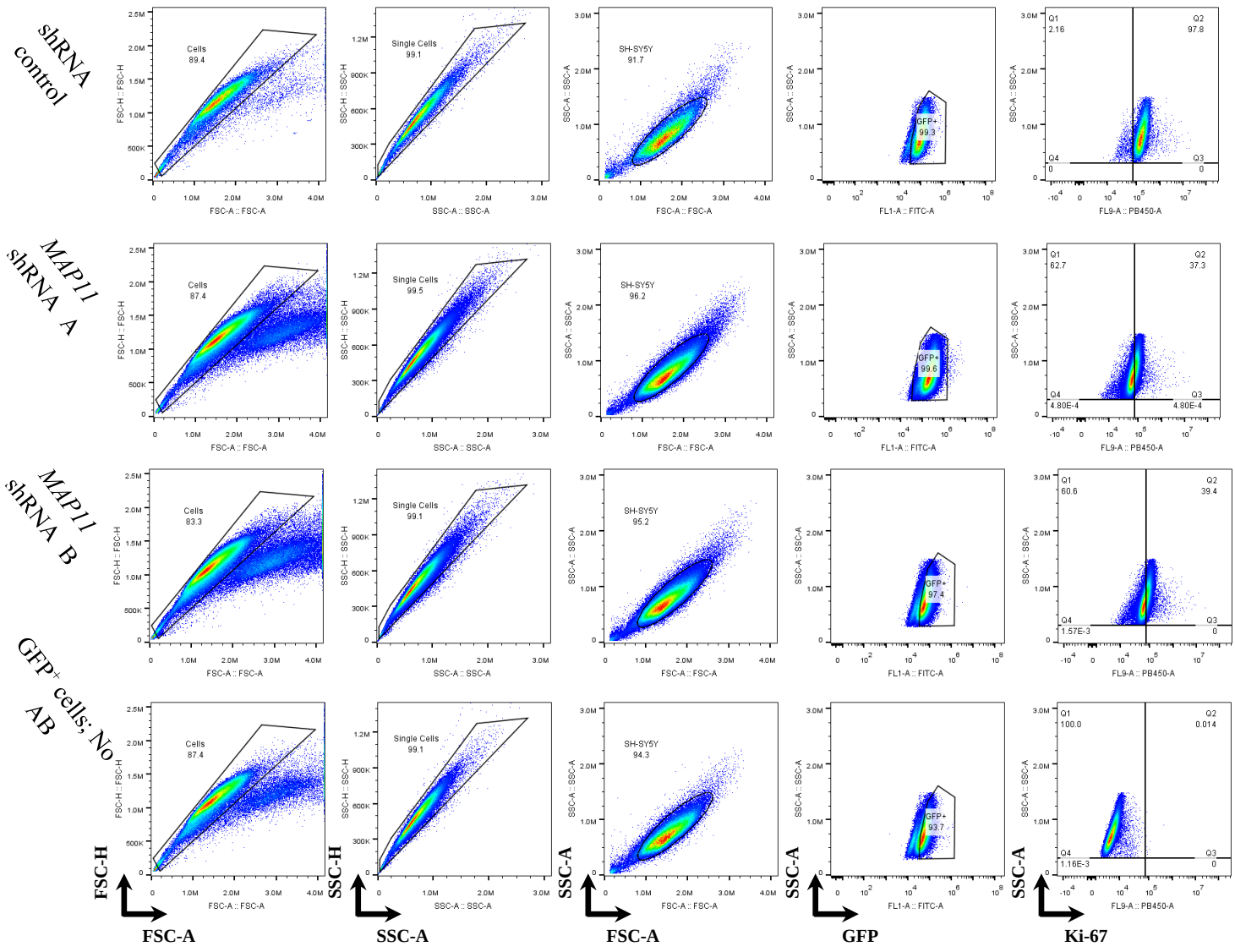
Supplementary figures:

Supplementary Fig. 1



Supplementary Fig. 1. Rabbit anti-human C7orf43 antibody (HPA019359; SIGMA) validation by western blot. SH-SY5Y cells were transfected with overexpressing constructs of wild-type and mutant MAP11 using lipofectamin 2000®. Twenty-four hours post transfection cells were lysed with protein sample buffer and run on polyacrylamide gel electrophoresis. Gel was transferred to nitrocellulose membranes using a wet transfer apparatus. Membrane was then incubated with the rabbit anti-C7orf43 antibody in a 1:5000 dilution concentration followed a secondary goat anti-rabbit IgG-HRP conjugated antibody (Santa Cruz Biotechnology Inc.; Sc-2004) in a 1:20000 dilution concentration. Membrane was washed 3 times in TTBS and visualized using an enhanced chemiluminescence detection kit (SuperSignal West Pico chemiluminescent substrate; Thermo scientific) in ChemiDoc MP imaging system (Bio Rad).

Supplementary Fig. 2

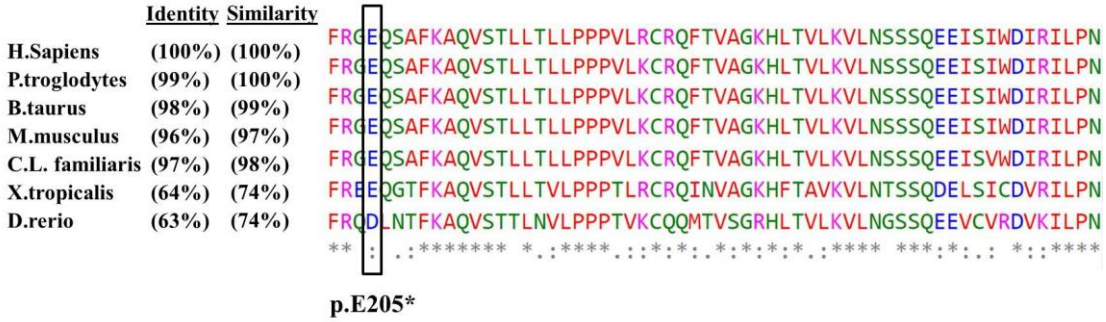


Supplementary Fig. 2. Proliferation assay of *MAP11* (C7orf43) knock-down cells. Cellular proliferation of *MAP11* knock-down cells was measured by fluorescence activated cell sorting (FACS) analysis of cells expressing the Ki-67 marker. FACS gating's were focused on minimizing cell debris while maintaining GFP positive single cells (the shRNA lentiviral cassette contains copGFP reporter) expressing the Ki-67 marker in the analysis. A negative control is represented by GFP positive cells not incubated with the Pacific blue Ki-67 antibody. A blue arrow shows the gating chronology.

Supplementary Fig. 3

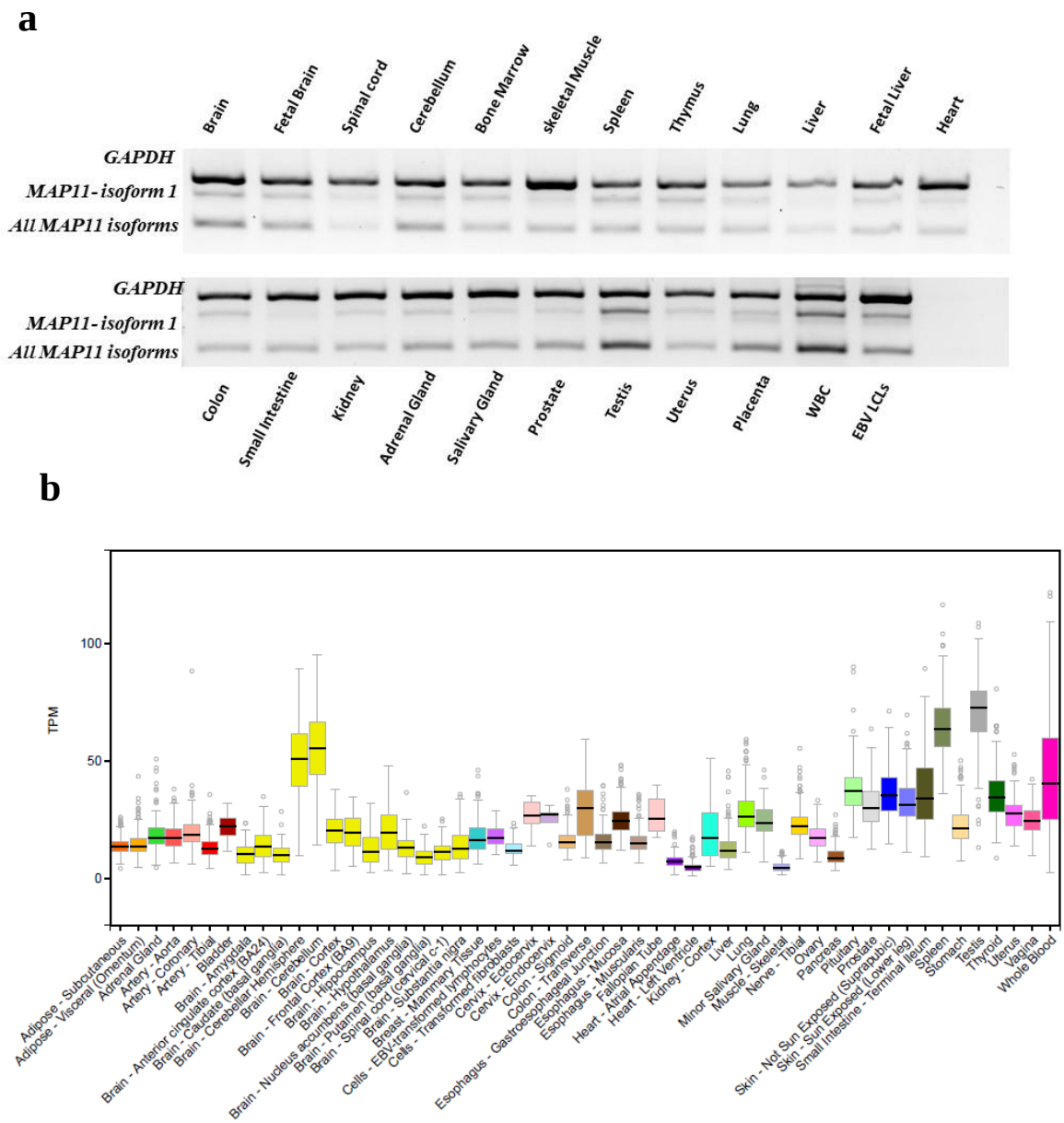
Supplementary Fig. 3. Frontal and lateral pictures of patient II:4, presenting microcephaly without facial dysmorphic features.

Supplementary Fig. 4



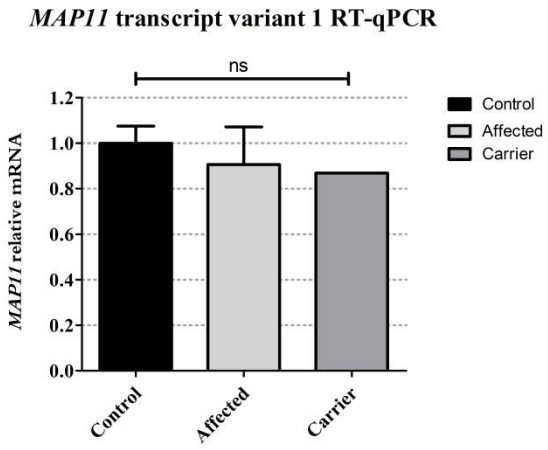
Supplementary Fig. 4. Multiple sequence alignment (MSA) of selected MAP11 vertebrate orthologues demonstrating its conservation. The putative p.(E205*) truncating mutation is boxed in black. MAP11 is highly conserved throughout evolution, as seen by values for identity and similarity of the full encoded protein (brackets, left)

Supplementary Fig. 5



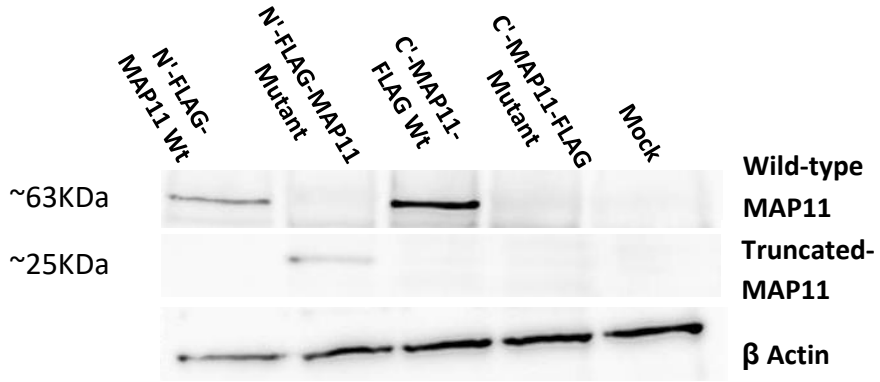
Supplementary Fig. 5. *MAP11 (C7orf43)* tissue expression. a) RT-PCR of 21 normal human tissues showing that *MAP11* is ubiquitously expressed in all tissues examined and is highly transcribed in brain, cerebellum, testis and whole blood. b) GTEx consortium of human *MAP11* data. Our in-house panel analysis demonstrates similar *MAP11* expression trends as in the GTEx data.

Supplementary Fig. 6



Supplementary Fig. 6. The mutant *MAP11 (C7orf43)* transcript is not degraded due to nonsense-mediated-mRNA-decay (NMD). A graph showing similar levels of the *MAP11* transcript variant 1 derived of patients and control lymphocytes. (Control: N=3; Affected: N=3; Carrier: N=1; ns= not statistically significant).

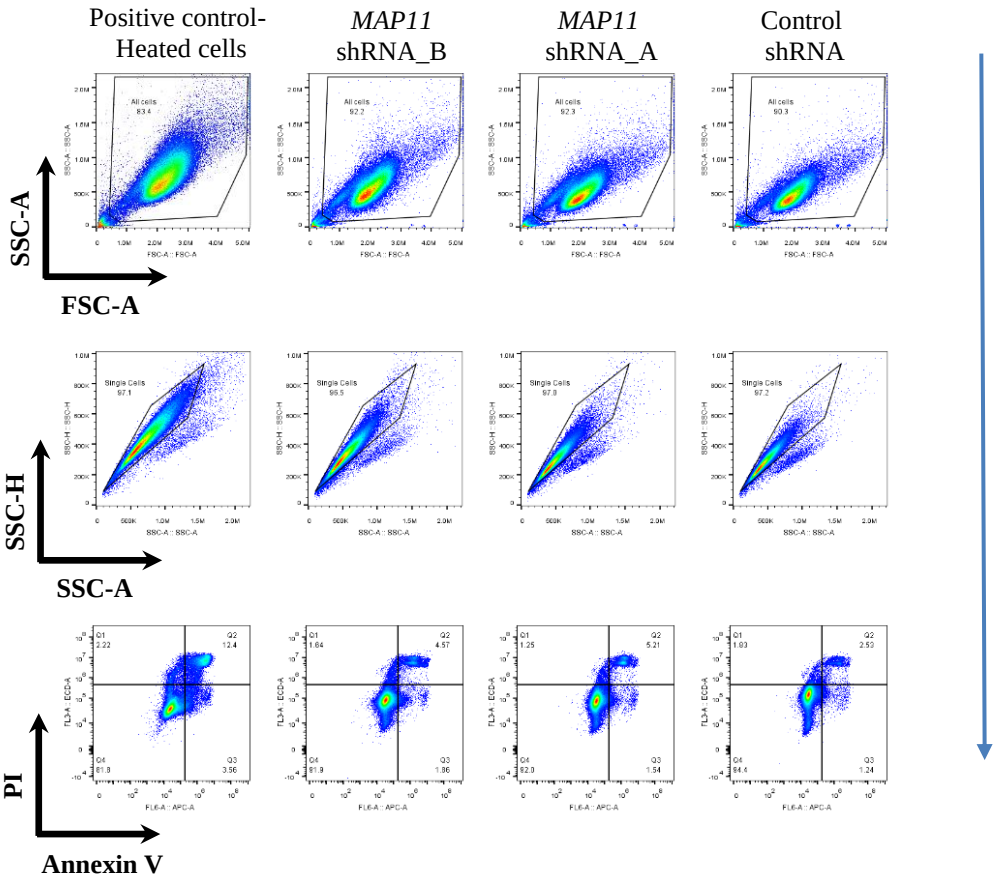
Supplementary Fig. 7



Supplementary Fig. 7. Western blot analysis of HEK293T cells overexpressing wild-type and mutant *MAP11*. N'-FLAG-MAP11 constructs are expressing the FLAG sequence fused to the N'-terminal part of MAP11. C'-MAP11-FLAG constructs are expressing the FLAG sequence fused to the C'-terminal part of MAP11. MAP11 fusion proteins were detected with a monoclonal mouse anti-FLAG primary antibody (F3165; Sigma Aldrich) and a goat anti-mouse HRP conjugated antibody (115-035-003; Jackson ImmunoResearch). Beta-actin was detected using a goat polyclonal anti-actin HRP conjugated antibody (Sc-1616; Santa Cruz). The band at molecular weight of ~25 KDa shows the presence of a truncated MAP11 protein in overexpressing cells.

Supplementary Fig. 8

a



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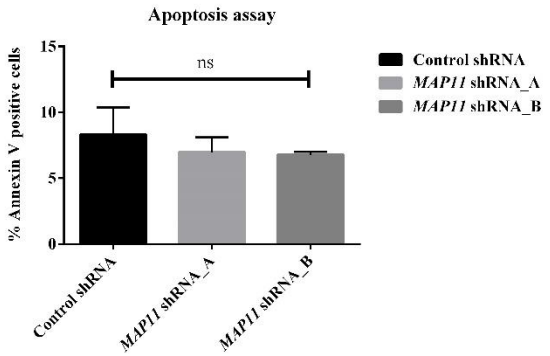

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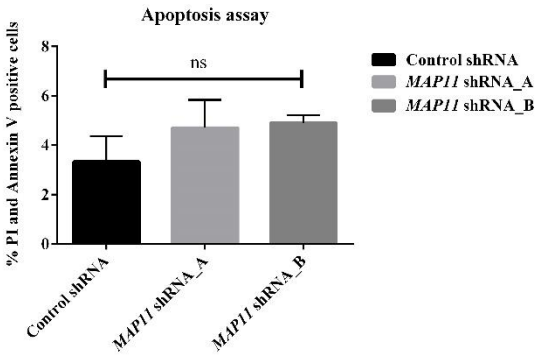
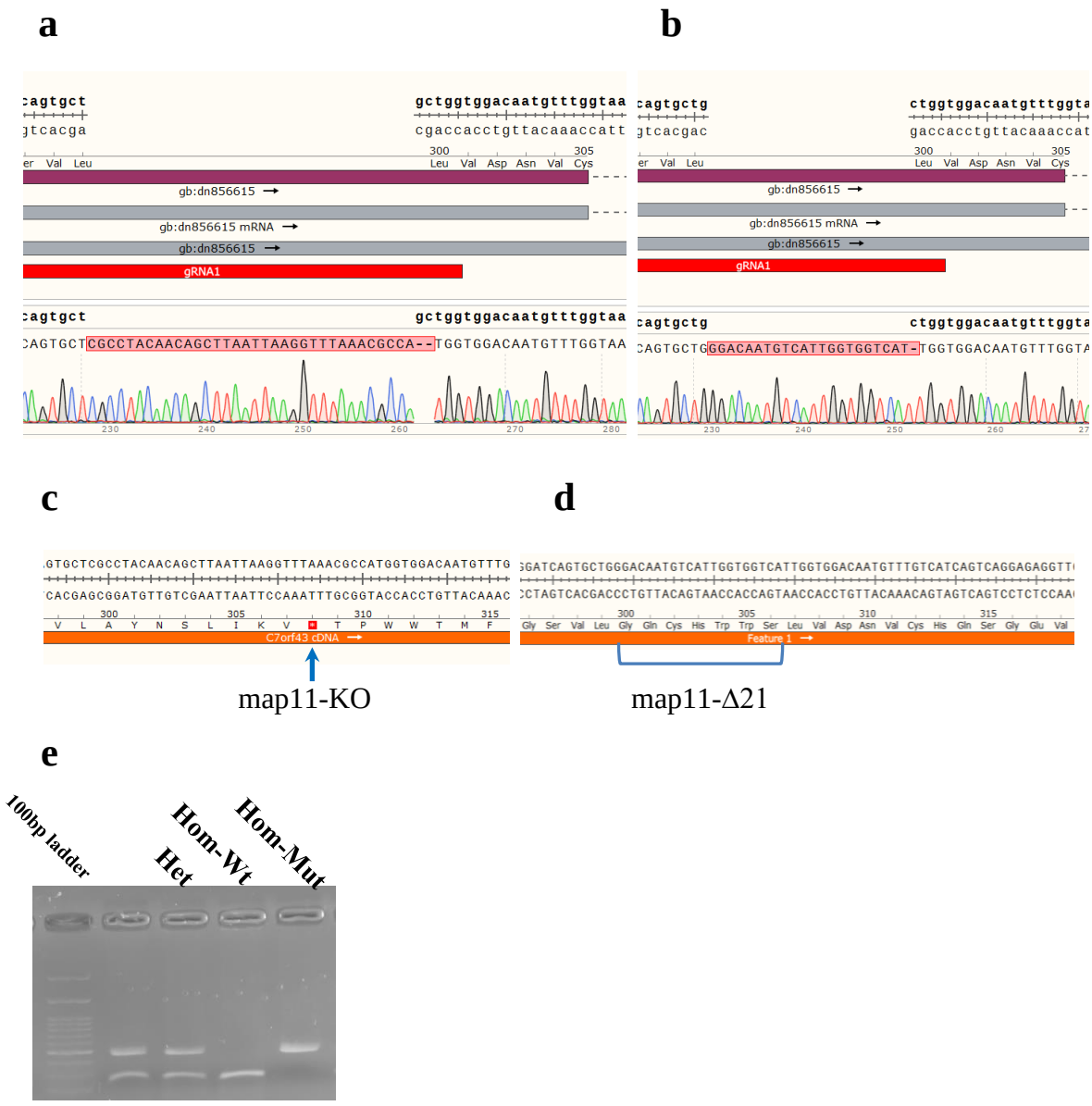


Supplementary Fig. 8. Apoptosis assay of *MAP11* knock-down SH-SY5Y cells. a) FACS analysis of Annexin V and propidium iodide (PI) positive cells. Analysis gating's were focused on minimizing cell debris while maintaining single cells positive for annexin V marker and PI. Positive control cells are normal SH-SY5Y cells heated to 55˚C for 10 minutes prior to annexin V and PI staining's. b) Percentage of annexin V positive cells (Q2+Q3) showing non-significant difference between *MAP11* knock-

down cells to shRNA controls in total apoptosis (early and late apoptosis). c) Percentage of cells positive for both annexin V and PI (Q2) showing non-significant difference between *MAP11* knock-down cells to shRNA controls in late apoptosis. A blue arrow demonstrates the gating chronology.

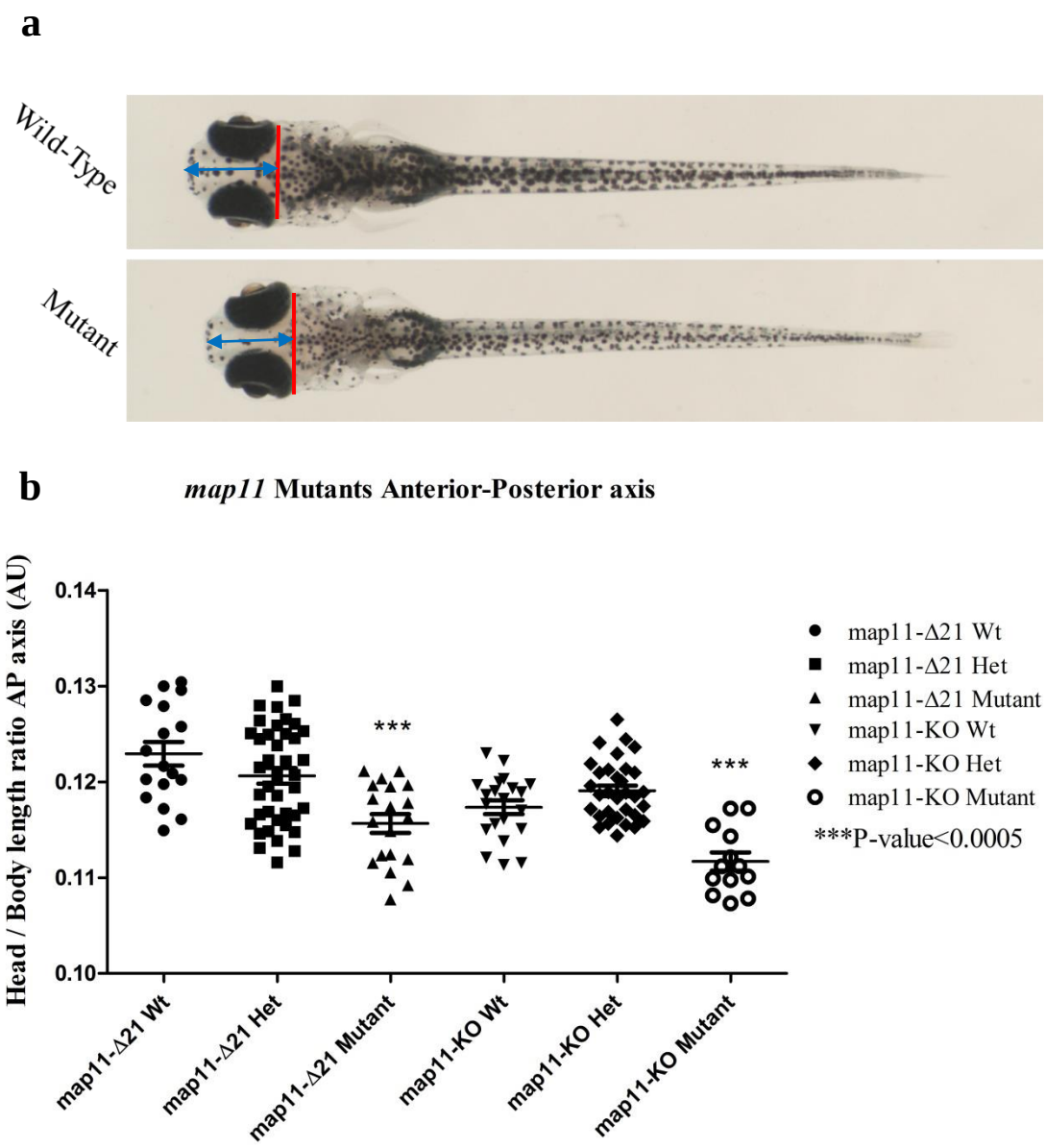
Supplementary Fig. 9



Supplementary Fig. 9. The zebrafish *MAP11*-orthologue mutant lines generated by CRISPR/Cas9 editing system. a) Sanger sequencing of the *map11*- KO mutant line aligned to the wild-type map11 reference sequence (above) showing the 34 nt insertion and a 2 nt deletion at the 5'-end of the insertion point. b) Sanger sequencing of the map11-Δ21 mutant line aligned to the wild-type *map11* reference sequence (above) showing the 22 nt insertion and a 1 nt deletion at the 5'-end of the insertion point. c)

Schematic view of the mutated *map11*-KO protein, demonstrating the putative stop codon at position 308 of the mature encoded protein (blue arrow). d) Schematic view of the mutated map11-Δ21 protein, demonstrating the putative 7 amino acid insertion (GQCHWWS) of the mature encoded protein (blue brackets). e) Agarose gel electrophoresis demonstrating the genotyping screens of F_2 offspring mutated zebrafish. BbvI restriction site is abrogated in both mutant *map11*-KO and map11-Δ21 amplicons (448bp amplicon of the wild-type allele; 480bp amplicon of the *map11*-KO mutant allele; 469bp amplicon of the map11-Δ21 mutant allele), enabling to screen for homozygous wild-types (234 + 214bp; appear as one band), heterozygous carriers (for *map11*-KO: 480bp + 234 + 214bp; for map11-Δ21: 469bp + 234 + 214bp) and homozygous mutant fish (for *map11*-KO: 480bp; for map11-Δ21: 469bp).

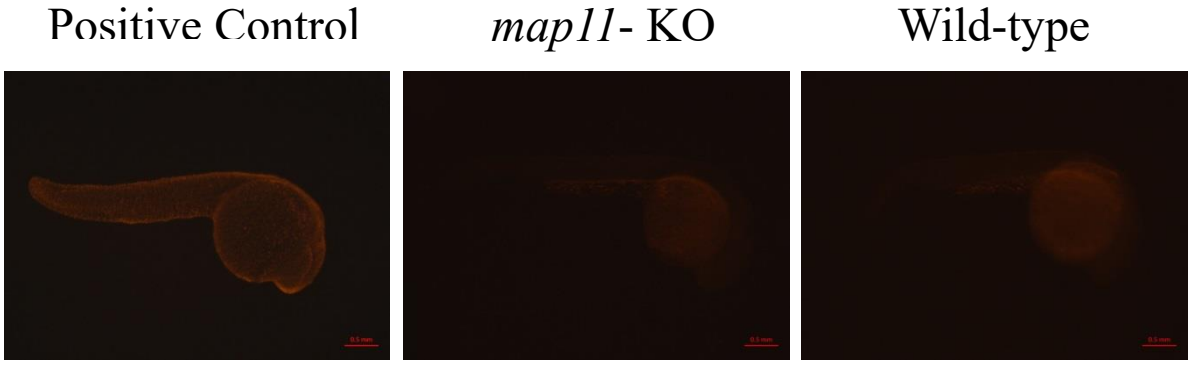
Supplementary Fig. 10



Supplementary Fig. 10. **Shortening of the anterior-posterior axis of mutant zebrafish heads**. a) A scheme demonstrating the anterior-posterior length measures done for zebrafish. The head length was defined as the length measured between the middle of a virtual line crossing the plan posterior to zebrafish eyes (in red) and the zebrafish most anterior part (blue arrowed line). Zebrafish body length was measured from the edge of the caudal fin to the zebrafish most anterior part (not shown in scheme). All measures were done using image J software. b) A graph showing a significant shortening of the anterior-posterior axis of mutant zebrafish heads.

Supplementary Fig 11.

a



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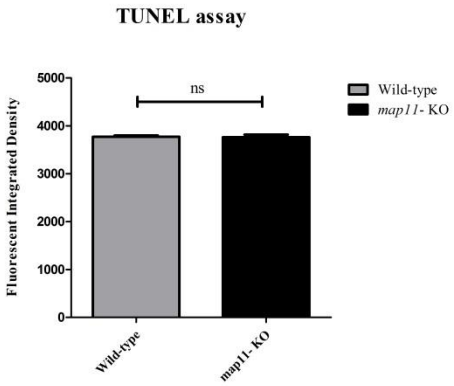

Supplementary Fig. 11. Zebrafish whole- mount TUNEL assay. a) Images of fluorescence microscopy showing 24hpf zebrafish embryos stained via TUNEL assay. Positive controls are wild-type zebrafish embryos incubated with DNAse I prior to TUNEL staining. Apoptotic cells are shown in red color. Scale bar is 0.5mm. b) A graph showing no difference in total apoptosis (measured as fluorescent integrated density) between wild-type and *map11*-KO zebrafish.