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Supplemental Data

Bi-allelic Variants in *DYNC1I2* Cause Syndromic Microcephaly with Intellectual Disability, Cerebral Malformations, and Dysmorphic Facial Features

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Supplemental Data

Supplementary Figures

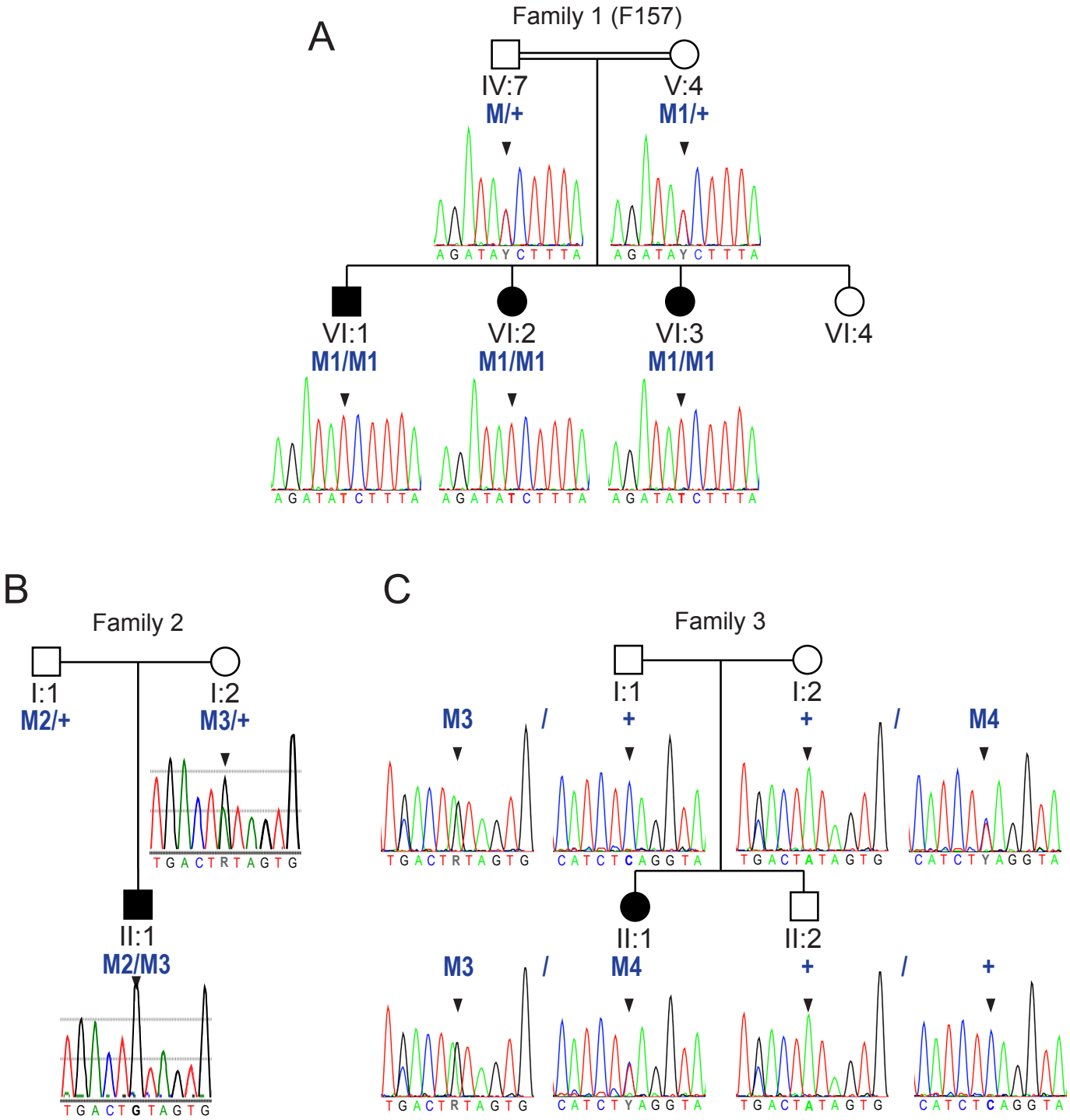

Figure S1. Validation of DYNC1I2 variants by Sanger sequencing. (A-C) Chromatograms showing validation of DYNC1I2 (NM_001378.2):c.607+1G>A in family 1 (M1, panel A); c.740A>G;p.(Tyr247Cys) in family 2 (M3, panel B); and c.740A>G;p.(Tyr247Cys) and c.868C>T; p.(Gln290*) in family 3 (M3 and M4, respectively, panel C). Black arrowheads indicate variant position for each individual.

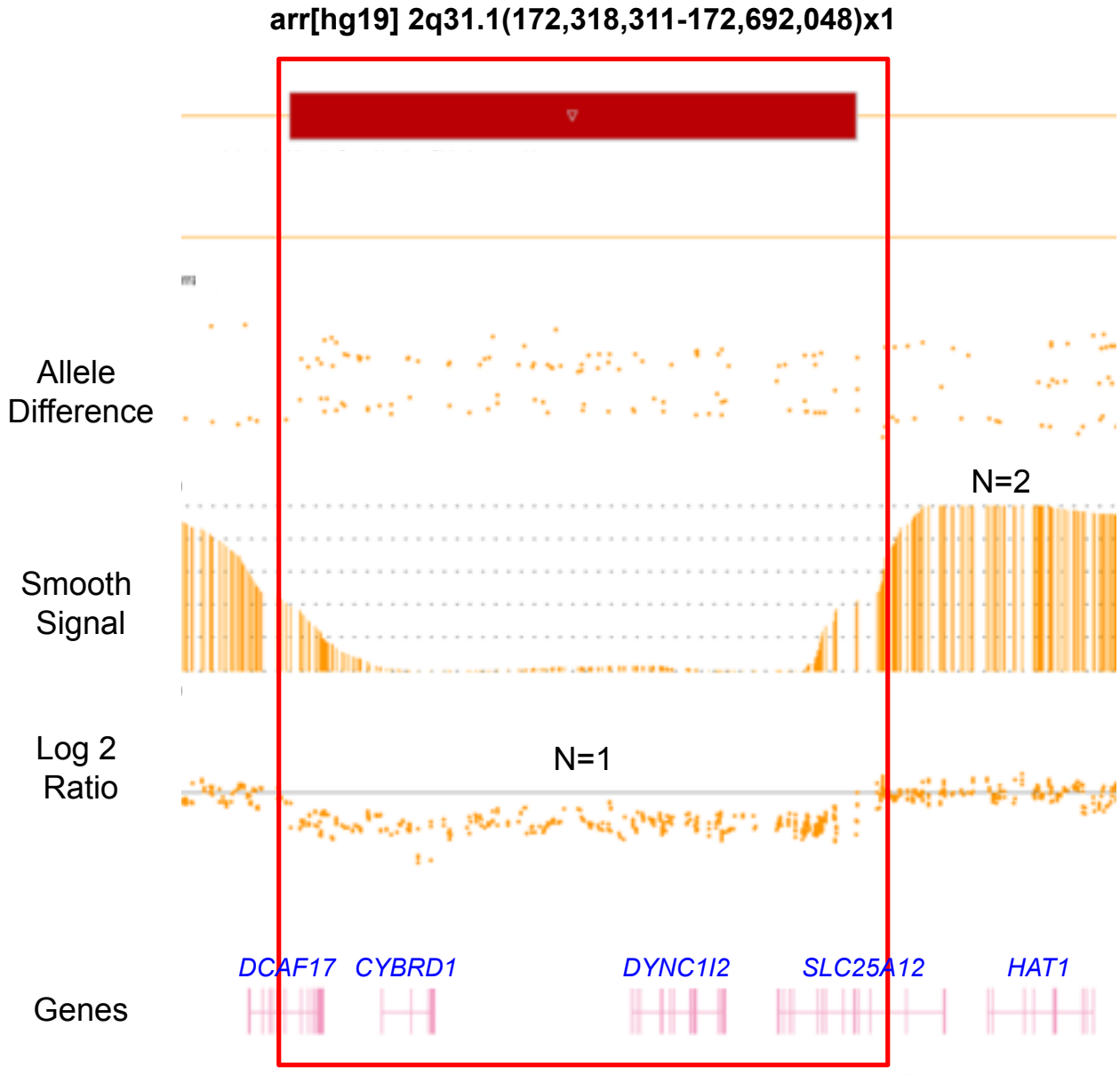

Figure S2. SNP array data showing the 374 kb deletion detected in the Family 2 proband. The heterozygous region was detected using the Affymetrix Cytoscan HD platform and encompasses four genes: DCAF17, CYBRD1, DYNC1I2, and SLC25A12. Data were analyzed using Chromosome Analysis Suite according to the GRCh37/hg19 assembly.

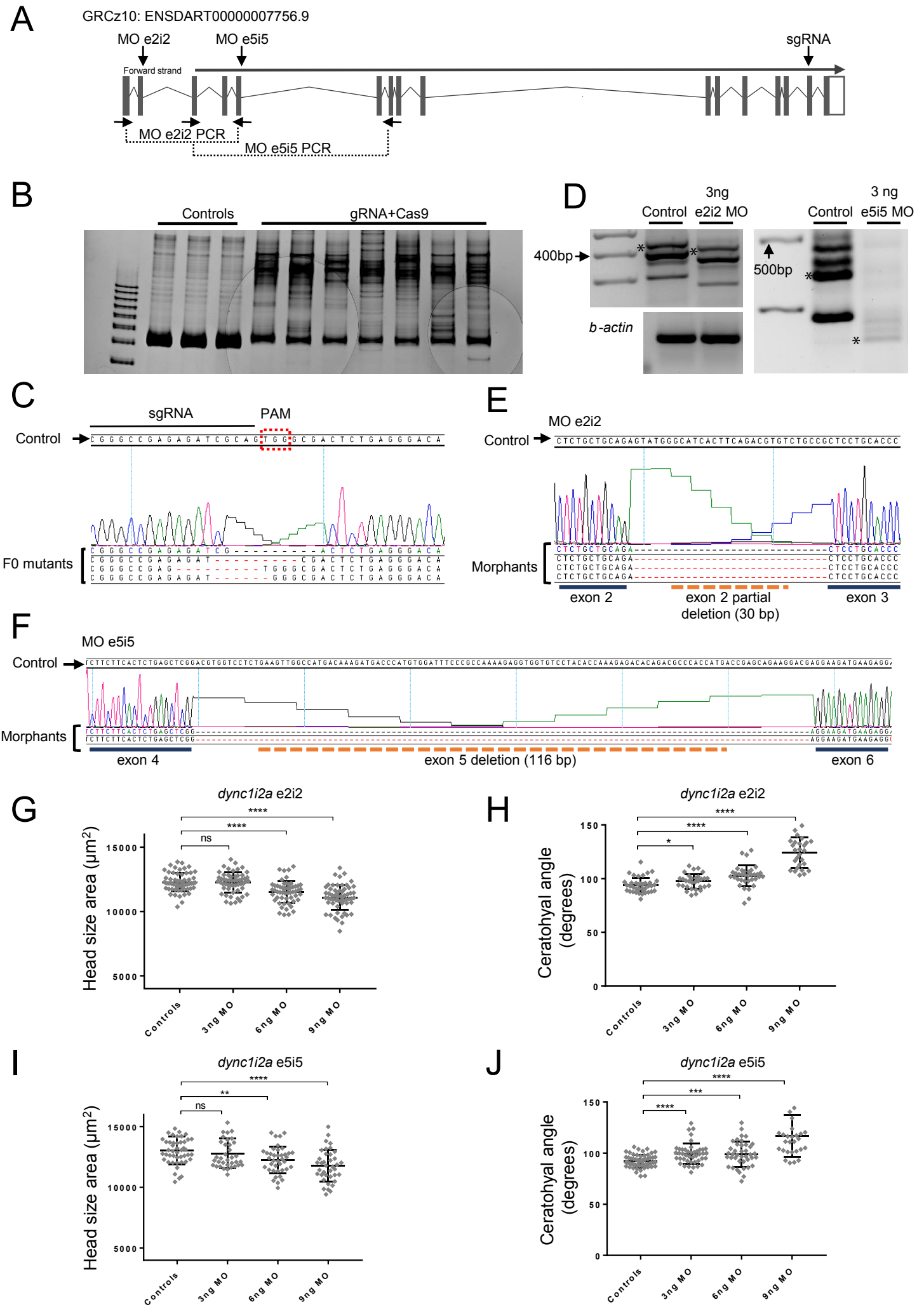
A
GRCz10: ENSDART00000007756.9
MO e2i2
MO e5i5
sgRNA
Forward strand
MO e2i2 PCR
MO e5i5 PCR
B
Controls
gRNA+Cas9
D
3ng
Control
e2i2 MO
3 ng
Control
e5i5 MO
400bp
500bp
b-actin
C
sgRNA
PAM
Control
F0 mutants
E
MO e2i2
Control
Morphants
exon 2
exon 2 partial
deletion (30 bp)
exon 3
F
MO e5i5
Control
Morphants
exon 4
exon 5 deletion (116 bp)
exon 6
G
dync1i2a e2i2
Head size area (µm2)
ns
Controls
3ng MO
6ng MO
9ng MO
H
dync1i2a e2i2
Ceratohyal angle (degrees)
I
dync1i2a e5i5
Head size area (µm2)
J
dync1i2a e5i5
Ceratohyal angle (degrees)

Figure S3. Efficiency of reagents used to ablate dync1i2a in zebrafish larvae. (A) Schematic of the candidate principal isoform transcribed from the dync1i2a locus (GRCz10, D. rerio). Filled rectangles, coding exons; unfilled rectangles, untranslated regions; gray lines, introns. Target position of reagents used are indicated with vertical arrows; primers to determine morpholino (MO) efficiency are indicated with horizontal arrows; sgRNA, single guide RNA. (B) Polyacrylamide gel image showing heteroduplex analysis of single control embryos or embryos injected with dync1i2a sgRNA plus Cas9 protein. (C) Representative sequence chromatograms generated from individual colonies that were TOPO cloned from PCR products amplified from individual control or dync1i2a F0 mutant larvae. PAM, protospacer adjacent motif (red dashed box); shRNA has estimated 70% mosaicism. (D) Agarose gel images show aberrantly spliced transcripts induced by two independent MOs targeting the splice donor sites of exons 2 and 5 of the canonical transcript. dync1i2a is subject to alternative splicing, likely explaining the presence of multiple amplification products; the expected wild-type fragments amplified from the isoform shown in panel A are indicated with asterisks (*) at 416 bp and 422 bp, respectively. We amplified b-actin to control for total RNA and cDNA integrity. (E, F) Representative sequence chromatograms generated from individual colonies that were TOPO cloned from RT-PCR products generated from controls, e2i2 and e5i5 morphants. (G, H) Representative dose response experiments for e2i2 MO on head size and craniofacial patterning, respectively. See Figure 3A and C for depiction of measurement strategies. (I, J) Representative dose response experiments for e5i5 MO on head size and craniofacial patterning, respectively. In panels G-J, phenotyping was performed at 3 days post fertilization; error bars represent standard deviation of the mean; ****, $p<0.0001$; ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$; ns, not significant; n=30-61/experimental condition, a total of three biological replicates gave similar results.

GRCz10: ENSDART00000159673.2

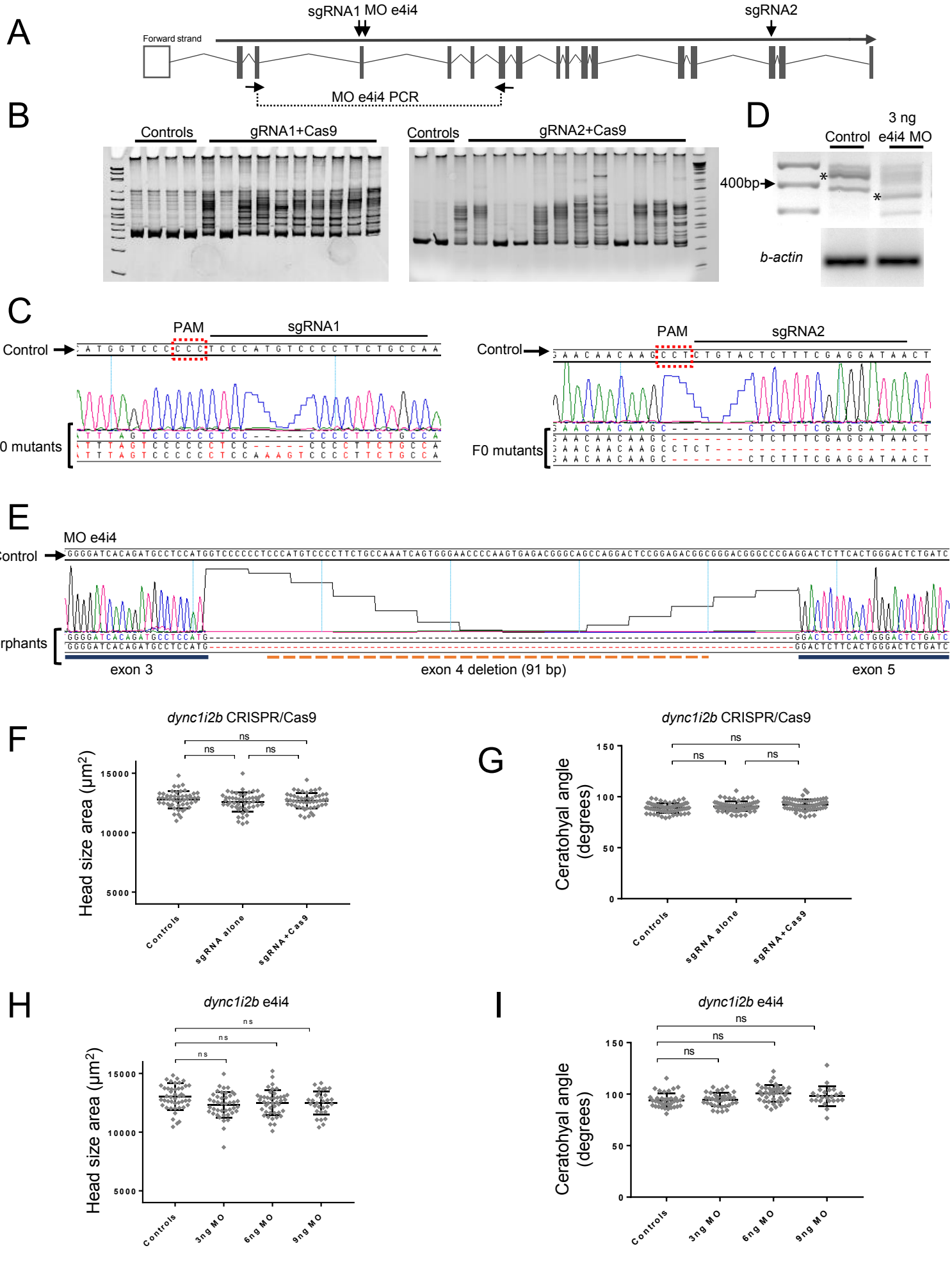

Figure S4. Efficiency of reagents used to ablate dync1i2b in zebrafish larvae. (A) Schematic of the candidate principal isoform transcribed from the dync1i2b locus (GRCz10, D. rerio). Filled rectangles, coding exons; unfilled rectangles, untranslated regions; gray lines, introns. Target position of reagents used are indicated with vertical arrows; primers to determine morpholino (MO) efficiency are indicated with horizontal arrows; sgRNA, single guide RNA. (B) Polyacrylamide gel images showing heteroduplex analysis of single control embryos or embryos injected with dync1i2b sgRNA1 or sgRNA2 plus Cas9 protein. (C) Representative sequence chromatograms generated from individual colonies that were TOPO cloned from PCR products amplified from individual control or dync1i2b F0 mutant larvae. PAM, protospacer adjacent motif (red dashed box); estimated mosaicism for sgRNA1 and sgRNA2: 84% and 50%, respectively. (D) Agarose gel images show aberrantly spliced transcripts induced by a MO targeting the splice donor site of exon 4. While there are no alternative transcripts annotated for dync1i2b in ENSEMBL, this likely explains the presence of multiple PCR products; the expected wild-type fragment amplified from the isoform shown in panel A is indicated with an asterisk (*) at 445bp. We amplified b-actin to control for total RNA and cDNA integrity. (E) Representative sequence chromatograms generated from individual colonies that were TOPO cloned from RT-PCR products generated from controls or e4i4 morphants. (F, G) Quantification of head size area and ceratohyal angle, respectively, in dync1i2b sgRNA2 F0 mutants (sgRNA1 showed similar results); we observed no significant phenotypes. (H, I) We observe no dose dependent response of e4i4 MO on head size and craniofacial patterning, respectively. See Figure 3A and C for depiction of measurement strategies. In panels F-I, phenotyping was performed at 3 days post fertilization; error bars represent standard deviation of the mean; ns, not significant; n=36-72/experimental condition, a total of two biological replicates gave similar results.

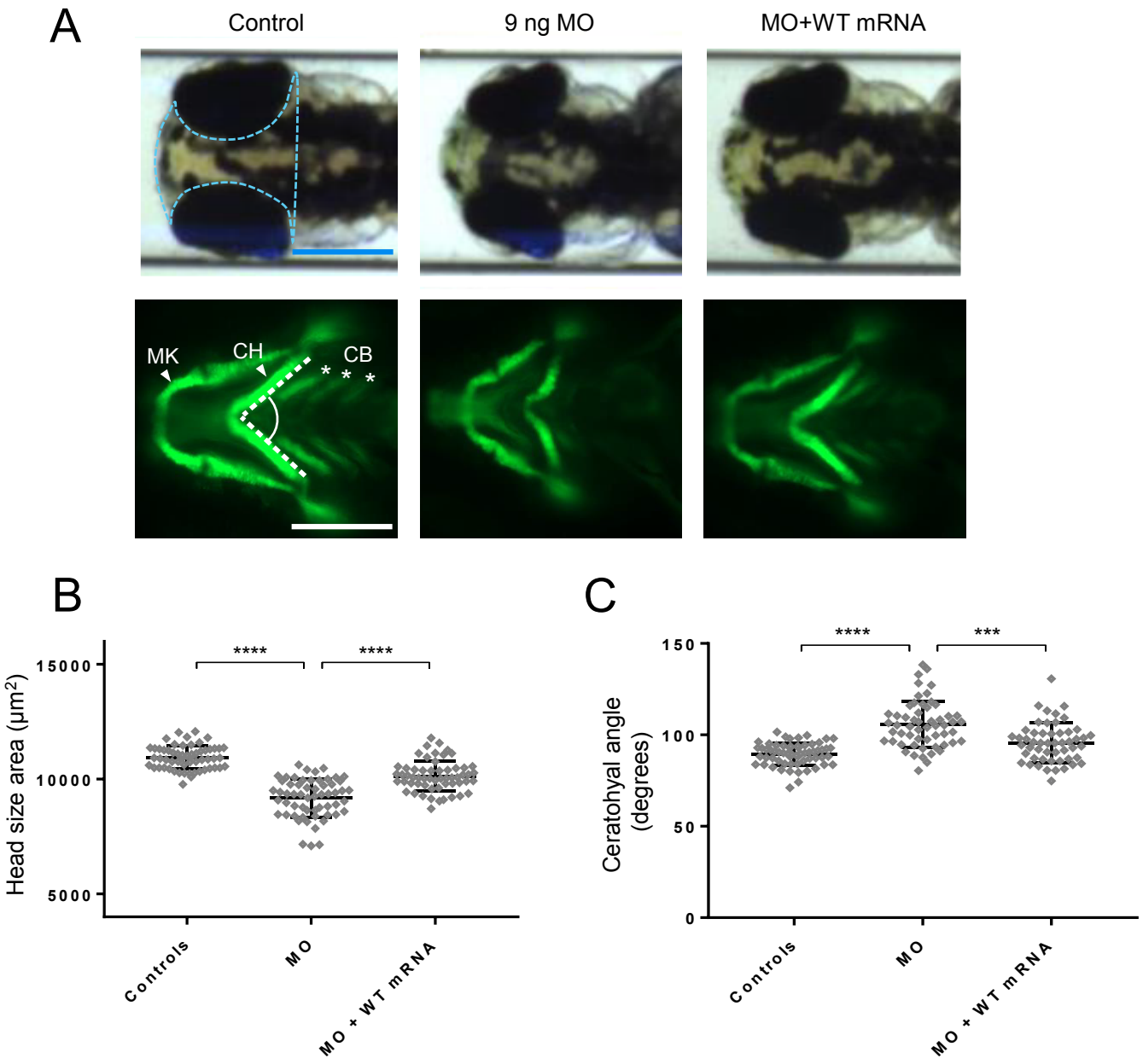

Figure S5. Morpholino-induced suppression with dync1i2a e5i5 MO and rescue with wild-type human mRNA. (A) Representative dorsal bright field images (top row); and ventral fluorescent images of -1.4col1a1:egfp (bottom row) controls or larvae injected with e5i5 MO with or without wild type human mRNA (NM_001271789.1). MK, Meckel's cartilage; CH, ceratohyal cartilage; CB, ceratobranchial arches. (B, C) Representative experiments quantifying head size area and ceratohyal angle, respectively. All phenotyping was performed at 3 days post-fertilization. Pale blue dotted line in panel A (top row) indicates area measured to obtain data in panel B. Angle indicated between dashed white lines in panel A (bottom row) indicates measurement used to obtain data for panel C. Scale bars in panel A: 300 m; image sizing is consistent across panels. ****, p<0.0001; ***, p<0.001; Error bars represent standard deviation of the mean; n=36-72/ experimental condition, total of two biological replicates gave similar results.

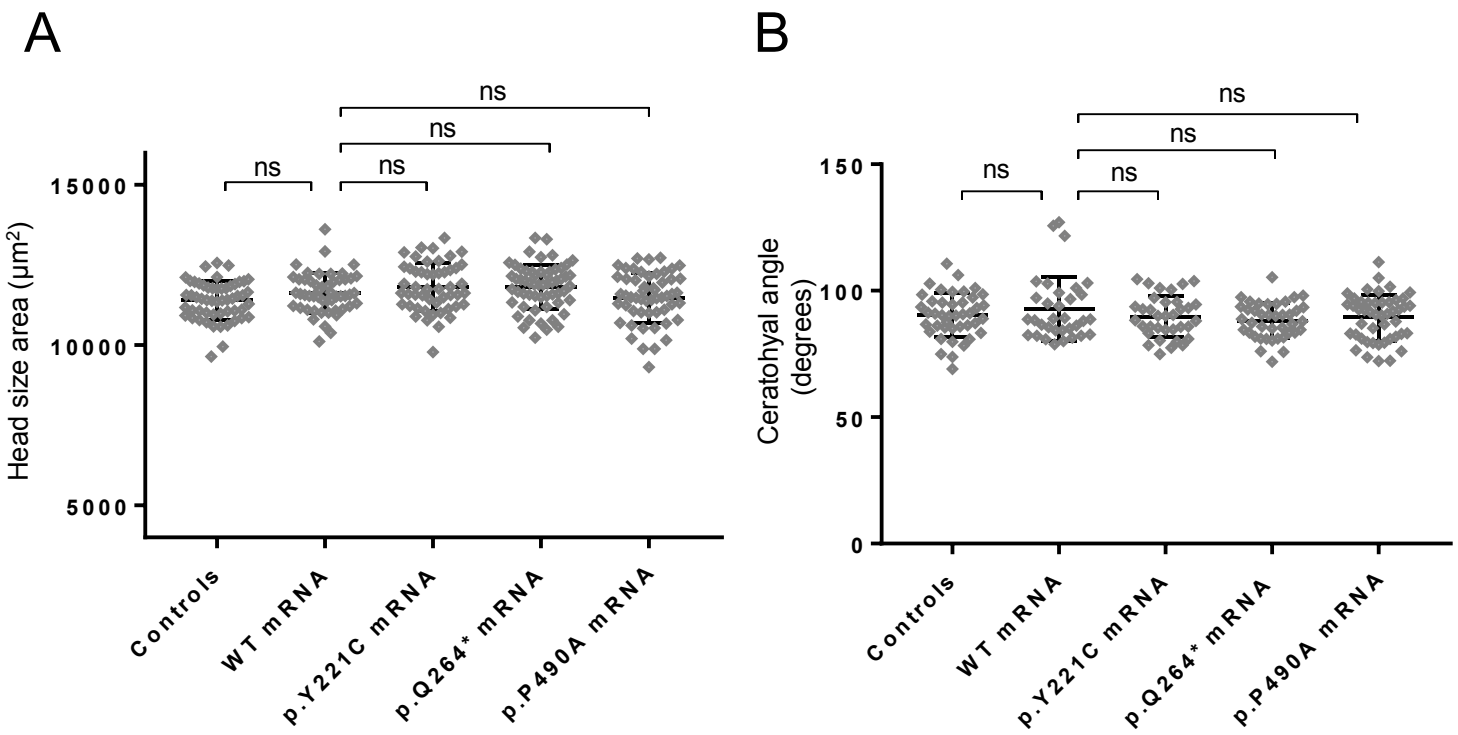

Figure S6. Ectopic expression of wild type or mutant human DYNC1I2 mRNA (NM_001271789.1) does not induce head size or cartilage patterning defects. (A, B) Quantification of dorsal head size and ceratohyal angle, respectively, in zebrafish larvae at 3 days post-fertilization. See Figure 3A and C for depiction of measurement strategies. Error bars represent standard deviation of the mean; ns, not significant; n=50-58/experimental condition; total of two biological replicates. Note that the amino acid codons Y221C, Q264* and P490A of the most abundant isoform (NM_001271789.1) used in the zebrafish experiments correspond to Y247C, Q290* and P516A of the longest isoform (NM_001378.2) of DYNC1I2.

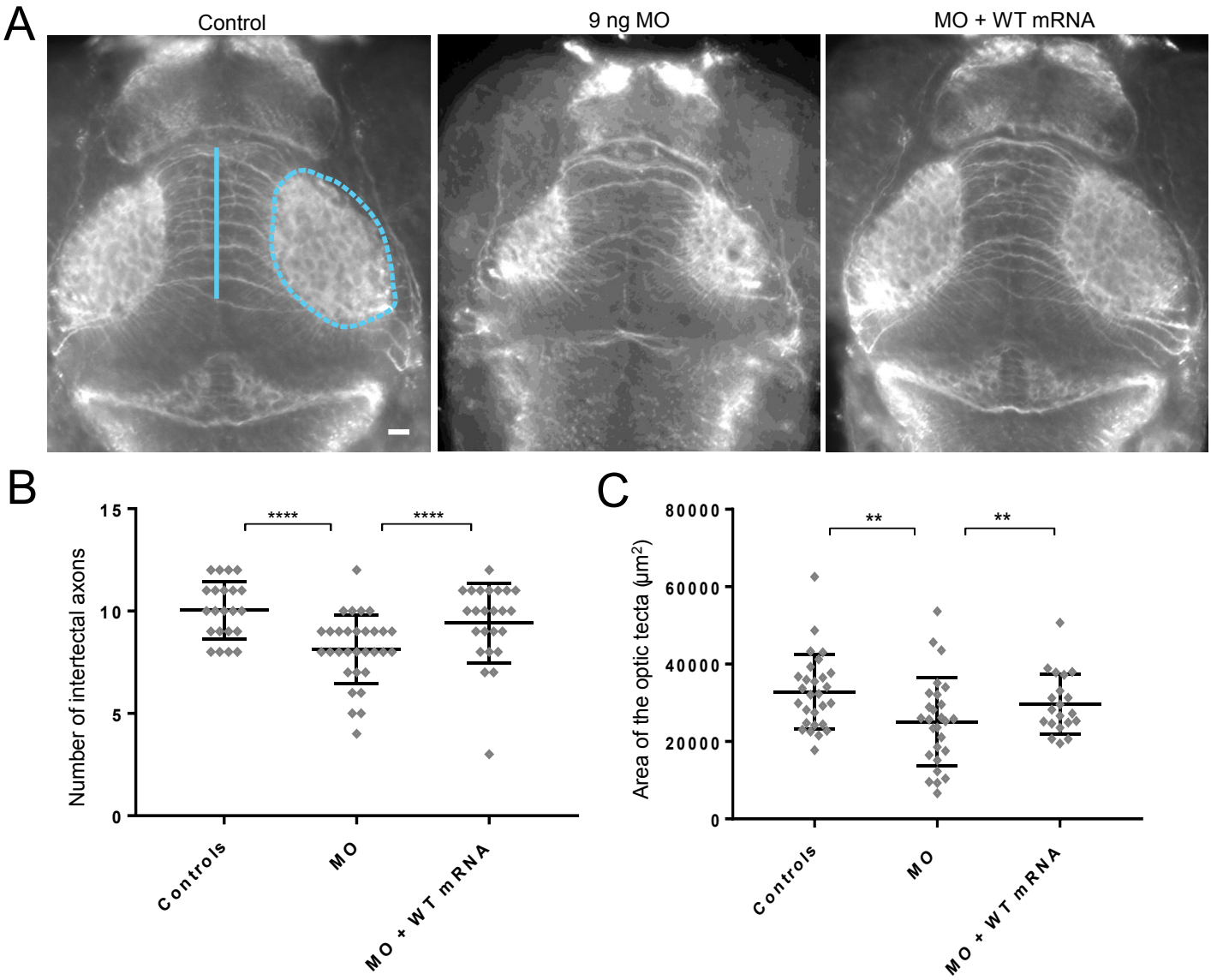

Figure S7. Evaluation of neuronal organization of anterior structures in dync1i2a zebrafish models. (A) Representative dorsal images of fluorescent signal in 3 day post fertilization larvae immunostained with anti-acetylated -tubulin to demarcate axon tracts. We counted the number of commissural axons crossing the midline (blue vertical line) between optic tecta; and area of the optic tecta (dashed blue oval). Top, anterior; bottom, posterior; scale bar: 30 µm; sizing is identical across panels. (B, C) Quantification of intertectal neuron number and optic tecta size, respectively. ****, p<0.0001; **, p<0.01; Error bars represent standard deviation of the mean; n=22-35/ experimental condition, total of three biological replicates produced similar results.

Supplementary Tables

Table S1. Expression of DYNC1I2 transcripts in discrete regions of the brain (Gtex; accessed January 2019). See accompanying excel file.

Table S2. Primers used for DYNC1I2 *in vivo* modeling studies.

Purpose	oligo name	Sequence
dync1i2a sgRNA1 CRISPR/Cas9	*dync1i2a* sgRNA 1	5'-GTCGGGCCGAGAGATCGCAGTGG-3'
dync1i2a sgRNA1 CRISPR/Cas9 efficiency	a-sgRNA1 PCR primer F	5'-CCATAATCCATAGGTCATTGGC-3'
dync1i2a sgRNA1 CRISPR/Cas9 efficiency	a-sgRNA1 PCR primer R	5'-ATCTCCTTCTGCTTTCATCCTG-3'
dync1i2a MO-induced suppression	*dync1i2a* e2i2 sb MO	5'-AGGATATAAATGTGACCTACCGGCA-3'
dync1i2a e2i2 sb MO efficiency	a-e2i2 PCR primer F	5'-TGTCGGATAAAAGTGAGCTGAA-3'
dync1i2a e2i2 sb MO efficiency	a-e2i2 PCR primer R	5'-GAAATCCACATGGGTCATCTTT-3'
dync1i2a MO-induced suppression	*dync1i2a* MO-e5i5	5'-CACATGAATTGTTGACTCACCGTCC-3'
dync1i2a e5i5 sb MO efficiency	a-e5i5 PCR primer F	5'-ATGTCTCCCACTGCCAAATC-3'
dync1i2a e5i5 sb MO efficiency	a-e5i5 PCR primer R	5'-CGTGTGCTGTGATCGAAAAA-3'
dync1i2b sgRNA1 CRISPR/Cas9	*dync1i2b* sgRNA 1	5'-TGGCAGAAGGGGACATGGGAGGG-3'
dync1i2b sgRNA1 CRISPR/Cas9 efficiency	b-sgRNA1 PCR primer F	5'-TTAACCACTGCCCTTTTTGTCT-3'
dync1i2b sgRNA1 CRISPR/Cas9 efficiency	b-sgRNA1 PCR primer R	5'-GCATCATGCACTATTGGAAAAA-3'
dync1i2b sgRNA2 CRISPR/Cas9	*dync1i2b* sgRNA 2	5'-TTATCCTCGAAAGAGTACAGAGG-3'
dync1i2b sgRNA2 CRISPR/Cas9 efficiency	b-sgRNA2 PCR primer F	5'-GGCTGTTTTTGTAAGGAGAGGA-3'
dync1i2b sgRNA2 CRISPR/Cas9 efficiency	b-sgRNA2 PCR primer R	5'-AGGAACCTGAAACAGCAGGTAA -3'
dync1i2b MO-induced suppression	*dync1i2b* MO-e4i4	5'-GTCAGTGTAAACAGTCTCACCTCGG-3'
dync1i2b e4i4 sb MO efficiency	b-e4i4 PCR primer F	5'-CACAGGAAGACTCTGATCTGGA-3'
dync1i2b e4i4 sb MO efficiency	b-e4i4 PCR primer R	5'-GGAGACGCTTCATCTTCTTCTTT-3'