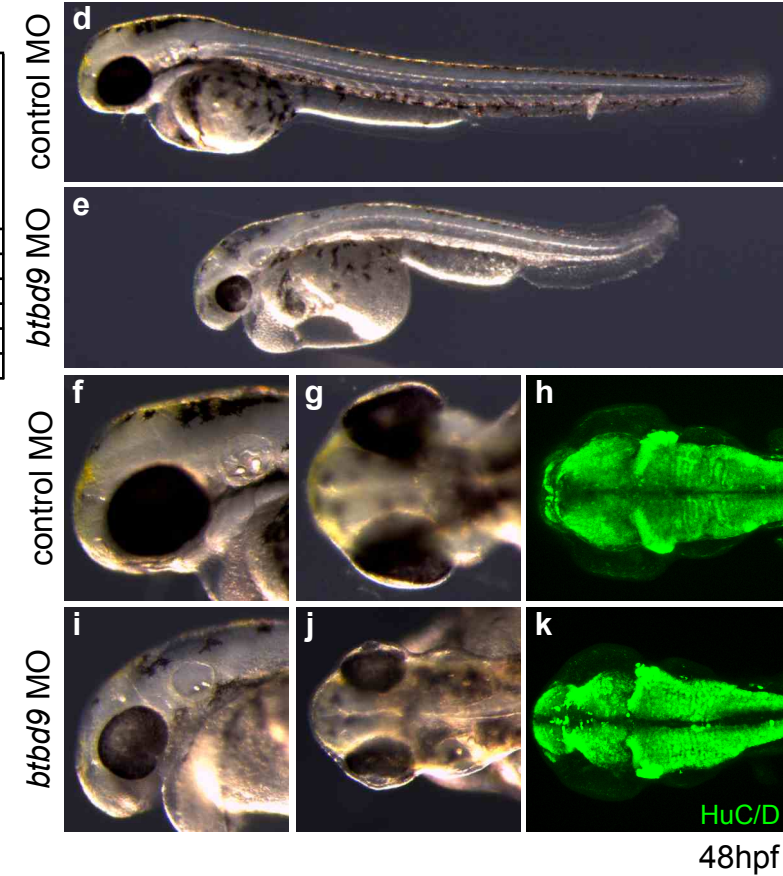


BTBD9

Proband phenotypes	Seizures Microcephaly Short philtrum	Microcephaly Prominent nasal bridge Hypertonia Bilateral single transverse palmar creases	Progressive microcephaly Intellectual disability Everted lower lip vermilion Spasticity Delayed speech and language development Abnormality of the eyelid Epicanthus
Inheritance	Autosomal recessive homozygous	Autosomal recessive homozygous	de novo
Consequence	frameshift_variant	frameshift_variant	missense_variant
Position	Chr6:g.38565777 CA>C	Chr6:g.38565777 CA>C	Chr6:g.38545415 C>T
Gene ID	ENSG00000183826	ENSG00000183826	ENSG00000183826
Transcript change	ENST00000498633.1:c.93delT	ENST00000498633.1:c.93delT	ENST00000328403.6:c.911G>A
Protein change	ENSP00000419382.1:p.Ile31MetfsTer22	ENSP00000419382.1:p.Ile31MetfsTer22	ENSP00000328328.6:p.Arg304His

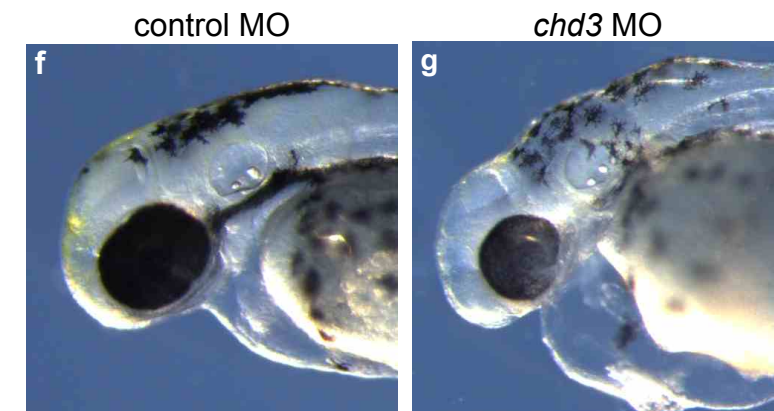
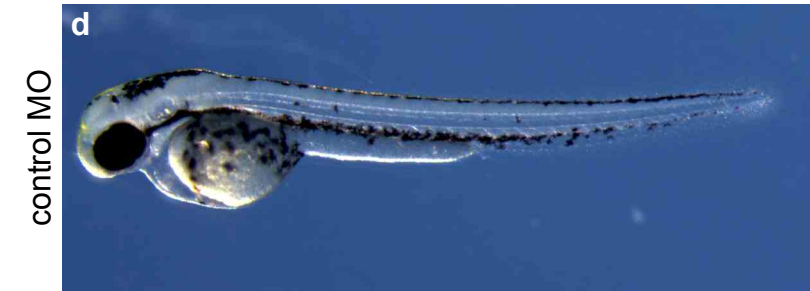


Zebrafish	
Gene	<i>btbd9</i>
Gene ID	ENSDARG00000068983
Morpholino	CATTTATTCCGCCACTCACCTTTGA
Knockdown confirmation	RT-PCR, 2nd Morpholino
Relevant phenotypes	Reduced head size (-16.6%, $p < 4 \times 10^{-6}$) Reduced brain size (-15.3%, $p < 0.002$)
Phenotypic concordance	Strong

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001094	Whole embryo	PATO:0000587	Decreased size
				ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0001115	Trunk	PATO:0001468	Curved dorsal
				ZFA:0000092	Axis	PATO:0000587	Decreased size
				ZFA:0000114	Heart	PATO:0000460	Abnormal
				ZFA:0000114	Heart	PATO:0001450	Edematous
low	5ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0000008	Brain	PATO:0000587	Decreased size
				ZFA:0001114	Head	PATO:0000587	Decreased size

CHD3

Proband phenotypes	Genu valgum	Submucous cleft hard palate	Broad thumb
	Hyperlordosis	Intellectual disability	Global developmental delay
	Constipation	Hypertelorism	Autism
	Global developmental delay		
	Hypermetropia		
	Abnormal facial shape		
Cafe-au-lait spot			
Inheritance	de novo	de novo	de novo
Consequence	stop_gained	missense_variant	missense_variant
Position	Chr17:g.7810193 G>T	Chr17:g.7806037 G>C	Chr17:g.7800459 A>G
Gene ID	ENSG00000170004	ENSG00000170004	ENSG00000170004
Transcript change	ENST00000330494.7:c.4510G>T	ENST00000330494.7:c.3362G>C	ENST00000330494.7:c.1766A>G
Protein change	ENSP00000332628.7:p.Glu1504Ter	ENSP00000332628.7:p.Arg1121Pro	ENSP00000332628.7:p.Glu589Gly



Zebrafish	
Gene	<i>chd3</i>
Gene ID	ENSDARG00000021405
Morpholino	ACTTCTAGTGAAAGTCAGTACCTGA
Knockdown confirmation	RT-PCR, 2nd Morpholino
Relevant phenotypes	Abnormal CNS and head shape
Phenotypic concordance	Strong

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001114	Head	PATO:0000460	Abnormal
				ZFA:0001094	Whole embryo	PATO:0000587	Decreased size
				ZFA:0001094	Whole embryo	PATO:0000502	Delayed
				ZFA:0000012	Central nervous system	PATO:0000592	Decreased thickness
				ZFA:0000008	Brain	PATO:0001450	Edematous
				ZFA:0000114	Heart	PATO:0000460	Abnormal

CNOT1

a	Proband phenotypes	Specific learning disability Autism spectrum disorder Short stature Feeding difficulties in infancy Ligamentous laxity Epicanthus Choroid plexus cyst Patent ductus arteriosus Short palm Bilateral single transverse palmar creases Microtia Narrow mouth	Generalized neonatal hypotonia Generalized joint laxity Micrognathia Wide nasal bridge Epicanthus Low-set ears Narrow nasal ridge Small chin Finger syndactyly Toe syndactyly Abnormality of limb bone morphology Abnormality of the eye Global developmental delay Hypotelorism Abnormality of the esophagus Unsteady gait Abnormality of the nipple
	Inheritance	de novo	de novo
	Consequence	stop_gained	missense_variant
	Position	Chr16:g.58633145 G>A	Chr16:g.58555172 G>A
	Gene ID	ENSG00000125107	ENSG00000125107
	Transcript change	ENST00000317147.5:c.97C>T	ENST00000317147.5:c.6967C>T
	Protein change	ENSP00000320949.5:p.Gln33Ter	ENSP00000320949.5:p.Leu2323Phe

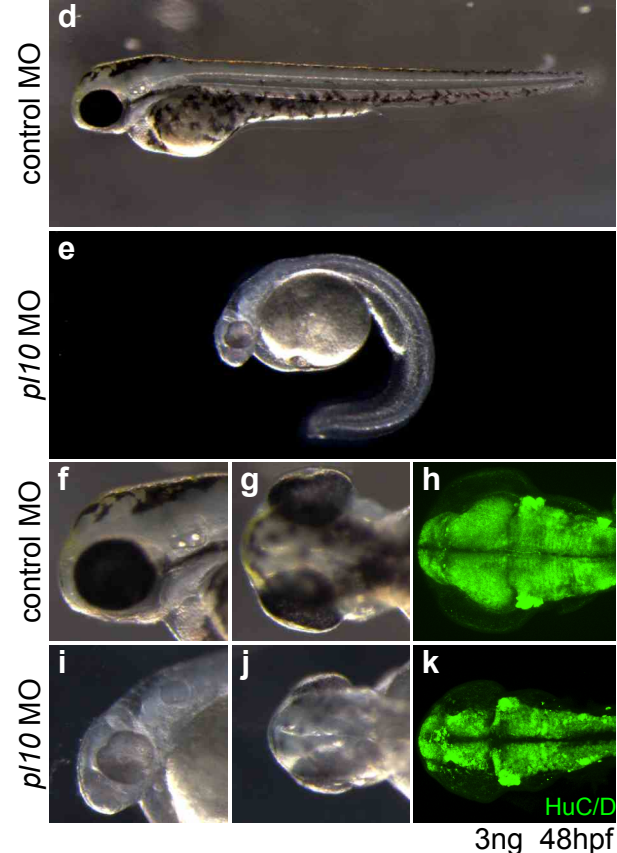


b	
Zebrafish	
Gene	<i>cnot1</i>
Gene ID	ENSDARG00000004174
Morpholino	TGTTGCTGCCACTGAACTTACATGT
Knockdown confirmation	RT-PCR
Relevant phenotypes	Growth delay
Phenotypic concordance	Weak

c		MO dose	Stage	Entity		Quality	
high	6ng	ZFS:0000029	day 1: Prim-5	ZFA:0001094	Whole embryo	PATO:0000587	Decreased size
				ZFA:0000051	Otic vesicle	PATO:0000587	Decreased size
				ZFA:0000051	Otic vesicle	PATO:0000646	Malformed
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0000092	Axis	PATO:0000574	Decreased length
				ZFA:0000051	Otic vesicle	PATO:0000646	Malformed

DDX3X

Proband phenotypes	Moderately short stature Microcephaly Specific learning disability	Ovarian cysts Global developmental delay	Intellectual disability severe Hyperactivity Ligamentous laxity Recurrent infections Poor motor coordination Sleep-wake cycle disturbance	Palmoplantar hyperkeratosis Global developmental delay Oral cleft Bilateral cleft lip and palate Ectopic anus Vesicoureteral reflux Conductive hearing impairment Sensorineural hearing impairment Joint hypermobility	
	Inheritance	de novo	de novo	de novo	
	Consequence	stop_gained	missense_variant	missense_variant	missense_variant
	Position	ChrX:g.41198321 C>T	ChrX:g.41202566 T>C	ChrX:g.41204533 C>T	ChrX:g.41205861 G>A
	Gene ID	ENSG00000215301	ENSG00000215301	ENSG00000215301	ENSG00000215301
	Transcript change	ENST00000399959.2:c.136C>T	ENST00000399959.2:c.641T>C	ENST00000399959.2:c.1126C>T	ENST00000399959.2:c.1601G>A
	Protein change	ENSP00000382840.2:p.Arg46Ter	ENSP00000382840.2:p.Ile214Thr	ENSP00000382840.2:p.Arg376Cys	ENSP00000382840.2:p.Arg534His



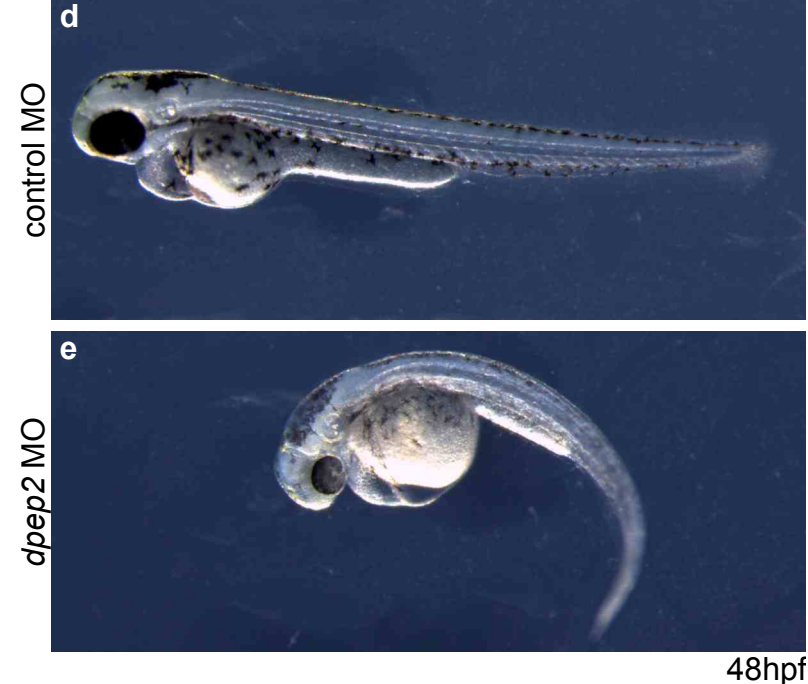
Zebrafish	
Gene	<i>p/10</i>
Gene ID	ENSDARG00000005774
Morpholino	TGAGGAACAGAACTCACTTCCAGT
Knockdown confirmation	RT-PCR, 2nd Morpholino
Relevant phenotypes	Reduced head size(-23.1%, $p < 1.8 \times 10^{-5}$) Reduced brain size(-27.4%, $p < 7.8 \times 10^{-10}$)
Phenotypic concordance	Strong

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001094	Whole embryo	PATO:0000587	Decreased size
				ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000092	Axis	PATO:0000574	Decreased length
				ZFA:0000107	Eye	PATO:0000587	Decreased size
				ZFA:0000107	Eye	PATO:0000460	Abnormal
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
low	3ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0000107	Eye	PATO:0000587	Decreased size
				ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000107	Eye	PATO:0000587	Decreased size
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
				ZFA:0000054	Pericardium	PATO:0001450	Edematous
				ZFA:0000008	Brain	PATO:0000587	Decreased size

DPEP2

a

Proband phenotypes	Global developmental delay Severe intrauterine growth retardation Peters anomaly Severe postnatal growth retardation
Inheritance	Autosomal recessive homozygous
Consequence	stop_gained
Position	Chr16:g.68025998 A>C
Gene ID	ENSG00000167261
Transcript change	ENST00000575203.1:c.489T>G
Protein change	ENSP00000459375.1:p.Tyr163Ter



b

Zebrafish	
Gene	<i>dpep2</i>
Gene ID	ENSDARG00000079216
Morpholino	AAGTAGGAGAATTTACCTGACCTCC
Knockdown confirmation	RT-PCR, 2nd Morpholino
Relevant phenotypes	Arrested/reduced embryonic growth
Phenotypic concordance	Moderate

c

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000107	Eye	PATO:0000587	Decreased size
				ZFA:0001115	Trunk	PATO:0000587	Decreased size
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
				ZFA:0001115	Trunk	PATO:0000592	Decreased thickness
				ZFA:0000054	Pericardium	PATO:0001450	Edematous

DTWD2

a

Proband phenotypes	Intellectual disability Seizures Prominent metopic ridge Dental crowding Abnormal hair whorl Specific learning disability Aggressive behavior
Inheritance	de novo
Consequence	stop_gained
Position	Chr5:g.118264282 G>A
Gene ID	ENSG00000169570
Transcript change	ENST00000304058.4:c.349C>T
Protein change	ENSP00000302892.4:p.Gln117Ter



b

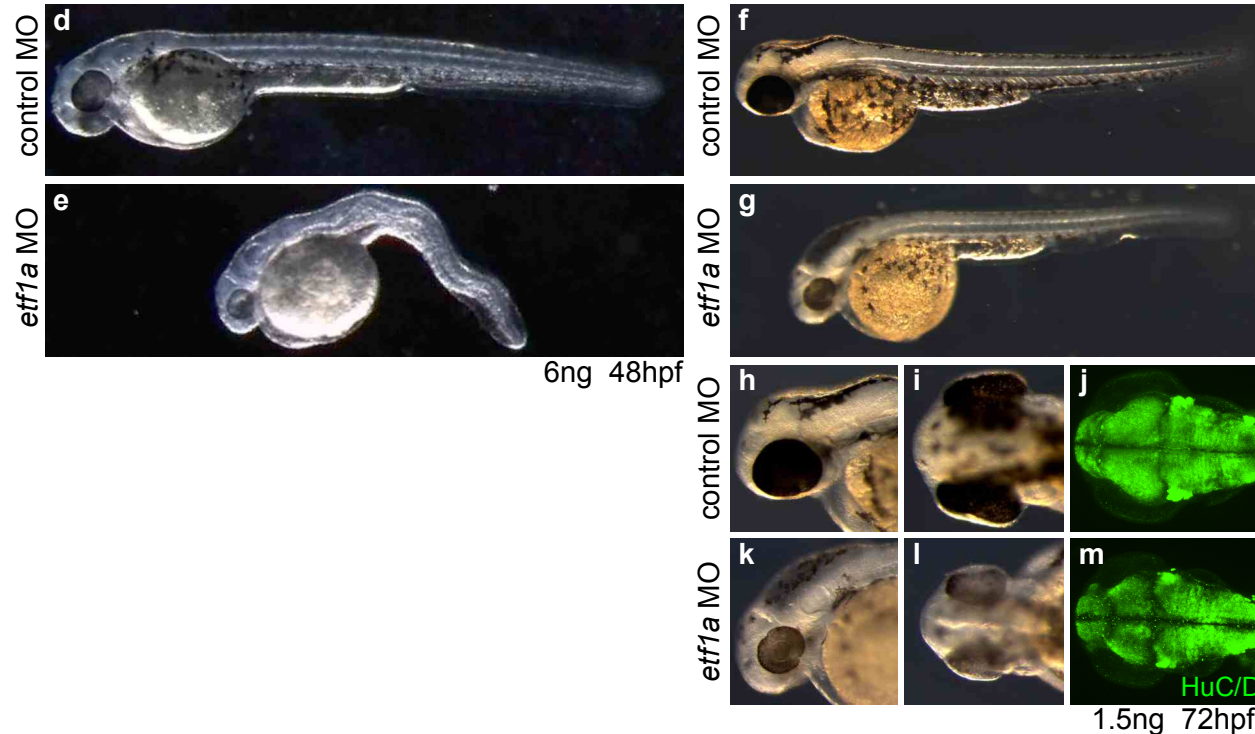
Zebrafish	
Gene	<i>dtwd2</i>
Gene ID	ENSDARG00000096425
Morpholino	ACTATACACACACTGACCTCAGC
Knockdown confirmation	RT-PCR
Relevant phenotypes	Severe multisystem defects
Phenotypic concordance	Weak

c

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001094	Whole embryo	PATO:0000638	Apoptotic
medium	4ng	ZFS:0000029	day 1: Prim-5	ZFA:0000155	Somite	PATO:0000460	Abnormal
				ZFA:0000092	Axis	PATO:0000574	Decreased length
				ZFA:0001114	Head	PATO:0000460	Abnormal
low	3ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001094	Whole embryo	PATO:0000587	Decreased size
				ZFA:0001115	Trunk	PATO:0000617	Bent
				ZFA:0000155	Somite	PATO:0000460	Abnormal

ETF1

Proband phenotypes	Global developmental delay
	Restrictive behavior
	Absence seizures
	Progressive microcephaly
	Broad-based gait
	Sleep-wake cycle disturbance
	Hypertelorism
Epicanthus	
Wide mouth	
Atonic seizures	
Inheritance	de novo
Consequence	splice_donor_variant
Position	Chr5:g.137848452 C>T
Gene ID	ENSG00000120705
Transcript change	ENST00000512198.1:c.*304+1G>A
Protein change	ENSP00000422272



Zebrafish	
Gene	<i>eff1(a)</i>
Gene ID	ENSDARG00000043976
Morpholino	TATGAGAGAACCAGCCTCACCTCT
Knockdown confirmation	RT-PCR
Relevant phenotypes	Reduced head size(-11.5%, $p < 2.3 \times 10^{-5}$) Reduced brain size(-13.4%, $p < 0.000014$)
Phenotypic concordance	Strong

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000029	day 1: Prim-5	ZFA:0001094	Whole embryo	PATO:0000460	Abnormal
medium	3ng	ZFS:0000029	day 1: Prim-5	ZFA:0001094	Whole embryo	PATO:0000587	Decreased size
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
				ZFA:0000135	Notocord	PATO:0000617	Bent
				ZFA:0000012	Central nervous system	PATO:0000592	Decreased thickness
				ZFA:0000155	Somite	PATO:0000460	Abnormal
low	1.5ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000107	Eye	PATO:0000587	Decreased size
				ZFA:0000008	Brain	PATO:0000587	Decreased size
low	1.5ng	ZFS:0000035	day 3: Protruding-mouth	ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000107	Eye	PATO:0000587	Decreased size

FRYL

a

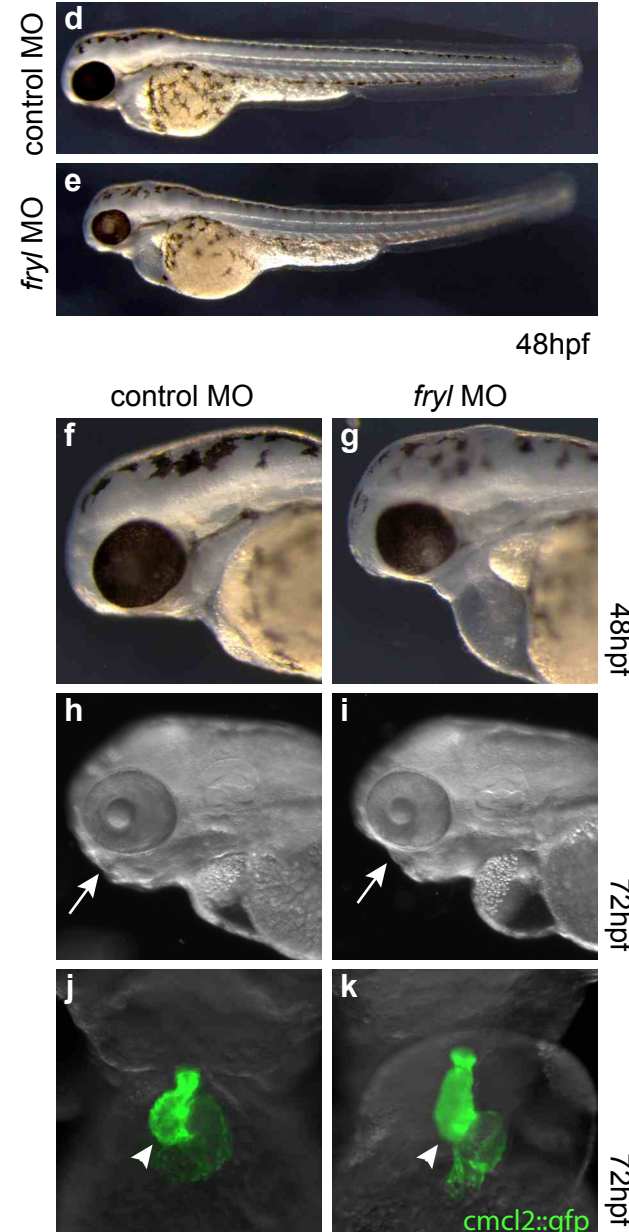
Proband phenotypes	Moderately short stature Abnormal facial shape Abnormality of the heart valves
Inheritance	de novo
Consequence	stop_gained
Position	Chr4:g.48542544 G>A
Gene ID	ENSG00000075539
Transcript change	ENST00000537810.1:c.6121C>T
Protein change	ENSP00000441114.1:p.Arg2041Ter

b

Zebrafish	
Gene	<i>fryl</i>
Gene ID	ENSDARG00000040001
Morpholino	GTATCAGGTCTCCTCACCCCTTTGGA
Knockdown confirmation	RT-PCR, 2nd Morpholino
Relevant phenotypes	Cardiac and craniofacial defects Reduced body axis (-7.6%, $p=3.25 \times 10^{-5}$)
Phenotypic concordance	Strong

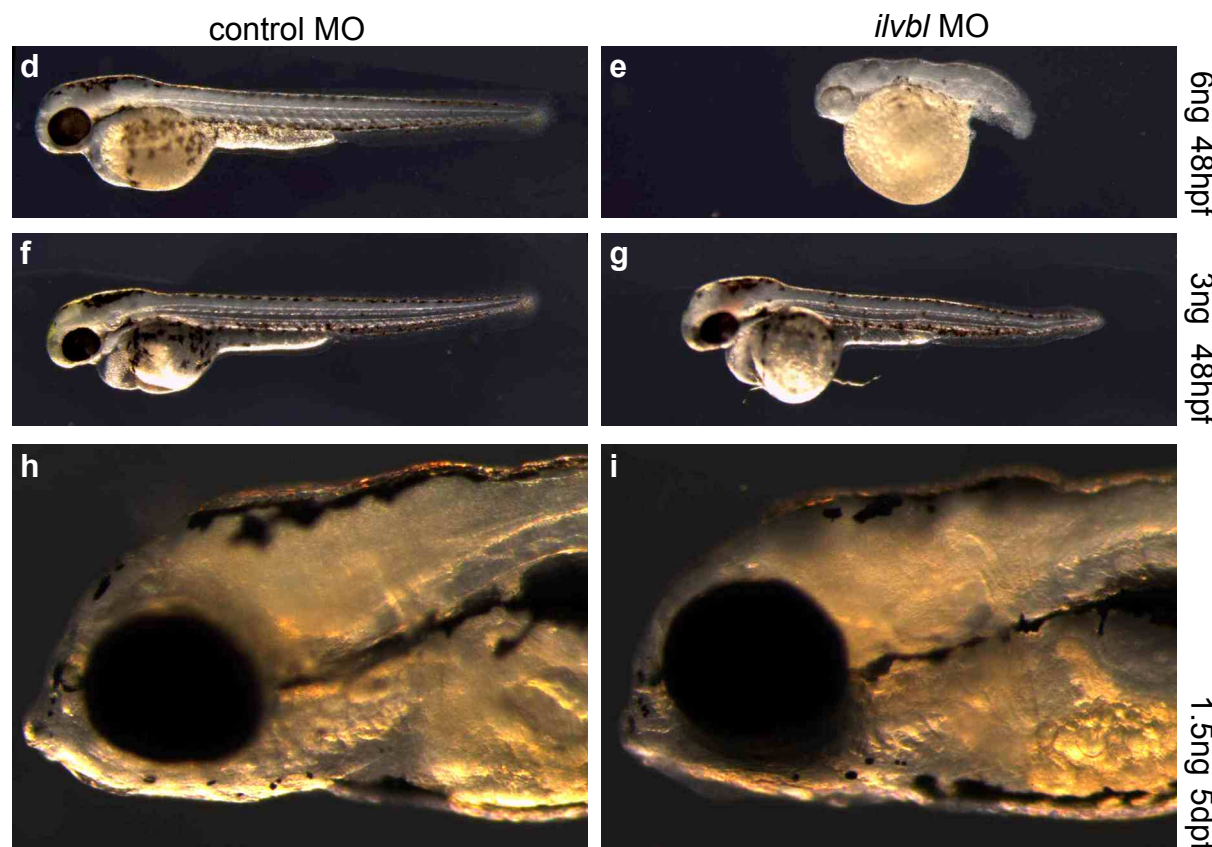
c

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0000092	Axis	PATO:0000574	Decreased length
				ZFA:0000054	Pericardium	PATO:0001450	Edematous
				ZFA:0000114	Heart	PATO:0000460	Abnormal
high	6ng	ZFS:0000035	day 3: Protruding-mouth	ZFA:0001114	Head	PATO:0000646	Malformed
				ZFA:0000054	Pericardium	PATO:0001450	Edematous
				ZFA:0000114	Heart	PATO:0000460	Abnormal



ILVBL

a	Proband phenotypes	Genu valgum Hyperlordosis Constipation Global developmental delay Hypermetropia Abnormal facial shape Cafe-au-lait spot
	Inheritance	de novo
	Consequence	stop_gained
	Position	Chr19:g.15226974 C>T
	Gene ID	ENSG00000105135
	Transcript change	ENST00000263383.3:c.1460G>A
	Protein change	ENSP00000263383.3:p.Trp487Ter



b	Zebrafish
Gene	<i>ilvbl</i>
Gene ID	ENSDARG00000017126
Morpholino	GCTGTTTGGGTTTAGTCTTACCAGA
Knockdown confirmation	RT-PCR
Relevant phenotypes	Severe multisystem defects Craniofacial defects at lower MO dose
Phenotypic concordance	Weak

c		MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000029	day 1: Prim-5	ZFA:0001094	Whole embryo	PATO:0000638	Apoptotic		
				ZFA:0001094	Whole embryo	PATO:0000587	Decreased size		
				ZFA:0001115	Trunk	PATO:0000462	Absent		
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001115	Trunk	PATO:0000646	Malformed		
				ZFA:0001114	Head	PATO:0000646	Malformed		
medium	3ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001114	Head	PATO:0001450	Edematous		
				ZFA:0000092	Axis	PATO:0000574	Decreased length		
				ZFA:0001114	Head	PATO:0000646	Malformed		
low	1.5ng	ZFS:0000037	Larval: Day 5	ZFA:0001114	Head	PATO:0000646	Malformed		

NONO

Proband phenotypes	Intellectual disability Neonatal hypotonia Joint hypermobility Bilateral sensorineural hearing impairment Myopia Abnormality of vision Bifid uvula High palate	Misalignment of teeth Feeding difficulties in infancy Bruising susceptibility Urinary incontinence Nystagmus Intrauterine growth retardation Hypoplasia of the corpus callosum
Inheritance	de novo	
Consequence	stop_gained	
Position	ChrX:g.70517750 C>T	
Gene ID	ENSG00000147140	
Transcript change	ENST00000535149.1:c.826C>T	
Protein change	ENSP00000441364.1:p.Arg276Ter	

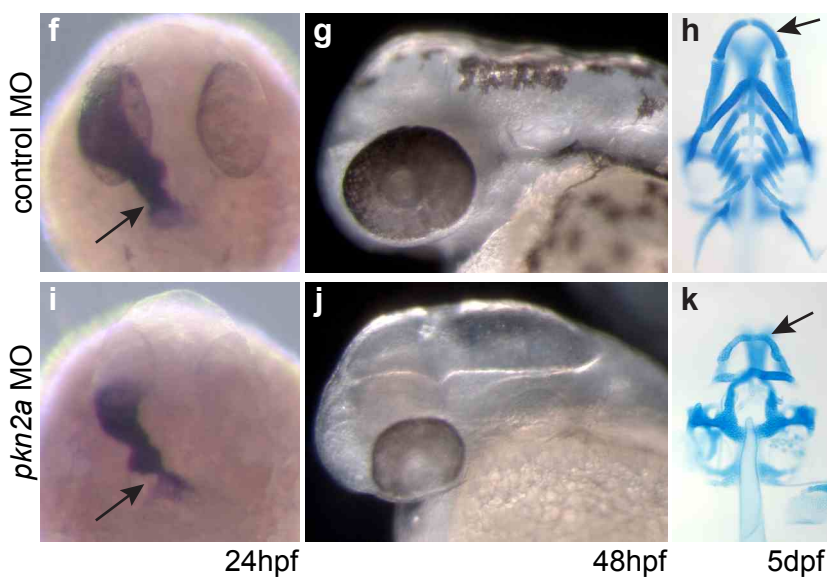
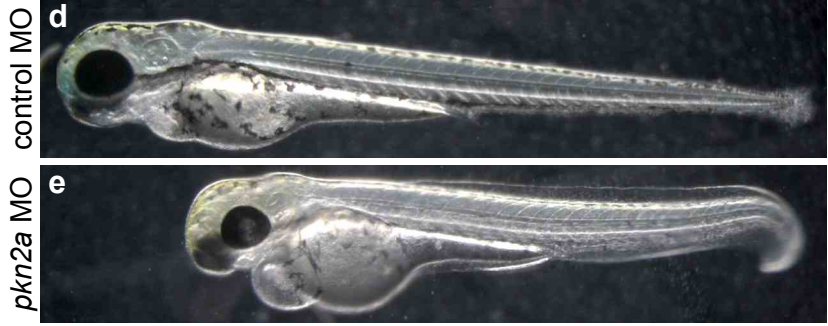


Zebrafish	
Gene	<i>nono</i>
Gene ID	ENSDARG00000020482
Morpholino	TGACGCCTAACCAAACCTCACCAGCC
Knockdown confirmation	RT-PCR
Relevant phenotypes	Severe multisystem defects including ear and growth defects
Phenotypic concordance	Weak

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000029	day 1: Prim-5	ZFA:0001094	Whole embryo	PATO:0000638	Apoptotic
medium	3ng	ZFS:0000029	day 1: Prim-5		Development	PATO:0000297	Arrested
				ZFA:0000155	Somite	PATO:0000462	Absent
				ZFA:0000012	Central nervous system	PATO:0000587	Decreased size
				ZFA:0000008	Brain	PATO:0000587	Decreased size
low	1ng	ZFS:0000029	day 1: Prim-5	ZFA:0001094	Whole embryo	PATO:0000460	Abnormal
				ZFA:0000092	Axis	PATO:0000574	Decreased length
				ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000051	Otic vesicle	PATO:0000587	Decreased size
				ZFA:0000008	Brain	PATO:0000587	Decreased size
				ZFA:0000054	Pericardium	PATO:0000587	Decreased size
				ZFA:0001115	Trunk	PATO:0000591	Increased thickness
				ZFA:0001115	Trunk	PATO:0000574	Decreased length
ZFA:0000155	Somite	PATO:0000460	Abnormal				
ZFA:0000107	Eye	PATO:0000587	Decreased size				

PKN2

a	Proband phenotypes	Muscular subaortic stenosis Proximal aortic coarctation Hypospadias Isolated growth hormone deficiency Bilateral sensorineural deafness Spina bifida occulta at l5 or s1 Upslanting palpebral fissures Mild micrognathia	Thick everted lower lip Thin upper lip Broad nose Arched sparse eyebrows Fleshy earlobes Small hands Inguinal hernia Mild-to-moderate growth deficiency	
	Inheritance	de novo		
	Consequence	missense_variant		
	Position	Chr1:g.89270569 T>C		
	Gene ID	ENSP00000317851		
	Transcript change	ENST00000316005.7:c.1466T>C		
	Protein change	ENSP00000317851.7:p.Leu489Pro		



b	Zebrafish
Gene	<i>pkn2 (pkn2a)</i>
Gene ID	ENSDARG00000006705
Morpholino	AGCTTTACTCATACCCAAGAGAAGA
Knockdown confirmation	RT-PCR, 2nd Morpholino
Relevant phenotypes	Cardiac, craniofacial cartilage, and growth defects
Phenotypic concordance	Strong

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0009091	melanocyte	PATO:0001997	Decreased number
				ZFA:0000107	Eye	PATO:0000587	Decreased size
				ZFA:0000008	Brain	PATO:0001450	Edematous
				ZFA:0000054	Pericardium	PATO:0001450	Edematous
				ZFA:0000114	Heart	PATO:0000460	Abnormal
high	6ng	ZFS:0000035	day 3: Protruding-mouth	ZFA:0001114	Head	PATO:0000460	Abnormal
				ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000092	Axis	PATO:0000574	Decreased length
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
				ZFA:0000054	Pericardium	PATO:0001450	Edematous
				ZFA:0000114	Heart	PATO:0000460	Abnormal
high	6ng	ZFS:0000037	Larval: Day 5	ZFA:0001114	Head	PATO:0000646	Malformed
medium	4.5ng	ZFS:0000037	Larval: Day 5	ZFA:0001458	Cranial cartilage	PATO:0000462	Absent
				ZFA:0001458	Cranial cartilage	PATO:0000587	Decreased size
				ZFA:0001273	Ventral mandibular arch	PATO:0000587	Decreased size

POGZ

Proband phenotypes	Bilateral sensorineural hearing impairment Generalized neonatal hypotonia Generalized hypotonia Decreased electroretinogram (ERG) amplitude Reduced amplitude of b-wave (ERG) Abnormality of vision evoked potentials Global developmental delay Cerebral atrophy Thin corpus callosum	Iris coloboma Global developmental delay Behavioural/Psychiatric Abnormality Hypoglycemic seizure Abnormality of the outer ear
Inheritance	de novo	de novo
Consequence	stop_gained	splice_donor_variant
Position	Chr1:g.151378800 A>T	Chr1:g.151378156 AGGG>AGG
Gene ID	ENSG00000143442	ENSG00000143442
Transcript change	ENST00000491586.1:c.2579T>A	ENST00000491586.1:c.3220delC
Protein change	ENSP00000418408.1:p.Leu860Ter	ENSP00000418408.1:p.Leu1075CysfsTer3



Zebrafish	
Gene	<i>pogza</i>
Gene ID	ENSDARG00000054778
Morpholino	GGTGTGGATGAAACTTACACAGAA
Relevant phenotypes	Severe multisystem defects including CNS defects
Phenotypic concordance	Weak

MO dose		Stage	Entity		Quality		
high	6ng		ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001094	Whole embryo	PATO:0000587
				ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000107	Eye	PATO:0000587	Decreased size
				ZFA:0000051	Otic vesicle	PATO:0000587	Decreased size
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
				ZFA:0000054	Pericardium	PATO:0001450	Edematous
				ZFA:0000008	Brain	PATO:0000587	Decreased size
				ZFA:0000008	Brain	PATO:0000592	Decreased thickness
				ZFA:0000008	Brain	PATO:0001450	Edematous
				ZFA:0009091	melanocyte	PATO:0001997	Decreased number
				ZFA:0001115	Trunk	PATO:0000647	Necrotic
				GO:0008345	Larval locomotory behavior	PATO:0000460	Abnormal
				ZFA:0000084	yolk	PATO:0001450	Edematous
				ZFA:0000075	Spinal cord	PATO:0000587	Decreased size
				ZFA:0000075	Spinal cord	PATO:0000592	Decreased thickness

PSD2

a	Proband phenotypes	Global developmental delay Lower limb hypertonia Muscular hypotonia Seizures
	Inheritance	de novo
	Consequence	splice_donor_variant
	Position	Chr5:g.139216587 G>C
	Gene ID	ENSG00000146005
	Transcript change	ENST00000274710.3:c.1594+1G>C
	Protein change	ENSP00000274710



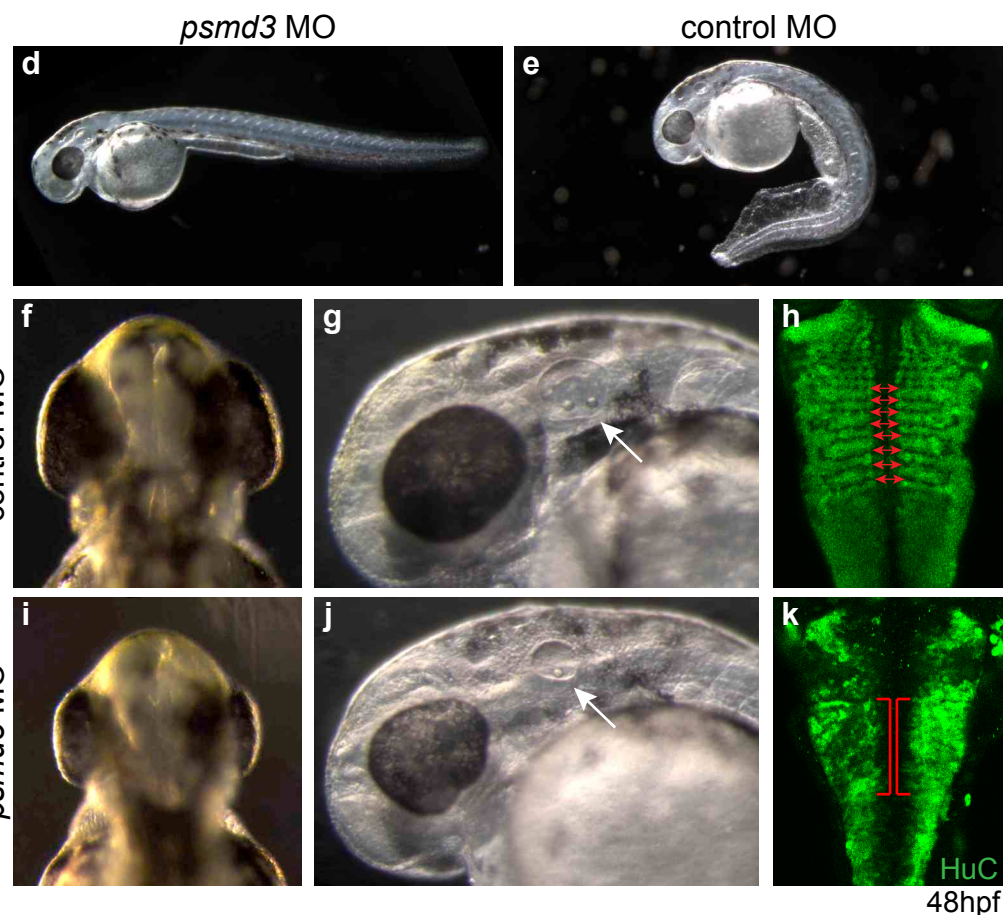
6ng 48hpf

b	Zebrafish	
	Gene	<i>BX005008.2 (psd2a)</i>
	Gene ID	ENSDARG00000063036
	Morpholino	TAAATGATAACTTGCCCTCTGAGGT
	Knockdown confirmation	2nd Morpholino
	Relevant phenotypes	Abnormal musculature, locomotion and CNS defects
	Phenotypic concordance	Moderate

c		MO dose	Stage	Entity	Quality		
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0000008	Brain	PATO:0001450	Edematous
				ZFA:0000054	Pericardium	PATO:0001450	Edematous
				ZFA:0000155	Somite	PATO:0000460	Abnormal
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
				ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000107	Eye	PATO:0000587	Decreased size
				GO:0008345	Larval locomotory behavior	PATO:0000460	Abnormal
				ZFA:0001094	Whole embryo	PATO:0000587	Decreased size
				ZFA:0000114	Heart	PATO:0000460	Abnormal
				ZFA:0000075	Spinal cord	PATO:0000592	Decreased thickness
low	3ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	GO:0008345	Larval locomotory behavior	PATO:0000460	Abnormal
				ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral

PSMD3

Proband phenotypes	Microcephaly	Frequent candida infections
	Muscular hypotonia	Migraine
	Strabismus	Stereotyped
	Gastroesophageal reflux	repetitive behaviour
	Constipation	Endocrine abnormality
	Epilepsy	Growth abnormality
	Drooling	
Inheritance	de novo	
Consequence	missense_variant	
Position	Chr17:g.38142858 C>T	
Gene ID	ENSG00000108344	
Transcript change	ENST00000264639.4:c.442C>T	
Protein change	ENSP00000264639.4:p.Arg148Cys	

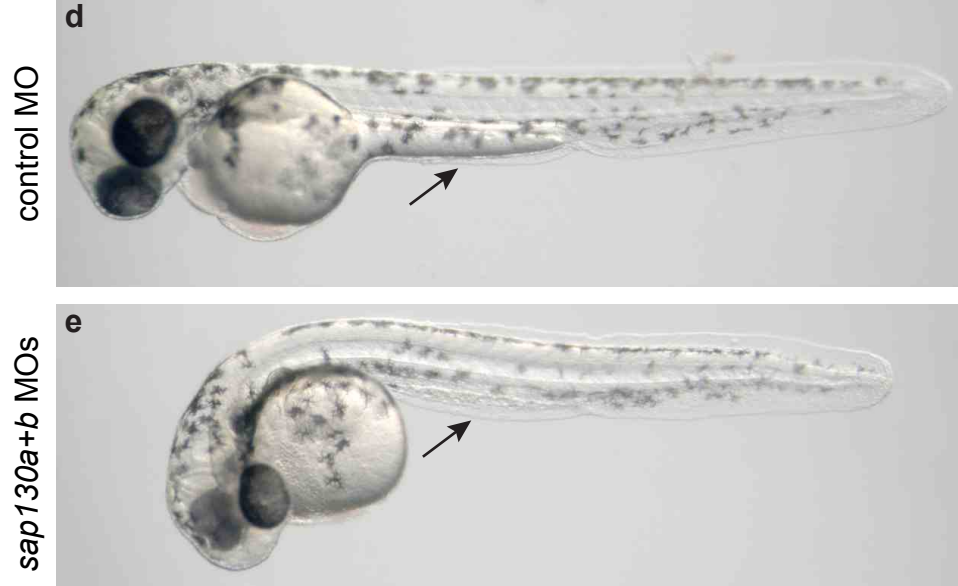


Zebrafish	
Gene	<i>psmd3</i>
Gene ID	ENSDARG00000018124
Morpholino	CTGATGAAGGGTGTGTTTACCTGCTC
Knockdown confirmation	RT-PCR
Relevant phenotypes	Reduced head size(-8.9%, p<0.0005) Reduced brain size(-16%, p<1.6 x 10 ⁻⁵) Defective CNS patterning
Phenotypic concordance	Strong

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000029	day 1: Prim-5	ZFA:0000051	Otic vesicle	PATO:0000587	Decreased size
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000008	Brain	PATO:0001450	Edematous
				ZFA:0000051	Otic vesicle	PATO:0000587	Decreased size
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
				ZFA:0000022	floorplate	PATO:0000462	Absent
				ZFA:0000008	Brain	PATO:0000460	Abnormal
				ZFA:0001617	otolith	PATO:0001997	Decreased number
				ZFA:0000114	Heart	PATO:0000460	Abnormal
ZFA:0000054	Pericardium	PATO:0001450	Edematous				

SAP130

a	Proband phenotypes	Prominent metopic ridge Craniosynostosis Short sternum Bifid uvula Velopharyngeal insufficiency Abnormality of dental enamel Carios teeth Patent foramen ovale Infantile muscular hypotonia Hypotelorism Delayed speech and language development
	Inheritance	de novo
	Consequence	stop_gained
	Position	Chr2:g.128750773 G>A
	Gene ID	ENSG00000136715
	Transcript change	ENST00000357702.5:c.1543C>T
	Protein change	ENSP00000350333.5:p.Arg515Ter



48hpf

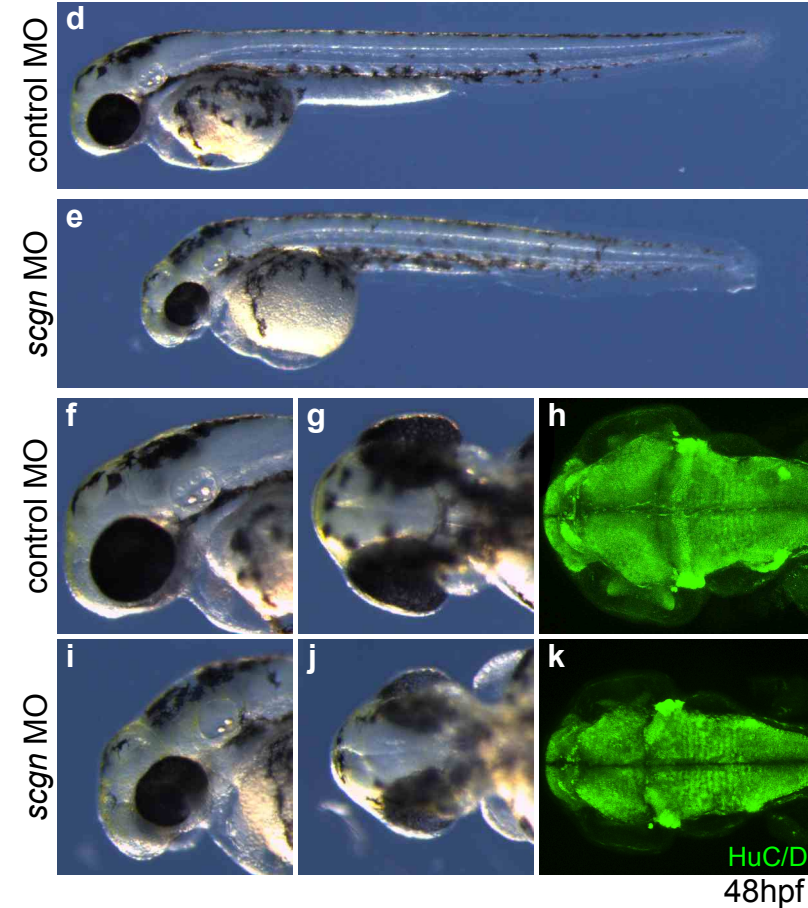
b		
Zebrafish		
Gene	<i>sap130a</i>	<i>sap130b</i>
Gene ID	ENSDARG00000056924	ENSDARG00000079352
Morpholino	GTGTATCACTATCCTCCTACCTGAA	ATTAAATGTGTCATAAACCTGTGCT
Knockdown confirmation	RT-PCR	RT-PCR
Relevant phenotypes	Abnormal locomotion in double knockdown	
Phenotypic concordance	Moderate	

c							
MO dose			Stage	Entity		Quality	
high	5ng+5ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0000106	yolk extension	PATO:0000462	Absent
				GO:0001966	Touch response	PATO:0000460	Abnormal
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
				ZFA:0001115	Trunk	PATO:0000591	Increased thickness
				GO:0008345	Larval locomotory behavior	PATO:0000460	Abnormal
				ZFA:0001114	Head	PATO:0001469	Curved ventral
high	5ng+5ng	ZFS:0000037	Larval: Day 5	ZFA:0000022	Floorplate	PATO:0000462	Absent
				GO:0035188	Hatching	PATO:0000462	Absent

SCGN

a

Proband phenotypes	Feeding difficulties in infancy Seizures Poor head control Microcephaly Specific learning disability Congenital cataract Global developmental delay
Inheritance	Autosomal recessive homozygous
Consequence	splice_donor_variant
Position	Chr6:g.25661873 G>A
Gene ID	ENSG00000079689
Transcript change	ENST00000377961.2:c.246+1G>A
Protein change	ENSP00000367197



b

Zebrafish	
Gene	<i>scgn</i>
Gene ID	ENSDARG00000058732
Morpholino	GTGGTTTATGTTTGATACCTTTGGC
Knockdown confirmation	RT-PCR, 2nd Morpholino
Relevant phenotypes	Reduced head size(-12.3%, p<0.0003) Reduced brain size(-17%, p<1.23 x 10 ⁻¹⁵)
Phenotypic concordance	Strong

c

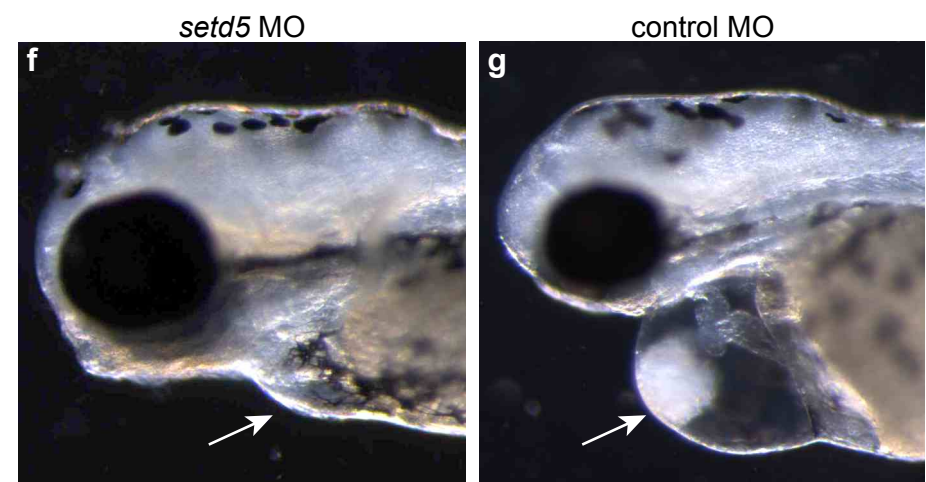
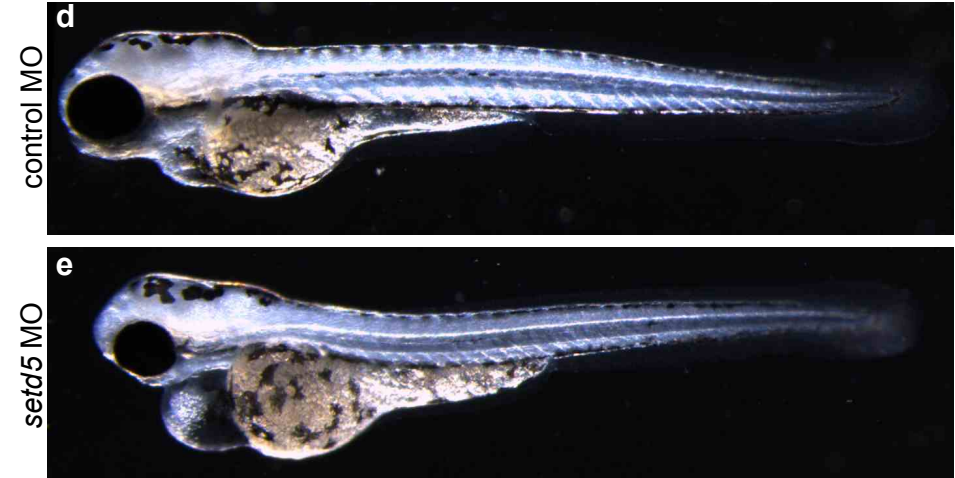
MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000008	Brain	PATO:0001450	Edematous
				ZFA:0000008	Brain	PATO:0000587	Decreased size
				ZFA:0000092	Axis	PATO:0000574	Decreased length
				ZFA:0000054	Pericardium	PATO:0001450	Edematous
high	6ng	ZFS:0000037	Larval: Day 5	ZFA:0000008	Brain	PATO:0000587	Decreased size
				ZFA:0000092	Axis	PATO:0000461	Normal length
low	4.5ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000092	Axis	PATO:0000574	Decreased length

SETD5

Proband phenotypes	Dilatation of the ascending aorta
	Focal clonic seizures
	Poor motor coordination
	Specific learning disability
Inheritance	de novo
Consequence	stop_gained
Position	Chr3:g.9512572 G>T
Gene ID	ENSG00000168137
Transcript change	ENST00000302463.6:c.2860G>T
Protein change	ENSP00000302028.6:p.Gly954Ter

b Zebrafish	
Gene	<i>setd5</i>
Gene ID	ENSDARG00000078576
Morpholino	ACGTCCAGCGCAGCAATGCAGGGAA
Knockdown confirmation	RT-PCR
Relevant phenotypes	Motility defect
	Reduced head size (-16.9%, p=0.005) Cardiac defect
Phenotypic concordance	Strong

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	GO:0008345	Larval locomotory behavior	PATO:0000460	Abnormal
				ZFA:0000054	Pericardium	PATO:0001450	Edematous
				ZFA:0000114	Heart	PATO:0000460	Abnormal
				ZFA:0001114	Head	PATO:0000587	Decreased size
high	6ng	ZFS:0000035	day 3: Protruding-mouth	GO:0008345	Larval locomotory behavior	PATO:0000460	Abnormal
				ZFA:0000054	Pericardium	PATO:0001450	Edematous
				ZFA:0000114	Heart	PATO:0000460	Abnormal
				ZFA:0001114	Head	PATO:0000587	Decreased size



SMARCD1

a	Proband phenotypes	Global developmental delay Neonatal hypotonia Plagiocephaly Deeply set eye Uplifted earlobe Low anterior hairline	Laryngomalacia Broad face Coarse facial features Downturned corners of mouth Deep philtrum Muscular hypotonia Abnormality of the cerebral white matter Abnormality of thyroid physiology
	Inheritance	de novo	de novo
	Consequence	stop_gained	missense_variant
	Position	Chr12:g.50492561 G>A	Chr12:g.50492587 T>C
	Gene ID	ENSG00000066117	ENSG00000066117
	Transcript change	ENST00000549274.1:c.255G>A	ENST00000549274.1:c.281T>C
	Protein change	ENSP00000447909.1:p.Trp86Ter	ENSP00000447909.1:p.Phe95Leu



b	Zebrafish
Gene	<i>smarcd1</i>
Gene ID	ENSDARG00000019004
Morpholino	TTGTTCTCCTCGCGCCTTACCGGGT
Knockdown confirmation	RT-PCR
Relevant phenotypes	Severe multisystem defects
Phenotypic concordance	Weak

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000029	day 1: Prim-5	ZFA:0001094	Whole embryo	PATO:0000638	Apoptotic
medium	3ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001094	Whole embryo	PATO:0000587	Decreased size
				ZFA:0000075	Spinal cord	PATO:0000587	Decreased size
				ZFA:0000075	Spinal cord	PATO:0000592	Decreased thickness
				ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
				ZFA:0000008	Brain	PATO:0000460	Abnormal
				ZFA:0000075	Spinal cord	PATO:0000587	Decreased size
				ZFA:0000075	Spinal cord	PATO:0000592	Decreased thickness
				ZFA:0009091	melanocyte	PATO:0001997	Decreased number
				ZFA:0000107	Eye	PATO:0000587	Decreased size
ZFA:0000155	Somite	PATO:0000460	Abnormal				
ZFA:0000114	Heart	PATO:0000460	Abnormal				

THNSL2

a

Proband phenotypes	Microcephaly Macrotia Soft doughy skin Long palpebral fissure Large eyes Highly arched eyebrow	Global developmental delay
Inheritance	Autosomal recessive homozygous	Autosomal recessive homozygous
Consequence	splice_donor_variant	splice_donor_variant
Position	Chr2:g.88478534 T>C	Chr2:g.88478534 T>C
Gene ID	ENSG00000144115	ENSG00000144115
Transcript change	ENST00000324166.5:c.802+2T>C	ENST00000324166.5:c.802+2T>C
Protein change	ENSP00000327323	ENSP00000327323



b

Zebrafish	
Gene	<i>thnsl2</i>
Gene ID	ENSDARG00000032584
Morpholino	AAGTAAACCTGAAGCGGCACCTGGA
Knockdown confirmation	RT-PCR, 2nd Morpholino
Relevant phenotypes	Reduced head size(-15.1, $p < 1.3 \times 10^{-12}$) Reduced brain size(-16.1%, $p < 1.6 \times 10^{-5}$)
Phenotypic concordance	Strong

c

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000107	Eye	PATO:0000587	Decreased size
				ZFA:0000008	Brain	PATO:0001450	Edematous
				ZFA:0000008	Brain	PATO:0000587	Decreased size
				ZFA:0000054	Pericardium	PATO:0001450	Edematous
				ZFA:0000114	Heart	PATO:0000460	Abnormal
				GO:0008345	Larval locomotory behavior	PATO:0000460	Abnormal

WWC1

a

Proband phenotypes	Delayed developmental milestones Mental retardation Hypertelorism Hypoplasia of the corpus callosum Absent or delayed speech
Inheritance	de novo
Consequence	missense_variant
Position	Chr5:g.167891774 G>A
Gene ID	ENSG00000113645
Transcript change	ENST00000265293.4:c.2957G>A
Protein change	ENSP00000265293.4:p.Arg986His



b

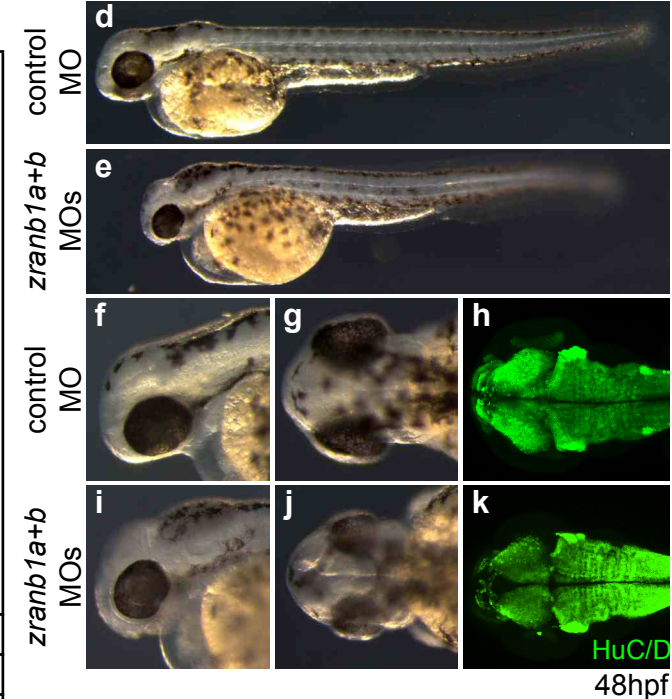
Zebrafish	
Gene	<i>wwc1</i>
Gene ID	ENSDARG00000076041
Morpholino	GCAGTACTCATCACATACCTATCCC
Knockdown confirmation	RT-PCR
Relevant phenotypes	none
Phenotypic concordance	None

c

MO dose		Stage		Entity		Quality	
high	6ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0000051	Otic vesicle	PATO:0000646	Malformed
				ZFA:0001617	Otolith	PATO:0001997	Decreased number
high	6ng	ZFS:0000035	day 3: Protruding-mouth	GO:0008345	Larval locomotory behavior	PATO:0000460	Abnormal
				ZFA:0001617	Otolith	PATO:0001997	Decreased number
high	6ng	ZFS:0000037	Larval: Day 5	ZFA:0001114	Head	PATO:0000646	Malformed
				GO:0008345	Larval locomotory behavior	PATO:0000461	Normal

ZRANB1

Proband phenotypes	Microcephaly	Microcephaly
	Constipation	Constipation
	Facial muscle weakness	Iris coloboma
	Moderate Seizures	Global developmental delay
	Tongue fasciculations	Delayed speech and language development
	Intracranial calcification by x-ray or ct	Joint hypermobility
		Hirsutism
	Long palpebral fissure	
	Depressed nasal bridge	
	Thick lower lip vermilion	
	Widely spaced teeth	
	Drooling	
	Smooth philtrum	
	Autism	
Inheritance	de novo	de novo
Consequence	missense_variant	missense_variant
Position	Chr10:g.126662852 C>T	Chr10:g.126662892 C>T
Gene ID	ENSG00000019995	ENSG00000019995
Transcript change	ENST00000359653.4:c.1312C>T	ENST00000359653.4:c.1352C>T
Protein change	ENSP00000352676.4:p.Arg438Trp	ENSP00000352676.4:p.Ala451Val



Zebrafish		
Gene	<i>CABZ01057907.1 (zranb1a)</i>	<i>zranb1b</i>
Gene ID	ENSDARG00000060257	ENSDARG00000063190
Morpholino	TAAATAACTTGGGTGCAGACCTGAC	TAATGGAGCGGATTTCTCACCAGCA
Knockdown confirmation	RT-PCR	RT-PCR
Relevant phenotypes	Reduced head size(-8.2%, $p < 5.4 \times 10^{-6}$) in double knockdown Reduced brain size(-9.2%, $p < 0.0015$) in double knockdown	
Phenotypic concordance	Strong	

MO dose		Stage		Entity		Quality	
high	5ng+5ng	ZFS:0000033	Hatching: Long-pec (48 hpf)	ZFA:0000008	Brain	PATO:0001450	Edematous
				ZFA:0001114	Head	PATO:0000646	Malformed
				ZFA:0001114	Head	PATO:0000587	Decreased size
				ZFA:0000008	Brain	PATO:0000587	Decreased size
high	5ng+5ng	ZFS:0000035	day 3: Protruding-mouth	ZFA:0000054	Pericardium	PATO:0001450	Edematous
				ZFA:0001115	Trunk	PATO:0001469	Curved ventral
high	5ng+5ng	ZFS:0000036	Larval:Day 4	ZFA:0001094	Whole embryo	PATO:0000587	Decreased size
high	5ng+5ng	ZFS:0000037	Larval:Day 5	ZFA:0001114	Head	PATO:0000646	Malformed