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Supplemental Data

UBR7 functions with UBR5 in the Notch signaling pathway and is involved in a neurodevelopmental syndrome with epilepsy, ptosis, and hypothyroidism

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SUPPLEMENTAL NOTE: CASE REPORTS

Individual 1 is a 9-year-old child who displayed a prominent forehead, hypothyroidism, developmental delay, significant growth delay, a congenital heart defect (operated PDA), a small penis, and strabismus. There were also hypoplastic patellae. No pancreatic has been identified. Family history is unrevealing and the parents are non-consanguineous. Two loss-of-function variants were identified in *UBR7*: NM_175748.4:c.37G>T, p.Glu13* and NM_175748.4:c.563_564insTT, p.Cys189Phefs*14.

Individual 2 is a 17-year-old male and is homozygous for a novel missense variant (NM_175748.4.3:c.914G>C, p.Trp305Ser), which is absent from gnomAD. No other candidate genes were identified on the clinical exome sequencing. The phenotype is overlapping with individual 1 in some areas: intellectual disability, severe global developmental delay, short stature, congenital heart disease (small VSD, now closed, dilated aortic arch), genital anomalies (cryptorchidism), and prominent forehead. However, he exhibited other phenotypic features that are not present in individual 1: seizures and myoclonias, macrocephaly and gastrointestinal dysmotility/constipation. Eyes are down-slanting and hyperteloric. Bushy and arched eyebrows. Ears are small and the left ear appears low set. Mouth normal. Long philtrum. Palate is high and arched. The nose is tubular in shape with a down-turned tip. Small penis, testicles and scrotum, being treated with testosterone for hypogonadism. He has scoliosis. Fingers show 3rd digit ulnar deviation bilaterally.

Individual 3 is a male aged 3 years and 9 months with intellectual disability, severe global developmental delay, absent speech, seizures, short stature, feeding difficulties (gastric tube), failure to thrive, constipation, congenital heart disease (patent ductus arteriosus and patent foramen ovale, spontaneously closed), genital anomalies (cryptorchidism), sacral dimple, strabismus, hyperopia, epicanthic folds, small and deep-set ears and a prominent forehead. He was born preterm in week 35. At the age of 3 years, he was diagnosed with a disease of the optic nerve / retina. He also had thrombopenia and leucopenia induced by antiepileptic drugs. Family history is

unrevealing and the parents are non-consanguineous. Trio exome sequencing identified biallelic loss of function variants in *UBR7*: a paternally inherited hemizygous splice site variant at the splice acceptor site of exon 6 (NM_175748.4.3:c.496-2A>G) and a maternally inherited deletion of exons 1-10 sparing the last exon. Subsequent genome sequencing confirmed the deletion as chr14:g.93,660,388_93,690,906del (hg19).

Individual 4 is a 7-year-old male born from consanguineous parents (first cousins). Family history is otherwise unremarkable. He has progressive encephalopathy and serial brain MRI revealed progressive volume loss and delayed myelination. At the age of 1 year, he developed seizures. He also had hypothyroidism diagnosed at 9 months of age and cryptorchidism. On last examination, his height was 118 cm (25-50th percentile), his weight was 24.8 kg (50-75th percentile) and his head circumference was 54 cm (10th percentile). Mild dysmorphic features were noted, including downslanting palpebral fissures, thick eyebrow, ptosis and unilateral single transverse palmar crease. He was referred to medical genetics at the age of 2 years in the context of hypotonia, epilepsy and developmental delay. Metabolic workup was normal and aCGH found no copy number variant but a region of homozygosity. Homozygous variant in *UBR7* (NM_175748.4:c.618delT, p.(Glu207Argfs*12)) was identified by genome sequencing.

Individual 5 is a male aged 3 years and 7 months. His parents are double first cousins and have three older children. They also had two miscarriages and an intrauterine fetal demise at 5 months of pregnancy. Among the siblings, one brother experienced speech delay. A cousin of the patient also had brain atrophy. Individual 5 displayed global developmental delay with hypotonia. He had recurrent febrile seizures and had a first unprovoked episode of status epilepticus at the age of 33 months. He also had congenital hypothyroidism detected by newborn screening and cryptorchidism. Brain MRI showed abnormal signal intensity with diffusion restriction in central tagmental tracts (suggesting metabolic disorder) and bilateral abnormal T2/FLAIR signal in the external capsule. Last physical examination revealed a height of 90 cm (10th percentile), a weight of 12.5 kg (10th percentile) and a head circumference of 48 cm (25th percentile). Dysmorphic features were noted: hypertelorism, deep nasal bridge, protruding and low-set ears, long fingers and hypertrichosis. Central hypotonia and hyperreflexia were present. He was referred to medical

genetics at the age of 19 months for hypotonia and developmental delay. Metabolic workup was normal and aCGH found no copy number variant but identified a region of homozygosity. Genome sequencing identified the same homozygous variant in *UBR7* (NM_175748.4:c.618delT, p.(Glu207Argfs*12)) as in individual 4. Interestingly, individuals 4 and 5 are both of Saudi Arabian descent. However, no relationship between their families was found for at least 3 generations.

Individuals 6 and 7 are siblings from first cousin Egyptian parents with no healthy children. Both children were delivered by cesarean section and perinatal histories were uneventful. Both presented with delayed developmental milestones and epilepsy. **Individual 6** is a 5 year old male, presenting with recurrent tonic seizures with cyanosis and atonic seizures with upward eye gaze at 6 months of age. Epilepsy was controlled at 4 years with leviteracetam. Upon clinical examination at 5 years, his weight was 9 kg (-4.5 SD), height 83 cm (-5.3 SD) and head circumference 44 cm (-5.7 SD). He was able to sit supported, had mild autistic behavior, severe intellectual disability and did not respond to simple commands. He had mild facial dysmorphism including flat forehead, straight thick eyebrows, hypertelorism, downslanting palpebral fissures, mild ptosis, prominent nose, long philtrum and low set ears. Long fingers, undescended testis, hypotonia of the limbs and brisk reflexes were also present. Karyotyping, metabolic workup and fundus examination were all normal. Echocardiogram showed atrial septal defect (5 mm) with moderate left to right shunt. Abdominopelvic ultrasound identified right and left testes in the lateral end of the inguinal canal. Brain MRI showed mild frontoparietal cortical changes, mild dilatation of lateral ventricles and thin corpus callosum. Exome sequencing identified a homozygous variant in *UBR7* (NM_175748.4.3:c.1186-1G>C). **Individual 7** presented at the age of 1 year and 10 months with delayed developmental milestones with moderate ability to support her head. Anthropometric measurement revealed weight 6.6 kg (-4.5 SD), length 72 cm (-3.6 SD) and head circumference 41.5 cm (-4.6 SD). She had closely similar facies as her brother and had similar long fingers. Neurological examination revealed severe cognitive delay, axial hypotonia, limb hypotonia and brisk reflexes. Echocardiogram revealed ventricular septal defect (7 mm). Brain CT showed mild cortical changes, mild dilated lateral ventricles (mainly frontal) and hypogenesis of the corpus callosum. The child died at the age of 2 years with sudden unexpected death in epilepsy (SUDEP) that was focal and occurred for the first time in her life.

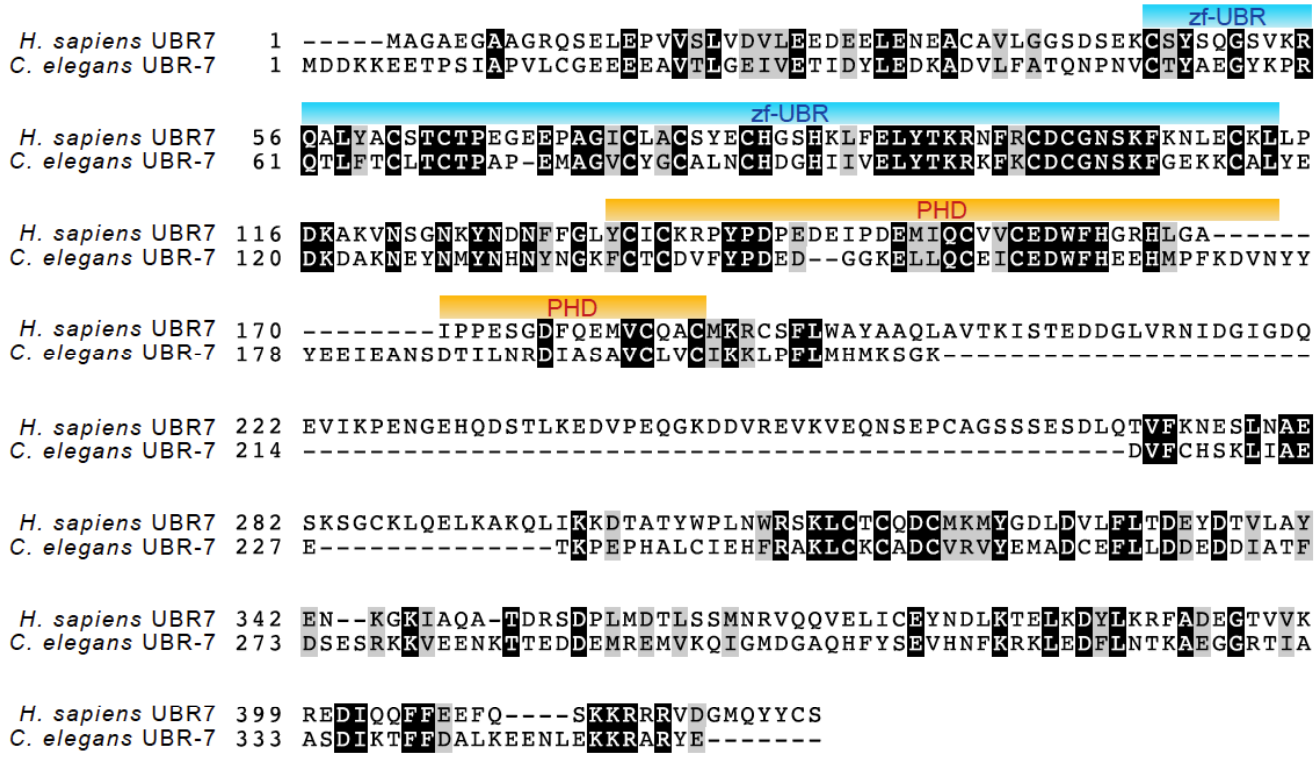

Figure S1

Figure S1. *H. sapiens* UBR7 and *C. elegans* UBR-7 have conserved zf-UBR and PHD domains. Amino acid sequence alignment of *H. sapiens* UBR7 and *C. elegans* UBR-7. Residue numbers are shown, and black and gray boxes indicate identical or similar residues, respectively. The conserved zf-UBR and PHD domains are highlighted.

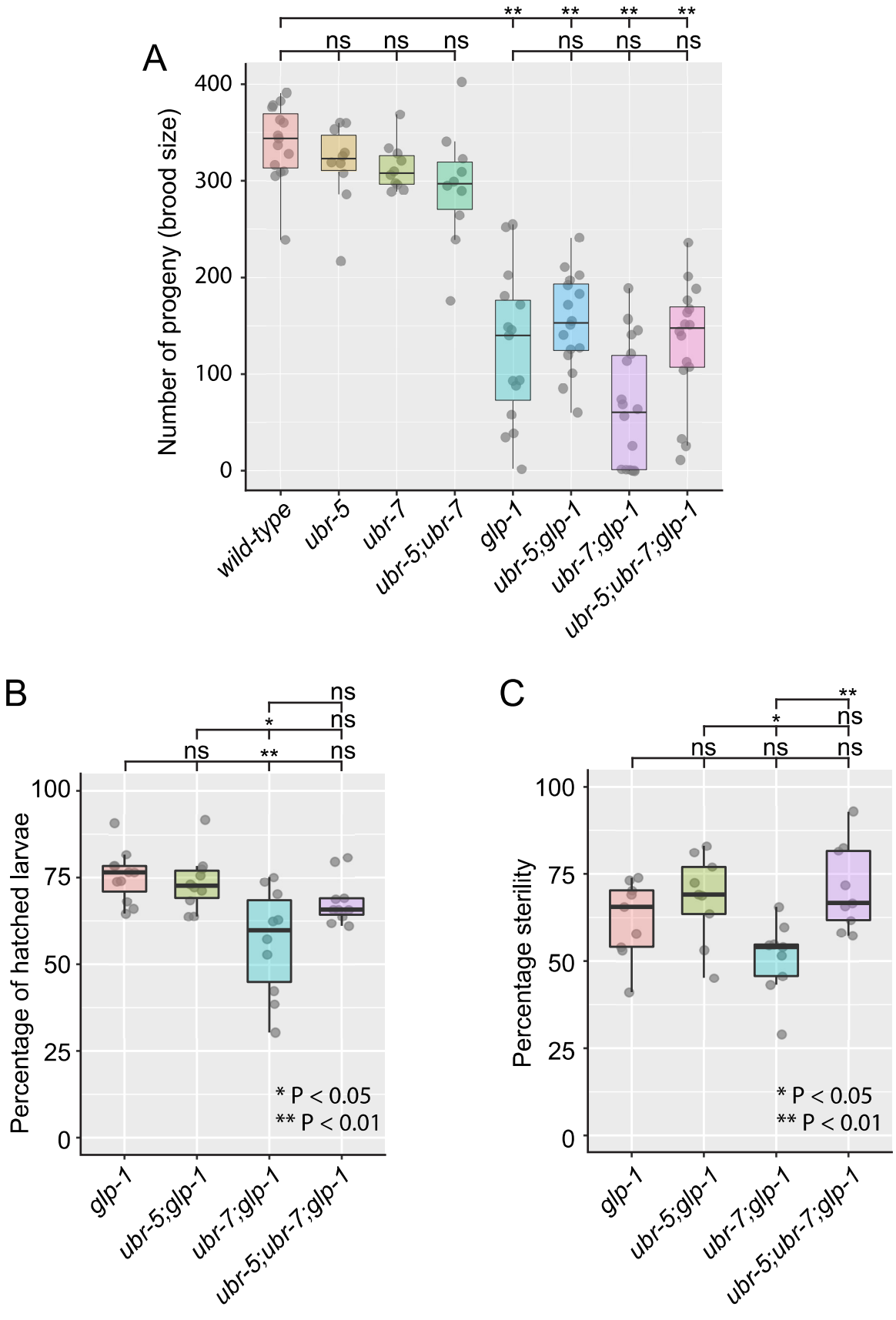
A
Number of progeny (brood size)
0
100
200
300
400
ns
ns
ns
**
**
**
**
ns
ns
ns
wild-type
ubr-5
ubr-7
ubr-5;ubr-7
glp-1
ubr-5;glp-1
ubr-7;glp-1
ubr-5;ubr-7;glp-1
B
Percentage of hatched larvae
0
25
50
75
100
ns
*
ns
ns
**
ns
* P < 0.05
** P < 0.01
glp-1
ubr-5;glp-1
ubr-7;glp-1
ubr-5;ubr-7;glp-1
C
Percentage sterility
0
25
50
75
100
**
*
ns
ns
ns
ns
* P < 0.05
** P < 0.01
glp-1
ubr-5;glp-1
ubr-7;glp-1
ubr-5;ubr-7;glp-1

Figure S2

Figure S2. Additional phenotypic analyses of the *ubr-5, ubr-7* and *ubr-5;ubr-7* mutants. A. Number of progeny (brood size) plotted for each of the indicated strains. The statistical significance (p value) was calculated by the Dunn's Kruskal-Wallis multiple comparisons with Holm-Sidak adjustment. B. Assessment of the proportion of larvae hatching from embryos for each of the indicated strains. Wild-type (N2), *ubr-5, ubr-7* and *ubr-5;ubr-7* animals exhibit ~100% larvae hatching (not shown). The statistical significance (p value) was calculated by the Dunn's Kruskal-Wallis multiple comparisons with Holm-Sidak adjustment. C. Graph of percentage sterility *for glp-1, ubr-5;glp-1, ubr-7;glp-1,* and *ubr-5;ubr-7;glp-1* mutant strains. Wild-type (N2), *ubr-5, ubr-7* and *ubr-5;ubr-7* animals exhibit ~0% sterility (not shown). The statistical significance (p value) was calculated with Tukey's honest significance test.

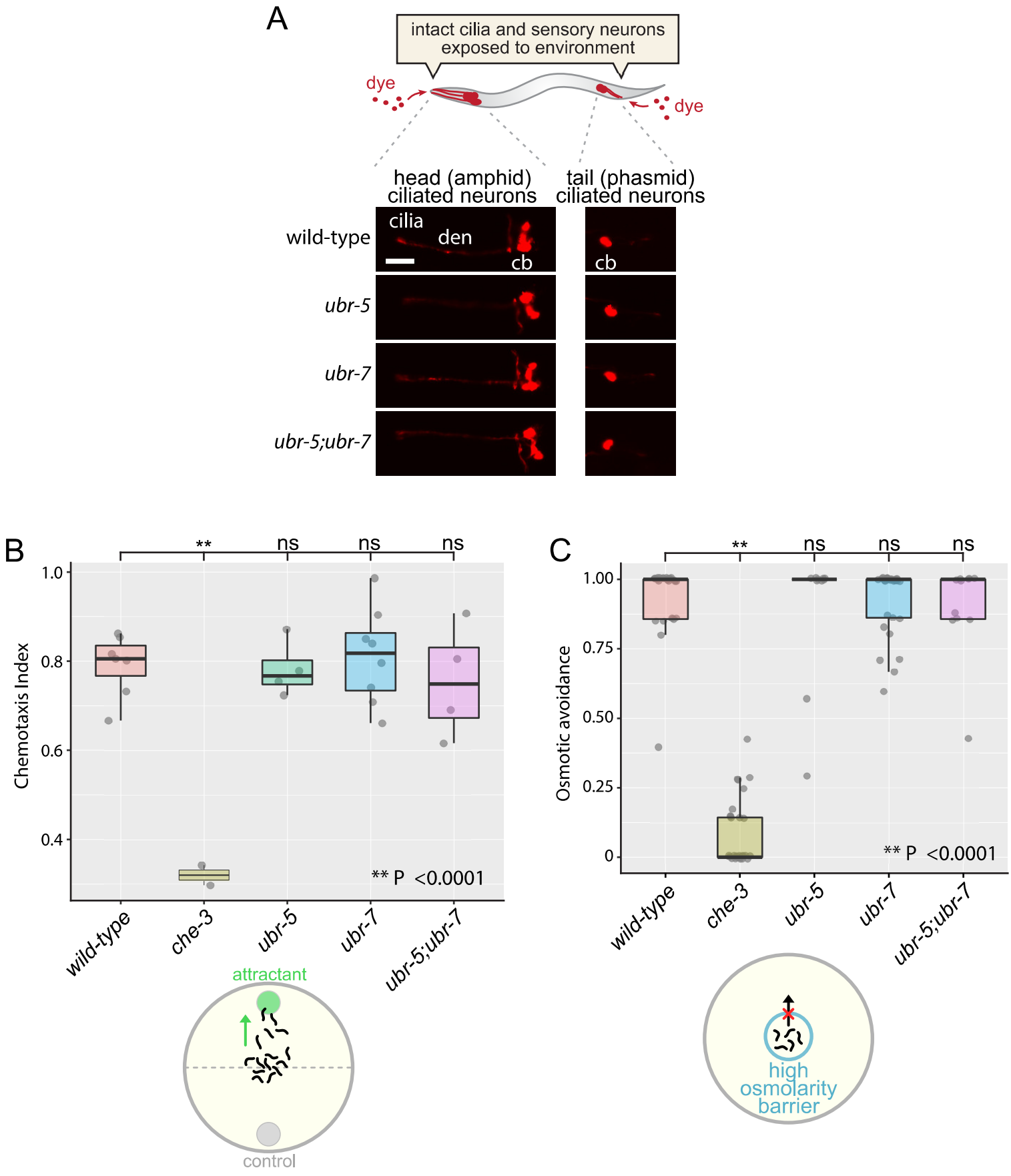
A
intact cilia and sensory neurons exposed to environment
dye
dye
head (amphid) ciliated neurons
tail (phasmid) ciliated neurons
wild-type
ubr-5
ubr-7
ubr-5;ubr-7
cilia
den
cb
cb
B
Chemotaxis Index
1.0
0.8
0.6
0.4
** ns ns ns
** P <0.0001
wild-type
che-3
ubr-5
ubr-7
ubr-5;ubr-7
attractant
control
C
Osmotic avoidance
1.00
0.75
0.50
0.25
0
** ns ns ns
** P <0.0001
wild-type
che-3
ubr-5
ubr-7
ubr-5;ubr-7
high osmolarity barrier

Figure S3

Figure S3. Assessment of ciliary integrity and function for the *ubr-5, ubr-7* and *ubr-5;ubr-7* mutants. A. Dye-filling assays to test for the structural integrity of cilia in various strains. In wild-type animals, red fluorescent dye enters through environmentally-exposed ciliary endings and permeate through sensory neurons. Cilia structure mutants do not take up dye. The *ubr-5*, *ubr-7* and *ubr-5;ubr-7* mutants take up dye normally. The statistical significance (p value) was calculated with Tukey's honest significance test. B. The *ubr-5, ubr-7* and *ubr-5;ubr-7* mutants are not defective in their cilium-dependent ability to chemotax towards an attractant, isoamyl alcohol. The control *che-3* mutant, impaired in cilium formation/function, displays a low chemotaxis index. The statistical significance (p value) was calculated by the Dunn's Kruskal-Wallis multiple comparisons with Holm-Sidak adjustment. C. The *ubr-5, ubr-7*, and *ubr-5;ubr-7* mutants can avoid crossing a 60% glycerol high-osmolarity ring, which requires intact ciliary function. The *che-3* cilia mutant fails to recognize this barrier and leaves the ring at a high frequency.

TABLE S1. Frequency of loss-of-function variants in gnomAD v2.1.1 (date of access: 2020-04-12).

Variant	Variant type	Allele count	Number of homozygotes
c.151-2A>G	Splice acceptor	1/251172	0
p.Asp146ArgfsTer6	Frameshift	1/247134	0
c.495+2T>C	Splice donor	1/250412	0
p.Pro172ArgfsTer35	Frameshift	2/202634	0
p.Glu173ArgfsTer14	Frameshift	2/203358	0
p.Arg188LeufsTer14	Frameshift	1/31388	0
p.Cys189PhefsTer14[b]	Frameshift	1/209572	0
c.602-2A>G	Splice acceptor	2/251292	0
p.Leu269ProfsTer6	Frameshift	1/250612	0
p.Lys346ArgfsTer12	Frameshift	1/251408	0
c.1124-5_1124-1delTTTAG	Splice acceptor	1/251238	0
c.1185+1G>T	Splice donor	1/251404	0
p.Glu400ThrfsTer46	Frameshift	1/251332	0
p.Gln404Ter	Stop gained	1/251410	0
p.Arg414LysfsTer23[a]	Frameshift	1/251428	0

[a]Low confidence pLoF due to the variant falling in the last filter position of the transcript. Variant annotation or quality dubious (as per gnomAD).[b]Same variant found in individual 1.

TABLE S2. *C. elegans* strains used in this study.

Strain	Genotype
N2 (Bristol)	wild-type
CB1124	*che-3(e1124)* [5X outcross]
EL34	*unc-32(e189)glp-1(q231ts) III*
EL619	*ubr-5(om2)*
GC833	*glp-1(ar202)*
MX2810	*ubr-7(gk3772)* [6x outcross]
MX2850	N2; nxEx2850[P*ubr-5*::gfp + P*osm-5*::*xbx-1*::tdTomato + *rol-6(su1006)*] strain 1
MX2859	N2; nxEx2859[P*ubr-7*::gfp + P*osm-5*::*xbx-1*::tdTomato + *rol-6(su1006)]* strain 1
MX2874	*unc-32(e189)glp-1(q231ts) III; ubr-7(gk3772)*
MX2891	*ubr-7(gk3772); glp-1(ar202)*
MX2892	*ubr-5(om2); glp-1(ar202)*
MX2895	N2; nxEx2895[*ubr-7*:gfp + P*osm-5*::*xbx-1*::tdTomato + *rol-6(su1006)*] strain 1
MX2912	*ubr-7(gk3772); ubr-5(om2)*
MX2921	*ubr-5(om2); ubr-7(gk3772); glp-1(ar202)*
MX2992	*ubr-5(om2); ubr-7(gk3772); glp-1(ar202);* nxEx2895[*ubr-7*::gfp + P*osm-5*::*xbx-1*::tdTomato + *rol-6(su1006)*]