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Supplemental Data

Dosage Changes of a Segment at 17p13.1 Leads to Intellectual Disability and Microcephaly due to Complex Genetic Interaction of Multiple Genes

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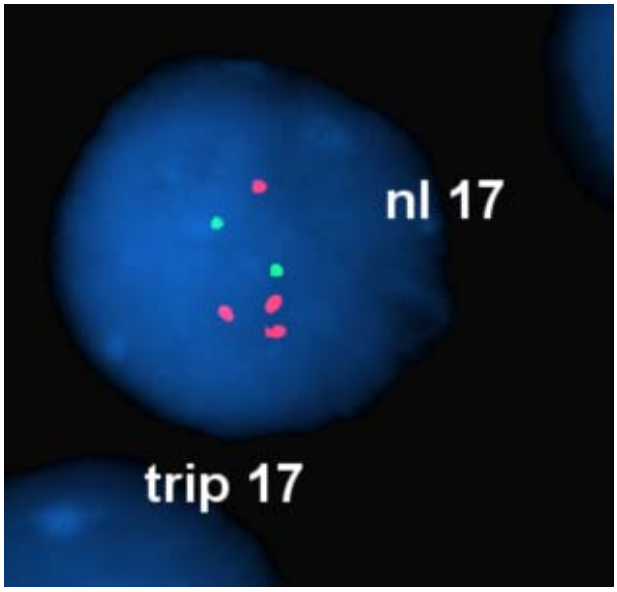
A



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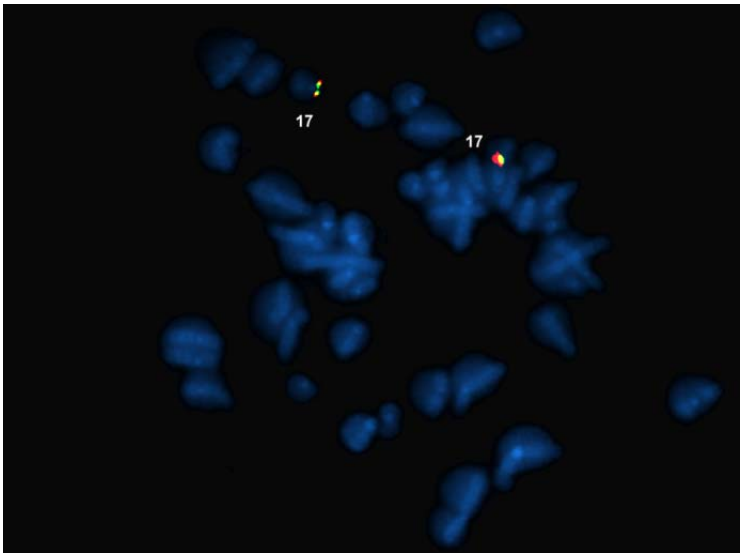
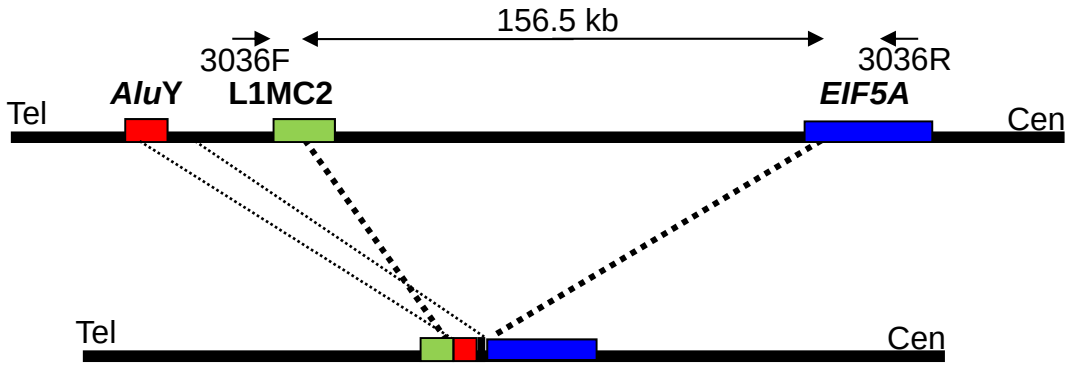


Figure S1 - Dual-color FISH supports presence of triplication in individual BAB3045
BAC clone RP11-599B13 (red) was used to target the triplicated segment and BAC RP11-601N13 (green) was used as control probe. **(A)** Interphase representative FISH **(B)** Metaphase representative FISH. Trip: triplication; nl: normal

A



L1MC2

interg (+) TCTATTAAAATGGCTAAAATCCAAAACATTGACAACACCAAATGTTGGCAAGGATGTGGAACAAGAG

BAB3036 TCTATTAAAATGGCTAAAATCCAACaCCCGCCACCACGCCCGGcTAATTTTTTGTATTTTTGGTAGA

interg (+) CTCCTGAGTAGCTGGGACTGGAGGCgCCCGCCACCACGCCCGGgTAATTTTTTGTATTTTTGGTAGA

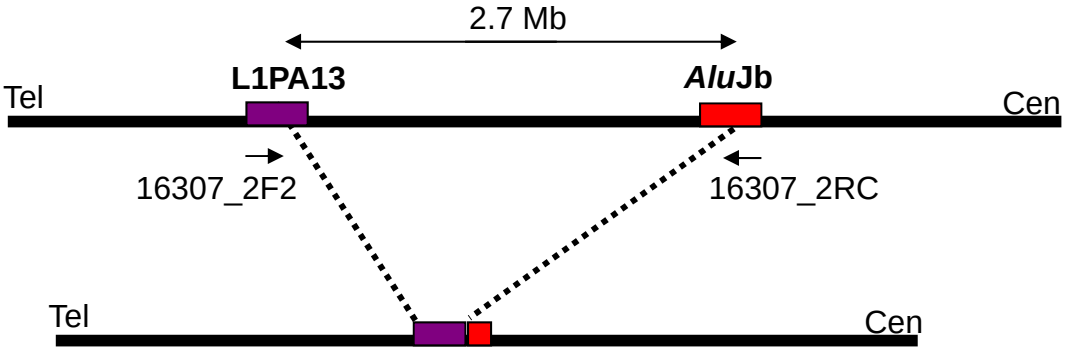
* *AluY* rs9894826

interg (+) GACAGGGTTTCACCATGTTAGCCAGGATGGTCTCGATCGTGTTTAAAATTTTTATACCAACTGGCAT

BAB3036 GACAGGGTTTCACCATGTTAGCCAGGATGGTCTCGATCGTGTTTAAACTAGCCTAGTATGATTTTCT

EIF5A (+) GTTGTAACTGGTTTGGGAGTTCCACGGTTTCGAAGTCCTTAACTCTACTAGCCTAGTATGATTTTCT

B



L1PA13

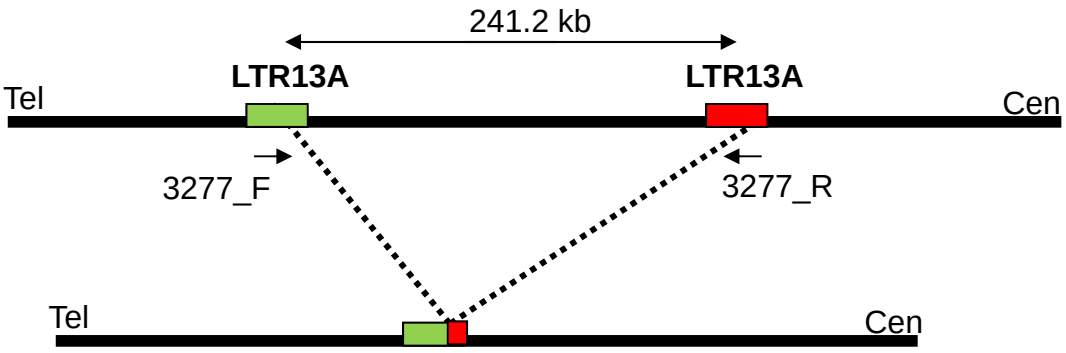
intron_ *NLRP1* (+) TAGGAATAATCAATATTGTTAAAATCGCCATACTACTCCAAGCAATTTACAGATTCAA

DECIPHER2009 TAGGAATAATCAATATTGTTAAAATCGCCATACTcctGCTGAGGCAGGAGTATCACTT

3'UTR_*SLC25A35* (+) GCCGGGCGTGGTGGCGGGAGCAGGAGCCACTCAAGAGGCTGAGGCAGGAGTATCACTT

*Alu*Jb

C



LTR13A

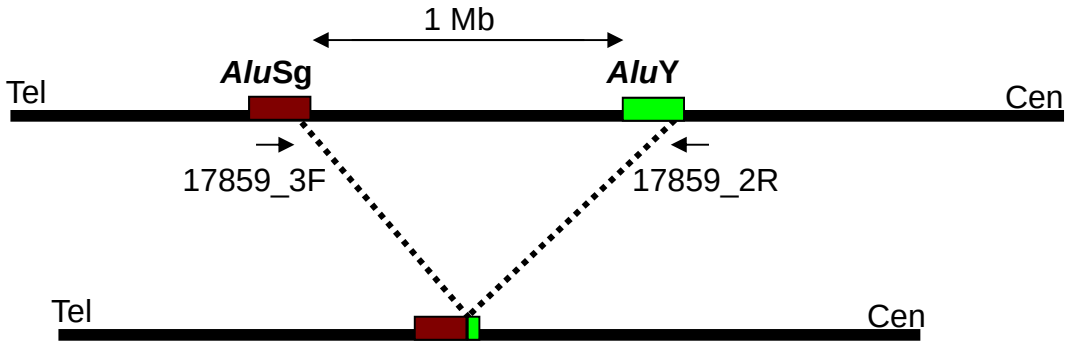
interg (+) CCTATACCtGCCGGTTATTCCTAGGTTATATTAGTAATGCAACAAgCAGTAATATTAAAAGCTAATG

BAB3277 CCTATACCtGCCGGTTATTCCTAGGTTATATTAGTAATGCAACAAaCAGTAATATTAAAAGCTAATG

interg (+) CCTATACCcGCCGGTTATTCCTAGGTTATATTAGTAATGCAACAAaCAGTAATATTAAAAGCTAATG

LTR13A

D



*Alu*Sg L2

interg (+) CCAAAGTGCTGGGATTACAGGTATGAGCCACCACGCCTGGTCAACCTTTTCTATTTCTCT

DECIPHER2173 CCAAAGTGCTGGGATTACAGGTATGAGCCACTGTACCCGGCTTTTTTTTTTTTTTTTTTT

intron_*DNAH2* (+) CCAAAGTGCTGGGATTACAGGTGTGAGCCACTGTACCCGGCTTTTTTTTTTTTTTTTTTT

AluY *AluSx*

Figure S2 - Breakpoint junction sequencing data for subjects with deletions spanning 17p13.1. (A) BAB3036 (B) DECIPHER2009 (C) BAB3277 (D) DECIPHER2173

Breakpoint junctions sequences are color-matched and aligned to respective proximal and distal genomic reference. Strand of alignment (+ or -) is indicated in parenthesis. Genomic sequences that are part of repetitive elements are underlined. Microhomology at the breakpoint is indicated by underlined black bold letters; mismatches (point mutations or insertions) between the breakpoint junction sequence and the reference genome are represented as black small letters; asterisks indicate location of point mutation. Deletion junction in subject BAB3036 presented with an insertion of part of an *Alu* element. No microhomology is immediately apparent at that junction but polymerase slippage between the short repeats present at both proximal and distal junctions (as indicated by blue arrows) would provide microhomology.

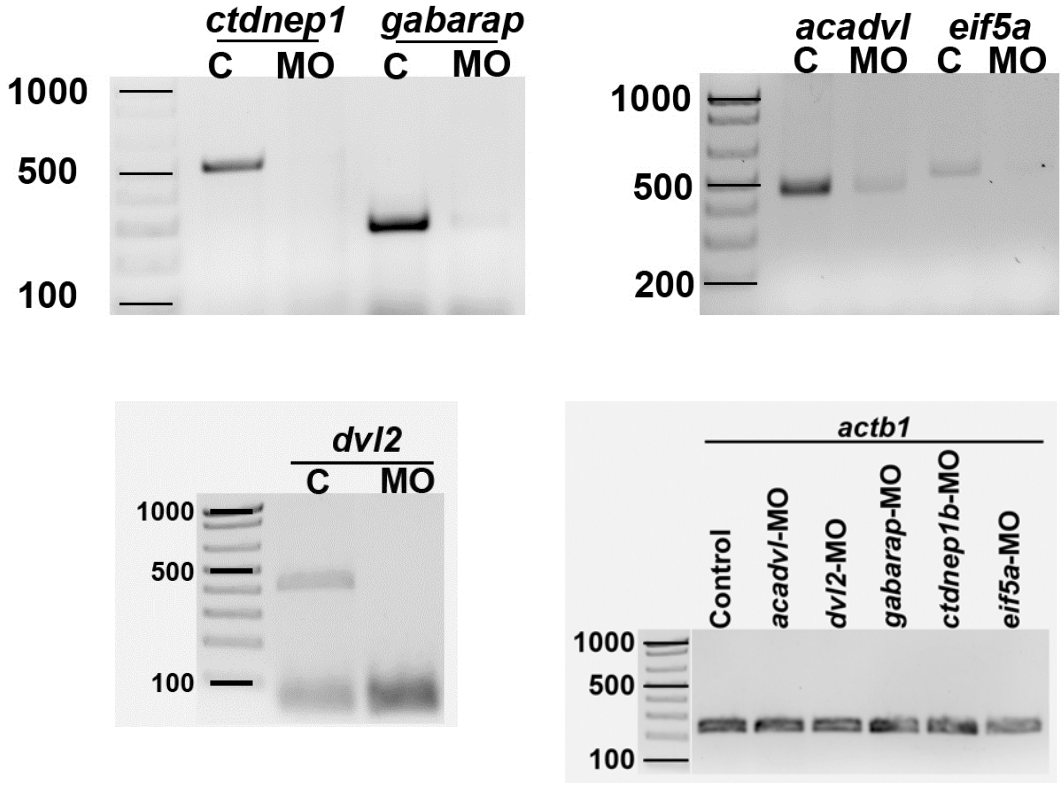
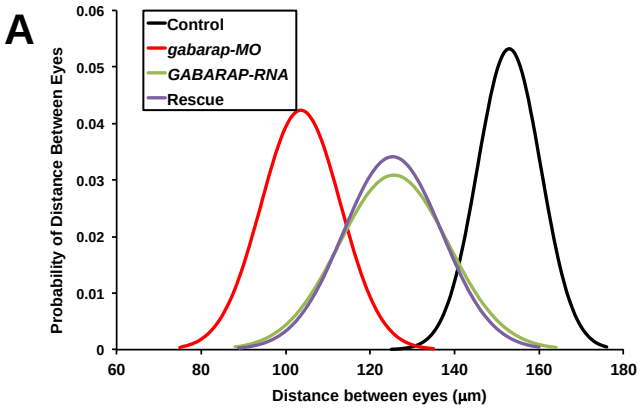
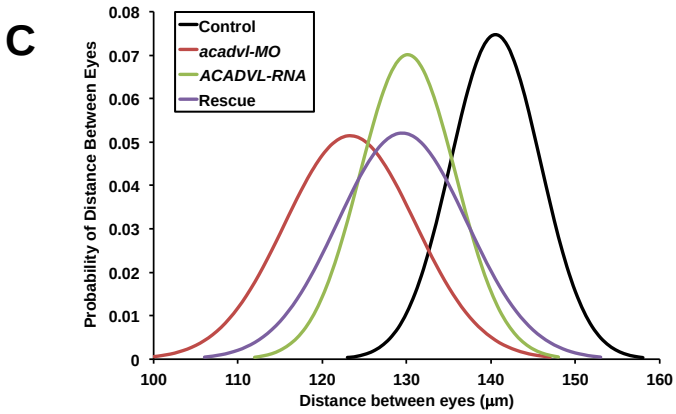


Figure S3 - RT-PCR to test the knockdown of the gene expression in zebrafish embryos on injection with morpholino

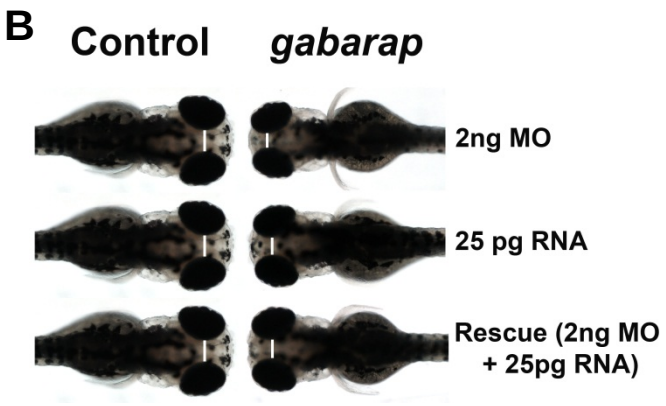
actb1: zebrafish beta actin 1 gene. Uninjected zebrafish embryos or injected with *ctdnep1b*, *gabarap*, *acadvl*, *eif5a*, and *dvl2* individually, were utilized for RNA extraction at 24dpf. The resultant cDNA from these RNA were tested for the knockdown of the gene expression of these respective genes in both control and morpholino injected RNA by RT-PCR. All the morpholinos result in either complete loss of the mRNA (*dvl2*, *ctdnep1,* and *eif5a*) or a partial loss (*acadvl* and *gabarap*). Bottom right panel shows the control PCR detecting the expression for β–actin.





	Z-score
***gabarap*-MO**	-6.573
***GABARAP*-RNA**	-3.640
***gabarap*-Rescue**	-3.677

	Z-score
***acadvl*-MO**	-3.235
***ACADVL*-RNA**	-1.953
***acadvl*-Rescue**	-2.075



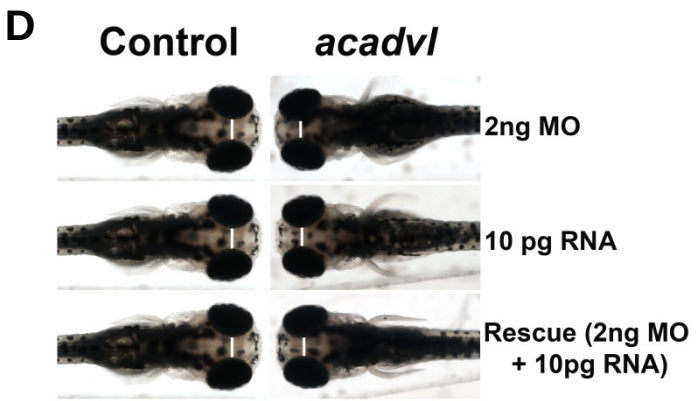
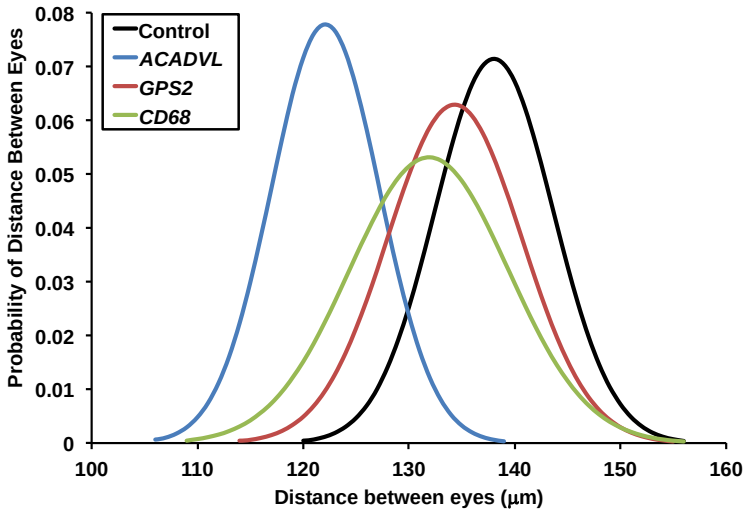


Figure S4 - Rescue of microcephaly induced by three severe loci. Microcephaly induced by morpholino against two of the three severe loci, namely, *gabarap* and *acadvl* were rescued by co-injecting their respective capped human mRNAs. Probability distribution curves of the distance between eyes in 4dpf zebrafish embryos are plotted for MO, human mRNA, and co-injection of morpholino and human mRNA for *GABARAP* **(A)** and *ACADVL* **(C)**. The co-injection of human mRNA with the morpholino rescued the microcephaly in both **(A)** and **(C)**. **(B, D)** Representative dorsal images of zebrafish embryos injected with the indicated morpholino and human mRNA scored for microcephaly at 4dpf. The white lines represent the distance calculated between eyes.



Human RNA	Z score
GPS2	-0.665
CD68	-1.099
ACADVL	-2.849

Figure S5 - Analysis of loci outside smallest region of overlap (SRO) for microcephaly in 4dpf zebrafish embryos

Probability distribution curves of the distance between eyes in 4dpf zebrafish embryos injected with 100pg of the indicated capped human mRNAs. Both *CD68* and *GPS2* mRNAs do not result in microcephaly, whereas, the injection of *ACADVL* as a positive control induced severe microcephaly.

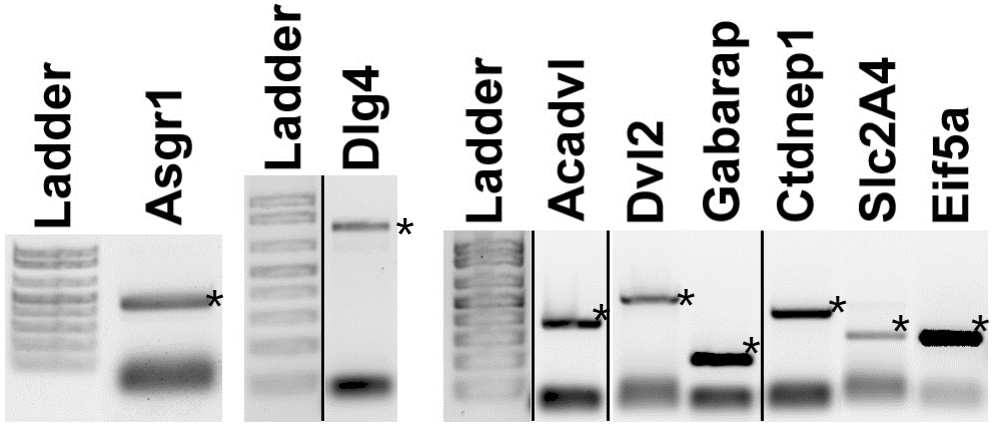


Figure S6 - RT-PCR analysis to test the expression of genes from SRO segment in Neuro2A cells prior to knockdown by shRNA with primers described in Supplementary Table 4

RNA extracted from Neuro2A cells was tested for the expression of nine genes in the SRO. Out of these, the expression of eight genes were detected by RT-PCR; *Cldn7* expression could not be detected in Neuro2A cells. * Indicates the gene-specific bands

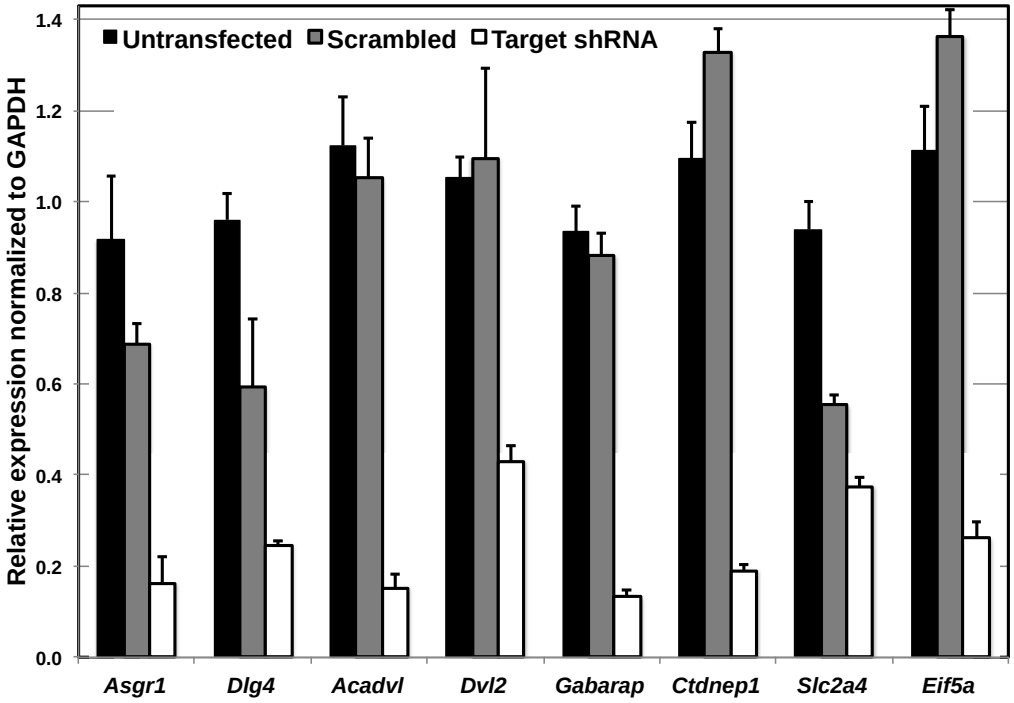


Figure S7 - Real-time PCR analyses to test the knockdown of indicated genes in Neuro2A cells

Neuro2A cells were transfected with a pool of four different siRNAs targeting *Asgr1* or non-targeting scrambled siRNA. The remaining seven genes were suppressed with a combination of five shRNAs in pLKO.1 vector for each gene and a non-targeting scrambled shRNA in pLKO.1 vector. RNA extracted from each condition shown was assayed for knockdown of the respective gene expression 48h post transfection. Bars represent the average fold expression and the error bars indicate the standard deviation from three experiments.

Table S1 - Primers used to amplify the breakpoint junctions by PCR

Sample	Primer	Primer sequence 5' - 3'
DECIPHER 2173	17859_2R	GGGCTCCACTGATTCTTCACTCT
	17859_3F	CTCACAGGCTGGGAATCTTGTTCT
DECIPHER 2009	16307_2RC	ACGGGATGGCAGAAAACTTC
	16307_2FC	ACAACCCACAGAATGGGAGA
BAB3036	3036_F	TGCCAGCCTACAGGATACTC
	3036_R	GCAGAGTTAAAGGAAAGTCAGCA
BAB3277	3277_F	TAGTTTGGCAAGCCTGTCCT
	3277_R	ACCAGAGGGGAGAAGCTCAG

Table S2 - Sequence of morpholino used for knockdown of the indicated genes in zebrafish

Human Gene	Zebrafish ortholog (Zv9)	Sequence of Morpholino	Target region
ACADVL	*acadvl* Chr7:21455329:21491712:1	ATGAAGCCAAGAGTCTCACCTCTGC	SB_exon3 (donor)
DVL2	*dvl2* Chr7:21417445:21453628	TGAGATTCACAGTAACGTACTGGAA	SB_exon3 (donor)
GABARAP	*gabarap* Chr7:72186770:72194682	TACGACACAAACCGACTTACAGGAA	SB_exon1 (donor)
CTDNEP1	*ctdnep1b* Chr7:72166393:72185168	TTATGAAGACTCACCGTTCGTATGT	SB_exon1 (donor)
CLDN7	*cldn7a* Chr7:23782703:23794544	ACAAACATACTGATACTCACTGTCC	SB_exon1 (donor)
EIF5A	*eif5A* Chr24:27277302:27282547	AACCCTATCCAAACATTACCTTTGC	SB_exon2 (donor)

Table S3 - List of RT-PCR primers to test the knockdown of gene expression by morpholino for the indicated zebrafish genes

Zebrafish Gene	Forward Primer	Reverse Primer
acadvl	CTGCAGAGGCTGTTCTGGACAAG	CAATGTTCTCTCCAGTAGCCAGCT
dvl2	CTTCAACGGAAGAGTTGTCTCCTG	GCTGCTGAACCTGCTCATGGTAT
gabarap	CTTTGAGAAGAGGCGATCAGAG	GTGATGTTCCTGGTACAACTGGCC
ctdnep1	CTCACGGACTCTGGTGTTATG	CCAGCGTACAGTGCTGTCTGTAG
eif5a	CGCGGTGACTTGACTGAAATA	GCTGAAAGCACAGTCACCAACA

Table S4 - Primer pairs to test the expression of the indicated genes in Neuro2A cells

Mouse Gene	Forward Primer	Reverse Primer	Product Size (bp)
Asgr1	CATCCCAAAATTCCCAACTCC	CTCCAATTCTGGAAGCCTGTC	392
Dlg4	GTGACAACCAAGAAATACCGC	ATATGAGGTTGTGATGTCTGGG	773
Acadvl	CTCTGCAAGGCTGTATGGAC	CCTCAATGCACCAGCTATCA	410
Dvl2	CGCAACATGGAGAAGTACAAC	CCACATCGGAGCCTAGAA	546
Gabarap	GTCCCGGTGATAGTGGAA	CATGGTGTTCCTGGTACAGC	230
Ctdnep1	CTGCCCTTATCTCCTTTGTCC	GATGGCATTGTCTGGGTG	472
Slc2a4	ACAGAAGGTGATTGAACAGAG	TGATGTTAGCCCTGAGTAGG	316
Eif5a	TTCGCGCGAGTTGGAATC	TGCCAATCAGCTGGAAGTC	313

Table S5 - Sequence of siRNAs targeting *Asgr1* and shRNAs for the remaining genes in the SRO cloned into pLKO.1 vector used for suppression of the indicated genes in Neuro2A cells

SYMBOL	GENE_DESCRIPTION	OLIGO SEQUENCE	REFSEQ_ID
Asgr1	asialoglycoprotein receptor 1	UUAAAGGACCGGUAAAGAA	NM_009714
Asgr1	asialoglycoprotein receptor 1	GGGCAAUGGCUCUGAAAGG	NM_009714
Asgr1	asialoglycoprotein receptor 1	GGAUAAGGCUAAUUAGGAA	NM_009714
Asgr1	asialoglycoprotein receptor 1	UGAAGUUAGUGGAGUCGAA	NM_009714
Dlg4	discs, large homolog 4 (Drosophila)	ACACGTCCTAAGCGGGAATAT	NM_007864
Dlg4	discs, large homolog 4 (Drosophila)	GATCAGTCATAGCAGCTACTT	NM_007864
Dlg4	discs, large homolog 4 (Drosophila)	GATCAGTCATAGCAGCTACTT	NM_007864
Dlg4	discs, large homolog 4 (Drosophila)	ACACGTCCTAAGCGGGAATAT	NM_007864
Dlg4	discs, large homolog 4 (Drosophila)	CCGTTTGAGTTCTCCTTTATT	NM_007864
Dvl2	dishevelled 2, dsh homolog (Drosophila)	CGAGCTTTCTTCGTACACCTA	NM_007888
Dvl2	dishevelled 2, dsh homolog (Drosophila)	GCTGCCTTTGTTACTCTATTT	NM_007888
Dvl2	dishevelled 2, dsh homolog (Drosophila)	CTGTGAGAGTTACCTAGTTAA	NM_007888
Dvl2	dishevelled 2, dsh homolog (Drosophila)	TAGGCGAGACGAAGGTGATTT	NM_007888
Dvl2	dishevelled 2, dsh homolog (Drosophila)	ACCCATCTTGAGGCCACATTG	NM_007888
Slc2a4	solute carrier family 2 (facilitated glucose transporter), member 4	CCAGTATGTTGCGGATCGTAT	NM_009204
Slc2a4	solute carrier family 2 (facilitated glucose transporter), member 4	GCTCCCTTCAGTTTGGCTATA	NM_009204
Slc2a4	solute carrier family 2 (facilitated glucose transporter), member 4	TGGCATCATTTCTCAATGGTT	NM_009204
Slc2a4	solute carrier family 2 (facilitated glucose transporter), member 4	CTTACGTCTTCCTTCTATTTG	NM_009204
Slc2a4	solute carrier family 2 (facilitated glucose transporter), member 4	GAAAGCTTCTGACCAACTAAG	NM_009204
Acadvl	acyl-Coenzyme A dehydrogenase, very long chain	GCGGTTGATCATGCTACTAAT	NM_017366
Acadvl	acyl-Coenzyme A dehydrogenase, very long chain	CGGATGGCTATTCTGCAGTAT	NM_017366
Acadvl	acyl-Coenzyme A dehydrogenase, very long chain	GCGATCTACTACTGTGCTTCA	NM_017366
Acadvl	acyl-Coenzyme A dehydrogenase, very long chain	CGGTTCTTTGAGGAAGTGAAT	NM_017366
Acadvl	acyl-Coenzyme A dehydrogenase, very long chain	CTTTGCCAAGACGCCAATTAA	NM_017366
Gabarap	gamma-aminobutyric acid receptor associated protein	CGAATTCATCTCCGTGCTGAA	NM_019749
Gabarap	gamma-aminobutyric acid receptor associated protein	CGAATTCATCTCCGTGCTGAA	NM_019749
Gabarap	gamma-aminobutyric acid receptor associated protein	TCTTTGTCAACAATGTCATTC	NM_019749
Gabarap	gamma-aminobutyric acid receptor associated protein	ACCATGAAGAAGACTTCTTTC	NM_019749
Gabarap	gamma-aminobutyric acid receptor associated protein	CTCTGTCACATCCGATGATTG	NM_019749
Ctdnep1	CTD nuclear envelope phosphatase 1	GCCAATTCAACTTTGTTGTGA	NM_026017
Dullard	Dullard homolog (Xenopus laevis)	CCGAAACCTTCACCAACATAG	NM_026017
Dullard	Dullard homolog (Xenopus laevis)	AGGCAGATCCGCACGGTAATT	NM_026017
Ctdnep1	CTD nuclear envelope phosphatase 1	CAGTATCAGACTGTTCGATAT	NM_026017
Dullard	Dullard homolog (Xenopus laevis)	TCAGACTGTTCGATATGATAT	NM_026017
Eif5a	eukaryotic translation initiation factor 5A	CCAATGCAGTGCTCAGCATTA	NM_181582
Eif5a	eukaryotic translation initiation factor 5A	CGTCGAGATGTCTACTTCGAA	NM_181582
Eif5a	eukaryotic translation initiation factor 5A	CCAACATCAAACGGAATGACT	NM_181582
Eif5a	eukaryotic translation initiation factor 5A	GAGCAGAAGTATGACTGTGGA	NM_181582
Eif5a	eukaryotic translation initiation factor 5A	CCAATGCAGTGCTCAGCATTA	NM_181582

Table S6 - Primer pairs used in real-time PCR analyses to test the knockdown of indicated genes in Neuro2A cells

Mouse Gene	Forward Primer	Reverse Primer
Asgr1	ACCCAGGGAAGTAGTGTGG	TCCTTTCAGAGCCATTGCC
Dlg4	CACGAAGCTGGAGCAGGAG	GGCCTGAGAGGTCTTCGATG
Acadvl	GGATTGTCAACGAGCAGTTCCT	CCTCAATGCACCAGCTATCA
Dvl2	TTTCAAGAGCGTTTTGCAGC	ACACAAGCCAGGAGACAAC
Gabarap	CCTGGTGCCTTCTGATCTTAC	CATGGTGTTCCTGGTACAGC
Ctdnep1	TAGAAGTGGTAAGCCAGTGGT	GTGCAGTGCTGTCTGTAGT
Slc2a4	CTGTGCCATCTTGATGACCGTG	GTTGGAGAAACCAGCGACAGC
Eif5a	CATGCCAAGGTCCATCTGG	TGCCAATCAGCTGGAAGTC