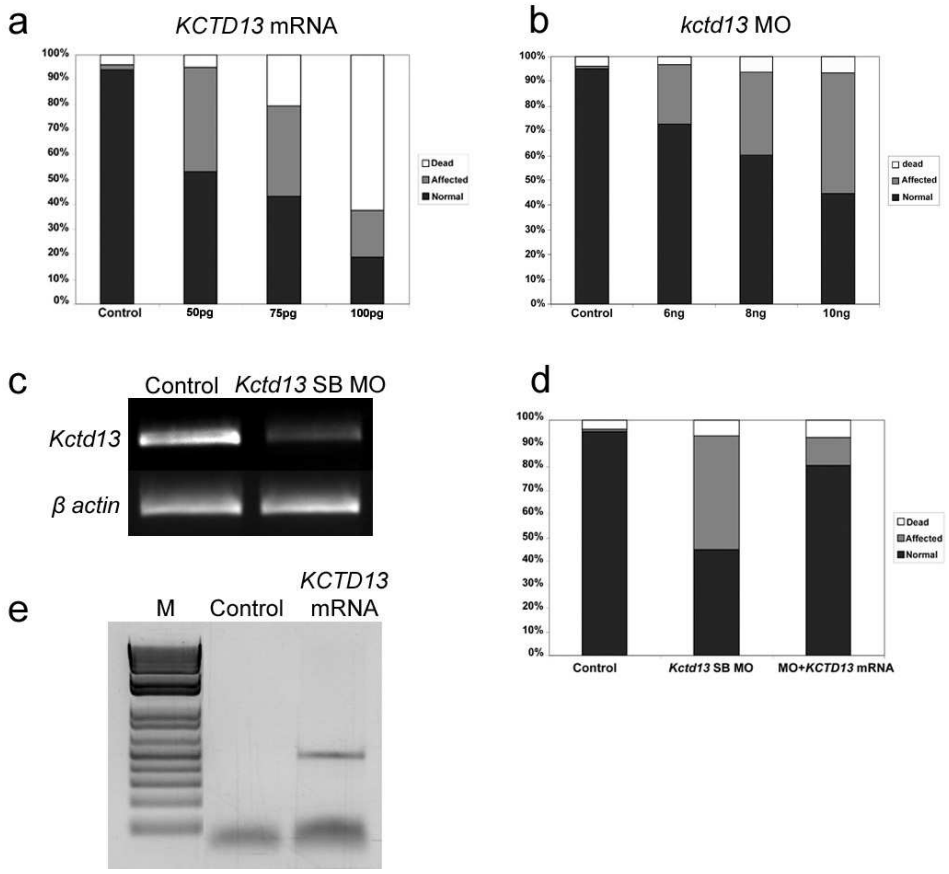
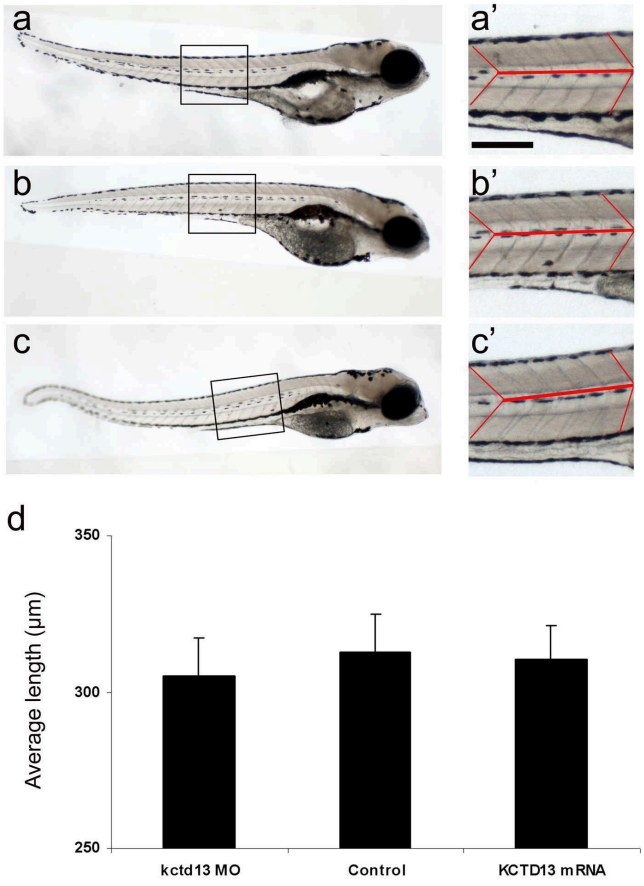
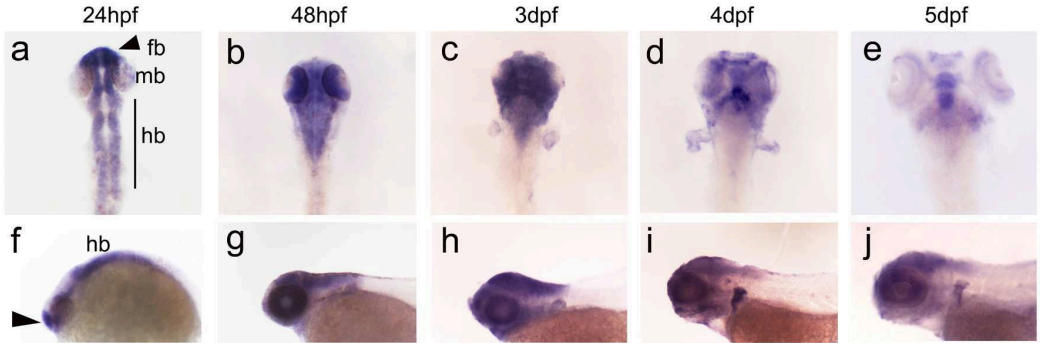


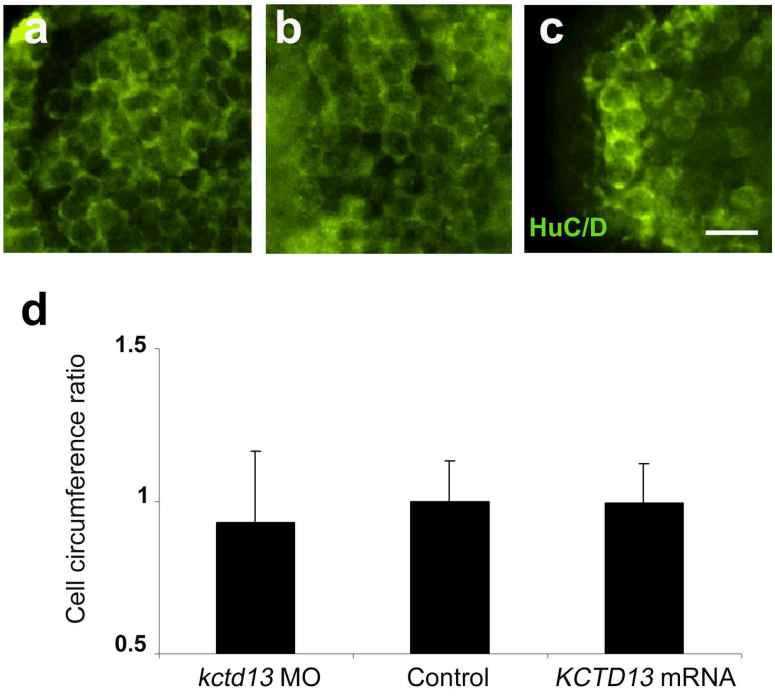
Suppl. Figure 1: Suppression and over-expression of KCTD13 gene in vivo produce head size phenotype. (a) Dose-response curve of KCTD13 mRNA. Wildtype zebrafish embryos were injected with increasing concentrations of KCTD13 mRNA (50, 75 and 100 pg) and were scored at 4.5 dpf for head size. (b) Dose-response curve of Kctd13 splice-blocking morpholino (SB-MO). Wildtype zebrafish embryos were injected at two-cell stage with increasing concentrations of SB-MO (6, 8 and 10 ng). Scoring was conducted as in (a). (c) Injection of Kctd13 SB-MO results in partial suppression of message as shown by PCR amplification of cDNA reverse transcribed from extracted total mRNA. β-actin was used as a control. (d) Reciprocal rescue of phenotypes: embryos injected with both 10 ng of SB-MO and with 50 pg of human KCTD13 mRNA rescue head size phenotypes seen in each of RNA alone and MO-alone injections. Control denotes embryos from the same clutch injected with a control morpholino at two-cell stage and scored as in (a). (e) RT-PCR using human KCTD13 primers on RNA extracted from control and KCTD13 mRNA injected-embryos at 4.25 dpf. M, marker (1kb plus ladder).



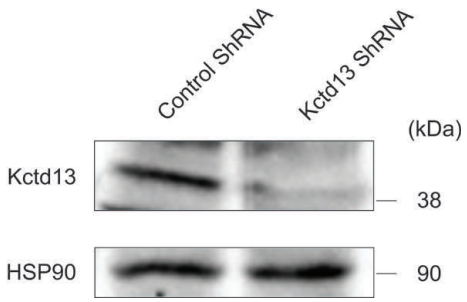
Suppl. Figure 2: Analysis of somitic length in KCTD13 morphants and overexpressants at 4.5 dpf. (a-c) Representative side views of embryos injected with kctd13 MO, control, injected with KCTD13 mRNA. (a'-c') Higher magnifications from boxes indicated in (a-c) showing five somites, the somitic chevrons are highlighted (red lines) and the length between five consecutive chevrons was measured for the three classes of embryos. (d) Bar graph represents the average length between five consecutive chevrons for 50 embryos injected with kctd13 MO, control, and injected with KCTD13 mRNA, scored blind to injection cocktail. Data are shown as mean ±SD. Scale bar, 160 □m.



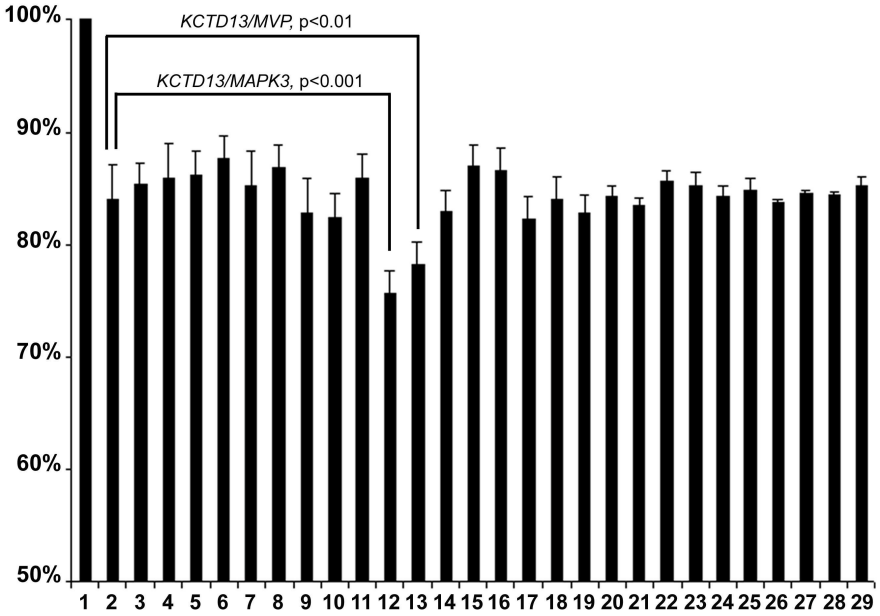
Suppl. Figure 3: Expression profile of *kctd13* during zebrafish development from 24 hpf to 5 dpf. (a-e) Dorsal flat-mount views and (f-j) side views of kctd13 expression in control embryos at 24 hpf, 48 hpf, 3 dpf, 4 dpf, and 5 dpf respectively. Black arrowheads (a, f) show robust and specific expression of kctd13 at 24 hpf in the anterior forebrain. Fb, Forebrain; mb, midbrain; hb, hindbrain.



Suppl. Figure 4: Analysis of cell circumference in the telencephalon of MO-injected, control and mRNA-injected embryos at 4.5 dpf. (a, b, c) Representative photographs of HuC/D positive cells in the telencephalon of 4.5 dpf embryos injected with MO, control, and injected with KCTD13 mRNA. Cell circumference of 50 cells was measured per class of embryos. (d) Graph shows the cell circumference ratio between control and either MO- or mRNA-injected embryos. Cell circumference for control is defined as 1. Data are shown as mean ±SD. Scale bar, 10 □m.

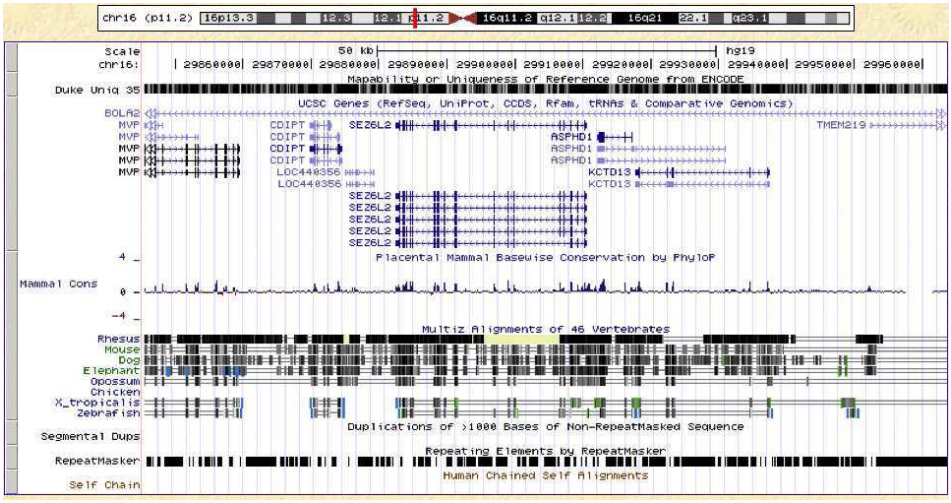


Suppl. Figure 5: Evaluation of the Kctd13 shRNA. Transfection of Neuro-2a cells with either control or Kctd13 shRNA plasmids followed by western blotting of whole cell lysates shows 70% knockdown in Kctd13 protein level. HSP90 was used as loading control.



Suppl. Figure 6: Pairwise injections of KCTD13 mRNA and the 28 other mRNAs encoded by the 16p11.2 CNV genes. Epistasis effect is observed in the cocktails of injections of KCTD13/MAPK3 (column 12) and KCTD13/MVP (column 13). The expressivity of the phenotype is increased significantly from 18% for KCTD13 alone (column 2) to 24% ($p<0.001$) and 22% ($p<0.1$) respectively. Mock injection is show in column 1. See Table S2 for details of the pairwise injections and p-values.

a


chr16 (p11.2)
Scale
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Mammal Cons
Multiz Alignments of 46 Vertebrates
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Mouse
Dog
Elephant
Opossum
Chicken
X_tropicalis
Zebrafish
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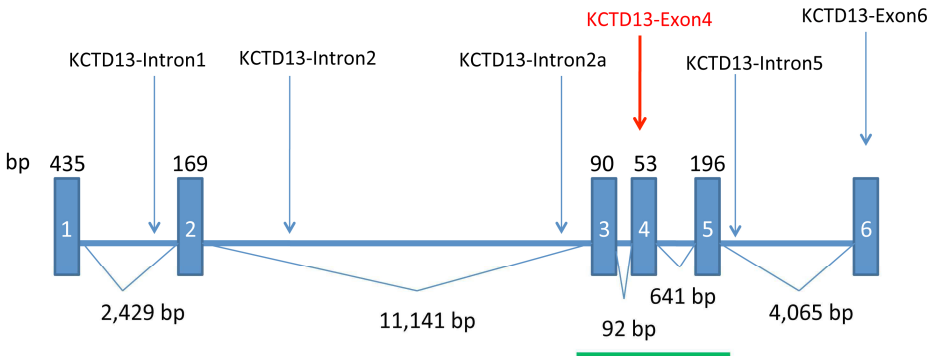
Duplications of >1000 Bases of Non-RepeatMasked Sequence
RepeatMasker
Repeating Elements by RepeatMasker
Self Chain
Human Chained Self Alignments

b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KCTD13-Exon6
KCTD13-Intron1
KCTD13-Intron2
KCTD13-Intron2a
KCTD13-Intron5
bp
435
169
90
53
196
1
2
3
4
5
6
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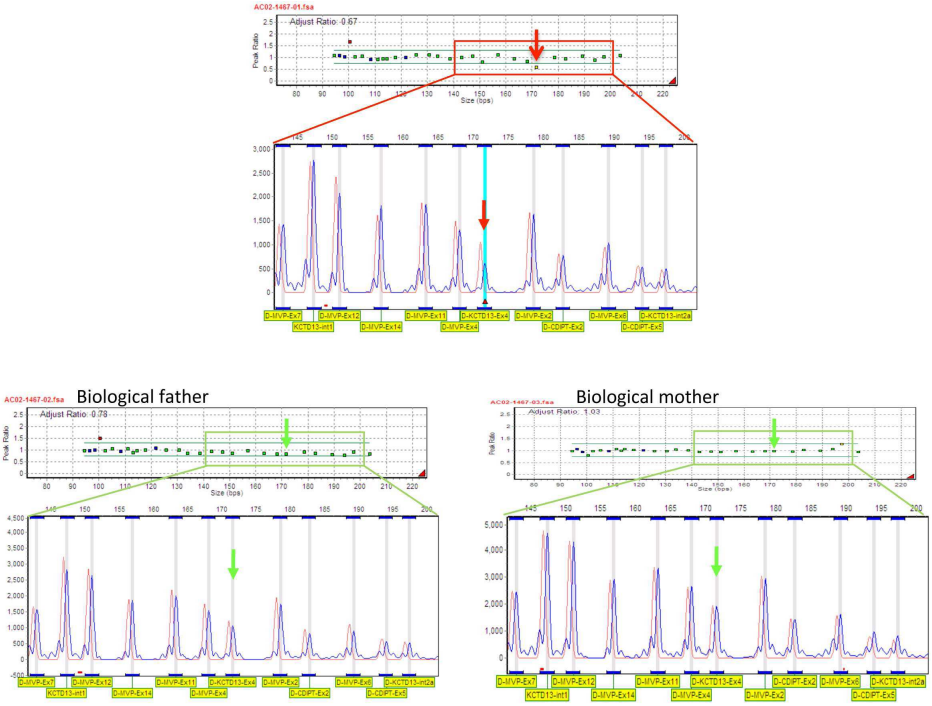
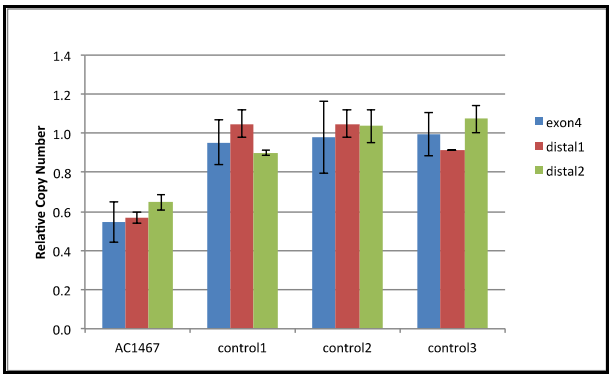
11,141 bp
641 bp
92 bp
4,065 bp

c


Biological father
Biological mother

d


Relative Copy Number
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1.2
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0.2
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AC1467
control1
control2
control3
exon4
distal1
distal2

Suppl. Figure 7: Analyses of a structural rearrangement altering *KCTD13* in an autism patient. (a) The putative restricted 118 kb critical region within the full 593 kb 16p11.2 microdeletion syndrome region was evaluated for small dosage changes by MLPA (Table S3), revealing a single probe alteration spanning exon 4 of *KCTD13*. (b) Genomic organization of KCTD13 with all exons, introns, and localization of six MLPA probes tested. The deleted probe is highlighted in red (exon 4). All other probes were intact, narrowing the maximum potentially deleted region to exons 3, 4, and 5 (underlined in green). (c) MLPA analyses in the proband (top) and both biological parents (bottom) reveal a single deleted probe in the proband that is diploid in both parents, indicating a de novo rearrangement. A subset of the intensity data is provided in the bottom panel. One probe is consistently duplicated in both the father and proband, likely representing either a common duplication inherited from the father or an artifact introduced by polymorphism in the MLPA probe. Follow-up custom tiling of the 16p11.2 region using Nimblegen aCGH platform and analysis revealed an atypical, distal microdeletion in the proband and mother in the previously described 16p11.2 region (Daniella et al., 2011). The deletion in the mother matched previous reports of the deleted region spanning approximately 28.72 Mb – 28.96 Mb of chromosome 16 (hg18). However, upon transmission to the child there was an apparent expansion of the breakpoints into the highly complex region of segmental duplications flanking this atypical deletion region (proband deletion region ~28.66 Mb – 29.02 Mb). This complex rearrangement of multiple including co-occuring inherited and de novo structural rearrangements of chromosome 16p11.2 warrants extensive additional investigation. (d) qPCR of both the KCTD13 region and the distal deletion region (two primer sets) in the proband and three control DNA samples ssc00129, ssc00251 and ssc00240, confirming dosage alteration in the proband in both regions evaluated.