| | aldoaa | asphd1 | c16orf53 | cdipt | coro1a | doc2a | fam57ba | gdpd3 | hirip3 | ino80e | kctd13 | kif22 | mapk3 | maz | ppp4ca | sez6l2 | taok2a | taok2b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *ypel3* | no | no | no | no | no | no | no | no | no | no | no | no | no | ND | no | no | no | no |
| *taok2b* | no | no | no | no | no | no | no | no | no | no | no | yes | no | no | no | yes | no | |
| *taok2a* | no | no | no | no | no | no | no | no | no | no | no | no | no | no | no | no | | |
| *sez6l2* | no | no | no | no | no | no | no | yes | no | no | no | no | yes | ND | no | | | |
| *ppp4ca* | no | yes | no | no | no | no | no | no | no | no | yes | no | no | ND | | | | |
| *maz* | no | ND | no | no | ND | no | no | no | ND | ND | no | ND | ND | | | | | |
| *mapk3* | no | no | no | no | no | no | no | no | no | no | no | no | | | | | | |
| *kif22* | no | no | no | no | no | yes | no | no | yes | yes | no | | | | | | | |
| *kctd13* | no | no | no | yes | no | no | yes | no | no | no | | | | | | | | |
| *ino80e* | no | yes | no | no | no | no | no | no | no | | | | | | | | | |
| *hirip3* | no | yes | no | no | no | no | yes | no | | | | | | | | | | |
| *gdpd3* | no | no | no | no | no | no | no | | | | | | | | | | | |
| *fam57ba* | yes | no | no | no | no | yes | | | | | | | | | | | | |
| *doc2a* | no | no | no | no | no | | | | | | | | | | | | | |
| *coro1a* | no | no | no | no | | | | | | | | | | | | | | |
| *cdipt* | no | no | no | | | | | | | | | | | | | | | |
| *c16orf53* | no | no | | | | | | | | | | | | | | | | |
| *asphd1* | no | | | | | | | | | | | | | | | | | |

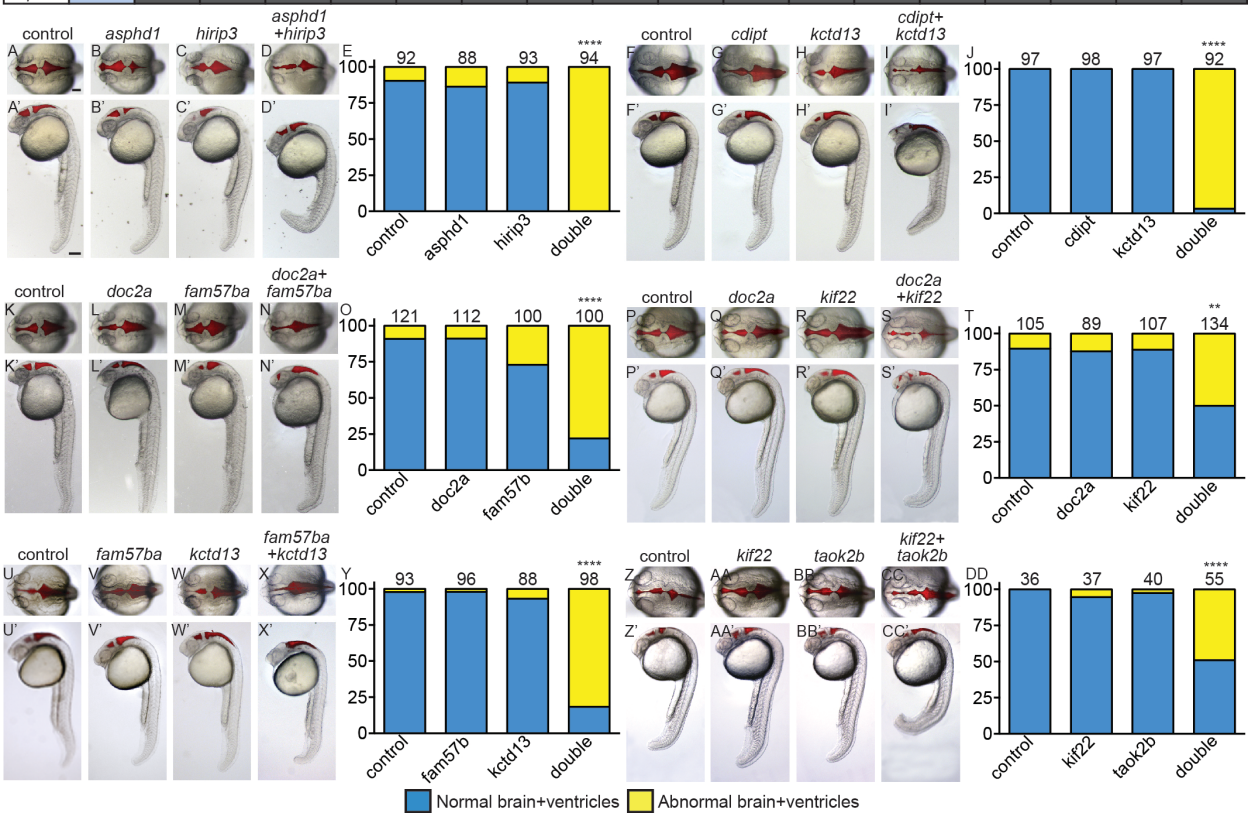


**Fig. S1. The majority of pairwise combinations of 16p11.2 homologs show no effect on brain and ventricle morphology.** Yellow "yes" is a positively interacting pair, "no" is for two genes that do not interact. ND: not determined, because *maz* interacts with p53, and some genes require *p53* MO co-injection. **Examples of interacting pairs affecting early brain development.** Six of sixteen interacting pairs are shown with dorsal (non-prime letters) and lateral (prime letters). Ventricular space was injected with Texas Red dextran to help visualize morphology. **A, F, K, P, U, Z**) control MO injected. **B, G, L, Q, V, AA**) Single LOF for Gene A, balanced with control MO. **C, H, M, R, W, BB**) Single LOF for Gene B, balanced with control MO. **D, I, N, S, X, CC**) Double LOF for Genes A and B together. **E, J, O, T, Y, DD**) Percent of embryos shown on y-axis with normal (blue bars) and abnormal (yellow bars) brain and ventricle shape was quantified, with the total number of embryos for each treatment above the bars. **p<1x10$^{-11}$, ****p<1X10$^{-15}$. Scale bars=100 um.

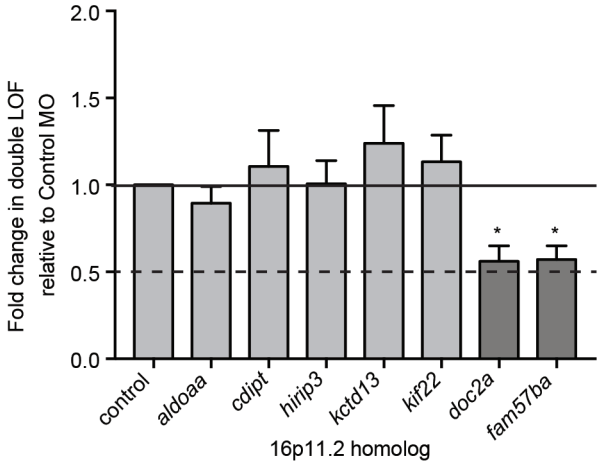


**Fig. S2. Other interacting 16p11.2 homolog expression not affected by *doc2a* and *fam57ba* double LOF.** qPCR was done to determine gene expression levels relative to control MO-injected embryos. 16p11.2 homologs selected for expression analysis interacted with either *doc2a* or *fam57ba* in the primary interaction screen. No significant changes from control expression levels (solid line) were seen for *aldoaa, cdipt, hirip3, kctd13,* or *kif22.* As a control, expression levels for *doc2a* and *fam57ba* were also determined, and were around 50% LOF (dashed line) as expected for the MO doses used. *$p<0.05$

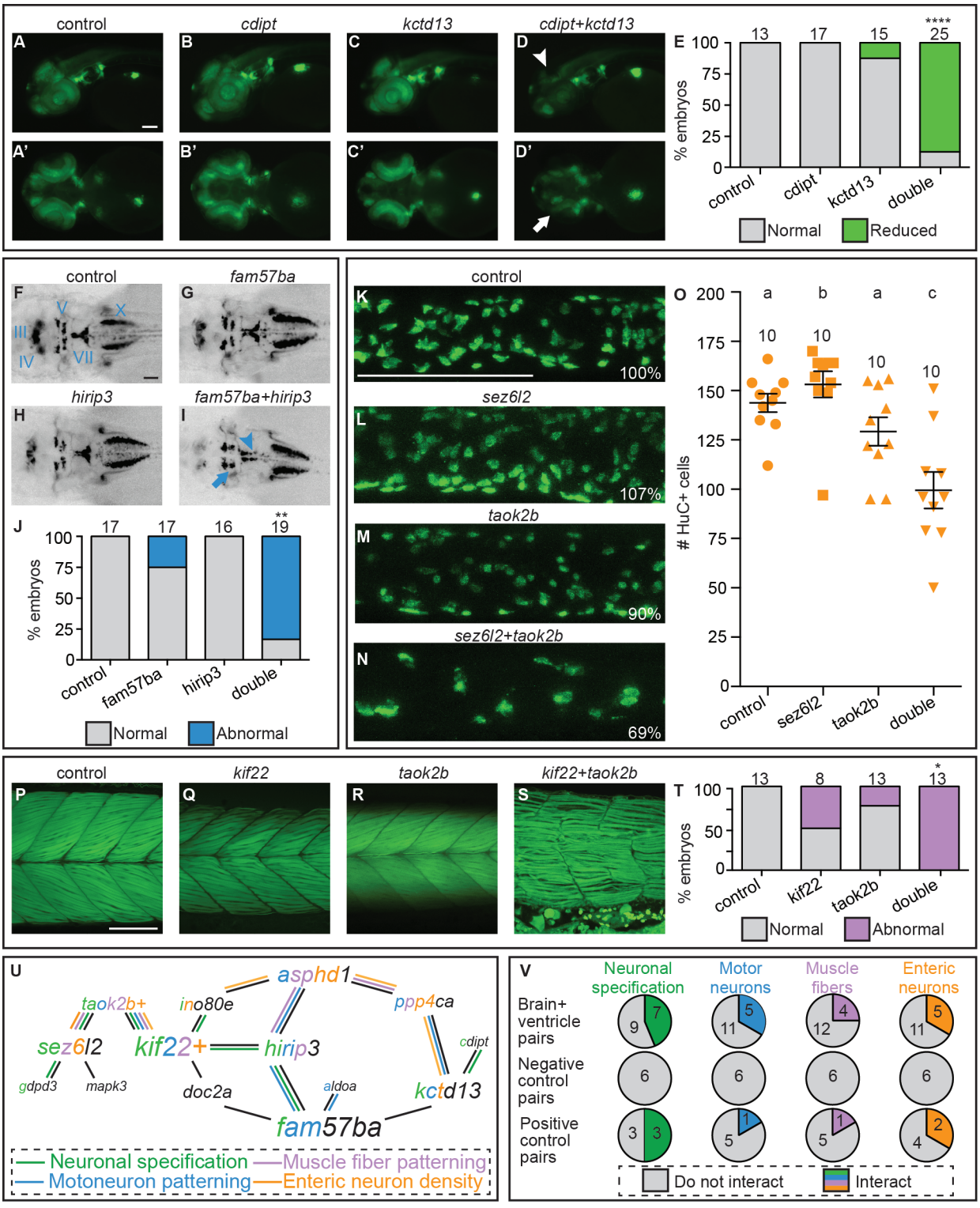


**Fig. S3. Interacting pairs affect multiple phenotypes in secondary screen.** One example of an interacting pair for each of the secondary screen assays. The rest of the interacting pairs are not shown. *To evaluate neuronal specification, single and double LOF was induced by injection of MOs into a*

*transgenic NeuroD:GFP line.* **A-C**) Control and single *cdipt* and *kctd13* LOF lateral views exhibit normal levels of *NeuroD:GFP* expression. **A'-C'**) Ventral views of same embryos. **D**) Lateral view of double LOF embryo shows reduced expression in the optic tectum (arrowhead). **D'**) Ventral view of double LOF embryo shows reduced expression in the retinal ganglion cells (arrow). **E**) Percent of embryos with normal (gray bars) and reduced (green bars) expression is quantified with total number of embryos scored above the bars. *We analyzed cranial motor neuron patterning using the transgenic islet1:GFP line.* **F-H**) Dorsal views of 2 dpf control and *fam57ba* and *hirip3* single LOF fish exhibit normal patterning of cranial motor neurons in *islet1:GFP* embryos. **I**) Double LOF embryos show reduced trigeminal (V, arrow) nerves in the medial-lateral direction, and a misshapen facial (VII, arrowhead) nerve. **J**) Percent of larvae showing normal (gray) versus abnormal (blue) cranial motor neuron patterning quantified, with total number of larvae imaged shown above each bar. *We examined enteric neuron density by antibody staining for the neuronal marker HuC.* HuC positive cells were counted in 3D reconstructions of the mid-intestine at 5 dpf. **K-M**) Control and *sez6l2* and *taok2b* single LOF images of HuC positive cells in the mid-intestine, with percentages of cells relative to control shown in bottom right corners. **N**) Double LOF larvae show reduced enteric neuron density. **O**) Quantification of number of enteric neurons, with each orange point representing a single larva, and total number of larvae assayed above each group. Different letters above each treatment indicate a statistically significant difference of $p<0.05$ as measured by t-test (Mann-Whitney). Error bars show standard error. *We evaluated muscle fiber patterning by phalloidin staining at 5 dpf.* **Q-R**) Single LOF for *kif22* and *taok2b* resemble the control. **S**) Double LOF larvae show an interaction where muscle fibers are no longer neatly arrayed into chevron shaped segments and instead show looser packing with some fibers crossing muscle boundaries. **T**) Percent of larvae showing normal (gray) versus abnormal (red) muscle fiber quantified, with total number of larvae imaged shown above each bar. **U**) Brain and ventricle interaction map overlaid with interactions from secondary screen, lines color coded for each assay, and letters colored in gene names for number of interactions for each assay. **V**) Number of pairs that interacted in brain and ventricle primary screen interacting in each of secondary assays (top row), negative control pairs (middle row), and positive control pairs (bottom row). *$p<0.005$, **$p<0.0001$, ****$p<1x10^{-15}$. Scale bars=100 um.

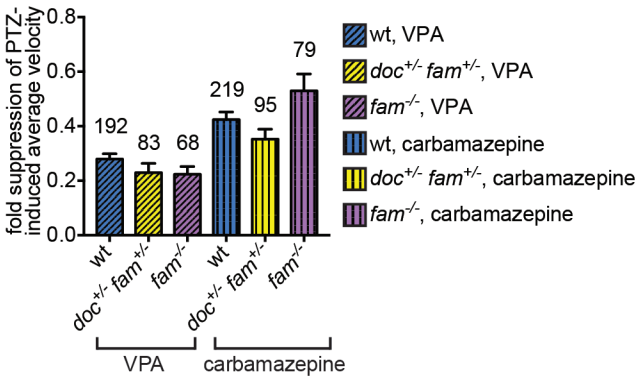


**Fig. S4. Human anti-epileptic drugs ameliorates seizure activity in *doc2a*$^{+/-}$ *fam57ba*$^{+/-}$ and *fam57ba*$^{-/-}$ mutants.** Average velocities of PTZ-treated larvae were compared with and without the anti-epileptics VPA and carbamazepine. Suppression = average PTZ velocity with AED ÷ average PTZ velocity without AED. Statistically significant suppression was observed with both drugs for all genotypes analyzed. Numbers of larvae tested for each treatment shown above the bars.

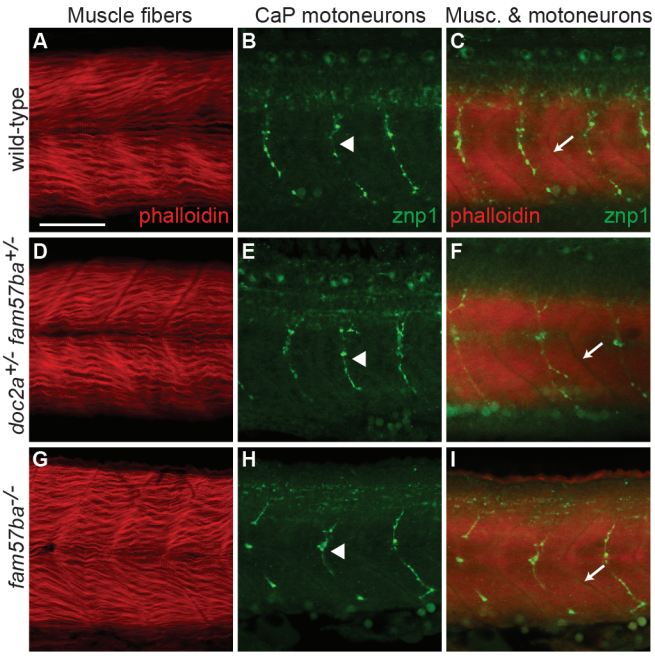


**Fig. S5. No apparent defects in musculature or primary motoneurons in *doc2a*$^{+/-}$ *fam57ba*$^{+/-}$ or *fam57ba*$^{-/-}$ mutants. A)** Muscle fibers in wild-type embryos stained with phalloidin 635. **B)** Wild-type caudal primary (CaP) motoneurons in a different embryo labeled with znp1 antibody, which binds to synaptotagmin. **C)** Labeling of both muscle fibers and CaP motoneurons in a different embryo shows one CaP motoneuron axon per muscle segment. Intersegmental boundaries indicated with arrows. **D-F)** Muscle fibers and CaP motoneurons in *doc2a*$^{+/-}$ *fam57ba*$^{+/-}$ double heterozygous embryos show no significant differences from wild-type. **G-I**) Muscle fibers and CaP motoneurons in *fam57ba*$^{-/-}$ homozygous mutant embryos show no significant differences from wild-type.

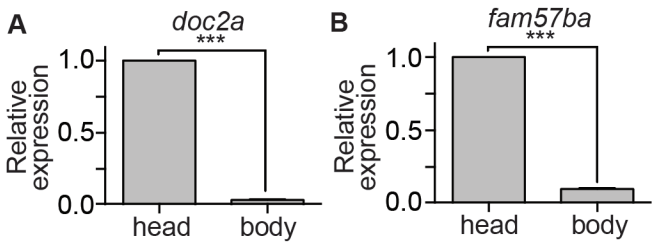


**Fig. S6. *doc2a* and *fam57ba* are expressed predominantly in the head.** Head/body tissue splits of 12 dpf larvae were analyzed for levels of *doc2a* or *fam57ba* expression by qPCR. Primer sequences and qPCR methods were as described previously (1). Five wild-type larvae were dissected per pool and 3 pools were analyzed in triplicate. **A**) Relative to expression in the head, 3.5% of *doc2a* expression was found in the body. **B**) Relative to expression in the head, 9.9% of *fam57ba* expression was found in the body. ***$p<0.001$.

| Gene Symbol | Amount MO used (ng) | Start or splice site MO* | p53 MO used |
|---|---|---|---|
| *aldoaa* | 0.5 | splice^ | no |
| *asphd1* | 1.5 | splice | yes |
| *c16orf53* | 0.2 | splice | no |
| *cdipt* | 3.0 | splice | no |
| *coro1a* | 1.5 | splice | yes |
| *doc2a* | 5.0 | splice | no |
| *fam57ba* | 0.3 | splice | no |
| *gdpd3* | 4.0 | start | yes |
| *hirip3* | 2.5 | start | yes |
| *ino80e* | 0.5 | start | yes |
| *kctd13* | 3.5 | splice | no |
| *kif22* | 2.0 | splice^ | yes |
| *mapk3* | 1.3 | start | yes |
| *maz* | 0.2 | splice | no |
| *ppp4ca* | 0.5 | splice | yes |
| *sez6l2* | 2.5 | start | yes |
| *taok2a* | 0.5 | splice | no |
| *taok2b* | 1.5 | splice | no |
| *ypel3* | 0.5 | splice | yes |

**Table S1. MO doses used for pair-wise interaction studies.**

*For splice site MOs, qPCR was used to determine the MO dose when ~50% normal RNA remained at 24 hpf. For start site MOs, sub-phenotypic doses were used.

^As these splice site MOs gave phenotypes at 50% LOF (1), a sub-phenotypic dose was used (~25% LOF).

*p53* MO was used if one or both genes required *p53* MO for single LOF experiments as determined previously (1).

| Gene Pair | Ventricle phenotype | NeuroD phenotype | Islet phenotype | HuC gut phenotype | Muscle phenotype |
|---|---|---|---|---|---|
| *aldoaa+fam57ba* | **Yes** | – | **Yes** | – | – |
| *asphd1+hirip3* | **Yes** | – | **Yes** | – | **Yes** |
| *asphd1+ino80e* | **Yes** | – | – | – | – |
| *asphd1+ppp4ca* | **Yes** | – | – | **Yes** | **Yes** |
| *cdipt+kctd13* | **Yes** | **Yes** | – | – | – |
| *doc2a+fam57ba* | **Yes** | – | – | – | – |
| *doc2a+kif22* | **Yes** | – | – | – | – |
| *fam57ba+hirip3* | **Yes** | **Yes** | **Yes** | – | – |
| *fam57ba+kctd13* | **Yes** | – | – | – | – |
| *gdpd3+sez6l2* | **Yes** | **Yes** | – | – | – |
| *hirip3+kif22* | **Yes** | **Yes** | – | **Yes** | – |
| *ino80e+kif22* | **Yes** | **Yes** | – | – | – |
| *kctd13+ppp4ca* | **Yes** | – | **Yes** | **Yes** | – |
| *kif22+taok2b* | **Yes** | **Yes** | **Yes** | **Yes** | **Yes** |
| *mapk3+sez6l2* | **Yes** | – | – | – | – |
| *sez6l2+taok2b* | **Yes** | **Yes** | – | **Yes** | **Yes** |

**Table S2. Summary of secondary screen positive interactions.** The 16 gene pairs that interacted in the brain and ventricle screen were tested in four secondary screen assays. Eleven of 16 pairs tested had a positive interaction in at least one of the secondary screen assays. Of those 11, there are 9 unique combinations of positive interactions among the secondary screen assays.

| Gene Pair | Ventricle phenotype | NeuroD phenotype | Islet phenotype | Muscle phenotype | HuC gut phenotype |
|---|---|---|---|---|---|
| *c16orf53+coro1a* | – | – | – | – | – |
| *c16orf53+maz* | – | – | – | – | – |
| *c16orf53+taok2a* | – | – | – | – | – |
| *c16orf53+ypel3* | – | – | – | – | – |
| *coro1a+ypel3* | – | – | – | – | – |
| *maz+taok2a* | – | – | – | – | – |
| *asphd1+fam57ba* | – | **Yes** | – | **Yes** | **Yes** |
| *asphd1+kif22* | – | – | **Yes** | – | – |
| *fam57ba+kif22* | – | **Yes** | – | – | – |
| *hirip3+kctd13* | – | – | – | – | – |
| *hirip3+taok2b* | – | **Yes** | – | – | **Yes** |
| *kctd13+taok2b* | – | – | – | – | **Yes** |

**Table S3. Summary of multiple MO assays on negative and positive control pairs.**

Using only the interacting pairs from the first pass screen could lead us to miss other gene interactions that could be affecting phenotypes in the secondary screen. To test whether gene interactions had been missed, we randomly chose 6 "negative control" pairs of genes (top half of table) that did not interact to affect brain development, nor did the individual genes of these pairs interact with any other genes in the primary screen. None of these double LOFs resulted in any phenotype, indicating that non-interacting genes from the brain and ventricle screen are unlikely to interact to give a phenotype in secondary assays.

To analyze whether genes that were part of the brain and ventricle interaction network were also interactive in secondary assays, we tested six "positive control" pairs (bottom half of table). These were pairs composed of genes that were part of the brain and ventricle interaction network, but did not directly interact in the primary screen. Between 1-3 pairs of the 6 interacted in each of the four secondary assays. These data indicate that it is likely that we have missed some interacting pairs by only looking at the 16 brain and ventricle interacting pairs. Interestingly, the percentage of interacting positive control pairs in each secondary assay was similar to the percentage of interacting brain and ventricle pairs in the same assay (i.e., 2 of 6 (33%) positive control pairs and 5 of 16 (31%) brain and ventricle pairs have reduced enteric neuron density).

| Gene symbol | *doc2a* | *fam57ba* |
|---|---|---|
| Left TALEN | HD NG HD HD NI NG HD HD NI NN NN NI NI HD NI NI | NN NN HD NI NG HD HD NG HD NG NN HD NG NN NN |
| Right TALEN | NI NG NN NN NN NN HD HD HD NN NN NI HD NI NN NN | HD HD NG HD NN NI NG NN NI NG NN NG HD NG NG NG NI HD NI NI NN NI NI |
| Left TALEN binding site | CTCCATCCAGGAACA | TGGCATCCTCTGCTGG |
| Right TALEN binding site | CTGTCCGGGCCCCAT | TTCTTGTAAAGACATCATCGAGGA |
| For genotyping primer | GCTCTATGACGGTGCGTAAG | AGCTTTTCTGCTTGGCTTG |
| Rev genotyping primer | ACAGAGTCCCACCTCCTCCT | TGTTCTCGGTTGAAATGCAG |
| Restriction enzyme | MscI | BtgZI |
| PCR product sizes | Uncut: 261bp  Cut: 204bp, 57bp | Uncut: 266bp  Cut: 185bp, 81bp |
| Mutant spacer sequence | CATG:::::::ACGT (-7bp) | ATACATC::CGCCTG (-2bp) |

**Table S4. TALEN information.** Sequences for designed TALENs targeting *doc2a* and *fam57ba*, binding sites in genome, and genotyping information. The restriction enzyme recognition site is absent after mutation; therefore, mutant PCR products will not be digested and wild-type will be cut. The spacer sequence lies between the left and right TALEN binding sites; both of these mutations are predicted to shift the reading frame and result in early stop codons and truncated proteins. All DNA sequences in 5' → 3' direction.

**Supplemental references**

1. Blaker-Lee, A., Gupta, S., McCammon, J.M., De Rienzo, G. and Sive, H. (2012) Zebrafish homologs of genes within 16p11.2, a genomic region associated with brain disorders, are active during brain development, and include two deletion dosage sensor genes. *Dis. Model. Mech.*, **5**, 834-851.