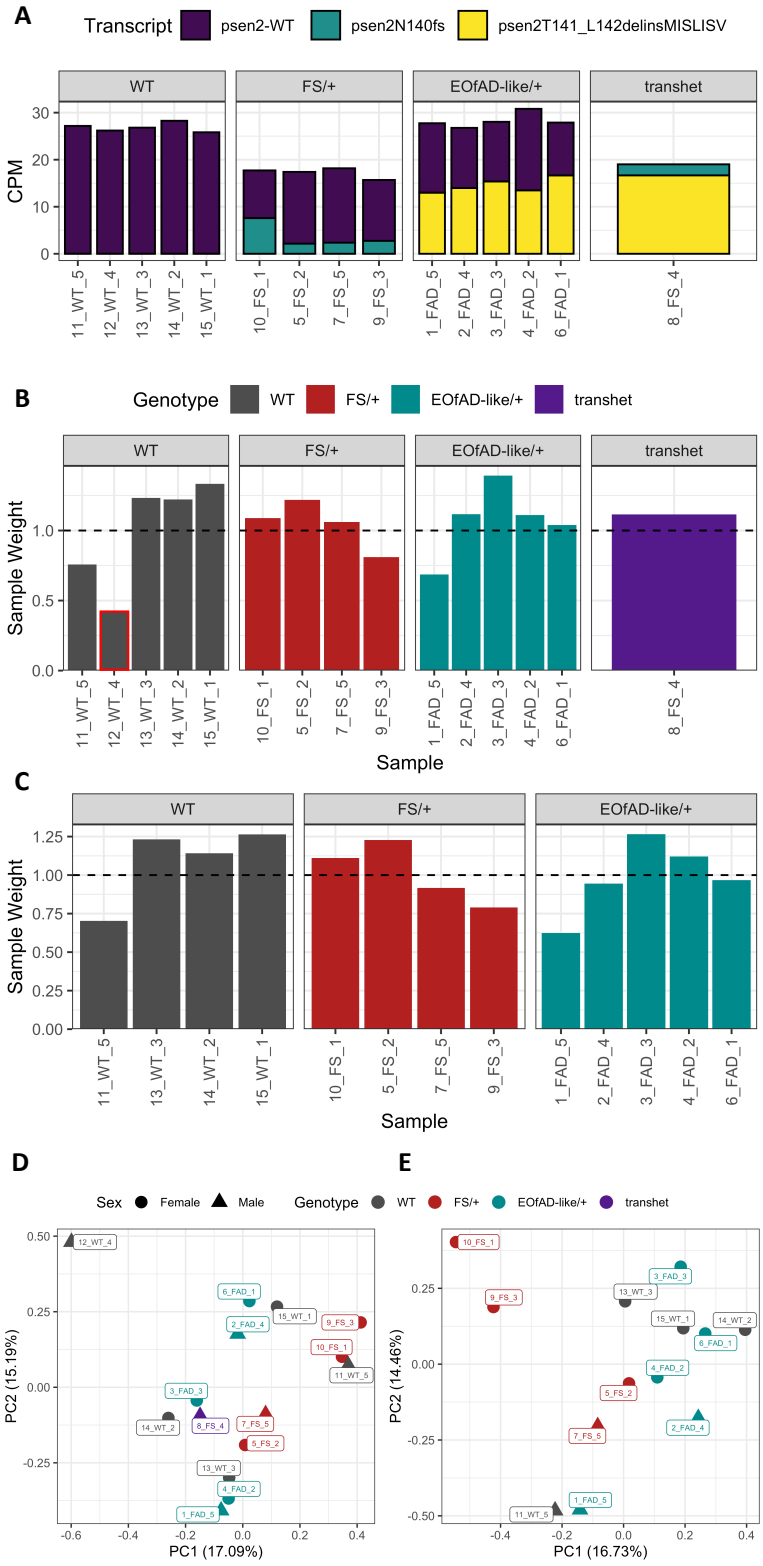
Supplementary Material

In-Frame and Frameshift Mutations in Zebrafish presenilin 2 Affect Different Cellular Functions in Young Adult Brains

Supplementary File 1. RNA-seq data quality control



A) Allele-specific expression (in counts per million, CPM) of *psen2* transcripts in young adult zebrafish brains. Reduced expression of the *psen2*^{N140fs} allele is observed in FS/+ brains, consistent with our previous observation that transcripts of this allele are subject to nonsense-mediated decay [1]. Sample 8_FS_4 does not express the wild type allele of *psen2* and is actually a transheterozygous (transhet) sample. Therefore, it was omitted from the analysis. B) Sample weights as calculated by the *voomWithQualityWeights* algorithm on all samples sequenced. Sample 12_WT_4 is highly downweighted relative to all other samples and was omitted from subsequent analysis. C) Sample weights recalculated after exclusion of samples 8_FS_4 and 12_WT_4. D) Principal component 1 (PC1) against PC2 from a principal component analysis (PCA) on all samples of the experiment. Sample 12_WT_4 does not cluster with the other samples. E. PCA plot of the *RUVseq*-normalised counts after exclusion of samples 8_FS_4 and 12_WT_4.

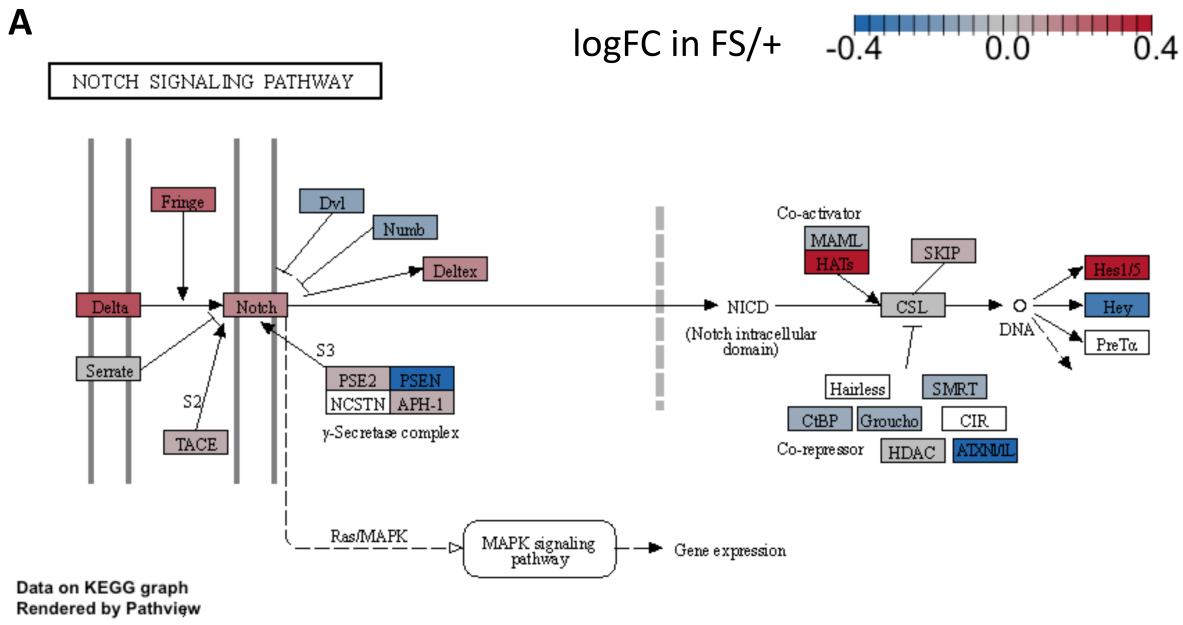
Supplementary File 2. Full results of differential gene expression analysis.
This is available separately from the main manuscript as a .csv file.

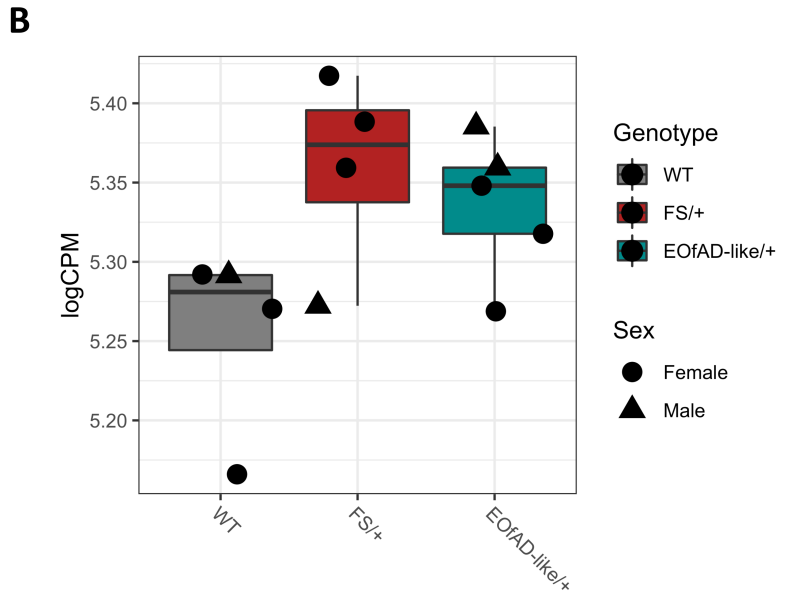
Supplementary File 3. Over-representation analysis using goseq.

Supplementary Table 1. The KEGG [2], GO [3, 4], and IRE [5] gene sets approaching over-representation in the DE genes list due to the FS mutation of *psen2*. The 5 DE genes due to the EOfAD-like mutation are not found in any of the gene sets. Therefore, the results of the over-representation analysis for the EOfAD-like mutation are not shown.

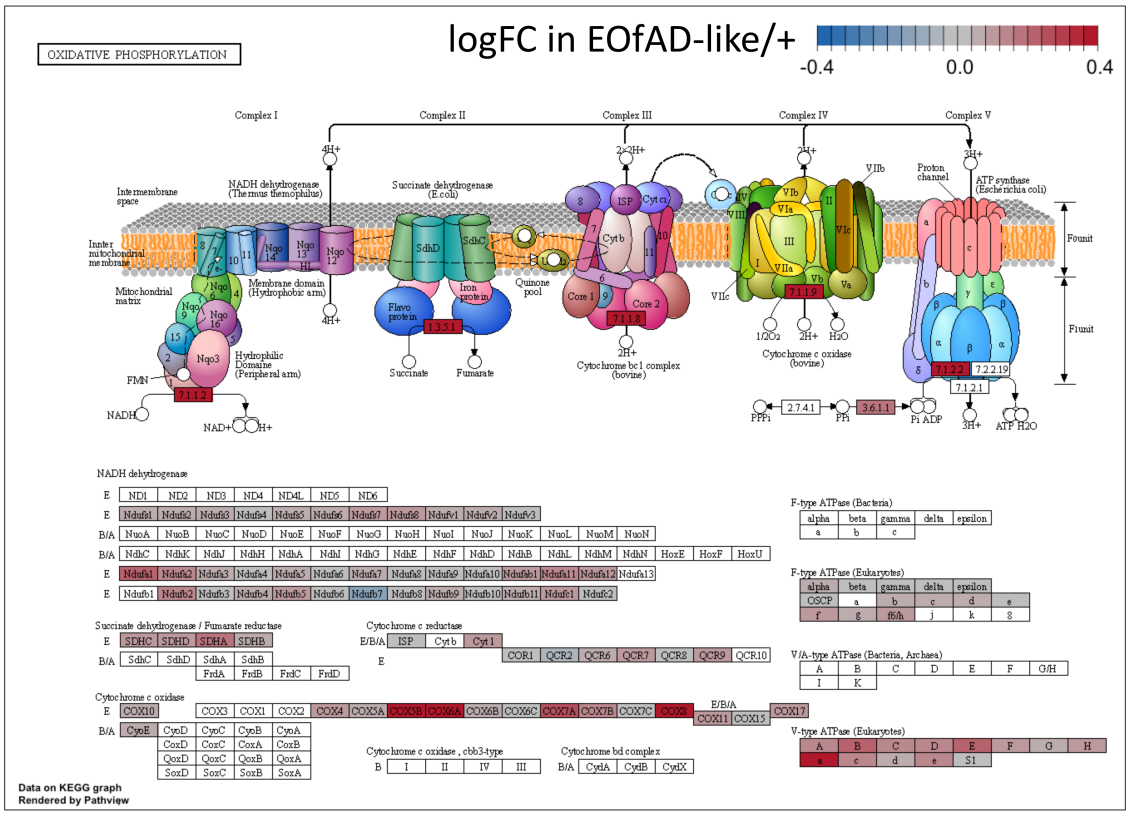
Gene set	**p**	**Number of DE genes in gene set**	**Number of genes in gene set**	**FDR adjusted p**
GO_PHOSPHOTRANSFERASE_ACTIVITY_NITROGENOUS_GROUP_AS_ACCEPTOR	1.94E-05	2	5	0.28952
GO_TRNA_3_END_PROCESSING	6.94E-05	2	9	0.517902
GO_NUCLEOSIDE_DIPHOSPHATE_METABOLIC_PROCESS	1.01E-03	3	142	1
KEGG_ALZHEIMERS_DISEASE	1.11E-03	3	146	1
GO_NCRNA_3_END_PROCESSING	1.39E-03	2	39	1
GO_PYRIMIDINE_NUCLEOBASE_TRANSPORT	2.85E-03	1	2	1
GO_UREA_TRANSMEMBRANE_TRANSPORTER_ACTIVITY	2.85E-03	1	2	1
GO_TRNA_BINDING	3.58E-03	2	63	1
GO_GLYCEROL_CHANNEL_ACTIVITY	4.26E-03	1	3	1
GO_UREA_TRANSPORT	4.27E-03	1	3	1

Supplementary File 4. Additional RNA-seq visualisations
A) Pathview [6] visualisation of the logFC of genes in the KEGG_NOTCH_SIGNALLING_PATHWAY gene set in FS/+ brains. Pathway Maps are displayed with copyright permission from KEGG. B) Expression of *psen1* in log counts per million (logCPM). C) Pathview [6] visualisation of the logFC of genes in the KEGG_OXIDATIVE_PHOSPHORYLATION gene set in EOfAD-like/+ brains. D) Pathview [6] visualisation of the logFC of genes in the KEGG_CELL_CYCLE gene set in EOfAD-like/+ brains. E. Heatmap of the logFC of genes in the KEGG_LONG_TERM_POTENTIATION gene set in EOfAD-like/+ brains.

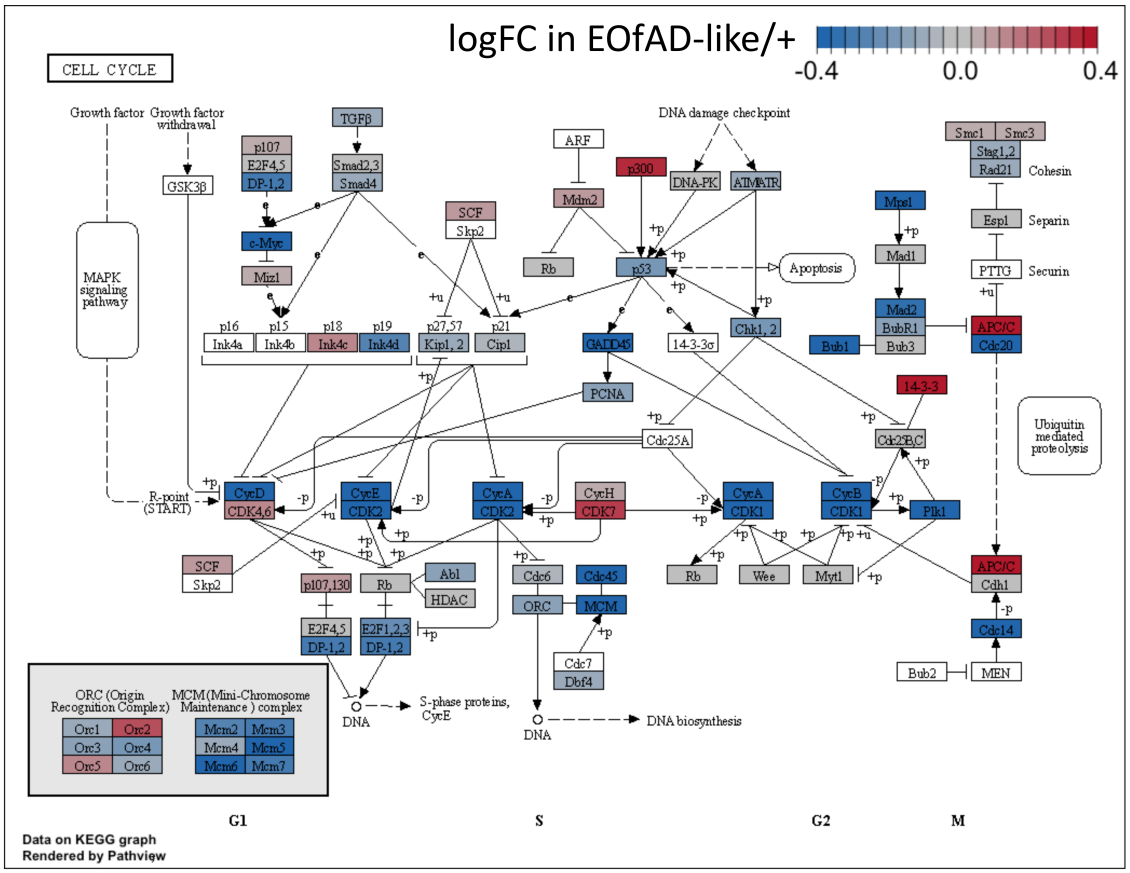




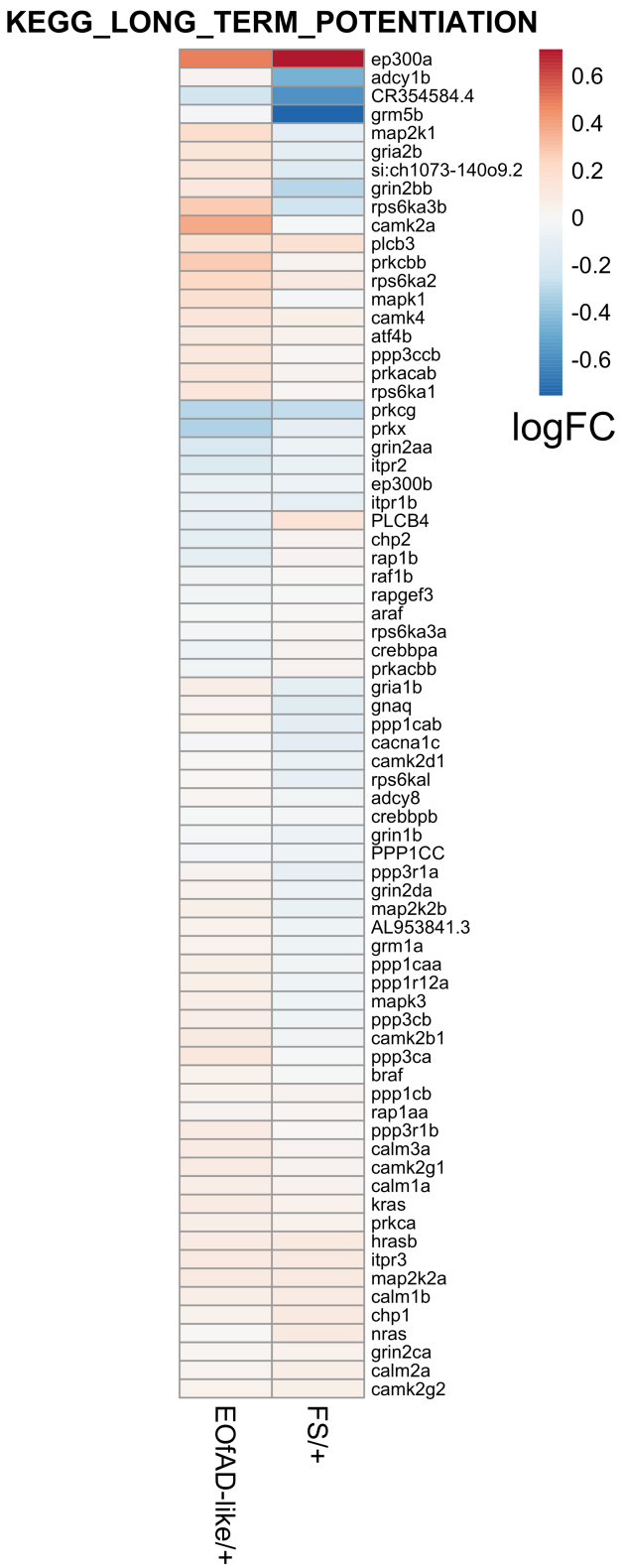
C


OXIDATIVE PHOSPHORYLATION
logFC in EOfAD-like/+
-0.4
0.0
0.4
Complex I
Complex II
Complex III
Complex IV
Complex V
Data on KEGG graph
Rendered by Pathview

D


CELL CYCLE
logFC in EOfAD-like/+
-0.4
0.0
0.4
Growth factor
Growth factor withdrawal
DNA damage checkpoint
MAPK signaling pathway
R-point (START)
Apoptosis
Cohesin
Separin
Securin
Ubiquitin mediated proteolysis
ORC (Origin Recognition Complex)
MCM (Mini-Chromosome Maintenance) complex
S-phase proteins, CycE
DNA biosynthesis
G1
S
G2
M
Data on KEGG graph
Rendered by Pathview

E


KEGG_LONG_TERM_POTENTIATION
ep300a
adcy1b
CR354584.4
grm5b
map2k1
gria2b
si:ch1073-140o9.2
grin2bb
rps6ka3b
camk2a
plcb3
prkcbb
rps6ka2
mapk1
camk4
atf4b
ppp3ccb
prkacab
rps6ka1
prkcg
prkx
grin2aa
itpr2
ep300b
itpr1b
PLCB4
chp2
rap1b
raf1b
rapgef3
araf
rps6ka3a
crebbpa
prkacbb
gria1b
gnaq
ppp1cab
cacna1c
camk2d1
rps6kal
adcy8
crebbpb
grin1b
PPP1CC
ppp3r1a
grin2da
map2k2b
AL953841.3
grm1a
ppp1caa
ppp1r12a
mapk3
ppp3cb
camk2b1
ppp3ca
braf
ppp1cb
rap1aa
ppp3r1b
calm3a
camk2g1
calm1a
kras
prkca
hrasb
itpr3
map2k2a
calm1b
chp1
nras
grin2ca
calm2a
camk2g2
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
logFC
EOfAD-like/+
FS/+

REFERENCES

[1] Jiang H, Newman M, Lardelli M (2018) The zebrafish orthologue of familial Alzheimer's disease gene PRESENILIN 2 is required for normal adult melanotic skin pigmentation. *PLoS One* **13**, e0206155.

[2] Kanehisa M, Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* **28**, 27-30.

[3] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene Ontology: tool for the unification of biology. *Nat Genet* **25**, 25-29.

[4] The Gene Ontology Consortium (2021) The Gene Ontology resource: enriching a GOld mine. *Nucleic Acids Res* **49**, D325-D334.

[5] Hin N, Newman M, Pederson SM, Lardelli M (2020) Iron responsive element (IRE)-mediated responses to iron dyshomeostasis in Alzheimer's disease. *bioRxiv*, 2020.2005.2001.071498.

[6] Luo W, Pant G, Bhavnasi YK, Blanchard SG, Jr., Brouwer C (2017) Pathview Web: user friendly pathway visualization and data integration. *Nucleic Acids Res* **45**, W501-W508.