Supplementary Material
-- Double mutant zebrafish model of TSC --

1 Supplementary Data

1.1 Supplementary Tables

Supplementary Table 1: List of primers (written in 5’ to 3’ direction) used for RT-qPCR for validation of RNA sequencing.

Supplementary Table 2: Differential expressed gene (DEG) lists for depdc5⁻/⁻, tsc2⁻/⁻ and depdc5⁻/⁻ x tsc2⁻/⁻ 5 dpf zebrafish compared to wild-type larvae.

Supplementary Table 3: KEGG enrichment analysis for depdc5⁻/⁻, tsc2⁻/⁻ and depdc5⁻/⁻ x tsc2⁻/⁻ 5 dpf zebrafish compared to wild-type larvae and GO enrichment analysis for tsc2⁻/⁻ and depdc5⁻/⁻ x tsc2⁻/⁻ 5 dpf zebrafish compared to wild-type larvae.

Supplementary Table 4: KEGG and GO enrichment analysis for depdc5⁻/⁻ x tsc2⁻/⁻ 5 dpf zebrafish compared to tsc2⁻/⁻ larvae.

Supplementary Table 5: GO enrichment analysis on overlapping genes between depdc5⁻/⁻ x tsc2⁻/⁻ 5 dpf zebrafish larvae and human SEGA lesions.

Supplementary Table 6: Hypergeometric testing of mitochondrial genes in tsc2⁻/⁻, depdc5⁻/⁻ x tsc2⁻/⁻, SEGA transcriptome profile and in the overlap between tsc2⁻/⁻ and SEGA and overlap between depdc5⁻/⁻ x tsc2⁻/⁻ and SEGA transcriptomes.

Supplementary Table 7: Summary of results derived with the DGIdb database using the overlapping up-regulated and down-regulated DEGs between depdc5⁻/⁻ x tsc2⁻/⁻ and SEGA transcriptomes as input.

1.2 Supplementary Figures

Supplementary Figure 1:

(A) Mating scheme for the generation of the double mutants. Mendelian ratio for the nine genotypes resulting of the double tsc2±/⁻ x depdc5±/⁻ heterozygotes is presented in bold. Made with Biorender.

(B) Representative image of confocal image of the GABAergic and glutamatergic neuronal networks in the optic tectum of 5 dpf wild-type larvae. GABAergic and glutamatergic cells are visualised by green and red fluorescence, respectively. Data are presented as mean ± SEM, n=12-20/condition. Significant values (one-way ANOVA) are noted as “ns” p>0.05.

(C) Total number of GABAergic and glutamatergic cells in the optic tectum of 5dpf wild-type, depdc5⁻/⁻, tsc2⁻/⁻ and double homozygous larvae.
(D) Representative 20x images of a forebrain, midbrain and early hindbrain section in wild-type, depdc5<sup>−/−</sup>, tsc2<sup>−/−</sup> and double homozygous larvae at 5 dpf, n=4 larvae/group. Ac = anterior commissure, Ce = cerebellum, lfb = lateral forebrain bundle, H = hypothalamus, P = pallium, Poc = postoptic commissure, Sd = dorsal part of subpallium, T = tegmentum, TeO = optic tectum, TVe = telencephalic ventricle

Supplementary Figure 2:

(A)-(B) RT-qPCR analysis for quantification of farsa1, stoml2, phb, rnf14, and ctsh (A) neurl1, hdac5, tsopoap1, reln and col28a1a (B) mRNA levels wild-type, depdc5<sup>−/−</sup>, tsc2<sup>−/−</sup>, and double homozygous larvae. Data are presented as mean ± SEM, n=3-4/condition. Significant values (Kruskal-Wallis) are noted as *** p ≤ 0.001, ** p ≤ 0.01 and * p ≤ 0.05