Expanded View Figures

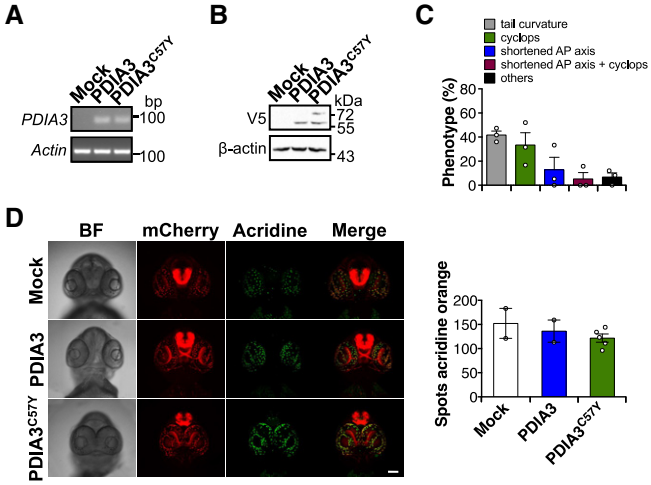

Figure EV1. Effects of PDIA3^{C57Y} expression in zebrafish.

A–D Zebrafish embryos at the one-cell stage were injected with sense mRNA coding for wild-type PDIA3-V5 or PDIA3^{C57Y}-V5. (A) *PDIA3* mRNA detection in embryos at 24 hpf by conventional PCR. *Actin* mRNA was employed as loading control. (B) PDIA3 protein levels at 24 hpf determined by Western blot analysis. β-actin was employed as loading control. (C) Quantification of abnormal morphologies in embryos expressing PDIA3^{C57Y} presented in Fig 2A. (D) Analysis of apoptosis in transgenic embryos with pan-neuronal expression of mCherry in the central nervous system at 72 hpf. Acridine orange staining (Acridine) shown in green and mCherry in red. BF, bright field. Scale bar 100 μm. Graph shows quantification of acridine orange staining (number of positive spots). Mock, 2; PDIA3, 2; PDIA3^{C57Y}, 5. Data are shown as mean ± s.e.m.

Source data are available online for this figure.

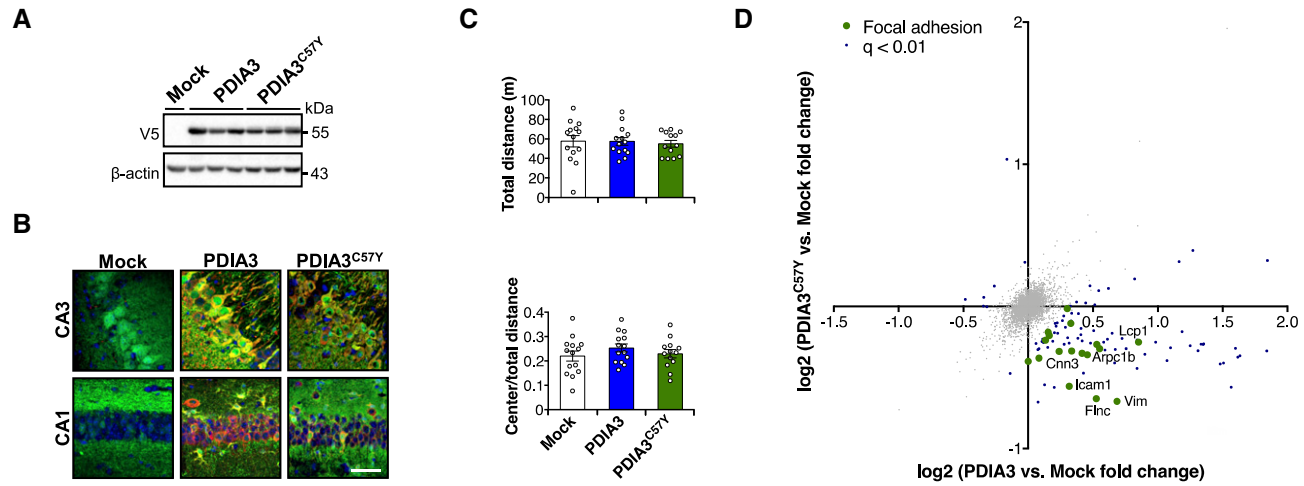

Figure EV2. Analysis of PDIA3^{C57Y} expression in the central nervous system of mice.

A–D Young mice at P55 received bilateral stereotaxis injection of adeno-associated virus serotype 9 (AAV9) to express wild-type PDIA3-V5 or PDIA3^{C57Y}-V5 and GFP or GFP alone (Mock) into the hippocampus for behavioral and proteomic analysis. (A) PDIA3 protein levels in hippocampus at P180 detected by Western blot analysis. β-actin employed as loading control. (B) Representative micrographs of hippocampus analyzed by immunofluorescence to GFP (green) and V5 (red) at P180 showing neuronal transduction in CA3 and CA1 regions. Nucleus stained with Hoechst (blue). Scale bar 50 μm. (C) Open field. Mice were placed in a squared arena and recorded for 15 min. Total distance (upper graph) and center to total distance ratio (lower graph) were measured as locomotor activity and exploratory behavior, respectively. Mock, n = 14; PDIA3, n = 14; PDIA3^{C57Y}, n = 13. Data are shown as mean ± s.e.m. and statistical analysis performed using one-way ANOVA with Tukey's *post hoc* test. No statistically significant differences exist between groups. (D) Proteomic analysis at P180. Mock, n = 3; PDIA3, n = 3; PDIA3^{C57Y}, n = 4. Scatter plot of proteomic analysis of hippocampus tissue of mice expressing wild-type PDIA3 or PDIA3^{C57Y}. The x-axis denotes logarithmic fold change in total protein levels in wild-type PDIA3 mice relative to Mock littermates. The y-axis denotes logarithmic fold change in total protein levels in PDIA3^{C57Y} mice relative to Mock littermates. Representative hits of focal adhesion are highlighted in green.

Source data are available online for this figure.

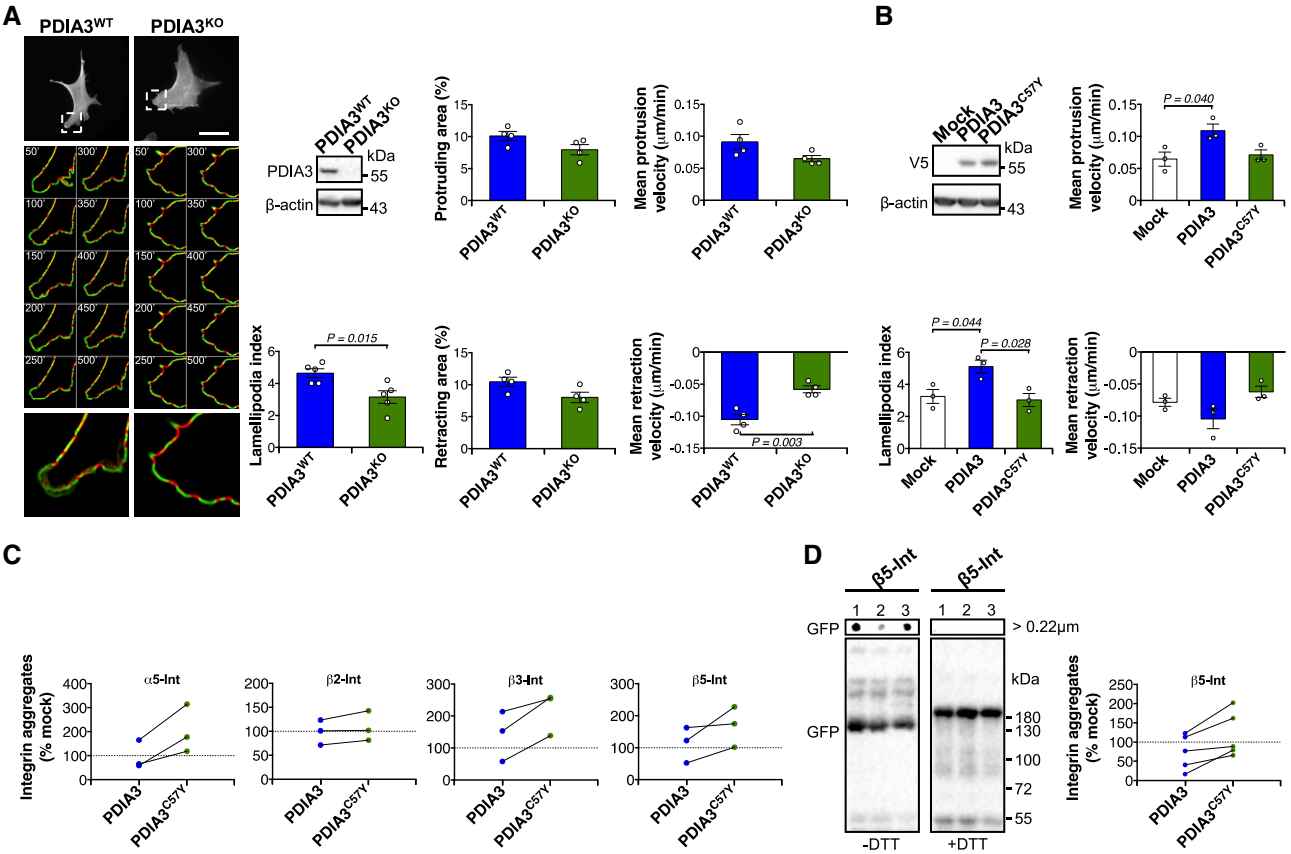

Figure EV3. Effects of PDIA3^{C57Y} expression on actin cytoskeleton and neuritogenesis.

A Mouse embryonic fibroblast (MEF) knock-out for *Pdia3* (*Pdia3*^{KO}) and wild-type MEF (*Pdia3*^{WT}) were transfected with construct for expression of EGFP-LifeAct. Representative micrographs of live cell imaging performed at 48 h after transfection are shown. Segmentation of time-lapse images was used to obtain protruding (green) and retracting (red) areas. Zoom of representative cell areas is shown. Lamellipodia number and protrusion/retraction movement were quantified using Fiji software. Scale bar 50 μm. $n = 4$ independent experiments. A total of 9 movies per group with 1 or 2 cells per movie were quantified. Data are shown as mean ± s.e.m. and statistical analysis performed using two-tailed Student's *t*-test. *Pdia3*^{KO} MEF was checked by Western blot analysis. β-actin was employed as loading control.

B *Pdia3*^{KO} MEF was co-transfected with constructs for expression of wild-type PDIA3-V5 or PDIA3^{C57Y}-V5, or empty vector (Mock), and EGFP-LifeAct. $n = 3$ independent experiments. A total of 10 movies per group with 1 or 2 cells per movie were quantified. Graphs show quantification of lamellipodia number and protruding/retracting velocity. Data are shown as mean ± s.e.m. and statistical analysis performed using one-way ANOVA with Tukey's *post hoc* test. Wild-type PDIA3-V5 or PDIA3^{C57Y}-V5 levels were checked by Western blot analysis. β-actin was employed as loading control.

C NSC-34 neuronal cell lines stably expressing wild-type PDIA3-V5 or PDIA3^{C57Y}-V5, or empty vector (Mock) were transfected with constructs for overexpression of α5-integrin (α5-int) fused to GFP, β2-integrin (β2-int) fused to YFP, β3-integrin (β3-int) fused to YFP, and β5-integrin (β5-int) fused to 2xGFP and analyzed by filter trap under non-reducing (−DTT, dithiothreitol) or reducing (+DTT) conditions as described in Fig 5I. The graphs show quantification of aggregates of each integrin paralog relative to mock control detected under non-reducing conditions. Lines connect aggregates quantified in the same experiment. $n = 3$ independent experiments.

D *Pdia3*^{KO} MEF was co-transfected with constructs for expression of wild-type PDIA3-V5 or PDIA3^{C57Y}-V5, or empty vector (Mock), and β5-integrin (β5-int) fused to 2xGFP and analyzed by Western blot and filter-trap under non-reducing (−DTT, dithiothreitol) or reducing (+DTT) conditions. 1, Mock, 2, PDIA3-V5, and 3, PDIA3^{C57Y}-V5. Representative image of five independent experiments. The graph shows quantification of integrin aggregates relative to mock control detected under non-reducing conditions. Lines connect aggregates quantification in the same experiment.

Source data are available online for this figure.

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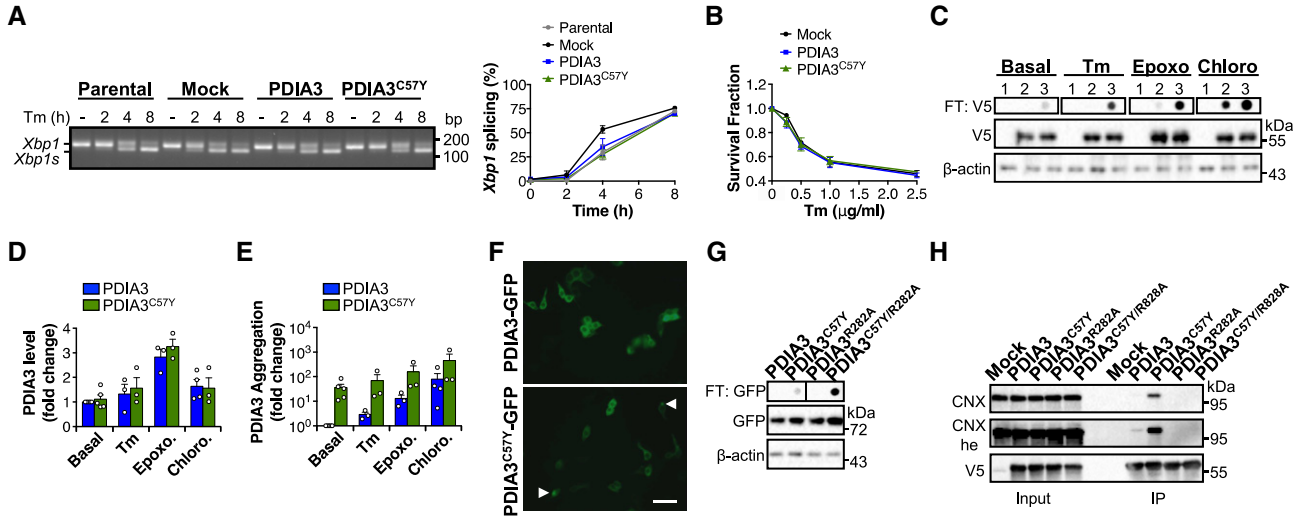


Figure EV4. Characterization of PDIA3^{C57Y} expression in cell culture.

A Kinetics of unfolded protein response activation measured by splicing of *X-box binding protein 1* (*Xbp1*) mRNA in NSC-34 cell lines stably expressing wild-type PDIA3-V5 or PDIA3^{C57Y}-V5, or empty vector (Mock) treated with 1 μg/ml of the ER stressor tunicamycin (Tm). *Xbp1s*, spliced form of *Xbp1* mRNA. Parental, non-transfected NSC-34. Graph shows quantification of percentage of *Xbp1* mRNA splicing in three independent experiments. Data are shown as mean ± s.e.m.

B NSC-34 cells were transfected with constructs for transient expression of wild-type PDIA3-V5 or PDIA3^{C57Y}-V5, or empty vector (Mock). Viability was determined after treatment with the indicated dose of Tm for 24 h in three independent experiments. Data are shown as mean ± s.e.m.

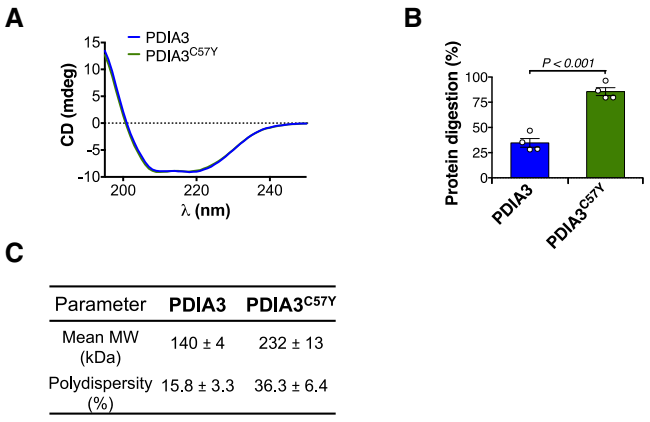
C–E NSC-34 cells were transfected with constructs for transient expression of wild-type PDIA3-V5 or PDIA3^{C57Y}-V5, or empty vector (Mock). (C) Filter-trap analysis of PDIA3-V5 aggregates under non-reducing conditions at 24 h after treatment with Tm (1 μg/ml), the proteasome inhibitor epoxomicin (Epoxo, 250 nM), or the lysosome inhibitor chloroquine (Chloro, 30 μM). β-actin was employed as loading control. 1, Mock, 2, PDIA3-V5, and 3, PDIA3^{C57Y}-V5. Representative image of three independent experiments. (D) Quantification of total levels of PDIA3 by Western blot under different stress conditions. PDIA3 levels expressed as fold change relative to the wild-type protein under basal condition. Data are shown as mean ± s.e.m. (E) Quantification of PDIA3 aggregates by filter trap under different stress conditions. PDIA3 aggregates levels expressed as fold change relative to the wild-type protein under basal condition. Data are shown as mean ± s.e.m.

F NSC-34 cells were transfected with constructs for transient expression of wild-type PDIA3-GFP or PDIA3^{C57Y}-GFP. Fluorescence micrographs show live cells at 48 h after transfection. Arrowheads point to puncta of PDIA3^{C57Y}-GFP. Scale bar 50 μm. The image is representative of a single experiment performed for construct validation.

G NSC-34 cells were transfected with constructs for transient expression of wild-type PDIA3-GFP, PDIA3^{C57Y}-GFP, PDIA3^{R282A}-GFP, or PDIA3$^{C57Y/R282A}$-GFP. Filter-trap analysis under non-reducing conditions was performed at 48 h after transfection. β-actin was employed as loading control. The image is representative of a single experiment performed for construct validation.

H HEK cell line was transfected with constructs for transient expression of wild-type PDIA3-V5, PDIA3^{C57Y}-V5, PDIA3^{R282A}-V5, or PDIA3$^{C57Y/R282A}$-V5, or empty vector (Mock). Immunoprecipitation of V5-tag was carried out at 48 h after transfection and PDIA3 interaction with calnexin (CNX) measured by Western blot; he, high exposure. The image is representative of a single experiment performed for construct validation.

Source data are available online for this figure.



C

Parameter	PDIA3	PDIA3^{C57Y}
Mean MW (kDa)	140 ± 4	232 ± 13
Polydispersity (%)	15.8 ± 3.3	36.3 ± 6.4

Figure EV5. Biophysical and biochemical properties of PDIA3^{C57Y}.

A Circular dichroism (CD) analysis of recombinant wild-type PDIA3 and PDIA3^{C57Y}. Average traces of CD spectroscopic scans are shown.

B Purified recombinant wild-type PDIA3 and PDIA3^{C57Y} were treated with proteinase K and analyzed by SDS–PAGE with Coomassie blue staining as shown in Fig 6H. $n = 4$ independent reactions. Bar graph shows quantification of protein digestion. Data are shown as mean ± s.e.m. and statistical analysis performed using two-tailed Student's *t*-test.

C Dynamic light scattering determination of mean molecular weight (MW) and percent polydispersity of wild-type PDIA3 and PDIA3^{C57Y}. Data are shown as mean ± s.d.

Source data are available online for this figure.

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