Expanded View Figures

Figure EV1. Peripheral DAPs are dispensable for ciliogenesis.

A RPE1 cells of the indicated genotypes were subjected to serum-starvation followed by immunofluorescence with the indicated antibodies to visualize the PC. Representative micrographs are shown. Blow-ups without Hoechst 33342 are magnified 2×. Scale bar: 5 μm.

B The percentage of ciliated cells was assessed by visual scoring of micrographs as in (A) across 3 biological replicates, with ≥ 50 cells per replicate. Individual values of biological replicates, their mean ± s.e.m. are reported. ANOVA test, comparing each sample to the wild type (**$P < 0.01$; n.s. = non-significant).

C Fluorescence micrographs of RPE1 cells of the indicated genotypes, either left untransduced (mock) or transduced with a lentiviral vector expressing Myc-SCLT1, and co- stained with the indicated antibodies. Blow-ups without Hoechst 33342 are magnified 2.5×. Scale bar: 5 μm.

D RPE1 cells of the indicated genotypes were either left untransduced (mock) or transduced with a lentiviral vector expressing Myc-SCLT1 and subjected to serum-starvation. Immunofluorescence with the indicated antibodies allowed visualization of the PC. Representative micrographs are shown. Blow-ups without Hoechst 33342 are magnified 2×. Scale bar: 5 μm.

E Ciliary length was measured from the same dataset described in (B), violin plots showing values for individual cilia obtained by pooling at least three biological replicates (≥ 150 cells); n.a. = not applicable due to absence of ciliated cells. Median values (red lines) and quartiles (black, dashed lines) are shown. Kruskal–Wallis test, comparing each sample to the wild type (****$P < 0.0001$; n.s. = non-significant).

F Scheme summarizing the epistatic interdependencies between DAPs emerging from data displayed in Fig 1 and the functional implications described in this figure. PC = primary cilium.

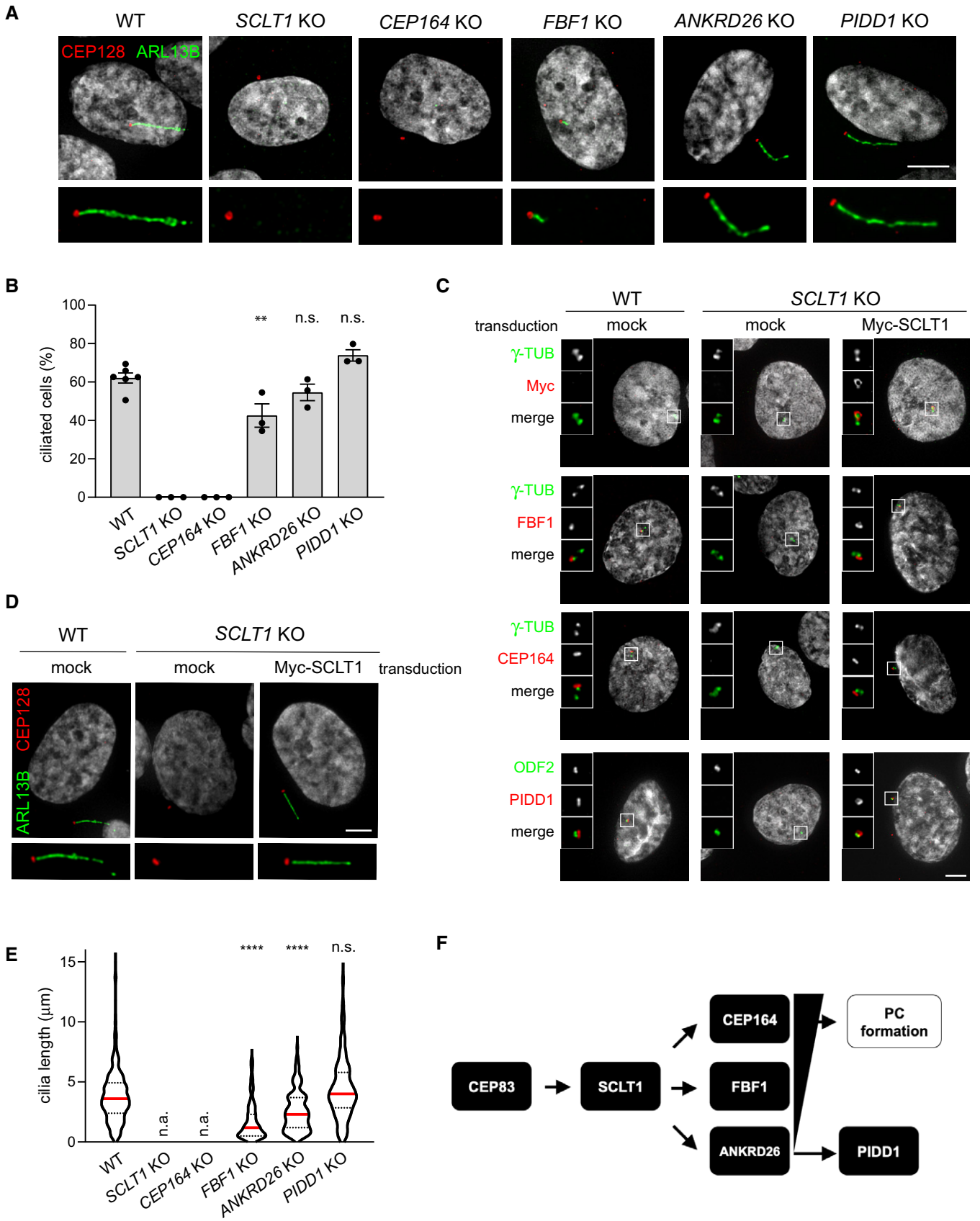


Figure EV1.

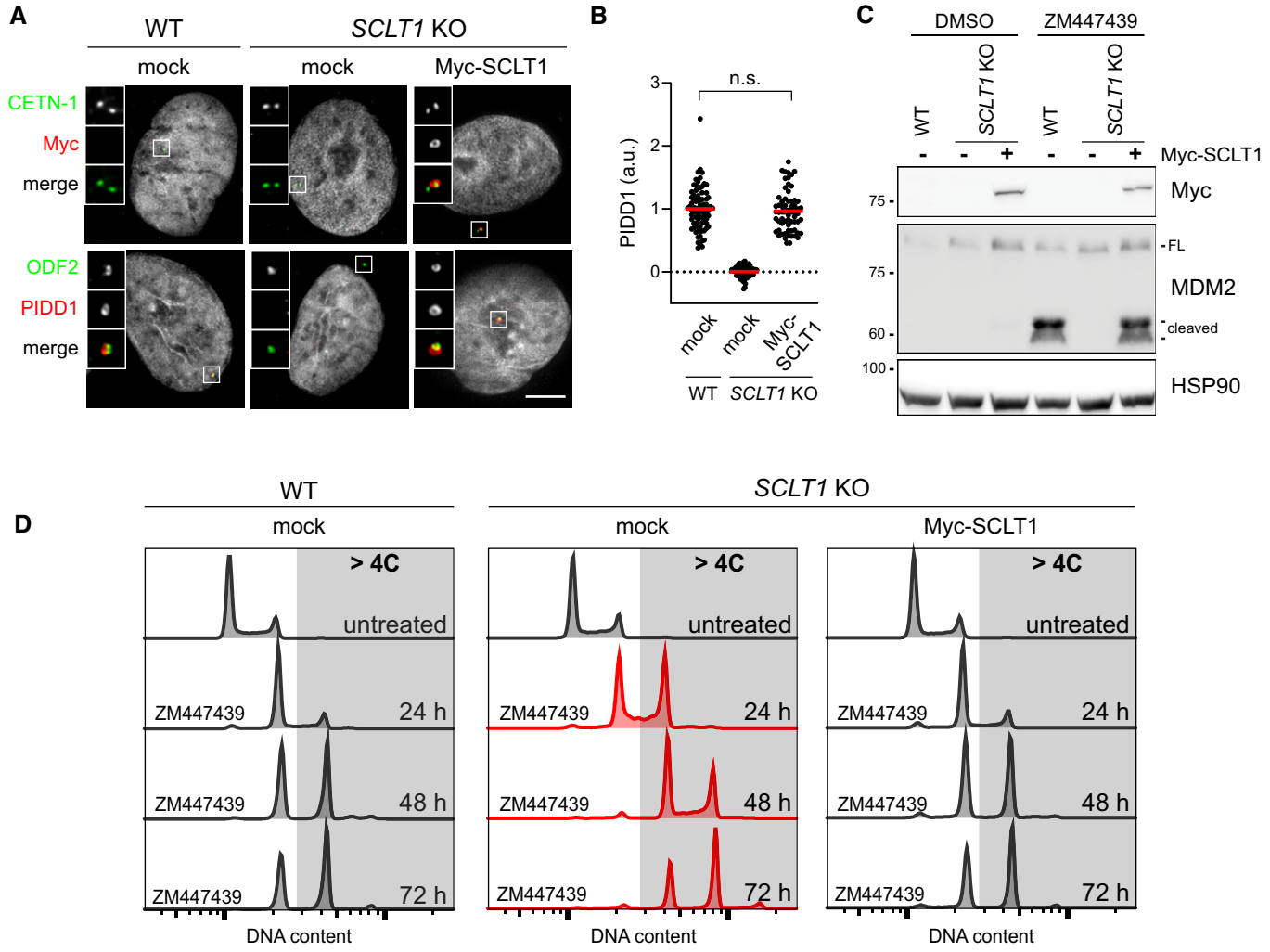


Figure EV2. Complementation of SCLT1 in *SCLT1* KO cells rescues PIDDosome activation also in A549 cells.

A Fluorescence micrographs of A549 cells of the indicated genotypes. Cells were either left untransduced (mock) or transduced with a lentiviral vector expressing Myc-SCLT1 and co-stained with the indicated antibodies. Blow-ups without Hoechst 33342 are magnified 2.5×. Scale bar: 5 μm.

B Dot plot showing the average pixel intensities of PIDD1 at individual parent centrioles in A549 cells of the indicated genotypes, treated as in (A). Mean values (red lines) ± s.e.m. are reported. $N > 50$ centrosomes were assessed for each condition in as many individual cells; a.u. = arbitrary units. Mann–Whitney test (n.s. = non-significant).

C A549 cells of the indicated genotypes were either left untransduced (–) or transduced with a lentiviral vector expressing Myc-SCLT1 (+). Cells were treated either with DMSO or with ZM447439 for 24 h and subjected to immunoblotting; $n = 3$ independent experiments.

D DNA content analysis of A549 cells of the indicated genotypes. Cells were either left untransduced (mock) or transduced with a lentiviral vector expressing Myc-SCLT1, treated with ZM447439 for up to 72 h and processed for DNA content analysis.

Source data are available online for this figure.

A

PRM PIDD1 reference peptides

Peptide #	Residues	Sequence	Contained in
1	183-198	LQTLPPALGALSTLQR	FL, N
2	491-497	VSMQVVR	FL, C
3	616-623	VNLIALQR	FL, C, CC
4	871-877	AVLELGR	FL, C, CC

B

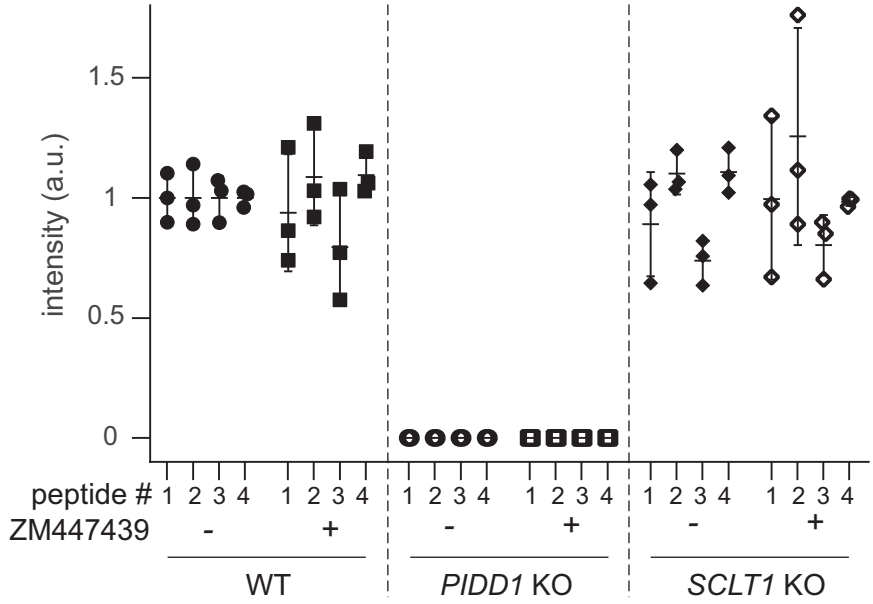
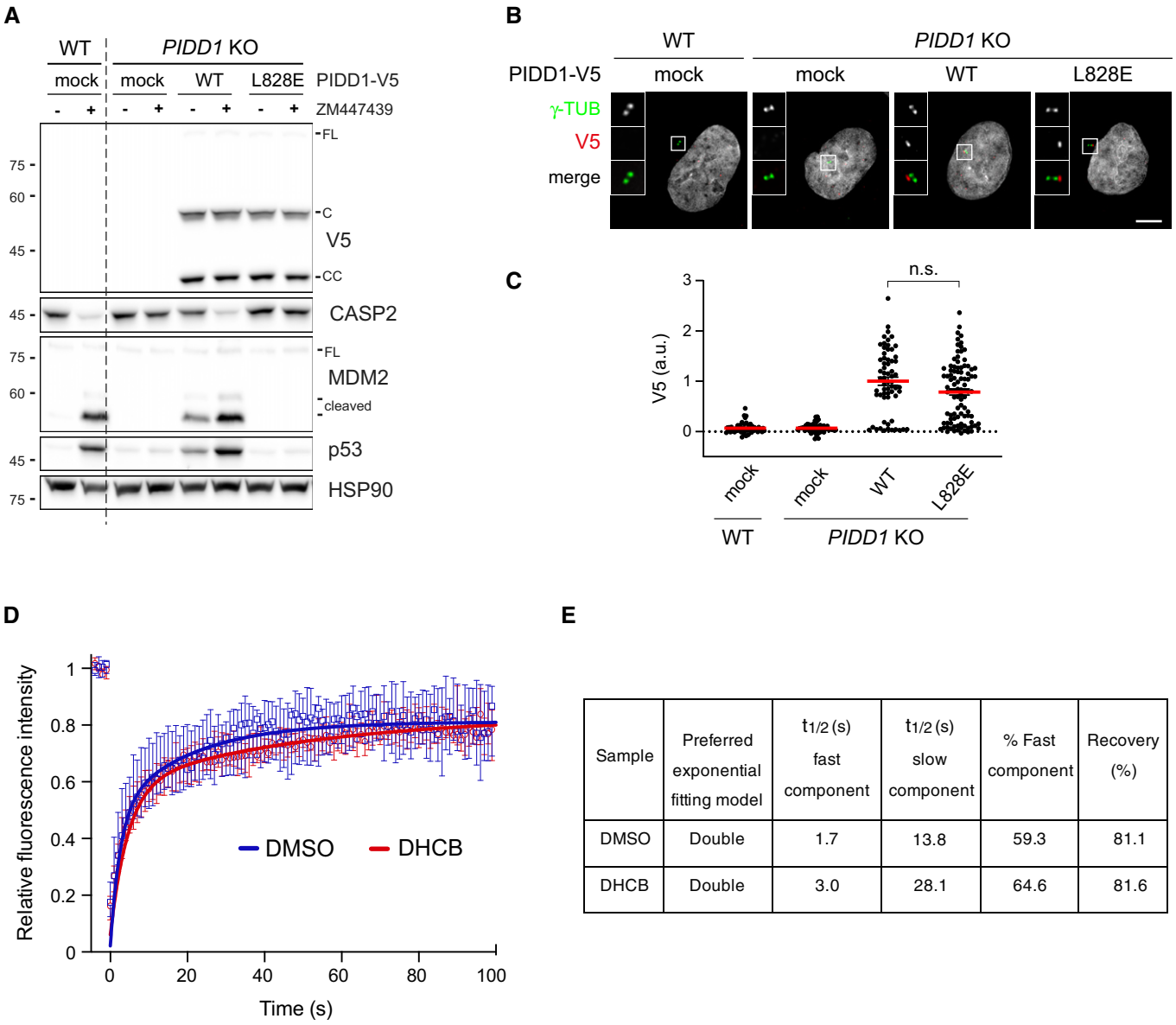


Figure EV3. Endogenous PIDD1 localization to DAs does not influence its proteostasis.

A Table reporting features of the reference (isotopically labelled) synthetic peptides that served as internal references for the quantification of the corresponding endogenous PIDD1 peptides.

B Quantification across a biological triplicate of four different PIDD1 peptides (described in A) in A549 cells of the indicated genotypes treated either with DMSO (−) or with ZM447439 (+) by means of parallel reaction monitoring. Mean values ± standard deviation are reported.



E

Sample	Preferred exponential fitting model	$t_{1/2}$ (s) fast component	$t_{1/2}$ (s) slow component	% Fast component	Recovery (%)
DMSO	Double	1.7	13.8	59.3	81.1
DHCB	Double	3.0	28.1	64.6	81.6

Figure EV4. PIDD1 displays a rapid exchange rate between the centrosomal and cytoplasmic pool.

A A549 cells of the indicated genotypes were either left untransduced (mock) or transduced with lentiviral vectors expressing PIDD1-V5 in its wild-type form or carrying the L828E mutation. Cells were treated either with DMSO or with ZM447439 for 24 h and subjected to immunoblotting; $n = 2$ independent experiments.

B Fluorescence micrographs of A549 cells of the indicated genotypes, treated as in (A) and co-stained with the indicated antibodies. Blow-ups without Hoechst 33342 are magnified 2.5×. Scale bar: 5 μm.

C Dot plot showing the average pixel intensities of V5-tag at individual parent centrioles in A549 cells, measured from micrographs as in (B). Mean values (red lines) ± s.e.m. are reported. $N > 50$ centrosomes were assessed for each condition in as many individual cells; a.u. = arbitrary units. Mann–Whitney test (n.s. = non-significant).

D FRAP analyses of centrosomal PIDD1^{L828E}-mNeonGreen turnover in PIDD1-deficient RPE1 cells treated with either DMSO or DHCB. Graph shows the median with interquartile range ($n = 12$ cells per condition). FRAP curves for both experimental conditions were best fitted with a double exponential curve.

E Relevant recovery parameters of FRAP experiments.

Source data are available online for this figure.

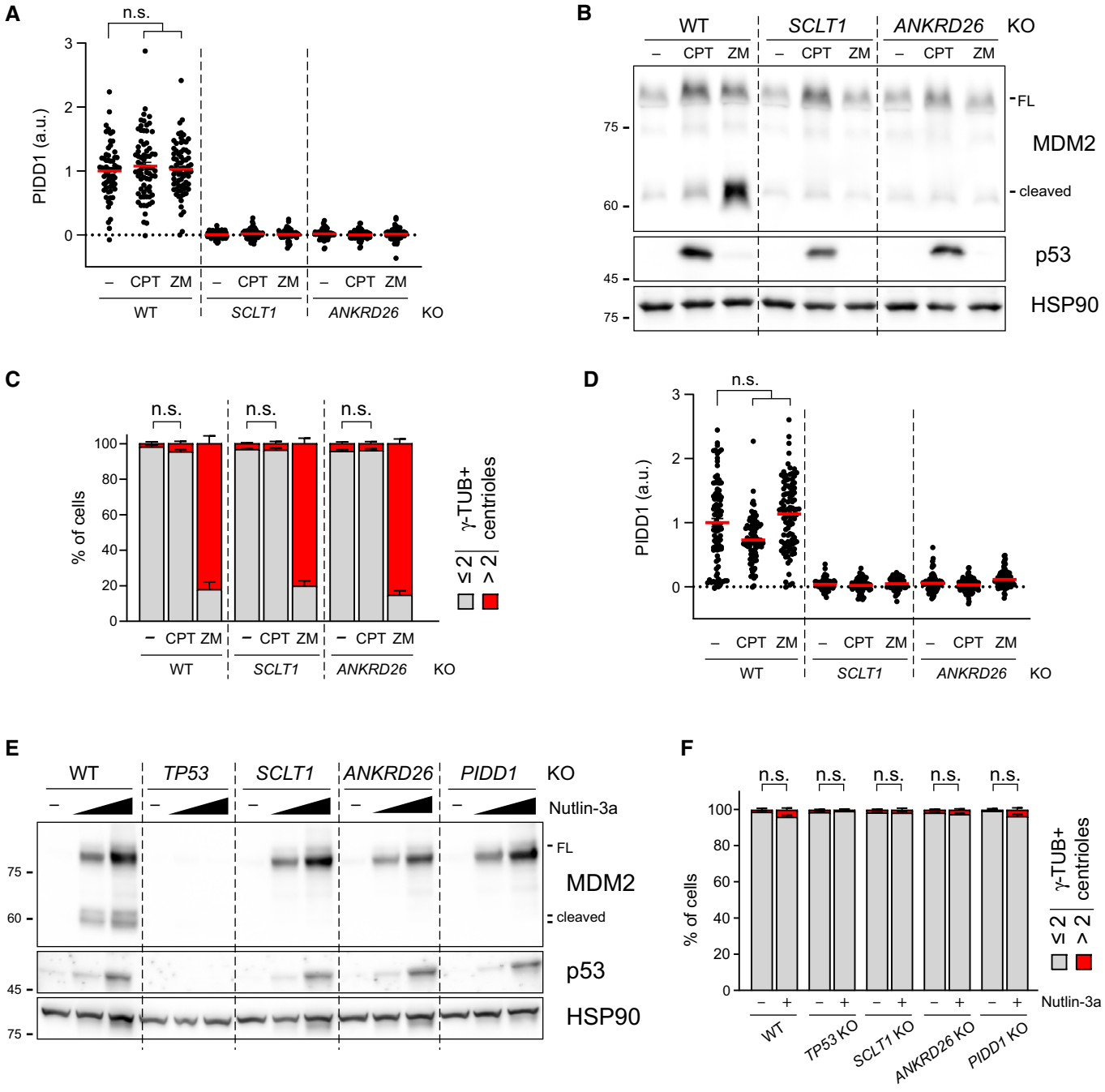


Figure EV5.

◀ **Figure EV5. PIDD1 localization to DAs is required for PIDDosome activation in response to DNA damage also in RPE1 cells.**

A Dot plot showing the average pixel intensities of PIDD1 at individual parent centrioles in A549 cells of the indicated genotypes and treatments (CPT = camptothecin, ZM = ZM447439). Mean values (red lines) ± s.e.m. are reported. $N > 50$ centrosomes were assessed for each condition in as many individual cells; a.u. = arbitrary units. Kruskal–Wallis test (n.s. = non-significant).

B RPE1 cells of the indicated genotypes were treated either with CPT or ZM or DMSO for 24 h. Samples were subjected to immunoblotting; $n = 3$ independent experiments.

C RPE1 cells treated as in (B) were subjected to fluorescence microscopy and centrosome abundance was assessed by counting the number of γ-tubulin-positive centrioles per cell across different genotypes and treatments. Mean values ± s.e.m. are reported. $N = 3, \geq 50$ cells from each independent experiment. ANOVA test (n.s. = non-significant).

D Dot plot showing the average pixel intensities of PIDD1 at individual parent centrioles in RPE1 of the indicated genotypes and treatments. Mean values (red lines) ± s.e.m. are reported. $N > 50$ centrosomes were assessed for each condition in as many individual cells; a.u. = arbitrary units. Kruskal–Wallis test (n.s. = non-significant).

E RPE1 cells of the indicated genotypes were either left untreated or treated with Nutlin-3a (3.3 μM or 10 μM). Cells were subjected to immunoblotting; $n = 3$ independent experiments.

F A549 cells were either treated with 10 μM Nutlin-3a for 24 h or left untreated. Cells were subjected to fluorescence microscopy and centrosome abundance was assessed by counting the number of γ-tubulin-positive centrioles per cell across different genotypes and treatments. Mean values ± s.e.m. are reported. $N = 3, \geq 50$ cells from each independent experiment. ANOVA test (n.s. = non-significant).

Source data are available online for this figure.