Expanded View Figures

Figure EV1. Amino acid sequence alignment of cavin family proteins and PLA analysis of cavin1 truncation mutants.

A Amino acid sequence alignment of cavin family proteins from mouse and zebrafish species highlighting helical regions (HR1 and HR2) and disordered regions (DR1, DR2 and DR3). Highly conserved residues are boxed in red.

B Each GFP-tagged construct (green) was co-expressed with cavin1-mCherry (red) in PC3 line, and PLA was performed as per manufacturer's protocol (described in Materials and Methods) between GFP and CAV1. Greyscale images shows PLA signal as white dots. Scale bars = 10 μm.

C Representative EM micrograph corresponding to Fig 1C showing caveolar morphology of PC3 cells transfected with GFP + cavin1-mCherry, GFP-cavin1 (45–230) + Cavin1-mCherry and GFP-cavin1 (45–280) + cavin1-mCherry. GFP fusions and cavin1-mCherry were co-expressed in PC3 cells and processed for ruthenium red staining. Scale bar = 100 nm.

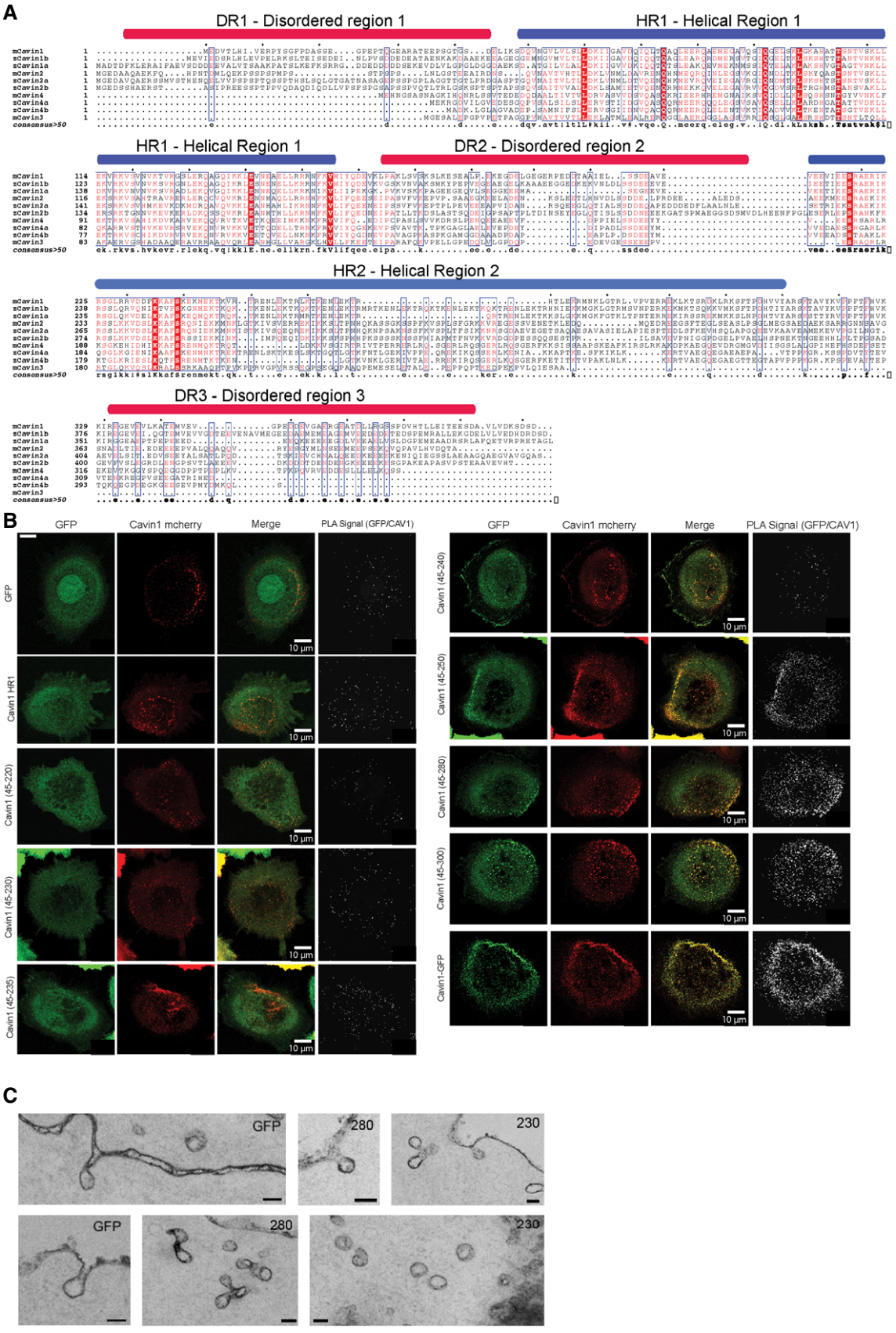

Figure EV1.

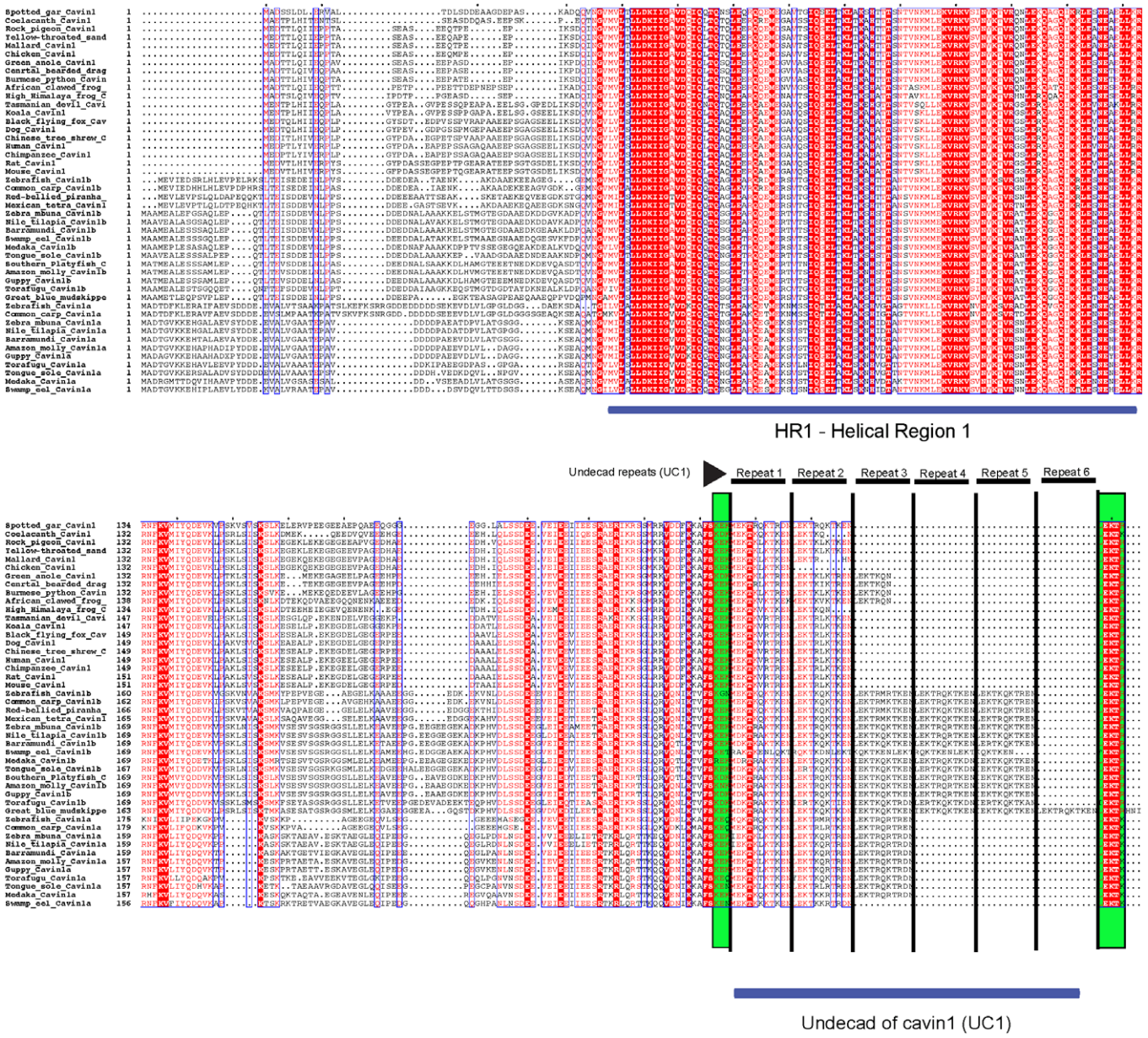

Figure EV2. Alignment of cavin1 protein sequences from various species.
Multiple protein sequence alignment of cavin1 was performed using Multialign program. Repeating units of the UC1 domain are marked with solid lines and the highly conserved amino acid sequences "KEK" and "LEKTR" are marked in light green.

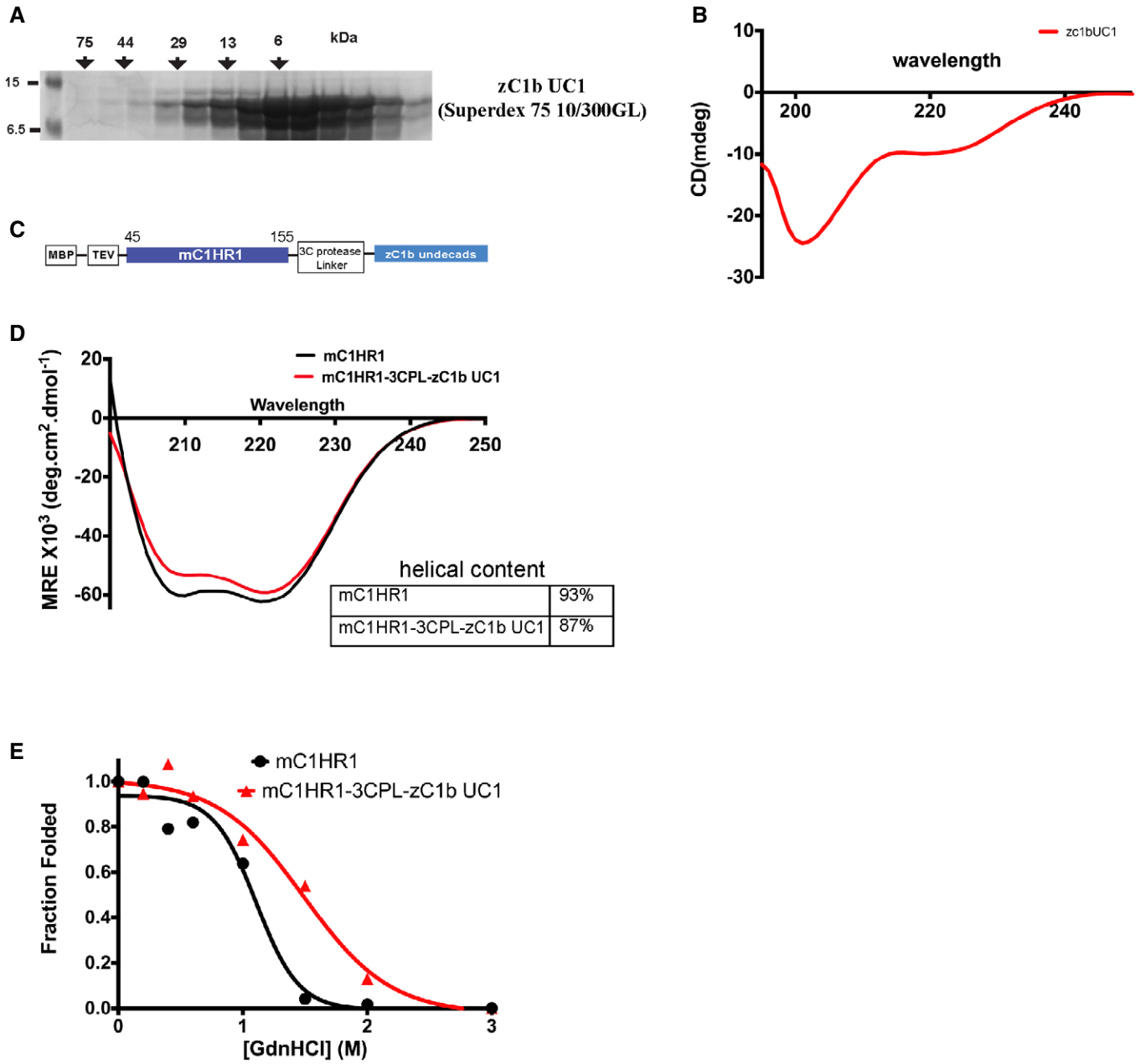

Figure EV3. ***In vitro*** **biochemical analysis of zebrafish cavin1b UC1 domain.**

A Size exclusion chromatography of zebrafish cavin1b UC1 domain expressed and affinity purified in *E. coli* analysed by SDS–PAGE and Coomassie Blue stain for protein. Molecular weight markers on the top with arrows represent size exclusion chromatography elution positions for standard globular proteins, while markers on the left indicate SDS–PAGE denatured protein markers.

B Circular dichroism spectroscopy analysis of zebrafish cavin1b UC1 domain showing spectra typical to largely random coil protein.

C Schematic representation of fusion construct used in (D) and (E).

D Circular dichroism spectroscopy analysis of mouse cavin1 HR1 domain and fusion construct mC1HR1-3CPL-zC1bUC1 with measured α-helical content using K2D3 server.

E Plot of fraction of folded protein for mC1HR1 and mC1HR1-3CPL-zC1b UC1 fusion using guanidine hydrochloride denaturant.

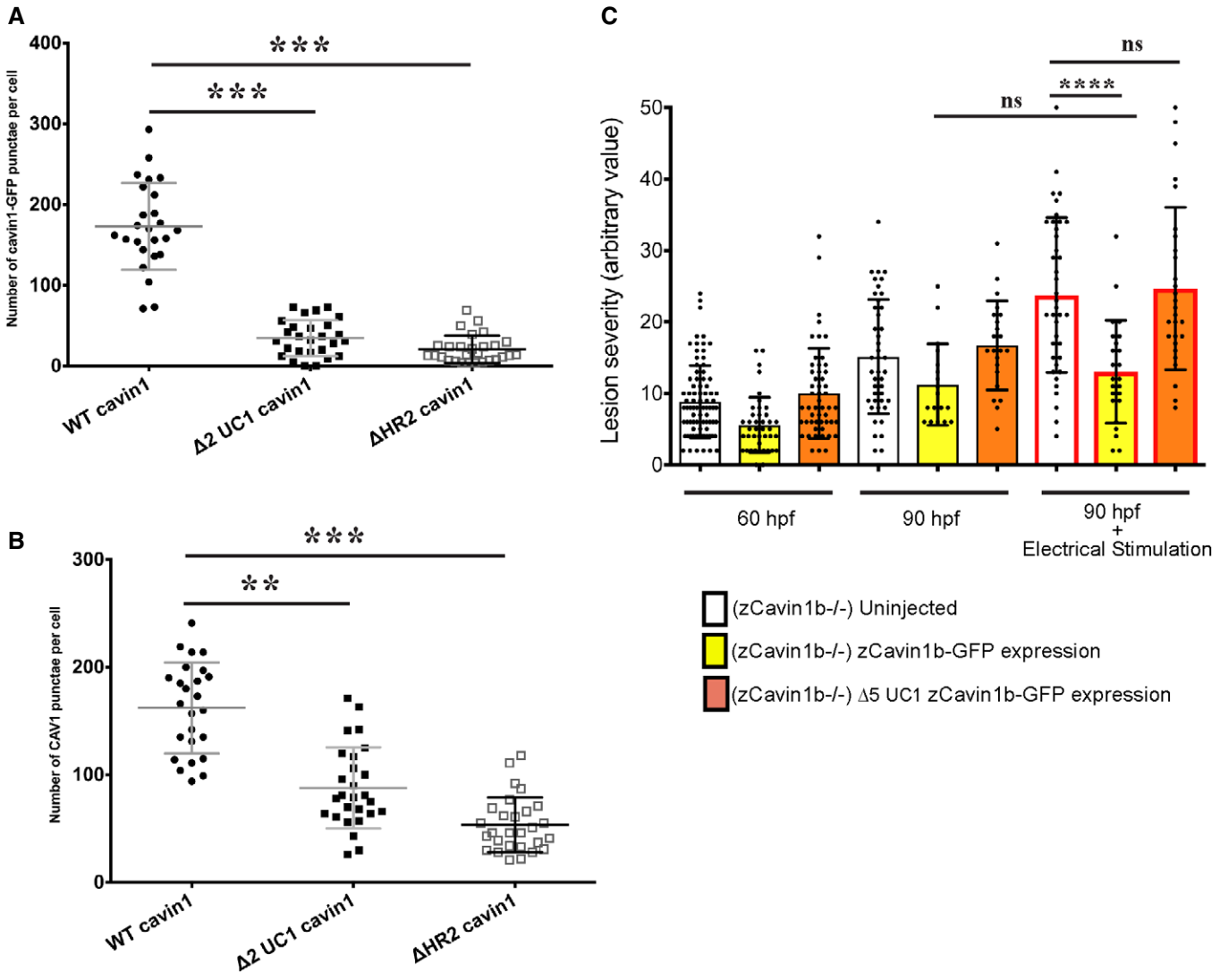

Figure EV4. Quantitative analysis (related to Fig 3B and E).

A Quantitation of cavin1-GFP punctae in A431 cells (related to Fig 3B). Data are presented as mean, and error bars indicate standard deviation. $N = 2$ (independent biological replicates), $n = 10–15$ (sample size) $^{***}P < 0.001$; one-way ANOVA followed by Bonferroni's multiple comparisons.

B Quantitation of CAV1 punctae in A431 cells (related to Fig 3B) after expression of GFP-tagged WT cavin1, Δ2 UC1 cavin1 and ΔHR2 cavin1. Data are presented as mean, and error bars indicate standard deviation. $N = 2$ (independent biological replicates), $n = 10–15$ (sample size) $^{***}P < 0.001$, $^{**}P < 0.01$; one-way ANOVA followed by Bonferroni's multiple comparisons.

C Plot of lesion severity index for 60 hpf embryos, 90 hpf embryos and 90 hpf embryos electrically stimulated at 60 hpf for 10 min. For 60 hpf, 90 hpf and 90 hpf stim; fish number (uninjected) = 84, 43, 39, respectively, fish number (zcavin1b-GFP) = 41, 18, 24, respectively, fish number (Δ5 UC1 zcavin1b-GFP) = 52, 22, 28, respectively. Data are presented as mean and error bars indicate standard deviation. ns = not significant, $^{****}P < 0.0001$; one-way ANOVA followed by Bonferroni's multiple comparisons.

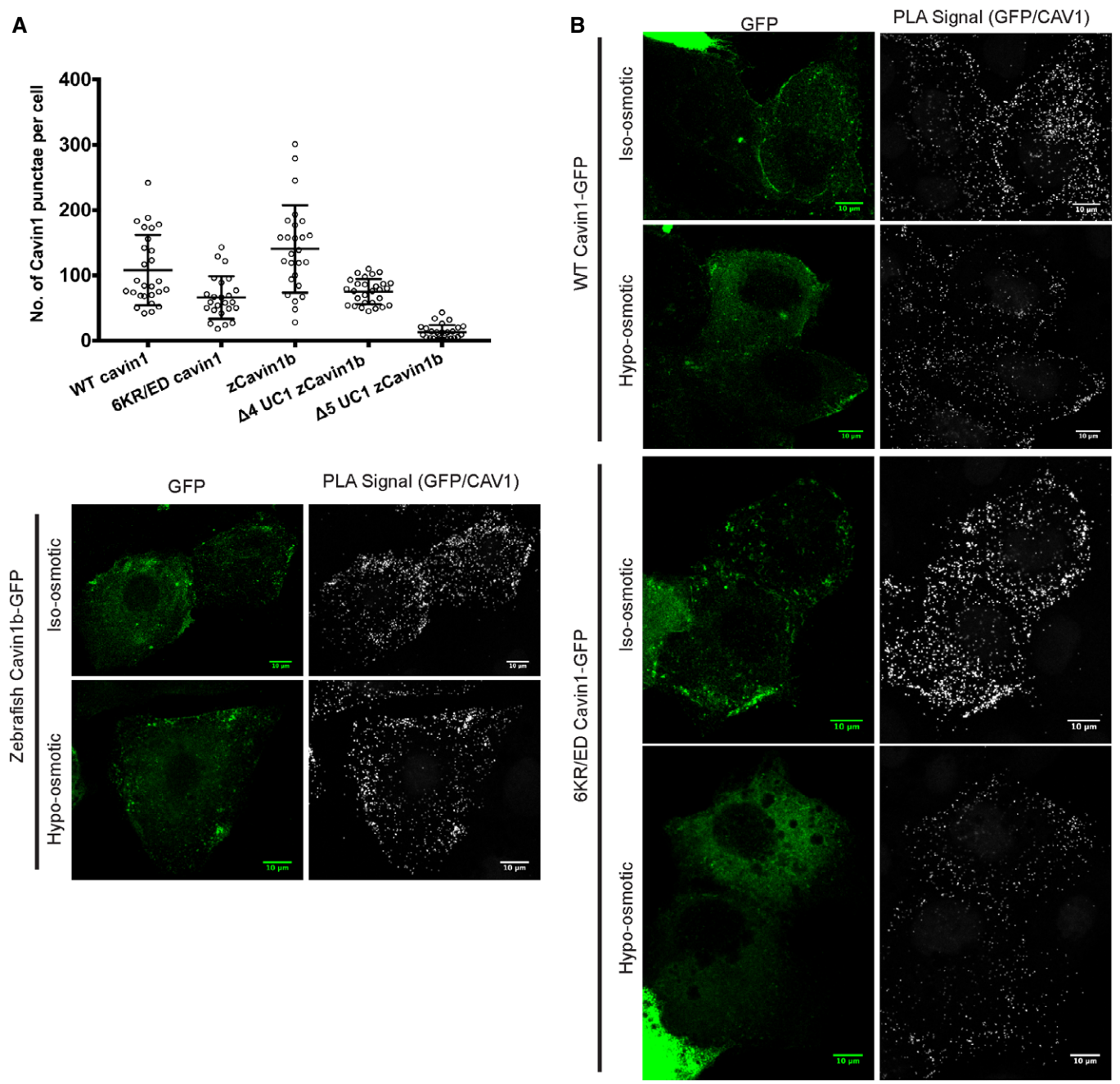

Figure EV5. Representative images related to Fig 4C.

A Quantitation of GFP-positive puncta in PC3 cells after expression of GFP-tagged constructs as shown. *N* = 2 (independent biological replicates), *n* = 10–15 (sample size). Data are presented as mean, and error bars indicate standard deviation. Scale bars = 10 μm.

B Representative images of PLA analysis presented in Fig 4C. Scale bars = 10 μm.