Expanded View Figures

Figure EV1. Compact and extended structural conformations of AcGFP1 concatemers.

A Dimensions (numerals) of AcGFP1 in Angstrom (Å) based upon GFP crystal structure. R and L indicate the radius and length of the protein, respectively. Geometric data are summarized in Table 1.
B Same as panel (A) but now for $AcGFP1^2$ displaying a minimal structural conformation ("compact").
C Same as panel (A) but now for $AcGFP1^2$ displaying a structural conformation of maximal size ("extended").
D Same as panel (A) but now for $AcGFP1^3$ displaying a compact structure.
E Same as panel (A) but now for $AcGFP1^3$ displaying an extended structure.
F Same as panel (A) but now for $AcGFP1^4$ displaying a compact structure.
G Same as panel (A) but now for $AcGFP1^4$ displaying an extended structure.

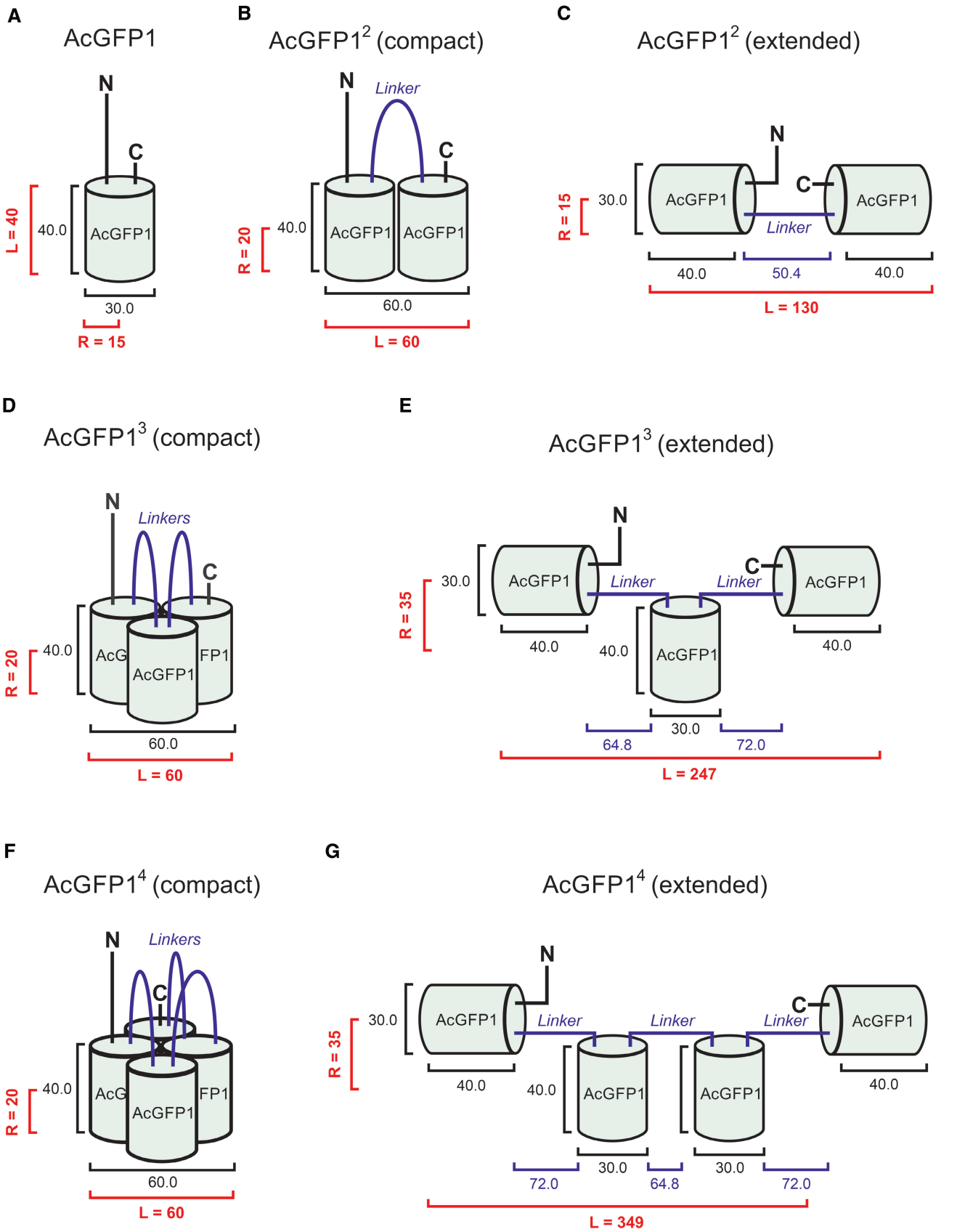


Figure EV1.

Figure EV2. Functional consequences of chloramphenicol (CAP) treatment in HeLa parental cells.

A Average oxygen consumption rate (OCR) in untreated cells, and cells treated with DOX, CAP, or DOX + CAP (for legend see panel B). Oligomycin (OLI), mitochondrial uncoupler (FCCP), and antimycin A + rotenone (AA/ROT) were added at the indicated time points.

B Same as panel (A) but now for the average extracellular acidification rate (ECAR).

C Statistical analysis of the data in panel (A and B) with respect to the basal OCR, maximal OCR and basal ECAR.

D Effect of CAP on the TMRM fluorescence signal in mitochondria and nucleus. Left panel: Typical example of fluorescence microscopy image of TMRM-stained cells. Fluorescence signals were manually determined in two regions of interest (yellow) defined in a mitochondria-dense ("m") and nucleoplasmic part ("n") of the cell and corrected for background using a close by ROI outside of the cell ("b"). Data panels: numerical values for the mitochondrial fluorescence signal (mito), nuclear fluorescence signal (nucleus) and fluorescence ratio value (mito/nucleus).

E Effect of DOX, CAP or DOX + CAP on the cellular protein levels of LONP1 (specific bands marked by red boxes), mtHSP70, mtHSP60, CLPP and CHOP. β-actin was used as cellular loading control. Arrowheads indicate molecular weight in kDa. Individual panels were contrast-optimized for visualization purposes. Original blots are presented in Appendix Fig S5B.

F Effect of CAP on mitochondrial DNA (mtDNA) levels expressed as number of mtDNA copies per cell and per nanogram (ng) of total DNA.

G Effect of CAP on the cellular levels of key mitochondrial fission and fusion proteins (specific bands are marked by red boxes): DRP1 (Dynamin-related protein 1, OPA1 (Optic atrophy protein 1), MFN2 (Mitofusin 2). β-actin and VDAC1 were used as cellular and mitochondrial loading controls, respectively. Arrowheads indicate molecular weight in kDa. Individual panels were contrast-optimized for visualization purposes. Original blots are presented in Appendix Fig S5A.

Data information: OCR and ECAR data (panels A–C) was obtained in a single ($N = 1$) experiment and the following number (n) of technical replicates: Untreated ($n = 5$), +DOX ($n = 5$), +CAP ($n = 4$), +DOX+CAP ($n = 6$). TMRM data (Panel D) was obtained in $N = 2$ independent experiments for $n = 991$ cells (−CAP) and $n = 668$ cells (+CAP). MtDNA data (panel F) was obtained in $N = 2$ independent experiments in $n = 7$ assays (−CAP) and $n = 6$ assays (+CAP). Each symbol represents an individual well (panel C), cell (panel D) or assay (panel F). In panels (A and B), individual data points reflect mean ± SEM. In panel (C), bars and errors reflect mean ± SEM. In panels (D and F), error bars mark the 95% (upper) and 5% (lower) percentile, the boundary boxes mark the 75% (upper) and 25% (lower) percentile, the square marks the mean value of the data, and the horizontal line within the box indicates the median value of the data. Significant differences, obtained using an independent Student's t-test, are indicated by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ between the marked conditions (a–c) in panel (C) and between the −CAP and +CAP condition (panel D and F). Not significant is marked by n.s. The exact P-values for panel (C) (basal OCR) were: Untreated (a) vs. +DOX (b): $P = 0.0126$; Untreated (a) vs. +CAP (c): $P = 1.554{\cdot}10^{-6}$; Untreated (a) vs. +DOX+CAP: $P = 4.082{\cdot}10^{-8}$; +DOX (b) vs. +CAP (c): $P = 2.671{\cdot}10^{-7}$; +DOX (b) vs. +DOX + CAP: $P = 3.806{\cdot}10^{-9}$. The exact P-values for panel (C) (maximal OCR) were: Untreated (a) vs. +DOX (b): $P = 6.509{\cdot}10^{-4}$; Untreated (a) vs. +CAP (c): $P = 2.019{\cdot}10^{-7}$; Untreated (a) vs. +DOX+CAP: $P = 4.900{\cdot}10^{-9}$; +DOX (b) vs. +CAP (c): $P = 4.558{\cdot}10^{-8}$; +DOX (b) vs. +DOX + CAP: $P = 5.713{\cdot}10^{-10}$. The exact P-values for panel (C) (basal ECAR) were: Untreated (a) vs. +DOX (b): $P = 0.0023$; Untreated (a) vs. +CAP (c): $P = 4.571{\cdot}10^{-8}$; Untreated (a) vs. +DOX+CAP: $P = 7.784{\cdot}10^{-10}$; +DOX (b) vs. +CAP (c): $P = 2.412{\cdot}10^{-8}$; +DOX (b) vs. +DOX+CAP: $P = 7.039{\cdot}10^{-10}$. The exact P-value for panel (C) (mito) was: $P = 7.145{\cdot}10^{-6}$. The exact P-value for panel (C) (nucleus) was: $P = 0.0237$. The exact P-value for panel (C) (mito/nucleus) was: $P = 5.702{\cdot}10^{-5}$. The exact P-value for panel (F) (per cell) was: $P = 0.556$. The exact P-value for panel (F) (per ng total DNA) was: $P = 0.139$.

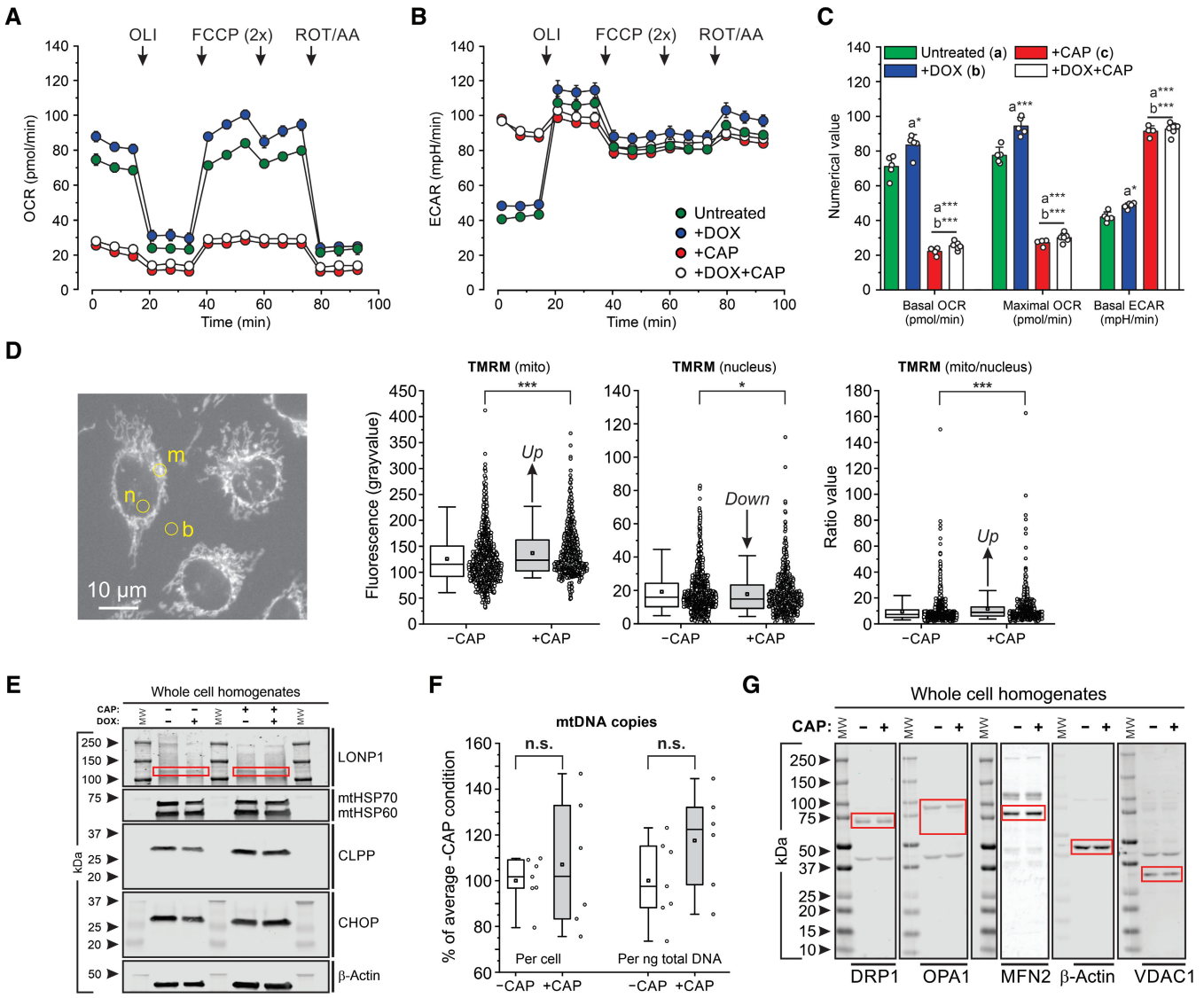


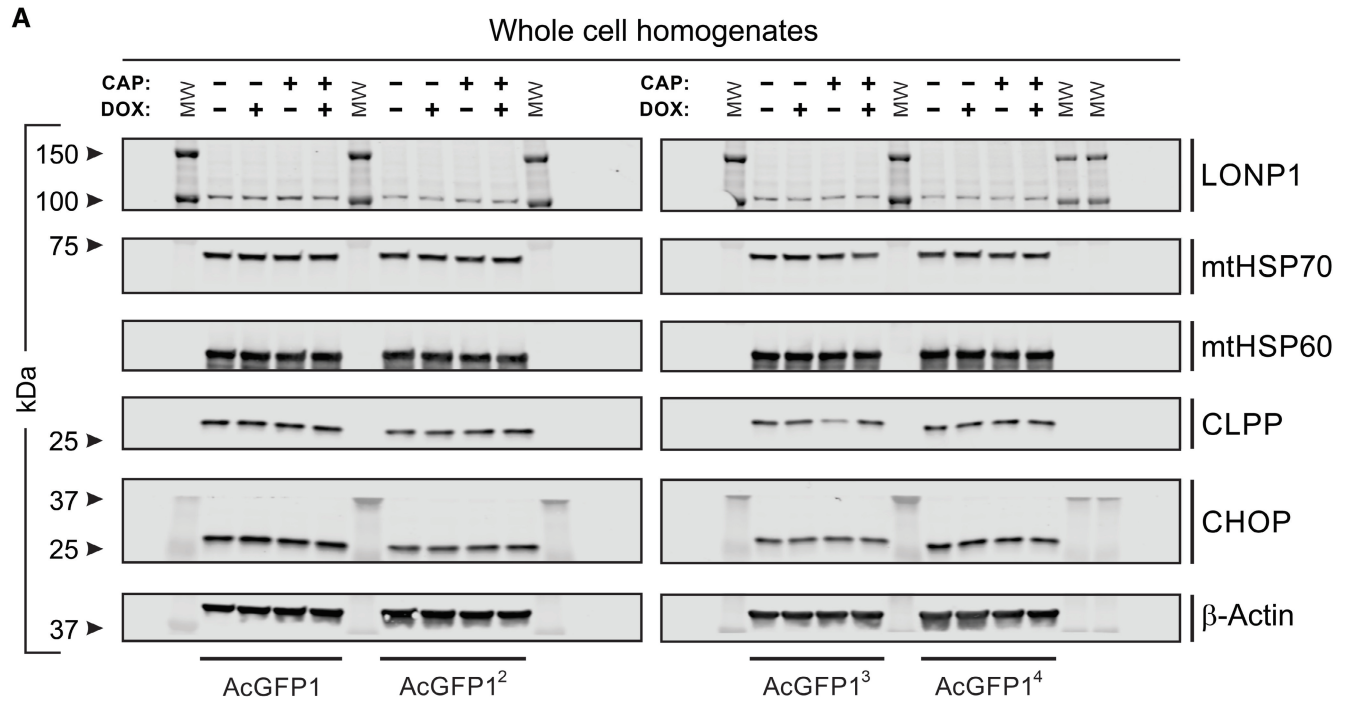
Figure EV2.

Figure EV3. Chloramphenicol (CAP) and doxycycline (DOX) do not increase protein markers typically associated with induction of the mitochondrial unfolded protein response (UPR^{mt}) in parental and FP-expressing cells.

A Western blot analysis (SDS–PAGE) of whole-cell homogenates was used to assess the levels of the following proteins: LONP1, mtHSP70, mtHSP60, CLPP, and CHOP. β-actin was used as a cellular loading control. MW indicates molecular weight in kDa. Effect of the expression inducer DOX (1 µg/ml, 24 h) and CAP (40 µg/ml, 72 h), alone and in combination (i.e., 48-h CAP treatment followed by 24 h CAP + DOX treatment), on protein levels in FP-expressing HeLa cell lines (AcGFP1, AcGFP1[2], AcGFP1[3], and AcGFP1[4]). Individual panels were contrast-optimized for visualization purposes. Original blots are presented in Appendix Fig S4.

B Quantitative analysis of protein levels in panel A and in HeLa parental cells (the open symbols reflect data for the parental cells in Appendix Fig S5B). All signals were normalized on β-actin and expressed as % of the condition without DOX and CAP ("−DOX−CAP"). Effects previously associated with UPR^{mt} induction are marked: "Up" indicates proteins that are expected to be upregulated upon UPR^{mt} induction, "Down "indicates proteins that are expected to be downregulated upon UPR^{mt} induction.

Data information: The effects of DOX, CAP, and DOX + CAP were compared with the "−DOX−CAP" (untreated) condition (i.e., with the dotted line marked "&") by testing whether the mean value for each protein (i.e., within each gray box in panel B) differed from 100% (using a one-sample Student's *t*-test). Comparisons with the DOX only condition (+DOX; marked "a") and CAP-only condition (+CAP; marked "b") were performed using an independent two-population *t*-test (i.e., between each gray box for each protein). Not significant is marked by n.s.



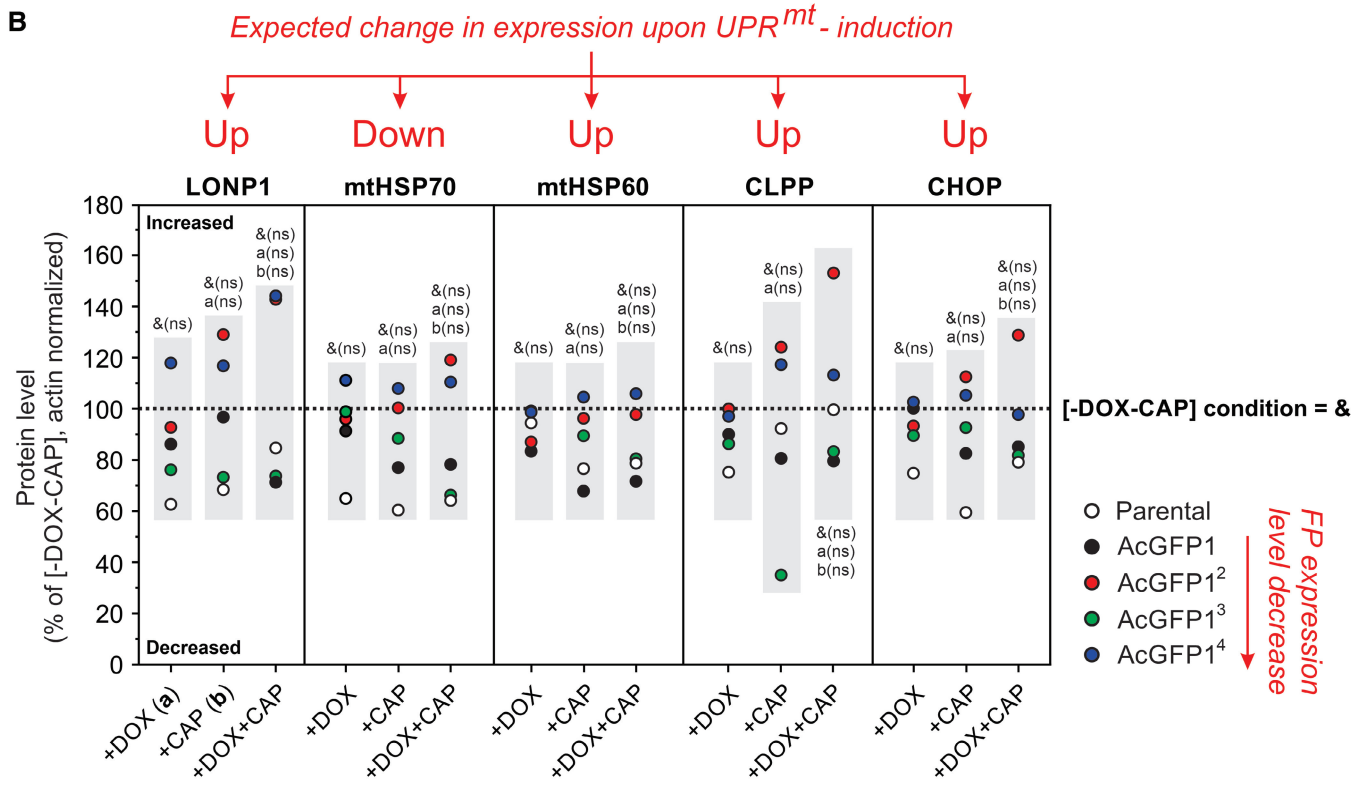


Figure EV3.