Expanded View Figures

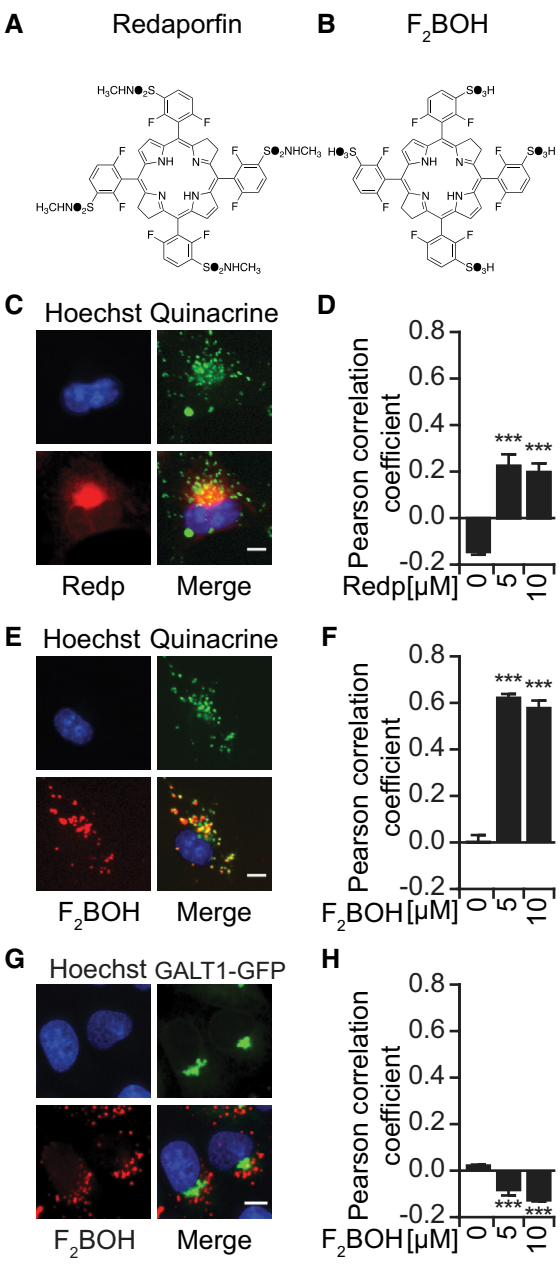

Figure EV1. F_2BOH preferentially accumulates at acidic vesicles.

A, B Molecular structures of the halogenated bacteriochlorins, redaporfin (LUZ11) (A), and F_2BOH (LUZ10) (B).

C–F Representative images of U2OS cells showing the co-occurrence of redaporfin (C, D) and F_2BOH (E, F) with acidic vesicles stained with quinacrine and the respective Pearson correlation coefficients. Scale bar: 10 μm.

G, H Representative images of U2OS-GALT1-GFP cells showing the lack of co-occurrence of F_2BOH with the GA (G) and the corresponding Pearson correlation coefficient (H). Scale bar: 10 μm.

Data information: Data are indicated as means ± SD of triplicates of one representative experiment out of 2–4 repeats. Asterisks indicate significant differences with respect to untreated cells. ***$P < 0.001$ (one-way ANOVA).

Figure EV2. Redaporfin-PDT induces an incomplete ER stress response characterized by the activation of ATF6 and IRE1 arms and phosphorylation of eIF2α, but no expression of the two transcription factors, ATF4 and CHOP.

A, B Representative images of U2OS cells stained for P-eIF2α 2.5 h after PDT with redaporfin (5 μM) or after 6 h of incubation with tunicamycin (2.5 μM) and thapsigargin (2.5 μM) (A), and its quantitative analysis that represents cytoplasmic P-eIF2α fluorescence signal (B). Scale bar: 10 μm.

C, D Representative images of P-eIF2α immunofluorescence in U2OS cells wild-type or KO for each eIF2α kinase after incubation with tunicamycin (2.5 μM, 6 h), thapsigargin (2.5 μM, 6 h), or arsenite trioxide (1 mM, 2 h) (C), and its quantitative analysis that represents cytoplasmic P-eIF2α fluorescence signal (D). Scale bar: 10 μm.

E–G Representative immunoblots (E) and densitometry of ATF4 (F) and CHOP (G) of protein extracts collected from U2OS cells at the indicated time points after redaporfin-PDT or after 12 h of incubation with tunicamycin (2.5 μM) or thapsigargin (2.5 μM).

H, I Representative images of U2OS cells expressing GFP-ATF6 8 h after PDT with redaporfin (5 μM), tunicamycin (2.5 μM), and thapsigargin (2.5 μM) (H), and the quantitative analysis that reflects ATF6 translocation from the cytoplasm into the nucleus (I). Scale bar: 10 μm.

J, K Representative images of U2OS cells expressing XBP1-GFP 20 h after PDT with redaporfin (5 μM), tunicamycin (2.5 μM), and thapsigargin (2.5 μM) (J), and quantitative analysis of XBP1 *de novo* expression based on cellular fluorescence (K). Scale bar: 10 μm.

L, M Impact of ATF6 and IRE1 silencing on the cytotoxicity of PDT with redaporfin (5 μM), which was evaluated at 6 h post-irradiation by double staining with PI and Hoechst 33342 (L) and the quantification of dying ($Hoechst^{bright}$ and PI^{-}) and dead cells (PI^{+} cells) (M). Scale bar: 20 μm.

Data information: Ctr indicates untreated cells and Redp* indicates irradiated cells. Data are indicated as means ± SD of triplicates of one representative experiment out of 2–4 repeats in panels (B), (D), (I), (K), and (M) and as means ± SEM of two independent experiments in panels (F) and (G). Asterisks indicate significant differences with respect to untreated cells, $^{**}P < 0.01$, $^{***}P < 0.001$ (one-way ANOVA).

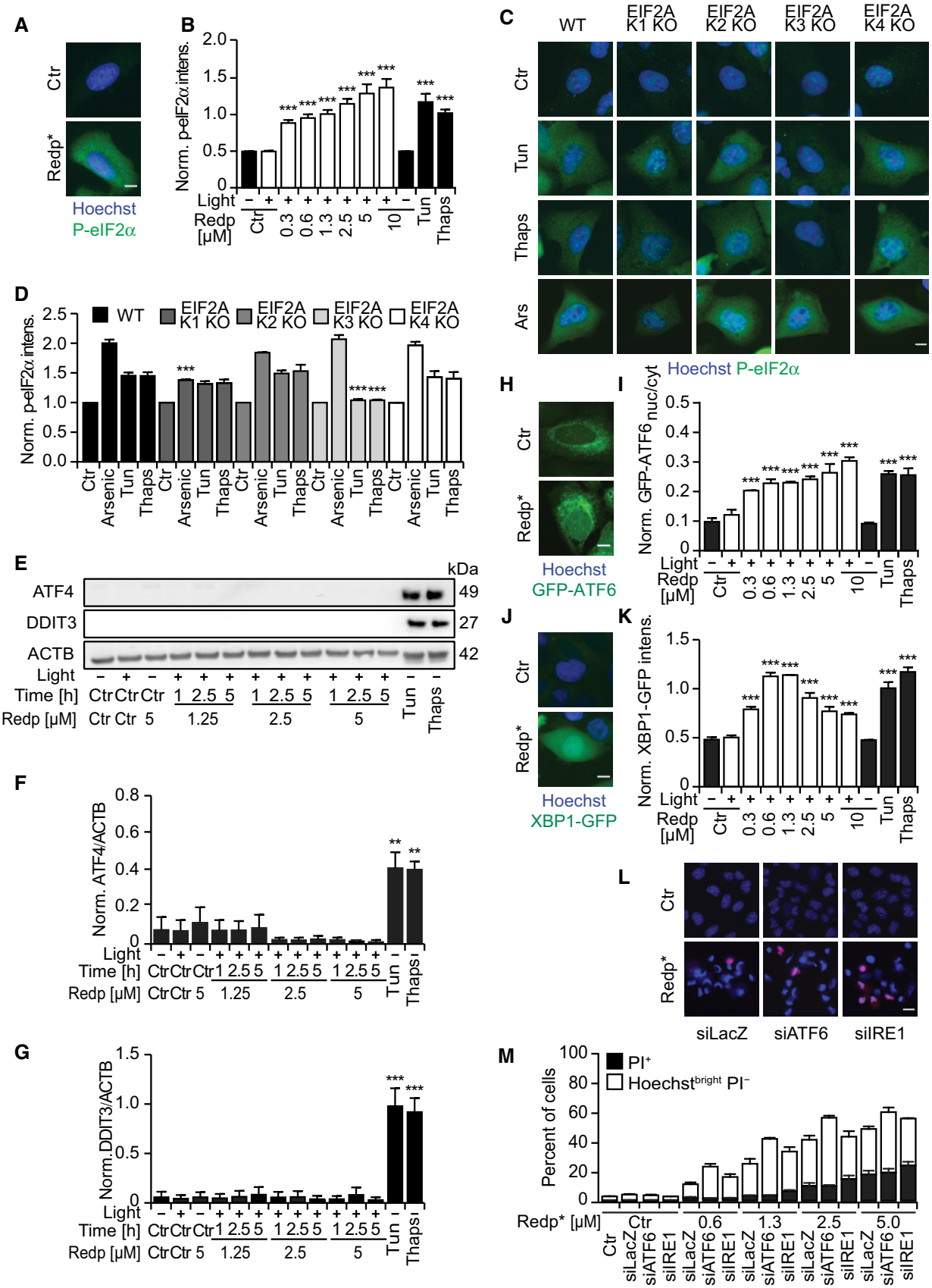
A
Ctr
Redp*
Hoechst
P-eIF2α
B
Norm. p-eIF2α intens.
Light
Redp [μM]
Ctr
0.3
0.6
1.3
2.5
5
10
Tun
Thaps
C
WT
EIF2A K1 KO
EIF2A K2 KO
EIF2A K3 KO
EIF2A K4 KO
Ctr
Tun
Thaps
Ars
Hoechst P-eIF2α
D
Norm. p-eIF2α intens.
WT
EIF2A K1 KO
EIF2A K2 KO
EIF2A K3 KO
EIF2A K4 KO
Ctr
Arsenic
Tun
Thaps
E
kDa
ATF4
DDIT3
ACTB
49
27
42
Light
Time [h]
Redp [μM]
F
Norm. ATF4/ACTB
G
Norm.DDIT3/ACTB
H
Ctr
Redp*
Hoechst
GFP-ATF6
I
Norm. GFP-ATF6 nuc/cyt
J
Ctr
Redp*
Hoechst
XBP1-GFP
K
Norm. XBP1-GFP intens.
L
Ctr
Redp*
siLacZ
siATF6
siIRE1
M
Percent of cells
PI+
Hoechstbright PI−
Redp* [μM]
Ctr
0.6
1.3
2.5
5.0

Figure EV2.

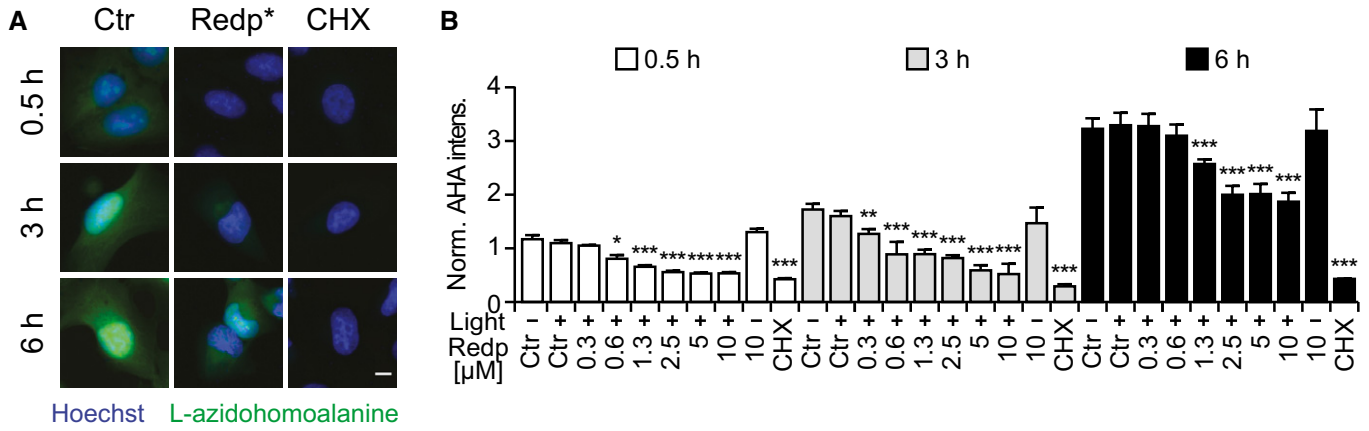

Figure EV3. Partial inhibition of protein synthesis by redaporfin-PDT.

A, B Representative images of newly synthesized proteins evaluated by detection of L-azidohomoalanine in U2OS cells submitted to PDT with redaporfin (5 μM) in methionine-free conditions and cycloheximide (CHX, 50 μM) (A), and quantitative analysis that depicts fluorescence signal on the cytoplasm (B).

Data information: Ctr indicates untreated cells and Redp* indicates irradiated cells. Data are indicated as means ± SD of triplicates of one representative experiment out of 2–4 repeats. Asterisks indicate significant differences with respect to untreated cells, ***$P < 0.001$ (one-way ANOVA). Scale bars: 10 μm.

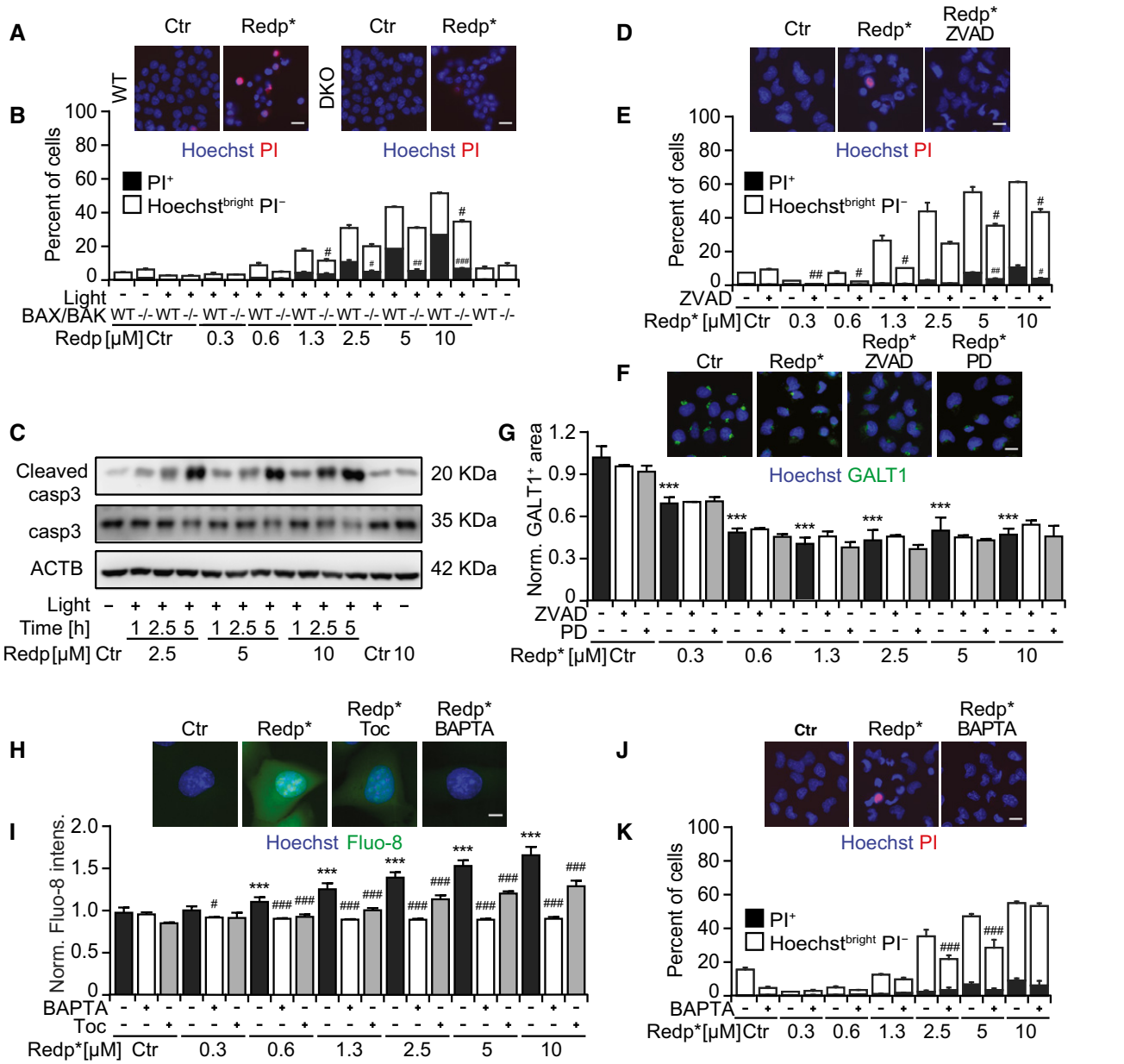

Figure EV4. Reduced cytotoxicity of redaporfin-PDT in the presence of the antioxidant Toc, the pan-caspase inhibitor ZVAD, the calcium chelator BAPTA, and BAX/BAK knockout.

A, B Viability of HCT116 and HCT116 BAX$^{-/-}$BAK$^{-/-}$ cells after treatment with redaporfin-based PDT. Scale bar: 20 μm.

C Representative immunoblot of cleaved caspase-3 in TC1 cells collected at the indicated time points after redaporfin-PDT.

D, E Viability of U2OS cells after treatment with redaporfin-PDT, in the presence of Z-VAD-fmk (ZVAD; 50 μM). Scale bar: 20 μm.

F, G Representative images of U2OS cells stained for GALT1, 6 h after PDT with redaporfin (5 μM), in the presence or absence of ZVAD (50 μM) or PD150606 (40 μM) (C), and the analysis that reflects the average area of GALT1$^+$ Golgi structures per cell (D). Scale bar: 20 μm.

H, I Representative images of calcium release evaluated by means of Fluo-8-AM in U2OS cells incubated with redaporfin (5 μM) for 20 h, with an additional incubation of 4 h with Toc (250 μM) or BAPTA-AM (5 μM) (H), and its quantification based on Fluo-8-AM fluorescence (I). Scale bar: 10 μm.

J, K Viability of U2OS cells after treatment with redaporfin-PDT, in the presence of BAPTA (5 μM). Scale bar: 20 μm.
Viability was assessed 6 h after irradiation by staining the cells with the vital dye propidium iodide (PI) and the chromatin dye Hoechst 33342. Cell death was quantified according to phenotypic groups divided into dying (Hoechst^{bright} PI$^-$) and dead cells (PI$^+$ cells).

Data information: Ctr indicates untreated cells and Redp* indicates irradiated cells. Data are indicated as means ± SD of triplicates of one representative experiment out of 2–4 repeats. Asterisks indicate significant differences with respect to untreated cells (one-way ANOVA), whereas hashes indicate significant differences for redaporfin-PDT in the presence or absence of the indicated inhibitor (two-way ANOVA), $^{***}P < 0.001$. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

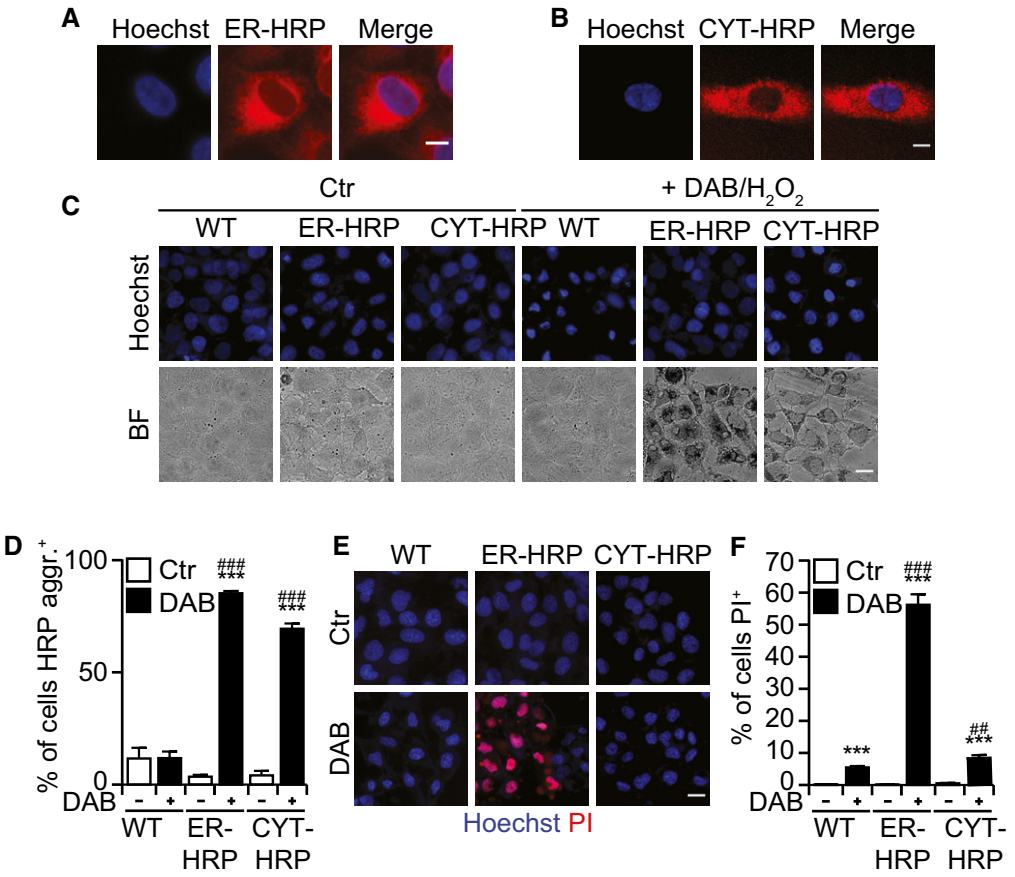

Figure EV5. ER-HRP and cytoplasmic HRP.

A, B Cells that stably express HRP in the ER (A) or at cytoplasm (B). Scale bar: 10 μm.

C–F DAB polymerization after treatment with DAB/H_2O_2 for 24 h at 37°C (C), percentage of cells with DAB aggregates (D), and quantification of dead cells (PI^+ cells) (E, F). Scale bar: 20 μm.

Data information: Bars indicate means ± SD of triplicates of one representative experiment out of two repeats. Asterisks indicate significant differences with respect to untreated cells, whereas hashes indicate significant differences between WT cells and HRP-expressing cells, $^{***}P < 0.001$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ (two-way ANOVA).

