Optogenetic Engineering of BAX to Control Mitochondrial Permeabilization and Prevent Apoptosis in Cells

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Supplementary information 1

Figure S1. Graphic representation of the sequences

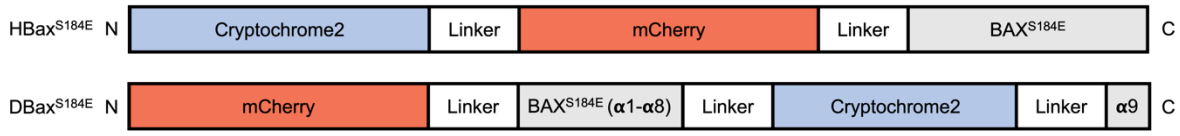

Table S1. Gene sequences

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DBT^{S184E} (mCh::BAX α1-α8::CRY2::α9^{S184E})

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Table S2. Experimental Ingredients

Antibodies			
Product Name	**Supplier**	**Identifier**	**Concentration**
Anti-Bax monoclonal antibody	Santa Cruz Biotechnology	sc-7480	1:200
Anti-Tomm20 polyclonal antibody	Abcam	ab78547	1:1000
Recombinant Anti-Bax antibody	Abcam	ab182733	1:1000
mCherry (E5D8F) Rabbit mAb	Cell Signaling Technology	43590S	1:500
DsRed Antibody (E-8)	Santa Cruz Biotechnology	sc-390909	1:200
GFP Antibody (B-2)	Santa Cruz Biotechnology	sc-9996	1:200
Apaf-1 (D5C3) Rabbit mAb	Cell Signaling Technology	8969	1:200
Cleaved Caspase-3 (Asp175) Antibody	Cell Signaling Technology	9661	1:200
Cytochrome c (6H2.B4) Mouse mAb	Cell Signaling Technology	12963	1:200
Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor 488	Invitrogen	A11001	1:200
Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody Alexa Fluor 568	Invitrogen	A11011	1:200
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody Alexa Fluor™ 350	Invitrogen	A-11046	1:200
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody Alexa Fluor™ 350	Invitrogen	A-11045	1:200
mounting medium with DAPI	Ibidi	50011	Dropwise
Chemicals			
Dimethyl Sulfoxide (DMSO)	Sigma Aldrich	317275	
Cisplatin	Sellekhem	SELLECK_S1166	
Plasmids			
Tomm20-Cib1-GFP	Addgene	#117242	
DBT^{S184E}	This study		
Software			
GraphPad Prism	GraphPad Software		
ImageJ	National Institutes of Health		
AlphaFold2	neurosnap.ai/		
RCSB PDB	rcsb.org		

Figure S2. Blue light chamber design (A–G) A custom-built light irradiation box with a 450 nm LED board was generated. Polystyrene covers placed on top of the LEDs acted as illumination surfaces for the culture plates. (H) Transfected cells were prepared according to the written procedures. Twenty-four hours after seeding on a plate, the cells were transfected with plasmids. FAD was added to cells 24 h post-transfection. Finally, 48 h post-transfection, the cells were irradiated with blue light in the light chamber.

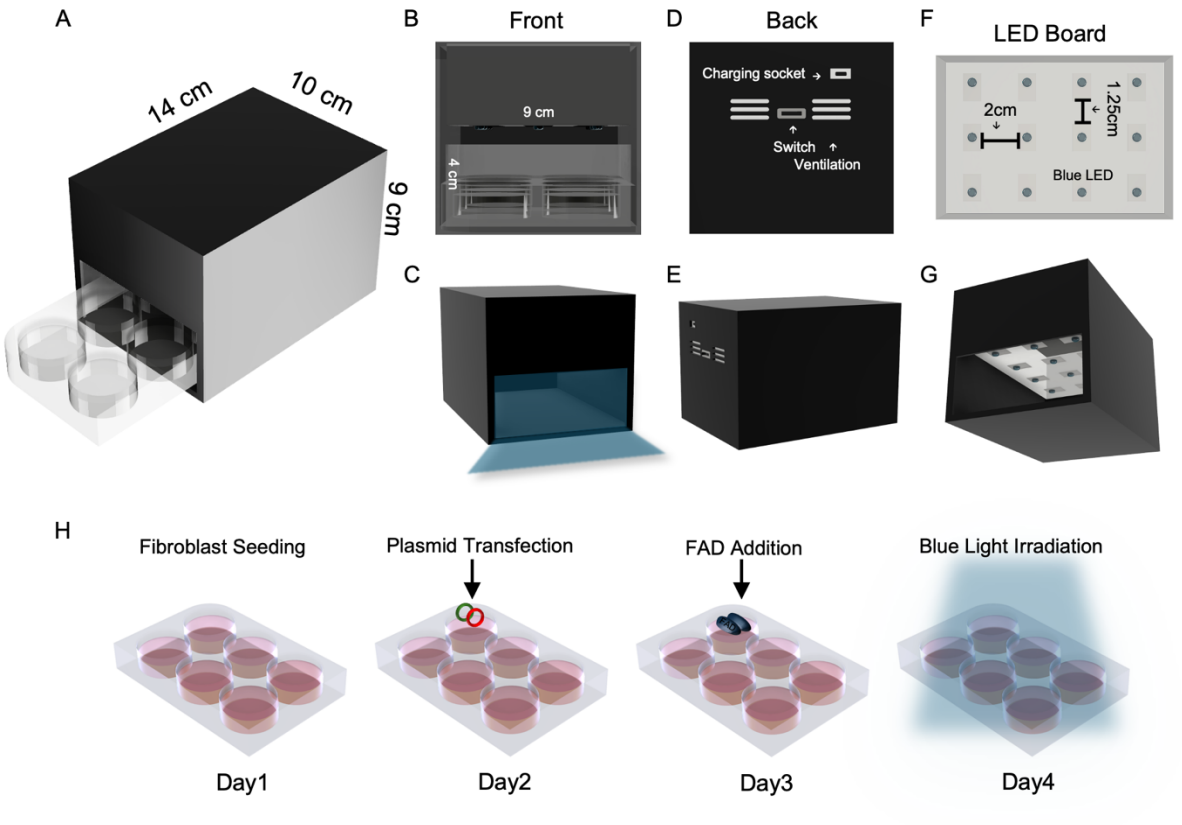

Figure S3. 3D rendered images of rBAX-rTOMM20 conjugated structures from multiple perspectives. endo-BAX is shown in red, rBAX in yellow, and rTOMM20 in cyan. These images, related to Figure 2, illustrate the spatial distribution and interactions among these mitochondrial proteins under each conditions: (A) 3D-rendered images of CTRL (rTOMM20-only transfected fibroblasts), (B) HBT-transfected fibroblasts, and (C) DBT-transfected fibroblasts. Scale bar: 1 μm

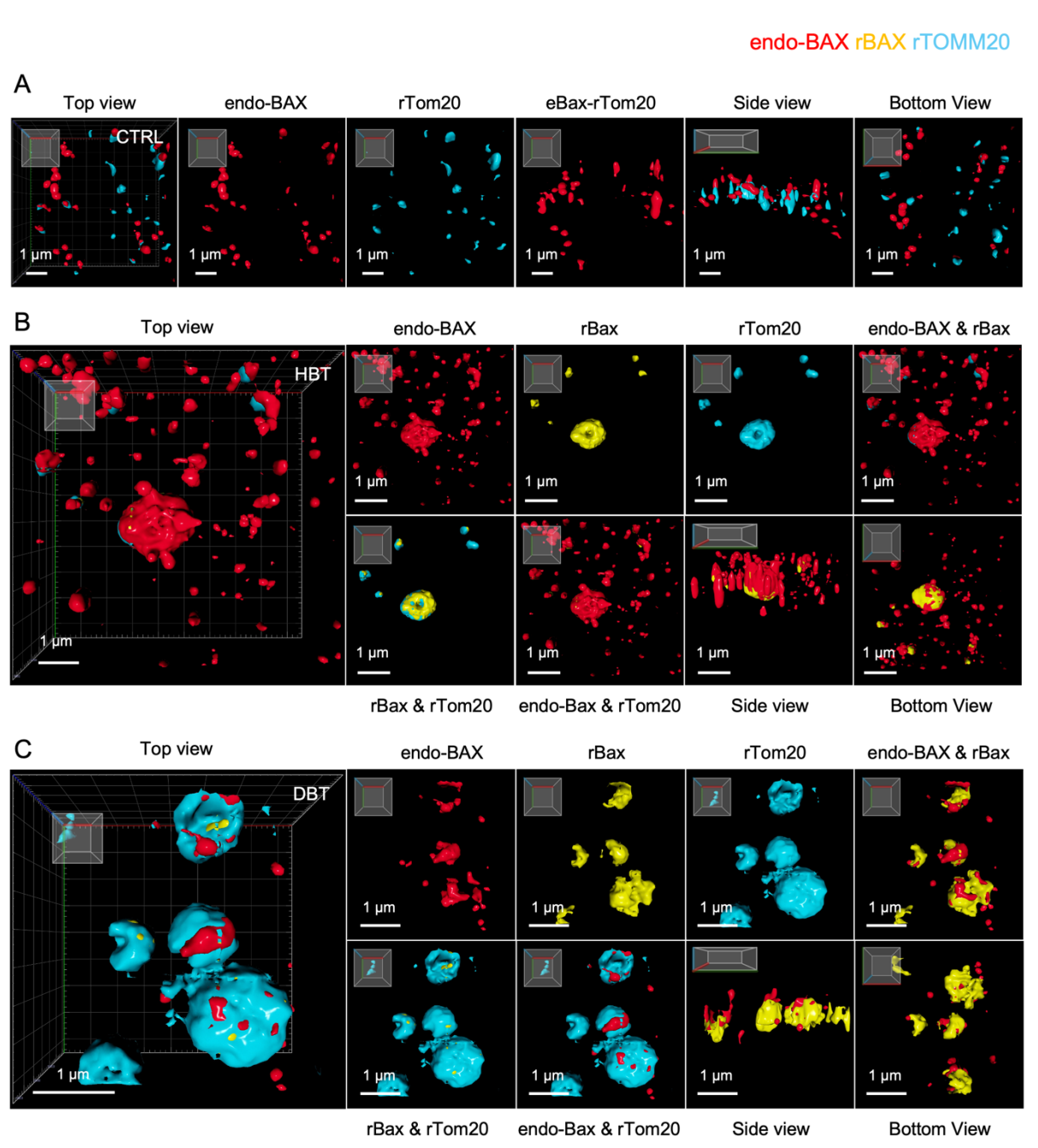

Figure S4. Quantification of the average gray value for the indicated cellular components is shown (related to Figure 3) (A–D) Average intensity profiles from multiple ROIs in the microscopy images under each condition are presented. The red line represents endo-BAX intensity, the cyan line denotes rTOMM20 intensity, and the yellow line indicates rBAX intensity (Mean ± SEM). (E) Correlation analysis was performed to evaluate the colocalization of endo-BAX and rBAX in the selected regions (n = 205 for all conditions): for HBT under blue-light irradiation (+HBT), R^2 = 0.5084 ($p < 0.0001$); for HBT in the dark (-HBT), R^2 = 0.1974 ($p < 0.0001$); for DBT under blue-light irradiation (+DBT), R^2 = 0.4192 ($p < 0.0001$); and for DBT in the dark (-DBT), R^2 = 0.4043 ($p < 0.0001$). Notably, the overall endo-BAX intensity was low in DBT conditions. (F) Correlation analysis of rTOMM20 and rBAX in the same regions (n = 205 for all) yielded the following: for +HBT, R^2 = 0.8735 ($p < 0.0001$); for -HBT, R^2 = 0.3018 ($p < 0.0001$); for +DBT, R^2 = 0.8523 ($p < 0.0001$); and for -DBT, R^2 = 0.118 ($p < 0.0001$). Overall, blue-light irradiation increased the colocalization of rBAX and rTOMM20, reflecting enhanced spatial association of these proteins. HBT, HBT-transfected fibroblasts; DBT, DBT-transfected fibroblasts; mean ± SEM for all

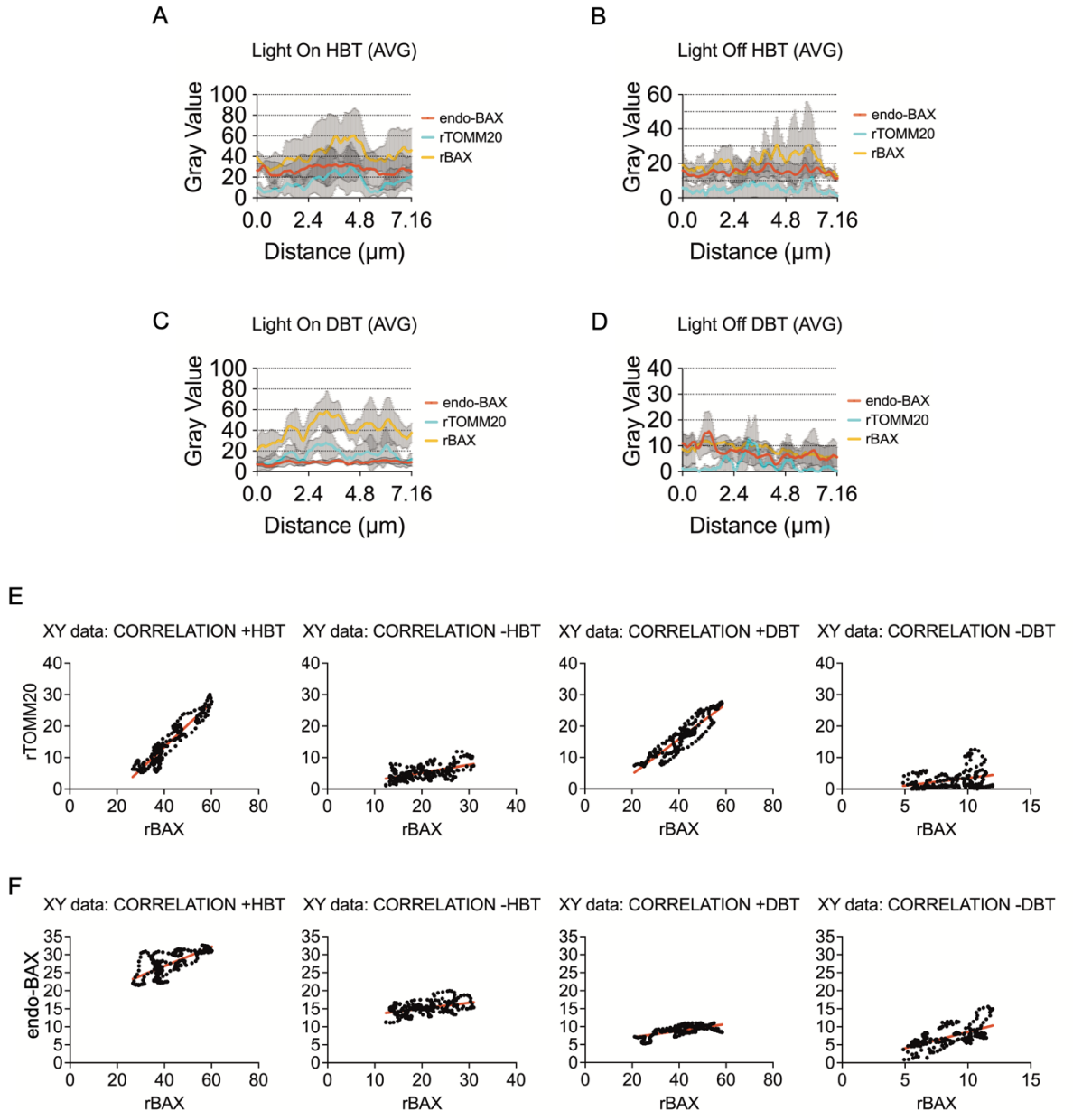
A
Light On HBT (AVG)
Gray Value
Distance (µm)
endo-BAX
rTOMM20
rBAX
B
Light Off HBT (AVG)
C
Light On DBT (AVG)
D
Light Off DBT (AVG)
E
XY data: CORRELATION +HBT
XY data: CORRELATION -HBT
XY data: CORRELATION +DBT
XY data: CORRELATION -DBT
rTOMM20
rBAX
F
endo-BAX

Figure S5. Co-localization analysis For the co-localization analysis, the immunostained images were color-thresholded to highlight specific regions: red and green for individual targets and orange (overlay regions) for areas of co-localization. Consistent settings were applied across all experimental groups.

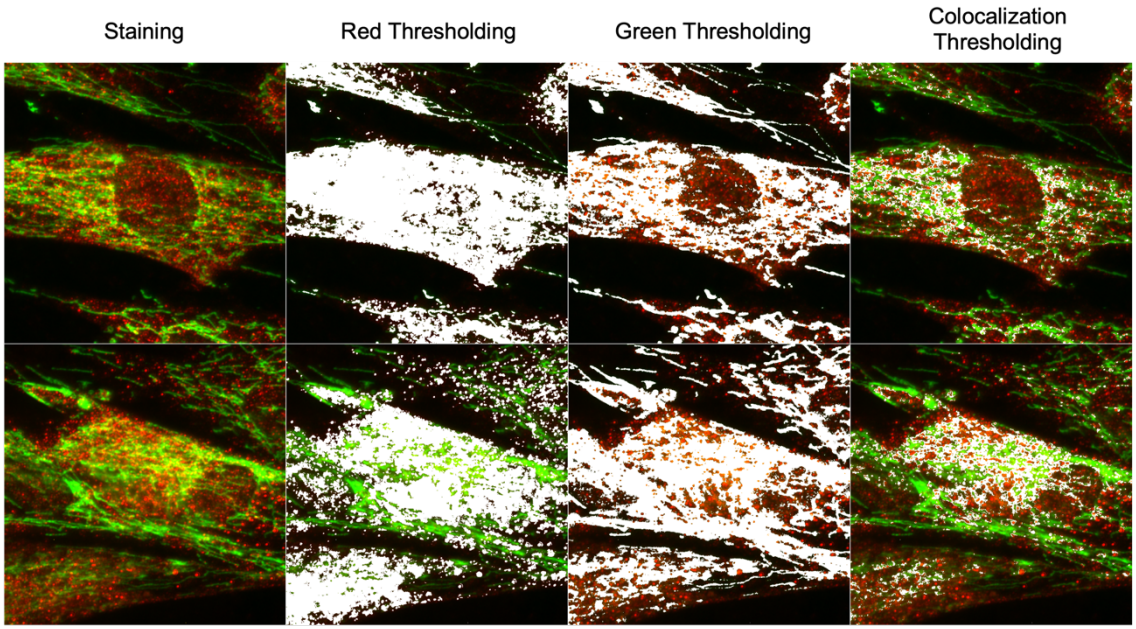

Figure S6. BAX size analysis For measurements of BAX aggregation size, immunostained 8-bit images were thresholded using the Shanbhag mode (100–255) in the ImageJ software. The particles were size-dependently filtered and counted, and the highly saturated areas were excluded from the analysis. Consistent settings were applied across all experimental groups.

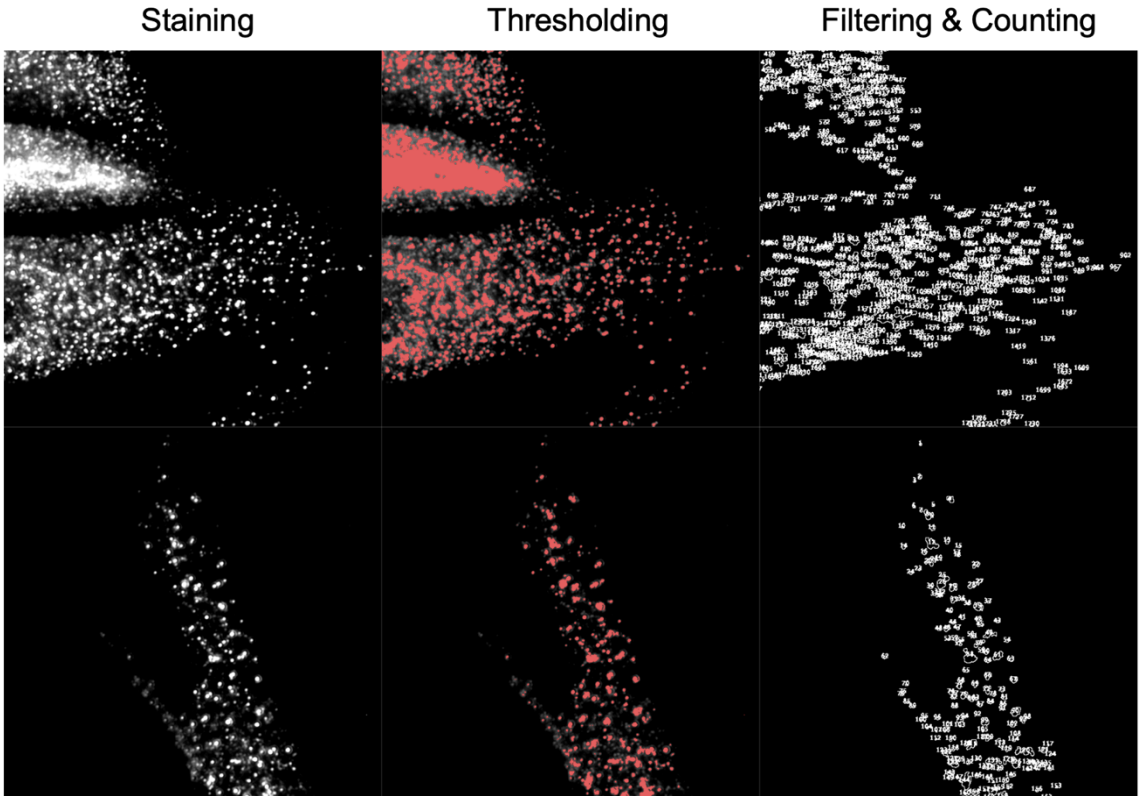

Figure S7. Mitochondrial fission analysis For the mitochondrial fission analysis, immunostained 8-bit images were thresholded using the MaxEntropy mode (35–255) in the ImageJ software. The particles were size-dependently filtered and counted, with consistent settings applied across all experimental groups for the analysis.

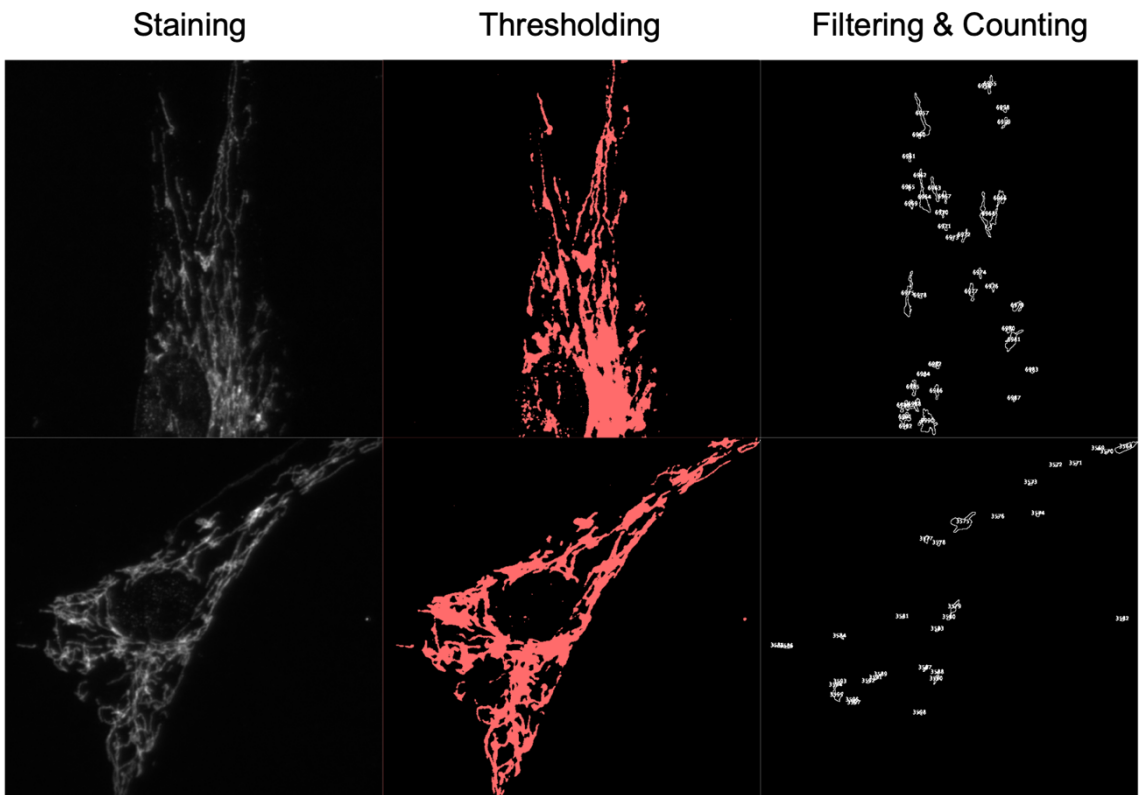

Figure S8. Color-merged immunostained images (dark-incubated conditions) Dark-incubated cells without blue light irradiation were fixed 48 h post-transfection and stained with anti-BAX and anti-TOMM20 antibodies to analyze cellular and subcellular morphology and localization. Scale bar: 100 μm; Red: endo-BAX; Green: endo-TOMM20; Blue: DAPI

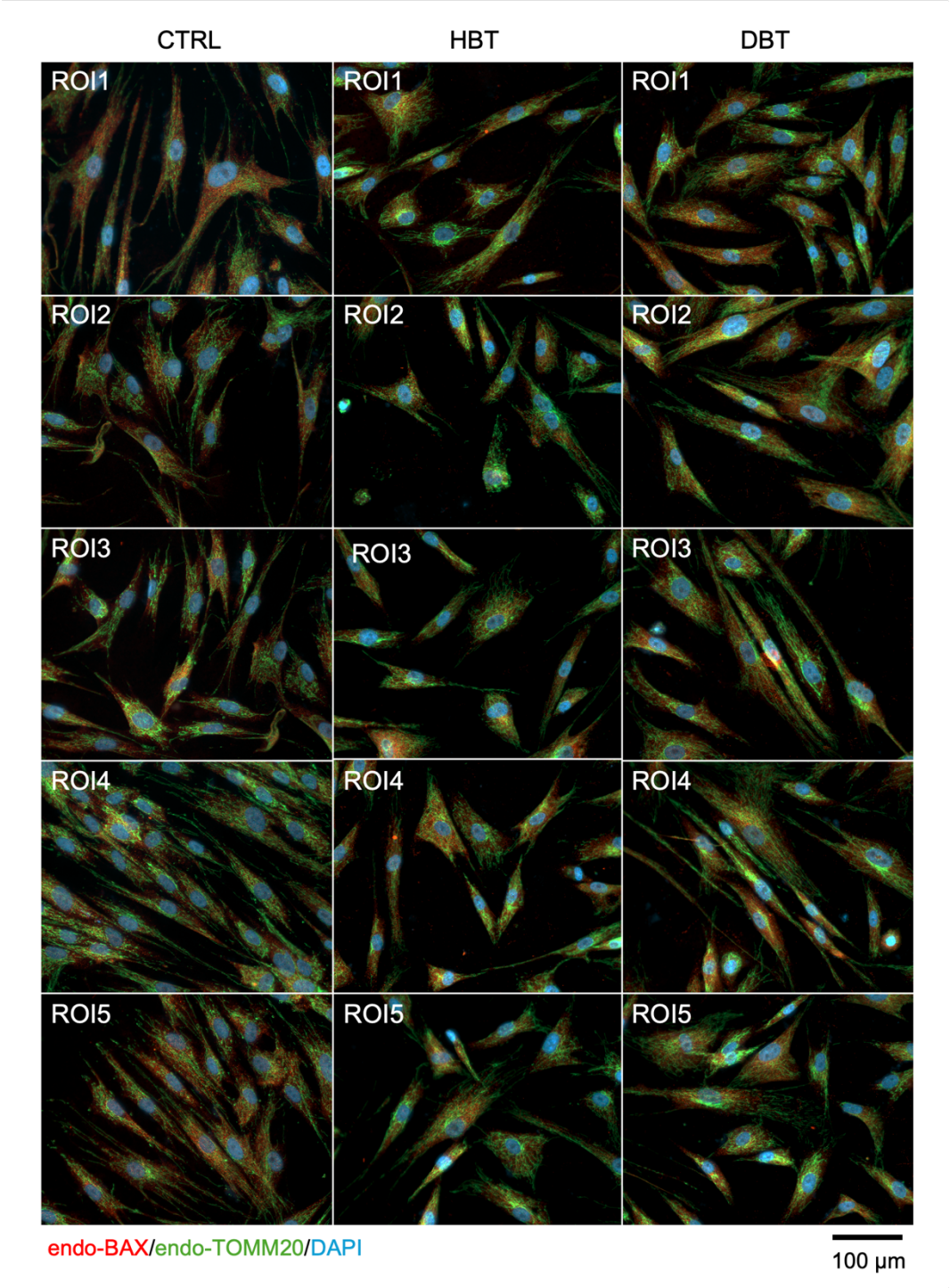

Figure S9. Immunostained images of endo-BAX in cells and their analysis (dark-incubated conditions) (A) Dark-incubated cells without blue light irradiation were stained with anti-BAX antibody to label endo-BAX in cells for analysis of their aggregation size. Scale bar: 20 μm; Red: BAX; Blue: DAPI (B, C) Bar graphs showing the aggregation size and perimeter of BAX measured by the fluorescently labelled dots in each cell (n = 300 for all, one-way ANOVA, post hoc: Tukey's multiple comparisons test). ns, non-significant; CTRL, rTOMM20 only-transfected fibroblasts; HBT, HBT-transfected fibroblasts; DBT, DBT-transfected fibroblasts; mean ± SEM for all

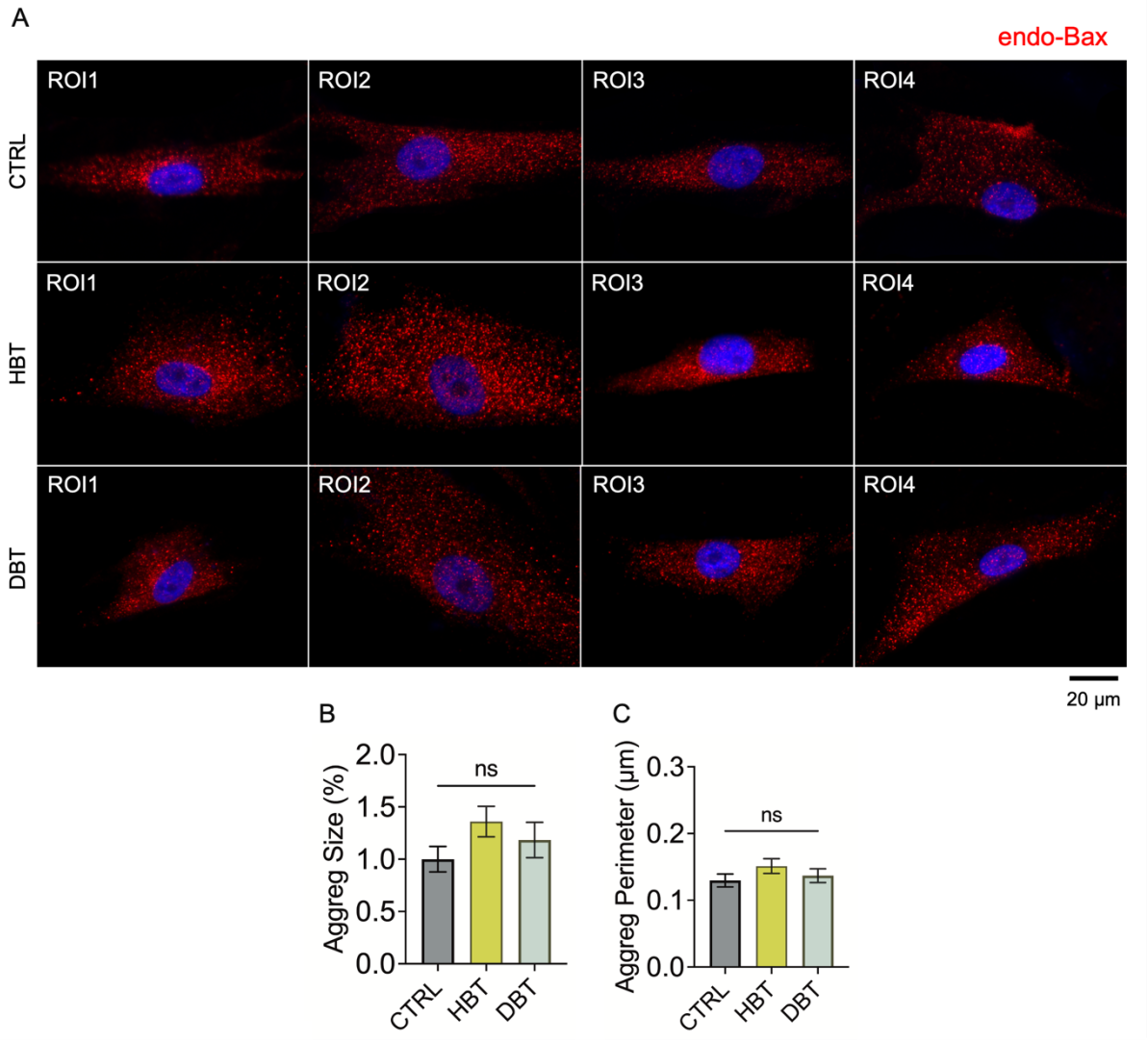

Figure S10. DAPI-stained images and analysis (dark-incubated conditions) (A) Dark-incubated cells without blue light irradiation were DAPI-stained to analyze their number and subcellular morphology. Scale bar: 100 μm. (B) Bar graph showing the number of DAPI counts in each ROI image per condition (ns, non-significant; n = 8 for all, one-way ANOVA, post hoc: Tukey's multiple comparisons test). (C) Bar graph showing the morphological analysis of nuclear solidity in each condition (n = 40 for all, one-way ANOVA, post-hoc: Tukey's multiple comparison test). ns, non-significant; CTRL, rTOMM20 only-transfected fibroblasts; HBT, HBT-transfected fibroblasts; DBT, DBT-transfected fibroblasts; mean ± SEM for all

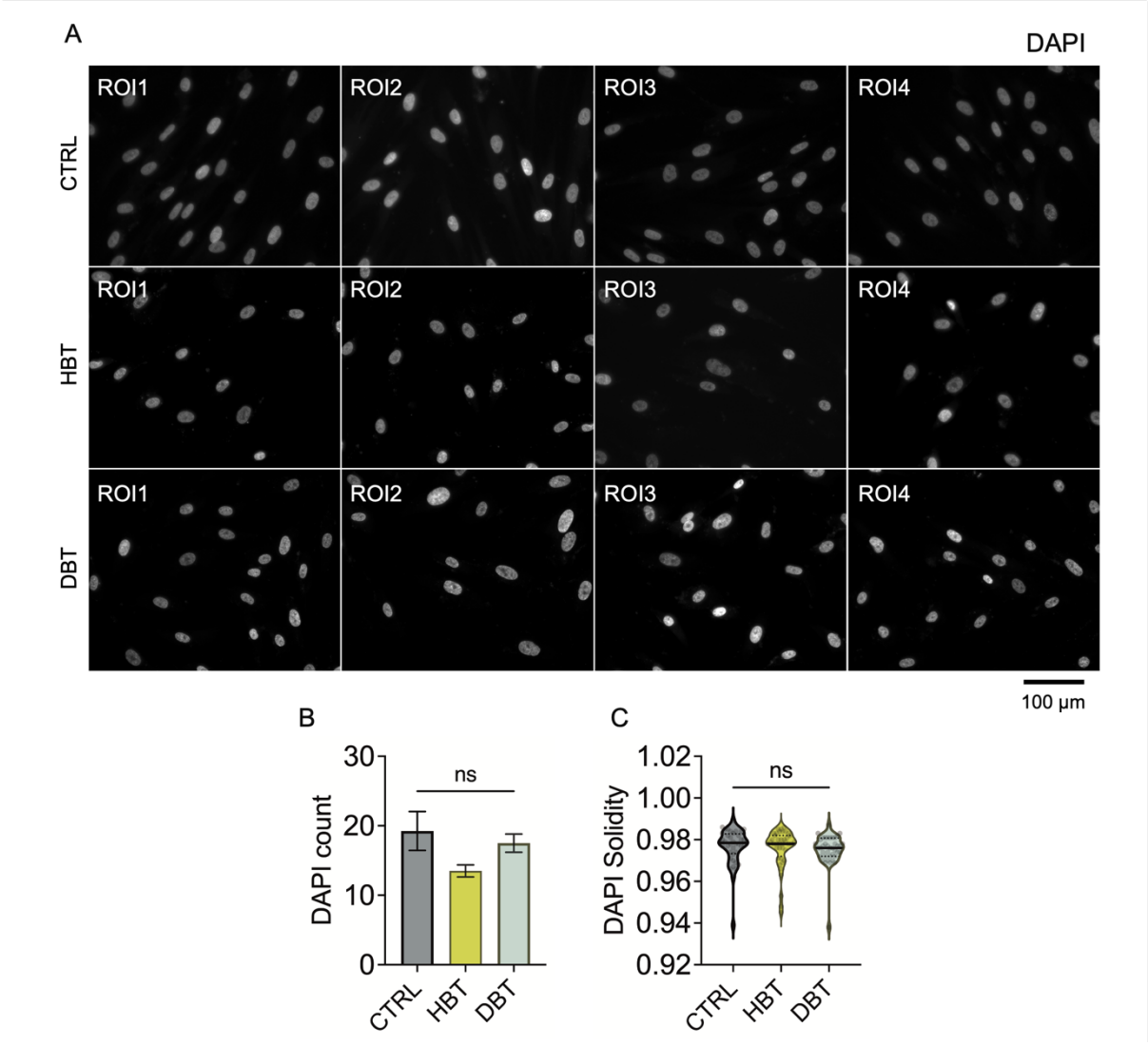

Figure S11. Immunostained images of endo-TOMM20 in cells and their analysis (dark-incubated conditions) (A) Dark-incubated cells without blue light irradiation were stained with anti-TOMM20 antibody to label endo-TOMM20 in cells for analyzing the mitochondrial fission. Scale bar: 20 μm; Green: TOMM20; Blue: DAPI. (B–D) Bar graphs showing the size measurements of the mitochondrial perimeter, aspect ratio, and circularity after dark incubation (n = 300 for all, one-way ANOVA, post hoc: Tukey's multiple comparisons test). ns, non-significant; CTRL, rTOMM20 only-transfected fibroblasts; HBT, HBT-transfected fibroblasts; DBT, DBT-transfected fibroblasts; mean ± SEM for all

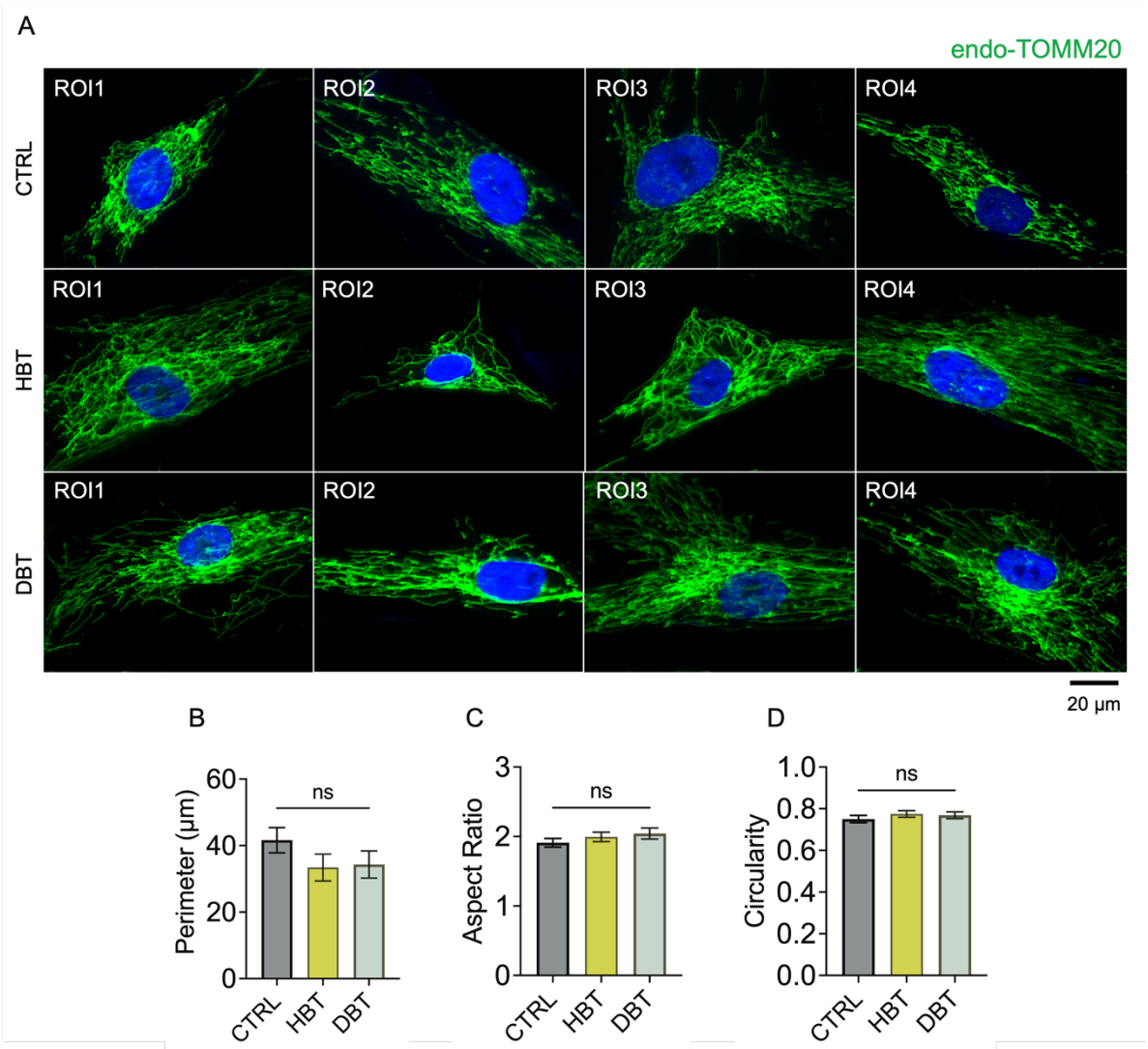

Figure S12. Immunostained images of CytC in cells and their analysis under drug-induced apoptotic conditions (blue light-incubated conditions) (A) (A) Blue-light incubated cells were stained with CytC and rBAX to assess their distribution and colocalization. Scale bar: 10 µm. (B–C) Intensity plot profiles along the X-axis of boxed regions from representative images of HBT (ROI1) and DBT (ROI4) are shown. (D) A bar graph displays the morphological analysis of CytC integrity using the aspect ratio (AR) in each condition (n = 300 for all; one-way ANOVA with Tukey's post hoc test). *p < 0.05; **p < 0.01; ns, non-significant; mean ± SEM

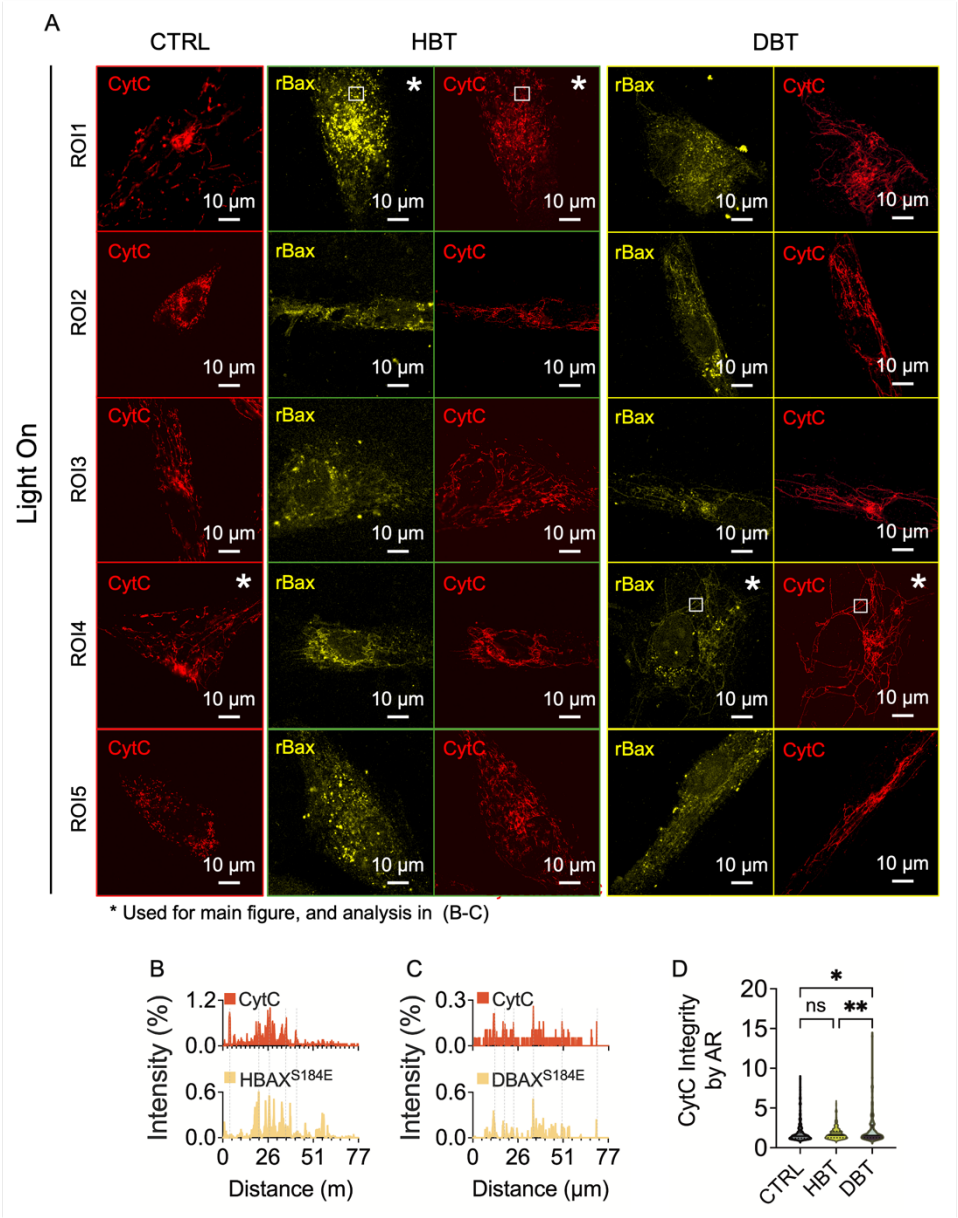

Figure S13. Immunostained images of CytC in cells and their analysis under drug-induced apoptotic conditions (dark-incubated conditions) (A) Dark-incubated cells were stained with CytC and rBax to evaluate their distribution and colocalization. Scale bar: 10 µm. (B) A bar graph illustrates CytC integrated density, with statistical analysis via one-way ANOVA and Tukey's post hoc (n = 7 for all). ns, non-significant (C) A bar graph presents the morphological analysis of CytC integrity, measured by aspect ratio (AR) in each condition (n = 300 for all; one-way ANOVA with Bonferroni's multiple comparisons test); ns, non-significant; mean ± SEM for all

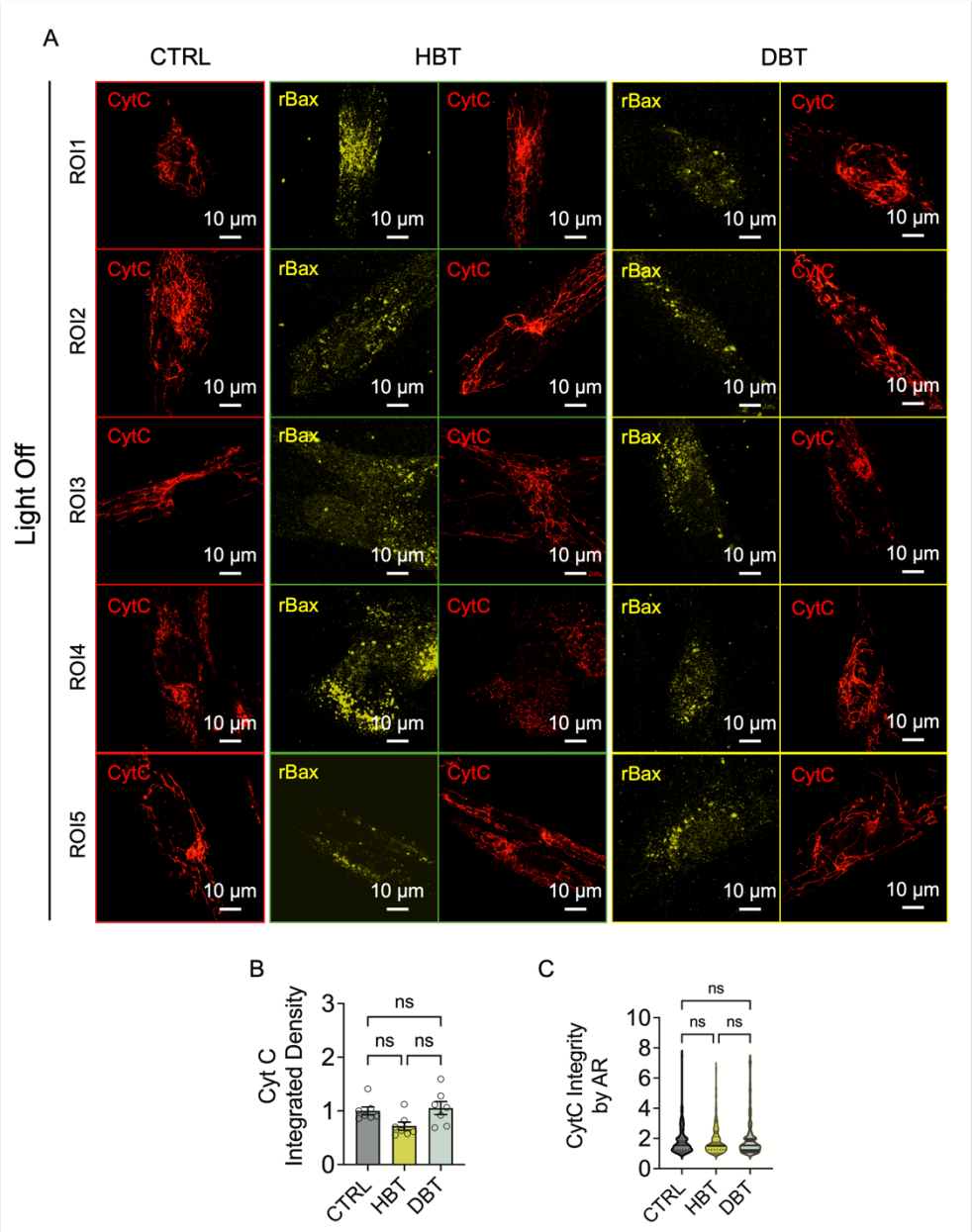

Figure S14. 3D-rendered immunofluorescence images showing the colocalization of CC3 with rBAX and rTOMM20 under drug-induced apoptotic conditions. The merged channels reveal overlapping signals that highlight the spatial association between apoptotic markers and mitochondrial structures. In these images, CC3 is shown in red, rBAX in yellow, and rTOMM20 in cyan. Specifically, (A) shows CTRL conditions (rTOMM20-only transfected fibroblasts), (B) depicts HBT-transfected fibroblasts, and (C) displays DBT-transfected fibroblasts, providing a comparative view of protein distribution across the different experimental setups (related to Figure 5).

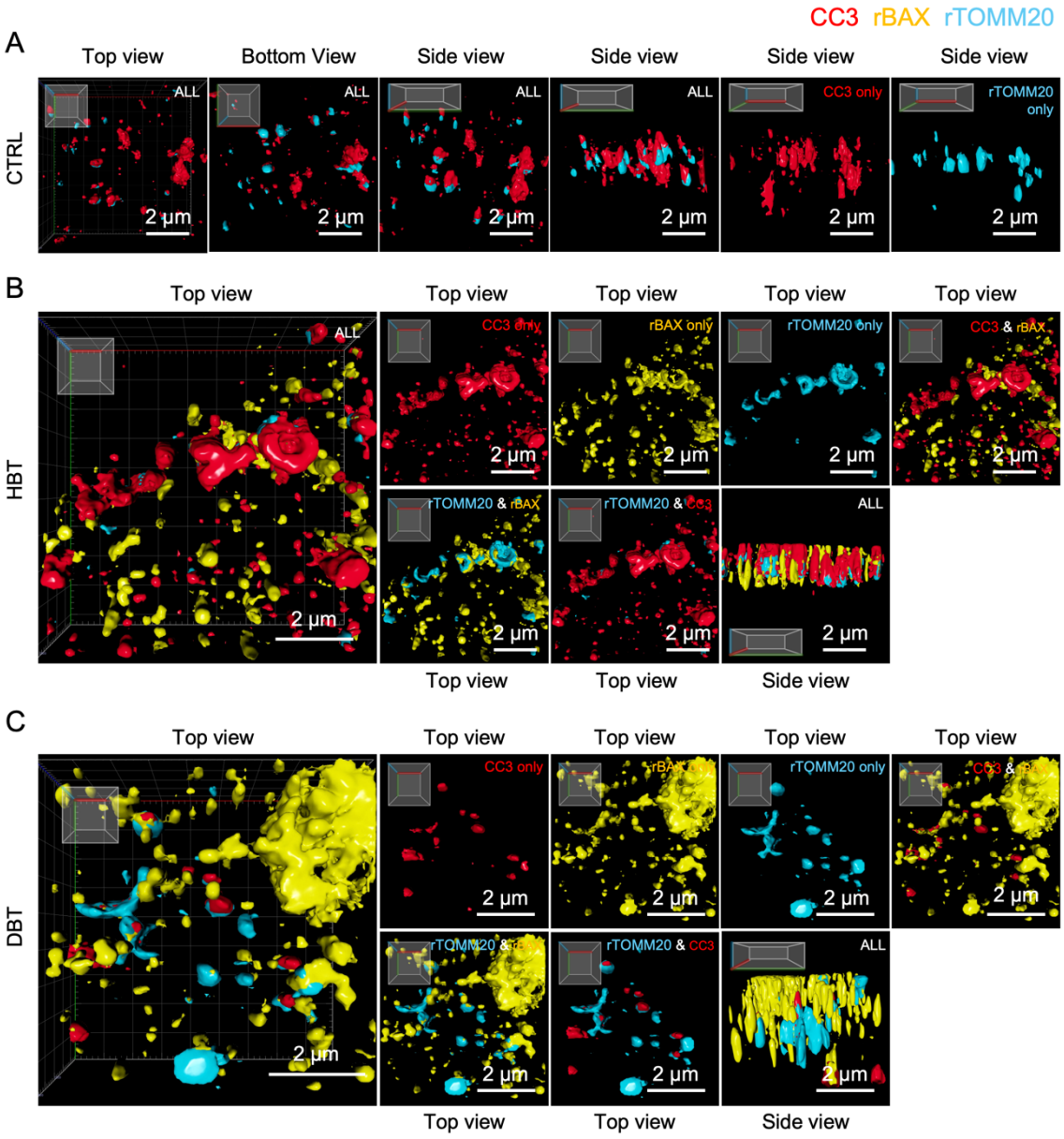

Figure S15. Immunostained images of CC3 in cells under drug-induced apoptotic conditions and their analysis (blue light-incubated conditions) (A) Blue -Light incubated cells were stained with CC3 and rBax to evaluate their distribution and colocalization. Scale bar: 10 μm.

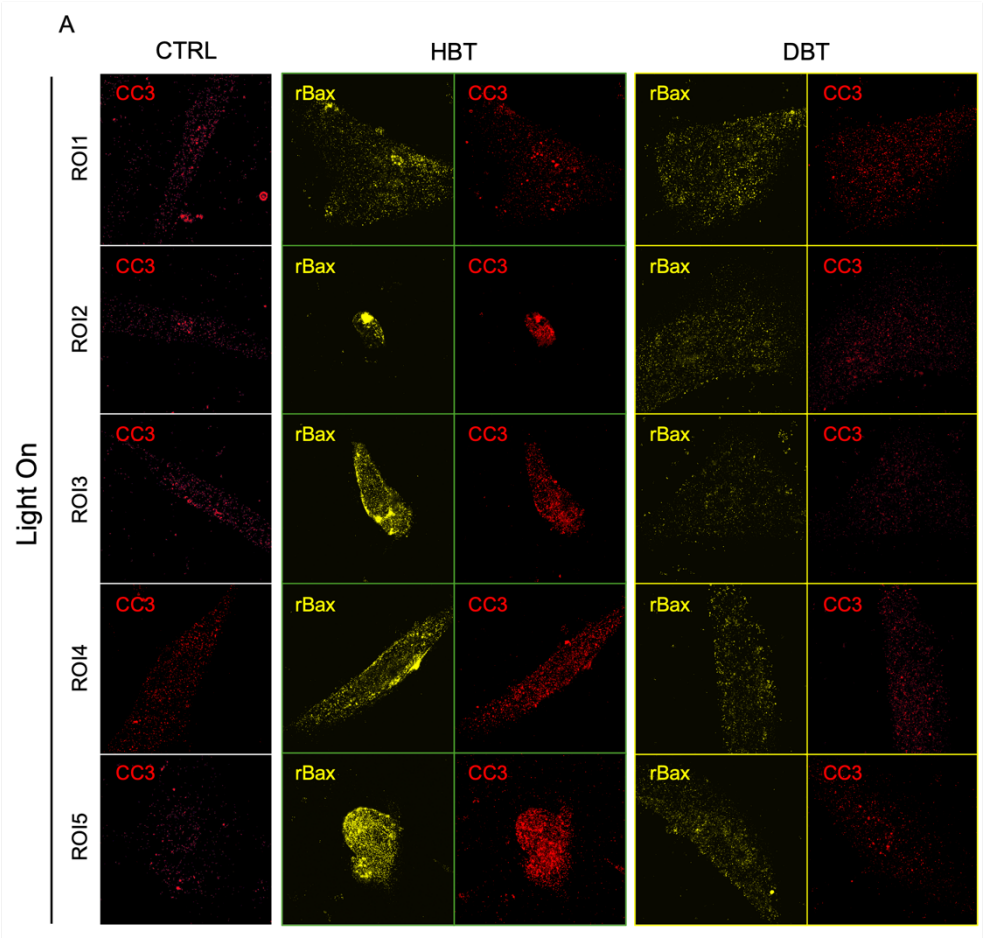

Figure S16. Immunostained images of CC3 in cells under drug-induced apoptotic conditions and their analysis (dark-incubated conditions) (A) Dark-incubated cells stained with CC3 and rBax to evaluate their distribution. Scale bar: 10 μm. (B) A correlation analysis of rBAX–CC3, with the R^2, slope, and p-value reported (n = 8 for all). The results indicate no significant distributional relevance between the two proteins. (C) Analysis of normalized CC3 levels per cell was conducted using one-way ANOVA with Tukey's post hoc test (n = 9 for all). ns, non-significant; mean ± SEM for all