Supplementary Information

RAF1 activation drives epithelial cell competition through parallel mitochondrial dysfunction and GAS7-dependent protrusion formation

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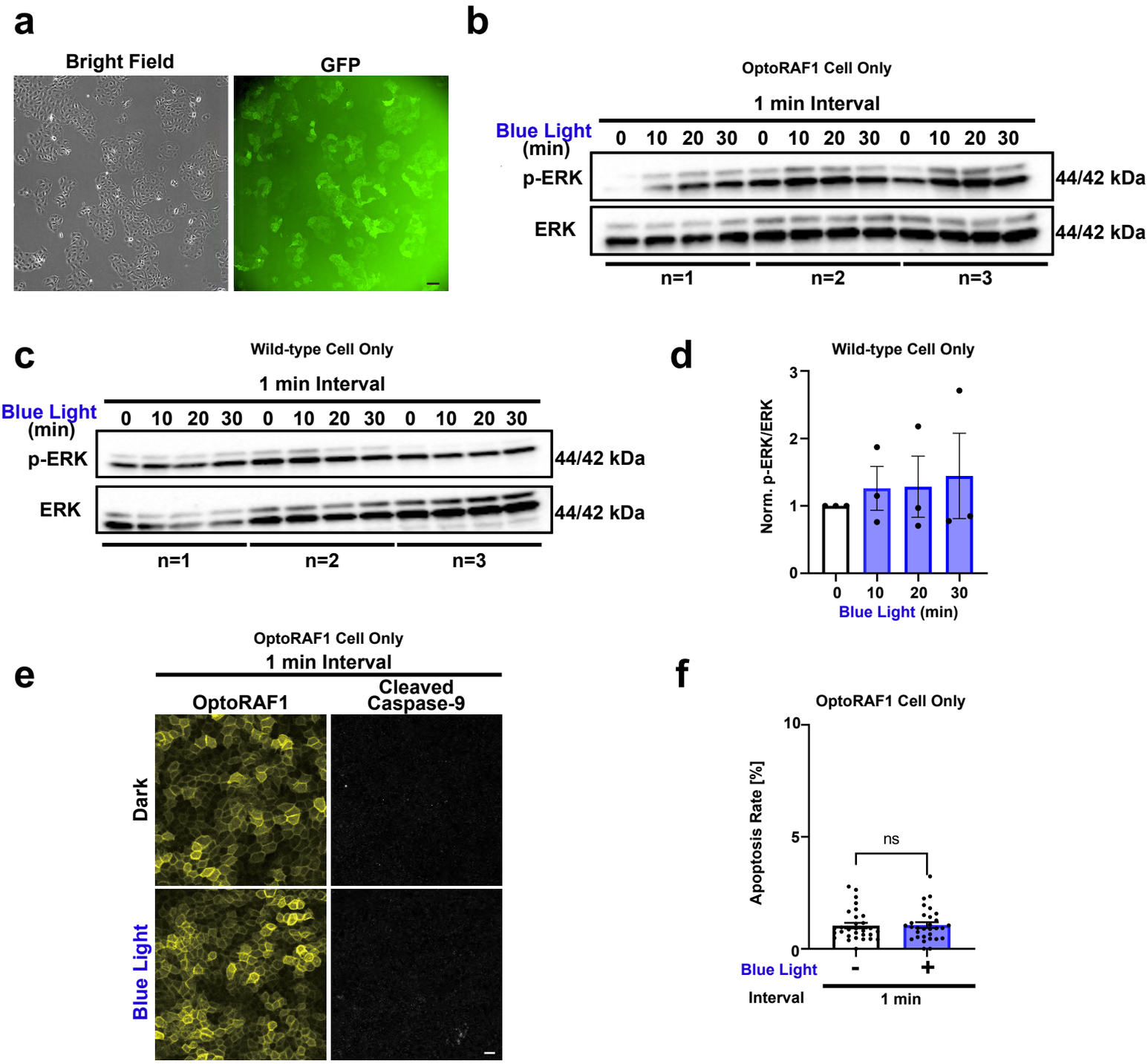


Supplementary Fig. 1 Establishment of OptoRAF1 cell lines and validation of blue light-induced effects in monoculture

a. Establishment of OptoRAF1 cells. Successfully transduced cells were GFP-positive. Scale bar, 100 µm.

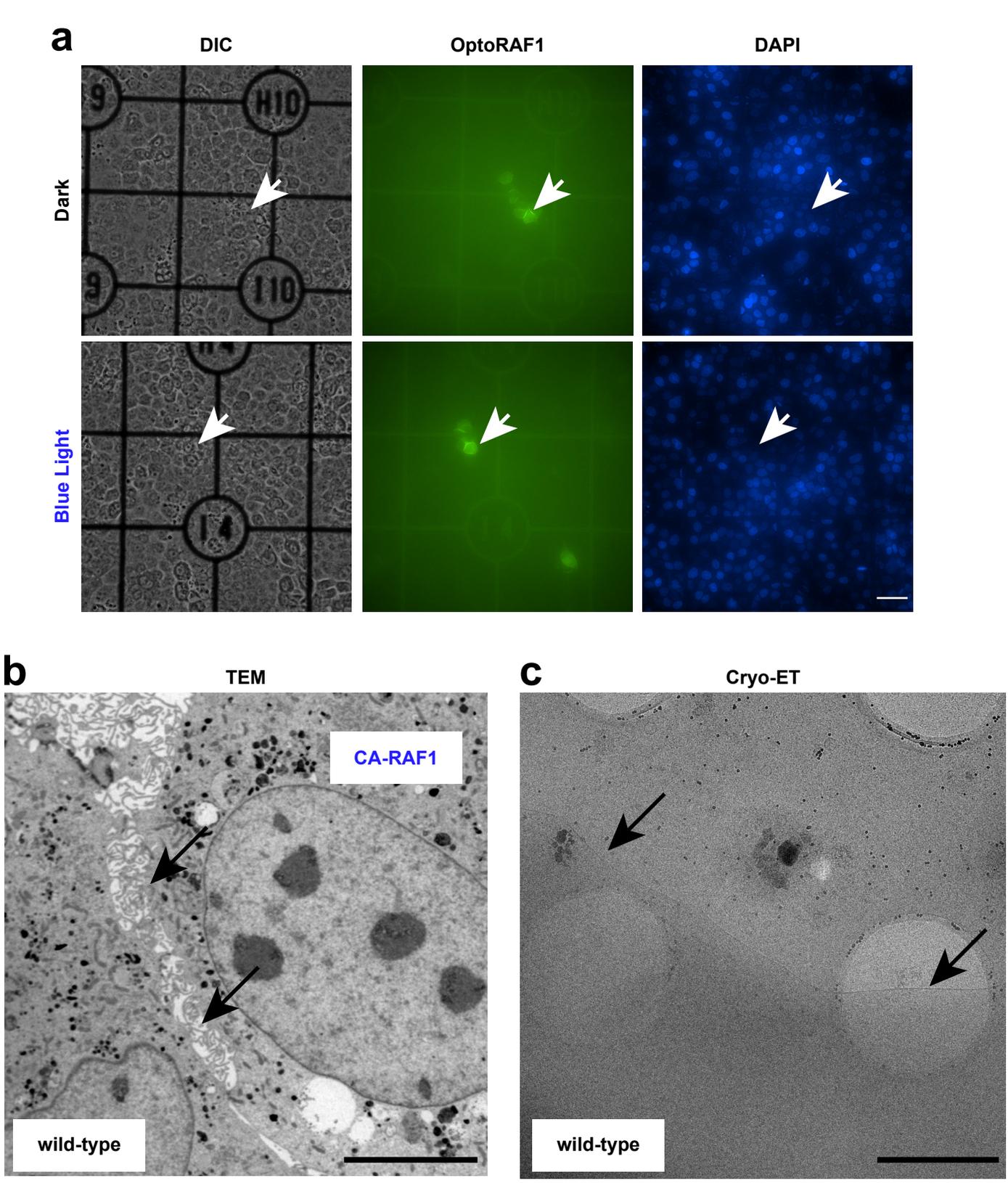
b. Immunoblot analysis of phosphorylated ERK (pERK at Thr202 and Tyr204) upon blue light stimulation in OptoRAF1 cells. Original blots are presented in **Supplementary Fig. 6**.

c. Immunoblot analysis of phosphorylated ERK (pERK at Thr202 and Tyr204) upon blue light stimulation in wild-type cells. Original blots are presented in **Supplementary Fig. 6**.

d. Quantification of the pERK/ERK levels in wild-type cells from immunoblots. One-way ANOVA with Dunnett's multiple comparisons test; n = 3.

e. Representative immunofluorescence images of OptoRAF1 cells stained with an antibody against cleaved caspase-9. Scale bar, 20 µm.

f. Quantification of cleaved caspase-9 positive cells. Data are presented as mean ± SEM from three independent biological replicates, with 8–10 randomly selected fields analyzed per replicate. ns, no significance, Welch's t test.

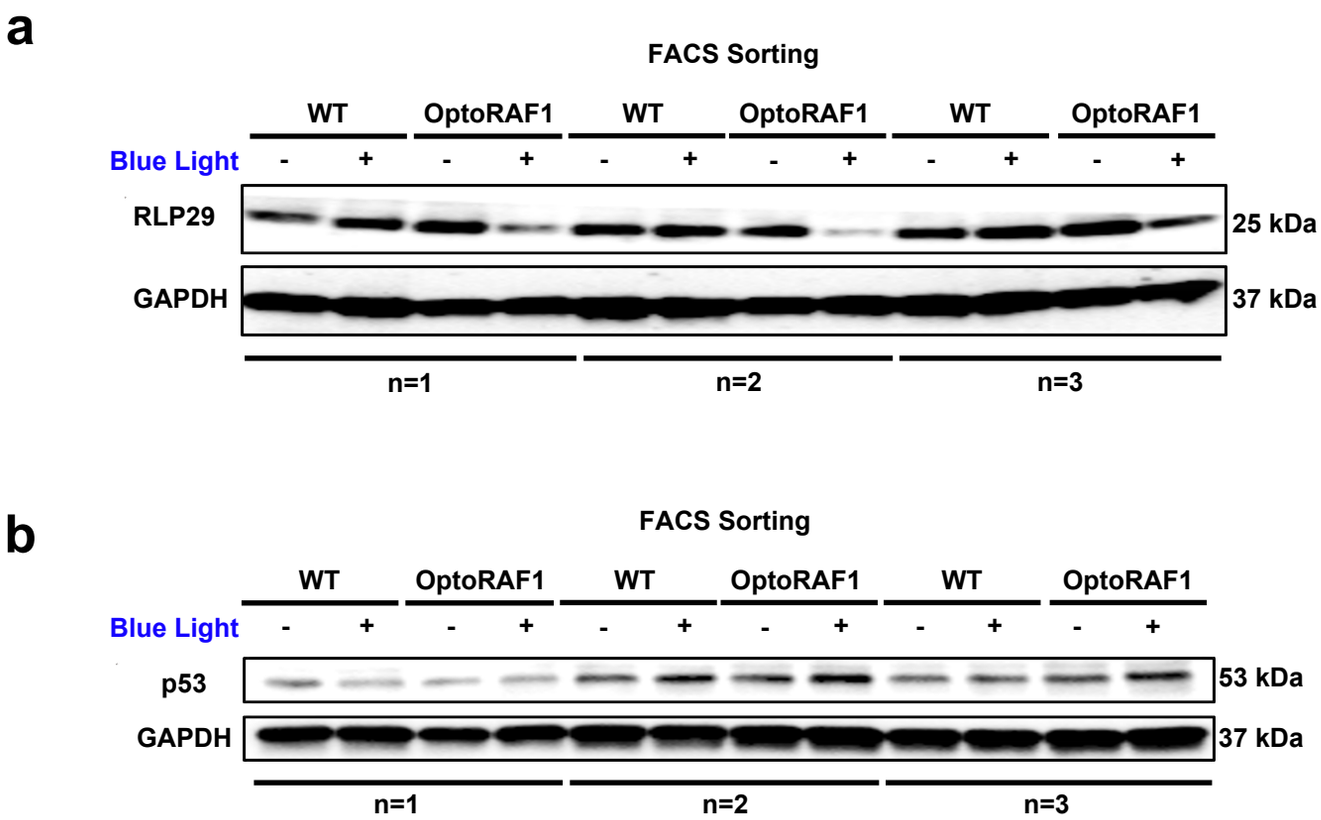


Supplementary Fig. 2 Determining the observation site for transmission electron microscopy and observation of the control group for cryo-ET

a. The cells indicated by the arrows were observed by transmission electron microscopy in **Fig. 2e**. Scale bar, 50 μm.

b. Representative transmission electron micrograph of constitutive active RAF1 expression cells. Arrows indicated the short protrusions. Scale bar, 10 μm.

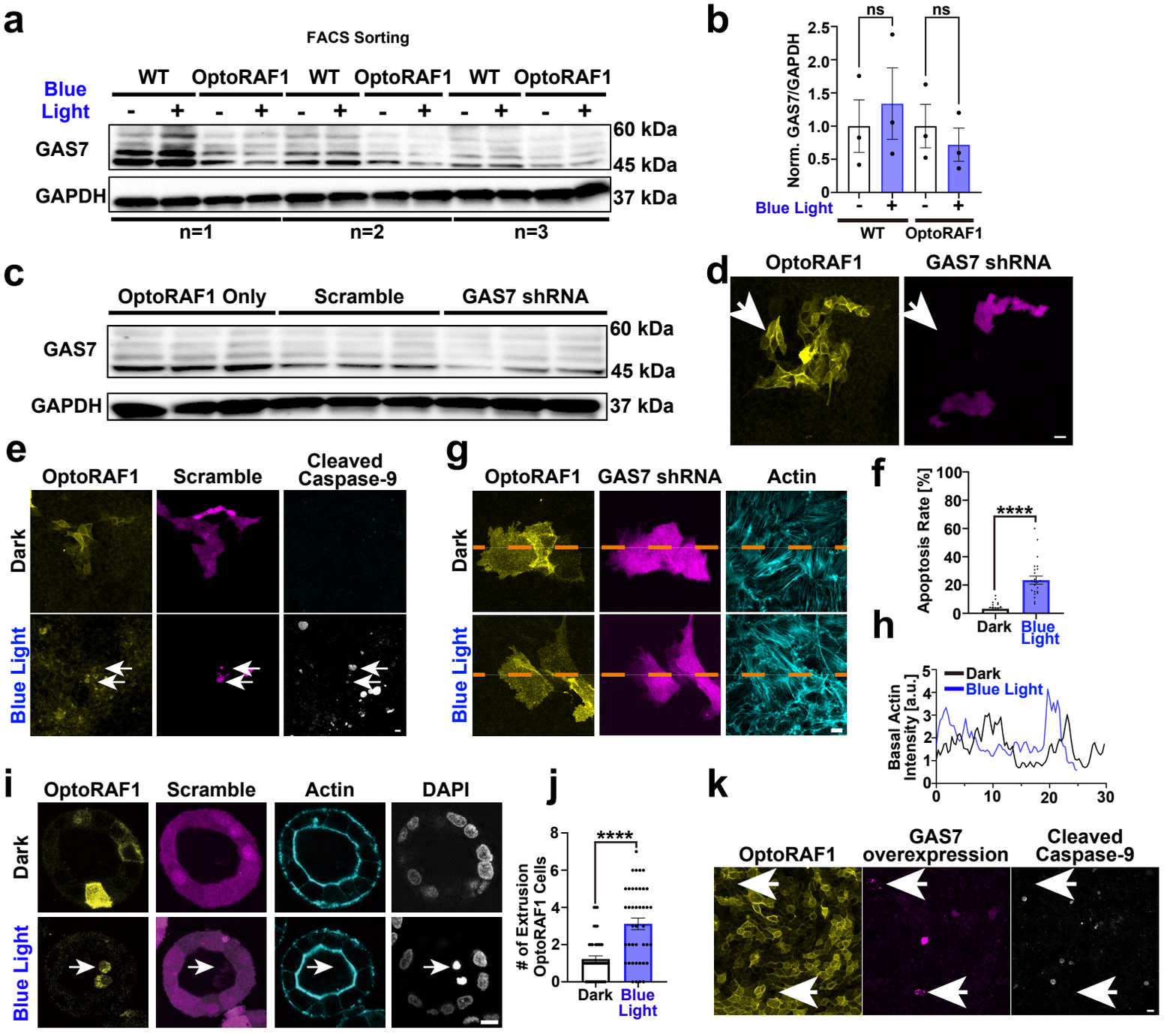
c. Representative wild-type cryo electron tomography image showing a smooth cell periphery without short protrusions. Arrows indicated the cell periphery. Scale bar, 1 μm.



Supplementary Fig. 3 All date of immunoblot analysis in Fig. 4

a. All data from immunoblot analysis of RPL29 and GAPDH with or without blue light stimulation corresponding to **Fig. 4c**. Original blots are presented in **Supplementary Fig. 6**.

b. All data from immunoblot analysis of p53 and GAPDH with or without blue light stimulation corresponding to **Fig. 4e**. Original blots are presented in **Supplementary Fig. 6**.



Supplementary Fig. 4 Control measurement of the effects of GAS7 corresponding to Fig. 5

a. Immunoblot analysis of GAS7. Cells were irradiated with or without a 470-nm laser every 1 min for 2 days, and then sorted with a MoFlo XDP cell sorter to obtain wide type cells and OptoRAF1 cells separately before immunoblot analysis. GAS7 has numerous isoforms that might yield multiple GAS7 bands in immunoblotting. Original blots are presented in **Supplementary Fig. 6**.

b. Quantification of the GAS7/GAPDH levels from immunoblots.

c. All data from immunoblot analysis of GAS7 corresponding to **Fig. 5e, f**. Original blots are presented in **Supplementary Fig. 6**.

d. Representative fluorescence images of OptoRAF1-GAS7 shRNA cells. White arrows indicated that the mCherry signal in OptoRAF1 cells does not interfere with the detection of the mCherry signal in GAS7 shRNA cells. Scale bar, 20 μm

e. Representative immunofluorescence images of scramble control cells mixed with wild-type MDCK cells stained with an antibody against cleaved caspase-9. White arrows indicated the cleaved caspase-9 positive cells. Scale bar, 20 μm.

f. Quantification of the cleaved caspase-9 positive cells in scramble control. Data are represented as mean ± SEM from three independent biological replicates, with 8 randomly selected fields analyzed per replicate. ****$p < 0.0001$, Welch's t test.

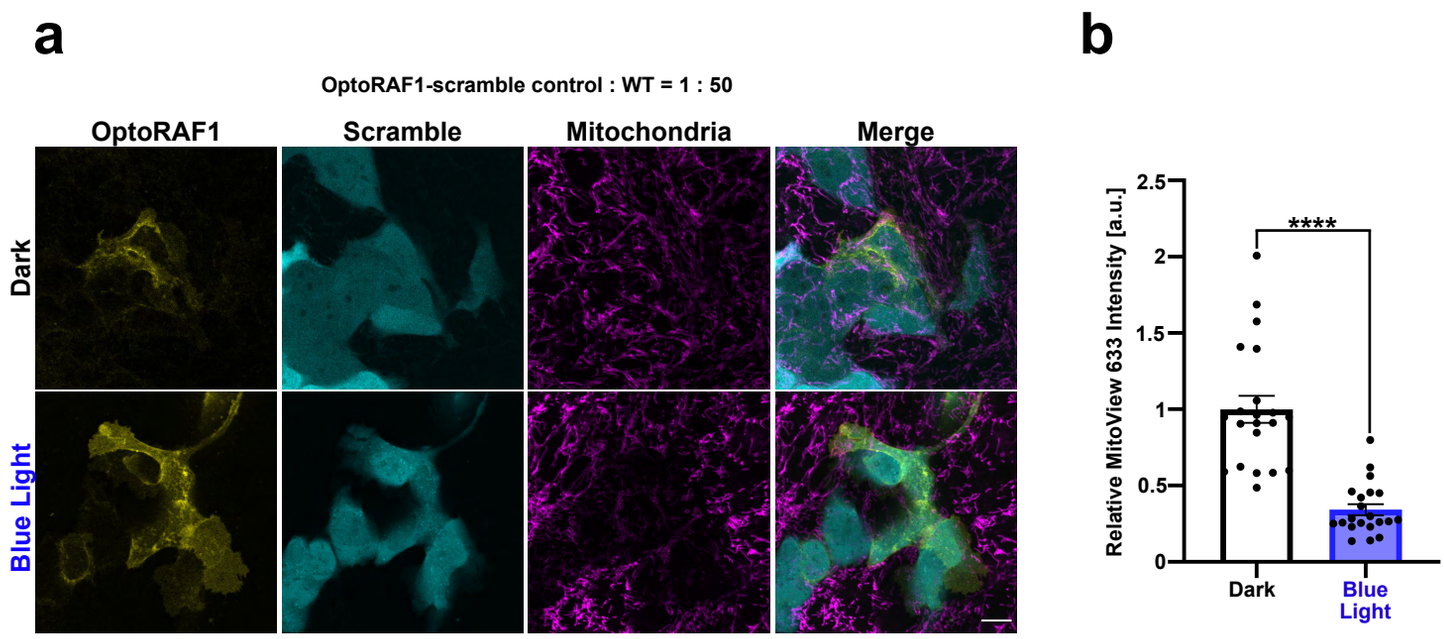
g. Representative immunofluorescence images of OptoRAF1-GAS7 shRNA cells mixed with wild-type MDCK cells stained with fluorescent phalloidin. Scale bar, 10 μm

h. Basal actin fluorescence intensity along the orange line indicated in (**g**).

i. Representative fluorescence images of cysts containing scramble control cells stained with DAPI (gray) and fluorescent phalloidin (cyan). White arrows indicated the extrusion OptoRAF1 cell from cyst. Scale bar, 10 μm

j. Quantification of the number of extruding cells in OptoRAF1-GAS7 shRNA. Data are represented as mean ± SEM from three independent biological replicates, with 8–18 randomly selected cysts analyzed per replicate. ****$p < 0.0001$, Welch's t test.

k.Representative immunofluorescence images of GAS7 overexpressing OptoRAF1 cells stained with an antibody against cleaved caspase-9. White arrows indicated the apoptotic GAS7-overexpressing OptoRAF1 cells without cleaved caspase-9 signal. Scale bar, 20 μm



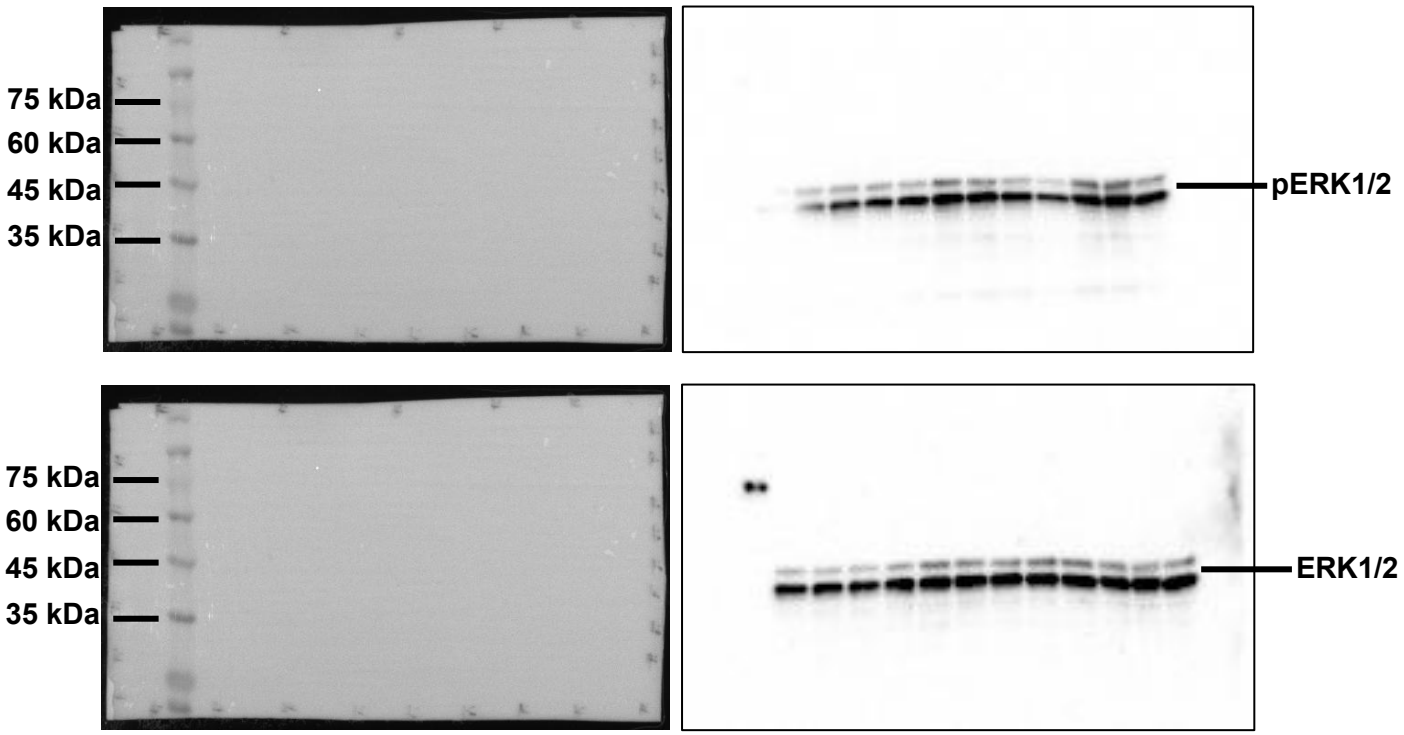
Supplementary Fig. 5 Mitochondrial membrane potential detection in scramble control group corresponding to Fig. 6

a. Representative fluorescence images of mitochondrial membrane potential in OptoRAF1-scramble control cells mixed with wild-type MDCK cells stained with MitoView 633. Scale bar, 10 μm.

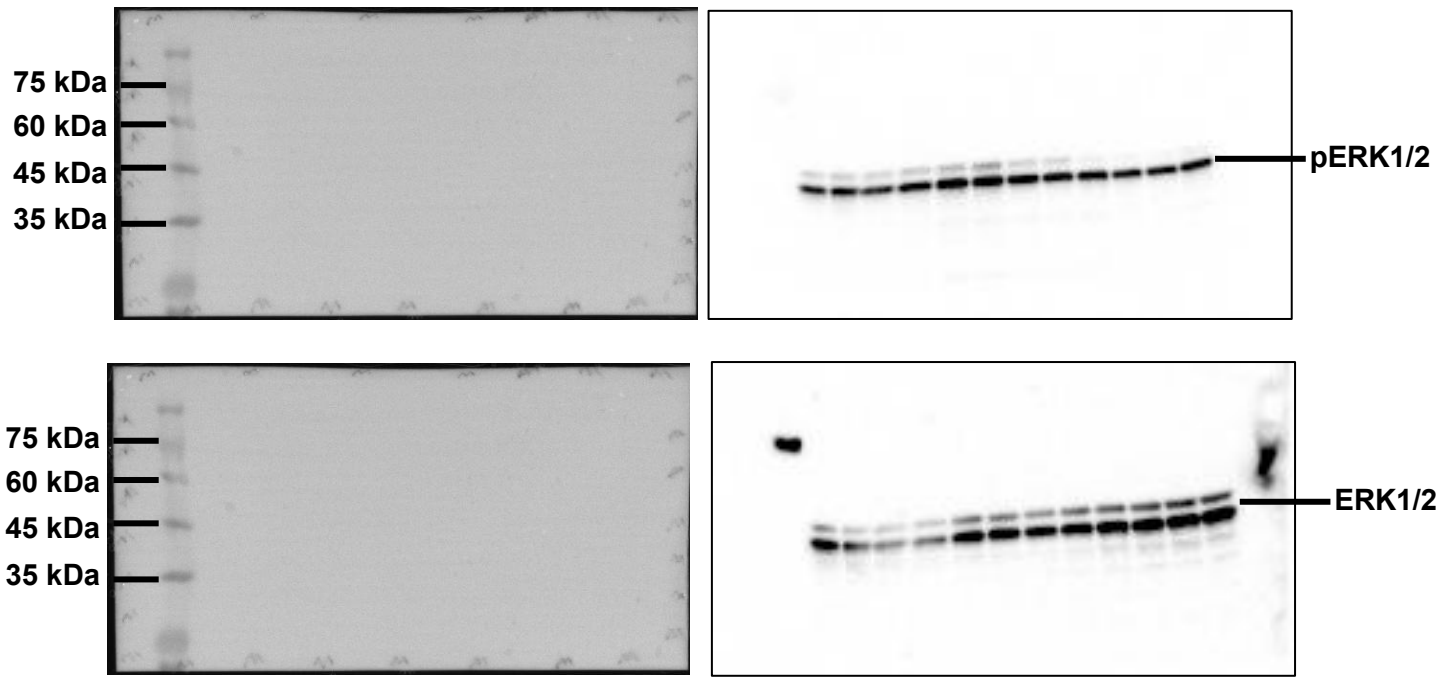
b. Quantification of the MitoView 633 fluorescence intensity in OptoRAF1-scramble control cells. Data are represented as mean ± SEM from three independent biological replicates, with 7 randomly selected cells analyzed per replicate. ****$p < 0.0001$, Welch's t test.

Immunoblotting uncropped images

Fig. 1b and Supplementary Fig. 1b



Supplementary Fig. 1c



Immunoblotting uncropped images

Fig. 4c and Supplementary Fig. 3a

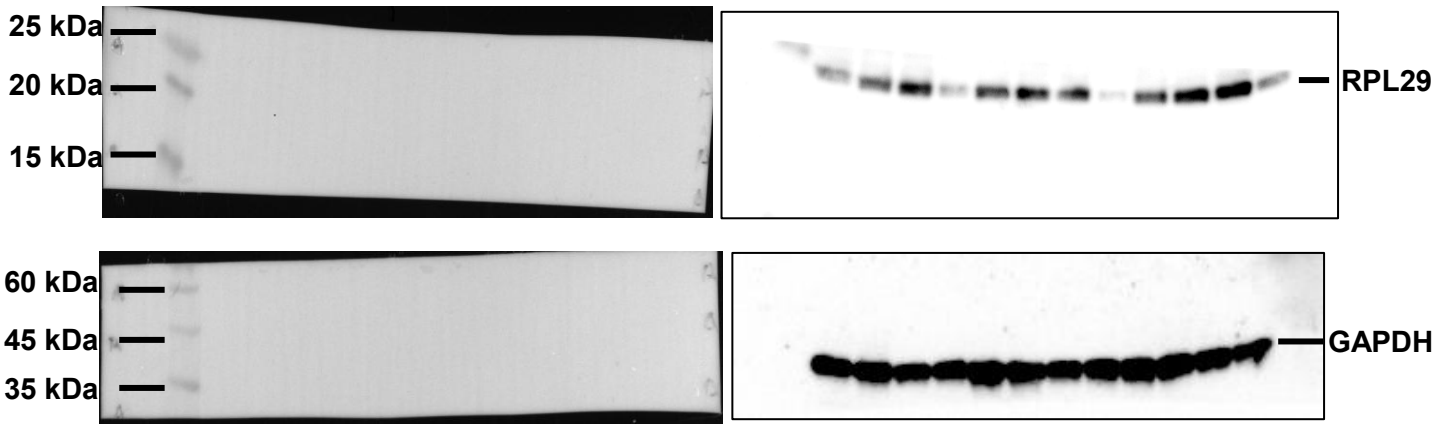


Fig. 4e and Supplementary Fig. 3b

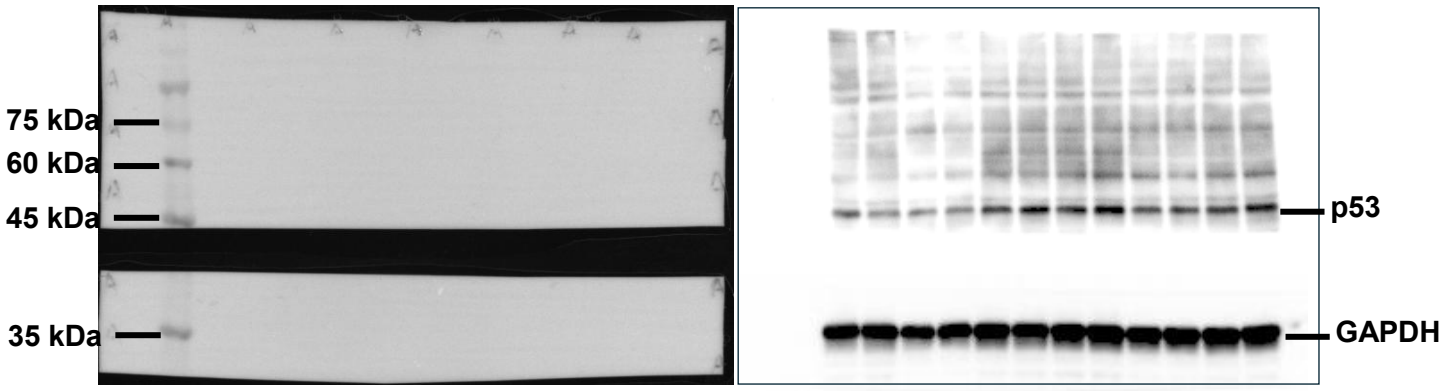
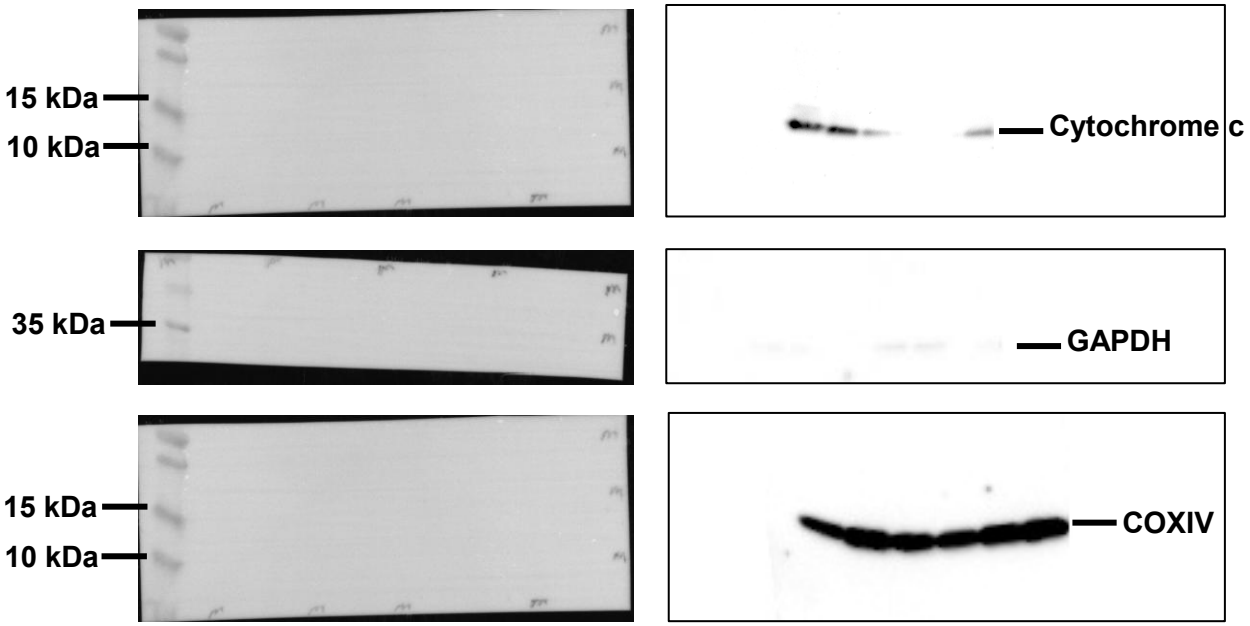


Fig. 4i



Immunoblotting uncropped images

Fig. 4j

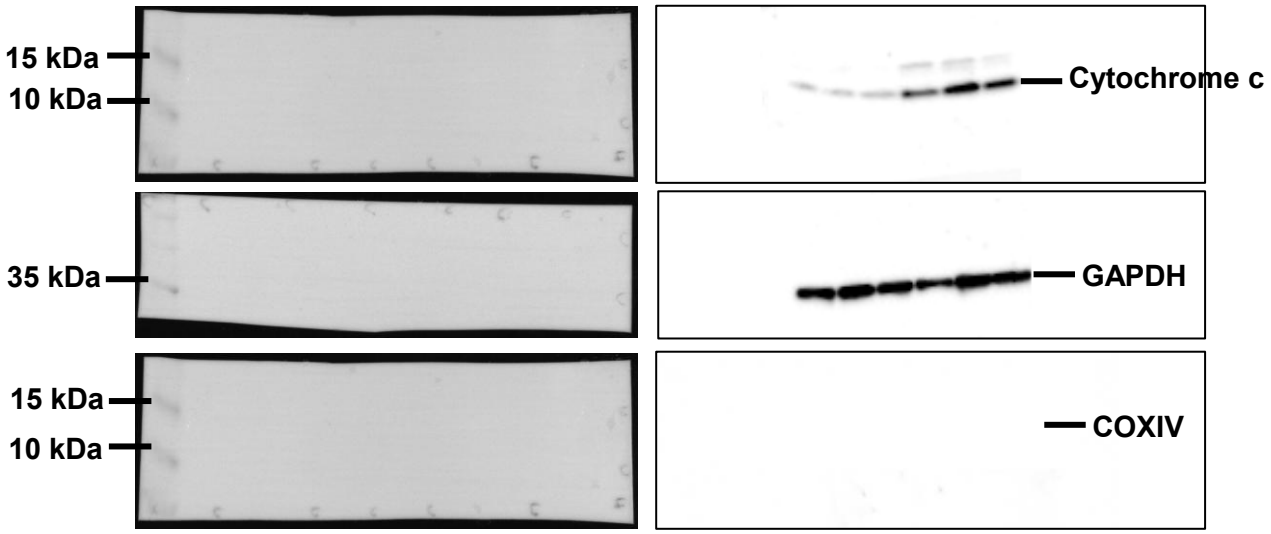
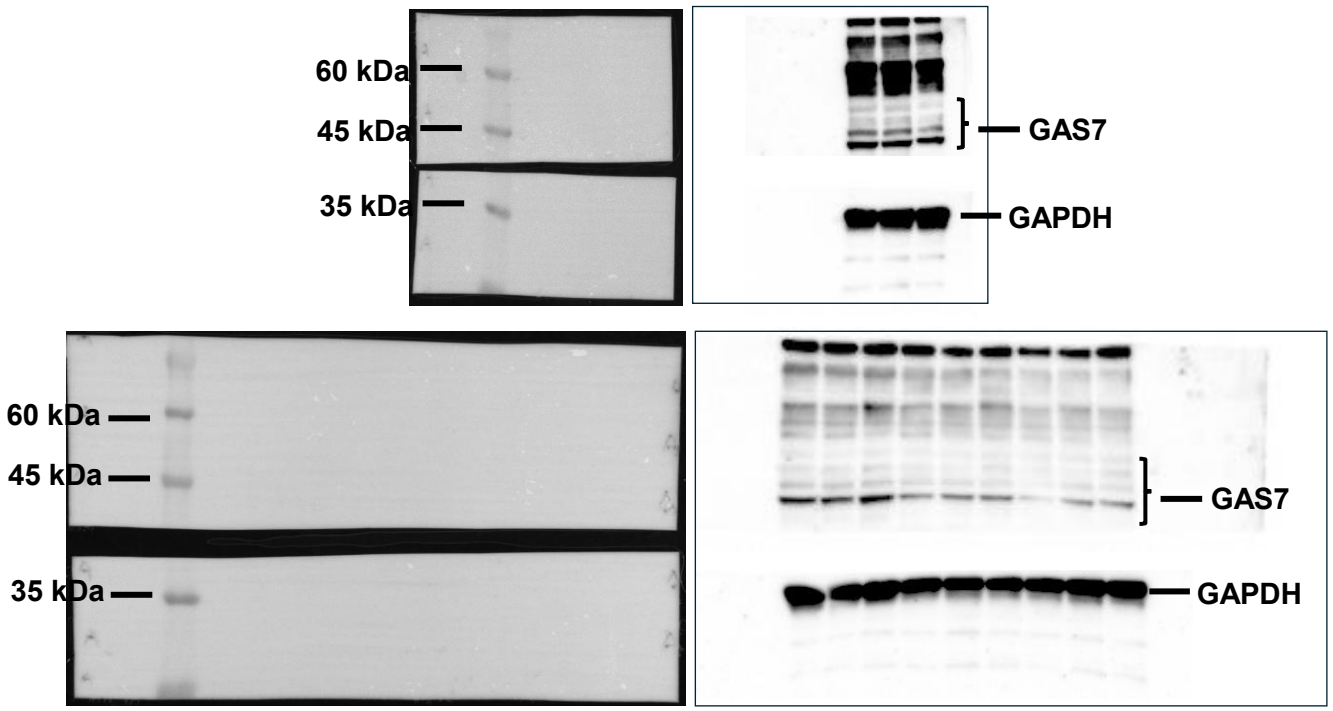
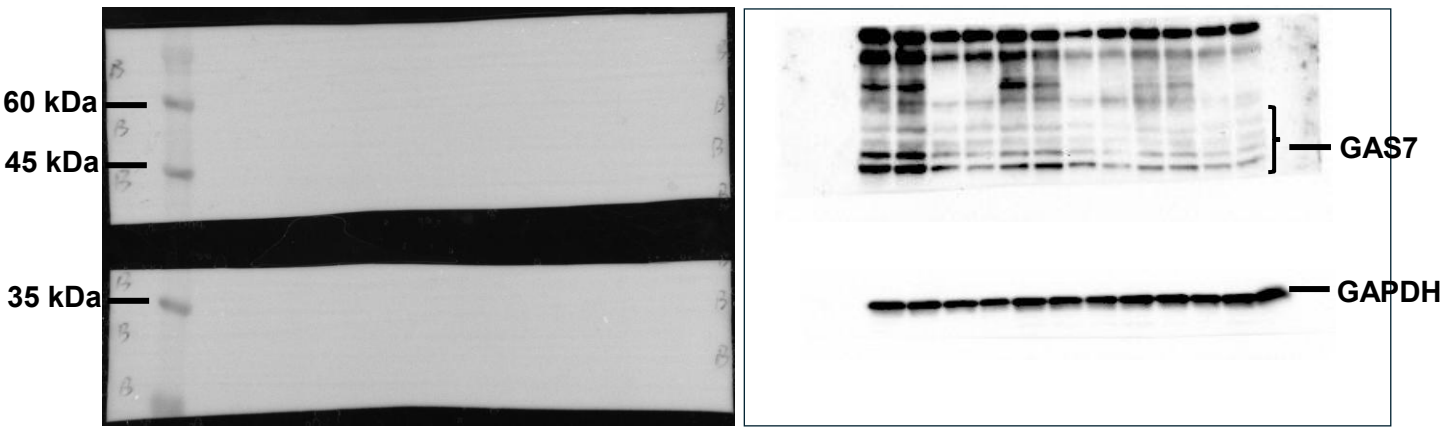


Fig. 5e and Supplementary Fig. 4c



Supplementary Fig. 4a



Supplementary Fig. 6 Uncropped immunoblot images in main Figures
Full-length immunoblot images used for indicated Figures are shown.