Expanded View Figures

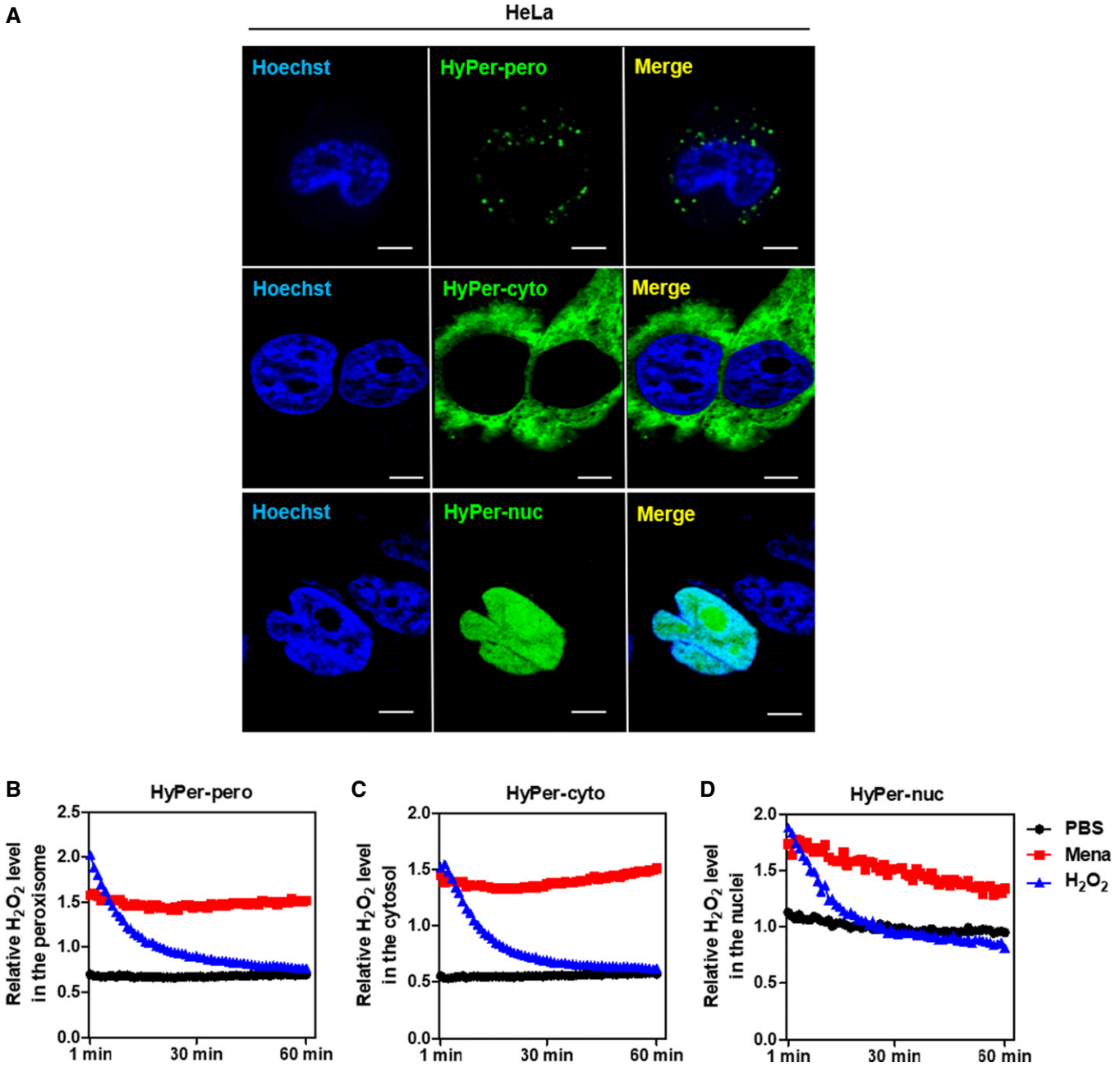


Figure EV1. Application and identification of a genetically encoded sensor to detect H_2O_2 in the peroxisome, cytosol, and nucleus.

A HyPer-pero, HyPer-cyto, and HyPer-nuc were ectopically expressed in HeLa cells, and their subcellular localization was determined by immunofluorescence staining. Representative immunofluorescence images (original magnification, 630×; a single focal plane, scale bar, 5 μm) are shown.

B–D HEK293 cells overexpressing the Hyper biosensor were treated with PBS, 500 μM H_2O_2, or 50 μM menadione for the indicated periods. The H_2O_2 level in the peroxisome (B), cytosol (C), and nucleus (D) was monitored as described in Materials and Methods.

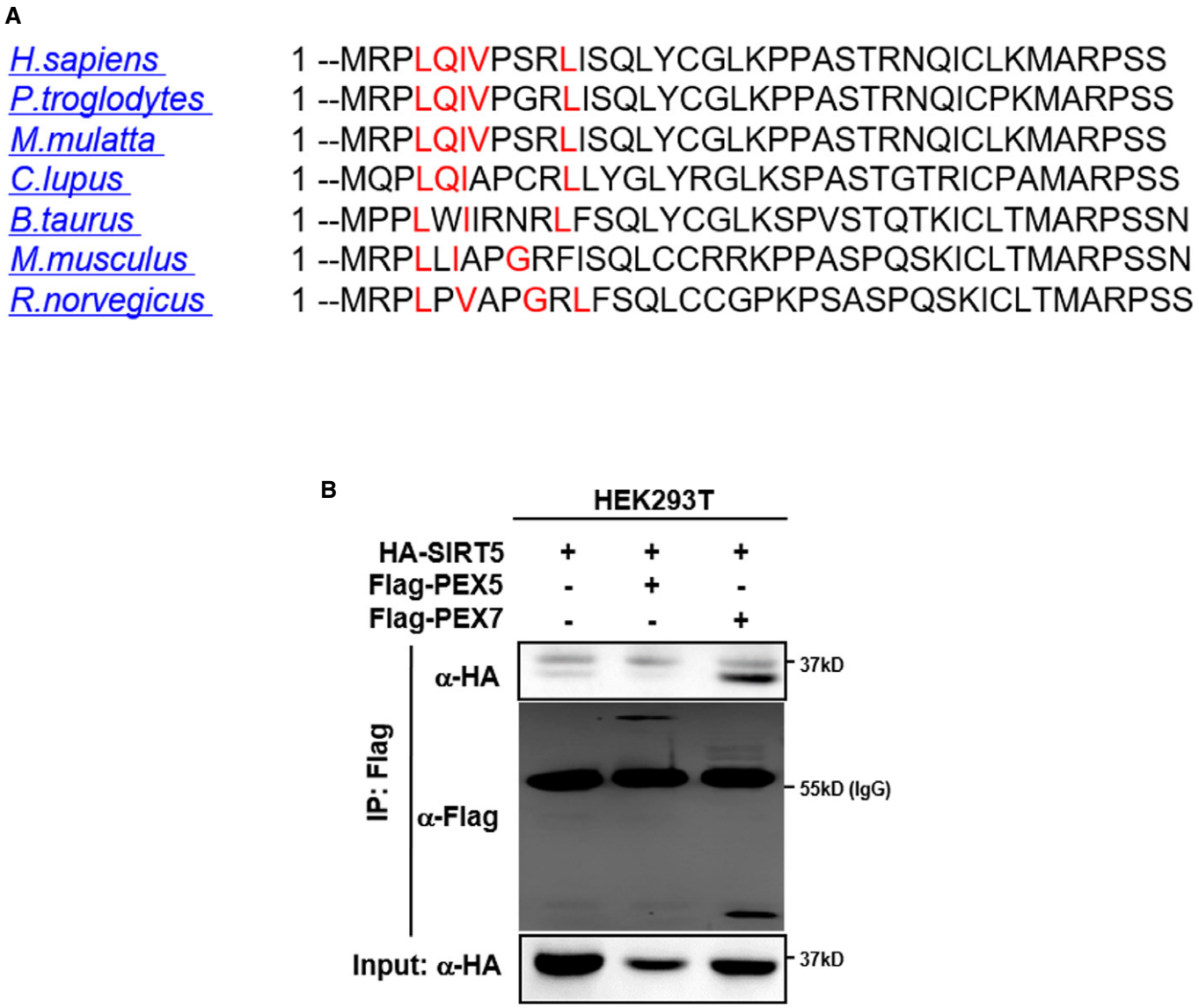


Figure EV2. SIRT5 harbors a predicted PTS2 sequence and interacts with PEX7 but not PEX5.

A The amino acid sequence of SIRT5 protein from the indicated species was imported into "PTSs predictor" of PEROXISOME database (*PeroxisomeDB*). As shown, SIRT5 contains a predicted PTS2 sequence (marked in red).

B SIRT5 interacts with PEX7, but not PEX5. HA-SIRT5 was co-expressed with Flag-PEX5 or Flag-PEX7 in HEK293T cells. Proteins were purified by immunoprecipitation (IP) with Flag beads, followed by Western blot to detect PEX5 or PEX7 with an HA antibody.

Source data are available online for this figure.

Figure EV3. ACOX1 is localized in the peroxisome.

A ACOX1 interacts with PEX5. HA-ACOX1 was co-overexpressed with Flag-PEX5 or Flag-PEX7 in HEK293T cells. PEX proteins were purified by IP with Flag beads, followed by Western blot to detect ACOX1 with an HA antibody.

B Both ACOX1 and ACOX1 SKL^{del} interact with SIRT5. HA-SIRT5 was co-overexpressed with Flag-ACOX1 or Flag-ACOX1 SKL^{del} in HEK293T cells, followed by IP with Flag beads and Western blot to detect SIRT5 with an HA antibody.

C ACOX1 but not ACOX1 SKL^{del} mutant is localized in peroxisomes. Flag-tagged wild-type or mutant ACOX1 was transiently overexpressed in HeLa cells. Immunofluorescence assay was performed to detect the indicated proteins as described in Materials and Methods. Representative immunofluorescence images (original magnification, 630×; a single focal plane, scale bar, 5 μm) are shown.

D ACOX1 co-localizes with the peroxisomal marker PMP70, but not the mitochondrial marker SDHA. Flag-ACOX1 was transiently overexpressed in HeLa cells. Immunofluorescence assay was performed to detect the indicated proteins as described in Materials and Methods. Representative immunofluorescence images (original magnification, 630×; a single focal plane, scale bar, 5 μm) are shown.

Source data are available online for this figure.

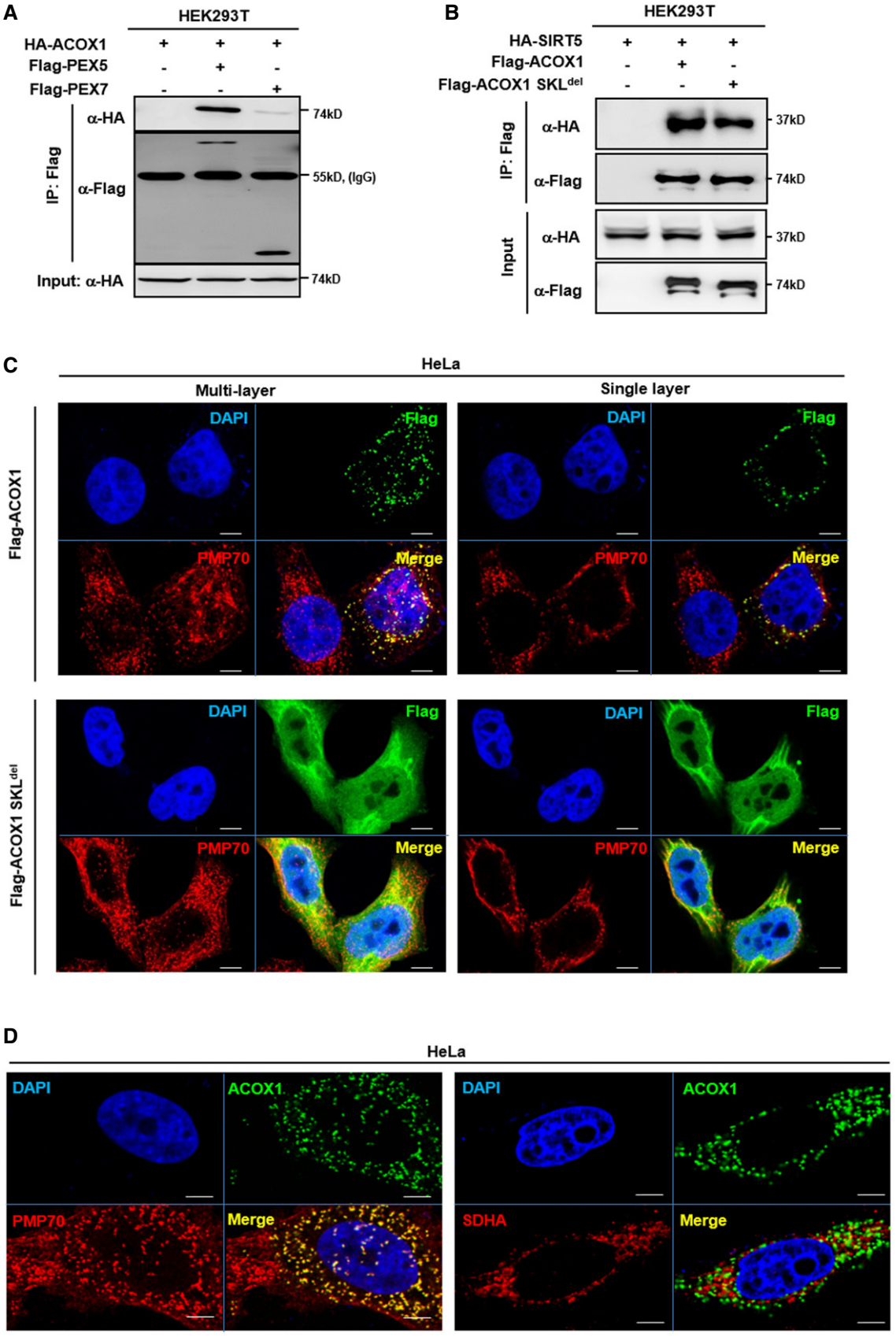
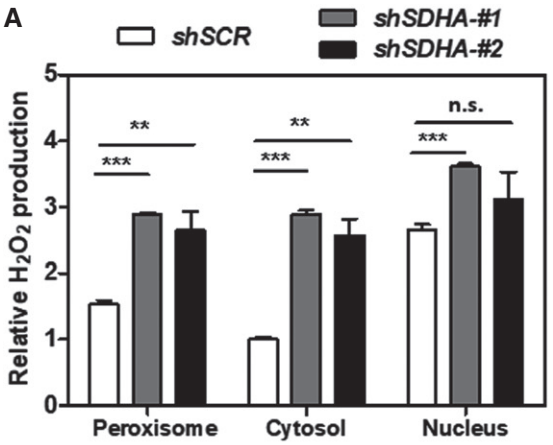
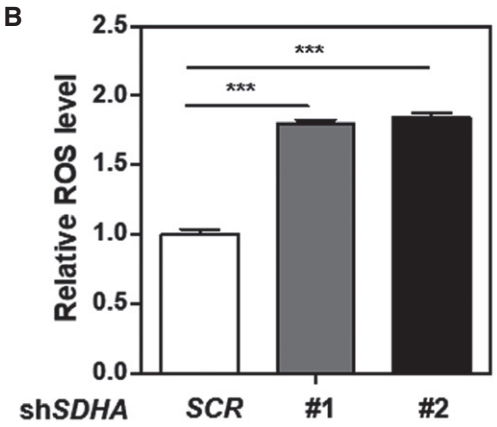


Figure EV3.





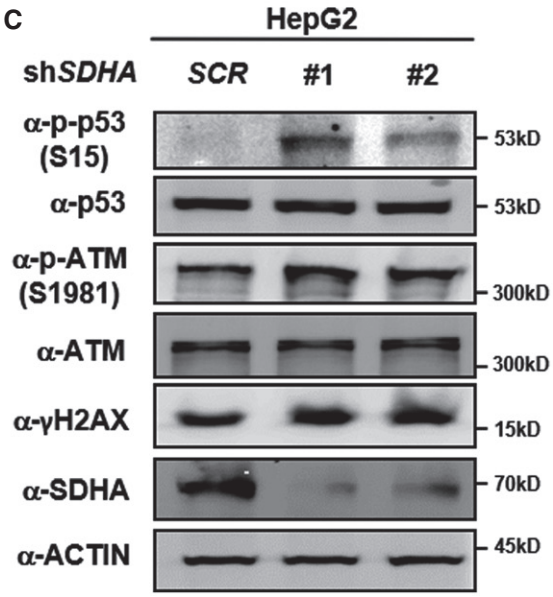


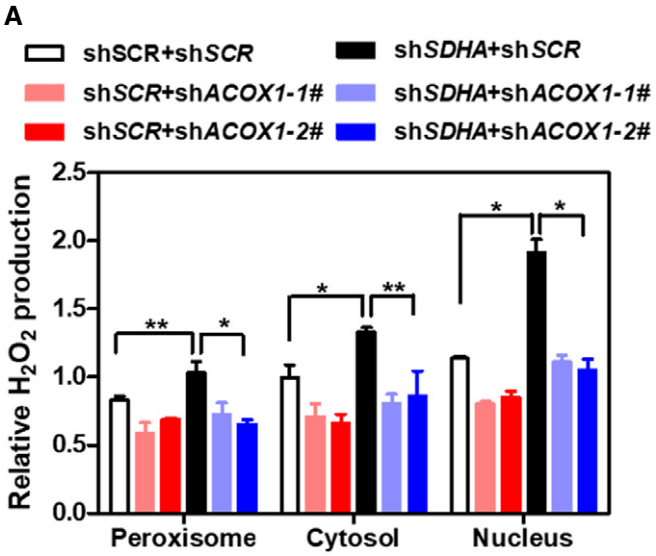
Figure EV4. *SDHA* knockdown leads to increased H_2O_2 and ROS, and induced oxidative DNA damage response.

A Knockdown of *SDHA* stimulates H_2O_2 production in the peroxisome, cytosol, and nucleus. In HepG2 cells with stable *SDHA* knockdown, endogenous H_2O_2 production in the indicated cellular compartments was determined by using the Hyper biosensor as described in Materials and Methods. Note: Given that the level of endogenous H_2O_2 does not change over time, we have collected the excitation ratio (490/420 nm) at single time point (at 5 min).

B Knockdown of *SDHA* increases cellular ROS. In HepG2 cells with stable *SDHA* knockdown, ROS level was determined in cell extracts as described in Materials and Methods.

C Knockdown of *SDHA* induces oxidative DNA damage response. In HepG2 cells with stable *SDHA* knockdown, the indicated classical oxidative DNA damage response markers were determined by Western blot analysis.

Data information: Shown are average values with standard deviation (SD) of triplicated experiments. $^{**}P < 0.01$, and $^{***}P < 0.001$ for the indicated comparisons by two-tailed unpaired Student's *t*-test. n.s. = not significant. Source data are available online for this figure.



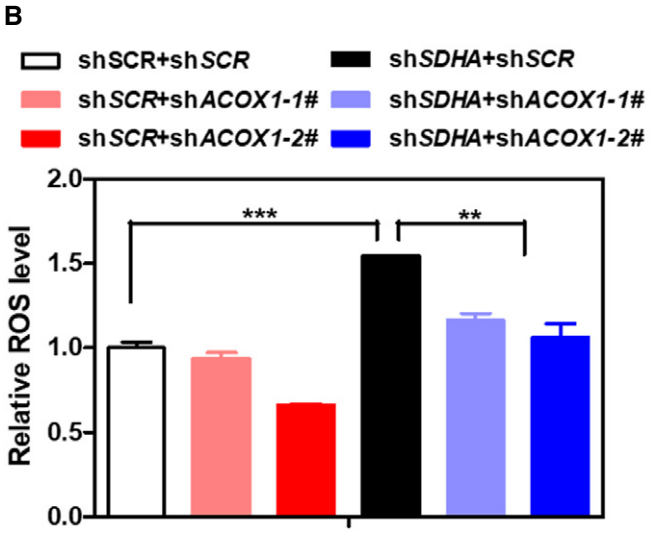


Figure EV5. *ACOX1* knockdown partially blocks the effect of *SDHA* knockdown on increasing cellular H_2O_2 and ROS in HepG2 cells.

A *ACOX1* knockdown partially diminishes the effect of *SDHA* knockdown on increasing H_2O_2. In HepG2 stable cells with single or double knockdown of *SDHA*/*ACOX1*, endogenous H_2O_2 level in the indicated cellular compartments was determined by using the Hyper biosensor as described in Materials and Methods. Note: given that the level of endogenous H_2O_2 does not change over time, we have collected the excitation ratio (490/420 nm) at single time point (at 5 min).

B *ACOX1* knockdown partially impairs the effect of *SDHA* knockdown on increasing ROS. In HepG2 stable cells with single or double knockdown of *SDHA*/*ACOX1*, ROS level was determined in cell extracts as described in Materials and Methods.

Data information: Shown are average values with standard deviation (SD) of triplicated experiments. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ for the indicated comparisons by two-tailed unpaired Student's *t*-test. n.s. = not significant.