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Supplemental Material to:

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Autophagy promotes resistance to photodynamic therapy-induced apoptosis selectively in colorectal cancer stem-like cells

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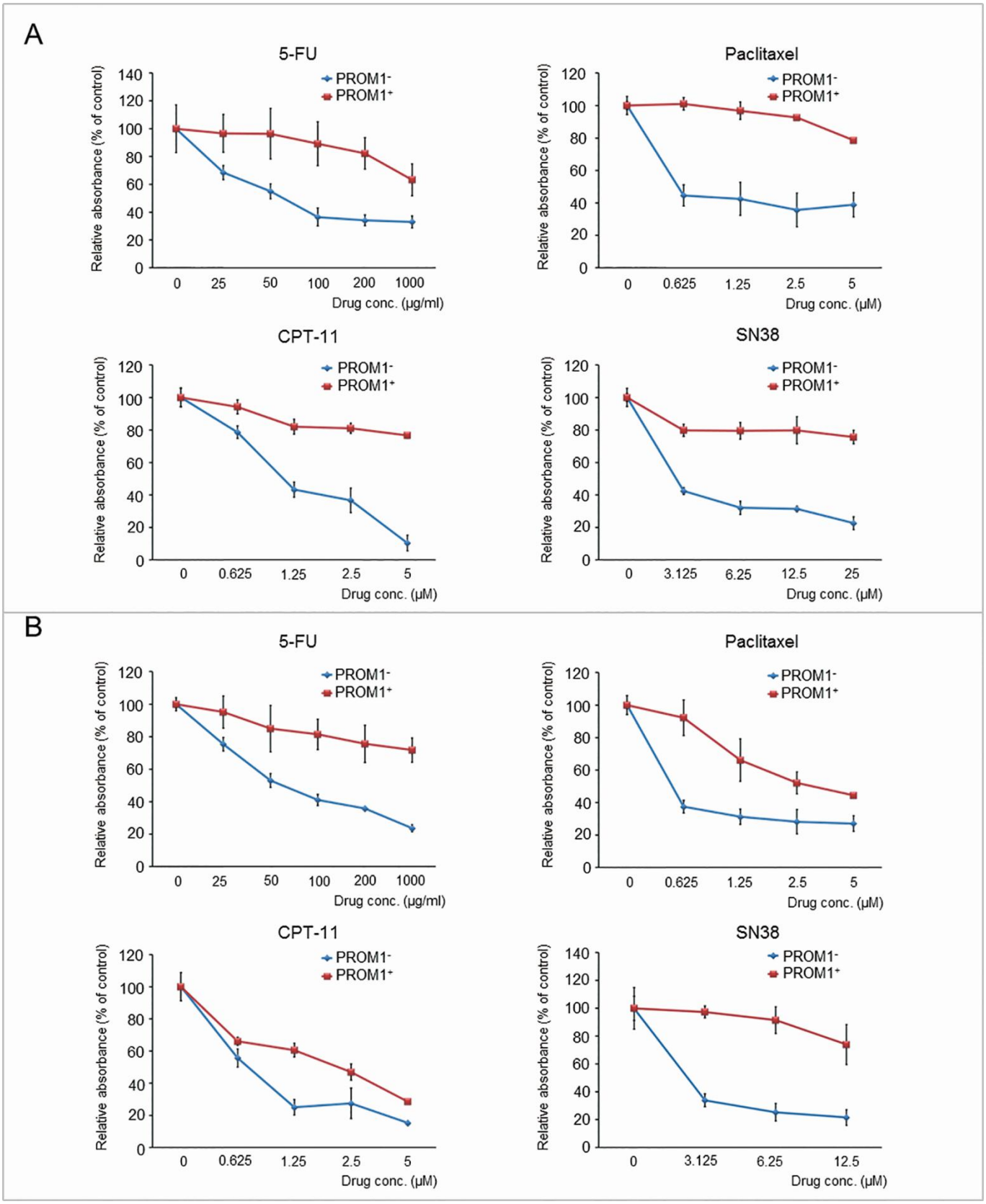

Figure S1. CSCs are more resistant to conventional chemotherapy compared to non-CSCs. PROM1/CD133^{+} and PROM1/CD133^{-} cells obtained from (A) PCCs and (B) HT29 cell line were first treated separately with increasing concentrations of various chemotherapeutic compounds for 72 h. These compounds included 5-FU, paclitaxel, CPT-11, and SN38. Thereafter, cytotoxicity was measured using the WST-1 assay. Cytotoxicity of treated cells was expressed as a percentage of the control. Each condition was performed at least in triplicate.

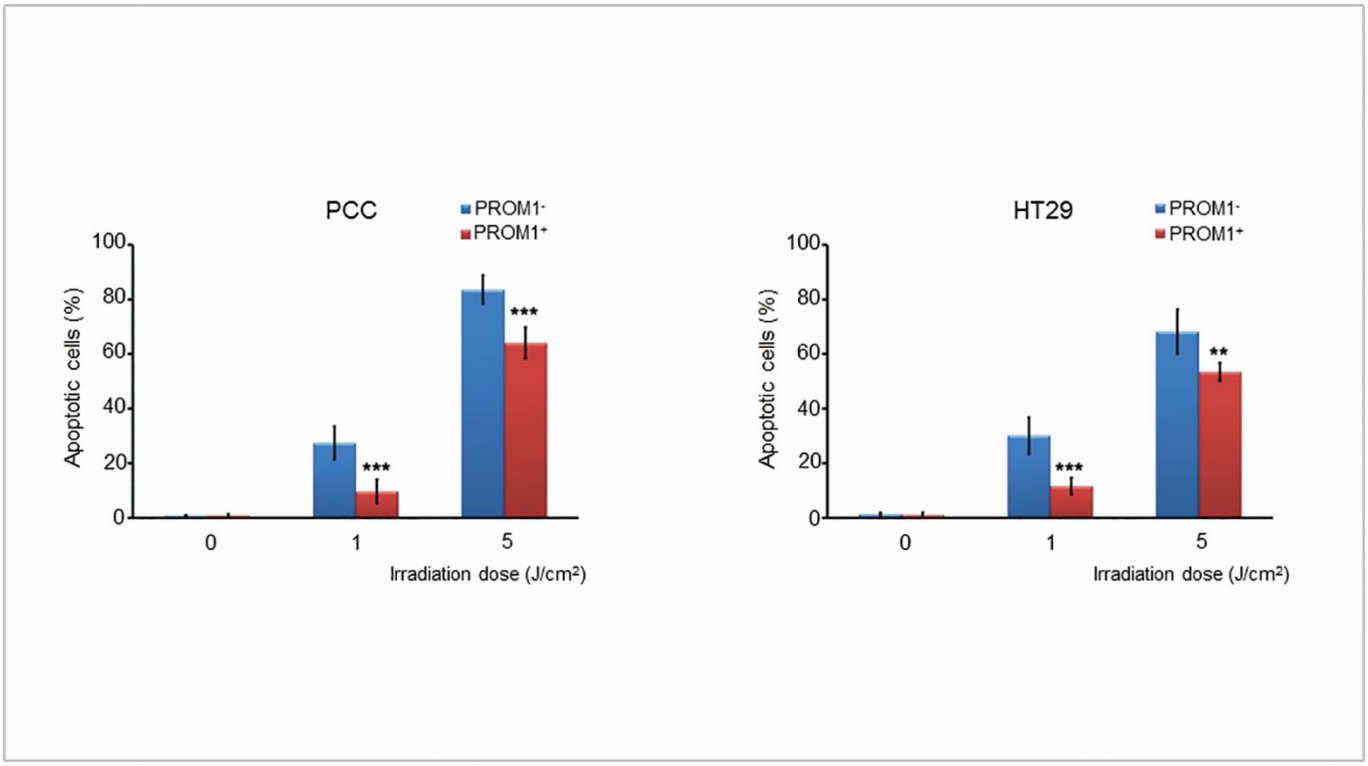

Figure S2. PpIX-mediated PDT causes significantly less apoptosis in CSCs compared to non-CSCs. Graphic representation of the percentages of apoptotic PROM1/CD133^{+} and PROM1/CD133^{-} cells after PpIX-mediated PDT at various light doses. These results are expressed as the mean ± SE of 3 different experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

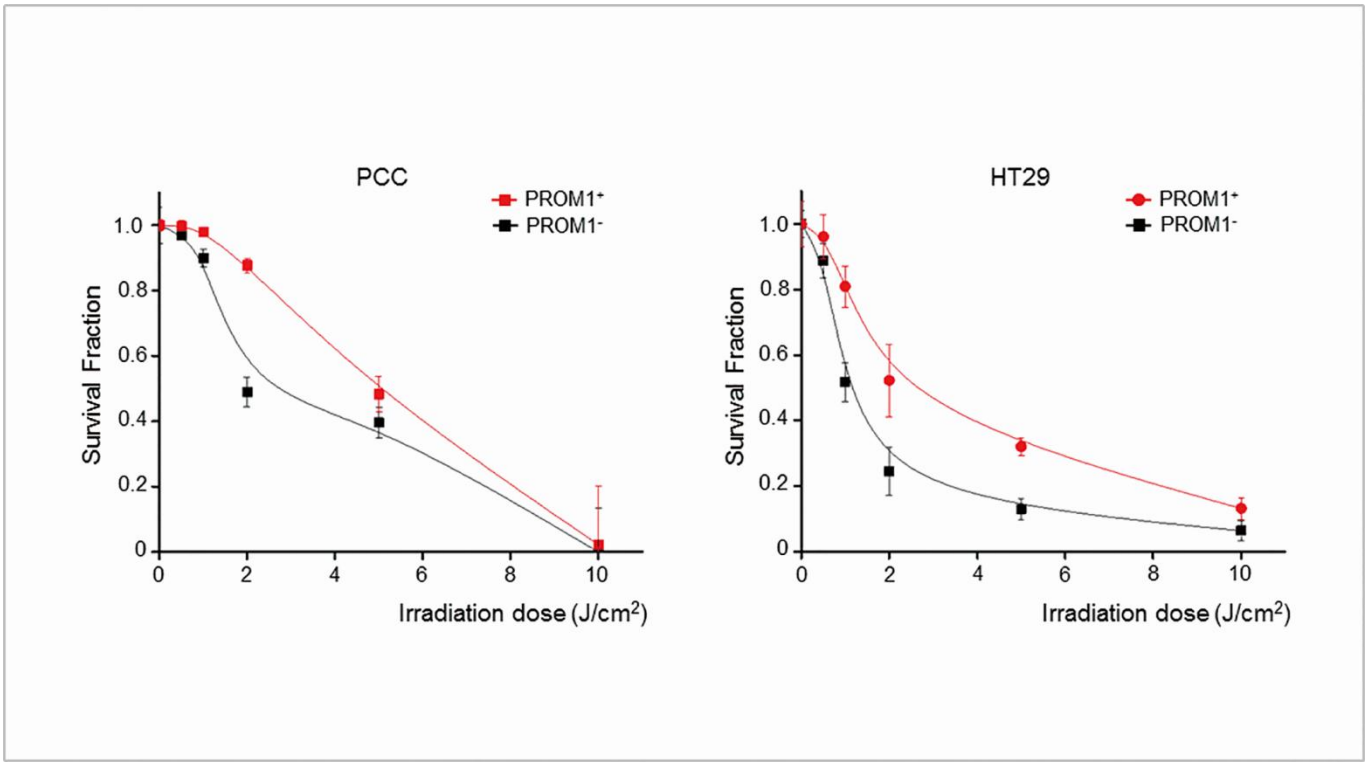

Figure S3. CSCs are less sensitive to PpIX-mediated PDT than non-CSCs. PROM1/CD133^{+} and PROM1/CD133^{-} cells from PCCs and HT29 cell lines were plated in 2 ml of medium at a concentration of 500 cells/well in 6-well plates and treated with PDT at various light doses. The treated cells were incubated at 37 ˚C for 8 to 10 days to allow colony formation. Colonies were fixed with methanol and stained with 0.5% crystal violet. Colonies having more than 50 cells were counted, and the plating efficiency (PE) and surviving fraction (S.F.) were calculated. Each condition was performed at least in triplicate.

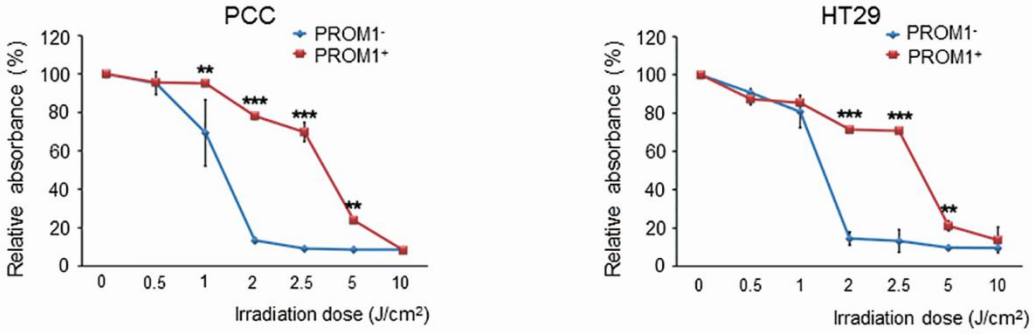

Figure S4. CSCs are more resistant to PpIX-mediated PDT compared to non-CSCs. Following the administration of PpIX-mediated PDT at various light doses, the PROM1/CD133^{+} and PROM1/CD133^{-} cytotoxicity was evaluated using the WST-1 assay. Cytotoxicity of treated cells was expressed as a percentage of the control. These results are expressed as the mean ± SE of 3 different experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

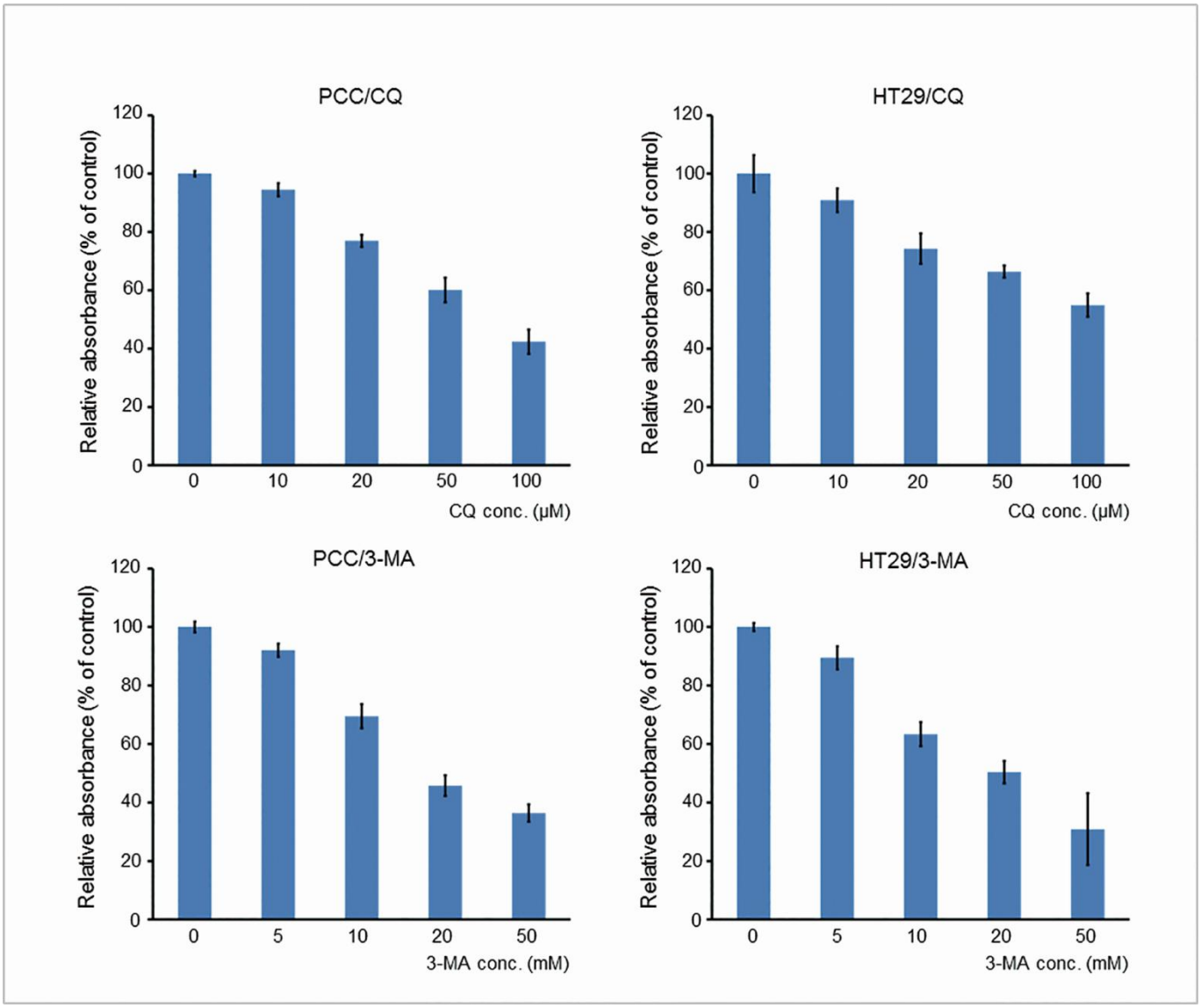

Figure S5. The optimal concentrations of autophagy inhibitors that effectively suppress autophagy but only with minimal cytotoxicity. PCCs and HT29 cells were treated with increasing concentrations of CQ and 3-MA for 72 h. Then, cytotoxicity was measured using the WST-1 assay. Cytotoxicity of treated cells was expressed as a percentage of the control. Each condition was performed at least in triplicate.

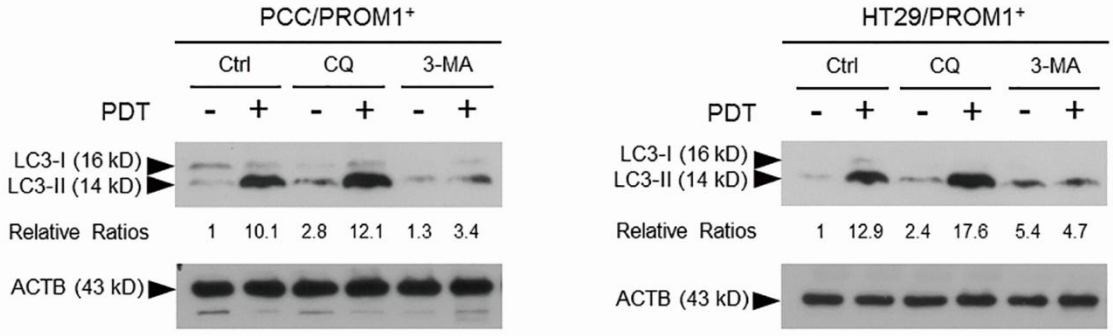

Figure S6. Effects of autophagy inhibitors on PDT-induced LC3-II accumulation in CSCs. The PROM1/CD133$^+$ cells were treated with CQ (10 μM) or 3-MA (5 mM) for 24 h, followed by PDT (1.3 J/cm^2). Cell lysates were harvested at 24 h post-PDT, and the expression of LC3 was detected by western blot. The band intensities on films were analyzed by ImageJ software. The relative amounts of LC3-II were quantified as ratios to ACTB, indicated underneath each gel. The relative ratio of LC3-II in Ctrl without PDT treatment is arbitrarily presented as 1.

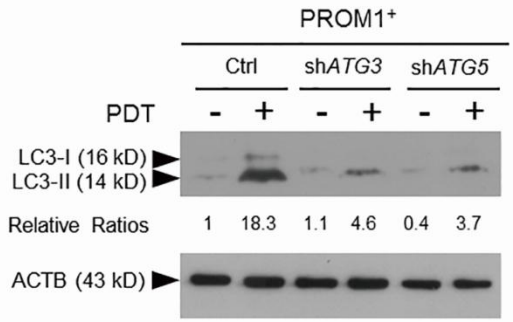

Figure S7. Effects of *ATG* silencing on PDT-induced LC3-II accumulation in CSCs. The PROM1/CD133^{+} PCCs that expressed sh*ATG3* or sh*ATG5* were treated with PDT (1.3 J/cm^{2}). Cell lysates were harvested at 24 h post-PDT, and the expression of LC3 was detected by western blot. The band intensities on films were analyzed by ImageJ software. The relative amounts of LC3-II were quantified as ratios to ACTB, indicated underneath each gel. The relative ratio of LC3-II in Ctrl without PDT treatment is arbitrarily presented as 1.