Figure EV1. HPV infection induces tumor suppression and cell death in response to cisplatin.

A-D Effects of cisplatin (3.5 mg/kg at 72-h intervals) on UM-SCC-22A (A) versus UM-SCC-47 (B)-derived xenograft tumors grown in the flanks of SCID mice were measured at days 0 and 14 (n = 5–8 mice/group, and *P < 0.05 by unpaired Student’s t-test). After the measurement of tumor volumes, CerS1-6 mRNAs were measured in extracted tumor tissues from individual SCID mice treated with cisplatin at day 0 versus day 14 in UM-SCC-22A (C) or UM-SCC-47 (D)-derived xenograft tumors. CerS1-6 mRNAs were normalized to 28S rRNA.

E, F Effects of cisplatin (E) and C18-pyr-cer (F) on cell death (48 h) in UM-SCC-22A [HPV(−)] versus UM-SCC-47 [HPV(+)] cells were measured by trypan blue exclusion assay. Data are means ± SD from three independent experiments, analyzed by unpaired Student’s t-test (**P < 0.05).
Figure EV2. Drp1 knockdown prevents mitophagy.

A Extended time course of experiment shown in Fig 2D. Effects of shRNA-mediated knockdown of Drp1 on mitophagy were measured using live cell imaging for co-localization of MTR/LTG in UM-SCC-1A cells transfected with vector (V) or HPV-E7 (E7 o/v) in the absence/presence of cisplatin (10 μM) for 15, 45, 60, and 120 min. Vehicle-treated cells were used as controls. Images represent at least three independent studies, and scale bar represents 100 μm.

B Ectopic expression of HPV-E7 was confirmed by qPCR (left panel), whereas Drp1 knockdown was confirmed by Western blotting (right panel) in cells presented in (A). Data represent three independent studies ± SD (n = 3).

C Representative graph obtained from Seahorse measurement of OCR in UM-SCC-47 cells grown in the absence/presence of C18-pyr-cer (20 μM, 2 h) with appropriate inhibitors (as described in Materials and Method) is shown. Data represent three independent studies ± SD (n = 3).
Figure EV2.
Figure EV3. Knockdown of autophagy inducer LC3 inhibits cisplatin-mediated cell death in HPV (+) HNSCC cells.
A, B siRNA-mediated knockdown of LC3B was confirmed using Western blotting with anti-LC3B antibody compared to Scr-siRNA-transfected controls (A). Actin was used as a loading control, and blots are representative of three independent experiments. Effects of siRNA-mediated knockdown of LC3B on cisplatin-mediated cell death were measured, and IC₅₀ concentrations were calculated by MTT assays (B) compared to Scr-siRNA-transfected controls (48 h). Data are means ± SD from three independent experiments, analyzed by unpaired Student’s t-test (*P < 0.05).

Figure EV4. HPV-E7 enhances mitochondrial ceramide-dependent lethal mitophagy.
A Effects of siRNA-mediated knockdown of HPV6/E7 on UM-SCC-47 growth inhibition in response to C₁₈-pyr-cer (72 h) or vehicle (DMSO) were measured by MTT assay. Scr-siRNA-transfected cells were used as controls. Data are means ± SD from three independent experiments, analyzed by unpaired Student’s t-test (*P < 0.05).
B Effects of ectopic expression of HPV-E6 versus HPV-E7 on HPV(−) UM-SCC-22A growth inhibition in response to C₁₈-pyr-cer (72 h) or vehicle (DMSO) were measured by MTT assay. Vector-transfected cells were used as controls. Data are means ± SD from three independent experiments, analyzed by unpaired Student’s t-test (*P < 0.05).
Figure EV5. Analysis of subcellular localization and function of E2F5 in HPV(+) HNSCC cells.

A Effects of shRNA-mediated knockdown on E2F5 on ATG5, Drp1, and LC3B mRNAs were measured using qRT-PCR in UM-SCC-47 cells compared to Scr-shRNA-transfected controls. Data are means ± SD from three independent experiments.

B Subcellular localization of E2F5 was assessed in the presence/absence of C18-pyr-cer (20 μM, 1 h) by immunofluorescence using fixed confocal micrographs of UM-SCC47 cells stained with DAPI, anti-F-actin, and anti-E2F5 antibodies. Images represent at least three independent experiments. Scale bars represent 100 μm.

C Protein abundance of E2F5 in cytoplasm versus nucleus in the presence/absence of cisplatin (20 μM, for 0, 1, 2, and 4 h) was detected by Western blotting using cytoplasm- versus nuclei-enriched subcellular fractions of UM-SCC-47 cells using anti-E2F5 antibody. Anti-clathrin antibody was used to validate cytoplasmic fractions, whereas anti-lamin B antibody was used to validate nuclear fractions. Western blot images represent at least three independent experiments.