Supplementary Figures & Figures Legends

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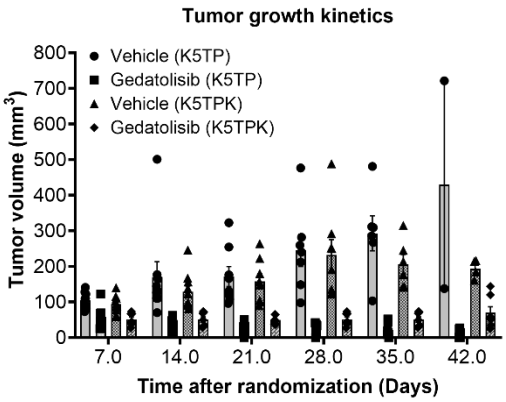

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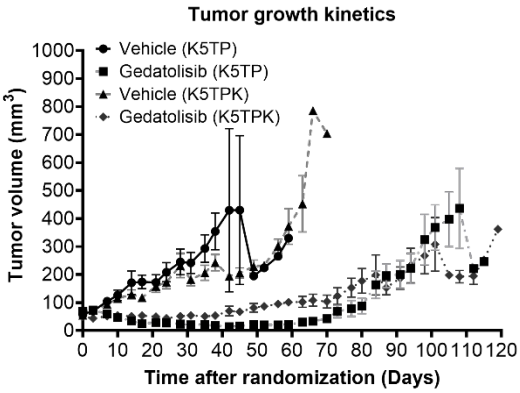
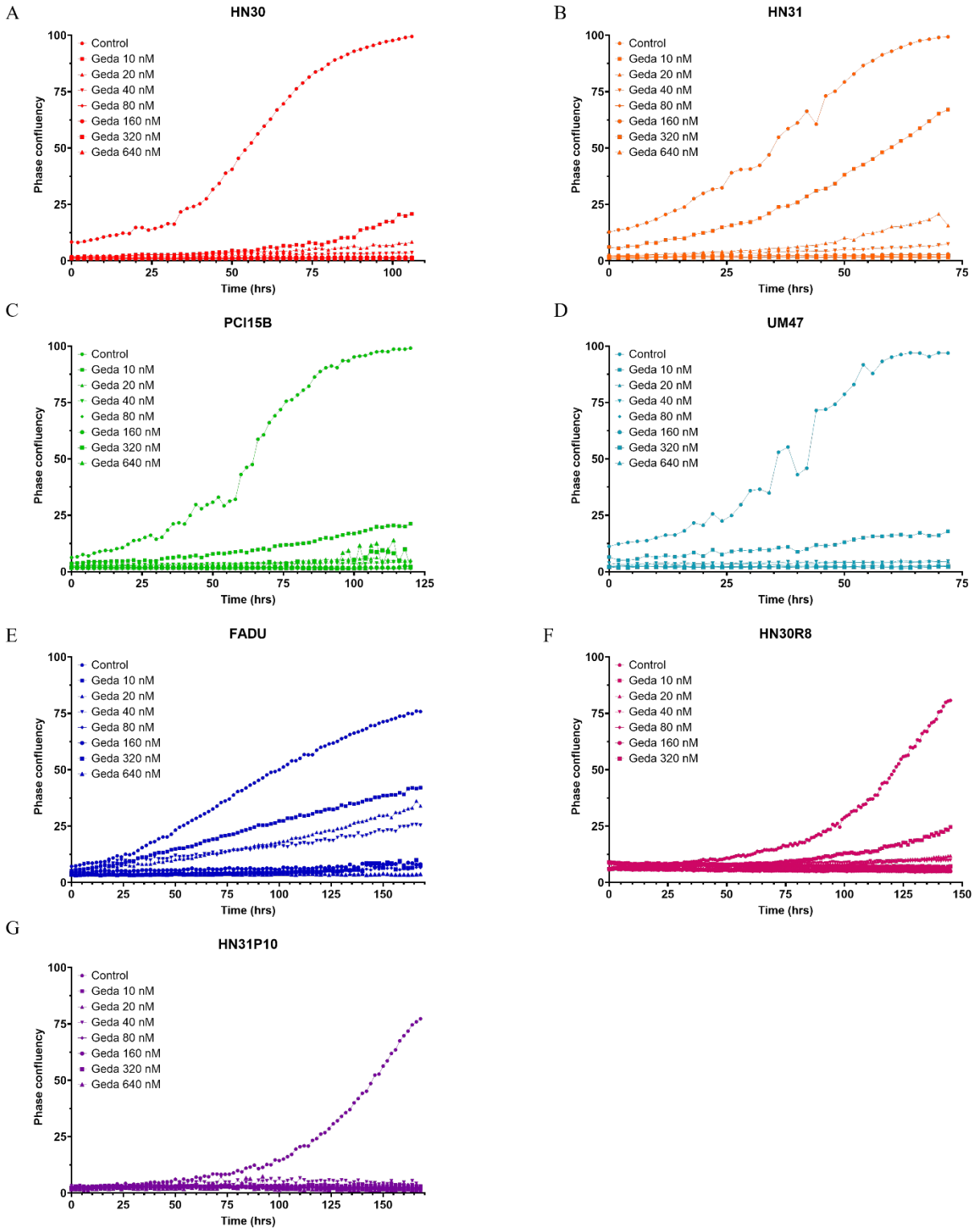
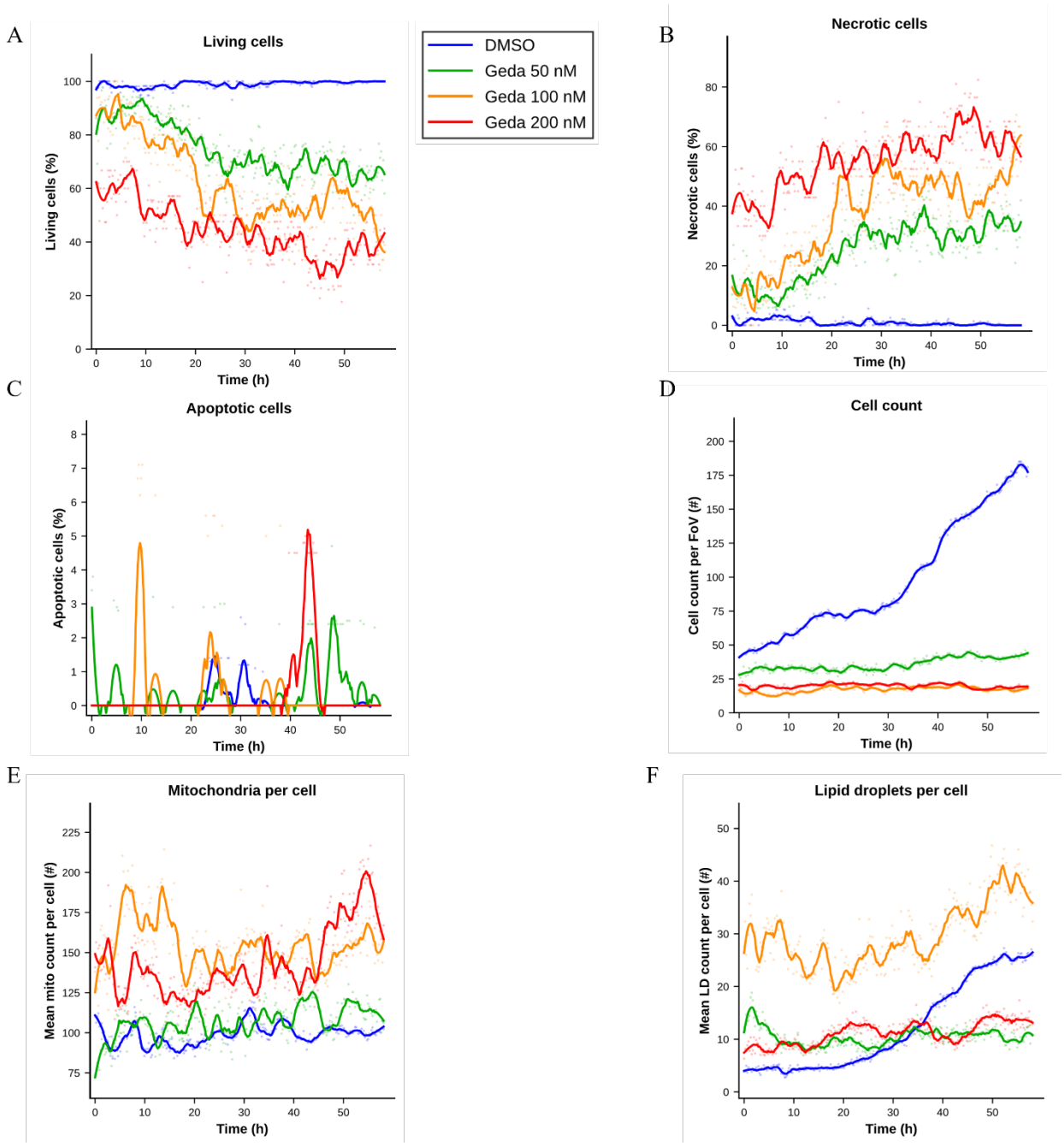


Supplementary Figure 1. Gedatolisib inhibits tumor growth in PI3K-driven genetically engineered mouse models of HNSCC. (A, B) Mean (+/- SEM) tumor growth kinetics for all four groups (Vehicle K5TP, Gedatolisib K5TP, Vehicle K5TPK, Gedatolisib K5TPK), plotted as absolute tumor volume over time.



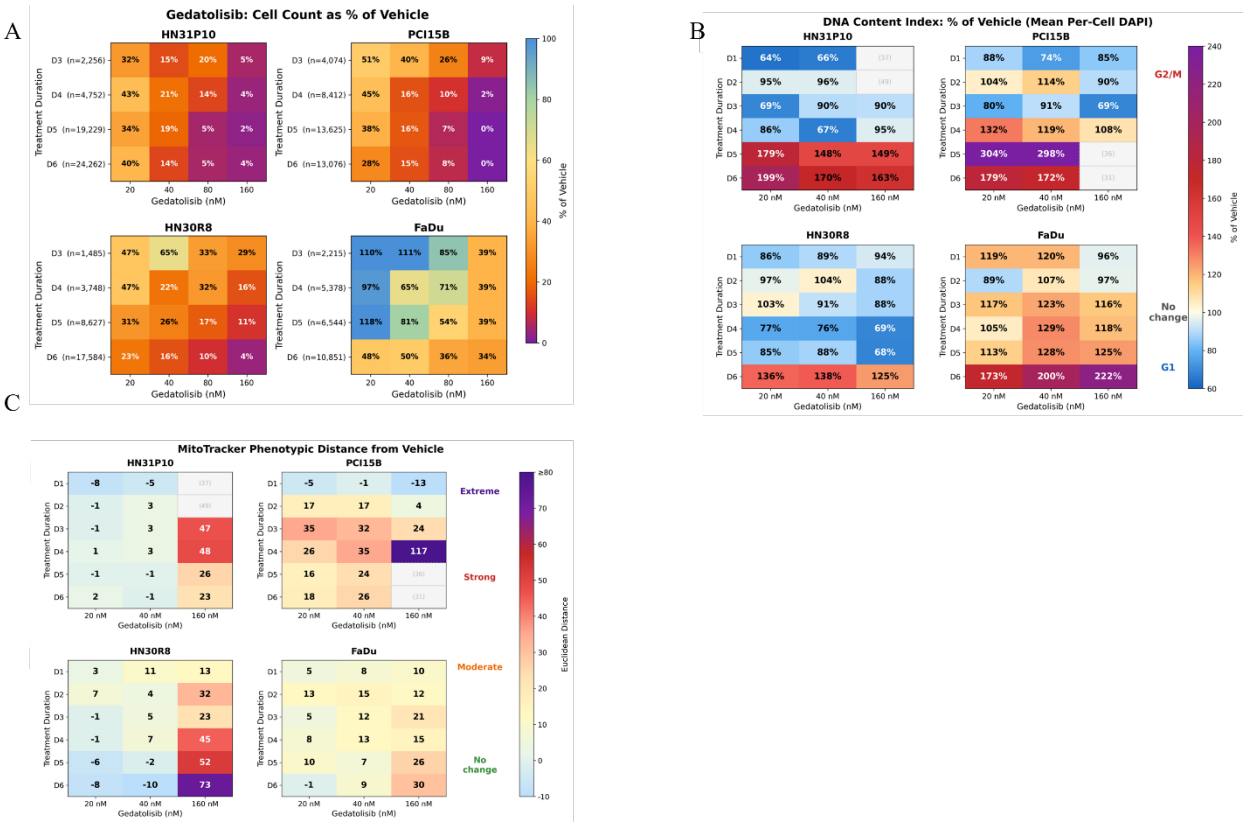
Supplementary Figure 2. Gedatolisib inhibits proliferation in a concentration-dependent manner across HNSCC cell lines. Real-time proliferation monitoring of HNSCC cell lines treated

with increasing concentrations of gedatolisib. Phase confluency was measured over time using the IncuCyte live-cell imaging system. (A) HN30, (B) HN31, (C) PCI15B, (D) UM47, (E) FaDu, (F) HN30R8, and (G) HN31P10. Cells were treated with vehicle control or gedatolisib at the indicated concentrations (10, 20, 40, 80, 160, 320, or 640 nM). HN30R8 (F) was tested up to 320 nM. Data are plotted as phase confluency (%) over time (hours).



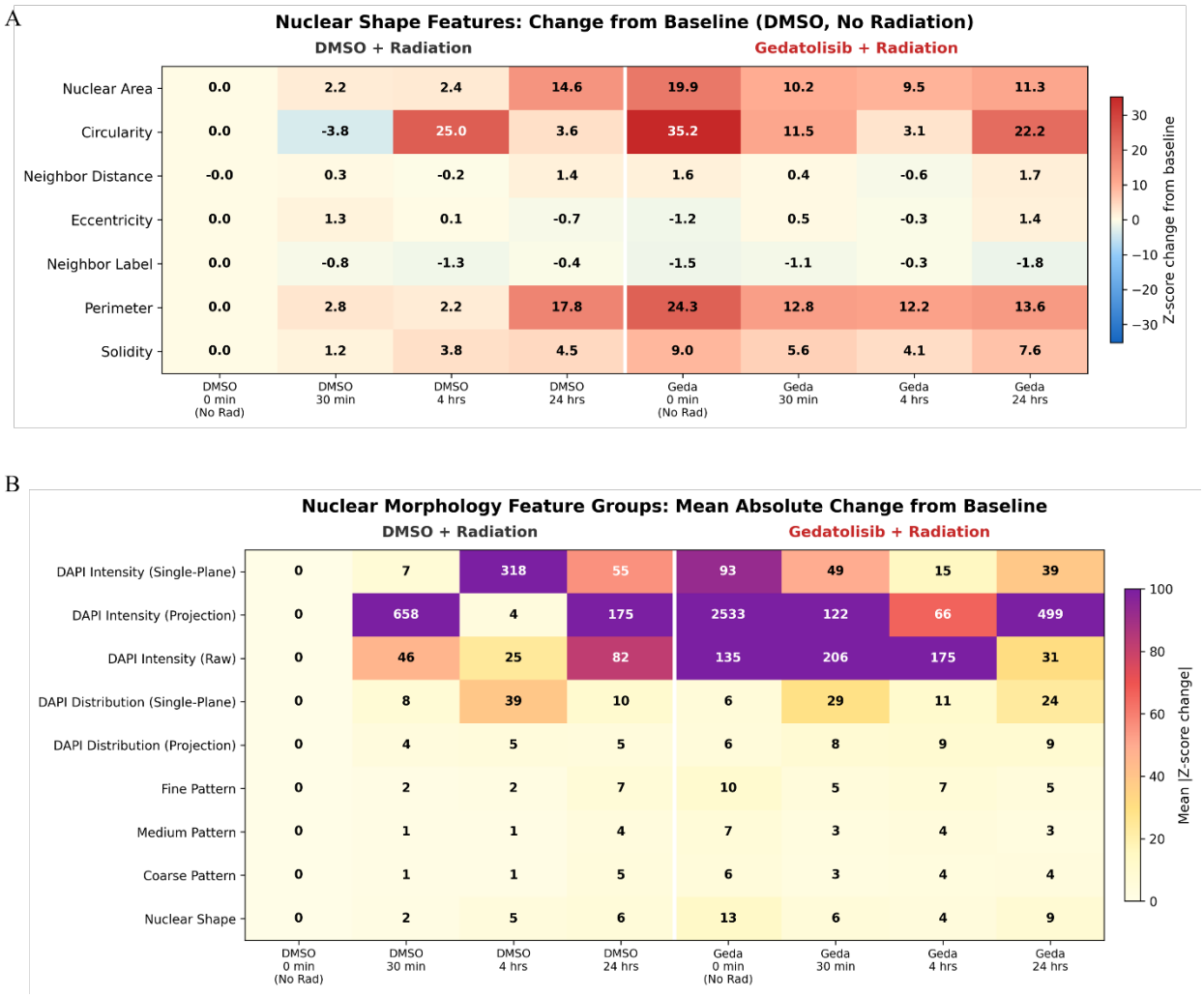
Supplementary Figure 3. Holotomographic live-cell imaging of gedatolisib-induced cell death and metabolic alterations in HN30R8 cells. HN30R8 cells were treated with DMSO (vehicle), gedatolisib 50 nM, 100 nM, or 200 nM and imaged continuously using Nanolive holotomographic microscopy beginning on day 3 post-treatment. Images were acquired at approximately 13.25-minute intervals for up to 58 hours (264 frames). Savitzky-Golay smoothed trend lines (window = 15,

polynomial order = 2) are shown with semi-transparent raw data points. (A) Percentage of living cells over time. (B) Percentage of necrotic cells over time, as classified by Nanolive Live Cell Analysis. (C) Percentage of apoptotic cells over time. (D) Total cell count per field of view. (E) Mean mitochondria count per cell, determined by Nanolive Sub-cellular Mitochondria Analysis. (F) Mean lipid droplet count per cell, determined by Nanolive Sub-cellular Lipid Droplet Analysis.



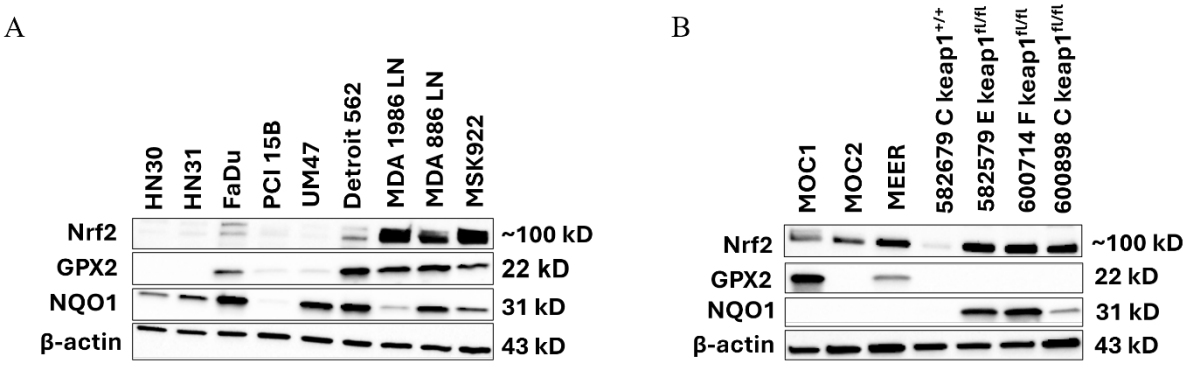
Supplementary Figure 4. Cell Painting Assay (CPA) morphological profiling of gedatolisib-treated HNSCC cell lines. HNSCC cell lines were treated with gedatolisib at the indicated concentrations (20, 40, or 160 nM) for increasing treatment durations (D1 through D6) and analyzed using the Cell Painting Assay (CPA). (A) Cell count expressed as a percentage of vehicle control for HN31P10, PCI15B, HN30R8, and FaDu. Values in parentheses indicate the number of cells counted per condition. Concentrations with fewer than 100 treated cells were excluded. (B) DNA content index, shown as the mean per-cell DAPI intensity expressed as a percentage of vehicle, for HN31P10, PCI15B, HN30R8, and FaDu. Values above 100% indicate increased DNA content consistent with cell cycle arrest in S or G2/M phase. The color scale represents percent of vehicle, with 100% (no change) displayed in white. (C) MitoTracker phenotypic distance from vehicle for HN31P10, PCI15B, HN30R8, and FaDu, calculated as the Euclidean distance across MitoTracker morphological

features relative to the matched vehicle condition. The color scale represents the magnitude of phenotypic change, with higher values (red) indicating greater deviation from vehicle.

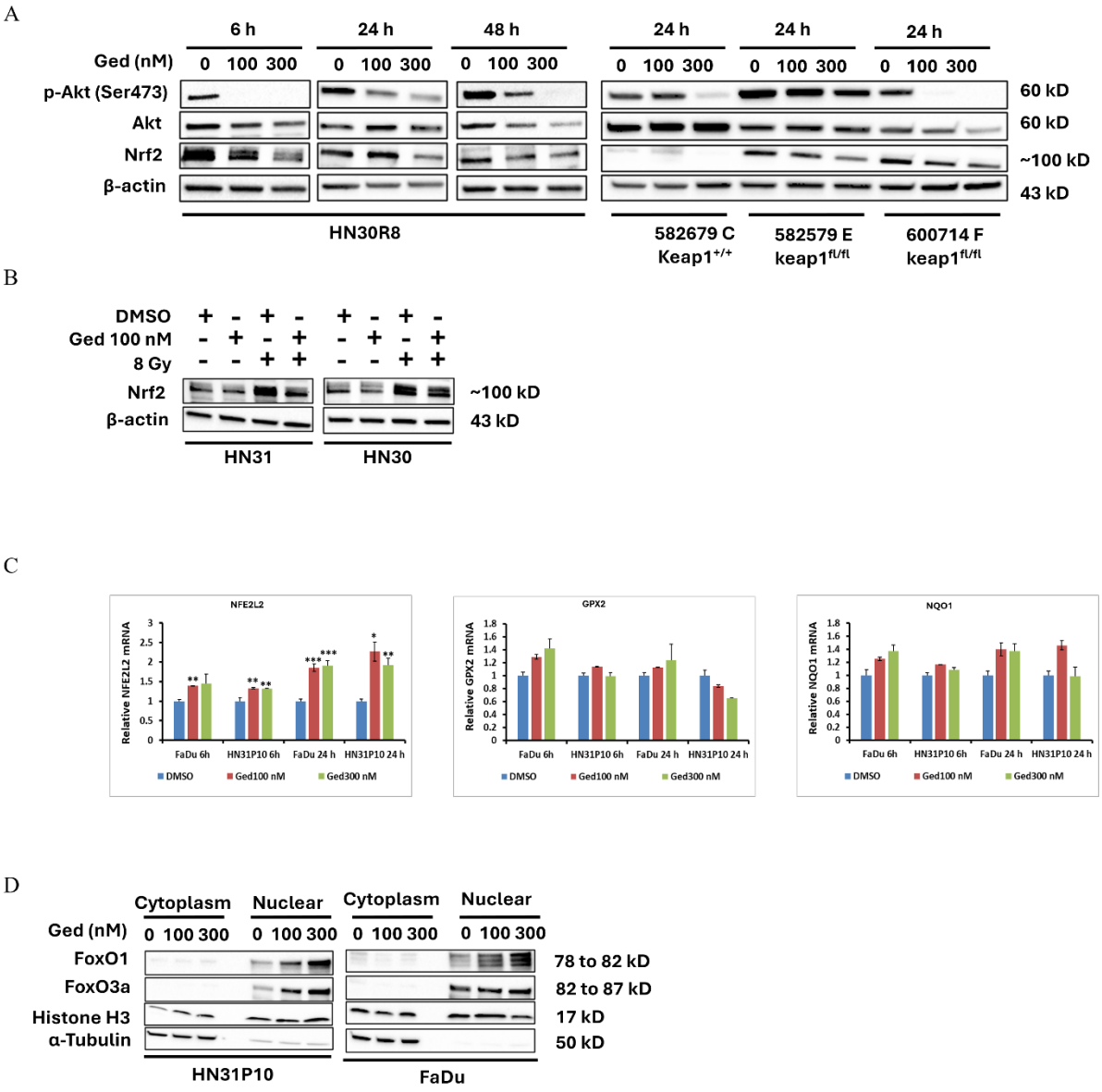


Supplementary Figure 5. Gedatolisib induces substantial nuclear morphological changes in HN30R8 cells. HN30R8 cells were pretreated with DMSO or gedatolisib and then irradiated with a single dose of 8 Gy. Cells were fixed and analyzed by high-content imaging at 0 minutes (unirradiated control), 30 minutes, 4 hours, and 24 hours post-irradiation. All values are expressed as change from the DMSO unirradiated baseline. (A) Heatmap of nuclear shape feature changes (Z-score change from baseline), including nuclear area, circularity, neighbor distance, eccentricity, neighbor label count, perimeter, and solidity. Gedatolisib pretreatment alone (0 min, no radiation) produced substantial changes in nuclear area, circularity, and perimeter relative to the DMSO baseline. (B) Heatmap of nuclear morphology feature groups (mean absolute Z-score change from baseline),

including DAPI intensity (single-plane, projection, and raw), DAPI distribution (single-plane and projection), texture features (fine, medium, and coarse pattern), and nuclear shape.



Supplementary Figure 6. Baseline NRF2 pathway protein expression across HNSCC and murine cell lines. (A) Western blot analysis of NRF2, GPX2, NQO1, and β-actin in a panel of human HNSCC cell lines: HN30, HN31, FaDu, PCI15B, UM47, Detroit 562, MDA 1986 LN, MDA 886 LN, and MSK922. (B) Western blot analysis of the same markers in murine cell lines (MOC1, MOC2, MEER) and GEMM-derived cell lines (582679 C, Keap1$^{+/+}$; 582579 E, Keap1$^{fl/fl}$; 600714 F, Keap1$^{fl/fl}$; 600898 C, Keap1$^{fl/fl}$). All cells were grown under standard conditions without drug treatment.



Supplementary Figure 7. Gedatolisib reduces NRF2 protein levels without suppressing NFE2L2 transcription. (A) Western blot analysis of p-AKT (Ser473), total AKT, NRF2, and β-actin in HN30R8, and GEMM-derived cell lines (582679 C, Keap1$^{+/+}$; 582579 E, Keap1$^{fl/fl}$; 600714 F, Keap1$^{fl/fl}$) treated with gedatolisib (0, 100, or 300 nM) for 6, 24, or 48 hours. (B) Western blot analysis of NRF2 and β-actin in HN31, and HN30 cells treated with DMSO or gedatolisib (100 nM), with or without a single dose of 8 Gy radiation, for 24 hours. (C) Quantitative RT-PCR analysis of NFE2L2, GPX2, and NQO1

mRNA expression in FaDu and HN31P10 cells treated with DMSO, gedatolisib 100 nM, or gedatolisib 300 nM for 6 or 24 hours. Data are presented as fold change relative to DMSO control. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. (D) Western blot analysis of FoxO1, FoxO3a, Histone H3, and α-tubulin in cytoplasmic and nuclear fractions from HN31P10, and FaDu cells treated with gedatolisib (0, 100, or 300 nM) for 6 hours.

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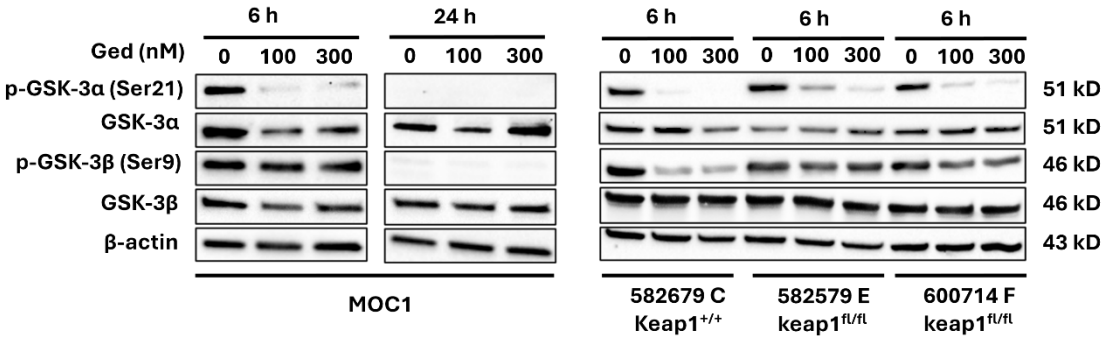

Supplementary Figure 8. Gedatolisib activates GSK3 and promotes proteasome-dependent NRF2 degradation. (A) Western blot analysis of the same markers in MOC1 (6 and 24 hours) and GEMM-derived cell lines (582679 C, Keap1$^{+/+}$; 582579 E, Keap1$^{fl/fl}$; 600714 F, Keap1$^{fl/fl}$) treated with gedatolisib for 6 hours.

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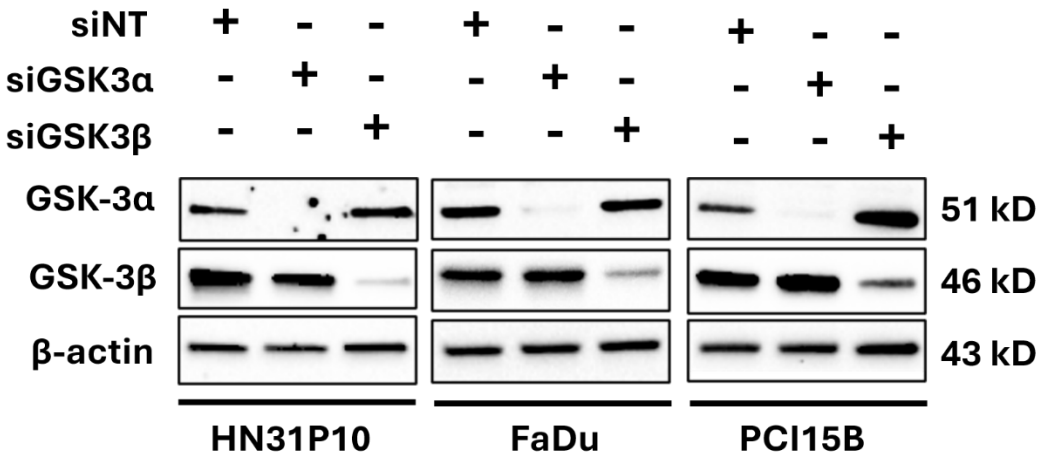

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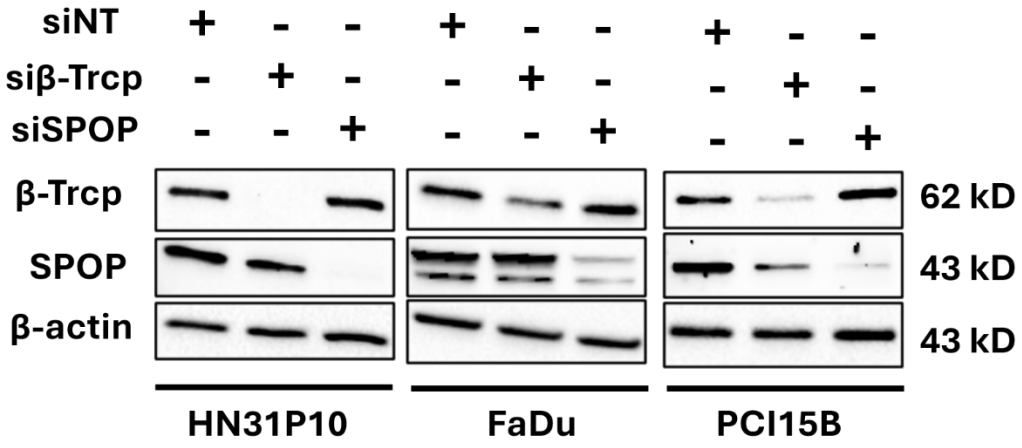
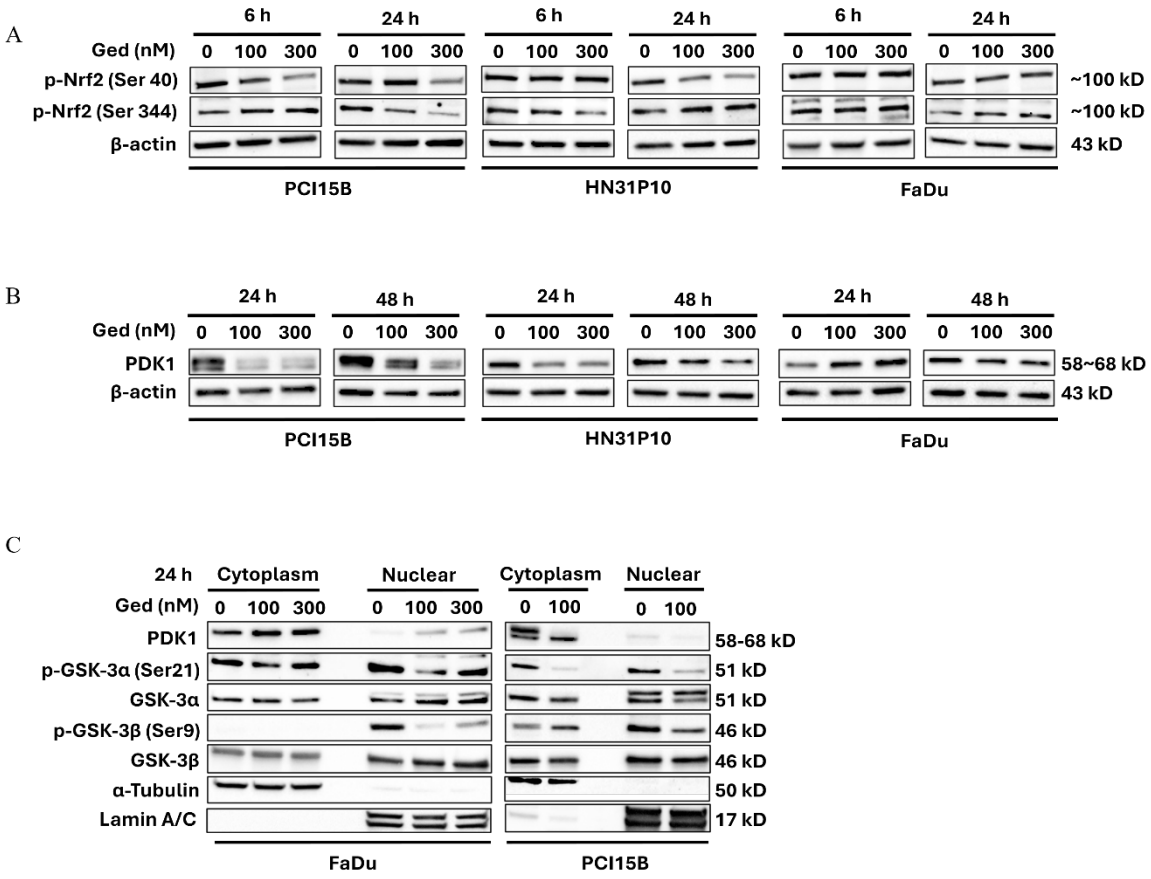
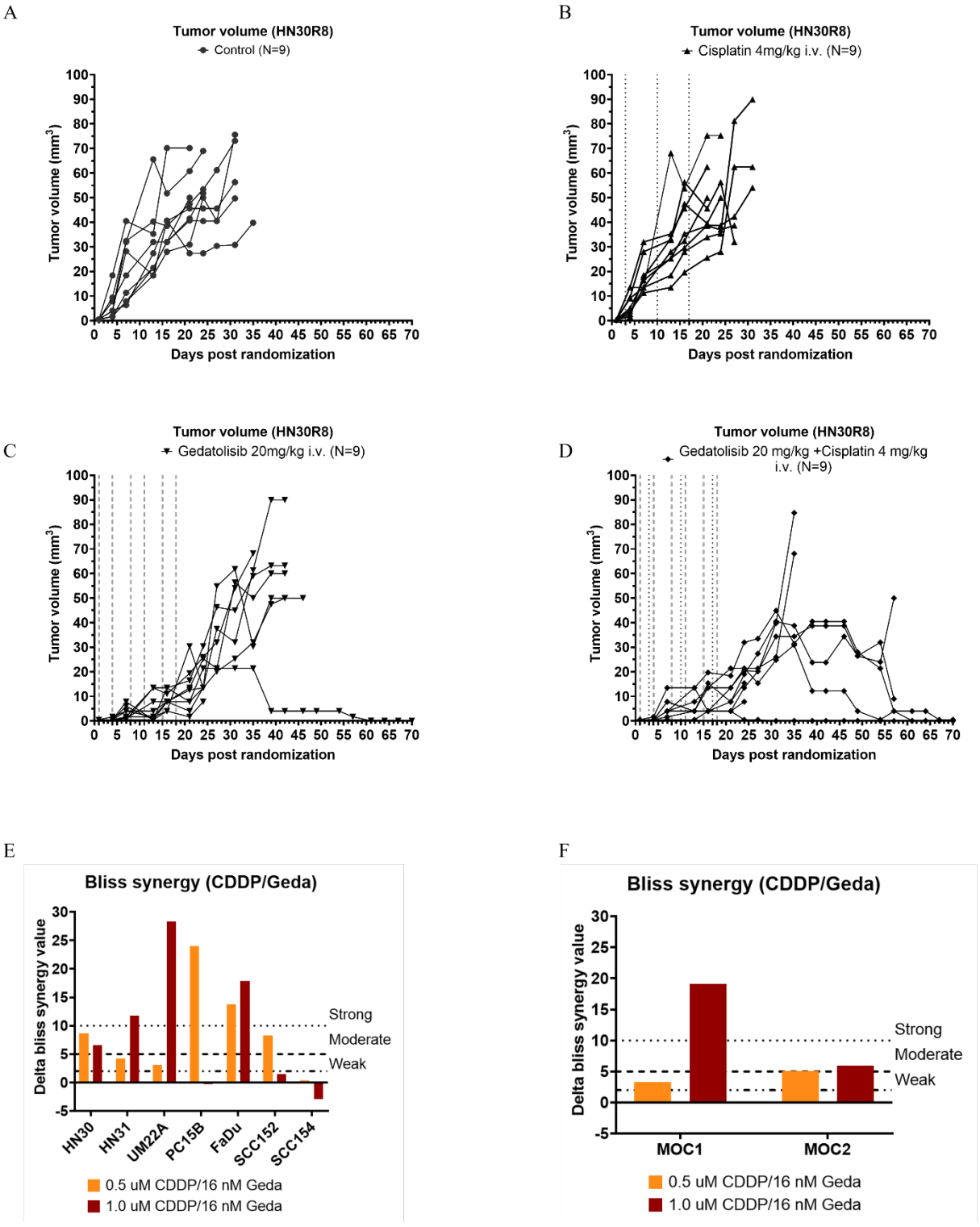


Supplementary Figure 9. GSK3 and the E3 ligase adaptors β-TrCP and SPOP mediate gedatolisib-induced NRF2 degradation. (A) Western blot confirmation of siRNA-mediated knockdown of GSK3-α and GSK3-β, individually and in combination, in HN31P10, FaDu, and PCI15B cells. Cells were transfected with non-targeting siRNA (siNT) or siRNAs targeting GSK3-α and/or GSK3-β. (B) Western blot confirmation of siRNA-mediated knockdown of β-TrCP and SPOP in HN31P10, FaDu, and PCI15B cells.

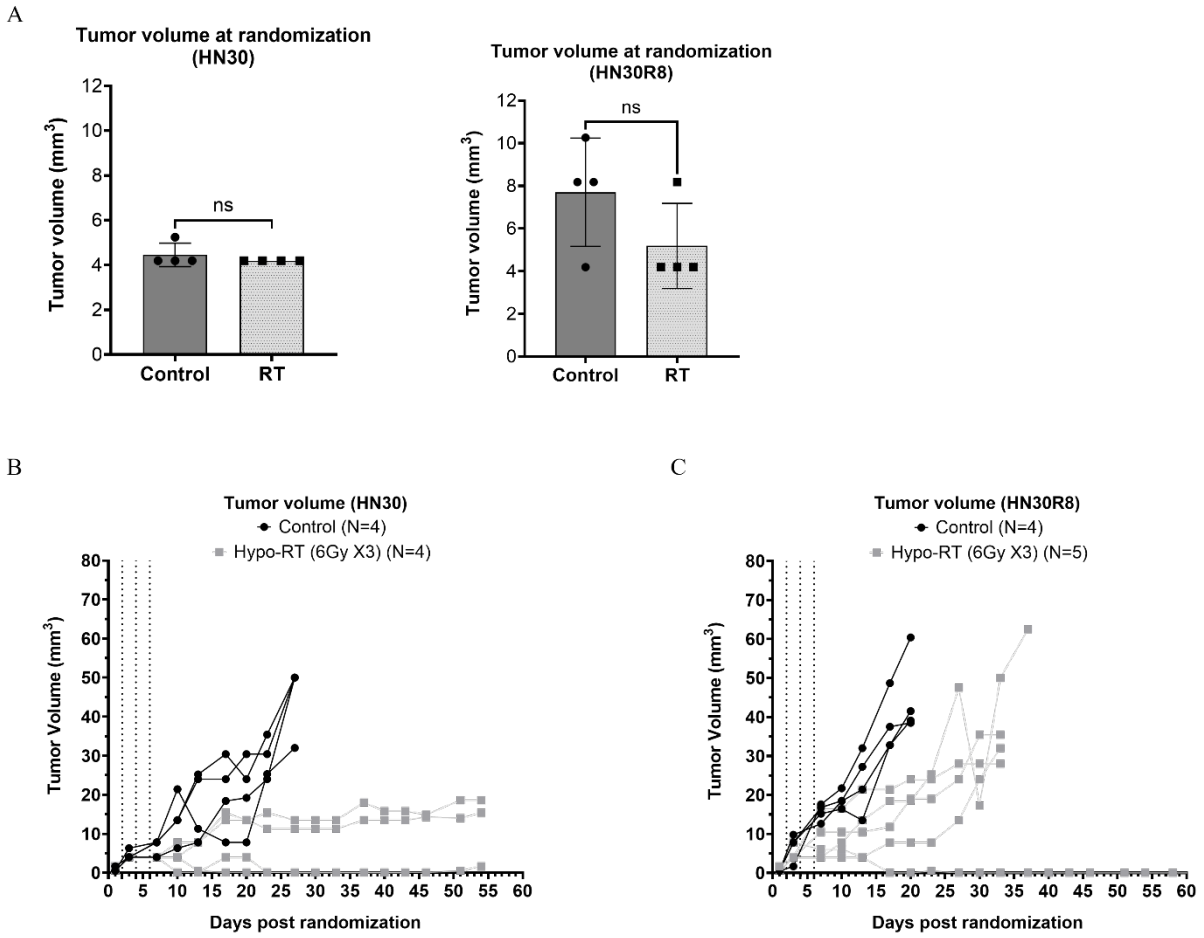


Supplementary Figure 10. Gedatolisib reduces NRF2 Ser40 phosphorylation and modulates subcellular distribution of GSK3 pathway components. (A) Western blot analysis of p-NRF2 (Ser40), p-NRF2 (Ser344), and β-actin in PCI15B, HN31P10, and FaDu cells treated with gedatolisib (0, 100, or 300 nM) for 6 or 24 hours. Phosphorylation at Ser40 stabilizes NRF2, while phosphorylation at Ser344 promotes degradation. (B) Western blot analysis of PDK1 and β-actin in PCI15B, HN31P10, and FaDu cells treated with gedatolisib (0, 100, or 300 nM) for 24 or 48 hours. PDK1 downregulation serves as confirmation of sustained PI3K pathway inhibition. (C) Western blot analysis of cytoplasmic and nuclear fractions from FaDu and PCI15B cells treated with gedatolisib (0, 100, or 300 nM for FaDu; 0 or 100 nM for PCI15B) for 24 hours, probed for PDK1, p-GSK3-α (Ser21), total GSK3-α, p-GSK3-β (Ser9), total GSK3-β, NRF2, α-tubulin (cytoplasmic marker), and Lamin A/C (nuclear marker).

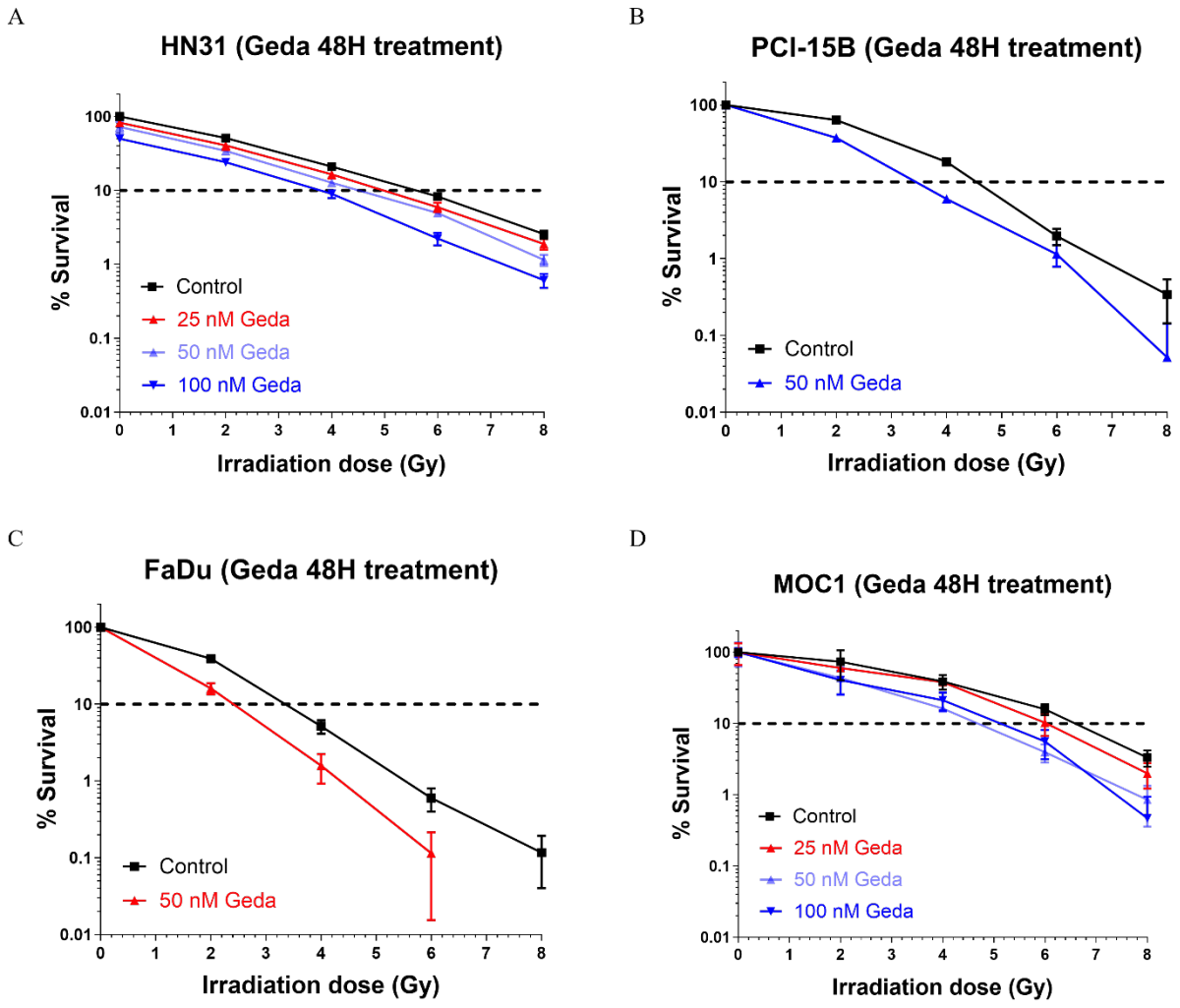


Supplementary Figure 11. Gedatolisib combined with cisplatin suppress NRF2 hyperactivated cisplatin-resistant HN30R8 tumor growth. Nude mice bearing HN30R8 orthotopic tongue

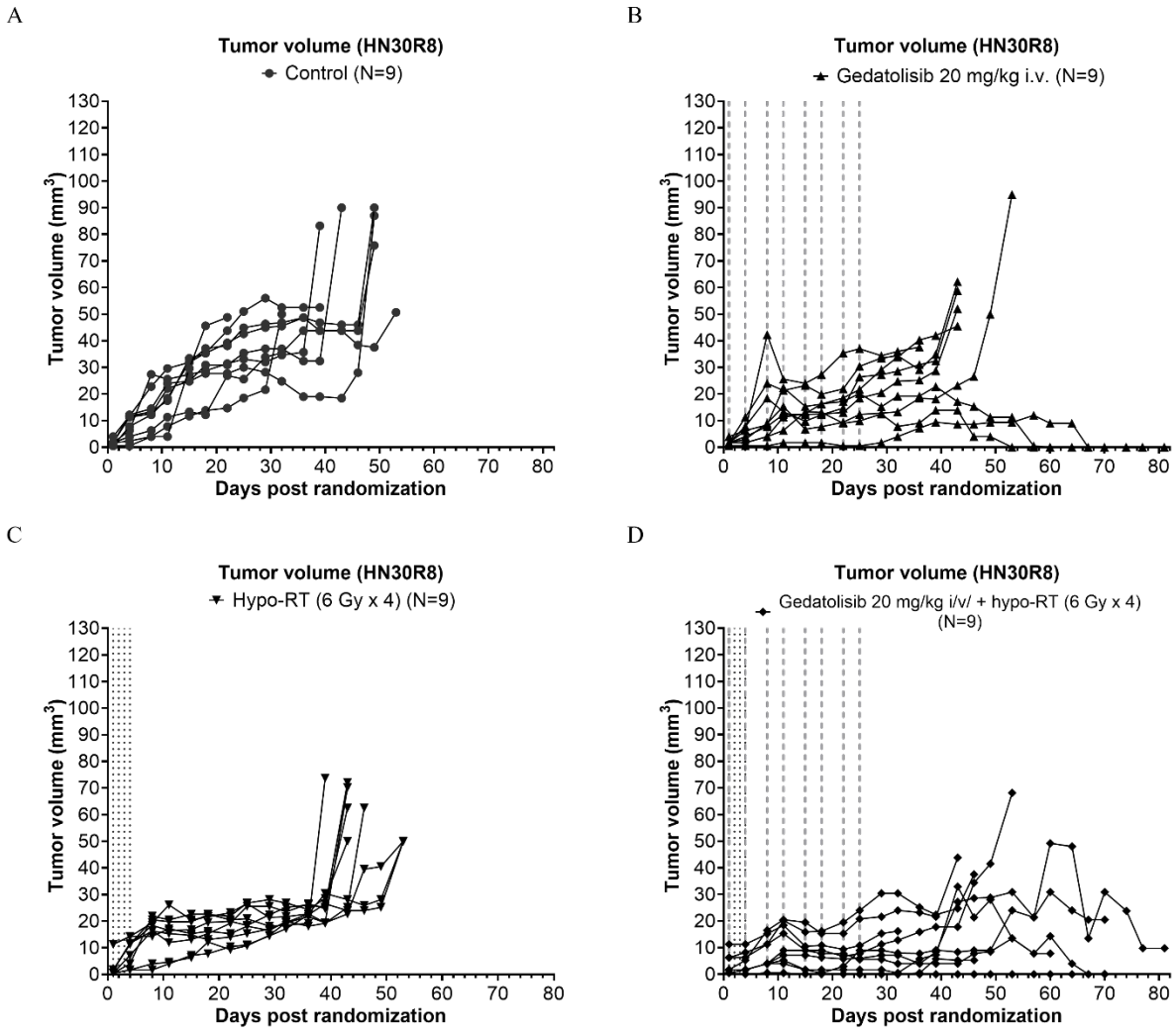
xenografts were randomized and treated as indicated. Gedatolisib was administered at 20 mg/kg i.v. twice weekly. Cisplatin was administered at 4 mg/kg i.v. Dashed grey vertical lines indicate drug dosing days. (A) vehicle control (N = 9), (B) cisplatin alone (N = 9), (C) gedatolisib alone (N = 9), and (D) gedatolisib plus cisplatin (N = 9). (E-F) Cells were treated with gedatolisib and cisplatin (CDDP) at the indicated concentrations, and viability was assessed after 72 hours. Synergy was calculated using the Bliss independence model. Delta Bliss synergy values above 0 indicate synergistic interaction; values within defined thresholds are classified as weak (dashed line), moderate (dotted line), or strong. (E) Delta Bliss synergy values for human HNSCC cell lines (HN30, HN31, UM22A, PCI15B, FaDu, SCC152, SCC154) at two cisplatin concentrations (0.5 and 1.0 uM) combined with gedatolisib (16 nM). (F) Delta Bliss synergy values for murine cell lines (MOC1, MOC2) at the same drug concentrations.



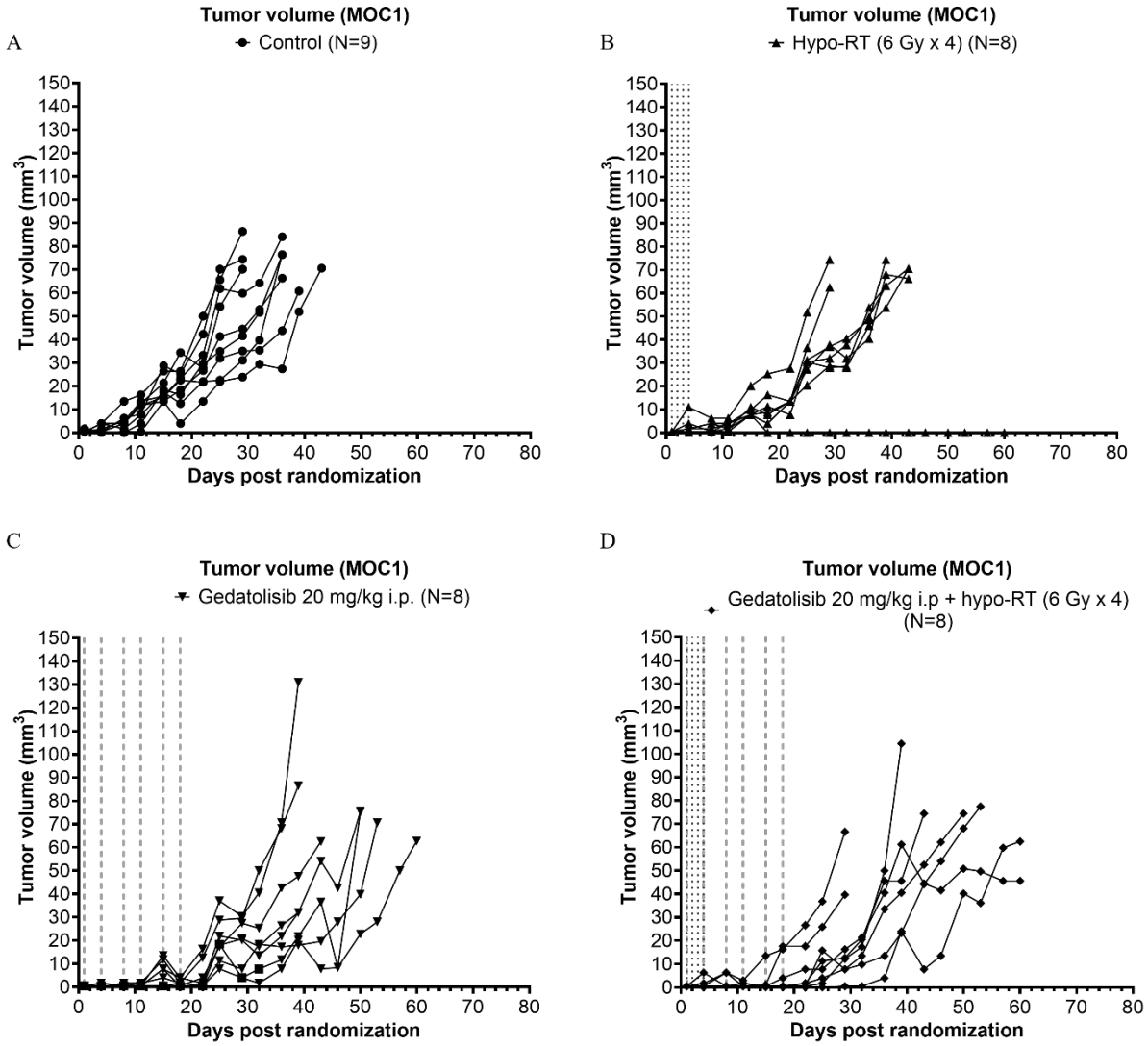
Supplementary Figure 12. HN30R8 cisplatin-resistant tumors are radioresistant compared to parental HN30 in an orthotopic model. Nude mice bearing HN30 or HN30R8 orthotopic tongue xenografts were randomized to vehicle control or hypofractionated radiation (6 Gy × 3 fractions). (A) Tumor volumes at the time of randomization for HN30 (left) and HN30R8 (right), confirming comparable tumor burden between treatment groups for both cell lines (ns, not significant). (B) Individual tumor growth traces for HN30 control (N = 4) and hypo-RT (N = 4). Radiation produced growth suppression in the parental HN30 model. (C) Individual tumor growth traces for HN30R8 control (N = 4) and hypo-RT (N = 5). HN30R8 tumors continued to grow despite radiation, consistent with NRF2-driven radioresistance. Dashed vertical lines indicate radiation dosing days.



Supplementary Figure 13. Gedatolisib enhances radiation sensitivity across KEAP1 wild-type HNSCC cell lines. Clonogenic survival of HN31 (A), PCI-15B (B), FaDu (C), and MOC1 (D) cells pretreated with the indicated concentrations of gedatolisib for 48 hours prior to irradiation (0-8 Gy). HN31 and MOC1 were tested at 25, 50, and 100 nM gedatolisib; PCI-15B and FaDu were tested at 50 nM. Dashed line indicates 10% survival. Data represent mean ± SEM.



Supplementary Figure 14. Gedatolisib combined with hypofractionated radiation in cisplatin-resistant HN30R8 xenografts. Nude mice bearing HN30R8 orthotopic tongue xenografts were randomized into four treatment arms: vehicle control, gedatolisib (20 mg/kg i.v., twice weekly), hypofractionated radiation (hypo-RT; 6 Gy x 4 fractions), or gedatolisib plus hypo-RT. Two independent experiments were performed. Individual tumor growth traces for (A) vehicle control (N = 9), (B) gedatolisib alone (N = 9), (C) hypo-RT (N = 9), and (D) gedatolisib plus hypo-RT (N = 9). Dashed gray vertical lines and solid vertical lines indicate gedatolisib and radiation dosing days, respectively.



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289 **Supplementary Figure 15. Gedatolisib and hypofractionated radiation in the syngeneic MOC1**
290 **model.** C57BL/6 mice bearing MOC1 syngeneic tumors were randomized into four treatment arms.
291 (A) Vehicle control (N = 9). (B) Hypofractionated radiation (hypo-RT; 6 Gy x 4 fractions) (N = 8). (C)
292 Gedatolisib (20 mg/kg i.p.) (N = 8). (D) Gedatolisib plus hypo-RT (N = 8). Individual tumor growth
293 traces are shown for each treatment arm. Dashed gray vertical lines and solid vertical lines indicate
294 gedatolisib and radiation dosing days, respectively.