**SUPPLEMENTAL FIGURES (Uzunparmak *et. al*.)**

**Figure S1.** Effects of knockdown of *CASP8* on *in vitro* cell proliferation and clonogenicity in HNSCCs.

**Figure S2.** Effects of treatment with Birinapant and zVAD-FMK with or without TNFα on cell viability in HNSCCs under *CASP8* loss

**Figure S3.** Effects of treatment with Birinapant and zVAD-FMK with or without TRAIL on cell viability in HNSCCs under *CASP8* loss

**Figure S4.** Effects of treatment with Birinapant and zVAD-FMK on clonogenicity in HNSCCs under *CASP8* loss

**Figure S5.** Necroptotic effects of treatment with Birinapant and zVAD-FMK are enhanced under *CASP8* loss in HNSCCs.

**Figure S6.** Loss of *CASP8* increases the radiosensitizing effects of Birinapant or Birinapant plus zVAD-FMK through induction of necroptosis.

**Figure S7.** Loss of *CASP8* enhances radiation killing by Birinapant or Birinapant plus zVAD-FMK through induction of necroptosis.

**Figure S8.** Necroptosis sensitivity in HNSCC cell lines

**Figure S9.** Validation of *in vitro* and *in vivo CASP8* knockdown using Tetracycline-Regulated Inducible RNA interference (RNAi) system

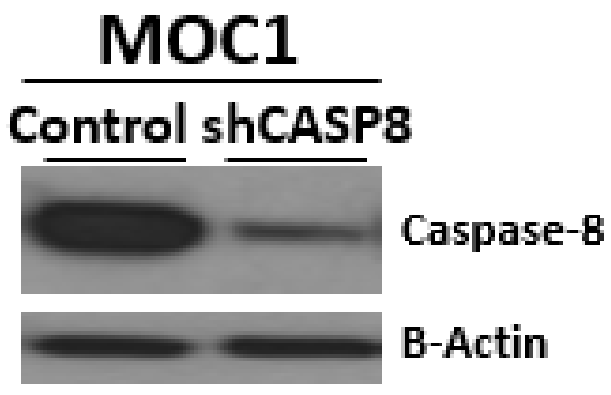
**Figure S10.** Treatment schema for *in vivo* experiments

**Figure S11.** Inhibition of *CASP8* function with Emricasan increases radiation killing by Birinapant in HNSCCs.

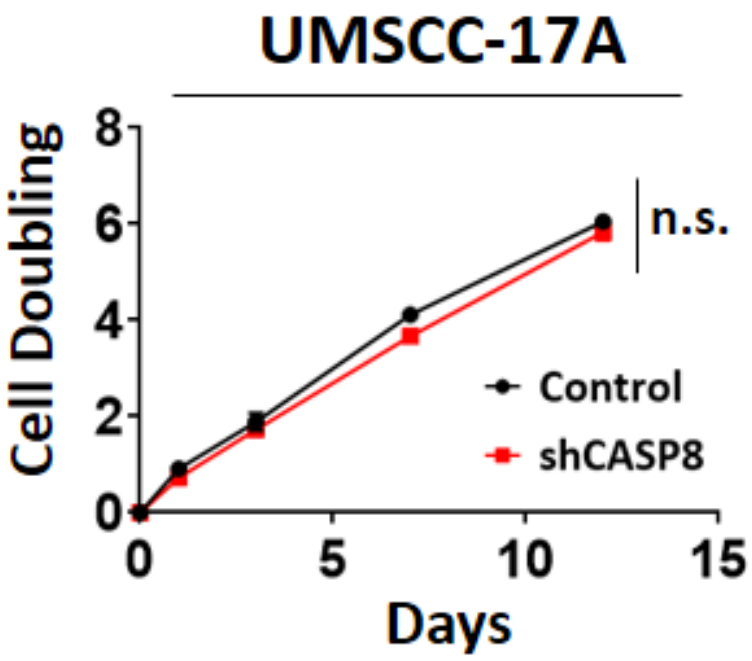
**Figure S12.** Inhibition of *CASP8* function with Emricasan enhances radiosensitizing effects of Birinapant in HNSCCs.

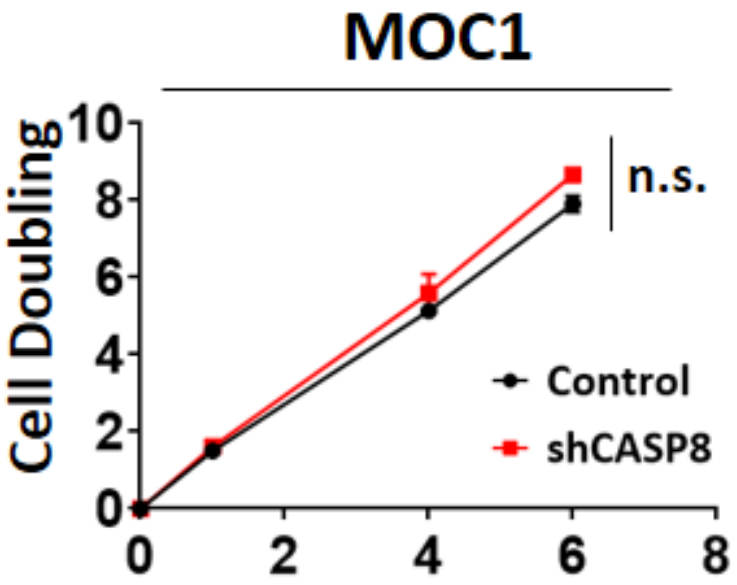
# Supplemental Figure 1

**A.**

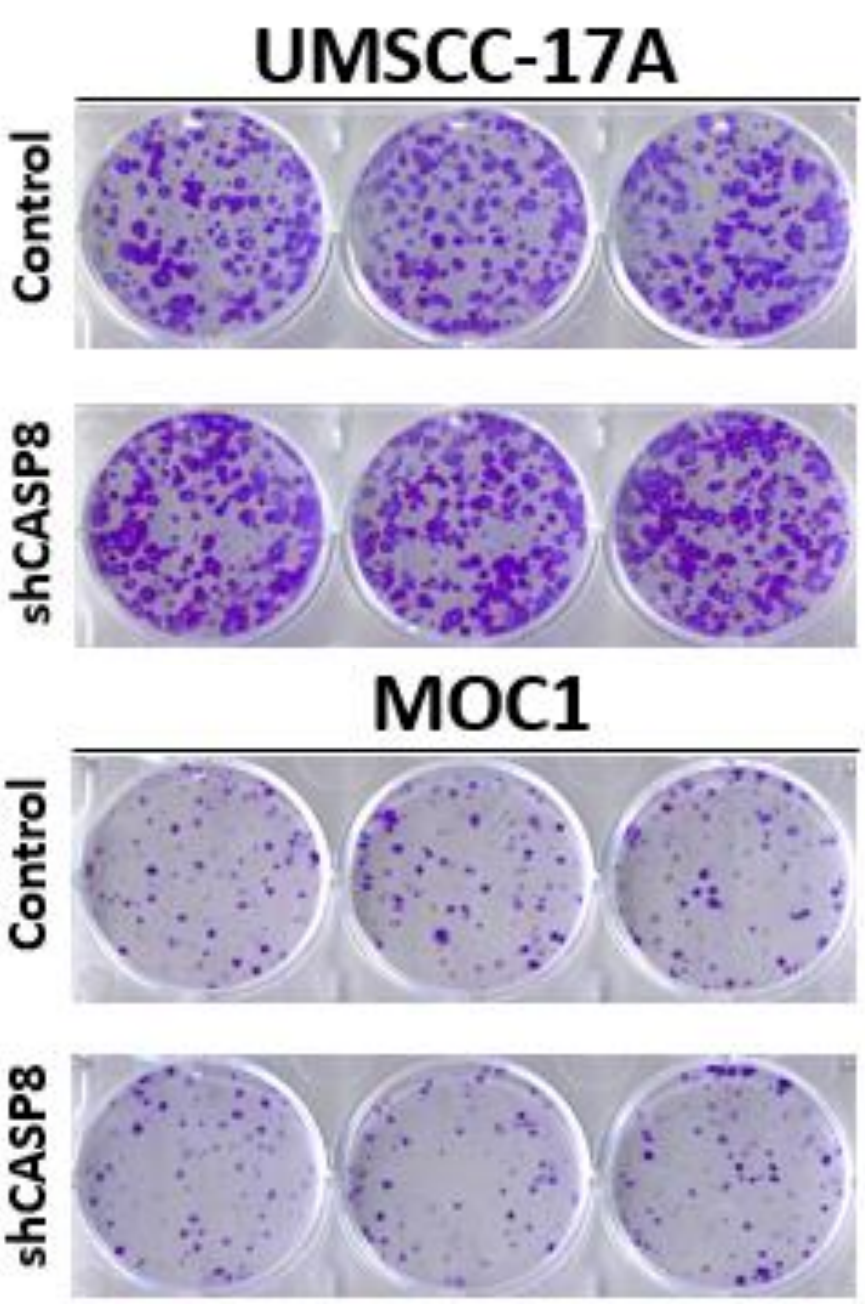



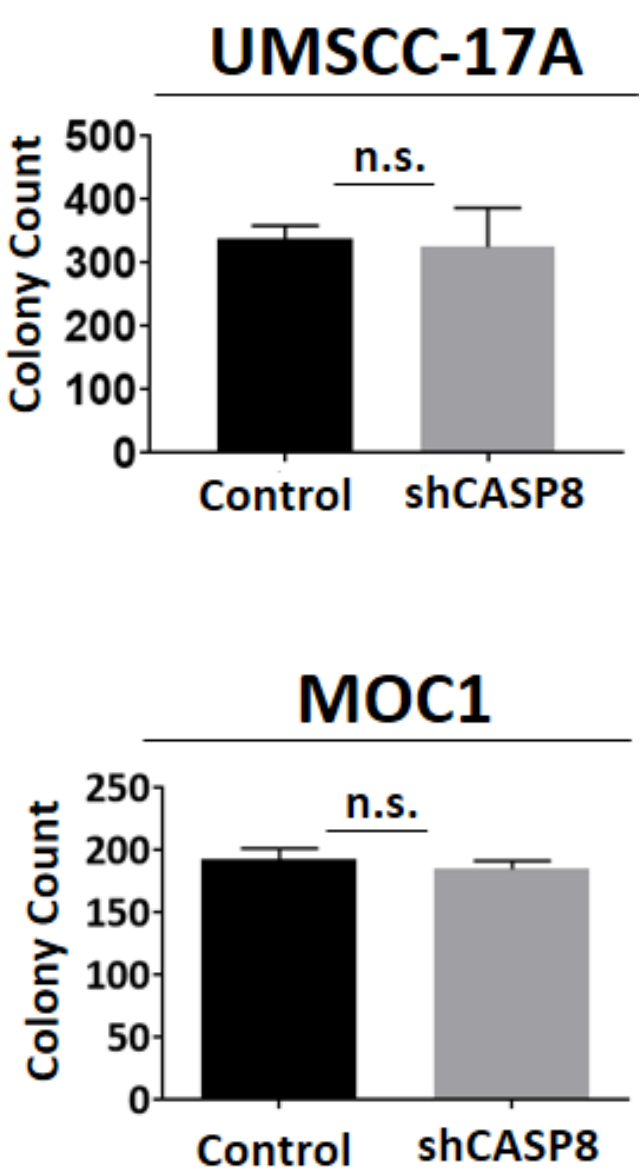

**B.**




**C.**

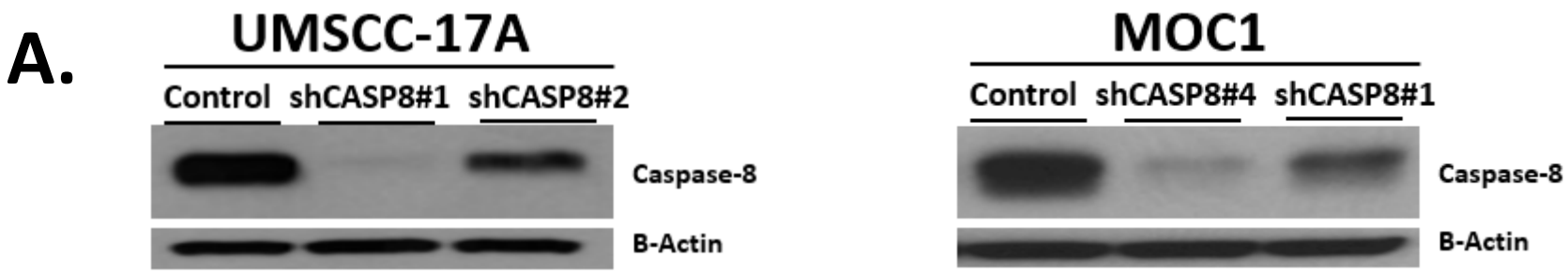


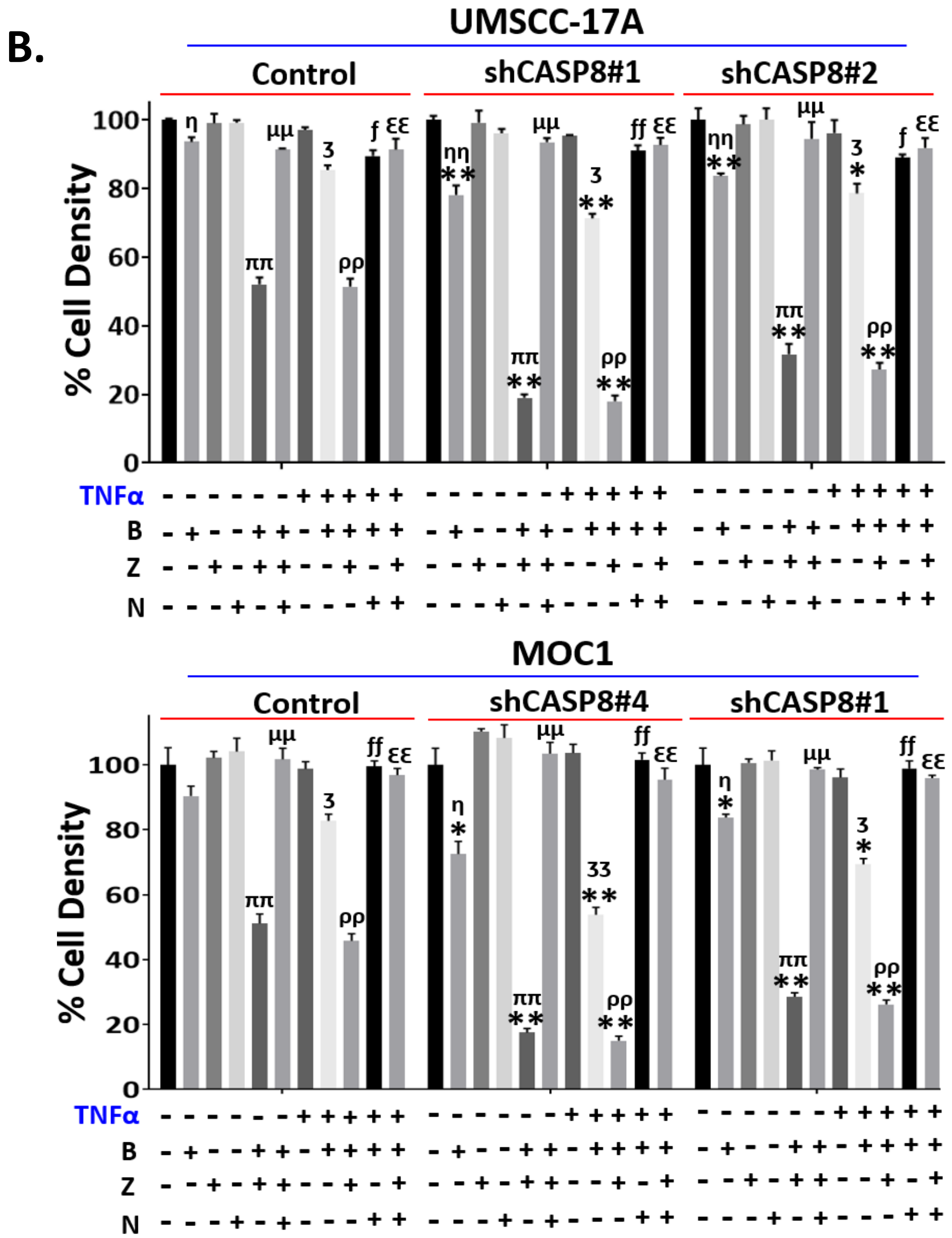



**Supplemental Figure 1. Effects of knockdown of *CASP8* on *in vitro* cell proliferation and clonogenicity in HNSCCs**

**A.** *CASP8* was knockdown using shRNA in UMSCC-17A and MOC1 HNSCC cell lines. Cell lysates obtained from the engineered control (scrambled shRNA) and shCASP8 cell lines were subjected to WB analysis for the validation of *CASP8* knockdown. β-Actin was used as loading control. **B.** Control and shCASP8 UMSCC-17A and MOC1 cell lines were subjected to cell proliferation analysis by Cell-Titer Glo. Luminescence reads were taken at the indicated time points and normalized to Day 0 reads to calculate cell doublings. Samples were run in replicates of four. 2-way ANOVA was used for statistical analysis. **C.** Control and shCASP8 UMSCC-17A and MOC1 cell lines were subjected to clonogenic survival analysis. Colony counts were used to assess baseline clonogenicity. Samples were run in triplicates. Student *t* test was used for statistics. All experiments detailed above were repeated three times with similar results.

# Supplemental Figure 2

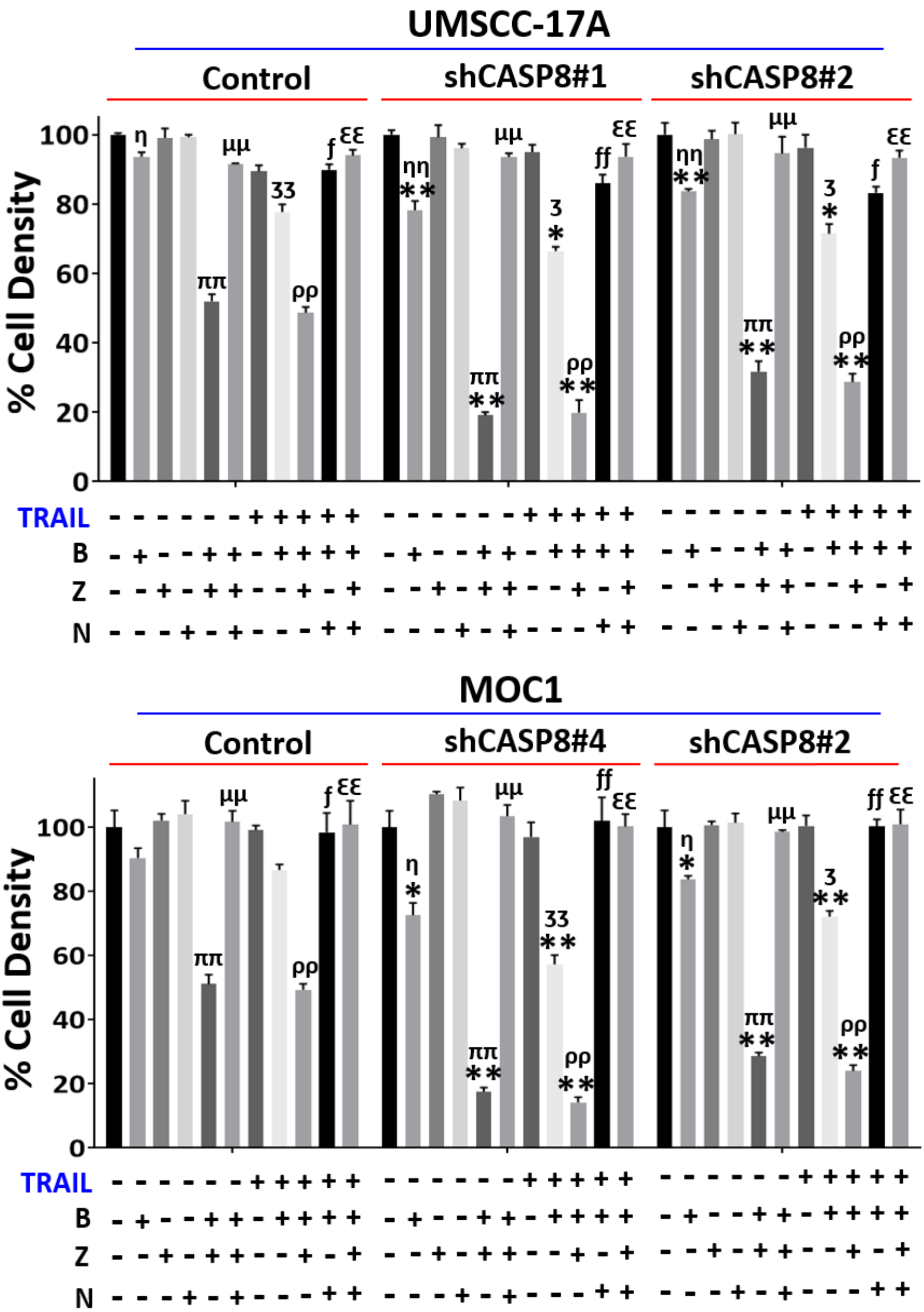


**Supplemental Figure 2. Effects of treatment with Birinapant and zVAD-FMK with or without TNFα on cell viability in HNSCCs under *CASP8* loss**

**A.** *CASP8* was knockdown using shRNA in UMSCC-17A and MOC1 HNSCC lines using 2 independent shRNA constructs. Knockdown of *CASP8* was validated by WB analysis. **B.** UMSCC-17A and MOC1 control and shRNA knockdown (shCASP8) cells were treated with Birinapant (**B** [200nmol/L for the UMSCC-17A cells; 1μmol/L for the MOC1 cells]), zVAD-FMK (**Z** [5μmol/L for both the cell lines]), Necrostatin-1s (**N** [10μmol/L for both the cell lines]) in the presence and absence of TNFα (**T** [50ng/mL for both the cell lines]) or the combinations as indicated for 24 hours. Cell viability was assessed using Cell-Titer Glo. Values normalized to nontreated cells from the same experiment to calculate % cell density. (This supplemental figure is related to **Figure 2A).** All treatments were carried out in replicates of four. Student *t* test was used for statistics. *****, *P*<0.05; ******, *P*<0.001 when comparing the effects of B, B+Z , T+B or T+B+Z conditions between isogenic control and shCASP8 cell lines. <u>The following symbols are used to make comparisons between the indicated treatment conditions for each individual cell line</u>: **η**, *P*<0.05; **ηη**, *P*<0.001 to compare no treatment (NT) vs B. **ʒ,** *P*<0.05; **ʒʒ**,*P*<0.001 to compare B vs T+B. ***ƒ***, *P*<0.05; ***ƒƒ***, *P*<0.001 to compare T+B vs T+B+N. **ε,** P<0.05; **εε,** *P*<0.001 to compare T+B+Z vs T+B+Z+N. **μ,** *P*<0.05; **μμ,** *P*<0.001 to compare B+Z vs B+Z+N. **π,** *P*<0.05; **ππ,** *P*<0.001 to compare B vs B+Z. **ρ,** *P*<0.05; **ρρ,** *P*<0.001 to compare T+B vs T+B+Z. All experiments detailed above were repeated three times with similar results.

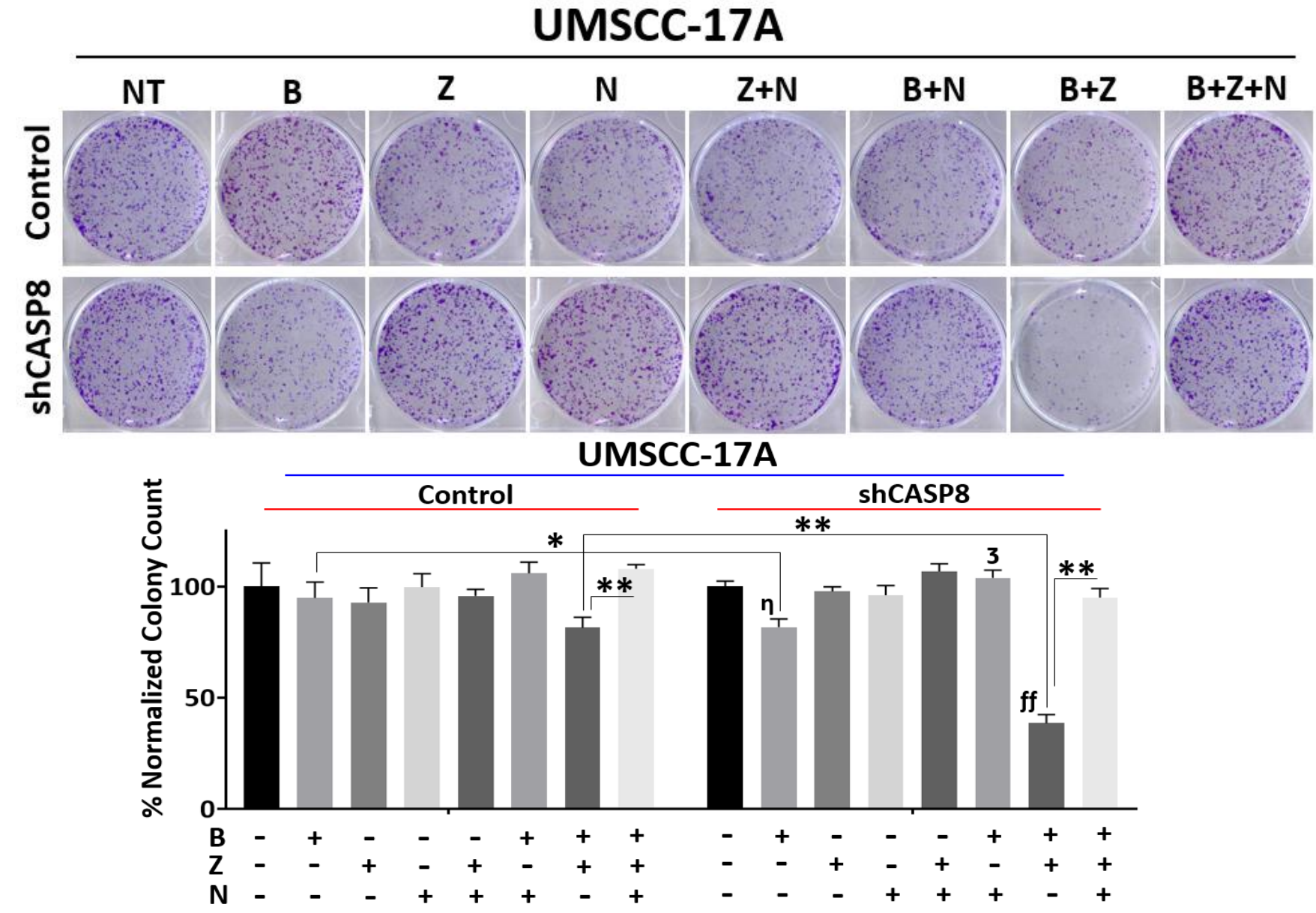
# Supplemental Figure 3



**Supplemental Figure 3. Effects of treatment with Birinapant and zVAD-FMK with or without TRAIL on cell viability in HNSCCs under *CASP8* loss**

UMSCC-17A and MOC1 control and shRNA knockdown (shCASP8) cells were treated with Birinapant (**B** [200nmol/L for the UMSCC-17A cells; 1μmol/L for the MOC1 cells]), zVAD-FMK (**Z** [5μmol/L for both the cell lines]), Necrostatin-1s (**N** [10μmol/L for both the cell lines]) in the presence and absence of TRAIL (**T** [10ng/ml for the UMSCC-17A cells; 50ng/mL for the MOC1 cells]) or the combinations as indicated for 24 hours. Cell viability was assessed using Cell-Titer Glo. Values normalized to nontreated cells from the same experiment to calculate % cell density. (This supplemental figure is related to **Figure 2A).** All treatments were carried out in replicates of four and experiments were repeated three times with similar results. Student *t* test was used for statistics. *****, *P*<0.05; ******, *P*<0.001 when comparing the effects of B, B+Z , T+B or T+B+Z conditions between isogenic control and shCASP8 cell lines. The following symbols are used to make comparisons between the indicated treatment conditions for each individual cell line: **η**, *P*<0.05; **ηη**, *P*<0.001 to compare no treatment (NT) vs B. **ʒ,** *P*<0.05; **ʒʒ**,*P*<0.001 to compare B vs T+B. ***ƒ***, *P*<0.05; ***ƒƒ***, *P*<0.001 to compare T+B vs T+B+N. **ε,** P<0.05; **εε,** *P*<0.001 to compare T+B+Z vs T+B+Z+N. **μ,** *P*<0.05; **μμ,** *P*<0.001 to compare B+Z vs B+Z+N. **π,** *P*<0.05; **ππ,** *P*<0.001 to compare B vs B+Z. **ρ,** *P*<0.05; **ρρ,** *P*<0.001 to compare T+B vs T+B+Z.

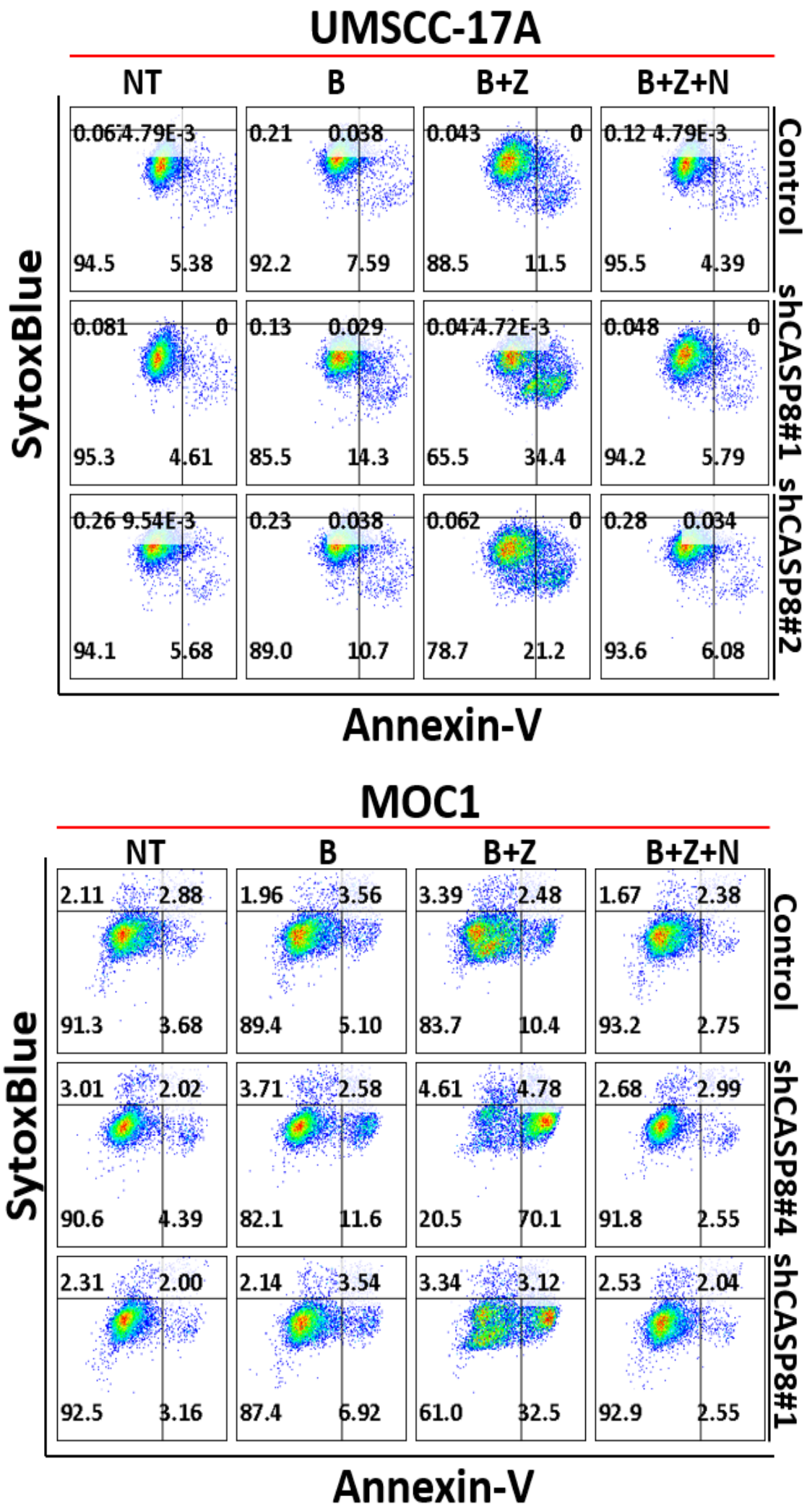
# Supplemental Figure 4



**Supplemental Figure 4. Effects of treatment with Birinapant and zVAD-FMK on clonogenicity in HNSCCs under *CASP8* loss**

UMSCC-17A and MOC1 control and shRNA knockdown (shCASP8) cells were treated with Birinapant (**B** [200nmol/L for the UMSCC-17A cells; 1μmol/L for the MOC1 cells]), zVAD-FMK (**Z** [5μmol/L for both the cell lines]), Necrostatin-1s (**N** [10μmol/L for both the cell lines]) or the combinations as indicated. 24 hour after treatments, drug dilutions were washed out, colonies were allowed to form for 5-12 days, after which they were stained and counted. Surviving colonies were normalized to nontreated cells from the same experiment. % normalized colony counts were plotted (This supplemental figure is related to **Figure 2B).** All treatments were carried out in triplicates and experiments were repeated three times with similar results. Student *t* test was used for statistics. *****, *P*<0.05; ******, *P*<0.001 for the indicated pairwise comparisons. The following symbols are used to make comparisons between the indicated treatment conditions for each individual cell line: **η**, *P*<0.05; **ηη**, *P*<0.001 to compare no treatment (NT) vs B. **ʒ**, *P*<0.05; **ʒʒ**, *P*<0.001 to compare B vs B+N. ***ƒ***, *P*<0.05; ***ƒƒ***, *P*<0.001 to compare B vs B+Z.

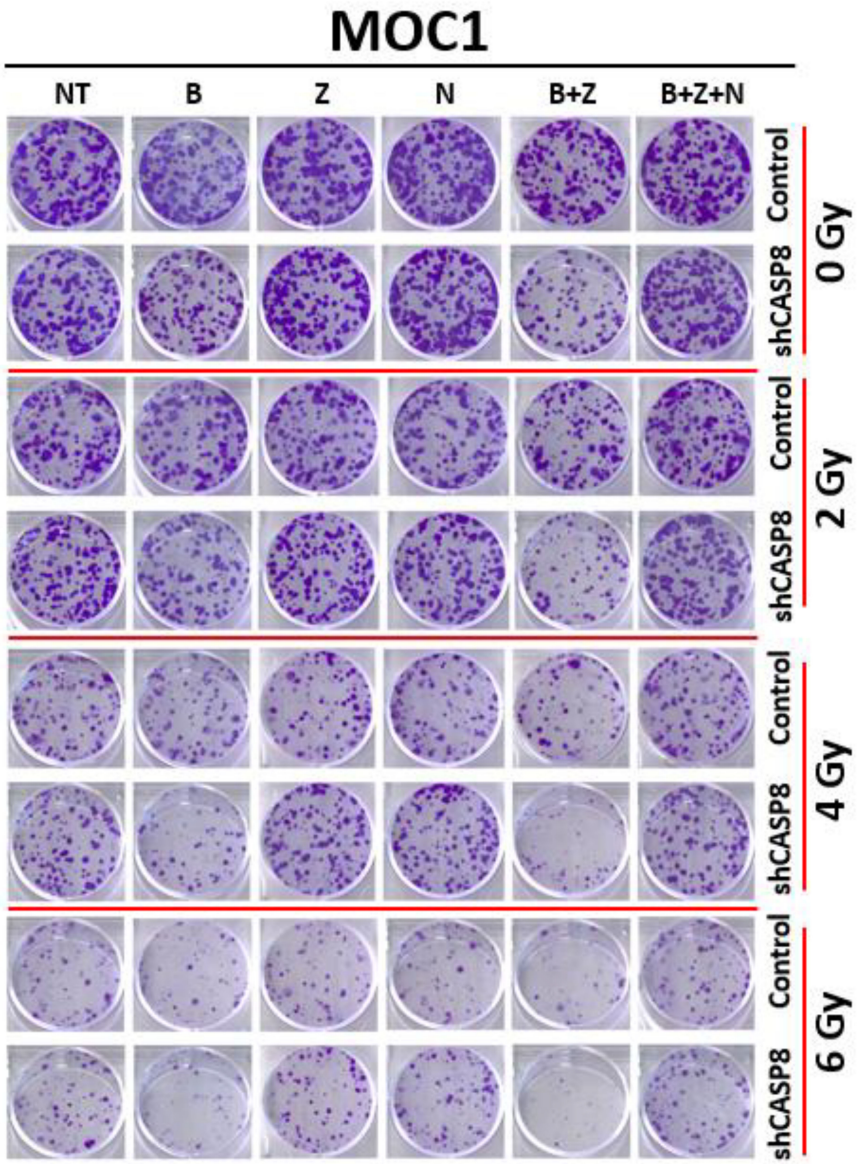
# Supplemental Figure 5



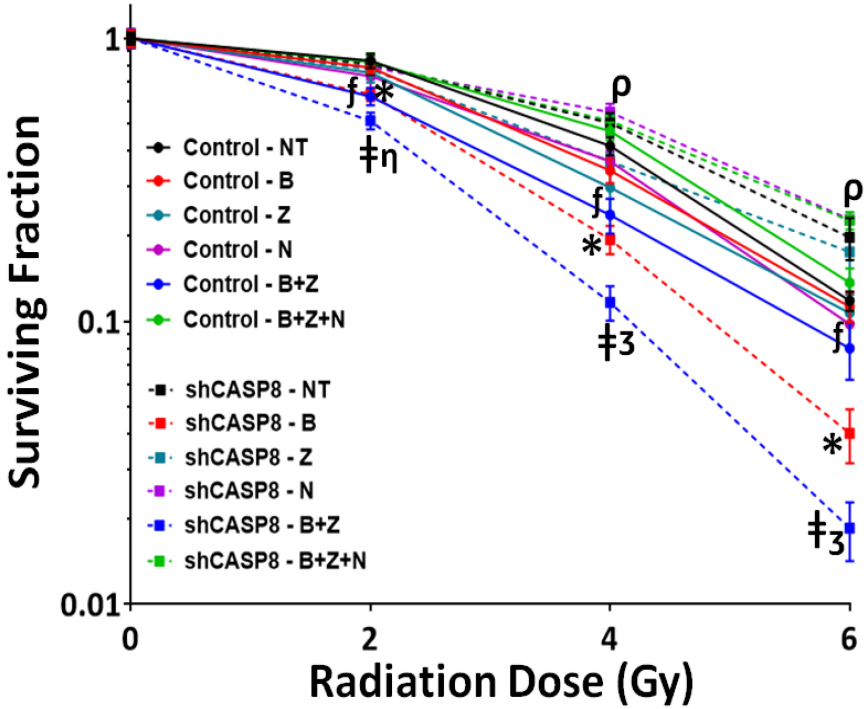
**Supplemental Figure 5. Necroptotic effects of treatment with Birinapant and zVAD-FMK are enhanced under *CASP8* loss in HNSCCs.**

UMSCC-17A and MOC1 control and shRNA knockdown (shCASP8) cells (2 independent shRNA clones for each cell line) were treated with Birinapant (**B** [200nmol/L for the UMSCC-17A cells; 1μmol/L for the MOC1 cells]), zVAD-FMK (**Z** [5μmol/L for both the cell lines]), Necrostatin-1s (**N** [10μmol/L for both the cell lines]) or the combinations as indicated. AnnexinV-APC/SytoxBlue staining was performed 24 hour after treatments. % Annexin-V positivity was used as a measure to assess cell death (This supplemental figure is related to **Figure 2C**). All treatments were carried out in triplicates and experiments were repeated three times with similar results. Student *t* test was used for statistics. *, $P<0.05$; **, $P<0.001$ for the indicated pairwise comparisons.

## Supplemental Figure 6



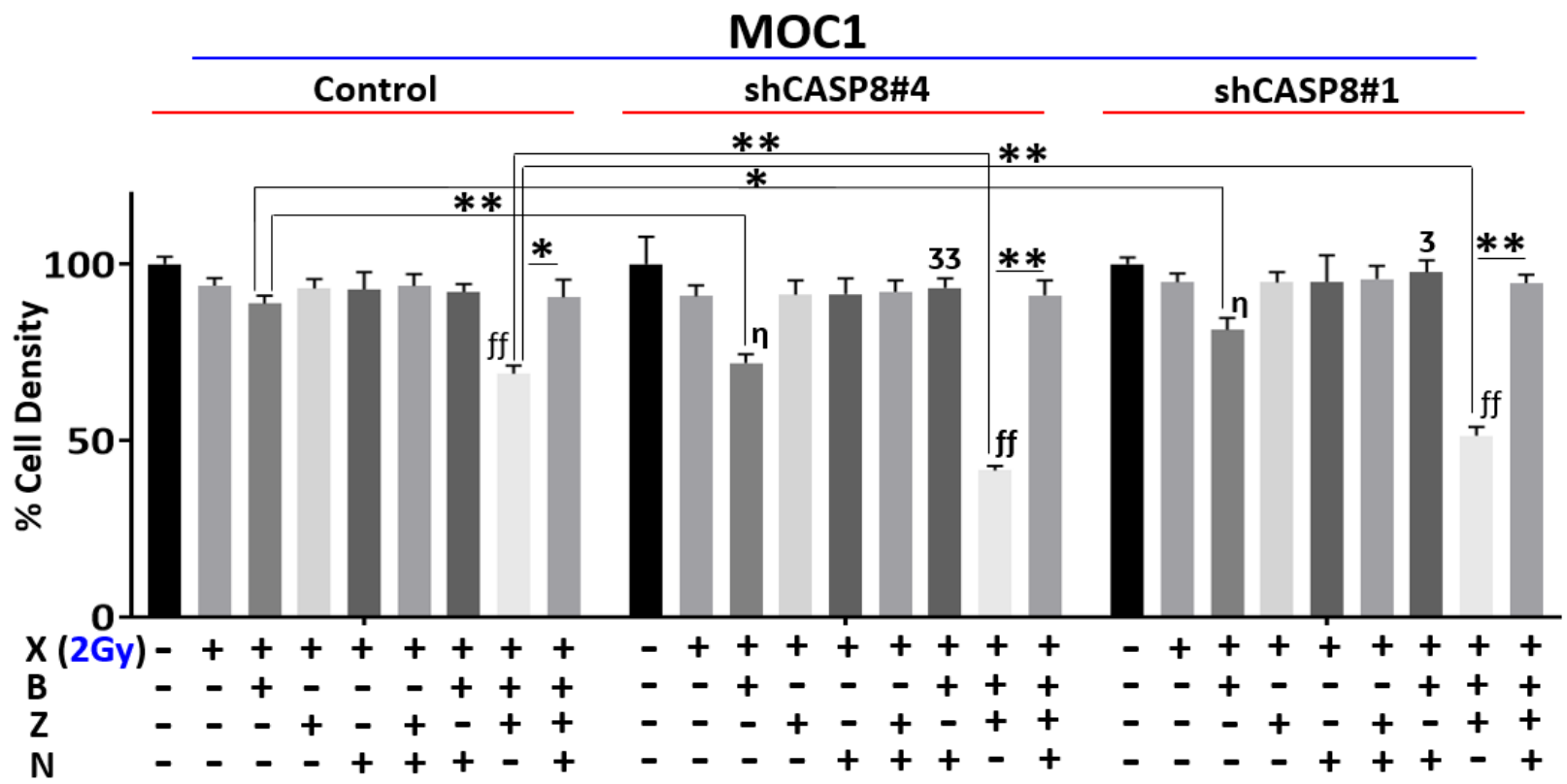

| Dose Enhancement Ratio (DER) values for the cell lines | | |
|---|---|---|
| Treatment Conditions | MOC1 Control | MOC1 shCASP8 |
| B | 1.07 | 1.53 |
| Z | 1.13 | 1.12 |
| N | 1.06 | 0.94 |
| B+Z | 1.24 | 1.81 |
| B+Z+N | 0.95 | 0.96 |



**Supplemental Figure 6. Loss of *CASP8* increases the radiosensitizing effects of Birinapant or Birinapant plus zVAD-FMK through induction of necroptosis.**

MOC1 control and shCASP8 cells were treated with radiation (**X** [2, 4 and 6 Gy]), Birinapant (**B** [250nmol/L]), zVAD-FMK (**Z** [5μmol/L]), Necrostatin-1s (**N** [10μmol/L]) or the combinations as indicated. 24 hour after treatments, drug dilutions were washed out, colonies were allowed to form for 5 days, after which they were stained and counted. Surviving colony counts were normalized to nontreated cells (cells treated with no drugs) of each radiation dose from the same experiment. *Log10* of surviving fractions were plotted (This supplemental figure is related to **Figure 3A**). All treatments were carried out in triplicates and experiments were repeated three times with similar results. Student *t* test was used for statistics. *, $P<0.05$; when comparing surviving fractions following X+B treatments between control and shCASP8 cells **ǂ,** $P<0.05$; when comparing surviving fractions following X+B+Z treatments between control and shCASP8 cells **ƒ,** $P<0.05$; when showing reversal of death upon addition of N to X+B+Z for the control cells, **η,** $P<0.05$; when showing reversal of death upon addition of N to X+B+Z for the shCASP8 cells, **ʒ,** $P<0.001$; when showing reversal of death upon addition of N to X+B+Z for the shCASP8 cells. Symbols are placed at the radiation doses they refer to. A more detailed version of the Dose Enhancement Ratio (DER) table can be found in **Supplemental Table-6.**

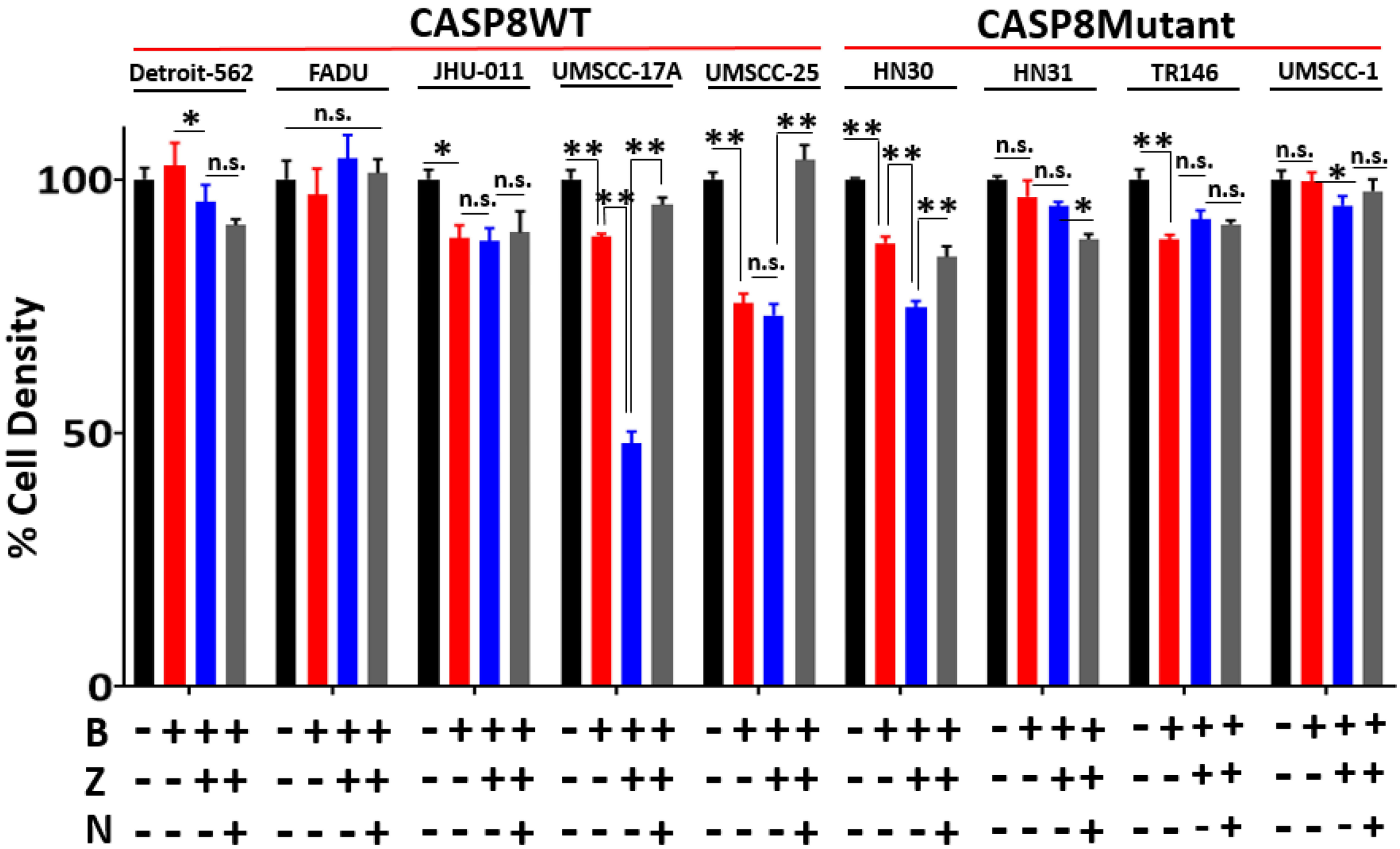
# Supplemental Figure 7



**Supplemental Figure 7. Loss of *CASP8* enhances radiation killing by Birinapant or Birinapant plus zVAD-FMK through induction of necroptosis.**

MOC1 control and shCASP8 cells were treated with radiation (**X** [2, 4 and 6 Gy]), Birinapant (**B** [250nmol/L]), zVAD-FMK (**Z** [5μmol/L]), Necrostatin-1s (**N** [10μmol/L]) or the combinations as indicated. 24 hour after treatments, cell viability was assessed by Cell-Titer Glo. Values normalized to nontreated cells from the same experiment to calculate % cell density (This supplemental figure is related to **Figure 3B**). All treatments were carried out in triplicates and experiments were repeated three times with similar results. Student *t* test was used for statistics. *, P<0.05; **, P<0.001 for the indicated pairwise comparisons. The following symbols are used to make comparisons between the indicated treatment conditions for each individual cell line: **η**, P<0.05; **ηη,** P<0.001 to compare X vs X+B. **ʒ,** P<0.05; **ʒʒ,** P<0.001 to compare X+B vs X+B+N. ***ƒ*,** P<0.05; ***ƒƒ*,** P<0.001 to compare X vs X+B+Z.

## Supplemental Figure 8

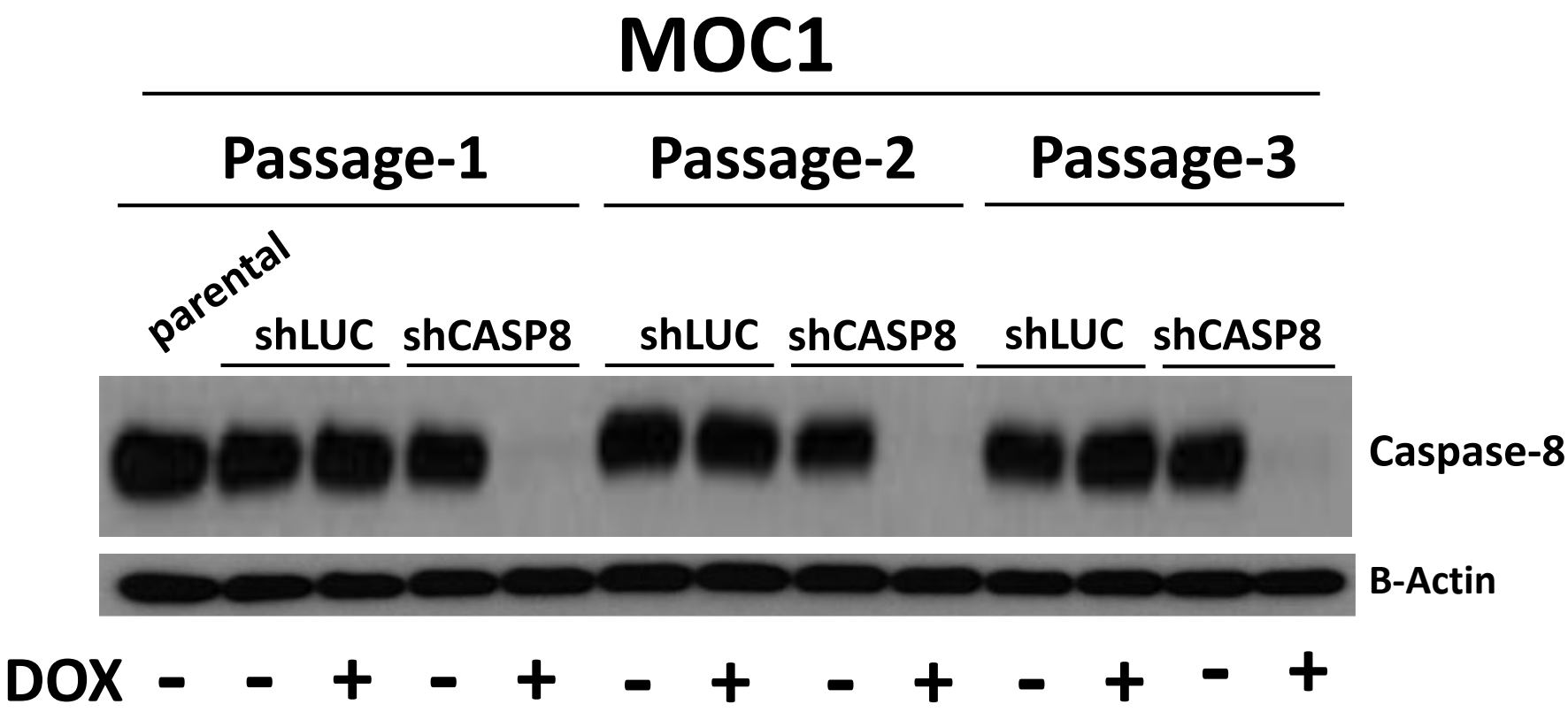


**Supplemental Figure 8. Necroptosis sensitivity in HNSCC cell lines**

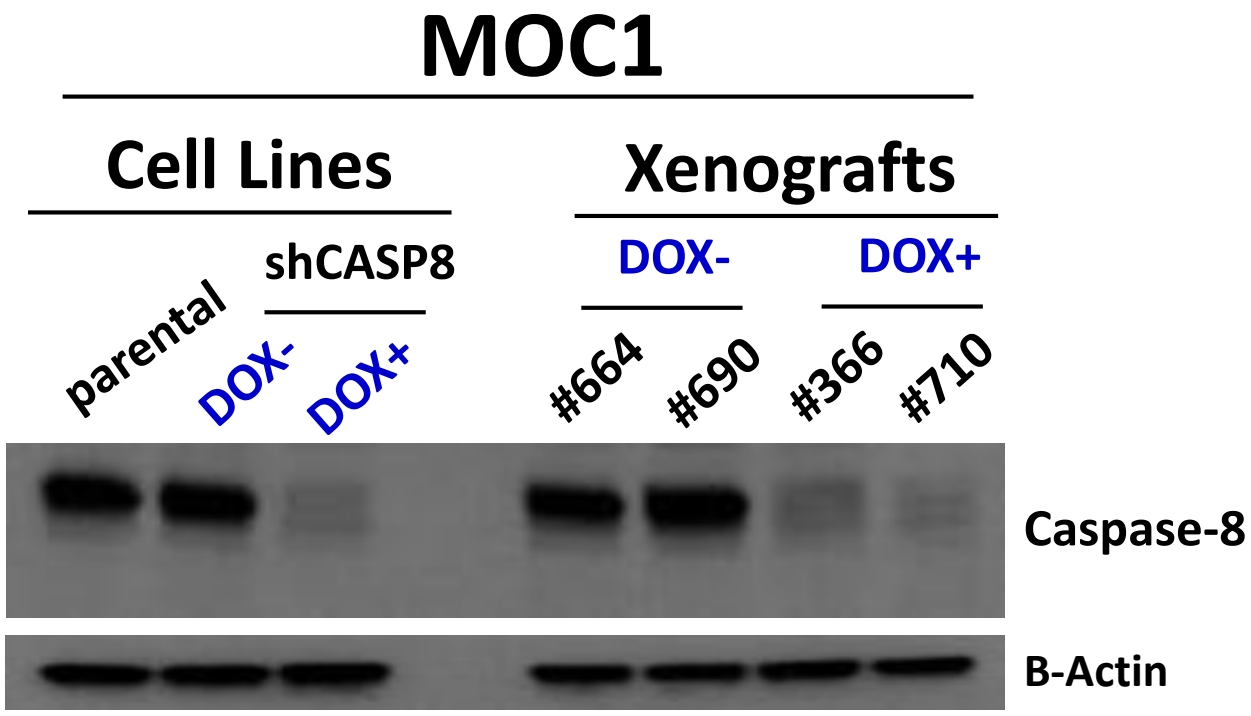
A panel of **9** human-derived HNSCC cell lines, **5** *CASP8*WT (Detroit-562, FADU, JHU-011, UMSCC-17A and UMSCC-25) and **4** *CASP8* mutant (HN30, HN31, TR146 and UMSCC-1) were treated with Birinapant (**B** [1μmol/L]), zVAD-FMK (**Z** [5μmol/L]), Necrostatin-1s (**N** [10μmol/L]) or the combinations. 24 hour after treatments, cell viability was assessed by Cell-Titer Glo. Values normalized to nontreated cells from the same experiment to calculate % cell density (This supplemental figure is related to **Figure 5A**). All treatments were carried out in triplicates and experiments were repeated three times with similar results. Student *t* test was used for statistics. *, P<0.05; **, P<0.001 for the indicated pairwise comparisons.

## Supplemental Figure 9

**A.**



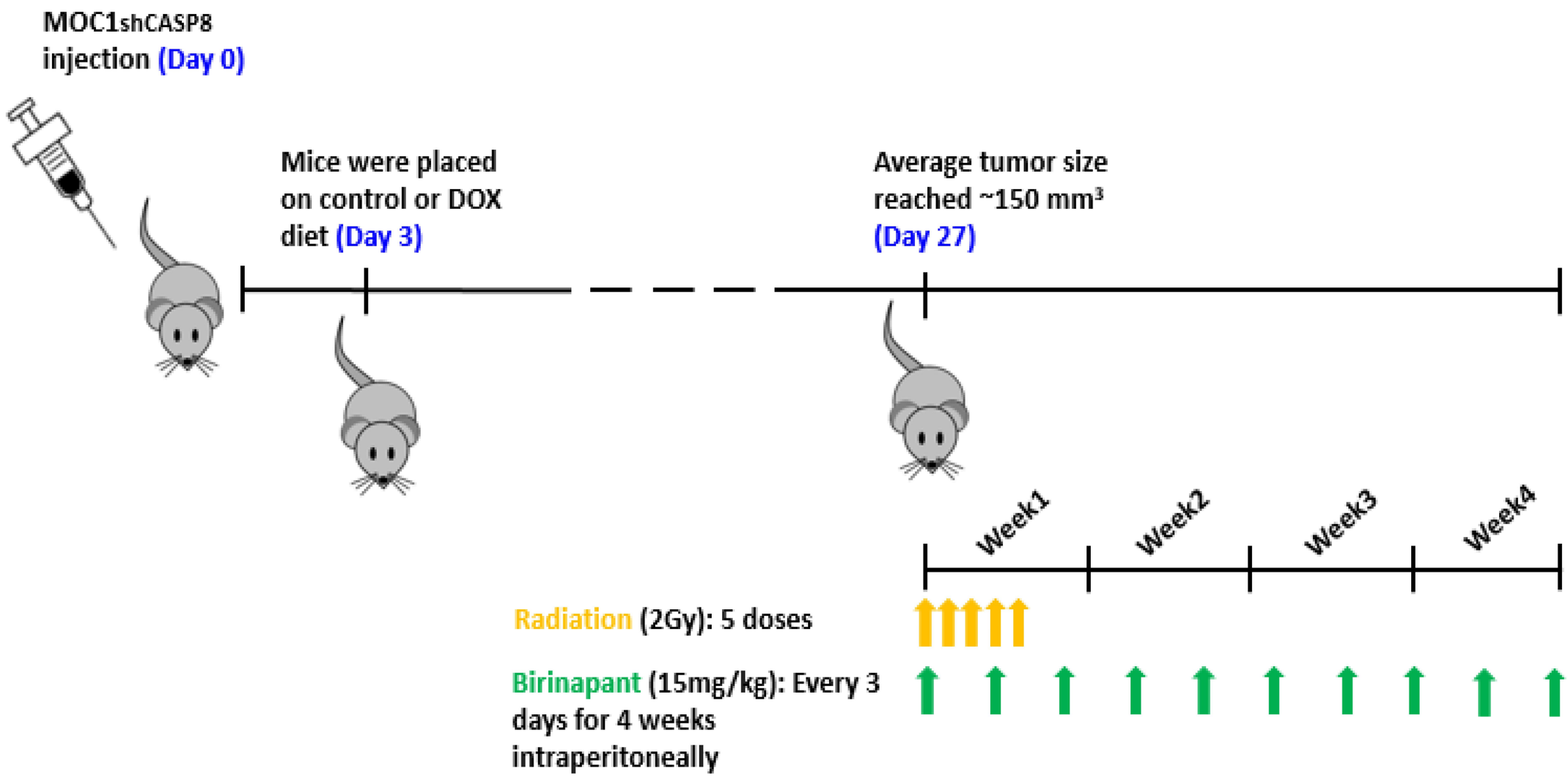

**B.**



**Supplemental Figure 9. Validation of *in vitro* and *in vivo CASP8* knockdown using Tetracycline-Regulated Inducible RNA interference (RNAi) system**

This supplemental figure is related to **Figures 6A-B.** **A.** MOC1 cells were transduced with lentiviral constructs designed against Luciferase (shLUC) and *Casp8 (*shCASP8*)*. The engineered shLUC and shCASP8 cells were cultured in the presence and absence of Doxycycline (25ng/ml). 72 hours after treatment, cells were passaged and cell lysates were obtained. This cycle was repeated 3 times. Cell lysates obtained from each cycle along with that from MOC1 parental cells were subjected to WB analysis for Caspase-8. β-Actin was used as loading control. **B.** Tumor samples collected from control (#664, #690) and Doxycycline (DOX)-fed (#366, #710) mice were minced and cultured in medium for 48 hours. Cells shed from the indicated xenografts that have attached to culture dishes were collected and lysed. Cell lysates obtained from the tumor samples along with those from the indicated cell lines were subjected to WB analysis for Caspase-8. β-Actin was used as loading control.

# Supplemental Figure 10



**Supplemental Figure 10. Treatment schema for *in vivo* experiments**

This supplemental figure is related to **Figures 6A-B.**

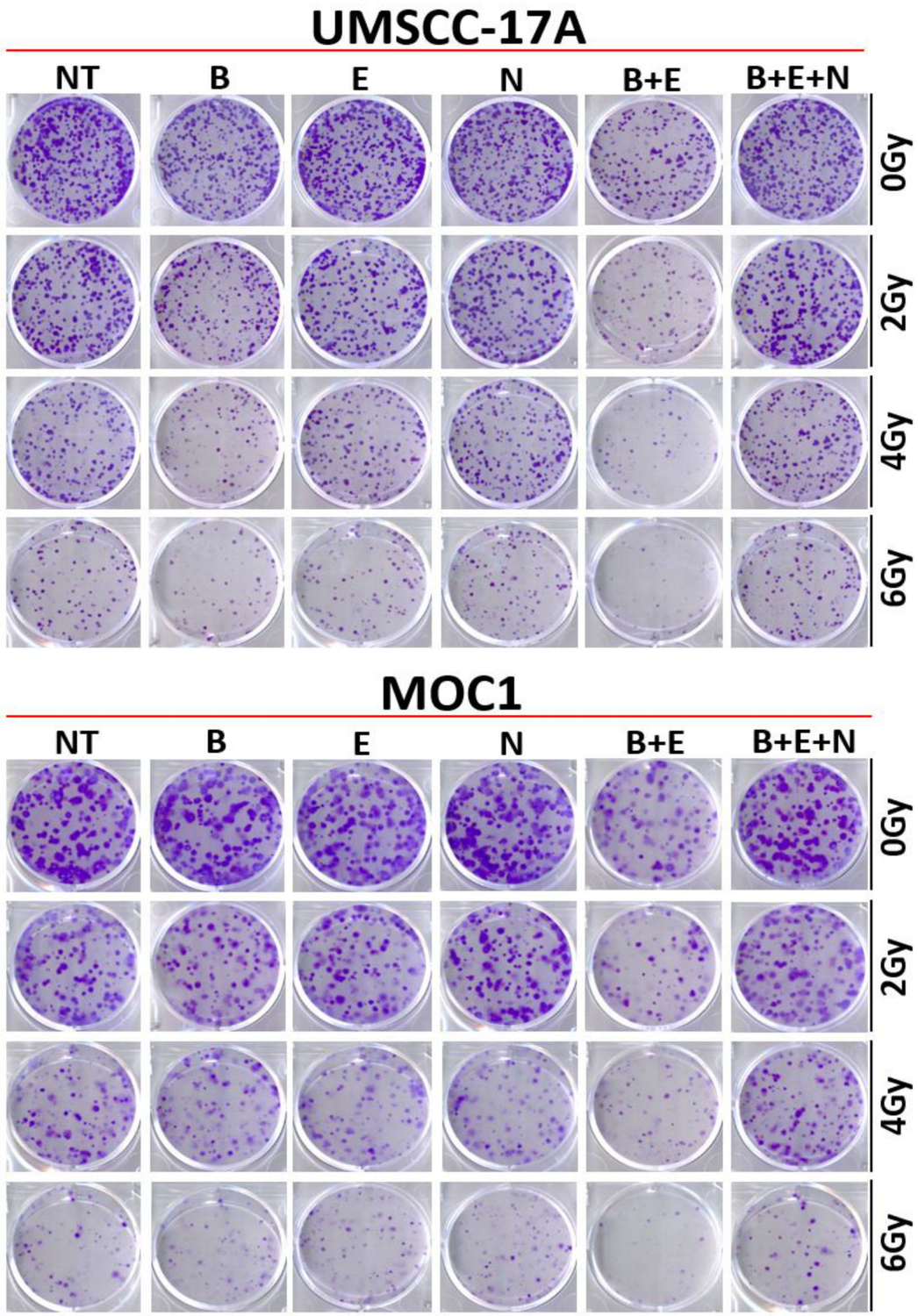
**Procedure shown on the treatment schema:** $2 \times 10^6$ MOC1 cells transduced with an inducible shRNA against *CASP8* were injected into the right flank of WT female C57BL/6 mice. Mice were randomized and placed on control or DOX diet (doxycycline hyclate added at 625 mg/kg) 3 days post injection to induce knockdown of *CASP8 in vivo* (Please refer to **Supplemental Figure 9** for the WB images). Control and *CASP8* knockdown mice were randomized into 4 treatment groups (vehicle control, 15mg/kg Birinapant, 5X2Gy radiation or combination, *n*=7-10/each) 27 days post inoculation when the average tumor volume reached ~150 $mm^3$. Radiation started on Day 27: 2Gy of radiation given Monday to Friday for 1 week. Birinapant started on Day 27: 15mg/kg Birinapant given intraperitoneally every 3 days for 4 weeks.

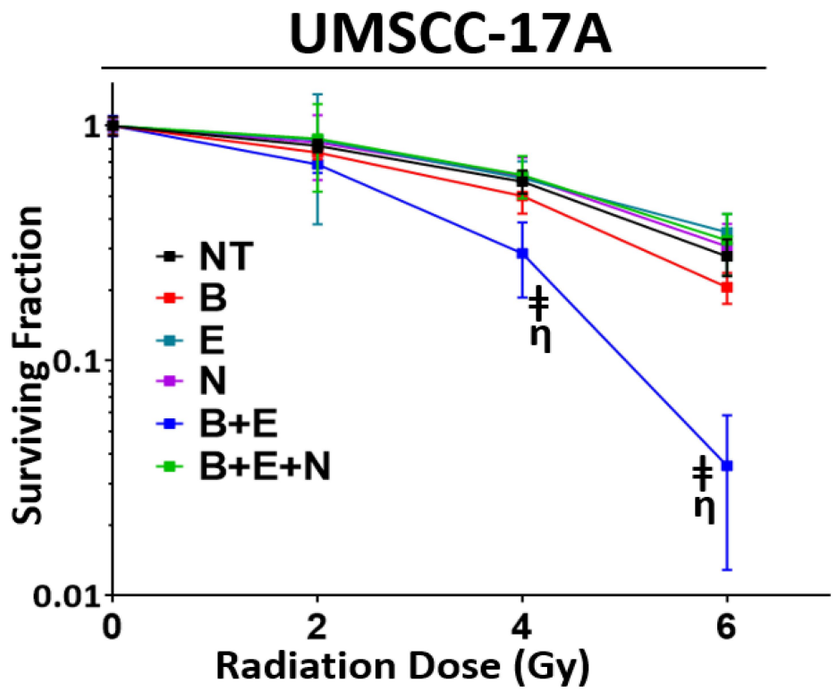
# Supplemental Figure 11

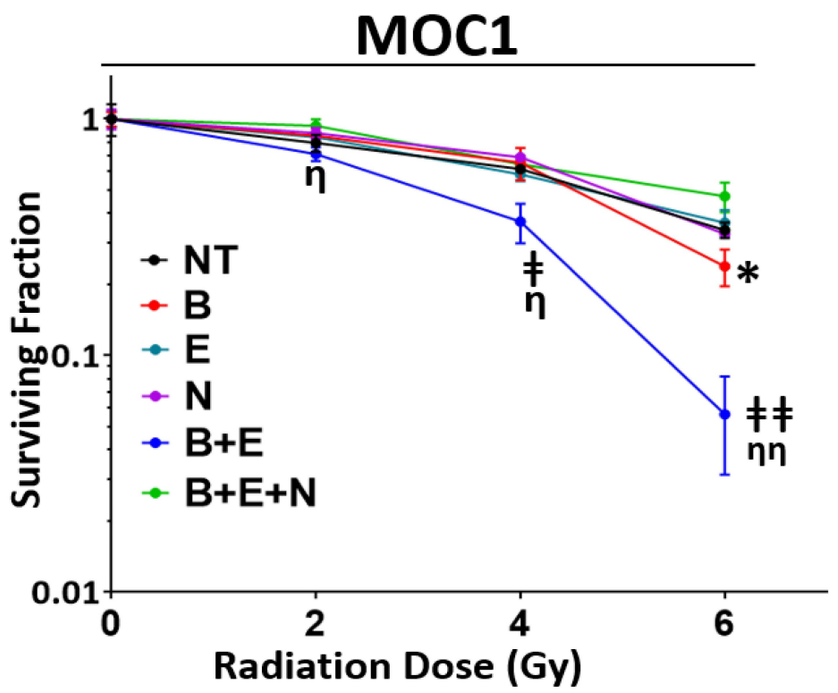
**Supplemental Figure 11. Inhibition of *CASP8* function with Emricasan increases radiation killing by Birinapant in HNSCCs.**

MOC1 and UMSCC-17A parental cells were treated with radiation (**X** [2, 4 and 6 Gy for both the cell lines]), Birinapant (**B** [200nmol/L for the UMSCC-17A cells and 1μmol/L for the MOC1 cells]), Emricasan (**E** [1μmol/L for both the cell lines]), Necrostatin-1s (**N** [10μmol/L for both the cell lines]) or the combinations as indicated. 24 hour after treatments, cell viability was assessed by Cell-Titer Glo. Values normalized to nontreated cells from the same experiment to calculate % cell density (This supplemental figure is related to **Figure 6C**). All treatments were carried out in replicates of four and experiments were repeated three times with similar results. Student *t* test was used for statistics. The following symbols are used to make comparisons between the indicated treatment conditions for each individual cell line: **η**, P<0.05; **ηη,** P<0.001 to compare X vs X+B. **ʒ,** P<0.05; **ʒʒ,** P<0.001 to compare X+B vs X+B+N. ***ƒ*,** P<0.05; ***ƒƒ*,** P<0.001 to compare X+B vs X+B+E. **μ,** P<0.05; **μμ,** P<0.001 X vs X+B+E. **ρ,** P<0.05; **ρρ,** P<0.001 to compare X+B+E vs X+B+E+N.

## Supplemental Figure 12



**Supplemental Figure 12. Inhibition of *CASP8* function with Emricasan enhances radiosensitizing effects of Birinapant in HNSCCs.**

MOC1 and UMSCC-17A parental cells were treated with radiation (**X** [2, 4 and 6 Gy for both the cell lines]), Birinapant (**B** [200nmol/L for the UMSCC-17A cells and 1μmol/L for the MOC1 cells]), Emricasan (**E** [1μmol/L for both the cell lines]), Necrostatin-1s (**N** [10μmol/L for both the cell lines]) or the combinations as indicated. 24 hour after treatments, drug dilutions were washed out, colonies were allowed to form for 5-12 days, after which they were stained and counted. Surviving colony counts were normalized to nontreated cells (cells treated with no drugs) of each radiation dose from the same experiment. Log10 of surviving fractions were plotted (This supplemental figure is related to **Figure 6D**). All treatments were carried out in triplicates and experiments were repeated three times with similar results. Student *t* test was used for statistics. *, P<0.05; when comparing X+B to X alone for the indicated radiation dose. **ǂ,** *P*<0.05 and **ǂǂ,** *P*<0.001; when comparing X+B+E to X alone for the indicated radiation doses. **η,** *P*<0.05 and **ηη,** *P*<0.001; when comparing X+B+E to X+B+E+N for the indicated radiation doses.

**SUPPLEMENTAL TABLES (Uzunparmak *et. al*.)**

**Table S1.** Univariate and multivariate Cox-regression analyses of selected factors for overall survival (OS) in HPV-negative oral cancers

**Table S2.** HNSCC cell lines used in the study

**Table S3.** shRNA and CRIPSR-Cas9 sgRNA oligo sequences

**Table S4.** List of reagents/drugs used in the study

**Table S5.** List of antibodies used for western blotting

**Table S6.** Dose enhancement ratio (DER) values for the cell lines used in Figure 3A and Supplemental Figure 6

**Table S7.** *p* values for key pairwise comparisons in mouse tumor growth analysis

**Table S8.** *p* values for key pairwise comparisons in mouse survival analysis

**Table S9.** Key tumor growth parameters assessed in the mouse study presented in Figure 6

**Supplemental Table 1:** Univariate and multivariate Cox-regression analyses of selected factors for overall survival (OS) in HPV-negative oral cancers

| **Univariate and Multivariate Cox-Regression Analysis** | | | | | | |
|---|---|---|---|---|---|---|
| | **Univariate** | | | **Multivariate** | | |
| **Variable** | **HR** | **CI95%** | **Wald p-value** | **HR** | **CI95%** | **Wald p-value** |
| *CASP8* / *HRAS* status | | | | | | |
| *CASP8* WT or *HRAS* Mut | | ref | **0.023** | | ref | **0.015** |
| *CASP8* Mut and *HRAS* WT | 0.56 | 0.34-0.93 | | 0.47 | 0.26-0.87 | |
| Age | | | | | | |
| continuous | 1.02 | 1-1.03 | **0.020** | 1.01 | 0.89-1.03 | 0.572 |
| Neoplasm Histologic Grade | | | | | | |
| *G1* | | ref | | | ref | |
| *G2* | 0.62 | 0.35-1.1 | 0.089 | 0.59 | 0.29-1.21 | 0.116 |
| *G3* | 0.49 | 0.25-0.92 | | 0.43 | 0.19-0.96 | |
| Anatomic subdivision | | | | | | |
| *Oral tongue* | | ref | | | | |
| *Oral cavity* | 0.86 | 0.54-1.38 | | | | |
| *Lip* | 1.07 | 0.14-7.79 | | | | |
| *Hard palate* | 1.45 | 0.35-6.02 | 0.690 | | | |
| *Floor of the mouth* | 0.67 | 0.42-1.07 | | | | |
| *Buccal mucosa* | 0.92 | 0.44-1.88 | | | | |
| *Alveolar ridge* | 1.2 | 0.47-3.05 | | | | |
| Tumor stage | | | | | | |
| I | | ref | | | ref | |
| II | 0.6 | 0.17-2.06 | | 1.26 | 0.32-4.9 | |
| III | 0.46 | 0.13-1.57 | **0.004** | 0.51 | 0.14-1.79 | **<0.0001** |
| IVA | 0.27 | 0.08-0.86 | | 0.16 | 0.05-0.53 | |
| IVB | 0.17 | 0.04-0.77 | | 0.06 | 0.01-0.34 | |
| Radiation therapy | | | | | | |
| *No* | | ref | **0.029** | | ref | **<0.0001** |
| *Yes* | 1.55 | 1.04-2.31 | | 3.57 | 2.19-5.83 | |

Multivariate model included the variables: CASP8 / HRAS status; Age; Neoplasm Histologic Grade; Tumor Stage; and Radiation therapy. HR = Hazard ratio; CI95% = confidence interval at 95%

**Supplemental Table 2:** HNSCC cell lines used in the study

| Cell Line | Culture Media | Primary Source |
|---|---|---|
| Detroit-562 | DMEM, 10% FBS | ATCC |
| FADU | DMEM, 10% FBS | ATCC |
| HN30 | DMEM, 10% FBS | Dr. John Ensley, Wayne State University |
| HN31 | DMEM, 10% FBS | Dr. John Ensley, Wayne State University |
| JHU-011 | RPMI 1640, 10% FBS | Dr. David Sidransky, Johns Hopkins University |
| TR146 | DMEM, 10% FBS | Dr. Thomas Rupniak, Imperial Cancer Research Fund, London |
| UMSCC-1 | DMEM, 10% FBS | Dr. Thomas E. Carey, University of Michigan |
| UMSCC-17A | DMEM, 10% FBS | Dr. Thomas E. Carey, University of Michigan |
| UMSCC-25 | DMEM, 10% FBS | Dr. Thomas E. Carey, University of Michigan |
| MOC1 | IMDM, 5% FBS | Dr Ravindra Uppaluri, Washington University School of Medicine |

**Supplemental Table 3:** shRNA and CRIPSR-Cas9 sgRNA oligo sequences

| **mouse *Casp8* CRISPR-Cas9 sgRNA sequences** | **DNA Sequence (5' to 3')** |
|---|---|
| *Casp8* for-1 | CACCGGTGTCGTCTATGGAACGGAT |
| *Casp8* rev-1 | AAACATCCGTTCCATAGACGACACC |
| *Casp8* for-2 | CACCGCAAGAACTATATTCCGGATG |
| *Casp8* rev-2 | AAACCATCCGGAATATAGTTCTTGC |
| *Casp8* for-3 | CACCGTAGCTTCTGGGCATCCTCGA |
| *Casp8* rev-3 | AAACTCGAGGATGCCCAGAAGCTAC |
| **human *CASP8* shRNA oligo sequences** | |
| *CASP8* shRNA#1 (shCASP8#1) | TAATTCGGAAGAGCAGCTC |
| *CASP8* shRNA#1 (shCASP8#2) | TTCCTTCTCCCAGGATGAC |
| **mouse *Casp8* shRNA oligo sequences** | |
| *Casp8* shRNA#1 (shCASP8#1) | AAACTTTGTCTGAAGTCCG |
| *Casp8* shRNA#4 (shCASP8#4) | TTTCATTTGCAGTGCAGTC |
| **mouse *Casp8* inducible shRNA oligo sequences** | |
| *Casp8* inducible shRNA#10 (shCASP8) | TTTCATTTGCAGTGCAGTC |
| **Luciferase inducible shRNA oligo sequences** | |
| luciferase inducible shRNA (shLUC) | CGCTGAGTACTTCGAAATGTC |
| **mouse *Ripk3* shRNA oligo sequences** | |
| *Ripk3* shRNA (shRIP3) | TACCTCGGAGACAGCAGCA |

**Supplemental Table 4:** List of reagents/drugs used in the study

| No. | Reagent/drug name | Catalog number | Company |
|---|---|---|---|
| 1 | Birinapant | S7015 | Selleckchem |
| 2 | zVAD-FMK | FMK001 | R&D Systems |
| 3 | human recombinant TNFα | 210-TA | R&D Systems |
| 4 | human recombinant TRAIL | 375-TEC | R&D Systems |
| 5 | murine recombinant TNFα | 315-01A | Peprotech |
| 6 | murine recombinant TRAIL | 315-19 | Peprotech |
| 7 | Necrostatin-1s (7-Cl-O-Nec1) | 10-4544 | Focus Biomolecules |
| 8 | Puromycin dihydrochloride from Streptomyces alboniger | P8833 | Sigma-Aldrich |
| 9 | Doxycycline | 631311 | Takara Bio USA |

**Supplemental Table 5:** List of antibodies used for western blotting

| No. | Antibody name | Catalog number | Source | Company | Dilution |
|---|---|---|---|---|---|
| 1 | human CASP8 | 551242 | mouse | BD Biosciences | 1:1000 |
| 2 | human/mouse CASP8 | AF1650 | rabbit | R&D Systems | 1:400 |
| 3 | RIP1 | 610458 | mouse | BD Biosciences | 1:1000 |
| 4 | human RIP3 (E1Z1D) | 13526 | rabbit | Cell Signaling | 1:1000 |
| 5 | mouse RIP3 (D4G2A) | 95702 | rabbit | Cell Signaling | 1:1000 |
| 6 | MLKL (D216N) | 14993 | rabbit | Cell Signaling | 1:1000 |
| 7 | MLKL (mouse specific) | 28640 | rabbit | Cell Signaling | 1:1000 |
| 8 | phospho-RIP1 (S166) | 31122 | rabbit | Cell Signaling | 1:1000 |
| 9 | phospho-MLKL (S358) (D6H3V) | 91689 | rabbit | Cell Signaling | 1:1000 |
| 10 | PARP | 9542 | rabbit | Cell Signaling | 1:1000 |
| 11 | Anti-HA | H3663 | mouse | Sigma-Aldrich | 1:2000 |
| 12 | β-actin | A1978 | mouse | Sigma-Aldrich | 1:10000 |
| 13 | Anti-rabbit IgG | 1706515 | goat | BioRad | 1:5000 |
| 14 | Anti-mouse IgG | 1706516 | goat | BioRad | 1:5000 |

**Supplemental Table 6:** Dose enhancement ratio (DER) values for the cell lines used in Figure 3A and Supplemental Figure 6

| **Dose Enhancement Ratio (DER) values for the cell lines used** | | |
|---|---|---|
| | **MOC1 Control** | **MOC1 shCasp8** |
| **Treatment Conditions** | | |
| B | 1.07 | 1.53 |
| Z | 1.13 | 1.12 |
| N | 1.06 | 0.94 |
| B+Z | 1.24 | 1.81 |
| B+Z+N | 0.95 | 0.96 |

**B:** Birinapant; **Z:** zVAD FMK; **N:** Necrostatin 1s; **$SF_{0.25:}$** Surviving fraction of 0.25;

**DER =** Mean radiation dose to reach $SF_{0.25}$ for control/Mean radiation dose to reach $SF_{0.25}$ for each of the given treatments

**DER>1.2** indicates radiosensitization

**Supplemental Table 7:** *p* values for key pairwise comparisons in mouse tumor growth analysis

| Compared Animal Cohorts | | *p* value |
|---|---|---|
| **Cohort#1** | **Cohort#2** | |
| Control-NT | Control-B | 0.7628 |
| Control-NT | Control-X | 0.0093 |
| Control-NT | Control-X+B | <0.001 |
| Control-B | Control-X | 0.0795 |
| Control-B | Control-X+B | <0.001 |
| Control-X | Control-X+B | 0.1357 |
| shCasp8-NT | shCasp8-B | <0.001 |
| shCasp8-NT | shCasp8-X | <0.001 |
| shCasp8-NT | shCasp8-X+B | <0.001 |
| shCasp8-B | shCasp8-X | <0.001 |
| shCasp8-B | shCasp8-X+B | <0.001 |
| shCasp8-X | shCasp8-X+B | <0.001 |
| | | |
| Control-NT | shCasp8-NT | <0.001 |
| Control-B | shCasp8-B | <0.001 |
| Control-X | shCasp8-X | 0.9714 |
| Control-X+B | shCasp8-X+B | <0.001 |

**Supplemental Table 8:** *p* values for key pairwise comparisons in mouse survival analysis

| Compared Animal Cohorts | | *p* value |
|---|---|---|
| **Cohort#1** | **Cohort#2** | |
| Control-NT | Control-B | 0.4345 |
| Control-NT | Control-X | <0.001 |
| Control-NT | Control-X+B | <0.001 |
| Control-B | Control-X | <0.001 |
| Control-B | Control-X+B | <0.001 |
| Control-X | Control-X+B | 0.0206 |
| shCasp8-NT | shCasp8-B | <0.001 |
| shCasp8-NT | shCasp8-X | <0.001 |
| shCasp8-NT | shCasp8-X+B | <0.001 |
| shCasp8-B | shCasp8-X | <0.001 |
| shCasp8-B | shCasp8-X+B | <0.001 |
| shCasp8-X | shCasp8-X+B | <0.001 |
| | | |
| Control-NT | shCasp8-NT | 0.4846 |
| Control-B | shCasp8-B | <0.001 |
| Control-X | shCasp8-X | 0.4747 |
| Control-X+B | shCasp8-X+B | <0.001 |

**Supplemental Table 9:** Key tumor growth parameters assessed in the mouse study presented in Figure 6

**Key tumor growth parameters calculated as part of tumor growth analysis in the in vivo study**

| Treatment Conditions | Tumor Doubling Times (TDT) | | Absolute Growth Delay (AGD) | Normalized Growth Delay (NGD) | Radiation Enhancement Factor (REF) |
|---|---|---|---|---|---|
| | Mean | SD | | | |
| MOC1 Control - NT | 8.3 | 3.2 | | | |
| MOC1 Control - B | 11.5 | 2.1 | 3.2 | | |
| MOC1 Control - X | 14.4 | 2.1 | 6.1 | | |
| MOC1 Control - X+B | 14.6 | 1.6 | 6.3 | 3.1 | 0.5 |
| | | | | | |
| MOC1 shCasp8 - NT | 7.1 | 1.2 | | | |
| MOC1 shCasp8 - B | 18.4 | 1.8 | 11.3 | | |
| MOC1 shCasp8 - X | 15.8 | 1.7 | 8.7 | | |
| MOC1 shCasp8 - X+B | 38 | 7.8 | 30.9 | 19.6 | 2.3 |

**NT:** No treatment; **B:** Birinapant; **X:** Radiation.

**Tumor doubling time (TDT):** number of days required for the doubling of tumor volumes from the day of randomization (Day27)

**Absolute growth delay (AGD) =** (Mean TDT for each treatment group – Mean TDT for the NT group)

**Normalized growth delay (NGD)** = (AGD for the X+B group – AGD for the B group)

**Radiation Enhancement Factor (REF) =** (NGD for the X+B group / AGD for the X group)

**REF>1.2** indicates enhancement of radiation sensitivity