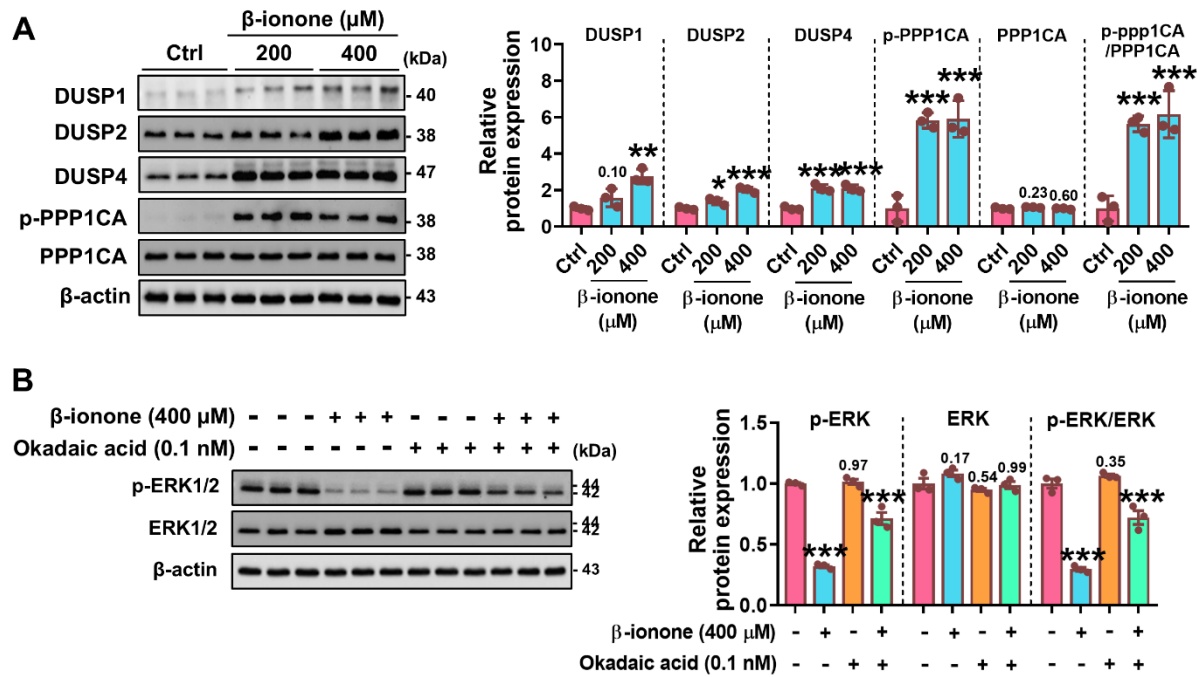


## SUPPLEMENTARY DATA

# **Olfactory Receptor 51E2 Activation by $\beta$ -Ionone Induces a Senescence-Like Phenotype and Increases Susceptibility to Senolytic Treatment in Colorectal Cancer**

**Ji-Sun Kim, Jin-A Kim, Young Hun Jung, Jung-Won Choi, Sung Won Shin, Byeongkyu Kang, Byung Soh Min, Hunsang Lee, Seok Chung, Sung-Joon Lee**

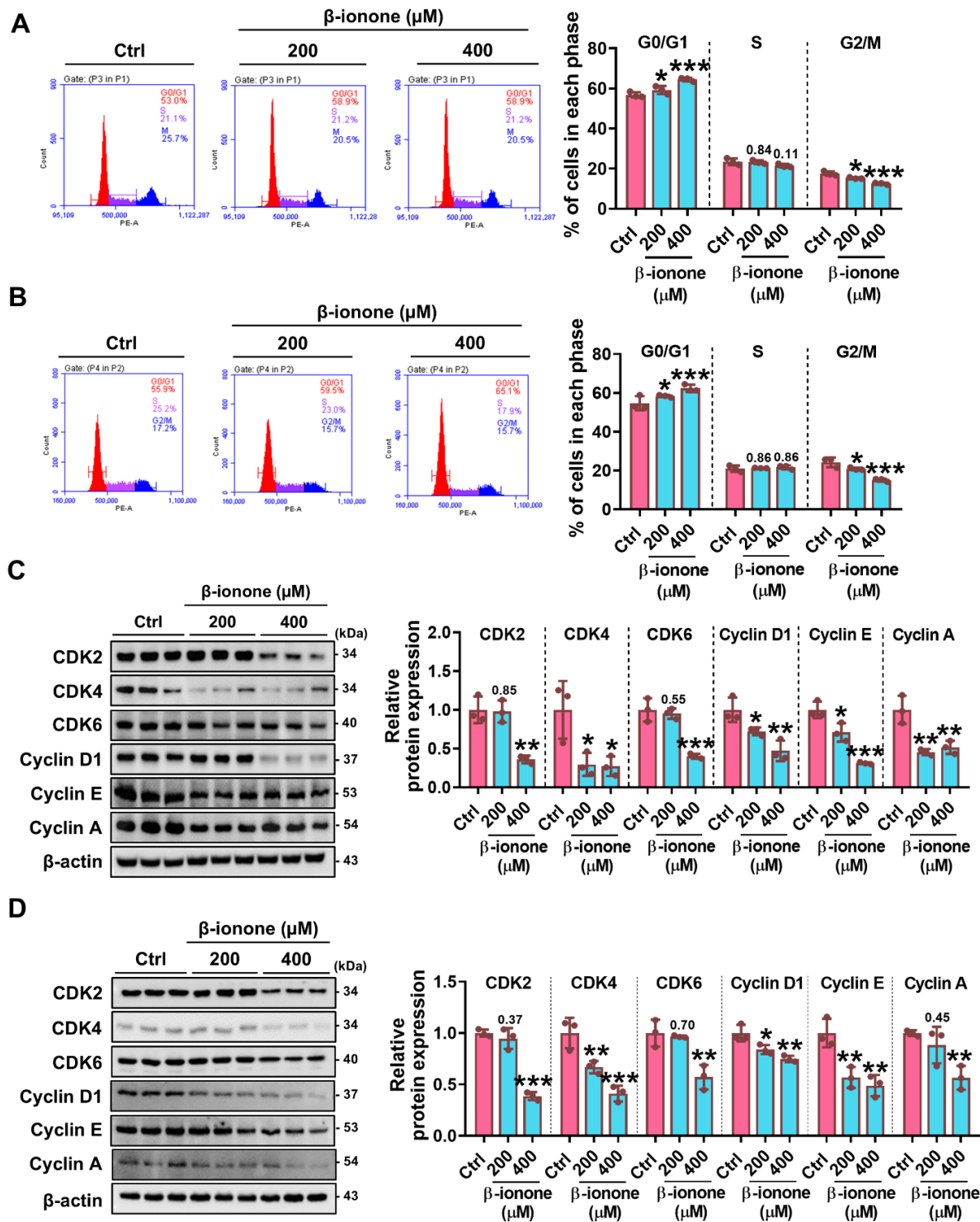
SUPPLEMENTARY DATA



**Supplementary Figure 1. Effects of  $\beta$ -ionone on phosphatase expression and ERK signaling in SW480 cells.**

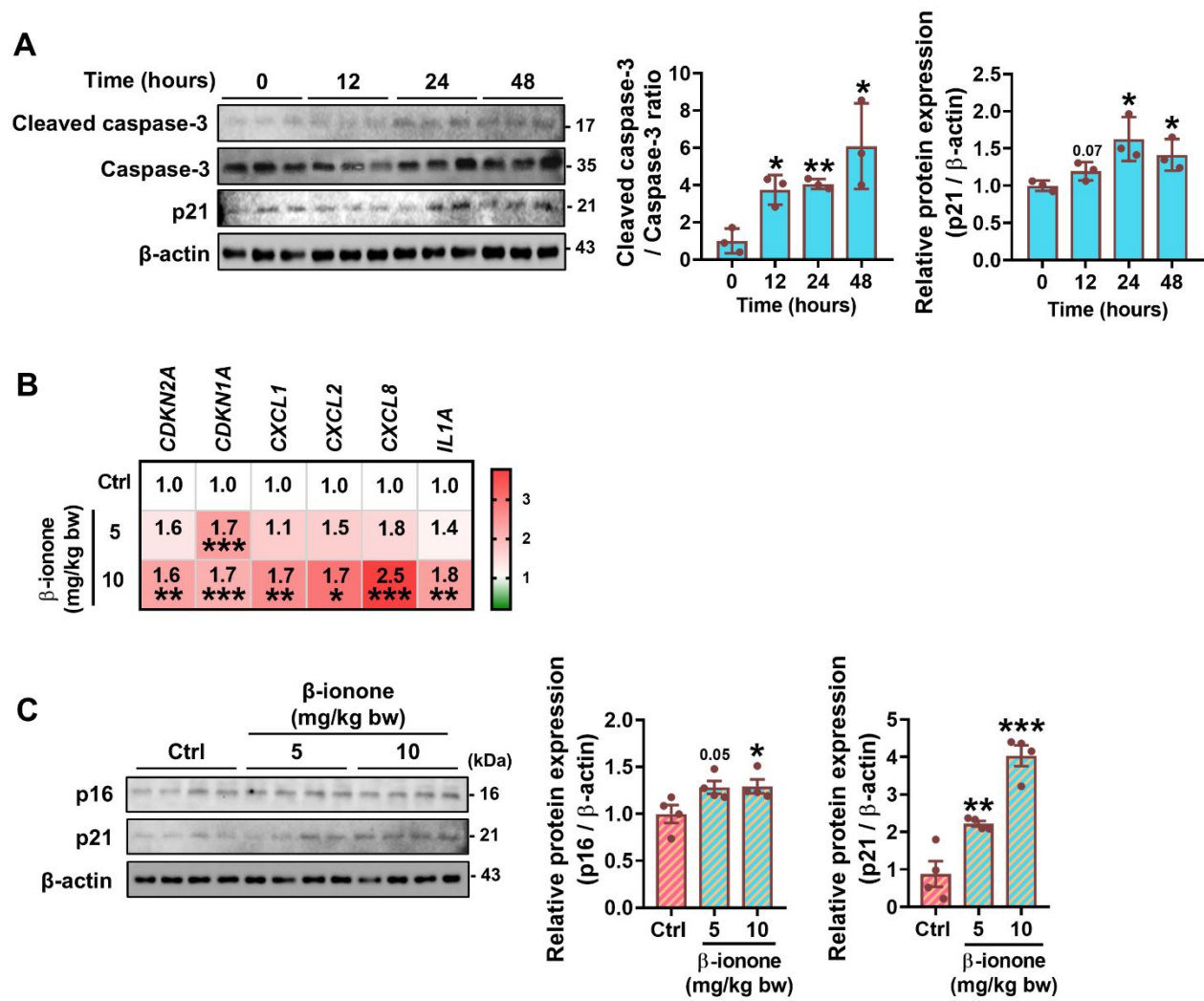
(A) Immunoblot analysis of DUSP1, DUSP2, DUSP4, phosphorylated PPP1CA (p-PPP1CA), and total PPP1CA in SW480 cells treated with  $\beta$ -ionone (200 and 400  $\mu$ M). Quantification of protein levels (normalized to  $\beta$ -actin) is shown in the right panel. (B) Immunoblot analysis of p-ERK1/2 and ERK1/2 in SW480 cells treated with  $\beta$ -ionone (400  $\mu$ M) in the presence or absence of okadaic acid (0.1 nM). Quantification of protein levels is shown in the right panel. Data are presented as mean  $\pm$  SD from three independent biological experiments. Statistical significance was evaluated using one-way ANOVA followed by Dunnett's multiple comparisons test. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

# SUPPLEMENTARY DATA



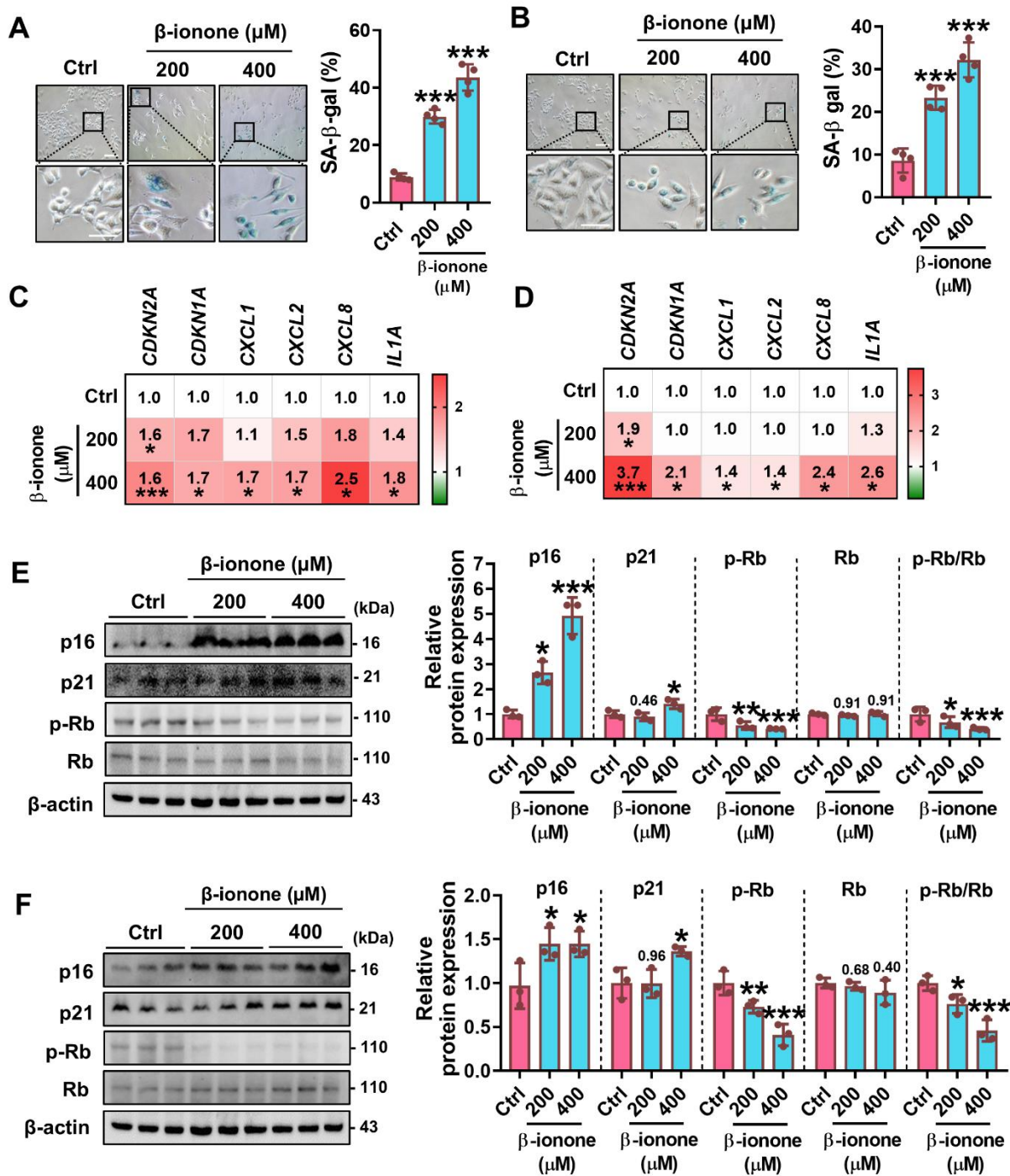
**Supplementary Figure 2. OR51E2 activation by β-ionone induces cell cycle arrest in RKO and SW620 CRC cells.** (A) Cell cycle distribution was analyzed by flow cytometry (left), and quantification is on the right in RKO cells. (B) Immunoblot analysis of CDK2, CDK4, CDK6, Cyclin D1, Cyclin E, and Cyclin A in RKO cells (left), with densitometric quantification shown on the right. (C) Cell cycle distribution was analyzed by flow cytometry (left), and quantification is on the right in SW620 cells. (D) Immunoblot analysis of CDK2, CDK4, CDK6, Cyclin D1, Cyclin E, and Cyclin A in SW620 cells (left), with densitometric quantification shown on the right. Data are presented as mean ± SD from three independent biological experiments. Statistical significance was evaluated using one-way ANOVA followed by Dunnett's multiple comparisons test. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

SUPPLEMENTARY DATA



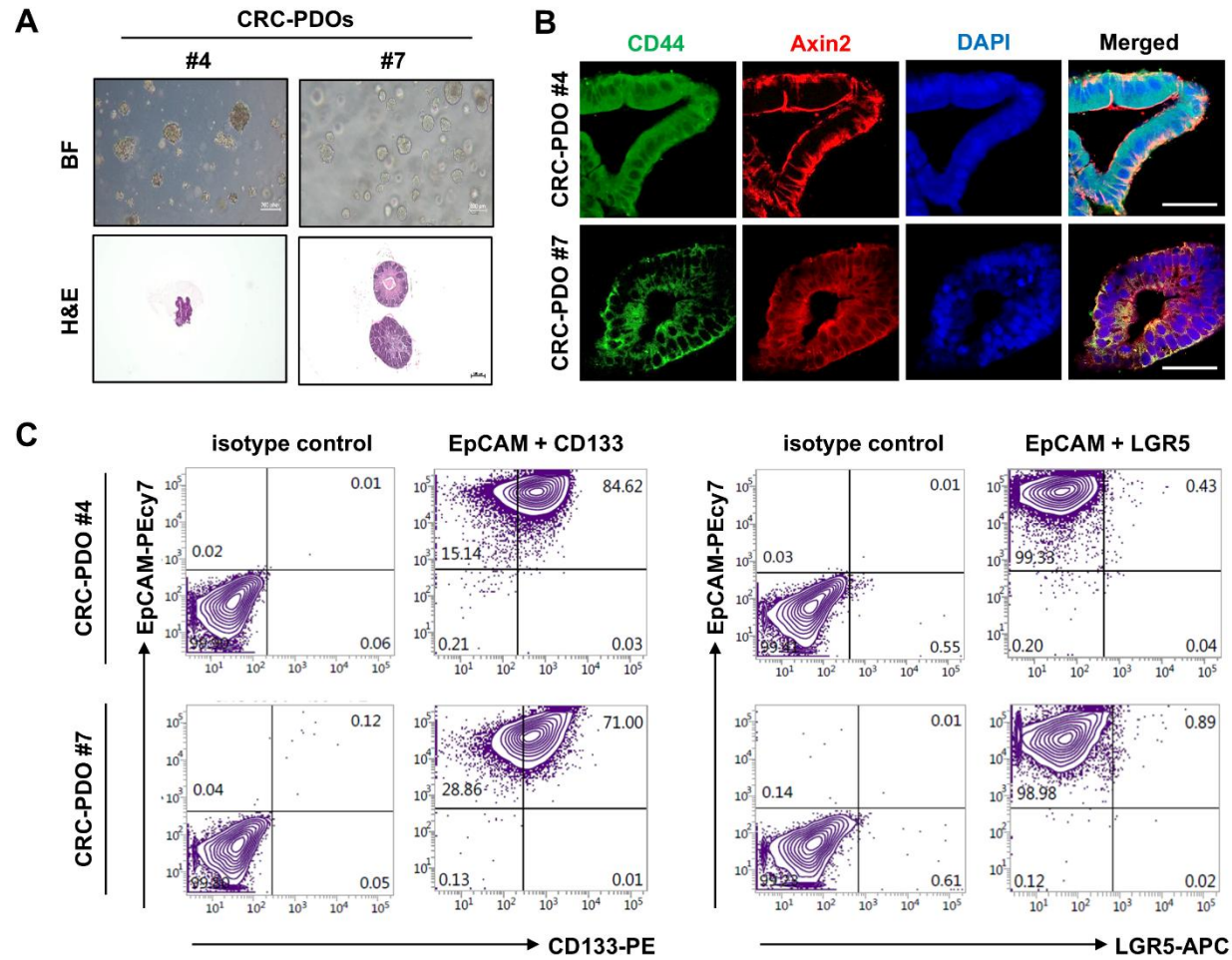
**Supplementary Figure 3. OR51E2 activation by  $\beta$ -ionone induces time-dependent apoptosis and senescence-associated responses in CRC cells and xenograft tumors.** (A) Immunoblot analysis of cleaved caspase-3, total caspase-3, and p21 in SW480 cells treated with  $\beta$ -ionone for the indicated times (0, 12, 24, and 48 h). Densitometric Quantification of cleaved caspase-3/total caspase-3 ratio and p21 protein levels normalized to  $\beta$ -actin is shown on the right. (B, C) Senescence-associated responses in SW620 xenograft tumors following  $\beta$ -ionone treatment. (B) Relative mRNA expression levels of senescence-associated genes (*CDKN2A* and *CDKN1A*) and SASP factors (*CXCL1*, *CXCL2*, *CXCL8*, and *IL1A*) in tumors treated with  $\beta$ -ionone (5 and 10 mg/kg body weight). (C) Immunoblot p16 and p21 (left), with densitometric quantification normalized to  $\beta$ -actin shown on the right. Data are presented as mean  $\pm$  SD from three independent biological experiments (A) and mean  $\pm$  SEM from four animals per group (B, C). Statistical significance was evaluated using one-way ANOVA followed by Dunnett's multiple comparisons test. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

# SUPPLEMENTARY DATA



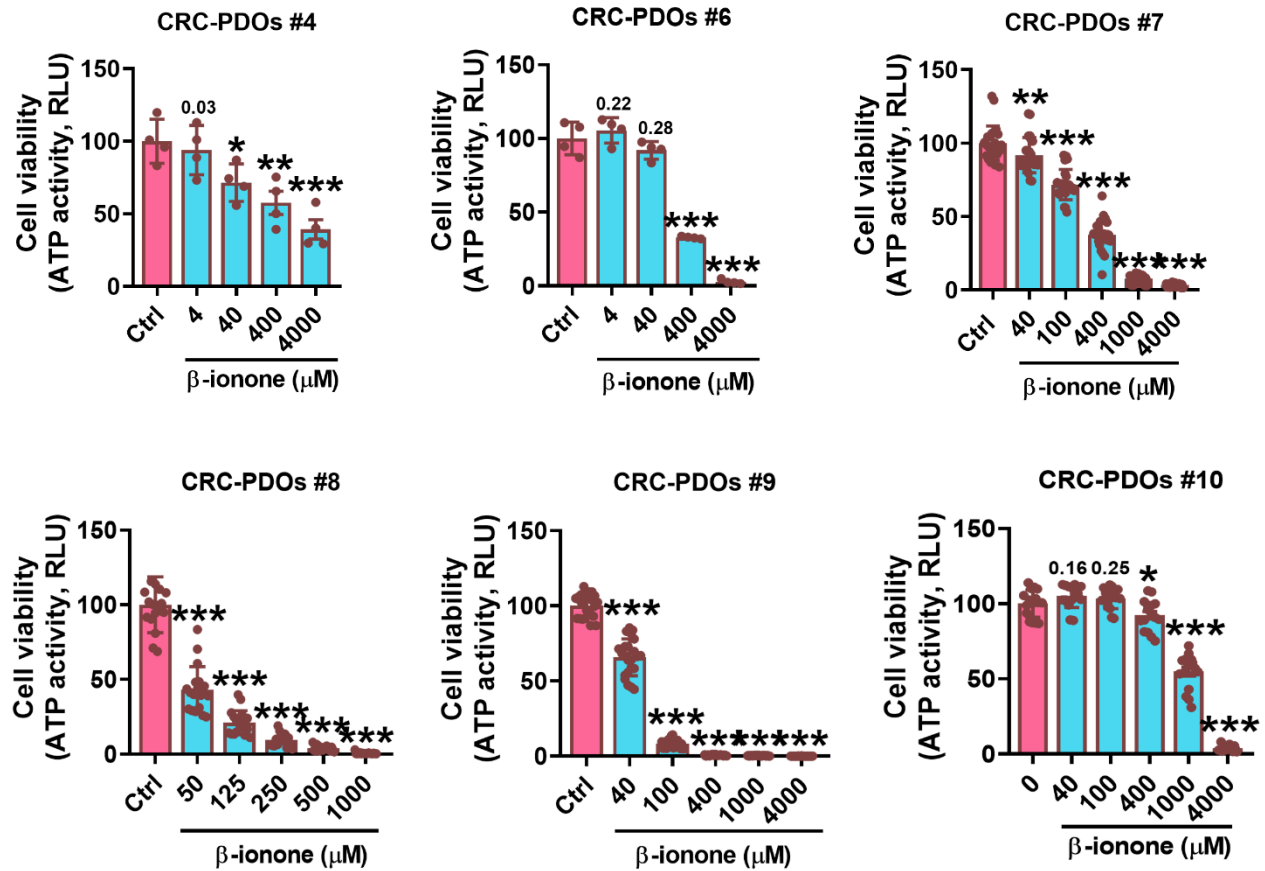
**Supplementary Figure 4. OR51E2 activation by  $\beta$ -ionone promotes senescence in RKO and SW620 CRC cells.** (A, B) Senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) staining (left) and quantification of SA- $\beta$ -gal-positive cells (right) following  $\beta$ -ionone treatment in RKO (A) and SW620 (B) cells. Quantification was performed using all analyzed fields from independent biological replicates. Scale bars, 100  $\mu$ m. (C, D) qRT-PCR analysis of senescence markers (*CDKN2A* [P16] and *CDKN1A* [P21]) and SASP factors (*CXCL1*, *CXCL2*, *CXCL8*, and *IL1A*) in RKO (C) and SW620 (D) cells. (E, F) Immunoblot analysis of p16, p21, phosphorylated Rb (p-Rb), and total Rb (Rb), in RKO (E) and SW620 (F) cells (left) in response to  $\beta$ -ionone treatment. Densitometric quantification is on the right. Data are presented as mean  $\pm$  SD from four independent biological experiments (A–D) and three independent biological experiments (E, F). Statistical significance was evaluated using one-way ANOVA followed by Dunnett’s multiple comparisons test. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

# SUPPLEMENTARY DATA



**Supplementary Figure 5. Morphological and molecular characterization of CRC-PDOs.** (A) Representative bright-field images (upper) and hematoxylin and eosin (H&E) staining (lower) showing the morphology and architecture of CRC-PDOs (#4 and #7). Scale bars, 100  $\mu$ m. (B) Immunofluorescence staining of CRC-PDOs (#4 and #7) demonstrating expression of CD44 (green), Axin2 (red), and nuclei counterstained with DAPI (blue). Scale bar, 50  $\mu$ m. (C) Flow cytometric analysis of dissociated CRC-PDOs (#4 and #7) showing distinct subpopulations based on CD133, LGR5, and EpCAM expression. Representative data from 3 independent experiments.

# SUPPLEMENTARY DATA



**Supplementary Figure 6.  $\beta$ -Ionone suppresses cell growth in CRC-PDOs.** CellTiter-Glo® assays demonstrate dose-dependent inhibition of cell growth in CRC-PDO lines following  $\beta$ -ionone treatment. CRC-PDO lines exhibited heterogeneous sensitivity to  $\beta$ -ionone over a range of concentrations (4-4000, 50-1000, or 40-4000  $\mu$ M) following 6 days of treatment. Data are presented as mean  $\pm$  SD from independent biological replicates ( $n = 4$  for CRC-PDOs #4 and #6;  $n = 24$  for CRC-PDOs #7-#10). Statistical significance was evaluated using one-way ANOVA followed by Dunnett's multiple comparisons test. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

# SUPPLEMENTARY DATA

**Supplementary Table 1.** Primer sequences used for real-time quantitative PCR.

| Genes <sup>1</sup> | Forward (5'-3')       | Reverse (5'-3')       |
|--------------------|-----------------------|-----------------------|
| <i>OR51E2</i>      | CATTGGTGTGGTACTCGCCT  | CAGCACCCGTGTTCTGATCT  |
| <i>GNAL</i>        | GGCCAACCTGAAAACCAAT   | GGTCTGTGGGTGTGTAGTCA  |
| <i>ADCY3</i>       | TCAAAACCATTTGGCAGCACG | TGTCGTAGTGTGGTTTCCGG  |
| <i>MKI67</i>       | GCCCCTAAAGTAGAACCCGT  | GGGTTCGGATGATTTGCCTC  |
| <i>CDKN2A</i>      | CCTCGTGCTGATGCTACTGA  | TTCTTTCAATCGGGGATGTC  |
| <i>CDKN1A</i>      | ACTTCCTCCTCCCCACTTGT  | CACCCTGCCCCAACCTTAGAG |
| <i>CXCL1</i>       | AGCTTGCCTCAATCCTGCAT  | CCTCTGCAGCTGTGTCTCTC  |
| <i>CXCL2</i>       | ATCAATGTGACGGCAGGGAA  | ACCCTCATACCTGACCGTGA  |
| <i>CXCL8</i>       | CAGAGACAGCAGAGCACACA  | CCTTCACACAGAGCTGCAGA  |
| <i>IL1A</i>        | TCTTCTGGGAAACTCACGGC  | GGCTCAGTACATGCTCAGCA  |
| <i>RPL32</i>       | AGAAGTTCATCCGGCACCAG  | CACTTCCAGCTCCTTGACGT  |

<sup>1</sup>*OR51E2*, olfactory receptor 51E2; *GNAL*, guanine nucleotide-binding protein (G protein) G(olf) subunit  $\alpha$ ; *ADCY3*, adenylate cyclase 3; *MKI67*, marker of proliferation Ki-67; *CDKN2A*, cyclin dependent kinase inhibitor 2A; *CDKN1A*, cyclin dependent kinase inhibitor 1A; *CXCL1*, C-X-C motif chemokine ligand 1; *CXCL2*, C-X-C motif chemokine ligand 2; *CXCL8*, C-X-C motif chemokine ligand 8; *IL1A*, interleukin 1 alpha; *RPL32*, ribosomal protein L32

# SUPPLEMENTARY DATA

**Supplementary Table 2.** List of primary and secondary antibodies for CRC-PDOs.

| Target protein                                                                 | Antibody (clone/type) | Company                         | Dilution (IF/IHC) |
|--------------------------------------------------------------------------------|-----------------------|---------------------------------|-------------------|
| Cleaved caspase-3                                                              | Rabbit mAb            | Cell Signaling Technology (USA) | 1:200             |
| EpCAM                                                                          | Mouse mAb             | Abcam (UK)                      | 1:200             |
| OR51E2                                                                         | Rabbit pAb            | Invitrogen (USA)                | 1:100             |
| CD44                                                                           | Mouse mAb             | Abcam (UK)                      | 1:100             |
| Axin2                                                                          | Rabbit pAb            | Abcam (UK)                      | 1:100             |
| ATP1A1                                                                         | Mouse mAb             | Santa Cruz Biotechnology (USA)  | 1:50              |
| KI-67                                                                          | Rabbit pAb            | Thermo Fisher Scientific (USA)  | 1:100             |
| EpCAM-PE-cyanine7                                                              | Rat mAb               | Thermo Fisher Scientific (USA)  | 1:1000            |
| LGR5-Alexa647                                                                  | Rat mAb               | BD Biosciences (USA)            | 1:50              |
| CD133-PE                                                                       | Mouse mAb             | BioLegend (USA)                 | 1:100             |
| Isotype control-PE                                                             | Mouse mAb             | Thermo Fisher Scientific (USA)  | 1:100             |
| Isotype control-Alexa647                                                       | Mouse mAb             | Thermo Fisher Scientific (USA)  | 1:100             |
| Isotype control PE cyanine7                                                    | Mouse mAb             | Thermo Fisher Scientific (USA)  | 1:1000            |
| Secondary antibody                                                             | Host species          | Company                         | Dilution (IF/IHC) |
| Alexa Fluor 488 anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody         | Goat                  | Thermo Fisher Scientific (USA)  | 1:100             |
| Alexa Fluor 488 anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody | Goat                  | Thermo Fisher Scientific (USA)  | 1:100             |
| Alexa Fluor 568 anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody  | Goat                  | Thermo Fisher Scientific (USA)  | 1:100             |
| Alexa Fluor 568 anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody        | Goat                  | Thermo Fisher Scientific (USA)  | 1:100             |

# SUPPLEMENTARY DATA

**Supplementary Table 3.** CRC patient-derived organoids.

| Patient No. | Diagnosis                                      | TNM stage <sup>1</sup> | Histology                        | KRAS                                                                                           | NRAS | MSI      |
|-------------|------------------------------------------------|------------------------|----------------------------------|------------------------------------------------------------------------------------------------|------|----------|
| 1           | Rectal                                         | T2, N0, M0             | Well differentiated              | KRAS G12D                                                                                      | Wild | MSS      |
| 2           | Ascending                                      | T2, M1c, M1            | Moderate differentiated          | KRAS G13D                                                                                      | Wild | MSS      |
| 3           | Rectal                                         | N1, M0                 | Moderate differentiated          | -                                                                                              | Wild | MSS      |
| 4           | Rectal, Adenocarcinoma                         | T2, N0, M0             | Moderate differentiated          | PIK3CA, FBXW7, MAP3K1, APC, FGFR1, TP53, SMARCA4                                               | Wild | MSS      |
| 5           | Rectal, Adenocarcinoma                         | T2, N1, M0             | Moderate differentiated Mucinous | PIK3CA, TET2, FGFR4, BRD2, PTCH1, TSC1, BRD3, KRAS G13D, TSC2, RNF43, SMAD2, SMAD4, PTEN, IDH2 | Wild | MSS      |
| 6           | Rectal, Adenocarcinoma                         | T2, N1, M1             | Poor differentiated              | CTNNB1, PIK3CA, RICTOR, ARID1B, EPHB4, BRAF, JAK2, ARID2, TP53, RNF43, SMAD4                   | Wild | MSS      |
| 7           | Ascending                                      | T2, N0, M0             | Moderate differentiated          | Wild                                                                                           | Wild | MSI high |
| 8           | Colon cancer, splenic flexure                  | T3, N1a, M1            | Moderate differentiated          | KRAS G13D                                                                                      | Wild | MSS      |
| 9           | liver metastasis, rectosigmoid junction cancer | T3, N1a, M1            | Moderate differentiated          | KRAS G12D                                                                                      | Wild | MSS      |
| 10          | Rectal                                         | T3, N1, M0             | Moderate differentiated          | KRAS G12D                                                                                      | Wild | MSS      |

<sup>1</sup>Tumor, node and metastasis (TNM) stage; T2, Primary tumor invades muscularis propria or size >1 cm; T3, Primary tumor invades subserosa; N0, No regional lymph node metastasis; N1, Regional lymph node metastasis; N1a, Cancer cells in 1 nearby lymph node; M0, No Distant metastasis; M1, Distant metastasis