

Supplementary Figure 1 As₂O₃ alters expression of TGFβ signaling mediators in OVCA429 ovarian cancer cell line.

(a) OVCA429 cells were seeded at 250,000 cells per well in 6-well plates. Following overnight attachment, the cells were treated with varying concentrations of As₂O₃ (2-50μM). After 18 hour incubation, the cell lysates were harvested and western analysis was performed using the following antibodies: (1) EVI1, (2) SnoN, (3) TAK1, (4) TGFβRII, (5) SMAD2/3, (6) AKT, and (7) GAPDH as a loading control. The data shown are representative of 2 independent experiments. **(b)** OVCA429 cells were seeded at 250,000 cells in 6-well plates. After 24 hours, the cells were treated with: (1) 5μM As₂O₃, (2) 5μM MG132, and (3) 5μM As₂O₃ and 5μM MG132. After 18 hours, the cell lysates were harvested and western analysis was performed using the following antibodies: (1) EVI1, (2) SnoN, (3) TGFβRII, (4) SMAD2/3, (5) AKT, and (6) GAPDH as a loading control. The data shown are representative of 2 independent experiments. **(c)** OVCA429 and HEY cells were seeded at 250,000 cells per well in 6-well plates. Following 24 hours, cells were transfected with non-targeting (control) siRNA or siRNAs targeting SMURF2. Twenty-four hours post-transfection, the cells were treated for 18 hours with 10μM As₂O₃. Cell lysates were collected and western analysis was performed using the following antibodies: (1) EVI1, (2) SnoN, (3) TGFβRII, (4) SMURF2, and (5) GAPDH as a loading control. The data shown are representative of 4 independent experiments. **(d)** OVCA429 cells were seeded at 250,000 cells per well in 6-well plates. After 24 hours, the cells were treated at different time points (1, 3, 18h) with 5μM MG132. Cell lysates were harvested and western analysis was performed using the following antibodies: (1) EVI1, (2) SnoN, (3) TGFβRII, (4) SMAD2/3, (5) AKT, and (6) GAPDH as a loading control. The data shown are representative of 2 independent experiments. **(e)** OVCA429 cells were seeded at 500,000 cells per well in 6-well plates. Following 24 hours, the cells were treated with (1) DMSO (control), (2) 5μM As₂O₃ and DMSO, or (3) 5μM MG132 for 18 hours. RNA was then isolated and used for qPCR. Relative RNA-fold changes are presented for EVI1, SnoN, and TGFβRII. The data shown are representative of 2 independent experiments.

Supplementary Figure 2 As₂O₃ alters cellular migration. HEY cells were treated for 6 hours with 5μM As₂O₃ and seeded at 25,000 cells into Boyden chamber inserts. Following 18 hours incubation, the cells that migrated through the pores were stained with crystal violet and counted. The results are displayed as number of cells migrated in the absence and presence of As₂O₃. The data shown are representative of 2 independent experiments.

Supplementary Figure 3 Resveratrol treatment of HEY cells induces autophagy in a beclin-1 independent manner. **(a)** HEY cells were seeded at 250,000 cells per well in 6-well plates. After overnight attachment, the cells were treated with varying concentrations of resveratrol (10-100μM). After 18 hour incubation, the cell lysates were harvested and western analysis was performed using the following antibodies: (1) SnoN, (2) Beclin-1, (3) p62, (4) LC3, (5) PARP, and (6) GAPDH as a loading control. The data shown are representative of 2 independent experiments. **(b)** HEY cells were seeded at 250,000 cells per well. After 24 hours, the cells were treated with: (1) 100μM resveratrol, (2) 100μM resveratrol and 1mM 3-MA, (3) 100μM resveratrol and 2.0mM 3-MA, (5) 100μM resveratrol and 5.0mM 3-MA, and (6) 5.0mM 3-MA only for 18 hours. Cell lysates were harvested and western analysis was performed using the following antibodies: (1) Beclin-1, (2) p62, (3) LC3, (4) PARP, and (5) GAPDH as a loading control. The data shown are representative of 2 independent experiments.

Supplementary Figure 4 EBSS treatment of HEY cells induces autophagy in a Beclin-1 dependent manner. **(a)** HEY cells were seeded at 250,000 cells per well in 6-well plates. After overnight attachment, the cells were treated with EBSS across a time course (15 minutes, 30 minutes, 45 minutes, 60 minutes, and 3 hours). Cell lysates were harvested and western analysis was performed using the following antibodies: (1) SnoN, (2) Beclin-1, (3) p62, (4) LC3, and (5) GAPDH as a loading control. The data shown are representative of 4 independent experiments. **(b)** HEY cells were seeded at 250,000 cells per well. After 24 hours, the cells were treated with: (1) EBSS (30 minutes), (2) EBSS (30 minutes) and 1.0mM 3-MA, (3) EBSS (30 minutes) and 2.0mM 3-MA, (5) EBSS (30 minutes) and 5.0mM 3-MA, and (6) 5.0mM 3-MA alone. Cell lysates were harvested and western analysis was performed using the following antibodies: (1) Beclin-1, (2) p62, (3) LC3, and (4) GAPDH as a loading control. The data shown are representative of 2 independent experiments. **(c)** HEY cells were seeded at 250,000 cells per well in 6-well plates. After 24 hours, the cells were treated

with (1) non-targeting (control) siRNA or (2) Beclin-1 siRNA. Twenty-four hours post-transfection, cells were again transfected with siRNA. Following 72 hours after the first transfection, cells were treated with EBSS for 30 minutes at which time cell lysates were harvested and western analysis was performed using the following antibodies: (1) Beclin-1, (2) LC3, and (3) GAPDH as a loading control. The data shown are representative of 2 independent experiments.