Supplementary figure legends

Figure S1. Dissemination and mass formation of xenografted cancer cells in the zebrafish embryo. **(A)** DiI-stained cancer cells (YD10B) were injected into yolk sac of Tg (fli1:EGFP) zebrafish embryos at 2 dpf. The xenografts were imaged at 4 days post injection (dpi) by the upright microscopy. Bar=200 μm. Red fluorescence (DiI)-labeled cancer cells migrated from injection site into the green fluorescent-labeled vasculature. Arrows indicate cancer cells located outside of the vasculature (extravasated). **(B)** Tumor mass formation. Bar=200 μm. Approximately 10 % of YD10B or HCT116 xenografted larvae formed a tumor mass that protruded from the abdomen. **(C)** Fluorescent beads (diameter: 15 μm) were injected into the yolk sac of embryos at 2 dpf. Four days after injection, embryos were observed and imaged.

Figure S2. Effect of incubation temperature on survival of xenografted larvae. Approximately one hundred YD10B cells visually counted were injected into yolk sac. After 4 days of incubation at 28, 31, and 35 °C, survival rates were assessed (24 larvae/group). Data are average ± SD of two independent assays. *=P<0.05.

Figure S3. Effect of paclitaxel treatment on xenografted cell proliferation. **(A)** Approximately one hundred YD10B cells were injected into yolk sac. At 4 dpi, the xenografted cancer cells were counted under a fluorescent microscope after enzymatic dissociation of the transplanted embryos (5 larvae/group). Data are representative of three independent assays (average ± SD of triplicate assays). #=P<0.01. **(B)** Representative phase contrast, fluorescent, and merged images of extracted cancer cells from transplanted embryos.

Figure S4. Effect of vincristine, a tubulin destabilizer, on cancer cell dissemination. Approximately one hundred YD10B cells were injected into yolk sac of embryos. 300 nM vincristine was treated to the xenografted embryos. **A)** Representative fluorescent and merged DIC:fluorescent images. Bar=200 μm. **B)** At 4dpi, the number of embryos exhibiting cancer cell dissemination were counted by microscopic observation (24 larvae/group). Data are average ± SD of three independent assays. #=P<0.01. **C)** Effect of vincristine on *in vitro* cancer cell proliferation. YD10B cells were seeded in a 96 well plate and incubated overnight. The culture media was changed to serum-free media prior to drug treatment. After 48 h of the drug treatment, cell proliferation was assessed by MTT assay. DMSO served as control. Data are representative of three independent assays (average ± SD of triplicate assays).

Figure S5. Effect of various anti-cancer drugs on HCT116 colon cancer cell dissemination in the zebrafish xenograft model. HCT116 human colon cancer cells (approximately 300 cells) were injected into the yolk sac of embryos. After distributing the embryos in 96 well plates, the xenografted embryos were treated with drugs for 4 days. **(A)** Effect of 100 nM paclitaxel on cancer cell dissemination. **i)** Representative fluorescent images and merged DIC/fluorescent images. Bar=200 μm. **ii)** At 4 dpi, the number of embryos exhibiting cancer cell dissemination were counted by microscopic observation (24 larvae/group). Data are average ± SD of three independent assays. #=P<0.01. **iii)** Anti-proliferative effect of paclitaxel on the *in vitro* culture of HCT116 colon cancer cells were assessed using the MTT assay (48h of drug treatment). Data are representative of three independent assays (average ± SD of triplicate assays). **(B)** Effect of 300 nM vincristine on cancer cell dissemination. **i)** Representative fluorescent images and merged DIC/fluorescent images. Bar=200 μm. **ii)** At 4

dpi, the number of embryos exhibiting cancer cell dissemination were counted by microscopic observation (24 larvae/group). Data are average ± SD of three independent assays. #=P<0.01. **iii)** Anti-proliferative effect of vincristine on the *in vitro* culture of HCT116 cells was assessed using the MTT assay (48h of drug treatment). Data are representative of three independent assays (average ± SD of triplicate assays). **(C)** Effect of 17-DMAG, an Hsp90 inhibitor, on cancer cell dissemination. 1 μM 17-DMAG was treated to the xenografted embryos**. i)** Representative fluorescent images and merged images from DIC images and the fluorescent images. Bar=200 μm. **ii)** At 4 dpi, the number of embryos exhibiting cancer cell dissemination were counted by microscopic observation (24 larvae/group). Data are average ± SD of three independent assays. #=P<0.01. **iii)** Effect of 17-DMAG on *in vitro* cancer cell proliferation. YD10B cells were seeded in a 96 well plate and incubated overnight. The culture media was changed to serum-free media prior to drug treatment. After 48 h of the drug treatment, cell proliferation was assessed by MTT assay. DMSO served as a control. Data are representative of three independent assays (average ± SD of triplicate assays).

Figure S6. Effect of AP-I-h7, a novel triazine-based anti-cancer drug candidate, on *in vivo* cancer cell dissemination. **(A)** Chemical structure of AP-I-h7**. (B)** Effect of AP-I-h7 on *in vitro* cancer cell proliferation. YD10B or HCT116 cells were seeded in a 96 well plate and incubated overnight. The culture media was changed to serum-free media prior to drug treatment. After 48 h of the drug treatment, cell proliferation was assessed by MTT assay. DMSO served as control. Data are representative of three independent assays (average ± SD of triplicate assays). **(C)** Effect of AP-I-h7 on *in vivo* cancer cell dissemination. Approximately one hundred YD10B cells or 300 HCT116 cells were injected into yolk sac of

embryos. 10 μM AP-I-h7 was treated to the xenografted embryos for 4 days**.** At 4 dpi, number of embryos exhibiting cancer cell dissemination was counted by microscopic observation (24 larvae/group). As positive controls, 50 nM and 100 nM paclitaxel were treated to YD10B-injected larvae and HCT116-injected larvae, respectively. **i)** Representative fluorescent images and merged DIC:fluorescent images (YD10B-injected larvae with or without drug treatment). Bar=200 μm. **ii)** Data are average ± SD of three independent assays. #=$P<0.01$.

Figure S7. Screening of novel anti-cancer compounds that inhibit cancer cell dissemination from 200 triazine library compounds. **(A)** Chemical structure of the 'hit' compounds. **(B)** Effects of BIII-B2 and BII-B9 on *in vivo* cancer cell dissemination. Approximately one hundred YD10B cells were injected into yolk sac of embryos. 5, 10, or 20 μM of each drug was treated to the xenografted embryos for 4 days**.** At 4 dpi, number of embryos exhibiting cancer cell dissemination was counted by microscopic observation (10 larvae/group). DMSO served as a control. Data are average ± SD of two independent assays.

Supplementary Materials and Methods

Injection of fluorescent beads

Green fluorescent polystyrene beads (Molecular Probes, CA, USA) were washed twice with PBS at room temperature and suspended in 10% FBS for injection. Approximately 50 beads were injected into the yolk sac (at 2 dpf) using an injector (PV820 pneumatic picopump, World Precision Instruments, FL, USA) equipped with borosilicate glass capillaries (World

Precision Instruments). Injected embryos were transferred to a 96-well plate (1 embryo/well) containing 200 μL E3 media (without methylene blue) and maintained at 31°C. Embryos were observed and imaged at 4 dpi by upright microscopy (Leica DM2500 Microscope, Germany).

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Tg (fli1:EGFP) zebrafish were maintained in accordance with standard guidelines. Care and treatment of the zebrafish were conducted in accordance with guidelines established by the Animal Care and Ethics Committees of the Gwangju Institute of Science and Technology, Republic of Korea. Embryos were obtained using standard mating conditions and staged for cell xenograft experiments at 48 hour post fertilization.