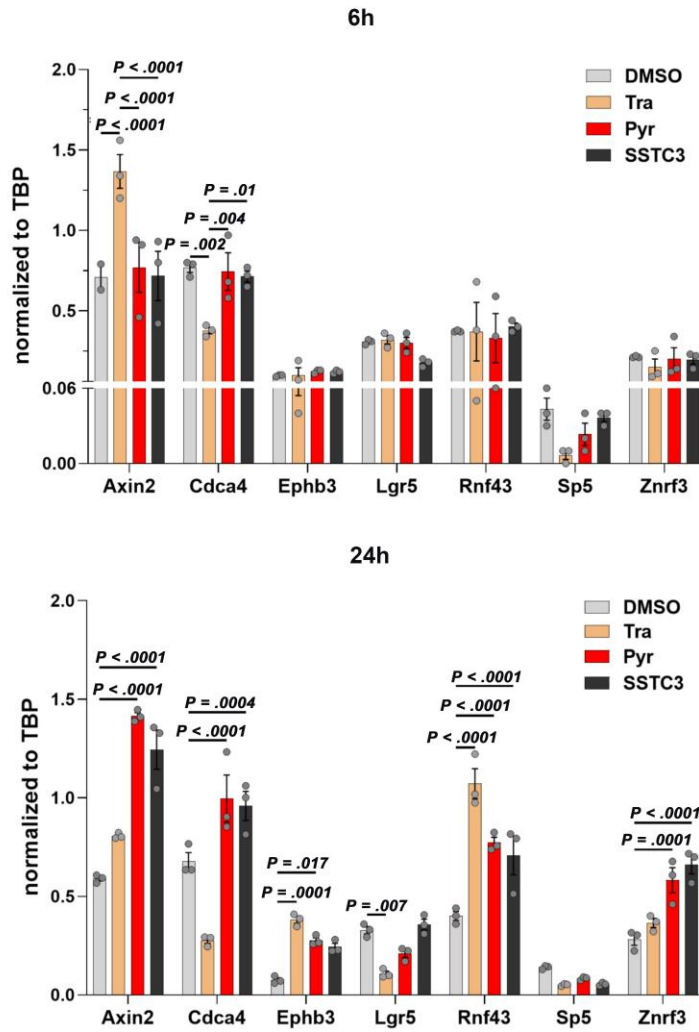
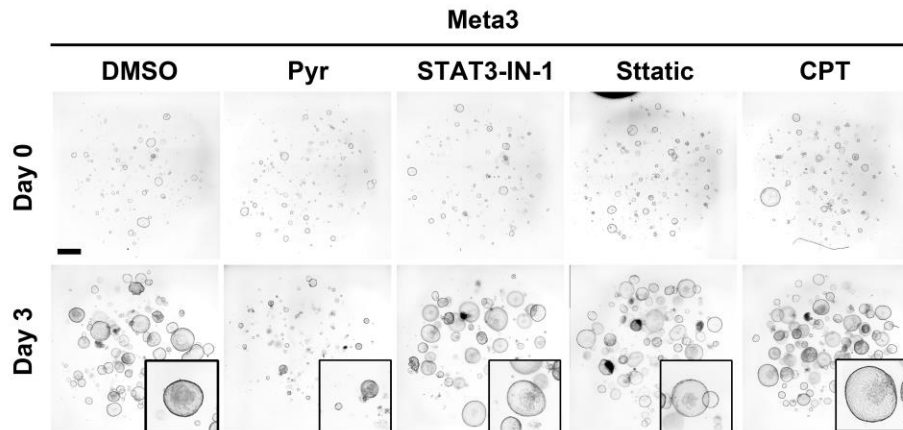
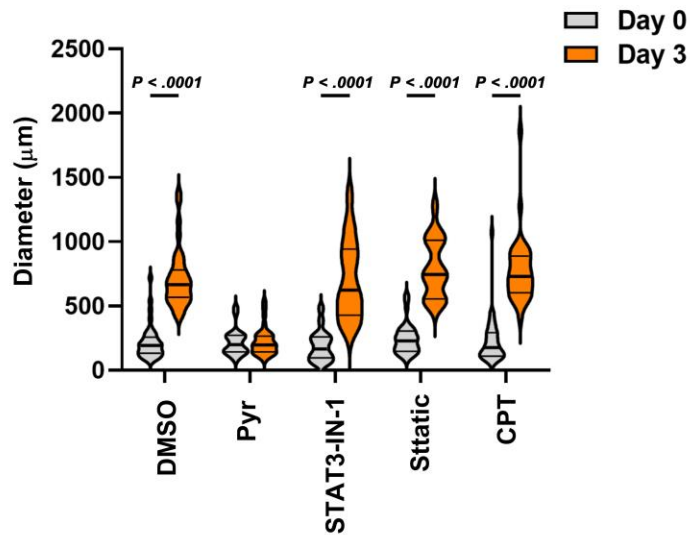


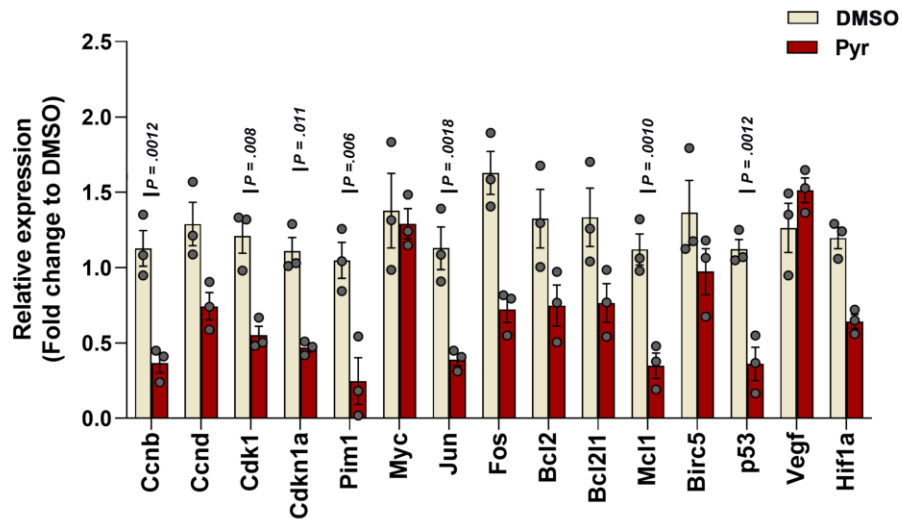
Supplementary Figure 1. A linear gastric mucosa strip (indicated by yellow dotted line) was obtained from a surgical specimen of gastric cancer patient. This section was adjacent to gastric cancer area (indicated by white dotted lines between clips). To prevent contamination from cancer cells, the area near the cancer lesion was marked with a wire (indicated by a green arrow). The mucosa enclosed within the red dotted box was utilized for the generation of gastric organoids.



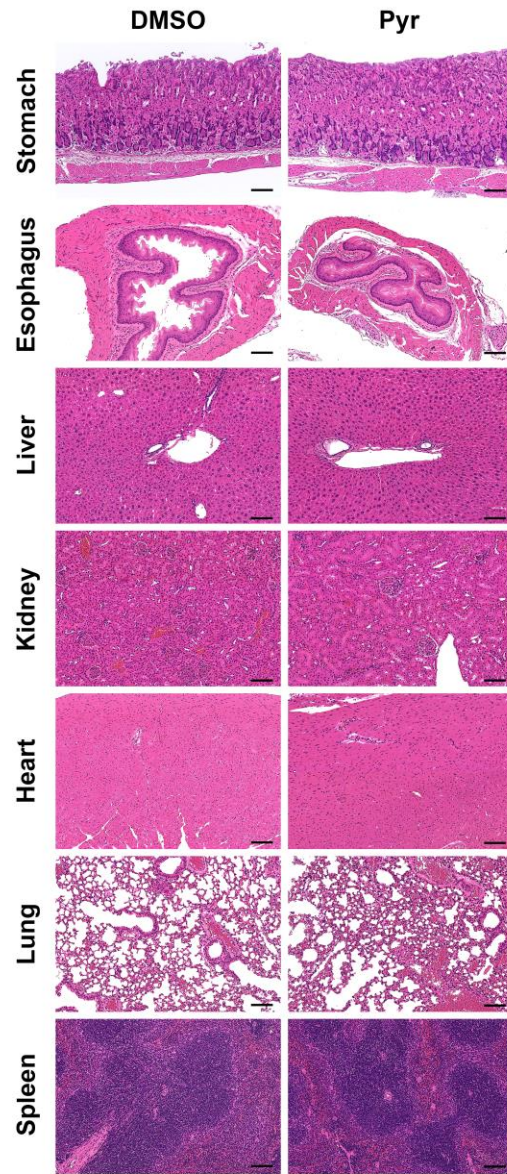
Supplementary Figure 2. Expression of Wnt/ β -catenin related genes upon treatment of MEK inhibitor, Pyrvinium and CK1 α inhibitor in dysplastic organoids. Real-time PCR analysis for Wnt-target genes including Axin2, Cdca4, Ephb3, Lrg5, Rnf43, Sp5, and Znrf3 in Meta4 organoids after treatment of DMSO vehicle, Trametinib (Tra, 1 μ M), Pyrvinium (Pyr, 100nM), and SSTC3 (1 μ M) for 6 hours or 24 hours, respectively. Mean \pm SD. One-way ANOVA with Tukey's multiple comparisons.

A**B**

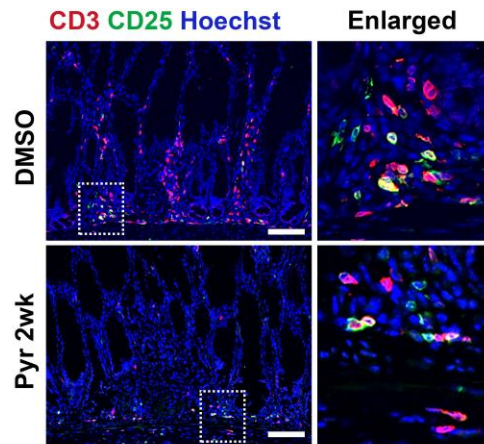
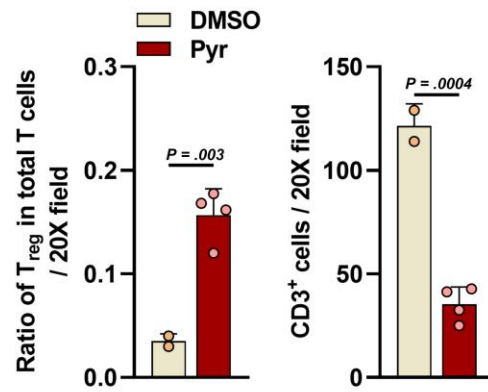
Supplementary Figure 3. Effects of STAT3 inhibitors in Meta3 organoids. **(A)** Phase-contrast images of Meta3 organoids treated with either DMSO vehicle, 100nM of Pyrvinium (Pyr), or three STAT3 inhibitors, including STAT3-IN-1 (1µM), Sttatic (2µM), and Cryptotanshione (CPT, 5µM) for 3 days. **(B)** Quantitation of organoid diameters before and after treatment. Mean ± SD. Two-way ANOVA with Tukey test for pairwise comparisons. Scale bar = 500µm



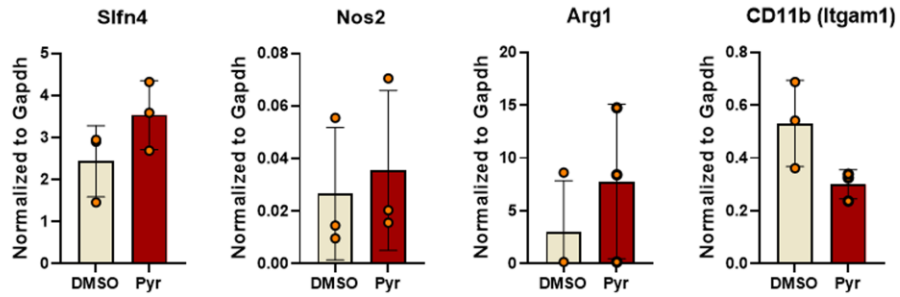
Supplementary Figure 4. Expression of STAT3-target genes upon treatment of Pyrvinium (Pyr) in dysplastic organoids. Real-time PCR analysis for 15 STAT3-target genes in Meta4 organoids after treatment of Pyrvinium (100nM) for 48 hours. Three replicates were performed. Two-tailed Mann-Whitney test.



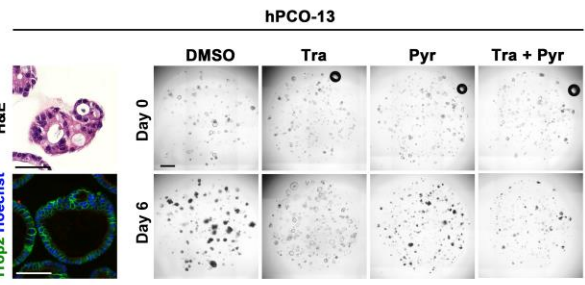
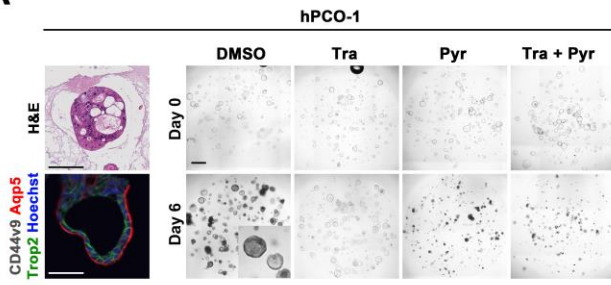
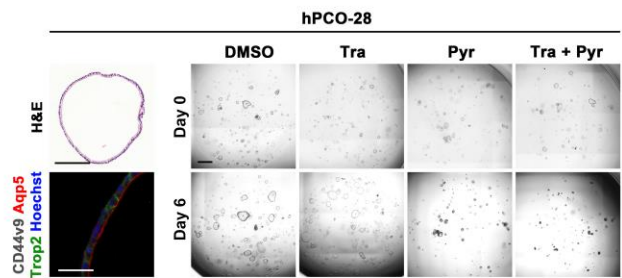
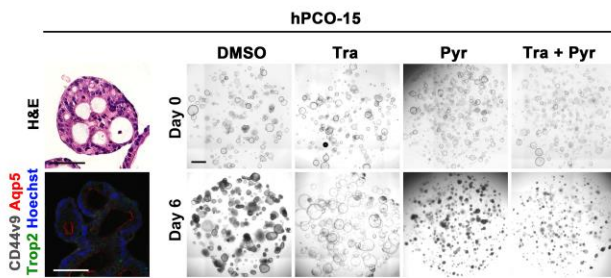
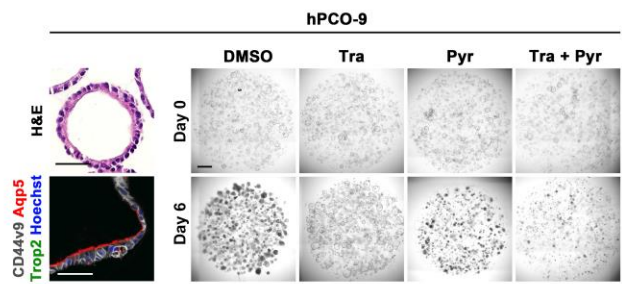
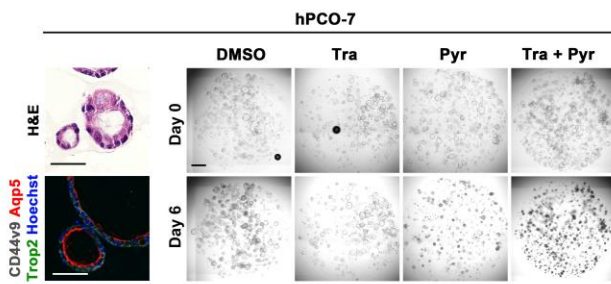
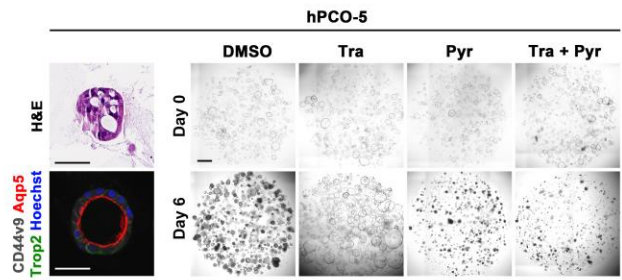
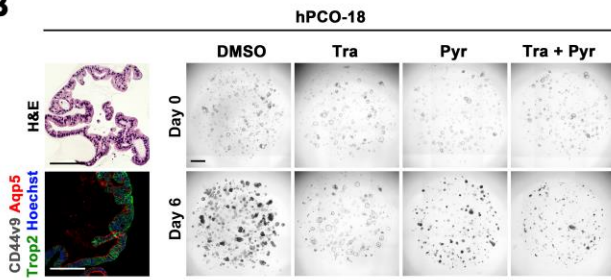
Supplementary Figure 5. Normal tissues in multiple organs, including the stomach, esophagus, liver, kidney, heart, lung, and spleen were unaffected by Pyrvinium (Pyr) treatment in vivo. Hematoxylin and eosin images were obtained from wild-type mice treated with either dimethyl sulfoxide (DMSO) or Pyr (4mg/L in drinking water) for a duration of 2 weeks. Scale bar = 100 μ m.

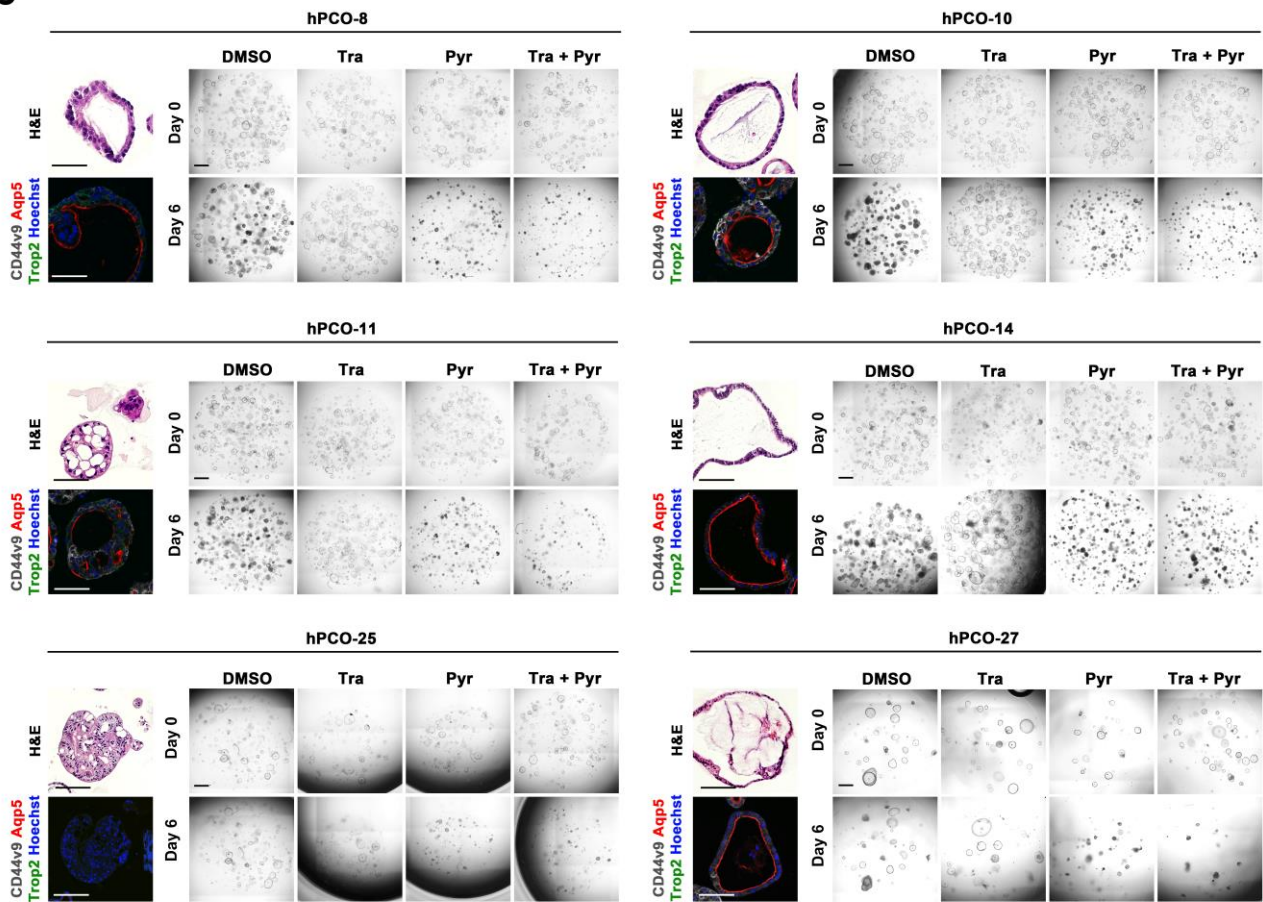
A**B**

Supplementary Figure 6. Alterations in regulatory T cells by Pyrvinium treatment in Mist1-Kras mice. **(A)** Immunostaining for CD3 and CD25 in the gastric corpus from Mist1-Kras mice treated with DMSO or Pyr for 2 weeks. **(B)** Quantitation of various immune cells per 20X field and ratio of regulatory T cells (Treg) per total T cells in the wild type mice and Mist1-Kras mice treated with DMSO or Pyr. Mean \pm SD. Two-tailed Mann-Whitney test. Scale bar = 100 μ m

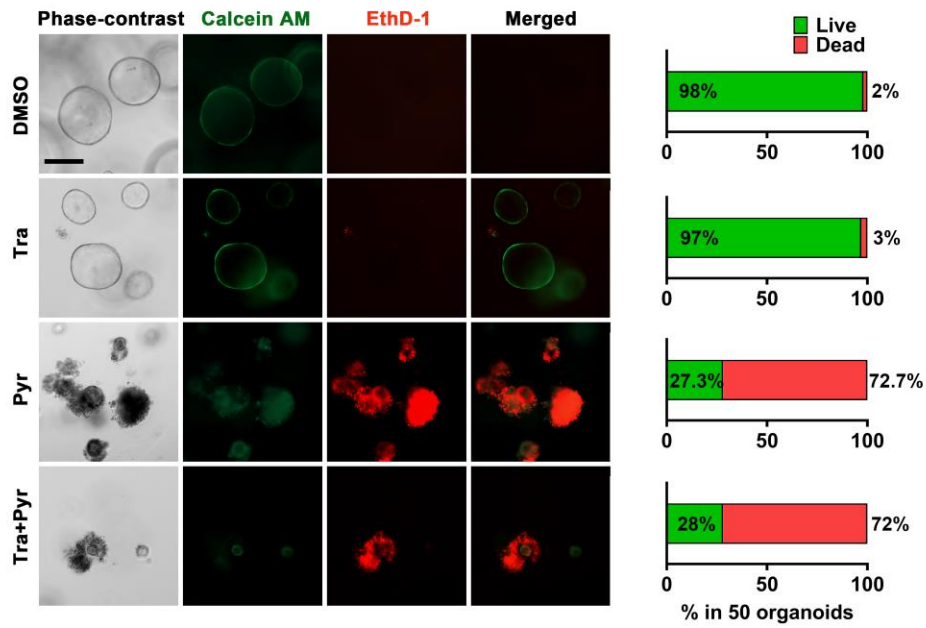


Supplementary Figure 7. Mist1-Kras mice at 3 months after Tamoxifen injection were administered either DMSO or Pyrvinium (Pyr) for 2 weeks. Quantitative real time-PCR analysis was performed for myeloid derived suppressor cell markers, including Slfn4, Nos2, and Arg1 and Itgam1. Relative quantification of each gene expression level was normalized to Gapdh gene expression. Mean \pm standard deviation. Two-tailed Mann-Whitney test.

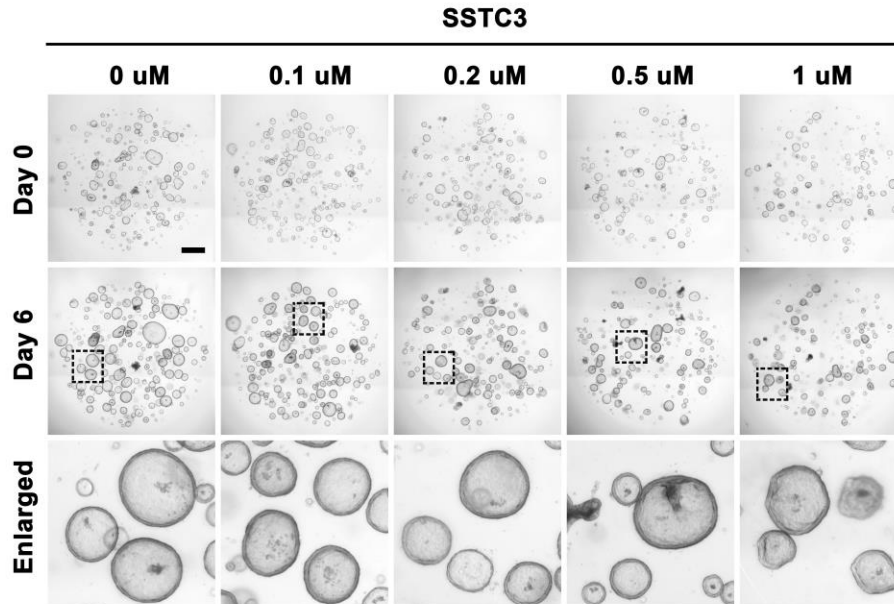
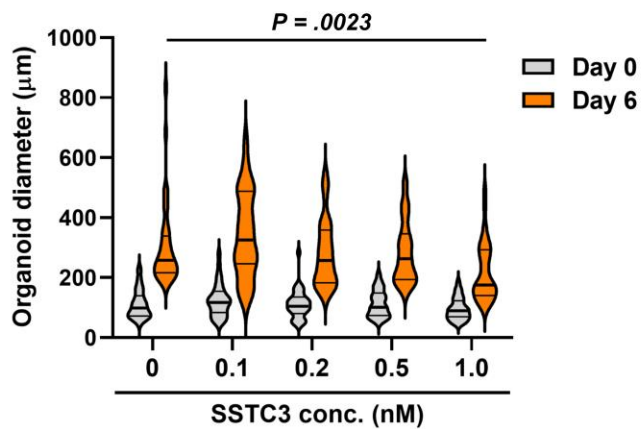
A**B**

C

Supplementary Figure 8. Effects of Pyruvium (Pyr) treatment in fourteen various human precancerous organoid (hPCO) lines established from human stomach samples of gastric cancer patients. H&E, co-immunostaining for CD44v9, Aqp5 and Trop2, and phase-contrast images in two Pyr-sensitive-(A), six Pyr-moderate (B), and six Pyr-resistant hPCO lines (C) treated with DMSO, 1 μ M of Trametinib (Tra), 100nM of Pyr and their combinations for 6 days. Scale bar = 100 μ m



Supplementary Figure 9. Cell death induced by Pyrvinium (Pyr) in human precancerous organoid (hPCO) line. Live/dead cell analysis using double staining of Calcein AM and Ethidium Homodimer-1 (EthD-1) after DMSO, Trametinib (Tra, 1 μ M), Pyrvinium (Pyr, 100nM), and combination of Tra and Pyr in Pyr-sensitive hPCO-34 line and percentages of live and dead cells after treatment. Scale bar = 500 μ m

A**B**

Supplementary Figure 10. Effects of Casein Kinase 1 α (CK1 α) activator treatment in human precancerous organoid (hPCO) line. **(A)** Phase-contrast images of Pyruvinium-sensitive hPCO-34 treated with DMSO vehicle or varying concentrations (0.1, 0.2, 0.5, and 1.0 μM) of SSTC3, a specific CK1 α activator, for 6 days. **(B)** Quantitation of organoid diameters before and after treatment. Mean \pm SD. Two-way ANOVA (with Tukey test for pairwise comparisons). Scale bar = 500 μm