Figure S1. Inhibition of cell growth by lapatinib in pancreatic ductal adenocarcinoma cells. Cell viability was assessed at 24 and 48 h following treatment with lapatinib in (A) BxPC-3, (B) KP-4, (C) PANC-1, (D) MIA PaCa-2, (E) KP-2, (F) KP-3, (G) 293 and (H) LP101 cells. Data are presented as the mean ± standard deviation, n=6.

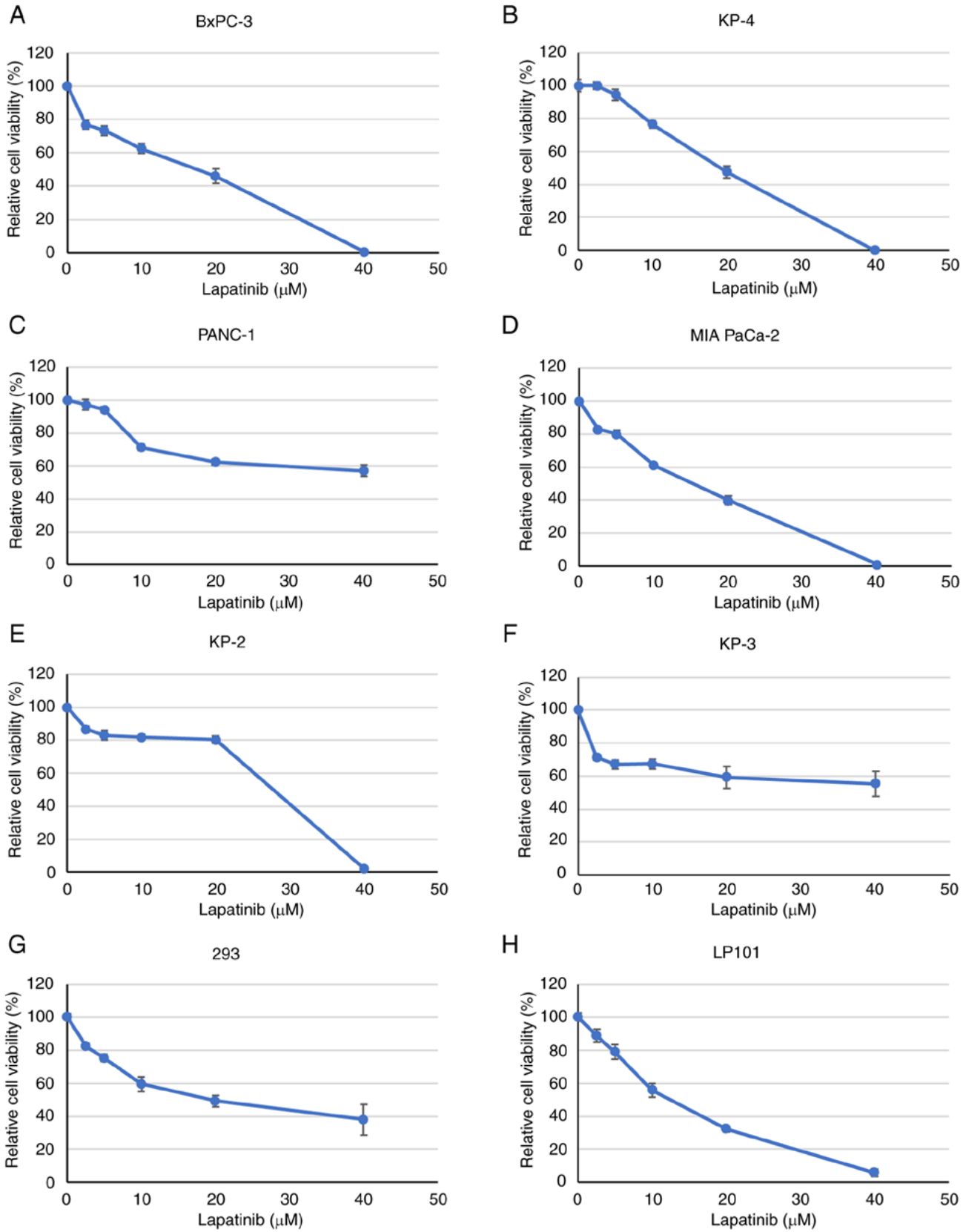

Figure S2. Inhibition of cell growth by FTY720 in pancreatic ductal adenocarcinoma cells. Cell viability was assessed at 24 and 48 h following treatment with FTY720 in (A) BxPC-3, (B) KP-4, (C) PANC-1, (D) MIA PaCa-2, (E) KP-2, (F) KP-3, (G) 293 and (H) LP101 cells. Data are presented as the mean ± standard deviation, n=6.

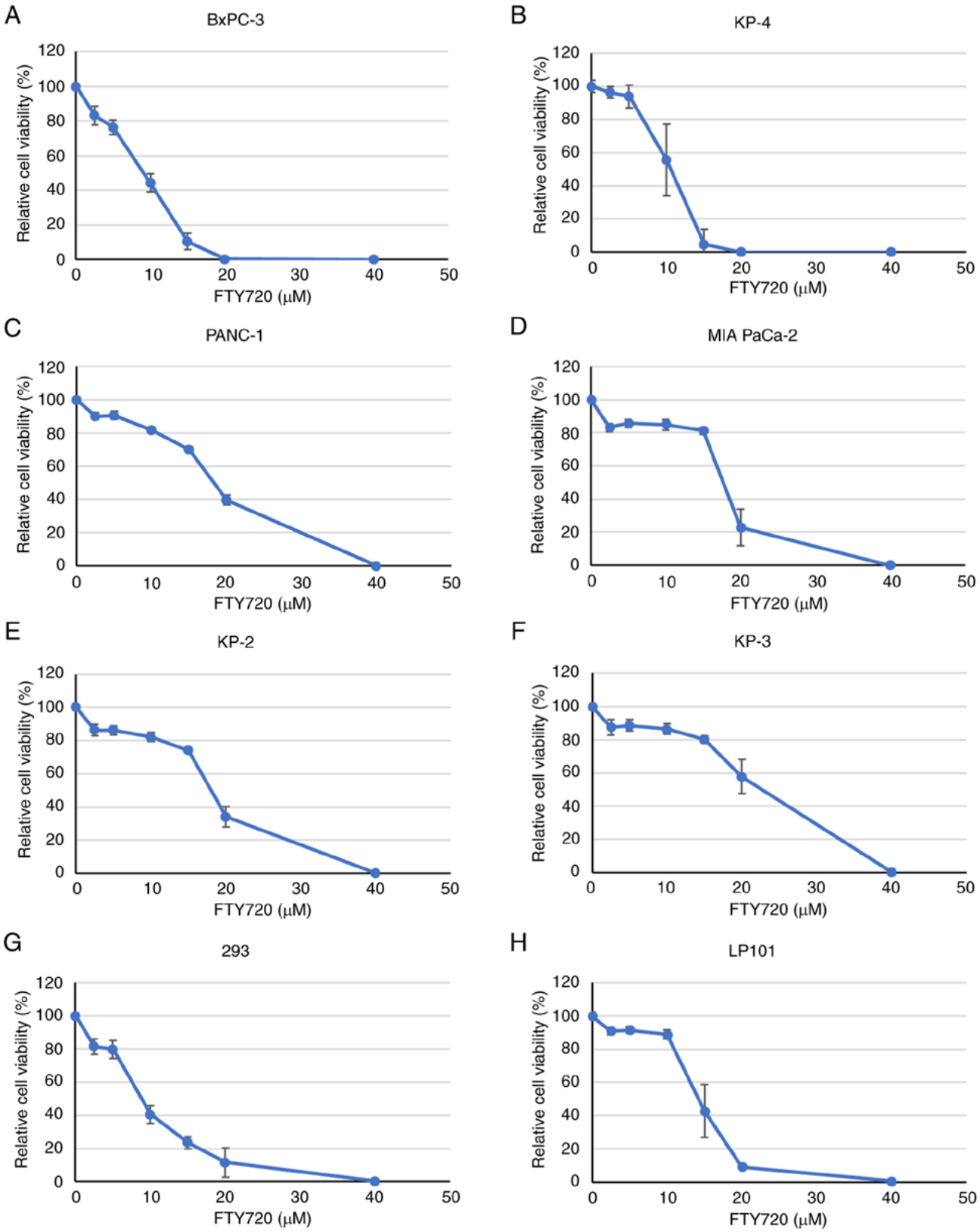

Figure S3. Inhibition of cell growth in pancreatic ductal adenocarcinoma and non-cancerous cells. Cell viability was assessed at 24 and 48 h following treatment with indicated drugs in (A) KP-2, (B) KP-3, (C) 293 and (D) LP101 cells. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Data are presented as the mean ± standard deviation, n=6. **P<0.01 vs. FTY 0 μM/Lap 0 μM. $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. each respective FTY 0 μM. Lap, lapatinib; FTY, FTY720.

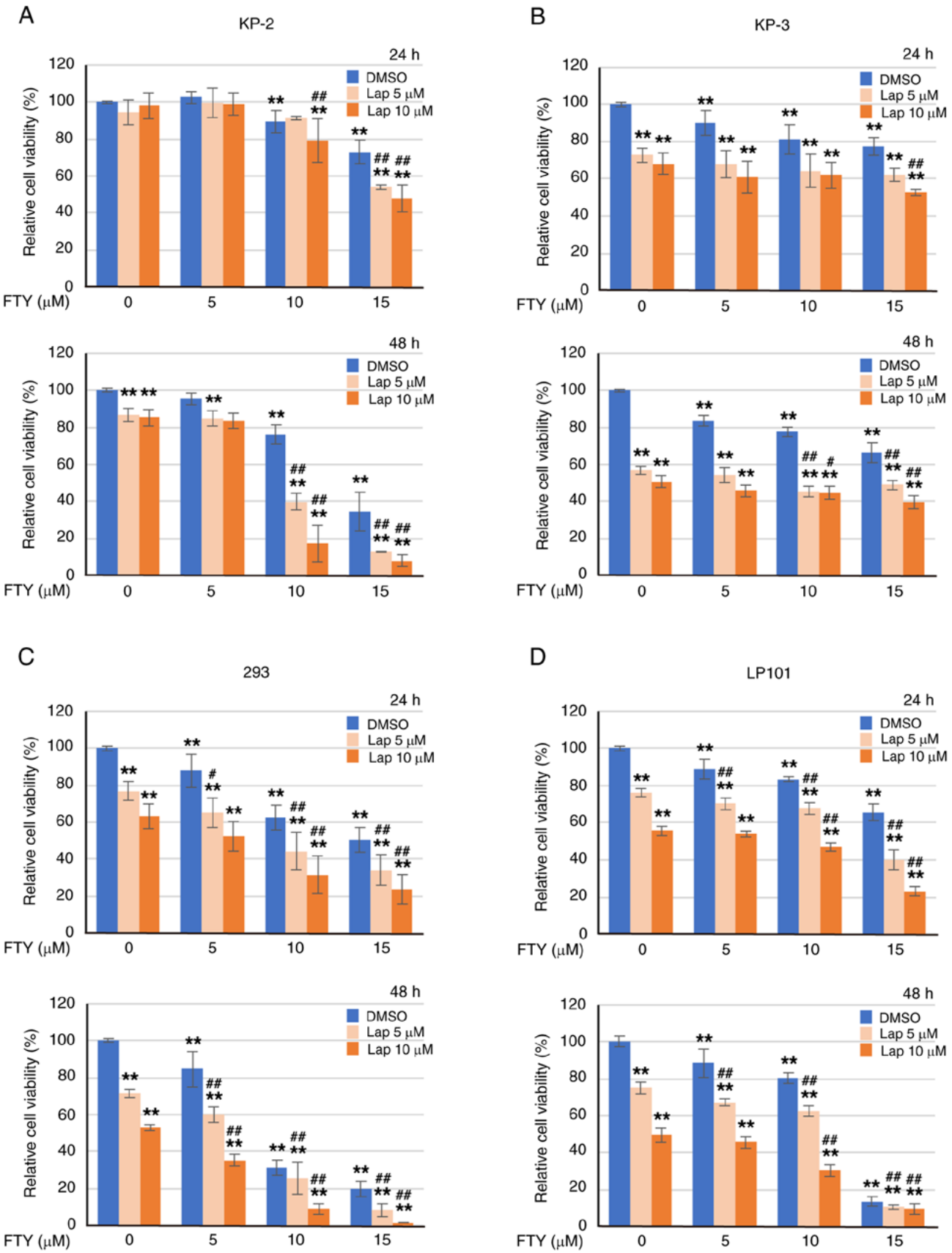

Figure S4. Immunoblot analysis of EGFR, p-EGFR, HER2 and p-HER2 24 h after treatment with Lap (10 μM) and FTY (10 μM) in BxPC-3, KP-4, PANC-1, MIA PaCa-2 and BT-474 (positive control) cells. ACTB and GAPDH were used as loading controls. EGFR, epidermal growth factor receptor; p-, phosphorylated; Lap, lapatinib; FTY, FTY720.

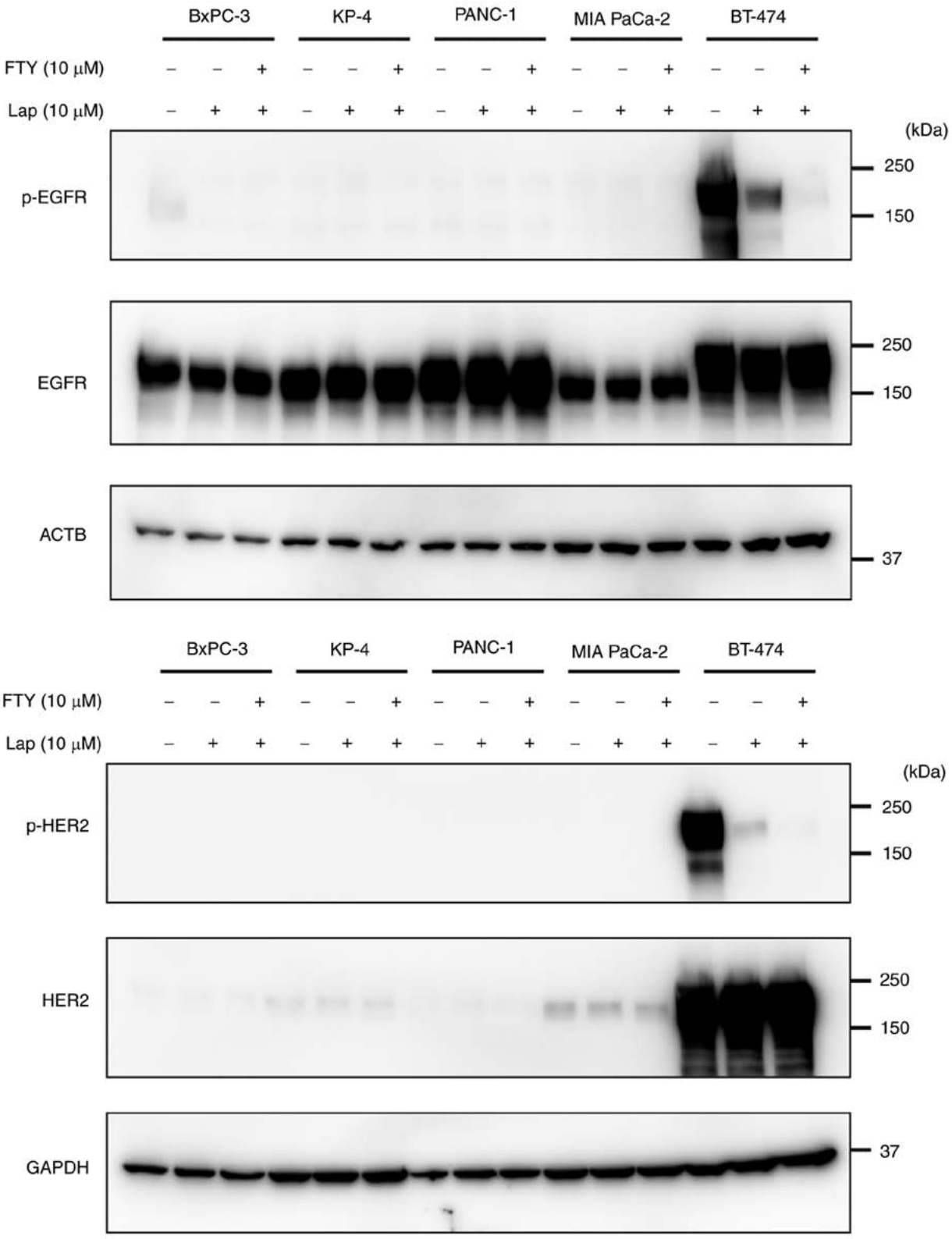

Figure S5. DNA fragmentation assay. Genomic DNA was extracted from BxPC-3, KP-4 and MIA PaCa-2 cells 24 h after treatment with Lap (10 μM) and FTY (10 μM). Extracted DNA was separated by electrophoresis on 2% agarose gel. M, 1 kbp DNA ladder marker; Lap, lapatinib; FTY, FTY720.

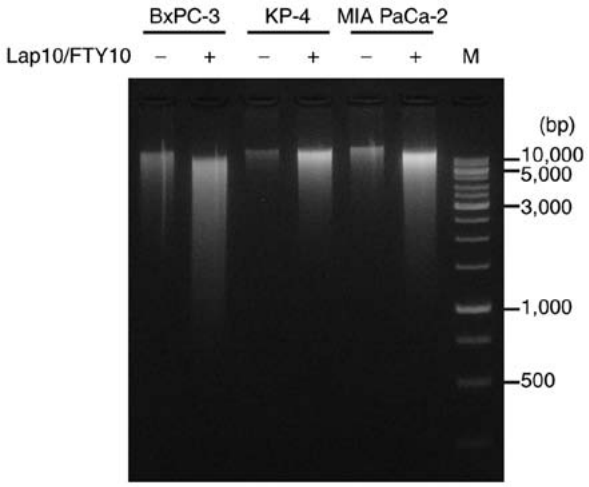

Figure S6. z-VAD suppresses apoptosis and Nec-1 suppresses necroptosis. (A and B) As a positive control experiment for apoptosis, cell viability was assessed 24 and 48 h following treatment with STS in the presence (25 or 50 μM) or absence of z-VAD in (A) BxPC-3 and (B) KP-4 cells. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Data are presented as the mean ± standard deviation, n=6. (C and D) As a positive control experiment for necroptosis, cell viability was assessed 24 and 48 h after treatment with Gef under amino acid starvation conditions in the presence (25 μM) or absence of Nec-1 in (C) BxPC-3 and (D) KP-4 cells. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Data are presented as the mean ± standard deviation, n=6. **P<0.01 vs. DMSO. STS, staurosporine; Gef, gefitinib; z-VAD, z-VAD-FMK; Nec-1, necrostatin-1; DMSO, dimethyl sulfoxide.

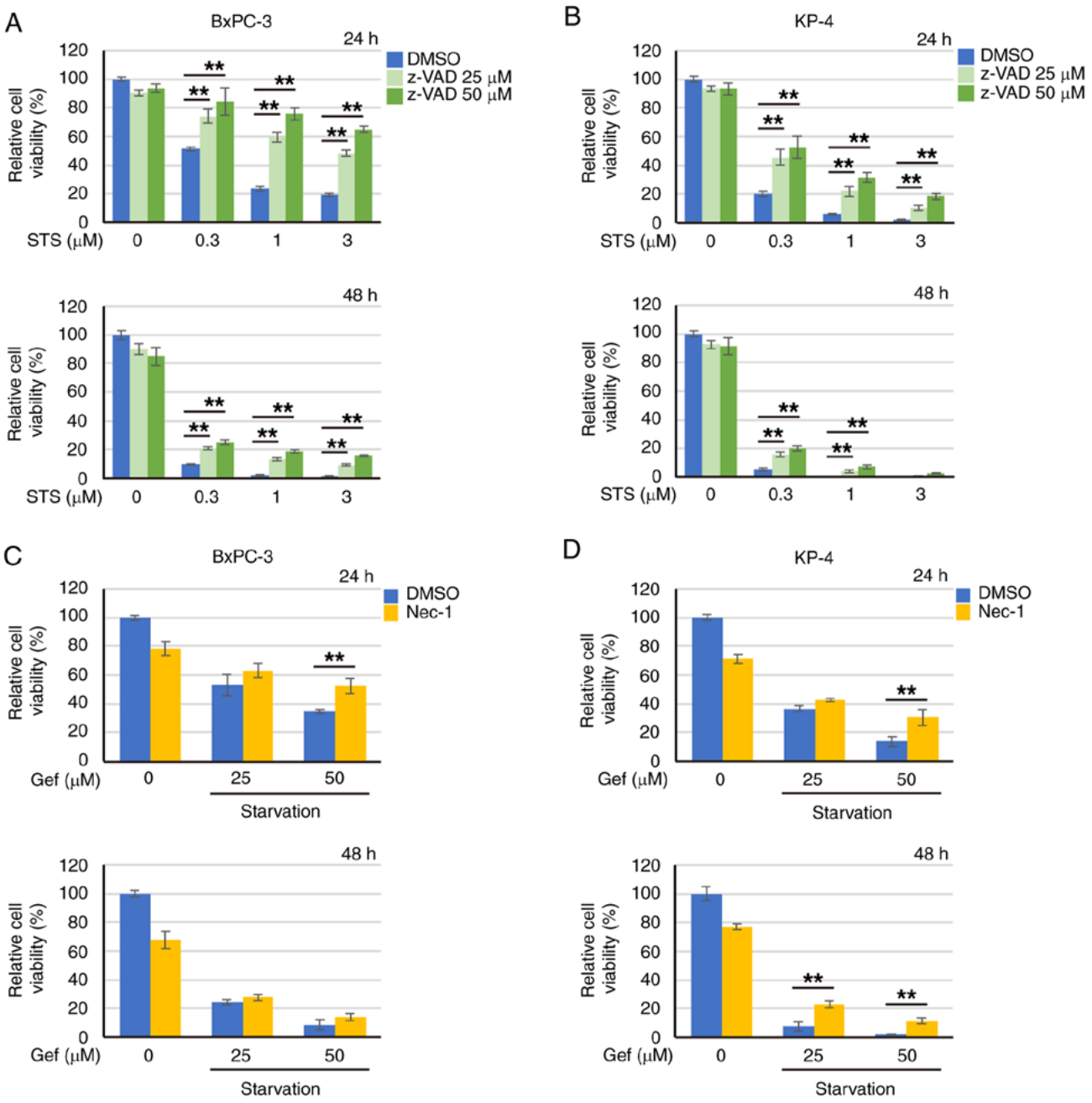

Figure S7. Non-canonical death induced in pancreatic ductal adenocarcinoma cells. (A and B) Cell viability was assessed 24 and 48 h after treatment with Lap and/or FTY in the presence (5 mM) or absence of NAC in (A) BxPC-3 and (B) KP-4 cells. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Data are presented as the mean ± standard deviation, n=4. **P<0.01 vs. DMSO. (C and D) Cell viability was assessed 24 and 48 h after treatment with Lap and/or FTY in the presence or absence of E64d (30 μM) and PepA (15 μM) in (C) BxPC-3 and (D) KP-4 cells. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Data are presented as the mean ± standard deviation, n=4. (E) PANC-1/GFP-LC3-RFP-LC3ΔG cells were treated with BafA1 (10 nM), HBSS, Lap (10 or 20 μM) or FTY720 (10 or 20 μM). Fluorescence intensities derived from GFP-LC3B and RFP-LC3ΔG were monitored over 24 h. Autophagic flux was assessed as alterations in the relative intensities of GFP/RFP, using DMSO-treated groups as a control. Data were analyzed using two-way ANOVA followed by Bonferroni's post hoc test. Data are presented as the mean ± standard deviation, n=4. **P<0.01 vs. DMSO. Lap, lapatinib; FTY, FTY720; NAC, N-acetyl-L-cysteine; PepA, pepstatin A; BafA1, bafilomycin A_1; HBSS, Hanks' balanced salt solution; DMSO, dimethyl sulfoxide; GFP, green fluorescent protein; RFP, red fluorescent protein.

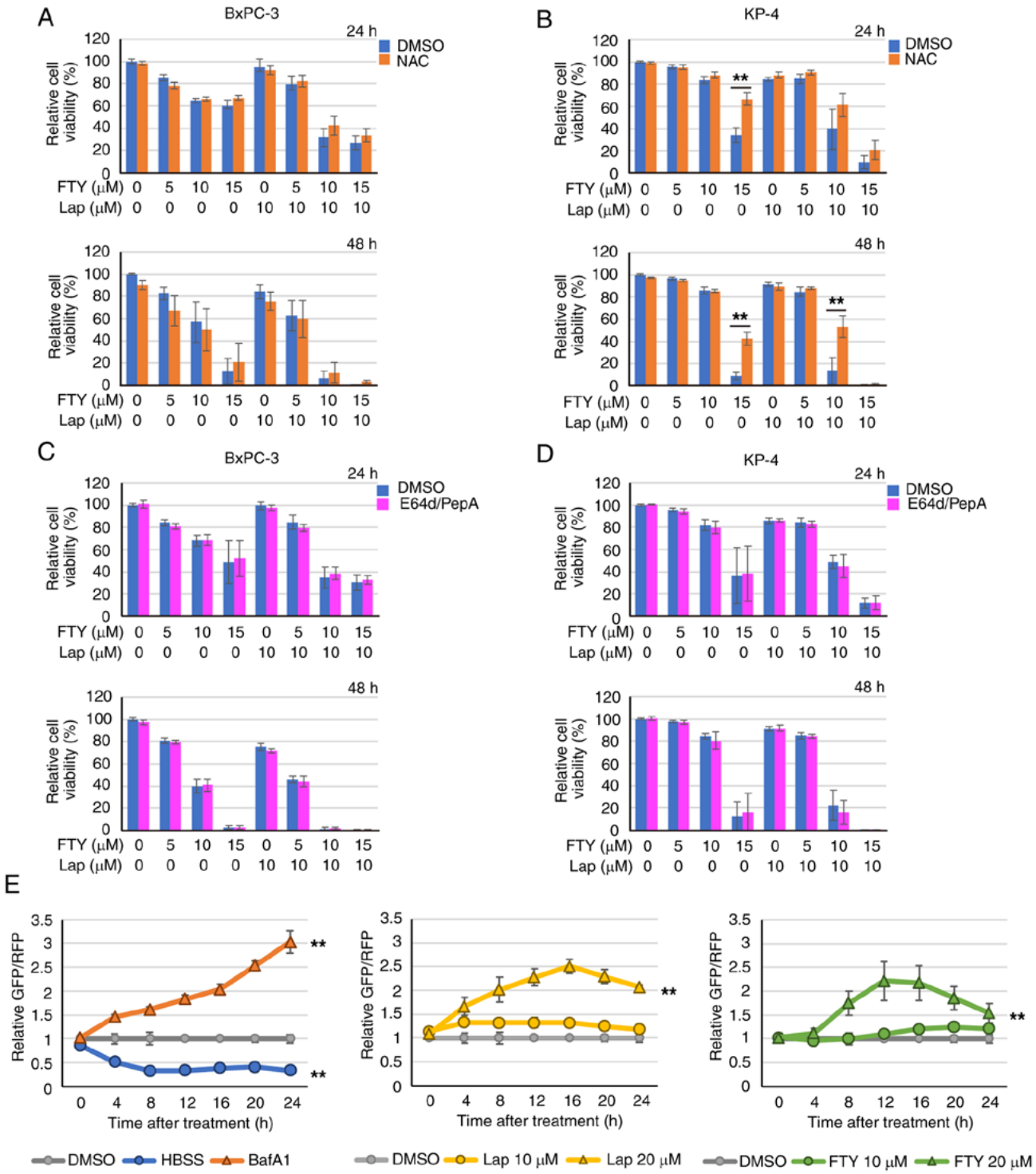

Figure S8. Impact of treatment with Lap and FTY on lysosomes in pancreatic ductal adenocarcinoma cells. (A) LysoTracker staining (magenta) at the indicated time point (0, 1, 2 and 4 h) following combination treatment with Lap (10 μM) and FTY (5 μM) in BxPC-3 and KP-4 cells. Scale bar, 20 μm. (B) Relative mean fluorescence intensities of LysoTracker at each time point for BxPC-3 and KP-4 cells from (A) are shown. Data were analyzed using Kruskal-Wallis with Dunn's post hoc test. The box extends from the lower to upper quartiles. The middle line represents the median value. X indicates the mean value. The whiskers represent the minimum to maximum values, except for outliers, which are indicated by dots. BxPC-3, n=26; KP-4, n=15. *P<0.05 and **P<0.01 vs. 0 h. Lap, lapatinib; FTY, FTY720.

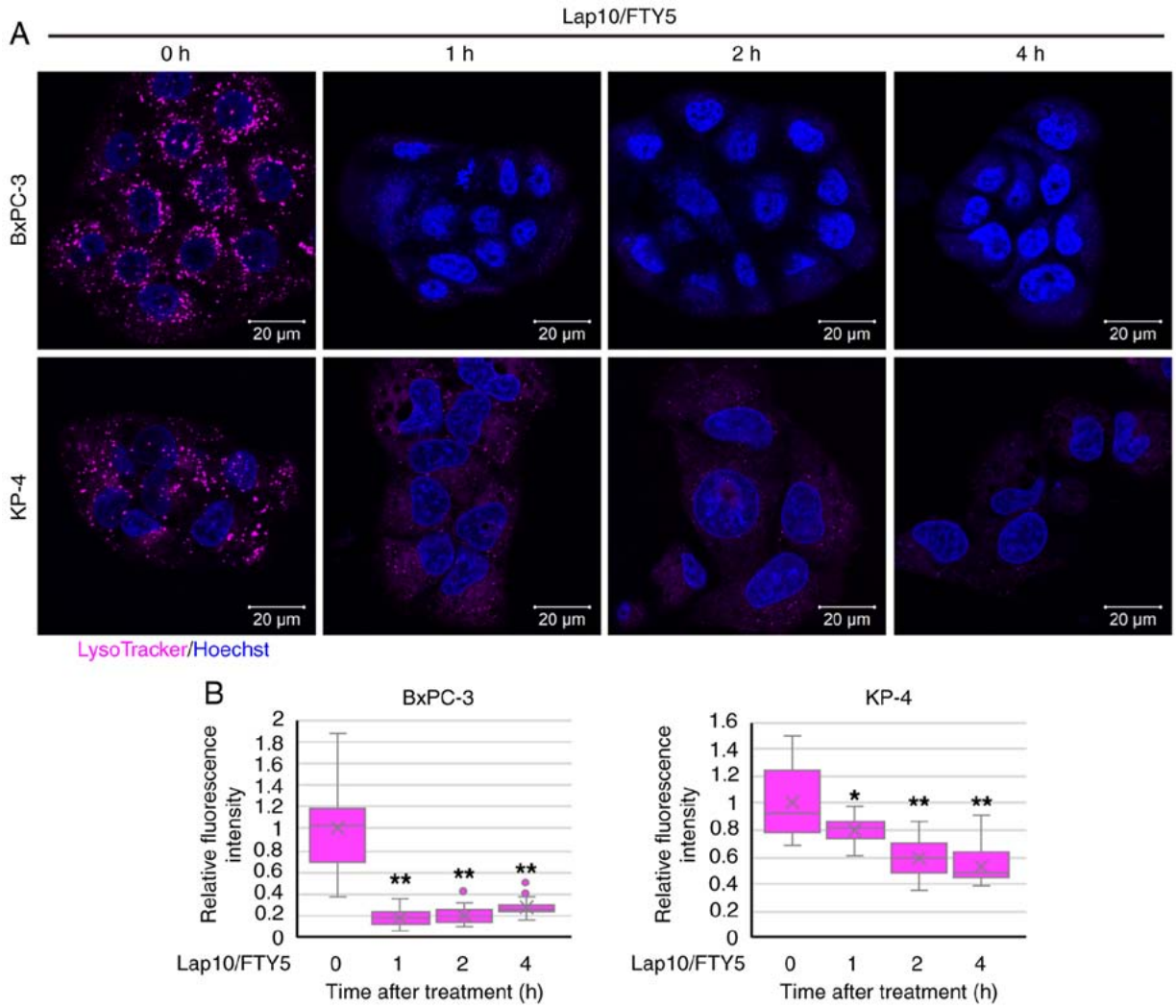

Figure S9. Combination treatment with Lap and FTY induces lysosomal membrane permeabilization. (A) Immunofluorescence analysis of LAMP2 (magenta) and LGALS3 (green) 10 h after combination treatment with Lap (10 μM) and FTY (5 μM) in KP-4 cells. The dashed boxed regions are shown at high magnification at the bottom. Scale bar, 20 μm. (B) Colocalization of fluorescence between LGALS3 and LAMP2 in KP-4 cells from (A) was analyzed using ImageJ. Data were analyzed using Mann Whitney U test. The box extends from the lower to upper quartiles. The middle line represents the median value. X indicates the mean value. The whiskers represent the minimum to maximum values, except for outliers, which are indicated by dots. n=19. **P<0.01 vs. DMSO. Lap, lapatinib; FTY, FTY720; LAMP, lysosome-associated membrane protein; LGALS3, galectin-3; DMSO, dimethyl sulfoxide.

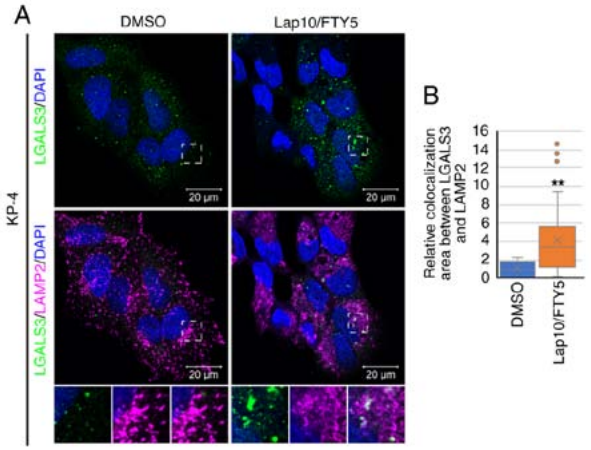

Figure S10. Impact of combination treatment with Lap and FTY on the morphology of the trans-Golgi network. (A) Immunofluorescence analysis of TGOLN2 (green) at indicated time points (0, 4, 10 and 24 h) after combination treatment with Lap (10 μM) and FTY (5 μM) in BxPC-3 and KP-4 cells. Dashed boxed regions are shown at high magnification in the inset. Scale bar, 20 μm. (B) Relative mean fluorescence kurtosis of TGOLN2 at each time point for BxPC-3 and KP-4 cells from (A) are shown. Data were analyzed using Kruskal-Wallis with Dunn's post hoc test. The box extends from the lower to upper quartiles. The middle line represents the median value. X indicates the mean value. The whiskers represent the minimum to maximum values, except for outliers, which are indicated by dots. BxPC-3, n=46; KP-4, n=25. **P<0.01 vs. 0 h. Lap, lapatinib; FTY, FTY720; TGOLN2, trans-Golgi network integral membrane protein 2.

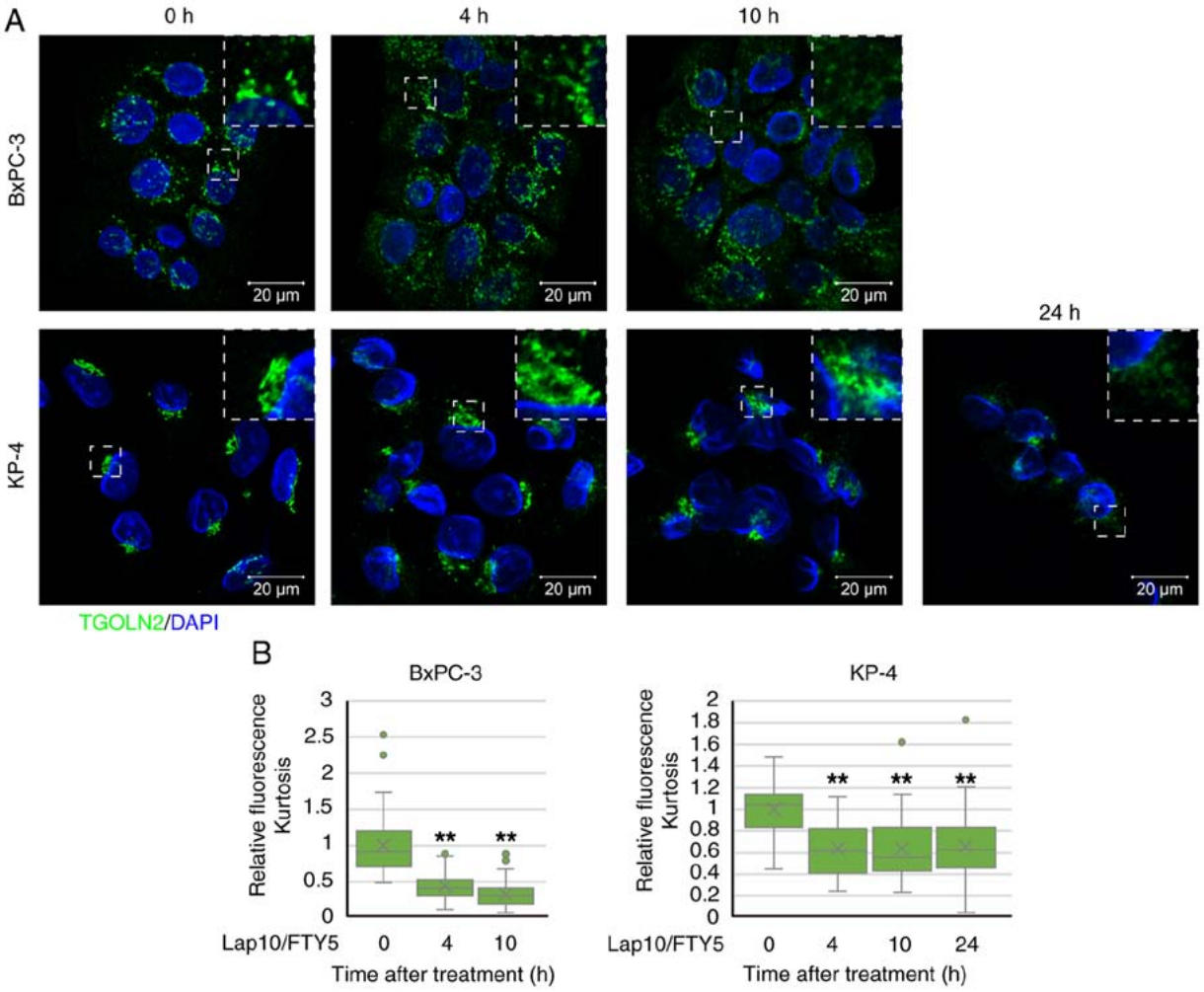

Figure S11. Cell growth inhibitory effects of the lysosome-targeted drug combination in pancreatic ductal adenocarcinoma cells. (A and B) Cell viability was assessed 24 and 48 h after treatment with Abe and/or FTY in (A) BxPC-3 and (B) KP-4 cells. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Data are presented as the mean ± standard deviation, n=6. **P<0.01 vs. FTY 0 μM/Abe 0 μM. $^{\#\#}$P<0.01 vs. DMSO. (C and D) Cell viability was assessed 24 and 48 h after treatment with Abe and/or HCQ in (A) BxPC-3 and (B) KP-4 cells. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Data are presented as the mean ± standard deviation, n=6. **P<0.01 vs. HCQ 0 μM/Abe 0 μM. $^{\#\#}$P<0.01 vs. DMSO. FTY, FTY720; Abe, abemaciclib; DMSO, dimethyl sulfoxide; HCQ, hydroxychloroquine.

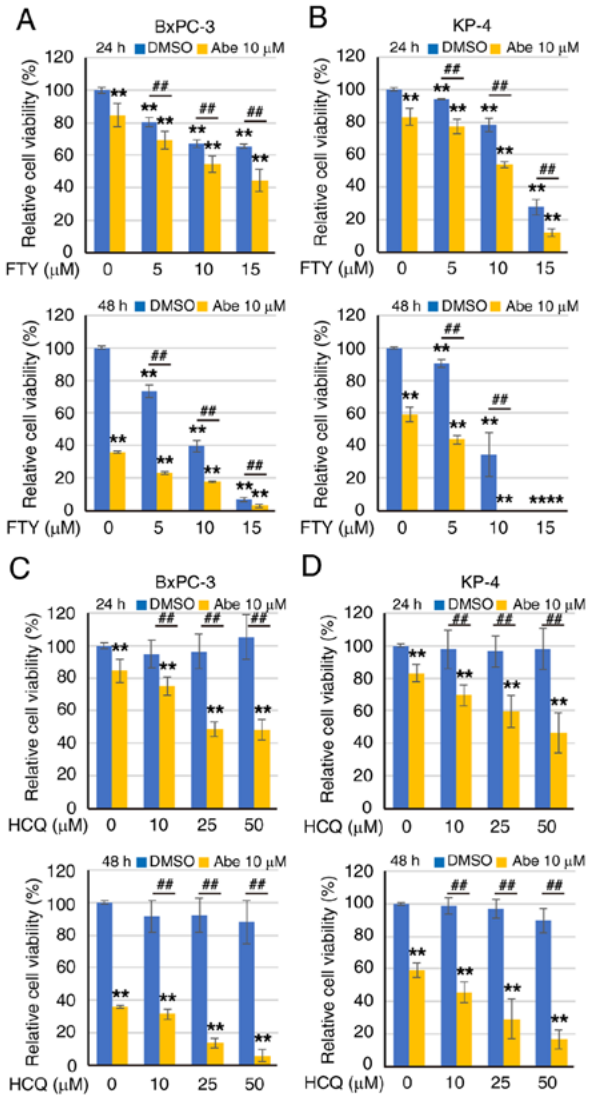

Figure S12. U18666A mitigates the effects of combination treatment with Lap and FTY in pancreatic ductal adenocarcinoma cells. (A) Filipin III staining (blue) 24 h after treatment with U18666A (1 μM) in KP-4 cells. Scale bar, 50 μm. (B-E) Cell viability was assessed 24 h after treatment with Lap and FTY in the presence (1 or 3 μM) or absence of U18666A in (B) KP-4, (C) PANC-1, (D) MIA PaCa-2 and (E) BxPC-3 cells. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Data are presented as the mean ± standard deviation, n=6. **P<0.01 vs. DMSO. (F) LysoTracker staining (magenta) 1 h after treatment with Lap (10 μM) and FTY (5 μM) in the presence (1 μM) or absence of U18666A in BxPC-3 cells. Scale bar, 20 μm. (G) Relative mean fluorescence intensities of LysoTracker in each condition of BxPC-3 cells from (F) are shown. Data were analyzed using Kruskal-Wallis with Dunn's post hoc test. The box extends from the lower to upper quartiles. The middle line represents the median value. X indicates the mean value. The whiskers represent the minimum to maximum values, except for outliers, which are indicated by dots. n=28. (H) The PI fluorescence intensity obtained from dead cells was monitored over 48 h following combination treatment with Lap and FTY in the presence (0.1, 0.3 or 1 μM) or absence of U18666A in BxPC-3 cells. Data were analyzed using two-way ANOVA followed by Bonferroni's post hoc test. Data are presented as the mean ± standard deviation, n=3. **P<0.01 vs. Lap 10 μM/FTY 10 μM. Lap, lapatinib; FTY, FTY720; DMSO, dimethyl sulfoxide; PI, propidium iodide.

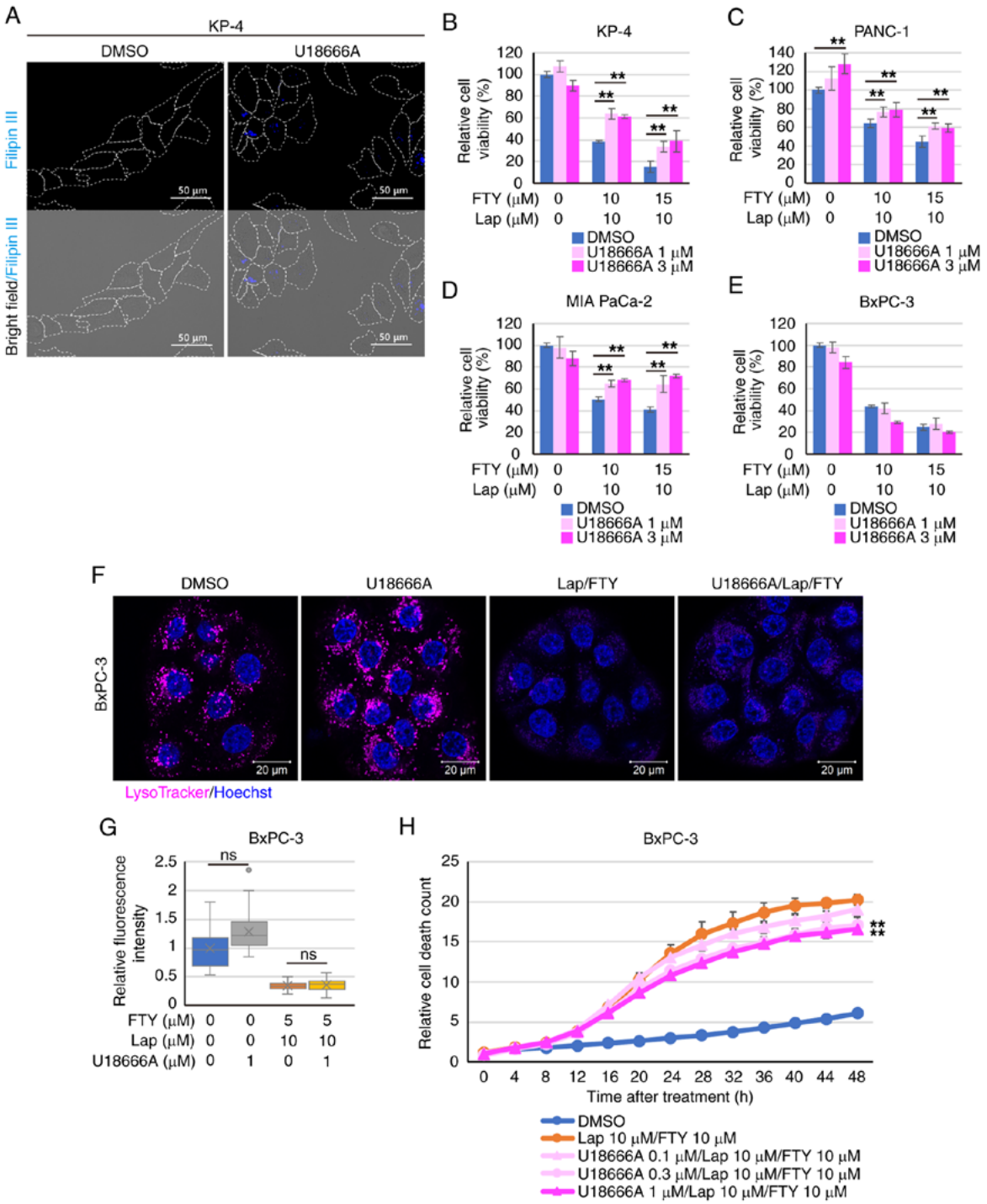

Figure S13. Schematic model presenting the cytotoxic activity of the lysosome-targeted drug combination in PDAC cells. The lysosome-targeted drug combination induced lysosomal membrane permeabilization, mitochondrial depolarization, ER stress and intracellular Ca^{2+} dysregulation, resulting in lysosome-dependent death in PDAC cells. PDAC, pancreatic ductal adenocarcinoma; Ca^{2+}, calcium; ER, endoplasmic reticulum.