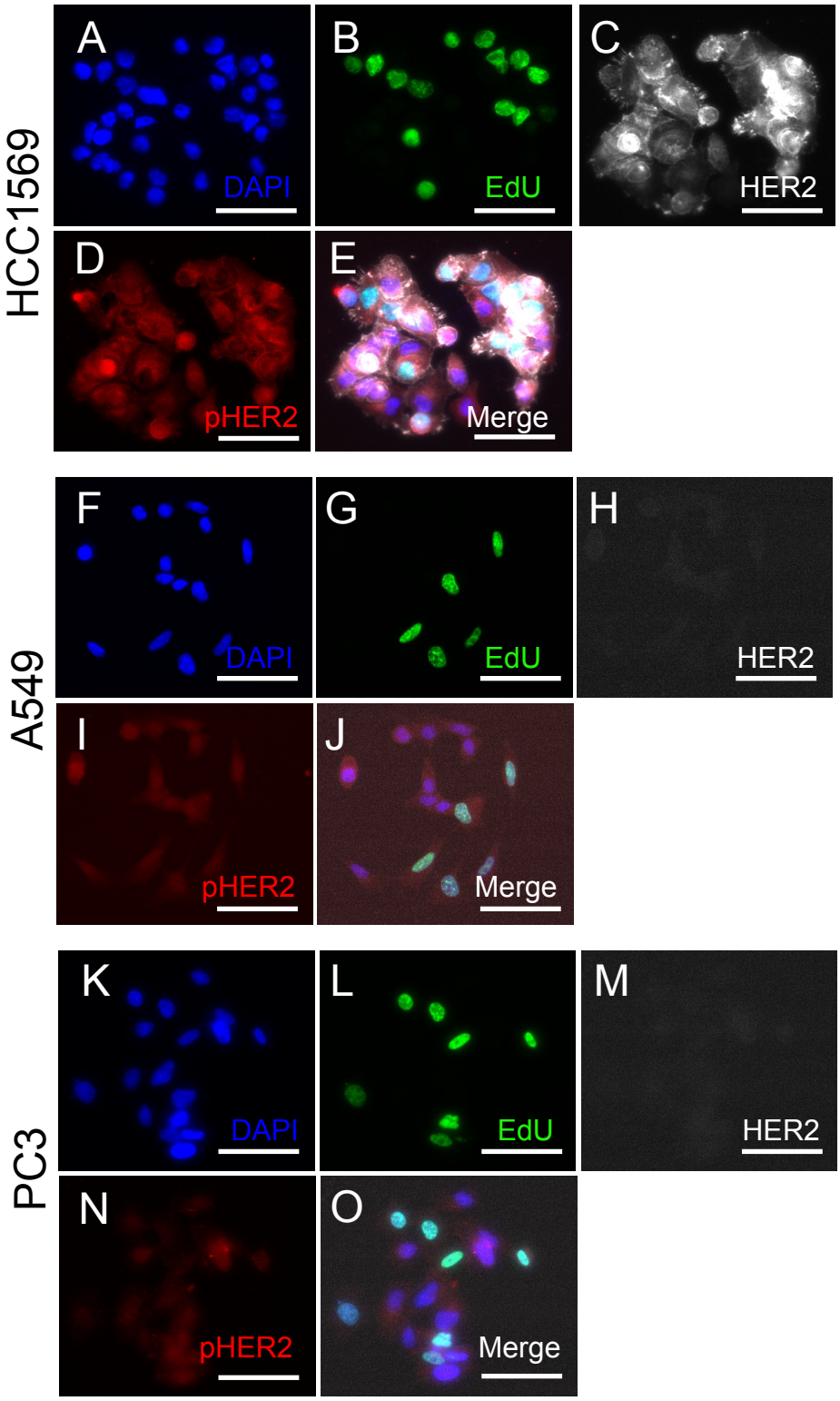

Figure S1 Representative images of HCC1569, A549, and PC3 cancer cell lines in MEMA experiment. Related to Figure 1.

Representative IF images of (A-E) HCC1569, (F-J) A549, and (K-O) PC3 cancer cell lines. Scale bars, 50 μm.

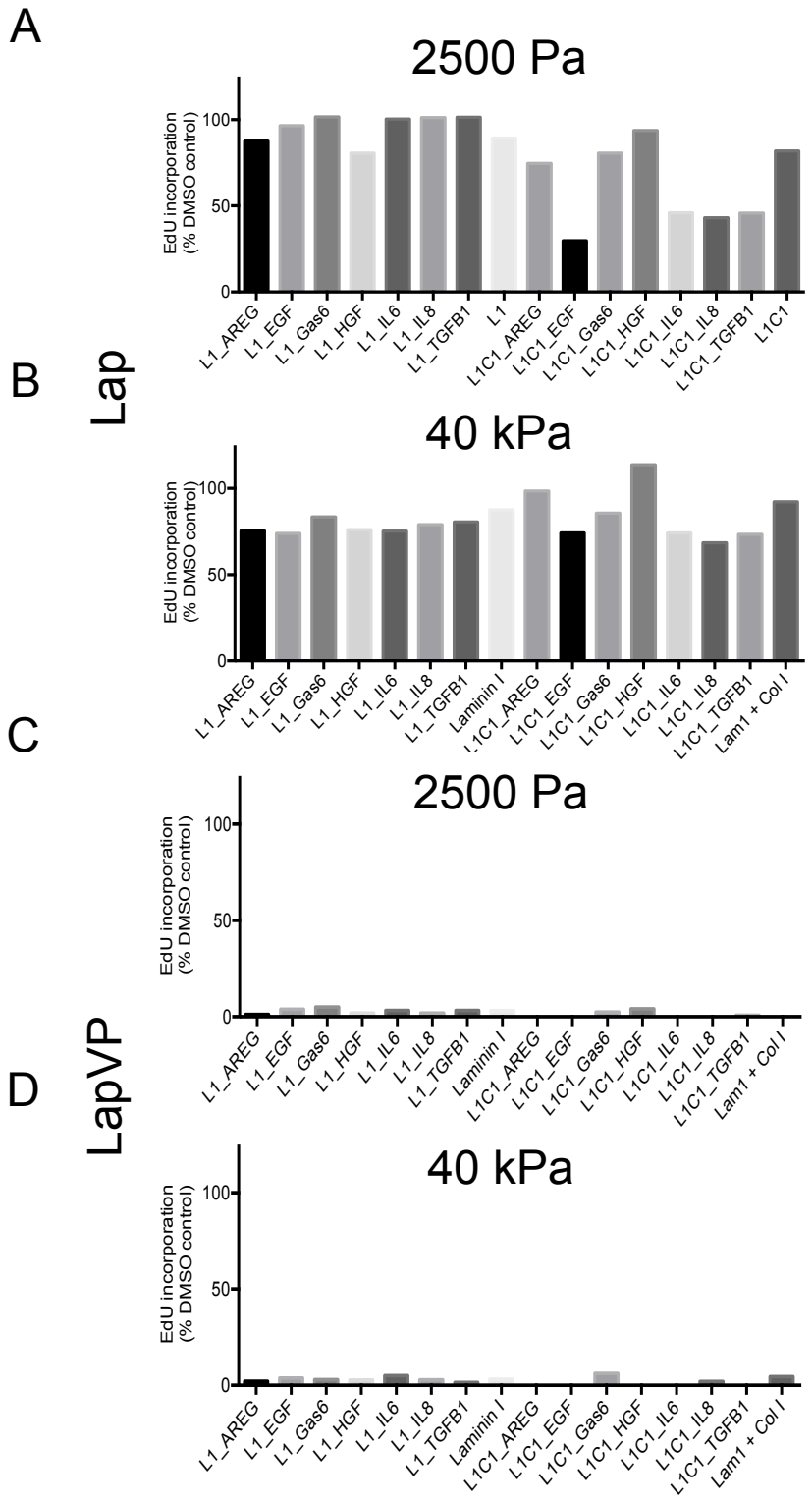

Figure S2 BT549 showed resistance to Lap but was sensitive to verteporfin. Related to Figure 2.

(A-D) Bar graphs represent drug responses in (A, C) 2500 Pa or (B, D) 40 kPa with treatment of (A, B) lapatinib or (C, D) lapatinib together with verteporfin. Data are relative incorporation of EdU expressed as a percentage of DMSO-treated cells, and represented as mean ± SEM (n = 60 for each microenvironment).

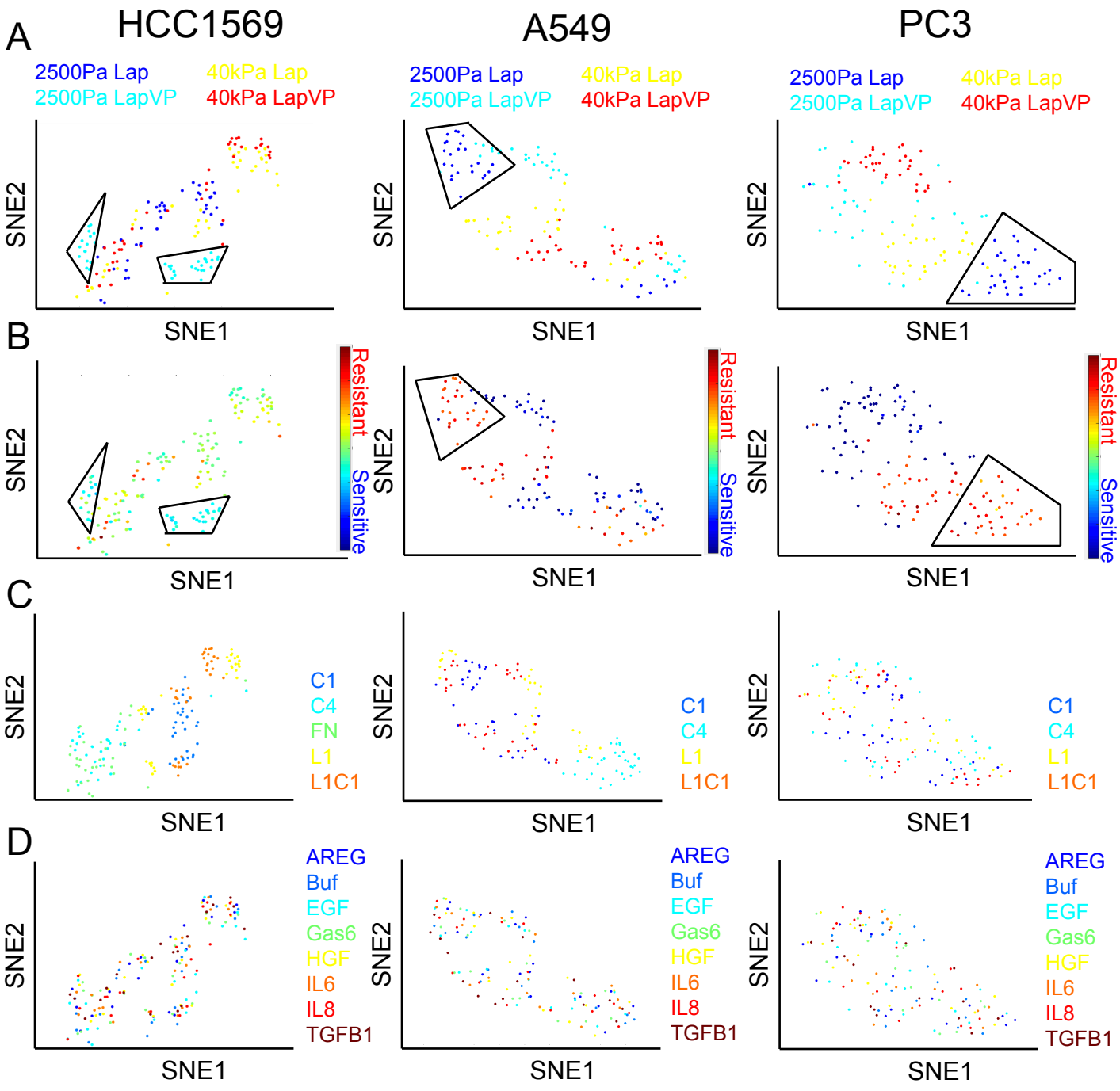

Figure S3 Changes in cell morphology co-organize with drug responses using t-SNE visualization. Related to Figure 3.

(A-D) MEMA data are visualized by t-SNE. Each data point represents one molecular composition and all morphological features were projected on a new 2D scatter plot. Colors represent (A) different treatment and stiffness, (B) drug responses, (C) ECM, and (D) ligand in HCC1569, A549, and PC3. Gated area represents well clustered data points based on stiffness and drug treatment (n = 60 for each microenvironment).

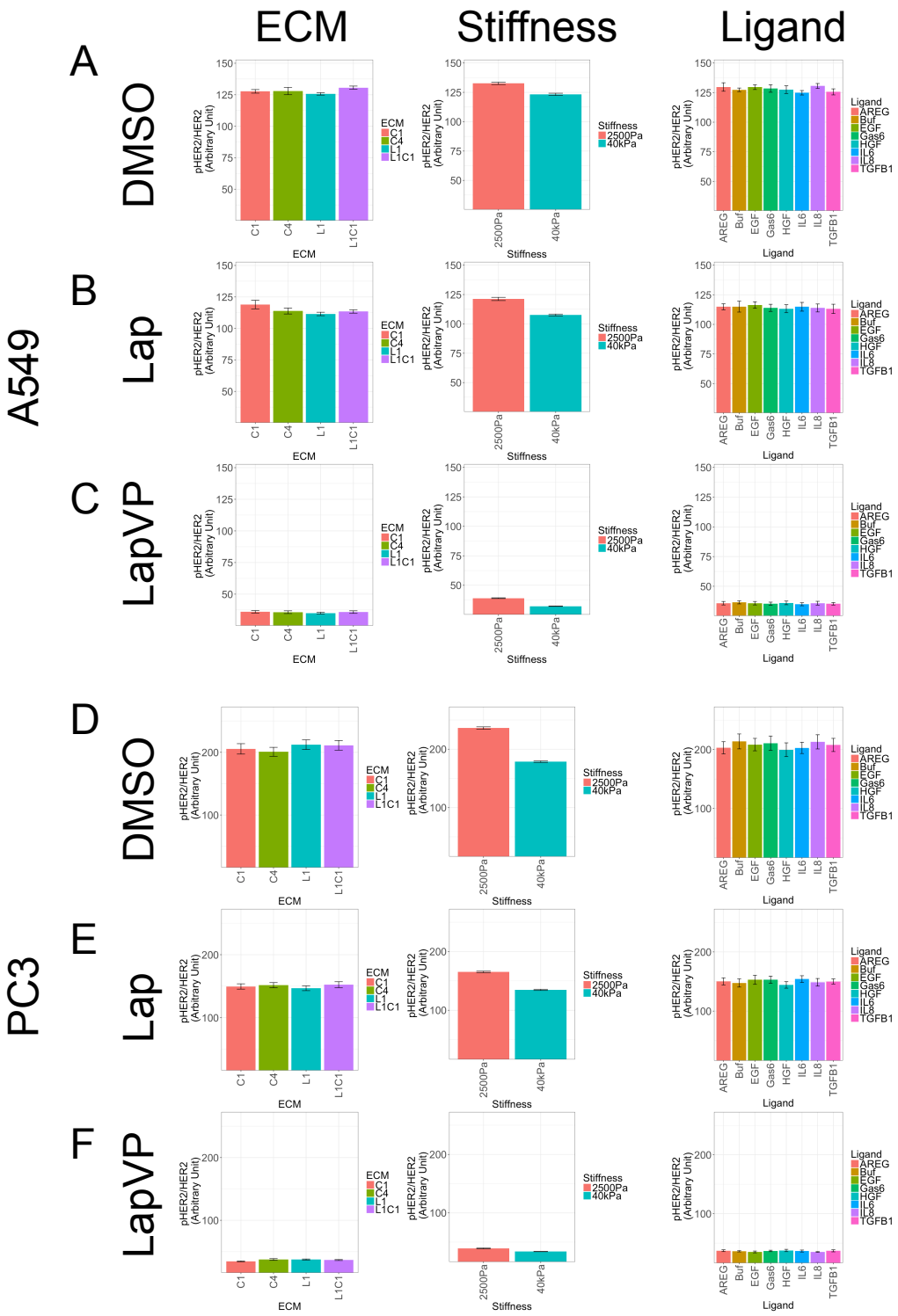

Figure S4 pHER2 and HER2 levels were not significantly modulated by microenvironment components in A549 and PC3. Related to Figure 4.

(A-F) Bar graphs represent pHER2/HER2 in (A, D) DMSO, (B, E) lapatinib, and (C, F) lapatinib with verteporfin treatment, modulated by individual component of microenvironment, including ECM, ligand, and stiffness of A549 and PC3. Data are analyzed by generalized linear model (GLM), and represented as mean ± SEM (n = 60 for each microenvironment).

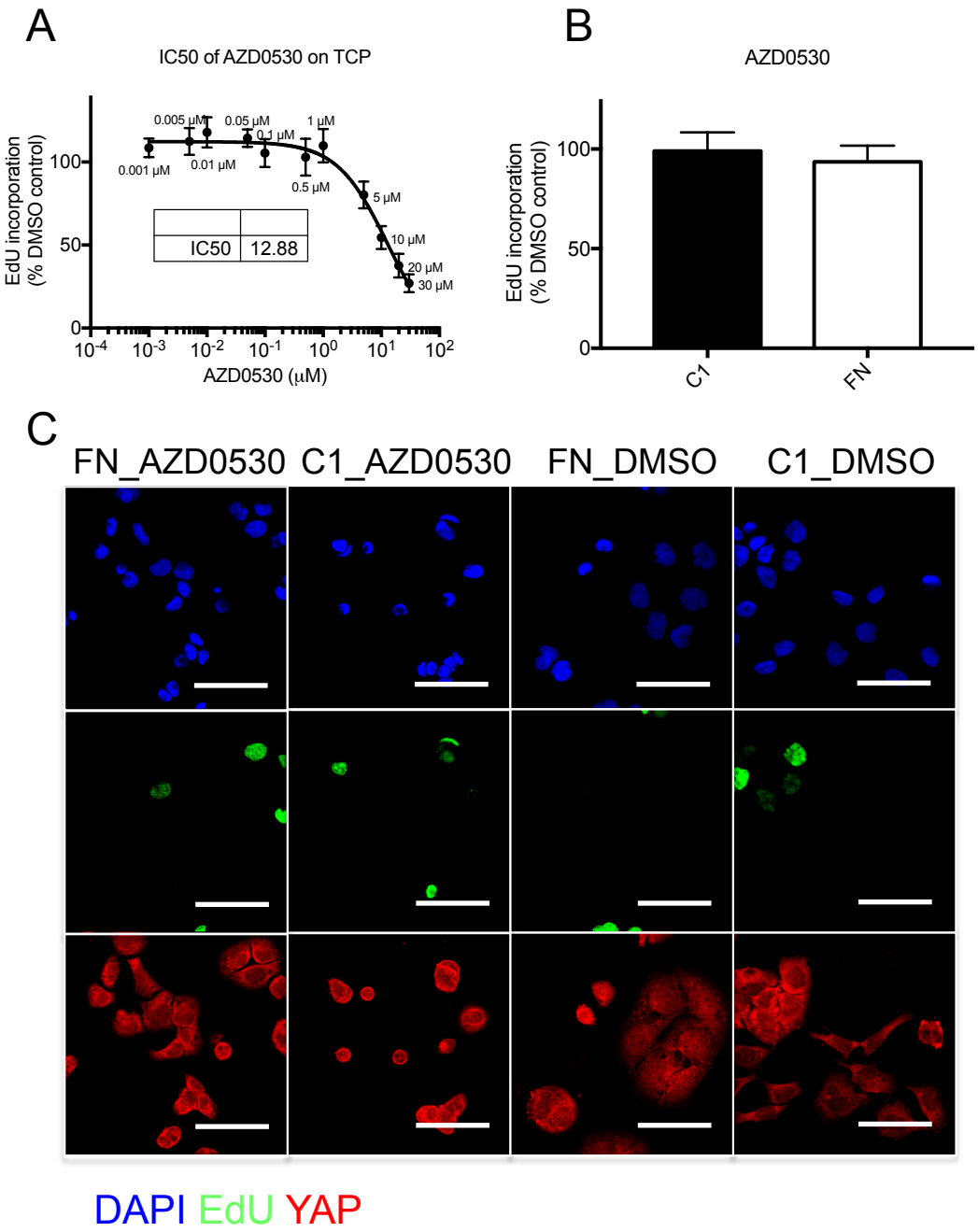

Figure S5 Src inhibitor, AZD0530, had inhibitory effect to proliferation of HCC1569 at concentration higher than 1 μM and decreased fibronectin-dependent nuclear YAP translocation. Related to Figure 5.

(A) Line graph represents the AZD0530 responses (Relative incorporation of EdU expressed as a percentage of DMSO-treated cells) of HCC1569. IC50 is calculated as 12.88 μM (n = 3, 500 cells/condition per experiment).
(B) Bar graph represents the AZD0530 response at 1 μM to HCC1569 cultured on either type I collagen- or fibronectin-coated 24-well plate. Data are represented as mean ± SEM (n = 3, 500 cells/condition per experiment).
(C) Representative IF images of DAPI (blue), EdU (green), and YAP (red) from HCC1569 cultured on type I collagen (C1) or fibronectin (FN), treated with or without AZD0530. Scale bars, 50 μm.