Supplementary Figures

ECT2 promotes lung adenocarcinoma progression through extracellular matrix dynamics and focal adhesion signaling

Figure S1

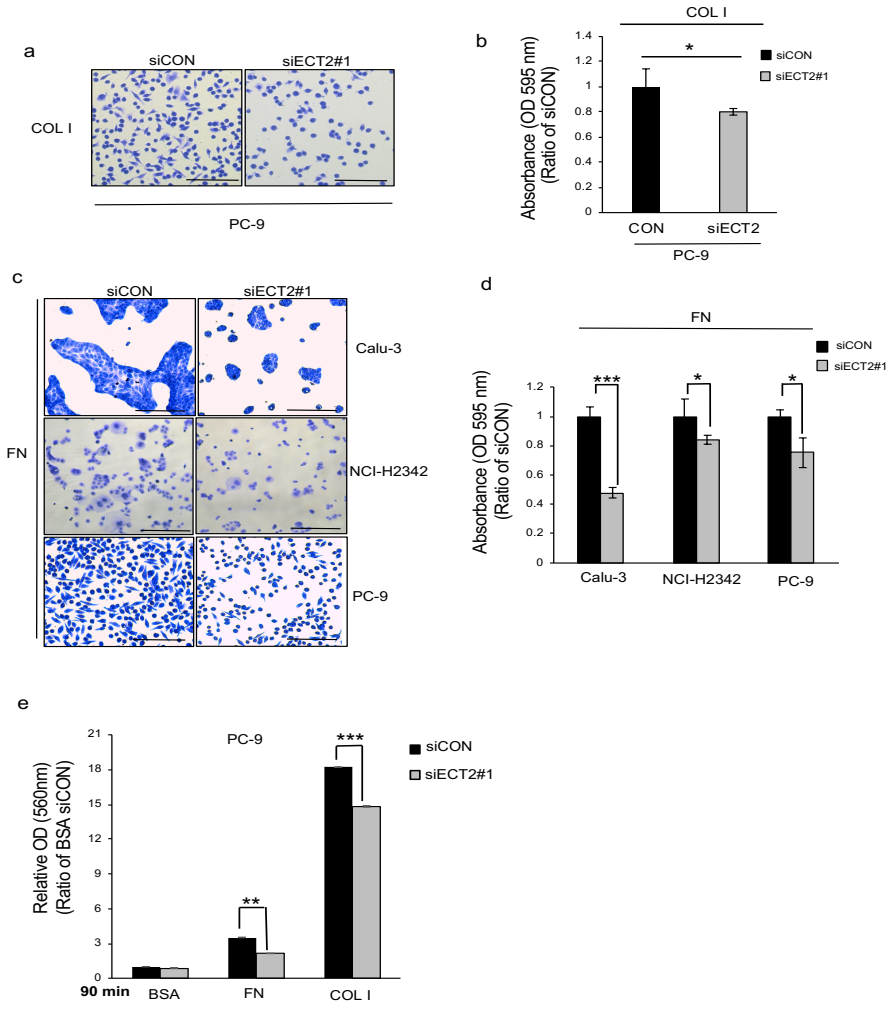

Figure S1. ECT2 suppression reduces the viability and adhesion of LAC cell lines. (a, b) PC-9 cells were transfected with siECT2#1 or siCON and seeded on COL l-coated plates. Non-attached cells were removed, and the viability of adherent cells was measured and quantified. Error bars represent mean±SD from more than four independent experiments. Scale bar 100 μm. (c, d) Calu-3, NCI-H2342, and PC-9 cells were transfected with siECT2#1 or siCON and seeded onto FN. Error bars represent mean±SD from more than four independent experiments. Scale bar 100 μm. (e) PC-9 cells were transfected with siECT2#1 or siCON for 24 h. After siRNA treatment, the cells were plated on FN-, COL l-, or 1% BSA-coated plates for 90 minutes. Error bars represent mean±SD, n = 4. * P <0.05, ** P <0.01, *** P <0.001.

Figure S2

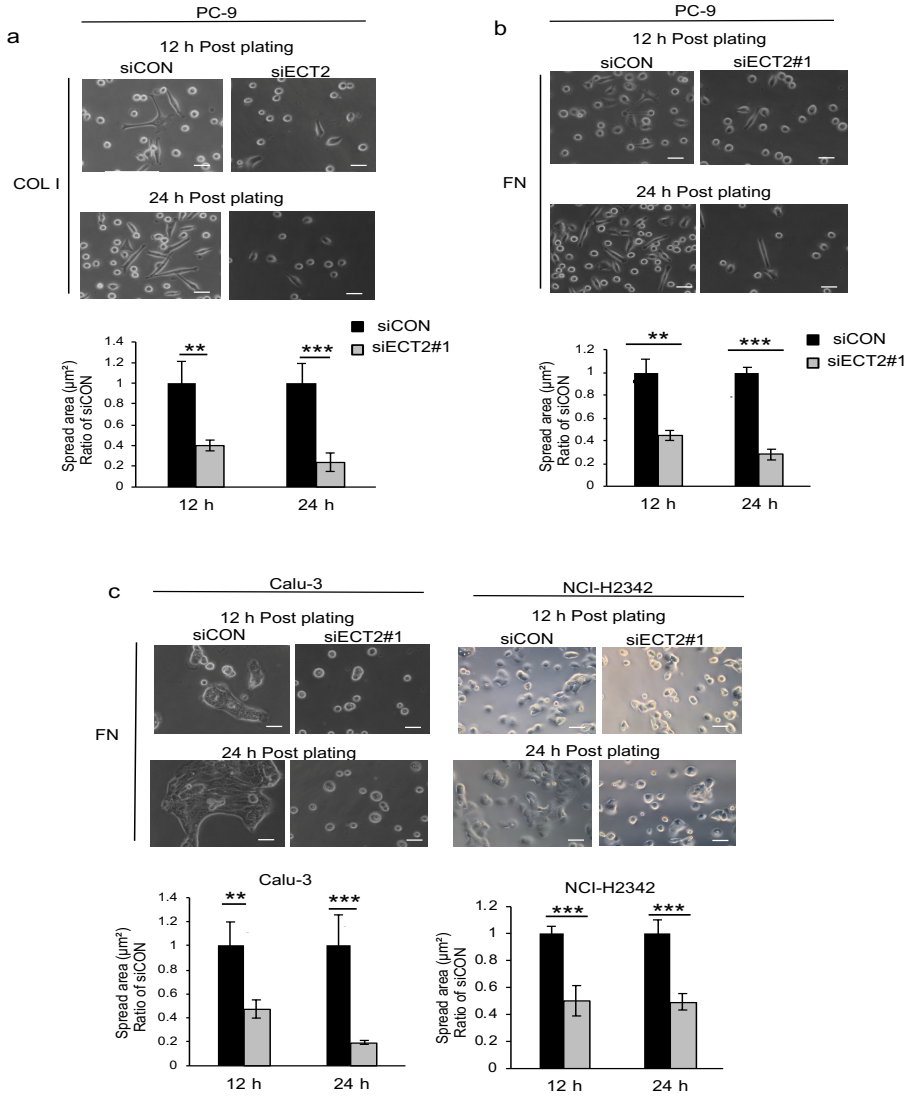

Figure S2. Suppression of ECT2 inhibits the spreading of LAC cells.

PC-9 cells transfected with siECT2#1 or siCON were spread on COL l- (a) and FN (b) -coated plates for 12 h and 24 h. The area of spread of siECT2#1- and siCON-transfected cells was analyzed and quantified using ImageJ software. Error bars represent mean±SD, n = 4. (c) Calu-3 and NCI-H2342 cells transfected with siECT2#1 or siCON were spread on FN-coated plates for 12 h and 24 h. Phase-contrast micrographs show that cells transfected with siECT2#1 exhibit delayed or absent spreading relative to control cells. The area of spread of siECT2#1- and siCON-transfected cells was analyzed and quantified using ImageJ software. Error bars represent mean±SD, n = 4, scale bar 100 μm. $^{**}P$ <0.01, $^{***}P$ <0.001.

Figure S3

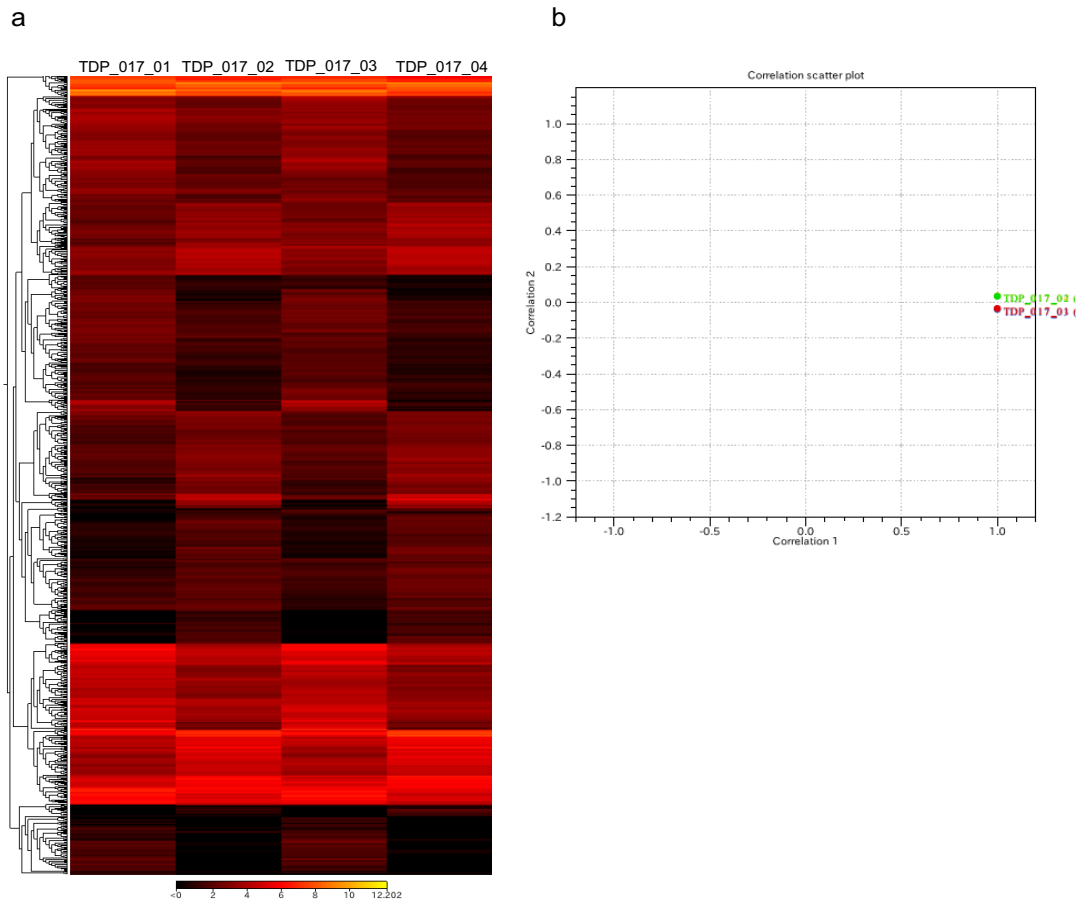

Figure S3. RNA-seq profiling of ECT2 silencing in Calu-3 cells.

Calu-3 cells were seeded on COL l-coated dishes and transfected with siECT2#1 or siCON for 48 h and 72 h. (a) Clustered heatmap shows a total of 2913 differentially expressed genes (GE) (absolute fold change and a difference of >2 fold) in Calu-3 cells at 48 h and 72 h. Data represent one sample per condition. (b) Correlation scatter plot of differentially expressed genes in Calu-3 cells transfected with siECT2#1 and siCON at 48 h and 72 h. The scatter plot indicates that similar expression patterns were observed among the differentially expressed genes in TDP_017_01 (GE) (Red) and TDP_017_03 (GE) (Blue), and TDP_017_02 (GE) (Green) and TDP_017_04 (GE) (Yellow). Abbreviations: TDP_017_01 (GE) (Red) represents siCON at 48 h, TDP_017_02 (GE) (Green) for siECT2#1 at 48 h, TDP_017_03 (GE) (Blue) for siCON at 72 h, and TDP_017_04 (GE) (Yellow) for siECT2#1 at 72 h.

Figure S4

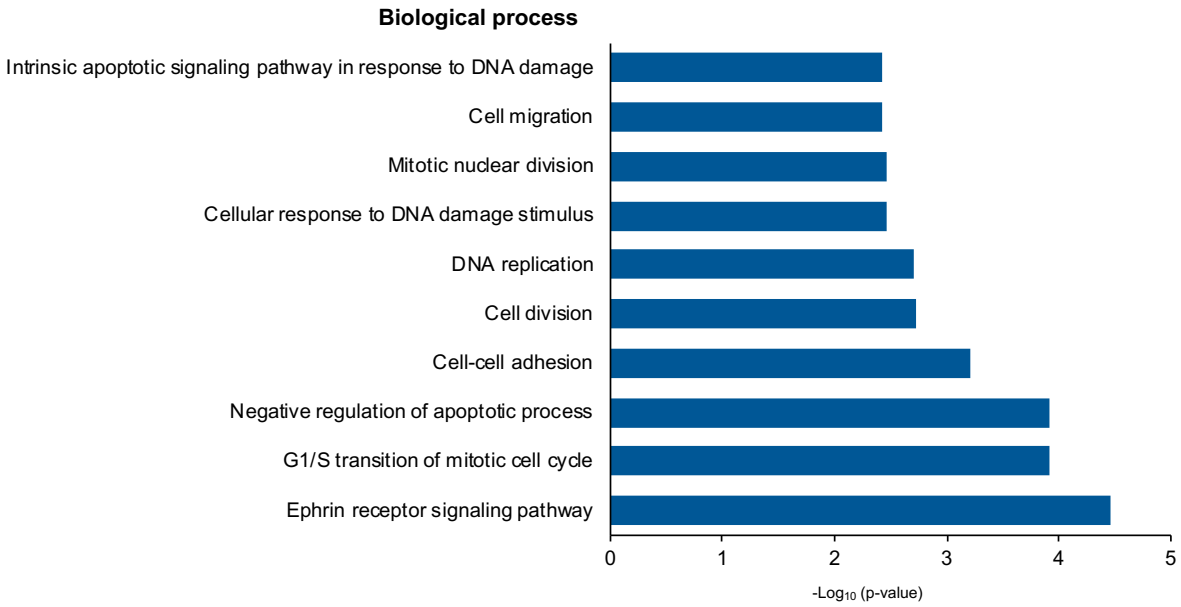

Figure S4. Functional analysis of GO biological process terms for common genes. Data representing the top 10 significantly enriched GO terms for biological processes were defined as $P < 0.05$ and more than 5 genes.

Figure S5

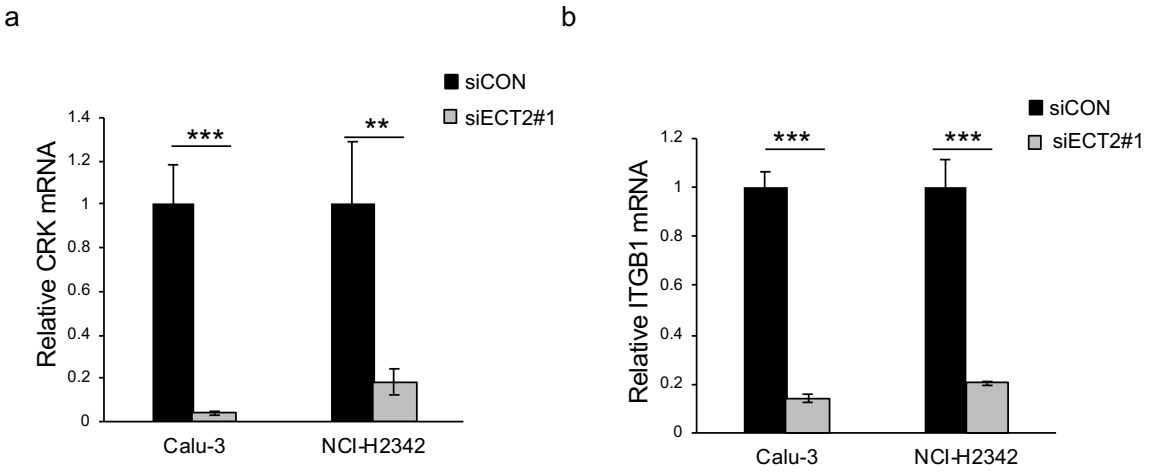

Figure S5. Confirmation of RNA-seq results for selected representative genes.

CRK (a) and ITGB1 (b) mRNA levels were determined by individual RT-PCR reactions. mRNA expression levels were assessed for both siECT2 and siCON in Calu-3 and NCI-H2342 cells. Error bars represent mean±SD, n = 4, $^{**}P < 0.01$, $^{***}P < 0.001$.

Figure S6

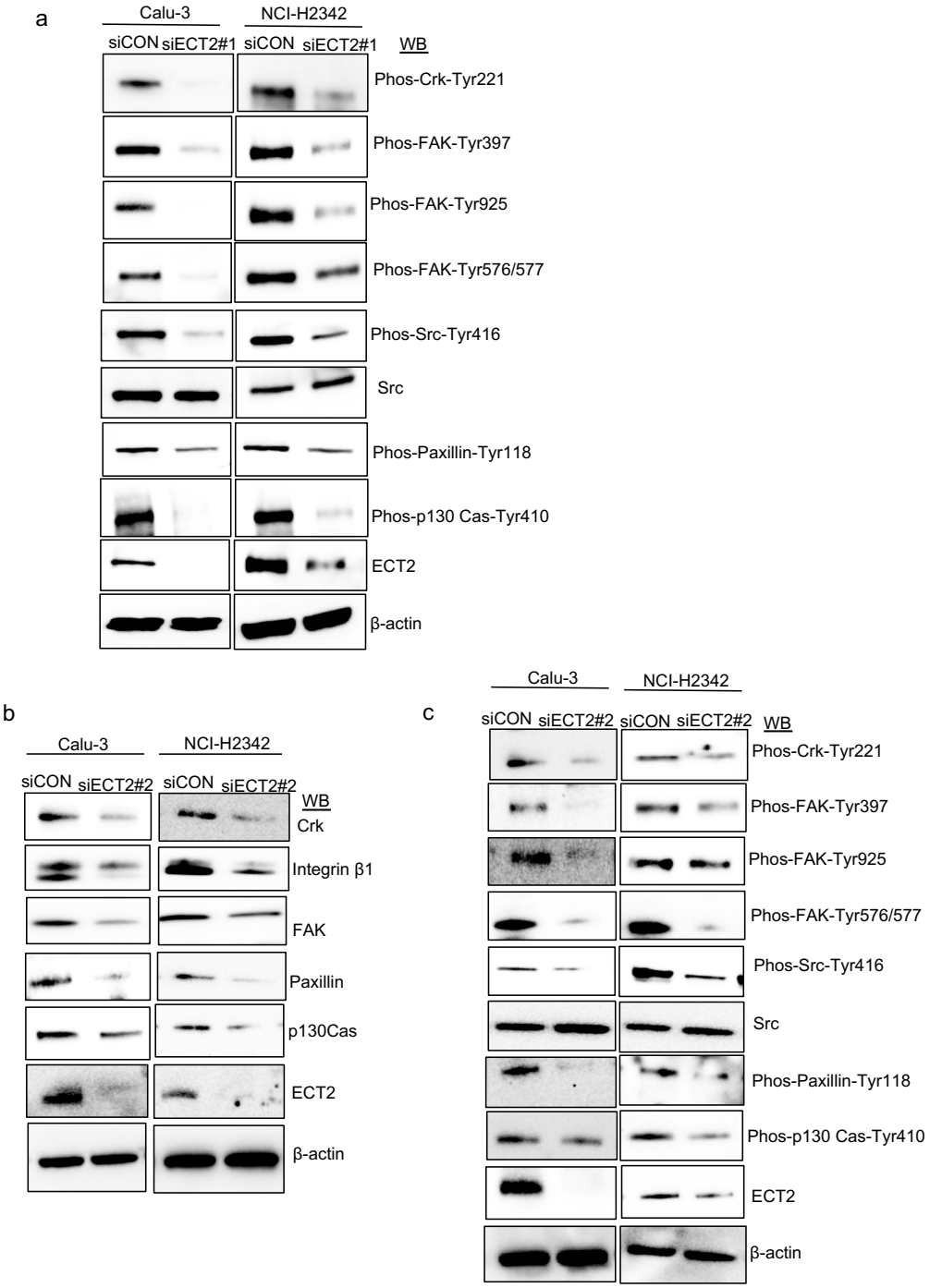

Figure S6. Effect of ECT2 knockdown on proteins involved in focal adhesion signaling. Expression of total and phosphorylated focal adhesion signaling for proteins such as Crk, FAK, integrin β1, and p130Cas and their downstream factors, including Src and paxillin, was assessed by Western blotting using Calu-3 and NCI-H2342 cells transfected with siECT2#1 (a) and siECT2#2 (b, c).

Figure S7

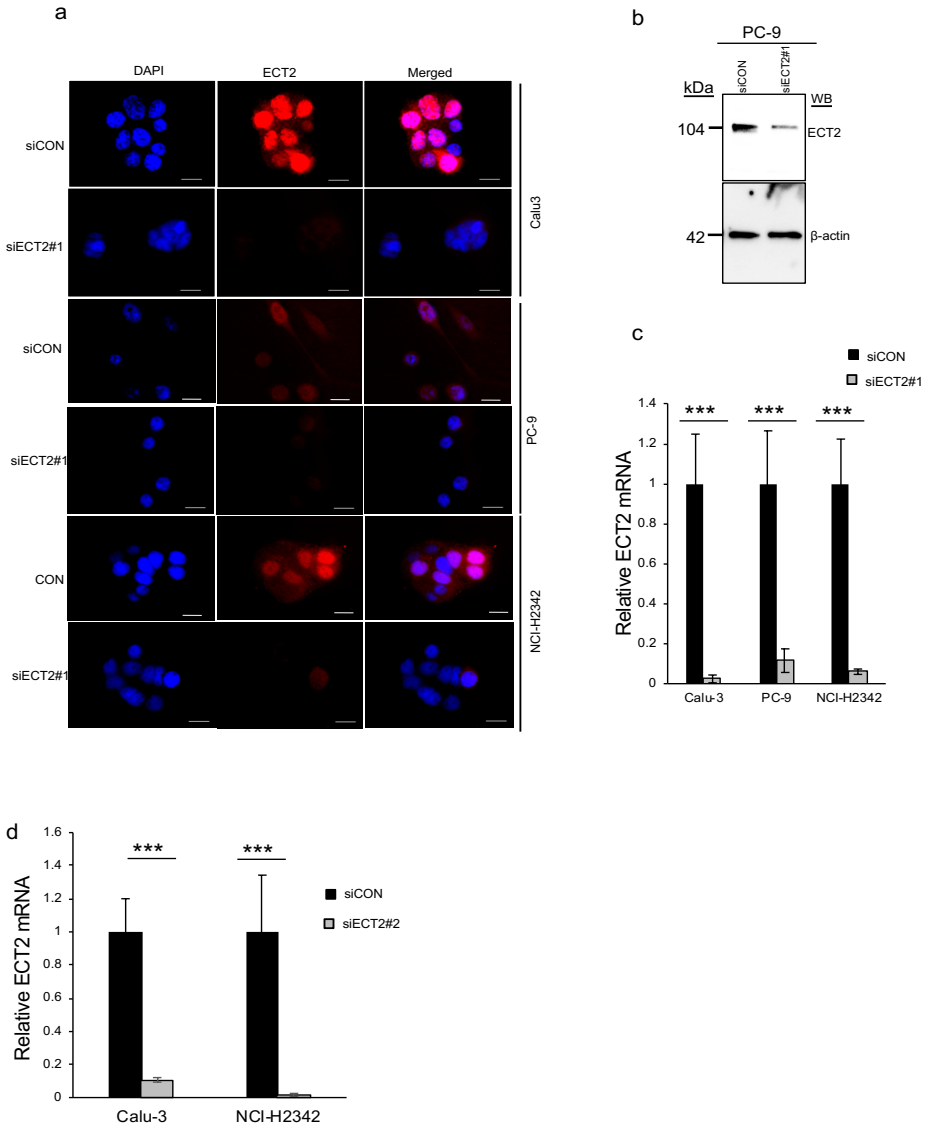

Figure S7. Validation of ECT2 silencing efficiency. (a) Representative images of Calu-3, PC-9, and NCI-H2342 cells transfected with siECT2#1 or siCON. The cells were fixed and reacted with an anti-ECT2 antibody. Nuclear DNA was counterstained with DAPI. Images were acquired using fluorescence microscopy. x1000 magnification, scale bar 10 μm. (b) Equal amounts of whole-cell lysate from PC-9 cells were transfected with siECT2#1 for 24 h. Western blots show effective ECT2 suppression by siECT2#1 in PC-9 cells. RT-PCR was used to validate the suppression of ECT2#1 (c) and ECT2#2 (d) at the mRNA level. Error bars represent mean±SD, n=4, ***P <0.001.

Figure S8

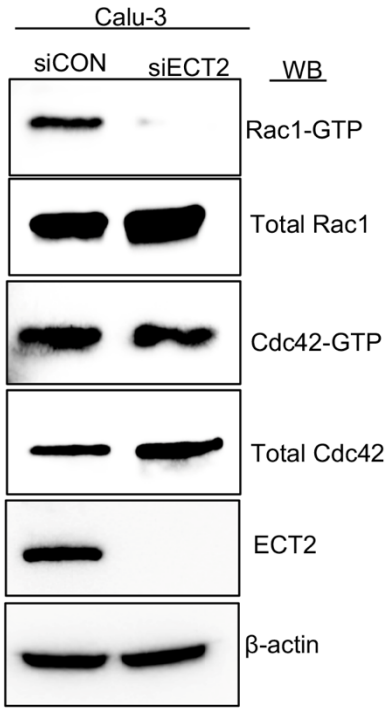

Figure S8. Effect of ECT2 suppression on Rho GTPases in LAC cells.

Total or active Rac1 (Rac1-GTP) and active Cdc42 (Cdc42-GTP) were examined using lysates from Calu-3 cells transfected with siECT2#1 or siCON for 72 h on COL l-coated dishes.