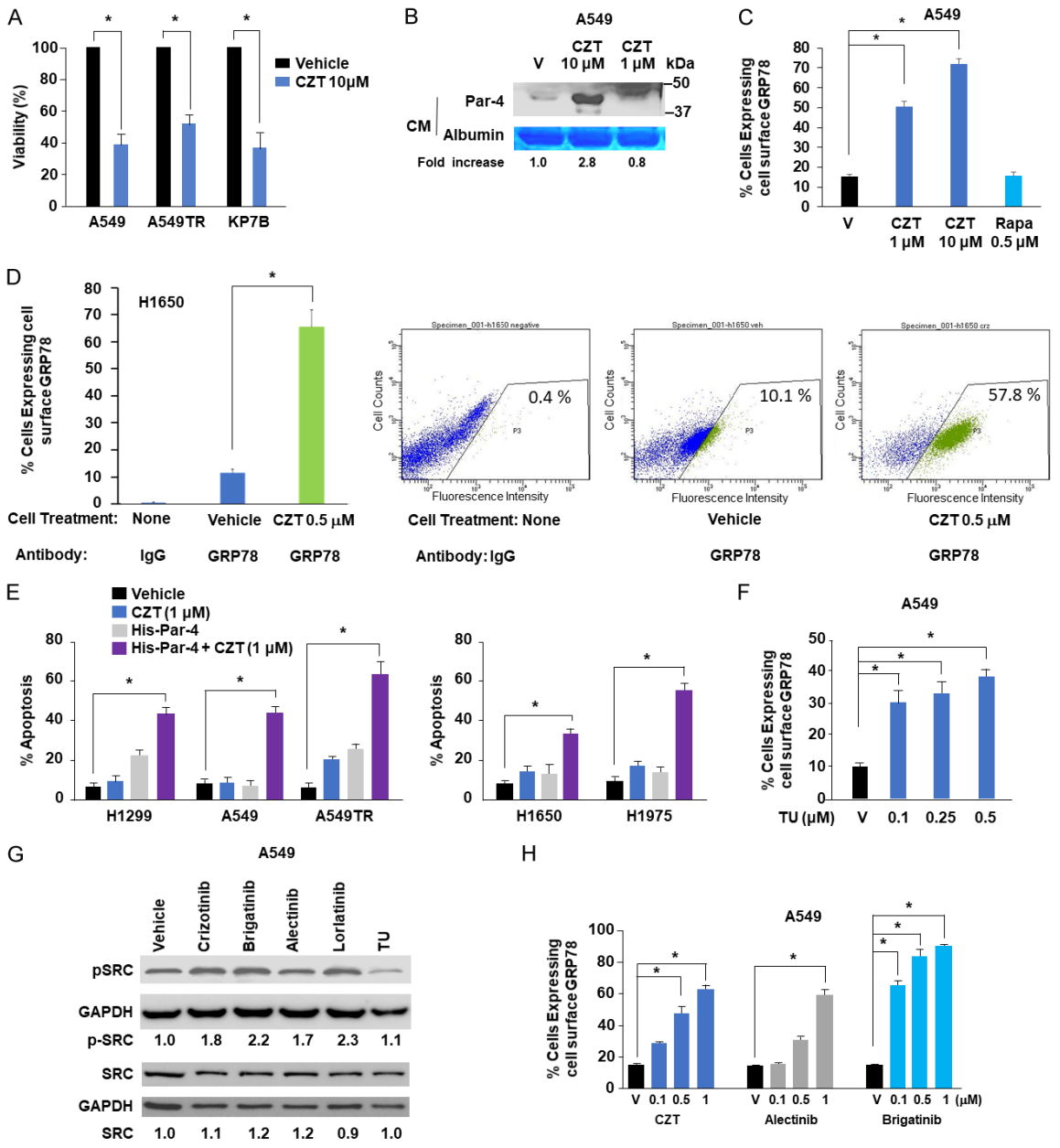

Figure S1. Validation of CZT, the top-ranking candidate drug in this screen. A. CZT at 10 μM concentrations inhibits ALK-negative lung cancer cells. A549, A549TR, and KP7B cells were treated with vehicle or CZT (10 μM) for 48 h and cell viability was determined by resazurin assays. Mean + SD is shown. *P < 0.001 by Student's t test. B. CZT at 10 μM but not 1 μM concentration induces Par-4 secretion in ALK-negative lung cancer cells. A549 cells were treated with vehicle (V) or CZT (1 or 10 μM) for 48 h, and the CM was examined for Par-4 secretion by Western blot analysis. C. CZT at 10 μM as well as 1 μM concentrations induces csGRP78 expression in ALK-negative lung cancer cells. A549 cells were treated with vehicle (V), CZT (1 or 10 μM), or autophagy-inducer rapamycin (Rapa, 0.5 μM), and csGRP78 expression was determined. Percentage of csGRP78-positive cells was calculated and Mean + SD values are shown. *P < 0.001 by Student's t test. D. CZT at 0.5 μM concentrations induces csGRP78 expression in ALK-negative lung cancer cells. H1650 cells were treated with vehicle or CZT (0.5 μM) and csGRP78 expression was determined. Right Panels present an example of the Flow Cytometry images from one of the three replicates. Each treatment was performed in triplicate to arrive at the Mean of csGRP78-positive cells + SD shown in the Left Panel. *P < 0.001 by Student's t test. E. CZT at 1 μM concentration sensitizes lung cancer cells to apoptosis by recombinant Par-4. Various lung cancer cells were treated with vehicle, recombinant Par-4 (100 nM), CZT (1 μM), and a combination of CZT (1 μM) and Par-4 (100 nM) for 24 h, and subjected to ICC analysis for active caspase-3. Apoptotic cells were scored by confocal microscopy. Mean + SD shown. *P < 0.01 by ANOVA test. F. Tunicamycin (TU) induces csGRP78 in cancer cells. Lung cancer cells were treated with the indicated concentrations of TU or vehicle (V). Unfixed cells were analyzed for csGRP78 expression. Percentage of csGRP78-positive cells was calculated and Mean + SD values are shown. *P < 0.001 by Student's t test. G. CZT and other ALK-inhibitors, but not TU, activate

SRC. A549 cells were treated with 0.5 μM amounts of various indicated drugs or vehicle for 24 h. The whole-cell lysates were subjected to Western blot analysis for pSRC, total SRC, or GAPDH, and fold increase in phosphorylation of Y419-SRC (pSRC) or SRC relative to vehicle is indicated. H. CZT and other ALK-inhibitors induce csGRP78 expression in ALK-negative lung cancer cells. A549 cells were treated with vehicle (V), or various concentrations of CZT, alectinib, or brigatinib for 24 h, and csGRP78 expression was determined in unfixed cells. Percentage of csGRP78-positive cells was calculated and Mean + SD values are shown. *P < 0.001 by Student's t test.

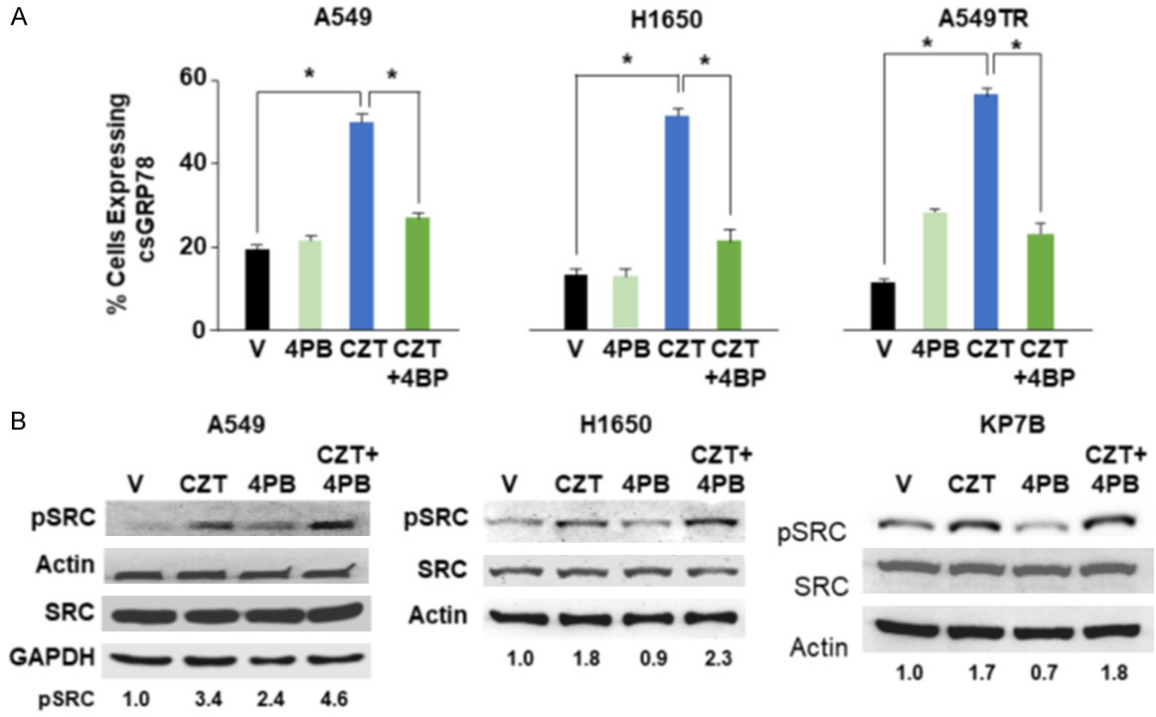

Figure S2. CZT induces SRC phosphorylation and cell surface GRP78 in an ER-stress-dependent manner. A. CZT induces cell surface GRP78 in an ER-stress-dependent manner. Lung cancer cells were treated with 1 μM CZT or vehicle (V) in the presence or absence of 4-phenylbutyrate (4PB, 5 μM) for 24 h. Unfixed cells were analyzed for cell surface GRP78 expression. Percentage of csGRP78-positive cells was calculated and Mean + SD values are shown. *P < 0.001 by Student's t test. B. CZT induces SRC activation by ER-stress-independent mechanism. Lung cancer cells were treated with 0.5 μM CZT or vehicle (V) in the presence or absence of 4PB (5 μM) for 24 h, and cell lysates were subjected to Western blot analysis.

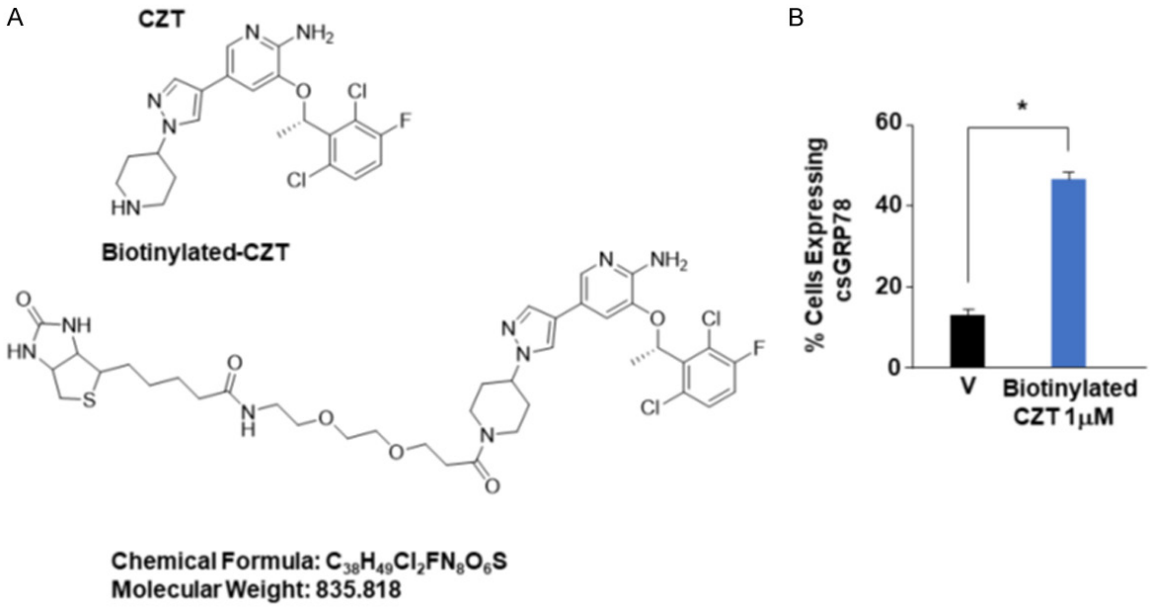

Figure S3. Biotinylated-CZT induces transport of GRP78 to the cell surface. A. Structure of biotinylated-CZT. The chemical structure of CZT and the biotinylated analog that was used for csGRP78 analysis and pull-down experiment is shown. B. Biotinylated-CZT induces csGRP78. A549 cells were treated with 1 μM biotinylated-CZT or vehicle (V) for 24 h. Unfixed cells were analyzed for cell surface GRP78 expression. Percentage of csGRP78-positive cells was calculated and Mean + SD values are shown. *$P < 0.001$ by Student's t test.

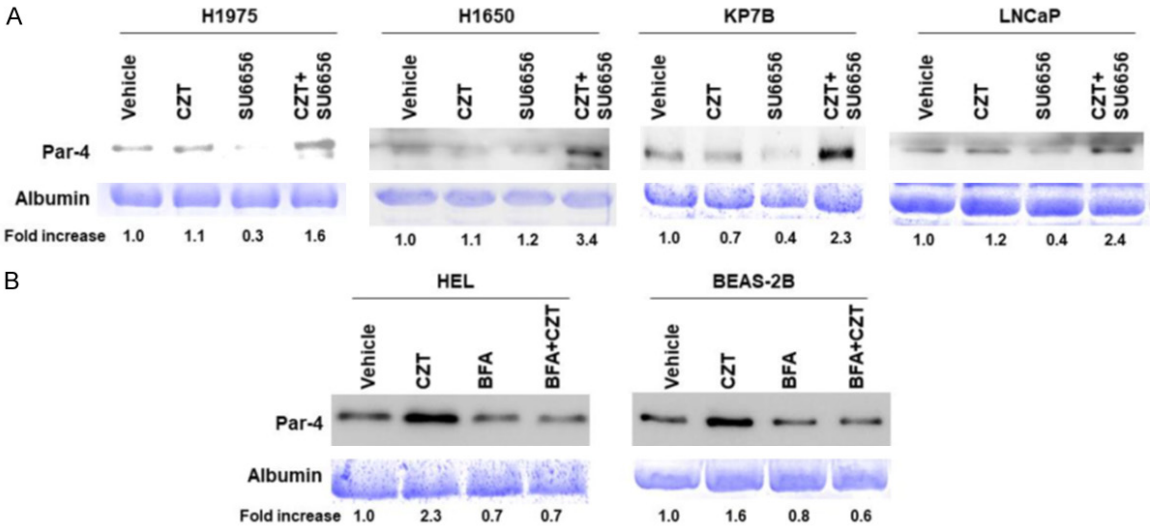

Figure S4. CZT inducible Par-4 secretion is inhibited by SRC or BFA. A. Activated SRC prevents induction of Par-4 secretion by CZT. H1650, H1975, KP7B and LNCaP cells were treated with CZT (1 μM), SU6656 (5 μM), vehicle or CZT (1 μM) plus SU6656 (5 μM) for 24 h, and the CM was examined for Par-4 secretion by western blot analysis. The levels of albumin in the CM corresponding to each sample were determined by staining the gels with Coomassie blue and used to normalize the Par-4 levels in the CM. Fold induction of Par-4 was then calculated. B. CZT induces Par-4 secretion in normal/immortalized cells by BFA-sensitive pathway. HEL or BEAS-B2 cells were treated with vehicle or CZT (500 nM) for 18 h, and then treated with BFA (1 mM) for 3 h. Aliquots of the CM from the cells were subjected to western blot analysis for Par-4. The corresponding gels were stained with Coomassie blue, and Par-4 bands were normalized relative to albumin. Fold change in Par-4 in response to the drugs relative to vehicle is shown.

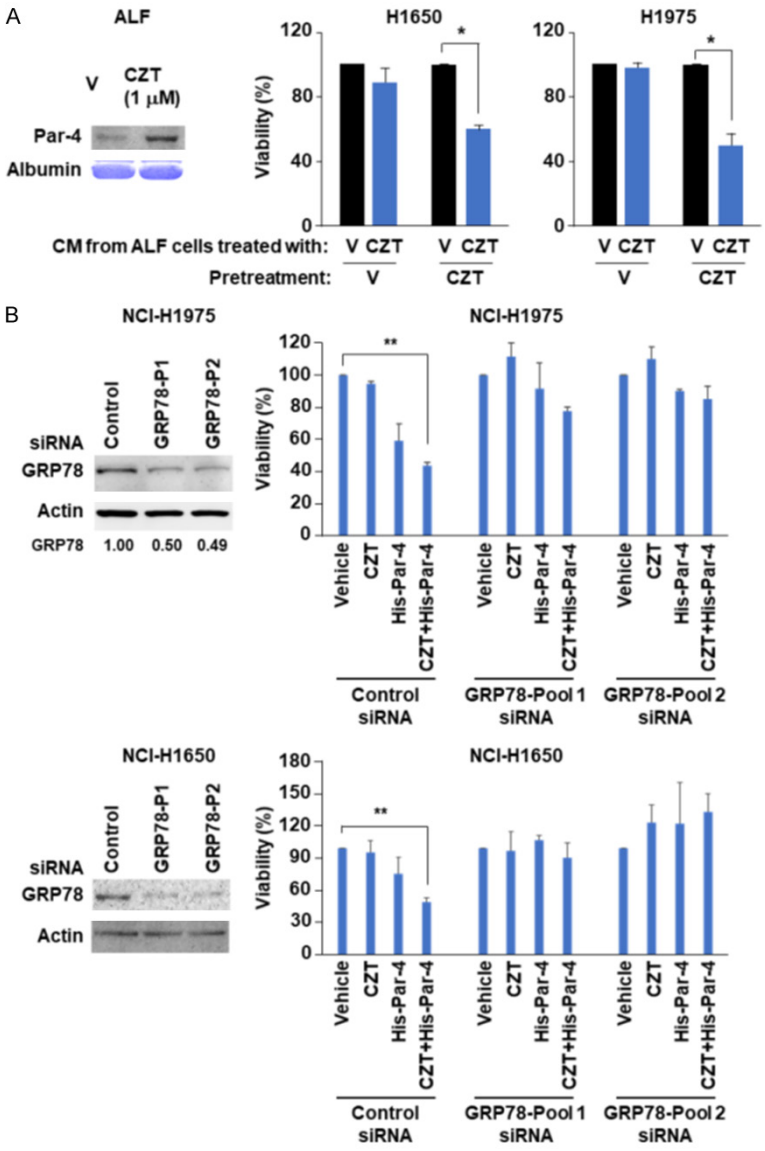

Figure S5. CZT induced Par-4 secretion from adult lung fibroblast (ALF) and recombinant Par-4 inhibits viability in CZT-treated lung cancer cells by a GRP78-dependent mechanism. A. Par-4 secreted by CZT from adult lung fibroblast (ALF) cells in the CM inhibits lung cancer cell viability. (Left Panel) ALF cells were treated with CZT (1 μM) or vehicle (V) for 24 h, and the CM was examined for Par-4 secretion by western blot analysis. (Right Panels) Lung cancer cells H1650 and H1975 were pretreated with CZT or vehicle (V) for 24 h and then after three washes, the cells were treated with the CM from ALF cells that were treated with CZT or vehicle. After 24 h, the cells were subjected to resazurin assays. Mean + SD values are shown. *$P < 0.001$ by Student's t test. B. GRP78 expression in response to CZT is essential for growth inhibition by Par-4. (Left Panels) Lung cancer cells H1650 and H1975 were treated with two different GRP78 siRNAs or control siRNA for 24 h, and the cells were examined for knockdown of GRP78 expression by western blot analysis. (Right Panels) After knockdown of GRP78 in the H1650 and H1975 lung cancer cells, the cells were treated with CZT or vehicle for 24 h in the presence or absence of recombinant Par-4 (His-Par-4, 100 nM). After 24 h, the cells were subjected to resazurin assays. Mean + SD values are shown. *$P < 0.001$ by Student's t test.

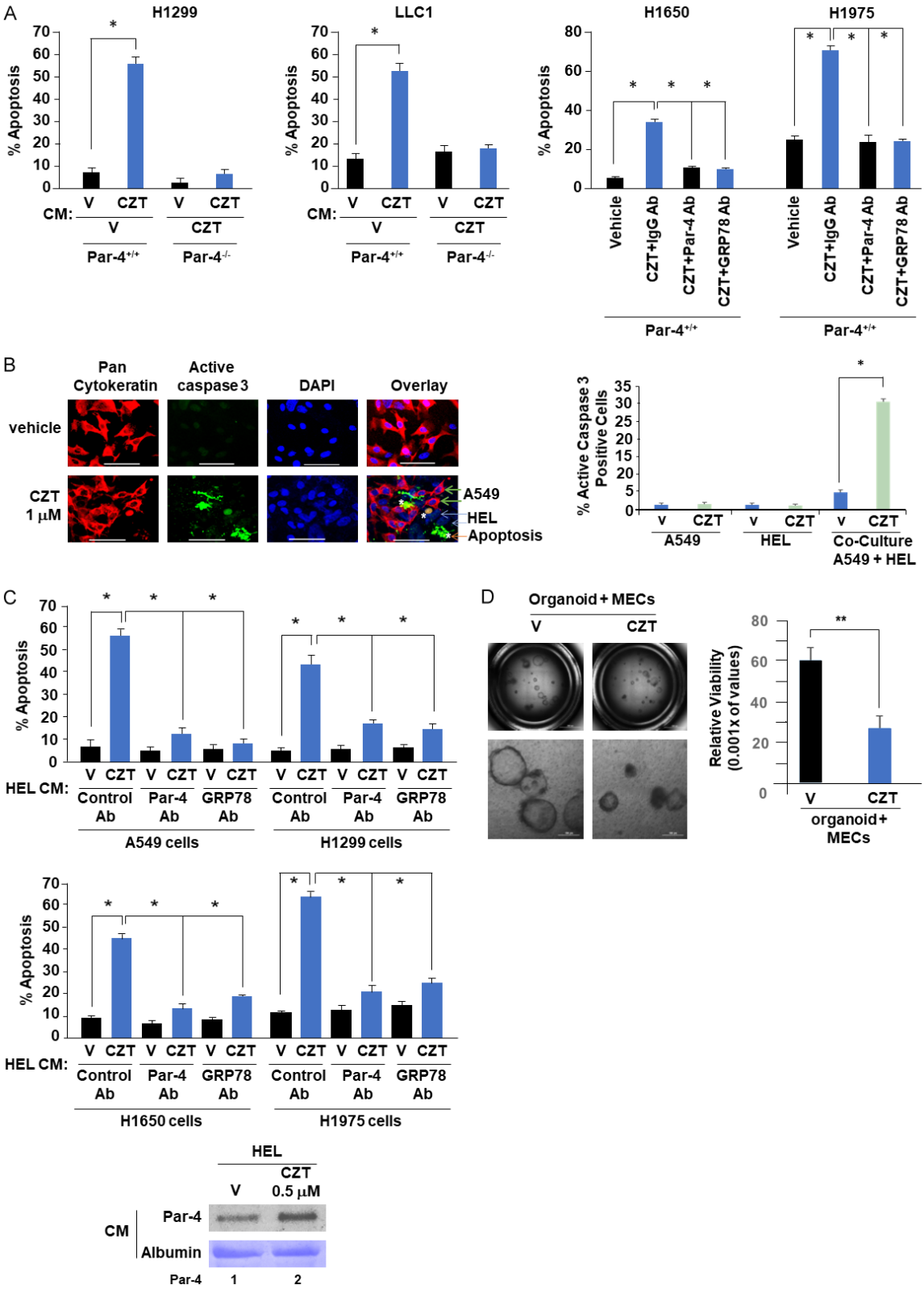

Figure S6. CZT induced Par-4 secretion in the CM of normal cells causes apoptosis in CZT-treated lung cancer cells by a Par-4- and GRP78-dependent mechanism. A. Par-4 secreted in CM of Par-4$^{+/+}$ MEFs but not Par-4$^{-/-}$ MEFs treated with CZT induces apoptosis of lung cancer cells. Par-4$^{+/+}$ or Par-4$^{-/-}$ MEFs were treated with vehicle (V) or CZT (500 nM) for 24 h. The CM was directly transferred to the indicated lung cancer cells pretreated with CZT (Left Pan-

els), or the CM was preincubated with Par-4 antibody, GRP78 antibody or IgG control antibody before adding it to the lung cancer cells (Right Panel). After 24 h, the lung cancer cells were subjected to ICC analysis for active caspase-3. Apoptotic cells were scored by confocal microscopy. Mean + SD shown. *$P < 0.01$ by Student's t test. Expression of Par-4 in the CM of the Par-$4^{+/+}$ or Par-$4^{-/-}$ MEFs was examined by western blot analysis as described in **Figure 4A**. B. CZT inhibits lung cancer cells cocultured with normal/immortalized lung fibroblast cells. Human lung cancer cells A549 were co-cultured with human normal lung fibroblast cells HEL, or grown separately, and treated with CZT (1 μM) or vehicle (V) for 48 h. The cells were then subjected to ICC analysis with the pan-cytokeratin antibody (red fluorescence) to detect A549 epithelial cells, active-caspase 3 antibody (green fluorescence) to detect apoptotic cells and DAPI (blue) to stain the nucleus. The cells were imaged (Left Panels), and active caspase 3-positive cells and pan-cytokeratin-positive cells were scored. White asterisks indicate apoptotic cells. Percent apoptotic cells (active caspase 3-positive cells) are expressed relative to the total number of pan-cytokeratin-positive cells (Right Panel). Mean + SD values are shown. *$P < 0.001$ by Student's t test. C. Par-4 secreted in CM of HEL cells treated with CZT induces apoptosis of lung cancer cells. (Top Two Panels) HEL cells were treated with vehicle (V) or CZT (500 nM) for 24 h. The CM from HEL cells was incubated with control IgG antibody (Ab), Par-4 Ab, or GRP78 Ab and added to the indicated lung cancer cells. After 24 h, the cancer cells were subjected to ICC analysis for active caspase-3. Apoptotic cells were scored by confocal microscopy. Mean + SD shown. *$P < 0.01$ by Student's t test. (Bottom Panel) Expression of Par-4 in the CM was examined by Western blot analysis. D. CZT inhibits organoid growth. To determine whether CZT inhibits the growth of lung cancer organoids, we prepared organoids from the bronchiolar mutant-EGFR (L858R; T790M) lung bronchiolar tumor cells (2,000 cells) obtained from lung tumors that do not express activated ALK, MET, or ROS1 in genetically engineered mice in the presence of mouse endothelial cells (MECs) (100,000 cells), and treated them with CZT (500 nM) or vehicle (V) at day 7 of culture. EGFR-T790M:L858R mutant murine lung cancer organoids were developed and propagated as previously described [Chen F, Liu J, Flight RM, Naughton KJ, Lukyanchuk A, Edgin AR, Song X, Zhang H, Wong KK, Moseley HNB, Wang C, and Brainson CF. Cellular origins of EGFR-driven lung cancer cells determine sensitivity to therapy. Advanced Science 2021; 8(22):2101999]. Growth of the organoids was analyzed 7 days after the treatments. Organoid growth was imaged (Left Panel) and quantified (Right Panel). n=3 wells per treatment. Scale bars are 2000 μm (Top Panels) and 300 μm (Bottom Panels). Mean + SD values are shown. **$P = 0.002$ by Student's t test.

Table S1. Lung cancer and normal/immortalized lung cell lines in this study

Cell Line	Description Driver Mutation/Tumor Suppressor Loss	Source/Ref
Human lung cancer cells		
A549	NSCLC K-Ras (G12S), CDKNA2del, LKB1del	ATCC
A549TR	Taxane resistant derivative of A549 cells	[1]
H460	NSCLC K-Ras (Q61H), PI3KCA(E545K), CDKNA2del	ATCC
H1975	NSCLC EGFR (L858R,T790M), PI3KCA (G118D), p53 mutant	ATCC
H2030	NSCLC K-Ras (G12C), p53 mutant	ATCC
H1650	NSCLC EGFR (E746X), p53del, CDKN2Adel	ATCC
H1299	NSCLC N-Ras (Q61K), p53del	ATCC
H2009	NSCLC K-Ras (G12A), p53 mutant	ATCC
Normal/Immortalized human lung cells		
BEAS-2B	lung epithelial cells	ATCC
HEL	lung epithelial cells	ATCC
Mouse tumor cells		
KP7B	NSCLC K-Ras (G12D), p53del	[1]
LLC1	NSCLC K-Ras (G12S)	ATCC
Normal mouse cells		
C57BL/6 Par-4$^{+/+}$	MEFs Wild type	[2]
C57BL/6 Par-4$^{-/-}$	MEFs Par-4$^{-/-}$	[2]
C57BL/6 Par-4$^{+/+}$	ALF Wild type	[2]

MEFs, mouse embryonic fibroblasts; ALF, mouse adult lung fibroblasts.

References

[1] Hebbar N, Burikhanov R, Shukla N, Qiu S, Zhao Y, Elenitoba-Johnson KSJ, et al. A naturally generated decoy of the prostate apoptosis response-4 protein overcomes therapy resistance in tumors. Cancer Res 2017; 77: 4039-50.

[2] Araujo N, Sledziona J, Noothi SK, Burikhanov R, Hebbar N, Ganguly S, et al. Tumor suppressor Par-4 regulates complement factor C3 and obesity. Front Oncol 2022; 12: 860446.