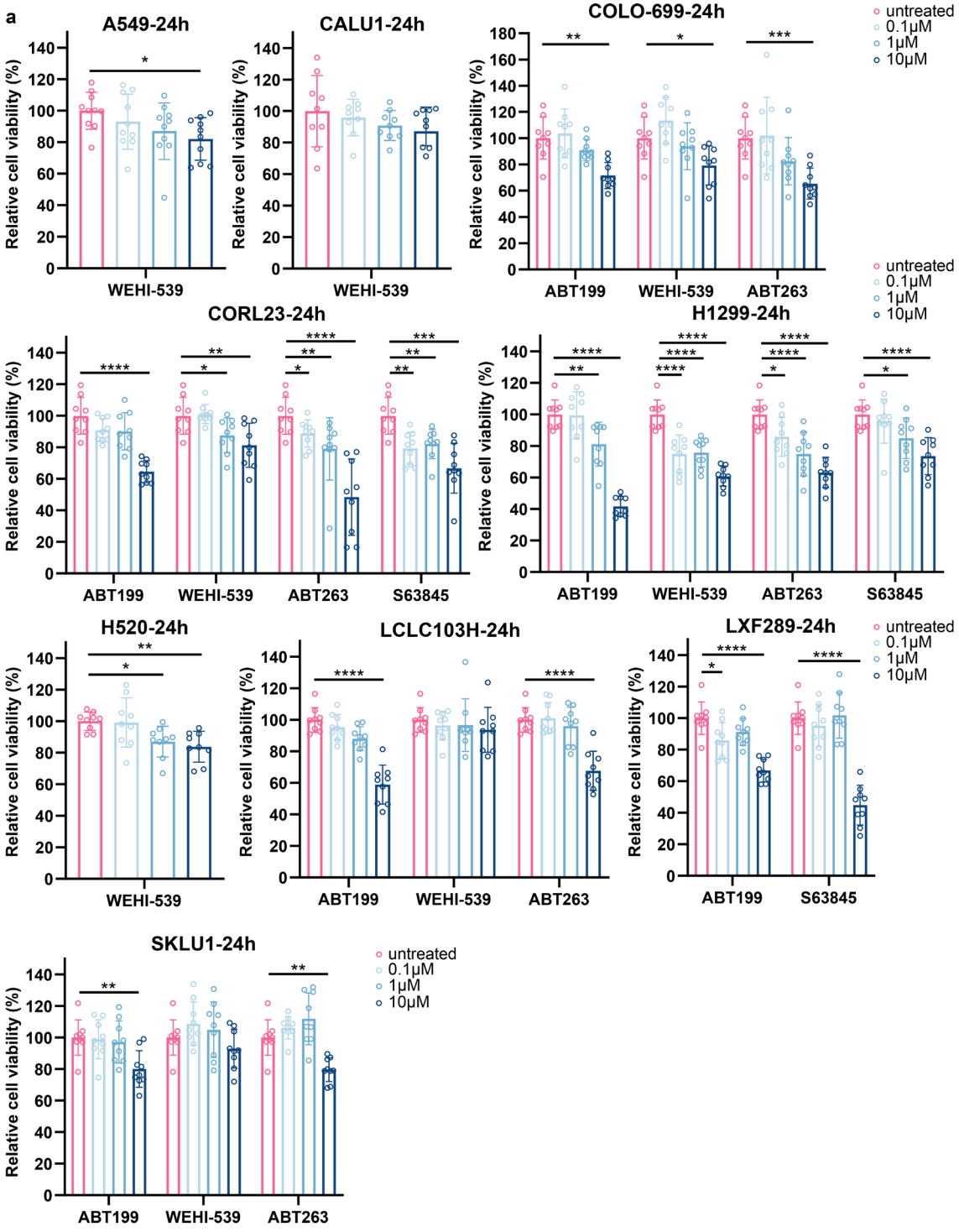
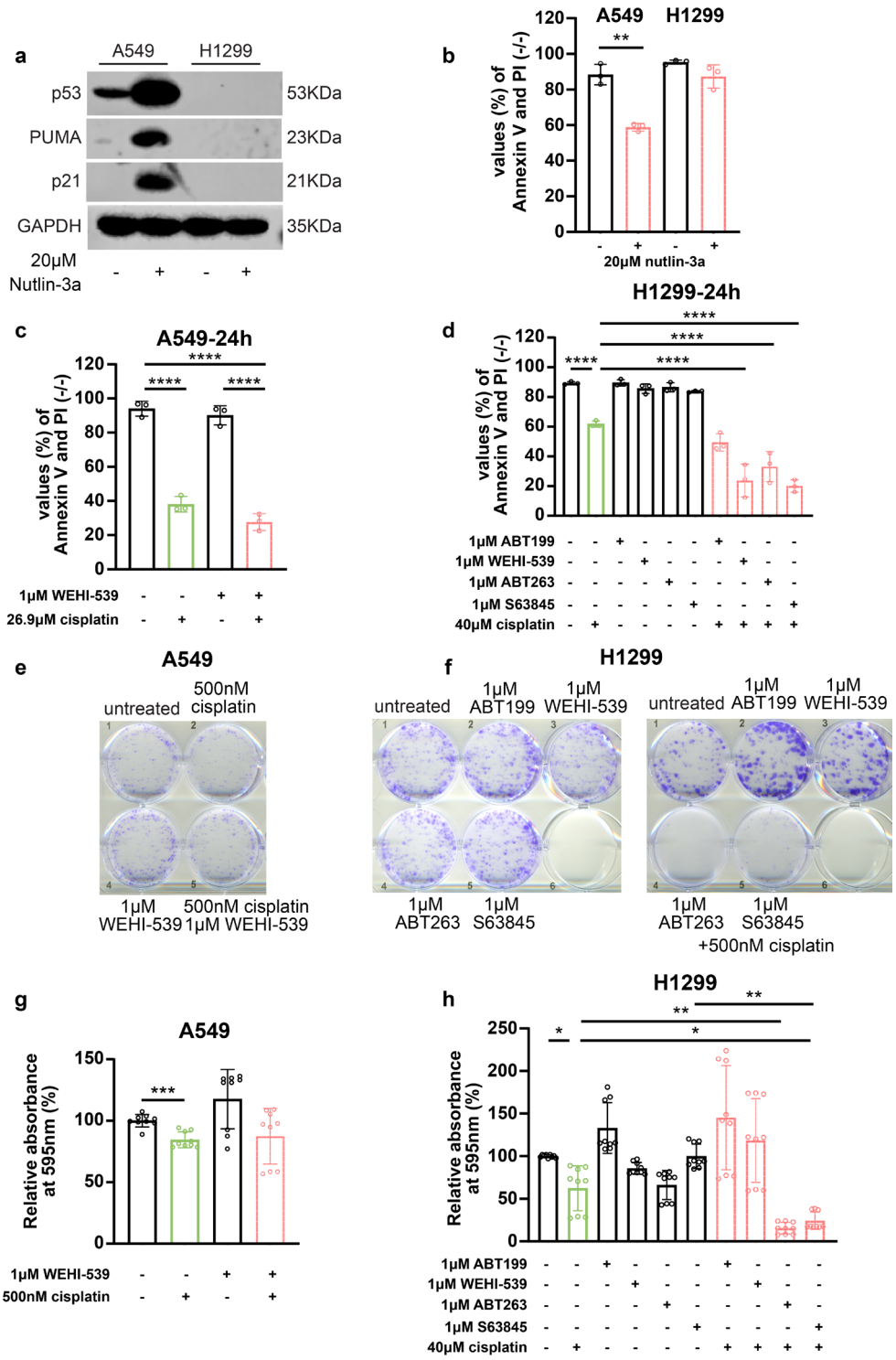
ONLINE SUPPLEMENTARY MATERIAL

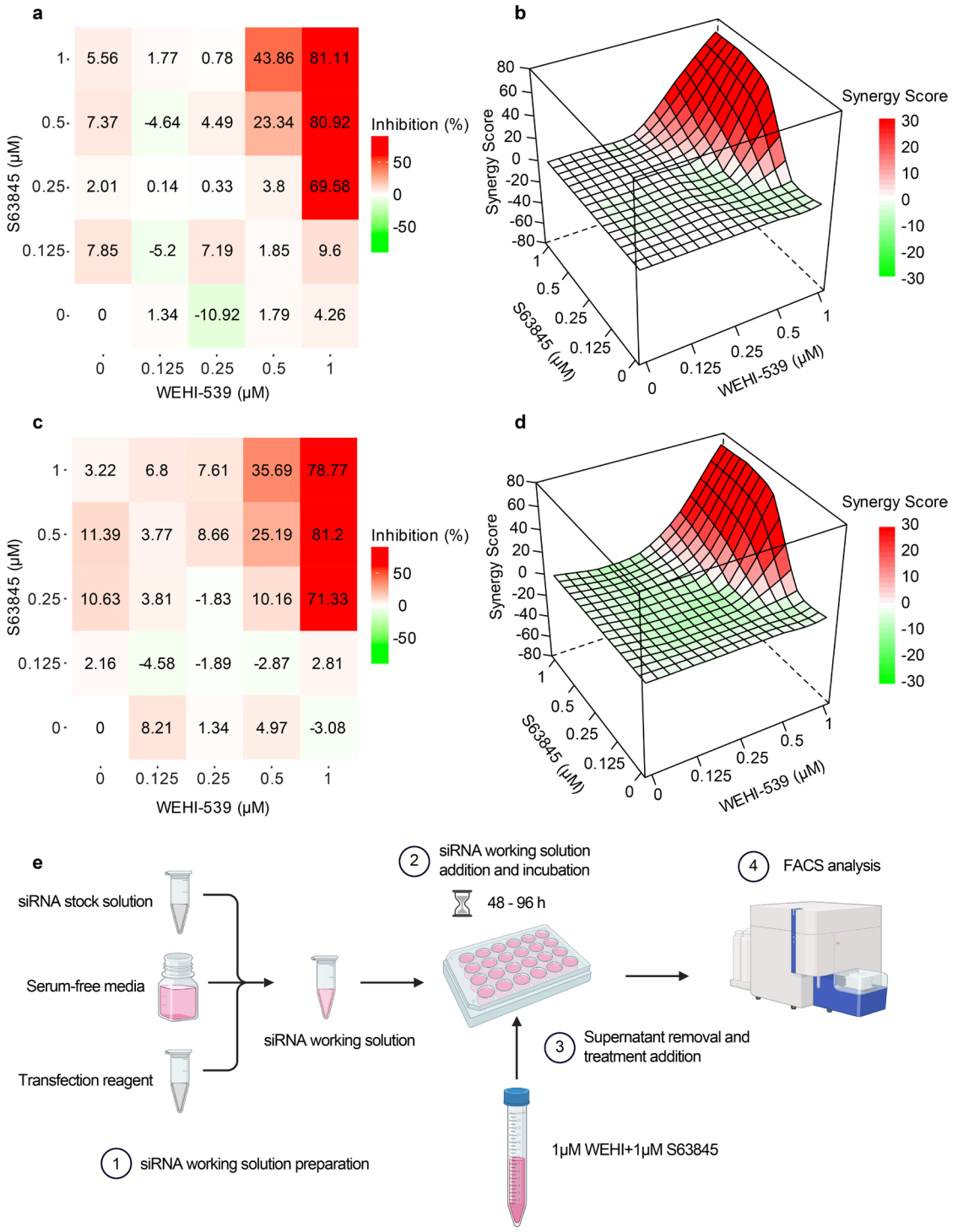
Wu. et al., Dual targeting of BCL-XL and MCL-1 exposes a rapid and exploitable apoptotic vulnerability in non-small cell lung cancer



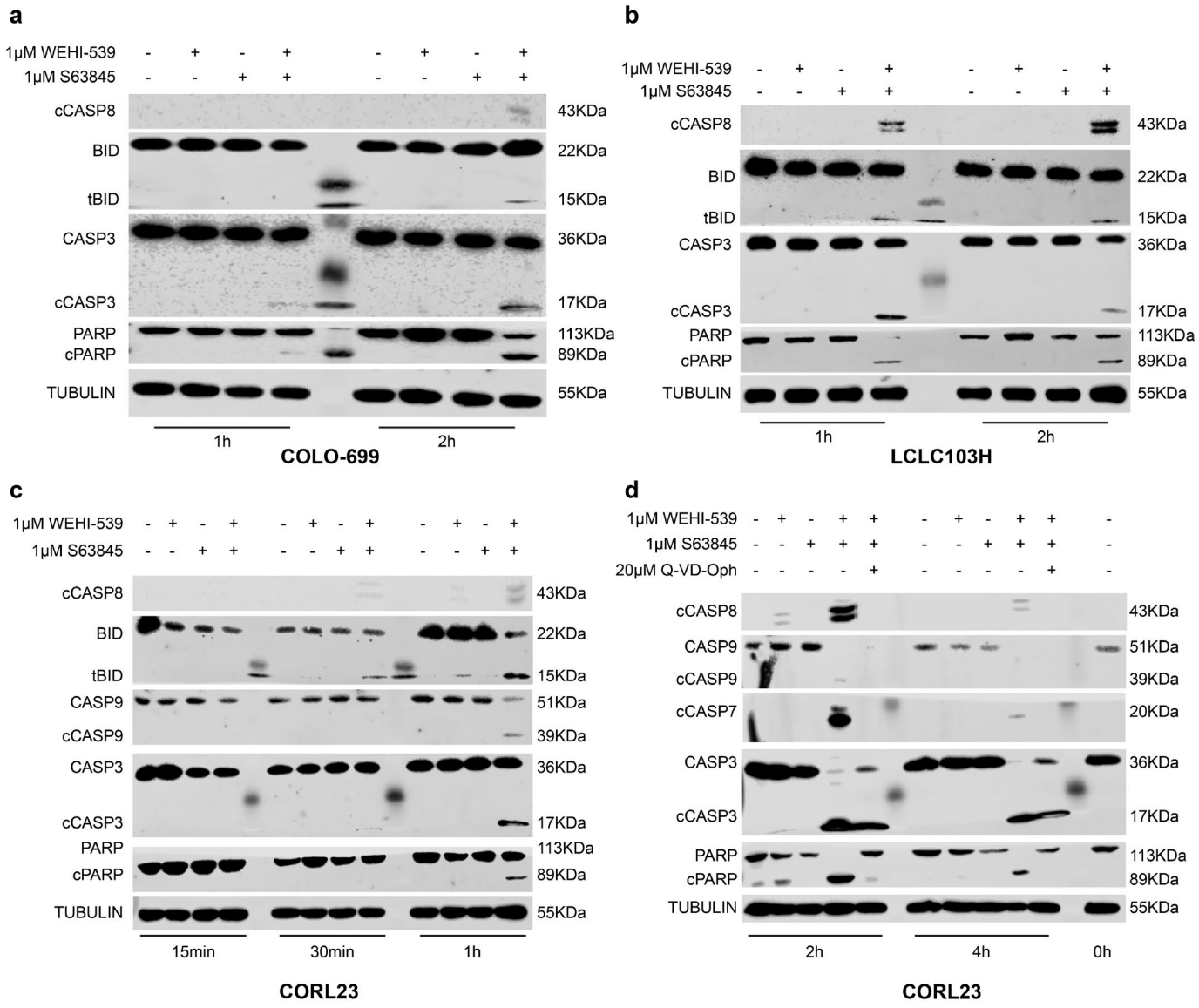
Supplementary Figure S1. PrestoBlue viability assay in NSCLC cell lines. a. BH3 mimetic screening in different NSCLC lines based on western blot results (Fig. 1a) (n = 3, biological replicates with 3 technical replicates, each). Quantitative data were presented as mean ± SEM. Statistical analysis was performed by one-way ANOVA for A549, CALU1 and H520, and two-way ANOVA for the other cell lines. * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



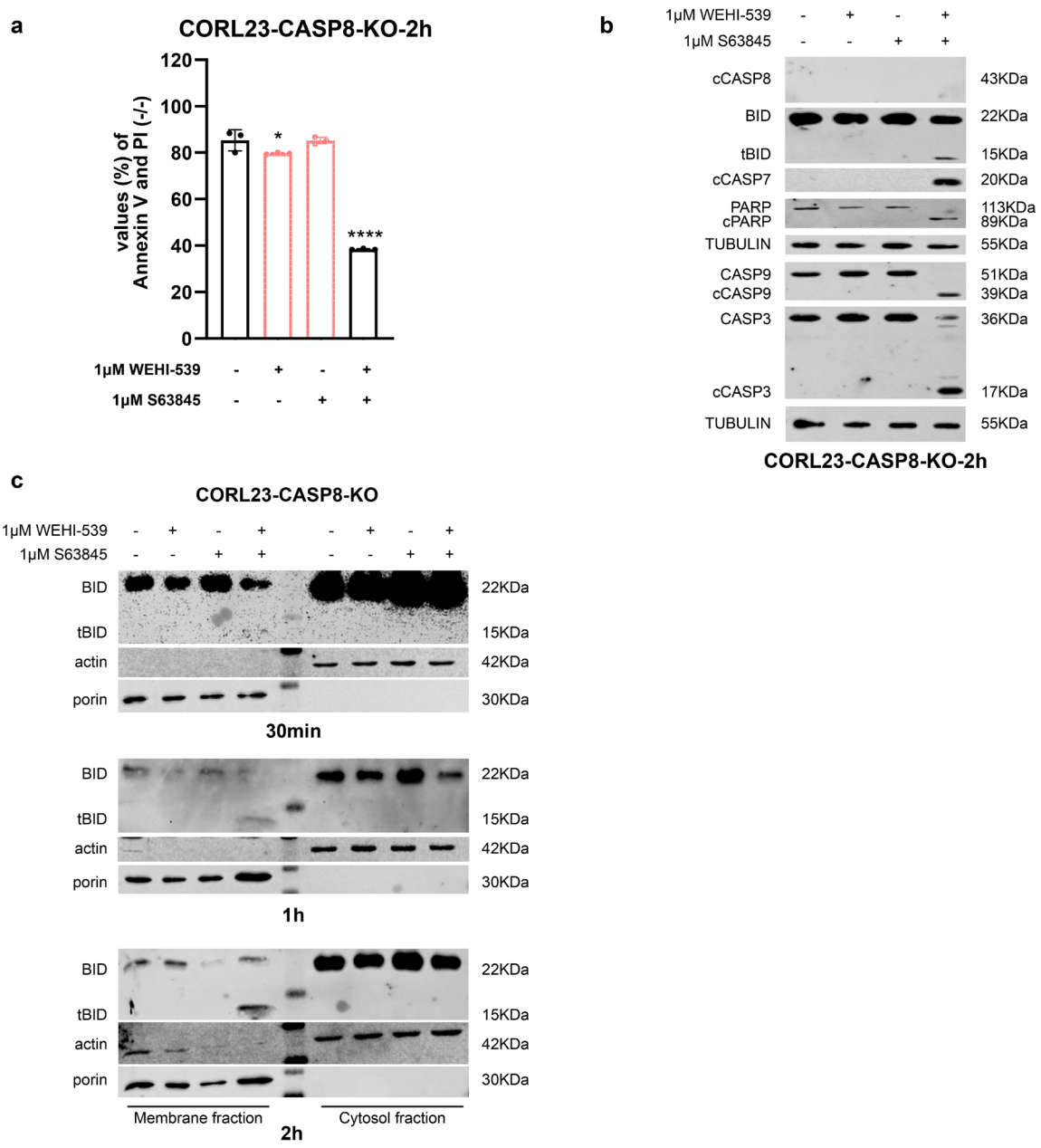
Supplementary Figure S2. Effects upon combination of BH3 mimetics with cisplatin in A549 and H1299 cells. a-b. Verification of p53 status of A549 and H1299 cells upon 20 μM nutlin-3a treatment for 48 h by western blotting (a) and flow cytometry (b) ($n = 3$, biological replicates) by. **c-d.** Evaluation of cell viability by flow cytometry (AnV/PI exclusion) using cisplatin combined with BH3 mimetics in A549 (c) and H1299 (d) cells for 24 hours ($n = 3$, biological replicates). **e-h.** Colony formation assay in A549 (e, g) and H1299 (f, h) cells upon combined treatment with cisplatin and indicated BH3 mimetics ($n = 3$, biological replicates per group). Figure **a** is representative of 2 independent repetitions. Quantitative data were presented as mean ± SEM, statistical analysis was performed by one-way ANOVA for **b**, **c**, **d**, **g**, **h**. * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



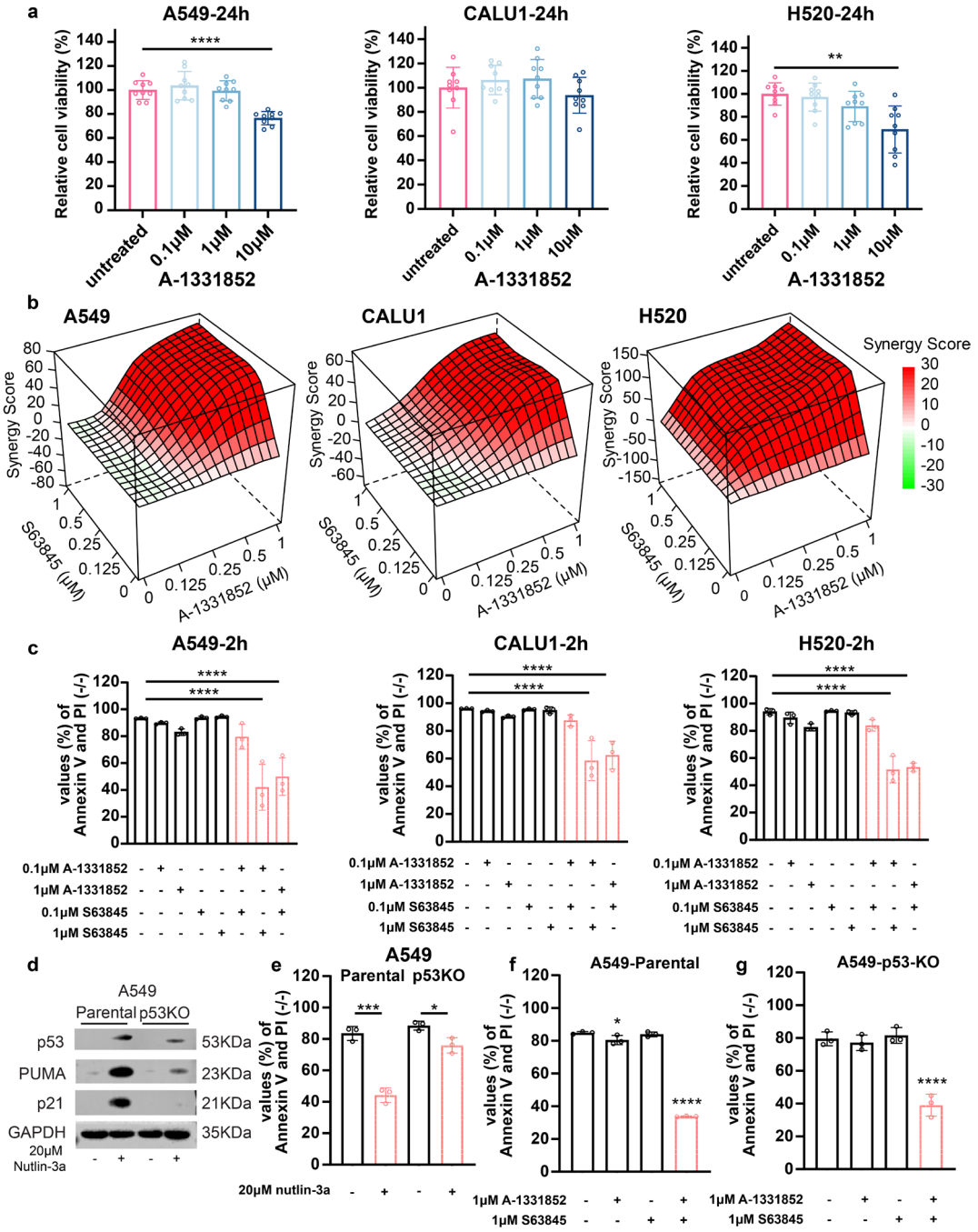
Supplementary Figure S3. Two independent biological repetitions of drug synergy analysis using SynergyFinder and workflow for esiRNA. a-d. H1299 cells were treated with the BCL-XL inhibitor WEHI-539 and the MCL-1 inhibitor S63845 at different concentrations for 24 hours. **e.** Workflow (image created by BioRender) for adding BH3 mimetics combinations after esiRNA-mediated gene knockdown followed by flow cytometric analysis. Heatmaps (**a, c**) (n = 3, biological replicates with 3 technical replicates, each) and 3D drug synergy score plots (**b, d**) were processed by SynergyFinder using data from PrestoBlue viability assay (n = 3, biological replicates).



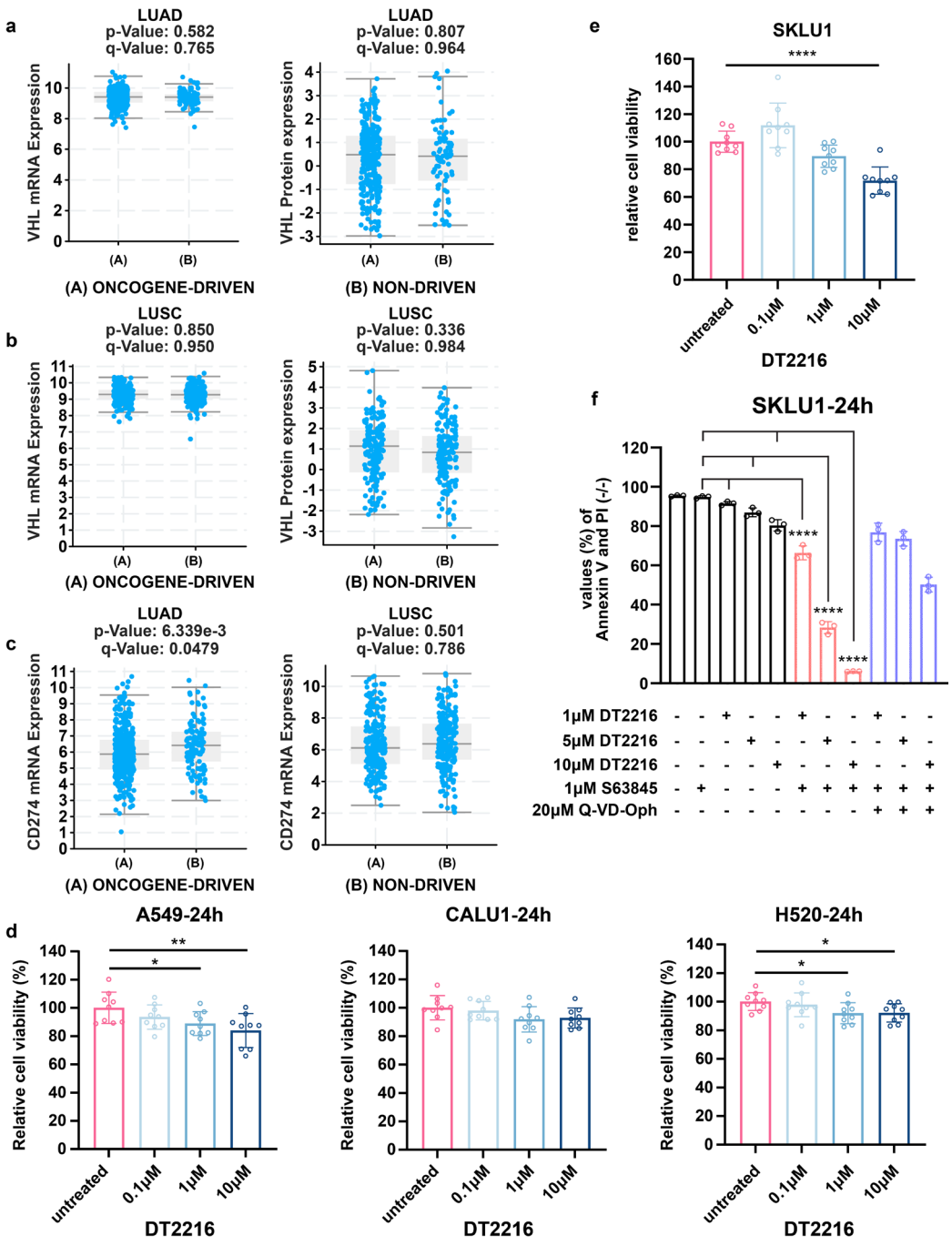
Supplementary Figure S4. Validation of more NSCLC cell lines using BH3 mimetics treatment. a-b. Combined treatment with WEHI-539 and S63845 of COLO-699 (**a**) and LCLC103H (**b**) cells for 1 and 2 hours, respectively. Protein levels of caspase-3, caspase-8, BID and PARP were detected by using western blotting. **c-d.** CORL23 was treated with the same combination or additionally with Q-VD-Oph at different time points to follow apoptosis induction over time. Figure **c-f** are representative results at least two independent repetitions.



Supplementary Figure S5. Performance of BH3 mimetics combinations in CORL23- caspase-8-knockout cells. a-b. Flow cytometry (n = 3, biological replicates) and western blot analyses were performed after the addition of WEHI-539 and S63845 combination in CASP8 KO CORL23 cells. **c.** After treating CASP8 KO CORL23 with WEHI-539 and S63845 combinations at three time points, subcellular fractionation was performed and analyzed by western blot. Figure **b-c** are representative results at least two independent repetitions. Quantitative data are presented as mean ± SEM, statistical analysis was performed by one-way ANOVA for **a**. * $p < 0.05$; **** $p < 0.0001$.



Supplementary Figure S6. Synergistic effect of the next generation BCL-XL inhibitor A-1331852 with the MCL-1 inhibitor S63845. a. After treatment with different concentrations of the BCL-XL inhibitor A-1331852 for 24 hours, PrestoBlue assay was performed to assess cell viability in A549, CALU1and H520 cells (n = 3, biological replicates with 3 technical replicates, each). **b.** 3D drug synergy images of A549, CALU1 and H520 analyzed and created by SynergyFinder (ZIP model) (n = 3, biological replicates per group). **c.** Determination of cell survival after 2 h treatment with reduced amounts of A-1331852 plus S63845 by flow cytometry (n = 3, biological replicates). **d-e.** Validation of the efficiency of p53 knockout in A549 cells by western blotting upon Nutlin-3a treatment (**d**) (data are representative of 2 independent repetitions) and flow cytometry (**e**) (n = 3, biological replicates). **f-g.** A549 parental (**f**) and p53 knockout cells (**g**) were treated with A-1331852 plus S63845 for 1 hour, and flow cytometry (AnV/PI exclusion) was used to determine cell survival rates (n = 3, biological replicates). Quantitative data were presented as mean ± SEM, statistical analysis was performed by one-way ANOVA for **a**, **c**, **e**, **f, g**. * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



Supplementary Figure S7. Feasibility of using DT2216 to Target BCL-XL. a-b. The groups driven by oncogenes (point mutations: EGFR, KRAS, BRAF, HER2, KEAP1, and STK11; structural variants (fusions): ALK, ROS1, RET, and NTRK1/2/3; copy number alterations (amplifications): MET, HER2, CCNE1, CCND1, FGFR1, MYC, and NKX2-1) and non-oncogenes were derived from lung adenocarcinoma (**a**) and lung squamous cell carcinoma (**b**) datasets in the cBioPortal database for VHL mRNA (log2) and protein analysis. **c.** The expression levels of CD274 mRNA (log2) in the above groups. **d.** Different concentrations of DT2216 were used in A549, CALU1 and H520 cells to determine the optimal working concentration. Cell viability was assessed by PrestoBlue assay (n = 3 biological replicates with 3 technical replicates, each). **e.** Viability assay by PrestoBlue in SKLU1 cells to determine the optimal concentration of DT2216 (n = 3, biological replicates with 3 technical replicates). **f.** Survival rates were measured 24 hours after combination treatment with DT2216 at various concentrations and 1μM S63845 in SKLU1 by flow cytometry (n = 3 biological replicates). Quantitative data are presented as mean ± SEM, statistical analysis was performed by one-way ANOVA for **d, e, f**. * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.