Supplementary Figures:

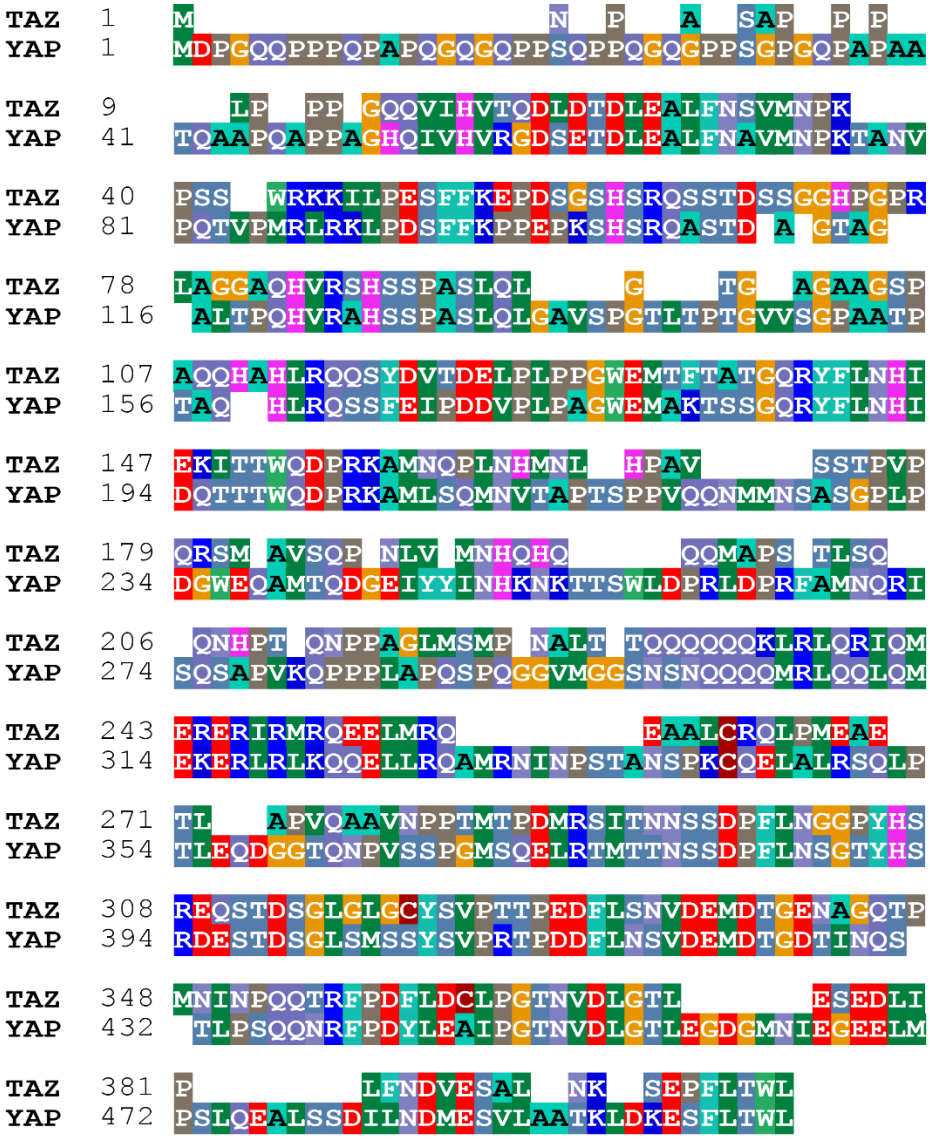

Figure S1: Comparison of YAP and TAZ protein sequences

Pairwise sequence alignment of the protein sequences of TAZ and YAP. Numbers at the left relate to amino acid positions. Color shading denotes different groups of amino acids. Alignment was generated by using BioEdit software.

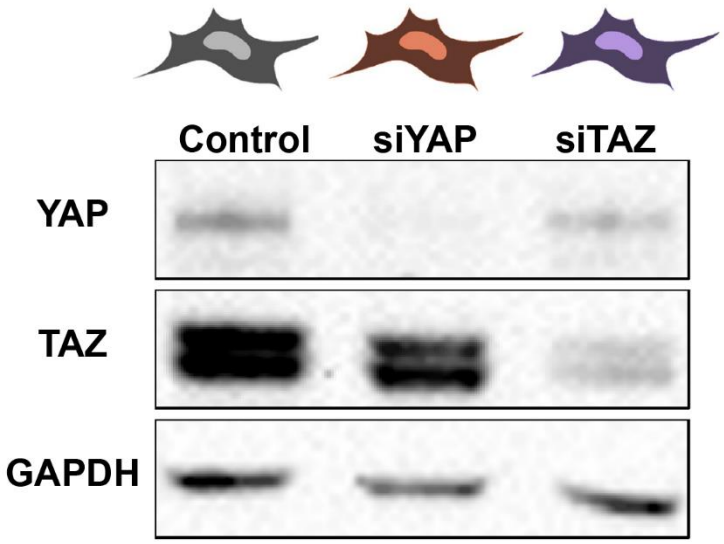

Figure S2, related to figure 1: Validation of YAP and TAZ knockdown in H1299 cells
YAP and TAZ protein levels in H1299 cells. Upper panel: Cartoon describing the approach taken to determine YAP and TAZ transcriptional programs. Lower panel: Representative immunoblot with the indicated antibodies.

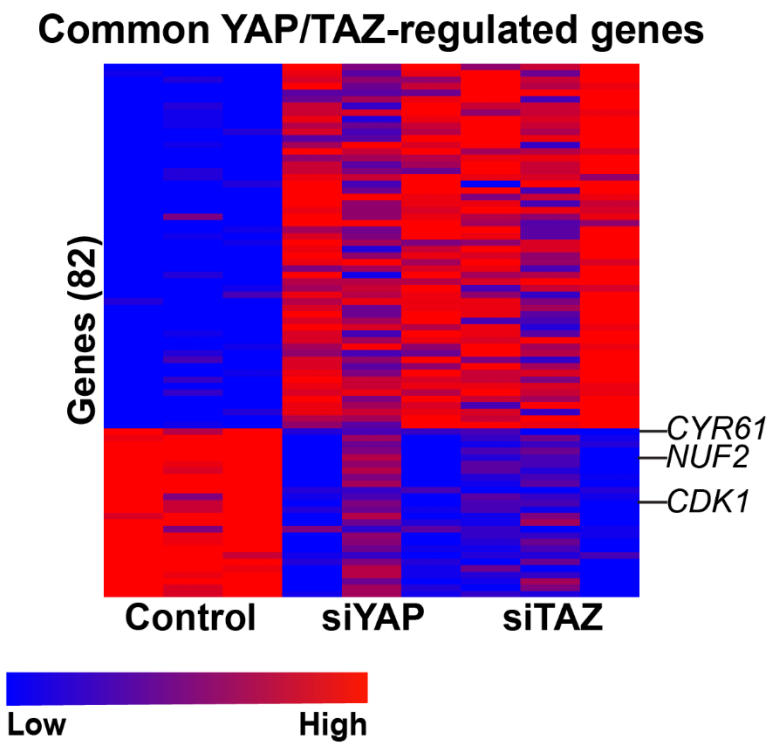

Figure S3, related to figure 1: Common YAP/TAZ-regulated genes

Heatmap of gene expression levels of the 82 common siYAP and siTAZ significantly differentially expressed genes compared to control (see Venn diagram in figure 1B). Absolute fold change≥2, adjusted p-value ≤0.05.

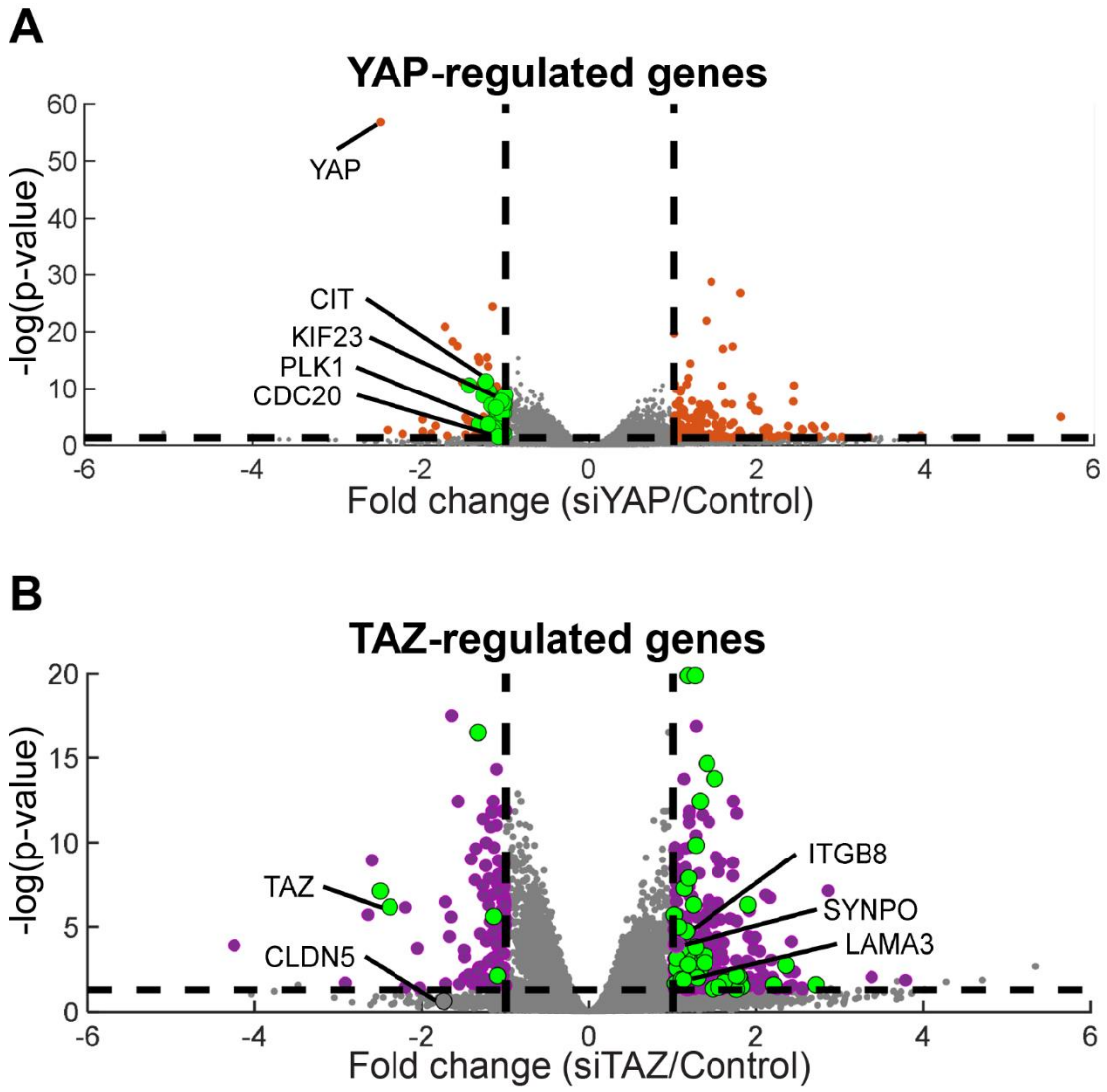

Figure S4, related to figure 2: Volcano plots of YAP-regulated and TAZ-regulated genes
A and B. Volcano plots of 18,977 genes detected in the RNA-seq analysis. Colored dots represent genes whose expression was significantly altered by either siYAP (orange) or siTAZ (purple), relative to control siRNA. Genes related to the top enriched GO terms for each siRNA (black columns in Fig. 2A, B) are marked in green. P-value represents corrected p-value and is -log10 transformed. Dashed lines represent p-value of 0.05 (bottom), and log2 fold change of 2 (right) and 0.5 (left).

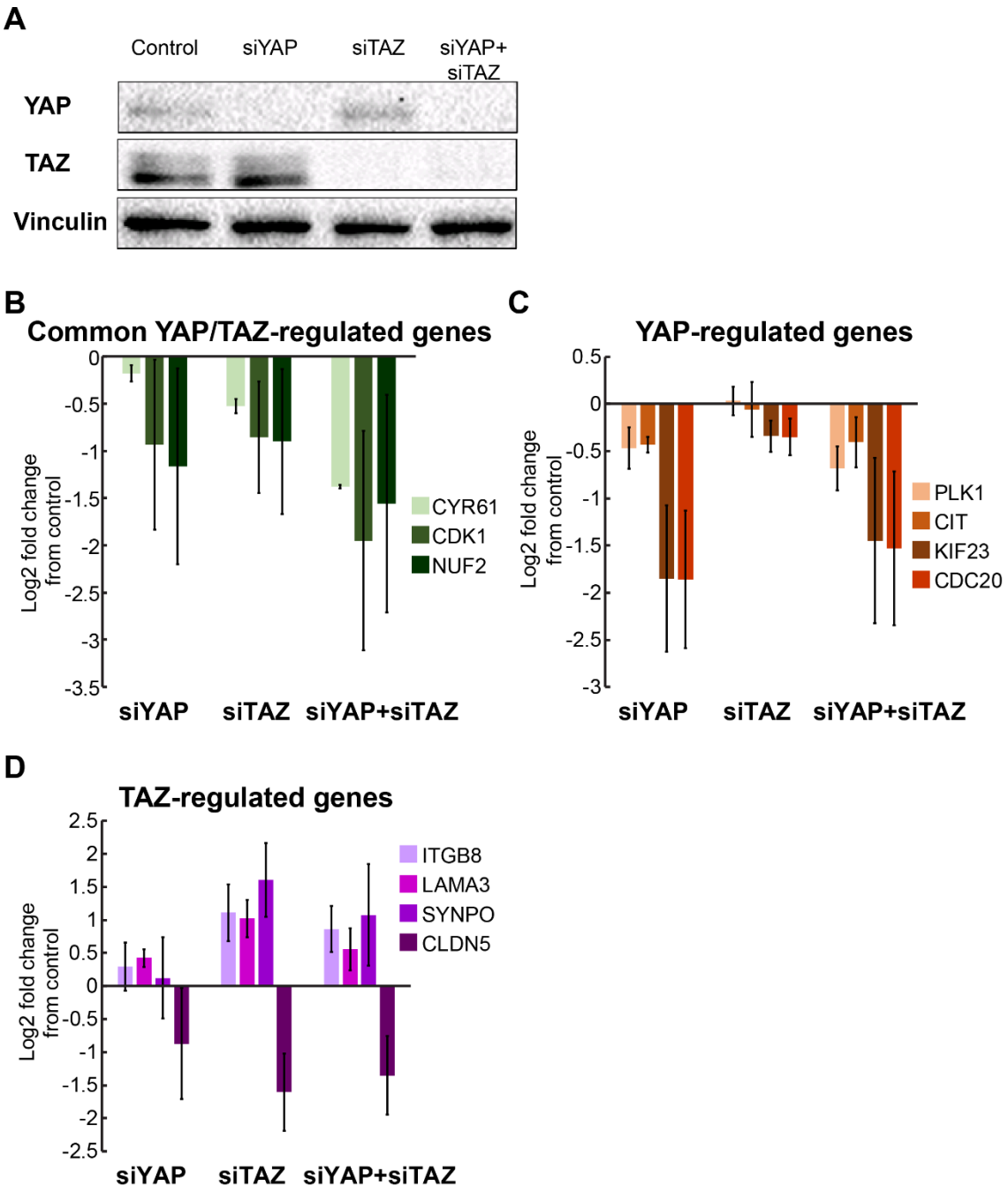

Figure S5, related to figure 2: Combined YAP and TAZ silencing preferentially affects common YAP/TAZ-regulated genes, but does not affect YAP-regulated or TAZ-regulated genes

A. Validation of knockdown with antibodies specific to YAP, TAZ or Vinculin as loading control. H1299 cells were transfected with the indicated siRNAs. Cultures were harvested 48 hours post-transfection.

B, C, D. H1299 cells were transiently transfected with siRNA against either YAP (siYAP), or TAZ (siTAZ), or both YAP and TAZ together (siYAP+siTAZ). RNA was extracted 48 hours later

and subjected to RT-qPCR analysis of the indicated genes. Data represents log2 mRNA expression (mean±SEM) normalized to HPRT and control transfected cells, from three independent biological repeats.

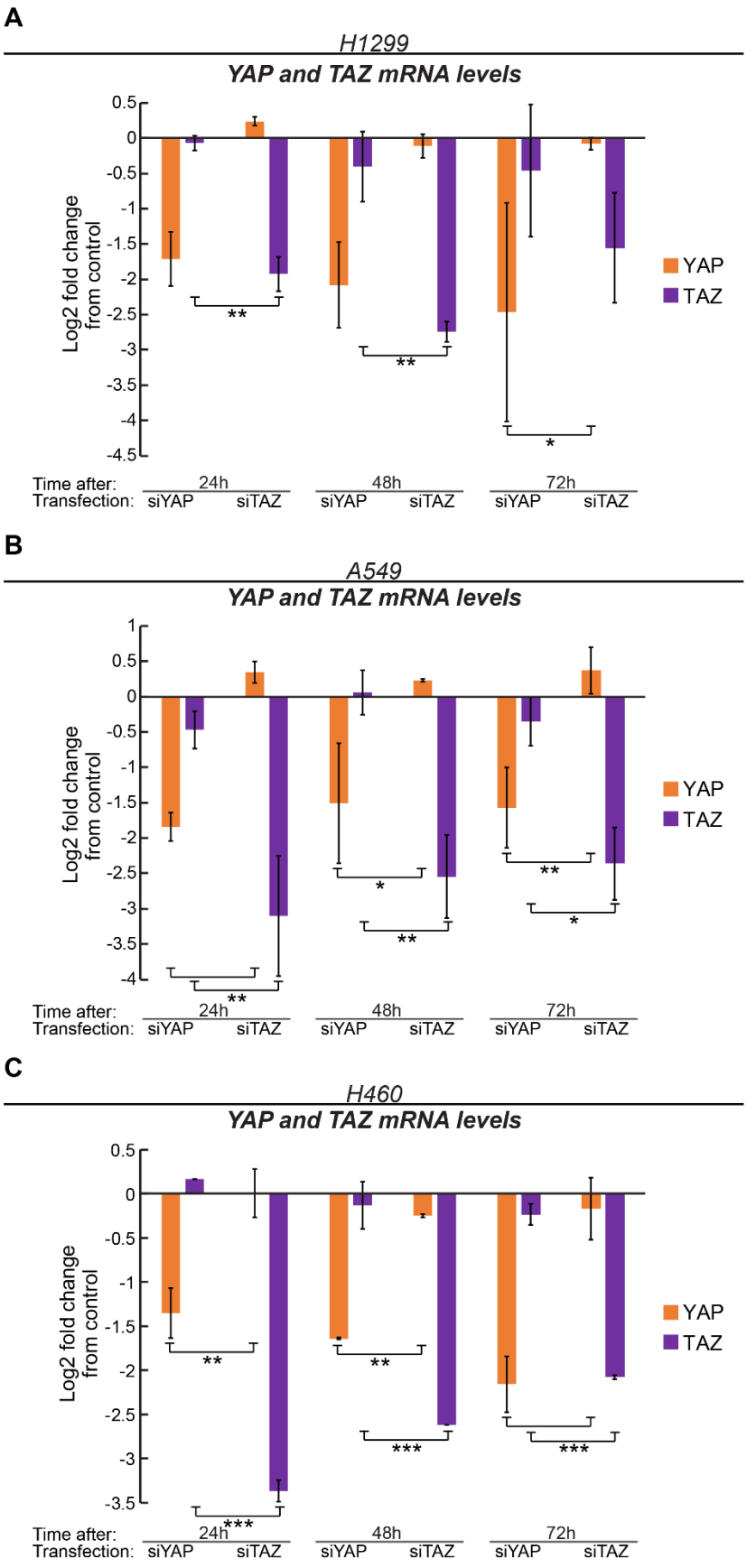
A
H1299
YAP and TAZ mRNA levels
Log2 fold change from control
YAP
TAZ
Time after:
Transfection:
24h
48h
72h
siYAP
siTAZ
B
A549
YAP and TAZ mRNA levels
C
H460
YAP and TAZ mRNA levels

Figure S6, related to figure 3 and figure 4: Validation of YAP and TAZ knockdown in NSCLC-derived cell lines

*H1299 (A), A549 (B) and H460 (C) cells were transfected with siRNA SMARTpools targeting either YAP (siYAP) or TAZ (siTAZ) or control siRNA. Cultures were harvested 24, 48 or 72 hours post-transfection, and subjected to RNA extraction and RT-qPCR analysis of YAP and TAZ mRNA levels. Data represents log2 mRNA expression (mean+STD) normalized to GAPDH and control transfected cells, from two independent biological repeats. *p<0.05; **p<0.01; ***p<0.001 one-way ANOVA and Tukey's post hoc test of the indicated comparisons.*

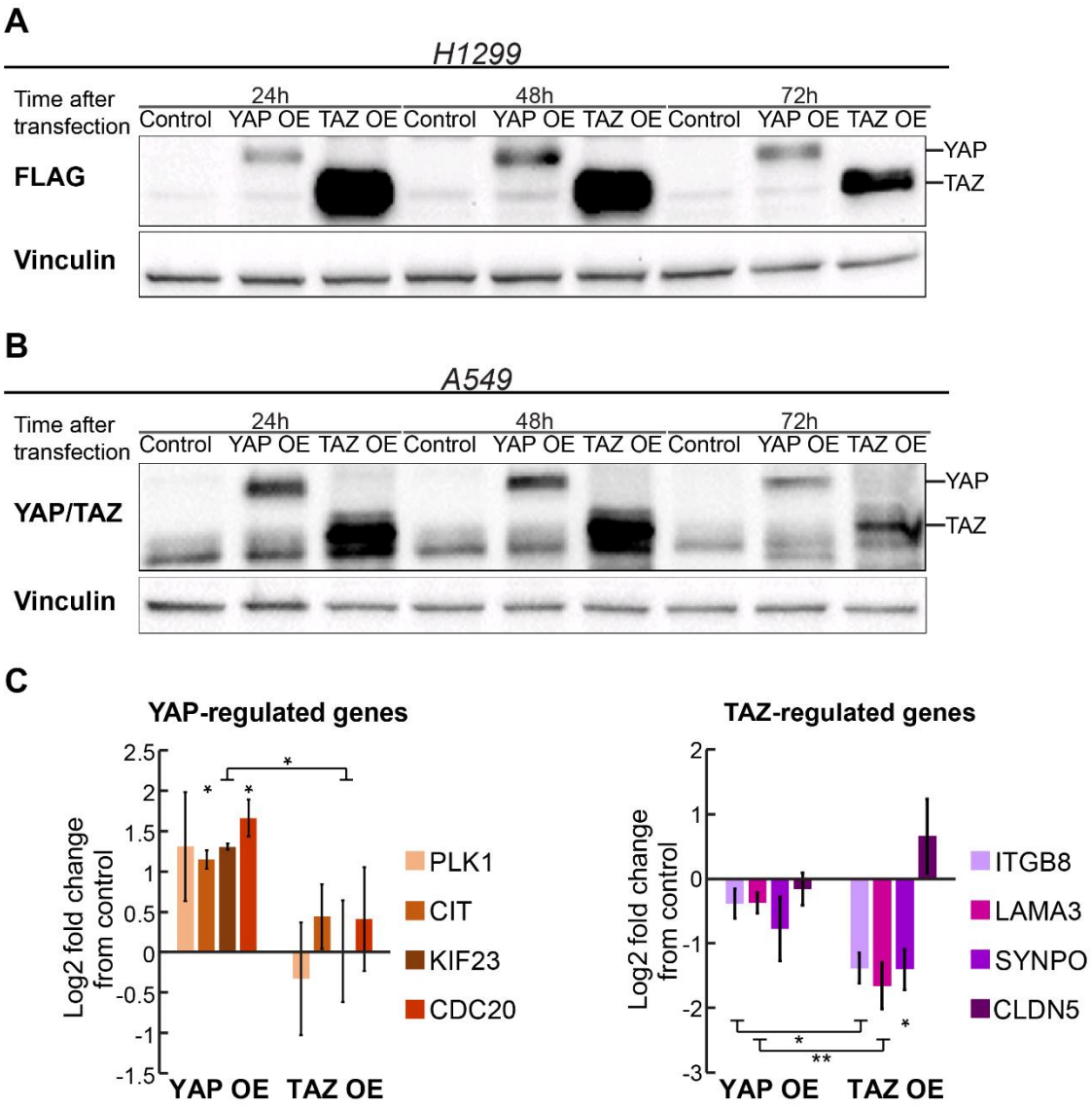

Figure S7, connected to figure 3 and figure 4: YAP and TAZ protein levels and candidate target gene expression upon YAP or TAZ overexpression

H1299 and A549 cells were transfected with plasmids encoding either YAP-flag (YAP OE) or TAZ-flag (TAZ OE). Cultures were harvested 24, 48 or 72 hours post-transfection.

A and B. Representative Western blot analysis with antibodies specific to FLAG, YAP, TAZ or Vinculin as loading control.

*C. RT-qPCR analysis of the indicated genes 48 hours post-transfection. Data represents log2 mRNA expression (mean±SEM) normalized to GAPDH and control transfected cells, from three independent biological repeats in H1299 cells. *p<0.05; **p<0.01 one-way ANOVA and Tukey's post hoc test for the indicated comparisons or vs. control.*

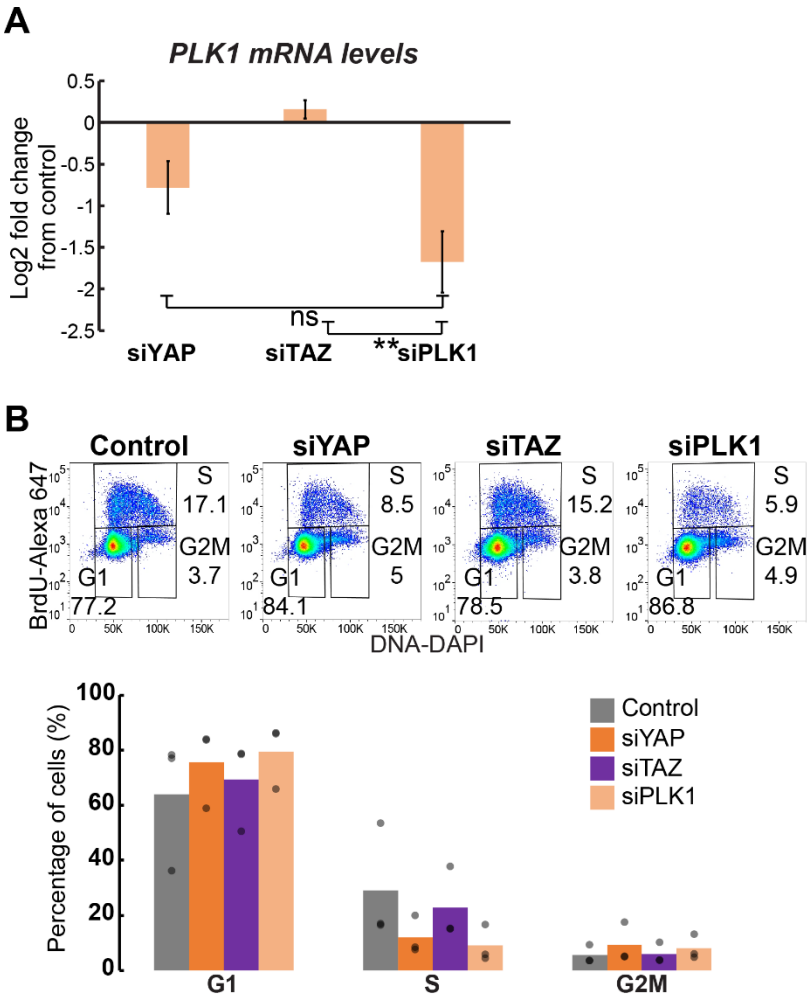

Figure S8, related to figure 3: Silencing of PLK1 mimics the cell cycle effects of siYAP

*A. RT-qPCR analysis of PLK1 mRNA levels in H1299 cells transfected with the indicated siRNAs for 48 hours. Data represents log2 mRNA expression (mean±SEM) normalized to GAPDH and control transfected cells, from three independent biological repeats. ns = not significant; **p<0.01 one-way ANOVA and Tukey's post hoc test of the indicated comparison.*

B. Cell cycle profiling by BrdU + DAPI of H1299 cells transfected with the indicated siRNAs, 48 hours post-transfection. Upper panel: representative FACS analysis images. Lower panel: Average percentages of cells in each cell cycle phase, from three biological repeats; each dot represents an independent biological repeat.

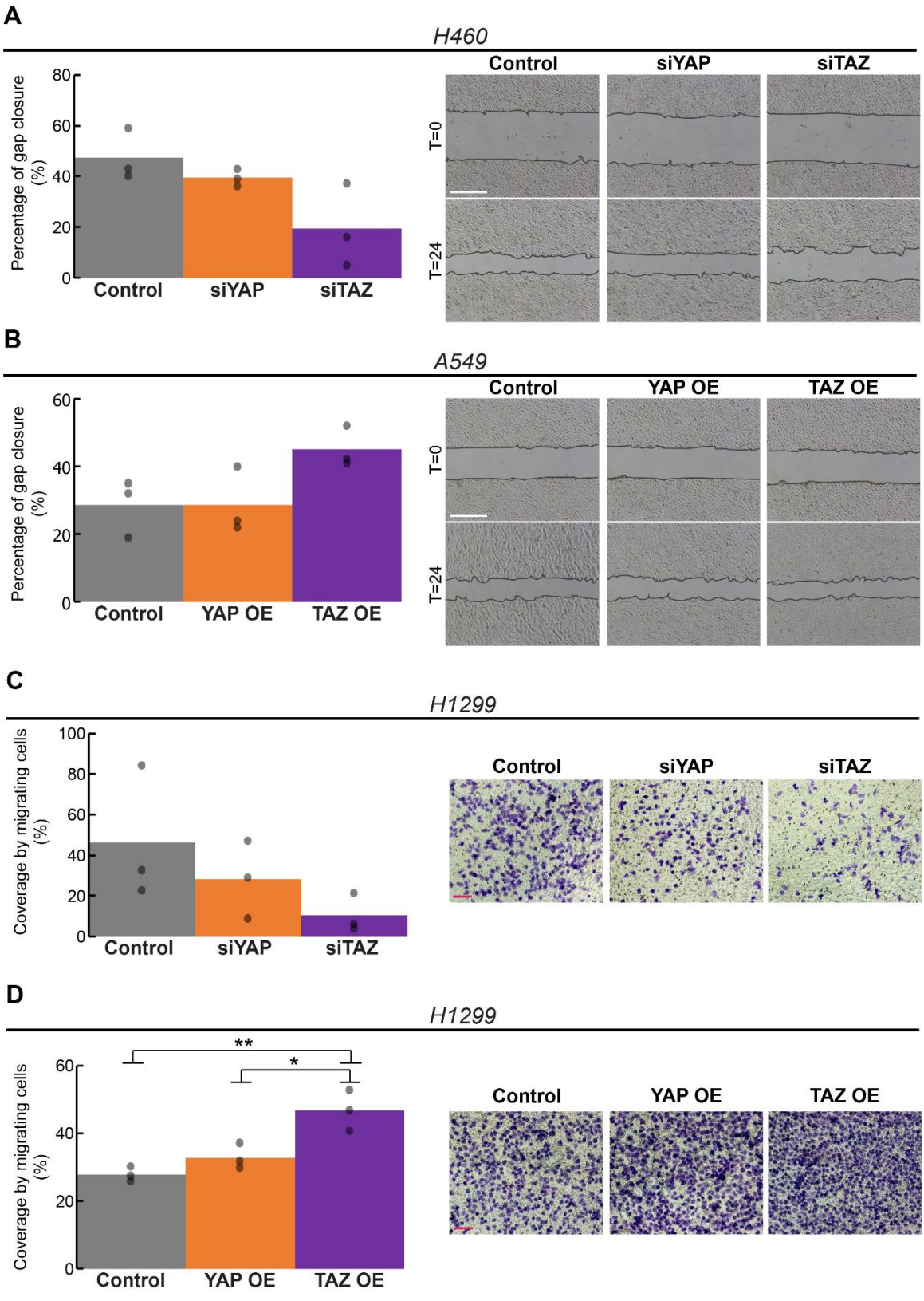
A
H460
Control
siYAP
siTAZ
Percentage of gap closure (%)
0
20
40
60
80
Control
siYAP
siTAZ
T=0
T=24
B
A549
Control
YAP OE
TAZ OE
Percentage of gap closure (%)
0
20
40
60
Control
YAP OE
TAZ OE
T=0
T=24
C
H1299
Control
siYAP
siTAZ
Coverage by migrating cells (%)
0
20
40
60
80
100
Control
siYAP
siTAZ
D
H1299
Control
YAP OE
TAZ OE
Coverage by migrating cells (%)
**
*
0
20
40
60
Control
YAP OE
TAZ OE

Figure S9, connected figure 4: TAZ preferentially regulates cell migration in NSCLC-derived cell lines

A and B. Gap closure ("Scratch") assays of H460 (n=3) and A549 (n=3) cell cultures transfected with the indicated siRNAs or plasmids, assayed 48 hours post-transfection.
Left panels: Average percentage of gap closure calculated from all biological repeats; each dot represents an independent biological repeat. Right panels: representative images of gap closure at T=0 and T=24 hours. Scale bar = 500µM.
C and D. Transwell migration assay of H1299 (n=3) cell cultures transfected with the indicated siRNAs or plasmids for 48 hours. Cells were seeded in transwell inserts. 16 hours later, cells that had migrated across the membrane were stained with crystal violet and photographed.
*Left panels: Average percentage of coverage by migrating cells calculated from all biological repeats; each dot represents an independent biological repeat. Right panels: representative images of the stained migrating cells. *p<0.05; **p<0.01 determined by one-way ANOVA and Tukey's post hoc test of the indicated comparisons. Scale bar = 100µM.*

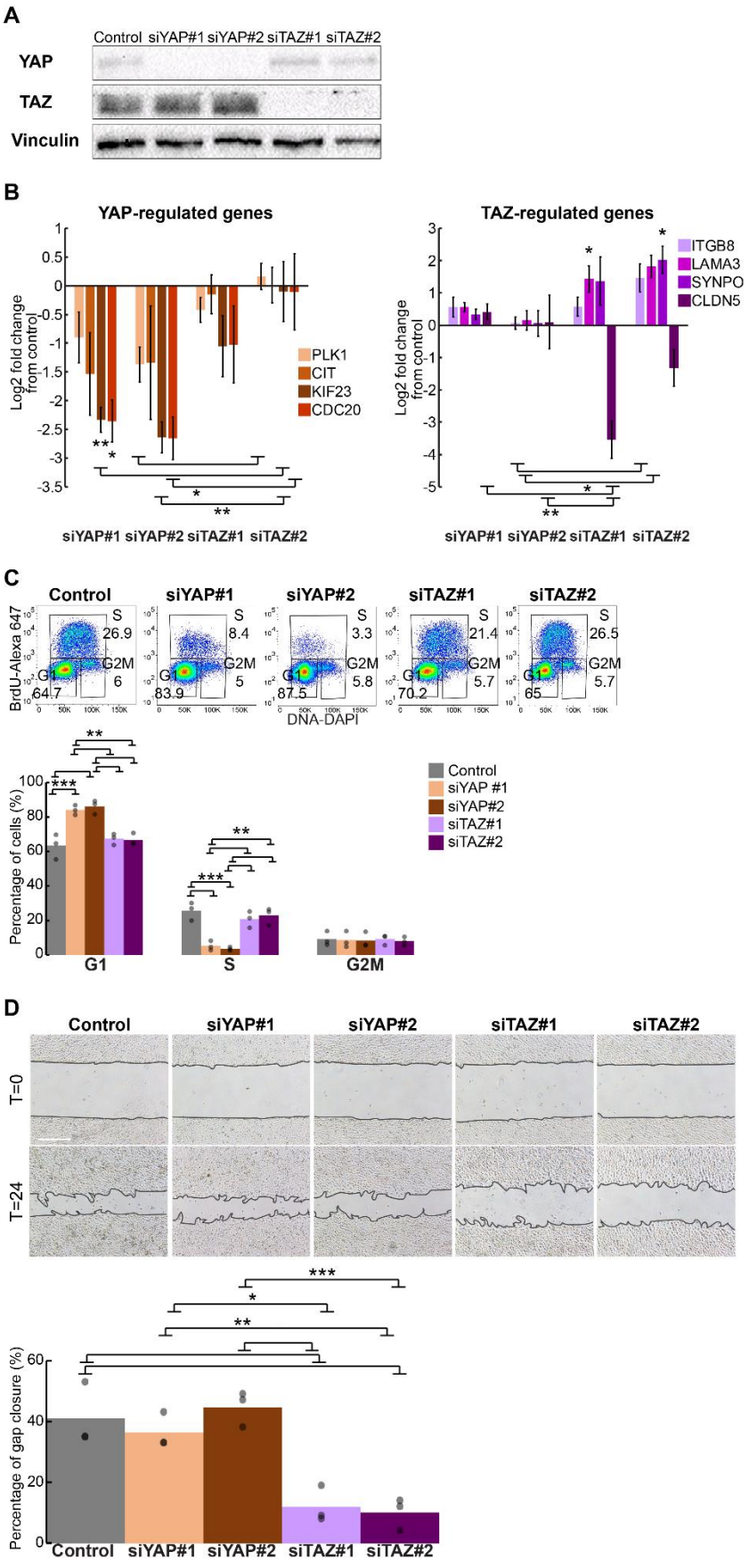
A
Control siYAP#1 siYAP#2 siTAZ#1 siTAZ#2
YAP
TAZ
Vinculin
B
YAP-regulated genes
TAZ-regulated genes
Log2 fold change from control
PLK1
CIT
KIF23
CDC20
ITGB8
LAMA3
SYNPO
CLDN5
siYAP#1 siYAP#2 siTAZ#1 siTAZ#2
C
Control
siYAP#1
siYAP#2
siTAZ#1
siTAZ#2
BrdU-Alexa 647
DNA-DAPI
S 26.9 G2M 6 G1 64.7
S 8.4 G2M 5 G1 83.9
S 3.3 G2M 5.8 G1 87.5
S 21.4 G2M 5.7 G1 70.2
S 26.5 G2M 5.7 G1 65
Percentage of cells (%)
G1
S
G2M
Control
siYAP #1
siYAP#2
siTAZ#1
siTAZ#2
D
Control
siYAP#1
siYAP#2
siTAZ#1
siTAZ#2
T=0
T=24
Percentage of gap closure (%)
Control siYAP#1 siYAP#2 siTAZ#1 siTAZ#2

Figure S10, connected to figure 3 and figure 4: YAP or TAZ silencing by single siRNA oligonucleotides phenocopies the effects of YAP or TAZ silencing by siRNA SMARTpools

H1299 cells were transfected with single siRNA oligonucleotides targeting either YAP (siYAP#1 or siYAP#2) or TAZ (siTAZ#1 or siTAZ#2) or control. Cultures were subjected to analysis 48 hours post-transfection.

A. Representative Western blot analysis with antibodies specific to YAP, TAZ or Vinculin as loading control.

*B. RT-qPCR analysis of the indicated genes. Data represents log2 mRNA expression (mean±SEM) normalized to GAPDH and control transfected cells, from three independent biological repeats. *p<0.05; **p<0.01 one-way ANOVA and Tukey's post hoc test for the indicated comparisons or vs. control.*

*C. Cell cycle profiling by BrdU + DAPI of H1299 cell cultures (n=3) transfected with the indicated single siRNA oligonucleotides. Upper panel: representative FACS analysis images. Lower panel: Average percentages of cells in each cell cycle phase, from three biological repeats; each dot represents an independent biological repeat. **p<0.01; ***p<0.001 determined by one-way ANOVA and Tukey's post hoc test of the indicated comparisons.*

*D. Gap closure assay of H1299 cell cultures (n=3) transfected with the indicated siRNAs. Upper panel: representative images of gap closure at T=0 and T=24 hours. Lower panel: Average percentage of gap closure calculated from all biological repeats; each dot represents an independent biological repeat. *p<0.05; **p<0.01; ***p<0.001 one-way ANOVA and Tukey's post hoc test of the indicated comparisons. Scale bar is 500µM.*

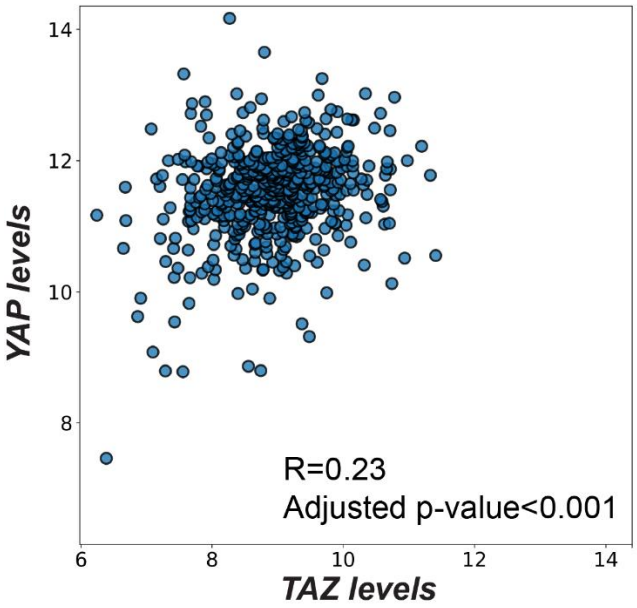

Figure S11, connected to figure 5: Levels of YAP and TAZ mRNA are only partially correlated across lung adenocarcinoma tumors

Scatter plot of YAP vs. TAZ mRNA levels across LUAD, derived from TCGA dataset. R = Pearson correlation coefficient. P-value was adjusted for multiple testing, using the procedure of Benjamini and Hochberg.

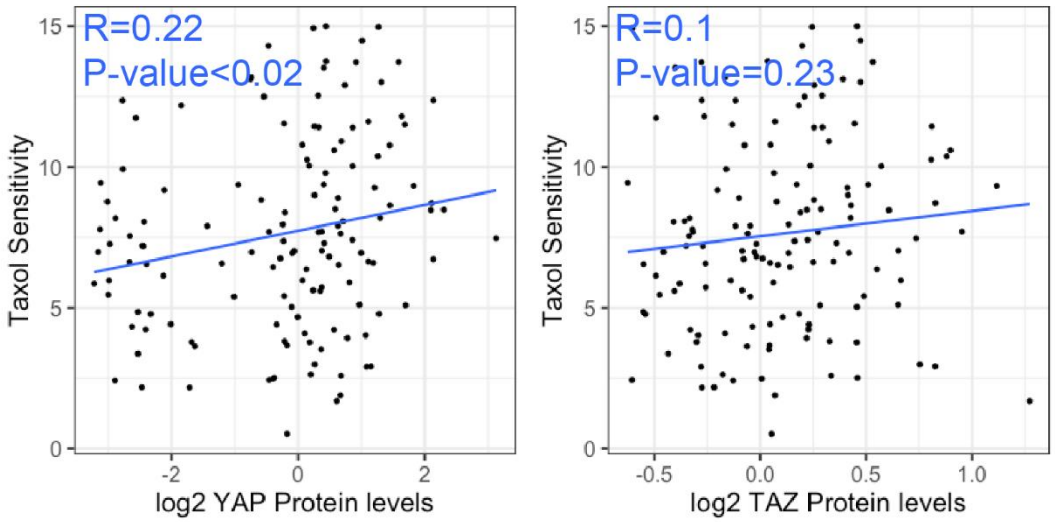

Figure S12, connected to figure 6: Taxol sensitivity is better correlated with YAP protein levels than with TAZ protein levels

Scatter plot of Taxol sensitivity in the GDSC dataset [35], measured by area under the dose-response curve, plotted against YAP (left) or TAZ (right) protein levels (determined by RPPA in the CCLE) across lung cancer cell lines (n=136). Protein levels are log-transformed. R represents Spearman correlation coefficient, and blue line represents the indicated R value.

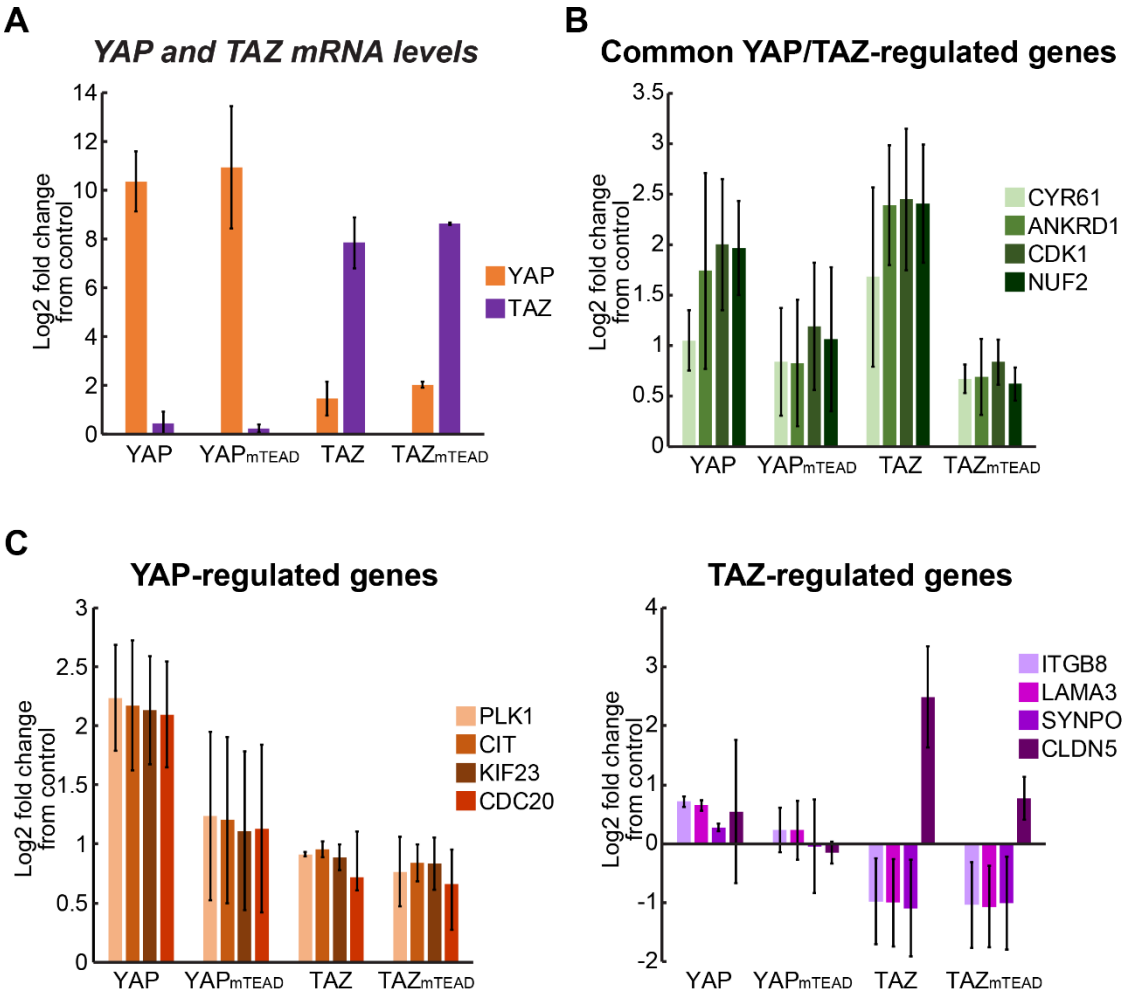

Figure S13: TEAD is partially involved in regulating YAP- and TAZ-regulated genes

H1299 cells were transfected with plasmids encoding either YAP-flag (YAP), YAP-flag in which residues 90, 91, 94, 95, 96 were replaced with alanine (YAP_{mTEAD}; defective in TEAD binding), TAZ-GFP (TAZ) or TAZ-GFP in which residue 51 was replaced with alanine (TAZ_{mTEAD}; defective in TEAD binding). Cultures were maintained in serum-free medium and harvested 48 hours post-transfection.

A. Validation of YAP and TAZ mRNA overexpression.

B and C. RT-qPCR analysis of the indicated genes. Data represents log2 mRNA expression (mean±STD) normalized to HPRT and control transfected cells, from two independent biological repeats.

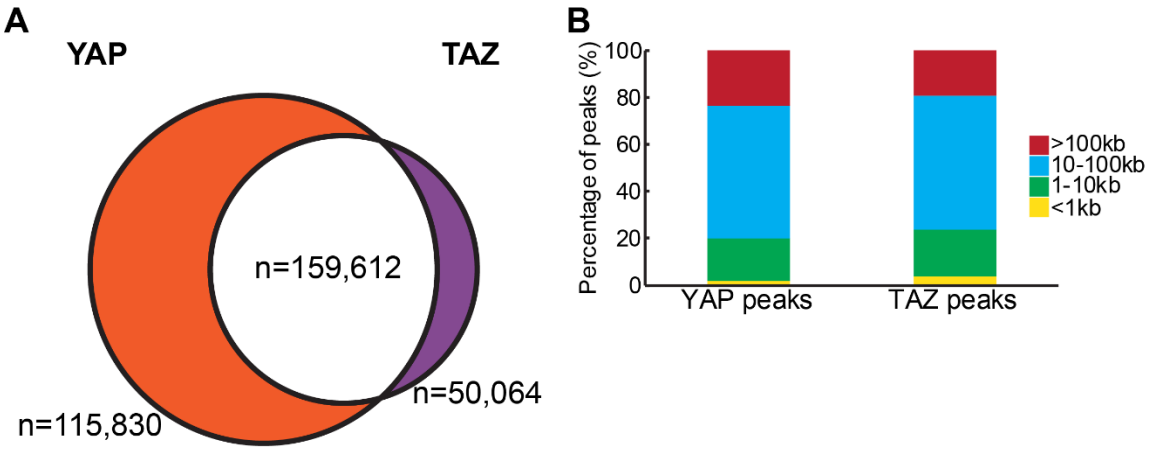

Figure S14, connected to figure 7: YAP and TAZ show both common and differential genome-wide binding distribution

Chromatin from H1299 cells was immunoprecipitated with either anti-YAP or anti-TAZ antibodies and subjected to DNA sequencing (ChIP-seq). In total, 275,442 YAP peaks and 209,676 TAZ peaks were called.

A. Venn diagram showing the overlap of peaks detected by anti-YAP and anti-TAZ antibodies.

B. Distribution of absolute distances from the nearest TSS of the indicated peaks, as determined by HOMER.