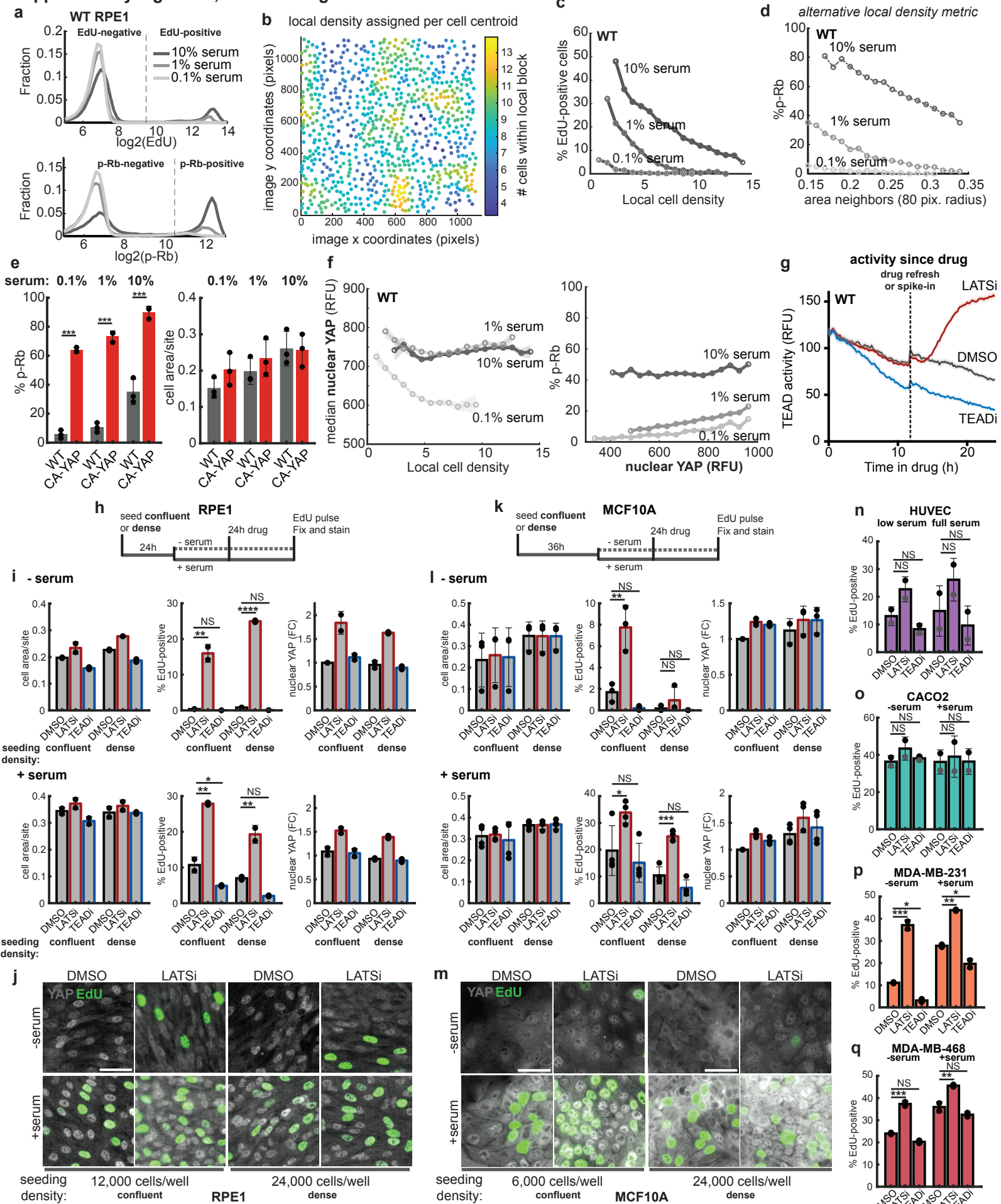


Supplementary Figure S1, related to Figure 1



Supplementary Figure S1, related to Figure 1

a) Histogram plots of binary distribution of EdU (top) and Rb phosphorylation (p-Rb 807/811, bottom) in wild-type (WT) RPE1 cells with 10%, 1%, or 0.1% serum. Dashed line indicates threshold values used to design whether cells are EdU-positive or have p-Rb. Data are representative of 3 independent experiments (same experiment as in Fig. 1c).

b) Representative local density estimation in an imaging site, where circles represent the location of the centroids of cell nuclei. Cell location coordinates were divided into a 10x10, equally-spaced grid (local density blocks). The z-scale color indicates the number of cell centroids within a block. Cells were assigned local densities based on the number of cells in their block, scaled by the image size to give a relative density unit. See also Methods.

c) Percent EdU-positive cells per local density bin for WT cells in titrated serum levels. Data are representative of 3 independent experiments.

d) Percent p-Rb per binned area of neighboring cell nuclear pixels within an 80-pixel (~50 μ m) radius for WT cells (as in Fig. 2c), with similarly graded density.

e) Mean percent p-Rb (left) and final cell density (right, total area of segmented nuclei/imaging site area) for WT and CA-YAP cells maintained in increasing serum levels (as in Fig. 2d) for 36h. N = 3 independent experiments. Student's t-test: % p-Rb, $p = 6.86 \times 10^{-6}$ (0.1% serum), $p = 2.50 \times 10^{-5}$ (1% serum), $p = 5.90 \times 10^{-4}$ (10% serum).

f) Median nuclear YAP calculated per local density bin (left) and percent p-Rb calculated per bin of nuclear YAP levels (right) for WT cells (as in Fig. 1e). Shaded error are 95% confidence intervals.

g) Mean TEAD reporter activity (real fluorescent units) over time since drug treatment in cycling RPE1 (TEADi at 0h, LATSi at 12h). Dashed line marks time of LATSi treatment or drug refresh (DMSO and TEADi conditions). N > 500 cells/condition, shaded error bars are $\pm$ SEM (data representative of 2 independent experiments).

h-i) Schematic (**h**) of confluent (12,000 cells/well) and dense (24,000 cells/well) seeding of RPE1 and (**i**) associated final density (fraction of total nuclei area/site area, left), percent EdU-positive (middle), and fold-change in nuclear YAP levels (normalized to confluent DMSO condition, right) in serum-starved (top) or serum conditions (bottom). N = 2 independent experiments. Ordinary one-way ANOVA with Dunnett's multiple comparisons, % EdU: **-serum**, $p = 0.0029$ (LATSi/confluent), 0.97 (TEADi/confluent), $< 1 \times 10^{-4}$ (LATSi/dense), 0.078 (TEADi/dense); **+serum**, $p = 0.0018$ (LATSi/confluent), 0.038 (TEADi/confluent), 0.0064 (LATSi/dense), 0.076 (TEADi/dense).

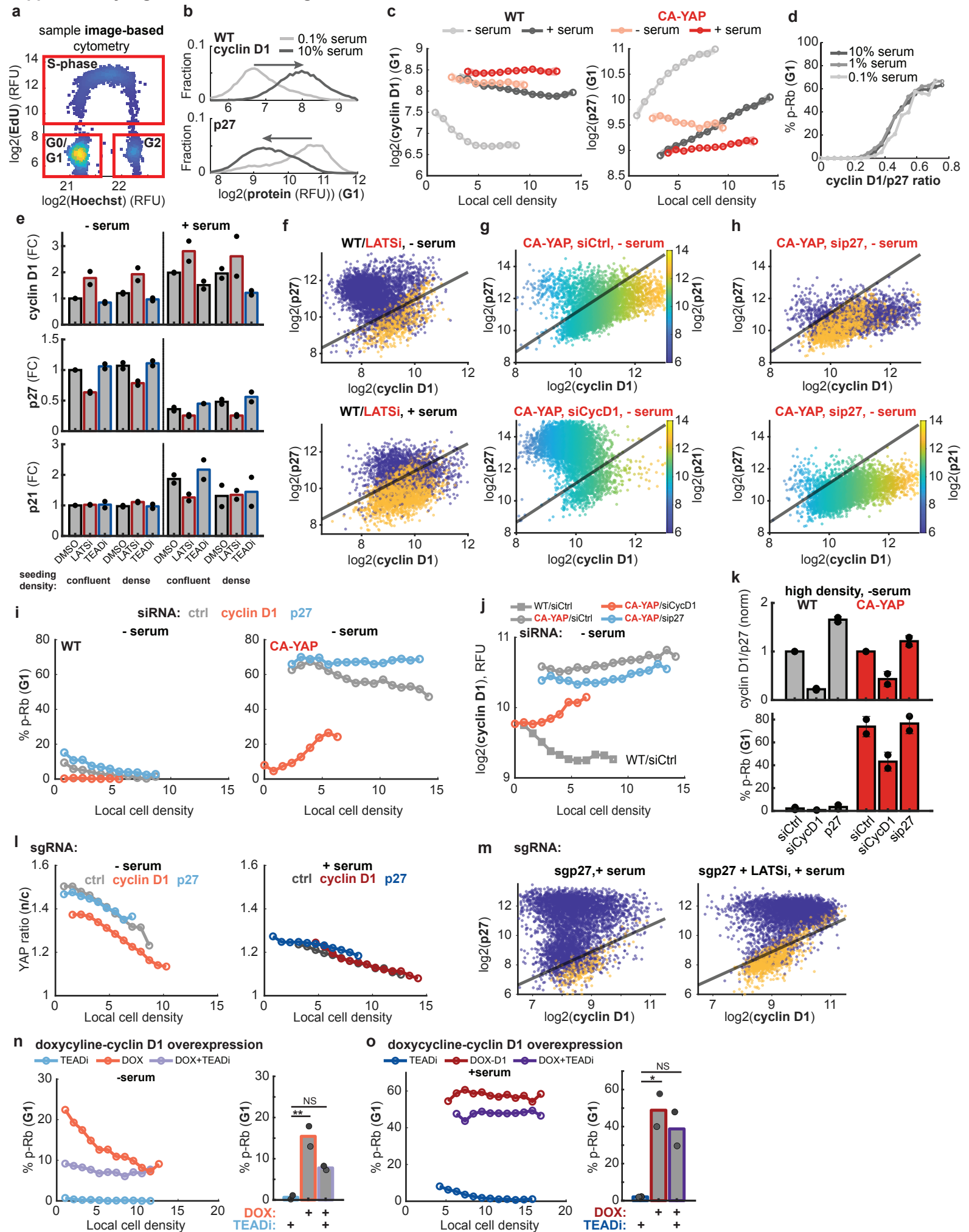
k-l) Schematic (**k**) of confluent (6,000 cells/well) and dense (24,000 cells/well) seeding of MCF10A and (**l**) final density (left), percent EdU-positive (middle), and fold-change in nuclear YAP levels (normalized to confluent DMSO condition, right) in serum-starved (top) or serum conditions (bottom). N = 3-4 independent experiments. Ordinary one-way ANOVA with Dunnett's multiple comparisons, % EdU: **-serum**, $p = 0.0023$ (LATSi/confluent), 0.34 (TEADi/confluent), 0.38 (LATSi/dense), 0.95 (TEADi/dense); **+serum**, $p = 0.037$ (LATSi/confluent), 0.59 (TEADi/confluent), $< 1 \times 10^{-4}$ (LATSi/dense), 0.069 (TEADi/dense). Dense data include those in Fig. 1j-k.

j, m) Representative immunofluorescence (IF) images of RPE1 (**j**) and MCF10A (**m**) with density and serum treatment as in (**h, k**), with overlay of YAP staining (grey) and EdU incorporation (green). Scale bar = 50 μ m.

n-q) Mean percent EdU-positive for HUVEC (**n**), CACO2 (**o**), MDA-MB-231 (**p**), and MDA-MB-468 (**q**) cells treated with DMSO, LATSi, or TEADi for 24h in pre-conditioned low/serum-starved or serum conditions (see also Methods). Cells seeded dense (24,000 cells/well), N = 2 independent experiments. Matched colors (**n-o**) indicate data from matching experimental replicate. Ordinary one-way ANOVA with Dunnett's multiple comparisons: HUVEC, all $p > 0.11$; CACO2, all $p > 0.29$; MDA-MB-231, $p = 7 \times 10^{-4}$ (LATSi/-serum), 0.022 (TEADi/-serum), 0.0027 (LATSi/+serum), 0.019 (TEADi/+serum); MDA-MB-468, $p = 0.0015$ (LATSi/-serum), 0.053 (TEADi/-serum), 0.017 (LATSi/+serum), 0.20 (TEADi/+serum).

All binned and histogram data are from one representative replicate of 3 independent experiments, x-data in 20 bins, $n > 100$ cells/bin and $> 15,000$ cells/condition.

Supplementary Figure S2, related to Figure 2

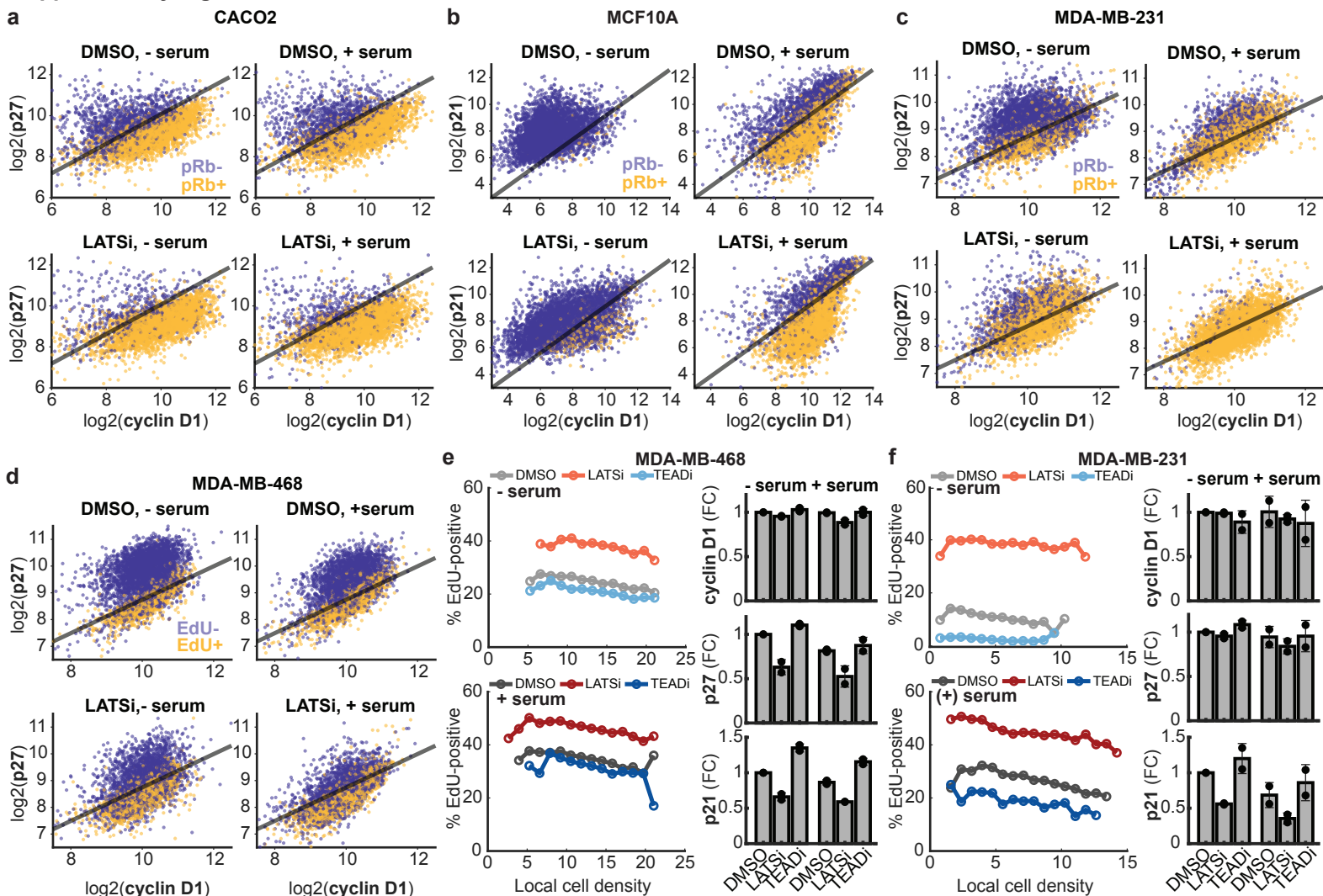


Supplementary Figure S2, related to Figure 2

- a)** Image-based cytometry of cells by DNA content (Hoechst staining, real fluorescence units (RFU)) and EdU-incorporation for G0/G1 gating (2N DNA/EdU-negative). All-cell gating reflects cells within the outer red boundary ($n = 8,000$ random cells).
- b)** Representative histogram distribution of cyclin D1 protein (top) and p27 protein (bottom) stain in G1-gated, WT cells in 0.1% or 10% serum.
- c)** Median protein levels (RFU) of cyclin D1 (left) and p27 (right) per local cell density bin for G1-gated WT and CA-YAP cells, with or without serum (as in Figure 2a-b). Shaded error bars are 95% confidence intervals, $n > 15,000$ cells per condition, $n > 100$ cells/bin. Data are representative of 2 independent experiments.
- d)** Percent p-Rb per cyclin D1/p27 ratio bin for serum titration in WT cells ($n > 15,000$ cells/condition).
- e)** Fold-change in cyclin D1, p27, and p21 protein levels for G1-gated WT cells seeded at confluent (12,000 cells/well) or dense (24,000 cells/well) densities. Cells without or with serum were treated with DMSO, LATSi, or TEADi for 24h. Data are normalized to confluent/DMSO condition. $N = 2$ independent experiments.
- f)** Cyclin D1/p27 single-cell scatterplots, colored by binary p-Rb state, in LATSi-treated cells in starvation (top) or serum (bottom). Data are G1-gated and representative of 3 independent experiments.
- (left) or p21 stain (right, parula scale) in starved WT cells.
- g-h)** Cyclin D1/p27 single-cell scatterplots (**g**), colored by p21 protein levels, of serum-starved CA-YAP cells with control (top) or cyclin D1 knockdown (bottom). Cyclin D1/p27 single-cell scatterplots (**h**), colored by p-Rb (top) or p21 protein levels (bottom), in serum-starved CA-YAP cells with p27 knockdown. Data are G1-gated and representative of 3 independent experiments, as in 2f.
- i-j)** Percent p-Rb per local density bin for starved WT (left) and CA-YAP (right) cells with siRNA knockdown of control, cyclin D1, or p27 (**i**). Median cyclin D1 protein levels per local density bin comparing knockdown in CA-YAP with control in WT (**j**), showing partial knockdown in CA-YAP. Data are G1-gated and representative of 3 independent experiments.
- k)** Normalized change in cyclin D1/p27 ratio (top) and percent p-Rb (bottom) for starved WT and CA-YAP cells seeded at dense (24,000 cells/well) density and siRNA knockdown of control, cyclin D1, or p27 expression (48h). $N = 2$ independent experiments.
- l)** Mean YAP ratio (nuclear/cytoplasmic) per local density bin for WT cells with sgRNA-mediated overexpression of control, cyclin D1, or p27 in starved (left) or serum (right) conditions, as in Figure 2h-i. Data are representative of 2 independent experiments.
- m)** Cyclin d1/p27 single-cell scatterplots, colored by p-Rb, for WT cells in serum with sgRNA-mediated overexpression of p27 and DMSO (left) or LATSi (right).
- n-o)** Percent p-Rb per local density bin (left) and mean p-Rb for cells with doxycycline(DOX)-inducible expression of cyclin D1 treated with TEADi only, DOX only, or DOX+TEADi in starved (**n**) or serum (**o**) conditions. Ordinary one-way ANOVA with Dunnett's multiple comparisons test (to TEADi condition): -serum, $p = 0.0093$ (DOX), 0.067 (TEADi+DOX); +serum, $p = 0.034$ (DOX), 0.063 (TEADi+DOX). NS = not significant.

All data representative of 2-3 independent experiments and gated for G1-phase. Single-cell scatterplots show $n = 5,000$ cells unless noted otherwise. Binary p-Rb state is depicted as purple or orange (below or above phosphorylation threshold, respectively).

Supplementary Figure S3



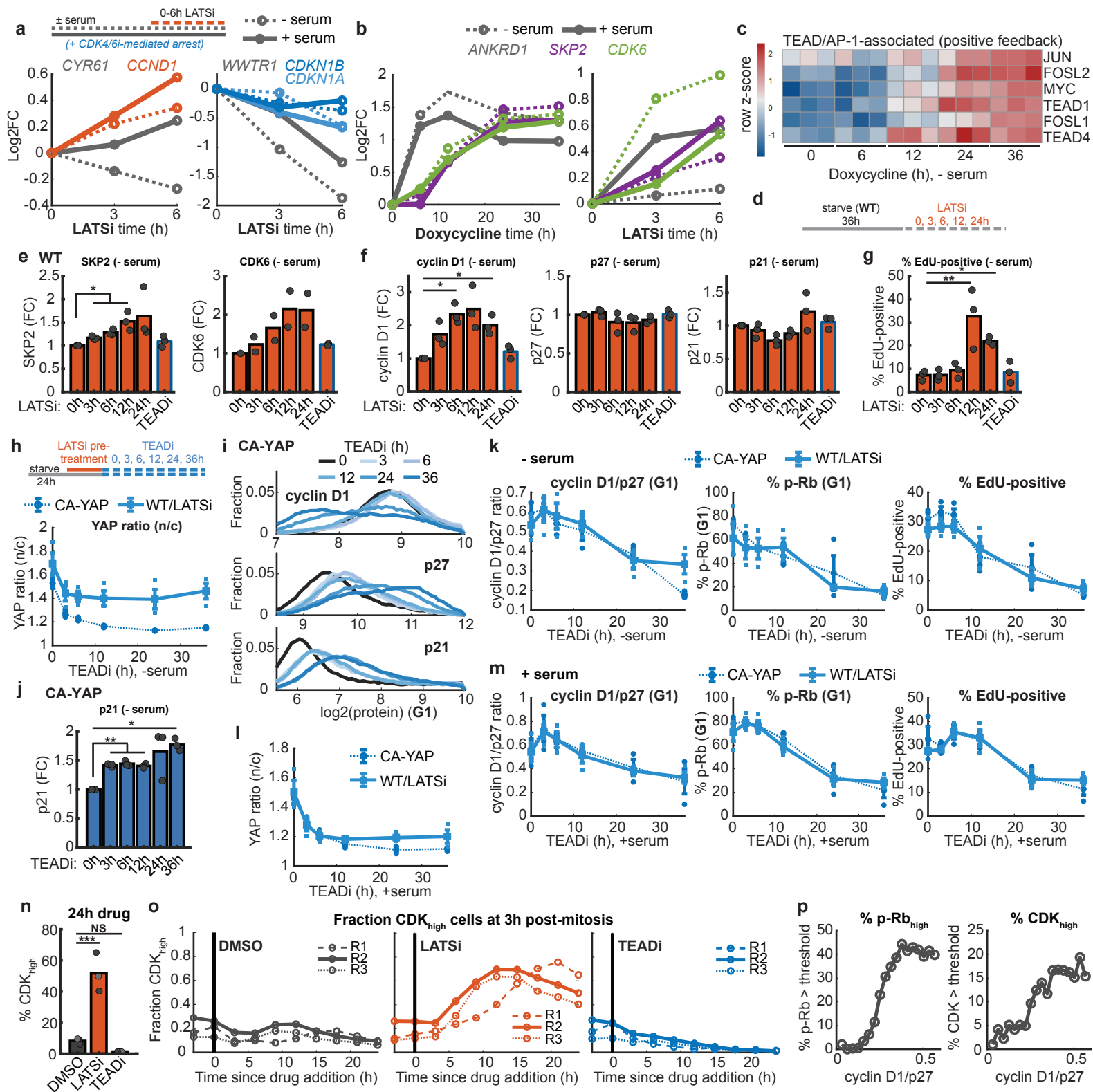
Supplementary Figure S3.

a-d) Cyclin D1/(p27 or p21) single-cell scatterplots, colored by binary p-Rb state in CACO2 (**a**), MCF10A (**b**), and MDA-MB-231 cells (**c**), or colored by binary EdU-state in MDA-MB-468 cells (**d**). Cells were in starved or serum conditions and DMSO- or LATSi-treated. Data are G1-gated for cells with p-Rb, or all-cell gating for MDA-MB-468 cells (Rb-negative cells), $n = 5,000$ random cells/plot. Data are representative of 2 independent experiments.

e-f) Percent EdU-positive cells per local density bin (left) for MDA-MB-468 (**e**) and MDA-MB-231 cells (**f**) treated with DMSO, LATSi, or TEADi for 24h in starved or serum conditions. Fold-change in cyclin D1, p27, and p21 protein levels (right) for 2N-gated cells, normalized to DMSO/starved condition. $N = 2$ independent experiments. Cells were seeded at dense (24,000 cells/well) density for 24h and pre-conditioned in respective media for 24h prior to drug treatment.

Binned data contain $n > 100$ cells/bin. Binary p-Rb and EdU states are depicted as purple or orange (below or above cutoff threshold, respectively).

Supplementary Figure S4, related to Figure 3



Supplementary Figure S4, related to Figure 3.

a-b) Top: Kinetics of sample YAP target gene regulation with 0-6h LATS inhibition (5 μ M TRULI) in starved or serum conditions in the presence of CDK4/6 inhibitor. (a) Log2 fold-change (Log2FC) of *CYR61* and *CCND1* (left), and of *WWTR1*, *CDKN1A*, and *CDKN1B* (right). (b) Log2FC in *ANKRD1*, *CDK6*, and *SKP2* in CA-YAP cells with doxycycline induction (left) and WT cells with LATS inhibition (right). Data from n = 3 independent experiments. Adjusted p-values and fold-change reported in **Table S3** as data include non-significant gene expression changes.

c) Heatmap of TEAD/AP-1-associated positive feedback genes in doxycycline-treated, starved CA-YAP cells (- serum). Color scale indicates row z-score.

d) Schematic for LATSi treatment (0.5 μ M TDI-011536) of serum-starved WT cells for 0-24h.

e-g) Mean fold-change in protein levels of (e) SKP2 (left) and CDK6 (right), (f) cyclin D1 (left), p27 (middle), and p21 (right), and (g) percent EdU for cells treated as in (d) or co-inhibited with TEADi (1 μ M GNE-7883). N = 2 (CDK6) or 3 independent experiments (all other proteins). Student's one-sample t test: **SKP2**, p = 0.016 (3h), 0.018 (6h), 0.047 (12h); **cyclin D1**, p = 0.017 (6h), 0.027 (24h), all other conditions p > 0.05. Ordinary one-way ANOVA with Dunnett's multiple comparisons to 0h: % EdU, p = 9.0×10^{-4} (12h), 0.039 (24h).

h) Top: experimental conditions for TEADi treatment (1 μ M GNE-7883) of serum-starved CA-YAP cells and LATSi pre-treated WT cells. Bottom: Mean YAP ratio $\pm$ SEM in CA-YAP (solid line) and LATSi-treated (dotted line) cells. CA-YAP data is same as Figure 3h.

i) Histogram distribution of cyclin D1, p27, and p21 protein levels over TEADi time course in representative experiment (- serum).

j) Mean fold-change in p21 with TEADi of CA-YAP as in (h). Student's one-sample t test: p = 0.0015 (3h), 0.0030 (6h), 0.0030 (12h), 0.12 (24h), 0.0057 (36h).

k) Mean cyclin D1/p27 ratio (left), percent p-Rb (middle) and EdU-positive (right) cells for treatment as in (h). CA-YAP data is same as in Figure 3j. Mean $\pm$ SEM, n = 3 independent experiments. Student's two-sample t test, LATSi: cyclin D1/p27, p = 0.035 (24h, 36h); % p-Rb, p = 0.038 (24h), 0.033 (36h); % EdU, p = 0.0038 (24h), 0.0012 (36h).

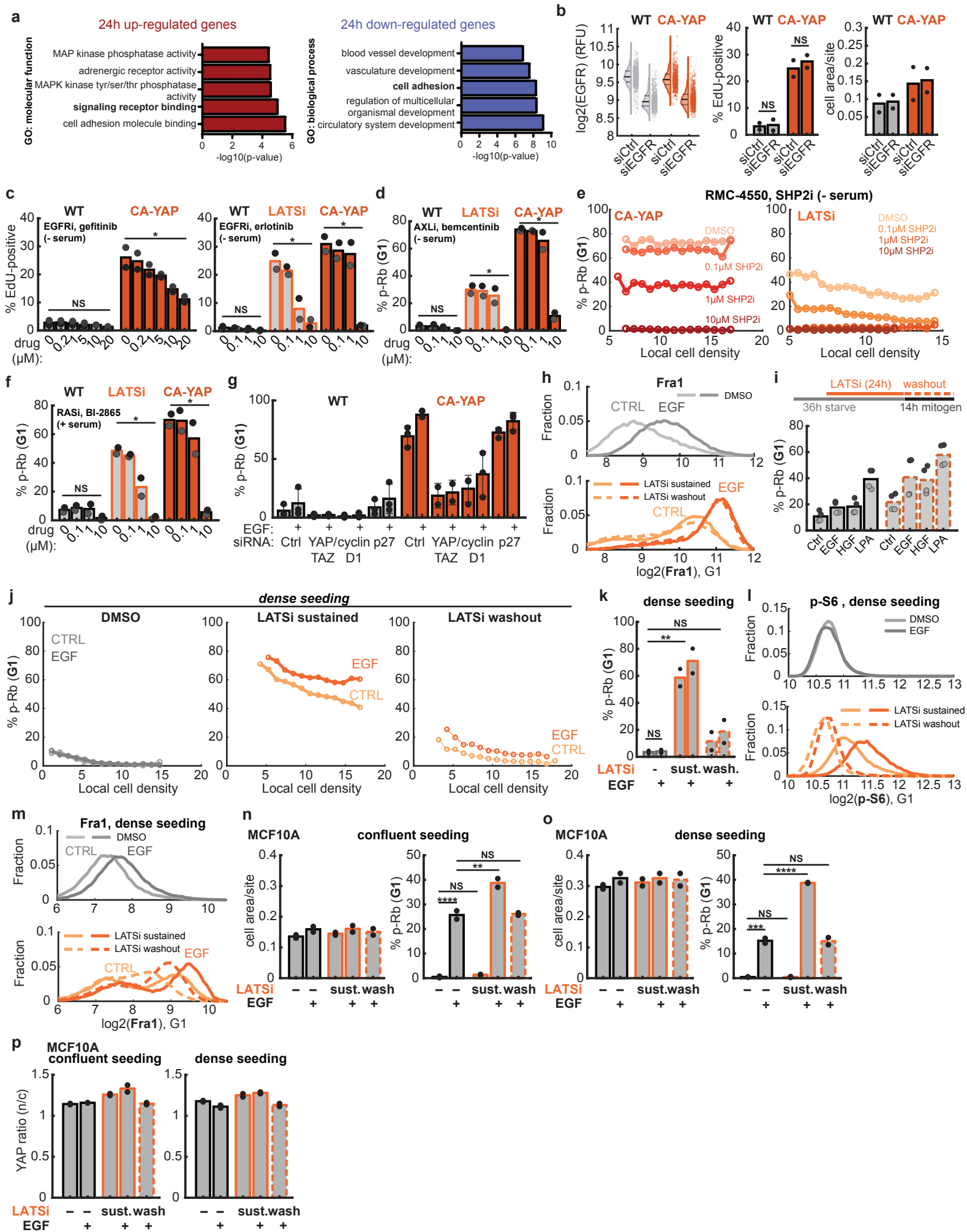
l-m) Mean YAP ratio (l), cyclin D1/p27 ratio (m, left), percent p-Rb (m, middle) and EdU-positive (m, right) for CA-YAP and LATSi-treated WT cells with TEADi in serum conditions. Mean $\pm$ SEM, n = 3 independent experiments.

n-o) Mean percentage CDK_{high} cells at 3h post-mitosis for cells reentering the cell cycle 24h after DMSO, LATSi, or TEADi treatment (n, n = 3 independent experiments). Fraction of CDK_{high} cells at 3h post-mitosis (mid-G1 phase) relative to drug treatment (o). Cells were aligned by mitosis and binned based on time spent in control (DMSO), LATSi, or TEADi (n > 100 cells/bin, n > 5,000 cells/condition). Each line represents an independent experiment (n = 3), where matched line styles indicate data from the same experiment. Ordinary one-way ANOVA (n): p = 6.0×10^{-4} (LATSi), 0.45 (TEADi).

p) Percent p-Rb (left) and CDK2 activity (right) above bifurcation threshold per bin of cyclin D1/p27 ratio. Cells were tracked during live-cell imaging and aligned after fixation and IF staining to match final cyclin D1/p27 ratio to CDK2 activity of the final live-cell frame (see also Methods). Data are representative of 3 independent experiments, n > 100 cells/bin.

All fixed-cell data are G1-gated, except for percent EdU-positive (all-cell gating).

Supplementary Figure S5, related to Figure 4



Supplementary Figure S5, related to Figure 4

a) Top: GO terms enriched for significantly up-regulated genes after 24-hour YAP induction ($q < 0.05$, $\text{Log2FC} > 1.0$) relative to control (0.1 % FBS). Bottom: GO process terms enriched for significantly down-regulated genes ($q < 0.05$, $\text{Log2FC} < -1.0$).

b) EGFR knockdown (48h) in starved WT and CA-YAP cells. Median and IQR of EGFR levels (left), percent EdU-positive (middle), and final cell density (cell area/site area, right). Student's t test: $p = 0.85$ (WT), 0.57 (CA-YAP).

c) Mean percent EdU-positive for serum-starved WT and CA-YAP cells treated with EGFR inhibitor, gefitinib (left), or WT, LATSi-treated WT, and CA-YAP treated with erlotinib (right) at indicated doses (μM) for 24h. $N = 2$ independent experiments. Ordinary one-way ANOVA: gefitinib, $p = 0.46$ (WT), 0.014 (CA-YAP); erlotinib, $p = 0.30$ (WT), 0.0091 (LATSi), 0.0054 (CA-YAP).

d) Mean percent p-Rb (G1-gated) for serum-starved WT, LATSi-treated WT, and CA-YAP cells treated with 0-10 μM RASi (BI-2865). $N = 2$ independent experiments, where matched colors indicate data from the same experiment. Ordinary one-way ANOVA: $p = 0.21$ (WT), 0.012 (LATSi), 5×10^{-4} (CA-YAP).

e) Percent p-Rb (G1-gated) per local density bin for serum-starved CA-YAP (left) and LATSi-treated WT (right) treated with 0-10 μM SHP2i (RMC-4550) for 24h. Data are representative of 2 independent experiments.

f) Mean percent p-Rb (G1-gated) for WT, LATSi-treated WT, and CA-YAP cells treated with 0-10 μM RASi (BI-2865) in serum. $N = 2$ independent experiments, where matched colors indicate data from the same experiment. Ordinary one-way ANOVA: $p = 0.22$ (WT), 0.0019 (LATSi), 0.0075 (CA-YAP).

g) Mean percent p-Rb (G1-gated) for serum-starved WT and CA-YAP cells with siRNA knockdown (48h) targeting control, YAP/TAZ, cyclin D1, and p27, and treated with control or EGF release for 14h.

h) Histogram distribution of Fra1 protein levels for DMSO-treated (top, CTRL) or LATSi-pretreated cells (bottom, LATSi sustained and washout). Data are representative of 3 independent experiments.

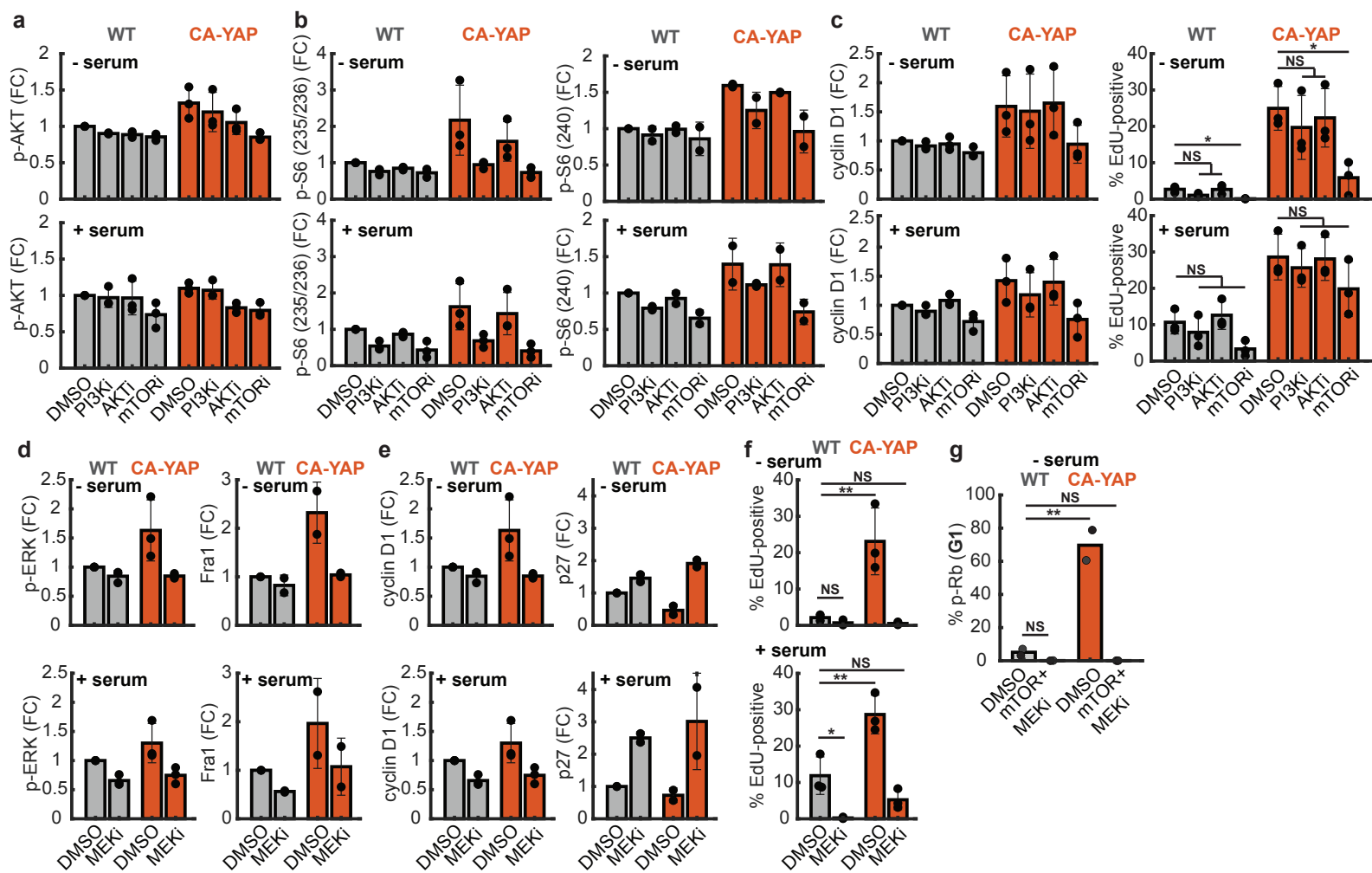
i) Top: experimental conditions for starvation of WT cells and pre-treatment with DMSO or LATSi (5 μM , TRULI) for 24h prior to washout and 14h mitogen treatment (25 ng/mL EGF and HGF, 1 μM LPA). Bottom: Mean percent p-Rb (G1-gated) with mitogen treatment. $N = 2$ independent experiments, where matched colors indicate well-replicates from the same experiment.

j-m) Percent p-Rb (G1-gated) per local density bin (**j**), mean percent p-Rb (**k**), and histogram distribution of p-S6(240/244) (**l**) and Fra1 (**m**) for serum-starved WT cells pre-treated with DMSO prior to 14h EGF (25 ng/mL) or control (CTRL) treatment, seeded at dense density (24,000 cells/well). Data are representative of 2 independent experiments ($n > 25,000$ cells/condition). Ordinary one-way ANOVA with Dunnett's multiple comparisons to DMSO/CTRL: DMSO, $p > 0.99$ (EGF); LATSi sustained, $p = 0.0033$ (CTRL), 0.0011 (EGF); LATSi washout, $p = 0.84$ (CTRL), 0.40 (EGF).

n-p) MCF10A seeded for confluent (6,000 cells/well) or dense (24,000 cells/well) final density, pre-conditioned with starvation (24h), then pre-treated with LATSi (24h), and released with or without LATSi and EGF (20 ng/mL). Mean final cell density (**n-o**, left), percent p-Rb (G1-gated, **n-o**, right), and YAP ratio (**p**) with drug treatments ($n = 2$ independent experiments). Ordinary one-way ANOVA with Dunnett's multiple comparison: to DMSO/CTRL, $p = 0.0006$ (DMSO/EGF, confluent), 0.79 (LATSi/CTRL, confluent), 0.0006 (DMSO/EGF, dense), 0.99 (LATSi/CTRL, dense); to DMSO/EGF, $p = 0.013$ (LATSi/EGF, confluent), 0.97 (LATSi wash/EGF, confluent), 0.0008 (LATSi/EGF, dense), 0.99 (LATSi wash/EGF, dense).

All single-cell data are G1-gated cells and representative of 2-3 independent experiments.

Supplementary Figure S6, related to Figure 5



Supplementary Figure S6, related to Figure 5

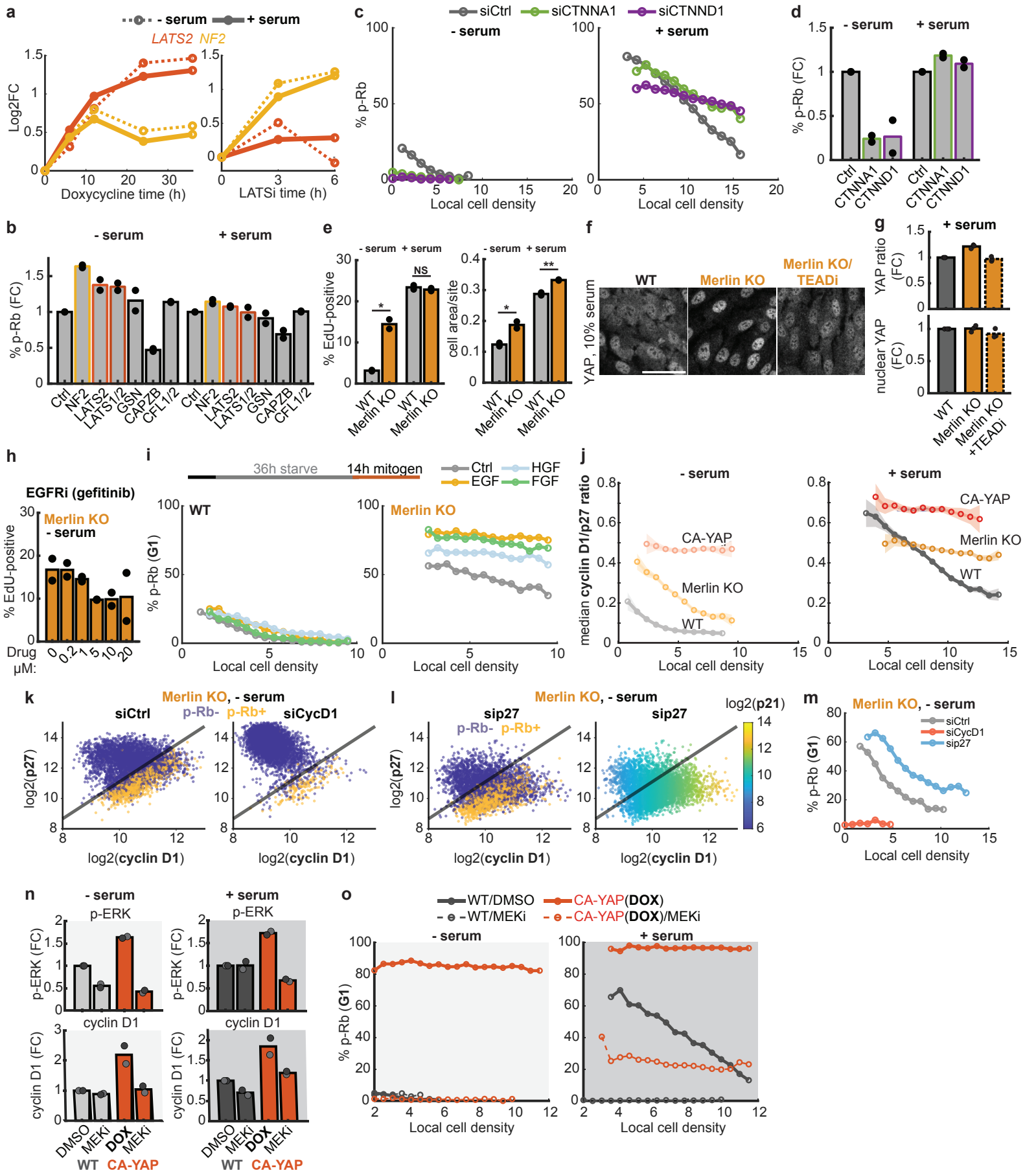
a-c) Fold-change in protein levels of **(a)** p-AKT(S473), **(b)** p-S6 (left: S235/236; right: S240/244), **(c)** cyclin D1 (left) and percent EdU-positive (right) for WT and CA-YAP cells treated with PI3Ki, AKTi, or mTORi, as in Figure 5e with starvation (top) or serum (bottom). N = 2-3 independent experiments. Ordinary one-way ANOVA with Dunnett's multiple comparisons to DMSO: **% EdU-positive, -serum**, $p = 0.080$ (WT/PI3Ki), >0.99 (WT/AKTi), 0.0095 (WT/mTORi), 0.70 (CA-YAP/PI3Ki), 0.94 (CA-YAP/AKTi), 0.027 (CA-YAP/mTORi); **+serum**, $p = 0.66$ (WT/PI3Ki), 0.84 (WT/AKTi), 0.079 (WT/mTORi), 0.89 (CA-YAP/PI3Ki), >0.99 (CA-YAP/AKTi), 0.28 (CA-YAP/mTORi).

d-f) Mean fold-change in protein levels of p-ERK and Fra1 **(d)**, cyclin D1 and p27 **(e)** for WT and CA-YAP cells treated with MEKi in starvation (top) or serum (bottom) conditions, as in Figure 5f. N = 2-3 independent experiments. Ordinary one-way ANOVA with Dunnett's multiple comparisons to DMSO: **% EdU-positive, -serum**, $p = 0.96$ (WT/MEKi), 0.0014 (CA-YAP/DMSO), 0.95 (CA-YAP/MEKi); **+serum**, $p = 0.018$ (WT/MEKi), 0.0021 (CA-YAP/DMSO), 0.17 (CA-YAP/MEKi).

g) Mean percent p-Rb in serum-starved WT and CA-YAP cells treated with DMSO (same as in Figure 5e) or combined mTOR+MEKi (100 nM Torin2, 100 nM PD0325901). N = 2 independent experiments. Ordinary one-way ANOVA with Dunnett's multiple comparisons to DMSO: $p = 0.81$ (WT/mTOR+MEKi), 0.0014 (CA-YAP/DMSO), 0.81 (CA-YAP/mTOR+MEKi).

All data are from G1-gated cells, except for percent EdU-positive (all-cell gating).

Supplementary Figure S7, related to Figure 6



Supplementary Figure S7, related to Figure 6

a) Differential expression (Log2FC) in *LATS2* and *NF2* since doxycycline CA-YAP induction (left) or LATSi treatment (right) in starved (dashed line) and serum conditions (solid line), as in Figure 3a and S4a. Adjusted p-values are in Supplementary Table 3.

b) Mean fold-change in percent p-Rb (all-cell gating) relative to each control for WT cells with siRNA knockdown (48h) of control (Ctrl), *NF2*, *LATS2*, *LATS1/2*, *GSN*, *CAPZB*, *CFL1/2* in starvation or serum conditions. N = 2 independent experiments.

c-d) Percent p-Rb (all-cell gating) per local density bin for WT cells with siRNA knockdown (48h) of control, *CTNNA1* (α -catenin), and *CTNND1* (p120-catenin) in starvation (left) or serum (right) conditions (**c**). Mean fold-change in percent p-Rb for cells, normalized to control of each serum condition (**d**). N = 2 independent experiments.

e) Different percent EdU levels (left) and final density (right) for Merlin KO relative to WT cells in starvation and serum conditions (seeded 8,000 cells/well). N = 2 independent experiments. Student's t test: % EdU-positive, p = 0.010 (Merlin KO, -serum), 0.46 (Merlin KO, +serum); cell area/site, p = 0.0086 (Merlin KO, -serum), 0.0014 (Merlin KO, +serum).

f-g) Representative immunofluorescence images (**f**) of YAP staining and mean fold-change (**g**) in YAP ratio (top) and nuclear YAP levels (bottom) in WT, Merlin KO, and TEADi-treated Merlin KO (0.5 μ M GNE-7883, 24h) in 10% serum. Scale bar = 50 μ m. N = 3 independent experiments.

h) Mean EdU-positive for serum-starved Merlin KO cells treated with gefitinib (EGFRi) at 0-20 μ M for 24h. N = 2 independent experiments. Ordinary one-way ANOVA: NS, p = 0.29.

i) Top: starvation-release protocol for WT or Merlin KO cells with control (CTRL), EGF, HGF, or FGF (all 25 ng/mL) for 14h. Bottom: percent p-Rb (G1-gated) per bin comparing WT (left) and Merlin KO (right) treated with CTRL or mitogens. N > 12,000 cells/condition, data are representative of 2 independent experiments.

j) Median cyclin D1/p27 ratio (G1-gated) per local density bin for WT (grey), Merlin KO (yellow), and CA-YAP cells (red) in 0.1% (left) or 10% serum (right). Shaded error are 95% confidence intervals, data are representative of 2 independent experiments.

k-m) Cyclin D1/p27 single-cell scatterplots of serum-starved Merlin KO with siRNA knockdown (48h) of control, cyclin D1, and p27, colored by binary p-Rb state (**k-l**) or p21 levels (**l**, right). Percent p-Rb per local density bin (**m**). Data are G1-gated and representative of 2 independent experiments.

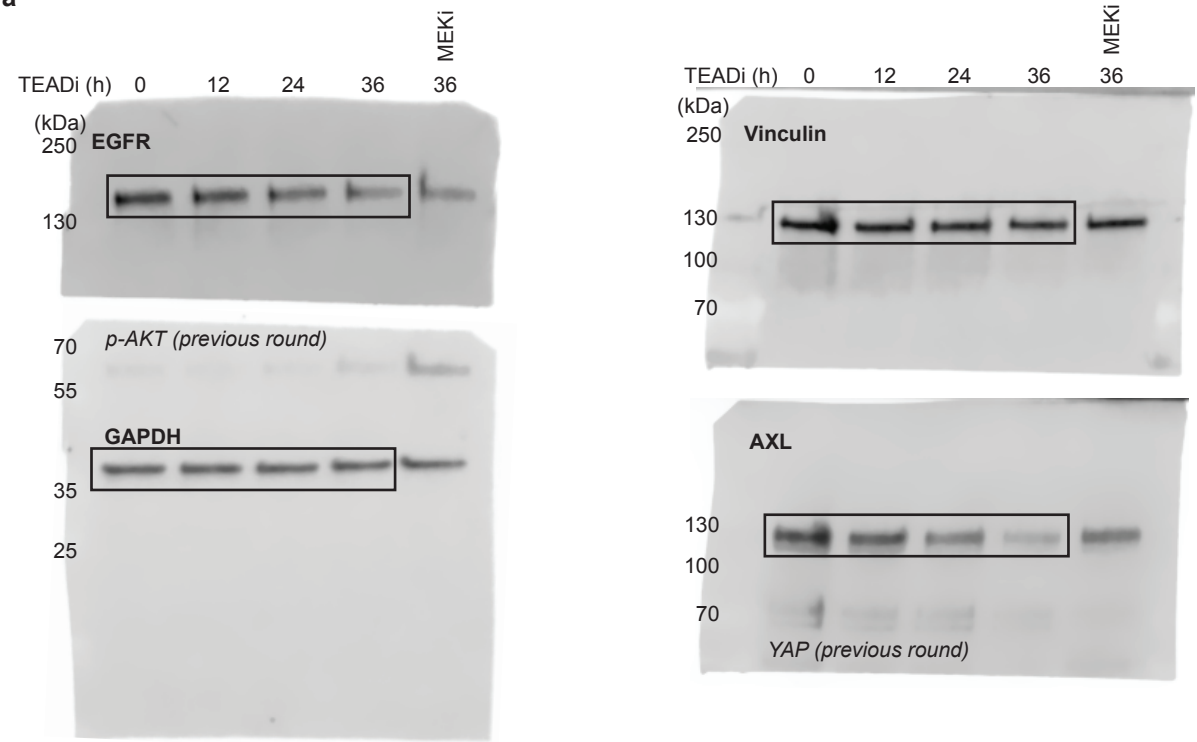
n) Mean fold-change in p-ERK (top) and cyclin D1 (bottom) for WT and DOX-induced CA-YAP cells treated as in Figure 5f in starvation (left) and 10% serum conditions (right). N = 2 independent experiments, where matched colors indicate values from the same experiment.

o) Percent p-Rb (G1-gated) per local density bin for WT and DOX-induced CA-YAP cells treated as in (**n**). Starved: n > 5,000 cells (WT), n > 20,000 cells/condition (CA-YAP); 10% serum: n > 10,000 cells (WT), n > 20,000 cells/condition. Data are representative of 2 independent experiments.

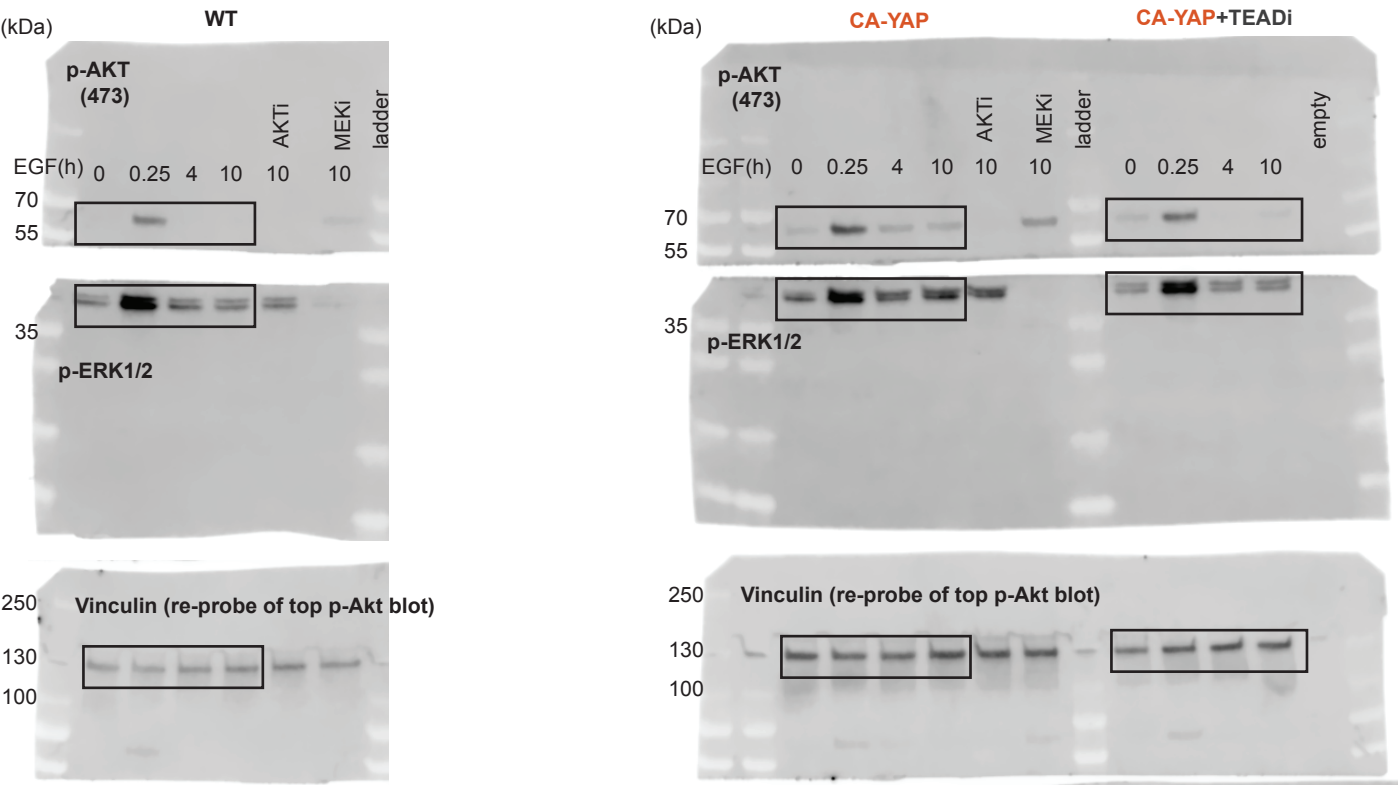
All cyclin D1, p27, and p21 data are G1-gated. P-Rb is G1-gated when indicated, otherwise includes all cells. Binned data are from a representative experiment and contain n > 100 cells/bin.

Supplementary Figure S8, related to Figure 4

a



b



Supplementary Figure S8, related to Figure 4

- a)** Uncropped western blots related to Figure 4b. CA-YAP cells treated with TEAD inhibitor for 0-36h or MEK inhibitor (not included in the main figure). Additional bands on lower blots are from immunostaining of additional proteins (not included in the main figure).
- b)** Uncropped western blots related to Figure 4f. WT (left blot) and DMSO or TEAD inhibitor pre-treated CA-YAP cells (right blot) treated with EGF for 0-10h, or EGF with AKT and MEK inhibitors (not included in the main figure). The blot with WT samples was cropped to remove sample conditions unrelated to the manuscript.

Supplementary Table S1. Oligonucleotide sequences and application.

Oligo	Sequence (5' → 3')		Application
Merlin KO sgRNA (cloned into px459)	Forward: CACCGCACAGTGGCCTGGCTCAAAA Reverse: AAACTTTTGAGCCAGGCCACTGTGC		Merlin KO generation
Pooled sgRNA non-targeting control	GATACGTCGGTACCGGACCG GTAACGCGAACTACGCGGGT GTCGACGTTATTGCCGGTCG GGAAACCTACGTCGACGAAT	U-009500- 10-05	Transient transfection
Pooled sgRNA CCND1	CCAAACGCCGGGAGCAGCGA GAGCCCAAAGCCATCCCTG ATCAAAGCCCGGCAGAGAAT GCCTGGGGAGACCACGAGAA	P-003210- 01-0005	Transient transfection
Pooled sgRNA CDKN1B (p27)	CAGCTCGCTAGGAGCCGGGG GGTCCAGGTCCCGGCTTCCC GCGACCAGCCAATCTCCCGG CGCTCGGGAACGAGGGGAGG	P-003472- 01-0005	Transient transfection
Pooled siRNA control	GCCCUUCUCUGCAGUCAAG UGGUUUACAUGUUGUGUGA UGGUUUACAUGUUUUCUGA UGGUUUACAUGUUUCCUA	D-001810- 10-05	Transient transfection
Pooled siRNA CCND1	ACAACUCCUGUCCUACUA GUUCGUGGCCUCUAAGAUG GCAUGUAGUCACUUUAUAA GCGUGUAGCUAUGGAAGUU	L-003210-00- 0005	Transient transfection
Pooled siRNA CDKN1B (p27)	CAGCTCGCTAGGAGCCGGGG GGTCCAGGTCCCGGCTTCCC GCGACCAGCCAATCTCCCGG CGCTCGGGAACGAGGGGAGG	L-040178-00- 0005	Transient transfection
Pooled siRNA LATS1	GGUGAAGUCUGUCUAGCAA UAGCAUGGAUUUCAGUAAU GGUAGUUCGUCUAUAUUAU GAAUGGUACUGGACAAACU	L-004632-00	Transient transfection
Pooled siRNA LATS2	GCACGCAUUUUACGAAUUC ACACUCACCUCGCCCAAUA AAUCAGAUAUCCUUGUUG GAAGUGAACCGGCAAUUGC	L-003865-00	Transient transfection
Pooled siRNA CFL1	CCUCUAUGAUGCAACCUAU CAUGGAAGCAGGACCAGUA UAAUGGAAUGUUGUGGAG ACUCUGUGCUUGUCUGUUU	L-012707-00	Transient transfection
Pooled siRNA CFL2	AAAGCAAGAUGAUUUUAGC AAACAUGAGUGGCAAGUAA GAGUUACAGUGAAUGAUGA AAGGAAAUCUUCUACACAA	L-019078-00	Transient transfection
Pooled siRNA CAPZB	GGAGUGAUCCUCAUAAAGA GAAGUACGCUGAACGAGAU CAAAGGAUAUCGUCAAUGG	L-011990-00	Transient transfection

	CACCAUGGAGUAACAAGUA		
Pooled siRNA GSN	CUGUUGAGGUUUUGCCUAA GCUAAGCGGUACAUCGAGA GCACUGAACUCCAACGAUG GAACGGAAAUCUGCAGUAU	L-007775-00	Transient transfection
Pooled siRNA CTNNA1	GAAGAGAGGUCGUUCUAAG GAUGGUAUCUUGAAGUUGA GUGGAUAAGCUGAACAUUA AGUAAAGGGCCCUCUAAUA	L-010505-00	Transient transfection
Pooled siRNA CTNND1	GGAAUGUGAUGGUUUAGUU UAGCUGACCUCCUGACUAA GGACCUUACUGAAGUUAUU GAGUGAAGCUCGCCGAAA	L-012572-00	Transient transfection
siRNA NF2 (Merlin)	UGGCCAACGAAGCACUGAUUU	IDT (Okada et al., 2005)	Transient transfection
siRNA YAP	GACAUCUUCUGGUCAGAGA dTdT	IDT (Dupont et al., 2011)	Transient transfection
siRNA WWTR1 (TAZ)	ACGUUGACUUAGGAACUUU dTdT	IDT (Dupont et al., 2011)	Transient transfection

Supplementary Table S2. Antibody dilutions and application.

Antibody	Vendor, Catalog #	Dilution	Application
Goat anti-mouse polyclonal secondary antibody (IRDye 800CW)	LI-COR Biosciences, CAT#925-32210	1:10,000	Western blotting
Goat anti-mouse polyclonal secondary antibody (IRDye 680LT)	LI-COR Biosciences, CAT#925-68020	1:10,000	Western blotting
Goat anti-rabbit polyclonal secondary antibody (IRDye 680LT)	LI-COR Biosciences, CAT#925-68021	1:10,000	Western blotting
Goat anti-mouse polyclonal secondary antibody (IRDye 800CW)	LI-COR Biosciences, CAT#925-32211	1:10,000	Western blotting
Goat anti-rabbit, HRP-linked secondary antibody	Cell Signaling Technology, CAT#7074	1:5,000	Western blotting
Goat anti-mouse, HRP-linked secondary antibody	Cell Signaling Technology, CAT#7076	1:5,000	Western blotting
Goat anti-mouse secondary antibody, Alexa Fluor 488	Thermo Scientific, CAT#A-11029	1:1,000	Immunofluorescence
Goat anti-mouse secondary antibody, Alexa Fluor 514	Thermo Scientific, CAT#A-31555	1:1,000	Immunofluorescence
Goat anti-mouse secondary antibody, Alexa Fluor 568	Thermo Scientific, CAT#A-11004	1:1,000	Immunofluorescence
Goat anti-rabbit secondary antibody, Alexa Fluor 488	Thermo Scientific, CAT#A-11008	1:1,000	Immunofluorescence
Goat anti-rabbit secondary antibody, Alexa Fluor 568	Thermo Scientific, CAT#A-11011	1:1,000	Immunofluorescence
Goat anti-rabbit secondary antibody, Alexa Fluor 647	Thermo Scientific, CAT#A-21245	1:1,000	Immunofluorescence
ANKRD1, rabbit polyclonal	Abcam, CAT#ab272894	1:2,000	Western blotting
AXL (C89E7, rabbit monoclonal)	Cell Signaling Technology, CAT#86616	1:1000	Western blotting
CDK6, rabbit monoclonal	Abcam CAT#ab124821	1:1000	Immunofluorescence

c-myc (D84C12, rabbit monoclonal)	Cell Signaling Technology, CAT#5605	1:800	Immunofluorescence
Cyclin D1 (A-12, mouse monoclonal)	Santa Cruz Biotechnology, CAT#sc-8396	1:200	Immunofluorescence
EGFR (D38B1, XP rabbit monoclonal)	CAT#4267	1:1,000	Western blotting
Fra-1 (C-12, mouse monoclonal)	Santa Cruz Biotechnology CAT#sc-28310	1:200	Immunofluorescence
GAPDH Rabbit monoclonal	Cell Signaling Technology, Cat#5174	1:5,000	Western blotting
Myc epitope, mouse monoclonal	Cell Signaling Technology, Cat#2276	1:8,000	Immunofluorescence
N-cadherin (H-2, mouse monoclonal)	Santa Cruz Biotechnology CAT#sc-393933	1:300	Immunofluorescence
P21 Waf1/Cip1 (SX118, mouse monoclonal)	BD Biosciences CAT#556430	1:500 (IF, round 1 only)	Immunofluorescence
P21 Waf1/Cip1 (12D1, rabbit monoclonal)	Cell Signaling Technology, CAT#2947	1:2500 (IF, any round)	Immunofluorescence
P27 Kip1 (SX53G8.5, mouse monoclonal)	Cell Signaling Technology, CAT#3698	1:1000 (IF, WB)	Immunofluorescence
P27 Kip1 (D69C12, XP rabbit monoclonal)	Cell Signaling Technology, CAT#3686	1:1600	Immunofluorescence
Phospho-Akt (Ser473, D9E, XP rabbit monoclonal)	Cell Signaling Technology, CAT#4060	1:200 (IF)	Immunofluorescence, western blotting
Phospho-Erk1/2 (P44/42 MAPK Thr202/Tyr204)	Cell Signaling Technology, CAT#4370	1:400 (IF) 1:1000 (WB)	Immunofluorescence, western blotting
Phospho-Rb (Ser807/811, D10B12, XP rabbit monoclonal)	Cell Signaling Technology, CAT#8516	1:2500 (IF) 1:1000 (WB)	Immunofluorescence, western blotting
Phospho S6 (Ser235/236, E2R10, mouse monoclonal)	Cell Signaling Technology, CAT#62016	1:800	Immunofluorescence
Phospho S6 (Ser240/244, D68F8, XP rabbit monoclonal)	Cell Signaling Technology, CAT#5364	1:2500 (IF)	Immunofluorescence
Skp2 (D3G5, XP rabbit monoclonal)	Cell Signaling Technology, CAT#2652	1:800 (IF)	Immunofluorescence
Vinculin (7F9, mouse monoclonal)	Thermo Scientific, CAT#14-97777-80	1:1000	Western blotting

YAP (63.7, mouse monoclonal)	Santa Cruz Biotechnology, CAT#sc-101199	1:200 (IF, any round)	Immunofluorescence
YAP (D8H1X, XP rabbit monoclonal)	Cell Signaling Technology, CAT#14074	1:400 (IF, round 1 only)	Immunofluorescence