Supplemental Materials and Methods

Cell Culture

Cells were maintained at 37°C in a 5% CO_2 incubator. HEK293 (human embryonic kidney cell line), IMR90 (primary human fetal lung fibroblasts, ATCC Cat#CCL-186), HFF (human foreskin fibroblasts, NDRI Lot#ND03285), BRK (baby rat kidney cells) and BRK E1A and E1B-transformed cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum. Human Bronchial/Tracheal Epithelial Cells (HBTEC, Lifeline Cell Technology Cat# FC-0035; lot# 02196) in BronchiaLife Medium Complete Kit (Lifeline Cell Technology catalog number: LL-0023).

RNA-seq Procedure and Data Analysis

$1X10^6$ HEK293 were transfected with indicated siRNAs for 4, 8, or 16 days. RNA was isolated using QIAGEN RNeasy Plus Mini Kit (Cat#74134). Eluted RNA was treated with Ambion DNA-free™ DNA Removal Kit (Cat#1906) and then Ambion TRIzol reagent, precipitated with isopropanol, and dissolved in sterile water. RNA concentration was measured with a Qubit fluorometer. One microgram of RNA was fragmented and copied into DNA then PCR amplified with bar-coded primers for separate samples to prepare sequencing libraries using the Illumina TruSeq RNA Sample Preparation procedure (Cat#RS-122-2101). Libraries were sequenced using the Illumina Hiseq-4000 to obtain single end 50-base-long reads. Sequences were aligned to the hg19 human genome sequence using TopHat v2. FPKM (fragments per kb per million mapped reads) for each annotated hg19 RefSeq gene ID was determined using Cuffdiff v2 from Cufflinks RNA-Seq analysis tools at http://cufflinks.cbcb.umd.edu. All RNA-seq was performed using biological duplicates and FPKM values represent normalized averages of duplicates. Published RNA-seq datasets from infected IMR90 (Ferrari et al. 2014) and infected HBTEC (Zemke and Berk 2017) are available on GEO: GSE59688 & GSE105039. RNA-seq from infected HFF was generated similarly as above except confluent HFF cells were infected at an MOI of 60 with a small E1A expressing Ad5 vector described below for 24 hours prior to RNA preparation. Gene ontology analysis was performed using DAVID Functional Annotation Tool (Huang et al., 2009a, 2009b). The web-based tool, SaVanT (Lopez et al., 2017) was used to determine the cell types with expression signatures most closely resembling expression from 16 day E1AKD HEK293.

ChIP-Seq

ChIP-seq was performed using $1x10^7$ siRNA transfected HEK293. Cells were transfected for 1 or 4 days for H3K18ac (814) and H3K27ac (Active Motif) ChIP, 4 days for H3K4me1 (abcam), RAD21 (abcam), and 8 days for YAP (CST D8H1X), TEAD1 (BD Transduction Laboratories), and TEAD4 (Santa Cruz N-G2). For H3K18ac, H3K27ac, H3K4me1 and RAD21 ChIP-seqs cells were cross-linked for 1% formaldehyde for 10 minutes at room temperature on rotator. Formaldehyde crosslinking was quenched with 0.14M glycine for 30 minutes at room temperature on rotator. Cells were washed with PBS and scraped from plates in PBS with Roche protease inhibitor cocktail (Cat#04693132001). Cells were pelleted and lysed in 400uL lysis buffer (1% SDS, 50mM Tris-HCl pH8, 20mM EDTA, Roche complete protease inhibitors) and sonicated at 4°C using the Qsonica Q800R2 at 20% amplitude 10s on 30s off until DNA fragments from sheared chromatin were mostly between the sizes of mostly ~200-600 base pairs. Samples were normalized for equal amounts of DNA as measured by Qubit fluorometer in sonicated, cross-linked chromatin prior to pre-clear and IP. Up to 100uL of sonicated chromatin was diluted in 10X lysis dilution buffer (16.7 mM Tris-HCl, 1.1% Triton X-100, 1.2mM EDTA, 167mM NaCl) and and precleared for 1h 4°C with 30uL of protein A or G dynabeads (ThermoFisher Cat#10001D or Cat#10003D) washed 10X lysis dilution buffer on nutator. IPs were performed O/N at 4°C on nutator with precleared chromatin and 2ug of antibody or 5uL of H3K18ac anti-rabbit sera. 50uL of dynabeads were added for 4h on nutator at 4°C. Bead-immunocomplexes were washed for 5min 2X with each of the following buffers in order: wash buffer A (50mM Hepes pH 7.9, 0.1% SDS, 1% Triton X-100, 0.1% Deoxycholate, 1mM EDTA, 140mM NaCl), wash buffer B (50mM Hepes pH 7.9, 0.1% SDS, 1% Triton X-100, 0.1% Deoxycholate, 1mM EDTA, 500mM NaCl), LiCl buffer (20mM Tris-HCl pH8, 0.5% NP-40, 0.5% Deoxycholate, 1mM EDTA, 250mM LiCl), TE (50mM Tris-HCl pH8, 1mM EDTA). Elution was performed in 150uL of elution buffer (50mM Tris HCl pH8, 1mM EDTA, 1% SDS) then ChIP samples and inputs (10uL of precleared chromatin lysis plus 140uL elution buffer) were reverse crosslinked O/N at 65°C. Samples were RNase A (10ug) treated for 1h at 37°C then proteinase K (20ug) 2h at 56°C and DNA was purified and extracted with phenol/chloroform and ethanol precipitated. DNA pellets were resuspended in 12uL of TE and measured using Qubit

fluorometer. YAP, TEAD1, and TEAD4 ChIP-seqs were performed similarly with the following modifications: cells were double-crosslinked with 4mM DSG in PBS for 30min then 1% formaldehyde for 10 min, crosslinking was quenched in 500mM Tris pH7.9 for 20min and cell pellets were lysed in 1mL lysis buffer 1 (50mM HEPES-KOH, pH 7.5, 140mM NaCl, 1mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100, Roche cOmplete protease inhibitors) for 10min on ice. Lysate was pelleted at 3000 rpm 5min 4°C then resuspended in 1mL lysis buffer 2 (10mM Tris-HCl, pH 8.0, 200mM NaCl, 1mM EDTA, 0.5mM EGTA, Roche complete protease inhibitors) and placed on nutator 10min 4°C and pelleted as before, then resuspended in 125uL of lysis buffer 3 (10mM Tris-HCl, pH 8.0, 100mM NaCl, 1mM EDTA, 0.5mM EGTA, 0.1% Na-Deoxycholate, 0.5% N-lauroylsarcosine, Roche complete protease inhibitors) and sonicated, 2ug of antibody was used, magnetic beads were washed and blocked in 0.5% BSA in PBS. Sequencing libraries were constructed from 1ng of immunoprecipitated and input DNA using the KAPA Hyper Prep Kit (KAPA Biosystems Cat#KK8504) and NEXTflex ChIP-Seq (BIOO Scientific Cat#NOVA-514121).

Data Analysis of ChIP-seq

ChIP-seq libraries were sequenced using Hiseq-2500 or 4000 systems for single-end 50 base pair reads. Reads were mapped to the hg19 human genome reference using Bowtie2 software. Only reads that aligned to a unique position in the genome with no more than two sequence mismatches were retained for further analysis. Duplicate reads that mapped to the same exact location in the genome were counted only once to reduce clonal amplification effects. A custom algorithm executed by MATLAB was used for further processing including peak calling (p-value < 10^{-6} were considered significant). The genome was tiled into 50 base pair windows and each read was extended by 150 bases and was counted as one read to each window to which it partially or fully matched. The total counts of the input and ChIP samples were normalized to each other. Samples were normalized for equal number of uniquely mapped reads. The input sample was used to estimate the expected counts in a window. Wiggle files were generated using a custom algorithm and present the data as normalized tag density as seen in all figures with genome browser shots. Metagene plots displaying normalized average relative ChIP-seq signals were generated using CEAS software. For determining super-enhancers with H3K27ac ChIP-seq in 4 day E1AKD293 cells we used ROSE software (Lovén et al., 2013; Whyte et al., 2013). Using the ROSE-generated list of super-enhancers we generated a list of super-enhancer associated genes in E1AKD HEK293 by assigning the closest TSS of an expressed gene (>1 FPKM) to the center of a super-enhancer.

ATAC-seq and data analysis

ATAC-seq (Omni-ATAC protocol) was performed as described in Corces et al. 2017. HEK293 cells siRNA transfected for 4 days were lifted from plates using Accutase (Innovative Cell Technologies, Inc.) and 50,000 viable cells were gently pelleted at 500 RCF 4°C for 5min and resuspended in 50 uL cold ATAC-Resuspension Buffer (RSB) containing 0.1% NP40, 0.1% Tween-20, and 0.01% Digitonin. Cells were incubated on ice for 3min then washed with 1 ml of cold ATAC-RSB containing 0.1% Tween-20 to lyse plasma membranes. Nuclei were gently pelleted at 500 RCF for 10min at 4°C and resuspended in 50 uL of transposition mixture: 25 uL 2x TD buffer and 2.5 uL transposase (both supplied in the Nextera DNA Library Prep Kit Cat#FC-121-1030), 16.5 uL PBS, 0.5 uL 1% digitonin, 0.5 uL 10% Tween-20, 5 uL H2O. DNA was isolated using DNA Clean and Concentrator-5 Kit (Zymo Cat#D4014) and eluted in 21uL of elution buffer. 20uL of eluted DNA was amplified for 5 cycles Amplify for 5 cycles using NEBNext 2x Master Mix (New England BioLabs Cat#M0541S) with 25uM primer Ad1 + 25uM primer Ad2 or 25uM Ad1 + 25uM Ad3. Refer to Corces et al. 2017 for PCR protocol and primer sequences. 5uL of preamplified sample was used in qPCR to determine additional cycles needed. Libraries were sequenced using Hi-seq-4000 single-end 50bp reads. Reads were aligned to hg19 reference genome using bowtie2. Macs14 was used for calling peaks with the following parameters: --space=50 --keep-dup all --nomodel -p 1e-6. The summits of peaks were used as the location for the center of peaks when doing motif analysis and metagene profiling. Motif analysis was performed on ATAC-seq peaks that increased >5X in signal with siE1A treatment compared to siCtrl using Homer (http://homer.salk.edu/homer PMID: 20513432) to search for enriched motifs +/- 300 bp from peak summits.

Confocal microscopy

250,000 cells were plated on fibronectin coated glass cover slips in 6-well plates. Cells were transfected or infected as indicated prior to fixing with 4% formaldehyde in PBS for 10min. Cells were then

permeabilized with 0.1% Triton-X 100 in PBS for 10min. Next, cells were incubated in block buffer (1% BSA, 0.1% Tween 20 in PBS) for 30 min. Cells were incubated with primary antibody, YAP (CST D8H1X), TAZ (Thermo CL0371), AMOT (CST D2O4H) at 1:200 dilutions and incubated for 1h. Cells were washed 3X with PBS then incubated with secondary Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) Cat#4412 or Anti-mouse IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 555 Conjugate) Cat#4409 (CST 1:1000) and if indicated Cytopainter phalloidin-ifluor 555 reagent (abcam ab176756, 1:1000) for 1h diluted in block buffer. DAPI was added for 5 minutes at 1 ug/mL. Slides were mounted with VECTASHIELD Hardset Antifade Mounting Medium (Vector Laboratories Cat. No: H-1400). Micrographs were taken using confocal scanning laser microscopy on a Leica TCS SP8 100X or 40X numerical aperture oil immersion objective. All scare bars represent 20 μm.

Ad Vector infections

*dl*1500, an Ad2 mutant vector containing a deletion removing the 13S E1A mRNA 5' splice site (Montell et al., 1984) or the ΔE1A vector *dl*312 (Jones and Shenk 1979) were used for a 4 day infection of IMR90 at an MOI of 10 prior to fixing for confocal microscopy (fig S2D). Ad5 small E1A vectors (e1aWT or P300b$^-$) expressed Ad2 WT or mutant small E1A from the normal E1A promoter with the *dl*1500 deletion removing the 13S E1A mRNA 5' splice site (Ferrari et al. 2014). The vectors were constructed using the Ψ5 vector and in vivo Cre24 mediated recombination (Hardy et al., 1997), and consequently contain an out of frame insertion of a LoxP site at the Bgl II site in the region encoding the carboxy-terminus of E1B-55K. These vectors were used to infect IMR90, HFF, and HBTEC for 24 hours at an MOI 40 in IMR90 (Ferrari et al. 2014), MOI 60 in HFF, and MOI 60 in HBTEC (Zemke and Berk 2017) prior to RNA-seq (fig S1A).

siRNA and plasmid Transfections and small molecule treatment

siRNA KD was performed in HEK293, HBTEC, and IMR90 using Invitrogen RNAiMAX reverse transfection protocol. Cells were plated in antibiotic free 10% FBS DMEM containing indicated Ambion/ThermoFisher Silencer Select siRNA for a final concentration of 10nM that was pre-incubated in 7.5uL of lipofectamine RNAiMAX (ThermoFisher Cat#13778075) reagent in 750uL of Opti-MEM in 6cm^2. Media and reagents were scaled up accordingly for 15cm^2 and scaled down when using 6-well plates based on surface areas. For a complete list of Ambion/ThermoFisher Silencer Select siRNAs used and sequences see key resources table. Ambion/ThermoFisher Silencer Negative Control no.1 AM4611 was used as a negative control (siCtrl). Double, triple and quadruple knock-downs were performed with a total siRNA concentration of 10nM. Any parallel knock-downs where cells were treated with fewer siRNAs they were used at the same concentration as any individual siRNA from the cells treated with the most number of siRNAs but were supplemented with negative control siRNA (siCtrl) to have the same total siRNA concentration of 10nM. pRK5-myc-RhoA-Q63L was a gift from Gary Bokoch (Addgene plasmid # 12964) pRK5-myc-RhoA-Q63L or a control empty vector, pAdlox, was transfected into HEK293. 2.5 ug of DNA was transfected into a HEK293 confluent monolayer in 6-well plates on glass coverslips. Plasmid DNA was pre-incubated with 10uL of lipofectamine 2000 transfection reagent (ThermoFisher Cat#11668019) in 250 uL in Opti-MEM for 5 minutes before adding it to cells. Transfections proceeded for 3 days prior to fixing and confocal microscopy (fig 4D). To block WNT signaling in E1AKD HEK293 cells were treated with 5uM IWP-2 Wnt Antagonist II (EMD Millipore Cat#681671) or DMSO (negative control) for 4 days beginning at time of siRNA transfection. To block ubiquitin-mediated degradation HEK293 cells were treated with 5uM MLN4942 NEDD8 E1 Activating Enzyme Inhibitor (NAE Inhibitor R&D Systems, Inc. Cat#I-502-01M) or DMSO (negative control) for 8 hours.

Western blot

Proteins were extracted from indicated cells by lysis in EBC (120 mM NaCl, 0.5% NP-40, 50 mM Tris-Cl pH 8.0, and Roche cOmplete protease inhibitors Cat#04693132001). Protein concentration was quantified by Bradford assay and normalized in Laemmli buffer and heated for 10min at 65°C then resolved in a 9% SDS-polyacrylamide gel. Proteins were electrotransferred to a polyvinylidene difluoride (PVDF) membrane then blocked in 5% milk in TBS-Tween 0.1% (blocking buffer) for 30 minutes. Primary antibody (M58 (anti-E1A), H3K18ac (814), H3K27ac, KU-86 (H-300), Beta-Actin anti-YAP (D8H1X), TAZ (D3I6D), anti-Phospho-YAP (D9W2I), anti-LATS1 (C66B5), anti-LATS2 (D83D6), anti-Phospho-LATS1 (D57D3), AMOT (D2O4H), AMOTL1 (PA5-42267), AMOTL2 (PA5-78770), or Wnt5a/b (C27E8)) was added at manufacturer recommended dilutions for 1h at room temperature or O/N at 4°C. Membranes were washed 3X in TBS-Tween (0.1%) then HRP conjugated anti-mouse or anti-rabbit secondary

antibodies were added for 1h room temperature in blocking buffer. Membranes were then washed 3X in TBS-Tween (0.1%) prior to addition of ECL reagent for detection of chemiluminescence. Western blots were validated with replicates of two or more with representative western blots presented.

qRT-PCR

Cells were collected at indicated times following transfection and RNA was isolated using QIAGEN RNeasy Plus Mini Kit (Cat#74134). 1ug of RNA, as measured by Qubit fluorometer, was used for reverse transcription with SuperScript III First-Strand Synthesis SuperMix using random hexamer primers. qRT-PCR was performed with 5uL of cDNA, diluted 1:10. Runs were done using an ABI 7500 Real Time Thermocycler and reactions took place in optical-grade, 96-well plates (Applied Biosystems, Carlsbad, CA, USA) 25uL total volume with primers at a concentration of 900nM and 12.5uL of 2X FastStart Universal SYBR Green Master (Rox) (Roche Cat#04913850001). Relative mRNA levels were calculated as $2^{\Delta Ct}$. Equal cDNA loading was confirmed with primers to 18s rRNA cDNA. Data are presented as average of 3 or more biological replicates ± standard deviation.

Sequenced Data Availability

ChIP-seq, mRNA-seq, and ATAC-seq data were deposited in the Gene Expression Omnibus (GEO) database under the accession number GSE130137

List of Antibodies and Oligonucleotides

Antibodies		
Rabbit polyclonal anti-H3K27ac	Active Motif	Cat#39133; RRID: AB_2561016; Lot#31814008
Rabbit polyclonal anti-H3K18ac (814)	Grunstein/Kurdistani laboratories	(Suka et al., 2001)
Mouse monoclonal anti-E1A (M58) hybridoma supernatant	Produced in house	(Harlow et al., 1985)
Mouse monoclonal anti-WWTR1 (TAZ) (CL0371)	ThermoFisher	Cat#MA5-24604
Rabbit polyclonal anti-Beta-Actin	GeneTex	Cat#GTX16039; RRID: AB_367276
Rabbit polyclonal anti-KU-86 (H-300)	Santa Cruz	Cat#sc-9034; RRID: AB_2218743
Mouse monoclonal anti-TEF-3 a.k.a. TEAD4 (N-G2)	Santa Cruz	Cat#sc-101184
Rabbit polyclonal anti-H3K4me1	abcam	Cat#ab8895
Mouse monoclonal anti-TEF1 a.k.a. TEAD1 (31/TEF1)	BD Transduction Laboratories	Cat#610922
Rabbit polyclonal anti-RAD21	abcam	Cat#ab992
Rabbit monoclonal anti-YAP (D8H1X)	Cell Signaling Technology	Cat#14074
Rabbit monoclonal anti-Wnt5a/b (C27E8)	Cell Signaling Technology	Cat#2530
Rabbit monoclonal anti-Angiomotin (D2O4H)	Cell Signaling Technology	Cat#43130
Rabbit monoclonal anti-Phospho-YAP (Ser127) (D9W2I)	Cell Signaling Technology	Cat#13008
Rabbit monoclonal anti-LATS1 (C66B5)	Cell Signaling Technology	Cat#3477
Rabbit monoclonal anti-LATS2 (D83D6)	Cell Signaling Technology	Cat#5888
Rabbit monoclonal anti-TAZ (D3I6D)	Cell Signaling Technology	Cat#70148

Rabbit monoclonal anti-Phospho-LATS1 (Thr1079) (D57D3)	Cell Signaling Technology	Cat#8654
Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate)	Cell Signaling Technology	Cat#4412
Anti-mouse IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 555 Conjugate)	Cell Signaling Technology	Cat#4409
Rabbit polyclonal anti-AMOTL1	ThermoFisher	Product#PA5-42267 RRID:AB_2606805
Rabbit polyclonal anti-AMOTL2	ThermoFisher	Product#PA5-78770 RRID: AB_2745886
Oligonucleotides		
Silencer Select siRNA YAP1 Sense: AGAUACUUCUUAAAUCACAtt Antisense: UGUGAUUUAAGAAGUAUCUct	ThermoFisher	Assay ID: s534572
Silencer Select siRNA CDC42 Sense: AGAUCUAGUUUAGAAAACAtt Antisense: UGUUUUCUAAACUAGAUCUag	ThermoFisher	Assay ID: s227090
Silencer Select siRNA WWTR1 (TAZ) Sense: AAACACCCAUGAACAUCAAtt Antisense: UUGAUGUUCAUGGGUGUUUgt	ThermoFisher	Assay ID: s24788
Silencer Select siRNA RHOA Sense: CACAGUGUUUGAGAACUAUtt Antisense: AUAGUUCUCAAACACUGUGgg	ThermoFisher	Assay ID: s758
Silencer Select siRNA RAC1 Sense: ACAGAUUAAUUUUUCCAUAtt Antisense: UAUGGAAAAAUUAAUCUGUaa	ThermoFisher	Assay ID: s11712
Silencer Select siRNA AMOTL1 Sense: CAACUUUUCUUCCACGGAAtt Antisense: UUCCGUGGAAGAAAAGUUGtt	ThermoFisher	Assay ID: s45895
Silencer Select siRNA AMOT Sense: CAUCGUUUGUCUAUACCAAtt Antisense: UUGGUAUAGACAAACGAUGtg	ThermoFisher	Assay ID: s45887
Silencer Select siRNA AMOTL2 Sense: AGACCAUGCGGAACAAGAUtt Antisense: AUCUUGUUCCGCAUGGUCUtc	ThermoFisher	Assay ID: s28109
Custom Select siRNA E1A Sense: GGUACUGGCUGAUAAUCUUtt Antisense: AAGAUUAUCAGCCAGUACCtt	ThermoFisher	Design ID: ADLJIAM
Silencer™ Negative Control No. 2 siRNA	ThermoFisher	Cat#AM4613
KRT80 Forward: ACCAGGAGAAGGAGGAGATGA KRT80 Reverse: TCGAAGATGGCTGAGTCCTG		
KRT7 Forward: CATCGAGATCGCCACCTACC KRT7 Reverse: ATATTCACGGCTCCCACTCC		
PTHLH Forward: AAATCAGAAAGTGTTCGAGGTTCA PTHLH Reverse: TTGAAAACCGAGCGGAGGAA		
COL1A1 Forward: CCTGGAGCCCCTGGC COL1A1 Reverse: CGGGGCCCTTGGGGA		
COL3A1 Forward: GCAGGGTCTCCTGGTTCAAA COL3A1 Reverse: CGGGACCCATTTCGCCTTTA		
GREM1 Forward: AGCGCCACGCGTCGAA GREM1 Reverse: TGGGACCCTTTCTTTTTCCCTT		
LBH Forward: GCCCGTGTCATCCTCACT LBH Reverse: CCTCAGTCATCTTGGCCGAT		

18s Forward: GTAACCCGTTGAACCCCATT
18s Reverse: CCATCCAATCGGTAGTAGCG