**SUPPLEMENTARY METHODS**

**Immunohistochemical analysis of mouse samples**

Lungs were fixed in 4% formalin for 24 hours, transferred into 70% ethanol and embedded in paraffin blocks (FFPE). Lung sections were cut and stained with an anti-cleaved caspase-3 antibody (Cell Signaling Technology, 9661) at 1:100 dilution and ki67 (Abcam, ab1558) at 1:500 dilution. Immunohistochemistry (IHC) was run on the Leica bond Rx using the Refine Kit and including a casein blocking step with the antigen retrieval ER1 at pH 6 for 20 minutes. Slides were scanned on the Olympus VS120-L100-W-12 and the staining quantified with HALO 3.0.331.299. Briefly, cells were segmented and the staining intensity was calculated using the Multiplex IHC v2.3.4 module. Haematoxylin-stained nuclei were identified to generate a total cell count. A ratio between the number of stained-positive cells and the total cell number was used to calculate the percentage of cleaved caspase-3 positive cells. The analysis was performed on individual slides and all lung sections were analysed by the same algorithm simultaneously.

**Co-immunoprecipitation**

Cells were washed twice with ice-cold PBS and lysed *in situ* with lysis buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 5% glycerol) containing fresh cOmplete protease inhibitor cocktail (Roche). Lysates were incubated on ice for 20 minutes and then clarified by centrifugation at 13,000 rpm for 10 minutes at 4 °C. Protein concentrations were estimated by BCA protein assay kit (Pierce) and lysates were pre-cleared with 50 μl of Dynabeads (Thermo Fisher Scientific) for 1 hour at 4 °C with gentle rotation. Exogenous FLAG-CKAP2L was immunoprecipitated overnight by incubation of the protein lysates with anti-FLAG M2 Affinity gel (Sigma-Aldrich). Endogenous CKAP2L was immunoprecipitated overnight at 4 °C from 1 mg total protein using anti-CKAP2L antibody (Thermo Fisher Scientific, PA5-58778) and normal rabbit IgG antibody (Cell Signaling Technology, 2729) was used as control. RPB1 was immunoprecipitated overnight at 4 °C from 3 mg total protein using anti-RPB1 antibody (Abcam #ab31106 or Thermo Fisher Scientific #49-1033). Immunocomplexes of endogenous proteins were incubated with Dynabeads Protein G (Thermo Fisher Scientific) for 1 hour at 4 °C with gentle rotation and both exogenous and endogenous immunocomplexes were then washed twice with Lysis Buffer containing 0.15 m NaCl, twice with 50 mm Tris-HCl (pH 7.5). Samples were finally eluted in Laemmli sample buffer (Biorad) and boiled at 95 °C for 10 minutes for western blot analysis.

**LC-MS analysis**

CKAP2L binding partners were identified by performing immunoprecipitation of endogenous protein coupled to mass spectrometry. Analysis of peptides was performed on an LTQ Orbitrap Velos-Pro (Thermo Scientific) mass spectrometer coupled with a Dionex Ultimate 3000 RS (Thermo Scientific). LC buffers were the following: buffer A (2% acetonitrile and 0.1% formic acid in Milli-Q water (v/v)) and buffer B (80% acetonitrile and 0.08% formic acid in Milli-Q water (v/v). 7 μL of each sample were loaded at 5 μL/min onto a trap column (100 μm × 2 cm, PepMap nanoViper C18 column, 5 μm, 100 Å, Thermo Scientific) equilibrated in buffer A for 17 min. The trap column was washed for 6 minutes at the same flow rate and then the trap column was switched in-line with a Thermo Scientific, resolving C18 column (75 μm × 50 cm, PepMap RSLC C18 column, 2 μm, 100 Å) kept at a constant temperature of 50oC. Peptides were eluted from the column at a constant flow rate of 300 nL/min with a linear gradient from 5% buffer B to 35% buffer B within 64 min, and from 35% buffer B to 98% buffer B for 2 min. The column was then washed for 20 min at 98% buffer B and re-equilibrated in 2% buffer B for 17 min. LTQ-Orbitap Velos Pro was operated in data dependent positive ionization mode. The source voltage was set to 2.50 Kv and the capillary temperature was 250oC. A scan cycle comprised MS1 scan (m/z range from 335-1800) in the velos pro-orbitrap followed by 15 sequential dependant MS2 scans, in LTQ using collision induced dissociation with threshold value set at 5000, the minimum injection time 200 ms, default charge state 2, isolation width 2 (m/z), normalised collision energy 35, activation Q 0.25, activation time 10 ms. The resolution of the Orbitrap Velos was set at to 60,000 after accumulation of 1,000,000 ions. Precursor ion charge state screening was enabled, with all unassigned charge states and singly charged species rejected. The lock mass option was enabled for survey scans to improve mass accuracy.

**Plasmids and other reagents**

pLKO.1 (Addgene, #8453), H2B-GFP (Addgene, #11680), CKAP2L (Genecopoeia, EX-H4839-Lv105), Empty Vector (Genecopoeia, EX-NEG-Lv105), KRAS (Addgene, #75282), MYC (Addgene, #102626). All plasmids were transfected using Lipofectamine 3000 (Thermo Fisher Scientific). Trametinib and Alvocidib were purchased from Selleckchem, (+)-JQ-1 from MedChem Express and Actinomycin D from Sigma-Aldrich.

**RNA interference**

For transient knockdown experiments, cells were transfected with siRNA targeting CKAP2L#1 (Qiagen, SI04168871), CKAP2L#2 (Qiagen, SI04281228), KRAS (Ambion, 57939), MYC (Horizon Discovery, L-003282-02-0005) or Negative control (Qiagen, SI03650318), using Lipofectamine RNAiMAX (Thermo Fisher Scientific). For stable knockdown experiments, shRNAs sequences (Supplementary Table S1) were obtained from the RNAi consortium and cloned into pLKO.1 lentiviral vector for the generation of lentiviral particles.

**Lentivirus transduction**

293T cells were co-transfected with 3 μg of lentiviral vectors and the lentiviral packaging plasmids psPAX2 and pMD2.G in antibiotic-free medium using Lipofectamine 3000. Lentiviral medium was collected after 48 and 72 hours of transfection, passed through a 0.45 μm filter and stored at -80°C until use.

**RNA isolation and qRT-PCR**

Total RNA was isolated by TRIzol according to manufacturer’s instructions. Complementary DNA (cDNA) was synthetized from 500 ng RNA using the Verso cDNA synthesis kit (Thermo Fisher Scientific) and PCR performed using FastStart Universal SYBR Green Master (Roche) on a LightCycler machine (Roche). Expression was normalized to B2M or ACTB. The qPCR primers used are listed in Supplementary Table S1.

**Proliferation assay**

Cell proliferation was assessed using IncuCyte Zoom or IncuCyte S3 (Essen Bioscience, Ann Arbor, MI). For proliferation studies following gene knockdown, cells were firstly transfected with siRNA for depletion of the gene of interest and after 48 hours re-seeded in a 96-well plate for analysis. For proliferation studies in response to drug treatments, cells were seeded in a 96-well plate and the following day treated with the drugs as indicated in the figure legends.

**Immunoblotting**

Protein lysates from cells were isolated using ice-cold RIPA buffer (Sigma-Aldrich) supplemented protease (cOmplete, Roche) and phosphatase (PhosSTOP, Roche) inhibitors. Lysates were incubated on ice for 20 minutes and clarified by centrifugation at 13,000 rpm for 10 minutes at 4 °C. Protein concentrations were estimated by BCA protein assay kit (Pierce) and samples were then resolved on a 4%-12% Bis-Tris gel (NuPAGE, Thermo Fisher Scientific) and transferred to nitrocellulose membrane for subsequent incubation with primary antibodies. Chemiluminescent signal was detected using Western Bright ECL Spray substrate and ChemiDoc imaging system (BioRad).

**Antibodies**

Antibodies used for western blot are as follows: anti-CKAP2L (Thermo Fisher Scientific, PA5-58778), anti-GAPDH (Cell Signaling Technology, 2118), anti-NELF-A (Abcam, ab85852), anti-RPB1 (Abcam, ab31106), anti-RPB1 Ser2 (Abcam, ab193468), anti-RPB1 Ser5 (Abcam, ab5131), anti-MYC (Abcam, ab32072), anti-BRD4 (Bethyl lab, A301-985A100), anti-CDK9 (Abcam, ab76320), anti-Cyclin T1 (Abcam, ab184703), anti-Flag (F1804, Sigma-Aldrich).

Antibodies used for ChIP: anti- RPB1 (Abcam, ab31106), anti-MYC (Novus Biologicals, NB600-302).

**Colony formation**

1x103 cells were seeded in triplicates per each condition in 6-well plates and incubated in a CO2 incubator at 37 °C for 8-12 days, depending on the cell line. Cells were then washed with PBS, fixed in ice-cold methanol and stained with 0.5 % crystal violet solution (Sigma-Aldrich) at room temperature for 20 minutes. Colonies were imaged and counted using the GelCount System (Oxford Optronix).

**Cell cycle analysis**

For analysis of cell cycle distribution, cells were harvested by trypsinization, washed with cold PBS and fixed in ice-cold 70% ethanol for 1 hour prior to incubation with FxCycleTM PI/RNase Staining Solution (Thermo Fisher Scientific) for 15 minutes at room temperature. Samples were then analysed by flow cytometry (Novocyte, ACEA Biosciences).

**Apoptotic cell death analysis**

Apoptotic cells, following treatments indicated in the figure legends, was assessed using the TACS® Annexin V/PI kit (Travigen). Cells were harvested by trypsinization, washed once in cold PBS and then incubated in Annexin V-FITC Incubation reagent, as per manufactures’ instructions. Samples were analysed by flow cytometry (Novocyte, ACEA Biosciences).