Supporting Information

Materials and Methods

Proliferation and Apoptosis Assays. Logarithmically-growing cells were seeded at optimized densities for each examined cell line onto individual wells of 24-well tissue culture plates for proliferation and 6-well tissue culture plates for apoptosis assays. Cells were treated with drug or the vehicle dimethyl sulfoxide (DMSO) 24 hours later. Proliferation was measured using the CellTiter-Glo Luminescent Assay (Promega). A panel of 20 human lung cancer cell lines was independently analyzed for proliferative changes after CFI-400945 treatment using the Sulforhodamine B (SRB) assay. Apoptosis assays were performed with the FITC Annexin V Apoptosis Detection Kit (BioLegend).

Washout Assays. Logarithmically-growing cells were seeded at optimized densities onto individual 10cm tissue culture plates and were subsequently treated with drug or vehicle. Cells were harvested at 24 hours and seeded at optimized densities for each cell line onto individual wells of 24-well tissue culture plates after three washings with phosphate buffered saline (PBS). Drug or vehicle (DMSO) was then added, as described in the text. Proliferation was measured 48 hours later using the CellTiter-Glo Assay (Promega).

Measurement of Cellular DNA Contents. Cells were fixed in ice-cold 70% ethanol and stained with PI/RNase Staining Solution (F10797, Thermo Fisher Scientific) with RNase A solution (70856-3, Novagen) added at 100 µg/ml after fixation. DNA contents were measured using a Gallios flow cytometer (Beckman Coulter) and were analyzed using FlowJo software (FlowJo, LLC).

Centrosome and Mitotic Analyses. Cells were fixed, stained with the anti-α-tubulin-specific antibody along with DAPI and then mounted with Pro-Long Gold antifade reagent. Stained cells were scored for multipolar anaphase cells using an Eclipse TE 2000-E microscope (Nikon). Primary antibodies were α-tubulin (for single stain: T6199; Sigma Aldrich; 1:1,000, for double staining with γ-tubulin: NB600-506; Novus Biologicals; 1:1,500), γ-tubulin (T5326; Sigma Aldrich; 1:1,000). Secondary antibodies were Texas red anti-mouse IgG (H + L) (TI-2000; Vector Laboratories; 1:500), Alexa fluor 594 anti-rat IgG (A21209; Invitrogen; 1:1,000), and Fluorescein anti-mouse IgG (FI-2000; Vector Laboratories; 1:10). Hoechst 33342 (62249; Thermo Scientific; 1:10,000) stained for DNA. Pro-Long Gold anti-fade reagent (P36934; Invitrogen) preserved immunofluorescence.

RNA *In Situ* Hybridization (ISH). A tissue microarray (TMA) was prepared using three 1.0-mm tissue cores obtained respectively from the center, middle and periphery of

formalin-fixed and paraffin-embedded histological sections from 235 surgically-resected primary lung cancer cases spanning stages I to IV. All lung cancer tissues were evaluated and underwent surgical resection at The University of Texas MD Anderson Cancer Center. Informed consent was obtained from all patients under a protocol approved by the Institutional Review Board (IRB). PLK4 RNA ISH assays were performed on lung cancer TMAs using a target probe for PLK4 (479488, Advanced Cell Diagnostic). An automated RNAscope assay was performed using the Leica Bond RX autostainer (Leica Biosystems). The tissue sections were deparaffinized and rehydrated following the Leica Bond protocol. Antigen retrieval was performed with Bond Solution #2 (Leica Biosystems) for 15 min at 95°C. Protease digestion was for 15 min at 40°C. Signals were visualized with 3,3'-Diaminobenzidine (DAB) and hematoxylin counterstaining. Probes for the housekeeping gene PPIB (313908, Advanced Cell Diagnostic) and for the bacterial gene DapB (312038, Advanced Cell Diagnostic) served as positive and negative controls, respectively. Images were digitized (Aperio Technologies). PLK4 RNA expression was evaluated using the Aperio Image Toolbox[™] by a RNA ISH algorithm. Findings were reviewed and scored by a pathologist, who was unaware of clinical data.

3

Cancer	Gene_1	Gene_2	Spearman Rho	p-value
LUAD	PLK4	CTLA4	0.169	0.000109
	PLK4	PD1	0.205	2.50E-06
	PLK4	PDL1	0.332	8.44E-15
	PLK4	PDL2	0.219	5.24E-07
LUSC	PLK4	CTLA4	0.017	0.710
	PLK4	PD1	0.034	0.444
	PLK4	PDL1	0.140	0.00172
	PLK4	PDL2	0.024	0.600

 Table S1. Correlation of PLK4 and immune checkpoint markers

The correlation of Gene_1 and Gene_2 were analyzed using TCGA data. CTLA4 and PD1 expression in these data reflect those of infiltrating T cells surrounding tumors.

LUAD: lung adenocarcinoma, LUSC: lung squamous cell carcinoma

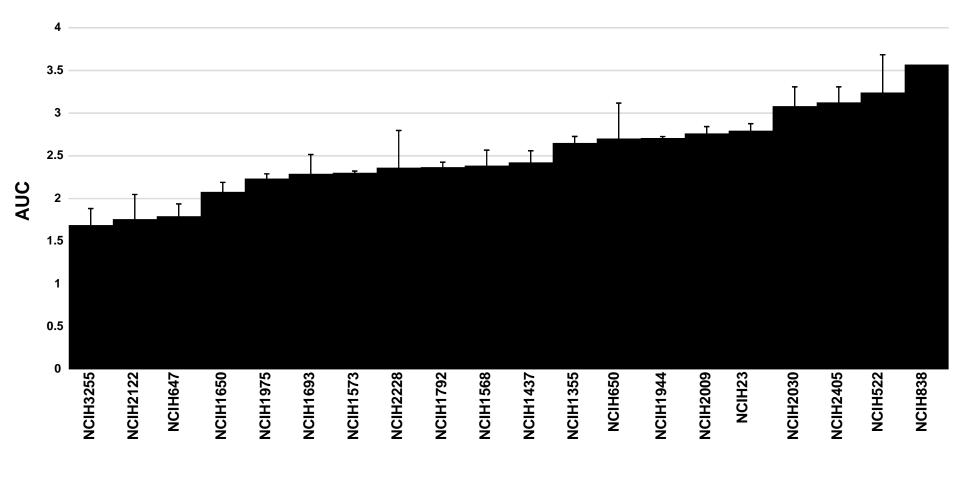


Fig. S1. The area under the curve (AUC) of a panel of lung cancer cell lines following treatment with CFI-400945. AUC was calculated based on the proportion of control values and log10 (dosages, nM) values to generate dose-response curves. Error bars are standard deviations.

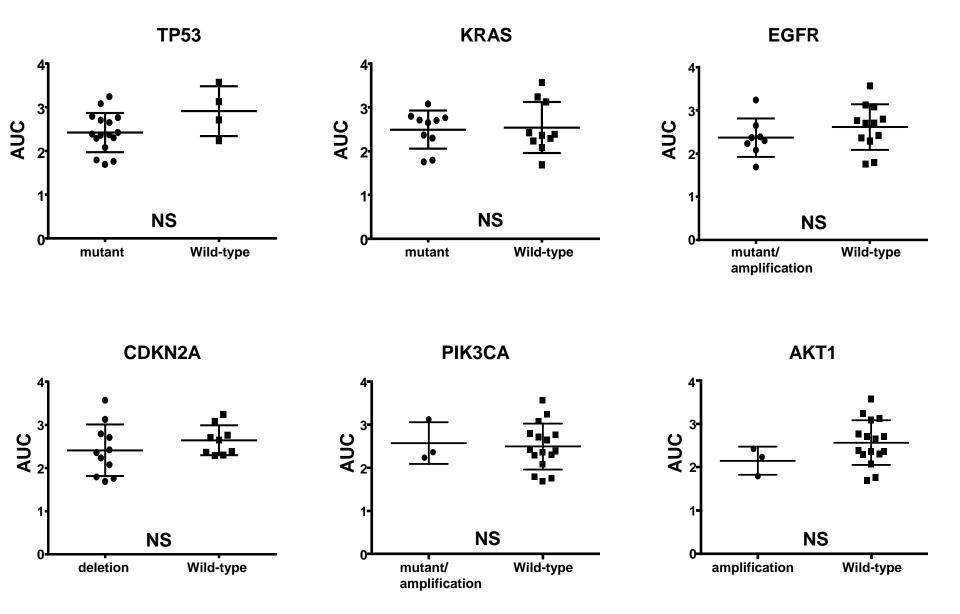


Fig. S2. Comparisons of the area under the curve (AUC) between the displayed lung cancer cell lines with the presence of common genetic alterations. Each circle or square represents an individual cell line. Bars represent mean with standard deviation and with NS referring to a comparison that is not statistically significant.

Cells with clustered centrosomes

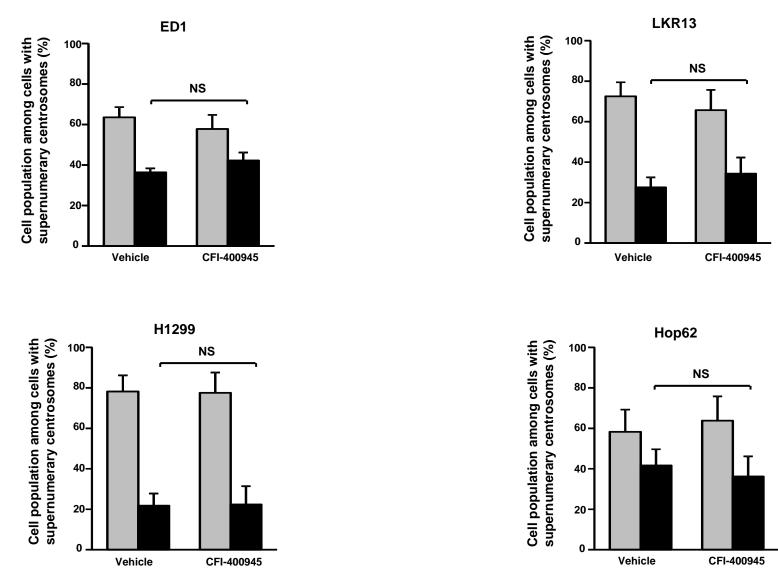


Fig. S3. Comparisons of percentages of cells with clustered centrosomes and multipolar cells among the indicated lung cancer cell lines having supernumerary centrosomes following vehicle or CFI-400945 treatments. Error bars are standard deviations. The abbreviation NS refers to a non-significant comparison.

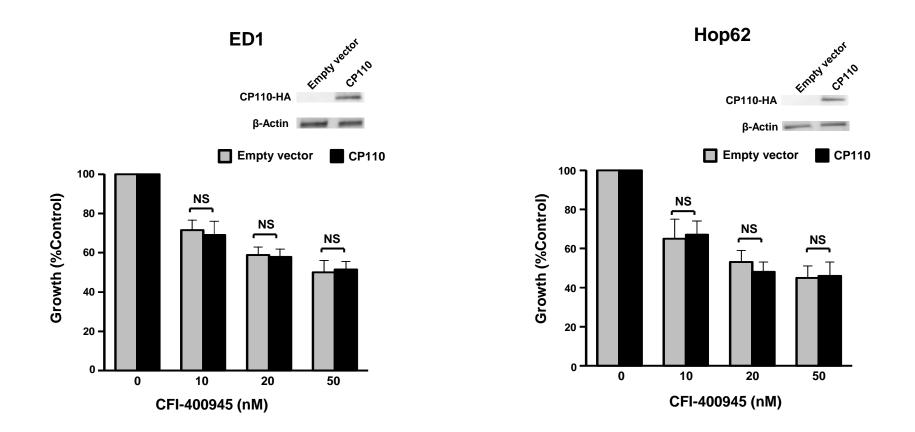
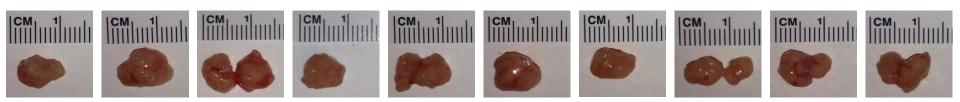
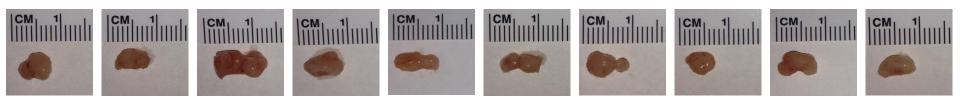


Fig. S4. Effects of engineered HA-tagged CP110 (CP110-HA) overexpression on growth inhibition by CFI-400945 treatment of the examined lung cancer cells. Immunoblots confirmed CP110 overexpression and they are displayed in the upper panels. Error bars are standard deviations with the abbreviation NS referring to a non-significant comparison.

Vehicle



CFI400945 (3mg/kg)



CFI400945 (7.5mg/kg)



Fig. S5. Comparisons of excised lung tumor sizes after completion of the treatments of individual mice with vehicle or CFI-400945 (3mg/kg or 7.5mg/kg). Photographs of resected lung tumors after these respective treatments are shown.

	Muscle - Skeletal	Liver Heart – Left Venticle	Whole Blood	Pancreas Brain – Cere Hemisphere	Heart – Atrial Appendage	Brain - Cerebellum Pituitarv	Esophagus – Gast Junction	Esophagus - Muscularis	Artery - Aorta Brain - Putamen	Colon - Sigmoid	Brain - Hippocampus	LINC - LIVEr Brain – Substantia nigra	Artery - Tibial	PCPG - Pheochromocytoma	KICH – Kidney chromophobe	Brain – Ant Cing cortex	Kidney - Cortex Brain - Amygdala	Stomach Artery - Coronary		Brain - Cortex Brain - Spinal cord	PRAD – Prostate adeno	Adipose - Omentum	Brain – Frontal Cortex Minor Salivary Gland	Fallopian Tube	Brain – Nuc accumbens Prostate	Uterus	I nyroia Bladder	Adrenal Gland KIRC – Kidnev clear cell	5	THCA - Thyroid Nerve - Tibial	LGG-Brain glioma	Lung Breast – Mammary Tissue	Cervix - Ecto Ovary	Adipose - Subcutaneous	CHOL - Cholangiocarcinoma	MESO - Mesothelioma ACC - Adrenocortical	Colon - Transverse	Vagina	Skin – Not Sun Exposed	Skin – Sun Exposed LUAD – Lung adeno	DLBC – B-cell lymphoma Transformed fibroblasts	SKCM – Skin cutaneous melanoma GRM - Glichlastoma	READ - Rectum adeno	Esophagus – Mucosa BRCA - Breast	COAD – Colon adeno UCEC – Uterine endometrial	Ov - Ovarian	SARC - Sarcoma BLCA - Bladder	LUSC – Lung squamous	низо – пеаа апа песк ТНҮМ - Thymoma	UCS – Uterine carcinosarcoma TGCT – Testicular germ	CESC - Cervical I AMI - I eutemia	Testis EBV – transformed lymphocytes	
PLK4 mRNA (TPM)	0											Participation of the				A. Martin Martin S.		stratement and track the light	- "					:		1 CTOCK MALE	· · · ·		<u>.</u>				· · · · · · · · · · · · · · · · · · ·		· ····································		· · · · · · · · · · · · · · · · · · ·	· Summer Superior			「「「「「「」」」」		- AND ANALY STREET		- Contract Internet (1971-1-1)	STREET BEAUTING						ţţ	
• PLK4											91. * 1					••	• •								•		•	•	•	•			•	• •		:	•								••								

green: normal tissue, red: cancers

Fig. S6. PLK4 mRNA expression profiles were examined among different cancer types (versus corresponding normal tissues) using The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) databases. Green displays normal tissues and red indicates malignant tumors.

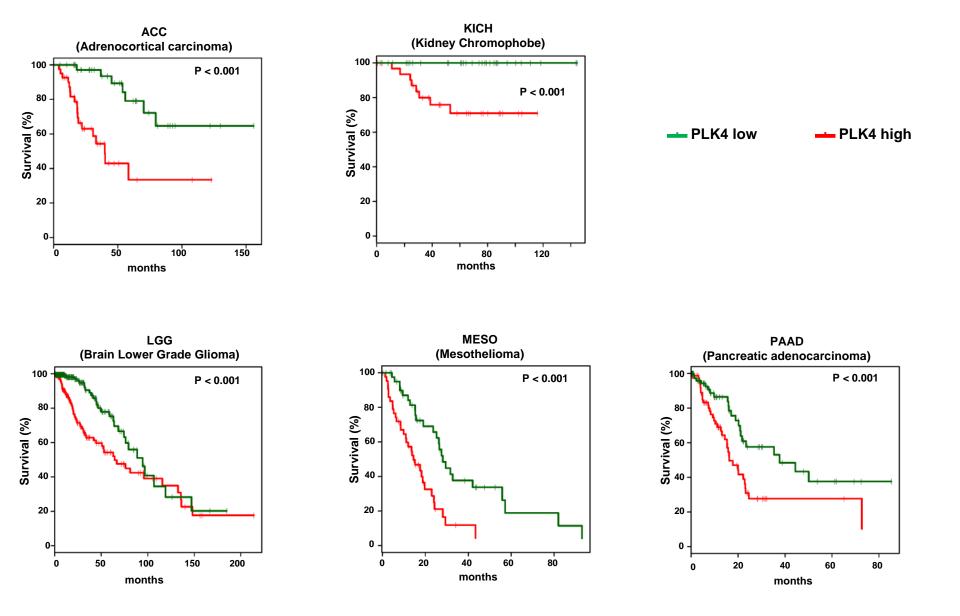


Fig. S7. Comparisons of overall survival between PLK4 mRNA high expression versus low expression groups in these different cancers were conducted using The Cancer Genome Atlas (TCGA) database.