

Supplementary

Table S1 List of cell lines used in this study

Cell line	Species	Source	Time	Culture medium (with 10% FBS)
A549	Human LUAD	Purchased from ATCC	September 2010	DMEM
Calu-1	Human epidermoid carcinoma	Purchased from ATCC	December 2015	RPMI
NCI-H292	Human mucoepidermoid carcinoma	Purchased from ATCC	February 2019	RPMI
HCC4006	Human LUAD	Purchased from ATCC	July 2019	RPMI
NCI-1573	Human LUAD	Purchased from ATCC	March 2019	RPMI
NCI-H1650	Human LUAD	Purchased from ATCC	January 2019	RPMI
NCI-H1975	Human LUAD	Purchased from ATCC	January 2014	RPMI
NCI-H3255	Human LUAD	Purchased from ATCC	November 2013	RPMI
NCI-H358	Human bronchioalveolar carcinoma	Purchased from ATCC	September 2010	RPMI
NCI-H441	Human LUAD	Purchased from ATCC	September 2010	RPMI
HCC827	Human LUAD	Purchased from ATCC	November 2013	RPMI

FBS, fetal bovine serum; LUAD, lung adenocarcinoma; ATCC, American Type Culture Collection; DMEM, Dulbecco's modified Eagle's medium; RPMI, Roswell Park Memorial Institute.

Table S2 List of antibodies used in this study

Antigen	Company	Catalog #	Purpose	Dilution ratio
Clathrin	BD Biosciences	610500	WB	1:1,000
HSP90	BD Biosciences	610418	WB	1:1,000
DKK1	Cell Signaling Technology	48367S	WB	1:200
DKK1	R&D Systems	MAB10962	IHC	1:200
AKT	Cell Signaling Technology	9272	WB	1:1,000
pAKT (S473)	Cell Signaling Technology	9271	WB	1:1,000
CD81	Cosmobio	SHI-EXO-M03	WB	1:1,000
TSG101	Sigma-Aldrich	HPA006161	WB	1:1,000
HSP90	BD Biosciences	610418	WB	1:2,000
pEGFR	Cell Signaling Technology	3777	WB	1:1,000
EGFR	BD Biosciences	610016	WB	1:1,000
LAMP1	Santa Cruz Biotechnology	Sc-20011	WB	1:1,000
Rab5	BD Biosciences	610724	WB	1:1,000
Rab7	Cell Signaling Technology	9367	WB	1:1,000
LDLR	Abcam	Ab52818	WB	1:1,000

WB, western blotting; IHC, immunohistochemistry.

Table S3 List of chemicals used in this study

Product	Company	Catalog #
DexoMAG	Liquids Research	
MagCapture Exosome isolation kit	Fujifilm Wako Pure Chemical Corporation	293-77601
Osimertinib	Selleck Chemicals	AZD9291
Bafilomycin A1	AdipoGen Life Sciences	BVT-0252-C100

Table S4 Target sequences for shRNA and siRNA used in this study

Gene	Sequence
Human DKK1 #1 (shRNA)	GGATCTCTTGAATGACAA
Human DKK1 #2 (shRNA)	CGGTTCTCAATTCCAACGCTA
Human DKK1 #1 (siRNA)	GCTCTCATGGACTAGAAAT
Human DKK1 #2 (siRNA)	CCTGGAGTGTAAGAGCTTT
Human DKK3 #1 (shRNA)	GGGTAGATGTGCAATAGAA
Human DKK3 #2 (shRNA)	GCATGAGGTGTTGTGCATT
Human Clathrin #1 (siRNA)	CCTGCGGTCTGGAGTCAAC
Human Clathrin #2 (siRNA)	CATTGGCTTCAGTACCCTG
Human ALIX #1 (siRNA)	GCTGAGTACCATCAGTCTA
Human ALIX #2 (siRNA)	GTACCTCAGTCTATATTGA
Human Rab27a #1 (siRNA)	GCTGCCAATGGGACAAACA
Human Rab27a #2 (siRNA)	CCTGAGAGTTAGGAGAAAT
Human Rab27b #1 (siRNA)	GCTGCCAATGGGACAAACA
Human Rab27b #12 (siRNA)	GCTGCCAATGGGACAAACA
Human VPS26a #1 (siRNA)	GCTAGAACACCAAGGAATT
Human VPS26a #2 (siRNA)	GGAAACAGAGAACAACTT
Human VPS35 #1 (siRNA)	CCTTTGGTATTTGCAGCTT
Human VPS35 #2 (siRNA)	CCATCCATTAAGAACCCAT
Human CD63 #1 (siRNA)	GCCTGCAAGGAGAACTATT
Control (shRNA)	GTGCGTTGCTAGTACCAAC
Control (siRNA)	CAGTCGCGTTTGCGACTGG

Table S5 Primer sequences for quantitative PCR used in this study

Gene	Forward	Reverse
Human <i>ALIX</i>	TGGCTGCAAAGCACTGTATC	AGGGCACGATTGATTTTGTGTC
Human <i>Rab27A</i>	TGGGAGACTCTGGTGTAGGG	CCCTGCTGTGTCCCATAACT
Human <i>Rab27B</i>	CACAAGGACCGAATGGATCT	GCAGTTGGCTCATCCAGTTT
Human <i>VPS26A</i>	ATGACGGAGAATCCGTTTCA	TGGCACCGATGTAAGATTCA
Human <i>VPS35</i>	CGTGAAGATGGACCTGGAAT	TCCACACGATCAGGGTAACA
Human <i>CD63</i>	TGGGTGTCTTCTCTTCTG	TCGGGTAATTCTCCATCTGC

PCR, polymerase chain reaction.

Table S6 EGFR mutation status of the lung cancer cell lines used in this study

Cell line	Histology	EGFR mutation type	Other mutations
NCI-H1650	Adenocarcinoma	E746-A750del	
NCI-H1975	Adenocarcinoma	L858R	T790M
NCI-H3255	Adenocarcinoma	L858R	
HCC827	Adenocarcinoma	E746-A750del	
HCC4006	Adenocarcinoma	L747-E749del	

EGFR, epidermal growth factor receptor.

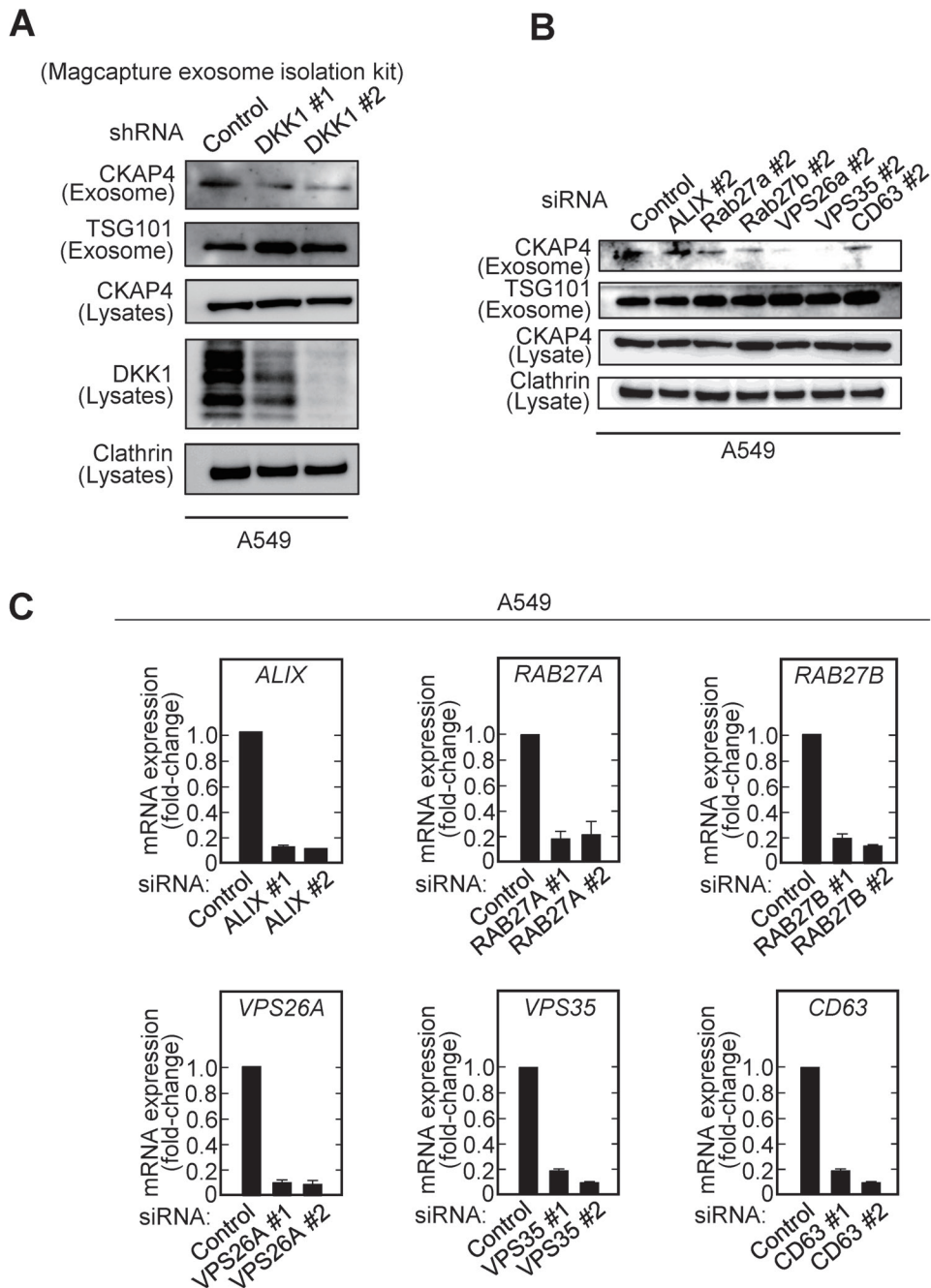


Figure S1 CKAP4 is secreted with SEVs in a DKK1-dependent manner. (A) SEVs were prepared from CM of A549 cells transfected with control (scramble) or the indicated shRNAs using MagCapture. SEVs and lysates were probed with the indicated antibodies. (B,C) SEVs were prepared from CM of A549 cells transfected with control (scramble) or indicated siRNAs. The mRNA levels of indicated genes were assayed by quantitative RT-PCR, and relative mRNA levels were normalized to *GAPDH*. Results are shown as fold-change compared to control cells and expressed as the mean \pm SD from three independent experiments. SEVs, small extracellular vesicles; CM, conditioned medium; RT-PCR, real-time polymerase chain reaction; SD, standard deviation.

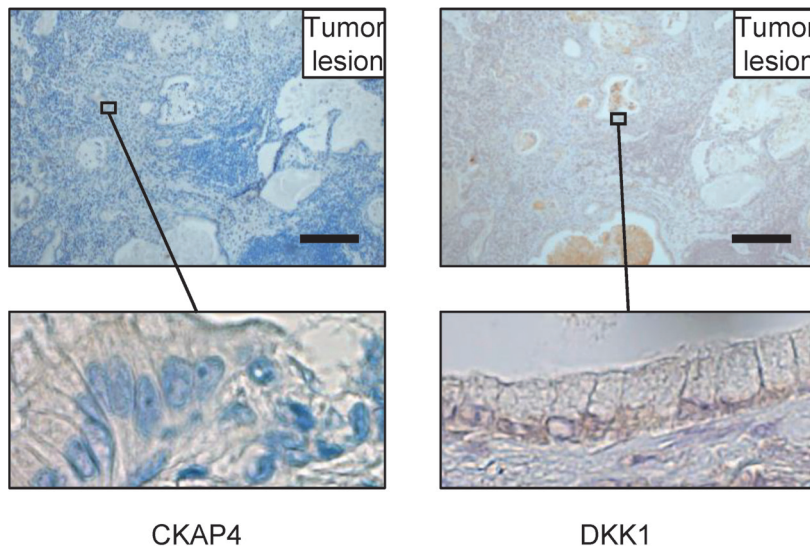


Figure S2 Neither DKK1 nor CKAP4 was stained immunohistochemically in some non-small lung cancer cases. Representative images of IHC staining negative for DKK1 or CKAP4. Tumor tissues were stained with indicated antibodies and hematoxylin. Scale bar: 200 μ m. IHC, immunohistochemistry.

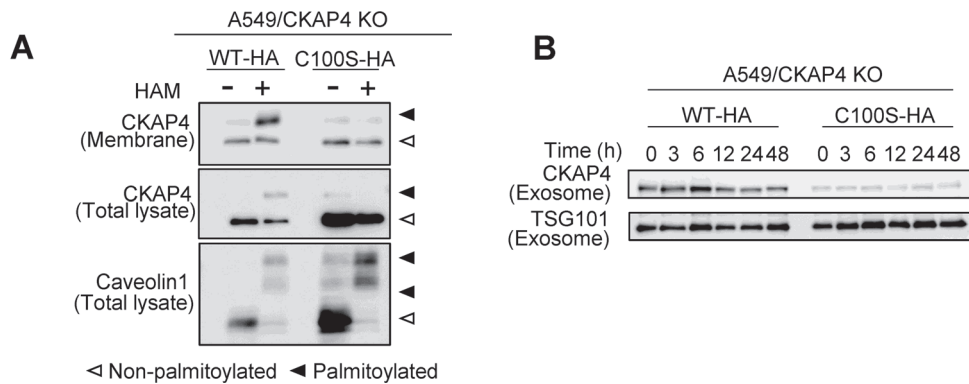


Figure S3 CKAP4 is secreted with exosomes in a palmitoylation-dependent manner. (A) A549/CKAP4 KO/CKAP4-HA (WT-HA) and A549/CKAP4 KO/CKAP4 C100S-HA (C100S-HA) cells were biotinylated and subjected to APEGS assay. Reactions performed without hydroxylamine [lanes of HAM (-)] were negative controls. Total lysates and biotinylated precipitates (Membrane) were immunoblotted for CKAP4. (B) The CMs from A549/WT-HA or A549/C100S-HA cells were collected and incubated at RT for indicated times, after which SEVs were prepared and probed with indicated antibodies. KO, knockout; WT-HA, wild-type CKAP4 with HA tag; C100S-HA, CKAP4^{C100S} mutant with HA tag; HAM, hydroxylamine; CM, conditioned medium; RT, room temperature; SEVs, small extracellular vesicles.