

Supplementary Materials: Molecular signature of small cell lung cancer after treatment failure: The *MCM* complex as therapeutic target

Shunsuke Misono, Keiko Mizuno, Takayuki Suetsugu, Kengo Tanigawa, Nijiro Nohata, Akifumi Uchida, Hiroki Sanada, Reona Okada, Shogo Moriya, Hiromasa Inoue and Naohiko Seki

Construction of the mRNA expression signature for treatment failure of SCLC

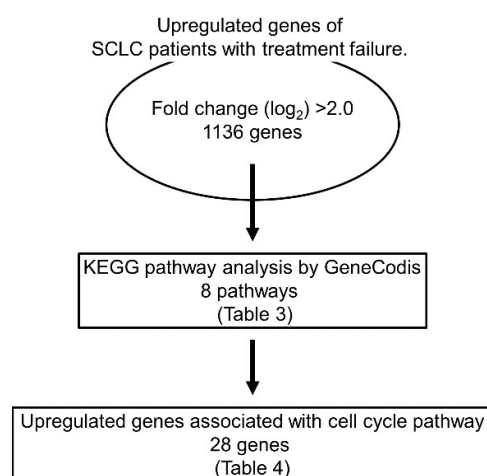


Figure S1. Flowchart of the genes upregulated in SCLC compared with normal tissues classified by KEGG pathway analysis.

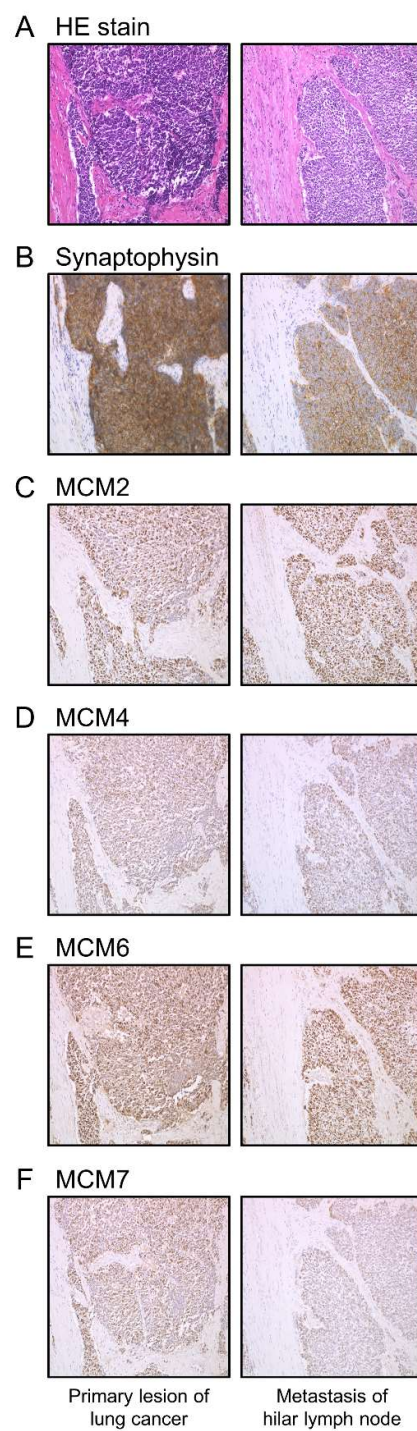


Figure S2. Histopathological findings and immunohistochemical analyses of MCM2, MCM4, MCM6, and MCM7 in case no. 2. (A-F) Overexpression of MCM2, MCM4, MCM6, and MCM7 was observed in the nuclei of cancer cells.

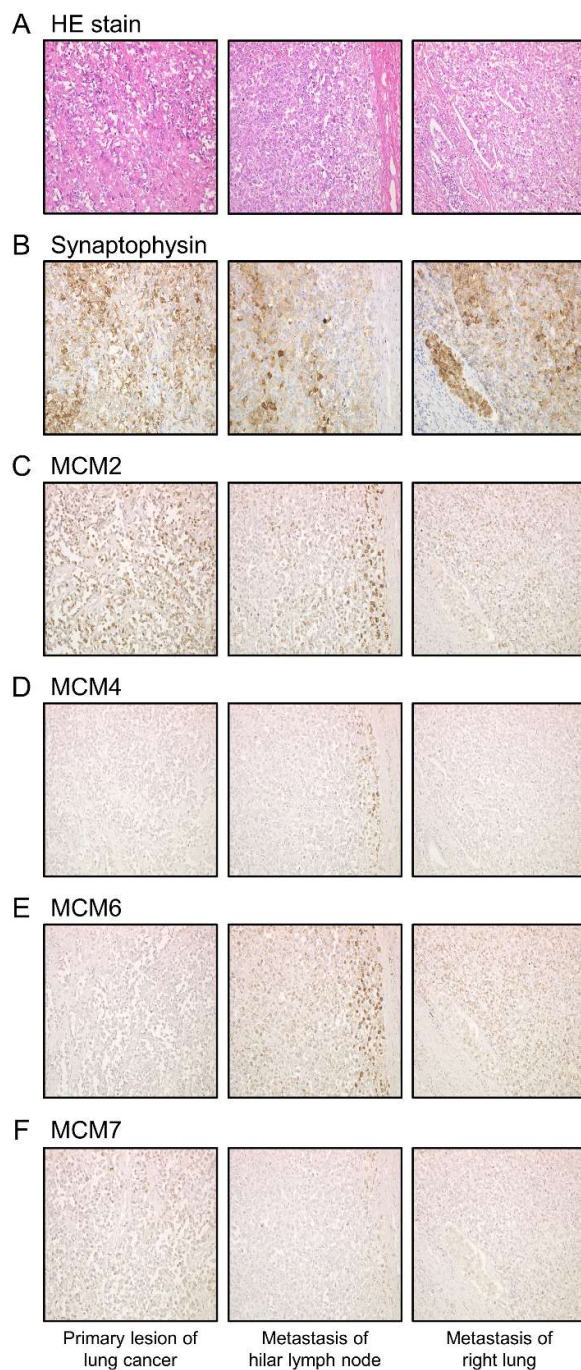


Figure S3. Histopathological findings and immunohistochemical analyses of MCM2, MCM4, MCM6, and MCM7 in case no. 3. (A-F) Overexpression of MCM2, MCM4, MCM6, and MCM7 was observed in the nuclei of cancer cells.

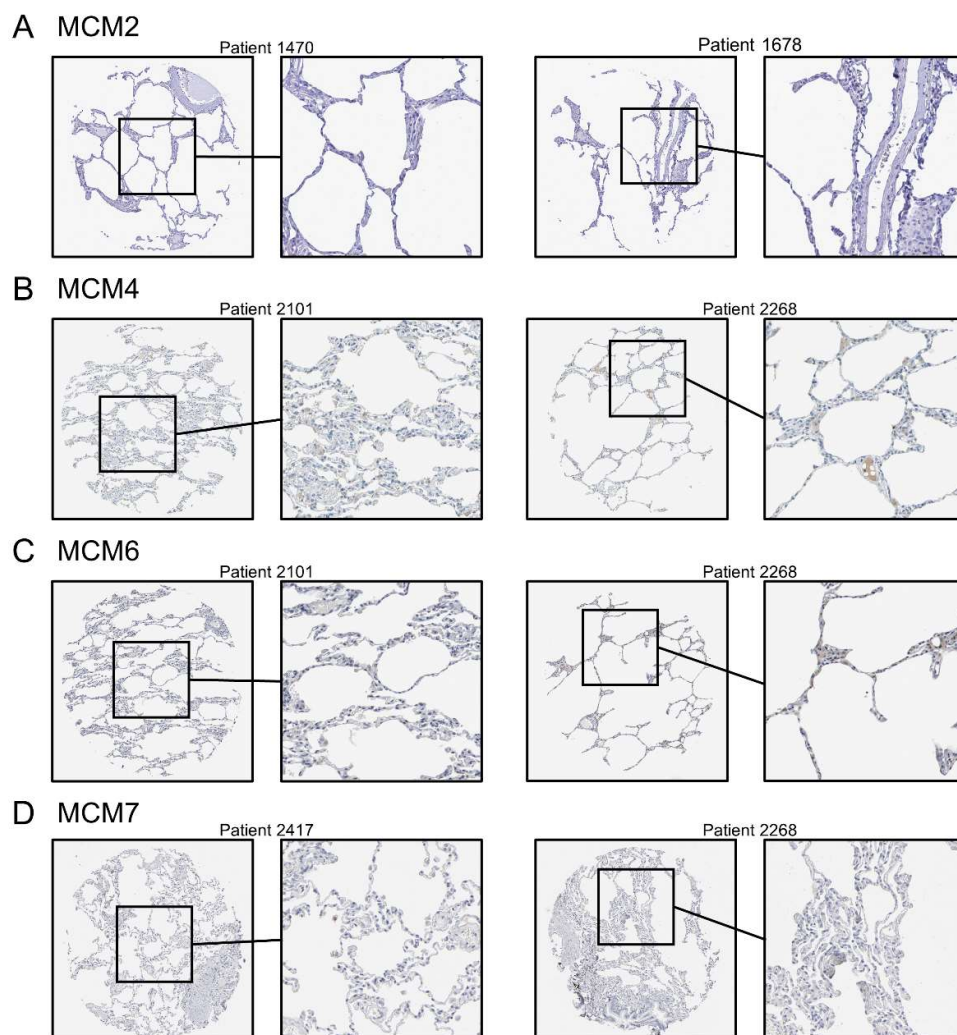
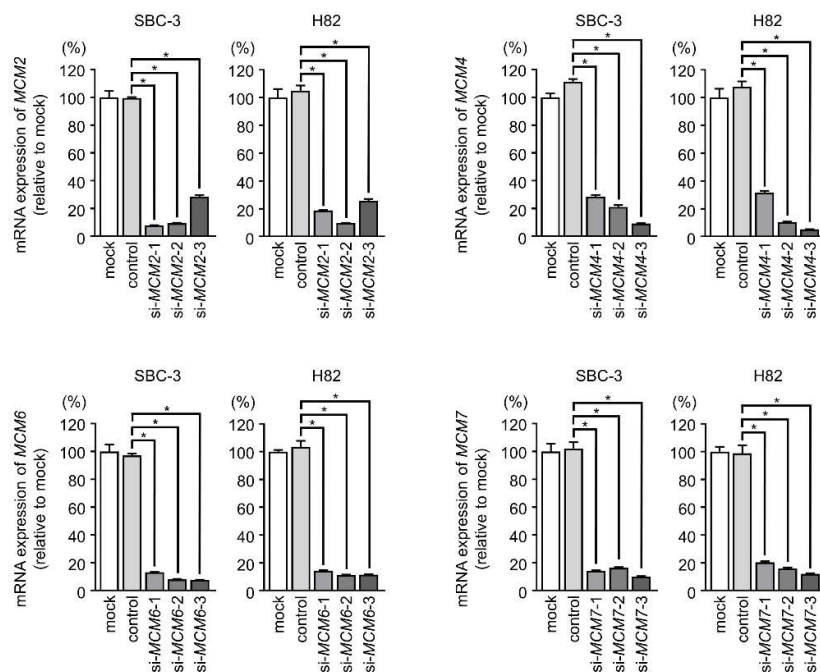
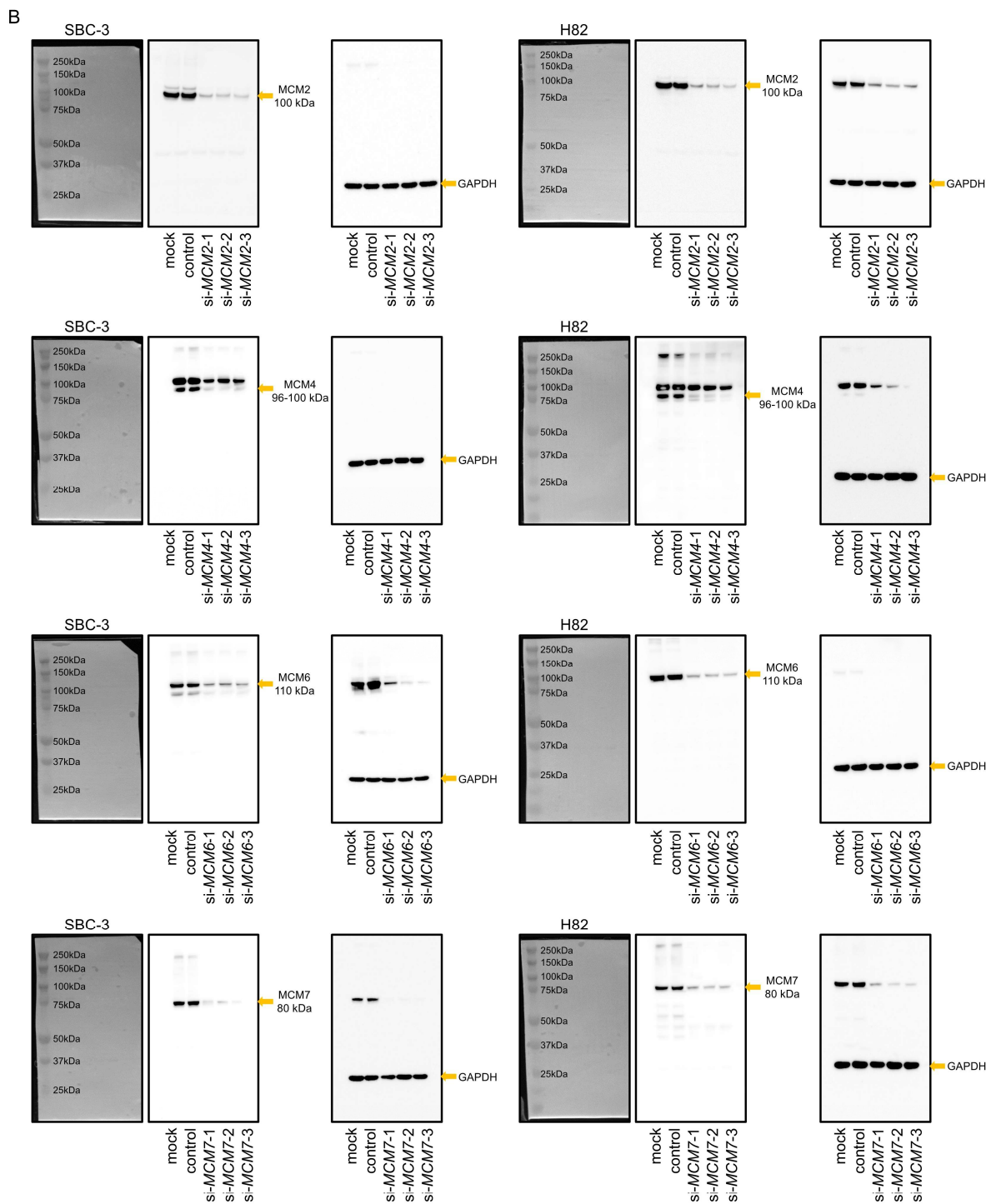


Figure S4. Histopathological findings and immunohistochemical analyses of MCM2, MCM4, MCM6, and MCM7 in normal lung tissue. (A-D) MCM family members were almost not detected in normal lung tissues (Credit: Image from the Human Protein Atlas).

A





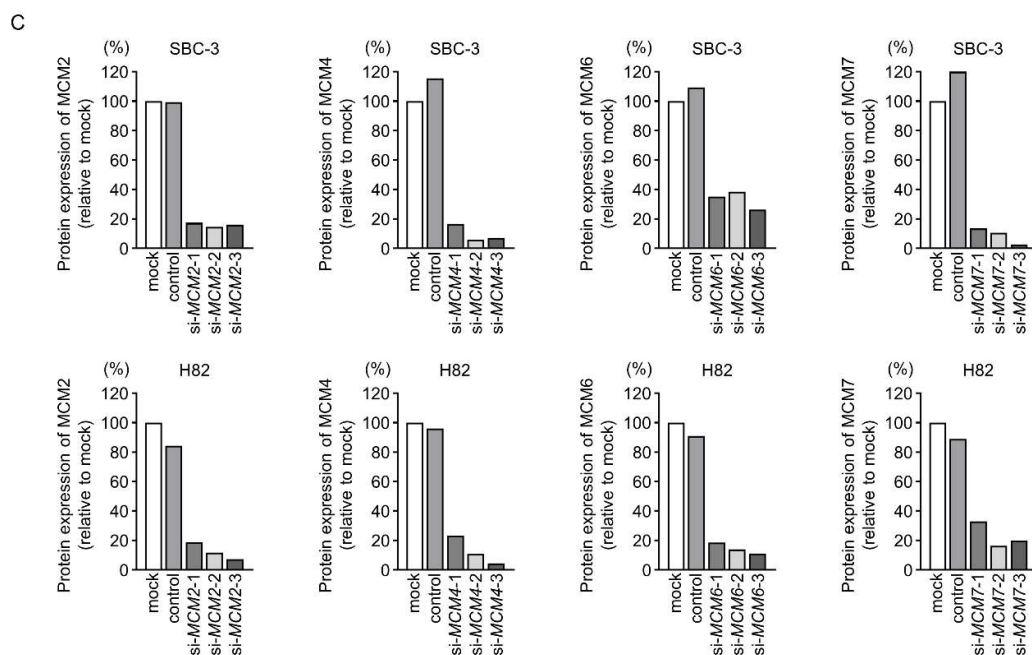
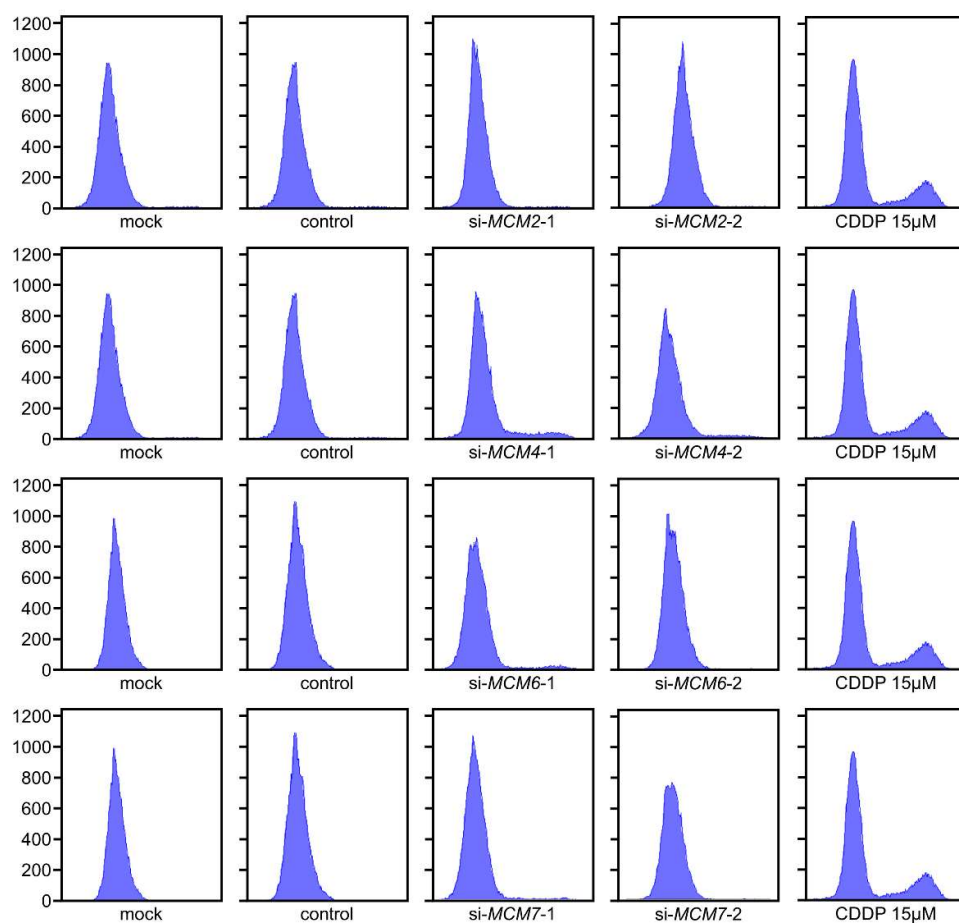


Figure S5. Downregulation of MCM family members by siRNA transfection in SCLC cells. Western blots for each MCM protein are shown. *, $p < 0.0001$. (A, B) RT-PCR and western blotting showed that expression levels of both mRNA and protein were markedly reduced by both siRNAs. (C) The western blotting results were quantified by ImageJ.

A



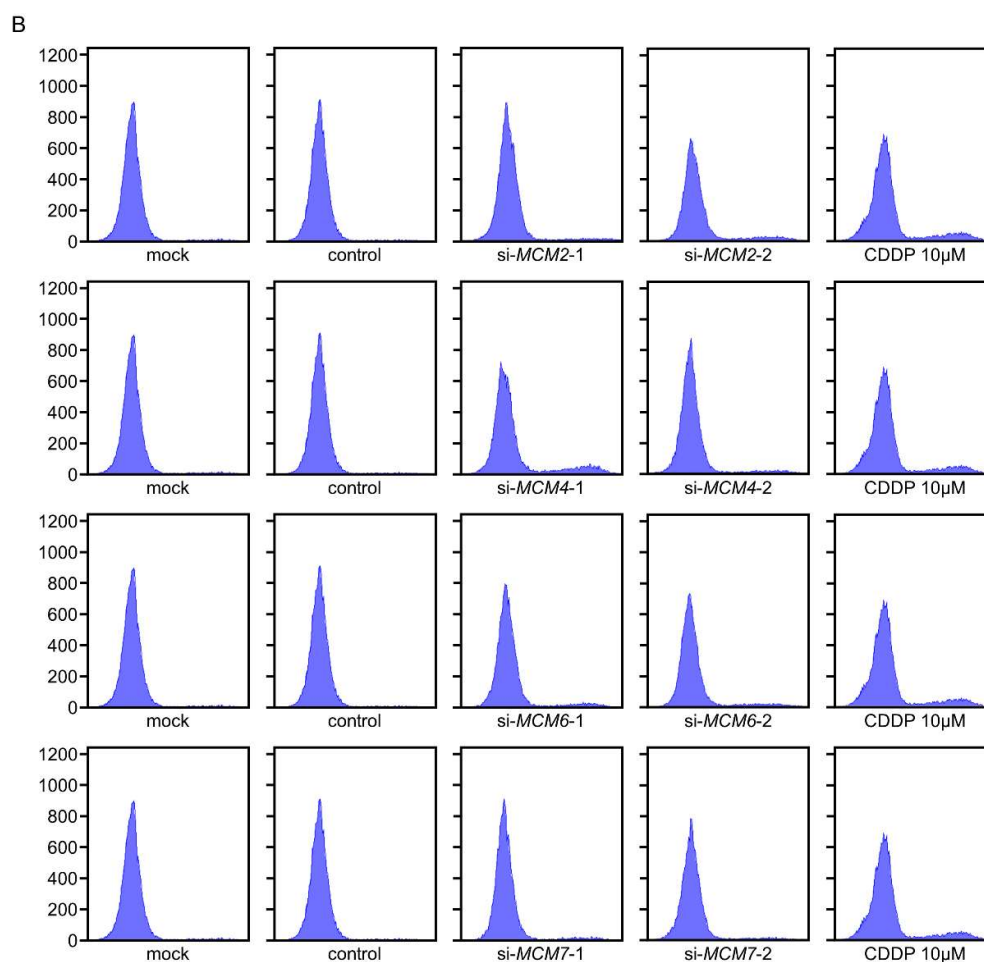


Figure S6. Apoptosis data obtained by flow cytometry from (A) SBC-3 and (B) H82 cells transfected with siRNAs targeting MCM2, MCM4, MCM6, and MCM7.

Table S1. Reagent used in this study.

Supplemental Table 1. Reagent used in this study			
Antibody	Dilution	Catalog number	Company
Synaptophysin (27G12)	IHC 1:100	413831	Nichirei Biosciences, Inc. (Tokyo, Japan)
Chromogranin	IHC 1:400	412751	Nichirei Biosciences, Inc.
CD56 (1B6)	IHC 1:25	413331	Nichirei Biosciences, Inc.
MCM2	IHC 1:1000 WB 1:1000	ab4461	Abcam (Cambridge, UK)
MCM4	IHC 1:1000 WB 1:2000	ab4459	Abcam

MCM6	IHC 1:100 WB 1:2000	ab4458	Abcam
MCM7	IHC 1:50 WB 1:200	ab2360	Abcam
GAPDH	WB 1:20000	MAB374	EMD Millipore (Billerica, MA, USA)
siRNA	Concentration	Catalog number	Company
si-MCM2	10 nM	HSS106390, HSS106392, HSS181044	Invitrogen (Carlsbad, CA, USA)
si-MCM4	10 nM	HSS106396, HSS106397, HSS106398	Invitrogen
si-MCM6	10 nM	HSS106402, HSS106403, HSS106404	Invitrogen
si-MCM7	10 nM	HSS106405, HSS106406, HSS181048	Invitrogen
probe	Assay ID		Company
MCM2	Hs01091564_m1		Applied Biosystems (Foster City, CA, USA)
MCM4	Hs00907398_m1		Applied Biosystems
MCM6	Hs00962418_m1		Applied Biosystems
MCM7	Hs00428518_m1		Applied Biosystems
GUSB	Hs00939627_m1		Applied Biosystems

Table S2: Upregulated genes associated with Homologous recombination pathway.

Entrez GeneID	Gene Symbol	GeneName	Location	Normalized read count (Log ₂)			p-value
				Log ₂ fold change	Normal lung tissues	SCLC tissues	
8438	<i>RAD54L</i>	RAD54-like	1p34.1	3.76	0.06	3.82	0.0078
83990	<i>BRIP1</i>	BRCA1 interacting protein C-terminal helicase 1	17q23.2	3.42	0.05	3.47	0.0010
25788	<i>RAD54B</i>	RAD54 homolog B	8q22.1	2.84	0.03	2.87	0.0002
672	<i>BRCA1</i>	breast cancer 1, early onset	17q21.31	2.46	0.11	2.57	0.0006
5888	<i>RAD51</i>	RAD51 recombinase	15q15.1	2.46	0.12	2.58	0.0055
7516	<i>XRCC2</i>	X-ray repair complement- ing defective repair in Chi- nese hamster cells 2	7q36.1	2.37	0.06	2.42	0.0261
675	<i>BRCA2</i>	breast cancer 2, early onset	13q13.1	2.30	-0.07	2.23	0.0005
11073	<i>TOPBP1</i>	topoisomerase (DNA) II binding protein 1	3q22.1	2.12	0.05	2.18	0.0003
641	<i>BLM</i>	Bloom syndrome, RecQ helicase-like	15q26.1	2.03	0.06	2.10	0.0240

7517	XRCC3	X-ray repair complement- ing defective repair in Chi- nese hamster cells 3	14q32.33	0.15	0.04	0.20	0.8186
------	-------	--	----------	------	------	------	--------

Table S3: Upregulated genes associated with DNA replication pathway.

Entrez GeneID	Gene Symbol	GeneName	Location	Log ₂ fold change	Normalized read count (Log ₂)		p-value
					Normal lung tissues	SCLC tissues	
4173	MCM4	minichromosome maintenance complex component 4	8q11.21	3.33	0.20	3.54	0.0004
4171	MCM2	minichromosome maintenance complex component 2	3q21.3	2.96	0.01	2.97	0.0008
5427	POLE2	polymerase (DNA di- rected), epsilon 2, ac- cessory subunit	14q21.3	2.69	-0.06	2.62	0.0016
5984	RFC4	replication factor C (activator 1) 4	3q27.3	2.31	-0.01	2.30	0.0007
5983	RFC3	replication factor C (activator 1) 3	13q13.2	2.26	0.01	2.27	0.0013
4176	MCM7	minichromosome maintenance complex component 7	7q22.1	2.20	0.13	2.33	0.0002
10535	RNASEH2A	ribonuclease H2, sub- unit A	19p13.2	2.20	0.08	2.27	0.0020
4175	MCM6	minichromosome maintenance complex component 6	2q21.3	2.05	-0.11	1.94	0.0002
5111	PCNA	proliferating cell nu- clear antigen	20p13	2.04	0.01	2.05	0.0017

Table S4: Upregulated genes associated with p53 signaling pathway.

Entrez GeneID	Gene Symbol	GeneName	Location	Log ₂ fold change	Normalized read count (Log ₂)		p-value
					Normal lung tissues	SCLC tissues	
9134	CCNE2	cyclin E2	8q22.1	3.57	0.05	3.62	0.0002

51512	<i>GTSE1</i>	G-2 and S-phase expressed 1	22q13.31	3.20	0.04	3.24	0.0011
9133	<i>CCNB2</i>	cyclin B2	15q22.2	3.05	0.05	3.10	0.0055
1029	<i>CDKN2A</i>	cyclin-dependent kinase inhibitor 2A	9p21.3	3.01	0.24	3.25	0.0047
6241	<i>RRM2</i>	ribonucleotide reductase M2	2p25.1	3.00	-0.29	2.71	0.0021
891	<i>CCNB1</i>	cyclin B1	5q13.2	2.82	-0.06	2.76	0.0008
898	<i>CCNE1</i>	cyclin E1	19q12	2.61	0.06	2.67	0.0041
1111	<i>CHEK1</i>	CHK1 checkpoint homolog	11q24.2	2.57	0.00	2.57	0.0088
983	<i>CDK1</i>	cyclin-dependent kinase 1	10q21.2	2.40	0.05	2.45	0.0058
56475	<i>RPRM</i>	represso, TP53 dependent G2 arrest mediator candidate	2q23.3	2.30	0.10	2.40	0.0499
