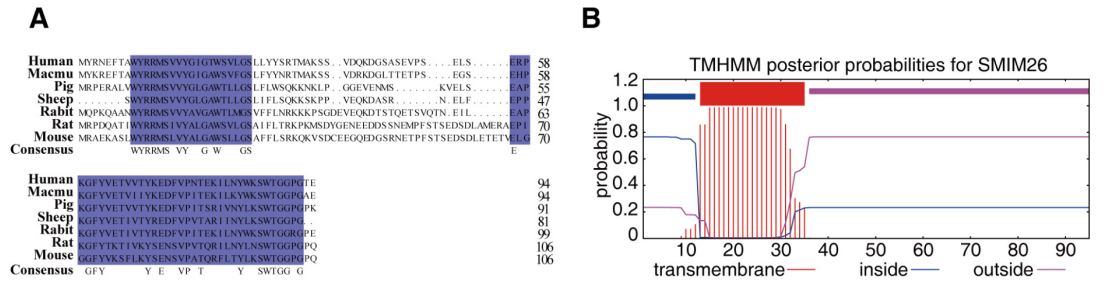
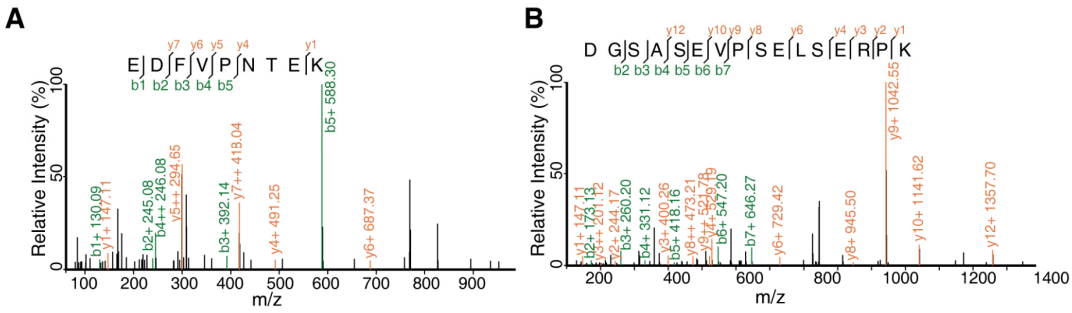
Appendix for: LINC00493-encoded microprotein SMIM26 exerts antimetastatic activity in renal cell carcinoma

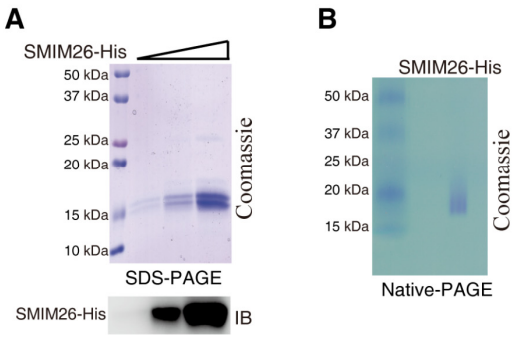
Contents:



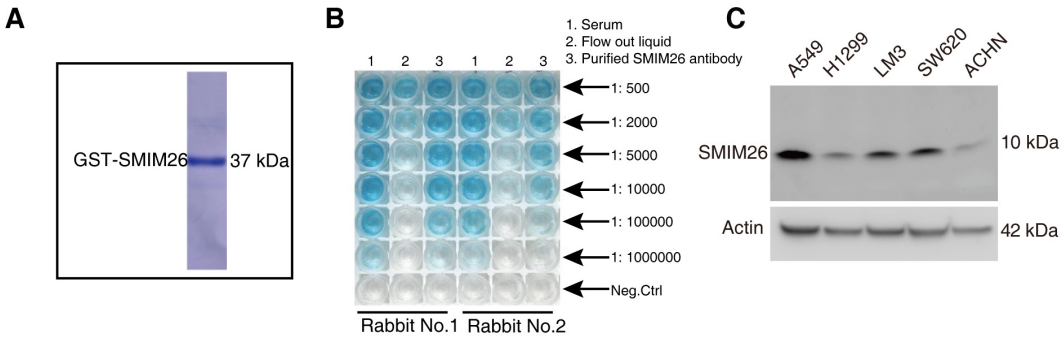
Appendix Fig S1. The sequence signature of SMIM26. (A) The homology of SMIM26 in different species. The dark blue sequence is the homologous sequence, and the white part is the sequence without homology. **(B)** The sequence characteristics of SMIM26 protein were predicted by TMHMM, 14-35 amino acids construct the transmembrane region.



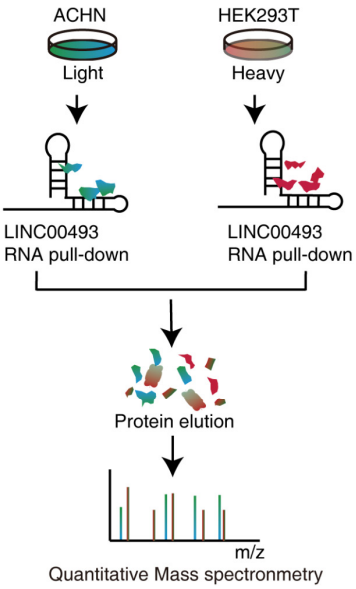
Appendix Fig S2. Two unique peptides of SMIM26 are identified by shotgun MS (A-B)



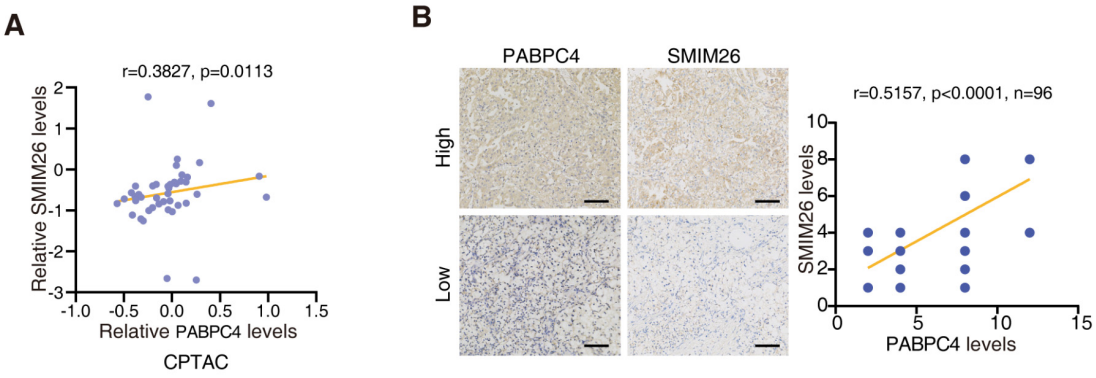
Appendix Fig S3. SMIM26 can't form multimers. (A) Coomassie blue staining and western blotting showing purified SMIM26-His. **(B)** Coomassie blue staining of SMIM26-His with Native-PAGE electrophoresis.



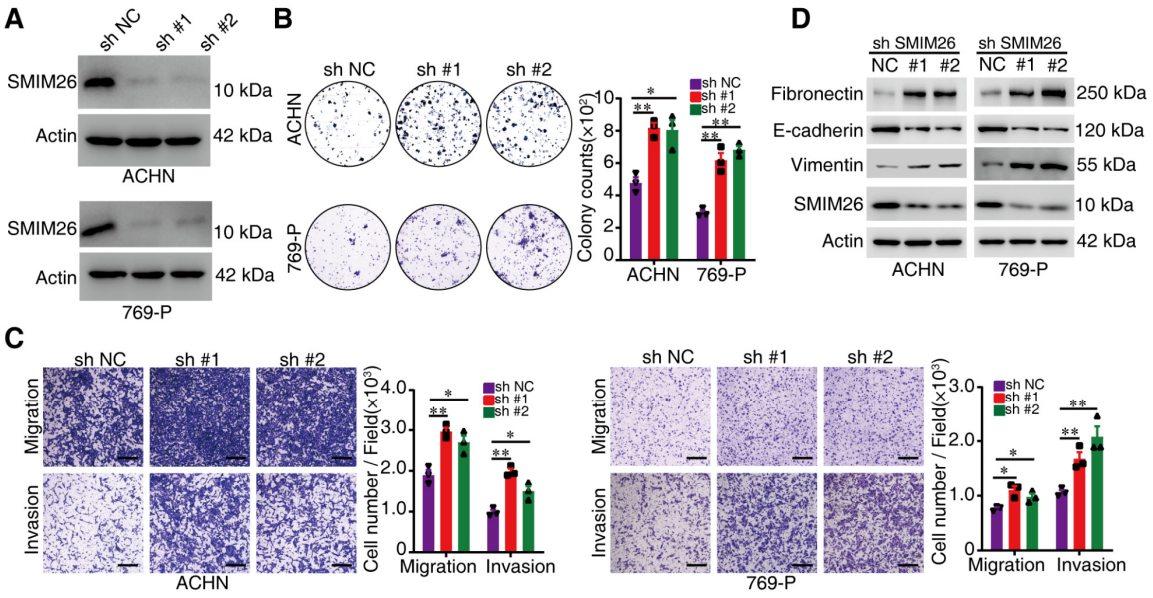
Appendix Fig S4. Generation of anti-SMIM26 antibody. (A) Coomassie blue staining showing purified GST-SMIM26. **(B)** The generation of anti-SMIM26 antibody from rabbits. **(C)** Immunoblotting verification of SMIM26 expression in multiple human cell lines.



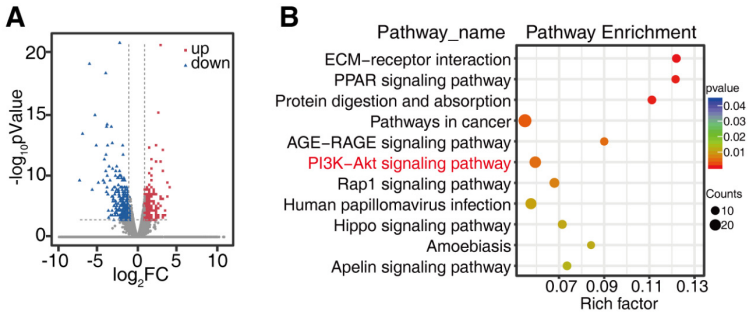
Appendix Fig S5. Schematic of the SILAC-based quantitative RNA–protein interactomics workflow.



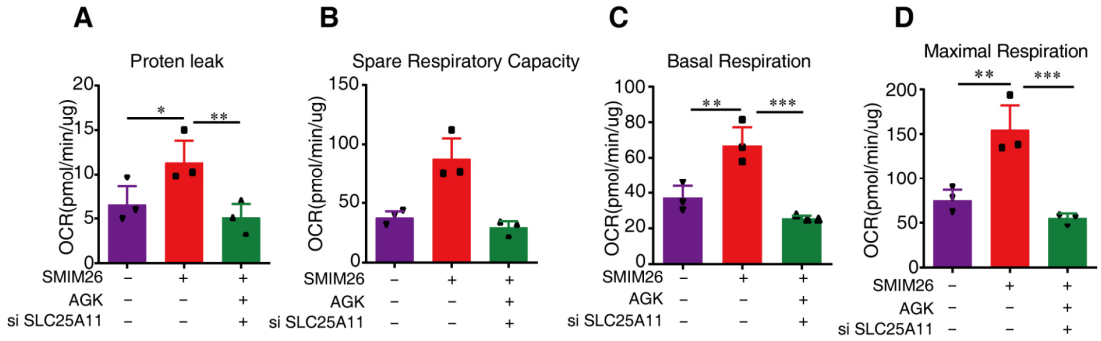
Appendix Fig S6. PABPC4 and SMIM26 expression is correlated. (A) SMIM26 is positively correlated with PABPC4 in CPTAC database. Spearman correlation is 0.3827, p=0.0113. **(B)** SMIM26 is positively correlated with PABPC4 in 96 ccRCC samples immunohistochemistry staining. Spearman correlation is 0.5157, p<0.0001. Scale bar, 100 μm.



Appendix Fig S7. Silencing of SMIM26 promotes tumorigenesis and metastasis. (A) Immunoblotting validation of the knockdown effect of SMIM26 in ccRCC cells. **(B)** Knockdown of SMIM26 promotes the clone formation capacity of ccRCC cells. Data are representative of three biological replicates. Unpaired two-tailed Student's t-test, $*P < 0.05$, $**P < 0.01$. Bars, SEM. **(C)** Transwell assays were used to test the migration and invasion abilities of both ccRCC cells with knockdown of SMIM26. Data are representative of three biological replicates. Scale bar, 600 μm. Unpaired two-tailed Student's t-test, $*P < 0.05$, $**P < 0.01$. Bars, SEM. **(D)** Immunoblotting validation of EMT marker of Vimentin, Fibronectin, and E-cadherin.



Appendix Fig S8. RNA-seq reveals that SMIM26 is involved in PI3K/AKT signaling. (A) RNA-seq was performed to determine the differentially expressed genes in ACHN cells with or without SMIM26 overexpression. **(B)** Bubble plot showing the KEGG pathways enriched by SMIM26-regulated differentially expressed genes.



Appendix Fig S9. Monitoring of the oxygen consumption profile in ACHN cells with the indicated treatment using a Seahorse XF24 analyzer. Proton leak **(A)**, spare respiratory capacity **(B)**, basal respiration **(C)**, and maximal respiration **(D)** were statistically analyzed. Data are representative of three biological replicates. Unpaired two-tailed Student's t-test, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Bar, SD.

Appendix Table S1. Clinical characteristics of patients with ccRCC in this study. Related to Fig. 2.

Characteristics	Total	No. (%)
Patients	368	
Age at diagnosis (years)	53 (4-83)	
Gender		
Male	247	67.1
Female	120	32.6
unknown	1	0.3
Fuhrman grading		
1	43	11.7
2	177	48.1
3	55	15.0
4	18	4.9
unknow	75	20.3
Necrosis		
No	219	59.5
Yes	74	20.1
unknow	75	20.4
Vascular invasion		
No	269	73.1
Yes	24	6.5
unknow	75	20.4
T stage		
1	226	61.4
2	85	23.1
3	45	12.2
4	12	3.3
Lymph nodes invasion		
No	341	92.7
Yes	26	7.1
unknow	1	0.2

Appendix Table S2. Foldchange of LINC00493 binding proteins identified by SMIM26 RNA-pulldown SILAC MS.

Related to Fig. 3.

Protein Name	Rep.1	Rep.2	Rep.3
XRCC6	2.337	2.8154	3.1794
CLH1	2.2705	3.144	3.7346
RS18	1.89	2.3513	2.6375
XRCC5	1.8035	2.3533	2.9547
LMNA	1.7867	3.9632	3.4633
PABPC4	1.6576	1.3157	1.269
HS71B	1.6392	2.0548	1.8278
HS71A	1.6392	2.0548	1.8278
NPM	1.5685	1.8711	1.5153
BIP	1.5416	1.473	1.9487
RS4X	1.4576	1.6194	2.2548
RS19	1.4279	1.6852	1.4967
LAP2B	1.421	1.8893	2.3174
HNRPU	1.4005	1.6404	1.3703
TPM3	1.3792	1.7362	2.4368
TPM4	1.3524	1.9048	2.13
NCL	1.3023	1.3931	1.3054
EF1G	1.3013	1.7955	1.7815
RS11	1.2657	1.9991	1.9762
RS3A	1.2465	1.4409	1.7175
RL8	1.2359	1.2401	1.4344
CH60	1.2309	1.3816	1.6673
HNRPM	1.2196	1.3123	1.264
HSP7C	1.2132	1.3312	1.2236
RL7	1.2001	1.565	1.8412

Appendix Table S3. SMIM26 binding proteins identified by Co-IP MS

Related to Fig. 5.

Proteins							
ABCB7	COPA	FANCI	MAGED2	PDLIM5	RPL18A	SLC25A4	TRIM27
ABCD3	COPB1	FARSA	MAGT1	PDS5A	RPL24	SLC3A2	TRIM28
ABCF2	COPB2	FKBP8	MAP1B	PFKP	RPL27A	SMC2	TRIP13
ACAT1	COPE	GALK1	MARS	PHGDH	RPL3	SMC3	TRIP6
AGK	COPG2	GANAB	MBOAT7	PPP6R3	RPL30	SMC4	TUBB2B
IFM1	CSDA	GCN1L1	MCM3	PRDX4	RPL31	SNRNP200	TUBB6
ALDH1B1	CSE1L	GEMIN4	MCM7	PRPF8	RPL35A	SNRNP40	TUFM
ANKHD1	CYC1	GEMIN5	MDN1	PRPS1	RPL9	SNRPD3	U2AF2
ARF4	DARS	HAX1	MGST3	PSMD3	RPLP1	SPTLC1	UBAP2L
ATP1A1	DDOST	HEATR2	MMS19	PTPLAD1	RPN1	SQSTM1	UNC45A
ATP2A2	DDX20	HNRNPA0	MOV10	QARS	RPN2	SRSF7	UQCRC2
ATP5L	DDX21	HNRNPF	MSH6	QSOX2	RPS12	SSR4	USP10
ATP5O	DDX39B	HSD17B12	MTHFD1	RALY	RPS27	STAU1	VDAC2
ATXN10	DHCR7	IARS	MYBBP1A	RARS	RPS28	SURF4	VDAC3
BCLAF1	DHX15	IDH3A	MYO1C	RCC2	RPS6	TARDBP	WDR61
BYSL	DNAJA1	ILF2	NCAPD2	RCN1	RPSA	TARS2	XPO1
C1orf57	DNAJA2	IPO4	NCLN	RCN2	SAMHD1	TBC1D15	XPO5
CAD	DNAJA3	IPO7	NDUFA13	RFC3	SAMM50	TBCD	XPO7
CALU	DNAJC11	IPO8	NDUFA5	RFC4	SCAMP3	TCP1	XPOT
CAND1	DRG1	IPO9	NDUFA9	RFC5	SCO2	TELO2	YME1L1
CCDC47	DSP	IRAK1	NDUFB10	RHOT2	SDF4	TIMM44	YTHDF2
CCT3	DYNC1H1	KIFC1	NOLC1	RIC8A	SEC13	TIMM50	YWHAQ
CCT5	ECM29	KPNA2	NPEPPS	RNF114	SFXN1	TM9SF3	ZC3HAV1
CCT6A	EEF2	KPNB1	NUP133	RPL10	SLC16A1	TMEM33	ZW10
CCT7	EFTUD2	LARS	NUP93	RPL10A	SLC1A5	TNPO1	SMIM26
CDK1	EIF5A	LASP1	OAT	RPL12	SLC25A10	TNPO3	
CDK2	EPRS	LBR	OCIAD2	RPL13A	**SLC25A11**	TOMM40	
CHCHD3	EXOC5	LRPPRC	PCBP1	RPL15	SLC25A12	TOP1	
CKAP5	FAF2	LRRC59	PCBP2	RPL17	SLC25A13	TRAFD1	
CNOT1	FAM98A	MAGED1	PDK3	RPL18	SLC25A3	TRIM25	

Appendix Table S4. SMIM26 binding proteins identified by SILAC-IP MS

Related to Fig. 5.

proteins	H/L Ratio	L/H Ratio	proteins	H/L Ratio	L/H Ratio	proteins	H/L Ratio	L/H Ratio
CYC1	1.55	1.56	FANCI	2.52	2.17	SLC3A2	2.35	3.00
RPN2	1.51	1.58	HSD17B12	2.55	2.18	ECM29	4.75	3.04
C20orf4	1.98	1.63	MDN1	4.60	2.20	EMD	2.33	3.07
EEF1G	1.58	1.64	ZW10	1.83	2.23	FAR1	2.25	3.08
AFG3L2	2.36	1.64	IPO9	5.81	2.25	GCN1L1	4.59	3.11
TUBA1C	2.11	1.69	SEC16A	2.31	2.26	ERLIN2	3.68	3.23
TUBB6	3.08	1.77	NUP93	1.82	2.27	PPP6R3	2.87	3.26
ESYT2	2.26	1.79	UQCRC2	2.41	2.27	IPO7	3.96	3.30
RCN1	2.02	1.81	TMEM165	4.41	2.27	ATP2A2	3.78	3.36
TNPO3	3.01	1.81	SEC61A1	2.97	2.28	TELO2	4.72	3.44
NUP205	1.66	1.81	NUP107	1.78	2.29	SLC25A5	3.20	3.53
MAGED2	2.37	1.86	KPNB1	2.91	2.31	PRKDC	4.25	3.71
TIMM50	1.90	1.92	PSMC2	1.82	2.34	SLC25A1	4.55	3.86
CSE1L	2.13	1.93	IPO11	3.35	2.40	TECR	4.82	3.90
EXOC4	2.42	1.95	SDF4	2.02	2.42	TRAFD1	2.83	3.99
TUBB	2.33	1.97	OXA1L	1.69	2.42	ATP1A1	4.05	4.20
CAD	2.27	1.98	DHCR7	3.97	2.48	SLC25A10	3.31	4.24
PPP6R1	2.12	1.98	IRAK1	2.21	2.55	**SLC25A11**	**6.29**	**4.27**
IPO5	2.87	2.01	XPO5	2.38	2.60	MON2	2.80	4.34
AGK	**1.81**	**2.02**	RCN2	3.56	2.73	SLC25A3	4.87	4.86
PHGDH	2.29	2.03	SLC25A13	3.72	2.78	XPOT	4.48	5.49
AIFM1	2.52	2.04	MMS19	4.55	2.82	SLC16A1	10.13	6.08
RHOT2	2.61	2.07	SLC1A5	2.08	2.89	SMIM26	10.13	8.20
FADS2	1.57	2.11	MAGED1	2.50	2.94			
TRIP13	1.51	2.15	XPO1	3.76	2.99			

Appendix Table S5. SMIM26 binding proteins identified by GST-SMIM26 pulldown MS

Related to Fig. 5.

Proteins					
KRT9	YBX1	ACADM	NFS1	SLC25A3	SNAP47
KRT1	EEF1B2	METTL15	RUVBL2	MAP2K7	MAP2K2
KRT2	SMIM26	TES	SLC25A24	GBAS	RPL3
KRT10	TUBB6	SLC16A1	PYCR2	PIP	SSB
HRNR	CDK1	HNRNPH1	GCDH	PREB	EIF5A
GST	RUVBL1	ALB	SCAMP3	DSG1	DIRAS2
SLC25A11	ATP5A1	FLOT1	EARS2	DDOST	SRSF8
ACTB	ATP5B	DNAJA2	HNRNPA1	NONO	SNRPA
KRT6B	PCNA	EMD	VAT1	TRIP13	TMPRSS13
KRT6A	DNAJA1	KRT77	DSC1	PELO	ABHD12
TUBB4B	KRT8	KRT18	HNRNPA0	PRMT6	GAPDH
KRT5	TUFM	IDH2	MAT2A	AZGP1	RPS7
CBR1	EEF1A1	NIPSNAP1	LANCL2	KRT78	EIF2S3
KRT14	PDK3	RPL10A	NDUFS2	FAM98B	EIF3F
TUBB	UQCRC2	FLOT2	ANXA2	KPRP	EEF1G
TUBA1C	EEF1A2	ENO1	OAT	RPL5	
FLG2	LANCL1	CLPX	DCD	SFXN3	
GSTP1	HNRNPF	CYC1	IGKC	PSMA1	
KRT16	SERPINH1	RPL4	**AGK**	PSMC5	
HSPB1	PYCR1	NDUFV1	ACOT9	RPL13	

Appendix Table S6. Sequences of primers, shRNAs, siRNAs.

Name	Sense sequence (5'-3')	Antisense sequence (5'-3')
qSMIM26	ACTATAGCCGGACAATGGCG	TGGGCGTTCAGAGAGTTCAC
qActin	ACGTGGACATCCGCAAAG	GACTCGTCATACTCCTGCTTG
qPABPC4	CTCTCGTGGCCTCCCTACTA	CAGCTGTCAGTCAGCCCTTG
qNCL	AAGCGTTGGAACTCACTGGT	AAGTGTTCTCGCATCTCGCT
GST-SMIM26 (pGEX-4T-1)	GCGAATTCATGTATCGAAATGAGTTCACGG	GCCTCGAGTCATGGTTCTGTACCAGGGCCACCAG
SMIM26-GFP (pEGFP-N1)	ATAAAACTCGAGCTGCGAGAATCGAGGCACTCG	ATAAAAGAATTCGTGGTTCTGTACCAGGGCCACCAG
SMIM26-flag (pLVX-Puro)	GCGAATTCATGTATCGAAATGAGTTCACGG	GCGGATCCTTACTTATCGTCGTCATCCTTGTAATCTGGTTCTGTACCAGGGCCAC
SMIM26-Mut (pLVX-Puro)	GCGAATTCGGATATCGAAATGAGTTCACGG	GCGGATCCAGCCTTATCGTCGTCATCCTTGTAATCTGGTTCTGTACCAGGGCCAC
sh-SMIM26-#1 (pLKO.1)	CCGGGATGGCTCAGCAAGTGAAGTACTCGAGTACTTCACTTGCTGAGCCATCTTTT	AAAAGATGGCTCAGCAAGTGAAGTACTCGAGTACTTCACTTGCTGAGCCATCCCGG
sh-SMIM26-#2 (pLKO.1)	CCGGGGAAACAGTTGTCACATATAACTCGAGTTATATGTGACAACTGTTTCCTTTTT	AAAAAGGAAACAGTTGTCACATATAACTCGAGTTATATGTGACAACTGTTTCCCCGG
HA-AGK (pLVX-Puro)	TCGAGCTCAAGCTTCGAATTCATGACGGTGTTCTTTAAAACGCT	GTACCGTCGACTGCAGAATTCTCAGGCGTAGTCAGGCACGTCGTAAGGATACTGGGT

		GGGGCTTGTGAGCAT
HA-AGK^{G126E} (pLVX-Puro)	AGGAGATGAAACACTGCA GGAGGTTGTTACTGG	GCAGTGTTTCATCTCCTCCT GCAACAATGATCA
si-SMIM26	CCAUGACUGGCUGCUGA AU	AUUCAGCAGCCAGUCAUG G
si-PABPC4-#1	GGGUCUGCCGCGAUAUG AUTT	AUCAUAUCGCGGCAGACCC TT
si-PABPC4-#2	GCUCCUCAAUGACCGCAA ATT	UUUGCGGUCAUUGAGGAG CTT
si-SLC25A11-#1	CCCGCCUUGGCAUCUAUA CTT	GUAUAGAUGCCAAGGCGG GUA
si-SLC25A11-#2	CAAUCCAAGCAGUUCUU ACTT	GUAAGAACUGCUUGGAUU GGG
si-SLC25A11-#3	GGUUUCUUCCUCUGCGG UATT	UACCGCAGAGGAAGAAAC CTT
si-NCL	CCUGCCAAGAAGACAGU UATT	UAACUGUCUUCUUGGCAG GTT