

Supplementary Information for:

**MDK Promotes Lymph Node Metastasis of Cervical Squamous Cell Carcinoma
by Activating the PI3K/AKT and p38 MAPK Pathway**

Running title : MDK promotes lymph node metastasis of cervical cancer

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**Table S1 to S3
Supplementary Legends
Figures S1 to S3**

Table S1. Expression of MDK in samples from 12 NC and 11 CSCC patients

Variant	Total	MDK		<i>P</i>
		-	+	
NC	12	11(91.67%)	1(8.33%)	<0.01
CSCC	11	4(36.36%)	7(63.64%)	

Table S2. Primary antibody information for Immunofluorescence, WB and IHC

Antibody	Concentration			Catalog number and Manufacturer
	Immunofluorescence	WB	IHC	
Rabbit anti-human MDK antibody		1:800	1:100	# ab236781, Abcam, USA
Mouse anti-human Bax antibody		1:1000		# AF0054, Beyotime, China
Rabbit anti-human Bcl-2 antibody		1:1000		# AF0060, Beyotime, China
Rabbit anti-human ZO-1 antibody		1:1000		# AF5145, Affinity, USA
Rabbit anti-human Claudin5 antibody	1:200	1:1000		# AF5216, Affinity, USA
Rabbit anti-human Occludin antibody	1:200	1:1000		# DF7504, Affinity, USA
Rabbit anti-human MMP9 antibody		1:1000		# ab76003, Abcam, USA
Rabbit anti-human MMP2 antibody		1:1000		# AF1420, Beyotime, China
Rabbit anti-human AKT antibody		1:1000		#9272S, Cell Signaling Technology (CST), USA
Rabbit anti-human p-AKT (Ser 473) antibody		1:1000		#4058L, CST, USA
Rabbit anti-human p38 MAPK antibody		1:1000		#9212S, CST, USA
Rabbit anti-human p-p38 MAPK antibody		1:1000		#9215L, CST, USA
Rabbit anti-mouse LYVE-1 antibody			1:200	#ab14917, Abcam, USA
Rabbit anti-GAPDH antibody		1:5000		# ab9485, Abcam, USA
Goat Anti-Rabbit IgG H&L (HRP)		1:5000	1:2000	# ab6721, Abcam, USA
Goat Anti-Mouse IgG H&L (HRP)		1:5000	1:2000	# ab6789, Abcam, USA

Table S3. Oligonucleotide sequences used for Realtime-PCR analysis.

Genes names	Sense primers (5' to 3')
hMDK-F	CGCGGTCGCCAAAAAGAAAG
hMDK-R	TACTTGCAGTCGGCTCCAAAC
hGAPDH-F	GGAGCGAGATCCCTCCAAAAT
hGAPDH-R	GGCTGTTGTCATACTTCTCATGG
hZO-1-F	CAACATACAGTGACGCTTCACA
hZO-1-R	CACTATTGACGTTTCCCCACTC
hClaudin5-F	CTCTGCTGGTTCGCCAACAT
hClaudin5-R	CAGCTCGTACTTCTGCGACA
hOccludin -F	ACAAGCGGTTTTATCCAGAGTC
hOccludin -R	GTCATCCACAGGCGAAGTTAAT

Supplementary Figure S1. MDK inhibits apoptosis of cervical cancer cells, and promotes tumor growth

A. The expression of MDK in five cervical cancer cell lines was analyzed by RT-PCR and normalized to the levels measured in the MS751 cell line (=1).

B. The IHC staining results showed the efficiencies of knockdown of MDK in C33a cells and the efficiencies of overexpress of MDK in MS751 cells.

C. FACS analysis of active caspase-3 in MS751 MDK and MS751 NC cells.

D. The footpad transplanted tumor volume in MS751 NC group and MS751 MDK group on day 28.

The data represented the median with 95% CI. Each experiment was repeated independently at least three times. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplementary Figure S2. ZEB2 was Upregulated in EOC-CSLCs and Correlated with their Mesenchymal Feature and Invasive Potential

A. Typical diagram of sprouting of HLECs pretreated with conditioned medium of C33a shMDK-1/C33a shMDK-3/C33a NC cells. Scale bar =10 μm .

B. The expressions of ZO-1, Claudin5 and Occludin in HLECs cultured by conditioned medium of MS751 MDK/MS751 NC cells were analyzed by RT-PCR.

C. The expressions of ZO-1, Claudin5 and Occludin in HLECs cultured by conditioned medium of C33a shMDK-1/C33a shMDK-3/C33a NC cells were analyzed by RT-PCR.

D. The lymph nodes with metastasis (-) (upper) and metastasis (+) (lower) were observed under confocal microscope. The purplish red is lymphoid sinuses labeled by the injected dextran, and the green fluorescence is the tumor cells with GFP fluorescence. Scale bar =100 μ m.

E. Lymph node metastasis was detected by frozen section, the green fluorescence is the tumor cells with GFP fluorescence. Scale bar =100 μ m.

The data represented the median with 95% CI. Each experiment was repeated independently at least three times. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplementary Figure S3. MDK activates the PI3K/AKT and p38 MAPK pathways to promote lymphangiogenesis.

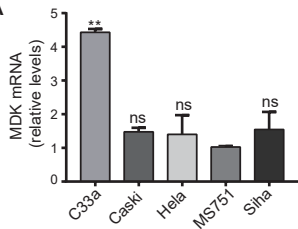
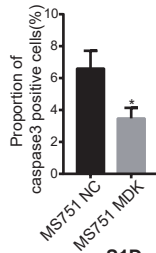
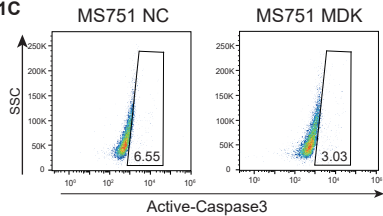
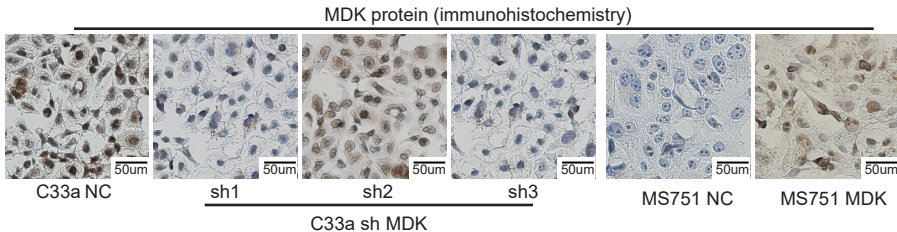
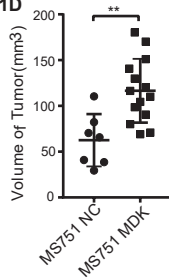
A. The effects of different concentrations of recombinant human MDK protein(0-400ng/ml) on the proliferation of HLECs for 48h.

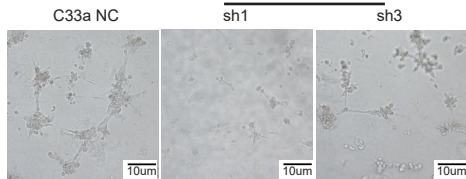
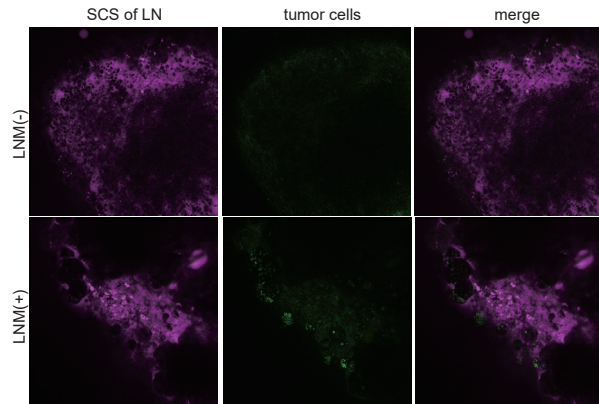
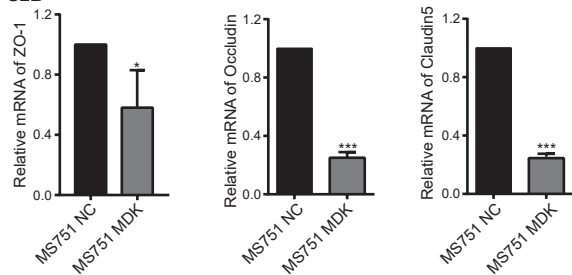
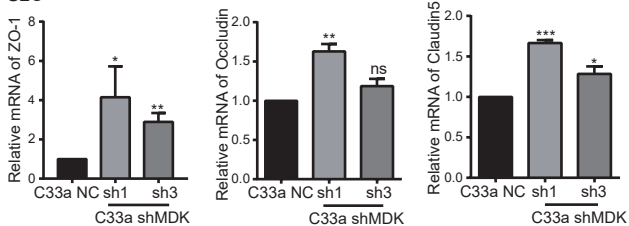
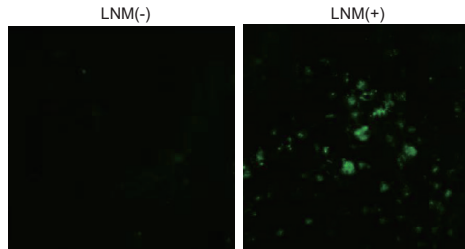
B. WB was used to detect the phosphorylation of AKT and p38 MAPK when HLECs were cultured by conditioned medium.

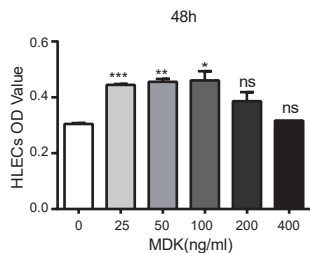
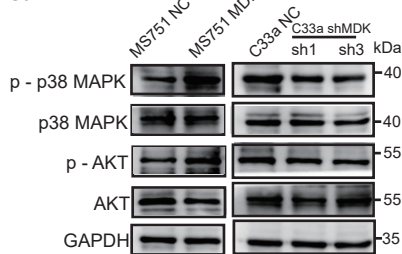
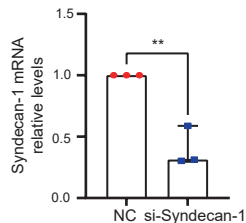
C. Point diagram of GO pathway enrichment of differential genes detected by transcriptomic sequencing.

D. The expressions of Syndecan-1 mRNA were analyzed by RT-PCR.

The data represented the median with 95% CI. Each experiment was repeated independently at least three times. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

S1A**S1C****S1B****S1D**

S2A**S2D****S2B****S2C****S2E**

S3A**S3B****S3D****S3C**