

Table S1. The clinicopathological relevance analysis of the expression of exosomal miR-23a-3p in the serum of HCC patients.

Characteristic	Low group	High group	p
n	20	20	
gender, n (%)			0.333(ns)
Female	10 (25%)	6 (15%)	
Male	10 (25%)	14 (35%)	
Age, n (%)			0.041(*)
<55	10 (25%)	3 (7.5%)	
≥55	10 (25%)	17 (42.5%)	
Differentiation, n (%)			0.010(**)
poor	6 (15%)	15 (37.5%)	
Well to	14 (35%)	5 (12.5%)	
Tumor size, n (%)			0.010(**)
>5	7 (17.5%)	16 (40%)	
≤5	13 (32.5%)	4 (10%)	
N stage, n (%)			0.010(**)
N0	13 (32.5%)	4 (10%)	
N1	7 (17.5%)	16 (40%)	
M stage, n (%)			0.020(*)
M0	19 (47.5%)	12 (30%)	
M1	1 (2.5%)	8 (20%)	
AJCC stage, n (%)			<0.001(***)
stage I	10 (25%)	1 (2.5%)	
stage II	6 (15%)	0 (0%)	
stage III	3 (7.5%)	11 (27.5%)	

Characteristic	Low group	High group	p
stage IV	1 (2.5%)	8 (20%)	

Table S2. The primer sequences used in real-time PCR

Name	Sequence
IL-10	Forward:GTTGTTAAAGGAGTCCTTGCTG Reverse:TTCACAGGGAAGAAATCGATGA
TNF- α	Forward:CCTCTCTCTAATCAGCCCTCTG Reverse:GAGGACCTGGGAGTAGATGAG
CD163	Forward:TTTGTCAACTTGAGTCCCTTAC Reverse:TCCCGCTACACTTGTTTTTAC
miR-23a-3p	ATCACATTGCCAGGGATTTC
HnRNPA1	Forward:TCAGAGTCTCCTAAAGAGCCC Reverse:ACCTTGTGTGGCCTTGCAT
PTEN	Forward:TGGATTCGACTTAGACTTGACCT Reverse:GGTGGGTTATGGTCTTCAAAGG
TJP1	Forward:CAACATACAGTGACGCTTACA Reverse:CACTATTGACGTTTCCCCACTC
CCL2	Forward:CAGCCAGATGCAATCAATGCC Reverse:TGGAATCCTGAACCCACTTCT
CCR2	Forward:CCACATCTCGTTCTCGGTTTATC Reverse:CAGGGAGCACCGTAATCATAATC
GAPDH	Forward:GGAGCGAGATCCCTCCAAAAT Reverse:GGCTGTTGTCATACTTCTCATGG

Table S3. Antibody

Antibody	Assay	Product code	Dilution
CD163	IHC	Ab182422, Abcam, USA	1/500
CD63	WB	Ab134045, Abcam, USA	1/1000
CD81	WB	Ab109201, Abcam, USA	1/1000
CD9	WB	Ab195422, Abcam, USA	1:1000
E-cadherin	WB	#3195, CST, USA	1:1000
N-cadherin	WB	#13116, CST,USA	1:1000
Vimentin	WB	#5741, CST,USA	1:1000
TJP1	WB	#13663, CST, USA	1:1000
Occludin	WB	#91131, CST,USA	1:1000
Claudin5	WB	ab236066, Abcam, USA	1:1000
hnRNPA1	WB	#8443, CST, USA	1:1000
CCR2	WB	#12199, CST,USA	1:1000
GAPDH	WB	#5174, CST,USA	1:1000

Table S4. The sequences of siRNAs used in this study

Name	Sequence
miR-23a-3p mimics	5'-AUCACAUUGCCAGGGAUUUC-3'
NC	5'-UUCUCCGAACGUGUCACGUTT-3'
miR-23a-3p Inhibitor	5'-UAGUGUAACGGUCCCUAAAGG-3'
NC	5'-GCUUAAGACAUUCCGAGGAAU-3'

Supporting Fig. legends

Supplementary Fig1. Polarized macrophage from Thp-1 was set up *in vitro*. **a:** The change of cell morphology was observed by microscope. **b, c:** Expression of CD68 and CD163 was determined by flow cytometry and IF.

Supplementary Fig2. Expression of miR-23a-3p in different groups was tested by RT-PCR. **a:** GW4869 decreased the upregulation of miR-23a-3p in HCC cells and HUVECs induced by M2 macrophage co-culture. **b:** Knockdown of miR-23a-3p in macrophages and tested by RT-PCR. **c:** The expression of miR-23a-3p in M2 macrophage exosomes after miR-23a-3p knockdown was induced by M2 macrophage exosomes or miR-23a-3p mimic and tested by RT-PCR. *P < 0.05, **P < 0.01, ***P < 0.001.**d.** The upregulation of miR-23a-3p expression in HCC cells and HUVECs caused by M2 macrophage coculture was also inhibited and tested by RT-PCR. *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Fig3. M2 macrophage exosomes promote angiogenesis and increase vascular permeability through transferring miR-23a-3p. **a:** The effect of M2 macrophage exosomal miR-23a-3p on the release of VEGFA as determined by ELISA. **b, c:** The effect of M2 macrophage exosomal miR-23a-3p on the proliferation and migration of HUVECs was tested by the CCK-8 and Transwell[®] assay. **d:** The effect of M2 macrophage exosomal miR-23a-3p on angiogenesis as tested by the tube formation assay. **e, f:** The effect of M2 macrophage exosomal miR-23a-3p on vascular permeability as tested by transendothelial invasion and endothelial permeability. **g:** The effect of M2 macrophage exosomal miR-23a-3p on the expression of vascular adhesion molecules as tested by western blotting. *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Fig4. miR-23a-3p knockdown suppressed HCC cell metastasis and angiogenesis in vivo. **A.** Different groups of cells were injected into the caudal vein of nude mice to induce lung metastasis. typical bioluminescence images and lung tissues were obtained, and liver metastases were stained with HE.**B.**

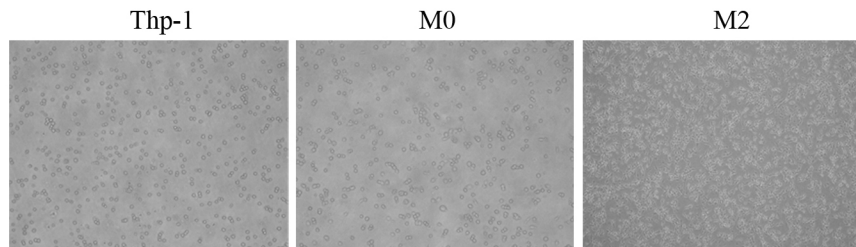
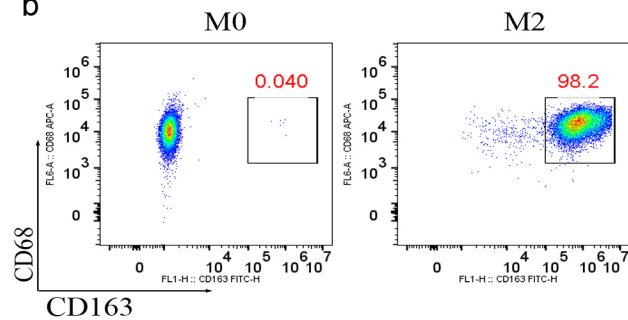
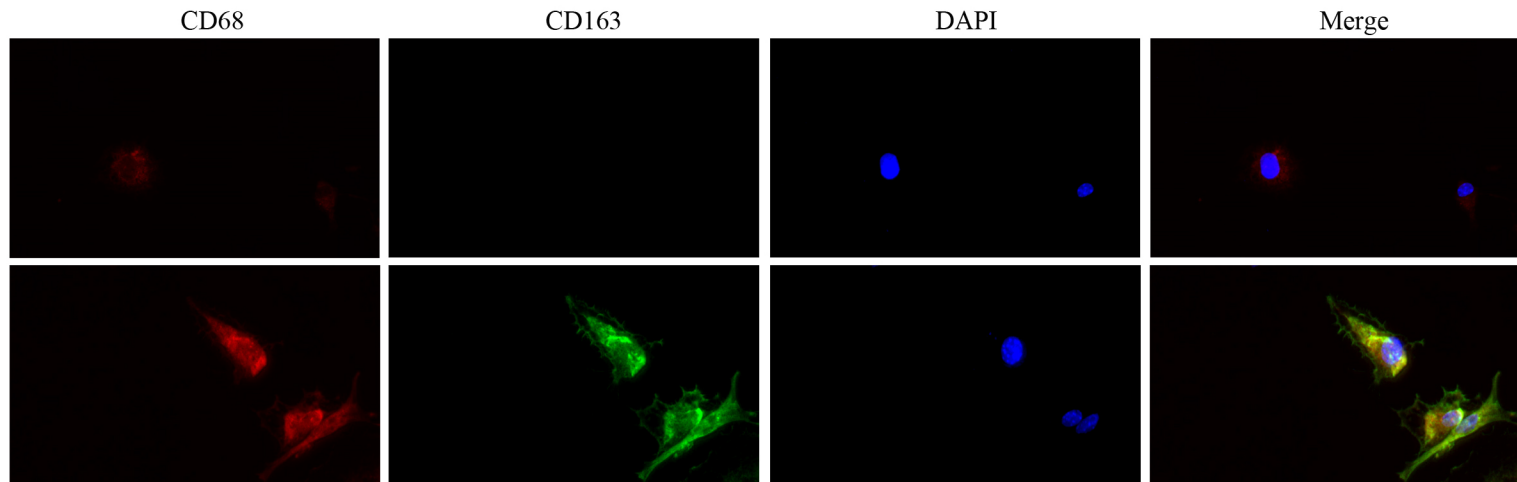
Supplementary Fig5. HnRNPA1 mediates miR-23a-3p packaging into M2 macrophage-derived exosomes. **a:** The effect of hnRNPA1 knock down on the release of VEGFA induced by M2 exosomes and tested by ELISA. **b, c:** The effect of hnRNPA1 knock down on the proliferation and migration of HUVECs induced by M2 exosomes and tested by CCK-8 and Transwell[®] assay. **d:** The effect of hnRNPA1 knock down on angiogenesis induced by M2 exosomes and tested by a tube formation assay. **e, f:** The effect of hnRNPA1 knock down on vascular permeability induced by M2 exosomes and tested by transendothelial invasion and endothelial permeability. **g:** The effect of hnRNPA1 knock down on the expression of vascular adhesion molecules induced by M2 exosomes as tested by western blotting. *P < 0.05, **P < 0.01,***P < 0.001.

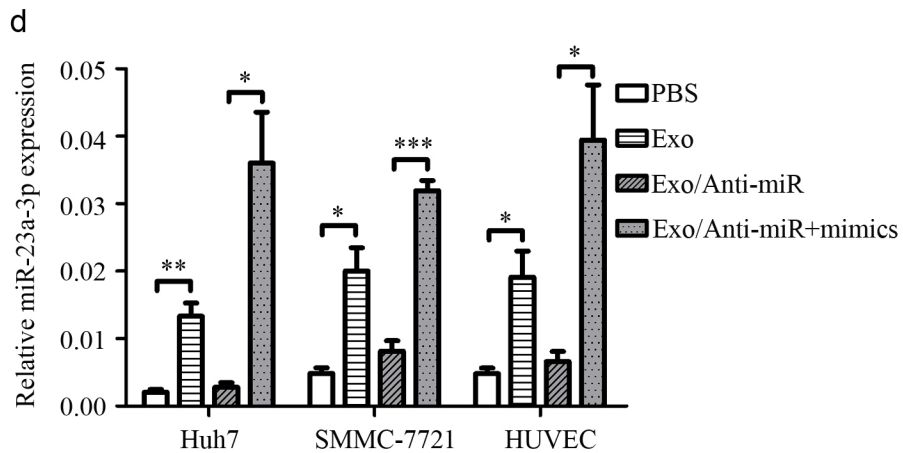
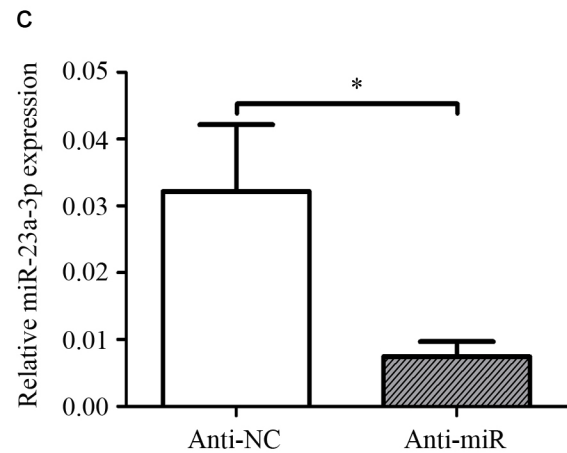
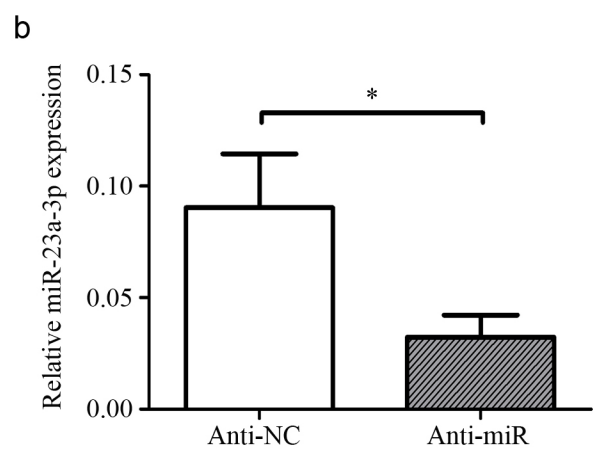
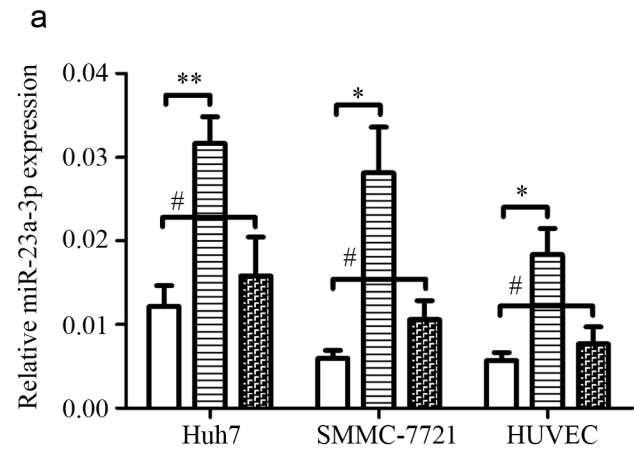
Supplementary Fig6. PTEN and TJP1 is the direct target of miR-23a-3p. **a** and **c:** The sequences of miR-23a-3p and the potential miR-23a-3p-binding sites at the 3'UTR of PTEN and TJP1 was predicted by targetscan. **b** and **d:** The effect of miR-23a-3p overexpression on the luciferase activity of PTEN and TJP1 was assessed using a luciferase assay. **e–h:** The effect of miR-23a-3p overexpression on the expression of PTEN and TJP1 was analyzed by qRT-PCR and western blotting. *P < 0.05, **P < 0.01, ***P < 0.001.

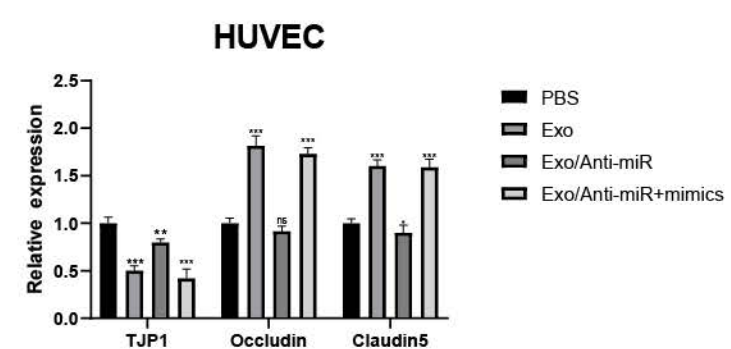
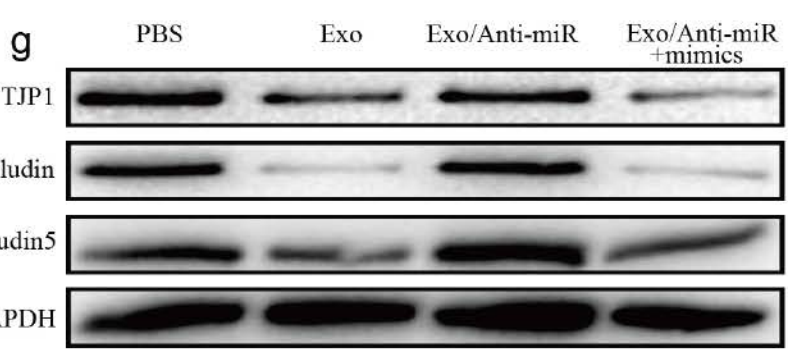
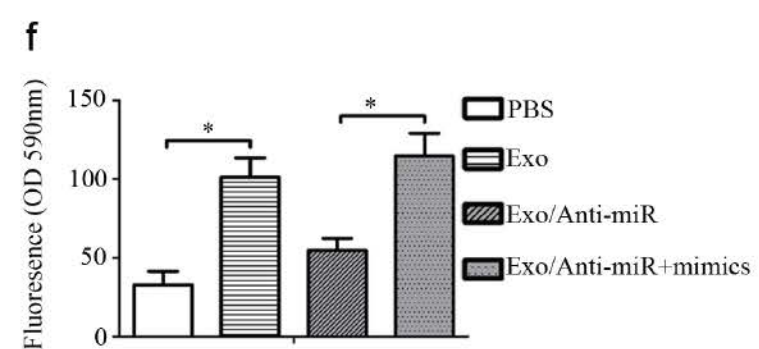
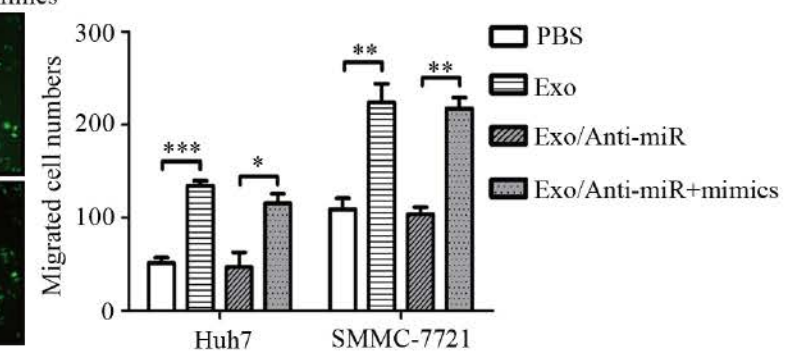
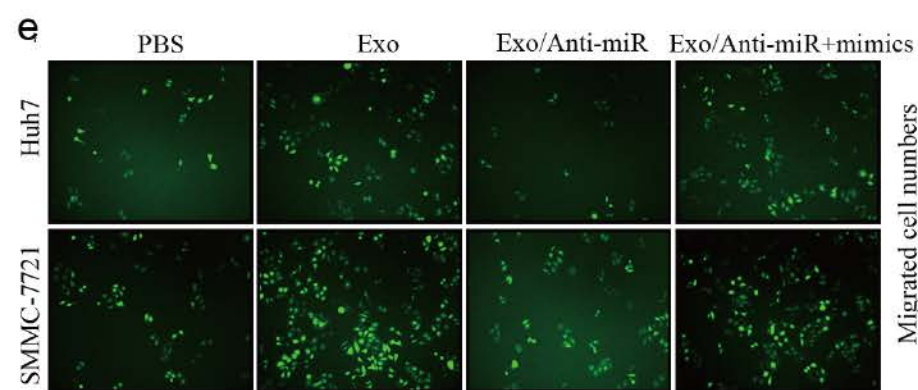
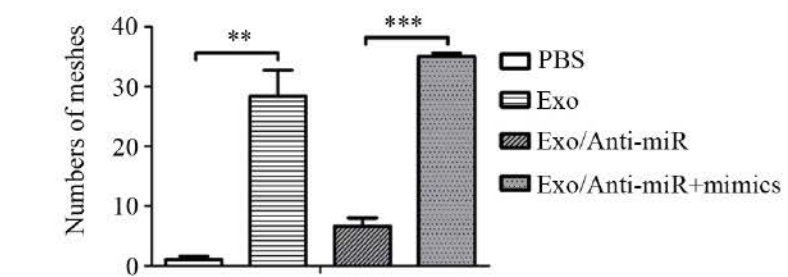
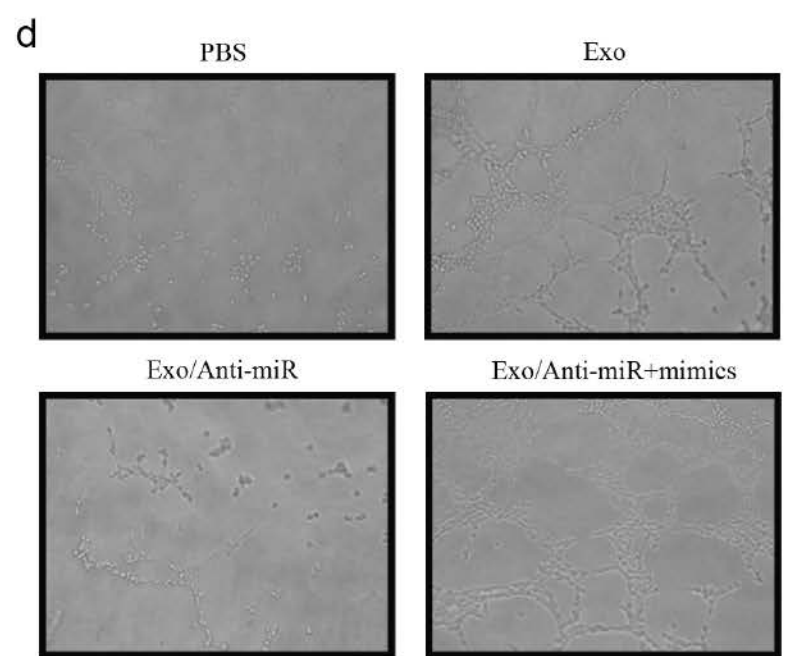
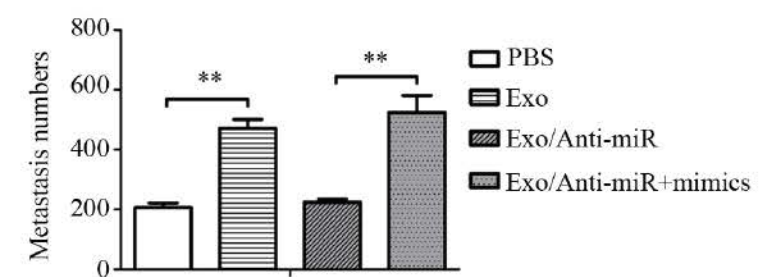
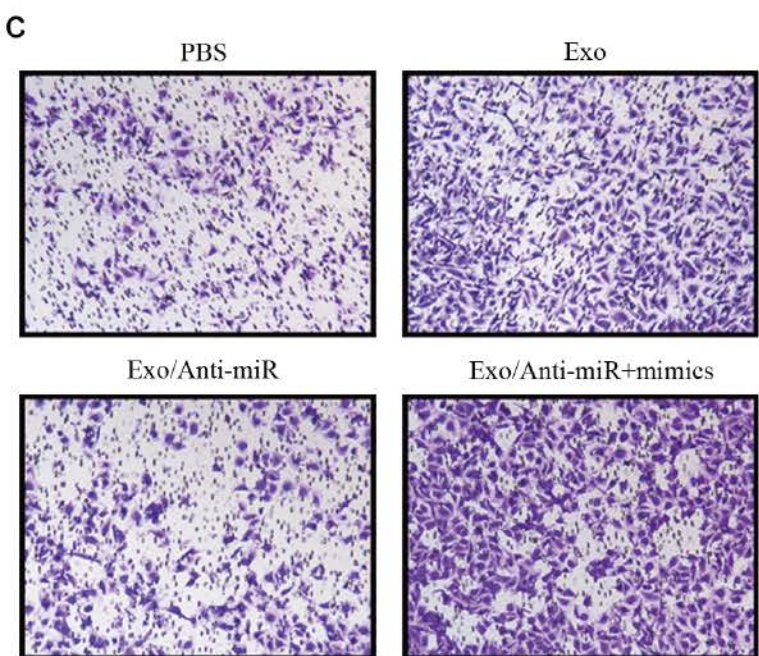
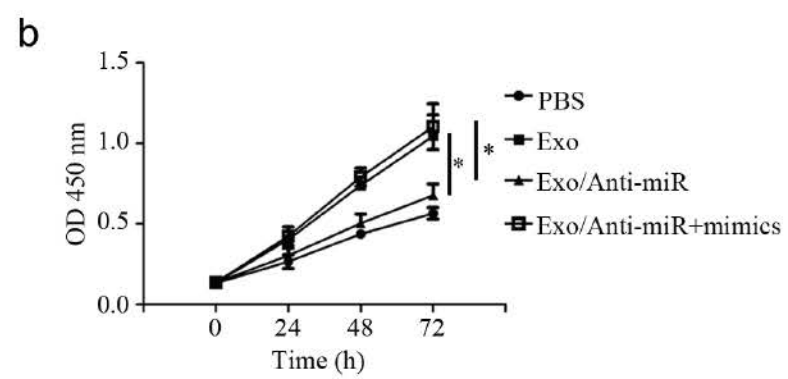
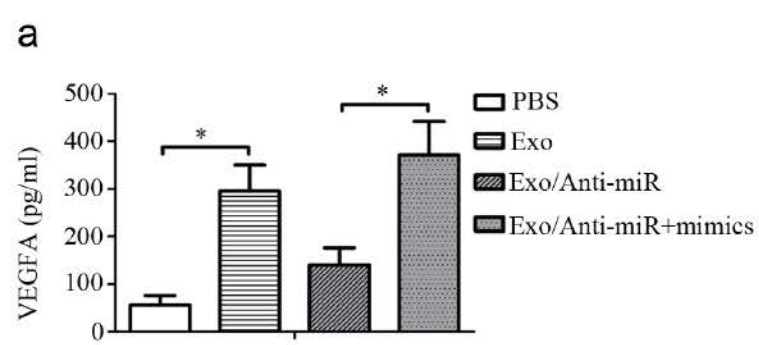
Supplementary Fig7. M2 macrophage exosomal miR-23a-3p promotes angiogenesis and increased vascular permeability by targeting PTEN and TJP1. **a:** The effect of PTEN on

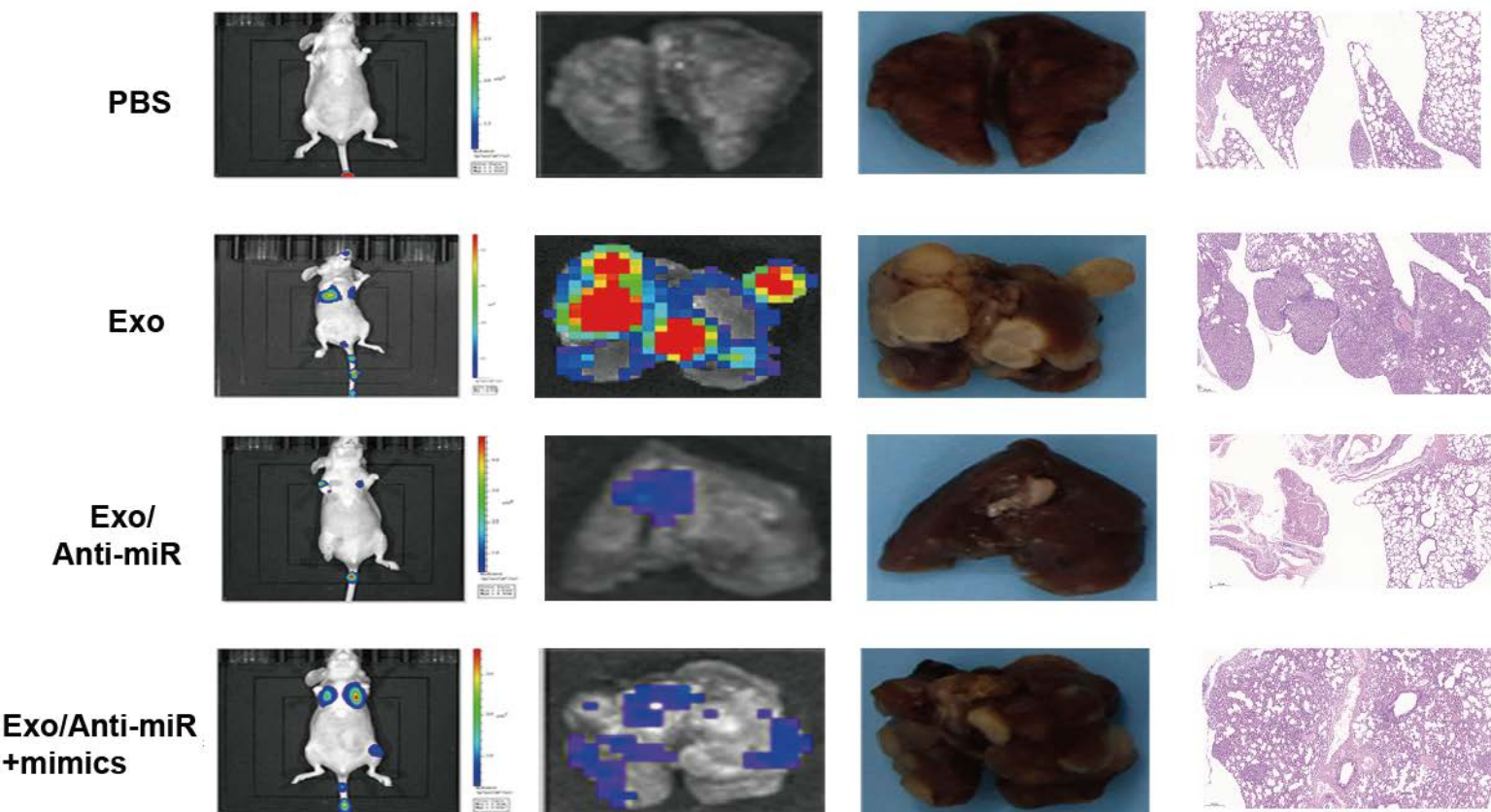
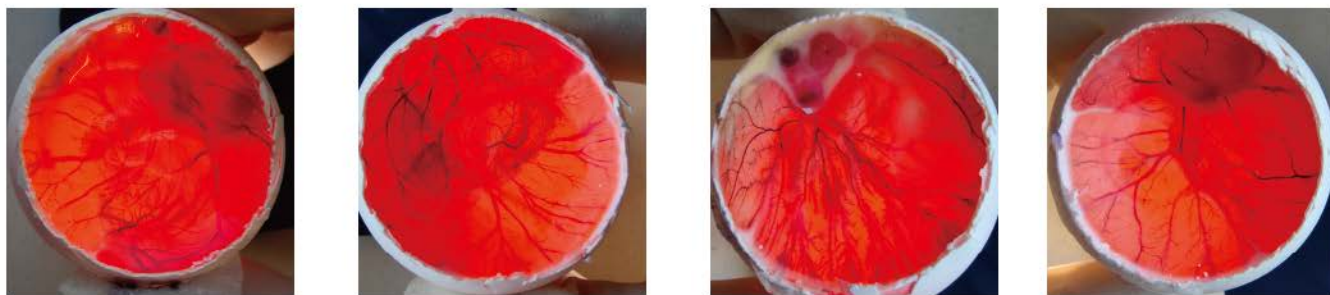
VEGFA secretion cells induced by M2 macrophage exosomal miR-23a-3p and tested by ELISA. **b-c:** The effect of PTEN on the proliferation and migration of HUVECs induced by M2 macrophage exosomal miR-23a-3p and tested by CCK-8 and a Transwell[®] assay. **d:** The effect of PTEN on angiogenesis as induced by M2 macrophage exosomal miR-23a-3p and tested by the tube formation assay. **e, f:** The effect of TJP1 on vascular permeability as induced by M2 macrophage exosomal miR-23a-3p and tested by transendothelial invasion and endothelial permeability. **g:** The effect of TJP1 on the expression of vascular adhesion molecules as induced by M2 macrophage exosomal miR-23a-3p and tested by western blotting. *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Fig8. High level of exosomal miR-23a-3p in the serum or plasma of HCC patients indicated a poor prognosis. a.Relative expression of exosomal miR-23a-3p in the serum of HCC patients and normal subjects was detected. **b.**The OS of two groups was plotted.

a**b****c**





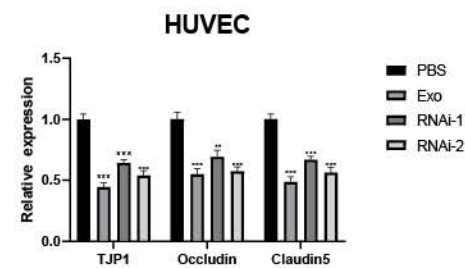
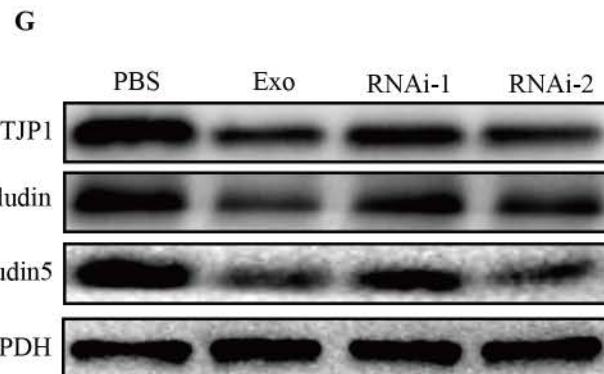
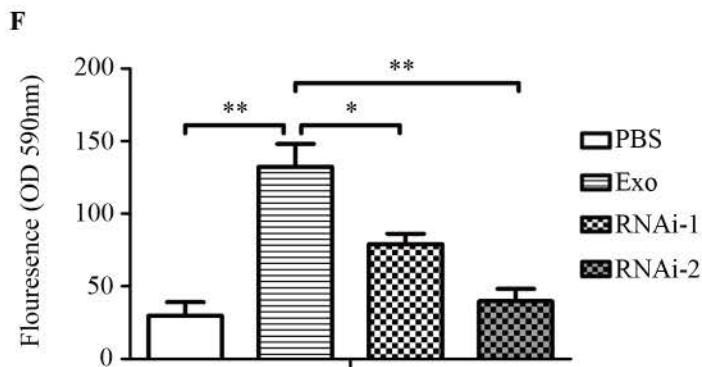
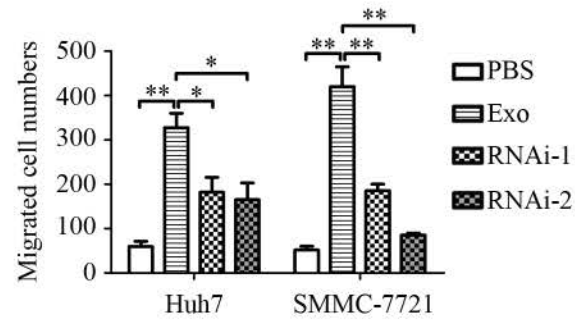
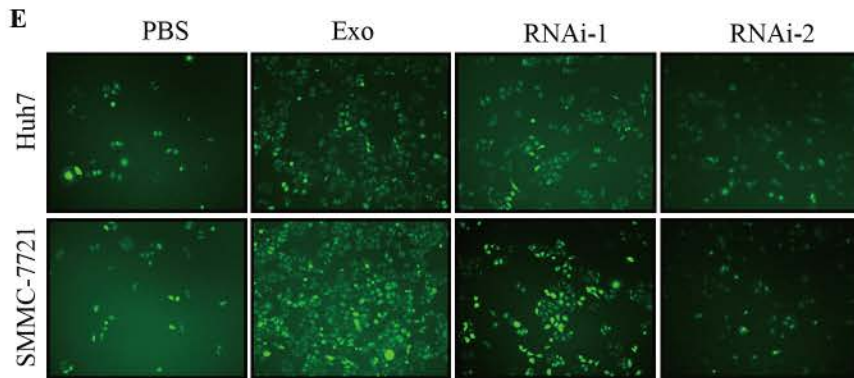
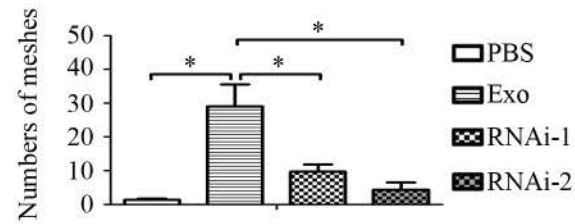
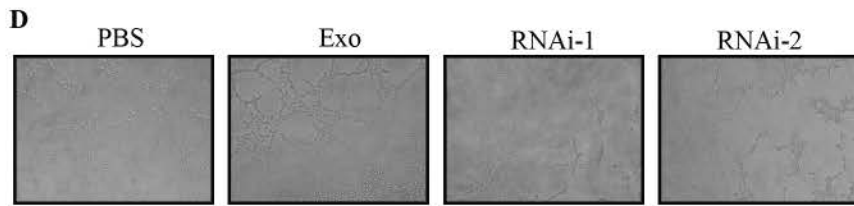
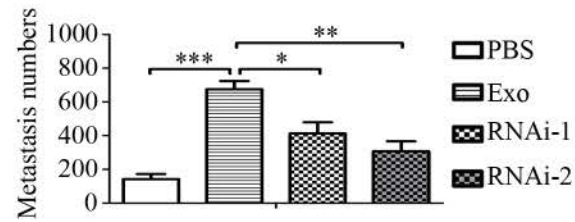
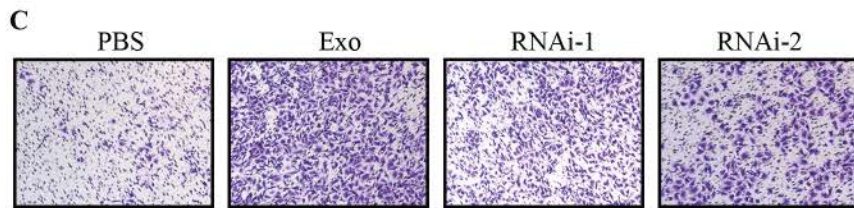
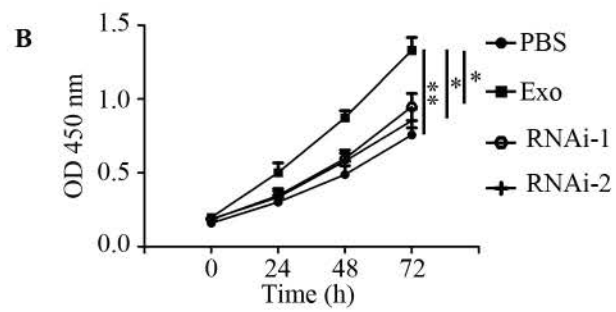
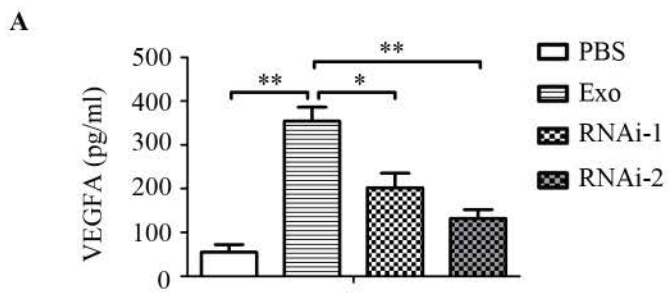
A**B**

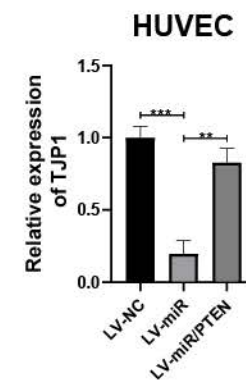
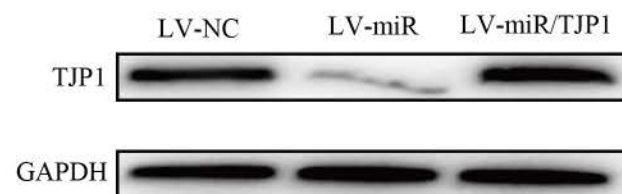
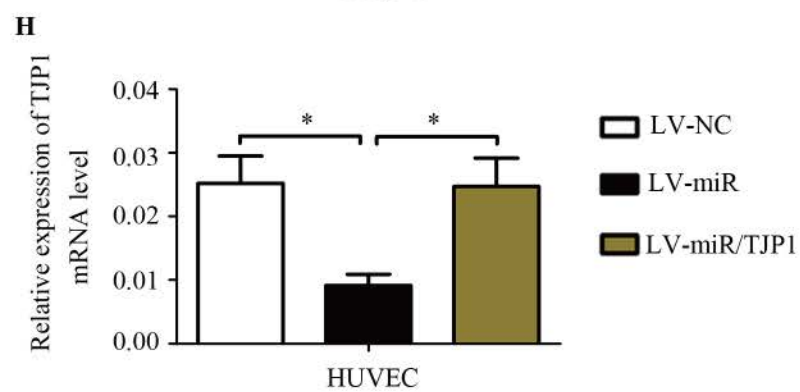
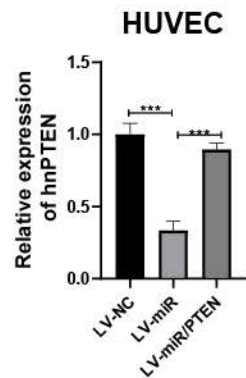
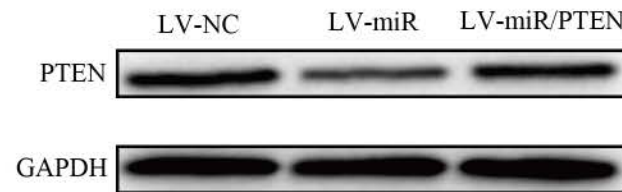
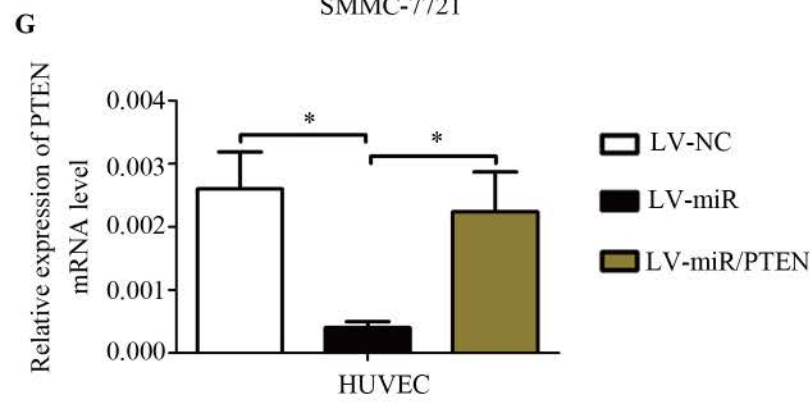
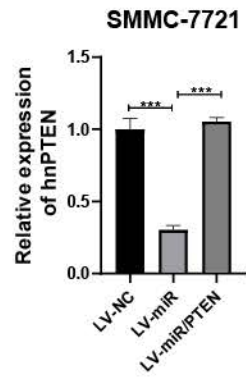
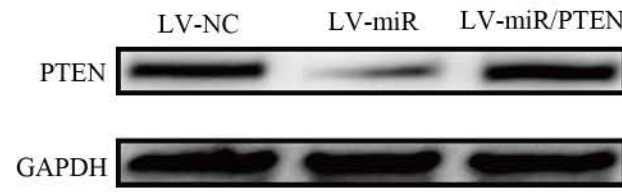
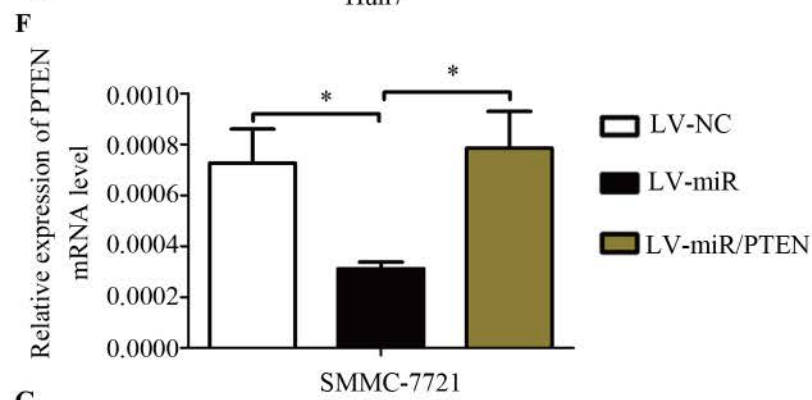
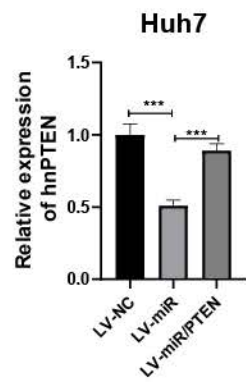
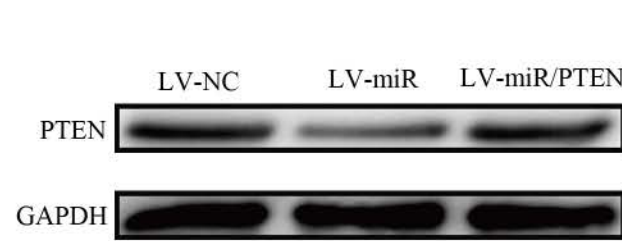
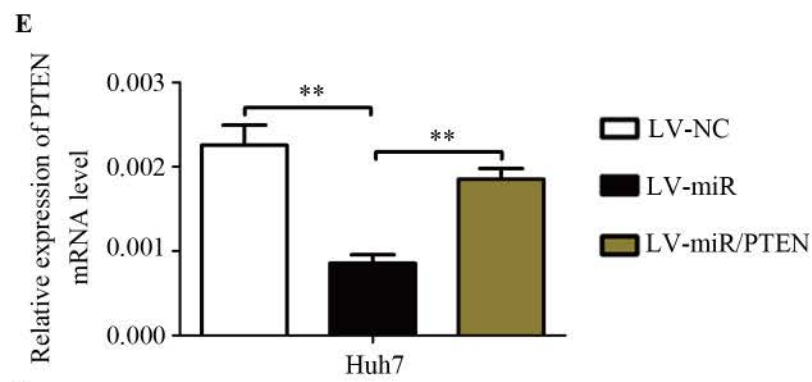
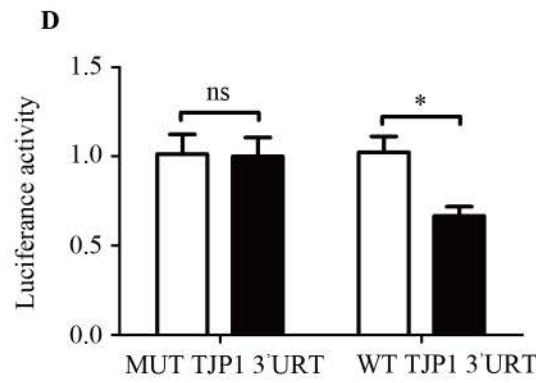
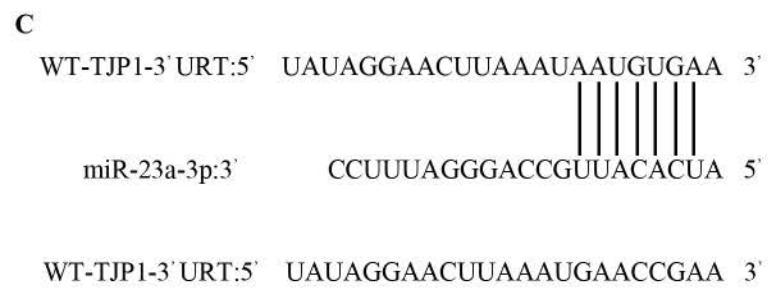
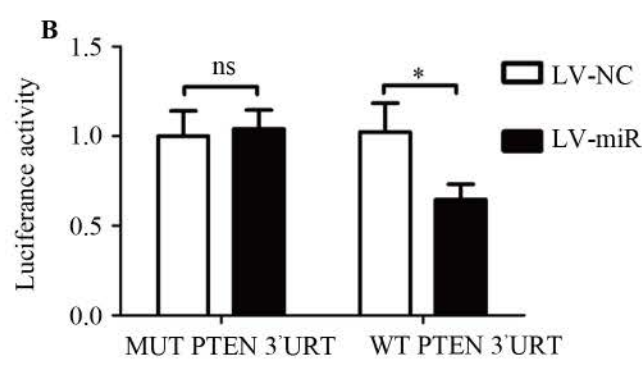
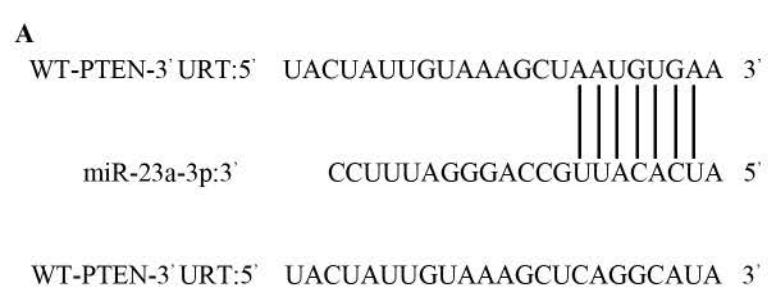
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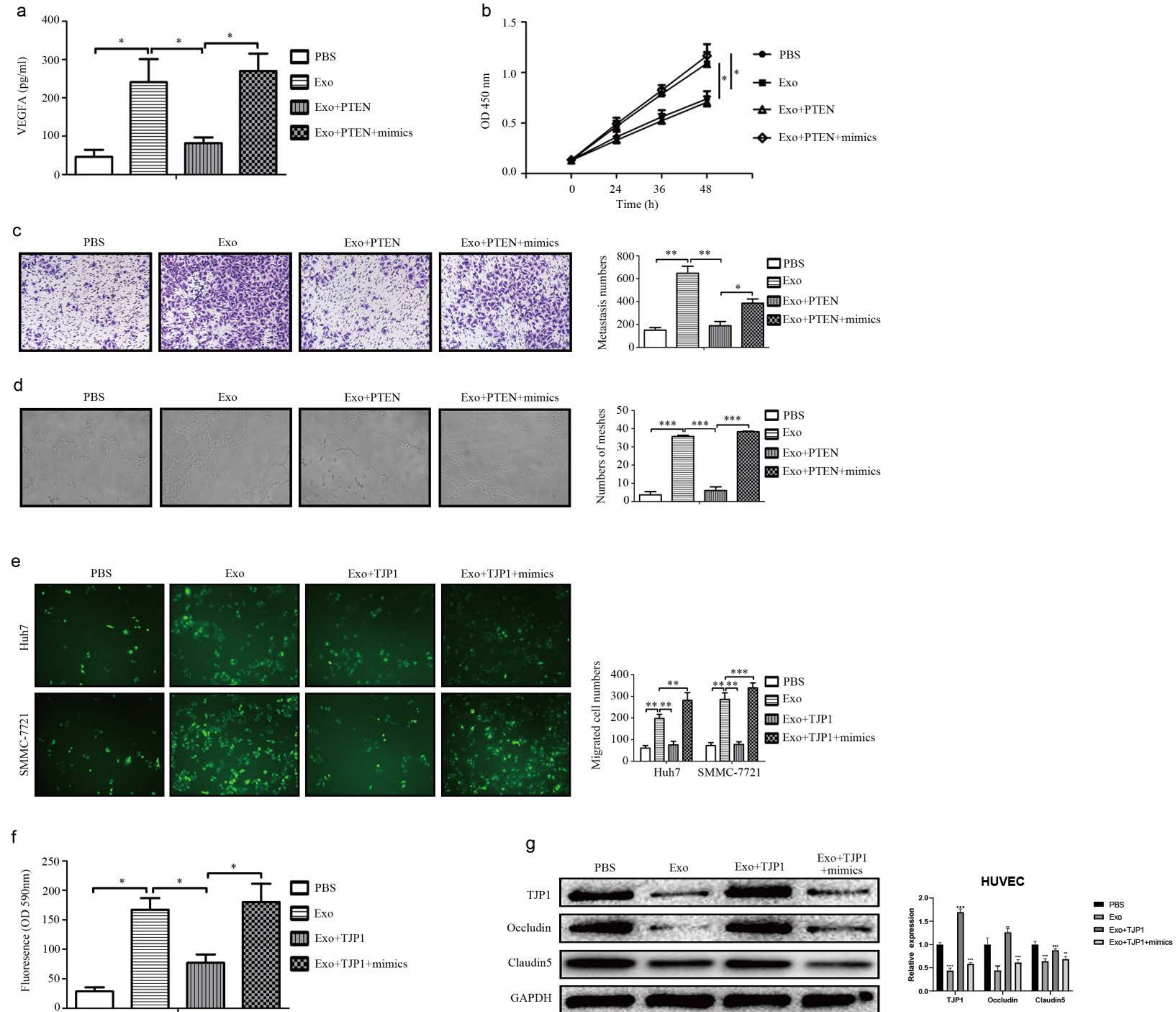
Exo

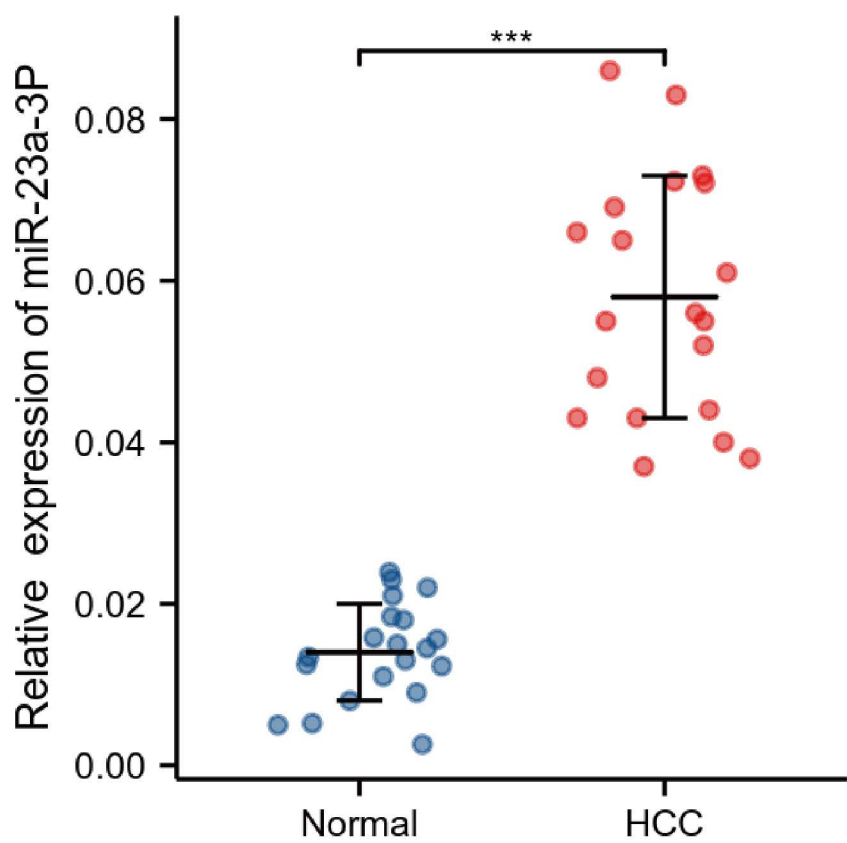
Exo/Anti-miR

Exo/Anti-miR+mimics







A**B**