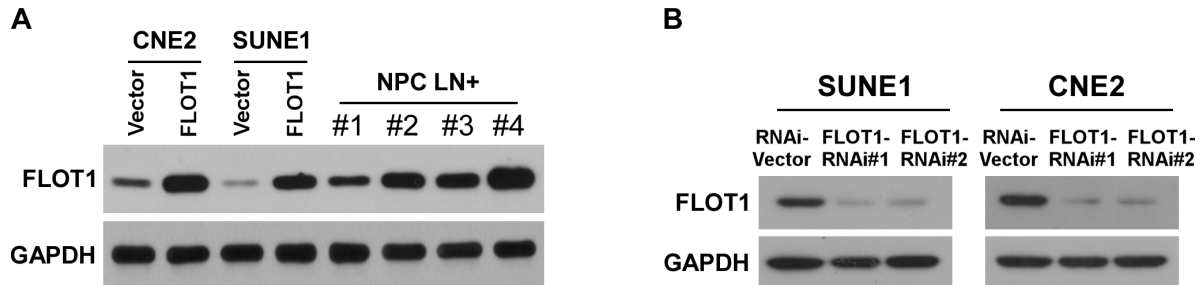
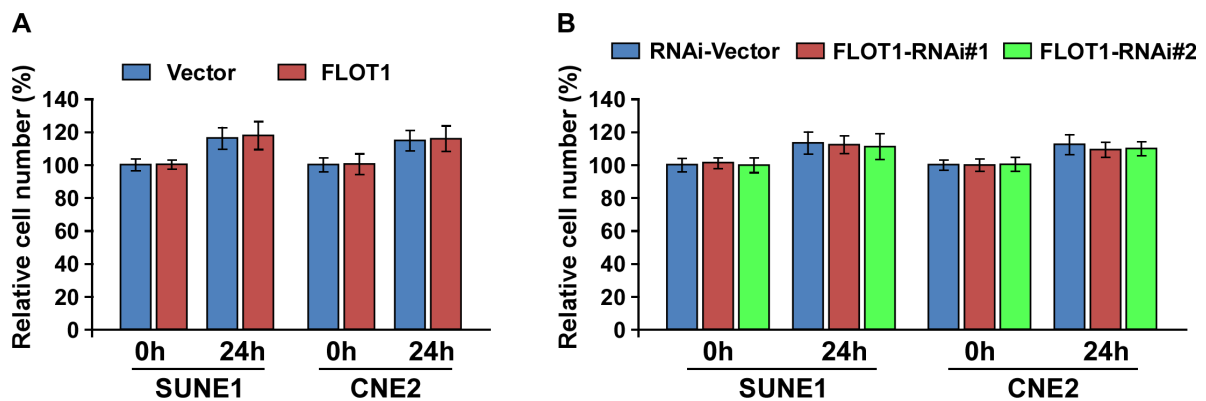


Upregulation of flotillin-1 promotes invasion and metastasis by activating TGF- β signaling in nasopharyngeal carcinoma

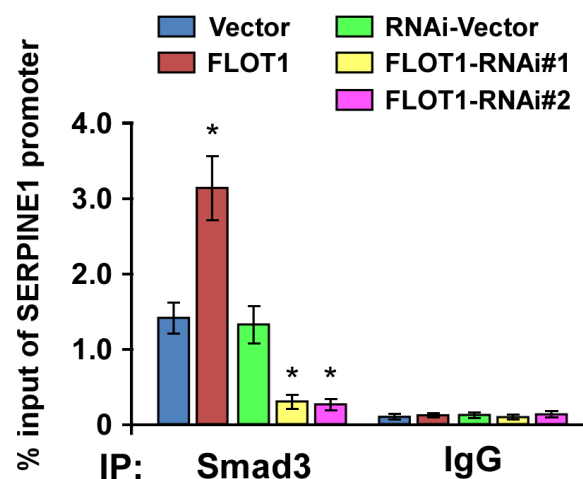
Supplementary Materials



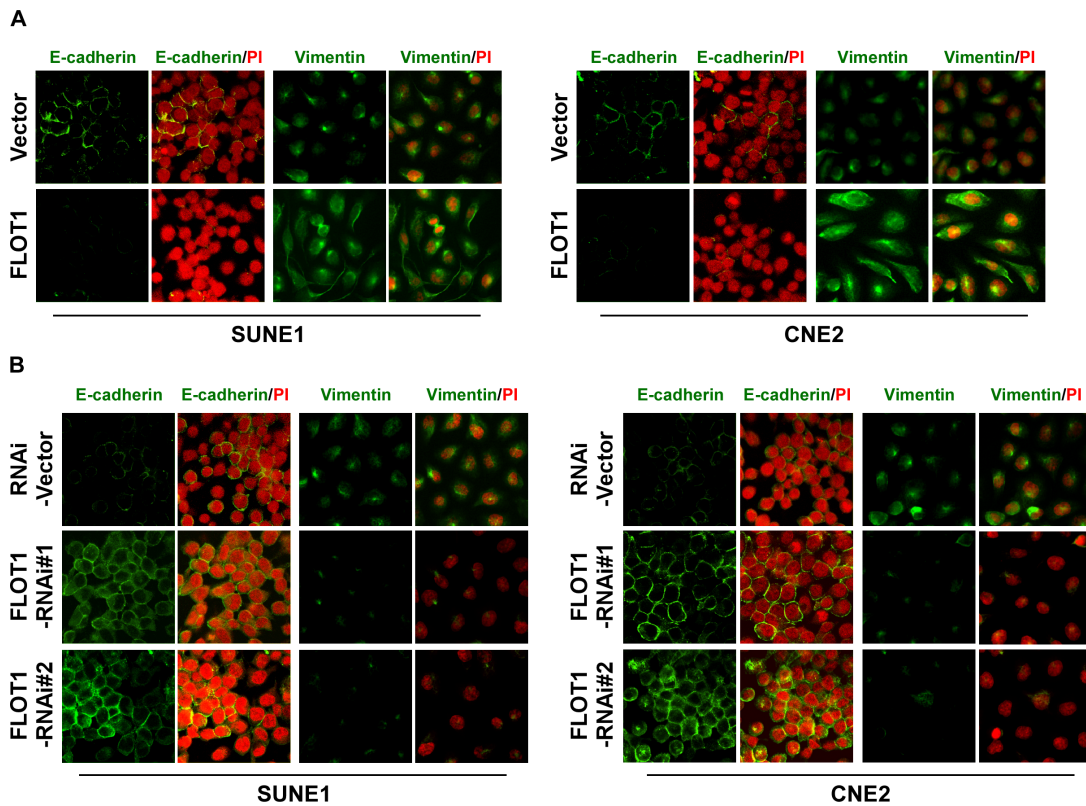
Supplementary Figure S1: Western blot analysis of FLOT1 in Vector control cells and FLOT1-transduced cells as well as 4 NPC LN+ tissues, or RNAi-vector control cells and FLOT1-silenced cells. GAPDH was used as a loading control.



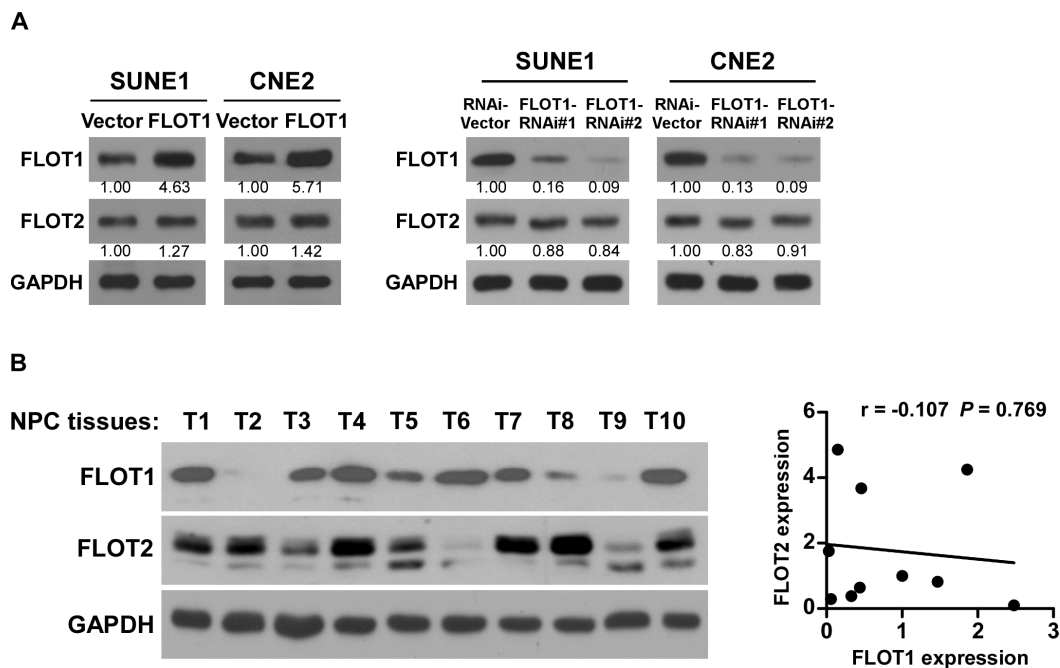
Supplementary Figure S2: The cell proliferation assay showed that neither FLOT1 overexpression (A) nor silencing (B) significantly change NPC cell numbers compared to controls within 24 h.



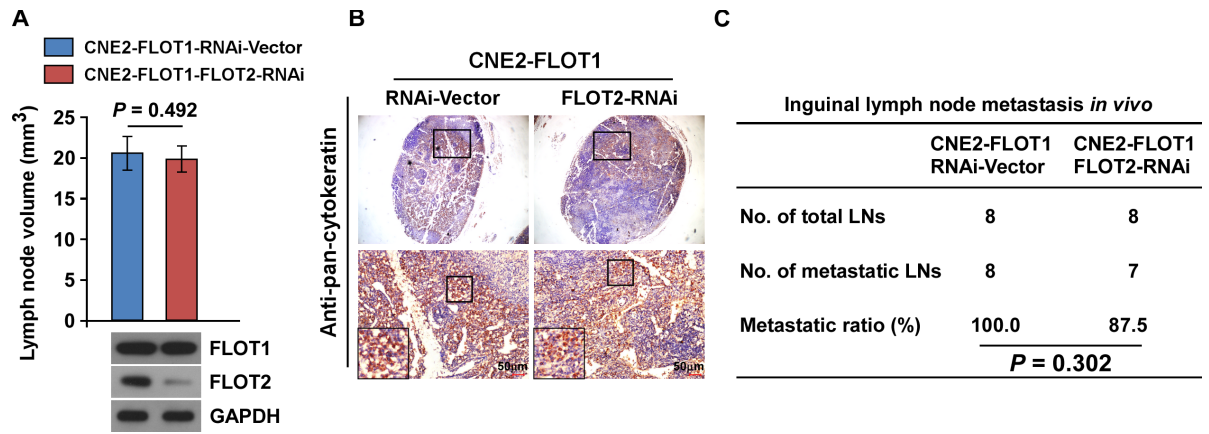
Supplementary Figure S3: ChIP assay revealed that the binding efficacy of Smad3 on SERPINE1 promoter was significantly increased in FLOT1-overexpressing CNE2 cells but decreased in FLOT1-silenced cells. * $P < 0.05$.



Supplementary Figure S4: FLOT1 overexpression facilitated, while silencing FLOT1 abrogated TGF- β -induced EMT in SUNE1 and CNE2 cells. (A and B) Fluorescent immunostaining of E-cadherin, Vimentin and Propidium iodide (PI) in the FLOT1-overexpressing (A) and FLOT1-silenced (B) NPC cells compared to controls. The green signal represents staining for E-cadherin or Vimentin, while the red signal signifies nuclear DNA staining with Propidium iodide.



Supplementary Figure S5: (A) Western blotting analysis of FLOT2 in the FLOT1-overexpressing and FLOT1-silenced cells compared to controls. **(B)** Expression analysis (left) and correlation (right) of FLOT1 expression and FLOT2 protein expression in ten freshly collected human NPC tissue samples (T). The ratio of first sample T1 (FLOT1/GAPDH and FLOT2/GAPDH) was considered as 1.0. GAPDH was used as a loading control.



Supplementary Figure S6: FLOT1 promoted lymph node metastasis independent of FLOT2 in NPC. (A) The average volumes of inguinal lymph nodes in each group. (B) Representative micrographs of the inguinal lymph nodes immunostained with an anti-pan-cytokeratin antibody. Scale bars: 50 μm. (C) The ratios of metastatic to total dissected inguinal lymph nodes from mice inoculated with the indicated cells, the Chi-square test was used for statistical analysis. Error bars represent mean ± SD.

Supplementary Table S1: Clinicopathological characteristics of studied patients and expression of FLOT1 in NPC

Factor	NO.	(%)
Gender		
Male	126	74.6
Female	43	25.4
Age (years)		
≤ 45	86	50.9
> 45	83	49.1
WHO histologic classification		
NKUC	115	68.0
NKDC	51	30.2
KSCC	3	1.8
Clinical stage		
I	13	7.7
II	40	23.7
III	67	39.6
IVa-b	49	29.0
N classification		
N ₀	57	33.7
N ₁	72	42.6
N ₂	32	18.9
N ₃	8	4.8
T classification		
T ₁	28	16.6
T ₂	47	27.8
T ₃	69	40.8
T ₄	25	14.8
VCA-IgA		
< 1:80	26	15.4

≥ 1:80	143	84.6
EA-IgA		
< 1:10	40	23.7
≥ 1:10	129	76.3
Recurrence or uncontrolled		
NO	100	59.2
Yes	69	40.8
Vital status		
Alive	117	69.2
Dead	52	30.8
Expression of FLOT1		
Low expression	70	41.4
High expression	99	58.6

KSCC, keratinizing squamous cell carcinoma; NKDC, nonkeratinizing differentiated carcinoma; NKUC, nonkeratinizing undifferentiated carcinoma.

Supplementary Table S2: Univariate analysis of different prognostic parameters in patients with NPC

Factor	No. patients	Mean survival (months)	Hazard ratio	95% CI	P-Value
Expression of FLOT1					
Low expression	70	80.1	1.0	Reference	
High expression	99	62.8	2.631	1.403–4.933	0.003
Gender					
Male	126	69.5	1.0	Reference	
Female	43	73.3	0.755	0.388–1.469	0.407
Age (years)					
≤ 45	86	75.5	1.0	Reference	
> 45	83	65.5	1.778	1.020–3.099	0.042
WHO histologic classification					
NKDC/KSCC	54	71.8	1.0	Reference	
NKUC	115	70.2	1.126	0.624–2.029	0.694
Clinical stage					
I–II	53	79.2	1.0	Reference	
III–IV	116	66.9	2.164	1.076–4.277	0.030
N classification					
N ₀	57	82.2	1.0	Reference	
N ₁₋₃	112	65.2	2.771	1.350–5.686	0.005
T classification					
T ₁₋₂	75	71.9	1.0	Reference	
T ₃₋₄	94	69.8	1.101	0.635–1.910	0.731
VCA-IgA					
< 1:80	26	74.3	1.0	Reference	
≥ 1:80	143	69.7	1.481	0.632–3.470	0.366
EA-IgA					
< 1:10	40	74.5	1.0	Reference	
≥ 1:10	129	69.0	1.652	0.778–3.510	0.192

Supplementary Table S3: Multivariate analysis of different prognostic parameters in patients with NPC

Factor	Hazard ratio	95% CI	P-Value
Expression of FLOT1			
Low expression	1.0	Reference	
High expression	2.089	1.099–3.968	0.024
Age (years)			
≤ 45	1.0	Reference	
> 45	1.169	0.628–2.177	0.622
Clinical stage			
I–II	1.0	Reference	
III–IV	1.772	0.881–3.562	0.109
N classification			
N ₀	1.0	Reference	
N ₁₋₃	2.347	1.002–5.498	0.049