Exosomal long noncoding RNA *LNMAT2* promotes lymphatic metastasis in bladder cancer

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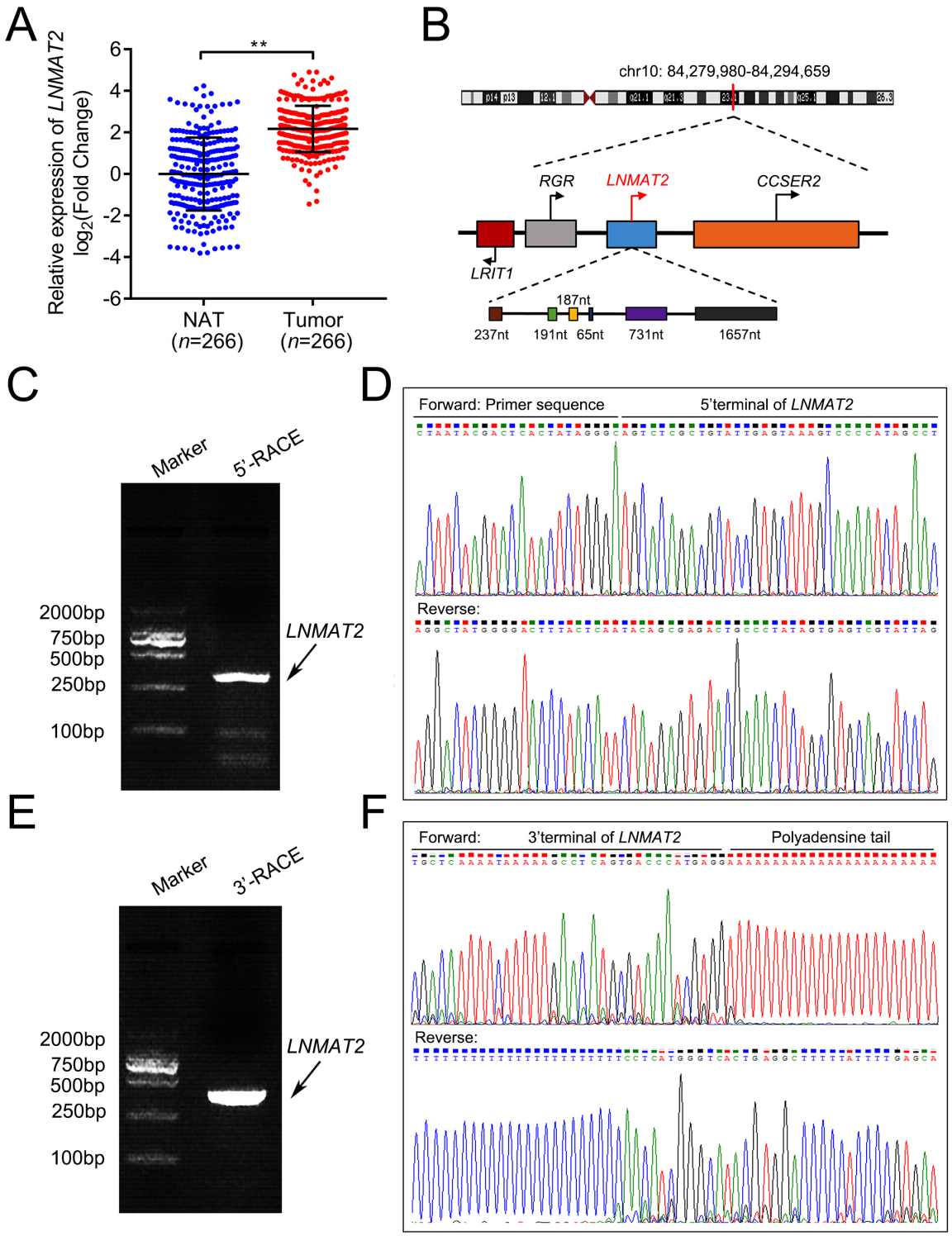
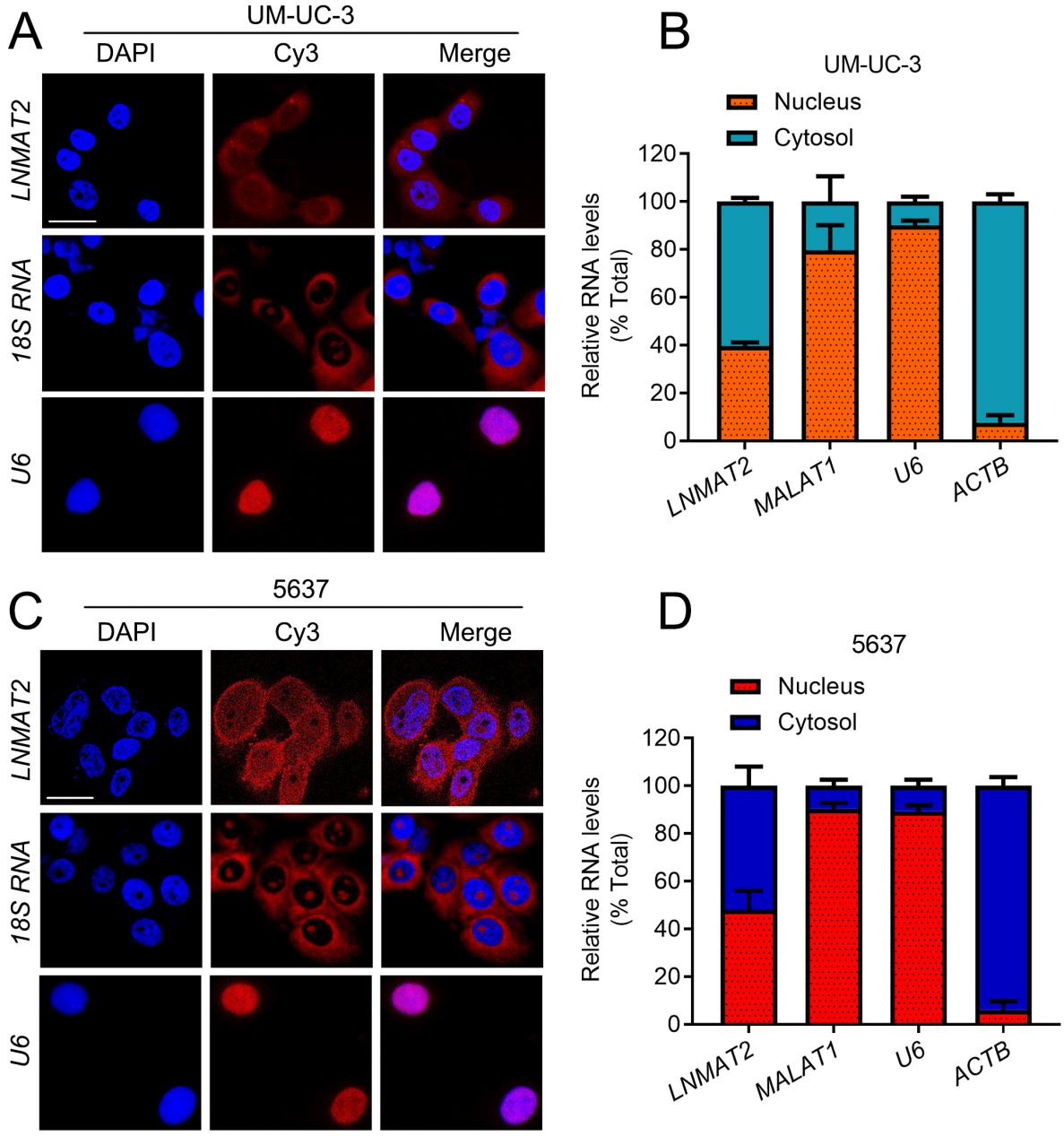
The authors have declared that no conflict of interest exists.



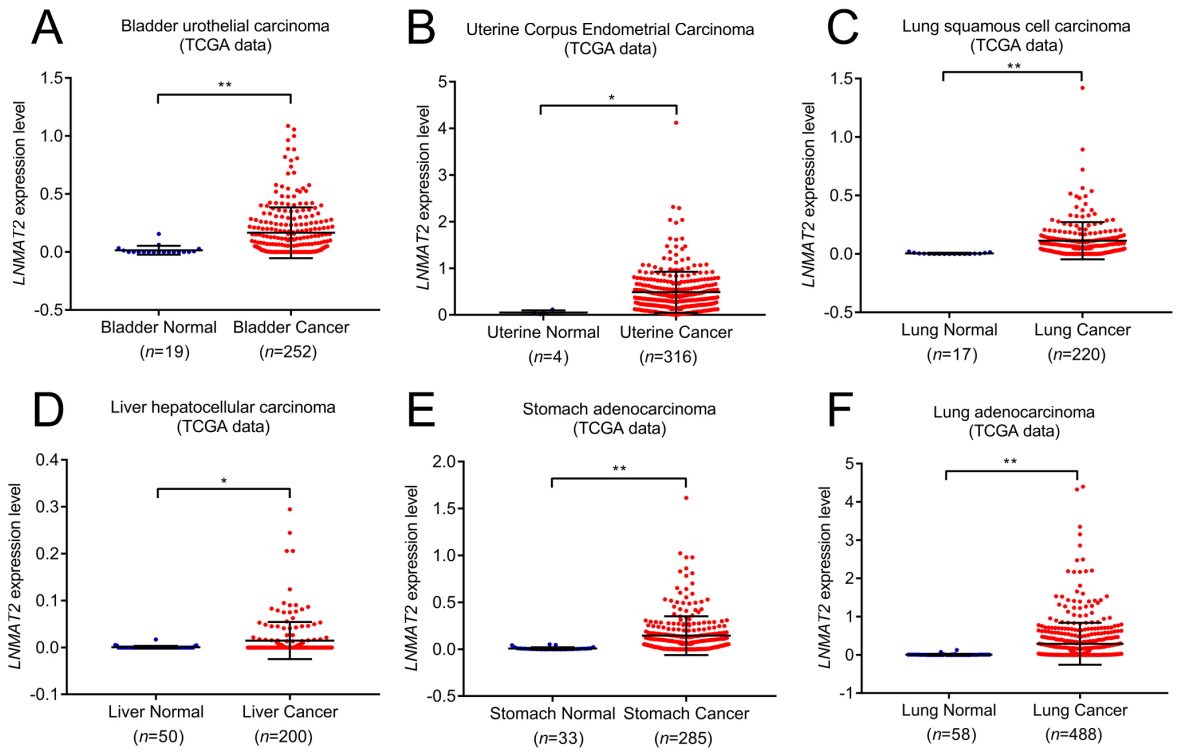
Supplemental Figure 1. *LNMAT2* is upregulated in BCa. (**A**) qRT-PCR analysis of *LNMAT2* expression in a cohort of 266 BCa patient samples paired with their respective NATs. *GAPDH* was used as an internal control. Groups were compared using the Nonparametric Mann-Whitney *U* test. (**B**) Schematic representation for the genomic locus of *LNMAT2* on chromosome and the neighboring protein coding genes of *LNMAT2* were

determined by RACE analysis. (**C and D**) The cap sequences and 5'-terminal sequences of *LNMAT2* were identified by 5'-RACE PCR. Representative image of agarose gel electrophoresis (C) and bidirectional sequencing of 5'-RACE products (D) are shown. (**E and F**) The tail sequences and 3'-terminal sequences of *LNMAT2* were identified by 3'-RACE PCR. Representative image of agarose gel electrophoresis (E) and bidirectional sequencing of 3'-RACE products (F) are shown. **P* < 0.05, ***P* < 0.01.

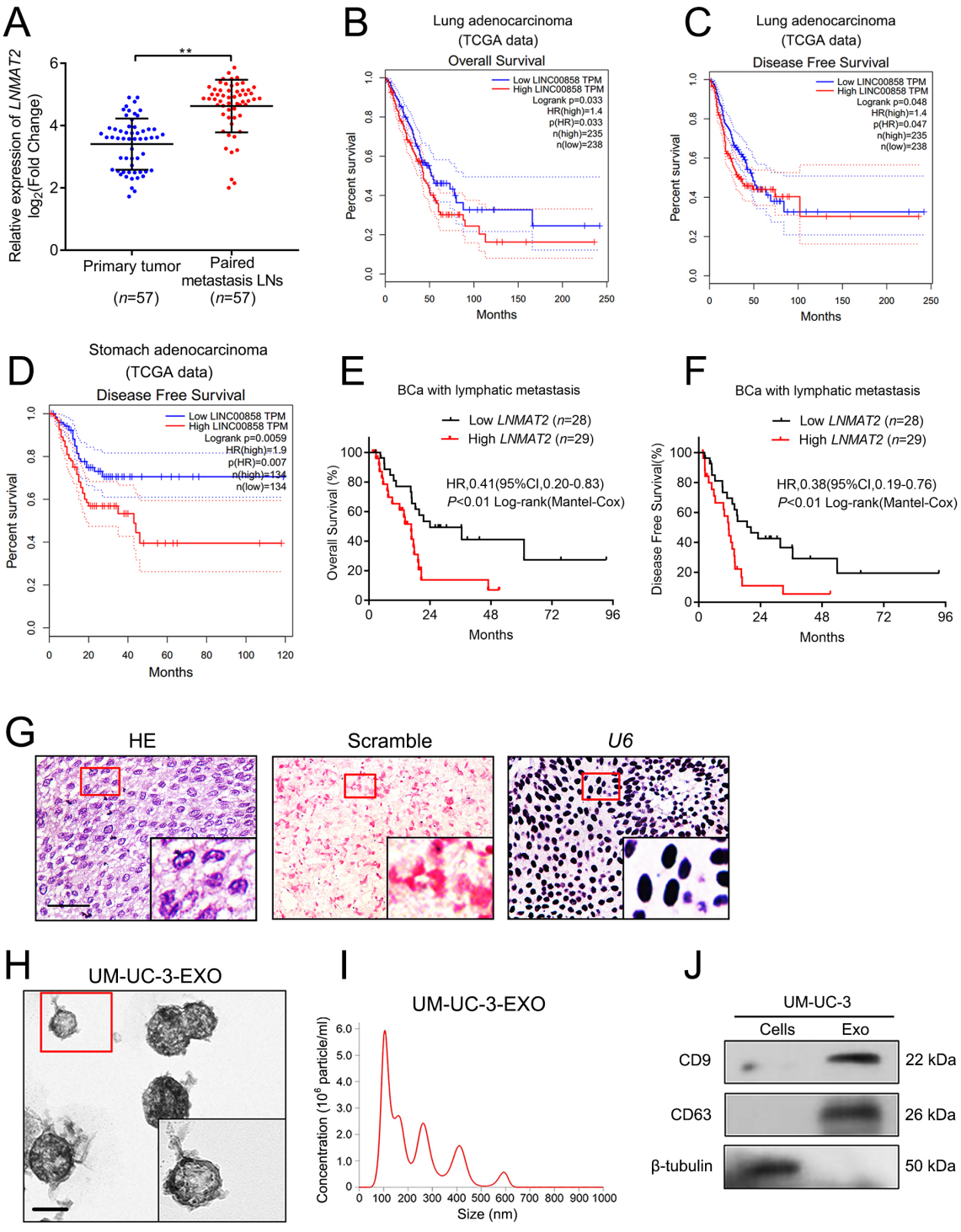


Supplemental Figure 2. Identification of the subcellular distribution of *LNMAT2*. (**A and B**) Representative image of FISH analysis (A) and subcellular fractionation assays (B) showed the subcellular distribution of *LNMAT2* in UM-UC-3 cells. *U6*, *MALAT1* and *U1* were used as nuclear control. *18S rRNA* and *ACTB* were used as cytoplasmic control. Scale bar: 5 μm. (**C and D**) Representative image of FISH analysis (C) and subcellular fractionation assays (D) showed the subcellular distribution of *LNMAT2* in 5637 cells. *U6*, *MALAT1* and *U1* were used as nuclear control. Scale bar: 5 μm. *18S rRNA* and *ACTB* were used as cytoplasmic control. Error bars represent the SD of three independent experiments.

1 *$P < 0.05$, **$P < 0.01$.

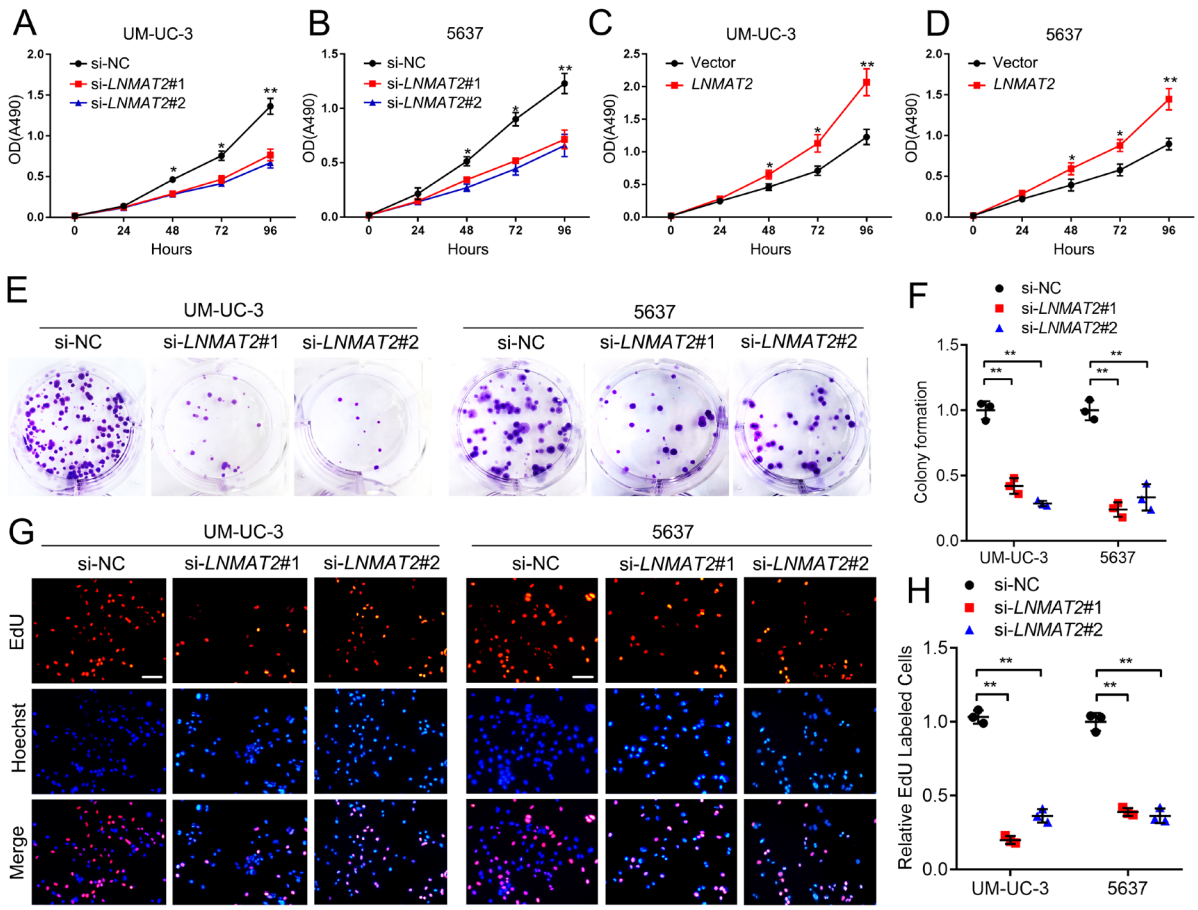


Supplemental Figure 3. *LNMAT2* is overexpressed in multiple human cancers. (**A-F**) *LNMAT2* expression were analyzed in different types of human cancers, including BCa from TCGA database. Groups were compared using the Nonparametric Mann-Whitney *U* test. The data was obtained from GEPIA (http://gepia.cancer-pku.cn/index.html). **P* < 0.05, ***P* < 0.01.

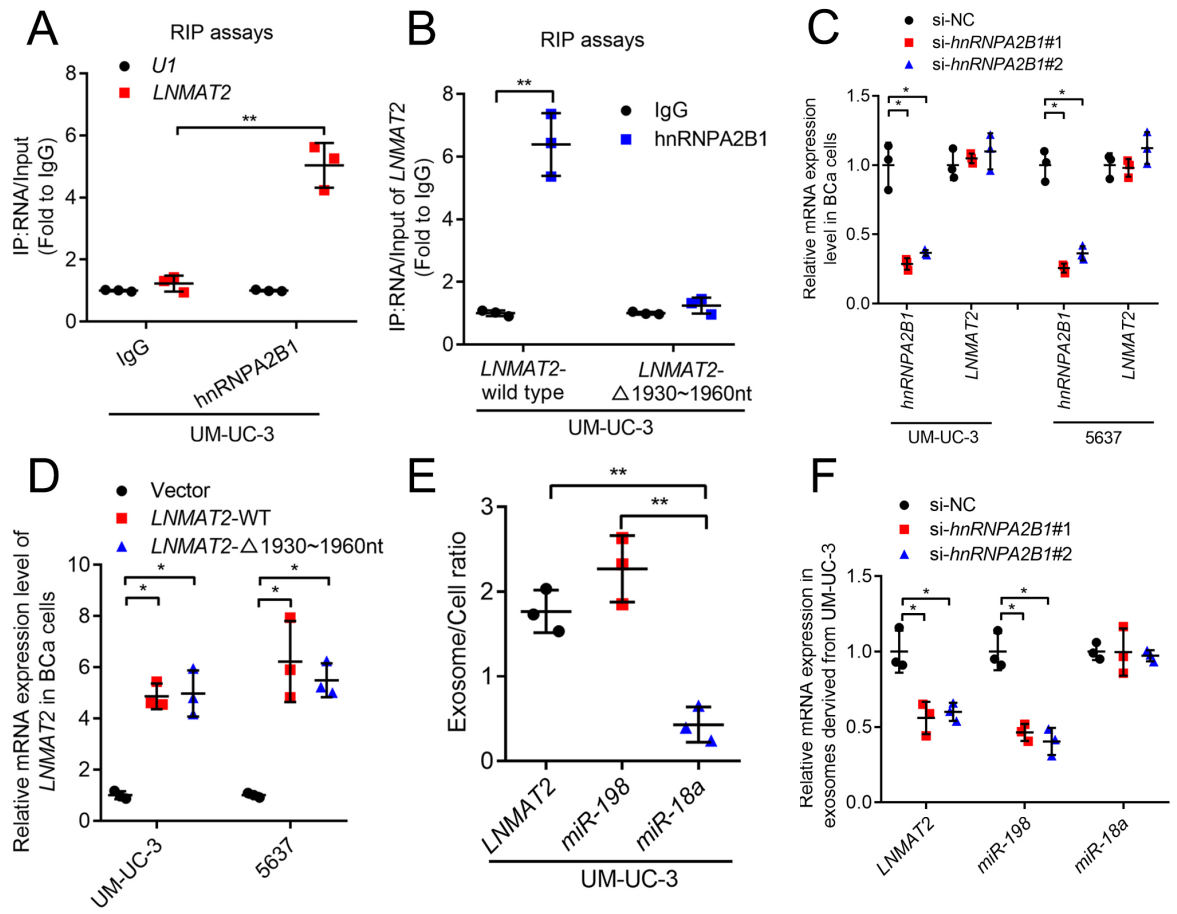


Supplemental Figure 4. *LNMAT2* positively correlates with LN-metastasis of BCa. (**A**) qRT-PCR analysis of *LNMAT2* expression in primary BCa tissues and paired metastatic LNs (n = 266). *GAPDH* was used as an internal control. Groups were compared using the Nonparametric Mann-Whitney *U* test. (**B-D**) Kaplan-Meier curves of OS and DFS were

determined for patients with high *LNMAT2* expression compared with low *LNMAT2* expression in various types of cancers. The data was obtained from GEPIA (http://gepia.cancer-pku.cn/index.html). (**E and F**) The Kaplan-Meier curve for OS and DFS of BCa patients with LN metastasis based on low vs. high expression of *LNMAT2*. The median *LNMAT2* expression was used as the cutoff value. (**G**) Representative ISH images of the scramble probe (red) as negative control and *U6* probe (blue) as positive control in BCa tissues. Scale bar: 50 μm. (**H and I**) Purified UM-UC-3-EXO were identified by TEM (H) and NanoSight (I). Scale bar: 100 nm. (**J**) Western Blot analysis of exosomal protein marker in UM-UC-3 cell lysates or UM-UC-3-EXO. **P* < 0.05, ***P* < 0.01.

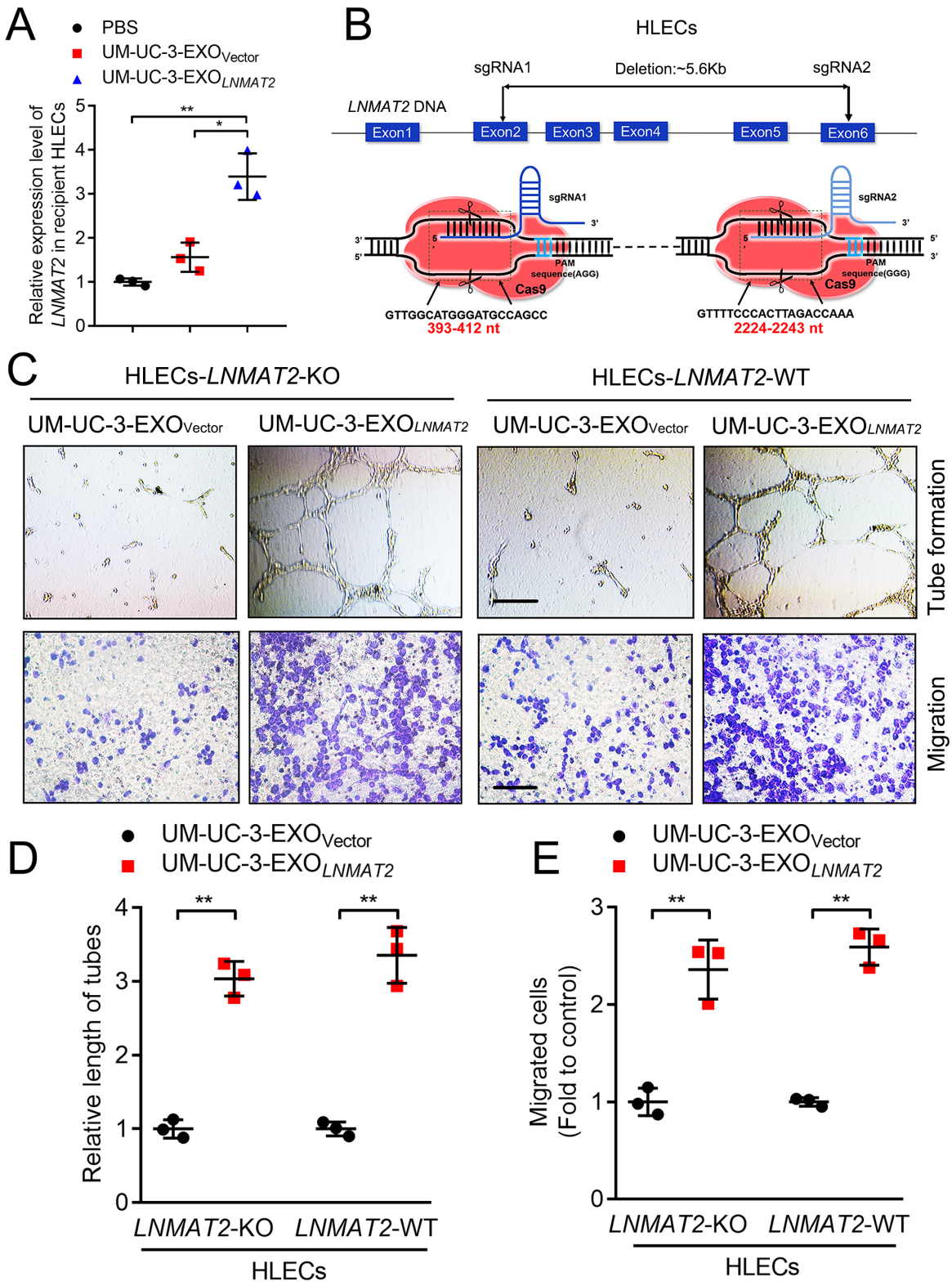


Supplemental Figure 5. *LNMAT2* promotes proliferation of BCa cells. (**A-D**) CCK-8 assay assessed the cell viability after knockdown (A and B) or overexpression (C and D) of *LNMAT2* in UM-UC-3 or 5637 cells. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. (**E and F**) Representative images (E) and quantifications (F) of Colony formation assay after *LNMAT2* knockdown in UM-UC-3 or 5637 cells. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. (**G and H**) Representative images (G) and quantifications (H) of EdU assay after *LNMAT2* knockdown in UM-UC-3 or 5637 cells. Scale bars: 100 μm. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. Error bars represent the SD of three independent experiments. $*P < 0.05$, $**P < 0.01$.



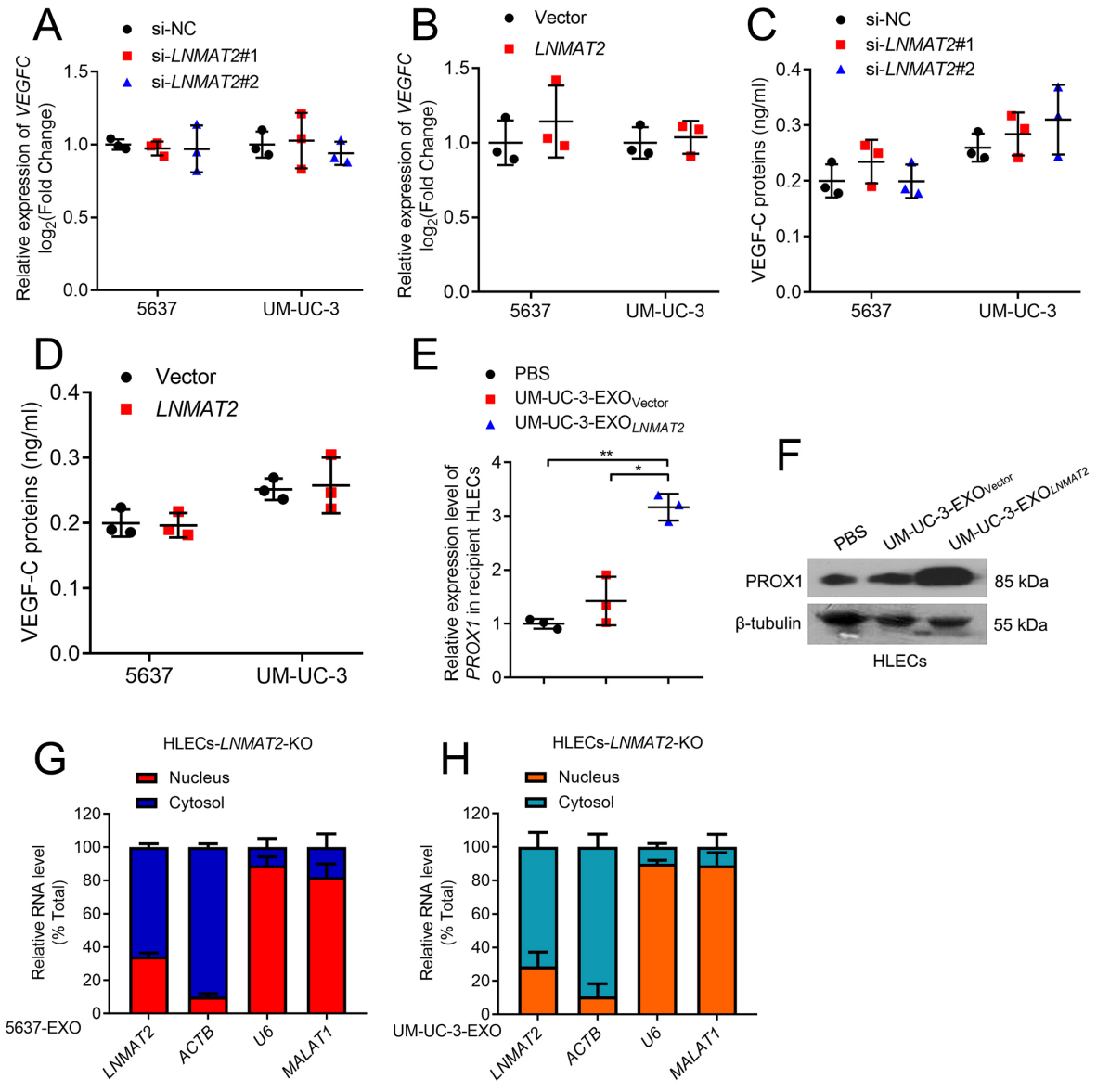
Supplemental Figure 6. *LNMAT2* is packaged into exosomes in hnRNPA2B1-dependent manner. (**A**) RIP analysis using the anti-hnRNPA2B1 antibody revealed that *LNMAT2* interacted specifically with hnRNPA2B1 in UM-UC-3 cells. IgG was used as the negative control and *U1* was used as the non-specific control. Statistical significance was assessed using two-tailed Student's *t*-test. (**B**) RIP assays performed after site-directed mutagenesis of 1930-1960 nt of *LNMAT2* in UM-UC-3 cells. Statistical significance was assessed using two-tailed Student's *t*-test. (**C**) qRT-PCR analysis of the expression of hnRNPA2B1 and *LNMAT2* in hnRNPA2B1 knockdown cells. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. (**D**) qRT-PCR analysis of *LNMAT2* expression in respective BCa cells. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. (**E**) qRT-PCR analysis of exosomes/cells ratio of

respective RNAs in UM-UC-3 cells. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. (**F**) qRT-PCR analysis of the expression of indicated RNAs in exosomes secreted by from hnRNPA2B1 knockdown UM-UC-3 cells. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. *GAPDH* was used as an internal control for qRT-PCR analysis in Figure C-F. Error bars represent the SD of three independent experiments. *$P < 0.05$, **$P < 0.01$.



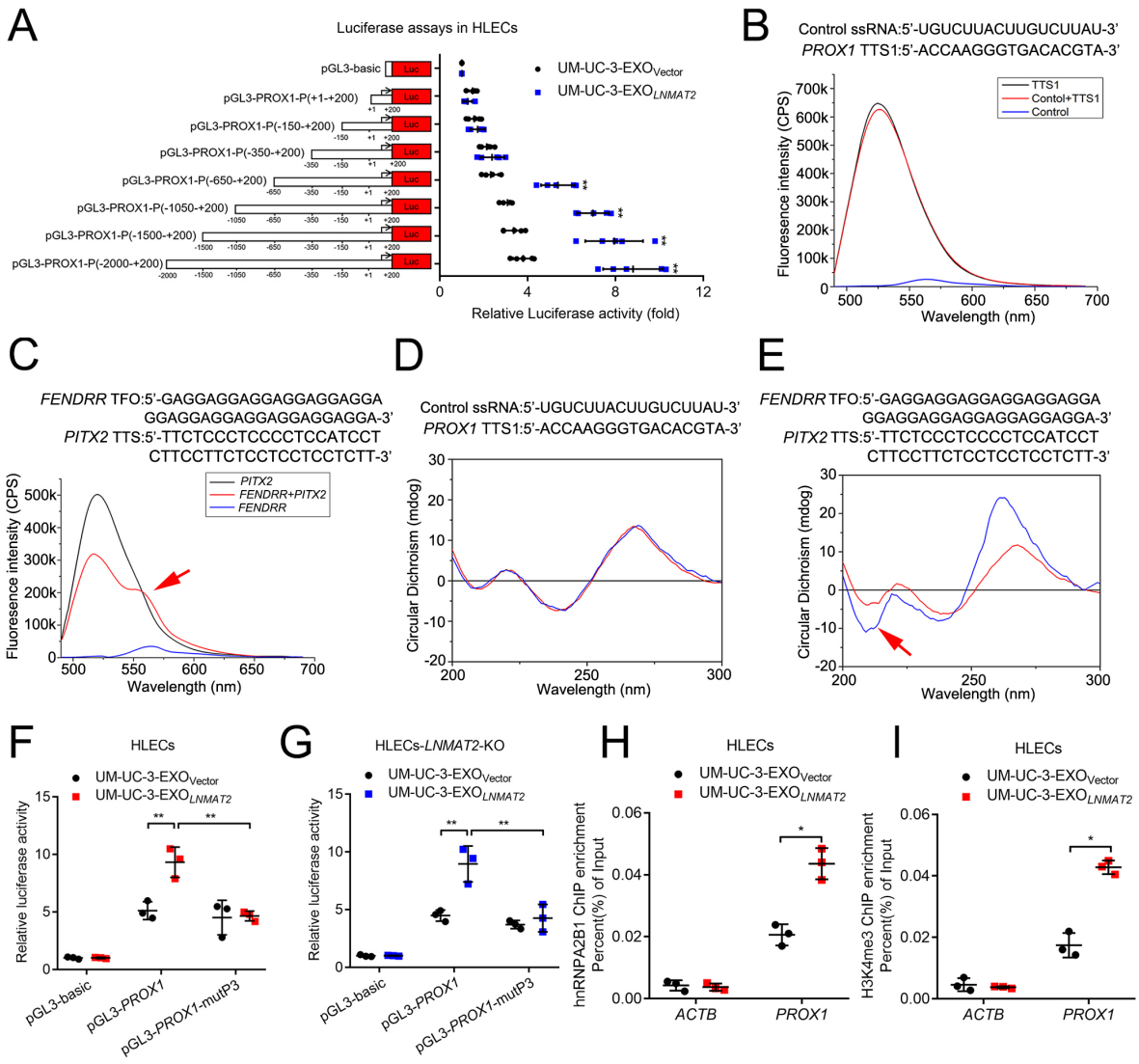
Supplemental Figure 7. BCa-secreted exosomes induce lymphangiogenesis without activating the expression of endogenous *LNMAT2* in HLECs. (A) qRT-PCR analysis of

LNMAT2 expression in HLECs treated with PBS, UM-UC-3-EXO$_{Vector}$ or UM-UC-3-EXO$_{LNMAT2}$. *GAPDH* was used as an internal control. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. (**B**) Schematic diagram of *LNMAT2*-KO cells from HLECs were established by CRISPR/Cas9. (**C-E**) Representative images (C) and quantifications of tube formation (D) and Transwell (E) migration by HLECs (*LNMAT2*-KO or *LNMAT2*-WT) after treating with UM-UC-3-EXO$_{Vector}$ or UM-UC-3-EXO$_{LNMAT2}$. Scale bars: 100 μm. Statistical significance was assessed using two-tailed Student's *t*-test. Error bars represent the SD of three independent experiments. **P* < 0.05, ***P* < 0.01.



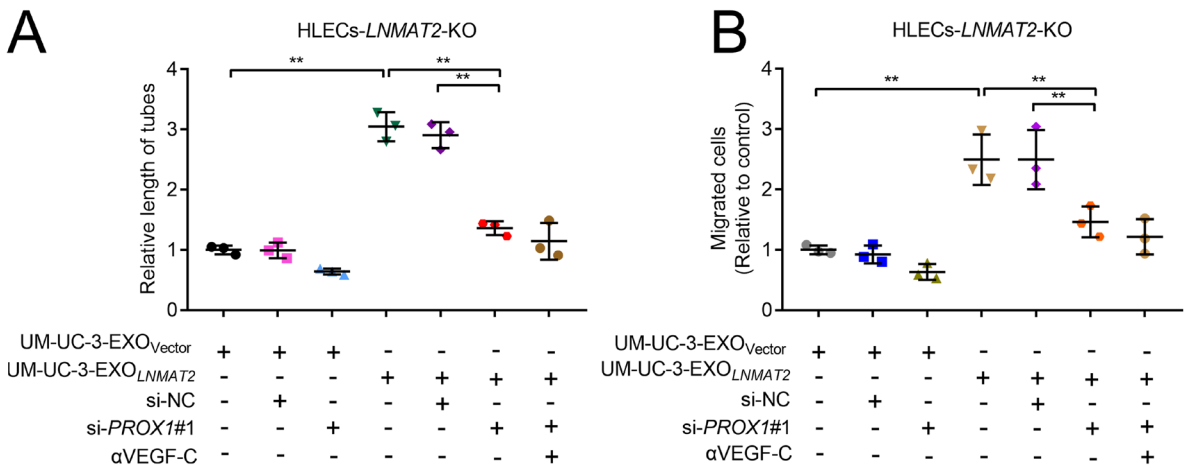
Supplemental Figure 8. Exosomal *LNMAT2* upregulates PROX1 in HLECs independent of VEGF-C. (A and B) qRT-PCR analysis of *VEGFC* expression in *LNMAT2* knockdown (A) and overexpressing (B) cells. *GAPDH* was used as an internal control. Statistical significance was assessed using two-tailed Student's *t*-test and one-way ANOVA followed by Dunnett's tests for multiple comparisons. **(C and D)** Histograms of ELISA for VEGF-C level detected in *LNMAT2* knockdown (C) and overexpressing (D) cells. Statistical significance was assessed using two-tailed Student's *t*-test and one-way ANOVA followed by Dunnett's

tests for multiple comparisons. **(E)** The expression of *PROX1* in HLECs after incubation with PBS, UM-UC-3-EXO$_{Vector}$ or UM-UC-3-EXO$_{LNMAT2}$ were detected by qRT-PCR. *GAPDH* was used as an internal control. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. **(F)** Western Blot analysis of PROX1 expression in HLECs after incubation with PBS, UM-UC-3-EXO$_{Vector}$ or UM-UC-3-EXO$_{LNMAT2}$. (**G and H**) Subcellular fractionation assays showed the subcellular distribution of *LNMAT2* in *LNMAT2*-KO HLECs treated with 5637-EXO (G) or UM-UC-3-EXO (H). *MALAT1* and *U1* were used as nuclear control and *ACTB* was used as cytoplasmic control. Error bars represent the SD of three independent experiments. *$P < 0.05$, **$P < 0.01$.

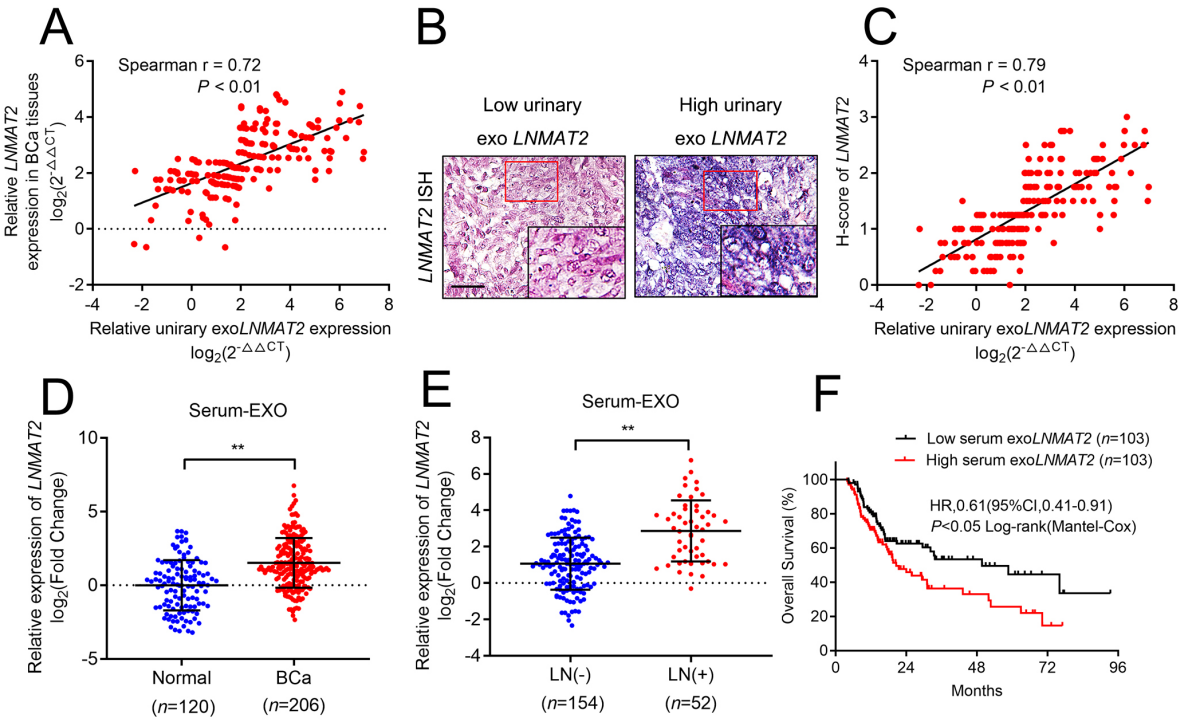


Supplemental Figure 9. Exosomal *LNMAT2* activates lymphatic PROX1 expression via formation of triplex with the promoter region of *PROX1*. (**A**) Sequential deletions for evaluating the transcriptional activity of the *PROX1* promoter linked to Renilla luciferase activity in HLECs treated with UM-UC-3-EXO$_{Vector}$ or UM-UC-3-EXO$_{LNMAT2}$. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. (**B and C**) FRET analysis of Control ssRNA/*PROX1* (B) and *FENDRR*/*PITX2* (C). (**D and E**) CD spectroscopy of Control ssRNA/*PROX1* (D) and *FENDRR*/*PITX2* (E). (**F and G**) WT or *LNMAT2* binding site mutated *PROX1* promoter were subjected to luciferase reporter assays in *LNMAT2*-WT (F) or *LNMAT2*-KO (G) HLECs, respectively, treated with

UM-UC-3-EXO$_{Vector}$ or UM-UC-3-EXO$_{LNMAT2}$. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. (**H and I**) ChIP-qPCR of hnRNPA2B1 occupancy (H) and H3K4me3 status (I) in *PROX1* promoter after HLECs incubation with UM-UC-3-EXO$_{Vector}$ or UM-UC-3-EXO$_{LNMAT2}$. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. Error bars represent the SD of three independent experiments. *$P < 0.05$, **$P < 0.01$.



Supplemental Figure 10. Exosomal *LNMAT2* promotes lymphangiogenesis by upregulating PROX1 in HLECs. (**A and B**) Histogram analysis of tube formation (A) and Transwell migration (B) by *LNMAT2*-KO HLECs cells treated with UM-UC-3-EXO$_{Vector}$ or UM-UC-3-EXO$_{LNMAT2}$, transfected with si-NC or si-*PROX1*#1, or in which VEGF-C was inhibited. Statistical significance was assessed using one-way ANOVA followed by Dunnett's tests. Error bars represent the SD of three independent experiments. *$P < 0.05$, **$P < 0.01$.



Supplemental Figure 11. Exosomal *LNMAT2* positively correlated with LN metastasis in BCa patients. (**A**) Correlation analysis of *LNMAT2* expression detected by qRT-PCR in urinary-EXO and tumor tissues of BCa patients (n = 206). (**B**) Representative images of ISH of *LNMAT2* expression in 206 cases of BCa tissues with different exosomal *LNMAT2* level. Scale bar: 50 μm. (**C**) Correlation analysis of exosomal *LNMAT2* expression in patients' urine and *LNMAT2* expression assessed by ISH in BCa tissues (n = 206). (**D**) qRT-PCR analysis of *LNMAT2* expression in serum-EXO isolated from 206 BCa patients and 120 healthy participants. *GAPDH* was used as an internal control. Groups were compared using the Nonparametric Mann-Whitney *U* test. (**E**) *LNMAT2* expression was analyzed in a 206-patient cohort of serum-EXO isolated from BCa patients with or without LN metastasis by qRT-PCR. *GAPDH* was used as an internal control. Groups were compared using the Nonparametric Mann-Whitney *U* test. **(F)** Kaplan-Meier curve of OS for BCa patients according to the relative serum exosomal *LNMAT2* expression. The median expression of

serum exosomal *LNMAT2* was used as the cutoff value (n = 206). Error bars represent the SD of three independent experiments. *$P < 0.05$, **$P < 0.01$.

Supplemental Tables

Supplemental Table 1. Correlation between *LNMAT2* expression and clinicopathologic characteristics of BCa patients

Characteristics	No. of cases	*LNMAT2* expression level		
		Low	High	*P*-value[A]
Total cases	266	133	133	
Gender				0.476
Male	201	98	103	
Female	65	35	30	
Age				0.456
< 65	112	59	53	
≥ 65	154	74	80	
T stage				0.508
T1	83	44	39	
T2-4	183	89	94	
T grade				0.890
Low	71	36	35	
High	195	97	98	
Lymphatic metastasis				**0.001****
Negative	209	118	91	
Positive	57	15	42	

Abbreviations: No. of cases = number of cases; T stage = tumor stage; T grade = tumor grade.

[A] Chi-square test, * P <0.05, ** P <0.01.

Supplemental Table 2. Univariate and multivariate analysis of Overall Survival (OS) for *LNMAT2* expression in BCa patients (*n* = 266)

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P*-value[A]	HR	95%CI	*P*-value[A]
Age (<65 vs. ≥65)	1.087	0.737-1.602	0.675			
Gender (Male vs. Female)	0.782	0.505-1.211	0.270			
T stage (T2-4 vs. T1)	1.603	1.015-2.530	0.043*	1.548	0.972-2.464	0.066
T grade (High vs. Low)	1.312	0.840-2.050	0.233			
Lymphatic metastasis (positive vs. negative)	1.730	1.131-2.645	0.011*	1.431	0.919-2.229	0.112
LNMAT2 expression (High vs. Low)	1.625	1.098-2.405	0.015*	1.553	1.038-2.324	0.032*

Abbreviations: HR = hazard ratio; 95%CI =95% confidence interval; T stage = tumor stage; T grade = tumor grade. [A] Cox regression analysis, * P <0.05, ** P <0.01.

Supplemental Table 3. Univariate and multivariate analysis of Disease-Free Survival (DFS) in for *LNMAT2* expression in BCa patients (*n* = 266)

Variables	Univariate analysis			Multivariate analysis		
	HR	**95%CI**	***P*-value[A]**	**HR**	**95%CI**	***P*-value[A]**
Age (<65 vs. ≥65)	0.992	0.705-1.397	0.965			
Gender (Male vs. Female)	0.766	0.521-1.127	0.176			
T stage (T2-4 vs. T1)	1.807	1.205-2.710	0.004**	1.737	1.150-2.625	0.009**
T grade (High vs. Low)	1.407	0.948-2.089	0.090			
Lymphatic metastasis (positive vs. negative)	1.843	1.270-2.675	0.001**	1.535	1.042-2.259	0.030*
LNMAT2 expression (High vs. Low)	1.502	1.069-2.111	0.019*	1.442	1.017-2.045	0.040*

Abbreviations: HR = hazard ratio; 95%CI =95% confidence interval; T stage =tumor stage; T grade = tumor grade. [A] Cox regression analysis, * P <0.05, ** P <0.01.

Supplemental Table 4. Effect of *LNMAT2* exosomes on popliteal LN metastasis in vivo (*n* = 12).

Xenograft	No. metastasis LNs	No. Non-metastasis LNs	Metastasis ratio	*P*-value[A] (vs. UM-UC-3-EXO$_{LNMAT2}$)
PBS	4	8	33.33%	0.022*
UM-UC-3-EXO$_{Vector}$	5	7	41.67%	0.035*
UM-UC-3-EXO$_{LNMAT2}$	10	2	83.33%	

[A] Chi-square test. * P <0.05, ** P <0.01.

Supplemental Table 5. The possible TFO predicted by LongTarget for *LNMAT2* and *PROX1* promoter.

Oligo ID	TFO (5'-3')	Oligo ID	TTS (5'-3')	Score
TFO1	TGGCTTCCCAATCTATC	**TTS1**	ACCAAGGGTGACACGTA	67
TFO2	TCATCCACGAACCAGA	**TTS2**	ATTGGGTGCTGGGGCC	56
TFO3	GAGTCCCTCATCCGG	**TTS3**	CCGGGGATGGAGGCC	52
TFO4	CCACCCAGCTAATCAG	**TTS4**	GGCGAGTGAATTAGTG	45
TFO5	TGAAACTTCACTTC	**TTS5**	ATTTGTAACTTGCA	41

Abbreviation: TFO, Triplex-forming oligos; Score, triplex-forming potential score.

Supplemental Table 6. Effect of *LNMAT2* exosomes on popliteal LN metastasis in vivo (*n* = 16).

Xenograft	No. metastasis LNs	No. Non-metastasis LNs	Metastasis ratio	*P*-value[A] (vs. UM-UC-3-EXO$_{LNMAT2}$+αVEGF-C)
PBS	5	11	31.25%	0.004**
UM-UC-3-EXO$_{Vector}$+αVEGF-C	7	9	43.75%	0.028*
UM-UC-3-EXO$_{LNMAT2}$+αVEGF-C	13	3	81.25%	

[A] Chi-square test. * P <0.05, ** P <0.01.

Supplemental Table 7. Correlation between exosomal *LNMAT2* expression and clinicopathologic characteristics of BCa patients

Characteristics	No. of cases	Exosomal *LNMAT2* expression level		
		Low	High	*P*-value[A]
Total cases	206	103	103	
Gender				0.757
Male	148	73	75	
Female	58	30	28	
Age				0.888
< 65	81	40	41	
≥ 65	125	63	62	
T stage				0.080
T1	53	32	21	
T2-4	153	71	82	
T grade				0.878
Low	59	30	29	
High	147	73	74	
Lymphatic metastasis				**0.001****
Negative	154	94	60	
Positive	52	9	43	

Abbreviations: No. of cases = number of cases; T stage = tumor stage; T grade = tumor grade.

[A] Chi-square test, * P <0.05, ** P <0.01.

Supplemental Table 8. Univariate and multivariate analysis of Overall Survival (OS) for exosomal *LNMAT2* expression in BCa patients (*n* = 206)

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P*-value [A]	HR	95%CI	*P*-value [A]
Age (<65 vs. ≥65)	1.044	0.692-1.576	0.837			
Gender (Male vs. Female)	1.204	0.762-1.902	0.426			
T stage (T2-4 vs. T1)	1.062	0.676-1.667	0.794			
T grade (High vs. Low)	0.919	0.604-1.400	0.695			
Lymphatic metastasis (positive vs. negative)	2.029	1.329-3.097	0.001**	1.580	0.997-2.502	0.051
Urinary exosomal *LNMAT2* expression (High vs. Low)	2.078	1.375-3.141	0.001**	1.773	1.132-2.778	0.012*

Abbreviations: HR = hazard ratio; 95%CI =95% confidence interval; T stage = tumor stage; T grade = tumor grade. [A] Cox regression analysis, * P <0.05, ** P <0.01.

Supplemental Table 9. Univariate and multivariate analysis of Disease-Free Survival (DFS) for exosomal *LNMAT2* expression in BCa patients (*n* = 206)

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P*-value [A]	HR	95%CI	*P*-value [A]
Age (<65 vs. ≥65)	0.986	0.666-1.406	0.864			
Gender (Male vs. Female)	1.169	0.775-1.764	0.455			
T stage (T2-4 vs. T1)	1.195	0.786-1.819	0.404			
T grade (High vs. Low)	0.814	0.558-1.188	0.286			
Lymphatic metastasis (positive vs. negative)	1.736	1.170-2.575	0.006**	1.368	0.893-2.094	0.150
Urinary exosomal *LNMAT2* expression (High vs. Low)	1.913	1.323-2.766	0.001**	1.726	1.158-2.571	0.007*

Abbreviations: HR = hazard ratio; 95%CI =95% confidence interval; T stage = tumor stage; T grade = tumor grade. [A] Cox regression analysis, * P <0.05, ** P <0.01.

Supplemental Table 10. Primer sequences used in this manuscript.

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')	Application
GAPDH	ATCACCATCTTCCAGGAGCGA	CCTTCTCCATGGTGGTGAAG AC	qRT-PCR
LNMAT2	GGTTCAGTTGGGCAAAAGGC	TCATTCAGTCACAGGGTGGC	qRT-PCR
hnRNPA2B1	CAACCTTCTAACTACGGTCCA A	CAGTATCGGCTCCTCCCAC	qRT-PCR
PROX1	CAGCCCGAAAAGAACAGAAG	GGGTCTAGCTCGCACATCTC	qRT-PCR
U1	GGGAGATACCATGATCACGAA GGT	CCACAAATTATGCAGTCGAG TTTC	qRT-PCR
miR-198	GACAGAGGTCCAGAGGGGAG	Universal primer CAGTGCGTGTCGTGGAGT	qRT-PCR
miR-18a	GATAGCAGCACAGAAATATTG GC	Universal primer CAGTGCGTGTCGTGGAGT	qRT-PCR
PROX1-P1	CCCCCACCCCTTTTATATTT	ACAGGAAGACTGCACGTCAC	ChIRP
PROX1-P2	GCGTCCTGGAAGAGCTAGTG	GGAAGAGAGGAGGGGAGAG G	ChIRP
PROX1-P3	ATGTGAAACCTCTGGCACCT	GGAGAAAAAGTGGGGGTTTT	ChIRP
PROX1-P4	CTGCGATTTATGCGTTTGAA	TTGCAAACATCTGGCGATTA	ChIRP
PROX1-P5	GGTGAAAGGGACGTTCTAGC	GGTGAAAGGGACGTTCTAGC	ChIRP
GAPDH-RNA	CAAGGCTGAGAACGGGAAG	AGGTAGTTTCGTGGATGCCA	ChIRP
GAPDH-DNA	GTTTCCAGGAGTGCCTTTGTG	ATTAGGGCAGACAATCCCGG C	ChIRP
PROX1	ATGTGAAACCTCTGGCACCT	GGAGAAAAAGTGGGGGTTTT	ChIP-qPCR
ACTB	GAAGCTAAGTCCTGCCCTCA	CAGTGAGGACCCTGGATGTG	ChIP-qPCR
LNMAT2 5'RACE	Universal primer in SMARTer kit	ATTCAGCACTAGGACTAGGA CAGC	Nested PCR (Outer)

LNMAT2 5'RACE	Universal primer in SMARTer kit	CCTTGCAATTTGTCTCTTGTGGCC	(Inner)
LNMAT2 3'RACE	AAGGGATTGAGAAGCTTTC	Universal primer in SMARTer kit	(Outer)
LNMAT2 3'RACE	CCATGGACTTCTGAGCCTTC	Universal primer in SMARTer kit	(Inner)
si-*LNMAT2*#1	CCAGCUUCUAAGGUGGUUATT	UAACCACCUUAGAAGCUGGTT	siRNAs
si-*LNMAT2*#2	CCUAGUCCUAGUGCUGAAUTT	AUUCAGCACUAGGACUAGGTT	siRNAs
si-*hnRNPA2B1*#1	GCAAUUCAUUGAGCGCAUUTT	GCAAUUCAUUGAGCGCAUUTT	siRNAs
si-*hnRNPA2B1*#2	GCUCUUUAUUGGUGGCUUATT	UAAGCCACCAAUAAAGAGCTT	siRNAs
si-*PROX1*#1	UGGAGAAGUAUGCGCGUCATT	UGACGCGCAUACUUCUCCATT	siRNAs
LNMAT2-sgRNA#1	caccGTTGGCATGGGATGCCAGCCgttt		CRISPR/Cas9
LNMAT2-sgRNA#2	caccGTTTTCCCACTTAGACCAAAgttt		CRISPR/Cas9

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Supplemental Table 11. Probes used in in situ hybridization (ISH) and Chromatin isolation by RNA purification (ChIRP).

`	Probe sequences (5'-3')	Label	Application
***LNMAT2*_odds1**	GACACCAATGGGATCATTCA	3'-Biotin	ChIRP
***LNMAT2*_odds2**	CTGGTAAGGAATGGTTCCAG	3'-Biotin	ChIRP
***LNMAT2*_odds3**	AAAACCAGGACAGATGGGCA	3'-Biotin	ChIRP
***LNMAT2*_odds4**	TCACAGAGACAACTGGCACA	3'-Biotin	ChIRP
***LNMAT2*_odds5**	GACCTCAGCAATGACACATT	3'-Biotin	ChIRP
***LNMAT2*_even1**	GATCTCCAACTCTGCTAGAA	3'-Biotin	ChIRP
***LNMAT2*_even2**	CCAAAAGAGGTTTGGCTTGG	3'-Biotin	ChIRP
***LNMAT2*_even3**	CAGACAAGTGTCCAGTAGTG	3'-Biotin	ChIRP
***LNMAT2*_even4**	GATTGGGAAGCCAGATTAGG	3'-Biotin	ChIRP
***LNMAT2*_even5**	CAAGTTTATCTGCAAGACCC	3'-Biotin	ChIRP
LNMAT2	AGGTAATCACAGAGCTGGAGCA	5'-DIG labeled and 3'-DIG labeled	ISH
U6	CACGAATTTGCGTGTCATCCTT	5'-DIG labeled and 3'-DIG labeled	ISH
Scramble	GTGTAACACGTCTATACGCCCA	5'-DIG labeled and 3'-DIG labeled	ISH

Abbreviation: DIG, Digoxigenin.

Supplemental Methods

Antibodies

Anti-LYVE-1 antibody (ab33682) for IHC, anti-PROX1 antibody (ab38692) for IHC, anti-luciferase antibody (ab181640) for IHC, anti-CD63 antibody (ab134054) for immunoblot, anti-hnRNPA2B1 antibody (ab31645) for immunofluorescence, RIP and ChIP were purchased from Abcam (MA, USA). Anti-hnRNPA2B1 antibody (9304) for immunoblot, anti-CD9 antibody (13403) for immunoblot, anti-PROX1 antibody (14963) for immunoblot, anti-β-tubulin antibody (2146) for immunoblot, anti-H3K27me3 antibody (9733) for ChIP, HRP-linked anti-mouse IgG antibody (7076) and HRP-linked anti-rabbit IgG antibody (7074) for immunoblot were purchased from Cell Signaling Technology (MA, USA). Anti-Ki67 antibody (ZM-0166) for IHC was purchased from Sino Biological Inc. (Beijing, China). Goat anti-rabbit IgG-HRP antibody (SA00001-15) and Goat anti-mouse IgG-HRP antibody (SA00001-1) for IHC were purchased from Proteintech Group (Chicago, USA). Control mouse IgG antibody, control rabbit IgG antibody and anti-RNA pol II antibody were provided in the EZ-Magna RIP kit or EZ-Magna ChIP A/G kit (Millipore, MA, USA). Alexa Fluor™ 555 Phalloidin antibody for immunofluorescence were purchased from Invitrogen (CA, USA).

Isolation and purification of exosomes

To isolate the exosomes from cell cultured media, BCa cells were grown in media supplemented with 10% exosome-depleted FBS for 72 h. The supernatant was collected and sequentially centrifuged at 1,000 × g for 5 min, 2,000 × g for 10 min and 10,000 × g for 30 min. The obtained supernatant was filtered through 0.22 μm filter (PALL, New York, USA)

followed by ultracentrifugation at 120,000 × g for 70 min in a 70Ti rotor (Beckman, California, USA). The supernatant was discarded and pellets were resuspended with PBS. The suspension was subsequently ultra-centrifuged at 120,000 × g for another 70 min. The purified exosomes were subjected to the following experiments. All the centrifugations were conducted at 4°C。

To isolate the exosomes from urine, mid-stream urine from patients or heathy volunteers was collected in 50 ml centrifuge tubes (Corning, New York, USA) and centrifuged at 2,000 × g for 20 min followed by 10,000 × g for 30 min. The supernatant was transferred to a 0.22 μm filter and the flow was collected and purified by ultracentrifugation as mentioned above. All the centrifugations were conducted at 4°C。

To isolate the exosomes from serum, blood sample was collected from patients or heathy volunteers using Vacutainer (BD, USA) and centrifuged at 1,000 × g for 10 min. The supernatant was subsequently centrifuged at 2,000 × g for 20 min and filtered through a 0.22 μm filter. The flow was collected and purified by ultracentrifugation as mentioned above. All the centrifugations were conducted at 4°C。

Electron microscopy analysis

Exosomes were loaded on a Formvar-carbon-coated electron microscope grid (Polysciences) for 30 min. Then the grid was washed in PBS and fixed in 2% glutaraldehyde (Sigma Aldrich) for 10 min. The grid was subsequently washed in PBS for 5 times and counter-stained with 2% uranyl acetate (Sigma Aldrich) for 1 min. Air-dried grids were viewed with a Hitachi transmission electron microscope.

Exosomes Internalization

Exosomes were stained with PKH67 Green Fluorescent Cell Linker Kit (Sigma Aldrich, St Louis, USA) according to the manufacturer's instruction and the staining was terminated by adding 1% BSA. PKH67-labeled exosomes were precipitated by ultracentrifugation and resuspended in ECM containing 10% exosome-depleted FBS. Then exosomes were added to HLECs and incubated for 12 h After washing twice with PBS, cells were fixed with 4% formaldehyde. The nuclei were stained with DAPI. A Zeiss confocal microscope system was used to obtain the images.

Tube formation assays and Transwell assays of HLECs

In tube formation assays, 1:2 growth factor reduced Matrigel (BD Biosciences, CA, USA) and ECM mixture was pre-coated to 24-well plate and solidified at 37°C for 30 min. 1×10^5 HLECs were seeded on the 24-well plate and incubated with either PBS, or exosomes for 12 h. Images were recorded by an inverted microscope and the length of lymphatic tubes were determined.

The migration abilities of HLECs were evaluated by Transwell assays. HLECs treated with either PBS or exosomes for 48 h and were harvested and suspended in ECM without FBS and then seeded in the upper chamber of Transwell apparatus (Corning Costar Corp, MA, USA), at a density of 1×10^5 cells per well. In the lower chamber, medium containing 10% FBS was added. After 18 h incubation, the cells were fixed in 4% paraformaldehyde (PFA) and stained with crystal violet. The number of cells migrated from upper chamber to lower chamber were counted in five random areas under Nikon Eclipse Ti microscope (Nikon, Tokyo, Japan) after wiping off the remaining cells in the upper chamber.

CCK-8, colony formation and EdU assays

For CCK-8 assays, 4×10^3 BCa cells were seeded in 96-well plates and transfected with siRNA or overexpression plasmid. After culturing overnight, cells were treated with CCK-8 solution according to the manufacturer's instructions of the CCK-8 kit (APExBIO, USA). The cell viability was measured by OD450 with a microplate reader (Epoch, BioTek, USA) every 24 h for 4 days.

For EdU assays, siRNA transfected BCa cells were seeded in 24-well plates and incubated for 24 h. Cells were stained with EdU and DAPI according to the manufacturer's instructions of the EdU kit (RioboBio, Guangzhou, China). The images were obtained with an Olympus laser scanning microscope system (Tokyo, Japan).

For the colony formation assays, 1×10^3 siRNA transfected BCa cells were seeded into 6-well plates and cultured for 2 weeks. The colonies were stained with 0.1% crystal violet. Visible colonies were counted and wells were measured in triplicate for each treatment group.

Subcutaneous tumorigenicity assay

Subcutaneous tumorigenicity assay were performed by subcutaneously inoculating luciferase-labeled UM-UC-3 cells (5×10^6) into BALB/c nude mice (the Experimental Animal Center, Sun Yat-sen University, Guangzhou, China). Mice were randomly divided into three groups (n = 10) and were intratumorally injected with either (i) PBS, (ii) UM-UC-3-EXO$_{Vector}$ and (iii) UM-UC-3-EXO$_{LNMAT2}$ (20 μg per dose), respectively every 3 days. The subcutaneous tumors were analyzed using a PerkinElmer IVIS Spectrum In Vivo Imaging System. The tumors were excised to measure the weight and volume 5 weeks after the treatment, and fixed in 37% formalin overnight followed by paraffin-embedding. Serial sections were stained with HE and IHC. The sections were visualized with Nikon Eclipse Ti

microscope (Nikon, Japan).

Lentivirus-mediated transduction

The full-length *LNMAT2* was cloned into the pCDH-CMV-MCS-EF1-Puro (with or without luciferase) and the double-stranded oligonucleotides targeting *LNMAT2* was cloned into the pLKO.1-Puro vector. The lentivirus was produced by co-transfecting lentiviral vectors and packaging vectors, psPAX2 (Addgene #12260, MA, USA) and pMD2.G (Addgene #12259) into HEK-293T cells. After 72 h, the culture media contained virus were harvested and concentrated at 4°C overnight in a LentiX™ Concentrator. Purified lentivirus was then used to infect BCa cells with 8 mg/ml Polybrene (Sigma Aldrich). Transfected cells were selected by adding puromycin (Sigma Aldrich) for 2 weeks to obtain a stable cell line.

RNA extraction and quantitative real-time PCR (qRT-PCR) analysis

Total RNA from cells, tissues and exosomes was extracted using the TRIzol reagent (Life Technologies) and purified using RNeasy mini kit (QIAGENMD, USA) following the manufacturers' instructions. RNA quantity was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc). 500ng of total RNA was mixed with 2μl of 5×PrimeScript RT Master Mix (Takara Biotechnology Co., Ltd., Dalian, China) and reversely transcribed to cDNA. qRT-PCR analysis was conducted using the TBGreen II (Takara Biotechnology Co., Ltd.) and analyzed on a Roche Light-Cycler system (Roche, CA, USA). The results were shown as the fold change using the 2-ΔΔCT method. *GAPDH* served as an internal control. The primers sequences used in qRT-PCR analysis were provided in Supplemental Table 10.

5'and 3'Rapid amplification of cDNA ends (RACE) of *LNMAT2*

5' and 3' RACE assays were performed following the instructions of a SMARTer RACE 5'/3' kit (CLONTECH Laboratories, CA, USA). Briefly, gene specific RACE product was generated through PCR amplification. The PCR products were separated on a 1% agarose gel and the gel extraction products were further subjected to bidirectional sequencing using indicated primers. *LNMAT2*-specific nested PCR primers sequences used for 5' and 3' RACE analysis was provided in Supplemental Table 10.

ISH and IHC analysis

For ISH, the double-(5' and 3')-digoxin (DIG)-labeled probes targeted *LNMAT2* were used. Scramble probe was used as negative control. The slides were dewaxed with dimethylbenzene and rehydrated with gradient alcohol, followed by proteinase K digestion and fixation with 4% PFA. After hybridization with the *LNMAT2* probe at 42°C overnight, the slides were subsequently incubated in anti-digoxin antibody at 4°C overnight. Finally, the staining was performed using Nitroblue Tetrazolium/5-Bromo-4-Chloro-3-Indolylphosphate (Roche, CA, USA) and images were captured with a Nikon Eclipse Ti microscope (Nikon, Tokyo, Japan). The H-score for *LNMAT2* expression was calculated as follows: H-score=$\sum$ (P × I) where P represents the percentage of stained cells; I represents the intensity of the staining which was defined as: 0 (absent), 1 (weak), 2 (moderate) and 3 (strong). The probes for ISH assays are listed in Supplemental Table 11.

For IHC, the formalin-fixed, paraffin-embedded sections were dewaxed and rehydrated as mentioned above, treated with 3% hydrogen peroxide followed by EDTA buffer for antigen retrieval. The sections were then blocked in goat serum for 30 min, incubated with respective

primary antibodies at 4°C overnight and subsequent with horseradish peroxidase-conjugated secondary antibodies for 30 min at room temperature. Finally, the sections were stained with the DAB substrate and hematoxylin. Images were recorded by Nikon Eclipse Ti microscope.

Colocalization of *LNMAT2* and hnRNPA2B1

The colocalization of *LNMAT2* and hnRNPA2B1 were confirmed by fluorescence staining. Briefly, 5637 cells were seeded on a glass-bottomed confocal plate and cultured overnight. After fixation with 4% PFA and permeabilization with 0.5% Triton, hybridization was carried out overnight with the *LNMAT2* probes conjugated with Alexa Fluor 555 (Invitrogen, CA, USA) at 37°C in 2×SSC, 10% formamide and 10% dextran. Subsequently, Anti-hnRNPA2B1 was incubated in the dark overnight followed by incubation with secondary antibody for 1h. Finally, the nuclei were stained by DAPI and the images were captured under a confocal microscope.

Western Blot analysis

Protein extraction were performed using RIPA lysis buffer (Pierce, IL, USA) containing protease inhibitor (Roche, CA, USA). Protein extracts were subjected to 10% SDS-polyacrylamide gel electrophoresis followed by electro-transfer to polyvinylidene difluoride membrane. After 1h of pre-membrane blocking with 5% BSA, the proteins were incubated with respective primary antibodies at 4°C overnight followed by secondary antibodies incubation at room temperature for 1 h. The detection of proteins was carried out using ECL reagent.

Nuclear fractionation

1×10^7 cells were harvested and washed with RNase-free PBS. After incubation with 5×fraction buffer (1.28M Sucrose, 20mM $MgCl_2$, 40mM Tris-HCl, pH 7.5, 4% Triton X-100) diluted in RNase-free water for 15min on ice, cells were subjected to centrifugation at 2500 rpm for 15min, and the pellet containing the nuclear fraction was used for RNA extraction.

ELISA analysis

Cell culture supernatant of *LNMAT2*-overexpressing or knockdown BCa cells were harvested and centrifuged at 3000 rpm for 5 min to remove the pellets. The level of secreted VEGF-C in cell culture supernatant was quantified using the Human VEGF-C Quantikine ELISA Kit (Cat. No. DVEC00, R&D) according to the manufacturer's instructions.

Serial deletion analysis and site-directed mutagenesis

LNMAT2 with various deletions were amplified by using a series of paired 3' nested primers with common 5' primers or 5' nested PCR primers with common 3' primers and cloned into pcDNA3.0 for in vitro transcription. The resulting RNAs were further subjected to RNA pull-down assays. The mutant *LNMAT2* RNAs (Δ1930-1960) described in the manuscript were synthesized following the instruction of QuikChange Site-directed Mutagenesis Kit (Stratagene, CA, USA).

ChIP and ChIRP assays

The EZ-Magna ChIP A/G kit (Millipore, MA, USA) was used for ChIP analysis. HLECs (2×10^7) pretreated with exosomes were fixed in 1% formaldehyde and the nuclear extracts prepared with completed nuclear lysis buffer were sheared to 100 ~ 200 bp in lengths by ultrasonication. 10% of the total sample volume was removed as a sample input. The

remaining sonicated lysate was incubated with anti-hnRNPA2B1 antibody (Abcam, MA, USA) or anti-H3K4me3 antibody (Abcam, MA, USA) at 4°C overnight and followed by precipitation with streptavidin magnetic beads. The retrieved chromatin was subjected to qRT-PCR analysis. Mouse IgG and anti-RNA pol II antibody (Millipore, MA, USA) served as the negative and positive control respectively.

For ChIRP assays, the Magna ChIRP RNA Interactome Kit (Millipore, MA, USA) was used according to the manufacturer's instructions. 2×10^7 HLECs pretreated with exosomes were harvested and treated with 1% glutaraldehyde. Then the cells were lysed and sonicated into 100-200 bp fragments in a 4°C water bath. The biotinylated probes were separated into "odds" and "even" groups and hybridized with the sonicated cell lysates for 4 h at 37°C. Then, DNA and RNA were respectively extracted from post-ChIRP beads, and qRT-PCR was performed to analyze the enrichment of DNA and RNA retrieval. The probes for ChIRP assays are listed in Supplemental Table 11.

Dual-luciferase reporter assays

Luciferase assays were performed to examine the interaction between *PROX1* promoter and *LNMAT2*. The indicated *PROX1* promoter fragments were cloned into the pGL3 plasmid and transfected into the HLECs cells pretreated with exosomes from *LNMAT2*-overexpressing or untreated BCa cells. The pGL3 vector was used as a negative control. A reporter plasmid containing Renilla luciferase was used as the standard reference. The luciferase activities were detected following the instruction of the Dual-Luciferase Reporter Assay System (Promega, WI, USA) 24 h after transfection. Renilla luciferase intensity was normalized against Firefly luciferase intensity.

Bioinformatics Analysis

The enrichment of hnRNPA2B1 binding motifs in RNAs is obtained from POSTAR2. The secondary structure of *LNMAT2* is predicted using RNAalifold. The *LNMAT2* binding motifs in *PROX1* promoter and binding sequences in *LNMAT2* are predicted by LongTarget.