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Supplemental Information

Hypoxia-induced lncHILAR promotes renal cancer metastasis via ceRNA for the miR-613/206/1-1-3p/Jagged-1/Notch/CXCR4 signaling pathway

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Figure S1

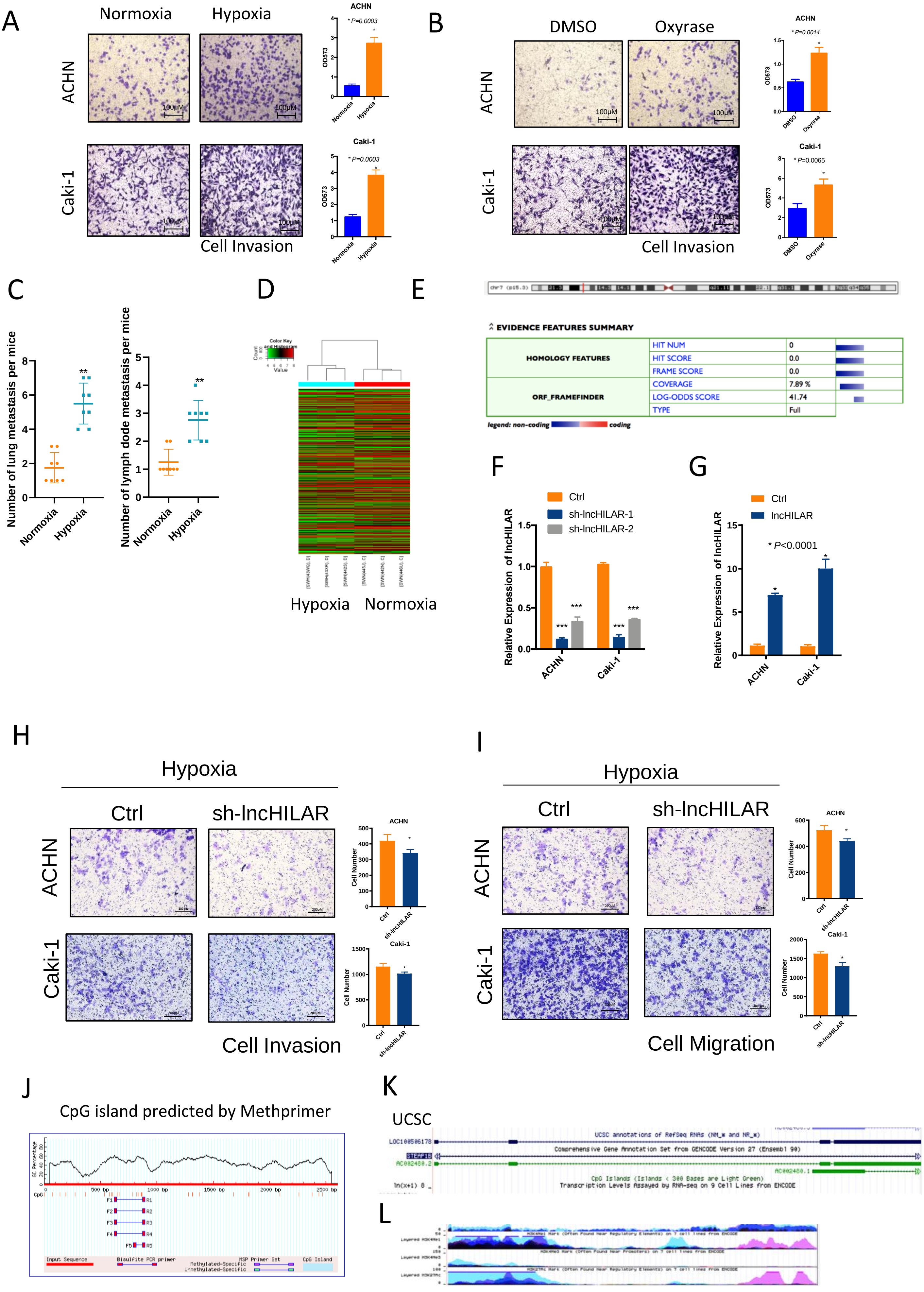

A
Normoxia
Hypoxia
ACHN
Caki-1
100μM
Cell Invasion
ACHN
* P=0.0003
OD573
Normoxia
Hypoxia
Caki-1
* P=0.0003
B
DMSO
Oxyrase
ACHN
Caki-1
100μM
Cell Invasion
ACHN
* P=0.0014
Caki-1
* P=0.0065
OD573
DMSO
Oxyrase
C
Number of lung metastasis per mice
Number of lymph dode metastasis per mice
Normoxia
Hypoxia
**
D
Color Key and Histogram
Count
Value
Hypoxia
Normoxia
E
chr7 (p15.3)
EVIDENCE FEATURES SUMMARY
HOMOLOGY FEATURES
HIT NUM 0
HIT SCORE 0.0
FRAME SCORE 0.0
ORF_FRAMEFINDER
COVERAGE 7.89 %
LOG-ODDS SCORE 41.74
TYPE Full
legend: non-coding coding
F
Ctrl
sh-lncHILAR-1
sh-lncHILAR-2
Relative Expression of lncHILAR
***
ACHN
Caki-1
G
Ctrl
lncHILAR
* P<0.0001
Relative Expression of lncHILAR
ACHN
Caki-1
H
Hypoxia
Ctrl
sh-lncHILAR
ACHN
Caki-1
Cell Number
Cell Invasion
I
Hypoxia
Ctrl
sh-lncHILAR
ACHN
Caki-1
Cell Number
Cell Migration
J
CpG island predicted by Methprimer
GC Percentage
CpG
F1 R1
F2 R2
F3 R3
F4 R4
F5 R5
Input Sequence
Bisulfite PCR primer
MSP Primer Set
Methylated-Specific
Unmethylated-Specific
CpG Island
K
UCSC
UCSC annotations of RefSeq RNAs (NM_* and NR_*)
Comprehensive Gene Annotation Set from GENCODE Version 27 (Ensembl 90)
STEAP1B
CpG Islands (Islands < 300 Bases are Light Green)
Transcription Levels Assayed by RNA-seq on 9 Cell Lines from ENCODE
L
Layered H3K4Me1
Layered H3K4Me3
Layered H3K27Ac

Figure S1.

A:Cell invasion of ACHN and Caki-1 under hypoxia (0.5% O_2). n=3, *P <0.001.

B:Cell invasion of ACHN and Caki-1 under hypoxia by using Oxyrase (100mM). n=3, *P <0.0001.

C: Nude mice were injected into tail vein with normoxic and hypoxic SN12PM6 cells ($1x10^6$ cells). Statistical analysis of lung and lymph node metastasis. n=8, **P<0.001

D: lncRNA microarray data of hypoxic and normoxic cells are presented in a heatmap.

E: Location of lncHILAR on chromosome 7 according to UCSC database (Upper panel) and analysis of coding potential of lncHILAR by Coding potential calculator (Lower panel).

F-G: qRT-PCR analysis of efficiency of shRNA-lncHILAR (F) and overexpression of lncHILAR(G), n=3, ***P <0.0001.

H-I: Cell invasion (H) and migration (I) of ACHN and Caki-1 cells under hypoxia after knockdown of lncHILAR. n=3, *P <0.05.

J-K: Analysis of CpG island in the promoter area of lncHILAR by Methprimer (H)and UCSC browser(K).

L: LncHILAR located at chromosome 7. Its promoter region was enriched with the H3K4Me1 and H3K27Ac histone mark presented with UCSC data.

Figure S2

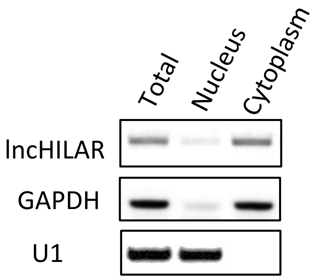
A

lncLocator Database

The subcellular location of the LncHILAR

Predicted Location	Score
Cytoplasm:	0.673102646638
Nucleus :	0.120802166227
Ribosome:	0.0327177183141
Cytosol:	0.139662668623

B



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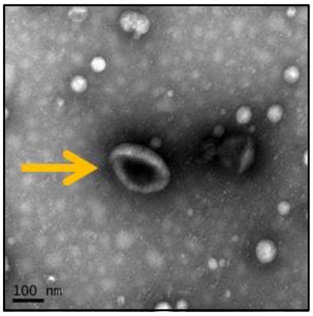

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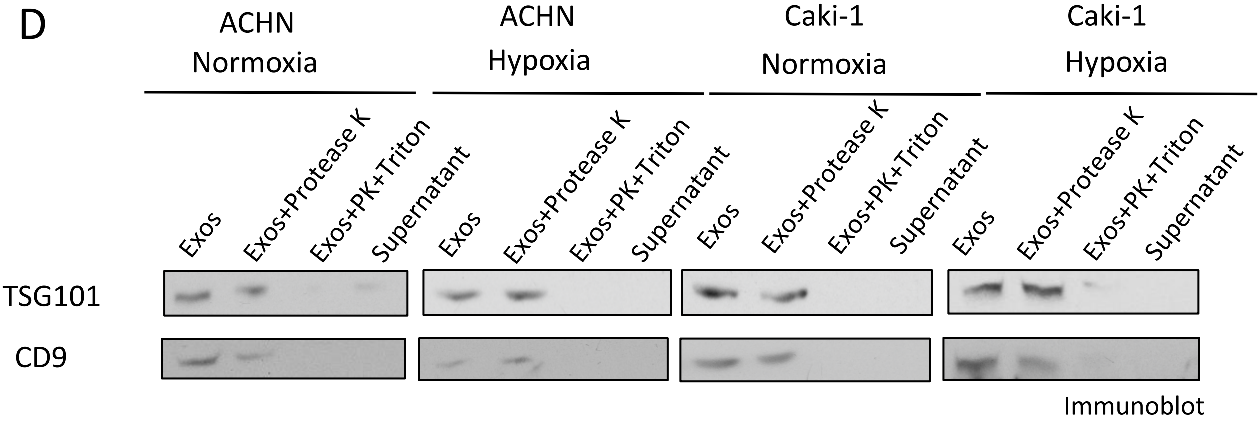

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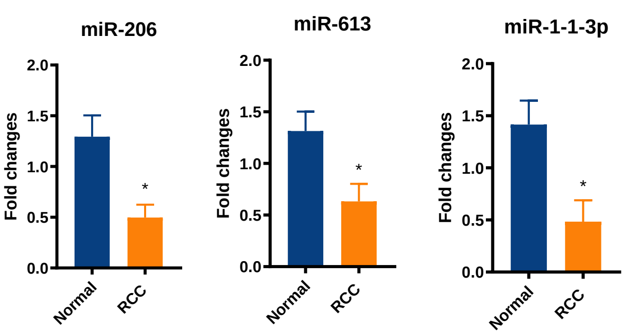

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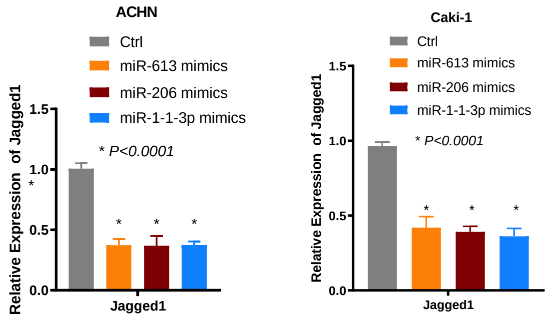

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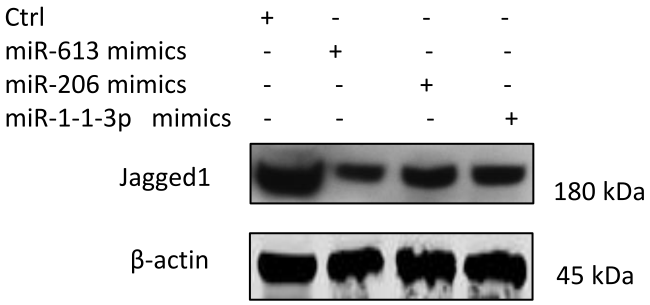

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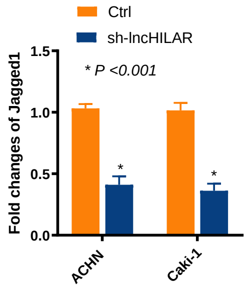
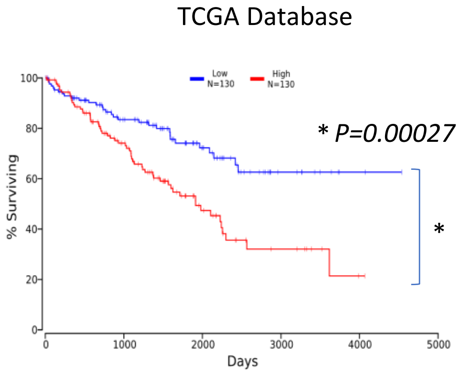


I



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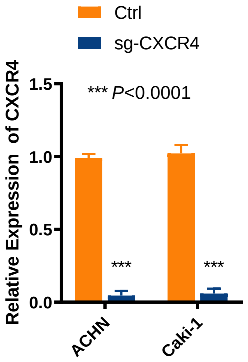

K

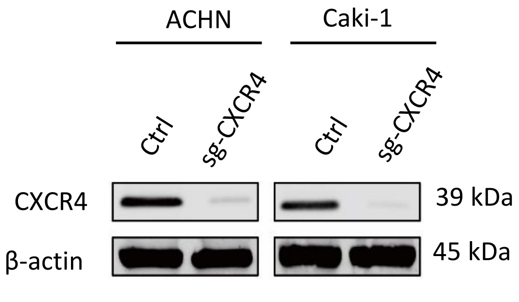

Figure S2.

A: The subcellular location of lncHILAR was predicted by lnclocator database, presented as a predicted score.

B: The cellular distribution of lncHILAR RNA by qRT-PCR showed that lncHILAR was predominately localized to the cytoplasm.

C: Representative electron microscopy images of exosomes secreted by RCC cells. Scale bar, 100 nm.

D: Immunoblot analysis of TSG101 and CD9 in isolated exosomes.

E: Expression of miR613/206/1-1-3p in RCC samples were determined by qRT-PCR, n=40, * $P<0.0001$.

F: qRT-PCR analysis of Jagged1 mRNA in ACHN and Caki-1 cells transfected with Control or miR-613/206/1-1-3p mimics (50nM) for 48 h, n=3, *$P <0.0001$.

G: Immunoblot analysis of Jagged1 expression of ACHN and Caki-1 cells transfected with Control or miR-613/206/1-1-3p mimics, n=3.

H: qRT-PCR analysis of Jagged1 mRNA of ACHN and Caki-1 cells after knock-down of lncHILAR, n=3, *$P <0.001$.

I: Overall survival of RCC patients in high CXCR4 expression group (n=130) and low CXCR4 expression group(n=130), *$P<0.001$. Data was acquired from TCGA database.

J: qRT-PCR analysis of CXCR4 mRNA of Caki-1 cells after knock-out of CXCR4(sg-CXCR4) by CRISPR/Cas9, n=3, *** $P<0.0001$.

K: Immunoblot analysis of CXCR4 protein of ACHN and Caki-1 cells after knock-out of CXCR4 by CRISPR/Cas9.

Table1. Oligonucleotide Sequences

Oligo		Sequence(5’-3’)
lncHILAR	shRNA-1	gatccGCTGTTCTAAGTCATGAATTTCAAGAGAATTCATGACTTAGAACAGCTTTTTTg
	shRNA-2	aattcAAAAAAGCTGTTCTAAGTCATGAATTCTCTTGAAATTCATGACTTAGAACAGCg
Jagged1 sgRNA	Forward	TACTATGGCTTTGGCTGCAATA
Jagged1 sgRNA	Reverse	CAACAGCATCTTCTACACTCCG
CXCR4 sgRNA	Forward	GGTCATGGGTTACCAGAAGAAA
CXCR4 sgRNA	Reverse	CAGGATGAGGACACTGCTGTAG
qPCR primer		
LncHILAR	Forward	CCAGCAGCAACCACATTAGG
	Reverse	CAAGTGGGTAAGGGGTGACAG
miR-613	Forward	GGAA TGTTCCTTCTTTGC
	Reverse	GAACATGTCTGCGTATCTC
miR-206	Forward	GAATGTAAGGAAGTGTGTG
	Reverse	GAACATGTCTGCGTATCTC
miR-1-1-3p	Forward	TGGAATGTAAAGAAGTATGT
	Reverse	GAACATGTCTGCGTATCTC
Jagged1	Forward	TGCTACAACCGTGCCAGTGACT
	Reverse	TCAGGTGTGTCGTTGGAAGCCA
E-cadherin	Forward	GCCTCCTGAAAAGAGAGTGGAAG
	Reverse	TGGCAGTGTCTCTCCAAA TCCG
N-cadherin	Forward	CCTCCAGAGTTTACTGCCATGAC
	Reverse	GTAGGATCTCCGCCACTGATTC

Zo-1	Forward	GTCCAGAATCTCGGAAAAGTGCC
	Reverse	CTTTCAGCGCACCATACCAACC
Vimentin	Forward	AGGCAAAGCAGGAGTCCACTGA
	Reverse	ATCTGGCGTTCCAGGGACTCAT
CXCR4	Forward	CTCCTCTTTGTCATCACGCTTCC
	Reverse	GGATGAGGACACTGCTGTAGAG
GAPDH	Forward	GTCTCCTCTGACTTCAACAGCG
	Reverse	ACCACCCTGTTGCTGTAGCCAA
β-actin	Forward	CACCATTGGCAATGAGCGGTTC
	Reverse	AGGTCTTTGCGGATGTCCACGT
U6	Forward	CTCGCTTCGGCAGCACA T
	Reverse	TTTGCGTGTCA TCCTTGCG
Chip primer		
Site1	Forward	GGTGAGGGGCCCTGATTTAT
	Reverse	GGTGAGGGGCCCTGATTTAT
Site2	Forward	CCAACTACCCAGGAACCCAT
	Reverse	CTGCTGAAGGGGTACGACG