LncRNA SNHG11 Facilitates Tumour Metastasis by Interacting with and Stabilizing HIF-1α

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Supplementary Information

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Cell culture

Human CRC cell lines (HCT-116, LoVo and RKO) and HEK293T cells were purchased from the American Type Culture Collection (ATCC, USA). Cell lines were authenticated by STR profiling and confirmed to be mycoplasma-free. HCT-116 cells were cultured in McCoy’s 5A medium, Lovo cells were cultured in Ham’s F-12K medium, RKO cells were cultured in RPMI-1640 medium and HEK293T cells were cultured in DMEM medium at 37°C in 95% air and 5% CO_2. All media were supplemented with 10% foetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin. 10 μM 5-Aza-2′-deoxycytidine (Sigma-Aldrich, MO, USA) was used to inhibit DNA methylation in CRC cells for indicated days. For hypoxia induction, cells were incubated in a hypoxic chamber with a gas mixture of 94% N_2/5% CO_2/1% O_2. The cells were incubated for 24h under hypoxic conditions.

Cell wound healing assay

RKO cells were seeded in 12-well plates and were cultured until they were 100% confluent. A scratch was created by scratching cell monolayers with a sterile 200 μl plastic pipette tip. Cells were then incubated with or without $CoCl_2$ for 12 h. Images were captured by phase contrast microscopy.

Migration and invasion assays

Migration and invasion assays were performed using a 24-well transwell chamber (Corning, NY, USA). Briefly, CRC cells (5×10^4 in 200 μl serum-free medium) were seeded into the upper chamber. The lower chamber was filled with medium containing 10% foetal bovine serum for 24 h with or without exposure to hypoxic conditions. Cells on the upper membrane surface were wiped off using a cotton swab. Migrated cells attached to the lower membrane surface were fixed with methanol and stained with crystal violet. For invasion assays, the upper chamber was precoated with Matrigel.

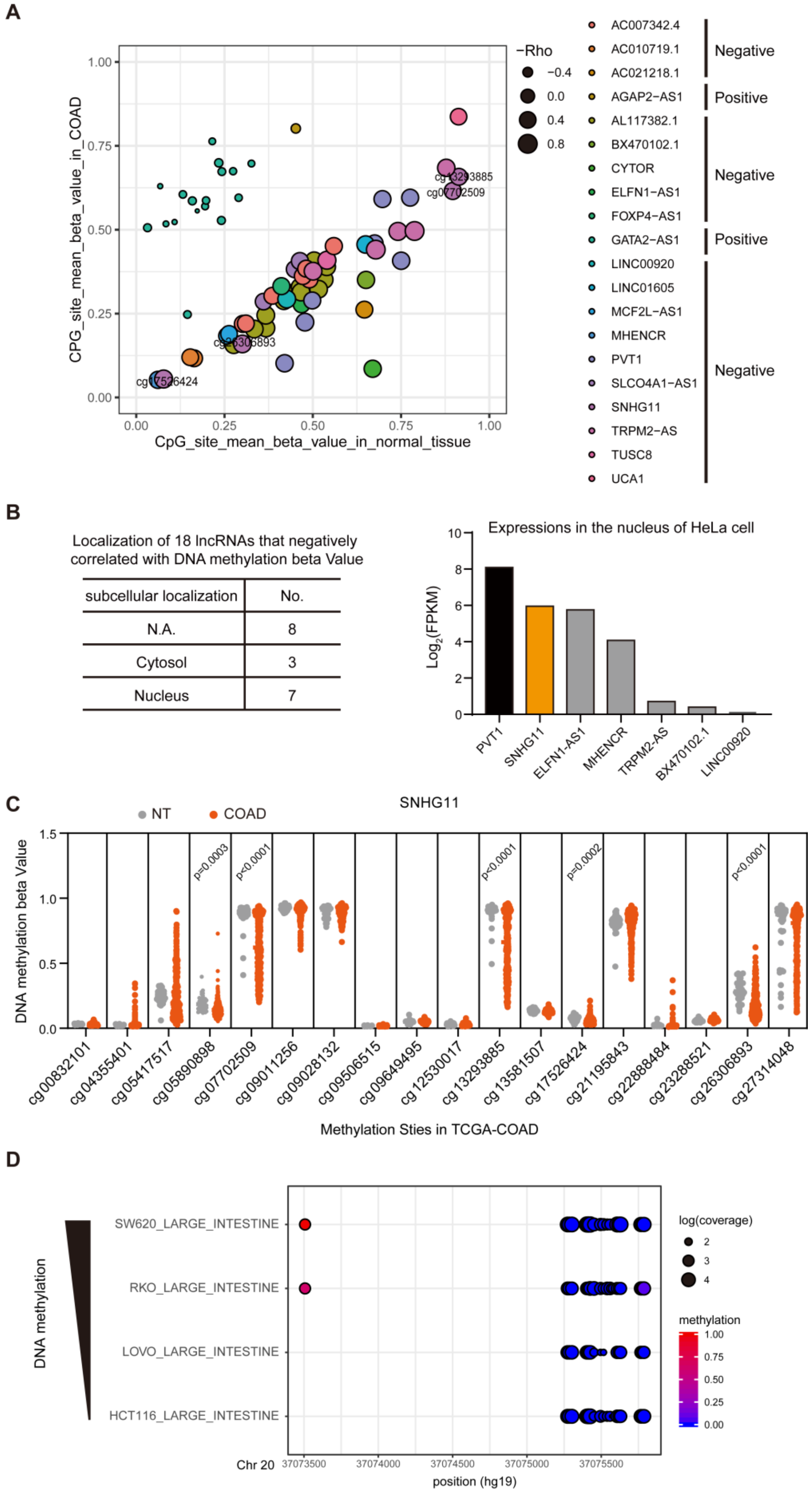
Fluorescence *in situ* hybridization

CRC cells were seeded on sterile glass coverslips and incubated with $CoCl_2$ for 24h. Then, the cells were fixed with 4% PFA for 10 min. Probes designed for ChIRP were used to detect SNHG11 and were incubated with cells for 1 h at 37°C. After brief washing with saline-sodium citrate, cells were incubated with an anti-HIF-1α antibody (Proteintech, IL, USA). After secondary antibody incubation, tyramide signal amplification (Thermo Fisher Scientific, IL, USA) and DAPI staining, images were captured by confocal microscopy (Leica, Germany).

Co-immunoprecipitation

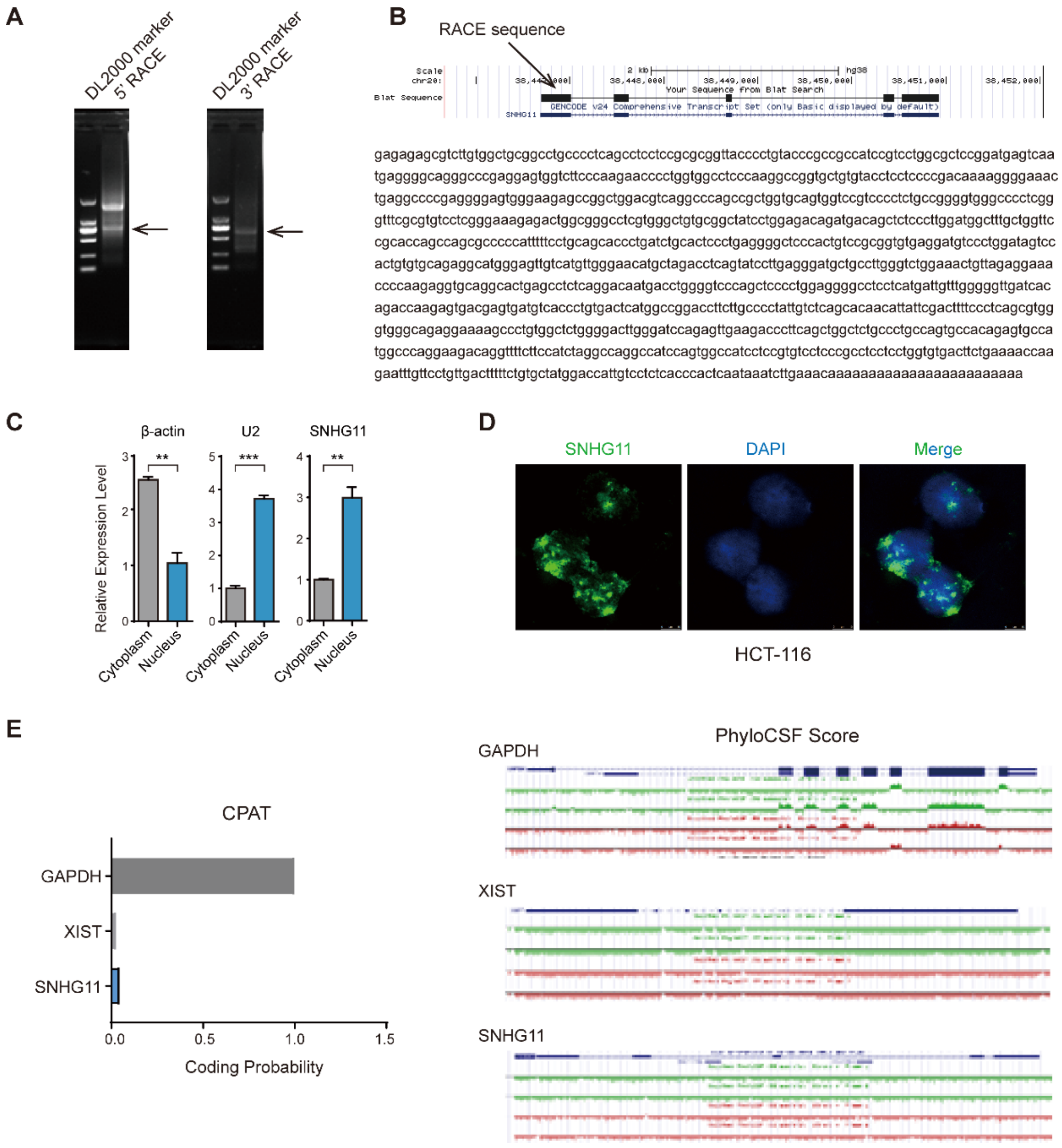
Cells grown in dishes were exposed to hypoxic conditions for 24 h and then were treated with MG-132 (Selleck, Shanghai, China) for 12 h before being lysed in IP lysis buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) supplemented with RNase and proteinase inhibitors. Anti-IgG and anti-HIF-1α were incubated with Protein A/G magnetic beads (Bimake, Shanghai, China) at room temperature for 30 min. Equal volumes of cell lysate supernatant were added to anti-IgG or the indicated antibody-labelled beads and incubated overnight at 4°C. The next day, beads were washed five times with NT2 buffer and then were boiled in SDS loading buffer.

Supplementary Figures



subcellular localization	No.
N.A.	8
Cytosol	3
Nucleus	7

Supplementary Fig 1. DNA Methylation of SNHG11. (A) CpG site methylation of 20 lncRNAs in TCGA COAD. (B) Distribution of lncRNAs in subcellular localization and expression level of lncRNA in the HeLa nuclear localization. (C) DNA methylation across SNHG11 locus from TCGA-COAD. Platform: Illumina Human Methylation 450. n=39 normal tissues and 294 CRC tissues in COAD, two-tailed Student’s t-test. (D) DNA methylation across SNHG11 loci in CRC cells were obtained from CCLE database.



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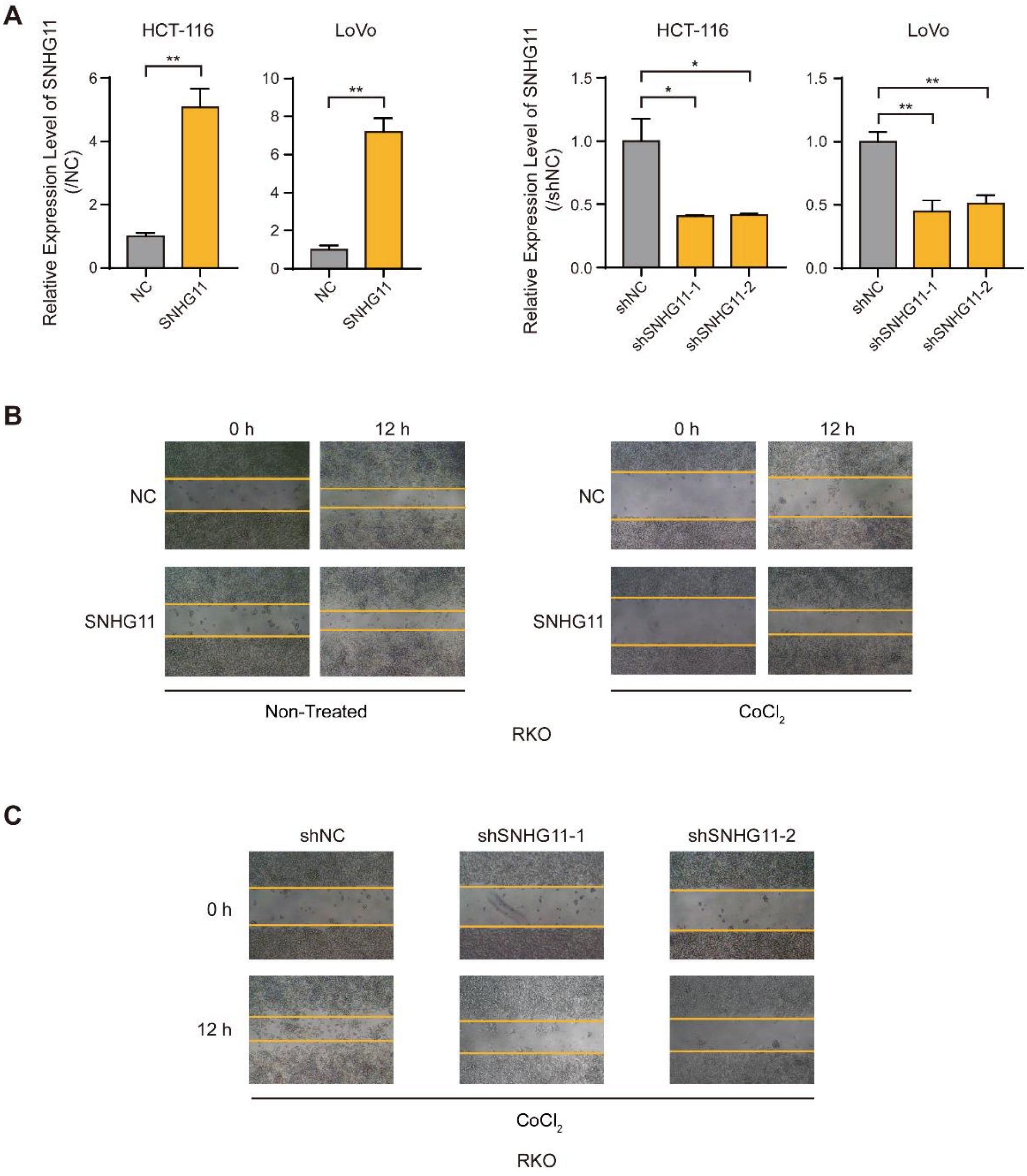
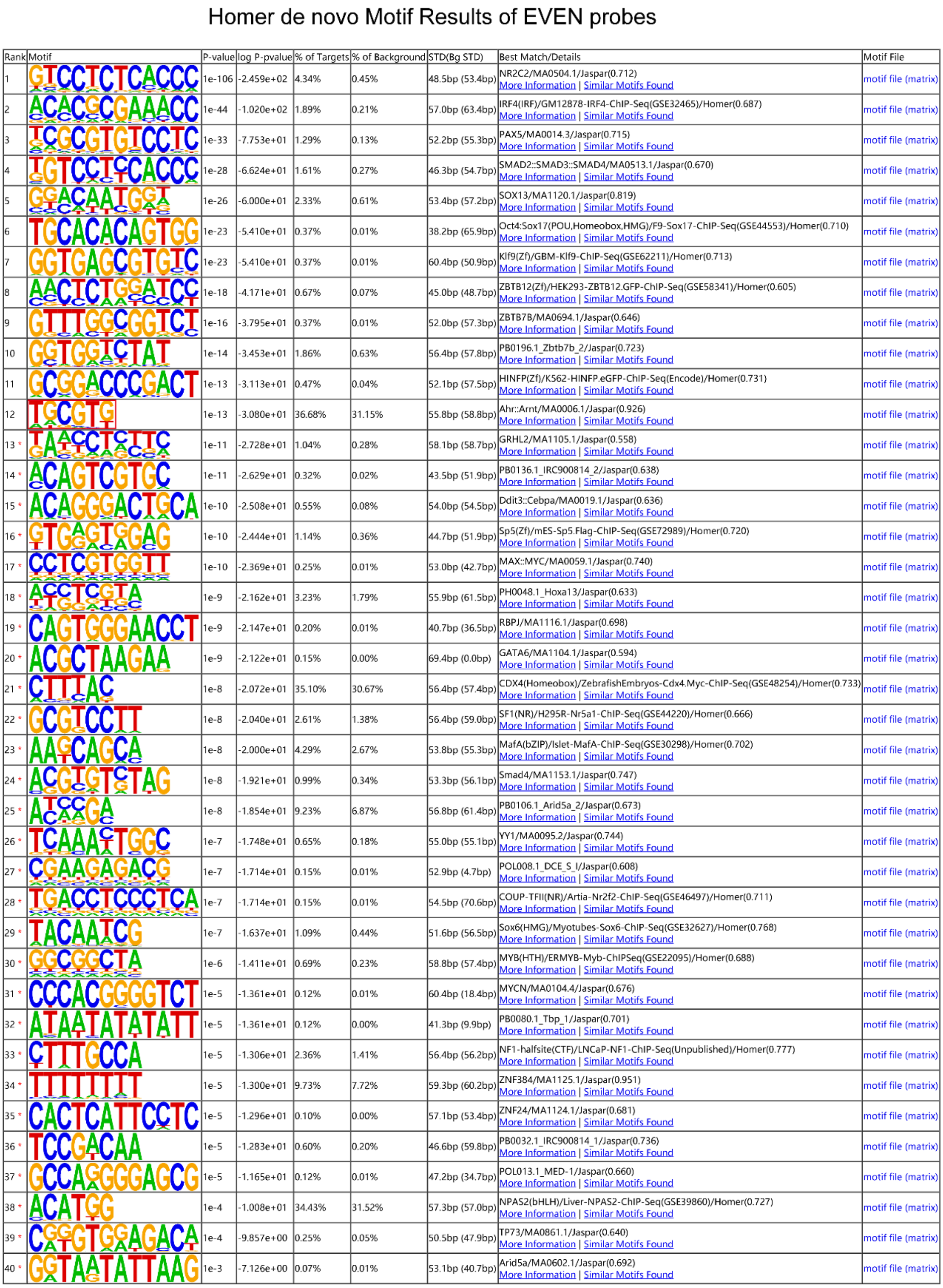
Supplementary Fig 2. Characteristic features of SNHG11. (A and B) 5’ and 3’ RACE assays of SNHG11. (C) Nuclear/cytoplasmic RNA fractionation analysis of the location of SNHG11 in HCT-116 cells. n=3 independent experiments, two-tailed Student’s t-test. (D) Confocal microscopic images of SNHG11 in HCT-116 cells treated with hypoxia. (E) Coding Potential Assessment Tool (CPAT, http://lilab.research.bcm.edu/cpat/) and PhyloCSF to estimate the coding probability of SNHG11. * p < 0.05, ** p < 0.01, and *** p < 0.001.



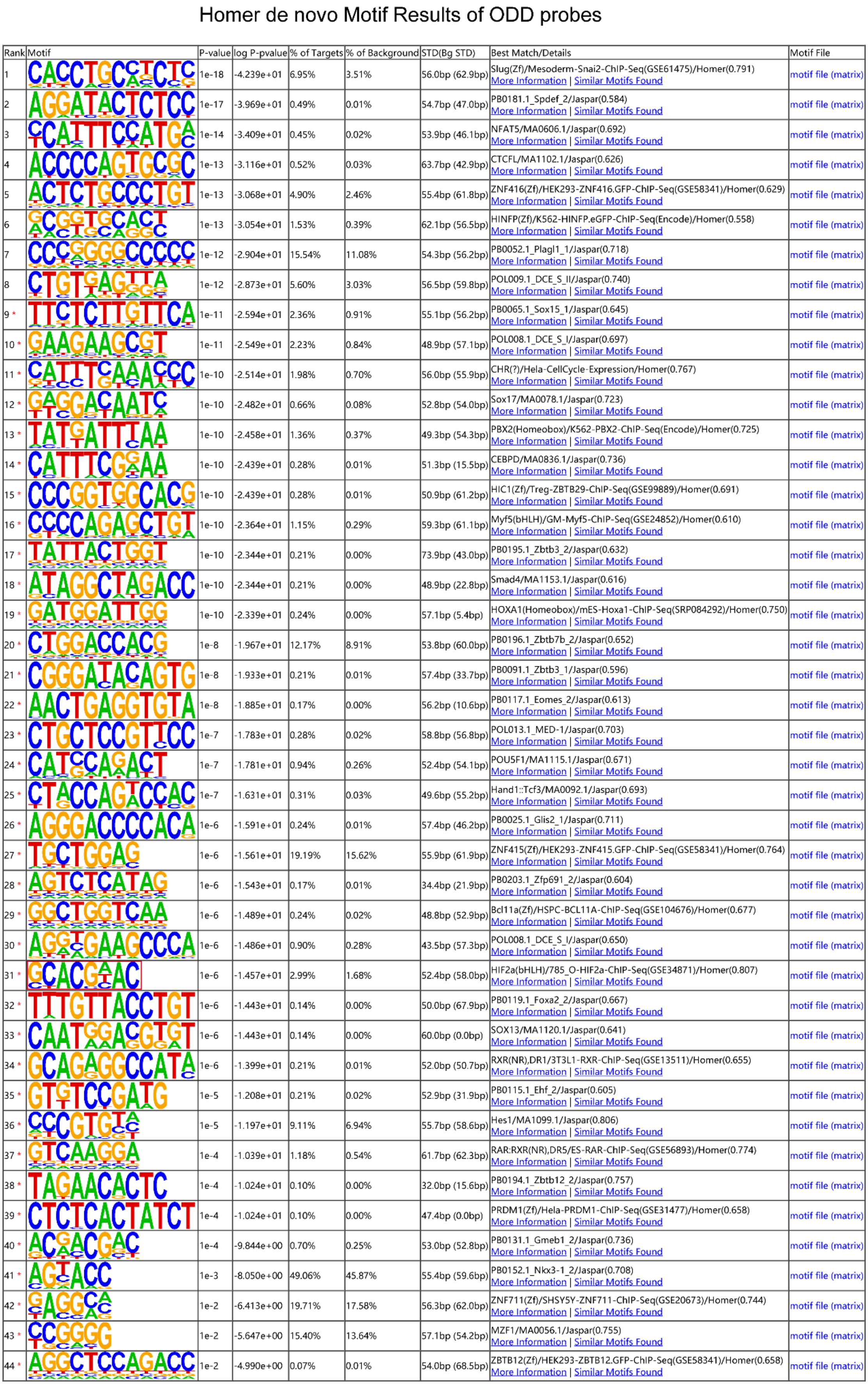
Supplementary Fig 3. SNHG11 facilitates the wound healing ability of CoCl$_2$-treated CRC cells. (A) Overexpression and knockdown efficiency of SNHG11 in CRC cells. n=3 independent experiments, two-tailed Student’s t-test. (B and C) Wound healing assays of CRC cells with indicated treatment. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.



Homer de novo Motif Results of EVEN probes

Rank	Motif	P-value	log P-pvalue	% of Targets	% of Background	STD(Bg STD)	Best Match/Details	Motif File
1	GTCCTCTCACCC	1e-106	-2.459e+02	4.34%	0.45%	48.5bp (53.4bp)	NR2C2/MA0504.1/Jaspar(0.712) More Information | Similar Motifs Found	motif file (matrix)
2	ACACGCGAAACC	1e-44	-1.020e+02	1.89%	0.21%	57.0bp (63.4bp)	IRF4(IRF)/GM12878-IRF4-ChIP-Seq(GSE32465)/Homer(0.687) More Information | Similar Motifs Found	motif file (matrix)
3	TCGCGTGTCCTC	1e-33	-7.753e+01	1.29%	0.13%	52.2bp (55.3bp)	PAX5/MA0014.3/Jaspar(0.715) More Information | Similar Motifs Found	motif file (matrix)
4	TGTCCTCCACCC	1e-28	-6.624e+01	1.61%	0.27%	46.3bp (54.7bp)	SMAD2::SMAD3::SMAD4/MA0513.1/Jaspar(0.670) More Information | Similar Motifs Found	motif file (matrix)
5	GGACAATGGT	1e-26	-6.000e+01	2.33%	0.61%	53.4bp (57.2bp)	SOX13/MA1120.1/Jaspar(0.819) More Information | Similar Motifs Found	motif file (matrix)
6	TGCACACAGTGG	1e-23	-5.410e+01	0.37%	0.01%	38.2bp (65.9bp)	Oct4:Sox17(POU,Homeobox,HMG)/F9-Sox17-ChIP-Seq(GSE44553)/Homer(0.710) More Information | Similar Motifs Found	motif file (matrix)
7	GGTGAGCGTGTC	1e-23	-5.410e+01	0.37%	0.01%	60.4bp (50.9bp)	Klf9(Zf)/GBM-Klf9-ChIP-Seq(GSE62211)/Homer(0.713) More Information | Similar Motifs Found	motif file (matrix)
8	AACTCTGGATCC	1e-18	-4.171e+01	0.67%	0.07%	45.0bp (48.7bp)	ZBTB12(Zf)/HEK293-ZBTB12.GFP-ChIP-Seq(GSE58341)/Homer(0.605) More Information | Similar Motifs Found	motif file (matrix)
9	GTTTGGCGGTCT	1e-16	-3.795e+01	0.37%	0.01%	52.0bp (57.3bp)	ZBTB7B/MA0694.1/Jaspar(0.646) More Information | Similar Motifs Found	motif file (matrix)
10	GGTGGTCTAT	1e-14	-3.453e+01	1.86%	0.63%	56.4bp (57.8bp)	PB0196.1_Zbtb7b_2/Jaspar(0.723) More Information | Similar Motifs Found	motif file (matrix)
11	GCGGACCCGACT	1e-13	-3.113e+01	0.47%	0.04%	52.1bp (57.5bp)	HINFP(Zf)/K562-HINFP.eGFP-ChIP-Seq(Encode)/Homer(0.731) More Information | Similar Motifs Found	motif file (matrix)
12	TGCGTG	1e-13	-3.080e+01	36.68%	31.15%	55.8bp (58.8bp)	Ahr::Arnt/MA0006.1/Jaspar(0.926) More Information | Similar Motifs Found	motif file (matrix)
13 *	TAACCTCTTC	1e-11	-2.728e+01	1.04%	0.28%	58.1bp (58.7bp)	GRHL2/MA1105.1/Jaspar(0.558) More Information | Similar Motifs Found	motif file (matrix)
14 *	ACAGTCGTGC	1e-11	-2.629e+01	0.32%	0.02%	43.5bp (51.9bp)	PB0136.1_IRC900814_2/Jaspar(0.638) More Information | Similar Motifs Found	motif file (matrix)
15 *	ACAGGGACTGCA	1e-10	-2.508e+01	0.55%	0.08%	54.0bp (54.5bp)	Ddit3::Cebpa/MA0019.1/Jaspar(0.636) More Information | Similar Motifs Found	motif file (matrix)
16 *	GTGAGTGGAG	1e-10	-2.444e+01	1.14%	0.36%	44.7bp (51.9bp)	Sp5(Zf)/mES-Sp5.Flag-ChIP-Seq(GSE72989)/Homer(0.720) More Information | Similar Motifs Found	motif file (matrix)
17 *	CCTCGTGGTT	1e-10	-2.369e+01	0.25%	0.01%	53.0bp (42.7bp)	MAX::MYC/MA0059.1/Jaspar(0.740) More Information | Similar Motifs Found	motif file (matrix)
18 *	ACCTCGTA	1e-9	-2.162e+01	3.23%	1.79%	55.9bp (61.5bp)	PH0048.1_Hoxa13/Jaspar(0.633) More Information | Similar Motifs Found	motif file (matrix)
19 *	CAGTGGGAACCT	1e-9	-2.147e+01	0.20%	0.01%	40.7bp (36.5bp)	RBPJ/MA1116.1/Jaspar(0.698) More Information | Similar Motifs Found	motif file (matrix)
20 *	ACGCTAAGAA	1e-9	-2.122e+01	0.15%	0.00%	69.4bp (0.0bp)	GATA6/MA1104.1/Jaspar(0.594) More Information | Similar Motifs Found	motif file (matrix)
21 *	CTTTAC	1e-8	-2.072e+01	35.10%	30.67%	56.4bp (57.4bp)	CDX4(Homeobox)/ZebrafishEmbryos-Cdx4.Myc-ChIP-Seq(GSE48254)/Homer(0.733) More Information | Similar Motifs Found	motif file (matrix)
22 *	GCGTCCTT	1e-8	-2.040e+01	2.61%	1.38%	56.4bp (59.0bp)	SF1(NR)/H295R-Nr5a1-ChIP-Seq(GSE44220)/Homer(0.666) More Information | Similar Motifs Found	motif file (matrix)
23 *	AAGCAGCA	1e-8	-2.000e+01	4.29%	2.67%	53.8bp (55.3bp)	MafA(bZIP)/Islet-MafA-ChIP-Seq(GSE30298)/Homer(0.702) More Information | Similar Motifs Found	motif file (matrix)
24 *	ACGTGTCTAG	1e-8	-1.921e+01	0.99%	0.34%	53.3bp (56.1bp)	Smad4/MA1153.1/Jaspar(0.747) More Information | Similar Motifs Found	motif file (matrix)
25 *	ATCCGA	1e-8	-1.854e+01	9.23%	6.87%	56.8bp (61.4bp)	PB0106.1_Arid5a_2/Jaspar(0.673) More Information | Similar Motifs Found	motif file (matrix)
26 *	TCAAACTGGC	1e-7	-1.748e+01	0.65%	0.18%	55.0bp (55.1bp)	YY1/MA0095.2/Jaspar(0.744) More Information | Similar Motifs Found	motif file (matrix)
27 *	CGAAGAGACG	1e-7	-1.714e+01	0.15%	0.01%	52.9bp (4.7bp)	POL008.1_DCE_S_I/Jaspar(0.608) More Information | Similar Motifs Found	motif file (matrix)
28 *	TGACCTCCCTCA	1e-7	-1.714e+01	0.15%	0.01%	54.5bp (70.6bp)	COUP-TFII(NR)/Artia-Nr2f2-ChIP-Seq(GSE46497)/Homer(0.711) More Information | Similar Motifs Found	motif file (matrix)
29 *	TACAATCG	1e-7	-1.637e+01	1.09%	0.44%	51.6bp (56.5bp)	Sox6(HMG)/Myotubes-Sox6-ChIP-Seq(GSE32627)/Homer(0.768) More Information | Similar Motifs Found	motif file (matrix)
30 *	GGCGGCTA	1e-6	-1.411e+01	0.69%	0.23%	58.8bp (57.4bp)	MYB(HTH)/ERMYB-Myb-ChIPSeq(GSE22095)/Homer(0.688) More Information | Similar Motifs Found	motif file (matrix)
31 *	CCCACGGGGTCT	1e-5	-1.361e+01	0.12%	0.01%	60.4bp (18.4bp)	MYCN/MA0104.4/Jaspar(0.676) More Information | Similar Motifs Found	motif file (matrix)
32 *	ATAATATATATT	1e-5	-1.361e+01	0.12%	0.00%	41.3bp (9.9bp)	PB0080.1_Tbp_1/Jaspar(0.701) More Information | Similar Motifs Found	motif file (matrix)
33 *	CTTTGCCA	1e-5	-1.306e+01	2.36%	1.41%	56.4bp (56.2bp)	NF1-halfsite(CTF)/LNCaP-NF1-ChIP-Seq(Unpublished)/Homer(0.777) More Information | Similar Motifs Found	motif file (matrix)
34 *	TTTTTTTT	1e-5	-1.300e+01	9.73%	7.72%	59.3bp (60.2bp)	ZNF384/MA1125.1/Jaspar(0.951) More Information | Similar Motifs Found	motif file (matrix)
35 *	CACTCATTCCTC	1e-5	-1.296e+01	0.10%	0.00%	57.1bp (53.4bp)	ZNF24/MA1124.1/Jaspar(0.681) More Information | Similar Motifs Found	motif file (matrix)
36 *	TCCGACAA	1e-5	-1.283e+01	0.60%	0.20%	46.6bp (59.8bp)	PB0032.1_IRC900814_1/Jaspar(0.736) More Information | Similar Motifs Found	motif file (matrix)
37 *	GCCAGGGGAGCG	1e-5	-1.165e+01	0.12%	0.01%	47.2bp (34.7bp)	POL013.1_MED-1/Jaspar(0.660) More Information | Similar Motifs Found	motif file (matrix)
38 *	ACATGG	1e-4	-1.008e+01	34.43%	31.52%	57.3bp (57.0bp)	NPAS2(bHLH)/Liver-NPAS2-ChIP-Seq(GSE39860)/Homer(0.727) More Information | Similar Motifs Found	motif file (matrix)
39 *	CGGGTGGAGACA	1e-4	-9.857e+00	0.25%	0.05%	50.5bp (47.9bp)	TP73/MA0861.1/Jaspar(0.640) More Information | Similar Motifs Found	motif file (matrix)
40 *	GGTAATATTAAG	1e-3	-7.126e+00	0.07%	0.01%	53.1bp (40.7bp)	Arid5a/MA0602.1/Jaspar(0.692) More Information | Similar Motifs Found	motif file (matrix)

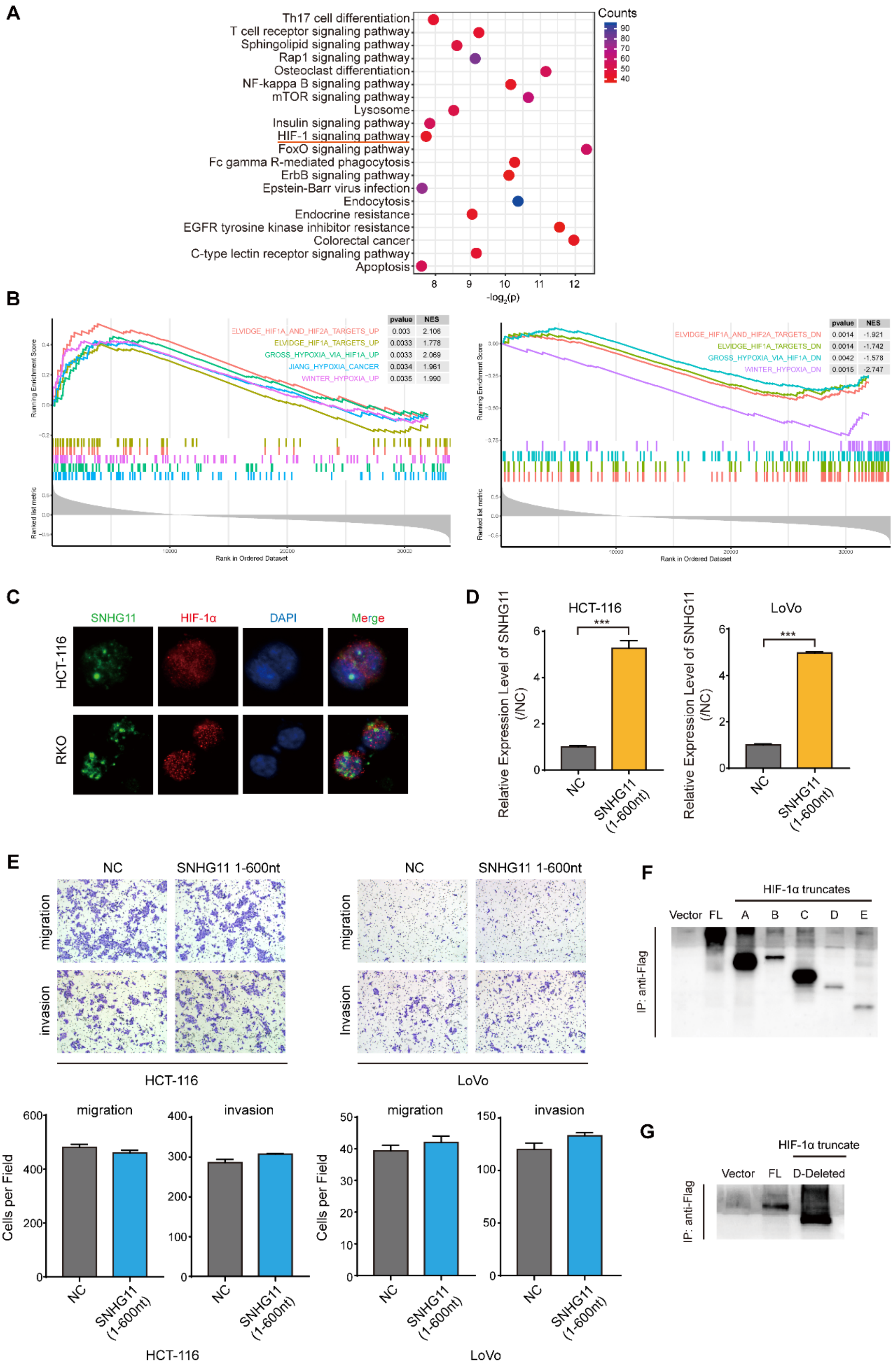
Supplementary Fig 4. Motif analysis of ChIRP-seq of SNHG11 by EVEN probes. ChIRP-seq experiment were conducted in hypoxia-treated HCT-116 cells. HOMER findmotifsgenome program was used to find motifs.



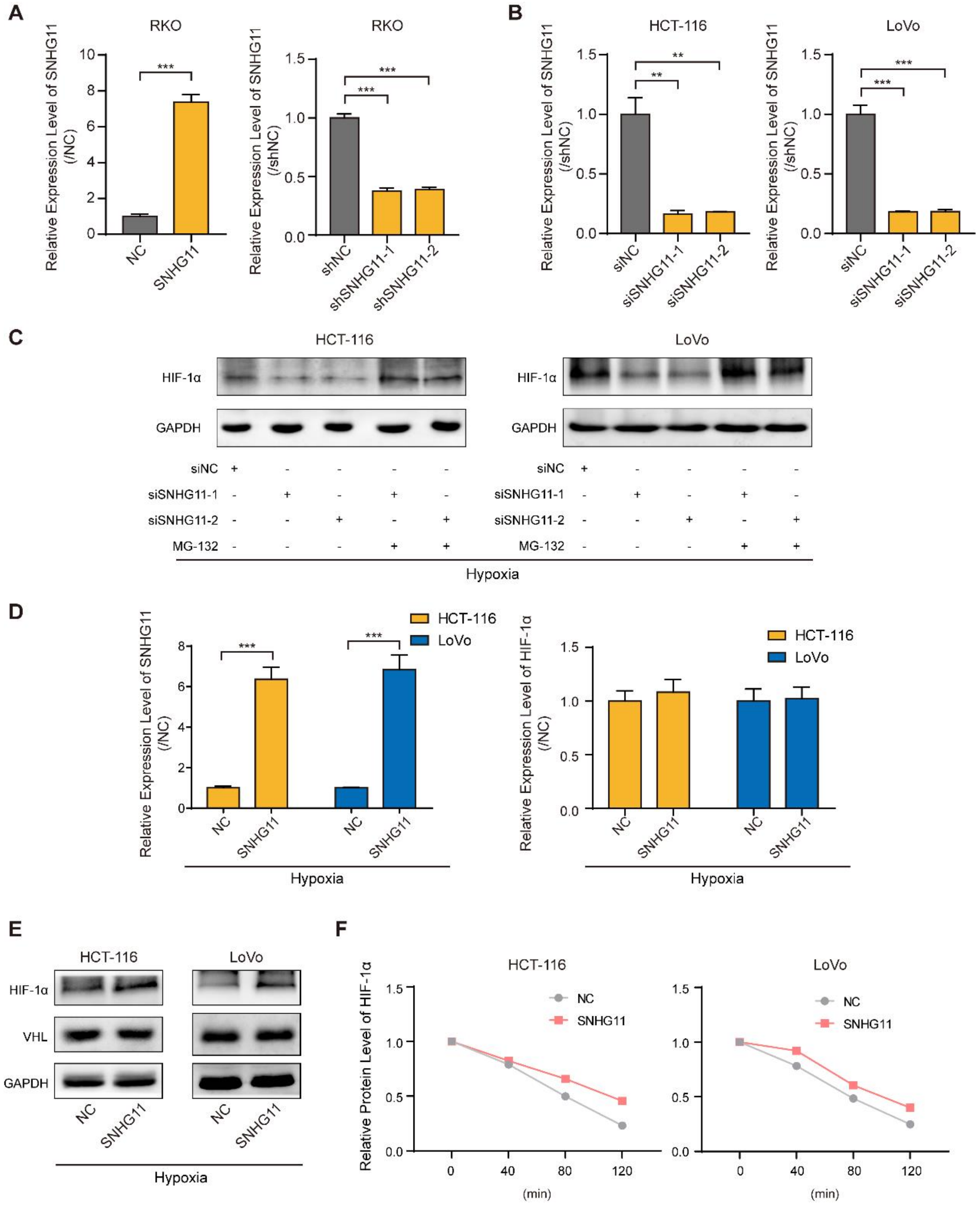
Homer de novo Motif Results of ODD probes

Rank	Motif	P-value	log P-pvalue	% of Targets	% of Background	STD(Bg STD)	Best Match/Details	Motif File
1	CACCTGCCTCTC	1e-18	-4.239e+01	6.95%	3.51%	56.0bp (62.9bp)	Slug(Zf)/Mesoderm-Snai2-ChIP-Seq(GSE61475)/Homer(0.791) More Information | Similar Motifs Found	motif file (matrix)
2	AGGATACTCTCC	1e-17	-3.969e+01	0.49%	0.01%	54.7bp (47.0bp)	PB0181.1_Spdef_2/Jaspar(0.584) More Information | Similar Motifs Found	motif file (matrix)
3	CCATTTCCATGA	1e-14	-3.409e+01	0.45%	0.02%	53.9bp (46.1bp)	NFAT5/MA0606.1/Jaspar(0.692) More Information | Similar Motifs Found	motif file (matrix)
4	ACCCCAGTGCGC	1e-13	-3.116e+01	0.52%	0.03%	63.7bp (42.9bp)	CTCFL/MA1102.1/Jaspar(0.626) More Information | Similar Motifs Found	motif file (matrix)
5	ACTCTGCCCTGT	1e-13	-3.068e+01	4.90%	2.46%	55.4bp (61.8bp)	ZNF416(Zf)/HEK293-ZNF416.GFP-ChIP-Seq(GSE58341)/Homer(0.629) More Information | Similar Motifs Found	motif file (matrix)
6	GCGGTGCACT	1e-13	-3.054e+01	1.53%	0.39%	62.1bp (56.5bp)	HINFP(Zf)/K562-HINFP.eGFP-ChIP-Seq(Encode)/Homer(0.558) More Information | Similar Motifs Found	motif file (matrix)
7	CCCGGGGCCCCC	1e-12	-2.904e+01	15.54%	11.08%	54.3bp (56.2bp)	PB0052.1_Plagl1_1/Jaspar(0.718) More Information | Similar Motifs Found	motif file (matrix)
8	CTGTGAGTTA	1e-12	-2.873e+01	5.60%	3.03%	56.5bp (59.8bp)	POL009.1_DCE_S_II/Jaspar(0.740) More Information | Similar Motifs Found	motif file (matrix)
9 *	TTCTCTTGTTCA	1e-11	-2.594e+01	2.36%	0.91%	55.1bp (56.2bp)	PB0065.1_Sox15_1/Jaspar(0.645) More Information | Similar Motifs Found	motif file (matrix)
10 *	GAAGAAGCGT	1e-11	-2.549e+01	2.23%	0.84%	48.9bp (57.1bp)	POL008.1_DCE_S_I/Jaspar(0.697) More Information | Similar Motifs Found	motif file (matrix)
11 *	CATTTCAAACCC	1e-10	-2.514e+01	1.98%	0.70%	56.0bp (55.9bp)	CHR(?)/Hela-CellCycle-Expression/Homer(0.767) More Information | Similar Motifs Found	motif file (matrix)
12 *	GAGGACAATC	1e-10	-2.482e+01	0.66%	0.08%	52.8bp (54.0bp)	Sox17/MA0078.1/Jaspar(0.723) More Information | Similar Motifs Found	motif file (matrix)
13 *	TATGATTTAA	1e-10	-2.458e+01	1.36%	0.37%	49.3bp (54.3bp)	PBX2(Homeobox)/K562-PBX2-ChIP-Seq(Encode)/Homer(0.725) More Information | Similar Motifs Found	motif file (matrix)
14 *	CATTTCGGAA	1e-10	-2.439e+01	0.28%	0.01%	51.3bp (15.5bp)	CEBPD/MA0836.1/Jaspar(0.736) More Information | Similar Motifs Found	motif file (matrix)
15 *	CCCGGTGGCACG	1e-10	-2.439e+01	0.28%	0.01%	50.9bp (61.2bp)	HIC1(Zf)/Treg-ZBTB29-ChIP-Seq(GSE99889)/Homer(0.691) More Information | Similar Motifs Found	motif file (matrix)
16 *	CCCCAGAGCTGT	1e-10	-2.364e+01	1.15%	0.29%	59.3bp (61.1bp)	Myf5(bHLH)/GM-Myf5-ChIP-Seq(GSE24852)/Homer(0.610) More Information | Similar Motifs Found	motif file (matrix)
17 *	TATTACTGGT	1e-10	-2.344e+01	0.21%	0.00%	73.9bp (43.0bp)	PB0195.1_Zbtb3_2/Jaspar(0.632) More Information | Similar Motifs Found	motif file (matrix)
18 *	ATAGGCTAGACC	1e-10	-2.344e+01	0.21%	0.00%	48.9bp (22.8bp)	Smad4/MA1153.1/Jaspar(0.616) More Information | Similar Motifs Found	motif file (matrix)
19 *	GATGGATTGG	1e-10	-2.339e+01	0.24%	0.00%	57.1bp (5.4bp)	HOXA1(Homeobox)/mES-Hoxa1-ChIP-Seq(SRP084292)/Homer(0.750) More Information | Similar Motifs Found	motif file (matrix)
20 *	CTGGACCACG	1e-8	-1.967e+01	12.17%	8.91%	53.8bp (60.0bp)	PB0196.1_Zbtb7b_2/Jaspar(0.652) More Information | Similar Motifs Found	motif file (matrix)
21 *	CGGGATACAGTG	1e-8	-1.933e+01	0.21%	0.01%	57.4bp (33.7bp)	PB0091.1_Zbtb3_1/Jaspar(0.596) More Information | Similar Motifs Found	motif file (matrix)
22 *	AACTGAGGTGTA	1e-8	-1.885e+01	0.17%	0.00%	56.2bp (10.6bp)	PB0117.1_Eomes_2/Jaspar(0.613) More Information | Similar Motifs Found	motif file (matrix)
23 *	CTGCTCCGTTCC	1e-7	-1.783e+01	0.28%	0.02%	58.8bp (56.8bp)	POL013.1_MED-1/Jaspar(0.703) More Information | Similar Motifs Found	motif file (matrix)
24 *	CATCCAGACT	1e-7	-1.781e+01	0.94%	0.26%	52.4bp (54.1bp)	POU5F1/MA1115.1/Jaspar(0.671) More Information | Similar Motifs Found	motif file (matrix)
25 *	CTACCAGTCCAC	1e-7	-1.631e+01	0.31%	0.03%	49.6bp (55.2bp)	Hand1::Tcf3/MA0092.1/Jaspar(0.693) More Information | Similar Motifs Found	motif file (matrix)
26 *	AGGGACCCCACA	1e-6	-1.591e+01	0.24%	0.01%	57.4bp (46.2bp)	PB0025.1_Glis2_1/Jaspar(0.711) More Information | Similar Motifs Found	motif file (matrix)
27 *	TGCTGGAG	1e-6	-1.561e+01	19.19%	15.62%	55.9bp (61.9bp)	ZNF415(Zf)/HEK293-ZNF415.GFP-ChIP-Seq(GSE58341)/Homer(0.764) More Information | Similar Motifs Found	motif file (matrix)
28 *	AGTCTCATAG	1e-6	-1.543e+01	0.17%	0.01%	34.4bp (21.9bp)	PB0203.1_Zfp691_2/Jaspar(0.604) More Information | Similar Motifs Found	motif file (matrix)
29 *	GGCTGGTCAA	1e-6	-1.489e+01	0.24%	0.02%	48.8bp (52.9bp)	Bcl11a(Zf)/HSPC-BCL11A-ChIP-Seq(GSE104676)/Homer(0.677) More Information | Similar Motifs Found	motif file (matrix)
30 *	AGGTGAAGCCCA	1e-6	-1.486e+01	0.90%	0.28%	43.5bp (57.3bp)	POL008.1_DCE_S_I/Jaspar(0.650) More Information | Similar Motifs Found	motif file (matrix)
31 *	GCACGAAC	1e-6	-1.457e+01	2.99%	1.68%	52.4bp (58.0bp)	HIF2a(bHLH)/785_O-HIF2a-ChIP-Seq(GSE34871)/Homer(0.807) More Information | Similar Motifs Found	motif file (matrix)
32 *	TTTGTTACCTGT	1e-6	-1.443e+01	0.14%	0.00%	50.0bp (67.9bp)	PB0119.1_Foxa2_2/Jaspar(0.667) More Information | Similar Motifs Found	motif file (matrix)
33 *	CAATGGACGTGT	1e-6	-1.443e+01	0.14%	0.00%	60.0bp (0.0bp)	SOX13/MA1120.1/Jaspar(0.641) More Information | Similar Motifs Found	motif file (matrix)
34 *	GCAGAGGCCATA	1e-6	-1.399e+01	0.21%	0.01%	52.0bp (50.7bp)	RXR(NR),DR1/3T3L1-RXR-ChIP-Seq(GSE13511)/Homer(0.655) More Information | Similar Motifs Found	motif file (matrix)
35 *	GTGTCCGATG	1e-5	-1.208e+01	0.21%	0.02%	52.9bp (31.9bp)	PB0115.1_Ehf_2/Jaspar(0.605) More Information | Similar Motifs Found	motif file (matrix)
36 *	CCCGTGTA	1e-5	-1.197e+01	9.11%	6.94%	55.7bp (58.6bp)	Hes1/MA1099.1/Jaspar(0.806) More Information | Similar Motifs Found	motif file (matrix)
37 *	GTCAAGGA	1e-4	-1.039e+01	1.18%	0.54%	61.7bp (62.3bp)	RAR:RXR(NR),DR5/ES-RAR-ChIP-Seq(GSE56893)/Homer(0.774) More Information | Similar Motifs Found	motif file (matrix)
38 *	TAGAACACTC	1e-4	-1.024e+01	0.10%	0.00%	32.0bp (15.6bp)	PB0194.1_Zbtb12_2/Jaspar(0.757) More Information | Similar Motifs Found	motif file (matrix)
39 *	CTCTCACTATCT	1e-4	-1.024e+01	0.10%	0.00%	47.4bp (0.0bp)	PRDM1(Zf)/Hela-PRDM1-ChIP-Seq(GSE31477)/Homer(0.658) More Information | Similar Motifs Found	motif file (matrix)
40 *	ACGACGAC	1e-4	-9.844e+00	0.70%	0.25%	53.0bp (52.8bp)	PB0131.1_Gmeb1_2/Jaspar(0.736) More Information | Similar Motifs Found	motif file (matrix)
41 *	AGTACC	1e-3	-8.050e+00	49.06%	45.87%	55.4bp (59.6bp)	PB0152.1_Nkx3-1_2/Jaspar(0.708) More Information | Similar Motifs Found	motif file (matrix)
42 *	GAGGCA	1e-2	-6.413e+00	19.71%	17.58%	56.3bp (62.0bp)	ZNF711(Zf)/SHSY5Y-ZNF711-ChIP-Seq(GSE20673)/Homer(0.744) More Information | Similar Motifs Found	motif file (matrix)
43 *	CCGGGG	1e-2	-5.647e+00	15.40%	13.64%	57.1bp (54.2bp)	MZF1/MA0056.1/Jaspar(0.755) More Information | Similar Motifs Found	motif file (matrix)
44 *	AGGCTCCAGACC	1e-2	-4.990e+00	0.07%	0.01%	54.0bp (68.5bp)	ZBTB12(Zf)/HEK293-ZBTB12.GFP-ChIP-Seq(GSE58341)/Homer(0.658) More Information | Similar Motifs Found	motif file (matrix)

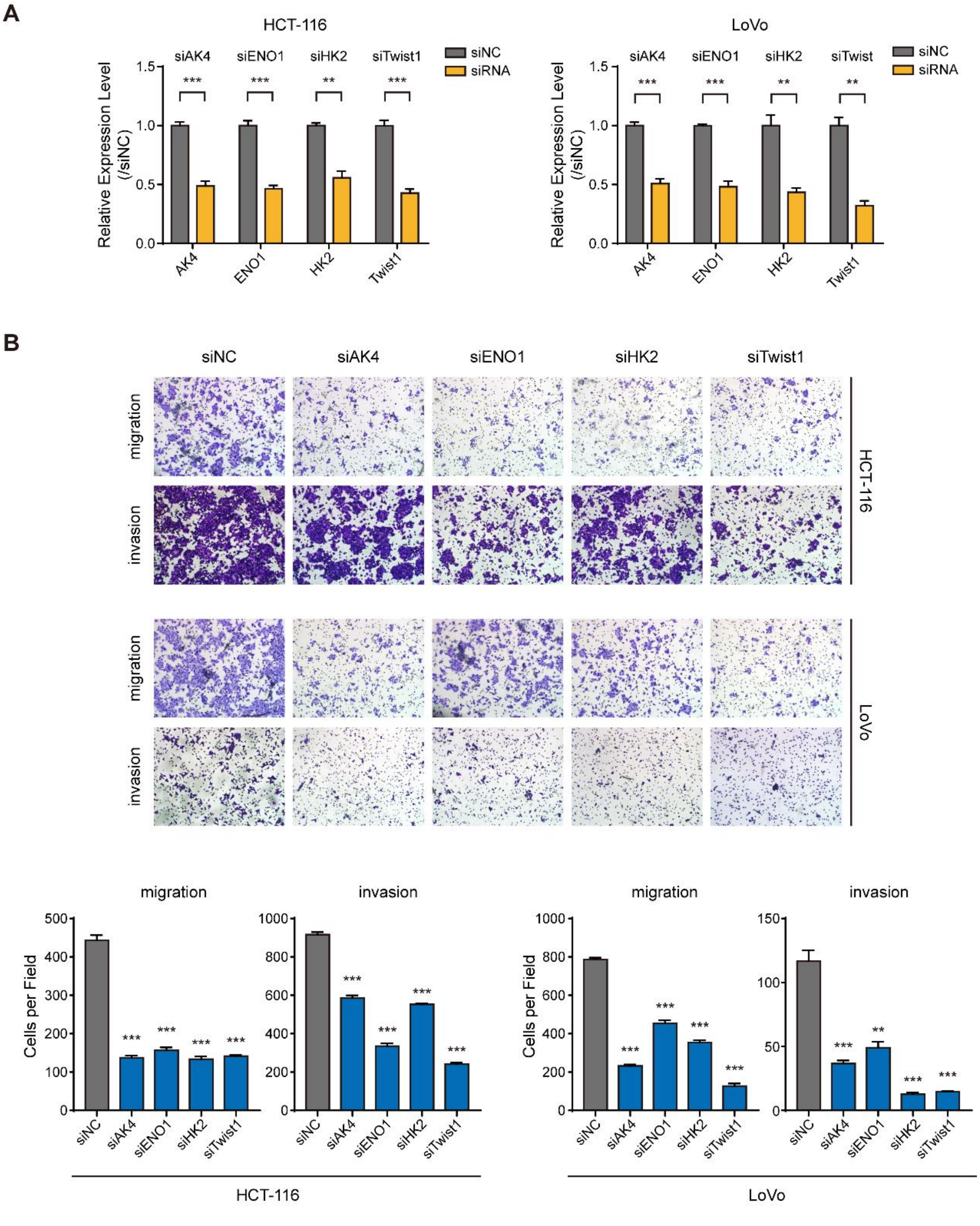
Supplementary Fig 5. Motif analysis of ChIRP-seq of SNHG11 by ODD probes. ChIRP-seq experiment were conducted in hypoxia-treated HCT-116 cells. HOMER findmotifsgenome program was used to find motifs.


A
Th17 cell differentiation
T cell receptor signaling pathway
Sphingolipid signaling pathway
Rap1 signaling pathway
Osteoclast differentiation
NF-kappa B signaling pathway
mTOR signaling pathway
Lysosome
Insulin signaling pathway
HIF-1 signaling pathway
FoxO signaling pathway
Fc gamma R-mediated phagocytosis
ErbB signaling pathway
Epstein-Barr virus infection
Endocytosis
Endocrine resistance
EGFR tyrosine kinase inhibitor resistance
Colorectal cancer
C-type lectin receptor signaling pathway
Apoptosis
8 9 10 11 12
-log2(p)
Counts
90 80 70 60 50 40
B
pvalue NES
ELVIDGE_HIF1A_AND_HIF2A_TARGETS_UP 0.003 2.106
ELVIDGE_HIF1A_TARGETS_UP 0.0033 1.778
GROSS_HYPOXIA_VIA_HIF1A_UP 0.0033 2.069
JIANG_HYPOXIA_CANCER 0.0034 1.961
WINTER_HYPOXIA_UP 0.0035 1.990
Running Enrichment Score
Ranked list metric
Rank in Ordered Dataset
10000 20000 30000
pvalue NES
ELVIDGE_HIF1A_AND_HIF2A_TARGETS_DN 0.0014 -1.921
ELVIDGE_HIF1A_TARGETS_DN 0.0014 -1.742
GROSS_HYPOXIA_VIA_HIF1A_DN 0.0042 -1.578
WINTER_HYPOXIA_DN 0.0015 -2.747
0.00 -0.25 -0.50 -0.75
C
SNHG11
HIF-1α
DAPI
Merge
HCT-116
RKO
D
HCT-116
LoVo
Relative Expression Level of SNHG11 (/NC)
***
NC
SNHG11 (1-600nt)
E
NC
SNHG11 1-600nt
migration
invasion
HCT-116
LoVo
Cells per Field
F
HIF-1α truncates
Vector FL A B C D E
IP: anti-Flag
G
HIF-1α truncate
Vector FL D-Deleted
IP: anti-Flag

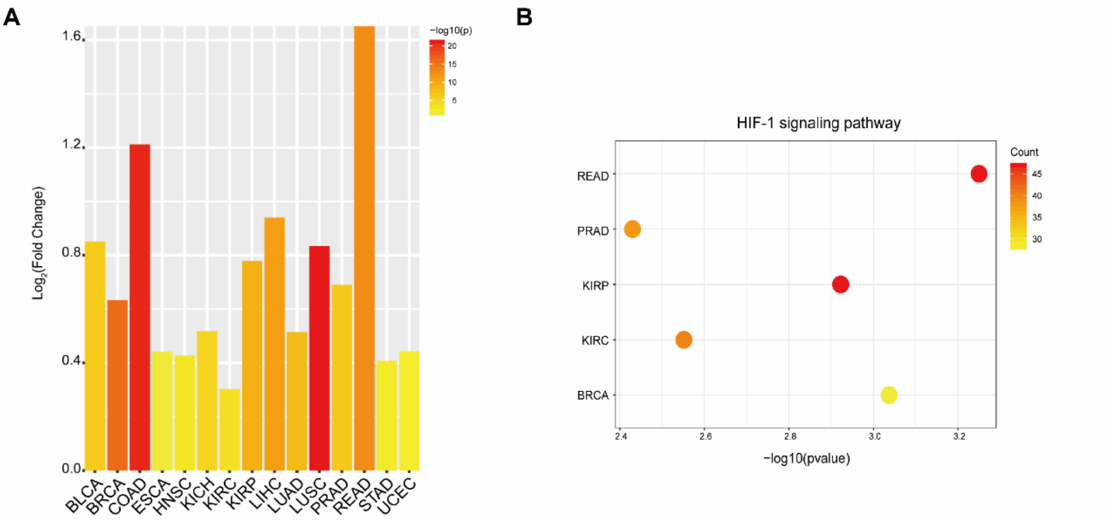
Supplementary Fig 6. SNHG11 interacts with HIF-1α in CRC cells. (A) SNHG11 correlated genes (R>0.3 or R<-0.3) in COAD enrich HIF-1 pathway. (B) GSEA analysis by correlations of genes with SNHG11 in TCGA-COAD. (C) Co-localization of SNHG11 and HIF-1α in CRC cells treated with hypoxia. (D) Overexpression efficiency of fragment-C-deleted SNHG11 in CRC cells. n=3 independent experiments, two-tailed Student's t-test. (E) Cell migration and invasion assays of CRC cells overexpressing fragment-C-deleted SNHG11 under hypoxia. (F and G) Deletion mapping to identify the HIF-1α binding domain. Western blotting to detect the immunoprecipitation efficiency of Flag-tagged full length or truncated HIF-1α in RIP assay. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.



Supplementary Fig 7. SNHG11 stabilizes HIF-1α in CRC cells. (A) Overexpression and knockdown efficiency of SNHG11 in RKO cells. n=3 independent experiments, two-tailed Student’s t-test. (B) Knockdown efficiency of SNHG11 in CRC cells transfected with siSNHG11. n=3 independent experiments, two-tailed Student’s t-test. (C) Expression of HIF-1α in CRC cells under hypoxia with indicated treatments. (D) Expression of SNHG11 and HIF-1α was analysed by qPCR in control and SNHG11 overexpressing CRC cells treated with hypoxia. (E) The HIF-1α and VHL expression level was analysed by western blotting in CRC cells overexpressing SNHG11 and exposed to hypoxia. (F) Relative expression level of HIF-1α related to Figure 4F was measured by integrated density value using ImageJ and normalized by the time point 0 min. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.



Supplementary Fig 8. Downstream targets of SNHG11/HIF-1α are involved in migration and invasion of CRC cells under hypoxia. (A) knockdown efficiency of SNHG11 in CRC cells treated with hypoxia. n=3 independent experiments, two-tailed Student's t-test. (B) Cell migration and invasion assays of hypoxia-treated HCT-116 and LoVo cells transfected with siNC or indicated siRNAs. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.



Supplementary Fig 9. SNHG11/HIF-1α may exert function in other cancers. (A) SNHG11 upregulates in several cancers based on TCGA data. (B) SNHG11 co-related genes (R>0.3 or R<-0.3) enrich HIF-1 signaling pathway in READ, PRAD, KIRP, KIRC and BRCA.

Supplementary Tables

Supplementary Table 1. Association between SNHG11 expression and clinicopathological variables of the studied CRC patients

Clinical Variables	Low SNHG11		High SNHG11		Total No.	Unknown No.	P
	Case No.	(%)	Case No.	(%)			
Age					78	0	0.233
<60	23	46.0	9	32.1			
≥60	27	54.0	19	67.9			
Gender							
Male	37	74.0	18	64.3	78	0	0.367
Female	13	26.0	10	35.7			
Tumor Volume							
≤5 cm	32	64.0	20	71.4	78	0	0.504
>5 cm	18	36.0	8	28.6			
Cancer Embolus							
Absent	36	72.0	15	53.6	78	0	0.101
Present	14	28.0	13	46.4			
Histologic Grade							
G1/G2	17	37.0	10	38.5	72	6	0.899
G3	29	63.0	16	61.5			
Metastasis							
Negative	30	60.0	7	40.0	78	0	**0.003**
Positive	20	40.0	21	75.0			
Relapse Metastasis							
Negative	35	70.0	9	32.1	78	0	**0.001**
Positive	15	30.0	19	67.9			

The bold *P* values are considered statistically significant

Supplementary Table 2. Sequences of primers for RNA pull down experiments

Identifier	Sequences (5'-3')
SHNG11-T7-F	GATCACTAATACGACTCACTATAGGGAGAGAGCGTCTTGTGGCTG
SHNG11-R	GTTTCAAGATTTATTGAGTGGG
SNHG11-F	GAGAGAGCGTCTTGTGGCTG
SNHG11-T7-R	GATCACTAATACGACTCACTATAGGGTTTCAAGATTTATTGAGTGGG
SHNG11-FL-F	GATCACTAATACGACTCACTATAGGGAGAGAGCGTCTTGTGGCTG
SNHG11-301-R	AGGCCCGCCAGTCTCTTTCC
SNHG11-301-F	GATCACTAATACGACTCACTATAGGGGAAAGAGACTGGCGGGCCT
SNHG11-601-R	GGTCATTGTCCTGAGAGGCT
SNHG11-601-F	GATCACTAATACGACTCACTATAGGAGCCTCTCAGGACAATGACC
SHNG11-FL-R	GTTTCAAGATTTATTGAGTGGG

Supplementary Table 3. Sequences of ChIRP probes

Identifier	Sequences (5'-3')
ODD-1	TCATTGACTCATCCGGAGCG
EVEN-1	GAGGACACGCGAAACCCGAG
ODD-2	AACCAGCAAAGCCATCCAAG
EVEN-2	TCTGCACACAGTGGACTATC
ODD-3	TTTCCTCTAACAGTTTCCAG
EVEN-3	CCCCAAACAATCATGAGGAG
ODD-4	TTGTGCTGAGACAATAGGGG
EVEN-4	CTTCAACTCTGGATCCCAAG
ODD-5	CCTGGCCTAGATGGAAGAAA
EVEN-5	AGTGGGTGAGAGGACAATGG

Supplementary Table 5. Sequences of qPCR primers to detect RNA expression

Identifier	Forward Primer	Reverse Primer
β-actin	TTGTTACAGGAAGTCCCTTGCC	ATGCTATCACCTCCCCTGTGTG
SNHG11	TGGAAACTGTTAGAGGAAAC	CTCTTGGTCTGTGATCAACC
HIF-1α	CTCGGCGAAGTAAAGAATCT	TGCTTTCATGTCATCTTCAA
U2	ATCGCTTCTCGGCCTTTTGG	AGGTCGATGCGTGGAGTGGA
AK4	TCGCCCAGAACTTTGGTCTC	TCTCACCAACTTCGGTGCTG
ENO1	GGGGAAGGGTGTCTCAAAGG	AGTTTCTTGCTAACCAGGGCA
HK2	CATCCAGAGGAGAGGGGACT	TCATCGCCTTCCACCATGTC
TWIST1	TCTCGGTCTGGAGGATGGAG	AATGACATCTAGGTCTCCGGC

Supplementary Table 6. Sequences of primers in RACE

Identifier	Sequences (5'-3')
SNHG11_GSP2ex	GCAGCACCCTGATCTGCACTCCCTGAG
SNHG11_GSP1ex	GGAGCTGGGACCCCAGGTCATTGTC
SNHG11_GSP2	ACTCCCTGAGGGGCTCCCACTGTCC
SNHG11_GSP1	GCCTGCACCTCTTGGGGTTTCCTCT

Supplementary Table 7. Sequences of siRNAs used in this study

Identifier	Sequences (5'-3')
siHIF-1α-1	GAAGGAACCUGAUGCUUUA
siHIF-1α-2	CUGAUGACCAGCAACUUGA
siHIF-1α-3	CAAUCAAGAAGUUGCAUUA
siSNHG11-1	GGAGTGGTCTTCCCAAGAA
siSNHG11-2	CCTCTCACCCACTCAATAA
siAK4	GGACTTCAATCCACCTCAT
siENO1	GGGTGTCTCAAAGGCTGTT
siHK2	GGAGATGGAGAAAGGGCTT
siTwist1	GCAAGATTCAGACCCTCAA

Supplementary Table 8. Sequences of primers used in this study

Identifier	Sequences (5'-3')
SNHG11_F	AAGCAGTGGTATCAACGCAG
SNHG11_R	GTTTCAAGATTTATTGAGTGGG
SNHG11-MS2-F	CACCGCGGGATCCGCTCTGAGAGAGCGTCTTGTGGCTG
SNHG11-MS2-R	CCGGTACCCCACTAGTCTCTGTTTCAAGATTTATTGAGTGGG
HIF-1α-FL-F	TGACGATGACAAGGAATTCATGGAGGGCGCCGGCGGC
HIF-1α-FL-R	GCTCGAGCCCGGGGGATCCTCAGTTAACTTGATCCAAAGC
HIF-1α-A-F	TGACGATGACAAGGAATTCATGGAGGGCGCCGGCGGC
HIF-1α-A-R	GCTCGAGCCCGGGGGATCCTCACAAGTCGTGCTGAATAATAC
HIF-1α-B-F	TGACGATGACAAGGAATTCGTCACCACAGGACAGTACAG
HIF-1α-B-R	GCTCGAGCCCGGGGGATCCTCAGGTGGCAGTGGTAGTGGTGG
HIF-1α-C-F	TGACGATGACAAGGAATTCGAGATGTTAGCTCCCTATATCC
HIF-1α-C-R	GCTCGAGCCCGGGGGATCCTCAGTTAACTTGATCCAAAGCTCTG
HIF-1α-D-F	TGACGATGACAAGGAATTCGTCACCACAGGACAGTACAG
HIF-1α-D-R	GCTCGAGCCCGGGGGATCCTCAACTAGGTGTCTGATCCTGAA
HIF-1α-E-F	TGACGATGACAAGGAATTCAGTCCTTCCGATGGAAGCAC
HIF-1α-E-R	GCTCGAGCCCGGGGGATCCTCATGTAACTGTGCTTTGAGGAC
HIF-1α-DMut-F1	TGACGATGACAAGGAATTCATGGAGGGCGCCGGCGGCGC
HIF-1α-DMut-R1	GTAATTTTTCATTCACACATACAATGC
HIF-1α-DMut-F2	ATGTGTGAATGAAAAATTACAGAATAT
HIF-1α-DMut-R2	GCTCGAGCCCGGGGGATCCTCAGTTAACTTGATCCAAAGC
shSNHG11-1-F	CACCGAGGAGTGGTCTTCCCAAGAATCAAGTTCTTGGGAAGACCACTCCTCTTTTTT
shSNHG11-1-R	AAACAAAAAAGAGGAGTGGTCTTCCCAAGAACTTGATTCTTGGGAAGACCACTCCTC
shSNHG11-2-F	CACCGCAGATGACAGCTCTCCCTTTCAAGAAGGGAGAGCTGTCATCTGCTTTTTT
shSNHG11-2-R	AAACAAAAAAGCAGATGACAGCTCTCCCTTCTTGAAAGGGAGAGCTGTCATCTGC

Supplementary Table 9. Antibodies used in this study

Identifier	Catalog Number	Company
GAPDH	60004-1-Ig	Proteintech
alpha Tubulin	66031-1-Ig	Proteintech
VHL	16538-1-AP	Proteintech
HIF1A	14179	Cell Signaling
FLAG M2	F1804	Sigma
β-actin	60008-1-Ig	Proteintech
PHD1	12984-1-AP	Proteintech
PHD2	20368-1-AP	Proteintech
PHD3	18325-1-AP	Proteintech
LAMIN A/C	10298-1-AP	Proteintech
UBIQUITIN	10201-2-AP	Proteintech
Goat anti-Mouse IgG (H+L), HRP conjugate	SA00001-1	Proteintech
Goat anti-Rabbit IgG (H+L), HRP conjugate	SA00001-2	Proteintech
VeriBlot for IP secondary antibody	ab131366	Abcam