Supplementary Materials
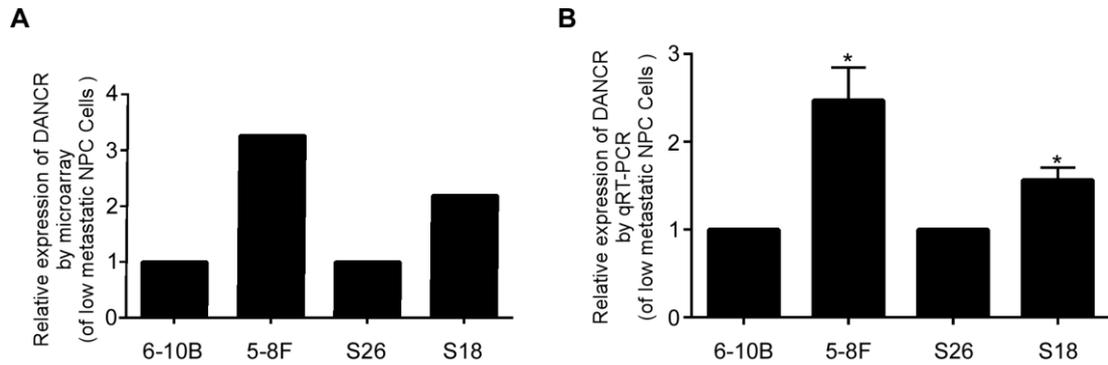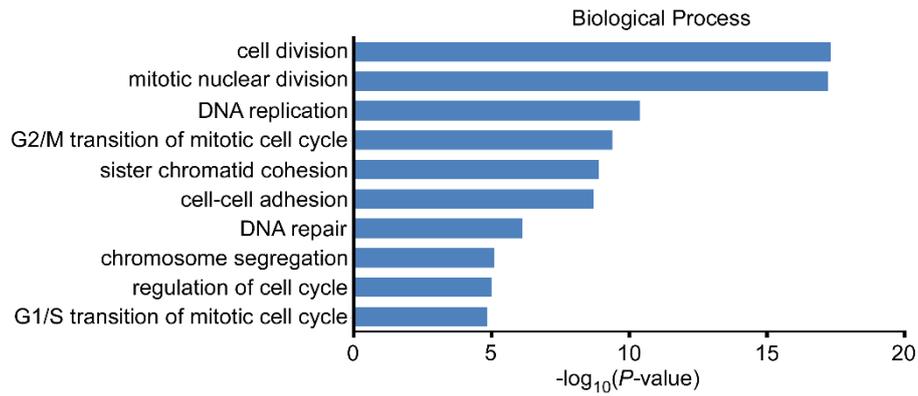

Figure S1. DANCR is upregulated in NPC cell lines with high metastatic potential.
(A) Relative expression of DANCR in NPC cell lines with high metastatic potential (5-8F and S18) and those with low metastatic potential (6-10B and S26) according to the microarray data (GSE89804). **(B)** Relative expression of DANCR in NPC cell lines with high or low metastatic potential by quantitative RT-PCR for validation. Data are presented as means ± SD. Student's *t*-tests, *$P<0.05$.

A

Biological Process

B

Cellular Component

C
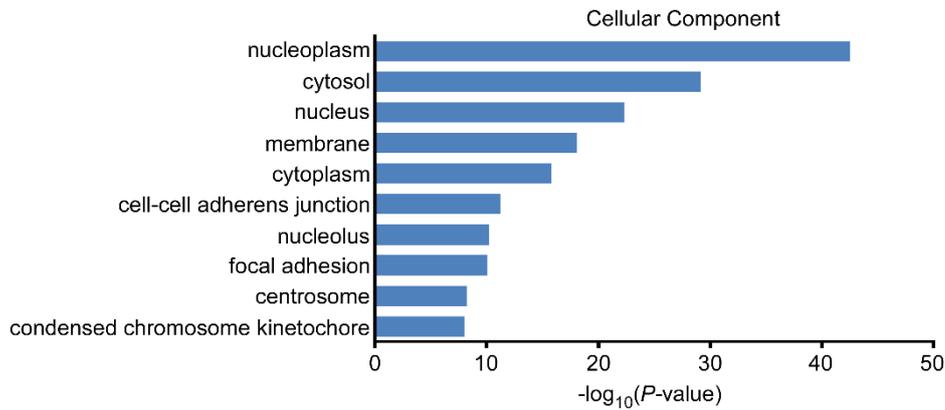
Molecular Function
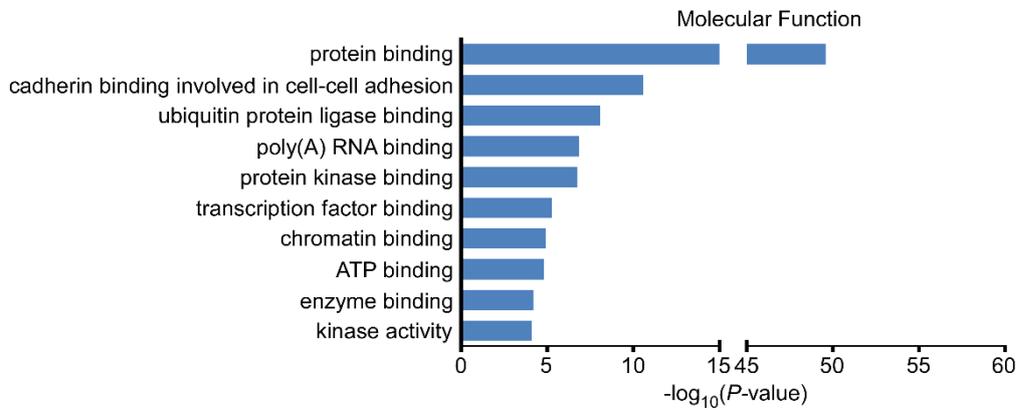
**Figure S2. Gene ontology (GO) analysis of dysregulated genes after silencing DANCR.**
(**A–C**) The top ten enrichment score values ($-\log 10$ (P-value)) for significantly enriched GO terms including Biological Process (A), Cellular Component (B) and Molecular Function (C).
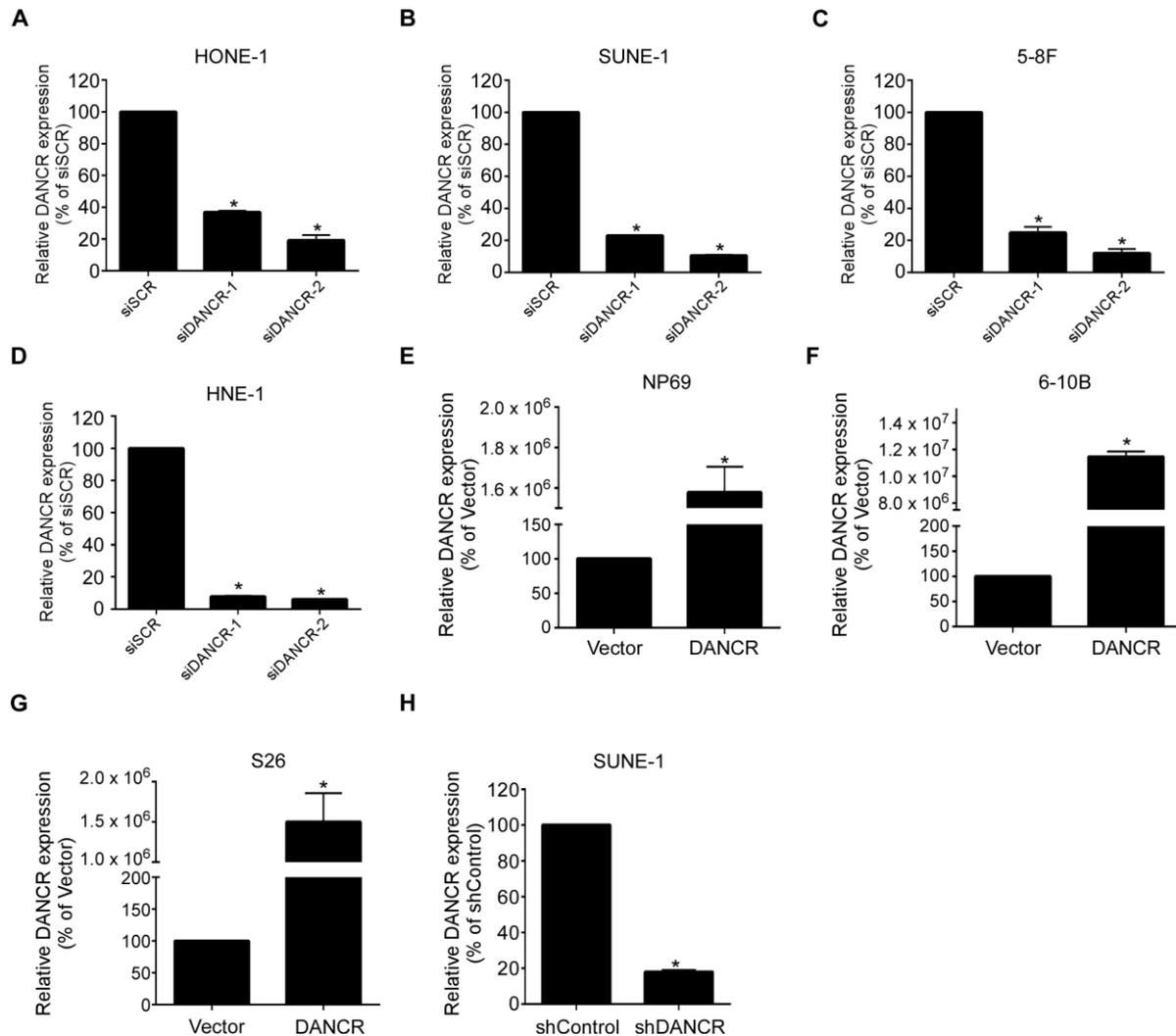
Figure S3. The efficiency of transfection in NPC cells.

(A-D), Relative expression of DANCR in HONE-1 (A), SUNE-1 (B), 5-8F (C) and HNE-1 (D) cells that transiently transfected with DANCR-siRNAs or scramble control. **(E-G)**, Relative expression of DANCR in NP69 (E), 6-10B (F) and S26 (G) cells that transfected with DANCR-overexpressing plasmid or the vector control. **(H)** Relative expression of DANCR in SUNE-1 cell that stably expressing DANCR-shRNA or control shRNA. Data are presented as means ± SD. Student's *t*-tests, *$P<0.05$. The experiments were independently repeated 3 times. (SiRNA to transiently knock-down DANCR in NPC cell lines was used for *in vitro* experiments, quantitative RT-PCR and western blotting assays. ShRNA to stably knock-down DANCR in SUNE-1 cells was used for *in vivo* experiments and RIP assays.)
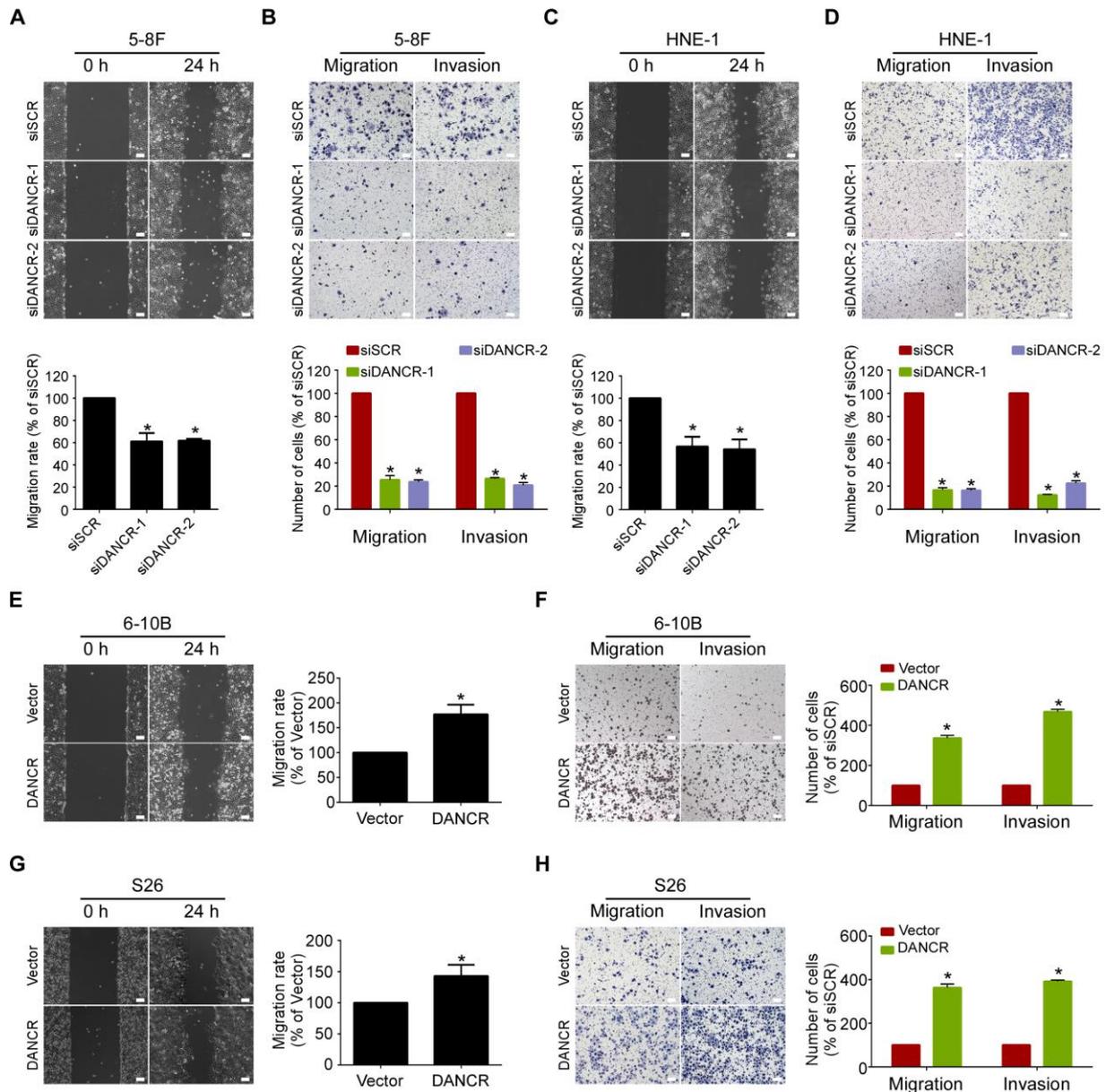
**Figure S4. DANCR promotes NPC cell migration and invasion *in vitro*.**
(A-B), Representative and quantification results of the wound healing assay (A), Transwell migration and invasion assays (B) for 5-8F cells that transfected with DANCR siRNAs or the scramble control. **(C-D)**, Representative and quantification results of the wound healing assay (C), Transwell migration and invasion assays (D) for HNE-1 cells that transfected with DANCR siRNAs or the scramble control. **(E-F)** Representative and quantification results of the wound healing assay (E), Transwell migration and invasion assays (F) for 6-10B cells that transfected with DANCR-overexpressing plasmid or the vector control. **(G-H)** Representative and quantification results of the wound healing assay (G), Transwell migration and invasion assays (H) for S26 cells that transfected with DANCR-overexpressing plasmid or the vector control. Scale bar, 100 μm. Data are presented as means ± SD. Student's *t*-tests, *$P<0.05$. The experiments were independently repeated 3 times.
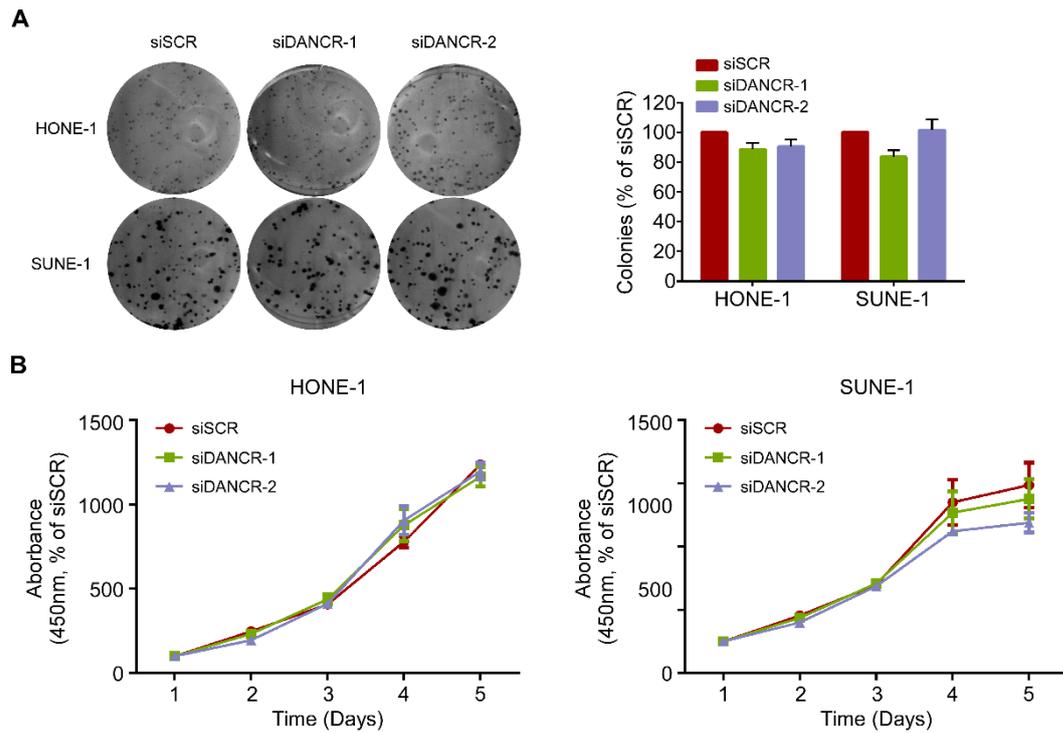
Figure S5. DANCR has little effect on NPC cell proliferation *in vitro*.
(A) Representative and quantification of the colony formation assay of HONE-1and SUNE-1 cells that transfected with DANCR siRNAs or scramble control. **(B)** CCK8 assays of HONE-1 and SUNE-1 cells that transfected with DANCR siRNAs or scramble control. Data are presented as means ± SD. Student's *t*-tests, all *P*>0.05. The experiments were independently repeated 3 times.

A
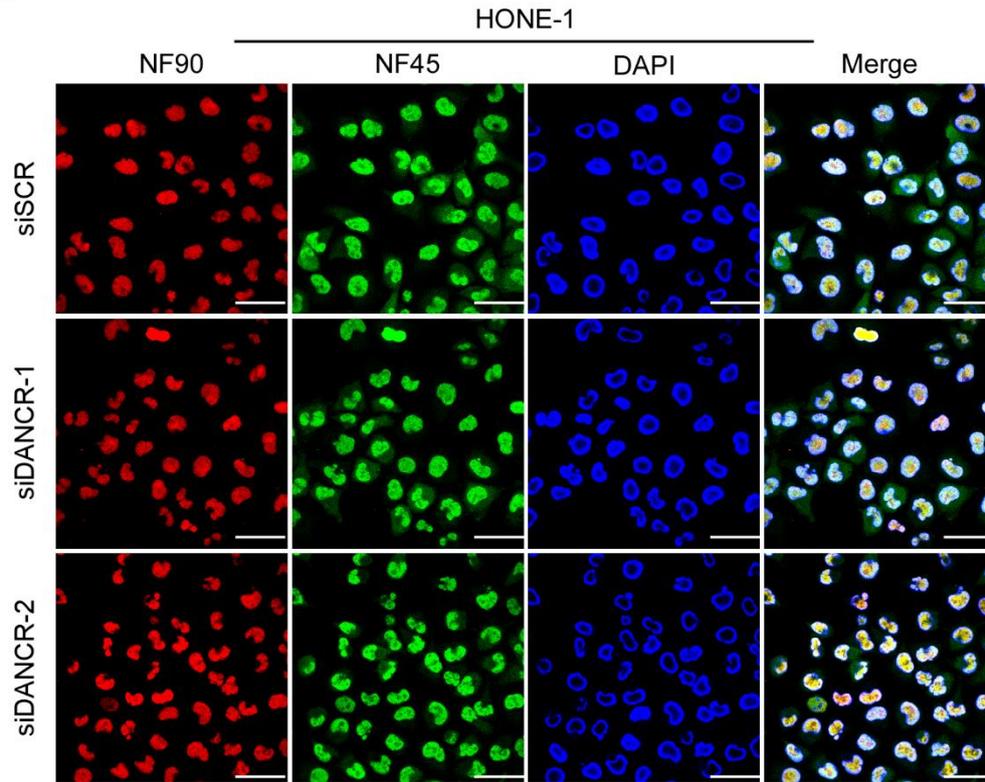
Figure S6. Localization and expression level of NF90 and NF45 after DANCR knockdown.
(A) Immunofluorescence images for DANCR expression in HONE-1 cells transfected with the scramble control or DANCR siRNAs. Scale bar, 50 μm.
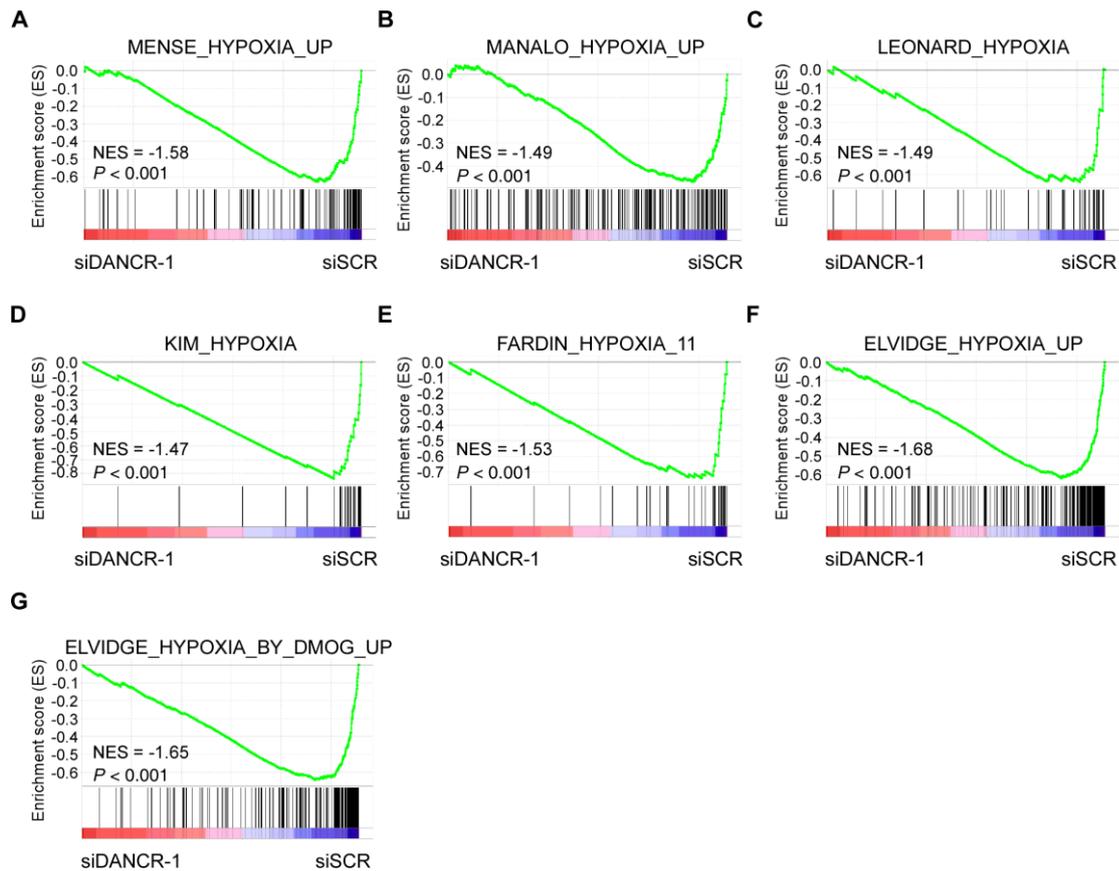
Figure S7. The correlation between hypoxic gene sets and DANCR expression assessing via Gene Set Enrichment Analysis (GSEA).

(A-G) Overview of GSEA used to identify the hypoxia related gene sets in SUNE-1 cells that transfected with DANCR siRNA or the scramble control, including Mense_Hypoxia_Up (A), Manalo_Hypoxia_Up (B), Leonard_Hypoxia (C), Kim_Hypoxia (D), Fardin_Hypoxia_11 (E), Elvidge_Hypoxia_Up (F) and Elvidge_Hypoxia_By_DMOG_Up (G). NES, normalized enrichment score.

A
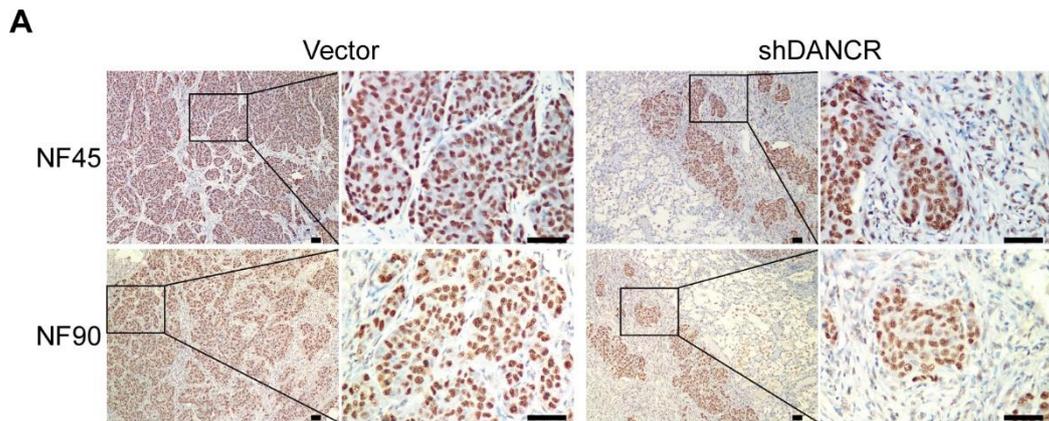
Figure S8. The expression of NF45 and NF90 *in vivo*.

(A) Immunohistochemical staining for HOPX and SNAIL expression in the lungs of mice xenograft (×100 and ×400). Scale bar, 50 mm.

Full unedited gel for Figure 3C
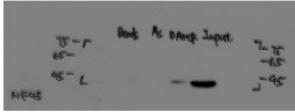
Full unedited gel for Figure 3D

Full unedited gel for Figure 3H
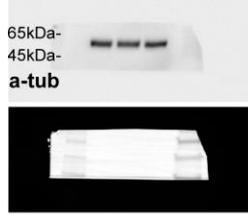
Full unedited gel for Figure 4D
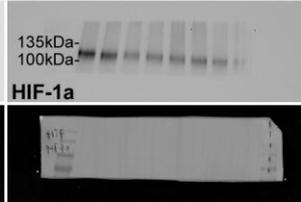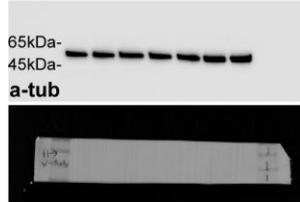
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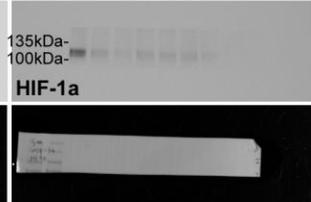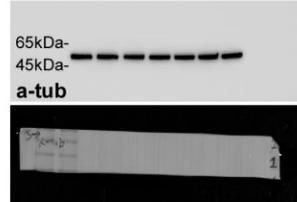
Figure S9. The full unedited gels of western blot assays.

Table S1. Primers used in this study.

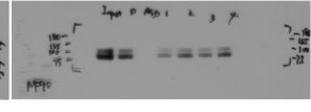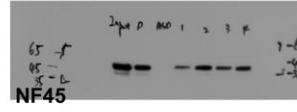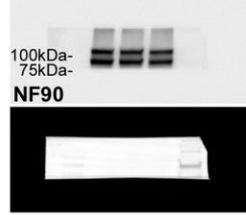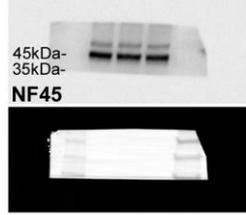| Gene	Sequence (5' to 3')
Primers for qRT-PCR from freshly-frozen tissues and cell lines	
GAPDH-F	CCATGAGAAGTATGACAACAGC
GAPDH-R	ATGGACTGTGGTCATGAGTC
DANCR-F	AGTTCTTAGCGCAGGTTGAC
DANCR-R	AAGGTGAACATGAAGCACCT
HIF1A-F	CAGCAACGACACAGAAACTG
HIF1A-R	AAAGTTCCAGTGACTCTGGA
Primers for qRT-PCR from FFPE tissues	
ACTB-F	GCATGGGTCAGAAGGATTCC
ACTB-R	AGGATGCCTCTCTTGCTCTG
DANCR-F	CTCTTACGTCTGCGGAAGTG
DANCR-R	CCTGTAGTTGTCAACCTGCG
siRNA sequences	
siSCR sense	UUCUCCGAACGUGUCACGUTT
siSCR antisense	ACGUGACACGUUCGGAGAATT
siDANCR-1 sense	CGGUCAUGAGAUUAUAUGUTT
siDANCR-1 antisense	ACAUAUAAUCUCAUGACCGTT
siDANCR-2 sense	GCCAUUGAAGCUGGAAUGUTT
siDANCR-2 antisense	ACAUUCCAGCUUCAAUGGCTT
siNF90-1 sense	GAUGCUGCGAUUGUGAUAATT
siNF90-1 antisense	UUAUCACAAUCGCAGCAUCTT
siNF90-2 sense	GGAGGUUGAUGGCAAUUCATT
siNF90-2 antisense	UGAAUUGCCAUCAACCUCCTT
siNF45-1 sense	CUUUGUACCACAUAUCCCATT
siNF45-1 antisense	UGGGAUAUGUGGUACAAAGTT
siNF45-2 sense	GAACUCCAUUUGGAUAUCATT
siNF45-2 antisense	UGAUAUCCAAAUGGAGUUCTT
shRNA sequences	
DANCR	GGAGCTAGAGCAGTGACAATG

Table S2. Antibodies used in this study.

Antibody	Company	Catalog no.	Dilution
Western blotting			
NF90	abcam	ab92355	1:2000
NF45	Santa Cruz	sc-271718	1:500
Mouse	CST	7076	1:5000
Rabbit	CST	7074	1:5000
HIF1A	CST	14179	1:250
Immunofluorescence			
NF90	abcam	ab92355	1:400
NF45	Santa Cruz	sc-271718	1:200
Immunohistochemistry			
NF90	abcam	ab92355	1:100
NF45	Santa Cruz	sc-271718	1:50
RNA-Immunoprecipitation			
NF90	abcam	ab92355	5 µg/ml
NF45	Santa Cruz	sc-271718	5 µg/ml

Table S3. Correlation between the clinicopathological features and DANCR expression in 212 patients with nasopharyngeal carcinoma.

Characteristic	No. of patients	DANCR expression		P Value*
		Low, *n* (%)	High, *n* (%)	
Age				
≤ 45 years	84	25 (33.8)	59 (42.8)	0.203
>45 years	128	49 (66.2)	79 (57.2)	
Gender				
Male	164	59 (79.7)	105 (76.1)	0.546
Female	48	15 (20.3)	33 (23.9)	
WHO Type				
II	0	0 (0)	0(0)	
III	212	74 (100)	138 (100)	
VCA-IgA				
< 1:80	20	7 (9.5)	13 (9.4)	1.000
≥ 1:80	192	67 (90.5)	125 (90.6)	
EA-IgA				
< 1:10	36	15 (20.3)	21 (15.2)	0.350
≥ 1:10	176	59 (79.7)	117 (84.8)	
T Stage				
T1-T2	29	8 (10.8)	21 (15.2)	0.496
T3-T4	183	66 (89.2)	117 (84.8)	
N Stage				
N0-N1	121	44 (59.5)	77 (55.8)	0.608
N2-N3	91	30 (40.5)	61 (44.2)	
TNM Stage				
III	127	42 (56.8)	85 (61.6)	0.493
IV	85	32 (43.2)	53 (38.4)	
Locoregional failure				
No	171	61 (82.4)	110 (79.7)	0.767
Yes	41	13 (17.6)	28 (20.3)	
Distant metastasis				
No	167	66 (89.2)	101 (73.2)	**0.011**
Yes	45	8 (10.8)	37 (26.8)	
Death				
No	139	56 (75.7)	83 (60.1)	**0.023**
Yes	73	18 (24.3)	55 (39.9)	

Abbreviations: VCA-IgA, viral capsid antigen immunoglobulin A; EA-IgA, early antigen immunoglobulin A. Bold values indicate $P< 0.05$, P value is determined by $\chi 2$ and Fisher's exact tests.

Table S4. Univariate and multivariable Cox regression analysis of prognostic factors in 212 patients with nasopharyngeal carcinoma.

Variable	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P*-value	HR	95%CI	*P*-value
Overall survival						
DANCR expression (high vs. low)	1.80	1.05-3.06	**0.031**	1.78	1.04-3.03	**0.034**
T stage (T3-T4 vs. T1-T2)	1.38	0.66-2.88	0.388			
N stage (N2-N3 vs. N0-N1)	2.05	1.29-3.26	**0.002**	2.04	1.28-3.25	**0.003**
Age (≥ 45 vs. <45 years)	1.33	0.82-2.17	0.249			
Gender (Male vs. female)	1.32	0.97-1.80	0.077			
VCA IgA (≥ 1:80 vs. < 1:80)	1.42	0.62-3.28	0.412			
EA IgA (≥ 1:10 vs. < 1:10)	1.05	0.58-1.92	0.863			
Disease-free survival						
DANCR expression (high vs. low)	1.70	1.02-2.84	**0.042**	1.68	1.01-2.81	**0.047**
T stage (T3-T4 vs. T1-T2)	1.19	0.59-2.38	0.631			
N stage (N2-N3 vs. N0-N1)	2.16	1.37-3.42	**0.001**	2.15	1.36-3.39	**0.001**
Age (≥ 45 vs. <45 years)	1.06	0.66-1.69	0.807			
Gender (Male vs. female)	0.86	0.65-1.14	0.283			
VCA IgA (≥ 1:80 vs. < 1:80)	1.35	0.58-3.10	0.486			
EA IgA (≥ 1:10 vs. < 1:10)	0.93	0.52-1.67	0.816			
Distant metastasis-free survival						
DANCR expression (high vs. low)	2.84	1.32-6.11	**0.007**	2.88	1.34-6.20	**0.007**
T stage (T3-T4 vs. T1-T2)	1.31	0.52-3.31	0.574			
N stage (N2-N3 vs. N0-N1)	2.83	1.54-5.22	**0.001**	2.63	1.42-4.85	**0.002**
Age (≥ 45 vs. <45 years)	1.04	0.57-1.90	0.906			
Gender (Male vs. female)	0.61	0.39-0.98	**0.039**	0.38	0.15-0.97	0.043
VCA IgA (≥ 1:80 vs. < 1:80)	1.62	0.50-5.23	0.420			
EA IgA (≥ 1:10 vs. < 1:10)	1.46	0.62-3.44	0.391			

Abbreviations: VCA-IgA, viral capsid antigen immunoglobulin A; EA-IgA, early antigen immunoglobulin A. Bold values indicate *P*< 0.05, *P* value is determined by Cox regression analysis.

Table S5. Proteins found by mass spectrometry analysis in the antisense-DANCR group (negative control).

Protein	Score
Pyruvate carboxylase, mitochondrial	231.75
Acetyl-CoA carboxylase 1	223.25
40S ribosomal protein S16	115.06
Tubulin beta chain	80.78
40S ribosomal protein S18	44.79
40S ribosomal protein S15	42.35
Putative beta-actin-like protein 3	33.55
60S ribosomal protein L23a	29.26
Fructose-bisphosphate aldolase A	29.09
Keratin, type I cytoskeletal 10	29.09

Table S6. Proteins found by mass spectrometry analysis in the DANCR group.

Protein	Score
Acetyl-CoA carboxylase 1	289.22
Interleukin enhancer-binding factor 3	181.91
Nuclease-sensitive element-binding protein 1	156.52
ATP-dependent RNA helicase A	156.39
Pyruvate carboxylase, mitochondrial	135.88
40S ribosomal protein S16	123.38
Interleukin enhancer-binding factor 2	103.79
Heterogeneous nuclear ribonucleoproteins A2/B1	93.65
60S ribosomal protein L27a	68.62
Nucleolin	65.69
Heterogeneous nuclear ribonucleoprotein K	61.96
40S ribosomal protein S7	61.58
Heterogeneous nuclear ribonucleoprotein A1-like 2	58.79
Leucine-rich PPR motif-containing protein, mitochondrial	57.10
Heterogeneous nuclear ribonucleoprotein A3	53.39
Polymerase I and transcript release factor	52.66
Matrin-3	49
Tubulin beta-1 chain	48.92
Rap guanine nucleotide exchange factor 2	45.29
Serine/arginine-rich splicing factor 1	44.11
Putative beta-actin-like protein 3	40.48
Fructose-bisphosphate aldolase A	40.44
Ezrin	39.48
Y-box-binding protein 3	39.15
Probable ATP-dependent RNA helicase DDX5	38.42
Far upstream element-binding protein 2	37.06
Dynein heavy chain 8, axonemal	36.95
Elongation factor 1-alpha 1	35.92
Protein unc-13 homolog C	35.43
Very-long-chain enoyl-CoA reductase	35.16
Transcription regulator protein BACH2	35.11
Nucleolar RNA helicase 2	35.03
40S ribosomal protein S11	34.85
Collagen alpha-1(XV) chain	34.06
Heterogeneous nuclear ribonucleoprotein A/B	31.56
Zinc finger E-box-binding homeobox 1	31.03
RAS protein activator like-3	30.94
Filaggrin	29.48
DNA-directed RNA polymerase I subunit RPA2	29.16