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Supplemental Information

Risk-Associated Long Noncoding RNA FOXD3-AS1

Inhibits Neuroblastoma Progression by Repressing

PARP1-Mediated Activation of CTCF

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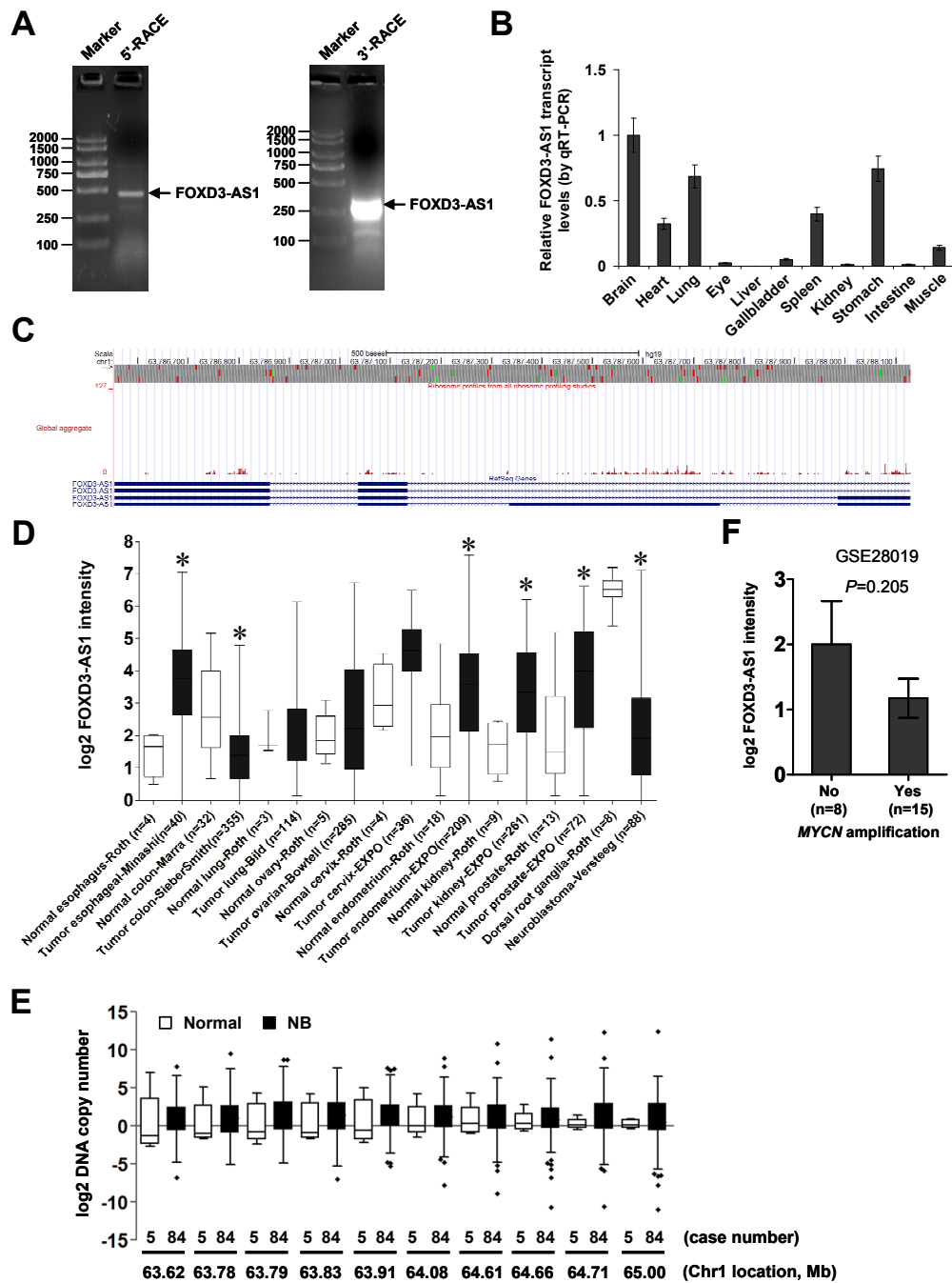


Figure S1 Characterization of a novel lncRNA FOXD3-AS1. (A) 5'- and 3'-RACE assays for identifying the full-length sequence of *FOXD3-AS1* in SH-SY5Y cells. (B) Real-time qRT-PCR indicating the *FOXD3-AS1* levels (normalized to GAPDH) in human embryonic tissues (at day 50 of gestation, mean \pm SD, n=3). (C) Ribosome profiling data showing protein-coding potential of *FOXD3-AS1*. (D) Mining of public TCGA datasets indicating the *FOXD3-AS1* levels in human tumors and their normal counterparts. (E) Representative CGH profile of *FOXD3-AS1* gene locus, locating at chr1:63786555-63788129, in NB tissues derived from Oncogenomics database (<https://pob.abcc.ncifcrf.gov/cgi-bin/JK>). (F) Mining of public dataset (GSE28019) revealing the *FOXD3-AS1* levels in NB cell lines with different status of *MYCN* amplification. Student's *t* test analyzed the difference in D-F. **P*<0.01 vs. normal.

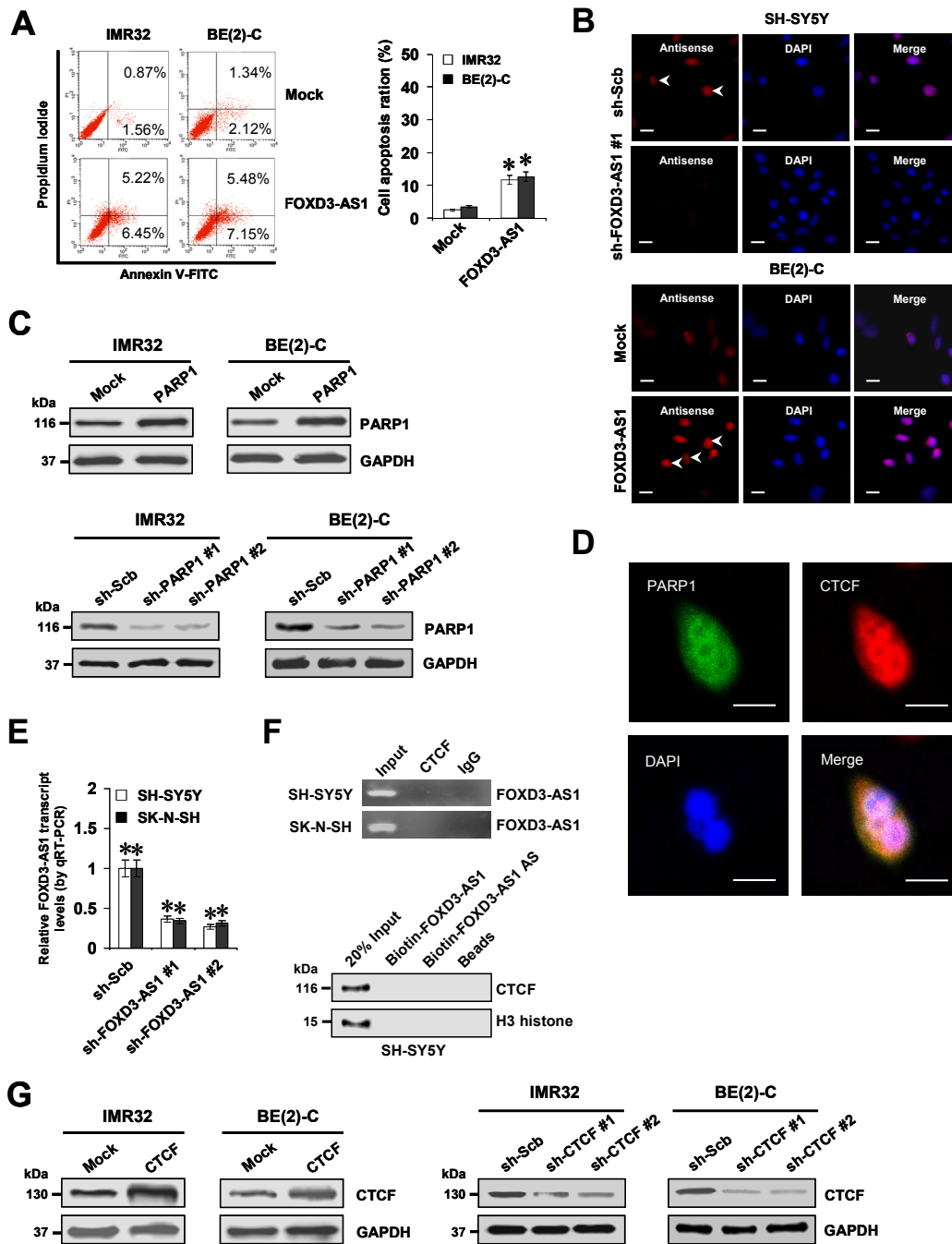


Figure S2 Expression and functions of PARP1, CTCF, and FOXD3-AS1 in NB cells. (A) Annexin V-FITC and propidium iodide staining flow cytometry (left panel) and quantification (right panel) depicting the change in apoptosis (after culture for 3 days) of NB cells stably transfected with *FOXD3-AS1*, than those transfected with empty vector (mock; mean \pm SD, n=5). (B) RNA fluorescence in situ hybridization with a 310-bp antisense probe (red) showing the nuclear localization of *FOXD3-AS1* in SH-SY5Y and BE(2)-C cells stably transfected with scramble shRNA (sh-Scb), sh-*FOXD3-AS1* #1, empty vector (mock), or *FOXD3-AS1*. Scale bars: 10 μ m. (C and G) Western blot assay showing the expression levels of PARP1 (C) and CTCF (G) (normalized to GAPDH) in IMR32 and BE(2)-C cells transfected with mock, *PARP1*, *CTCF*, sh-Scb, sh-*PARP1*, or sh-*CTCF* for 72 hrs. (D) Fluorescence immunocytochemical staining showing the co-localization of PARP1 and CTCF in BE(2)-C cells. Scale bars: 100 μ m. (E) Real-time qRT-PCR assay indicating the expression of *FOXD3-AS1* (normalized to GAPDH) in SH-SY5Y and SK-N-SH cells stably transfected with sh-Scb or sh-*FOXD3-AS1* (mean \pm SD, n=5). (F) RIP (upper panel) and biotin-labeled RNA pull-down (lower panel) assays showing the interaction between *FOXD3-AS1* and CTCF in SH-SY5Y cells. Student's *t* test analyzed the difference in A and E. **P*<0.01 vs. mock or sh-Scb.

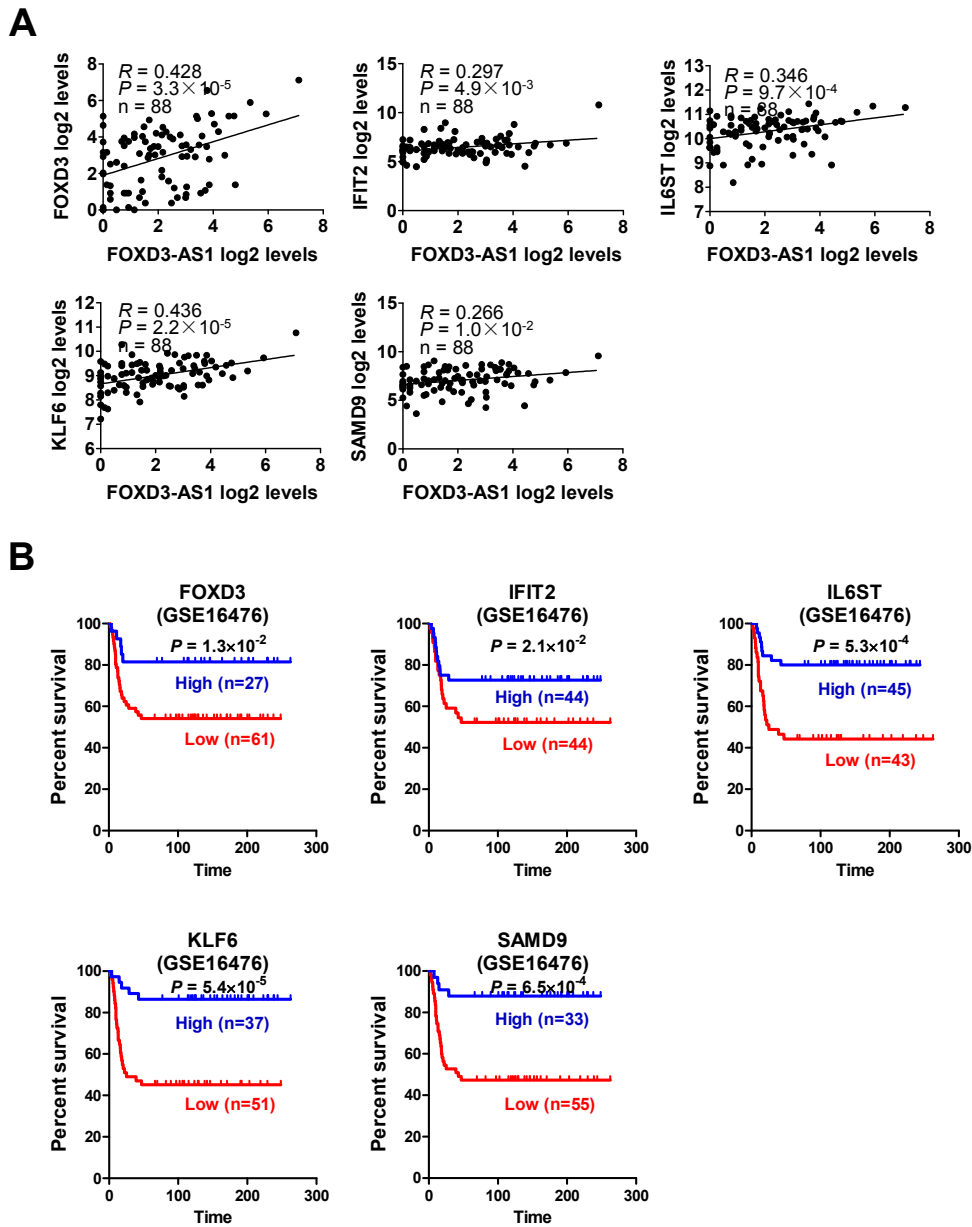


Figure S3 Expression profiles and Kaplan–Meier survival plots of *FOXD3-AS1* downstream genes in public datasets. (A) The expression correlation between *FOXD3-AS1* and downstream target genes *FOXD3*, *IFIT2*, *IL6ST*, *KLF6*, and *SAMD9* in 88 well-defined NB patients (GSE16476). **(B)** Mining of public dataset (GSE16476) indicating the survival curve of NB patients with high or low levels of *FOXD3* (cutoff value=13.5), *IFIT2* (cutoff value=86.5), *IL6ST* (cutoff value=1357.4), *KLF6* (cutoff value=559.2), and *SAMD9* (cutoff value=175.9). Pearson’s correlation coefficient analysis for gene expression in **A**. Log-rank test for survival comparison in **B**.

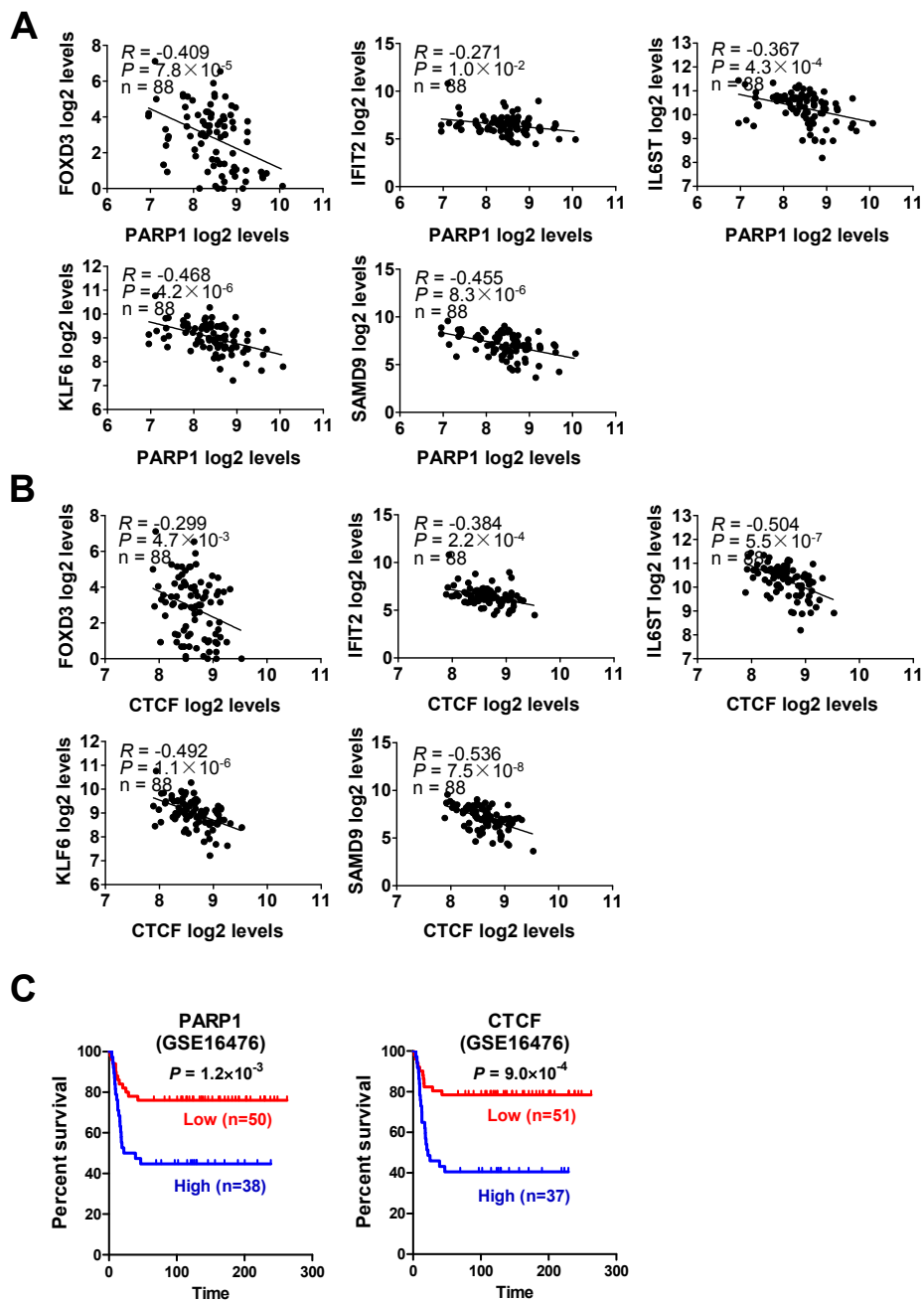


Figure S4 Expression profiles and Kaplan–Meier survival plots of PARP1 and CTCF in public datasets. (A and B) The expression correlation between *PARP1* (A) or *CTCF* (B) and downstream target genes *FOXD3*, *IFIT2*, *IL6ST*, *KLF6*, and *SAMD9* in 88 well-defined NB patients (GSE16476). **(C)** Mining of public dataset (GSE16476) indicating the survival curves of NB patients with high or low levels of *PARP1* (cutoff value=380.6) and *CTCF* (cutoff value=409.6). Pearson’s correlation coefficient analysis for gene expression in **A** and **B**. Log-rank test for survival comparison in **C**.

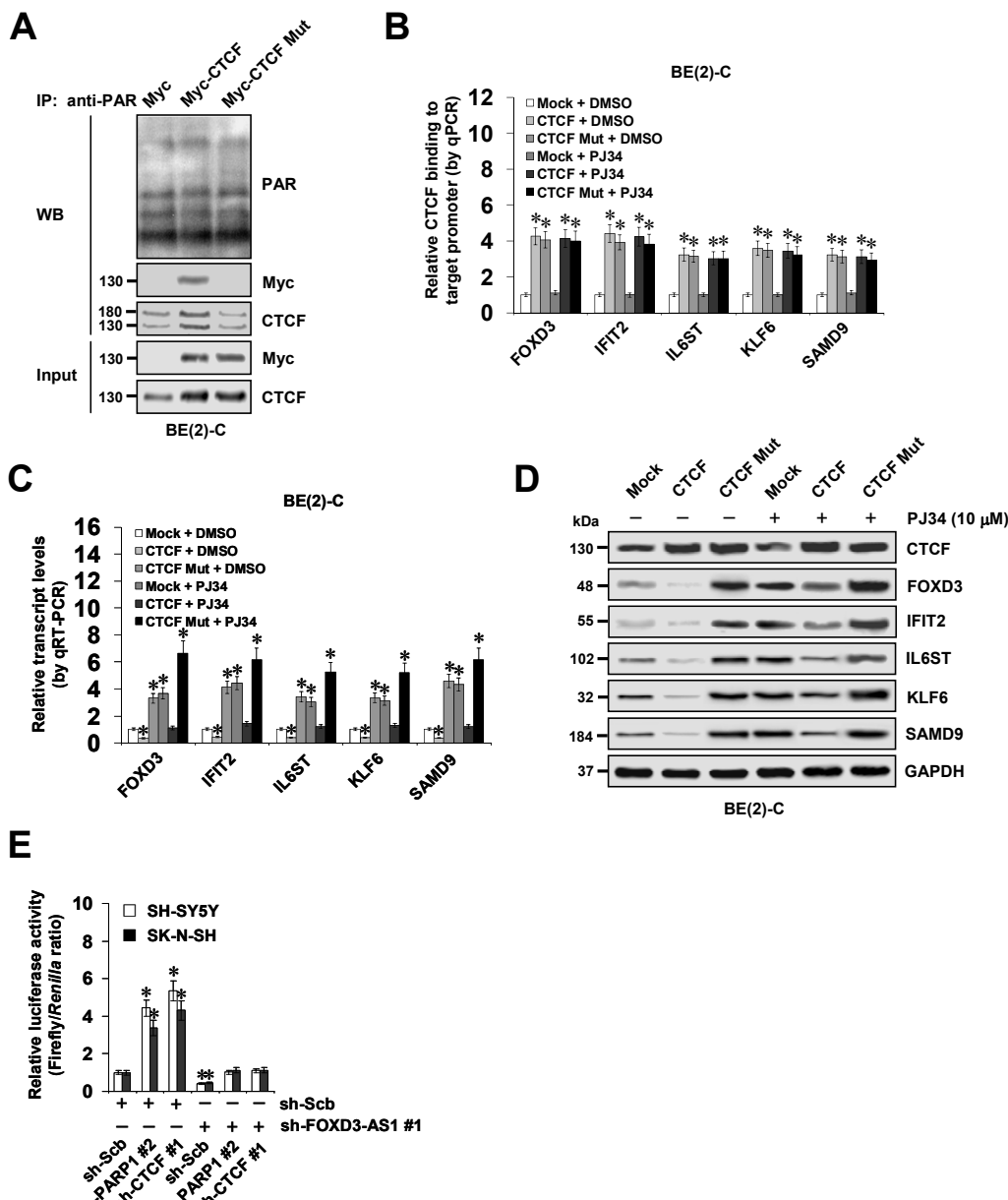


Figure S5 Roles of PARP1-mediated PARylation in regulating CTCF activity. (A) IP and western blot assays showing the expression of PARylated CTCF in NB cells transfected with empty vector (mock), wild type *CTCF*, or mutant *CTCF* (*CTCF Mut*) for 72 hrs. (B) ChIP and qPCR assay using a CTCF antibody indicating the binding of CTCF to target gene promoters in NB cells transfected with mock, *CTCF*, or *CTCF Mut* for 72 hrs, and those treated with PJ34 (10 μ mol/L) for 24 hrs. (C and D) Real-time qRT-PCR (C) and western blot (D) assays showing the transcript and protein levels of target genes in NB cells transfected with mock, *CTCF*, or *CTCF Mut* for 72 hrs, and those treated with PJ34 (10 μ mol/L) for 24 hrs. (E) Dual-luciferase assay indicating the relative activity of *FOXD3* promoter (24 hrs post-transfection) in NB cells stably transfected with sh-Scb or sh-*FOXD3*-AS1 #1, and those co-transfected with sh-PARP1 #2 or sh-*CTCF* #1 (mean \pm SD, n=5). Student's *t* test analyzed the difference in B, C and E. **P*<0.01 vs. mock+DMSO or sh-Scb.

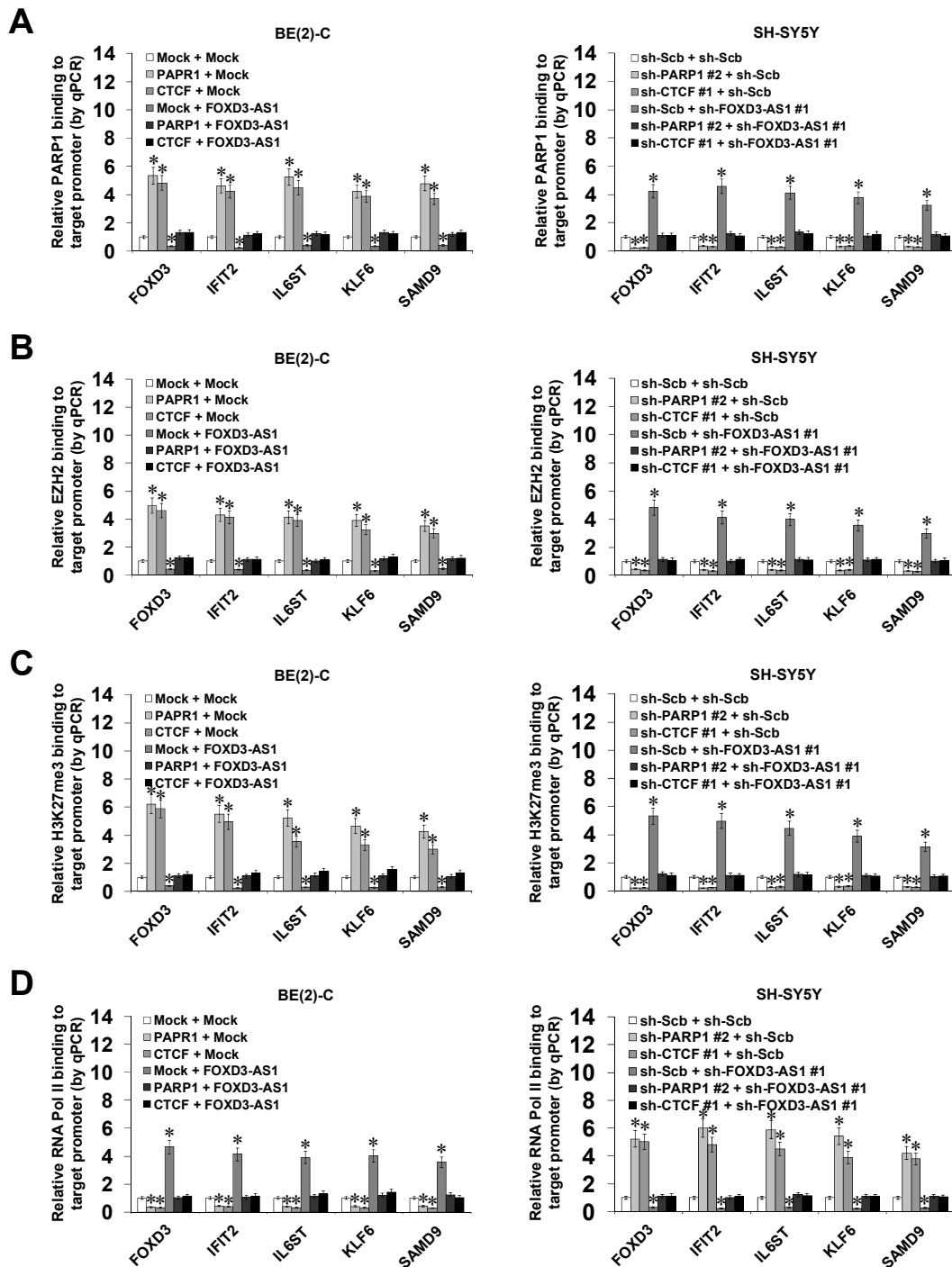


Figure S6 Binding of PARP1, EZH2, H3K27me3, and RNA Pol II to target gene promoters. ChIP and qPCR assay indicating the enrichment of PARP1 (A), EZH2 (B), H3K27me3 (C), and RNA Pol II (D) on target gene promoters (normalized to input DNA) in BE(2)-C and SH-SY5Y cells stably transfected with empty vector (mock), *FOXD3-AS1*, scramble shRNA (sh-Scb), or sh-*FOXD3-AS1* #1, and those co-transfected with *PAPR1*, *CTCF*, sh-*PAPR1* #2, or sh-*CTCF* #1 (mean \pm SD, n=5). Student's *t* test analyzed the difference in A-D. **P*<0.01 vs. mock or sh-Scb.

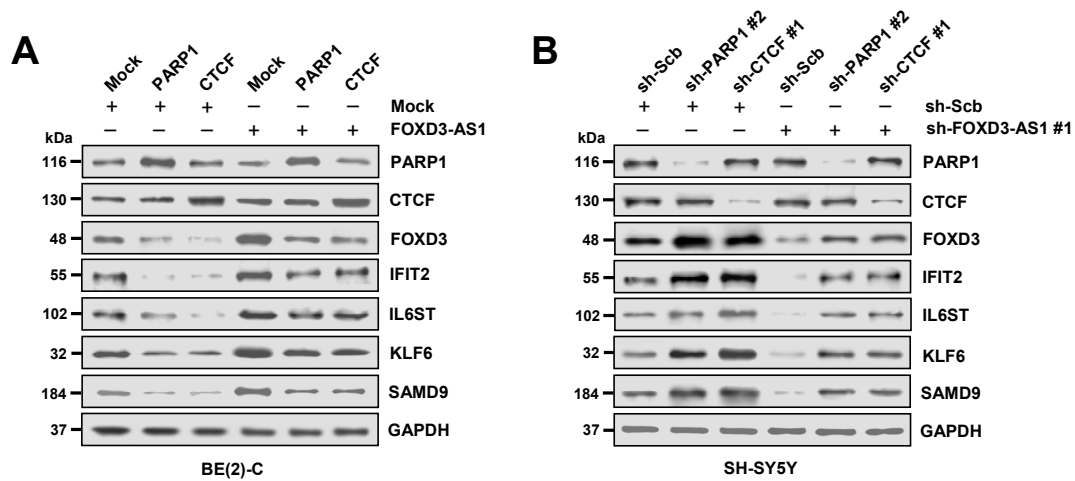


Figure S7 Effects of FOXD3-AS1, PARP1, and CTCF on downstream gene expression in NB cells. (A and B) Western blot assay showing the expression levels of PARP1, CTCF, and downstream genes *FOXD3*, *IFIT2*, *IL6ST*, *KLF6*, and *SAMD9* (normalized to GAPDH) in NB cells stably transfected with empty vector (mock), *FOXD3-AS1*, scramble shRNA (sh-Scb), or sh-*FOXD3-AS1* #1, and those co-transfected with *PARP1*, *CTCF*, sh-*PARP1* #2, or sh-*CTCF* #1.

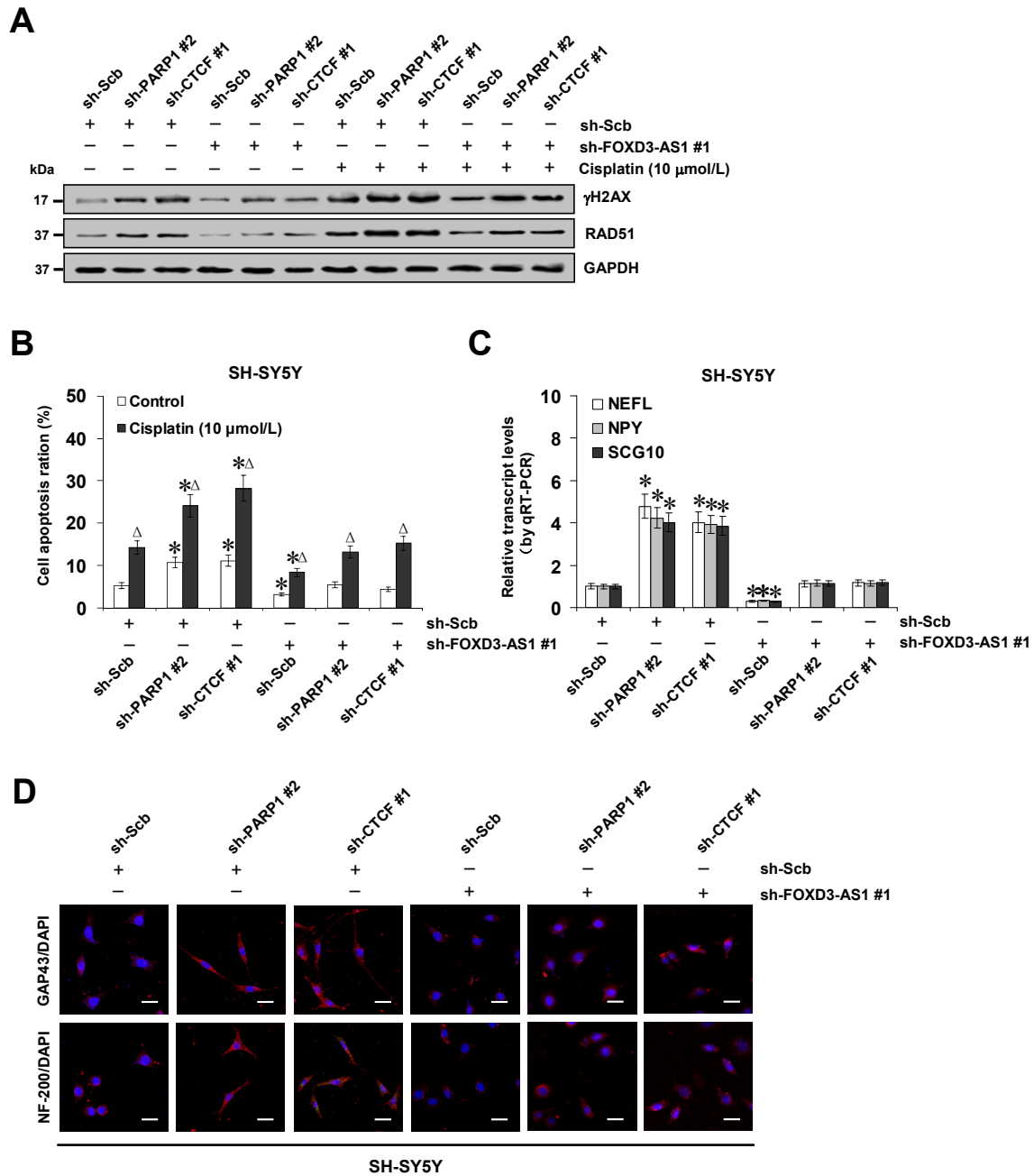


Figure S8 Effects of FOXD3-AS1, PARP1, and CTCF on DNA damage-induced apoptosis and neuronal differentiation of NB cells. (A and B) Western blot (A) and annexin V-FITC and propidium iodide staining flow cytometry (B) assays indicating the expression of γ H2AX and RAD51 and apoptosis in SH-SY5Y cells stably transfected with scramble shRNA (sh-Scb), sh-FOXD3-AS1 #1, sh-PARP1 #2, or sh-CTCF #1, with/without treatment with cisplatin for 24 hrs. **(C)** Real-time qRT-PCR showing the neuronal differentiation marker levels (normalized to GAPDH) in SH-SY5Y cells stably transfected with sh-Scb or sh-FOXD3-AS1 #1, and those co-transfected with sh-PARP1 #2 or sh-CTCF #1. **(D)** Fluorescence immunocytochemical staining assays indicating the neuronal differentiation of NB cells stably transfected with sh-Scb or sh-FOXD3-AS1 #1, and those co-transfected with sh-PARP1 #2 or sh-CTCF #1. Scale bars: 10 μ m. Student's *t* test analyzed the difference in **B** and **C**. **P*<0.01 vs. sh-Scb. Δ *P*<0.01 vs. control.

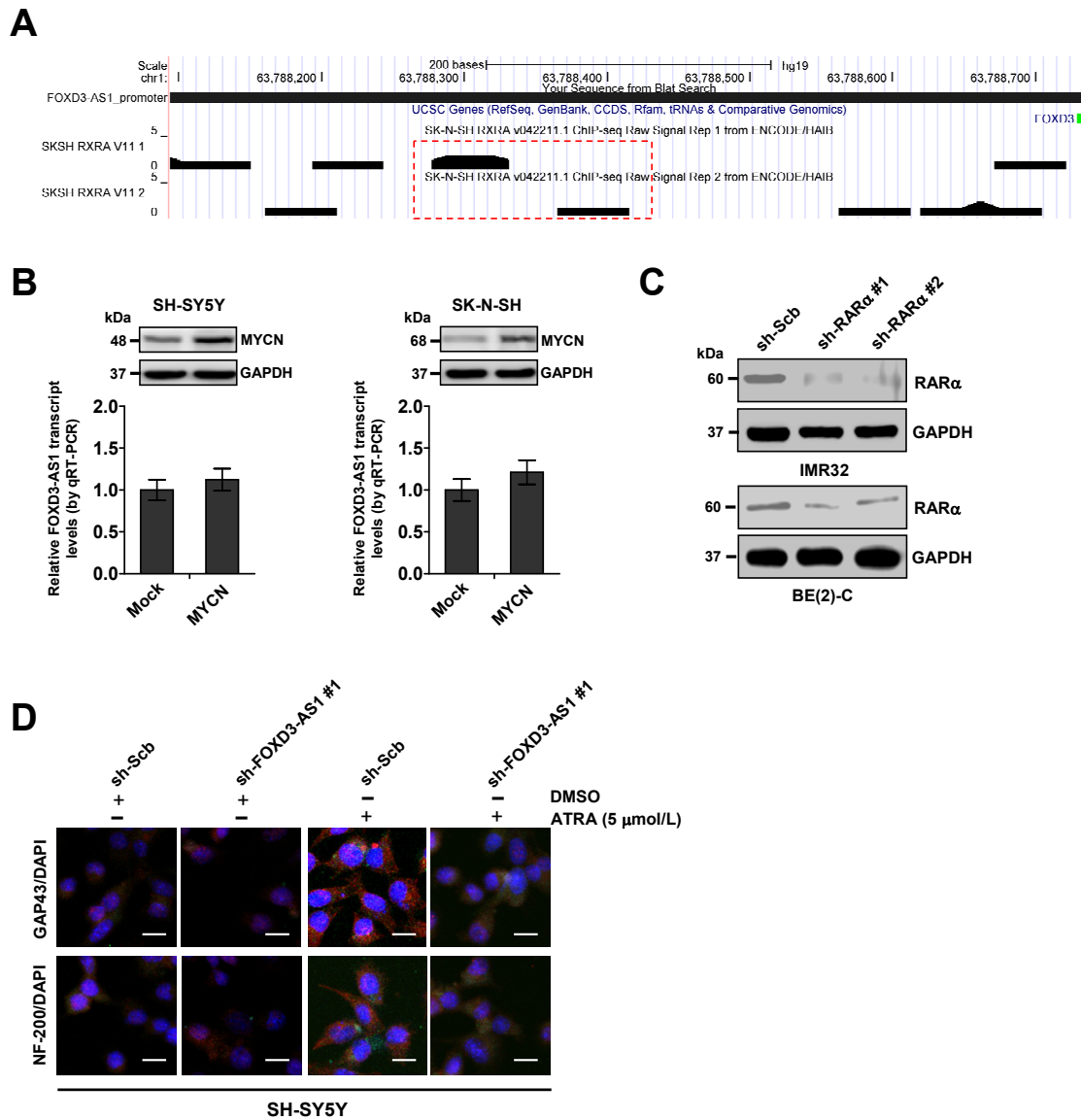


Figure S9 Effects of *RXR* α knockdown and neuronal differentiation of NB cells. (A) UCSC Genome Browser view indicating the endogenous binding of *RXR* α to *FOXD3-AS1* promoter in SK-N-SH cells. **(B)** Real-time qRT-PCR (lower panel) and western blot (upper panel) indicating the *FOXD3-AS1* levels (normalized to GAPDH) in NB cells transfected with mock or *MYCN* for 72 hrs (mean \pm SD, n=6). **(C)** Western blot assay showing the *RXR* α expression levels (normalized to GAPDH) in IMR32 and BE(2)-C cells transfected with scramble shRNA (sh-Scb) or sh-*RXR* α for 48 hrs. **(D)** Fluorescence immunocytochemical staining assays indicating the neuronal differentiation of NB cells stably transfected with sh-Scb or sh-*FOXD3-AS1* #1, and those treated with treated with ATRA (5 μ mol/L) for 48 hrs.

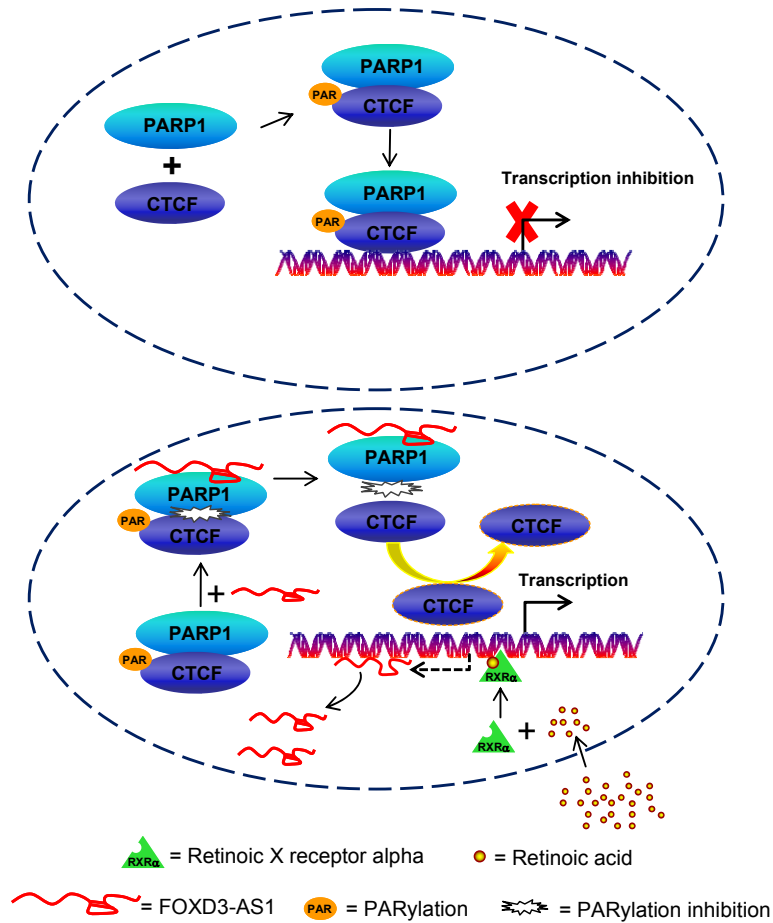


Figure S10 Mechanisms underlying FOXD3-AS1-inhibited progression of NB. As a retinoic acid-inducible lncRNA, FOXD3-AS1 binds to PARP1 protein to repress its physical interaction with CTCF, which in turn decreases the PARylation and activation of CTCF, resulting in derepressed expression of downstream target genes and suppression of NB progression.

Table S1 Log-rank test and Cox regression analyses of lncRNAs crucial for survival of NB patients (GSE16476)

LncRNA symbol	Full name of lncRNA	Log rank test <i>P</i>-value	Cox regression <i>P</i>-value	Hazard ratio	95% CI
FOXD3-AS1	FOXD3 antisense RNA 1	8.50×10^{-3}	0.004	0.472	0.313-1.446
LINC01268	long intergenic non-protein coding RNA 1268	3.90×10^{-3}	0.387	0.675	0.278-1.642
ZNF667-AS1	ZNF667 antisense RNA 1	1.20×10^{-3}	0.192	2.277	0.661-4.840
FOXCUT	FOXC1 upstream transcript	1.20×10^{-3}	0.006	2.189	1.386-4.334
NBAT1	neuroblastoma associated transcript 1	1.30×10^{-2}	0.139	0.550	0.249-1.215

95% CI, 95% confidence interval.

Table S2 *FOXD3-AS1* transcript levels in 64 NB patients (GSE12460)

Case	<i>MYCN</i> amplification	INSS Stage	Tumor type	<i>FOXD3-AS1</i> transcript levels
1	No	4	NB	3.9
2	Yes	1	NB	3.04
3	No	3	NB	1.81
4	No	1	GN	7.29
5	Yes	4	NB	4.51
6	Yes	4	NB	3.41
7	No	unknown	NB	5.07
8	Yes	unknown	NB	1
9	No	4	NB	1.07
10	No	4	NB	1.93
11	Yes	3	NB	5.06
12	No	1	NB	1.54
13	No	2b	NB	1.14
14	No	4	GNB	0.26
15	Yes	3	NB	4.13
16	No	unknown	NB	0.93
17	No	4	NB	0.26
18	Yes	unknown	NB	2.41
19	No	1	GN	7.41
20	No	1	NB	4.72
21	No	3	NB	2.1
22	No	1	GNB	5.26
23	Yes	4	NB	0
24	No	4S	NB	2.17
25	Yes	4	NB	4.84
26	No	3	NB	4.39
27	No	3	NB	4.05
28	No	2a	GNB	7.34
29	No	unknown	GNB	6.2
30	Yes	4	NB	1
31	No	2a	GNB	7.4
32	No	2b	NB	0.68
33	No	4S	NB	4.72
34	No	2	NB	3.35
35	No	4	NB	3.29
36	Yes	2b	NB	5.4
37	Yes	4	NB	2.74
38	No	1	NB	2.1
39	No	2	GN	6.65
40	No	unknown	NB	7.01
41	No	4S	NB	0.49
42	No	4S	NB	1.26
43	No	2b	NB	4.1
44	No	4S	NB	2.79
45	No	1	NB	5.35
46	No	4S	NB	3.49
47	No	1	NB	4.56
48	No	4S	NB	0
49	Yes	4	NB	1.72
50	No	2a	NB	1.89
51	No	2b	NB	4.45
52	No	4S	NB	4.78
53	Yes	unknown	NB	0.85
54	No	4	NB	4.1
55	No	1 or 2	NB	3.9
56	No	4	NB	3.05
57	No	1	NB	1.2
58	No	2b	NB	5.38
59	No	4	NB	4.26
60	No	2	GNB	5.64
61	No	2	unknown	5.78
62	No	2	unknown	1.2
63	No	4	NB	0.26
64	No	2	NB	0

Table S3 *FOXD3-AS1* transcript levels in 88 NB patients (GSE16476)

Case	Age (>18 months)	Gender	<i>MYCN</i> amplification	INSS Stage	Progression	Survival time (months)	Death	<i>FOXD3-AS1</i> transcript levels
1	No	F	No	2	No	262.5	No	1.14
2	No	M	No	2	No	163.8	No	1.68
3	No	F	No	4S	Yes	248.5	No	0.93
4	No	M	No	2	No	230.1	No	4.04
5	No	F	No	2	Yes	238.2	No	0.77
6	No	M	No	4	No	238.9	No	1.43
7	Yes	F	No	4	Yes	16.2	Yes	4.81
8	Yes	F	Yes	4	Yes	42.5	Yes	0.77
9	Yes	F	No	4	Yes	19.2	Yes	0
10	Yes	F	No	4	Yes	20.3	Yes	3.94
11	Yes	M	Yes	4	Yes	11.3	Yes	0
12	Yes	M	Yes	4	Yes	9.86	Yes	0.68
13	Yes	F	Yes	4	Yes	13.1	Yes	0.93
14	Yes	F	No	4	Yes	46.9	Yes	2.49
15	Yes	M	Yes	4	Yes	6.5	Yes	3.71
16	Yes	M	Yes	4	No	97.2	No	1.43
17	No	M	No	3	No	3.6	Yes	3.43
18	No	M	No	3	No	133.7	No	7.11
19	No	M	Yes	4	Yes	28.8	Yes	0.77
20	No	M	No	4	No	229.1	No	2.29
21	No	M	No	2	No	181.9	No	2.43
22	No	F	No	4S	No	200.4	No	1.63
23	Yes	M	No	4	Yes	5.5	Yes	2.14
24	No	M	No	4S	No	191.9	No	0
25	No	M	No	3	No	170.6	No	4.43
26	No	F	No	4S	Yes	156.4	No	2.58
27	No	F	No	2	No	138.4	No	2.14
28	No	M	No	3	No	136.3	No	1.32
29	No	M	No	4S	No	229.3	No	3.6
30	No	M	No	3	No	243.8	No	4.76
31	Yes	F	No	1	No	208.8	No	4.57
32	Yes	F	Yes	4	Yes	2.1	Yes	3.04
33	Yes	M	No	4	No	9.8	Yes	1.68
34	No	M	No	2	Yes	223.4	No	2.87
35	No	M	No	4	No	229.8	No	2.14
36	No	F	No	1	No	113.8	No	5.93
37	Yes	M	No	4	Yes	9.4	Yes	0.26
38	No	F	No	4S	Yes	82.1	No	1.14
39	No	F	No	2	No	100.7	No	3.77
40	No	M	No	1	No	115.4	No	3.79
41	Yes	M	No	4	Yes	4.2	Yes	0
42	No	M	No	3	No	186.6	No	3.49
43	No	F	No	4S	No	202.3	No	4.19
44	Yes	F	No	3	No	175.8	No	3.19
45	No	M	No	4	Yes	8.5	Yes	2
46	Yes	F	No	4	No	190.1	No	1.54
47	Yes	M	No	4	Yes	17.4	Yes	2.54
48	Yes	M	No	2	Yes	15.2	Yes	2.38
49	No	F	No	4S	No	152.3	No	0.26
50	No	M	No	2	No	118.7	No	3.05
51	No	M	No	1	No	124.9	No	1.49
52	No	F	No	2	No	123.4	No	1.72
53	No	M	No	2	No	146.4	No	5.35
54	Yes	M	No	4	No	129.5	No	1.89
55	No	M	No	3	No	141.5	No	0

56	No	M	No	4S	No	66.4	No	3.35
57	Yes	M	No	3	No	106.4	No	2.87
58	Yes	M	No	4	No	14.7	Yes	2.7
59	No	F	No	4S	No	203.2	No	1.85
60	No	F	No	4	No	128.5	No	0.85
61	Yes	M	Yes	4	Yes	18.4	Yes	3.02
62	No	F	No	4	No	202	No	1.14
63	No	M	No	1	No	146.1	No	0.77
64	No	M	No	3	No	170.8	No	0.14
65	Yes	M	No	4	Yes	7.3	Yes	3.86
66	Yes	M	Yes	4	Yes	39.2	Yes	0.14
67	No	F	No	2	No	123.5	No	3.86
68	No	M	No	1	No	125.7	No	2.17
69	Yes	F	No	4	Yes	9.8	Yes	0.93
70	Yes	M	No	4	Yes	13.2	Yes	0
71	No	F	Yes	4	No	120.8	No	3
72	No	M	No	1	No	102	No	0
73	Yes	M	Yes	4	Yes	8	Yes	0.49
74	Yes	M	No	4	No	77.1	No	3.39
75	Yes	F	No	4	Yes	18	Yes	2.17
76	No	M	No	2	No	69.7	No	2.81
77	Yes	M	No	3	Yes	16.3	Yes	1.32
78	Yes	M	No	1	No	191.7	No	0
79	No	M	No	4S	No	161.9	No	0.26
80	No	F	No	3	No	115	No	3.02
81	Yes	F	Yes	4	Yes	10.8	Yes	1.38
82	Yes	F	No	4	Yes	22.2	Yes	0.26
83	Yes	M	No	2	No	108.7	No	1.93
84	Yes	F	Yes	4	Yes	12.8	Yes	1.54
85	Yes	M	Yes	4	Yes	25.2	Yes	0
86	Yes	M	No	4	No	89.4	No	3.55
87	Yes	F	Yes	3	Yes	5.9	Yes	0
88	No	F	No	4S	No	219.2	No	0.49

F, female; M, male.

Table S4 *FOXD3-AS1* transcript levels in 42 NB patients

Case	Age (months)	Gender	<i>MYCN</i> amplification	INSS Stage	Histology	Survival time (months)	Death	<i>FOXD3-AS1</i> transcript levels *
1	6.2	F	No	4	PD	18.1	No	0.435
2	5.3	F	No	4S	PD	18.2	Yes	0.421
3	17.1	M	No	2	PD	18.0	Yes	0.421
4	10.2	M	No	4S	PD	17.3	Yes	0.412
5	16.3	F	No	4S	PD	17.1	Yes	0.381
6	8.1	F	No	4S	PD	16.0	Yes	0.381
7	9.2	M	No	2	PD	16.2	Yes	0.372
8	33.5	F	No	4S	PD	16.3	Yes	0.365
9	22.4	M	No	4	PD	15.1	Yes	0.365
10	58.2	M	Yes	4S	PD	15.3	Yes	0.265
11	8.1	M	Yes	3	PD	9.2	Yes	0.212
12	9	M	No	2	WD	48.1	No	0.842
13	18.3	M	No	2	WD	36.3	No	0.823
14	35.1	F	No	1	WD	41.4	No	0.808
15	23.3	M	No	2	WD	38.1	No	0.786
16	18.2	F	No	1	WD	21.2	No	0.765
17	10.4	M	No	3	WD	19.0	Yes	0.742
18	16.5	M	No	3	WD	13.2	Yes	0.712
19	8.2	M	No	2	WD	32.1	No	0.648
20	7.3	M	No	2	WD	24.1	No	0.632
21	11.3	F	Yes	4S	PD	15.3	Yes	0.121
22	7.4	M	No	4S	PD	12.2	Yes	0.436
23	5.2	M	Yes	4	PD	11.3	Yes	0.118
24	6.1	M	No	4	PD	11.4	Yes	0.352
25	9.3	M	No	3	PD	10.1	Yes	0.343
26	8.2	F	No	2	WD	35.3	No	0.616
27	7.2	M	Yes	1	WD	22.2	Yes	0.456
28	7.1	M	No	1	WD	19.1	No	0.565
29	10.2	M	Yes	3	WD	35.3	No	0.456
30	6.1	M	No	2	WD	18.1	No	0.564
31	19.4	F	Yes	3	PD	12.2	Yes	0.113
32	19.3	F	No	3	WD	10.1	Yes	0.534
33	28.2	M	No	3	PD	23.2	No	0.322
34	30.1	M	No	3	PD	13.3	Yes	0.302
35	36.3	M	No	3	PD	17.2	Yes	0.298
36	45.1	M	Yes	4	PD	11.2	Yes	0.101
37	18.5	F	No	2	WD	34.4	No	0.529
38	20.3	F	No	3	WD	33.1	No	0.495
39	26.5	M	No	3	WD	30.1	No	0.491
40	32.2	M	No	3	WD	33.0	No	0.486
41	41.6	M	No	3	PD	15.5	Yes	0.285
42	43.2	M	Yes	4	WD	34.2	No	0.354

F, female; M, male; PD, poor differentiation; WD, well differentiation. * normalized to transcript levels in normal dorsal ganglia.

Table S5 Mass spectrometry analysis of protein pulled down by biotin-labeled FOXD3-AS1

SH-SY5Y			SK-N-SH		
FOXD3-AS1 AS	FOXD3-AS1	Differential protein	FOXD3-AS1 AS	FOXD3-AS1	Differential protein
ACTN1	ACTN1	ACTR3	ADH5	ADH5	ATAD1
ACTN4	ACTN4	ANXA3	ADRM1	ADRM1	BTF3
ANXA1	ACTR3	BAG3	ADSL	ADSL	FABP5
ANXA2	ANXA1	CDK13	AHCY	AHCY	FAH
ANXA5	ANXA2	CDK15	AK2	AK2	FUBP3
BAG6	ANXA3	CHD4	AK3	AK3	HIST1H2BM
CDK1	ANXA5	CHD5	ALDOA	ALDOA	HNRNPA0
CDK12	BAG3	COPB2	ALDOC	ALDOC	HNRNPLL
CDK14	BAG6	DDX21	ATP2A1	ATAD1	KIF5B
CDK16	CDK1	DDX27	BUB3	ATP2A1	MAPK3
COPB1	CDK12	ENO1	CAP1	BTF3	PARP1
EEF1A1	CDK13	H2AFV	CAPN2	BUB3	PFDN6
EEF1A1P5	CDK14	H2AFX	CCT4	CAP1	PGAM5
EEF1B2	CDK15	HNRNPDL	CCT5	CAPN2	STMN2
ENO2	CDK16	INTS3	CDK15	CCT4	TUBA1C
H2AFJ	CHD4	INTS4	CDK16	CCT5	
H2AFZ	CHD5	KRT17	CDK17	CDK15	
H2BFS	COPB1	PARP1	DDX3Y	CDK16	
HIST1H2AA	COPB2	TUBA3C	DDX5	CDK17	
HIST1H2AB	DDX21		ELAVL1	DDX3Y	
HIST1H2AC	DDX27		FAM49B	DDX5	
HIST1H2AD	EEF1A1		HIST1H2BK	ELAVL1	
IDH1	EEF1A1P5		HIST1H2BN	FABP5	
IDH2	EEF1B2		HNRNPCL3	FAH	
KRT16	ENO1		HNRNPCL4	FAM49B	
KRT2	ENO2		KRT5	FUBP3	
PCNA	H2AFJ		KRT6A	HIST1H2BK	
S100A11	H2AFV		S100A11	HIST1H2BM	
TUBA1A	H2AFX		STIP1	HIST1H2BN	
TUBA1C	H2AFZ			HNRNPA0	
TUBA3E	H2BFS			HNRNPCL3	
YBX1	HIST1H2AA			HNRNPCL4	
	HIST1H2AB			HNRNPLL	
	HIST1H2AC			KIF5B	
	HIST1H2AD			KRT5	
	HNRNPDL			KRT6A	
	IDH1			MAPK3	
	IDH2			PARP1	
	INTS3			PFDN6	
	INTS4			PGAM5	
	KRT16			S100A11	
	KRT17			STIP1	
	KRT2			STMN2	
	PARP1			TUBA1C	
	PCNA				
	S100A11				
	TUBA1A				
	TUBA1C				
	TUBA3C				
	TUBA3E				
	YBX1				

Table S6 Mass spectrometry analysis of protein pulled down by PARP1 antibody

SH-SY5Y			BE(2)-C		
Mock	FOXD3-AS1	Differential protein	Mock	FOXD3-AS1	Differential protein
AKR1B1	AKR1B1	BANP	ACTA1	ACTA1	BMI1
BANP	DAD1	CTCF	ACTA2	ACTA2	CDK8
CTCF	DARS	DDX5	APTX	APTX	CTCF
DAD1	DDB1	DHFR	BANF1	BANF1	HIST1H1C
DARS	DDOST	EEF1A1	BMI1	DDX17	HIST1H1D
DDB1	DDX17	EEF1B2	CDK8	DDX39B	HIST1H1E
DDOST	EEF1A1P5	GDI1	CTCF	DDX3X	KRT16
DDX17	EWSR1	GDI2	DDX17	DDX3Y	KRT2
DDX5	FLNA	HIST2H2AC	DDX39B	E2F1	TUBA4A
DHFR	FLNB	HIST2H2BF	DDX3X	EEF1A1	TUBB
EEF1A1	H2AFJ	HIST2H3A	DDX3Y	EEF1A1P5	TUBB4A
EEF1A1P5	H2AFV	KRT1	E2F1	EEF1B2	TUBB4B
EEF1B2	H2AFX	KRT10	EEF1A1	H2AFJ	
EWSR1	HIST1H2AA	KRT14	EEF1A1P5	H2AFV	
FLNA	HIST1H2AB	NDUFV1	EEF1B2	H2AFX	
FLNB	HIST1H2AC	NEDD4	H2AFJ	HSPA4	
GDI1	HNRNPK	PCNA	H2AFV	HSPA5	
GDI2	KRT16	TUBB4B	H2AFX	HSPA8	
H2AFJ	KRT17	TUBB6	HIST1H1C	IDH1	
H2AFV	MCM6		HIST1H1D	IDH2	
H2AFX	MCM7		HIST1H1E	IDH3A	
HIST1H2AA	MDH1		HSPA4	KRT17	
HIST1H2AB	NEDD8		HSPA5	NMT1	
HIST1H2AC	PAK1		HSPA8	NONO	
HIST2H2AC	PAK2		IDH1	SP1	
HIST2H2BF	PARK7		IDH2		
HIST2H3A	PHB		IDH3A		
HNRNPK	SLC25A12		KRT16		
KRT1	SLC25A3		KRT17		
KRT10	TUBB4A		KRT2		
KRT14			NMT1		
KRT16			NONO		
KRT17			SP1		
MCM6			TUBA4A		
MCM7			TUBB		
MDH1			TUBB4A		
NDUFV1			TUBB4B		
NEDD4					
NEDD8					
PAK1					
PAK2					
PARK7					
PCNA					
PHB					
SLC25A12					
SLC25A3					
TUBB4A					
TUBB4B					
TUBB6					

Table S7 Primer sets used for RACE, qPCR, RIP, probe, and ChIP

Primer set	Primers	Sequence	Product size (bp)	Application
FOXD3-AS1	GSP1	5'-GACAGACAGGGATTGGGTT-3'		3'-RACE
	GSP2	5'-GAATAGTTGCCGAGAGAAA-3'		5'-RACE
	NGSP1	5'-CAACTAGGCGGCCTTGACAC-3'		3'-RACE
	NGSP2	5'-AGAAGCCATAACTGGCTAC-3'		5'-RACE
FOXD3-AS1	Forward	5'-GAATAGTTGCCGAGAGAAA-3'	322	qPCR
	Reverse	5'-GACAGACAGGGATTGGGTT-3'		
FOXD3-AS1	Forward	5'-GGAGGAGGCGAGGATGTGTG-3'	310	RIP, probe
	Reverse	5'-TGGTGTGTCTAGGCCAAGGA-3'		
FOXD3	Forward	5'-GACGACGGGCTGGAAGAGAA-3'	161	qPCR
	Reverse	5'-GCCTCCTTGGGCAATGTCA-3'		
IFIT2	Forward	5'-ATCTCTTCCGTGTCTGTTCC-3'	228	qPCR
	Reverse	5'-TCTCCCTTGATTTCTGGTTT-3'		
IL6ST	Forward	5'-CCAAAGGACCTACTGTTTCG-3'	235	qPCR
	Reverse	5'-TCATCTGTGTATGCTGCCA-3'		
KLF6	Forward	5'-CTCCACGCCTCCATCTTCT-3'	135	qPCR
	Reverse	5'-TCGCCATTTCCCTTGTAC-3'		
SAMD9	Forward	5'-GGCAGAGTGGAGATGTGTGGA-3'	137	qPCR
	Reverse	5'-AAAAGCGGGAGTGATGGGTAT-3'		
NEFL	Forward	5'-CTGCCTACGGCGGTTTAC-3'	156	qPCR
	Reverse	5'-CTTCAGAGGGGGGCTCAT-3'		
NPY	Forward	5'-GCTAGGTAACAAGCGACT-3'	186	qPCR
	Reverse	5'-CTCTGCCTGGTGATGAGG-3'		
SCG10	Forward	5'-GGCCAGGCTTTTGAGCTGATCTT-3'	181	qPCR
	Reverse	5'-CCCTCTTCTCTGCCAATTGTTTC-3'		
GAPDH	Forward	5'-AGAAGGCTGGGGCTCATTG-3'	258	qPCR
	Reverse	5'-AGGGGCCATCCACAGTCTTC-3'		
U1	Forward	5'-ACTTACCTGGCAGGGGAGATACC-3'	137	qPCR
	Reverse	5'-CCACTACCACAAATTATGCAGTCG-3'		
FOXD3 (-859/-610)	Forward	5'-AGAAGGGCGGAAGGGAGAGG-3'	250	ChIP
	Reverse	5'-TCAACAAAGGGACGAGAGAC-3'		
IFIT2 (-689/-524)	Forward	5'-AACTACTTTTGAATGCTTGCC-3'	166	ChIP
	Reverse	5'-CCCATCTTTGCTCTGCCTTATA-3'		
IL6ST (-334/-98)	Forward	5'-CTCCAGTTCATGACCCCGTT-3'	237	ChIP
	Reverse	5'-GCCCCTGAGAGACCTTTGC-3'		
KLF6 (-535/-319)	Forward	5'-GAAGGGGACGGGGGGGAA-3'	217	ChIP
	Reverse	5'-CGGCCGAGCTAAGGGAG-3'		
SAMD9 (-274/-51)	Forward	5'-TGGTTGCAAGACAAAGGAC-3'	224	ChIP
	Reverse	5'-GTGGAGGTAGCAGGGGAGT-3'		
FOXD3-AS1 Set 1 (-293/-62)	Forward	5'-CCCCTCACTTAGCCCGCCTT-3'	232	ChIP
	Reverse	5'-CCGCCTCCCGCTGGATCTTT-3'		
FOXD3-AS1 Set 2 (-716/-537)	Forward	5'-TCCTTCTCTTCCAGCCCGTC-3'	180	ChIP
	Reverse	5'-AGCCCCCGAACACCCTCATC-3'		

GSP, gene specific primer; NGSP, nested gene specific primer; FOXD3-AS1, forkhead box D3 antisense RNA 1; IFIT2, interferon induced protein with tetratricopeptide repeats 2; IL6ST, interleukin 6 signal transducer; KLF6, Kruppel like factor 6; SAMD9, sterile alpha motif domain containing 9; NEFL, neurofilament light chain; NPY, neuropeptid Y; SCG10, superior cervical ganglia-10; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; RACE, rapid amplification of cDNA ends; RIP, RNA immunoprecipitation; ChIP, chromatin immunoprecipitation.

Table S8 Oligonucleotide sets used for constructs

Oligo Set	Sequences
pcDNA3.1-FOXD3-AS1 (Exon 1+2+3+4)	5'-CGGGGTACCAGGGAATTGTCAACAAAGGGACGA-3' (sense); 5'-GCCGCTCGAGGATTTTTAAATTTTTATTTTTATTTTATTGAATTATTTTTGGTGTGTCT A-3' (antisense)
pcDNA3.1-FOXD3-AS1 (Exon 2+3+4)	5'-CGGGGTACCCTTTAAAGAGTAAGAGCAGCGCAC-3' (sense); 5'-GCCGCTCGAGGATTTTTAAATTTTTATTTTTATTTTATTGAATTATTTTTGGTGTGTCT A-3' (antisense)
pcDNA3.1-FOXD3-AS1 (Exon 3+4)	5'-CGGGGTACCGTGTGGACAAATCCTCCAAGATTT-3' (sense); 5'-GCCGCTCGAGGATTTTTAAATTTTTATTTTTATTTTATTGAATTATTTTTGGTGTGTCT A-3' (antisense)
pcDNA3.1-FOXD3-AS1 (Exon 4)	5'-CGGGGTACCGCCGCCTAGTTGGGAGCCGCAAGA-3' (sense); 5'-GCCGCTCGAGGATTTTTAAATTTTTATTTTTATTTTATTGAATTATTTTTGGTGTGTCT A-3' (antisense)
pcDNA3.1-FOXD3-AS1 (antisense)	5'-CGGGGTACCGATTTTTAAATTTTTATTTTTATTTTATTGAATTATTTTTGGTGTGTCTA -3' (sense); 5'-GCCGCTCGAGAGGGAATTGTCAACAAAGGGACGA-3' (antisense)
pCMV-FLAG-PARP1	5'-CGGAAGATCTGATGGCGGAGTCTTCGGATAAGCTCT-3' (sense); 5'-CTAGTCTAGATTACCACAGGGAGGTCTTAAAATTG-3' (antisense)
pCMV-FLAG-PARP1 (ZnF)	5'-CGGAAGATCTGATGGCGGAGTCTTCGGATAAGCTCT-3' (sense); 5'-CTAGTCTAGACGAGGCTGTGGAGGGCGGAGGCGT-3' (antisense)
pCMV-FLAG-PARP1 (BRCT-WGR)	5'-CGGAAGATCTGGCTCCTGCTGCTGTGAACTCCTCTG-3' (sense); 5'-CTAGTCTAGACTTGGTGCCAGGATTTACTGTCAGC-3' (antisense)
pCMV-FLAG-PARP1 (CAT)	5'-CGGAAGATCTGTCCAAGCTCCCAAGCCAGTTCAGG-3' (sense); 5'-CTAGTCTAGATTACCACAGGGAGGTCTTAAAATTG-3' (antisense)
pCMV-FLAG-PARP1 (Δ CAT)	5'-CGGAAGATCTGATGGCGGAGTCTTCGGATAAGCTCT-3' (sense); 5'-CTAGTCTAGACTTGGTGCCAGGATTTACTGTCAGC-3' (antisense)
pCMV-FLAG-PARP1 (Δ ZnF)	5'-CGGAAGATCTGGCTCCTGCTGCTGTGAACTCCTCTG-3' (sense); 5'-CTAGTCTAGATTACCACAGGGAGGTCTTAAAATTG-3' (antisense)
pGEX-6P-1-PARP1	5'-TCCCCCGGGGATGGCGGAGTCTTCGGATAAGCTCT-3' (sense); 5'-GCCGCTCGAGTTACCACAGGGAGGTCTTAAAATTG-3' (antisense)
pGEX-6P-1-PARP1 (ZnF)	5'-TCCCCCGGGGATGGCGGAGTCTTCGGATAAGCTCT-3' (sense); 5'-GCCGCTCGAGCGAGGCTGTGGAGGGCGGAGGCGT-3' (antisense)
pGEX-6P-1-PARP1 (BRCT-WGR)	5'-TCCCCCGGGGCTCCTGCTGCTGTGAACTCCTCTG-3' (sense); 5'-GCCGCTCGAGCTTGGTGCCAGGATTTACTGTCAGC-3' (antisense)
pGEX-6P-1-PARP1 (CAT)	5'-TCCCCCGGGGTCCAAGCTCCCAAGCCAGTTCAGG-3' (sense); 5'-GCCGCTCGAGTTACCACAGGGAGGTCTTAAAATTG-3' (antisense)
pGEX-6P-1-PARP1 (Δ CAT)	5'-TCCCCCGGGGATGGCGGAGTCTTCGGATAAGCTCT-3' (sense); 5'-GCCGCTCGAGCTTGGTGCCAGGATTTACTGTCAGC-3' (antisense)
pGEX-6P-1-PARP1 (Δ ZnF)	5'-TCCCCCGGGGCTCCTGCTGCTGTGAACTCCTCTG-3' (sense); 5'-GCCGCTCGAGTTACCACAGGGAGGTCTTAAAATTG-3' (antisense)
pCMV-Myc-CTCF	5'-CGCGGATCCATGGAAGGTGATGCAGTCAAGCCA-3' (sense); 5'-GCCGCTCGAGTCACCGGTCCATCATGCTGAGGATC-3' (antisense)
pCMV-Myc-CTCF (N-terminal)	5'-CGCGGATCCATGGAAGGTGATGCAGTCAAGCCA-3' (sense); 5'-GCCGCTCGAGCTCATCAGTGTGGCTTTTCATGTGA-3' (antisense)
pCMV-Myc-CTCF (ZnF)	5'-CGCGGATCCAGACCACACAAGTGCCATCTCTGTG-3' (sense); 5'-GCCGCTCGAGATTATCAGCATGTCTTGCCATGGTA-3' (antisense)
pCMV-Myc-CTCF	5'-CGCGGATCCTGTGCTGGCCAGATGGCGTAGAGG-3' (sense);

(C-terminal)	5'-GCCGCTCGAGTCACCGGTCCATCATGCTGAGGATC-3' (antisense)
pCMV-Myc-CTCF	5'-GCGTTATACAGCGGCGGGCAAAGATGTAGATGTGTCTGTCTACGATT-3' (sense-1);
(Mut)	5'-ACATCTTTGCCCGCCGCTGTATAACGCAGTTTGCTCTTTTTGGTTTT-3' (antisense-1)
	5'-CTACGATTTTGCGGCAGCACAGCAGGCGGGTCTGCTATCAGAGGTTAATGCAGAGA AAG -3' (sense-2);
	5'-GATAGCAGACCCGCCTGCTGTGCTGCCGCAAATCGTAGACAGACACATCTACATC TTT' (antisense-2)
	5'-TCTGCTATCAGAGGTTAATGCAGAGAAAGTGGTTGGTAATATGAAGCCTCCAAAGC-3' (sense-3);
	5'-CCAACCACTTTCTCTGCATTAACCTCTGATAGCAGACCCGCCTGCTGTGCTGCCGC -3' (antisense-3)
pBiFC-VN173-PARP1	5'-CGGAAGATCTGATGGCGGAGTCTTCGGATAAGCTCT-3' (sense);
	5'-CTAGTCTAGACCACAGGGAGGTCTTAAAATTGAAT-3' (antisense)
pBiFC-VC155-CTCF	5'-CCGGAATTCGGATGGAAGGTGATGCAGTCGAAGCCA-3' (sense);
	5'-CCGCTCGAGCCCGGTCCATCATGCTGAGGATCATC-3' (antisense)
pGL3-FOXD3 (-1870/+130)	5'-GGAAGATCTGCCTAAATGAGGGAGGAAAG-3' (sense);
	5'-CCCAAGCTTAACCTGCGTCGCTGTCCTTCTCTTCC-3' (antisense)
pGL3-FOXD3 (CTCF Mut)	5'-CGTGCCCATATATGCTGGCGCACAGTGCGGAGCGGAGTTG-3' (sense);
	5'-CGCCAGCATATATGGGGCACGGAGGGCGCTGCGGCCCGCCC-3' (antisense)
pGL3-FOXD3-AS1 (-607/+35)	5'-CGGGGTACCGGGTCATCCCTCCTCGGGTTGGT-3' (sense);
	5'-GCCGCTCGAGTTGCGCGTCTCTCGTCCCTTTGTT-3' (antisense)
pGL3-FOXD3-AS1 (RXR α Mut)	5'-GCCTTCCTCGATTGCTGGCCCTGGCGTGGGGCGCAGGAGCGGTCC-3' (sense);
	5'-GCCAGGGCCAGCAATCGAGGAAGGCGGGCTAAGTGAGGGGGCGCG-3' (antisense)

FOXD3-AS1, forkhead box D3 antisense RNA 1; PARP1, poly(ADP-ribose) polymerase 1; CTCF, CCCTC-binding factor; BiFC, bimolecular fluorescence complementation; RXR α , retinoic X receptor alpha.

Table S9 Oligonucleotide sets used for short hairpin RNAs and small interfering RNAs

Oligo Set	Sequences
sh-Scb	5'-AGGGATACAAGCATATACCACTCGAGTGGTATATGCTTGTATCCCTC-3' (sense); 5'-GAGGGATACAAGCATATACCACTCGAGTGGTATATGCTTGTATCCCT-3' (antisense)
sh-FOXD3-AS1 #1	5'-GATCCCGTGTGGACAAATCCTCCAAGACTCGAGTCTTGGAGGATTTGTCCACACTTTT TGGAT-3' (sense); 5'-AGCTATCCAAAAAGTGTGGACAAATCCTCCAAGACTCGAGTCTTGGAGGATTTGTCC ACACGG-3' (antisense)
sh-FOXD3-AS1 #2	5'-GATCCCGAGGAGTTCCGAGAGGAAATACTCGAGTATTTCTCTCGGAACTCCTCTTTT TGGAT-3' (sense); 5'-AGCTATCCAAAAAGAGGAGTTCCGAGAGGAAATACTCGAGTATTTCTCTCGGAACTC CTCGG-3' (antisense)
sh-PARP1 #1	5'-GATCCCGTCTCATCAAGATGATCTTTCTCGAGAAAGATCATCTTGATGAGGACTTTT TGGAT-3' (sense); 5'-AGCTATCCAAAAAGTCTCATCAAGATGATCTTTCTCGAGAAAGATCATCTTGATGAG GACGG-3' (antisense)
sh-PARP1 #2	5'-GATCCCGTGGAGTATGAGATCGACCTTCTCGAGAAGGTCGATCTCATACTCCACTTTT TGGAT-3' (sense); 5'-AGCTATCCAAAAAGTGGAGTATGAGATCGACCTTCTCGAGAAGGTCGATCTCATACT CCACGG-3' (antisense)
sh-CTCF #1	5'-CCGGGCCTCTTTCTTGGCAAAGTTTCTCGAGAACTTTGCCAAGAAAGAGGCTTTTT G-3' (sense); 5'-AATTCAAAAAGCCTCTTTCTTGGCAAAGTTTCTCGAGAACTTTGCCAAGAAAGAGG C-3' (antisense)
sh-CTCF #2	5'-CCGGGCTGTGTTTCATGAGCGCTATCTCGAGATAGCGCTCATGAAACACAGCTTTTT G-3' (sense); 5'-AATTCAAAAAGCTGTGTTTCATGAGCGCTATCTCGAGATAGCGCTCATGAAACACAG C-3' (antisense)
sh-RXR α #1	5'-CCGGGACCTACGTGGAGGCAAACATCTCGAGATGTTTGCCTCCACGTAGGTCTTTT TG-3' (sense); 5'-GATCCAAAAAGACCTACGTGGAGGCAAACATCTCGAGATGTTTGCCTCCACGTAGG TC -3' (antisense)
sh-RXR α #2	5'-CCGGGGGACATGCAGATGGACAAGACTCGAGTCTTGTCCATCTGCATGTCCCTTTT TG-3' (sense); 5'-GATCCAAAAAGGGACATGCAGATGGACAAGACTCGAGTCTTGTCCATCTGCATGTC CC-3' (antisense)
si-Scb	5'-GAACGAUCGAGUAAACGGAtt-3' (sense); 5'-UCCGUUUACUCGAUCGUUCtt-3' (antisense)
si-PARP1	5'-GUGGAGUAUGAGAUCGACctt-3' (sense); 5'-GGUCGAUCUCAUACUCCACTt-3' (antisense)
si-CTCF	5'-GCCUCUUUCUUGGCAAAGUtt-3' (sense); 5'-ACUUUGCCAAGAAAGAGGctt-3' (antisense)

FOXD3-AS1, forkhead box D3 antisense RNA 1; PARP1, poly(ADP-ribose) polymerase 1; CTCF, CCCTC-binding factor; RXR α , retinoic X receptor alpha; sh-Scb, scramble short hairpin RNAs.