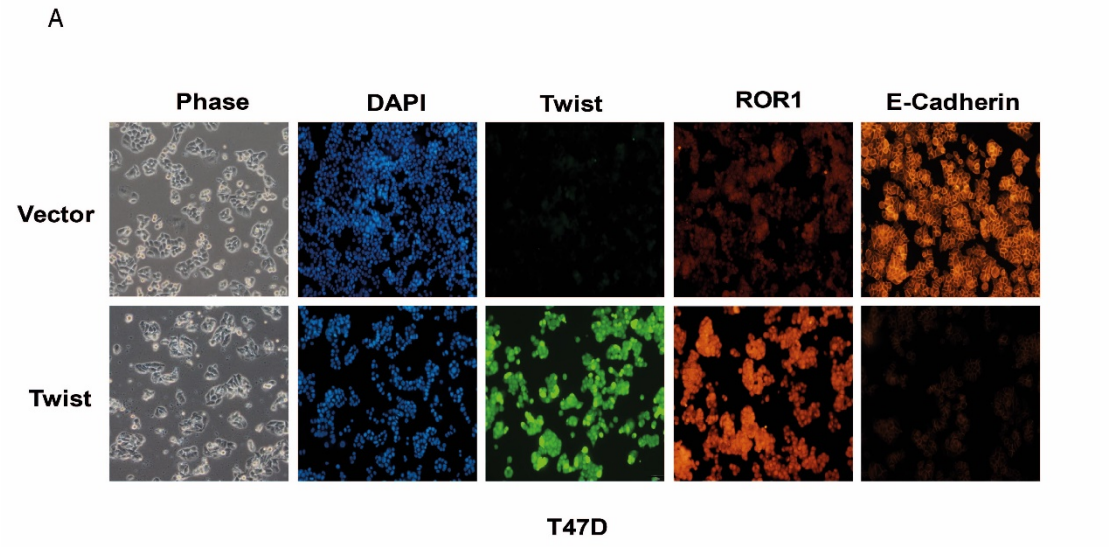
Supplementary Information

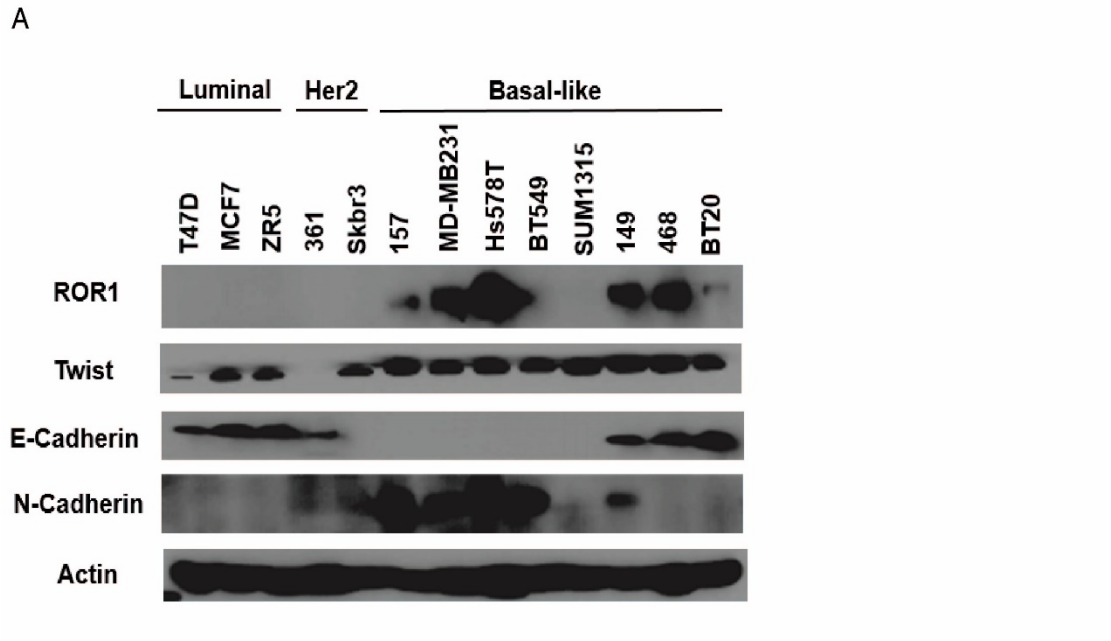
Supplementary Figures and Figure Legends

Figure S1



Supplemental Figure S1. Twist promotes the expression of ROR1. (A)T47D cells expressing the vector or the Twist were examined for morphological changes indicative of EMT by phase microscopy and the expression of ROR1, E-cadherin, and Twist (green) by immunofluorescence staining (40X).

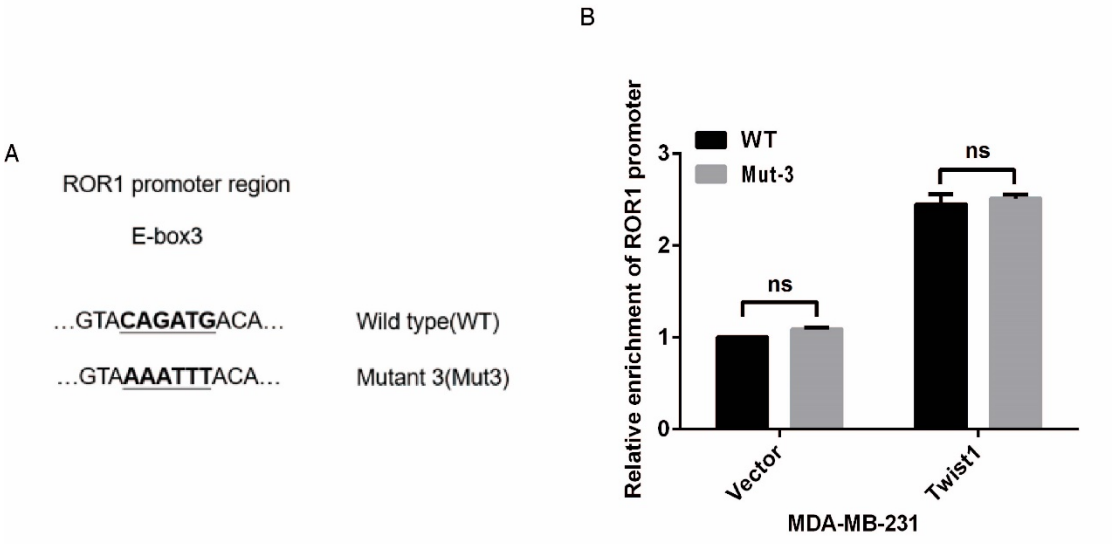
Figure S2



Supplemental Figure S2. Both Twist and ROR1 are highly expressed in BLBC cells.

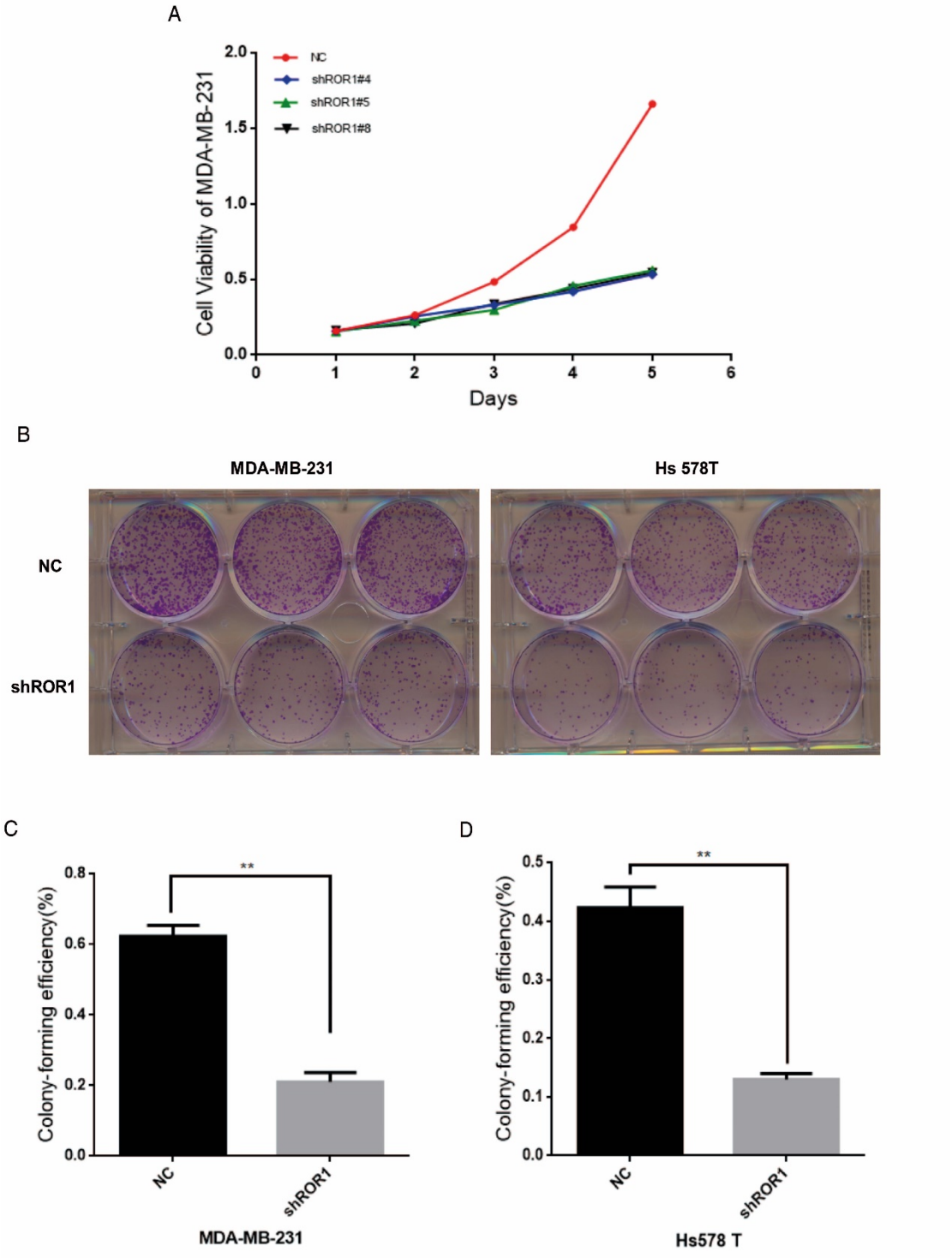
(A) The indicated proteins were analyzed by Western blotting in indicated cells.

Figure S3



Supplemental Figure S3. The promoter activity of ROR1 is marginally affected by mutating E-box in fragment 3. (A) Schematic illustration of wild-type ROR1 promoter and its mutant. **(B)** ROR1 promoters (wild-type or mutant) linked to luciferase were transfected into MDA-MB-231 cells, and after 48h luciferase activity was assayed.

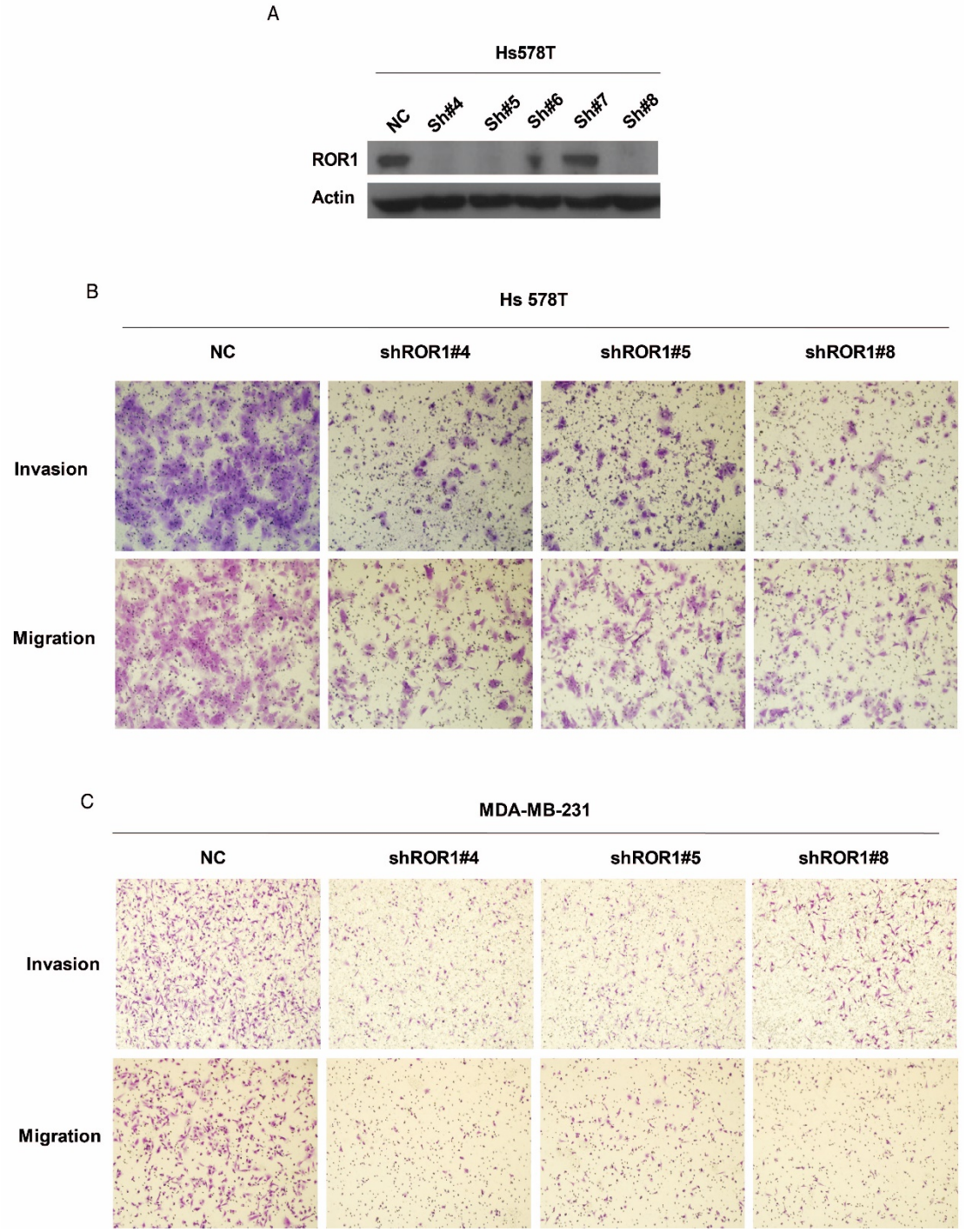
Figure S4



Supplemental Figure S4. Silencing ROR1 inhibits the growth of BLBC cells. (A) The viability of indicated cells stably expressing NC or shROR1 was measured with an MTT. The dots represent the means, and the bars indicate the SD. The results are

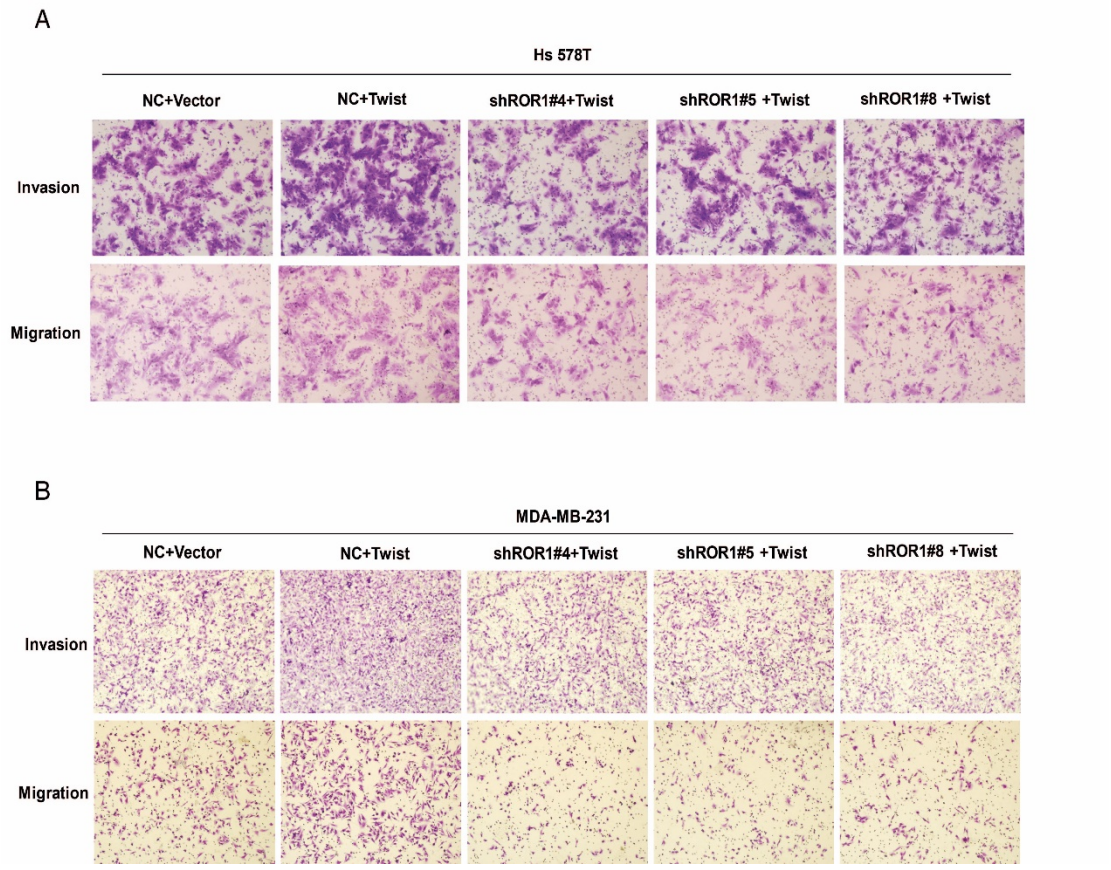
expressed as the mean ± SD of three independent experiments. **(B)** Images of colony formation of indicated cells. **(C)** colony-forming efficiencies were calculated after 12-day conventional culture. Measurements were carried out in triplicate, and experiments were repeated three times. Data are presented as mean ± SD. P values were calculated using Student's t test. **P < 0.01.

Figure S5



Supplemental Figure S5. The knockdown of ROR1 enhances migration and invasion of BLBC cells. (A) Western blot analyses of ROR1 expression in the Hs 578T cells with control or ROR1 shRNA. **(B-C)** Cell migration and invasion were assessed in Hs 578T **(B)** and MDA-MB-231 **(C)** cells stably expressing control or shRNA-ROR1, as described in the Materials and Methods (40X).

Figure S6



Supplementary Figure S6. The promotion of cell migration and invasion after Twist overexpression primarily depends on ROR1. Hs 578T(**A**) and MDA-MB-231(**B**) cells stably expressing control, shRNA-ROR1, Twist or both, as indicated, were subjected to cell migration and invasion assays as described in the Materials and Methods (40X).

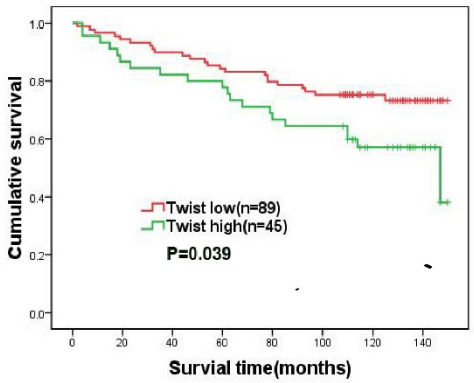
Figure S7

A

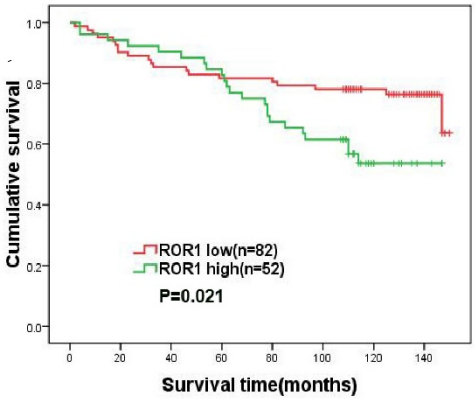
	Twist expression		Total
	Low	High	
ROR1 expression			
Low	60	22	82
High	29	23	52
Total	89	45	134

P=0.038,R=0.180

B



C



Supplementary Figure S7. The combination of Twist and ROR1 correlates with clinical prognosis in breast cancer. (A) A positive correlation was observed between Twist and ROR1 expression in 134 paraffin-embedded breast cancer tissues (p =0.038, $\chi 2$ tests. R: Spearman correlation coefficient). Overall survival curve was generated on the basis of the protein level of Twist (**B**) or ROR1(**C**) in 134 parraffin-embedded breast cancer tissues.

Supplementary Materials and Methods: Primers used in qRT-PCR, the luciferase reporter assay, and the chromatin immunoprecipitation (ChIP) assay.

RNA extraction and qRT-PCR

The primers used to amplify the indicated genes are as follows.

	qRT-PCR Primers	
Gene	**Forward Sequence (5′-3′)**	**Reverse sequence (5′-3′)**
Twist	GTCCGCAGTCTTACGAGGAG	GCTTGAGGGTCTGAATCTTGCT
ROR1	CAGTCAGTGCTGAATTAGTGCC	TCATCGAGG GTCAGGTAAGAAT
Vimentin	GACGCCATCAACACCGAGTT	CTTTGTCGTTGGTTAGCTGGT
ZO-1	GTATCCGATTGTTGTGTTCC	TCACTTGTAGCACCATCCGC
GAPDH	ACAGTCAGCCGCATCTTCTT	GACAAGCTTCCCGTTCTCAG

The luciferase reporter assay

The primers used for cloning the indicated promoters are as follows.

	Primers for promoter clone	
Gene	**Forward Sequence (5′-3′)**	**Reverse sequence (5′-3′)**
ROR1-Mut-1	CCCAGGGCGACTCACGCCCACT GGTGCGACCCGGACAGCCTGGG ACTGACCCGCC	CGTGAGTCGCCCTGGGTCGGA CTCCGAGAACAGCGAAAATTT CGCCCAGGGGCTG
ROR1-Mut-2	CCCAGGGCGACTCACGCCCACT GGTGCGACCCGGAAAATTTTGG ACTGACCCGCC	CGTGAGTCGCCCTGGGTCGGA CTCCGAGAACAGCGCACCCTG CGCCCAGGGGCTG
ROR1-D-Mut	CCCAGGGCGACTCACGCCCACT GGTGCGACCCGGAAAATTTTGG ACTGACCCGCC	CGTGAGTCGCCCTGGGTCGGA CTCCGAGAACAGCGAAAATTT CGCCCAGGGGCTG
ROR1-Mut-3	TAAATCTTGCCAGGAGGCAGTA AATTTACATTGA	CTCCTGGCAAGATTTATTGAGG ATTA

The chromatin immunoprecipitation (ChIP) assay

The primers for the indicated promoters are as follows.

	ChIP-qPCR Primers	
Gene	**Forward Sequence (5′-3′)**	**Reverse sequence (5′-3′)**
ROR1-1	GGTGCCAAGGAGTATGGA	TCGGGAGTTGTTGCTGTT
ROR1-2	CTGAGTGCTGGCTTGGCTAC	CTGGAGGAACAGGAAGAAGC
ROR1-3	AAAGTTAAACCAGGAGAAATGG	CTGGCAAGATTTATTGAGGATT
ROR1-4	CCTCGGTTTCCCCTTCTG	CTCCTCAAACTCGCCACC
ROR1-5	GACCCAGGGCGACTCACG	CAGAACATCCACGGGCTCTTC
Vimentin	CAGCCTATCACAGCCCAGAG	ATGTCTCCTGGAATGGGCAC
GAPDH	CCCAAAGTCCTCCTGTTTCA	GTCTTGAGGCCTGAGCTACG