SUPPLEMENTARY MATERIALS

Supplementary Methods:

RNA Isolation and Quantitative RT-PCR. Total RNA was isolated from 293DLX4, 293Vec cells, MDA-MB-231shVec control cells and DLX4 knockdown cells (shDLX453) using TRIZOL reagent (Invitrogen). Reverse transcription and PCR were performed using mRNA-specific primers as described Supplementary materials, Table S1. The GAPDH RNA was measured as an endogenous control to normalize the relative levels of mRNAs. Semi-Quantitative RT-PCR was performed. Briefly, 1 μg total RNA was reverse transcribed using Random primer method. RT-PCR with duplicate was performed using primers specific for TWIST and DLX4 gene. 10 μl PCRs contained 1 μl cDNA, 1μl 10×AccuPrime™ PCR Buffer I, 0.2 μl AccuPrime™ Taq DNA Polymerase (Invitrogen) and 0.5 μM each primer. Reaction conditions were 30 cycles of 30 s at 95ºC, 30 s at 60-62ºC and 30 s at 68ºC, followed by 5 min at 68°C. The number of cycles during the PCR was limited to the linear phase where the amount PCR product is proportional to the amount of starting material. Semi-quantitative RT-PCR using GAPDH primers under the same conditions, but limiting the number of cycles to 25 was carried out for comparison to the specific products. The sequences of primers used for Semi-Quantitative RT-PCR are shown in the Supplementary materials, Table S1.

Supplementary Figure S1. **TWIST and DLX4 mRNA expression levels in HEK293 DLX4 cell line.** Reverse transcription and PCR were performed using mRNA-specific primers as described Supplementary materials, Table S1. The GAPDH RNA was measured as an endogenous control to normalize the relative levels of mRNAs.

Supplementary Figure S2. **TWIST and DLX4 mRNA expression levels in HEK293 vector control cell line.** Reverse transcription and PCR were performed using mRNA-specific primers as described Supplementary materials, Table S1. The GAPDH RNA was measured as an endogenous control to normalize the relative levels of mRNAs.

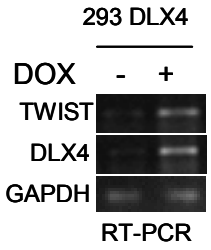
Supplementary Figure S3. **Knockdown efficiency of DLX4 in MDA-MB-231 cell line**. The knockdown efficiency was determined by western blotting with antibodies against DLX4 or Tubulin.

Supplementary Figure S4. **TWIST and DLX4 mRNA expression levels at control cells(shCtrl) and DLX4 knockdown cells (shDLX4/53) in MDA-MB-231 cell line.** Note that knocking down of DLX4 also decreased the expression of TWIST. Reverse transcription and PCR were performed using mRNA-specific primers as described Supplementary materials, Table S1. The GAPDH RNA was measured as an endogenous control to normalize the relative levels of mRNAs.

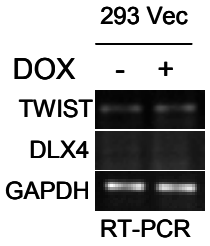
Supplementary Table S1. PCR primers and product sizes

Amplicons	Forward primers	Reverse primers	Size
Cloning primers	DLX4 EcoRV	DLX4 NheI	
DLX4 FL	gccGATATCacctctttgccctgcccc	caaGCTAGCcatcatctgaggcgaagcca	
RT-PCR primers			
TWIST	ACGAGCTGGACTCCAAGATG	CACGCCCTGTTTCTTTGAAT	291
DLX4	CAGAGCACCCTCAGGAACTC	GCAGGGAGACACAGAGAAGG	335
GAPDH	ACAGTCAGCCGCATCTTCTT	TTGATTTTGGAGGGATCTCG	312
ChIP primers			
ChIP primer1	AGCCGCAGAGACCTAAACAA	CAGAATGCAGAGGTGTGAGG	282
ChIP primer2	CGTCAGACTGGGTCGTTGTA	GCATGCCAAGAAAAGTGTCA	214
ChIP primer3	AGTAAGGCAGCGGCTCACTA	GCAGCTTTGGTCTTGGAAAC	230
ChIP primer4	CAGTTTGCAAAGCAGGGAGT	GACGCTCTGATGCCAGAATA	264
ChIP primer5	AATCCTGCCTGAGGAATGTG	GGAGCCAACAAACAGGTGAT	317
ChIP primer6	AAGTGAAGTGAGTGTGTTTTCCAA	CGAACAGTACCGCAAGTGAA	252
ChIP primer7	GAGGATGTCCCAGTTAAAACAAA	GGCTCCTGTAGACCCAGCTA	264

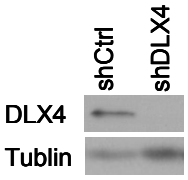
Supplementary Figure S1.



Supplementary Figure S2.



Supplementary Figure S3



Supplementary Figure S4