OMTN, Volume *21*

Supplemental Information

Transcriptional Factor Yin Yang 1 Promotes the Stemness of Breast Cancer Cells by Suppressing miR-873-5p Transcriptional Activity

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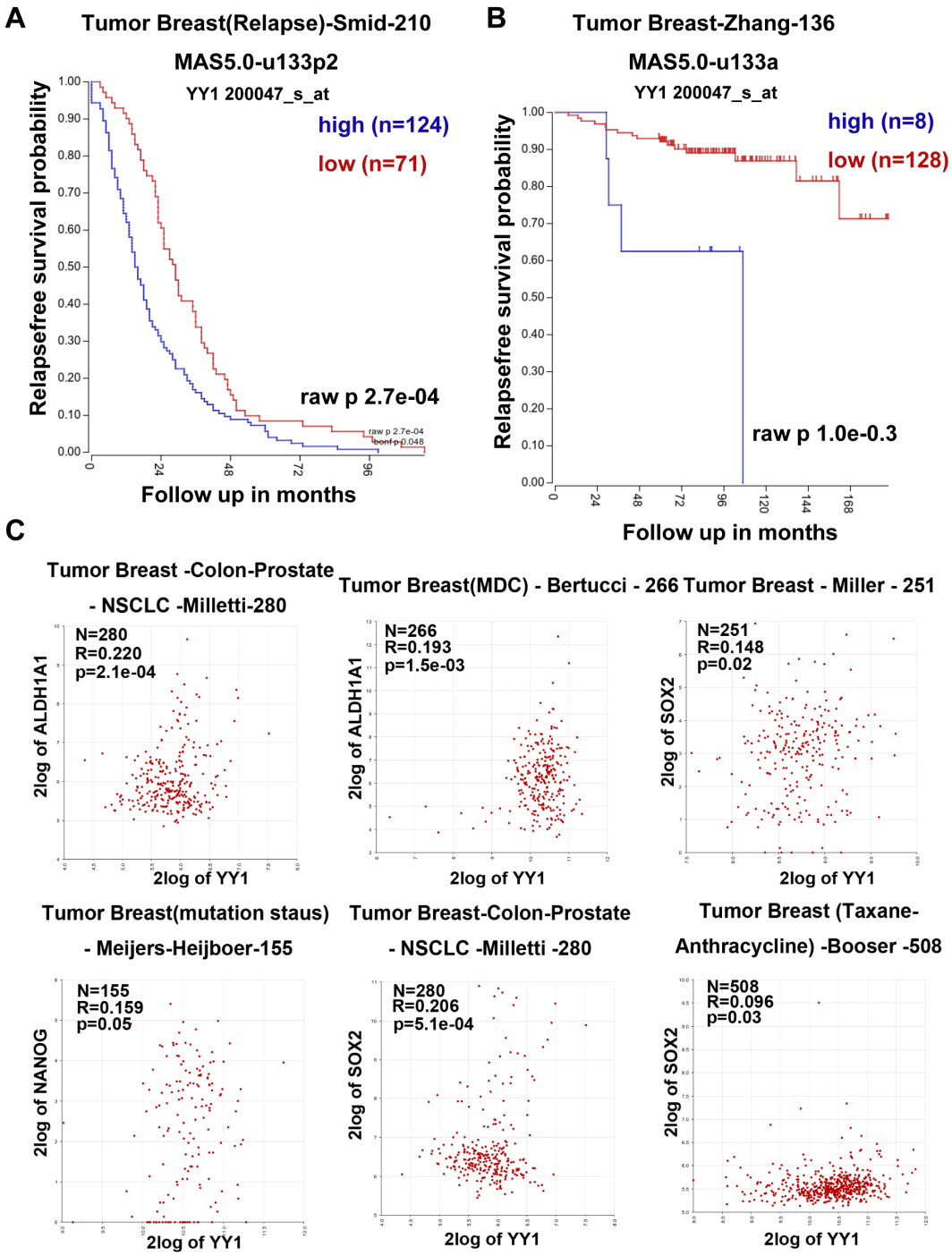

Figure S1 YY1 was positively correlated with the expression of stemness markers in breast cancer tissues via online database. (A and B) Correlation between YY1 expression and relapse free survival of breast cancer patients based on KM-Plotter analysis. **(C)** Correlation between YY1 expression and the expression of ALDH1A1, SOX2, NANOG in breast tumor tissues.

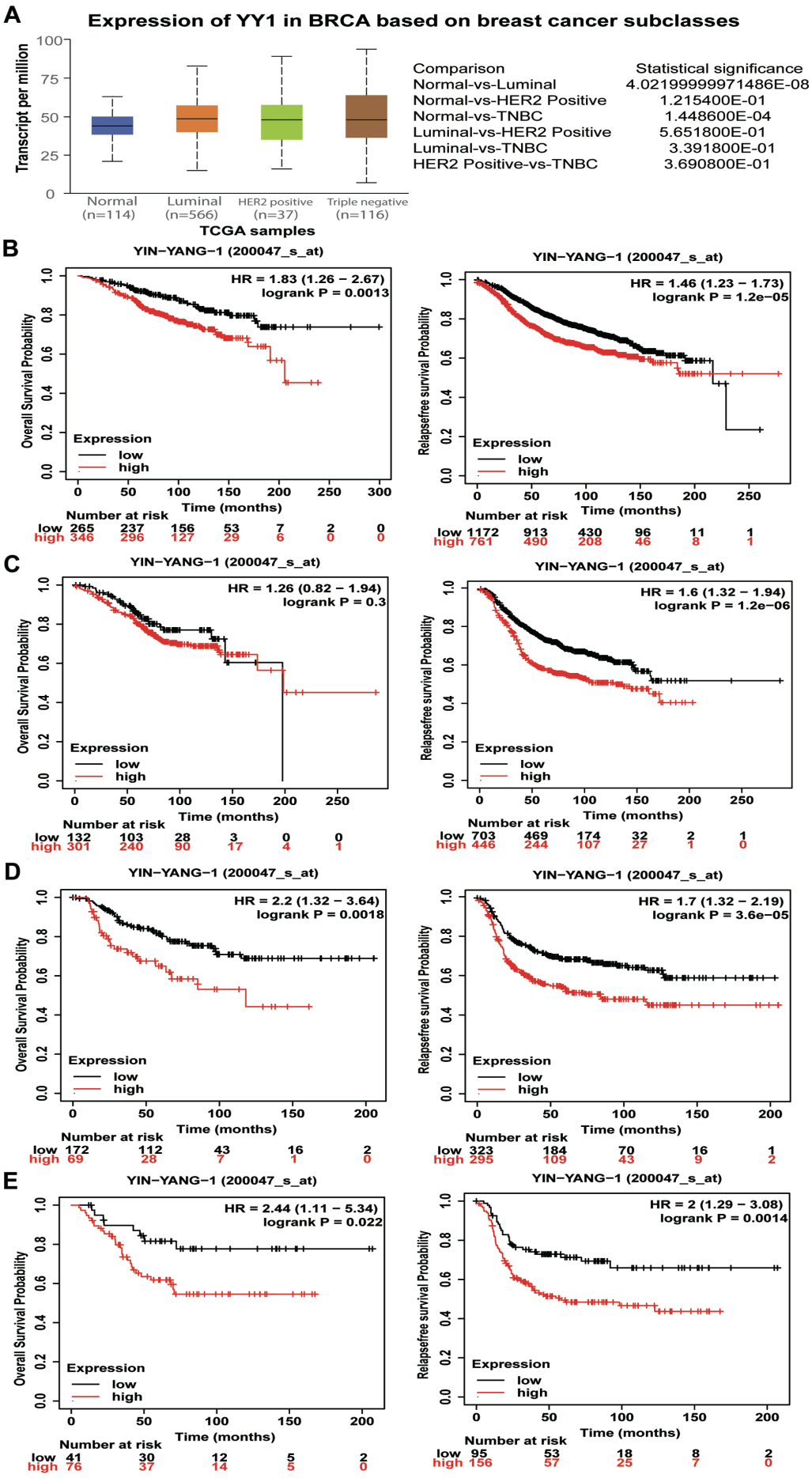
A
Expression of YY1 in BRCA based on breast cancer subclasses
Transcript per million
100
75
50
25
0
Normal (n=114)
Luminal (n=566)
HER2 positive (n=37)
Triple negative (n=116)
TCGA samples
Comparison
Statistical significance
Normal-vs-Luminal
4.02199999971486E-08
Normal-vs-HER2 Positive
1.215400E-01
Normal-vs-TNBC
1.448600E-04
Luminal-vs-HER2 Positive
5.651800E-01
Luminal-vs-TNBC
3.391800E-01
HER2 Positive-vs-TNBC
3.690800E-01
B
YIN−YANG−1 (200047_s_at)
HR = 1.83 (1.26 − 2.67)
logrank P = 0.0013
Overall Survival Probability
Expression
low
high
Time (months)
Number at risk
low 265 237 156 53 7 2 0
high 346 296 127 29 6 0 0
YIN−YANG−1 (200047_s_at)
HR = 1.46 (1.23 − 1.73)
logrank P = 1.2e−05
Relapsefree survival Probability
Expression
low
high
Time (months)
Number at risk
low 1172 913 430 96 11 1
high 761 490 208 46 8 1
C
YIN−YANG−1 (200047_s_at)
HR = 1.26 (0.82 − 1.94)
logrank P = 0.3
Overall Survival Probability
Expression
low
high
Time (months)
Number at risk
low 132 103 28 3 0 0
high 301 240 90 17 4 1
YIN−YANG−1 (200047_s_at)
HR = 1.6 (1.32 − 1.94)
logrank P = 1.2e−06
Relapsefree survival Probability
Expression
low
high
Time (months)
Number at risk
low 703 469 174 32 2 1
high 446 244 107 27 1 0
D
YIN−YANG−1 (200047_s_at)
HR = 2.2 (1.32 − 3.64)
logrank P = 0.0018
Overall Survival Probability
Expression
low
high
Time (months)
Number at risk
low 172 112 43 16 2
high 69 28 7 1 0
YIN−YANG−1 (200047_s_at)
HR = 1.7 (1.32 − 2.19)
logrank P = 3.6e−05
Relapsefree survival Probability
Expression
low
high
Time (months)
Number at risk
low 323 184 70 16 1
high 295 109 43 9 2
E
YIN−YANG−1 (200047_s_at)
HR = 2.44 (1.11 − 5.34)
logrank P = 0.022
Overall Survival Probability
Expression
low
high
Time (months)
Number at risk
low 41 30 12 5 2
high 76 37 14 5 0
YIN−YANG−1 (200047_s_at)
HR = 2 (1.29 − 3.08)
logrank P = 0.0014
Relapsefree survival Probability
Expression
low
high
Time (months)
Number at risk
low 95 53 18 8 2
high 156 57 25 7 0

Figure S2 The level of YY1 and the relationship between YY1 overexpression with patient survival in the four major breast cancer molecular subtypes. (A) The expression of YY1 in the four subtypes of basic breast cancer and normal breast cancer tissues via online clinical posted date. **(B-E)** Correlation between YY1 overexpression with overall survival **(left)** and relapse free survival **(right)** for Luminal A (**B)**, Luminal B **(C)**, base-like **(D)** and HER2 **(E)** on KM-Plotter analysis.

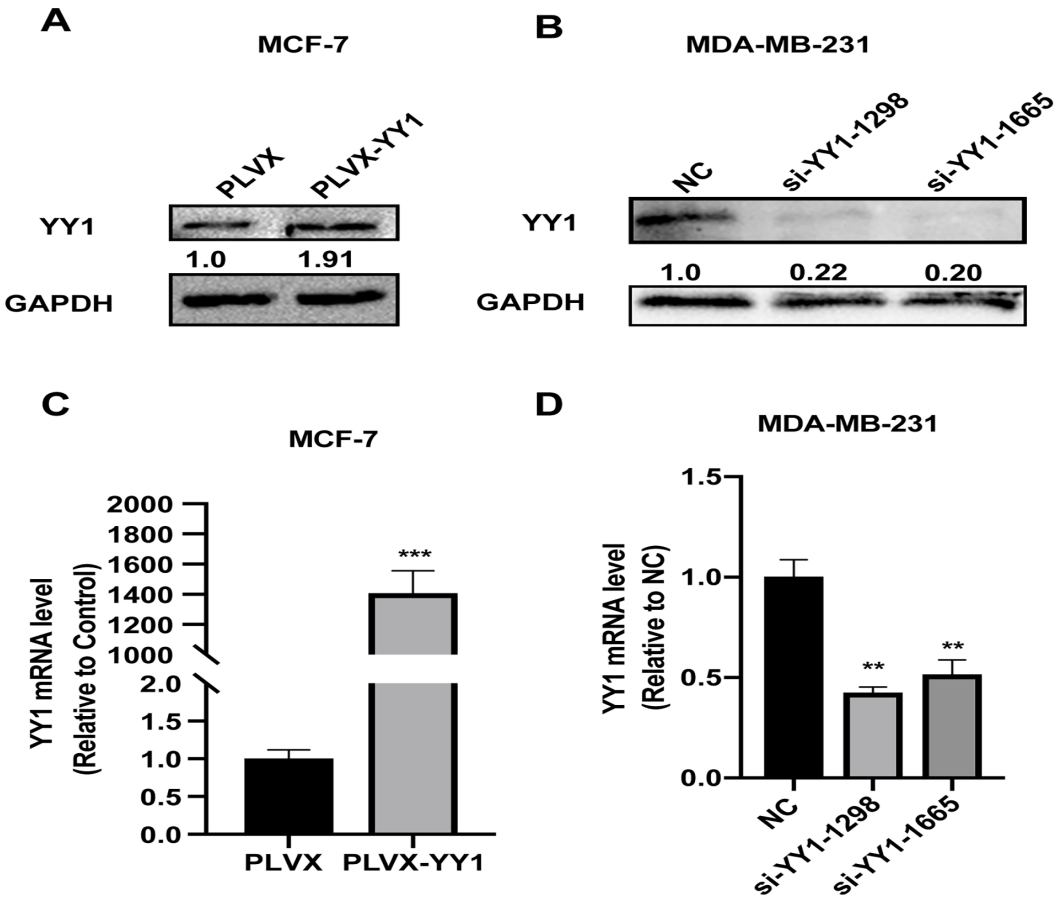

Figure S3 Overexpression and knockdown efficiency of YY1 in MCF-7 and MDA-MB-231 cells. (A-D) The overexpression and knockdown efficiency of YY1 was confirmed in MCF-7 cells with YY1 overexpression or MDA-MB-231 cells with YY1 knockdown by western blot **(A and B)** and qRT-PCR **(C and D)**. Samples derive from the same experiment and that blots were processed in parallel. The data are presented as the mean ± sd, n≥3, *p < 0.05, **p < 0.01, ***p < 0.001 vs PLVX or NC.

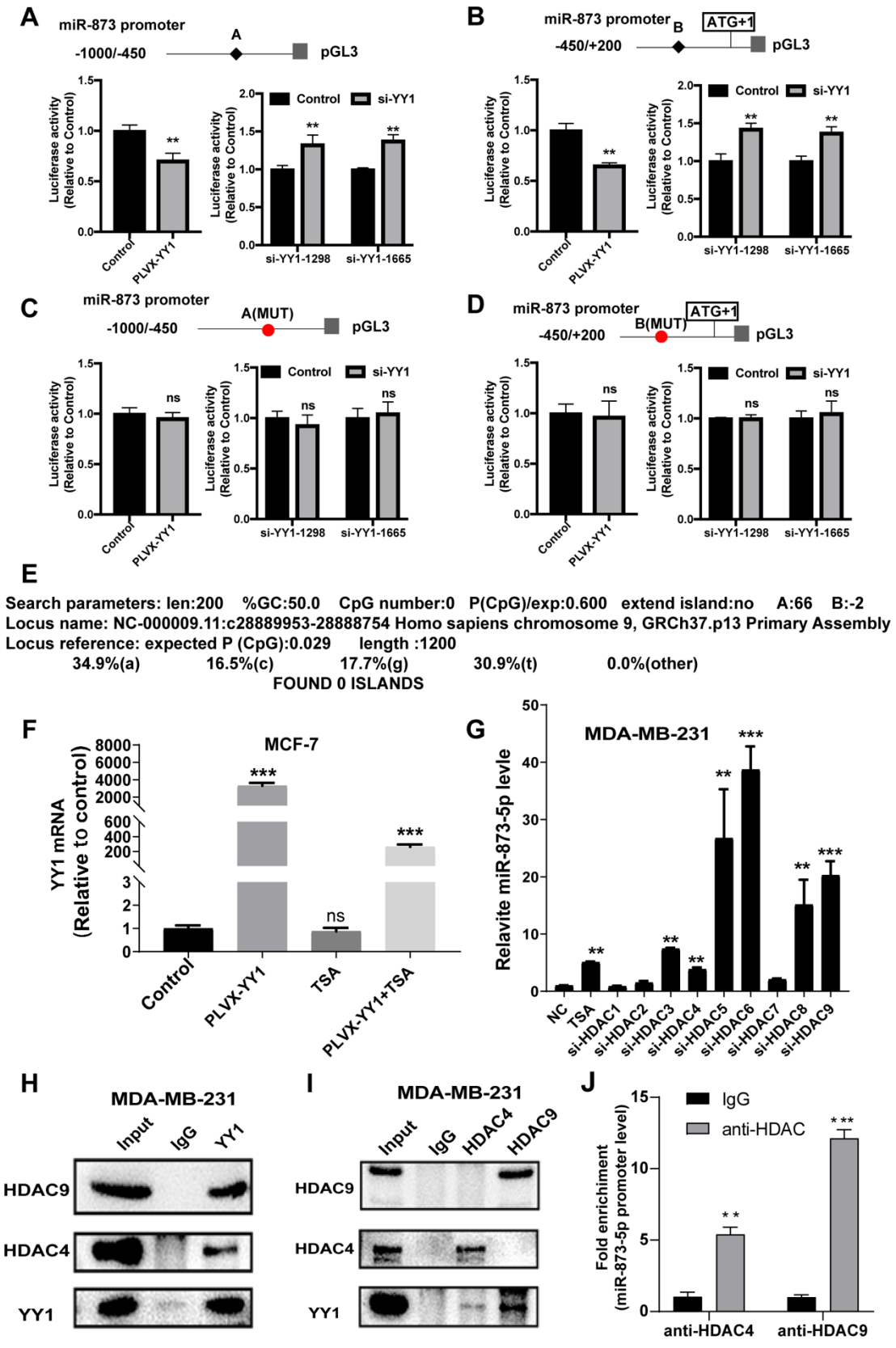

Figure S4 YY1 regulated miR-873-5p expression by regulating the deacetylation level of miR-873-5p promoter. (A-D) Relative luciferase activity of luciferase reporter plasmids (WT or MUT) and measured in HEK293T cells with YY1 overexpression or knockdown. **(E)** Software CpGFinder predicts

CpG islands situation in miR-873-5p promoter**. (F)** The mRNA level of YY1 detected via qRT-PCR in MCF-7 cells with different treatment**.** (**G**) miR-873-5p level was detected in MDA-MB-231 cells with TSA or si-HDAC 1-9, separately. MDA-MB-231 cell, followed by Co-immunoprecipitation **(Co-IP)** with anti-YY1, HDAC4, HDAC9, and rabbit IgG as control **(H-I)**. **(J)** ChIP assay was performed to detect the abundance of miR-873-5p promoter pulled down by Anti-HDAC4 and Anti-HDAC9.The data are presented as the mean ± sd, n≥3, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs PLVX or NC

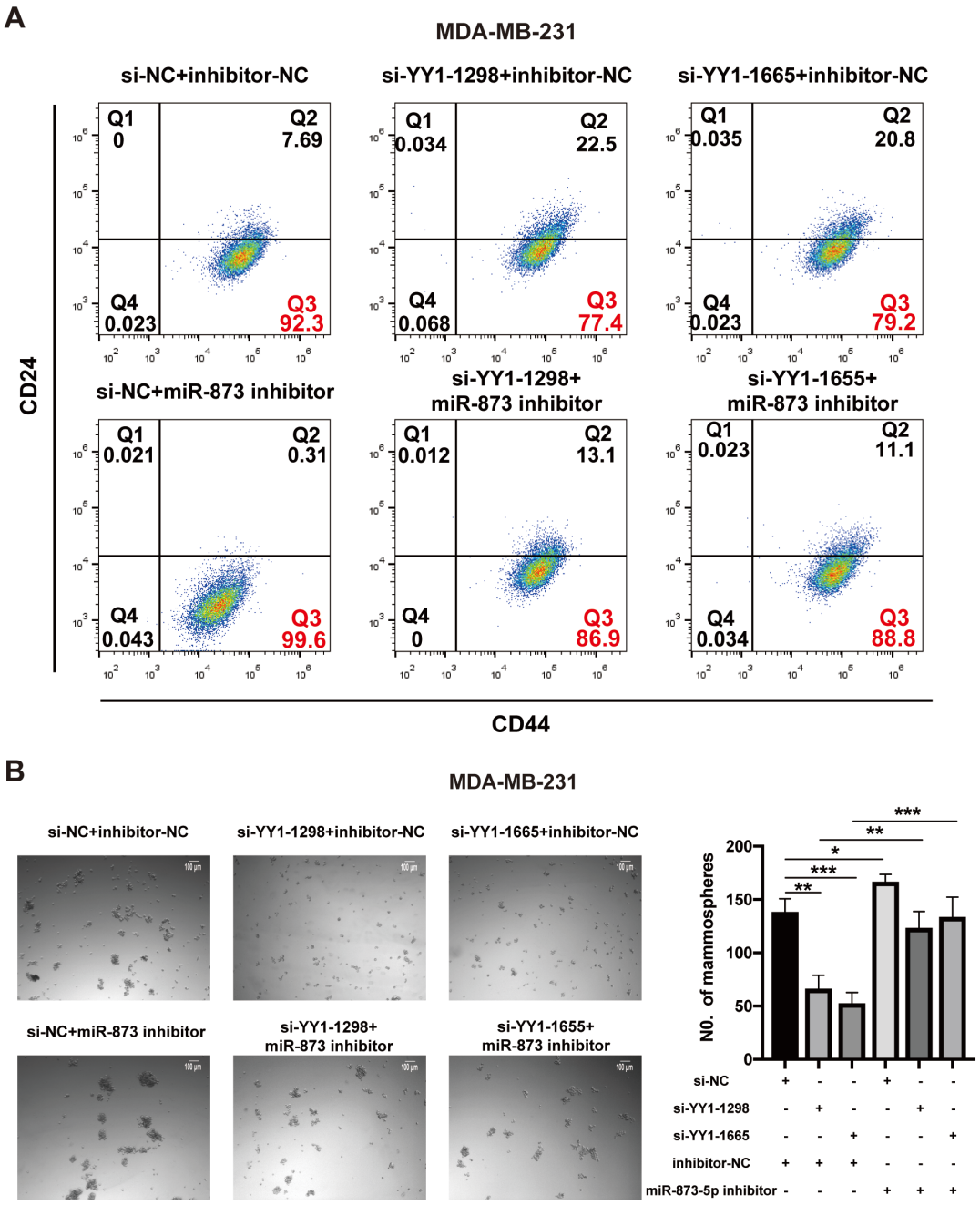

Figure S5 YY1 regulated the stemness of breast cancer cells by regulating the level of miR-873-5p. (A and B) $CD44^{+}/CD24^{-}$ population or spheroid formation (50X) was measured in cells with different treatment. The data are presented as the mean ± sd, n≥3, *p < 0.05, **p < 0.01, vs PLVX or PLVX-YY1

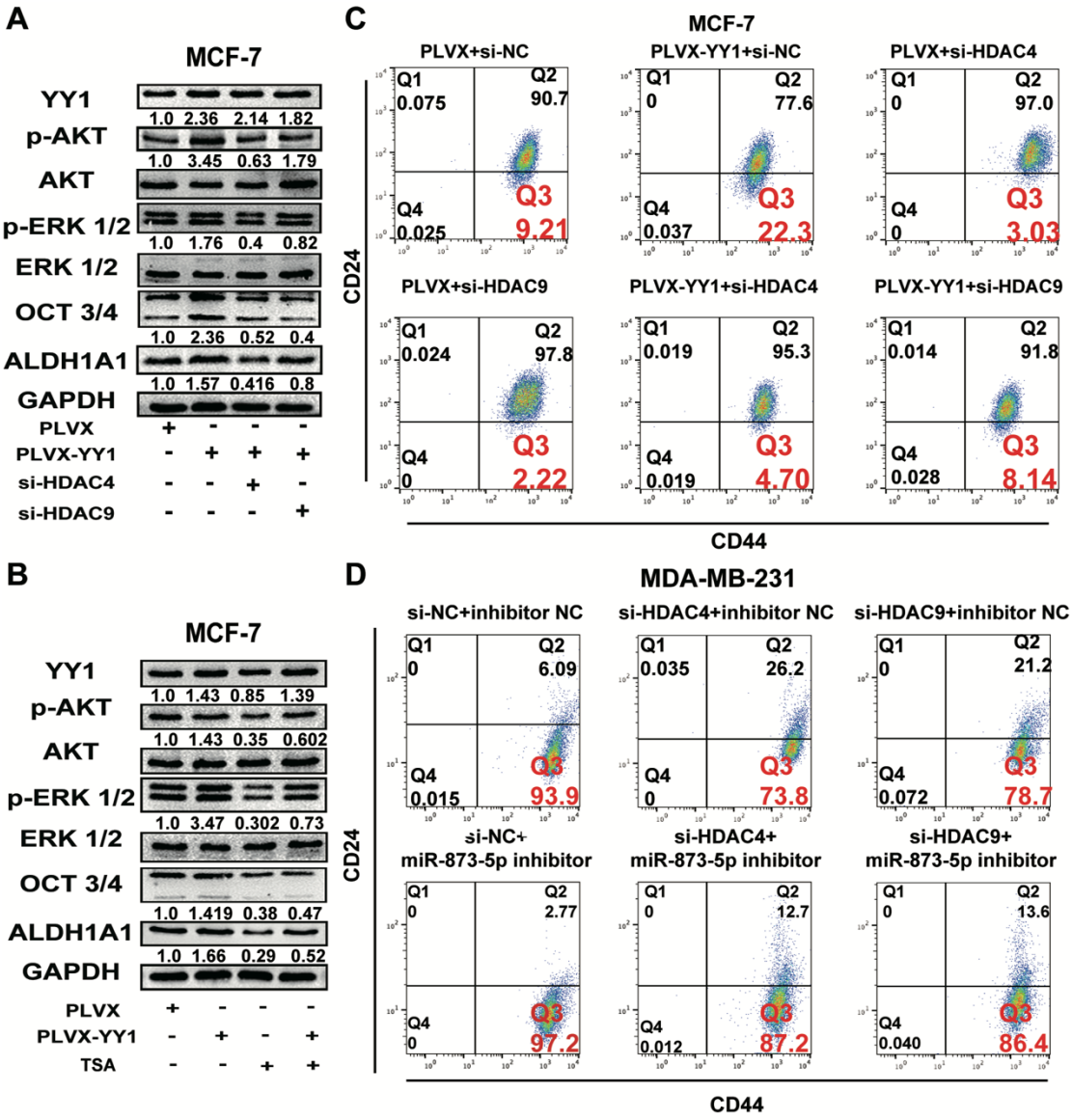

Figure S6 HDAC knockdown reduced the stemness of breast cancer cells dependent on miR-873-5p. (A and B) Expression of stemness markers (ALDH1A1 and OCT3/4), p-AKT and p-ERK1/2 was detected by western blot in MCF-7 cells with TSA **(A)** or HDAC siRNA **(B)**. **(C and D)** $CD44^+/CD24^-$ population was determined via flow cytometry analysis in MCF-7 cells and MDA-MB-231 cells with different treatment. Samples derive from the same experiment and that blots were processed in parallel. The data are presented as the mean ± sd, n≥3, *p < 0.05, **p < 0.01, ***p < 0.001 vs PLVX or NC.

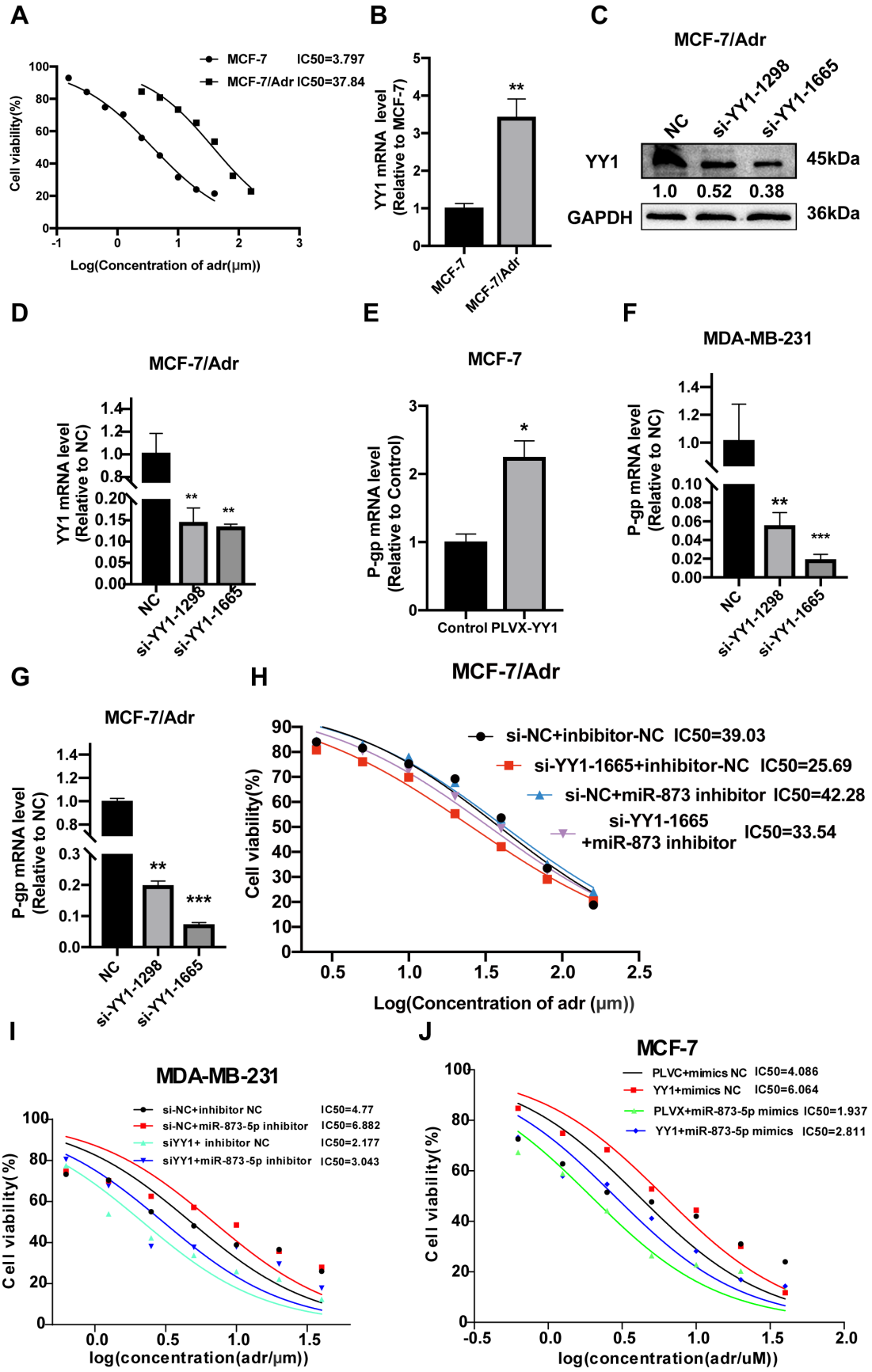

Figure S7 YY1 knockdown attenuated adriamycin resistance dependent on miR-873-5p. (A) IC50 values of adriamycin in MCF-7 and MCF-7/Adr cells. **(B)** YY1 expression was measured in MCF-

7 and MCF-7/Adr cells via qRT-PCR. **(C and D)** Knockdown efficiency of YY1 was confirmed in MCF-7/Adr cells by western blot and qRT-PCR assay. **(E)** PGP mRNA level was detected in MCF-7 cells with YY1 overexpression. **(F and G)** PGP mRNA level was examined in MDA-MB-231 and MCF/Adr cells with YY1 knockdown. (**H-J**) IC50 values of adriamycin in MCF-7/Adr, MDA-MB-231 and MCF-7 cells with YY1 knockdown plus miR-873-5p inhibitor transfection or not. The data are presented as the mean ± sd, $n \geq 3$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs PLVX or NC.

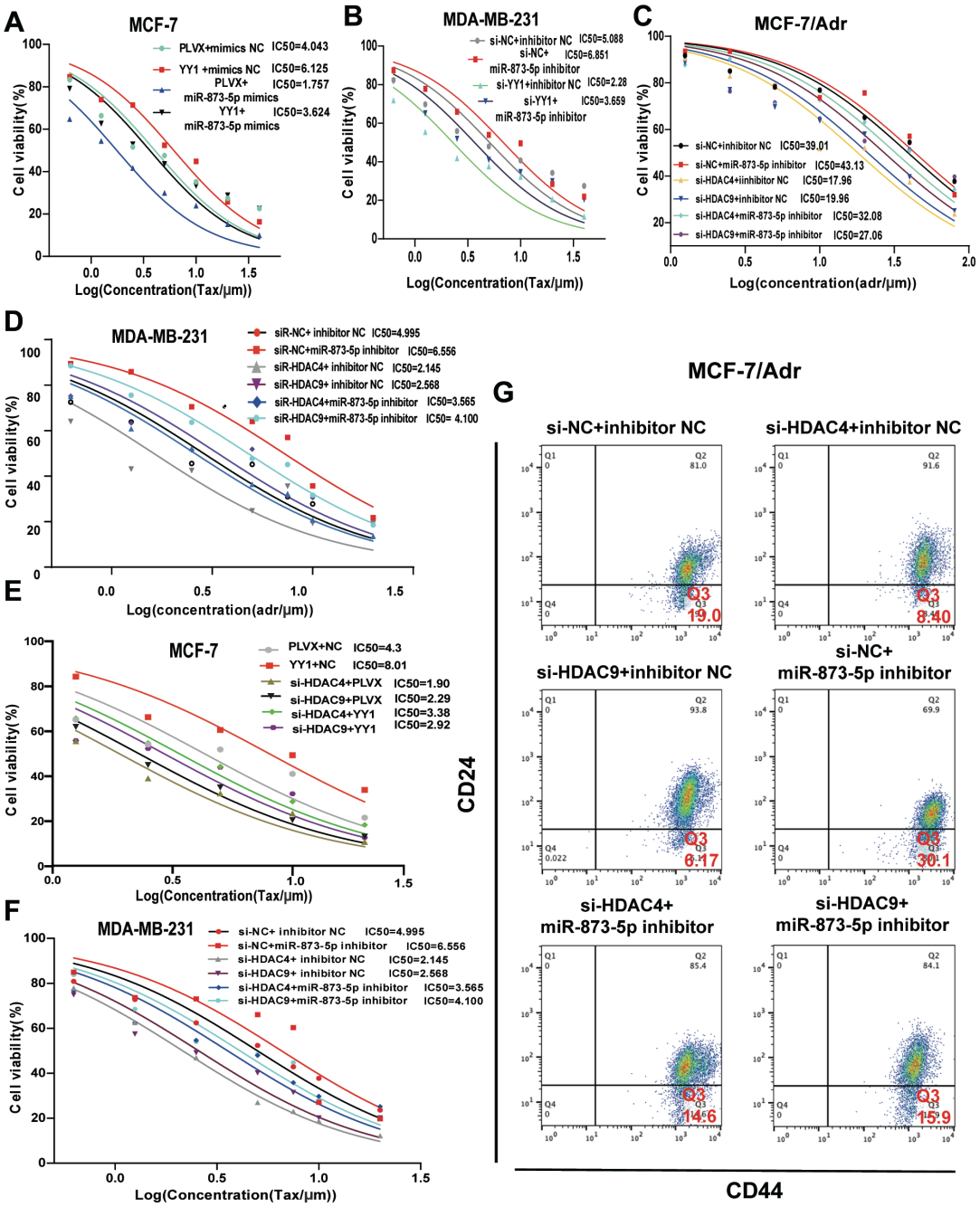

Figure S8 YY1 confers chemotherapeutic resistance through miR-873-5p and HDAC4/9. (A-B) IC50 values of taxol in MCF-7 and MDA-MB-231 cells with different treatment. **(C-F)** IC50 values of adriamycin in MCF-7, MDA-MB-231 and MCF-7/Adr cells with different treatment. **(G)** $CD44^{+}/CD24^{-}$ population was measured in cells with different treatment. Samples derive from the same experiment and that blots were processed in parallel. The data are presented as the mean ± sd, n≥3, *p < 0.05, **p < 0.01, ***p < 0.001 vs PLVX or NC.

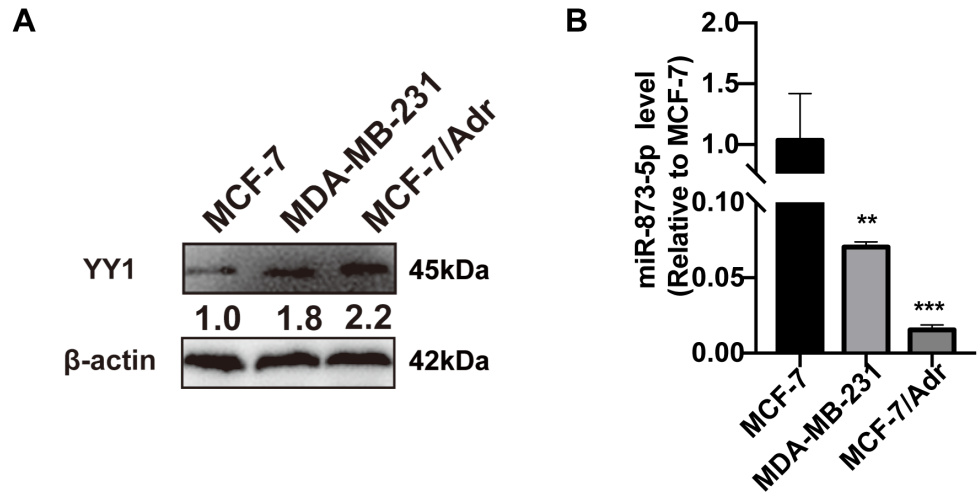

Figure S9 The level of YY1 and miR-873-5p in different breast cancer cells. (A) YY1 level was detected by Western blot in MCF-7, MDA-MB-231 and MCF-7/Adr cells. **(B)** miR-873-5p level detected by qRT-PCR in MCF-7, MDA-MB-231 and MCF-7/Adr cells. The data are presented as the mean ± sd, n≥3, **p < 0.01, ***p < 0.001 vs MCF-7.

Supplemental Table 1. Sequences of primers used for siRNA and miRNA

siR-YY1-1298	Forward (5’-3’)	CCAAACAACUGGCAGAAUU
	Reverse (5’-3’)	AAUUCUGCCAGUUGUUUGG
siR-YY1-1665	Forward (5’-3’)	UCAGUCAACUAACACUGAAA
	Reverse (5’-3’)	UUUCAGUGUUAGUUGACUGA
LV3-NC	Forward(5’-3’)	TTCTCCGAACGTGTCACGT
	Reverse (5’-3’)	ACGTGACACGTTCGGAGAA
LV3-has-miR-873-5p mimics	Forward (5’-3’)	GCAGGAACTTGTGAGTCTCCT
	Reverse (5’-3’)	AGGAGACTCACAAGTTCCTGC
LV3-has-miR-873-5p inhibitor	Forward (5’-3’)	AGGAGACTCACAAGTTCCTGC
	Reverse (5’-3’)	GCAGGAACTTGTGAGTCTCCT
siR-HDAC1	Forward (5’-3’)	CCCGGAGGAAAGUCUGUUA
	Reverse (5’-3’)	UAACAGACUUUCCUCCGGG
siR-HDAC2	Forward (5’-3’)	CCCAUAACUUGCUGUUAAA
	Reverse (5’-3’)	UUUAACAGCAAGUUAUGGG
siR-HDAC3	Forward (5’-3’)	CCUGCAUUACGGUCUCUAU
	Reverse (5’-3’)	AUAGAGACCGUAAUGCAGG
siR-HDAC4	Forward (5’-3’)	CGACUCAUCUUGUAGCUUAUU
	Reverse (5’-3’)	AAUAAGCUACAAGAUGAGUCG
siR-HDAC5	Forward (5’-3’)	CAUUGCCCACGAGUUCUCACCUGAU
	Reverse (5’-3’)	AUCAGGUGAGAACUCGUGGGCAAUG
siR-HDAC6	Forward (5’-3’)	GCACCAUGGUCAAGGAACA
	Reverse (5’-3’)	UGUUCCUUGACCAUGGUGC
siR-HDAC7	Forward (5’-3’)	AUCAGUUGCUGCGUCAUGUdTdT
	Reverse (5’-3’)	ACAUGACGCAGCAACUGAUdTdT
siR-HDAC8	Forward (5’-3’)	CAUUCAGGAUGGCAUACAA
	Reverse (5’-3’)	UUGUAUGCCAUCCUGAAUG
siR-HDAC9	Forward (5’-3’)	GCCAGUAGUCCUAGGUUAUUGUGUAdTdT
	Reverse (5’-3’)	UACACAAUAACCUAGGACUACUGGCdTdT
siR-NC	Forward (5’-3’)	**UUCUCCGAACGUGUCACGU**
	Reverse (5’-3’)	ACGUGACACGUUCGGAGAA

Supplemental Table 2. Sequences of primers used for qRT-PCR in this study

Name		Sequences
YY1	Forward (5'-3')	AAGAGCGGCAAGAAGAGTTAC
	Reverse (5'-3')	CAACCACTGTCTCATGGTCAATA
ALDH1	Forward (5'-3')	AGCCTTCACAGGATCAACAGA
	Reverse (5'-3')	GTCGGCATCAGCTAACACAA
Nanog	Forward (5'-3')	GCAGGCAACTCACTTTATCC
	Reverse (5'-3')	CCCACAAATCACAGGCATAG
OCT4	Forward (5'-3')	AGCGATCAAGCAGCGACTA
	Reverse (5'-3')	GGAAAGGGACCGAGGAGTA
Sox2	Forward (5'-3')	CATCACCCACAGCAAATGAC
	Reverse (5'-3')	CAAAGCTCCTACCGTACCACT
GAPDH	Forward (5'-3')	CTTAGTTGCGTTACACCCTTTCTTG
	Reverse (5'-3')	CTGTCACCTTCACCGTTCCAGTTT

Supplemental Table 3. Sequences of primers used for Luciferase reporter assay

Name		Sequences
PGL3-miR-873-5p -promoter (-1000/+200)	Forward (5'-3')	GGTACCGGAAAAAAGAAAAACAGGATGGTGC
	Reverse (5'-3')	CTCGAGCATTTGCAGATAAGCAGCTGTTCAT
PGL3-miR-873-5p -promoter (-1000/-450)	Forward (5'-3')	GGGGTACCTTGAACAAACTAACATAGGCAAAAT
	Reverse (5'-3')	CCGCTCGAGATAGCAGTATTTCCATGTCACACCA
PGL3-miR-873-5p -promoter (-450/+200)	Forward (5'-3')	GGGGTACCGAATTCTGATCTCGTAGTTCCCTTT
	Reverse (5'-3')	CCGCTCGAGTTCATTTTCAATAGGAGACTCACAA
PGL3-miR-873-5p -promoter A (MUT)	Forward (5'-3')	ACGGTGGTGGGGTAAAATAGCCCAAATAATAATTTT TAAAATT
	Reverse (5'-3')	TATTTTCCCCACCACCGTGTGATGATGATATAATAA CTGGAAACA
PGL3-miR-873-5p -promoter B (MUT)	Forward (5'-3')	TATTCACGGGACCTCCTTGAACAGCTGCTTATCTGC AAATG
	Reverse (5'-3')	AAGGAGGTCCCGTGAATACAGTAATCTGTTCCCCTG GA