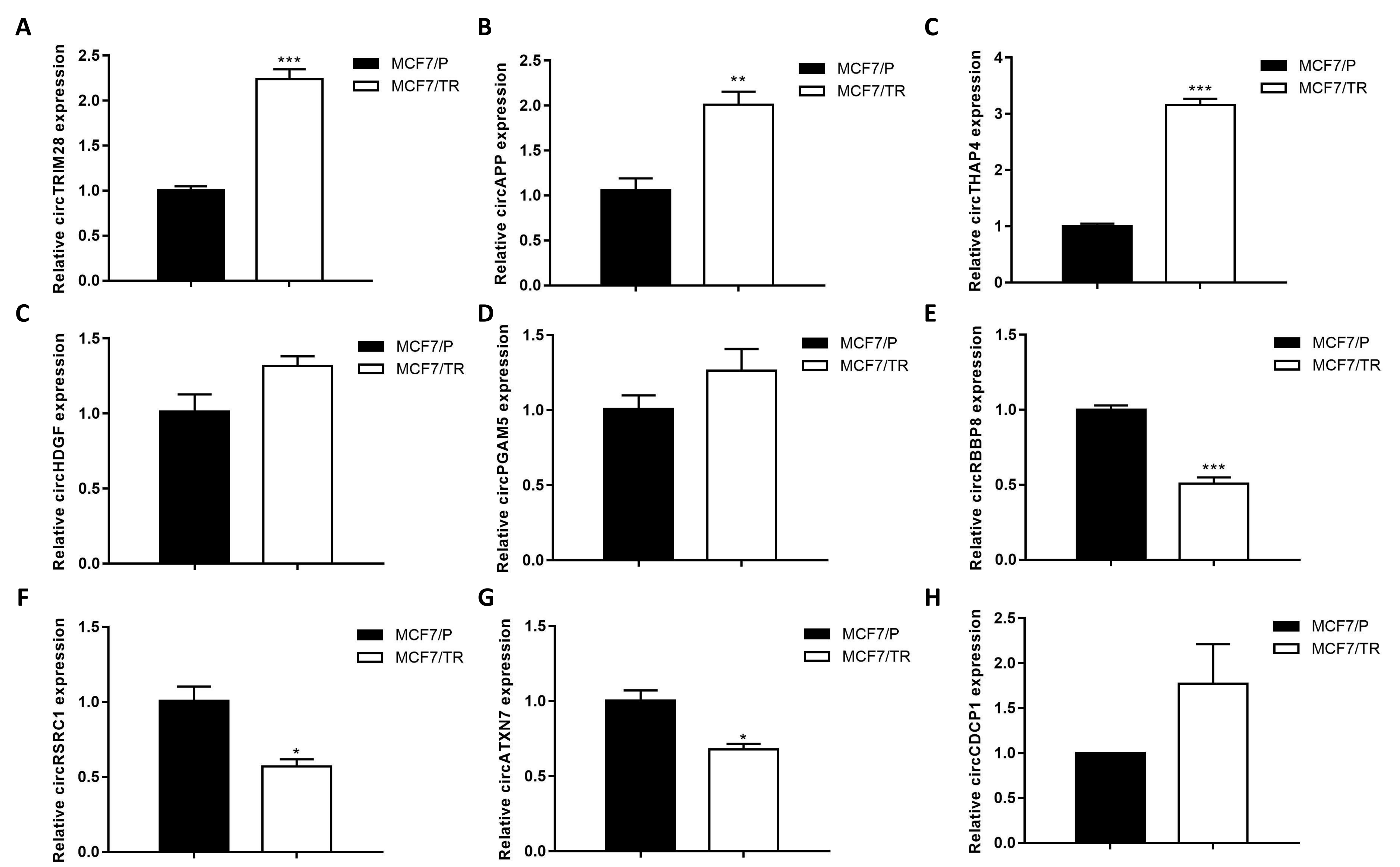


Supplemental Information

**circRNA_0025202 Regulates Tamoxifen Sensitivity
and Tumor Progression via Regulating
the miR-182-5p/FOXO3a Axis in Breast Cancer**

Yuting Sang, Bing Chen, Xiaojin Song, Yaming Li, Yiran Liang, Dianwen Han, Ning Zhang, Hanwen Zhang, Ying Liu, Tong Chen, Chen Li, Lijuan Wang, Wenjing Zhao, and Qifeng Yang



I

ID	hsa_circ_0025202					
Position	chr12:6646474-6647162					
Strand	+					
Genomic length	688					
Spliced sequence length	495					
Annotation	ANNOTATED CDS coding INTERNAL OVCODE OVERLAPTX OVEXON					
Repeats	NA					
Best transcript	NM_002046					
Gene symbol	GAPDH					
Scores	circRNA study	Sample	total reads circular junction	unique reads circular junction	unique reads linear-5' junctions	unique reads linear-3' junctions
		K562				
		Gm12878				
		Sknshra				
		H1hesc				
	Salzman2013	Nhek				
		Hepg2				
		A549				
	Ag04450					

Figure S1

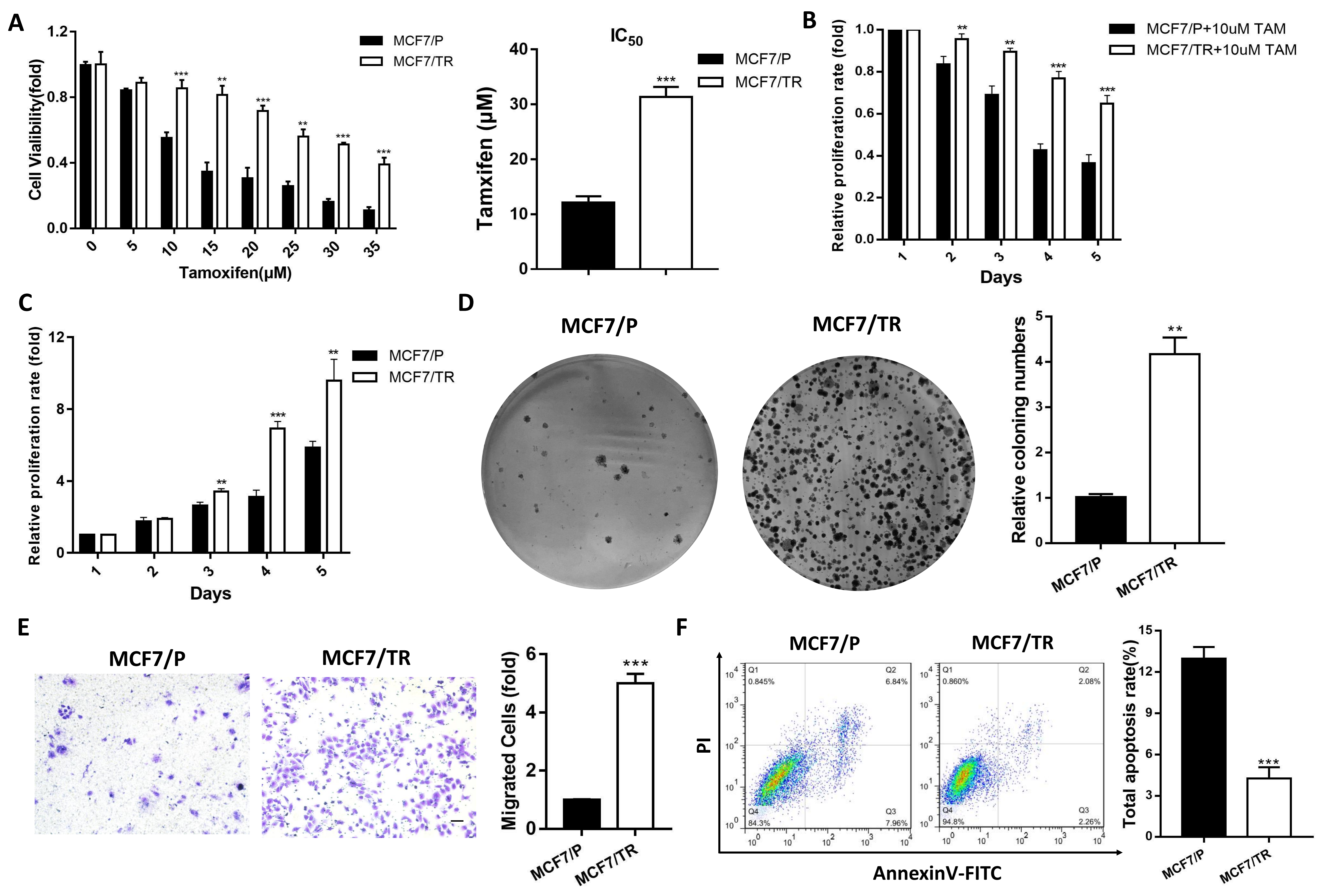


Figure S2

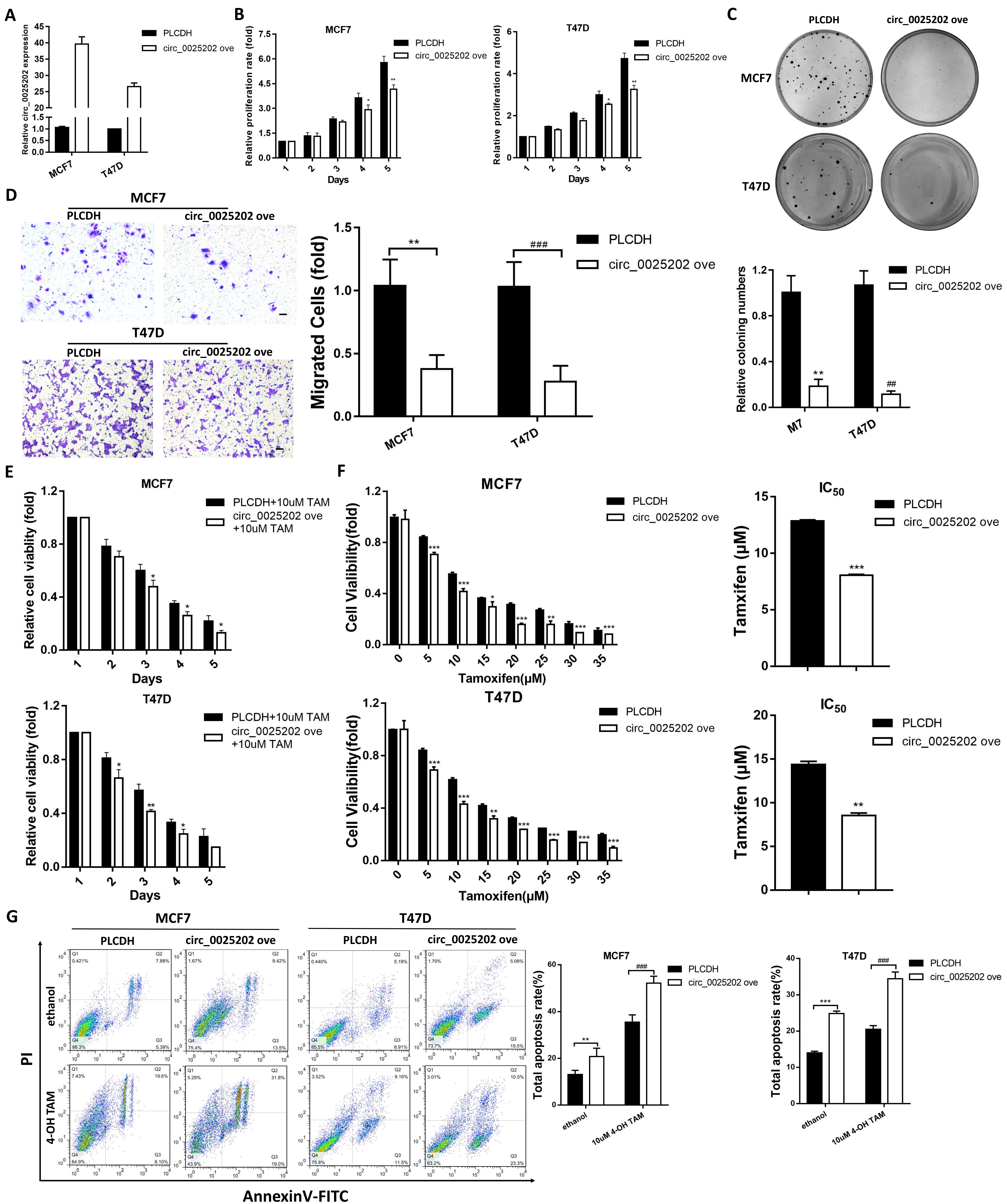


Figure S3

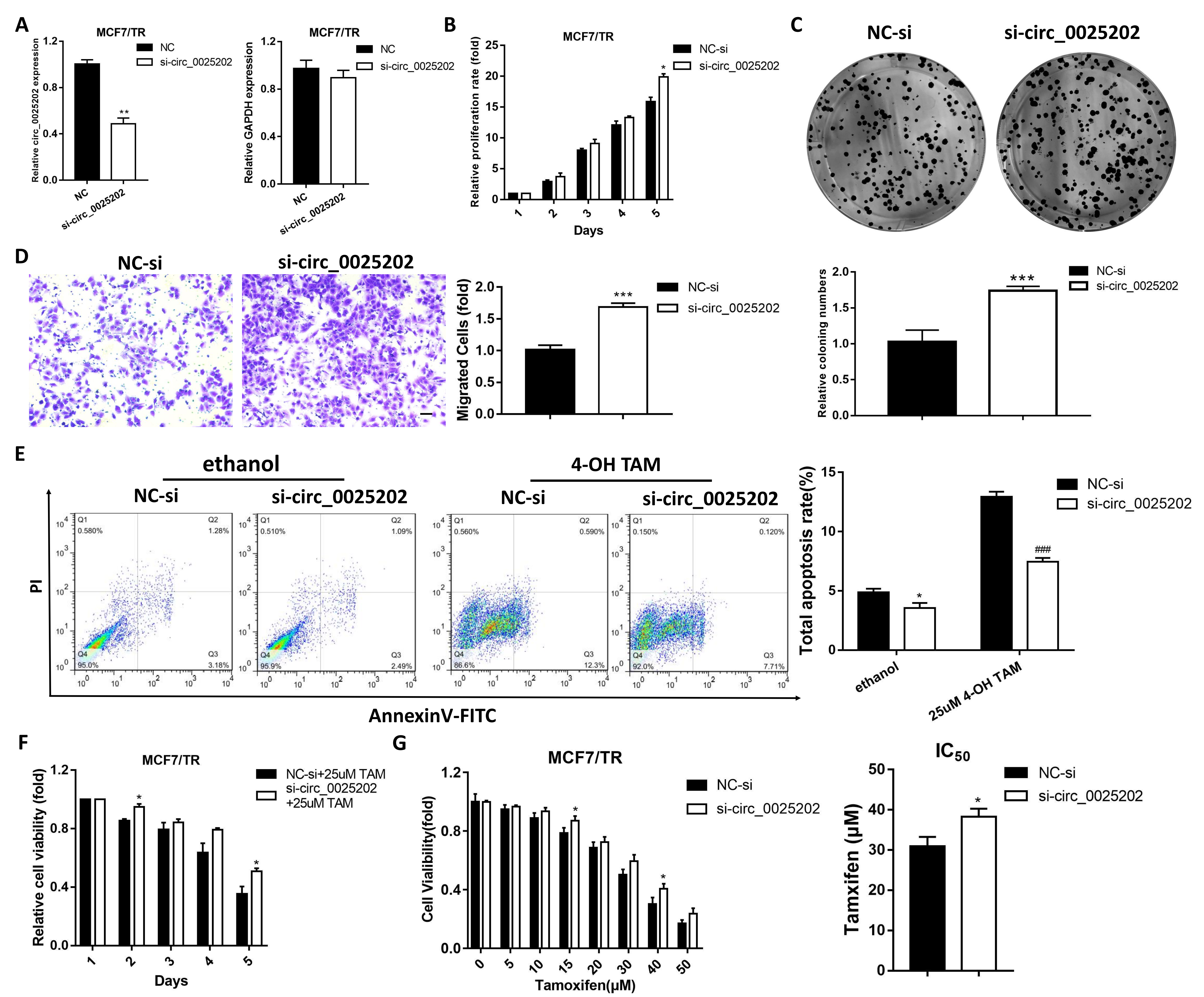


Figure S4

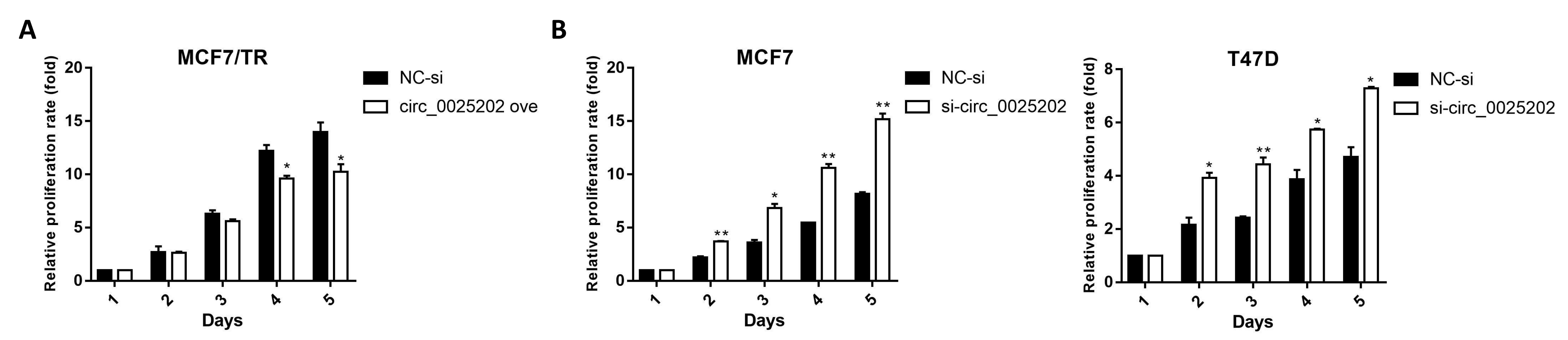


Figure S5

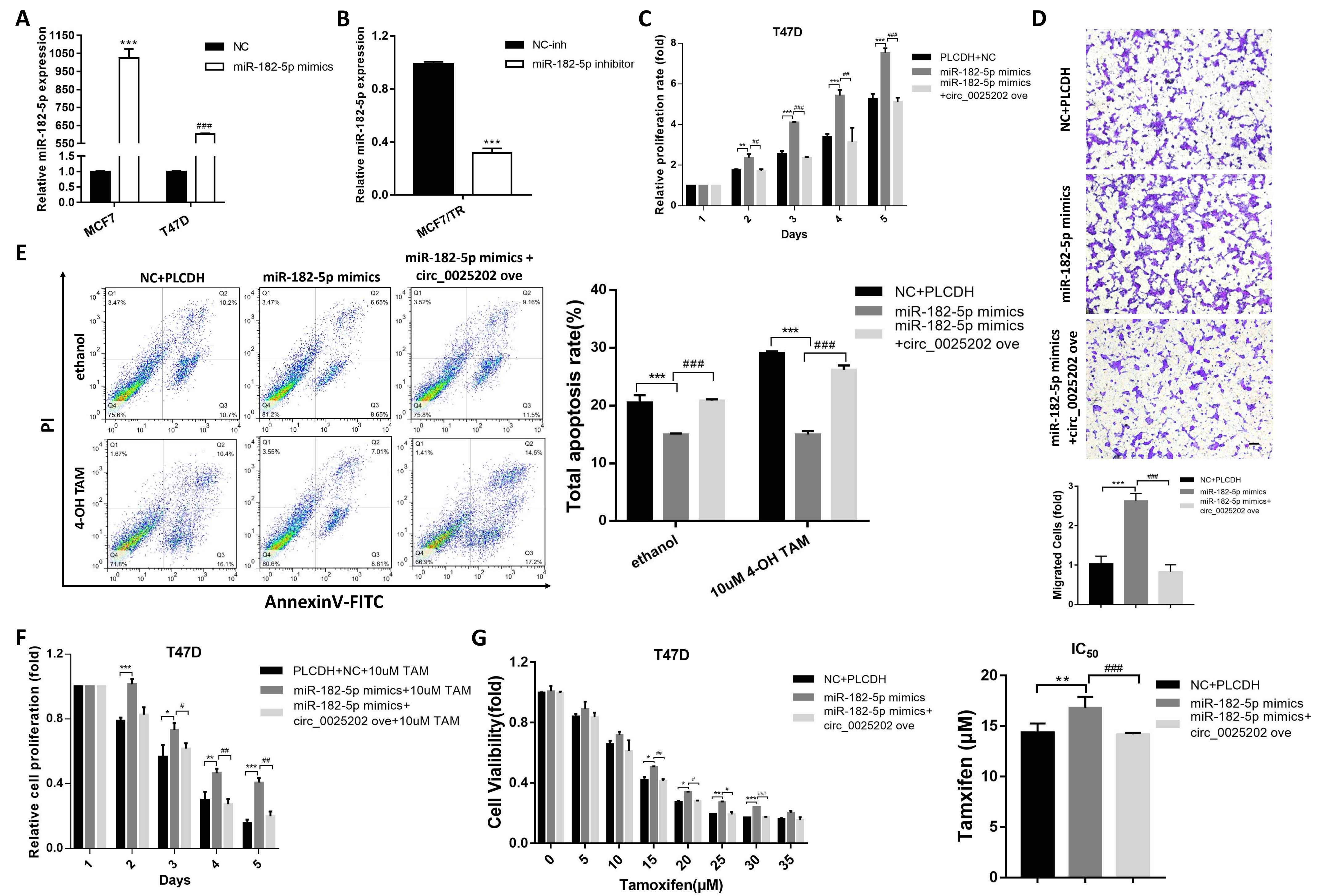


Figure S6

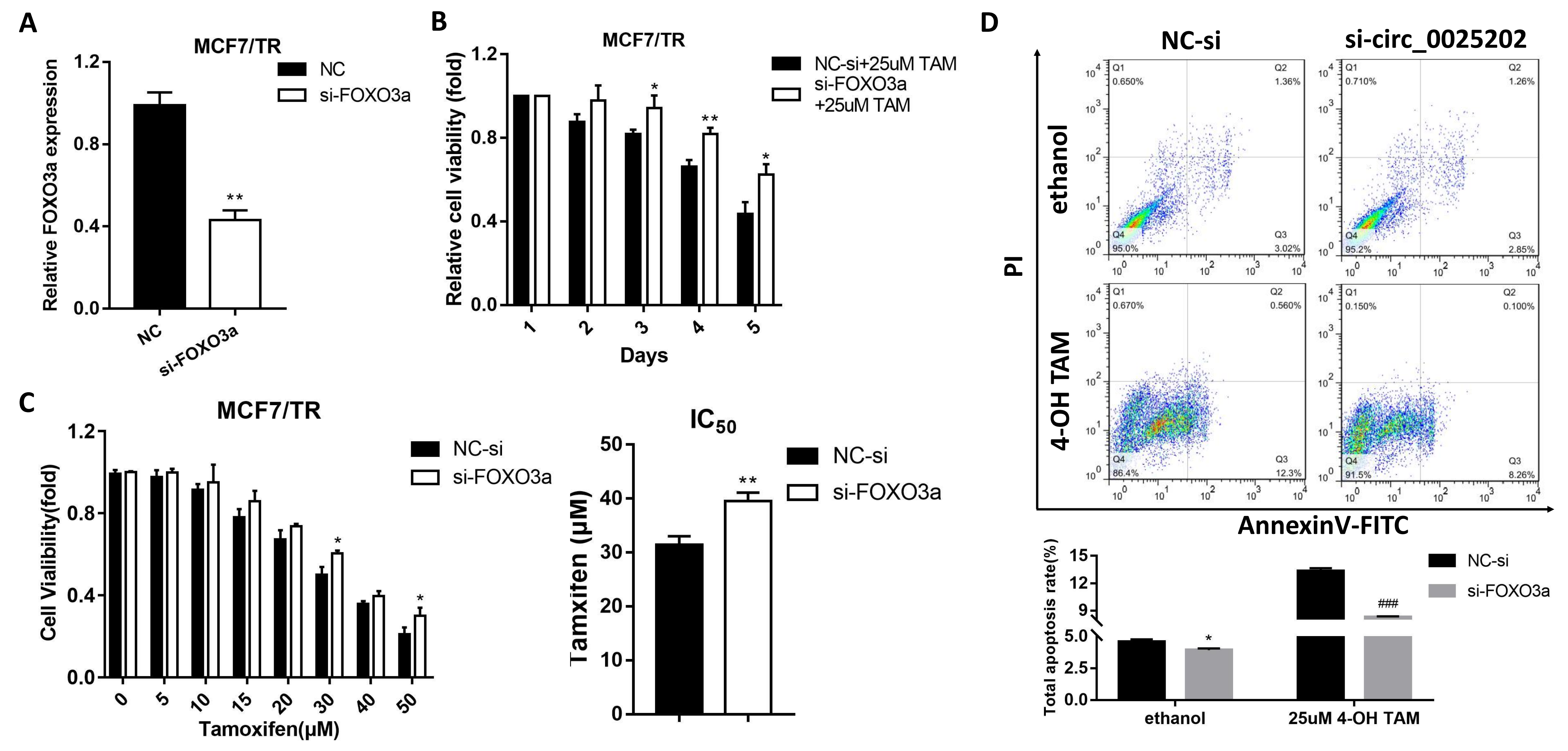


Figure S7

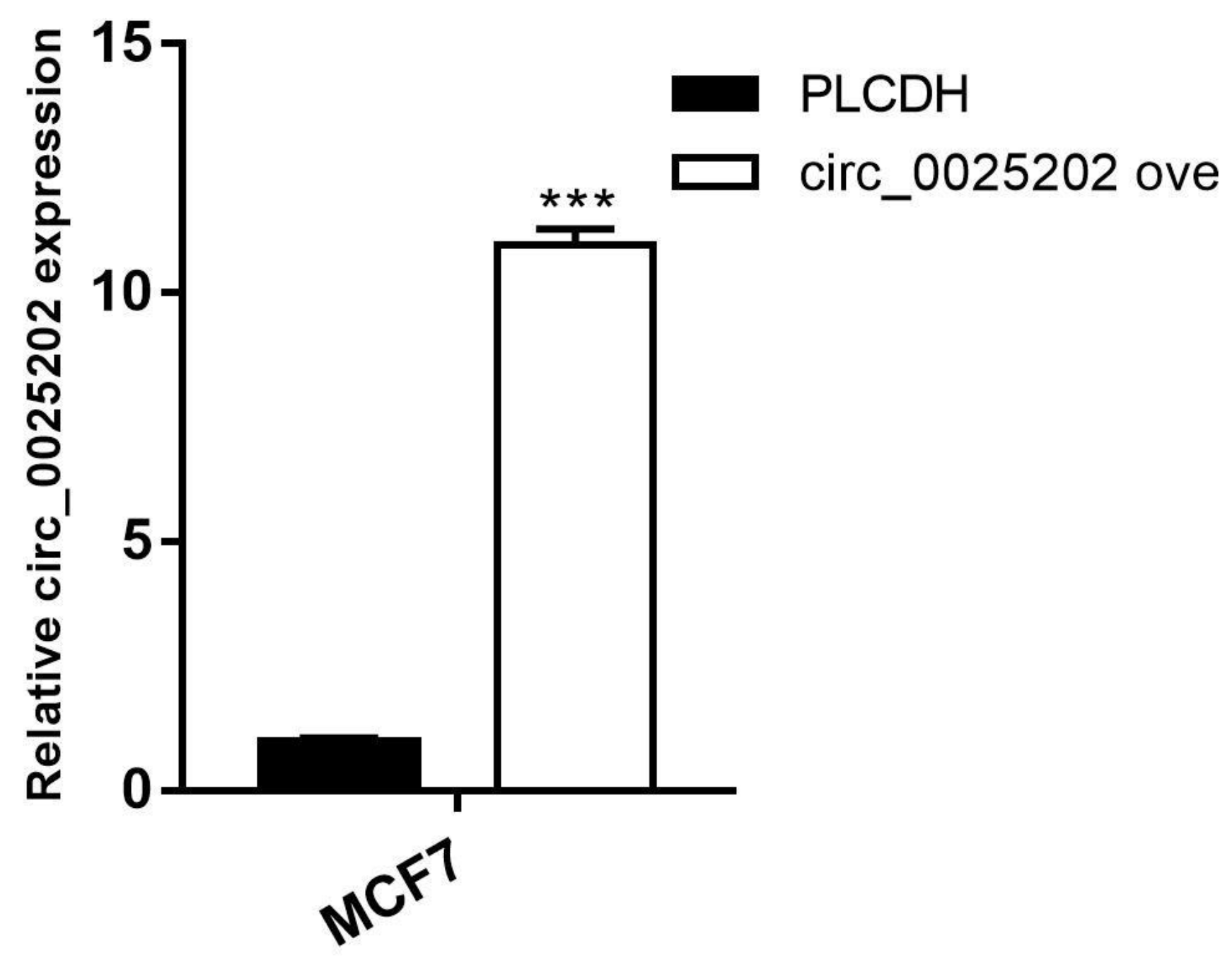


Figure S8

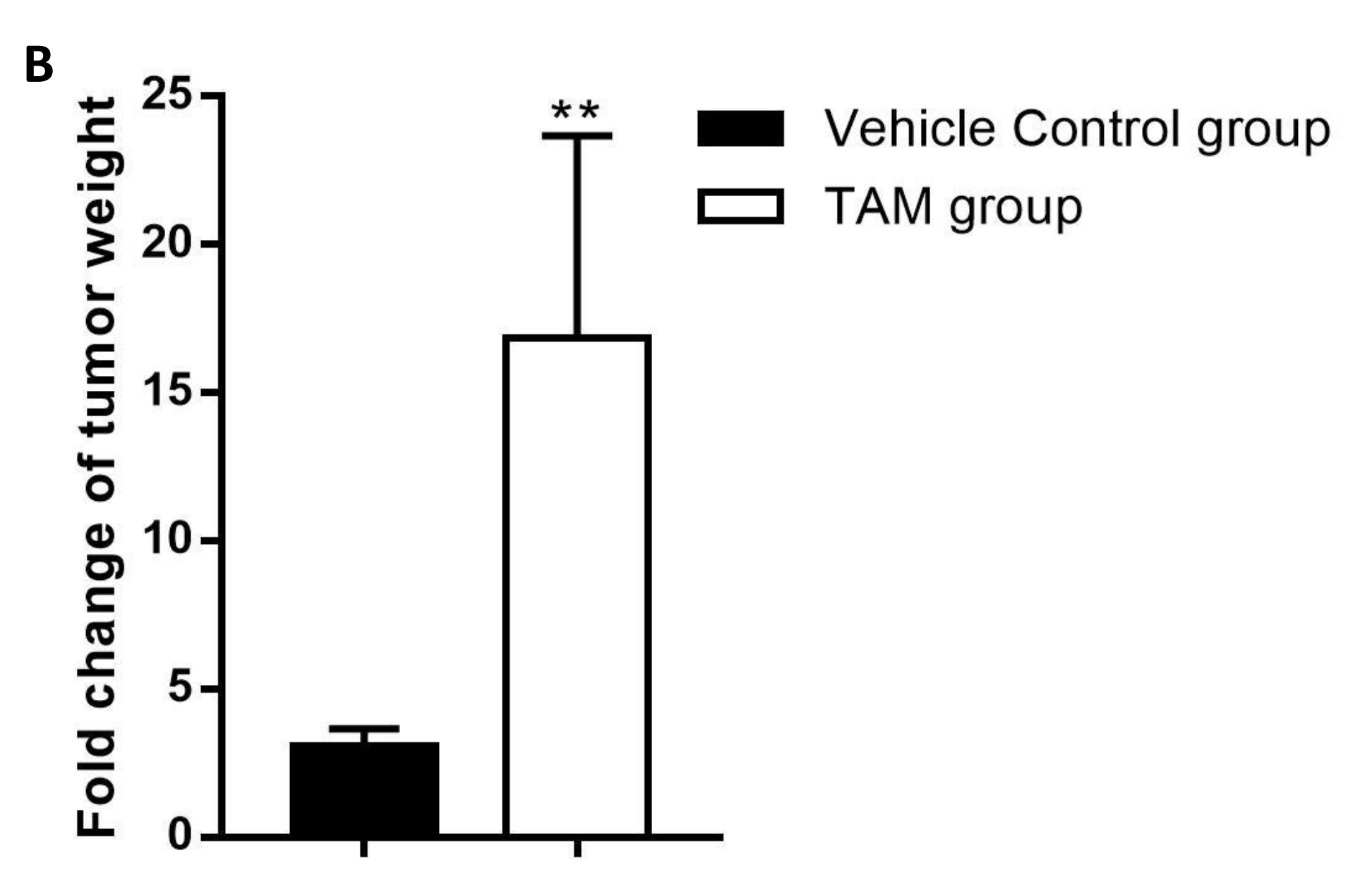
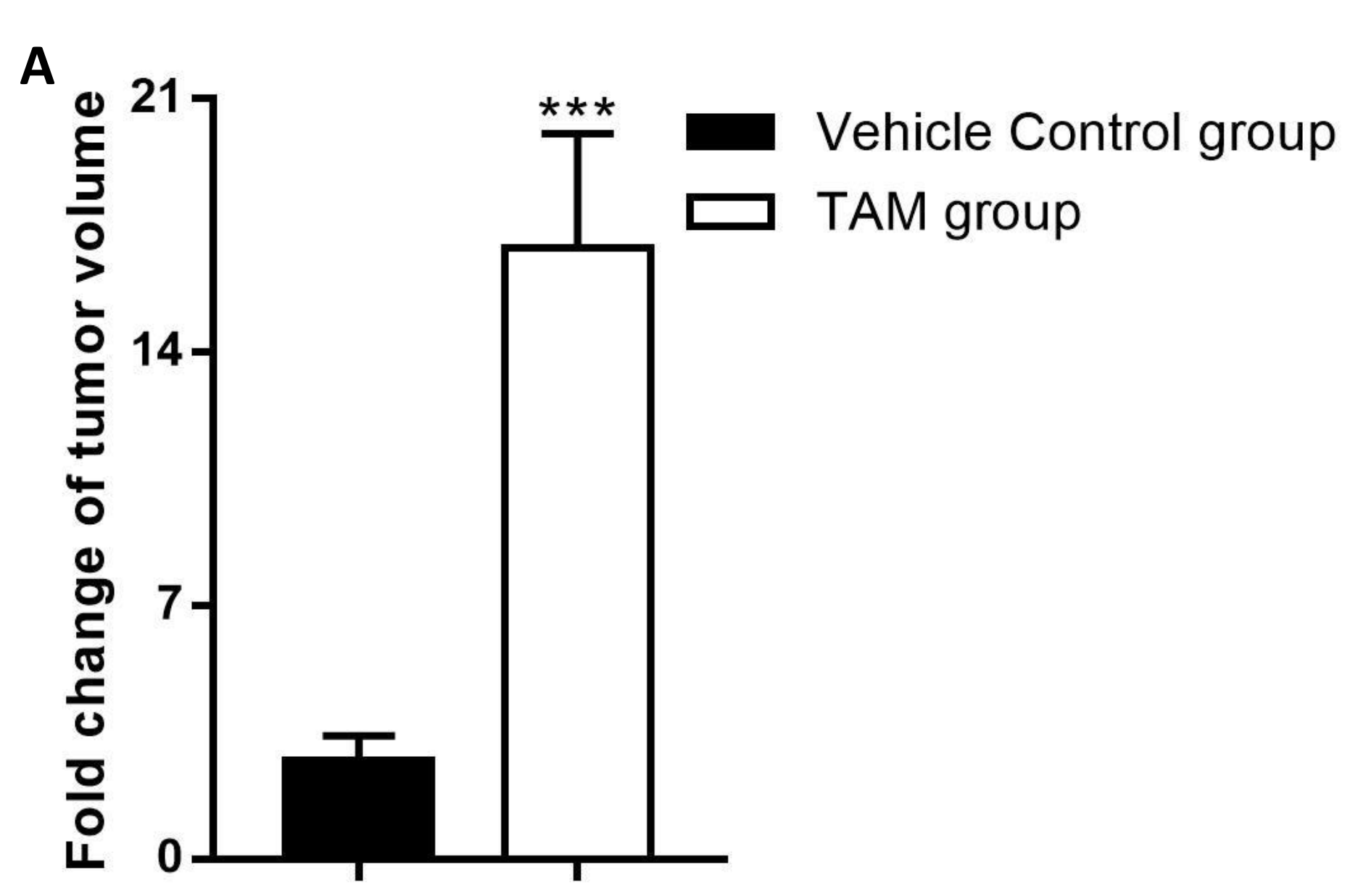


Figure S9

Supplementary Figure Legends

Figure S1: Screening of differently expressed circRNAs between MCF7/P and MCF7/TR cell lines. **A.-H.** Verification of the expression level of other 9 candidates circRNAs in MCF7/P and MCF7/TR cell lines by qRT-PCR. **I.** Information of hsa_circ_0025202 in CircBase database. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S2: Features of MCF7/P and MCF7/TR cell lines. **A.** IC_{50} and **B.** Cell viability assessed by MTT assay in MCF7/P and MCF7/TR treated with TAM or TAM at the indicated time points. **C.** Cell proliferation activity, **D.** colony formation capacity, **E.** cell migration, and **F.** cell apoptosis were measured respectively by MTT, cell colony formation, transwell assays and Annexin V-FITC assay in MCF7/P and MCF7/TR cell lines. Data are presented as means \pm SEM of at least three independent experiments. ** $p < 0.01$, *** $p < 0.001$. Scale bar: 50 μ m.

Figure S3: Hsa_circ_0025202 suppresses growth, metastasis and increases TAM sensitivity of HR-positive BC cells *in vitro*. **A.** Verification of the efficiency of hsa_circ_0025202 ove in MCF7 and T47D cells by qRT-PCR. **B.** Cell proliferation activity, **C.** colony formation capacity, **D.** cell migration potential and **G.** cell apoptosis were measured respectively by MTT, cell colony formation, transwell assay and Annexin V-FITC assay in MCF7 and T47D cells transfected with hsa_circ_0025202 ove. **E. and F.** cell cytotoxicity and IC_{50} assays in MCF7 and T47D cells transfected with or without hsa_circ_0025202 ove. Data are presented as means \pm SEM of at least three independent experiments. * $p < 0.05$, ** $p < 0.01$, ## $p < 0.01$, *** $p < 0.001$, ### $p < 0.001$. Scale bar: 50 μ m.

Figure S4: Knockdown of hsa_circ_0025202 promotes growth, metastasis and decreases TAM sensitivity of HR-positive BC cells *in vitro*. **A.** The expression of hsa_circ_0025202 and *GAPDH* were determined by qRT-PCR in MCF7/TR cells transfected with NC-si and si-circ_0025202. **B.** MTT assay was conducted to evaluate cell proliferative ability. **C.** Colony formation in MCF7/TR cells. **D.** Transwell migration was conducted to evaluate cell migration ability (magnification, $\times 100$). Scale bar: 50 μ m. **E.** Cells were treated with TAM (25 nM) or ethanol (control) and subjected to Annexin V-FITC and propidium iodine staining to detect apoptotic rate. **F.** MTT assay demonstrates

cell viability of MCF7/TR treated together with TAM. **G.** IC₅₀ assay shows the variation in TAM sensitivity. Data are presented as means ± SEM of at least three independent experiments. *p < 0.05, **p < 0.01, ***, ###p < 0.001.

Figure S5: CCK8 assay was utilized for analyzing the cell viability. **A.** Cell viability of MCF7/TR cells transfected with PLCDH or circ_0025202 ove. **B.** Cell viability of MCF7 and T47D cells transfected with NC-si or si-circ_0025202. Data are presented as means ± SEM of at least three independent experiments. *p < 0.05, **p < 0.01.

Figure S6: Has_circ_0025202 sponges miR-182-5p to regulate the tumor progression and TAM sensitivity of BC cells. **A.** Verification of the efficiency of miR-182-5p mimics in MCF7 and T47D cells by qRT-PCR. **B.** Verification of the efficiency of miR-182-5p inhibitor in MCF7/TR cells by qRT-PCR. **C.** Cell proliferation activity, **D.** cell migration potential and **E.** cell apoptosis measured respectively by MTT assay, transwell assay and Annexin V-FITC assay in T47D cells after transfection of miR-182-5p mimics combined with or without hsa_circ_0025202 ove. **F. and G.** MTT analysis for cell cytotoxicity and IC₅₀ in T47D cells treated with TAM after transfection of miR-182-5p mimics combined with or without hsa_circ_0025202 ove. Data are presented as means ± SEM of at least three independent experiments. *, #p < 0.05, **, ##p < 0.01, ***, ###p < 0.001. Scale bar: 50 μm.

Figure S7: FOXO3a downregulation decreases TAM sensitivity of MCF7/TR cells and protects cell from apoptosis. **A.** Verification of the efficiency of si-FOXO3a in MCF7/TR cells by qRT-PCR. **B.** MTT data for the proliferation of MCF7/TR cells treated with TAM after transfection of si-FOXO3a. **C.** MTT data for the cell cytotoxicity and IC₅₀ of MCF7/TR cells treated with TAM after transfection with si-FOXO3a. **D.** Annexin V-FITC assay was used to assess cell apoptosis in MCF7/TR cells transfected with si-FOXO3a. Data are presented as means ± SEM of at least three independent experiments. *p < 0.05, **p < 0.01, ###p < 0.001.

Figure S8: Verification of hsa_circ_0025202 level in the established stable M7/circ_0025202 ovc cells by qRT-PCR. Data are presented as means \pm SEM of at least three independent experiments. ***p < 0.001.

Figure S9: Fold changes of tumor volume and weight in PBS group and TAM group between M7/PLCDH and M7/circ_0025202 ovc group. **A.** Fold changes of tumor volume in PBS group and TAM group between M7/PLCDH and M7/circ_0025202 ovc group. **B.** Fold changes of tumor weight in PBS group and TAM group between M7/PLCDH and M7/circ_0025202 ovc group. Data are presented as means \pm SEM of at least three independent experiments. **p < 0.01, ***p < 0.001.

Table S1. Primers and oligoes used in this study

Name		Sequence
Plasmid construction		
PLCDH-ciR	F	CGGAATTCTGAAATATGCTATCTTACAGCAATGCCTCCTGCACCACCAA
	R	CGGGATCCTCAAGAAAAAATATATTCACCAGGAAATGAGCTTGACAAA
hsa_circ_0025202 wt	F	AGCTTTGTTTAAACCAATGCCTCCTGCACCACCAA
	R	CCGCTCGAGCAGGAAATGAGCTTGACAAAGTG
hsa_circ_0025202 mut	F	CTAGAAAAACCTCGGTTTTATGATGACATCAAG
	R	CTTGATGTCATCATAAAACCGAGGTTTTTCTAG
FOXO3a 3'UTR wt1	F	CCGCTCGAGAGGATCACTGAGGAAGGGGAAGTG
	R	GCTCTAGAGCTGTCCTCCACTGGCAGGCG
FOXO3a 3'UTR wt2	F	CCGCTCGAGACCCGTCCAGGACAGAACCGT
	R	GCTCTAGATGCCTCTCACTCATACTTCTAGC
FOXO3a 3'UTR mut1	F	CTTGCTGAGAGCAGAGGGTTACCAGGGTTTTCTCTGTA
	R	TACAGAGAAAACCCCTGGTAACCCTCTGCTCTCAGCAAG
FOXO3a 3'UTR mut2	F	AACCCTTTAGTGACAGGGTTACCTGAGTGG AGAGCTGA
	R	TCAGCTCTCCACTCAGGTAACCCTGTCACATAA
RT-PCR		
hsa_circ_0052375 (TRIM28)	div-F	CCAACCAGCGGAAATGTGAG
	div-R	TGCACACGCTTCTGTACGTC
hsa_circ_0007556 (APP)	div-F	GGTGGGCGGTGTTGTCATA
	div-R	GGCATGAGAGCATCGTTTCC
hsa_circ_0007647 (THAP4)	div-F	CTGTCCGCCAGCCGC
	div-R	CGGTCAGGTGGAAGATGGATG
hsa_circ_0014788 (HDGF)	div-F	TGCTGACTGTAGCTTTGGAAGT
	div-R	GGCTGTTGATTTACGGCAG
hsa_circ_0097922 (PGAM5)	div-F	ATGGCGTGGGGTTTAAGGTG
	div-R	ATCTTGTCCGGAGGCATGAA
hsa_circ_0000837 (RBBP8)	div-F	TCCAAGCAGCAGATGAAGAG
	div-R	TCTTGAACACCAAGTCCAAGTG
hsa_circ_0001355 (RSRC1)	div-F	GTCGAAGTCGTTCAAGGGT
	div-R	GCTCGAGGAGGACCGTCTA
hsa_circ_0007761 (ATXN7)	div-F	AATCTGTGGGTTGAGGC
	div-R	GCTCCGACATTCTTTCC
hsa_circ_0123878 (CDCP1)	div-F	TGTGACCAAGATGCCCAAAGT
	div-R	GGGTCCCCAGCTTTATGAGAA
ACTIN	F	CACCATTGGCAATGAGCGGTTT
	R	AGGTCTTTGCGGATGTCCACGT
GAPDH	F	GTCTCCTCTGACTTCAACAGCG
	R	ACCACCCTGTTGCTGTAGCCAA
hsa_circ_0025202 (GAPDH)	div-F	TCATTTCTGCAATGCCTCCT
	div-R	ATGATGTTCTGGAGAGCCCC
ACTIN	div-F	GATGTGGATCAGCAAGCAGG
	div-R	TACTTCAGGGTGAGGATGCC
FOXO3a	F	TCTACGAGTGGATGGTGCGTTG
	R	CTCTTGCCAGTTCCCTCATTCTG
miR-182-5p	F	TTTGGAATGGTAGAACTCACACT
miR-34a-5p	F	TGGCAGTGTCTTAGCTGGTTGT
miR-556-5p	F	GATGAGCTCATTGTAATATGAG
miR-490-5p	F	CCATGGATCTCCAGGTGGGT
miR-197-3p	F	TTCACCACCTTCTCCACCCAGC
miR-516b-5p	F	ATCTGGAGGTAAGAAGCACTTT
miR-885-3p	F	AGGCAGCGGGGTGTAGTGGATA
miR-1271-5p	F	CTTGGCACCTAGCAAGCACTCA
miR-382-5p	F	GAAGTTGTTTCGTGGTGGATTTCG
miR-449a	F	TGGCAGTGTATTGTTAGCTGGT
miR-183-5p	F	TATGGCACTGGTAGAATTCCT

miR-34c-5p	F	AGGCAGTGTAGTTAGCTGATTGC
miR-449b-5p	F	AGGCAGTGTATTGTTAGCTGGC
miR-1225-3p	F	TGAGCCCCTGTGCCGCCCCAG
miR-4306	F	TGGAGAGAAAGGCAGTA
miR-185-5p	F	TGGAGAGAAAGGCAGTTCCTGA
miR-940	F	AAGGCAGGGCCCCCGCTCCCC
miR-1827	F	TGAGGCAGTAGATTGAAT
miR-215-5p	F	ATGACCTATGAATTGACAGAC
miR-491	F	AGTGGGGAACCCTTCCATGAGG
miR-657	F	GGCAGGTTCTCACCTCTCTAGG
miR-609	F	AGGGTGTCTCTCATCTCT
miR-520h	F	ACAAAGTGCTTCCCTTTAGAGT
miR-520g	F	ACAAAGTGCTTCCCTTTAGAGTGT
snRNA U6 F	F	GCGCGTCGTGAAGCGTTC
snRNA U6 R	R	GTGCAGGGTCCGAGGT

RNA oligos

si-circ_0025202-1	sense	UCCUGCAAUGCCUCCUGCATT (5'-3')
	antisense	UGCAGGAGGCAUUGCAGGATT (5'-3')
si-circ_0025202-2	sense	AAGCUCAUUUCCUGCAAUGTT (5'-3')
	antisense	CAUUGCAGGAAAUGAGCUUTT (5'-3')
miR-182-5p mimics	sense	UUUGGCAAUGGUAGAACUCACACU (5'-3')
	antisense	UGUGAGUUCGACCAUUGCCAAAUU (5'-3')
miR-182-5p inhibitor	sense	AGUGUGAGUUCUACCAUUGCCAAA (5'-3')
si-FOXO3a	sense	CAACCUGUCACUGCAUAGU (5'-3')
	antisense	ACUAUGCAGUGACAGGUUG (5'-3')
NC-si	sense	UUCUCCGAACGUGUCACGUTT (5'-3')
	antisense	ACGUGACACGUUCGGAGAATT (5'-3')
NC	sense	CAGUACUUUUGUGUAGUACAA (5'-3')
